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Synthetic Studies of Complex Ansa-Bridged Prodiginines and Constrained Fluorinated Peptidic Polycycles

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Chemistry

by

Angel Mendoza

2023

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2023

ABSTRACT OF THE DISSERTATION

Synthetic Studies of Complex Ansa-Bridged Prodiginines and Constrained Fluorinated Peptidic Polycycles

by

Angel Mendoza

Doctor of Philosophy in Chemistry

University of California, Los Angeles, 2023

Professor Patrick G. Harran, Chair

Chapter 1 provides an introduction to the biological significance of natural product prodiginines such as streptorubin, roseophilin, and the marineosins. This includes a brief discussion of structural features and biological mechanisms of action. The prodiginine of interest in our synthetic studies concerned marineosin A. This chapter then discusses how we used our previously published route to scale up the synthesis of marineosin A, challenges encountered, and how we were able to accomplish one of our goals of accessing novel synthetic analogs. We then segue into mechanistic studies concerning an atypical acid-promoted migration of a hydroxy-

propyl sidechain that affords a new unprecedented class of synthetic rearranged prodiginines. We then postulate a potential operative mechanism based on data obtained experimentally and computationally.

Chapter 2, we show that unprotected peptides react with commercial octafluorocyclopentene (OFCP) to afford fluorinated macrocycles via successive ipso substitutions of vinyl fluoride. We quantify relative reaction rates and establish sequence variations having combinations of cysteine, tyrosine, histidine, and serine residues. Compounds bearing permutations of these functional groups are shown to react rapidly at room temperature to give stable, isolable products. We also introduce our initial discoveries involving polysubstitution cascades with OFCP.

Chapter 3, we further probe and diversify our polysubstitution cascades of linear unprotected peptides with OFCP to generate complex fluorinated polycycles. The reactions occur in a single flask at 0–25 ºC and require no catalysts or heavy metals. OFCP can directly polycyclize linear sequences using native functionality, or fluorospiroheterocyclic intermediates can be intercepted with exogenous nucleophiles. The latter tactic generates molecular hybrids composed of peptides, sugars, lipids, and heterocyclic components. Building on this strategy we also develop novel small-molecules as inserts to afford orthogonal cyclizations and diversify our synthetic outcomes. This platform can create stereoisomers of both single- and double-looped macrocycles. Calculations indicate that the latter can mimic diverse protein surface loops. Subsets of the molecules have low energy conformers that may shield their polar surface area through intramolecular hydrogen bonding. A significant fraction of OFCP-derived macrocycles tested show moderate to high passive permeability in parallel artificial membrane permeability assays (PAMPA).

The dissertation of Angel Mendoza is approved.

Ellen May Sletten

Jose Alfonso Rodriguez

Craig A. Merlic

Patrick G. Harran, Committee Chair

University of California, Los Angeles

2023

This dissertation is dedicated to my biggest supporter, my mom Minerva.

Aut viam inveniam aut faciam

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Curriculum Vitae

Education

University of California Los Angeles (UCLA), Los Angeles, CA — July 2018 – Present
Doctor of Philosophy (Ph.D.), Organic Chemistry — Expected September 2023
Candidate of Philosophy (C. Phil.), Organic Chemistry — September 2020
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University of California San Diego (UCSD), La Jolla, CA — August 2014 – June 2018
Bachelor of Science (B.S.), Chemistry/Molecular Synthesis (ACS certified)

Professional Experience

University of California Los Angeles (UCLA), Los Angeles, CA — July 2018 – Present
Graduate Student Researcher, Advisor: Prof. Patrick Harran
Worked on 3 main collaborative research projects: peptidomimetic methods development, medicinal chemistry, and mechanistic studies on 'prodiginine' small-molecules

- **Project 1:** Developed novel macrocyclization strategies to afford 5 new classes of peptidomimetic, fluorinated macrobicyclic structures
    - Developed novel small-molecules as inserts to enable orthogonal ring closings in macrocycles derived from Friedel-Craft cyclizations and macrolactam/cyclodepsipeptide formations
    - Determined membrane permeability of novel peptidomimetic structures using PAMPA and used this data to postulate structure-activity relationships
- **Project 2:** Isolated and characterized the 'prodiginine' natural product (+)-marineosin A and 3 of its novel synthetic analogs for biological testing in collaboration with the Jared Rutter Lab (University of Utah)
- **Project 3:** Investigated an atypical acid-promoted condensation reaction that generated a new class of synthetic prodiginines derived from a pseudo [1,7]-sigmatropic rearrangement in collaboration with the Ken Houk Lab (UCLA)

Takeda California, Inc., San Diego, CA — August 2016 – June 2018
Medicinal Chemistry Intern, Advisor: Thomas Nixey

- Synthesized novel small-molecule kinase inhibitors with applications towards a CNS disorder
- Library synthesis via amide couplings and cross-couplings; Suzuki-Miyaura couplings and Pd-catalyzed carbonylations
- Analyzed assay data to work towards establishing a structure-activity relationship with respect towards antagonist drug potency
- Small-molecule synthesis via convergent, linear, and parallel synthesis; ran up to 40 reactions in parallel

University of California San Diego (UCSD), La Jolla, CA — September 2016 – April 2018
Undergraduate Student Researcher, Advisor: Prof. Valerie Schmidt

- Developed catalysts to furnish cross-couplings between electron-rich π-systems and thiols
- Screened conditions to afford the intermolecular anti-Markovnikov hydroamination of alkenes
- Synthesized novel intermediates, N-heterocyclic carbenes, and cyclic alkyl amino carbene ligands for senior graduate students

Publications and Patents

1. **Mendoza, A.**[‡]; Bernardino, S. J.[‡]; Dweck, M. J.; Valencia, I.; Evans, E.; Tian, H.; Lee, W.; Li, Y.; Houk, K. N.; Harran, P. G. "Cascade Synthesis of Fluorinated Spiroheterocyclic Scaffolding for Peptidic Macrobicycles" *J. Am. Chem. Soc*., **2023**, 145, 15888–15895.
https://doi.org/10.1021/jacs.3c03071

2. Tsunemi, T.; Bernardino, S. J.; **Mendoza, A.**; Jones, C. G.; Harran, P. G. "Syntheses of atypically fluorinated peptidyl macrocycles via sequential vinylic substitutions" *Angew. Chem. Int. Ed.*, **2020**, 132, 684–688.
https://doi.org/10.1002/ange.201910136

3. Harran, P. G.; **Mendoza, A**; Bernardino, S. J.; Dweck. M. J. "Methods of Synthesizing Macrocycles" **2023**, Provisional Patent. US 62/489,603 (Attorney Docket No.: UCH-35960; UCLA Ref. No.: 2023-139-1).

Honors and Awards

- Chemists Association Dissertation Award — June 2023
- UCLA Chemistry Admissions Committee Member — December 2022
- Merck Research Award for Underrepresented Chemists of Color (Finalist) — May 2021
- NSF GRFP Fellowship — April 2020 – June 2023
- Ford Foundation Fellowship (Honorable Mention) — March 2020
- Christopher S. Foote Fellowship — September 2019
- Eugene V. Cota Robles Fellowship — March 2018 – Present
- Competitive Edge Summer Fellowship — July 2018
- UCSD Provost Honors — December 2016 – June 2018

Chapter 1 – Synthesis and Mechanistic Studies of Complex Ansa-Bridged Prodiginine Macrocycles

1.1 Introduction

Apoptosis is a genetically encoded biochemical pathway used by higher organisms to remove damaged or diseased cells. Deregulation of this process is a key factor in cancer development.[1] This form of programmed cell death is a highly regulated process moderated by two antagonistic classes of proteins which reside within the mitochondria. The two classes of proteins, termed *pro-death* and *pro-survival*, are members of the *B-cell lymphoma-2* (Bcl-2) family of proteins and interact with one another via a great number of protein-protein interactions (PPI's).[2-4] Overexpression of the Bcl-2 *pro-survival* proteins can lead to dysregulation of the apoptotic process resulting in the uncontrolled, over proliferation of cells such as cancer.[5] When put under stress, cells release a class of proteins known as *BH3-only* proteins. These α-helix proteins function to bind to *pro-survival* proteins in an inhibitive manner, allowing *pro-death* proteins to carry out a cascade of events which results in the eventual death of the cell. These *BH3-only* proteins bind to *pro-survival* proteins at a specific hydrophobic groove known as the BH3 receptor site.[6] The design of compounds which serve as *BH3-only* protein mimics offers a promising approach to fighting diseases caused by the dysregulation of apoptosis. The prodiginine family of compounds show great promise in serving as such mimics (**Fig. 1.1**). These compounds range from simple prodigiosins (**2**–**3**) to more complex ansa-bridged pyrrolophane structures (**4**–**6**, **1.1a**). Interest stems from Shore's findings that streptorubin B (**4**) encourages apoptosis in cell cultures, presumably from PPIs with Bcl-2 proteins.[7] Obatoclax (**1**) is a fully synthetic analog of streptorubin B that reached phase II clinical trials for leukemia and myeloma, where overexpression of Mcl-1 is critical for disease maintenance.[8,9]

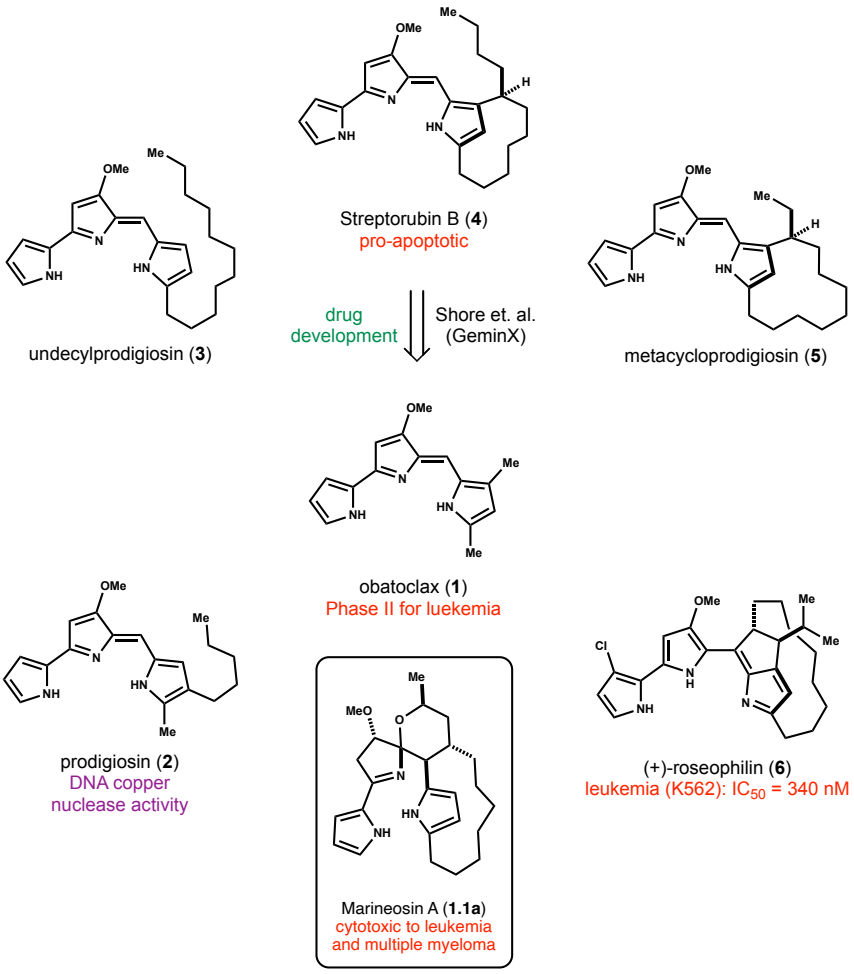


Fig. 1.1 Natural and Synthetic Prodiginine Alkaloids

Obatoclax was described as a BH3 helix mimetic. However, studies indicate obatoclax also exhibits off-mechanism cytotoxicity.[10] The development of improved compounds of this general chemotype with enhanced pharmacological properties and reduced off-target toxicity is desired. Moreover, our lab has also completed the total syntheses of complex prodiginines such as (+)-roseophilin (**6**) and Marineosin A (**1.1a**).[11,12] Both compounds possess sub micromolar toxicity against several human cancer cell lines.[13-15] Marineosin A was of particular interest as its mechanism of action is unknown, possesses 0.5 μM inhibition against HCT-116 cell lines, and its structure is atypical for prodiginines as it possesses an interrupted chromophore stemming from its spiroiminal motif.[14] Using our laboratory's previous published synthetic route, it was an interest

to scale up the synthesis of marineosin A and sythesize synthetic analogs to probe biological activity against various cancer cell lines.

1.2 Synthesis of Marineosin A and its synthetic analogs

We based our synthesis of **1.1a** on our lab's previous published 8-step route.[12] Our initial synthesis of marineosin A afforded 2.0 mg and none of its diastereomeric analogs. A synthetic goal during our optimization efforts was to isolate its synthetic diastereomeric product and potentially access natural product marineosin B (not shown). Starting with commercially available 2,6-dichloropyridine (**1.2**), we subjected this reagent to a large-scale Kumada cross-coupling (50 g of 1,8-dibromooctane x 2) to obtain pyridinophane **1.3** (**Fig. 1.2A**). We further optimized this step by incorporating a distillation and discarding a flash column chromatography purification (see SI for details). **1.3** was then subjected to an oxidation with mCPBA to afford pyridinophane N-oxide (**1.4**) as a crude oil. Previously, **1.4** was purified by a flash column chromatography, but after optimization we were able to incorporate a recrystallization with boiling pentane to afford **1.4** as a crystalline solid. **1.4** was then subjected to a flow photolysis under irradiation (254 nm) to afford desired ketopyrrolophane **1.5** in 27% isolated yield (further optimized from the previous 25%). Our colleagues (Dr. Evan Hurlow and Mr. Morris Dweck) have performed extensive mechanistic studies probing this transformation and have established an operative mechanism supported by DFT calculations. Data indicates the mechanism proceeds via formation of an oxaziridine intermediate followed by cleavage of this 3 membered intermediate into a 1,2-oxazepine structure. Ketopyrrolophane **1.5** was then treated with excess LDA (3.1 equiv) and this lithiated intermediate alkylated with excess S-propylene oxide to afford hydroxy-ketopyrrolophane product **1.9a** (40%). **1.9a** was then subjected to an acid-mediated condensation

with bis-pyrrole (see SI for synthetic scheme to access heterobiaryl nucleophiles) to afford desired synthon isopremarineosin **1.11a** and unexpected rearranged product **1.12a**.

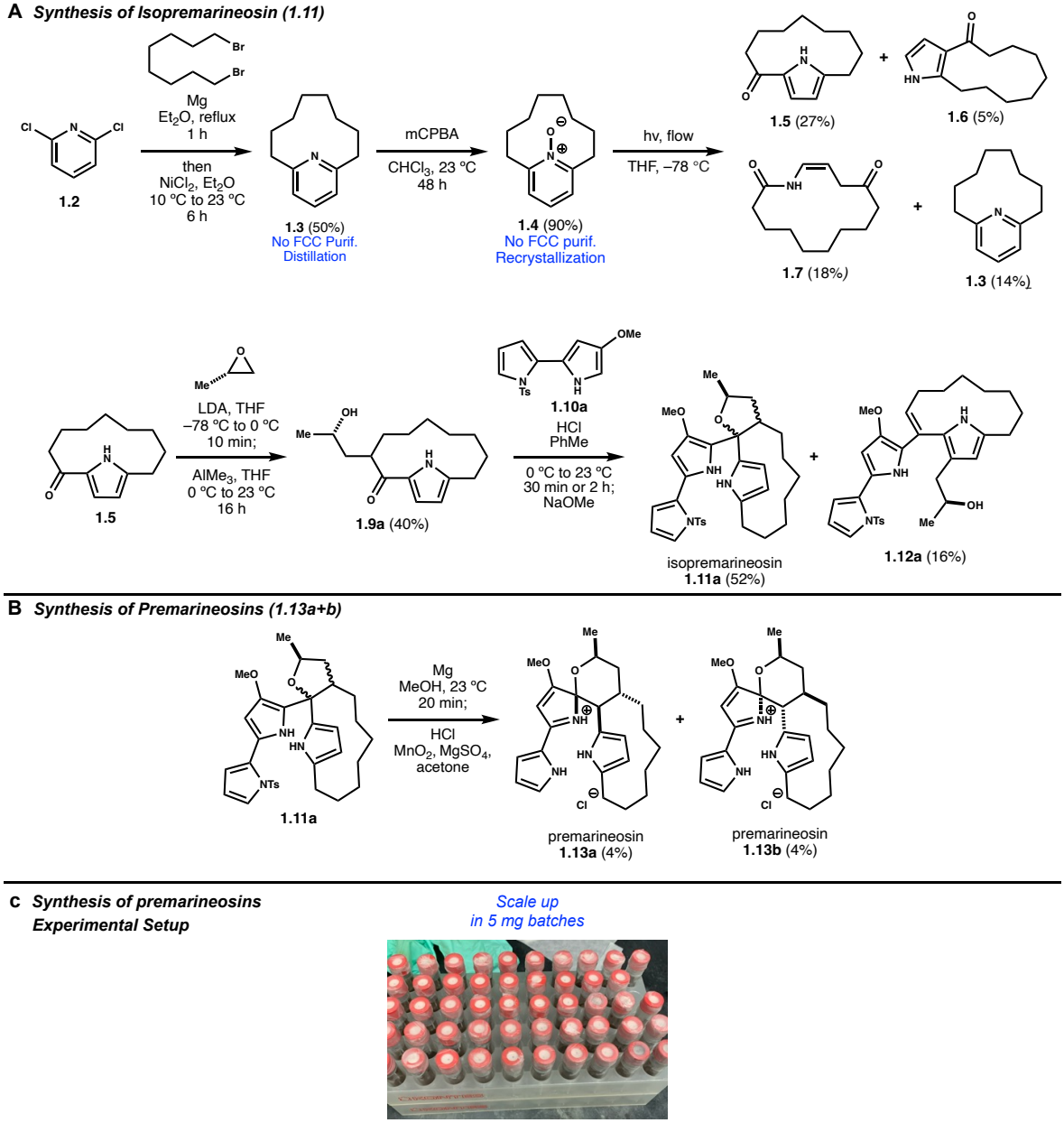


Fig. 1.2. Route Optimization (**A**) Synthesis of Isopremarineosin (**1.11a**). (**B**) Synthesis of Premarineosins (**1.13a**+**b**). (C) Synthesis of Premarineosins Experimental Setup. Isopremarinesoin isolated as a 1:1 mixture of diastereomers. For experimental procedures – see SI for details.

Isopremarineosin (**1.11**) was then subjected to a Mg-mediated detosylation and immediately acidified with HCl (**Fig. 1.2B**). This aza-fulvene intermediate was then treated with excess 'acidic' MnO_2 in 5 mg batches to initiate a redox-neutral cycloisomerization and access desired **1.13a** and diastereomeric **1.13b** premarineosins (8% total yield). This MnO_2 mediated cycloisomerization was a synthetic bottleneck and caused material throughput to be the major challenge. The yields for this cycloisomerization ranged from 0–8% in each trial run and we were limited to 5 mg batches of the acidified aza-fulvene intermediate (**Fig. 1.2C**). Higher batch loadings would afford 0% yields. Moreover, intermediates were very air / water sensitive and prone to decomposition. The reaction would only be productive if we 'freshly prepared acidic' MnO_2 (prepared via the oxidation of $MnSO_4$ with $KMnO_4$; see SI for details). Furthermore, we lacked adequate quality controls to check our 'freshly prepared acidic' MnO_2 reagent other than detecting for the presence of Mn^{2+} ions (see SI for details). The highest observed yield for the cycloisomerization was 8% but 0% yields consistently plagued this step with the average over multiple trials being <1% yield of premarineosins. Premarineosin products could not be separated via SiO_2 purifications. Originally, desired premarineosin **1.13a** was separated from its diastereomeric analog **1.13b** via preparative HPLC, but HPLC caused diastereomer **1.13b** to decompose presumably due to instability in solution with 0.1% TFA in H_2O/MeCN. In an effort to isolate both analogs we decided to telescope both compounds forward as mixtures and separate them in our final hydrogenation step.

Premarineosins **1.13a** and **1.13b** were then subjected to a hydrogenation with Rosenmund's catalyst ($Pd/BaSO_4$) to reduce the methyl enol ether moiety (**Fig. 1.3A**). Reduction afforded desired Marineosin A (**1.1a**) and its corresponding 'Diastereomer' analog **1.14a** as the major product in quantitative yield (40% and 60% respectively). Purification and separation of both of these structurally similar products proved to be very challenging as both compounds could not be separated by preparative reverse-phase HPLC, and both saw significant degradation upon concentration from 0.1% TFA in H_2O/MeCN. Separation of both these compounds was particularly challenging via preparative TLC as conventional solvent systems such as EtOAc/Hex, CH_2Cl_2/Hex, EtOAc/PhMe, etc. did not separate these products. After much empirical trial purification experiments, it was elucidated that a 5 component solvent system consisting of Hex:EtOAc:Acetone:MeOH:NEt_3 (ratio: 171:19:6:4:2) worked very well to separate this product mixture in quantitative yield (**Fig. 1.3B**).

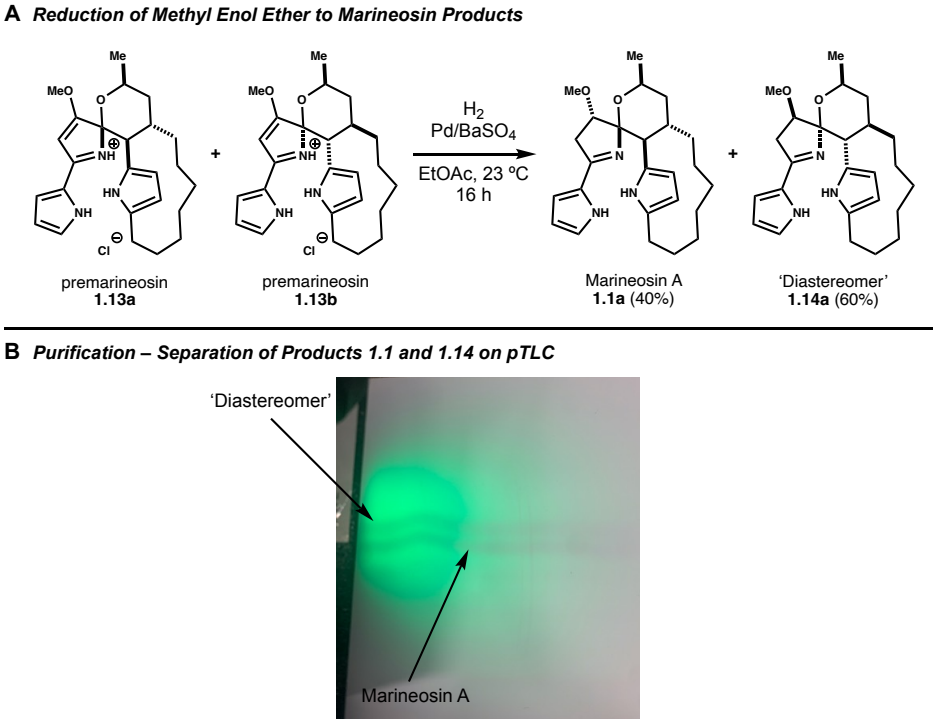


Fig. 1.3 Isolation and Purification of Marineosin A and 'Diastereomer' Analog (**A**) Reduction of Methyl Enol Ether to Marineosin Products. (**B**) Purification – Seperation of Products **1.1** and

1.14 on pTLC. Visualization of bands – 254 nm light. 5 component solvent system – Hex:EtOAc:Acetone:MeOH:NEt_3, ratio: 171:19:6:4:2.

With our optimized synthetic route and established purification conditions we decided to synthesize antipode analogs of marineosin A with this stereochemistry set as (R) on the methyl pyran instead of the natural (S) (**Fig. 1.4A**). This stereochemistry being set with (R)-propylene oxide instead of (S)-propylene oxide during our alkylation of ketopyrrolophane **1.5** (**Fig. 1.2A**). Identical processing of intermediates afforded ent-premarineosins (**1.13c** and **1.13d**) that were then reduced and subjected to our optimized purification with our 5 component solvent system. Novel antipode marineosin A (**1.1b**) and its 'Diastereomer' **1.14b** (quantitative yield of **1.1b** and **1.14b** from premarineosins) were readily purified and separated on pTLC (**Fig. 1.4B**).

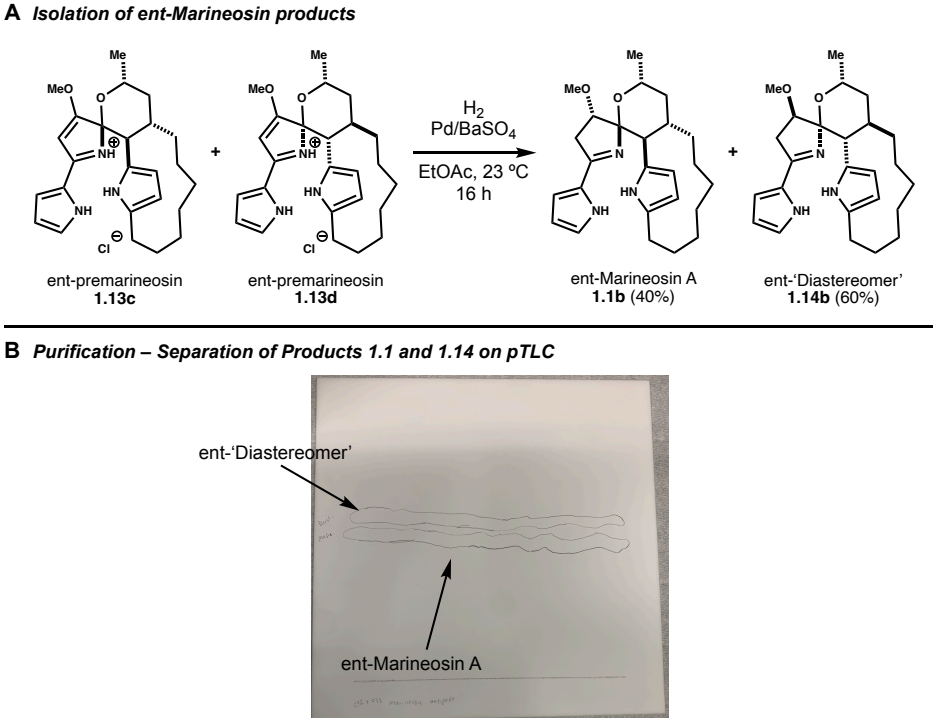


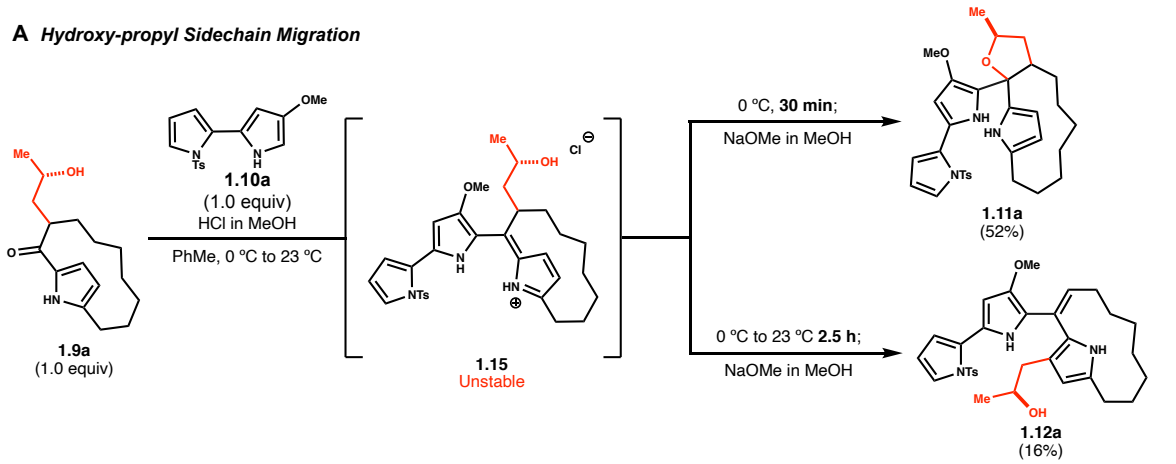
Fig. 1.4 Isolation and Purification of ent-Marineosin A and ent-'Diastereomer' Analog (**A**) Reduction of Methyl Enol Ether to ent-Marineosin Products. (**B**) Purification – Seperation of Products **1.1b** and **1.14b** on pTLC. Visualization of bands – 254 nm light. 5 component solvent system – Hex:EtOAc:Acetone:MeOH:NEt_3, ratio: 171:19:6:4:2.

In summary ~1 g of isopremarineosin (**1.11a**) was pushed through our cycloisomerization and ~200 dram vials (5 mg scale) used in our cycloisomerization step. Overall, 1.47 mg of marineosin A, 840 µg of its 'diastereomer', 870 µg of ent-marineosin A, and 1.15 mg of its 'ent-diastereomer' were isolated. All these spiroiminal products (**1.14a**, **1.1b**, and **1.14b**) with the exception of natural marineosin A (**1.1a**) are novel, previously unreported structures in the literature. These 4 structures were sent out to our collaborators at the Jared Rutter lab at the University of Utah for biological testing. Unfortunately, preliminary results were inconclusive as these samples were stored for months before assays were run. Degradation of these products is a major concern as related prodiginine structures tend to decompose under prolonged storage if not under air-free conditions and frozen in dry PhH.

1.3 Mechanistic Studies on the Migrating Hydroxy-Propyl Sidechain Moiety

En route to our synthesis of marineosin A we observed an atypical acid-promoted condensation reaction to afford expected isopremarineosin product **1.11a** but unexpected hydroxy-propyl side-chain migration product **1.12a** (**Fig. 1.5A**).[12] Rearranged product **1.12a** is a member of a new class of synthetic prodiginines with unknown biological activities. We initially hypothesized this transformation proceeded through a [1,7]-sigmatropic rearrangement via aza-fulvene intermediate **1.15**. Aza-fulvene intermediate **1.15** was very unstable (air and moisture sensitive) and efforts to isolate it resulted in complete degradation into intractable products, presumably due to the inherent instability of the electron-rich trispyrrolic π-system. We hypothesized the inherent instability of our aza-fulvene intermediate constituted it as a high energy species that was predisposed to undergo a sigmatropic rearrangement.

A ***Hydroxy-propyl Sidechain Migration***



B Timepoint Optimization

entry	time (h)	1.11a	1.12a
1	0.5	52%	12%
2	1.0	50%	13%
3	1.5	49%	13%
4	2.0	45%	14%
5	**2.5**	**43%**	**16%**
6	4.0	33%	12%
7	8.0	5%	8%
8	16	0%[a]	0%
9	24	0%[a]	0%

Fig. 1.5. Atypical migrating hydroxy-propyl moiety. (A) Hydroxy-propyl side-chain migration via unstable aza-fulvene intermediate **1.15**. (B) Optimization of time-point to obtain highest isolated yield of **1.12a**. Condensation of **1.9a** and **1.10a** affords aza-fulvene intermediate **1.15**. Immediate base quench with base affords predominantly **1.11a**. Base quench after 2.5 h affords appreciable amount of **1.12a** and diminished yield of **1.11a**.

Originally, we were focused with obtaining an optimal yield of marineosin synthon **1.11a** with the ideal timepoint established being 30 min (52%) although rearranged **1.12a** (12%) was observed. Unfortunately, intermediates could not be reliably monitored by analytical HPLC (0.1% TFA in MeCN/H_2O or 0.1% formic acid in MeCN/H_2O) as isopremarineosin would instantly convert to its aza-fulvene intermediate **1.15a** when exposed to acid which caused both **1.11a** and **1.12a** to have identical retention times. Efforts to establish HPLC methods not incorporating acid caused significant decomposition of all products into unidentifiable peaks and caused M/Z signals to be significantly attenuated. In lieu of reliable analytical methods to track intermediates we resorted to isolated yields of products. It was elucidated that a 2.5 h timepoint was optimal for rearranged **1.12a** (16%) and with a diminished yield of **1.11a** (43%) (entry 5; **Fig. 1.5B**). Longer reaction times of 8–24 h resulted in low yields and / or complete degradation into intractable products.

We initially hypothesized that the operative mechanism for observed sidechain migration was a unimolecular [1,7]-sigmatropic rearrangement of the hydroxy-propyl sidechain moiety. Treatment of **1.11a** to form aza-fulvene intermediate **1.15** should have readily transformed to rearranged **1.12a** if this transformation was indeed a sigmatropic rearrangement. Surprisingly, treatment of **1.11a** with acid and stirring for 2.5 h did not afford any detectable rearrangement product **1.12a** (**Fig. 1.6**). Stirring aza-fulvene intermediate **1.15** for longer reaction times (2.5–24 h) gave slow degradation of aza-fulvene into intractable products and no detectable **1.12a**. Additionally, we detosylated **1.11a** and treated this intermediate with acid to form its

corresponding aza-fulvene intermediate. This detosylated aza-fulvene intermediate likewise did not give any detectable rearranged product after 2.5 h and decomposed very readily. In addition to **1.11a** we acidified other tetrahydrofuran derivatives (**1.11d-1.11f**) which afforded identical results as isopremarineosin. We concluded that the operative mechanism was not unimolecular nor a sigmatropic rearrangement.

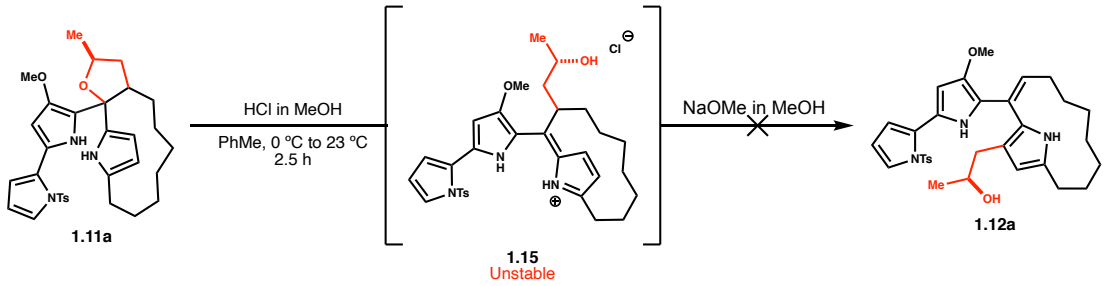


Fig. 1.6. Isopremarineosin does NOT participate in a [1,7]-sigmatropic rearrangement. (A) Hydroxy-propyl sidechain migration via aza-fulvene intermediate **1.15**.

After we concluded that the operative mechanism of this transformation did not proceed through a unimolecular mechanism, we then suspected that a bimolecular mechanism would be operative. When we acidified isopremarineosin **1.11a** (**1.0 equiv**) in the presence of hydroxy-ketopyrrolophane **1.9a** (**1.0 equiv**), after 2.5 h we were surprised to observe complete consumption of **1.9a** and formation of appreciable amounts of rearranged **1.12a** (36%) (**Fig. 1.7A**). We then postulated a bimolecular mechanism was operative where the hydroxy-propyl ketopyrrolophane **1.9a** served as a promotor and possibly the source of the hydroxy-propyl sidechain moiety present in the rearranged product. In further support of this hypothesis, when we condensed **1.9a** (**2.0 equiv**) with bispyrrole **1.10a** (**2.0 equiv**); complete consumption of **1.9a** and a quantitative yield of condensed products **1.12a** (40%) and **1.11a** (60%) was observed (**Fig. 1.7B**). This was markedly different when only **1.0 equiv** of **1.9a** was utilized to afford 59% yield of condensation products (**Fig.1.5B**). We also screened ketopyrrolophane **1.5** as a promoter; acidification in the presence of **1.11a** afforded no reaction and recovery of starting materials further indicating the importance of the hydroxy-propyl moiety present in **1.9a** in the mechanism (**Fig. 1.7C**). Expectedly, acidification of solely hydroxy-propyl ketopyrrolophane **1.9a** afforded no rearranged product **1.12b** further demonstrating the importance of both **1.9a** and **1.11a** being present to furnish rearrangement (**Fig. 1.7D**).

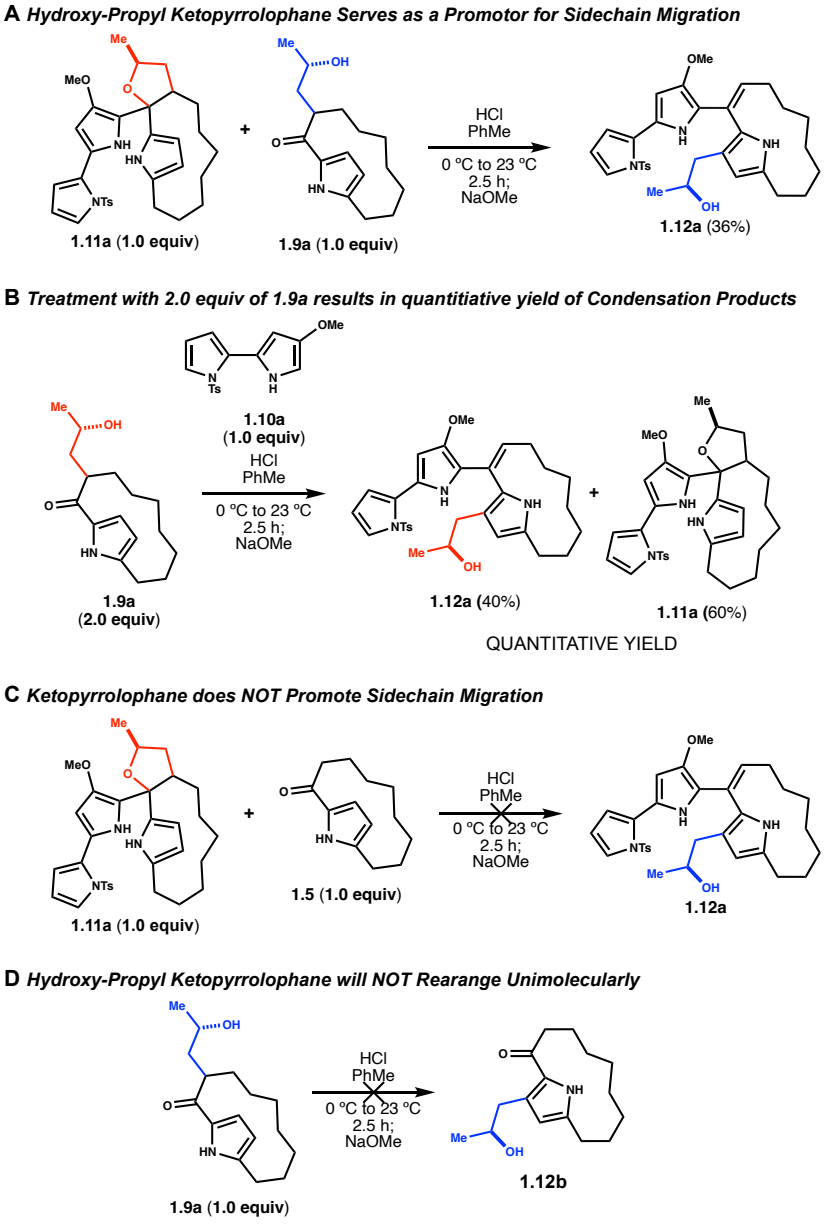
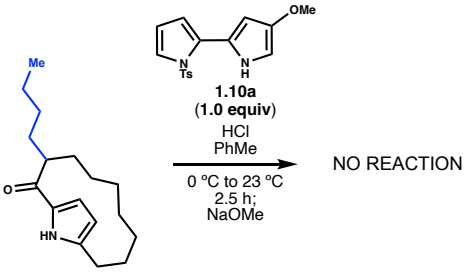


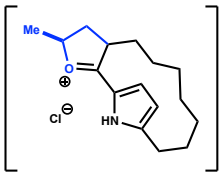
Fig. 1.7. **Control Reactions Probing a Bimolecular Mechanism**. (**A**) Hydroxy-Propyl Ketopyrrolophane Serves as a Promotor for Sidechain Migration. (**B**) Treatment with 2.0 equiv of **1.9a** results in quantitative yield of Condensation Products. (**C**) Ketopyrrolophane does NOT Promote Sidechain Migration. (**D**) Hydroxy-Propyl Ketopyrrolophane will NOT Rearrange Unimolecularly. For experimental procedures – see SI for details.

After we concluded **1.9a** was a promotor in sidechain migration, we then decided to probe this motif. We prepared des-hydroxy derivative **1.9b** and attempted to condense it onto bispyrrole **1.10a** (**Fig. 1.8A**). Surprisingly, no reaction was observed with the exception of recovered starting materials. We then concluded for alkylated ansa-bridged ketones that oxonium formation was necessary in the mechanism for productive condensation (**Fig. 1.8B**). We then proceeded to synthesize 1 carbon homolog derivative **1.9c** and condensed it with bispyrrole to afford isopremarineosin analog **1.11b** but surprisingly no isolable **1.12c** (**Fig. 1.8C**). Rearranged product **1.12c** may have been observed by analytical LC-MS and as a potential spot on analytical TLC. Unfortunately, attempts to isolate and characterize this spot resulted in intractable decomposition products. Surprisingly, attempts to utilize **1.9c** as a promotor in the presence of isopremarineosin **1.11a** (**Fig. 1.8D**) resulted in consumption of **1.9c**, recovery of **1.11a** (70%), but no detectable rearranged products. Lastly, we then prepared des-keto derivative **1.9d** and attempts to utilize it as a promotor afforded no reaction with isolation of only starting materials further indicating that oxonium formation is fundamental (**Fig. 1.8E** and **Fig. 1.8B**).

A ***Condensation with Dess-hydroxy Butyl Sidechain***

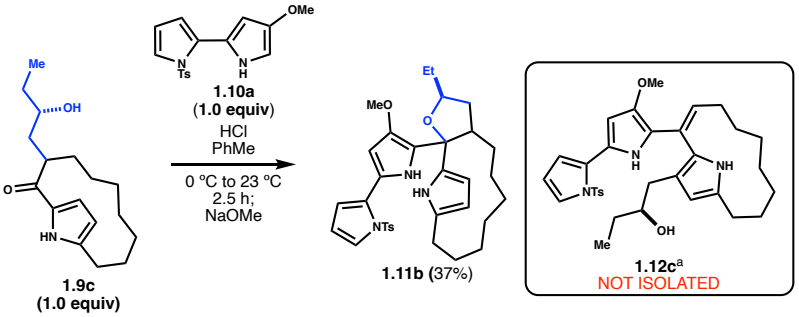


B ***Oxonium Intermediate may Fundamental in Mechanism***

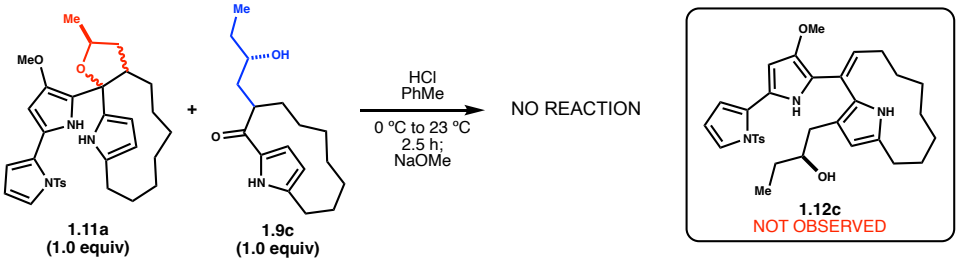


Oxonium Intermediate Fundamental in Mechanism

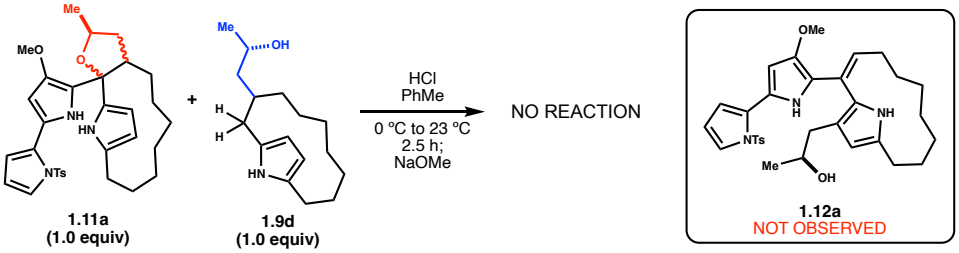
C ***Condensation with Hydroxy-Butyl Sidechain***



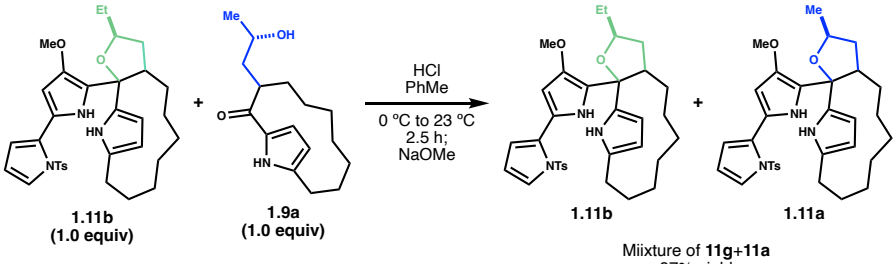
D ***Probing Hydroxy-Butyl 1.9c as a Promotor for Sidechain Migration***



E ***Probing Dess-keto 1.9d as a Promotor for Sidechain Migration***



F ***Probing Analog 1.11b for Sidechain Migration***



Miixture of **11g+11a**
97% yield

Fig. 1.8. (**A**) Condensation with Des-Hydroxy Butyl Sidechain. (**B**) Oxonium Intermediate may be Fundamental in Mechanism. (**C**) Condensation with Hydroxy-Butyl Sidechain. (**D**) Probing Hydoxy-Butyl **1.9c** as a Promotor for Sidechain Migration. (**E**) Probing Des-Keto **1.9d** as a Promotor for Sidechain Migration. (**F**) Probing Analog **1.11b** for Sidechain Migration. a) **1.12c** may have been observed on analytical LC-MS but isolation attempts of **1.12c** failed. For experimental procedures – see SI for details.

Remarkably, subjecting isopremarineosin analog **1.11b** (**1.0 equiv**) in the presence of known promoter **1.9a** (**1.0 equiv**), we observed complete consumption of **1.9a**, starting material **1.11b**, and formation of isopremarineosin **1.11a** (**Fig. 1.8F – ^{1}H NMR**; see SI for details). This result was markedly different to control reaction **Fig.1.8D** that afforded no reaction. This result gave support to our hypothesis that a bimolecular mechanism is productive in rearrangement chemistry, and transposition of the hydroxy-propyl sidechain. Initially we suspected formation of isopremarineosin **1.11a** stemmed from simple hydrolysis of **1.11b** followed by subsequent condensation with **1.9a**, but this is unlikely. In control experiments (not shown), attempts to hydrolyze isopremarineosin **1.11a** and rearrangement product **1.11b** failed to give any hydrolyzed products, presumably due to the stability induced by cross-conjugation of the trispyrrolic π-system.

In addition to the sidechain motif, we also decided to probe the heterobiaryl π-system. Although initially intended to furnish synthetic marineosin A analogs we prepared a series heterobiaryl nucleophiles with varying degrees of electron-density (**Fig.1.9A**). Electron poor 3,5-difluoro variant afforded isopremarineosin analog **1.11c** and rearranged product **1.12c** in the lowest yields while electron rich anisole derivative afforded predominantly rearranged **1.12e** in the highest yield (20%). A desmethoxy bispyrrole variant (**S2**; see SI for details) was also prepared and condensed to afford isopremarineosin analog **1.11f** but notably, no rearranged product. This result indicated that electron-richness on the pyrrole B-ring was necessary to furnish rearrangement. A series of identical control experiments (**Fig 1.7** and **Fig. 1.8**) were run investigating anisole derivative **1.11e** which resulted in identical results in which the only difference being that electron-rich **1.11e** gave higher yields of its corresponding rearranged product **1.12e**. A general trend observed was that increased electron-density on the heterobiaryl π-system increased the efficiency of the sidechain rearrangement.

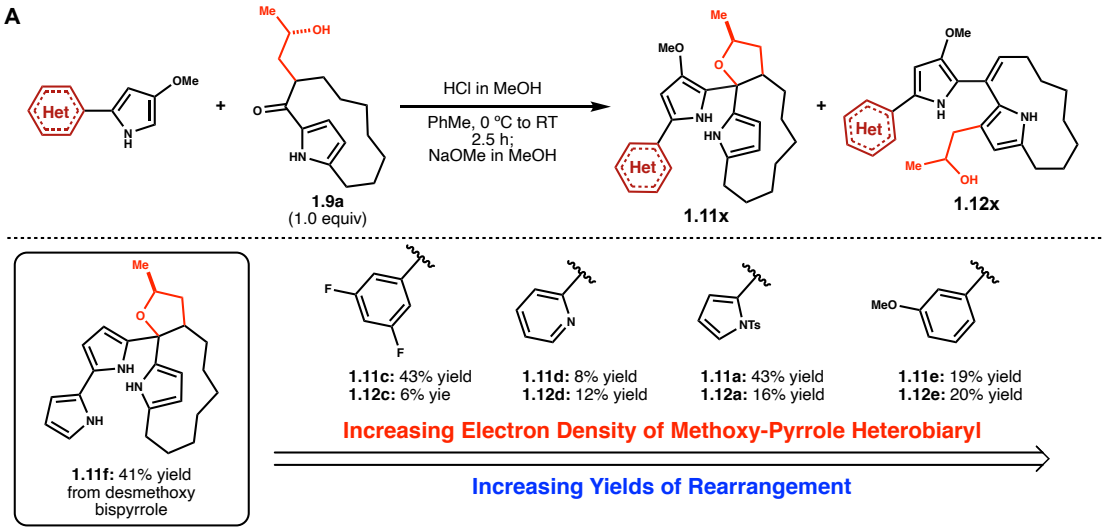


Fig. 1.9. (**A**) Electron-density on the heterobiaryl π-system is a principal factor for rearrangement efficiency. Desmethoxy bispyrrole indicates methoxy moiety is necessary to induce rearrangement. For preparation of heterobiaryls – see SI for details.

With the results obtained from our control experiments we then collaborated with our colleague Dr. Mark Maskeri in the Ken Houk Lab for computational insight into a plausible operative mechanism. DFT calculations confirmed experimental observations that isopremarineosin and its analogs when acidified (**Fig. 1.6**) would not rearrange via a sigmatropic rearrangement. DFT calculations (**Fig. 1.10**) indicated that a charged suprafacial [1,7]-sigmatropic rearrangement was prohibitively high in energy at $\Delta G^{\ddagger}$ = 69.0 kcal/mol. Furthermore, our [π6a+2σs] process would be expected to proceed through an antarafacial transformation but similarly DFT calculations indicated that an antarafacial mechanism was prohibitively higher in energy (not shown). Likewise, a neutral aza-fulvene intermediate was calculated to be prohibitively high in energy, either proceeding through a [1,3] or [1,7]-sigmatropic rearrangement (**Fig. S5**; see SI for details). DFT calculations confirmed experimental observations, further strengthening our hypothesis of a bimolecular mechanism being operative.

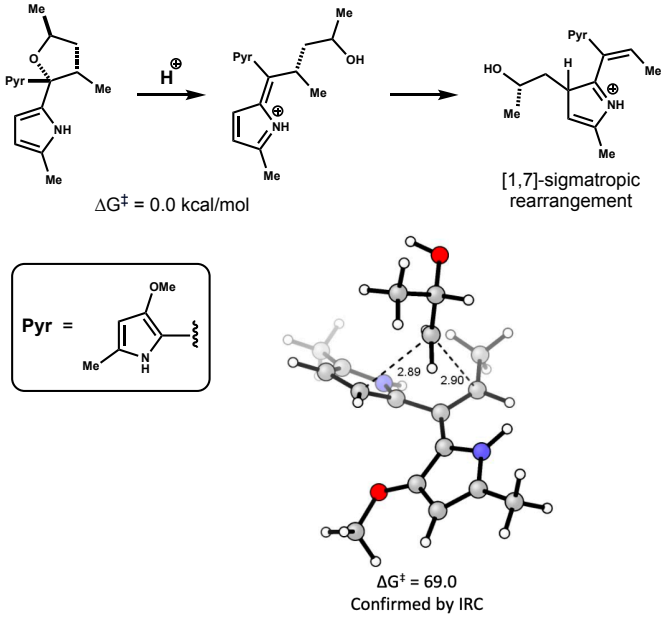


Fig. 1.10. DFT calculations support experimental results that a suprafacial [1,7]-sigmatropic rearrangement is not an operative mechanism via a charged aza-fulvene intermediate. wB97X-D/def2-TZVPP/SMD(PhMe)//wB97X-D/def2-SVP/SMD(PhMe), QH corrections (@298.15 K), energies in kcal/mol.

One mechanism with preliminary support from DFT invokes formation of oxonium intermediate **int-1** from **1.9a** (**Fig. 1.11**). **int-1** is then attacked by the pyrrole π-system from **1.11a** to form condensed **int-2**. **int-2** then rearomatizes to restore the pyrrole moiety present in **int-3**. **int-3** then fragments via a phenonium ion-type elimination to extrude enol tautomer **1.5** and **int-4**. The cyclopropyl motif is then cleaved with H_2O to form **int-5** followed the acid-mediated opening of the tetrahydrofuran moiety to form **int-6**. **int-6** then fragments via the loss of propylene oxide to form final product **1.12a**. Although this mechanism was initially postulated by DFT, calculations themselves indicate the fragmentation of **int-3** to **int-4** is prohibitively high in energy and we have never experimentally observed formation of ketopyrrolophane **1.5** as a side product. In conclusion, likely another as of yet unknown bimolecular mechanism is operative.

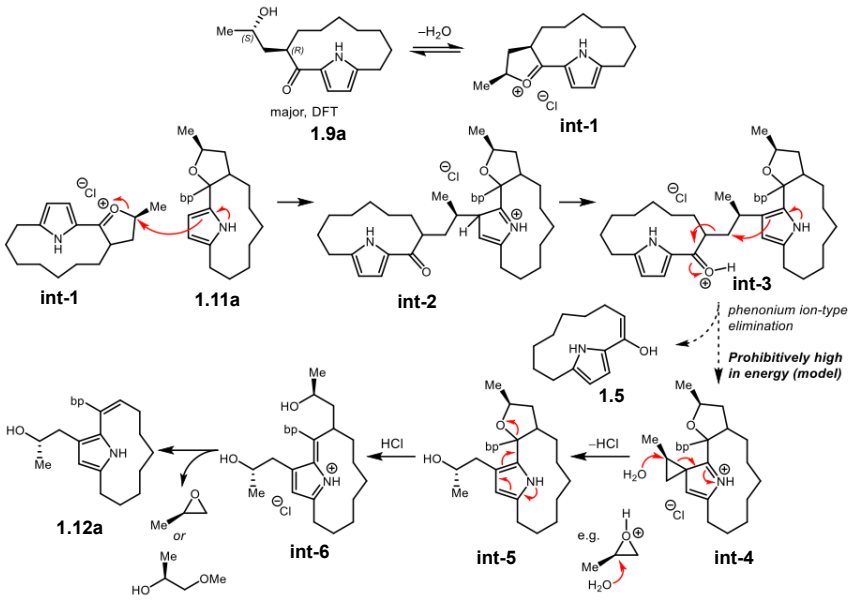


Fig. 1.11. Possible mechanism supported by DFT calculations involves the extrusion of propylene oxide. Although this mechanism is calculated to be prohibitively high. wB97X-D/def2-TZVPP/SMD(PhMe)//wB97X-D/def2-SVP/SMD(PhMe), QH corrections (@298.15 K), energies in kcal/mol

Simulated ^{13}C NMR spectra and ^{1}H NMR calculations indicate that structure **1.12a** may be misassigned and that a plausible structure may be **1.12a'** where the hydroxy-propyl sidechain is connected on the pyrrole B-ring (**Fig. 1.12A**, **Fig. S3** and **Fig. S4**; see SI for details). This is a concern due to us not being able to propose a reasonable operative mechanism supported by DFT calculations to afford structure **1.12a**. Furthermore, we have not yet unambiguously confirmed a structure by X-ray crystallography. Attempts to grow crystals from **1.12a** have not been successful as structure **1.12a** exists as an amorphous foam. Further derivatization via detosylation quickly result in decomposition of **1.12g (Fig. 1.12B)**. Other attempts such as growing crystals from powder **1.12d** and preparing salts from the pyridyl moiety have resulted in complete degradation. Derivatizations of the hydroxy motif has diversified our synthetic outcomes via urethanes, acetates, and silyl ethers (**1.12h**–**1.12j**) but unfortunately these products exist as amorphous powders and / or are unstable. Although preliminary efforts to grow a crystal from urethane **1.12h** resulted in the formation of a crystalline powder, unfortunately this compound did not sufficiently diffract to confirm a structure. Most promising has been rearranged anisole structure **1.12e** that exists as a crystalline solid. Preliminary analysis of **1.12e** by microED has been promising and efforts are currently ongoing to grow better quality crystals.

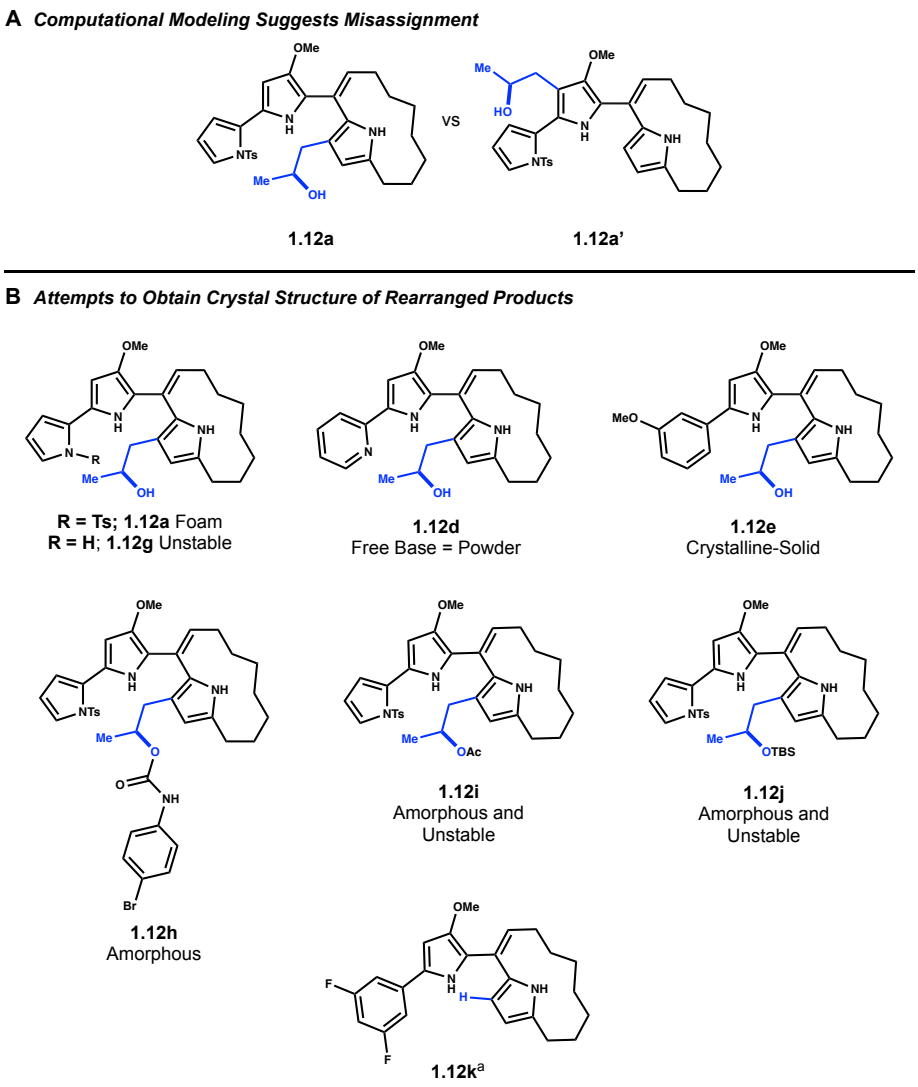


Fig. 1.12. **Possible misassignment of rearranged structure 1.12a**. (**A**) Computational Modeling Suggests Misassignment. (**B**) Attempts to Obtain Crystal Structure of Rearranged Products. DFT calculations indicate simulated ^{13}C NMR more closely matches that of structure **1.12a'** where the hydroxy-propyl sidechain is connected to the pyrrole B-ring. nmr=giao mPW1PW91/6-311+G(2d,p)/PCM(acetone)//B3LYP/6-31+G(d,p). See SI for details. a) Deshydroxy-propyl sidechain derivative **1.12k** was synthesized to further confirm structure **1.12a** by NMR analysis.

1.4 Conclusions

Using our lab's synthetic route to marineosin A; we further refined this route, synthesized marinesin A, and reported new synthetic analogs for biological testing. Unfortunately, results received from our collaborators were inconclusive as marineosin and its analogs were stored for months before testing. Degradation of these products is a concern as our stability tests indicate

poor stability of related compounds to prolonged storage. Future work will have to involve development of new methodologies (such as photoredox) to afford desired cycloisomerized premarineosin products from their isopremarineosin starting materials as this step is a synthetic bottleneck. Furthermore, our mechanistic studies into sidechain rearranged products will need to involve structural confirmation of products. Most promising are derivatizations of rearranged analogs into urethane derivatives. Overall, we are confident that a bimolecular mechanism is likely operative involving trans-positioning of the hydroxy-propyl sidechain. Lastly, we have established an optimal synthetic route to access a new class of synthetic rearranged prodiginines which is amendable to analog synthesis. In addition to establishing an operative mechanism, further investigation into rearranged hydroxy-propyl products' thus far unknown biological activity could hold promise.

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1.6 Chapter 1 – Experimental Appendix

1. Materials and Methods

Unless otherwise specified, reactions were performed in flame-dried glassware under an atmosphere of argon. Reagents were purchased from commercial vendors and used as received unless otherwise stated. Tetrahydrofuran (THF), diethyl ether (Et_2O), acetonitrile (CH_3CN) and toluene (PhMe) were passed through a Glass Contour solvent drying system. Methanol (MeOH) was distilled from $Mg(OMe)_2$, which was generated by refluxing methanol over magnesium turnings until all the turnings had converted into a white precipitate. The distilled MeOH was stored in a flame-dried flask over activated 3Å molecular sieves under argon. Magnesium turnings were polished by sequential rinsing with aqueous 1M HCl, absolute ethanol, and Et_2O. The washed turnings were placed under vacuum for several hours then stored under an atmosphere of argon.

Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous materials, unless otherwise stated. Thin-layer chromatography (TLC) was conducted on precoated plates (Sorbent Technologies, silica gel 60 PF254, 0.25 mm) visualized with UV 254 nm. For acid-sensitive compounds, TLC plates were neutralized with TEA/Hexane (1%) before use. Column chromatography was performed on silica gel 60 (SiliCycle, 240−400 mesh). For acid-sensitive compounds, silica gel was premixed with TEA/Hexane (1%) before being loaded into the column. Purifications with preparative HPLC were performed using an Agilent 1200 HPLC system equipped with G1361A preparative pumps, a G1314A autosampler, a G1314A VWD, a G1364B automated fraction collector, and a Waters Sunfire C18 column (5 μm, 19 mm × 250 mm), unless otherwise noted. Analytical HPLC was performed using the same system, but with a G1312A binary pump. Mass spectra were recorded using an Agilent 6130 LC/MS system equipped with an

ESI source. High resolution mass spectra were recorded using Thermo Fisher Scientific Exactive Plus with IonSense ID-CUBE DART source and Orbitrap analyzer. NMR spectra were recorded on Bruker Avance spectrometers (400/101 MHz and 500/126 MHz).

Flow photoreactions were carried out using an Ace Glass low temperature immersion well photoreactor using a Hanovia 450W mercury arc lamp. Photoreactions were also examined in a Rayonet photoreactor with a 254 and 300 nm bulb set using quartz test tubes. For setup of photoreaction in flow format, 1/16" ID x 1/8" OD x 1/32" Wall VersilonTM FEP Tubing was coiled around an Ace Glass 7858 triple-walled quartz immersion well in either one or three layers. The reaction solution was propelled by a peristaltic pump (Binaca model 1001) through the FEP tubing under irradiation by a Hanovia 450W mercury arc lamp.

Some NMR spectra of previously published compounds have been omitted. All omitted spectra can be found in published manuscript (*2*).

2. Experimental Procedures

2.1 General Procedures

Unless otherwise stated, heterobiaryl nucleophiles **1.10a**–**1.12e** (see page S13 for more details) were synthesized following modified procedures based on references (*1*) and (*2*). Furthermore, heterobiaryls were used as 'crude' reaction mixtures derived from reduction of the Cbz-group intermediate.

General Procedure A – Acid Promoted Condensation Reactions

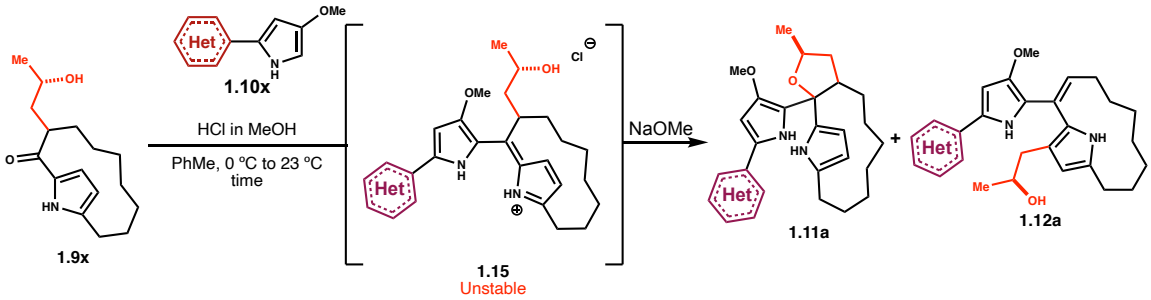


Fig. S1. General Procedure for Condensation Reactions.

Flame-dried Na_2SO_4 (100% wt. equiv) was added to an appropriately sized, flamed-dried reaction vessel equipped with a stir bar containing **1.9x** (1.0 equiv) stripped with dry PhMe (3 x). Then diluted with dry PhMe (0.1 M); cooled to 0 ºC and stirred. Then the solution of crude heterobiaryl (1.0 equiv) in dry PhMe (0.1 M) stripped with dry PhMe (3 x) was added over ~30 min dropwise while maintaining 0 ºC. After the first drop of **1.10x** was added, HCl in MeOH (1.0 molar, 1.5 equiv) was added quickly by syringe. Upon addition the mixture became bright–pink color. After addition was complete; reaction mixture was a dark-purple/red. After x h, the reaction was quenched with NaOMe in MeOH (1.0 M, 3.85 equiv) at 0 °C. The resulting orange solution was added dropwise into ice-water, then extracted with EtOAc (3 x). The organic layers were combined, washed with brine, dried over Na2SO4, filtered, and concentrated. Concentration afforded a dark-red oil as crude product. Crude product was purified via flash SiO_2

chromatography (SiO_2 was slurry packed with 1% NEt_3/Hex and then stripped with Hex) to afford title compounds. Compounds were then stored frozen in dry PhH or as stock solutions in dry PhMe. Moreover, compounds were stored under an argon atmosphere.

2.2 Synthesis of Fig. 1.2, 1.3, and 1.4 compounds

(2,6)pyridinophane (**1.3**)

Reaction was done in parallel with the exact same conditions (2 reactions; exact same stoichiometries in parallel).

To a flame-dried 500-mL three-necked round bottom flask equipped with a stir bar, reflux condenser, and 100-mL addition funnel was added anhydrous Et_2O (360 mL, 0.5 M) under argon. Then against argon was added Mg turnings (8.74 g, 2.0 equiv, 360 mmol) and a few iodine crystals (spatula tip). Then (~1 mL, ~1 g) of 1,8-dibromooctane was added and allowed to heat up to 40 ºC with a heating mantle. Grignard initiated after ~15 min at which point the heating mantle was removed and the rest of the 1,8-dibromooctane (49.9 g, 33.8 mL, 180 mmol, 98.0% wt.) was added dropwise (as to maintain a gentle reflux) via the addition funnel. After the addition was complete, the heating mantle was again fitted to the reaction vessel and allowed to heat to 40 ºC and stirred for 1 h. After 1 h, this grignard intermediate was allowed to cool to 23 ºC and stopped stirring.

Separately, to a flame-dried 5-L three-necked round bottom flask equipped with a stir bar and 500-mL addition funnel was added 2,6-dichloropyridine (1.2) (27.2 g, 1.0 equiv, 180 mmol, 98% wt.) and $NiCl_2$•DPPP (796 mg, 8 mol %, 1.44 mmol, 98% wt.). Then under argon, was diluted with anhydrous Et_2O (1.80 L, 0.1 M). Then the reaction mixture was cooled to 10 ºC with an ice-water bath and stirred. Then via cannula transfer the Grignard reagent was transferred under positive argon pressure to the 500-mL addition funnel. Once finished with transferring, the

Grignard reagent was added dropwise over the course of 6 h while maintaining stirring and 10 ºC ice-water bath. After 6 h, the reaction mixture was carefully poured into ice water then filtered through celite. The organic layer was separated and the aqueous layer was extracted with EtOAc (100 mL x 3). The organic layers were combined, washed with brine, dried over Na2SO4, filtered, and concentrated to an orange oil. The orange-oil was then transferred to a 250-mL round bottom flask equipped with a stir bar. The crude product was then equipped with a short path distillation head and heated to 150–170 ºC under vacuo (<5 mbar). Isolated desired product title compound (17.0 g, 50% isolated yield) as a colorless-oil. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.46 (t, J = 7.6 Hz, 1H), 6.89 (d, J = 7.6 Hz, 2H), 2.84 (t, J = 6.38 Hz, 4H), 1.83–1.77 (m, 4H), 1.42–1.36 (m, 4H), 1.06–0.98 (m, 4H) ^{13}C NMR ($CDCl_3$, 101 MHz) δ 160.6, 136.6, 120.0, 35.7, 26.3, 25.1, 23.2. HRMS calculated for [$C_{13}H_{19}N$+H] = 190.1590; found 190.1583; 3.7 ppm mass defect. For NMR spectras; see reference (*2*).

(2,6)pyridinophane N-oxide (**1.4**)

To a stirred solution of **1.3** (34.4 g, 182 mmol, 1.0 equiv) in CHCl3 (182 mL) in a 3 L flask submerged in a room temperature water bath was added mCPBA (62.8 g, 75% purity, 273 mmol, 1.5 equiv) in several portions. After 30 min, the reaction turned too thick to stir and another 100 mL of CHCl3 was added. The resulting mixture was then stirred at 23 ºC for 16 h, and then diluted with CHCl3 (2 L), followed by addition of K2CO3. The resulting white slurry was stirred at 23 ºC for 2 h then mixed with celite (200 g) and filtered through a layer of celite. The filter cake was rinsed with CHCl3 until no product could be detected by TLC. The filtrate was then concentrated under reduced pressure to afford crude product. This crude residue was then taken up in a minimal amount of pentane and heated to reflux until homogenous. The crude mixture was then allowed to

cool to 23 ºC and then stored in the freezer overnight to afford crystals. These crystals were then filtered and washed with cold pentane to afford title compound (30.6 g, 90% isolated yield) as a pale-yellow crystalline solid. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.46 (t, J = 7.6 Hz, 1H), 6.89 (d, J = 7.6 Hz, 2H), 2.84 (t, J = 6.4 Hz, 4H), 1.82–1;76 (m, 4H), 1.42–1.36 (m, 4H), 1.04–0.98 (m, 4H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 160.6, 136.6, 120.0, 35.7, 26.3, 25.1, 23.2; HRMS calculated for [$C_{13}H_{19}NO$+H] = 206.1539; 206.1534; 2.4 ppm mass defect. For NMR spectras; see reference (*2*).

Flow photolysis transformation from **1.4** to products **1.5**, **1.6**, **1.7**, and **1.3**.

To a quartz immersion well wrapped with 3 layers of FEP tubing was placed in a dewar and cooled to –75 °C with isopropanol and dry ice (254 nm light). The solution of **1.4** (6.32 g, 30.8 mmol) in anhydrous THF (0.01 M) was pumped through the FEP tubing with a residence time of 5 minutes. The resulting light yellow solution was added a minimal amount of SiO2 and concentrated under reduced pressure to afford crude product. The crude residue was then concentrated to form a dry-load with SiO_2. Crude product was purified via flash SiO_2 chromatography (gradient Hex → 10% EtOAc/Hex → 20% EtOAc/Hex → 22% EtOAc/Hex → 30% EtOAc/Hex → 40% EtOAc/Hex) afforded title compounds **1.5** (27%), **1.6** (5%), **1.7** (18%), and **1.3** (14%). For NMR spectras; see reference (*2*).

1*1*H-1(2,5)-pyrrolacyclodecaphan-2-one (**1.5**)

White solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.73 (s, 1H), 6.98 (t, J = 3.2 Hz, 1H), 6.09 (t, J = 3.2 Hz, 1H), 2.78–2.73 (m, 2H), 2.68 (t, J = 6.3 Hz, 2H), 1.79 (tq, J = 13.6, 6.8, 6.1 Hz, 4H), 1.60–1.49 (m, 2H), 1.37 (p, J = 6.0 Hz, 2H), 1.21 (p, J = 6.9 Hz, 2H), 1.08 (ddt, J = 12.3, 8.9, 5.4 Hz, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 190.3, 137.9, 132.3, 115.9, 111.5, 36.1, 27.0, 26.7, 26.2,

23.5, 23.4, 23.3, 21.7; HRMS calculated for [$C_{13}H_{19}NO$+H] = 206.1545; found 206.1553; 3.9 ppm mass defect. For NMR spectras; see reference (*2*)

5,6,7,8,9,10,11,12-octahydrocycloundeca[*b*]pyrrol-4(1*H*)-one (**1.6**)

White solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.14 (s, 1H), 6.63 (t, J = 2.7 Hz, 1H), 6.44 (t, J = 2.9 Hz, 1H), 3.09 (t, J = 6.4 Hz, 1H), 2.68 (t, J = 6.4 Hz, 1H), 1.75 (p, J = 6.2 Hz, 2H), 1.64–1.58 (m, 2H), 1.37 (p, J = 6.1 Hz, 2H), 1.23 (tt, J = 8.9, 4.6 Hz, 2H), 1.12 (ddt, J = 10.3, 7.7, 4.6 Hz, 2H), 1.09–1.01 (m, 2H); ^{1}H NMR ($CDCl_3$, 126 MHz) δ 202.3, 136.9, 124.3, 116.6, 110.1, 41.7, 28.1, 27.8, 25.4, 25.1, 24.6, 24.5, 22.8; HRMS calculated for [$C_{13}H_{19}NO$+H] = 206.1545; found 206.1532; 6.3 ppm mass defect. For NMR spectras; see reference (*2*).

(*Z*)-azacyclotetradec-13-ene-2,11-dione (**1.7**)

White solid. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.63 (d, J = 10.1 Hz, 1H), 6.90 (ddt, J = 10.4, 1.3 Hz, 1H), 4.80 (dt, J = 9.1, 7.7 Hz, 1H), 3.15 (dd, J = 7.7, 1.2 Hz, 2H), 2.45 (t J = 6.7 Hz, 2H), 2.36–2.33 (m, 2H), 1.69–1.61 (m, 4H), 1.35 (tt, J = 7.1, 3.0 Hz, 4H), 1.27 (q, J = 7.3 Hz, 2H), 1.17 (dq, J = 8.4, 6.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 210.7, 170.9, 125.4, 101.0, 41.9, 40.7, 36.0, 26.8, 26.0, 25.3, 24.3, 23.3; HRMS calculated for [$C_{13}H_{20}NO_2$+H] = 224.1651; 224.1637; 6.3 ppm mass defect. For NMR spectras; see reference (*2*).

3-((*S*)-2-hydroxypropyl)-1^1*H*-1(2,5)-pyrrolacyclodecaphan-2-one (**1.9a**)

To a flame-dried 3-neck 100 mL round bottom flask, equipped with a low-temperature thermometer and a stir bar, was added anhydrous THF (10 mL) and iPr2NH (0.9 mL, 6.2 mmol, 3.2 eq.) at 23 ºC, and then cooled to -78 °C with an acetone-dry ice bath, followed by addition of

nBuLi (2.5 M in hexane, 2.5 mL, 6.2 mmol, 3.1 eq.). After addition, the resulting colorless solution was stirred for 10 min at -78 °C, then warmed up to 0 °C in ice-water bath. A solution of **1.5** (410 mg, 2 mmol, 1.0 eq.) in anhydrous THF (30 mL) was added into the formed LDA solution via syringe pump over 3 h while maintaining the LDA solution at 0 °C. The resulting light-yellow solution was stirred for another 30 min at 0 °C, then cooled to - 78 °C with an acetone-dry ice bath. (*S*)-Propylene oxide (0.7 mL, 10 mmol, 5.0 eq.) was added, followed by $AlMe_3$ solution (2.0 M in hexane, 1.1 mL, 2.2 mmol, 1.1 eq.). The reaction mixture was stirred at -78 °C for another 30 min, and then slowly warmed up to 23 ºC over 1 h, then allowed to stir at 23 ºC overnight. TLC monitoring showed products were the major spots. To quench the reaction, the mixture was carefully added into a stirred solution of potassium sodium tartrate and ice, then extracted with EtOAc (50 mL x 3). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered, concentrated, and purified by flash column chromatography using TEA neutralized silica gel (Hex → 25% EtOAc/Hex → 30% EtOAc/Hex → 35% EtOAc/Hex → 40% EtOAc/Hex). The mixture of diastereomers **1.9a** (40%, d.r. ~ 1:1) was obtained as a light-yellow foam. HRMS calculated for $[C_{16}H_{24}NO_2+H]$ = 263.1885; found 263.1894; 3.4 ppm mass defect. For NMR spectra of each respective diastereomer product; see reference (*2*).

Isopremarineosin (**1.11a**)

Synthesized following General Procedure A; quenched reaction after 30 min. Isolated **1.11a** (52% isolated yield) as a purple-foam and as a 1:1 mixture of diastereomers. ^1H NMR ($CDCl_3$, 500 MHz) δ 8.96 (s, 1H), 8.84 (s, 1H), 8.76 (s, 1H), 8.69 (s, 1H), 7.39–7.36 (m, 2H), 7.26 (d, J = 3.0 Hz, 2H), 7.25 (d, J = 3.0 Hz, 2H), 6.92 (d, J = 8.0 Hz, 2H), 6.83 (d, J = 8.0 Hz, 2H), 6.23 (t, J = 3.3 Hz, 2H), 6.18 (ddd, J = J =3.3, 1.8, 9.1 Hz, 2H), 6.01 (dq, J = 3.1, 2.8 Hz, 2H), 6.01

(dd, J = 3.2, 7.4 Hz, 2H), 5.82 (dt, J = 14.8, 2.8 Hz, 2H), 4.45–4.38 (m, 1H), 4.20–4.14 (m, 1H), 3.83 (s, 3H), 3.80 (s, 3H), 2.87 (q, J = 7.5 Hz, 1H), 2.76 (t, J = 6.1 Hz, 1H), 2.72–2.51 (m, 4H), 2.28 (s, 3H), 2.24 (s, 3H), 2.22–2.16 (m, 1H), 1.88–1.83 (m, 1H), 1.79–1.72 (m, 1H), 1.69–1.58 (m, 2H), 1.57–1.42 (m, 6H), 1.39 (d, J = 6.2 Hz, 3H), 1.35 (d, J = 6.2 Hz, 3H), 1.32–0.84 (m, 15H), 0.82–0.75 (m, 1H), 0.74–0.65 (m, 1H); ^{13}C NMR ($CDCl_3$, 126 MHZ) δ 144.7, 144.6, 141.6, 139.7, 134.7, 134.5, 133.4, 132.2, 131.3, 130.9, 129.5, 129.4, 127.6, 127.6, 127.5, 127.4, 123.3, 123.1, 123.1, 121.6, 115.3, 114.9, 114.5, 114.1, 111.3, 111.1, 106.2, 105.3, 105.0, 104.9, 100.0, 99.9, 85.2, 84.5, 75.5, 73.7, 58.8, 58.8, 48.1, 48.0, 42.1, 41.0, 31.6, 31.5, 29.5, 28.2, 27.8, 27.3, 26.8, 26.7, 25.8, 25.5, 25.4, 23.8, 22.7, 21.6, 21.5, 21.3, 14.2, 14.1; HRMS calculated for [$C_{32}H_{39}N_3O_4S$+H] = 562.2734; found 562.2712; 3.9 ppm mass defect. For spectra of each respective diastereomer. See reference (*2*).

Rearranged Product **1.12a**

Synthesized following General Procedure A; quenched reaction after 2.5 h. Isolated **1.12a** (16% isolated yield) as a dark-red foam. ^{1}H NMR (acetone-d_6, 500 MHz) δ 9.30 (s, 1H), 9.11 (s, 1H), 7.38 (d, J = 8.4 Hz, 2H), 7.32 (dd, J= 3.2, 1.9 Hz, 1H), 7.30 (d, J = 8.4 Hz, 2H), 6.47 (t, J = 8.4 Hz, 1H), 6.26 (m, 2H), 6.08 (s, 1H), 5.79 (d, J = 2.8 Hz, 1H), 3.89 (q, J = 6.4 Hz, 1H), 3.75 (s, 3H), 2.64 (m, 2H), 2.44 (d, J = 7.3 Hz, 1H), 2.42 (d, J = 5.9 Hz, 1H), 2.36 (s, 3H), 1.92 (m, 2H), 1.60 (m, 2H), 1.53–1.13 (m, 7H), 1.11 (d, J = 6.2 Hz, 3H), 0.61 (m, 1H), 0.54 (m, 1H); ^{13}C NMR (acetone-d_6, 126 MHz) δ 146.1, 146.0, 136.2, 132.9, 130.7, 129.8, 129.2, 129.2, 127.9, 127.4, 127.4, 124.2, 123.9, 119.4, 118.5, 118.3, 117.3, 117.2, 115.2, 112.9, 106.3, 99.9, 99.8, 68.6, 58.1, 37.4, 30.1, 29.0, 28.5, 28.4, 27.7, 27.4, 26.3, 23.6, 21.5; HRMS calculated for [$C_{32}H_{39}N_3O_4S$+H] = 562.2734; found 562.2755; 3.7 ppm mass defect.

Preparation of *'freshly prepared acidic'* MnO_2

To a 500mL round bottom flask (equipped with a stir bar) was added $MnSO_4 \bullet H_2O$ (25.40 g, 150 mmol, 5.0 equiv) followed by de-ionized water (150 mL, 1.0 M). The resulting solution was then stirred until homogenous and then cooled to 0 °C (via an ice-water bath). To this $MnSO_4 \bullet H_2O$ solution was added dropwise (via an addition funnel) a solution of $KMnO_4$ (4.75g, 30 mmol, 1.0 equiv) dissolved in de-ionized water (150 mL, 0.2 M) while the reaction mixture was stirred at 0°C under an atmosphere of argon gas.

After addition of the $KMnO_4$ solution was complete, the reaction mixture (pH~3–4) was stirred for 1 hour at 0°C and then allowed to warm up to 23 ºC (done by removing the reaction vessel from the ice bath). After this 1 h, stirring was stopped and the resulting dark brown reaction mixture was left to stand for ~25 h at 23 ºC under an atmosphere of argon gas.

After standing for ~25 h, the reaction mixture was filtered (via vacuum filtration) and the resulting dark brown filter cake was rinsed with de-ionized water until testing of the filtrate failed to produce a yellow color when 28–30% aqueous NH_4OH was added; done to test for the presence of Mn^{2+} ion. The resulting dark brown solid was then rinsed with a minimal amount of acetone; just enough to cover the brown solids.

The resulting sticky brown solid was then transferred to a 250mL round bottom flask and stripped with PhH (5 x) with the bath set to 25°C. Between strips, the PhH suspension was sonicated for several minutes and, after 5 strips with PhH, the dry solid was vigorously stirred under an atmosphere of argon gas to break up the chunks of solid. The resulting product was isolated as a light-brown, fine powder and stored at 23 ºC under an argon atmosphere. No yield and / or characterization was performed for our prepared MnO_2. Our *'freshly prepared acidic*

MnO_2' had to be used in cycloisomerization reactions within ~2 weeks to be productive. Utilization of this reagent over more than 2 weeks would result in complete degradation and 0 % yields of our premarineosin products. Note: Reagent was checked after 2 weeks for presence of Mn^{2+} ions with NH_4OH which resulted in no Mn^{2+} ions being present.

Premarineosins **1.13a** and **1.13b**

To a flame-dried dram vial was charged with a toluene stock solution of compound **1.11a** (1.0 equiv), which was then concentrated and stripped with anhydrous toluene (1 mL x 3). The resultant oil was then mixed with polished Mg (5.0 equiv) under argon and diluted with MeOH (0.6 mL). The resulting mixture was sonicated at 23 ºC for 20 min, at which point TLC showed complete conversion. The clear orange solution was transferred into a new vial under argon and the reaction vial was rinsed with toluene (1 mL) while leaving the unreacted Mg behind. The orange solution was concentrated on a rotavapor to dryness and backfilled with argon. This process was repeated twice with toluene (1 mL x 2) (after each addition of toluene, the heterogeneous mixture was sonicated to minimize bumping). The residue was taken up in toluene, mixed with celite and filtered through a celite plug (monster pipette) into a flame-dried vial under argon (do NOT push air through celite pipette plug! Use argon to push through.). The plug was rinsed with toluene until the filtrate turned colorless. This provided a solution of detosylated isopremarineosin (**S1**) in toluene in assumed quantitative yield. Telescoped to the next step.

The solution of **S1** was then concentrated to a volume of ~2 mL, then cooled in an ice-water bath for 5 min. Dry HCl in MeOH solution (3.0 equiv) was added and the yellow solution turned dark purple-red immediately. This solution was carefully concentrated without heating on a rotavapor until the pressure reached 20 mbar to remove any MeOH and excess HCl. The vial

was placed back into an ice-water bath under argon and the remaining toluene solution of **S1** was diluted with dry acetone to a total volume of 4 mL.

Into each of a set of 4 flame dried dram vials was placed the 'acidic' MnO_2 reagent (68 mg) and $MgSO_4$ (30 mg) under argon. To each vial was added dry acetone (0.5 mL). Under vigorous stirring, 1 mL of the dark red solution was added into each vial dropwise. After addition, the mixture was filtered through a celite plug and rinsed with acetone until the filtrate turned colorless. The yellow filtrate was concentrated under reduced pressure to afford crude product. Crude product was then purified The crude residue was then concentrated to form a dry-load with SiO_2. Crude product was purified via flash SiO_2 chromatography (gradient Hex → 20% EtOAc/Hex) afforded title compounds (0–8% isolated yield). Premarineosin products isolated as a ~1:1 mixture of diastereomers. For NMR spectra of each respective diastereomer product; see reference (*2*).

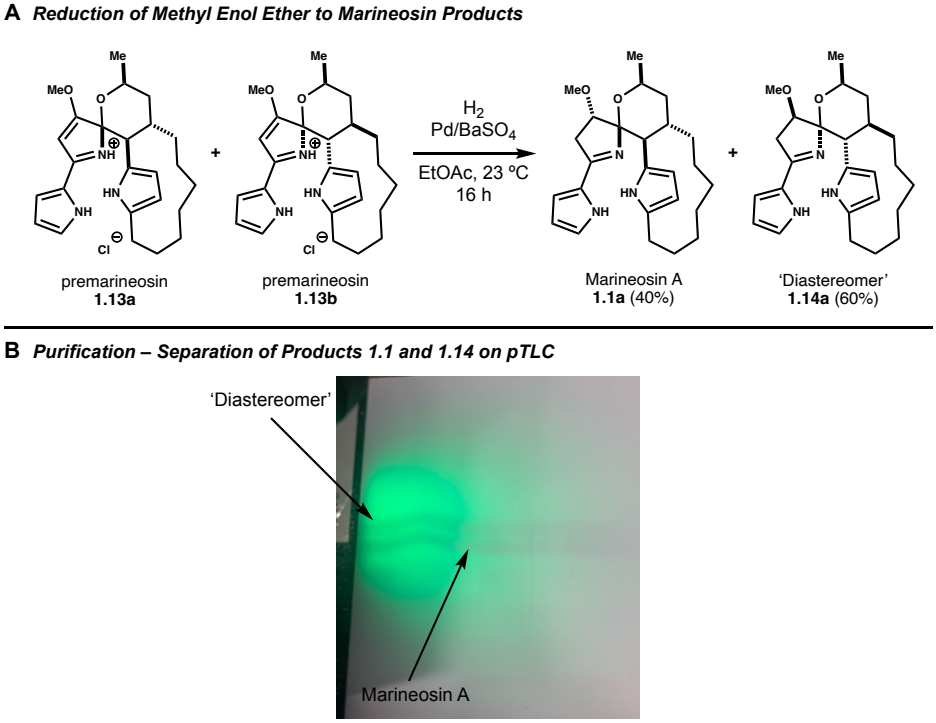


Fig. 1.3 Isolation and Purification of Marineosin A and 'Diastereomeric' Analog (**A**) Reduction of Methyl Enol Ether to Marineosin Products. (**B**) Purification – Seperation of Products **1.1** and **1.14** on pTLC. Visualization of bands – 254 nm light. 5 component solvent system – Hex:EtOAc:Acetone:MeOH:NEt_3, ratio: 171:19:6:4:2.

A mixture of compound **1.13a** and **1.13b** (1.52 mg, 3.42 μmol) and Pd/BaSO4 (2.15 mg) in EtOAc (300 μL) were stirred vigorously (1500 rpm) under a hydrogen atmosphere (1 atm.) for 16 h. LCMS showed reaction was complete. The mixture was filtered through celite and rinsed with EtOAc. The filtrate was concentrated to afford crude product, and then purified by pTLC; solvent system = Hex:EtOAc:Acetone:MeOH:NEt_3; ratio = 171:19:6:4:2. Isolated marineosin A **1.1a** (0.56 mg, 40% isolated yield) as a colorless film. Diastereomer **1.14a** (0.84 mg, 60% isolated yield) as a colorless film.

Marineosin A **1.1a**

^{1}H NMR (acetone-d_6, 500 MHz) δ 10.96 (s, 1H), 8.23 (s, 1H), 6.98 (s, 1H), 6.38 (s, 1H), 6.12 (s, 1H), 5.69 (s, 1H), 5.45 (s, 1H), 4.28–4.20 (m, 1H), 3.90–3.82 (m, 1H), 3.42 (s, 3H), 2.95–2.92 (m, 1H), 2.90–2.86 (m, 1H), 2.40–2.29 (m, 1H), 2.27–2.20 (m, 2H), 1.94–1.84 (m, 1H), 1.79 (d, J = 12.4 Hz, 1H), 1.70–1.58 (m, 2H), 1.53 (d, J = 6.6 Hz, 3H), 1.41–1.25 (m, 6H), 1.24–1.14 (m, 1H), 1.08–0.99 (m, 1H), 0.93–0.83 (m, 2H), 0.76–0.65 (m, 1H), 0.57–0.47 (m, 1H); HRMS calculated for [$C_{25}H_{35}N_3O_2$+H] = 410.2802; found 410.2798; 1.0 ppm mass defect. For ^{13}C NMR spectra; see reference (*2*).

Diastereomer **1.14a**

^{1}H NMR (acetone-d_6, 500 MHz) δ 10.95 (s, 1H), 7.00 (s, 1H), 6.41 (s, 1H), 6.14 (s, 1H), 5.73 (s, 1H), 5.46 (s, 1H), 4.44–4.36 (m, 1H), 3.87 (t, J = 8.7 Hz, 1H), 3.42 (s, 3H), 2.97 (dd, J = 15.9, 8.3 Hz, 1H), 2.86–2.83 (m, 1H), 2.25–2.20 (m, 2H), 1.90–1.85 (m, 2H), 1.75–1.68 (m, 2H), 1.64–1.57 (m, 2H), 1.54–1.47 (m, 3H), 1.40–1.34 (m, 3H), 1.25–1.23 (m, 1H), 1.08–1.04 (m, 1H), 0.96 (d, J = 6.7 Hz, 2H), 0.73–0.63 (m, 1H), 0.46–0.34 (m, 1H); HRMS calculated for [$C_{25}H_{35}N_3O_2$+H] = 410.2802; found 410.2810; 2.0 ppm mass defect.

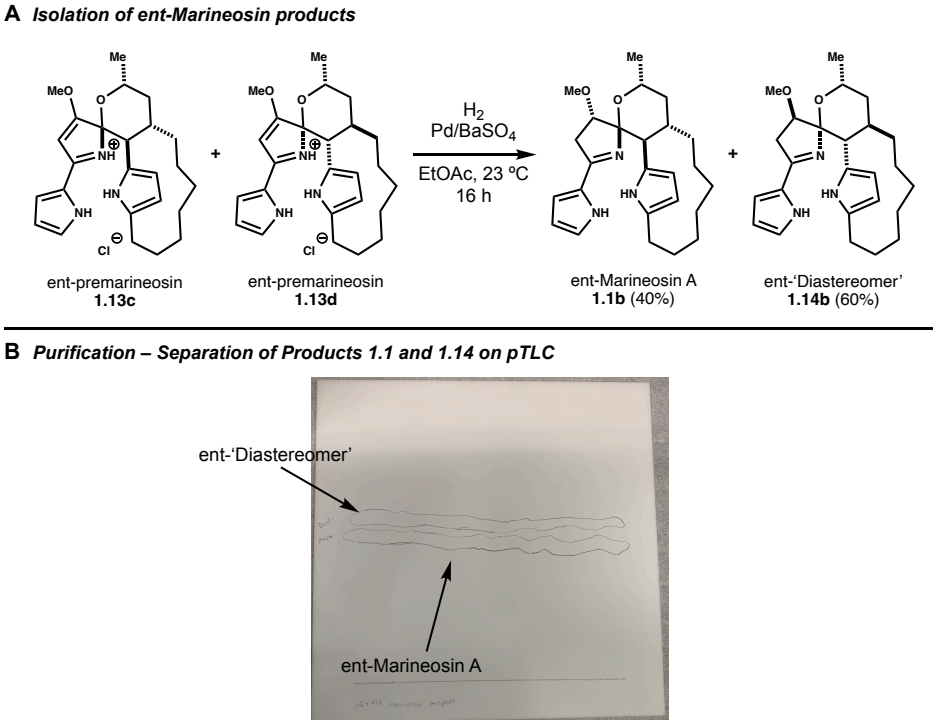


Fig. 1.4. Isolation and Purification of ent-Marineosin A and ent-'Diastereomeric' Analog (**A**) Reduction of Methyl Enol Ether to Marineosin Products. (**B**) Purification – Seperation of Products **1.1** and **1.14** on pTLC. Visualization of bands – 254 nm light. 5 component solvent system – Hex:EtOAc:Acetone:MeOH:NEt_3, ratio: 171:19:6:4:2.

Ent-marineosin A and ent-'diastereomer' were prepared in identical procedures to afford **1.1a** and **1.14a**. Ent-premarineosins (**1.13c** + **1.13d**) (2.10 mg, 4.72 μmol) afforded crude product. Crude product was then purified by pTLC; solvent system = Hex:EtOAc:Acetone:MeOH:NEt_3; ratio = 171:19:6:4:2. Isolated ent-marineosin A **1.1b** (0.77 mg, 40% isolated yield) as a colorless film. Diastereomer **1.14b** (1.15 mg, 60% isolated yield) as a colorless film.

ent-Marineosin A **1.1b**

^{1}H NMR (acetone-d_6, 500 MHz) δ 10.96 (s, 1H), 8.23 (s, 1H), 6.98 (s, 1H), 6.38 (s, 1H), 6.12 (s, 1H), 5.69 (s, 1H), 5.45 (s, 1H), 4.28–4.20 (m, 1H), 3.90–3.82 (m, 1H), 3.42 (s, 3H), 2.95–2.92 (m, 1H), 2.90–2.86 (m, 1H), 2.40–2.29 (m, 1H), 2.27–2.20 (m, 2H), 1.94–1.84 (m, 1H), 1.79 (d, J = 12.4 Hz, 1H), 1.70–1.58 (m, 2H), 1.53 (d, J = 6.6 Hz, 3H), 1.41–1.25 (m, 6H), 1.24–1.14 (m, 1H),

1.08–0.99 (m, 1H), 0.93–0.83 (m, 2H), 0.76–0.65 (m, 1H), 0.57–0.47 (m, 1H); HRMS calculated for [$C_{25}H_{35}N_3O_2$+H] = 410.2802; found 410.2814; 2.9 ppm mass defect.

ent-Diastereomer **1.14b**

^{1}H NMR (acetone-d_6, 500 MHz) δ 10.95 (s, 1H), 7.00 (s, 1H), 6.41 (s, 1H), 6.14 (s, 1H), 5.73 (s, 1H), 5.46 (s, 1H), 4.44–4.36 (m, 1H), 3.87 (t, J = 8.7 Hz, 1H), 3.42 (s, 3H), 2.97 (dd, J = 15.9, 8.3 Hz, 1H), 2.86–2.83 (m, 1H), 2.25–2.20 (m, 2H), 1.90–1.85 (m, 2H), 1.75–1.68 (m, 2H), 1.64–1.57 (m, 2H), 1.54–1.47 (m, 3H), 1.40–1.34 (m, 3H), 1.25–1.23 (m, 1H), 1.08–1.04 (m, 1H), 0.96 (d, J = 6.7 Hz, 2H), 0.73–0.63 (m, 1H), 0.46–0.34 (m, 1H); HRMS calculated for [$C_{25}H_{35}N_3O_2$+H] = 410.2802; found 410.2805; 0.7 ppm mass defect.

2.3 Synthesis of Fig. 1.8 Compounds

3-butyl-1^1*H*-1(2,5)-pyrrolacyclodecaphan-2-one (**1.9b**)

Synthesized in the same general procedure to afford **1.9a**. Isolated desired product as a white-film. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 8.77 (s, 1H), 6.98 (dd, J = 3.5, 2.8 Hz, 1H), 6.10–6.08 (m, 1H), 2.97–2.83 (m, 2H), 2.69–2.57 (m, 1H), 2.02–1.92 (m, 1H), 1.92–1.8 1(m, 1H), 1.74–1.63 (m, 2H), 1.62–1.57 (m, 2H), 1.50–1.38 (m, 3H), 1.35–1.28 (m, 3H), 1.26–1.19 (m, 3H), 1.17–1.09 (m, 1H), 0.88 (t, J = 7.1 Hz, 3H); ^{13}C NMR ($CDCl_3$, 101 MHz) δ 191.5, 137.9, 133.2, 116.0, 111.6, 44.7, 35.0, 32.1, 30.6, 26.8, 26.6, 24.6, 23.8, 23.7, 23.1, 22.2, 14.2. HRMS calculated for [$C_{17}H_{27}NO$+H] = 262.2171; found 262.2179; 3.1 ppm mass defect.

(2^5*S*)-2^5-ethyl-2^2-(4-methoxy-1'-tosyl-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)-2^2,2^3,2^4,2^5-tetrahydro-1^1*H*-1(2,5)-pyrrola-2(2,3)-furanacyclononaphane (**1.11b**)

Synthesized in the same general procedure to afford **1.11a**. Isolated desired product as a purple-film. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.90 (s, 2H), 8.54 (s, 1H), 8.69 (s, 1H), 7.38 (m, 2H), 7.27 (m, 2H), 7.25 (m, 2H), 6.90 (d, J = 8.2 Hz, 2H), 6.80 (d, J = 8.2 Hz, 2 H), 6.23 (t, J = 3.1 Hz, 2H), 6.19 (dd, J = 3.3, 1.8 Hz, 1H), 6.17 (dd, J = 3.3, 1.8 Hz, 1H), 6.02 (t, J = 2.8 Hz, 1H), 6.00 (t, J = 2.8 Hz, 1H), 5.88 (dd, J = 3.2, 1 Hz, 2H), 5.83 (t, J = 2.9 Hz, 1 H), 5.81 (t, J = 2.9 Hz, 1 H), 4.17 (m, 1H), 3.94 (m, 1H), 3.82 (s, 3H), 3.80 (s, 3H), 2.84 (q, J = 7.2 Hz, 1 H), 2.76 (m, 1H), 2.73–2.66 (m, 1H), 2.66–2.61 (m, 1H), 2.61–2.57 (m, 1H), 2.57–2.49 (m, 1H), 2.27 (s, 3H), 2.24 (s, 3H), 2.19–2.13 (m, 1H), 1.90–1.80 (m, 3 H), 1.80–1.69 (m, 2H), 1.68–1.60 (m, 4H), 1.53–1.43 (m, 6H), 1.18–1.12 (m, 4H), 1.11–1.06 (m, 2H), 1.02 (t, J = 7.5 Hz, 3H), 1.00–0.94 (m, 6H), 0.91–0.82 (m, 5H), 0.81–0.67 (m, 2H); ^{13}C-NMR ($CDCl_3$, 126 MHz) δ 144.7, 144.6, 141.6, 139.9, 136.4, 134.7, 134.5, 133.5, 132.5, 131.2, 130.9, 129.6, 129.5, 129.0, 128.2, 127.7, 127.7, 127.6, 126.8, 126.5,

123.3, 123.2, 123.1, 121.9, 115.4, 115.1, 114.5, 114.0, 111.3, 111.2, 106.2, 105.3, 105.2, 105.1, 99.9, 99.9, 84.9, 84.3, 81.3, 79.4, 59.0, 58.9, 47.9, 47.7, 40.3, 39.1, 34.8, 31.7, 30.1, 29.6, 28.9, 28.2, 27.9, 27.5, 26.9, 26.0, 26.0, 25.5, 25.4, 23.9, 22.8, 21.7, 14.3, 11.6, 11.0, 10.9; HRMS calculated for [$C_{33}H_{40}N_3O_4S$+H] = 576.2891, found 576.2879; 2.1 ppm mass defect.

(2*S*)-1-(1^1*H*-1(2,5)-pyrrolacyclodecaphane-3-yl)propan-2-ol (**1.9d**)

To a 5-mL round bottom flask equipped with a stir bar, reflux condenser was added **1.9a** (60 mg, 1.0 equiv, 228 μmol), KOH (51 mg, 87% Wt, 3.4 Eq, 0.78 mmol), diluted with diethylene glycol (1.74 mL, 0.13 M), and added hydrazine hydrate (120 mg, 117 μL, 35% Wt, 5.8 equiv, 1.32 mmol). The reaction mixture was then purged with argon and then the reaction mixture was allowed to heat up to 200 ºC and stir for 3 h. Afterwards, the reaction mixture was cooled to 23 ºC and allowed to stir overnight. The reaction mixture was then diluted with water, transferred to a sep. funnel, and then extracted with EtOAc (3 times). The organic layers were then washed with water, brine, dried over Na_2SO_4, filtered, and concentrated to afford crude product. Crude product was purified via flash column chromatography using TEA neutralized silica gel (Hex → 25% EtOAc/Hex → 30% EtOAc/Hex → 35% EtOAc/Hex → 40% EtOAc/Hex) to afford title compound (15.6 mg, 27% isolated yield) as a colorless-film. ^{1}H-NMR ($CDCl_3$, 500 MHz) δ 9.28 (s, 1H), 9.16 (s, 1H), 6.98–6.94 (m, 2H), 6.09–6.06 (m, 2H), 3.89–3.78 (m, 2H), 3.42 (tt, J = 11.2, 3.2 Hz, 1H), 3.23–3.16 (m, 1H), 2.88–2.80 (m, 2H), 2.64–2.55 (m, 2H), 2.22–2.10 (m, 2H), 1.91–1.78 (m, 6H), 1.75–1.60 (m, 7H), 1.43–1.31 (m, 8H), 1.20 (d, J = 6.1 Hz, 6H), 1.16–1.07 (m, 5H), 0.96–0.75 (m, 6H); HRMS calculated for [$C_{16}H_{27}NO$+H] = 250.2171; found 250.2178; 2.8 ppm mass defect.

2.4 Synthesis of Fig. 1.9 Compounds

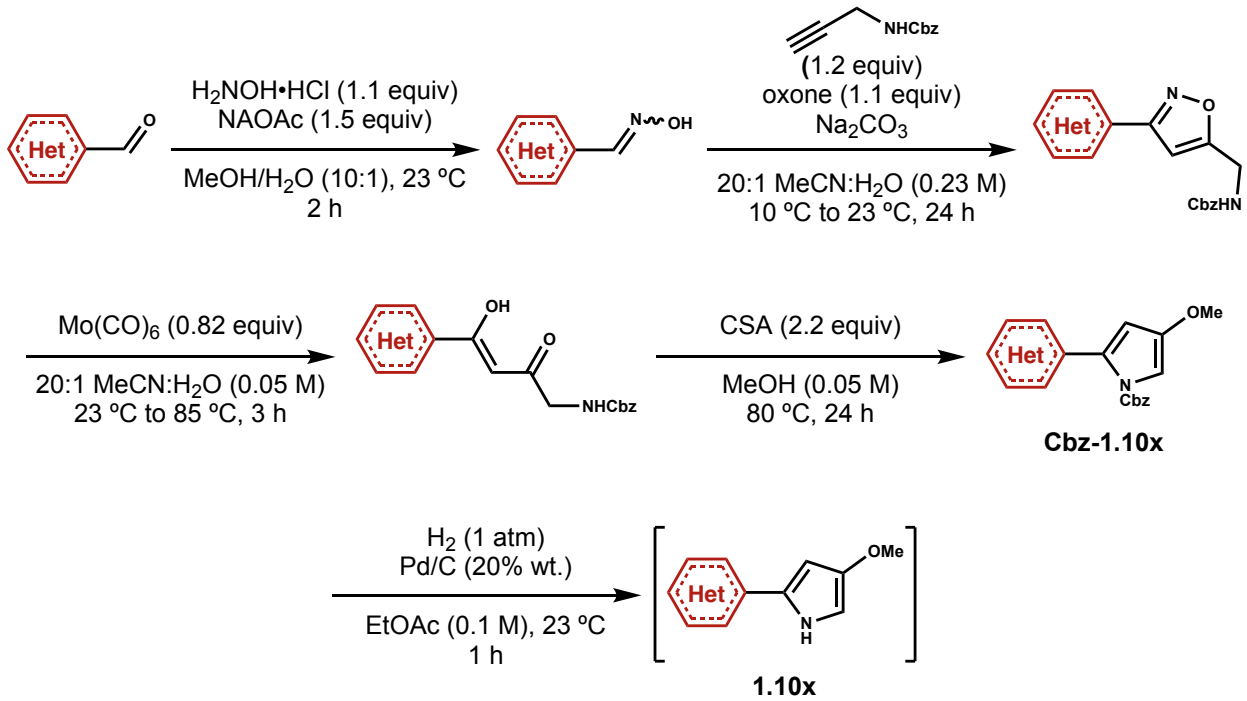


Fig. S2. General synthesis of heterobiaryl nucleophiles. Unless otherwise stated, all heterobiaryls were synthesized following this general scheme based on modified procedures from reference (*1*) and (*2*). Cbz-protected heterobiaryls (**Cbz-1.10x**) were characterized and are listed below. Cbz-*deprotected* products **1.10x** derived from reduction via hydrogenation were used as crudes in condensation reactions. Cbz-*deprotected* products **1.10x** were not characterized due to compound instabilities.

benzyl 2-(3,5-difluorophenyl)-4-methoxy-1*H*-pyrrole-1-carboxylate (**Cbz-1.10c**)

Synthesized following procedure outlined in **Fig. S2**. Product isolated as a white-powder. ^{1}H NMR ($CDCl_3$, 400MHz) δ 7.35 (t, J = 3.26 Hz, 3H), 7.26-7.23 (m, 1H), 6.84 (d, J = 2.15 Hz, 2H), 6.82 (d, J = 2.35 Hz, 1H), 6.71 (tt, J = 9.01, 2.35 Hz, 2.35 Hz, 1H), 6.03 (d, J = 2.15, 1H), 5.21 (s, 2H), 3.74 (s, 3H); HRMS calculated for [$C_{19}H_{15}F_2NO_3$+H] = 344.1098; found 344.1107; 2.6 ppm mass defect.

benzyl 4-methoxy-2-(pyridin-2-yl)-1*H*-pyrrole-1-carboxylate (**Cbz-1.10d**)

Synthesized following procedure outlined in **Fig. S2**. Product isolated as a light-green powder. ^{1}H NMR ($CDCl_3$, 500MHz) δ 9.23 (s, 1H), 8.32 (ddd, J = 4.9, 1.7, 1.0 Hz, 1H), 7.61 (td, J = 7.3, 1.8 Hz, 1H), 7.55 (dt, J = 8.0, 1.0 Hz, 1H), 7.39–7.34 (m, 3H), 7.33–7.27 (m, 1H), 7.03 (ddd, J = 7.4, 4.9, 1.1 Hz, 1H), 6.48 (dd, J = 2.7, 1.8 Hz 1H), 6.38 (dd, J = 2.8, 1.8 Hz, 1H), 4.71 (s, 2H), 3.78 (s, 3H); ^{13}C NMR ($CDCl_3$, 126 MHz) δ 171.3, 150.4, 150.3, 148.9, 141.0, 136.7, 128.9, 128.7, 127.8, 127.1, 120.7, 118.1, 102.2, 95.8, 65.5, 58.1; HRMS calculated for [$C_{18}H_{16}N_2O_3$+H] = 309.1239; found 309.1243; 1.3 ppm mass defect.

benzyl 4-methoxy-2-(3-methoxyphenyl)-1*H*-pyrrole-1-carboxylate (**Cbz-1.10e**)

Synthesized following procedure outlined in **Fig. S2**. Product isolated as an off-white powder. ^{1}H NMR ($CDCl_3$, 500MHz) δ7.54 (d, J = 8.18 Hz, 1H), 7.51-7.46 (m, 1H), 7.38 (t, J = 8.18 Hz, 1H), 7.34-7.28 (m, 2H), 7.23-7.16 (m, 2H), 7.11 (dd, J = 8.18, 2.56 Hz, 1H), 6.94-6.80 (m, 2H), 5.19 (s, 1H), 3.86 (s, 3H), 3.75 (d, J = 2.56 Hz, 2H), 2.60 (s, 3H); ^{13}C NMR ($CDCl_3$, 126MHz) δ 159.8, 149.8, 138.5, 129.6, 128.5, 128.3, 121.9, 121.1, 119.7, 114.9, 113.4, 112.3, 107.7, 101.4, 68.6, 57.6, 55.5, 55.2. HRMS calculated for [$C_{20}H_{19}NO_4$+H] = 338.1392; found 338.1399; 2.1 ppm mass defect.

1*H*,1'*H*-2,2'-bipyrrole (**S2**)

To a flame-dried 500-mL round bottom flask equipped with a stir bar was added 1H-pyrrole (1.00 g, 1.03 mL, 14.9 mmol) and diluted with dry CH_2Cl_2 (248 mL, 0.06 M) under argon. The solution was then allowed to cool to –78 ºC and quickly added bis(trifluoroacetoxy)iodo]benzene (2.16 g, 0.33 equiv, 4.92 mmol, 98% wt.) and bromotrimethylsilane (1.58 g, 1.34 mL, 0.67 equiv, 9.99

mmol, 97% wt.) under argon. The reaction mixture was then allowed to stir for 1 h at –78 ºC. After completion, the reaction mixture was allowed to warm up to 0 ºC and quenched with addition of ~50 mL of sat. $NaHCO_3$; then allowed to warm up to 23 ºC. The reaction mixture was then allowed to stir for 10 min at 23 ºC. The organic layer was then separated from the aqueous; the aqueous layer was then extracted with CH_2Cl_2 (3 x). The organic layers were then combined and dried over Na_2SO_4. Filtration and concentration of volatiles resulted in a black crude product. Crude product was purified via flash SiO_2 chromatography (gradient Hex → 10% EtOAc/Hex → 20% EtOAc/Hex) afforded title compound (348.8 mg, 35% isolated yield) as a dark-purple powder. Product was very unstable and had to be stored under argon in the freezer. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.27 (s, 2H), 6.79–6.76 (m, 2H), 6.25 (q, J = 2.9 Hz, 2H), 6.23–6.20 (m, 2H); HRMS calculated for [$C_8H_8N_2$+H] = 133.0766; found 133.0768; 1.5 ppm mass defect.

3,5-difluorophenyl-isopremarineosin analog **1.11c**

Synthesized following General Procedure A. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.89 (s, 1H), 8.53 (s, 1H), 8.46 (s, 1H), 8.39 (s, 1H), 6.92–6.80 (m, 4H), 6.62–6.52 (m, 2H), 6.33–6.28 (m, 2H), 6.00–5.96 (m, 1H), 5.95 (t, J = 2.8 Hz, 1H), 5.80 (t, J = 2.80 Hz, 1H), 5.76 (t, J = 2.80 Hz, 1H), 4.51–4.40 (m, 1H), 4.20–4.09 (m, 1H), 3.93 (s, 3H), 3.90 (s, 3H), 2.93–2.88 (m, 3H), 2.83–2.76 (m, 1H), 2.69–2.61 (m, 1H), 2.61–2.56 (m, 2H), 2.56–2.48 (m, 1H), 2.22–2.14 (m, 1H), 1.91–1.83 (m, 1H), 1.79–1.71 (m, 1H), 1.55–1.45 (m, 5H), 1.42 (d, J = 6.1 Hz, 3H), 1.41–1.38 (m, 5H), 1.33–1.25 (m, 4 H), 1.20–1.13 (m, 4H), 1.11–1.02 (m, 4H), 1.01–0.92 (m, 2H), 0.91–0.87 (m, 1H), 0.86–0.77 (m, 2H), 0.76–0.66 (m, 1H). ^{13}C-NMR ($CDCl_3$, 126 MHz) δ 150.3, 150.2, 149.1, 148.9, 143.2, 141.1, 136.2, 136.1, 132.8, 131.9, 131.5, 131.0, 128.6, 127.7, 127.5, 127.0, 125.5, 125.0, 124.2, 122.6, 120.1, 119.9, 117.3, 117.0, 106.2, 105.4, 105.0, 104.8, 95.5, 95.3, 85.2, 84.6,

75.5, 73.7, 65.4, 58.7, 58.6, 47.9, 47.5, 42.1, 40.7, 29.7, 28.0, 27.6, 27.0, 26.7, 26.6, 25.8, 25.3, 23.5, 22.7, 21.9, 21.4; HRMS calculated for [$C_{27}H_{32}F_2N_2O_2$+H] = 455.2110; found 455.2122; 2.6 ppm mass defect.

3,5-difluorophenyl-rearranged analog **1.12c**

Synthesized following General Procedure A. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.79 (s, 1H), 7.68 (s, 1H), 6.93–6.87 (m, 2H), 6.59 (t, J = 7.9 Hz, 1H), 6.51 (tt, J = 8.9, 2.1 Hz, 1H), 6.31 (d, J = 3.0 Hz, 1H), 5.85 (d, J = 2.8 Hz, 1H), 4.08–3.99 (m, 1H), 3.88 (s, 3H), 2.73–2.64 (m, 1H), 2.59 (dd, J = 14.3, 3.8 Hz, 1H), 2.56–2.47 (m, 1H), 2.37 (dd, J = 14.4, 9.4 Hz), 1.99–1.84 (m, 2H), 1.74–1.60 (m, 4H), 1.51–1.45 (2 H), 1.23 (d, J = 6.2 Hz, 3H), 0.98–0.95 (m, 1H), 0.86–0.83 (m, 1H), 0.80–0.72 (m, 1H), 0.65–0.51 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 164.6, 164.5, 162.7, 162.6, 146.4, 133.1, 132.2, 125.5, 123.7, 118.5, 106.0, 105.8, 100.6, 96.0, 68.8, 58.1, 35.9, 34.8, 31.7, 29.9, 28.7, 28.6, 28.6, 28.1, 27.2, 27.0, 25.6, 25.4, 23.5, 14.3; HRMS calculated for [$C_{27}H_{32}F_2N_2O_2$+H] = 455.2510; found 455.2515; 1.1 ppm mass defect.

Pyridyl-isopremarineosin analog **1.11d**

Synthesized following General Procedure A. Isolated analog **1.11d** possibly as an HCl salt due to over integration of 2 protons based on ^{1}H NMR. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.44 (s, 1H), 9.31 (s, 1H), 8.96 (s, 1H), 8.62 (s, 1H), 8.41–8.37 (m, 2H), 7.59–7.50 (m, 2H), 7.39–7.34 (m, 4H), 6.98–6.91 (m, 2H), 6.48–6.43 (m, 2H), 5.99–5.94 (m, 2H), 5.78 (t, J = 2.6 Hz, 1H), 5.74 (t, J = 2.9 Hz, 1H), 4.49–4.40 (m, 1H), 4.24–4.15 (m, 1H), 3.95 (s, 3H), 3.91 (s, 3H), 2.94–2.88 (m, 1H), 2.82–2.76 (m, 1H), 2.66–2.60 (m, 1H), 2.60–2.56 (m, 2H), 2.56–2.48 (m, 1H), 2.21–2.14 (m, 1H), 1.86–1.81 (m, 1H), 1.78–1.70 (m, 1H), 1.67–1.58 (m, 3H), 1.55–1.44 (m, 6H), 1.42–1.40 (m, 6H), 1.36–

1.29 (m, 3H), 1.19–1.13 (m, 3H), 1.09–1.03 (m, 2H), 0.99–0.93 (m, 2H), 0.86–0.78 (m, 3H), 0.75–0.69 (m, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 150.3, 150.2, 149.1, 148.9, 143.2, 141.1, 136.2, 136.1, 132.8, 131.9, 131.5, 131.0, 128.6, 127.7, 127.5, 127.0, 125.5, 125.0, 124.2, 122.6, 120.1, 119.9, 117.3, 117.0, 106.2, 105.4, 105.0, 104.8, 95.5, 95.4 85.2, 84.6, 75.5, 73.7, 65.4, 58.7, 58.6, 47.9, 47.6, 42.1, 40.7, 29.7, 29.5, 28.0, 27.6, 27.0, 26.7, 26.6, 25.8, 25.3, 23.5, 22.7, 21.9, 21.4; HRMS calculated for $[C_{26}H_{33}N_3O_{2+H}]$ = 420.2651; 420.2659; 1.9 ppm mass defect.

Pyridyl-rearranged analog **1.12d**

Synthesized following General Procedure A. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 9.32 (s, 1H), 8.35 (m, 1H), 7.70 (s, 1H), 7.55 (t, J = 7.4 Hz, 1H), 7.39 (d, J = 8.1 Hz, 1H), 6.94 (m, 1H), 6.58 (t, J = 8.5 Hz, 1H), 6.45 (s, 1H), 5.88 (d, J = 2.4 Hz, 1H), 4.01–3.92 (m, 1H), 3.87 (s, 1H), 2.71–2.61 (m, 1H), 2.60–2.51 (m, 1H), 2.39 (dd, J = 14.2, 9.2 Hz, 1H), 2.01–1.91 (m, 1H), 1.78–1.66 (m, 2H), 1.64–1.56 (m, 2H), 1.53–1.45 (m, 2H), 1.44–1.35 (m, 2H), 1.19 (d, J = 6.2 Hz, 3H), 0.79–0.71 (m, 1H), 0.70–0.59 (m, 1H) ^{13}C-NMR (126 MHz, $CDCl_3$) δ 150.2, 148.9, 146.6, 136.6, 132.8, 130.8, 126.0, 123.3, 120.1, 119.0, 118.6, 117.9, 106.2, 95.8, 68.3, 58.1, 36.4, 29.8, 28.7, 28.6, 28.5, 28.1, 25.6, 23.3; HRMS calculated for $[C_{26}H_{33}N_3O_{2+H}]$ = 420.2651; 420.2653; 0.5 ppm mass defect.

3-anisole-isopremarineosin analog **1.11e**

Synthesized following General Procedure A. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.96 (s, 1H), 8.52 (s, 1H), 8.45 (s, 1H), 8.35 (s, 1H), 7.23 (t, J = 7.9 Hz, 2H), 6.99–6.97 (m, 1H), 6.97–6.95 (m, 1H), 6.92 (t, J = 1.9 Hz, 1H), 6.91 (t, J = 1.7 Hz, 1H), 6.73–6.67 (m, 2H), 6.31–6.26 (m, 2H), 5.99 (t, J = 2.8 Hz, 1H), 5.96 (t, J = 2.8 Hz, 1H), 5.80 (t, J = 2.9 Hz, 1H), 5.75 (t, J = 2.9 Hz, 1H), 4.50–4.41 (m, 1H), 4.18–4.09 (m, 1H), 3.94 (s, 3H), 3.90 (s, 3H), 3.82 (s, 6H), 3.01–2.91 (m, 1H), 2.85–2.77

(m, 1H), 2.69–2.61 (m, 1H), 2.58 (t, J = 5.8 Hz, 2H), 2.55–2.46 (m, 1H), 2.23–2.13 (m, 1H), 1.89–1.82 (m, 2H), 1.80–1.71 (m, 3H), 1.66–1.60 (m, 2H), 1.56–1.50 (m, 2H), 1.48–1.44 (m, 2H), 1.42 (d, J = 6.6 Hz, 3H), 1.41–1.38 (m, 5H), 1.23–1.20 (m, 2H), 1.18–1.14 (m, 2H), 1.10–1.05 (m, 2H), 0.97 (d, J = 6.7 Hz, 2H), 0.89–0.87 (m, 3H), 0.85–0.81 (m, 2H), 0.76–0.67 (m, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 160.2, 160.1, 134.3, 134.2, 133.0, 132.1, 131.8, 131.3, 130.0, 129.9, 126.6, 125.8, 125.6, 125.1, 122.6, 116.4, 116.2, 115.8, 111.8, 111.6, 111.4, 109.2, 109.0 106.5, 106.4, 105.5, 105.0, 105.0, 95.2, 95.1, 85.4, 84.8, 75.7, 73.9, 58.8, 55.4, 55.4, 48.1, 47.5, 34.8, 31.7, 29.8, 28.1, 27.8, 26.9, 26.8, 26.6, 25.9, 25.6, 25.5, 25.4, 22.8, 22.1, 21.4, 14.3, 14.3. HRMS calculated for [$C_{28}H_{36}N_2O_3$+H] = 449.2804; found 449.2818; 3.1 ppm mass defect.

3-anisole-rearranged analog **1.11e**

Synthesized following General Procedure A. ^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.50 (s, 1H), 7.73 (s, 1H), 7.19 (t, J = 7.1 Hz, 1H), 6.97 (d, J = 7.7 Hz, 1H), 6.94 (t, J = 1.8 Hz, 1H), 6.73–6.62 (m, 1H), 6.52 (t, J = 8.5, 1H), 6.30 (d, J = 3.0, 1H), 5.84 (d, J = 2.9 Hz, 1 H), 4.03–3.94 (m, 1H), 3.88 (s, 3H), 3.80 (s, 3H), 2.69–2.61 (m, 1H), 2.60–2.49 (m, 2H), 2.44–2.35 (m, 1H), 1.98–1.90 (m, 1H), 1.90–1.82 (m, 2H), 1.76–1.68 (m, 1H), 1.66–1.57 (m, 2H), 1.55–1.46 (m, 2H), 1.42–1.38 (m, 2H), 1.20 (t, J = 6.2, 3H), 0.78–0.65 (m, 1H), 0.65–0.52 (m, 1H) ^{13}C-NMR (400 MHz, $CDCl_3$) δ 160.1, 146.4, 134.1, 132.9, 130.5, 129.8, 127.2, 125.7, 123.9, 118.5, 117.2, 116.2, 111.3, 109.3, 105.6, 95.1, 68.7, 58.1, 55.4, 36.1, 31.7, 28.1, 27.2, 26.9, 25.6, 25.4, 23.2, 22.8; HRMS calculated for [$C_{28}H_{36}N_2O_3$+H] = 449.2804; found 449.2807; 0.7 ppm mass defect.

Desmethoxy isopremarineosin analog **1.11f**

Synthesized following General Procedure A. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.57 (s, 1H), 8.50 (s, 1H), 7.75 (s, 1H), 7.44 (s, 1H), 6.78–6.71 (m, 2H), 6.28 (t, J = 3.0 Hz, 1H), 6.22–6.19 (m, 3H), 6.19–6.16 (m, 3H), 6.15 (t, J = 6.2 Hz, 1H), 6.04 (t, J = 2.9 Hz, 1H), 5.86 (t, J = 3.0 Hz, 1H), 5.83 (t, J = 2.8 Hz, 1H), 5.80 (t, J = 3.0 Hz, 1H), 4.48 (s, J = 6.3 Hz, 1H), 3.96–3.87 (m, 1H), 2.82–2.74 (m, 2H), 2.73–2.67 (m, 1H), 2.64–2.57 (m, 1H), 2.54–2.47 (m, 1H), 2.33–2.24 (m, 1H), 2.15–2.08 (m, 1H), 1.99–1.83 (m, 3H), 1.76–1.68 (m, 3H), 1.53–1.49 (m, 2H), 1.45–1.40 (m, 3H), 1.39–1.35 (m, 2H), 1.35–1.33 (m, 3H), 1.13 (d, J = 6.3 Hz, 3H), 1.08–0.99 (m, 6H), 0.97 (d, J = 6.6 Hz, 3H), 0.86–0.83 (m, 3H); HRMS calculated for $[C_{24}H_{31}N_3O+H]$ = 378.2545; found 378.2552; 1.9 ppm mass defect.

2.5 Synthesis of Fig. 1.12 Compounds

Urethane derivative **1.12h**

To a dram vial charged with **1.12a** (31.4 mg, 1.0 equiv, 55.9 μmol) (stripped 3 times with dry PhMe) was added a flame-dried stir bar, 1-bromo-4-isocyanatobenzene (22.1 mg, 2.0 equiv, 112 μmol), diluted with DCE (559 μL, 0.1M), and then added NEt_3 (31.2 μL, 2.0 equiv, 224 μmol) . The reaction mixture was then purged with argon, capped (with electrical tape) and the reaction mixture allowed to stir at 23 ºC for 3 h. After TLC indicated reaction completion, the reaction mixture was added onto water and then transferred to a separatory funnel. The aqueous layer was extracted with EtOAc (3 times). The organic layers combined and washed with brine (1 time), dried over Na_2SO_4, filtered, and concentrated to afford crude product. Crude product was purified via flash SiO_2 chromatography (SiO_2 slurry packed with 1% NEt_3/Hex and then stripped with Hex) (gradient Hex → 10% EtOAc/Hex) afforded title compound (14.8 mg, 34% isolated yield) as a dark-red film. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 8.50 (s, 1H), 7.67 (s, 1H), 7.35–7.31 (m, 4H), 7.19 (d, J = 8.8 Hz, 2H), 7.12 (d, J = 8.2 Hz, 2H), 6.52 (t, J = 8.4 Hz, 1H), 6.46 (s, 1H), 6.22 (t, J = 3.3 Hz, 1H), 6.18 (dd, J = 3.3, 1.7 Hz, 1H), 5.93 (d, J = 2.9 Hz, 1H), 5.83 (d, J = 2.6 Hz, 1H), 5.08 (sext, J = 6.2 Hz, 1H), 3.77 (s, 3H), 3.41 (q, J = 7.2 Hz, 1H), 2.96 (q, J = 7.2 Hz, 1H), 2.64 (d, J = 6.2 Hz, 2H), 2.61–2.52 (m, 2H), 2.32 (s, 3H), 2.01–1.93 (m, 2H), 1.56–1.45 (m, 2H), 1.43–1.36 (m, 2H), 1.19–1.12 (m, 3H), 0.86–0.80 (m, 2H), 0.64–0.55 (m, 1H), 0.55–0.45 (m, 1H); HRMS calculated for [$C_{39}H_{43}BrN_4O_5S$+H] = 759.2216; found 759.2223; 0.9 ppm mass defect.

Acetoxy derivative **1.12i**

To a dram vial equipped with a stir bar was charged with 1.12a (10.6 mg, 1.0 equiv, 18.9 μmol) and diluted with anhydrous CH_2Cl_2 (78.9 μL, 0.24 M). To this solution NEt_3 (5.31 μL, 2.0

equiv, 37.7 μmol), DMAP (233 μg, 0.1 equiv, 1.89 μmol), and Ac_2O (2.31 μL, 1.3 equiv, 24.5 μmol) were added and the mixture allowed at 0 ºC. The reaction mixture was allowed to gradually warm up to 23ºC and stirred for 16 h. Once, TLC indicated completion, the reaction mixture was quenched by addition of aqueous NH_4Cl and then the mixture was extracted with CH_2Cl_2(3 x). The organic layers were combined and washed with brine, sat. $NaHCO_3$, water, and dried over $NaSO_4$, filtered, and concentrated to afford crude product. Crude product was purified via flash SiO_2 chromatography (SiO_2 slurry packed with 1% NEt_3/Hex and then stripped with Hex) (gradient Hex → 15% EtOAc/Hex) afforded title compound (4.3 mg, 38% isolated yield) as a dark-red oil; R_f = 0.36 at 30% EtOAc/Hex. Compound was very unstable and notable degradation was observed via ^1H NMR over hours at 23 ºC in solution with $CDCl_3$ and / or acetone-d_6. Compound completely decomposed into intractable products upon repurification. ^1H NMR (acetone-d_6, 500 MHz) δ 9.53 (s, 1H), 7.75 (d, J = 8.3 Hz, 2H), 7.68 (dd, J = 3.2, 1.8 Hz, 1H), 7.08 (d, J = 8.0 Hz, 2H), 6.93 (dd, J = 3.6, 1.8 Hz, 1H), 6.43 (t, J = 4.2 Hz, 1H), 6.14 (s, 1H), 6.08 (s, 1H), 5.02–4.94 (m, 1H), 3.79 (s, 3H), 3.15–3.07 (m, 1H), 2.77–2.71 (m, 2H), 2.67–2.62 (m, 1H), 2.58–2.49 (m, 1H), 2.46–2.40 (m, 1H), 2.31 (s, 3H), 1.86 (s, 3H), 1.83–1.78 (m, 2H), 1.45–1.35 (m, 3H), 1.05 (d, J = 6.4 Hz, 3H), 0.77–0.70 (m, 2H); HRMS calculated for $[C_{34}H_{41}N_3O_5S+H]$ = 604.2845; found 604.2851; 1.0 ppm mass defect.

TBS derivative **1.12j**

To a flame-dried dram vial equipped with a stir bar was charged with **1.12a** (7.1 mg, 1.0 equiv, 13 μmol) (stripped with dry PhMe 3 x), imidazole (1.7 mg, 2.0 equiv, 25 μmol), and then diluted with dry CH_2Cl_2 (97 μL, 0.13 M). The reaction mixture was then allowed to stir at 0 ºC and then was added TBSCl (2.3 mg, 1.2 equiv, 15 μmol). The reaction mixture was then allowed to warm up to

23 ºC and stir for 16 h. When TLC indicated reaction completion; the reaction mixture was then added to ice-water, transferred to a sep. funnel, extracted with EtOAc (3 Xs), dried over Na_2SO_4, filtered, and concentrated to afford crude product. Crude product was purified via flash SiO_2 chromatography (SiO_2 slurry packed with 1% NEt_3/Hex and then stripped with Hex) (gradient Hex → 10% EtOAc/Hex) afforded title compound (2.6 mg, 30% isolated yield) as a dark-red film. Compound was very unstable and notable degradation was observed via ^{1}H NMR over hours at 23 ºC in solution with acetone-d_6. ^{1}H NMR (acetone-d_6, 500 MHZ) δ 9.30 (s, 1H), 8.83 (s, 1H), 7.35 (d, J = 8.4 Hz, 2H), 7.31–7.27 (m, 3H), 6.50 (t, J = 8.4 Hz, 1H), 6.28–6.23 (m, 2H), 6.05 (d, J = 2.9 Hz, 1H), 5.77 (d, J = 2.7 Hz, 1H), 4.28–4.21 (m, 1H), 4.21–4.15 (m, 1H), 4.07–4.0 (m, 1H), 3.97–3.91 (m, 1H), 3.74 (s, 3H), 2.66–2.61 (m, 2H), 2.34 (s, 3H), 2.31–2.26 (m, 2H), 1.56–1.53 (m, 2H), 1.42–1.39 (m, 3H), 1.15–1.10 (m, 3H), 1.05–1.02 (m, 2H), 0.65–0.59 (m, 2H), 0.11 (s, 9H), -0.04 (s, 6H); HRMS calculated for [$C_{38}H_{53}N_3O_4SSi$+H] = 676.3604; found 676.3609; 0.7 ppm mass defect.

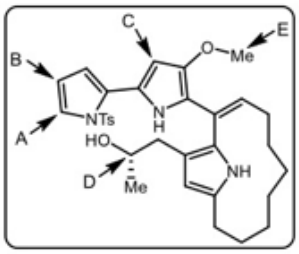
Deshydroxy-propyl condensation derivative **1.12k**

Synthesized following General Procedure A. Crude product was purified via flash neutralized Al_2O_3 chromatography (Al_2O_3 was slurry packed with 1% NEt_3/Hex and then stripped with Hex) (gradient Hex → 5% EtOAc/Hex). Isolated product (51.1 mg, 44% isolated yield) as a ruby-dusty solid; R_f = 0.37 at 8% EtOAc/Hex. ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.55 (s, 1H), 9.39 (s, 1H), 7.21–7.14 (m, 2H), 6.67 (m, 1H), 6.62 (s, 1H), 6.49 (t, J = 8.3 Hz, 1H), 5.87 (t, J = 2.9 Hz, 1H), 5.82 (t, J = 3.0 Hz, 1H), 3.81 (s, 3H), 2.66 (t, J = 6.9 Hz, 2H), 1.60–1.53 (m, 2H), 1.43–1.34 (m, 4H), 1.33–1.24 (m, 2H), 1.23–1.16 (m, 2H), 0.73 (quin, J = 7.7 Hz, 2H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 165.4, 165.3, 163.5, 163.4, 137.2, 137.1, 133.1, 129.7, 127.9, 126.2, 125.3, 120.3, 109.4, 106.6,

106.6, 106.5, 106.4, 100.7, 100.5, 100.3, 97.5, 58.2, 35.2, 28.3, 27.7, 27.6, 27.6, 27.6, 27.5, 25.7; HRMS calculated for [$C_{24}H_{26}F_2N_2O$+H] = 397.2091; found 397.2092; 0.3 ppm mass defect.

3. Computational Methods – Supplemental Figures

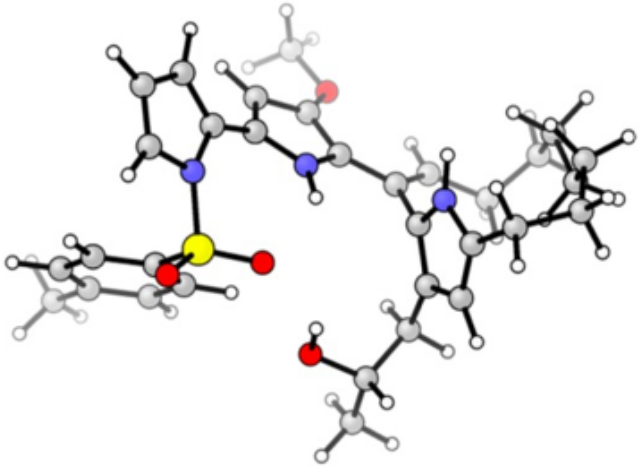
3.1 Supplemental Figures



Optimized 80 structures
57 unique stationary points

19 contribute > 1% Boltzmann population each

RMSD for this method ~2.1 ppm



Resonances aligned manually
SI spectra require additional assignments

Experimental (δ)	DFT (δ)	Exp-DFT	
146.1	147.8	-1.7	
146	144.8	1.2	
136.2	137.0	-0.8	
132.9	132.3	0.6	
130.7	132.1	-1.4	
129.8	129.4	0.4	
129.2	129.3	-0.1	
129.2	127.9	1.3	
127.9	127.8	0.1	
127.4	127.1	0.3	
127.4	126.9	0.5	
124.2	123.5	0.7	
123.9	121.6	2.3	A
119.4	120.1	-0.7	
118.5			
118.3			
117.3	117.6	-0.3	
117.2	116.8	0.4	
115.2	113.9	1.3	
112.9	110.2	2.7	B
106.3	105.7	0.6	
99.9			
99.8	96.8	3.0	C
68.6	71.2	-2.6	D
58.1	55.0	3.1	E
37.4	37.9	-0.5	
30.1	31.7	-1.6	
29	30.0	-1.0	
28.5	29.9	-1.4	
28.4	29.9	-1.5	
27.7	28.9	-1.2	
27.4	27.4	0.0	
26.3	26.0	0.3	
23.6	22.0	1.6	
21.5	21.8	-0.3	

Fig. S3. Computed ^{13}C NMR Shifts are Reasonable Match for Proposed Structure. nmr=giao mPW1PW91/6-311+G(2d,p)/PCM(acetone)//BLYP/6-31+G(d,p).

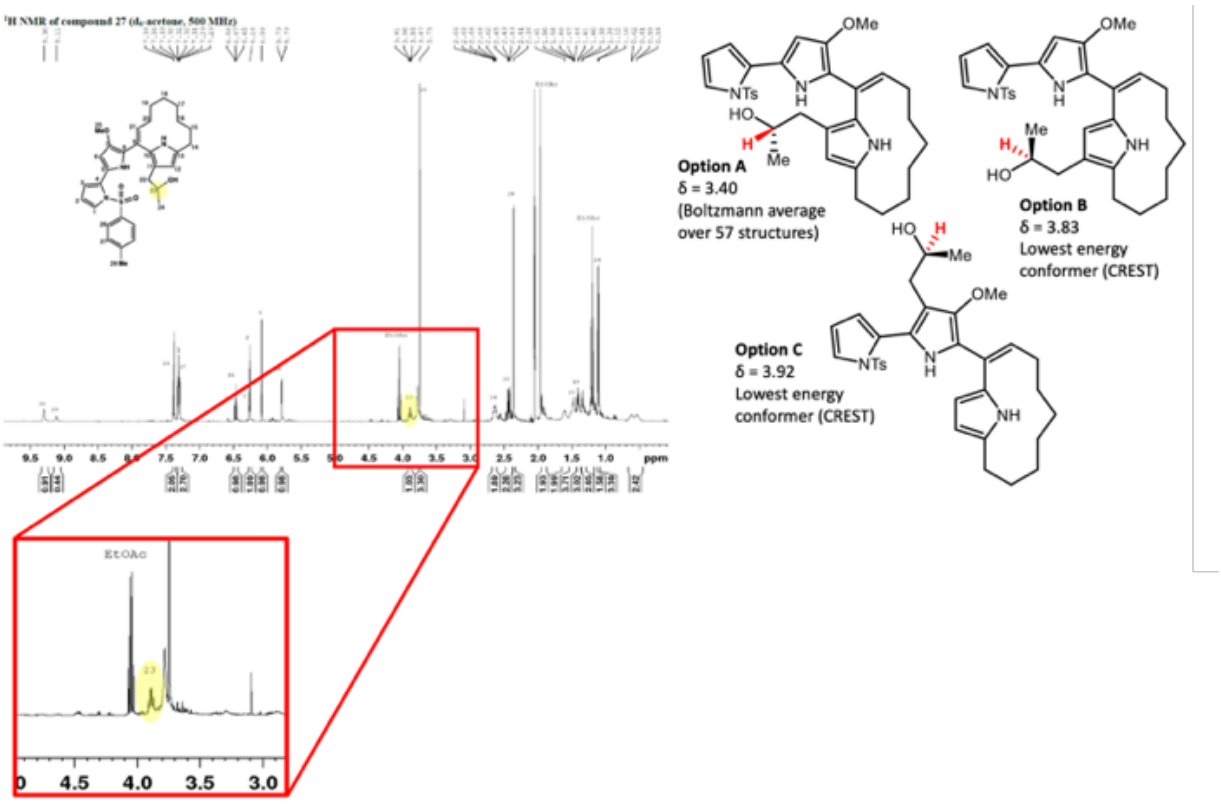


Fig. S4. Only computed ensemble for proposed connectivity (Option A). However, ^{1}H NMR calculations of other connectivity suggests there may be a better fit with Option B or Option C; more conformers needed. Highly complicated by massive conformational flexibility of this molecule. nmr=giao mPW1PW91/6-311+G(2d,p)/PCM(acetone)//BLYP/6-31+G(d,p).

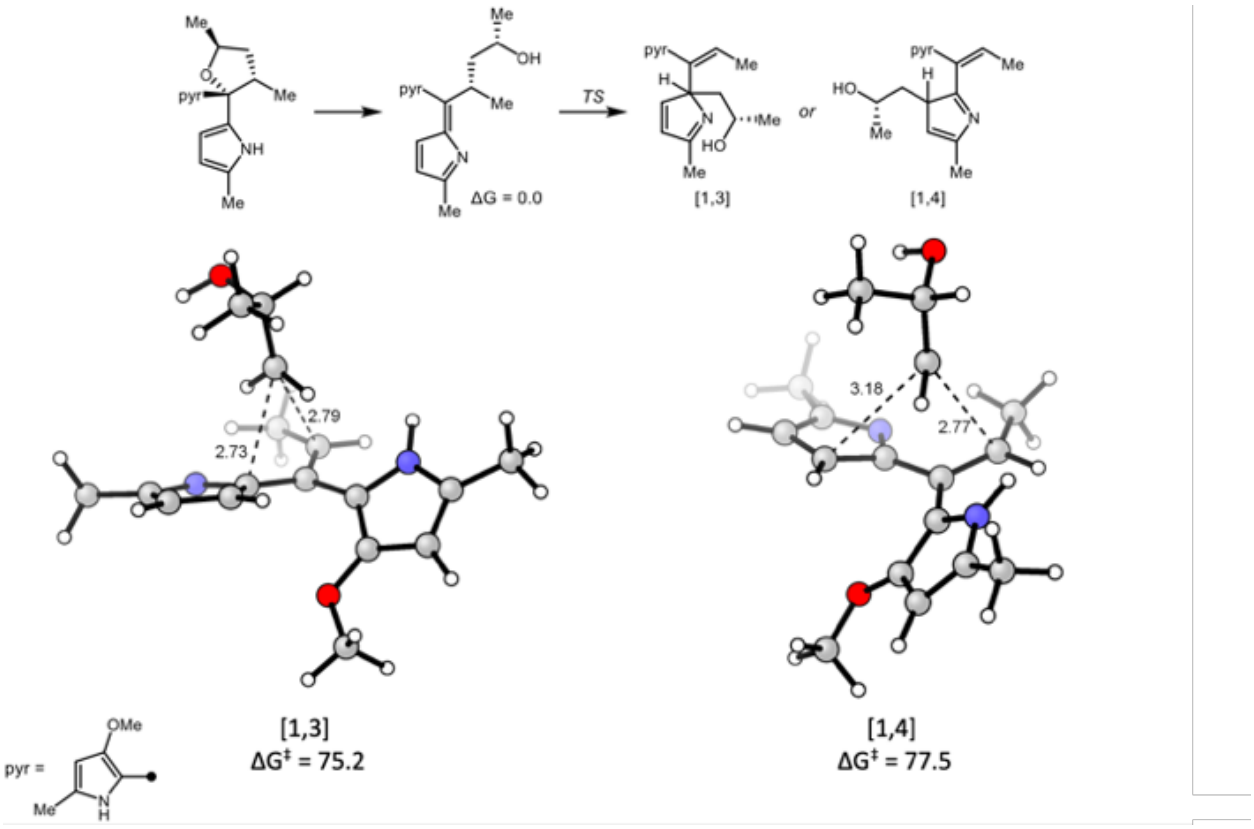
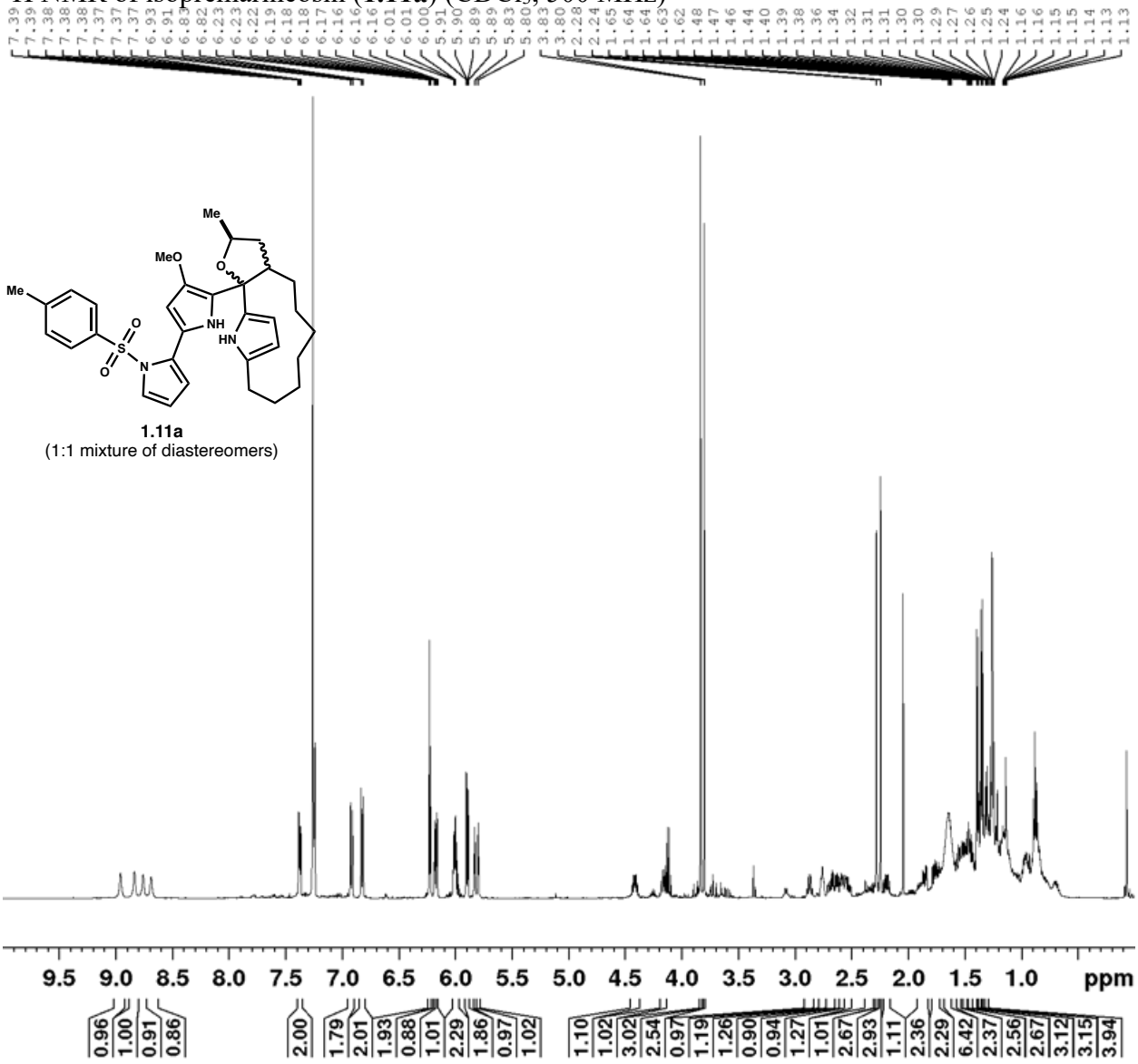


Fig. S5. Both a [1,3] and [1,4] / [1,7] sigmatropic rearrangement are prohibitively high from a Neutral Chromophore. wB797X-D/def2-TZVPP/SMD(PhMe)//wB97X-D/def2-SVP/SMD(PhMe), QH corrections (@ 298.15 K), energies in kcal/mol.

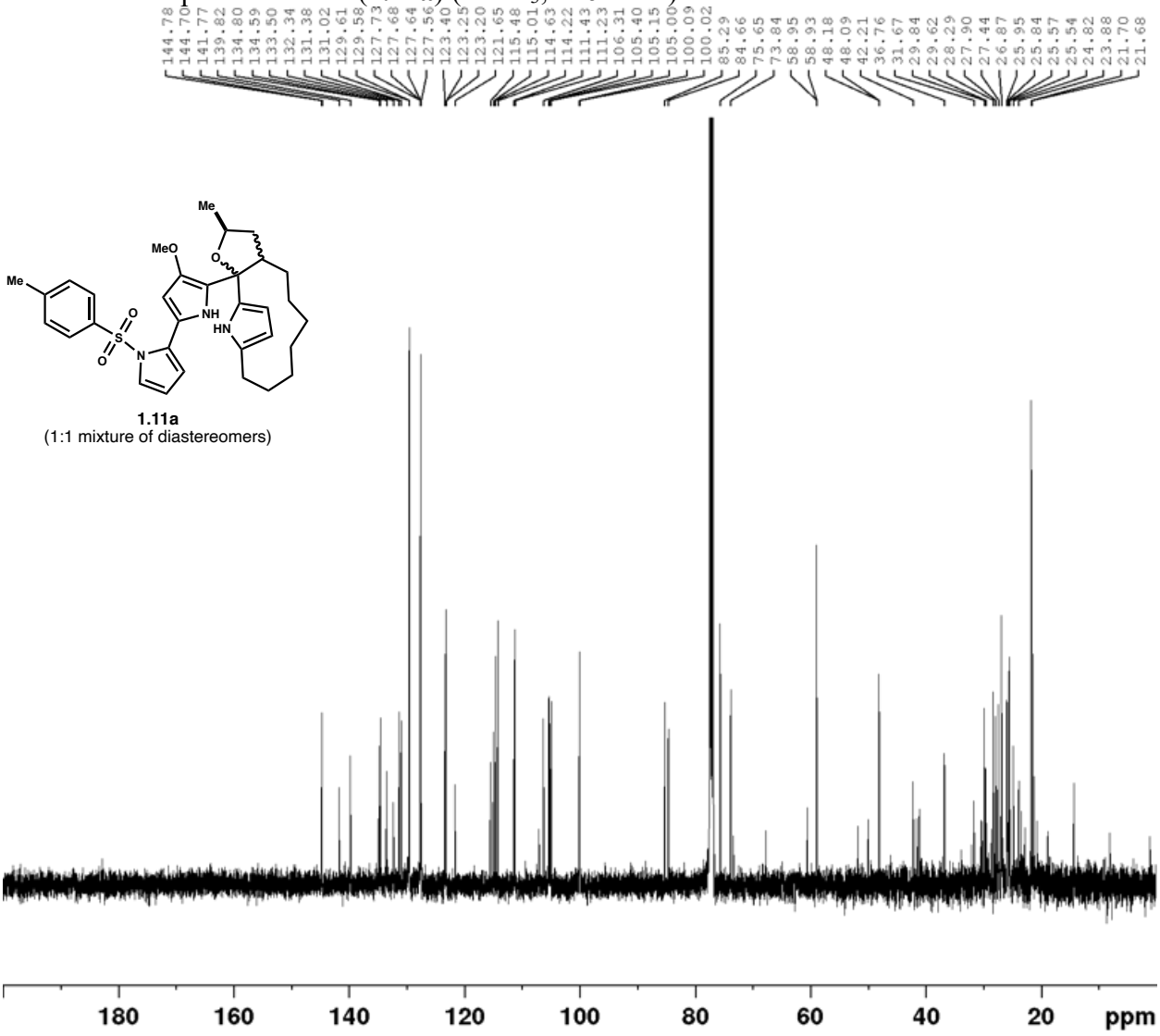
4. Product Characterization Data

4.1 NMR Spectra

^{1}H NMR of isopremarineosin (**1.11a**) ($CDCl_3$, 500 MHz)

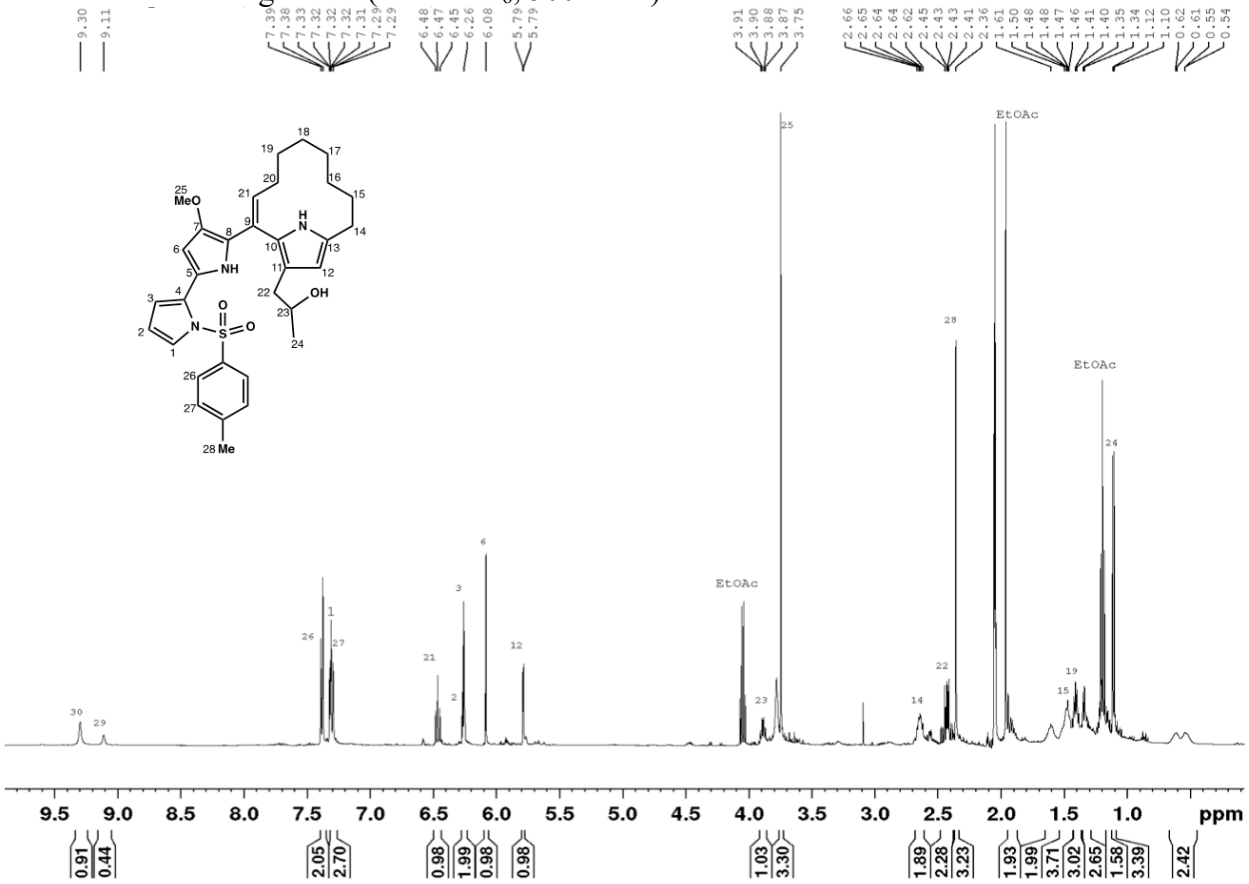


^{1}C NMR of isopremarineosin (**1.11a**) ($CDCl_3$, 126 MHz)

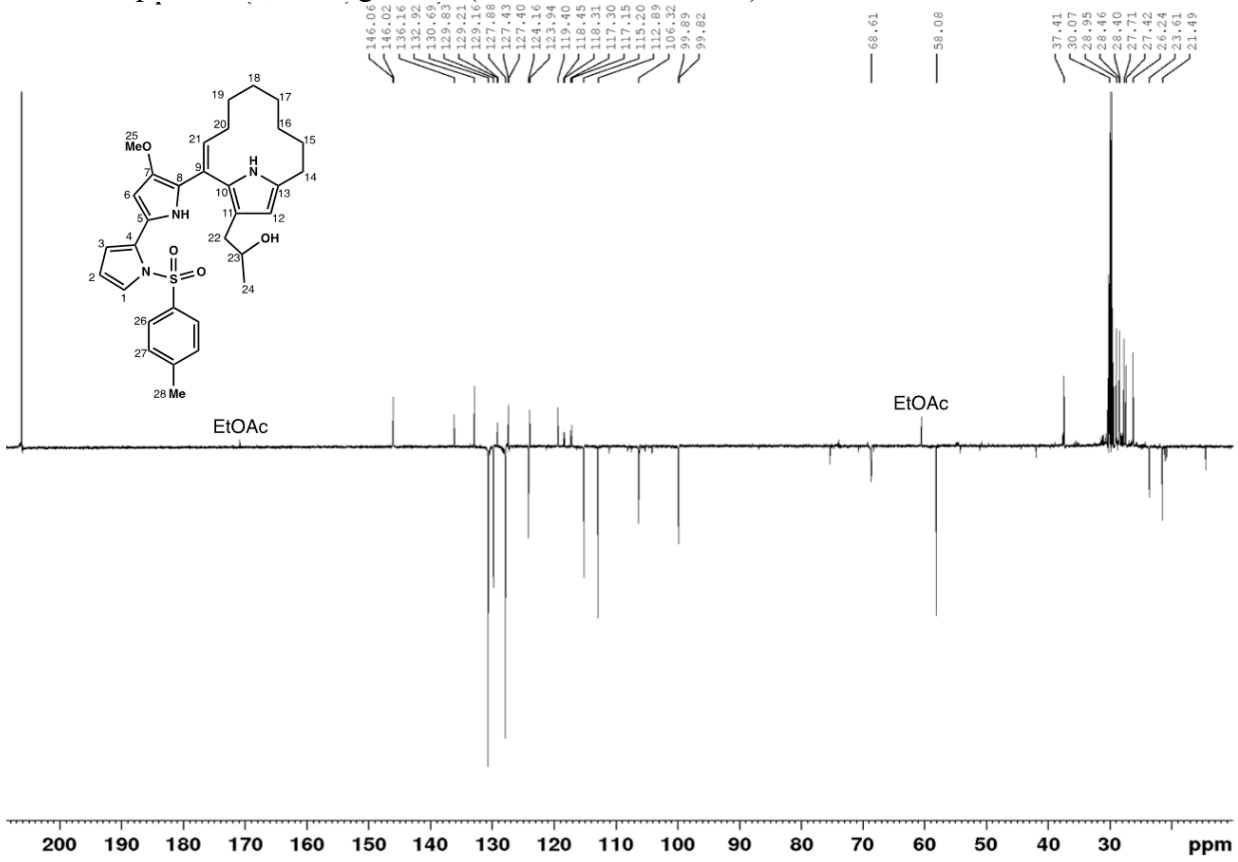


1.11a
(1:1 mixture of diastereomers)

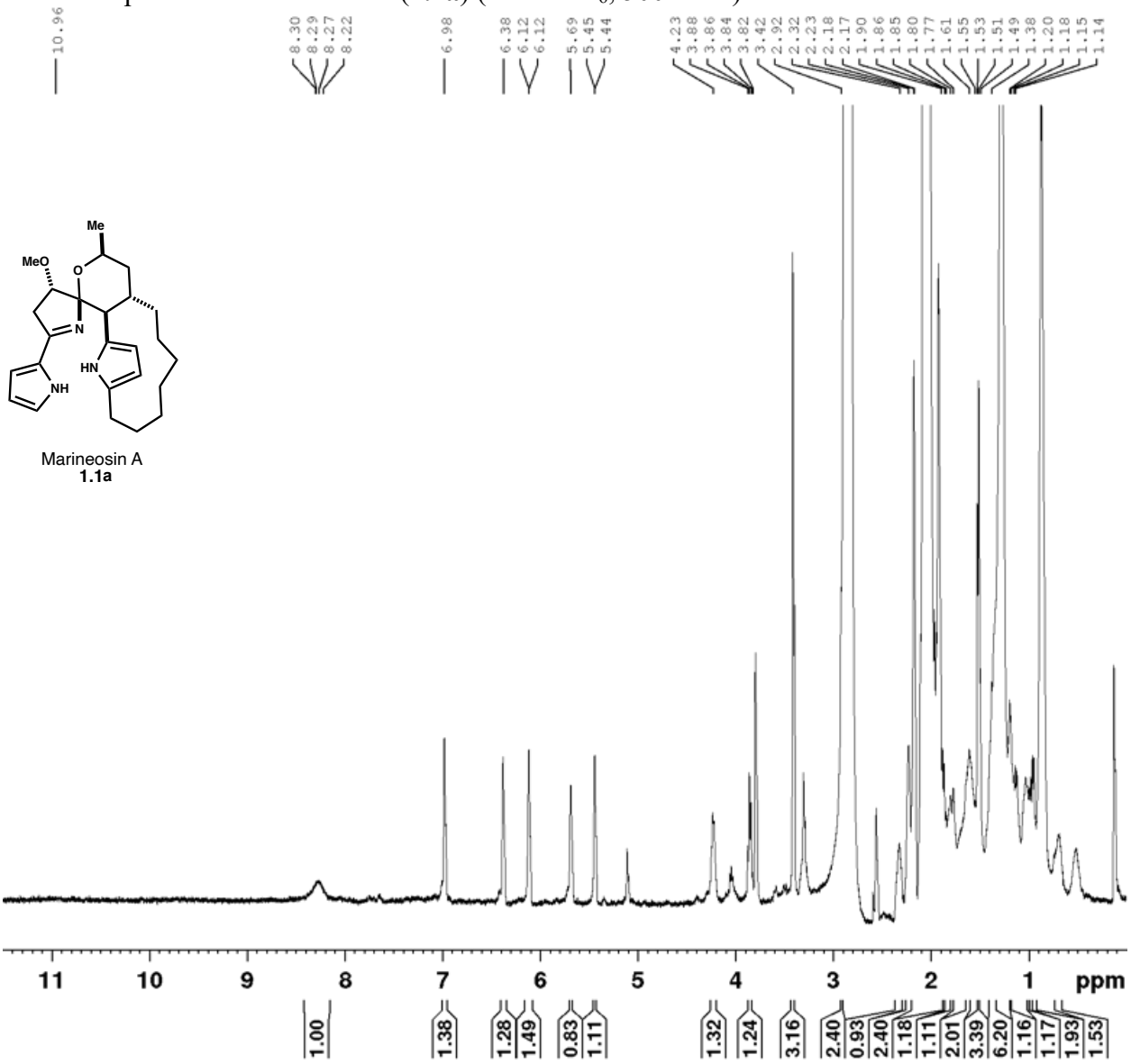
^{1}H NMR of Rearranged 12a (acetone-d_6, 500 MHz)



^{13}C NMR spectra of Rearranged 12a (acetone-d_6, 126 MHz)

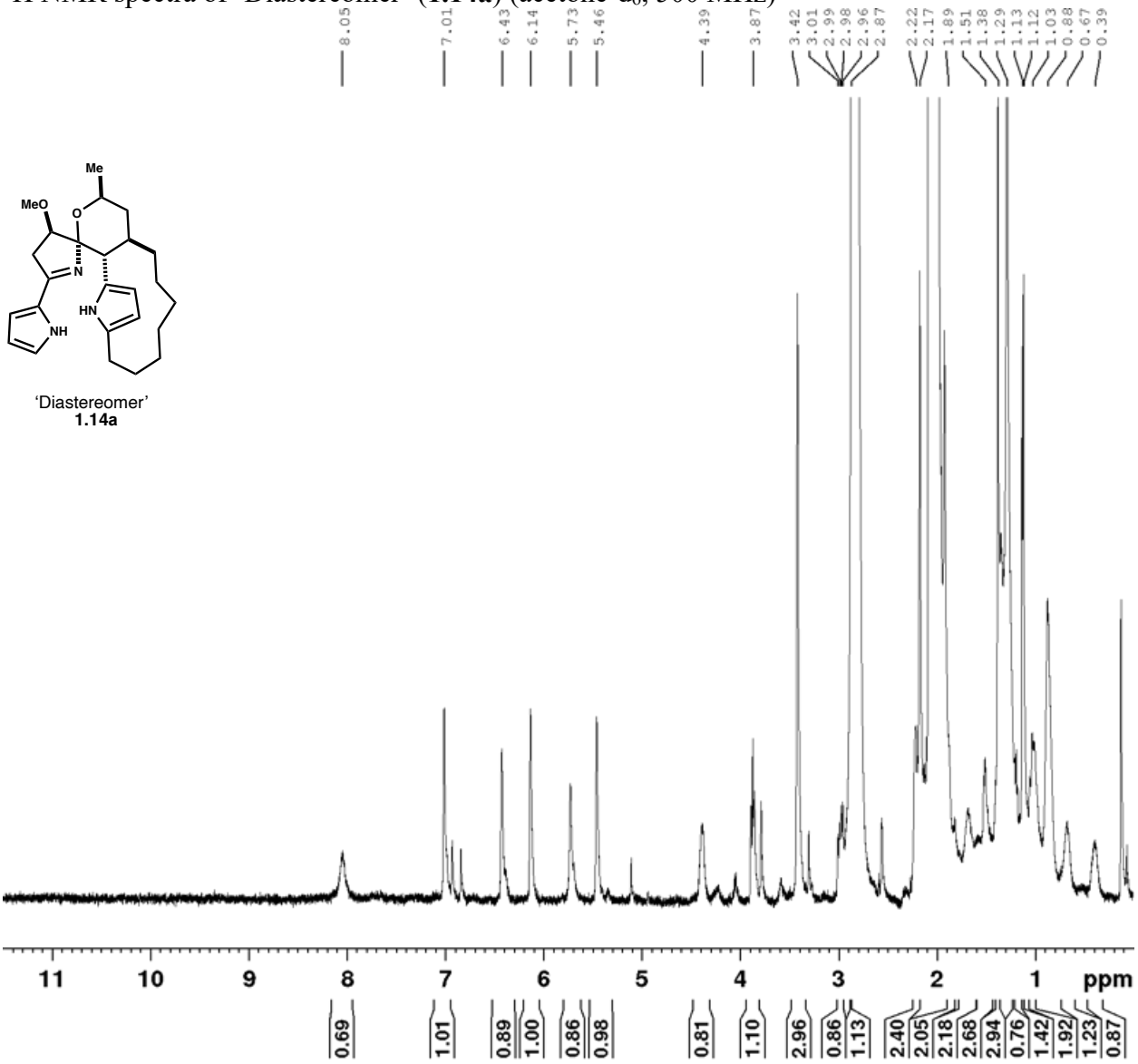


^{1}H NMR spectra of Marineosin A (**1.1a**) (acetone-d_6, 500 MHz)

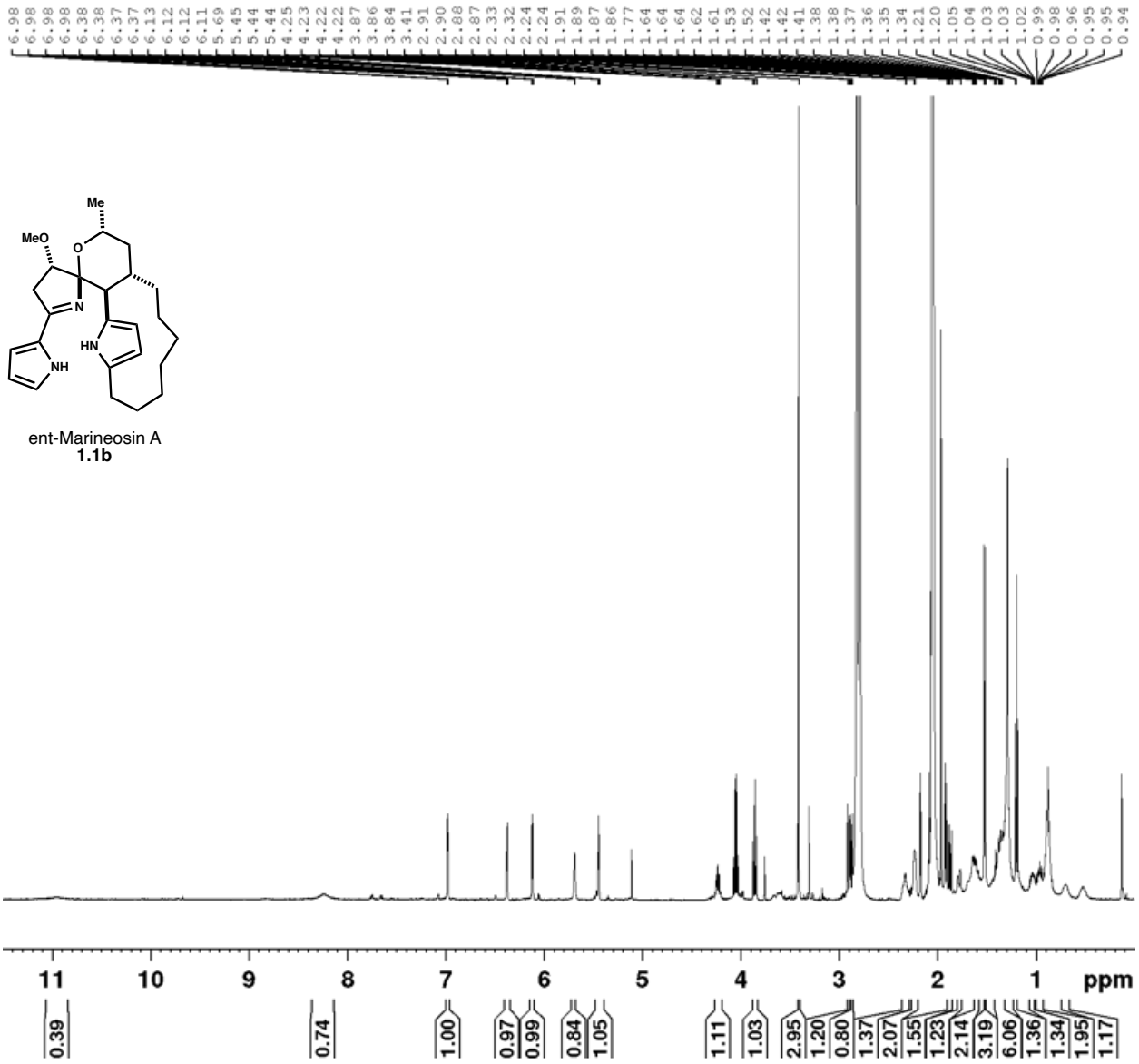


Marineosin A
1.1a

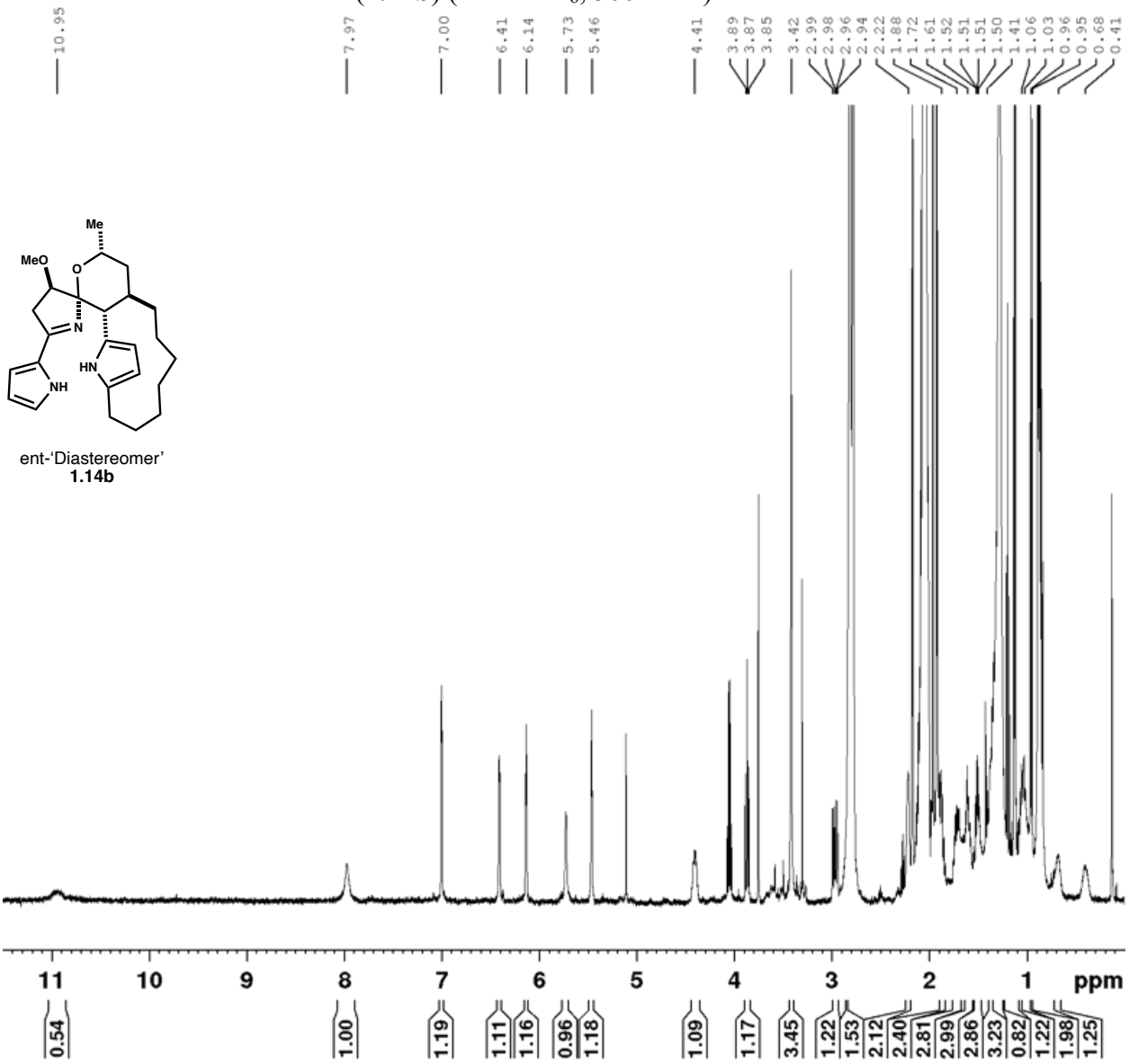
^{1}H NMR spectra of 'Diastereomer' (**1.14a**) (acetone-d_6, 500 MHz)



^{1}H NMR of ent-Marineosin A (**1.1b**) (acetone-d_6, 500 MHz)

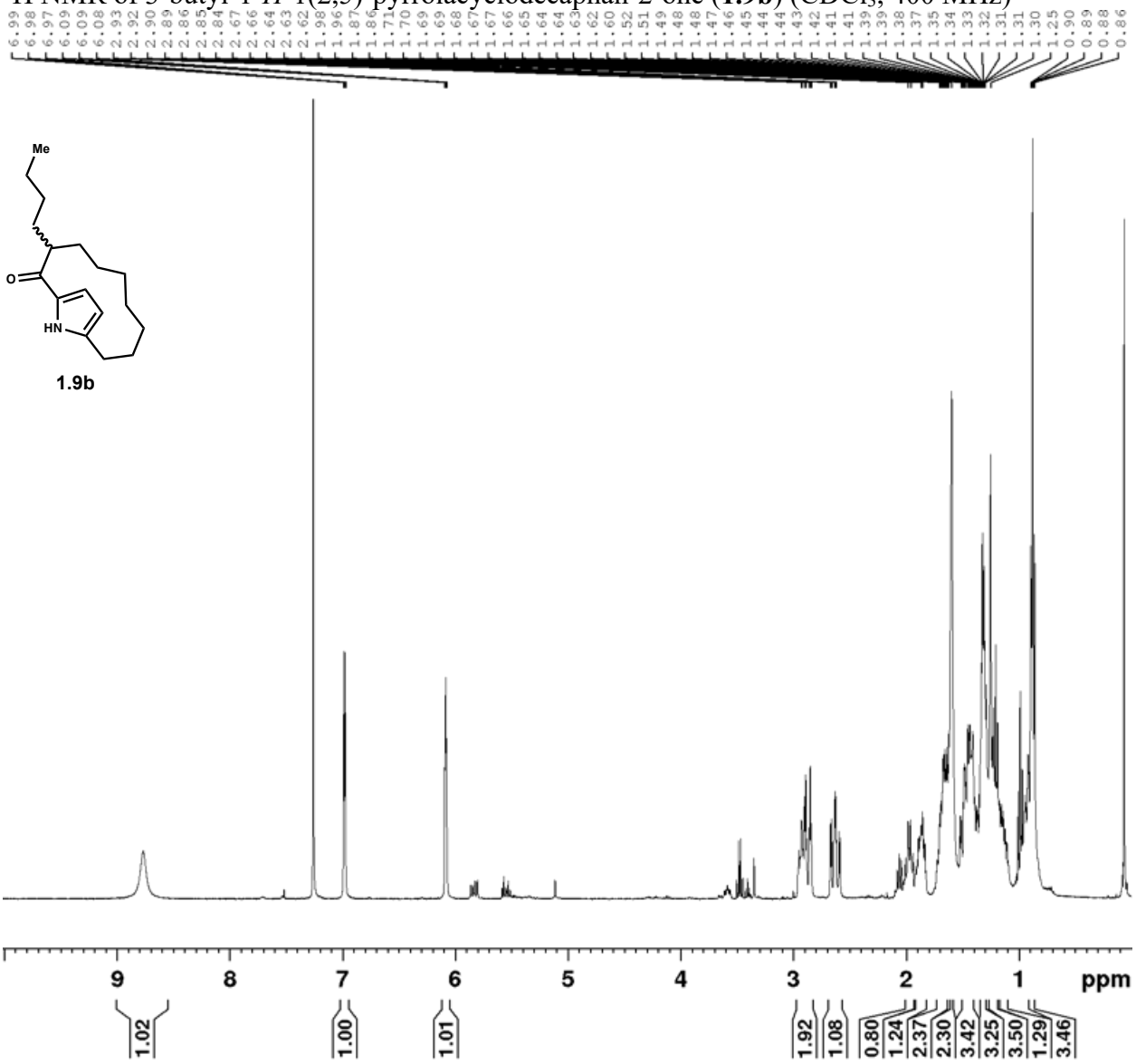


^{1}H NMR of ent-Marineosin A (**1.14b**) (acetone-d_6, 500 MHz)

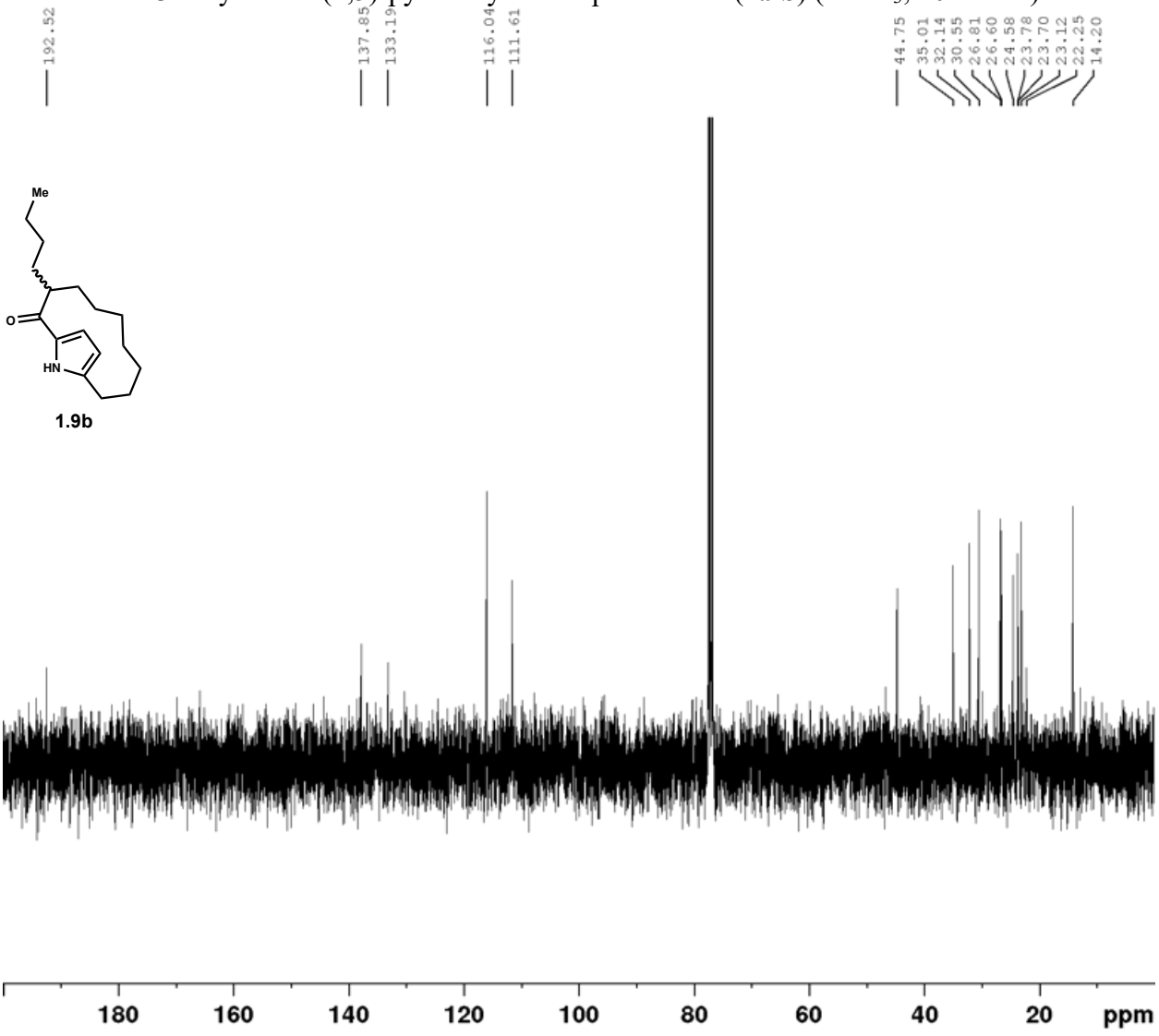


ent-'Diastereomer'
1.14b

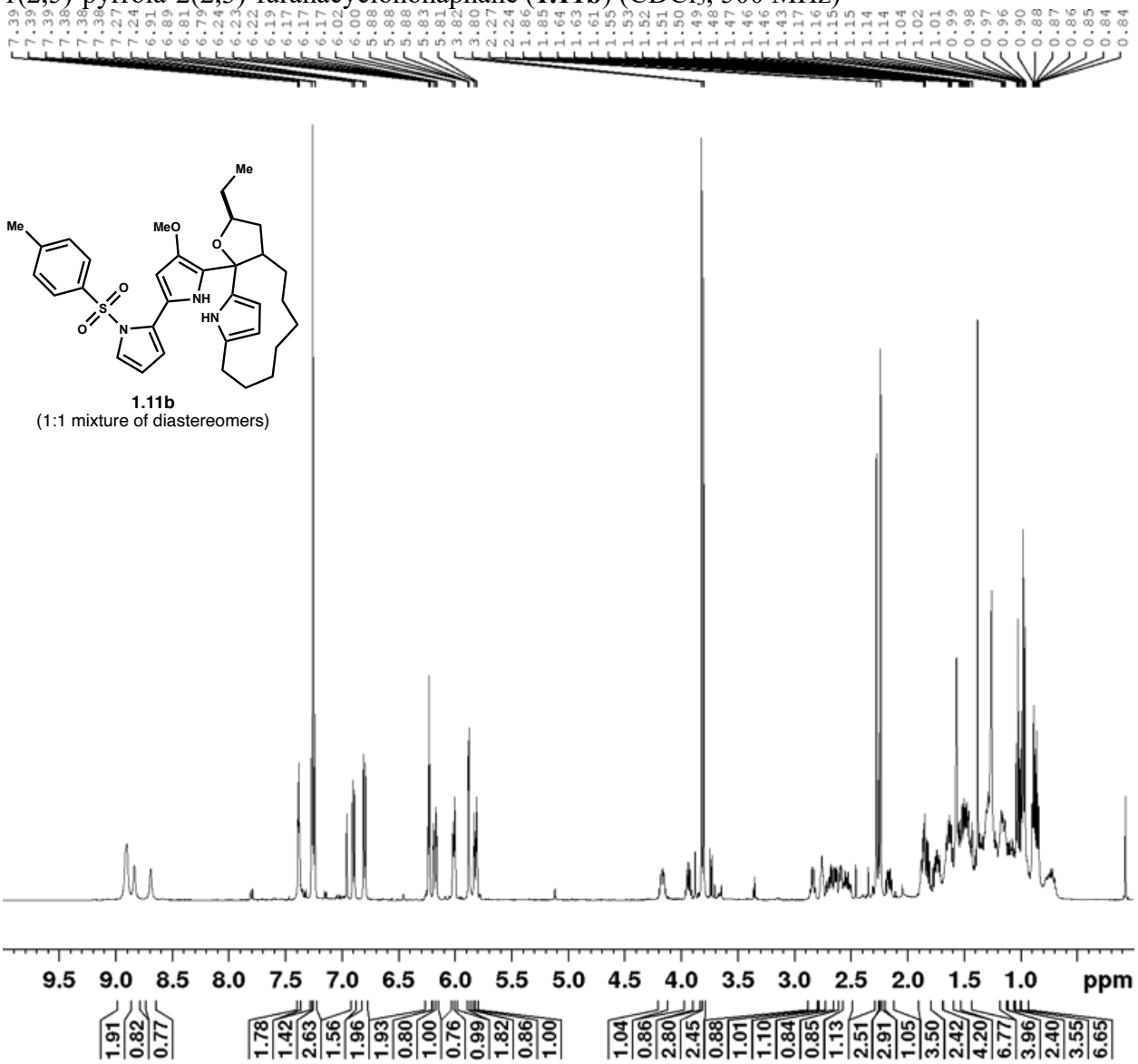
^{1}H NMR of 3-butyl-1^1*H*-1(2,5)-pyrrolacyclodecaphan-2-one (**1.9b**) ($CDCl_3$, 400 MHz)



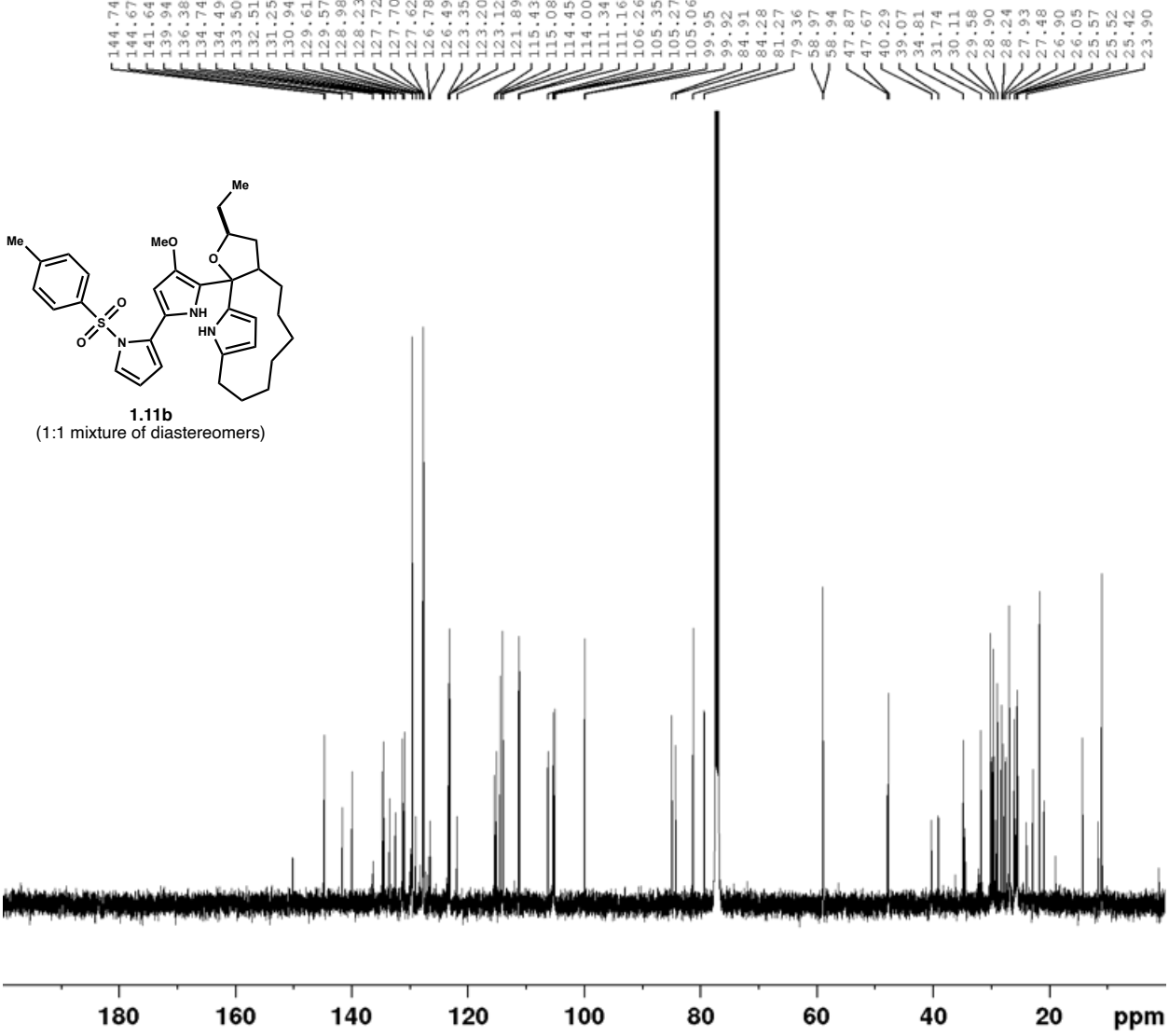
^{13}C NMR of 3-butyl-1^{1}*H*-1(2,5)-pyrrolacyclodecaphan-2-one (**1.9b**) ($CDCl_3$, 101 MHz)



(2^5S)-2^5-ethyl-2^2-(4-methoxy-1'-tosyl-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)-2^2,2^3,2^4,2^5-tetrahydro-1^1*H*-1(2,5)-pyrrola-2(2,3)-furanacyclononaphane (**1.11b**) ($CDCl_3$, 500 MHz)

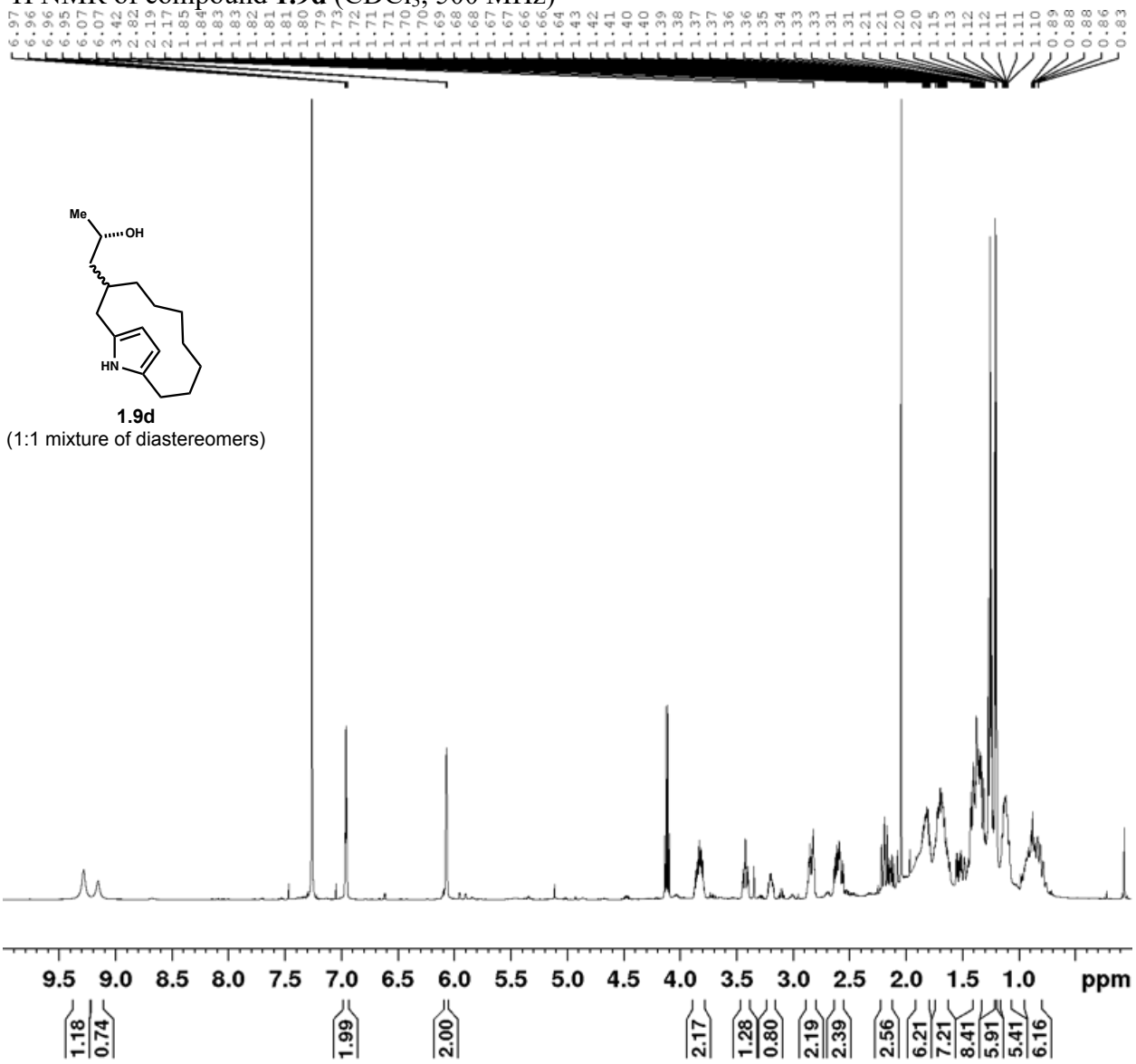


(2^5S)-2^5-ethyl-2^2-(4-methoxy-1'-tosyl-1*H*,1'*H*-[2,2'-bipyrrol]-5-yl)-2^2,2^3,2^4,2^5-tetrahydro-1^1H-1(2,5)-pyrrola-2(2,3)-furanacyclononaphane (**1.11b**) ($CDCl_3$, 126 MHz)

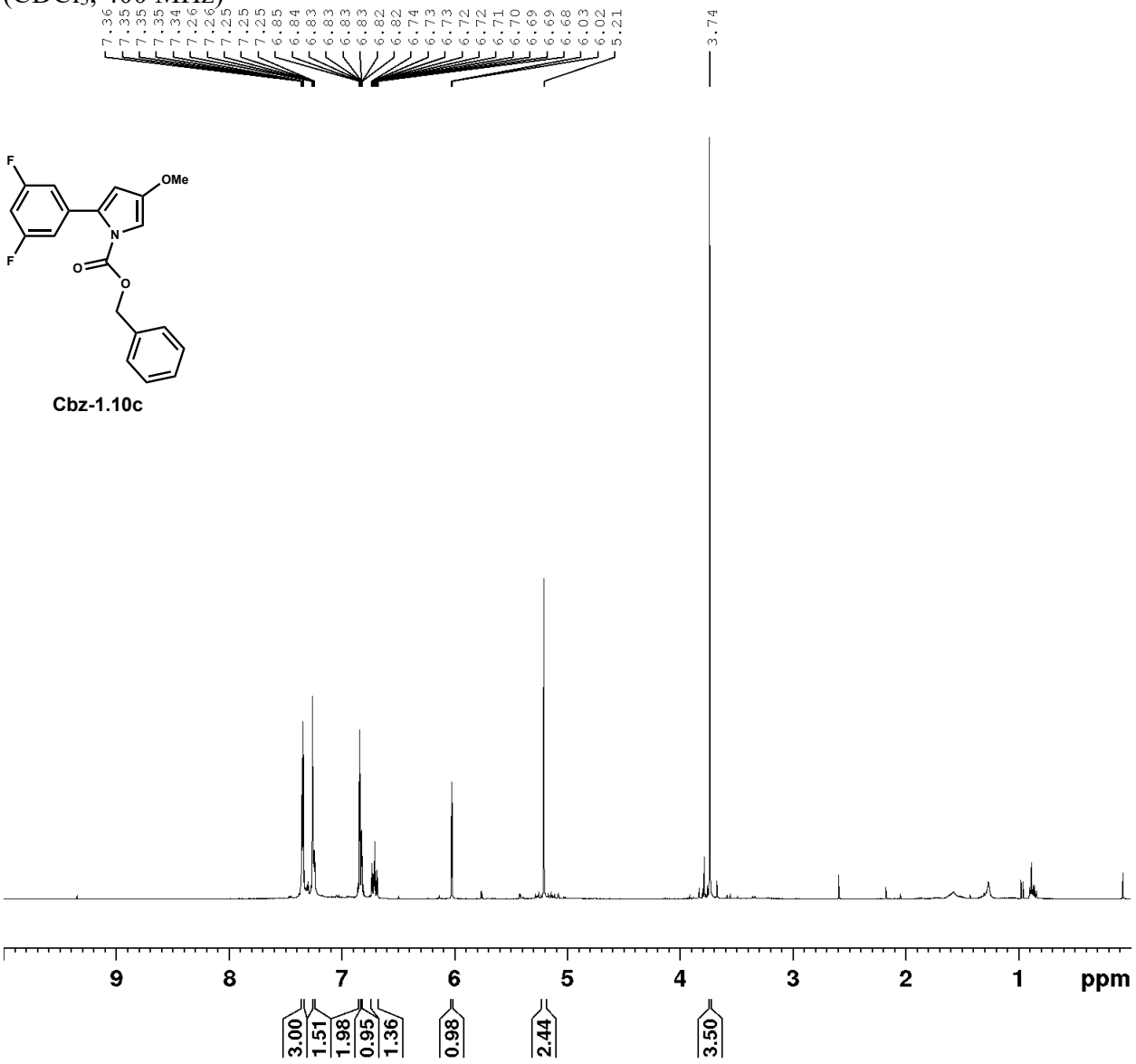


1.11b
(1:1 mixture of diastereomers)

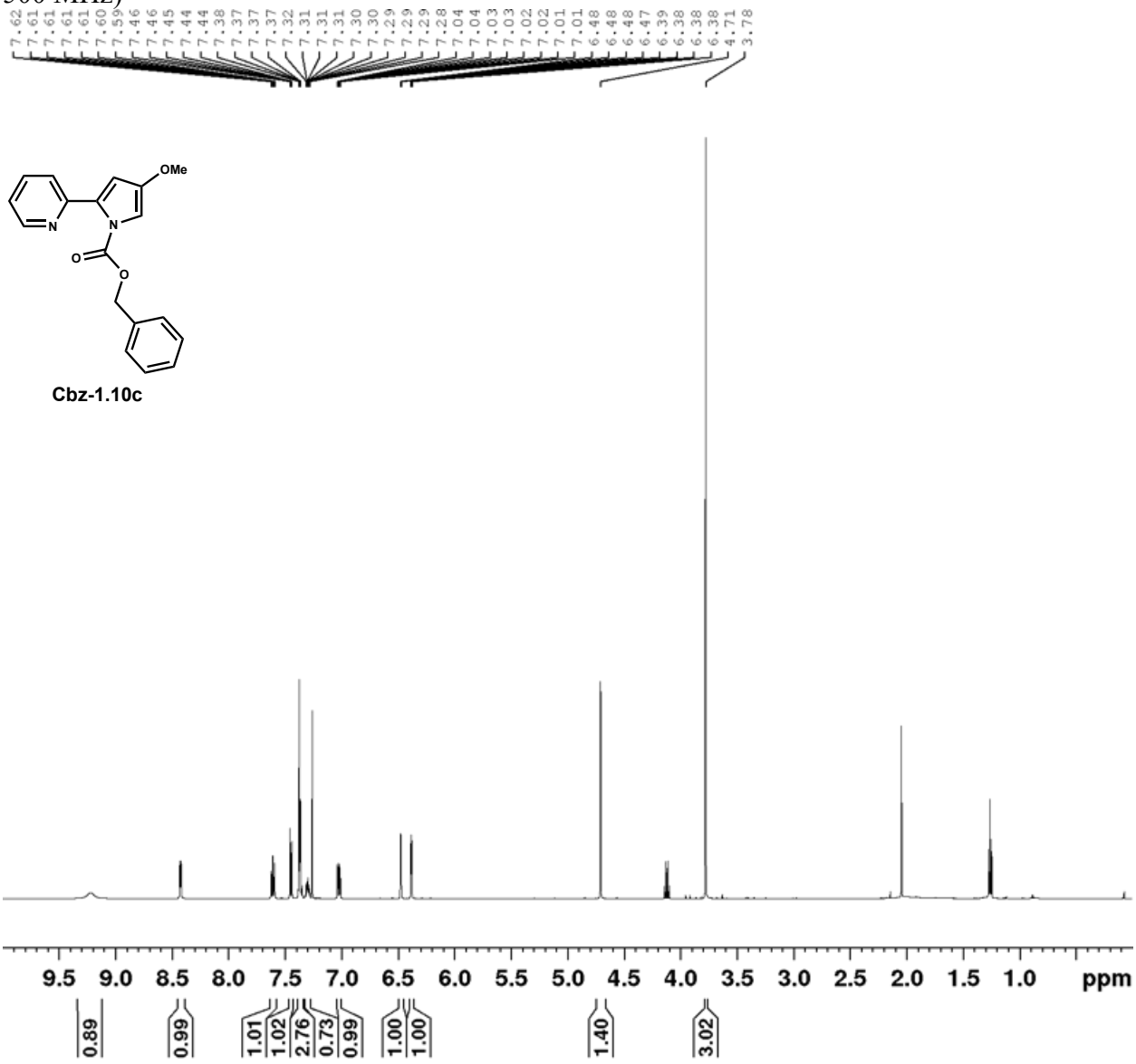
^{1}H NMR of compound **1.9d** ($CDCl_3$, 500 MHz)



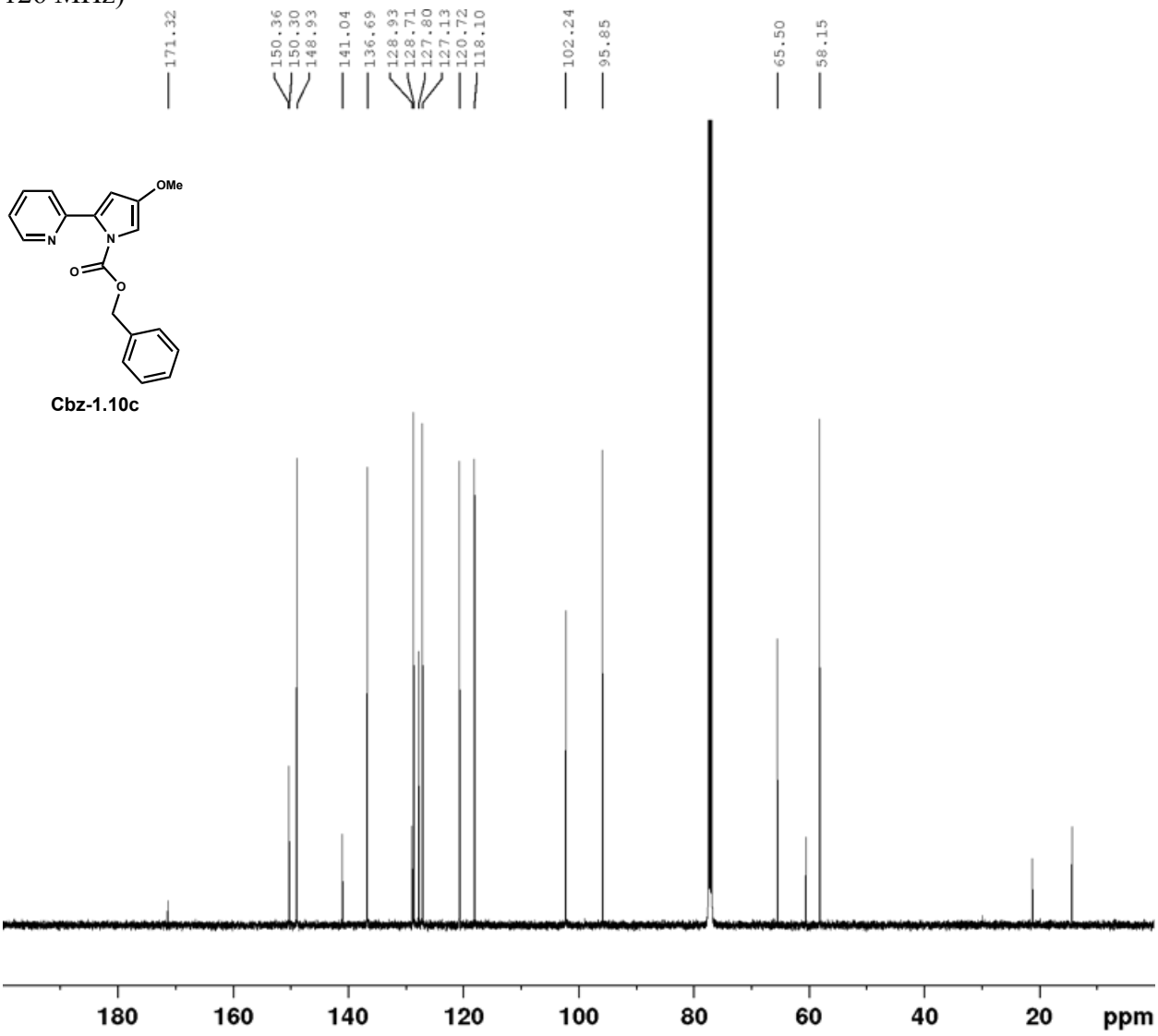
^{1}H NMR of benzyl 2-(3,5-difluorophenyl)-4-methoxy-1*H*-pyrrole-1-carboxylate (**Cbz-1.10c**) ($CDCl_3$, 400 MHz)



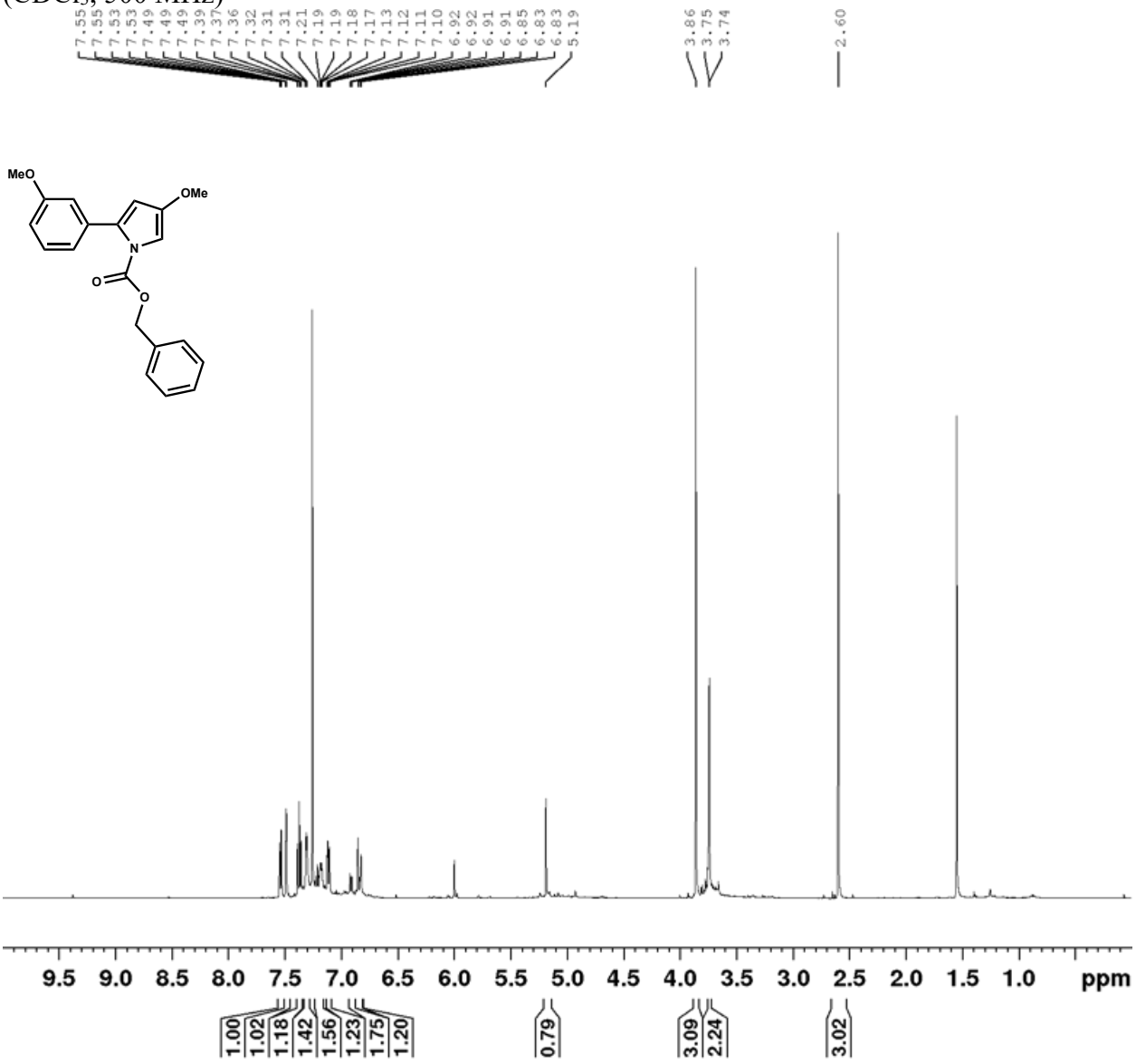
^{1}H NMR of benzyl 4-methoxy-2-(pyridin-2-yl)-1*H*-pyrrole-1-carboxylate (**Cbz-1.10d**) ($CDCl_3$, 500 MHz)



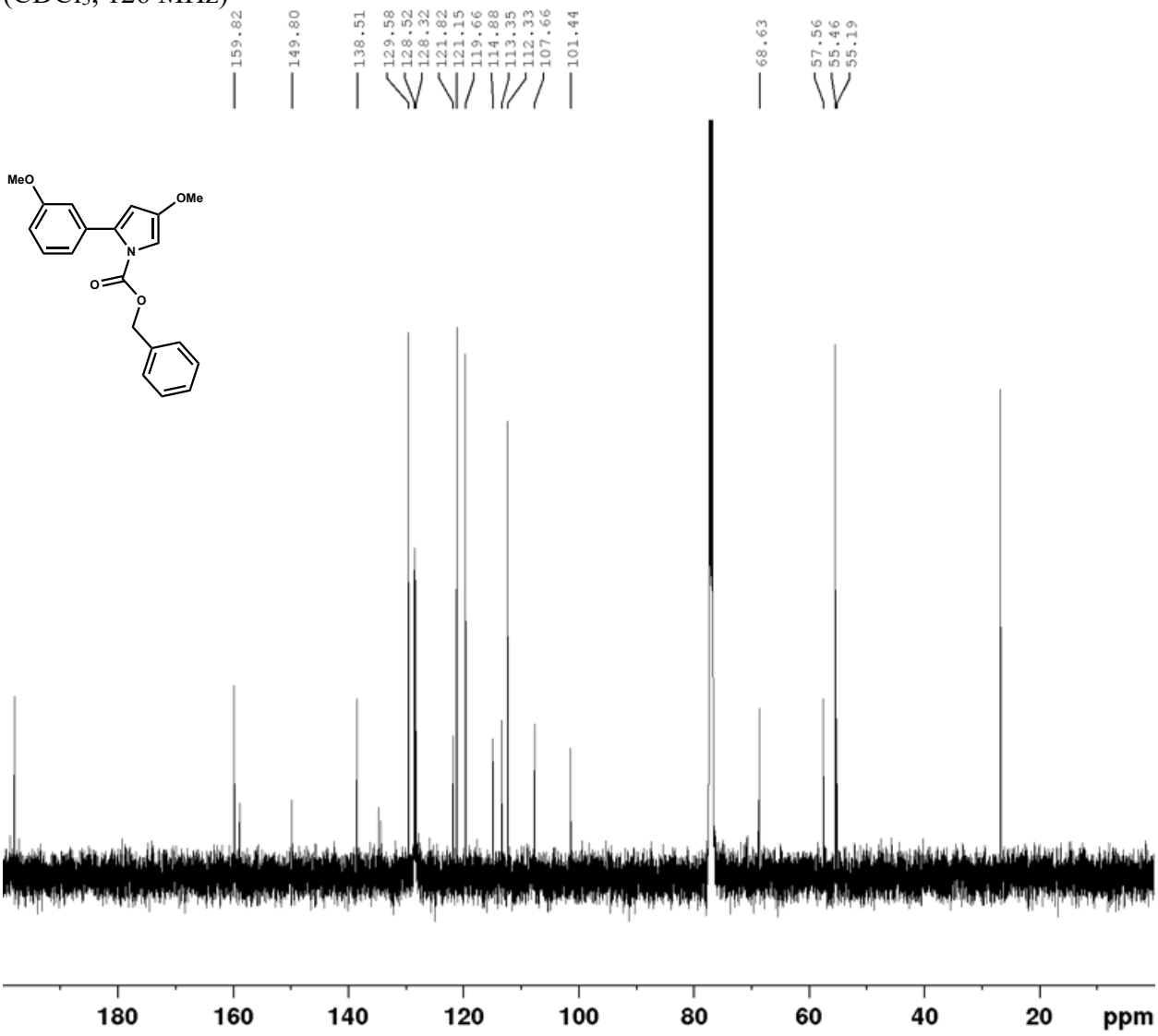
^{13}C NMR of benzyl 4-methoxy-2-(pyridin-2-yl)-1*H*-pyrrole-1-carboxylate (**Cbz-1.10d**) ($CDCl_3$, 126 MHz)



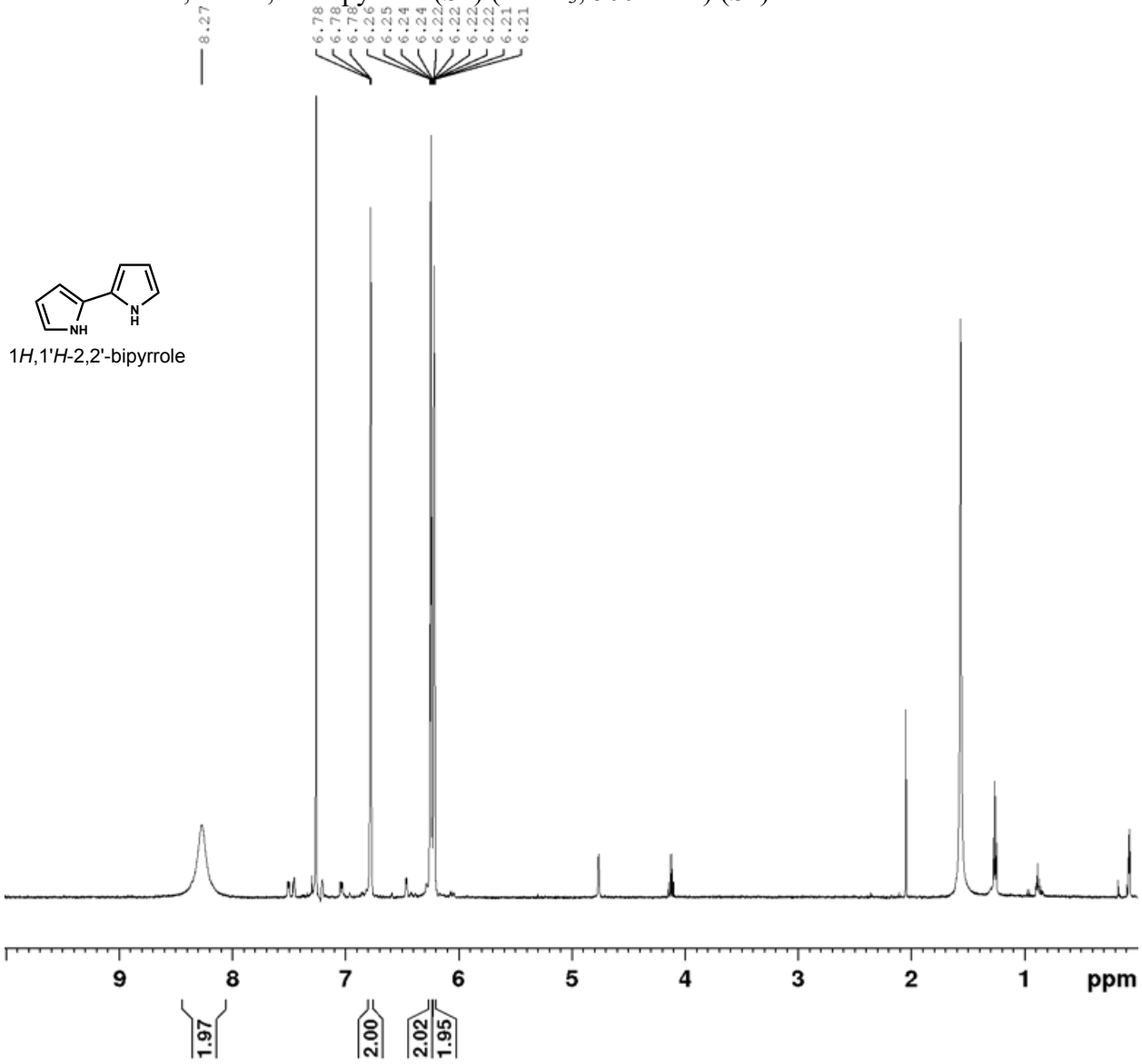
^{1}H NMR of benzyl 4-methoxy-2-(3-methoxyphenyl)-1*H*-pyrrole-1-carboxylate (**Cbz-1.10e**) ($CDCl_3$, 500 MHz)



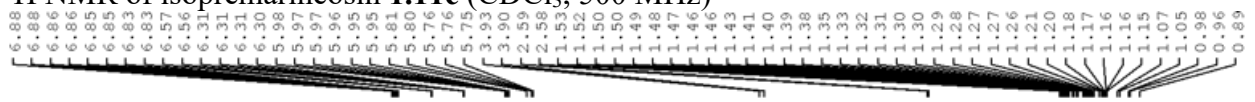
^{13}C NMR of benzyl 4-methoxy-2-(3-methoxyphenyl)-1*H*-pyrrole-1-carboxylate (**Cbz-1.10e**) ($CDCl_3$, 126 MHz)



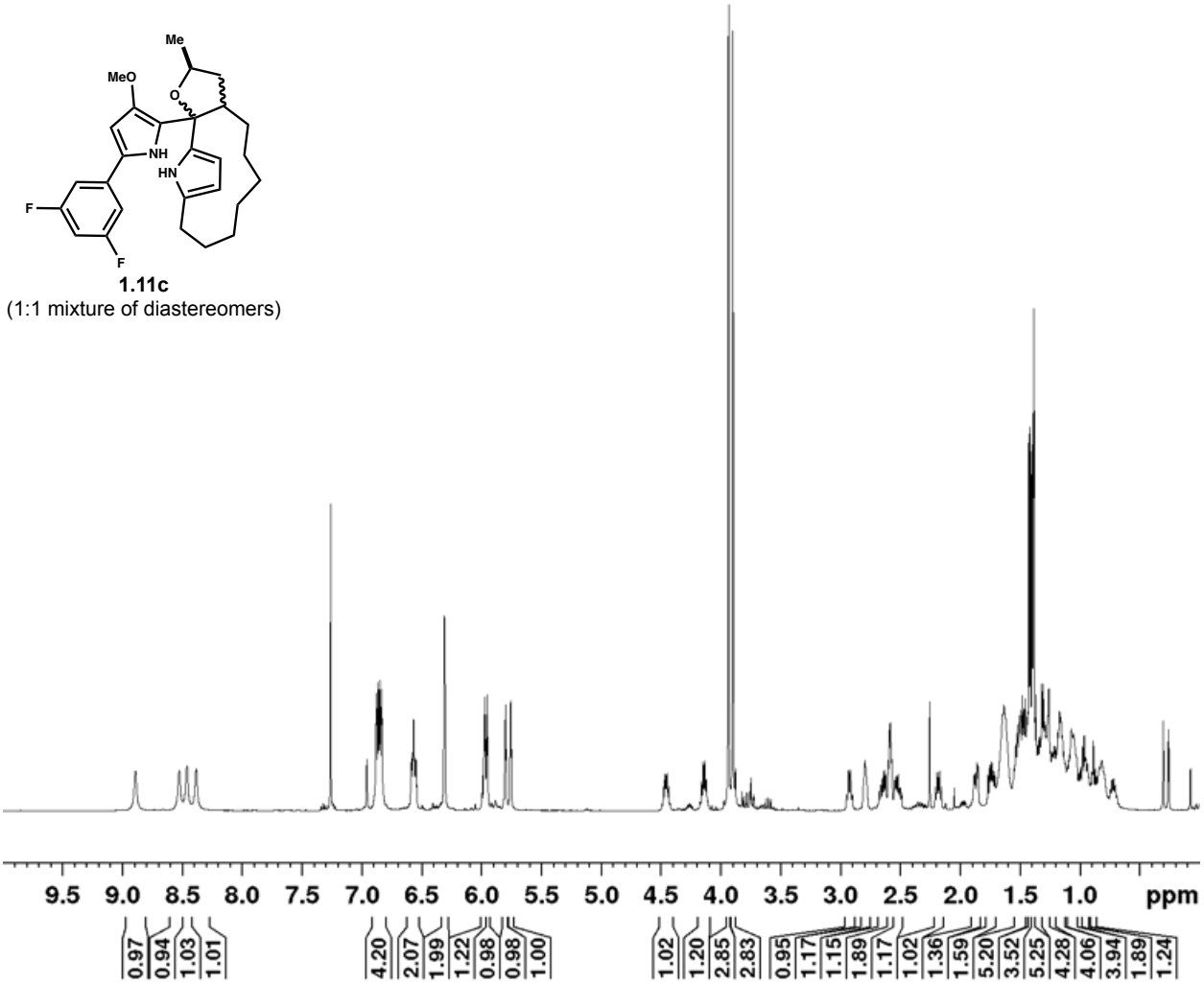
^{1}H NMR of 1H, 1’H-2,2’-bispyrrole (**S2**) ($CDCl_3$, 500 MHz) (**S2**)



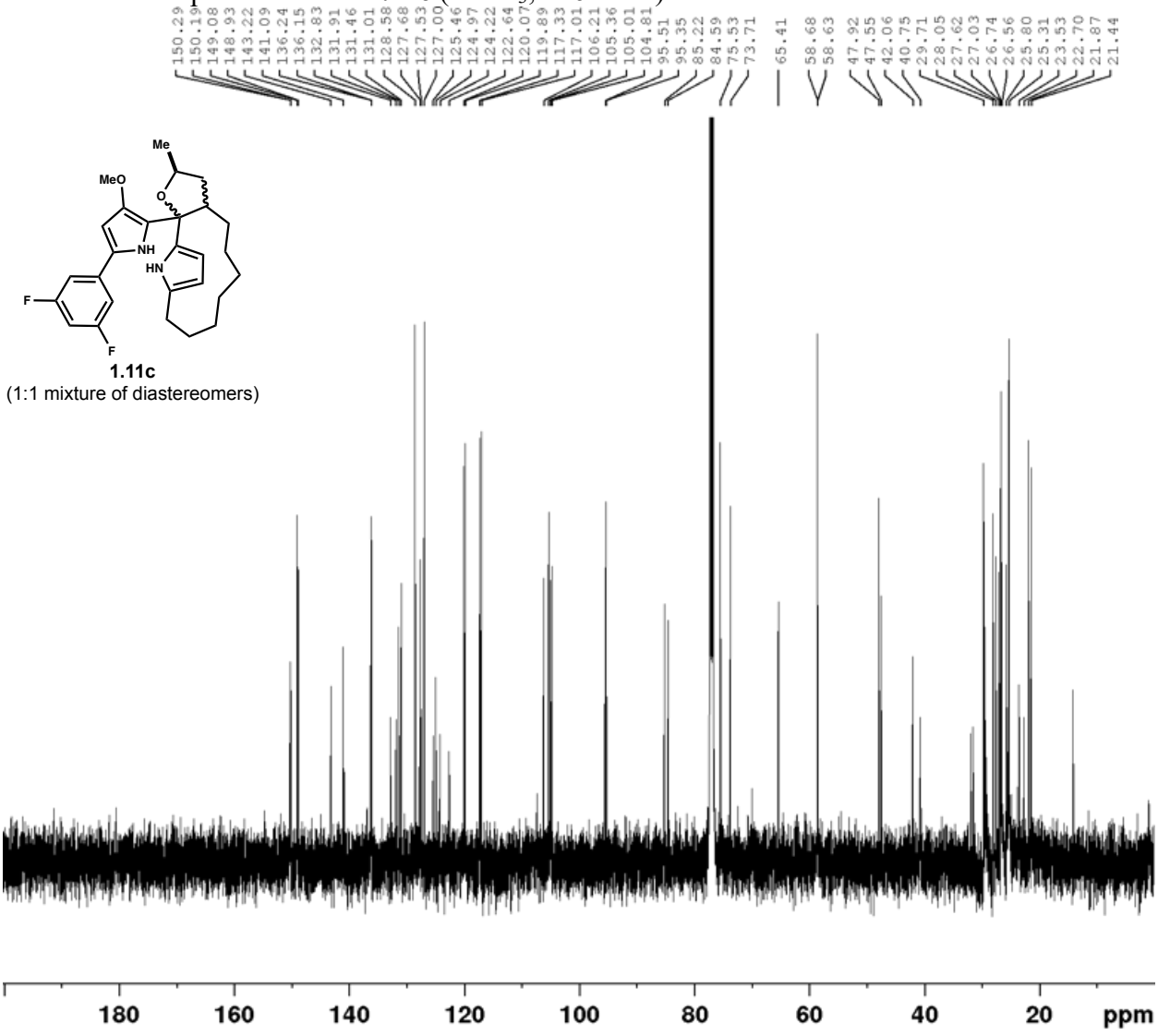
^{1}H NMR of isopremarineosin **1.11c** ($CDCl_3$, 500 MHz)



1.11c
(1:1 mixture of diastereomers)

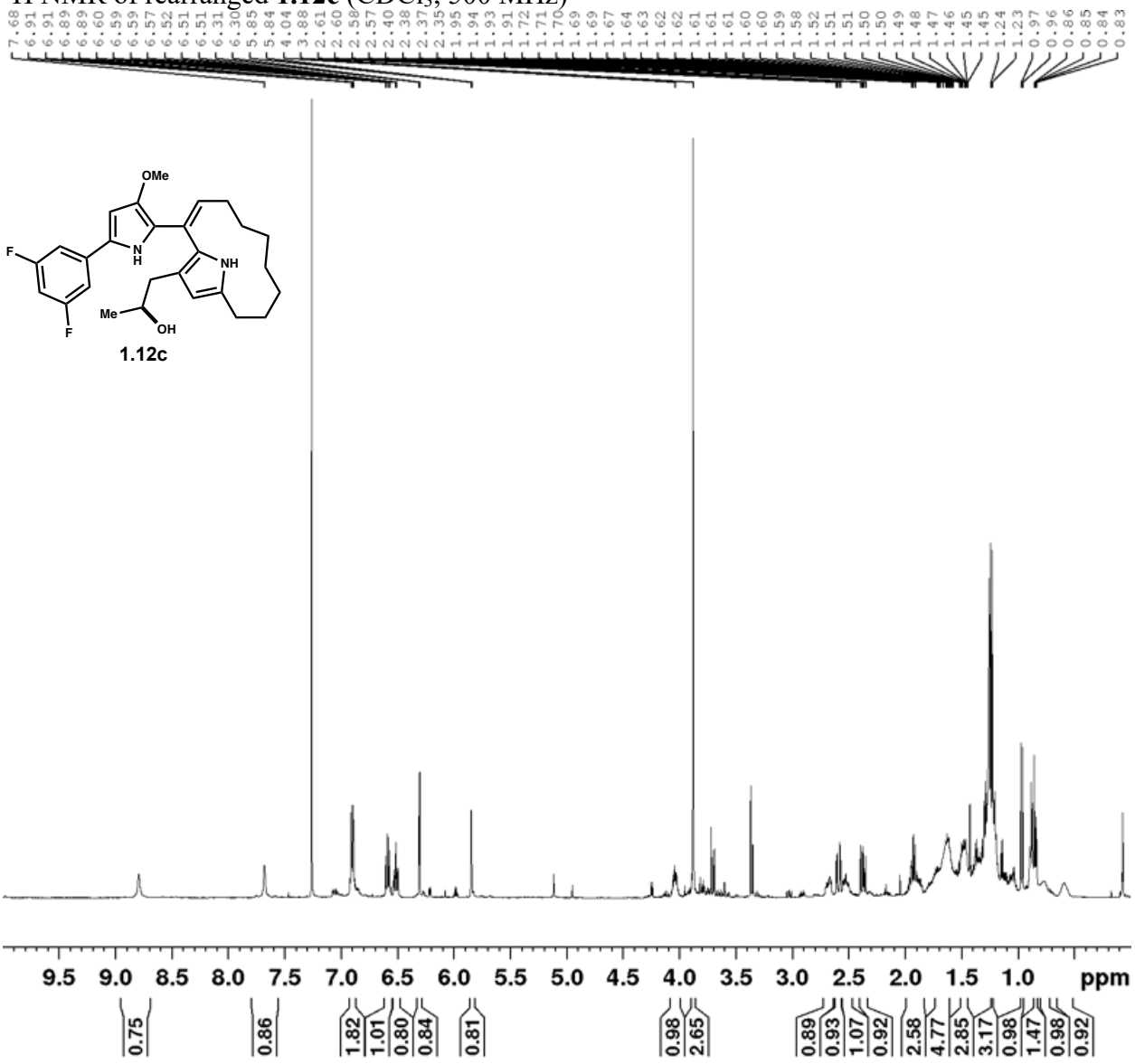


^{13}C NMR of isopremarineosin **1.11c** ($CDCl_3$, 126 MHz)

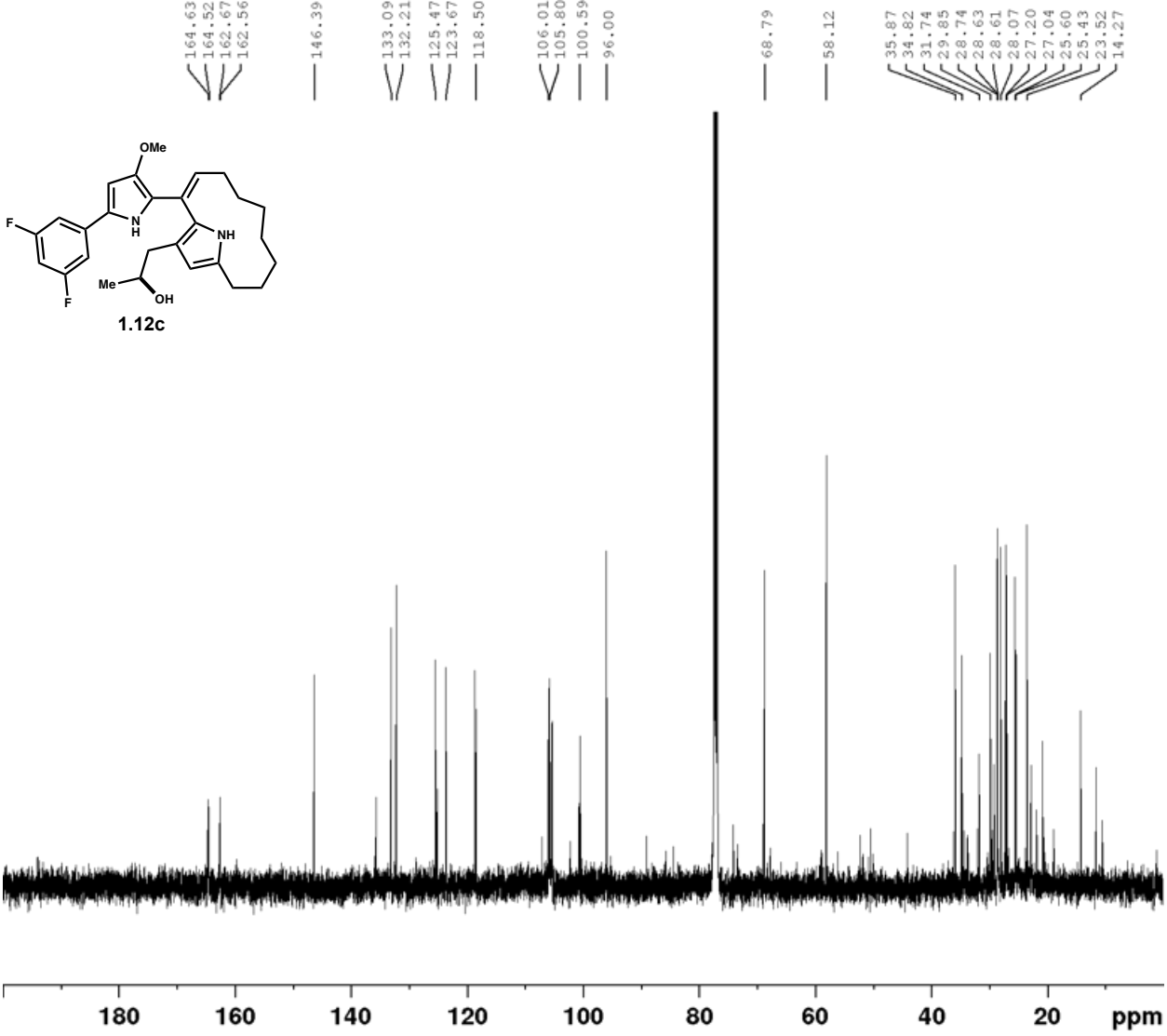


1.11c
(1:1 mixture of diastereomers)

^{1}H NMR of rearranged **1.12c** ($CDCl_3$, 500 MHz)



^{13}C NMR of rearranged **1.12c** ($CDCl_3$, 126 MHz)



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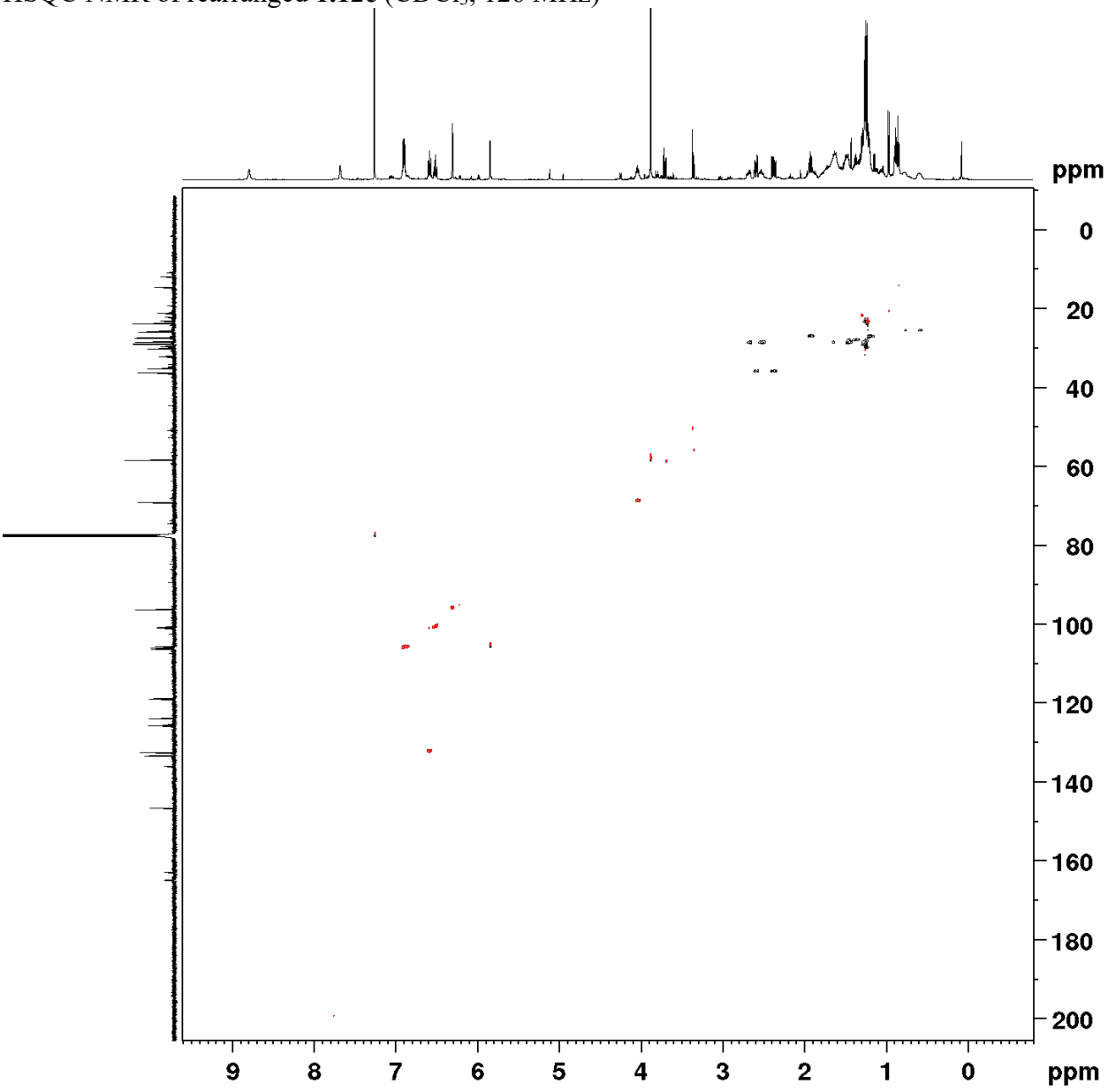

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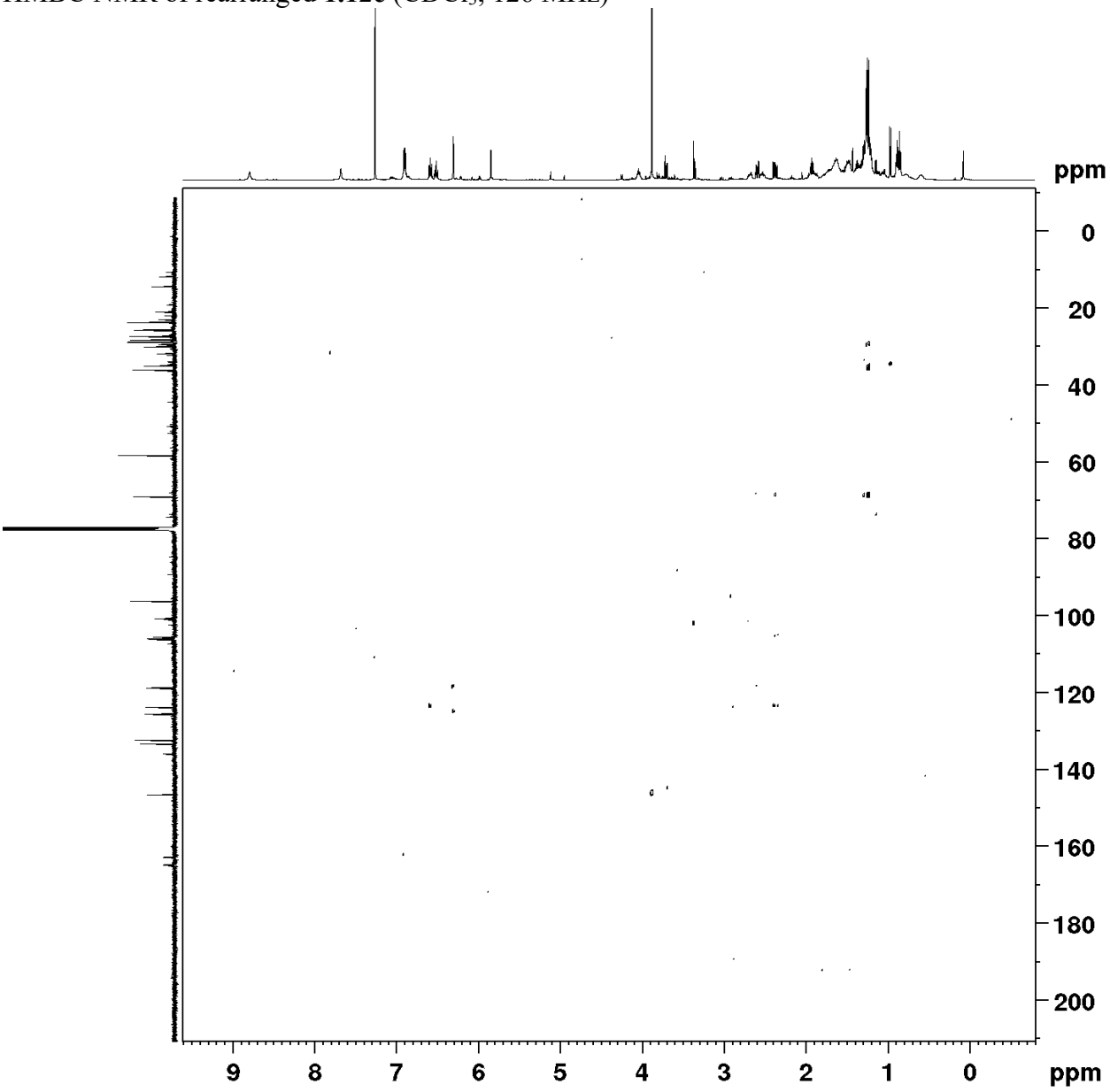
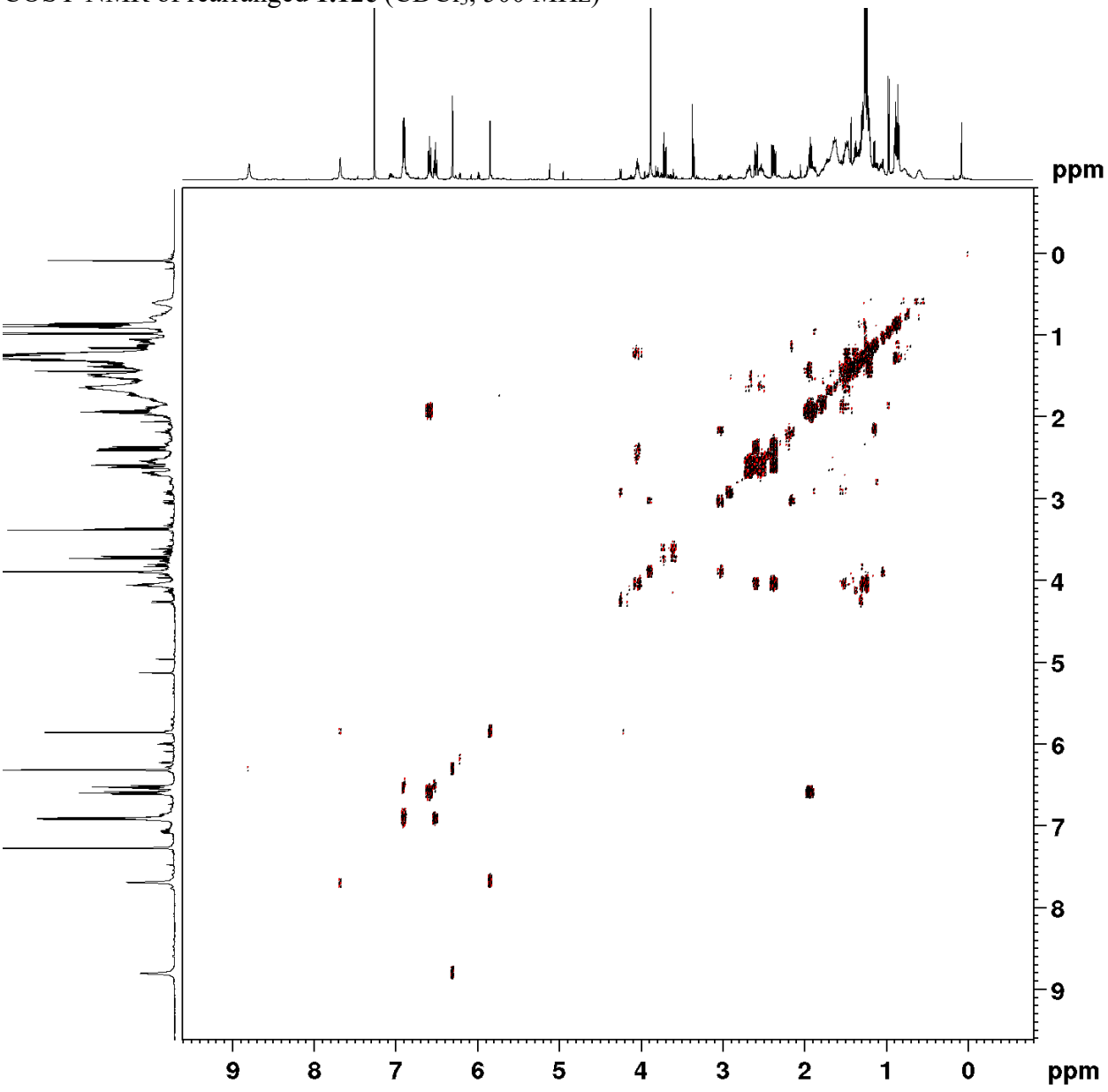
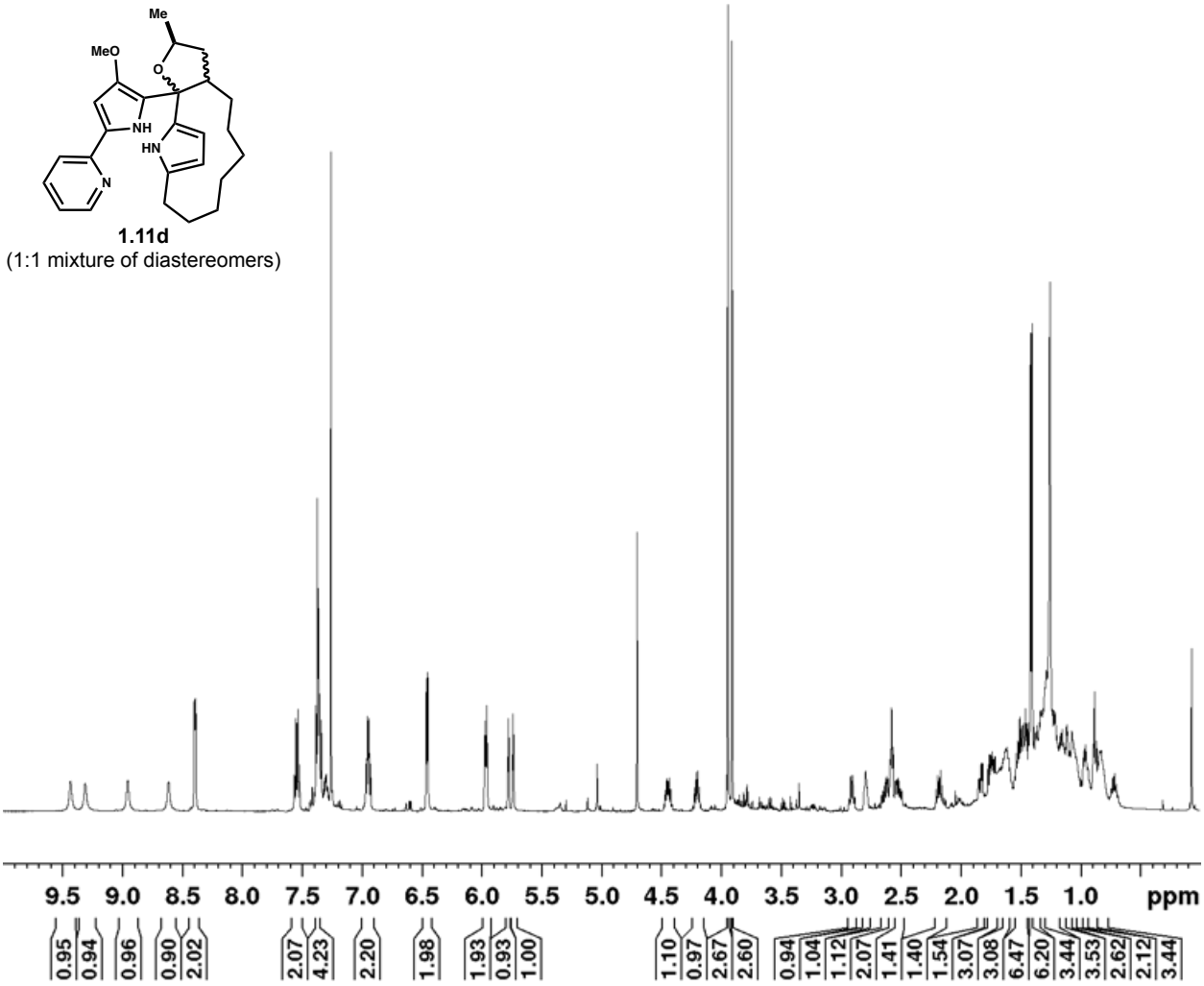


COSY NMR of rearranged **1.12c** ($CDCl_3$, 500 MHz)



^{1}H NMR of isopremarineosin analog **1.11d** ($CDCl_3$, 500 MHz)

1.11d
(1:1 mixture of diastereomers)



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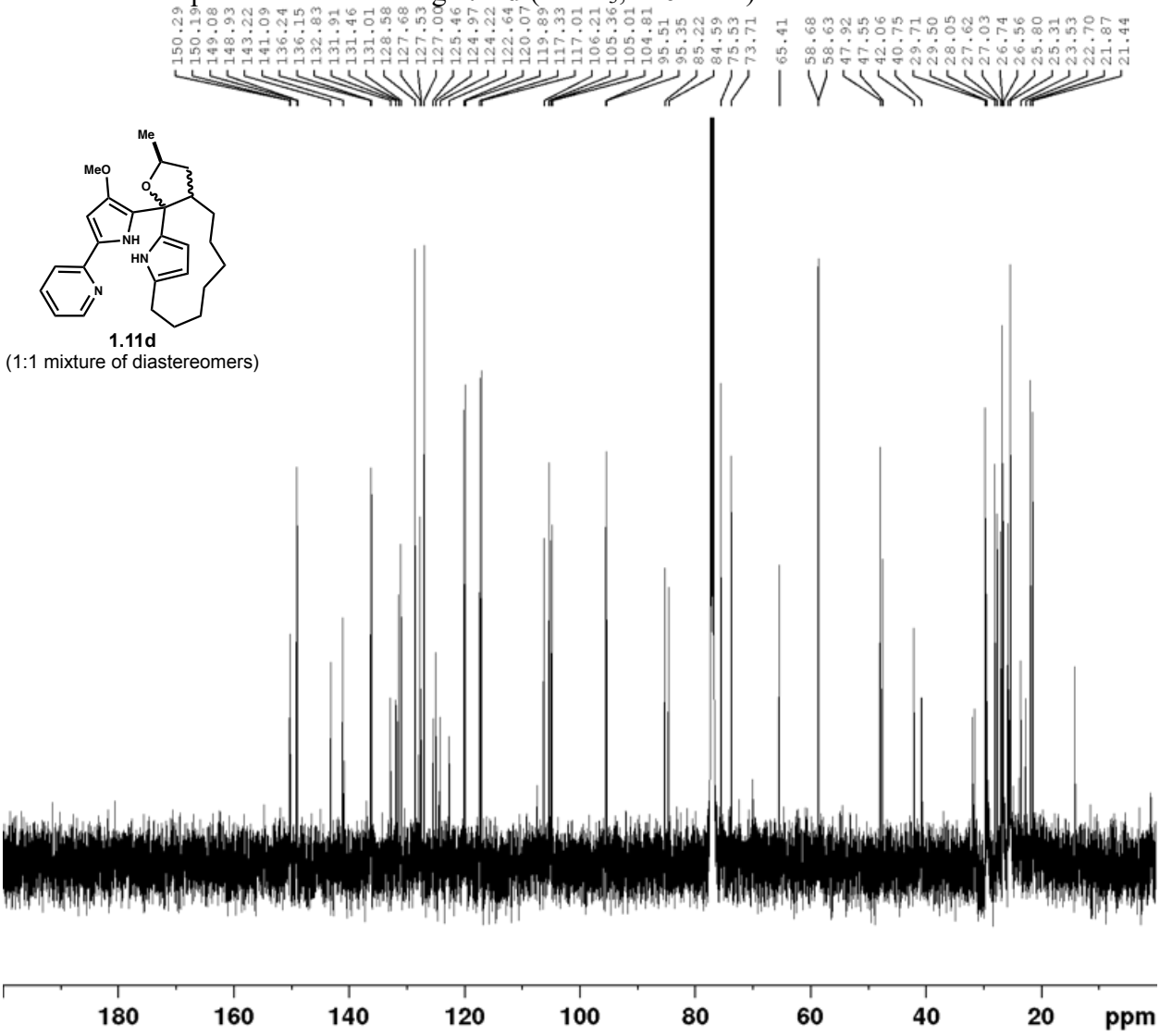
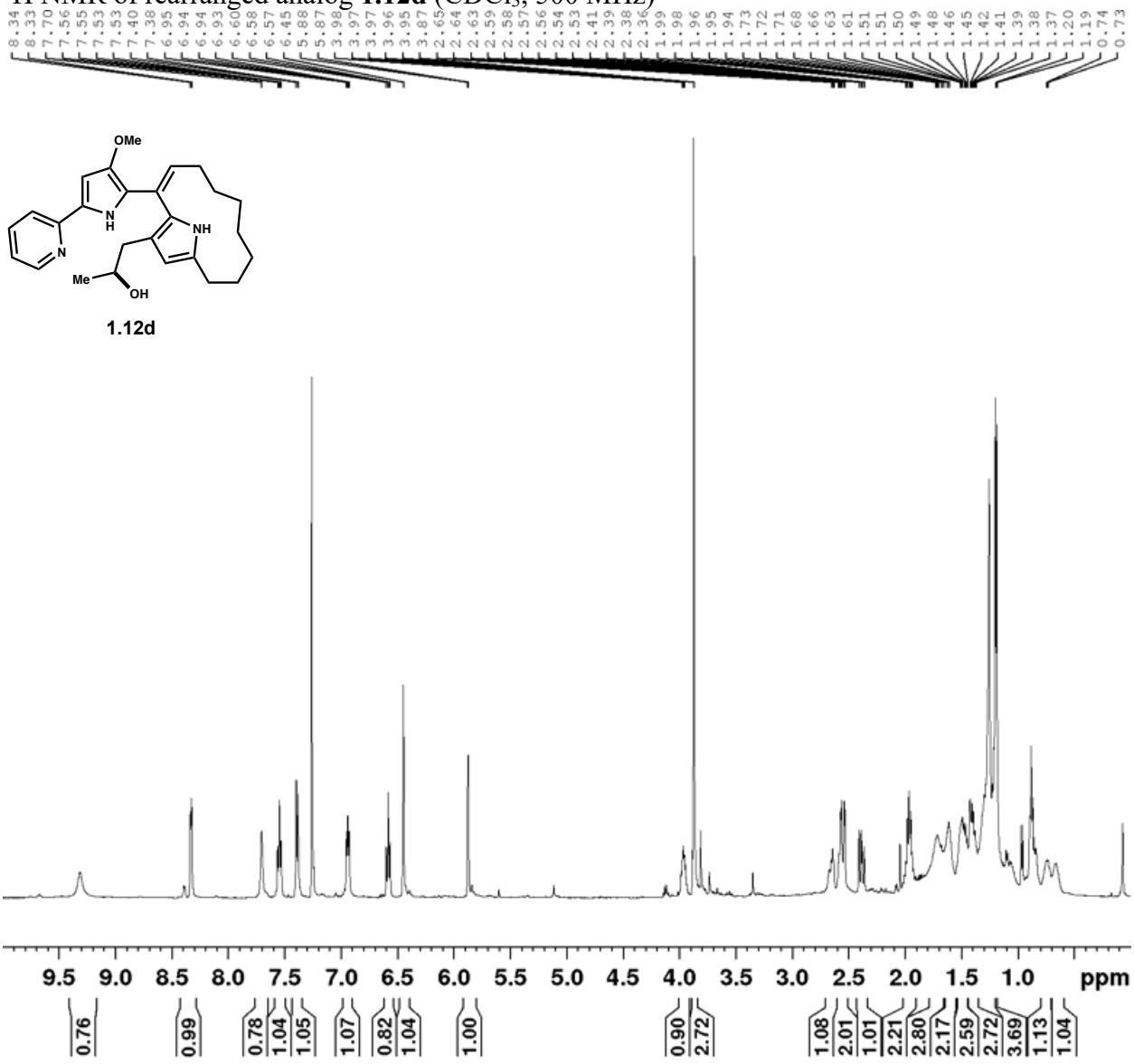
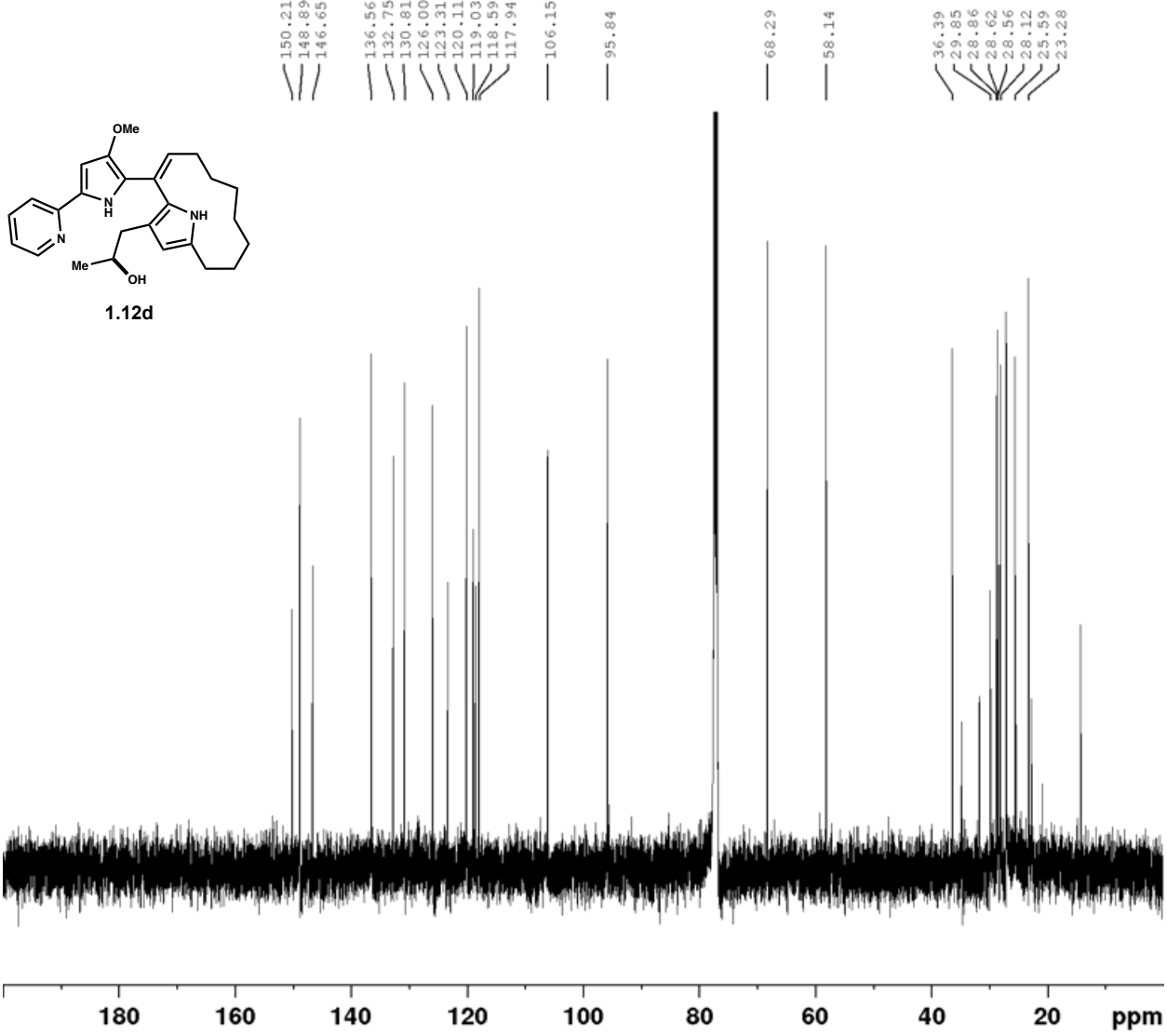


1.11d
(1:1 mixture of diastereomers)

^{1}H NMR of rearranged analog **1.12d** ($CDCl_3$, 500 MHz)



^{13}C NMR of rearranged analog **1.12d** ($CDCl_3$, 126 MHz)



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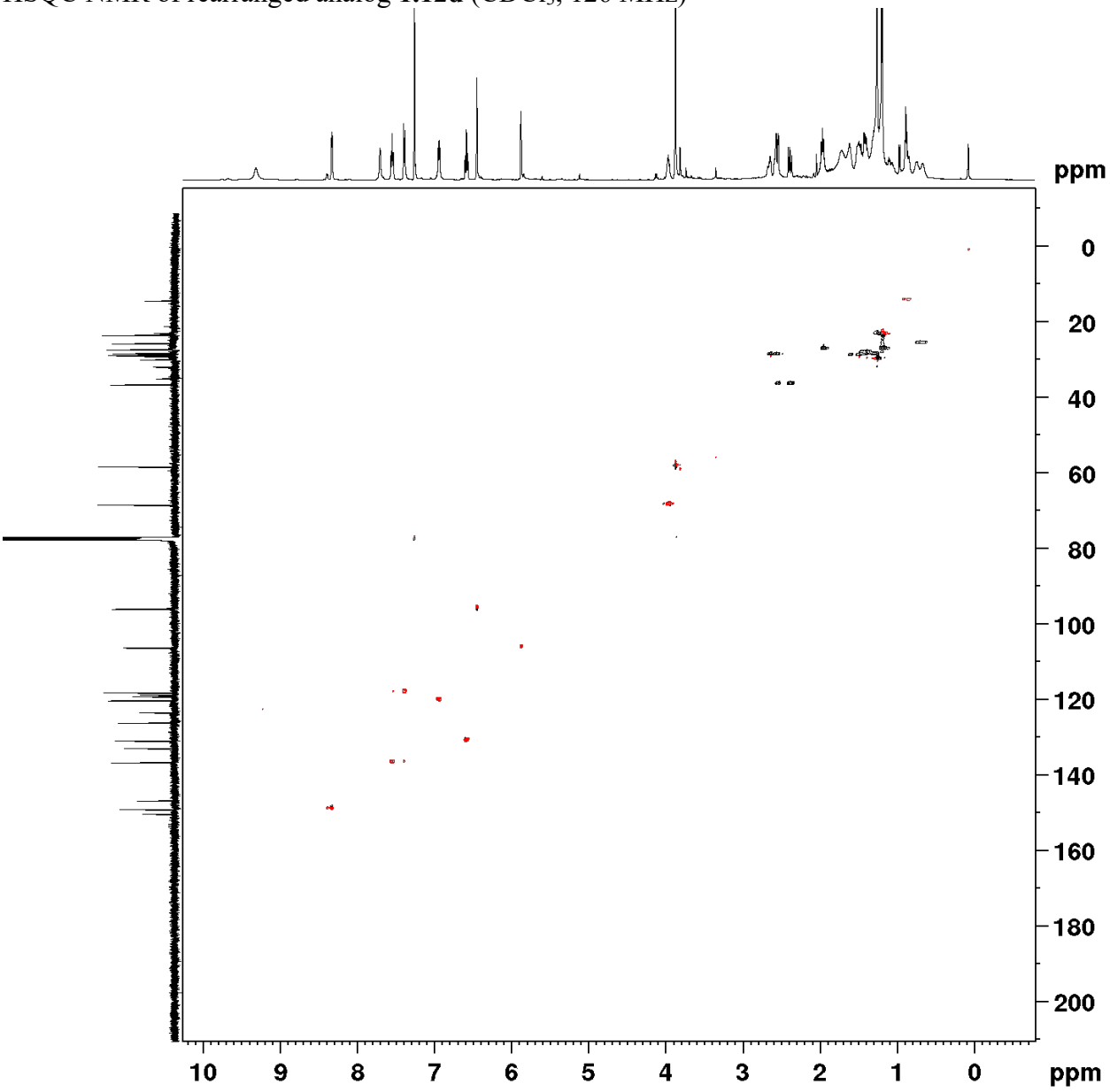

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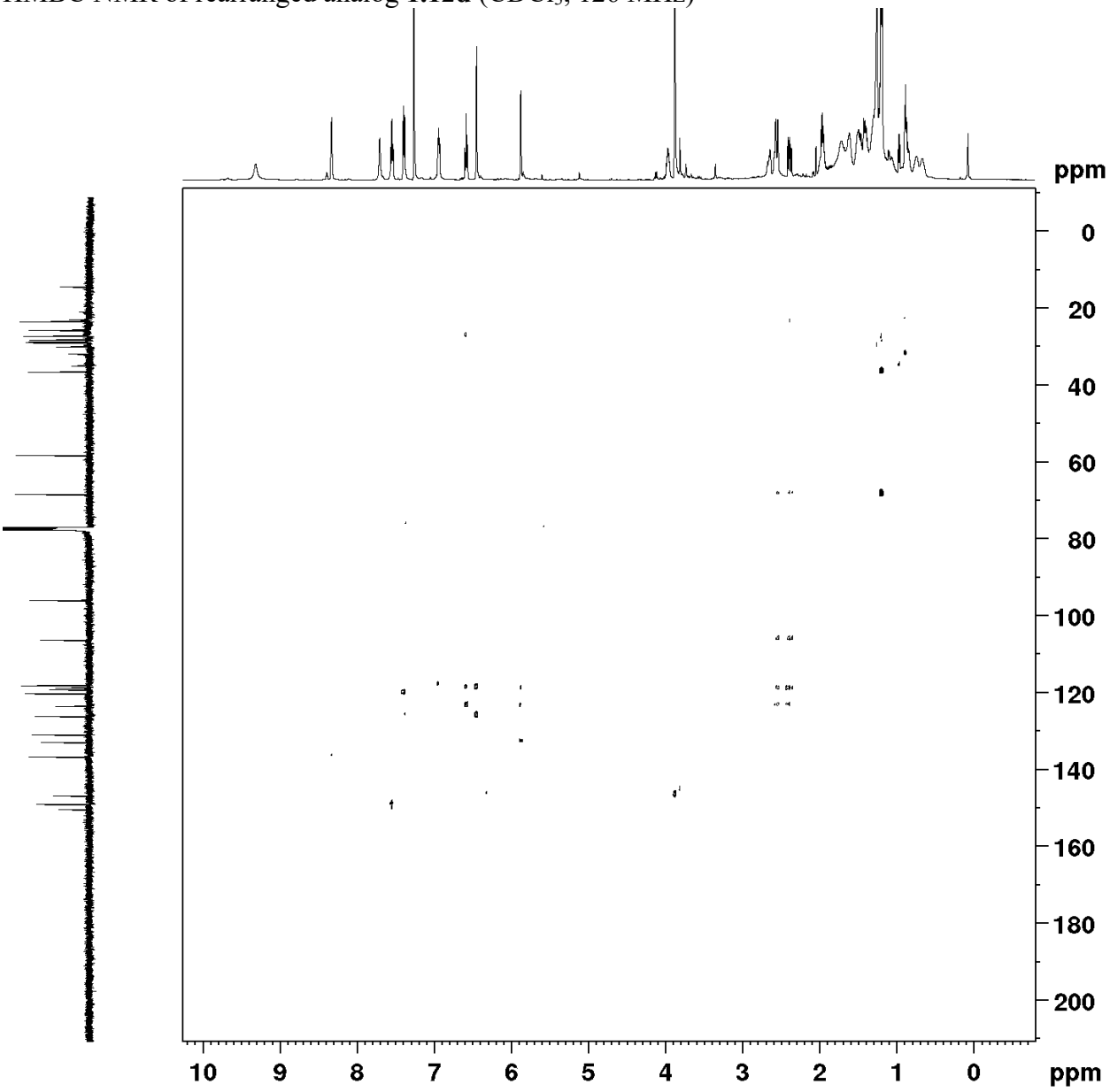
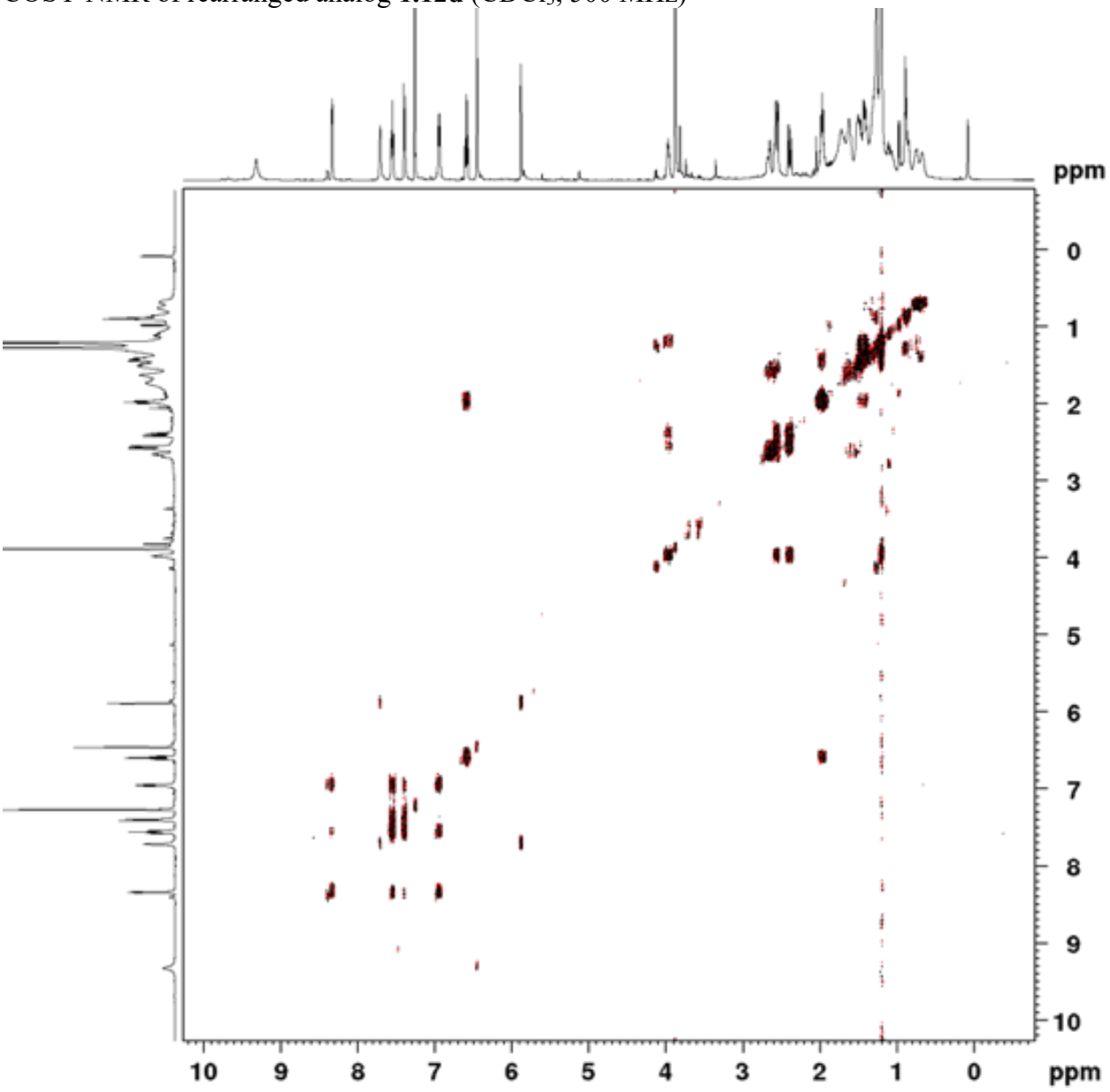
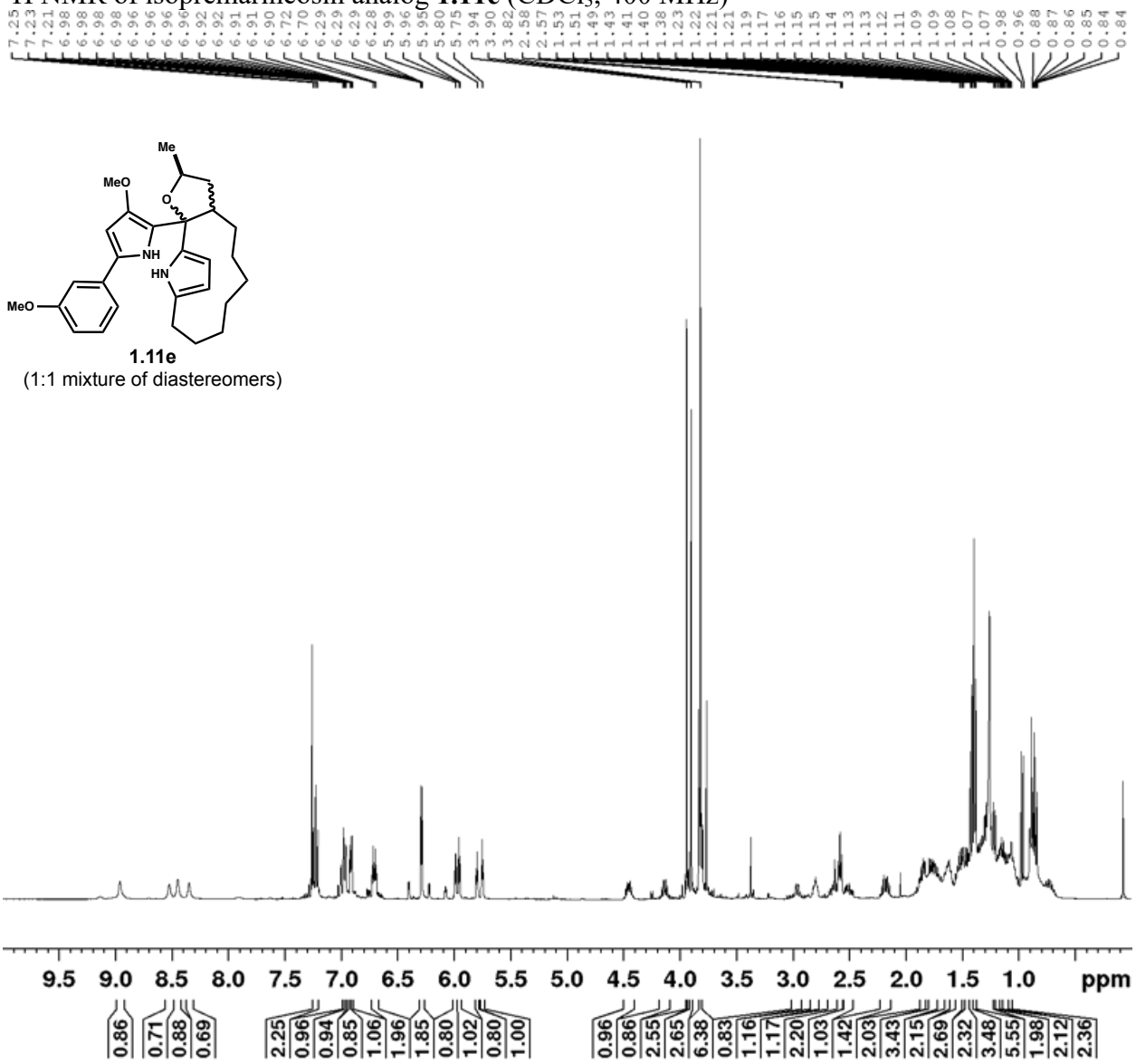


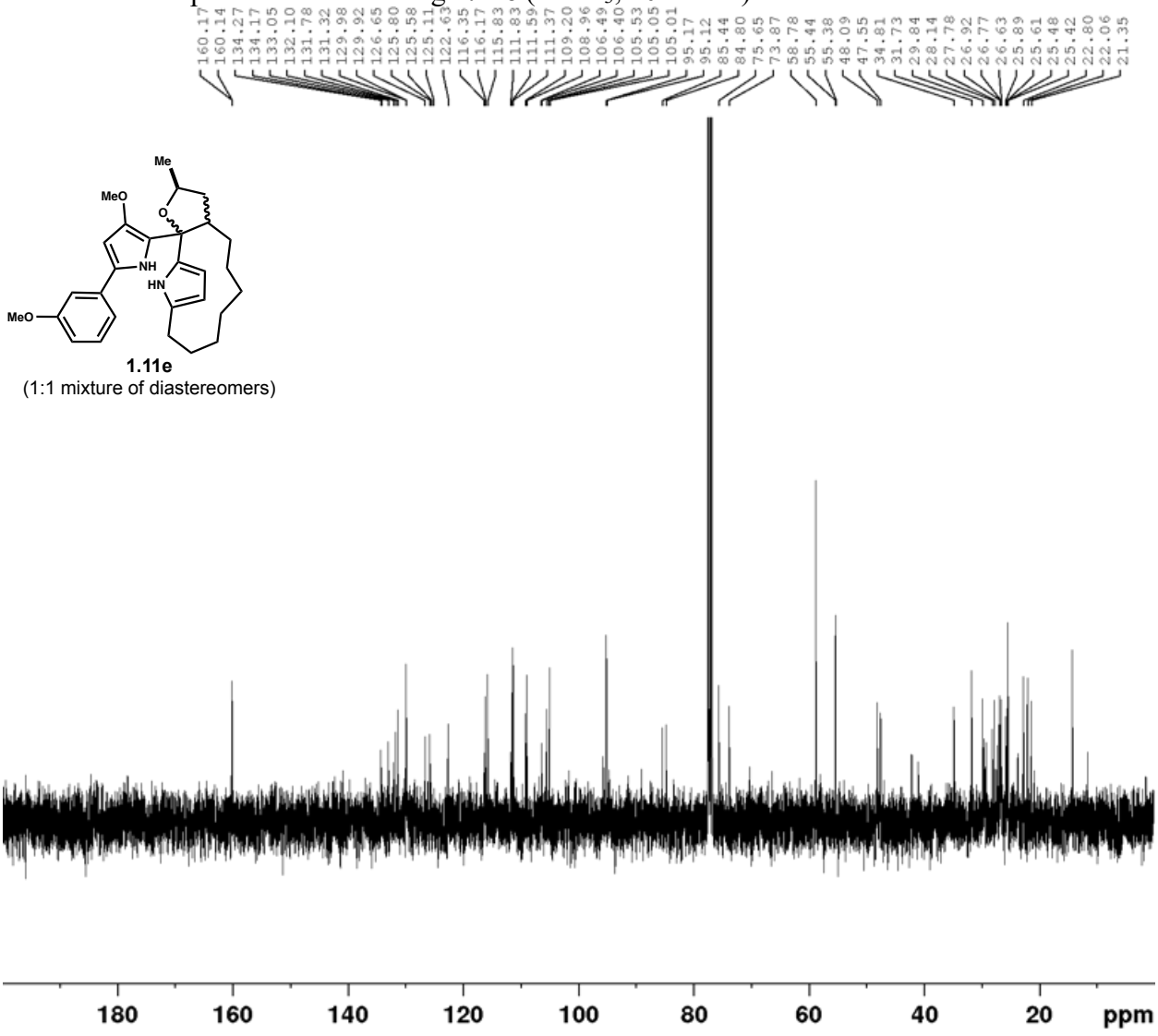
COSY NMR of rearranged analog **1.12d** ($CDCl_3$, 500 MHz)



^{1}H NMR of isopremarineosin analog **1.11e** ($CDCl_3$, 400 MHz)

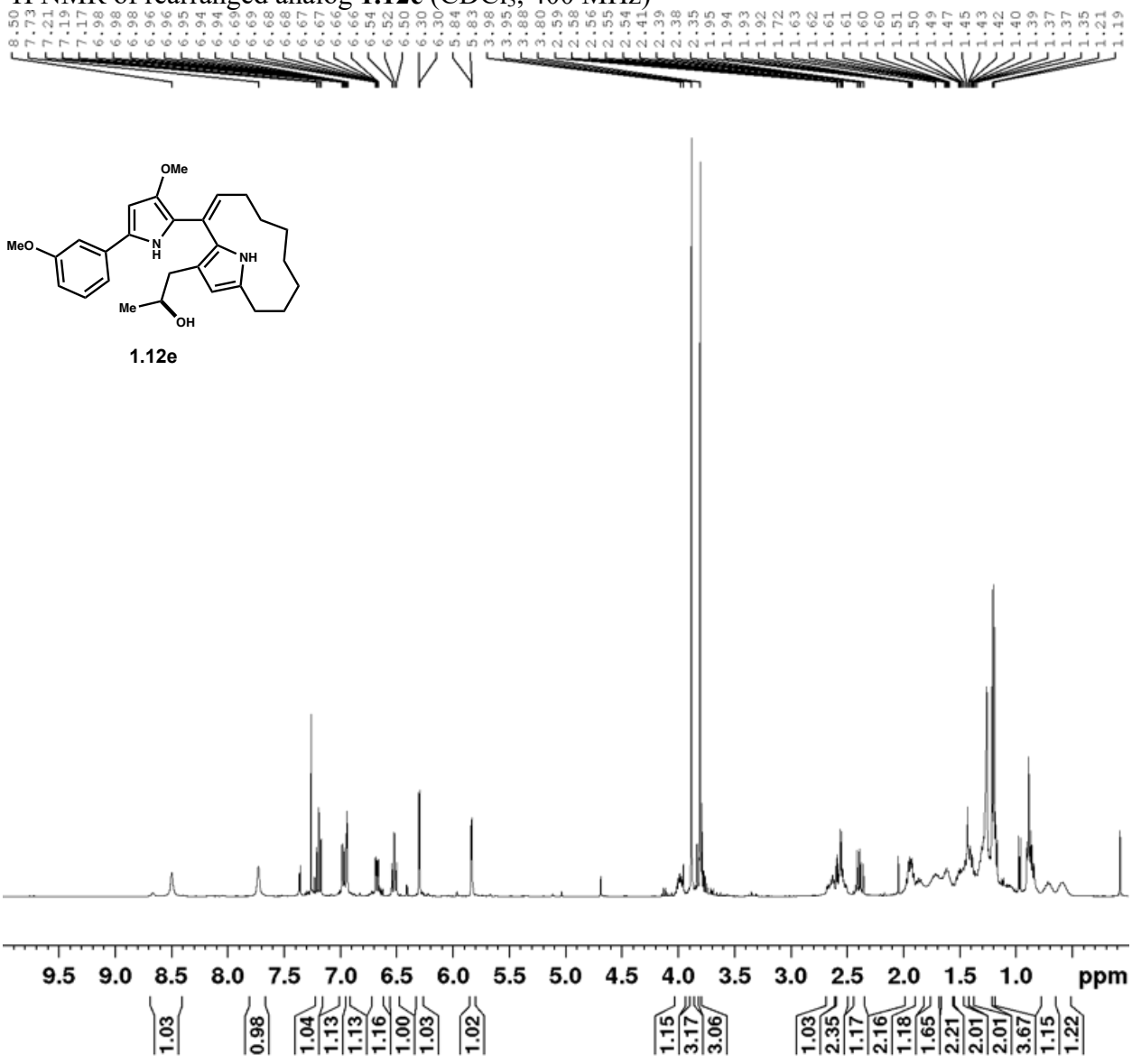


^{13}C NMR of isopremarineosin analog **1.11e** ($CDCl_3$, 101 MHz)

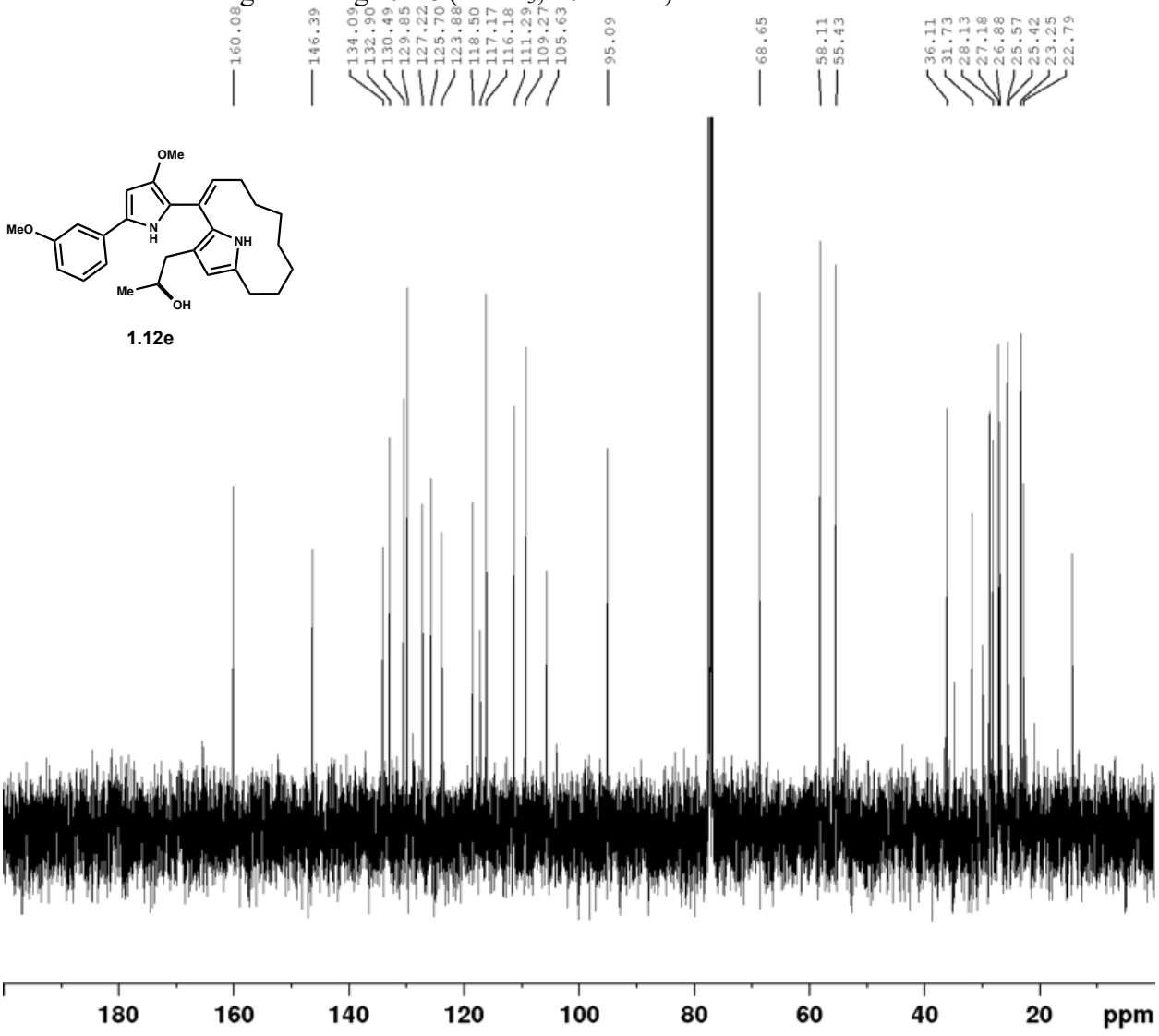


1.11e
(1:1 mixture of diastereomers)

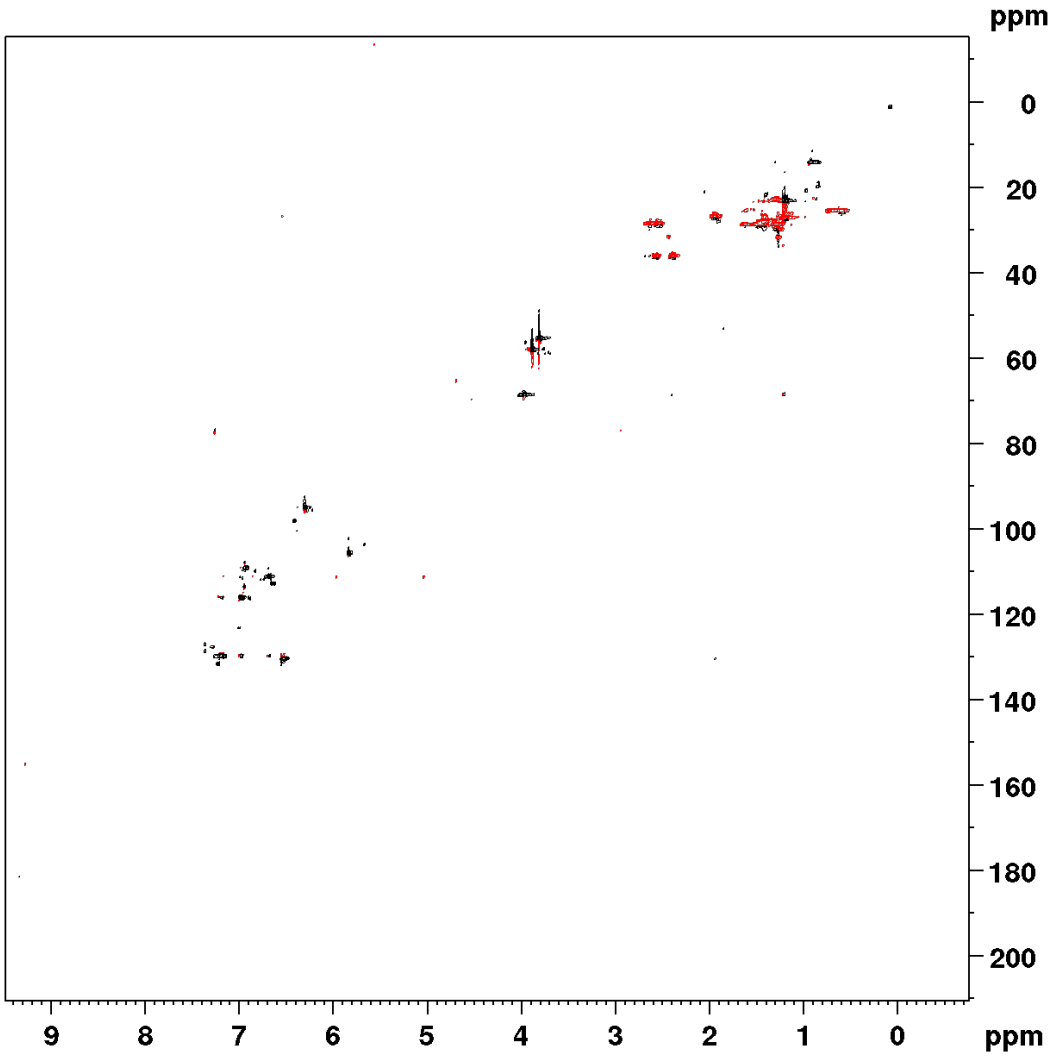
^{1}H NMR of rearranged analog **1.12e** ($CDCl_3$, 400 MHz)



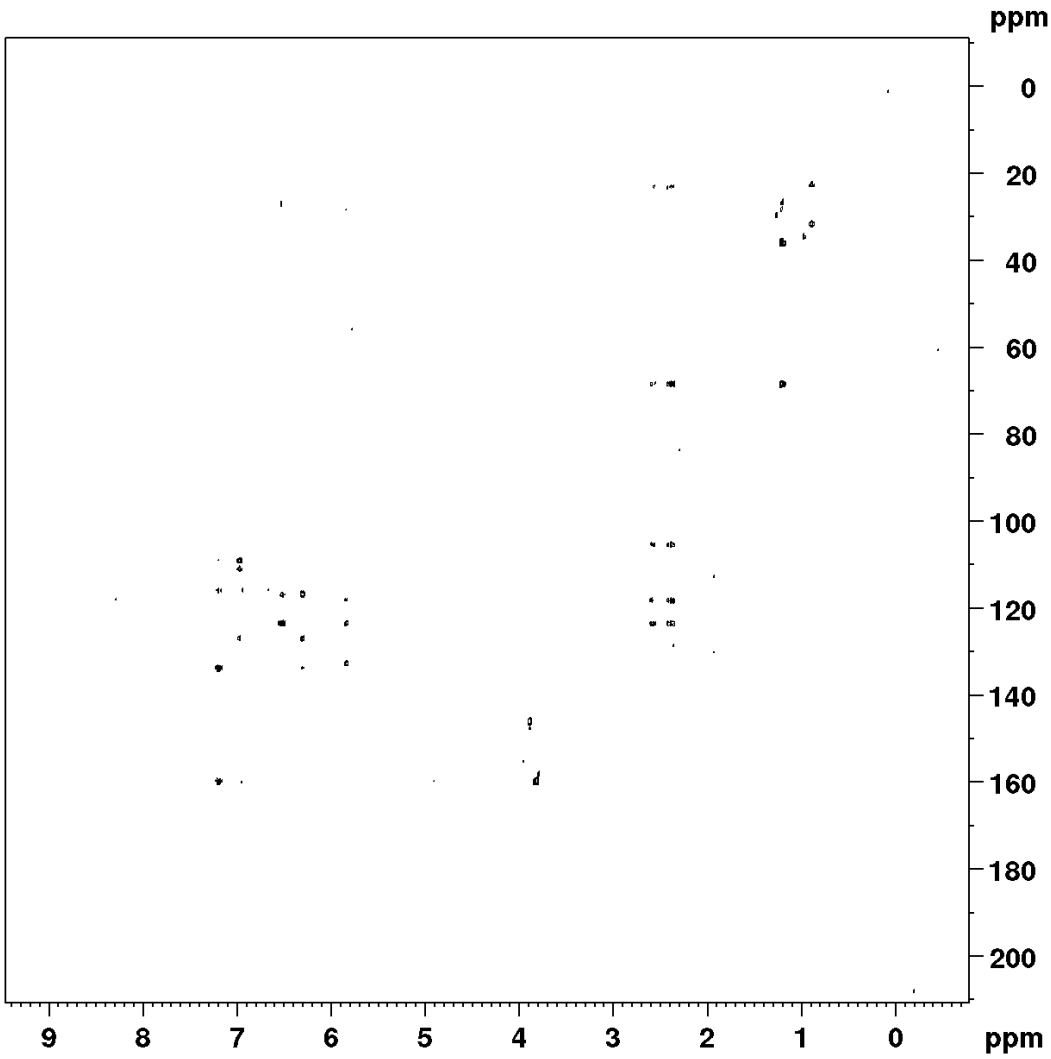
^{13}C NMR of rearranged analog **1.12e** ($CDCl_3$, 101 MHz)



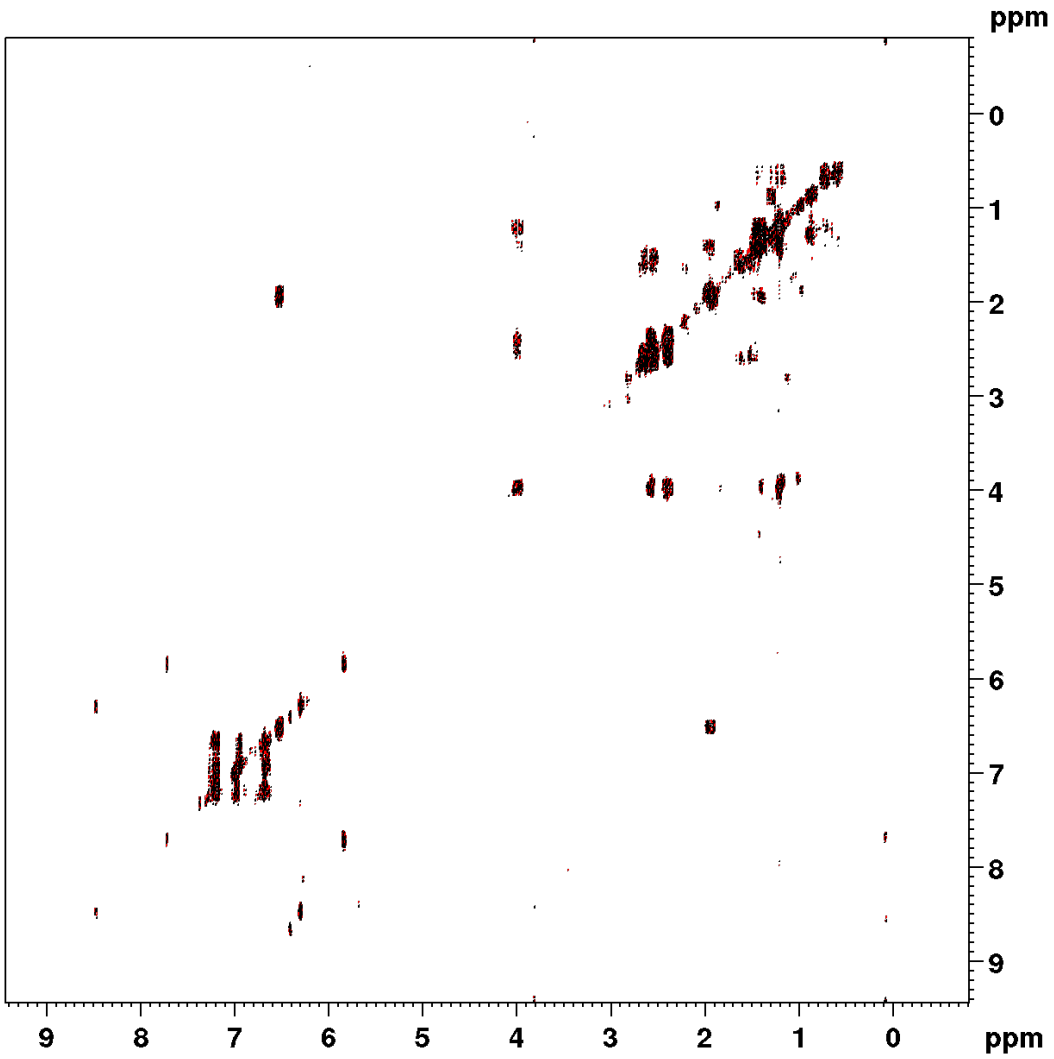
HSQC NMR of rearranged analog **1.12e** ($CDCl_3$, 101 MHz)



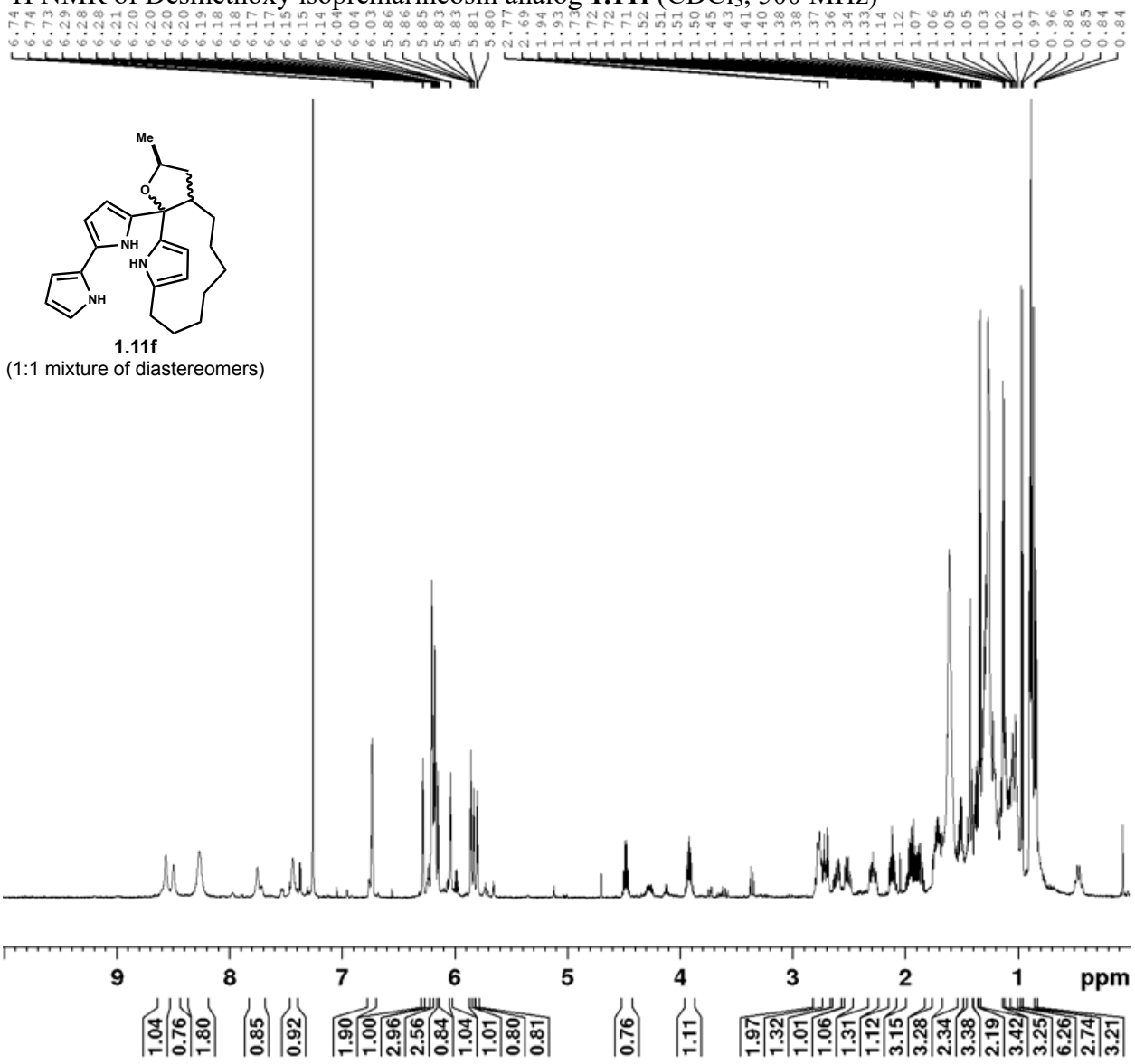
HMBC NMR of rearranged analog **1.12e** ($CDCl_3$, 101 MHz)



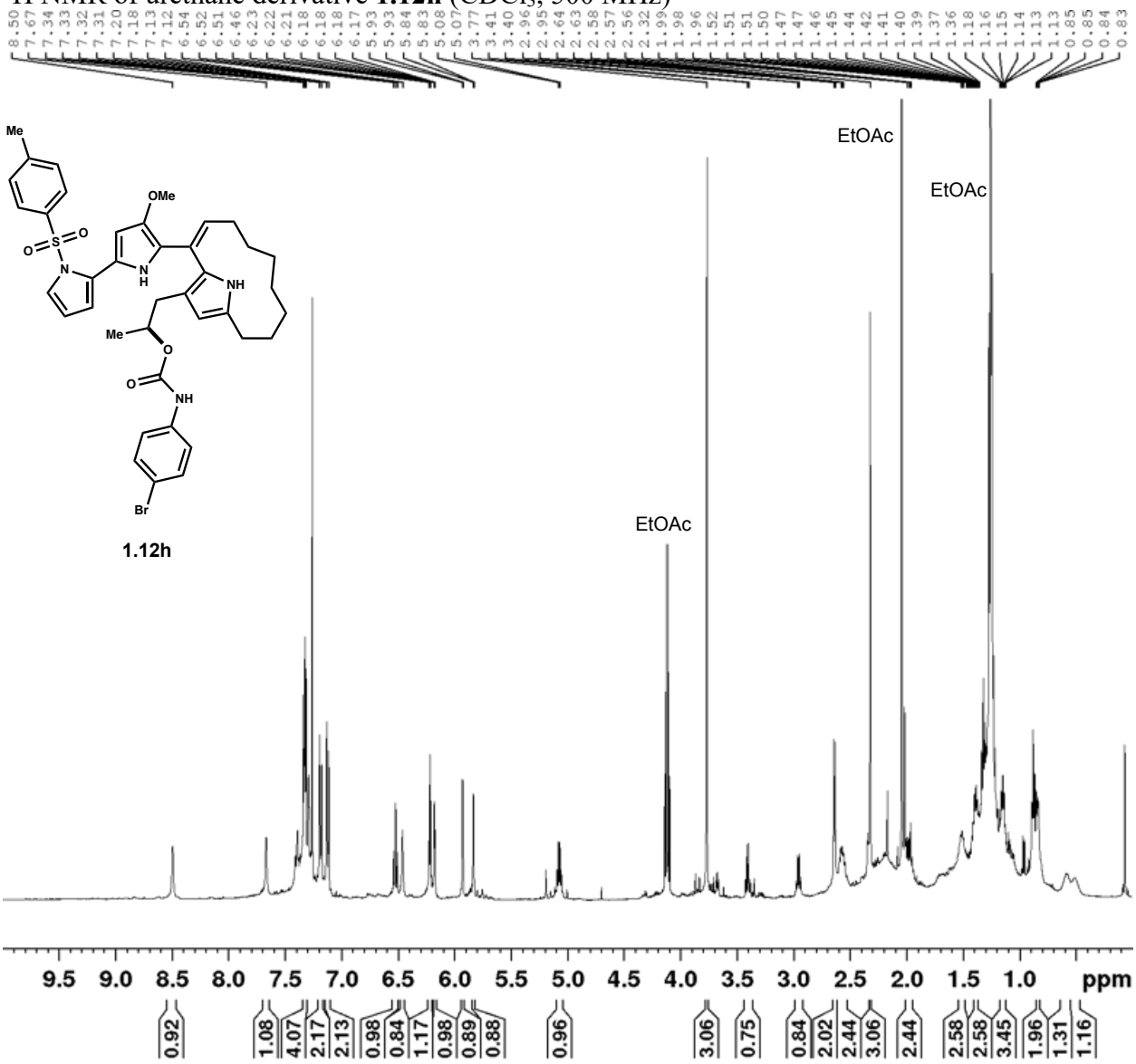
COSY NMR of rearranged analog **1.12e** ($CDCl_3$, 400 MHz)



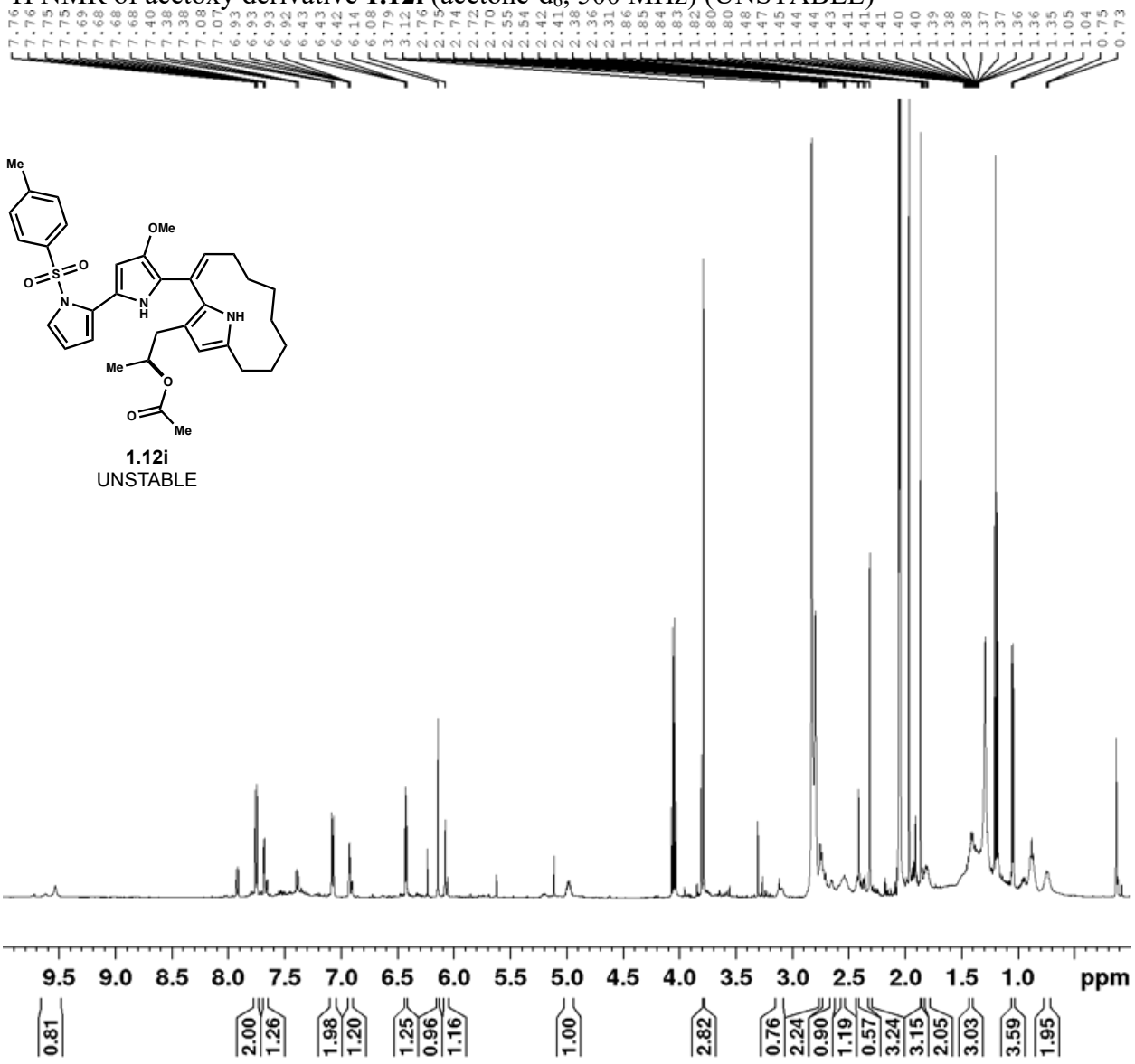
^{1}H NMR of Desmethoxy isopremarineosin analog **1.11f** ($CDCl_3$, 500 MHz)



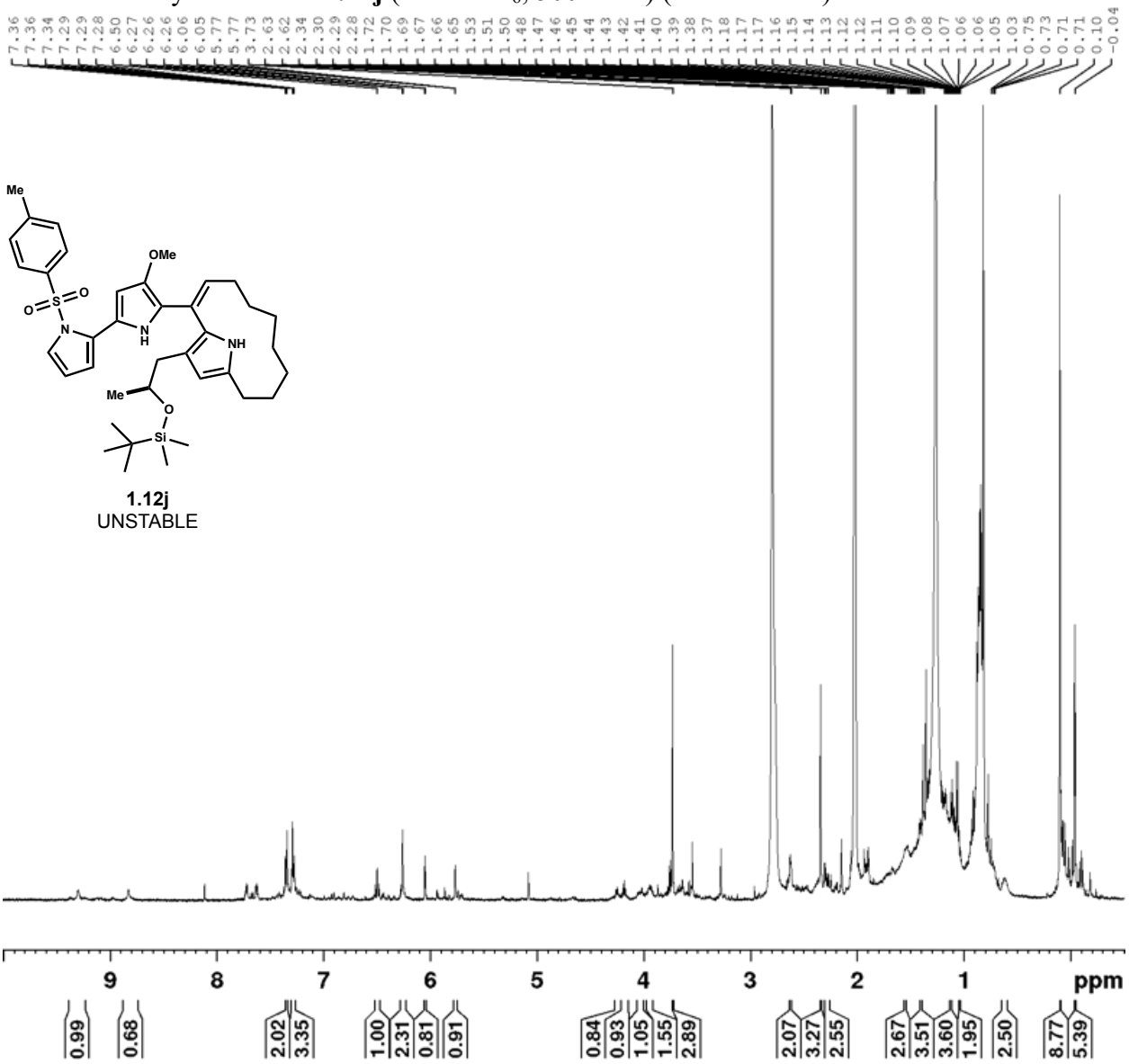
^{1}H NMR of urethane derivative **1.12h** ($CDCl_3$, 500 MHz)



^{1}H NMR of acetoxy derivative **1.12i** (acetone-d_6, 500 MHz) (UNSTABLE)

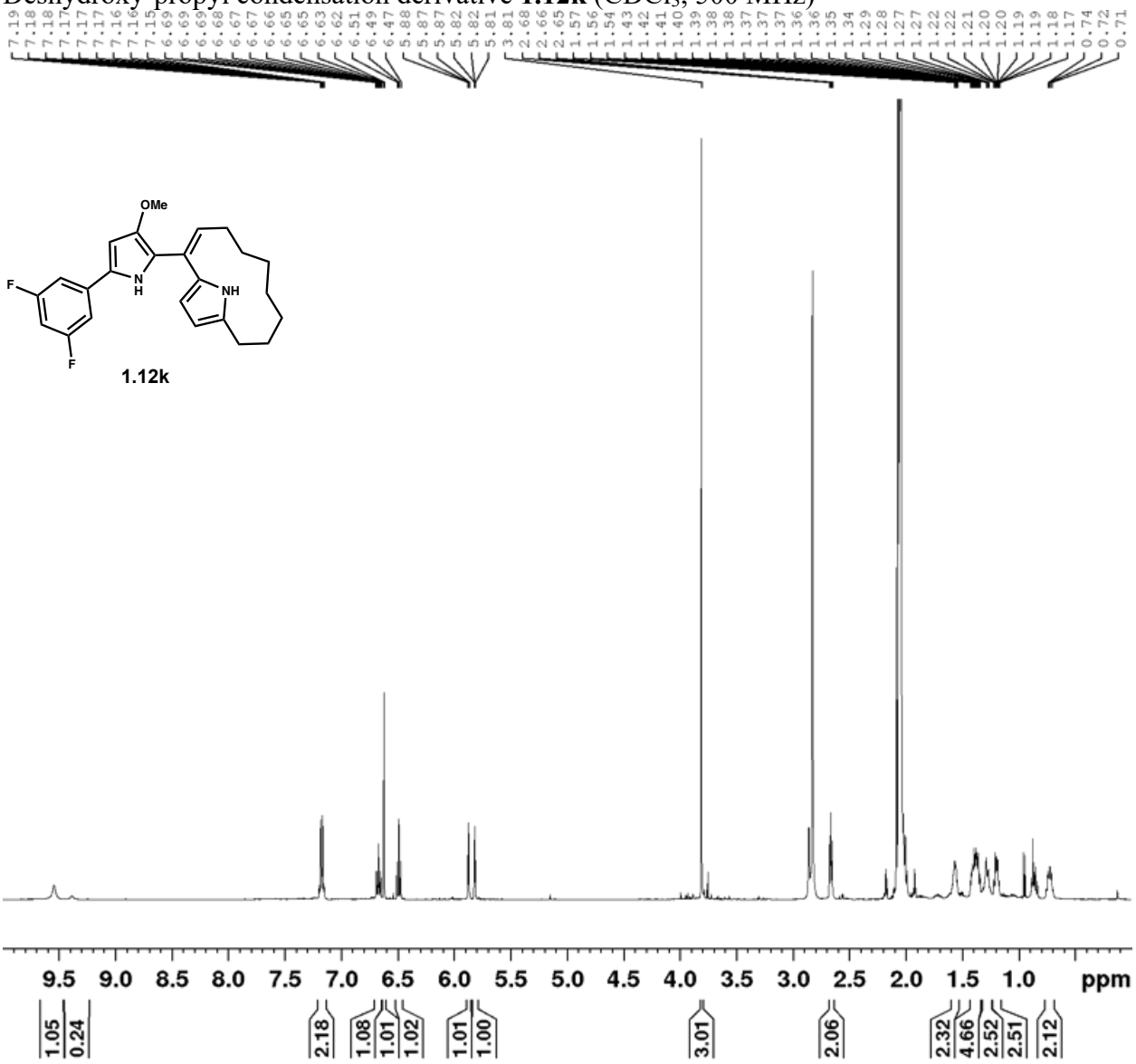


^{1}H NMR of silyl derivative **1.12j** (acetone-d_6, 500 MHz) (UNSTABLE)



1.12j
UNSTABLE

Deshydroxy-propyl condensation derivative **1.12k** ($CDCl_3$, 500 MHz)



Deshydroxy-propyl condensation derivative **1.12k** ($CDCl_3$, 126 MHz)

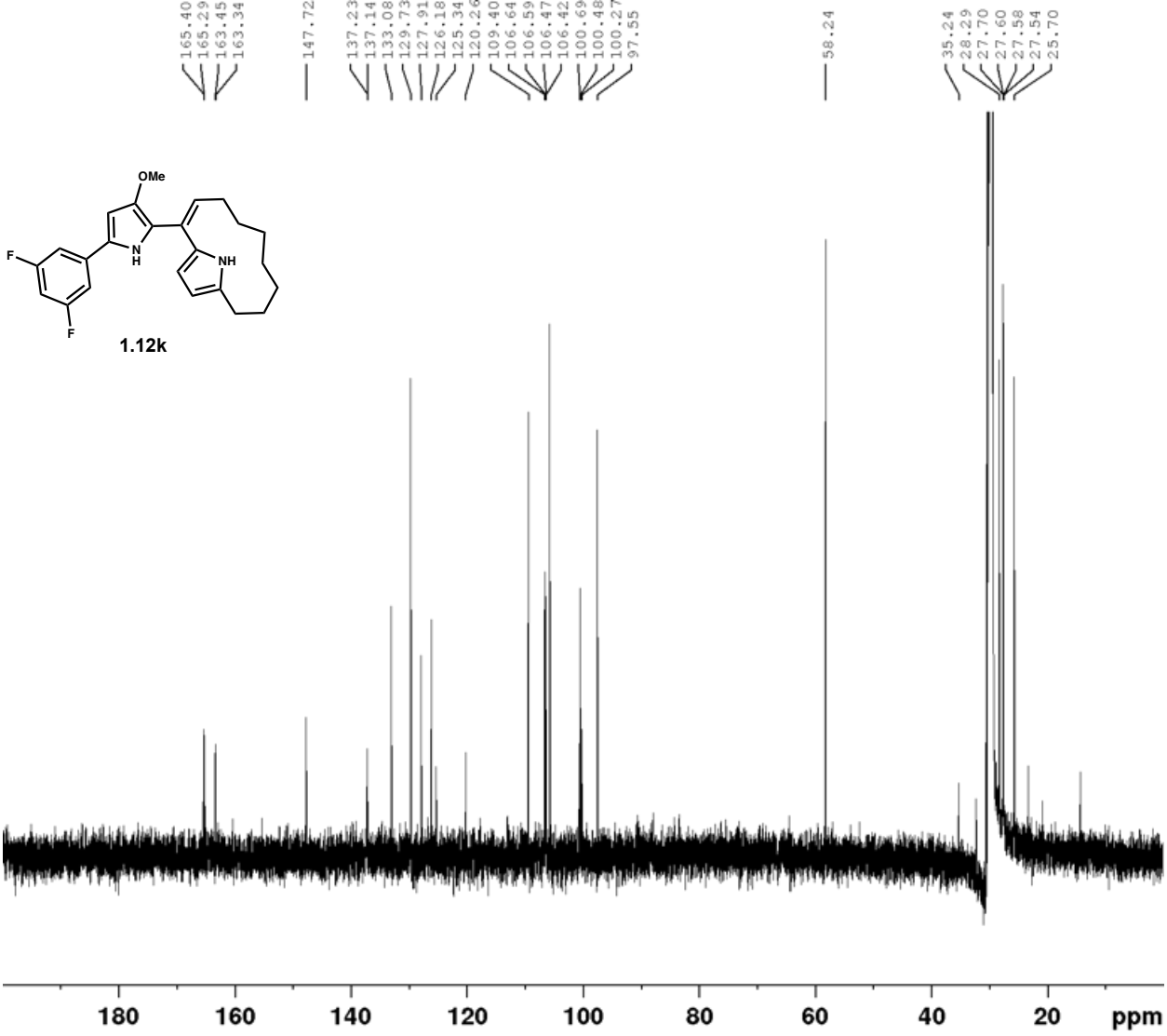


Fig. 1.8F – ^{1}H NMR ($CDCl_3$, 500 MHz)

NMR trace of purified product mixture from **Fig. 1.8F** reaction.

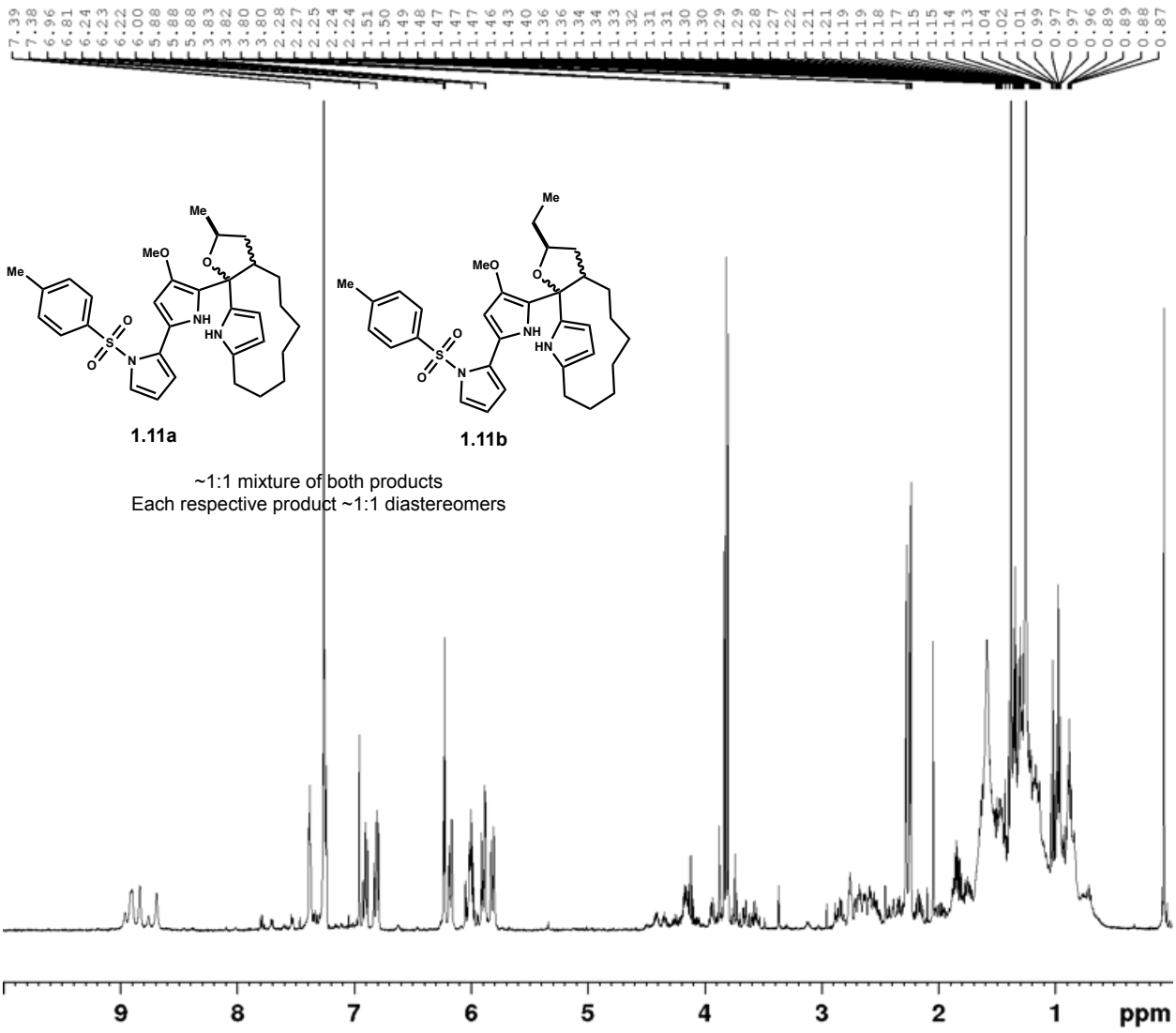


Fig. 1.8F – ^{1}H NMR ($CDCl_3$, 500 MHz) – Zoomed in

NMR trace of purified product mixture from **Fig. 1.8F** reaction.

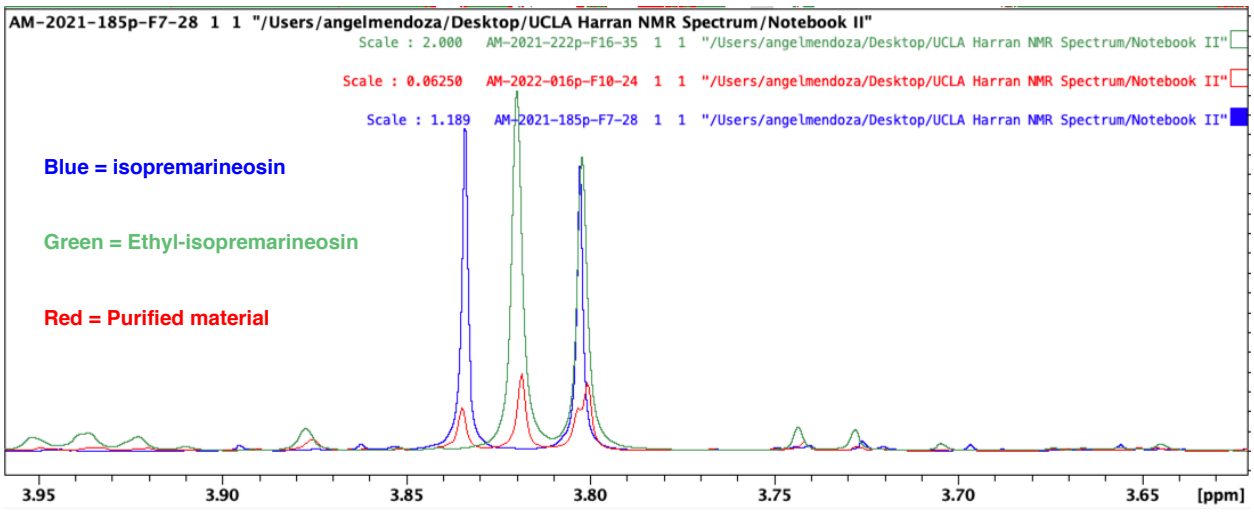


Fig. 1.8F – ^{1}H NMR ($CDCl_3$, 500 MHz) – HRMS trace of product mixture

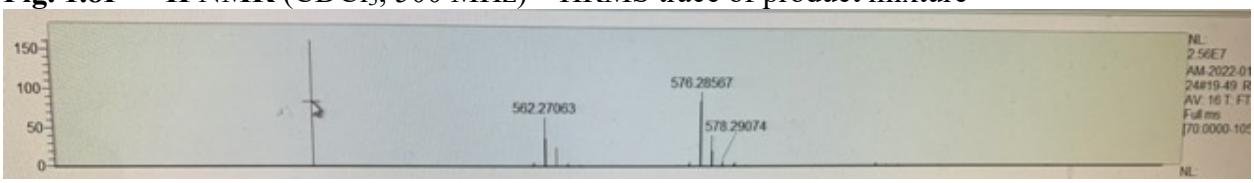


Fig. S6. HRMS trace of purified product mixture from **Fig. 1.8F** reaction. HRMS mass defect for detected **1.11a** = 5.0 ppm; HRMS mass defect for detected **1.11b** = 5.9 ppm.

5. Supplemental References

(1) Frederich, J. H.; Matsui, J. K.; Chang, R. O.; Harran, P. G. Substituted 2,2′-Bipyrroles and Pyrrolylfurans via Intermediate Isoxazolylpyrroles. *Tetrahedron Lett.* **2013**, *54* (21), 2645–2647.

(2) Feng, Z.; Allred, T. K.; Hurlow, E. E.; Harran, P. G. Anomalous Chromophore Disruption Enables an Eight-Step Synthesis and Stereochemical Reassignment of (+)-Marineosin A. *J. Am. Chem. Soc.* **2019**, *141* (6), 2274–2278.

Chapter 2 – Syntheses of Atypically Fluorinated Peptidyl Macrocycles through Sequential Vinylic Substitutions

2.1 Introduction

Ipso substitution of a halogen to form large rings was first demonstrated during synthetic studies on antibiotics of the vancomycin class. In those experiments, biarylether motifs were formed through unimolecular S_nAr reactions involving phenoxide addition to fluoro nitro arenes.[1] More recently, by exploiting reactivity of polyfluorinated aromatics, Spokoyny, Pentelute, and co-workers showed that perfluorobenzene (**2.1**) could engage peptides harboring proximal thiols in successive ipso substitutions to form p-dithiotetrafluorophenyl ether bridged macrocycles.[2] The same group showed that decafluorodiphenyl sulfone (**2.2**) reacts with diamine-bearing peptides to afford 4,4'-diamino octafluorodiphenyl sulfone bridged macrocycles.[3-5]

In our own studies on constrained peptidomimetics, we were considering ways to build bridged bicyclic structures by transannulating existing macrocycles.[6] The ease of fluoride ipso substitutions was appealing. However, relative to reagents **2.1** and **2.2**, we sought broader nucleophile participation and product geometries that are more compatible with smaller polyheterocyclic structures. Rather than aromatic substitutions, we choose to examine vinylic substitutions of fluoride from octafluorocyclopentene (OFCP, **2.3**; **Fig. 2.1**).[7-16] Simple phenols,[7]

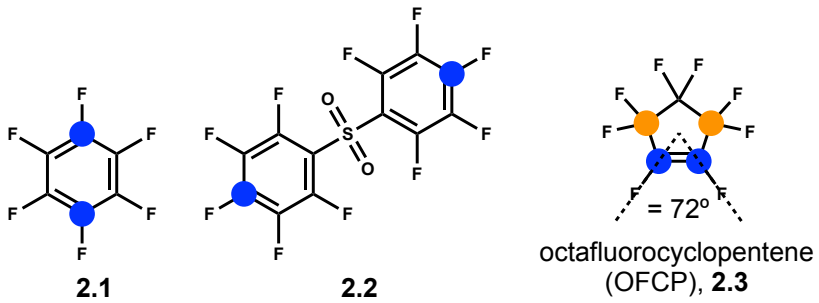


Fig. 2.1. Polyfluorinated arylation and vinylation reagents. Octafluorocyclopentene has the potential to react multiple times as an electrophile, at both primary (blue) and secondary (orange) positions.

amines,[10] mercptans,[14,15] and alcohols[14-16] are known to displace fluoride from this commercial reagent, and the extent of substitution could be varied. Herein, we report that successive, dissymmetric substitutions of fluoride on OFCP by functionalized peptides occurs readily, which provides a flexible means to generate constrained fluorinated macrocycles.[17]

2.2 Results and Discussion

To model ring-closure steps in projected macrocyclizations involving amino acid side chains, we first converted OFCP into monophenoxy derivative **2.5** (0.9 equiv PhOH, Et_3N, 74%). This molecule was found to react (Cs_2CO_3, DMF, 0 ºC) individually with tyrosine, cysteine, and histidine monomers to afford hexafluorocyclopentenylated adducts **2.8**, **2.9**, and **2.10**, respectively, in high yields (**Fig. 2.2**). The reaction to give **2.9** was near instantaneous (< 15 sec) and those affording **2.8** and **2.10** were complete within minutes (see SI for details). LCbz-Ser-NH_2 reacted more slowly. Moreover, initially formed substitution product **2.11c** was unstable and reacted further in situ via its carboxamide, displacing an allylic fluoride through 6-exo trig cyclization to afford spirooxazinones **2.11a/b** (d.r. = 1:1). Products **2.8**–**2.10** and **2.11a**/**b** were stable to handling and chromatography and showed diagnostic ^{19}F NMR spectra consistent with their assigned structures (See SI).

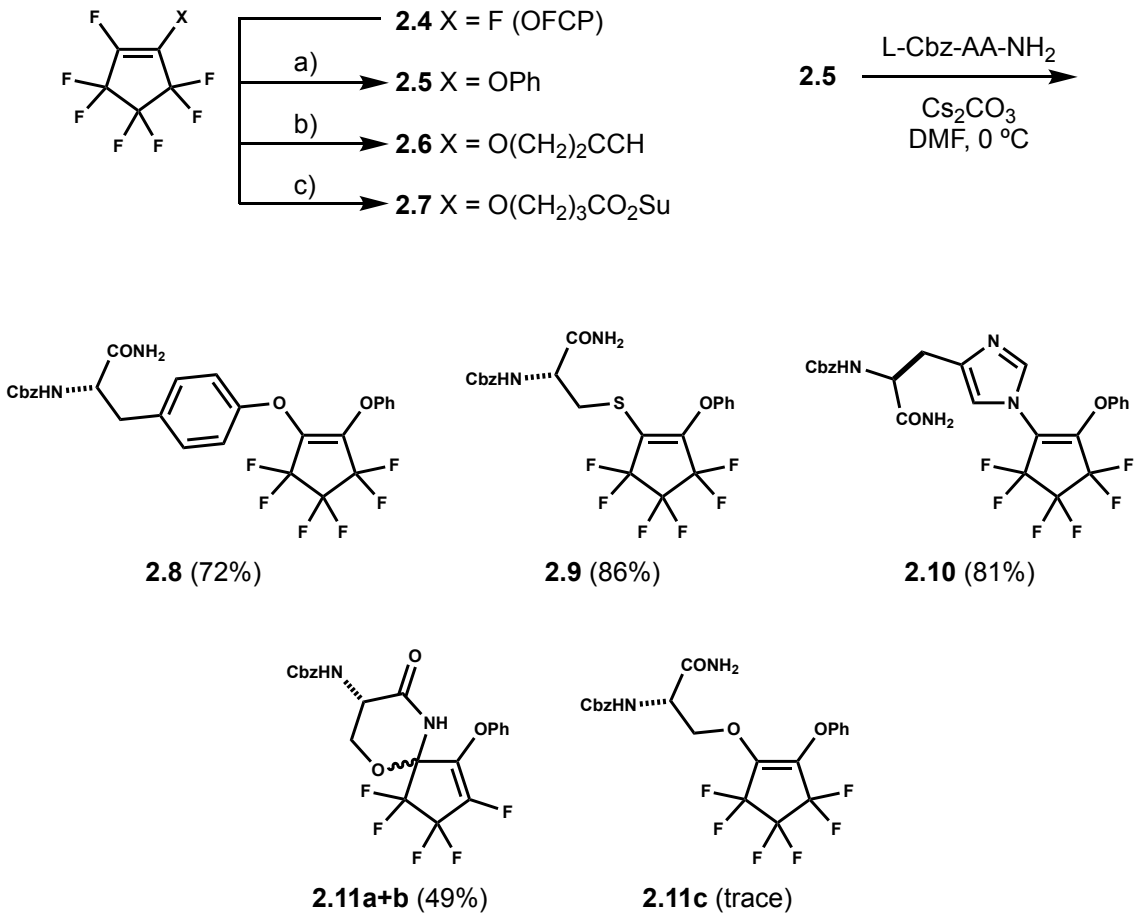


Fig. 2.2. Bimolecular amino acid derivatizations using OFCP. A) OFCP, PhOH, Et_3N, DMF, 0 ºC, 1 h, 74%. B) OFCP, 3-butyn-1-ol, DMF, 0 ºC to rt, 3 h, 57%. C) i) OFCP, $HO(CH_2)_3CO_2Bn$, Et_3N, DMF, 0 ºC to rt, 1 h, 61%. ii) H_2, 10% Pd/C, EtOH. iii) HOSu, EDC•HCl, CH_2Cl_2, rt, 3 h, 83% (2 steps). DMF = dimethylformamide, Su = succimidyl, EDC = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide.

To examine whether single vinyl fluoride substitutions on OFCP could be used to form peptidyl macrocycles, we prepared butynyloxy (**2.6**) and carboxypropyloxy (**2.7**) derivatives of OFCP (**Fig. 2.2**). Both structures were stable oils that could be purified by chromatography. Starting with the tyramide derivative of L-AF (**2.12**, **Fig. 2.3**), N-acylation with 4-pentynoic acid and exposure of the product to **2.6** in the presence of Cs_2CO_3 (DMF, rt) gave diyne (**2.13**) as a white solid. Subjecting this material to Verniest's conditions for Glaser-Hay coupling afforded aryloxy hexafluorocyclopentene containing macrocyclic diyne **2.14**.[18,19] We then tested whether

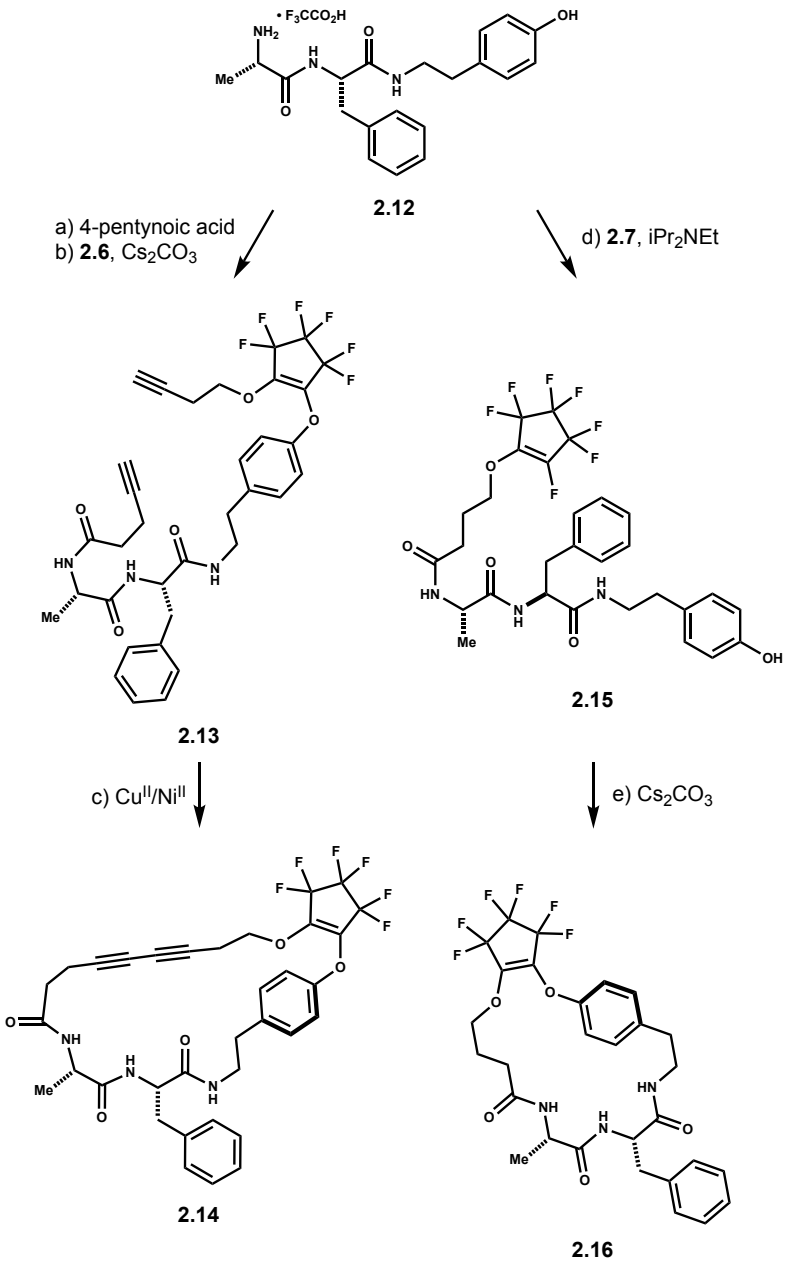


Fig. 2.3. Stepwise synthesis of macrocycles using vinylic substitutions. a) 4-pentenoic acid, EDC•HCl, HOBt, iPr_2EtN, DMF, rt, 3 h. b) **2.6**, Cs_2CO_3, DMF, rt, 3 h, 53%. c) $Cu(OAc)_2•H_2O$, $NiCl_2$, Et_3N, pyridine, EtOH, 50 ºC, 3 h, 52%. d) **2.7**, iPr_2EtN, DMF, 0 ºC, 1 h. e) Cs_2CO_3, DMF, 10 mM, 0 ºC to rt, 2 h, 47% from **2.12**. HOBt = hydroxybenzotriazole.

vinylic substitution could be applied as the ring-closing step. Acylation of **2.12** with **2.7** and treating the product with Cs_2CO_3 (1.5 equiv, DMF, 0 ºC to rt) gave fluorinated macrocycle **2.16** in

good overall yield. To our knowledge, the ring systems in **2.14** and **2.16** have not been described previously. We next tested whether linear peptide derivatives would react with OFCP itself en route to macrocycles. When a DMF solution of dipeptidyl alcohol **2.17** was treated with OFCP in the presence of Et_3N, ether conjugate **2.18** was formed. An N-terminal histidine residue was then appended to the molecule, during which the alkoxyheptafluorocyclopentene motif was stable (**Fig. 2.4**). Treatment of product **2.19** with Cs_2CO_3 in DMF selectively formed imidazole-containing macrocycle **2.20**. The primary amine was unaffected by the reaction. N1-C1 connectivity in **2.20** was assigned based on C1 showing an HMBC correlation to C35H, but not C34H (compare to **2.26**; **Fig 2.5**).

Taken, together, data from biomolecular models and incremental ring syntheses suggest that linear peptides harboring combinations of cysteine, tyrosine, histidine, and serine residues can participate in direct macrocyclizations mediated by OFCP. This proved facile for sequences

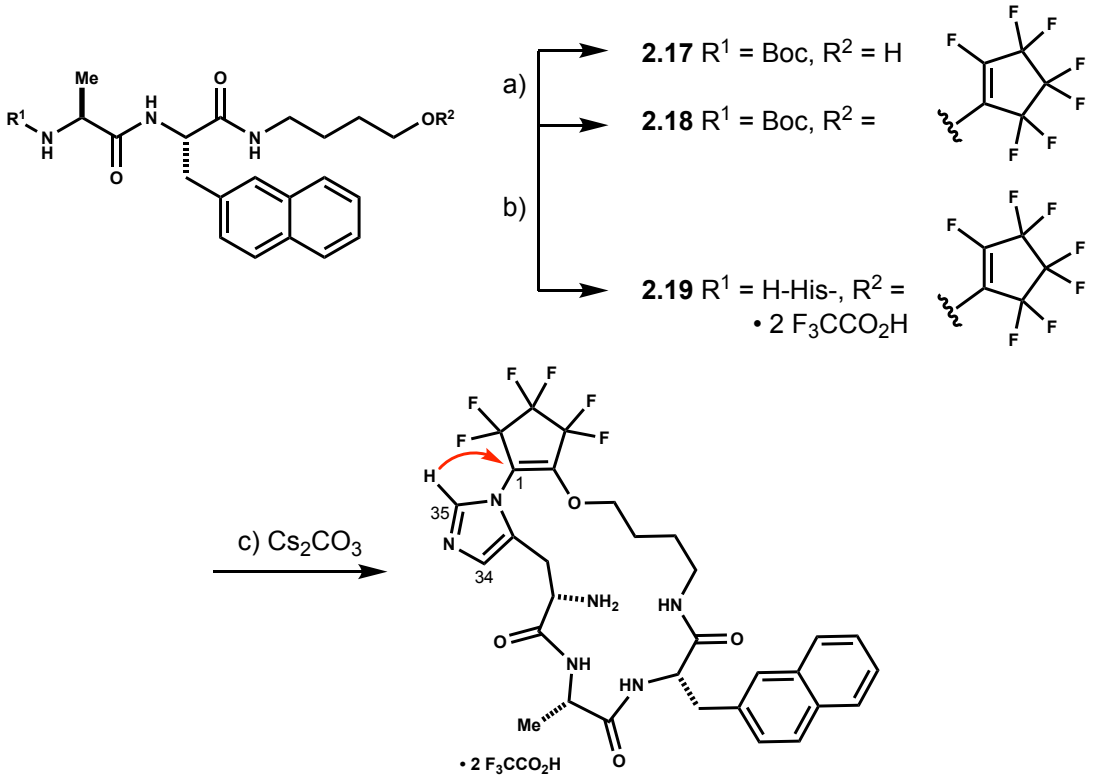


Fig. 2.4. a) OFCP, Et_3N, DMF, rt, 3 h, 40%. b) i) F_3CCO_2H, CH_2Cl_2, rt, 1 h. ii) Boc-His-OH, EDC•HCl, K-Oxyma, iPr_2EtN, DMF, rt, 3 h. iii) F_3CCO_2H, CH_2Cl_2, 2 h, 45% from **2.18**. c) Cs_2CO_3, DMF, 0 ºC to rt, 2 h, 2 h, 39%. Red arrow = HMBC correlation.

containing two cysteine residues. Stirring Ac-CFAC-NH_2 (**2.21**) with 1.5 equiv OFCP in the presence of Et_3N (DMF, 0 ºC) provided macrocycle **2.22** in 51% yield of isolated product. In mixed nucleophile systems, a two-stage, one-pot procedure proved most effective. Exposure of tetrapeptide **2.23**, which contains cysteine and tyrosine residues, to 5.0 equiv OFCP in the presence of Et_3N (DMF, 0 ºC) led to selective derivatization of the thiol within minutes. Subsequent removal of excess OFCP (b.p. 27 ºC, 1 atm) by rotary evaporation and adding solid Cs_2CO_3 to the solution gave unique cyclophane **2.24**. The same method applied to Ac-YFAH-NH_2 engaged the tyrosine and histidine side chains to afford macrocycle **2.26**. The histidine reacted with OFCP first, vinylating its distal nitrogen, which after tyrosine capture produced a regioisomeric variant of the imidazole linkage observed in macrocycle **2.20** (**Fig. 2.4**). HMBC correlation were observed

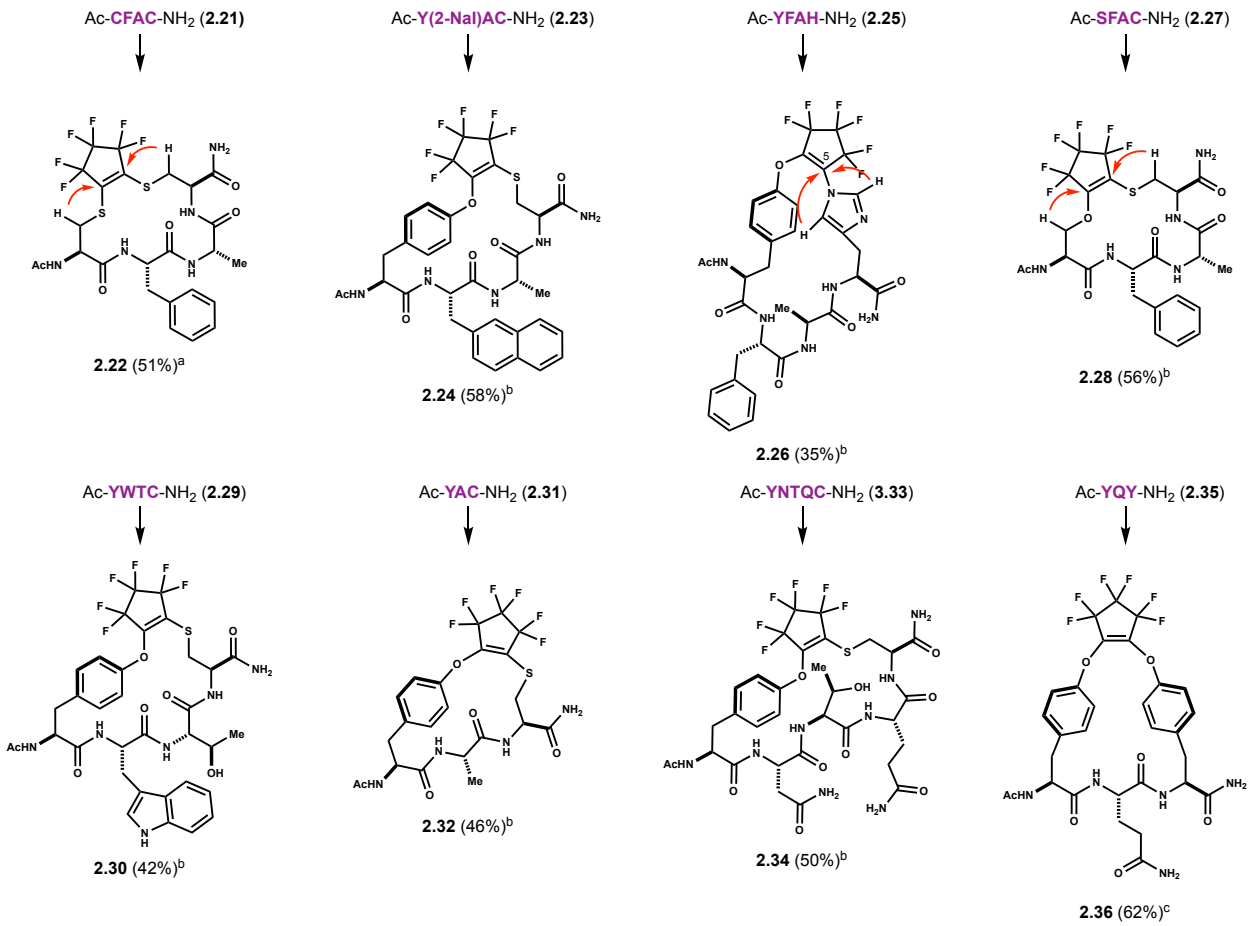


Fig. 2.5. Reagents and conditions: a) OFCP, NEt_3, DMF, 0 ºC, 2 h. b) OFCP (5.0 equiv), NEt_3, DMF, 0 ºC, 30 min, then Cs_2CO_3, 0 ºC, 30 min – 1 h. c) OFCP, Cs_2CO_3, rt, 1 h. Red arrow = HMBC correlations.

between C5 and both imidazole protons in **2.26**. **Fig. 2.5** lists additional fluorinated macrocycles prepared in the above manner, each representing a new ring system. Side-chain protecting groups were not required. Reactions proceeded rapidly at 0 ºC and no provisions were made to exclude oxygen or atmospheric moisture. In the three- to five-residue sequences examined, unprotected Trp, Asn, Gln, and Thr side chains did not participate or interfere with fluoride substitution reactions. Macrocyclic products were stable to handling, isolation, and extended storage except for serine-linked macrocycle **2.28**.[20] This molecule gradually underwent ring opening through net hydrolysis upon standing in solution at room temperature.[21]

In free-base form, the amino acid side chains of lysine and ornithine reacted with OFCP, but the resultant substitution products were generally not stable. In contrast, OFCP reacted with free N-terminal cysteine residues to form isoable hexafluorocyclopentathiazine derivatives (**Fig. 2.6**). The reactions examined were complete within minutes. The ability to rapidly hexafluorocyclopentenylate the N-terminus of peptides and peptidomimetics may have utility, for example, in probe syntheses for positron emission tomography (PET).[22]

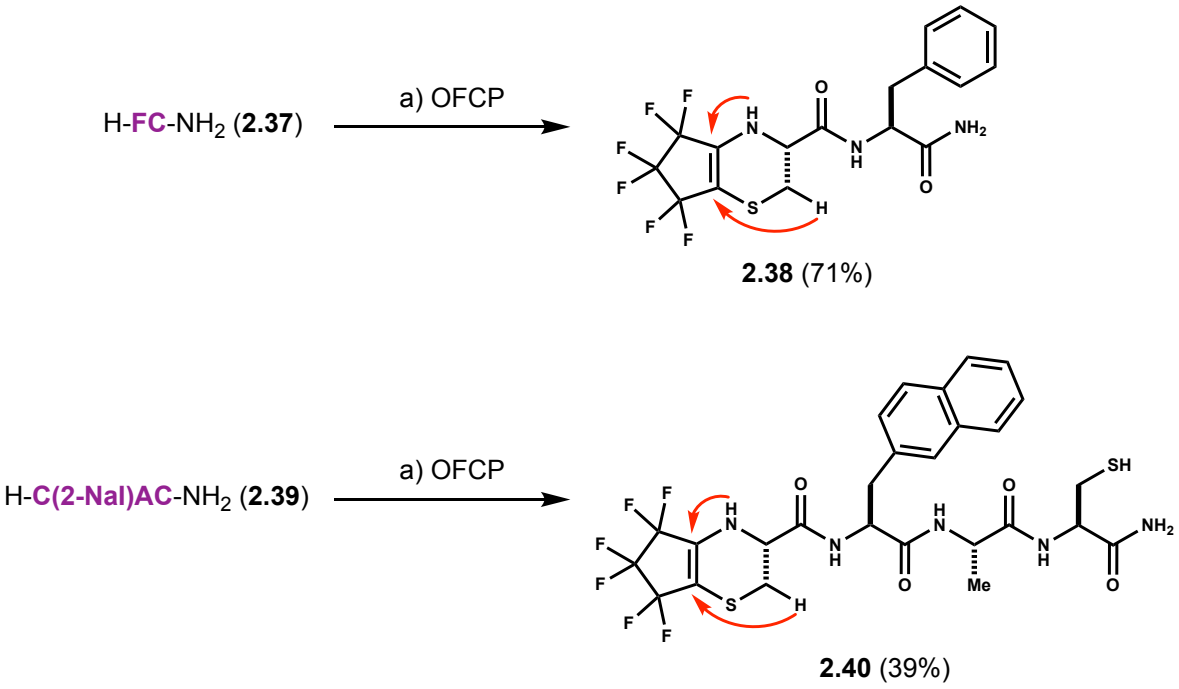


Fig. 2.6. Reagents and conditions: a) OFCP, NEt_3, DMF (10 mM), 0 ºC, 30 min. Red arrows = HMBC correlations.

Substituting the two vinyl positions on OFCP with heteroatoms gave products that are potentially susceptible to further internal reactions, as was observed during conversion of **2.11c** to **2.11a/b** (**Fig. 2.2**). Experimenting initially with **2.22**, we found that treatment with PhOH/Cs_2CO_3 in DMF gave spirocyclic thiazinone-containing phenyl ether **2.41**, wherin two additional fluorine atoms had been displaced from the cyclopentene core. This result implies that phenoxide could be captured intramolecularly. In fact, we discovered that hexapeptides of consensus sequence

YXCXXC undergo remarkable polysubstitution reactions with OFCP. Stirring Ac-YACFAC-NH_2 with 1.5 equiv OFCPin DMF at 0 ºc rapidly formed a disubstitution adduct (monitored by LC-MS). Excess Cs_2CO_3 was then added and the solution was warmed to rt and stirred for 4 h. This gave macrobicyclic structure **2.43**, wherin the peptide had displaced four fluorine atoms from OFCP, forming three new rings (**Fig. 2.7**) in the process.

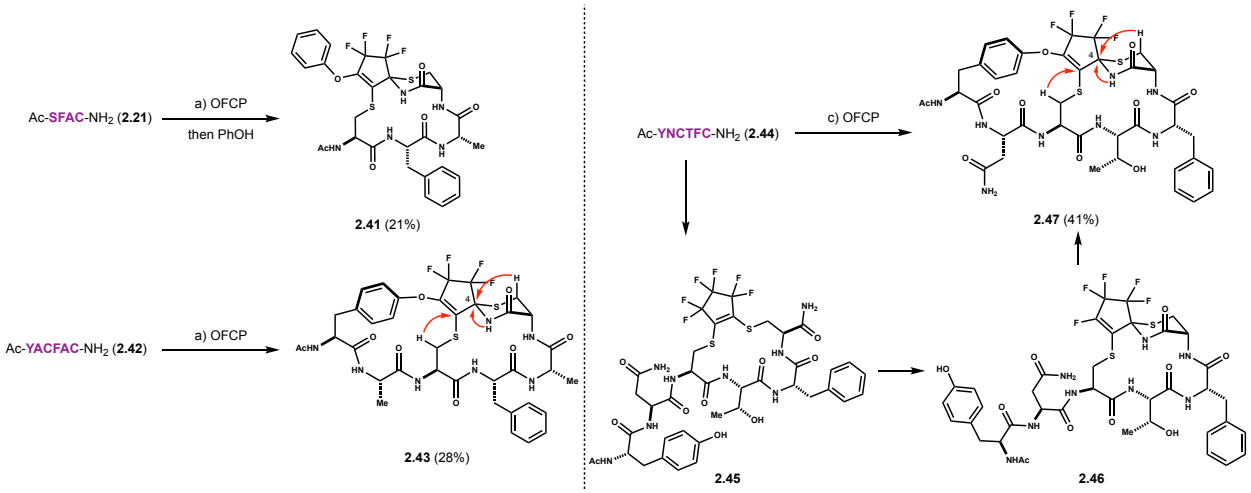


Fig. 2.7. Reagents and conditions: a) OFCP, NEt_3, DMF (5 mM), 0 ºC, 30 min, then Cs_2CO_3, rt, 6 h, 21% (unoptimized). b) OFCP, NEt_3, DMF (5 mM), 0 ºC, 30 min, then Cs_2CO_3, rt, 4 h, 28%. c) OFCP, NEt_3, DMF (5 mM), 0 ºC, 10 min, then Cs_2CO_3, rt, 6 h, 41%.[23] Red arrows = HMBC correlations.

The outcome was rationalized in terms of the C-terminal carboxamide cyclizing at C4 in an initially formed cyclic bis-thioether, generating a new vinyl fluoride that subsequently captured the pendant phenoxide. Stereochemistry at the newly formed spiro center (C4, dr = 10:1) was assigned by analogy to **2.47** (see below). Similar results were obtained starting with Ac-YNCTFC-NH_2 (**2.44**), and complex polycycle **2.47** was isolated in good yield. In that case, quenching the reaction with AcOH after 2 h provided a mixture of **2.47** and two intermediate structures (**Fig. 2.8A**). Data obtained for purified samples of those compounds (**Fig. 2.8B**) suggested they were cyclic bis-thioether **2.45** and spirocycle **2.46**, which is fully consistent with the progression of events proposed above.

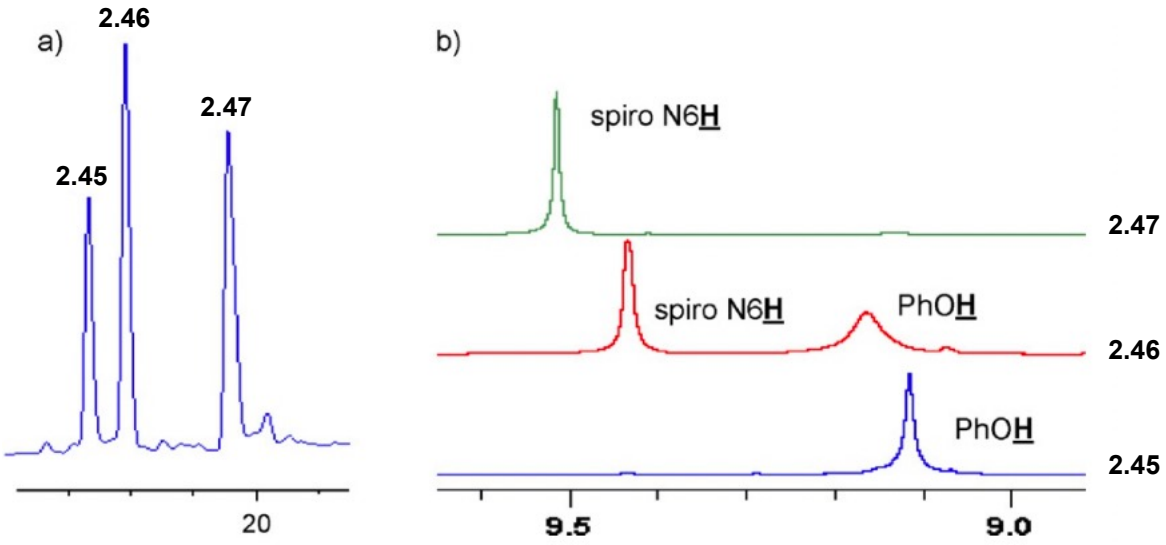


Fig. 2.8. A) Analytical HPLC trace (C18, 50 → 100% MeCN + 0.1% F_3CCO_2H) of mixture obtained upon quenching (AcOH) the reaction of **2.44** with Cs_2CO_3 after 2 h. B) Diagnostic ^{1}H-NMR signals indicative of structures of **2.45**, **2.46**, **2.47**, having m/z 963.3, 943.3, and 923.2 respectively. See the SI for details.

As expected, re-exposing **2.45** and **2.46** to Cs_2CO_3 in DMF produced **2.47**. Scrambled variants of **2.44**, namely Ac-CNYYFC-NH_2, Ac-NYCAFC-NH_2, and Ac-YCNTFC-NH_2, each reacted smoothly with OFCP, but none substituted more than three of its fluorine atoms (see SI for details). The YXCXXC consensus was required to form macrobicyclic structures through four substitutions.

After many failed attempts to grow quality crystals, the structure **2.47** was firmly established by X-ray analysis of a single crystal grown using the vapor diffusion method (CH_2Cl_2 into DMSO/PhH at 25 ºC). In the solid state, **2.47** exists in a single conformation. Large cavities in the unit cell are occupied by DMSO, with two water molecules bound along the polar face of the structure (**Fig. 2.9**).

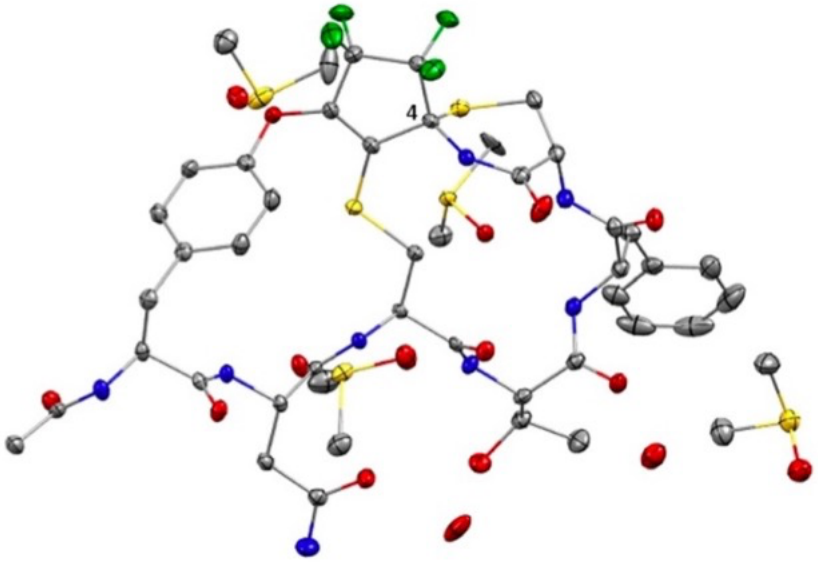


Fig. 2.9. X-ray structure of **2.47** [ORTEP, 50% probability thermal ellipsoids].[24] Four molecules of DMSO and two molecules of water (shown) per molecule of **2.47** exist in the unit cell. Hydrogen atoms omitted for clarity. See SI for details.

2.3 Conclusions

These reaction cascades convert linear, unprotected peptides into conformationally defined polyheterocycles with two turn surfaces scaffolded by a tetrafluorocyclopentene core. Constrained peptidomimetics of this kind have not been studied previously and may prove useful in medicinal chemistry programs. Experiments along these lines are ongoing, as are evaluations of the pharmacological properties of polyfluorocyclopentenylated macrocycles in general. The ease and simplicity of OFCP-based polycyclizations should greatly facilitate access to complex fluorinated macrocycles.

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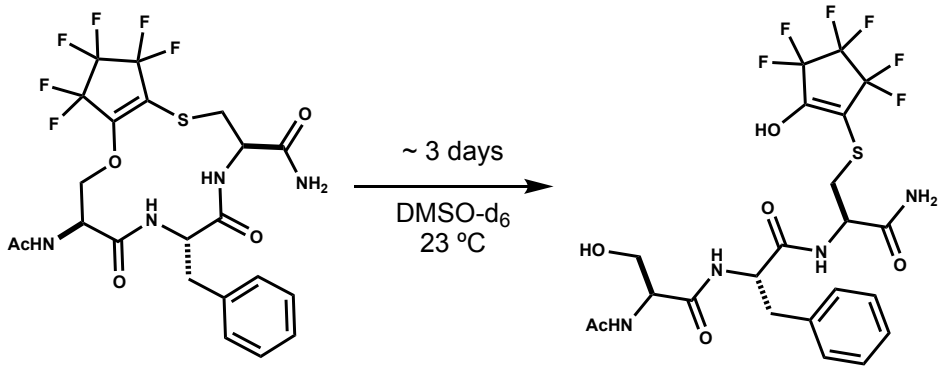
(20) For example, macrocycle **2.22** was stable in 1M aq. HCl/MeOH (1:9) solution for 24 h; whereas it decomposed within 1 h in basic media [1M aq. NaOH in MeOH (1:9)]. See **Fig. S5** and **S6**. In general, reactions promoted by Cs_2CO_3 were quenched with AcOH to avoid degradations during aqueous workup.

(21) Macrocycle **2.28** was stable as a solid, but upon standing in DMSO-d_6, it gradually (several weeks) underwent ring opening through hydrolysis (+2 H2O, -HF net). Related structure **2.48**, which has a smaller macrocycle size, hydrolyzed more rapidly. See SI for details.



2.48 **2.49**

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(23) Adding up to 20 vol % water to the reaction of 2.44 with OFCP in the presence of Et3N had no discernible impact on the formation of 2.45 (see Fig. S4).

(24) CCDC 1946197 contains the supplementary crystallographic data for this paper. This data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

2.5 Chapter 2 – Experimental Appendix

1. Materials and Methods

Reagents were purchased from commercial vendors and used as received unless otherwise stated. Tetrahydrofuran (THF), diethyl ether (Et_2O), acetonitrile (MeCN) and toluene (PhMe) were passed through a Glass Contour solvent drying system. Yields refer to chromatographically and spectroscopically (^1H NMR) homogeneous materials, unless otherwise stated. Thin-layer chromatography (TLC) was conducted on precoated plates (Sorbent Technologies, silica gel 60 PF254, 0.25 mm) visualized with UV 254 nm. Column chromatography was performed on silica gel 60 (SiliCycle, 240−400 mesh). Purification of peptides was performed using an Agilent 1200 HPLC system equipped with G1361A preparative pumps, a G1314A auto sampler, a G1314A VWD, a G1364B automated fraction collector, and a Waters Sunfire C18 column (5 μm, 19 mm × 250 mm), unless otherwise noted. Analytical HPLC was performed using the same system, but with a G1312A binary pump. Mass spectra were recorded using an Agilent 6130 LC/MS system equipped with an ESI source. High-resolution mass spectra were recorded on Thermo Scientific Exactive® Mass Spectrometer with DART ID-CUBE Waters GST Premier, and Waters LCT Premier. NMR spectra were recorded on Bruker Avance spectrometers (400/101 MHz and 500/126 MHz).

NMR spectra were recorded on Bruker Advance (300, 400 or 500 MHz) spectrometers. HSQC, HMBC NMR experiments were used to aid assignment of NMR peaks when required.

2. Experimental Procedures

General procedure A – Peptide Synthesis:

C-terminal carboxamide peptides were synthesized manually using standard Fmoc solid phase synthesis protocols on Rink Amide MBHA resin (200-400 mesh, 0.73 mmol/g, 1% DVB) on 0.37-0.50 mmolscale using a fritted glass reaction vessel. Fmoc-deprotection was achieved with 20% piperidine in DMF (2 x 30 min). The reaction vessel was washed with DMF (3x) and CH_2Cl_2 (2x). The vessel was then charged with the appropriate Fmoc-amino acid (4 eq.) and HBTU (4 eq.) followed by DMF (10-20 ml) and iPr_2NEt (10 eq). The resin was agitated for 2 hours, drained, and washed with DMF (3X). After all coupling were completed the resin was cleaved with TFA/ water/TIPS (90:5:5) for 1.5 hrs. The cleaved resin was removed by filtration and the filtrate was concentrated under vacuum. The peptide was precipitated with Et_2O and isolated by centrifugation. The peptide pellet was repeated triturated with EtOAc and isolated by centrifugation to ensure complete removal of cleavage reagents. X was used to prepare PFCP containing peptides.

General procedure B – Macrocyclization

A flask was charged with linear precursor (1 eq) and DMF (10 mM in substrate). The mixture was added Cs_2CO_3 (2 eq) at 0 °C, stirred for 2 hrs at rt. The mixture was diluted with EtOAc, washed with brine (5 times). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation. Purification condition — see details per example, below.

General procedure C – Direct Macrocyclization

A flask was charged with linear precursor (1 eq) and DMF (10 mM in substrate). The mixture was added octafluorocyclopentene (1M in MeCN, 5 eq) and Et_3N (1.5 eq) at 0 °C. The mixture was stirred for 30 minutes and then excess amount of octafluorocyclopentene and MeCN were removed by rotary evaporation. The mixture was added Cs_2CO_3 (1 eq) at 0 °C and stirred for 1 hr at rt. The mixture was diluted with EtOAc, washed with brine (5 times). The organic layer was dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation. Purification condition — see details per example, below.

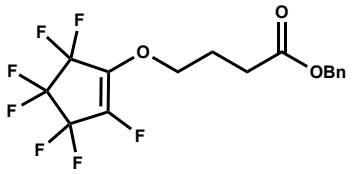
((Perfluorocyclopent-1-en-1-yl)oxy)benzene (**2.5**)

A solution of phenol (1.09 g, 11.6 mmol) in DMF (60 ml) was cooled to 0 °C and added octafluorocyclopentene (1.70 ml, 12.7 mmol) and Et_3N (2.66 ml, 19.1 mmol). The resulting solution was allowed to stir for 1 hr at 0 °C. The reaction mixture was added H_2O, extracted with EtOAc, washed with brine (x5), dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation to give a crude oil. Purification by column chromatography (SiO_2, hexane only) afforded the title compound (2.45 g, 8.56 mmol, 74%) as a colorless oil. H NMR (DMSO-d_6, 400 MHz) δ 7.53-7.45 (m, 4H), 7.40-7.35 (m, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 153.1, 136.5–133.6 (m, 1C), 134.3–133.6 (m, 1C). 130.2, 127.1, 119.1, 114.2–106.2 (m, 3C). ^{19}F NMR (282 MHz) δ -113.5 (d, J = 14.0 Hz, 2F). -114.0-(-114.1) (m, 2F), -128.1 (s, 2F), -150.4-(-150.6) (m, 1F). HRMS m/z $[M]^+$ calc'd for $C_{11}H_5F_7O$ 286.0, found 286.0.

1-(But-3-yn-1-yloxy)-2,3,3,4,4,5,5-heptafluorocyclopent-1-ene (**2.6**)

A solution of 3-butyn-1-ol (1 ml, 13.2 mmol) in DMF (100 ml) was cooled to 0 °C and added octafluorocyclopentene (2.11 ml, 15.9 mmol) and Et_3N (2.22 ml, 15.9 mmol). The resulting solution was allowed to stir for 3 hr at rt. The reaction mixture was diluted with Et_2O. The organic layer was washed with brine (5 times), dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation. Purification by column chromatography (SiO_2, hexane only) afforded the title compound (1.96 g, 7.48 mmol, 57%) as a pale yellow oil. ^{1}H NMR (DMSO-d6, 500 MHz) δ 4.59 (dt, J = 6.3, 2.9 Hz, 2H), 2.95-2.93 (m, 1H), 2.69 (dt, J = 6.3, 2.6 Hz, 2H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 135.2 (t, J = 23.6 Hz, 1C), 134.7–131.8 (m, 1C), 114.1–106.4 (m, 3C), 79.7, 73.0, 71.5 (d, J = 4.6 Hz, 1C), 19.0 (d, J = 1.5 Hz, 1C). ^{19}F (DMSO-d_6, 282 MHz) δ -112.8-(-112.9) (m, 2F), -114.6-(-114.8) (m, 2F), -128.2-(-128.4) (m, 2F), -159.6-(-159.8) (m, 1F) HRMS m/z $[M]^+$ calc'd for $C_9H_5F_7O$ 262.0223; found 262.0223.

Benzyl 4-((perfluorocyclopent-1-en-1-yl)oxy)butanoate (**S1**)



A solution of benzyl 4-hydroxybutanoate[1] (2.95 g, 15.2 mmol) in DMF (60 ml) was cooled to 0 °C and added octafluorocyclopentene (3.04 ml, 22.8 mmol) and Et_3N (4.24 ml, 30.4 mmol). The resulting solution was allowed to stir for 1 hr at rt. The reaction mixture was diluted with Et_2O. The organic layer was washed with brine (5 times), dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation to give a crude oil. Purification by column chromatography (SiO_2, gradient 10-20% EtOAc/hexane) afforded the title compound (3.59 g, 9.29 mmol, 61%) as

a pale yellow oil. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.39–7.32 (m, 5H), 5.14 (s, 2H), 4.43 (td, J = 6.2, 2.9 Hz, 2H), 2.53 (t, J = 7.2 Hz, 2H), 2.13 (quin, J = 6.6 Hz, 2H). ^{13}C NMR (DMSO-d_6 126 MHz) δ 172.3, 135.9, 135.9–135.6 (m, 1C), 135.6–133.6 (m, 1C), 128.8, 128.6, 128.5, 114.0–106.6 (m, 3C), 72.5 (d, J = 4.4 Hz, 1C), 66.8, 29.9, 24.6 (d, J = 1.4 Hz, 1C). ^{19}F NMR (DMSO-d_6, 282 MHz) δ -115.3 (brd, J = 12.7 Hz, 2F), -116.5-(-116.6) (m, 2F). -130.0 (s, 2F), -162.0-(-162.2) (m, 1F). HRMS m/z $[M]^+$ calc'd for $C_{16}H_{13}F_7O_3$ 386,9747; found 386.0748.

2,5-Dioxopyrrolidin-1-yl 4-((perfluorocyclopent-1-en-1-yl)oxy)butanoate (**2.7**)

A solution of **S1** (2.75 g, 9.30 mmol) in DCM (100 ml) was added N-hydroxysuccinimide (2.14 ml, 18.6 mmol) and EDC·HCl (3.57 ml, 18.6 mmol). The resulting solution was allowed to stir for 3 hrs at rt. The reaction mixture was added 1N HClaq and extracted with CHCl3. The organic layer was dried over Na2SO4, filtered and the solvent was removed by rotary evaporation. Purification by column chromatography (SiO_2, gradient 33-50% EtOAc/Hex) afforded the title compound (3.03 g, 7.72 mmol, 83%) as a pale yellow oil. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 4.61 (td, J = 6.2, 3.1 Hz, 2H), 2.83 (t, J = 7.4 Hz, 2H), 2.82 (brs, 4H), 2.09 (quin, J = 6.8 Hz, 2H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 170.2, 168.4, 135.5–135.2 (m, 1C), 134.3–132.1 (m, 1C), 113.7–106.7 (m, 3C), 72.4 (d, J = 4.4 Hz, 1C), 26.4, 25.4, 23.9 (d, J = 1.2 Hz, 1C); ^{19}F (DMSO-d_6, 282 MHz) δ -112.7-(-112.9) (m, 2F), -114.7-(-114.8) (m, 2F), -128.2-(-128.3) (m, 2F), -160.7-(-160.8) (m, 1F); HRMS $[M\text{-}OSu]^+$ calc'd for $C_9H_6F_7O_2$ 279.0251; found 279.0247.

Benzyl (S)-(1-amino-3-(4-((3,3,4,4,5,5-hexafluoro-2-phenoxycyclopent-1-en-1-yl)oxy)phenyl)-1-oxopropan-2-yl)carbamate (**2.8**)

A solution of **2.5** (96 mg, 0.336 mmol) in DMF (2.5 ml) was cooled to 0 °C and added Cbz-L-Tyr-NH_2 (81 mg, 0.258 mmol) and Cs_2CO_3 (110 mg, 0.336 mmol). The resulting solution was allowed to stir for 1 hr at 0 °C. The reaction mixture was added water, and then extracted with EtOAc. The organic layer was washed with brine (5 times), dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation to give a crude oil. Purification by column chromatography (SiO_2, gradient 0-5% MeOH/$CHCl_3$) afforded the title ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.46 (brs, 1H), 7.39 (d, J = 8.8 Hz, 1H), 4.93 (AB quartet, J = 12.8 Hz, 2H), 4.11–4.06 (m, 1H), 2.89 (dd, J = 13.7, 3.9 Hz, 1H), 2.64 (dd, J = 13.7, 10.8 Hz, 1H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.3, 155.9, 153.6, 152.0, 137.0, 135.5, 133.8-133.4 (m, 2C), 130.4, 129.8, 128.3, 127.7, 127.5, 125.2, 116.9, 116.6, 114.8–107.0 (m, 3C), 65.2, 56.1, 36.6; ^{19}F NMR (DMSO-d_6, 282 MHz) δ 112.7 (app. t, 4F), -128.6 (app. s, 2F); HRMS m/z $[M + H]^+$ calc'd for $C_{28}H_{22}F_6N_2O_2H$ 581.1511; found 581.1532.

Benzyl (R)-(1-amino-3-((3,3,4,4,5,5-hexafluoro-2-phenoxycyclopent-1-en-1-yl)thio)-1-oxopropan-2-yl)carbamate (**2.9**)

A solution of **2.5** (105 mg, 0.367 mmol) in DMF (2.8 ml) was cooled to 0 °C and added Cbz-L-Cys-NH_2 (72 mg, 0.282 mmol) and Cs_2CO_3 (110 mg, 0.367 mmol). The resulting solution was allowed to stir for 1 hr at 0 °C. The reaction mixture was added water, and then extracted with EtOAc. The organic layer was washed with brine (5 times), dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation to give a crude oil. Purification by column chromatography (SiO_2, gradient 0-5% MeOH/$CHCl_3$) afforded the title compound (126 mg, 0.242 mmol, 86%) as a white solid. H NMR (DMSO-d6, 500 MHz) δ 7.56 (d, J = 8.7 Hz, 1H), 7.52 (brs,

1H) , 7.44 (t, J = 8.3 Hz) , 7.26 - 7.36 (m, 7H) , 7.22 (d, J = 8.1 Hz, 2H)3.10 (dd, J = 9.4, 13 Hz, 1H), 3.37 (dd, J = 4.9, 13 Hz, 1H), 4.10 - 4.14 (m, 1H), 5.04 (dd, 1.6, 13 Hz, 2H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 170.9, 155.9, 153.3, 148.0 (m, 1C), 136.8, 130.4, 128.2, 127.5, 127.2, 126.2, 122.1 (m, 1C), 118.5, 117.5–107.6 (m, 3C), 65.7, 54.0, 33.4; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -105.3 and -105.4 (AB quartet, J = 249 Hz, 2F), -112.2 and -112.4 (AB quartet, J = 255 Hz, 2F), -128.6-(-128.7) (m, 2F). HRMS m/z $[M+H]^+$ calc'd for $C_{22}H_{18}F_6N_2O_4SH$ 521.0970; found 521.0952.

Benzyl (S)-(1-amino-3-(1-(3,3,4,4,5,5-hexafluoro-2-phenoxycyclopent-1-en-1-yl)-1H-imidazol-4-yl)-1-oxopropan-2- yl)carbamate (**2.10**)

A solution of **2.5** (100 mg, 0.350 mmol) in DMF (2.7 ml) was cooled to 0 °C and added Cbz-L-His-NH_2 (77 mg, 0.269 mmol) and Cs_2CO_3 (110 mg, 0.350 mmol). The resulting solution was allowed to stir for 1 hr at 0 °C. The reaction mixture was added water, and then extracted with EtOAc. The organic layer was washed with brine (5 times), dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation to give a crude oil. Purification by column chromatography (SiO_2, gradient 0-5% MeOH/$CHCl_3$) afforded the title compound (120 mg, 0.216 mmol, 81%) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.85 (s, 1H), 7.38–7.18 (m, 13H), 7.05 (brs, 1H), 4.98 (s, 2H), 4.16–4.14 (m, 1H), 2.82 (dd, J = 15.0, 4.2 Hz), 2.68 (dd, J = 15.0, 9.8 Hz); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.2, 155.8, 152.4, 140.2 (m, 1C), 140.0, 137.0, 136.9, 130.0, 128.3, 127.8, 127.8, 127.6, 126.6, 118.7, 116.5 (m, 1C), 115.3, 114.4-107.2 (m, 3C), 65.4, 54.0, 30.2. ^{19}F NMR (DMSO-d_6, 282 MHz) δ; -108.3, -111.4, -128.1. HRMS m/z [M + H] calc'd for $C_{25}H_{20}F_6N_4O_4H$ 555.1467; found 555.1492.

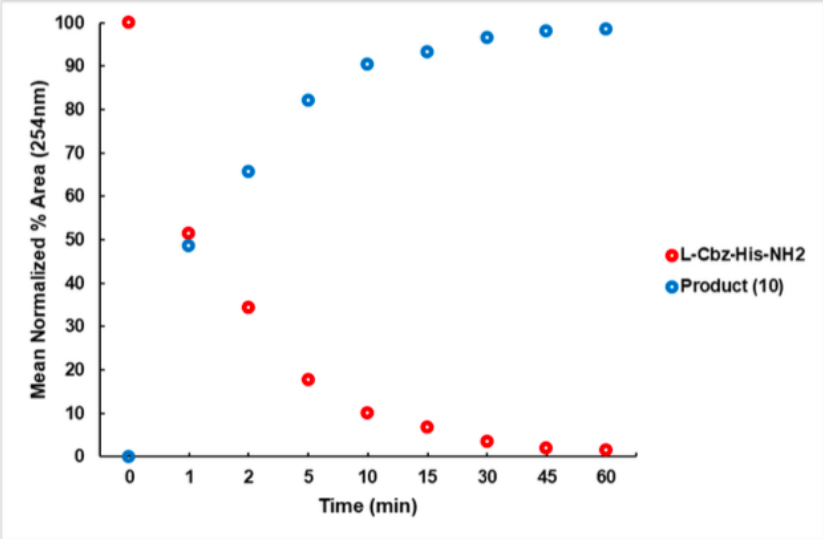
Benzyl ((8R)-2,3,3,4,4-pentafluoro-9-oxo-1-phenoxy-6-oxa-10-azaspiro[4.5]dec-1-en-8-yl)carbamate (**2.11 a**/**b**)

A solution of 5 (78 mg, 0.273 mmol) in DMF (2.1 ml) was cooled to 0 °C and added Cbz-L-Ser-NH_2 (50 mg, 0.21 mmol) and Cs_2CO_3 (89 mg, .273 mmol). The resulting solution was allowed to stir for 3 hr at 0 °C. The reaction mixture was quenched with AcOH (31 µl, 2.6 eq) and concentrated under reduced pressure to give a crude oil. Purification by column chromatography (SiO_2, gradient 10-20% MeCN/CH_2Cl_2) afforded the title compound as a white solid and as a mixture of diastereomers (1:1) and rotamers (50.2 mg, 0.104 mmol, 49%). The diastereomeric mixture was separated by preparative reverse-phase HPLC and identified by NMR. Cbz deprotection of 11a provided a single compound, confirming the presence the initial rotamer mixture. 11a: ^{1}H NMR (DMSO-d6, 500 MHz) δ 7.64 (brs, 1H), 7.50 – 7.22 (m, 9H), 7.18 (d, J = 7.5 Hz, 1.5H), 6.90 (d, J = 6.9 Hz, 0.5H), 5.30 – 5.10 (m, 2H), 4.64 – 4.50 (m, 2H), 4.1 – 4.02 (m, 1H); ^{13}C NMR (DMSO-d6, 126 MHz) δ 168.9 (major), 168.8 (minor), 153.5 (major), 153.2 (minor), 151.8 (major), 151.3 (minor), 138.5 - 137.9 (m, 1C), 135.9 (major), 135 (minor), 133.7 – 130.5 (m, 1C), 130, 128.5, 128.3, 127.9, 127.2, 126.4, 118.9, 118.5, 115.2 – 109.8 (m, 2C), 93.5 – 92.8 (m, 1C), 70.9 (major), 70.2 (minor), 68.2 (minor), 67.3 (major), 59.5 (minor), 58.9 (major). ^{19}F NMR (DMSO-d_6, 282 MHz) δ -106.4–(-108.5) (m, 1F), -116.4–(-120.2) (m, 2F), -127.0–(-128.8) (m, 1F), -158.7–(-159.9) (m, 1F); HRMS m/z $[M + Na]^+$ calc'd for $C_{22}H_{17}F_5N_2O_3Na$ 507.0955; found 507.0967. **1.11b**: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.44 (t, J = 7.23 Hz, 1.5H), 7.40 – 7.20 (m, 10H) 6.92 (d, J = 7.5, 0.5H), 5.31 – 5.03 (m, 2H), 4.65 – 4.56 (m, 1H), 4.45 – 4.31 (m, 2H). ^{13}C NMR (DMSO-d6, 126 MHz) δ 169.5 (major), 169.3 (minor), 154.1 (major), 153.5, 151.9 (major), 151.2 (minor), 138.4 – 137.8 (m, 1C), 135.8 (major), 134.8 (minor), 134.7 – 131.3 (m, 1C), 129.9, 129.8, 128.7, 128.6, 128.4, 128.3, 127.9, 127.3, 126.1, 126.1, 118.8, 118.6, 115.7

– 109.8 (m, 2C), 93.1 – 91.7 (m, 1C), 71.3 (major), 70.5 (minor), 68.2 (minor), 67.2 (major), 58.5 (minor), 58 (major); ^{19}F NMR (DMSO-d_6, 282 MHz) δ -109.0 (dd, J = 243.6, 17.7 Hz, 1F), -116.5–(-118.6) (m, 2F), -126.7 (dd, J = 230.3, 22.9 Hz, 1F), -157.3 (dd, J = 68.7, 13.3 Hz, 1F); HRMS m/z $[M + Na]^+$ calc'd for $C_{22}H_{17}F_5N_2O_3Na$ 507.0955, found 507.0956.

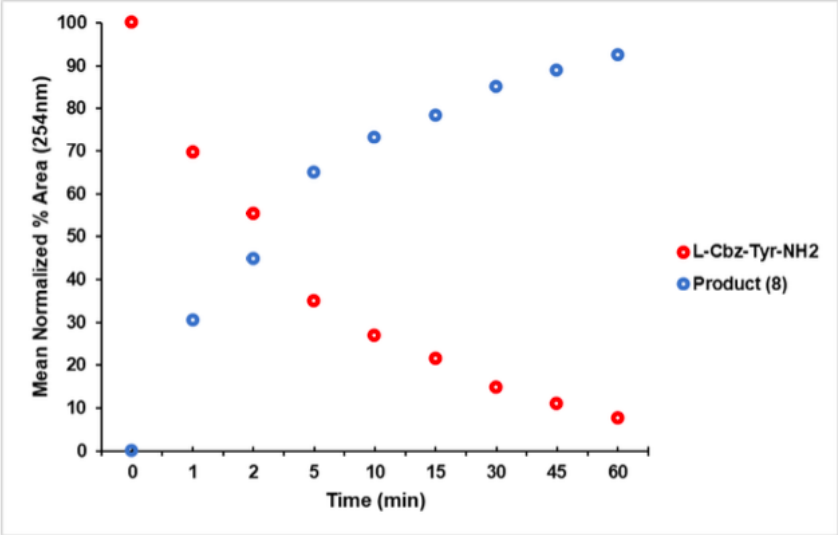
Rate determination for individual reactions of L-Cbz-His-NH2, L-Cbz-Tyr-NH2, L-Cbz-Ser-NH2, and L-Cbz-Cys-NH2 with mono phenoxy derivative 2.5

Amino acids (0.05 mmol) L-Cbz-Ser-NH2, L-Cbz-Tyr-NH2, L-Cbz-His-NH2, and L-Cbz-Cys-NH2 were individually subjected (as triplicates) to reactions with mono phenoxy derivative 5 (0.065 mmol, 1.3 eq) and Cs2CO3 (0.065 mmol, 1.3 eq) in DMF (0.1 M, 0.5 ml) at 0 °C. Aliquots (10 µl) were removed periodically, quenched in an HPLC vial containing 1M AcOH in MeCN, and analyzed by HPLC-UV. Time-course peak area data (254 nm) was used to construct the corresponding plots (below). L-Cbz-Cys-NH2 was observed to be fully consumed within 15 seconds.



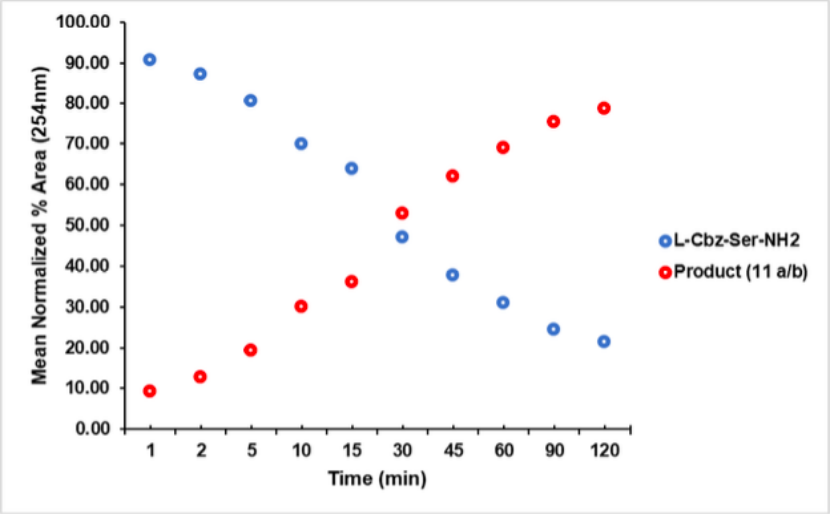
	Mean (n = 3) Normalized % Area (254 nm)	
Time (min)	L-Cbz-His-NH2	Product (**10**)
0	100	0
1	51.3	48.7
2	34.3	65.7
5	17.7	82.0
10	10.0	90.3
15	6.7	93.3
30	3.3	96.7
45	1.9	98.1
60	1.5	98.5

Fig. S1. Rate determination for the reaction of L-Cbz-His-NH2 with mono phenoxy derivative **2.5**.



	Mean (n = 3) Normalized % Area (254 nm)	
Time (min)	L-Cbz-Tyr-NH_2	Product (**8**)
0	99.99	0.01
1	69.5	30.5
2	55.2	44.8
5	35.0	65.0
10	26.9	73.1
15	21.6	78.4
30	14.9	85.1
45	11.1	89.0
60	7.6	92.4

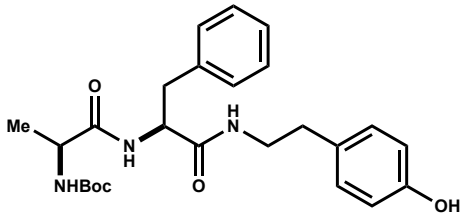
Fig. S2. Rate determination for the reaction of L-Cbz-Tyr-NH_2 with mono phenoxy derivative **2.5**.



	Mean (n = 3) Normalized % Area (254 nm)	
Time (min)	L-Cbz-Ser-NH_2	Product (**11 a/b**)
1	90.80	9.23
2	87.30	12.70
5	80.57	19.40
10	69.97	30.03
15	63.87	36.13
30	47.10	52.83
45	37.70	62.07
60	31.00	69.03
90	24.40	75.63
120	21.33	78.70

Fig. S3. Rate determination for the reaction of L-Cbz-Ser-NH_2 with mono phenoxy derivative **2.5**.

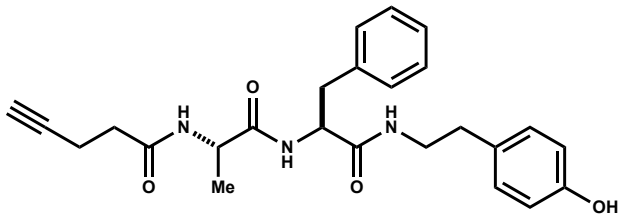
tert-Butyl ((S)-1-(((S)-1-((4-hydroxyphenethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**S2**)



Tyramine·HCl (1.70 g, 9.80 mmol), iPr2EtN (3.41ml, 19.6 mmol) and HBTU (3.72 g, 9.80 mmol) were added to a solution of Boc-L-Phe-OH (2.00 g, 7.54 mmol) in DMF (30 ml). The resulting solution was allowed to stir for 1 hr at rt. The reaction mixture was added H2O and extracted with EtOAc, washed with 1N HCl, sat. NaHCO3 and brine. The organic layer was dried over Na2SO4, filtered and the solvent was removed by rotary evaporation. The residue was dissolved in DCM (30 ml) and added TFA (15 ml). The resulting solution was allowed to stir for 1 hr at rt and concentrated by rotary evaporation to give a crude solid (3.11 g, 7.54 mmol analytical). The residue (1.72 g, analytical 4.16 mmol) was dissolved in DMF (40 ml) and treated with Boc-L-Ala-OH (1.02 g, 5.41 mmol), iPr2EtN (2.17 ml, 12.5 mmol) and then by HBTU (2.05 g, 5.41 mmol). After stirring 1 hr, added H2O and extracted with EtOAc. The combined organic phase was washed sequentially with 1N HCl, sat. NaHCO3 and brine. Dried over Na2SO4 and concentrated. Purification by column chromatography (SiO2, gradient 30-40% EtOAc/hexane) afforded the title compound (1.54 g, 3.37 mmol, 81% from Boc-L- Phe-OH) as a white foam. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.13 (s, 1H), 7.93 (t, J = 4.9 Hz, 1H), 7.73 (d, J = 8.4 Hz, 1H), 7.22-7.20 (m, 2H), 7.16-7.13 (m, 3H), 6.97-6.94 (m, 1H), 6.93 (d, J = 8.4, 2H), 6.63 (d, J = 8.4 Hz, 2H), 4.42-4.38 (m, 1H), 3.87-3.84 (m, 1H), 3.22-3.17 (m, 1H), 3.14-3.09 (m, 1H), 2.90 (dd, J = 13.6, 5.1 Hz 1H), 2.77 (dd, J = 13.6, 8.8 Hz, 1H), 2.53-2.48 (m, 2H), 1.35 (s, 9H), 1.04 (d, J = 7.2 Hz, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.3, 170.5, 155.6, 155.1, 137.6, 129.4, 129.3, 129.2, 128.0, 126.2,

115.1, 78.2, 53.6, 50.1, 40.6, 37.7, 34.2, 28.2, 18.0; HRMS m/z $[M + H]^+$ calc'd for $C_{25}H_{33}N_3O_5Na$ 478.2318; found 478.2343.

N-((S)-1-(((S)-1-((4-Hydroxyphenethyl)amino)-1-oxo-3-phenylpropan-2-yl)amino)-1-oxopropan-2-yl)pent-4-ynamide (**S3**)



TFA (10 ml) was added to a solution of S2 (1.75 g, 3.84 mmol) in DCM (20 ml) and stirred for 1hr at rt. The reaction mixture was concentrated by rotary evaporation to give a crude solid 12 (1.80 g, 3.84 mmol). The residue 12 (250 mg, 0.532 mmol, analytical) was dissolved in DMF (4 ml) and added 4-pentinoic acid (78 mg, 0.798 mmol), HOBt (108 mg, 0.798 mmol), iPr2EtN (277 µl, 1.60 mmol) and EDC·HCl (153 mg, 0.798 mmol). The mixture was stirred for 3 hrs and diluted with EtOAc. The organic phase was washed with 1N HClaq, sat NaHCO3 and brine, then dried over Na2SO4 and concentrated. The residue was triturated with Et2O, centrifuged, and the resulting solid was collected to give the title compound (220 mg, 0.505 mmol, 95%) as a pale yellow solid, used for next step without further purification. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 9.15 (s, 1H), 8.07 (d, J = 7.1 Hz, 1H), 7.88 (t, J = 5.6 Hz, 1H), 7.83 (d, J = 8.3 Hz, 1H), 7.26-7.21 (m, 2H), 7.20-7.14 (m, 3H), 6.96 (d, J = 8.4 Hz, 2H), 6.66 (d, J = 8.4 Hz, 2H), 4.43-4.36 (m, 1H), 4.25-4.16 (m, 1H), 3.26-3.10 (m, 2H), 2.95 (dd, J = 13.8, 5.2 Hz, 1H), 2.82-2.74 (m, 2H), 2.57-2.51 (m, 1H), 2.37-2.26 (m, 4H), 1.10 (d, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.9, 170.5, 170.4, 155.6, 137.8, 129.5, 129.4, 129.2, 128.0, 126.2, 115.1, 83.7 71.4, 53.8, 48.4,

40.6, 37.5 34.2, 33.9, 17.9, 14.0; HRMS m/z $[M + Na]^+$ calc'd for $C_{25}H_{29}N_3O_4Na$ 458.2056, found 458.2083.

N-((S)-1-(((S)-1-((4-((2-(But-3-yn-1-yloxy)-3,3,4,4,5,5-hexafluorocyclopent-1-en-1-yl)oxy)phenethyl)amino)-1-oxo-3- phenylpropan-2-yl)amino)-1-oxopropan-2-yl)pent-4-ynamide (**2.13**)

2.6 (95 mg, 0.361 mmol) and Cs_2CO_3 (110 mg, 0.361 mmol) were added to a solution of S3 (105 mg, 0.241 mmol) in DMF (2 ml). The resulting solution was allowed to stir for 3 hrs at rt. The reaction mixture was diluted with EtOAc, washed with brine, dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation to give a crude oil. Purification by column chromatography (SiO_2, EtOAc only) afforded the title compound (86 mg, 0.127 mmol, 53%) as a white solid. ^1H NMR (DMSO-d_6, 500 MHz) δ 8.07 (d, J = 7.0 Hz, 1H), 7.95 (t, J = 5.5 Hz, 1H), 7.85 (d, J = 8.3 Hz, 1H), 7.32-7.07 (m, 9H), 4.43 (m, 1H), 4.33 (t, J = 6.2 Hz, 1H), 4.20 (qn, J = 7.0 Hz, 1H), 2.95-2.89 (m, 2H), 2.80-2.74 (m, 2H), 2.69-2.64 (m, 2H), 2.54-2.51 (m, 2H), 2.36-2.27 (m, 4H), 1.10 (d, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.9, 170.6, 170.4, 153.7, 140.9-140.4 (m,1C), 137.8, 130.5-130.2 (m, 1C), 136.1, 130.4, 129.1, 128.0, 126.2, 116.5, 114.6-106.4 (m, 3C), 83.7, 79.8, 73.0, 71.3, 70.8, 53.8, 48.4, 40.1, 37.5, 34.0, 33.9, 19.1, 17.9, 14.0; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -110.2 (s, 2F), -114.1 (s, 2F), -129.1-(-129.2) (m, 2F). HRMS m/z $[M + H]^+$ calc'd for $C_{34}H_{33}F_6N_3O_5H$ 678.2403, found 678.2400.

Macrocyclic Product (**2.14**)

$Cu(OAc)_2 \cdot H_2O$ (6.7 mg, 0.0369 mmol), $NiCl_2$ (4.8 mg, 0.0369 mmol), Et_3N (15 µl, 0.111 mmol) and pyridine (15 µl, 0.185 mmol) were added to a solution of 13 (25 mg, 0.0369 mmol) in EtOH

(4 ml). The reaction mixture was vigorously stirred for 3 hrs under open air at 50 °C. After cooling to rt, diluted with EtOAc, washed with 1N HCl aq, sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation. Purification by column chromatography (SiO_2, gradient 80-100% EtOAc/Hex) afforded the title compound (13 mg, 0.0192 mmol, 52%) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.20 (d, J = 6.0 Hz, 1H), 7.81 (d, J = 8.4 Hz, 1H), 7.75 (t, J = 5.5 Hz, 1H), 7.31 (d, J = 8.6 Hz, 2H), 7.28-7.22 (m, 1H), 7.23 (d, J = 7.5 Hz, 2H), 7.19-7.15 (m, 1H), 7.12 (d, J = 7.2 Hz, 2H), 7.10 (d, J = 8.6 Hz, 1H), 4.31-4.25 (m, 1H), 4.23-4.16 (m, 2H), 4.02 (qn, J = 6.7 Hz, 1H), 3.39-3.27 (m, 2H), 2.95 (dd, J = 13.9, 4.7 Hz, 1H), 2.79-2.73 (m, 3H), 2.61-2.28 (m, 6H), 1.03 (d, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.9. 171.1, 170.5, 153.3, 138.9-138.5 (m, 1C), 138.7, 138.2, 136.5, 130.5, 129.2-129.0, 129.0, 128.0, 126.2, 116.7, 115.5-105.5 (m, 3C), 78.3, 72.9. 70.4, 66.7, 65.0, 54.2, 49.3, 36.8, 33.9, 32.8, 19.7, 17.1, 14.6; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -111.0 (d, J = 27.0 Hz, 2F), -112.3-(-112.4) (m, 2F), -128.7 (s, 2F). HRMS m/z $[M + H]^+$ calc'd for $C_{34}H_{31}F_6N_3O_5H$ 676.2246, found 676.2241.

Macrocyclic Product (**2.16**)

TFA (10 ml) was added to a solution of **S2** (1.75 g, 3.84 mmol) in DCM (20 ml) and stirred for 1hr at rt. The reaction mixture was concentrated by rotary evaporation to give a crude solid 12 (1.80 g, 3.84 mmol). The residue 12 (62 mg, 0.131 mmol, analytical) was dissolved in DMF (1.3 ml) and added 7 (43 mg, 0.144 mmol), cooled to 0 °C, and then added iPr_2EtN (34 ul, 0.197 mmol). The reaction mixture was stirred for 2 hrs at rt, then added H_2O, extracted with EtOAc, washed with 1N HCl, sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation to give crude 15. The residue 15 was dissolved

in DMF (13 ml) and added Cs_2CO_3 (64 mg, 0.196 mmol) at 0 °C, stirred for 1 hr at rt,. The reaction mixture was added H_2O, extracted with EtOAc. The combined extract was washed with brine (x5), dried over Na_2SO_4 and concentrated. Purification by column chromatography (SiO_2, gradient 1-5% MeOH/$CHCl_3$) afforded the title compound (38 mg, 0.0619 mmol, 47% from **S2**) as a white solid. ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.14 (d, J = 4.4 Hz, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.32 (t, J = 5.4 Hz, 1H), 7.26–7.24 (m, 2H), 7.18–7.14 (m, 5H), 7.02 (d, J = 8.6 Hz, 2H), 4.31–4.27 (m, 1H), 4.17–4.11 (t, J = 6.8 Hz, 2H), 3.73–3.71 (m, 1H), 3.60–3.57 (m, 1H), 3.19 (dd, J = 14.3, 4.0 Hz, 1H), 3.05-2.96 (m, 1H), 2.99 (dd, J = 14.3, 10.0 Hz, 1H), 2.80-2.75 (m, 1H), 2.68-2.63 (m, 1H), 2.02-1.89 (m, 2H), 1.43–1.28 (m, 2H), 1.00 (d, J = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.6, 172.3, 171.0, 153.2, 138.8-138.3 (m, 1C), 138.3, 136.7, 130.6, 129.6-129.1 (m, 1C), 128.8, 128.1, 126.2, 116.2, 115.6–105.7 (m, 3C), 72.9, 53.7, 50.5, 40.6, 35.8, 33.7, 30.5, 24.5, 16.9; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -111.8 (d, J = 54.6 Hz, 2F), -112.5 (d, J = 71.9 Hz, 2F), -128.6–(-128.8) (m, 2F); HRMS m/z $[M+H]^+$ calc'd for $C_{28}H_{30}F_6N_3O_5H$ 614.2084; found 614.2062.

tert-Butyl ((S)-1-(((S)-1-((4-hydroxybutyl)amino)-3-(naphthalen-2-yl)-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**2.17**)

K-Oxyma (2.58 g, 14.3 mmol), EDC·HCl (2.73 g, 14.3 mmol), iPr_2EtN (3.31ml, 19.0 mmol) and 4-amino-1-butanol (1.76 g, 19.0 mmol) were added to a solution of Boc-L-2-Nal-OH (3.00 g, 9.51 mmol) in DMF (60 ml). The resulting solution was allowed to stir for 2 hrs at rt. The reaction mixture was added H_2O and extracted with EtOAc, washed with 1N HCl, sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation. The residue was dissolved in DCM (20 ml) and added TFA (20 ml). The resulting

solution was allowed to stir for 1 hr at rt and concentrated by rotary evaporation to give a crude oil (8.78 g, 9.51 mmol analytical). The residue (6.51 g, analytical 7.05 mmol) was dissolved in DMF (50 ml) and treated with Boc-L-Ala- OH (1.73 g, 9.17 mmol), K-Oxyma (1.91 g, 10.6 mmol), iPr2EtN (2.45 ml, 14.1 mmol) and then by EDC·HCl (2.30 g, 12.0 mmol). After stirring 3 hrs, diluted with EtOAc, washed sequentially with 1N HCl, sat. $NaHCO_3$ and brine. Dried over Na_2SO_4 and concentrated. Purification by column chromatography (SiO_2, gradient 40-60% EtOAc/hexane) afforded the title compound (2.97 g, 6.49 mmol, 92% from Boc-L-2-Nal-OH) as a white solid. ^{1}H NMR (DMSO-d6, 500 MHz) δ 7.89-7.86 (m, 1H), 7.84-7.81 (m, 1H), 7.80-7.76 (m, 3H), 7.65 (s, 1H), 7.47-7.40 (m, 2H), 7.34 (d, J = 8.2 Hz, 1H), 6.98 (d, J = 7.1 Hz, 1H), 4.50 (q, J = 5.9 Hz, 1H), 4.32 (t, J = 5.1 Hz, 1H), 3.85 (t, J = 7.1, 1H), 3.31-3.26 (m, 2H), 3.11-2.93 (m, 4H), 1.38-1.23 (m, 4H), 1.33 (s, 9H), 1.03 (d, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.3, 170.3, 155.1, 135.3, 132.9, 131.8, 127.9, 127.5, 127.4, 127.4, 127.4, 125.9, 125.3, 78.2, 60.3, 53.6, 50.2, 38.5, 38.0, 29.7, 28.1, 25.6, 17.9. HRMS m/z $[M + H]^+$ calc'd for $C_{25}H_{35}N_3O_5H$ 458.2655, found 458.2661.

tert-Butyl ((S)-1-(((S)-3-(naphthalen-2-yl)-1-oxo-1-((4-((perfluorocyclopent-1-en-1-yl)oxy)butyl)amino)propan-2-yl)amino)-1- oxopropan-2-yl)carbamate (**2.18**)

A solution of **2.17** (569 mg, 1.24 mmol) in DMF (12 ml) was cooled to 0 °C and added octafluorocyclopentene (1M in MeCN, 3.73 ml, 3.73 mmol) and Et_3N (691 µl, 4.96 mmol). The resulting solution was allowed to stir for 3 hr at rt. The reaction mixture was diluted with EtOAc. The organic layer was washed with brine (5 times), dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation. Purification by column chromatography (SiO_2, gradient 50-80% EtOAc/hexane) afforded the title compound (420 g, 0.647 mmol, 52%) as a white solid. ^{1}H

NMR (DMSO-d_6, 500 MHz) δ 7.98–7.93 (m, 1H), 7.87–7.77 (m, 4H), 7.67 (s, 1H), 7.49–7.41 (m, 2H), 7.37 (d, J = 8.3 Hz, 1H), 7.00 (d, J = 7.0 Hz, 1H), 4.53 (q, J = 6.8 Hz, 1H), 4.40-4.30 (m, 2H), 3.92-3.86 (m, 1H), 3.17-3.08 (m, 2H), 3.06–2.92 (m, 2H), 1.43–1.15 (m, 4H), 1.35 (s, 9H), 1.07 (d, J = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.4, 170.4, 155.2, 135.7-135.2 (m, 1C), 135.2, 134.3-131.6 (m, 1C), 132.9, 131.8, 127.8, 127.5, 127.4, 127.4 (2C), 125.8, 125.3, 113.8-106.2 (m, 3C), 78.2, 73.6 (d, 4.1 Hz, 1C), 53.7, 50.1, 38.0, 37.8, 28.1 (3C), 25.9, 24.8, 17.9; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -112.5–(-112.9) (m, 2F), -114.6-(-115.0) (m, 2F), -128.1-(--128.5) (m, 2F), -161.0-(-161.5) (m, 1F). HRMS m/z $[M + H]^+$ calc'd for $C_{30}H_{34}F_7N_3O_5H$ 650.2465, found 650.2494.

(S)-2-Amino-3-(1H-imidazol-4-yl)-N-((S)-1-(((S)-3-(naphthalen-2-yl)-1-oxo-1-((4-((perfluorocyclopent-1-en-1-yl)oxy)butyl)amino)propan-2-yl)amino)-1-oxopropan-2-yl)propanamide (**2.19**)

TFA (1 ml) was added to a solution of **2.18** (324 g, 0.498 mmol) in DCM (3 ml) and stirred for 1hr at rt. The reaction mixture was concentrated by rotary evaporation to give a crude solid (331 mg, 0.498 mmol). The residue (55 mg, 0.0831 mmol, analytical) was dissolved in DMF (1 ml) and added Boc-L-His-OH (25 mg, 0.0997 mmol), K-Oxyma (16 mg, 0.0914 mmol), Et_3N (35 µl, 0.249 mmol) and EDC·HCl (24 mg, 0.125 mmol). The resulting solution was allowed to stir for 5 hrs at rt. The reaction mixture was cooled to 0 °C, added H_2O and then extracted with EtOAc. The organic layer was washed with brine (x5), dried over Na_2SO_4 and concentrated. The residue was dissolved in DCM (2 ml), added TFA (1 ml) and stirred for 1hr at rt. The reaction mixture was concentrated by rotary evaporation. Purification by preparative reverse-phase HPLC (45-75% MeCN/H_2O) to give the title compound (34 mg, 0.0372 mmol, 45% from **2.18**) as a white solid.

^{1}H NMR (DMSO-d$_6$, 500 MHz) δ 14.43 (brs, 1H), 8.91 (s, 1H), 8.68 (d, J = 2.3 Hz, 1H), 8.53-8.20 (m, 2H), 8.48 (d, J = 7.9 Hz, 1H), 8.03 (t, J = 5.2 Hz, 1H), 7.85-7.78 (m, 3H), 7.70 (s, 1H), 7.50-7.36 (m, 4H), 4.60-4.52 (m, 1H), 4.41-4.24 (m, 3H), 4.12 (t, J = 6.1Hz, 1H), 3.20–2.88 (m, 6H), 1.50–1.26 (m, 4H), 1.23 (d, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d$_6$, 126 MHz) δ 172.1, 170.2, 167.0, 158.4 (q, J = 31.6 Hz, 2CF_3CO_2H), 135.7–135.2 (m, 1C), 135.1, 134.5, 134.3–131.4 (m, 1C), 132.9, 131.8, 127.9, 127.6, 127.5, 127.4, 127.4, 125.9, 125.4, 120.7-106.1 (m, 3C and 2CF_3CO_2H), 73.6 (d, J = 4.1 Hz, 1C), 54.4, 51.2, 48.7, 38.1, 37.7, 26.5, 25.8, 24.8, 18.0; ^{19}F NMR (DMSO-d$_6$, 282 MHz) δ 73.3 (s, 6F), -112.7 (s, 2F), -114.9 (s, 2F), -128.3 (s, 2F). HRMS m/z [M + H]$^+$ calc'd for $C_{31}H_{33}F_7N_6O_4H$ 687.2530, found 687.2524.

Macrocyclic Product (**2.20**)

Cs_2CO_3 (14 mg, 0.0437 mmol) was added at 0 °C to a solution of 19 (10 mg, 0.0109 mmol) in DMF (1 ml) and stirred for 2 hrs at rt,. The reaction mixture was added H_2O at 0 °C, extracted with EtOAc. The combined extract was washed with brine (x5), dried over Na_2SO_4 and concentrated. Purification by column chromatography (SiO_2, gradient 5-15% MeOH/$CHCl_3$) afforded the title compound (3.8 mg, 0.00425 mmol, 39%) as a white solid. ^{1}H NMR (DMSO-d$_6$, 500 MHz) δ 8.64 (d, J = 6.5 Hz, 1H), 8.35 (d, J = 7.8 Hz, 8.19-8.12 (m, 3H), 8.07 (s, 1H), 7.87-7.76 (m, 4H), 7.67 (s, 1H), 7.44 (qn, J = 6.7 Hz, 2H), 7.40-7.36 (m, 2H), 4.57-4.51 (m, 1H), 4.33-4.16 (m, 3H), 4.14-4.09 (m, 1H), 3.37-3.22 (m, 2H), 3.19-3.13 (m, 1H), 3.08-3.02 (m, 1H), 2.94-2.88 (m, 1H), 1.75-1.67 (m, 2H), 1.55-1.48 (m, 2H), 1.11 (d, J = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d$_6$, 126 MHz) δ 171.6, 170.5, 167.7, 158.3 (q, J = 35.8 Hz, CF_3CO_2H), 143.8–143.0 (m, 1C), 138.2, 135.6, 135.6, 132.9, 131.8, 127.9, 127.5, 127.4, 127.3, 125.9, 125.4, 118.6-107.3 (m, 3C), 117.7, 115.8 (q, J = 292.8 Hz, CF_3CO_2H), 112.1-111.4 (m, 1C), 73.0, 54.4, 51.5, 49.2, 37.9, 36.8, 28.5,

25.9, 24.5, 17.6; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -74.2 (s, 6F), -106.7 and -107.6 (AB quartet, J = 244 Hz, 2F), -110.8 and -111.3 (AB quartet, J = 256 Hz, 2F), -127.5 (s, 2F). HRMS m/z $[M + H]^+$ calc'd for $C_{31}H_{32}F_6N_6O_4H$ 667.2468, found 667.2442.

Ac-CFAC-NH_2 (**2.21**)

Synthesized according to General Procedure A. ^{1}H NMR (DMSO-d6, 500 MHz) δ 8.19 (d, J = 7.0 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 8.00 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 8.0 Hz, 1H), 7.37 (s, 1H), 7.27-7.15 (m, 6H), 4.53-4.47 (m, 1H), 4.35-4.25 (m, 3H), 3.06 (dd, J = 14.0, 4.0 Hz), 2.84-2.66 (m, 4H), 2.61-2.54 (m, 1H), 2.24 (t, J = 8.5 Hz, 1H), 2.23 (t, J = 8.4 Hz, 1H), 1.83 (s, 3H), 1.24 (d, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.0, 171.3, 170.8, 169.9, 169.5, 137.7, 129.2, 128.0, 126.3, 55.0, 54.6, 53.9, 48.5, 37.1, 26.1. 26.1, 22.5, 17.8; HRMS m/z $[M + Na]^+$ calc'd for $C_{20}H_{29}N_5O_5S_2Na$ 506.1508, found 506.1528.

Macrocyclic Product (**2.22**)

To a solution of 21 (56 mg, 0.116 mmol) in DMF (12 ml) was added Et_3N (40 µl, 0.290 mmol) and octafluorocyclopentene (1M in MeCN, 174 µl, 0.174 mmol) over 3 minutes at 0 °C. The resulting solution was allowed to stir for 2 hr at 0 °C. The reaction mixture was diluted with EtOAc. The organic layer was washed with brine (5 times), dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation. Purification by preparative reverse-phase HPLC (55-90% MeCN/H_2O) afforded the title compound (39 mg, 0.0595 mmol, 51%) as a white powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.57 (d, J = 8.6 Hz, 1H), 8.42 (d, J = 8.8 Hz, 1H), 8.29 (d, J = 8.1 Hz, 1H), 7.69 (d, J = 7.2 Hz, 1H), 7.49 (s, 1H), 7.39 (s, 1H), 7.28–7.24 (m, 2H), 7.23–7.16 (m, 3H), 4.63–4.58 (m, 1H), 4.51–4.55 (m, 1H), 4.40-4.32 (m, 2H), 3.63 (dd, J = 13.9, 3.3 Hz, 1H), 3.36

(dd, J = 12.6, 8.4 Hz, 1H), 3.26 (dd, J = 12.6, 4.3 Hz, 1H), 3.02 (dd, J = 13.9, 10.7 Hz, 1H), 2.96 (dd, J = 13.9, 4.7 Hz, 1H), 2.90 (dd, J = 13.9, 10.0 Hz, 1H), 1.80 (s, 3H), 1.22 (d, J = 6.6 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 71.9, 170.6, 170.3, 169.0, 168.2, 138.2-137.7 (m, 1C), 137.3, 136.0-135.4 (m, 1C), 129.1, 128.2, 126.5, 118.6-108.2 (m, 3C), 55.7, 51.0, 51.0, 48.3, 37.2, 34.0, 31.8, 22.2, 18.7; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -100.0 and -100.9 (AB quartet, J = 68.4 Hz, 2F), -108.2 and -109.0 (AB quartet, 192.8 Hz, 2F), -127.9 (s, 2F). HRMS m/z $[M + H]^+$ calc'd for $C_{25}H_{27}F_6N_5O_5S_2H$ 656.1436 found 656.1415.

Ac-Y(2-NaI)AC-NH_2 (**2.23**)

Synthesized according to General Procedure A. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.13 (brs, 1H), 8.25 (d, J = 6.9 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 7.95 (d, J = 8.1, 1H), 7.93 (d, J = 8.3 Hz, 1H), 7.86-7.78 (m, 3H), 7.74 (s, 1H), 7.49-7.41 (m, 3H), 7.39 (s, 1H), 7.22 (s, 1H), 6.92 (d, J = 8.3 Hz, 2H), 6.58 (d, J = 8.3 Hz, 2H), 1.62 (td, J = 8.8, 4.3 Hz, 1H), 4.37-4.28 (m, 3H), 3.24 (dd, J = 13.9, 4.1 Hz, 1H), 2.99 (dd, J = 13.9, 9.4 Hz, 1H), 2.85-2.69 (m, 3H), 2.56-2.50 (m, 1H), 2.24 (t, J = 8.4 Hz, 1H), 1.67 (s, 3H), 1.26 (d, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.0, 171.5, 171.3, 170.8, 169.1, 155.7, 135.4. 133.0, 131.8, 129.9, 128.0, 127.6, 127.5, 127.4, 127.4, 125.9, 125.4, 114.8, 54.6, 54.3, 53.7, 48.6, 37.5, 36.5, 26.1, 22.4, 17.8. HRMS m/z $[M + H]^+$ calc'd for $C_{30}H_{35}N_5O_6SH$ 594.2386, found 594.2383.

Macrocyclic Product (**2.24**)

Synthesized according to General Procedure C. The reaction mixture was triturated by adding H_2O and MeOH instead of diluted with EtOAc, and isolated by centrifugation. The peptide pellet was repeated washed with EtOAc to give the title compound (24 mg, 0.0313 mmol, 58%) as a white

solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.52 (d, J = 8.5 Hz, 1H), 8.48 (d, J = 9.0 Hz, 1H), 8.08 (d, J = 8.4 Hz), 7.86 (d, J = 7.8 Hz, 1H), 7.81 (d, J = 7.8 Hz, 1H), 7.78 (d, J = 8.4 Hz, 1H), 7.63 (s, 1H), 7.57 (s, 1H), 7.54 (d, J = 7.7 Hz, 1H), 7.50- 7.43 (m, 2H), 7.33 (s, 1H), 7.29 (d, J = 8.4 Hz, 1H), 7.19 (d, J = 8.5 Hz, 2H), 7.11 (d, J = 8.5 Hz, 2H), 4.76-4.70 (m, 1H), 4.46 (q, J = 8.0 Hz, 1H), 4.38 (td, J = 8.9, 4.9 Hz, 1H), 4.28 (qn, J = 6.9 Hz, 1H), 3.21 (dd, J = 13.4, 7.6 Hz, 1H), 3.11-2.92 (m, 4H), 2.71 (dd, J = 13.9, 3.2 Hz, 1H), 1.61 (s, 3H), 1.12 (d, J = 6.8 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.1, 170.5, 170.1 169.5, 168.5, 151.5, 148.6–148.1 (m, 1C), 135.2, 134.8, 132.9, 131.9, 130.1, 127.8, 127.8, 127.6, 127.5, 127.4, 125.8, 125.4, 122.6–122.1 (m, 1C), 118.5, 116.8–107.4 (m, 3C), 54.9, 52.0, 51.8, 47.9, 38.1, 36.0, 32.7, 22.2, 19.1; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -103.6 (d, J = 249.7 Hz, 1F), -107.9 (d, J = 257.5 Hz, 1F), -109.2 (d, J = 249.7 Hz, 1F), -114.5 (d, J = 257.5 Hz, 1F), -128.2 and -129.6 (AB quartet, J = 235.4 Hz, 2F). HRMS m/z $[M + H]^+$ calc'd for $C_{35}H_{33}F_6N_5O_6SH$ 766.2134, found 766.2152.

Ac-YFAH-NH_2•TFA (**2.25**)

Synthesized according to General Procedure A. ^{1}H NMR (DMSO-d6, 500 MHz) δ 14.13 (br s, 2H), 9.16 (br s, 1H), 8.95 (s, 1H), 8.16 (d, J = 6.6 Hz, 1H), 8.09 (d, J = 8.2 Hz, 1H), 8.01 (s, J = 8.3 Hz, 1H), 7.99 (d, J = 8.3 Hz, 1H), 7.37-7.14 (m, 8H), 6.94 (d, J = 8.5 Hz, 2H), 6.60 (d, J = 8.5 Hz, 2H), 4.52-4.46 (m, 2H), 4.35-4.29 (m, 1H), 4.21 (qn, J = 6.9 Hz, 1H), 3.12 (dd, J = 15.3, 5.3 Hz, 1H), 3.04 (dd, J = 14.1, 4.1 Hz, 1H), 2.94 (dd, J = 15.3 Hz, 1H), 2.81-2.73 (m, 2H), 2.56-2.51 (m, 1H), 1.73 (s, 3H), 1.20 (d, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 500 MHz) δ 172.1, 171.6, 171.6, 171.1, 169.4, 158.0 (TFA), 155.7, 137.7, 133.7, 130.0, 129.4, 129.2, 128.0, 127.9, 126.3, 116.8, 114.8, 54.4, 53.7, 51.4, 48.7, 37.1, 36.4, 26.8, 22.5, 17.5. HRMS m/z $[M + H]^+$ calc'd for $C_{29}H_{35}N_7O_6H$ 578.2727, found 578.2745.

Macrocyclic Product (**2.26**)

Synthesized according to General Procedure C. Purification by preparative reverse-phase HPLC (50-85% MeCN/H2O) afforded the title compound (37 mg, 0.0494 mmol, 35%) as a white powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.64 (d, J = 6.9 Hz, 1H), 8.03 (d, J = 8.0 Hz, 1H), 7.96 (d, J = 5.3 Hz, 1H), 7.40 (s, 1H), 7.33 (s, 1H), 7.29–7.24 (m, 3H), 7.23–7.17 (m, 3H), 7.12 (s, 1H), 7.06 (s, 1H), 6.93 (d, J = 8.6 Hz, 2H), 6.74 (d, J = 8.6 Hz, 2H), 4.63–4.58 (m, 1H), 4.30–4.16 (m, 3H), 3.02–2.97 (m, 2H), 2.94–2.87 (m, 2H), 2.85–2.72 (m, 2H), 1.82 (s, 3H), 1.31 (d, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.1, 171.8, 171.2, 170.4, 168.9, 150.2 , 142.0–141.5 (m, 1C), 139.3, 137.9, 137.2, 134.5, 130.9, 129.0, 128.2, 126.5, 116.9, 115.2, 114.5-113.9 (m, 1C), 115.9-116.8 (m, 3C), 56.0, 52.3, 52.1, 48.7, 37.1, 36.7, 30.1, 22.3, 18.4; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -107.1 and -108.9 (AB quartet, J = 246.6 Hz, 2F), -114.1 and – 115.7 (AB quartet, J = 254.3 Hz, 2F), -128.4 (s, 2F). HRMS m/z $[M + H]^+$ calc'd for $C_{34}H_{33}F_6N_7O_6H$ 750.2475, found 750.2439.

Ac-SFAC-NH_2 (**2.27**)

Synthesized according to General Procedure A. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.16 (d, J = 6.9 Hz, 1H), 8.06 (d, J = 7.9 Hz, 1H), 7.92 (d, J = 7.6 Hz, 7.88 (d, J = 8.0 Hz, 1H), 7.35 (br s, 1H), 7.29-7.18 (m, 6H), 4.99 (br s, 1H), 4.54-4.48 (m, 1H), 4.36-4.24 (m, 3H), 3.49 (d, J = 5.9 Hz, 2H), 3.10 (dd, J = 14, 3.9 Hz, 1H), 2.87-2.80 (m, 2H), 2.78-2.71 (m, 1H), 2.27 (t, J = 8.4 Hz, 1H), 1.85 (s, 3H), 1.25 (d, J = 7.1 Hz, 3H). ^{13}C NMR (DMSO-d_6, 500 MHz) δ 172.0, 171.3, 170.9, 170.4, 169.5, 137.7, 129.2, 128.0, 126.2, 61.7, 55.1, 54.7, 53.9, 48.6, 36.9, 26.1, 22.5, 17.6; HRMS m/z $[M + Na]^+$ calc'd for $C_{20}H_{29}N_5O_6SNa$ 490.1736, found 490.1759.

Macrocyclic Product (**2.28**)

Synthesized according to General Procedure C. After work-up and concentration, the residue was triturated by with EtOAc, and isolated by centrifugation to give the title compound (41 mg, 0.0641 mmol, 56%) as a white solid. ^{1}H NMR (DMSO-d6, 500 MHz) δ 8.54 (d, J = 8.1 Hz, 1H), 8.33 (d, J = 9.1 Hz, 1H), 8.30 (d, J = 7.6 Hz, 1H), 7.71 (d, J = 7.6 Hz, 1H), 7.45 (s, 1H), 7.34 (s, 1H), 7.31-7.26 (m, 2H), 7.25-7.19 (m, 3H), 4.66-4.53 (m, 3H), 4.46-4.41 (m, 1H), 4.34-4.28 (m, 2H), 3.46 (dd, J = 13.9, 3.0 Hz, 1H), 3.00-2.90 (m, 3H), 1.80 (s, 3H), 1.19 (d, J = 6.8 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.1, 170.8, 170.7, 169.4, 168.4, 151.5–151.1 (m, 1C), 137.1, 129.0, 128.3, 126.6, 118.6-107.6 (m, 3C), 111.4-110.8 (m, 1C), 71.4, 56.2, 52.0, 51.4, 48.4, 36.9, 35.1, 22.2, 18.1; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -100.0 (d, J = 245.1 Hz, 1F), -107.2 (d, J = 245.1 Hz, 1H), -110.2 (d, J = 255.7 Hz, 1F), -115.7 (d, J = 255.7 Hz, 1F), -128.2 and -129.2 (AB quartet, J = 235.4 Hz, 2F). HRMS m/z $[M + H]^+$ calc'd for $C_{25}H_{27}F_6N_5O_6SH$ 640.1664, found 640.1668.

Ac-YWTC-NH_2 (**2.29**)

Synthesized according to General Procedure A. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.81 (d, J = 1.4 Hz, 1H), 9.13 (br s, 1H), 8.20 (d, J = 7.7 Hz, 1H), 7.98 (d, J = 8.3 Hz, 1H), 7.91 (d, J = 7.8 Hz, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.60 (d, J = 7.9 Hz, 1H), 7.35 (s, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.29 (br s, 1H), 7.17 (d, J = 1.8 Hz, 1H), 7.05 (t, J = 7.5 Hz, 1H), 7.00-6.94 (m, 3H), 6.60 (d, J = 8.4 Hz, 2H), 4.65- 4.60 (m, 1H), 4.42-4.34 (m, 2H), 4.30 (dd, J = 8.0, 3.9 Hz, 1H), 4.08-4.02 (m, 1H), 3.20 (dd, J = 15.0, 4.4 Hz, 1H), 3.00 (dd, J = 15.0, 9.3 Hz, 1H), 2.90-2.73 (m, 3H), 2.56 (dd, J = 14.0, 10.4 Hz, 1H), 2.32 (t, J = 8.5 Hz, 1H), 1.71 (s, 3H), 1.03 (d, J = 6.4 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.7, 171.7, 171.4, 169.7, 169.2, 155.7, 136.0, 130.0, 128.1, 127.3,

123.6, 120.8, 118.5, 118.2, 114.8, 111.2, 110.0, 66.5, 58.1, 54.7, 54.2, 53.4, 36.6, 27.2, 26.0, 22.4, 19.3. HRMS m/z $[M + H]^+$ calc'd for $C_{29}H_{36}N_6O_7S$ 613.2444, found 613.2441.

Macrocyclic Product (**2.30**)

A flask was charged with linear precursor 29 (24 mg, 0.0392 mmol) and DMF (6 ml). To the mixture octafluorocyclopentene (1M in MeCN, 196 µl, 0.196 mmol) and Et_3N (8 µl, 0.0588 mmol) were added at 0 °C. The mixture was stirred for 5 minutes and then excess amount of octafluorocyclopentene and MeCN were removed by rotary evaporation. The mixture was dropped to a suspension of Cs_2CO_3 (13 mg, 0.0392 mmol) in DMF (2 ml) at 0 °C over 3 min and stirred for 1 hr at 0 °C. The mixture was quenched with AcOH (7µl, 0.0588 mmol) and concentrated by rotary evaporation. Purification by preparative reverse-phase HPLC (55%-75% MeCN/H_2O) afforded the title compound (13 mg, 0.0166 mmol, 42%) as a white solid. ^1H NMR (DMSO-d_6, 500 MHz) δ 10.85 (d, J = 1.9 Hz, 1H), 8.57 (d, J = 9.2 Hz, 1H), 8.38 (d, J = 8.8 Hz, 1H), 8.25 (d, J = 8.4 Hz, 1H), 7.57 (s, 1H), 7.51 (d, J = 8.1 Hz, 1H), 7.46 (s, 1H), 7.45 (d, J = 8.0 Hz, 1H), 7.31 (d, J = 8.1 Hz, 1H), 7.20 (d, J = 8.6 Hz, 2H), 7.07 (d, J = 2.2 Hz, 1H), 7.06-7.02 (m, 3H), 6.96-6.92 (m, 1H), 5.51 (d, J = 4.7 Hz, 1H), 4.88-4.82 (m, 1H), 4.48-4.42 (m, 1H), 4.37-4.31 (m, 2H), 3.87-3.81 (m, 1H), 3.54 (dd, J = 14.0, 5.2 Hz, 1H), 3.08 (dd, J = 14.0, 9.6 Hz, 1H), 3.05-2.93 (m, 3H), 2.76 (dd,J = 14.4, 3.7 Hz, 1H), 1.83 (s, 3H), 0.81 (d, J = 6.4 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.1, 171.0, 169.4, 168.8, 168.3, 151.5, 148.8-148.2 (m, 1C), 136.1, 135.2, 130.0, 127.2, 124.3-123.7 (m, 1C), 120.8, 118.3, 118.1, 118.0, 115.8-109.6 (m, 3C), 111.2, 109.3, 79.2, 66.3, 56.8, 54.6, 52.2, 51.6, 35.9, 32.6, 28.4, 22.4, 17.9; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -101.7 (d, J = 249.5 Hz, 1F), -107.0 (d, J = 254.4 Hz, 1F), -110.1 (d, J = 248.3 Hz, 1F), -116.2 (d, J =

254.4 Hz, 1F), -128.4 and -129.5 (AB quartet, J = 235.3 Hz, 2F). HRMS m/z $[M + H]^+$ calc'd for $C_{34}H_{34}F_6N_6O_7SH$ 785.2192, found 785.2200.

Ac-YAC-NH_2 (**2.31**)

Synthesized according to General Procedure A. ^1H NMR (DMSO-d_6, 500 MHz) δ 9.15 (s, 1H), 8.26 (d, J = 6.9 Hz, 1H), 8.04 (d, J = 8.1 Hz, 1H), 7.83 (d, J = 8.0 Hz, 1H), 7.35 (s, 1H), 7.21 (s, 1H), 7.03 (d, J = 8.3 Hz, 2H), 6.63 (d, J = 8.3 Hz, 2H), 4.44-4.36 (m, 1H), 4.33-4.28 (m, 1H), 4.27-4.19 (qn, J = 6.9 Hz, 1H), 2.94-2.86 (m, 1H), 2.84-2.77 (m, 1H), 2.77-2.70 (m, 1H), 2.60 (dd, J = 13.6, 10.4 Hz, 1H), 2.24 (t, J = 8.4 Hz, 1H), 1.75 (s, 3H), 1.24 (d, J = 7.2 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.1, 171.7, 171.3, 169.3, 155.7, 130.1, 128.1, 114.8, 54.5, 54.3, 48.6, 36.6, 26.1, 22.5, 17.6. HRMS m/z $[M + Na]^+$ calc'd for $C_{17}H_{24}N_4O_5SNa$ 419.1365, found 419.1385

Macrocyclic Product (**2.32**)

Synthesized according to General Procedure C. Purification by trituration with DMF and $CHCl_3$ afforded the title compound (33 mg, 0.0580 mmol, 46%) as a white solid. ^1H NMR (DMSO-d_6, 500 MHz) δ 8.21 (d, J = 7.3 Hz, 1H), 7.95 (d, J = 8.2 Hz, 1H), 7.69 (d, J = 8.6 Hz, 1H), 7.43 (brs, 1H), 7.39-7.18 (brm, 2H), 7.31 (brs, 1H), 7.09-7.01 (m, 2H), 4.61-4.56 (m, 1H), 4.39 (td, J = 8.9, 3.5 Hz, 1H), 4.30 (qn, J = 7.1 Hz, 1H), 3.28 (dd, J = 12.0, 3.6 Hz, 1H), 3.10 (dd, J = 12.0, 9.5 Hz, 1H), 2.94-2.89 (m, 1H), 2.84-2.77 (m, 1H), 1.87 (s, 3H), 1.08 (d, J = 6.8 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.3, 170.2, 169.5, 169.2, 168.8, 151.1, 147.6–146.9 (m, 1C), 134.8, 130.7, 118.9-118.3 (m, 1C), 118.2, 116.8-108.9 (m, 3C), 54.0, 50.6, 47.5, 37.5, 34.3, 22.5, 18.3; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -102.8 and -103.9 (AB quartet, J = 245.0 Hz, 2F), -115.0 and -

115.5 (AB quartet, J = 192.0 Hz, 2F), -128.3 and -129.0 (AB quartet, J = 177.1 Hz, 2F). HRMS m/z $[M + Na]^+$ calc'd for $C_{22}H_{22}F_6N_4O_5SNa$ 591.1113, found 591.1141.

Ac-YNTQC-NH_2 (**2.33**)

Synthesized according to General Procedure A. ^1H NMR (DMSO-d_6, 500 MHz) δ 9.15 (brs, 1H), 8.42 (d, J = 7.4 Hz, 1H), 8.08 (d, J = 8.3 Hz, 1H), 8.04 (d, J = 7.6 Hz,1H), 7.79 (d, J = 8.2 Hz, 1H), 7.71 (d, J = 7.7 Hz, 1H), 7.50 (s, 1H), 7.28-7.19 (m, 3H), 7.04-6.99 (m, 1H), 7.03 (d, J = 8.4 Hz, 2H), 6.79 (brs, 1H), 6.62 (d, J = 8.4 Hz, 2H), 4.95 (brs, 1H), 4.60 (q, J = 6.9 Hz, 1H), 4.45-4.39 (m, 1H), 4.29- 4.24 (m, 1H), 4.24-4.17 (m, 1H), 4.13 (dd, J = 7.7, 3.5 Hz, 1H), 4.10-4.03 (m, 1H), 2.89-2.77 (m, 2H), 2.74-2.44 (m, 4H), 2.27 (t, J = 8.5 Hz, 1H), 2.19-2.09 (m, 2H), 2.01-1.93 (m, 1H), 1.87-1.78 (m, 1H), 1.75 (s, 3H), 1.06 (d, J = 6.4 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.9, 171.9, 171.9, 171.5, 171.4, 171.3, 170.3, 169.4, 155.7, 130.1, 128.0, 114.8, 66.3, 58.6, 55.0, 54.3, 52.8, 49.9, 36.8, 36.7, 31.6, 27.3, 26.0, 22.4, 19.7. HRMS m/z $[M + H]^+$ calc'd for $C_{27}H_{40}N_8O_{10}SH$ 669.2666, found 669.2653.

Macrocyclic Product (**2.34**)

A flask was charged with linear precursor 33 (40 mg, 0.0598 mmol) and DMF (6 mL). The mixture was added octafluorocyclopentene (1M in MeCN, 299 µl, 0.299 mmol) and Et_3N (12.5µl, 0.0897 mmol) at 0 °C. The mixture was stirred for 30 minutes and then excess amount of octafluorocyclopentene and MeCN were removed by rotary evaporation. The mixture was added Cs_2CO_3 (19 mg, 0.0598 mmol) at 0 °C and stirred for 1 hr at rt. The mixture was added AcOH (17 µl, 0.299 mmol) and concentrated by rotary evaporation. Purification by preparative reverse-phase HPLC (30%-70% MeCN/H_2O) afforded the title compound (25 mg, 0.0297 mmol, 50%) as

a whitesolid. ^{1}H NMR (DMSO-d6,500MHz) δ 8.56 (d, J = 5.3 Hz , 1H), 8.43 (d, J = 6.3 Hz, 1H), 7.75 (d, J = 7.0 Hz, 1H), 7.68 (d, J = 8.0 Hz, 1H), 7.52 (s, 1H), 7.44 (d, J = 6.6 Hz, 1H), 7.30 (brs, 1H), 7.28 (d, J = 8.6 Hz, 2H), 7.19 (brs, 1H), 7.11 (d, J = 8.6 Hz, 2H), 7.07 (brs, 2H), 6.77 (brs, 1H), 4.92 (brs, 1H), 4.39-4.33 (m, 2H), 4.27-4.21 (m, 1H), 4.05-3.97 (m, 2H), 3.93 (dd, J = 6.6, 5.2 Hz, 1H), 3.25 (dd, J = 12.7 4.3 Hz, 1H), 3.02-2.89 (m, 3H), 2.61-2.51 (m, 2H), 2.18-2.10 (m, 1H), 2.10-2.01 (m, 1H), 1.94 (s, 3H), 1.90-1.82 (m, 1H), 1.79–1.70 (m, 1H), 1.05 (d, J = 6.3 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.6, 171.9, 171.9, 171.7, 171.6, 170.7, 170.5, 170.3, 151.5, 148.5-147.9 (m, 1C), 135.0, 130.9, 119.2-118.6 (m, 1C), 118.9, 118.6-106.7 (m, 3C), 66.0, 60.3, 55.9, 53.5, 53.0, 51.3, 36.8, 35.7, 34.0, 31.4, 26.9, 22.5, 19.6; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -103.5 and -104.6 (AB quartet, J = 250.8 Hz, 2F), -113.7 and -114.8 (AB quartet, J = 252.8 Hz, 2F), -128.7 (brd, J = 5.2 Hz, 2F). HRMS m/z $[M + Na]^+$ calc'd for $C_{32}H_{38}F_6N_8O_{10}SNa$ 863.2233, found 863.2222.

Ac-YQY-NH_2 (**2.35**)

Synthesized according to General Procedure A. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.14 (brs, 2H), 8.22 (d, J = 7.5 Hz, 1H), 8.06 (d, J = 8.0 Hz, 1H), 7.73 (d, J = 8.1 Hz, 1H), 7.30 (s, 1H), 7.23 (s, 1H), 7.07 (s, 1H), 7.03 (d, J = 8.5 Hz, 2H), 6.99 (d, J = 8.5 Hz, 2H), 6.78 (s, 1H), 6.63 (d, J = 8.5 Hz, 2H), 6.62 (d, J = 8.5 Hz, 2H), 4.40-4.34 (m, 1H), 4.34-4.28 (m, 1H), 4.18-4.12 (m, 1H), 2.91-2.82 (m, 2H), 2.72 (dd, J = 8.3, 14.0 Hz, 1H), 2.60 (dd, J =10.5, 14.0 Hz, 1H), 2.12-1.99 (m, 2H), 1.88-1.80 (m, 1H), 1.76 (s, 3H), 1.77-1.67 (m, 1H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.9, 172.8, 171.9, 170.8, 169.5, 155.8, 155.7, 130.1, 130.0, 128.1, 127.7, 114.9, 114.8, 54.6, 54.0, 52.6, 36.7, 36.5, 31.4, 27.6, 22.5. HRMS m/z $[M + Na]^+$ calc'd for $C_{25}H_{31}N_5O_7Na$ 536.2121, found 536.2083.

Macrocyclic Product (**2.36**)

A flask was charged with linear precursor **3.35** (45 mg, 0.0870 mmol) and DMF (8.7 mL). The mixture was added Cs_2CO_3 (43 mg, 0.131 mmol) and octafluorocyclopentene (1M in MeCN, 131 µl, 0.0756 mmol) at rt. The mixture was stirred for 1 h. The mixture was added AcOH (25 µl, 0.435 mmol) and concentrated by rotary evaporation. Purification by preparative reverse-phase HPLC (45%-75% MeCN/H_2O) afforded the title compound (37 mg, 0.0539 mmol, 62%) as a white solid. H NMR (DMSO-d_6, 500 MHz) δ 8.25 (d, J = 7.7 Hz, 1H), 8.19 (d, J = 8.5 Hz, 1H), 7.70 (d, J = 7.2 Hz, 1H), 7.56 (s, 1H), 7.28 (s, 1H), 7.24 (d, J = 8.6 Hz, 2H), 7.17 (s, 1H), 6.96 (d, J = 8.6 Hz, 2H), 6.78 (s, 1H), 6.69 (d, J = 8.6 Hz, 2H), 6.45 (d, J = 8.6 Hz, 2H), 4.54 (td, J = 7.0, 3.3 Hz, 1H), 4.35 (td, J = 8.6, 3.5 Hz, 1H), 4.31-4.25 (m, 1H), 2.95 (dd, J = 13.6, 7.2 Hz, 1H), 2.87-2.80 (m, 3H), 2.16-2.05 (m, 2H), 2.00-1.91 (m, 1H), 1.95 (s, 3H), 1.80 (m, 1H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.9, 173.7, 173.0, 170.8, 169.2, 169.0, 152.5, 152.1, 135.3, 134.1-132.9 (m, 2C), 131.2, 131.0, 116.6-106.9 (m, 3C), 116.2, 116.0, 53.9, 52.8, 52.1, 36.6, 36.5, 31.4, 27.5, 22.7; ^{13}F NMR (DMSO-d_6, 376 MHz) δ -112.6 and - 113.4 (AB quartet, J = 249.6 Hz, 2F), -113.3 and -113.4 (AB quartet, J = 250.3 Hz, 2F), -128.5 (s, 2F). HRMS m/z $[M + Na]^+$ calc'd for $C_{30}H_{29}F_6N_5O_7Na$ 708.1869, found 708.1904.

Cyclic Product (**2.38**)

3.37[2] (100 mg, 329 mmol) was added to a 100-mL round bottom flask equipped with a stir bar, diluted with DMF (28 mL), and allowed to cool to 0 °C. Then was added Et_3N (69 uL, 494 mmol) followed by addition of octafluorocyclopentene (3M in MeCN, 110 uL, 494 mol). The mixture was then allowed to stir for 30 min at 0 °C Then the mixture was added water (10 mL), extracted

with EtOAc (3 x 10 mL). The organic layer was washed with brine (3 x 10 mL), dried over Na_2SO_4, filtered, and the volatiles removed under rotary evaporation to afford crude compound 38. Purification by preparative reverse-phase HPLC (40-80% MeCN/H_2O) afforded the title compound 38 (103 mg, 329 mmol, 71%) as a white-powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.24 (d, J = 4.8 Hz, 1H), 7.79 (d, J = 8.1 Hz, 1H), 7.55 (s, 1H), δ 7.22-7.17 (m, 4H), 7.15-7.14 (m, 2H), 4.51-4.47 (m, 2H), 3.32-3.29 (m, 1H), 2.98 (dd, J = 13.8, 4.6 Hz, 1H), 2.85 (dd, J = 13.8, 8.1 Hz, 1H), 2.79 (dd, J = 12.9, 3.1 Hz, 1H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.1, 168.2, 137.2, 137.0– 136.6 (m, 1C), 129.2, 128.0, 126.3, 118.5 (m, 1C), 116.7-116.4 (m, 1C), 115.0-114.6 (m, 1C), 113.1-112.7 (m, 1C), 110.9-110.6 (m 1C), 108.8-108.4 (m, 1C), 96.9 (m, 1C), 53.7, 52.8, 37.7, 24.7; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -100.6 (dd, J = 234.0, 12.2 Hz, 1F), - 101.7 (dd, J = 225.6, 28.2 Hz, 1F), -111.2 and -112.2 (AB quartet, J = 249.4 Hz, 2F), -125.4 and -128.0 (AB quartet J = 234.4, 2F). HRMS m/z [M + Na]+ calc'd for $C_{17}H_{15}F_6N_3O_2SNa$ 462.0687, found 462.0675.

H-C(2-NaI)AC-NH_2 (**2.39**)

Synthesized according to General Procedure A. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.68 (d, J = 7.9 Hz, 1H), 8.55 (d, J = 6.9 Hz, 1H), 8.23-8.02 (br m, 3H), 8.00 (d, J = 7.9 Hz, 1H), 7.89-7.80 (m, 4H), 7.54-7.41 (m, 4H), 7.23 (s, 1H), 4.77-4.70 (m, 1H), 4.37-4.30 (m, 2H), 3.97-3.91 (m, 1H), 3.33-3.26 (m, 1H), 3.07-2.93 (m, 2H), 2.91-2.78 (m, 2H), 2.76-2.69 (m, 1H), 2.23 (br s, 1H), 1.28 (d, J = 7.0 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.0, 171.3, 170.7, 166.9, 135.3, 133.0, 131.9, 127.7, 127.6, 127.6, 127.5, 127.4, 126.0, 25.5, 54.5, 54.3, 53.6, 48.6, 37.3, 26.2, 25.5, 17.9. HRMS m/z $[M + H]^+$ calc'd for $C_{22}H_{29}N_5O_4S_2H$ 492.1739, found 492.1787.

Cyclic Product (**2.40**)

To a solution of **2.39** (70 mg, 0.116 mmol) in DMF (10 ml) was added Et_3N (40 µl, 0.29 mmol) and octafluorocyclopentene (1M in MeCN, 173 µl, 0.173 mmol) over 3 minutes at 0 °C. The resulting solution was allowed to stir for 2 hr at 0 °C. The reaction mixture was diluted with EtOAc. The organic layer was washed with brine (5 times), dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation. Purification by preparative reverse-phase HPLC (65-80% MeCN/H_2O) afforded the title compound (35 mg, 0.0450 mmol, 39%) as a white powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.41 (d, J = 7.1 Hz, 1H), 8.14 (d, J = 4.6 Hz, 1H), 8.04 (d, J = 4.3 Hz, 1H), 8.02 (d, J = 4.1 Hz, 1H), 7.85–7.82 (m, 1H), 7.81–7.78 (m, 1H), 7.75 (d, J = 8.5 Hz, 1H), 7.71 (s, 1H), 7.48–7.42 (m, 3H), 7.38 (dd, J = 8.4, 1.4 Hz, 1H), 7.23 (s, 1H), 4.69 (dt, J = 8.6, 4.0 Hz, 1H), 4.44-4.40 (m, 1H), 4.40-4.30 (m, 2H), 3.26-3.20 (m, 2H), 2.99 (dd, J = 13.9, 9.1 Hz, 1H), 2.86–2.79 (m, 2H), 2.76–2.69 (m, 1H), 2.23 (t, J = 8.4 Hz, 1H), 1.27 (d, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.1, 171.3, 170.4, 168.4, 137.1-136.7 (m, 1C), 135.0, 133.0, 131.9, 127.8, 127.6, 127.4, 127.4, 125.9, 125.4, 118.7-108.5 (m, 3C), 96.6-96.1 (m, 1C), 54.6, 53.9, 52.4, 48.5, 37.9, 26.2, 25.0, 18.1; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -100.4 (dd, J = 240.4, 12.4 Hz, 1F), -101.7 (dd, J = 238.9, 15.1 Hz, 1F), -11.3 and -112.4 (AB quartet, J = 248.6 Hz, 2F), -125.4 and -128.1 (AB quartet, J = 235.8 Hz, 2F). HRMS m/z $[M + H]^+$ calc'd for $C_{27}H_{27}F_6N_5O_4S_2H$ 664.1487, found 664.1529.

Macrocyclic Product (**2.41**)

A flask was charged with linear precursor **2.22** (35 mg, 0.0724 mmol) and DMF (7 mL). The mixture was added octafluorocyclopentene (1M in MeCN, 109 µl, 0.109 mmol) and Et_3N (25 µl, 0.181 mmol) at 0 °C. The mixture was stirred for 30 minutes and then excess amount of

octafluorocyclopentene and MeCN were removed by rotary evaporation. The mixture was added PhOH (13.6 mg, 0.145 mmol) and Cs_2CO_3 (47 mg, 0.145 mmol) at 0 °C and stirred for 4 hr at rt. Then the mixture was added PhOH (13.6 mg, 0.145 mmol) and Cs_2CO_3 (47 mg, 0.145 mmol) and stirred for 2 hr at rt. The mixture was added AcOH (25 µl, 0.434 mmol) at 0 °C and concentrated by rotary evaporation. Purification by preparative reverse-phase HPLC (45%-80% MeCN/H_2O) afforded the title compound (11 mg, 0.0155 mmol, 21%) as an off-white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.46 (s, 1H), 8.53 (d, J = 8.6 Hz, 1H), 8.29 (d, J = 8.3 Hz, 1H), 8.03 (d, J = 8.8 Hz, 1H), 7.39 (t, J = 7.9 Hz, 2H), 7.29-7.12 (m, 8H), 7.04 (d, J = 9.4 Hz, 1H), 4.75 (dt, J = 9.4, 2.5 Hz, 1H), 4.58-4.51 (m, 1H), 4.49-4.41 (m, 1H), 4.29-4.20 (m, 1H), 3.62-3.53 (m, 1H), 2.98-2.88 (m, 2H), 2.88-2.75 (m, 3H), 1.72 (s, 13 3H), 1.20 (d, J = 7.1 Hz, 3H). C NMR (DMSO-d_6, 126 MHz) δ 171.3, 171.0, 169.2, 168.6, 168.1, 153.5, 142.7-142.0 (m, 1C), 136.9, 133.7-133.4 (m, 1C), 129.9, 129.1, 128.2, 126.5, 124.5, 118.8-113.7 (m, 2C), 116.4, 68.6-67.9 (m, 1C), 55.4, 51.7, 48.2, 46.4, 37.3, 30.7, 30.5, 22.3, 17.1; ^{19}F NMR (DMSO-d_6, 376 MHz) δ -103.0 (dd, J = 252.2, 13.8 Hz, 1F), -114.0 (dd, J = 223.6, 12.6 Hz, 1F), -119.6 (dd, J = 253.5, 11.9 Hz, 1F), -127.5 (dd, J = 223.0, 11.9 Hz, 1F). HRMS m/z $[M + Na]^+$ calc'd for $C_{31}H_{31}F_4N_5O_6S_2Na$ 732.1550, found 732.1545.

Ac-YACFAC-NH_2 (**2.42**)

Synthesized according to General Procedure A. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.14 (brs, 1H), 8.23 (d, J = 7.0 Hz, 1H), 8.19 (d, J= 7.1 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 8.01 (d, J = 8.3 Hz, 1H), 7.92 (d, J = 8.1 Hz, 1H), 7.89 (d, J = 8.1 Hz, 1H), 7.38 (brs, 1H), 7.27- 7.15 (m, 6H), 7.02 (d, J = 8.4 Hz, 2H), 6.63 (d, J = 8.4 Hz, 2H), 4.55-4.49 (m, 1H), 4.42-4.21 (m, 5H), 3.06 (dd, J = 14.1, 4.0 Hz, 1H), 2.89-2.64 (m, 6H), 2.58 (dd, J = 14.0 Hz, 1H), 2.25 (dd, J = 9.2, 8.0 Hz, 1H), 2.23 (dd, J

= 8.8, 8.0 Hz, 1H), 1.74 (s, 3H), 1.24 (d, J = 7.1 Hz, 3H), 1.18 (d, J = 7.1 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.1, 172.0, 171.5, 171.3, 170.8, 169.5, 169.2, 155.7, 137.6, 130.1, 129.2, 128.1, 128.1, 126.3, 114.8, 54.8, 54.6, 54.2, 53.9, 48.6, 48.2, 37.2, 36.7, 26.3, 26.1, 22.5, 17.8; HRMS m/z $[M + Na]^+$ calc'd for $C_{32}H_{43}N_7O_8S_2Na$ 740.2512, found 740.2492.

Macrocyclic Product (**2.43**)

A flask was charged with linear precursor **2.42** (36 mg, 0.0504 mmol) and DMF (10 mL). The mixture was added octafluorocyclopentene (1M in MeCN, 76 µl, 0.0756 mmol) and Et_3N (18 µl, 0.126 mmol) at 0 °C under argon. The mixture was stirred for 5 minutes and then excess amount of octafluorocyclopentene and MeCN were removed by rotary evaporation. The mixture was added Cs_2CO_3 (66 mg, 0.202 mmol) at 0 °C and stirred for 4 hr at rt. The mixture was added AcOH (17 µl, 0.302 mmol) and concentrated by rotary evaporation. Purification by preparative reverse-phase HPLC (40%-60% MeCN/H_2O) afforded the title compound (12 mg, 0.0141 mmol, 28%) as an off-white solid. Major isomer: ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.48 (s, 1H), 8.53 (d, J = 8.1 Hz, 1H), 8.37 (d, J = 8.0 Hz, 1H), 8.08 (d, J = 7.8 Hz, 1H), 8.06 (d, J = 7.4 Hz, 1H), 7.52 (d, J = 8.5 Hz, 1H), 7.30 (d, J = 8.9 Hz, 1H), 7.28-7.12 (m, 6H), 7.09-6.87 (m, 3H), 4.70 (dt, J = 8.9, 2.7 Hz, 1H), 4.68-4.61 (m, 1H), 4.54-4.47 (m, 2H), 4.23 (dt, J = 8.1, 7.0 Hz, 1H), 4.06-3.99 (m, 1H), 3.54 (dd, J = 14.6, 2.6 Hz, 1H), 3.39-3.33 (m, 1H), 2.99 (dd, J = 13.3, 5.5 Hz, 1H), 2.94-2.85 (m, 3H), 13 2.83 (dd, J = 14.6, 2.1 Hz, 1H), 2.61 (dd, J = 13.1, 10.9 Hz, 1H), 1.86 (s, 3H), 1.18 (d, J = 7.2 Hz, 3H), 0.96 (d, J = 6.8 Hz,3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.5, 171.4, 171.1, 168.8, 168.7, 168.1, 167.6, 151.9, 140.3-139.8 (m, 1C), 136.8, 134.6-134.3 (m, 1C), 132.3, 130.4, 129.0, 128.3, 126.6, 116.6-113.8 (m, 2C), 114.8, 69.0-68.2 (m, 1C), 54.8, 53.5, 50.5, 49.7, 46.7, 46.7, 37.7, 37.3 33.5, 30.3, 22.5, 19.5, 16.8. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -103.5

(dd, J = 250.4, 13.1 Hz, 1F), -114.3 (dd, J = 223.0, 12.7 Hz, 1F), 119.5 (dd, J = 250.4, 13.6 Hz, 1F), -127.0 (d, J = 223.0 Hz, 1F). HRMS m/z $[M + Na]^+$ calc'd for $C_{37}H_{39}F_4N_7O_8S_2Na$ 872.2136, found 872.2136.

Ac-YNCTFC-NH_2 (**2.44**)

Synthesized according to General Procedure A. ^1H NMR (DMSO-d_6, 500 MHz) δ 9.15 (brs, 1H), 8.41 (d, J = 7.6 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 8.04 (d, J = 8.2 Hz, 1H), 7.96 (d, J = 7.8 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 7.86 (d, J = 8.1 Hz, 1H), 7.47 (brs, 1H), 7.26- 7.14 (m, 7H), 7.03 (d, J = 8.5 Hz, 2H), 7.00 (brs, 1H), 6.63 (d, J = 8.5 Hz, 2H), 4.57-4.50 (m, 2H), 4.49-4.44 (m, 1H), 4.41-4.36 (m, 1H), 4.31-4.26 (m, 1H), 4.14 (dd, J = 8.0, 4.8 Hz, 1H), 3.95-3.90 (m, 1H), 3.07 (dd, J = 14, 5.1 Hz, 1H), 2.90-2.82 (m, 2H), 2.81-2.66 (m, 4H), 2.63-2.55 (m, 2H), 2.50-2.43 (m, 1H), 2.29 (t, J = 8.6 Hz, 1H), 2.22 (t, J = 8.5 Hz, 1H), 1.75 (s, 3H), 0.97 (d, J = 6.4 Hz, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.8, 171.8, 171.3, 171.0, 170.7, 169.8, 169.7, 169.5, 155.7, 137.5, 130.0, 129.2, 128.1, 128.0, 126.3, 114.8, 66.5, 58.7, 55.0, 55.0, 54.5, 54.0, 49.9, 37.0, 36.7, 36.6, 26.1, 26.0, 22.5, 19.5. HRMS m/z $[M + H]^+$ calc'd for $C_{34}H_{46}N_8O_{10}S_2H$ 791.2856, found 791.2849.

Intermediate **2.45** and **2.46**

A flask was charged with linear precursor **2.44** (100 mg, 0.126 mmol) and DMF (25 mL). The mixture was added octafluorocyclopentene (1M in MeCN, 190 µl, 0.190 mmol) and Et_3N (44 µl, 0.315 mmol) at 0 °C under argon. The mixture was stirred for 5 minutes and then excess amount of octafluorocyclopentene and MeCN were removed by rotary evaporation. The mixture was added Cs_2CO_3 (328 mg, 1.01 mmol) at rt and stirred for 2 hr. The mixture was added AcOH (108

μl, 1.89 mmol) and concentrated by rotary evaporation. Purification by preparative reverse-phase HPLC (36%-57% MeCN/H_2O) afforded **2.45** (9 mg, 0.00935 mmol, 7.4%) as an off-white solid., **2.46** (18 mg, 0.0191 mmol, 15%) as an off-white solid and **2.47** (23 mg, 0.0249 mmol, 20%) as an off-white solid.

2.45: ^{1}H NMR (DMSO-d6, 500 MHz) δ 9.11 (s, 1H), 8.70 (d, J = 7.8 Hz, 1H), 8.47 (d, J = 7.6 Hz, 1H), 8.43 (d, J = 9.2 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H), 7.89 (d, J = 7.2 Hz, 1H), 7.38 (s, 1H), 7.31 (s, 1H), 7.28 (d, J = 7.3 Hz, 1H), 7.25-7.08 (m, 5H), 6.97 (d, J = 8.3 Hz, 2H), 6.91 (s, 1H), 6.84 (s, 1H), 6.59 (d, J = 8.3 Hz, 2H), 5.07 (brs, 1H), 4.85-4.80 (m, 1H), 4.61-4.55 (m, 2H), 4.42-4.36 (m, 1H), 4.36-4.30 (m, 1H), 4.05–3.99 (m, 2H), 3.58–3.42 (m, 3H), 2.94 (d, J = 6.1 Hz, 2H), 2.87 (dd, J = 14.3, 11.5 Hz, 1H), 2.79 (dd, J = 14.0, 2.8 Hz, 1H), 2.60 (dd, J = 15.7, 6.6 Hz, 1H), 2.55-2.51 (m, 1H), 2.35 (dd, J = 15.7, 6.9 Hz, 1H), 1.70 (s, 3H), 0.98 (d, J = 5.8 Hz, 3H). ^{13}C NMR (DMSO-d6, 126 MHz) δ 172.1, 171.3, 170.7, 170.4, 170.2, 169.6, 169.1, 168.4, 155.6, 140.5-139.9 (m, 1C), 136.8, 135.5-134.8 (m, 1C), 129.9, 129.5, 128.2, 128.1, 126.4, 114.7, 65.8, 60.5, 54.0, 53.9, 51.0, 50.4, 49.3, 38.5, 36.9, 36.3, 34.2, 32.9, 22.5, 22.4, 20.6; ^{19}F NMR (DMSO-d6, 376 MHz) δ -98.4 and -98.9 (AB quartet, J = 180.2 Hz, 2F), -110.4 and -111.0 (AB quartet, J = 177.4 Hz, 2F), -127.3 and -128.8 (AB quartet, J = 234.6 Hz, 2F). HRMS m/z $[M + Na]^+$ calc'd for $C_{39}H_{44}F_6N_8O_{10}S_2Na$ 985.2424, found 985.2465.

2.46: ^{1}H NMR (DMSO-d6, 400 MHz) δ 9.43 (s, 1H), 9.16 (brs, 1H), 8.36 (d, J = 8.2 Hz, 1H), 8.31 (d, J = 7.7 Hz, 1H), 8.27 (d, J = 9.0 Hz, 1H), 8.04 (d, J = 8.0 Hz, 1H), 7.93 (d, J = 7.5 Hz, 1H), 7.49 (brs, 1H), 7.28-7.17 (m, 7H), 7.03 (brs, 1H), 7.02 (d, J = 8.5 Hz, 2H), 6.63 (d, J = 8.5 Hz, 2H), 4.98 (brs, 1H), 4.89-4.83 (m, 1H), 4.78-4.72 (m, 1H), 4.57 (td, J = 9.7, 4.0 Hz, 1H), 4.49 (q, J = 7.0 Hz, 1H), 4.41- 4.33 (m, 1H), 3.84 (t, J = 6.9 Hz, 1H), 3.58-3.48 (m, 3H), 3.28 (dd, J = 13.9,

4.0 Hz, 1H), 2.98 (dd, J = 13.5, 6.0 Hz, 1H), 2.89-2.79 (m, 2H), 2.75 (dd, J = 13.9, 10.6 Hz, 1H), 2.64-2.54 (m, 2H), 2.45 (dd, J = 15.4, 6.5 Hz, 1H), 1.75 (s, 3H), 0.63 (d, J = 6.2 Hz, 3H); ^{13}C NMR (DMSO-d$_6$, 126 MHz) δ 171.8, 171.7, 171.1, 170.5, 170.2, 169.5, 169.1, 167.9, 155.7, 148.8-146.1 (m, 1C), 138.0, 130.0, 129.2, 128.2, 128.1, 126.3, 118.6-108.2 (m, 3C), 114.8, 68.7-68.2 (m, 1C), 65.9, 61.7, 54.4, 53.9, 52.5, 49.7, 46.6, 37.3, 36.9, 36.5, 31.4, 30.5, 22.4, 20.1; ^{19}F NMR (DMSO-d$_6$, 376 MHz) δ -109.0 (dt, J = 250.9, 14.1 Hz, 1F), -113.8 (dd, J = 221.8, 10.0 Hz, 1F), -118.6 (dt, J = 250.9, 12.1 Hz, 1F), -123.6 (dd, J = 221.8, 11.3 Hz, 1F), -135.3 (t, J = 14.1 Hz, 1F). HRMS m/z $[M + Na]^+$ calc'd for $C_{39}H_{43}F_5N_8O_{10}S_2Na$ 965.2361, found 965.2401.

Macrocyclic Product (**2.47**)

A flask was charged with linear precursor **2.44** (60 mg, 0.0759 mmol) and DMF (15 mL). The mixture was added octafluorocyclopentene (1M in MeCN, 114 µl, 0.114 mmol) and Et_3N (26 µl, 0.190 mmol) at 0 °C under argon. The mixture was stirred for 5 minutes and then excess amount of octafluorocyclopentene and MeCN were removed by rotary evaporation. The mixture was added Cs_2CO_3 (99mg, 0.304 mmol) at 0 °C and stirred for 2 hr at rt. The mixture was added Cs_2CO_3 (99 mg, 0.304 mmol) and stirred for 4 hr at rt. The mixture was added AcOH (65 µl, 1.14 mmol) and concentrated by rotary evaporation. Purification by preparative reverse-phase HPLC (40%-55% MeCN/H_2O) afforded the title compound (29 mg, 0.0311 mmol, 41%) as an off-white solid. ^{1}H NMR (DMSO-d$_6$, 500 MHz) δ 9.51 (s, 1H), 8.33 (d, J = 9.5 Hz, 1H), 8.12 (d, J = 6.9 Hz, 1H), 8.05 (d, J = 8.2 Hz, 1H), 7.98 (d, J = 6.8 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.42 (s, 1H), 7.36 (d, J = 8.6 Hz, 1H), 7.30-7.17 (m, 6H), 7.06-6.84 (m, 3H), 7.03 (s, 1H), 4.98 (brs, 1H), 4.84-4.79 (m, 1H), 4.72 (dd, J = 14.2, 6.8 Hz, 1H), 4.60-4.55 (m, 1H), 4.55-4.48 (m, 1H), 4.41-1.34 (m, 1H), 3.74 (t, J = 6.9 Hz, 1H), 3.61 (d, J = 14.0 Hz, 1H), 3.48-3.41 (m, 2H), 3.22 (dd, J = 14.0, 3.7 Hz,

1H), 2.97 (dd, J = 13.2, 5.1 Hz, 1H), 2.89-2.82 (m, 3H), 2.70 (dd, J = 13.2, 10.1 Hz, 1H); 2.38 (dd, J = 15.6, 8.5 Hz, 1H), 2.32 (dd, J = 15.6, 5.0 Hz, 1H), 1.87 (s, 3H), 0.51 (d, J = 6.0 Hz, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.7, 170.8, 170.3, 169.5, 169.1, 168.8, 168.5, 168.0, 152.4, 139.2-138.8 (m, 1C), 137.8, 135.2-134. 9 (m, 1C), 132.1, 130.5, 129.1, 128.2, 126.4, 118.7-111.5 (m, 2C), 114.9, 68.6-68.1 (m, 1C), 65.9, 62.1, 54.8, 53.6, 50.4, 48.4, 46.5, 38.4, 37.3, 37.2, 32.7, 30.6, 22.4, 20.0; ^{19}F NMR (DMSO-d6, 282 MHz) δ -103.2 and -119.2 (AB quartet, J = 252.2 Hz, 2F), -113.8 and -127.0 (AB quartet, J = 223.0 Hz, 2F). HRMS m/z $[M + Na]^+$ calc'd for $C_{39}H_{42}F_4N_8O_{10}S_2Na$ 945.2299, found 945.2327.

Ac-SFC-NH_2 (**S4**)

Synthesized according to General Procedure A. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (d, J = 7.8 Hz, 1H), 7.98 (d, J = 7.7 Hz, 1H), 7.96 (d, J = 6.6 Hz, 1H), 7.26-7.21 (m, 5H), 7.20-7.16 (m, 2H), 4.48 (ddd, J = 9.2, 8.0, 4.6 Hz, 1H), 4.29 (td, J = 8.0, 4.9 Hz, 1H), 4.25 (dd, J = 7.4, 6.3 Hz, 1H), 3.48 (d, J = 6.3 Hz, 2H), 3.09 (dd, J = 14.0, 4.6 Hz, 1H), 2.87 (dd, J = 13.5, 8.9 Hz, 1H), 2.80 (ddd, J = 13.6, 9.3, 5.0 Hz, 1H), 2.70 (dt, J = 13.6, 7.7 Hz, 1H), 2.27 (dd, J = 9.3, 7.7 Hz, 1H), 1.83 (s, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.4, 170.8, 170.5, 169.6, 137.8, 129.2, 128.1, 126.3, 61.6, 55.2, 55.1, 54.3, 36.7, 26.0, 22.5. HRMS m/z $[M + Na]^+$ calc'd for $C_{17}H_{24}N_4O_5SNa$ 419.1365, found 419.1362.

Macrocyclic Product (**2.48**)

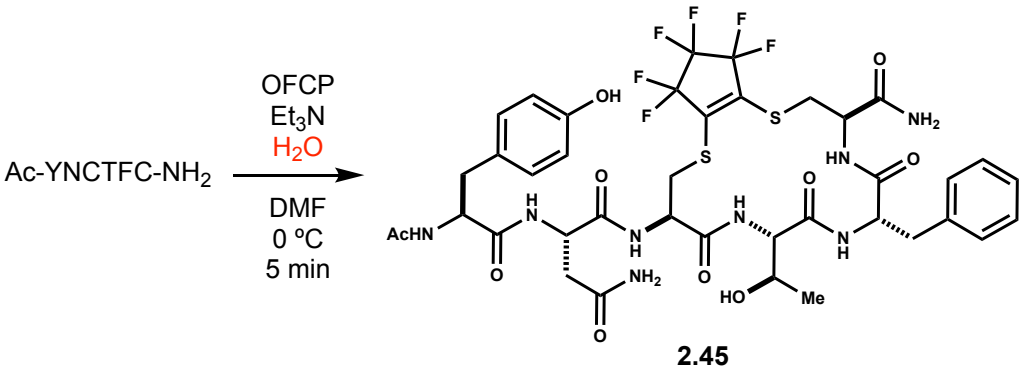
Synthesized according to General Procedure C. Purification by preparative reverse-phase HPLC (45%-65% MeCN/H_2O) afforded the title compound (24 mg, 0.0422 mmol, 28%) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.77 (d, J = 9.0 Hz, 1H), 8.23 (d, J = 7.7 Hz, 1H), 8.04 (d, J =

8.9 Hz, 1H), 7.31 (brs, 1H), 7.29–7.17 (m, 5H), 6.98 (brs, 1H), 4.76–4.67 (m, 2H), 4.65–4.59 (m, 1H), 4.43–4.33 (m, 2H), 3.32 (dd, J = 14.0, 4.3 Hz, 1H), 3.13 (dd, J = 14.0, 5.4 Hz, 1H), 2.98 (dd, J = 14.0, 9.5 Hz, 1H), 2.82 (d, J = 14.0, 9.8 Hz, 1H), 1.86 (s, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.3, 171.1, 169.5, 168.1, 154.9–154.2 (m, 1C), 137.9, 129.1, 128.2, 126.4, 117.7–108.4 (m, 4C), 72.7, 54.1, 51.4, 50.4, 36.1, 35.5, 22.3; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -102.7 and -108.2 (AB quartet, J = 244.0 Hz, 2F), -107.5 and -113.6 (AB quartet, J = 261.0 Hz, 2F), -127.4 and -128.1 (AB quartet, J = 200.0 Hz, 2F). HRMS m/z $[M + Na]^+$ calc'd for $C_{22}H_{22}F_6N_4O_5SNa$ 591.1113, found 591.1077.

Compound **2.49**

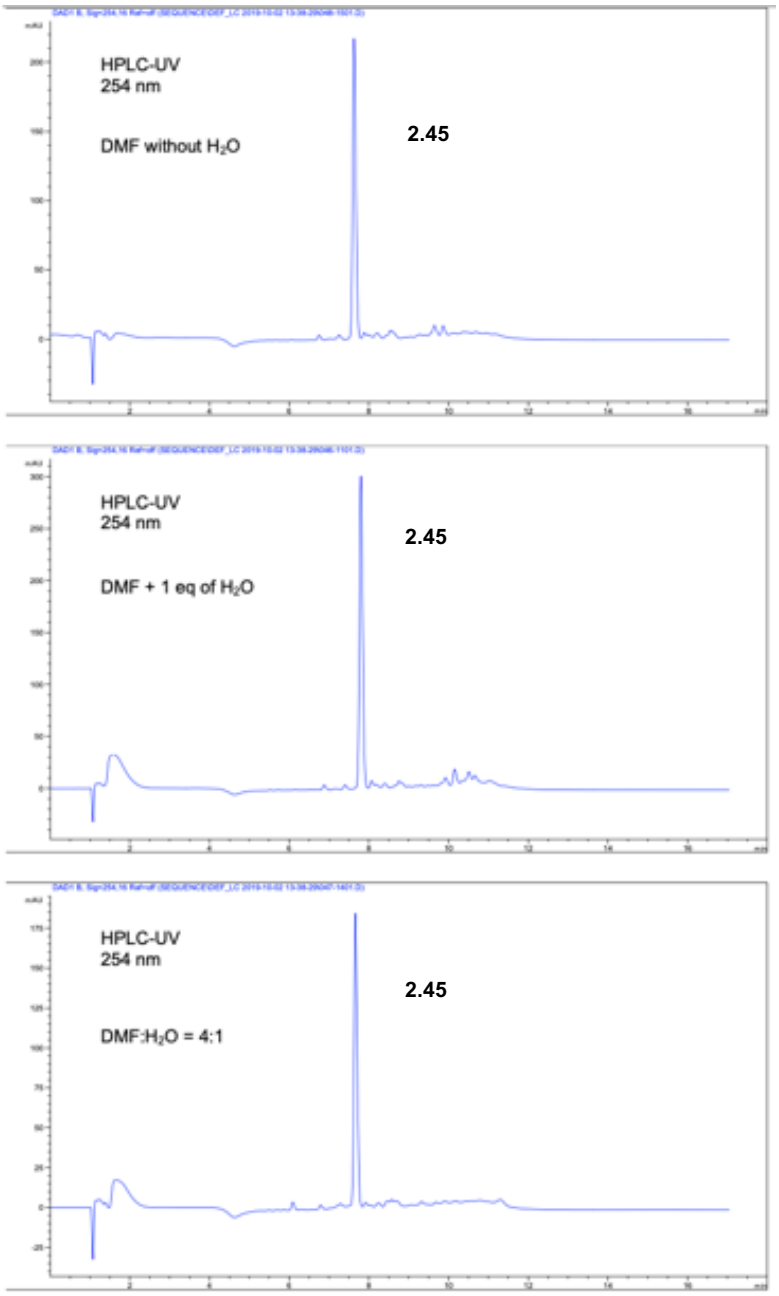
^{1}H NMR (DMSO-d6, 500 MHz) δ 8.68 (d, J = 7.9 Hz, 1H), 8.45 (d, J = 7.6 Hz, 1H), 8.28-8.18 (m, 3H), 7.42 (brs, 1H), 7.33-7.16 (m, 6H), 4.66-4.57 (m, 1H), 4.38-4.24 (m, 2H), 4.19 (dd, J = 7.0, 14.5 Hz, 1H), 4.11-4.02 (m, 1H), 3.12 (dd, J = 14.0, 4.3 Hz, 1H), 2.89- 2.76 (m, 3H), 2.00 (s, 3H); ^{13}C NMR (DMSO-d_6, 126 MHz) δ 179.9–179.2 (m, 2C), 171.6, 170.1, 170.0 165.6, 137.6, 129.2, 128.2, 126.4, 112.8-108.1 (m, 2C), 107.0, 62.1, 54.6, 52.8, 51.3, 37.2, 34.8, 20.5; ^{19}F NMR (DMSO-d_6, 282 MHz) δ -124.9 (s, 4F). HRMS m/z $[M + Na]^+$ calc'd for $C_{22}H_{24}F_4N_4O_7SNa$ 587.1199, found 587.1186.

Water Sensitivity Experiments



2.45

A flask was charged with linear precursor 44 (19 mg each, 0.0240 mmol) and DMF/H_2O (a; 5 ml of DMF without H_2O. b; 5ml of DMF + 1 eq. H_2O. c; 4 ml of DMF + 1 ml of H_2O). To the mixture octafluorocyclopentene (1M in MeCN, 36 µl, 0.036 mmol) and Et_3N (8.4 µl, 0.060 mmol) were added at 0 °C. The mixture was stirred for 5 minutes.



Analytical HPLC Method
Column: Agilent Zorbax SB-Aq
4.6 x 50 mm, 1.8 µm
Solvent A: H_2O + 0.1% TFA
Solvent B: MeCN + 0.1% TFA
Flow rate: 1.00 ml/min

Time (min)	%B
0	4
0.5	55
7.5	100
13.5	100

Fig. S4. Method for the Determination of aqueous stability in the reaction of **2.44** to **2.45**.

pH Sensitivity Experiments

Compound **2.22** was subjected to aq. NaOH and aq. HCl in MeOH separately to probe stability in basic/acidic conditions, respectively. Under basic conditions, decomposition of **2.22** was observed after one hour. Whereas under acidic conditions, **2.22** remained stable over 24 h. These results were determined bsed on HPLC traces obtained at various time points as outlined below.

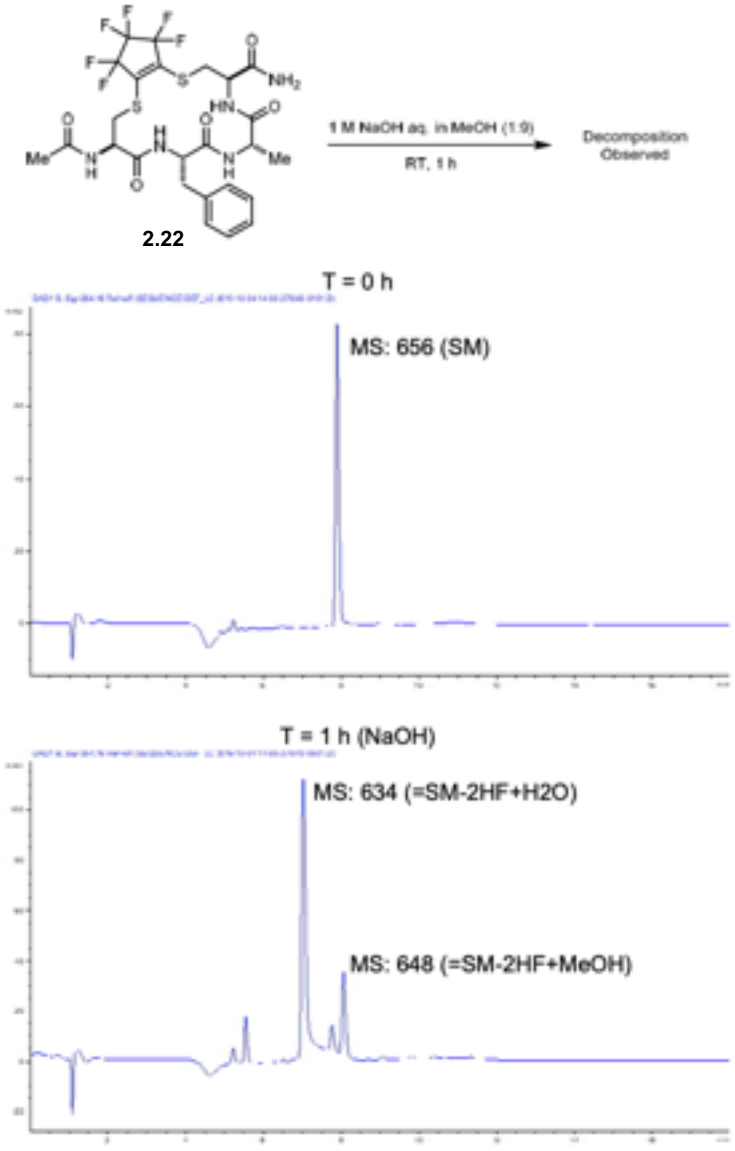


Fig. S5. Determination of basic pH stability of **2.22** by HPLC over 1 h.

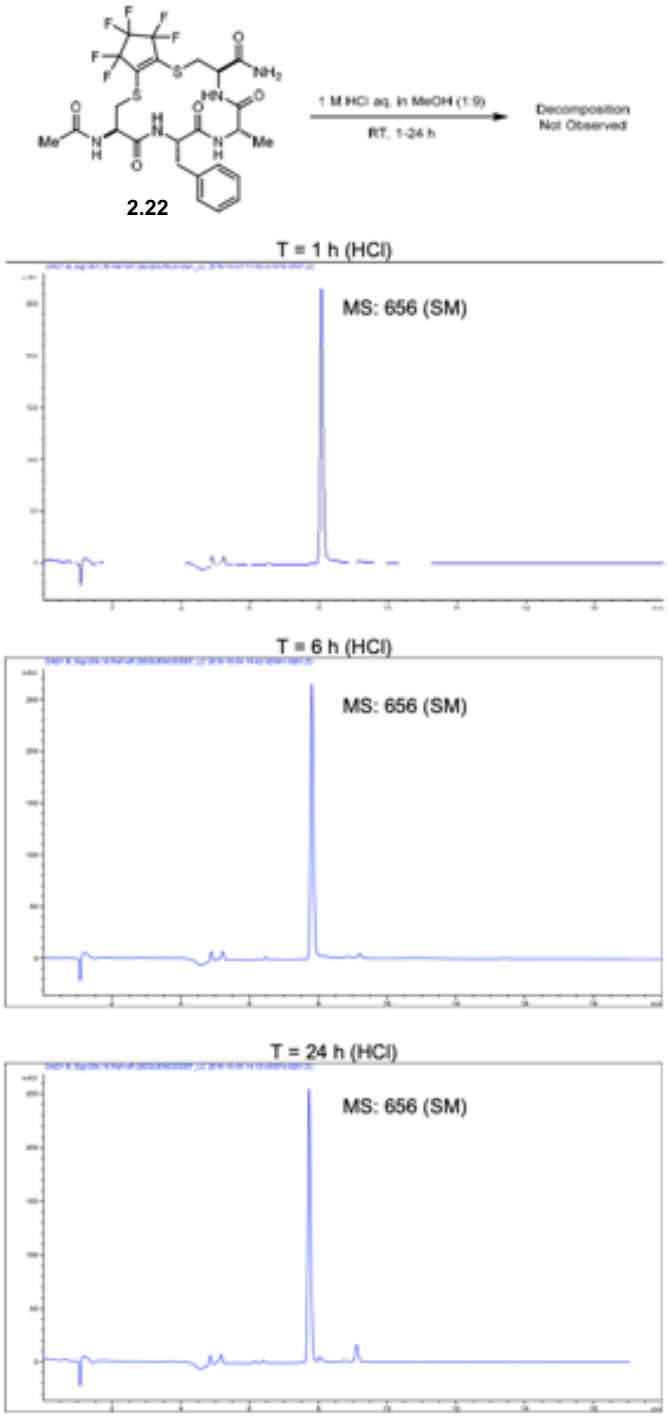


Fig. S6. Rate determination for the reaction of L-Cbz-His-NH_2 with mono phenoxy derivative **2.5**.

Probing the YXCXXC Consensus Sequence for Macrobicycle Synthesis

The following amino acids were reacted with OFCP to probe the consensus sequence YXCXXC. In all cases tentative HPLC-MS data suggested the formation of intermediates derived from three substitutions; however, the fourth substitution, as seen similarily in compounds 43 and 47, proved to be much slower and provided complex product mixtures compared to the originally outlined polycyclizations. In the cases of linear peptides Ac-NYCAFC-NH_2 and Ac-YCNTFC-NH_2, the difficulty involved in the fourth substitution likely stems from the adjacent cysteine and tyrosine residues preventing the formation of a strained bicyclic system. Regarding linear peptide Ac-CNYTFC-NH_2, the bulkiness of tyrosine as well as the sterically hindering spirocycle intermediate likely disallows the fourth substitution based on this unfavorable approach from its position within the ring.

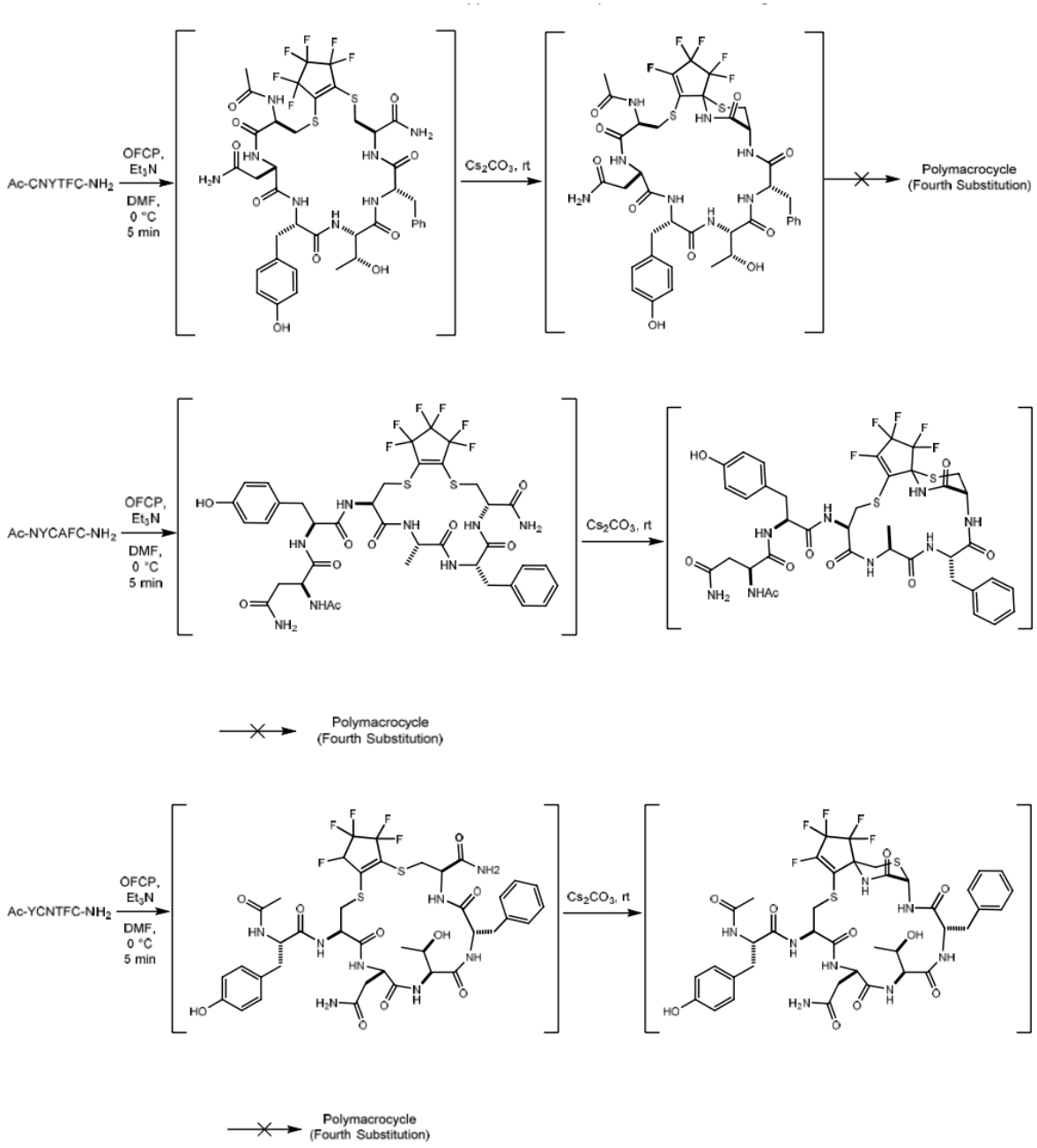


Fig. S7. Probing the YXCXXC consensus sequence for macrobicycle syntheses using scrambled variations of Ac-YNCTFC-NH_2

Crystal Structure Analysis

Deposition Number 1946197 contains the supplementary crystallographic data for this paper. These data are provided free of charge by the joint Cambridge Crystallographic Data Centre and Fachinformationszentrum Karlsruhe Access Structures service www.ccdc.cam.ac.uk/structures.

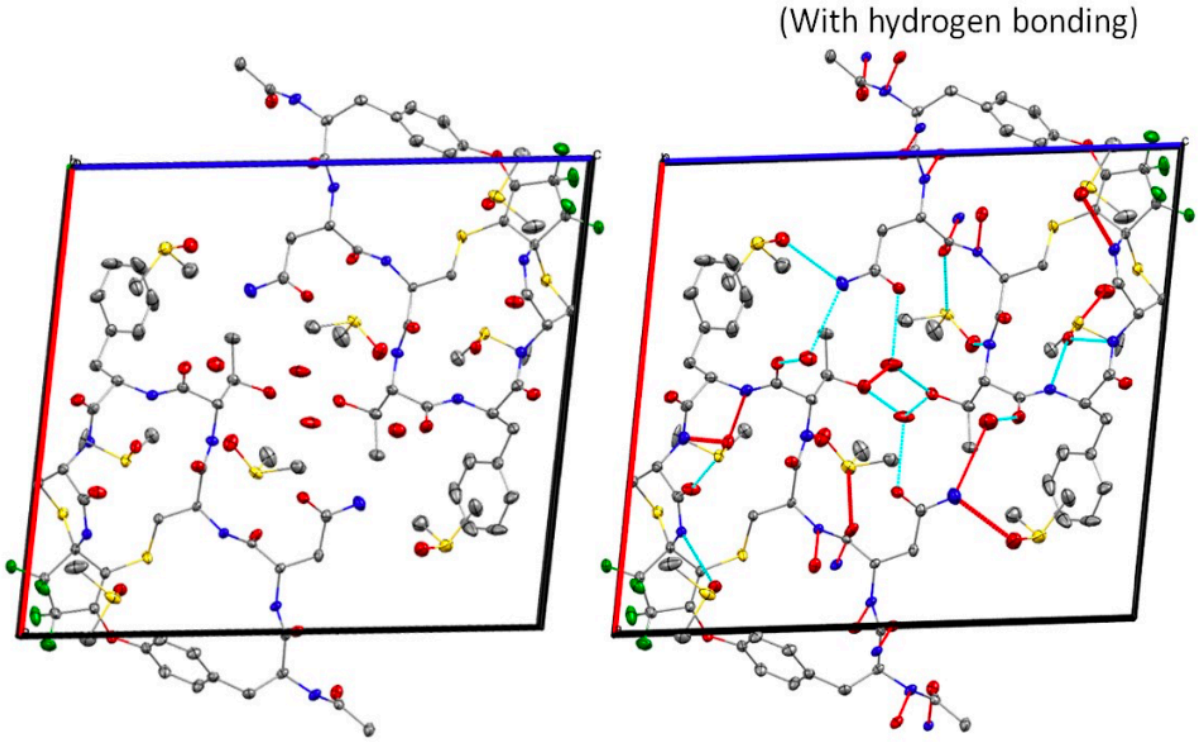
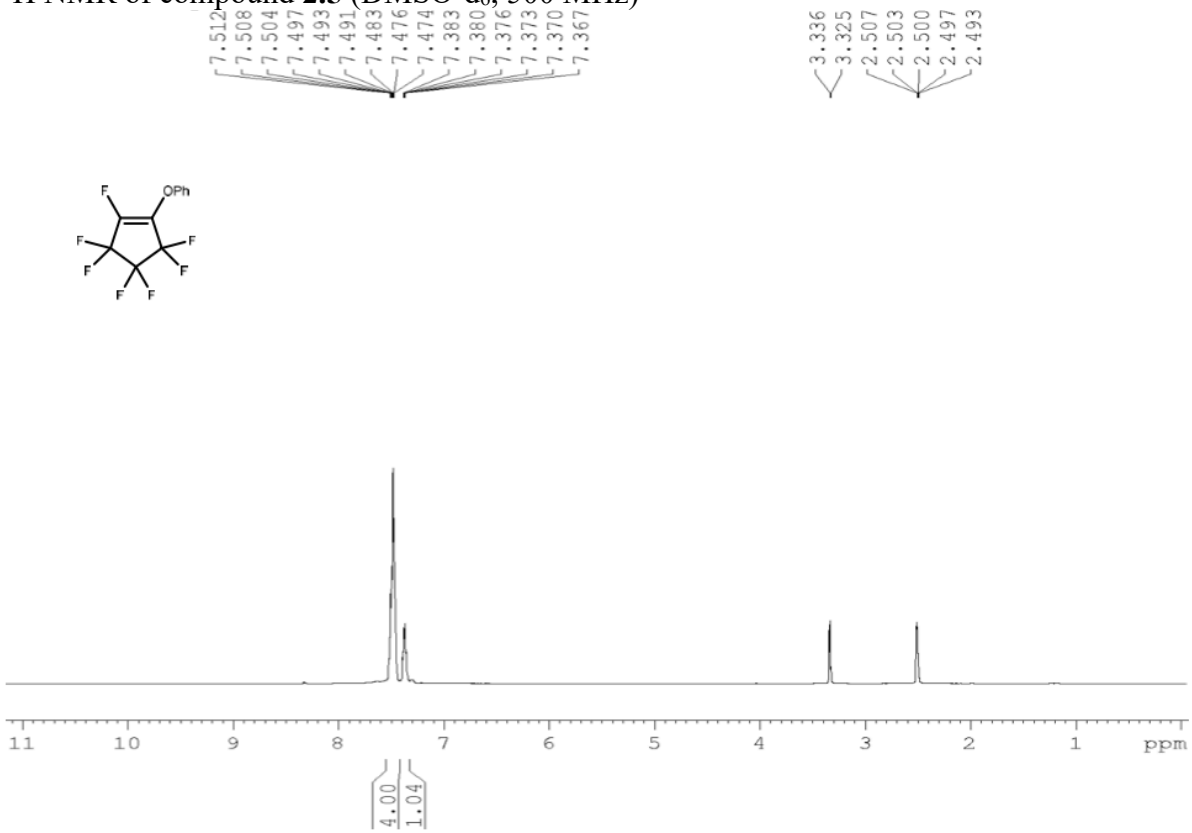


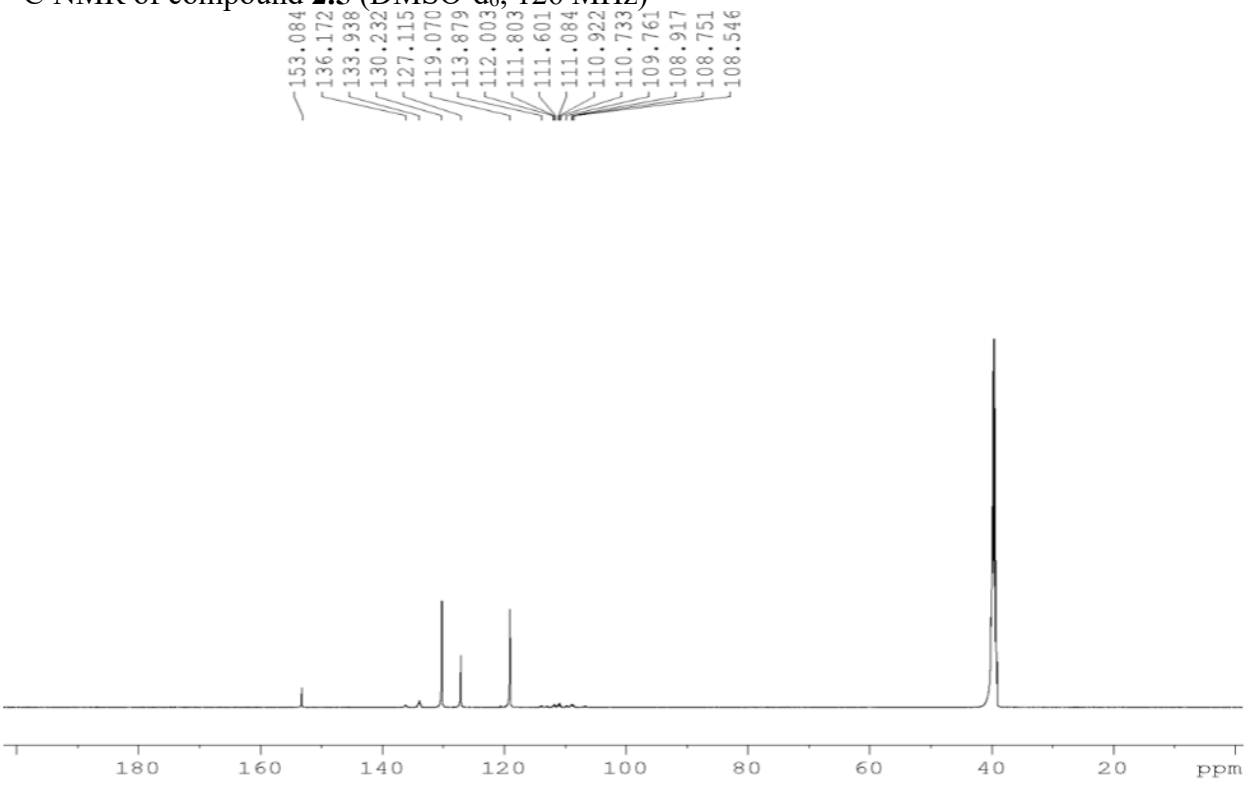
Fig. S8. Structure was solved in space group P2(1) with ORTEP showing 50% probability. Both residual DMSO and water molecules can be observed within the unit cell. Water atoms can be seen localized between the two asymetric units of the of the unit cell and may be providing additional stabilization effects through hydrogen bonding.

3. NMR Spectra

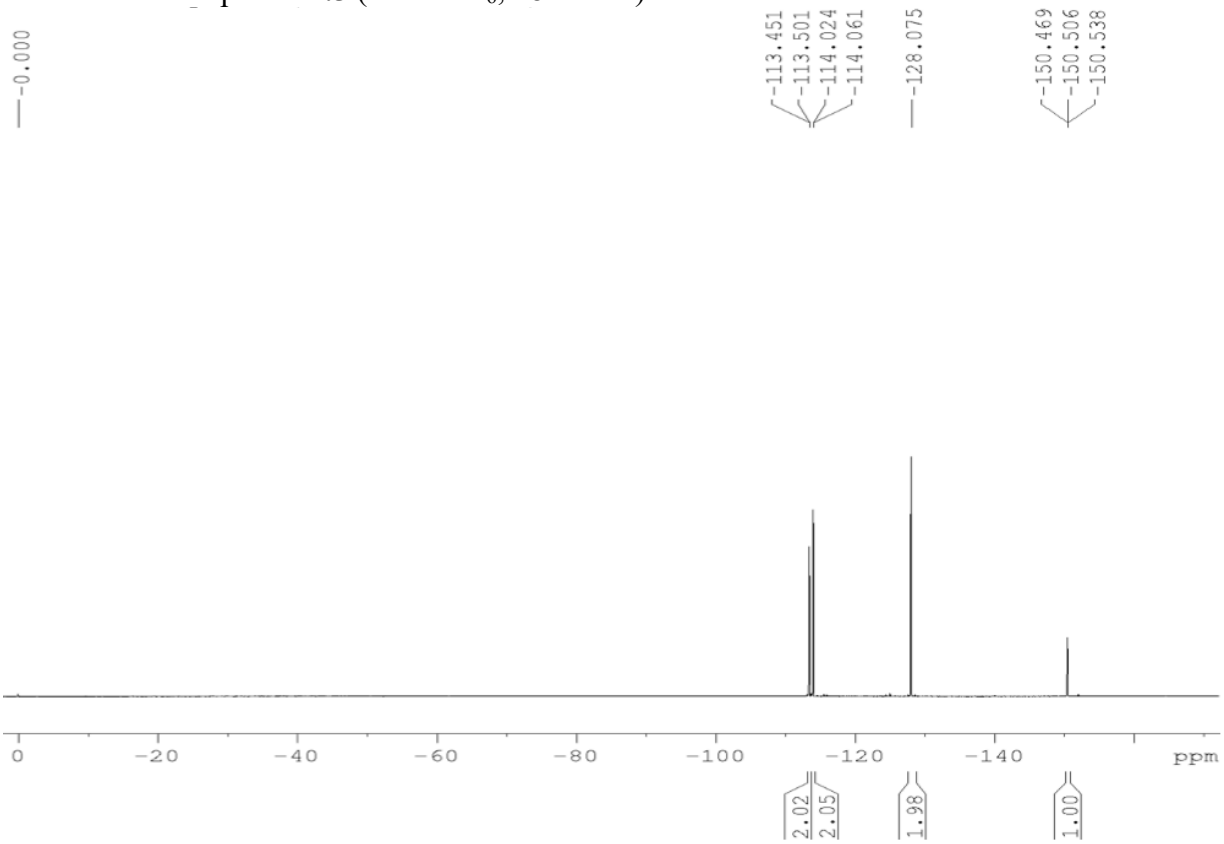
^{1}H NMR of compound **2.5** (DMSO-d_6, 500 MHz)



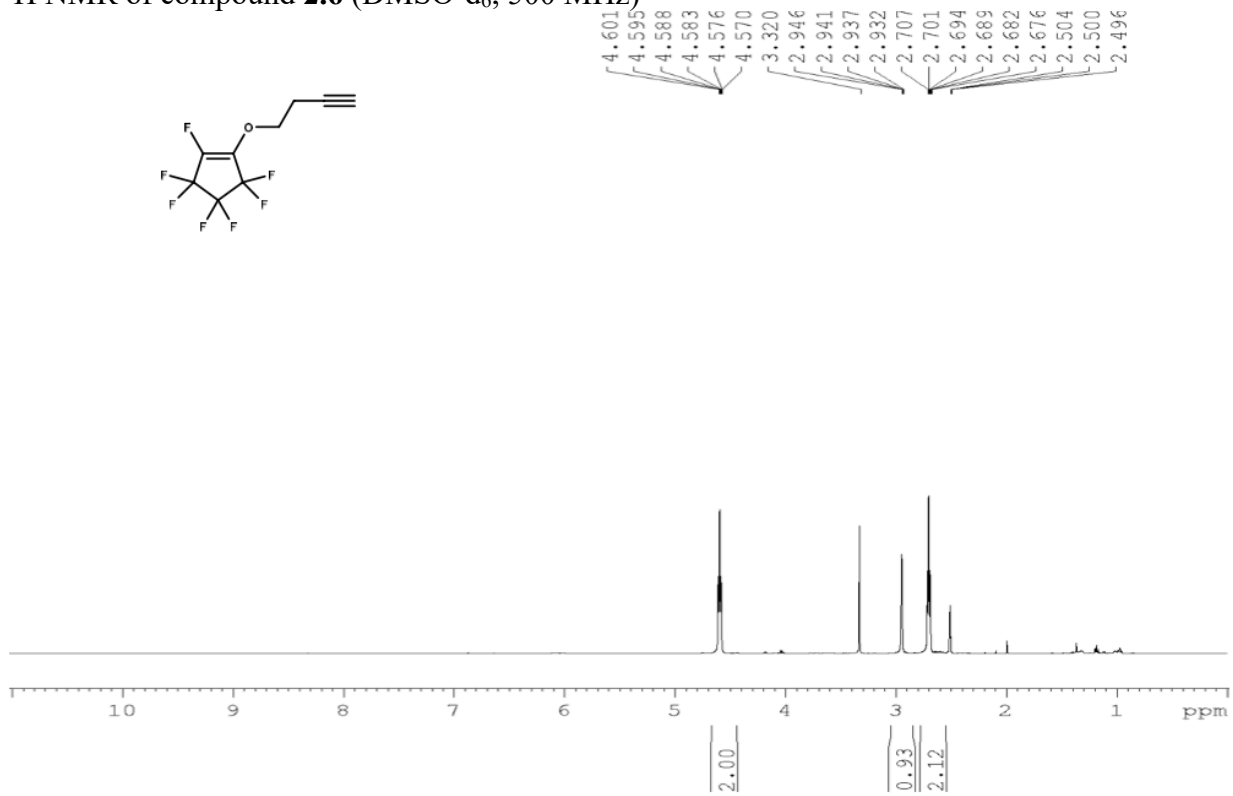
^{13}C NMR of compound **2.5** (DMSO-d_6, 126 MHz)



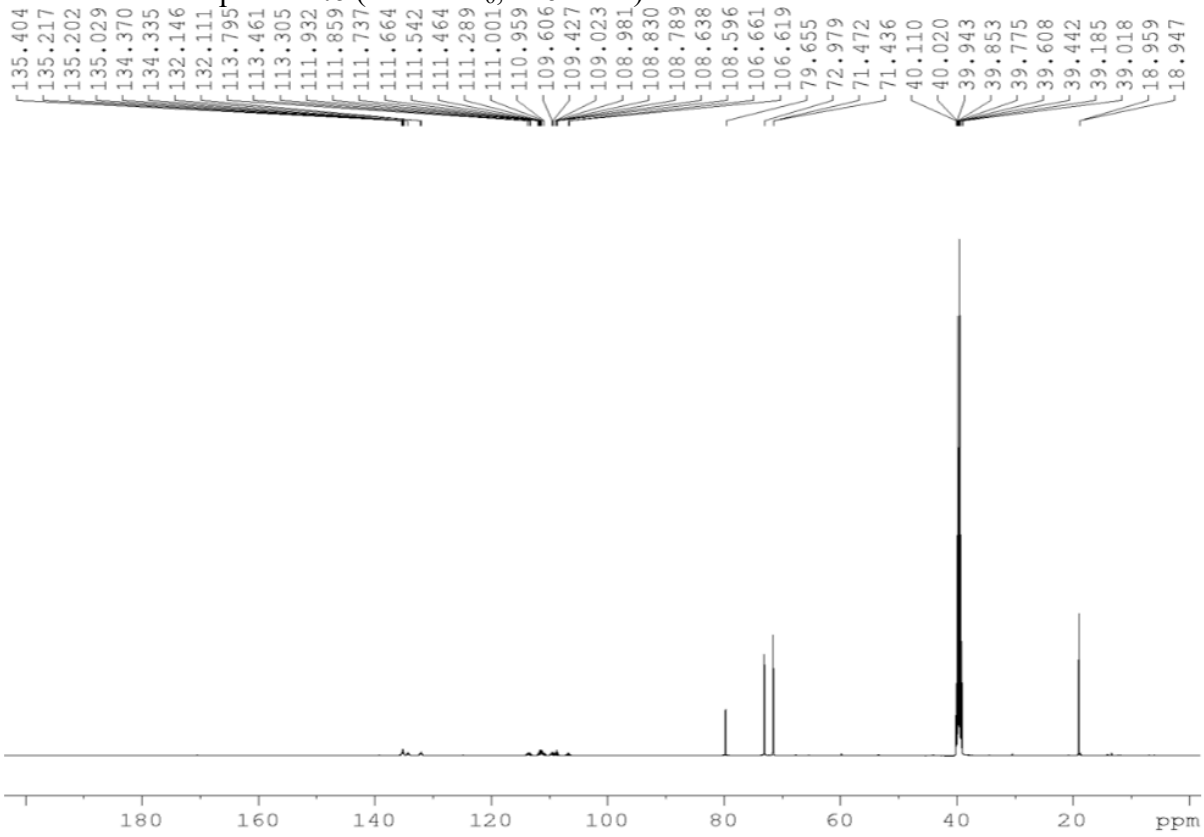
^{19}F NMR of compound **2.5** (DMSO-d_6, 282 MHz)



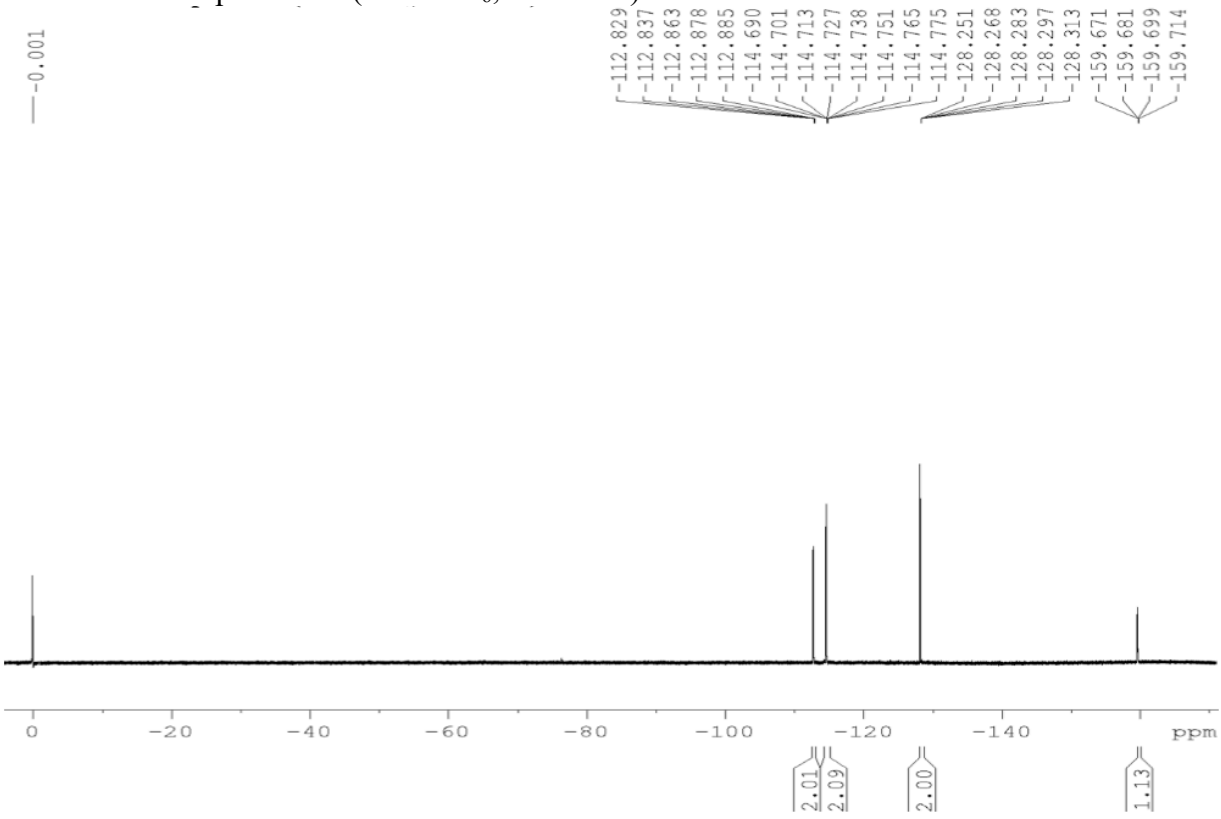
^{1}H NMR of compound **2.6** (DMSO-d_6, 500 MHz)



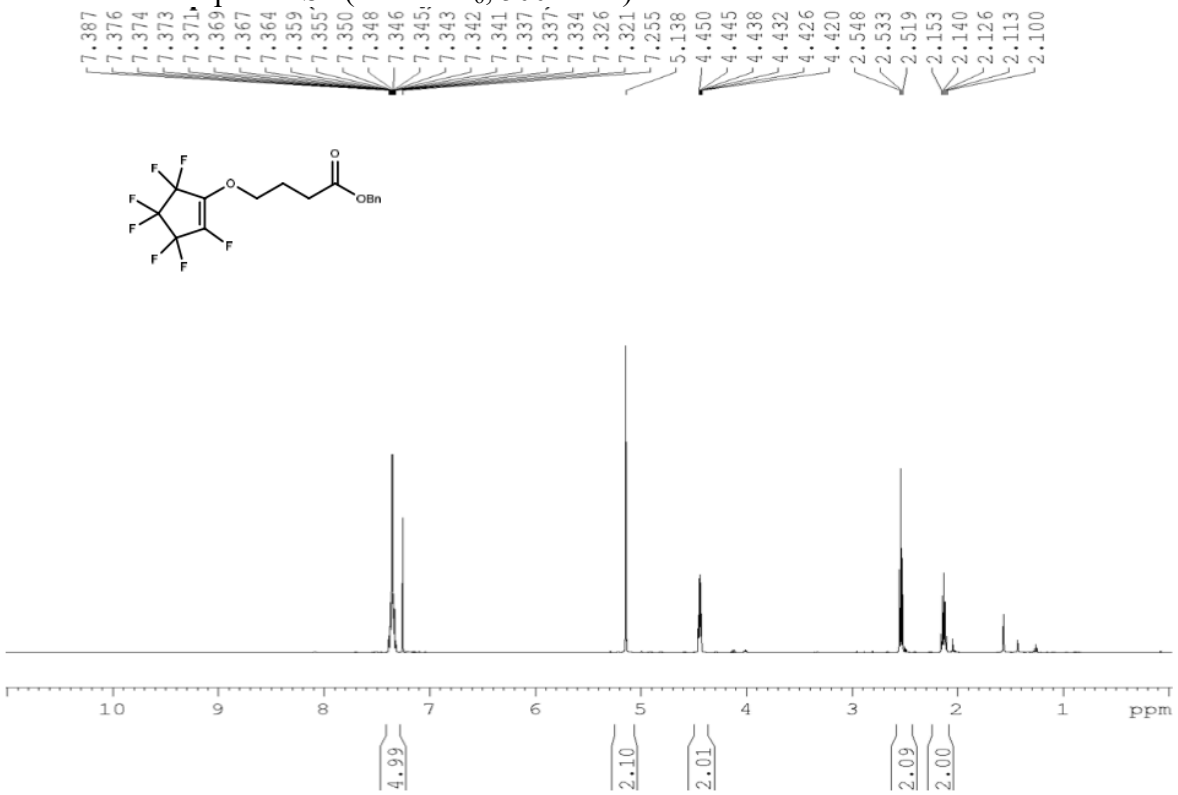
^{13}C NMR of compound **2.6** (DMSO-d_6, 126 MHz)



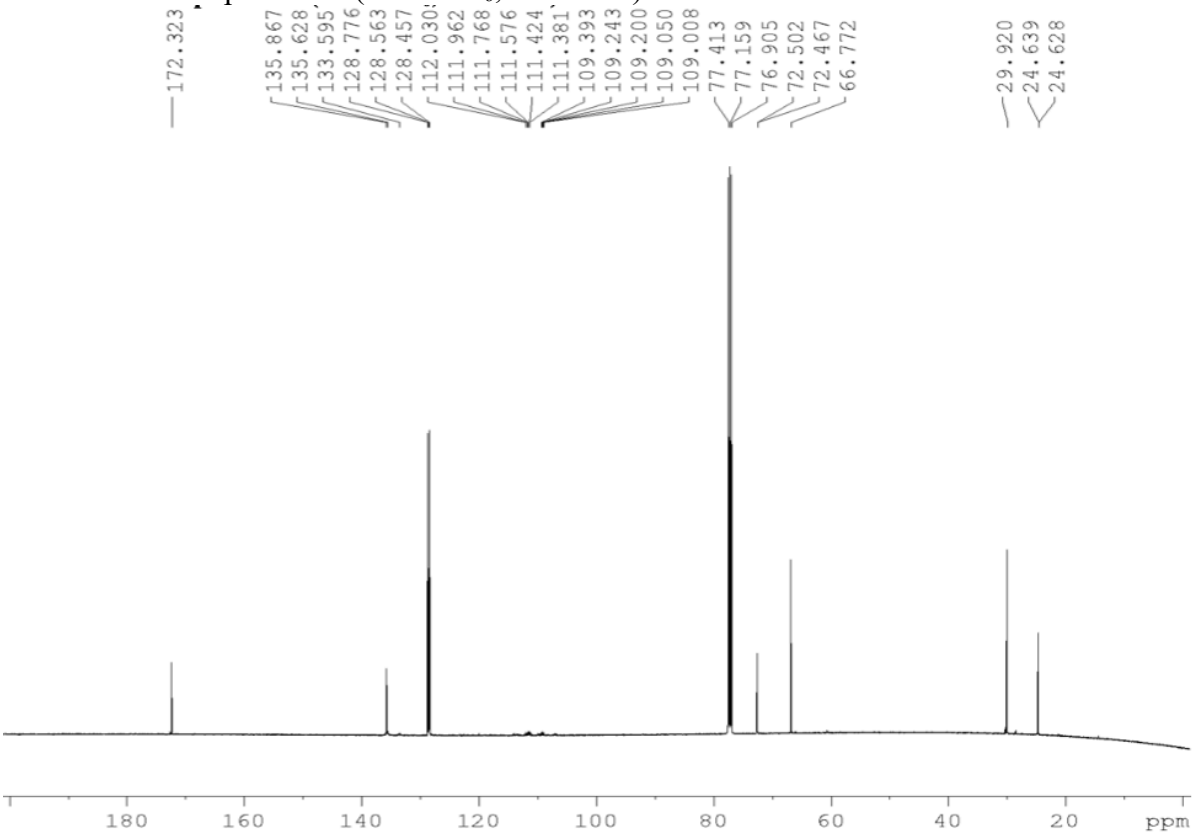
^{19}F NMR of compound **2.6** (DMSO-d_6, 282 MHz)



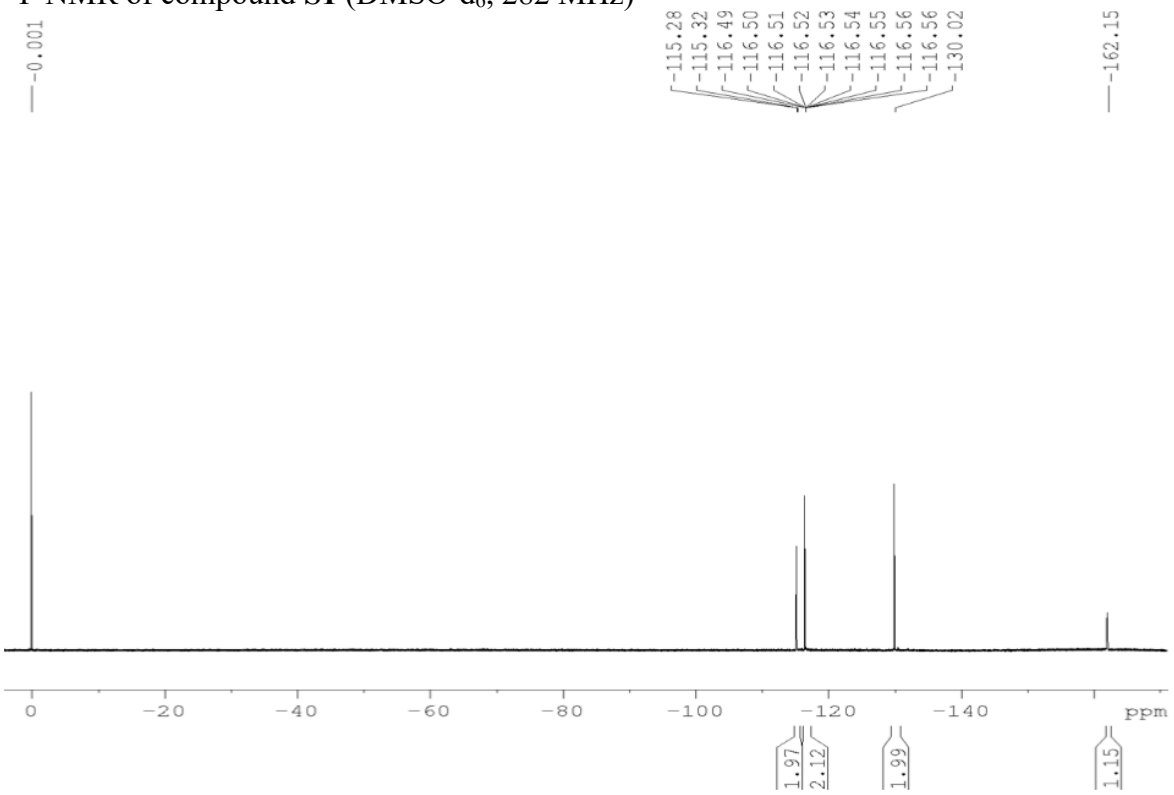
^{1}H NMR of compound **S1** (DMSO-d_6, 500 MHz)



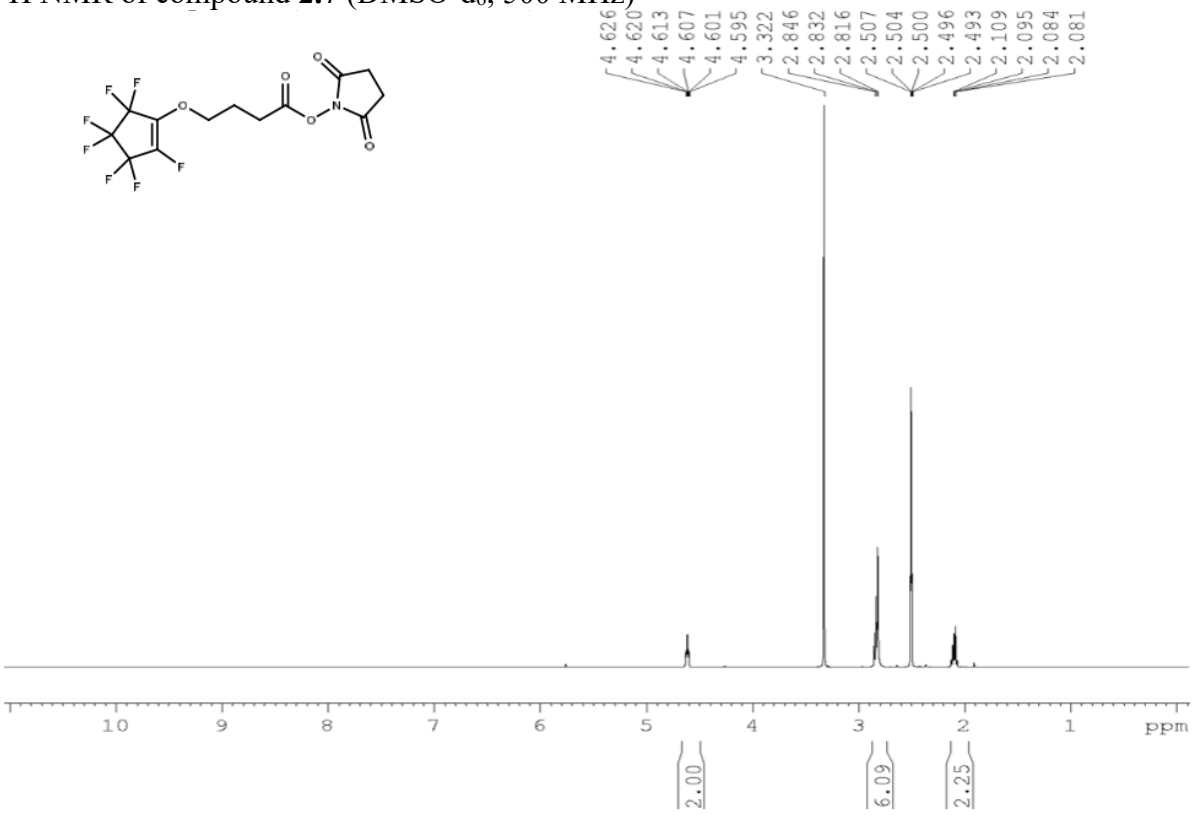
^{13}C NMR of compound **S1** (DMSO-d_6, 126 MHz)



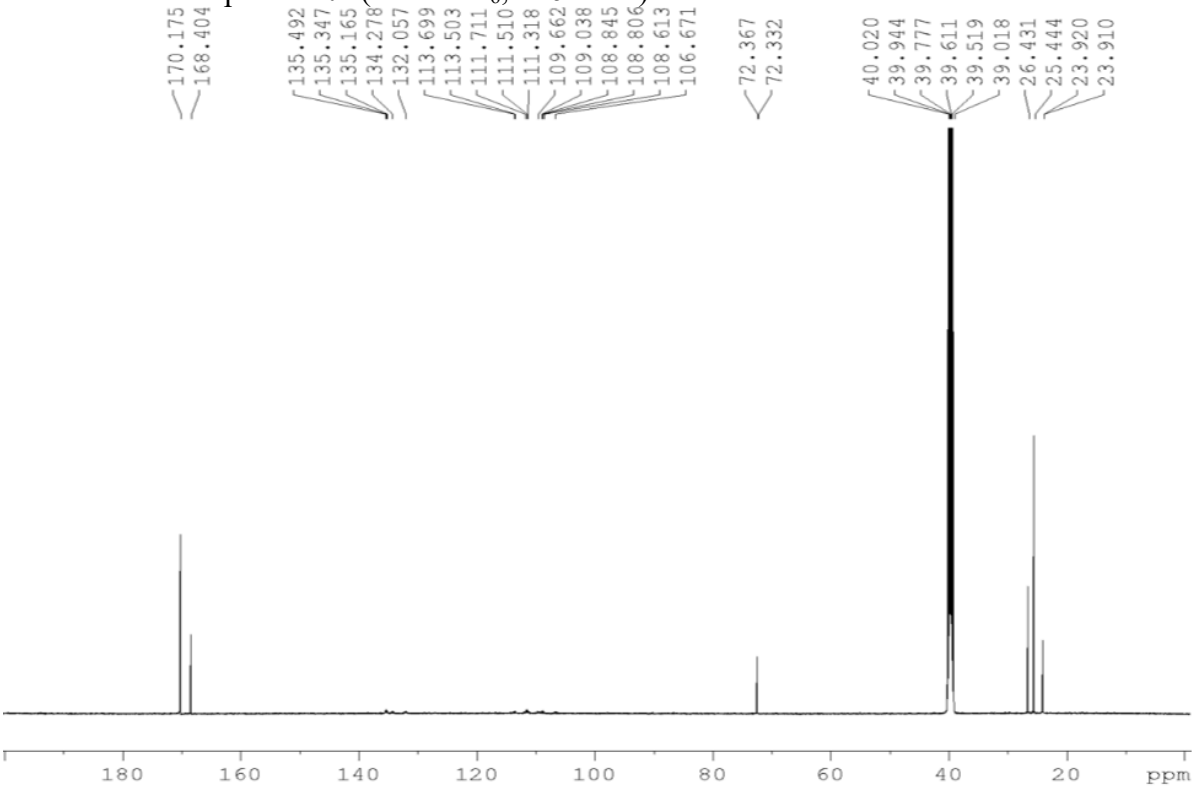
^{19}F NMR of compound **S1** (DMSO-d_6, 282 MHz)



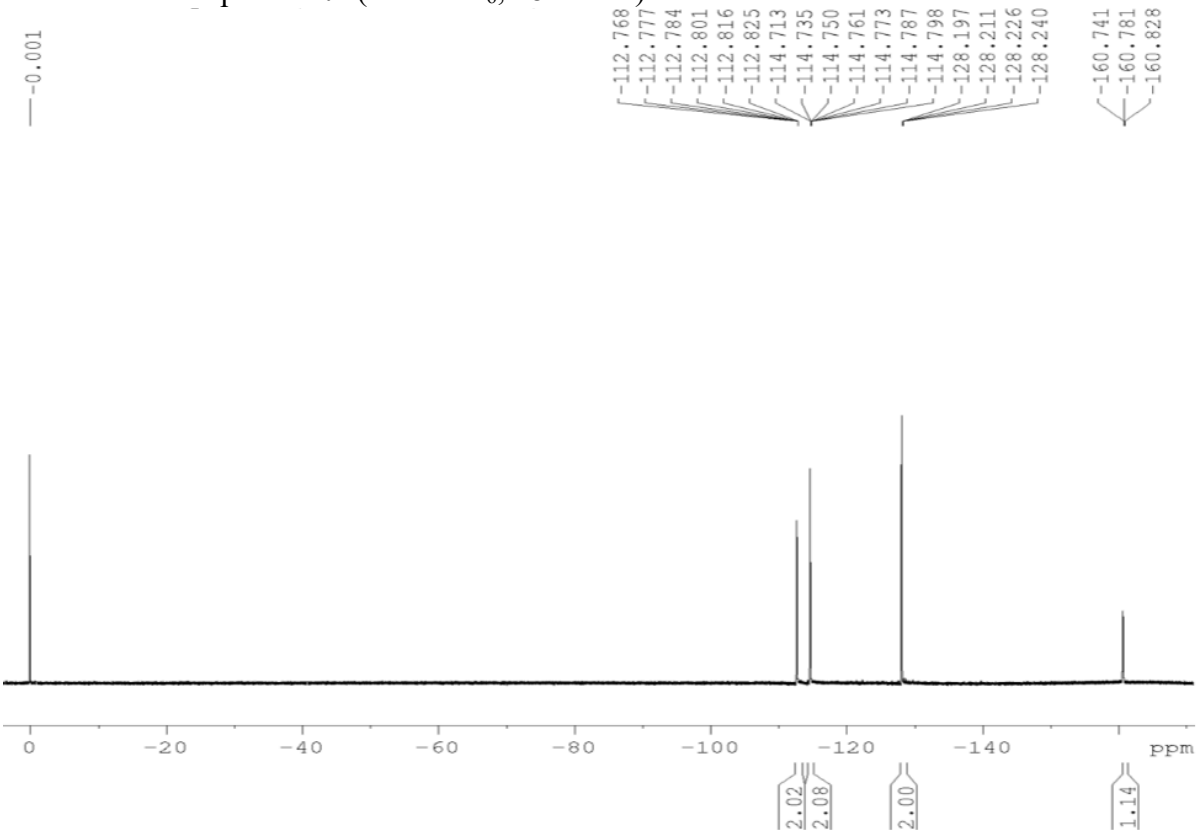
^{1}H NMR of compound **2.7** (DMSO-d_6, 500 MHz)



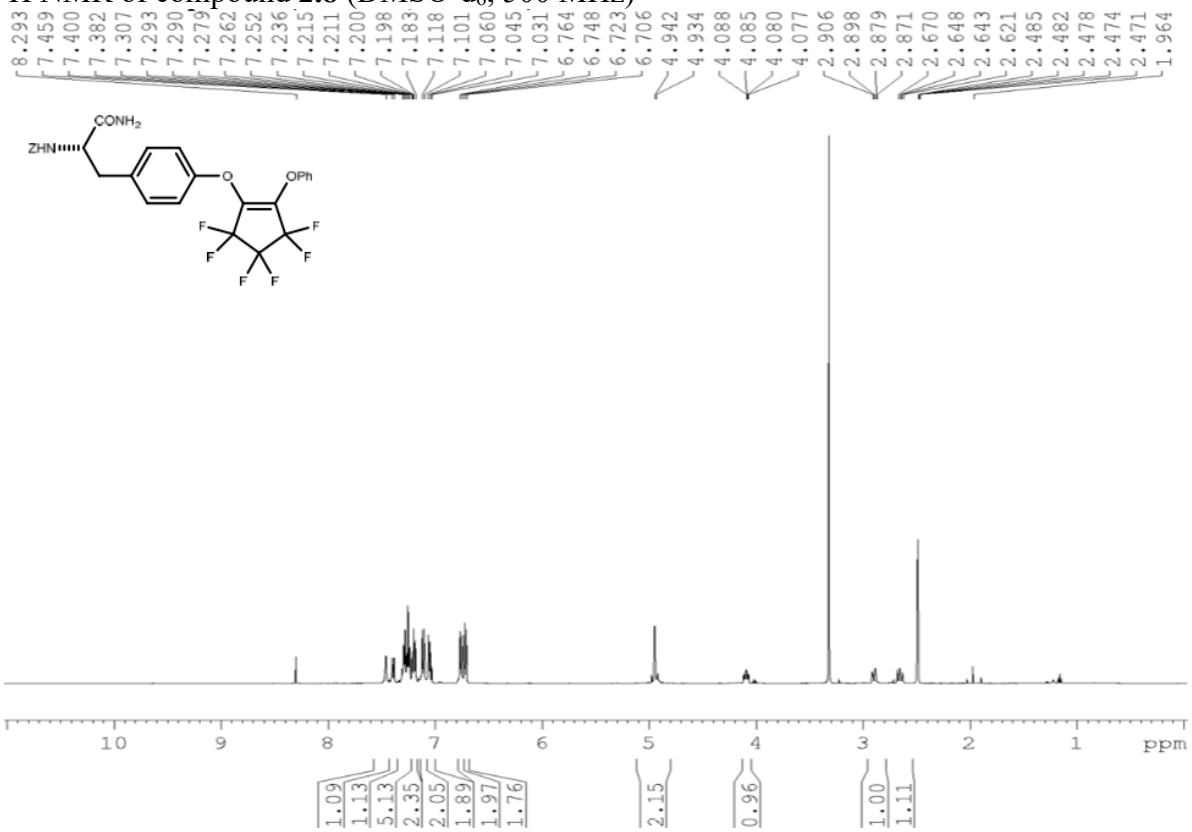
^{13}C NMR of compound **2.7** (DMSO-d_6, 126 MHz)



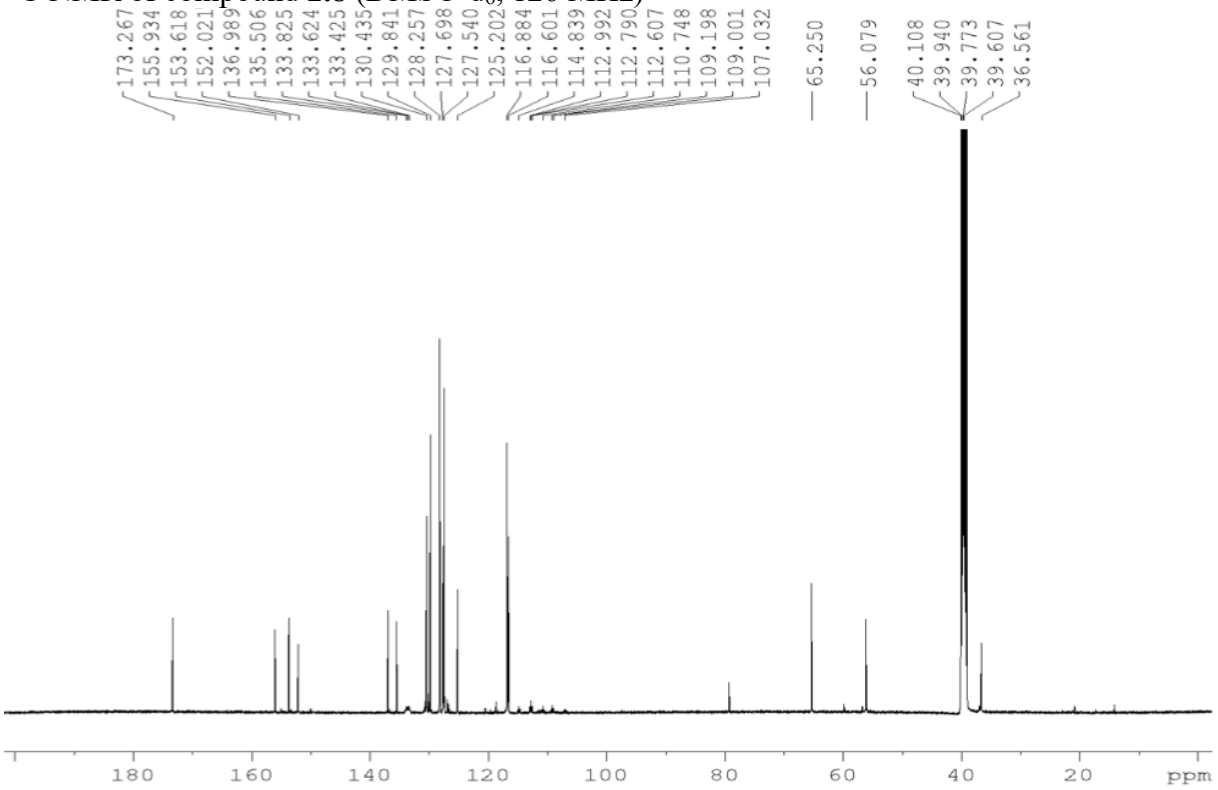
^{19}F NMR of compound **2.7** (DMSO-d_6, 282 MHz)



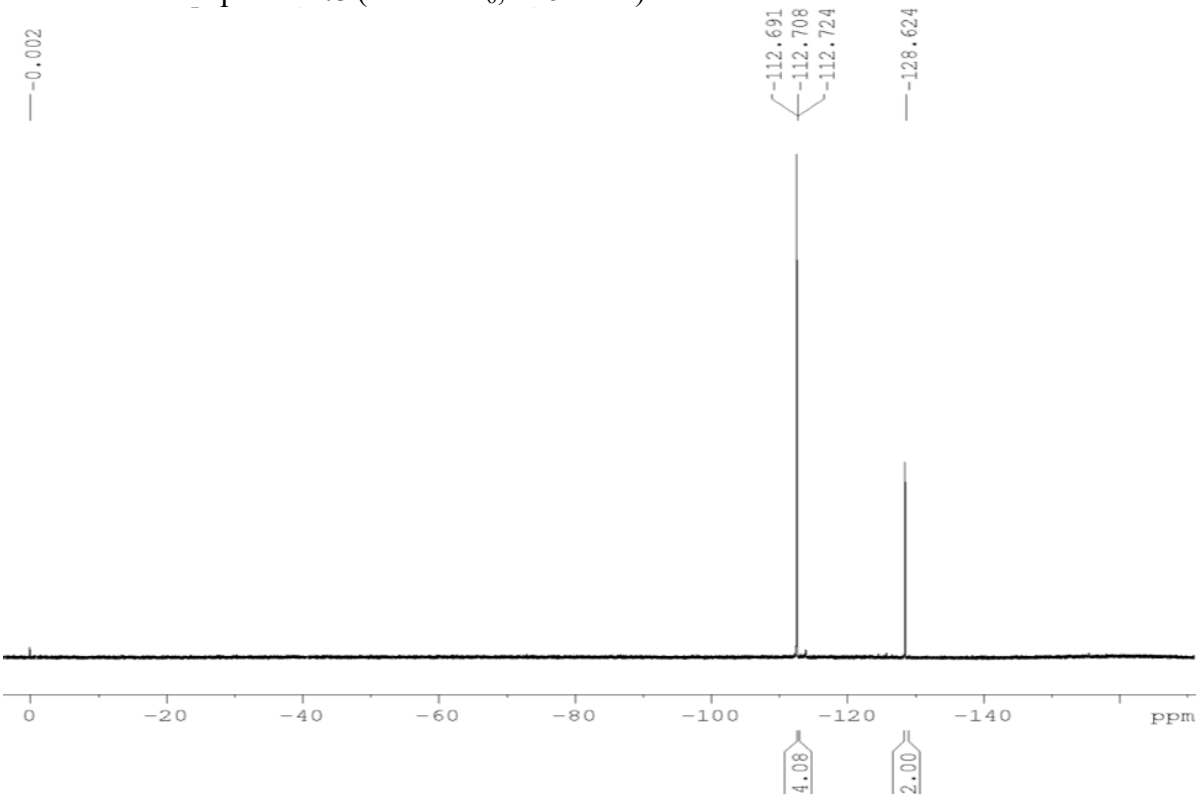
^{1}H NMR of compound **2.8** (DMSO-d_6, 500 MHz)



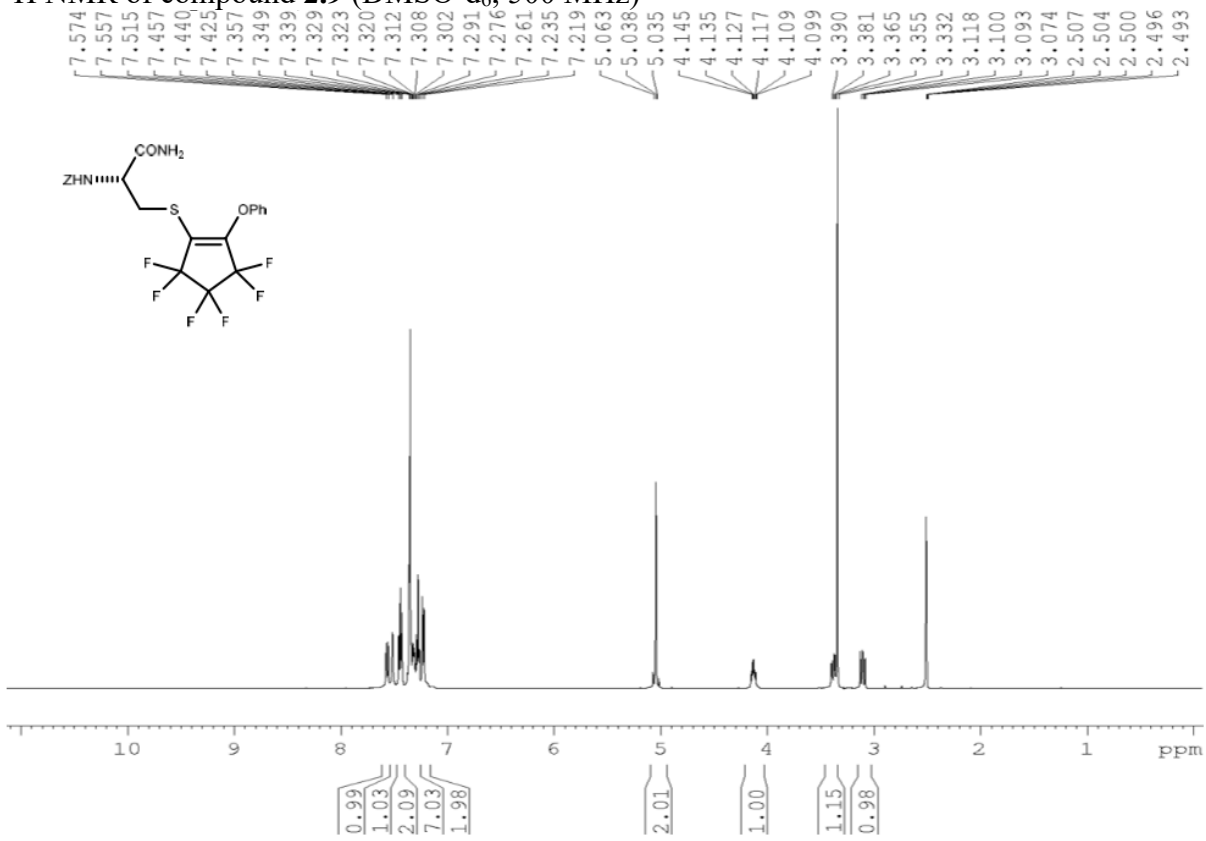
^{13}C NMR of compound **2.8** (DMSO-d_6, 126 MHz)



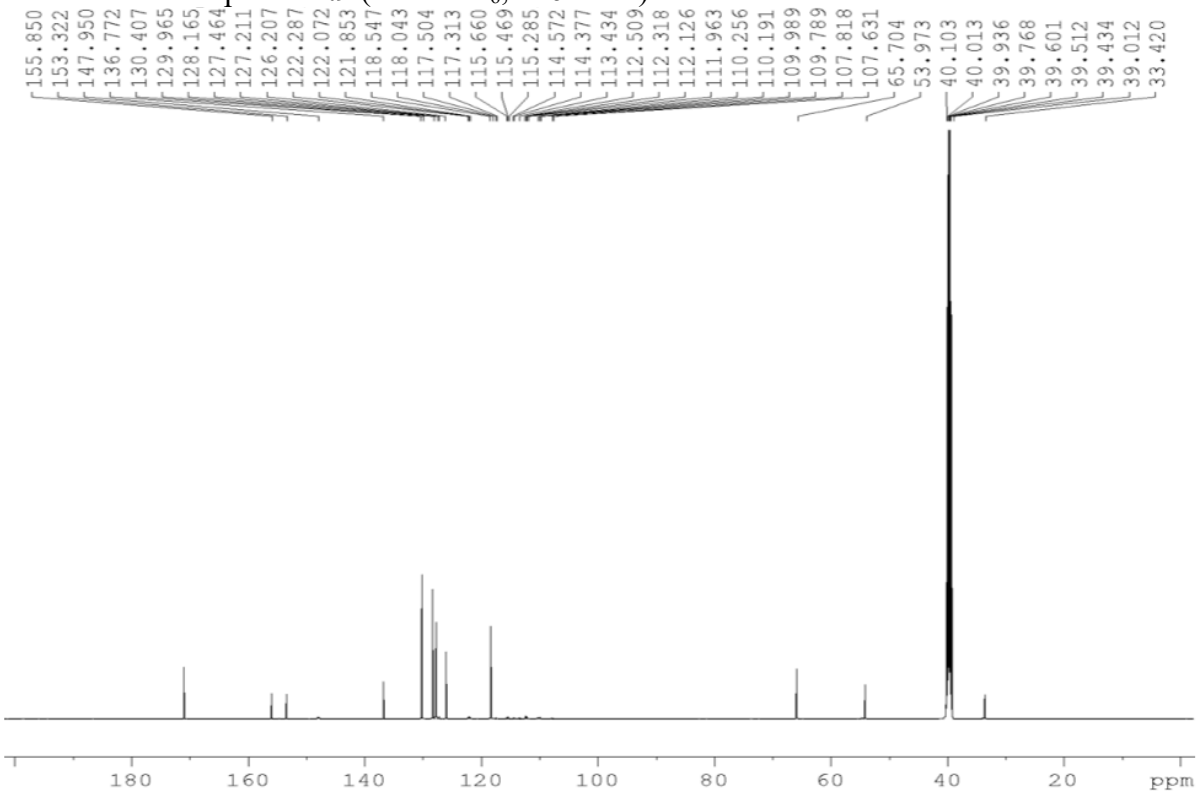
^{19}F NMR of compound **2.8** (DMSO-d_6, 126 MHz)



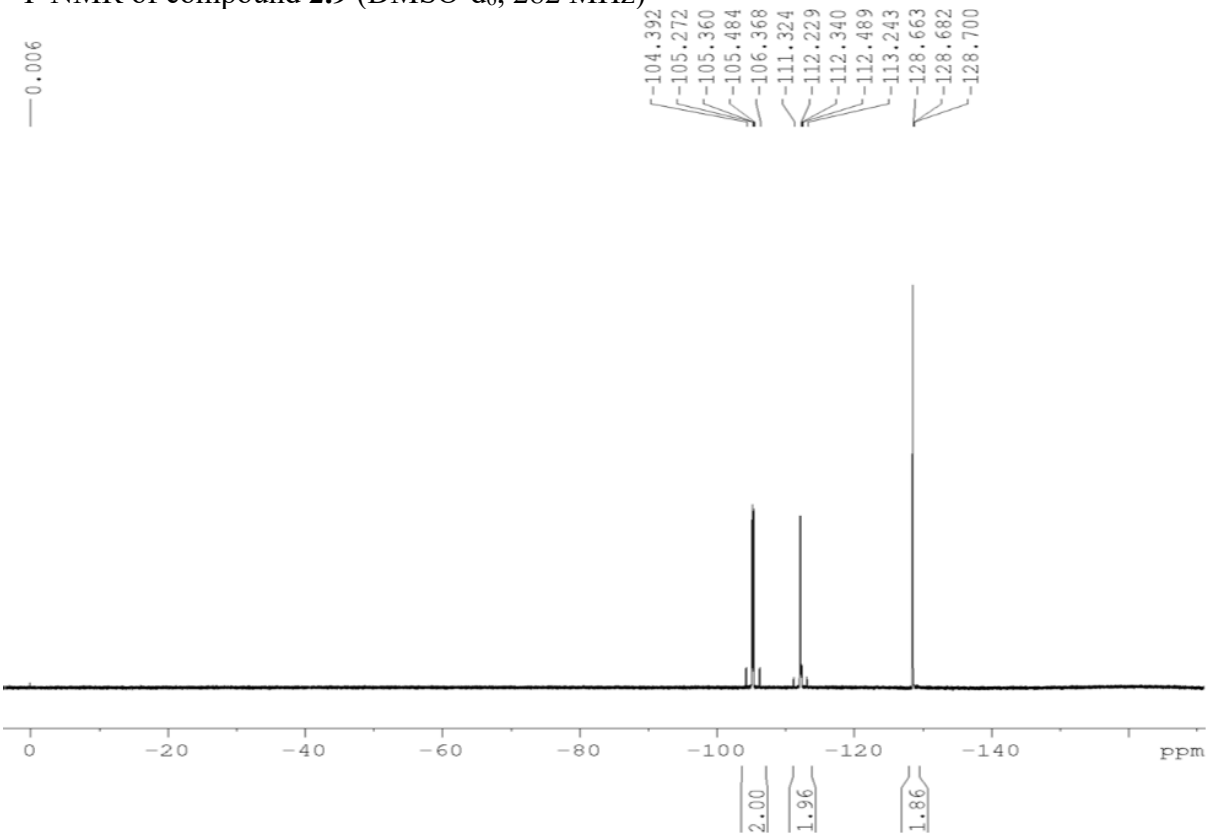
^{1}H NMR of compound **2.9** (DMSO-d_6, 500 MHz)



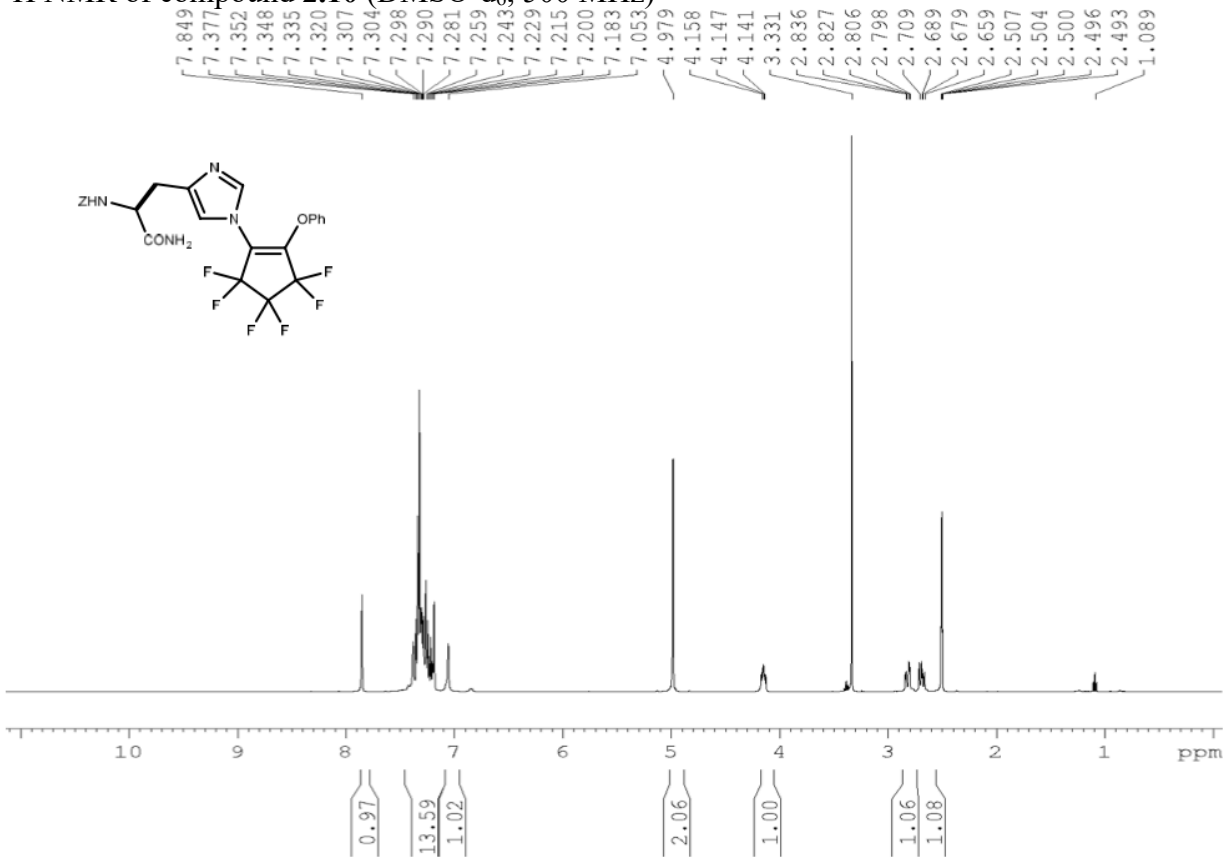
^{13}C NMR of compound **2.9** (DMSO-d_6, 126 MHz)



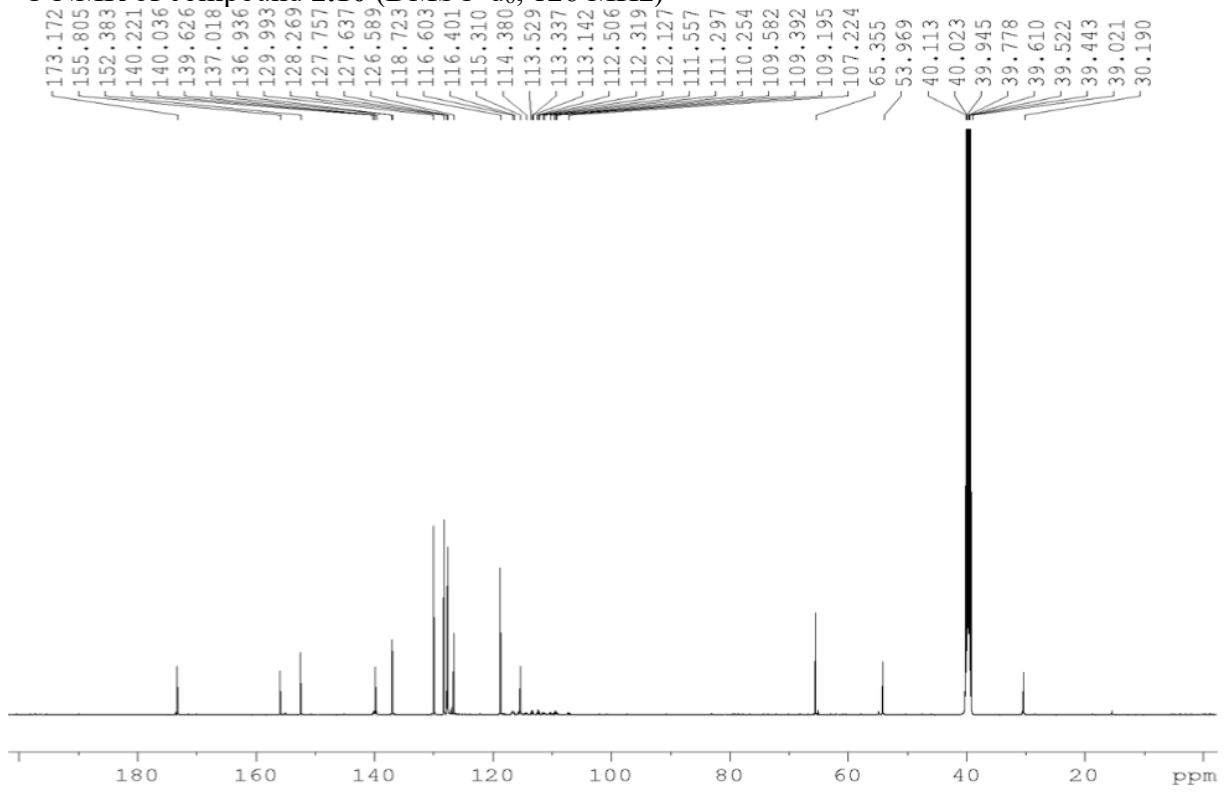
^{19}F NMR of compound **2.9** (DMSO-d_6, 282 MHz)



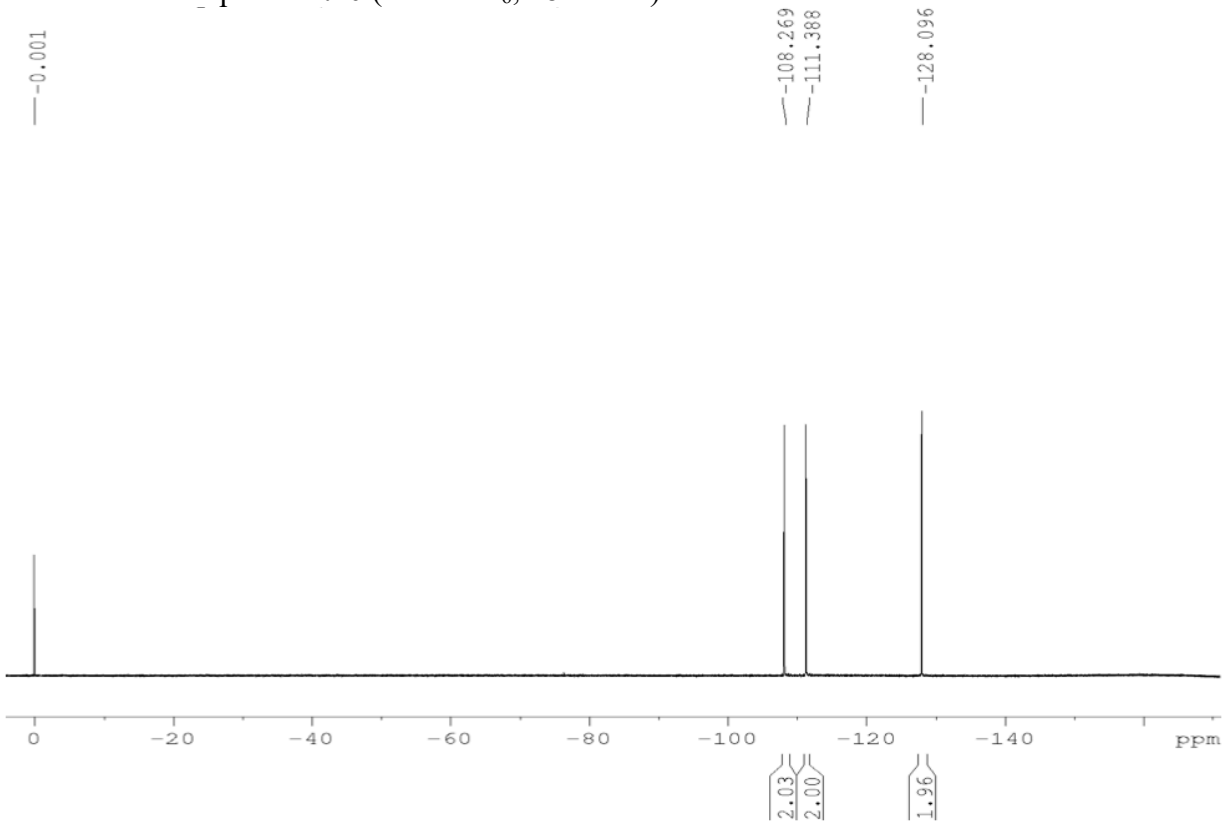
^{1}H NMR of compound **2.10** (DMSO-d_6, 500 MHz)



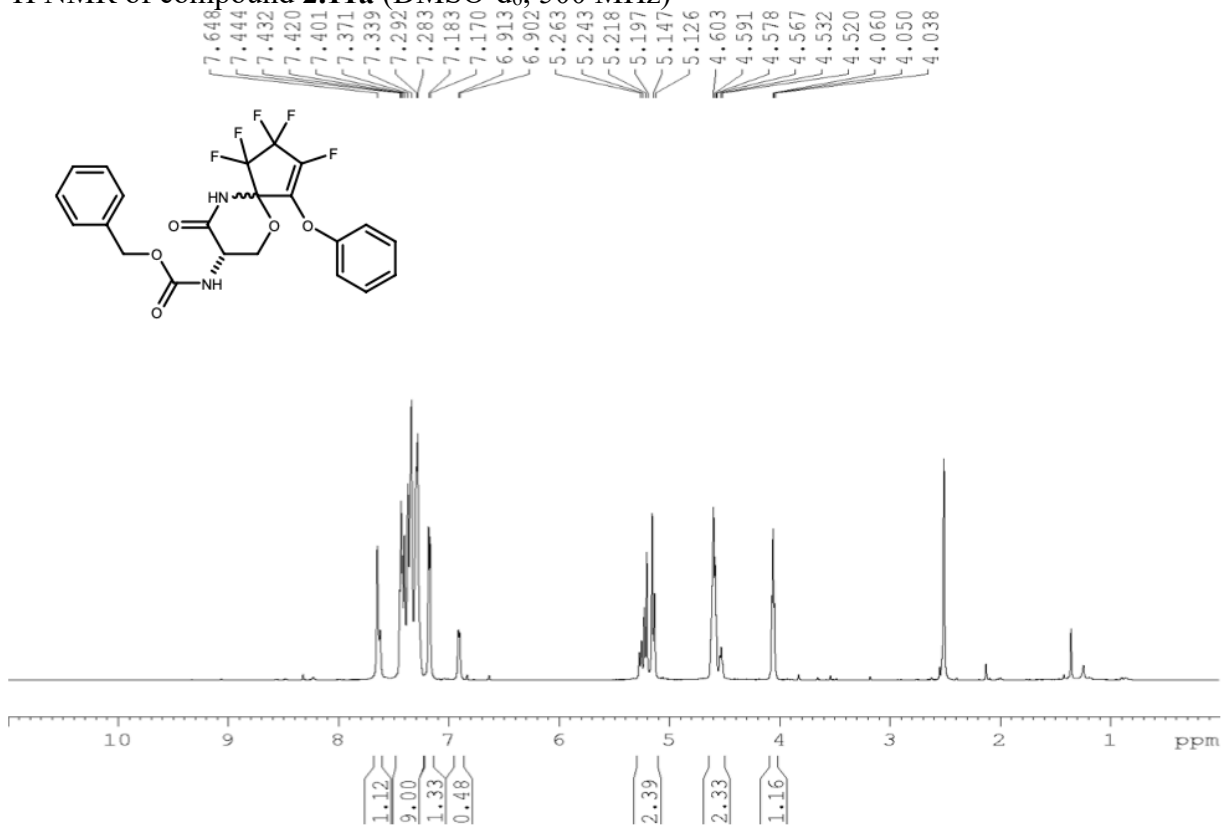
^{13}C NMR of compound **2.10** (DMSO-d_6, 126 MHz)



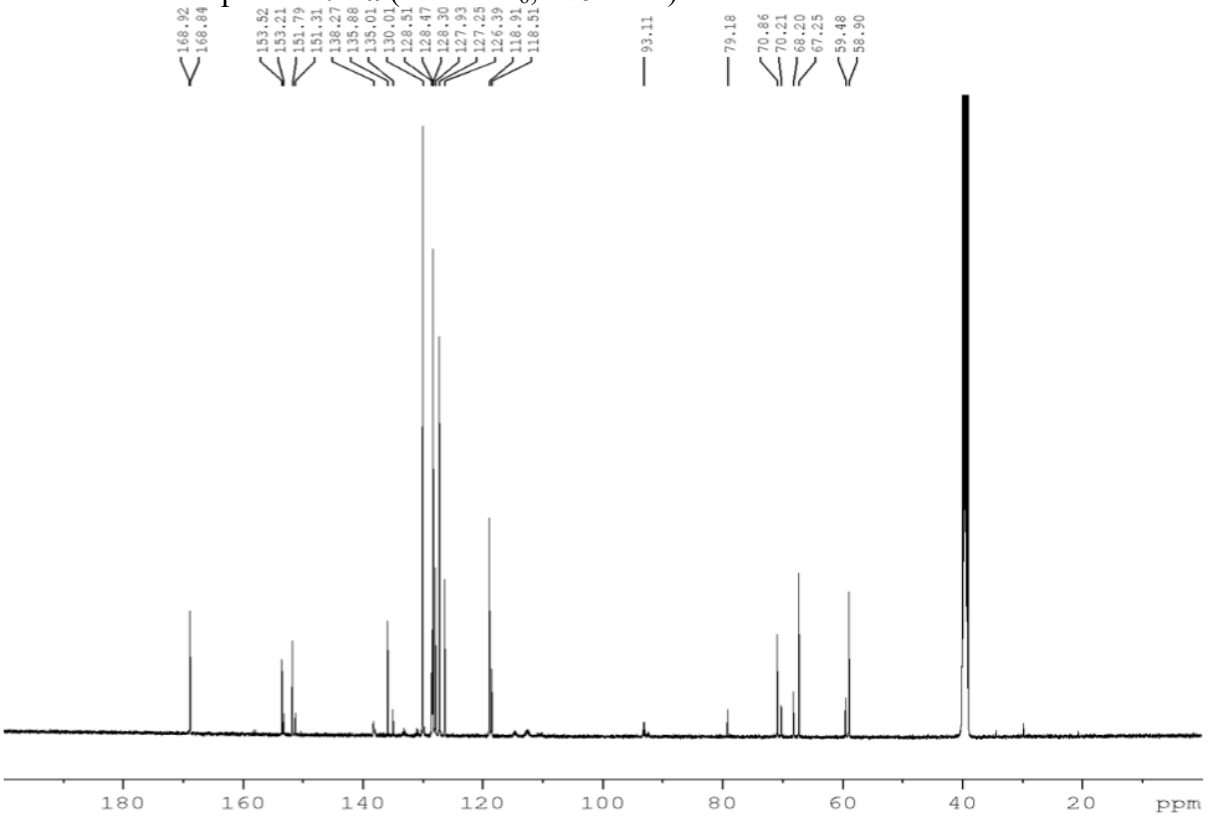
^{19}F NMR of compound **2.10** (DMSO-d_6, 282 MHz)



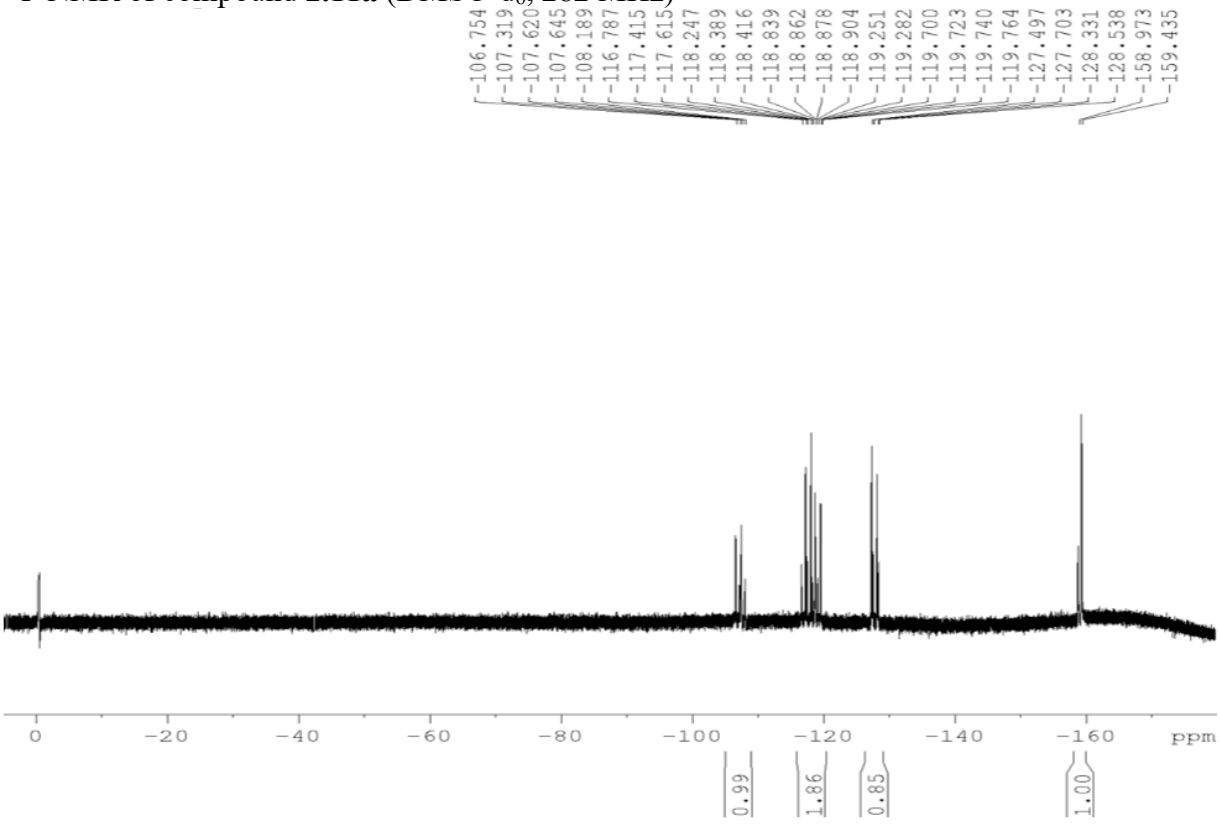
^{1}H NMR of compound **2.11a** (DMSO-d_6, 500 MHz)



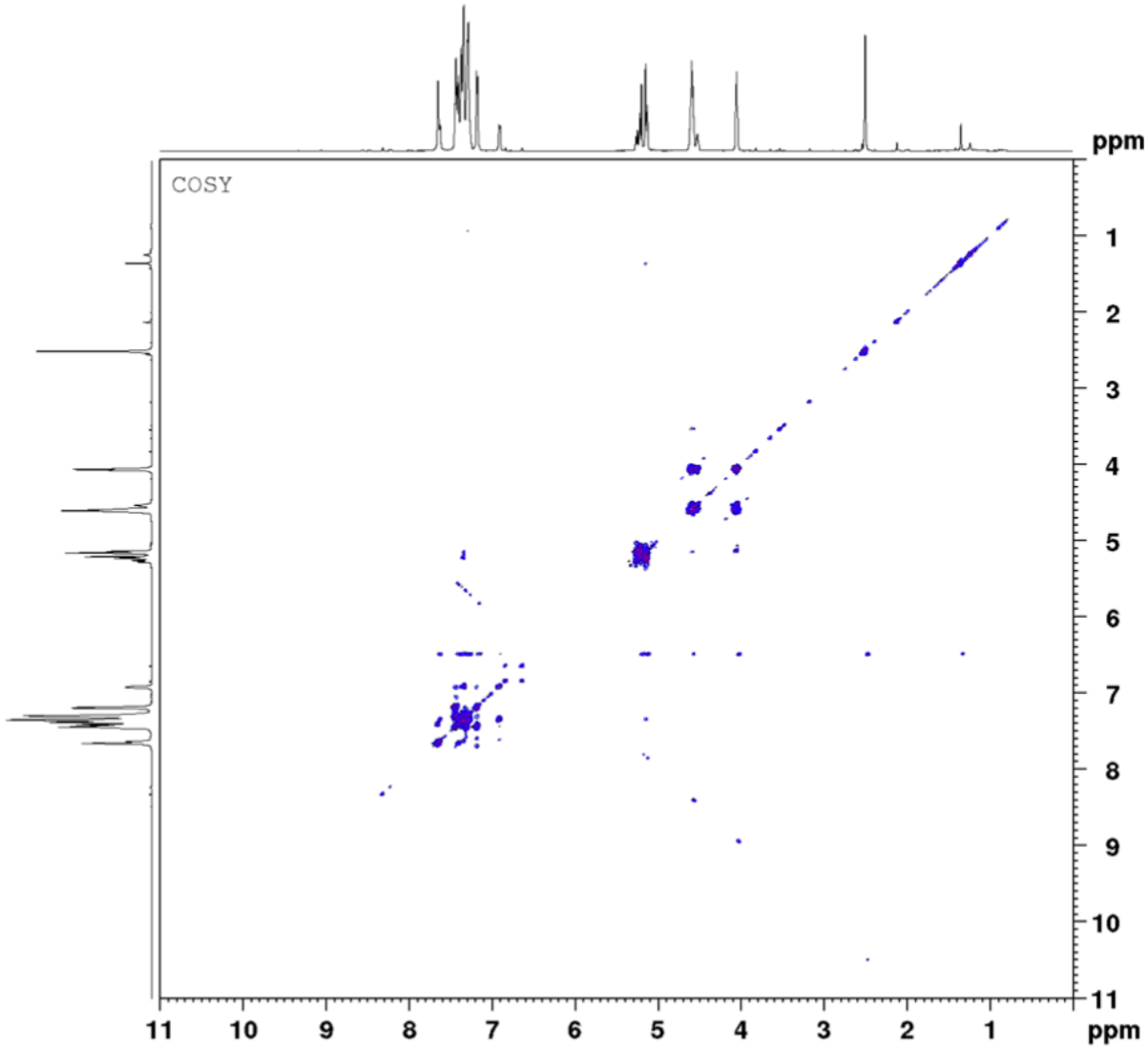
^{13}C NMR of compound **2.11a** (DMSO-d_6, 126 MHz)



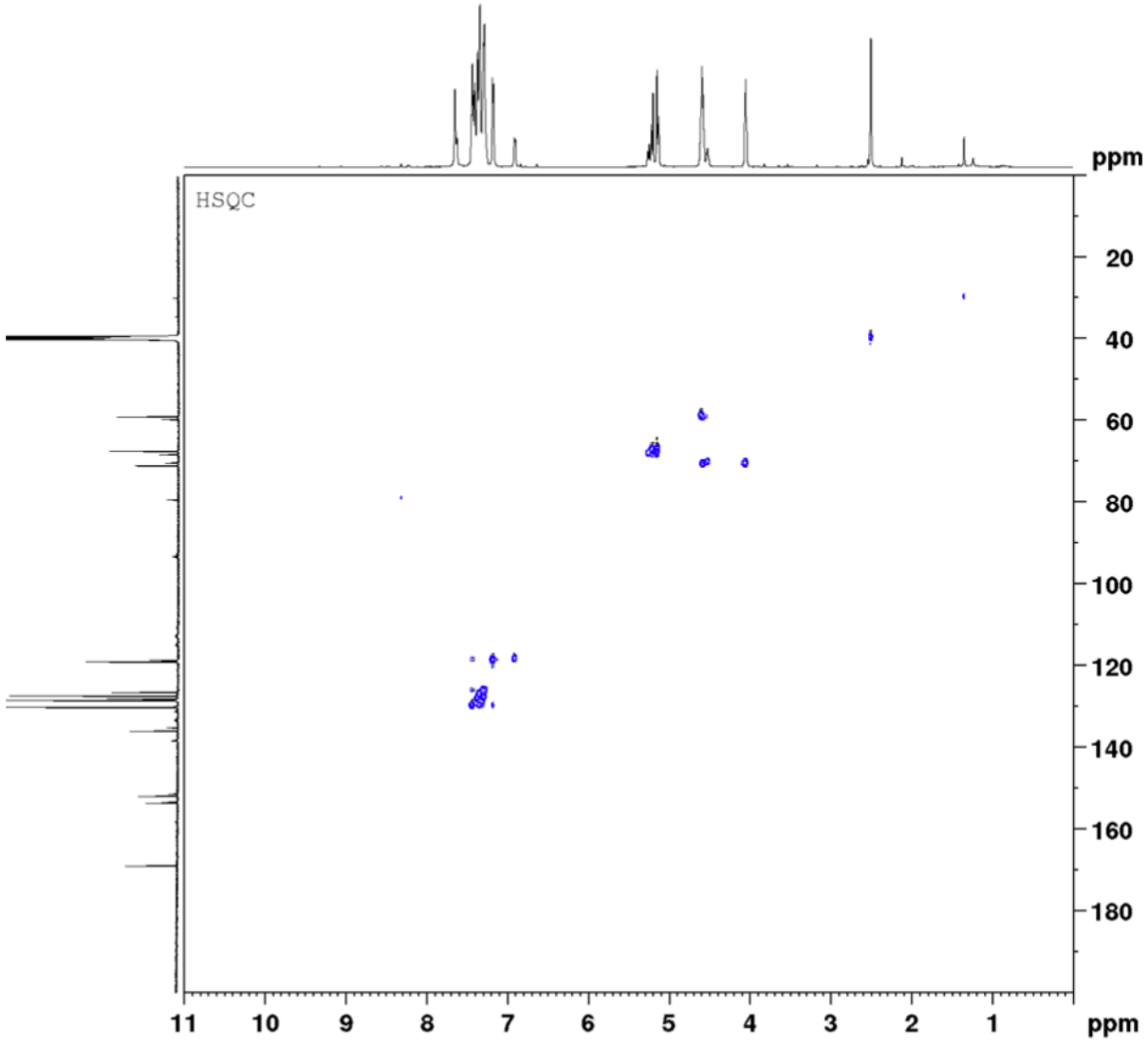
^{19}F NMR of compound **2.11a** (DMSO-d_6, 282 MHz)



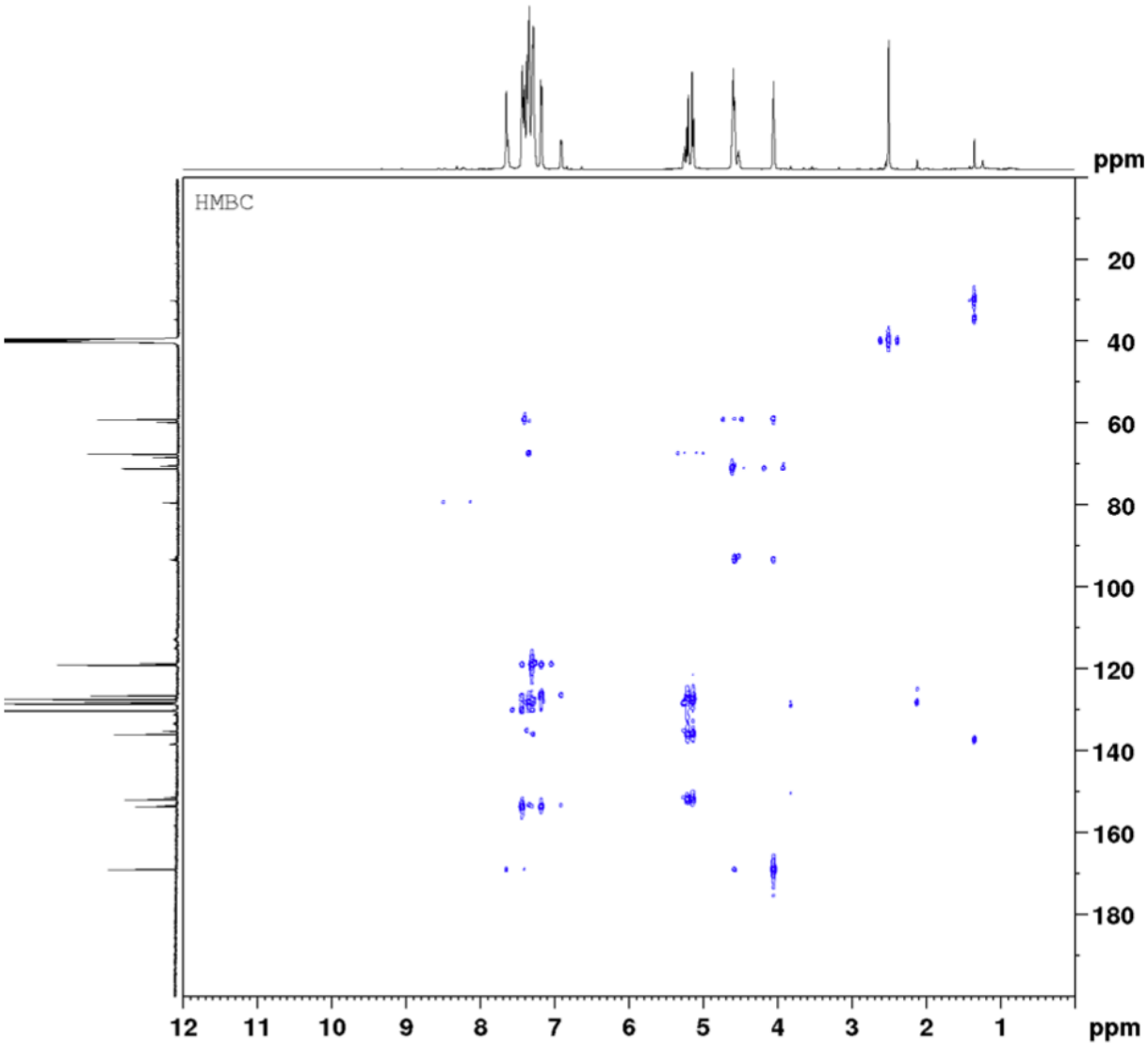
COSY spectrum of compound **2.11a** (DMSO-d_6)



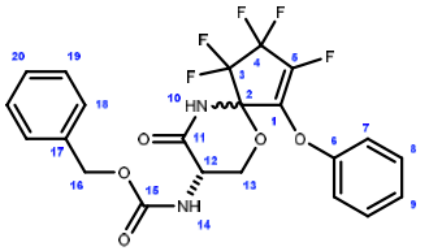
HSQC spectrum of compound **2.11a** (DMSO-d_6)



HMBC spectrum of compound **2.11a** (DMSO-d_6)

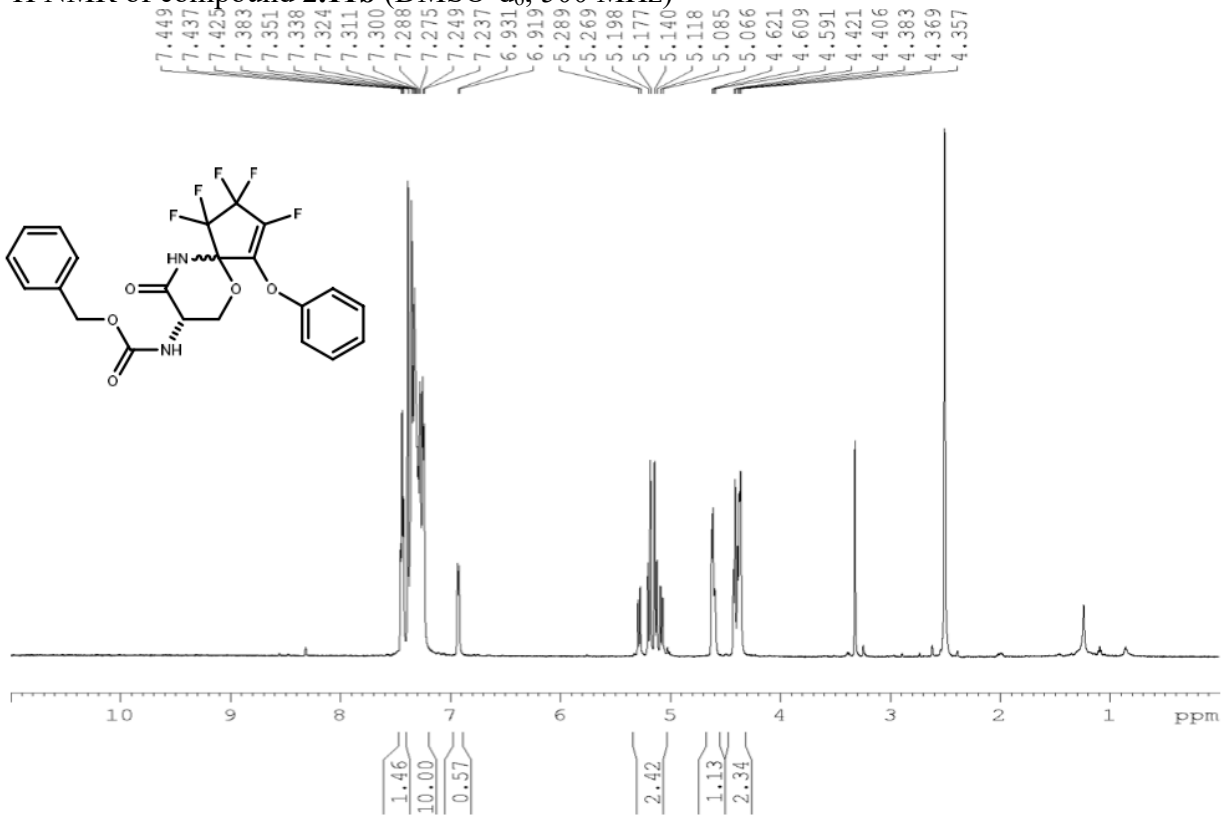


Compound **2.11a** (DMSO-d_6)

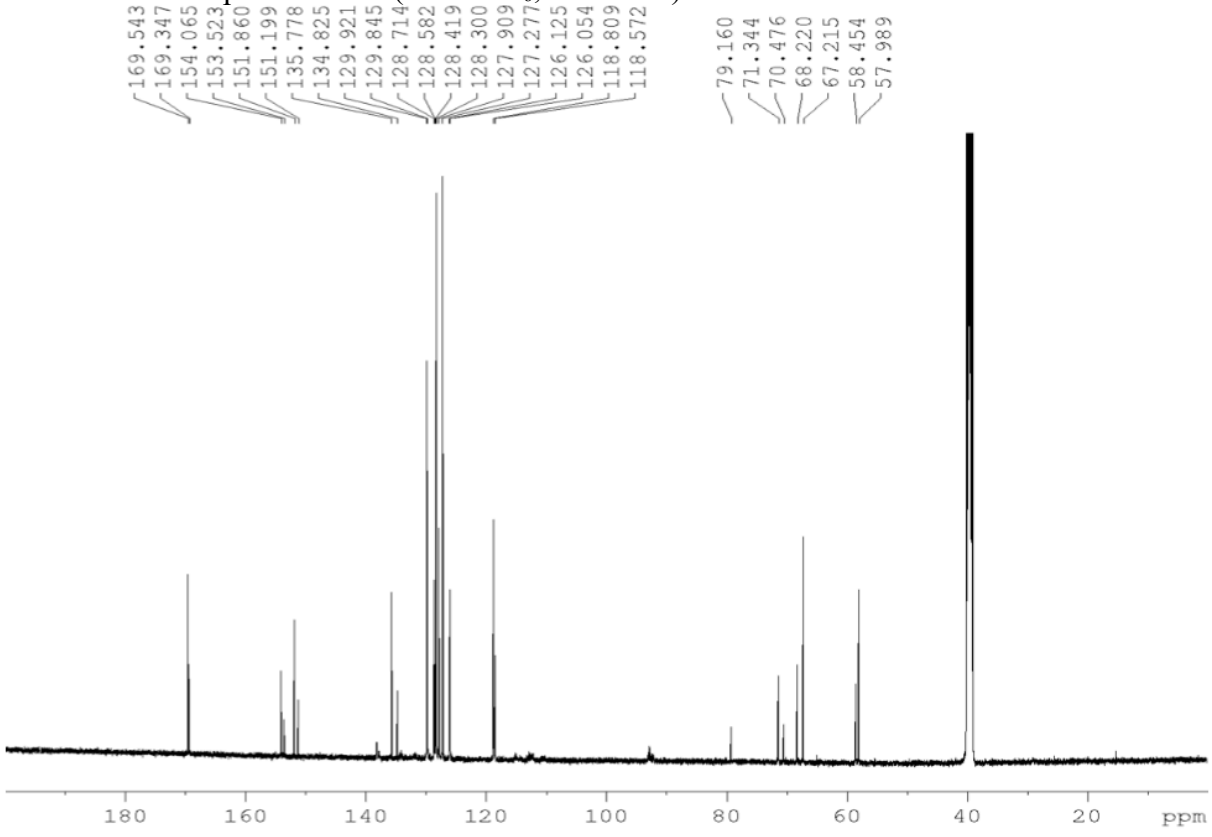


	13C	1H	Key Correlation
1	138.5 – 137.9 (m)	-	-
2	93.5 – 92.8 (m)	-	HMBC 13 -> 2
3	115.2 – 109.8 (m)	-	-
4	115.2 – 109.8 (m)	-	-
5	133.7 – 130.5 (m)	-	-
6	153.5	-	HMBC 7 -> 6
7	118.9, 118.5	7.18 (d, J = 7.5 Hz, 1.5H), 6.90 (J = 6.9 Hz, 0.5H)	HMBC 7 -> 6
8	130.0	7.50 -7.22 (m, 2H) overlap	HMBC 7 -> 8
9	126.4	7.50 -7.22 (m, 1H) overlap	HMBC 7 -> 9
10	-	7.4 (bs, 1H)	HMBC 10 -> 11,12
11	168.9 (major), 168.8 (minor)	-	HMBC 12,13 -> 11
12	58.9	4.64 – 4.50 (m, 2H)	COSY 12 -> 13; HMBC 13 ->12
13	70.9 (major), 70.2 (minor)	4.64 – 4.50 (m, 2H), 4.10 – 4.02 (m, 1H)	COSY 12 -> 13; HMBC 13 ->12
14	-	7.64 (bs, 1H)	HMBC 14 -> 11
15	151.8 (major), 151.3 (minor)	-	HMBC 16 -> 15
16	68.2 (minor), 67.3 (major)	5.30 – 5.10 (m, 2H)	HMBC 16 -> 15,17
17	135.9 (major), 135 (minor)	-	HMBC 16 -> 17
18	127.2	7.50 -7.22 (m, 2H) overlap	HMBC 18 -> 16
19	128.3	7.50 -7.22 (m, 2H) overlap	HMBC 19 -> 15,17
20	127.9	7.50 -7.22 (m, 1H) overlap	HMBC 20 -> 15,17

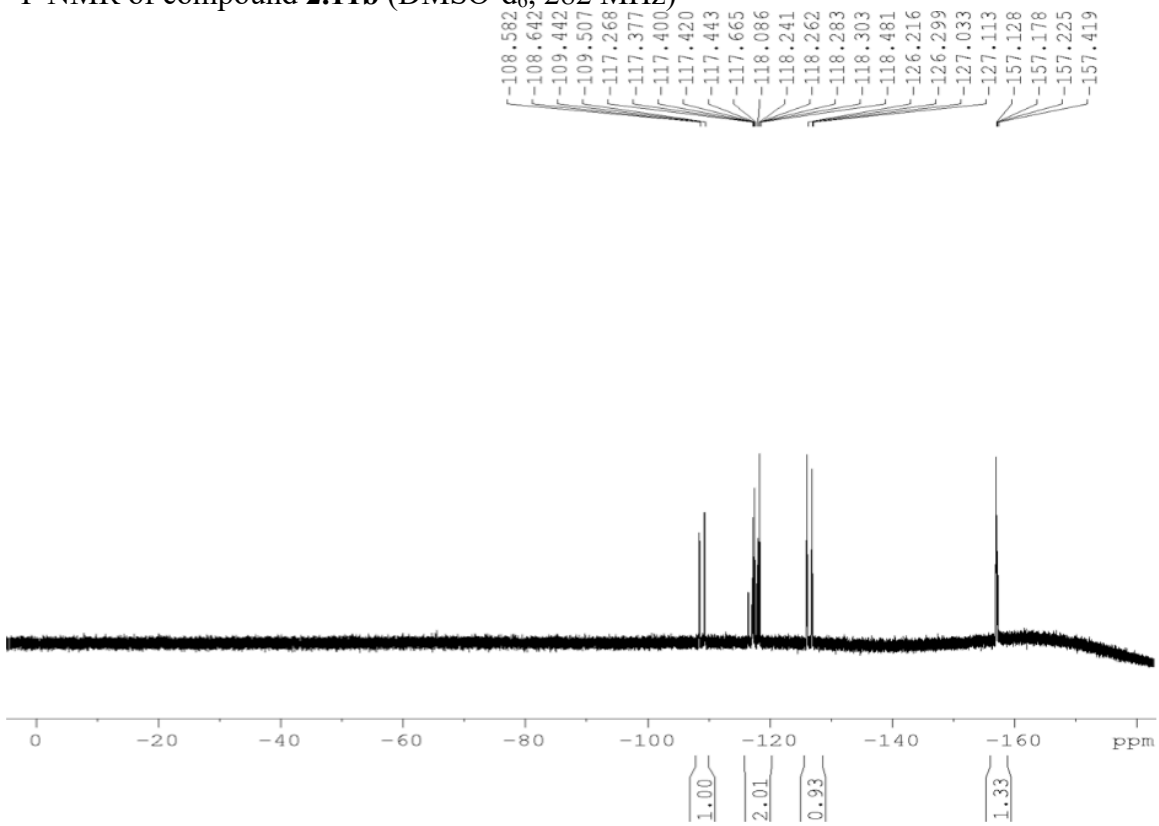
^{1}H NMR of compound **2.11b** (DMSO-d_6, 500 MHz)



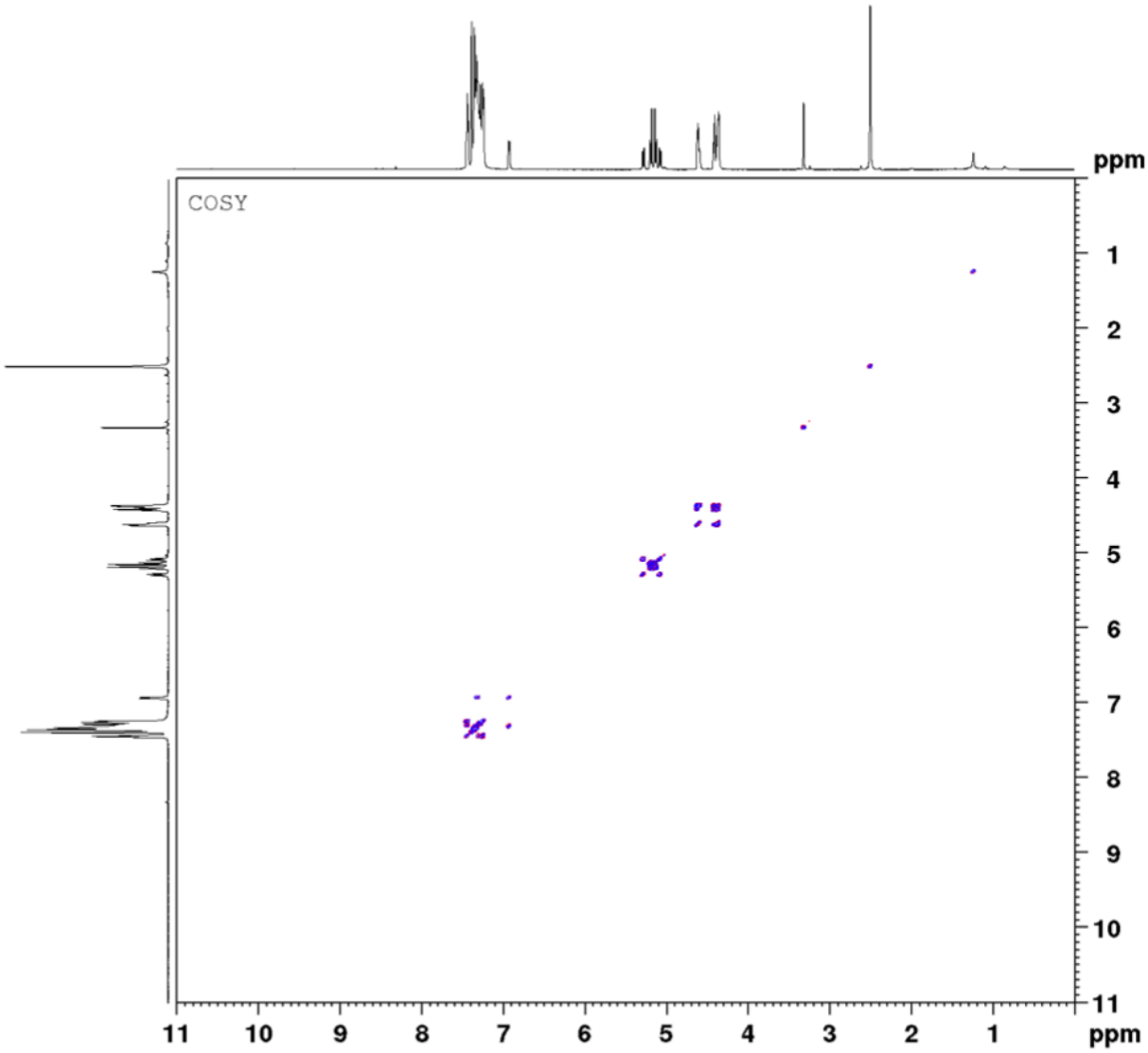
^{13}C NMR of compound **2.11b** (DMSO-d_6, 126 MHz)



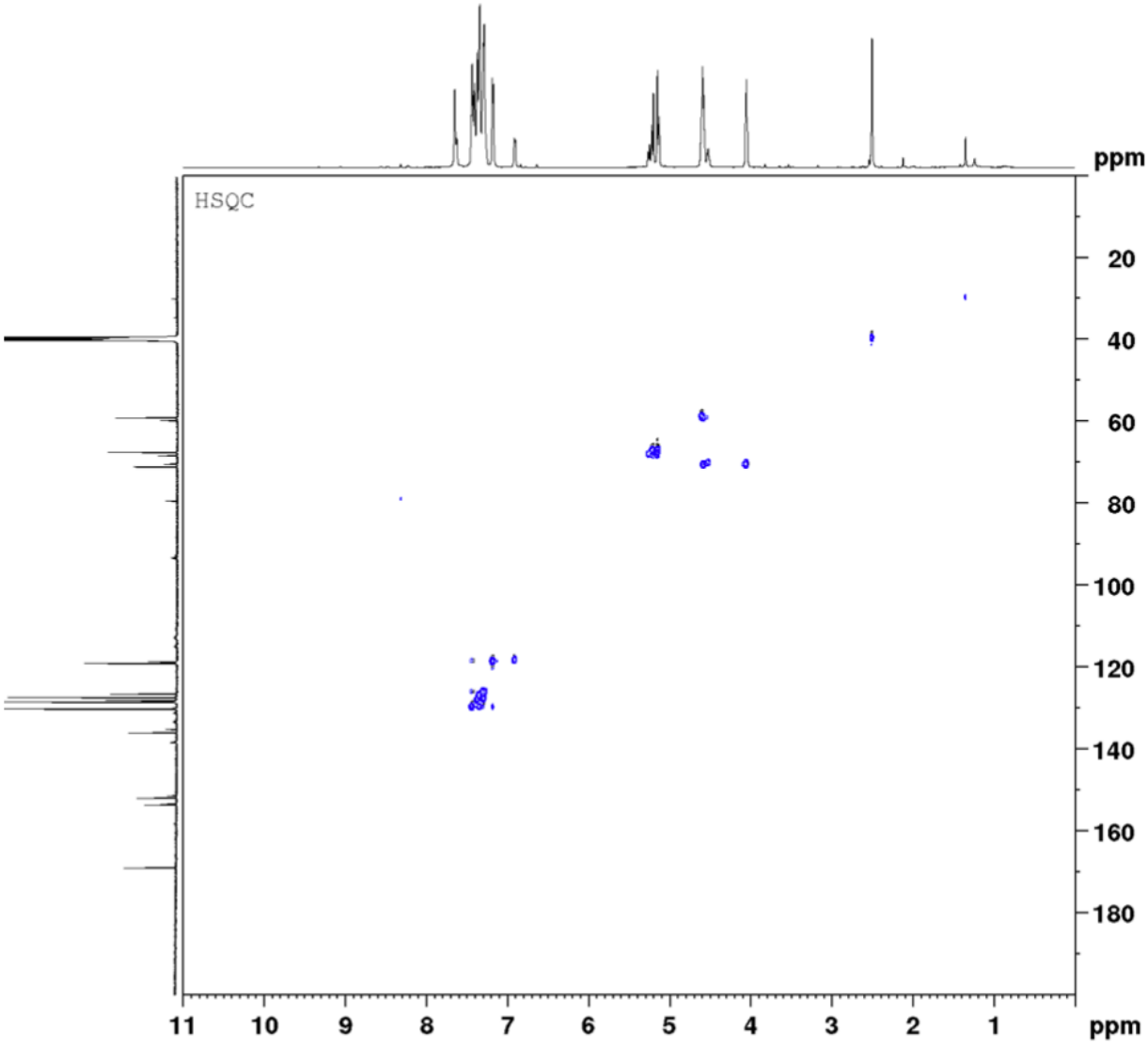
^{19}F NMR of compound **2.11b** (DMSO-d_6, 282 MHz)



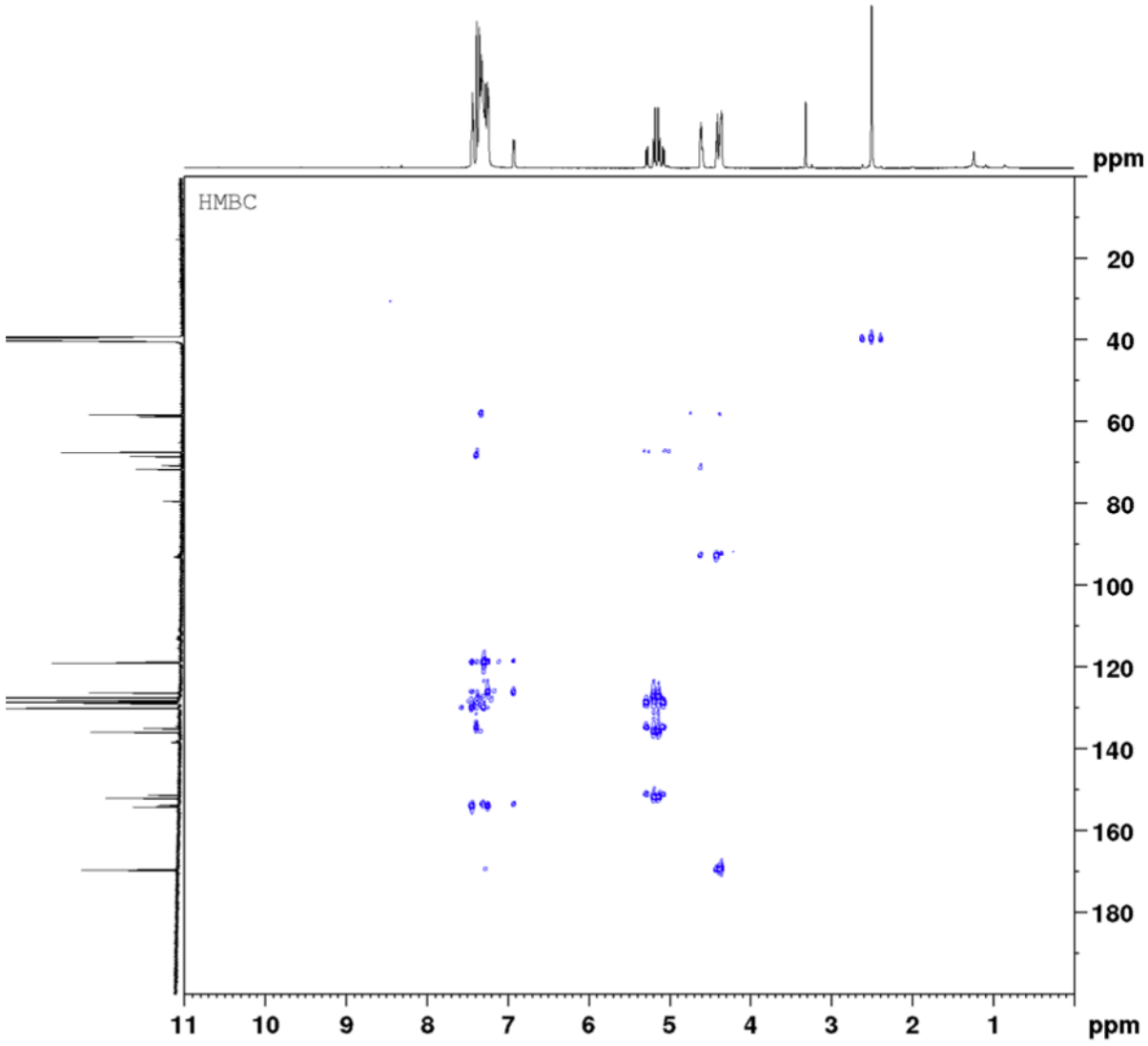
COSY spectrum of compound **2.11b** (DMSO-d_6)



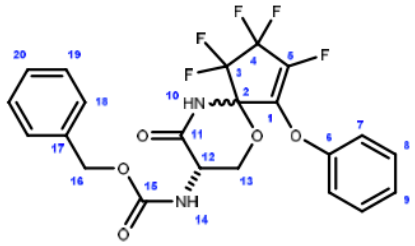
HSQC spectrum of compound **2.11b** (DMSO-d_6)



HMBC spectrum of compound **2.11b** (DMSO-d_6)

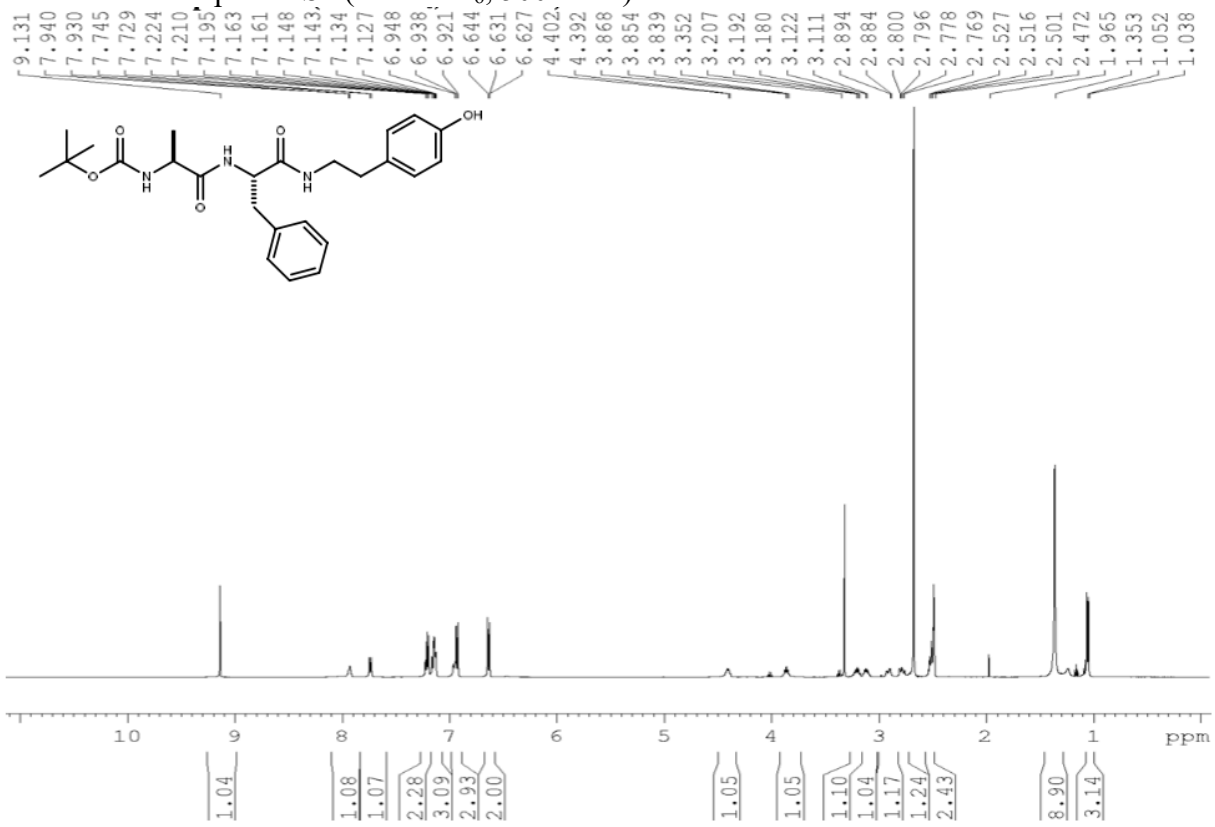


Compound **2.11b** (DMSO-d_6)

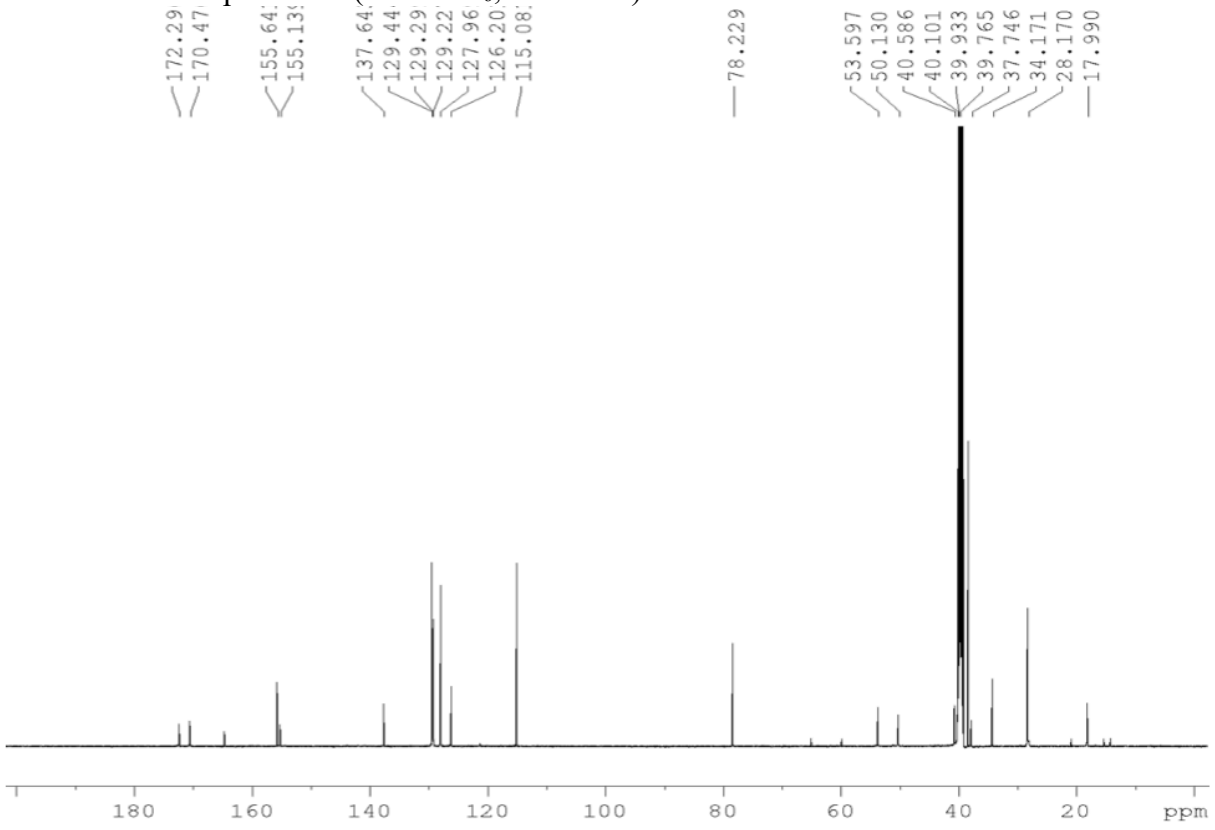


	13C	1H	Key Correlation
1	138.4 - 137.8 (m)	-	-
2	93.1 – 91.7 (m)	-	HMBC 13 -> 2
3	115.7 – 109.8 (m)	-	-
4	115.7 – 109.8 (m)	-	-
5	134.7 – 131.3 (m)	-	-
6	154.1 (major), 153.5 (minor)	-	HMBC 7 -> 6
7	118.8, 118.6	7.40 – 7.20 (m, 1.5H) overlap, 6.92 (d, J = 7.5, 0.5H)	HMBC 7 -> 6
8	129.9, 129.8	7.40 – 7.20 (m, 0.5H) overlap, 7.44 (t, J = 7.23 Hz, 1.5H)	HMBC 7 -> 8
9	126.1, 126.1	7.40 – 7.20 (m, 1H) overlap	HMBC 7 -> 9
10	-	7.40 – 7.20 (m, 1H) overlap	HMBC 10 -> 11
11	169.5 (major), 169.3 (minor)	-	HMBC 10,12 -> 11
12	58.5 (minor), 58 (major)	4.65 – 4.56 (m, 1H)	HMBC 12 -> 11,13
13	71.3 (major), 70.5 (minor)	4.45 – 4.31 (m, 2H)	HMBC 13 -> 12,11
14	-	7.40 – 7.20 (m, 1H) overlap	HMBC 14 -> 12
15	151.9 (major), 151.2 (minor)	-	HMBC 16 -> 15
16	68.2 (minor), 67.2 (major)	5.31 – 5.03 (m, 2H)	HMBC 16 -> 15,17
17	135.8 (major), 134.8 (minor)	-	HMBC 16 -> 17
18	128.6 (minor), 127.3 (major)	7.40 – 7.20 (m, 2H) overlap	HMBC 18 -> 15,17
19	128.4, 128.3	7.40 – 7.20 (m, 2H) overlap	HMBC 19 -> 15,17
20	127.9	7.40 – 7.20 (m, 2H) overlap	HMBC 20 -> 15,17

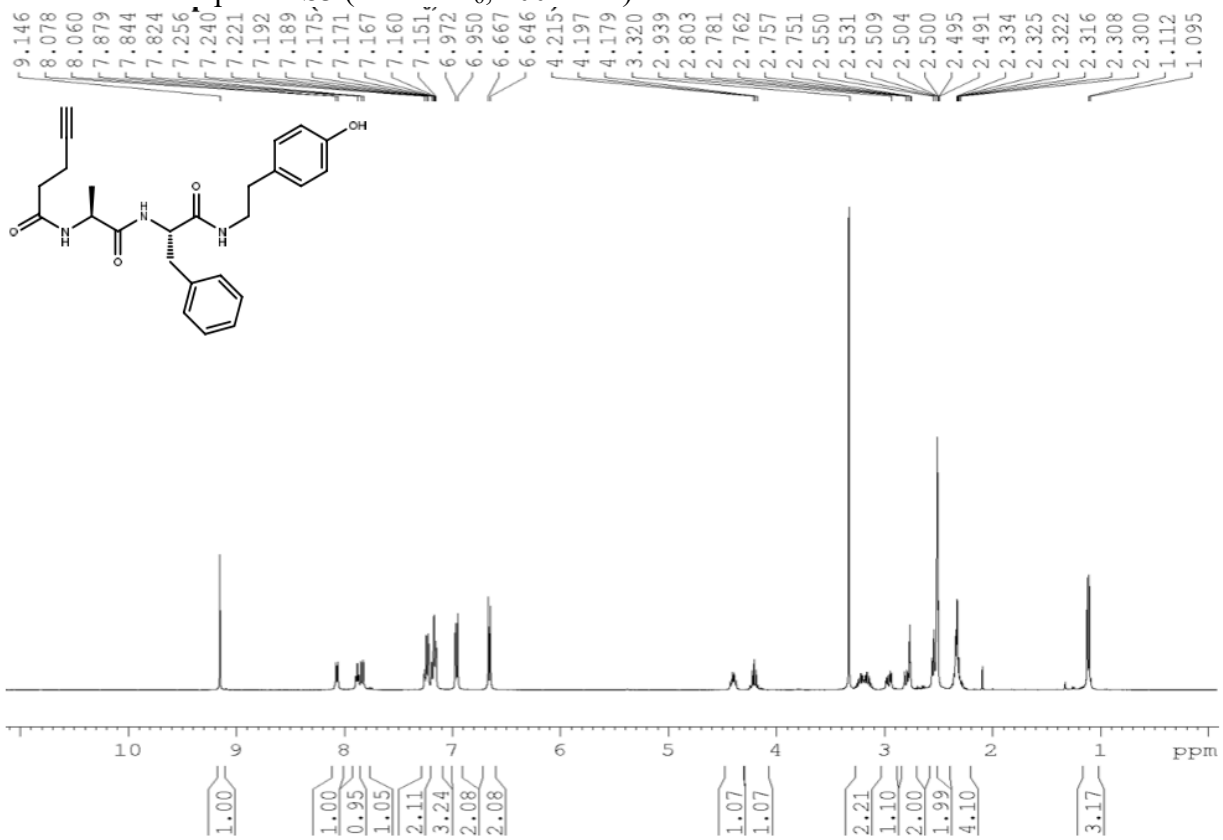
^{1}H NMR of compound **S2** (DMSO-d_6, 500 MHz)



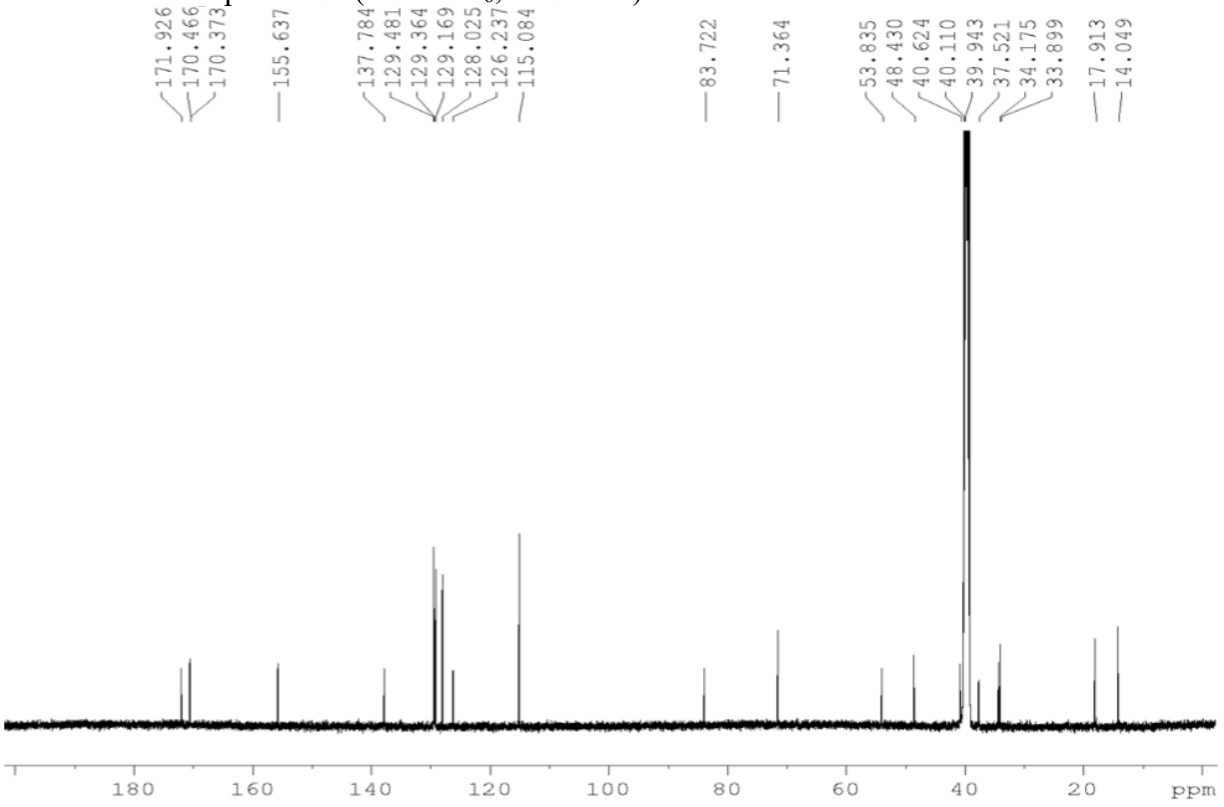
^{13}C NMR of compound **S2** (DMSO-d_6, 126 MHz)



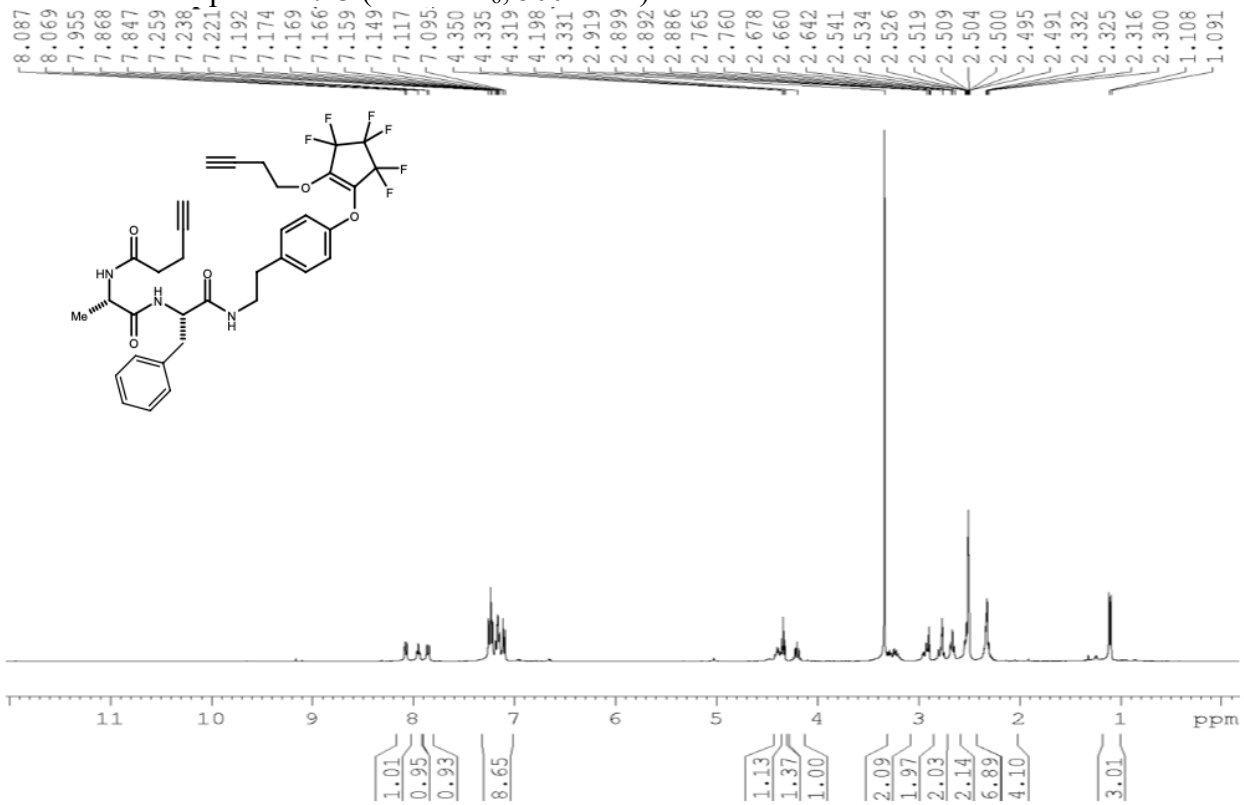
^{1}H NMR of compound **S3** (DMSO-d_6, 400 MHz)



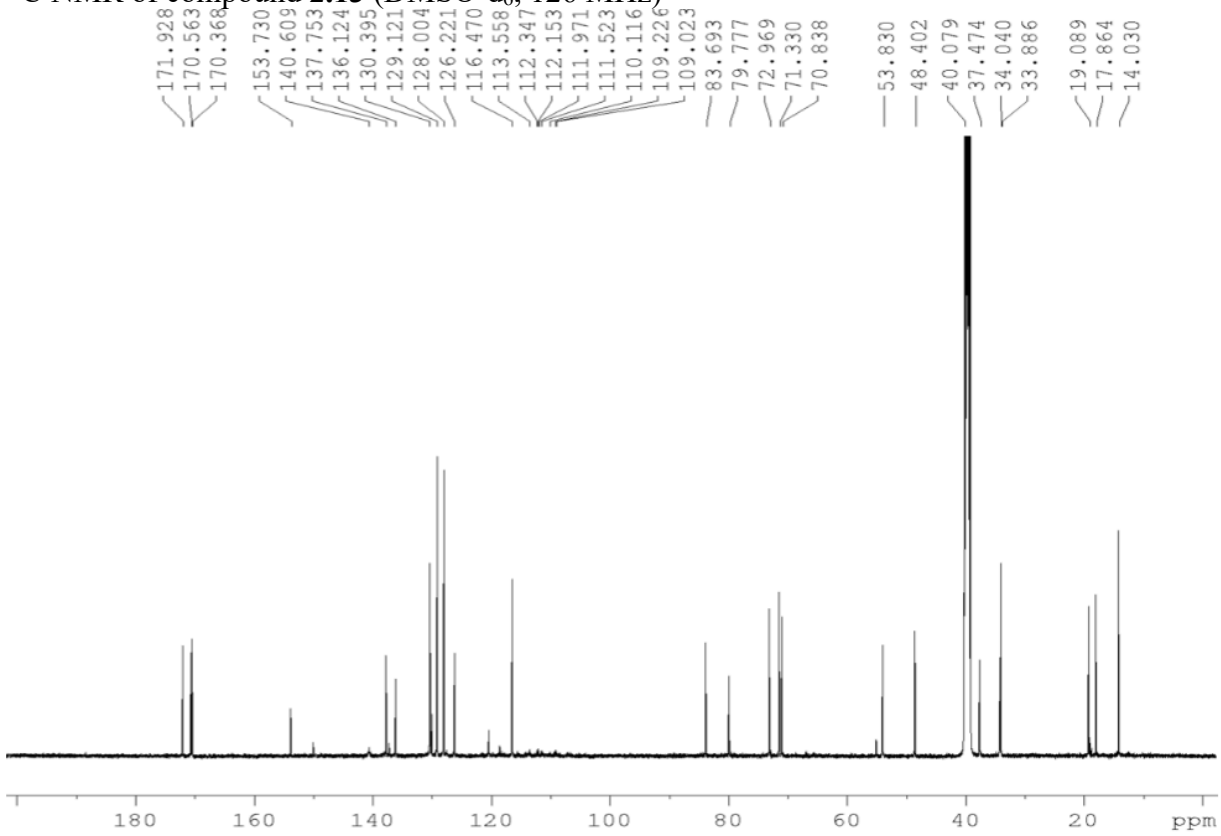
^{13}C NMR of compound **S3** (DMSO-d_6, 126 MHz)



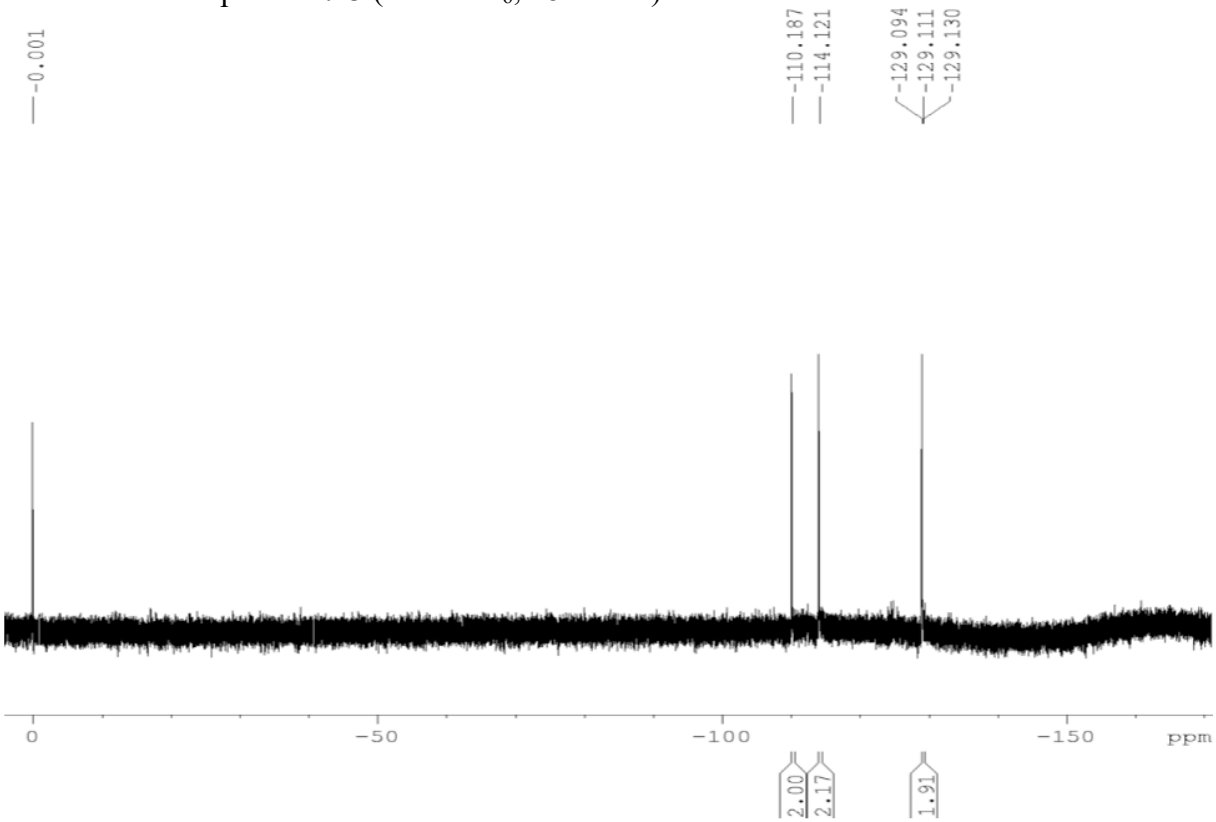
^{1}H NMR of compound **2.13** (DMSO-d_6, 500 MHz)



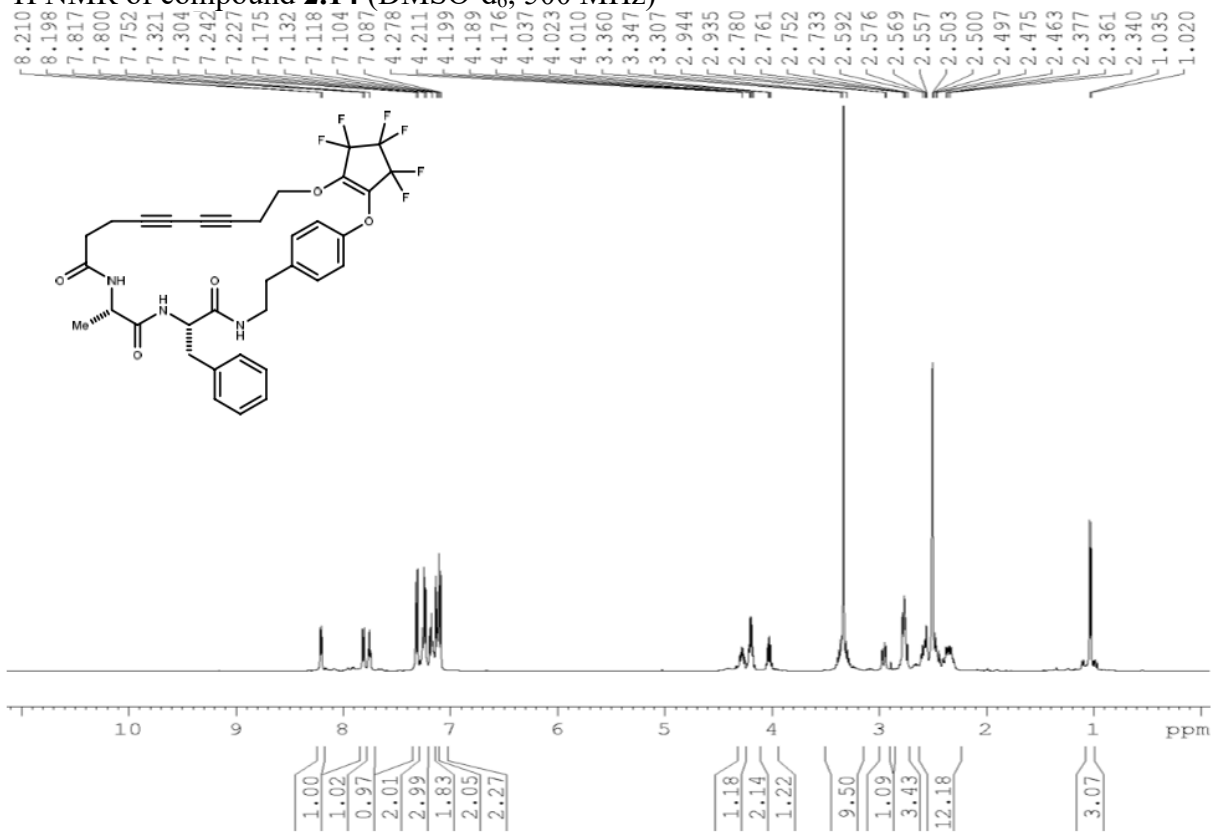
^{13}C NMR of compound **2.13** (DMSO-d_6, 126 MHz)



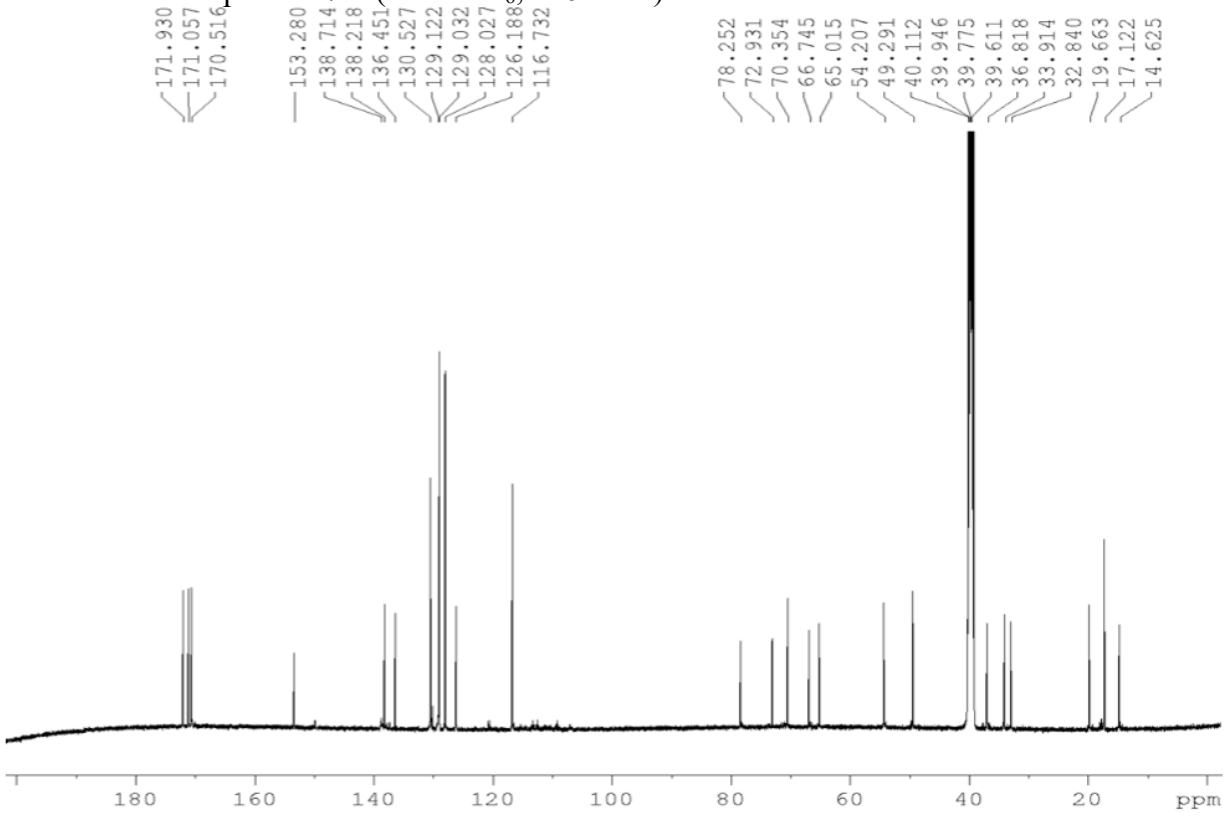
^{19}F NMR of compound **2.13** (DMSO-d_6, 282 MHz)



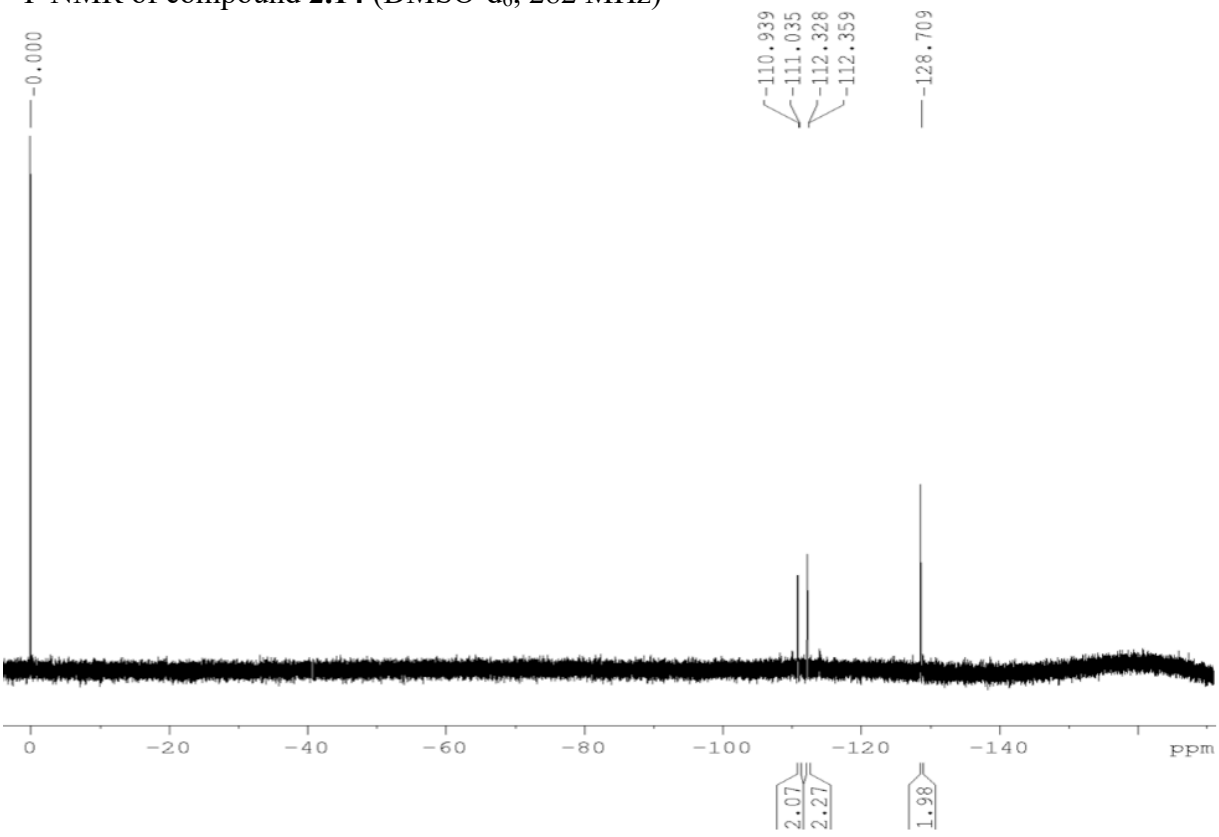
^{1}H NMR of compound **2.14** (DMSO-d_6, 500 MHz)



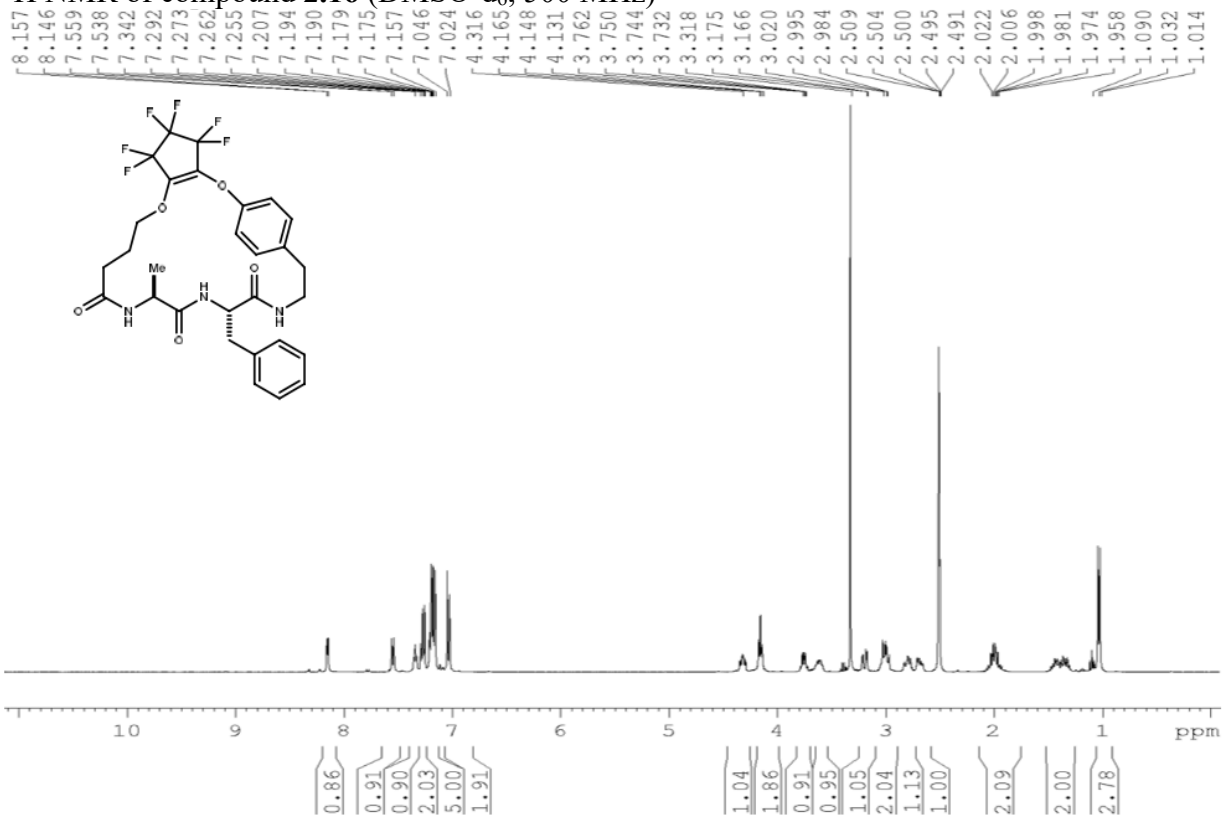
^{13}C NMR of compound **2.14** (DMSO-d_6, 126 MHz)



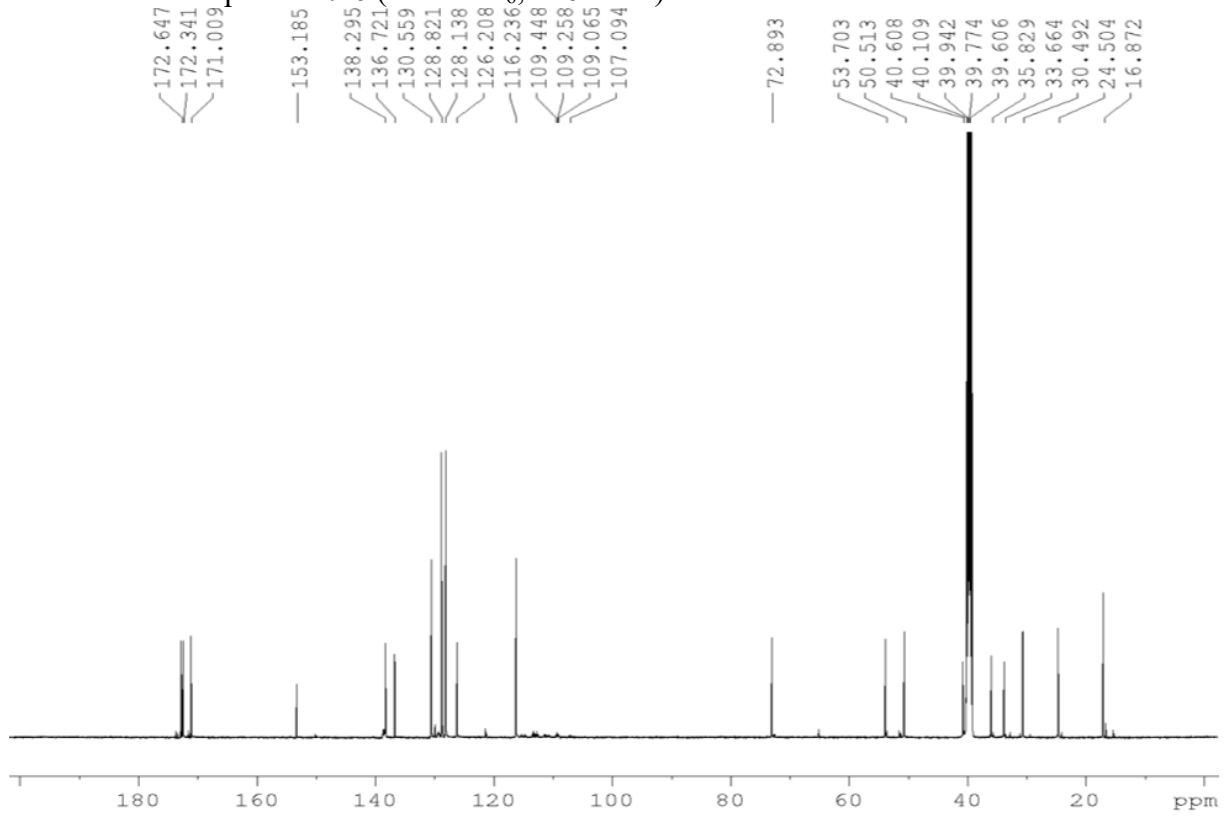
^{19}F NMR of compound **2.14** (DMSO-d_6, 282 MHz)



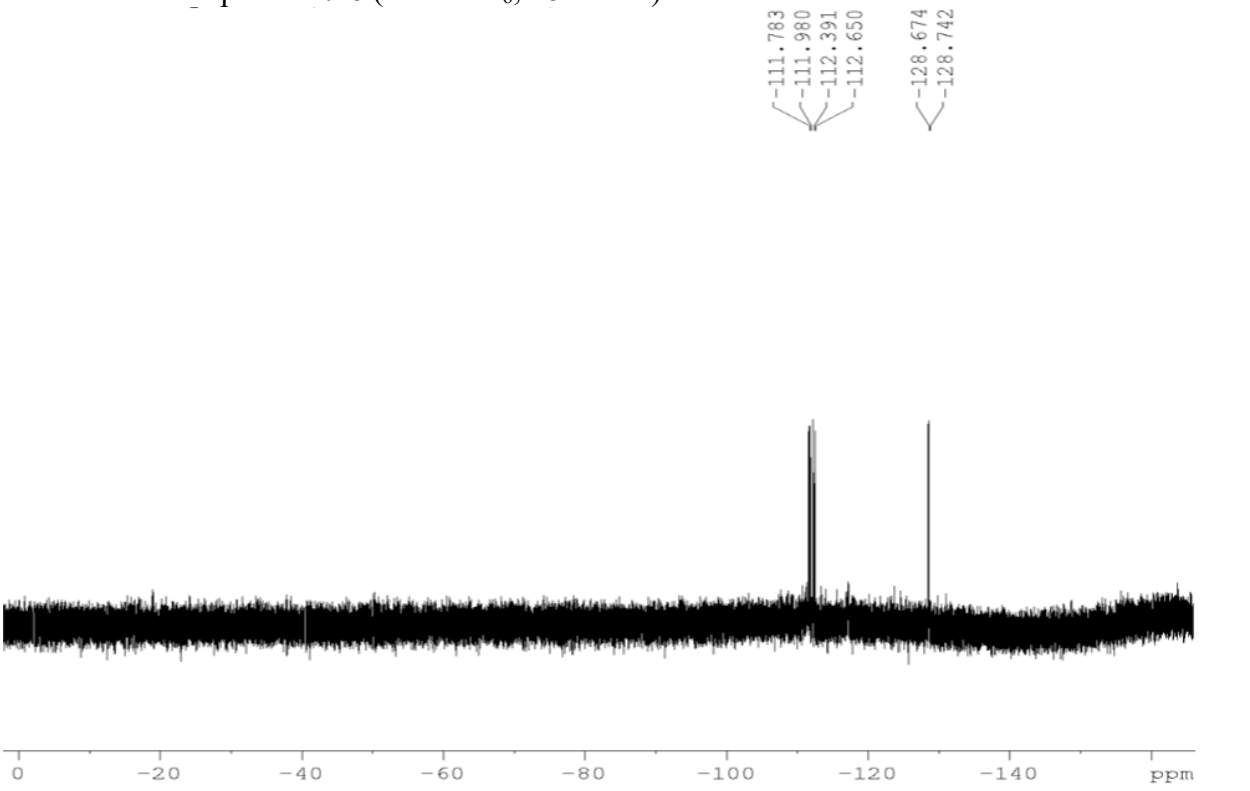
^{1}H NMR of compound **2.16** (DMSO-d_6, 500 MHz)



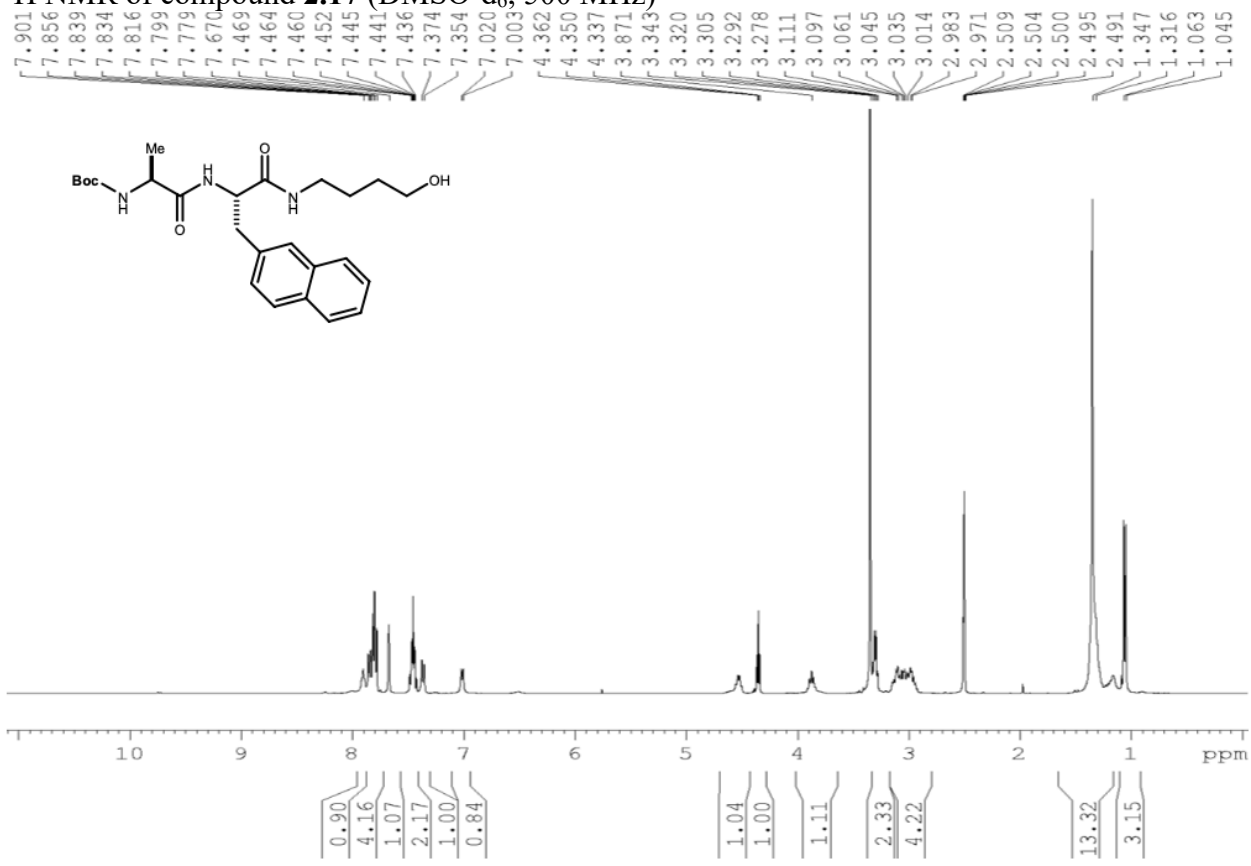
^{13}C NMR of compound **2.16** (DMSO-d_6, 126 MHz)



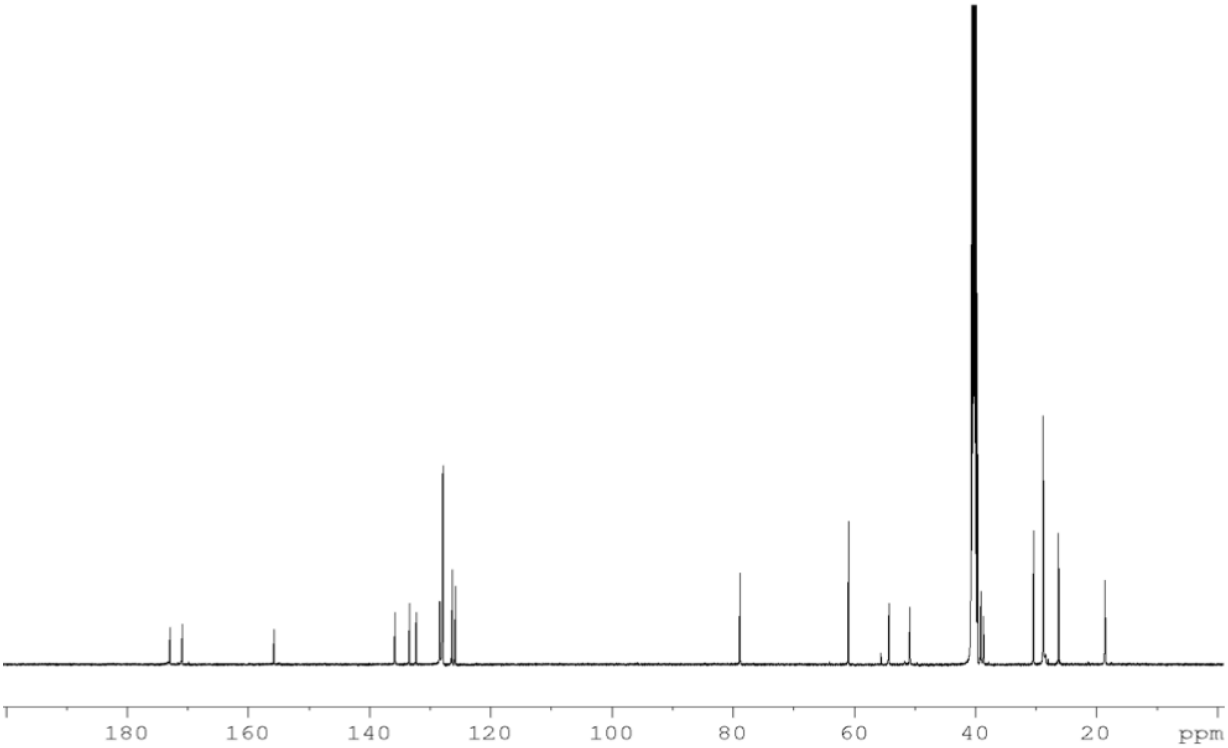
^{19}F NMR of compound **2.16** (DMSO-d_6, 282 MHz)



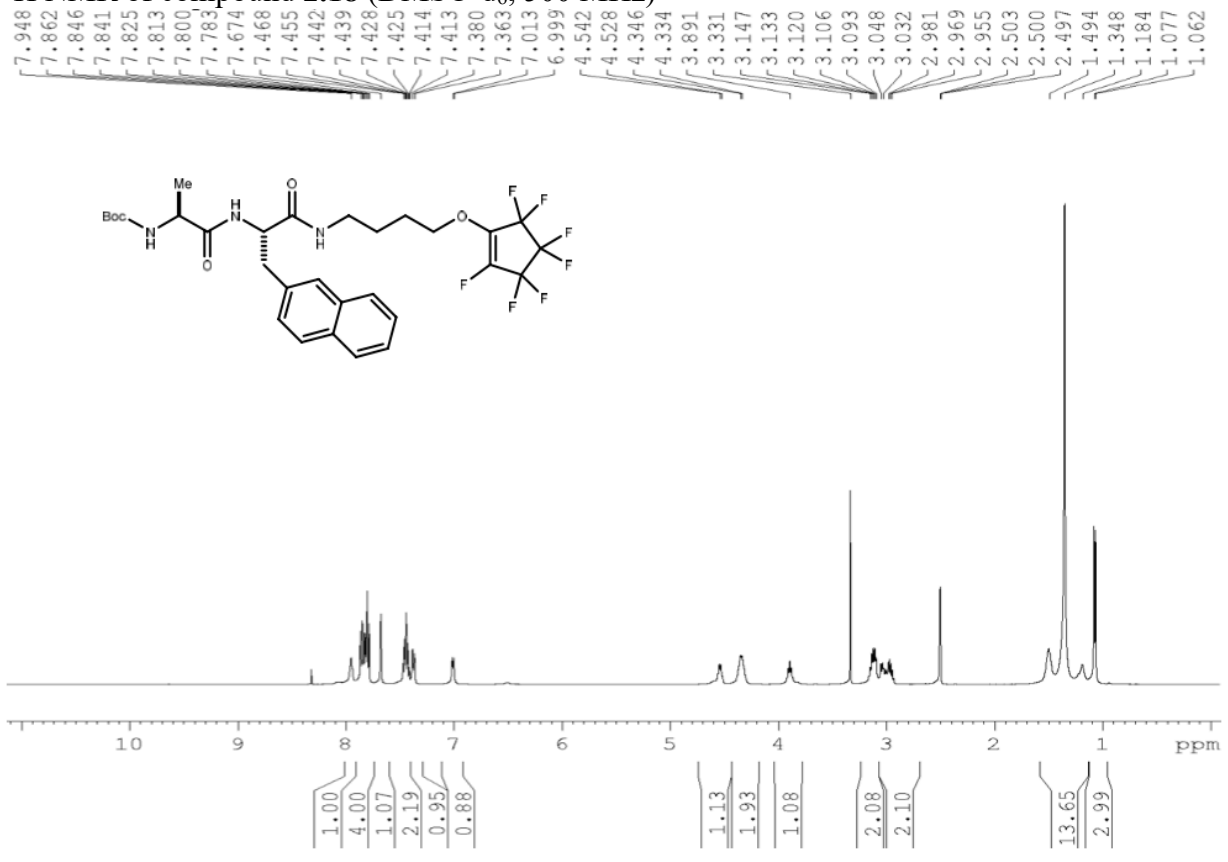
^{1}H NMR of compound **2.17** (DMSO-d_6, 500 MHz)



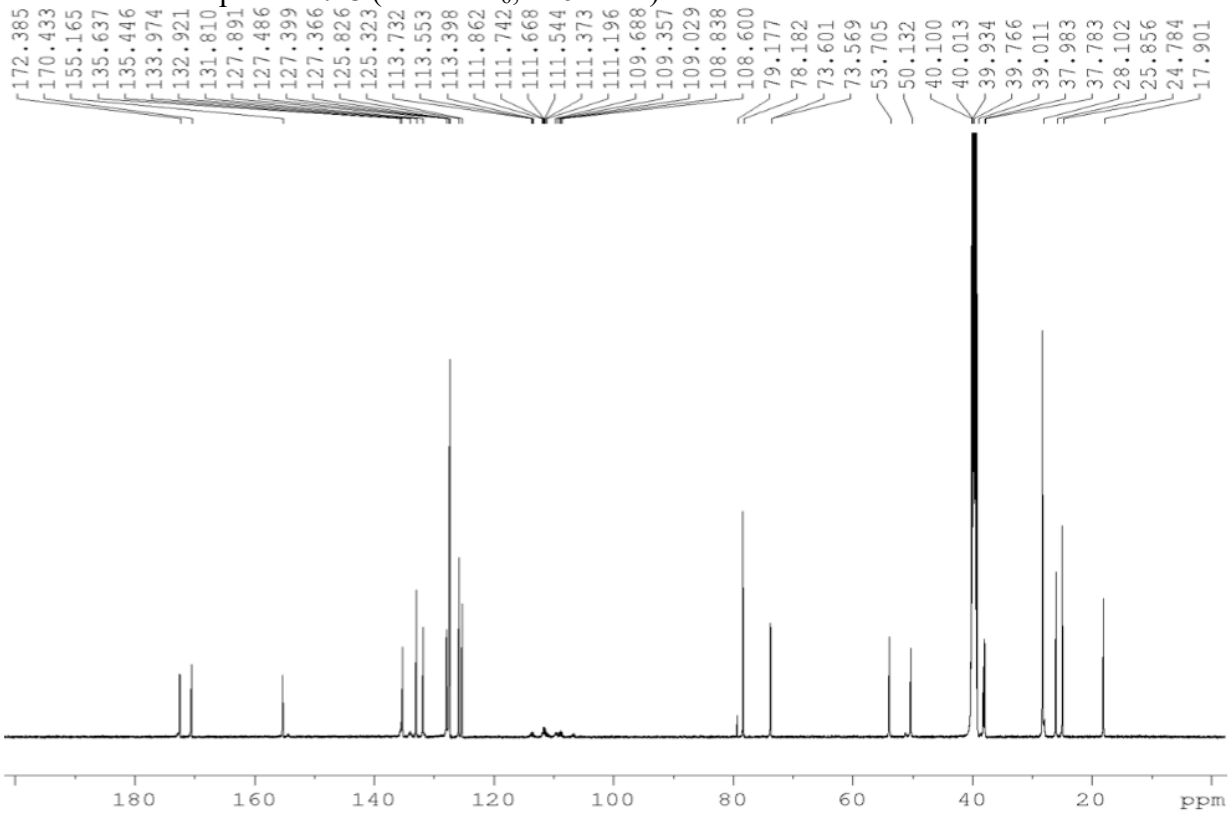
^{13}C NMR of compound **2.17** (DMSO-d_6, 126 MHz)



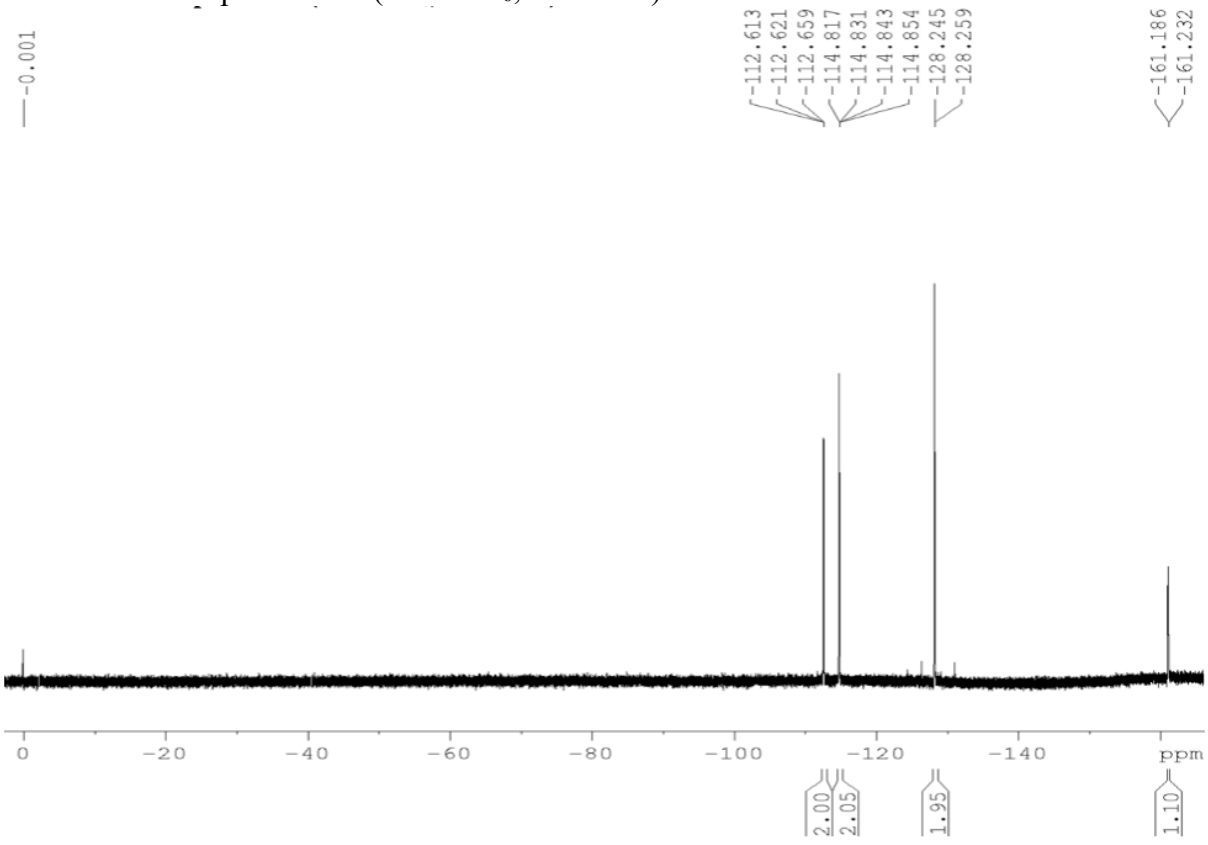
^{1}H NMR of compound **2.18** (DMSO-d_6, 500 MHz)



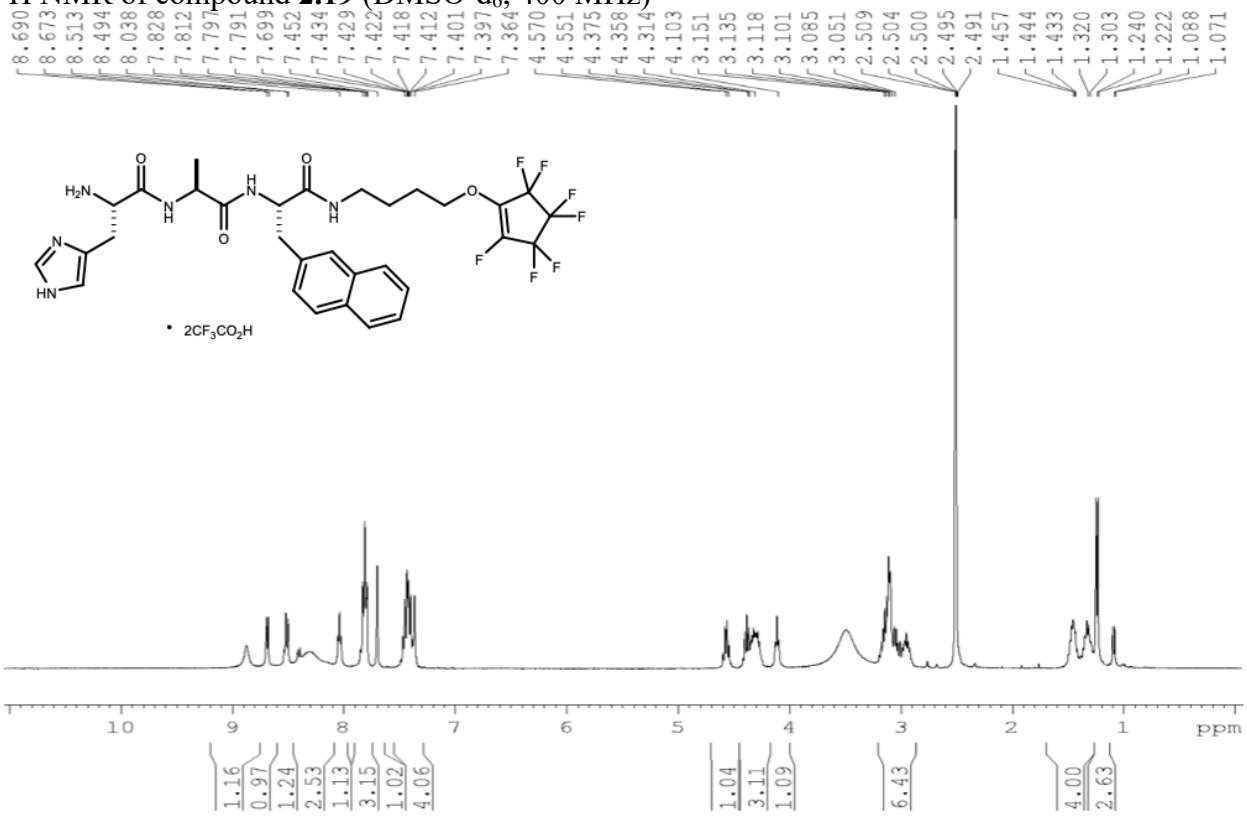
^{13}C NMR of compound **2.18** (DMSO-d_6, 126 MHz)



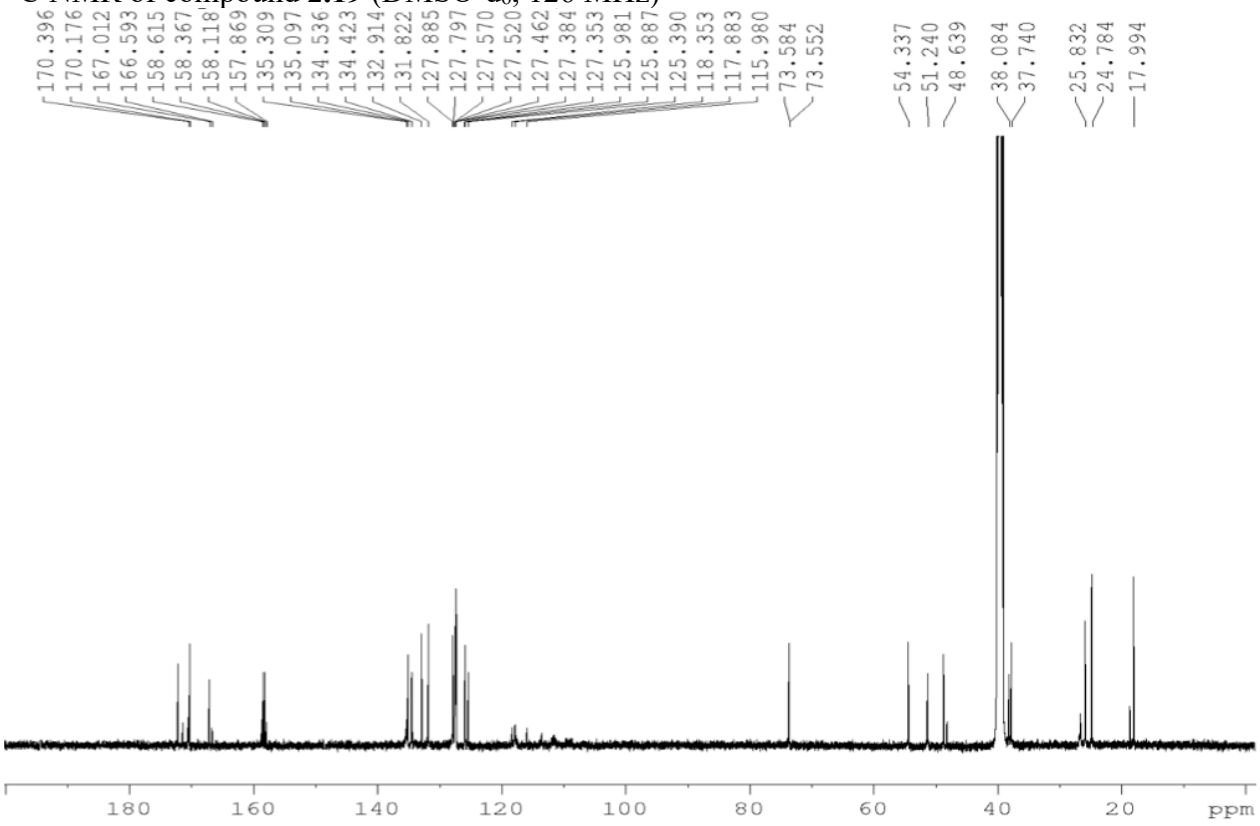
^{19}F NMR of compound **2.18** (DMSO-d_6, 282 MHz)



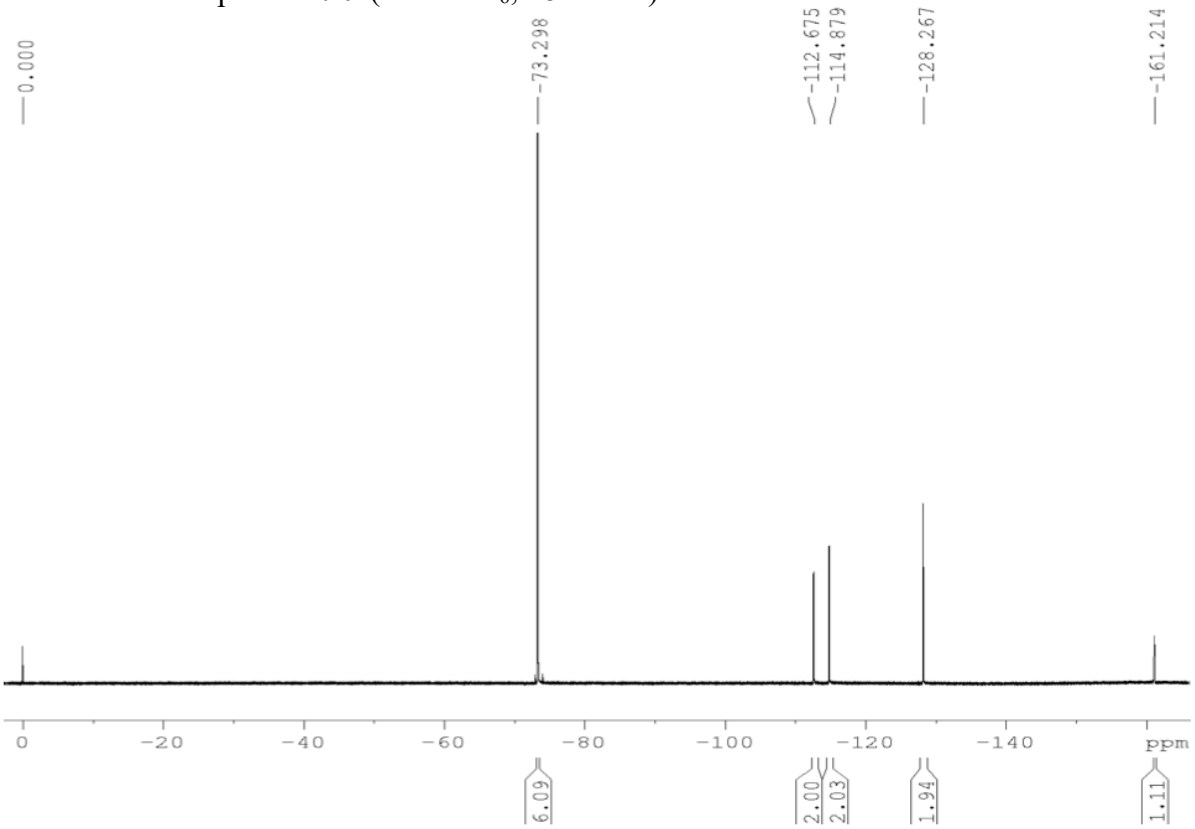
^{1}H NMR of compound **2.19** (DMSO-d_6, 400 MHz)



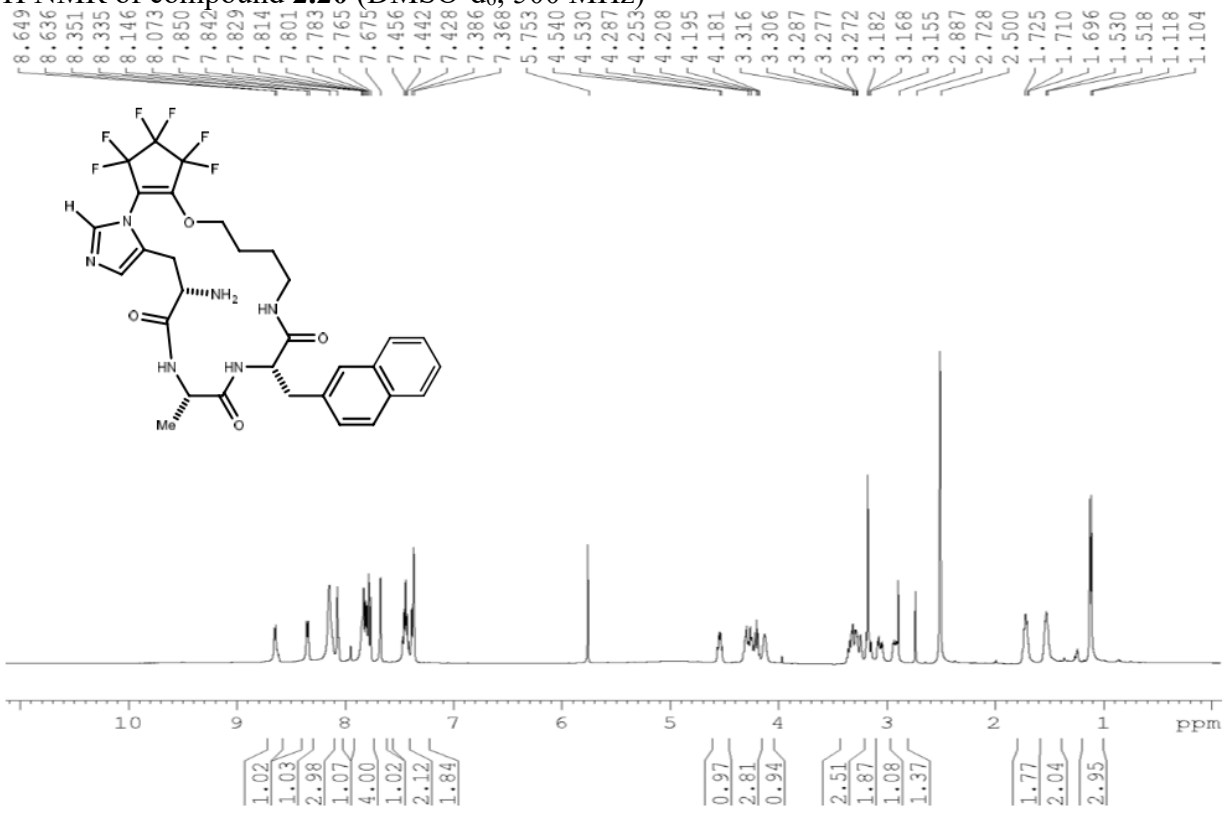
^{13}C NMR of compound **2.19** (DMSO-d_6, 126 MHz)



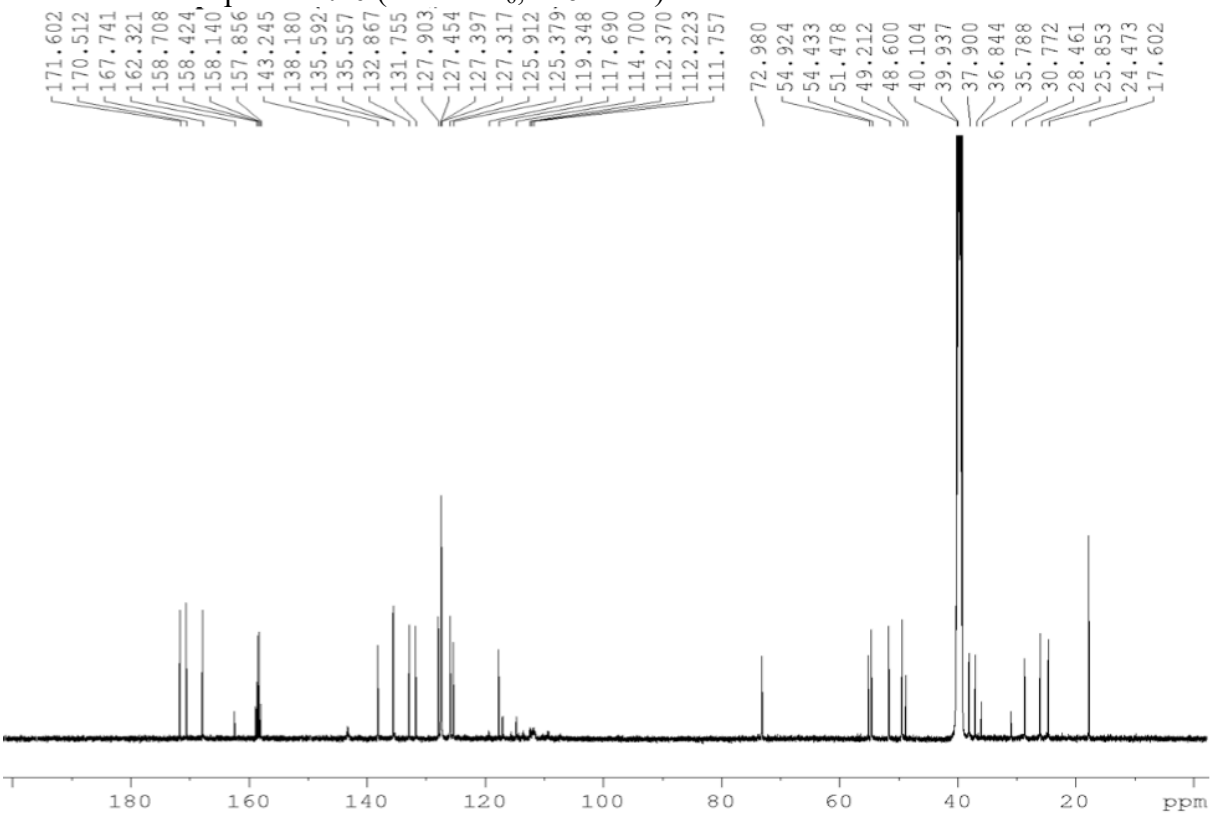
^{19}F NMR of compound **2.19** (DMSO-d_6, 282 MHz)



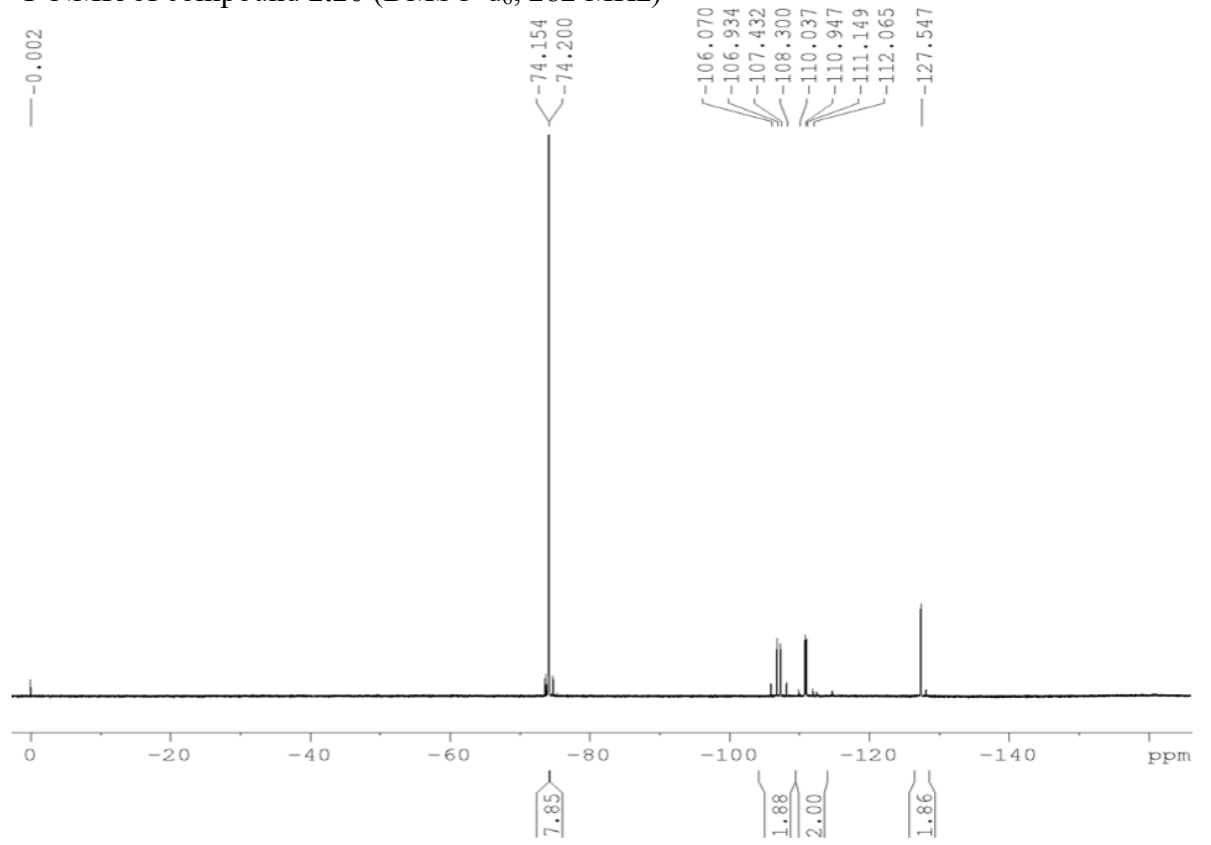
^{1}H NMR of compound **2.20** (DMSO-d_6, 500 MHz)



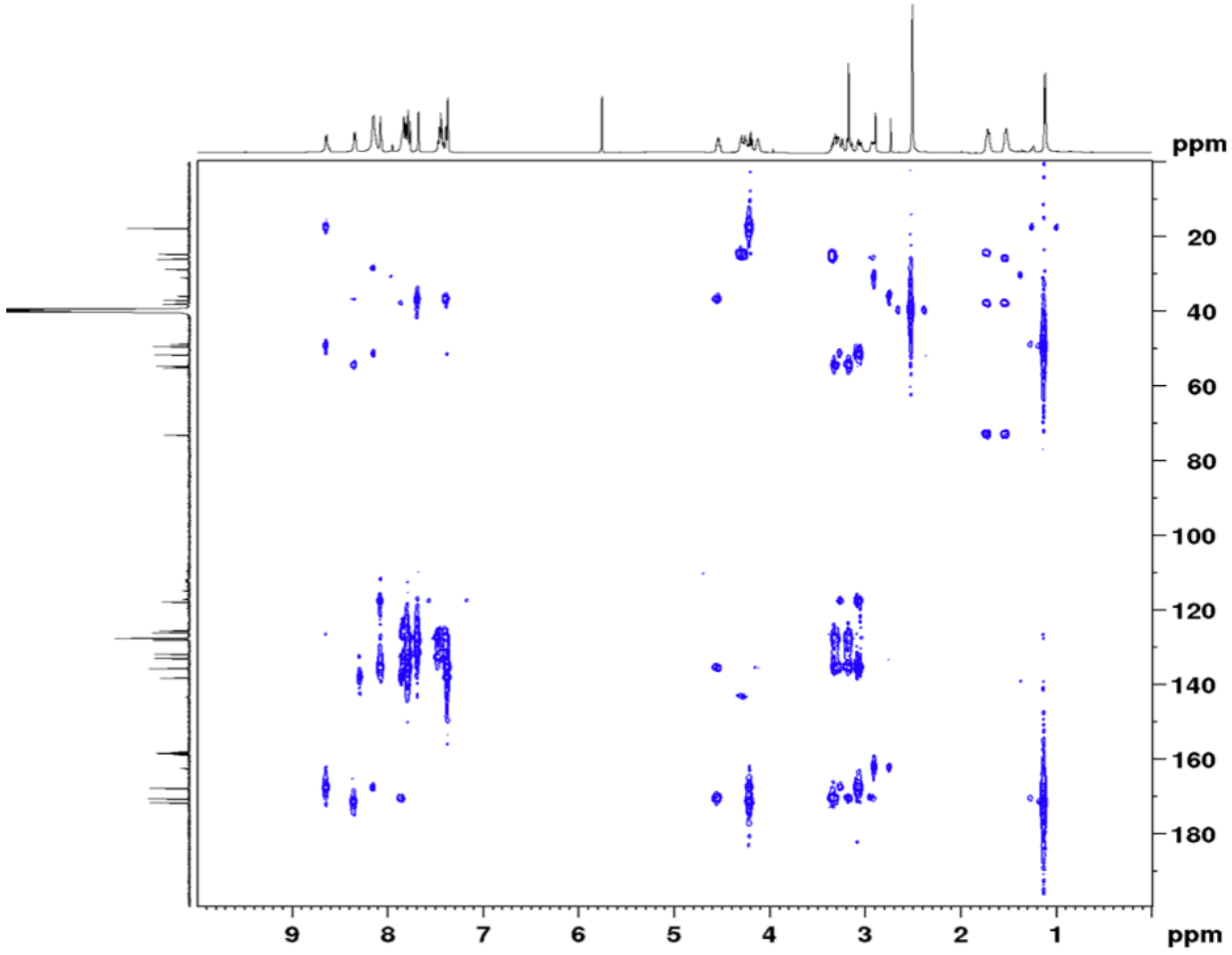
^{13}C NMR of compound **2.20** (DMSO-d_6, 126 MHz)



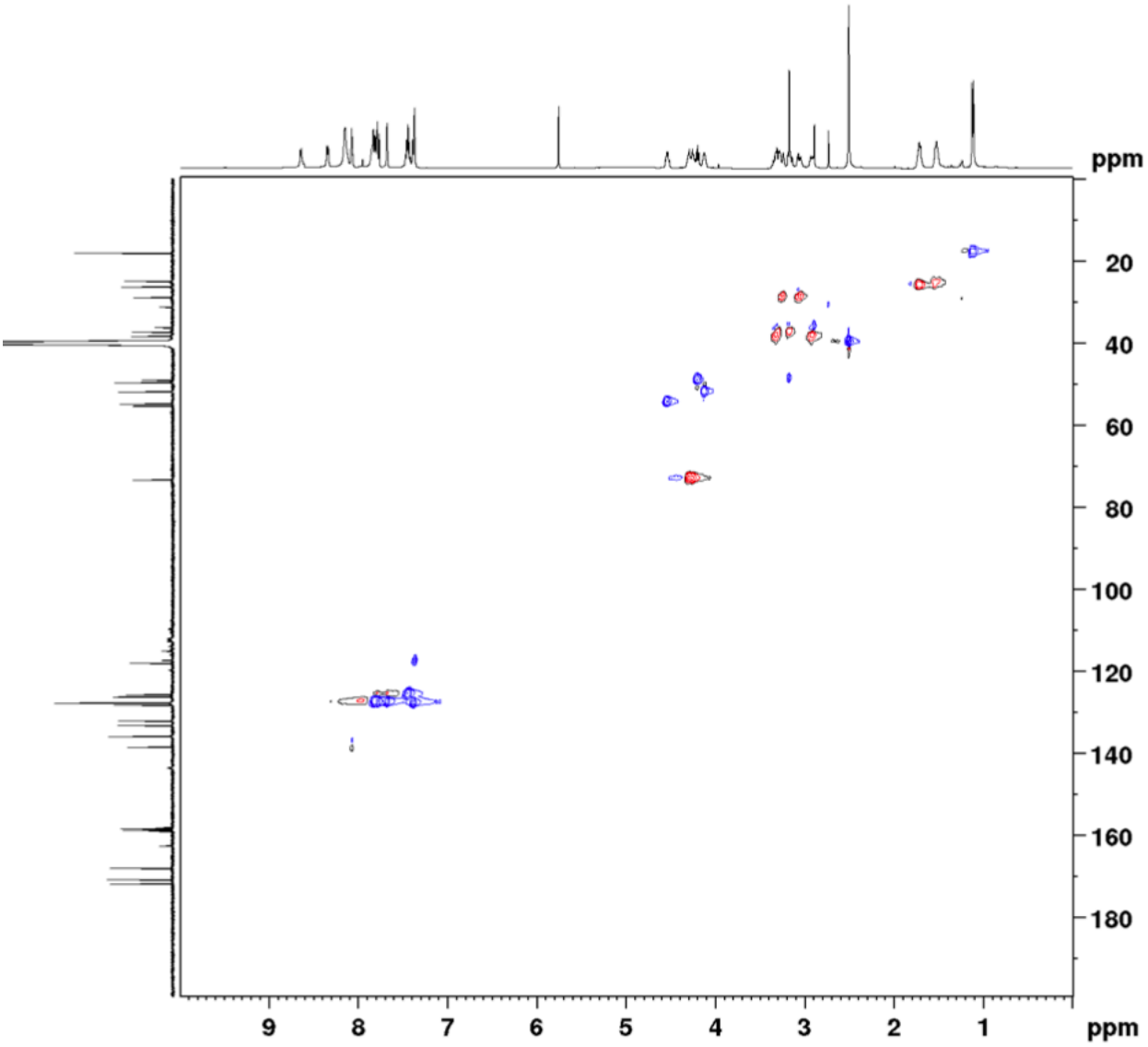
^{19}F NMR of compound **2.20** (DMSO-d_6, 282 MHz)



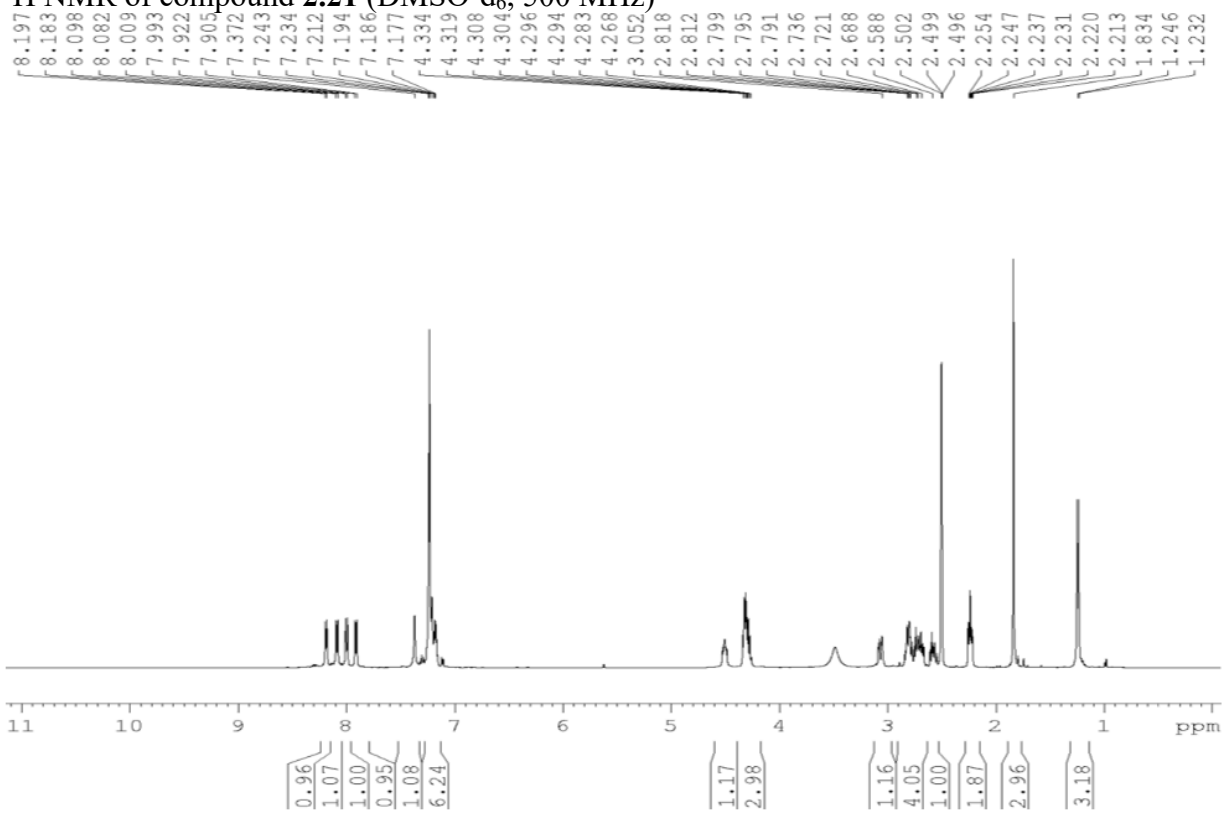
HMBC spectrum of compound **2.20** (DMSO-d_6)



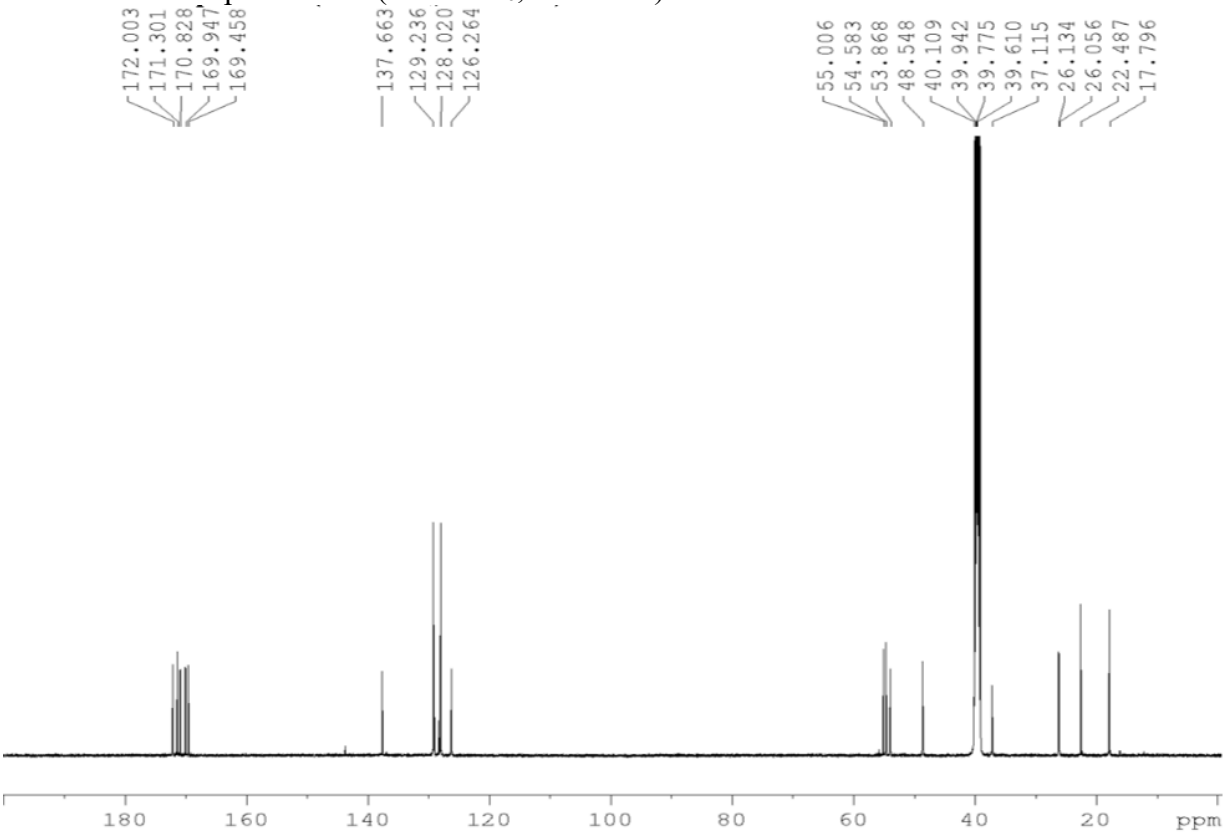
HSQC spectrum of compound **2.20** (DMSO-d_6)



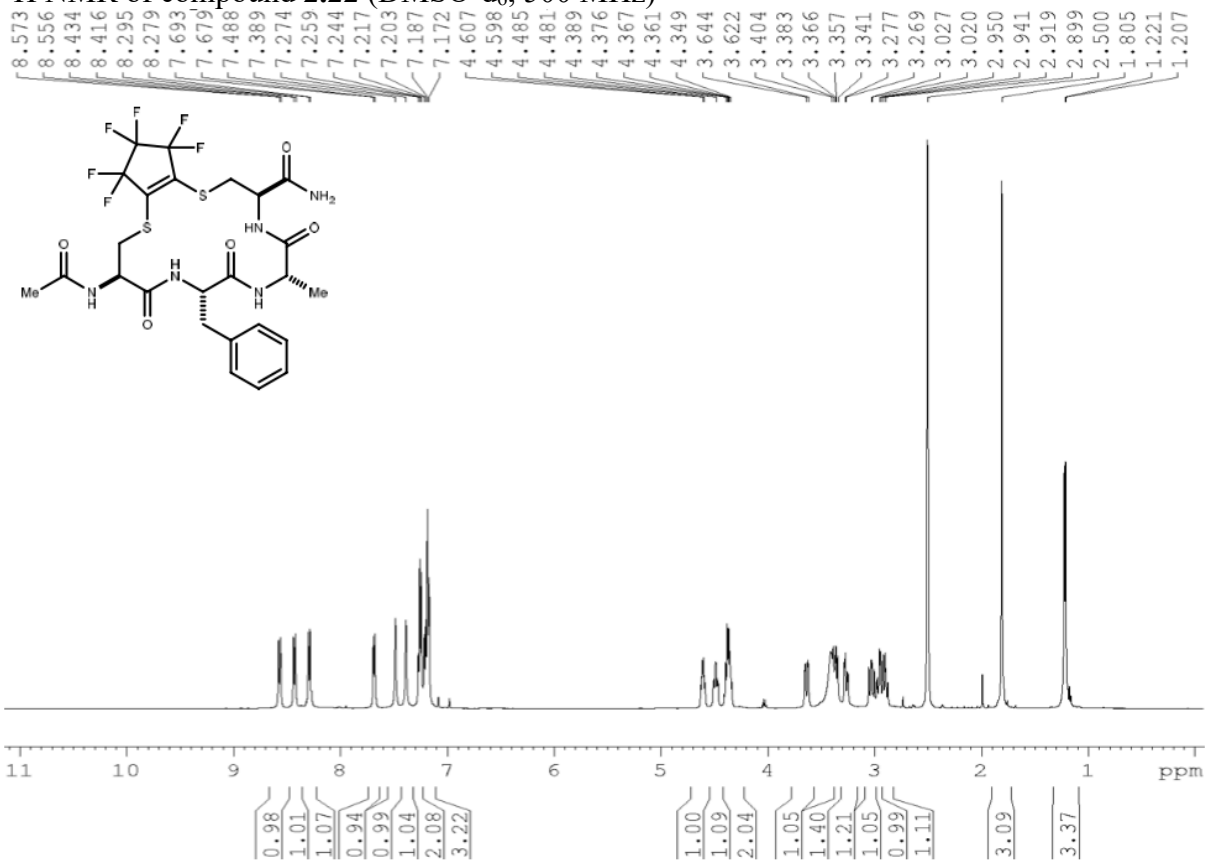
^{1}H NMR of compound **2.21** (DMSO-d_6, 500 MHz)



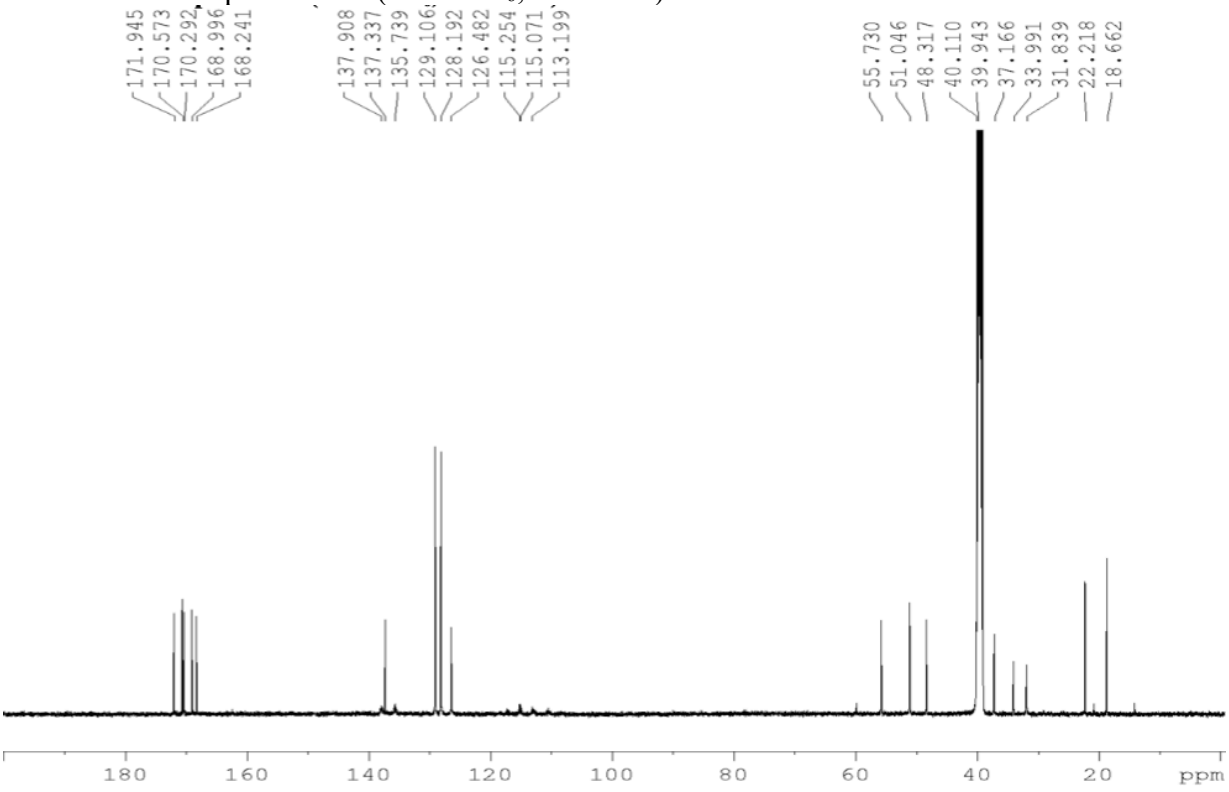
^{13}C NMR of compound **2.21** (DMSO-d_6, 126 MHz)



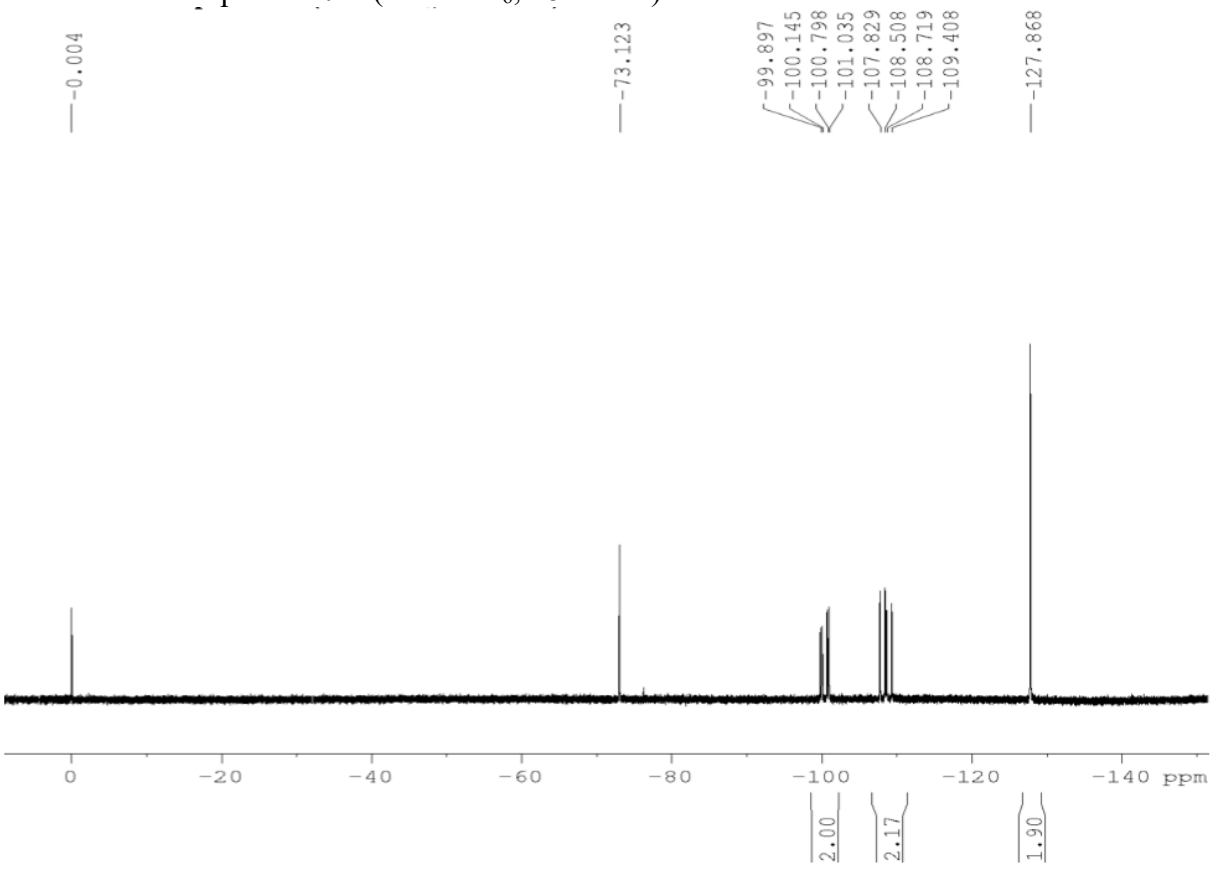
^{1}H NMR of compound **2.22** (DMSO-d_6, 500 MHz)



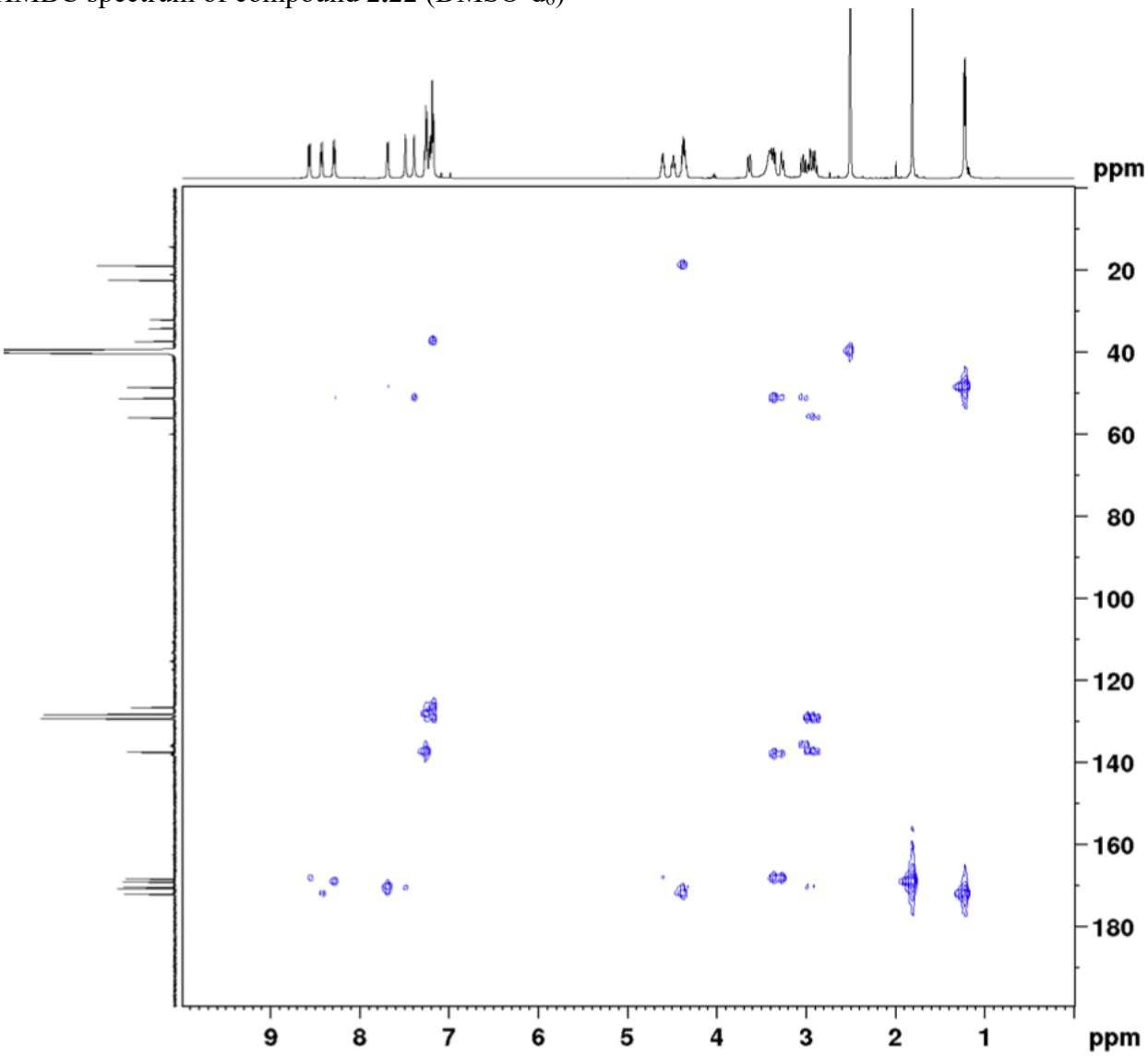
^{13}C NMR of compound **2.22** (DMSO-d_6, 126 MHz)



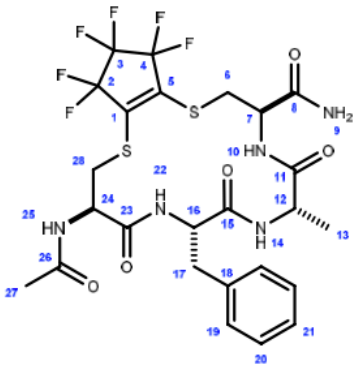
^{19}F NMR of compound **2.22** (DMSO-d_6, 282 MHz)



HMBC spectrum of compound **2.22** (DMSO-d_6)

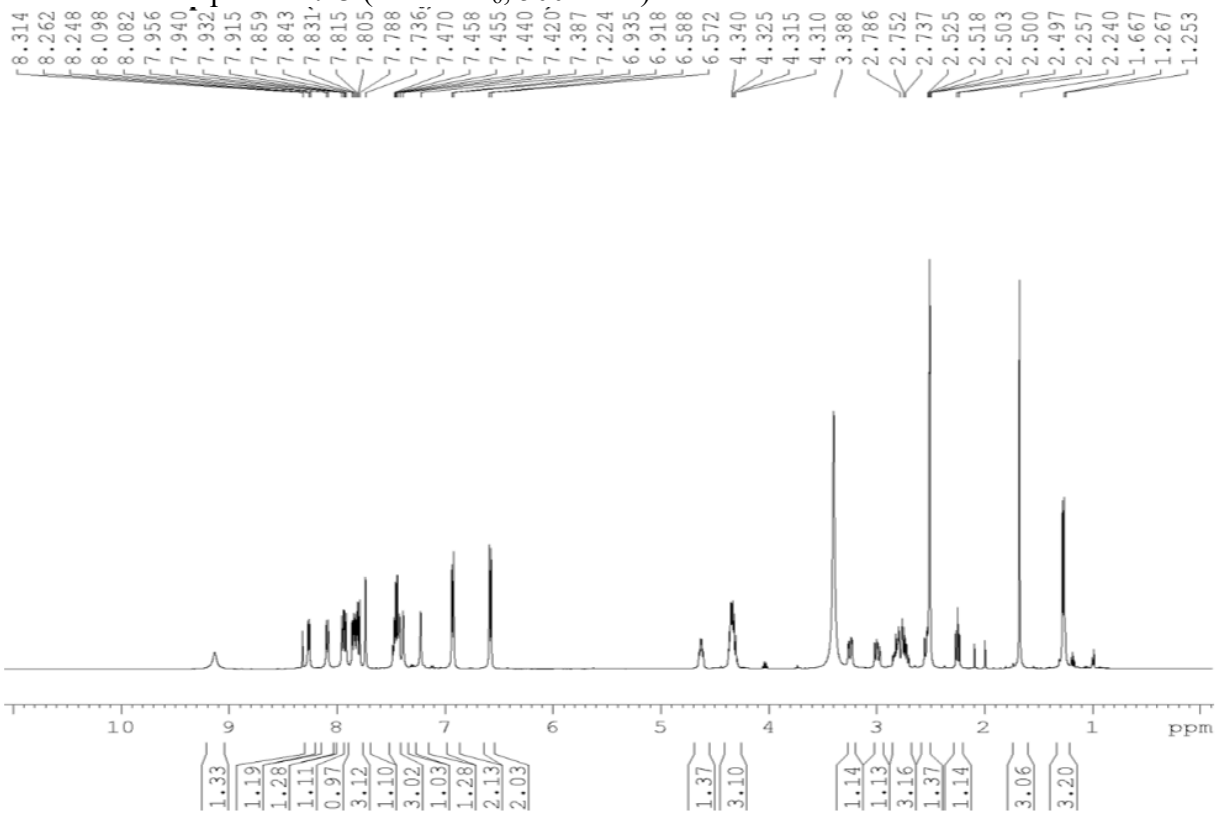


Compound **2.22** (DMSO-d_6)

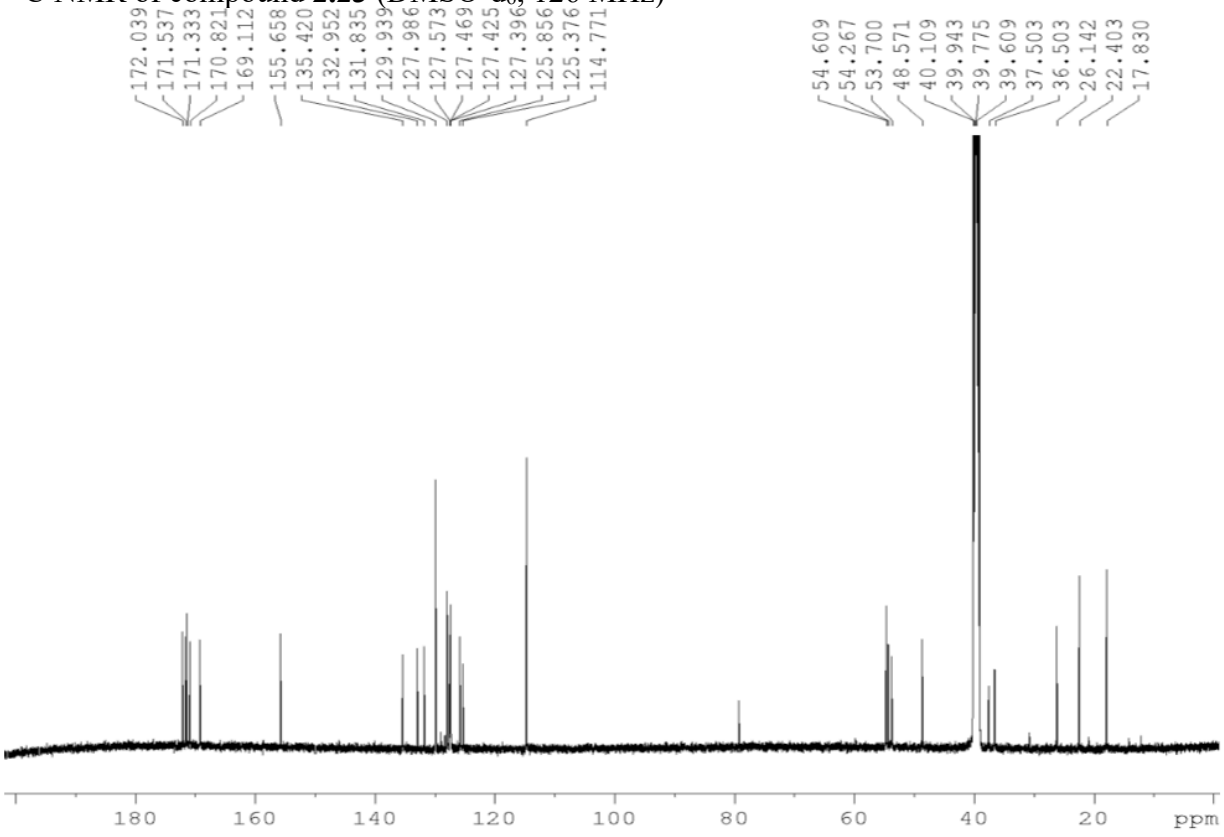


	13C	1H	Key Correlation
1	138.2–137.7 (m)	–	HMBC 28 –> 1
2	118.6–108.2 (m)	–	
3	118.6–108.2 (m)	–	
4	118.6–108.2 (m)	–	
5	136.0–135.4 (m)	–	HMBC 6 –> 5
6	34	3.63 (dd, J = 13.9, 3.3 Hz, 1H), 3.02 (dd, J = 13.9, 10.7 Hz, 1H)	HMBC 6 –> 5,8
7	51	4.51–4.45 (m, 1H)	HMBC 9 –> 7, HMBC 7 –> 6
8	170.3	–	HMBC 10 –> 8
9	–	7.49 (s, 1H), 7.39 (s, 1H)	HMBC 9 –> 7,8
10	–	7.69 (d, J = 7.2 Hz, 1H)	HMBC 10 –> 12,8
11	171.9	–	HMBC 13,14 –> 11
12	48.3	4.40–4.32 (m, 2H)	HMBC 13,14 –> 12; HMBC 12 –> 11,13
13	18.7	1.22 (d, J = 6.6 Hz, 3H)	HMBC 13 –> 11,12; HMBC 12 –> 13
14	–	8.42 (d, J = 8.8 Hz, 1H)	HMBC 14 –> 11,12
15	170.6	–	HMBC 17 –> 15
16	55.7	4.40–4.32 (m, 2H)	HMBC 17 –> 16; HMBC 16 –> 15,18
17	37.2	2.96 (dd, J = 13.9, 4.7 Hz, 1H), 2.90 (dd, J = 13.9, 10.0 Hz, 1H)	HMBC 19 –> 17; HMBC 17 –> 15,16,18,19
18	137.3	–	HMBC 20 –> 18
19	129.1	7.23–7.16 (m, 3H)	HMBC 19 –> 21
20	128.2	7.28–7.24 (m, 2H)	HMBC 20 –> 18
21	126.5	7.23–7.16 (m, 3H)	HMBC 21 –> 19
22	–	8.57 (d, J = 8.6 Hz, 1H)	HMBC 22 –> 23
23	168.2	–	HMBC 28 –> 23
24	51	4.63–4.58 (m, 1H)	HMBC 24 –> 23,28; HMBC 25,28 –> 24
25	–	8.29 (d, J = 8.1 Hz, 1H)	HMBC 25 –> 26
26	169	–	HMBC 27 –> 26
27	22.2	1.80 (s, 3H)	HMBC 27 –> 26
28	31.8	3.36 (dd, J = 12.6, 8.4 Hz, 1H), 3.26 (dd, J = 12.6, 4.3 Hz, 1H)	HMBC 28 –> 23,1

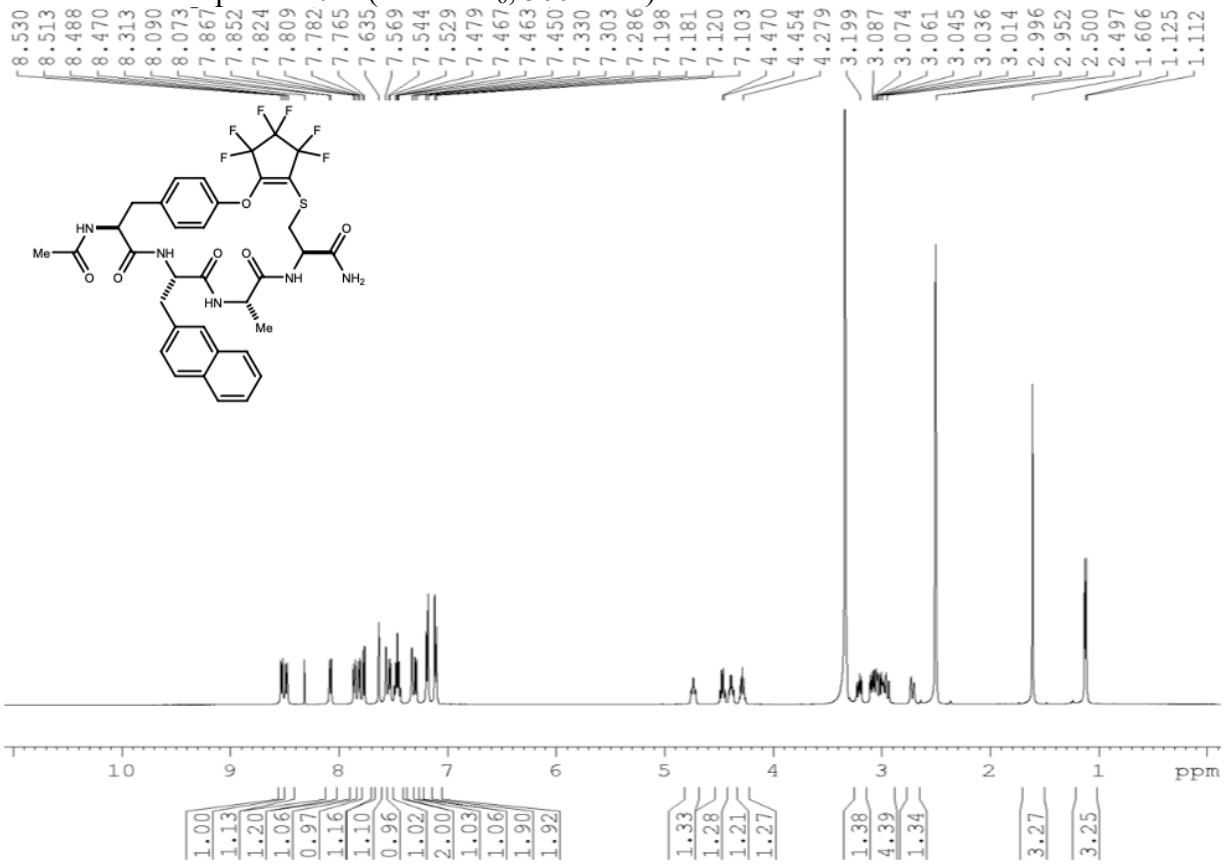
^{1}H NMR of compound **2.23** (DMSO-d_6, 500 MHz)



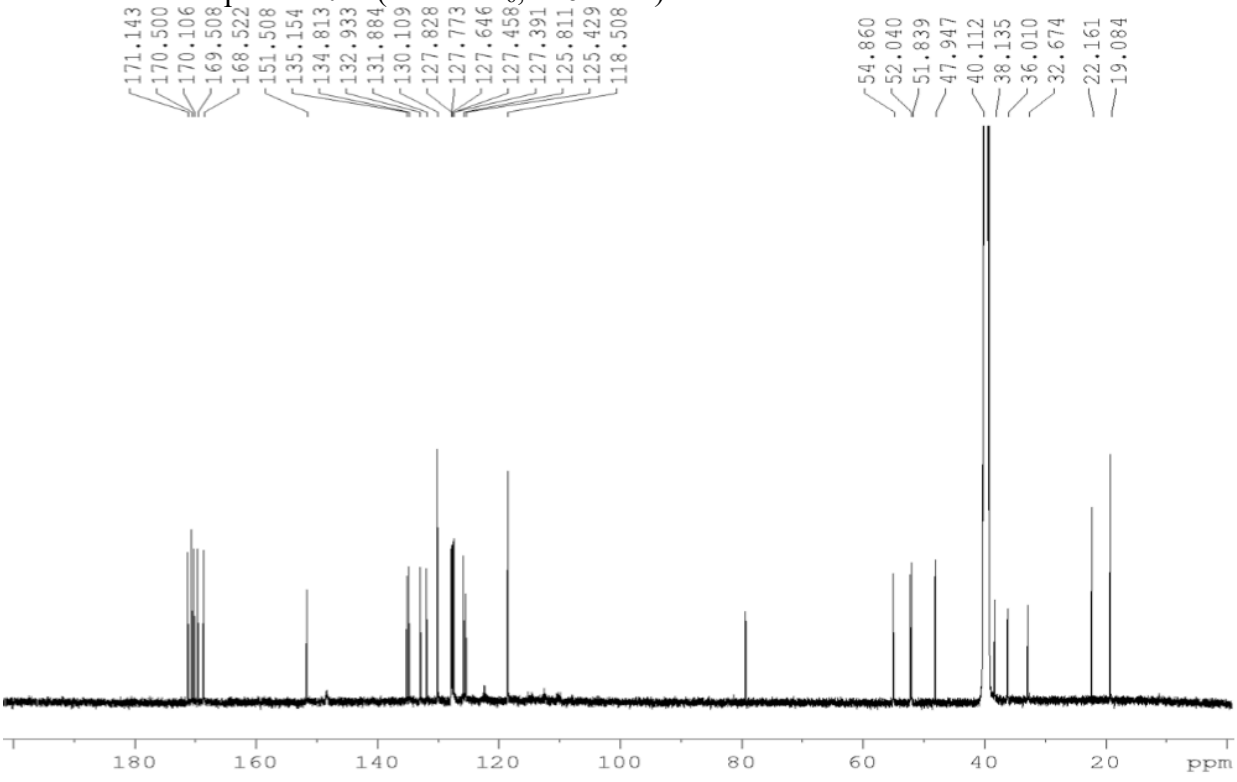
^{13}C NMR of compound **2.23** (DMSO-d_6, 126 MHz)



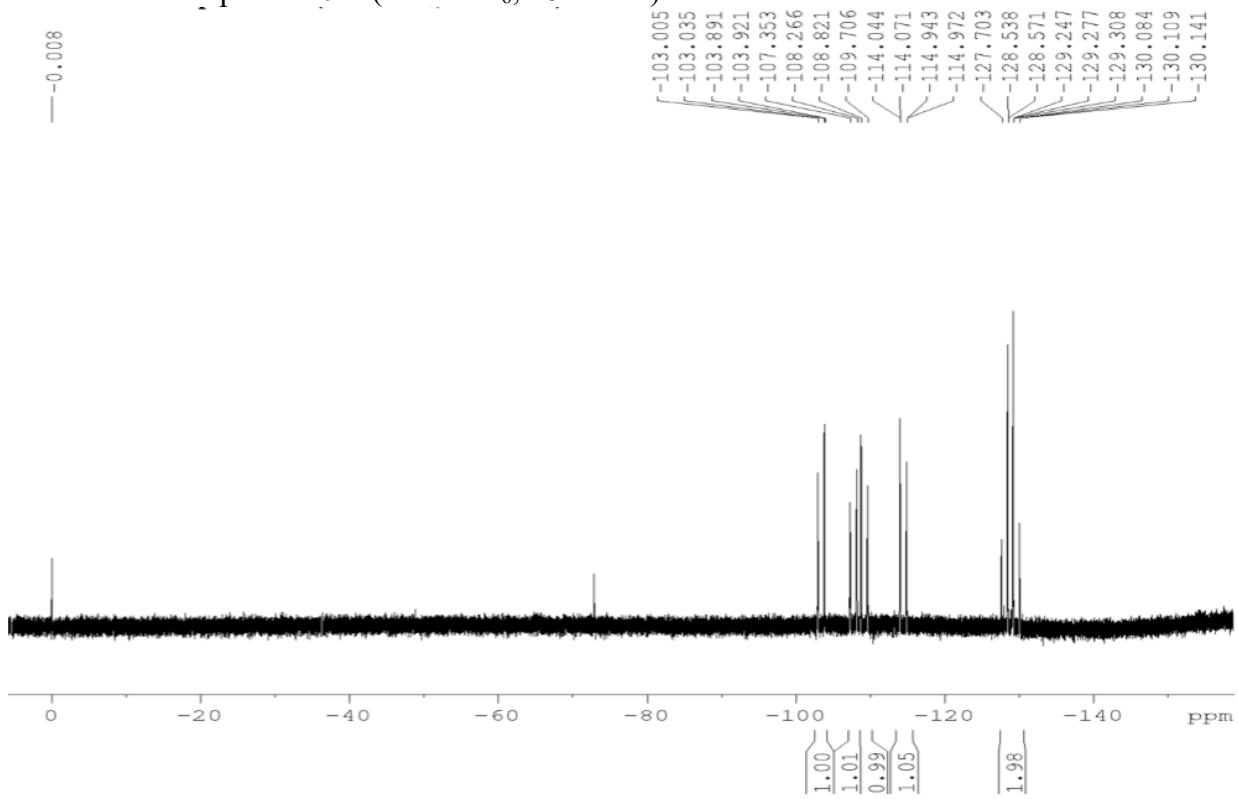
^{1}H NMR of compound **2.24** (DMSO-d_6, 500 MHz)



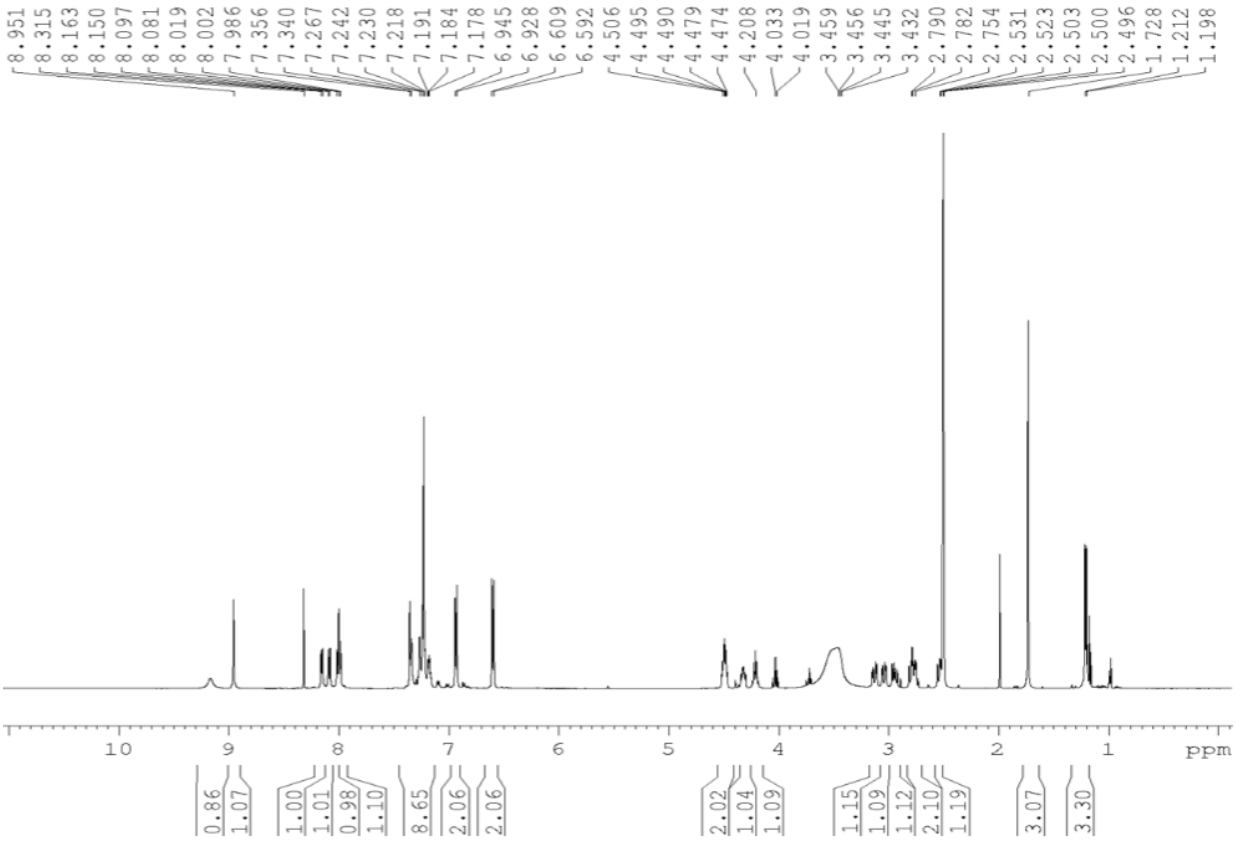
^{13}C NMR of compound **2.24** (DMSO-d_6, 126 MHz)



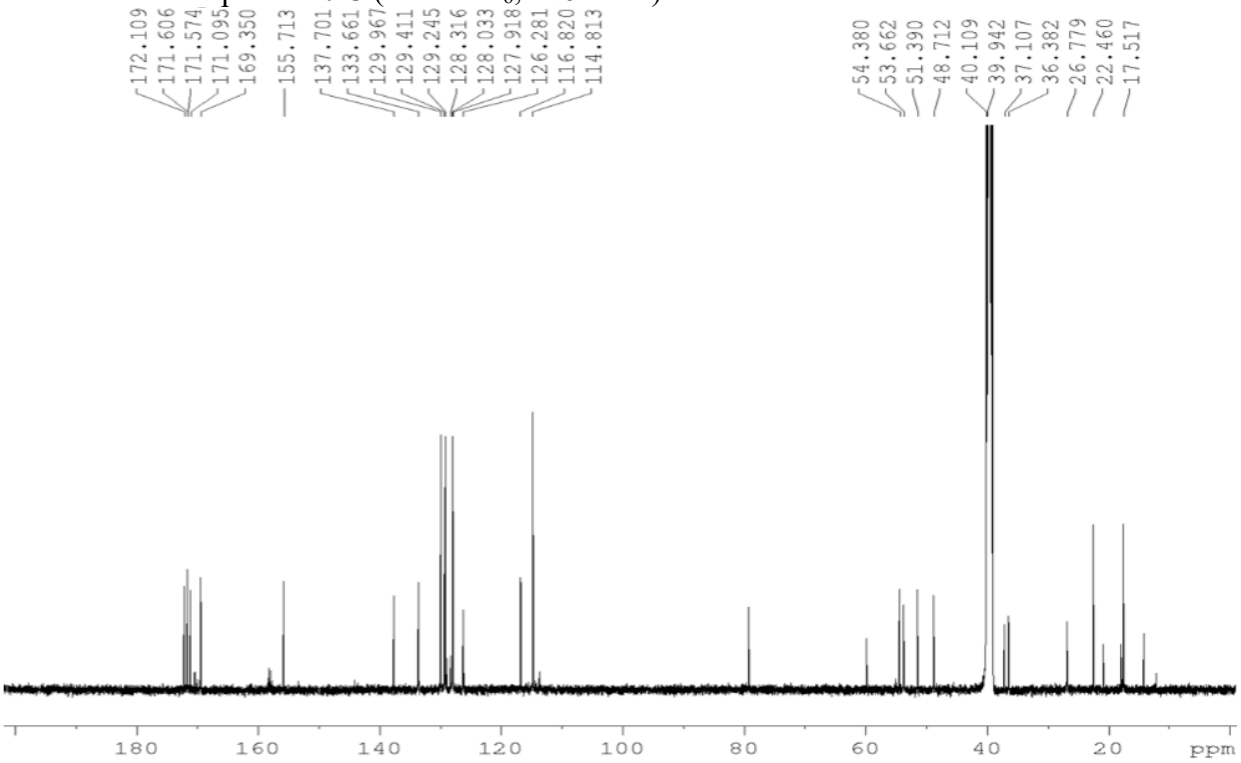
^{19}F NMR of compound **2.24** (DMSO-d_6, 282 MHz)



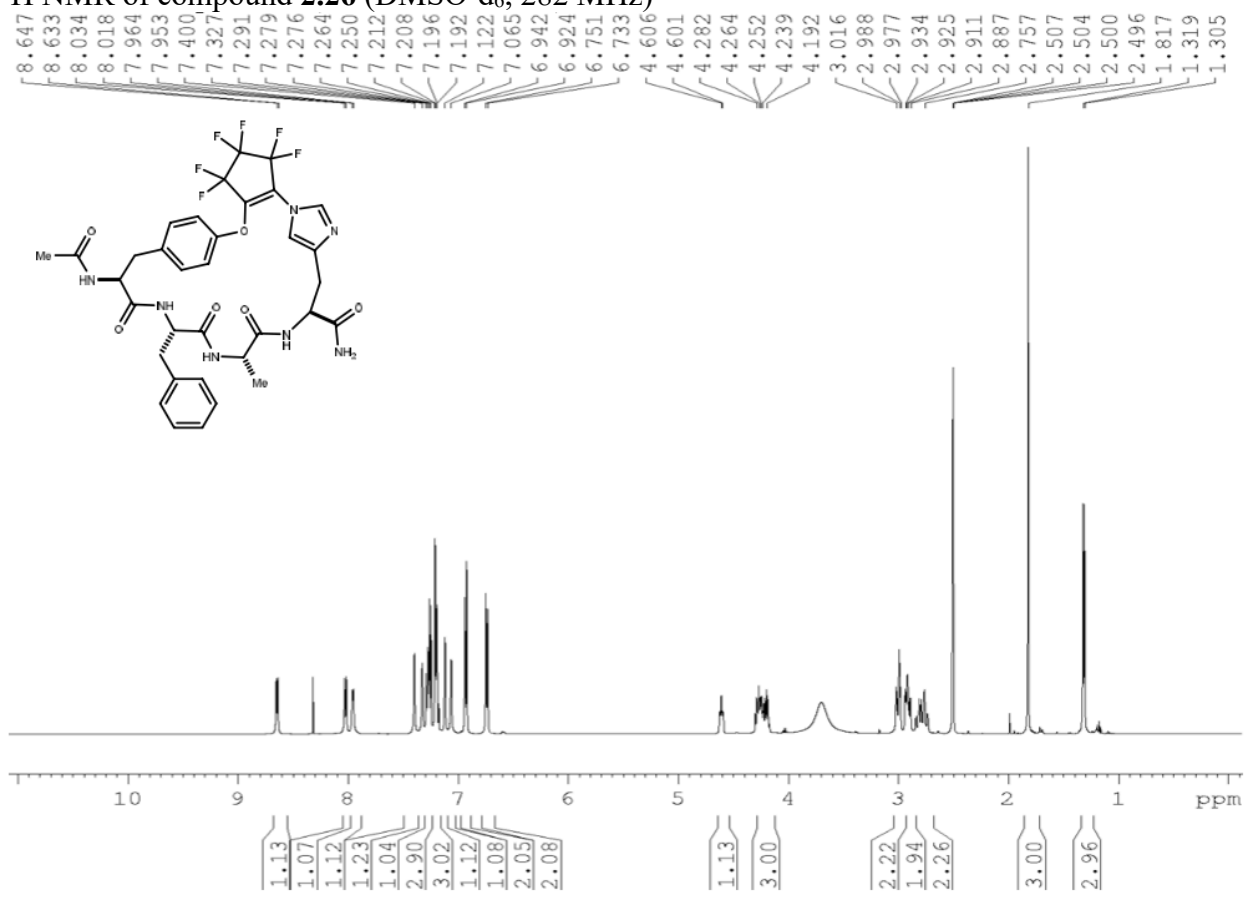
^{1}H NMR of compound **2.25** (DMSO-d_6, 500 MHz)



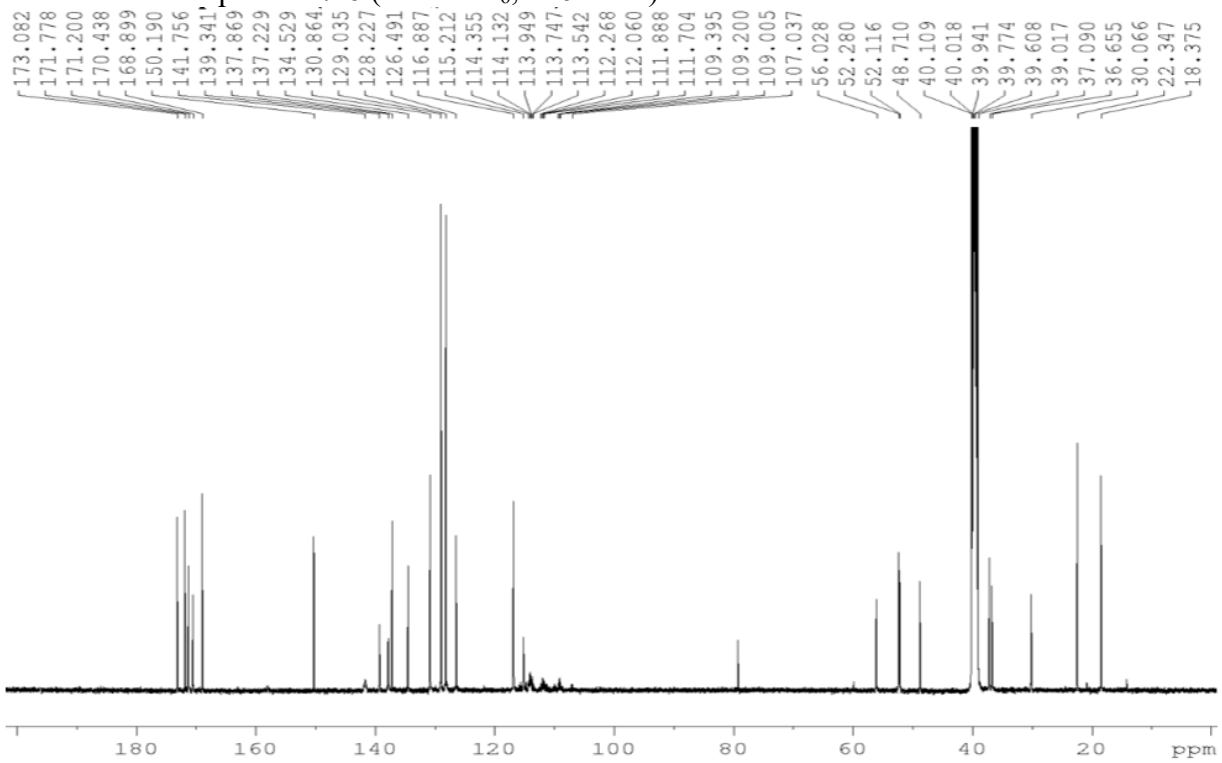
^{13}C NMR of compound **2.25** (DMSO-d_6, 126 MHz)



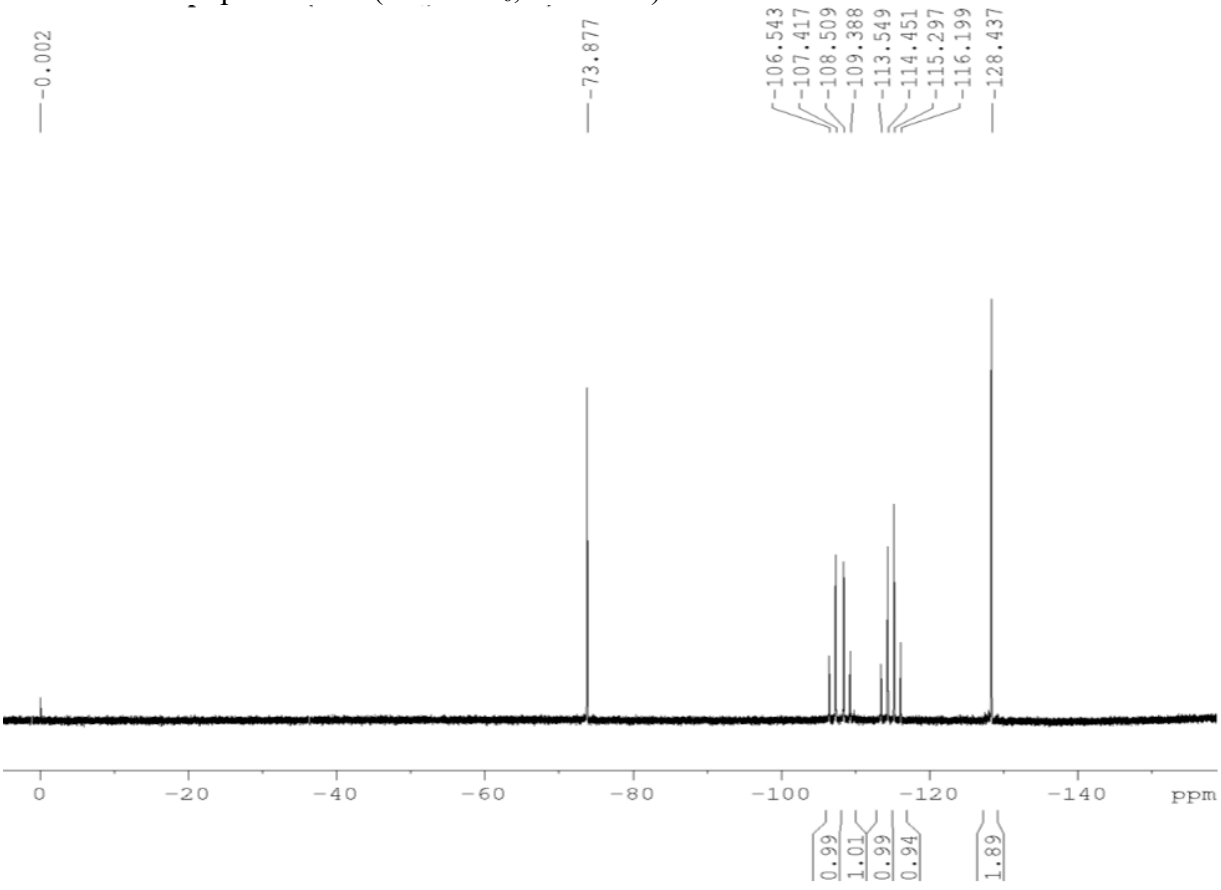
^{1}H NMR of compound **2.26** (DMSO-d_6, 282 MHz)



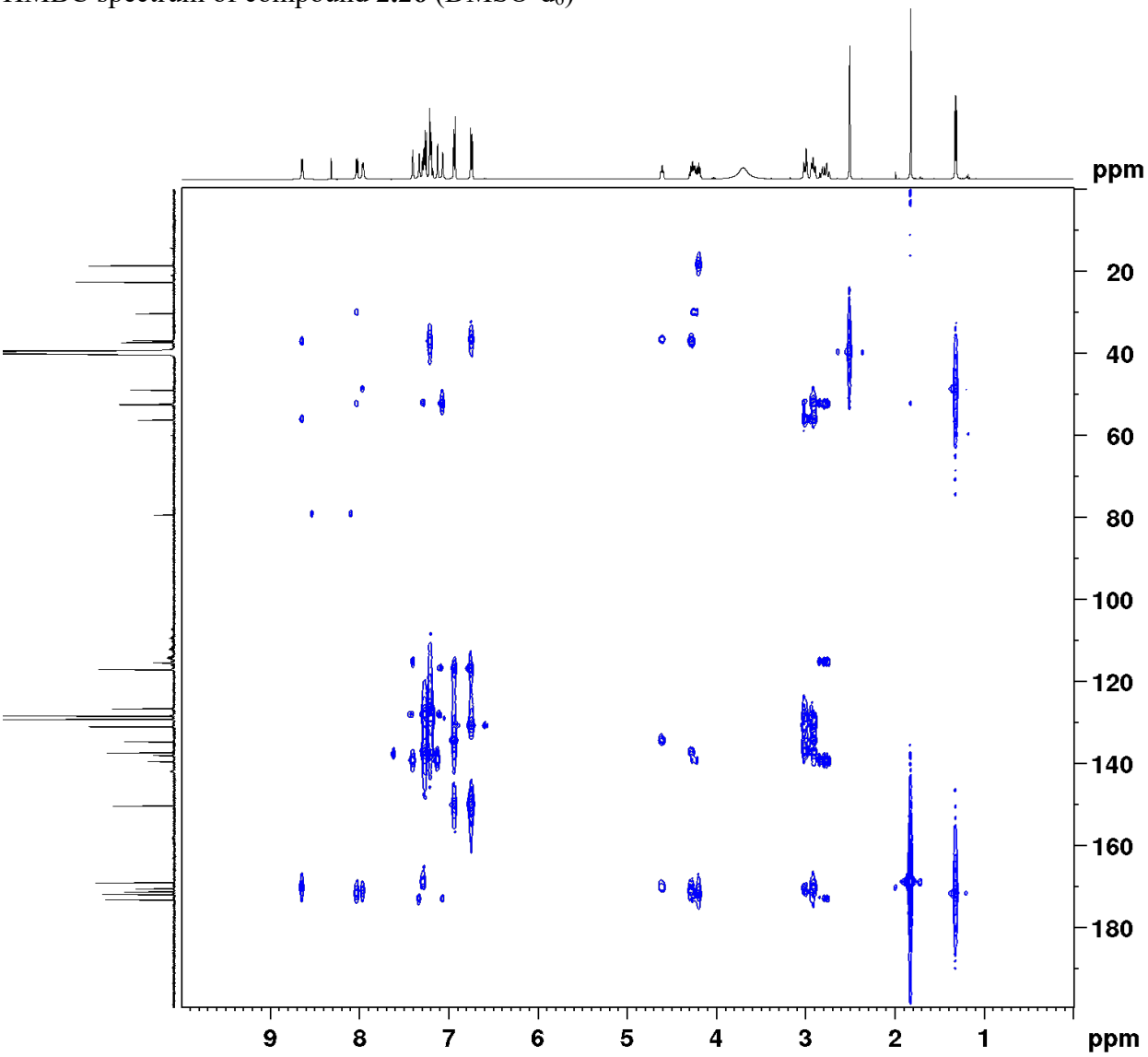
^{13}C NMR of compound **2.26** (DMSO-d_6, 126 MHz)



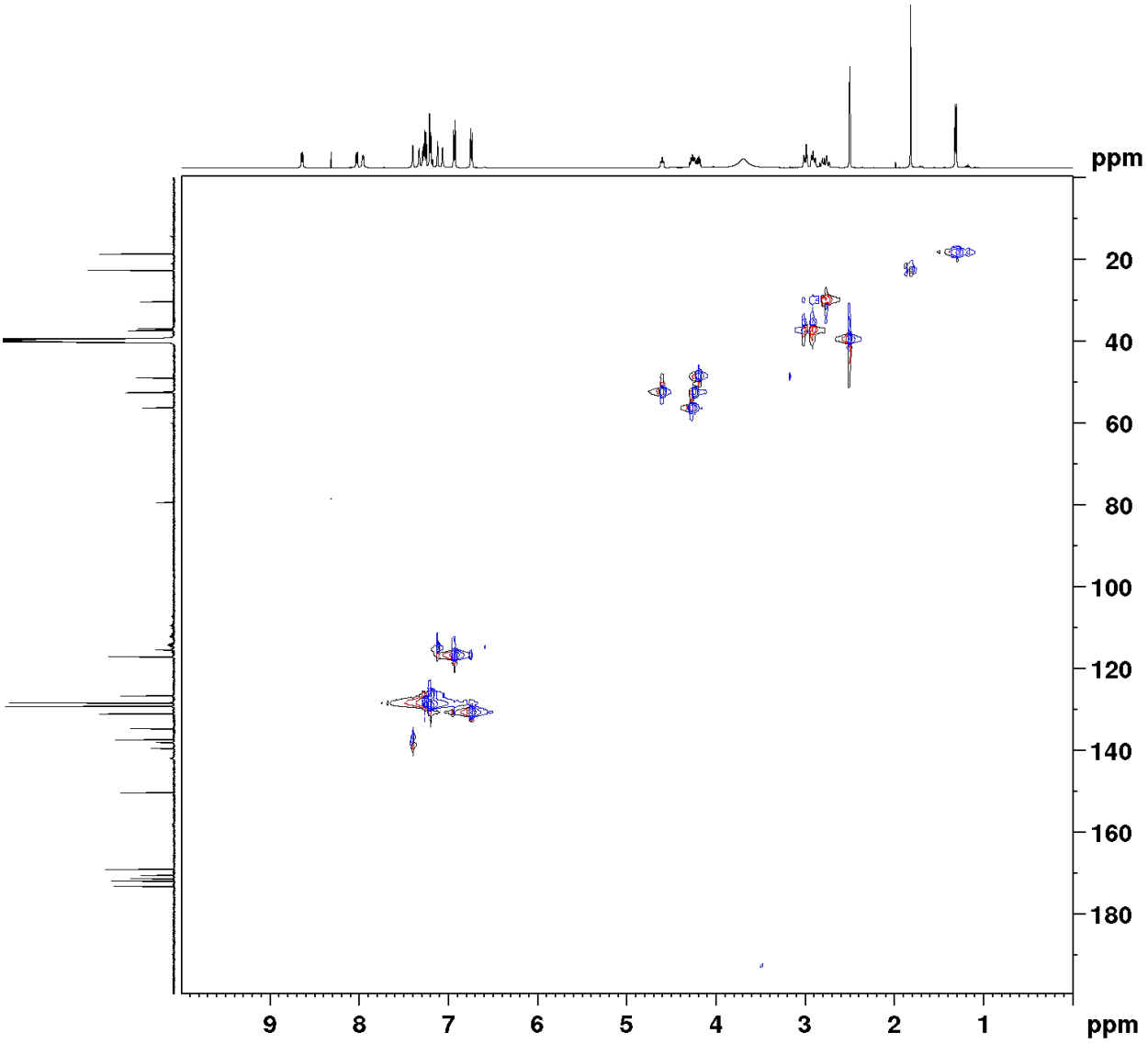
^{19}F NMR of compound **2.26** (DMSO-d_6, 282 MHz)



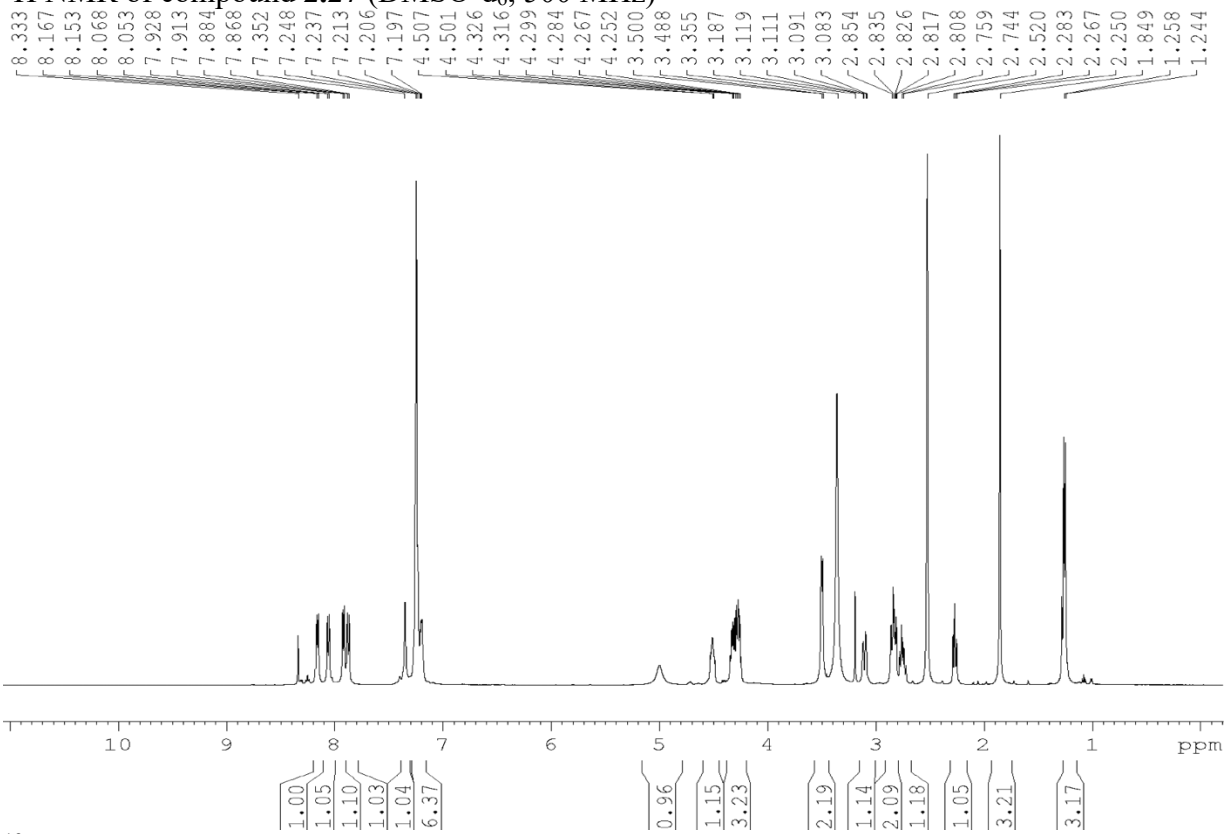
HMBC spectrum of compound **2.26** (DMSO-d_6)



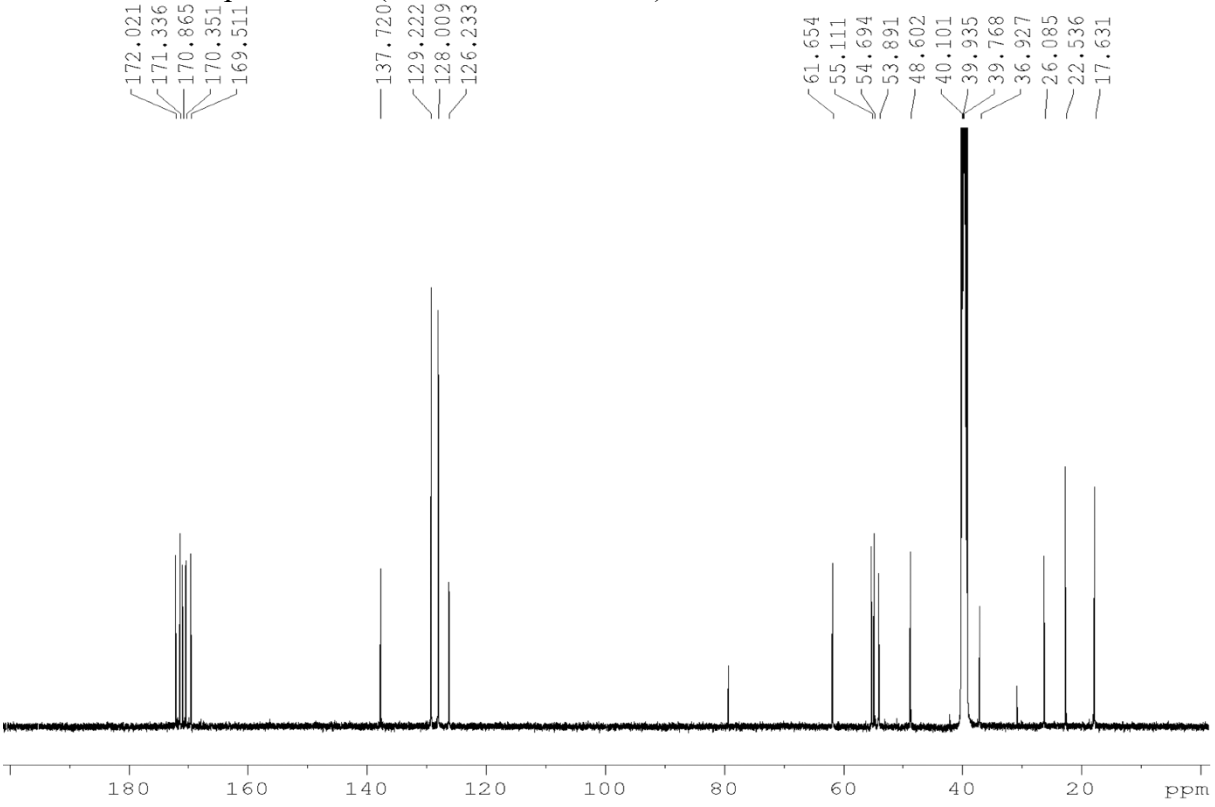
HSQC spectrum of compound **2.26** (DMSO-d_6)



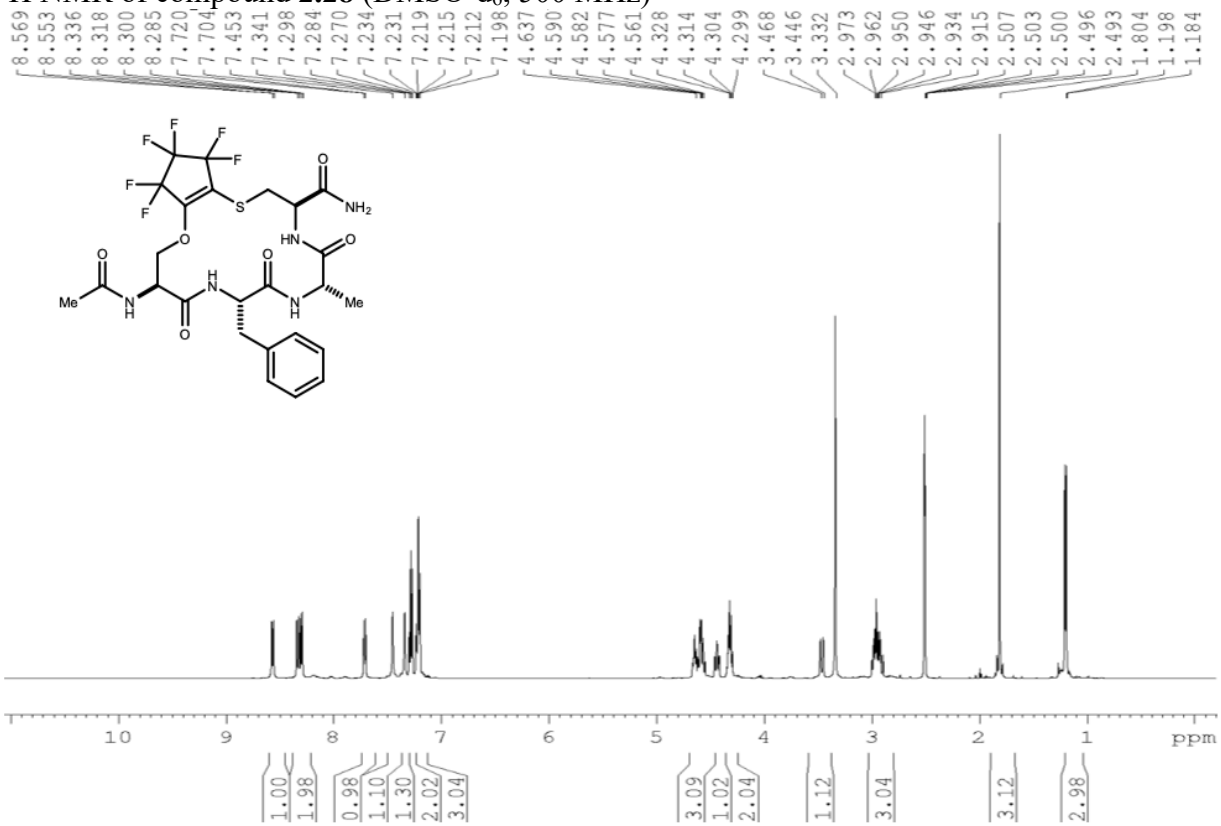
^{1}H NMR of compound **2.27** (DMSO-d_6, 500 MHz)



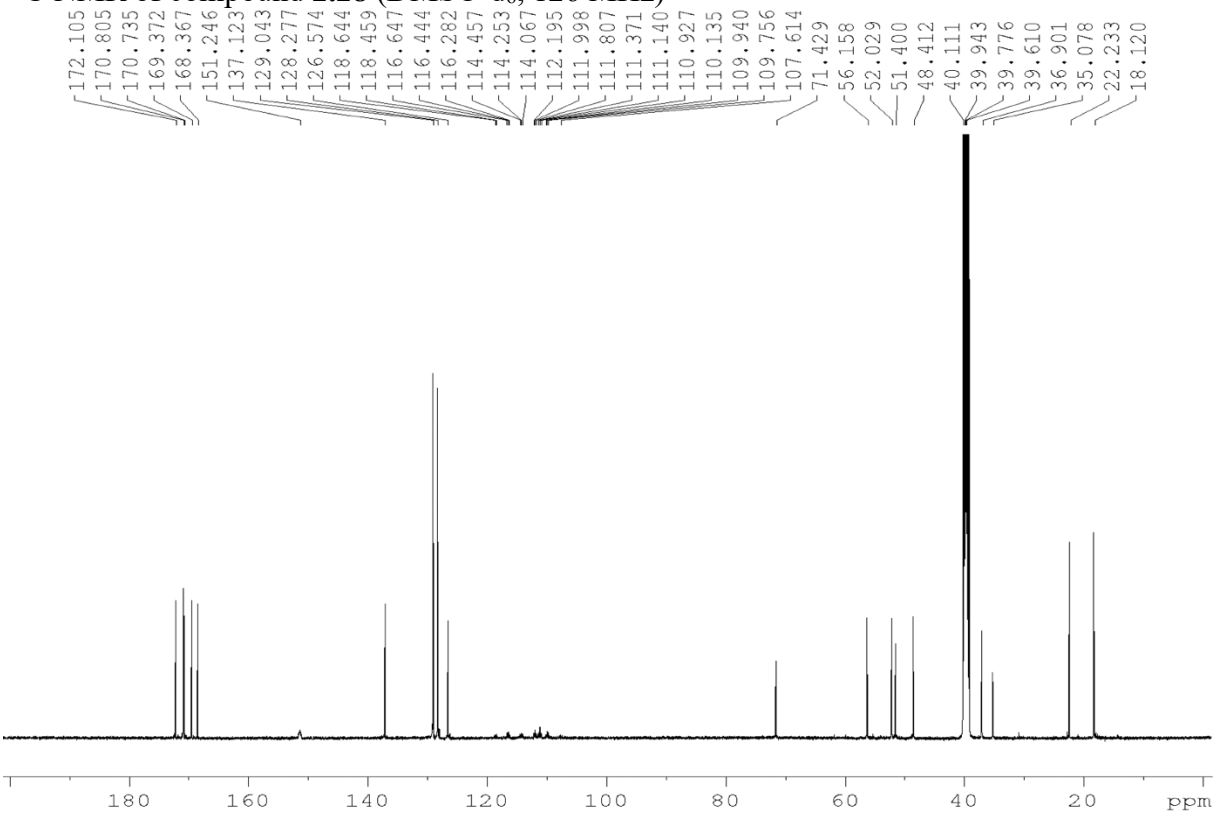
^{13}C NMR of compound **2.27** (DMSO-d_6, 126 MHz)



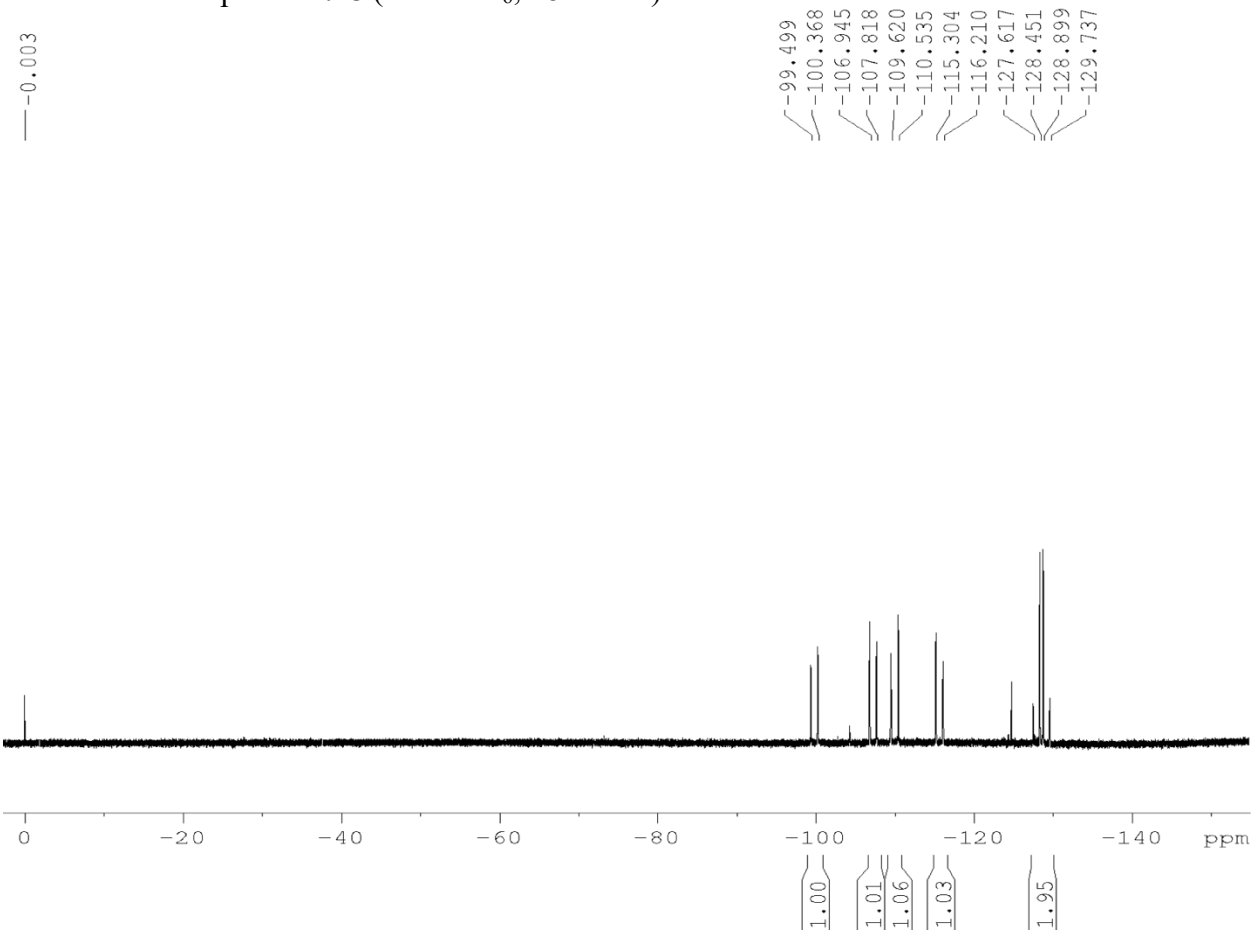
^{1}H NMR of compound **2.28** (DMSO-d_6, 500 MHz)



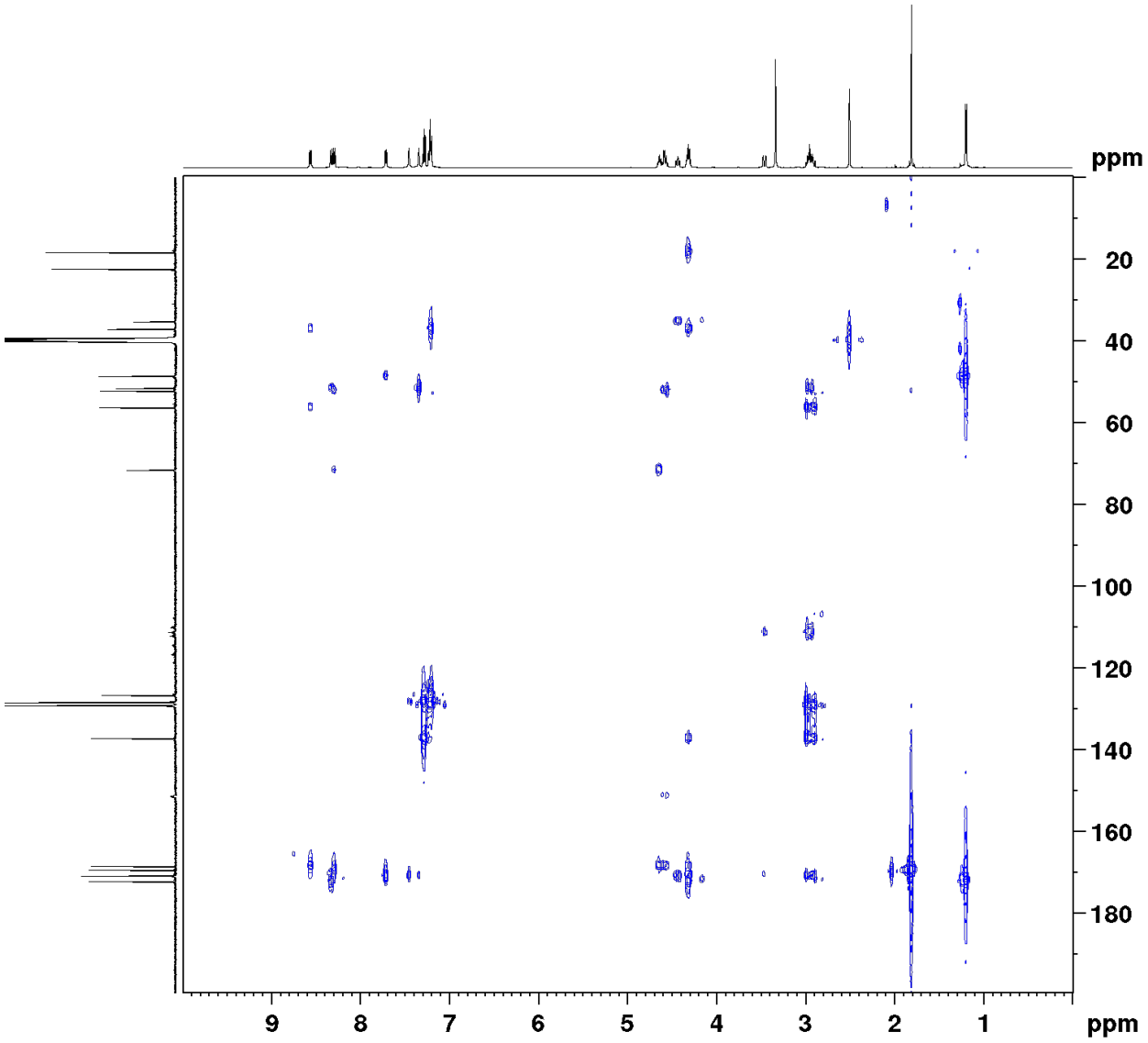
^{13}C NMR of compound **2.28** (DMSO-d_6, 126 MHz)



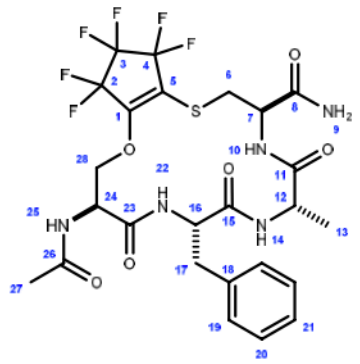
^{19}F NMR of compound **2.28** (DMSO-d_6, 282 MHz)



HMBC spectrum of compound **2.28** (DMSO-d_6)

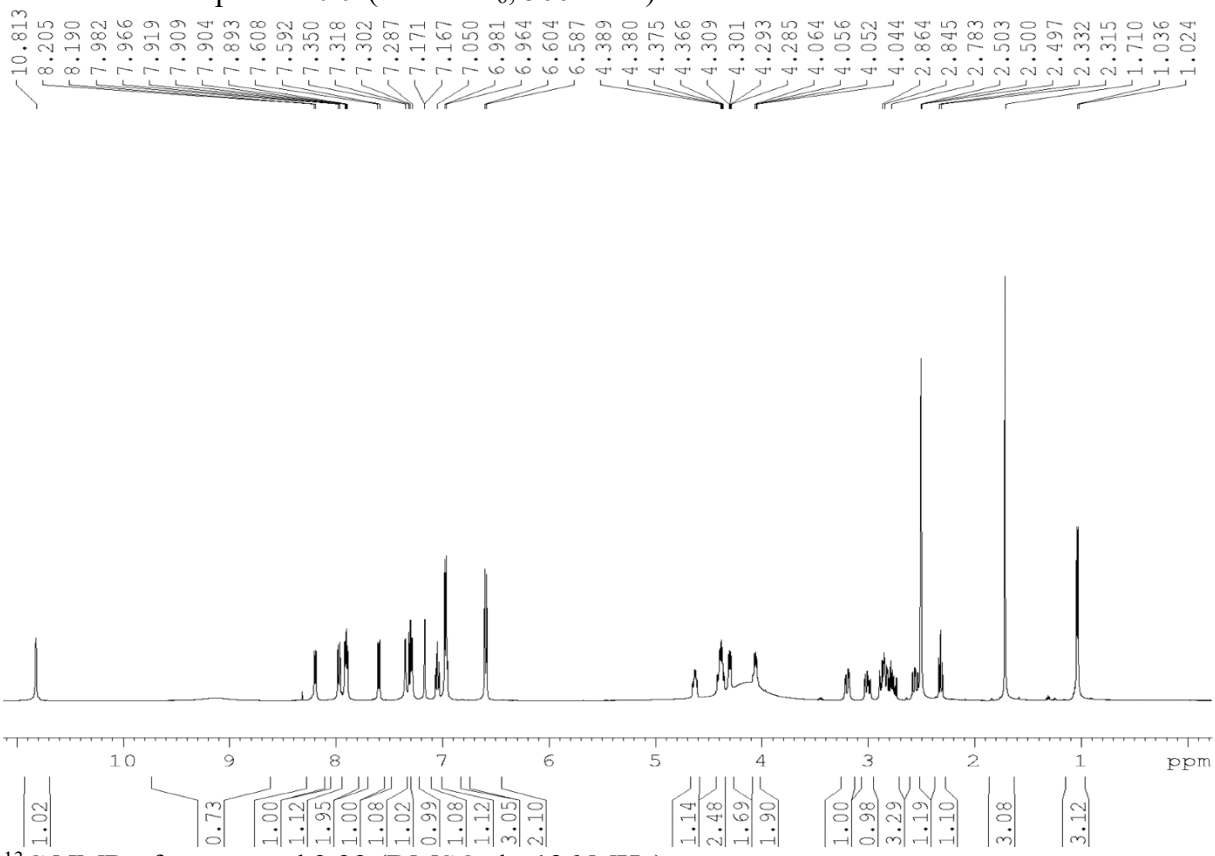


Compound **2.28** (DMSO-d_6)

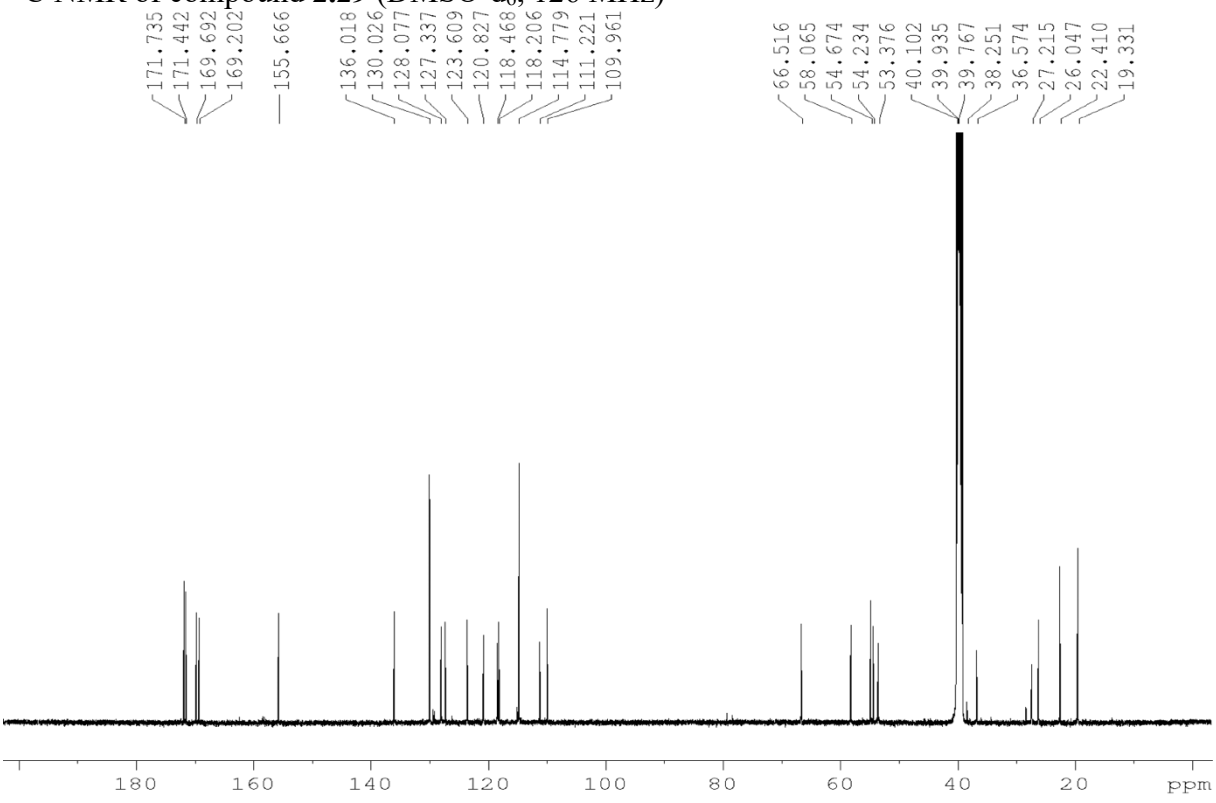


	13C	1H	Key Correlation
1	151.5–151.1 (m, 1C)	–	HMBC 28 -> 1
2	126.6, 118.6–107.6 (m, 3C)	–	
3	126.6, 118.6–107.6 (m, 3C)	–	
4	126.6, 118.6–107.6 (m, 3C)	–	
5	111.4–110.8 (m, 1C)	–	HMBC 6 -> 5
6	35.1	3.46 (dd, J = 13.9, 3.0 Hz, 1H), 3.00–2.90 (m, 3H)	HMBC 6 -> 5,7; HMBC 7,10 -> 6
7	51.4	4.46–4.41 (m, 1H)	HMBC 9,10 -> 7; HMBC 7 -> 6
8	170.7/170.8	–	HMBC 6 -> 8
9	–	7.45 (s, 1H), 7.34 (s, 1H)	HMBC 9 -> 8
10	–	8.33 (d, J = 9.1 Hz, 1H)	HMBC 10 -> 7,11
11	172.1	–	HMBC 13 -> 11
12	48.4	4.34–4.28 (m, 2H)	HMBC 13 -> 12; HMBC 12 -> 13, 11
13	18.1	1.19 (d, J = 6.8 Hz, 3H)	HMBC 12 -> 13
14	–	7.71 (d, J = 7.6 Hz, 1H)	HMBC 14 -> 12
15	170.7/170.8	–	HMBC 14 -> 15
16	56.2	4.34–4.28 (m, 2H)	HMBC 17 -> 16, HMBC 16 -> 17
17	36.9	3.00–2.90 (m, 3H)	HMBC 17 -> 18,19; HMBC 19 -> 17
18	137.1	–	HMBC 16,17 -> 18
19	129	7.25–7.19 (m, 3H)	HMBC 17 -> 19
20	128.3	7.31–7.26 (m, 2H)	HMBC 20 -> 18
21	126.6	7.25–7.19 (m, 3H)	HMBC 19 -> 21
22	–	8.54 (d, J = 8.1 Hz, 1H)	HMBC 22 -> 16,17
23	168.4	–	HMBC 28 -> 23
24	52	4.66–4.53 (m, 3H)	HMBC 24 -> 23
25	–	8.30 (d, J = 7.6 Hz, 1H)	HMBC 25 -> 26
26	169.4	–	HMBC 27 -> 26
27	22.2	1.80 (s, 3H)	HMBC 27 -> 26
28	71.4	4.66–4.53 (m, 3H)	HMBC 28 -> 1

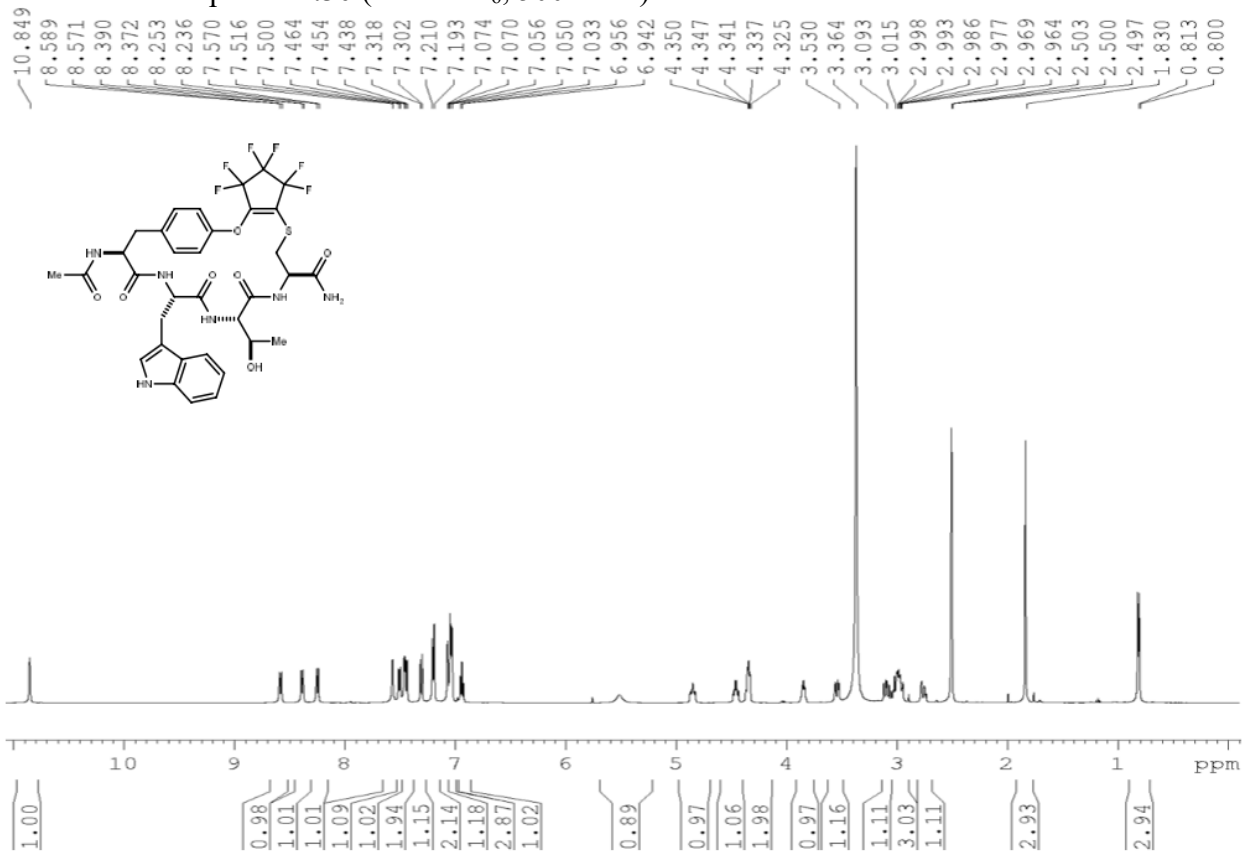
^{1}H NMR of compound **2.29** (DMSO-d_6, 500 MHz)



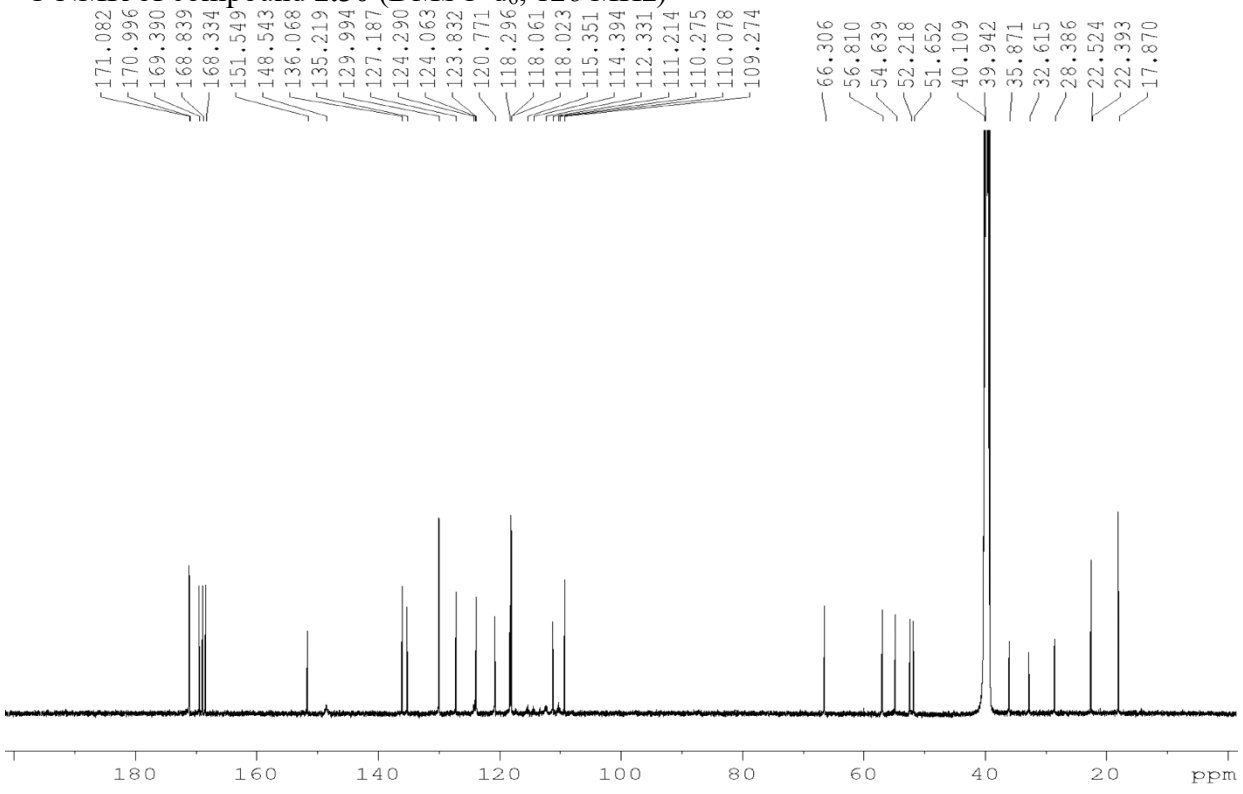
^{13}C NMR of compound **2.29** (DMSO-d_6, 126 MHz)



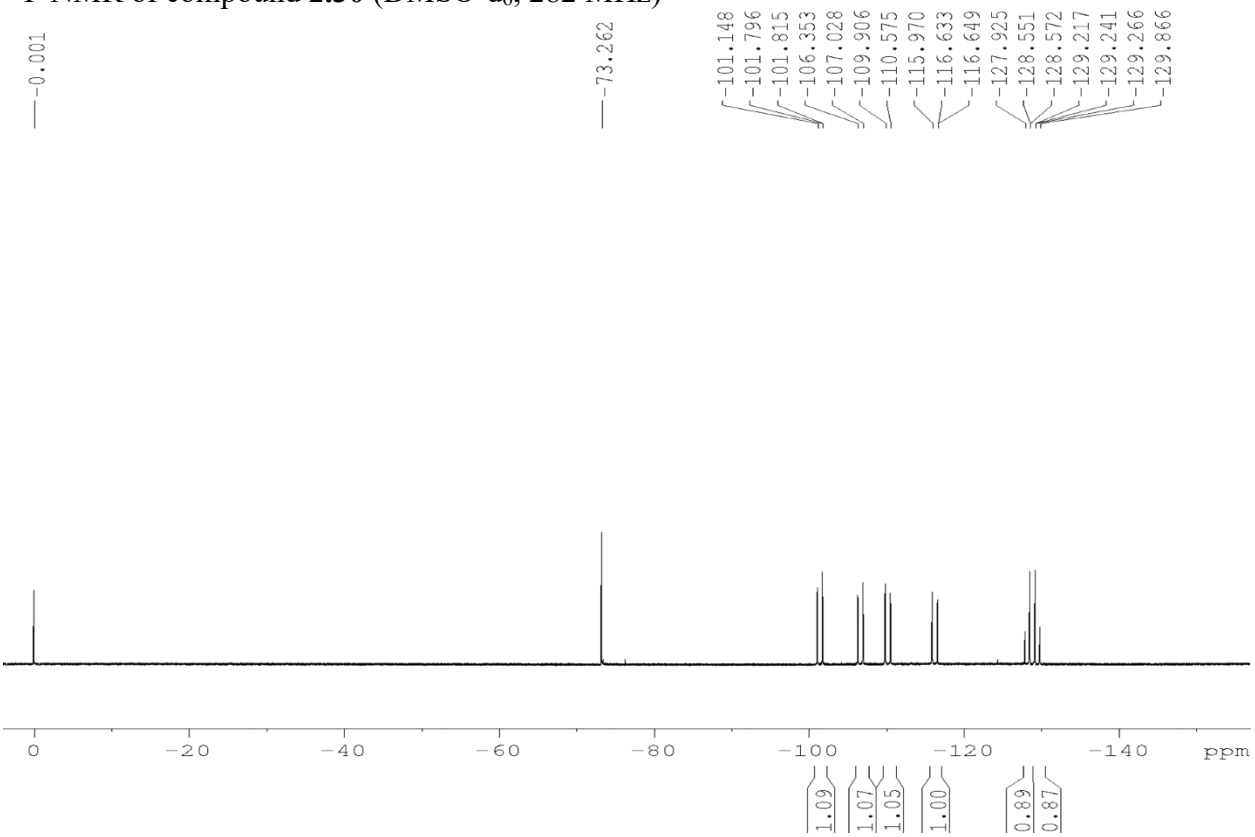
^{1}H NMR of compound **2.30** (DMSO-d_6, 500 MHz)



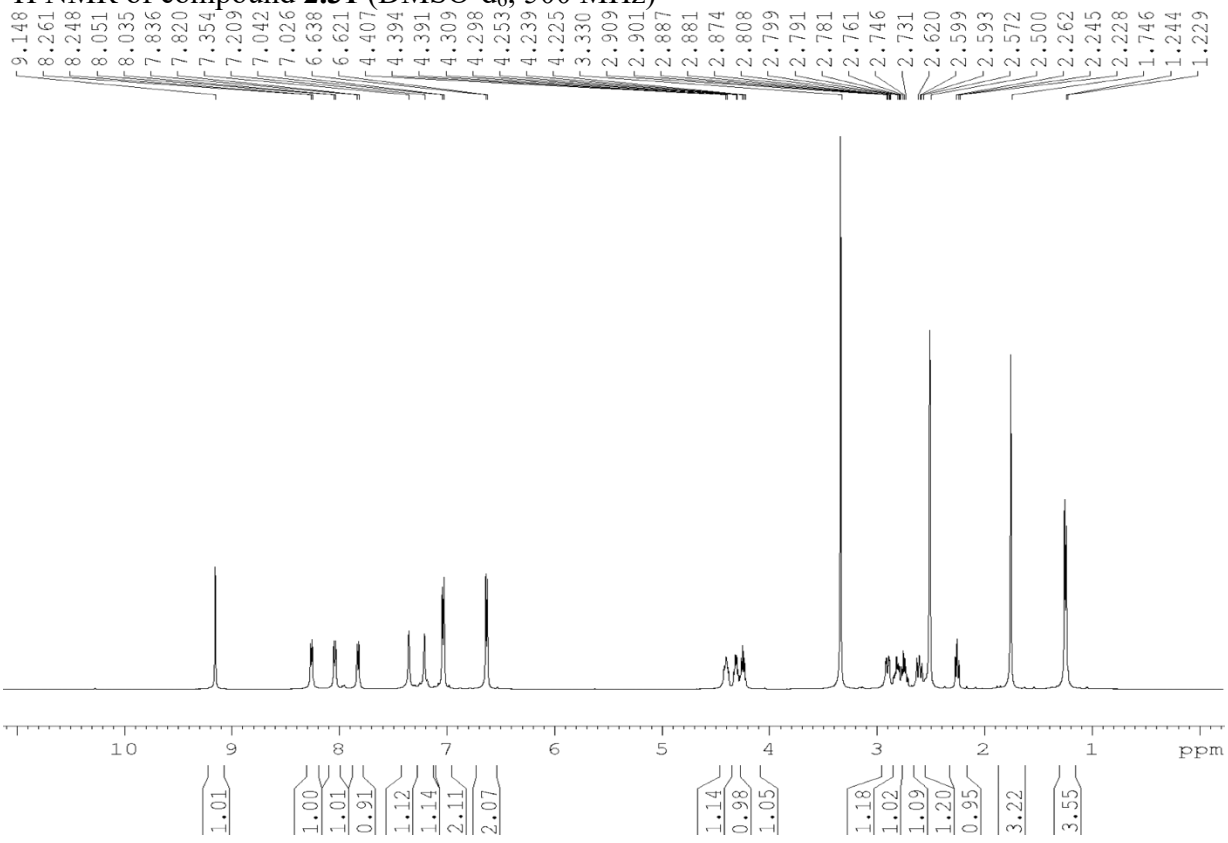
^{13}C NMR of compound **2.30** (DMSO-d_6, 126 MHz)



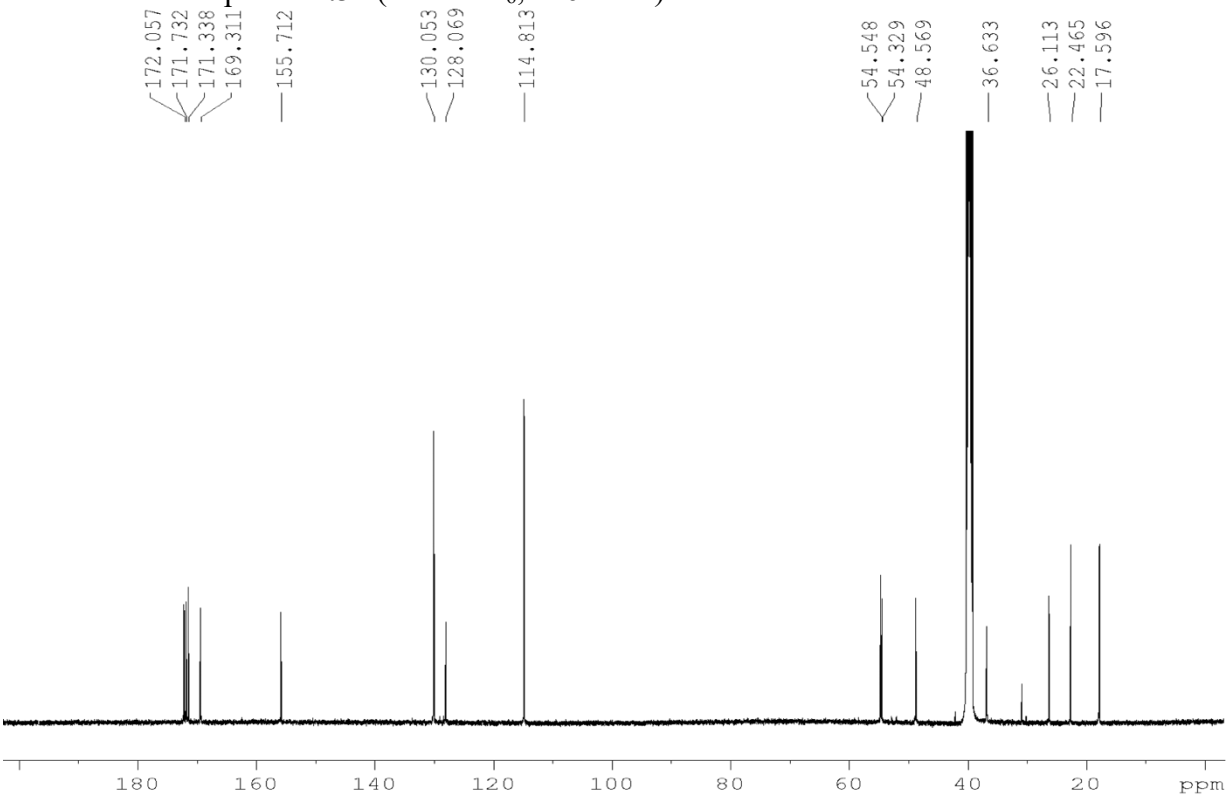
^{19}F NMR of compound **2.30** (DMSO-d_6, 282 MHz)



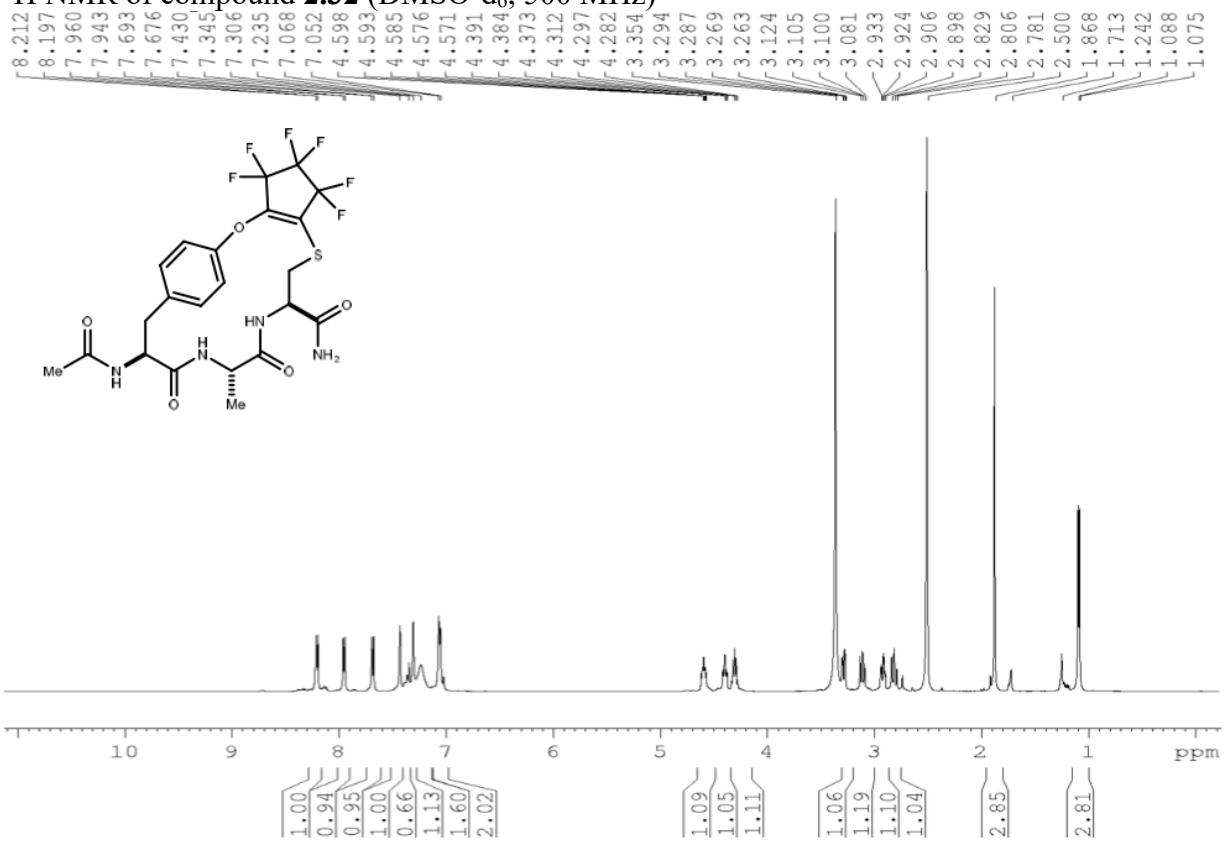
^{1}H NMR of compound **2.31** (DMSO-d_6, 500 MHz)



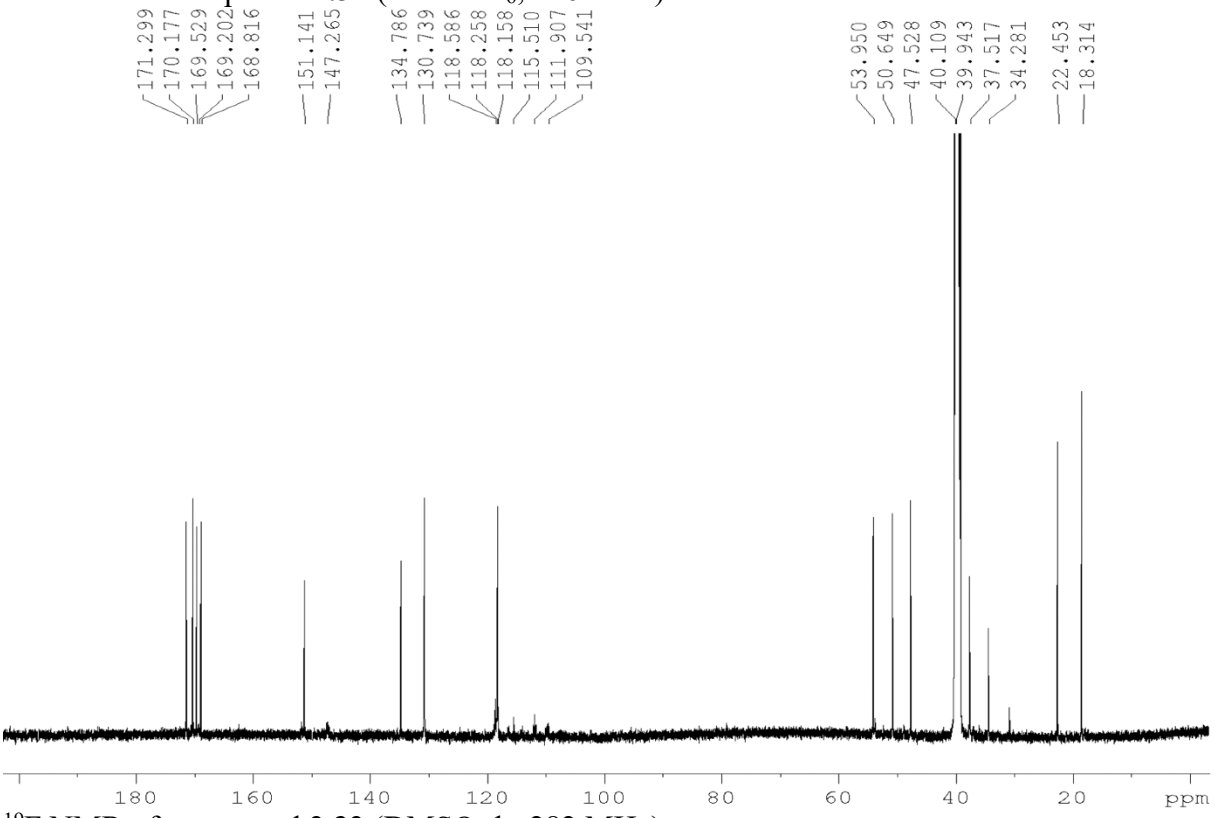
^{13}C NMR of compound **2.31** (DMSO-d_6, 126 MHz)



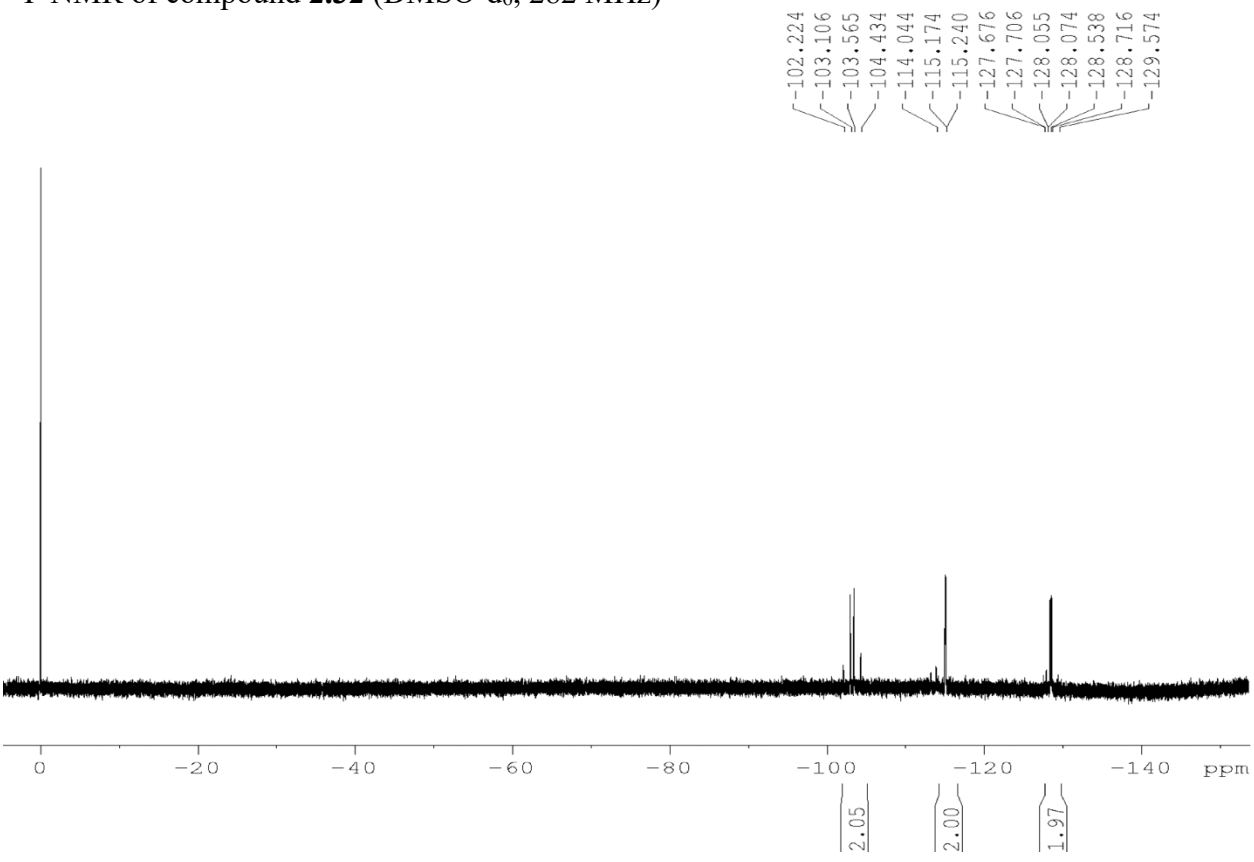
^{1}H NMR of compound **2.32** (DMSO-d_6, 500 MHz)



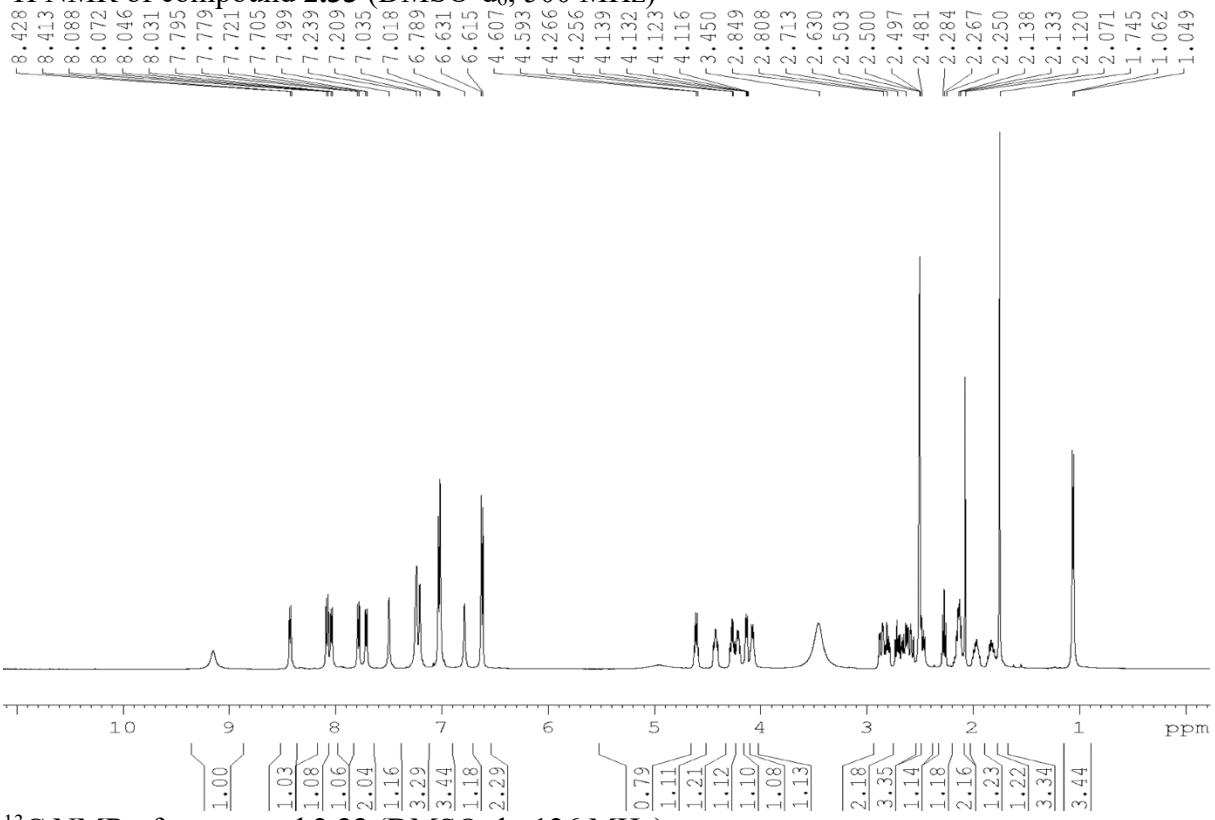
^{13}C NMR of compound **2.32** (DMSO-d_6, 126 MHz)



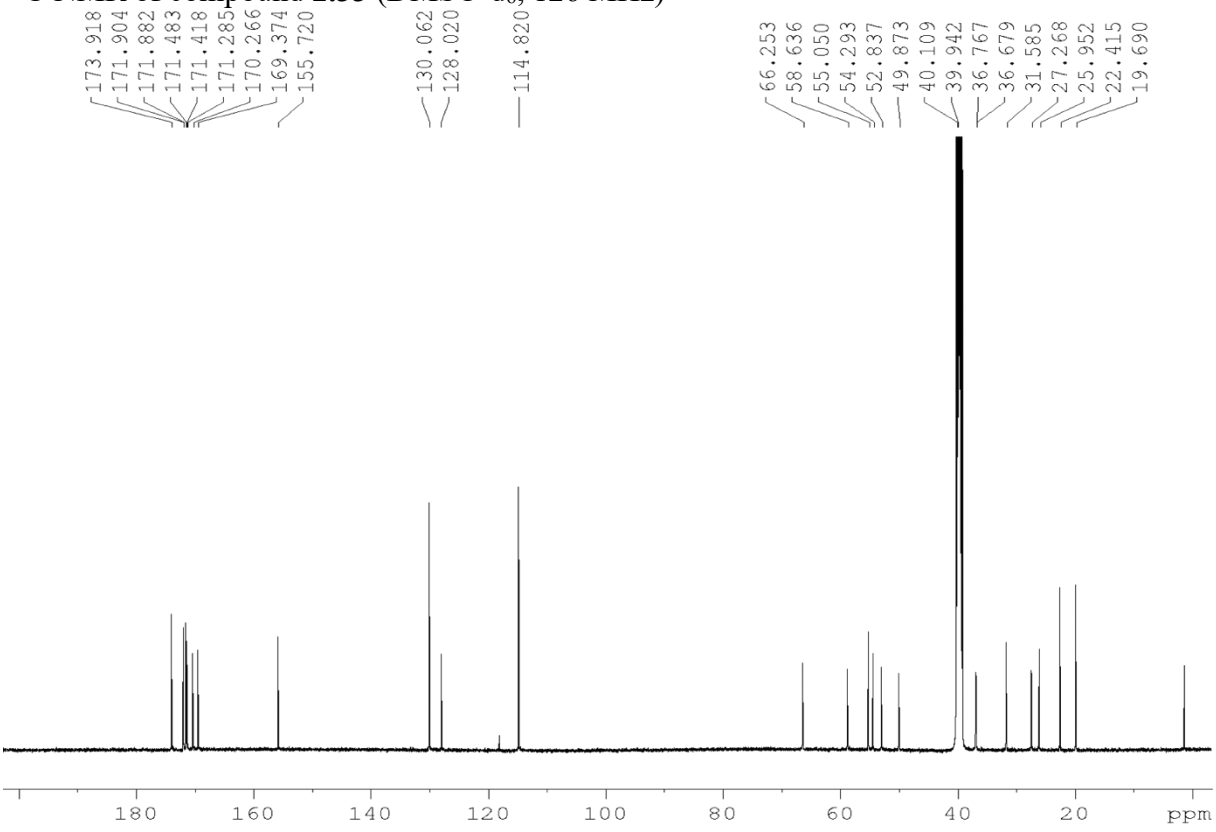
^{19}F NMR of compound **2.32** (DMSO-d_6, 282 MHz)



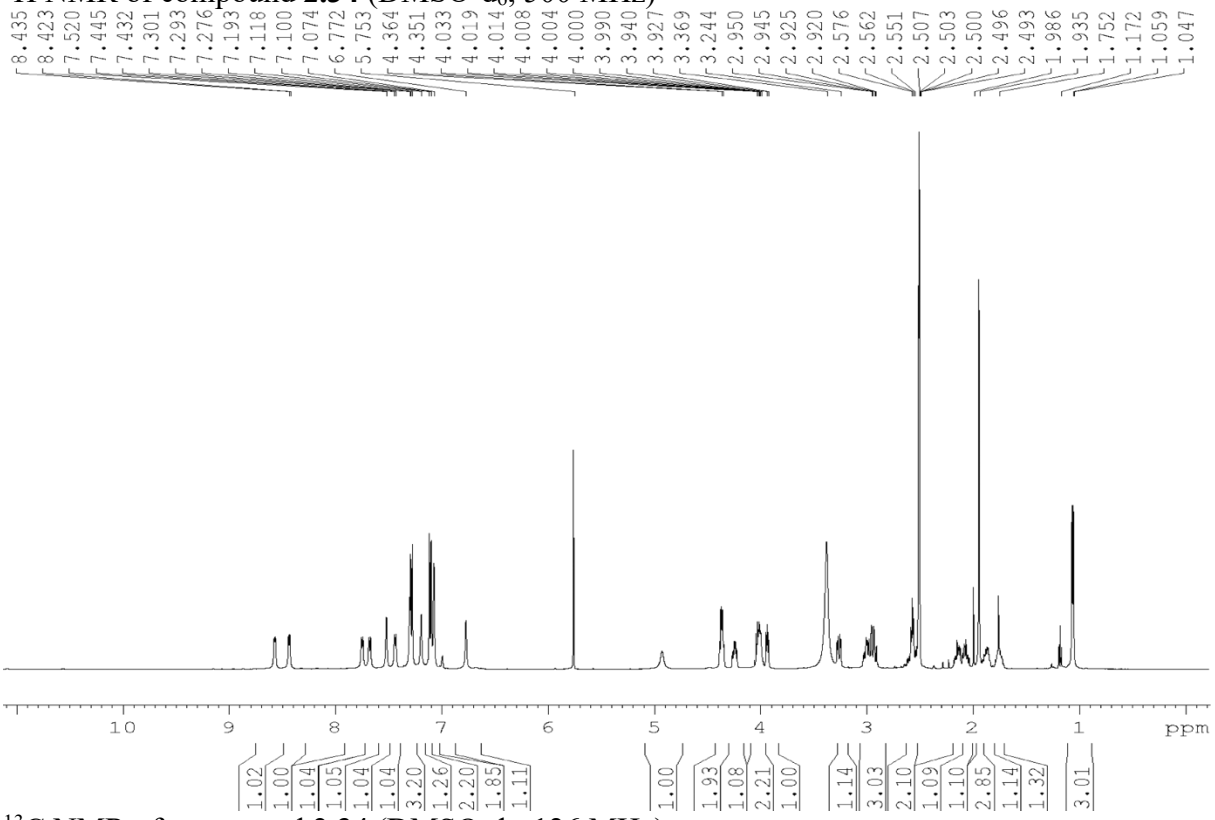
^{1}H NMR of compound **2.33** (DMSO-d_6, 500 MHz)



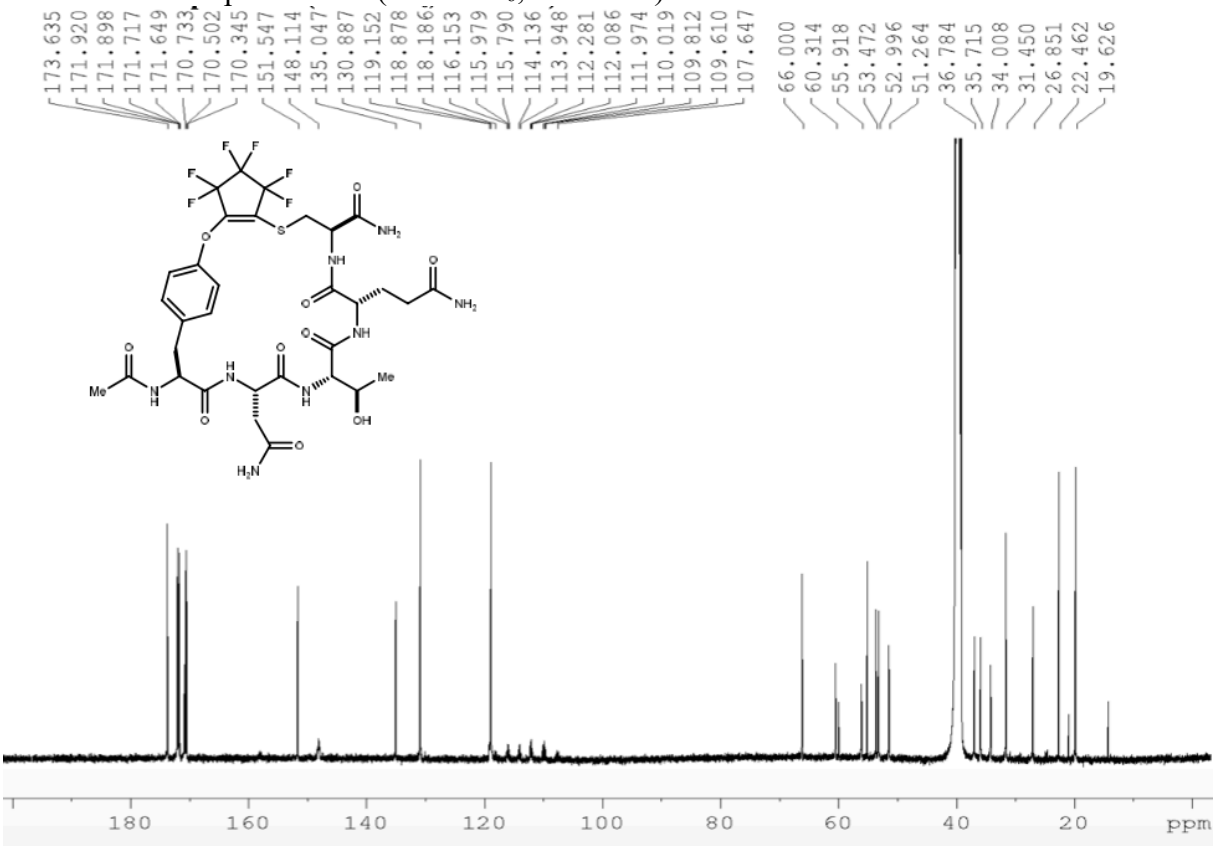
^{13}C NMR of compound **2.33** (DMSO-d_6, 126 MHz)



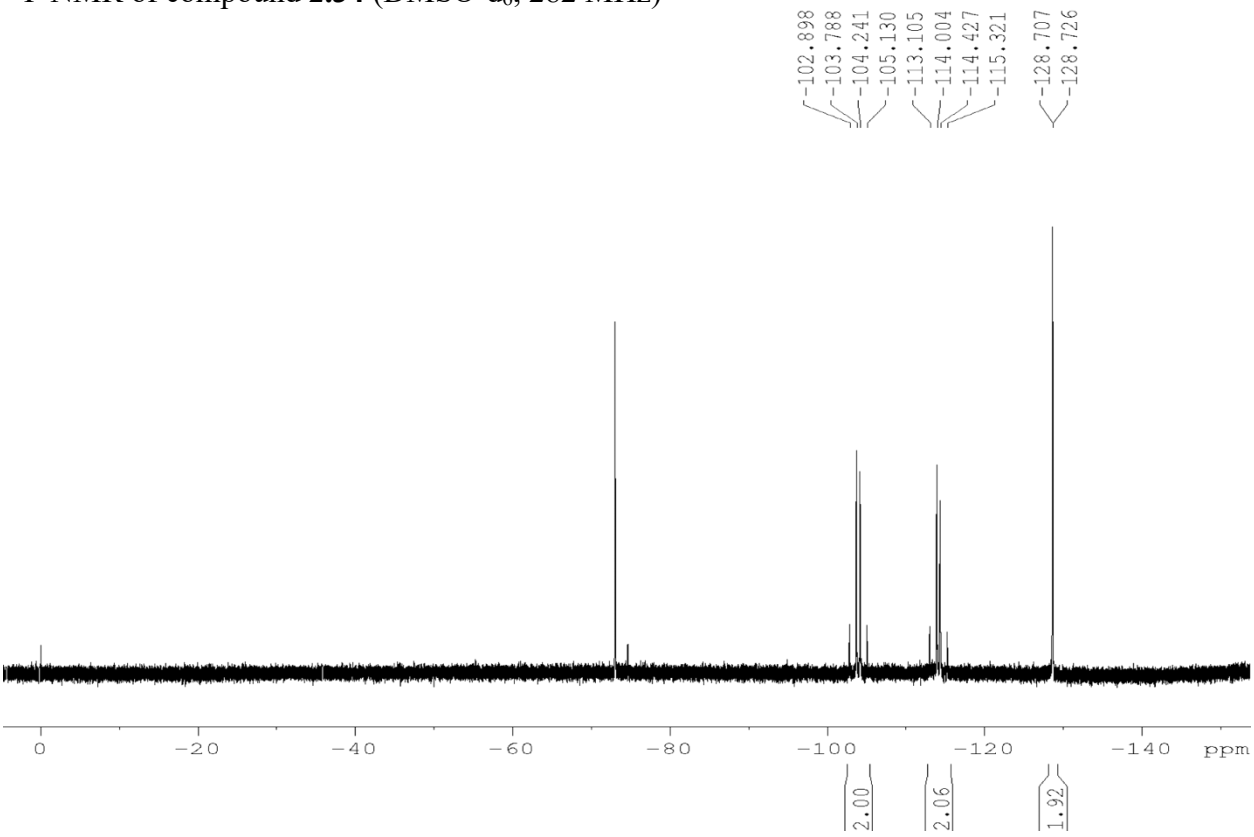
^{1}H NMR of compound **2.34** (DMSO-d_6, 500 MHz)



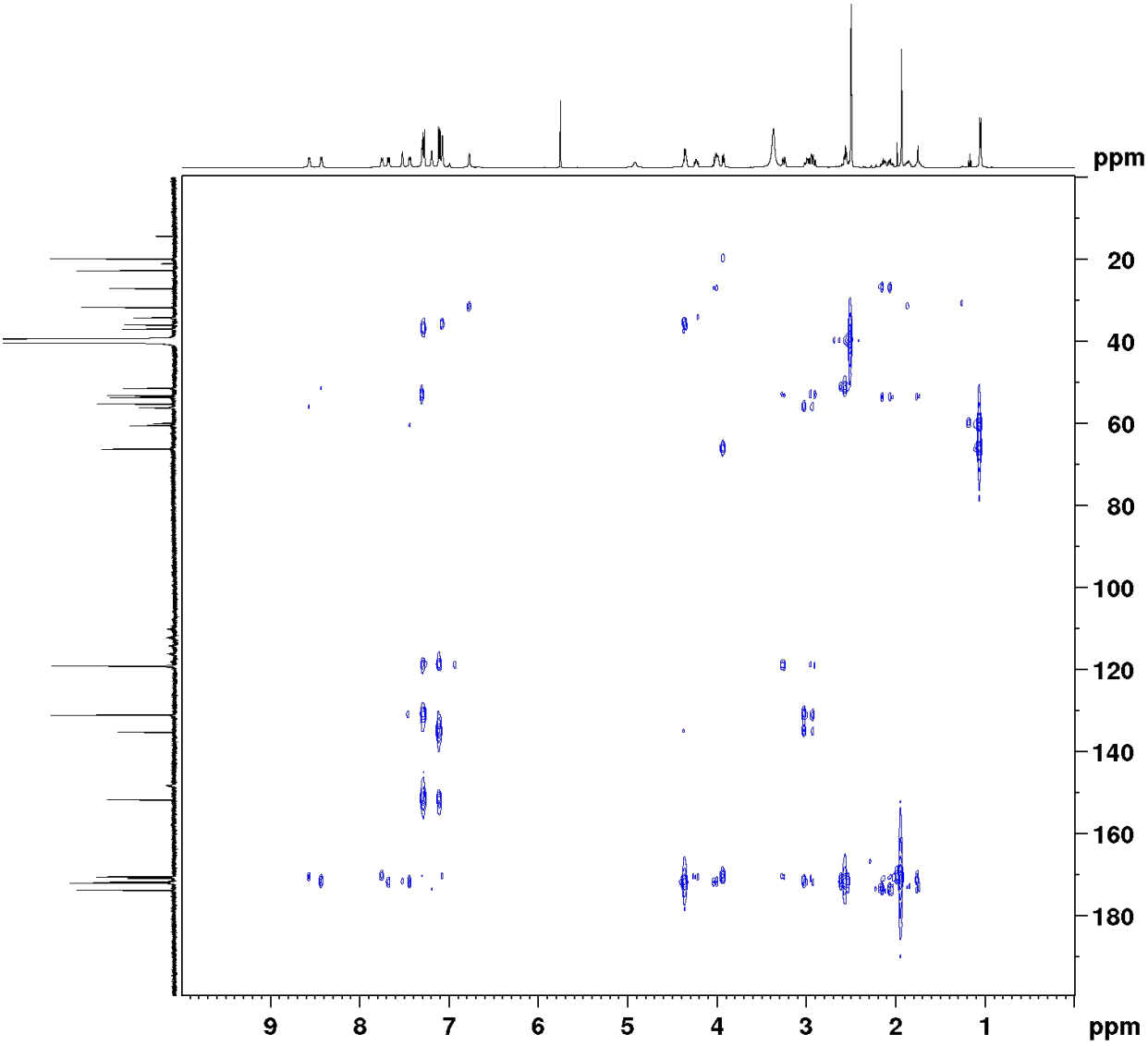
^{13}C NMR of compound **2.34** (DMSO-d_6, 126 MHz)



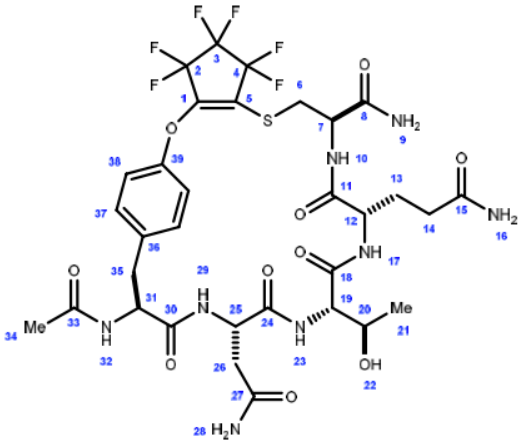
^{19}F NMR of compound **2.34** (DMSO-d_6, 282 MHz)



HMBC spectrum of compound **2.34** (DMSO-d_6)

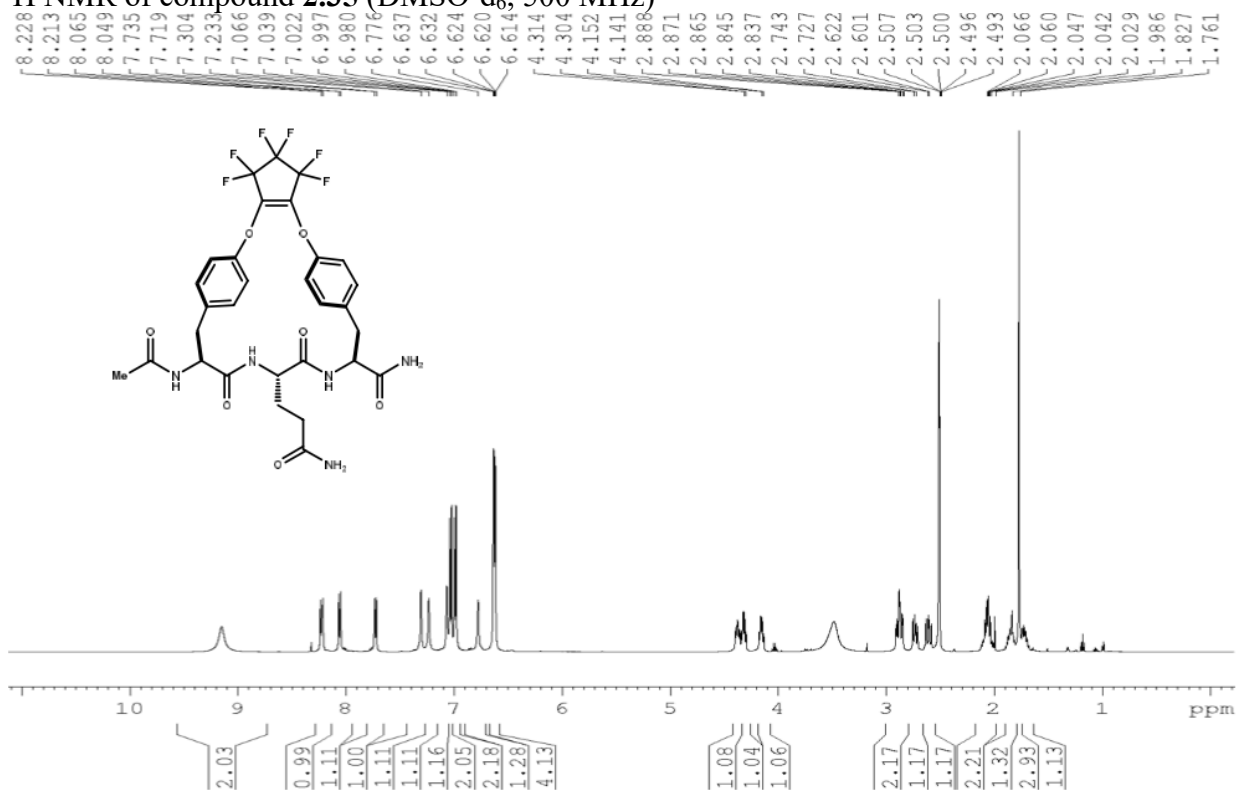


HMBC spectrum of compound **2.34** (DMSO-d_6)

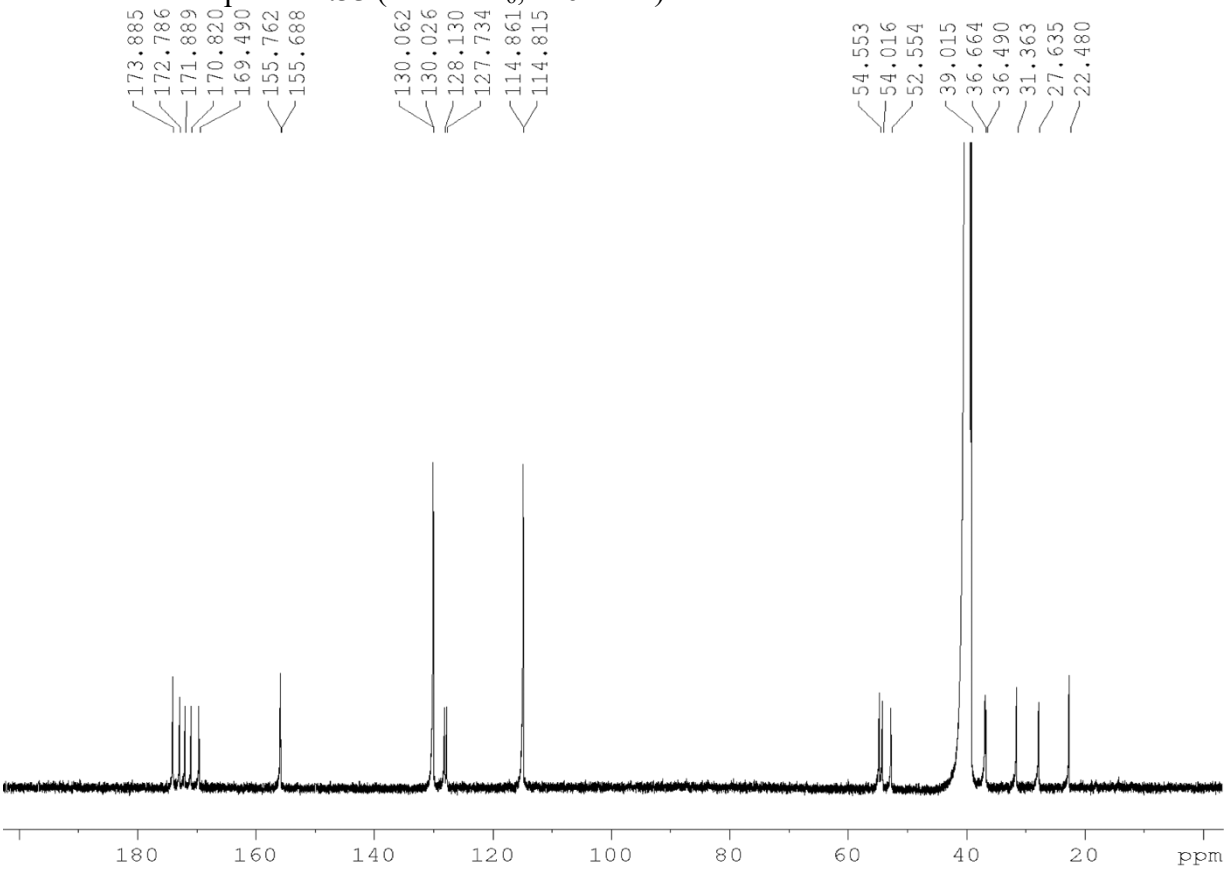


	13C	1H	Key Correlation
1	148.5–147.9 (m, 1C)	–	
2	118.6–106.7 (m, 3C)	–	
3	118.6–106.7 (m, 3C)	–	
4	118.6–106.7 (m, 3C)	–	
5	119.2–118.6 (m, 1C)	–	HMBC 6 –> 5
6	34.0	3.25 (dd, J = 12.7 4.3 Hz, 1H), 3.02–2.89 (m, 1H) overlap	HMBC 6 –> 5; HMBC 7 –> 6, HSQC
7	53.0	4.27–4.21 (m, 1H)	HMBC 6 –> 7; HMBC 7 –> 8, HSQC
8	170.5	–	HMBC 6 –> 8
9	–	7.30 (brs, 1H), 7.52 (s, 1H)	HMBC 9 –> 8
10	–	7.68 (d, J = 8.0 Hz, 1H)	HMBC 10 –> 7,8
11	171.9 overlap	–	HMBC 10 –> 11
12	53.5	4.05–3.97 (m, 1H) overlap	HMBC 12 –> 11,13,14, HSQC
13	26.9	1.90–1.82 (m, 1H), 1.79–1.70 (m, 1H)	HMBC 14,12 –> 13; HMBC 13 –> 12,14, HSQC
14	31.4	2.18–2.10 (m, 1H), 2.10–2.01 (m, 1H)	HMBC 14 –> 12,13,15; HMBC 12,13,16 –> 14
15	173.6	–	HMBC 16 –> 15
16	–	7.19 (brs, 1H), 6.77 (brs, 1H)	HMBC 16 –> 15,14
17	–	7.75 (d, J = 7.0 Hz, 1H)	HMBC 17 –> 12,13
18	170.3	–	HMBC 19 –> 18
19	60.3	3.93 (dd, J = 6.6, 5.2 Hz, 1H)	HMBC 19 –> 20,21, HSQC
20	66.0	4.05–3.97 (m, 1H) overlap	HMBC 21,19 –> 20, HSQC
21	19.6	1.05 (d, J = 6.3 Hz, 3H)	HMBC 19 –> 21, HSQC
22	–	4.92 (brs, 1H)	
23	–	7.44 (d, J = 6.6 Hz, 1H)	HMBC 23 –> 19,20
24	171.9 overlap	–	HMBC 23 –> 24
25	51.3	4.39–4.33 (m, 1H) overlap	HMBC 26 –> 25; HMBC 26 –> 25, HSQC
26	35.7	2.61–2.51 (m, 2H)	HMBC 29 –> 26; HMBC 25 –> 26, HSQC
27	171.6	–	HMBC 26 –> 27
28	–	7.07 (brs, 2H)	HMBC 28 –> 27
29	–	8.43 (d, J = 6.3 Hz, 1H)	HMBC 29 –> 25
30	171.7	–	HMBC 35,31 –> 30
31	55.9	4.39–4.33 (m, 1H) overlap	HMBC 35 –> 31; HMBC 31 –> 36, HSQC
32	–	8.56 (d, J = 5.3 Hz, 1H)	HMBC 32 –> 33
33	170.7	–	HMBC 34 –> 33
34	22.5	1.94 (s, 3H)	HMBC 34 –> 33, HSQC
35	36.8	3.02–2.89 (m, 2H) overlap	HMBC 35 –> 31; HMBC 37–> 35, HSQC
36	135.0	–	HMBC 35 –> 36
37	130.9	7.28 (d, J = 8.6 Hz, 2H)	HMBC 35 –> 37, HSQC
38	118.9	7.11 (d, J = 8.6 Hz, 2H)	HMBC 38 –> 39,36, HSQC
39	151.5	–	HMBC 37 –> 39

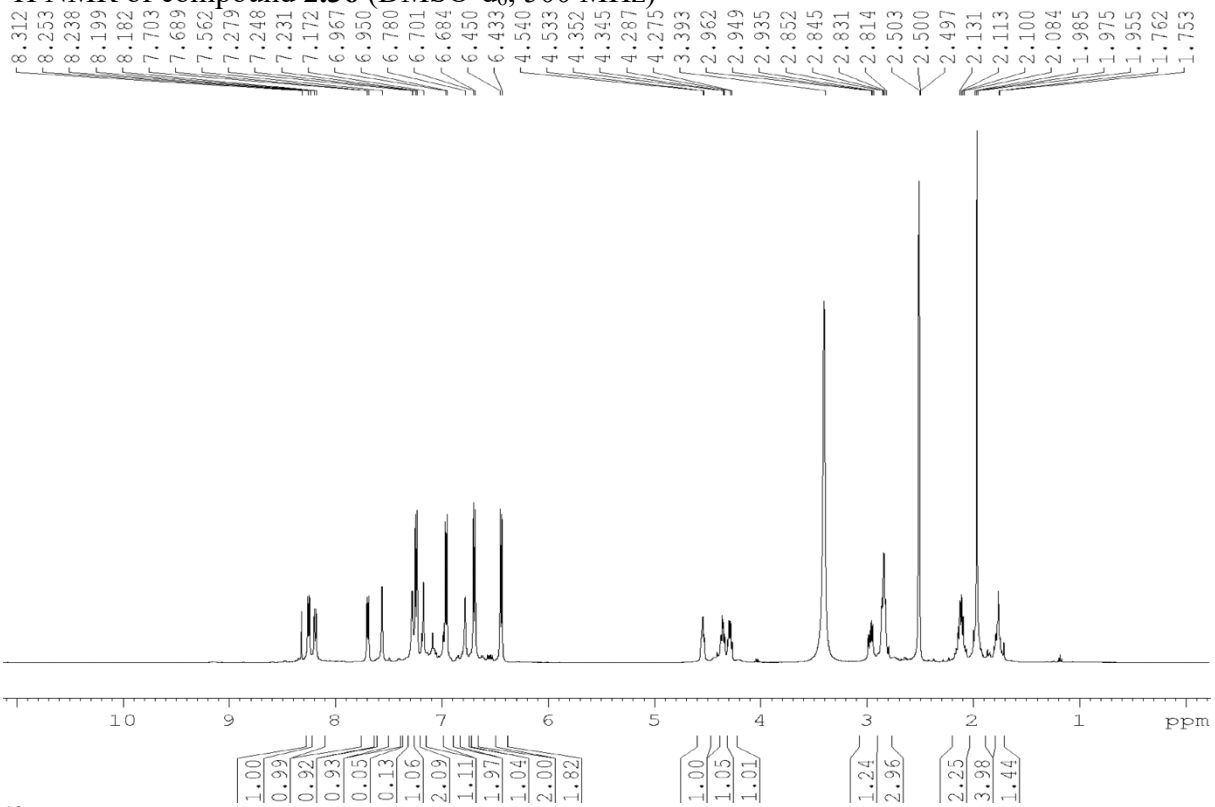
^{1}H NMR of compound **2.35** (DMSO-d_6, 500 MHz)



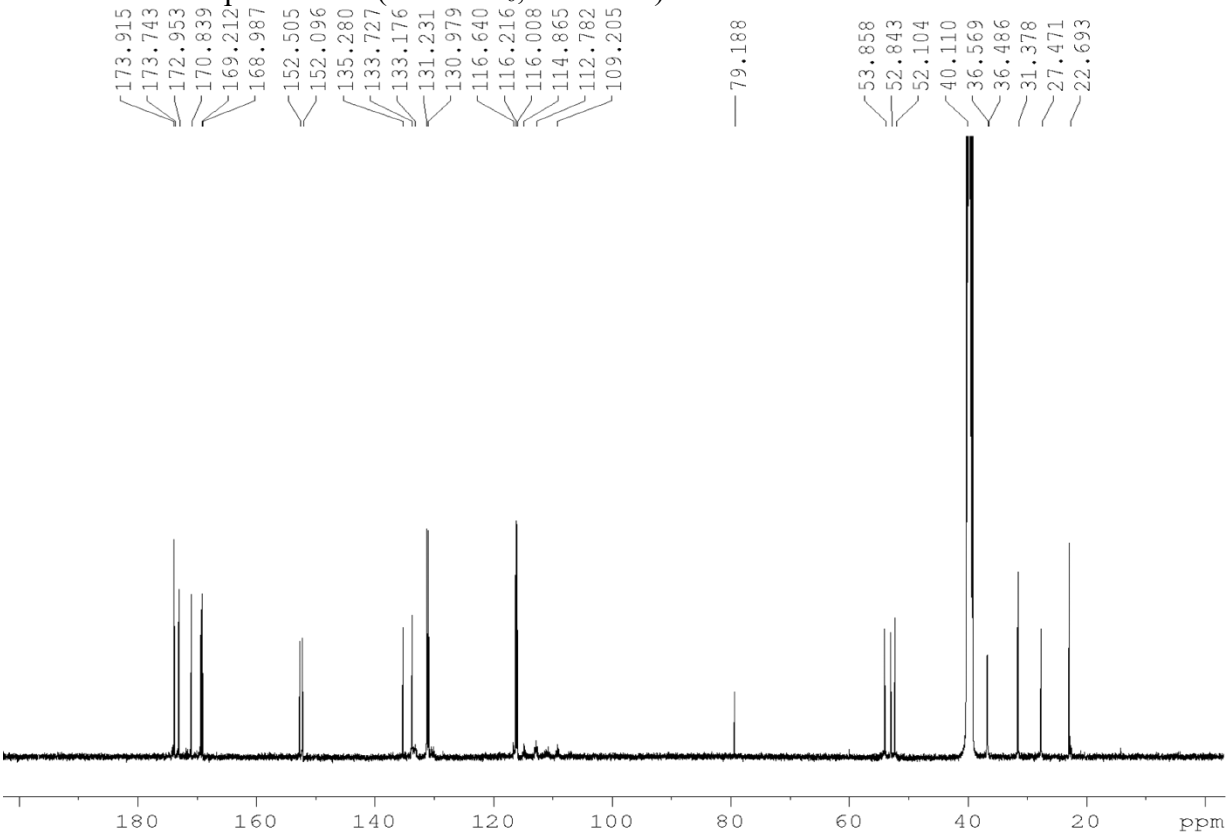
^{13}C NMR of compound **2.35** (DMSO-d_6, 126 MHz)



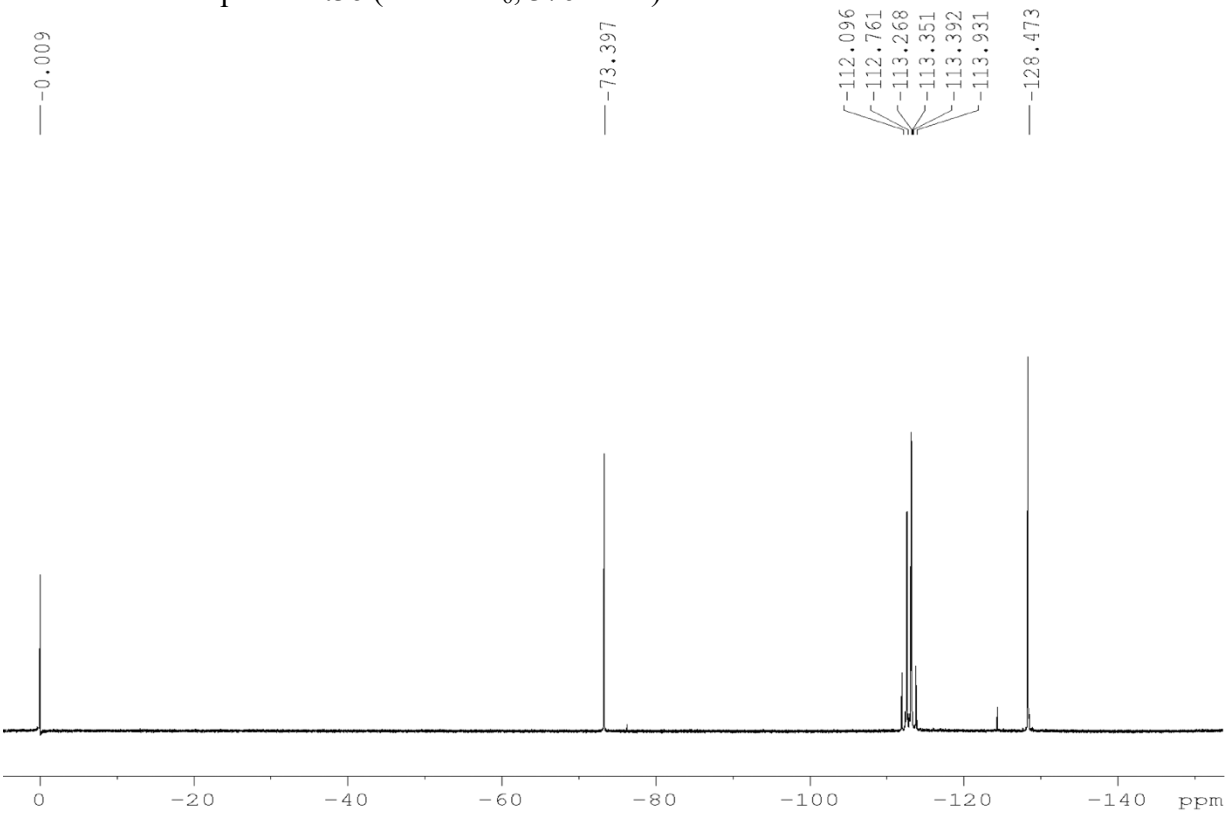
^{1}H NMR of compound **2.36** (DMSO-d_6, 500 MHz)



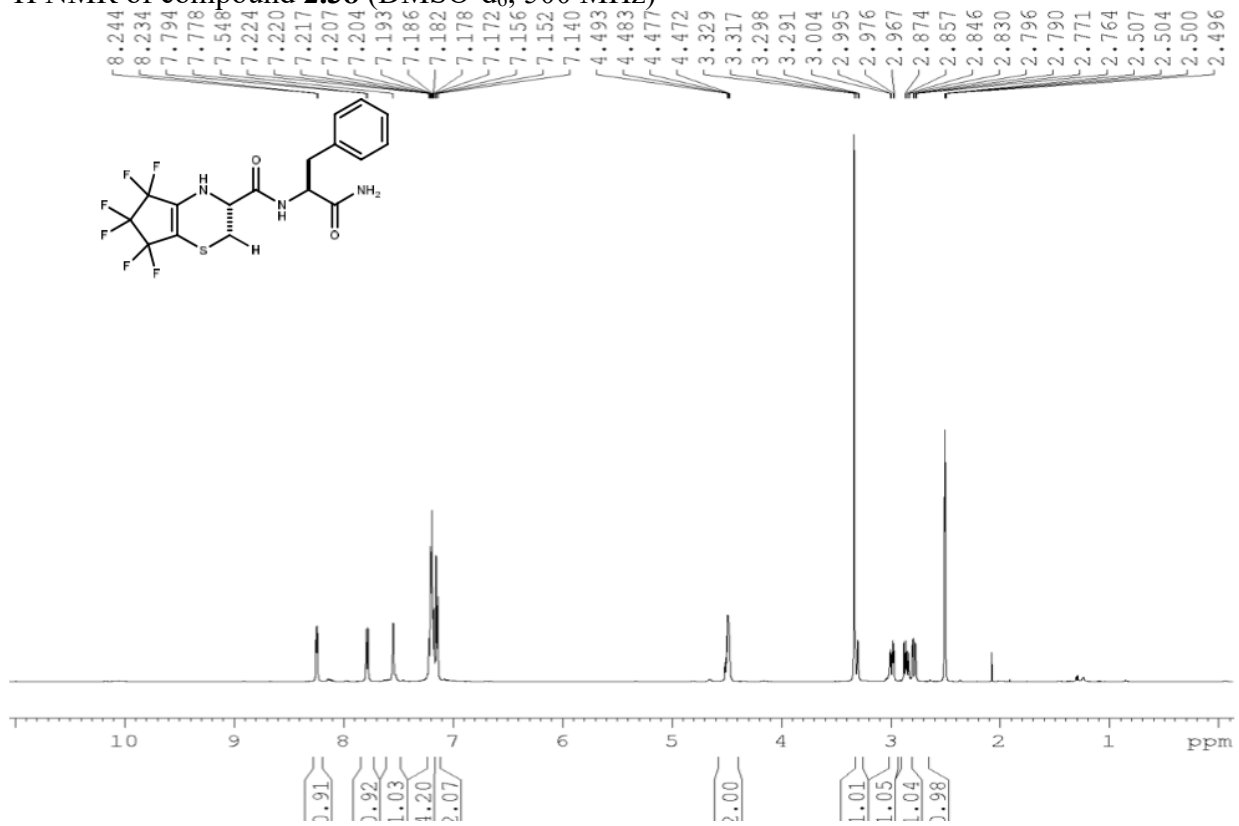
^{13}C NMR of compound **2.36** (DMSO-d_6, 126 MHz)



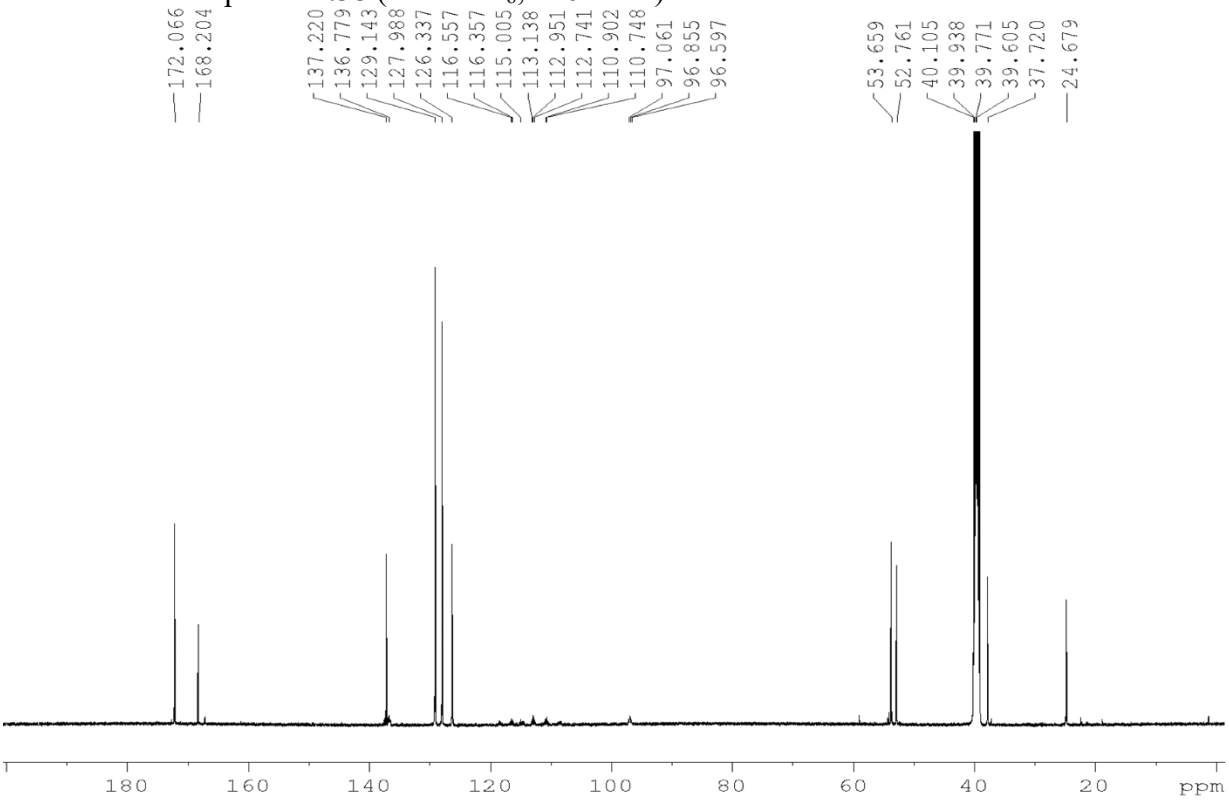
^{19}F NMR of compound **2.36** (DMSO-d_6, 376 MHz)



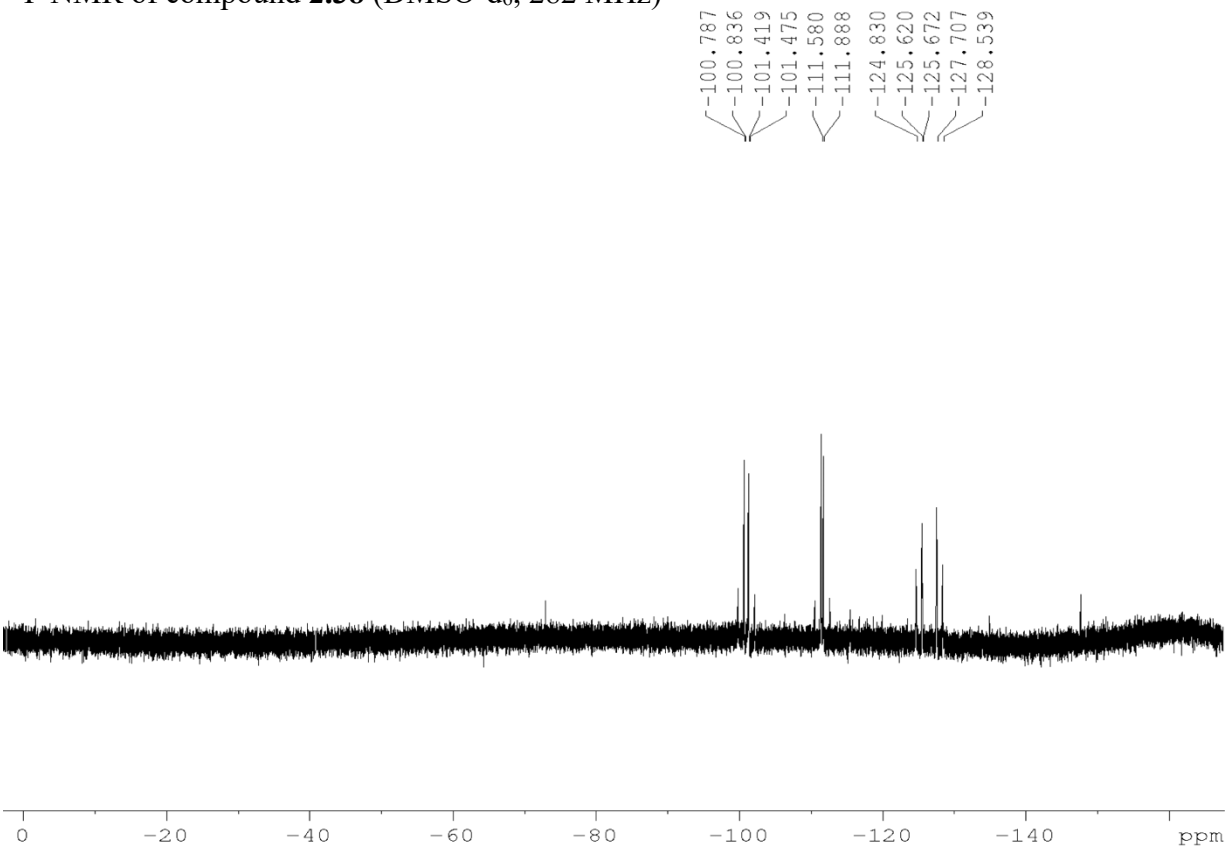
^{1}H NMR of compound **2.38** (DMSO-d_6, 500 MHz)



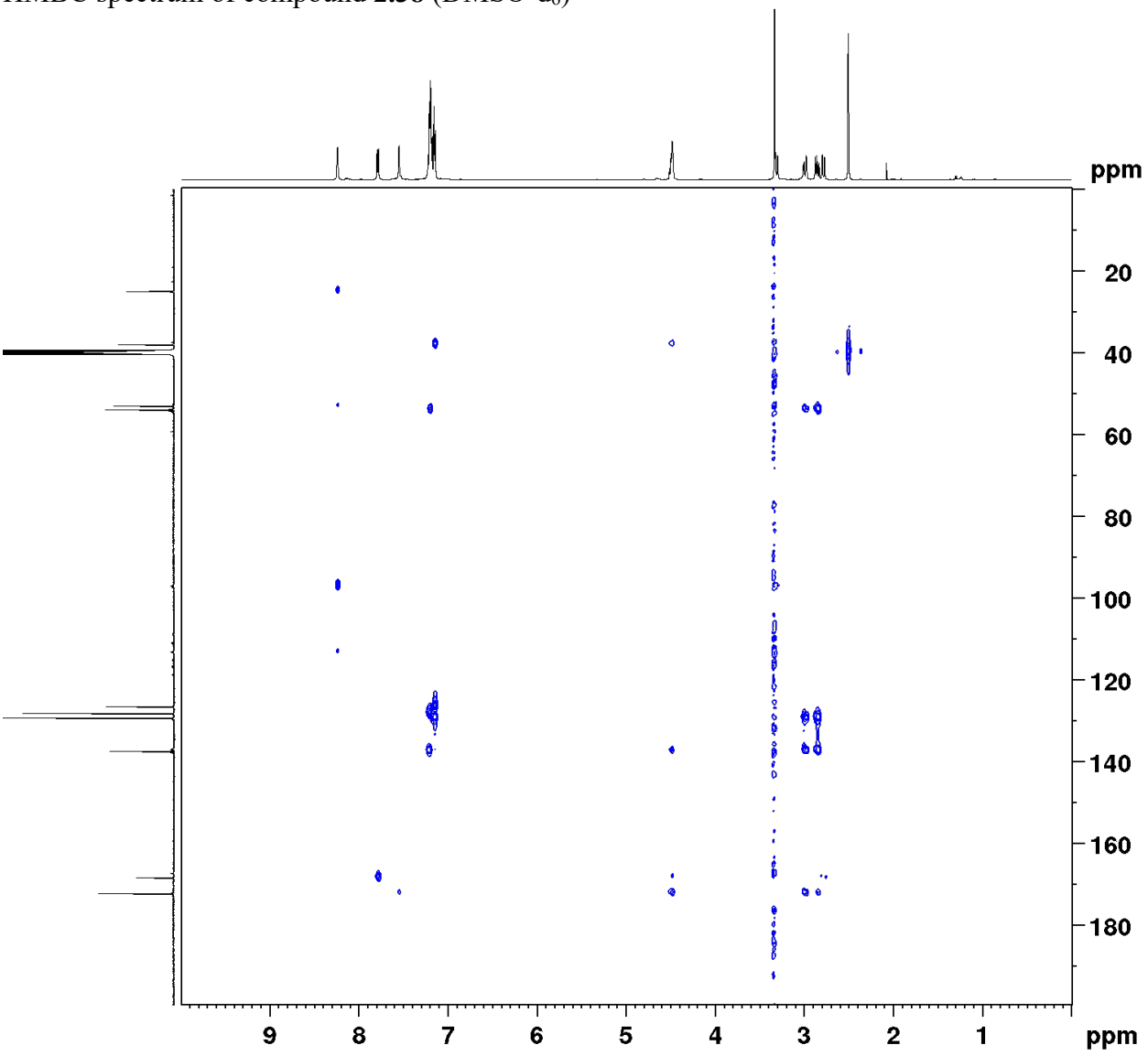
^{13}C NMR of compound **2.38** (DMSO-d_6, 126 MHz)



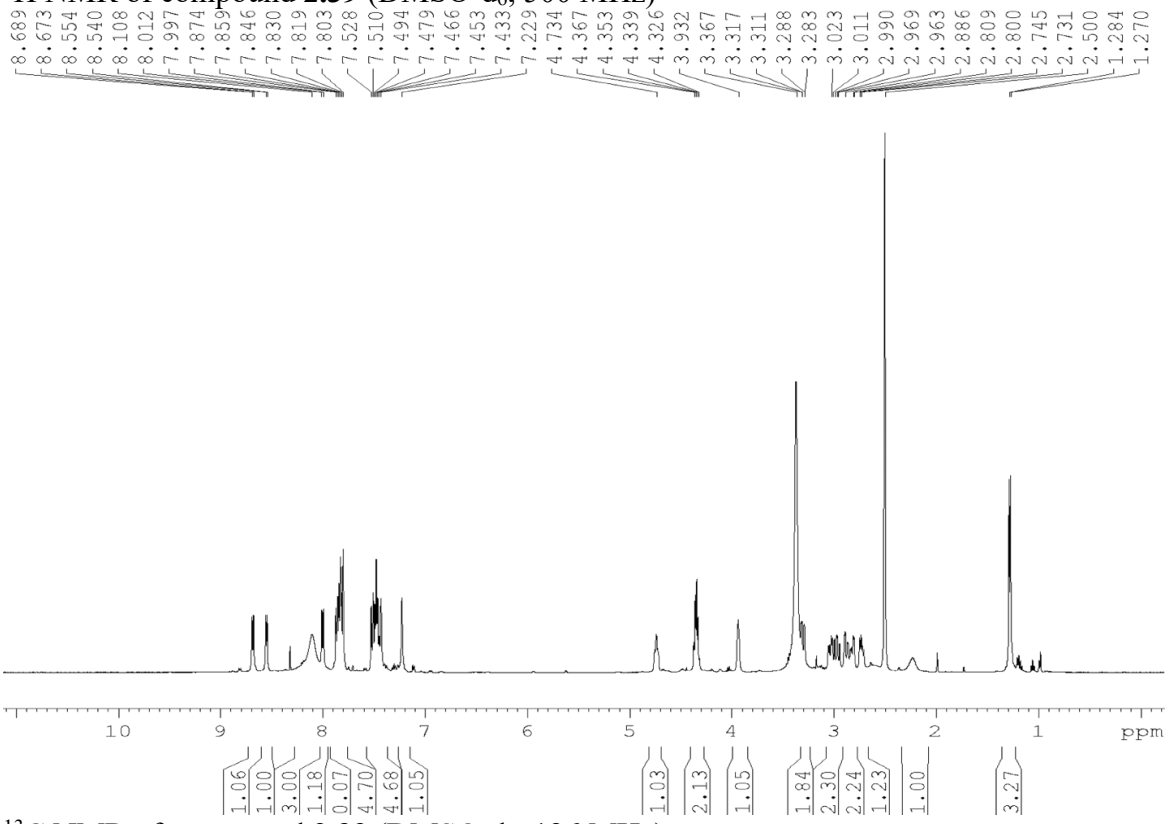
^{19}F NMR of compound **2.38** (DMSO-d_6, 282 MHz)



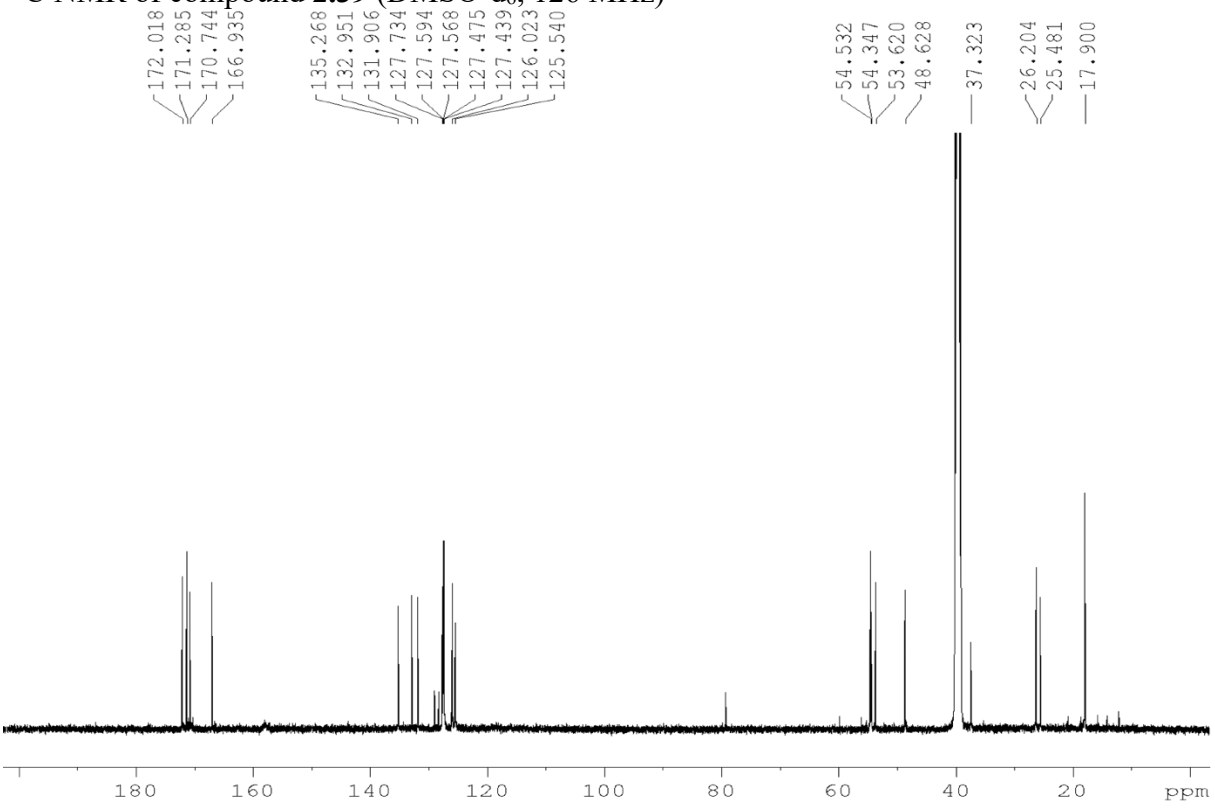
HMBC spectrum of compound **2.38** (DMSO-d_6)



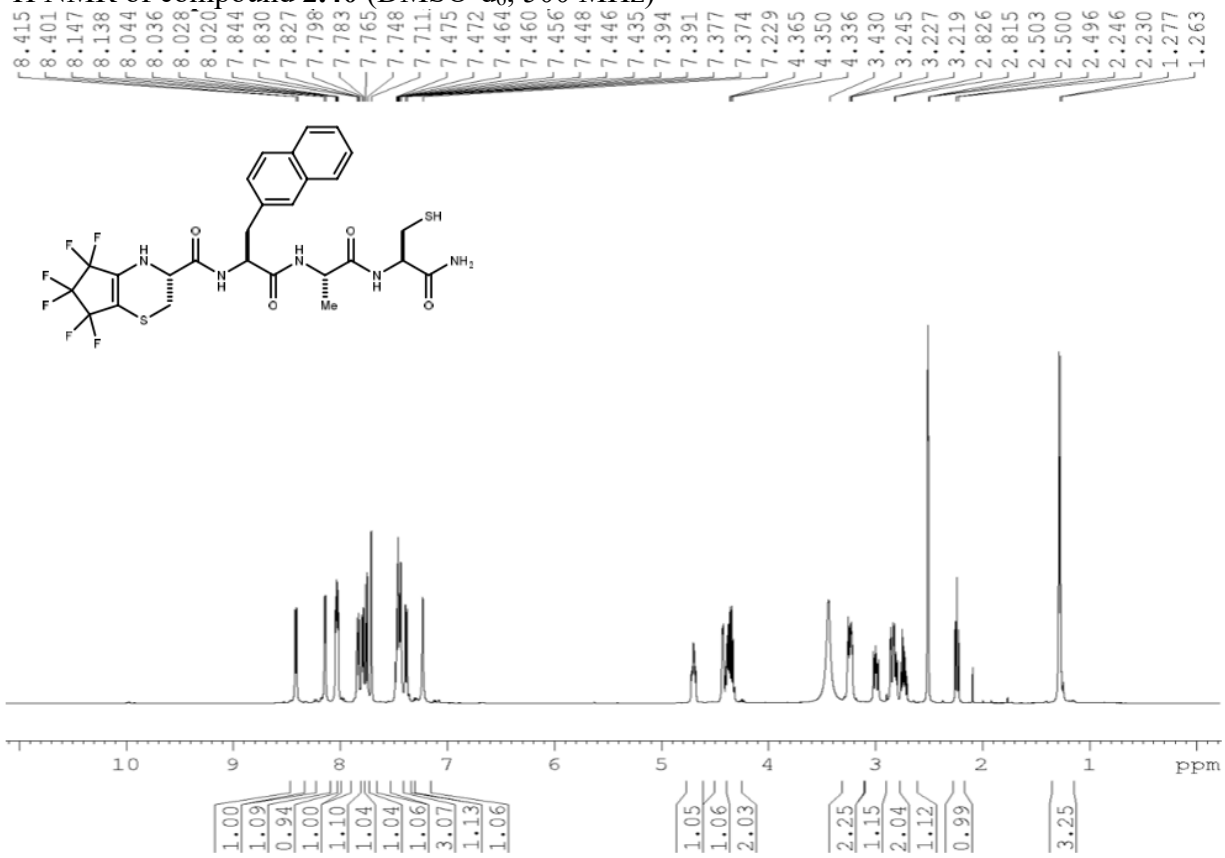
^{1}H NMR of compound **2.39** (DMSO-d_6, 500 MHz)



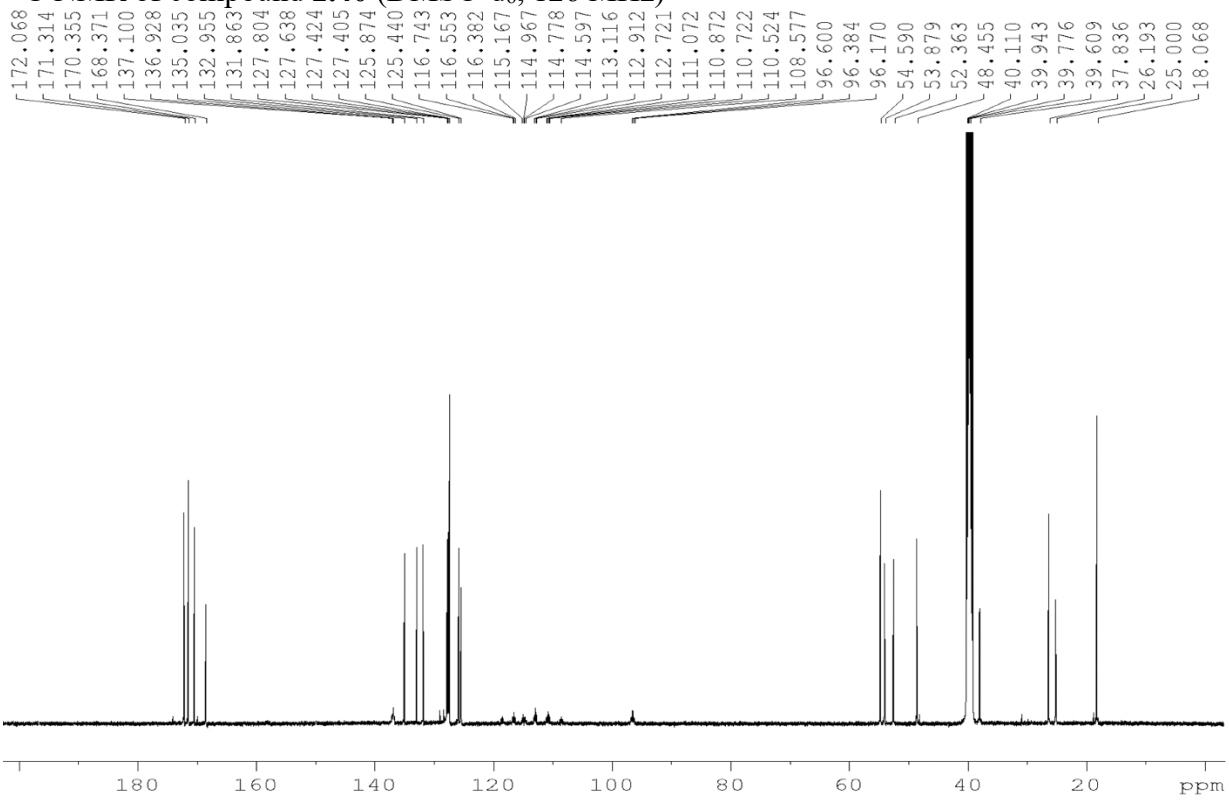
^{13}C NMR of compound **2.39** (DMSO-d_6, 126 MHz)



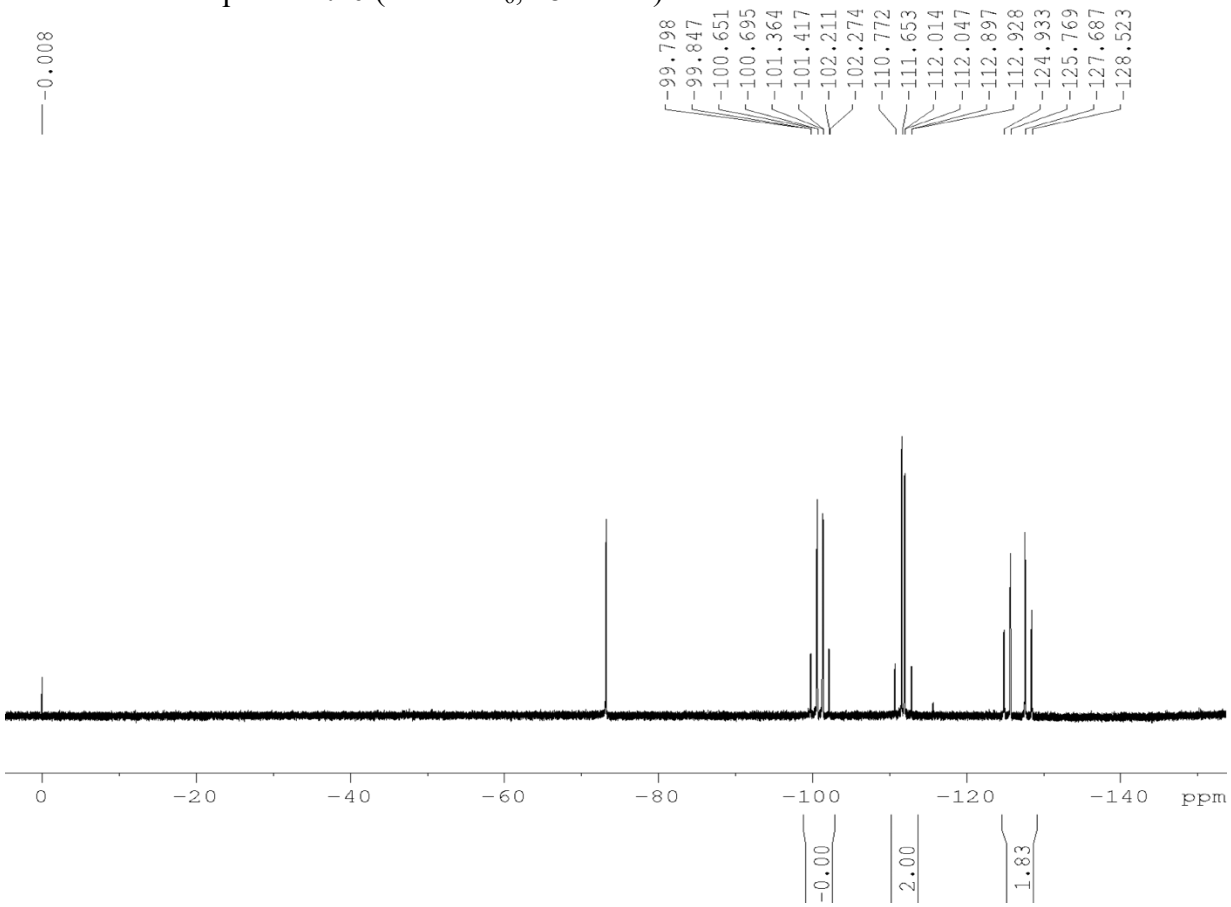
^{1}H NMR of compound **2.40** (DMSO-d_6, 500 MHz)



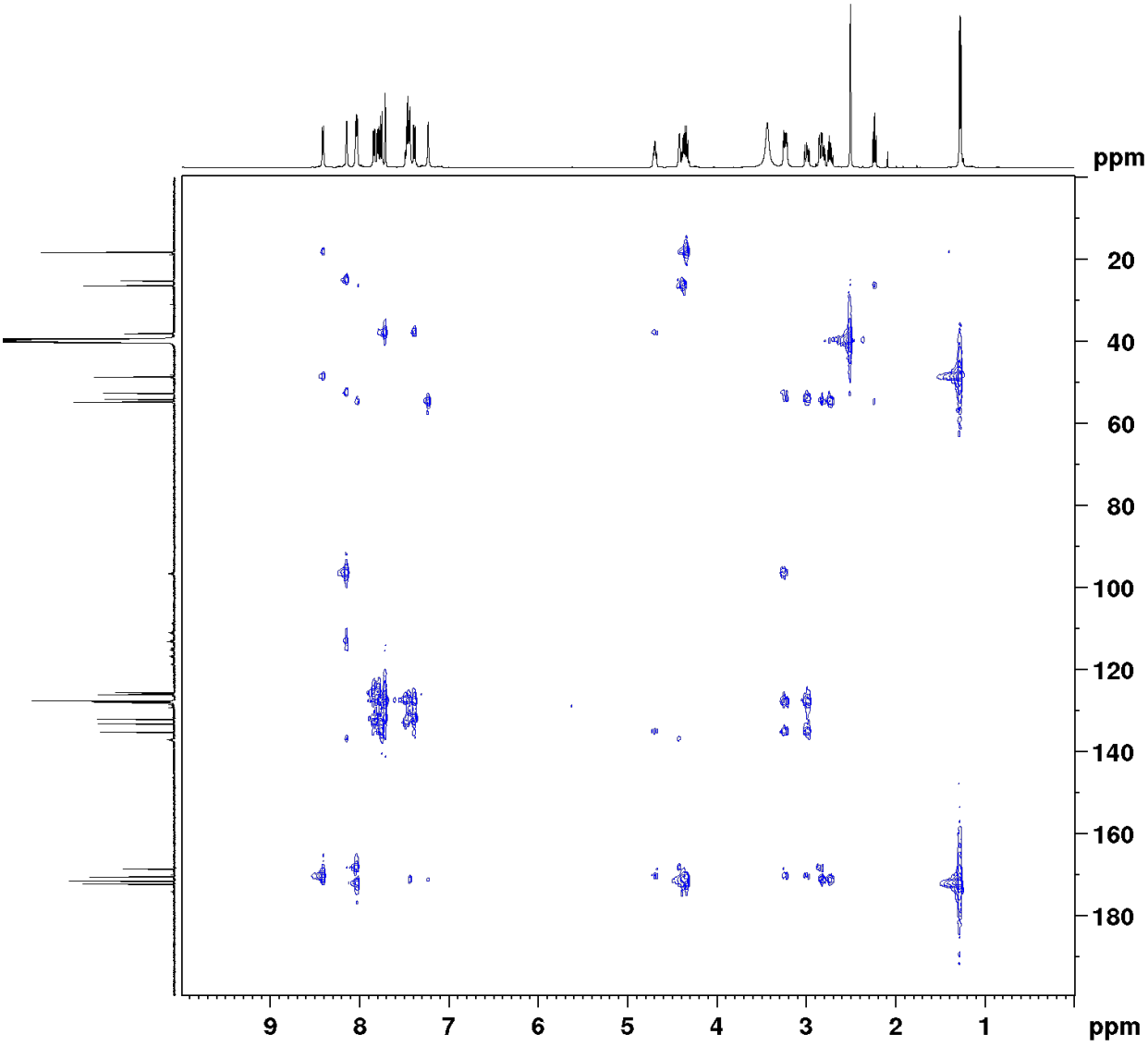
^{13}C NMR of compound **2.40** (DMSO-d_6, 126 MHz)



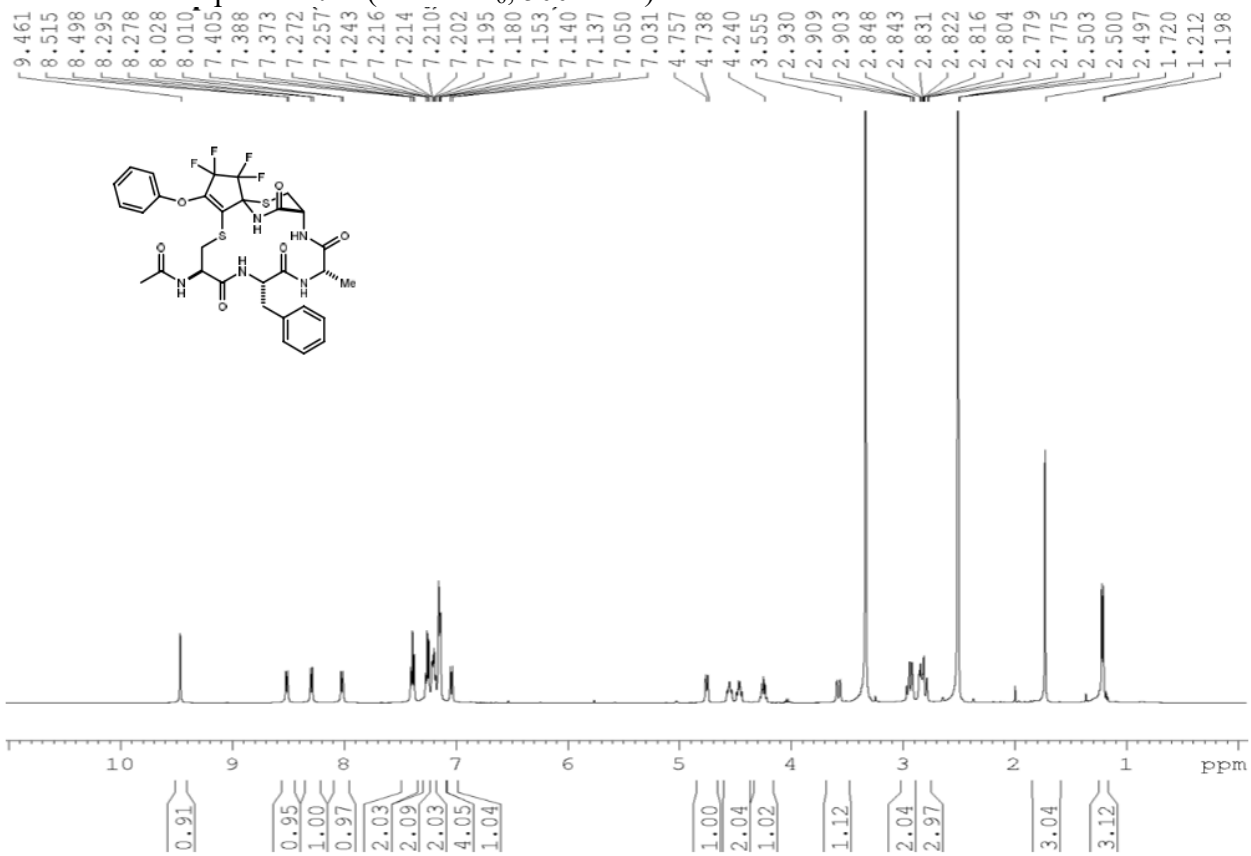
^{19}F NMR of compound **2.40** (DMSO-d_6, 282 MHz)



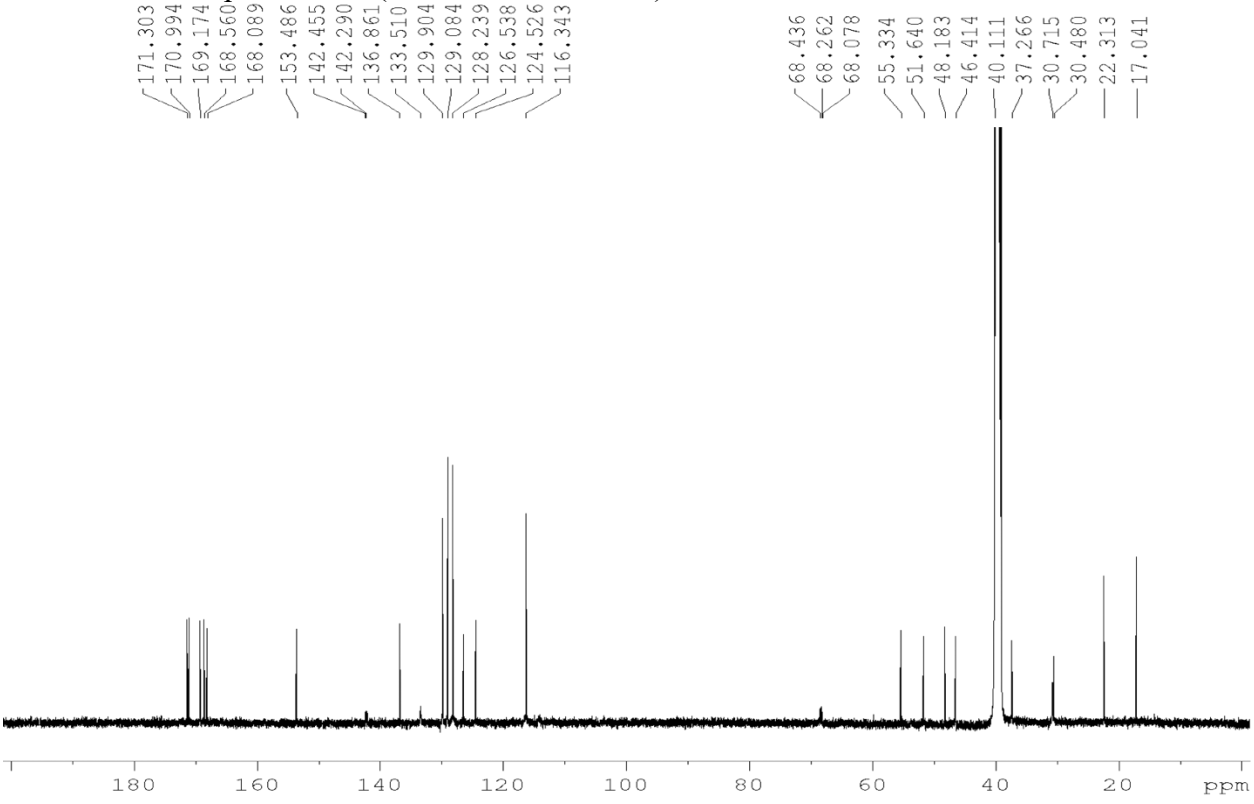
HMBC spectrum of compound **2.40** (DMSO-d_6)



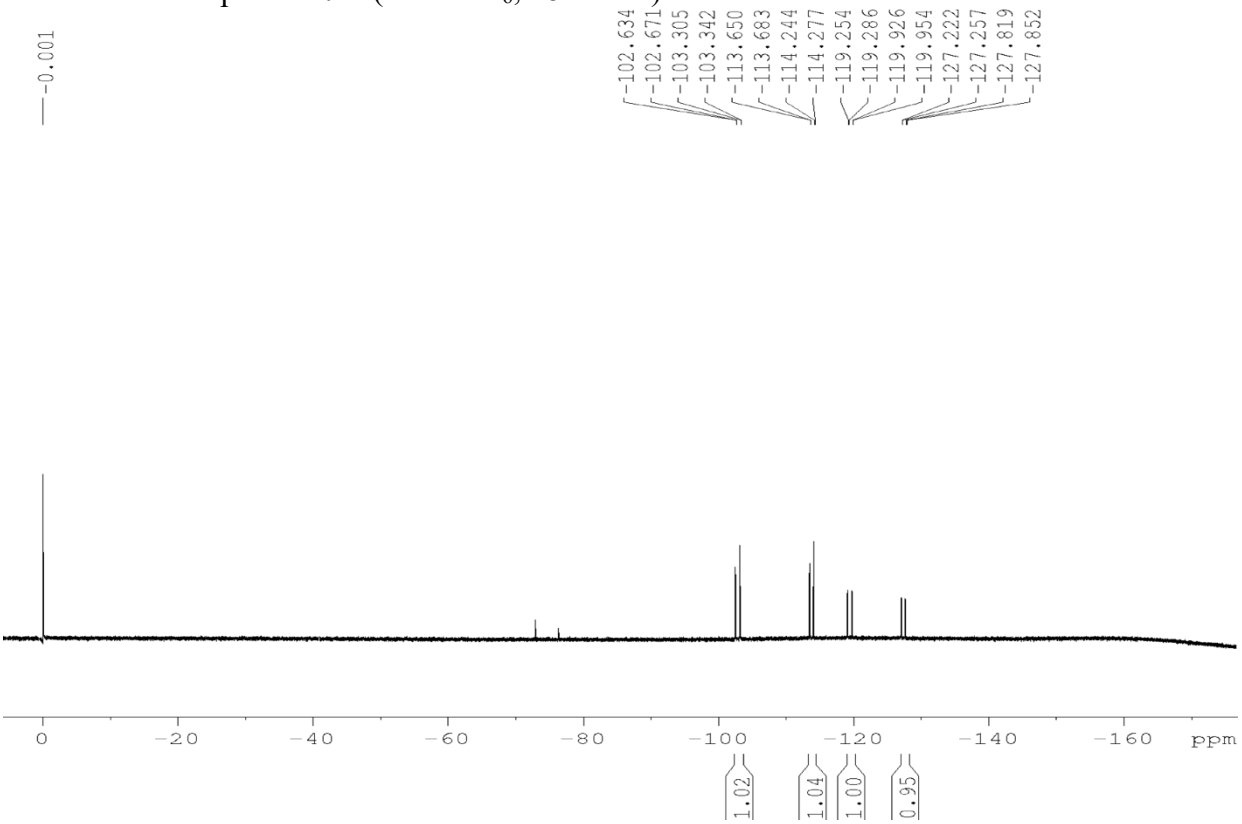
^{1}H NMR of compound **2.41** (DMSO-d_6, 500 MHz)



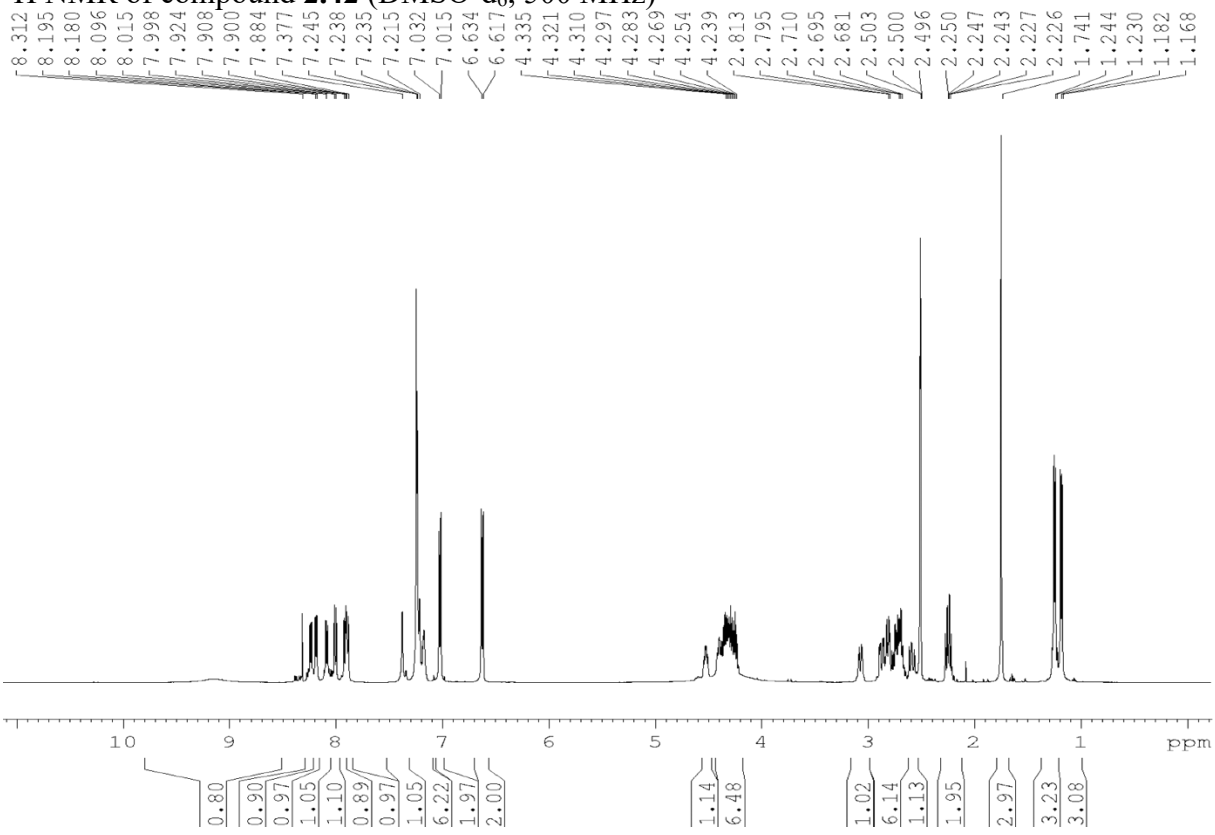
^{13}C NMR of compound **2.41** (DMSO-d_6, 126 MHz)



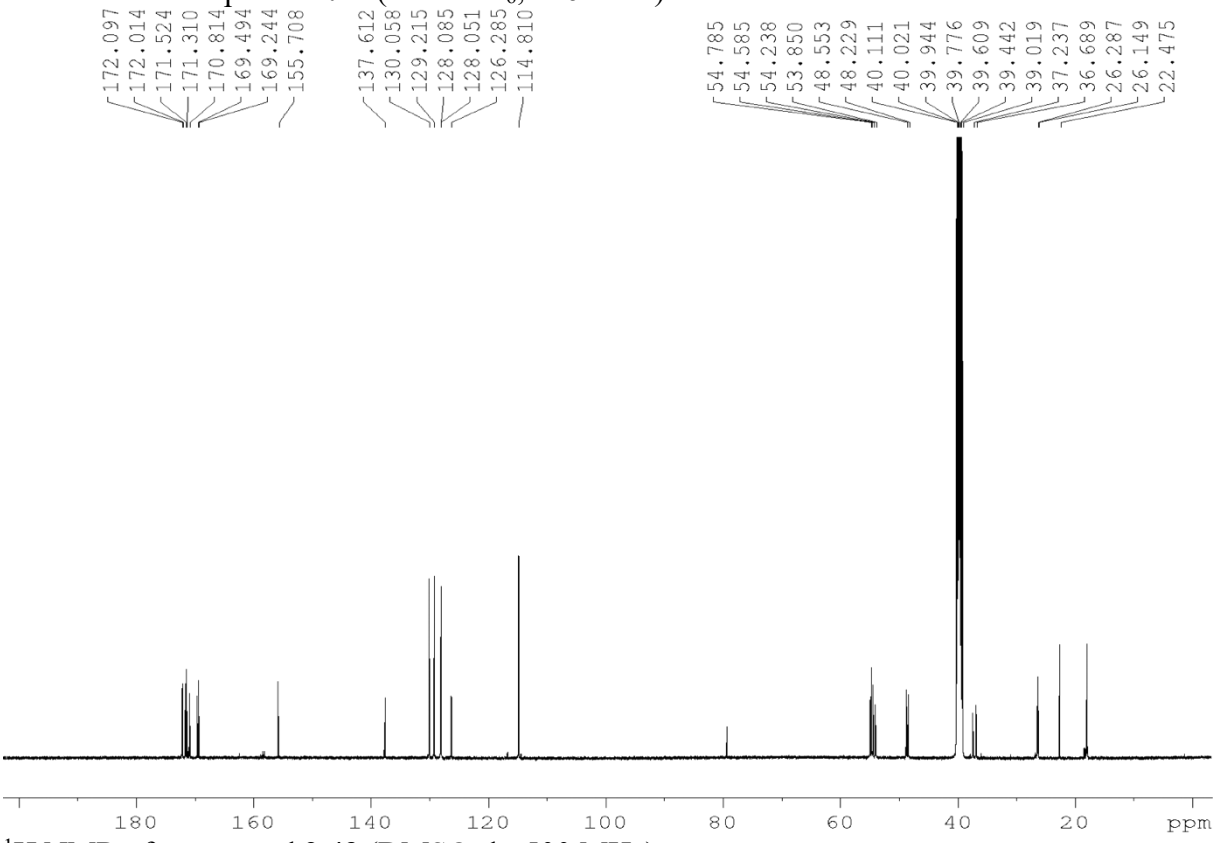
^{19}F NMR of compound **2.41** (DMSO-d_6, 282 MHz)



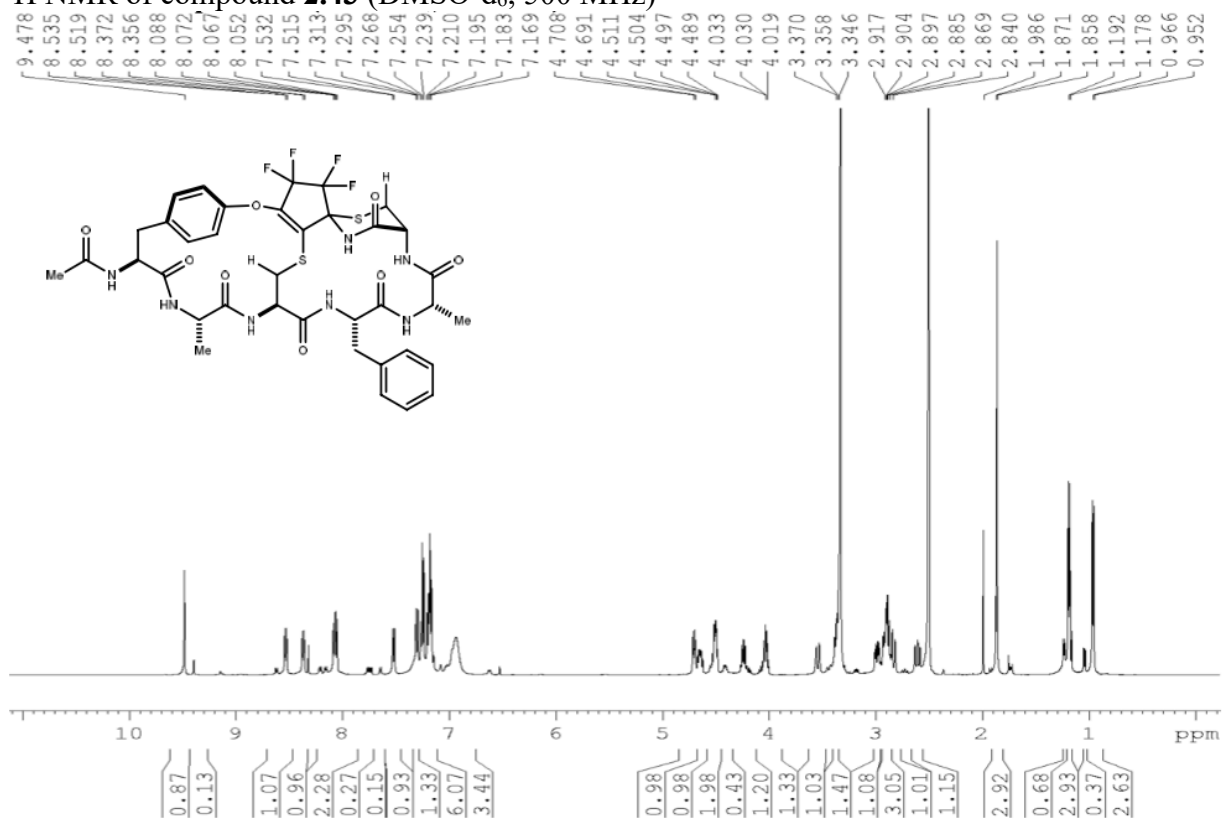
^{1}H NMR of compound **2.42** (DMSO-d_6, 500 MHz)



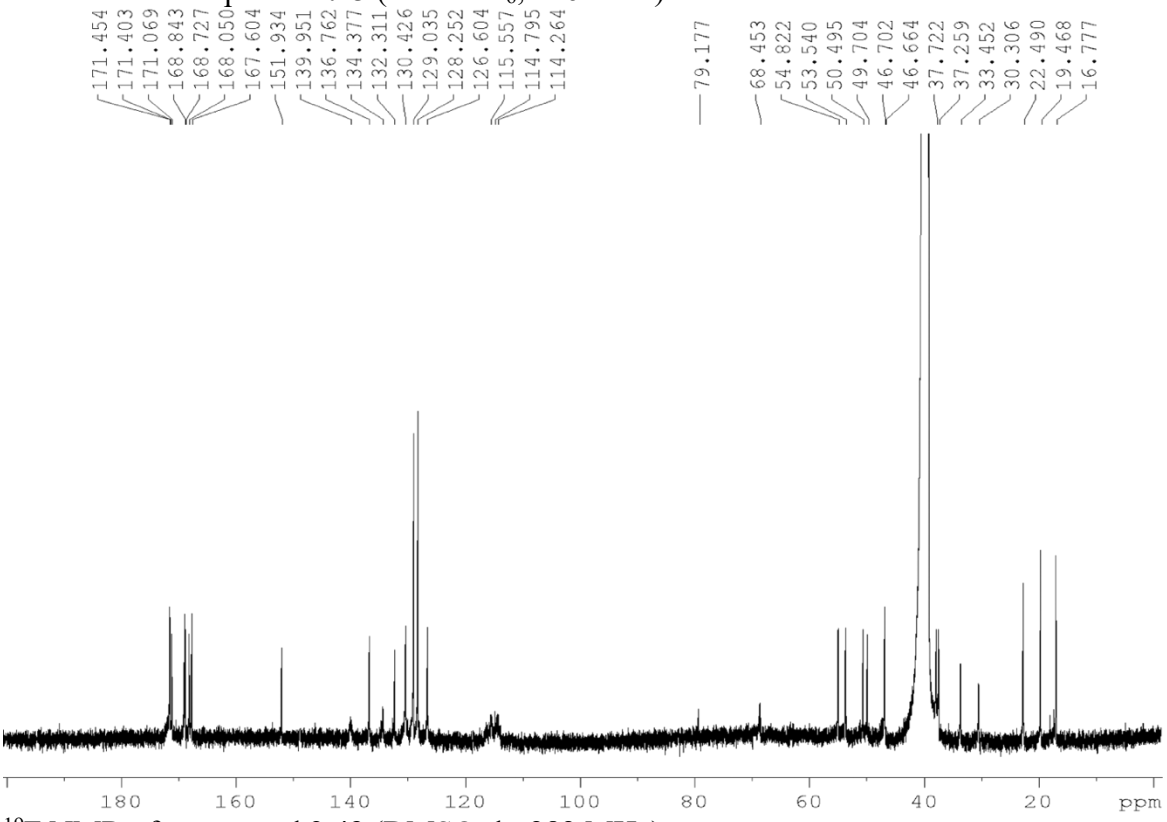
^{13}C NMR of compound **2.42** (DMSO-d$_6$, 126 MHz)



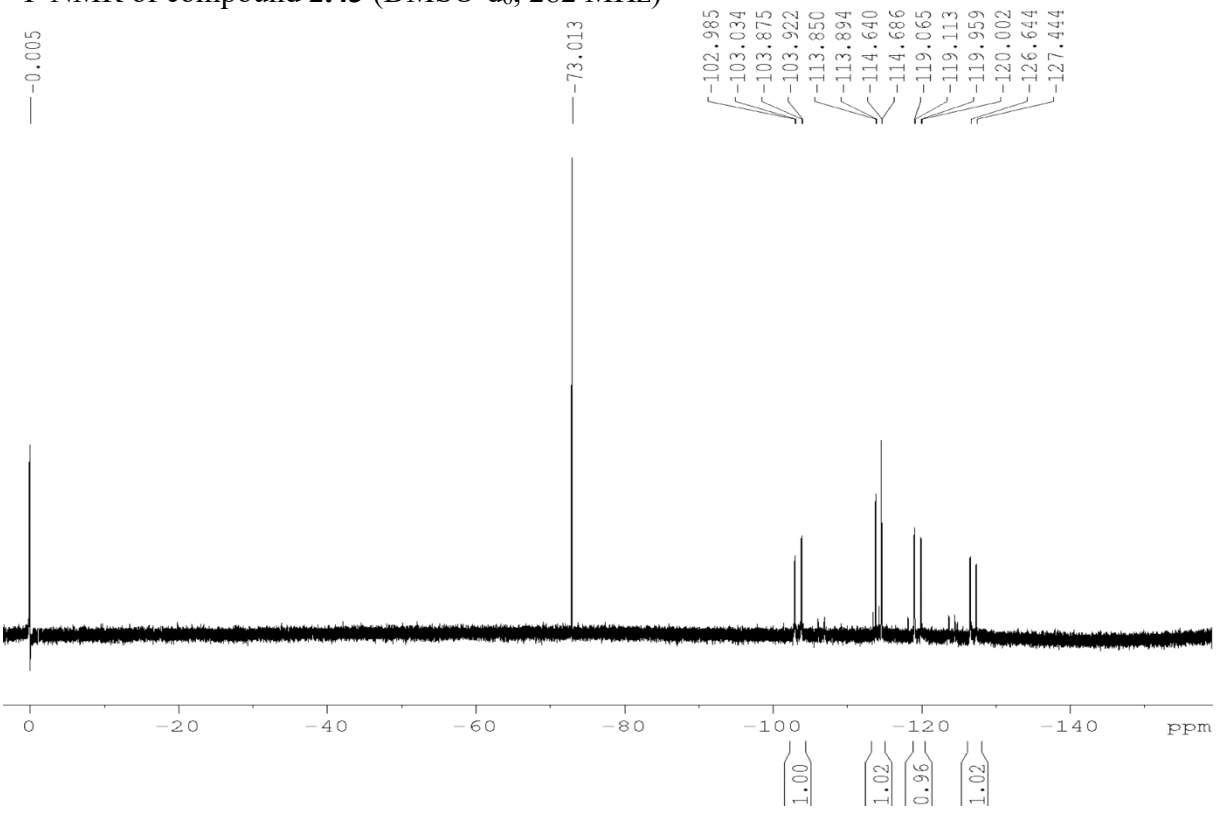
^{1}H NMR of compound **2.43** (DMSO-d$_6$, 500 MHz)



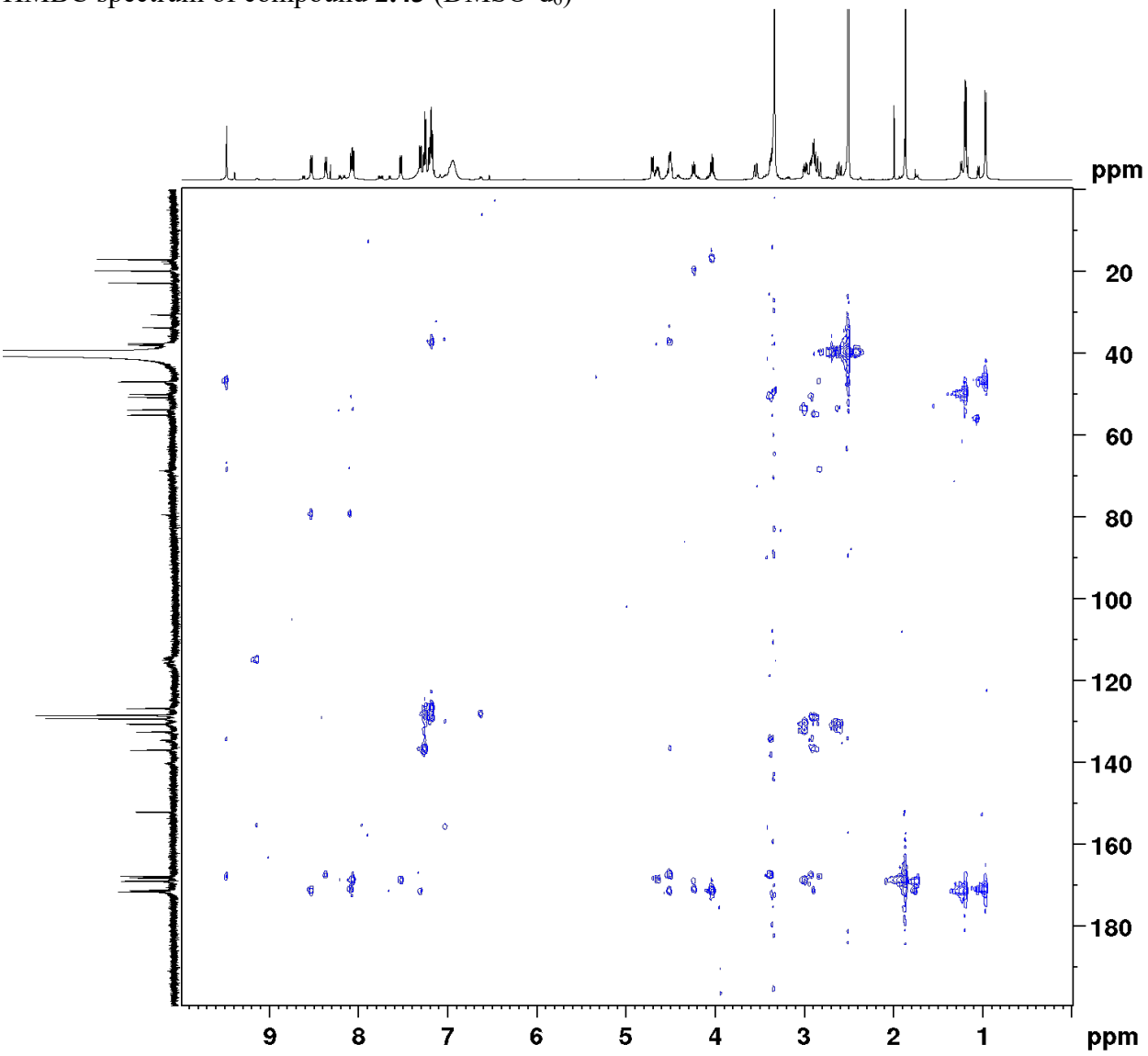
^{13}C NMR of compound **2.43** (DMSO-d_6, 126 MHz)



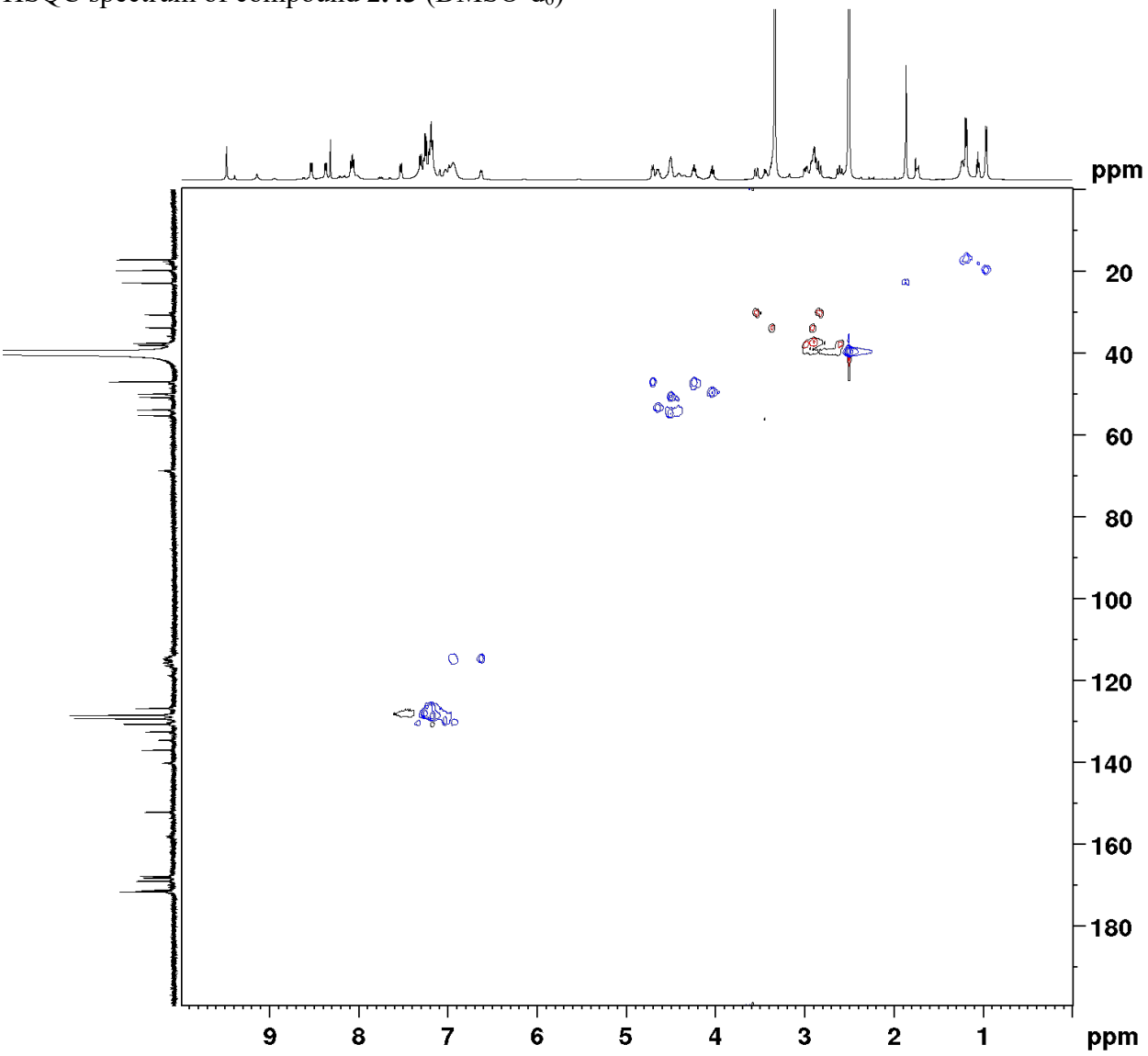
^{19}F NMR of compound **2.43** (DMSO-d_6, 282 MHz)



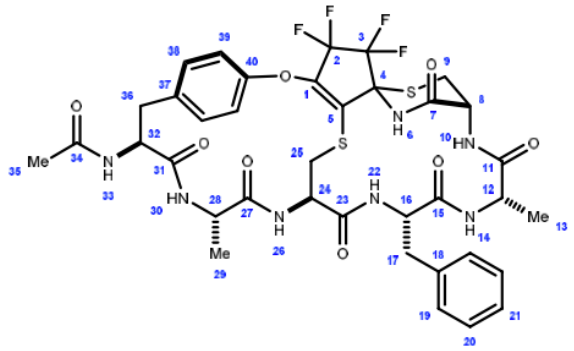
HMBC spectrum of compound **2.43** (DMSO-d_6)



HSQC spectrum of compound **2.43** (DMSO-d_6)

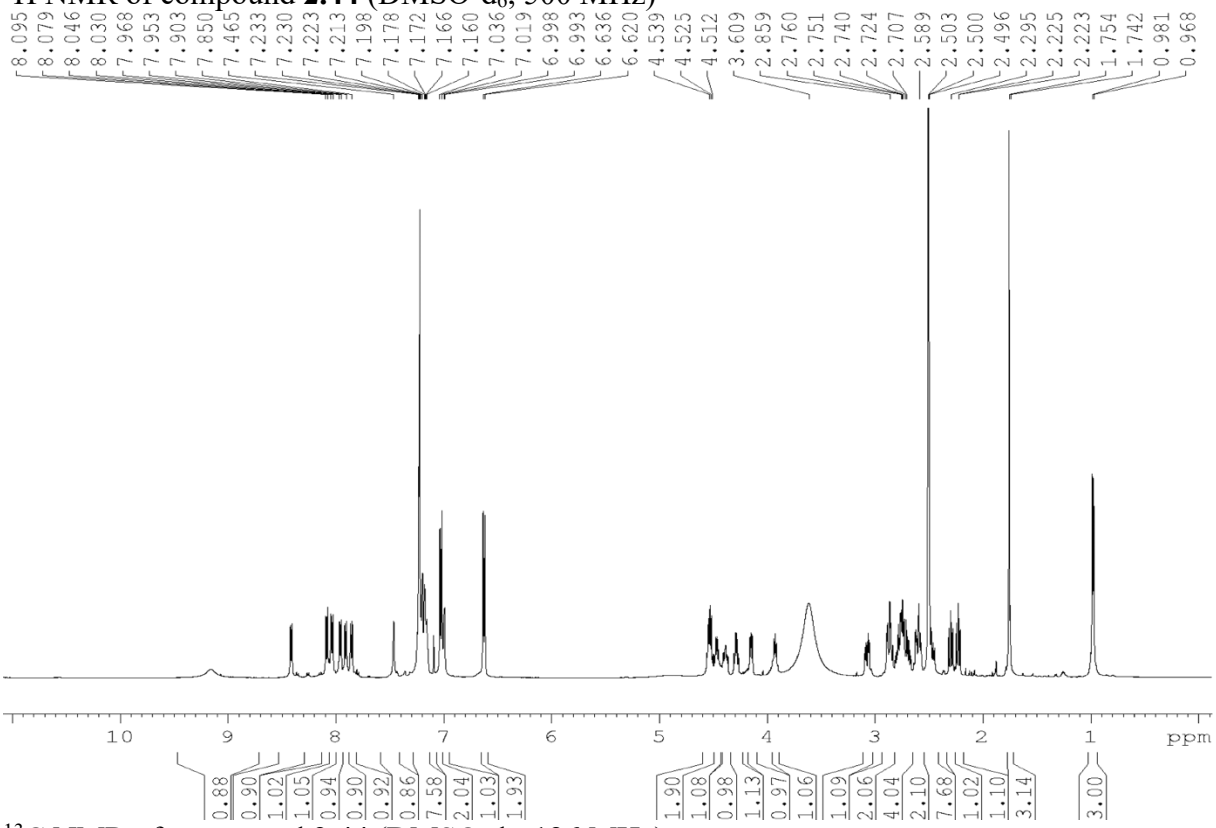


Compound **2.43** (DMSO-d_6)

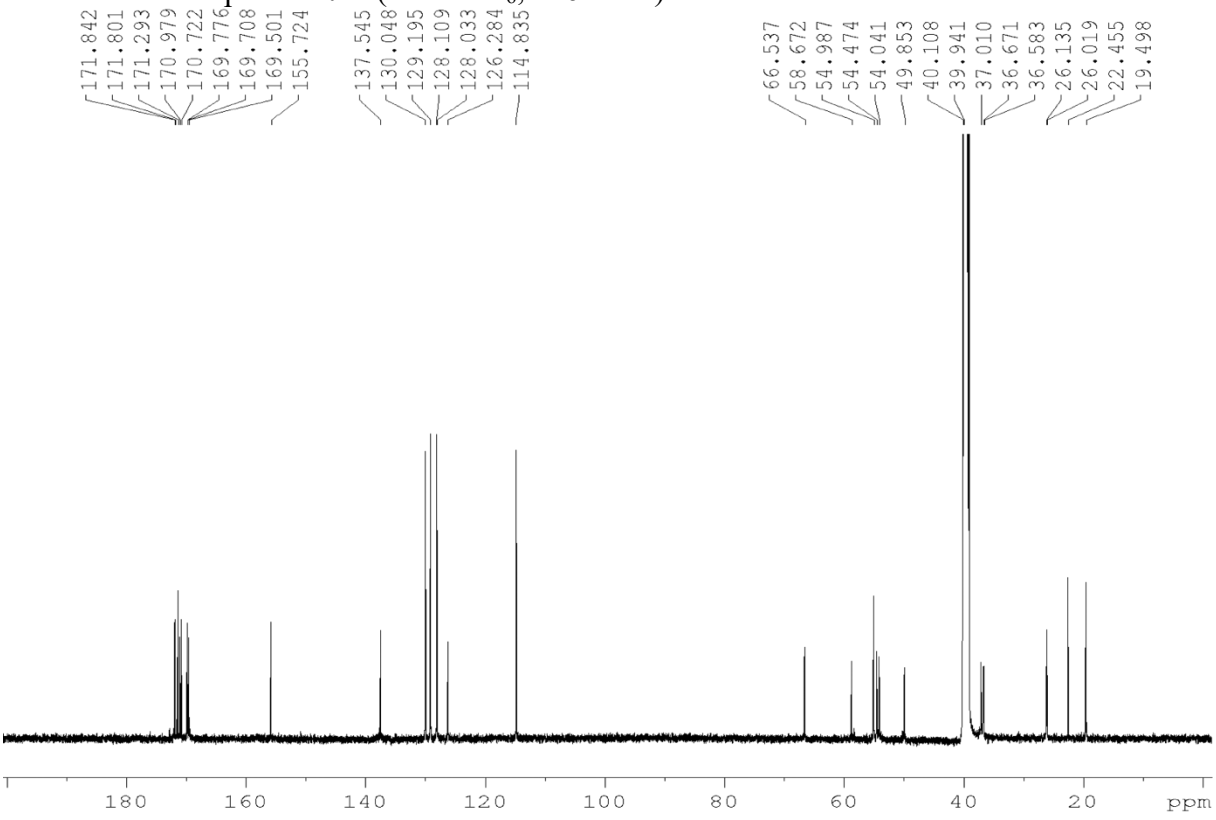


	13C	1H	Key Correlation
1	140.3–139.8 (m, 1C)	–	
2	116.6–113.8 (m, 1C)	–	
3	116.6–113.8 (m, 1C)	–	
4	69.0–68.2 (m, 1C)	–	HMBC 6, 9 –> 4
5	134.6–134.3 (m, 1C)	–	HMBC 6, 25 –> 5
6	–	9.48 (s, 1H)	HMBC 6 –> 7
7	168.1	–	HMBC 6 –> 7
8	46.7	4.70 (dt, J = 8.9, 2.7 Hz, 1H)	HMBC 8 –> 7, HSQC
9	30.3	3.54 (dd, J = 14.6, 2.6 Hz, 1H), 2.83 (dd, J = 14.6, 2.1 Hz,	HMBC 9 –> 4, 8, HSQC
10	–	7.30 (d, J = 8.9 Hz, 1H)	HMBC 10 –> 11
11	171.5	–	HMBC 12 –> 11
12	49.7	4.06–3.99 (m, 1H)	HMBC 13 –> 12, HSQC
13	16.8	1.18 (d, J = 7.2 Hz, 3H)	HMBC 12 –> 13, HSQC
14	–	8.53 (d, J = 8.1 Hz, 1H)	HMBC 14 –> 15
15	171.4	–	HMBC 14, 16 –> 15
16	54.8	4.54–4.47 (m, 1H) overlap	HMBC 17 –> 16, HSQC
17	37.3	2.94–2.85 (m, 2H) overlap	HMBC 17 –> 18, HSQC
18	136.8	–	HMBC 17, 19 –> 18
19	129.0	7.28–7.12 (m, 2H) overlap	HMBC 19 –> 18, 20, HSQC
20	128.3	7.28–7.12 (m, 2H) overlap	HMBC 19, 21 –> 20, HSQC
21	126.6	7.28–7.12 (m, 1H) overlap	HMBC 21 –> 20, HSQC
22	–	8.37 (d, J = 8.0 Hz, 1H)	HMBC 22 –> 16, 23
23	167.6	–	HMBC 22, 24 –> 23
24	50.5	4.54–4.47 (m, 1H) overlap	HMBC 25, 26 –> 24, HSQC
25	33.5	3.39–3.33 (m, 1H), 2.94–2.85 (m, 1H) overlap	HMBC 25 –> 5, 24, HSQC
26	–	8.08 (d, J = 7.8 Hz, 1H)	HMBC 26 –> 24, 27
27	171.1	–	HMBC 26, 28 –> 27
28	46.7	4.23 (dt, J = 8.1, 7.0 Hz, 1H)	HMBC 28 –> 27, 29
29	19.5	0.96 (d, J = 6.8 Hz, 3H)	HMBC 28 –> 29, HSQC
30	–	7.52 (d, J = 8.5 Hz, 1H)	HMBC 30 –> 28, 31
31	168.8	–	HMBC 30, 32 –> 31
32	53.5	4.68–4.61 (m, 1H)	HMBC 32 –> 31, 36, HSQC
33	–	8.06 (d, J = 7.4 Hz, 1H)	HMBC 33 –> 32, 34
34	168.7	–	HMBC 33, 35 –> 34
35	22.5	1.86 (s, 3H	HMBC 35 –> 34, HSQC
36	37.7	2.99 (dd, J = 13.3, 5.5 Hz, 1H), 2.61 (dd, J = 13.1, 10.9 Hz	HMBC 36 –> 32, 37, HSQC
37	132.3	–	HMBC 36, 38 –> 37
38	130.4	7.28–7.12 (m, 1H) overlap, 7.09–6.87 (m, 1H) overlap	HMBC 38 –> 37, 39, HSQC
39	114.8	7.09–6.87 (m, 2H) overlap	HMBC 39 –> 38, 40, HSQC
40	151.9	–	HMBC 39 –> 40

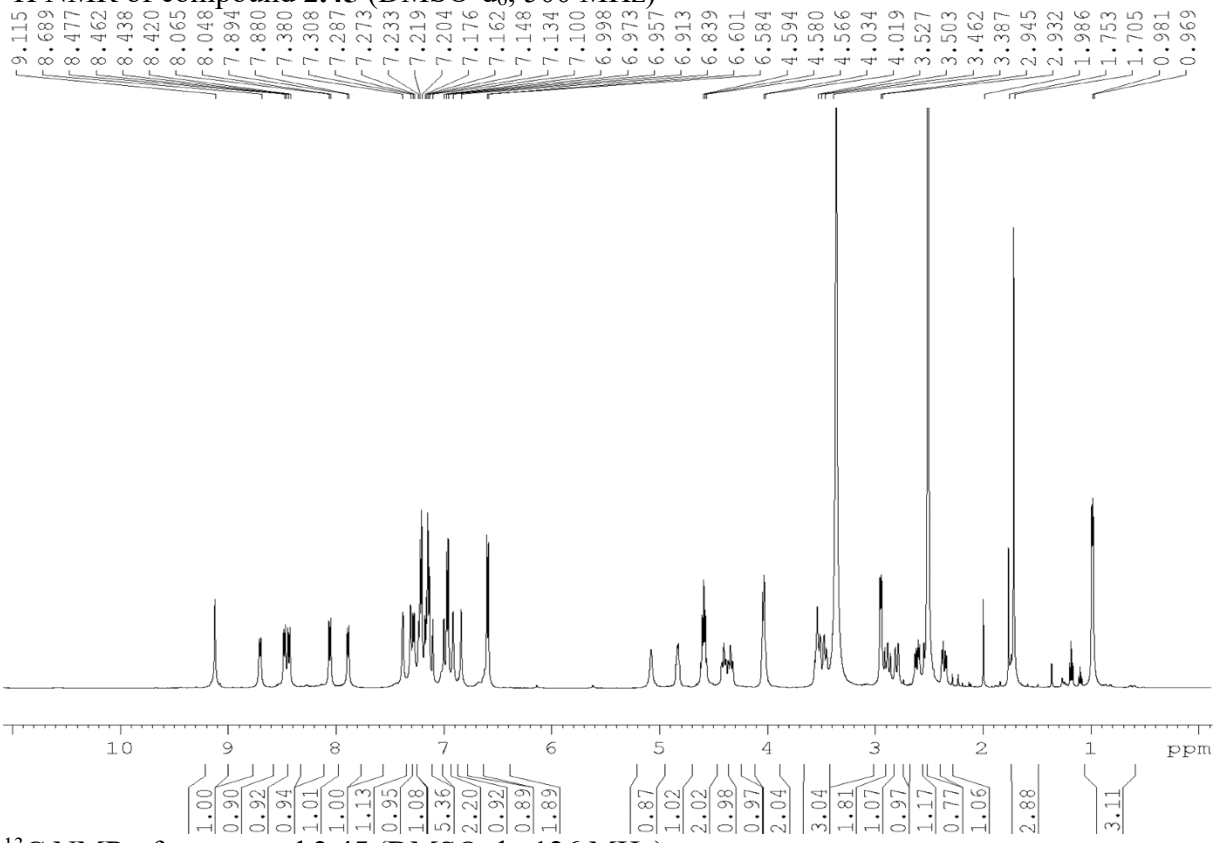
^{1}H NMR of compound **2.44** (DMSO-d_6, 500 MHz)



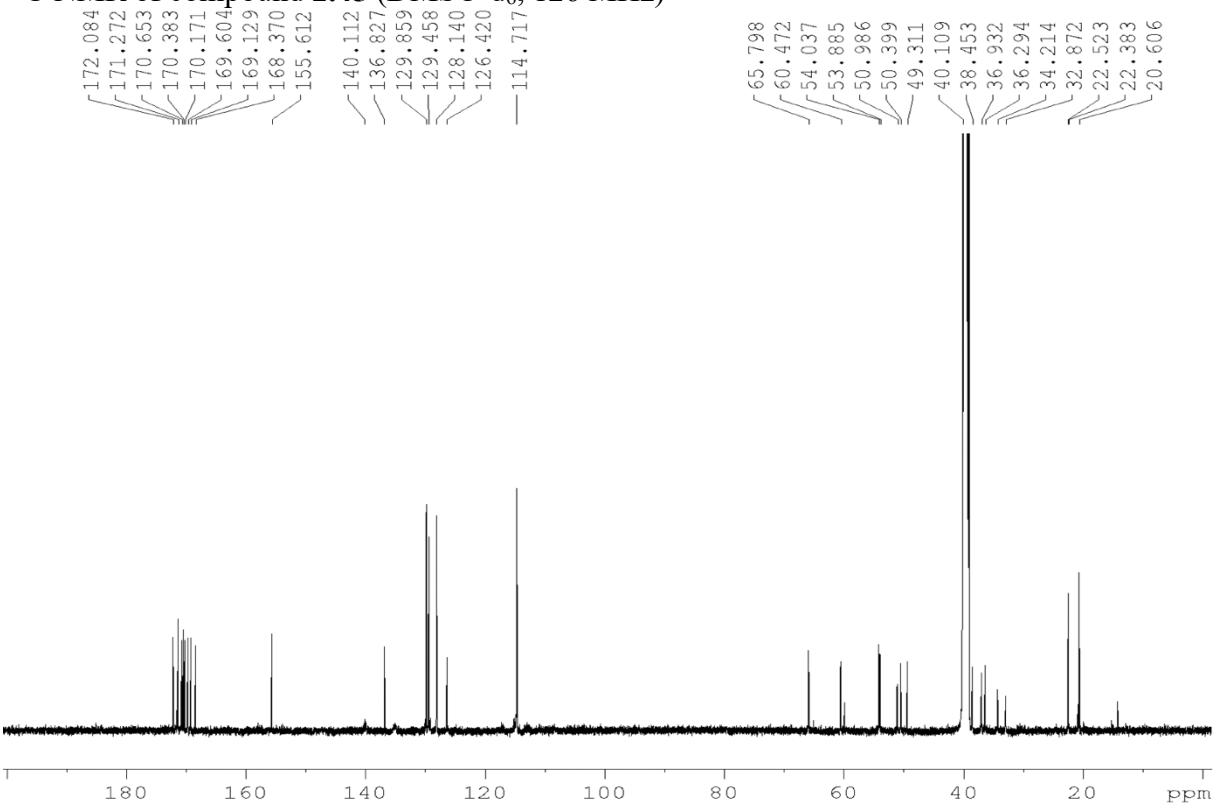
^{13}C NMR of compound **2.44** (DMSO-d_6, 126 MHz)



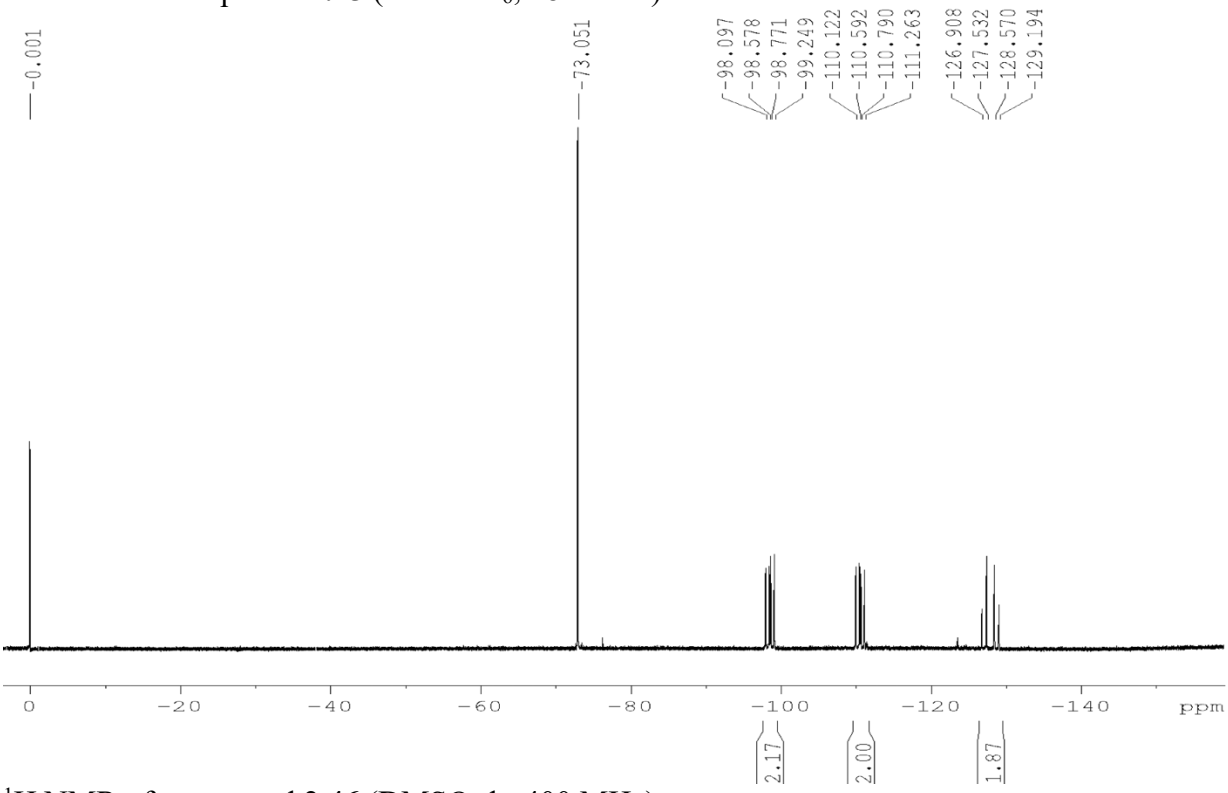
^{1}H NMR of compound **2.45** (DMSO-d_6, 500 MHz)



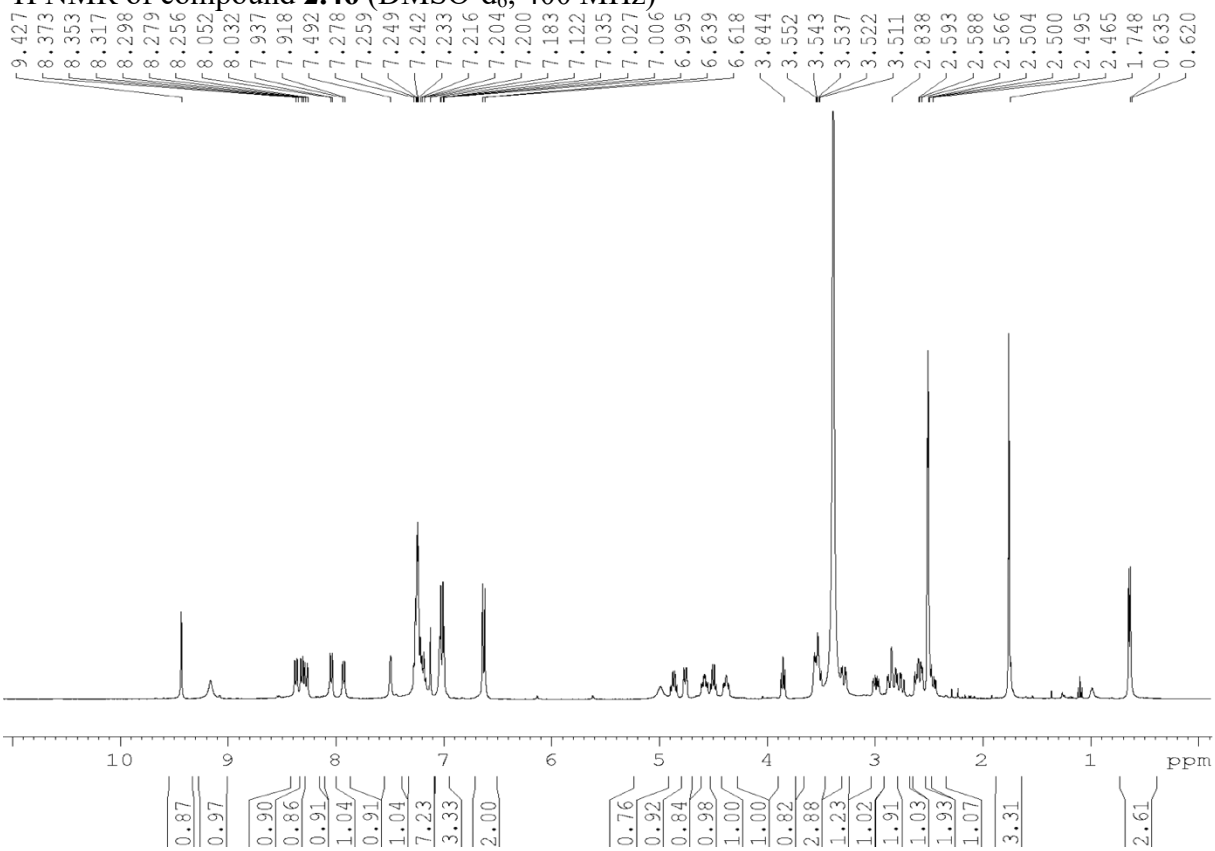
^{13}C NMR of compound **2.45** (DMSO-d_6, 126 MHz)



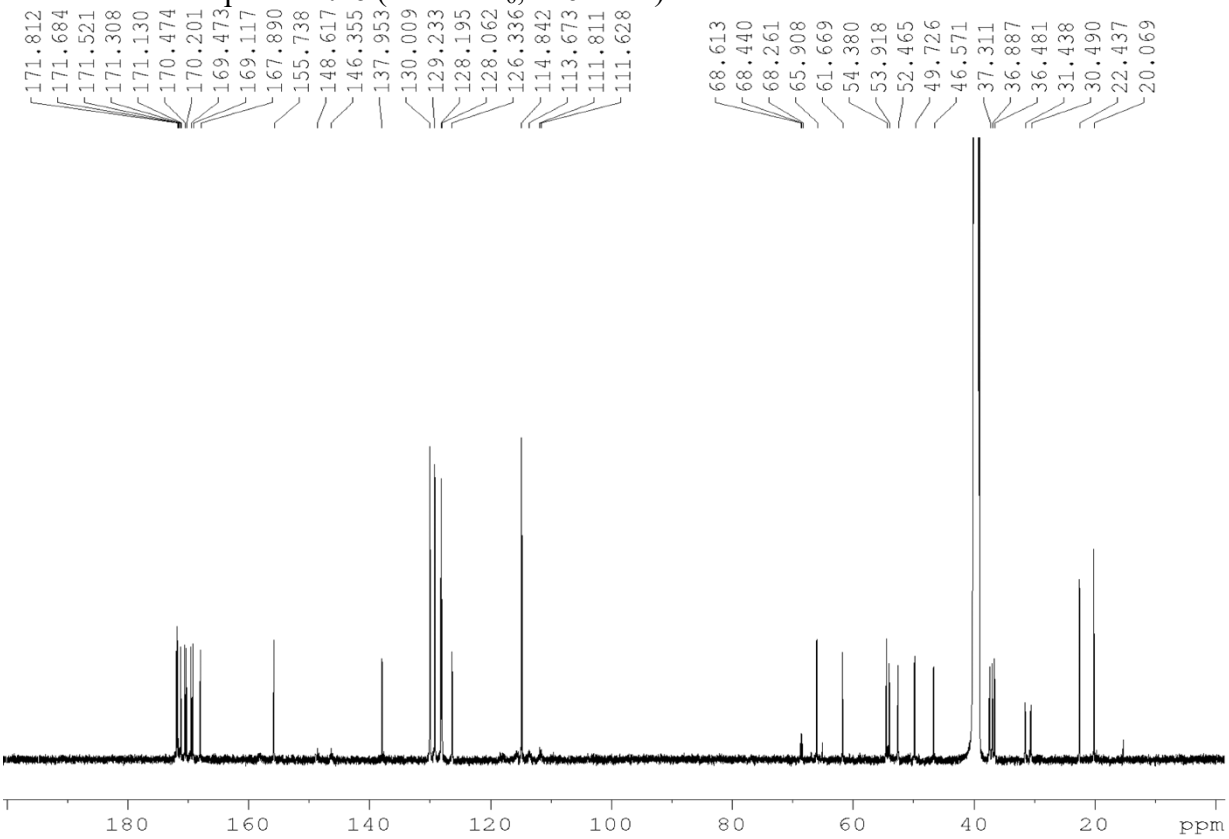
^{19}F NMR of compound **2.45** (DMSO-d_6, 282 MHz)



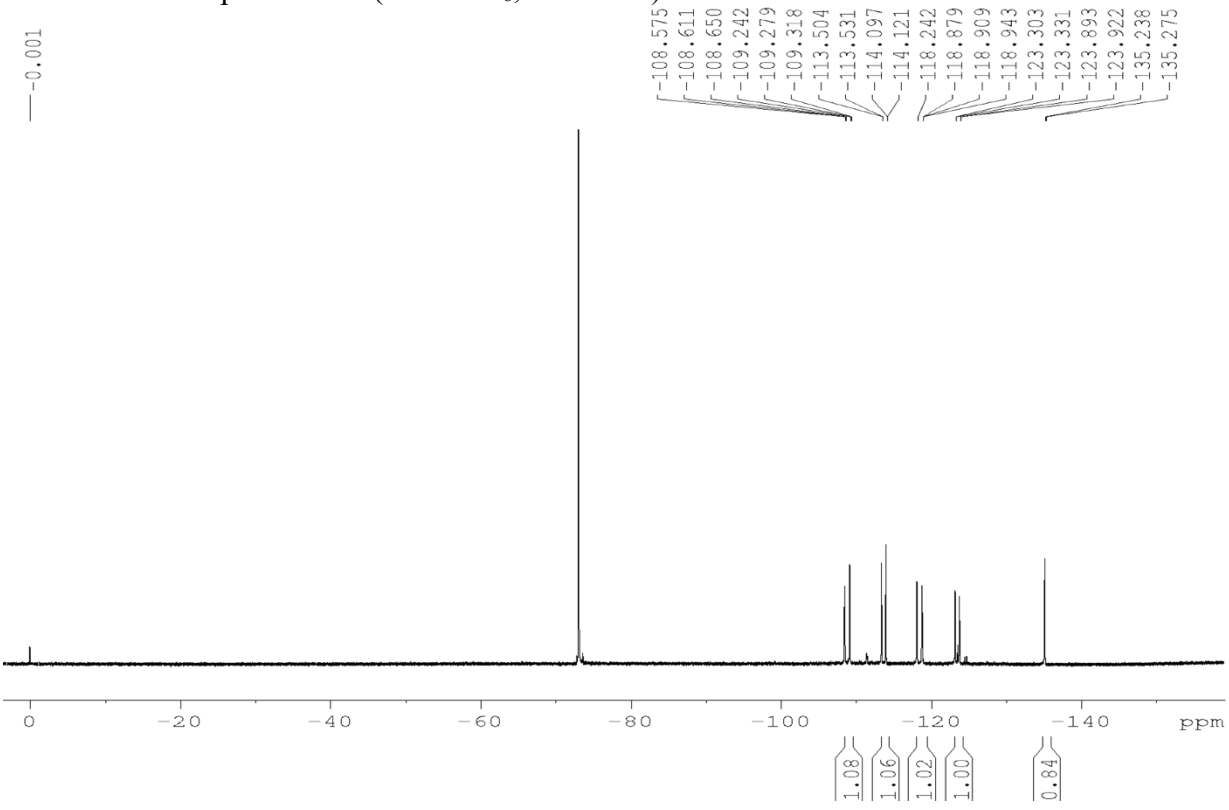
^{1}H NMR of compound **2.46** (DMSO-d_6, 400 MHz)



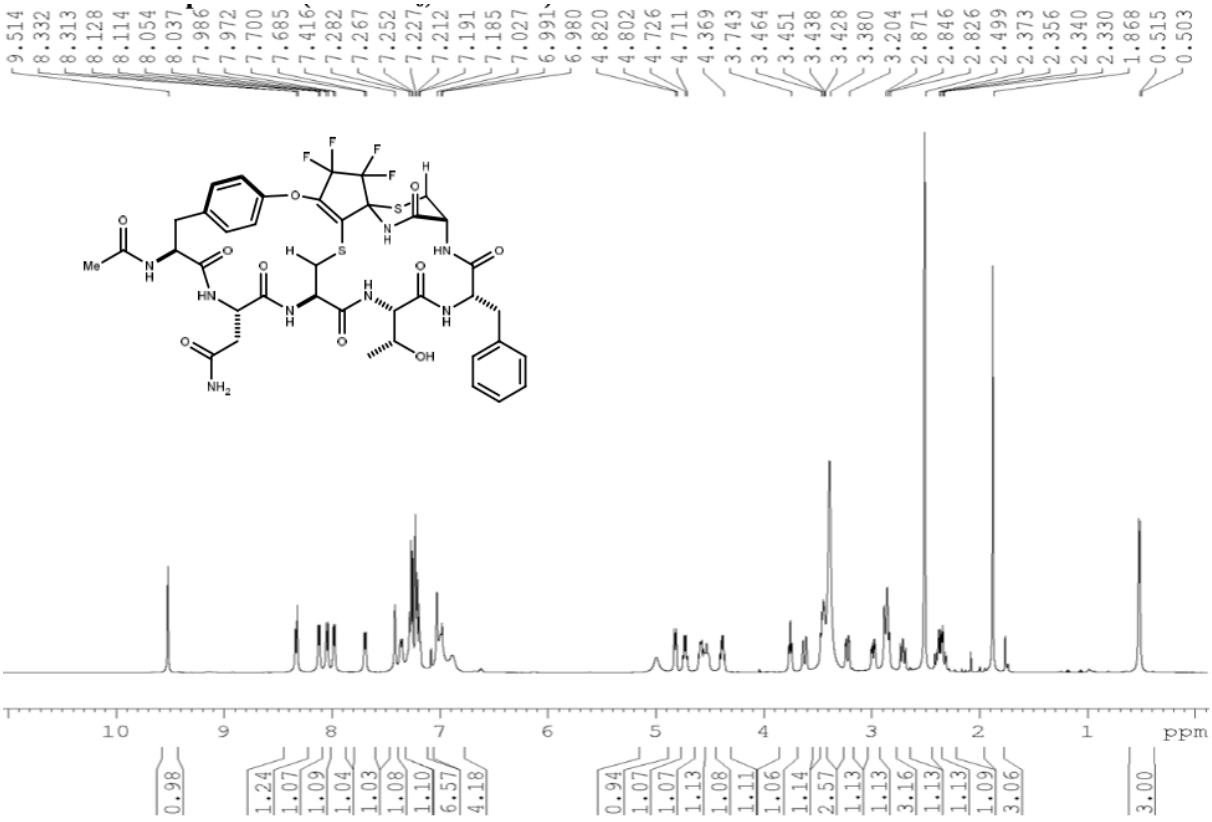
^{13}C NMR of compound **2.46** (DMSO-d_6, 126 MHz)



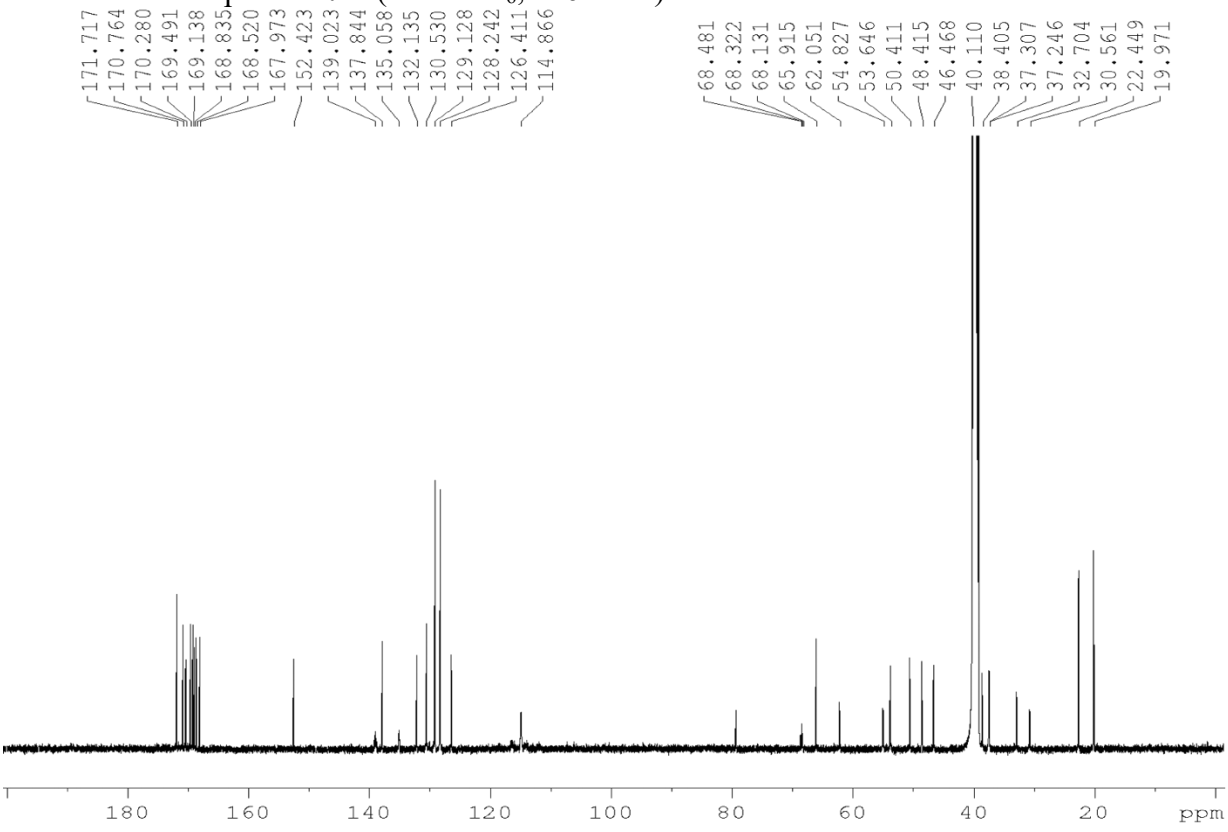
^{19}F NMR of compound **2.46** (DMSO-d_6, 376 MHz)



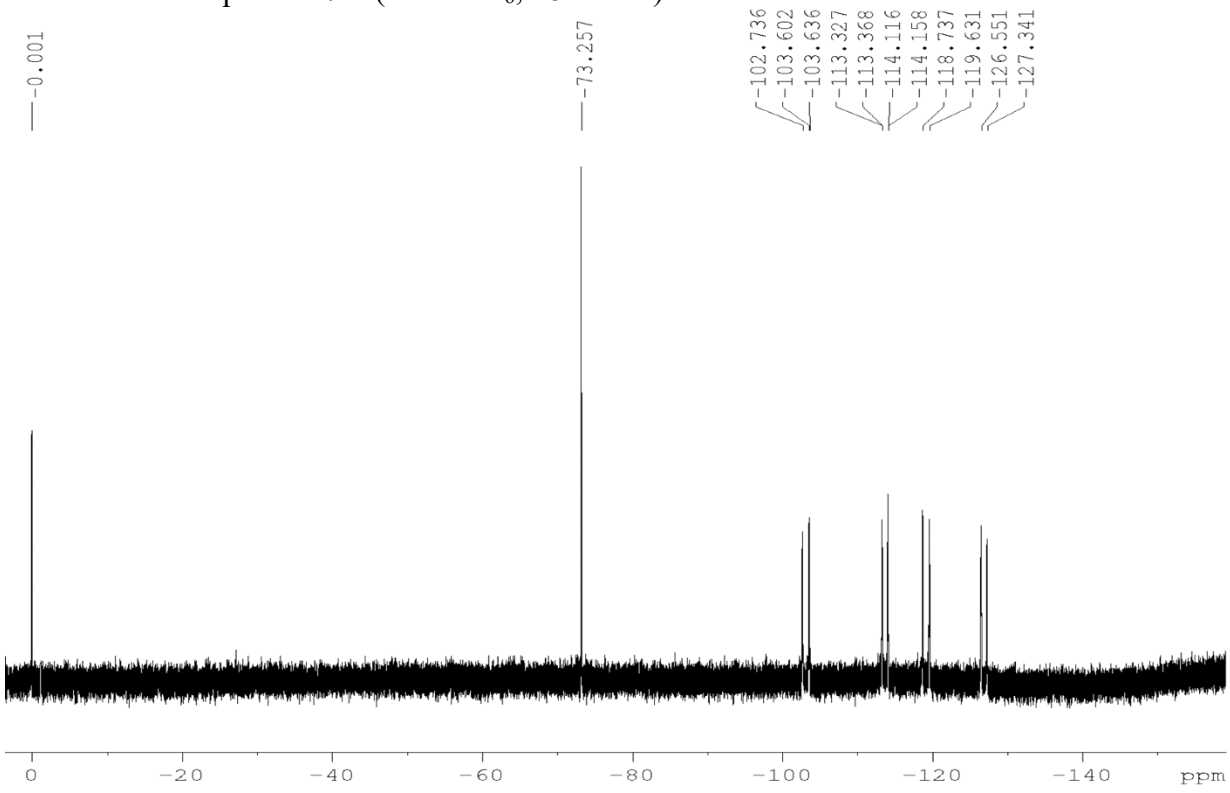
^{1}H NMR of compound **2.47** (DMSO-d_6, 500 MHz)



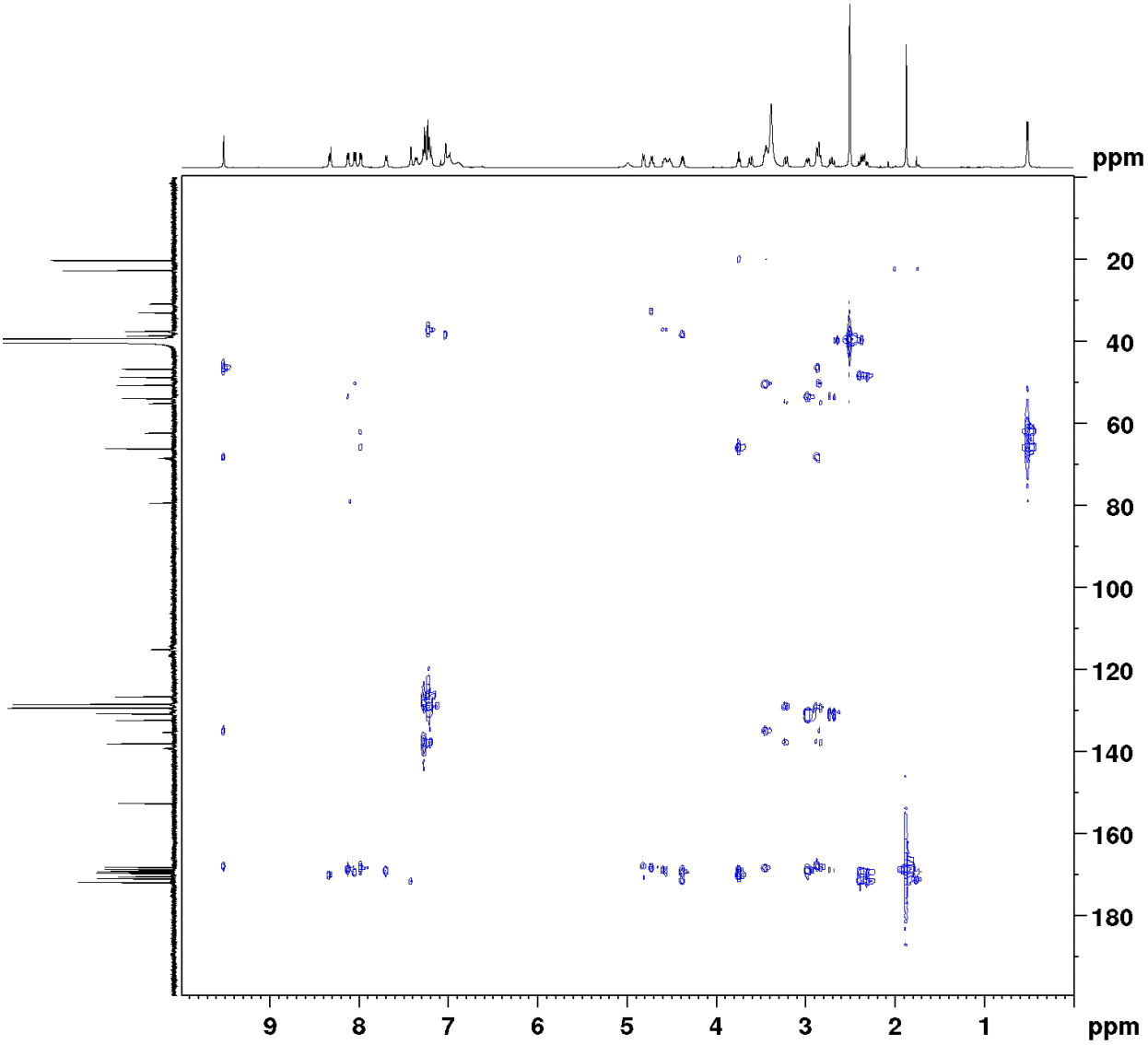
^{13}C NMR of compound **2.47** (DMSO-d_6, 126 MHz)



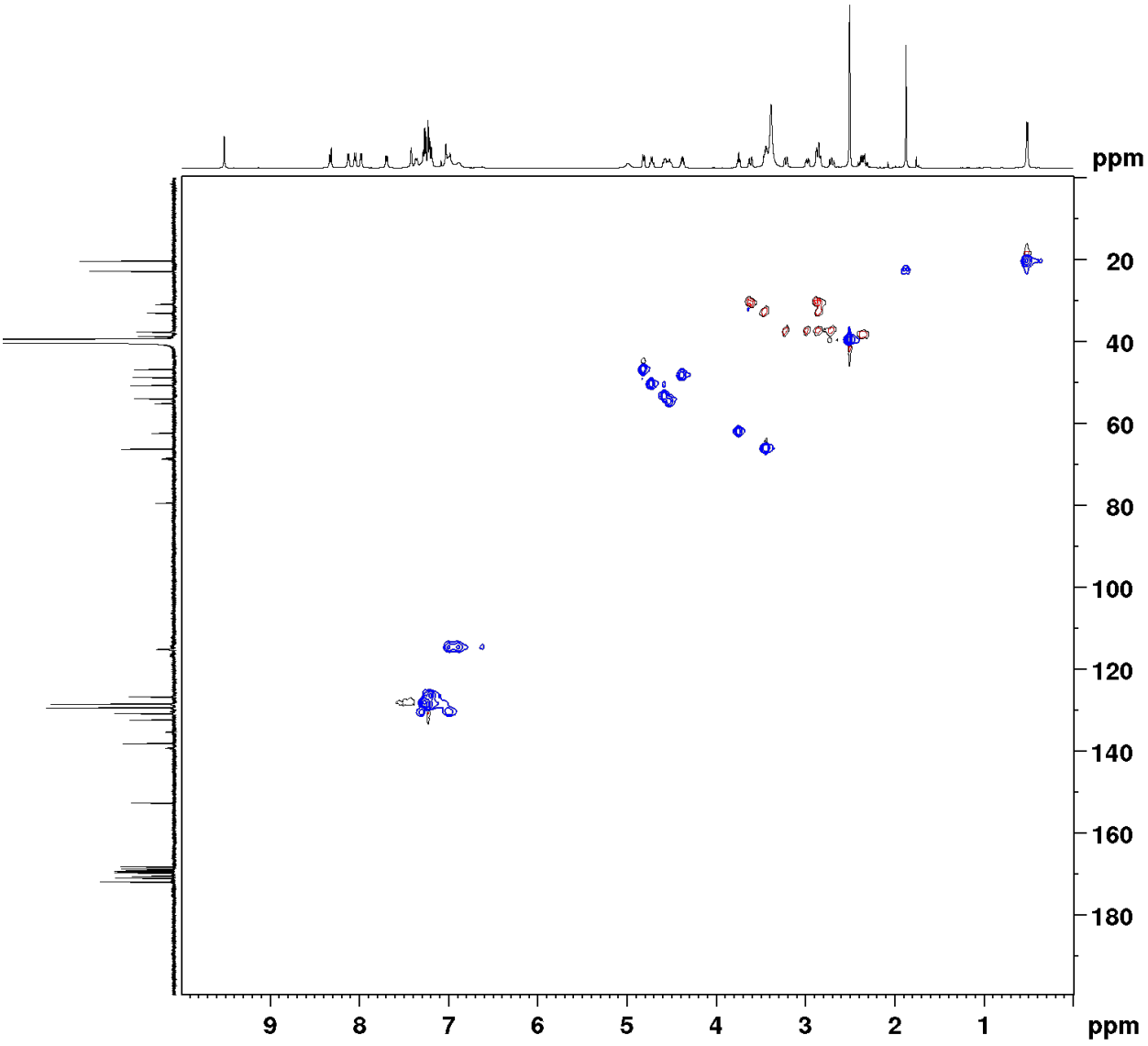
^{19}F NMR of compound **2.47** (DMSO-d_6, 282 MHz)



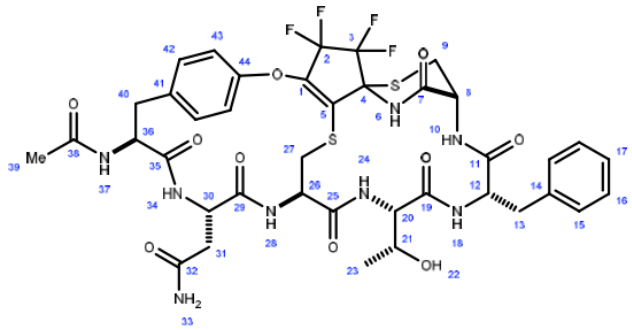
HMBC spectrum of compound **2.47** (DMSO-d_6)



HSQC spectrum of compound **2.47** (DMSO-d_6)

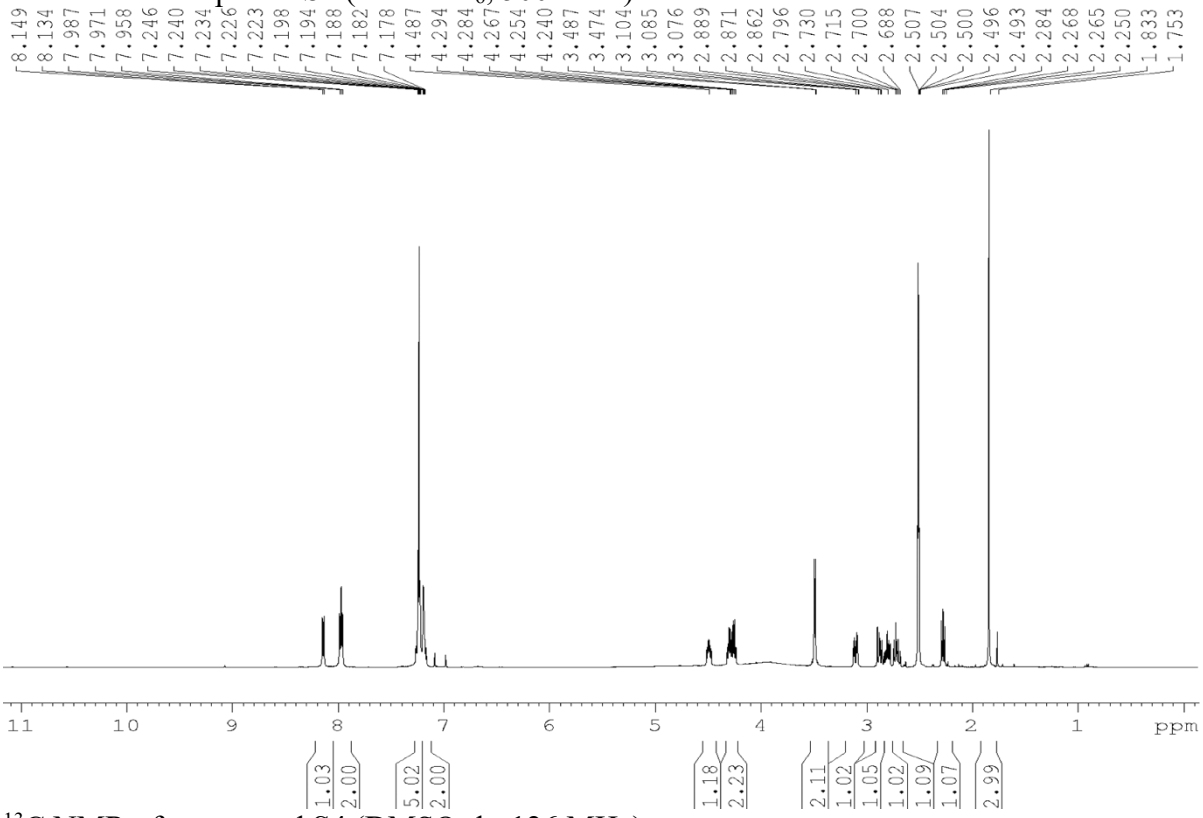


Compound **2.47** (DMSO-d_6)

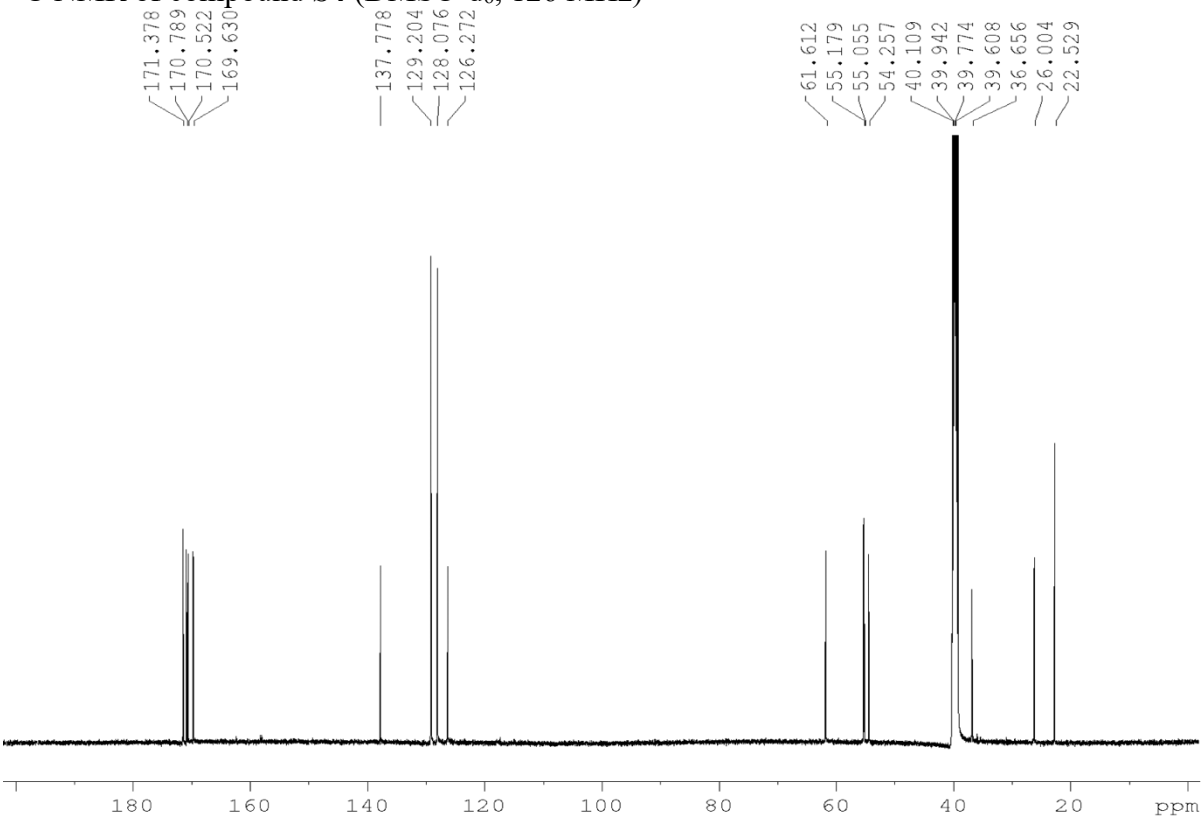


	13C	1H	Key Correlation
1	139.2–138.8 (m, 1C)	–	
2	118.7–111.5 (m, 2C)	–	
3	118.7–111.5 (m, 2C)	–	
4	68.6–68.1 (m, 1C)	–	HMBC 6, 9 -> 4
5	135.2–134.9 (m, 1C)	–	HMBC 6, 9, 27 -> 5
6	–	9.51 (s, 1H)	HMBC 6 -> 7
7	168.0	–	HMBC 6 -> 7
8	46.5	4.84–4.79 (m, 1H)	HMBC 8 -> 7, HSQC
9	30.6	3.61 (d, J = 14.0 Hz, 1H), 2.89–2.82 (m, 1H) overlap	HMBC 9 -> 4, 8, HSQC
10	–	7.36 (d, J = 8.6 Hz, 1H)	HMBC 10 -> 11
11	170.8	–	HMBC 12 -> 11
12	54.8	4.55–4.48 (m, 1H)	HMBC 13 -> 12
13	37.3 or 37.2	3.22 (dd, J = 14.0, 3.7 Hz, 1H)	HMBC 12 -> 13, HSQC
14	137.8	–	HMBC 13 -> 14
15	128.2	7.30–7.17 (m, 2H) overlap	HMBC 15 -> 14, HSQC
16	129.1	7.30–7.17 (m, 2H) overlap	HMBC 15 -> 16, HSQC
17	126.4	7.30–7.17 (m, 1H) overlap	HMBC 17 -> 16, HSQC
18	–	8.33 (d, J = 9.5 Hz, 1H)	HMBC 18 -> 19
19	170.3	–	HMBC 18, 20 -> 19
20	62.1	3.74 (t, J = 6.9 Hz, 1H)	HMBC 20 -> 19, 24, HSQC
21	65.9	3.48–3.41 (m, 1H) overlap	HMBC 20, 23 -> 21, HSQC
22	–	4.98 (brs, 1H)	
23	20.0	0.51 (d, J = 6.0 Hz, 3H)	HMBC 21 -> 23, HSQC
24	–	7.98 (d, J = 6.8 Hz, 1H)	HMBC 24 -> 20, 25
25	168.5	–	HMBC 24, 26 -> 25
26	50.4	4.72 (dd, J = 14.2, 6.8 Hz, 1H)	HMBC 26 -> 25, 27, HSQC
27	32.7	3.48–3.41 (m, 1H) overlap	HMBC 27 -> 5, HSQC
28	–	8.05 (d, J = 8.2 Hz, 1H)	HMBC 28 -> 26, 29
29	169.5	–	HMBC 28, 30 -> 29
30	48.4	4.41–1.34 (m, 1H)	HMBC 30 -> 29, 31, HSQC
31	38.4	2.38 (dd, J = 15.6, 8.5 Hz, 1H), 2.32 (dd, J = 15.6, 5.0 Hz,	HMBC 31 -> 32, HSQC
32	171.7	–	HMBC 31, 33 -> 32
33	–	7.42 (s, 1H), 7.03 (s, 1H)	HMBC 33 -> 32
34	–	7.69 (d, J = 7.7 Hz, 1H)	HMBC 34 -> 30, 35
35	169.1	–	HMBC 34, 36 -> 35
36	53.6	4.60–4.55 (m, 1H)	HMBC 37, 40 -> 36, HSQC
37	–	8.12 (d, J = 6.9 Hz, 1H)	HMBC 37 -> 36, 38, HSQC
38	168.8	–	HMBC 37, 39 -> 38
39	22.4	1.87 (s, 3H)	HMBC 39 -> 38, HSQC
40	37.3 or 37.2	2.97 (dd, J = 13.2, 5.1 Hz, 1H), 2.70 (dd, J = 13.2, 10.1 Hz	HMBC 40 -> 36, 41
41	132.1	–	HMBC 42 -> 41
42	130.5	7.30–7.17 (m, 1H) overlap, 7.06–6.84 (m, 1H) overlap	HMBC 42 -> 41, HSQC
43	114.9	7.06–6.84 (m, 2H) overlap	HMBC 43 -> 42, 44, HSQC
44	152.4	–	HMBC 43 -> 44, HSQC

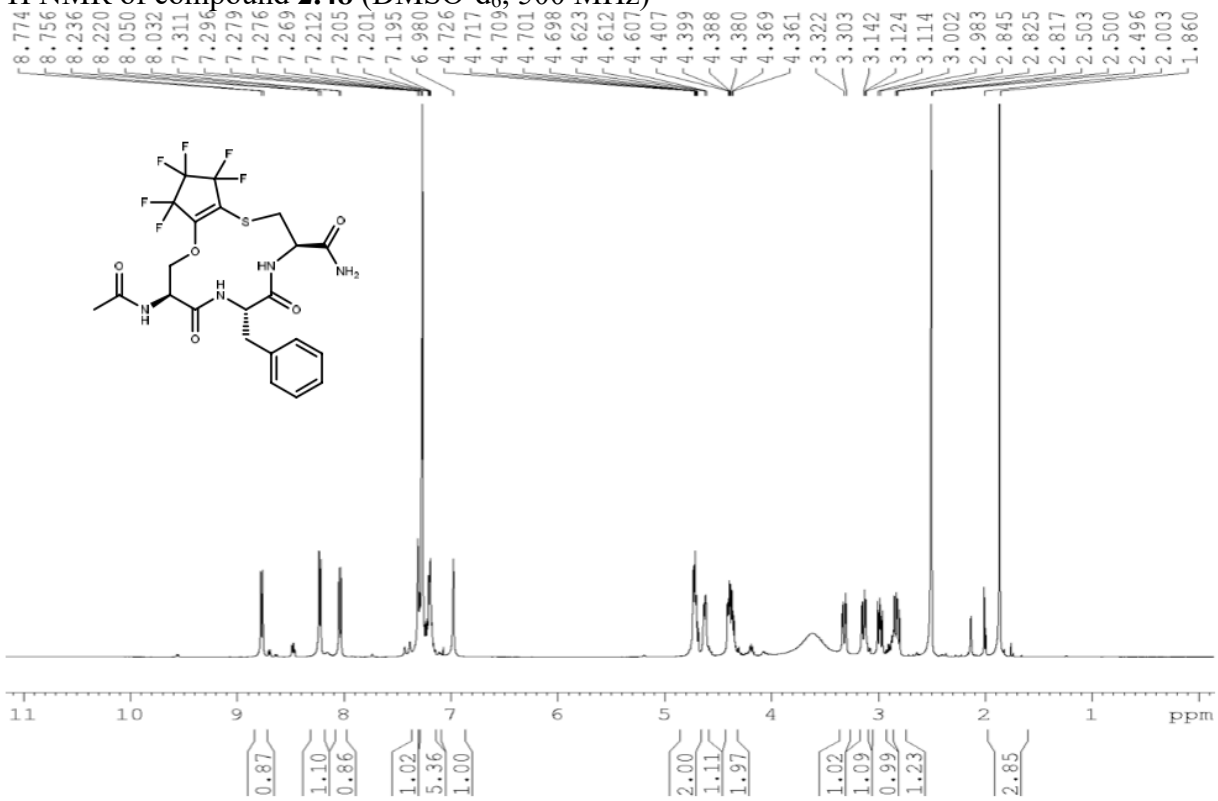
^{1}H NMR of compound **S4** (DMSO-d_6, 500 MHz)



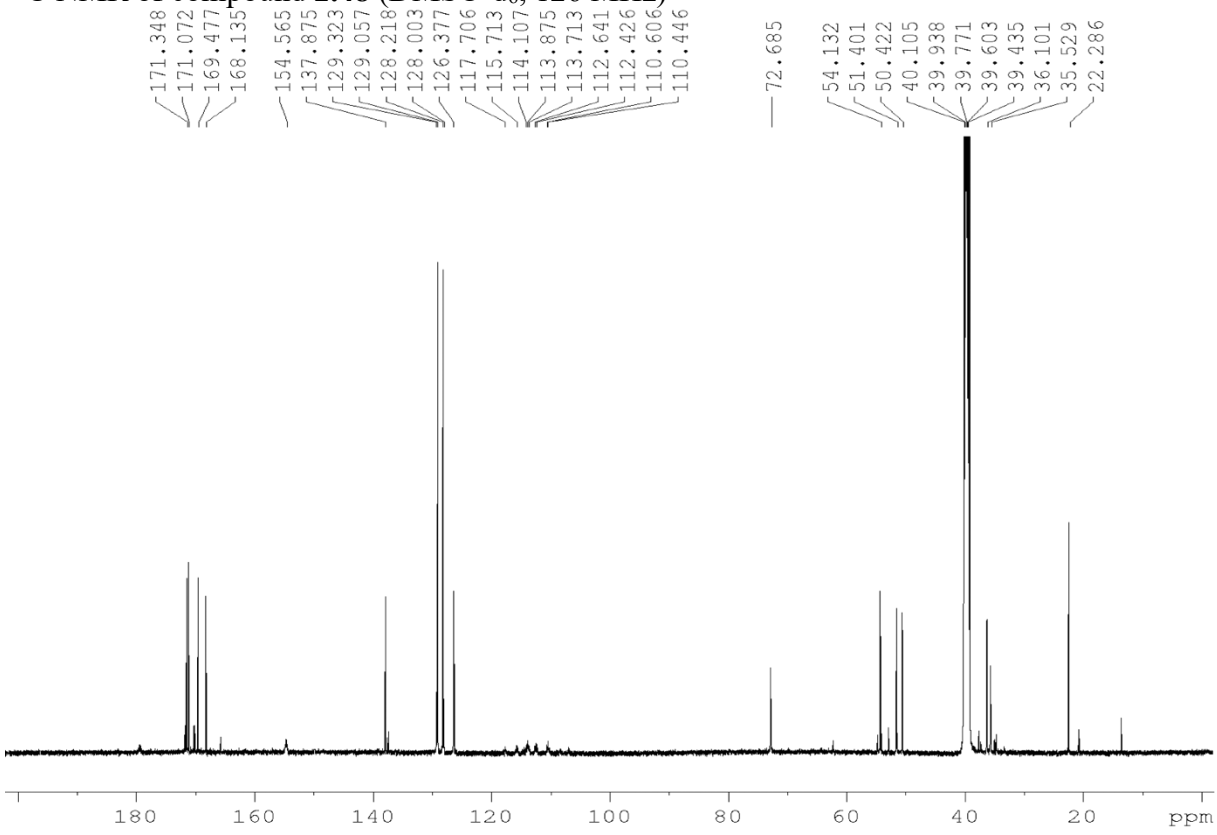
^{13}C NMR of compound **S4** (DMSO-d_6, 126 MHz)



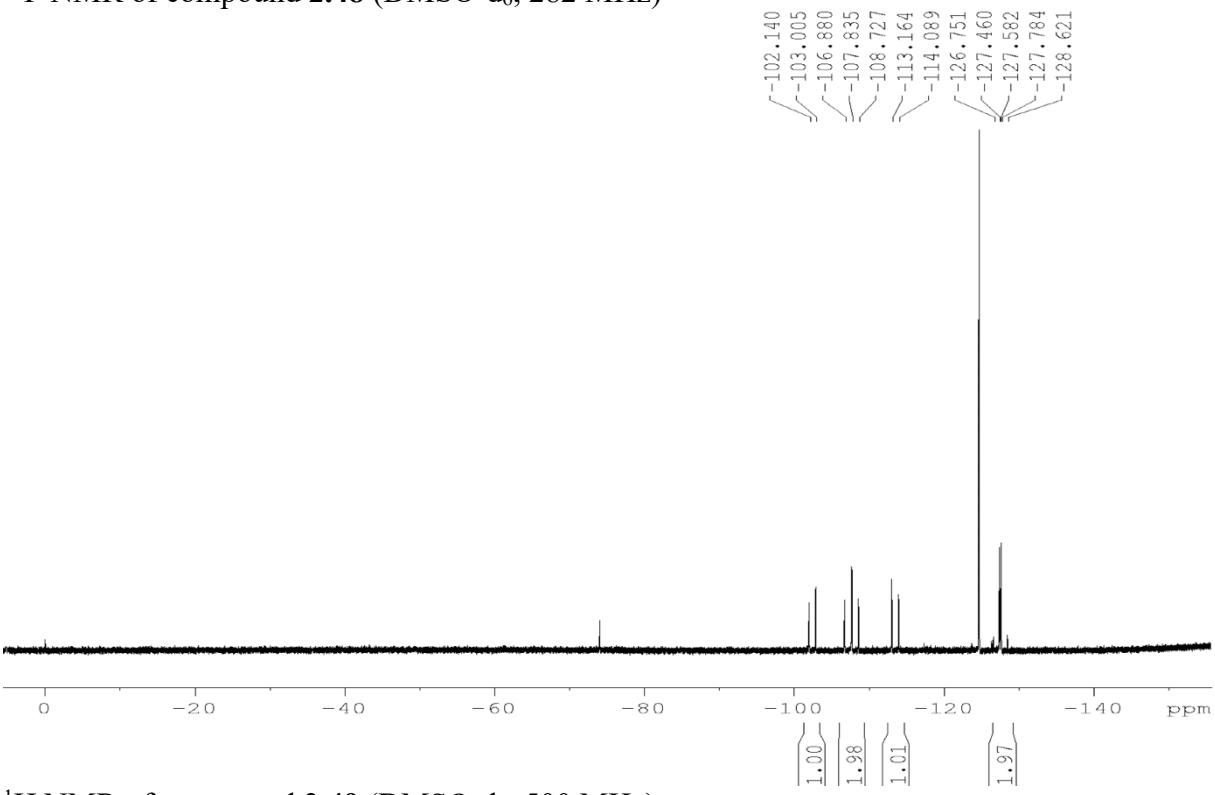
^{1}H NMR of compound **2.48** (DMSO-d_6, 500 MHz)



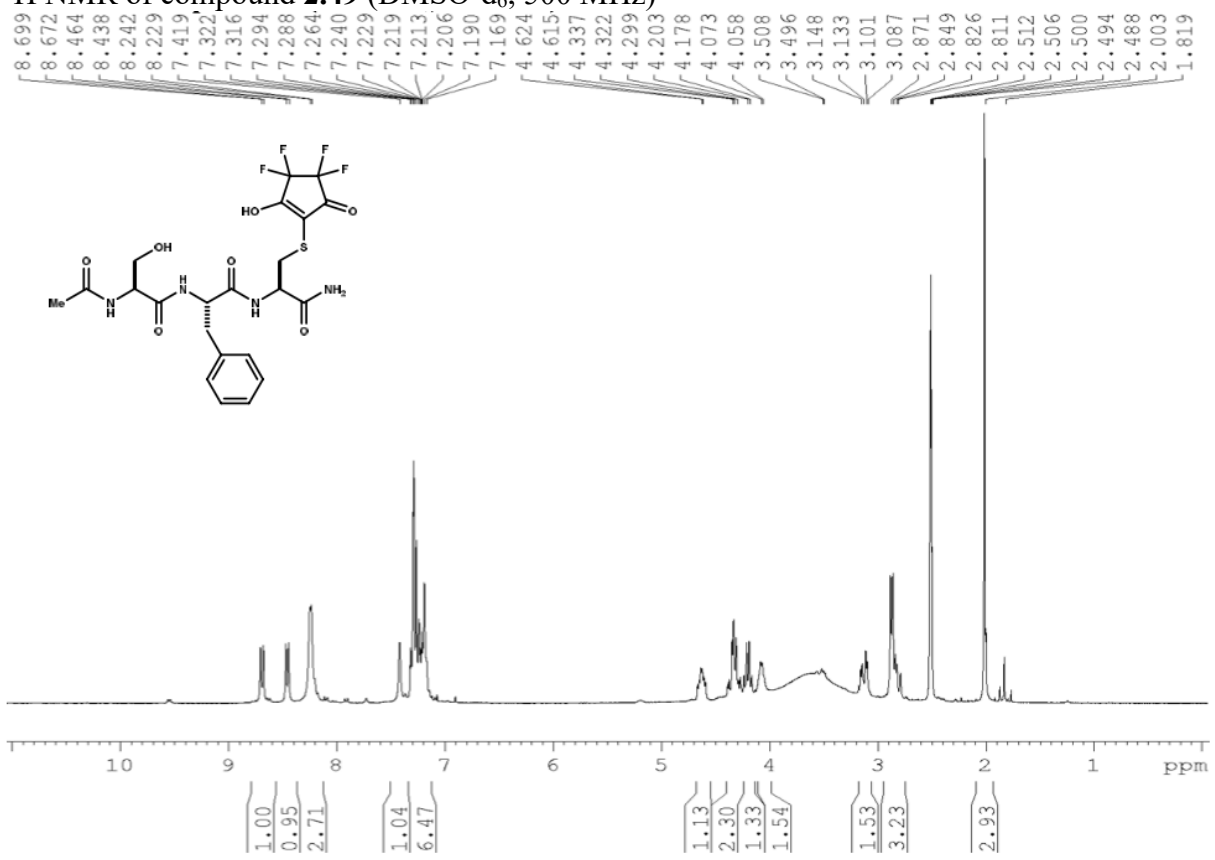
^{13}C NMR of compound **2.48** (DMSO-d_6, 126 MHz)



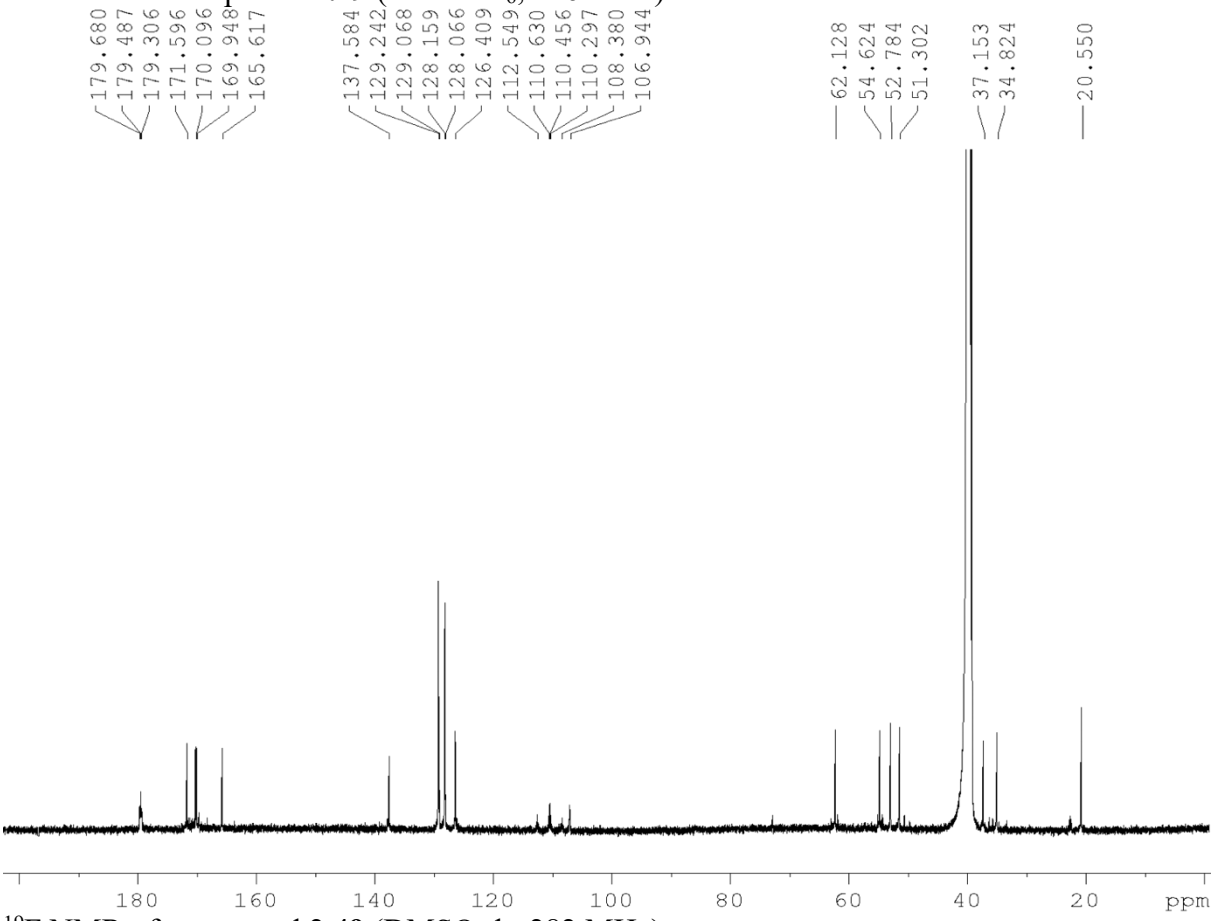
^{19}F NMR of compound **2.48** (DMSO-d_6, 282 MHz)



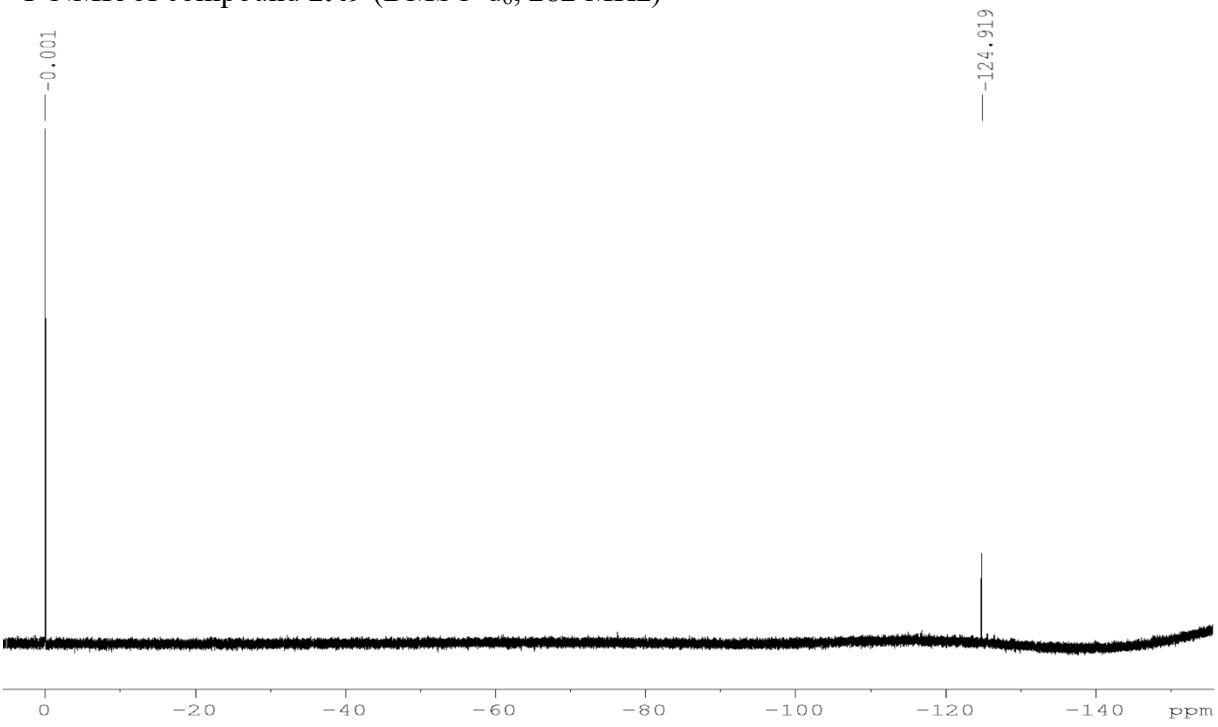
^{1}H NMR of compound **2.49** (DMSO-d_6, 500 MHz)



^{13}C NMR of compound **2.49** (DMSO-d_6, 126 MHz)



^{19}F NMR of compound **2.49** (DMSO-d_6, 282 MHz)



4. Supplemental References

(1) Hada, N.; Shida, Y.; Negishi, N.; Schweizer, F.; Takeda, T. *Chem. Pharm. Bull.* **2009**, 57, 1081–1088.

(2) Albrich, H.; Vahrenkamp, H. *Chem. Ber*. **1994**, 127, 1223–1233.

Chapter 3 – Cascade Synthesis of Fluorinated Spiroheterocyclic Scaffolding for Peptidic Macrobicycles

3.1 Introduction

Reaction processes that form multiple covalent bonds while creating new rings and stereocenters are inherently valuable in organic synthesis. They advance an overarching goal of the field, which is to enable syntheses of value-added products from abundant raw materials in the most direct manner possible. Reaction cascades have been used effectively for the synthesis of diverse carbocyclic and heterocyclic ring systems, using both catalytic and stoichiometric methods.[1,2] The typical objective is to streamline the assembly of molecules. However, multi bond-forming processes can also manipulate the form and properties of pre-assembled structures. For example, we have developed reagents that can engage small peptides in successive ring forming reactions to afford polycyclic derivatives.[3-11] In the example shown in **Fig. 3.1B**, three consecutive operations imbed the core of synthetic reagent **3.1** into unprotected WWY. Four new bonds (red) are formed between the oligomer and the scaffolding reagent, resulting in four new rings and three new stereocenters.[8] Other methods to cyclize unprotected peptides using native functional groups (e.g. **Fig. 3.1A**) achieve less bonding with the scaffold and install fewer conformational constraints.[12-16] Using **3.1** and related reagents, it is possible to convert machine made oligomers systematically into stable composite macrocycles having diverse shapes and improved pharmacological properties.[17]

The scaffolding imparted by residual **3.1** (colored blue in **3.2**) is hydrophobic. It was of interest to develop methods that could install scaffolding having polar elements and the potential for

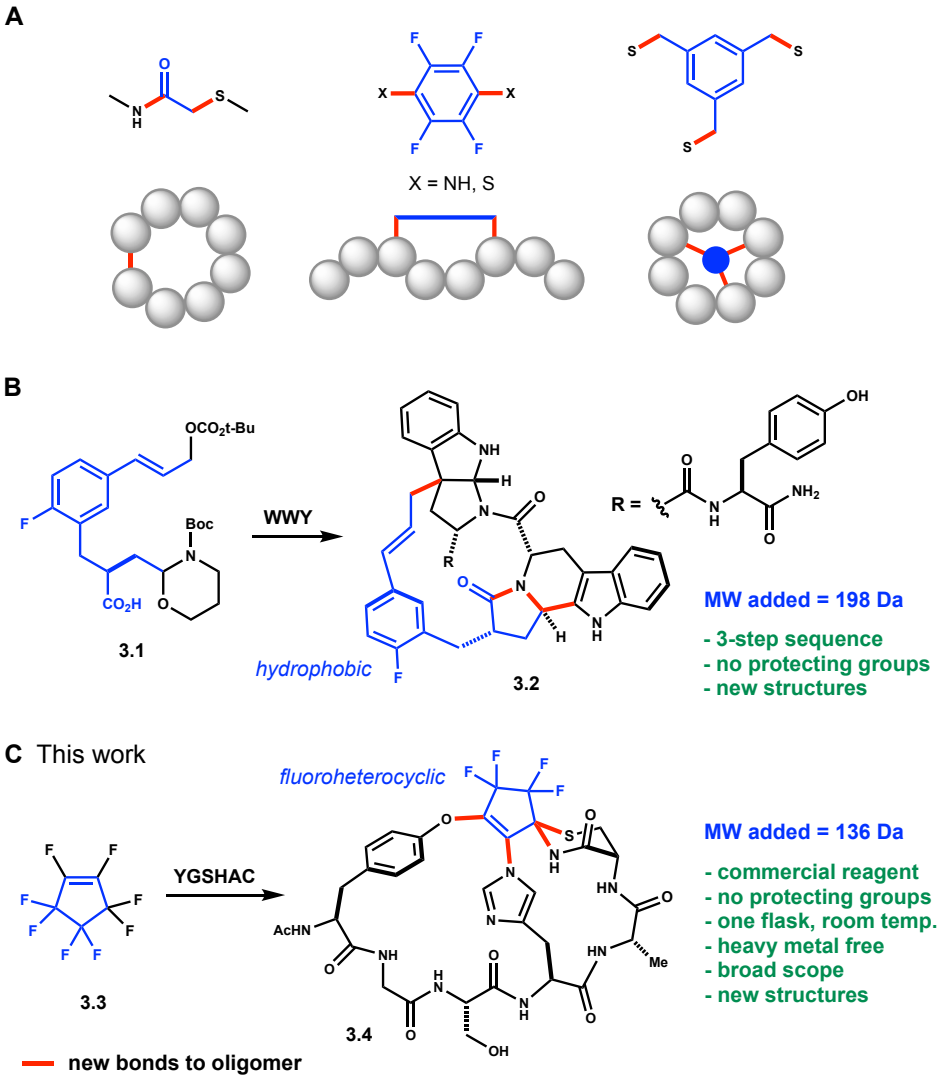


Fig. 3.1. Cyclizations of linear, unprotected peptides (**A**) Common side chain engagements using native functional groups (*refs. 12-15*). (**B**) Previous work - sequenced main chain and side chain reactions using multi-contact scaffolding. (**C**) A new discovery: relative rate controlled polysubstitution cascades using perfluoro-cyclopentene.

transannular hydrogen bonding. Here we describe new polysubstitution cascades that achieve this outcome. The reactions modify linear, unprotected peptides in a single flask at room temperature. They require no catalysts or heavy metals, and they generate a wealth of previously unknown heterocyclic ring systems with tunable properties.

Electron deficient aryl fluorides participate in nucleophilic ipso substitution (S_NAr) reactions. Sanger's method for N-terminal peptide sequencing is a seminal example.[18] More

heavily fluorinated aromatics such as hexafluorobenzene can engage two nucleophiles successively at ring positions 1 and 4. The reaction is efficient with sulfur nucleophiles and has been used to generate macrocyclic peptides via cysteine 'stapling'.[13] Polysubstitutions become possible when using perfluorinated cycloalkenes. For example, commercial octafluorocyclopentene (OFCP) reacts with simple nucleophiles at both its vinyl positions, and subsequently at its allylic positions to afford adducts having up to six fluorine atoms replaced.[19] We have discovered how to translate this incremental electrophilicity of OFCP into relative rate-controlled substitution cascades. Using peptides as polynucleophilic partners, OFCP generates multicyclic products from linear precursors in a sequence dependent manner.

We show how fluorinated thiazaspirodecenone intermediates can be intercepted to generate novel glycoconjugates, functionalization products and macrobicyclic composites using molecular inserts. We study the molecular properties of end products and computationally probe how fluorospirocyclic scaffolding can generate new structural mimics of major loop types observed at protein contact surfaces in the Protein Data Bank.

3.2 Direct Polysubstitution Cascades of Unprotected Peptides

Linear peptides having three proximal nucleophilic residues react with OFCP (1.5 eq, 25˚C, Et_3N, DMF) according to the progression shown in **Fig. 3.2A**. Two successive vinylic substitutions occur rapidly (k_{rel} for Ser:Tyr:His:Cys ~ 1:30:45:1000) to afford macrocycles **3.6**.[20] DFT calculations have been performed to elaborate details of mechanism. The potent electrophilicity of OFCP is related to the high stabilization of anionic intermediates by negative hyperconjugation.

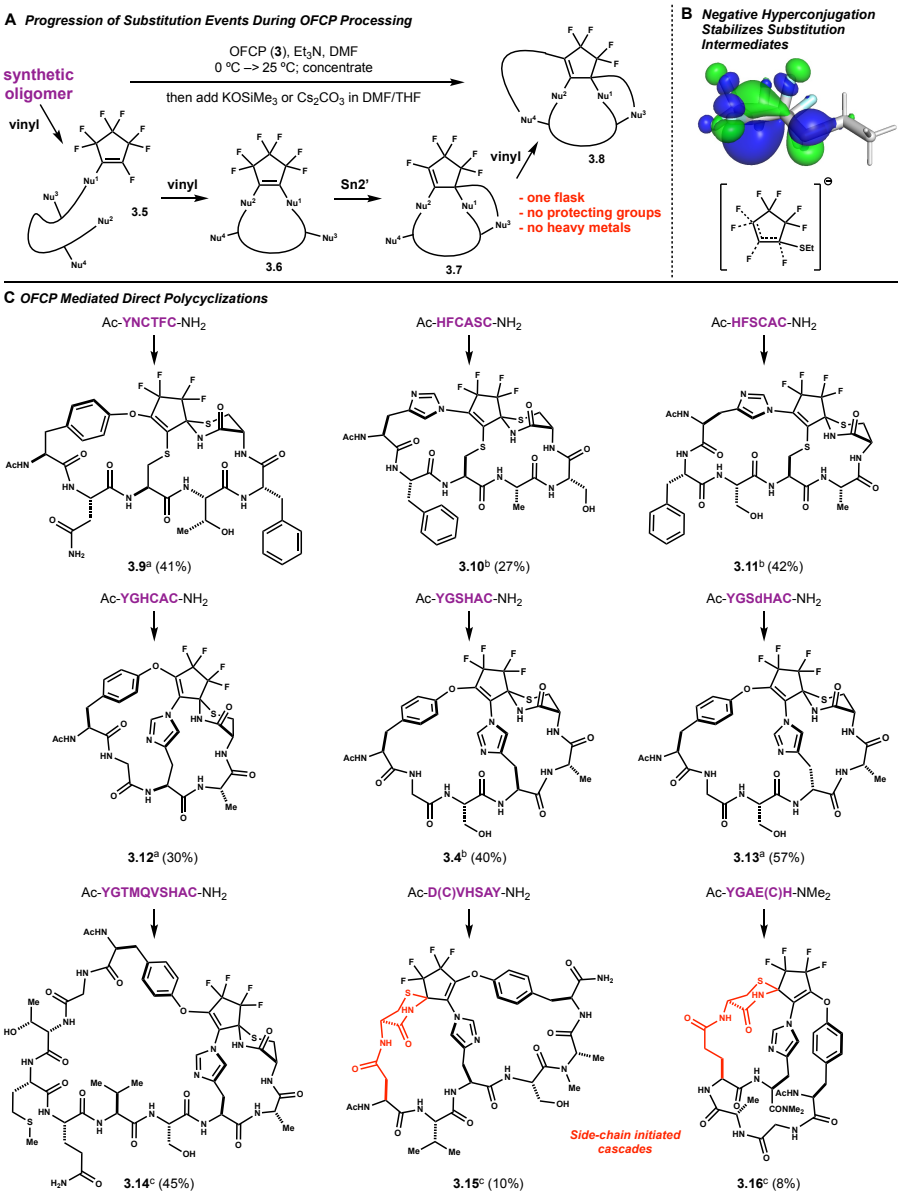


Fig. 3.2. Direct macrobicyclization of linear unprotected peptides. (**A**) Progression of substitution events during OFCP processing. (**B**) Negative hyperconjugation stabilizes substitution intermediates. Computational rendering of delocalized HOMO present in intermediate formed by model reaction of ethyl mercaptide + OFCP (M06-2x-D3/def2TZVP). See SI for details. (**C**) OFCP mediated direct polycyclizations. Linear peptides were synthesized on solid phase as N-terminal acetamides and cleaved as primary C-terminal carboxamides. Yields reflect preparative HPLC isolation (C18 Sunfire, using 0.1% TFA in H_2O/MeCN) wherein all histidine derived products were isolated as TFA salts. Reagent and Conditions: a) OFCP (1.5 equiv), Et_3N (2.5 equiv), DMF (5.0 mM), 0 °C, 30 min; conc. then Cs_2CO_3 (6.0 equiv), 1:4 DMF/THF (5.0 mM), 0 °C –> RT, 3 h, (>10:1 d.r.). b) OFCP (1.5 equiv), Et_3N (2.5 equiv), DMF (5 mM), 0 °C, 30 min; conc. then $KOSiMe_3$ (4.0 equiv), 1:4 DMF/THF (5.0 mM), 0 °C –> RT, 3 h, (>10:1 d.r.). c) same as b) except using solely DMF as solvent.

This is reflected, for example, in the highly delocalized HOMO calculated for the intermediate formed in the model reaction of ethyl mercaptide with OFCP (**Fig. 3.2B**). Fluoride loss from this intermediate is calculated to have a very low barrier (See SI). Excess OFCP (b.p. = 27˚C @ 1 atm) is removed from **3.6** in vacuo and the residue is redissolved in a DMF/THF solution containing excess Cs_2CO_3 or $KOSiMe_3$. This initiates spirocyclization via Sn2' displacement of a third fluorine atom (when Nu^3 is a C-terminal carboxamide) to afford a new vinyl fluoride that is captured by a fourth competent nucleophile to give polycycles **3.8**. Compounds **3.8** are stable, soluble and purified using standard chromatographic techniques. Ac-YNCTFC-NH_2 reacts with OFCP at its two cysteine residues within minutes at 25˚C. Subsequent Cs_2CO_3 treatment installs the thiazaspirodecenone motif while forming a new vinyl fluoride that then captures the N-terminal tyrosine residue to afford **3.9** in 41% isolated yield (**Fig. 3.2C**). By initiating reaction cascades at a C-terminal cysteine amide, it is possible to synthesize a range of macrobicyclic structures that are bridged by sulfide and imidazole linkages. There is flexibility in ring size on either side of the bridging residue and, in the case of histidine derivative **3.4**, bridge position epimer **3.13** can be prepared readily. Diastereoselectivity at the newly formed spiro center is high (>10:1) across the series. Products show sharp, well resolved ^1H NMR spectra at ambient temperature. By repositioning the cysteinyl amide off of a glutamate or aspartate side chain, it is possible to initiate alternate macrobicyclization cascades. For example, branched peptide Ac-YGAE(C)H-NMe_2 affords the striking, double looped polycycle **3.16** when reacted successively with OFCP and $KOSiMe_3$. To our knowledge, each of the ring systems shown in **Fig. 3.2C** are without precedent in the literature.

3.3 Vinyl Fluoride Derivatization

It is possible to isolate and characterize vinyl fluorides **3.7**. As shown in **Fig. 3.3A**, treatment of Ac-CWSC-NH_2 with OFCP followed by $KOSiMe_3$ affords spiro tricyclic compound **3.17** (35%, >10:1 d.r.). Under identical conditions, the same sequence containing D-cysteine affords epimeric

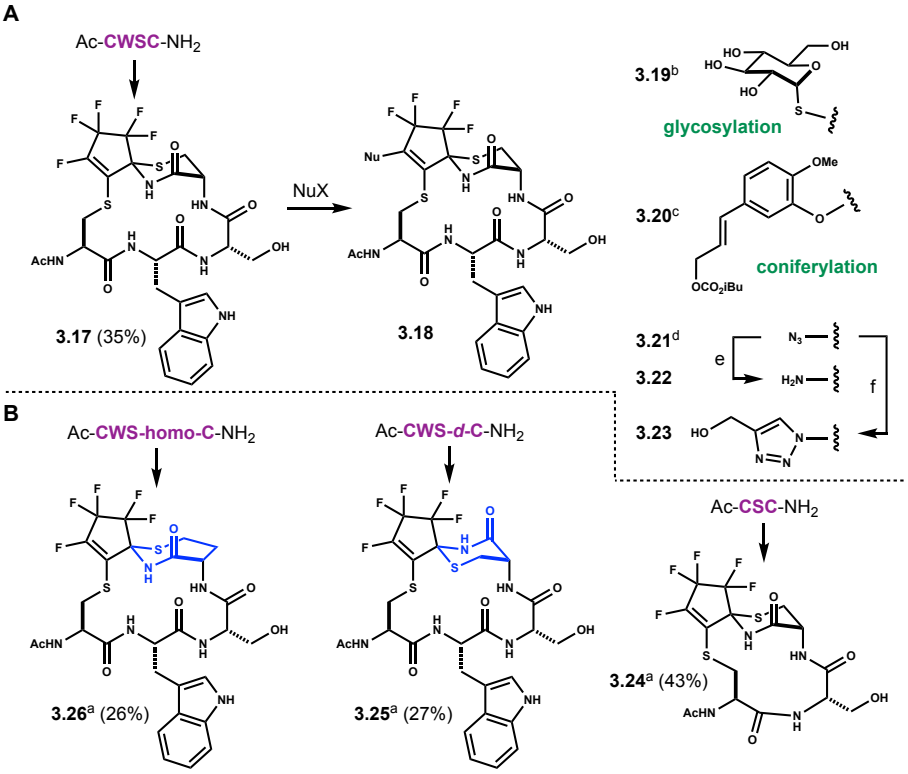


Fig. 3.3. Intermediate spirocyclic vinyl fluorides can be isolated in isomeric forms and react efficiently with exogenous nucleophiles. (**A**) Bimolecular substitution reactions using compound **3.17**. (**B**) Linear peptides were synthesized on solid phase as N-terminal acetamides and cleaved as primary C-terminal carboxamides. Yields reflect preparative HPLC isolation (C18 Sunfire, using 0.1% TFA in H_2O/MeCN). Reagents and conditions: a) OFCP (1.5 equiv), Et_3N (2.5 equiv), DMF (5.0 mM), 0 °C, 30 min; conc. then $KOSiMe_3$ (2.0 equiv), 1:4 DMF/THF (5.0 mM), 0 °C –> RT, 1 h, (>10:1 d.r.). b) beta-D-Thioglucose sodium salt (1.0 equiv), DMF (50 mM), 0 °C, 1 h, >95%. c) Coniferyl Carbonate **3.33** (2.0 equiv), $KOSiMe_3$ (4.0 equiv), DMF (50 mM), 0 °C –> RT, 12 h, 53%. d) NaN_3 (1.0 equiv), DMF (50 mM), 0 °C, 1 h, >95%. e) H_2, Pd/C (10 wt %), EtOH, 35 °C, 12 h, 87%. f) Propargyl Alcohol (1.0 equiv), Phenylenediamine (15 mol %), Sodium ascorbate (10 mol%), $CuSO_4$•$5H_2O$ (5 mol%), 2:3 H_2O:tBuOH (0.2 M), 12 h, 75%.

macrocycle **3.25** (**Fig. 3.3B**), while its homo cysteine variant affords spiro thiazepinone **3.26** in good yield and diastereoselectivity (>10:1 d.r.). Smaller ring analogs are also accessible. Omitting the tryptophan residue results in the formation of spiro tricyclic substance **3.24**.

The vinyl fluoride in **3.17** can be intercepted in bimolecular reactions to give a variety of substituted and homologated derivatives. It reacts with commercial β-D-thioglucose sodium salt within minutes at 25˚C to afford unprotected glycoconjugate **3.19** in near quantitative yield. By varying the peptide sequence and the thioglycoside used, amalgamations with OFCP could generate a new class of glycosylated cyclic peptidomimetics. Compound **3.17** also reacts with a coniferyl alcohol derivative to afford **3.20**. The cinnamyl carbonate in **3.20** provides means to form an additional large ring via electrophilic capture of amines, carboxylates, imidazoles and pi basic aromatic residues (e.g. see **Fig. 3.1B**).[3-11] Azidation of **3.17** with NaN_3 in DMF provides vinyl azide **3.21** in >95% yield. This molecule participates in Sharpless/Huisgen 'click' cycloadditions with terminal alkynes. Stirring **3.21** with 1.0 equiv. of propargyl alcohol in the presence of a phenylenediamine ligated Cu^{II} catalyst affords triazole **3.23** in 75% yield.[21] Alternatively, azide **3.21** can be hydrogenolyzed to afford β-thio enamine **3.22**. This molecule can be purified without hydrolysis. Its amino group strongly resists acylation, even with Ac_2O / DMAP. Proximal fluorination attenuates its nucleophilicity. In fact, inductive deactivation appears to have an impact on the stability of these structures in general. For example, **3.13** and **3.24** are stable (<5% loss by HPLC analysis) in aqueous buffer of varying pH for extended periods of time (72h at pH 4.5, 7.0 and 10.0, 25°C). In addition, both compounds were stable to superstoichiometric amounts of N-chlorosuccinimide (NCS) and 30% aq. H_2O_2 (2.0 eq. oxidant, pH 4.5 buffer / CH_3CN, 25˚C) over 72 h. The alkene, the thioaminoketal, the imidazole and the sulfur atoms were unaffected in these

experiments. For comparison, the disulfide derived from hexapeptide HFCASC (**S30**, the linear precursor to **3.10**) decomposed at pH 10.0 and reacted readily with NCS (See SI for details).

3.4 Investigation of Thiazole Small-Molecule Inserts for Orthogonal Cyclizations

The reactivity of intermediate vinyl fluorides **3.7** provides options to form a second large ring using molecular inserts. This markedly expands the diversity of possible outcomes. The first target we were interested in harnessing onto our peptidyl system was that of 2-aminothiazole-5-carbinol (**3.27**, **Fig. 3.4A**) developed by Saulnier and coworkers.[22] **3.27** was prepared for its propensity to ionize under acidic conditions and form a methylenethiazol-iminium intermediate (**3.28**). **3.28** would then rearomatize via the capture of small-molecule nucleophiles such as phenols, indoles, alcohols, and amides. These nucleophiles could be extrapolated to peptidic systems; more specifically, amino acid residues such as tyrosine and tryptophan (via C-C bond

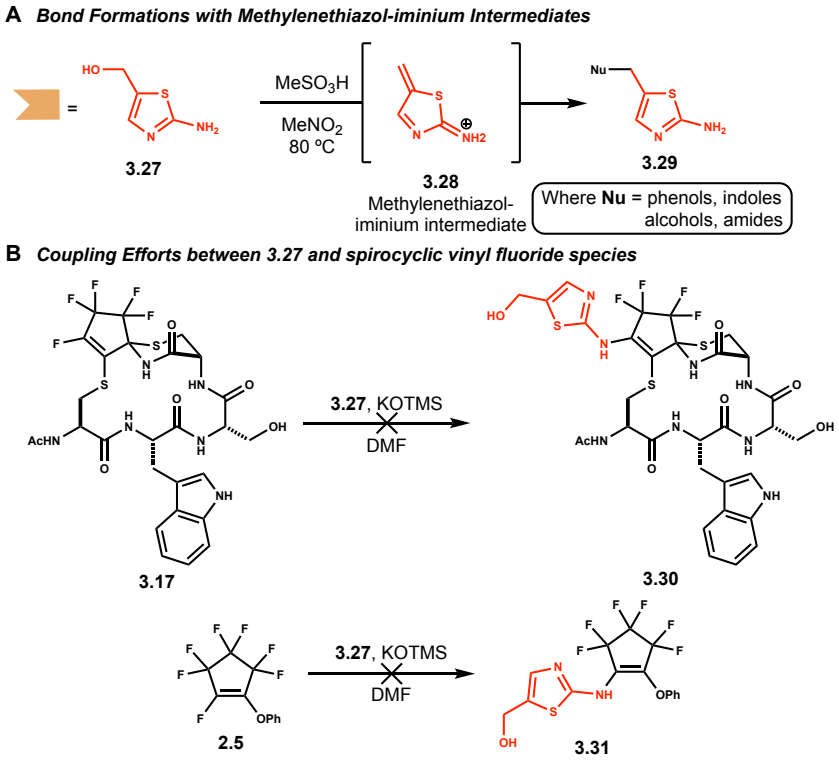


Fig. 3.4. (**A**) Literature Precedent demonstrates broad nucleophile participation with **3.27** (*Ref 22*). (**B**) Attenuated nucleophilicity on amino motif (**3.27**) prevents coupling prevents ipso-displacement of electrophilic spirocyclic vinyl fluoride species **3.17** and **2.5**.

formations), serine and threonine, and glutamine, asparagine, and backbone amides. These outcomes would markedly expand the scope of possible reactivities to obtain macrobicyclic structures. After much optimization, we prepared **3.27** and tested the nucleophilicity of the amino-motif with a model electrophile. We attempted to couple **3.27** onto electrophilic vinyl fluoride species **3.17** (**Fig. 3.4B**). Stirring both species for prolonged time (> 72 h), excess base, and elevated temperature (23 ºC -> 100 ºC) yielded no detectable desired product **3.30**. Predominantly unreacted starting materials **3.17** and **3.27** were observed. Furthermore, we then attempted to couple **3.27** onto a more electrophilic vinyl fluoride species **2.5** and observed no detectable product **3.31**. In conclusion, given thiazole's (**3.27**) poor nucleophilicity it was necessary to reroute and prepare thiazole inserts with sufficient nucleophilicity to couple onto our electrophilic intermediates.

We prepared 5-(hydroxymethyl)thiazole-2(3H)-thione (**3.32**), the exo-sulfur based nucleophile in 3 steps (see SI for details) and then modeled desired reactivity bimolecularly (**Fig. 3.5**). **3.32** showed markedly increased nucleophilicity and coupled onto vinyl fluoride **2.5** in quantitative yield and nearly instantaneous to afford **3.33** (> 99% yield, 1 min, 23 ºC). Using the conditions reported by Saulnier and coworkers; $MeSO_3H$, $MeNO_2$, and p-cresol as a surrogate for

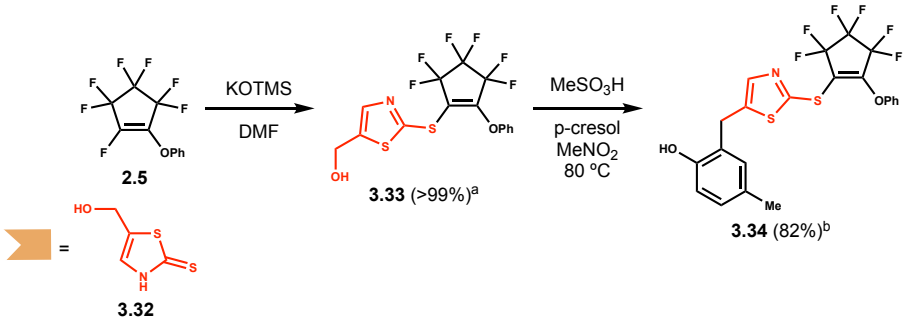


Fig. 3.5. Novel small-molecule insert **3.31** shows good nucleophilicity and readily ionizes under acidic conditions. a) thiazole **3.32** (1.1 equiv), KOTMS (1.1 equiv), DMF (50 mM), 23 ºC, 1 min. b) $MeSO_3H$ (5.0 equiv), p-cresol (5.0 equiv), $MeNO_2$ (0.05 M), 80 °C, 4 h.

tyrosine; complete conversion and good isolated yield (82%) of the C-C bond linked product **3.34** was readily afforded.[22] We then transitioned **3.32** onto real peptidyl systems.

In these experiments (**Fig. 3.6A**), initial OFCP mediated macrocyclizations and base-induced spirocyclizations occur in one flask as before. The incipient spirocyclic vinyl fluorides are then captured in situ with a nucleophile that can be subsequently activated as an electrophile. For example, treating Ac-YACFAC-NH_2 with 1.5 eq. OFCP (Et_3N, DMF) forms a macrocycle at 0°C

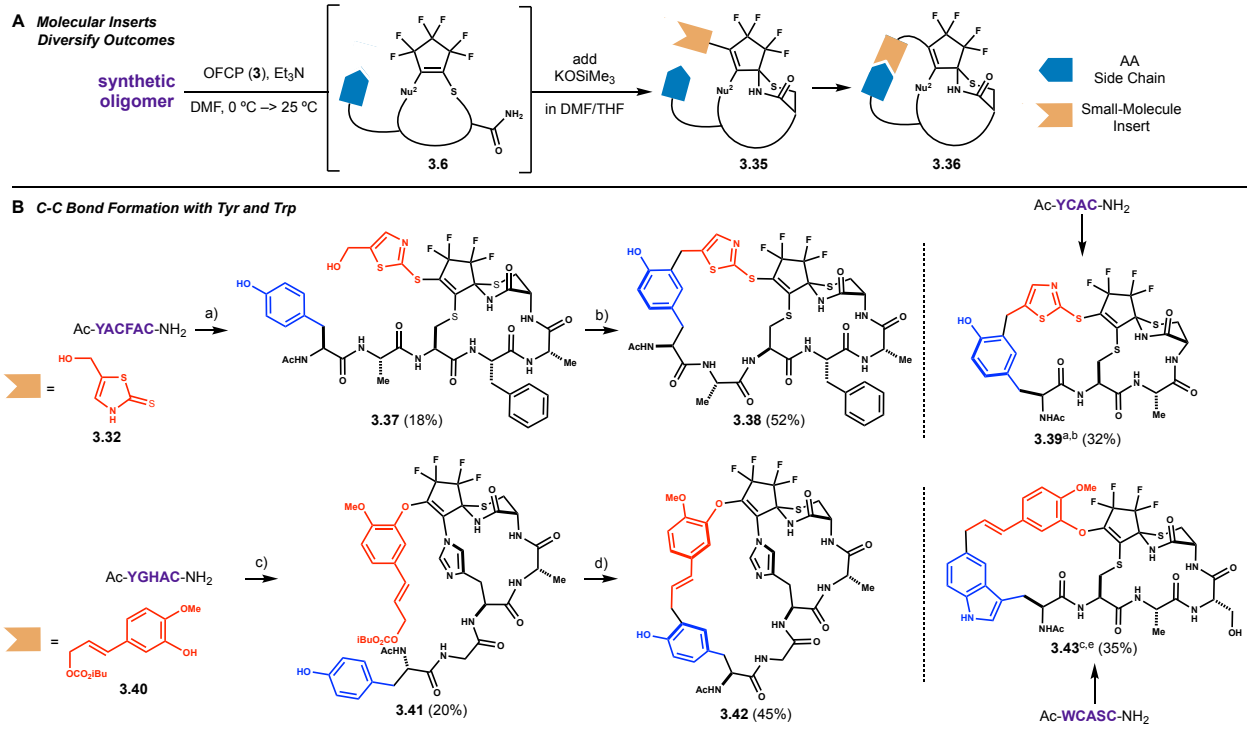


Fig. 3.6. Synthesis of OFCP scaffolded macrobicyclic structures using small-molecule inserts. (**A**) Molecular inserts diversify outcomes. (**B**) C-C bond formation with Tyr and Trp Linear peptides were synthesized on solid phase as N-terminal acetamides and cleaved as primary C-terminal carboxamides. Yields reflect preparative HPLC isolation (C18 Sunfire, using 0.1% TFA in H_2O/MeCN) wherein all histidine derived products were isolated as TFA salts. Reagents and conditions: a) OFCP (1.5 equiv), Et3N (2.5 equiv), DMF (10 mM), 0 °C, 30 min; conc. then $KOSiMe_3$ (3.5 equiv), thiazole **3.32** (1.2 equiv), 1:4 DMF/THF (30 mM), 0 °C –> rt, 3 h. b) 7.5% TFA, $MeNO_2$ (5.0 mM), 80 °C, 12 h. c) OFCP (1.5 equiv), NEt_3 (2.5 equiv), DMF (10 mM), 0 ºC, 30 min; conc. then Cs_2CO_3 (6.0 equiv) 1:4 DMF/THF (30 mM) 0 ºC, 1 h; then **3.40** (2.0 equiv) 0 ºC –> rt, 48 h. d) Tf_2NH (5.0 equiv), $MeNO_2$ (2.5 mM), RT, 1 h. e) $Sc(OTf)_3$ (1.0 equiv), $MeNO_2$ (5.0 mM), rt, 1 h.

(**Fig. 3.6B**). Subsequent exposure to $KOSiMe_3$ generates a spirocyclic vinyl fluoride that is intercepted in situ with hydroxymethylated thiazole thione **3.32** to afford **3.37** in good yield.[22] The strong nucleophilicity of the exocyclic sulfur atom in **3.32** permits biomolecular substitution to outcompete internal macrocyclization of the tyrosyl phenol in this system. After much reaction screening; it was discovered that excess TFA outperformed $MeSO_3H$ in peptidyl systems. Warming a $MeNO_2$ solution of **3.37** containing 7.5 vol % TFA ionizes the primary hydroxyl group and the incipient methyldene thiazolium cation captures the tethered phenol via C-C bonding to generate macrobicyclic structure **3.38** in good yield.

This alkylative macrocyclization is tolerant of varying ring sizes and substitution patterns. Reacting the 4-mer Ac-YCAC-NH_2 with OFCP and **3.32** in the same process as above affords macrobicycle **3.39** wherein each large ring contains one less amino acid residue. Replacing **3.32** with coniferyl alcohol derivative **3.40** provides for further options. For example, treating Ac-YGHAC-NH_2 with OFCP (Et_3N, DMF) followed by an excess of Cs_2CO_3 generates a macrocyclic vinyl fluoride that reacts with added **3.40** (2.0 equiv) to provide phenolic ether **3.41**. Relative to **3.32**, the capture rate by **3.40** is slower but the product is stable under the extended reaction conditions and is easily purified. When **3.41** is treated with Tf_2NH in $MeNO_2$ at 25˚C for 1 h, preparative HPLC provides imidazolium bridged macro-bicyclic structure **3.42** in good yield. As we have shown previously, the cinnamyl cation, generated either as a solvated ion pair or in metal stabilized form, is adept at large ring formations. When Ac-WCASC-NH_2 is treated successively with OFCP and **3.40** as before and the resultant product is ionized with $Sc(OTf)_3$ in $MeNO_2$, novel indole-linked macrobicycle **3.43** is produced. Internal cinnamylation engages the otherwise unreactive (towards OFCP) tryptophan side chain in C-C bond formation.

In addition to C-C bond formed products (**Fig. 3.6**) we became interested if we could expand our reaction scope to C-X bonds with free serine, threonine, glutamine, and asparagine side chains via in-situ capture of the methylene thiazol-iminium (**3.28**) species akin to literature precedent for **3.29**.[22] We prepared oligomer Ac-SGCFC-NH_2 bearing a proximal alcohol at the N-terminus (**Fig. 3.7**). Processing of this oligomer with our optimized conditions readily afforded acyclic diol **3.44**. Subjecting **3.44** to a warmed TFA solution afforded no detectable product with unreacted starting material being predominant. Stronger acids such as super acids TfOH and Tf_2NH resulted in comparable outcomes with no detectable product **3.45**. This result indicated that trapping of O- or N- nucleophiles was inherently more difficult as these products could reversibly convert back to starting materials when compared to irreversible C-C bond linked products (**Fig. 3.6**). In addition to acidolysis, extensive Mitsunobu screens were run, but unfortunately those experiments resulted in decomposition into intractable products as soon as phosphines / azodicarboxylates were added to **3.44**. We then hypothesized we could readily form C-X bonds by making more reactive derivatives based the hydroxyl-moiety present in **3.32**.

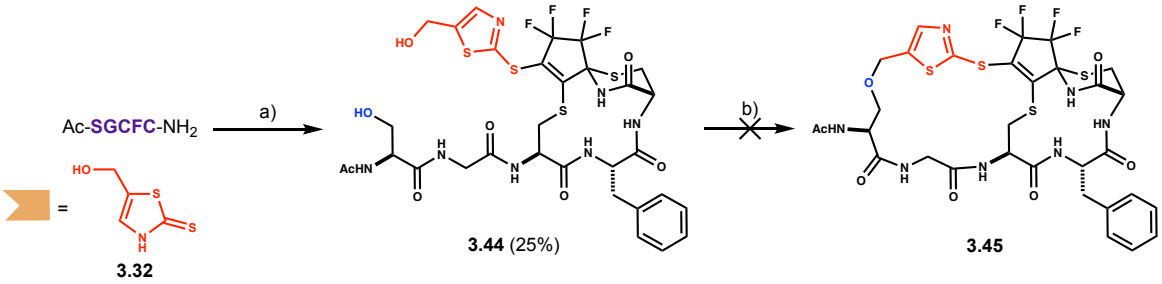


Fig. 3.7. Attempt to synthesize OFCP scaffolded macrobicyclic structures via C-X bonds. a) OFCP (1.5 equiv), Et3N (2.5 equiv), DMF (10 mM), 0 °C, 30 min; conc. then $KOSiMe_3$ (3.5 equiv), thiazole **3.32** (1.2 equiv), 1:4 DMF/THF (30 mM), 0 °C –> RT, 3 h. b) 7.5% TFA, $MeNO_2$ (5.0 mM), 80 °C, 12 h.

Inspired by our previous work with our cinnamyl alcohol scaffolding reagents we hypothesized that carbonate esters would readily ionize in comparison to their parent primary alcohol (**3.33**).[3-11] In this reaction pathway we would extrude tBuOH / iBuOH and CO_2 gas. We prepared the model tBu-carbonate ester **3.47** via Boc anhydride (**Fig. 3.8**). Subjecting **3.47** to 7.5 vol% TFA, p-cresol, and $MeNO_2$ at room temperature resulted in undesired and complete reversion to **3.33** within 1 h, presumably via the loss of isobutene and CO_2 gas. In an effort to prevent this reaction pathway we prepared isobutyl-carbonate ester **4.46** via isobutyl chloroformate. After optimization, complete conversion of **4.47** in the presence of 7.5 vol% TFA, p-cresol, and $MeNO_2$ to desired product **3.34** was observed to be markedly faster (1 h) and required lower reaction temperature (60 ºC) than the parent primary alcohol (**3.32**) with the stronger mesic acid (see **Fig. 3.5**). Additionally, we also prepared alkyl chloride derivative **3.48** which ionized and captured p-cresol to afford **3.34** at 60 ºC and 1 h although with diminished 61% yield. Protonation of the alkyl chloride **3.48** is unlikely which hints that a possible mechanistic pathway to afford **3.34** proceeds via thermal decomposition of this chloride. Notably, **3.48** was unstable to prolonged storage at room temperature over multiple days (< 72 h) and decomposed to intractable products, presumably

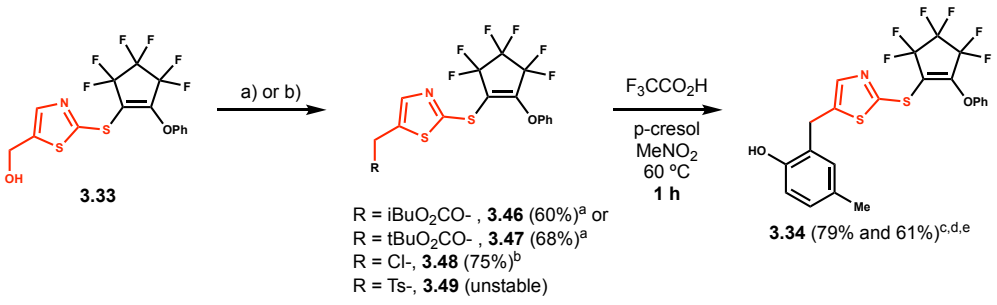


Fig. 3.8. Derivatization of model primary alcohol into carbonate esters and alkyl chloride. a)Boc_2O or $iBuO_2CCl$ (1.25 equiv), pyridine (1.25 equiv), CH_2Cl_2 (0.2 M), 0 ºC to 23 ºC, 2 h. b) $SOCl_2$ (1.5 equiv), 1:5 CH_2Cl_2:PhMe (0.17 M), 0 ºC to 23 ºC, 16 h. c) 7.5 vol% F_3CCO_2H, p-cresol (3.0 equiv), $MeNO_2$ (0.05 M), 60 °C, 1 h. d) Acidolysis with **3.47** resulted in complete conversion to **3.33** within 1 h. 79% yield shown reflects that of **3.46**, subjection to acidolysis. e) 61% yield reflects that of **3.48**, subjection to acidolysis.

due to loss of chloride. Likewise, full conversion to tosyl derivative **3.49** was observed (via LC-MS) but this reagent was hard to handle and readily decomposed (< 30 min at 23 ºC).

Based on promising results observed for **3.46** (**Fig. 3.8**), we then prepared **3.50** in 6 steps (see SI for details). We then proceeded to test **3.50** on real peptidyl systems and subjected this thiazole derivative to OFCP processing with oligomer Ac-YACFAC-NH_2 (**Fig. 3.9A**). Unfortunately, the exo-sulfur atom present in **3.50** would not couple onto our vinyl fluoride intermediates. Predominantly decomposition and undesired macrobicylic **2.43** (via intramolecular ipso-displacement from the proximal Tyr residue) was observed. We then refocused on simpler **2.5** (**Fig. 3.9B**), coupling mediated with NEt_3 resulted in unreacted **2.5** and decomposition of **3.50** into intractable products. We hypothesize that generation of the thiazole-thiolate anion by

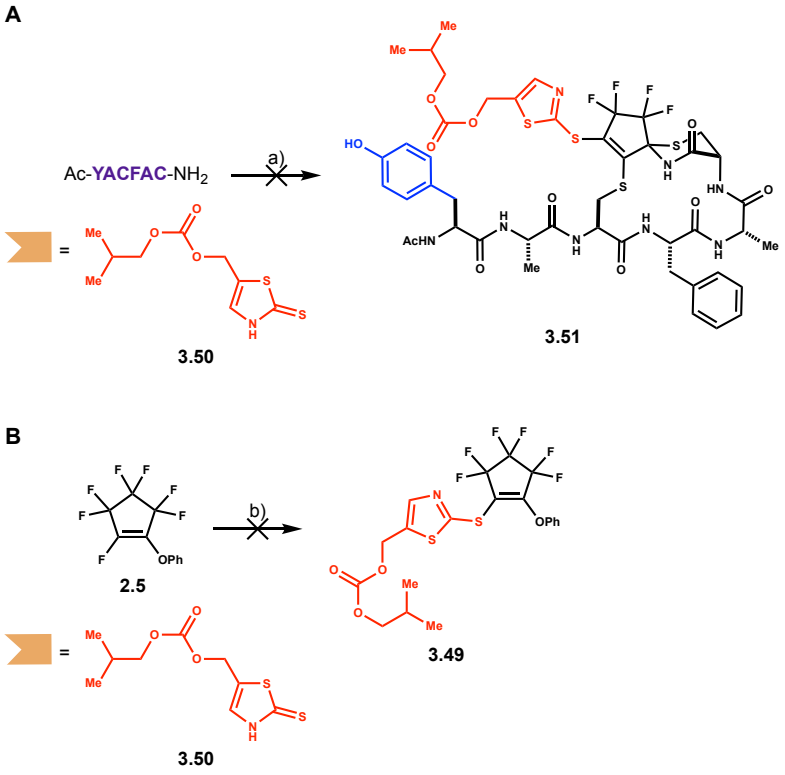


Fig. 3.9. Coupling efforts of carbonate ester 3.50 with vinyl fluorides. (A) **3.50** did not couple in real system (B) **3.50** decomposed upon attempting to couple onto model **2.5** a) OFCP (1.5 equiv), Et_3N (2.5 equiv), DMF (10 mM), 0 °C, 30 min; conc. then $KOSiMe_3$ (3.5 equiv), thiazole **3.50** (1.2 equiv), 1:4 DMF/THF (30 mM), 0 °C –> RT, 3 h. b) thiazole **3.50** (1.2 equiv), NEt_3 (3.0 equiv), DMF (0.05 M), 23 ºC, 1 h.

deprotonation with base resulted in decomposition via extrusion CO_2 gas and iBuOH which outcompeted thiolate nucleophilic displacement of vinyl fluoride intermediates.

In an effort to make parent thiazole **3.32** more suspectable to ionize under milder conditions and with consideration that carbonate esters were inherently unstable, we prepared tertiary alcohol derivative **3.52** in 3 steps (see SI for details). **3.52** performed well in OFCP processing; presumably since under basic conditions, elimination of hydroxy anion is unlikely. Acyclic diol **3.53** was readily prepared (28% yield) (**Fig. 3.10A**). **3.53** readily ionized with 5 vol % TFA at room temperature within 1 hour. This is consistent with substituent effects for S_N1/solvolysis reactions as more substituted tertiary carbocations readily form in comparison to their primary counterparts.[23] Unfortunately, desired ether linked C-X product **3.54** was not observed. Instead,

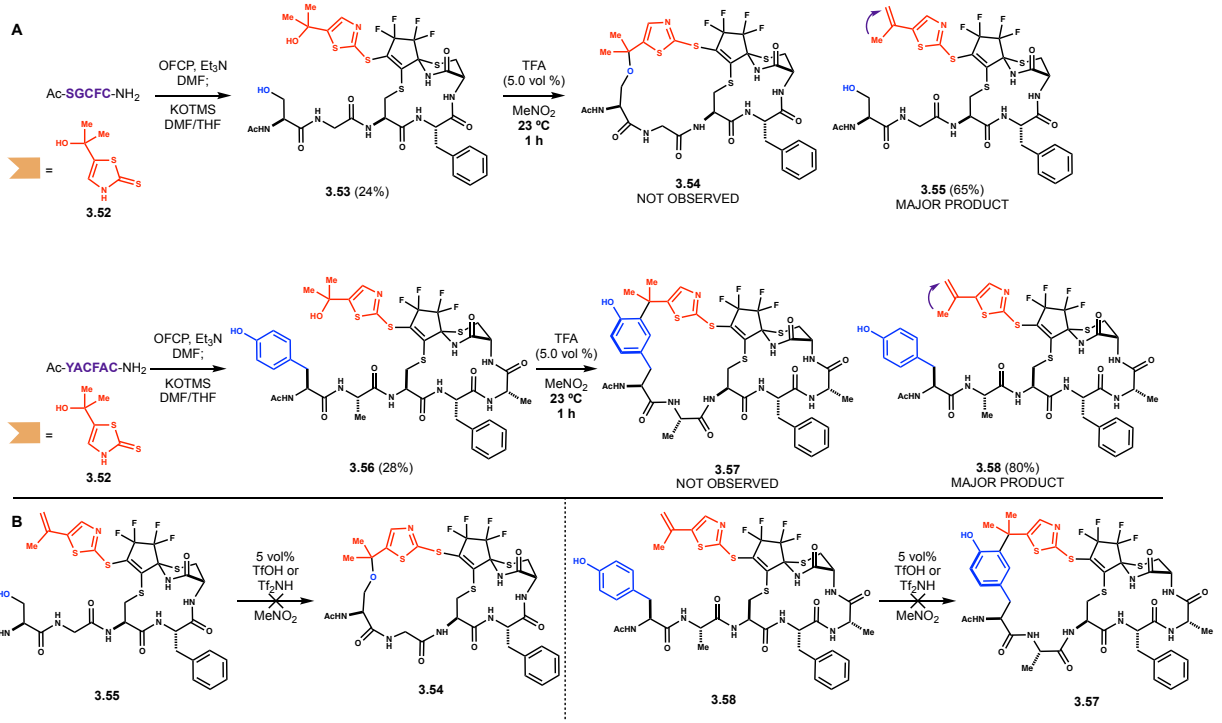


Fig. 3.10. Acidolysis with tertiary alcohol 3.52. (A) Attempt at making C-C and C-X bonds resulted in alkene formation (B) Alkene products could not be subsequently ionized to form C-C and C-X bonds a) OFCP (1.5 equiv), Et_3N (2.5 equiv), DMF (10 mM), 0 °C, 30 min; conc. then $KOSiMe_3$ (3.5 equiv), thiazole **3.52** (1.2 equiv), 1:4 DMF/THF (30 mM), 0 °C –> RT, 3 h. b) 5 vol% F_3CCO_2H, $MeNO_2$ (5.0 mM), 23 ºC, 1 h.

undesired alkene product **3.58** was isolated in good yield (65%) as the only observable product. We then hypothesized potential reaction pathways: 1. Formation of carbocation and then subsequent elimination to undesired alkene product **3.55** that outcompeted heteroatom capture (**3.54**). 2. Ether formed C-X bonds were inherently unstable to acidolysis / solvolysis reactions as the ether linked oxygen (**3.54**) could be protonated and then reversed back to unreacted starting material **3.53** and/or eliminated to alkene product **3.55**. We were then interested if we could form C-C bonds with a known oligomer sequence that readily reacted with parent thiazole **3.32**. More specifically, we wanted to see if could form C-C bonds with thiazole **3.52** as formation of **3.57** would be irreversible and would not convert to alkene **3.58**. Capture of the carbocation intermediate with a tyrosine residue would indicate capture would be faster than alkene formation via elimination. We subjected known oligomer Ac-YACFAC-NH_2 that previously readily reacted with **3.32** to give C-C bond formed product **3.38** (see **Fig. 3.6**) to OFCP processing with thiazole **3.52** to afford acyclic intermediate **3.56**. Acyclic **3.56** was then subjected to acidolysis with excess TFA to afford undesired alkene product **3.58** (80% yield) at room temperature within 1 h. Unfortunately, desired product **3.57** was not detected which indicated that the rate of alkene formation markedly outcompeted capture of the carbocation by the proximal tyrosine moiety. Additionally, we hypothesized we could readily ionize alkenes **3.55** and **3.58** with stronger superacids such as TfOH and Tf_2NH and have subsequent capture of the benzylic cationic intermediate by a respective proximal hydroxyl / phenol group. Subjecting **3.55** and **3.58** to subsequent acidolysis (**Fig. 3.10B**) resulted in only unreacted starting material and no detectable products **3.54** and **3.57**. In conclusion, the tertiary alcohol present in thiazole **3.52** was not an adequate insert to form any C-C let alone C-X bonds. Although intermediates derived from this insert readily ionized relative to **3.32**; these cationic intermediates formed only alkene products.

These alkene products were very stable and could not be derivatized further to make C-C and C-X bonds. Currently, formation of C-X bonds with thiazoles via acidolysis remains elusive.

3.5 Investigation of TEMPO-derived Inserts for Carbocation Generation

In our efforts to make macrobicyclic structures derived from C-X bonds we then hypothesized that generation of these bonds would be most optimal under neutral conditions. Knowles has recently shown that branched nitroxyl ethers undergo mesolytic cleavage when subjected to photo-redox catalysis to generate secondary and tertiary benzylic cations.[24] These species can be trapped bimolecularly in situ with both carbon (C-C) and heteroatom (C-X) nucleophiles. We propose to use this catalysis to initiate intramolecular reactions (**Fig. 3.11B**). We synthesized novel TEMPO-derived phenol insert **3.59** in 3 steps (**Fig. 3.11A**; see SI for details) as we predicted this compound to readily couple onto our vinyl fluoride intermediates and then upon

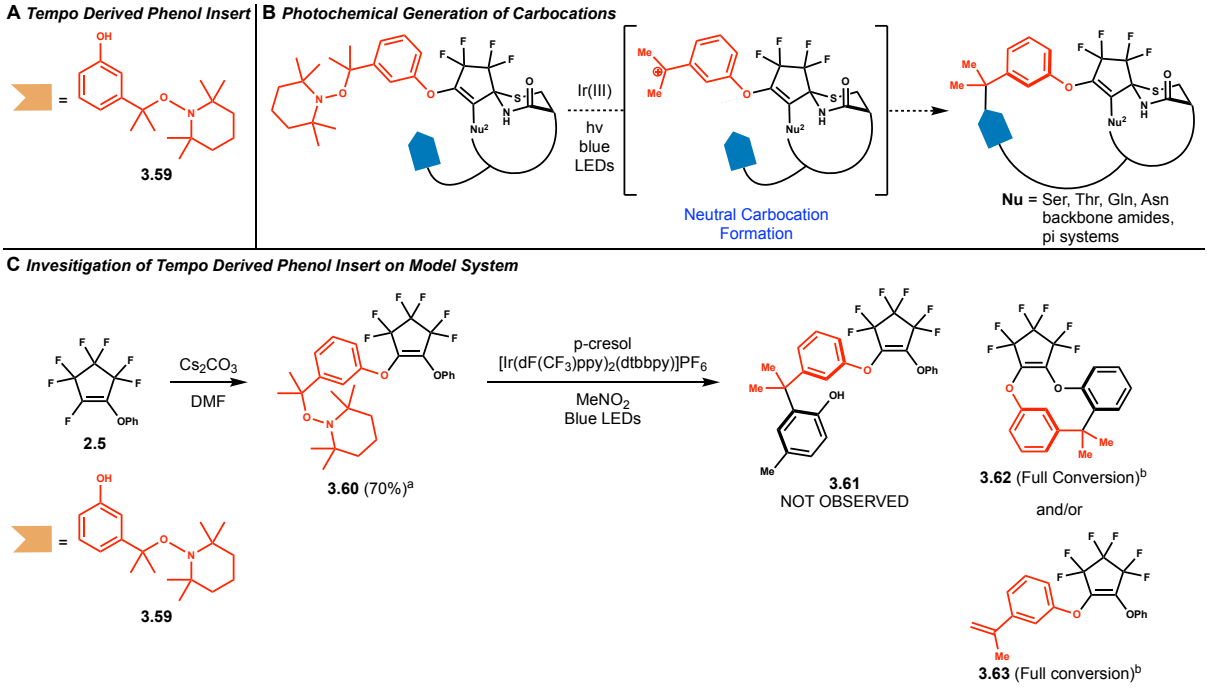


Fig. 3.11. Neutral Generation of Carbocations with TEMPO-derived Phenol 3.59. (A) Tempo Derived Phenol Insert (B) Photochemical Generation of Carbocations (C) Investigation of Tempo-Derived Phenol Insert on Modeal System a) phenol **3.59** (1.1 equiv), Cs_2CO_3 (1.1 equiv), DMF (0.05 M), 23 ºC, 1 h. b) p-cresol (5.0 equiv), $[Ir(dF(CF_3)ppy)_2(dtbbpy)]PF_6$ (2 mol %), $MeNO_2$ (0.1 M), 23 ºC, 12 h. Product **3.62** / **3.63** was unstable to SiO_2 purification and decomposed.

irradiation form a carbocation. This carbocation would then subsequently be captured by nucleophilic residues such as serine, threonine, glutamine, tryptophan, etc. With phenol **3.59** prepared we coupled it onto our model **2.5** to produce intermediate **3.60** in good yield (70%). We then subjected **3.60** to the optimized conditions reported by Knowles and coworkers for their bimolecular photo-redox couplings.[24] Subjecting **3.60** to excess p-cresol, catalytic $[Ir(dF(CF_3)ppy)_2(dtbbpy)]PF_6$, $MeNO_2$ solvent, and Blue LEDs produced full conversion to products **3.62** and/or **3.63** based on LC-MS / HRMS. Desired bimolecular coupling product **3.61** incorporating p-cresol was not observed. Based on LC-MS data, clean conversion to products **3.62** or **3.63** was observed but unfortunately these product/s were unstable to SiO_2 purification and decomposed into intractable products. Two possible outcomes are hypothesized: 1. Intramolecular capture of the carbocation by the proximal phenyl ether moiety (OPh) present in **3.60**. 2. Full conversion to alkene product **3.63** which was the predominant product in previous tertiary carbocation chemistry with thiazole (see **Fig. 3.10**). Furthermore, a reaction (**Fig. 3.11C**) was also performed with exogenous cyclohexanol in place of p-cresol that resulted in the identical reaction outcome. Unfortunately, further reaction screens were not performed due to the low quantities of phenol **3.59** that could be prepared (3 steps, unoptimized). As of yet, it is unknown if tertiary cations under neutral photo-redox conditions favor C-C / C-X bond formation over alkene formation. Future experiments will need to involve the preparation of more phenol **3.59** and coupling it onto real peptidyl systems. Potential products derived from these experiments should be stable and conclusions can be made if phenol **3.59** favors desired macrobicycle formation or undesired alkene formation.

3.6 Investigations for the Synthesis of Macrolactams and Cyclodepsipeptides

The side chains of aspartic and glutamic acid were also potential sites for ring closures. Our initial hypothesis was to generate spirocyclic vinyl fluorides followed by ipso-displacement with NaN_3, and subsequent hydrogenation would afford the enamine moiety in the presence of a free acid residue (**Fig. 3.12A**). However, free carboxylate groups caused OFCP reactions to be chaotic and low yielding; seco-acid **3.64** was isolated in trace amount which made material throughput a challenge. A potential solution was to protect free acid side-chains via methyl esters. Acyclic **3.65** was prepared where the Glu residue was masked via a methyl ester and incorporation

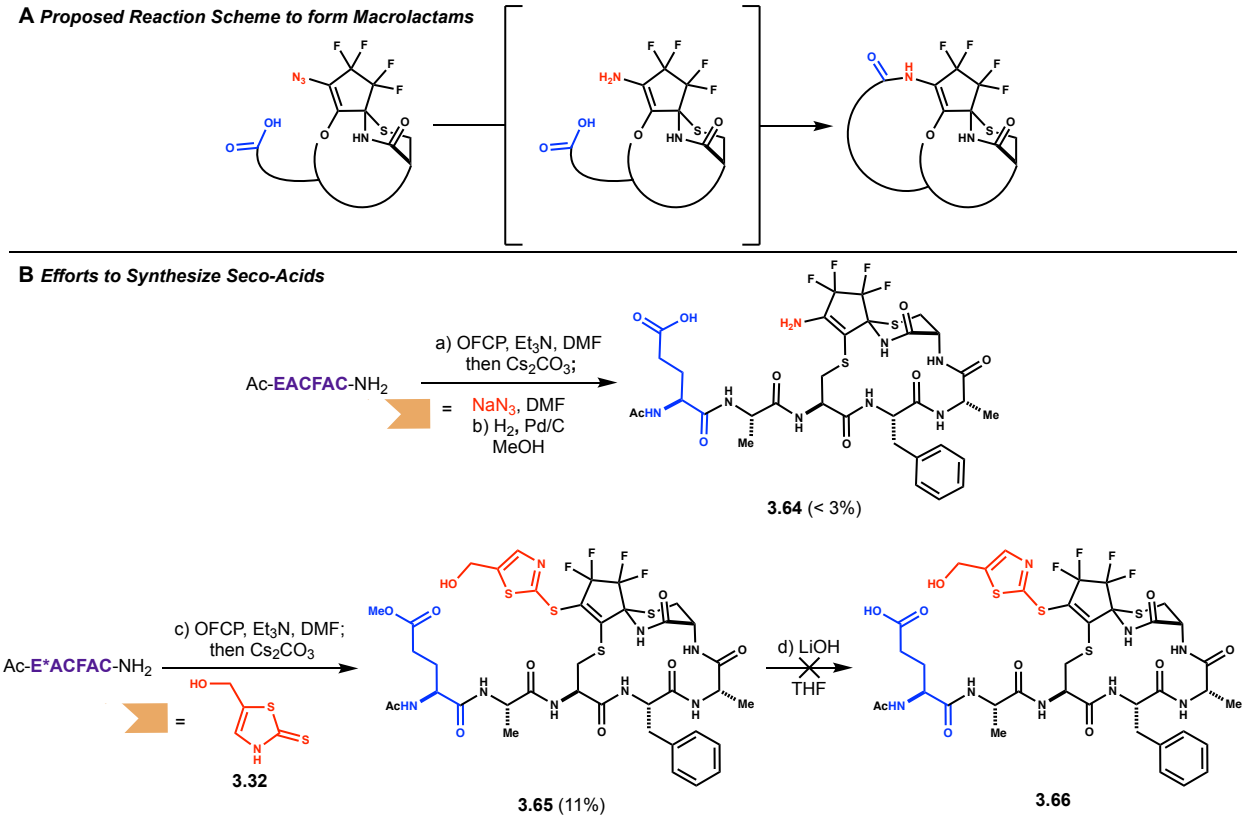


Fig. 3.12. (A) Proposed Reaction Scheme to form Macrolactams (B) Efforts to Synthesize Seco-Acids a) OFCP (1.5 equiv), Et_3N (2.5 equiv), DMF (10 mM), 0 °C, 30 min; conc. then Cs_2CO_3 (6.0 equiv), NaN_3 (1.2 equiv), 1:4 DMF/THF (30 mM), 0 °C –> RT, 3 h. b) H_2, Pd/C (10 wt %), MeOH, 35 °C. c) FCP (1.5 equiv), Et_3N (2.5 equiv), DMF (10 mM), 0 °C, 30 min; conc. then KOTMS (3.5 equiv), thiazole **3.32** (1.2 equiv), 1:4 DMF/THF (30 mM), 0 °C –> RT, 3 h. d) LiOH (3.0 equiv), THF (0.1 M), 23 ºC, 16 h.

of **3.32** insert. Attempts to saponify acyclic **3.65** did not afford any detectable seco-acid **3.66**. Predominantly epimerized acyclic **3.65** and intractable decomposition products were observed (**Fig. 3.12B**).

Bode had developed a cyano sulfurylide masked form of Asp (D*) to avoid aspartimide forming truncations during SPPS.[25] We synthesized monomer **3.67** and its new one carbon homolog **3.68**. Each proved to be an excellent surrogate for Asp and Glu, respectively, during peptide synthesis and OFCP processing. We solved our material throughput problem with these masked acids and readily accessed acyclic intermediate **3.69** (**Fig. 3.13A**). Oxidative workup with excess NCS and dehydration with EDC•HCl did not yield desired macrolactam product **3.70**.

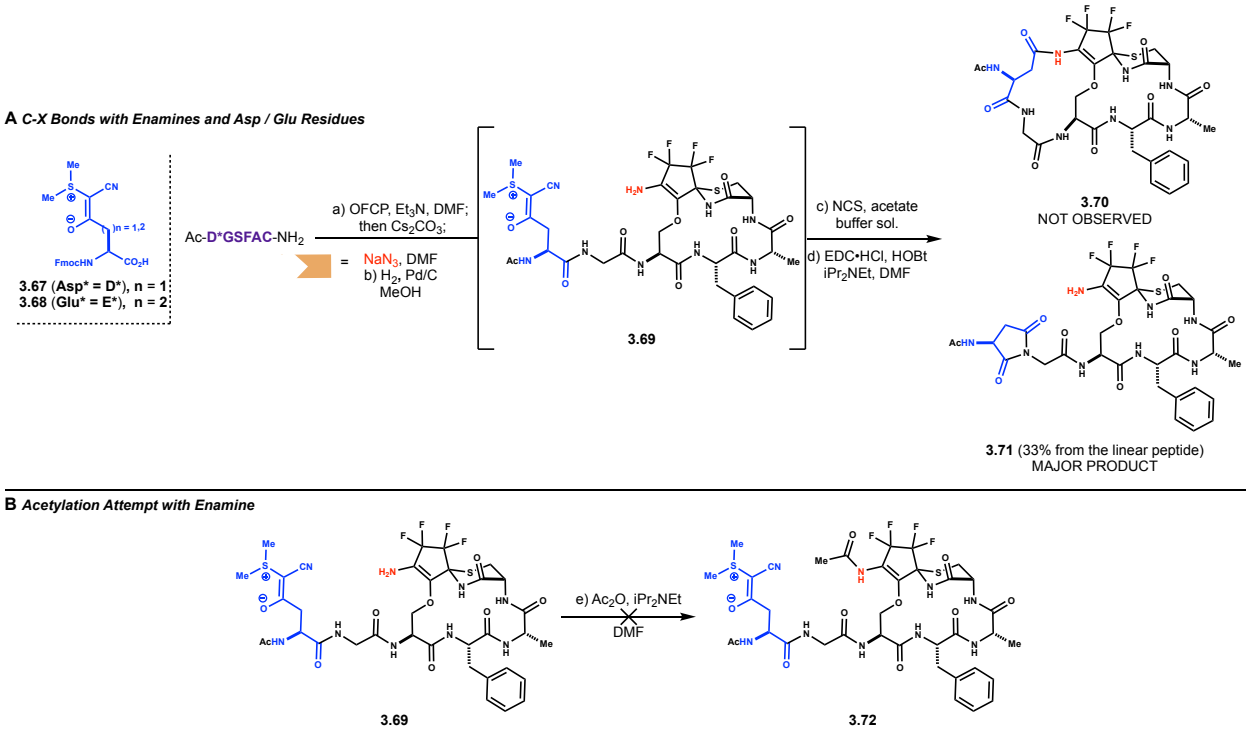


Fig. 3.13. (A) C-X Bonds with Enamines and Asp / Glu Residues (B) Acetylation Attempt with Enamine a) OFCP (1.5 equiv), Et_3N (2.5 equiv), DMF (10 mM), 0 °C, 30 min; conc. then Cs_2CO_3 (6.0 equiv), NaN_3 (1.2 equiv), 1:4 DMF/THF (30 mM), 0 °C –> RT, 3 h. b) H_2, Pd/C (10 wt %), MeOH, 35 °C. c) N-Chlorosuccinimide (1.5–3.0 equiv), 2:8 MeCN:NaOAc/AcOH (acetate buffer, pH 4.5) (1.0 mM), RT, 1 h. d) EDC•HCl (1.5 equiv), HOBt (1.0 equiv), Et_3N (3.0 equiv), DMF (5.0 mM), 0 ºC –> RT, 16 h. d) Ac_2O (1.5 equiv), iPr_2NEt (3.0 equiv), DMF (0.05 M), 23 ºC, 72 h.

Instead, the major product was undesired imide **3.71** in good yield (33% overall yield from the linear peptide) which aspartic acid residues are notably susceptible to forming. After numerous control experiments it was elucidated that the enamine moiety was not sufficiently nucleophilic. In a seminal screen, attempts were made to acetylate **3.69** (**Fig. 3.13B**). Surprisingly, after 72 h no detectable desired enamide product **3.72** was observed except for unreacted starting material **3.69**. This attenuated nucleophilic issue was solved with amine nucleophiles that possess sufficient nucleophilicity such as those derived from commercially available cysteamine.

For example, Ac-D*GSFAC-NH_2 reacts successively with OFCP and Cs_2CO_3 to give a spirocyclic vinyl fluoride that is captured in situ with commercial cysteamine to form adduct **3.73** (**Fig. 3.14A**; >10:1 d.r.). It is not necessary to isolate this species. Rather, mild oxidative hydrolysis of the cyano sulfurylide (NCS, CH_3CN /pH 4.5 acetate buffer, 25 ˚C) provides the corresponding carboxylic acid in high yield. Lactamization of the incipient amino acid then affords macrobicyclic compound **3.74** in 30% overall yield from the starting peptide. Product **3.74** contains a bridging serine residue. Identical OFCP processing of cyano sulfurylide residue containing peptides tolerates variations in peripheral functionality (see **3.75**, **3.78**, **3.79**) as well as threonine bridging (**3.76** and **3.80**). The synthesis of imidazolium salt bridged macrobicycle **3.77** directly from Ac-D*PNHFTC-NH_2 highlights the complexity forming potential of the methods. Replacing cysteamine with 2-mercaptoethanol or thiazole thione **3.32** permits the EDC mediated synthesis of macrolactones **3.81**–**3.83**. Structures of this kind are prototypes for a new class of hybrid cyclodepsipeptides.

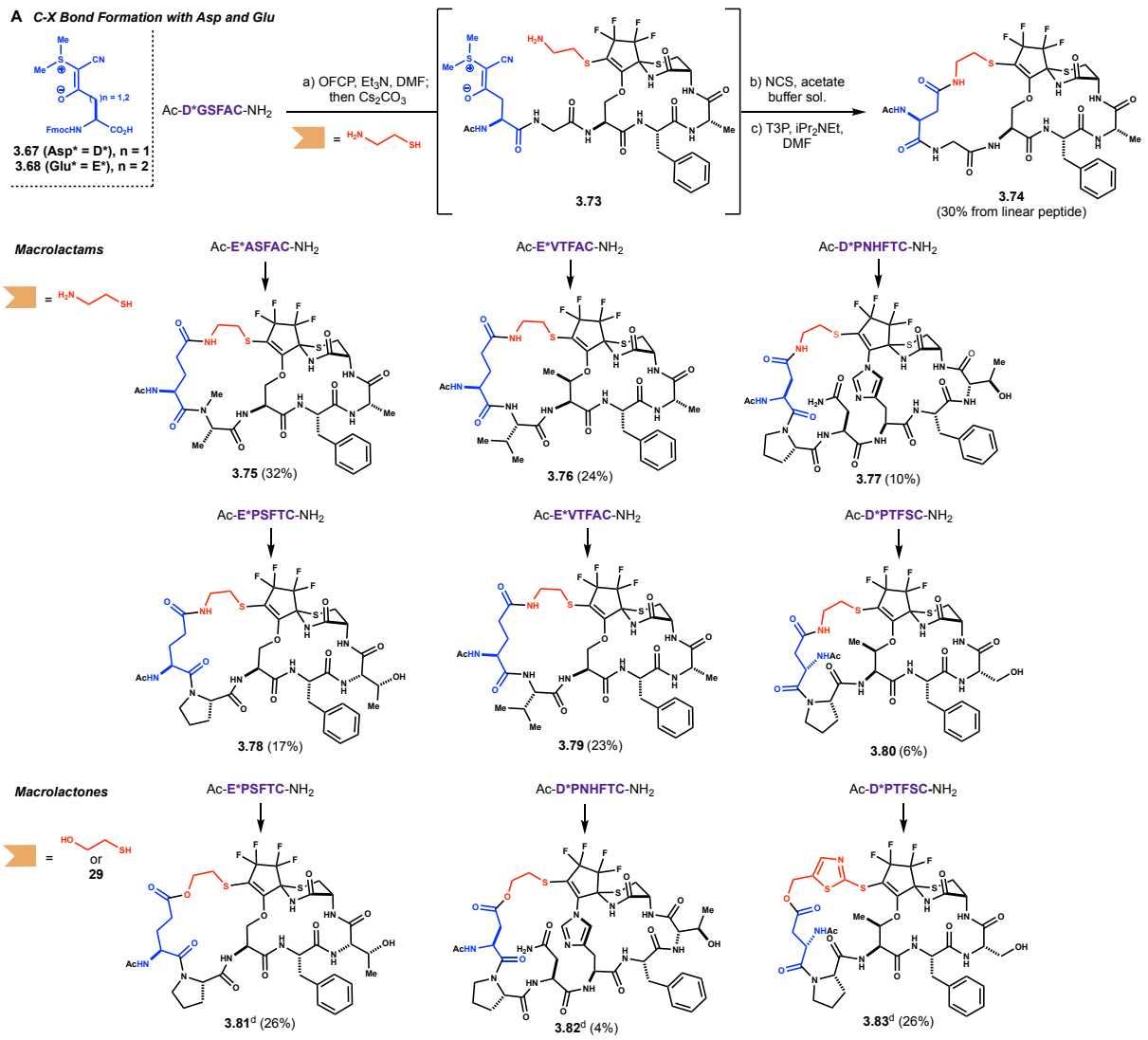


Fig. 3.14. Synthesis of OFCP scaffolded macrobicyclic structures using small-molecule inserts. (**A**) C-X bond formation with Asp and Glu. Linear peptides were synthesized on solid phase as N-terminal acetamides and cleaved as primary C-terminal carboxamides. Yields reflect preparative HPLC isolation (C18 Sunfire, using 0.1% TFA in H_2O/MeCN) wherein all histidine derived products were isolated as TFA salts. Yields shown for **3.75**–**3.83** refer to isolated yields from their respective linear oligomers. Reagents and conditions: a) OFCP (1.5 equiv), NEt_3 (2.5 equiv), DMF (10 mM), 0 ºC, 30 min; conc. then Cs_2CO_3 (5.0 equiv), 1:4 DMF/THF (30 mM), 0 ºC, 1 h; then cysteamine, 2-mercaptoethanol, or **3.32** (1.2 equiv), 0 ºC, 1 h. b). N-Chlorosuccinimide (1.5–3.0 equiv), 2:8 MeCN:NaOAc/AcOH (acetate buffer, pH 4.5) (1.0 mM), RT, 1 h. c) propanephosphonic acid anhydride (T3P) (1.5 equiv), iPr_2NEt (3.5 equiv), DMF (5.0 mM), 0 ºC, 1 h. d) Macrolactonization done using EDC•HCl (3.0 equiv), HOBt (2.0 equiv), Et_3N (5.0 equiv), DMF (5.0 mM), 0 ºC –> RT, 44–64 h.

3.7 Computational 'Hot' Loop Backbone Mimicry

Cyclic peptides and peptidomimetics are potential ligands for protein surfaces involved in protein-protein interactions (PPIs).[26] The majority of characterized protein-protein interactions (PPIs) are mediated by non-helical, non-strand surface loops.[27] Kritzer et al analyzed more than 1400 contiguous 'hot' loops mediating PPIs in the Protein Data Bank and found the large majority clustered into just 11 backbone structural types.[28] Because spectroscopic data indicated the thiazaspirodecenone scaffolded double looped structures in **Fig. 3.2C** were conformationally well defined, we hypothesized that selective mimics of Kritzer's 'hot' loops might be devised by varying ring sizes on either side of the bridging residue.[29] To probe this idea, we analyzed a hypothetical library of macrobicycles resultant from OFCP processing of Ac-X–$(Ala)_m$–Y–$(Ala)_n$–Cys-NH_2 sequences: where X = Cys, His and Tyr, Y = Cys and His and m, n = 1–5. Each of those 150 structures was geometry optimized using molecular force fields and low energy conformations were identified using a Monte Carlo search algorithm (see SI). Conformers within 5 kcal/mol the calculated minimum for each library member were overlaid onto a representative of the 11 Kritzer loop types using the PyMOL® superposition function.[30] The conformer that aligned best in each case was assigned a MatchAlign score. These were then normalized relative to loop self-superimposed maximal values to yield 'percent similarity' scores for each loop type. This method to evaluate structural mimicry permits meaningful comparisons across macrobicycle and representative loop size variations. Library members that best mimic each representative loop type are plotted along the diagonal (top right to bottom left) in the heat map shown in **Fig. 3.15**. This core data set identifies close mimics (percent similarity > 75%) for six of the eleven major PPI mediating loop types. Of these six, three appear quite selective, despite the analysis lacking side chain annotations. Interestingly, the data shows mimicry can occur at the N-terminal loop (see

2WAM/H5H2C overlay), the C-terminal loop (see 2OL1/H1H5C overlay), or at the composite surface created at the double loop junction (see 3N3R/Y4C1C overlay). In general, the double looped nature of OFCP derived products allows one turn surface to be conformationally geared by small perturbations in the second. For example, the 4J07/H4H4C overlay has a similarity score of

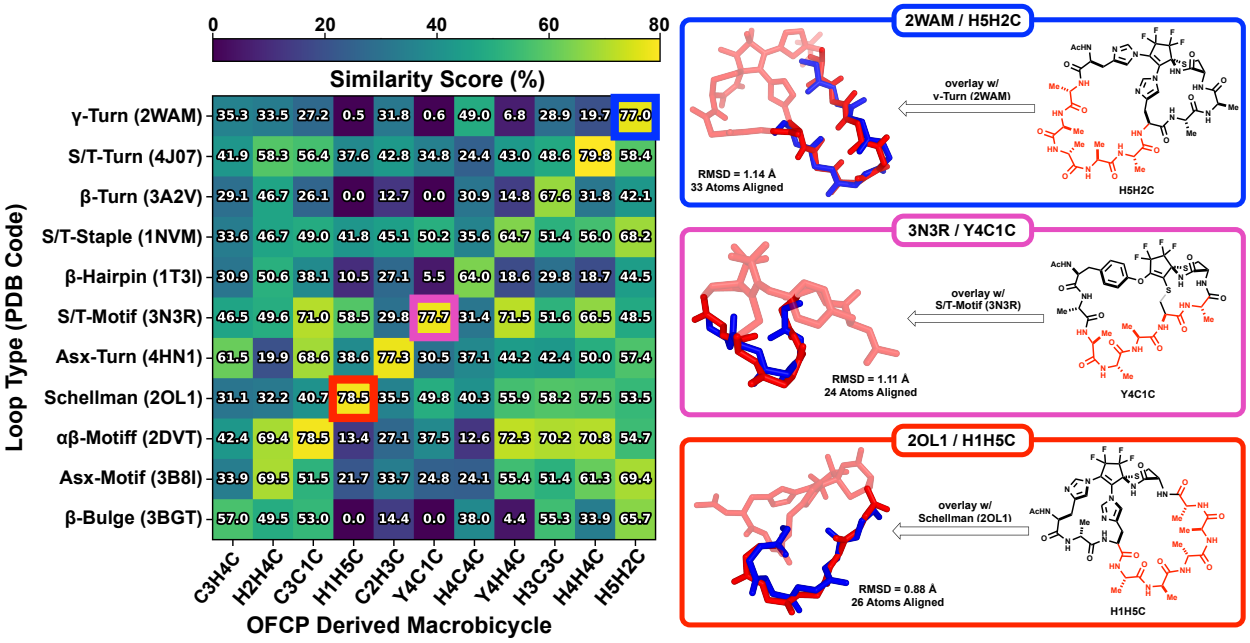


Fig. 3.15. 'Hot' Loop backbone mimcry within a library of computed OFCP derived poly-A macrobicycles. Heat map showing percent similarity scores between select OFCP derived macrobicycles (x-axis) and various representative loop types (y-axis). Data along the diagonal (top right to bottom left) shows those macrobicycles which best structurally mimics each loop type. OFCP processed macrobicycles are named based on the amino acid sequence of the peptide from which they were derived with letters corresponding to 1 letter amino acid abbreviations and numbers representing the number of intervening L-alanine residues between nucleophilic residues (See SI for details). Reaction schemes to synthesize CXCXC and HXHXC derived macrobicycles are described in the SI. Each of the 150 OFCP derived macrobicycles used in this study were geometry optimized using molecular force fields and low energy conformations were identified using a Monte Carlo search algorithm (see SI for details). Low energy conformers of each library member were overlaid onto a representative of the 11 Kritzer loop types using the Pymol® superposition function. The resulting generated MatchAlign scores were normalized relative to loop self-superimposed maximal values to yield 'similarity scores'. OFCP derived macrobicycles demonstrate good loop mimicry utilizing their N-terminal loop (2WAM/H5H2C), C-terminal loop (2OL1/H1H5C) as well as the composite surface created at the double loop junction (3N3R/Y4C1C) of the macrobicycle.

79.8 while the closely related 4J07/H4H5C overlay has a similarity score of 8.6 (see SI for further details).

3.8 Parallel Artificial Membrane Permeability Assay (PAMPA) Experimentation

Computed backbone overlays generate hypotheses that can be validated in future experiments. The utility of those experiments will be broader if the macrocyclic compounds are membrane permeant. Intracellular PPIs are particularly challenging to target with peptidic structures. Cyclic peptides having mw > 1 kDa are rarely taken up passively into cells.[31] Below 1 kDa, numerous factors must be considered. The fluorinated polycycles generated in this study are new chemotypes that have not been evaluated for permeability in any format. OFCP derived macrocycles were

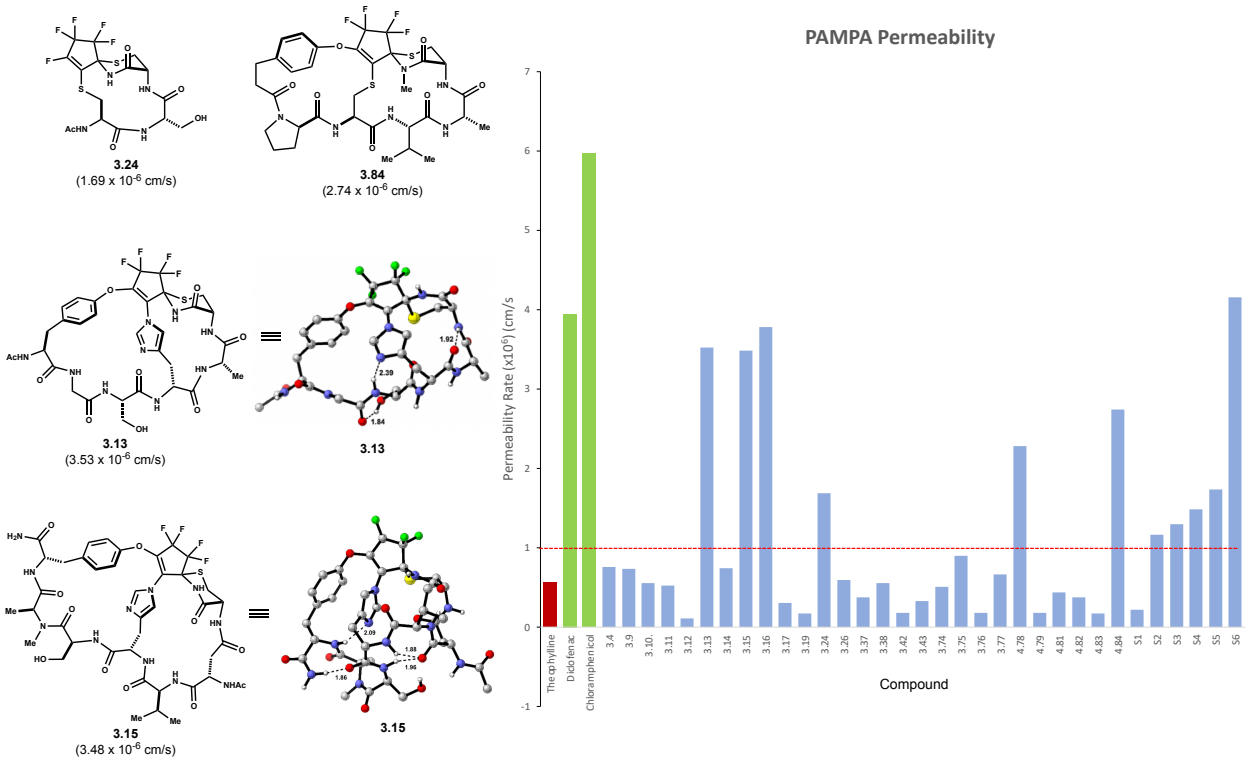


Fig. 3.16. Evaluation of passive membrane permeability in vitro using PAMPA. Bar graph shows averaged data from two independent experiments, each performed in technical replicate in a 96-well format (UV-vis detection, see SI for details). Theophylline, diclofenac and chloramphenicol are low, medium and high permeability standards, respectively. See SI for a complete listing of molecules tested. PAMPA analyses of linear peptide precursors was complicated by their insolubility in assay buffer. Calculated low energy conformers of **3.13** and

3.15 rendered using CylView® and annotated with intramolecular hydrogen bonds (bond lengths in angstroms) identified by Schrodinger Maestro Macromodel® (see SI for details).

analyzed in parallel artificial membrane permeability assays. Averaged data is plotted in **Fig. 3.16**. Nearly 30% of the molecules tested showed $P_{app} > 1.0 \times 10^{-6}$cm/sec, a 'significant' permeability benchmark recently advocated by Baker et al. in studies of computationally designed peptidyl macrocycles.[32] Notably, P_{app} measured for several compounds approached that of diclofenac and chloramphenicol – low molecular weight, orally bioavailable drugs used as permeability standards. Both mono and bimacrocyclic structures were among the most permeable (see **Fig. 3.16**). In general, macrobicyclic products from **Fig. 3.14** show greater passive membrane permeability than their seco precursors. Imidazole bridged macrobicycle **3.13** has mass = 804 Da, 8 H-bond donors and a calculated tPSA = 249 A^2, yet it is one of the most permeable compounds tested. Calculated low energy conformations of **3.13** (free base) in a low dielectric field appear to be stabilized by transannular hydrogen bonding in both large rings (shown in **Fig. 3.16**). Low energy conformers of doubled looped structure **3.15** are also calculated to be structured by internal hydrogen bonding, involving both the bridging imidazole and peripheral functional groups. 'Chameleonic' conformational behavior that can transiently shield polar surface area is thought a contributing factor for passive permeability observed in other 'beyond rule of 5' macrocycles.[33] However, experimental studies on larger compound sets will be needed to define parameters, including internal hydrogen bonding and peripheral fluorination, most correlated with passive membrane permeability in these systems.

For future PAMPA experiments we are currently synthesizing a library of compounds to establish a structure activity relationship between our fluorinated chemotypes and observed passive membrane permeability. The first strategy that we employ is derivatization of spirocyclic vinyl fluorides like **3.24** (**Fig. 3.17**). We took compound **3.24** (1.69 x 10^{-6} cm/s) possessing good passive membrane permeability and compound **3.17** (3.10 x 10^{-7} cm/s) with poor passive membrane permeability to see if potential trends would be observed for both derivative types. Although initially synthesized as a control experiment to see if we could couple secondary amines onto vinyl fluorides; we readily prepared tertiary enamine product **3.85** (40%) derived from N-methylbenzylamine. The tertiary enamine presents a potential hydrogen atom bond acceptor site to further shield polar surface area. We suspect heteroatoms participating in transannular hydrogen bonding is a key pharmacophore interaction and as such we prepared compounds incorporating both mercaptopyridine (1 hydrogen bond acceptor) and mercaptopyrimidine (2 hydrogen bond acceptors) moieties, respectively for products **3.88**, **3.89**, **3.91**. Initially we were interested to

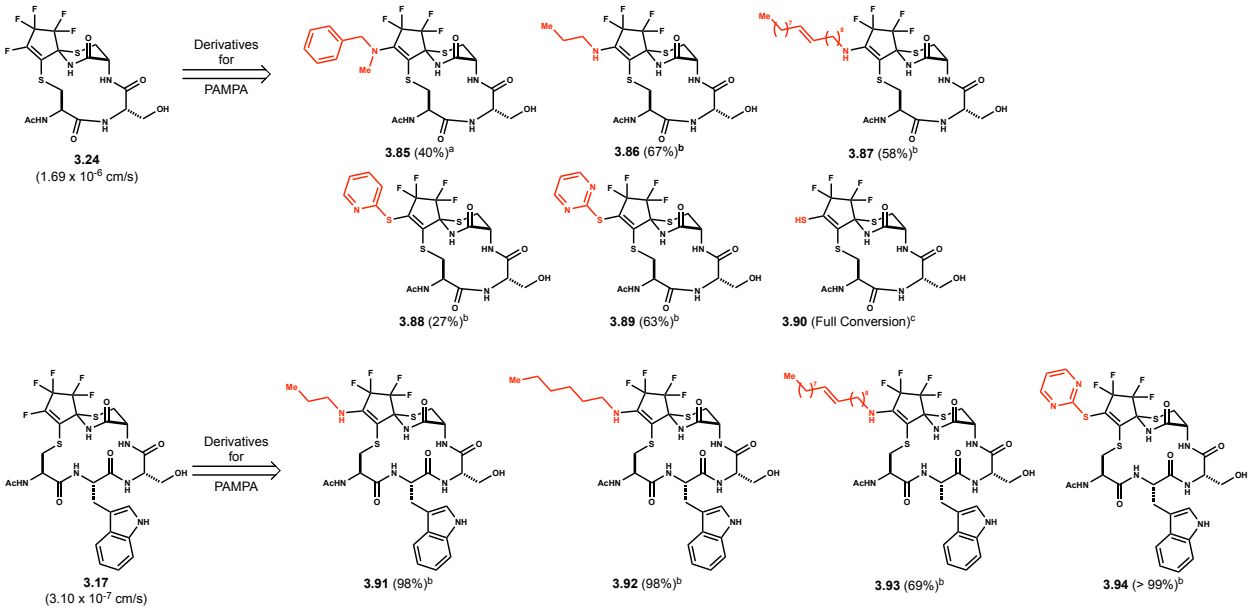


Fig. 3.17. Derivatization of spirocyclic vinyl fluorides for future in vitro PAMPA Experiments. a) BnNHMe (1.1 equiv), Cs_2CO_3 (1.5 equiv), DMF (0.05 M), 23 ºC, 16 h. b) 1º amine or mercaptan (1.1 equiv), Cs_2CO_3 (1.5 equiv), DMF (0.05 M), 23 ºC, 30 min.

couple lipid thioacetates onto our vinyl fluorides such as those derived from oleic acid, but products isolated from these couplings were unstable and completely converted to thioenols; product **3.90** being an example. Furthermore, we plan to incrementally probe lipophilicity in our structures via couplings of increasingly greasy amines derived from propyl amine, hexylamine, and oleyl amine (**3.86**, **3.87**, **3.91**, **3.92**, and **3.93**).

In addition to macrocycles, in future PAMPA assays we plan to establish an SAR trend for our macrobicyclic chemotypes. Double-looped structures possessing high passive permeabilities previously shown in **Fig. 3.16** tend to have >8 bond donors and masses approaching 1000 Da. We suspect this double-looped chemotype is exceptional in predisposing compounds to transannular hydrogen bonding. Initially prepared to see if could form macrolactam products with diamine inserts; double-looped product **3.95** was readily prepared that introduces both an extra hydrogen bond donor and a hydrogen bond acceptor relative to parent structure **3.74** (see **Fig. 3.18** for select examples). Most significant is that all 'side-chain initiated cascade' products **3.13**, **3.16**, and **S5** possess the highest observed passive-membrane permeabilities for our fluorinated products. The highly constrained nature of these 'side-chain initiated' products might be a contributing factor. For example, we plan to take highest performing macrobicyclic **3.16** and probe its structure. We suspect that the bridging imidazole moiety present in highest performing structures **3.16** and **3.13** is key pharmacophore as it might serve as an extra hydrogen bond acceptor in transannular hydrogen bonding. In efforts to probe this hyothesis we plan to prepare bridging triazole derivative **3.96** as it introduces two extra sites for potential hydrogen bond acceptors that can interact on both sides of the double-looped motif. Proposed structures such as **3.96** and **3.99** bearing this triazole might possess significantly higher permeabilities. Similarly, we plan to install an imidazole moiety into well performing macrolactam **3.78** as this introduces an extra H-bond acceptor relative to the

parent structure possessing a bridging ether. Furthermore, we plan on synthesizing derivatives bearing an N-Methyl alanine residue (**3.97** and **3.100**) or incorporating small-molecule inserts that ablate H-bond donors (**3.103**). Lastly, spirocyclic epimers (**3.102**) and ablation of the N-terminus (**3.101**) will be probed in future PAMPA experiments to elucidate all key pharmacophores.

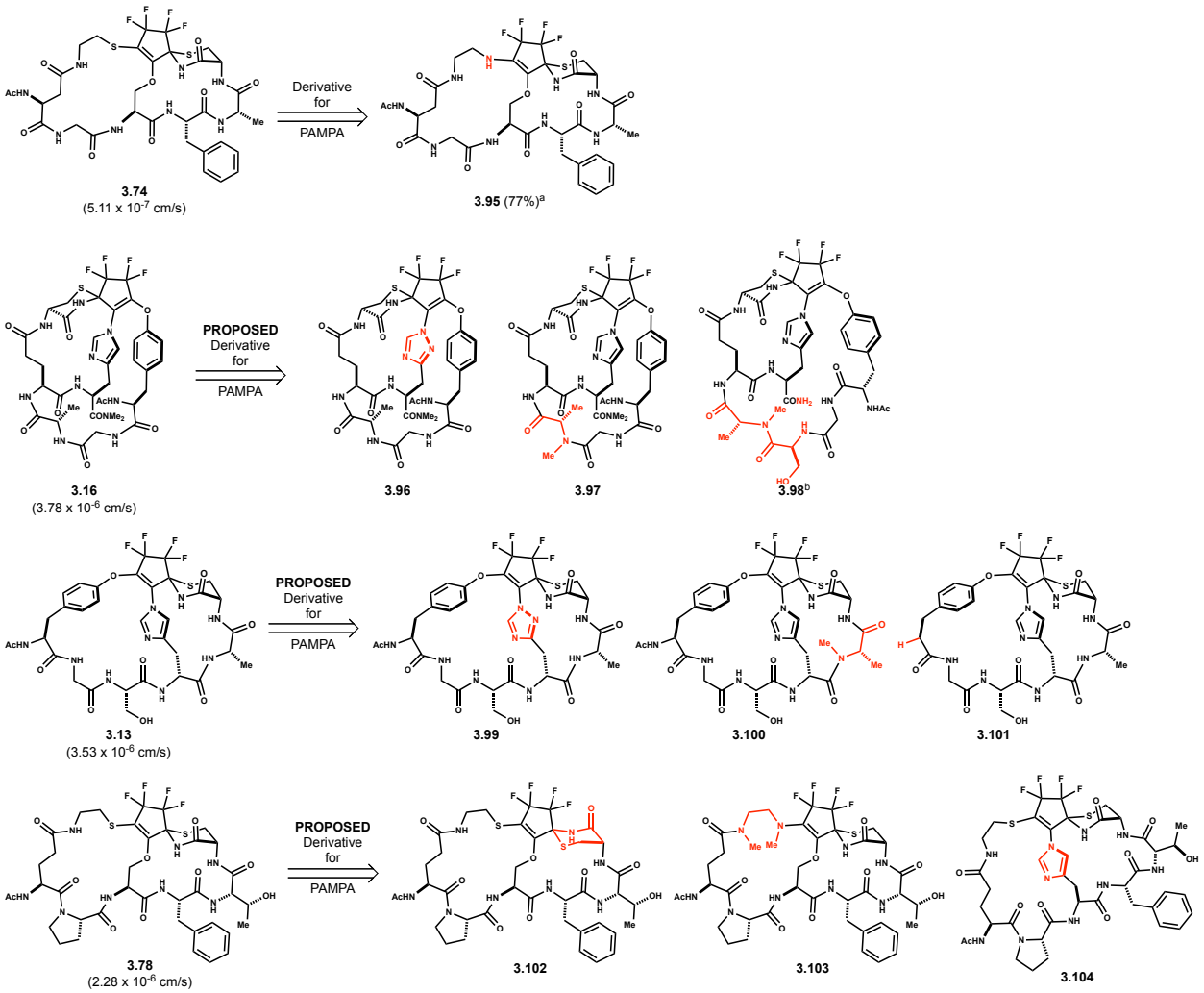


Fig. 3.18. Select Proposed Structures to Establish Structure Activity Relationship. a) propanephosphonic acid anhydride (T3P) (1.5 equiv), iPr_2NEt (3.5 equiv), DMF (5.0 mM), 0 ºC, 1 h. Yield shown reflects isolated yield from its seco-acid precursor. b) characterization of product in progress. Not prepared as a direct analog for SAR study but to obtain a higher yield for a side-chain initiated cascade product.

3.9 Future Work – Benzyne Intermediates towards Peptidic Cyclizations

We believe benzyne-type intermediates via small-molecule inserts have the potential to drastically broaden the range of participating amino acid residues (**Fig. 3.19A**). Benzyne intermediates are known to react with alcohols, phenols, carboxamides, amines, etc; functional groups that can be extrapolated to our peptidic systems. Aryl silyl triflates (Kobayashi Aryne Precursors) are the most common benzyne precursors known.[34] My colleague, Dr. Salvador Bernardino decided to initially prepare phenolic aryl silyl triflates but these compounds were unstable to prolonged storage so the aryl silyl bromide derivative (**3.105**) was prepared instead.[35] This insert reacted well to afford acyclic intermediate **3.107** but unfortunately desired

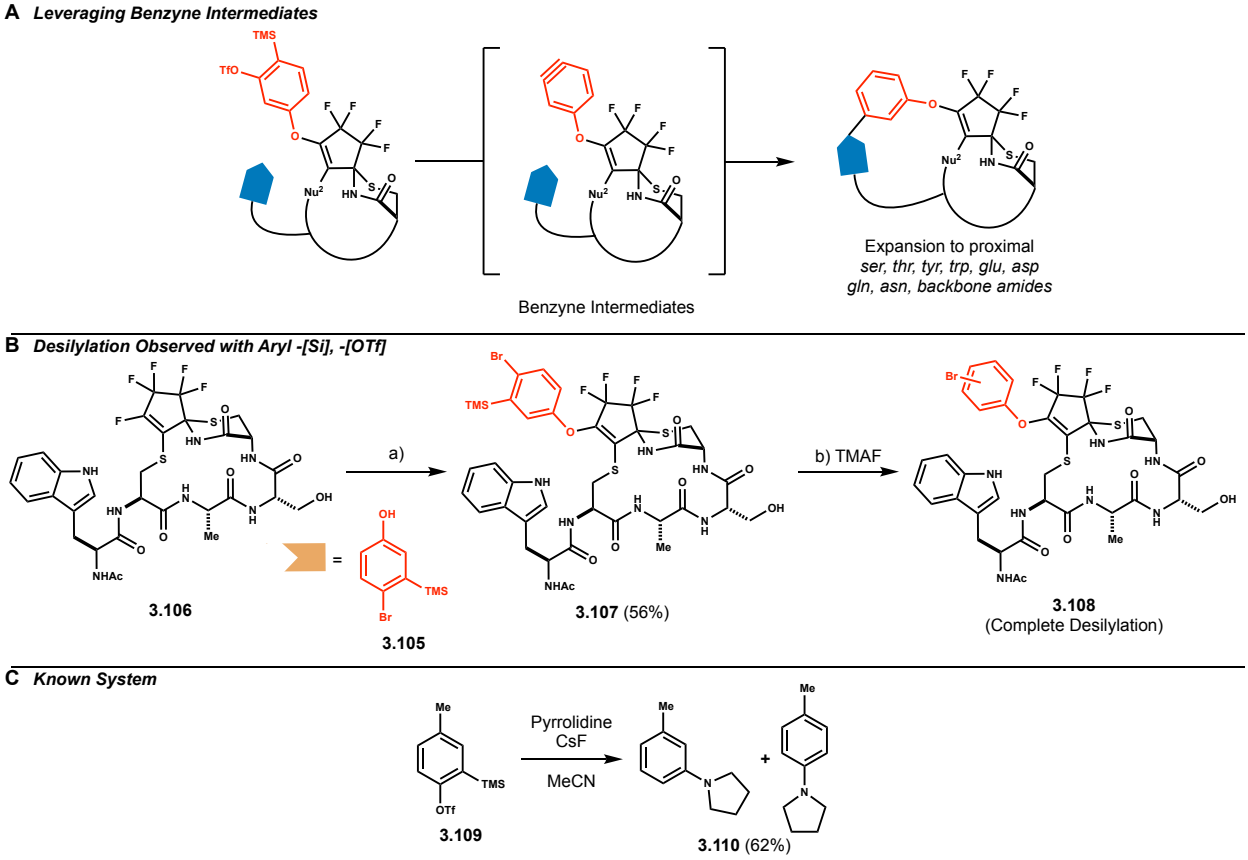


Fig. 3.19. Background – Dr. Salvador Bernardino's efforts towards Benzyne Formation via Kobayashi Aryne Precursors (*Ref 35***).** (**A**) Leveraging Benzyne Intermediates (**B**) Desiylation Observed with Aryl -[Si], -[Br] (**C**) Known System a) phenol 3.105 (2.0 equiv), KOTMS (2.0 equiv), DMF (0.05 M), 23 ºC, 24 h. b) TMAF (4.0 equiv), THF/MeCN (5.0 mM), 40 ºC, 7 h.

macrobicycle product was never observed except for undesired desilylation product **3.108**. Known system **3.109** readily reacted to give products **3.110**. We then hypothesized that electron-richness of the π-system was perturbing benzyne intermediates. We then explored other strategies to form benzynes on our fluorinate structures.

With our lack of successful results with Kobayashi aryne precursors (**Fig. 3.19**) we were then interested to see if we could develop anthranilic acids as these reagents are known to decompose in the presence of nitrites into benzynes via the extrusion of N_2 and CO_2 gas (**Fig. 3.20A**).[36] We synthesized novel anthranilic acid insert **3.111** based on literature precedent (3 steps, see SI for details).[37] This reagent performed well to OFCP processing with no aberrant reactivity observed from the carboxylic acid / aniline moieties to afford acyclic intermediate **3.112** (**Fig. 3.20B**). Reacting **3.112** with amyl nitrite and heat caused the reaction mixture to bubble vigorously, associated with the extrusion of gas and generation of benzyne. Unfortunately, desired product **3.113** was not observed except for complete conversion of **3.112** into intractable products. To the best of our knowledge, anthranilic acids are not known to react with simple nucleophiles like alcohols / phenols but react exclusively in cycloadditions. In probing cycloaddition reactivity, we prepared model **3.114** derived from **2.5** in quantitative yield (**Fig. 3.20C**; < 99%). Anthranilic acids when decomposed into benzynes are known to react with CS_2 via a [3+2] cycloaddition to form a carbene intermediate.[38] **3.114** was decomposed in this manner to form a carbene that inserted into BnOH to afford **3.115** in complete conversion. Thio-orthoester **3.115** was not stable to SiO_2 purification and introduced a racemic carbon site. It was not an interest to further probe anthranilic acid derived cycloadditions in our peptidyl systems as we believed it would not be general to simple amino acid residues. Furthermore, carbenes derived from CS_2 would introduce

two additional sulfur atoms and install a racemic center which would result in diastereomeric product mixtures in our fluorinated peptidyl systems.

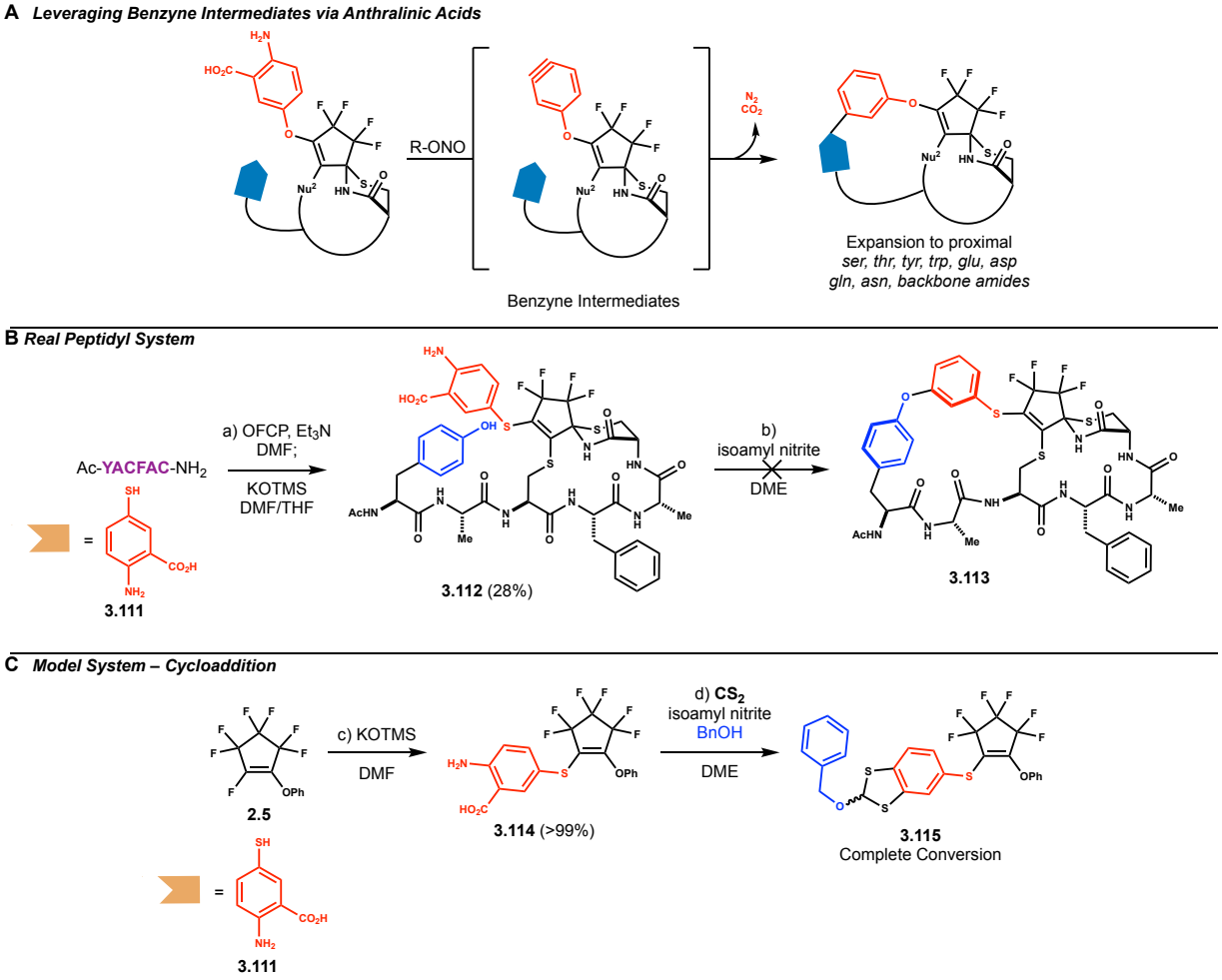


Fig. 3.20. Anthranilic Acids as Benzyne Precursors. (**A**) Leveraging Benzyne Intermediates via Anthranilic Acids (**B**) Real Peptidyl System (**C**) Model System – Cycloaddition a) OFCP (1.5 equiv), Et3N (2.5 equiv), DMF (10 mM), 0 °C, 30 min; conc. then $KOSiMe_3$ (3.5 equiv), mercaptan **3.111** (1.2 equiv), 1:4 DMF/THF (30 mM), 0 °C –> RT, 3 h. b) amyl nitrite (1.5 equiv), DME (5.0 mM), 70 ºC, 1 h. c) mercaptan 3.111 (1.1 equiv), KOTMS (1.5 equiv), DMF (0.05 M), 23 ºC, 1 h. d) CS_2 (8.0 equiv), isoamyl nitrite (1.5 equiv), BnOH (2.0 equiv), DME (0.1 M), 70 ºC, 1 h.

Previously, phenol aryl silyl bromide inserts such as **3.105** yielded predominantly desilylated products and phenol aryl silyl triflates were unstable to storage.[35] We hypothesized this aberrant reactivity stemmed from the electron-rich π-system via the phenyl ether moiety. Previously we demonstrated we could readily couple secondary amines onto our spirocyclic vinyl fluorides with the isolation of tertiary enamine **3.85** (**Fig. 3.17**). In an effort to probe this hypothesis we synthesized novel secondary amine Kobayashi aryne precursor inserts such as **3.116** and **3.117**

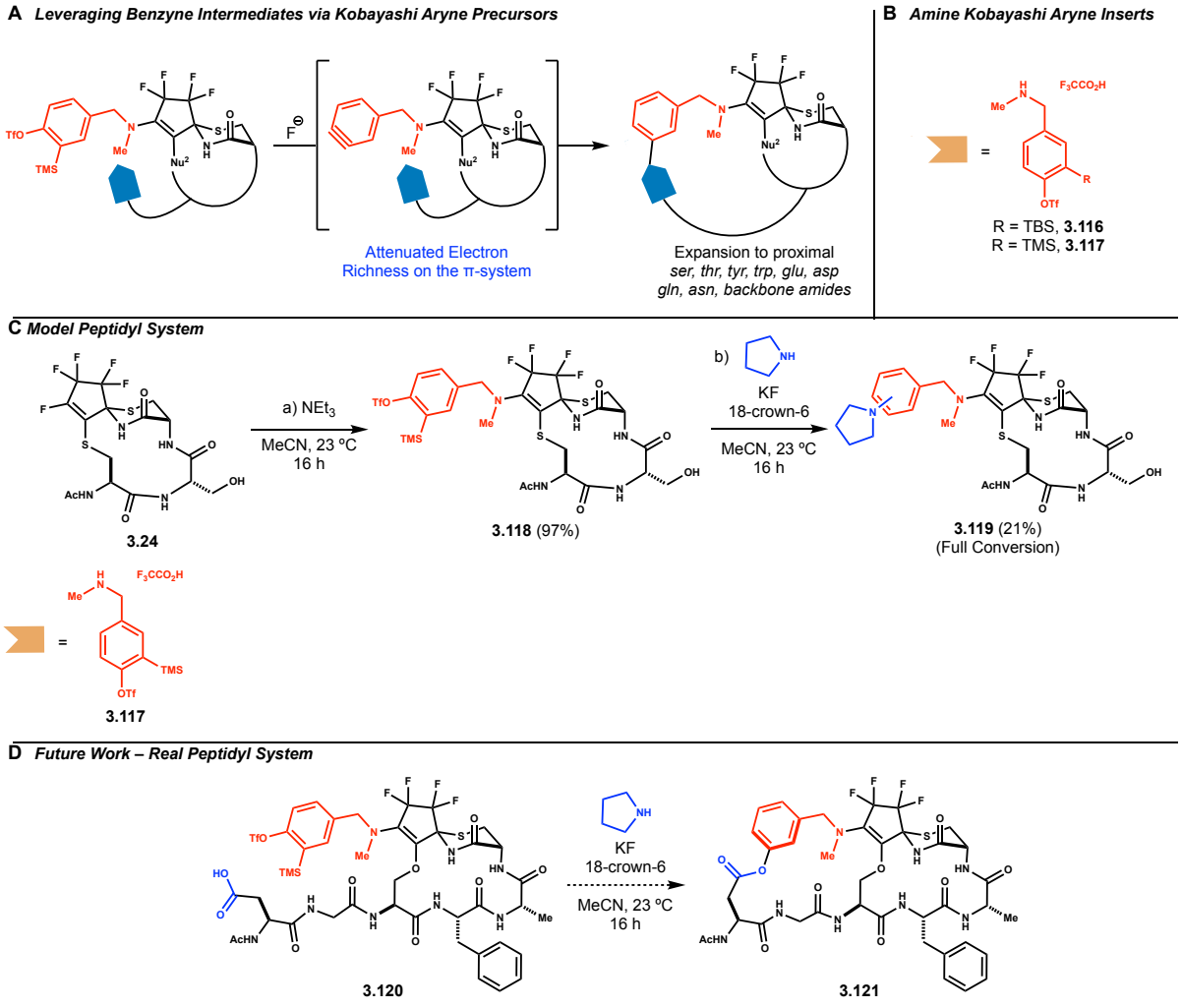


Fig. 3.21. Amine Inserts as Kobayashi Aryne Precursors. (**A**) Leveraging Benzyne Intermediates via Kobayashi Aryne Precursors (**B**) Amine Kobayashi Aryne Inserts (**C**) Model Peptidyl System (**D**) Future Work – Real Peptidyl System a) 2º amine **3.117** (1.3 equiv), NEt_3 (5.0 equiv), MeCN (0.05 M), 23 ºC, 16 h. b) pyrrolidine (2.0 equiv), KF (2.0 equiv), 18-crown-6 ether (2.0 equiv), MeCN (0.05 M), 23 ºC, 16 h.

(**Fig. 3.21A**–**B**; each in 4 steps, see SI for details) where an sp^3 methylene unit was situated between the π-system and the nitrogen heteroatom. Relative to phenol **3.105**, amines **3.116** and **3.117** are remarkably stable to prolonged storage.

We prepared secondary amine **3.117** following the scheme outlined in **Fig. 3.22** after much optimization. Commercially available 3-bromo-4-hydroxybenzaldehyde (**3.122**) was protected to afford crude acetal product **3.123** in assumed quantitative yield. Crude **3.123** was then subjected to a Brooke rearrangement followed by deprotection of the acetal moiety by treatment with excess 1M HCl to afford desired aryl silyl product **3.124** (30% yield). **3.124** was then treated with N-phenyl-bistriflimide and base to afford the desired Kobayashi product **3.125** in good yield. Treatment of **3.124** with Tf_2O resulted in low yield of **3.125** accompanied with undesired decomposition products that made purification difficult. **3.125** was then subjected to a reductive-amination with $MeNH_2$•HCl. Surprisingly, treatment of the imine intermediate with a slight excess of $NaBH_4$ did not cause reduction of the aryl triflate moiety. This crude amine product was then purified via preparative reverse-phase HPLC to afford to afford **3.117** (37% yield) as a TFA salt

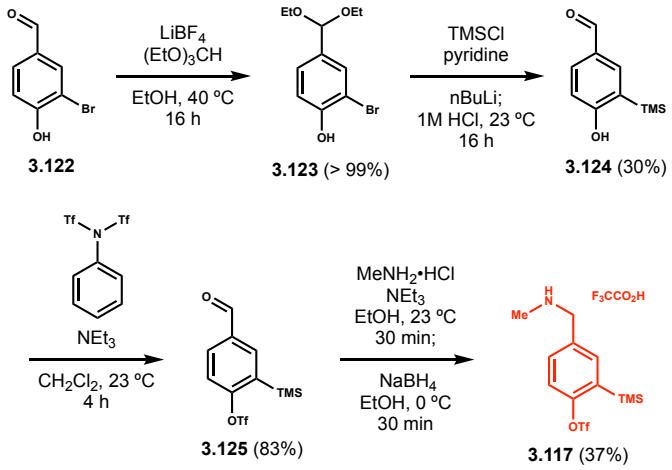


Fig. 3.22. Synthesis of Amine Insert as an Aryne Precursors. For experimental procedures – see SI for details.

and as a white-powder. TBS derivative **3.116** was synthesized following schemes outlined above (**Fig. 3.22** – see SI for details).

Recently, we coupled aryl silyl triflates **3.116** and **3.117** onto model spirocyclic vinyl fluoride **3.24** to afford **3.118** in good yield (97%). Secondary amine **3.116** bearing an aryl TBS coupled very slowly (unoptimized) and its respective product was not isolated (**Fig. 3.21C**). Model substrate **3.118** was then subjected to a fluoride source / general conditions for aryne formation in the presence of pyrrolidine to afford product **3.119**. Product **3.119** was isolated as a mixture of regioisomers that could not be separated on SiO_2. Notably, **3.119** marks the first instance of our lab generating a benzyne intermediate in our fluorinated peptidyl system after 2 years worth of efforts. In the near future we plan to probe benzynes on real peptidyl systems (**Fig. 3.21D**), specifically we will prepare acyclic intermediate **3.120** bearing a proximal carboxylic acid. **3.120** will be exposed to a fluoride source to see if the proximal carboxylate will react with benzyne to give product **3.121**. According to literature, reaction of aliphatic carboxylates with benzynes is limited.[34] If we can readily access **3.121**, this will have profound promise in us being able to capture benzynes with simple amino acid residues such as serine, threonine, tyrosine, tryptophan, methionine, aspartate, asparagine, glutamine, backbone amides, etc.

3.10 Conclusions

In summary, relative rate controlled polysubstitution cascades using perflurorocycloalkenes is new methodology to rapidly generate complex fluorinated composite macrocycles. OFCP can directly polycyclize linear peptide sequences using native functionality, or fluorospiroheterocyclic intermediates can be intercepted with exogenous nucleophiles. The latter tactic can generate a range of molecular hybrids composed of peptides, sugars, lipids and heterocyclic components. The platform can create both single and double looped macrocycles in multiple stereoisomeric forms. Subsets of these molecules will have low energy conformers that shield polar surface area through intramolecular hydrogen bonding, a behavior that may correlate with passive membrane permeability. Ongoing studies are focused on OFCP mediated polycyclizations that generate fluorinated molecular probes for chemical biology research, new methodology development strategies derived from small-molecule inserts, new classes of glycopeptide conjugates, novel fluorinated antimicrobials and molecular ‘glues’, as well as stable shape mimics of diverse loop structures mediating intracellular PPIs.[39]

3.11 References

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3.12 Chapter 3 – Experimental Appendix

1. Materials and Methods

Reagents were purchased from commercial vendors and used as received unless otherwise stated. Tetrahydrofuran (THF), diethyl ether (Et_2O), acetonitrile (MeCN) and toluene (PhMe) were passed through a Glass Contour solvent drying system. Anhydrous N,N dimethylformamide (DMF) was purchased from Supelco, Inc – Sigma Aldrich. All reactions were run under an argon atmosphere using anhydrous solvents unless otherwise stated. Yields refer to chromatographically and spectroscopically (^{1}H NMR) homogeneous materials, unless otherwise stated. Thin-layer chromatography (TLC) was conducted on precoated plates (Sorbent Technologies, silica gel 60 PF254, 0.25 mm) visualized with UV 254 nm. Column chromatography was performed on silica gel 60 (SiliCycle, 240−400 mesh). Purification of peptides was performed using an Agilent 1200 HPLC system equipped with G1361A preparative pumps, a G1314A auto sampler, a G1314A VWD, a G1364B automated fraction collector, and a Waters Sunfire C18 column (5 μm, 19 mm × 250 mm), unless otherwise noted. 0.1% TFA in MeCN/H_2O solvent system. Analytical HPLC was performed using the same system, but with a G1312A binary pump. 0.1% TFA in MeCN/H_2O solvent system. Mass spectra were recorded using an Agilent 6130 LC/MS system equipped with an ESI source. High-resolution mass spectra were recorded on Thermo Scientific Exactive® Mass Spectrometer with DART ID-CUBE Waters GST Premier, Waters LCT Premier, and Agilent 6545 LC-QTOF. NMR spectra were recorded on Bruker Avance spectrometers (400/100 MHz, 500/125 MHz, and 600/150 MHz). NMR spectra were recorded on Bruker Advance (300, 400, 500, or 600 MHz) spectrometers. HSQC, HMBC, and COSY NMR experiments were used to aid assignment of NMR peaks when required. All ^{19}F NMR spectrums used $CFCl_3$ as a calibration standard.

2. Experimental Procedures

2.1 General Experimental Procedures

Peptide Synthesis for primary C-terminal carboxamides:

C-terminal carboxamide peptides were synthesized manually using standard Fmoc solid phase synthesis protocols on Rink Amide MBHA resin (200-400 mesh, 0.73 mmol/g, 1% DVB) on 0.37–0.50 mmol scale using a fritted glass reaction vessel. Fmoc-deprotection was achieved with 2%/5% DBU/piperazine in DMF (1 x 15 min). The reaction vessel was washed with DMF (2 x), CH_2Cl_2 (2 x), and DMF (2 x). The vessel was then charged with the appropriate Fmoc-amino acid (3.0 equiv.) and HBTU (3.0 equiv.) followed by DMF (10–20 mL) and iPr_2NEt (10.0 equiv.). The resin was shaken for 45 minutes, drained, and washed with DMF (2 x), CH_2Cl_2 (2 x), and DMF (2 x). Couplings involving secondary amines such as Pro or Asp*/Glu* were left to shake for 90 minutes. After all couplings were completed, the resin was cleaved by shaking with a solution of 90:5:5 TFA/H_2O/TIPS (27 mL of TFA, 1.5 mL of H_2O and TIPS) for 90 minutes. The cleaved resin was filtered and rinsed with Et_2O until ~200 mL of volume. The peptide was then triturated, and this process repeated with Et_2O (2 x) to afford a crude peptide pellet. This peptide pellet was then stripped with anhydrous PhMe (3 x) to afford the desired linear peptide.

Peptide synthesis for secondary C-terminal carboxamides:

C-terminal carboxamide peptides were synthesized manually using standard Fmoc solid phase synthesis protocols on 2-chlorotrityl chloride resin (100–200 mesh, 1.02 mmol/g, 1% DVB) on 1.02 mmol scale using a fritted glass reaction vessel. Fmoc-deprotection was achieved with 2%/5% DBU/piperazine in DMF (1 x 15 min). The reaction vessel was washed with DMF (2 x), CH_2Cl_2 (2 x), and DMF (2 x). The vessel was then charged with the appropriate Fmoc-amino acid (3.0

equiv.) and HBTU (3.0 equiv.) followed by DMF (10–20 mL) and iPr_2NEt (10.0 equiv.). The resin was shaken for 45 minutes, drained, and washed with DMF (2 x), CH_2Cl_2 (2 x), and DMF (2 x). Couplings involving secondary amines such as Pro or Asp*/Glu* were left to shake for 90 minutes. After all couplings were completed, the resin was cleaved by shaking with a solution of 1:99 TFA:CH_2Cl_2 (3 x 33 mL, total of 100 mL). Filtered onto a solution filled with 1:9 pyridine:MeOH (100 mL). Concentration under reduced pressure afforded desired crude peptide. This crude peptide was then capped with $MeNH_2$. C-terminus capping was achieved following general EDC•HCl/HOBt coupling protocol. After capping, the crude peptide was purified via preparative HPLC.

Fig. 3.2: General Procedure A: Direct Macrobicyclization

To a flame-dried round bottom flask equipped with a stir bar was charged with linear peptide (1.0 equiv.), diluted with anhydrous DMF (5.0 mM), and then allowed to stir at 0 ºC. Then OFCP (1.5 equiv., 1.0 M in MeCN) was added followed by NEt_3 (2.5 equiv.). The reaction mixture was allowed to stir at 0 ºC for 30 min. When HPLC indicated macrocycle intermediate formation, the reaction mixture was concentrated under reduced pressure to dryness. The reaction residue was then diluted with 1:4 DMF/THF (5.0 mM) and stirred at 0 ºC. Then Cs_2CO_3 (6.0 equiv.) was added, and the reaction mixture allowed to gradually warm up to 23 ºC for 3 h. When HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (10.0 equiv.) and then concentrated under reduced pressure to afford crude product. The crude product was then purified via preparative HPLC – see details for specific example below.

Fig. 3.2: General Procedure B: Direct Macrobicyclization

To a flame-dried round bottom flask equipped with a stir bar was charged with linear peptide (1.0 equiv), diluted with anhydrous DMF (5.0 mM), and then allowed to stir at 0 ºC. Then OFCP (1.5 equiv, 1.0 M in MeCN) was added followed by NEt_3 (2.5 equiv.). The reaction mixture was allowed to stir at 0 ºC for 30 min. When HPLC indicated macrocycle intermediate formation, the reaction mixture was concentrated under reduced pressure to dryness. The reaction residue was then diluted with 1:4 DMF/THF (5.0 mM) and stirred at 0 ºC. Then KOTMS (4.0 equiv.) was added, and the reaction mixture allowed to gradually warm up to 23 ºC for 3 h. When HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (10.0 equiv.) and then concentrated under reduced pressure to afford crude product. The crude product was then purified via preparative HPLC – see details for specific example below.

Fig. 3.2: General Procedure C: Direct Macrobicyclization

To a flame-dried round bottom flask equipped with a stir bar was charged with linear peptide (1.0 equiv), diluted with anhydrous DMF (5.0 mM), and then allowed to stir at 0 ºC. Then OFCP (1.5 equiv, 1.0 M in MeCN) was added followed by NEt_3 (2.5 equiv.). The reaction mixture was allowed to stir at 0 ºC for 30 min. When HPLC indicated macrocycle intermediate formation, the reaction mixture was concentrated under reduced pressure to dryness. The reaction residue was then diluted with DMF (5.0 mM) and stirred at 0 ºC. Then KOTMS (4.0 equiv.) was added, and the reaction mixture allowed to gradually warm up to 23 ºC for 3 h. When HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (10.0 equiv.) and then concentrated

under reduced pressure to afford crude product. The crude product was then purified via preparative HPLC – see details for specific example below.

Fig. 3.3: General Procedure A:

To a flame-dried round bottom flask equipped with a stir bar was charged with linear peptide (1.0 equiv.), diluted with anhydrous DMF (5.0 mM), and then allowed to stir at 0 ºC. Then OFCP (1.5 equiv., 1.0 M in MeCN) was added followed by NEt_3 (2.5 equiv.). The reaction mixture was allowed to stir at 0 ºC for 30 min. When HPLC indicated macrocycle intermediate formation, the reaction mixture was concentrated under reduced pressure to dryness. The reaction residue was then diluted with 1:4 DMF/THF (5.0 mM) and stirred at 0 ºC. Then KOTMS (2.0 equiv.) was added, and the reaction mixture allowed to gradually warm up to 23 ºC for 1 h. When HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (10.0 equiv.) and then concentrated under reduced pressure to afford crude product. The crude product was then purified via preparative HPLC – see details for specific example below.

Fig. 3.14: General Procedure F: Macrobicyclization via bond formations with Asp and Glu

To a flame-dried round bottom flask equipped with a stir bar was charged with linear peptide (1.0 equiv), diluted with anhydrous DMF (10.0 mM), and then allowed to stir at 0 ºC. Then OFCP (1.5 equiv, 1.0 M in MeCN) was added followed by NEt_3 (2.5 equiv.). The reaction mixture was allowed to stir at 0ºC for 30 min. When HPLC indicated macrocycle intermediate formation, the reaction mixture was concentrated under reduced pressure to dryness. The reaction residue was then added 1:4 DMF:THF (30 mM) and cooled to 0 ºC. Then Cs_2CO_3 (5.0 equiv.) was added, and the reaction mixture allowed to stir at 0 ºC for 1 h. When HPLC indicated full conversion to the

desired vinyl fluoride then cysteamine, 2-mercaptoethanol, or **3.29** were added and the reaction mixture allowed to stir at 0 ºC for 1 h. When HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (10.0 equiv.) and then concentrated under reduced pressure to afford crude product. The crude product was then telescoped to the next step.

Fig. 3.14: General Procedure G: Macrobicyclization via bond formations with Asp and Glu

Assumed a 50% yield from General Procedure f when calculating stoichiometry. To the same round bottom flask used for General Procedure f, the crude product was diluted with a solution 2:8 MeCN:aqueous buffer pH 4.5 (NaOAc/AcOH, 0.1 M). The reaction mixture was then allowed to stir at 23 ºC and then was added NCS in MeCN (1.0 M) portionwise (0.5 equiv.). Total NCS varied from 1.5–3.0 equiv. depending on substrate. When HPLC indicated full conversion to the desired free acid, the reaction mixture was quenched with $Na_2S_2O_3 \bullet 5H_2O$ (6.0 equiv.). The reaction mixture was concentrated under reduced pressure to afford crude product. The crude product was then purified via preparative HPLC – see details for specific example below. Yields shown refer to yields from their linear peptides.

Fig. 3.14: General Procedure H: Macrobicyclization via bond formations with Asp and Glu

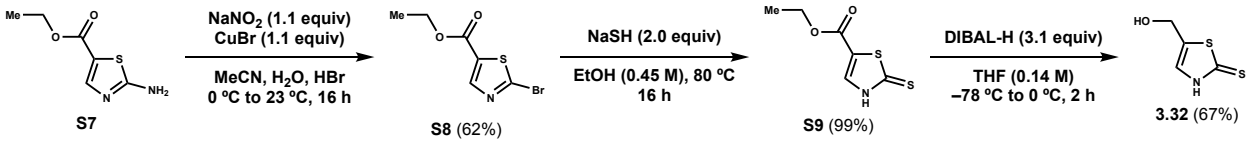
To a flame-dried round bottom flask equipped with a stir bar was charged with the incipient amino acid (1.0 equiv.) and diluted with anhydrous DMF (5.0 mM). The solution was added iPr_2NEt (3.5 equiv.) and the reaction mixture allowed to cool to 0 ºC. Then propanephosphonic acid anhydride in EtOAc (1.5 equiv., 50% wt.) was added and the reaction mixture allowed to stir at 0 ºC for 1 h. After HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (5

equiv.) and then concentrated under reduced pressure to afford crude product. The crude product was then purified via preparative HPLC – see details for specific example below.

Fig. 3.14: General Procedure I: Macrobicyclization via bond formations with Asp and Glu

To a flame-dried round bottom flask equipped with a stir bar was charged with the incipient seco acid (1.0 equiv.), EDC•HCl (2.5 equiv.), HOBt (1.5 equiv.), and then diluted with DMF (5.0 mM). The reaction mixture was then allowed to stir at 0 ºC and then added NEt_3 (5.0 equiv.). The reaction mixture was then allowed to warm up to 23 ºC and stirred for 44–64 h. After HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (5.0 equiv.) and then concentrated under reduced pressure to afford crude product. The crude product was then purified via preparative HPLC – see details for specific example below.

2.2 Synthesis of Thiazole Thione Linker (3.32)



Scheme 1. Synthesis of thiazole thione **3.32**

Ethyl 2-bromothiazole-5-carboxylate (**S8)**:

To a flame-dried 500 mL round bottom flask equipped with a stir bar was charged with **S7** (10.00 g, 56.91 mmol, 1.0 equiv.), $NaNO_2$ (4.32 g, 62.60 mmol, 1.1 equiv.), diluted with MeCN (58.68 mL, 0.97 M), H_2O (30.76 mL, 1.85 M), and the reaction mixture allowed to cool to 0 ºC. Then CuBr (8.98 g, 62.60 mmol, 1.1 equiv.) was added portion-wise followed by dropwise addition of HBr (46.27 mL, 1.23 M) at 0 ºC. The reaction mixture was then allowed to warm up to 23 ºC and stirred for 16 h. After 16 h, the reaction mixture was diluted with ice water, CH_2Cl_2, and transferred to an Erlenmeyer flask. The solution was kept cool and gradually quenched with solid $NaHCO_3$ and then transferred to a separatory funnel. The aqueous layer was extracted with CH_2Cl_2 (3 x), organic layers combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated to afford crude product. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 2.5% EtOAc/Hex) afforded title compound as a faint-yellow oil (8.26 g, 62% isolated yield), R_f = 0.28 at 5% EtOAc/Hex. ^{1}H NMR (DMSO-d_6, 500 MHz) δ8.31 (s, 1H), 4.32 (q, 2H), 1.29 (t, 3H) ^{13}C-NMR (500 MHz, DMSO-d_6) δ 159.47, 148.17, 142.19, 132.75, 61.94, 14.04 HRMS m/z calc'd for $[C_6H_6BrNO_2S+H]^+$ 236.9375; found 236.9375; 0 ppm mass defect.

Ethyl 2-thioxo-2,3-dihydrothiazole-5-carboxylate (**S9)**:

To a 100-mL round bottom flask equipped with a stir bar and a reflux condenser was charged with **S8** (2.86 g, 12.11 mmol, 1.0 equiv,), NaSH (2.26 g, 24.23 mmol, 2.0 equiv., 60% wt.), and then

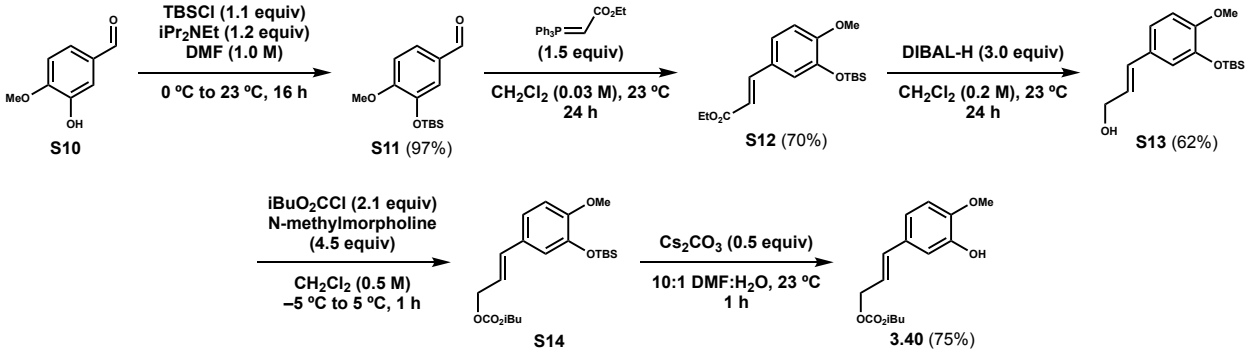
diluted with anhydrous EtOH (26.92 mL, 0.45 M). The reaction mixture was then heated to 80 ºC and stirred for 16 h. After 16 h, the reaction mixture was allowed to cool to 23 ºC and then poured onto an iced solution of aqueous 1 N HCl. The mixture was then transferred to a separatory funnel and the aqueous layer extracted with CH_2Cl_2 (4 x). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated to afford title compound as a white-solid (2.26 g, 99% isolated yield) as a white-solid. ^{1}H-NMR (500 MHz, DMSO-d_6) δ 13.80 (s, 1H), 8.08 (s, 1H), 4.24 (q, 2H), 1.25 (t, 3H). ^{13}C-NMR (500 MHz, DMSO-d_6) δ 190.93, 158.87, 136.83, 118.59, 61.43, 14.08. HRMS m/z calc'd for $[C_6H_7NO_2S_2+H]^+$ 188.991823; found 188.991208; 3.3 ppm mass defect.

5-(hydroxymethyl)thiazole-2(3H)-thione **(3.32)**:

To a flame-dried 250-mL round bottom flask equipped with a stir bar was charged with **S9** (2.26 g, 11.9 mmol, 1.0 equiv.) and diluted with anhydrous THF (85.3 mL, 0.14 M). The reaction solution was then allowed to cool down to –78 ºC and stirred. Then at –78 ºC was added dropwise DIBAL-H (37.0 mL, 37.0 mmol, 3.1 equiv., 1.0 M in hexanes). The reaction mixture was then allowed to stir at –78 ºC for 30 min. Then after 30 min, the reaction mixture was allowed to warm up to 0 ºC and stirred for 1.5 h. After 1.5 h, the reaction mixture was cooled to 0 ºC and was added dropwise 1 N HCl and the mixture stirred. The reaction mixture was then transferred to a separatory funnel and the aqueous layer extracted with EtOAc (4 x). The organic layers were combined (NOT washed with brine), dried over Na_2SO_4, filtered, and concentrated to afford crude product as a yellow-powder. The crude product was purified via flash SiO_2 chromatography (gradient 35% EtOAc/Hex → 75% EtOAc/Hex) afforded title compound as a white-powder (1.18 g, 67% isolated yield). ^{1}H-NMR (500 MHz, DMSO-d_6) δ 12.97 (s, 1H), 7.16 (s, 1H), 5.46 (s, 1H),

4.37 (s, 2H) ^{13}C-NMR (126 MHz, DMSO-d_6) δ 188.59, 132.59, 125.19, 55.88. HRMS [M+1] calc'd for $C_4H_5NOS_2H$ 147.9891, found 147.9894.

2.3 Synthesis of Cinnamyl Phenol (3.40)



Scheme 2. Synthesis of cinnamyl phenol **3.40**

3-((tert-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde **(S11)**:

To a flame-dried round bottom flask equipped with a stir bar was charged with 3-hydroxy-4-methoxybenzaldehyde (10.00 g, 65.77 mmol, 1.0 equiv., **S10**) and diluted with anhydrous DMF (6.58 mL, 1.0 M) and added iPr_2NEt (13.7 mL, 78.92 mmol, 1.2 equiv.). The reaction was cooled to 0 °C and added TBSCl (10.86 g, 72.3 mmol, 1.1 equiv.) slowly then stirred for 16 h at 23 ºC. The reaction was diluted with H2O then extracted with EtOAc (3 x). The combined organic phases were washed with brine (3 x), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford crude product. The residue was purified by flash SiO_2 column chromatography (gradient Hex → 15% EtOAc/Hexanes) to give **S11** (16.98 g, 97%) as a clear oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ9.81 (s, 1H), 7.47 (dd, J – 8.3, 2.0 Hz, 1H), 7.36 (d, J = 2.0 Hz, 1H), 6.94 (d, J = 8.3 Hz, 1H), 3.88 (s, 3H), 1.00 (s, 9H), 0.16 (s, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ191.0, 156.7, 145.7, 130.3, 126.4, 120.1, 111.3, 55.6, 25.7, 18.5, -4.6 HRMS m/z calc'd for $[C_{14}H_{22}O_3Si+H]^+$ 267.1416; found 267.1424; 3.0 ppm mass defect.

ethyl (E)-3-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)acrylate **(S12)**:

To flame-dried round bottom flask equipped with a stir bar was charged with **S11** (10.00 g, 37.58 mmol, 1.0 equiv.) and diluted with anhydrous CH_2Cl_2 (1252 mL, 0.03 M). Then to this stirred solution was added ethyl 2-(triphenyl-l5- phosphaneylidene)acetate (19.62 g, 56.37 mmol, 1.5 equiv.) at 23 ºC under inert atmosphere. The reaction mixture was stirred at 23 ºC for 24 h. Solvent was removed under reduced pressure to afford crude product. The residue was purified by flash SiO_2 column chromatography (gradient Hex → 15% EtOAc/Hexanes) to give **S12** (8.84 g, 70% isolated yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ7.58 (d, J = 15.9 Hz, 1H), 7.09 (dd, J = 8.2, 2.1 Hz, 1H), 7.05 (d, J – 2.1 Hz, 1H), 6.83 (d, J = 8.2 Hz, 1H), 6.25 (d, J = 15.9 Hz, 1H), 4.25 (q, J = 7.12 Hz, 2H), 3.83 (s, 1H), 1.33 (t, J = 7.00 Hz, 3H), 1.00 (s, 3H), 0.16 (s, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ167.5, 153.2, 145.3, 144.6, 127.6, 123.2, 119.8, 115.9, 111.8, 60.5, 55.6, 25.8, 18.6, 14.5, -4.5. HRMS m/z calc'd for $[C_{18}H_{28}O_4Si+H]^+$ 337.1835; found 337.1836; 0.3 ppm mass defect.

(E)-3-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)prop-2-en-1-ol **(S13)**:

To a flame-dried round bottom flask equipped with a stir bar was charged with **S12** (5.00 g, 14.87 mmol, 1.0 equiv.) and diluted with anhydrous CH_2Cl_2 (74.36 mL, 0.2 M). To this stirred solution was added DIBAL-H (46.1 mL, 46.1 mmol, 3.1 equiv., 1.0 M in hexanes) dropwise at 23 ºC under inert atmosphere. The reaction mixture was stirred at 23 ºC for 24 h. After completion the reaction was concentrated under reduced pressure to afford crude product. The resulting residue was purified by flash SiO_2 column chromatography (gradient Hex → 20% EtOAc/Hex) to give **S13** (2.71 g, 62% isolated yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ6.95–6.90 (m, 2H), 6.79 (d, J = 8.9 Hz, 1H), 6.49 (d, J = 15.9 Hz, 1H), 6.20 (dt, J = 15.6, 5.9 Hz, 1H), 4.29 (dd, J = 6.1, 1.4 Hz, 2H), 3.80 (s, 1H), 1.00 (s, 9H), 0.16 (s, 6H). ^{13}C NMR ($CDCl_3$, 126 MHz) δ151.0, 145.2, 131.3, 129.9, 126.4,

120.6, 118.8, 112.0, 64.1, 55.6, 25.9, 18.6, -4.5. HRMS m/z calc'd for $[C_{16}H_{27}O_3Si+H]^+$ 295.1729; found 295.1735; 2.0 ppm mass defect.

(E)-3-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)allyl isobutyl carbonate **(S14)**:

To a flame-dried round bottom flask equipped with a stir bar was charged with **S13** (2.50 g, 1.0 equiv., 8.50 mmol), diluted with anhydrous CH_2Cl_2 (17.0 mL, 0.5 M), and N-methylmorpholine (4.21 mL, 38.25 mmol, 4.5 equiv.). The reaction mixture was then cooled to 5 ºC under argon. Isobutyl chloroformate (2.44g, 17.85 mmol, 2.1 equiv.) was added dropwise and continued to stir at -5 °C until completion. After completion as observed by TLC (1 h), the reaction was quenched with $NaHCO_3$, extracted with EtOAc (3 x), washed with $NaHCO_3$ (2 x), brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford crude product. The crude product was pushed through a plug of silica (50% EtOAc/Hex) and pushed forward without further purification.

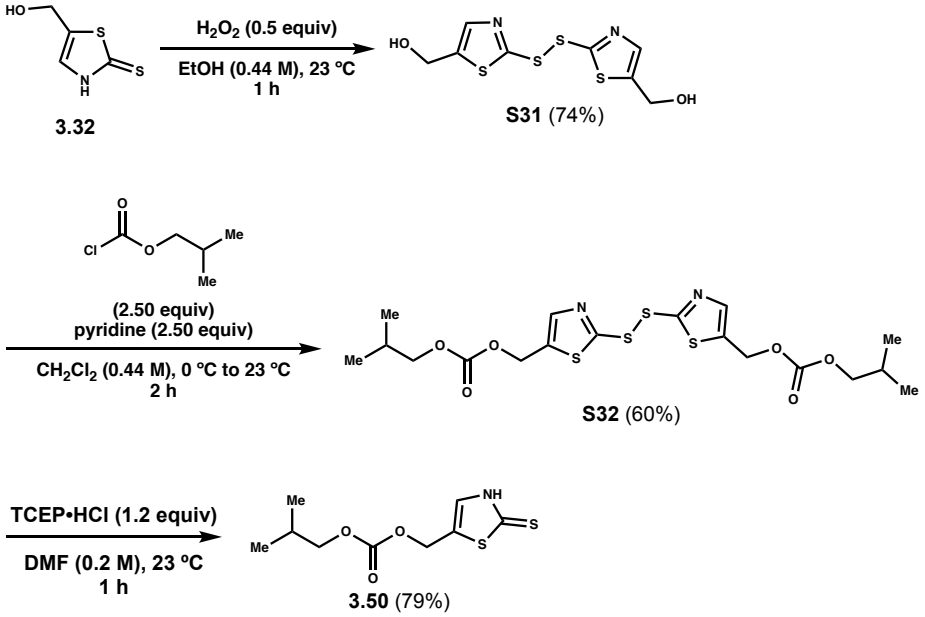
(E)-3-(3-hydroxy-4-methoxyphenyl)allyl isobutyl carbonate **(3.40)**:

To a flame-dried round bottom flask equipped with a stir bar was charged with **S14** and diluted with 10:1 DMF:H_2O and added Cs_2CO_3 (1.38 g, 4.25 mmol, 0.5 equiv.). The reaction mixture was then allowed to stir at 23 ºC until complete by TLC. The reaction mixture was then diluted with EtOAc, washed with brine (2 x), dried over Na_2SO_4, concentrated under reduced pressure to afford crude product. The crude product was purified by flash SiO_2 column chromatography (gradient Hex → 30% EtOAc/Hex) to give **33** as a clear oil (1.79 g, 75% isolated yield). ^{1}H NMR ($CDCl_3$, 500 MHz) δ7.01 (d, J = 2.0 Hz, 1H), 6.86 (dd, J = 8.3, 1.8, 1H), 6.79 (d, J = 8.4, 1H), 6.58 (d, J = 15.7 Hz, 1H), 6.15 (dt, J =15.8, 6.6 Hz, 1H), 5.62 (s, 1H), 4.75 (d, J = 6.6 Hz, 2H), 3.94 (d, J = 6.7 Hz, 2H), 3.88 (s, 1H), 1.98 (sept, J = 6.7 Hz, 1H), 0.95 (d, J = 6.7 Hz, 6H). ^{13}C NMR ($CDCl_3$, 126

MHz) δ 155.4, 146.9, 145.8, 134.7, 129.9, 120.9, 119.5, 112.3, 110.6, 74.3, 68.6, 56.1, 27.9, 19.0.

HRMS m/z calc'd for $[C_{15}H_{20}O_5+H]^+$ 281.1389; found 281.1394; 1.8 ppm mass defect.

2.4 Synthesis of Thiazole Thione Carbonate Ester Linker (3.50)



Scheme 3. Synthesis of thiazole thionee carbonate ester **3.50**

(disulfanediylbis(thiazole-2,5-diyl)dimethanol (**S31**)

To an oven-dried dram vial equipped with a stir bar was charged with **3.32** (200. mg, 1 equiv, 1.36 mmol), diluted with EtOH (3.09 mL, 0.44 M). The mixture was then added 30% H_2O_2 aqueous (69.4 µL, 30% Wt, 0.5 Eq, 679 µmol) and the mixture stirred at 23 ºC for 1 h. Once TLC indicated reaction completion, the reaction mixture was concentrated under reduced pressure to afford crude product. The crude product was purified via flash SiO_2 chromatography (80% EtOAc/Hex) afforded title compound as a yellow-solid (147.1 mg, 74% isolated yield); R_f = 0.27 at 70% EtOAc/Hex. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.62 (s, 2 H), 4.84 (s, 4H).

(disulfanediylbis(thiazole-2,5-diyl))bis(methylene) diisobutyl bis(carbonate) (**S32**)

To a flame-dried 10-mL round bottom flask equipped with a stir bar was charged with **S31** (73.5 mg, 1 equiv, 251 µmol), diluted with dry CH_2Cl_2 (1.26 mL, 0.2 M), followed by pyridine (50.8 µL, 2.5 Eq, 628 µmol). The reaction mixture was then allowed to stir at 0 ºC and then was added

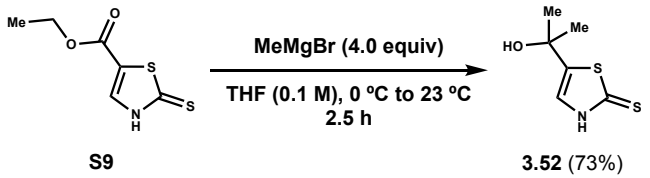
isobutyl chloroformate (87.6 mg, 83.2 µL, 98% Wt, 2.5 equiv, 628 µmol). The reaction mixture was then allowed to warm up to 23 ºC and stirred for 2 h. When TLC indicated reaction completion, the reaction mixture was quenched with H_2O and then transferred to a sep. funnel. The aqueous was extracted with CH_2Cl_2 (2 times), the organic layers were combined, washed with brine (1 x), dried over $MgSO_4$, filtered, and concentrated to afford crude product. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 15% EtOAc/Hex) afforded title compound as a white-flaky solid (74.8 mg, 60% isolated yield); R_f = 0.28 at 20% EtOAc/Hex. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.73 (s, 2 H), 5.25 (s, 4 H), 3.94 (d, J = 6.6 Hz, 4 H), (sept, J = 6.8 Hz, 2 H), 0.94 (d, J = 6.7 Hz, 12 H) ^{13}C NMR (126 MHz, $CDCl_3$) δ 167.2, 155.1, 145.1, 135.3, 74.8, 60.9, 27.9, 19.0 HRMS calculated for [$C_{18}H_{24}N_2O_6S_4$+H] = 493.0590; found 493.0606; 3.0 ppm mass defect.

isobutyl ((2-thioxo-2,3-dihydrothiazol-5-yl)methyl) carbonate (**3.50**)

To a 5 mL round bottom flask equipped with a stir bar was added **S32** (74.8 mg, 1.0 equiv, 152 µmol), diluted with DMF (759 µL, 0.2 M), and then added a solution of aqueous TCEP•HCl (52.2 mg, 364 µL, 0.5 M,m 1.2 equiv, 182 µmol). The reaction mixture was then allowed to at 23 ºC for 15 min. After HPLC indicated reaction completion, the mixture was then quenched with AcOH (130 µL, 15.0 equiv, 2.28 mmol), and concentrated under reduced pressure to afford crude as a yellow oil. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 30% EtOAc/Hex) afforded title compound as a white-yellowish powder (59.1 mg, 79% isolated yield); R_f = 0.32 at 30% EtOAc/Hex. ^{1}H NMR (500 MHz, $CDCl_3$) δ 10.43 (s, 1H), 7.05 (s, 1H), 5.01 (s, 2H), 3.95 (d, J = 6.6 Hz, 2H), 1.98 (sept, J = 6.7 Hz, 1H), 0.95 (d, J = 6.7 Hz, 6H) ^{13}C NMR (126

MHz, $CDCl_3$) δ 155.1, 127.2, 125.8, 74.9, 60.9, 27.9, 19.0 HRMS calculated for [$C_9H_{13}NO_3S_2$+H] = 248.0410; found 248.0412; 0.8 ppm mass defect.

2.5 5-(2-hydroxypropan-2-yl)thiazole-2(3*H*)-thione (3.52)



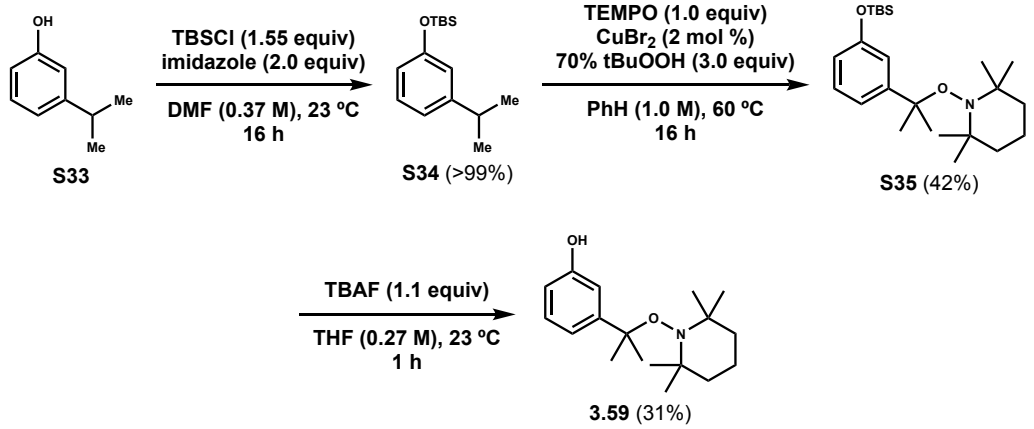
S9 **3.52** (73%)

Scheme 4. Synthesis of thiazole thione **3.52**

5-(2-hydroxypropan-2-yl)thiazole-2(3*H*)-thione (**3.52**)

To a flame-dried 250-mL round bottom flask equipped with a stir bar was diluted with dry Et_2O (32.2 mL, 0.2 M) followed by MeMgBr (3.07 g, 9.92 mL, 2.6 M, 4.0 equiv, 25.8 mmol). The reaction mixture was then allowed to stir at 0 ºC and then was added dropwise a solution of **S9** (1.22 g, 1.0 equiv, 6.45 mmol) (stripped 3 x with dry PhMe) in dry Et_2O (32.2 mL, 0.2 M). The reaction mixture was then gradually allowed to warm up to 23 ºC and stirred for 2.5 h. Once TLC indicated reaction completion; the mixture was quenched with 0.5 M HCl (aq) and then transferred to a seperatory funnel. The aqueous layer was then extracted with EtOAc (3 x), organic layers combined, dried over $MgSO_4$, filtered, and concentrated to afford crude product. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 20% EtOAc/Hex → 50% EtOAc/Hex) afforded title compound as a yellow powder (829.3 mg, 73% isolated yield); R_f = 0.31 at 50% EtOAc/Hex. ^{1}H NMR (400 MHz, $CDCl_3$) δ 12.89 (s, 1H), 7.04 (s, 1H), 5.33 (s, 1H), 1.40 (s, 6H) ^{1}H NMR (100 MHz, $CDCl_3$) δ 187.8, 142.0, 122.5, 68.3, 31.0 HRMS calculated for [$C_9H_{13}NO_3S_2$+H] = 176.0204; found 176.0207; 1.7 ppm mass defect.

2.6 3-(2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)propan-2-yl)phenol (3.59)



Scheme 5. Synthesis of 3-(2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)propan-2-yl)phenol (**3.59**)

tert-butyl(3-isopropylphenoxy)dimethylsilane (**S34**)

To a flame-dried 50 mL round bottom flask equipped with a stir bar was charged with S33 (0.995 g, 1.00 mL, 98% wt., 1.0 equiv, 7.16 mmol) diluted with anhydrous DMF (19,4 mL, 0.37 M) and then added TBSCl (1.67 g, 1.55 equiv, 11.1 mmol) followed by imidazole (975 mg, 2.0 equiv, 14.3 mmol). The reaction mixture was then allowed to stir at 23 ºC for 16 h. After TLC indicated reaction completion, the mixture was diluted with 1:1 EtOAc/Heptane and then washed with aqueous sat. $NaHCO_3$. The aqueous layer was then extracted with 1:1 EtOAc/Heptane (3 x). The organic layers were combined, washed with brine, dried over $MgSO_4$, filtered, and concentrated to afford a colorless oil as crude product. The crude residue was then purified. The crude product was purified via flash SiO_2 chromatography (Hex) afforded title compound as a colorless oil (1.79 g, quantitative yield); R_f = 0.52 at 1% EtOAc/Hex. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (t, J = 7.9 Hz, 1H), 6.82 (dt, J = 7.6, 0.6 Hz, 1H), 6.70 (t, J = 1.9 Hz, 1H), 6.65 (dd, J = 8.0, 1.0 Hz, 1H), 2.84 (sept, J = 7.0 Hz, 1H), 1.23 (d, J = 6.9 Hz, 6H), 0.99 (s, 9H), 0.20 (s, 6H) ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.7, 150.7, 129.2, 119.6, 118.3, 117.4, 34.1, 25.9, 24.1, 18.4, -4.2.

1-((2-(3-((*tert*-butyldimethylsilyl)oxy)phenyl)propan-2-yl)oxy)-2,2,6,6-tetramethylpiperidine (**S35**)

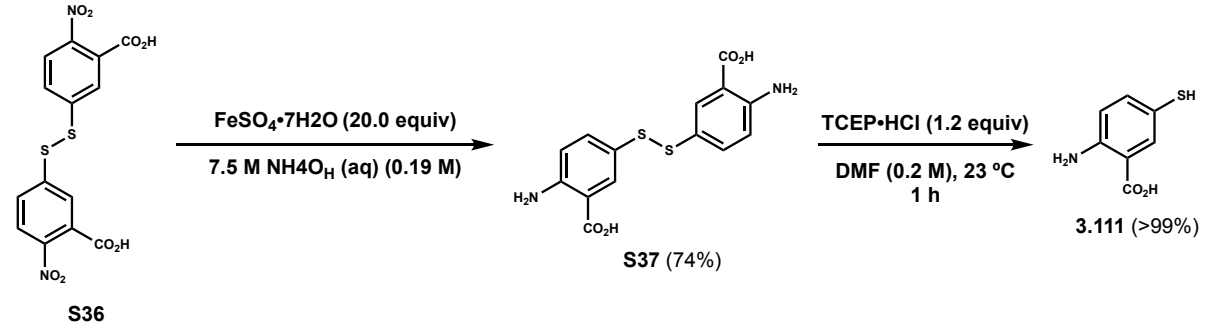
To a flame-dried dram vial equipped with a stir bar was charged with $CuBr_2$ (1.44 mg, 99% wt., 2 mol %, 6.40 μmol), TEMPO (51.0 mg, 98% wt., 1.0 equiv, 320 μmol), followed by S34 (401 mg, 5.0 equiv, 1.60 mmol) in solution with PhH (320 μL, 1.0 M). The reaction mixture was then allowed to heat to 60 ºC and stirred. The reaction mixture was then added tBuOOH (131 μL, 70% wt., 3.0 equiv, 960 μmol) dropwise; the reaction mixture was then allowed to stir at 60 ºC for 16 h. Afterwards, the reaction mixture was then diluted with Et_2O and then washed with brine (2 x). The aqueous layers were combined and then quenched with sat. aqueous $Na_2S_2O_3$. The organic layer was then dried over Na_2SO_4, filtered, and then concentrated to afford crude product. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 3% EtOAc/Hex → 10% EtOAc/Hex) afforded title compound as a pale-green oil (53.8 mg, 42% isolated yield). Purified product was not very clean by ^{1}H NMR. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.13 (m, 1H), 7.06 (m, 1H), 6.96 (s, 1H), 6.69 (m, 1H), 1.56 (m, 6H), 1.45 (m, 4H), 1.27 (m, 2H), 1.09 (s, 6H), 0.98 (s, 9H), 0.85 (s, 6H).

3-(2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)propan-2-yl)phenol **(3.59)**

To a dram vial equipped a stir bar was charged with **S35** (53.8 mg, 1.0 equiv, 133 μmol) and then diluted with THF (491 μL, 0.27 M) followed by addition of 1M TBAF in THF (146 μL, 1.1 equiv, 146 μmol). The reaction mixture was then allowed to stir at 23 ºC for 1 h. Once TLC/HPLC indicated reaction completion; the reaction mixture was diluted with EtOAc and then washed with aqueous 1M HCl. The aqueous layer was then extracted with EtOAc (3 x). The organic layers were combined; washed with brine, dried over Na_2SO_4, filtered, and concentrated to afford crude

product as a dark-orange oil. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 10% EtOAc/Hex → 25% EtOAc/Hex) afforded title compound as a pale-yellow solid (12.1 mg, 31% isolated yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.16 (t, J = 7.7 Hz, 1H), 7.04 (d, J = 7.8 Hz, 1H), 6.99 (s, 1H), 6.67 (d, J = 7.8 Hz, 1H), 4.60 (s, 1H), 1.57 (s, 6H), 1.45–1.42 (m, 2H), 1.42–1.39 (m, 1H), 1.30–1.23 (m, 3H), 1.09 (s, 6H), 0.86 (s, 6H) ^{13}C NMR (126 MHz, $CDCl_3$) δ 155.1, 153.2, 128.9, 118.0, 113.1, 112.9, 79.7, 59.6, 40.9, 34.0, 28.3, 20.7, 17.2. HRMS calculated for [$C_{18}H_{29}NO_2$+H] = 292.2271; found 292.2261; 3.4 ppm mass defect.

2.7 2-amino-5-mercaptobenzoic acid (3.59)



Scheme 6. Synthesis of 2-amino-5-mercaptobenzoic acid (**3.59**)

5,5'-disulfanediylbis(2-aminobenzoic acid) **(S37)**

To a 250-mL round bottom flask equipped with a stir bar was charged with 7.5 M NH4OH (133 mg, 102 mL, 0.037 molar, 1 Eq, 3.78 mmol) and the solution thoroughly sparged with argon. The solution was then heated to 95 ºC with stirring and then added FeSO4•7H2O (21.0 g, 20 Eq, 75.7 mmol) under positive argon. Then a solution of 5,5'-disulfanediylbis(2-nitrobenzoic acid) (1.50 g, 1 Eq, 3.78 mmol) in 7.5 M NH_4OH (133 mg, 19.9 mL, 0.19 molar, 1 Eq, 3.78 mmol) was added dropwise to the reaction mixture. After 2 h, the oil bath was removed and the reaction mixture allowed to cool to 23 ºC. Then decolorizing carbon (1.29 g) was added to the black reaction mixture, which was then filtered through filter paper. The yellow-orange filtrate was collected and cooled to 5 ºC. The filtrate was acidified with concentrated phosphoric acid to pH ~ 3. The aqueous mixture was extracted with 2-propanol/$CHCl_3$ (1:3, 4 x 200 mL). The organic layers were combined, washed with water, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford crude product. For ^{1}H NMR see manuscript reference (*37*).

2-amino-5-mercaptobenzoic acid **(3.59)**

To a 5 mL round bottom flask equipped with a stir bar was added **S37** (74.8 mg, 1.0 equiv, 152 μmol), diluted with DMF (759 μL, 0.2 M), and then added a solution of solid TCEP•HCl (52.2 mg, 1.2 equiv, 182 μmol). The reaction mixture was then allowed to at 23 ºC for 15 min. After HPLC indicated reaction completion, the mixture was then quenched with AcOH (130 μL, 15.0 equiv, 2.28 mmol), and concentrated under reduced pressure to afford crude as a yellow oil that was assumed to be quantitative yield of product **3.59**. Crude **3.59** was telescoped to OFCP processing reactions without further purification.

2.8 Synthesis of Amine Kobayashi Benzyne Precursors (3.116 and 3.117)

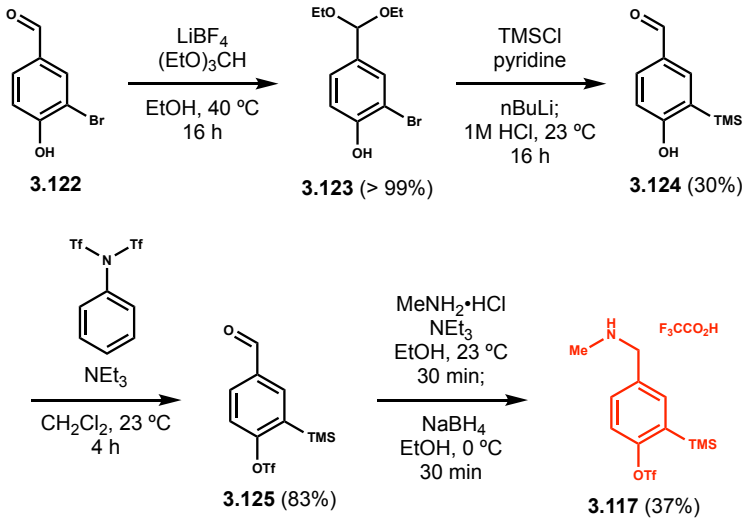


Fig. 3.21. Synthesis of Amine Insert as an Aryne Precursors. For experimental procedures – see SI for details.

2-bromo-4-(diethoxymethyl)phenol (**3.123**)

To a flame-dried 100-mL round bottom flask equipped with a stir bar and a reflux condenser was charged with 3-bromo-4-hydroxybenzaldehyde (12.00 g, 1.0 equiv, 59.70 mmol), $LiBF_4$ (79.60 mg, 98% Wt, 6.0 mol %, 3.582 mmol), and then diluted with EtOH (29.85 mL, 2.0 M). The reaction mixture was allowed to stir at 23 ºC until homogenous and then triethyl orthoformate (23.47 g, 26.3 mL, 98% Wt, 2.6 equiv, 155.2 mmol) was added. The reaction mixture was then allowed to heat to 40 ºC and stirred for 30 min. Reaction was monitored by TLC. When TLC indicated reaction completion, the reaction mixture was cooled to 23 ºC and quenched by the addition of sat. aq $NaHCO_3$. The reaction mixture was then transferred to a sep. funnel and extracted with EtOAc (3 x). The organic layers were combined, washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure to afford crude product as an orange-oil. Assumed a quantitative yield; crude product was telescoped to the next step without further purification.

4-hydroxy-3-(trimethylsilyl)benzaldehyde (**3.124**)

To a flame-dried 25-mL round bottom flask equipped with a stir bar was charged with **3.123** (16.42 g, 1.0 equiv, 59.68 mmol) (stripped with dry PhMe x3), diluted with dry CH_2Cl_2 (67.82 mL, 0.88 M, 1.0 equiv, 59.68 mmol), the reaction mixture was then allowed to stir at 0 ºC, and then TMS-Cl (7.780 g, 9.09 mL, 1.2 equiv, 71.61 mmol) was added. The reaction mixture was allowed to warm up to 23 ºC and then allowed to stir for 16 h.

The reaction mixture was then concentrated to dryness to afford crude product. The reaction mixture was then diluted with dry THF (170.5 mL, 0.35 M) and the reaction mixture allowed to cool to –78 ºC. Then nBuLi (3.823 g, 23.87 mL, 2.5 molar, 1.0 equiv, 59.68 mmol) was slowly added, the reaction mixture allowed to warm up to 23 ºC, and the reaction allowed to stir for 2 h. The reaction was then quenched with sat. NH_4Cl and 1M HCl. The reaction mixture was then allowed to stir at 23 ºC overnight. The reaction mixture was then extracted with EtOAc (3 x). The organic layers were then combined, wash with brine (1 x), dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford crude product. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 15% EtOAc/Hex → 25% EtOAc/Hex) afforded title compound as a white-solid (3.48 g, 30% isolated yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.88 (s, 1H), 7.90 (d, J = 2.0 Hz, 1H), 7.79 (d, J = 8.3, 2.1 Hz, 1H), 6.82–6.77 (m, 1H), 0.35 (s, 9H) ^{13}C NMR (126 MHz, $CDCl_3$) δ 191.7, 163.4, 138.8, 133.3, 131.2, 129.6, 126.8, 115.2, -1.1. HRMS calculated for [$C_{10}H_{14}O_2Si$+H] = 195.0841; found 195.0849; mass defect 4.1 ppm.

4-formyl-2-(trimethylsilyl)phenyl trifluoromethanesulfonate (**3.125**)

To a flame-dried dram vial equipped with a stir bar was charged with **3.124** (100. mg, 1 Eq, 515 μmol), diluted with dry CH_2Cl_2 (919 μL, 0.56 M, 1.0 equiv, 515 μmol), added 1,1,1-trifluoro-N-phenyl-N-((trifluoromethyl)sulfonyl)methanesulfonamide (206 mg, 98% Wt, 1.1 equiv, 566 μmol), and the reaction allowed to stir at 0 ºC. Then NEt_3 (57.3 mg, 78.9 μL, 1.1 equiv, 566 μmol) was added and the reaction mixture allowed to stir at 0 ºC. When TLC indicated reaction completion, the reaction mixture was added H_2O. The reaction mixture was then transferred to a sep. funnel and the aqueous layer extracted with CH_2Cl_2 (3 x). The organic layers combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated to afford crude product as an orange-oil. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 3.5% EtOAc/Hex) afforded title compound as a colorless-oil (129.5 mg, 83% isolated yield); R_f = 0.29 at 5% EtOAc/Hex. ^{1}H NMR (600 MHz, $CDCl_3$) δ 10.04 (s, 1H), 8.05 (d, J = 2.2 Hz, 1H), 7.96 (dd, J = 8.6, 2.2 Hz, 1H), 7.54 (d, J = 8.5 Hz, 1H), 0.41 (s, 9H) ^{13}C NMR (151 MHz, $CDCl_3$) δ 190.8, 158.7, 138.0, 134.9, 134.2, 132.8, 130.1, 120.2, -0.9. ^{19}F NMR (565 MHz, $CDCl_3$) δ -73.66 (s, 3F). HRMS calculated for [$C_{11}H_{13}F_3O_4SSi$+H] = 327.0334; found 327.0338; 1.2 ppm mass defect.

4-((methylamino)methyl)-2-(trimethylsilyl)phenyl trifluoromethanesulfonate (**3.117**)

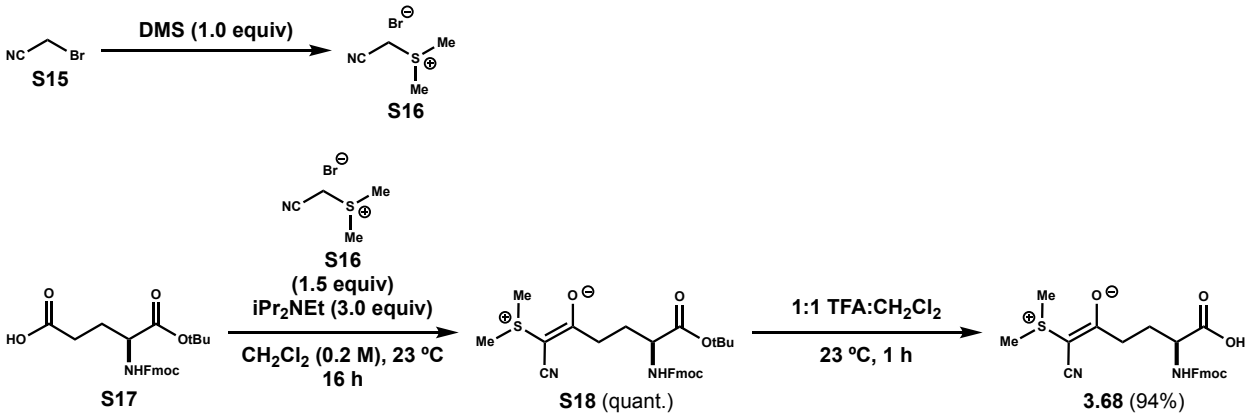
To a scintillation vial equipped with a stir bar was charged with **3.125** (139.5 mg, 1.0 equiv, 427.4 μmol), diluted with dry EtOH (2.137 mL, 0.2 M), then added $MeNH_2$•HCl (72.15 mg, 2.5 equiv, 1.069 mmol) followed by NEt_3 (94.29 mg, 130 μL, 2.18 equiv, 931.8 μmol), and then the reaction mixture allowed to stir at 23 ºC for 30 min. When LCT-premier indicated reaction completion, the reaction mixture was concentrated under reduced pressure to afford a white-powder as crude product.

The crude product then diluted with EtOH (2.137 mL, 0.2 M, 1.0 equiv, 427.4 μmol) and then added $NaBH_4$ (16.17 mg, 1.0 equiv, 427.4 μmol) and the reaction mixture allowed to stir at 0 ºC for 30 min. After TLC and LCT-premier indicated reaction completion, the reaction mixture was quenched with ice-water. The reaction mixture was then diluted with 1M NaOH, extracted with CH_2Cl_2(3 x), washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure to afford crude product as a faint-yellow oil. The crude product was then purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 80% MeCN/H_2O → 90% MeCN/H_2O) afforded title compound as a TFA salt (72.5 mg, 37% isolated yield) and as a white-powder. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.90 (s, 1H), 7.58 (d, J = 2.2 Hz, 1H), 7.50 (dd, J = 8.6, 2.2 Hz, 1H), 7.38 (d, J = 8.5 Hz, 1H), 4.14 (s, 2H), 2.64 (s, 3H), 0.35 (s, 9H) ^{13}C NMR (151 MHz, $CDCl_3$) δ 155.8, 138.0, 134.9, 132.8, 129.0, 120.3, 52.1, 32.5, -1.2 ^{19}F NMR (565 MHz, $CDCl_3$) δ -73.86 (s, 3H), -75.87 (s, 3H). HRMS of [$C_{12}H_{18}F_3NO_3SSi$+H] = 342.0807; found 342.0822; 4.4 ppm mass defect.

2-(*tert*-butyldimethylsilyl)-4-((methylamino)methyl)phenyl trifluoromethanesulfonate (**3.116**)

Amine Kobayashi Benzyne Precursor **3.116** was prepared in the same sequential procedure outlined for **3.117**. **3.116** was isolated as a free base, yellow oil. **3.116** was isolated as crude product and not purified further. ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.43 (d, J = 2.3 Hz, 1H), 7.39 (dd, J = 8.6, 2.3 Hz, 1H), 7.32 (d, J = 8.5 Hz, 1H), 3.76 (s, 2H), 2.44 (s, 3H), 0.89 (s, 9H), 0.38 (s, 6H) ^{13}C NMR (151 MHz, $CDCl_3$) δ 154.7, 138.9, 137.2, 131.0, 129.5, 119.1, 55.3, 36.2, 26.7, 17.7, -4.5 ^{19}F NMR (565 MHz, $CDCl_3$) δ -73.91 (s, 3H). HRMS of [$C_{15}H_{24}F_3NO_3SSi$+H] = 384.1277; found 384.1282; 1.3 ppm mass defect.

2.9 Synthesis of Fmoc-Glu*-OH (3.68)



Scheme 7. Synthesis of **3.68**

Compounds **3.67** and **S16** were synthesized following manuscript reference (*25*).

Fmoc-Glu*-OtBu (**S18**):

To a flame-dried 250-mL round bottom flask equipped with a stir bar was charged with Fmoc-Glu-OtBu (15.0 g, 1.0 equiv.), 35.3 mmol) and then dissolved in CH_2Cl_2 (176 mL, 0.2 M) until homogeneous. The solution was then added HBTU (16.2 g, 1.21 equiv., 42.7 mmol), **S16** (9.63 g, 1.5 equiv., 52.9 mmol), and iPr_2NEt (18.4 mL, 3.0 equiv., 106 mmol). The reaction mixture was then allowed to stir at 23 ºC for 16 h. The reaction mixture was then added sat. $NaHCO_3$ (aq.) and then transferred to a sep. funnel. The aqueous layer was extracted with EtOAc (3 x) and the organic layers combined. The organic layers were then washed with brine, dried over Na_2SO_4, filtered, and concentrated to afford crude product. The crude product was purified by flash SiO_2 column chromatography (gradient 50% EtOAc/Hex → 80% EtOAc/Hex → EtOAc) to give **S18** as a hydroscopic orange oil (17.9 g, quantitative yield).

Fmoc-Glu*-OH (**3.68**):

To a 100-mL round bottom flask equipped with a stir bar was charged with **S17** (17.9 g, 35.3 mmol) and added 1:1 TFA:CH_2Cl_2 (20:20 mL). The reaction mixture was then allowed to stir at 23 ºC for 1 h. After HPLC indicated reaction completion; the reaction mixture was concentrated under reduced pressure to afford a crude oil which was solubilized with a minimal amount of CH_2Cl_2. Then MeOH was added and evaporated several times, to afford crude product as a yellow solid. Then the crude product was subjected to a trituration with Et_2O (3 x) to afford **38** (15.0 g, 94% isolated yield) as a white solid. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 12.57 (s, 1H), 7.89 (d, J = 7.50 Hz, 2H), 7.73 (dd, J = 7.38 Hz, J = 3.06 Hz, 2H), 7.66 (d, J = 8.04 Hz, 1H), 7.42 (t, J = 7.32 Hz, 2H), 7.37-7.31 (m, 2H), 4.28-4.22 (m, 2H), 3.98-3.93 (m, 1H), 2.78 (s, 6H), 2.47-2.36 (m, 2H), 2.04-1.99 (m, 1H), 1.82-1.73 (m, 1H), 1.29-1.21 (m, 1H) 13C NMR (DMSO-d_6, 151 MHz) δ 188.56, 173.74, 156.11, 143.85, 140.70, 127.64, 127.10, 125.29, 120.11, 119.57, 65.7, 54.7, 53.4, 46.6, 34.4, 27.6, 26.2, 16.7 HRMS m/z calculated for $[C_{24}H_{24}N_2O_5S+H]^+$ = 453.1484; found 453.1487; 0.7 ppm mass defect.

2.10 Synthesis of Fig. 3.2 Compounds

Polycycle **3.9**:

Synthesized according to general procedure 2a with crude peptide Ac-YNCTFC-NH_2 (60.0 mg, 1.0 equiv., 75.9 μmol). Purification by preparative-reverse phase HPLC (gradient 40% MeCN/H_2O → 55% MeCN/H_2O) afforded the title compound (29.1 mg, 41% isolated yield) as an off-white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.51 (s, 1H), 8.33 (d, J = 9.5 Hz, 1H), 8.12 (d, J = 6.9 Hz, 1H), 8.05 (d, J = 8.2 Hz, 1H), 7.98 (d, J = 6.8 Hz, 1H), 7.69 (d, J = 7.7 Hz, 1H), 7.42 (s, 1H), 7.36 (d, J = 8.6 Hz, 1H), 7.30-7.17 (m, 6H), 7.06-6.84 (m, 3H), 7.03 (s, 1H), 4.98 (brs, 1H), 4.84-4.79 (m, 1H), 4.72 (dd, J = 14.2, 6.8 Hz, 1H), 4.60-4.55 (m, 1H), 4.55-4.48 (m, 1H), 4.41-1.34 (m, 1H), 3.74 (t, J = 6.9 Hz, 1H), 3.61 (d, J = 14.0 Hz, 1H), 3.48-3.41 (m, 2H), 3.22 (dd, J = 14.0, 3.7 Hz, 1H), 2.97 (dd, J = 13.2, 5.1 Hz, 1H), 2.89-2.82 (m, 3H), 2.70 (dd, J = 13.2, 10.1 Hz, 1H), 2.38 (dd, J = 15.6, 8.5 Hz, 1H), 2.32 (dd, J = 15.6, 5.0 Hz, 1H), 1.87 (s, 3H), 0.51 (d, J = 6.0 Hz, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.7, 170.8, 170.3, 169.5, 169.1, 168.8, 168.5, 168.0, 152.4, 139.2-138.8 (m, 1C), 137.8, 135.2-134.9 (m, 1C), 32.1, 130.5, 129.1, 128.2, 126.4, 118.7-111.5 (m, 2C), 114.9, 68.6-68.1 (m, 1C) 65.9, 62.1, 54.8, 53.6, 50.4, 48.4, 46.5, 38.4, 37.3, 37.2, 30.6, 22.4, 20.0 ^{19}F NMR (DMSO-d_6, 282 MHz) δ -103.2 and -119.2 (AB quartet, J = 252.2 Hz, 2F), -113.8 and -127.0 (AB quartet, J = 223.0 Hz, 2F). HRMS m/z calculated for $[C_{39}H_{42}F_4N_8O_{10}S_2+Na]^+$ = 945.2299, found 945.2327, 3.0 ppm mass defect.

Polycycle **3.10**:

Synthesized according to general procedure 2b with crude peptide Ac-HFCASC-NH_2 (50.0 mg, 1.0 equiv., 53.43 μmol). Purification by preparative-reverse phase HPLC – (gradient 10% MeCN/H_2O → 50% MeCN/H_2O) afforded title compound (13.76 mg, 27% isolated yield) as an

off-white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.38 (s, 1H), 8.42 (d, J = 8.9 Hz, 1H), 8.36 – 8.28 (m, 3H), 7.93 (t, J = 8.2, 1H), 7.31 (d, J = 9.2 Hz, 2H), 7.28 – 7.20 (m, 7H), 7.20 – 7.16 (m, 1H), 4.85 – 4.80 (m, 1H), 4.63 – 4.53 (m, 3H), 4.31 (quint, J = 4.5 Hz, 1H), 4.20 (quint, J = 7.4 Hz, 1H), 3.82 (dd, J = 10.85, 5.1 Hz, 1H), 3.52 (dd, J = 10.85, 3.80 Hz, 1H), 3.34 (dd, J = 12.6, 6.7 Hz, 1H), 2.92 – 2.86 (m, 2H), 2.85 – 2.79 (m, 1H), 2.79 – 2.74 (m, 1H) 1.82 (s, 3H), 1.24 (d, J = 7.2 Hz, 3H). ^{13}C (DMSO-d_6, 126 MHz) δ 172.9, 171.4, 169.7, 169.6, 168.8, 167.8, 167.7, 137.3, 133.8, 129.2, 128.0, 126.3, 61.2, 55.3, 53.5, 51.1, 51.0, 50.3, 48.6, 48.4, 46.4, 36.8, 33.7, 30.5, 30.1, 22.5, 17.6. ^{19}F (DMSO-d_6, 282 MHz) δ -103.2 – (-104.7) (m, 1F), -114.0 – (-116.4) (m, 2F), -136 – (-125.2) (m, 2F). HRMS m/z calculated for $[C_{34}H_{37}F_4N_9O_8S_2+H]^+$ = 840.2221; found 840.2233; 1.4 ppm mass defect.

Polycycle **3.11**:

Synthesized according to general procedure 2b with crude peptide Ac-HFSCAC-NH_2 (50.0 mg, 1.0 equiv., 53.43 μmol). Purification by preparative-reverse phase HPLC (gradient 10% MeCN/H_2O → 55% MeCN/H_2O) afforded title compound (21.4 mg, 42% isolated yield) as an off-white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.10 (d, J = 7.7, 1H), 8.84 (s, 1H), 8.41 (d, J = 8.7 Hz, 1H), 8.07 - 8.01 (m, 2H), 7.63 (d, J = 8.1 Hz, 1H), 7.54 (d, J = 7.65 Hz, 1H), 7.38 (s, 1H), 7.28 - 7.17 (m, 6H), 4.75 - 4.70 (m, 1H), 4.68 - 4.62 (m, 1H), 4.62 - 4.56 (m, 2H) 4.52 (quint, J = 7.2, 1H), 4.43 - 4.36 (m, 1H), 3.80 - 3.73 (m, 1H), 3.57 - 3.39 (m, 3H), 3.47 - 3.41 (m, 1H), 3.16 - 3.05 (m, 2H), 3.03 - 2.94 (m, 2H), 2.94 - 2.81 (m, 3H), 1.77 (s, 3H), 1.31 (d, J = 7.3 Hz, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 170.6, 170.3, 170.1, 170.0, 168.9, 167.9, 167.5, 141.3 - 140.9 (m, 1C), 139.0, 137.5, 135.6, 132.8 - 132.6 (m, 1C), 129.0, 128.2, 126.4, 117.6 - 115.8 (m, 2C), 117.6 - 115.8 (m, 2C), 114.5, 70.4 - 69.7 (m, 1C), 61.3, 55.1, 53.8, 51.6, 51.1, 48.5, 47.1, 38.6, 37.8, 30.8,

29.8, 22.5, 16.0. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -98.8 and -120.7 (AB quartet, J = 254.1 Hz, 2F), -117.2 and -127.3 (AB quartet, J = 222.4 Hz, 2F). HRMS m/z calculated for $[C_{44}H_{37}F_4N_9O_8S_2+H]^+$ = 840.2221; found 840.2213; 1.0 ppm mass defect.

Polycycle **3.12**:

Synthesized according to General Procedure 2A using crude peptide Ac-YGHCAC-NH_2 (100. mg, 1.0 equiv., 142 μmol). Reaction completion took 24 h. Purification by preparative-reverse phase HPLC – (10% MeCN/H_2O → 57.5% MeCN/H_2O → 62.5% MeCN/H_2O) afforded desired product. Desired product was then repurified via pTLC; 10% MeOH/$CHCl_3$ solvent system; afforded title compound (35.7 mg, 30% isolated yield) as an off-white powder. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.33 (s, 1H), 8.55 (d, J = 6.5 Hz, 1H), 8.26 (t, J = 5.5 Hz, 1H), 8.10 (d, J = 8.2 Hz, 1H), 8.04 (d, J = 7.7 Hz, 1H), 7.51 (s, 1H), 7.10 (d, J = 8.6 Hz, 2H), 6.97 (d, J = 8.6 Hz, 2H), 6.91 (s, 1H), 6.64–6.60 (m, 1H), 4.64 (d, J = 8.8 Hz, 1H), 4.60–4.54 (m, 1H), 4.40–4.32 (m, 1H), 4.08 (m, 1H), 3.72–3.64 (m, 2H), 3.00–2.91 (m, 2H), 2.88–2.78 (m, 2H), 2.66–2.58 (m, 2H), 1.72 (m, 3H), 1.18 (d, J = 7.4 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.6, 170.9, 170.6, 169.5, 168.1, 167.2, 139.5, 138.8–138.7 (m, 1C), 138.6, 135.2, 130.2, 130.0–129.9 (m, 1C), 122.3–122.2 (m, 2C), 116.0, 114.9, 113.5, 67.8–67.7 (m, 1C), 53.9, 52.0, 49.6, 45.5, 42.1, 36.1, 30.2, 25.1, 22.5, 17.3 ^{19}F NMR (DMSO-d_6, 565 MHz) δ -105.9 and -120.1 (AB quartet, J = 254.0 Hz, 2F), -114.1 and -126.1 (AB quartet, J = 225.8 Hz, 2F) HRMS calculated for $[C_{33}H_{35}F_4N_9O_9S+H]^+$ = 810.2293; found 810.2329; 4.4 ppm mass defect.

Polycycle **3.4**:

Synthesized according to General Procedure 2B using crude peptide Ac-YGSHAC-NH_2 (50.0 mg, 1.0 equiv., 63.15 μmol). Purification by preparative-reverse phase HPLC (gradient 10% MeCN/H_2O → 60% MeCN/H_2O) afforded title compound (23.3 mg, 40% isolated yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.93 (s, 1H), 8.58 (d, J = 7.0 Hz, 1H), 8.42 (d, J = 6.6 Hz, 2H), 8.34 (s, 1H), 7.78 (d, J = 7.8 Hz, 1H), 7.54 (s, 1H), 7.19 (s, 1H), 7.15 - 7.10 (m, 2H), 7.10 - 7.00 (m, 2H), 6.67 - 6.58 (m, 1H), 4.71 - 4.64 (m, 1H), 4.58 - 4.51 (m, 1H), 4.27 (q, J = 6.4 Hz, 1H), 4.17 (q, J = 7.0 Hz, 1H), 4.09 (quint, J = 7.1 Hz, 1H), 3.95 - 3.87 (m, 1H); 3.72 - 3.56 (m, 3H),, 3.45 (d, J = 13.1 Hz, 1H), 2.90 - 2.83 (m, 2H); 2.82 - 2.73 (m, 1H), 2.68 - 2.58 (m, 2H), 1.79 (s, 1H), 1.24 (d, J = 7.2 Hz, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ171.6, 171.0, 169.7, 169.7, 169.3, 169.0, 167.2, 151.0, 143.1 - 142.5 (m, 1C), 139.4, 136.8, 135.7, 130.8, 128.2 - 127.8 (m, 1C), 119.7 - 113.0 (m, 2C), 119.7 - 113.0 (m, 2C), 119.2, 118.09, 67.3 - 66.4 (m, 1C), 60.9, 55.8, 54.7, 50.8, 49.3, 46.3, 42.8, 36.7, 30.1, 29.8, 22.3, 17.3. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -105.9 and -123.8 (AB quartet, J = 257.6 Hz, 2F), -117.1 and -127.2 (AB quartet, J = 223.6 Hz, 2F). HRMS m/z calculated for $[C_{33}H_{35}F_4N_9O_9S + H]^+$ = 810.2293; found 810.2294; 1.0 ppm mass defect; 0.1 ppm mass defect.

Polycycle **3.13**:

Synthesized according to General Procedure 2B using crude peptide Ac-YGSdHAc-NH_2 (100. mg, 1.0 equiv., 126 μmol). Purification by preparative-reverse phase HPLC (gradient 10% MeCN/H_2O → 55% MeCN/H_2O → 60% MeCN/H_2O) afforded title compound (66.0 mg, 57% isolated yield) as a yellow-powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.25 (s, 1H), 8.75 (d, J = 6.6 Hz, 1H), 8.44 (d, J = 6.8 Hz, 1H), 8.23 (t, J = 5.1 Hz, 1H), 7.33 – 7.28 (m, 1H), 7.26 (d, J = 5.76 Hz, 1H), 7.09 – 7.05 (m, 2H), 7.93 – 6.98 (m, 4H), 6.19 (d, J = 8.6 Hz, 1H), 4.66 – 4.61 (m, 1H), 4.58 – 4.53 (m,

1H), 4.41 – 4.36 (m, 1H), 4.08 (quint, J = 3.3 Hz, 1H), 4.0 (quint, J = 6.9 Hz, 1H), 3.91 (dd, J = 16.2, 5.2 Hz, 1H), 3.85 (dd, J = 10.5, 3.8 Hz, 1H), 3.71 – 3.66 (m, 1H), 3.56 (d, J = 5.2 Hz, 1H), 3.54 – 3.48 (m, 1H), 3.06 (dd, J = 15.4, 3.5 Hz, 1H), 2.96 (dd, J = 13.4, 3.78 Hz, 1H), 2.81 – 2.75 (m, 4H), 1.93 (s, 3H), 1.13 (d, J = 7.3 Hz, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) 171.2, 170.7, 170.6, 170.1, 169.4, 168.9, 167.0, 153.8, 139.6, 138.1–138.0 (m, 1C), 134.0, 131.2, 128.3, 127.6, 123.2–120.0 (m, 1C), 116.8, 115.3, 114.7–114.4 (m, 2C), 67.3– 67.0 (m, 1C), 61.5, 56.3, 52.9, 52.5, 50.0, 46.0, 43.0, 37.8, 30.2, 28.1, 22.7, 16.7. ^{19}F NMR (DMSO-d_6, 565 MHz) -105.1 and -120.2 (AB quartet, J = 257.2 Hz, 2F), -114.7 and -125.8 (AB quartet, J = 227.3 Hz, 2F) HRMS m/z calculated for $[C_{33}H_{35}F_4N_9O_9+H]^+$ = 810.2293; found 810.2293; 0 ppm mass defect.

Polycycle **3.14**:

Synthesized according to General Procedure 2C using crude peptide Ac-YGTMQVSHAC-NH_2 (50.0 mg, 40.0 μmol, 1.0 equiv.). Purification by preparative-reverse phase HPLC (gradient 10% MeCN/H_2O → 55% MeCN/H_2O → 60% MeCN/H_2O) afforded title compound (24.6 mg, 45% isolated yield) as a yellow-crystalline powder. NMR of **14** contained complex mixture of rotational isomers; ratio ~ 1 : 8.5 . Major rotational isomer was characterized. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.19 (s, 1H), 8.44 (t, J = 5.6 Hz, 1H), 8.38 (d, J = 7.0 Hz, 1H), 8.15–8.11 (m, 2H), 7.99 (d, J = 7.1 Hz, 1H), 7.91–7.85 (m, 3H), 7.79 (d, J = 7.4 Hz, 1H), 7.32 (d, J = 7.3 Hz, 1H), 7.13 (d, J = 8.6 Hz, 2H), 7.02–6.99 (m, 2H), 6.84 (s, 1H), 6.65–6.60 (m, 1H), 4.62 (dt, J = 9.1, 2.9 Hz, 1H), 4.53–4.48 (m, 1H), 4.40–4.34 (m, 3H), 4.27–4.21 (m, 2H), 4.17 (dd, J = 7.6, 3.5 Hz, 1H), 4.16–4.10 (m, 3H), 4.10–4.05 (m, 1H), 3.86 (dd, J = 16.4, 5.3 Hz, 1H), 3.80–3.74 (m, 1H), 3.61–3.55 (m, 2H), 3.55–3.50 (m, 2H), 3.13–3.08 (m, 1H), 2.97–2.91 (m, 2H), 2.85–2.80 (m, 1H), 2.70–2.62 (m, 1H), 2.45–2.40 (m, 1H), 2.13–2.09 (m, 2H), 2.01 (s, 3H), 1.90–1.79 (m, 2H), 1.74 (m, 3H),

1.18 (d, J = 2.6 Hz, 3H), 1.16 (d, J = 2.8 Hz, 1H), 1.06 (d, J = 6.3 Hz, 3H), 0.87 (d, J = 6.8 Hz, 3H), 0.82 (d, J = 6.8 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 174.0, 172.0, 171.5, 171.2, 170.7, 170.5, 170.1, 170.1, 169.8, 169.8, 169.4, 167.2, 151.3, 139.1, 136.5–136.3 (m, 1C) 135.6, 130.4, 130.0, 128.1–128.0 (m, 1C), 116.8, 115.8–115.5 (m, 2C), 114.9, 67.3–67.2 (m, 1C), 67.0, 66.1, 58.6, 57.6, 55.3, 54.2, 53.0, 52.3, 52.0, 52.0, 49.3, 45.7, 45.6, 42.5, 35.8, 31.5, 30.8, 30.7, 29.7, 22.3, 19.8, 19.2, 17.7, 17.3, 14.5 ^{19}F NMR (DMSO-d_6, 565 MHz) δ -106.8 and -120.8 (AB quartet, J = 254.9 Hz, 2F), -114.9 and -126.0 (AB quartet, J = 227.3 Hz, 2F). HRMS m/z calculated for $[C_{52}H_{68}F_4N_{14}O_{15}S_2+H]^+$ = 1269.4444; found 1269.4449; 0.4 ppm mass defect.

Polycycle **3.15**:

Synthesized according to General Procedure 2C using crude peptide Ac-D(C)VHSAY-NH_2 (90.0 mg, 1.0 equiv., 94.9 μmol). Purification by preparative-reverse phase HPLC – (10% MeCN/H_2O → 50% MeCN/H_2O → 55% MeCN/H_2O) afforded desired product. Desired product was repurified via SiO_2 pTLC; 10% MeOH/$CHCl_3$ solvent system (plate run up twice) afforded (10.6 mg, 10% isolated yield) as an off-white film. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.42 (s, 1H), 8.79 (d, J = 6.0 Hz, 1H), 8.57 (d, J = 8.8 Hz, 1H), 8.30 (d, J = 5.7 Hz, 1H), 8.07 (d, J = 7.9 Hz, 1H), 7.89 (s, 2H), 7.64 (d, J = 5.9 Hz, 1H), 7.54 (s, 1H), 7.83 (s, 1H), 7.14 (d, J = 8.6 Hz, 2H), 6.96 (d, J = 8.6 Hz, 2H), 6.90 (s, 1H), 4.86 (t, J = 5.4 Hz, 1H), 4.74 (td, J = 7.5, 3.3 Hz, 1H), 4.67–4.63 (m, 1H), 4.53–4.48 (m, 1H), 4.39–4.33 (m, 1H), 4.26–4.22 (m, 1H), 4.18–4.11 (m, 2H), 3.65–3.59 (m, 2H), 3.51 (s, 1H), 3.47–3.41 (m, 2H), 3.17–3.13 (m, 1H), 3.03–2.98 (m, 1H), 2.98–2.91 (m, 2H), 2.87–2.77 (m, 2H), 2.66–2.62 (m, 1H), 2.02–1.97 (m, 1H), 1.97–1.90 (m, 1H), 1.83 (s, 3H), 1.23 (d, J = 6.6 Hz, 3H), 0.83 (d, J = 6.8 Hz, 3H), 0.80 (d, J = 6.8 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.8, 171.1, 170.5, 170.1, 169.8, 169.6, 169.3, 169.1, 168.9, 153.8–153.6 (m, 1C), 138.1,

136.6, 135.4–135.3 (m, 1C), 130.8, 129.7, 121.0, 116.5, 116.4–116.3 (m, 2C), 113.8, 69.8, 67.0, 61.3, 58.4, 56.0, 54.6, 53.9, 51.5, 49.2, 48.4, 30.7, 29.0, 25.1, 22.5, 22.1, 19.0, 19.0, 17.5 HRMS m/z calculated for $[C_{40}H_{47}F_4N_{11}O_{11}S+H]^+$ = 966.3192; found 966.3155; 3.8 ppm mass defect.

Polycycle **3.16**:

Synthesized according to General Procedure 2C using crude peptide Ac-YGAE(C)H-NMe_2 (50.0 mg, 1.0 equiv., 59.0 μmol). Linear peptide was prepared via general solution phase protocols. Purification by preparative-reverse phase HPLC – (gradient 10% MeCN/H_2O → 65% MeCN/H_2O) afforded desired product. Desired product was repurified via pTLC; 10% MeOH/$CHCl_3$ solvent system afforded title compound (4.7 mg, 8% isolated yield) as a white-powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.26 (s, 1H), 8.60 (s, 1H), 8.49 (d, J = 8.1 Hz, 1H), 8.37 (d, J = 7.7, 1H), 7.7 (s, 1H), 7.65 (d, J = 10.1 Hz, 1H), 7.18 (s, 1H), 6.99 (d, J = 8.7 Hz, 2H); 6.91 (d, J = 8.7, 2H), 6.70 (d, J = 6.2, 1H), 6.62 (s, 1H), 5.24 (quint, J = 7.1 Hz, 1H), 4.82 - 4.73 (m, 1H), 4.79 - 4.72 (m, 1H), 4.36 - 4.26 (m, 1H), 4.15 (dd, J = 17.3, 7.4, 1H), 4.03 - 3.97 (m, 1H), 3.53 (dd, J = 17.3, 3.4, 1H), 3.16 (s, 3H), 3.12 - 2.98 (m, 1H), 3.04 - 2.99 (m, 1H), 2.90 - 2.82 (m, 1H), 2.85 - 2.79 (m, 2H), 2.77 (s, 3H), 2.53 - 2.47 (m, 1H), 2.02 - 1.96 (m, 1H), 1.72 - 1.66 (m, 1H), 1.96 - 1.91 (m, 1H), 1.71 - 1.67 (m, 1H), 1.84 (s, 3H), 1.22 (d, J = 6.70, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.8, 171.2, 171.0, 170.8, 170.0, 169.7, 168.9, 167.4, 153.2, 146.5 - 144.6 (m, 1C), 137.6, 136.9, 135.1, 130.2, 129.5 - 128.2 (m, 1C), 118.4, 118.4, 118.1 - 117.0 (m, 2C), 118.1 - 117.0 (m, 2C). ^{19}F NMR (DMSO-d_6, 282 MHz) δ -111.1 –(-112.2) (m, 1F), -115.8 – (-119.6) (m, 3F). HRMS m/z calculated for $[C_{37}H_{42}F_4N_{10}O_9S+H]^+$ = 879.2871; found 879.2868; 0.3 ppm mass defect.

2.11 Synthesis of Fig. 3 Compounds

Macrocycle **3.17**:

Synthesized according to General Procedure 3A using crude peptide Ac-CWSC-NH_2 (300. mg, 1.0 equiv., 0.557 mmol). Purification by preparative-reverse phase HPLC afforded title compound (134.6 mg, 35% isolated yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.88 (s, 1H), 8.61 (s, 1H), 7.82 (s, 1H), 7.64 – 7.54 (m, 1H), 7.54 – 7.47 (m, 1H), 7.46 – 7.38 (m, 1H), 7.38 – 7.28 (m, 1H), 7.26 – 7.16 (m, 1H),7.11 – 7.02 (m, 1H), 7.02 – 6.93 (m, 1H), 6.75 (bs, 1H), 5.22 – 5.11 (m, 1H), 5.07 – 4.99 (m, 1H), 4.55 – 4.45 (m, 1H), 4.37 – 4.25 (m, 1H), 4.15 – 4.00 (m, 1H), 3.95 – 3.87 (m, 1H), 3.72 – 3.55 (m, 2H), 3.52 – 3.43 (m, 1H), 3.26 – 3.13 (m, 3H), 2.74 – 2.65 (m, 1H), 2.02 – 1.93 (m, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.2, 171.1, 170.8, 169.8, 169. 4, 136.1, 127.2, 123.5, 121.0, 118.4, 118.0, 111.4, 109.4, 64.9, 60.3, 57.4, 56.8, 51.7, 36.5, 34.4, 26.4, 24.3. HRMS m/z calculated for $[C_{27}H_{28}F_5N_6O_6S_2+H]^+$ = 691.1432; found 691.1431; 0.1 ppm mass defect.

Macrocycle **3.19**:

To a flame-dried 5-mL round bottom flask equipped with a stir bar was charged with **3.17** (20.0 mg, 1.0 equiv., 29.0 μmol), diluted with anhydrous DMF (0.58 mL, 50 mM), and cooled to 0 ºC. To the flask was added beta-D-thioglucose sodium salt (1.0 equiv.) and stirred for 1 h. After completion the reaction was concentrated under reduced pressured and purified by reverse-phase to give **3.19** (25.1 mg, >95%).^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.89 (s, 1H), 9.19 (bs, 1H), 9.11 – 8.84 (m, 2H), 8.26 (d, J = 8.6 Hz, 1H), 7.50 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.19 (d, J = 9.3 Hz, 1H), 7.06 (t, J = 7.26 Hz, 1H), 6.98 (t, J = 7.2 Hz, 1H), 4.79 (d, J = 9.2 Hz, 1H), 4.74 (d, J = 9.6 Hz, 1H), 4.66 (q, J = 7.6 Hz, 1H), 4.48 (q, J = 7.6 Hz, 1H), 4.38 – 4.31 (m, 1H), 3.85 – 3.77 (m, 1H), 3.64 – 3.54 (m, 3H), 3.53 – 3.43 (m, 3H), 3.30 – 3.21 (m, 3H), 3.11 – 2.98

(m, 3H), 1.83 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) 172.1, 169.9, 169.5, 168.8, 167.6, 136.1, 127.1, 123.8, 120.9, 118.4, 118.0, 111.4, 109.3, 84.6, 81.3, 78.0, 73.5, 69.4, 61.9, 60.7, 55.7, 55.3, 51.5, 46.1, 33.4, 30.7, 27.8, 22.5. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -98.6 and -112.7 (AB quartet, J = 248.8 Hz, 2F), -111.3 and -127.0 (AB quartet, J = 223.6 Hz, 2F). HRMS m/z calculated for $[C_{33}H_{38}F_4N_6O_{11}S_3+H]^+$ = 867.1775; found 867.1776; 0.1 ppm mass defect.

Macrocycle **3.20**:

To a flame-dried 5-mL round bottom flask equipped with a stir bar was charged with compound **3.17** (20.0 mg, 1.0 equiv., 29.0 μmol), diluted with DMF (0.58 mL, 50 mM), and cooled to 0 °C. To the flask was added coniferyl carbonate **3.40** (2.0 equiv.), $KOSiMe_3$ (2.0 equiv.), and stirred until completion observed by HPLC. After completion the reaction was quenched with AcOH (5 equiv.) and concentrated under reduced pressured and purified by reverse-phase HPLC to give **3.20** (14.6 mg, 53%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ10.87 (s, 1H), 9.30 (s, 1H), 8.62 (d, J = 9.3 Hz, 1H), 8.32 (t, J = 9.6 Hz, 2H), 7.49 (1H, J = 7.9 Hz, 1H), 7.33 (d, J = 7.9 Hz, 2H), 7.27 (d, J = 1.8 Hz, 1H), 7.16 (J = 9.9 Hz, 1H), 7.11 (1H, J = 8.7Hz, 1H), 7.08 – 7.03 (m, 2H), 6.98 (t, J = 7.1 Hz, 1H) 6.62 (1H, J = 15.9 Hz, 1H), 6.31 (dt, J = 15.9, 6.3 Hz, 1H), 4.87 (dt, J = 9.9, 2.6 Hz, 1H), 4.73 (d, J = 5.7 Hz, 2H), 4.66 – 4.53 (m, 2H), 4.46 – 4.44 (m, 1H), 3.89 (d, J = 6.6 Hz, 2H), 3.77 (s, 3H), 3.63 (d, J = 12.1 Hz, 1H), 3.55 – 3.49 (m, 1H), 3.38 (bs, 1H), 3.12 – 2.98 (m, 3H), 2.93 (t, J = 12.2, 1H), 2.75 – 2.68 (m, 1H), 1.95 – 1.85 (m, 2H), 1.73 (s, 3H), 1.46 – 1.41 (m, 1H), 0.89 (d, J = 6.6 Hz, 6H). ^{13}C NMR (DMSO-d_6, 126 MHz) 172.7, 169.7, 169.5, 169.1, 168.5, 155.0, 150.3, 142.2, 136.6, 132.9, 129.3, 127.4, 125.4, 124.1, 122.8, 121.4, 118.9, 118.3, 117.8, 113.6, 111.8, 109.6, 73.8, 68.2, 61.9, 61.2, 56.2, 56.0, 54.9, 52.5, 46.3, 30.5, 29.7, 28.4, 27.7, 22.7, 19.2. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -103.1 and -119.0 (AB quartet, J = 252.3 Hz, 2F), -114.2 and -

127.7 (AB quartet, J = 222.7 Hz, 2F). HRMS m/z calculated for $[C_{42}H_{46}F_4N_6O_{11}S_2+H]^+$ = 951.2680; found 951.2686; 0.6 ppm mass defect.

Macrocycle **3.21**:

To a flame-dried 5-mL round bottom flask equipped with a stir bar was charged with compound **3.17** (40.0 mg, 1.0 equiv., 58.0 µmol), diluted with DMF (0.58 mL, 50 mM), and cooled to 0 °C. To the flask was added NaN_3 (1.0 equiv.) and stirred for 1 h. After completion the reaction was concentrated under reduced pressured and purified by reverse-phase HPLC to give **21** (41.4 mg, >95%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.88 (s, 1H), 9.16 (s, 1H), 8.52 (d, 1H), 8.35 (t, J = 10.0, 2H), 7.49 (d, J = 7.8 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.12 (d, J = 9.5 Hz, 1H), 7.10 – 7.03 (m, 2H), 6.98 (t, J = 7.4 Hz, 1H), 4.97 (bs, 1H), 4.81 (d, J = 9.2 Hz, 1H), 4.64 – 4.53 (m, 2H), 4.38 – 4.30 (m, 1H), 3.89 – 3.80 (m, 1H), 3.59 (d, J = 13.2 Hz, 1H), 3.54 – 3.46 (m, 1H), 3.23 – 3.13 (m, 2H), 3.09 – 3.02 (m, 2H), 1.79 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) 172.1, 169.4, 169.1, 168.6, 167.8, 136.1, 127.1, 123.7, 120.9, 118.4, 117.9, 111.4, 109.2, 61.3, 55.3, 54.8, 51.9, 45.9, 32.1, 27.8, 22.4, 21.1. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -102.8 and -115.7 (AB quartet, J = 250.6 Hz, 2F), -113.5 and -126.5 (AB quartet, J = 224.7 Hz, 2F). HRMS m/z calculated for $[C_{27}H_{27}F_4N_9O_6S_2+H]^+$ = 714.1540; found 714.1542; 0.3 ppm mass defect.

Macrocycle **3.22**:

To a flame-dried 5-mL round bottom flask equipped with a stir bar was charged with compound **3.21** (15.0 mg, 1.0 equiv., 21.0 µmol), diluted with EtOH (0.420 mL, 50 mM) and flushed with argon. Pd/C (10 wt. %) was then added, flushed with hydrogen gas, and warmed to 35 °C. The reaction mixture was then stirred at 35 ºC for 16 h. After completion the reaction was filtered with

celite and concentrated under reduced pressure. Purification by reverse-phase HPLC gave **3.22** (12.6 mg, 87%) as a solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.88 (s, 1H), 8.63 (s, 1H), 8.54 (d, J = 8.6 Hz, 1H), 8.41 (d, J = 9.2 Hz, 1H), 8.25 (d, J = 7.7 Hz, 1H), 7.50 (d, J = 7.5 Hz, 1H), 7.33 (d, J = 7.75 Hz, 1H), 7.16 – 7.03 (m, 3H), 6.98 (t, J = 7.0 Hz, 1H), 6.55 (s, 2H), 4.74 (d, J = 9.2 Hz, 1H), 4.70 – 4.58 (m, 2H), 4.41 – 4.32 (m, 1H), 3.51 – 3.42 (m, 2H), 3.17 (s, 1H), 3.06 (bs, 2H), 2.70 – 2.64 (m, 1H), 2.62 – 2.55 (m, 1H), 1.80 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) 172.2, 169.1, 168.9, 168.6, 168.0, 136.1, 127.2, 123.7, 120.9, 118.4, 118.0, 111.4, 109.1, 61.4, 55.1, 54.7, 51.9, 45.7, 34.4, 31.3, 30.4, 29.8, 28.3, 22.4, 20.7. ^{19}F NMR (DMSO-d_6, 282 MHz) δ – 104.3 – (-106.0) (m, 2F), -111.5 – (-113.7) (m, 2F), -120.6 – (-122.5) (m, 2F), -126.7 – (-128.6) (m, 2F). HRMS m/z calculated for $[C_{27}H_{29}F_4N_7O_6S_2+H]^+$ = 688.1635; found 688.1637; 0.3 ppm mass defect.

Macrocycle **3.23**:

To a flame-dried 5-mL round bottom flask equipped with a stir bar was charged with compound **3.21** (15.0 mg, 1.0 equiv., 21.0 μmol), propargyl alcohol (1.0 equiv.), phenylenediamine (15 mol %), sodium ascorbate (10 mol%), $CuSO_4$•$5H_2O$ (5 mol%) in 2:3 H_2O:tBuOH (0.2 M). The reaction was let stir at 23 ºC for 12 h until observed completion by HPLC. After completion the reaction was concentrated under reduced pressure and purified by reverse-phase HPLC gave **3.23** (12.1 mg, 75% isolated yield) as a solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.89 (s, 1H), 9.48 (s, 1H), 8.44 (d, J = 9.1 Hz, 1H), 8.32 (d, J = 8.32 Hz, 2H), 8.27 (d, J = 8.6 Hz, 1H), 7.49 (d, J = 7.9 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H), 7.22 (d, J = 9.3 Hz, 1H), 7.10 (d, J = 2.2 Hz, 1H), 7.06 (t, J = 7.3 Hz, 1H), 6.97 (t, J = 7.4 Hz, 1H), 4.87 (dt, J = 9.3, 2.5 Hz, 1H), 4.61 (s, 2H), 4.47 (q, J = 7.7 Hz, 2H), 4.37 (quint, J = 4.2 Hz, 2H), 3.84 (dd, J = 10.8, 4.4 Hz, 1H), 3.72 – 3.65 (m, 1H), 3.47 (dd, J = 10.8,

3.5 Hz, 1H), 3.26 (dd, J = 12.5, 8.1 Hz, 1H), 3.11 – 2.98 (m, 2H), 2.87 – 3.83 (m, 1H), 2.36 (dd, J = 12.5, 7.0 Hz, 1H), 1.74 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) 171.9, 169.6, 169.3, 168.6, 167.6, 149.1, 136.1, 127.1, 123.8, 123.1, 121.0, 118.4, 118.0, 111.4, 109.0, 61.4, 55.6, 55.0, 54.5, 51.6, 46.1, 32.6, 30.8, 27.6, 22.3. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -101.7 – (-103.2) (m, 1F), -113.1 – (-118.0) (m, 2F), -124.0 – (-125.3) (m, 1F). HRMS m/z calculated for $[C_{30}H_{31}F_4N_9O_7S_2+H]^+$ = 770.1802; found 770.1806; 0.5 ppm mass defect.

Macrocycle **3.24**:

Synthesized according to General Procedure 3A using crude peptide Ac-CSC-NH_2 (100. mg, 1.0 equiv., 0.297 mmol). Purification by reverse-phase preparative HPLC to give **3.24** (62.0 mg, 43% isolated yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.09 (d, J = 8.0 Hz, 1H), 8.90 (bs, 1H), 8.29 (d, J = 7.2 Hz, 1H), 7.76 (d, J = 9.8 Hz, 1H), 5.09 – 5.02 (m, 1H), 4.71 – 4.63 (m, 1H), 4.45 – 4.37 (m, 1H), 3.83 (dd, J = 11.2, 5.6 Hz, 1H), 3.68 (dd, J = 11.1, 3.0 Hz, 1H), 3.64 – 3.57 (m, 1H), 3.53 (dd, J = 11.2, 4.0 Hz, 1H), 3.48 (d, J = 13.7 Hz, 1H), 3.43 – 3.36 (m, 1H), 2.87 (dd, J = 13.9, 3.4 Hz, 1H), 1.92 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) 169.5, 168.7, 168.0, 167.5, 150.6 – 150.1 (m, 1C), 127.5 – 125.8 (m, 3C), 68.8 - 68.6 (m, 1C), 60.7, 60.3, 55.5, 51.1, 46.6, 31.3, 29.2, 22.4. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -105.7 (dt, J = 255.1, 15.9 Hz, 1F), -120.2 (dd, J = 221.5, 6.99 Hz, 1F), -123.9 (dt, J = 255.4, 11.1 Hz, 1F), -125.0 (dd, J = 220.6, 13.3 Hz, 1F), -131.8 – (-132.1) (m, 1F). HRMS m/z calculated for $[C_{16}H_{17}F_5N_4O_5S_2+H]^+$ = 505.0639; found 505.0640; 0.2 ppm mass defect.

Macrocycle **3.25**:

Synthesized according to General Procedure 3A using crude peptide AcCWS-*d*-C-NH_2 (100. mg, 1.0 equiv., 0.186 mmol). Purification by reverse-phase HPLC gave **3.25** (34.7 mg, 27% isolated yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.87 (s, 1H), 9.25 (s, 1H), 8.49 (d, J = 8.49 Hz, 1H), 8.40 (d, J = 8.4 Hz, 1H), 8.29 (d, J = 7.2 Hz, 1H), 7.45 (d, J = 7.9 Hz, 1H), 7.32 (d, J = 8.1 Hz, 1H), 7.29 (d, J = 9.0 Hz, 1H), 7.10 – 7.03 (m, 2H), 6.96 (t, J = 7.6 Hz, 1H), 5.07 (t, J = 5.3 Hz, 1H), 4.67 – 4.59 (m, 2H), 4.53 – 4.45 (m, 1H), 4.23 – 4.18 (m, 1H), 3.72 (quint, J = 5.7 Hz, 1H), 3.55 – 3.49 (m, 1H), 3.42 – 3.37 (m, 1H), 3.27 (dd, J = 11.0 Hz,, 4.5 Hz, 1H), 3.17 (d, J = 5.15 Hz, 1H), 3.12 – 3.05 (m, 1H), 3.04 – 2.96 (m, 1H), 2.75 – 2.67 (m, 1H), 1.81 (s, 1H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.5, 169.5, 169.0, 168.0, 167.4, 136.1, 127.1, 123.7, 120.9, 118.4, 117.9, 111.3, 109.1, 60.8, 57.0, 54.8, 51.1, 46.4, 32.2, 30.5, 28.7, 22.2. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -106.4 (dt, J = 250.8, 14.7 Hz, 1F), -114.9 (dd, J = 222.0, 10.2 Hz, 1F), -121.5 (dt, J = 250.2, 11.7 Hz, 1F), -125.6 – (-126.6) (m, 1F), -142.4 (t, J = 14.3 Hz, 1F). HRMS m/z calculated for $[C_{27}H_{27}F_5N_6O_6S_2+H]^+$ = 691.1432; found 691.1432; 0 ppm mass defect.

Macrocycle **3.26**:

Synthesized according to General Procedure 3A using crude peptide Ac-CWS-homo-C-NH_2 (100. mg, 1.0 equiv., 0.181 mmol). Purification by reverse-phase HPLC gave **3.26** (33.2 mg, 26% isolated yield) as a solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.89 (s, 1H), 9.47 (s, 1H), 8.58 (s, 1H), 7.51 (d, J = 8.6 Hz, 1H), 7.32 (t, J = 8.55 Hz, 2H), 7.26 (s, 1H), 7.17 (s, 1H), 7.06 (t, J = 7.4 Hz, 1H), 6.84 (s, 1H), 6.88 (d, J = 5.2 Hz, 1H), 5.02 (s, 1H), 4.33 – 4.26 (m, 2H), 4.12 – 4.07 (m, 1H), 3.83 – 3.78 (m, 1H), 3.74 – 3.61 (m, 3H), 1.95 (s, 3H), 1.83 (s, 1H). ^{19}F NMR (DMSO-d_6, 282 MHz) δ -108.8 (dt, J = 252.1, 14.8 Hz, 1F), -112.6 (d, J = 218.1 Hz, 1F), -116.2 (d, J = 219.0

Hz, 1F), -120.1 – (-121.2) (m, 1F), -136.4 (d, J = 14.8 Hz, 1F). HRMS m/z calculated for $[C_{28}H_{29}F_5N_6O_6S_2+H]^+$ = 705.1588; found 705.1590; 0.3 ppm mass defect.

2.12 Synthesis of Fig. 3.5 Compounds

(2-((3,3,4,4,5,5-hexafluoro-2-phenoxycyclopent-1-en-1-yl)thio)thiazol-5-yl)methanol (**3.33**)

To a flame-dried dram vial equipped with a stir bar was added **3.32** (28.3 mg, 1.1 equiv, 192 μmol), diluted with DMF (3.49 mL, 0.05 M), added **2.5** (50.0 mg, 1.0 equiv, 175 μmol) and allowed to stir. Then KOTMS (30.7 mg, 95% wt., 1.3 equiv, 227 μmol) was added at 23 ºC and the reaction mixture allowed to stir at 23 ºC for 1 h.. The reaction mixture was then quenched with the addition of AcOH (30.0 μL, 3.0 equiv, 524 μmol) and allowed to stir at 23 ºC for 5 min. The reaction mixture was then concentrated under vacuo to afford an orange oil as crude product. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 15% EtOAc/Hex → 40% EtOAc/Hex) afforded title compound as a yellow-oil (72.2 mg, quantitative yield); R_f = 0.35 at 40% EtOAc/Hex. HRMS calculated for $[C_{15}H_9F_6NO_2S_2+H]$ = 414.0057; found 414.0064; 1.7 ppm mass defect.

2-((2-((3,3,4,4,5,5-hexafluoro-2-phenoxycyclopent-1-en-1-yl)thio)thiazol-5-yl)methyl)-4-methylphenol (**3.34**)

To a flame-dried dram vial equipped with **3.33** (9.0 mg, 1.0 equiv, 22 μmol) and p-cresol (12.0 mg, 5.0 equiv, 0.11 mmol) was diluted with $MeNO_2$ (0.44 mL, 0.05 M). The reaction mixture was then added $MeSO_3H$ (6.3 mg, 4.2 μL, 3.0 equiv, 65 μmol) and the mixture allowed to stir at 80 ºC for 4 h. After HPLC indicated reaction completion; the mixture was concentrated under reduced pressure to afford crude product. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 10% EtOAc/Hex → 15% EtOAc/Hex) afforded title compound as a white-film (9.0 mg, 82% isolated yield); R_f = 0.24 at 15% EtOAc/Hex. ^1H NMR ($CDCl_3$, 500 MHz) δ 9.43 (s, 1H), 7.56 (s, 1H), 7.32–7.28 (m, 2H), 7.27–7.22 (m, 1H), 7.10 (d, J = 8.0 Hz, 2H), 6.91 (s, 1H),

6.88 (dd, J = 8.1, 1.7 Hz, 1H), 6.72 (d, J = 8.1 Hz, 1H), 3.98 (s, 2H), 2.16 (s, 3H) ^{13}C NMR ($CDCl_3$, 126 MHz) δ 152.4, 152.1, 151.9, 143.5, 140.9, 130.3, 129.9, 128.4, 127.6, 126.8, 125.4, 119.2, 115.0, 27.1, 20.1. HRMS calculated for [$C_{22}H_{15}F_6NO_2S_2$+H] = 504.0527; found 504.0537; 2.0 ppm mass defect.

2.13 Synthesis of Fig. 3.6 Compounds

Macrocycle **3.37**:

To a flame-dried round bottom flask equipped with a stir bar was charged with crude linear Ac-YACFAC-NH_2 (219.9 mg, 1.0 equiv., 0.3063 mmol), diluted with anhydrous DMF (30.63 mL, 10.0 mM), and then allowed to stir at 0 ºC. Then OFCP (229.7 mL, 1.5 equiv., 1.0 M in MeCN) was added followed by NEt_3 (107 µL, 2.5 equiv., 0.7658 mmol). The reaction mixture was allowed to stir at 0 ºC for 30 min. When HPLC indicated macrocycle intermediate formation, the reaction mixture was concentrated under reduced pressure to dryness. The reaction residue was added thiazole **29**, diluted with 1:4 DMF/THF (30 mM), and then stirred at 0 ºC. The reaction solution was then added KOTMS (3.5 equiv.) and the reaction mixture allowed to gradually warm up to 23 ºC for 3 h. When HPLC indicated reaction completion, the reaction mixture was added AcOH (10 equiv.) and then concentrated under reduced pressure to afford crude product. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (54.8 mg, 18% isolated yield) as a yellow-powder; R_f = 0.33 at 15% MeOH/$CHCl_3$.

Polycycle **3.38**:

To a flame-dried scintillation vial equipped with a stir bar was charged with **30** (22.8 mg, 1.0 equiv, 22.9 µmol), diluted with anhydrous $MeNO_2$ (4.57 mL, 5.0 mM), and then added TFA (344 µL, 7.5 vol %). The solution was then then purged with argon and added a screwtop cap. The reaction was then allowed to stir at 80 ºC for 12 h. After HPLC indicated reaction completion, the reaction mixture was concentrated under reduced pressure to afford crude product. The crude product was then purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O →

60% MeCN/H_2O) to afford title compound (11.7 mg, 52% isolated yield) as an orange-powder. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.49 (s, 1H), 8.46 (d, J = 7.5 Hz, 1H), 8.39 (d, J = 8.3 Hz, 1H), 8.26 (s, 1H), 8.15 (d, J = 7.7 Hz, 1H), 8.07 (d, J = 7.5 Hz, 1H), 7.65 (d, J = 8.0 Hz, 1H), 7.30–7.25 (m, 2H), 7.23–7.18 (m, 2H), 7.18–7.15 (m, 2H), 6.86 (d, J = 7.3 Hz, 1H) 6.68 (d, J = 7.9 Hz, 1 H), 4.70 (d, J = 8.2 Hz), 4.61–4.56 (m, 1H), 4.56–4.51 (m, 1H), 4.44 (dd, J = 14.1, 7.2 Hz, 1H), 4.25–4.19 (m, 1H), 4.19–4.11 (m, 1H), 4.03 (d, J = 15.6 Hz, 1H), 3.90 (d, J = 15.6 Hz, 1H), 3.74–3.67 (dd, J = 12.2, 7.1 Hz, 1H), 3.57 (d, J = 13.1 Hz, 1H), 3.53 (dd, J = 12.4, 8.1 Hz, 1H), 2.94–2.81 (m, 4H), 2.74–2.61 (m, 1H), 1.86 (s, 3H), 1.18 (d, J = 6.6 Hz, 3H), 1.00 (d, J = 6.5 Hz, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.6, 171,3, 171.0, 169.3, 169.0, 168.8, 167.6, 153.2, 152.8, 143.3, 141.3, 137.9–137.9 (m, 1C), 136.6, 130.6, 129.5, 129.0, 128.3, 127.8, 126.6, 125.6–125.5 (m, 1C), 124.4, 119.4–119.3 (m, 1C), 117.1–117.0 (m, 1C), 114.8, 70.1–70.0 (m, 1C), 55.4, 53.6, 52.1, 48.5, 47.4, 46.5, 37.2, 36.1, 32.2, 30.4, 26.9, 22.5, 19.1, 17.3 ^{19}F NMR (DMSO-d_6, 565 MHz) ^{19}F (DMSO-d_6, 565 MHz) δ -98.3 and -112.2 (AB quartet, J = 248.8 Hz, 2F), -113.0 and -128.2 (AB quarter, J = 223.8 Hz, 2F) HRMS m/z calculated for **[**$C_{41}H_{42}F_4N_8O_8S_4$+H] = 979.2023; found 979.2048; found 2.6 ppm mass defect.

Polycycle **3.39**:

Synthesized in same sequential procedure to make **3.37** and **3.38** using crude peptide Ac-YCAC-NH_2. Acyclic macrocycle intermediate (36.9 mg, 1.0 equiv., 47.4 μmol) afforded crude product which was then purified via reverse-phase preparative HPLC (gradient 10% MeCN/H_2O → 65% MeCN/H_2O → 70% MeCN/H_2O) to afford desired product. Desired product was repurified via pTLC; 10% MeOH/$CHCl_3$ solvent system to afford title compound (11.6 mg, 32% isolated yield) as a white-powder. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.48 (s, 1H), 9.11 (s, 1H), 8.77 (d, J = 9.2

Hz, 1H), 7.96 (d, J = 7.3 Hz, 1H), 7.65 (s, 1H), 7.58 (d, J = 8.9 Hz, 1H), 7.53 (d, J = 9.6 Hz, 1H), 6.79–6.74 (m, 2H), 6.69 (d, J = 8.0 Hz, 1H), 4.73–4.67 (m, 1H), 4.58–4.52 (m, 2H), 4.50–4.46 (m, 1H), 4.26 (d, J = 15.6 Hz, 1H), 3.83 (dd, J = 14.0, 4.8 Hz, 1H), 3.70 (d, J = 15.5 Hz, 1H), 3.65–3.58 (m, 1H), 3.44–3.40 (m, 1H), 3.24 (dd, J = 13.1, 3.2 Hz, 1H), 2.91 (dd, J = 13.6, 3.7 Hz, 1H), 2.42 (dd, J = 13.1, 7.9 Hz, 1H), 1.87 (s, 3H), 1.23 (d, J = 5.2 Hz, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 170.8, 170.6, 169.5, 168.5, 167.2, 154.2, 153.2, 143.9, 141.3, 136.9 (m, 1C), 135.5 (m, 1C), 129.9, 129.8, 127.0, 125.6, 114.9, 67.0 (m, 1C), 53.8, 53.5, 48.0, 47.3, 35.7, 32.7, 31.0, 26.7, 22.9, 15.5. ^{19}F NMR (DMSO-d_6, 565 MHz) -96.3, -114.0 (AB quartet, J = 250.6 Hz, 2F) -119.1, -127.7 (AB quartet, J = 219.4 Hz, 2F) HRMS m/z calculated for $[C_{29}H_{28}F_4N_6O_6S_4+H]^+$ = 761.0968; found 761.1005; 4.9 ppm mass defect.

Polycycle **3.41**:

To a flame-dried 50-mL round bottom flask equipped with a stir bar was charged with crude Ac-YGHAC-NH_2 (135.0 mg, 1.0 equiv., 192 μmol), diluted with anhydrous DMF (19.2 mL, 10.0 mM), and then allowed to stir at 0 ºC. Then OFCP (287 μL, 1.5 equiv., 287 μmol, 1M in MeCN) followed by NEt_3 (134 μmol, 2.5 equiv., 958 μmol) were added and the reaction mixture allowed to stir at 0 ºC for 30 min. After HPLC indicated full conversion to macrocycle intermediate, the reaction mixture was concentrated to dryness under reduced pressure to remove excess OFCP, NEt_3, and DMF. The reaction mixture was then dissolved in 1:4 DMF:THF (7.66 mL, 30 mM) and cooled to 0 ºC. Then Cs_2CO_3 (375 mg, 6.0 equiv., 1.15 mmol) was added and the reaction mixture stirred at 0 ºC for 1 h until full conversion to spirocycle intermediate was observed. The reaction mixture was then added phenol **33** (107.9 mg, 2.0 equiv., 129 μmol) and then allowed to stir at 23 ºC for 48 h. After HPLC indicated reaction completion, the reaction mixture was quenched with

AcOH (165 µL, 15.0 equiv., 2.87 mmol), and then concentrated under reduced pressure to afford crude product. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) to afford **34** (37.8 mg, 20% isolated yield) as a yellow-powder, R_f = 0.32 at 15% MeOH/$CHCl_3$. HRMS m/z calculated for $[C_{45}H_{50}F_4N_8O_{12}S+Na]^+$ = 1025.3103; found 1025.3121; 1.8 ppm mass defect.

Polycycle **3.42**:

To a flame-dried 15-mL round bottom flask equipped with a stir bar was charged with **34** (18.0 mg, 1.0 equiv., 17.9 µmol) and diluted with anhydrous $MeNO_2$ (3.59 mL, 5.0 mM). The mixture was then added Tf_2NH (25.2 mg, 5.0 equiv., 89.7 µmol) in dry $MeNO_2$ (3.59 mL, 5.0 mM) (total volume of 2.5 mM). The reaction mixture was then allowed to stir at 23 ºC for 1 h. After HPLC indicated reaction completion, the reaction mixture was quenched with iPr_2NEt (31.3 µL, 10.0 equiv., 379 µmol) and concentrated under reduced pressure to afford crude product. The crude product was then purified via preparative HPLC – (gradient 5% MeCN/H_2O → 60% MeCN/H_2O → 70% MeCN/H_2O) to afford desired product. Desired product was then repurified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) to afford title compound (8.0 mg, 45% isolated yield) as a colorless-film. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.15 (s, 1H), 9.03 (s, 1H), 8.65 (d, J = 7.6 Hz, 1H), 8.39 (t, J = 5.6 Hz, 1H), 8.05 (d, J = 7.6 Hz, 1H), 7.89 (m, 1H), 7.39 (m, 1H), 7.04–7.01 (m, 2H), 6.96 (s, 1H), 6.88 (m, 1H), 6.87 (m, 1H), 6.85 (m, 1H), 6.70 (d, J = 8.3 Hz, 1H), 6.54 (m, 1H), 6.18 (dt, J = 15.9, 6.4 Hz, 1H), 6.07 (d, J = 15.9 Hz, 1H), 4.58 (m, 2H), 4.35 (q, J = 6.9 Hz, 1H), 4.14 (m, 1H), 4.01 (dd, J = 15.8, 7.0 Hz, 1H), 3.80 (s, 3H), 3.47 (m, 2H), 3.42–3.34 (m, 4H), 2.91 (m, 1H), 2.77–2.71 (m, 2H), 1.84 (s, 3H), 1.21 (d, J = 7.4 Hz, 3H) ^{13}C (DMSO-d_6, 126 MHz) δ 171.7, 170.7, 169.8, 169.0, 168.7, 167.2, 153.5, 147.9,

140.7, 138.4, 137.1–136.9 (m, 1C), 130.8, 130.5, 130.1, 129.7, 129.0, 127.9, 127.9, 127.6, 125.4, 124.4, 117.3–117.1 (m, 1C), 117.0, 116.3–116.2 (m, 2C), 114.6, 112.9, 67.0–66.7 (m, 1C), 55.9, 54.1, 51.3, 48.9, 45.8, 42.6, 37.3, 32.2, 29.8, 29.0, 22.6, 17.7 ^{19}F (DMSO-d_6, 565 MHz) δ -106.0 and -121.6 (AB quartet, J = 248.4 Hz, 2F), -116.0 and -126.1 (AB quartet, J = 215.6 Hz, 2F) HRMS m/z calculated for $[C_{40}H_{40}F_4N_8O_9S+H]^+$ = 885.2653; found 885.2694; 3.1 ppm mass defect.

Polycycle **3.43**:

A flame-dried round bottom flask equipped with a stir bar was charged with compound crude peptide Ac-WCASC-NH_2, diluted with DMF (10 mM), added Et_3N (2.5 equiv.), and cooled to 0 °C. To the flask was added OFCP (3.0 equiv.) dropwise and let stir for 30 min. Then the flask was concentrated under reduced pressure. The crude intermediate macrocycle was then diluted with a DMF/THF mixture (1:4, 30 mM) a cooled to 0 °C. To the flask was added **3.40** (2.0 equiv.), Cs_2CO_3 (6.0 equiv.), and let stir until completion observed by HPLC. After completion the reaction was quenched with AcOH (15.0 equiv.), concentrated under reduced pressured and purified by RP-HPLC. The acyclic intermediate (34.0 mg, 1.0 equiv., 33.3 μmol) was then diluted with dry $MeNO_2$ (6.65 mL, 5 mM). To the flask was added $ScOTf_3$ (32.7 mg, 2.0 equiv., 66.5 μmol) and let stir until completion observed by HPLC. Upon completion, the mixture was concentrated under reduced pressure and purified by reverse-phase HPLC – (gradient 10% MeCN/H_2O → 65% MeCN/H_2O) to obtain **36** (12.0 mg, 35% isolated yield) and two other regioisomeric products for a 70% combined yield. Only one of the regioisomeric products were characterized for NMR. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.9 (s, 1H), 9.22 (s, 1H), 8.24 (d, J = 7.2 Hz, 2H), 7.94 (d, J = 8.8 Hz, 1H), 7.60 (d, J = 7.6 Hz, 1H), 7.50 (s, 1H), 7.26 – 7.07 (m, 6H), 6.96 (dt, J = 29.7, 7.3 Hz, 2H), 6.64 – 6.45 (m, 2H), 4.86 (d, J = 5.7 Hz, 1H), 4.80 (d, J = 9.6 Hz, 1H), 4.64 – 4.54 (m, 2H),

4.38 – 4.33 (m, 1H), 4.30 (t, J = 7.8 Hz, 1H), 3.82 (s, 3H), 2.44 – 2.16 (m, 2H), 3.13 – 3.06 (m, 4H), 2.90 – 2.80 (m, 1H), 2.79 – 2.70 (m, 1H), 2.67 (d, J = 13.9 Hz, 1H), 1.70 (s, 3H), 1.24 (d, J = 7.2 Hz, 3H). ^{13}C-NMR (500 MHz, DMSO-d_6) δ 173.2, 172.0, 169.3, 169.2, 168.1, 168.0, 148.9, 141.8, 137.4, 134.7, 133.1, 130.2, 130.0, 129.2, 128.9, 128.2, 126.8, 125.3, 124.6, 120.2, 118.3, 118.0, 117.2, 113.2, 110.6, 108.4, 74.2, 61.3, 55.9, 54.6, 54.2,52.7, 50.3, 32.9, 31.1, 30.7, 26.8, 22.8, 17.9. HRMS [M+1] calculated for $[C_{40}H_{41}F_4N_7O_9S+H]^+$ = 904.2422, found 904.2423; 0.1 ppm mass defect.

2.14 Synthesis of Fig. 3.8 Compounds

2-((3,3,4,4,5,5-hexafluoro-2-phenoxycyclopent-1-en-1-yl)thio)thiazol-5-yl)methyl isobutyl carbonate (**3.46**)

To a flame-dried dram vial equipped with a stir bar was charged with **3.33** (14.8 mg, 1.0 equiv, 35.8 μmol), diluted with dry CH_2Cl_2 (179 μL, 0.2 M), followed by pyridine (3.62 μL, 1.25 equiv, 44.8 μmol). The reaction mixture was then allowed to stir at 0 ºC and then was added isobutylchloroformate (5.92 μL, 98% wt., 2.25 equiv, 44.8 μmol). The reaction mixture was then allowed to warm up to 23 ºC and stirred for 2 h. When TLC indicated reaction completion, the reaction mixture was quenched with H_2O and then transferred to a sep. funnel. The aqueous was extracted with CH_2Cl_2 (2 x), the organic layers were combined, dried over Na_2SO_4, filtered, and concentrated to afford crude product. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 10% EtOAc/Hex) afforded title compound as a colorless oil (11.0 mg, 60% isolated yield); R_f = 0.54 at 20% EtOAc/Hex. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.65 (s, 1H), 7.31 (t, J = 7.6 Hz, 2H), 7.23 (t, J = 7.4 Hz, 1H), 7.03 (d, J = 8.3 Hz, 2H), 5.23 (s, 2H), 3.96 (d, J = 6.7 Hz, 2H), 1.98 (sept, J = 6.7 Hz, 1H), 0.95 (d, J = 6.7 Hz, 6H) ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.8, 155.1, 152.8, 144.3, 135.4, 129.9, 126.8, 119.5, 74.8, 60.8, 27.9, 19.0

tert-butyl ((2-((3,3,4,4,5,5-hexafluoro-2-phenoxycyclopent-1-en-1-yl)thio)thiazol-5-yl)methyl) carbonate (**3.47**)

To a dram vial equipped with a stir bar was added **3.33** (18.5 mg, 1.0 equiv, 44.8 μmol), DMAP (1.09 mg, 0.2 equiv, 8.95 μmol), diluted with CH_2Cl_2 (448 μL, 0.1 M), and then the reaction mixture allowed to stir at 0 ºC. Then Boc_2O (19.5 mg, 2.0 equiv, 89.5 μmol) and NEt_3 (12.5 μL, 2.0 equiv, 89.5 μmol) was added at 0 ºC, the reaction allowed to gradually warm up to 23 ºC, and

stir for 2 h. When TLC/HPLC indicated reaction completion, the reaction mixture was diluted with CH_2Cl_2 and quenched with the addition of water. The reaction mixture was then transferred to a sep. funnel, the aqueous layer extracted with CH_2Cl_2 (three times), dried over Na_2SO_4, filtered, and then concentrated to afford crude product. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 15% EtOAc/Hex) afforded title compound as a pale-yellow oil (15.6 mg, 68% isolated yield); R_f = 0.51 at 20% EtOAc/Hex. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.63 (s, 1H), 7.32 (t, J = 7.8 Hz, 2H), 7.24 (t, J = 7.5 Hz, 1H), 7.03 (d, J = 7.9 Hz, 2H), 5.16 (s, 2H), 1.50 (s, 9H) ^{13}C NMR ($CDCl_3$, 126 MHz) δ 158.6, 153.2, 152.8, 144.1, 135.8, 129.9, 126.8, 119.5, 83.5, 60.1, 27.8.

5-(chloromethyl)-2-((3,3,4,4,5,5-hexafluoro-2-phenoxycyclopent-1-en-1-yl)thio)thiazole (**3.48**)

To a flame-dried dram vial equipped with a stir bar was added 3.33 (14.8 mg, 1.0 equiv, 35.8 μmol) and diluted with dry PhMe (174 μmol, 0.206 M) and CH_2Cl_2 (34.8 μL, 1.03 M). The reaction mixture was then allowed to stir at 0 ºC and then was added $SOCl_2$ (4.04 μL, 97% wt., 1.5 equiv, 53.7 μmol). The reaction mixture was then allowed to warm up to 23 ºC and allowed to stir overnight. After TLC indicated reaction completion; the reaction mixture was concentrated under reduced pressure to afford crude product which was then partitioned between sat. $NaHCO_3$ and EtOAc and transferred to a sep. funnel. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated to afford crude product. The crude product was purified via flash SiO_2 chromatography (gradient Hex → 10% EtOAc/Hex) afforded title compound as a pale-yellow oil. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.58 (s, 1H), 7.33 (t, J = 8.2 Hz, 2H), 7.25 (t, J = 7.3 Hz, 1H), 7.03 (d, J = 7.9 Hz, 2H), 4.72 (s, 2H) ^{13}C NMR ($CDCl_3$, 126 MHz)

δ 158.5, 152.7, 142.8, 138.7, 129.9, 126.8, 119.5, 37.1. HRMS calculated for [$C_{15}H_8ClF_6NOS_2$+H] = 431.9713; found 431.9734; 4.9 ppm mass defect.

2.15 Synthesis of Fig. 3.10 Compounds

Macrocycle **3.53**

To a flame-dried round bottom flask equipped with a stir bar was charged with crude linear Ac-SGCFC-NH_2 (137.9 mg, 1.0 equiv., 0.2477 mmol), diluted with anhydrous DMF (30.63 mL, 10.0 mM), and then allowed to stir at 0 ºC. Then OFCP (445.9 mL, 1.5 equiv., 1.0 M in MeCN) was added followed by NEt_3 (107 μL, 2.5 equiv., 0.7658 mmol). The reaction mixture was allowed to stir at 0 ºC for 30 min. When HPLC indicated macrocycle intermediate formation, the reaction mixture was concentrated under reduced pressure to dryness. The reaction residue was added thiazole **3.52**, diluted with 1:4 DMF/THF (30 mM), and then stirred at 0 ºC. The reaction solution was then added KOTMS (3.5 equiv.) and the reaction mixture allowed to gradually warm up to 23 ºC for 3 h. When HPLC indicated reaction completion, the reaction mixture was added AcOH (10 equiv.) and then concentrated under reduced pressure to afford crude product. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (50.0 mg, 24% isolated yield) as a yellow-powder; R_f = 0.34 at 15% MeOH/$CHCl_3$. HRMS calculated for [$C_{33}H_{37}F_4N_7O_8S_4$+H] = 864.1601; found 864.1612; 1.3 ppm mass defect.

Macrocycle **3.55**

To a scintillation vial equipped with a stir bar and charged with 3.53 (20.0 mg, 1.0 equiv, 23.2 μmol) (stripped with PhH 3 x) was diluted with dry $MeNO_2$ (4.63 mL, 5.0 mM) and then added TFA (232 μL, 5 vol%). The reaction mixture was then allowed to stir at 23 ºC for 1 h. After HPLC indicated reaction completion, the reaction mixture was concentrated under reduced pressure to afford crude product. The crude product was then purified via flash SiO_2

chromatography (gradient $CHCl_3$ → 7% MeOH/$CHCl_3$) afforded title compound (12.7 mg, 65% isolated yield) as a yellow-powder; R_f = 0.44 at 15% MeOH/$CHCl_3$. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.06 (s, 1H), 9.00 (d, J = 8.3 Hz, 1H), 8.20 (d, J = 7.1 Hz, 1H), 8.08 (t, J = 5.6 Hz, 1H), 7.95 (d, J = 7.6 Hz, 1H), 7.88 (s, 1H), 7.67 (d, J = 9.1 Hz, 1H), 7.30–7.22 (m, 5H), 7.21–7.17 (m, 1H), 5.37 (m, 1H), 5.19 (m, 1H), 4.85–4.81 (m, 1H), 4.66–4.58 (m, 2H), 4.22–4.17 (m, 1H), 3.91 (dd, J = 11.6, 4.6 Hz, 1H), 3.74 (qd, J = 15.6, 5.7 Hz, 2H), 3.59 (d, J = 7.5, 4.1 Hz, 1H), 3.56–3.50 (m, 2H), 3.48–3.43 (m, 1H), 3.09 (q, J = 7.0 Hz, 1H), 2.97 (dd, J = 10.6, 3.4 Hz, 1H), 2.79–2.72 (m, 1H), 2.11 (s, 3H), 1.85 (s, 3H), 1.25–1.22 (m, 1H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 170.5, 169.5, 169.2, 168.8, 168.4, 167.3, 152.4, 145.0, 140.8, 138.0, 134.1, 128.9, 128.3, 126.4, 116.2, 70.6, 61.7, 55.3, 51.3, 47.2, 45.7, 41.8, 22.6, 20.9, 8.6 ^{19}F NMR (DMSO-d_6, 282 MHz) δ -96.7, -115.0 (AB quartet, J = 254.0 Hz, 2F), -120.4, -127.9 (AB quartet, J = 219.7 Hz, 2F). HRMS calculated for [$C_{33}H_{35}F_4N_7O_7S_4$+H] = 846.1495; found 846.1509; 1.7 ppm mass defect.

Macrocycle (**3.56**)

To a flame-dried round bottom flask equipped with a stir bar was charged with crude linear Ac-YACFAC-NH_2 (250.0 mg, 1.0 equiv., 0.348 mmol), diluted with anhydrous DMF (10.0 mM), and then allowed to stir at 0 ºC. Then OFCP (1.5 equiv., 1.0 M in MeCN) was added followed by NEt_3 (2.5 equiv). The reaction mixture was allowed to stir at 0 ºC for 30 min. When HPLC indicated macrocycle intermediate formation, the reaction mixture was concentrated under reduced pressure to dryness. The reaction residue was added thiazole **3.52**, diluted with 1:4 DMF/THF (30 mM), and then stirred at 0 ºC. The reaction solution was then added KOTMS (3.5 equiv.) and the reaction mixture allowed to gradually warm up to 23 ºC for 3 h. When HPLC indicated reaction completion, the reaction mixture was added AcOH (10 equiv.) and then concentrated under reduced pressure

to afford crude product. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (98.8 mg, 28% isolated yield) as a yellow-powder; R_f = 0.45 at 15% MeOH/$CHCl_3$. HRMS calculated for [$C_{43}H_{48}F_4N_8O_9S_4$+H] = 1025.2442; found 1025.2457; 1.5 ppm mass defect.

Macrocycle (**3.58**)

To a scintillation vial equipped with a stir bar and charged with **3.56** (20.0 mg, 1.0 equiv, 19.5 μmol) (stripped with PhH 3 x) was diluted with dry $MeNO_2$ (3.90 mL, 5.0 mM) and then added TFA (195 μL, 5 vol%). The reaction mixture was then allowed to stir at 23 ºC for 1 h. After HPLC indicated reaction completion, the reaction mixture was concentrated under reduced pressure to afford crude product. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (15.7 mg, 80% isolated yield) as an off-white powder; R_f = 0.51 at 15% MeOH/$CHCl_3$. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.61 (s, 1H), 9.18 (s, 1H), 8.52–8.43 (m, 2H), 8.23 (d, J = 8.0 Hz, 1H), 8.15 (d, J = 6.8 Hz, 1H), 8.01 (d, J = 8.1 Hz, 1H), 7.73 (s, 1H), 7.28–7.15 (m, 7H), 7.02 (d, J = 7.6 Hz, 2H), 6.62 (d, J = 7.6 Hz, 2H), 5.30–5.22 (m, 1H), 5.19–5.09 (m, 1H), 4.74–4.62 (m, 2H), 4.44–4.33 (m, 2H), 4.33–4.16 (m, 2H), 3.77–3.69 (m, 1H), 3.65–3.57 (m, 2H), 2.97–2.82 (m, 5H), 2.06 (s, 3H), 1.73 (s, 3H), 1.20-1.12 (m, 7H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.5, 171.6, 171.5, 170.7, 169.2, 168.9, 167.7, 143.2, 140.4, 136.8, 134.2, 130.0, 129.0, 128.3, 128.2, 126.6, 118.5, 116.1, 115.6, 114.8, 67.0, 55.6, 54.2, 51.4, 48.5, 48.2, 46.6, 36.8, 36.7, 25.1, 22.5, 20.8, 18.1, 17.3. HRMS calculated for [$C_{43}H_{46}F_4N_8O_8S_4$+H] = 1007.2336; found 1007.2359; 2.3 ppm mass defect.

2.16 Synthesis of Fig. 3.11 Compounds

1-((2-(3-((3,3,4,4,5,5-hexafluoro-2-phenoxycyclopent-1-en-1-yl)oxy)phenyl)propan-2-yl)oxy)-2,2,6,6-tetramethylpiperidine (**3.60**)

To a flame-dried dram vial equipped with a stir bar was charged with **2.5** (10.8 mg, 1.0 equiv, 37.7 μmol), diluted DMF (755 μL, 0.05 M), and then added **3.59** (12.1 mg, 1.1 equiv, 41.5 μmol) followed by Cs_2CO_3 (13.5 mg, 1.1 equiv, 41.5 μmol). The reaction mixture was then allowed to stir at 23 ºC for 1 h. When HPLC indicated reaction completion; the reaction mixture quenched with AcOH (10.8 μL, 5.0 equiv, 189 μmol). The reaction mixture was then concentrated under reduced pressure to afford crude product as a yellow oil. The crude product was then purified via flash SiO_2 chromatography (gradient Hex → 1% EtOAc/Hex) afforded title compound (14.8 mg, 70% isolated yield) as a colorless oil; R_f = 0.35 at 1% EtOAc/Hex. ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.20–7.14 (m, 3H), 7.10–7.01 (m, 2H), 6.91 (q, J = 1.7 Hz, 1H), 7.78–7.76 (m, 1H), 7.76–7.74 (m, 1H), 6.55 (ddd, J = 8.1, 2.6, 0.8 Hz, 1H), 1.47 (s, 6H), 1.44–1.40 (m, 2H), 1.40–1.37 (m, 1H), 1.31–1.20 (m, 3H), 1.06 (s, 6H), 0.81 (s, 6H) ^{13}C NMR ($CDCl_3$, 126 MHz) δ 154.5, 154.2, 153.4, 129.6, 128.8, 124.9, 122.0, 117.1, 114.9, 114.6, 79.6, 59.6, 40.9, 34.0, 28.3, 20.7, 17.2. HRMS calculated for [$C_{29}H_{33}F_6NO_3$+H] = 558.2437; found 558.2459; 3.9 ppm mass defect.

Product **3.62** or **3.63**

To an oven dried dram vial equipped with a stir bar was charged with **3.60** (14.8 mg, 1.0 equiv, 26.5 μmol), Ir[(dF(CF3)ppy)$_2$dtbbpy]PF6 (602 μg, 99% wt., 2 mol %, 0.531 μmol), diluted with anhydrous MeNO2 (265 μL, 0.1 M), followed by p-cresol (5.0 equiv). The reaction mixture was then sparged for 5 min with argon and subsequent cap switch to a screw cap. The reaction mixture was then allowed to stir at 23 ºC under blue LEDs. Reaction was checked at 12 h which indicated complete consumption of **3.60** into either intramocular Friedel-crafts product **3.62** or alkene

formation **3.63**. Concentration afforded crude product that was unstable to SiO_2 purification and decomposed. HRMS calculated for $[C_{26}H_{26}F_6O_3+H]$ = 501.1864; found 501.1879; 3.0 ppm mass defect.

2.17 Synthesis of Fig. 3.12 Compounds

Macrocycle **3.65**

To a flame-dried 25-mL round bottom flask equipped with a stir bar was charged with Ac-EACFAC-NH_2, diluted with dry DMF (7.17 mL, 0.05 M), and then allowed to stir at 0 ºC. Then NEt_3 (150 µL, 3 Eq, 1.07 mmol) was added followed by OFCP (114 mg, 537 µL, 1.0 molar, 1.5 Eq, 537 µmol). The reaction mixture was allowed to stir at 0 ºC for 30 min (until HPLC indicated completion). Once the initial macrocycle intermediate was formed, to the reaction mixture was removed excess NEt_3, OFCP, and MeCN under reduced pressure. The reaction mixture was then added REACTANT followed by KOTMS (121 mg, 95% Wt, 2.5 Eq, 896 µmol) at 23 ºC and the mixture allowed to stir at 23 ºC for x h. Once HPLC indicated completion, the reaction mixture was quenched with AcOH. The reaction mixture was then removed excess AcOH under reduced pressure to afford crude product. HRMS calculated for $[C_{38}H_{44}F_4N_8O_{10}S_4+H]$ = 977.2078; found 977.2090; 1.2 ppm mass defect.

2.18 Synthesis of **Fig. 3.13** Compounds

Macrocycle **3.71**

To a flame-dried round bottom flask equipped with a stir bar was charged with crude Ac-D*GSFAC-NH_2 (1.0 equiv), diluted with anhydrous DMF (10.0 mM), and then allowed to stir at 0 ºC. Then OFCP (1.5 equiv., 1.0 M in MeCN) was added followed by NEt_3 (196 µL, 2.5 equiv., 1.41 mmol). The reaction mixture was allowed to stir at 0 ºC for 30 min. When HPLC indicated macrocycle intermediate formation, the reaction mixture was concentrated under reduced pressure to dryness. The reaction residue was then added 1:4 DMF:THF (15.65 mL, 30 mM) and cooled to 0 ºC. Then Cs_2CO_3 (6.0 equiv) was added, and the reaction mixture allowed to stir at 0 ºC for 1 h. When HPLC indicated full conversion to the desired vinyl fluoride then NaN_3 (1.2 equiv) was added and the reaction mixture allowed to stir at 0ºC for 1 h. When HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (10.0 equiv) and then concentrated under reduced pressure to afford crude product. The crude product was then telescoped to the next step.

Assumed a 50% yield from General Procedure F when calculating stoichiometry. To the same round bottom flask used for General Procedure F, the crude product was diluted with a solution 2:8 MeCN:aqueous buffer pH 4.5 (NaOAc/AcOH, 0.2 M). The reaction mixture was then allowed to stir at 23 ºC and then was added NCS in MeCN (1.0 M) portionwise (0.5 equiv.). Total NCS varied from 1.5–3.0 equiv. (1.5 equiv. for precursor that led to **40**) depending on substrate. When HPLC indicated full conversion to the desired free acid, the reaction mixture was quenched with $Na_2S_2O_3$•$5H_2O$ (6.0 equiv.). The reaction mixture was concentrated under reduced pressure to afford crude product.

To a flame-dried 5-mL round bottom flask equipped with a stir bar was charged with compound crude azide**,** diluted with EtOH (50 mM) and flushed with argon. Pd/C (10 wt. %) was then added, flushed with hydrogen gas, and warmed to 35 °C. The reaction mixture was then stirred at 35 ºC for 16 h. After completion the reaction was filtered with celite and concentrated under reduced pressure. Purification by reverse-phase HPLC gave crude seco acid.To a flame-dried round bottom flask equipped with a stir bar was charged with the acyclic amino acid (8.72 mg, 1.0 equiv., 11.1 μmol) and diluted with anhydrous DMF (2.08 mL, 5.0 mM). The solution was added NEt_3 (5.0 equiv) and the reaction mixture allowed to cool to 0 ºC. Then EDC•HCl (1.5 equiv) was added followed by HOBt (1.5 equiv)and the reaction mixture allowed to stir at 23 ºC for 16 h. After HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (10.0 equiv) and then concentrated under reduced pressure to afford crude product. The crude product was then purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 65% MeCN/H_2O) afforded title compound as a colorless film (33% overall yield from the linear peptide). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.65–8.58 (m, 2H), 8.47 (d, J = 6.4 Hz, 1H), 7.38 (d, J = 7.5 Hz, 1H), 7.32–7.26 (m, 2H), 7.26–7.18 (m, 4H), 7.13 (m, 1H), 6.85 (s, 1H), 6.66 (s, 2H), 5.09 (d, J = 6.7 Hz, 1H), 4.73 (d, J = 9.8 Hz, 1H), 4.60–4.51 (m, 1H), 4.30 (d, J = 4.7 Hz, 1H), 4.27–4.16 (m, 1H), 4.15–4.07 (m, 1H), 4.07–3.99 (m, 1H), 3.69 (pent, J = 6.8 Hz, 1H), 3.09–3.00 (m, 1H), 3.00–2.89 (m, 3H), 2.70 (t, J = 11.4 Hz, 1H), 2.57–2.52 (m, 1H), 1.83 (s, 3H), 1.16 (d, J = 7.1 Hz, 3H) ^{13}H NMR (DMSO-d_6, 126 MHz) δ 175.4, 174.1, 171.7, 171.4, 170.5, 170.0, 169.8, 164.3, 148.0–147.6 (m, 1C), 136.7, 129.2, 128.2, 126.6, 116.9–116.0 (m, 1C), 114.8–114.3 (m, 1C), 98.7–98.3 (m, 1C), 97.3–97.0 (m, 1C), 68.8, 58.3, 57.3, 52.1, 49.6, 48.3, 40.7, 36.5, 34.9, 31.9, 22.1, 15.2. HRMS calculated for [$C_{31}H_{34}F_4N_8O_9S$+H] = 771.2184; found 771.2198; 1.8 ppm mass defect.

2.19 Synthesis of Fig. 3.14 Compounds

Polycycle **3.40**:

Followed General Procedure 4F–4H.

To a flame-dried round bottom flask equipped with a stir bar was charged with crude Ac-D*GSFAC-NH_2 (339.3mg, 1.0 equiv., 469.4 μmol), diluted with anhydrous DMF (46.94 mL, 10.0 mM), and then allowed to stir at 0 ºC. Then OFCP (704.1 μL, 1.5 equiv., 1.0 M in MeCN) was added followed by NEt_3 (196 μL, 2.5 equiv., 1.41 mmol). The reaction mixture was allowed to stir at 0 ºC for 30 min. When HPLC indicated macrocycle intermediate formation, the reaction mixture was concentrated under reduced pressure to dryness. The reaction residue was then added 1:4 DMF:THF (15.65 mL, 30 mM) and cooled to 0 ºC. Then Cs_2CO_3 (917.6 mg, 6.0 equiv., 2.82 mmol) was added, and the reaction mixture allowed to stir at 0 ºC for 1 h. When HPLC indicated full conversion to the desired vinyl fluoride then cysteamine (64.0 mg, 1.2 equiv., 563.3 μmol) was added and the reaction mixture allowed to stir at 0ºC for 1 h. When HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (268.7 μL, 10.0 equiv., 4.694 mmol) and then concentrated under reduced pressure to afford crude product. The crude product was then telescoped to the next step.

Assumed a 50% yield from General Procedure F when calculating stoichiometry. To the same round bottom flask used for General Procedure F, the crude product was diluted with a solution 2:8 MeCN:aqueous buffer pH 4.5 (NaOAc/AcOH, 0.2 M). The reaction mixture was then allowed to stir at 23 ºC and then was added NCS in MeCN (1.0 M) portionwise (0.5 equiv.). Total NCS varied from 1.5–3.0 equiv. (1.5 equiv. for precursor that led to **40**) depending on substrate. When HPLC indicated full conversion to the desired free acid, the reaction mixture was quenched with $Na_2S_2O_3$•$5H_2O$ (6.0 equiv.). The reaction mixture was concentrated under reduced pressure to

afford crude product. The crude product was then purified via preparative HPLC – (gradient 10% MeCN/H_2O → 55% MeCN/H_2O) afforded the acyclic amino acid (161.5 mg, 41% isolated yield) as a white-powder.

To a flame-dried round bottom flask equipped with a stir bar was charged with the acyclic amino acid (10.0 mg, 1.0 equiv., 10.4 μmol) and diluted with anhydrous DMF (2.08 mL, 5.0 mM). The solution was added iPr_2NEt (6.3 μL, 3.5 equiv., 36.4 μmol) and the reaction mixture allowed to cool to 0 ºC. Then propanephosphonic acid anhydride in EtOAc (9.3 μL, 1.5 equiv., 50% wt.) was added and the reaction mixture allowed to stir at 0 ºC for 1 h. After HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (2.98 μL, 5.0 equiv., 52 μmol) and then concentrated under reduced pressure to afford crude product. The crude product was then purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 65% MeCN/H_2O) afforded title compound (6.4 mg, 74% isolated yield, 30% from linear peptide) as a white-powder. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.61 (d, J = 4.1 Hz, 1H), 8.07 (d, J = 7.5 Hz, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.80–7.74 (m, 1H), 7.56–7.51 (m, 1H), 7.50 (d, J = 7.6 Hz, 1H), 7.41 (s, 1H), 7.32–7.24 (m, 2H), 7.26–7.21 (m, 3H), 4.97–4.91 (m, 1H), 4.86–4.80 (m, 1H), 4.55–4.49 (m, 1H), 4.26–4.21 (m, 1H), 4.20–4.14 (m, 2H), 4.09–4.04 (m, 1H), 3.58–3.50 (m, 2H), 3.37–3.25 (m, 2H), 3.18–3.06 (m, 3H), 3.03–2.95 (m, 2H), 2.87–2.83 (m, 1H), 2.47–2.40 (m, 1H), 2.32–2.26 (m, 1H), 1.80 (s, 3H), 1.16 (d, J = 6.8 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.4, 171.2, 170.9, 168.9, 168.6, 168.3, 167.8, 162.3, 140.9–140.2 (m, 1C), 136.9, 129.0, 128.3, 126.6, 120.6–120.3 (m, 1C), 118.5–118.1 (m, 1C), 116.1–115.7 (m, 1C), 71.3, 69.0–68.9 (m, 1C) 57.3, 56.4, 52.6, 50.4, 48.5, 41.6, 41.1, 36.1, 35.8, 32.0, 30.3, 22.5, 16.0 ^{19}F NMR (DMSO-d_6, 282 MHz) δ -103.5, -107.6 (AB quartet, J = 247.0 Hz, 2F), -115.3, -120.2 (AB quartet, J = 229.0 Hz, 2F). HRMS [M+1] calculated for $[C_{33}H_{38}F_4N_8O_9S_2+H]^+$ = 831.2218; found 831.2225; 0.8 ppm mass defect.

Polycycle **3.75**:

Followed general procedure 4F, 4G, and 4H.

Followed General Procedure 4F and 4G using crude peptide Ac-E*ASFAC-NH_2 (126. mg, 1.0 equiv., 165 μmol) afforded crude acyclic amino acid. Crude product was purified via reverse-phase HPLC – (gradient 5% MeCN/H_2O → 45% MeCN/H_2O) to afford acyclic amino acid (65.5 mg, 40% isolated yield) as a yellow-film. HRMS calculated for $[C_{36}H_{46}F_4N_8O_{10}S_2+H]^+$ = 890.2715; found 890.2734; 2.1 ppm mass defect. Followed General Procedure 4H to obtain macrolactam using acyclic amino acid (40.0 mg, 1.0 equiv., 39.8 μmol) afforded crude product. Crude product was purified via reverse-phase HPLC – (gradient 5% → 70% MeCN/H_2O → 85% MeCN/H_2O) to afford title compound (27.8 mg, 80% isolated yield, 32% from linear peptide) as an off-white powder. NMR of **41** contained complex mixture of rotational isomers; ratio ~ 2:3 + minor isomers. All rotational isomers were characterized. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.21 (d, J = 6.45 Hz, 0.62H), 8.78 (d, J = 5.75 Hz, 1H), 8.39 (d, J = 8.10 Hz, 1H), 8.29 (d, J = 8.45 Hz, 1H), 8.23 (d, J = 7.20 Hz, 1H), 8.07 (d, J = 8.65 Hz, 1H), 7.95 (s, 1.7H), 7.71 (d, J = 7.60 Hz, 1H), 7.62 (d, J = 6.45 Hz, 1H), 7.42 (s, 1.7H), 7.32 (d, J = 17.55 H, 2H), 7.25-7.18 (m, 6H), 7.15-7.07 (m, 5H), 6.23 (d, J = 3.95 Hz, 1H), 4.98-4.82 (m , 1H), 4.81-4.71 (m, 2H), 4.67-4.59 (m, 2H), 4.41-4.30 (m, 1H), 4.24-4.08 (m, 6H), 3.60-3.42 (m, 4H), 3.38-3.28 (m, 2H), 3.28-3.16 (m, 3H) 3.16-3.04 (m, 4H), 3.03 (s, 3H), 2.99 (s, 3H), 2.97-2.90 (m, 3H), 1.96 (s, 2H), 1.90 (s, 3H), 1.86-1.79 (m, 3H), 1.78-1.65 (m, 4H), 1.56-1.46 (m, 2H), 1.23 (d, J = 6.55 Hz, 3H), 1.17 (d, J = 2.33 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.7, 172.3, 172.5, 171.8, 171.3, 171.3, 171.3, 171.3, 171.2, 171.1, 170.7, 170.6, 169.5, 169.3, 168.9, 168.6, 147.0–146.9 (m, 1C), 141.1–141.0 (m, 1C), 137.3, 137.0, 128.9, 128.9, 128.2, 128.0, 126.3, 118.9–118.8 (m, 1C), 116.6, 114.3, 112.0–111.9 (m, 1C), 71.3–

71.1 (m, 2C), 59.3, 58.6, 58.4, 53.5, 53.4, 53.2, 52.9, 48.3, 48.2, 47.5, 42.4, 35.8, 35.6, 34.8, 34.7, 34.0, 31.1, 30.3, 30.0, 29.9, 29.3, 29.2, 28.5, 27.7, 26.6 HRMS calculated for $[C_{36}H_{44}F_4N_8O_9S_2+H]^+$ = 873.2687; found 873.2691; 0.5 ppm mass defect.

Polycycle **3.76**:

Followed general procedure 4F, 4G, and 4H.

Followed General Procedure 4F and 4G using crude peptide Ac-E*VTFAC-NH_2 (100. mg, 1.0 equiv., 126 μmol) afforded crude acyclic amino acid. Crude product was purified via reverse-phase HPLC – (gradient 5% MeCN/H_2O → 55% MeCN/H_2O) to afford acyclic amino acid (42.5 mg, 33% isolated yield) as a yellow-film. HRMS calculated for $[C_{38}H_{50}F_4N_8O_{10}S_2+H]^+$ = 919.3106; found 918.3110; 0.4 ppm mass defect. Followed General Procedure 4H to obtain macrolactam using acyclic amino acid (31.5 mg, 1.0 equiv., 30.5 μmol) afforded crude product. Crude product was purified via reverse-phase HPLC – (gradient 5% → 65% MeCN/H_2O → 80% MeCN/H_2O) to afford title compound (19.8 mg, 72% isolated yield, 24% from linear peptide) as an off-yellow powder. NMR of **42** contained complex mixture of rotational isomers; ratio ~ 2:3 + minor isomers. All rotational isomers were characterized. See VT NMR experiment. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.36–8.30 (m, 1.5 H), 8.09 (d, J = 7.8 Hz, 1H), 8.00 (d, J = 7.9 Hz, 0.5 H), 7.50 (s, 0.5H), 7.92–7.87 (m, 1.5H), 7.83 (d, J = 7.8 Hz, 1H), 7.79 (d, J = 7.9 Hz, 1H), 7.63 (d, J = 8.3 Hz, 0.5H), 7.40 (s, 1H), 7.34 (s, 1H), 7.30–7.24 (m, 4H), 7.24–7.17 (m, 5H), 6.47 (t, J = 4.8 Hz, 1H), 5.12–5.04 (m, 1H), 4.61–4.50 (m, 2H), 4.44–4.27 (m, 2H), 4.19–4.11 (m, 2H), 4.11–4.04 (m, 1H), 3.61–3.52 (m, 0.5H), 3.52–3.47 (m, 1H), 3.45–3.32 (m, 1H), 3.30–3.18 (m, 1.5 H), 3.18–3.09 (m, 2H), 3.07–3.00 (m, 1.5 H), 2.99–2.90 (m, 1H), 2.33–2.21 (m, 1H), 2.07–2.00 (m, 1H), 1.98–1.87 (m, 1.5H), 1.83 (s, 3H), 1.80 (s, 2H), 1.44–1.39 (m, 4H), 1.25–1.22 (m, 1H), 1.20 (d, J = 6.7 Hz, 2H),

1.15 (d, J = 6.8 Hz, 3H), 0.98–0.92 (m, 1.5H), 0.89 (d, J = 6.7 Hz, 3H), 0.86–0.82 (m, 4H), 0.81 (d, J = 6.7 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.8, 172.4, 171.9, 171.8, 171.7, 171.7, 171.1, 171.7, 171.4, 170.9, 170.7, 170.4, 168.7, 168.4, 167.7, 167.3, 147.2–147.1 (m, 1C), 145.8–145.7 (m, 1C), 140.1–140.0 (m, 1C), 137.0, 136.8, 128.7, 128.5, 128.3, 128.3, 126.5, 126.4, 119.0–118.9 (m, 1C), 116.8–116.7 (m, 1C), 114.5–114.2 (m, 1C), 112.2–112.0 (m, 1C), 64.3, 50.1, 56.3, 55.8, 53.3, 53.3, 51.8, 50.7, 48.5, 48.3, 41.5, 41.3, 30.8, 35.5, 34.4, 33.6, 30.6, 30.3, 30.0, 29.9, 29.3, 29.2, 29.0, 28.7, 28.4, 27.9, 26.8, 22.5, 21.0, 20.9, 19.8, 19.6, 17.0, 16.5, 16.4, 16.3, 16.2, 16.0 HRMS calculated for $[C_{38}H_{48}F_4N_8O_9S_2+H]^+$ = 901.3000; found 901.3004; 0.4 ppm mass defect.

Polycycle **3.77**:

Followed general procedure 4F, 4G, and 4H.

Followed General Procedure 4F and 4G using crude peptide Ac-D*PTFSC-NH_2 (194.3 mg, 1.0 equiv., 181 μmol) afforded crude acyclic amino acid. Crude product was purified via reverse-phase HPLC – (gradient 5% MeCN/H_2O → 45% MeCN/H_2O) to afford acyclic amino acid (80.2 mg, 34% isolated yield) as a yellow-film. HRMS calculated for $[C_{44}H_{54}F_4N_{12}O_{12}S_2+H]^+$ = 1083.3440; found 1083.3448; 0.7 ppm mass defect. Followed General Procedure 4H to obtain macrolactam using acyclic amino acid (40.0 mg, 1.0 equiv., 30.5 μmol) afforded crude product. Crude product was purified via reverse-phase HPLC – (gradient 5% → 45% MeCN/H_2O → 50% MeCN/H_2O) to afford title compound (10.8 mg, 30% isolated yield, 10% from linear peptide) as a yellow-powder. NMR of **43** contained complex mixture of rotational isomers; ratio ~ 1:10:35. Major rotational isomer was characterized. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.31 (s, 1H), 8.25–8.21 (m, 2H), 8.05 (d, J = 7.2 Hz, 1H), 7.84 (d, J = 8.3 Hz, 1H), 7.74 (d, J = 6.4 Hz, 1H), 7.61 (t, J = 4.8 Hz, 1H), 7.57

(s, 1H), 7.39 (s, 1H), 7.31–7.24 (m, 5H), 7.22–7.19 (m, 2H), 6.97 (s, 1H), 4.85–4.74 (m, 3H), 4.58–4.49 (m, 1H), 4.44–4.32 (m, 2H), 4.29 (dd, J = 9.3, 2.1 Hz, 1H), 4.21 (dd, J = 8.5, 4.4 Hz, 1H), 4.18–4.12 (m, 1H), 3.71–3.63 (m, 1H), 3.62–3.55 (m, 2H), 3.24–3.08 (m, 4H), 3.07–2.99 (m, 3H), 2.86–2.78 (m, 3H), 2.71–2.67 (m, 1H), 2.28 (dd, J = 15.2, 8.9 Hz, 1H), 2.12–2.04 (m, 1H), 1.83 (s, 3H), 1.82–1.79 (m, 2H), 1.77–1.74 (m, 2H), 0.96 (d, J = 6.3 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.8, 171.3, 171.2, 171.1, 170.9, 170.4, 170.2, 169.5, 168.9, 168.4, 131.4–131.3 (m, 1C), 129.3–129.2 (m, 1C), 129.0, 128.3, 126.5, 117.1, 115.8–115.7 (m, 1C), 114.7, 112.5–112.4 (m, 1C), 67.5–67.3 (m, 1C), 67.0, 66.5, 60.5, 58.8, 56.2, 53.6, 52.4, 49.7, 47.8, 47.1, 37.3, 36.3, 35.8, 33.0, 30.8, 29.0, 25.1, 24.3, 22.3, 20.1 ^{19}F NMR (DMSO-d_6, 565 MHz) δ -94.3 and -114.1 (AB quartet, J = 251.4 Hz, 2F), -114.1 and -127.0 (AB quartet, J = 230.0 Hz, 2F) HRMS calculated for $[C_{44}H_{51}F_4N_{12}O_{11}S_2+H]^+$ = 1065.3334; found 1065.3350; 1.5 ppm mass defect.

Polycycle **3.78**:

Followed general procedure 4F, 4G, and 4H.

Followed General Procedure 4F–4G using crude peptide Ac-E*PSFTC-NH_2 (126. mg, 1.0 equiv, 165 μmol) afforded crude acyclic incipient amino acid. Crude product was purified via reverse-phase HPLC – (gradient 5% MeCN/H_2O → 55% MeCN/H_2O) to afford acyclic incipient amino acid (64.3 mg, 33% isolated yield) as a yellow-film. Followed General Procedure 4H to obtain macrolactam using acyclic amino acid (20.0 mg, 1.0 equiv., 19.1 μmol) afforded crude product. Crude product was purified via reverse-phase HPLC – (gradient 5% MeCN/H_2O → 65% MeCN/H_2O → 75% MeCN/H_2O) followed by a repurification via flash column chromatography SiO_2 – (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (8.7 mg, 50% isolated yield, 17% isolated yield from linear peptide) as a white-film. NMR of **44**

contained complex mixture of rotational isomers. Major rotational isomer was characterized. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.13 (d, J = 7.5 Hz, 1H), 8.06 (d, J = 8.4 Hz, 1H), 7.97 (t, J = 5.6 Hz, 1H), 7.94 (d, J = 8.2 Hz, 1H), 7.47 (d, J = 9.3 Hz, 1H), 7.28–7.22 (m, 4H), 7.21–7.17 (m, 2H), 7.06 (s, 1H), 4.90 (dd, J = 6.3, 3.2 Hz, 1H), 4.85 (t, J = 5.5 Hz, 1H), 4.61 (dd, J = 9.4, 3.1 Hz, 1H), 4.54 (td, J = 8.8, 3.3 Hz, 1H), 4.45 (q, J = 7.3 Hz, 1H), 4.38 (dd, J = 8.2, 3.4 Hz, 1H), 4.35–4.29 (m, 1H), 3.73–3.66 (m, 2H), 3.60 (m, 1H), 3.50–3.41 (m, 4H), 3.26–3.22 (m, 1H), 3.15–3.00 (m, 3H), 3.00–2.90 (m, 2H), 1.92–1.89 (m, 1H), 1.89–1.84 (m, 2H), 1.81 (s, 3H), 1.77–1.69 (m, 4H), 1.04 (d, J = 6.4 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.8, 171.5, 171.1, 170.4, 169.9, 169.1, 168.0, 167.9, 138.0, 134.8–134.6 (m, 1C), 134.6–134.4 (m, 1C), 129.1, 128.2, 126.3, 119.1–118.9 (m, 2C), 71.0, 71.0–70.9 (m, 1C), 61.6, 59.0, 55.9, 54.7, 54.3, 51.7, 49.8, 46.9, 45.6, 35.9, 30.7, 29.0, 28.9, 28.6, 25.1, 24.3, 22.2, 15.4 ^{19}F NMR (DMSO-d_6, 282 MHz) δ -99.6, -118.1 (AB quartet, J = 252 Hz, 2F), -120.3, -133.0 (AB quartet, J = 232 Hz, 2F). HRMS m/z calculated for $[C_{38}H_{46}F_4N_8O_{10}S_2+H]^+$ = 915.2783; found 915.2799; 1.8 ppm mass defect.

Polycycle **3.79**:

Followed general procedure 4F, 4G, and 4H.

Crude peptide Ac-E*VTFAC-NH_2 (123. mg, 1.0 equiv., 158 μmol) was subjected to general procedure 4F, 4G and reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 55% MeCN/H_2O) to afford acyclic amino acid (50.3 mg, 31% isolated yield) as a yellow-film. HRMS calculated for $[C_{37}H_{46}F_4N_8O_{11}S_2+H]^+$ = 905.2949; found 905.2978; 3.2 ppm mass defect. Followed General Procedure 4H to obtain macrolactam **45**. Acyclic amino acid (40.0 mg, 1.0 equiv., 39.3 μmol) afforded crude product. Crude product was purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 70% MeCN/H_2O → 85% MeCN/H_2O) to afford

title compound (25.2 mg, 72% isolated yield, 23% yield from peptide). NMR of **46** contained complex mixture of rotational isomers; ratio ~ 2:1 + minor isomers. Both rotational isomers were characterized. See VT NMR experiment. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.65 (d, J = 6.2 Hz, 0.5 H), 8.37 (d, J = 7.4 Hz, 1H), 8.31 (s, 0.5H), 8.24 (d, J = 5.7 Hz, 1H), 8.11 (d, J = 6.0 Hz, 1H), 8.03 (dd, J = 11.8, 6.7 Hz, 1.4H), 7.93–7.88 (m, 1.5H), 7.86 (s, 0.5H), 7.82 (d, J = 7.9 Hz, 1H), 7.50–7.44 (m, 1.5 H), 7.33 (s, 1H), 7.30–7.21 (m, 4H), 7.20–7.15 (m, 5H), 6.31 (t, J = 4.0 Hz, 1H), 4.83 (d, J = 7.0 Hz, 1H), 4.74 (d, J = 6.8 Hz, 1H), 4.72–4.66 (m, 1.5H), 4.59–4.48 (m, 2H), 4.38–4.31 (2H), 4.30–4.21 (m, 2.5H), 4.14–4.09 (m, 1H), 4.08–4.01 (m, 1H), 3.98–3.92 (m, 2H), 3.89–3.84 (m, 1H), 3.75–3.71 (m, 0.5H), 3.60 (t, J = 6.5 Hz, 1H), 3.54–3.48 (m, 1H), 3.48–3.32 (m, 4H), 3.31–3.14 (m, 4H), 3.13–3.05 (m, 2H), 3.04–2.98 (m, 2H), 2.90–2.83 (m, 1.5H), 2.24–2.16 (m, 1H), 2.14 (m, 1H), 1.85 (s, 1.5 H), 1.83 (s, 3H), 1.19 (d, J = 6.6 Hz, 1.5H), 1.09 (d, J = 6.8 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H), 0.86 (d, J = 6.8 Hz, 3H), 0.80 (d, J = 6.7 Hz, 1.5H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.4, 173.4, 172.8, 172.4, 172.2, 172.1, 171.8, 171.8, 171.4, 171.2, 170.5, 170.4, 170.2, 169.2, 169.0, 168.9, 148.6–148.5 (m, 1C), 145.7–145.6 (m, 1C), 139.6–139.5 (m, 1C), 137.5, 137.4, 129.3, 129.0, 128.7, 126.9, 119.9, 118.5, 117.4–117.1 (m, 1C), 115.1–114.9 (m, 1C), 112.6–112.5 (m, 1C), 70.3–70.2 (m, 1C), 58.7, 58.2, 57.5, 57.0, 53.6, 53.5, 51.3, 51.1, 49.8, 49.0, 48.8, 46.2, 42.1, 36.4, 35.5, 34.5, 33.0, 31.0, 30.3, 30.2, 29.5, 28.8, 28.6, 27.9, 27.7, 26.4, 25.6, 25.0, 24.2, 22.9, 20.3, 20.1, 16.7, 16.6, 16.1, 16.1 ^{19}F NMR (DMSO-d_6, 282 MHz) δ -103.7 and -106.0 (AB quartet, J = 251.1 Hz, 2F), -116.6 and -122.0 (AB quartet, J = 229.2 Hz, 2F), -106.2 and -120.8 (AB quartet, J = 250.5 Hz, 2F minor)-106.4 and -115.7 (AB quartet, J = 227.4 Hz, 2F minor) HRMS calculated for $[C_{37}H_{46}F_4N_8O_9S_2+H]^+$ = 887.2844; found 887.2851; 0.8 ppm mass defect.

Polycycle **3.80**:

Followed general procedure 4F, 4G, and 4H.

Crude peptide Ac-D*PTFSC-NH_2 (130. mg, 1.0 equiv., 164 μmol) was subjected to general procedure 4F, 4G and reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 55% MeCN/H_2O) to afford acyclic amino acid (44.1 mg, 26% isolated yield) as a red-foam. HRMS calculated for $[C_{37}H_{46}F_4N_8O_{11}S_2+H]^+$ = 919.2742; found 919.2754; 1.3 ppm mass defect. Followed General Procedure 4H to obtain macrolactam **46**. Acyclic amino acid (35.0 mg, 1.0 equiv, 33.9 μmol) afforded crude product. Crude product was purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 55% MeCN/H_2O → 65% MeCN/H_2O) to afford desired product. Desired product was then repurified via pTLC; 10% MeOH/$CHCl_3$ solvent system to afford title compound (7.2 mg, 24% isolated yield, 6% yield from linear peptide) as a colorless-film. NMR of **46** contained complex mixture of rotational isomers; ratio ~ 1:1 + minor isomers. Both rotational isomers were characterized. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.61 (d, J = 6.8 Hz, 1H), 8.37 (d, J = 8.9 Hz, 1H), 8.30–8.26 (m, 1H), 8.15 (t, J = 5.0 Hz, 1H), 8.03 (d, J = 7.6 Hz, 1H), 7.95 (t, J = 5.3 Hz, 1H), 7.73–7.65 (m, 4H), 7.44–7.41 (m, 1H), 7.26–7.21 (m, 9H), 7.20–7.16 (m, 2H), 7.08–7.02 (m, 2H), 5.32 (t, J = 4.7 Hz, 1H), 4.91–4.87 (m, 1H), 4.80–4.76 (m, 1H), 4.76–4.67 (m, 4H), 4.62–4.55 (m, 2H), 4.51 (m, 1H), 4.42–4.36 (m, 2H), 4.03 (dd, J = 11.4, 5.5 Hz, 1H), 3.91 (dd, J = 12.2, 6.1 Hz, 1H), 3.78–3.74 (m, 3H), 3.73–3.69 (m, 3H), 3.65–3.62 (m, 1H), 3.61–3.59 (m, 1H), 3.51–3.57 (m, 1H), 3.45–3.42 (m, 1H), 3.42–3.39 (m, 2H), 3.34–3.31 (m, 2H), 3.17–3.11 (m, 4H), 3.10–3.06 (m, 3H), 3.05–2.98 (m, 2H), 2.95–2.90 (m, 2H), 2.77 (dd, J = 14.0, 11.3 Hz, 1H), 2.46–2.42 (m, 2H), 2.34 (dd, J = 16.8, 5.4 Hz, 1H), 2.26 (dd, J = 15.7, 8.8 Hz, 1H), 2.04–1.96 (m, 3H), 1.94–1.90 (m, 1H), 1.86–1.80 (m, 4H), 1.76 (s, 6H), 0.85 (d, J = 6.3 Hz, 3H), 0.63 (d, J = 6.1 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 174.3, 173.5, 172.2, 171.6, 170.7, 169.9,

169.9, 169.5, 169.4, 169.1, 169.0, 168.0, 167.7, 167.7, 167.6, 167.4, 138.4, 138.0, 129.7, 129.1, 128.2, 128.0, 126.3, 126.1, 67.0, 66.7–66.6 (m, 2C), 66.3, 65.9, 65.7, 64.9, 59.1, 55.3, 53.4, 53.0, 48.4, 47.7, 46.8, 46.7, 46.5, 37.4, 37.1, 36.5, 35.1, 33.1, 31.6, 31.3, 31.3, 30.7, 29.1, 29.0, 28.8, 28.7, 28.6, 25.1, 25.1, 22.3, 22.3, 22.1, 19.8, 19.7, 14.0 HRMS m/z ratio calculated for $[C_{37}H_{44}F_4N_8O_{10}S_2+H]^+$ = 901.2636; found 901.2702; 4.1 ppm mass defect.

Polycycle **3.81**:

Followed general procedure 4F, 4G, and 4I.

Crude peptide Ac-E*PSFTC-NH_2 (150. mg, 1.0 equiv., 186 μmol) was subjected to general procedure 4F, 4G and reverse-phase preparative HPLC – (gradient 5% MeCN/H_2O → 55% MeCN/H_2O → 65% MeCN/H_2O) afforded acyclic seco-acid (108.2 mg, 62% isolated yield) as a light-yellow foam. HRMS calculated for $[C_{38}H_{47}F_4N_7O_{12}S_2+H]^+$ = 934.2739; found 934.2773; 3.6 ppm mass defect. Followed General Procedure 4I to obtain macrolactone **47**. Acyclic seco-acid (40.0 mg, 1.0 equiv., 42.8 μmol) was afforded crude product after 44 h. Crude product was purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 60% MeCN/H_2O → 65% MeCN/H_2O) afforded title compound (16.4 mg, 42% isolated yield, 26% yield from linear peptide). NMR of **47** contained complex mixture of rotational isomers. Major rotational isomer was characterized. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.52 (t, J = 7.6 Hz, 1H), 8.29 (d, J = 8.5 Hz, 1H), 8.22 (dd, J = 7.3, 2.3 Hz, 1H), 8.02 (d, J = 8.4 Hz, 1H), 7.32–7.22 (m, 5H), 7.19 (t, J = 7.0 Hz, 1H), 7.12 (s, 1H), 4.93–4.83 (m, 3H), 4.77–4.70 (m, 1H), 4.62–4.56 (m, 2H), 4.54 (t, J = 6.0 Hz, 1H), 4.44–4.40 (m, 1H), 3.88–3.77 (m, 2H), 3.60–3.56 (m, 1H), 3.55– 3.47 (m, 2H), 3.41–3.35 (m, 1H), 3.35–3.29 (m, 2H), 3.10–3.04 (m, 1H), 3.04–2.99 (m, 1H), 2.82 (dd, J = 13.8, 10.4 Hz, 1H), 2.48–2.44 (m, 1H), 2.34–2.27 (m, 2H), 2.23–2.17 (m, 1H), 1.94–1.88 (m, 2H), 1.84 (s,

3H), 1.82–1.79 (m, 1H), 1.66–1.58 (m, 1H), 1.08 (dd, J = 6.0, 1.4 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.7, 170.1, 168.9, 168,8, 168.3, 167.5, 167.4, 167.3, 148.3–148.2 (m, 1C), 144.5–144.4 (m, 1C), 137.6, 129.3, 128.1, 126.4, 117.1–116.9 (m, 1C), 116.1–115.9 (m, 1C), 72.0–71.7 (m, 1C), 66.4, 61.2, 60.3, 58.2, 53.7, 51.4, 50.5, 49.4, 46.0, 37.4, 34.9, 30.5, 28.8, 28.1, 25.1, 24.7, 24.3, 22.3, 16.2 ^{19}F NMR (DMSO-d_6, 565 MHz) δ -98.53 and -118.6 (AB quartet, J = 251.1 Hz, 2F), -117.0 and -131.7 (AB quartet, J = -223.8 Hz, 2F) HRMS calculated for $[C_{38}H_{45}F_4N_7O_{11}S_2+H]^+$ = 916.2633; found 916.2654; 2.3 ppm mass defect.

Polycycle **3.82**:

Followed general procedure 4F, 4G, and 4I.

Crude peptide Ac-D*PNHFTC-NH_2 (150. mg, 1.0 equiv., 157 μmol) was subjected to general procedure 4F, 4G and reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 50% MeCN/H_2O) afforded acyclic seco-acid (73.1 mg, 43% isolated yield) as a yellow-powder. HRMS m/z calculated for $[C_{44}H_{53}F_4N_{11}O_{13}S_2+H]^+$ = 1084.3281; found 1084.3295; 1.3 ppm mass defect. Followed General Procedure 4I to obtain macrolactone **48**. Acyclic seco-acid (40.0 mg, 1.0 equiv., 33.4 μmol) afforded crude product after 64 h. Crude product was purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 55% MeCN/H_2O → 60% MeCN/H_2O) afforded desired product. Desired product was repurified via pTLC; 10% MeOH/$CHCl_3$ solvent system (plate was run up twice) to afford title compound (5.0 mg, 13% isolated yield, 6% yield from linear peptide) as an opaque-film. NMR of **48** contained complex mixture of rotational isomers. Major rotational isomer was characterized. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 9.41 (s, 1H), 8.15 (d, J = 7.6 Hz, 1H), 8.12 (d, J = 7.6 Hz, 1H), 8.07 (d, J = 8.3 Hz, 1H), 7.94 (d, J = 8.9 Hz, 1H), 7.84 (d, J = 1.1 Hz, 1H), 7.60–7.52 (m, 2H), 7.44 (s, 1H), 7.42 (s, 1H), 7.30–7.26 (m, 2H), 7.22–7.17 (m,

3H), 5.16 (d, J = 5.2 Hz, 1H), 4.87–4.81 (m, 2H), 4.76–4.71 (m, 1H), 4.38–4.32 (m, 1H), 4.28 (dd, J = 12.8, 7.4 Hz, 1H), 4.24 (dd, J = 9.1, 2.4 Hz, 1H), 4.21–4.16 (m, 2H), 4.15–4.10 (m, 1H), 3.76–3.69 (m, 1H), 3.65–3.58 (m, 2H), 3.58–3.51 (m, 1H), 3.19–3.13 (m, 1H), 3.05–2.97 (m, 2H), 2.92–2.82 (m, 3H), 2.72–2.69 (m, 1H), 2.44 (dd, J = 15.4, 7.8 Hz, 1H), 2.24 (dd, J = 15.4, 10.4, 1H), 2.07–2.01 (m, 1H), 2.01–1.96 (m, 1H), 1.96–1.83 (m, 3H), 1.81 (s, 3H), 0.97 (d, J = 6.4 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) 172.2, 171.5, 171.2, 170.7, 170.5, 169.7, 169.2, 168.9, 168.0, 140.8, 138.9, 137.4, 131.3–131.1 (m, 1C), 129.8–129.6 (m, 1C), 129.0, 128.3, 126.4, 115.2, 112.2–112.1 (m, 2C), 67.7–67.4 (m, 1C), 60.6, 60.3, 59.0, 56.0, 51.5, 49.7, 47.0, 46.9, 46.6, 38.1, 35.9, 35.4, 32.1, 30.4. 29.7, 29.0, 28.8, 24.3, 22.3. 20.2 ^{19}F NMR (DMSO-d_6, 565 MHz) δ -99.9 and -113.3 (AB quartet, J = 248.3 Hz, 2F), -114.5 and -127.2 (AB quartet, J = 225.8 Hz, 2F) HRMS calculated for $[C_{44}H_{51}F_4N_{11}O_{12}S_2+H]^+$ = 1066.3175; found 1066.3180; 0.5 ppm mass defect.

Polycycle **3.83**:

Followed general procedure 4F, 4G, and 4I.

Crude peptide Ac-D*PTFSC-NH_2 (150. mg, 1.0 equiv., 189 μmol) was subjected to general procedure 4F, 4G and reverse-phase preparative HPLC (gradient 10% → 60% MeCN/H_2O → 65% MeCN/H_2O) afforded acyclic seco-acid (67.2 mg, 36% isolated yield) as a yellow-foam. HRMS m/z calculated for $[C_{39}H_{44}F_4N_8O_{12}S_3+H]^+$ = 989.2255; found 989.2239; 3.7 ppm mass defect. Followed General Procedure 4I to obtain macrolactone **49**. To a flame-dried 25-mL round bottom flask equipped with a stir bar was charged with the incipient seco acid (24.5 mg, 1.0 equiv., 24.8 μmol), EDC•HCl (12.1 mg, 2.5 equiv., 61.9 μmol), HOBt (7.11 mg, 1.5 equiv., 37.2 μmol, 80%), and then diluted with DMF (4.95 mL, 5.0 mM). The reaction mixture was then allowed to stir at 0 ºC and then added NEt_3 (17.3 μL, 5.0 equiv., 124 μmol). The reaction mixture was then allowed

to warm up to 23 ºC and stirred for 48 h. After HPLC indicated reaction completion, the reaction mixture was quenched with AcOH (14.2 µL, 10.0 equiv., 248 µmol) and then concentrated under reduced pressure to afford crude product. The crude product was then purified via preparative HPLC – (gradient 10% MeCN/H_2O → 70% MeCN/H_2O → 75% MeCN/H_2O) afforded title compound (17.4 mg, 72% isolated yield, 26% from linear peptide) as a white-powder. NMR of **49** contained complex mixture of rotational isomers. Major rotational isomer was characterized. ^{1}H NMR (DMSO-d_6, 600 MHz) δ 8.78 (d, J = 6.9 Hz, 1H), 8.29 (d, J = 8.0 Hz, 1H), 7.84 (m, 1H), 7.85 (m, 1H), 7.84 (s, 1H), 7.38 (d, J = 7.8 Hz, 1H), 7.27–7.23 (m, 3H), 7.22–7.19 (m, 2H), 7.09 (s, 1H), 5.31 (d, J = 13.0 Hz, 1H), 5.16 (d, J = 13.0 Hz, 1H), 4.87 (m, 1H), 4.70–4.66 (m, 1H), 4.66–4.62 (m, 1H), 4.53–4.50 (m, 1H), 4.29–4.26 (m, 1H), 4.06 (dd, J = 7.9, 4.2 Hz, 1H), 3.98 (dd, J = 11.3, 2.7 Hz, 1H), 3.91–3.88 (m, 1H), 3.85 (dd, J = 12.0, 3.1 Hz, 1H), 3.65–3.62 (m, 2H), 3.51–3.46 (m, 1H), 3.35–3.32 (m, 1H), 2.94 (dd, J = 13.8 Hz, 5.5 Hz, 1H), 2.84–2.75 (m, 3H), 2.54 (m, 1H), 2.06–2.02 (m, 1H), 1.81–1.79 (m, 1H), 1.79 (s, 3H), 0.94 (d, J = 6.4 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.4, 171.1, 169.9, 169.3, 169.1, 167.3, 166.8, 161.3, 144.5, 137.6, 137.3–137.2 (m, 1C), 135.3, 135.2–135.1 (m, 1C), 129.1, 128.2, 126.4, 116.7–116.6 (m, 2C), 114.3, 67.5–67.4 (m, 1C), 66.7, 59.9, 58.3, 58.1, 57.0, 55.8, 53.6, 48.8, 47.0, 46.9, 37.0, 36.0, 35.8, 30.8, 29.2, 22.2, 19.7 ^{19}F NMR (DMSO-d_6, 565 MHz) δ -99.5 and -118.5 (AB quartet, J = 255.5 Hz, 2F), -119.0 and -132.0 (AB quartet, J = 218.0 Hz, 2F) HRMS m/z calculated for $[C_{39}H_{42}F_4N_8O_{11}S_3+H]^+$ = 971.2150; 971.2174; 2.5 ppm mass defect.

2.20 Synthesis of Fig. 3.16 and PAMPA Supplemental Compounds

Polycycle **3.84**:

Followed general procedure 2A.

Crude peptide Ac-Dat-PCVAC-NHMe (50.0 mg, 1.0 equiv., 76.6 μmol) (where Dat = des-amino tyrosine) afforded crude product. Crude product was then purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 75% MeCN/H_2O) afforded title compound (15.7 mg, 26% isolated yield) as an opaque-film. NMR of **50** contained complex mixture of rotational isomers; ratio 1:1:4. Major rotational isomer was characterized. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.31 (d, J = 9.4 Hz, 1H), 7.90 (d, J = 7.7 Hz, 1H), 7.70 (d, J = 6.9 Hz, 1H), 7.56 (d, J = 8.9 Hz, 1H), 7.30 (d, J = 8.2 Hz, 2H), 7.19 (dd, J = 8.2, 2.2 Hz, 2H), 4.71 (d, J = 6.6 Hz, 1H), 4.40 (d, J = 6.6 Hz, 1H), 4.34–4.29 (m, 1H), 4.25–4.18 (m, 1H), 3.96 (s, 3H), 3.94–3.88 (m, 1H), 3.63 (m, 1H), 3.49–3.41 (m, 2H), 3.20–3.16 (m, 1H), 3.06–2.99 (m, 1H), 2.97–2.90 (m, 1H), 2.82 (m, 1H), 2.44–2.37 (m, 2H), 2.21–2.17 (m, 1H), 2.08–2.02 (m, 1H), 2.02–1.92 (m, 2H), 1.89–1.82 (m, 1H), 1.70–1.65 (m, 1H), 1.31 (d, J = 7.4 Hz, 3H), 0.81 (d, J = 6.4 Hz, 3H), 0.78 (d, J = 6.4 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.2, 171.9, 171.4, 170.4, 168.5, 167.6, 148.3–148.1 (m, 1C), 137.8, 131.5, 129.3–129.2 (m, 1C), 118.6, 117.2–116.9 (m, 1C), 115.6, 114.9–114.6 (m, 1C), 74.7–74.5 (m, 1C), 60.1, 58.8, 55.1, 52.1, 49.5, 48.4, 47.3, 35.5, 35.3, 33.8, 30.4, 29.5, 25.9, 24.3, 19.0, 18.5, 17.9 ^{19}F NMR (DMSO-d_6, 282 MHz) δ -106.8 and -119.7 (AB quartet, J = 257.6 Hz, 2F), -113.5 and -120.3 (AB quartet, J = 229.7 Hz, 2F) HRMS m/z calculated for $[C_{33}H_{40}F_4N_6O_7S_2+H]^+$ = 785.2414; found 785.2419; 0.6 ppm mass defect.

Compound **S1**

To a stirred solution of **17** in DMF (50 mM) was added Et_3N (2.2 equiv.) and 1,4-benzenedithiol (0.5 equiv.) at 0 °C and let stir and warm to rt. Upon completion as observed by HPLC, the reaction was quenched with AcOH (5.0 equiv.) and concentrated under reduced pressure. Purification by RP-HPLC afforded the dimerized product **S1** (20%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.91 (s, 2H), 9.51 (s, 2H), 8.50 (d, J = 9.1 Hz, 2H), 8.39 (d, J = 8.0 Hz, 2H), 8.31 (d, J = 8.5 Hz, 2H), 7.69 (d, J = 19.5 Hz, 2H), 7.50 (d, J = 7.7 Hz, 2H), 7.38 – 7.29 (m, 6H), 7.22 (d, J = 9.0 Hz, 2H), 7.13 (s, 2H), 7.07 (t, J = 7.3 Hz, 2H), 6.98 (t, J = 7.3 Hz, 2H), 5.02 (s, 2H), 4.83 (d, J = 8.9 Hz, 2H), 4.66 (q, J = 7.7 Hz, 2H), 4.47 (q, J = 7.4 Hz, 2H), 4.42 – 4.33 (m, 2H), 4.26 – 4.17 (m, 2H), 3.88 – 3.76 (m, 2H), 3.66 (d, 2H), 3.55 – 3.42 (m, 5H), 3.11 – 3.03 (m, 6H), 2.82 (d, J = 14.0 Hz, 2H), 1.79 (s, 6H). ^{13}C-NMR (500 MHz, DMSO-d_6) δ 171.8, 169.8, 169.5, 168.7, 167.6, 167.0, 136.1, 131.6, 130.4, 129.7, 128.7, 127.1, 123.7, 121.0, 118.5, 118.0, 111.4, 109.1, 79.2, 65.7, 61.6, 55.7, 55.0, 51.5, 46.1, 45.6, 36.0, 35.9, 35.8, 33.7, 33.0, 32.6, 31.9, 31.3, 30.7, 29.0, 28.9, 28.7, 28.7, 28.7, 28.0, 27.6, 26.2, 25.8, 25.5, 25.4, 25.3, 22.5, 22.4, 22.3, 22.1, 19.4, 19.1, 14.0, 14.0, 12.0, 11.2, 8.5. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -96.8 – (-98.8) (m, 2F), - 111.3 – (-113.8) (m, 4F), -127.0 – (-128.7) (m, 2F).

Compound **S5**

To a stirred solution of crude peptide Cbz-Glu(Cys)-Ala-Histamine in DMF (5 mM) was added Et_3N (2.5 equiv.), and OFCP (1.5 equiv.) at 0 °C and let stir for 30 min. After initial macrocyclization was complete, the mixture was concentrated under reduced pressure to remove excess Et_3N, OFCP, and MeCN. The reaction mixture was then cooled once again to 0 °C and added Me_3SiOK (4.0 equiv.) and let stir at rt. After reaction completion as observed by HPLC the

mixture was quenched with AcOH (10.0 equiv.) and concentrated under reduced pressure. Purification by FCC (0 - 10% MeOH/$CHCl_3$) afforded **S5** (41%). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.96 (bs, 1H), 9.88 (bs, 1H), 8.48 (s, 1H), 8.01 (s, 1H), 7.67 (s, 1H), 7.46 (s, 1H), 7.42 - 7.24 (m, 5H), 7.07 (s, 1H), 5.07 - 4.92 (m, 2H), 4.89 (s, 1H), 4.4 - 4.25 (m, 1H), 4.04 (q, J = 12.3 Hz 1H); 3.09 (d, J = 13.0 Hz, 1H), 3.8 - 3.64 (m, 1H), 2.98 (d, J = 15.0 Hz, 1H), 2.85 - 2.63 (m, 2H), 2.29 - 2.16 (m, 1H); 2.13 - 1.98 (m, 1H), 1.97 - 1.72 (m, 2H), 1.18 - 1.02 (m, 3H). ^{13}C-NMR (500 MHz, DMSO-d_6) δ 174.2, 171.3, 170.6, 170, 155.3, 140.6, 139.8 - 139.1 (m, 1C), 138.4, 137, 128.4, 127.9, 127.8, 123.3 - 122.9 (m, 1C), 115.5 - 109.5 (m, 2C), 113.4, 65.4, 53.5, 47.5, 46, 35.5, 31.4, 30.9, 29.9, 28.2, 17.9. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -110.7 (dt, J = 251.3, 13.5 hz, 1F), -112.2 (dd, J = 223.7, 8.9 Hz, 1F), -116.9 (dt, J = 251.5, 12.5 Hz, 1F), -119.8 (dd, J = 224.0, 10.5 Hz, 1F), -146.4 (t, J = 13.9 Hz, 1F). HRMS [M+1] calc'd for $C_{29}H_{30}F_5N_7O_6SH$ 700.1977, found 700.1998.

Compound **S6**

TFA (10 ml) was added to a solution of SM (1.75 g, 3.84 mmol) in DCM (20 ml) and stirred for 1h at rt. The reaction mixture was concentrated by rotary evaporation to give a crude solid 12 (1.80 g, 3.84 mmol). The residue 12 (62 mg, 0.131 mmol, analytical) was dissolved in DMF (1.3 ml) and added 7 (43 mg, 0.144 mmol), cooled to 0 °C, and then added iPr_2EtN (34 ul, 0.197 mmol). The reaction mixture was stirred for 2 h at rt, then added H_2O, extracted with EtOAc, washed with 1N HCl, sat. $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation to give crude **15**. The residue **15** was dissolved in DMF (13 ml) and added Cs_2CO_3 (64 mg, 0.196 mmol) at 0 °C, stirred for 1 h at rt,. The reaction mixture was added H_2O, extracted with EtOAc. The combined extract was washed with brine (x5), dried over Na_2SO_4 and concentrated. Purification by column chromatography (SiO_2, gradient 1-5%

MeOH/$CHCl_3$) afforded the title compound (38 mg, 0.0619 mmol, 47% from SM) as a white-solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.14 (d, J = 4.4 Hz, 1H), 7.53 (d, J = 8.1 Hz, 1H), 7.32 (t, J = 5.4 Hz, 1H), 7.26–7.24 (m, 2H), 7.18–7.14 (m, 5H), 7.02 (d, J = 8.6 Hz, 2H), 4.31–4.27 (m, 1H), 4.17–4.11 (t, J = 6.8 Hz, 2H), 3.73–3.71 (m, 1H), 3.60–3.57 (m, 1H), 3.19 (dd, J = 14.3, 4.0 Hz, 1H), 3.05–2.96 (m, 1H), 2.99 (dd, J = 14.3, 10.0 Hz, 1H), 2.80–2.75 (m, 1H), 2.68–2.63 (m, 1H), 2.02–1.89 (m, 2H), 1.43–1.28 (m, 2H), 1.00 (d, J = 7.2 Hz, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.6, 172.3, 171.0, 153.2, 138.8–138.3 (m, 1C), 138.3, 136.7, 130.6, 129.6–129.1 (m, 1C), 128.8, 128.1, 126.2, 116.2, 116.2, 116.2, 115.6–105.7 (m, 3C), 72.9, 53.7, 50.5, 40.6, 35.8, 33.7, 30.5, 24.5, 16.9 ^{19}F NMR (DMSO-d_6, 282 MHz) δ -111.8 (d, J = 54.6 Hz, 2F), -112.5 (d, J = 71.9 Hz, 2F), -128.6-(-128.8) (m, 2F). HRMS m/z calculated for $[C_{28}H_{30}F_6N_3O_5+H]^+$ = 614.2084; found 614.2062; 3.6 ppm mass defect.

Compound **S30**

Crude peptide Ac-HFCASC-NH_2 (50.0 mg, 1.0 equiv., 60.8 μmol) was diluted with H_2O (1.0 mM, 60.8 mL). The solution was allowed to stir until the peptide was homogenous and then 30% aqueous H_2O_2 (31.1 μL, 5.0 equiv., 304 μmol) was added. The reaction mixture was then allowed to stir at 23 ºC over 16 h. When HPLC indicated reaction completion, the reaction mixture was concentrated under reduced pressure to afford product. Crude product was then purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 32.5% MeCN/H_2O → 37.5% MeCN/H_2O) afforded title compound (10.8 mg, 22% isolated yield) as a white-film. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.96–8.90 (s, 2H), 8.54 (d, J = 6.0 Hz, 1H), 8.52 (d, J = 7.5 Hz, 1H), 8.13 (d, J = 8.3 Hz, 1H), 8.07 (d, J = 7.6 Hz, 1H), 7.81 (d, J = 7.6 Hz, 1H), 7.38 (d, J = 7.6 Hz, 1H), 7.28–7.23 (m, 1H), 7.22–7.18 (m, 3H), 7.07–7.03 (m, 1H), 4.59–4.54 (m, 2H), 4.53–4.47 (m, 2H),

4.35–4.30 (m, 1H), 4.22–4.18 (m, 1H), 4.10 (q, J = 6.8 Hz, 1H), 3.74 (dd, J = 10.8, 5.4 Hz, 1H), 3.63 (dd, J = 6.7, 4.2 Hz, 1H), 3.22–3.15 (m, 1H), 3.09–3.03 (m, 2H), 3.02–2.96 (m, 2H), 2.87–2.80 (m, 3H), 1.80 (s, 3H), 1.30 (d, J = 7.3 Hz, 3H) ^{13}C NMR (DMSO-d_6, 126 MHz) δ 173.2, 171.3, 170.6, 170.0, 170.0, 169.5, 169.4, 137.2, 133.8, 129.4, 129.2, 128.1, 126.4, 116.7, 60.1, 55.9, 55.2, 53.8, 51.3, 51.1, 51.1, 45.7, 43.0, 26.9, 22.5, 20.4, 17.0 HRMS m/z calculated for $[C_{29}H_{39}N_9O_8S_2+H]^+$ = 706.2441; found 706.2472; 4.4 ppm mass defect.

2.21 Synthesis of Fig. 3.17 and Fig. 3.18 Follow Up PAMPA Experiment Compounds

Compound **3.85**

To a dram vial equipped with a stir bar was charged with **3.24** (5.0 mg, 1 Eq, 9.9 μmol), diluted with dry DMF (.20 mL, 0.05 M, 1 Eq, 9.9 μmol), added N-methyl-1-phenylmethanamine (1.9 mg, 2.0 μL, 97% Wt, 1.5 Eq, 15 μmol), and then Cs_2CO_3 (4.8 mg, 1.5 Eq, 15 μmol). The reaction mixture was then allowed to stir at 23 ºC for 2 h. HPLC indicated full conversion. The reaction mixture was quenched with AcOH (3.0 mg, 2.8 μL, 5 Eq, 50 μmol). The reaction mixture was then concentrated under reduced pressure to afford crude. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (2.4 mg, 40% isolated yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.93 (d, J = 8.4 Hz, 1H), 8.59 (s, 1H), 8.05 (d, J = 7.5 Hz, 1H), 7.98 (d, J = 9.9 Hz, 1H), 7.73 (t, J = 7.5 Hz, 2H), 7.31 (t, J = 7.3 Hz, 1H), 7.23 (d, J = 7.0 Hz, 2H), 5.21 (d, J = 15.3 Hz, 1H), 4.98 (t, J = 5.3 Hz, 1H), 4.93 (pent, J = 3.7 Hz, 1H), 4.63 (ddd, J = 10, 3.6, 1.7 Hz, 1H), 4.45–4.39 (m, 1H), 4.19 (d, J = 15.3 Hz, 1H), 3.83 (pent, J = 5.5 Hz, 1H), 3.71–3.65 (m, 1H), 3.43 (dd, J = 11.1, 4.2 Hz, 1H), 3.17 (dd, J = 11.1, 3.4 Hz, 1H), 2.92 (s, 3H), 2.67 (dd, J = 14.0, 3.8 Hz, 1H), 1.85 (s, 3H) ^{1}H NMR (DMSO-d_6, 126 MHz) δ 169.2, 169.0, 167.9, 167.7, 137.0, 129.6, 128.6, 127.6, 79.2, 60.3, 55.3, 53.9, 50.9, 46.6, 42.9, 31.5, 22.3 ^{19}F NMR (DMSO-d_6, 282 MHz) δ -96.9, -116.4 (AB quartet, J = 250.2 Hz, 2F), -119.2, -127.2 (AB quartet, J = 221.3 Hz, 2F). HRMS calculated for [$C_{24}H_{27}F_4N_5O_5S_2$+H] = 606.1468; found 606.1481; 2.1 ppm mass defect.

Compound **3.86**

To a dram vial equipped with a stir bar was charged with **3.24** (5.0 mg, 1 Eq, 9.9 μmol), diluted with dry DMF (.20 mL, 0.05 M, 1 Eq, 9.9 μmol), added n-propylamine (1.5 Eq, 15 μmol), and

then Cs_2CO_3 (4.8 mg, 1.5 Eq, 15 μmol). The reaction mixture was then allowed to stir at 23 ºC for 30 min. HPLC indicated full conversion. The reaction mixture was quenched with AcOH (3.0 mg, 2.8 μL, 5 Eq, 50 μmol). The reaction mixture was then concentrated under reduced pressure to afford crude. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (3.6 mg, 67% isolated yield). HRMS calculated for [$C_{19}H_{25}F_4N_5O_5S_2$+H] = 544.1312; found 544.1339; 5.0 ppm mass defect.

Compound **3.87**

To a dram vial equipped with a stir bar was charged with **3.24** (5.0 mg, 1 Eq, 9.9 μmol), diluted with dry DMF (.20 mL, 0.05 M, 1 Eq, 9.9 μmol), added oleylamine (1.5 Eq, 15 μmol), and then Cs_2CO_3 (4.8 mg, 1.5 Eq, 15 μmol). The reaction mixture was then allowed to stir at 23 ºC for 30 min. HPLC indicated full conversion. The reaction mixture was quenched with AcOH (3.0 mg, 2.8 μL, 5 Eq, 50 μmol). The reaction mixture was then concentrated under reduced pressure to afford crude. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (4.3 mg, 58% isolated yield). HRMS calculated for [$C_{34}H_{53}F_4N_5O_5S_2$+H] = 752.3503 found 752.3520; 2.3 ppm mass defect.

Compound **3.88**

To a dram vial equipped with a stir bar was charged with **3.24** (5.0 mg, 1 Eq, 9.9 μmol), diluted with dry DMF (.20 mL, 0.05 M, 1 Eq, 9.9 μmol), added 2-mercaptopyridine (1.5 Eq, 15 μmol), and then Cs_2CO_3 (4.8 mg, 1.5 Eq, 15 μmol). The reaction mixture was then allowed to stir at 23

ºC for 30 min. HPLC indicated full conversion. The reaction mixture was quenched with AcOH (3.0 mg, 2.8 µL, 5 Eq, 50 µmol). The reaction mixture was then concentrated under reduced pressure to afford crude. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (1.6 mg, 27% isolated yield). HRMS calculated for [$C_{21}H_{21}F_4N_5O_5S_3$+H] = 596.0719; found 596.0728; 1.5 ppm mass defect.

Compound **3.89**

To a dram vial equipped with a stir bar was charged with **3.24** (5.0 mg, 1 Eq, 9.9 µmol), diluted with dry DMF (.20 mL, 0.05 M, 1 Eq, 9.9 µmol), added 2-mercaptopyrimidine (1.5 Eq, 15 µmol), and then Cs_2CO_3 (4.8 mg, 1.5 Eq, 15 µmol). The reaction mixture was then allowed to stir at 23 ºC for 30 min. HPLC indicated full conversion. The reaction mixture was quenched with AcOH (3.0 mg, 2.8 µL, 5 Eq, 50 µmol). The reaction mixture was then concentrated under reduced pressure to afford crude. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (1.6 mg, 27% isolated yield). HRMS calculated for [$C_{20}H_{20}F_4N_6O_5S_3$+H] = 597.0672; found 597.0700; 4.7 ppm mass defect.

Compound **3.90**

To a dram vial equipped with a stir bar was charged with **3.24** (5.0 mg, 1 Eq, 9.9 µmol), diluted with dry DMF (.20 mL, 0.05 M, 1 Eq, 9.9 µmol), added sodium thioacetate (1.5 Eq, 15 µmol), and then Cs_2CO_3 (4.8 mg, 1.5 Eq, 15 µmol). The reaction mixture was then allowed to stir at 23 ºC for 30 min. HPLC indicated full conversion. The reaction mixture was quenched with AcOH (3.0 mg,

2.8 µL, 5 Eq, 50 µmol). The reaction mixture was then concentrated under reduced pressure to afford crude. The crude product was unstable to SiO_2 purification. HRMS calculated for [$C_{16}H_{18}F_4N_4O_5S_3$+H] = 519.0454; found 519.0470; 3.1 ppm mass defect.

Compound **3.91**

To a dram vial equipped with a stir bar was charged with **3.17** (5.0 mg, 1 Eq, 7.2 µmol), diluted with dry DMF (.20 mL, 0.05 M, 1 Eq, 9.9 µmol), added n-propylamine (1.5 Eq, 25 µmol), and then Cs_2CO_3 (4.8 mg, 1.5 Eq, 15 µmol). The reaction mixture was then allowed to stir at 23 ºC for 30 min. HPLC indicated full conversion. The reaction mixture was quenched with AcOH (3.0 mg, 2.8 µL, 5 Eq, 50 µmol). The reaction mixture was then concentrated under reduced pressure to afford crude. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (5.2 mg, 98% isolated yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.87 (d, J = 1.9 Hz, 1H), 8.67 (s, 1H), 8.43 (t, J = 7.7 Hz, 2H), 8.22 (d, J = 8.1 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.33 (d, J = 8.1 Hz, 1H), 7.14–7.03 (m, 3H), 6.97 (td, J = 7.7, 0.8 Hz, 1H), 6.51 (t, J = 6.5 Hz, 1H), 5.05–4.98 (m, 1H), 4.75–4.59 (m, 3H), 4.37–4.30 (m, 1H), 3.92–3.84 (m, 1H), 3.51–3.42 (m, 1H), 3.14–3.08 (m, 1H), 3.07–3.02 (m, 1H), 2.89 (dd, J = 11.9, 9.8 Hz, 1H), 2.77–2.70 (m, 1H), 2.59 (dd, J = 14.4, 2.4 Hz, 1H), 1.80 (s, 3H), 1.52–1.43 (m, 2H), 1.26–1.22 (m, 3H), 0.83 (t, J = 7.4 Hz, 3H). HRMS calculated for [$C_{30}H_{35}F_4N_7O_6S_2$+H] = 730.2105; found 730.2114; 1.2 ppm mass defect.

Compound **3.92**

To a dram vial equipped with a stir bar was charged with **3.17** (5.0 mg, 1 Eq, 7.2 µmol), diluted with dry DMF (.20 mL, 0.05 M, 1 Eq, 9.9 µmol), added n-hexylamine (1.5 Eq, 25 µmol), and then

Cs_2CO_3 (4.8 mg, 1.5 Eq, 15 μmol). The reaction mixture was then allowed to stir at 23 ºC for 30 min. HPLC indicated full conversion. The reaction mixture was quenched with AcOH (3.0 mg, 2.8 μL, 5 Eq, 50 μmol). The reaction mixture was then concentrated under reduced pressure to afford crude. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (5.0 mg, 89% isolated yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.87 (d, J = 1.9 Hz, 1H), 8.67 (s, 1H), 8.41 (t, J = 11.0 Hz, 2H), 8.22 (d, J = 7.7 Hz, 1H), 7.49 (d, J = 8.3 Hz, 1H), 7.33 (d, J = 7.8 Hz, 1H), 7.14–7.03 (m, 3H), 6.97 (t, J = 6.9 Hz, 1H), 6.48 (t, J = 6.0 Hz, 1H), 5.03–4.97 (m, 1H), 4.76–4.70 (m, 1H), 4.70–4.59 (m, 2H), 4.38–4.31 (m, 1H), 3.92–3.84 (m, 1H), 3.49–3.41 (m, 2H), 3.07–2.99 (m, 2H), 1.80 (s, 3H), 1.30–1.16 (m, 10H), 0.85 (t, J = 6.6 Hz, 1H). HRMS calculated for [$C_{33}H_{41}F_4N_7O_6S_2$+H] = 772.2574; found 772.2589; 1.9 ppm mass defect.

Compound **3.93**

To a dram vial equipped with a stir bar was charged with **3.17** (5.0 mg, 1 Eq, 7.2 μmol), diluted with dry DMF (.20 mL, 0.05 M, 1 Eq, 9.9 μmol), added oleylamine (1.5 Eq, 25 μmol), and then Cs_2CO_3 (4.8 mg, 1.5 Eq, 15 μmol). The reaction mixture was then allowed to stir at 23 ºC for 30 min. HPLC indicated full conversion. The reaction mixture was quenched with AcOH (3.0 mg, 2.8 μL, 5 Eq, 50 μmol). The reaction mixture was then concentrated under reduced pressure to afford crude. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (4.7 mg, 69% isolated yield). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.86 (s, 1H), 8.66 (s, 1H), 8.40 (d, J = 8.9 Hz, 1H), 8.37 (d, J = 9.6 Hz, 1H), 8.23 (d, J = 8.2 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.32 (d, J = 8.0 Hz, 1H), 7.14–7.03 (m, 3H), 6.97 (t, J = 6.9 Hz, 1H), 6.46 (t, J = 5.9 Hz, 1H), 5.36–5.30 (m, 1H), 4.98 (t, J

= 5.4 Hz, 1H), 4.75–4.58 (m, 3H), 4.37–4.31 (m, 1H), 3.92–3.85 (m, 1H), 3.51–3.40 (m, 2H), 3.17–3.09 (m, 1H), 3.09–3.03 (m, 2H), 2.91–2.83 (m, 1H), 2.74–2.66 (m, 1H), 2.02–1.93 (m, 4H), 1.79 (s, 3H), 1.30–1.22 (m, 26H), 0.88–0.83 (m, 4H). HRMS calculated for [$C_{45}H_{63}F_4N_7O_6S_2$+H] = 938.4296; found 938.4331; 3.7 ppm mass defect.

Compound **3.94**

To a dram vial equipped with a stir bar was charged with **3.17** (5.0 mg, 1 Eq, 7.2 μmol), diluted with dry DMF (.20 mL, 0.05 M, 1 Eq, 9.9 μmol), added 2-mercaptopyrimidine (1.5 Eq, 25 μmol), and then Cs_2CO_3 (4.8 mg, 1.5 Eq, 15 μmol). The reaction mixture was then allowed to stir at 23 ºC for 30 min. HPLC indicated full conversion. The reaction mixture was quenched with AcOH (3.0 mg, 2.8 μL, 5 Eq, 50 μmol). The reaction mixture was then concentrated under reduced pressure to afford crude. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (5.7 mg, quantitative yield). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 10.89 (d, J = 1.9 Hz, 1H), 8.63 (d, J = 4.9 Hz, 2H), 8.48 (d, J = 9.4 Hz, 1H), 8.28 (d, J = 8.0 Hz, 1H), 8.20 (d, J = 8.7 Hz, 1H), 7.48 (d, J = 7.8 Hz, 1H), 7.38–7.24 (m, 4H), 7.11 (d, J = 2.4 Hz, 1H), 7.06 (td, J = 8.2, 1.0 Hz, 1H), 6.97 (td, J = 7.7, 0.9 Hz, 1H), 5.02 (t, J = 5.9 Hz, 1H), 4.83 (dq, J = 9.2, 2.2 Hz, 1H), 4.59–4.51 (m, 1H), 4.45–4.37 (m, 2H), 3.80 (quint, J = 5.4 Hz, 1H), 3.67 (dd, J = 14.5, 3.3 Hz, 1H), 3.57 (dd, J = 11.7, 5.5 Hz, 1H), 3.47–3.41 (m, 1H), 3.05 (d, J = 7.4 Hz, 2H), 2.85 (dd, J = 15.0, 2.0 Hz, 1H), 1.72 (s, 3H). HRMS calculated for [$C_{31}H_{30}F_4N_8O_6S_3$+H] = 783.1465; found 783.1482; 2.1 ppm mass defect.

Compound **3.95**

Followed general procedure 4F, 4G, and 4I.

Seco-acid (15.2 mg, 1.0 equiv, 16.1 μmol) afforded crude product. The crude product was then purified via preparative HPLC – (gradient 10% MeCN/H_2O → 70% MeCN/H_2O → 75% MeCN/H_2O) afforded title compound (10.1 mg, 77% isolated yield) as a white-powder. HRMS calculated for [$C_{33}H_{19}F_4N_9O_9S$+H] = 814.2606; found 814.2622; 2.0 ppm mass defect.

2.22 Synthesis of Fig. 3.20 and Fig. 3.21 – Future Benzyne Experiments – Compounds

Macrocycle **3.112**

To a flame-dried round bottom flask equipped with a stir bar was charged with crude linear Ac-YACFAC-NH_2 (150.0 mg, 1.0 equiv., 0.209 mmol), diluted with anhydrous DMF (10.0 mM), and then allowed to stir at 0 ºC. Then OFCP (.5 equiv., 1.0 M in MeCN) was added followed by NEt_3 (2.5 equiv.). The reaction mixture was allowed to stir at 0 ºC for 30 min. When HPLC indicated macrocycle intermediate formation, the reaction mixture was concentrated under reduced pressure to dryness. The reaction residue was added thiazole **3.32**, diluted with 1:4 DMF/THF (30 mM), and then stirred at 0 ºC. The reaction solution was then added KOTMS (3.5 equiv.) and the reaction mixture allowed to gradually warm up to 23 ºC for 3 h. When HPLC indicated reaction completion, the reaction mixture was added AcOH (10 equiv.) and then concentrated under reduced pressure to afford crude product. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (54.8 mg, 18% isolated yield) as a yellow-powder; R_f = 0.33 at 15% MeOH/$CHCl_3$. Crude product was then purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 60% MeCN/H_2O → 70% MeCN/H_2O) afforded title compound (60.0 mg, 28% isolated yield) as a yellow-powder. HRMS calculated for [$C_{44}H_{46}F_4N_8O_{10}S_3$+H] = 1019.2513; found 1019.2528; 1.5 ppm mass defect.

2-amino-5-((3,3,4,4,5,5-hexafluoro-2-phenoxycyclopent-1-en-1-yl)thio)benzoic acid (**3.114**)

To a flame-dried dram vial equipped with a stir bar was added **3.11** (1.1 equiv), diluted with DMF (0.05 M), added **2.5** (50.0 mg, 1.0 equiv, 175 µmol) and allowed to stir. Then KOTMS (30.7 mg, 95% wt., 1.3 equiv, 227 µmol) was added at 23 ºC and the reaction mixture allowed to stir at 23 ºC for 1 h.. The reaction mixture was then quenched with the addition of AcOH (30.0 µL, 3.0

equiv, 524 μmol) and allowed to stir at 23 ºC for 5 min. Crude product was then purified via reverse-phase preparative HPLC – (gradient 10% MeCN/H_2O → 80% MeCN/H_2O → 90% MeCN/H_2O) afforded title compound (quantitative) as a yellow-powder. HRMS calculated for [$C_{18}H_{11}F_6NO_3S$+H] = 436.0442; found 436.0458; 3.7 ppm mass defect.

2-(benzyloxy)-5-((3,3,4,4,5,5-hexafluoro-2-phenoxycyclopent-1-en-1-yl)thio)benzo[*d*][1,3]dithiole (**3.115**)

To a flame-dried 5 mL round bottom flask equipped with a stir bar was charged 3.114 (7.1 mg, 1.0 equiv, 16 μmol) and diluted with DCE (0.16 mL, 0.1 M). The mixture was then added BnOH (3.4 μL, 2.0 equiv, 33 μmol) and then allowed to heat at 85 ºC for 5 min. Then the reaction mixture was then allowed to cool to 23 ºC and isoamyl nitrite (3.0 μL, 95% wt., 1.3 equiv, 21 μmol) was added and vigorous bubbling immediately occurred. The reaction mixture was then allowed to heat at 85 ºC for 2 h. HPLC indicated complete conversion to desired product. Reaction mixture was then concentrated under reduced pressure to afford crude product. Product 3.115 was unstable to SiO_2 purification and decomposed. HRMS calculated for [$C_{25}H_{16}F_6O_2S_3$+H] = 559.0295; 559.0298; 0.5 ppm mass defect. Major ion corresponded to loss of proton from orthothioester $[C_{25}H_{15}F_6O_2S_3]^+$.

Compound **3.118**

To a dram vial equipped with a stir bar was charged with **3.24** (6.3 mg, 1 Eq, 12 μmol), diluted with dry MeCN (.25 mL, 0.05 M), added **3.117** (7.4 mg, 1.3 Eq, 16 μmol), and then NEt_3 (8.7 μL, 5.0 equiv, 62 μmol). The reaction mixture was then allowed to stir at 23 ºC for 24 h. HPLC indicated full conversion. The reaction mixture was quenched with AcOH (10 equiv). The reaction

mixture was then concentrated under reduced pressure to afford crude. The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (9.7 mg, 97% isolated yield). HRMS calculated for [$C_{28}H_{34}F_7N_5O_8S_3Si$+H] = 826.1305; found 826.1291; 1.7 ppm mass defect.

Compound **3.119**

To a dram vial equipped with a stir bar was charged with **3.118** (9.7 mg, 1 Eq, 12 μmol) (stripped 3 x with dry PhMe), diluted with dry MeCN (0.23 mL, 0.05 MM), added pyrrolidine (1.0 mg, 1.2 μL, 99% Wt, 1.2 Eq, 14 μmol) followed by KF (4.0 equiv) and 18-crown-6 ether (1.2 equiv). After 16 h, HPLC indicated reaction completion and the reaction was quenched with AcOH (11 mg, 10 μL, 15 Eq, 0.18 mmol). The crude product was then purified via flash SiO_2 chromatography (gradient $CHCl_3$ → 5% MeOH/$CHCl_3$ → 10% MeOH/$CHCl_3$) afforded title compound (1.7 mg, 21% isolated yield). Purification did not separate regioisomeric products. HRMS calculated for [$C_{28}H_{34}F_4N_6O_5S_2$+H] = 675.2046; found 675.2019; 4.0 ppm mass defect.

3. Stability Experiments

3.1 Stability Studies on Macrobicycle 13

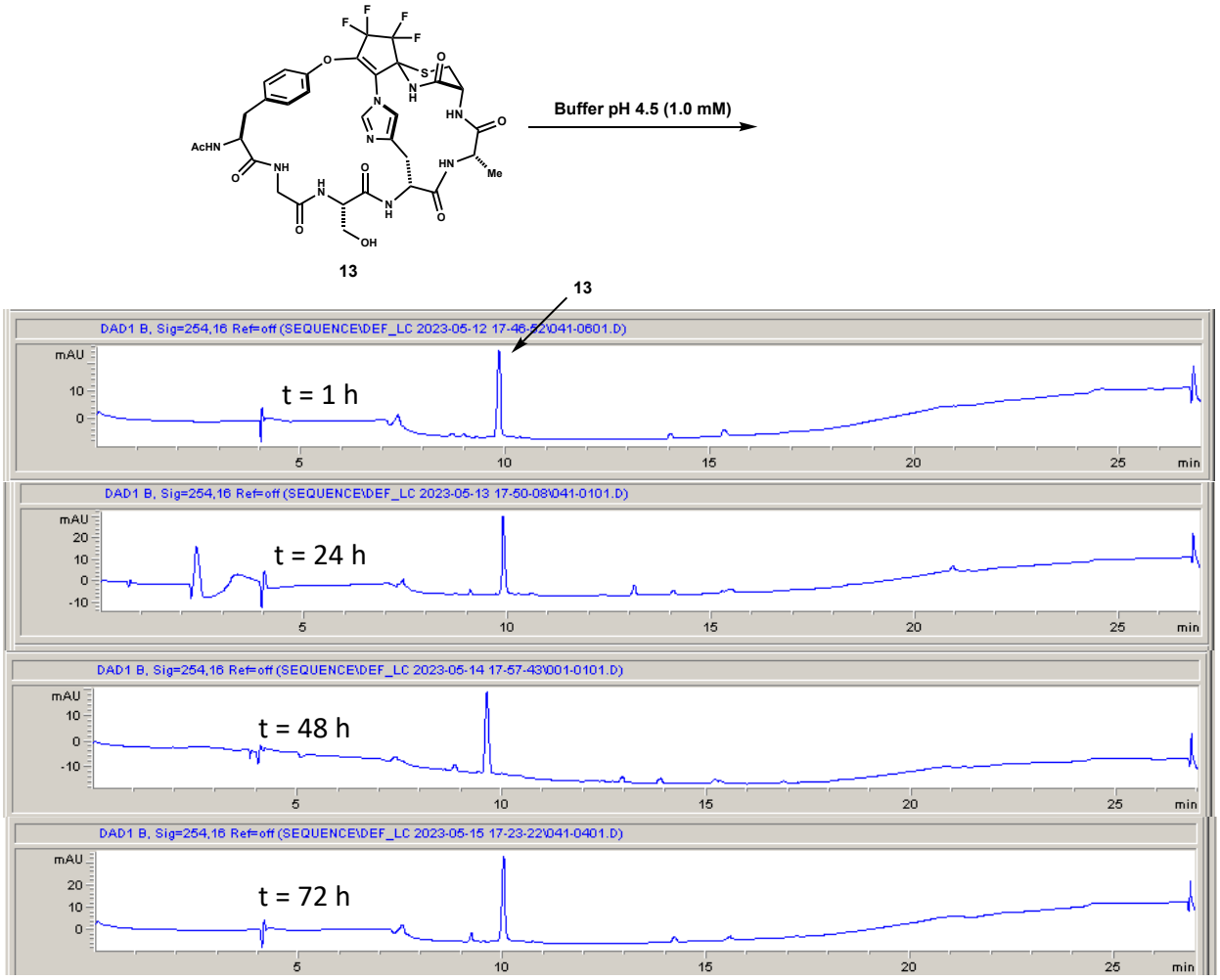


Fig. S1. Degradation Experiment of Macrobicyle 13 at pH 4.5. Compound **13** was subjected a degradation experiment at pH 4.5 over a 72 h time course. No noticeable decomposition of **13** was observed. **13** (1.0 mg, 1.0 equiv., 1.2 µmol) was diluted with NaOAc/AcOH (0.2 M) buffer pH 4.5 (1.0 mM, 1.2 mL). The solution was then allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (254 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

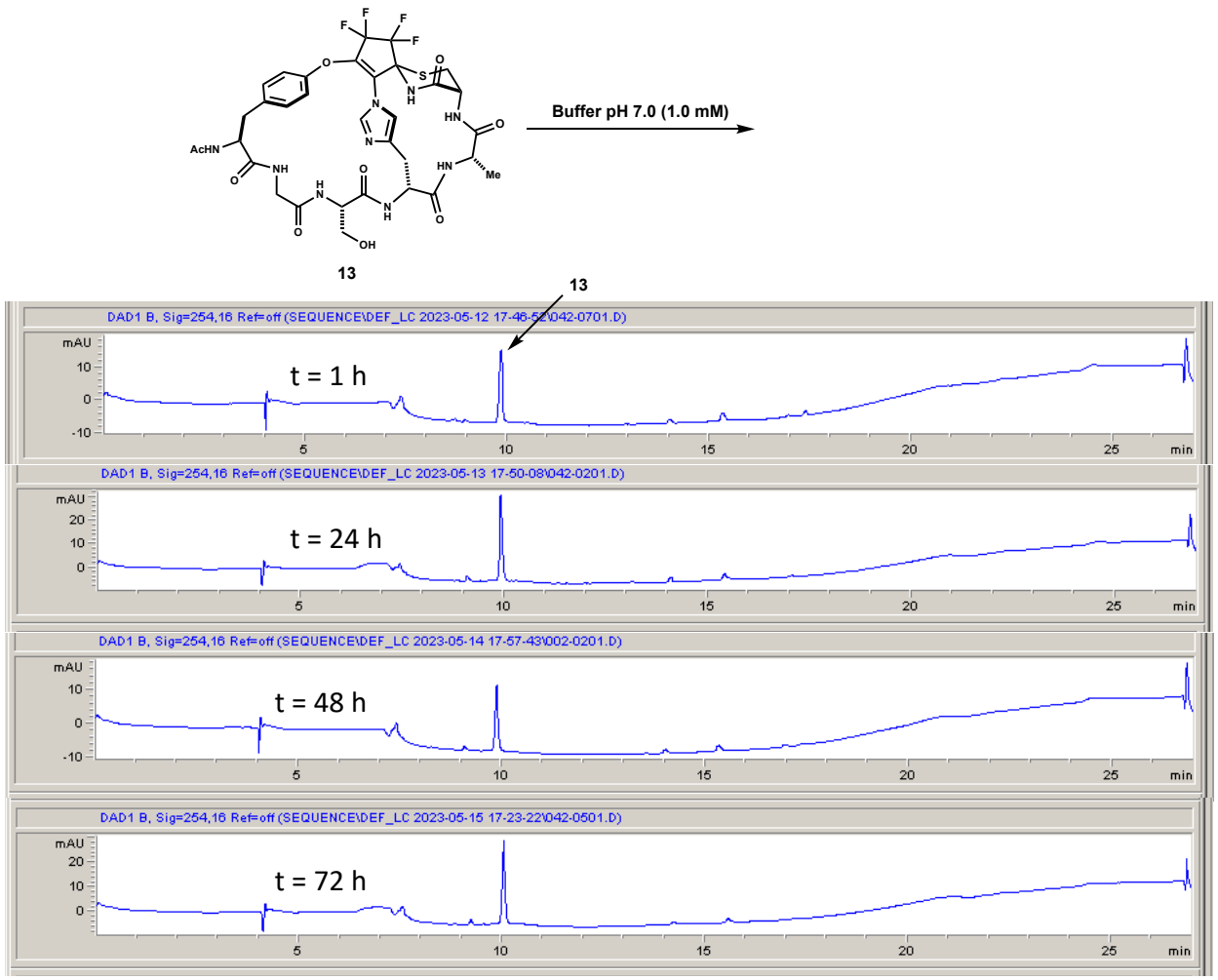


Fig. S2. Degradation Experiment of Macrobicycle 13 at pH 7.0. Compound **13** was subjected a degradation experiment at pH 7.0 over a 72 h time course. No noticeable decomposition of **13** was observed. **13** (1.0 mg, 1.0 equiv., 1.2 µmol) was diluted with KH_2PO_4/Na_2HPO_4 (0.05 M) buffer pH 7.0 (1.0 mM, 1.2 mL). The solution was then allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (254 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

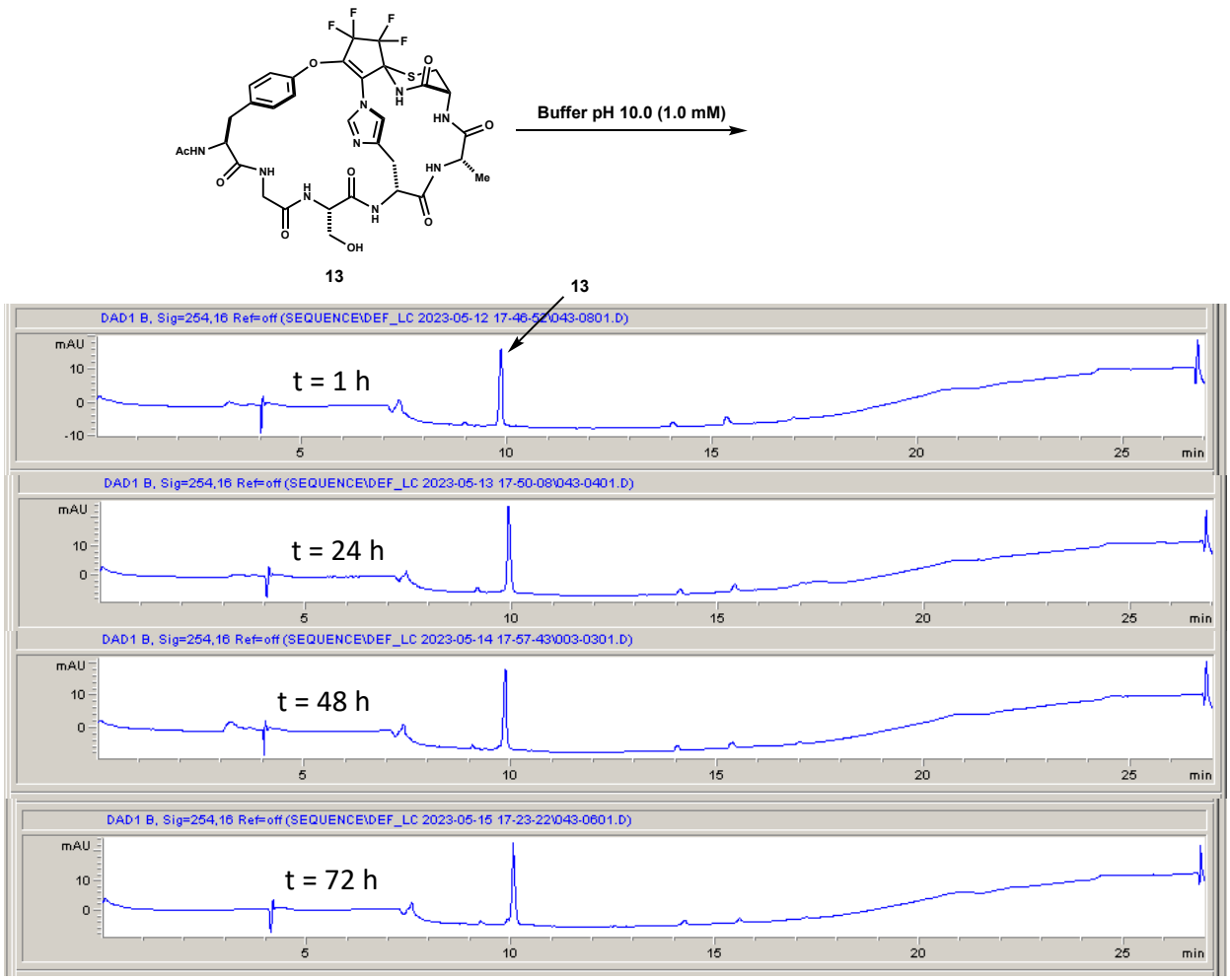


Fig. S3. Degradation Experiment of Macrobicycle 13 at pH 10.0. Compound **13** was subjected a degradation experiment at pH 10.0 over a 72 h time course. No noticeable decomposition of **13** was observed. **13** (1.0 mg, 1.0 equiv., 1.2 µmol) was diluted with $K_2CO_3/BK_3O_3/KOH$ (0.05 M) buffer pH 10.0 (1.0 mM, 1.2 mL). The solution was then allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (254 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

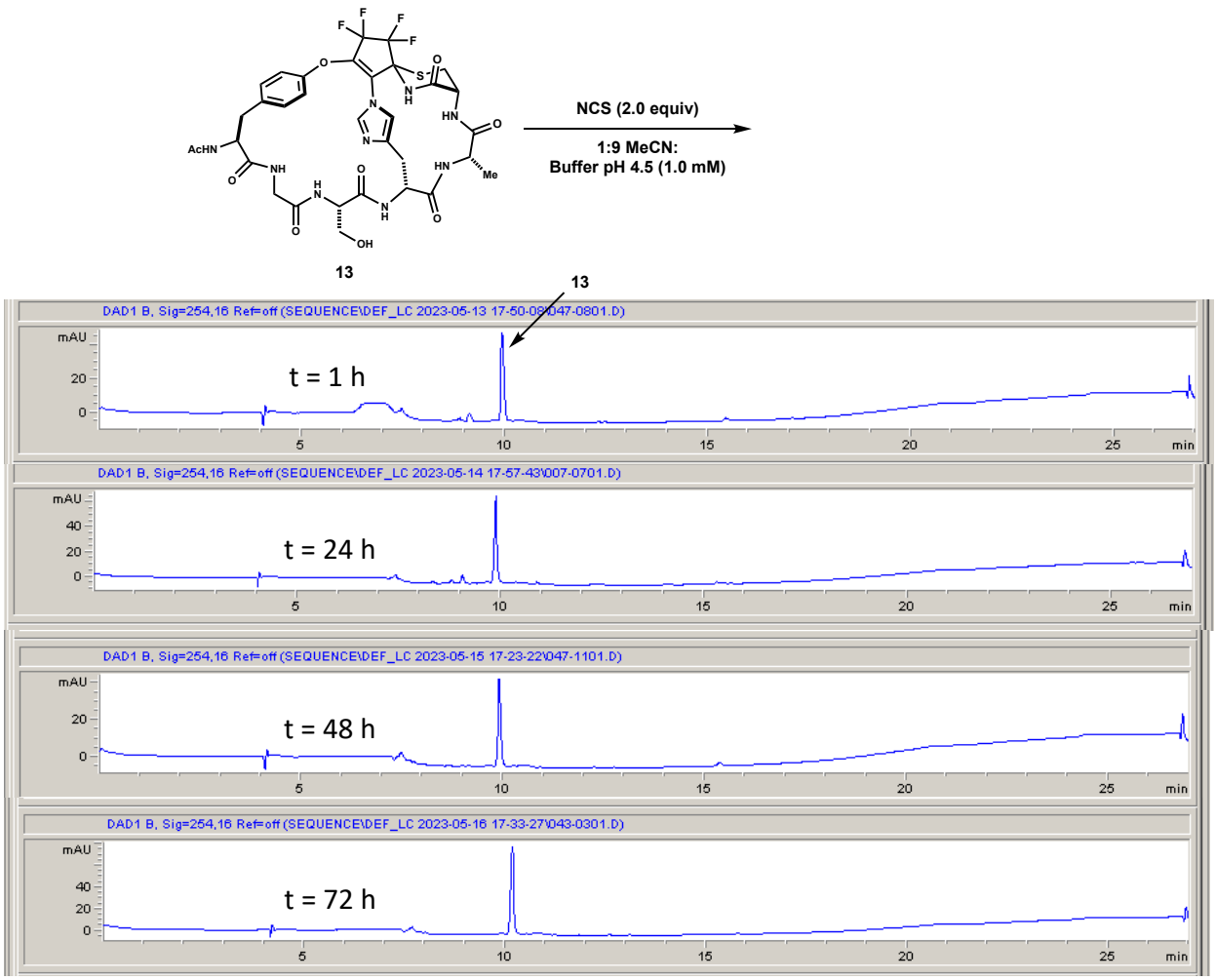


Fig. S4. Degradation Experiment of Macrobicycle 13 with NCS. Compound **13** was subjected a degradation experiment with NCS over a 72 h time course. No noticeable decomposition of **13** was observed. **13** (1.0 mg, 1.0 equiv.., 1.2 µmol) was diluted with 1:9 MeCN : NaOAc/AcOH (0.2 M) buffer pH 4.5 (1.0 mM, 1.2 mL). The solution was then added NCS (25 µL, 2.0 equiv, 2.5 µmol, 0.1 M in MeCN) and the reaction mixture allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (254 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

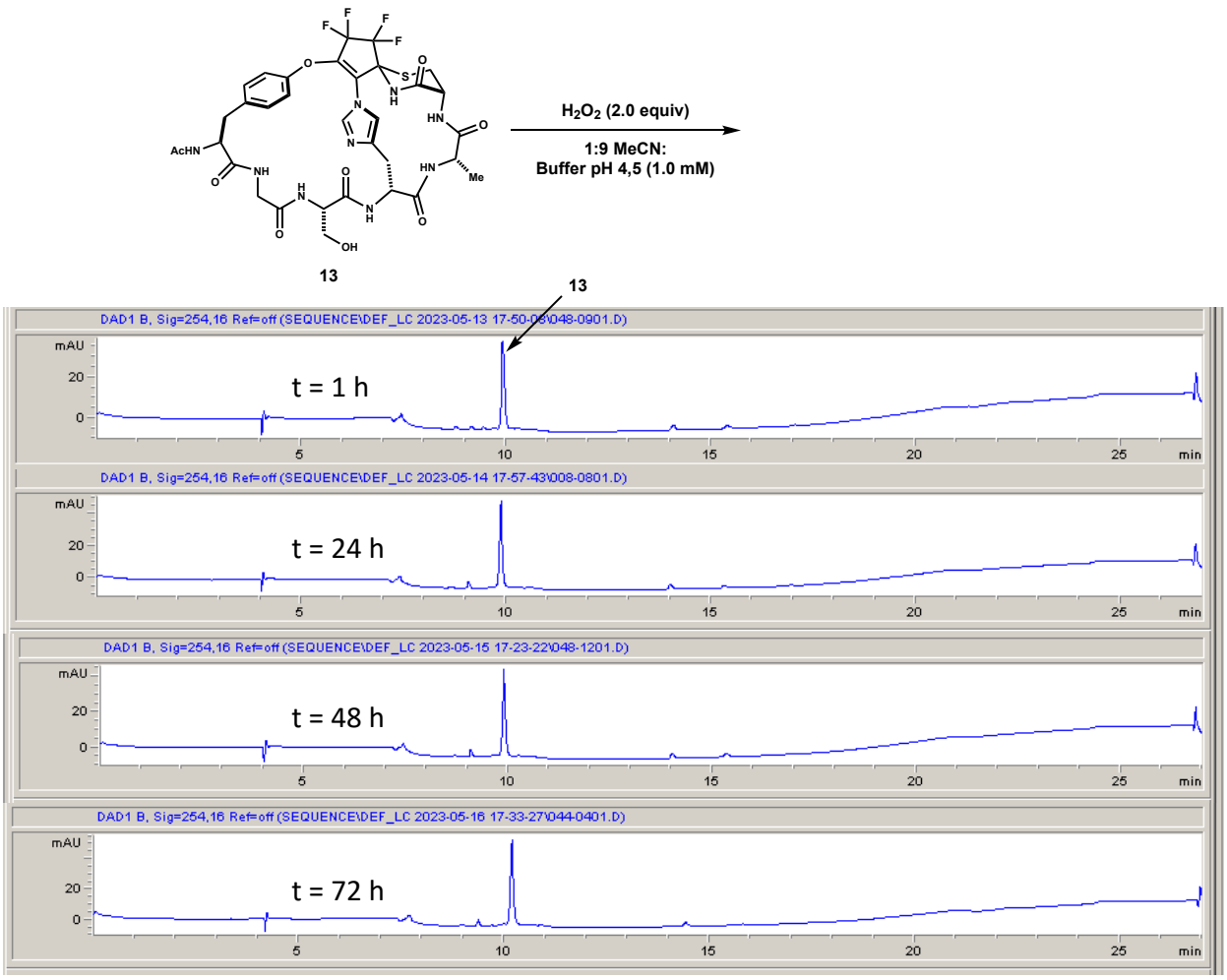
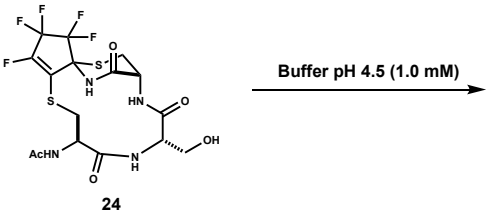


Fig. S5. Degradation Experiment of Macrobicycle 13 with H_2O_2. Compound **13** was subjected a degradation experiment with H_2O_2 over a 72 h time course. No noticeable decomposition of **13** was observed. **13** (1.0 mg, 1.0 equiv., 1.2 µmol) was diluted with 1:9 MeCN : NaOAc/AcOH (0.2 M) buffer pH 4.5 (1.0 mM, 1.2 mL). The solution was then added H_2O_2 (25 µL, 2.0 equiv., 2.5 µmol, 0.1 M in MeCN) and the reaction mixture allowed to stir at 25 ºC for 72 h. H_2O_2 solution prepared from 30% aqueous H_2O_2. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (254 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

3.2 Stability Studies on Macrocycle 24



24

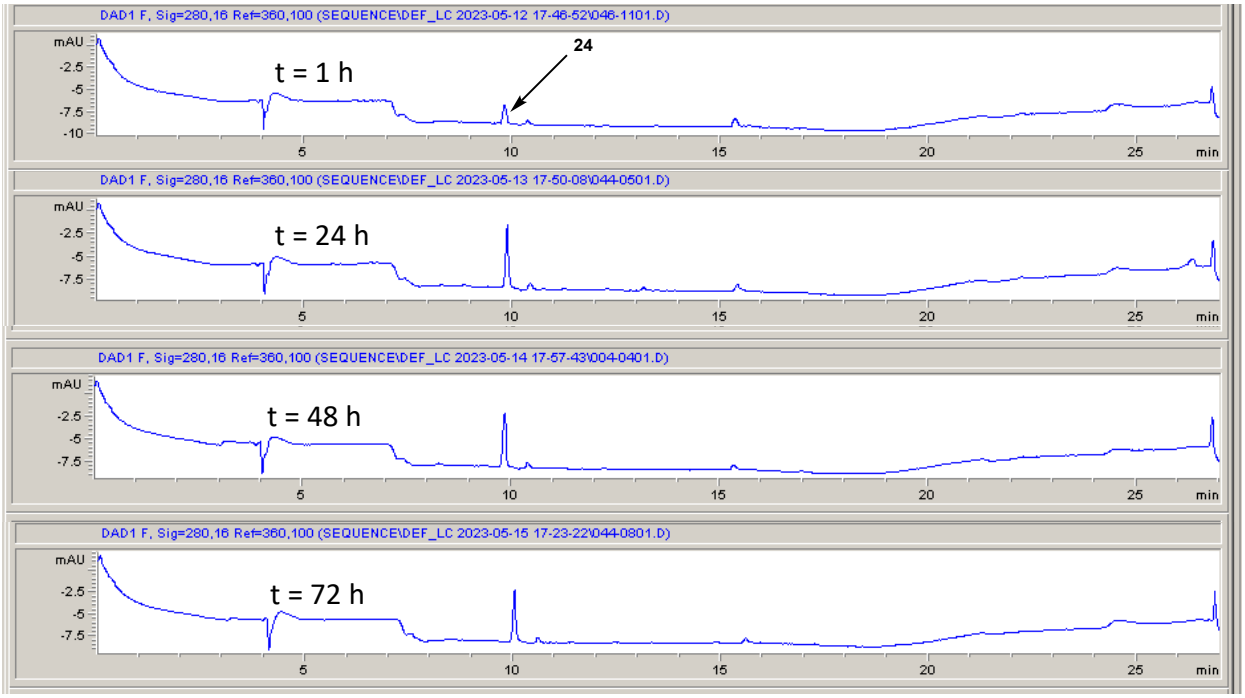


Fig. S6. Degradation Experiment of Macrocycle 24 at pH 4.5. Compound **24** was subjected a degradation experiment at pH 4.5 over a 72 h time course. No noticeable decomposition of **24** was observed. **24** (1.0 mg, 1.0 equiv., 2.0 µmol) was diluted with NaOAc/AcOH (0.2 M) buffer pH 4.5 (1.0 mM, 2.0 mL). The solution was then allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (280 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

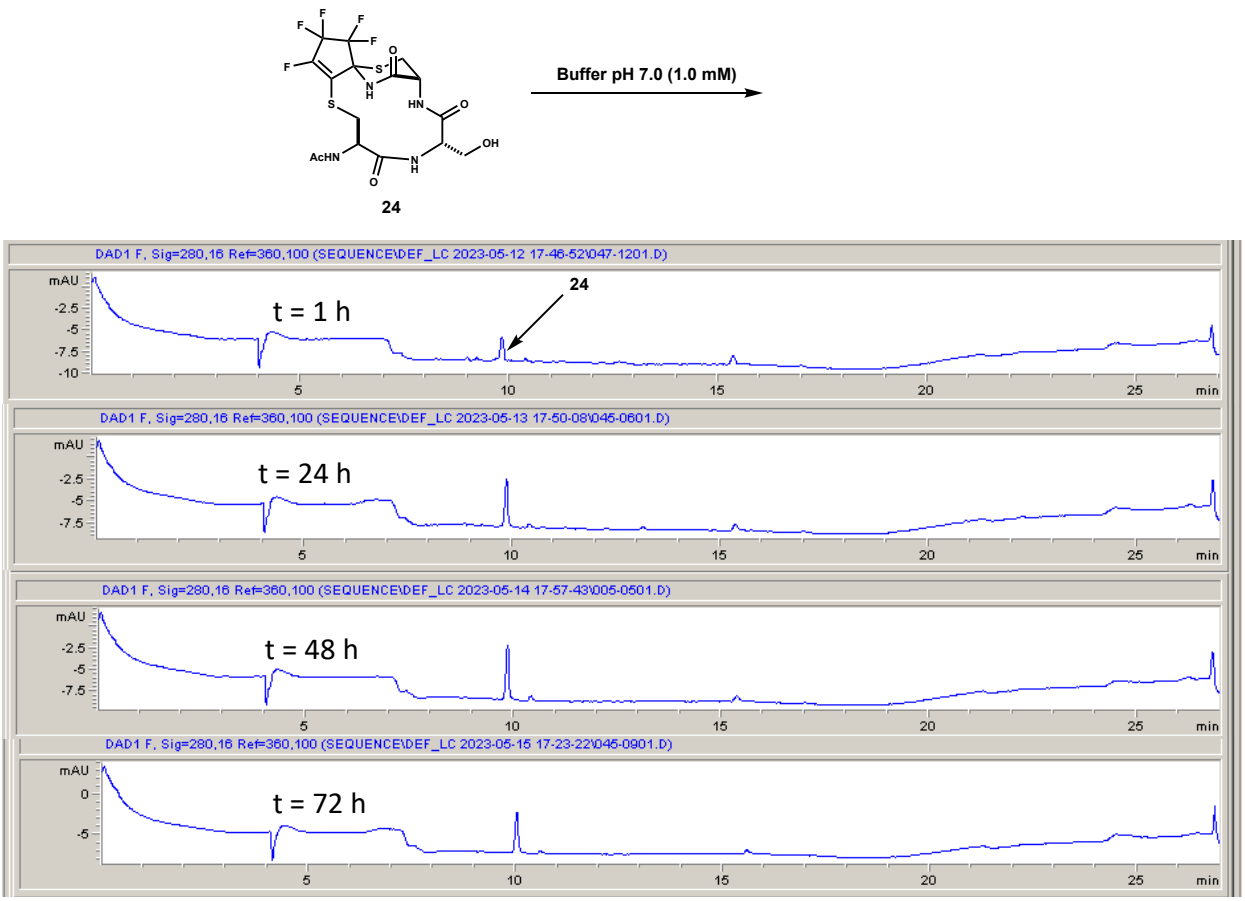


Fig. S7. Degradation Experiment of Macrocycle 24 at pH 7.0. Compound **24** was subjected a degradation experiment at pH 7.0 over a 72 h time course. No noticeable decomposition of **24** was observed. **24** (1.0 mg, 1.0 equiv., 2.0 µmol) was diluted with KH_2PO_4/Na_2HPO_4 (0.05 M) buffer pH 7.0 (1.0 mM, 2.0 mL). The solution was then allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (280 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

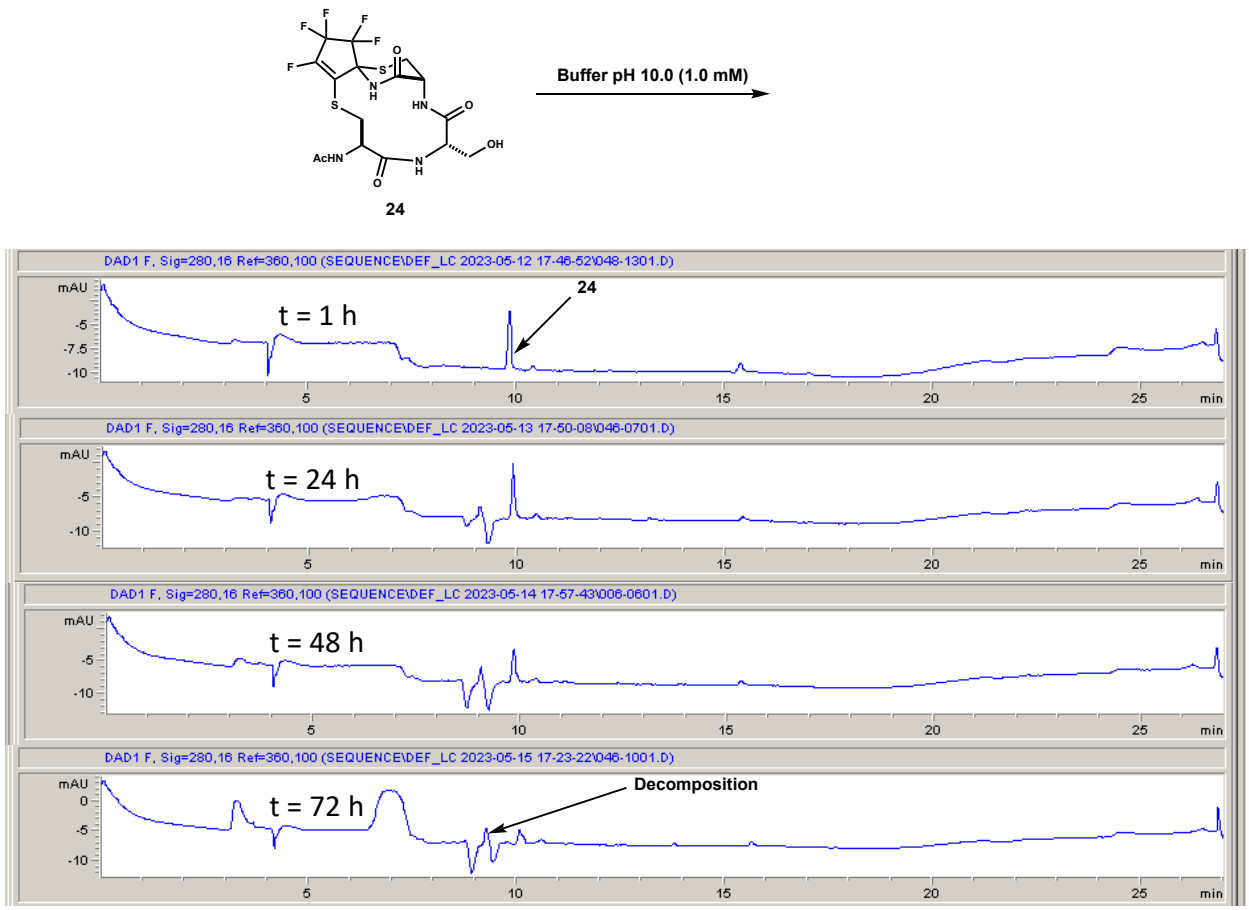


Fig. S8. Degradation Experiment of Macrocycle 24 at pH 10.0. Compound **24** was subjected a degradation experiment at pH 10.0 over a 72 h time course. Noticeable decomposition of **24** was observed starting at 24 h. **24** (1.0 mg, 1.0 equiv., 2.0 µmol) was diluted with $K_2CO_3/BK_3O_3/KOH$ (0.05 M) buffer pH 10.0 (1.0 mM, 2.0 mL). The solution was then allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (280 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

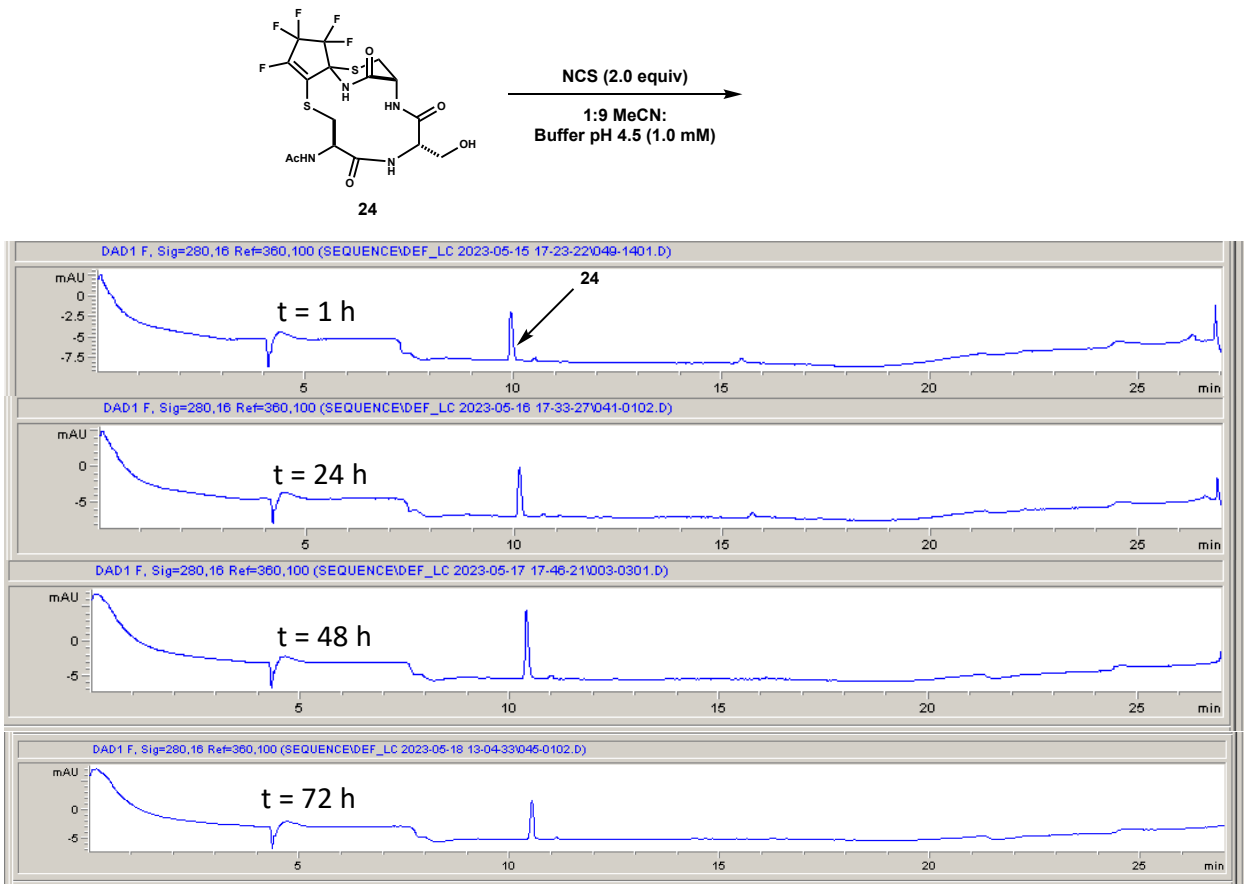


Fig. S9. Degradation Experiment of Macrocycle 24 with NCS. Compound **24** was subjected a degradation experiment with NCS over a 72 h time course. No noticeable decomposition of **24** was observed. **24** (1.0 mg, 1.0 equiv., 2.0 µmol) was diluted with 1:9 MeCN : NaOAc/AcOH (0.2 M) buffer pH 4.5 (1.0 mM, 2.0 mL). The solution was then added NCS (40 µL, 2.0 equiv., 4.0 µmol, 0.1 M in MeCN) and the reaction mixture allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (280 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

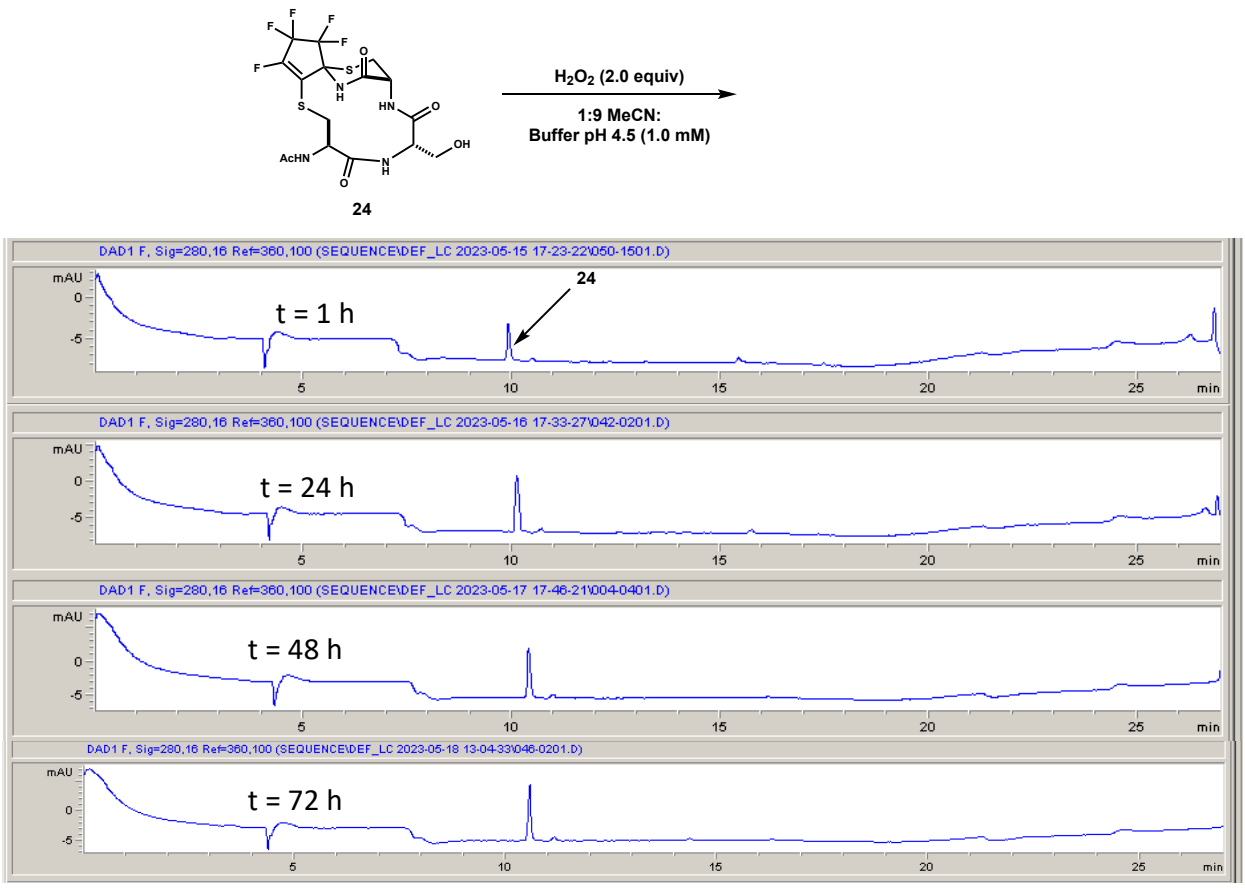


Fig. S10. Degradation Experiment of Macrocycle 24 with H_2O_2. Compound **24** was subjected a degradation experiment with H_2O_2 over a 72 h time course. No noticeable decomposition of **24** was observed. **24** (1.0 mg, 1.0 equiv., 2.0 µmol) was diluted with 1:9 MeCN : NaOAc/AcOH (0.2 M) buffer pH 4.5 (1.0 mM, 2.0 mL). The solution was then added H_2O_2 (40 µL, 2.0 equiv., 4.0 µmol, 0.1 M in MeCN) and the reaction mixture allowed to stir at 25 ºC for 72 h. H_2O_2 solution prepared from 30% aqueous H_2O_2. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (280 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

3.3 Stability Studies on Disulfide S30

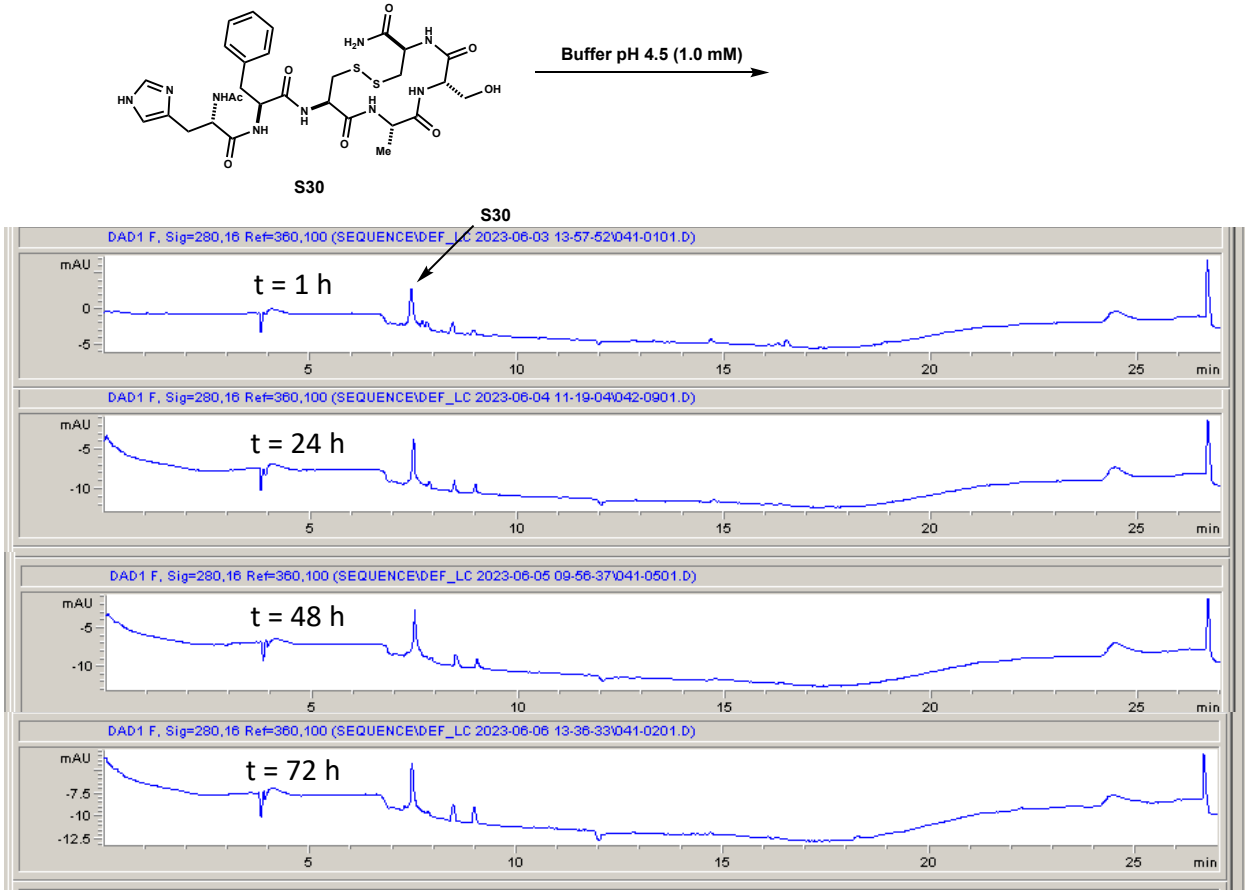


Fig. S11. Degradation Experiment of Macrocycle S30 at pH 4.5. Compound **S30** was subjected a degradation experiment at pH 4.5 over a 72 h time course. Noticeable decomposition of **S30** was observed starting at 1 h. **S30** (1.0 mg, 1.0 equiv., 1.2 µmol) was diluted with NaOAc/AcOH (0.2 M) buffer pH 4.5 (1.0 mM, 1.2 mL). The solution was then allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (280 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

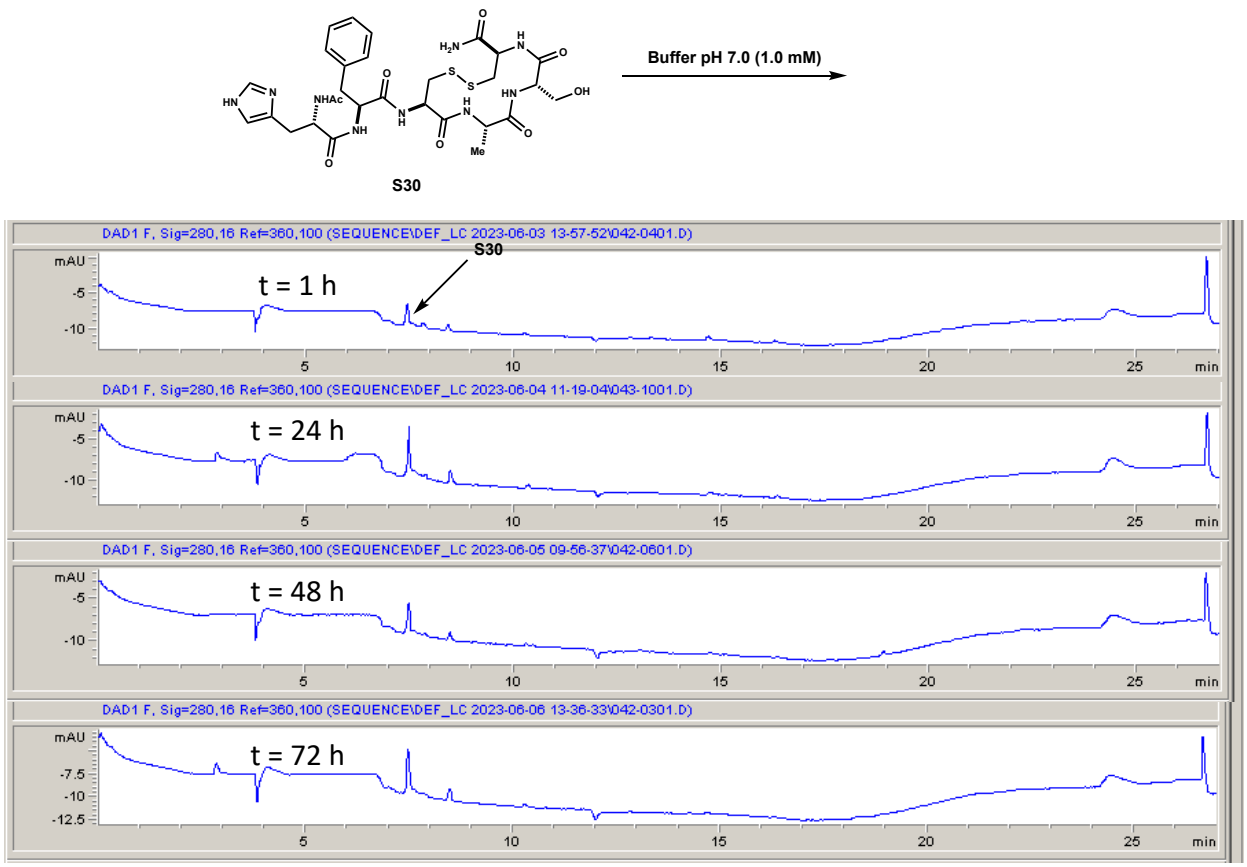


Fig. S12. Degradation Experiment of Macrocycle S30 at pH 7.0. Compound **24** was subjected a degradation experiment at pH 7.0 over a 72 h time course. No noticeable decomposition of **S30** was observed. **S30** (1.0 mg, 1.0 equiv., 1.2 µmol) was diluted with KH_2PO_4/Na_2HPO_4 (0.05 M) buffer pH 7.0 (1.0 mM, 1.2 mL). The solution was then allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (280 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

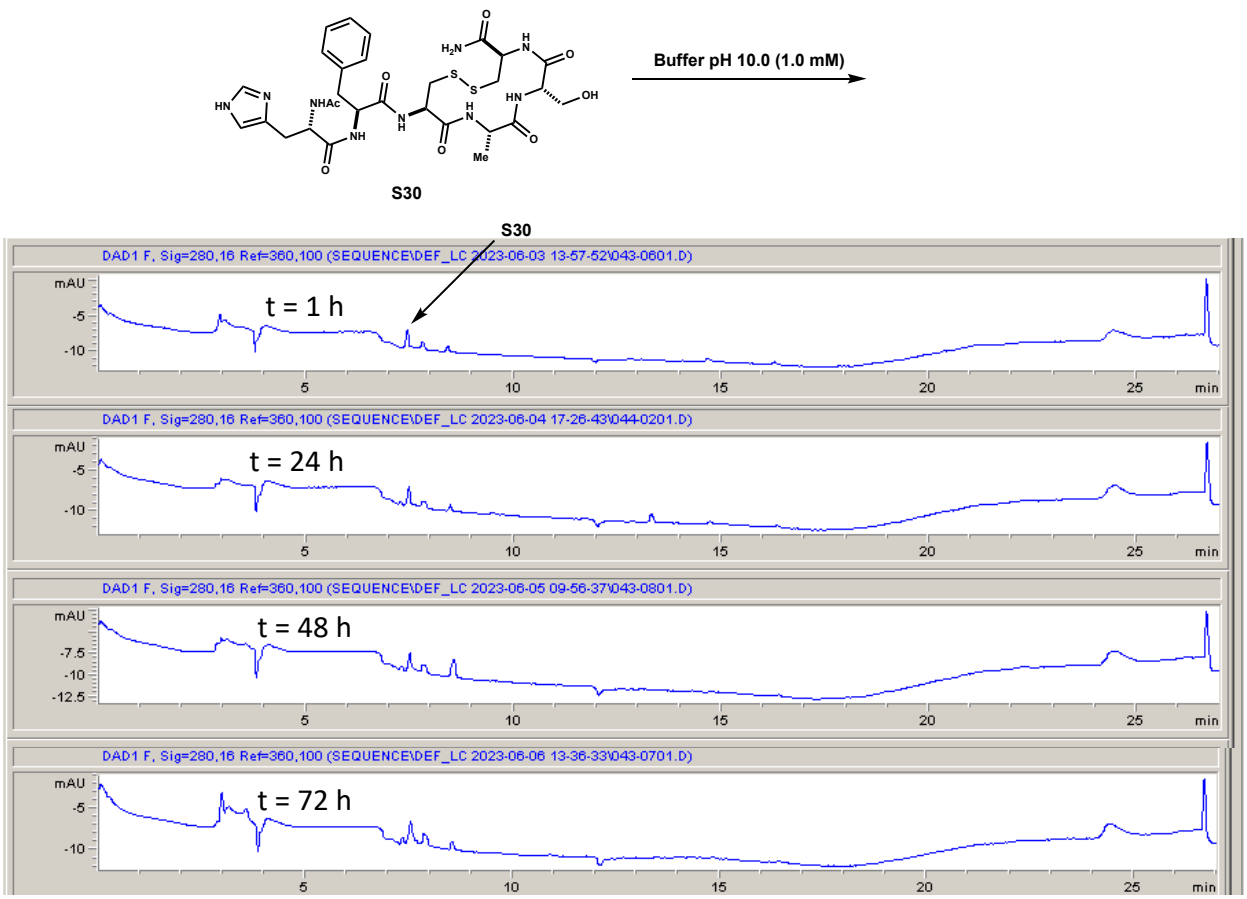


Fig. S13. Degradation Experiment of Macrocycle S30 at pH 10.0. Compound **S30** was subjected a degradation experiment at pH 10.0 over a 72 h time course. Noticeable decomposition of **24** was observed starting at 1 h. **S30** (1.0 mg, 1.0 equiv., 1.2 µmol) was diluted with K_2CO_3/BK_3O_3/KOH (0.05 M) buffer pH 10.0 (1.0 mM, 1.2 mL). The solution was then allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (280 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

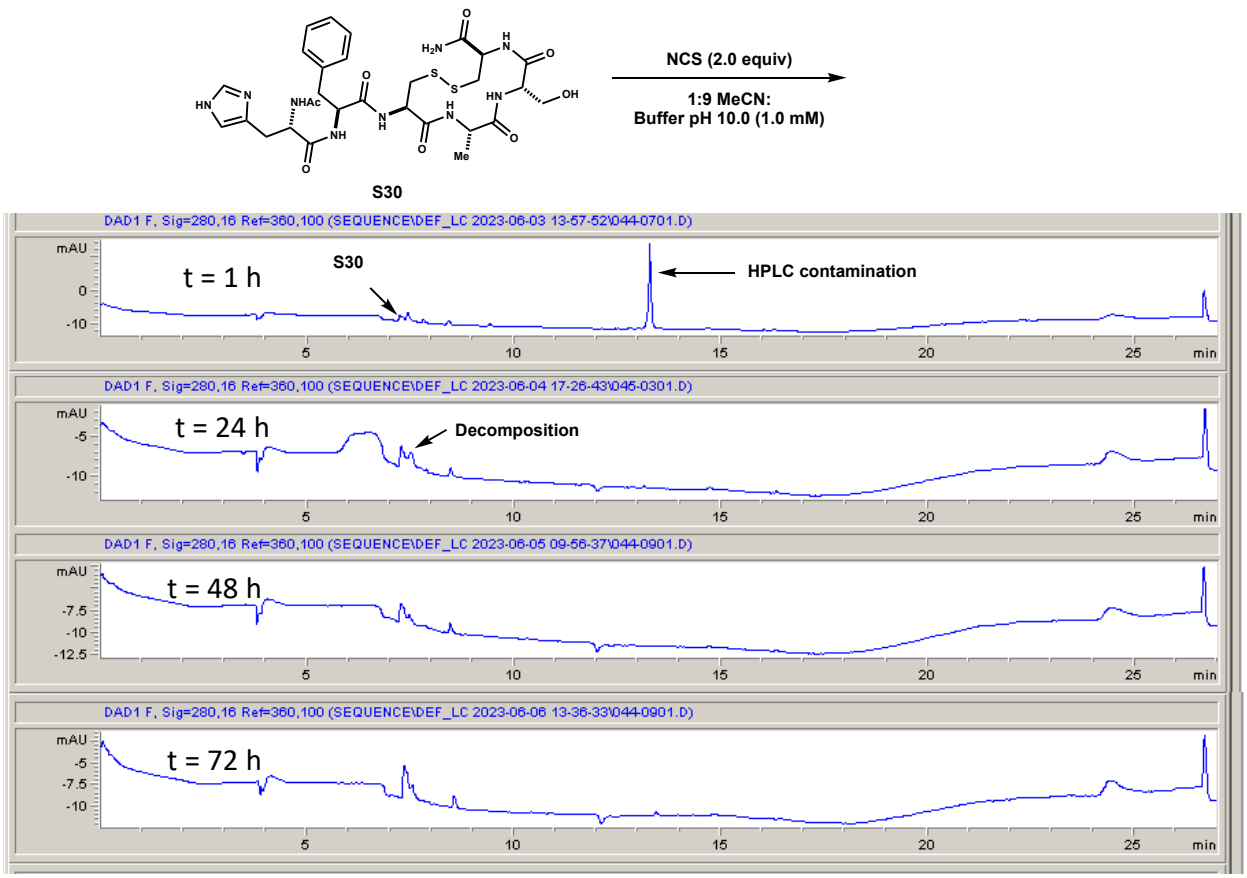


Fig. S14. Degradation Experiment of Macrocycle S30 with NCS. Compound **S30** was subjected a degradation experiment with NCS over a 72 h time course. Noticeable decomposition of **S30** was observed after 1 h and complete decomposition observed after 24 h. **S30** (1.0 mg, 1.0 equiv, 1.2 µmol) was diluted with 1:9 MeCN : NaOAc/AcOH (0.2 M) buffer pH 4.5 (1.0 mM, 1.2 mL). The solution was then added NCS (25 µL, 2.0 equiv., 2.5 µmol, 0.1 M in MeCN) and the reaction mixture allowed to stir at 25 ºC for 72 h. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (280 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

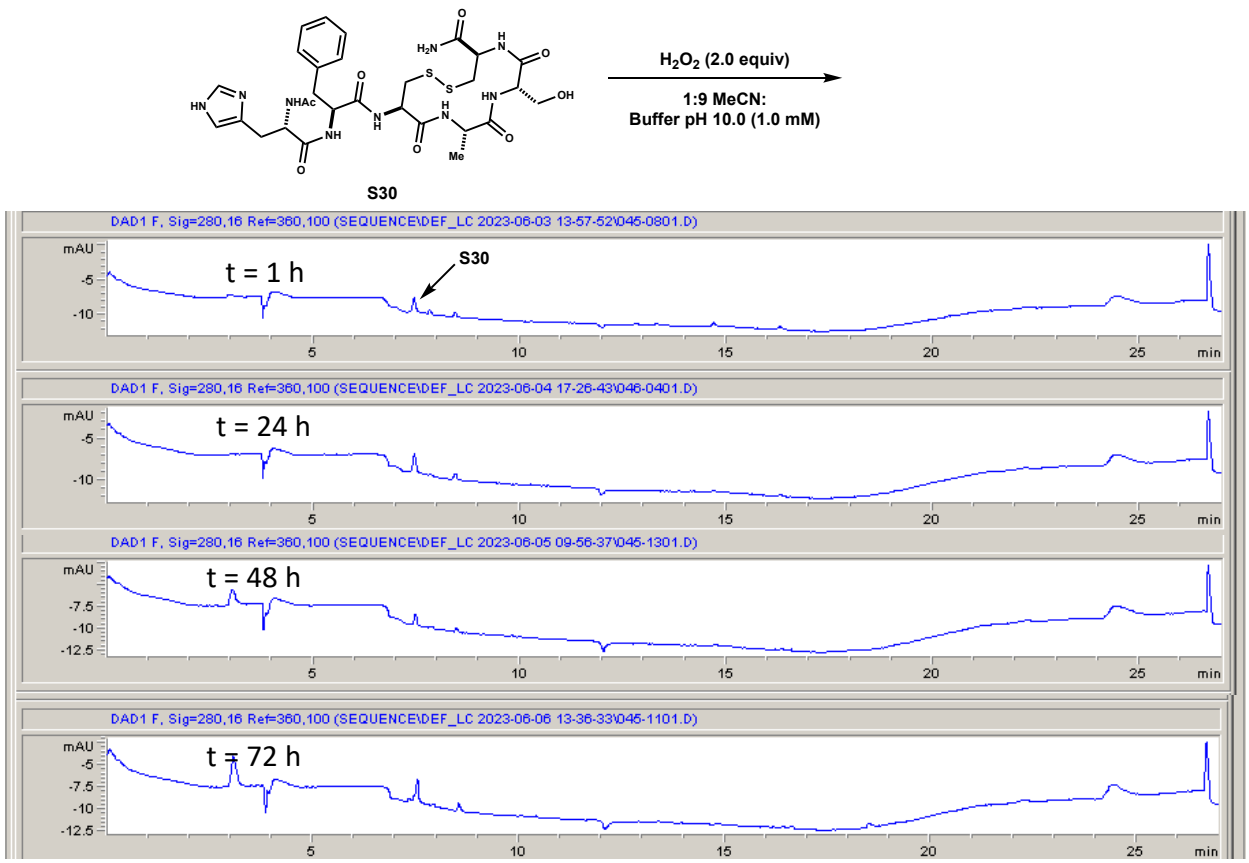


Fig. S15. Degradation Experiment of Macrocycle S30 with H_2O_2. Compound **S30** was subjected a degradation experiment with H_2O_2 over a 72 h time course. No noticeable decomposition of **S30** was observed. **S30** (1.0 mg, 1.0 equiv., 1.2 µmol) was diluted with 1:9 MeCN : NaOAc/AcOH (0.2 M) buffer pH 4.5 (1.0 mM, 1.2 mL). The solution was then added H_2O_2 (25 µL, 2.0 equiv., 4.0 µmol, 0.1 M in MeCN) and the reaction mixture allowed to stir at 25 ºC for 72 h. H_2O_2 solution prepared from 30% aqueous H_2O_2. Time points were taken at 1 h, 24 h, 48 h, and 72 h. Monitored via analytical HPLC (280 nm). The reaction was monitored by taking 10 µL aliquots at of the reaction mixture at the stated time points and diluting into 130 µL of 1% AcOH in MeCN.

4. Computational Evaluation of Loop Mimicry

4.1 Computational Methods

Computational models of macrocycles and macrobicycle as well as all calculations performed on them (geometry optimization and conformational searches) were generated and carried out using Schrödinger's Maestro Macromodel®.[1,2] Geometry optimizations were carried out using the AMBER* force field minimization in water with default settings (Method: PRGC, Maximum iterations: 2500, Convergence threshold: 0.05). Low energy conformations of each geometry optimized structure were identified using a Monte Carlo search algorithm using the AMBER* force field in water with default settings (Method: PRGC, Maximum iteration: 2500, Convergence threshold: 0.05, Maximum number of steps: 1000, 21.0 kJ/mol energy window for saving structures). Low energy conformers of each library member were overlaid onto each of the 11 Kritzer loop type representatives using the PyMOL® superposition function; generating MatchAlign scores for each confirmation-loop type pair. The generated MatchAlign scores, which are a function of root-mean-square deviation (RMSD) and the number of atoms used in the superposition, were normalized relative to loop self-superimposed maximal values to yield 'similarity scores (%)'. Normalizing each MatchAlign score in this way allows for appropriate comparison of confirmation-loop type pair overlays regardless of the length of the loop and/or macrobicycle. Determination of intramolecular hydrogen bonding (shown in **Fig. 6**) was done using Schrödinger's Maestro MacroModel® and verified using PyMOL®.

4.2 Supplemental Figures and Tables

Table S1.

Loop Type	PDB Code	Chain	Length	First AA	Last AA	Sequence	Max Align. Score
β-Turn	3A2V	A	6	S207	F212	SLDWWF	30.7
β-Hairpin	1T3I	A	6	V264	F269	VFFDHF	30.7
β-Bulge	3BGT	A	6	H174	P179	HVDGSP	20.8
Schellman Loop	2OL1	B	6	S73	D78	SLKGID	20.8
αβ-Motiff	2DVT	D	5	I274	G278	ILEIG	19.8
γ-Turn	2WAM	A	7	T89	F95	TFKTDHF	36.7
Asx-Turn	4HN1	A	6	S18	R23	SDHRGR	20.8
Asx-Motif	3B8I	C	5	G94	N98	GNALN	19.8
S/T-Turn	4J07	A	7	R84	F90	RGDTPHF	26.7
S/T-Motif	3N3R	A	5	R40	P44	RDWWP	24.8
S/T-Staple	1NVM	A	6	V278	L283	VDRETL	30.7

Table S1. Representative Loop Type Information. Selected representatives for each of the 11 loop types identified by Kritzer. Selected representatives were chosen on the basis that they have backbone torsional angles close to that of the average for each loop type.

Fig. S16.

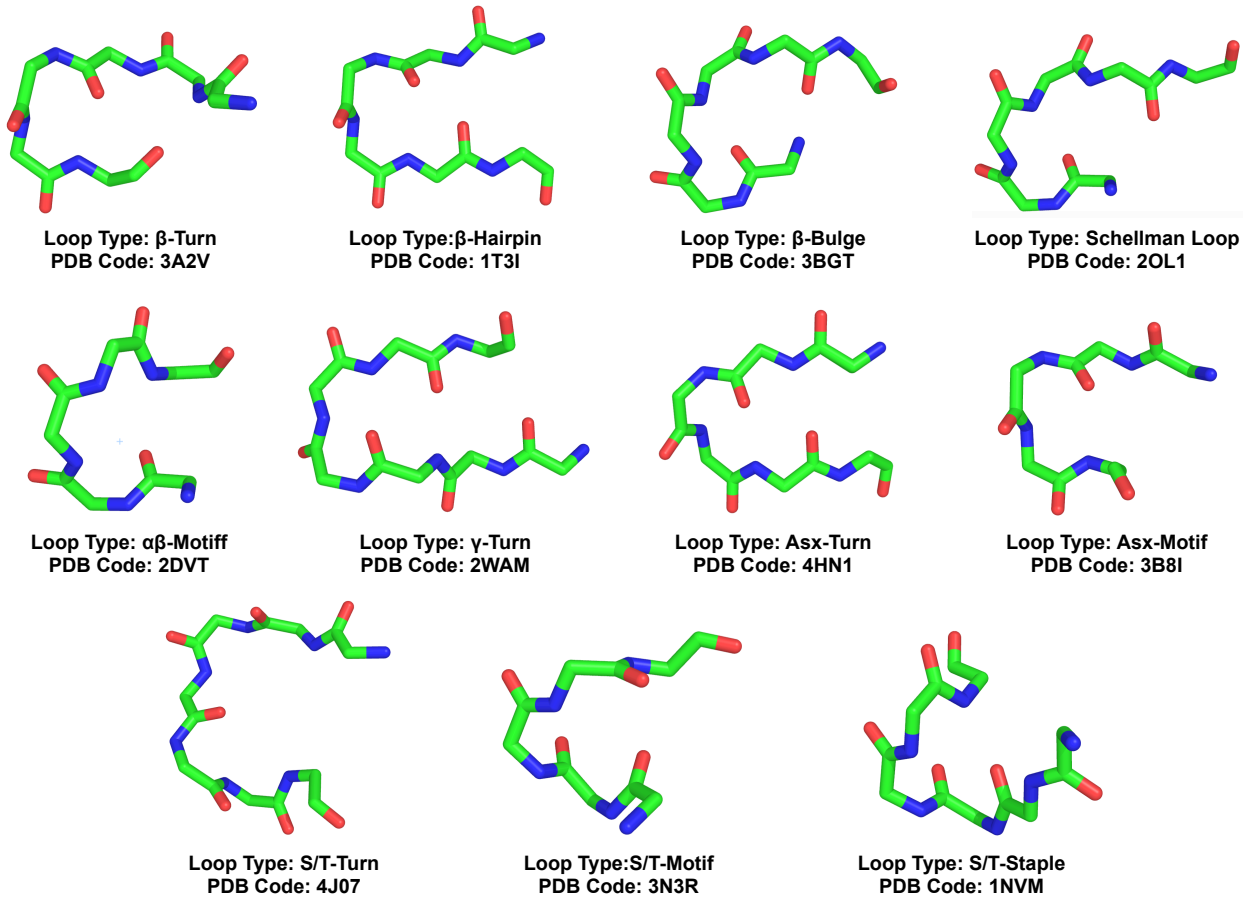


Fig. S16. Images of Loop Type Representatives. Depicted loops were extracted from protein crystal structures obtained from the Protein Data Base (PDB) and are rendered using PyMOL®.

Fig. S17.

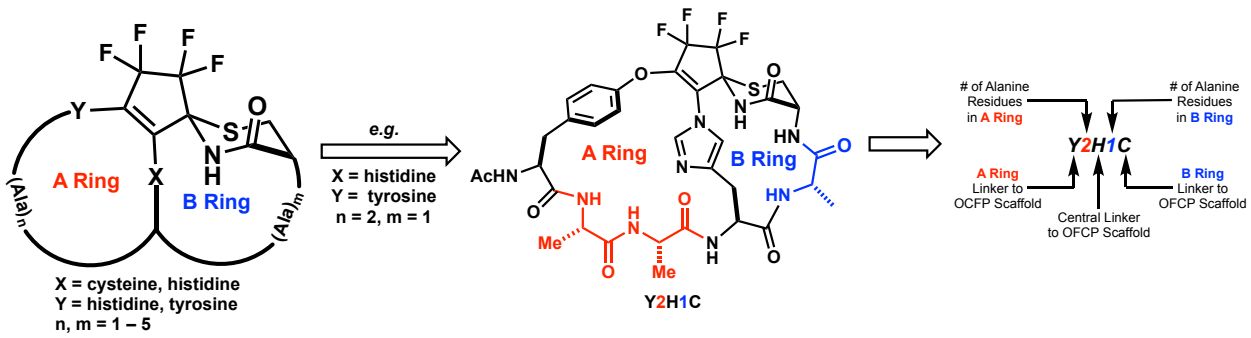


Fig. S17. General Structure and Naming Convention for OFCP Processed Poly-Alanine Macrobicycles. General structure of OFCP derived poly-alanine macrobicycles (Left), a select example of OFCP derived poly-alanine macrobicycle (center) and an explanation of the naming convention (right). OFCP processed macrobicycles are named based on the amino acid sequence of the peptide from which they are derived with letters corresponding to 1 letter amino acid abbreviations and numbers representing the number of intervening L-alanine residues between nucleophilic residues.

Table S2.

Entry #	Group	Family	Code	Conformations
1	X#X1X	X1X1X	C1C1C	6
2			H1C1C	7
3			Y1C1C	5
4			C1H1C	5
5			H1H1C	8
6			Y1H1C	7
7		X2X1X	C2C1C	85
8			H2C1C	10
9			Y2C1C	11
10			C2H1C	18
11			H2H1C	11
12			Y2H1C	5
13		X3X1X	C3C1C	34
14			H3C1C	15
15			Y3C1C	20
16			C3H1C	8
17			H3H1C	28
18			Y3H1C	25
19		X4X1X	C4C1C	99
20			H4C1C	65
21			Y4C1C	35
22			C4H1C	6
23			H4H1C	43
24			Y4H1C	22
25		X5X1X	C5C1C	52
26			H5C1C	54
27			Y5C1C	29
28			C5H1C	5
29			H5H1C	51
30			Y5H1C	44

Entry #	Group	Family	Code	Conformations
31	X#X2X	X1X2X	C1C2C	25
32			H1C2C	10
33			Y1C2C	13
34			C1H2C	18
35			H1H2C	10
36			Y1H2C	9
37		X2X2X	C2C2C	29
38			H2C2C	25
39			Y2C2C	58
40			C2H2C	10
41			H2H2C	23
42			Y2H2C	27
43		X3X2X	C3C2C	106
44			H3C2C	30
45			Y3C2C	29
46			C3H2C	31
47			H3H2C	33
48			Y3H2C	56
49		X4X2X	C4C2C	23
50			H4C2C	55
51			Y4C2C	44
52			C4H2C	48
53			H4H2C	27
54			Y4H2C	43
55		X5X2X	C5C2C	19
56			H5C2C	97
57			Y5C2C	55
58			C5H2C	39
59			H5H2C	54
60			Y5H2C	19

Entry #	Group	Family	Code	Conformations
61	X#X3X	X1X3X	C1C3C	35
62			H1C3C	13
63			Y1C3C	50
64			C1H3C	55
65			H1H3C	22
66			Y1H3C	10
67		X2X3X	C2C3C	6
68			H2C3C	64
69			Y2C3C	88
70			C2H3C	65
71			H2H3C	24
72			Y2H3C	36
73		X3X3X	C3C3C	60
74			H3C3C	42
75			Y3C3C	36
76			C3H3C	22
77			H3H3C	31
78			Y3H3C	25
79		X4X3X	C4C3C	34
80			H4C3C	68
81			Y4C3C	20
82			C4H3C	81
83			H4H3C	63
84			Y4H3C	20
85		X5X3X	C5C3C	6
86			H5C3C	70
87			Y5C3C	44
88			C5H3C	33
89			H5H3C	46
90			Y5H3C	6

Entry #	Group	Family	Code	Conformations
91	X#X4X	X1X4X	C1C4C	65
92			H1C4C	27
93			Y1C4C	41
94			C1H4C	88
95			H1H4C	30
96			Y1H4C	15
97		X2X4X	C2C4C	24
98			H2C4C	44
99			Y2C4C	23
100			C2H4C	48
101			H2H4C	10
102			Y2H4C	24
103		X3X4X	C3C4C	130
104			H3C4C	56
105			Y3C4C	21
106			C3H4C	38
107			H3H4C	21
108			Y3H4C	37
109		X4X4X	C4C4C	24
110			H4C4C	86
111			Y4C4C	21
112			C4H4C	34
113			H4H4C	78
114			Y4H4C	95
115		X5X4X	C5C4C	25
116			H5C4C	79
117			Y5C4C	74
118			C5H4C	7
119			H5H4C	4
120			Y5H4C	104

Entry #	Group	Family	Code	Conformations
121	X#X5X	X1X5X	C1C5C	35
122			H1C5C	19
123			Y1C5C	27
124			C1H5C	7
125			H1H5C	54
126			Y1H5C	36
127		X2X5X	C2C5C	36
128			H2C5C	23
129			Y2C5C	21
130			C2H5C	64
131			H2H5C	64
132			Y2H5C	45
133		X3X5X	C3C5C	123
134			H3C5C	57
135			Y3C5C	39
136			C3H5C	33
137			H3H5C	48
138			Y3H5C	53
139		X4X5X	C4C5C	16
140			H4C5C	19
141			Y4C5C	57
142			C4H5C	60
143			H4H5C	52
144			Y4H5C	33
145		X5X5X	C5C5C	93
146			H5C5C	13
147			Y5C5C	73
148			C5H5C	15
149			H5H5C	39
150			Y5H5C	51

Table. S2. Complete OFCP Derived Poly-Alanine Macrobicycle Library. OFCP derived poly-alanine macrobicycles were categorized into groups based on the number of intervening L-alanine residues composing the B Ring and then into families based on the number of intervening L-alanine residues composing the A Ring. Also, shown are the number of low-energy conformations identified for each of the 150 OFCP derived poly-alanine macrobicycles used I the study.

Fig. S18.

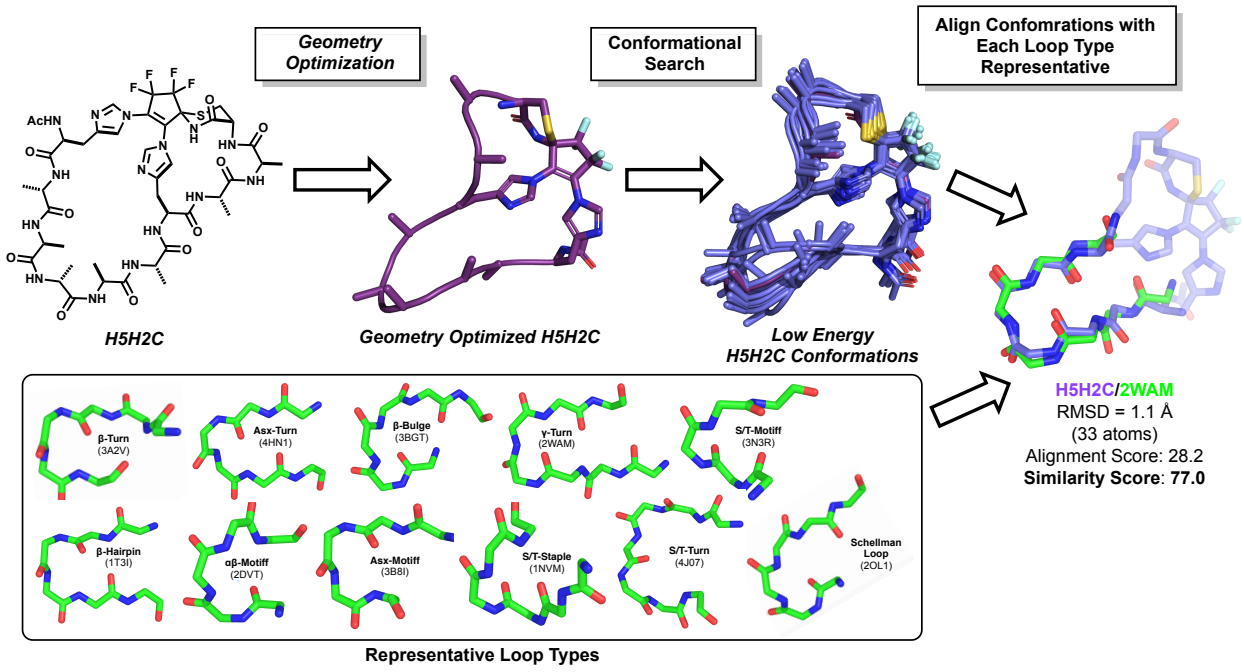


Fig. S18. Workflow for Determination of OFCP Derived Poly-Alanine Macrobicycle/Loop Type Similarity Scores. Each of the 150 OFCP derived macrobicycles used in this study were geometry optimized using molecular force fields and low energy conformations were identified using a Monte Carlo search algorithm. Low energy conformers of each library member were overlaid onto a representative of the 11 Kritzer loop types using the PyMOL® superposition function. The resulting generated MatchAlign scores were normalized relative to loop self-superimposed maximal values to yield 'similarity scores'.

Fig. S19.

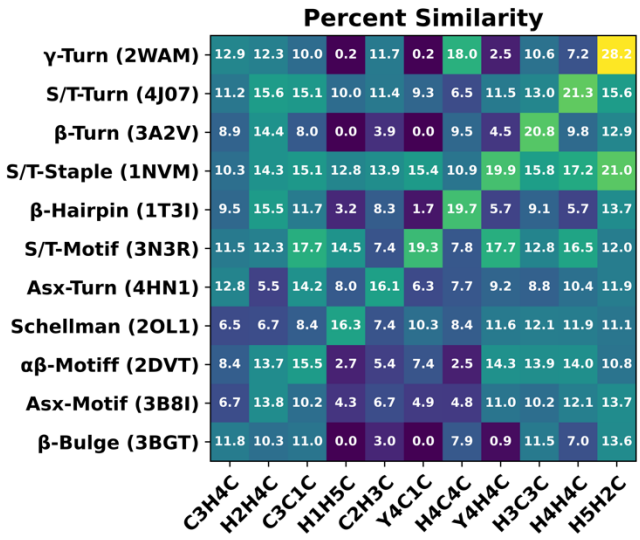


Fig. S19. Non-Normalized Version of Heat Map Shown in Fig. 5. Heatmap above shows non-normalized data displayed in **Fig. 5** heatmap.

Table S3.

Loop	Macrobicycle	Conf. #	RMSD	# of Atoms	Align. Score	Max Align.	% Similarity
γ-Turn	H5H2C	4	1.14	33	28.2	36.7	77.0
S/T-Turn	H4H4C	77	0.72	25	21.3	26.7	79.8
β-Turn	H3C3C	23	0.36	24	20.8	30.7	67.6
S/T-Staple	Y4H4C	70	0.89	28	19.9	30.7	64.7
β-Hairpin	H4C4C	33	0.79	24	19.7	30.7	64.0
S/T-Motif	Y4C1C	22	1.11	24	19.3	24.8	77.7
Asx-Turn	C2H3C	9	0.27	16	16.1	20.8	77.3
Schellman	H1H5C	25	0.88	26	16.3	20.8	78.5
αβ-Motiff	C3C1C	2	1.39	23	15.5	19.8	78.5
Asx-Motif	H2H4C	3	0.77	18	13.8	19.8	69.5
β-Bulge	C3H4C	29	0.24	12	11.8	20.8	57.0

Table S3. Data for Best Mimics of Loop Type Representatives. Data in the table above correspond to the OFCP derived poly-alanine macrobicycles/representative loop type overlays displayed along the diagonal (top right to bottom left) in **Fig. 5.**

Fig. S20.

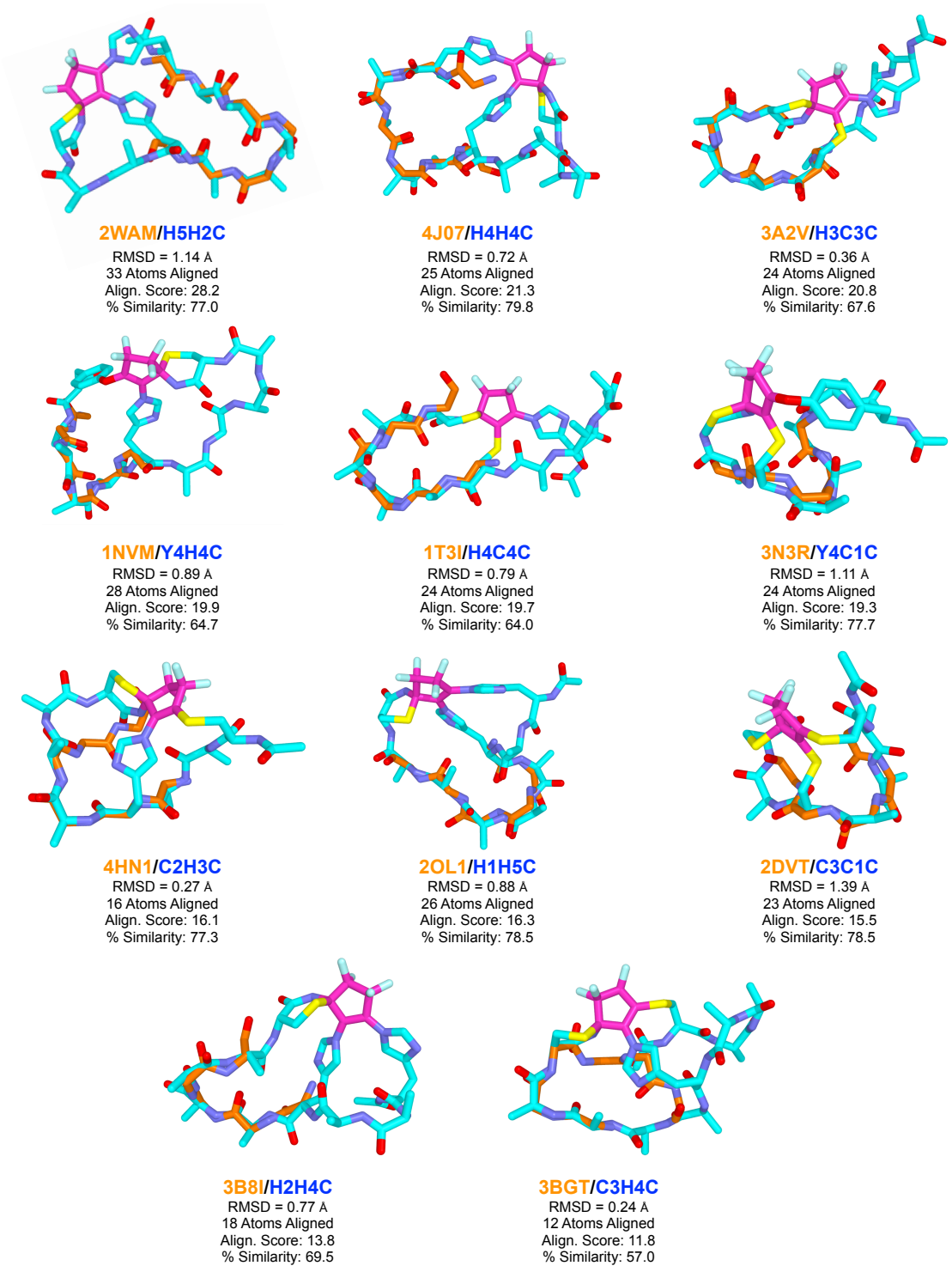


Fig. S20. Images of Best Macrobicycle Loop/Type Representatives. Depicted are the 11 loop type representatives super imposed onto their OFCP derived poly-alanine macrobicycle optimal mimic. Overlays were performed using the PyMOL® super position function.

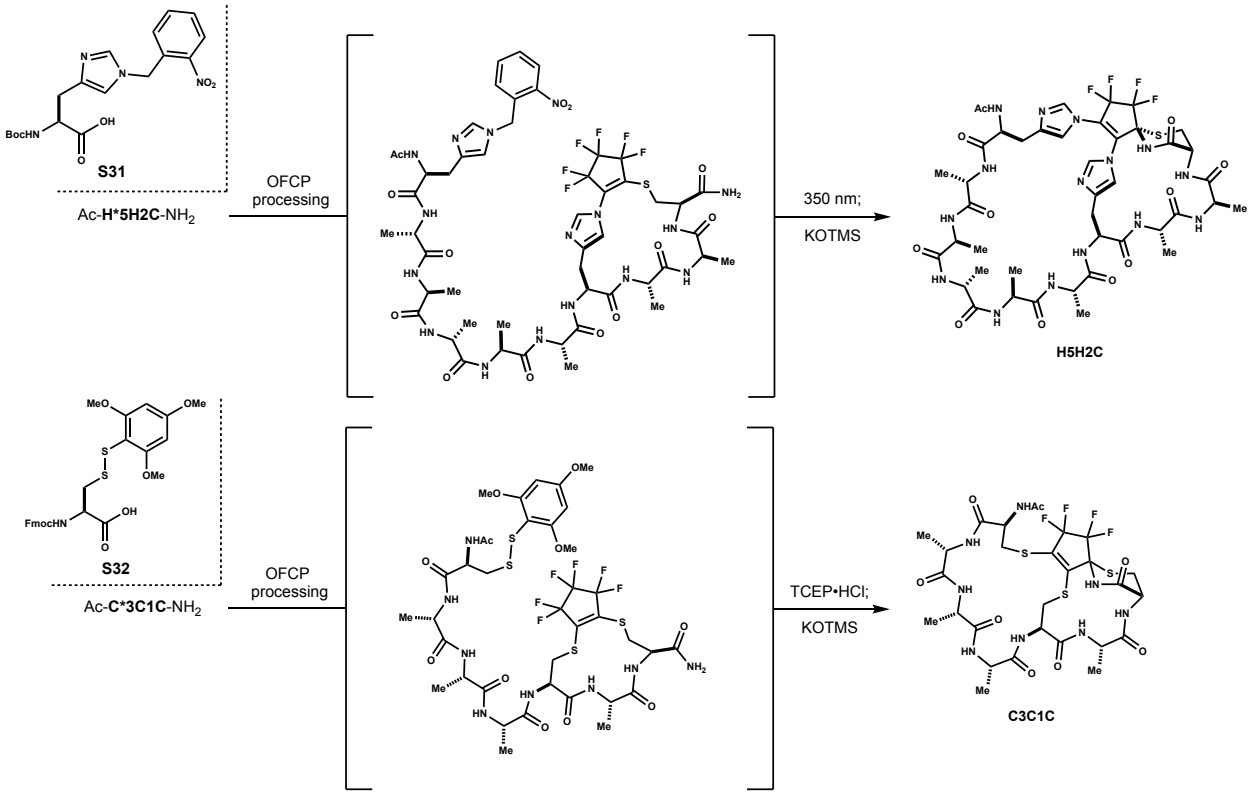


Fig. S21. **Differential Protecting Group Schemes to Access Macrobicycles Derived from HXHXH and CXCXC.** Macrobicycles derived from linear peptides such as Ac-H5H2C-NH_2 can be accessed from differential protection of the histidine residue proximal to the N-terminus. Differential protection via known monomer **S31**.[3] Macrobicycles derived from linear peptides such as Ac-C3C1C-NH_2 can be accessed from differential protection of the cysteine residue proximal to the N-terminus. Differential protection via known monomer **S32**.[4]

5. Computations Related to Reactivity of OFCP

5.1 Computational Methods

All computations related to the calculations of the delocalized HOMO present in the intermediate formed by model reaction of ethyl mercaptide with OFCP (shown in **Fig. 2B**) were carried out using the Gaussian 16 program[5] and performed at the level of M062X-D3/def2TZVP/SMD.[6-12] Homo cube file was obtained using Multiwfn[13] with picture rendering being done in PyMOL®. After detailed calculations of different nucleophiles, it can be shown that negative charge will always be delocalized which reflects the importance of negative hyperconjugation in lowering the activation energy of the reaction.

5. PAMPA Experiment – Supplemental Information

This assay was performed as outlined in the protocol of the parallel artificial permeability assay kit (BioAssay Systems Cat. # PAMPA-096). Incubation was performed at rt for 18 h. Data analysis was performed using a Tecan M1000 plate reader and 96-well UV plates purchased from BioAssay Systems (Cat. # P96 UV). UV absorbance was measured from 230 to 500 nm in 10 nm intervals to determine peak absorbance of test compounds and controls. The following equation used to determined Permeability Rate (P_e):

$$P_e = C \times -\ln\left(1 - \frac{OD_A}{OD_E}\right) \text{ cm/s}$$

Where OD_A is the absorbance of Acceptor Solution minus Blank, OD_e is the absorbance of Equilibrium Standard minus Blank, and, using an 18 h incubation, $C = 7.72 \times 10^{-6}$. Two PAMPA assays were run each in respective technical replicates.

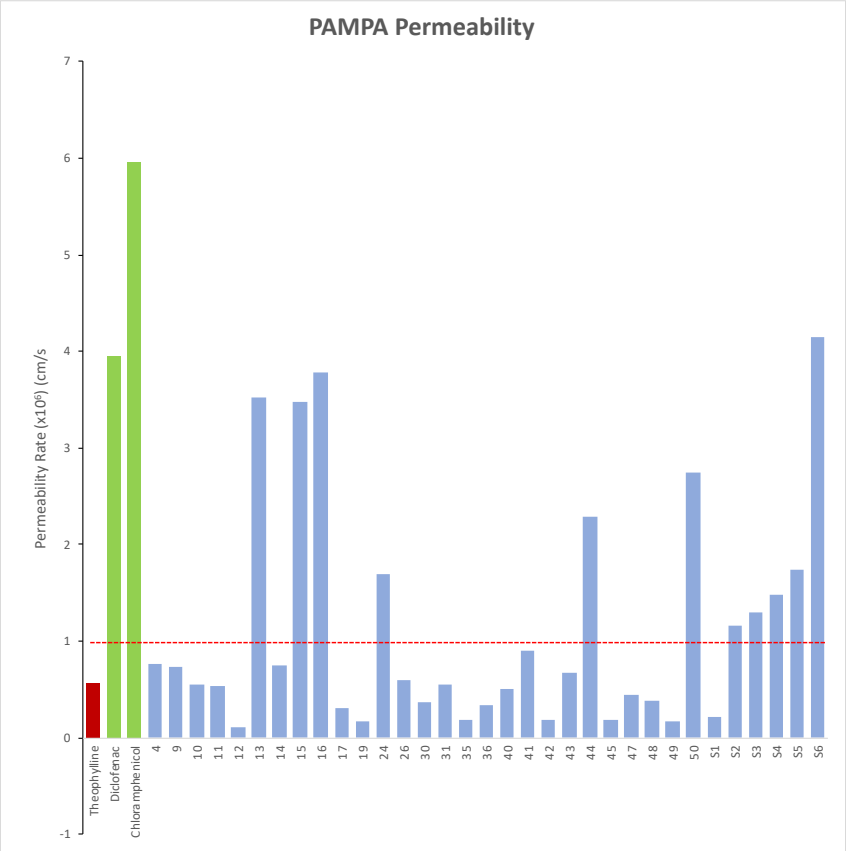


Figure 5: PAMPA average data set of select structures from two PAMPA assays.

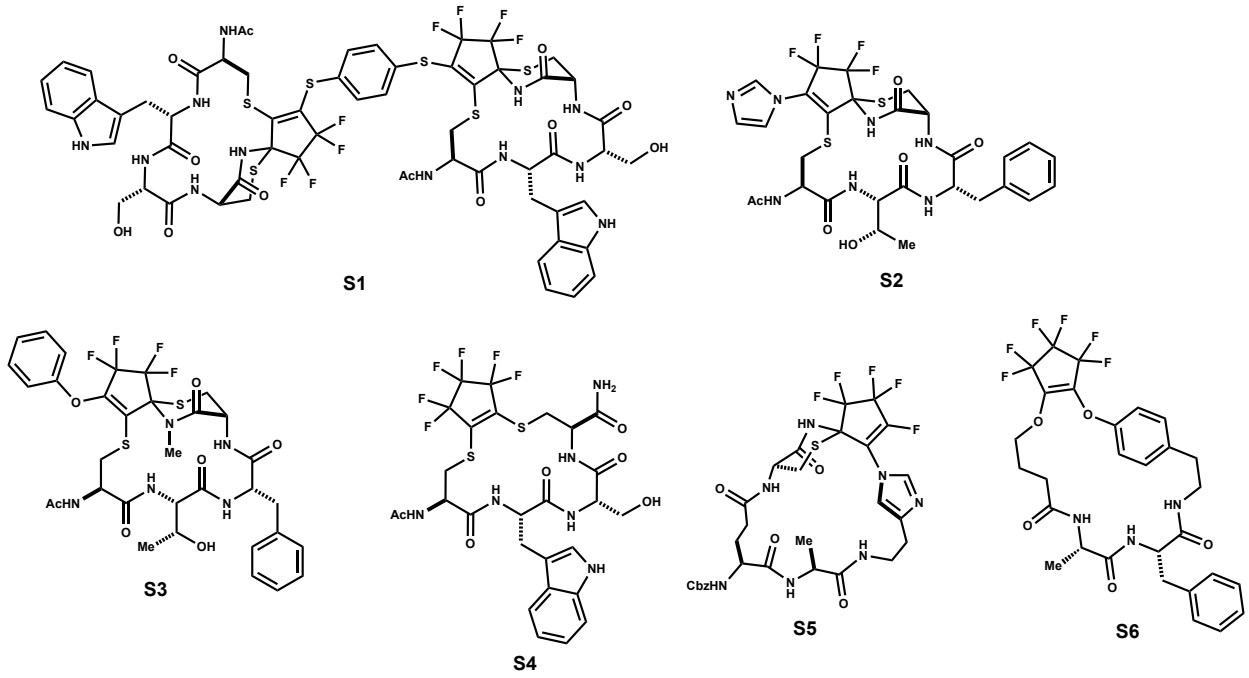


Fig. S22. Figure legend depicting structures of **S1**–**S6**.

Compound #	Average Permeability
Theophylline	5.62389E-07
Diclofenac	3.94501E-06
Chloramphenicol	5.96548E-06
4	7.56292E-07
9	7.32926E-07
10	5.54279E-07
11	5.28474E-07
12	1.12019E-07
13	3.52671E-06
14	7.44164E-07
15	3.48058E-06
16	3.78308E-06
17	3.10102E-07
19	1.74545E-07
24	1.68976E-06
26	5.97371E-07
30	3.73806E-07
31	5.53492E-07
35	1.82988E-07
36	3.28936E-07
40	5.11253E-07
41	8.978E-07
42	1.81437E-07
43	6.65527E-07
44	2.28393E-06
45	1.77868E-07
47	4.39143E-07
48	3.76608E-07
49	1.74244E-07
50	2.73852E-06
S1	2.20475E-07
S2	1.16524E-06
S3	1.30106E-06
S4	1.48167E-06
S5	1.73852E-06
S6	4.15331E-06

Table S4. Mean permeability rates (cm/sec) of select structures from two PAMPA assays. For more detailed breakdown see **Table S5**.

Compound #	Value 1 (1st Assay)	Value 2 (1st Assay)	Value 3 (2nd Assay)	Value 4 (2nd Assay)	Mean Permeability	standard deviation
Theophylline	5.50506E-07	4.83665E-07	5.966E-07	6.18784E-07	5.62389E-07	5.96921E-08
Diclofenac	3.42111E-06	3.00558E-06	4.0553E-06	5.29804E-06	3.94501E-06	9.99975E-07
Chloramphenicol	4.4116E-06	3.77755E-06	9.6062E-06	6.06656E-06	5.96548E-06	2.61194E-06
24	1.21507E-06	1.12784E-06	9.09285E-07	3.50685E-06	1.68976E-06	1.2182E-06
26	1.03955E-06	5.67439E-07	4.78383E-07	3.04109E-07	5.97371E-07	2.72296E-07
S5	2.13268E-06	2.67784E-06	6.91806E-07	1.45176E-06	1.73852E-06	8.59366E-07
17	4.83377E-07	4.12442E-07	1.05394E-07	2.39193E-07	3.10102E-07	1.70715E-07
S1	9.67674E-08	2.31138E-08	3.5097E-07	4.11048E-07	2.20475E-07	1.89387E-07
16	7.37655E-06	2.83884E-06	2.45846E-06	2.45846E-06	3.78308E-06	2.40235E-06
4	1.19846E-06	1.02366E-06	4.28999E-07	3.74052E-07	7.56292E-07	4.16422E-07
11	4.60269E-07	4.86662E-07	8.92448E-07	2.74518E-07	5.28474E-07	2.60366E-07
10	8.1878E-07	7.11556E-07	4.01875E-07	2.84903E-07	5.54279E-07	2.51983E-07
13	6.70897E-06	6.13686E-06	7.24281E-07	5.36741E-07	3.52671E-06	3.35327E-06
36	3.83341E-07	2.30465E-07	3.5097E-07	3.5097E-07	3.28936E-07	6.73979E-08
S20	2.50116E-07	1.70253E-07	2.6509E-07	1.6248E-07	2.11985E-07	5.31241E-08
50	3.21103E-06	2.66217E-06	2.36361E-06	2.71726E-06	2.73852E-06	3.51238E-07
40	7.72644E-07	6.71728E-07	3.16438E-07	2.84204E-07	5.11253E-07	2.47374E-07
41	6.23807E-07	5.47747E-07	1.07896E-06	1.34068E-06	8.978E-07	3.77082E-07
S19	7.79745E-07	7.4254E-07	2.74762E-07	8.14067E-08	4.69613E-07	3.46093E-07
S27	1.01154E-06	1.1675E-06	9.66259E-07	6.30554E-07	9.43964E-07	2.26023E-07
9	8.3408E-07	7.82441E-07	1.09354E-06	2.21642E-07	7.32926E-07	3.67033E-07
S2	6.01863E-07	5.32625E-07	1.57178E-06	1.95467E-06	1.16524E-06	7.08538E-07
S3	1.37509E-06	1.13606E-06	1.43846E-06	1.25465E-06	1.30106E-06	1.33841E-07
S4	3.13019E-06	2.54358E-06	1.12293E-07	1.40623E-07	1.48167E-06	1.58313E-06
S28	6.58694E-07	4.42951E-07	1.05919E-06	1.52721E-06	9.22013E-07	4.77467E-07
S6	7.74549E-06	7.07376E-06	1.01577E-06	7.78212E-07	4.15331E-06	3.77131E-06
S29	9.88791E-07	8.93482E-07	1.47417E-05	1.23785E-06	4.46545E-06	5.93431E-06
30	Not Tested	Not Tested	3.5365E-07	3.93963E-07	3.73806E-07	2.85059E-08
49	Not Tested	Not Tested	2.05331E-07	1.43156E-07	1.74244E-07	4.39642E-08
14	Not Tested	Not Tested	6.57418E-07	8.30909E-07	7.44164E-07	1.22677E-07
31	Not Tested	Not Tested	7.63816E-07	3.43168E-07	5.53492E-07	2.97444E-07
44	Not Tested	Not Tested	2.28393E-06	2.28393E-06	2.28393E-06	0
48	Not Tested	Not Tested	2.61088E-07	4.92127E-07	3.76608E-07	1.6337E-07
47	Not Tested	Not Tested	5.03657E-07	3.74629E-07	4.39143E-07	9.12366E-08
S22	Not Tested	Not Tested	2.55718E-07	3.15952E-07	2.85835E-07	4.25921E-08
S24	Not Tested	Not Tested	3.35705E-07	3.93103E-07	3.64404E-07	4.05865E-08
15	Not Tested	Not Tested	1.84053E-06	5.12063E-06	3.48058E-06	2.31938E-06
43	Not Tested	Not Tested	1.07582E-06	2.5523E-07	6.65527E-07	5.80248E-07
S23	Not Tested	Not Tested	4.05224E-07	1.77479E-07	2.91352E-07	1.6104E-07
S21	Not Tested	Not Tested	4.18909E-07	5.45585E-08	2.36734E-07	2.57635E-07
42	Not Tested	Not Tested	1.71775E-07	1.91099E-07	1.81437E-07	1.36642E-08
45	Not Tested	Not Tested	1.94224E-07	1.61512E-07	1.77868E-07	2.31308E-08
35	Not Tested	Not Tested	7.75886E-08	2.88387E-07	1.82988E-07	1.49057E-07
S26	Not Tested	Not Tested	1.03086E-06	3.87156E-07	7.09009E-07	4.55169E-07
S25	Not Tested	Not Tested	5.14857E-07	5.91294E-07	5.53076E-07	5.40486E-08
12	Not Tested	Not Tested	1.28136E-07	9.59019E-08	1.12019E-07	2.27928E-08
19	3.73191E-07	3.47748E-08	1.66028E-07	1.24185E-07	1.74545E-07	1.433E-07

Table S5. Mean permeability rates (cm/sec) of all compounds from 2nd PAMPA assay averaged out with permeability values from 1st PAMPA assay. Value 1 and 2 reflect first independent PAMPA assay. Value 3 and 4 reflect 2nd independent PAMPA assay.

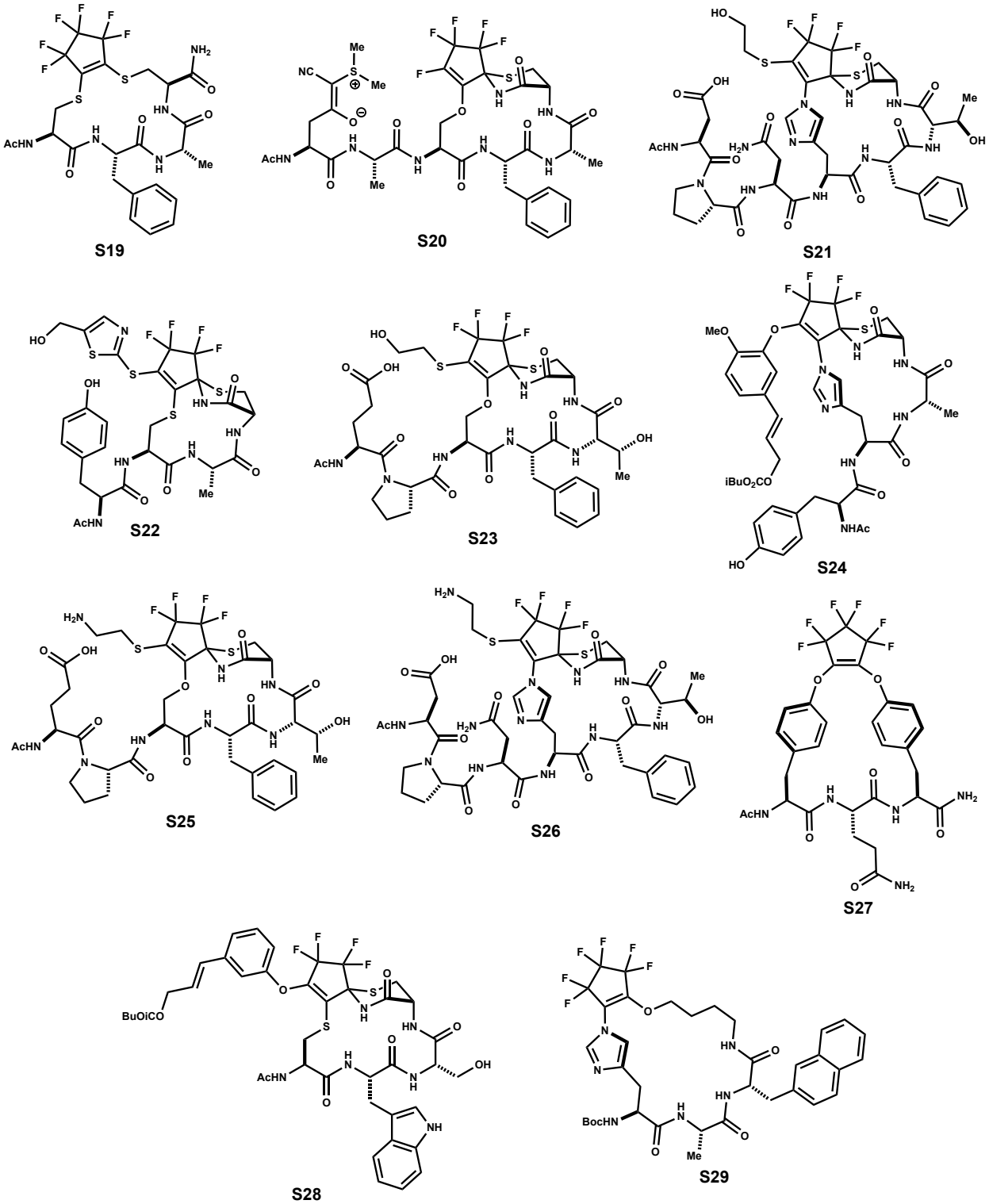


Fig. SX. Structures of S19–29.

Fig. S23. Figure legend depicting structures **S19**–**S29**.

Compound #	Value 1	Value 2	Mean Permeability	standard deviation
Theophylline	5.966E-07	6.18784E-07	6.07692E-07	1.56864E-08
Diclofenac	4.0553E-06	5.29804E-06	4.67667E-06	8.7875E-07
Chloramphenicol	9.60618E-06	6.06656E-06	7.83637E-06	2.50289E-06
12	1.28136E-07	9.59019E-08	1.12019E-07	2.27928E-08
S4	1.12293E-07	1.40623E-07	1.26458E-07	2.00326E-08
19	1.66028E-07	1.24185E-07	1.45106E-07	2.95874E-08
17	1.05394E-07	2.39193E-07	1.72294E-07	9.46101E-08
49	2.05331E-07	1.43156E-07	1.74244E-07	4.39642E-08
45	1.94224E-07	1.61512E-07	1.77868E-07	2.31308E-08
S19	2.74762E-07	8.14067E-08	1.78084E-07	1.36723E-07
42	1.71775E-07	1.91099E-07	1.81437E-07	1.36642E-08
35	7.75886E-08	2.88387E-07	1.82988E-07	1.49057E-07
S20	2.6509E-07	1.6248E-07	2.13785E-07	7.25569E-08
S21	4.18909E-07	5.45585E-08	2.36734E-07	2.57635E-07
S22	2.55718E-07	3.15952E-07	2.85835E-07	4.25921E-08
S23	4.05224E-07	1.77479E-07	2.91352E-07	1.6104E-07
40	3.16438E-07	2.84204E-07	3.00321E-07	2.27928E-08
10	4.01875E-07	2.84903E-07	3.43389E-07	8.27116E-08
36	3.5097E-07	3.5097E-07	3.5097E-07	0
S24	3.35705E-07	3.93103E-07	3.64404E-07	4.05865E-08
30	3.5365E-07	3.93963E-07	3.73806E-07	2.85059E-08
48	2.61088E-07	4.92127E-07	3.76608E-07	1.6337E-07
S1	3.5097E-07	4.11048E-07	3.81009E-07	4.24816E-08
26	4.78383E-07	3.04109E-07	3.91246E-07	1.2323E-07
4	4.28999E-07	3.74052E-07	4.01526E-07	3.88533E-08
47	5.03657E-07	3.74629E-07	4.39143E-07	9.12366E-08
S25	5.14857E-07	5.91294E-07	5.53076E-07	5.40486E-08
31	7.63816E-07	3.43168E-07	5.53492E-07	2.97444E-07
11	8.92448E-07	2.74518E-07	5.83483E-07	4.36942E-07
13	7.24281E-07	5.36741E-07	6.30511E-07	1.32611E-07
9	1.09354E-06	2.21642E-07	6.57592E-07	6.16526E-07
43	1.07582E-06	2.5523E-07	6.65527E-07	5.80248E-07
S26	1.03086E-06	3.87156E-07	7.09009E-07	4.55169E-07
14	6.57418E-07	8.30909E-07	7.44164E-07	1.22677E-07
S27	9.66259E-07	6.30554E-07	7.98407E-07	2.37379E-07
S6	1.01577E-06	7.78212E-07	8.96991E-07	1.67978E-07
S5	6.91806E-07	1.45176E-06	1.07178E-06	5.37371E-07
41	1.07896E-06	1.34068E-06	1.20982E-06	1.85064E-07
S28	1.05919E-06	1.52721E-06	1.2932E-06	3.30942E-07
S3	1.43846E-06	1.25465E-06	1.34656E-06	1.29979E-07
S2	1.57178E-06	1.95467E-06	1.76323E-06	2.70743E-07
24	9.09285E-07	3.50685E-06	2.20807E-06	1.83676E-06
44	2.28393E-06	2.28393E-06	2.28393E-06	0
24	2.45846E-06	2.45846E-06	2.45846E-06	0
50	2.36361E-06	2.71726E-06	2.54043E-06	2.50068E-07
15	1.84053E-06	5.12063E-06	3.48058E-06	2.31938E-06
S29	1.47417E-05	1.23785E-06	7.98976E-06	9.54864E-06

Table S6. Permeability rates (cm/sec) of full library for 2nd PAMPA assay (including technical duplicates, permeability average, and standard deviations).

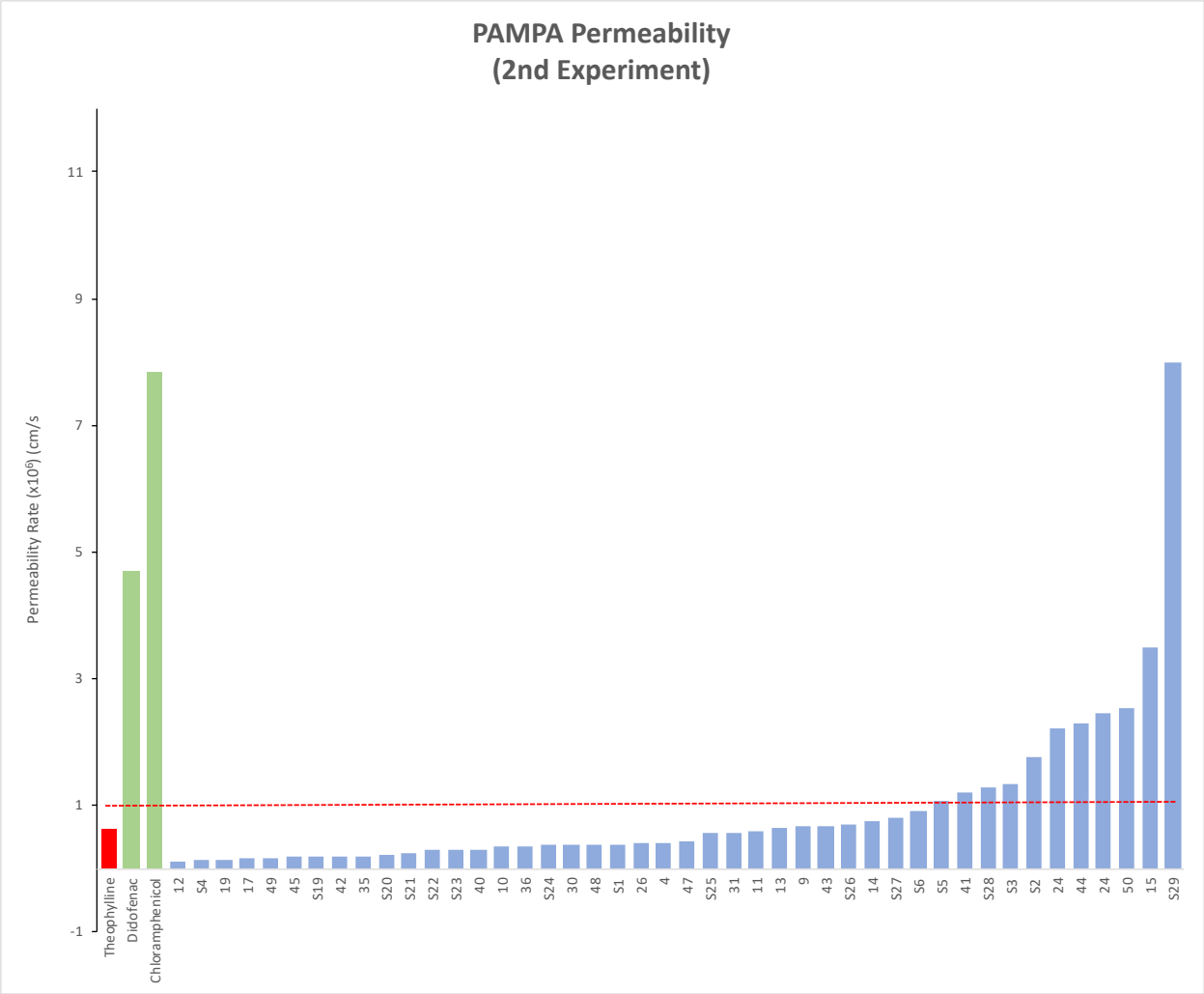


Fig. S24. Graph represents mean permeability values (cm/sec) (depiction of **Table S6**) for all compounds tested in 2nd PAMPA assay.

Raw Absorbance Data for 2nd PAMPA assay

Wavelength	A1	A2	A3	A4	A5	A6	A7	A8
230	0.106	0.097	0.093	0.094	0.145	0.238	0.093	0.085
240	0.077	0.07	0.068	0.074	0.093	0.094	0.076	0.067
250	0.068	0.061	0.06	0.065	0.077	0.074	0.068	0.059
260	0.059	0.052	0.049	0.056	0.064	0.057	0.059	0.049
270	0.051	0.044	0.042	0.048	0.056	0.048	0.053	0.041
280	0.048	0.04	0.038	0.043	0.05	0.044	0.05	0.037
290	0.044	0.037	0.036	0.041	0.044	0.04	0.044	0.036
300	0.042	0.035	0.034	0.038	0.041	0.037	0.042	0.034
310	0.04	0.033	0.033	0.036	0.038	0.035	0.04	0.032
320	0.039	0.031	0.032	0.034	0.036	0.034	0.038	0.031
330	0.037	0.029	0.03	0.033	0.034	0.032	0.036	0.03
340	0.035	0.028	0.029	0.031	0.032	0.031	0.036	0.029
350	0.034	0.027	0.028	0.03	0.031	0.029	0.034	0.027
360	0.032	0.026	0.026	0.029	0.029	0.028	0.033	0.026
370	0.032	0.026	0.026	0.029	0.029	0.028	0.033	0.026
380	0.042	0.035	0.036	0.038	0.038	0.038	0.042	0.036
390	0.03	0.024	0.025	0.027	0.027	0.027	0.032	0.025
400	0.03	0.024	0.025	0.027	0.027	0.027	0.032	0.025
410	0.03	0.024	0.025	0.027	0.027	0.027	0.031	0.025
420	0.03	0.024	0.025	0.027	0.027	0.027	0.031	0.025
430	0.03	0.025	0.025	0.027	0.027	0.027	0.031	0.025
440	0.03	0.025	0.025	0.027	0.027	0.027	0.031	0.025
450	0.029	0.024	0.025	0.026	0.026	0.026	0.03	0.025
460	0.029	0.024	0.025	0.026	0.026	0.026	0.03	0.025
470	0.029	0.024	0.025	0.026	0.026	0.026	0.03	0.025
480	0.029	0.024	0.025	0.026	0.026	0.026	0.03	0.024
490	0.029	0.024	0.025	0.026	0.026	0.026	0.03	0.025
500	0.029	0.024	0.025	0.026	0.026	0.026	0.03	0.024

A9	A10	A11	A12	B1	B2	B3	B4	B5	B6
0.096	0.087	0.131	0.244	0.102	0.094	0.104	0.092	0.096	0.095
0.07	0.069	0.087	0.112	0.074	0.071	0.077	0.069	0.07	0.07
0.061	0.06	0.073	0.09	0.065	0.062	0.066	0.059	0.061	0.061
0.051	0.049	0.062	0.073	0.055	0.053	0.054	0.048	0.051	0.049
0.044	0.042	0.056	0.062	0.047	0.046	0.046	0.041	0.043	0.041
0.04	0.037	0.051	0.056	0.042	0.042	0.043	0.036	0.039	0.037
0.038	0.034	0.048	0.052	0.04	0.038	0.04	0.033	0.036	0.033
0.036	0.033	0.045	0.049	0.038	0.036	0.038	0.032	0.034	0.031
0.034	0.031	0.042	0.046	0.036	0.034	0.036	0.03	0.032	0.03
0.033	0.029	0.041	0.043	0.034	0.033	0.034	0.029	0.031	0.029
0.032	0.028	0.038	0.041	0.033	0.031	0.031	0.027	0.03	0.027
0.031	0.026	0.037	0.039	0.032	0.03	0.03	0.026	0.029	0.026
0.029	0.025	0.035	0.037	0.03	0.029	0.028	0.025	0.027	0.025
0.028	0.024	0.033	0.036	0.029	0.027	0.027	0.024	0.026	0.024
0.028	0.024	0.033	0.035	0.029	0.027	0.026	0.024	0.026	0.024
0.038	0.033	0.043	0.044	0.038	0.037	0.036	0.033	0.036	0.033
0.027	0.023	0.031	0.033	0.028	0.026	0.025	0.023	0.025	0.023
0.026	0.022	0.031	0.032	0.028	0.026	0.025	0.023	0.024	0.023
0.027	0.023	0.031	0.032	0.028	0.026	0.025	0.023	0.025	0.024
0.026	0.023	0.03	0.032	0.028	0.026	0.025	0.023	0.025	0.023
0.027	0.023	0.031	0.032	0.027	0.026	0.025	0.023	0.025	0.024
0.027	0.023	0.03	0.031	0.027	0.026	0.025	0.023	0.025	0.024
0.027	0.023	0.03	0.031	0.027	0.026	0.025	0.023	0.025	0.024
0.026	0.023	0.029	0.031	0.027	0.026	0.025	0.023	0.025	0.024
0.026	0.023	0.029	0.03	0.027	0.026	0.025	0.023	0.025	0.024
0.026	0.023	0.029	0.03	0.027	0.026	0.025	0.023	0.025	0.024
0.027	0.023	0.029	0.03	0.027	0.026	0.025	0.023	0.025	0.024
0.026	0.023	0.029	0.03	0.027	0.026	0.024	0.023	0.025	0.024

B7	B8	B9	B10	B11	B12	C1	C2	C3
0.096	0.095	0.106	0.108	0.096	0.097	0.095	0.099	0.098
0.071	0.069	0.078	0.071	0.071	0.076	0.074	0.071	0.073
0.062	0.06	0.068	0.06	0.062	0.068	0.066	0.062	0.065
0.052	0.051	0.057	0.05	0.052	0.058	0.056	0.052	0.056
0.044	0.044	0.049	0.042	0.045	0.051	0.049	0.044	0.05
0.04	0.039	0.046	0.037	0.041	0.046	0.046	0.039	0.045
0.04	0.036	0.043	0.034	0.039	0.042	0.042	0.036	0.04
0.038	0.034	0.041	0.033	0.037	0.039	0.04	0.034	0.037
0.036	0.032	0.039	0.031	0.035	0.037	0.038	0.033	0.035
0.034	0.03	0.037	0.03	0.034	0.035	0.036	0.031	0.034
0.032	0.029	0.034	0.028	0.032	0.033	0.035	0.03	0.032
0.031	0.028	0.032	0.027	0.031	0.032	0.034	0.029	0.031
0.03	0.027	0.03	0.025	0.03	0.031	0.032	0.028	0.03
0.028	0.025	0.029	0.024	0.029	0.03	0.031	0.027	0.029
0.028	0.026	0.029	0.024	0.029	0.03	0.031	0.026	0.029
0.038	0.034	0.038	0.033	0.038	0.039	0.04	0.035	0.038
0.027	0.024	0.027	0.023	0.028	0.029	0.03	0.025	0.028
0.026	0.024	0.027	0.023	0.027	0.028	0.029	0.025	0.028
0.027	0.024	0.027	0.023	0.028	0.029	0.029	0.025	0.028
0.026	0.024	0.027	0.023	0.027	0.029	0.029	0.025	0.028
0.026	0.024	0.027	0.023	0.027	0.028	0.029	0.025	0.028
0.026	0.024	0.027	0.024	0.027	0.028	0.029	0.025	0.028
0.026	0.024	0.027	0.023	0.027	0.028	0.028	0.025	0.028
0.026	0.024	0.027	0.023	0.027	0.028	0.028	0.025	0.027
0.026	0.024	0.026	0.023	0.027	0.028	0.028	0.025	0.028
0.026	0.025	0.026	0.023	0.026	0.028	0.028	0.025	0.028
0.026	0.024	0.027	0.023	0.026	0.028	0.028	0.025	0.027
0.026	0.025	0.026	0.023	0.026	0.028	0.028	0.025	0.028

C4	C5	C6	C7	C8	C9	C10	C11	C12
0.1	0.106	0.08	0.083	0.092	0.084	0.1	0.106	0.136
0.07	0.074	0.063	0.064	0.066	0.063	0.071	0.072	0.073
0.061	0.064	0.055	0.056	0.057	0.055	0.062	0.063	0.062
0.05	0.053	0.045	0.045	0.047	0.044	0.052	0.052	0.052
0.042	0.046	0.037	0.038	0.039	0.036	0.044	0.044	0.045
0.039	0.042	0.033	0.034	0.035	0.032	0.04	0.04	0.041
0.036	0.039	0.029	0.032	0.032	0.03	0.038	0.037	0.038
0.034	0.036	0.028	0.03	0.031	0.028	0.036	0.035	0.036
0.032	0.034	0.027	0.029	0.029	0.027	0.034	0.033	0.034
0.03	0.033	0.026	0.027	0.028	0.026	0.033	0.031	0.032
0.029	0.031	0.025	0.026	0.027	0.025	0.031	0.03	0.031
0.028	0.03	0.024	0.025	0.026	0.024	0.03	0.028	0.03
0.026	0.028	0.023	0.024	0.024	0.023	0.029	0.027	0.028
0.025	0.027	0.022	0.023	0.023	0.022	0.027	0.026	0.026
0.025	0.027	0.022	0.022	0.023	0.022	0.027	0.026	0.026
0.034	0.036	0.031	0.032	0.032	0.031	0.036	0.035	0.036
0.024	0.025	0.022	0.022	0.022	0.021	0.026	0.024	0.025
0.024	0.025	0.021	0.022	0.022	0.021	0.026	0.024	0.025
0.024	0.025	0.022	0.022	0.023	0.021	0.026	0.025	0.025
0.024	0.025	0.022	0.022	0.023	0.022	0.026	0.025	0.025
0.024	0.025	0.022	0.022	0.023	0.022	0.026	0.025	0.025
0.024	0.025	0.022	0.022	0.023	0.022	0.026	0.025	0.025
0.024	0.025	0.022	0.022	0.023	0.022	0.025	0.024	0.025
0.024	0.025	0.022	0.022	0.023	0.022	0.025	0.024	0.025
0.024	0.025	0.022	0.022	0.023	0.022	0.025	0.024	0.025
0.024	0.025	0.022	0.022	0.023	0.022	0.025	0.025	0.025
0.024	0.025	0.022	0.022	0.023	0.022	0.025	0.024	0.025
0.024	0.025	0.022	0.022	0.023	0.022	0.025	0.025	0.025

D1	D2	D3	D4	D5	D6	D7	D8	D9
0.118	0.1	0.125	0.097	0.096	0.12	0.093	0.105	0.122
0.086	0.072	0.083	0.072	0.068	0.081	0.067	0.075	0.085
0.076	0.063	0.073	0.063	0.059	0.07	0.059	0.066	0.075
0.066	0.053	0.065	0.053	0.049	0.058	0.049	0.057	0.067
0.058	0.047	0.059	0.045	0.041	0.048	0.043	0.05	0.062
0.053	0.044	0.053	0.039	0.037	0.044	0.039	0.046	0.056
0.048	0.04	0.043	0.035	0.034	0.039	0.036	0.045	0.046
0.044	0.039	0.037	0.033	0.032	0.038	0.033	0.042	0.04
0.041	0.036	0.032	0.032	0.03	0.035	0.031	0.039	0.035
0.04	0.033	0.03	0.03	0.029	0.035	0.029	0.036	0.033
0.038	0.03	0.029	0.029	0.028	0.033	0.028	0.033	0.031
0.036	0.029	0.027	0.027	0.027	0.033	0.027	0.032	0.03
0.034	0.027	0.026	0.026	0.025	0.031	0.026	0.031	0.029
0.031	0.026	0.025	0.025	0.024	0.029	0.024	0.03	0.028
0.032	0.026	0.025	0.025	0.024	0.029	0.024	0.029	0.028
0.042	0.035	0.034	0.034	0.034	0.04	0.033	0.038	0.037
0.03	0.025	0.024	0.024	0.023	0.028	0.023	0.028	0.026
0.031	0.025	0.024	0.024	0.023	0.029	0.023	0.028	0.026
0.031	0.025	0.024	0.024	0.024	0.029	0.023	0.028	0.026
0.03	0.025	0.024	0.024	0.023	0.028	0.023	0.028	0.026
0.03	0.025	0.024	0.024	0.024	0.03	0.024	0.028	0.026
0.03	0.025	0.024	0.024	0.024	0.03	0.023	0.028	0.026
0.03	0.025	0.024	0.024	0.024	0.029	0.023	0.028	0.026
0.03	0.025	0.024	0.024	0.024	0.03	0.023	0.028	0.026
0.03	0.025	0.024	0.024	0.024	0.029	0.024	0.028	0.026
0.03	0.025	0.024	0.024	0.024	0.029	0.023	0.028	0.026
0.03	0.025	0.024	0.024	0.024	0.03	0.023	0.027	0.026
0.029	0.025	0.024	0.024	0.024	0.029	0.023	0.028	0.026

D10	D11	D12	E1	E2	E3	E4	E5	E6
0.096	0.12	0.122	0.098	0.096	0.098	0.091	0.1	0.095
0.07	0.085	0.076	0.069	0.068	0.068	0.067	0.068	0.067
0.061	0.076	0.062	0.06	0.058	0.059	0.058	0.058	0.059
0.051	0.066	0.05	0.049	0.048	0.049	0.047	0.047	0.051
0.044	0.059	0.042	0.041	0.04	0.042	0.04	0.039	0.043
0.039	0.053	0.039	0.036	0.036	0.038	0.036	0.034	0.038
0.036	0.049	0.037	0.033	0.033	0.034	0.034	0.033	0.035
0.034	0.046	0.035	0.032	0.032	0.032	0.032	0.032	0.033
0.033	0.043	0.033	0.03	0.03	0.031	0.031	0.03	0.031
0.031	0.041	0.031	0.029	0.029	0.029	0.03	0.029	0.03
0.029	0.04	0.03	0.027	0.028	0.028	0.028	0.027	0.028
0.028	0.038	0.029	0.026	0.027	0.027	0.027	0.026	0.027
0.027	0.037	0.027	0.025	0.026	0.026	0.026	0.024	0.026
0.026	0.035	0.026	0.024	0.024	0.024	0.025	0.023	0.025
0.026	0.035	0.026	0.024	0.025	0.025	0.025	0.023	0.025
0.035	0.044	0.035	0.033	0.034	0.034	0.034	0.032	0.034
0.024	0.033	0.024	0.023	0.024	0.024	0.024	0.022	0.024
0.024	0.033	0.024	0.023	0.024	0.023	0.024	0.022	0.024
0.025	0.033	0.024	0.023	0.024	0.024	0.024	0.022	0.024
0.025	0.033	0.024	0.023	0.024	0.024	0.024	0.022	0.024
0.025	0.032	0.024	0.023	0.024	0.024	0.024	0.022	0.024
0.025	0.032	0.024	0.024	0.024	0.024	0.024	0.022	0.024
0.025	0.032	0.024	0.023	0.024	0.024	0.024	0.022	0.024
0.025	0.031	0.024	0.023	0.024	0.024	0.024	0.022	0.024
0.025	0.031	0.024	0.024	0.024	0.024	0.024	0.022	0.024
0.025	0.031	0.024	0.023	0.024	0.024	0.024	0.022	0.024
0.025	0.031	0.024	0.023	0.024	0.024	0.024	0.022	0.024
0.025	0.03	0.024	0.024	0.024	0.024	0.024	0.022	0.024

E7	E8	E9	E10	E11	E12	F1	F2	F3
0.098	0.09	0.097	0.101	0.101	0.094	0.107	0.099	0.118
0.069	0.066	0.067	0.072	0.07	0.067	0.081	0.068	0.077
0.059	0.058	0.058	0.064	0.061	0.06	0.074	0.059	0.068
0.049	0.048	0.048	0.054	0.051	0.05	0.064	0.049	0.059
0.041	0.04	0.04	0.046	0.043	0.042	0.058	0.041	0.051
0.037	0.036	0.036	0.042	0.039	0.037	0.055	0.037	0.044
0.033	0.033	0.033	0.037	0.036	0.034	0.047	0.034	0.04
0.032	0.032	0.031	0.035	0.034	0.032	0.046	0.033	0.038
0.03	0.03	0.03	0.033	0.033	0.03	0.046	0.032	0.034
0.029	0.029	0.029	0.032	0.032	0.029	0.046	0.031	0.033
0.028	0.028	0.027	0.031	0.03	0.027	0.045	0.03	0.031
0.026	0.026	0.026	0.03	0.029	0.026	0.044	0.029	0.03
0.025	0.025	0.025	0.029	0.028	0.025	0.043	0.028	0.028
0.024	0.024	0.024	0.028	0.027	0.024	0.042	0.026	0.026
0.024	0.024	0.024	0.028	0.026	0.024	0.042	0.026	0.026
0.033	0.033	0.033	0.037	0.036	0.033	0.052	0.036	0.035
0.023	0.023	0.023	0.027	0.025	0.023	0.041	0.026	0.025
0.023	0.023	0.023	0.027	0.025	0.023	0.04	0.026	0.024
0.024	0.024	0.023	0.027	0.026	0.023	0.04	0.026	0.024
0.023	0.024	0.023	0.027	0.025	0.023	0.04	0.026	0.024
0.024	0.024	0.023	0.027	0.026	0.023	0.04	0.026	0.024
0.024	0.024	0.023	0.027	0.025	0.023	0.04	0.026	0.024
0.024	0.024	0.023	0.028	0.025	0.023	0.039	0.026	0.024
0.023	0.024	0.023	0.027	0.025	0.023	0.039	0.026	0.024
0.024	0.024	0.023	0.027	0.025	0.023	0.039	0.026	0.024
0.024	0.024	0.023	0.028	0.025	0.023	0.038	0.026	0.024
0.024	0.024	0.023	0.027	0.025	0.023	0.039	0.026	0.024
0.024	0.024	0.023	0.027	0.025	0.023	0.038	0.026	0.024

F4	F5	F6	F7	F8	F9	F10	F11	F12
0.123	0.094	0.091	0.094	0.099	0.11	0.126	0.096	0.093
0.069	0.068	0.065	0.068	0.069	0.076	0.071	0.068	0.066
0.06	0.059	0.056	0.06	0.059	0.068	0.062	0.06	0.057
0.052	0.049	0.046	0.05	0.048	0.059	0.055	0.05	0.046
0.048	0.04	0.038	0.043	0.04	0.051	0.05	0.041	0.039
0.044	0.035	0.034	0.038	0.035	0.044	0.047	0.037	0.035
0.034	0.033	0.031	0.035	0.03	0.039	0.036	0.034	0.035
0.031	0.031	0.03	0.033	0.029	0.037	0.034	0.032	0.033
0.03	0.03	0.028	0.031	0.027	0.034	0.032	0.03	0.032
0.028	0.028	0.028	0.03	0.026	0.032	0.031	0.029	0.032
0.027	0.027	0.026	0.029	0.025	0.03	0.029	0.028	0.029
0.026	0.026	0.025	0.027	0.024	0.029	0.028	0.026	0.029
0.025	0.024	0.024	0.026	0.023	0.027	0.027	0.025	0.026
0.024	0.023	0.023	0.025	0.022	0.026	0.026	0.024	0.023
0.024	0.023	0.023	0.025	0.022	0.026	0.026	0.024	0.024
0.033	0.032	0.032	0.034	0.031	0.034	0.035	0.033	0.033
0.023	0.022	0.022	0.024	0.021	0.024	0.025	0.023	0.023
0.023	0.022	0.022	0.024	0.021	0.024	0.025	0.023	0.023
0.023	0.023	0.022	0.024	0.021	0.024	0.025	0.023	0.022
0.023	0.022	0.022	0.024	0.021	0.024	0.025	0.023	0.022
0.023	0.022	0.022	0.024	0.022	0.024	0.025	0.023	0.023
0.023	0.023	0.022	0.024	0.022	0.024	0.025	0.023	0.023
0.023	0.023	0.022	0.024	0.022	0.024	0.025	0.023	0.023
0.023	0.023	0.022	0.024	0.022	0.024	0.025	0.024	0.023
0.023	0.023	0.022	0.024	0.022	0.024	0.025	0.024	0.023
0.023	0.022	0.022	0.024	0.022	0.024	0.025	0.024	0.023
0.023	0.023	0.022	0.024	0.022	0.024	0.025	0.024	0.023
0.023	0.023	0.022	0.024	0.022	0.024	0.025	0.024	0.023

G1	G2	G3	G4	G5	G6	G7	G8	G9
0.101	0.152	0.118	0.131	0.13	0.105	0.092	0.112	0.101
0.073	0.107	0.07	0.085	0.086	0.069	0.064	0.07	0.067
0.064	0.094	0.059	0.069	0.074	0.06	0.056	0.06	0.057
0.054	0.082	0.049	0.056	0.065	0.05	0.045	0.05	0.046
0.048	0.076	0.041	0.044	0.055	0.043	0.038	0.042	0.038
0.044	0.076	0.036	0.04	0.047	0.039	0.033	0.038	0.034
0.04	0.068	0.034	0.036	0.04	0.036	0.03	0.034	0.032
0.038	0.066	0.032	0.032	0.036	0.034	0.029	0.032	0.03
0.036	0.064	0.03	0.03	0.033	0.032	0.028	0.031	0.029
0.035	0.066	0.029	0.028	0.032	0.031	0.027	0.029	0.028
0.033	0.061	0.027	0.027	0.03	0.029	0.026	0.028	0.026
0.032	0.06	0.026	0.026	0.029	0.028	0.024	0.027	0.025
0.031	0.055	0.025	0.025	0.028	0.027	0.023	0.026	0.024
0.03	0.05	0.024	0.024	0.027	0.026	0.022	0.024	0.023
0.03	0.049	0.024	0.023	0.027	0.026	0.022	0.024	0.023
0.039	0.059	0.032	0.033	0.036	0.035	0.031	0.033	0.032
0.029	0.046	0.022	0.023	0.026	0.025	0.021	0.023	0.022
0.029	0.045	0.022	0.023	0.025	0.024	0.021	0.023	0.022
0.029	0.043	0.023	0.023	0.026	0.025	0.022	0.023	0.022
0.028	0.042	0.023	0.023	0.025	0.025	0.022	0.023	0.022
0.028	0.042	0.023	0.023	0.026	0.025	0.022	0.023	0.022
0.028	0.041	0.023	0.023	0.026	0.024	0.022	0.023	0.022
0.028	0.04	0.023	0.023	0.025	0.025	0.022	0.023	0.022
0.028	0.039	0.023	0.023	0.025	0.025	0.022	0.023	0.022
0.028	0.038	0.023	0.023	0.025	0.024	0.022	0.024	0.023
0.028	0.037	0.023	0.023	0.025	0.024	0.022	0.023	0.023
0.028	0.038	0.023	0.023	0.025	0.025	0.022	0.023	0.023
0.028	0.036	0.023	0.023	0.025	0.025	0.022	0.024	0.023

G10	G11	G12	H1	H2	H3	H4	H5	H6
0.098	0.121	0.097	0.098	0.093	0.248	0.367	0.138	0.098
0.073	0.083	0.067	0.069	0.066	0.188	0.226	0.093	0.066
0.06	0.071	0.058	0.06	0.057	0.218	0.178	0.082	0.057
0.049	0.061	0.048	0.05	0.046	0.302	0.215	0.084	0.047
0.038	0.052	0.041	0.042	0.039	0.384	0.262	0.091	0.04
0.033	0.045	0.037	0.038	0.034	0.408	0.267	0.081	0.035
0.03	0.038	0.035	0.035	0.031	0.356	0.211	0.054	0.032
0.028	0.034	0.033	0.033	0.03	0.269	0.133	0.046	0.03
0.027	0.032	0.031	0.031	0.028	0.18	0.074	0.047	0.029
0.026	0.031	0.03	0.03	0.027	0.109	0.047	0.04	0.027
0.025	0.029	0.028	0.028	0.026	0.07	0.038	0.032	0.026
0.024	0.028	0.027	0.027	0.025	0.052	0.034	0.026	0.025
0.023	0.027	0.026	0.026	0.023	0.042	0.033	0.025	0.024
0.021	0.026	0.025	0.025	0.022	0.036	0.031	0.023	0.022
0.022	0.026	0.025	0.025	0.022	0.031	0.031	0.023	0.022
0.031	0.035	0.034	0.034	0.031	0.038	0.04	0.032	0.031
0.021	0.025	0.024	0.023	0.021	0.026	0.03	0.022	0.021
0.021	0.025	0.024	0.023	0.021	0.025	0.029	0.022	0.021
0.021	0.025	0.024	0.024	0.022	0.025	0.029	0.022	0.022
0.021	0.025	0.024	0.023	0.022	0.025	0.029	0.022	0.022
0.022	0.025	0.024	0.024	0.022	0.025	0.029	0.022	0.022
0.022	0.025	0.024	0.024	0.022	0.025	0.029	0.023	0.022
0.022	0.025	0.024	0.024	0.022	0.025	0.029	0.023	0.022
0.022	0.025	0.024	0.024	0.022	0.024	0.028	0.022	0.022
0.022	0.025	0.024	0.024	0.022	0.025	0.028	0.022	0.022
0.022	0.025	0.024	0.024	0.022	0.024	0.028	0.023	0.022
0.022	0.025	0.024	0.024	0.022	0.024	0.028	0.022	0.022
0.022	0.025	0.024	0.023	0.022	0.024	0.028	0.022	0.022

H7	H8	H9	H10	H11	H12
0.092	0.204	0.43	0.138	0.073	0.073
0.065	0.157	0.258	0.091	0.063	0.063
0.056	0.178	0.2	0.08	0.054	0.055
0.046	0.24	0.25	0.084	0.044	0.044
0.038	0.302	0.312	0.093	0.036	0.037
0.033	0.319	0.318	0.082	0.032	0.033
0.031	0.28	0.249	0.051	0.028	0.029
0.029	0.213	0.15	0.042	0.027	0.028
0.028	0.146	0.074	0.044	0.026	0.027
0.027	0.09	0.04	0.038	0.025	0.026
0.026	0.059	0.029	0.029	0.024	0.025
0.024	0.044	0.025	0.024	0.023	0.024
0.023	0.037	0.023	0.022	0.022	0.022
0.022	0.031	0.022	0.021	0.021	0.021
0.022	0.028	0.022	0.021	0.021	0.021
0.031	0.035	0.031	0.03	0.03	0.031
0.021	0.023	0.021	0.02	0.02	0.021
0.021	0.023	0.021	0.02	0.02	0.021
0.022	0.023	0.022	0.021	0.021	0.022
0.022	0.023	0.022	0.021	0.021	0.022
0.022	0.023	0.022	0.021	0.021	0.022
0.022	0.023	0.022	0.021	0.021	0.022
0.022	0.023	0.022	0.021	0.021	0.022
0.022	0.023	0.022	0.021	0.021	0.022
0.022	0.023	0.022	0.021	0.021	0.022
0.022	0.023	0.022	0.021	0.022	0.022
0.022	0.023	0.022	0.021	0.021	0.022
0.022	0.023	0.022	0.021	0.022	0.022

Raw Equilibrium Solutions for 2nd PAMPA assay

Wavelength	A1	A2	A3	A4	A5	A6
230	1.501	1.376	1.55	1.014	1.327	#SAT
240	0.389	0.346	0.418	0.21	0.202	0.745
250	0.323	0.298	0.31	0.163	0.143	0.447
260	0.353	0.341	0.248	0.161	0.127	0.431
270	0.404	0.396	0.209	0.173	0.117	0.451
280	0.432	0.411	0.173	0.181	0.125	0.451
290	0.403	0.392	0.126	0.166	0.134	0.391
300	0.346	0.327	0.095	0.142	0.131	0.291
310	0.292	0.26	0.071	0.121	0.11	0.208
320	0.226	0.195	0.058	0.098	0.082	0.162
330	0.158	0.138	0.052	0.075	0.058	0.142
340	0.111	0.101	0.052	0.06	0.048	0.13
350	0.086	0.081	0.052	0.051	0.044	0.12
360	0.072	0.066	0.051	0.045	0.041	0.11
370	0.063	0.057	0.047	0.042	0.04	0.102
380	0.066	0.06	0.052	0.05	0.048	0.104
390	0.051	0.046	0.038	0.038	0.037	0.089
400	0.048	0.044	0.037	0.036	0.036	0.086
410	0.046	0.042	0.035	0.035	0.035	0.083
420	0.044	0.041	0.034	0.034	0.034	0.081
430	0.042	0.04	0.034	0.034	0.034	0.079
440	0.041	0.039	0.033	0.033	0.033	0.077
450	0.039	0.038	0.033	0.032	0.033	0.075
460	0.038	0.037	0.032	0.032	0.032	0.074
470	0.037	0.036	0.032	0.031	0.032	0.072
480	0.036	0.036	0.032	0.031	0.032	0.072
490	0.035	0.035	0.031	0.031	0.031	0.07
500	0.035	0.035	0.031	0.03	0.031	0.07

B1	B2	B3	B4	B5	B6
1.505	1.566	1.834	1.451	1.201	1.84
0.292	0.361	0.496	0.332	0.138	0.186
0.22	0.263	0.394	0.271	0.089	0.106
0.204	0.217	0.393	0.265	0.088	0.083
0.225	0.194	0.425	0.235	0.096	0.071
0.289	0.164	0.429	0.171	0.099	0.063
0.326	0.119	0.367	0.125	0.09	0.056
0.277	0.085	0.311	0.105	0.075	0.052
0.191	0.064	0.262	0.087	0.057	0.048
0.112	0.054	0.21	0.065	0.042	0.046
0.061	0.052	0.151	0.056	0.036	0.044
0.045	0.053	0.106	0.052	0.032	0.041
0.042	0.054	0.086	0.049	0.03	0.039
0.04	0.05	0.075	0.046	0.029	0.037
0.04	0.043	0.07	0.045	0.029	0.037
0.048	0.047	0.076	0.052	0.038	0.046
0.036	0.033	0.061	0.04	0.028	0.035
0.035	0.032	0.054	0.039	0.027	0.035
0.034	0.031	0.048	0.038	0.027	0.034
0.034	0.03	0.045	0.037	0.027	0.033
0.033	0.03	0.043	0.037	0.027	0.033
0.033	0.03	0.04	0.036	0.026	0.032
0.032	0.029	0.039	0.035	0.026	0.032
0.032	0.029	0.038	0.034	0.026	0.032
0.032	0.028	0.036	0.034	0.026	0.031
0.032	0.028	0.035	0.033	0.026	0.031
0.031	0.028	0.035	0.033	0.026	0.031
0.031	0.028	0.034	0.032	0.026	0.03

C1	C2	C3	C4	C5	C6
0.952	1.425	1.495	1.61	1.829	3.523
0.2	0.262	0.359	0.325	0.382	0.558
0.152	0.211	0.302	0.26	0.3	0.448
0.15	0.2	0.315	0.325	0.356	0.52
0.155	0.211	0.328	0.407	0.443	0.461
0.153	0.256	0.314	0.439	0.513	0.33
0.134	0.282	0.258	0.403	0.52	0.202
0.104	0.242	0.181	0.348	0.456	0.136
0.079	0.171	0.115	0.264	0.324	0.107
0.065	0.105	0.08	0.177	0.192	0.076
0.059	0.062	0.066	0.116	0.114	0.064
0.054	0.047	0.059	0.087	0.076	0.06
0.05	0.041	0.056	0.072	0.06	0.058
0.046	0.037	0.052	0.063	0.052	0.055
0.044	0.035	0.049	0.062	0.05	0.054
0.052	0.043	0.055	0.07	0.057	0.062
0.04	0.031	0.042	0.057	0.045	0.051
0.039	0.031	0.039	0.054	0.043	0.05
0.038	0.03	0.038	0.05	0.042	0.049
0.037	0.029	0.036	0.048	0.039	0.048
0.037	0.03	0.035	0.048	0.039	0.048
0.036	0.029	0.034	0.046	0.038	0.047
0.035	0.028	0.033	0.045	0.036	0.046
0.035	0.028	0.033	0.045	0.036	0.046
0.034	0.028	0.032	0.044	0.036	0.045
0.034	0.028	0.032	0.043	0.035	0.045
0.033	0.028	0.031	0.044	0.035	0.044
0.033	0.028	0.031	0.042	0.034	0.044

D1	D2	D3	D4	D5	D6
1.439	1.442	1.041	1.294	1.554	1.607
0.274	0.264	0.177	0.224	0.214	0.327
0.223	0.205	0.133	0.184	0.153	0.255
0.233	0.198	0.127	0.232	0.145	0.263
0.235	0.213	0.126	0.284	0.128	0.276
0.214	0.247	0.111	0.252	0.093	0.263
0.171	0.256	0.082	0.196	0.064	0.209
0.126	0.213	0.059	0.17	0.052	0.114
0.096	0.157	0.044	0.125	0.046	0.087
0.083	0.107	0.038	0.082	0.043	0.076
0.076	0.074	0.035	0.057	0.041	0.069
0.069	0.061	0.033	0.046	0.04	0.065
0.061	0.054	0.032	0.042	0.039	0.062
0.053	0.046	0.031	0.038	0.034	0.059
0.049	0.041	0.031	0.038	0.03	0.056
0.055	0.047	0.04	0.047	0.036	0.062
0.042	0.035	0.029	0.036	0.024	0.047
0.041	0.034	0.03	0.035	0.024	0.044
0.039	0.033	0.03	0.034	0.024	0.043
0.037	0.031	0.029	0.034	0.023	0.041
0.036	0.03	0.029	0.033	0.023	0.04
0.035	0.029	0.029	0.033	0.023	0.039
0.034	0.028	0.028	0.032	0.023	0.038
0.033	0.028	0.028	0.032	0.023	0.037
0.032	0.028	0.028	0.031	0.023	0.036
0.032	0.027	0.028	0.031	0.023	0.036
0.031	0.027	0.028	0.031	0.023	0.035
0.031	0.027	0.028	0.03	0.023	0.035

E1	E2	E3	E4	E5	E6
1.396	1.4	1.857	1.412	1.853	1.634
0.25	0.19	0.371	0.253	0.336	0.31
0.184	0.127	0.293	0.193	0.27	0.212
0.175	0.097	0.329	0.171	0.321	0.192
0.172	0.071	0.38	0.135	0.356	0.196
0.165	0.052	0.406	0.1	0.325	0.178
0.155	0.043	0.372	0.074	0.229	0.13
0.137	0.039	0.253	0.059	0.081	0.093
0.126	0.036	0.185	0.05	0.05	0.061
0.115	0.035	0.116	0.047	0.046	0.044
0.101	0.034	0.07	0.045	0.042	0.037
0.086	0.033	0.054	0.043	0.038	0.034
0.073	0.031	0.049	0.039	0.035	0.032
0.063	0.028	0.043	0.035	0.032	0.03
0.057	0.028	0.04	0.034	0.032	0.029
0.062	0.036	0.046	0.043	0.04	0.038
0.049	0.026	0.034	0.032	0.029	0.027
0.047	0.026	0.032	0.032	0.028	0.026
0.045	0.026	0.031	0.031	0.027	0.026
0.043	0.025	0.03	0.03	0.026	0.026
0.042	0.025	0.03	0.03	0.026	0.026
0.041	0.025	0.029	0.03	0.026	0.026
0.04	0.025	0.029	0.03	0.026	0.025
0.039	0.025	0.028	0.029	0.025	0.025
0.038	0.024	0.028	0.029	0.025	0.025
0.037	0.024	0.028	0.029	0.025	0.025
0.037	0.024	0.027	0.029	0.025	0.025
0.036	0.024	0.027	0.029	0.025	0.025

F1	F2	F3	F4	F5	F6
1.299	1.437	1.789	1.65	1.3	1.525
0.401	0.284	0.502	0.147	0.166	0.363
0.35	0.195	0.405	0.083	0.113	0.298
0.312	0.171	0.408	0.087	0.097	0.298
0.286	0.164	0.395	0.107	0.082	0.306
0.259	0.168	0.355	0.106	0.069	0.307
0.221	0.17	0.307	0.044	0.062	0.348
0.173	0.149	0.242	0.033	0.049	0.305
0.117	0.11	0.19	0.031	0.042	0.254
0.076	0.074	0.152	0.029	0.039	0.205
0.058	0.055	0.12	0.028	0.038	0.186
0.051	0.048	0.098	0.027	0.037	0.169
0.047	0.045	0.081	0.026	0.037	0.164
0.044	0.042	0.071	0.024	0.035	0.158
0.041	0.041	0.066	0.024	0.034	0.151
0.047	0.049	0.071	0.034	0.041	0.15
0.035	0.037	0.056	0.024	0.029	0.139
0.034	0.036	0.052	0.024	0.028	0.135
0.033	0.035	0.048	0.024	0.027	0.132
0.032	0.034	0.044	0.023	0.026	0.129
0.032	0.034	0.042	0.024	0.026	0.122
0.031	0.033	0.039	0.024	0.026	0.119
0.031	0.032	0.037	0.024	0.026	0.117
0.03	0.032	0.036	0.023	0.026	0.114
0.03	0.032	0.034	0.023	0.025	0.112
0.03	0.031	0.033	0.023	0.025	0.11
0.03	0.031	0.033	0.023	0.025	0.104
0.03	0.031	0.032	0.023	0.025	0.107

G1	G2	G3	G4	G5	G6
1.289	1.443	1.28	1.664	1.302	1.523
0.218	0.186	0.28	0.419	0.242	0.18
0.18	0.122	0.193	0.343	0.186	0.109
0.188	0.104	0.149	0.337	0.178	0.108
0.178	0.094	0.129	0.329	0.166	0.118
0.136	0.084	0.1	0.316	0.13	0.103
0.08	0.073	0.079	0.286	0.083	0.065
0.054	0.06	0.073	0.254	0.054	0.054
0.044	0.053	0.07	0.21	0.044	0.046
0.041	0.049	0.066	0.146	0.04	0.041
0.039	0.045	0.064	0.125	0.04	0.037
0.038	0.042	0.061	0.11	0.04	0.036
0.038	0.04	0.06	0.109	0.041	0.035
0.037	0.038	0.058	0.11	0.04	0.034
0.036	0.037	0.057	0.104	0.039	0.034
0.043	0.046	0.065	0.105	0.045	0.043
0.032	0.035	0.055	0.096	0.032	0.032
0.032	0.034	0.054	0.092	0.031	0.033
0.032	0.034	0.053	0.091	0.03	0.033
0.031	0.033	0.052	0.09	0.03	0.033
0.031	0.033	0.052	0.086	0.03	0.033
0.03	0.032	0.051	0.085	0.029	0.032
0.03	0.031	0.05	0.083	0.029	0.032
0.03	0.031	0.05	0.081	0.028	0.032
0.03	0.03	0.049	0.079	0.028	0.032
0.03	0.03	0.048	0.078	0.028	0.032
0.03	0.03	0.048	0.075	0.028	0.032
0.029	0.03	0.047	0.075	0.028	0.032

					DMSO-blank
H1	H2	H3	H4	H5	H6
1.626	1.831	1.644	2.043	2.018	1.565
0.494	0.683	0.302	0.508	0.371	0.128
0.587	0.538	0.296	0.35	0.346	0.061
0.599	0.434	0.409	0.451	0.575	0.044
0.466	0.35	0.524	0.586	0.788	0.035
0.267	0.273	0.561	0.61	0.665	0.03
0.111	0.193	0.49	0.475	0.234	0.028
0.071	0.14	0.367	0.279	0.078	0.027
0.059	0.1	0.244	0.126	0.071	0.026
0.053	0.079	0.144	0.057	0.058	0.025
0.049	0.071	0.087	0.036	0.041	0.024
0.046	0.065	0.061	0.029	0.031	0.022
0.044	0.06	0.048	0.027	0.028	0.022
0.04	0.054	0.039	0.026	0.026	0.02
0.038	0.048	0.033	0.026	0.026	0.02
0.046	0.053	0.038	0.035	0.034	0.03
0.034	0.04	0.026	0.024	0.025	0.02
0.032	0.038	0.025	0.024	0.024	0.02
0.031	0.037	0.024	0.025	0.024	0.02
0.03	0.036	0.024	0.024	0.024	0.02
0.03	0.035	0.024	0.025	0.024	0.02
0.03	0.034	0.024	0.025	0.024	0.02
0.03	0.034	0.024	0.024	0.024	0.021
0.029	0.033	0.024	0.024	0.024	0.021
0.029	0.032	0.024	0.024	0.024	0.021
0.029	0.032	0.024	0.024	0.024	0.02
0.029	0.032	0.024	0.024	0.023	0.021
0.028	0.031	0.024	0.024	0.024	0.021

Raw Absorbance Data for 1st PAMPA assay

Wavelength/Well	A1	A2	A3	A4	A5	A6	A7	A8
230	0.11	0.097	0.096	0.104	0.139	0.107	0.106	0.103
240	0.084	0.074	0.076	0.08	0.1	0.082	0.08	0.08
250	0.071	0.063	0.067	0.068	0.084	0.072	0.07	0.07
260	0.061	0.053	0.057	0.058	0.071	0.061	0.06	0.061
270	0.053	0.045	0.051	0.05	0.064	0.054	0.053	0.054
280	0.048	0.041	0.046	0.045	0.058	0.049	0.048	0.05
290	0.044	0.038	0.041	0.041	0.054	0.044	0.044	0.047
300	0.041	0.035	0.038	0.038	0.048	0.041	0.04	0.043
310	0.039	0.033	0.037	0.036	0.044	0.039	0.037	0.04
320	0.038	0.032	0.036	0.034	0.042	0.037	0.034	0.038
330	0.036	0.03	0.034	0.033	0.039	0.036	0.032	0.036
340	0.035	0.029	0.033	0.032	0.038	0.034	0.031	0.034
350	0.034	0.028	0.032	0.03	0.036	0.033	0.03	0.033
360	0.032	0.026	0.031	0.029	0.034	0.031	0.028	0.031
370	0.032	0.026	0.031	0.029	0.034	0.031	0.028	0.032
380	0.042	0.036	0.041	0.038	0.043	0.041	0.038	0.041
390	0.031	0.025	0.03	0.028	0.032	0.03	0.027	0.03
400	0.031	0.026	0.03	0.028	0.032	0.03	0.027	0.03
410	0.03	0.025	0.029	0.028	0.031	0.03	0.027	0.03
420	0.03	0.025	0.029	0.027	0.031	0.03	0.027	0.03
430	0.03	0.025	0.029	0.027	0.031	0.03	0.027	0.03
440	0.03	0.025	0.029	0.027	0.03	0.029	0.027	0.029
450	0.029	0.025	0.028	0.027	0.03	0.029	0.026	0.029
460	0.029	0.025	0.028	0.027	0.03	0.029	0.026	0.029
470	0.029	0.025	0.028	0.027	0.029	0.029	0.026	0.029
480	0.029	0.025	0.028	0.027	0.029	0.028	0.026	0.028
490	0.029	0.025	0.028	0.026	0.029	0.028	0.026	0.029
500	0.028	0.025	0.028	0.026	0.029	0.028	0.026	0.028

Wavelength/Well	**A9**	**A10**	**A11**	**A12**	**B1**	**B2**	**B3**	**B4**
230	0.097	0.103	0.109	0.095	0.103	0.095	0.096	0.097
240	0.074	0.08	0.084	0.074	0.08	0.075	0.076	0.076
250	0.065	0.07	0.074	0.066	0.071	0.067	0.068	0.068
260	0.054	0.06	0.065	0.055	0.061	0.058	0.058	0.058
270	0.047	0.053	0.058	0.048	0.054	0.051	0.051	0.052
280	0.042	0.047	0.053	0.044	0.049	0.046	0.047	0.047
290	0.04	0.042	0.047	0.039	0.045	0.042	0.042	0.043
300	0.037	0.04	0.043	0.037	0.042	0.039	0.04	0.04
310	0.036	0.038	0.041	0.035	0.04	0.037	0.038	0.038
320	0.035	0.036	0.039	0.034	0.038	0.036	0.036	0.036
330	0.033	0.035	0.037	0.032	0.037	0.034	0.034	0.034
340	0.032	0.034	0.035	0.031	0.036	0.033	0.033	0.033
350	0.03	0.032	0.033	0.03	0.034	0.032	0.032	0.032
360	0.028	0.031	0.031	0.029	0.033	0.03	0.03	0.031
370	0.028	0.031	0.031	0.029	0.033	0.031	0.031	0.031
380	0.038	0.04	0.041	0.039	0.042	0.04	0.04	0.04
390	0.027	0.03	0.03	0.028	0.032	0.03	0.03	0.03
400	0.027	0.03	0.03	0.028	0.032	0.03	0.029	0.03
410	0.027	0.029	0.03	0.028	0.031	0.03	0.029	0.029
420	0.027	0.029	0.029	0.028	0.031	0.029	0.029	0.029
430	0.027	0.029	0.029	0.028	0.031	0.029	0.029	0.029
440	0.027	0.029	0.029	0.028	0.03	0.029	0.028	0.029
450	0.027	0.029	0.029	0.028	0.03	0.028	0.028	0.028
460	0.027	0.029	0.029	0.028	0.03	0.028	0.028	0.028
470	0.027	0.028	0.028	0.027	0.03	0.028	0.028	0.028
480	0.026	0.028	0.028	0.027	0.029	0.028	0.028	0.028
490	0.026	0.028	0.028	0.027	0.029	0.028	0.028	0.028
500	0.026	0.028	0.028	0.027	0.029	0.028	0.027	0.028

Wavelength/Well	B5	B6	B7	B8	B9	B10	B11	B12
230	0.094	0.097	0.108	0.103	0.101	0.142	0.094	0.096
240	0.075	0.077	0.084	0.084	0.08	0.099	0.075	0.074
250	0.067	0.071	0.076	0.076	0.071	0.091	0.067	0.065
260	0.057	0.058	0.067	0.067	0.061	0.087	0.058	0.056
270	0.05	0.046	0.06	0.06	0.053	0.081	0.051	0.049
280	0.046	0.038	0.054	0.055	0.048	0.068	0.047	0.045
290	0.042	0.033	0.047	0.049	0.043	0.054	0.042	0.041
300	0.039	0.032	0.044	0.047	0.04	0.046	0.04	0.038
310	0.037	0.031	0.04	0.045	0.038	0.038	0.037	0.036
320	0.035	0.029	0.039	0.043	0.036	0.036	0.036	0.034
330	0.034	0.027	0.037	0.041	0.034	0.034	0.034	0.032
340	0.033	0.026	0.036	0.04	0.034	0.033	0.033	0.031
350	0.031	0.024	0.034	0.039	0.032	0.032	0.032	0.03
360	0.03	0.022	0.032	0.039	0.031	0.03	0.03	0.029
370	0.03	0.021	0.032	0.038	0.031	0.03	0.031	0.029
380	0.04	0.031	0.042	0.047	0.04	0.04	0.04	0.038
390	0.029	0.021	0.031	0.037	0.03	0.029	0.03	0.028
400	0.029	0.021	0.031	0.037	0.03	0.029	0.03	0.028
410	0.028	0.021	0.031	0.036	0.029	0.029	0.029	0.027
420	0.028	0.021	0.03	0.036	0.029	0.028	0.029	0.027
430	0.028	0.021	0.031	0.036	0.029	0.029	0.029	0.027
440	0.028	0.021	0.03	0.036	0.029	0.028	0.029	0.027
450	0.028	0.021	0.03	0.035	0.029	0.028	0.028	0.027
460	0.028	0.021	0.03	0.035	0.029	0.028	0.028	0.027
470	0.027	0.021	0.03	0.035	0.028	0.028	0.028	0.026
480	0.027	0.021	0.029	0.035	0.028	0.027	0.028	0.026
490	0.027	0.021	0.029	0.034	0.028	0.028	0.028	0.026
500	0.027	0.021	0.029	0.034	0.028	0.027	0.028	0.026

Wavelength/Well	C1	C2	C3	C4	C5	C6	C7	C8
230	0.111	0.097	0.102	0.122	0.098	0.095	0.095	0.076
240	0.086	0.077	0.076	0.088	0.077	0.072	0.075	0.062
250	0.075	0.07	0.065	0.075	0.069	0.064	0.068	0.053
260	0.065	0.06	0.055	0.063	0.059	0.054	0.059	0.043
270	0.058	0.053	0.046	0.054	0.051	0.046	0.052	0.034
280	0.053	0.048	0.04	0.05	0.047	0.041	0.047	0.029
290	0.048	0.044	0.034	0.045	0.042	0.037	0.042	0.026
300	0.044	0.041	0.032	0.041	0.039	0.035	0.039	0.025
310	0.042	0.039	0.031	0.037	0.037	0.033	0.037	0.024
320	0.04	0.038	0.03	0.036	0.036	0.032	0.036	0.024
330	0.038	0.036	0.029	0.033	0.034	0.031	0.034	0.022
340	0.037	0.035	0.028	0.032	0.033	0.03	0.034	0.021
350	0.035	0.034	0.026	0.031	0.032	0.029	0.032	0.02
360	0.033	0.032	0.024	0.03	0.03	0.027	0.031	0.019
370	0.034	0.032	0.024	0.03	0.03	0.028	0.032	0.019
380	0.043	0.042	0.035	0.04	0.04	0.037	0.041	0.029
390	0.032	0.031	0.024	0.029	0.03	0.026	0.03	0.019
400	0.032	0.031	0.025	0.029	0.03	0.026	0.031	0.02
410	0.031	0.031	0.024	0.029	0.029	0.026	0.03	0.02
420	0.031	0.03	0.025	0.028	0.029	0.026	0.03	0.02
430	0.031	0.03	0.025	0.028	0.029	0.026	0.03	0.02
440	0.03	0.03	0.025	0.028	0.028	0.026	0.03	0.02
450	0.03	0.03	0.025	0.028	0.028	0.026	0.03	0.02
460	0.03	0.029	0.025	0.028	0.028	0.026	0.029	0.02
470	0.03	0.029	0.025	0.028	0.028	0.026	0.029	0.02
480	0.03	0.029	0.025	0.027	0.028	0.026	0.029	0.02
490	0.03	0.029	0.025	0.027	0.028	0.026	0.029	0.02
500	0.029	0.029	0.025	0.027	0.027	0.025	0.029	0.02

Wavelength/Well	C9	C10	C11	C12	D1	D2	D3	D4
230	0.098	0.094	0.129	0.102	0.115	0.096	0.133	0.136
240	0.07	0.074	0.092	0.081	0.091	0.076	0.082	0.1
250	0.06	0.066	0.081	0.07	0.083	0.068	0.073	0.097
260	0.049	0.057	0.071	0.06	0.073	0.058	0.063	0.089
270	0.041	0.05	0.063	0.052	0.063	0.051	0.055	0.074
280	0.035	0.046	0.054	0.047	0.054	0.047	0.05	0.059
290	0.03	0.041	0.045	0.041	0.048	0.042	0.044	0.051
300	0.028	0.039	0.039	0.039	0.043	0.04	0.04	0.043
310	0.027	0.037	0.036	0.037	0.04	0.038	0.038	0.039
320	0.026	0.036	0.035	0.036	0.038	0.037	0.037	0.038
330	0.025	0.034	0.033	0.035	0.036	0.035	0.035	0.036
340	0.024	0.033	0.032	0.034	0.035	0.034	0.034	0.034
350	0.023	0.032	0.031	0.032	0.034	0.032	0.033	0.033
360	0.022	0.03	0.03	0.031	0.032	0.031	0.031	0.031
370	0.022	0.031	0.03	0.031	0.032	0.031	0.032	0.031
380	0.032	0.04	0.039	0.04	0.042	0.041	0.041	0.041
390	0.022	0.03	0.029	0.03	0.031	0.03	0.03	0.03
400	0.022	0.03	0.029	0.03	0.031	0.03	0.03	0.03
410	0.022	0.029	0.029	0.03	0.03	0.03	0.03	0.029
420	0.022	0.029	0.028	0.03	0.03	0.029	0.03	0.029
430	0.022	0.029	0.028	0.031	0.03	0.029	0.03	0.029
440	0.022	0.029	0.028	0.03	0.029	0.029	0.029	0.028
450	0.022	0.029	0.028	0.03	0.029	0.029	0.029	0.028
460	0.023	0.028	0.028	0.031	0.029	0.028	0.029	0.028
470	0.022	0.028	0.028	0.03	0.029	0.028	0.029	0.028
480	0.022	0.028	0.027	0.03	0.029	0.028	0.029	0.028
490	0.022	0.028	0.027	0.03	0.029	0.028	0.029	0.028
500	0.022	0.028	0.027	0.03	0.028	0.028	0.028	0.028

Wavelength/Well	D5	D6	D7	D8	D9	D10	D11	D12
230	0.292	0.164	0.123	0.358	0.219	0.094	0.093	0.094
240	0.201	0.127	0.097	0.232	0.175	0.073	0.073	0.073
250	0.231	0.133	0.087	0.187	0.194	0.066	0.066	0.065
260	0.221	0.126	0.083	0.222	0.252	0.056	0.056	0.056
270	0.148	0.092	0.083	0.266	0.31	0.049	0.049	0.049
280	0.082	0.068	0.078	0.27	0.327	0.046	0.045	0.045
290	0.059	0.055	0.06	0.213	0.285	0.04	0.041	0.04
300	0.047	0.045	0.054	0.138	0.219	0.038	0.038	0.037
310	0.039	0.04	0.056	0.084	0.155	0.037	0.037	0.036
320	0.037	0.037	0.048	0.056	0.1	0.036	0.035	0.035
330	0.034	0.036	0.039	0.041	0.067	0.035	0.034	0.033
340	0.033	0.034	0.033	0.034	0.051	0.034	0.033	0.032
350	0.032	0.034	0.03	0.031	0.044	0.032	0.032	0.031
360	0.03	0.032	0.029	0.03	0.038	0.031	0.031	0.03
370	0.03	0.032	0.029	0.03	0.036	0.031	0.031	0.03
380	0.04	0.041	0.038	0.039	0.043	0.041	0.04	0.04
390	0.029	0.031	0.028	0.029	0.032	0.03	0.03	0.029
400	0.029	0.031	0.028	0.028	0.031	0.03	0.03	0.029
410	0.029	0.03	0.028	0.028	0.03	0.03	0.03	0.029
420	0.029	0.03	0.027	0.028	0.03	0.03	0.029	0.028
430	0.029	0.03	0.028	0.028	0.03	0.03	0.029	0.028
440	0.028	0.03	0.027	0.028	0.03	0.029	0.029	0.028
450	0.028	0.03	0.027	0.028	0.03	0.029	0.029	0.028
460	0.028	0.029	0.027	0.028	0.03	0.029	0.029	0.028
470	0.028	0.029	0.027	0.027	0.029	0.029	0.029	0.028
480	0.028	0.029	0.027	0.027	0.029	0.028	0.028	0.027
490	0.028	0.029	0.027	0.027	0.029	0.029	0.028	0.027
500	0.028	0.029	0.026	0.027	0.029	0.028	0.028	0.027

Wavelength/Well	E1	E2	E3	E4	E5	E6	E7	E8
230	0.089	0.108	0.084	0.097	0.096	0.094	0.096	0.089
240	0.068	0.078	0.062	0.073	0.075	0.073	0.074	0.067
250	0.058	0.068	0.054	0.066	0.066	0.066	0.066	0.058
260	0.047	0.057	0.043	0.057	0.057	0.056	0.057	0.047
270	0.04	0.05	0.034	0.049	0.05	0.05	0.05	0.039
280	0.035	0.045	0.03	0.044	0.044	0.045	0.046	0.034
290	0.032	0.042	0.026	0.039	0.041	0.041	0.041	0.03
300	0.031	0.039	0.026	0.037	0.038	0.039	0.039	0.029
310	0.029	0.037	0.025	0.035	0.036	0.037	0.037	0.028
320	0.028	0.036	0.024	0.033	0.035	0.036	0.036	0.026
330	0.027	0.034	0.022	0.031	0.033	0.034	0.034	0.025
340	0.026	0.033	0.021	0.03	0.032	0.033	0.033	0.024
350	0.025	0.032	0.02	0.029	0.031	0.032	0.032	0.023
360	0.023	0.03	0.019	0.028	0.03	0.03	0.03	0.021
370	0.023	0.031	0.019	0.028	0.03	0.03	0.03	0.021
380	0.033	0.04	0.028	0.037	0.039	0.04	0.04	0.031
390	0.023	0.03	0.019	0.027	0.029	0.03	0.03	0.021
400	0.023	0.03	0.019	0.027	0.029	0.03	0.03	0.021
410	0.023	0.029	0.019	0.027	0.029	0.03	0.029	0.021
420	0.023	0.029	0.019	0.026	0.029	0.029	0.029	0.021
430	0.023	0.029	0.02	0.026	0.029	0.029	0.029	0.021
440	0.023	0.029	0.02	0.026	0.028	0.029	0.029	0.022
450	0.023	0.028	0.02	0.026	0.028	0.029	0.029	0.021
460	0.023	0.028	0.02	0.026	0.028	0.029	0.028	0.022
470	0.023	0.028	0.02	0.026	0.028	0.029	0.028	0.021
480	0.023	0.028	0.02	0.026	0.028	0.028	0.028	0.021
490	0.023	0.028	0.02	0.026	0.028	0.028	0.028	0.022
500	0.023	0.028	0.02	0.025	0.028	0.028	0.028	0.022

Wavelength/Well	E9	E10	E11	E12	F1	F2	F3	F4
230	0.086	0.096	0.096	0.094	0.091	0.089	0.094	0.096
240	0.067	0.075	0.075	0.074	0.072	0.073	0.077	0.073
250	0.059	0.067	0.067	0.065	0.065	0.066	0.072	0.065
260	0.048	0.057	0.058	0.056	0.056	0.056	0.061	0.057
270	0.041	0.051	0.051	0.049	0.049	0.049	0.05	0.049
280	0.036	0.046	0.047	0.045	0.044	0.045	0.045	0.042
290	0.033	0.041	0.042	0.04	0.04	0.04	0.041	0.038
300	0.031	0.039	0.04	0.038	0.038	0.039	0.039	0.036
310	0.03	0.037	0.038	0.036	0.036	0.037	0.037	0.034
320	0.029	0.035	0.037	0.035	0.034	0.037	0.036	0.032
330	0.027	0.034	0.035	0.033	0.033	0.035	0.034	0.031
340	0.026	0.033	0.034	0.032	0.032	0.034	0.033	0.029
350	0.025	0.032	0.033	0.031	0.031	0.033	0.031	0.028
360	0.024	0.03	0.031	0.03	0.03	0.032	0.029	0.027
370	0.024	0.03	0.032	0.03	0.03	0.032	0.028	0.026
380	0.034	0.04	0.041	0.039	0.04	0.042	0.038	0.036
390	0.023	0.03	0.031	0.029	0.029	0.031	0.028	0.025
400	0.024	0.03	0.031	0.029	0.029	0.031	0.028	0.025
410	0.023	0.03	0.03	0.029	0.029	0.031	0.027	0.025
420	0.023	0.029	0.03	0.028	0.028	0.031	0.027	0.025
430	0.024	0.029	0.03	0.028	0.028	0.031	0.027	0.025
440	0.023	0.029	0.03	0.028	0.028	0.03	0.027	0.025
450	0.023	0.029	0.03	0.028	0.028	0.03	0.026	0.025
460	0.023	0.028	0.029	0.028	0.028	0.03	0.026	0.025
470	0.023	0.028	0.029	0.028	0.028	0.03	0.026	0.024
480	0.023	0.028	0.029	0.027	0.027	0.029	0.026	0.024
490	0.023	0.028	0.029	0.028	0.028	0.03	0.026	0.024
500	0.023	0.028	0.029	0.027	0.027	0.029	0.026	0.024

Wavelength/Well	F5	F6	F7	F8	F9	F10	F11	F12
230	0.09	0.089	0.128	0.088	0.088	0.105	0.09	0.097
240	0.072	0.071	0.092	0.071	0.071	0.081	0.07	0.072
250	0.065	0.064	0.085	0.064	0.063	0.071	0.062	0.061
260	0.055	0.055	0.08	0.055	0.054	0.061	0.052	0.05
270	0.049	0.048	0.074	0.049	0.048	0.054	0.045	0.043
280	0.044	0.044	0.063	0.044	0.043	0.049	0.04	0.038
290	0.04	0.04	0.05	0.04	0.04	0.045	0.036	0.034
300	0.038	0.038	0.044	0.037	0.037	0.042	0.034	0.032
310	0.036	0.036	0.038	0.036	0.035	0.04	0.032	0.031
320	0.035	0.034	0.035	0.034	0.034	0.038	0.031	0.03
330	0.033	0.033	0.033	0.033	0.033	0.036	0.03	0.028
340	0.032	0.032	0.032	0.032	0.032	0.035	0.029	0.027
350	0.031	0.031	0.031	0.031	0.03	0.034	0.028	0.026
360	0.03	0.029	0.03	0.029	0.029	0.032	0.027	0.025
370	0.03	0.03	0.03	0.03	0.029	0.032	0.027	0.025
380	0.039	0.039	0.039	0.039	0.039	0.042	0.036	0.034
390	0.029	0.029	0.029	0.028	0.028	0.031	0.026	0.024
400	0.029	0.028	0.029	0.028	0.028	0.031	0.026	0.024
410	0.028	0.028	0.028	0.028	0.028	0.031	0.026	0.024
420	0.028	0.028	0.028	0.028	0.028	0.03	0.026	0.024
430	0.028	0.028	0.028	0.028	0.028	0.031	0.026	0.024
440	0.028	0.028	0.028	0.028	0.028	0.03	0.025	0.024
450	0.028	0.027	0.028	0.027	0.027	0.03	0.025	0.024
460	0.028	0.028	0.028	0.027	0.027	0.03	0.025	0.024
470	0.027	0.027	0.027	0.027	0.027	0.03	0.025	0.024
480	0.027	0.027	0.027	0.027	0.027	0.03	0.025	0.024
490	0.027	0.027	0.027	0.027	0.027	0.03	0.025	0.024
500	0.027	0.027	0.027	0.027	0.027	0.029	0.025	0.024

Wavelength/Well	**G1**	**G2**	**G3**	**G4**	**G5**	**G6**	**G7**	**G8**
230	0.12	0.097	0.093	0.097	0.089	0.095	0.092	0.118
240	0.088	0.077	0.072	0.077	0.072	0.074	0.072	0.087
250	0.074	0.068	0.065	0.068	0.064	0.065	0.064	0.076
260	0.062	0.058	0.056	0.058	0.055	0.056	0.055	0.066
270	0.053	0.05	0.048	0.052	0.049	0.049	0.048	0.058
280	0.048	0.045	0.044	0.046	0.044	0.045	0.044	0.051
290	0.043	0.041	0.04	0.042	0.04	0.04	0.04	0.043
300	0.039	0.038	0.038	0.039	0.038	0.037	0.037	0.038
310	0.037	0.036	0.036	0.037	0.036	0.036	0.036	0.036
320	0.035	0.035	0.035	0.036	0.034	0.034	0.034	0.034
330	0.034	0.033	0.033	0.034	0.033	0.033	0.033	0.033
340	0.033	0.032	0.032	0.033	0.032	0.032	0.032	0.032
350	0.032	0.031	0.031	0.032	0.031	0.031	0.031	0.031
360	0.03	0.03	0.03	0.031	0.03	0.03	0.03	0.03
370	0.03	0.03	0.03	0.031	0.03	0.03	0.03	0.03
380	0.04	0.039	0.039	0.04	0.039	0.039	0.039	0.039
390	0.029	0.029	0.029	0.03	0.029	0.029	0.029	0.029
400	0.029	0.029	0.029	0.03	0.029	0.029	0.029	0.029
410	0.029	0.029	0.028	0.029	0.029	0.029	0.028	0.028
420	0.028	0.029	0.028	0.029	0.028	0.028	0.028	0.028
430	0.029	0.028	0.028	0.029	0.028	0.028	0.028	0.028
440	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028
450	0.028	0.028	0.028	0.028	0.028	0.028	0.028	0.028
460	0.028	0.028	0.027	0.028	0.028	0.028	0.028	0.028
470	0.028	0.028	0.027	0.028	0.028	0.028	0.028	0.028
480	0.028	0.028	0.027	0.028	0.028	0.028	0.027	0.027
490	0.028	0.027	0.027	0.028	0.028	0.027	0.027	0.027
500	0.027	0.027	0.027	0.028	0.027	0.027	0.027	0.027

Wavelength/Well	G9	G10	G11	G12	H1	H2	H3	H4	H5	H6
230	0.089	0.093	0.096	0.106	0.11	0.32	0.161	0.12	0.334	0.199
240	0.071	0.074	0.075	0.078	0.083	0.218	0.126	0.095	0.218	0.16
250	0.064	0.067	0.066	0.068	0.077	0.252	0.129	0.084	0.175	0.176
260	0.055	0.057	0.057	0.058	0.068	0.241	0.121	0.078	0.204	0.227
270	0.048	0.05	0.05	0.052	0.058	0.158	0.09	0.077	0.243	0.279
280	0.044	0.046	0.046	0.048	0.05	0.081	0.069	0.072	0.246	0.295
290	0.039	0.041	0.042	0.042	0.044	0.055	0.056	0.056	0.196	0.257
300	0.037	0.038	0.039	0.039	0.039	0.042	0.046	0.051	0.128	0.198
310	0.035	0.036	0.037	0.038	0.037	0.033	0.041	0.053	0.08	0.141
320	0.034	0.035	0.035	0.036	0.035	0.031	0.039	0.047	0.054	0.091
330	0.033	0.033	0.034	0.035	0.034	0.029	0.038	0.038	0.041	0.06
340	0.032	0.032	0.033	0.034	0.032	0.028	0.036	0.032	0.034	0.046
350	0.031	0.031	0.032	0.033	0.032	0.027	0.035	0.03	0.032	0.039
360	0.029	0.03	0.03	0.032	0.03	0.025	0.034	0.029	0.03	0.034
370	0.029	0.03	0.03	0.032	0.03	0.025	0.034	0.029	0.03	0.032
380	0.039	0.039	0.04	0.041	0.04	0.034	0.043	0.038	0.04	0.039
390	0.029	0.029	0.03	0.031	0.029	0.024	0.032	0.028	0.029	0.028
400	0.028	0.029	0.029	0.031	0.029	0.024	0.032	0.028	0.029	0.027
410	0.028	0.028	0.029	0.031	0.029	0.024	0.032	0.027	0.029	0.027
420	0.028	0.028	0.029	0.03	0.028	0.024	0.031	0.027	0.028	0.027
430	0.028	0.028	0.029	0.03	0.028	0.024	0.031	0.027	0.028	0.027
440	0.028	0.028	0.028	0.03	0.028	0.024	0.031	0.027	0.028	0.026
450	0.028	0.028	0.028	0.03	0.028	0.024	0.031	0.027	0.028	0.026
460	0.028	0.028	0.028	0.03	0.028	0.024	0.03	0.027	0.028	0.026
470	0.028	0.028	0.028	0.03	0.028	0.024	0.03	0.027	0.028	0.026
480	0.027	0.028	0.028	0.029	0.027	0.024	0.03	0.026	0.028	0.026
490	0.027	0.028	0.028	0.029	0.028	0.024	0.03	0.026	0.027	0.026
500	0.027	0.028	0.028	0.029	0.027	0.024	0.03	0.026	0.027	0.026

Raw Equilibrium Solutions for 1st PAMPA assay

Wavelength/Well	A1	A2	A3	A4	A5	A6	A7	A8
230	1.934	1.313	1.648	1.488	1.529	1.92	1.656	1.487
240	0.62	0.2	0.22	0.302	0.339	0.39	0.389	0.327
250	0.527	0.196	0.155	0.232	0.266	0.313	0.329	0.293
260	0.531	0.265	0.145	0.238	0.274	0.368	0.351	0.31
270	0.521	0.363	0.124	0.248	0.286	0.437	0.414	0.341
280	0.478	0.443	0.085	0.232	0.274	0.473	0.478	0.389
290	0.374	0.412	0.052	0.175	0.217	0.43	0.467	0.392
300	0.241	0.248	0.039	0.08	0.12	0.283	0.37	0.332
310	0.199	0.098	0.035	0.056	0.092	0.198	0.239	0.238
320	0.176	0.051	0.033	0.05	0.082	0.119	0.158	0.163
330	0.169	0.043	0.031	0.048	0.075	0.07	0.127	0.12
340	0.158	0.042	0.03	0.047	0.07	0.054	0.11	0.105
350	0.156	0.042	0.029	0.045	0.067	0.049	0.102	0.102
360	0.154	0.041	0.027	0.041	0.063	0.046	0.096	0.097
370	0.143	0.04	0.025	0.038	0.06	0.043	0.089	0.086
380	0.139	0.048	0.033	0.045	0.067	0.05	0.091	0.084
390	0.123	0.034	0.022	0.033	0.054	0.037	0.078	0.064
400	0.114	0.033	0.022	0.032	0.051	0.036	0.074	0.059
410	0.108	0.033	0.022	0.032	0.049	0.034	0.071	0.055
420	0.103	0.032	0.022	0.031	0.047	0.034	0.069	0.052
430	0.096	0.032	0.022	0.03	0.046	0.033	0.066	0.051
440	0.092	0.031	0.022	0.03	0.044	0.032	0.064	0.049
450	0.088	0.03	0.022	0.029	0.043	0.031	0.062	0.047
460	0.085	0.03	0.022	0.029	0.041	0.031	0.06	0.045
470	0.081	0.03	0.022	0.029	0.041	0.03	0.058	0.044
480	0.078	0.029	0.022	0.029	0.04	0.03	0.057	0.043
490	0.075	0.029	0.021	0.028	0.039	0.03	0.055	0.042
500	0.073	0.029	0.022	0.028	0.038	0.03	0.055	0.042

Wavelength/Well	A9	A10	A11	A12	B1	B2	B3	B4
230	1.354	1.777	3.416	1.436	1.641	1.612	1.456	1.354
240	0.248	0.351	2.159	0.201	0.371	0.554	0.297	0.246
250	0.191	0.288	1.947	0.137	0.243	0.475	0.202	0.208
260	0.167	0.342	1.948	0.104	0.198	0.398	0.178	0.219
270	0.125	0.378	2.028	0.075	0.185	0.355	0.169	0.236
280	0.086	0.346	2.035	0.054	0.16	0.339	0.177	0.226
290	0.06	0.24	1.778	0.043	0.12	0.307	0.185	0.177
300	0.047	0.091	1.713	0.039	0.091	0.242	0.163	0.128
310	0.041	0.06	1.662	0.036	0.067	0.16	0.118	0.082
320	0.038	0.055	1.56	0.035	0.054	0.101	0.075	0.055
330	0.036	0.051	1.464	0.034	0.047	0.075	0.054	0.044
340	0.035	0.047	1.303	0.032	0.044	0.065	0.046	0.04
350	0.033	0.043	1.17	0.03	0.041	0.06	0.042	0.038
360	0.031	0.04	1.058	0.028	0.039	0.055	0.04	0.036
370	0.031	0.039	0.942	0.027	0.038	0.05	0.038	0.035
380	0.04	0.048	0.867	0.037	0.046	0.055	0.046	0.043
390	0.029	0.036	0.816	0.026	0.035	0.04	0.034	0.031
400	0.029	0.035	0.762	0.026	0.035	0.038	0.033	0.031
410	0.029	0.034	0.723	0.025	0.034	0.037	0.032	0.03
420	0.028	0.032	0.689	0.025	0.034	0.036	0.031	0.029
430	0.028	0.032	0.65	0.025	0.034	0.035	0.031	0.03
440	0.028	0.032	0.622	0.025	0.033	0.034	0.03	0.029
450	0.028	0.031	0.595	0.025	0.032	0.033	0.03	0.029
460	0.028	0.03	0.57	0.025	0.032	0.032	0.029	0.028
470	0.027	0.03	0.545	0.025	0.032	0.032	0.029	0.028
480	0.027	0.03	0.524	0.024	0.032	0.032	0.029	0.028
490	0.027	0.03	0.501	0.024	0.032	0.031	0.029	0.028
500	0.027	0.03	0.484	0.024	0.032	0.031	0.028	0.027

Wavelength/Well	B5	B6	B7	B8	B9	B10	B11	B12
230	1.01	1.414	2.041	1.666	1.544	1.188	1.206	1.15
240	0.136	0.274	0.748	0.364	0.517	0.222	0.189	0.217
250	0.089	0.292	0.605	0.278	0.627	0.177	0.132	0.167
260	0.072	0.352	0.618	0.277	0.638	0.18	0.137	0.194
270	0.063	0.318	0.61	0.289	0.492	0.176	0.159	0.231
280	0.053	0.225	0.552	0.285	0.278	0.145	0.181	0.247
290	0.041	0.167	0.464	0.239	0.116	0.102	0.194	0.228
300	0.037	0.14	0.369	0.147	0.075	0.078	0.175	0.187
310	0.035	0.114	0.283	0.108	0.063	0.055	0.129	0.136
320	0.033	0.094	0.214	0.081	0.057	0.047	0.082	0.092
330	0.031	0.083	0.166	0.061	0.053	0.043	0.059	0.075
340	0.03	0.077	0.133	0.05	0.05	0.04	0.048	0.066
350	0.028	0.067	0.109	0.043	0.047	0.039	0.044	0.064
360	0.027	0.051	0.094	0.038	0.043	0.037	0.042	0.064
370	0.027	0.039	0.085	0.036	0.041	0.037	0.041	0.061
380	0.036	0.043	0.087	0.043	0.048	0.046	0.049	0.066
390	0.026	0.03	0.071	0.031	0.036	0.035	0.039	0.056
400	0.026	0.029	0.066	0.03	0.034	0.034	0.038	0.055
410	0.026	0.028	0.06	0.029	0.033	0.034	0.038	0.055
420	0.025	0.027	0.056	0.028	0.032	0.033	0.038	0.054
430	0.025	0.026	0.052	0.028	0.032	0.033	0.037	0.052
440	0.025	0.026	0.049	0.028	0.031	0.032	0.036	0.052
450	0.025	0.025	0.046	0.027	0.03	0.032	0.036	0.051
460	0.025	0.025	0.044	0.027	0.03	0.032	0.036	0.05
470	0.025	0.024	0.042	0.027	0.029	0.031	0.035	0.05
480	0.025	0.024	0.04	0.026	0.029	0.031	0.035	0.049
490	0.024	0.024	0.039	0.026	0.029	0.031	0.034	0.048
500	0.025	0.024	0.038	0.026	0.029	0.031	0.035	0.049

Wavelength/Well	C1	C2	C3	C4	C5	C6	C7	C8
230	2.807	1.697	1.716	1.306	1.499	1.33	1.327	1.399
240	1.324	0.618	0.498	0.325	0.342	0.292	0.232	0.232
250	1.16	0.61	0.41	0.272	0.314	0.208	0.196	0.194
260	1.161	0.566	0.45	0.266	0.35	0.167	0.208	0.207
270	1.246	0.426	0.466	0.261	0.295	0.146	0.197	0.179
280	1.251	0.256	0.416	0.247	0.191	0.114	0.148	0.118
290	1.017	0.11	0.313	0.227	0.1	0.098	0.085	0.069
300	0.7	0.062	0.165	0.205	0.064	0.089	0.054	0.049
310	0.544	0.048	0.112	0.175	0.049	0.082	0.043	0.044
320	0.475	0.042	0.096	0.125	0.042	0.073	0.039	0.04
330	0.446	0.04	0.085	0.108	0.038	0.07	0.037	0.038
340	0.414	0.039	0.077	0.096	0.036	0.066	0.037	0.036
350	0.39	0.038	0.069	0.096	0.034	0.065	0.036	0.035
360	0.369	0.036	0.062	0.095	0.032	0.063	0.035	0.035
370	0.326	0.036	0.059	0.09	0.032	0.061	0.033	0.033
380	0.301	0.045	0.067	0.092	0.041	0.066	0.041	0.041
390	0.268	0.034	0.054	0.084	0.03	0.056	0.03	0.031
400	0.24	0.034	0.052	0.082	0.03	0.055	0.029	0.031
410	0.219	0.034	0.05	0.082	0.029	0.054	0.029	0.031
420	0.202	0.033	0.048	0.083	0.029	0.052	0.028	0.031
430	0.186	0.033	0.047	0.078	0.029	0.05	0.028	0.031
440	0.172	0.033	0.046	0.079	0.028	0.049	0.028	0.031
450	0.158	0.032	0.044	0.078	0.028	0.048	0.028	0.031
460	0.147	0.032	0.044	0.078	0.028	0.047	0.027	0.031
470	0.135	0.032	0.042	0.076	0.028	0.046	0.027	0.031
480	0.126	0.031	0.041	0.079	0.027	0.046	0.027	0.031
490	0.118	0.031	0.041	0.073	0.027	0.044	0.027	0.03
500	0.111	0.031	0.04	0.075	0.027	0.045	0.027	0.031

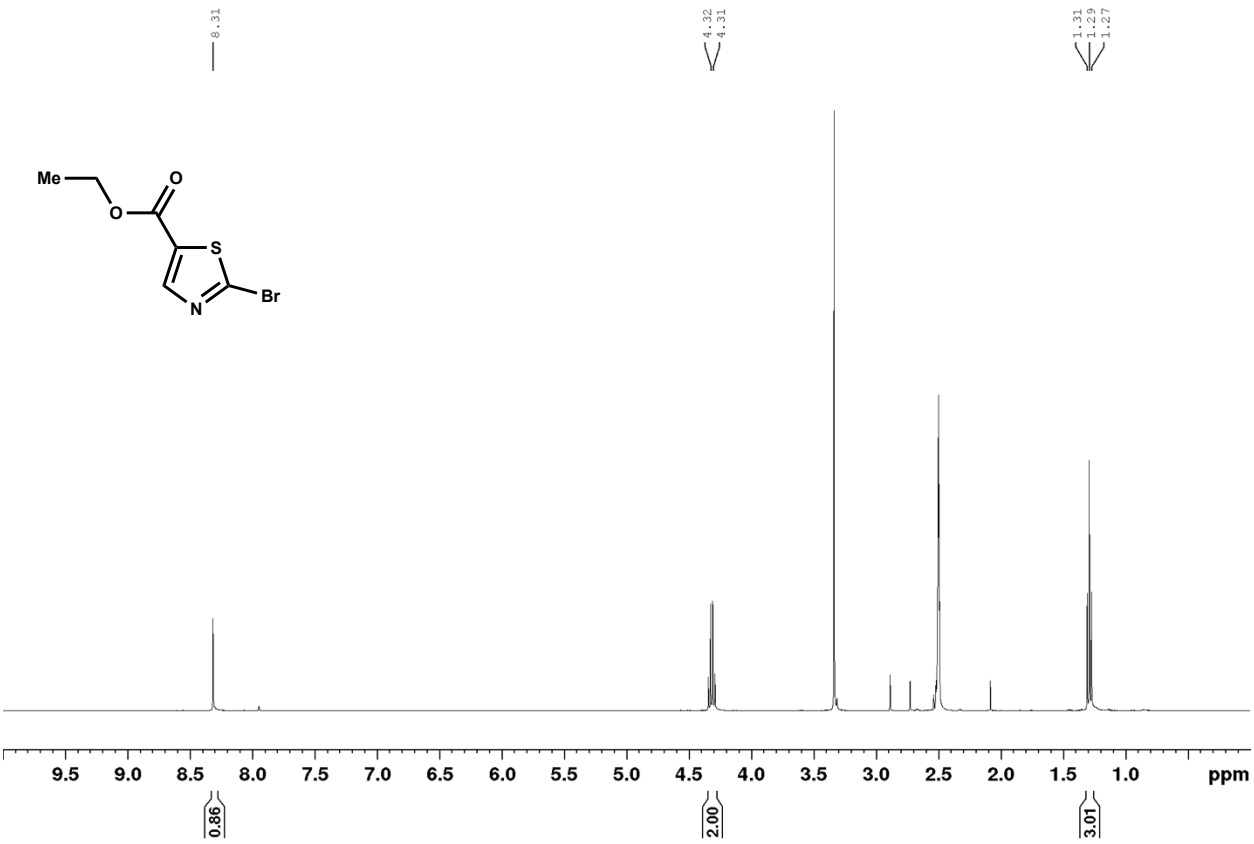
Wavelength/Well	C9	C10	C11	C12	D1	D2	D3	D4
230	1.413	1.837	1.418	1.349	1.628	1.572	1.637	2.542
240	0.334	0.481	0.299	0.15	0.481	0.144	0.332	1.433
250	0.28	0.331	0.241	0.095	0.418	0.077	0.26	1.386
260	0.292	0.285	0.236	0.085	0.412	0.064	0.24	1.417
270	0.287	0.264	0.222	0.085	0.364	0.062	0.229	1.384
280	0.217	0.268	0.173	0.085	0.288	0.053	0.211	1.289
290	0.118	0.261	0.112	0.078	0.295	0.038	0.158	1.037
300	0.065	0.211	0.073	0.06	0.223	0.034	0.124	0.85
310	0.049	0.13	0.059	0.047	0.17	0.031	0.108	0.66
320	0.044	0.065	0.052	0.04	0.125	0.027	0.093	0.567
330	0.042	0.04	0.051	0.037	0.12	0.025	0.086	0.522
340	0.04	0.034	0.051	0.034	0.108	0.024	0.08	0.475
350	0.038	0.031	0.053	0.033	0.11	0.023	0.078	0.447
360	0.037	0.029	0.053	0.031	0.11	0.022	0.075	0.424
370	0.036	0.029	0.05	0.031	0.1	0.021	0.071	0.389
380	0.045	0.039	0.054	0.04	0.093	0.031	0.076	0.37
390	0.034	0.028	0.042	0.029	0.083	0.021	0.065	0.342
400	0.033	0.028	0.041	0.029	0.077	0.021	0.062	0.317
410	0.032	0.027	0.04	0.029	0.075	0.021	0.059	0.298
420	0.032	0.027	0.039	0.029	0.072	0.021	0.058	0.282
430	0.032	0.027	0.038	0.028	0.066	0.021	0.055	0.265
440	0.031	0.027	0.037	0.028	0.064	0.022	0.054	0.252
450	0.031	0.027	0.036	0.028	0.062	0.021	0.052	0.238
460	0.03	0.026	0.036	0.028	0.059	0.021	0.05	0.227
470	0.03	0.026	0.035	0.028	0.058	0.021	0.049	0.214
480	0.03	0.026	0.035	0.028	0.057	0.021	0.047	0.204
490	0.03	0.026	0.034	0.027	0.053	0.021	0.046	0.195
500	0.03	0.026	0.034	0.028	0.054	0.021	0.045	0.186

Wavelength/Well	D5	D6	D7	D8	D9	D10
230	1.603	1.828	1.936	2.232	1.801	1.493
240	0.393	0.713	0.361	0.586	0.368	0.122
250	0.42	0.715	0.343	0.411	0.373	0.06
260	0.402	0.732	0.568	0.525	0.519	0.042
270	0.26	0.639	0.773	0.676	0.668	0.032
280	0.135	0.467	0.649	0.704	0.718	0.028
290	0.09	0.392	0.221	0.548	0.621	0.026
300	0.068	0.341	0.074	0.327	0.462	0.025
310	0.05	0.3	0.071	0.162	0.31	0.024
320	0.044	0.247	0.057	0.079	0.178	0.023
330	0.038	0.247	0.04	0.046	0.103	0.022
340	0.034	0.221	0.029	0.032	0.068	0.021
350	0.032	0.225	0.026	0.029	0.052	0.02
360	0.03	0.228	0.024	0.027	0.041	0.018
370	0.03	0.203	0.024	0.026	0.033	0.018
380	0.04	0.184	0.033	0.035	0.038	0.028
390	0.029	0.179	0.023	0.025	0.025	0.018
400	0.029	0.165	0.024	0.025	0.024	0.019
410	0.028	0.161	0.024	0.025	0.023	0.019
420	0.028	0.154	0.024	0.025	0.023	0.019
430	0.028	0.141	0.024	0.025	0.023	0.019
440	0.028	0.136	0.024	0.025	0.023	0.019
450	0.027	0.13	0.024	0.025	0.023	0.019
460	0.028	0.125	0.024	0.025	0.023	0.019
470	0.027	0.12	0.024	0.025	0.023	0.019
480	0.027	0.116	0.023	0.025	0.023	0.019
490	0.027	0.108	0.023	0.024	0.023	0.019
500	0.027	0.108	0.023	0.024	0.023	0.02

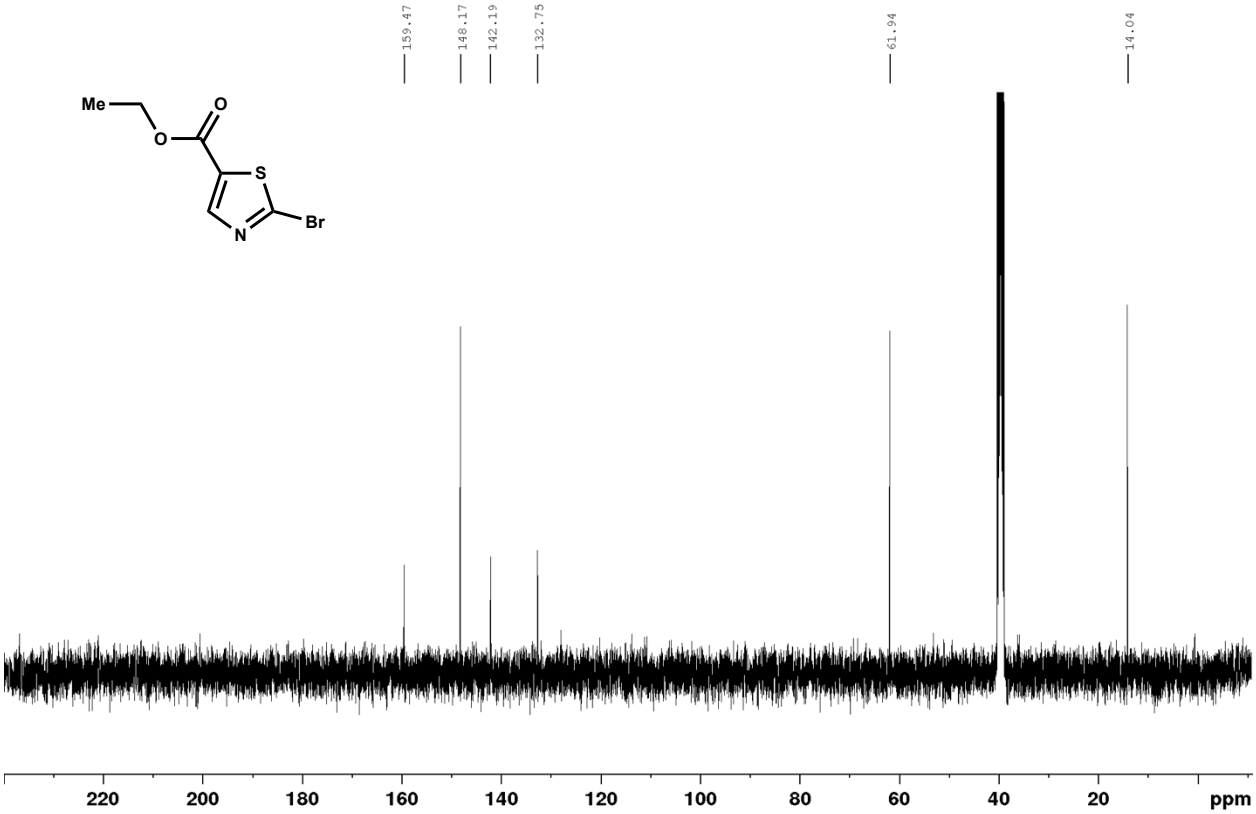
7. Product Characterization Data

7.1 NMR Spectra / LC-MS Analytical Traces

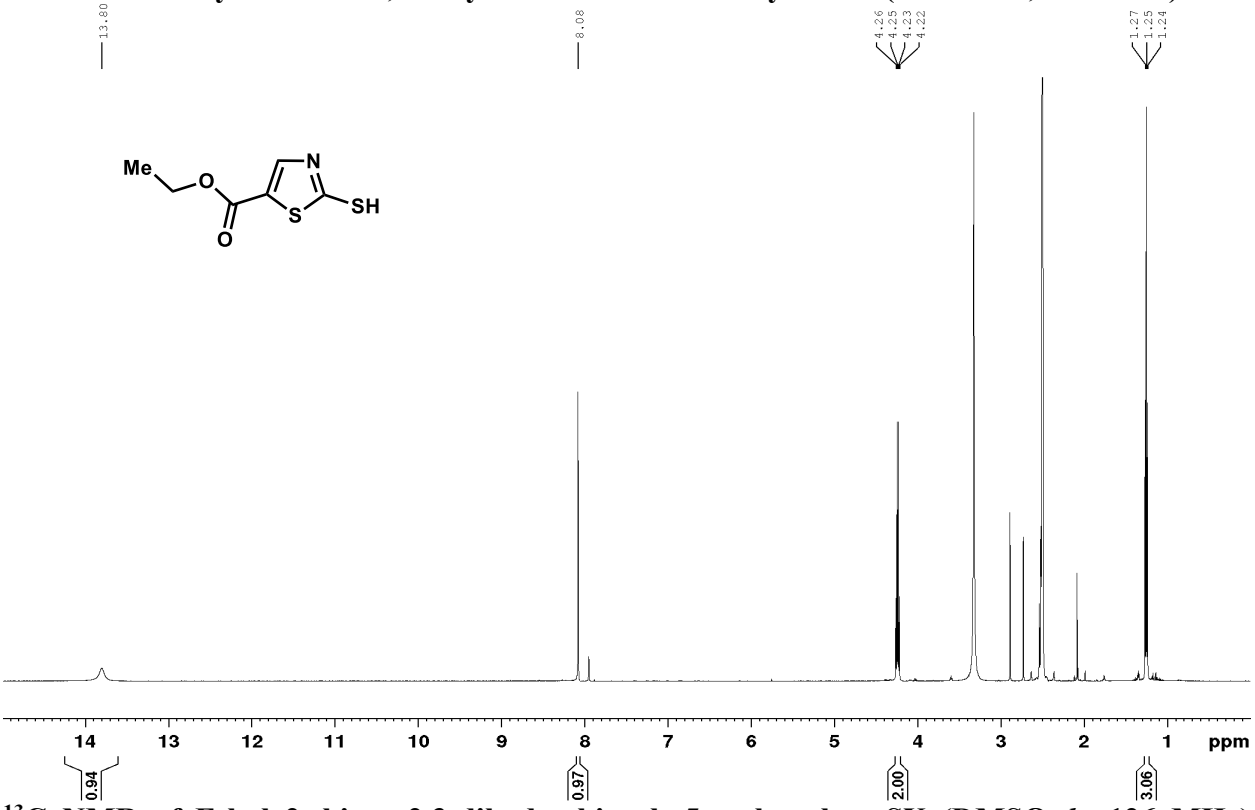
^{1}H NMR of Ethyl 2-bromothiazole-5-carboxylate S8 (DMSO-d_6, 500 MHz)



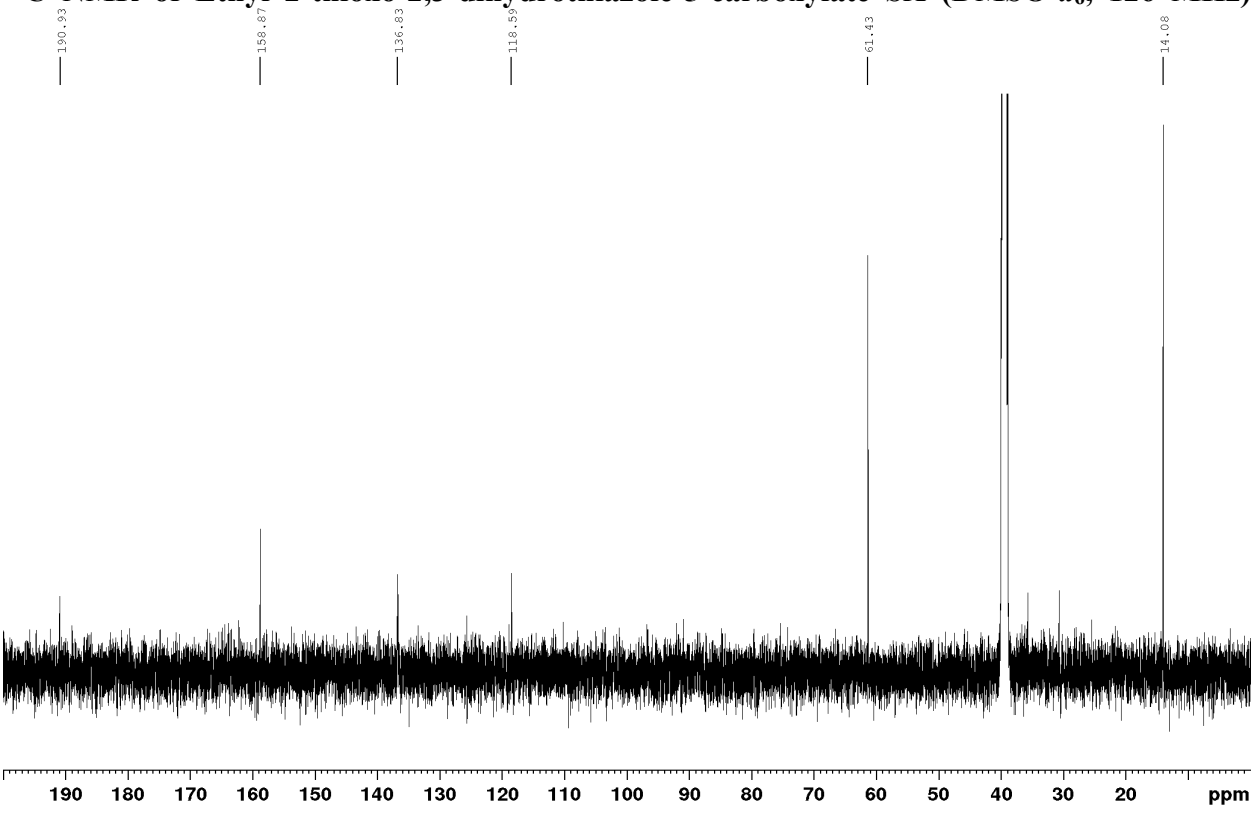
^{13}C NMR of Ethyl 2-bromothiazole-5-carboxylate S8 (DMSO-d_6, 126 MHz)



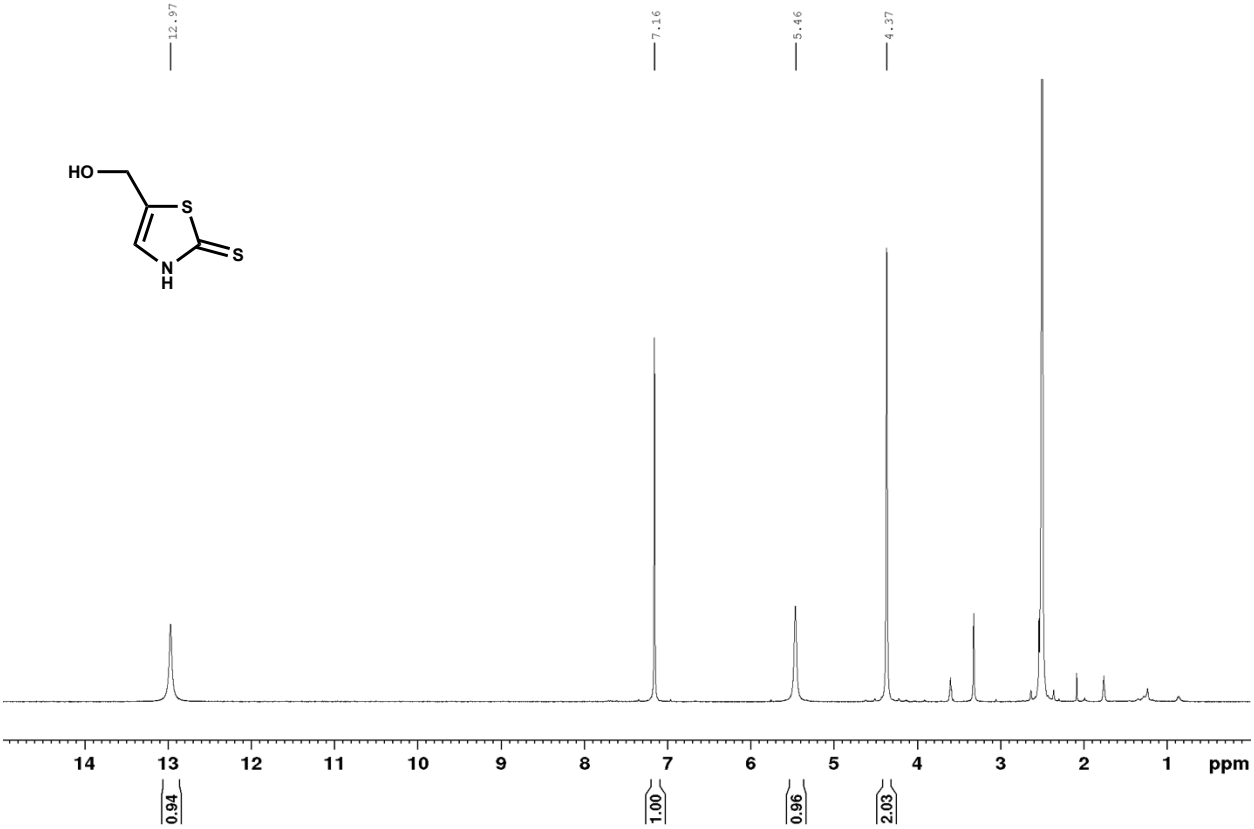
^{1}H NMR of Ethyl 2-thioxo-2,3-dihydrothiazole-5-carboxylate S9 (DMSO-d_6, 500 MHz)



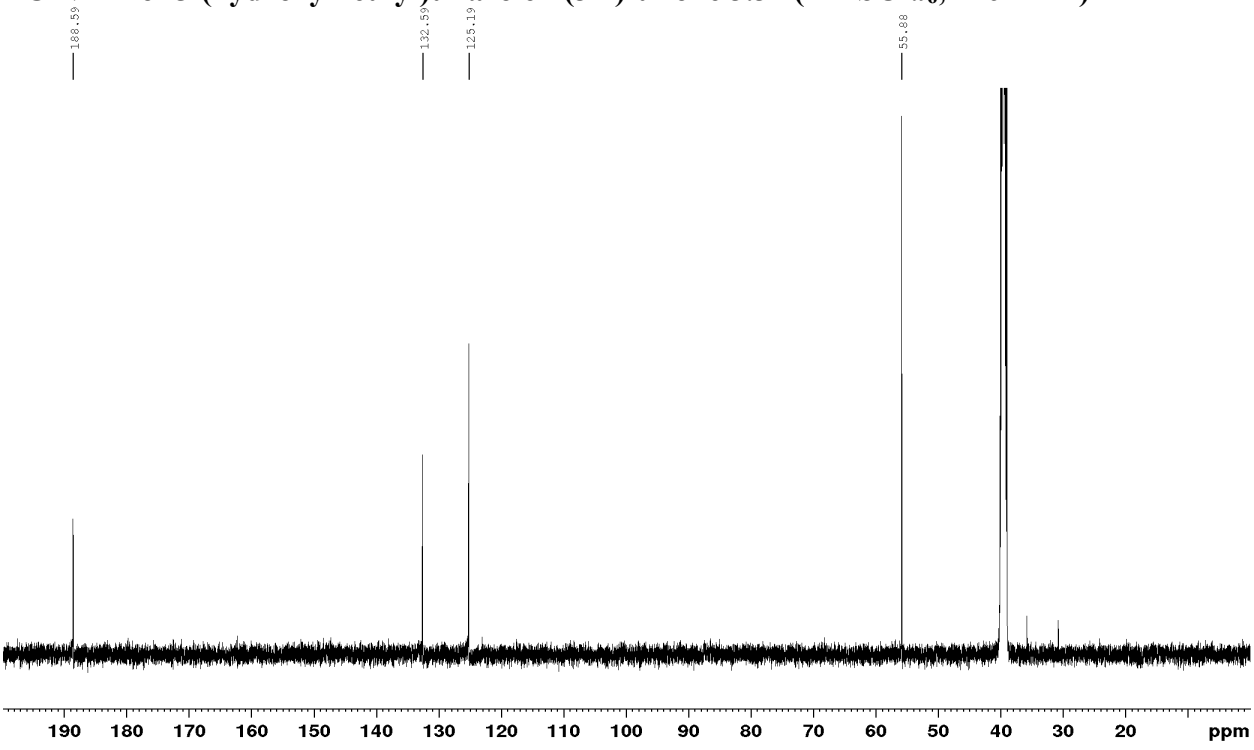
^{13}C NMR of Ethyl 2-thioxo-2,3-dihydrothiazole-5-carboxylate SX (DMSO-d_6, 126 MHz)



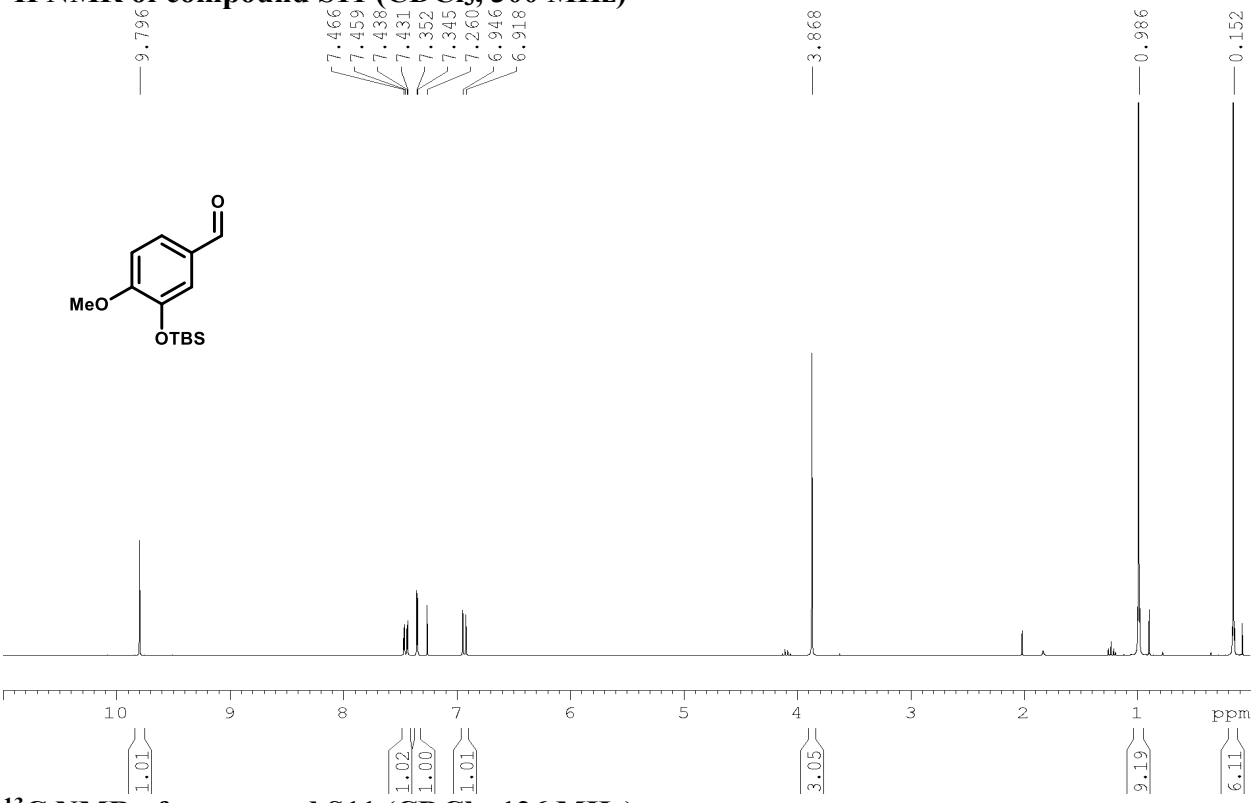
^{1}H NMR of 5-(hydroxymethyl)thiazole-2(3H)-thione 3.32 (DMSO-d_6, 500 MHz)



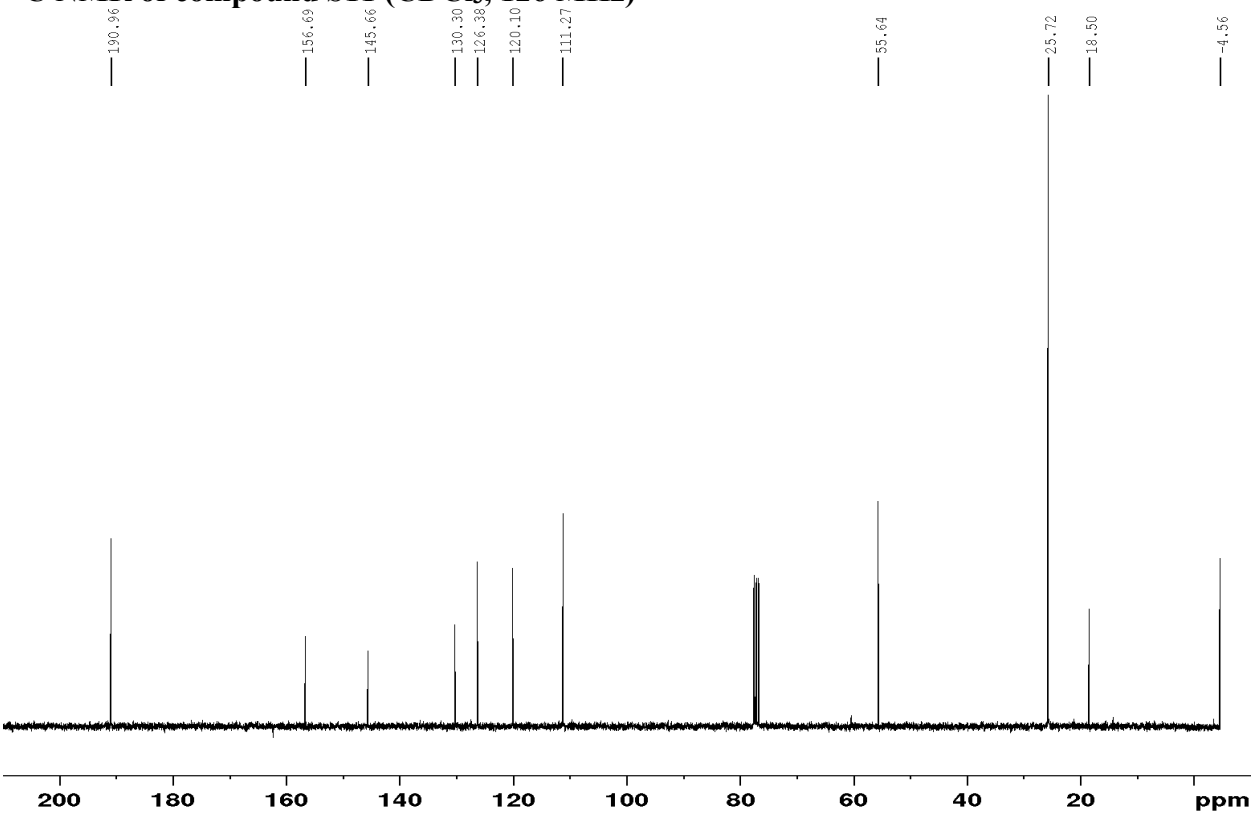
^{13}C NMR of 5-(hydroxymethyl)thiazole-2(3H)-thione 3.32 (DMSO-d_6, 126 MHz)



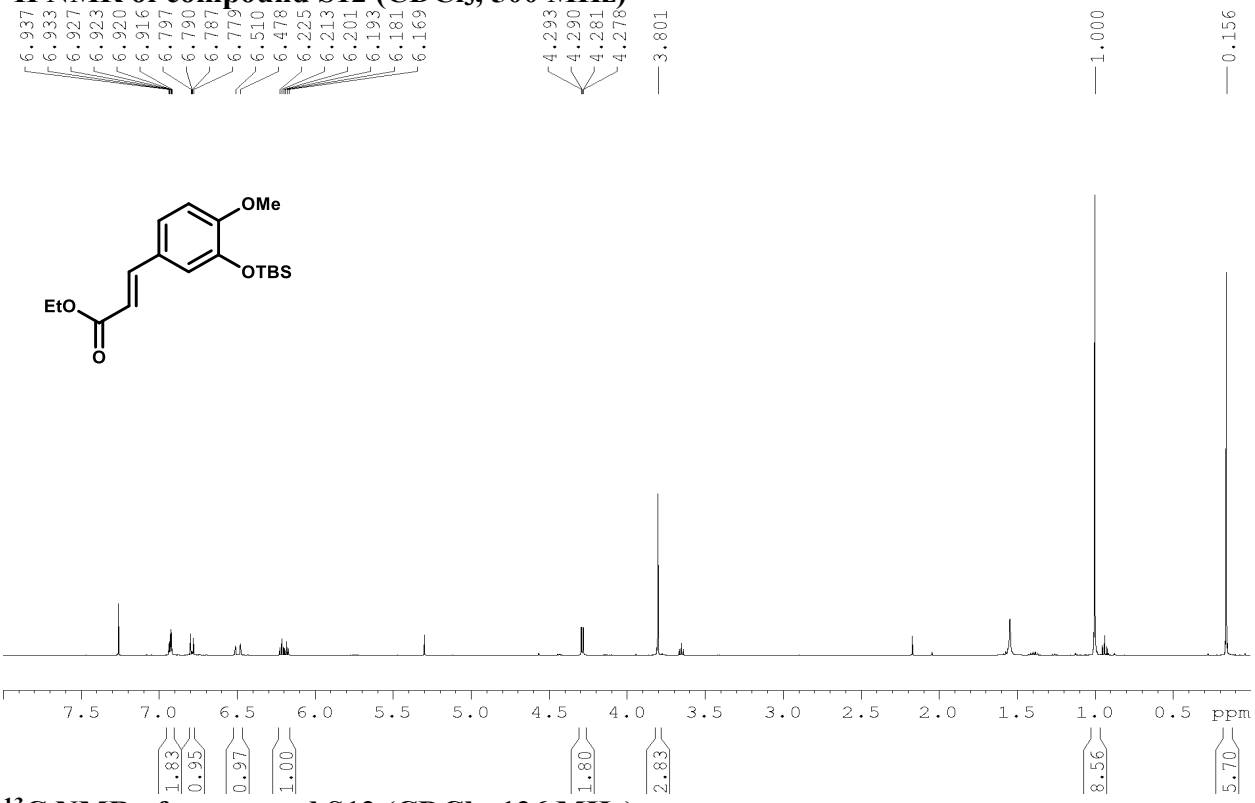
^{1}H NMR of compound S11 ($CDCl_3$, 300 MHz)



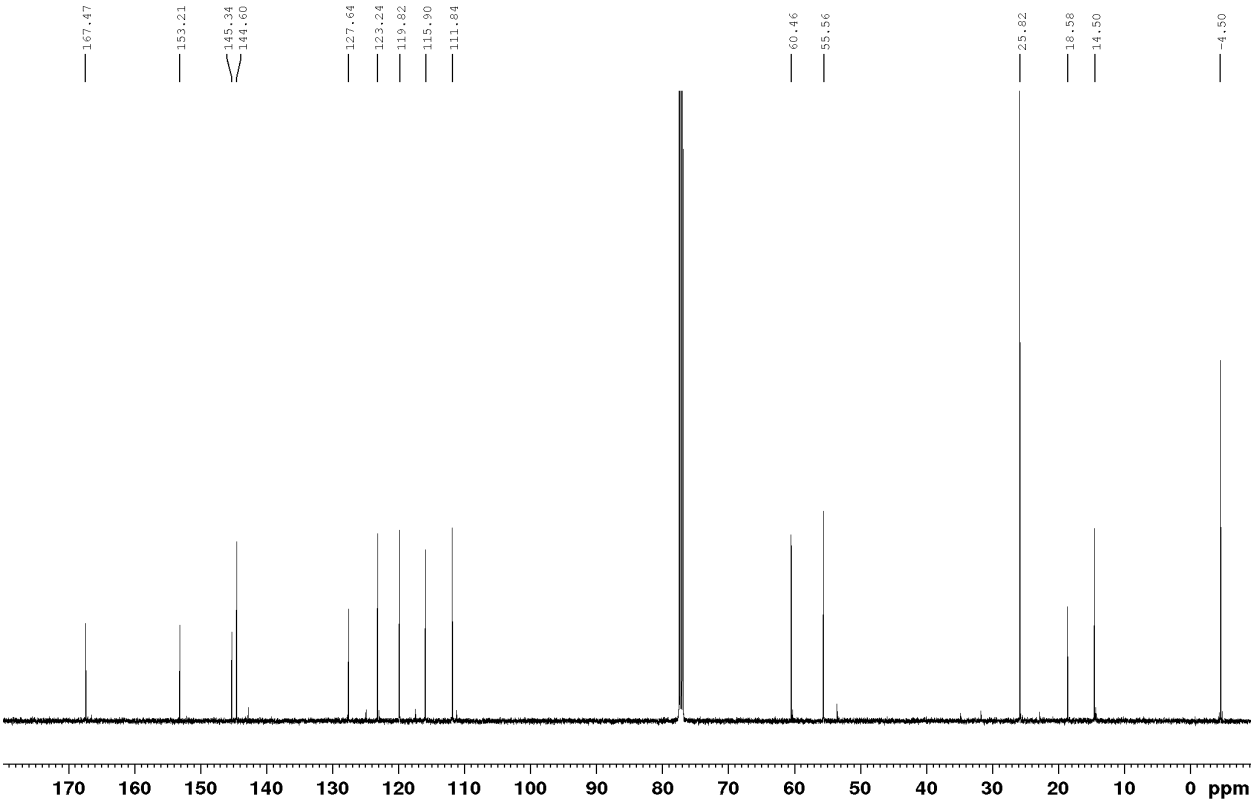
^{13}C NMR of compound S11 ($CDCl_3$, 126 MHz)



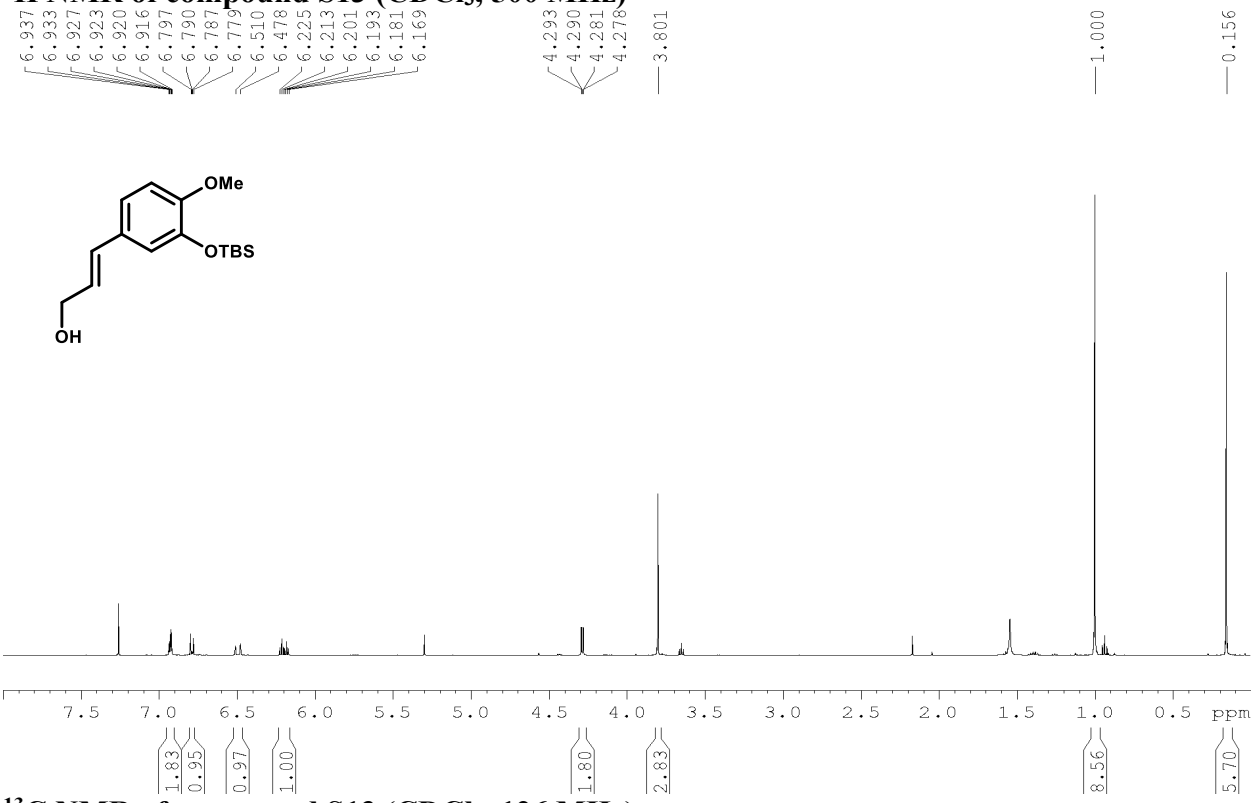
^{1}H NMR of compound S12 ($CDCl_3$, 500 MHz)



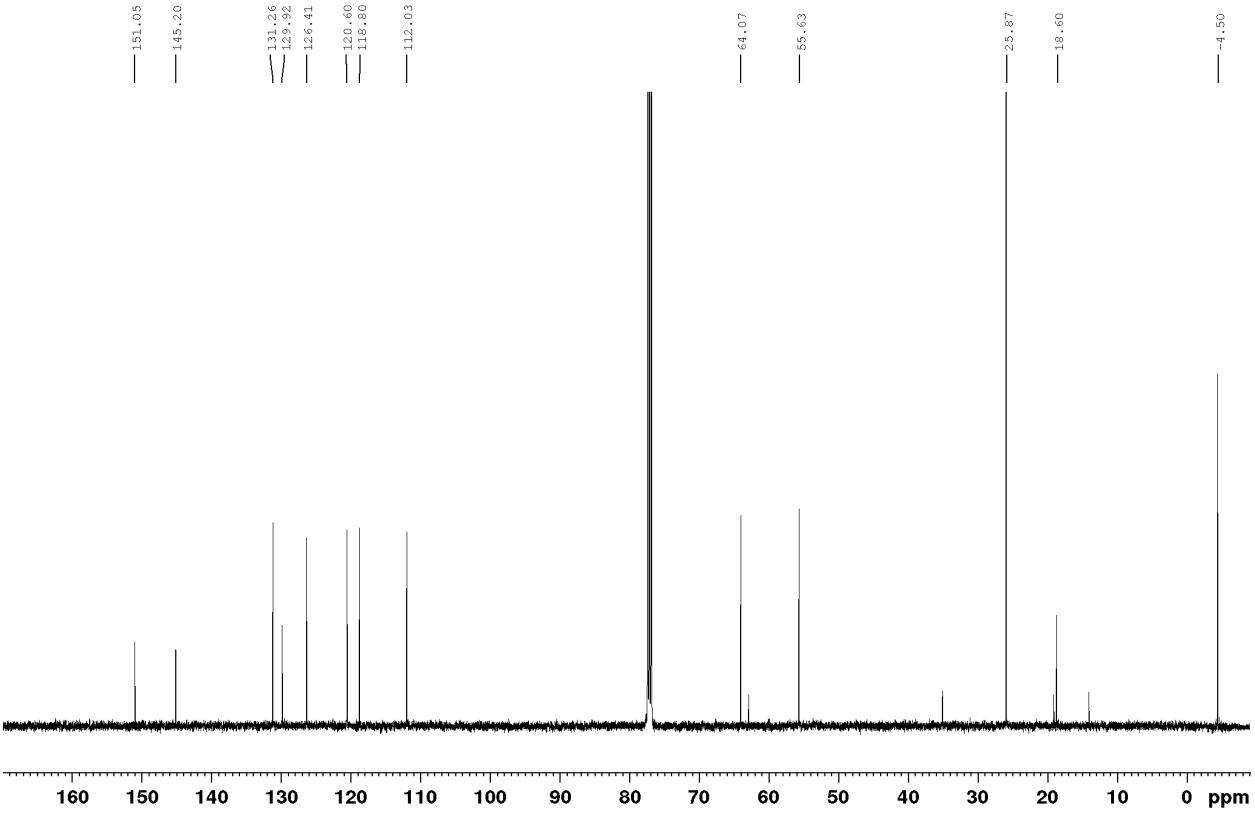
^{13}C NMR of compound S12 ($CDCl_3$, 126 MHz)



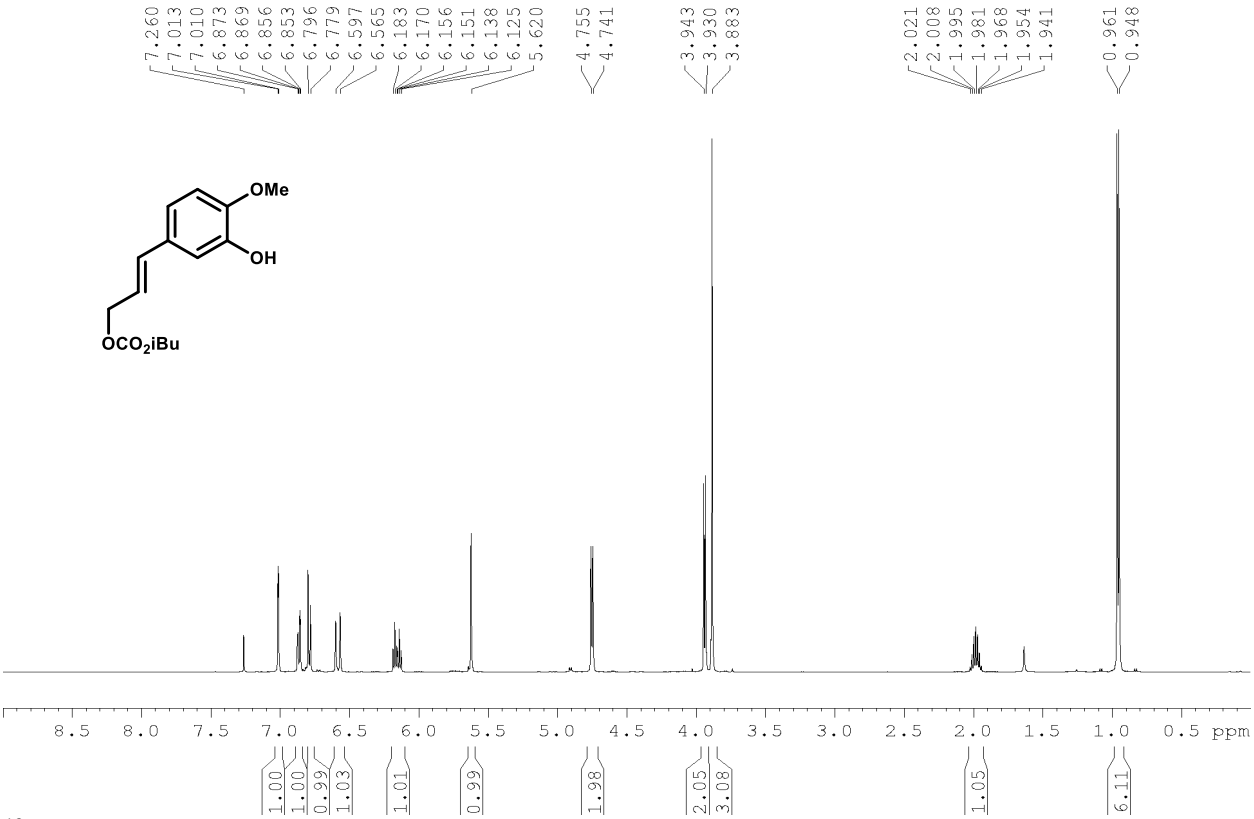
^{1}H NMR of compound S13 ($CDCl_3$, 500 MHz)



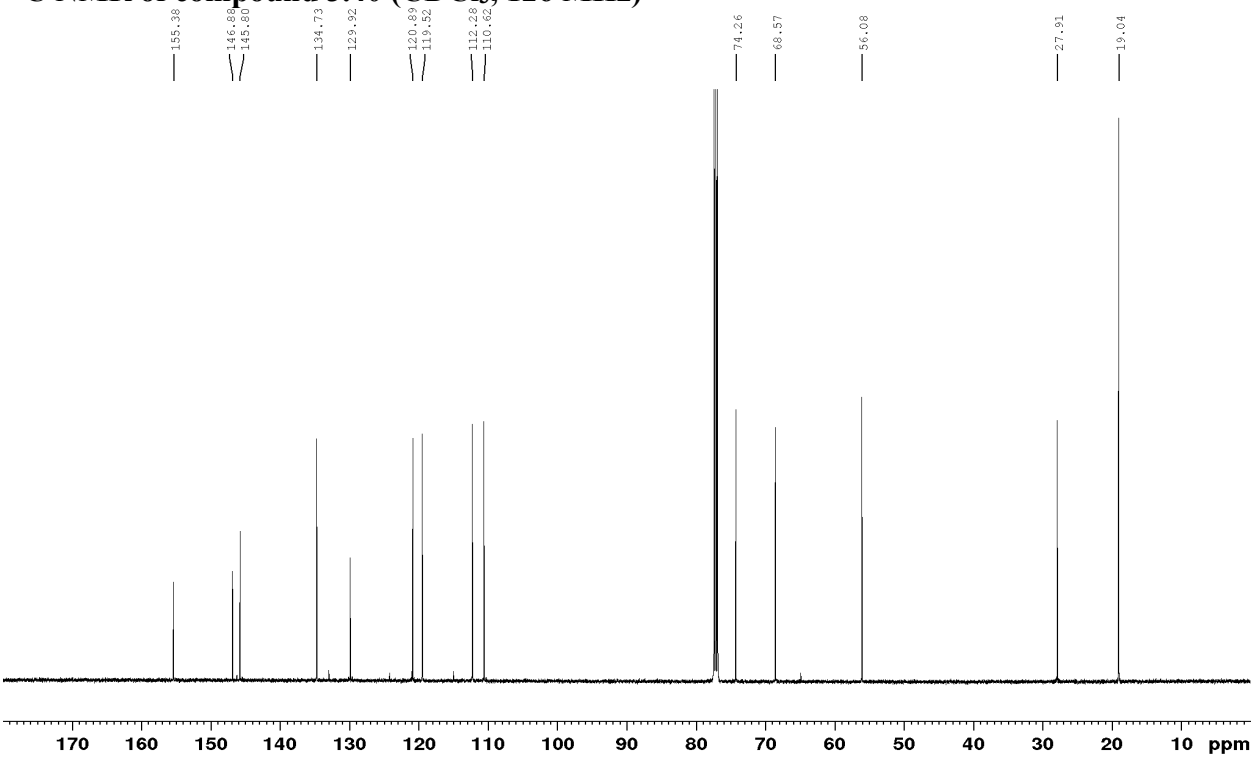
^{13}C NMR of compound S13 ($CDCl_3$, 126 MHz)



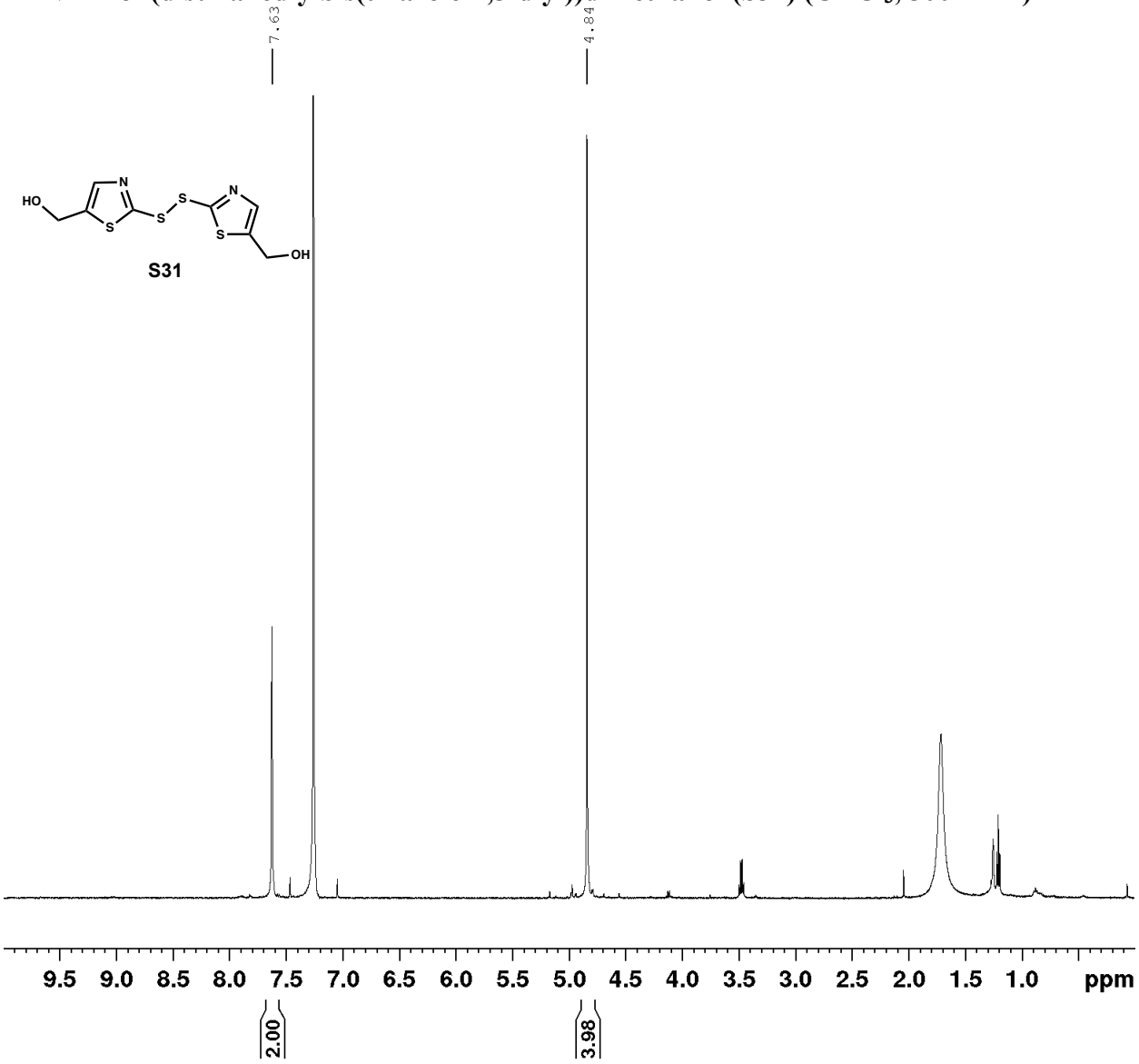
^{1}H NMR of compound 3.40 ($CDCl_3$, 500 MHz)



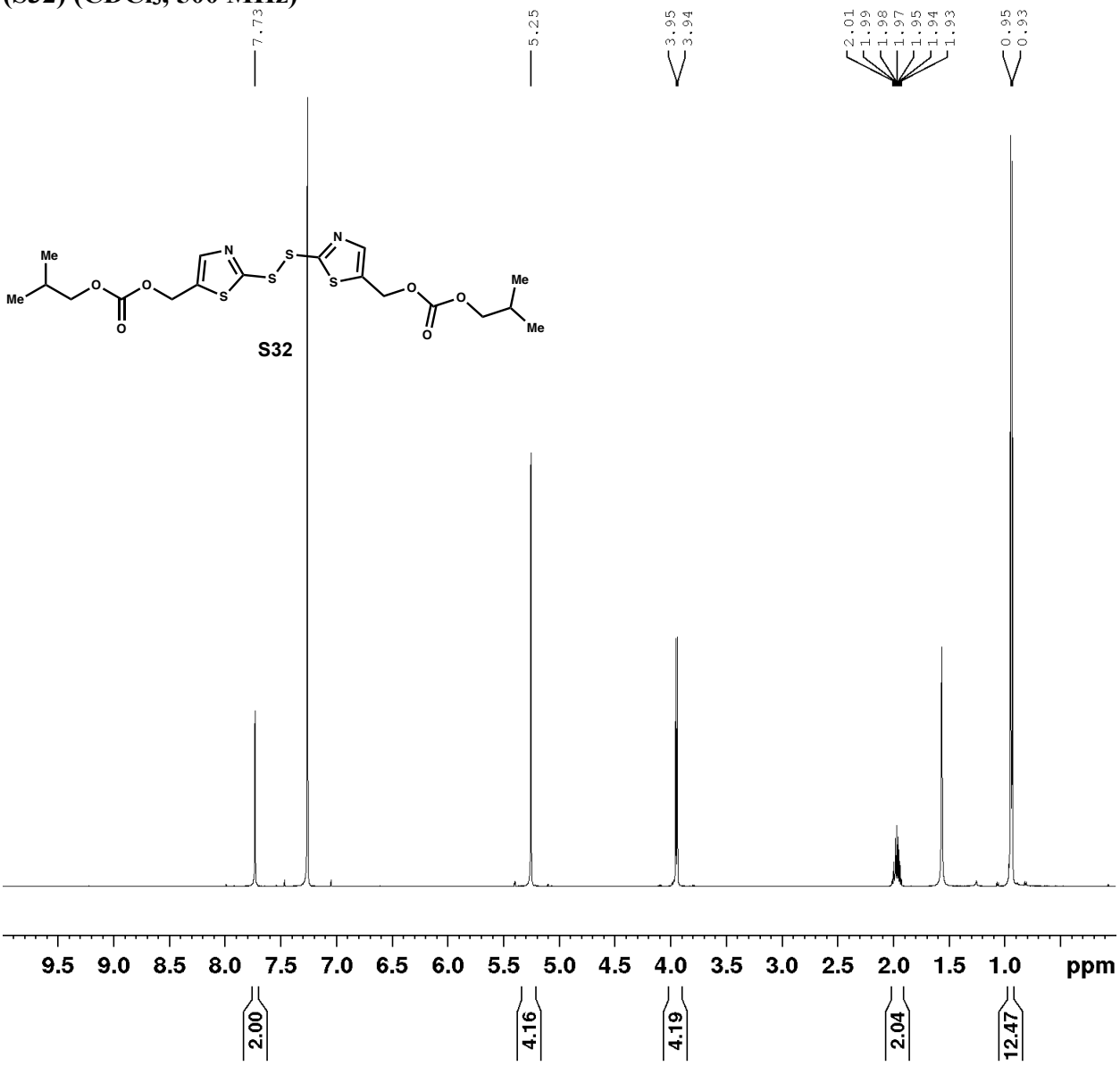
^{13}C NMR of compound 3.40 ($CDCl_3$, 126 MHz)



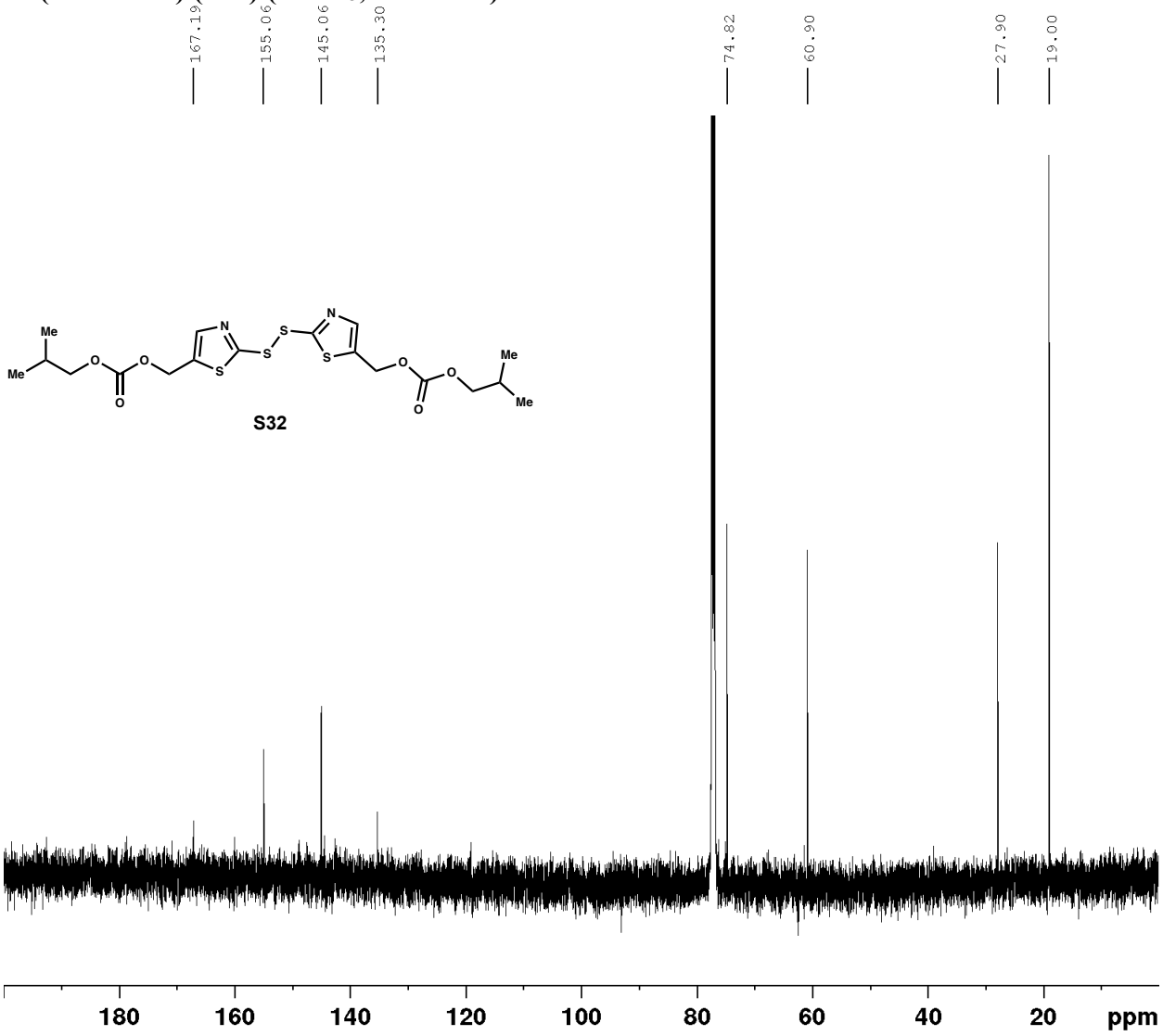
^{1}H NMR of (disulfanediylbis(thiazole-2,5-diyl))dimethanol (S31) ($CDCl_3$, 500 MHz)



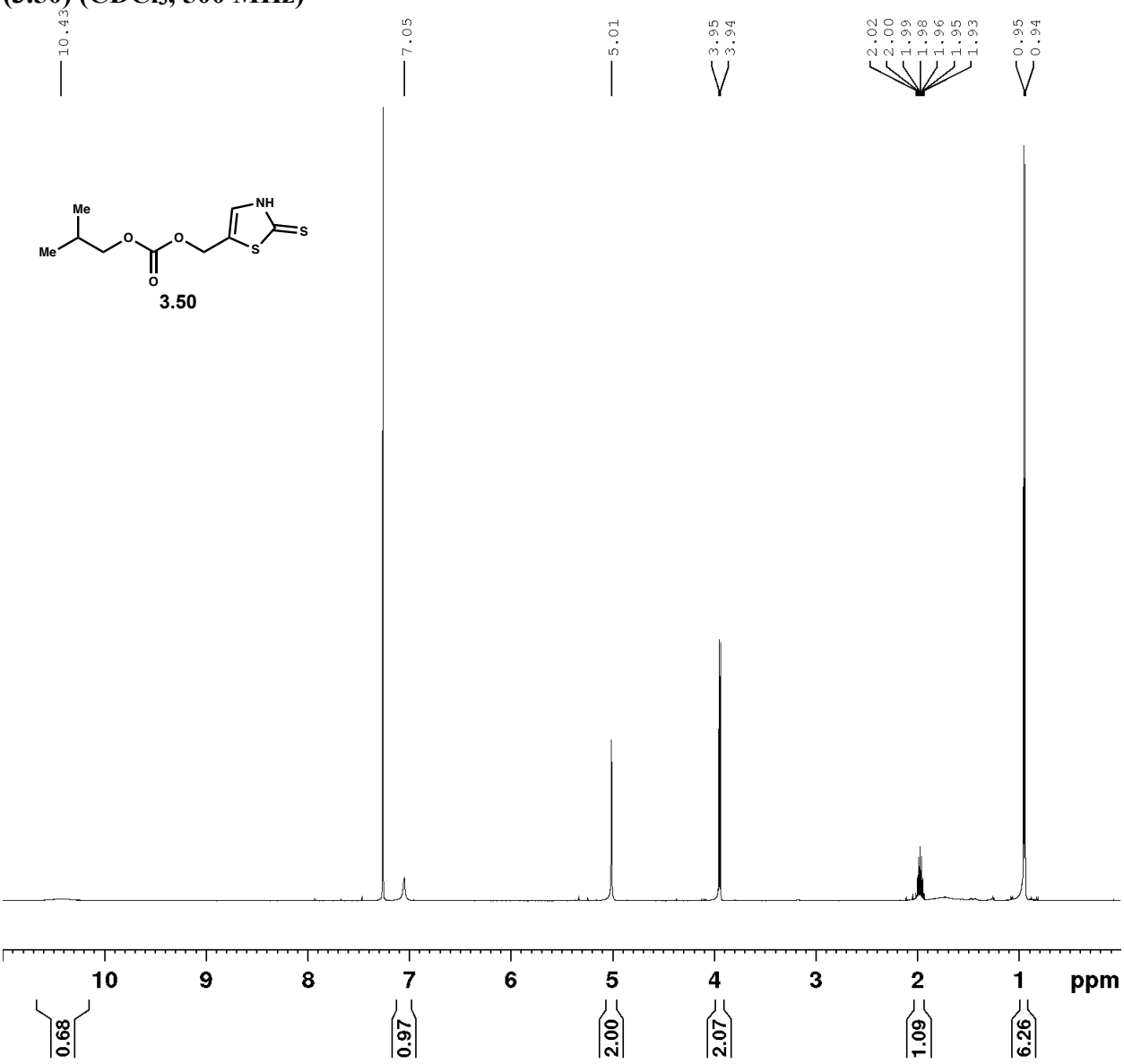
^{1}H NMR of (disulfanediylbis(thiazole-2,5-diyl))bis(methylene) diisobutyl bis(carbonate) (S32) ($CDCl_3$, 500 MHz)



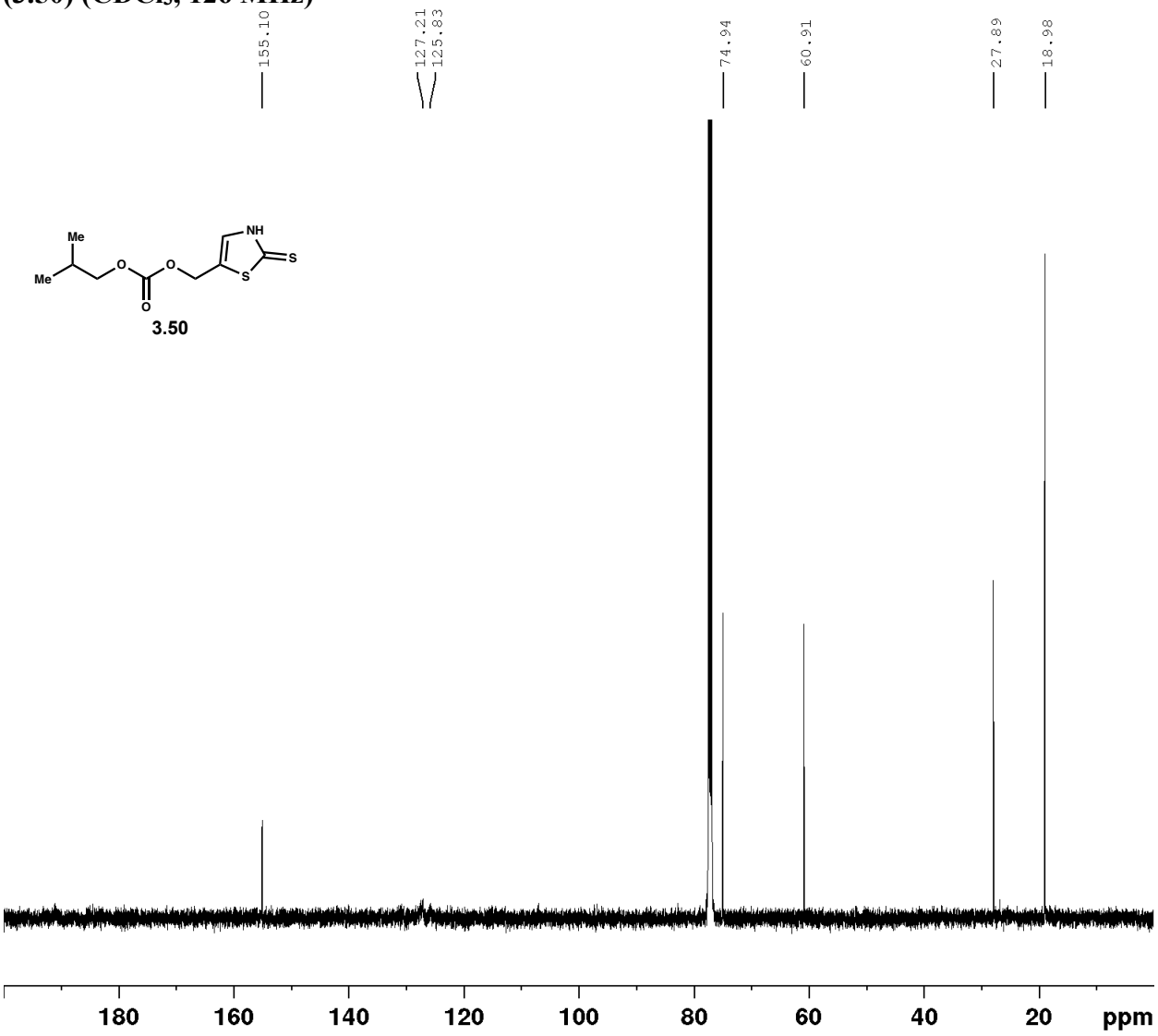
^{13}C NMR spectra of (disulfanediylbis(thiazole-2,5-diyl))bis(methylene) diisobutyl bis(carbonate) (S32) ($CDCl_3$, 126 MHz)



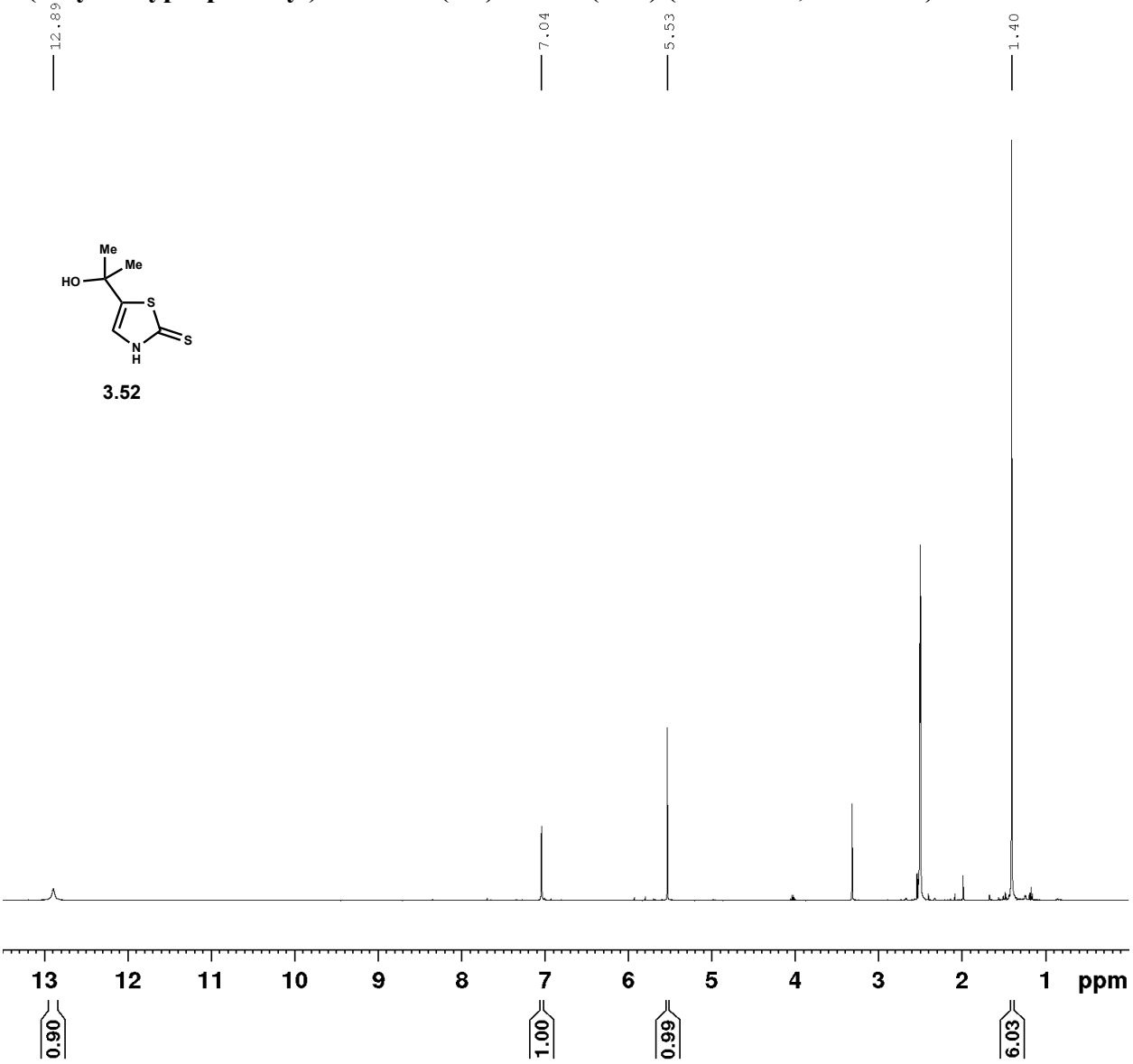
^{1}H NMR of (disulfanediylbis(thiazole-2,5-diyl))bis(methylene) diisobutyl bis(carbonate) (3.50) ($CDCl_3$, 500 MHz)



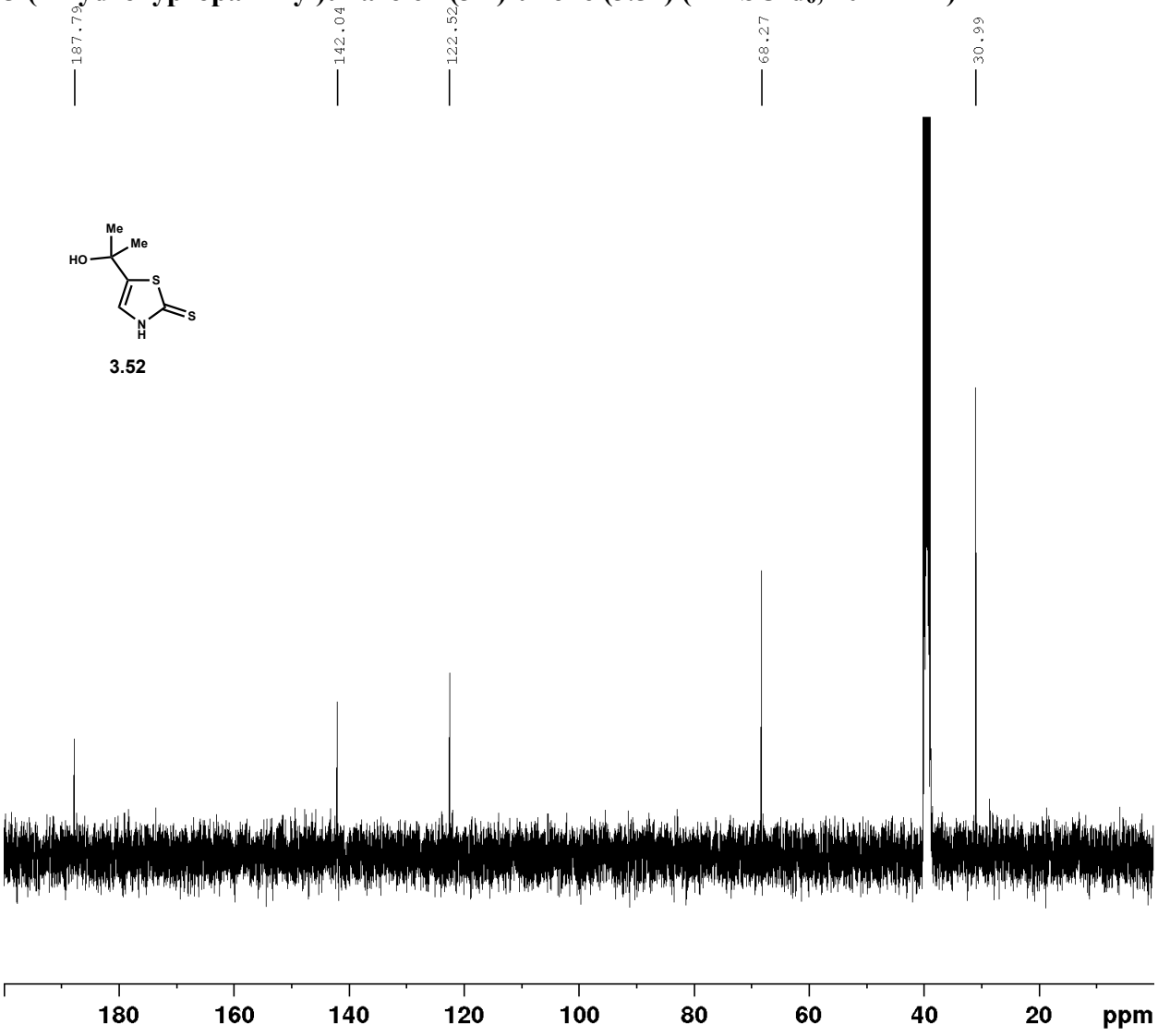
^{13}C NMR of (disulfanediylbis(thiazole-2,5-diyl))bis(methylene) diisobutyl bis(carbonate) (3.50) ($CDCl_3$, 126 MHz)



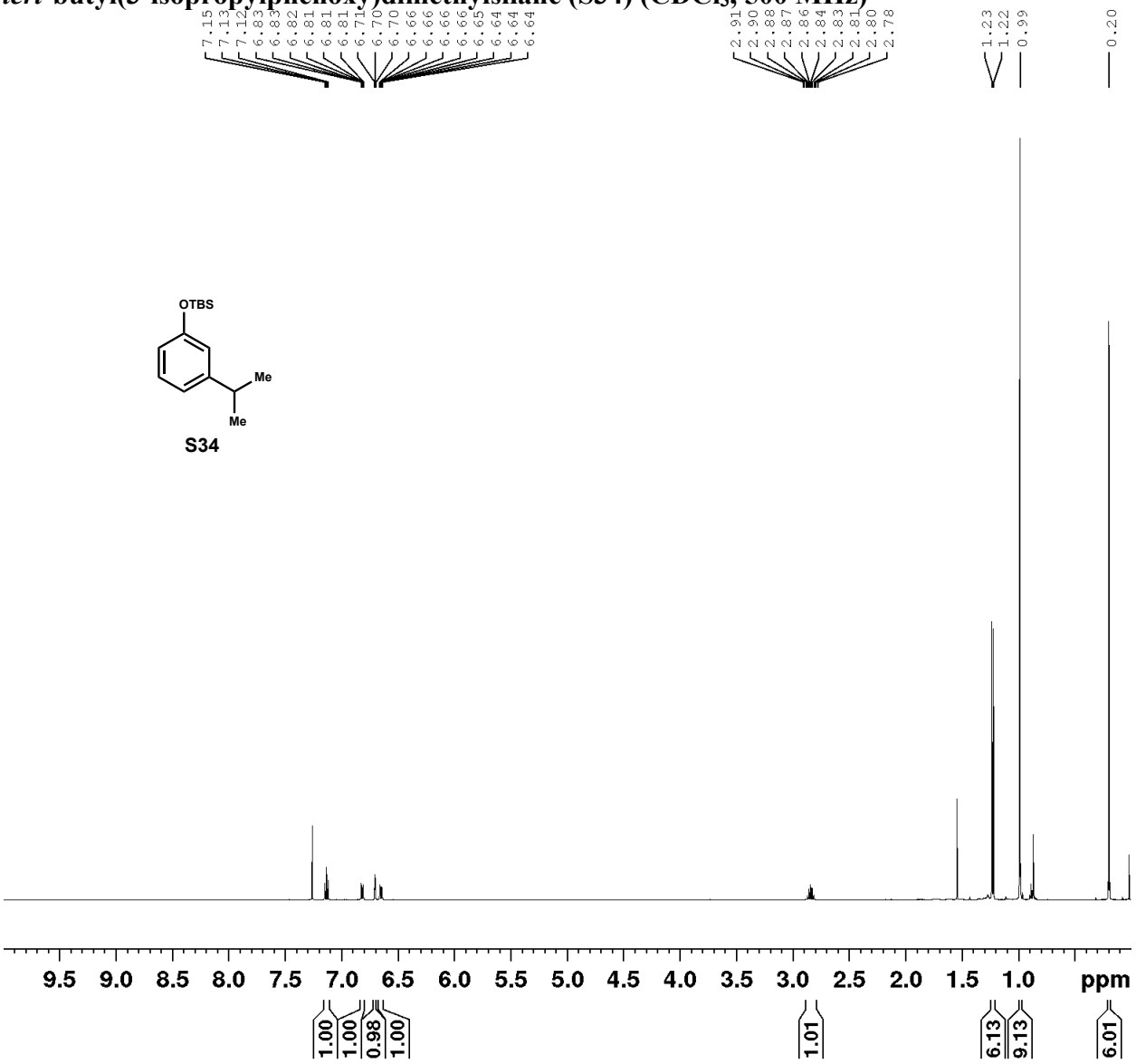
5-(2-hydroxypropan-2-yl)thiazole-2(3*H*)-thione (3.52) (DMSO-d_6, 400 MHz)



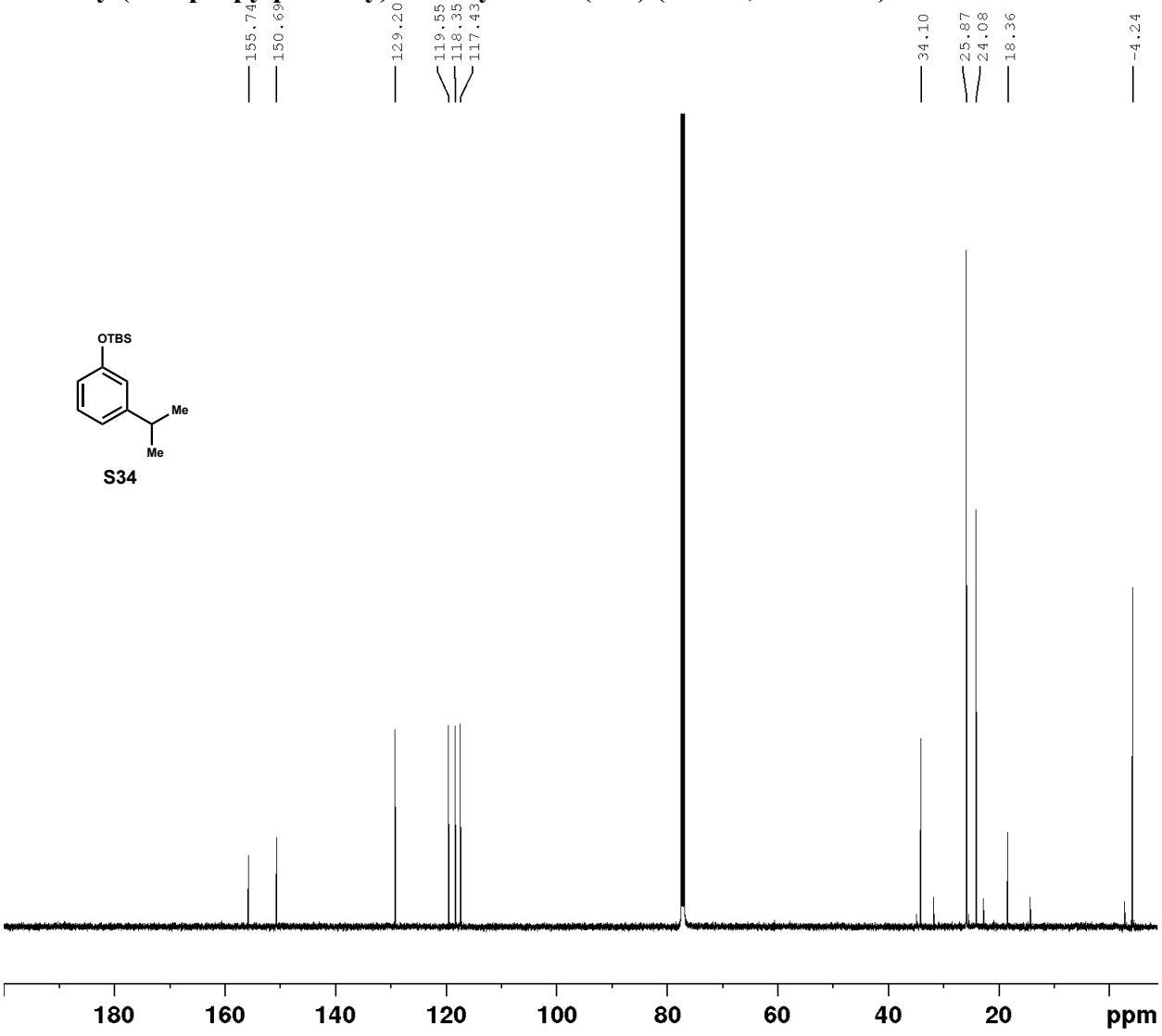
5-(2-hydroxypropan-2-yl)thiazole-2(3*H*)-thione (3.52) (DMSO-d_6, 101 MHz)



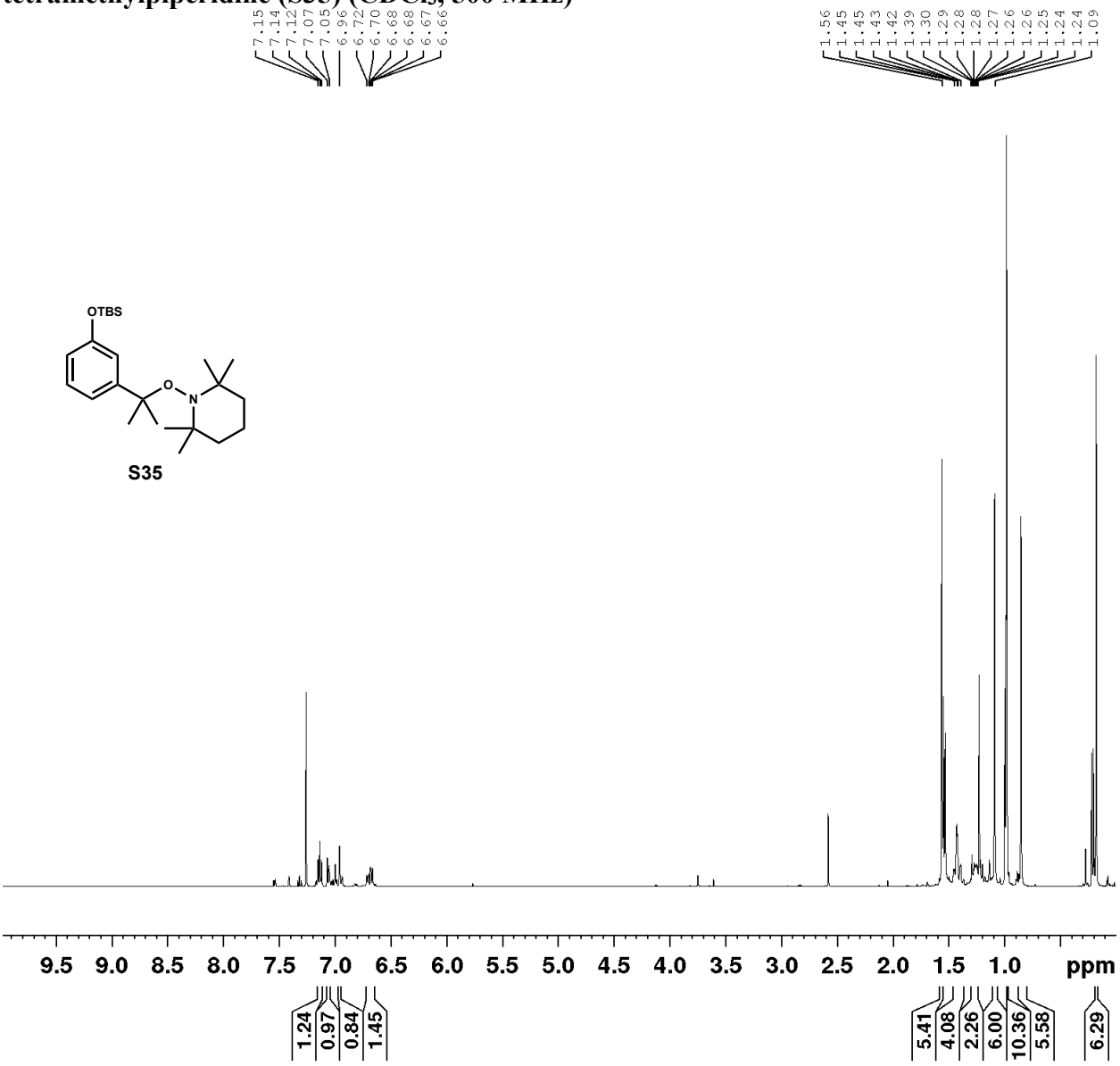
tert-butyl(3-isopropylphenoxy)dimethylsilane (S34) ($CDCl_3$, 500 MHz)



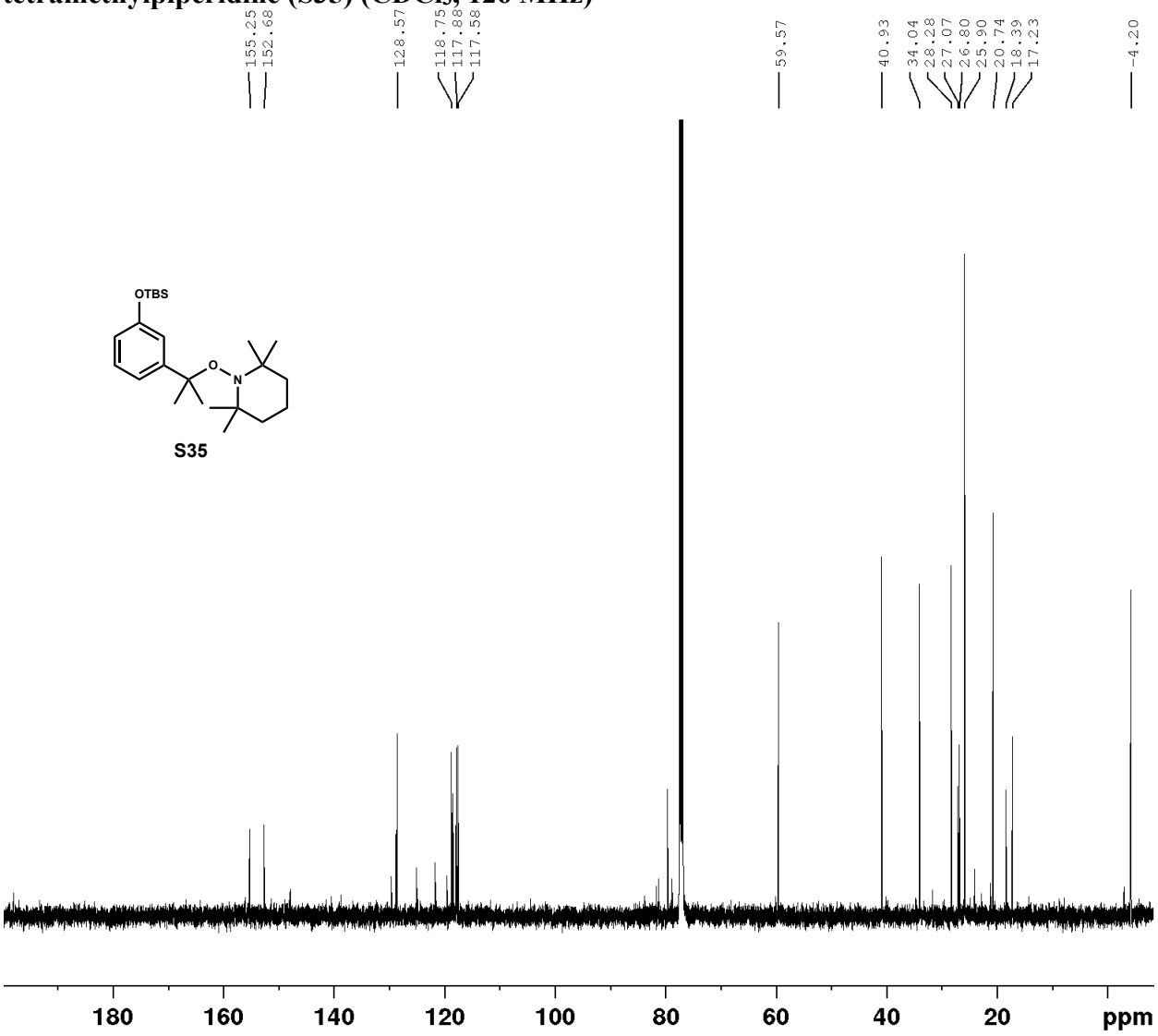
***tert*-butyl(3-isopropylphenoxy)dimethylsilane (S34) ($CDCl_3$, 126 MHz)**



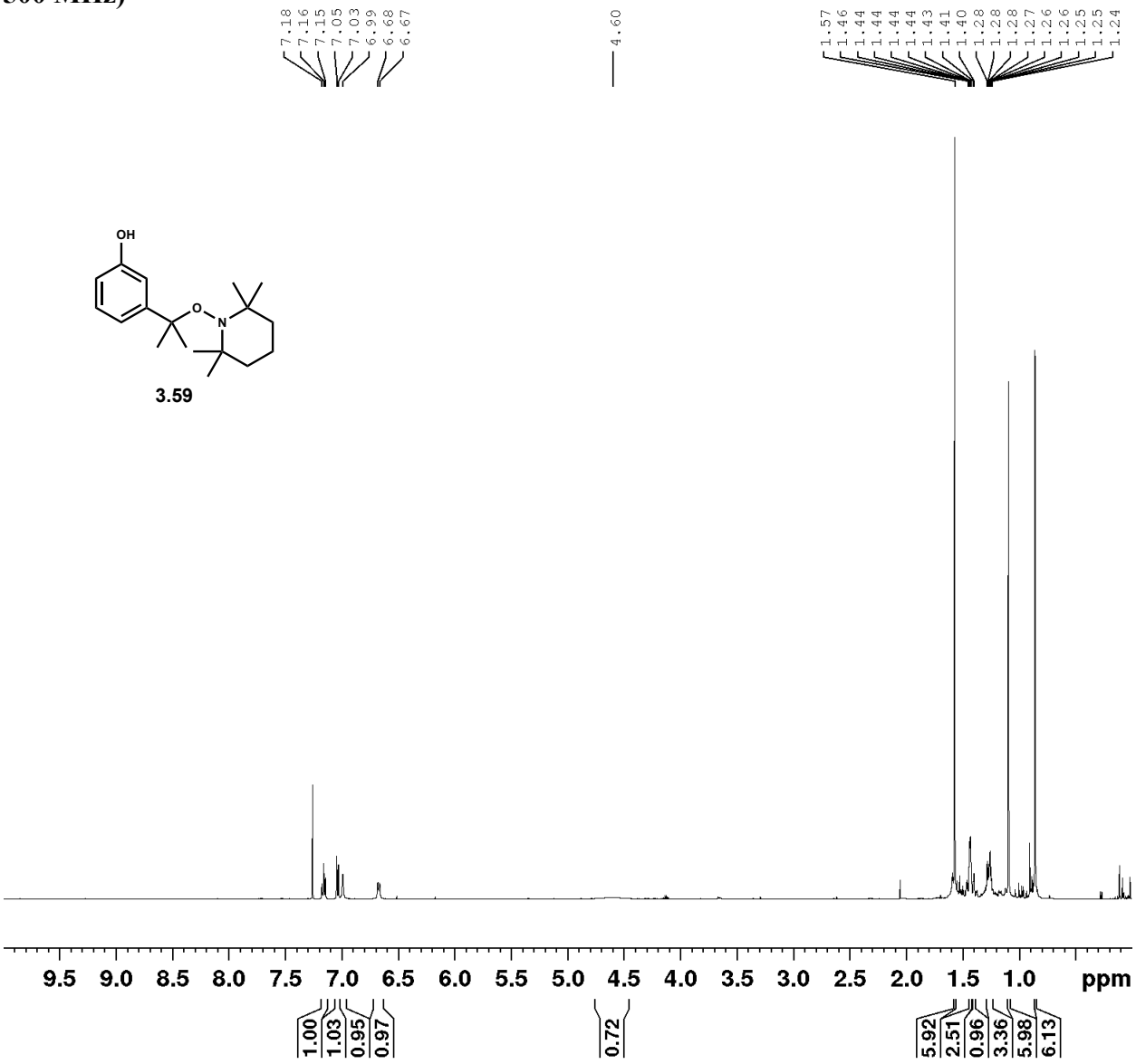
^{1}H NMR of 1-((2-(3-((*tert*-butyldimethylsilyl)oxy)phenyl)propan-2-yl)oxy)-2,2,6,6-tetramethylpiperidine (S35) ($CDCl_3$, 500 MHz)



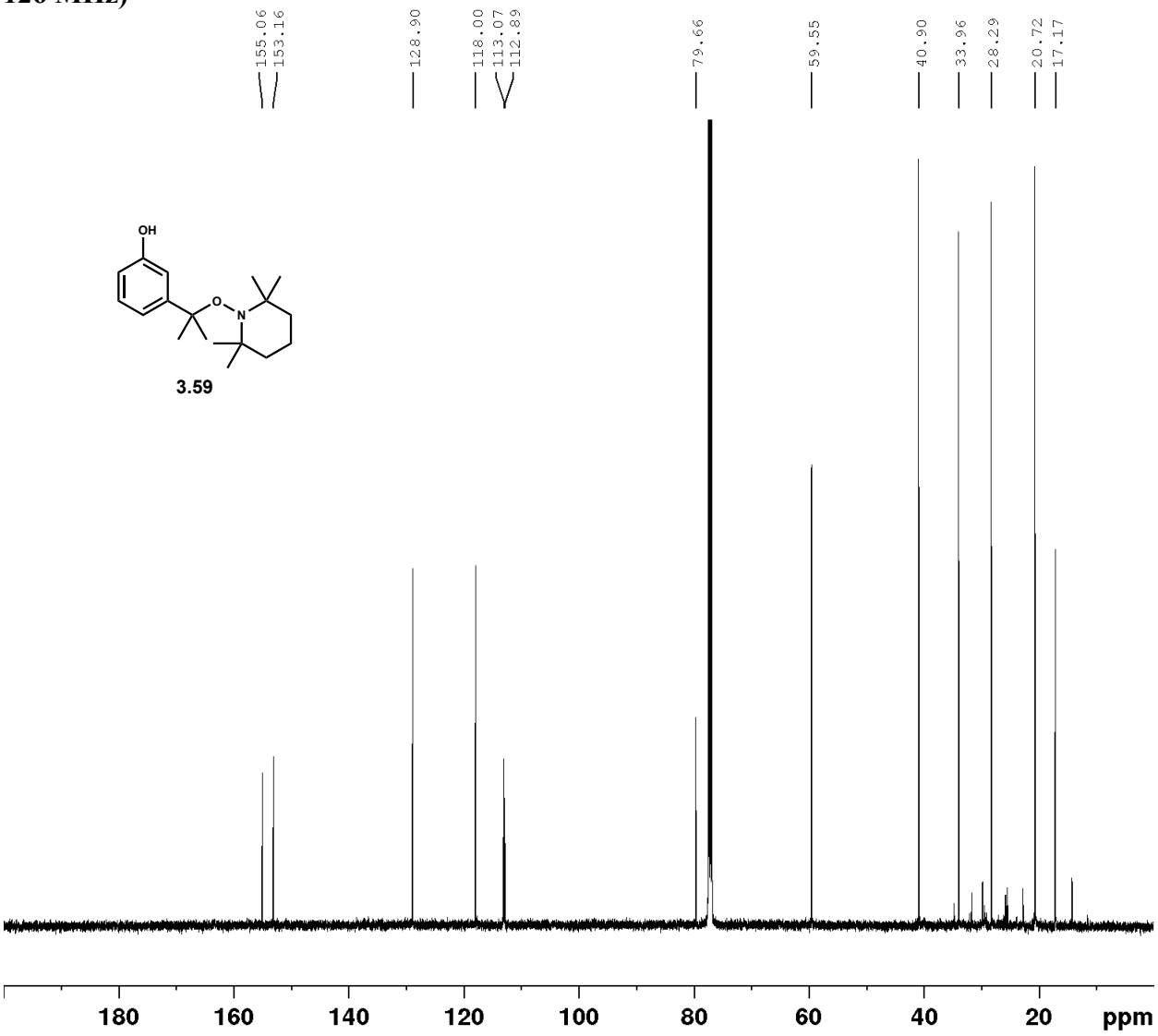
^{13}C NMR of 1-((2-(3-((*tert*-butyldimethylsilyl)oxy)phenyl)propan-2-yl)oxy)-2,2,6,6-tetramethylpiperidine (S35) ($CDCl_3$, 126 MHz)



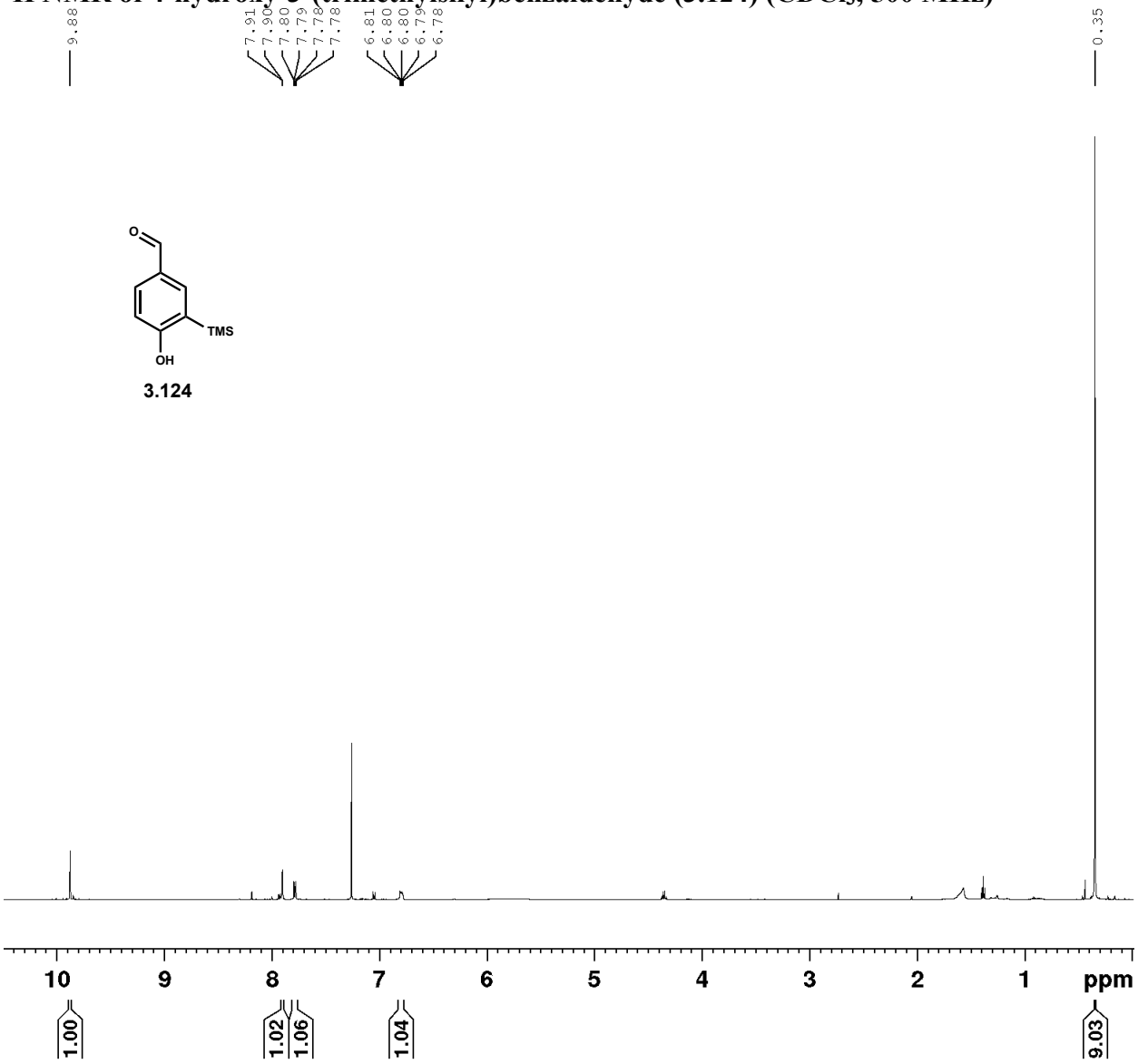
^{1}H NMR of 3-(2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)propan-2-yl)phenol (3.59) ($CDCl_3$, 500 MHz)



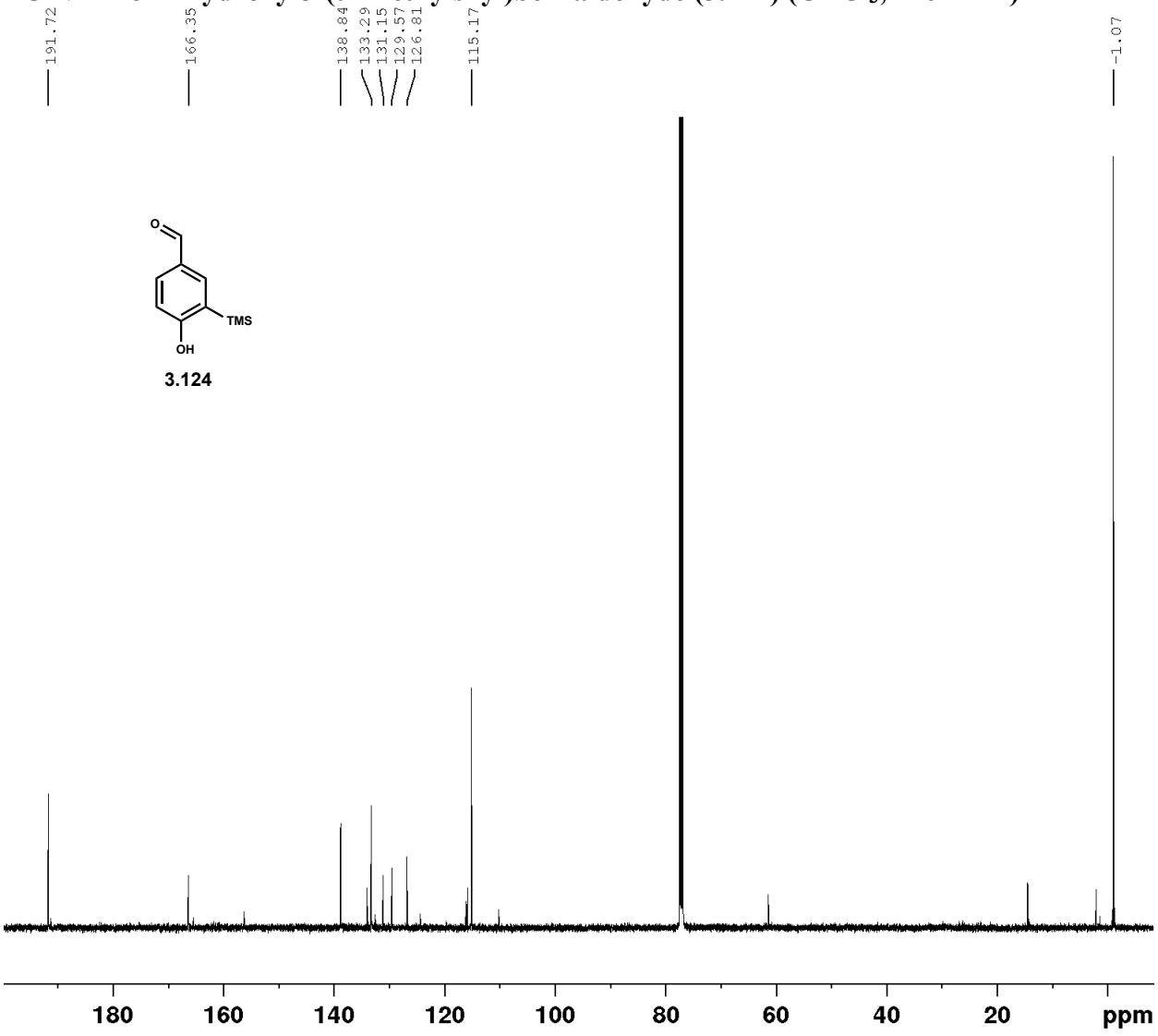
^{13}C NMR of 3-(2-((2,2,6,6-tetramethylpiperidin-1-yl)oxy)propan-2-yl)phenol (3.59) ($CDCl_3$, 126 MHz)



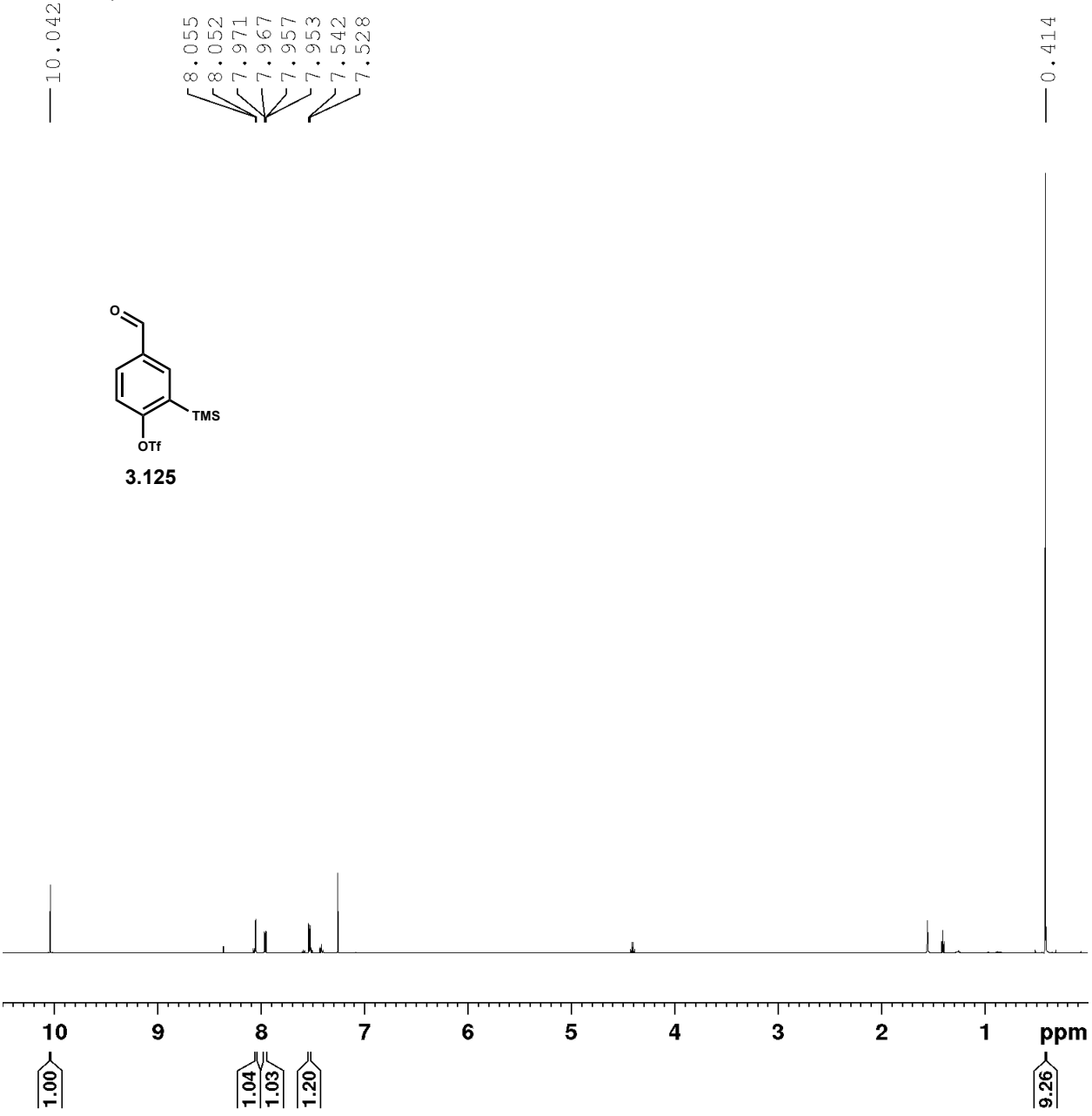
^{1}H NMR of 4-hydroxy-3-(trimethylsilyl)benzaldehyde (3.124) ($CDCl_3$, 500 MHz)



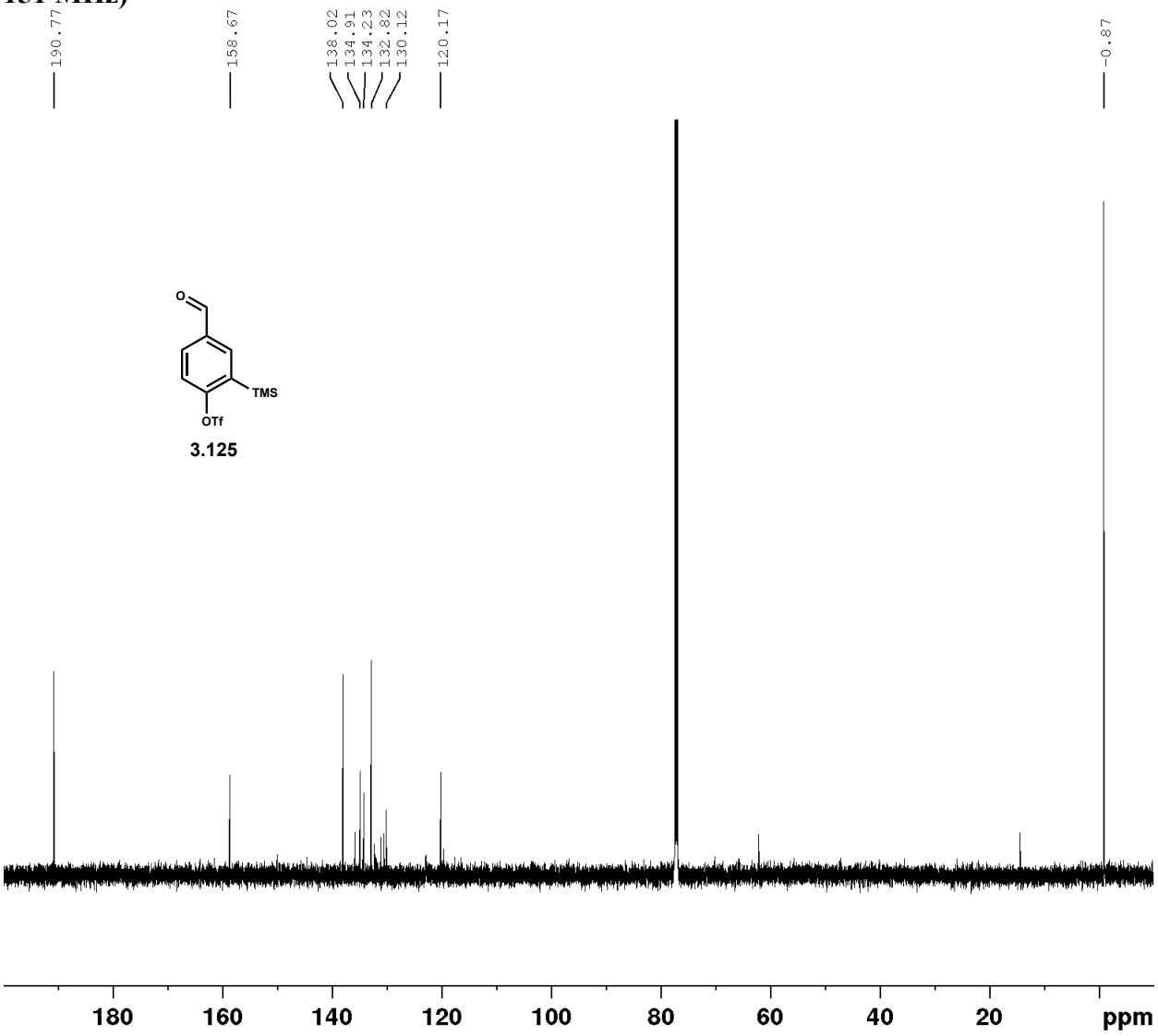
^{13}C NMR of 4-hydroxy-3-(trimethylsilyl)benzaldehyde (3.124) ($CDCl_3$, 126 MHz)



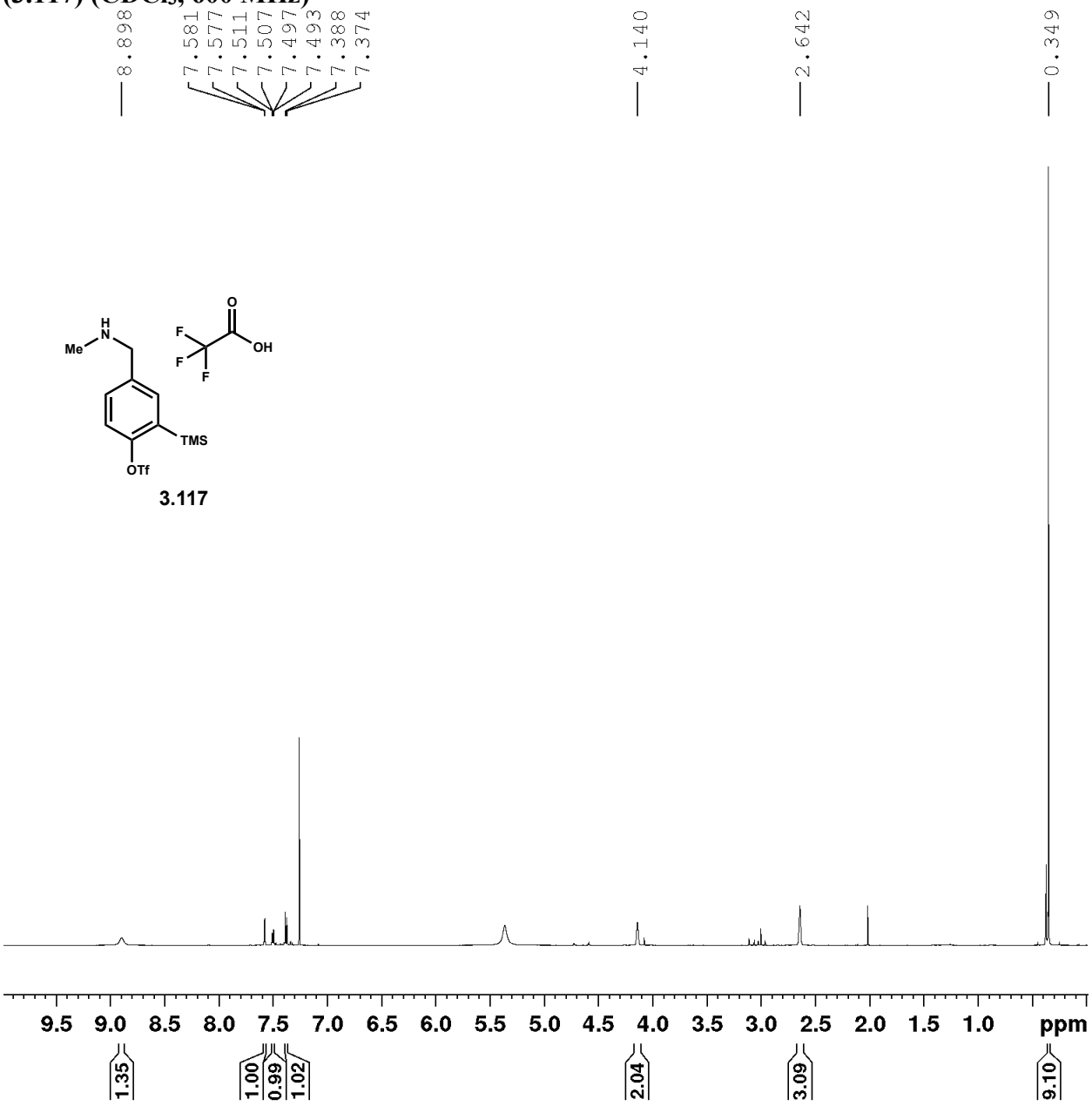
^{1}H NMR of 4-formyl-2-(trimethylsilyl)phenyl trifluoromethanesulfonate (3.125) ($CDCl_3$, 600 MHz)



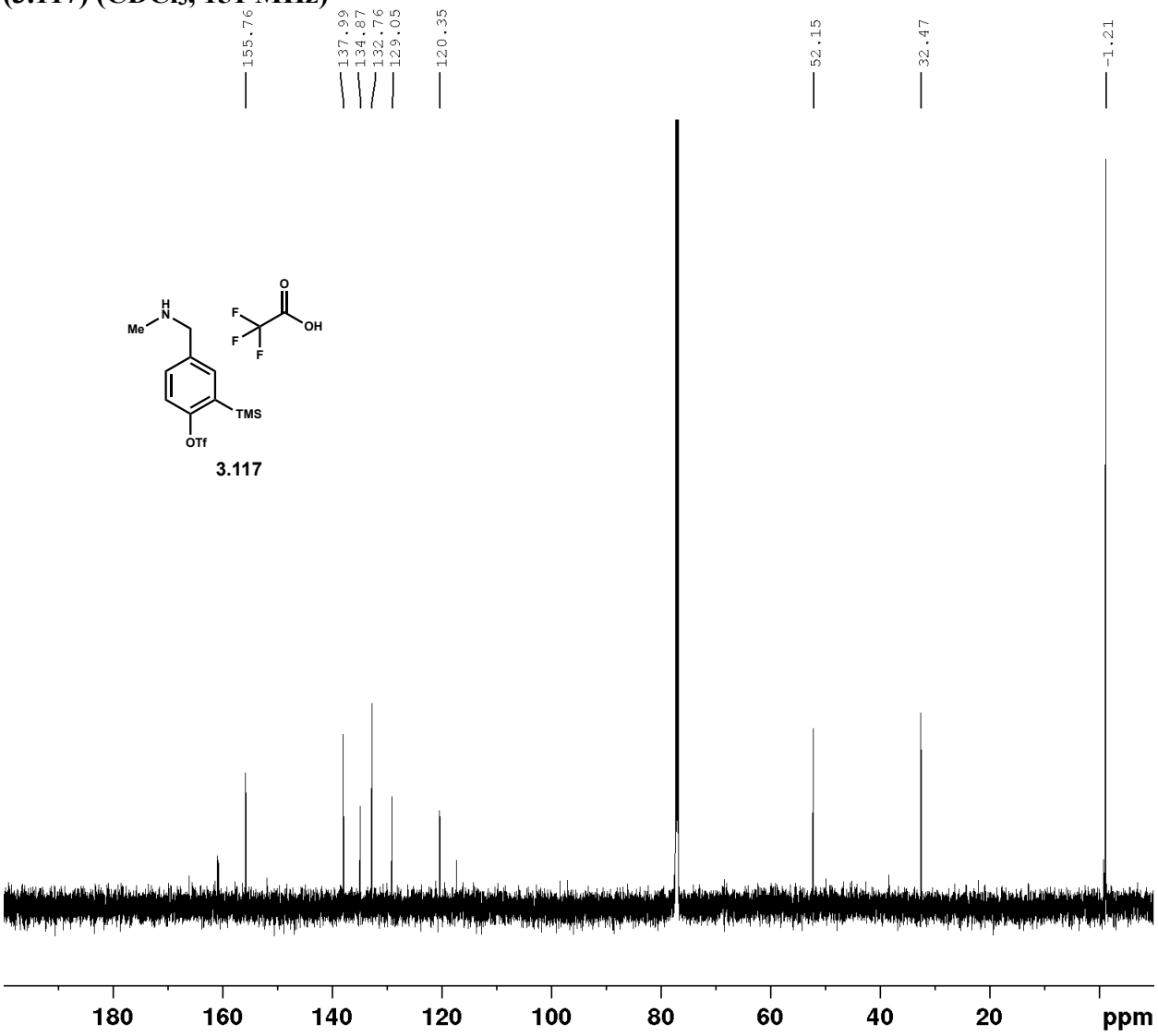
^{13}C NMR of 4-formyl-2-(trimethylsilyl)phenyl trifluoromethanesulfonate (3.125) ($CDCl_3$, 151 MHz)



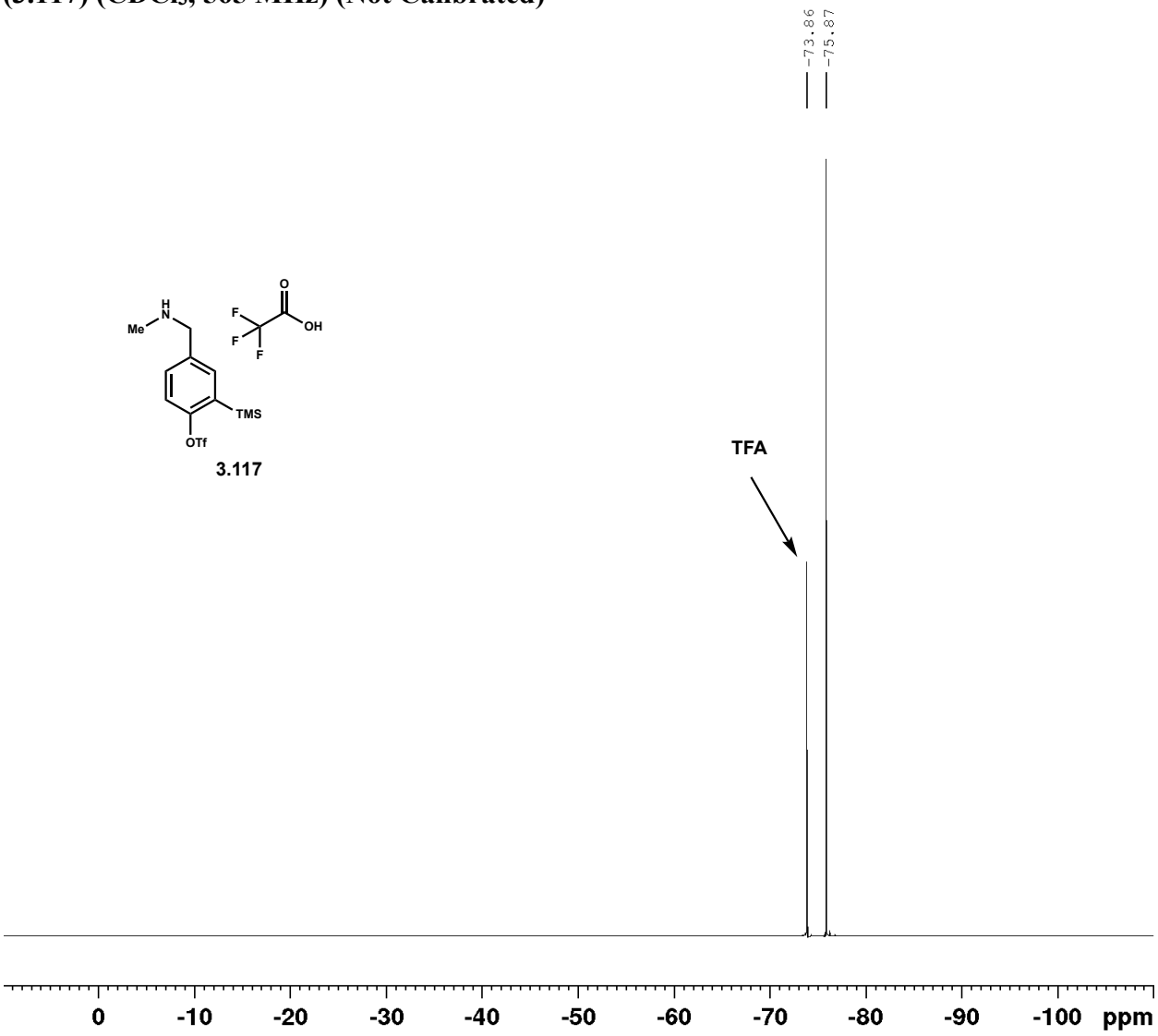
^{1}H NMR of 4-((methylamino)methyl)-2-(trimethylsilyl)phenyl trifluoromethanesulfonate (3.117) ($CDCl_3$, 600 MHz)



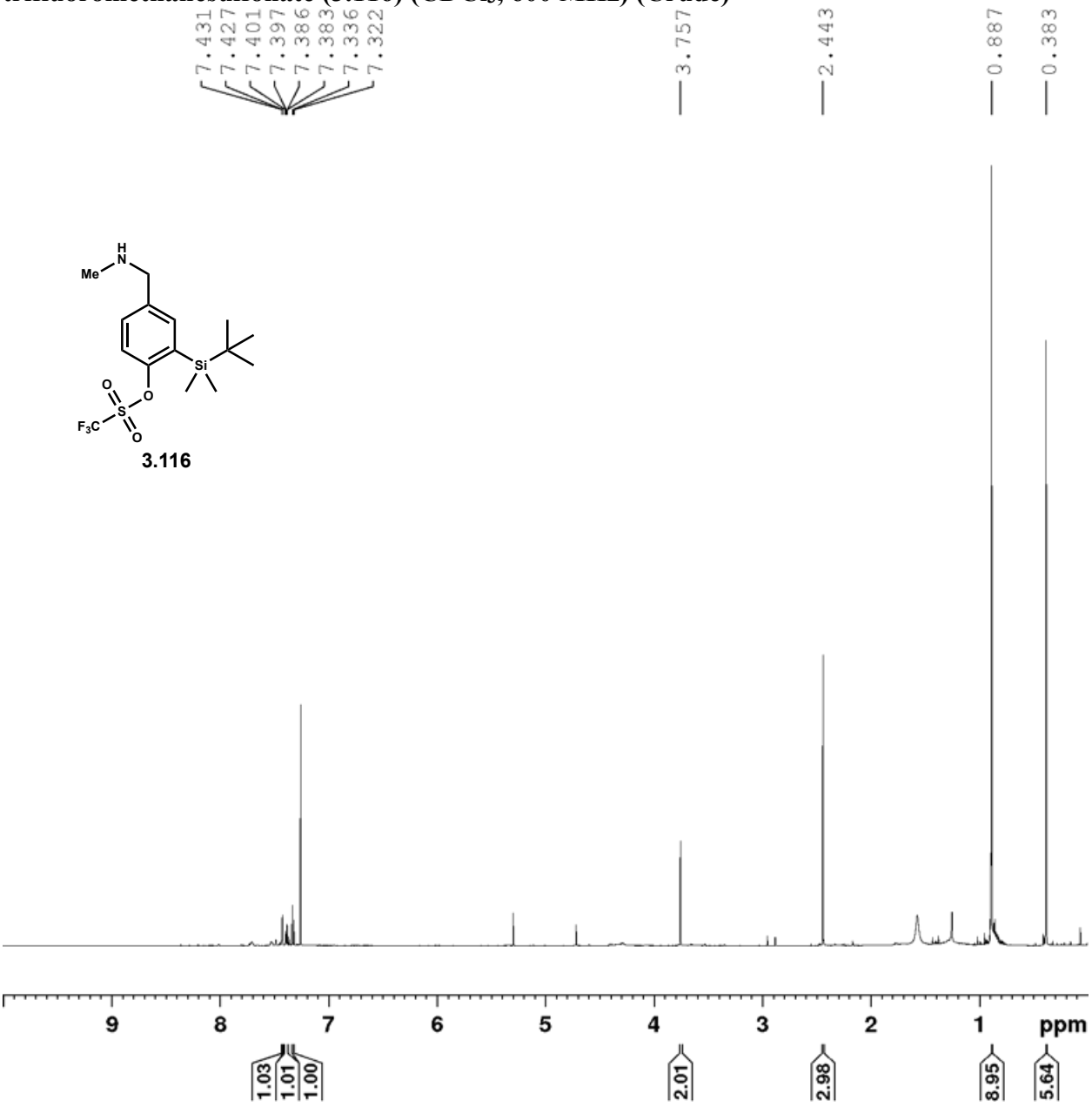
^{13}C NMR of 4-((methylamino)methyl)-2-(trimethylsilyl)phenyl trifluoromethanesulfonate (3.117) ($CDCl_3$, 151 MHz)



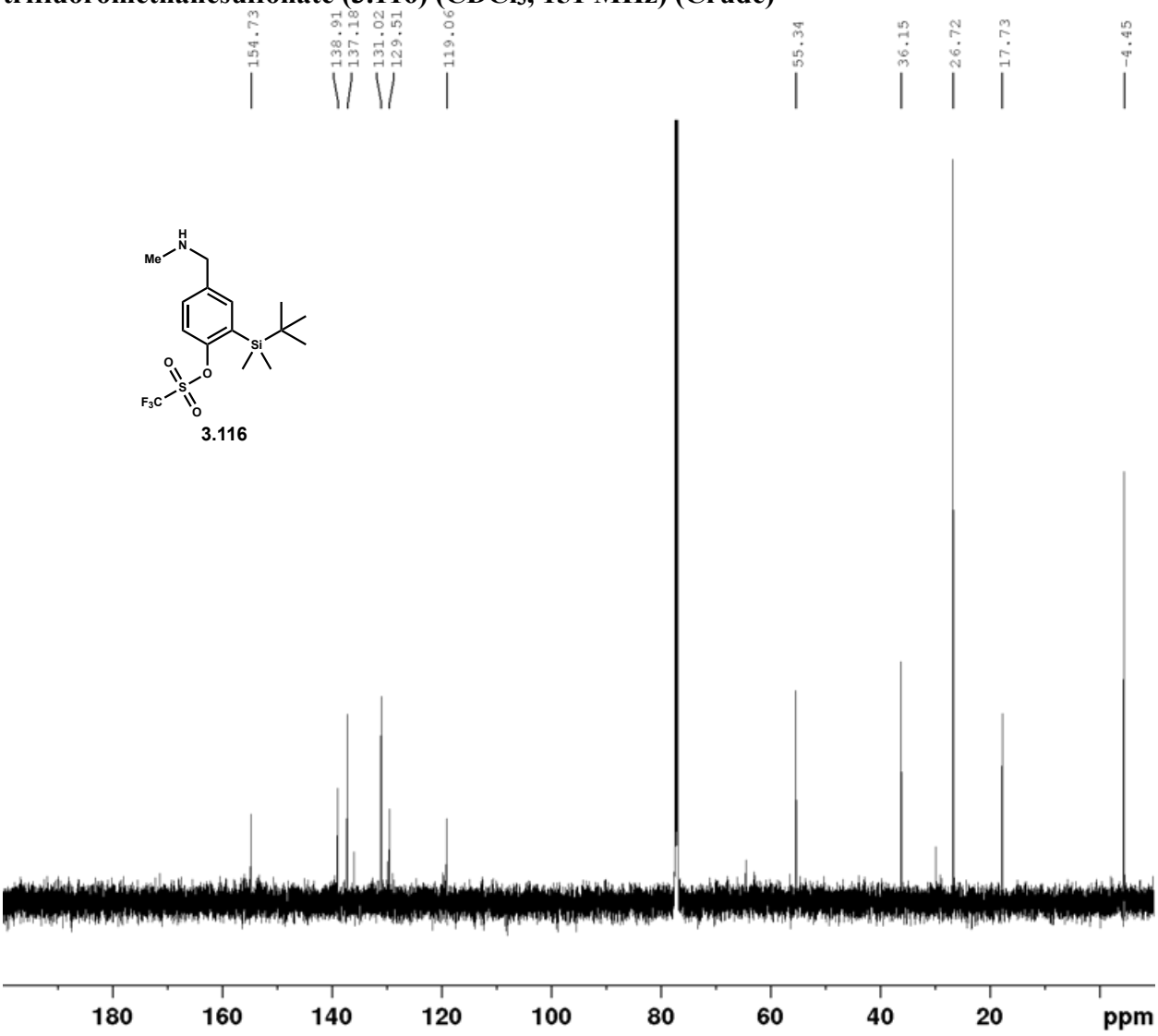
^{19}F NMR of 4-((methylamino)methyl)-2-(trimethylsilyl)phenyl trifluoromethanesulfonate (3.117) ($CDCl_3$, 565 MHz) (Not Calibrated)



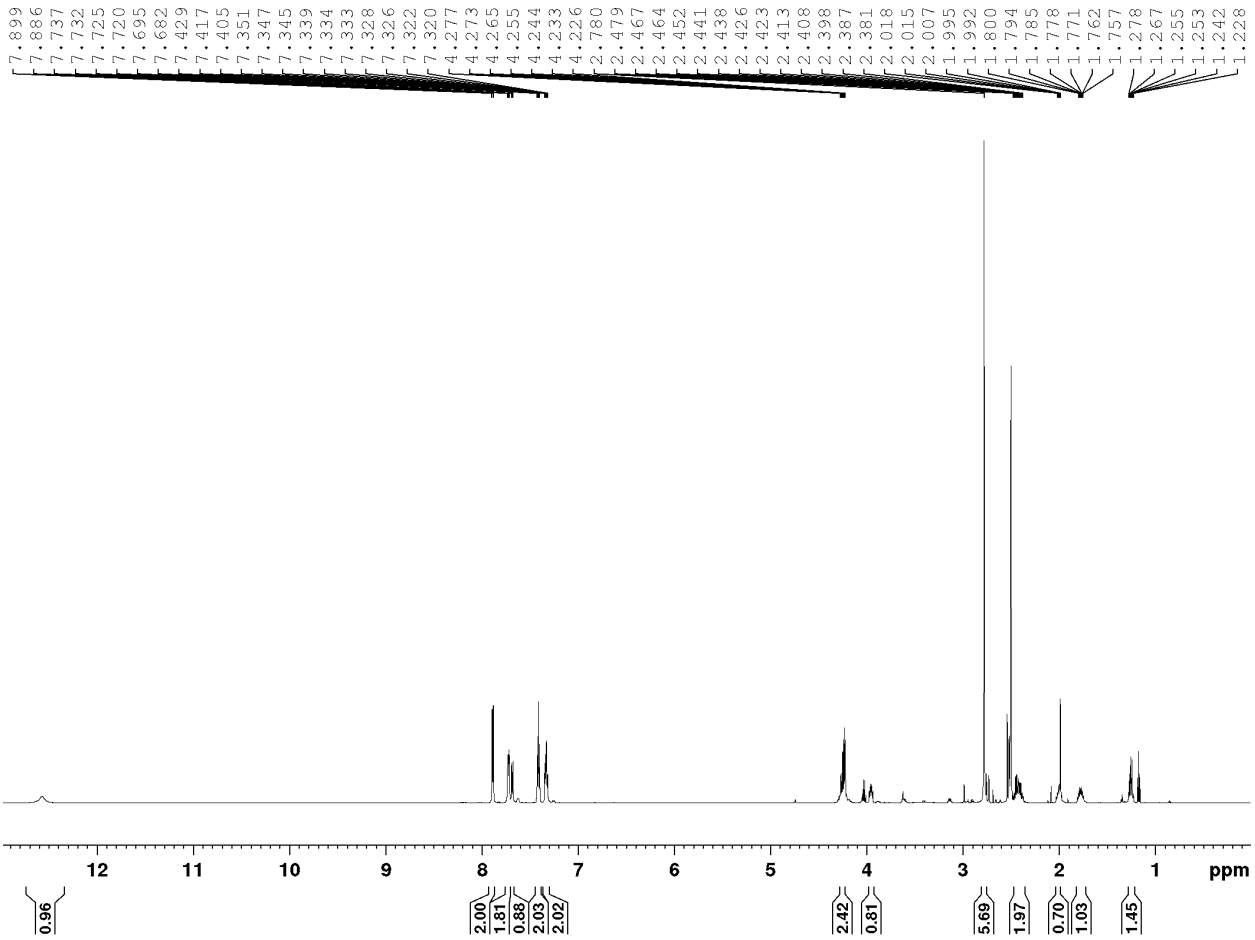
^{1}H NMR of 2-(*tert*-butyldimethylsilyl)-4-((methylamino)methyl)phenyl trifluoromethanesulfonate (3.116) ($CDCl_3$, 600 MHz) (Crude)



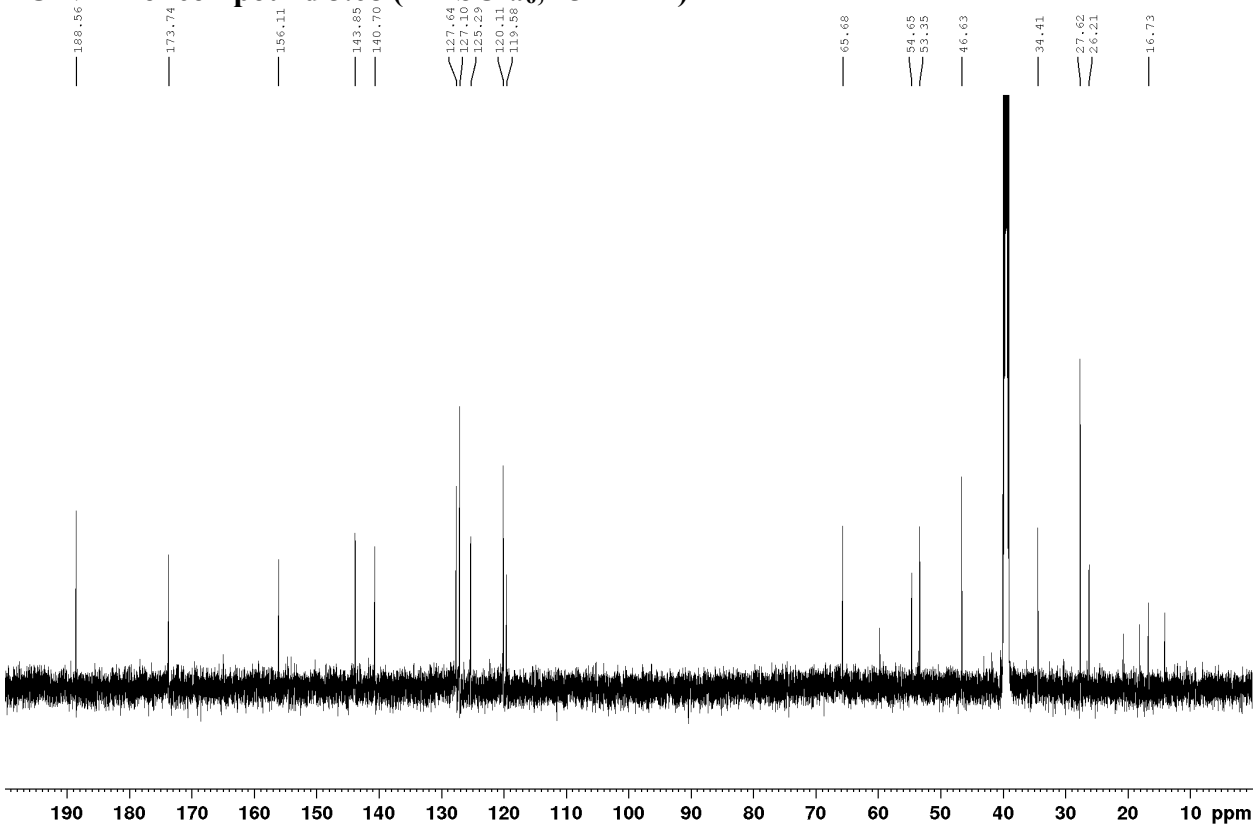
^{13}C NMR of 2-(*tert*-butyldimethylsilyl)-4-((methylamino)methyl)phenyl trifluoromethanesulfonate (3.116) ($CDCl_3$, 151 MHz) (Crude)



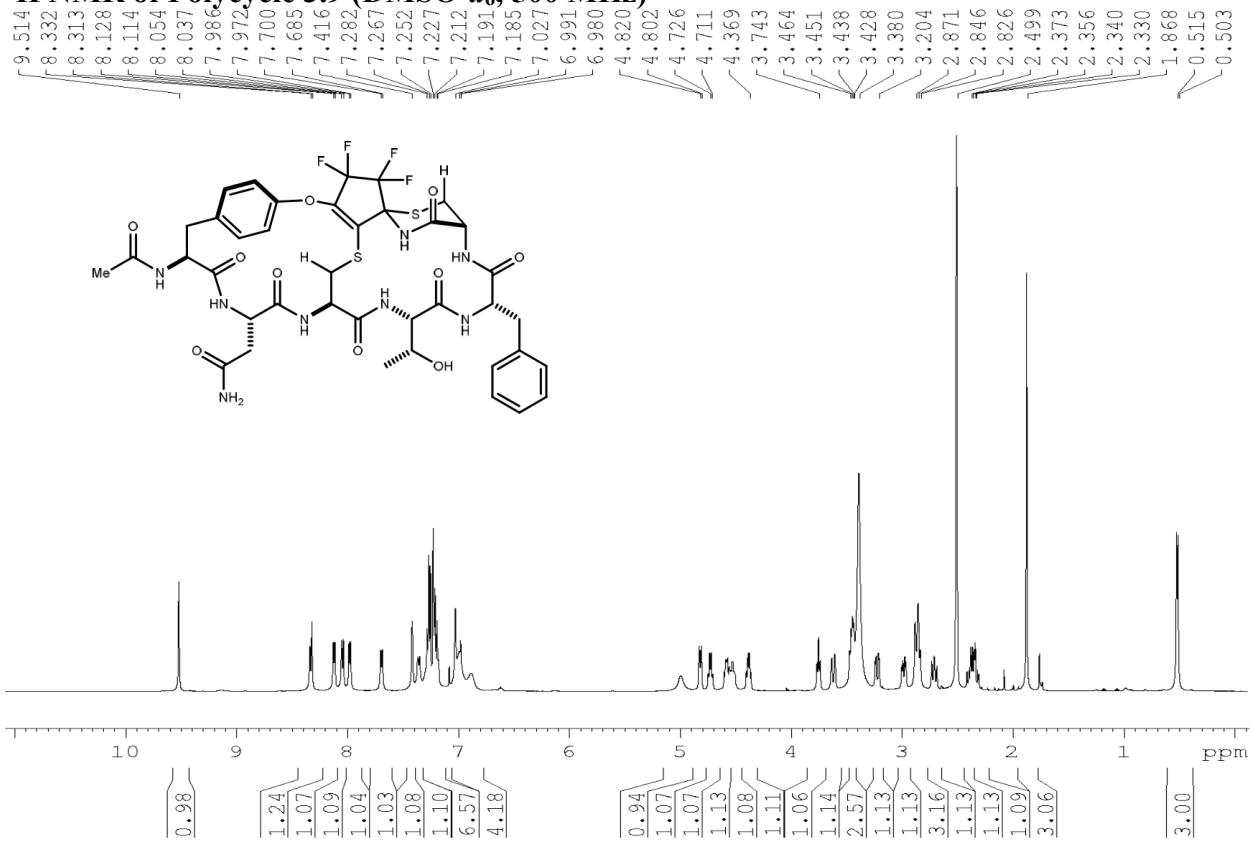
^{1}H NMR of compound 3.68 (DMSO-d_6, 600 MHz)



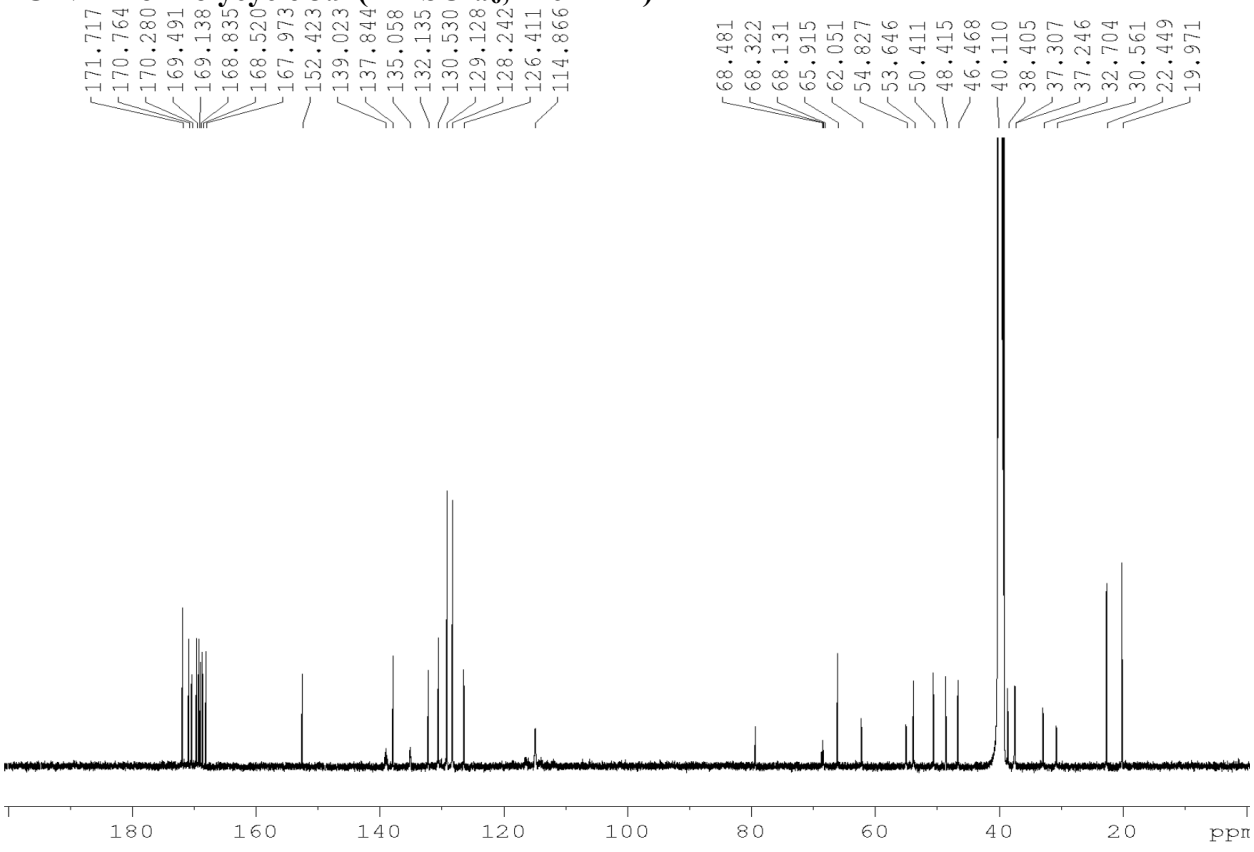
^{13}C NMR of compound 3.68 (DMSO-d_6, 151 MHz)



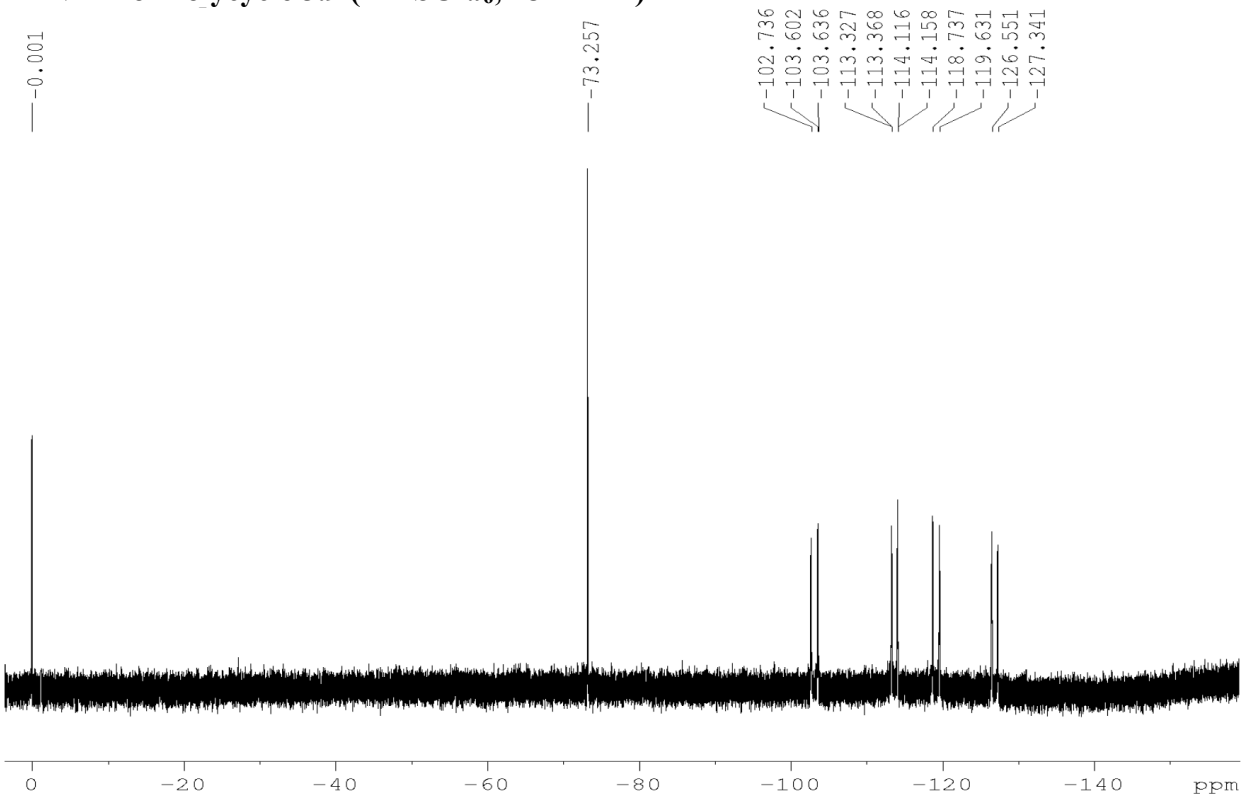
^{1}H NMR of Polycycle 3.9 (DMSO-d_6, 500 MHz)



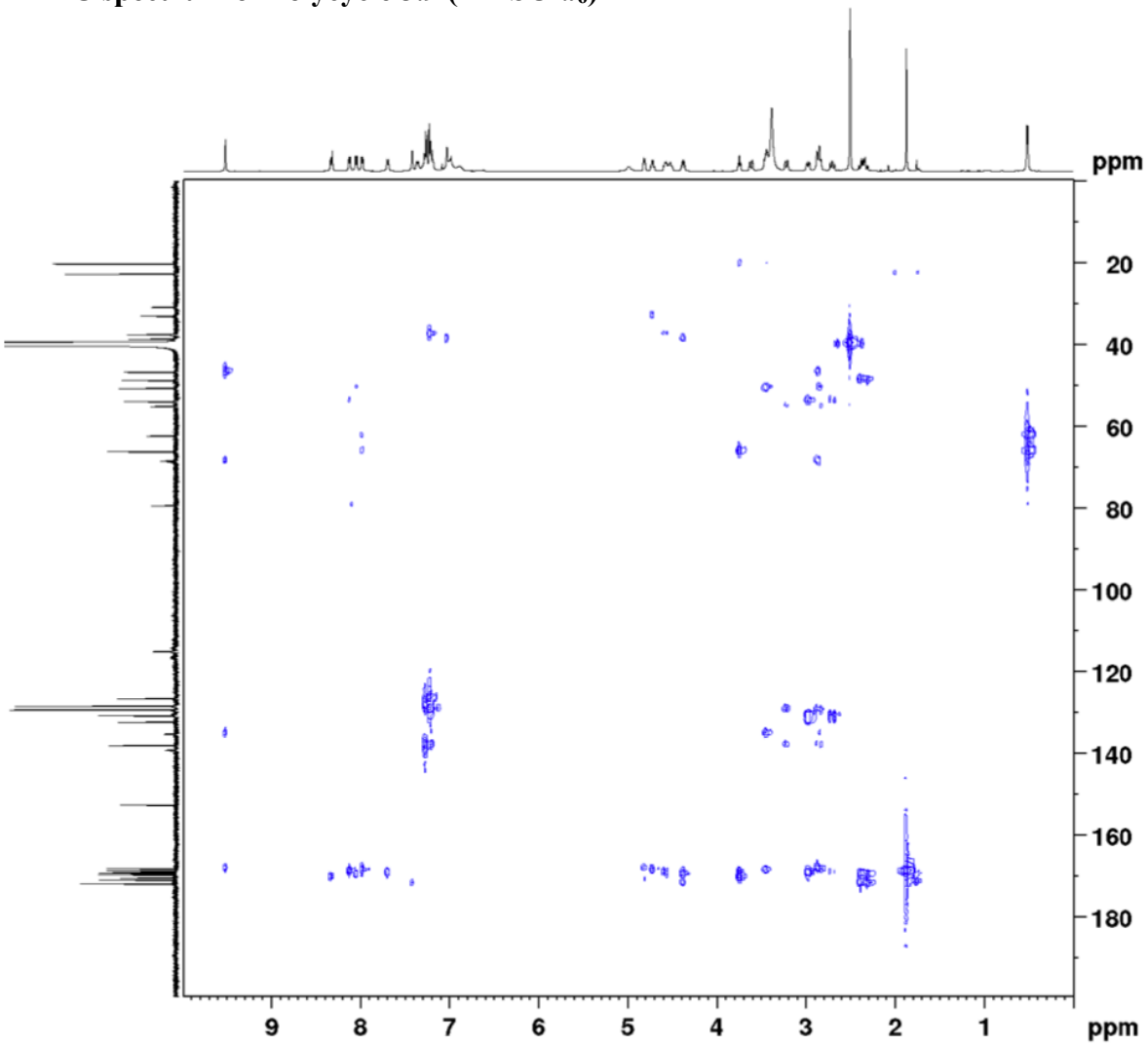
^{13}C NMR of Polycycle 3.9 (DMSO-d_6, 126 MHz)



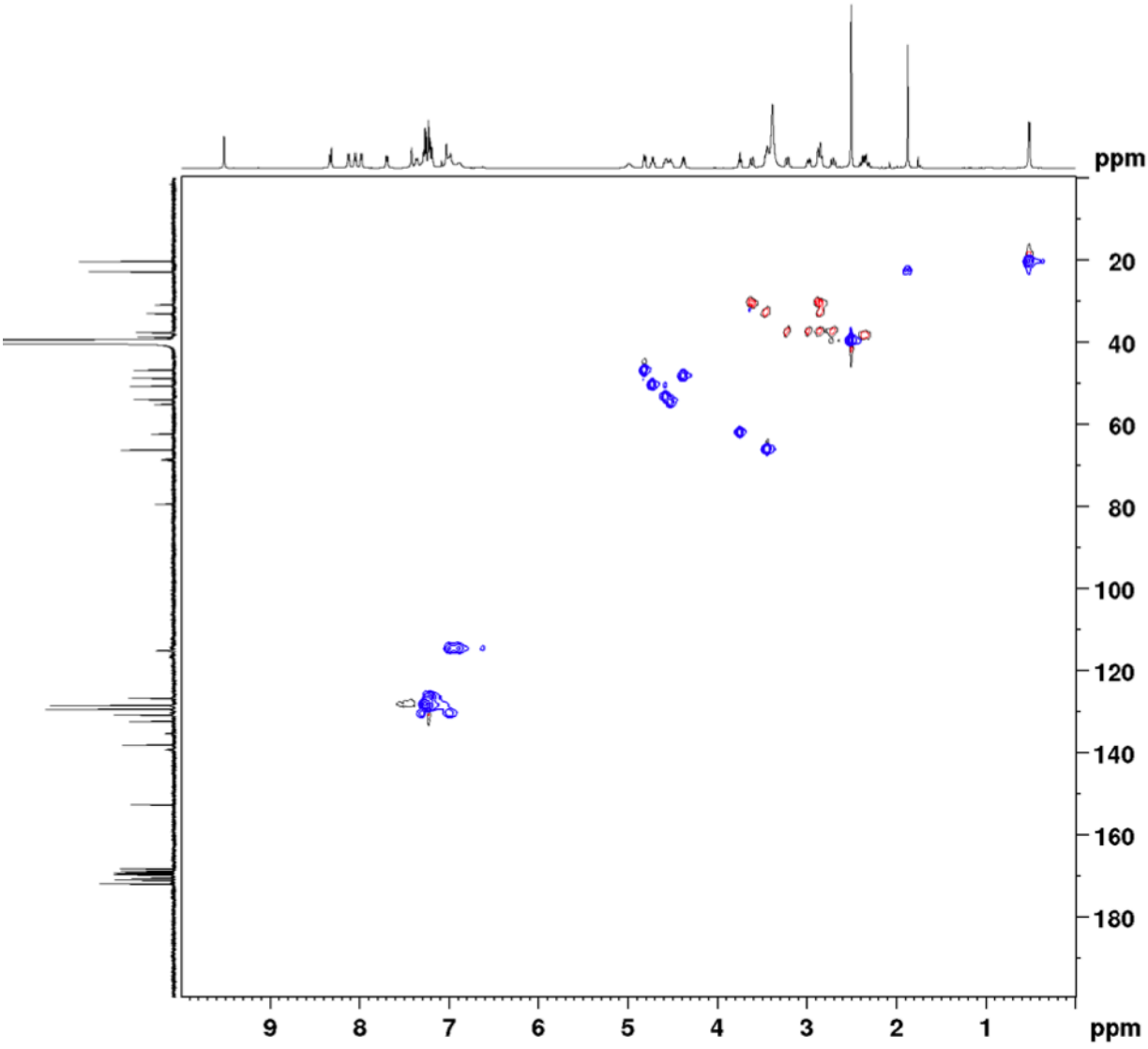
^{19}F NMR of Polycycle 3.9 (DMSO-d_6, 282 MHz)



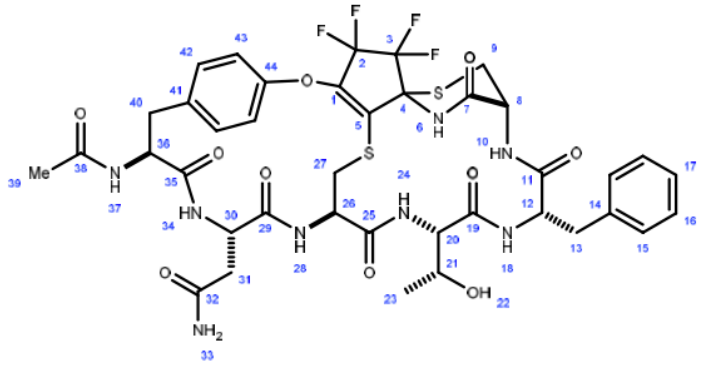
HMBC spectrum of Polycycle 3.9 (DMSO-d_6)



HSQC spectrum of Polycycle 3.9 (DMSO-d_6)

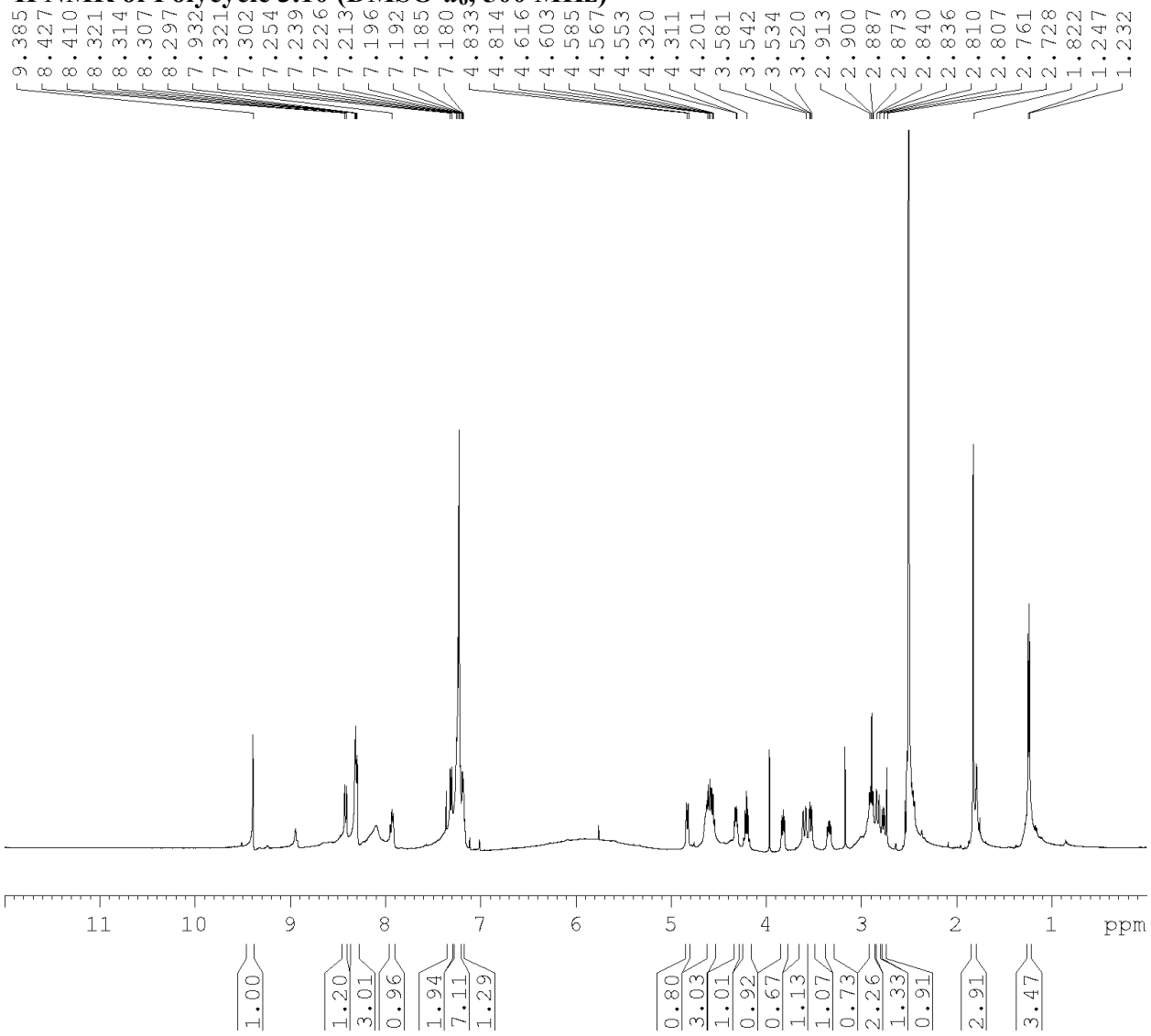


Polycycle 3.9 (DMSO-d_6)

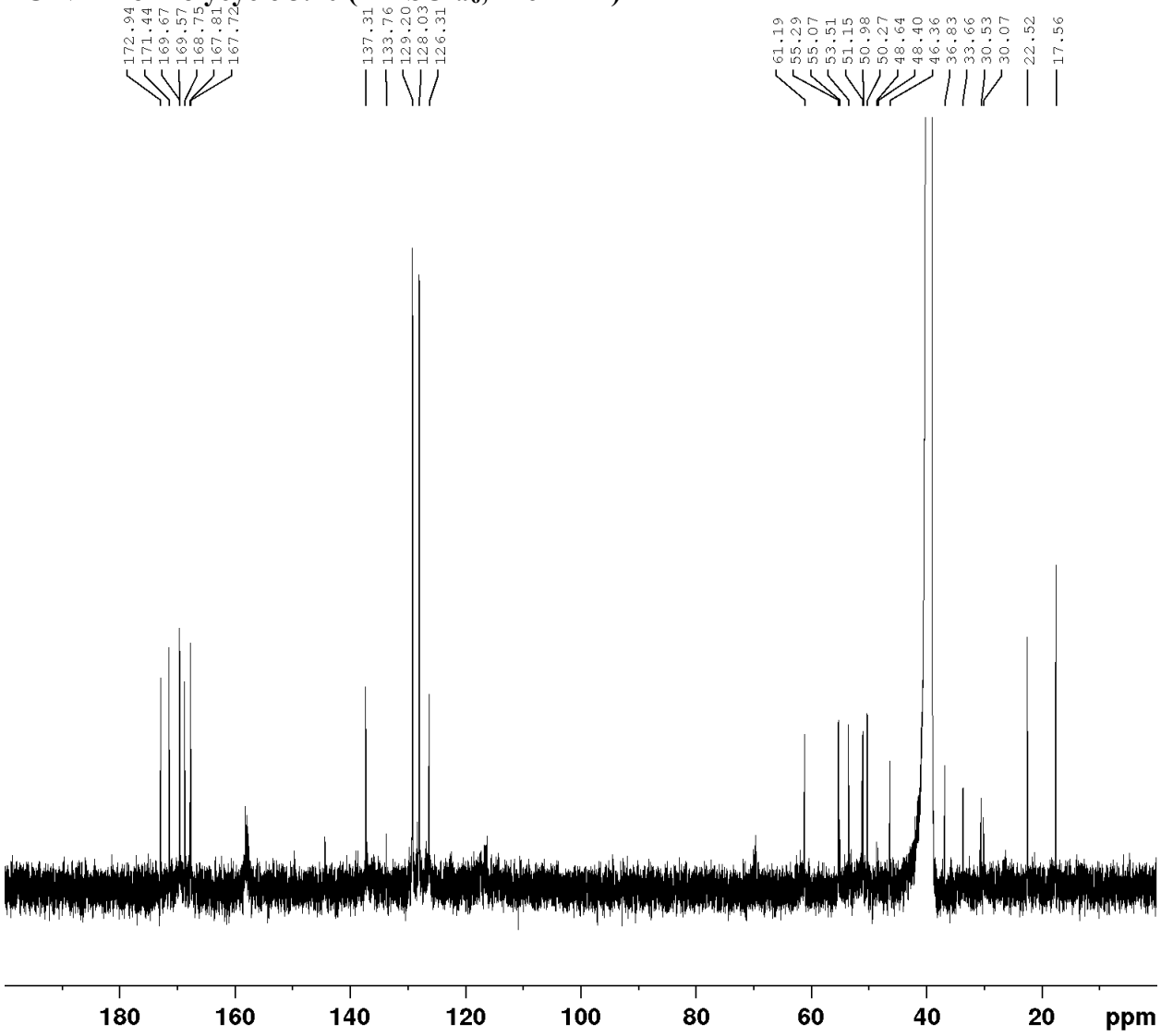


	13C	1H	Key Correlation
1	139.2–138.8 (m, 1C)	–	
2	118.7–111.5 (m, 2C)	–	
3	118.7–111.5 (m, 2C)	–	
4	68.6–68.1 (m, 1C)	–	HMBC 6, 9 -> 4
5	135.2–134.9 (m, 1C)	–	HMBC 6, 9, 27 -> 5
6	–	9.51 (s, 1H)	HMBC 6 -> 7
7	168.0	–	HMBC 6 -> 7
8	46.5	4.84–4.79 (m, 1H)	HMBC 8 -> 7, HSQC
9	30.6	3.61 (d, J = 14.0 Hz, 1H), 2.89–2.82 (m, 1H) overlap	HMBC 9 -> 4, 8, HSQC
10	–	7.36 (d, J = 8.6 Hz, 1H)	HMBC 10 -> 11
11	170.8	–	HMBC 12 -> 11
12	54.8	4.55–4.48 (m, 1H)	HMBC 13 -> 12
13	37.3 or 37.2	3.22 (dd, J = 14.0, 3.7 Hz, 1H)	HMBC 12 -> 13, HSQC
14	137.8	–	HMBC 13 -> 14
15	128.2	7.30–7.17 (m, 2H) overlap	HMBC 15 -> 14, HSQC
16	129.1	7.30–7.17 (m, 2H) overlap	HMBC 15 -> 16, HSQC
17	126.4	7.30–7.17 (m, 1H) overlap	HMBC 17 -> 16, HSQC
18	–	8.33 (d, J = 9.5 Hz, 1H)	HMBC 18 -> 19
19	170.3	–	HMBC 18, 20 -> 19
20	62.1	3.74 (t, J = 6.9 Hz, 1H)	HMBC 20 -> 19, 24, HSQC
21	65.9	3.48–3.41 (m, 1H) overlap	HMBC 20, 23 -> 21, HSQC
22	–	4.98 (brs, 1H)	
23	20.0	0.51 (d, J = 6.0 Hz, 3H)	HMBC 21 -> 23, HSQC
24	–	7.98 (d, J = 6.8 Hz, 1H)	HMBC 24 -> 20, 25
25	168.5	–	HMBC 24, 26 -> 25
26	50.4	4.72 (dd, J = 14.2, 6.8 Hz, 1H)	HMBC 26 -> 25, 27, HSQC
27	32.7	3.48–3.41 (m, 1H) overlap	HMBC 27 -> 5, HSQC
28	–	8.05 (d, J = 8.2 Hz, 1H)	HMBC 28 -> 26, 29
29	169.5	–	HMBC 28, 30 -> 29
30	48.4	4.41–1.34 (m, 1H)	HMBC 30 -> 29, 31, HSQC
31	38.4	2.38 (dd, J = 15.6, 8.5 Hz, 1H), 2.32 (dd, J = 15.6, 5.0 Hz,	HMBC 31 -> 32, HSQC
32	171.7	–	HMBC 31, 33 -> 32
33	–	7.42 (s, 1H), 7.03 (s, 1H)	HMBC 33 -> 32
34	–	7.69 (d, J = 7.7 Hz, 1H)	HMBC 34 -> 30, 35
35	169.1	–	HMBC 34, 36 -> 35
36	53.6	4.60–4.55 (m, 1H)	HMBC 37, 40 -> 36, HSQC
37	–	8.12 (d, J = 6.9 Hz, 1H)	HMBC 37 -> 36, 38, HSQC
38	168.8	–	HMBC 37, 39 -> 38
39	22.4	1.87 (s, 3H)	HMBC 39 -> 38, HSQC
40	37.3 or 37.2	2.97 (dd, J = 13.2, 5.1 Hz, 1H), 2.70 (dd, J = 13.2, 10.1 Hz	HMBC 40 -> 36, 41
41	132.1	–	HMBC 42 -> 41
42	130.5	7.30–7.17 (m, 1H) overlap, 7.06–6.84 (m, 1H) overlap	HMBC 42 -> 41, HSQC
43	114.9	7.06–6.84 (m, 2H) overlap	HMBC 43 -> 42, 44, HSQC
44	152.4	–	HMBC 43 -> 44, HSQC

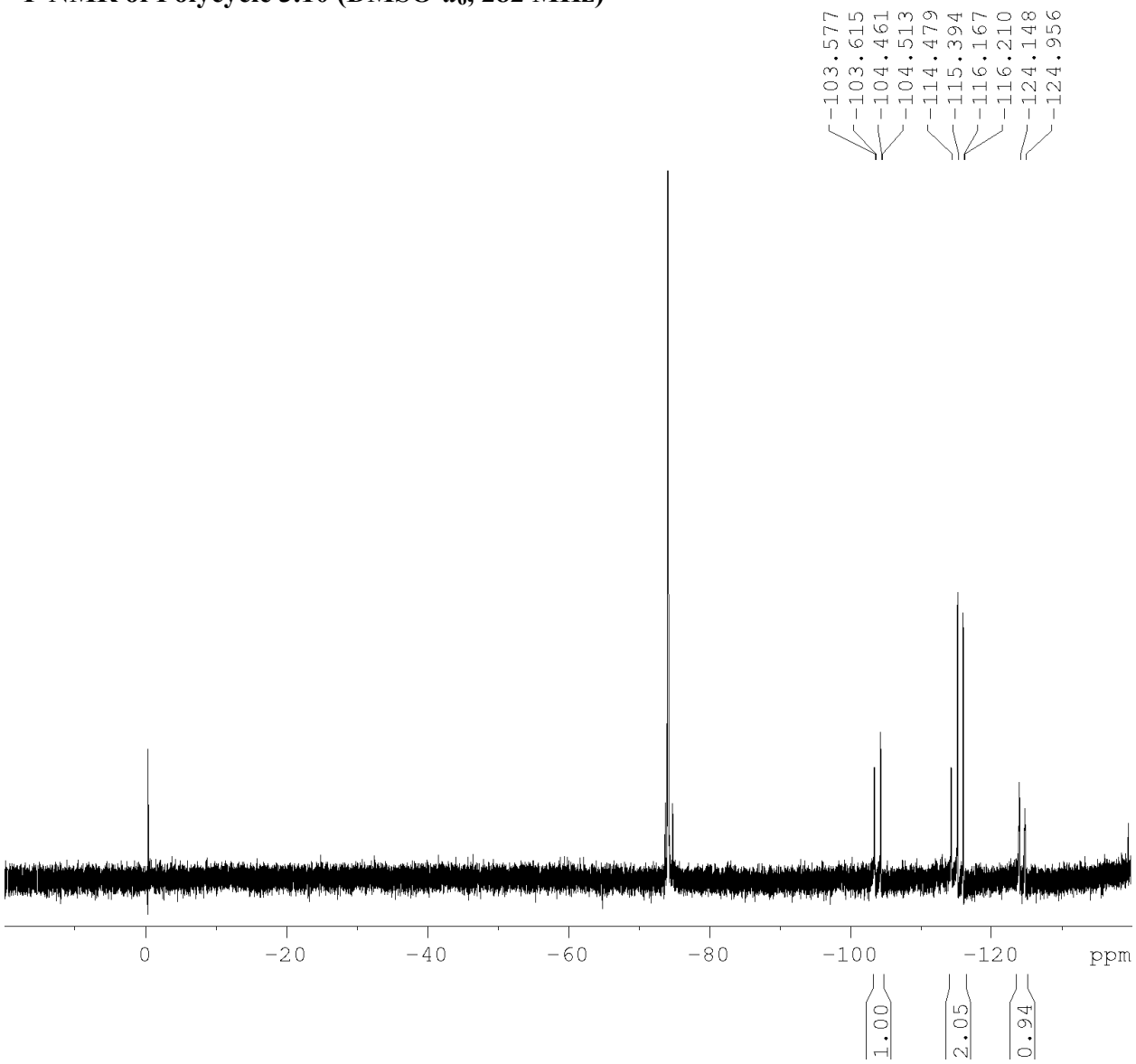
^{1}H NMR of Polycycle 3.10 (DMSO-d_6, 500 MHz)



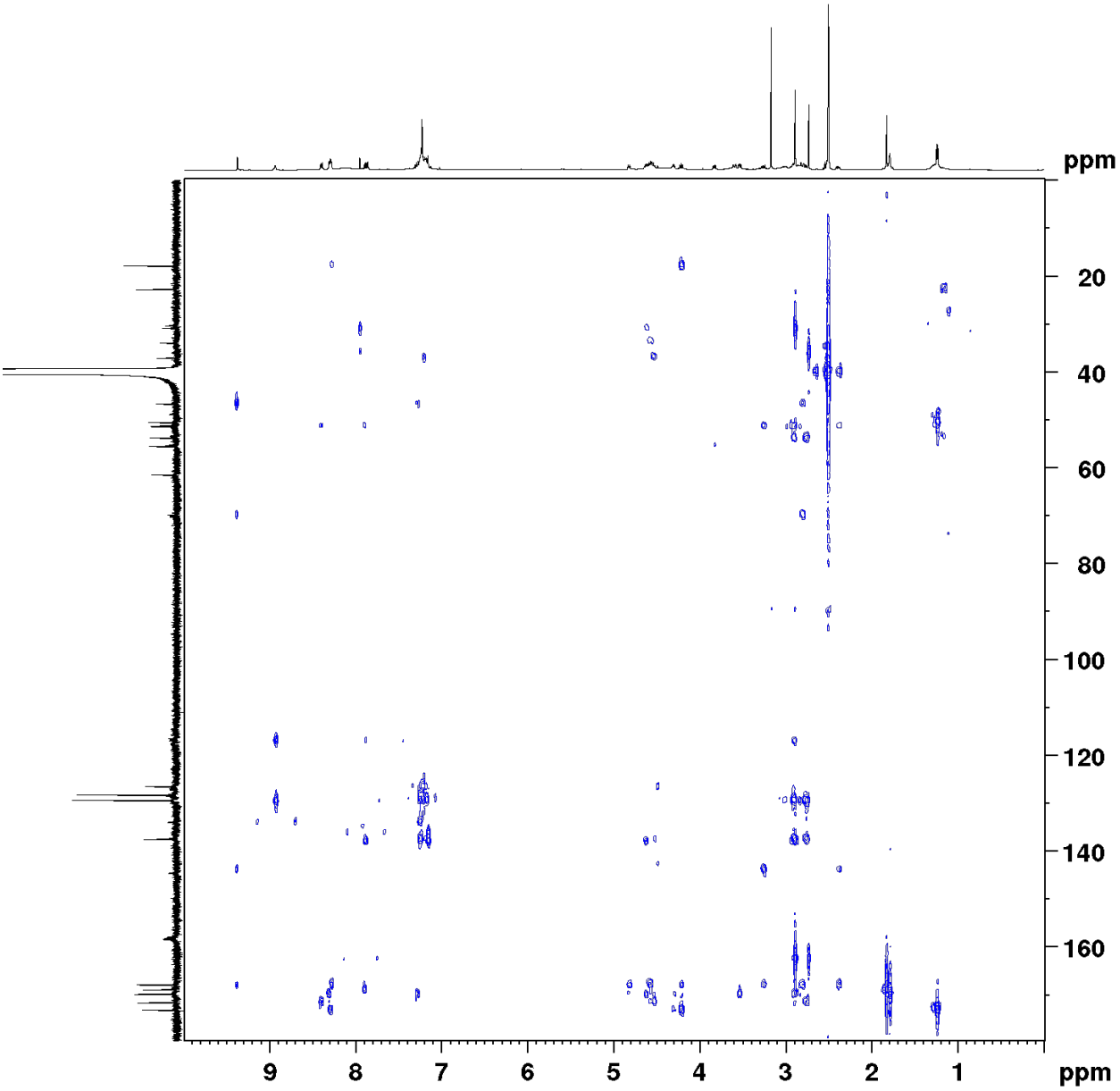
^{13}C NMR of Polycycle 3.10 (DMSO-d_6, 126 MHz)



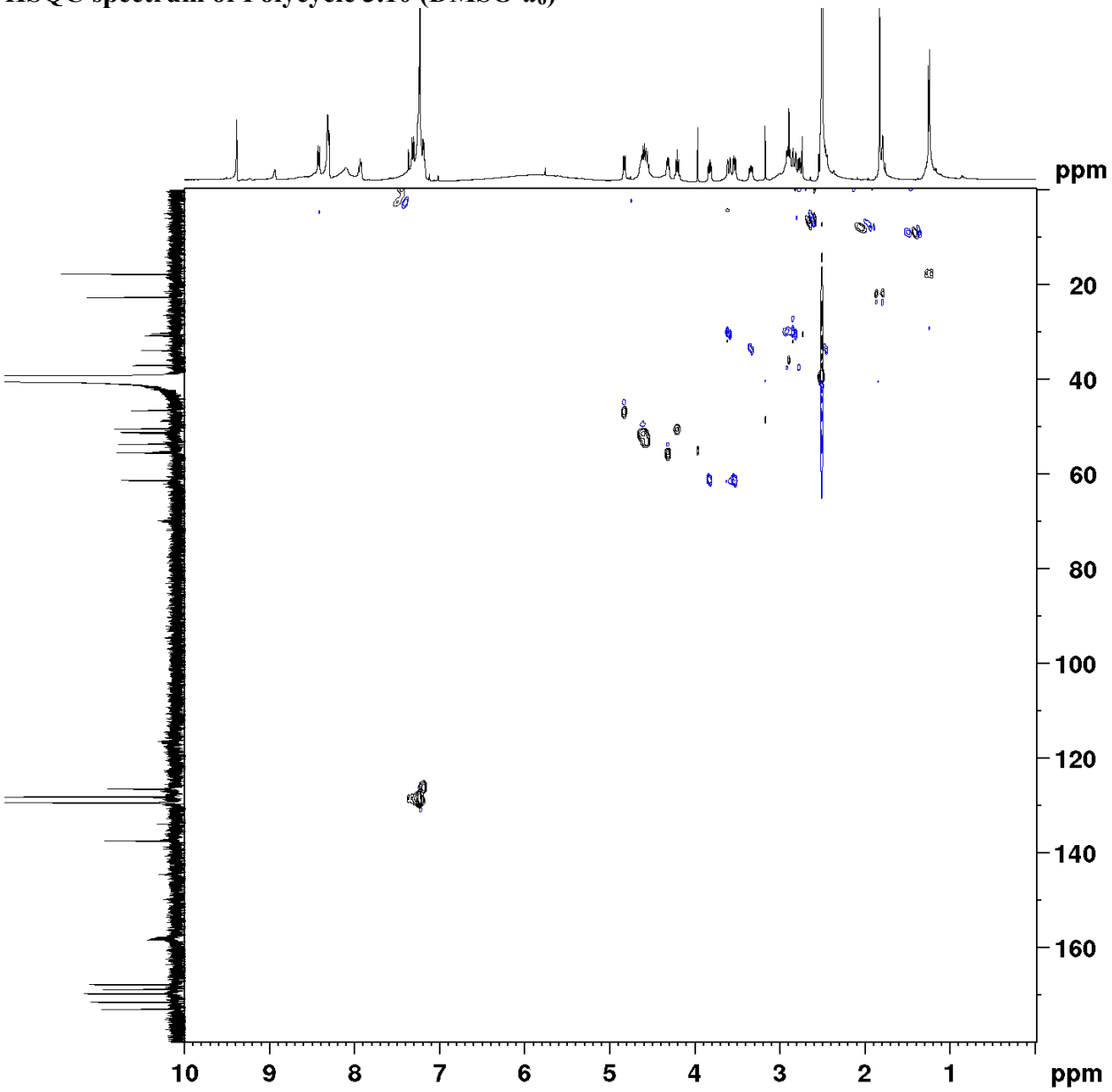
^{19}F NMR of Polycycle 3.10 (DMSO-d_6, 282 MHz)



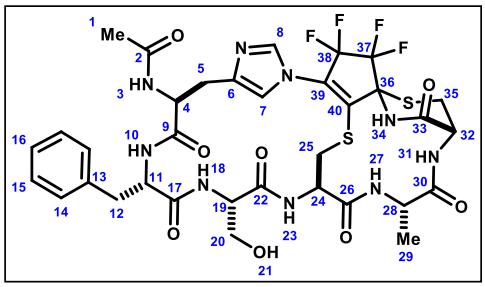
HMBC spectrum of Polycycle 3.10 (DMSO-d_6)



HSQC spectrum of Polycycle 3.10 (DMSO-d_6)

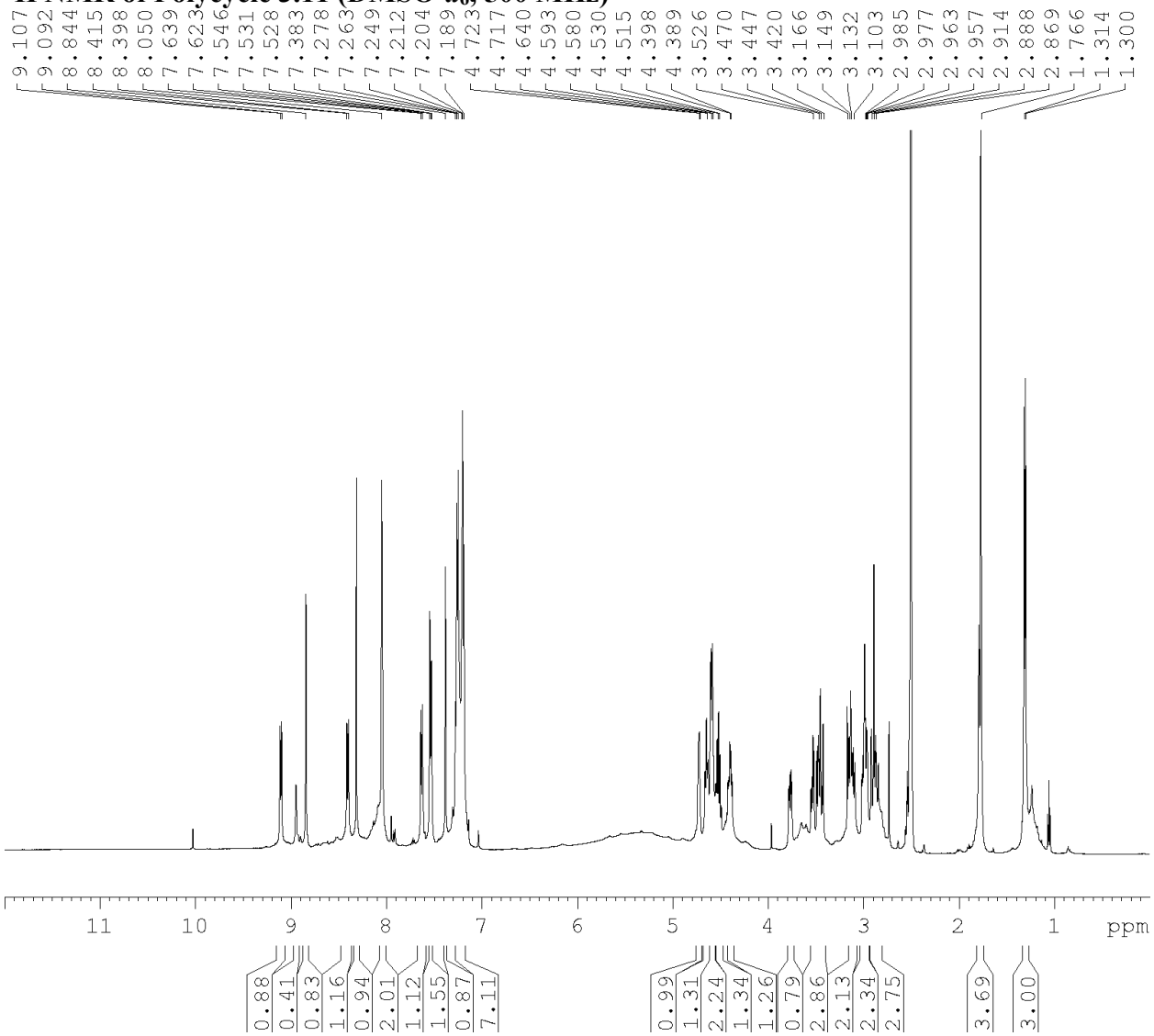


Polycycle 3.10 (DMSO-d_6, 298K)

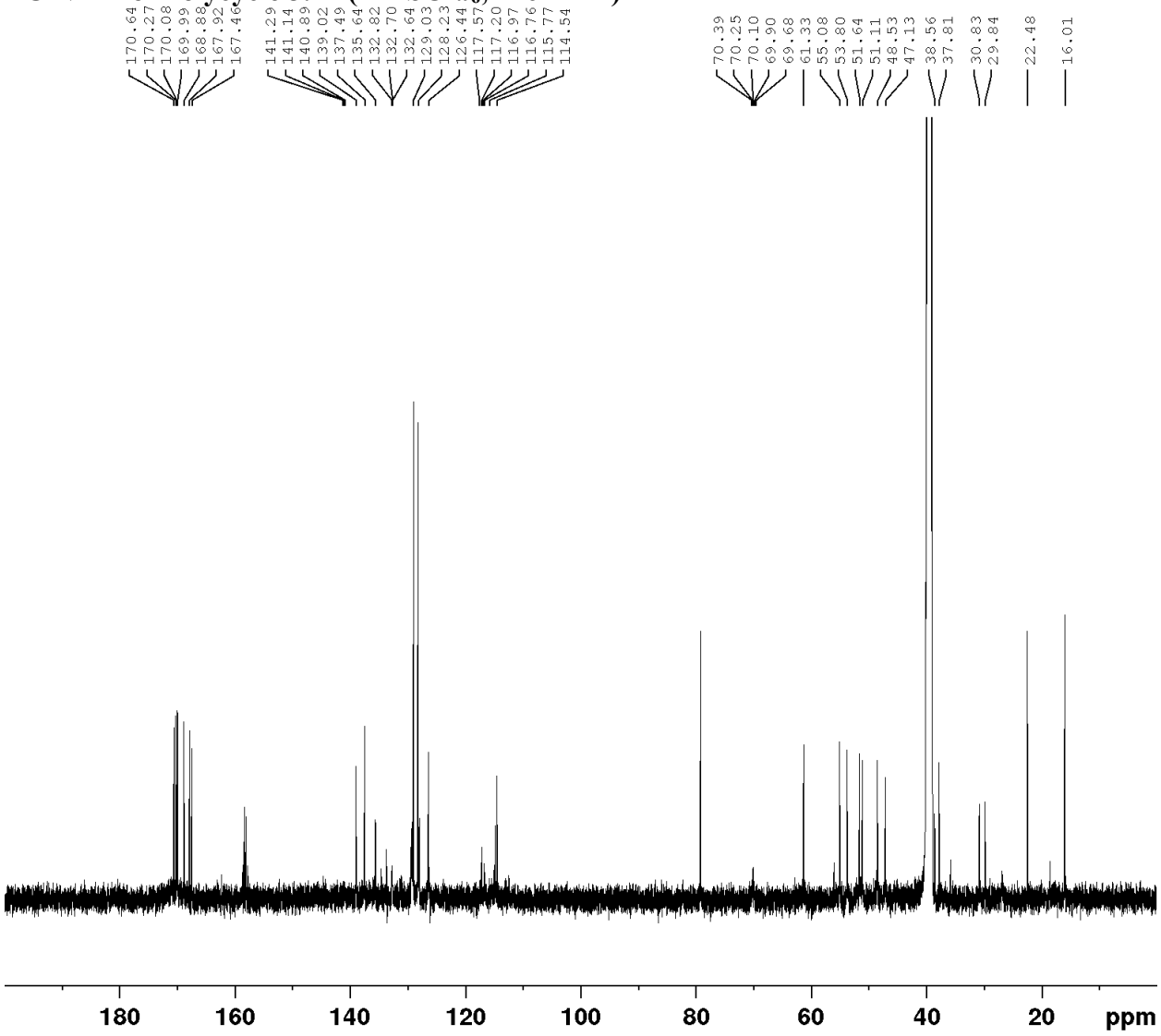


	13C	1H	Key Correlation
1	22.5	1.77 (s, 3H)	1 -> 2 HMBC
2	168.9	-	1 -> 2 HMBC
3	-	7.63 (d, J = 8.1 Hz, 1H)	3 -> 2, 4 HMBC
4	51.1	4.68 - 4.62 (m, 1H)	3 -> 4 HMBC
5	29.8	3.16 - 3.05 (m, 2H); 2.94 - 2.81 (m, 3H)	4 -> 5 HMBC
6	139.0	-	4, 5 -> 6 HMBC
7	114.5	7.38 (s, 1H)	8 -> 7 HMBC
8	135.6	8.07 - 8.01 (m, 2H)	8 -> 7 HMBC
9	170.0	-	4 -> 9 HMBC
10	-	8.41 (d, J = 8.7 Hz, 1H)	10 -> 9 HMBC
11	55.1	4.43 - 4.36 (m, 1H)	10 -> 11 HMBC
12	37.8	3.03 - 2.94 (m, 2H); 2.90 - 2.81 (m, 3H)	11 -> 12 HMBC
13	137.5	-	12 -> 13 HMBC
14	129.0	7.28 - 7.17 (m, 6H)	12 -> 14 HMBC
15	128.2	7.28 - 7.17 (m, 6H)	14 -> 15 HMBC
16	126.4	7.28 - 7.17 (m, 6H)	15 -> 16 HMBC
17	170.1	-	11, 19 -> 17 HMBC
18	-	7.28 - 7.17 (m, 6H)	-
19	53.8	4.62 - 4.56 (m, 2H)	20 -> 19 HMBC
20	61.3	3.57 - 3.39 (m, 3H)	19 -> 20 HMBC
21	-	-	-
22	170.3	-	19, 23 -> 22 HMBC
23	-	8.07 - 8.01 (m, 2H)	23 -> 24 COSY
24	51.6	4.75 - 4.70 (m, 1H)	23 -> 24 COSY
25	38.6	3.80 - 3.73 (m, 1H); 3.16 - 3.05 (m, 2H)	24 -> 25 HMBC
26	167.9	-	27 -> 26 HMBC
27	-	9.10 (d, J = 7.7, 1H)	27 -> 28 HMBC
28	48.5	4.52 (quint, J = 7.2, 1H)	29 -> 28 HMBC
29	16.0	1.31 (d, J = 7.3 Hz, 3H)	28 -> 29 HMBC
30	170.6	-	31 -> 30 HMBC
31	-	7.54 (d, J = 7.65 Hz, 1H)	32 -> 32, 35 HMBC
32	47.1	4.62 - 4.56 (m, 2H)	HMBC 34 -> 32 HMBC
33	167.5	-	34 -> 35 HMBC
34	-	8.84 (s, 1H)	34 -> 36 HMBC
35	30.8	3.47 - 3.41 (m, 1H) 3.03 - 2.94 (m, 2H)	35 -> 36 HMBC
36	70.4 - 69.7 (m, 1C)	-	35 -> 36 HMBC
37	117.6 - 115.8 (m, 2C)	-	
38	117.6 - 115.8 (m, 2C)	-	
39	141.3 - 140.9 (m, 1C)	-	
40	132.8 - 132.6 (m, 1C)	-	

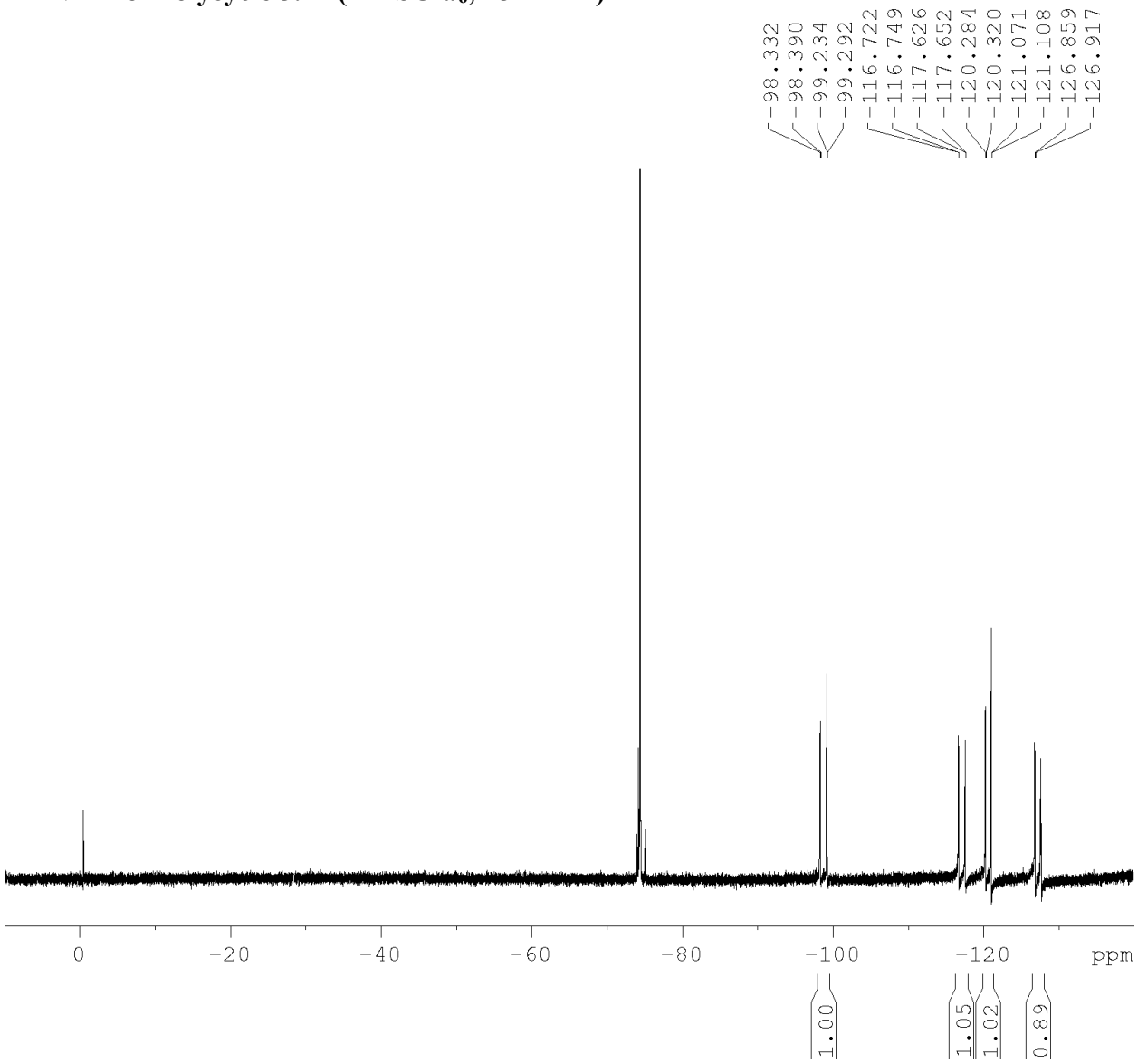
^{1}H NMR of Polycycle 3.11 (DMSO-d_6, 500 MHz)



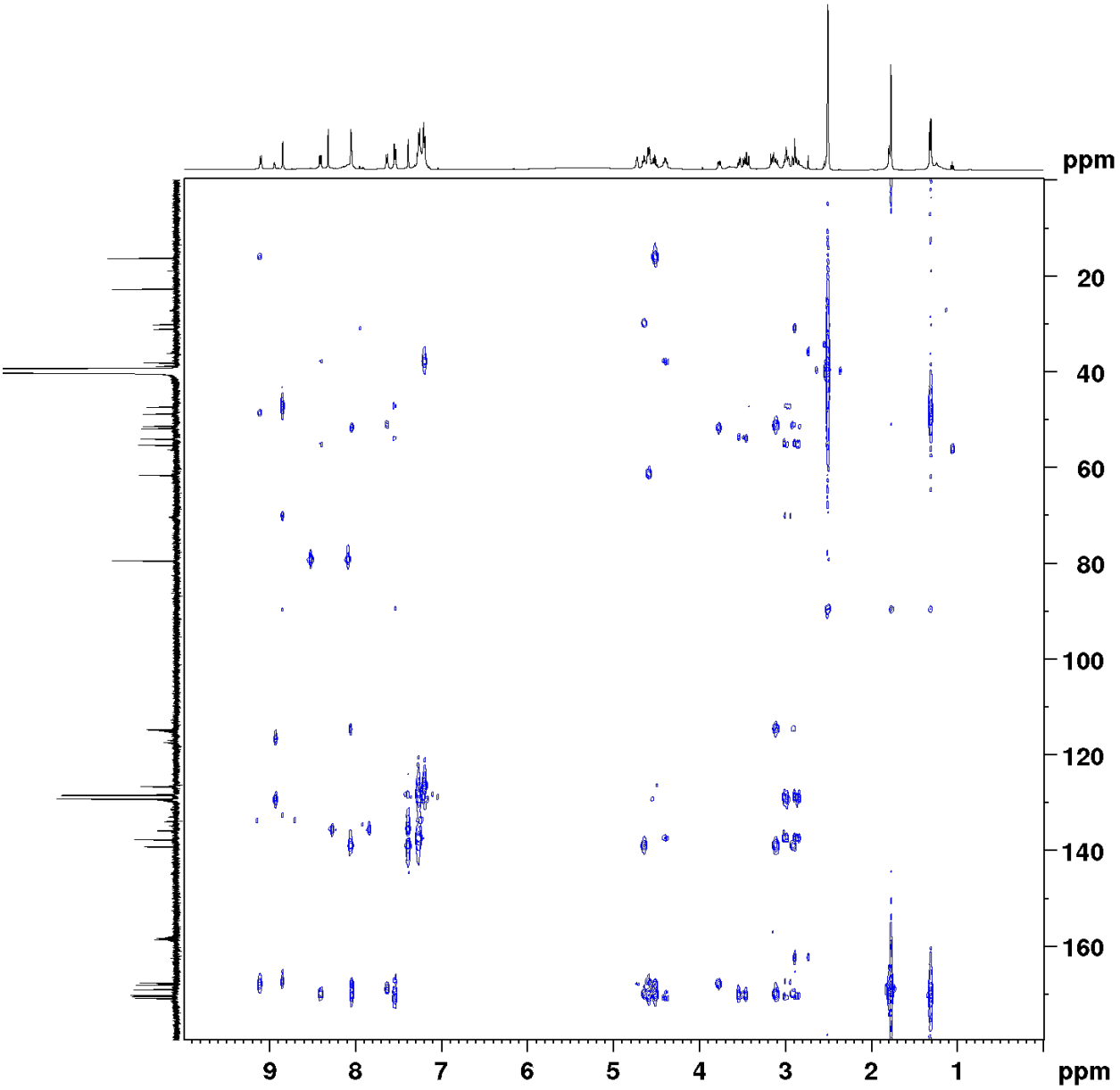
^{13}C NMR of Polycycle 3.11 (DMSO-d_6, 126 MHz)



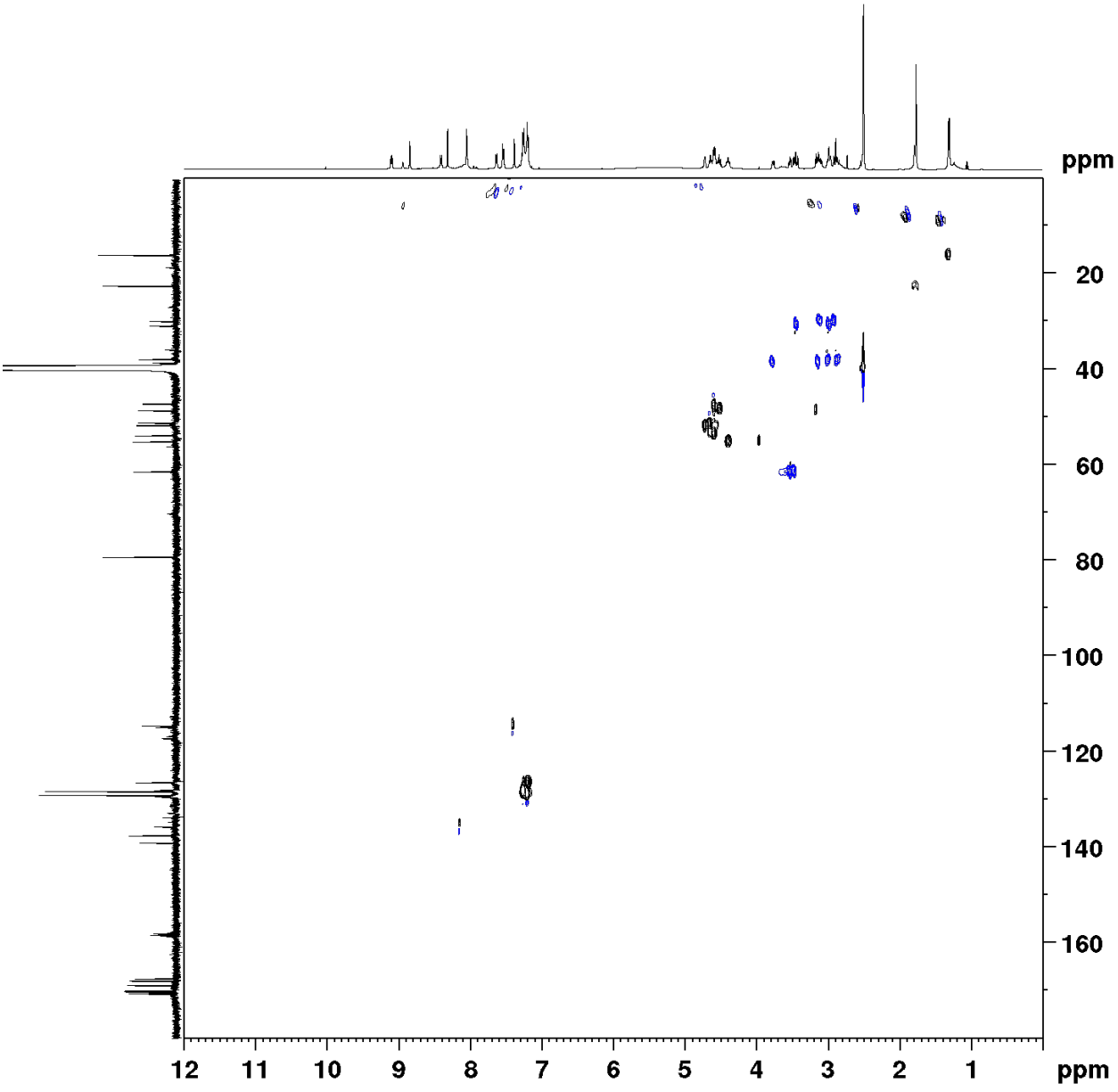
^{19}F NMR of Polycycle 3.11 (DMSO-d_6, 282 MHz)



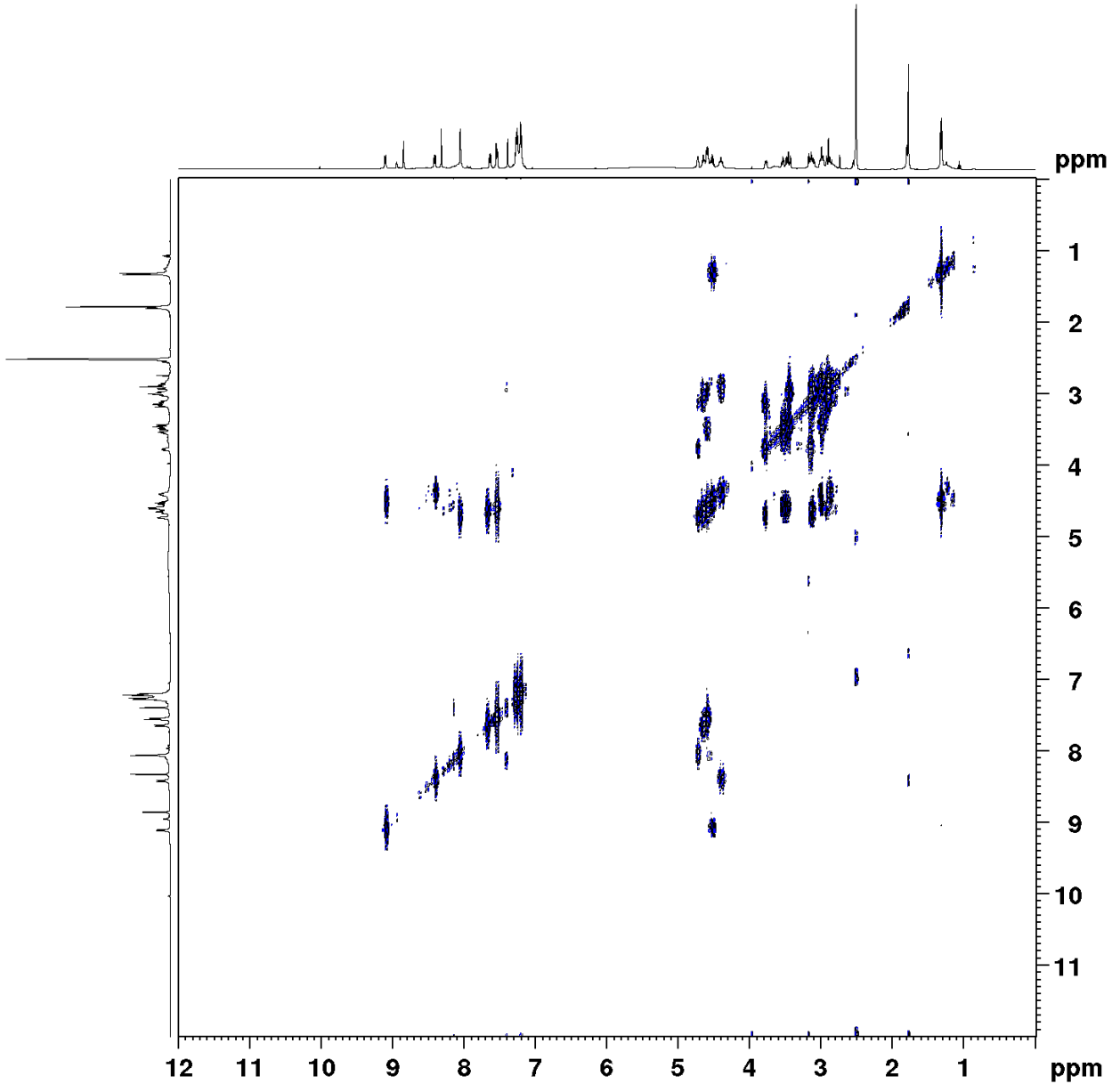
HMBC spectrum of polycycle 3.11 (DMSO-d_6)



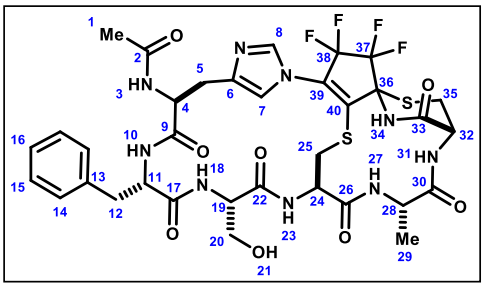
HSQC spectrum of polycycle 3.11 (DMSO-d_6)



COSY spectrum of polycycle 3.11 (DMSO-d_6)

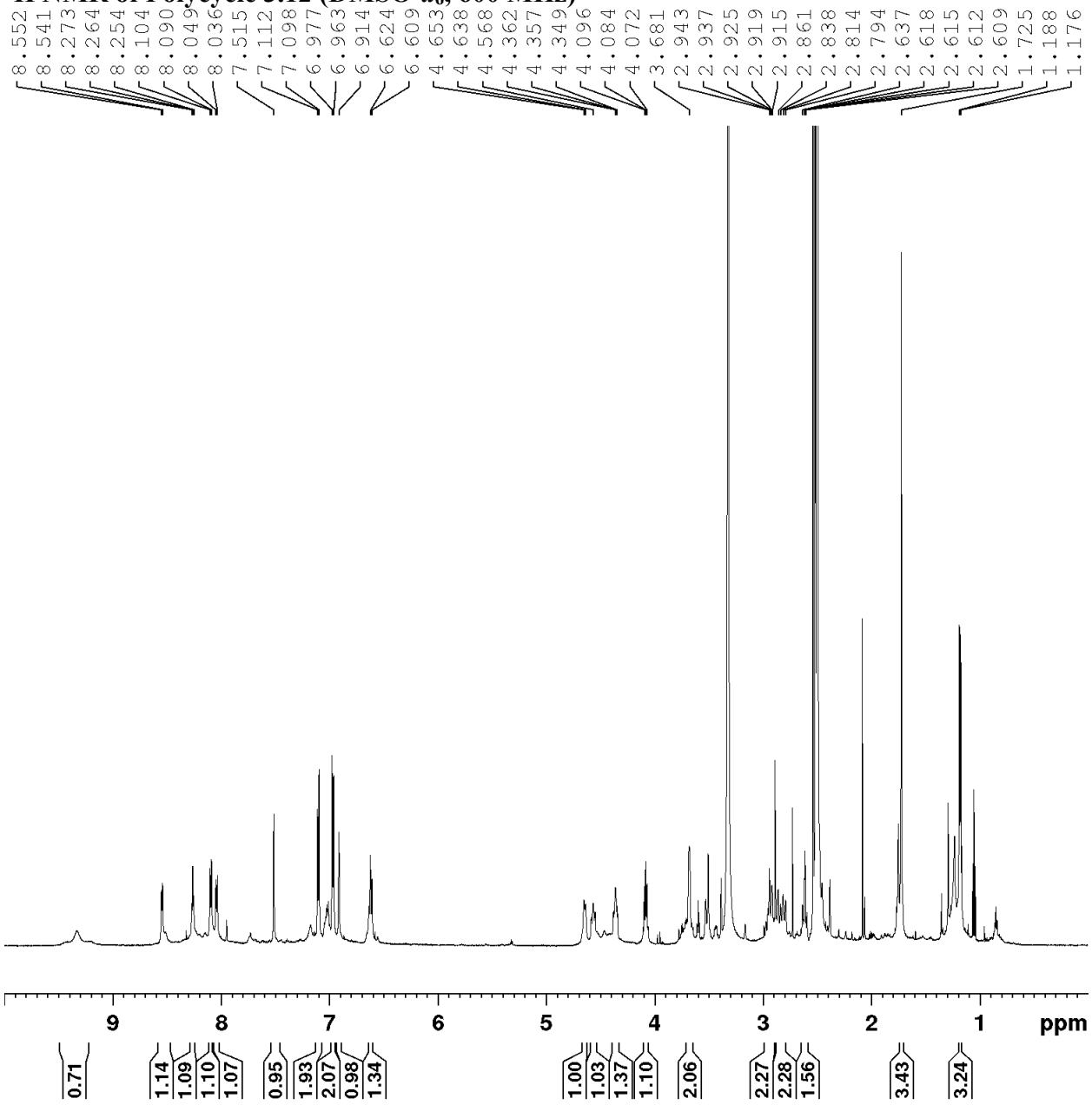


Polycycle **3.11** (DMSO-d_6, 298K)

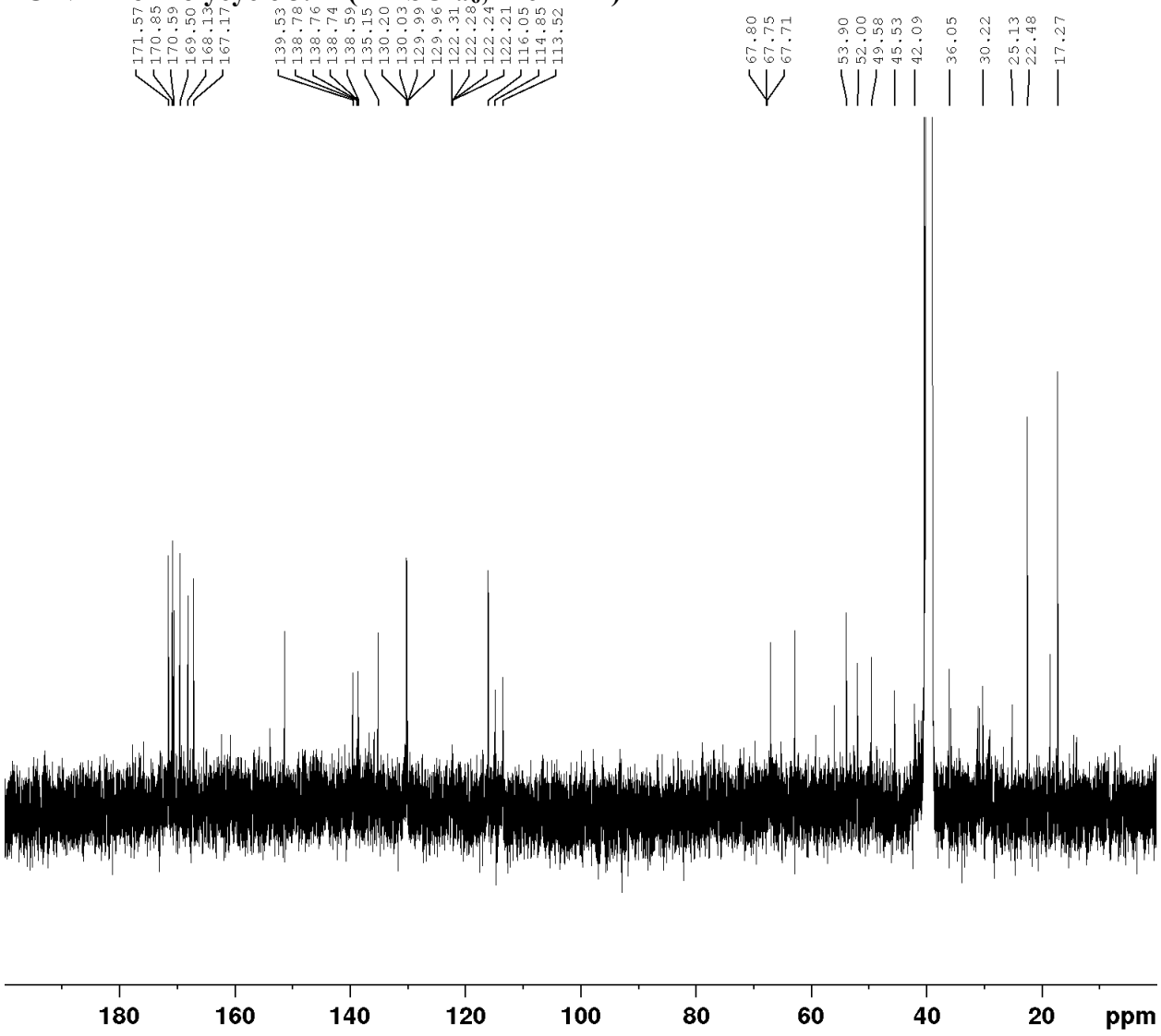


	13C	1H	Key Correlation
1	22.5	1.77 (s, 3H)	1 -> 2 HMBC
2	168.9	-	1 -> 2 HMBC
3	-	7.63 (d, J = 8.1 Hz, 1H)	3 -> 2, 4 HMBC
4	51.1	4.68 - 4.62 (m, 1H)	3 -> 4 HMBC
5	29.8	3.16 - 3.05 (m, 2H); 2.94 - 2.81 (m, 3H)	4 -> 5 HMBC
6	139.0	-	4, 5 -> 6 HMBC
7	114.5	7.38 (s, 1H)	8 -> 7 HMBC
8	135.6	8.07 - 8.01 (m, 2H)	8 -> 7 HMBC
9	170.0	-	4 -> 9 HMBC
10	-	8.41 (d, J = 8.7 Hz, 1H)	10 -> 9 HMBC
11	55.1	4.43 - 4.36 (m, 1H)	10 -> 11 HMBC
12	37.8	3.03 - 2.94 (m, 2H); 2.90 - 2.81 (m, 3H)	11 -> 12 HMBC
13	137.5	-	12 -> 13 HMBC
14	129.0	7.28 - 7.17 (m, 6H)	12 -> 14 HMBC
15	128.2	7.28 - 7.17 (m, 6H)	14 -> 15 HMBC
16	126.4	7.28 - 7.17 (m, 6H)	15 -> 16 HMBC
17	170.1	-	11, 19 -> 17 HMBC
18	-	7.28 - 7.17 (m, 6H)	-
19	53.8	4.62 - 4.56 (m, 2H)	20 -> 19 HMBC
20	61.3	3.57 - 3.39 (m, 3H)	19 -> 20 HMBC
21	-	-	-
22	170.3	-	19, 23 -> 22 HMBC
23	-	8.07 - 8.01 (m, 2H)	23 -> 24 COSY
24	51.6	4.75 - 4.70 (m, 1H)	23 -> 24 COSY
25	38.6	3.80 - 3.73 (m, 1H); 3.16 - 3.05 (m, 2H)	24 -> 25 HMBC
26	167.9	-	27 -> 26 HMBC
27	-	9.10 (d, J = 7.7, 1H)	27 -> 28 HMBC
28	48.5	4.52 (quint, J = 7.2, 1H)	29 -> 28 HMBC
29	16.0	1.31 (d, J = 7.3 Hz, 3H)	28 -> 29 HMBC
30	170.6	-	31 -> 30 HMBC
31	-	7.54 (d, J = 7.65 Hz, 1H)	32 -> 32, 35 HMBC
32	47.1	4.62 - 4.56 (m, 2H)	HMBC 34 -> 32 HMBC
33	167.5	-	34 -> 35 HMBC
34	-	8.84 (s, 1H)	34 -> 36 HMBC
35	30.8	3.47 - 3.41 (m, 1H) 3.03 - 2.94 (m, 2H)	35 -> 36 HMBC
36	70.4 - 69.7 (m, 1C)	-	35 -> 36 HMBC
37	117.6 - 115.8 (m, 2C)	-	
38	117.6 - 115.8 (m, 2C)	-	
39	141.3 - 140.9 (m, 1C)	-	
40	132.8 - 132.6 (m, 1C)	-	

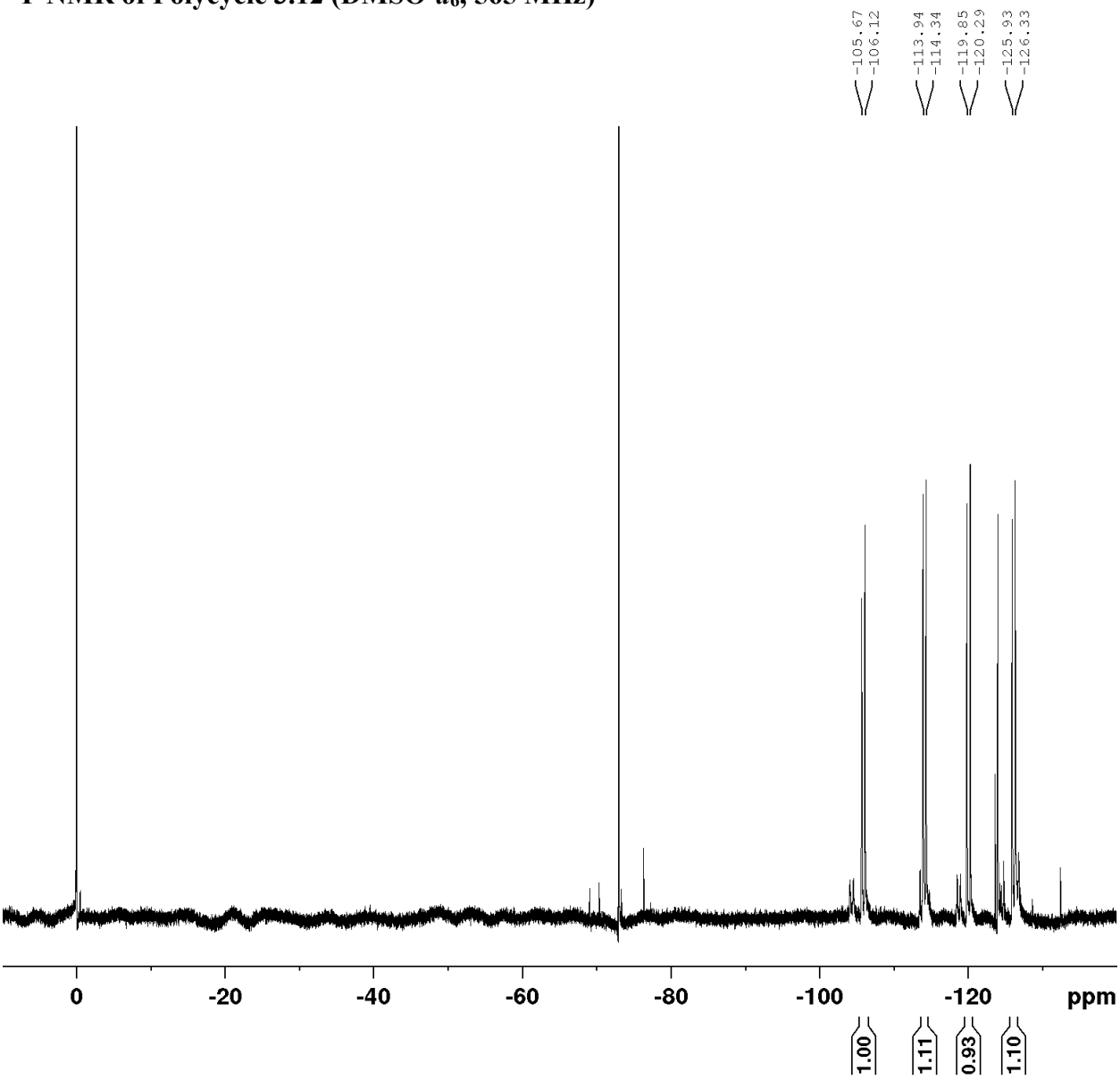
^{1}H NMR of Polycycle 3.12 (DMSO-d_6, 600 MHz)



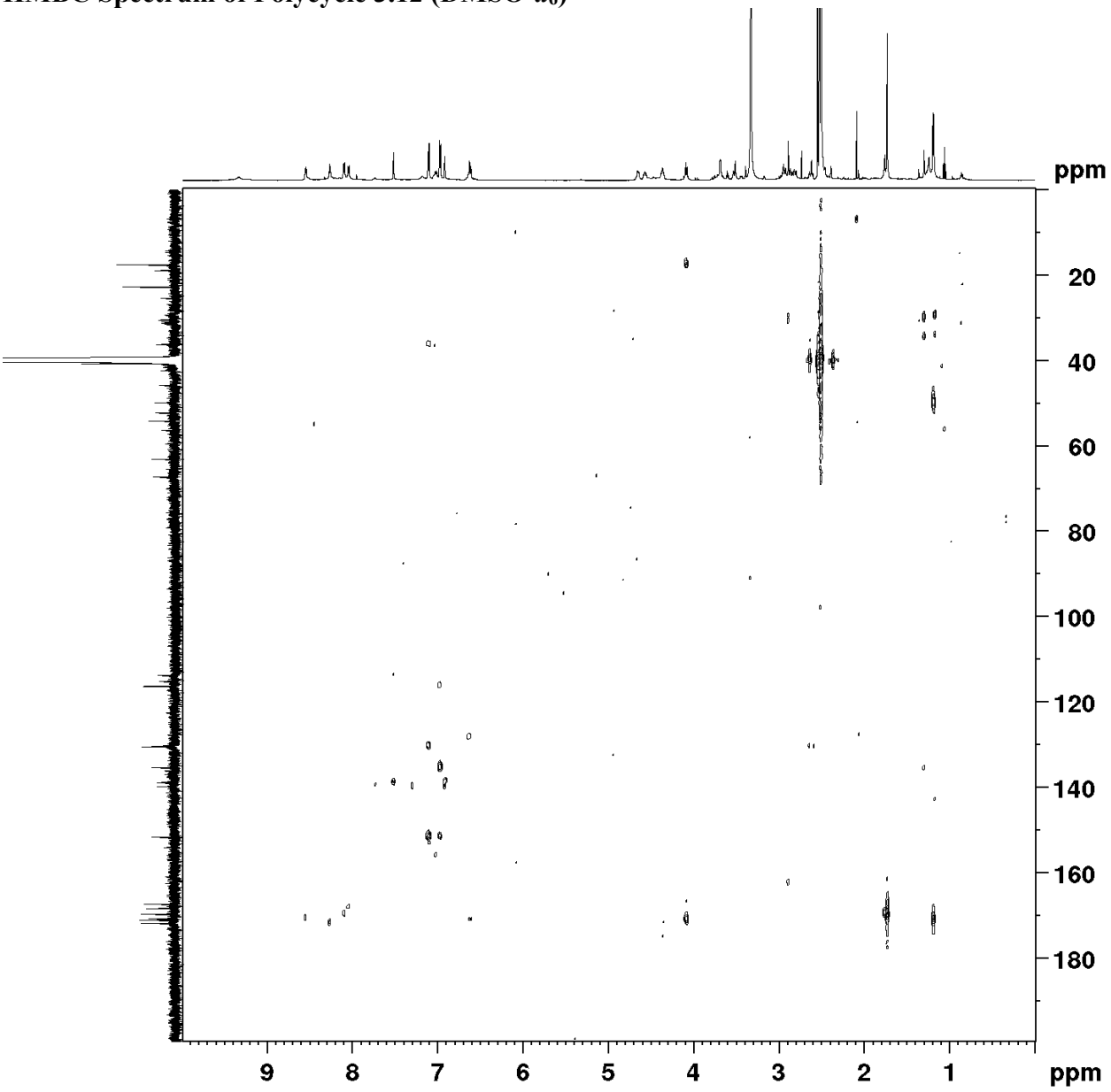
^{13}C NMR of Polycycle 3.12 (DMSO-d_6, 126 MHz)



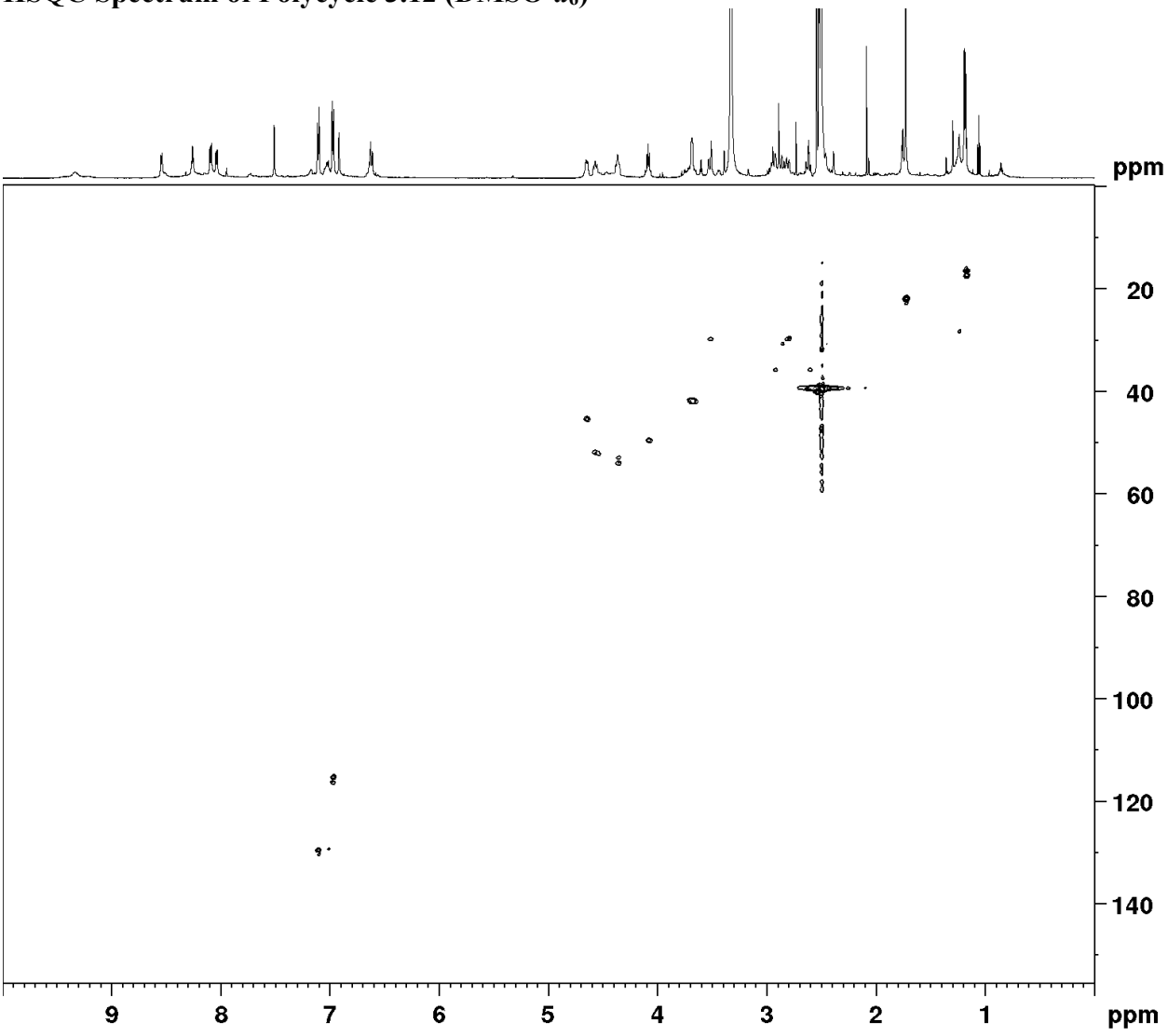
^{19}F NMR of Polycycle 3.12 (DMSO-d_6, 565 MHz)



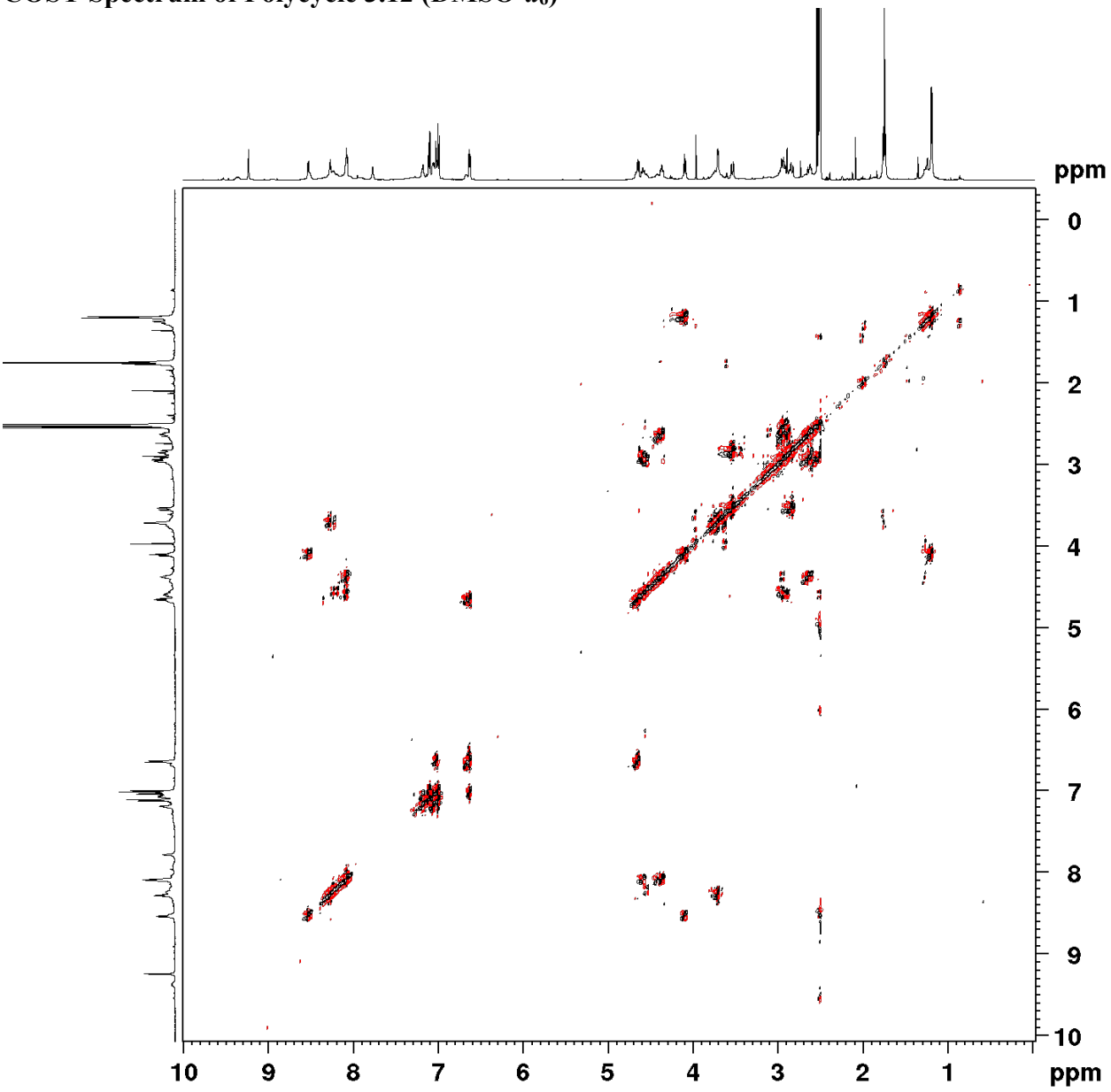
HMBC Spectrum of Polycycle 3.12 (DMSO-d_6)



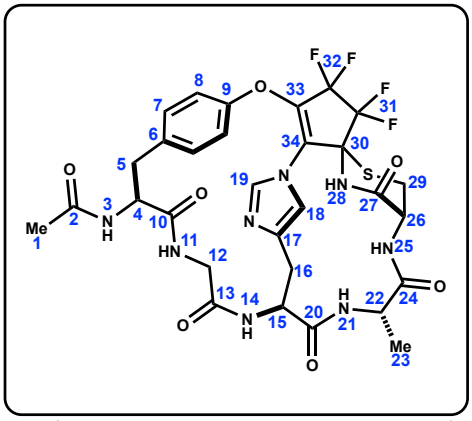
HSQC Spectrum of Polycycle 3.12 (DMSO-d_6)



COSY Spectrum of Polycycle 3.12 (DMSO-d_6)

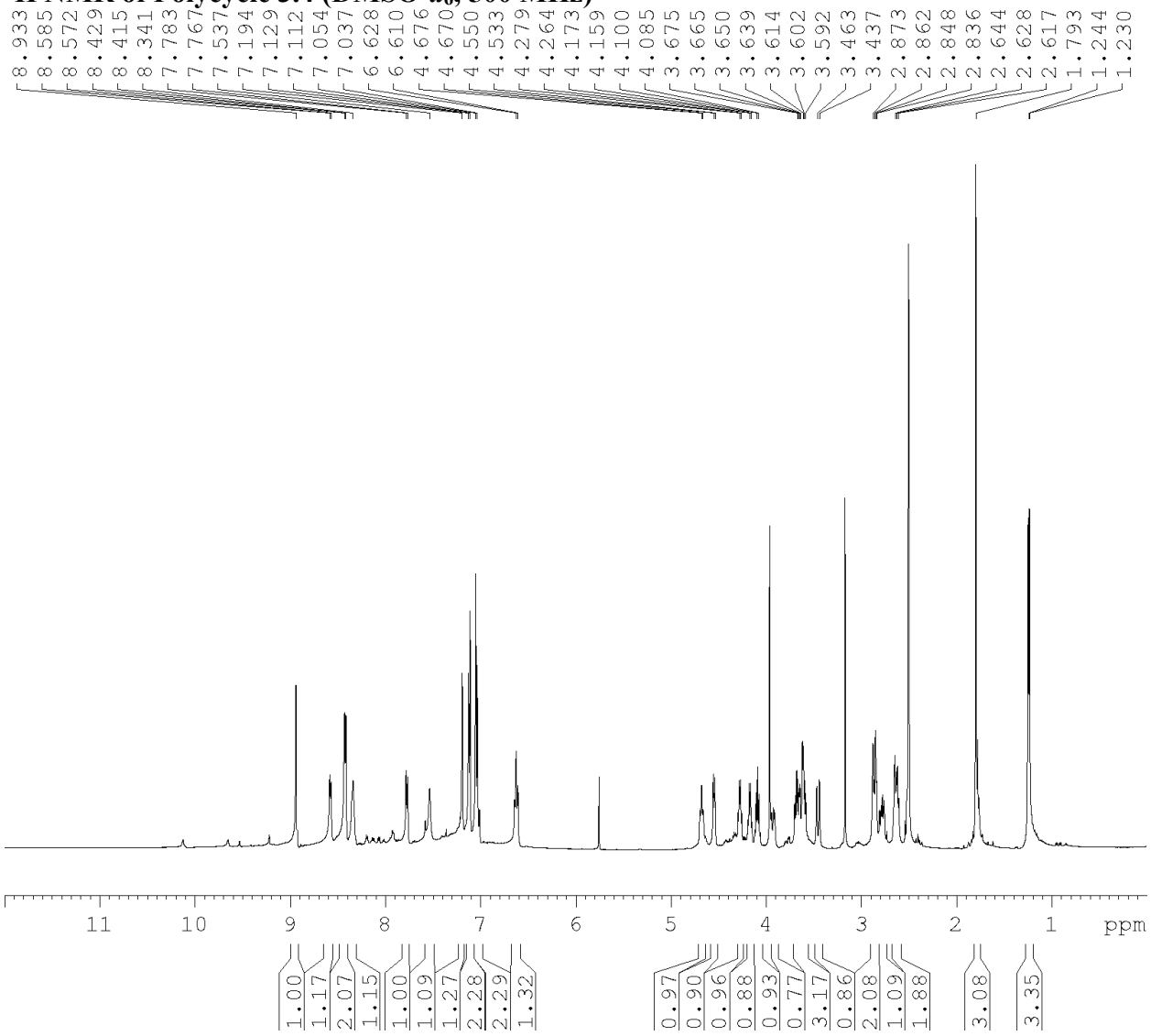


Polycycle **3.12** (DMSO-d_6, 298K)

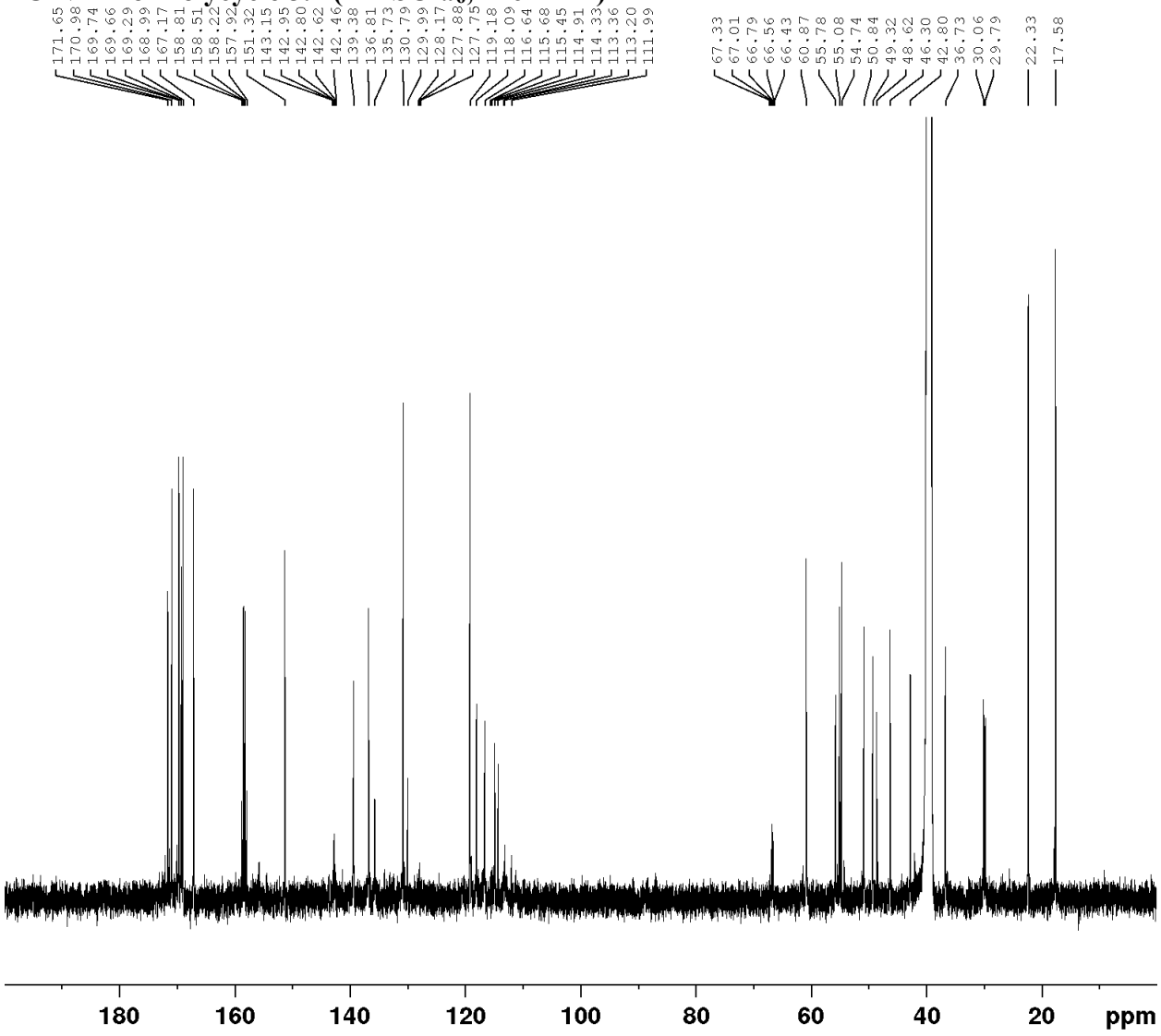


	13C	1H	Key Correlation
1	22.48	1.72 (s, 1H)	HSQC
2	169.50	–	1->2 HMBC
3	–	8.10 (d, J = 8.16 Hz, 1H)	3 ->2 HMBC
4	53.90	4.40-4.32 (m, 1H)	3 ->4 COSY, HSQC
5	36.05	2.66-2.58 (m, 2H)	4->5 COSY, HSQC
6	135.15	–	5 ->6 HMBC
7	130.20	7.10 (d, J = 8.58 Hz, 1H)	7->6 HMBC, HSQC
8	116.04	6.97 (d, J = 8.58 Hz, 1H)	7 ->8 HMBC, 7-> 8 COSY, HSQC
9	151.37	–	8 ->9 HMBC
10	171.57	–	11 ->10, 4->10HMBC
11	–	8.26 (t, J = 5.46 Hz, 1H)	11->10 HMBC, 11->12 COSY
12	42.10	3.72-3.64 (m, 2H)	11->12 HMBC, HSQC
13	168.13	–	14->13, 12->13 HMBC
14	–	8.04 (d, J = 7.74 Hz, 1H)	14->13 HMBC
15	52.00	4.60-4.54 (m, 1H)	15->16 HMBC, 15>16 COSY, HSQC
16	25.13	3.00-2.91 (m, 2H)	16->15 HMBC, 15->16 COSY, HSQC
17	138.59	–	16->17, 18->17 HMBC
18	113.52	6.91 (s, 1H)	18->17 HMBC, HSQC
19	139.53	7.51 (s, 1H)	HSQC
20	170.85	–	21->20, 15->20 HMBC
21	–	6.64-6.60 (m, 1H)	21 ->20 HMBC
22	49.58	4.08 (m, 1H)	22->23 COSY, HSQC
23	17.26	1.18 (d, J = 7.38 Hz, 1H)	HSQC
24	170.59	–	22->24 HMBC
25	–	8.55 (d, J = 6.54 Hz, 1H)	25->24 HMBC
26	45.53	4.64 (d, J = 8.82 Hz, 1H)	25 ->27 HMBC, HSQC
27	167.17	–	28->27, 26->27 HMBC
28	–	9.33 (s, 1H)	–
29	30.22	2.88-2.78 (m, 2H)	26->29 HMBC, 26->29 COSY, HSQC
30	67.8-67.7 (m, 1C)	–	28->30 HMBC
31	122.20-122.30 (m, 1C)	–	–
32	122.20-122.30 (m, 1C)	–	–
33	138.74-138.77 (m, 1C)	–	–
34	129.96-130.03 (m, 1C)	–	–

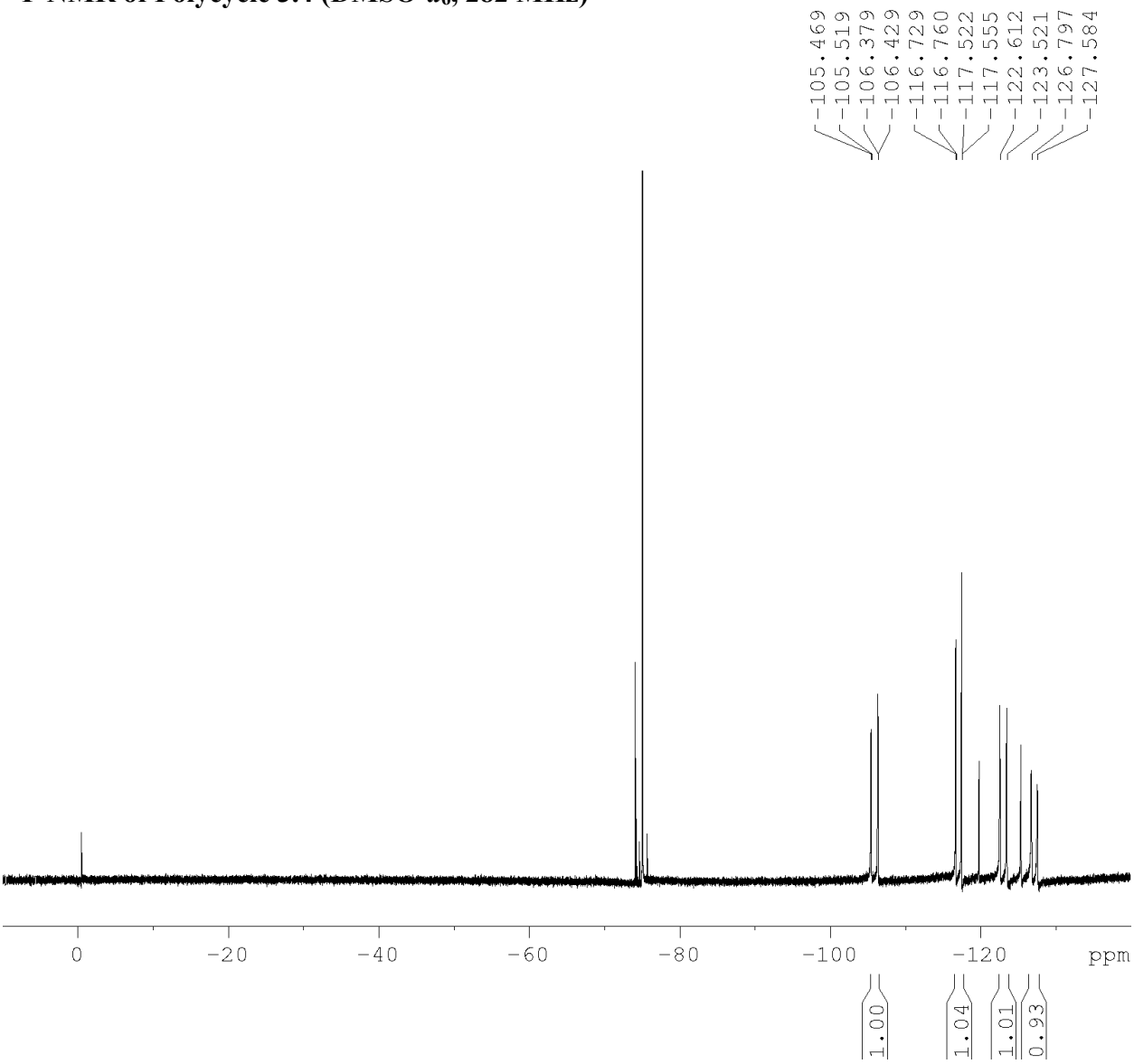
^{1}H NMR of Polycycle 3.4 (DMSO-d_6, 500 MHz)



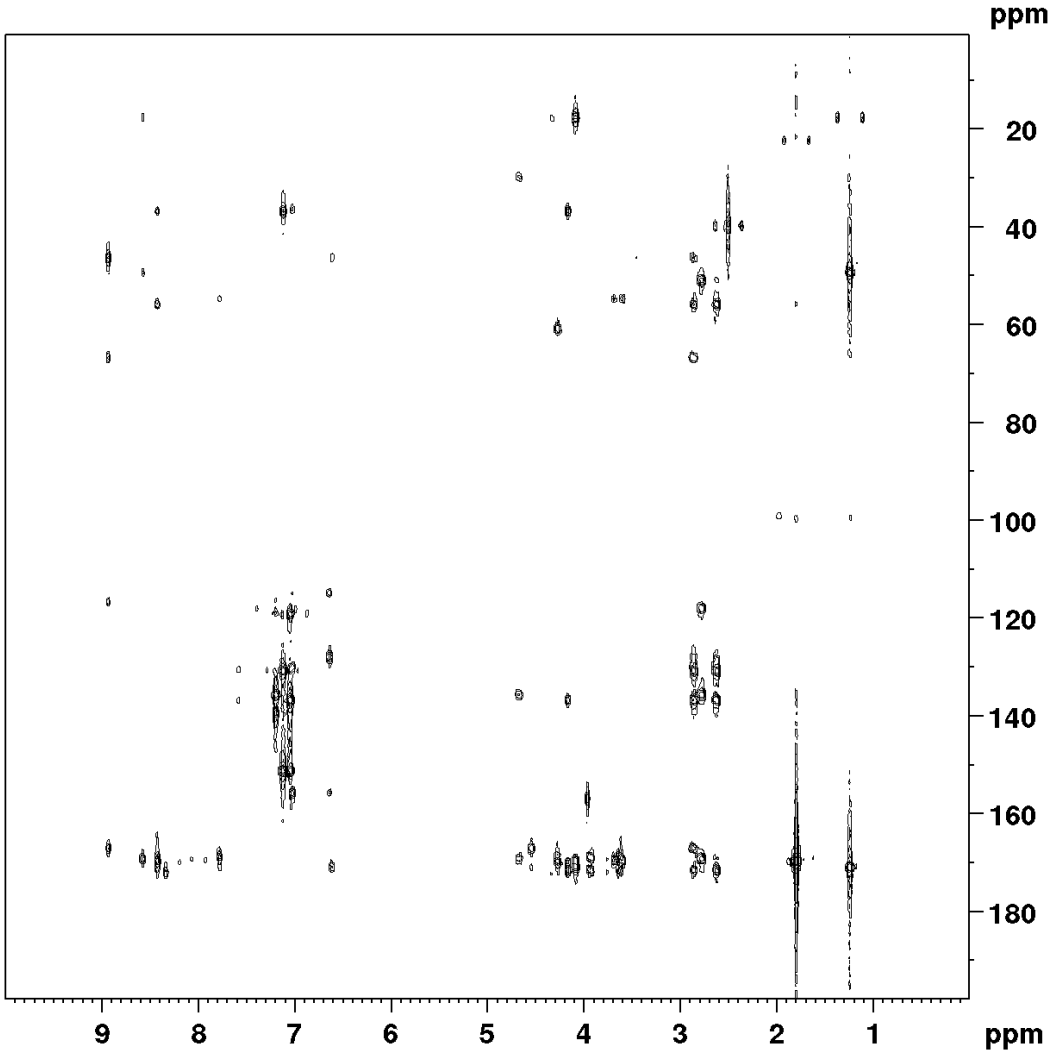
^{13}C NMR of Polycycle 3.4 (DMSO-d_6, 126 MHz)



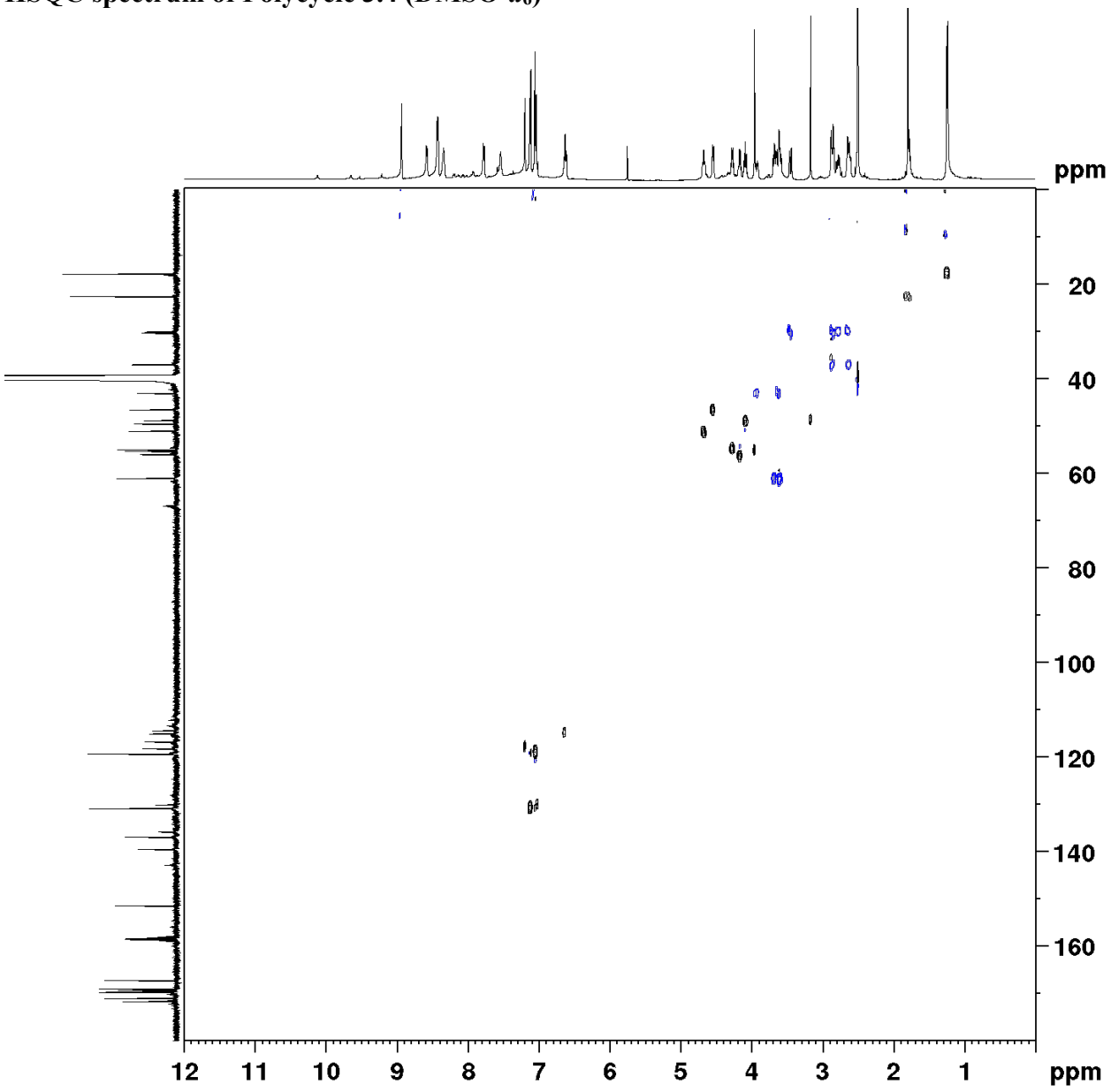
^{19}F NMR of Polycycle 3.4 (DMSO-d_6, 282 MHz)



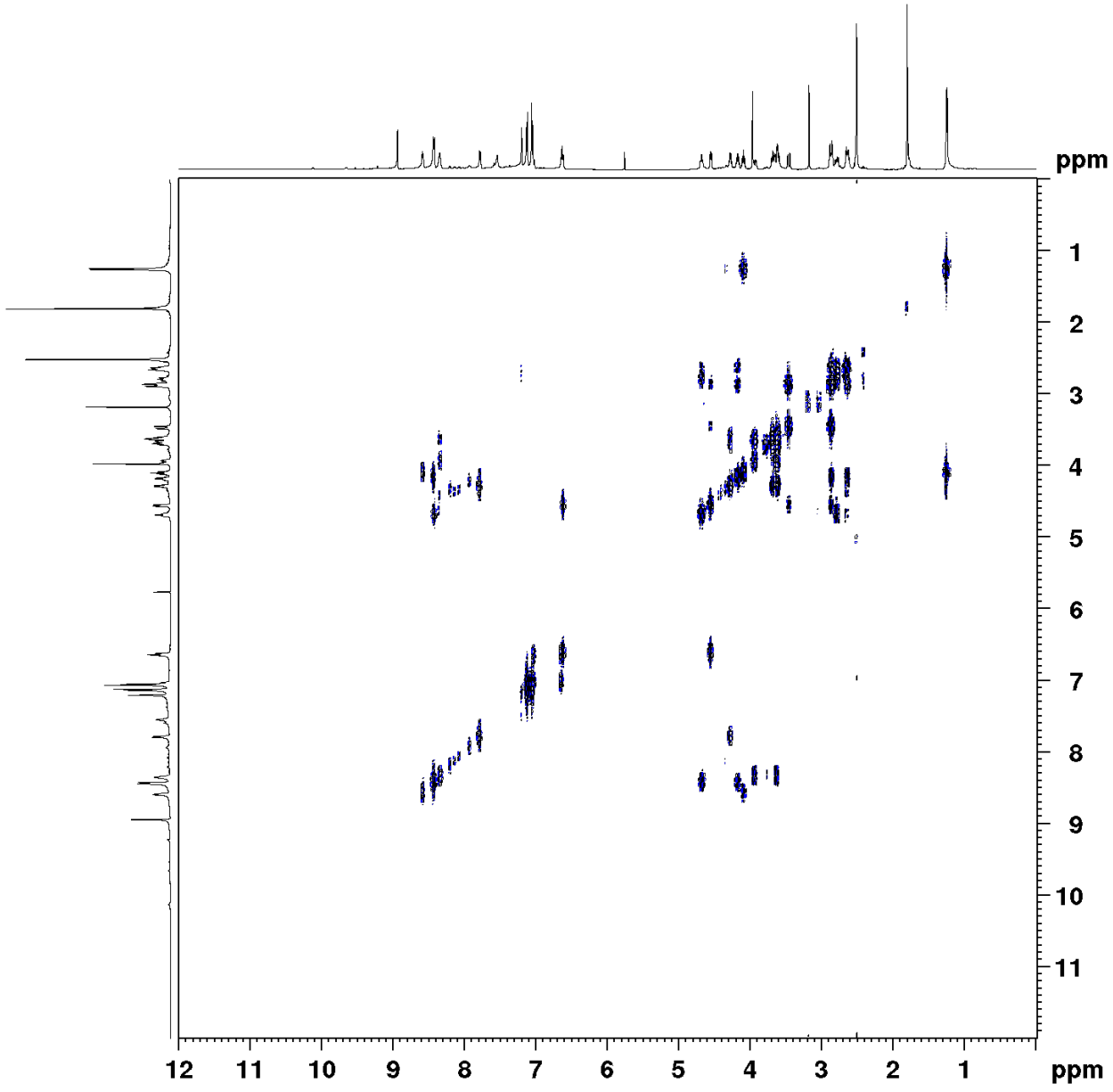
HMBC of Polycycle 3.4 (DMSO-d_6)



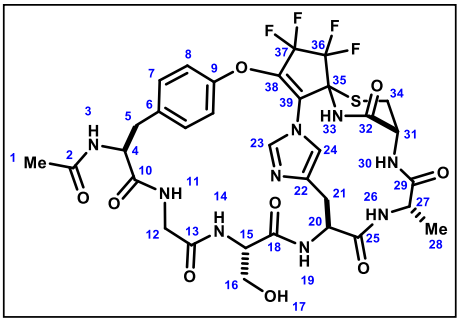
HSQC spectrum of Polycycle 3.4 (DMSO-d_6)



COSY spectrum of Polycycle 3.4 (DMSO-d_6)

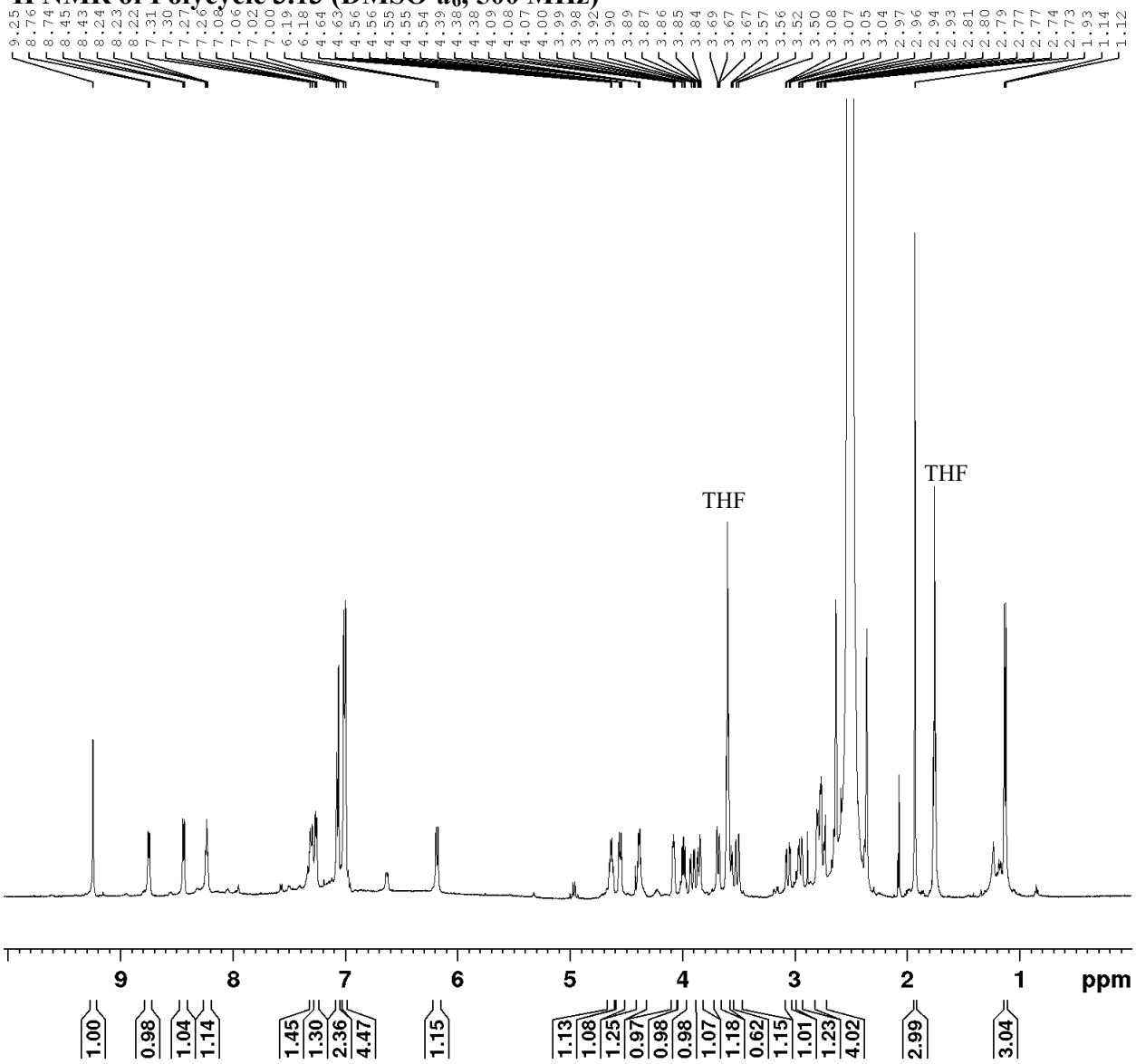


Polycycle **3.4** (DMSO-d_6, 298K)

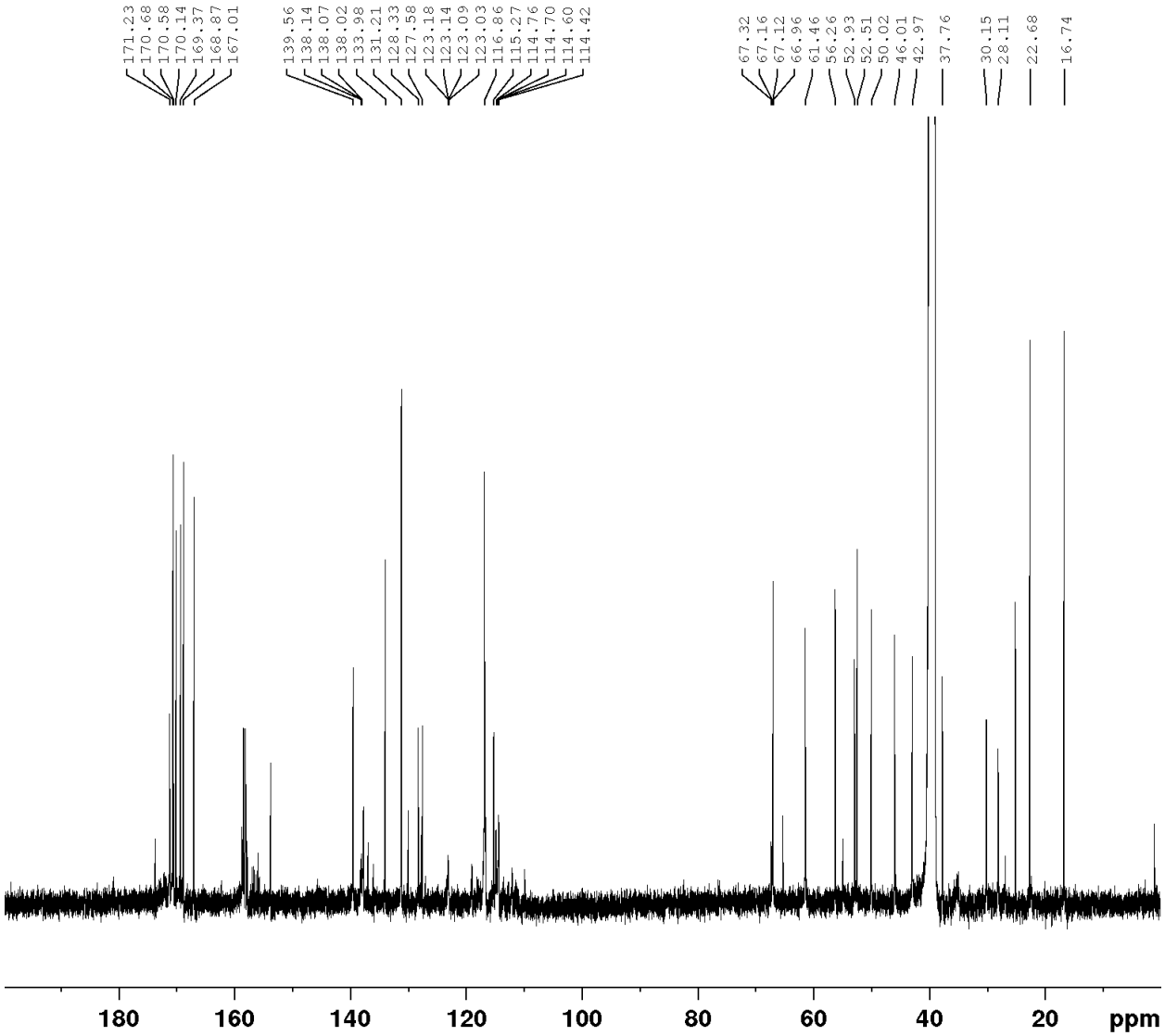


	13C	1H	Key Correlation
1	22.3	1.79 (s, 1H)	1 -> 2 HMBC
2	169.7	-	1, 3 -> 2 HMBC
3	-	8.42 (d, J = 6.6 Hz, 2H)	3 -> 2, 4, 5 HMBC
4	55.8	4.17 (q, J = 7.0 Hz, 1H)	3, 5 -> 4
5	36.7	2.90 - 2.83 (m, 2H); 2.68 - 2.58 (m, 2H)	5 -> 4, 6 HMBC
6	136.8	-	4, 5 -> 6 HMBC
7	130.8	7.15 - 7.10 (m, 2H)	5 -> 7 HMBC
8	119.2	7.10 - 7.00 (m, 2H)	7 -> 8 HMBC
9	151.0	-	7 -> 9 HMBC
10	171.6	-	4 -> 10 HMBC
11	-	8.34 (s, 1H)	11 -> 10 HMBC
12	42.8	3.95 - 3.87 (m, 1H); 3.72 - 3.56 (m, 3H)	11 -> 12 HMBC
13	169.0	-	12 -> 13 HMBC
14	-	7.78 (d, J = 7.8 Hz, 1H)	14 -> 13 HMBC
15	54.7	4.27 (q, J = 6.4 Hz, 1H)	14 -> 15 HMBC
16	60.9	3.72 - 3.56 (m, 3H)	15 -> 16 HMBC
17	-	-	-
18	169.7	-	15, 16 -> HMBC
19	-	8.42 (d, J = 6.6 Hz, 2H)	19 -> 20 HMBC
20	50.8	4.71 - 4.64 (m, 1H)	19 -> 20 HMBC
21	29.8	2.82 - 2.73 (m, 1H); 2.68 - 2.58 (m, 2H)	20 -> 21 HMBC
22	135.7	-	21 -> 22 HMBC
23	139.4	7.54 (s, 1H)	24 -> 23 HMBC
24	118.09	7.19 (s, 1H)	23 -> 24 HMBC
25	169.3	-	26 -> 25 HMBC
26	-	8.58 (d, J = 7.0 Hz, 1H)	26 -> 25 HMBC
27	49.3	4.09 (quint, J = 7.1 Hz, 1H)	28 -> 27 HMBC
28	17.6	1.24 (d, J = 7.2 Hz, 3H)	27 -> 28 HMBC
29	171.0	-	28 -> 29 HMBC
30	-	6.67 - 6.58 (m, 1H)	30 -> 31, 32 HMBC
31	46.3	4.58 - 4.51 (m, 1H)	32 -> 32, 34 HMBC
32	167.2	-	33 -> 32 HMBC
33	-	8.93 (s, 1H)	33 -> 35 HMBC
34	30.1	3.45 (d, J = 13.1 Hz, 1H) ; 2.90 - 2.83 (m, 2H)	31 -> 34 HMBC
35	67.3 - 66.4 (m, 1C)	-	34 -> 35 HMBC
36	119.7 - 113.0 (m, 2C)	-	33 -> 36 HMBC
37	119.7 - 113.0 (m, 2C)	-	
38	143.1 - 142.5 (m, 1C)	-	
39	128.2 - 127.8 (m, 1C)	-	

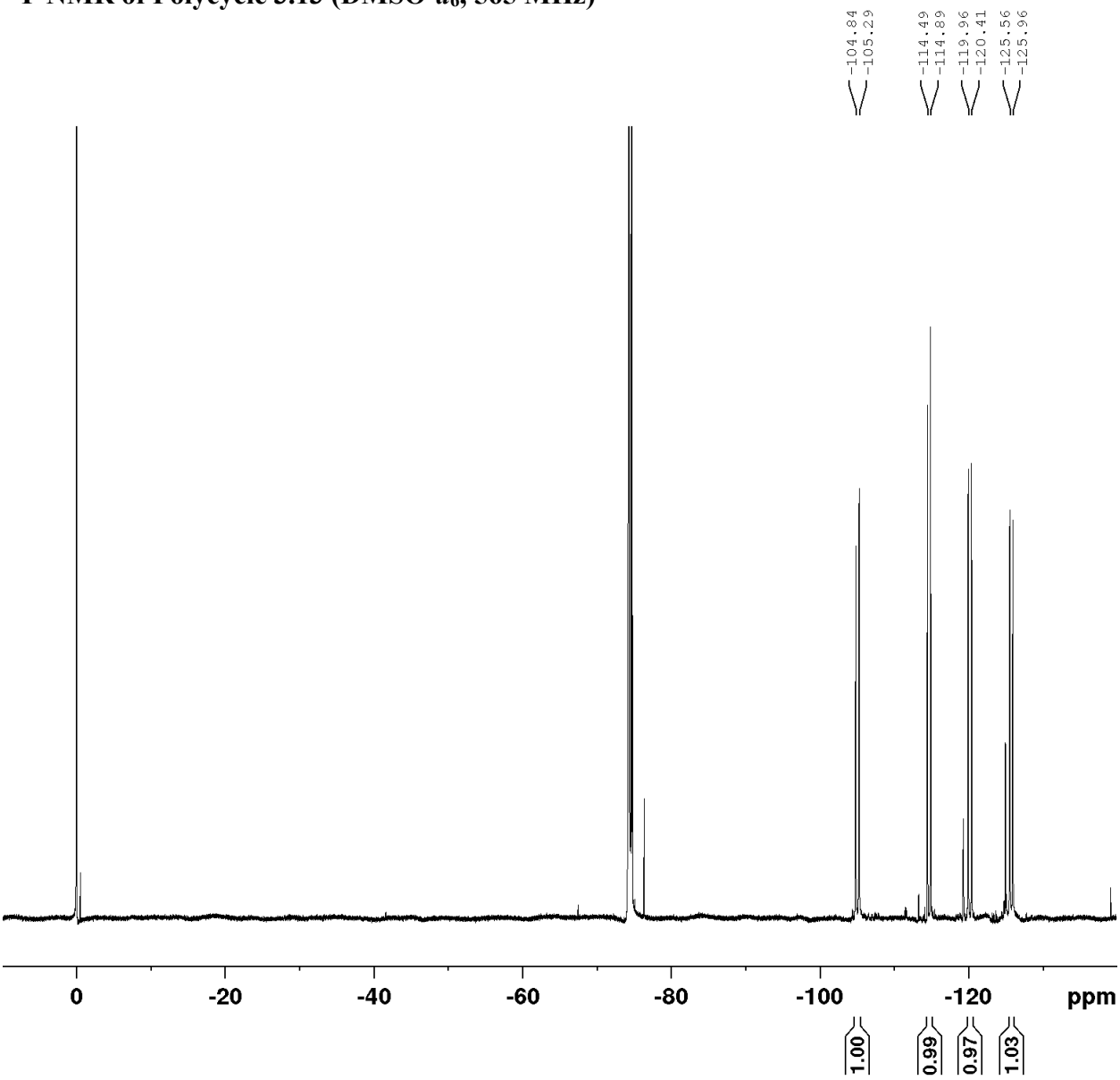
^{1}H NMR of Polycycle 3.13 (DMSO-d_6, 500 MHz)



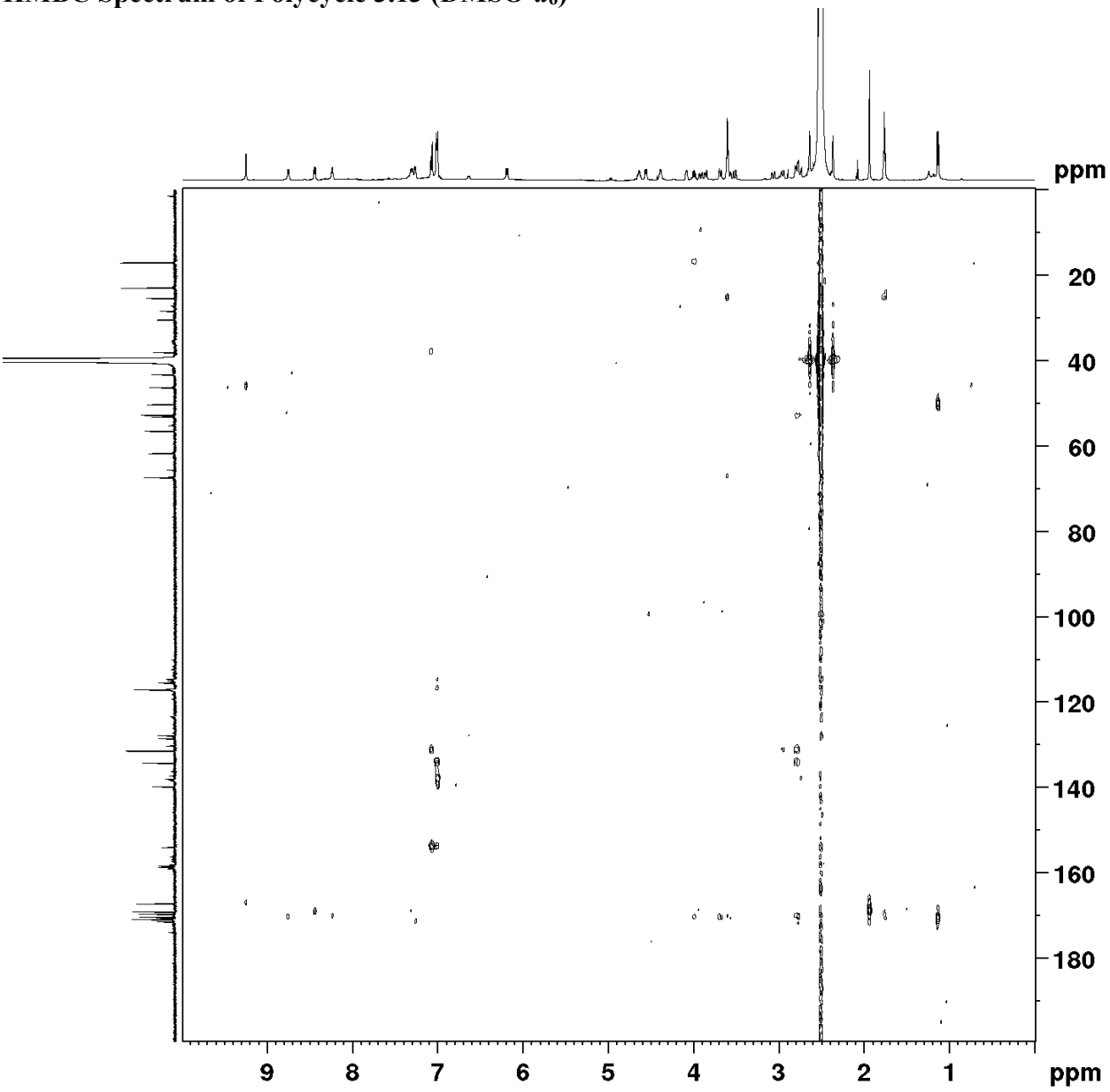
^{13}C NMR of Polycycle 3.13 (DMSO-d_6, 126 MHz)



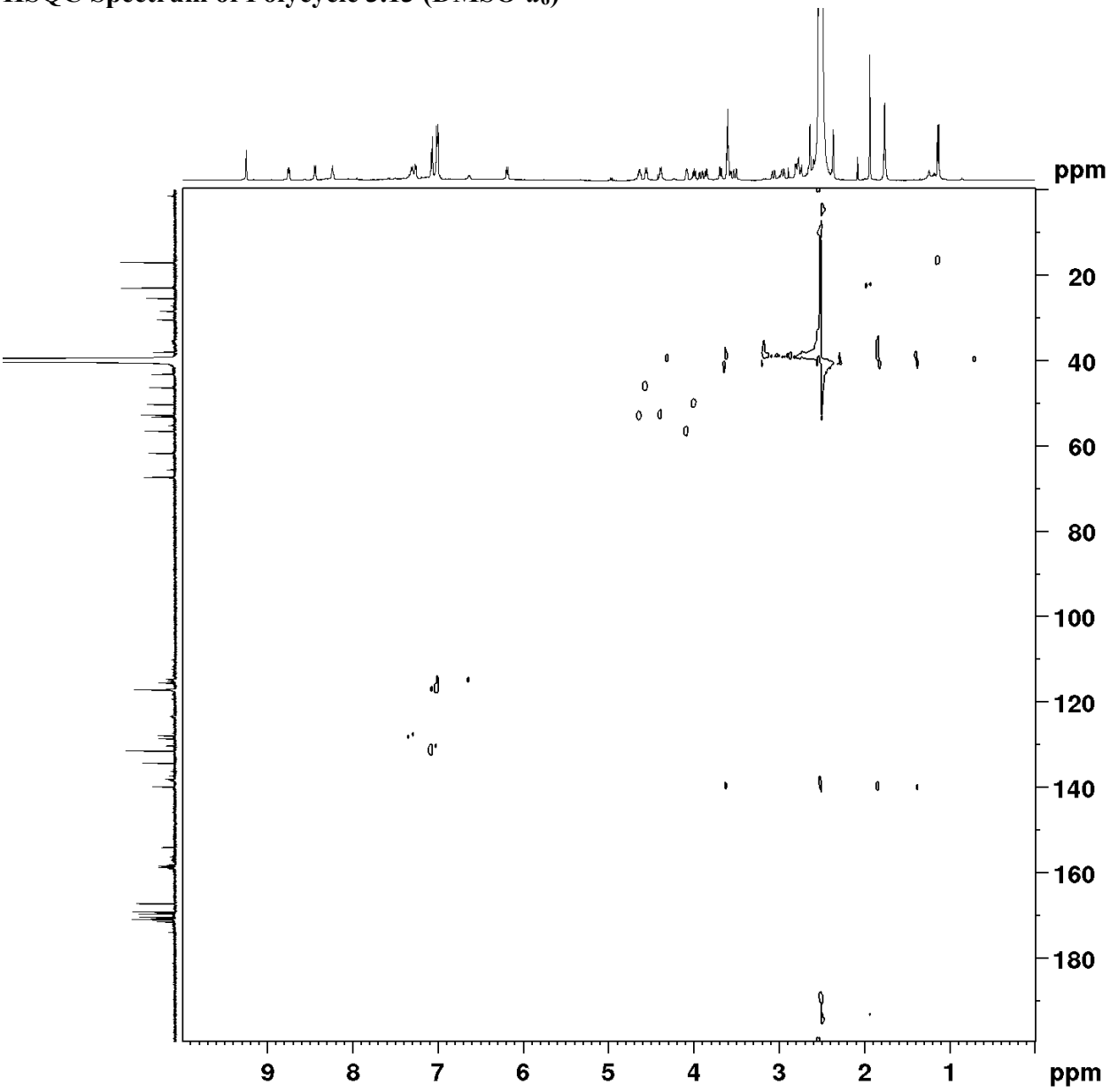
^{19}F NMR of Polycycle 3.13 (DMSO-d_6, 565 MHz)



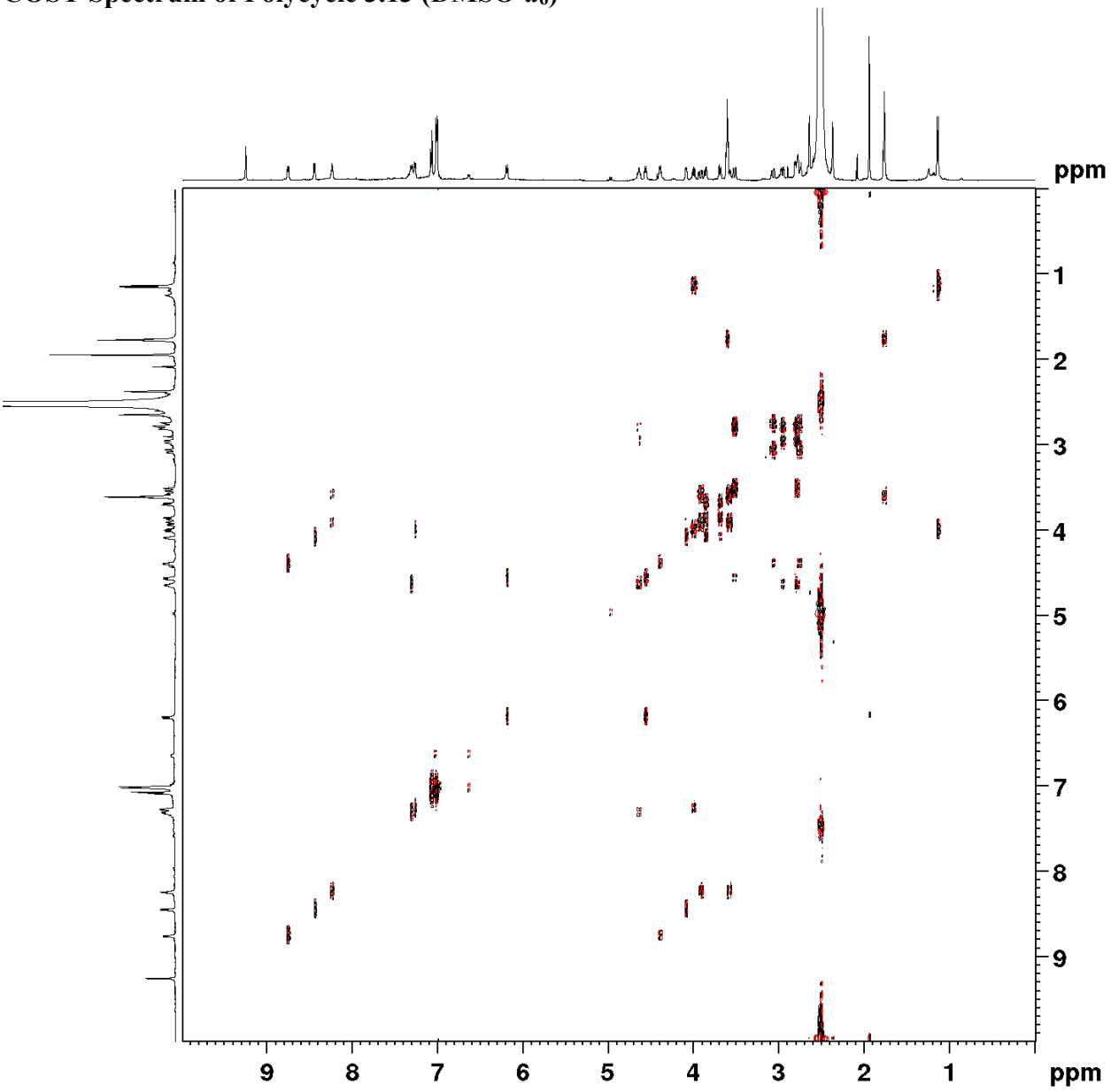
HMBC Spectrum of Polycycle 3.13 (DMSO-d_6)



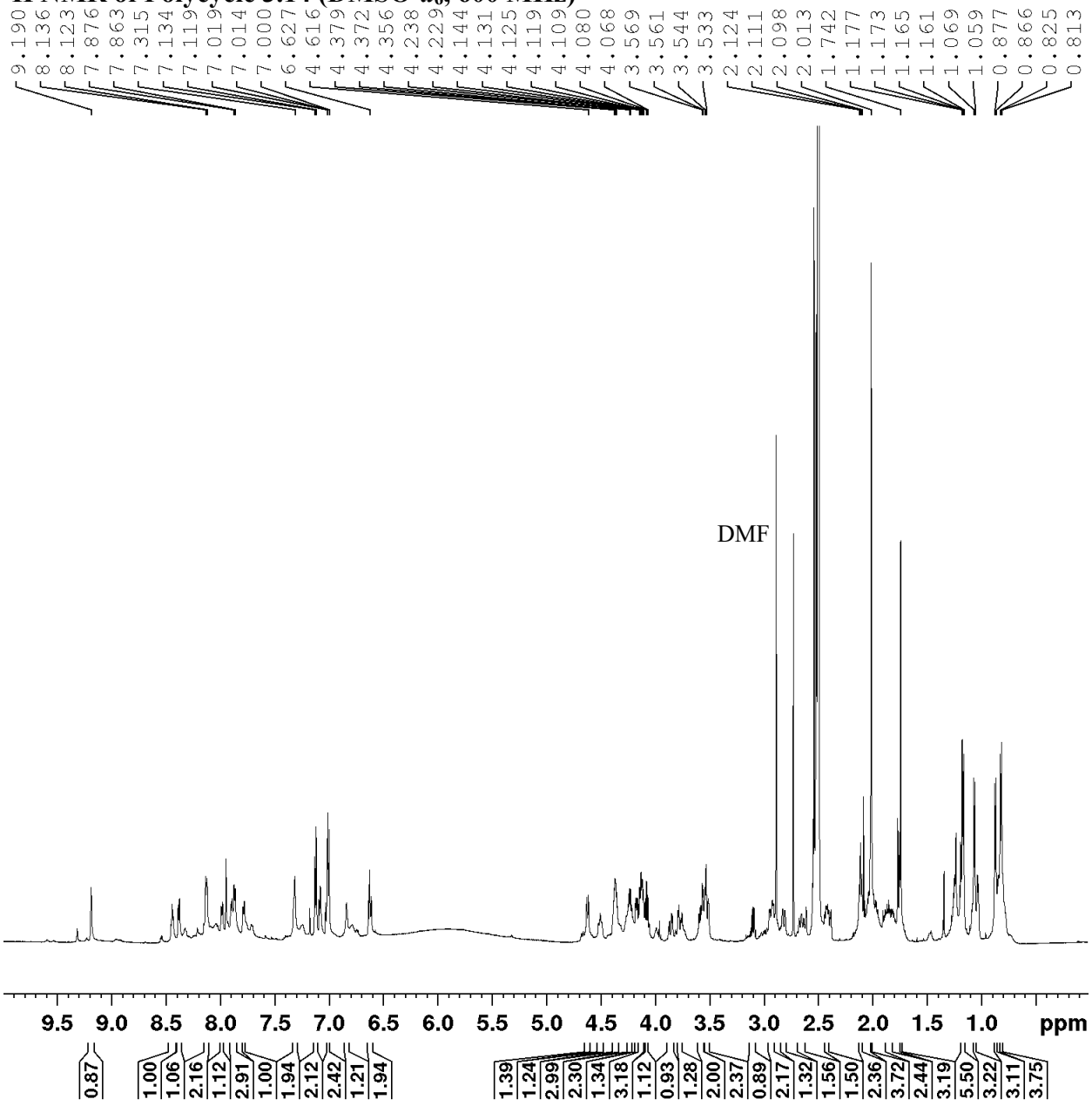
HSQC Spectrum of Polycycle 3.13 (DMSO-d_6)



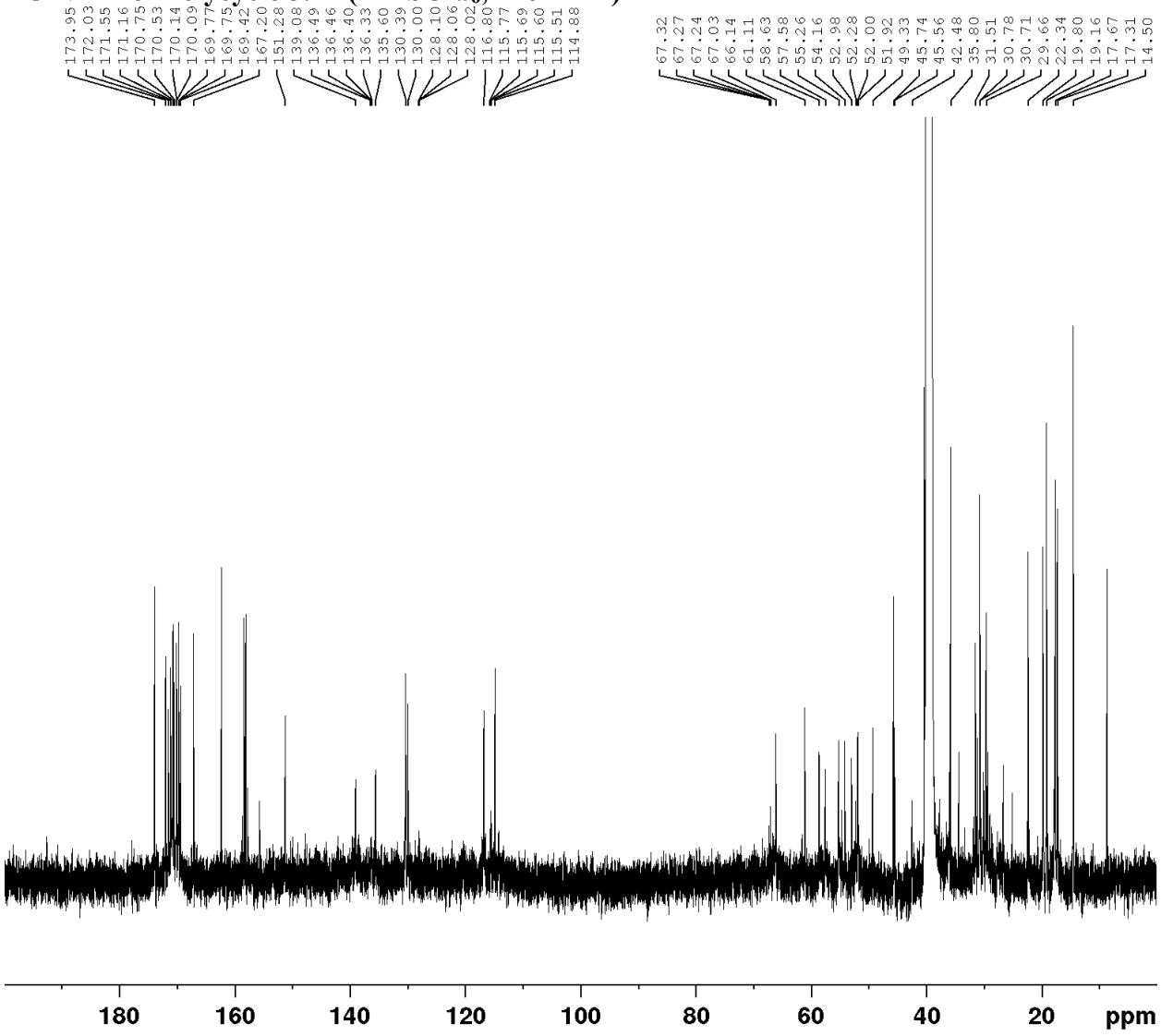
COSY Spectrum of Polycycle 3.13 (DMSO-d_6)



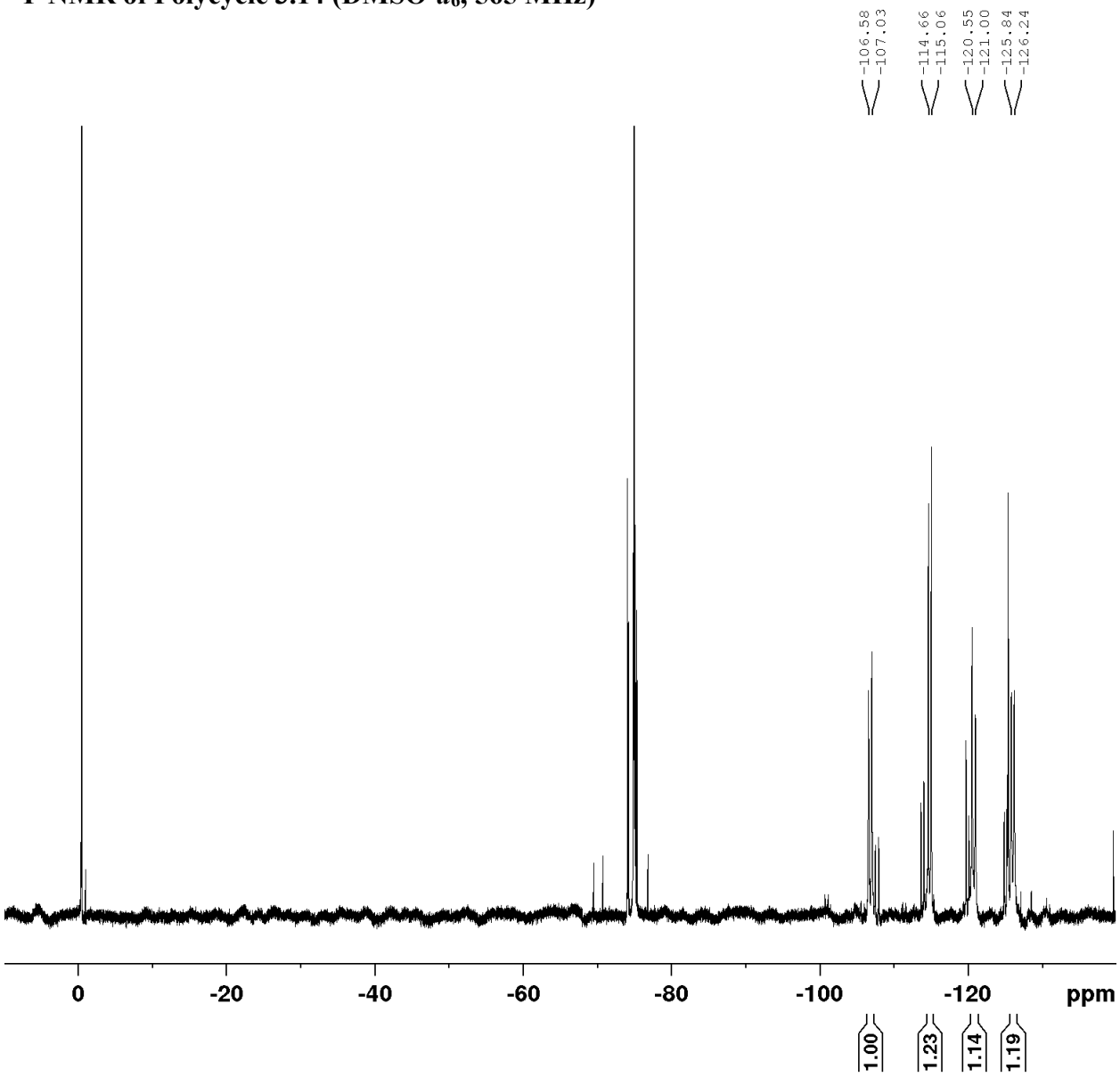
^{1}H NMR of Polycycle 3.14 (DMSO-d_6, 600 MHz)



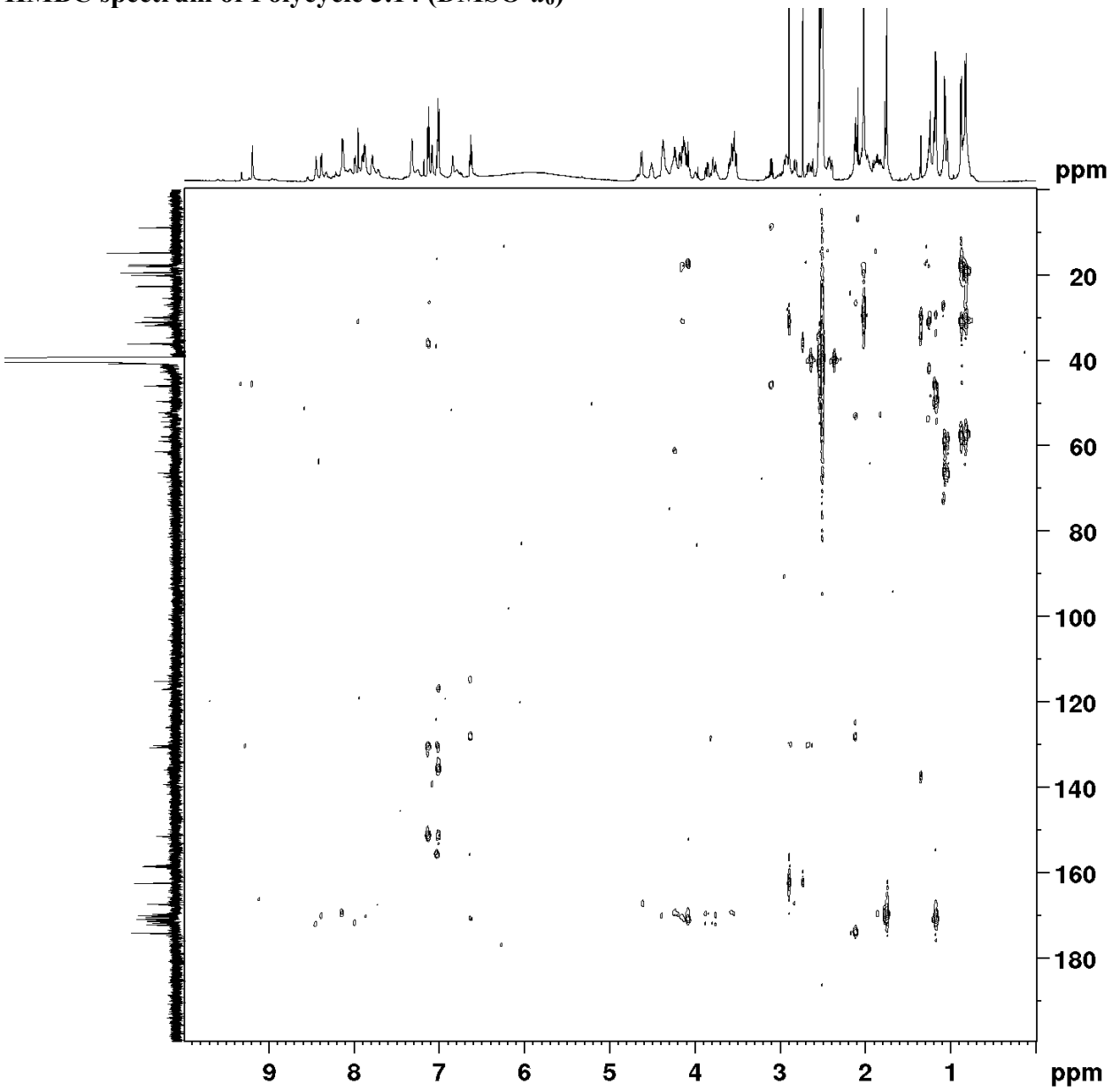
^{13}C NMR of Polycycle 3.14 (DMSO-d_6, 126 MHz)



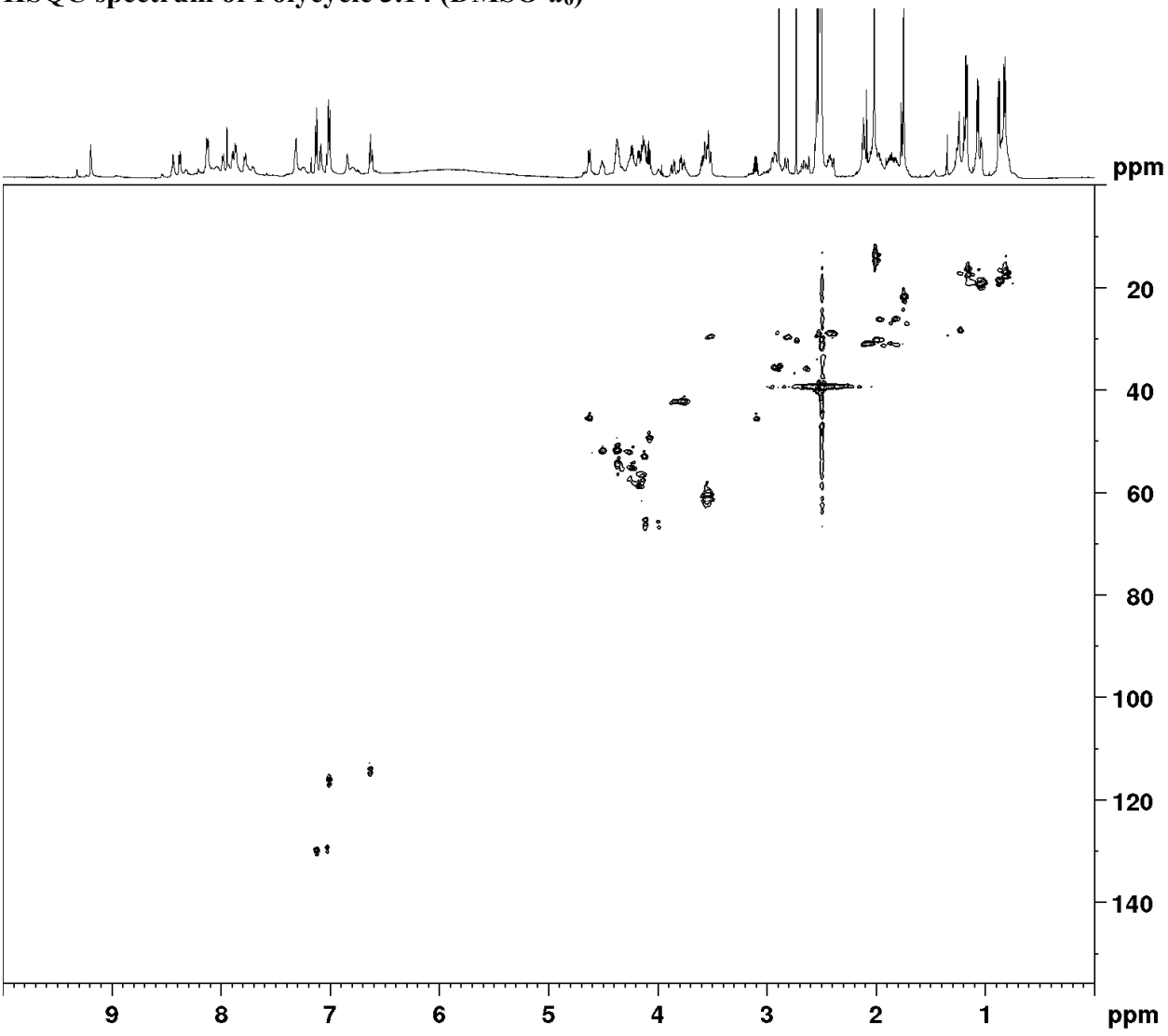
^{19}F NMR of Polycycle 3.14 (DMSO-d_6, 565 MHz)



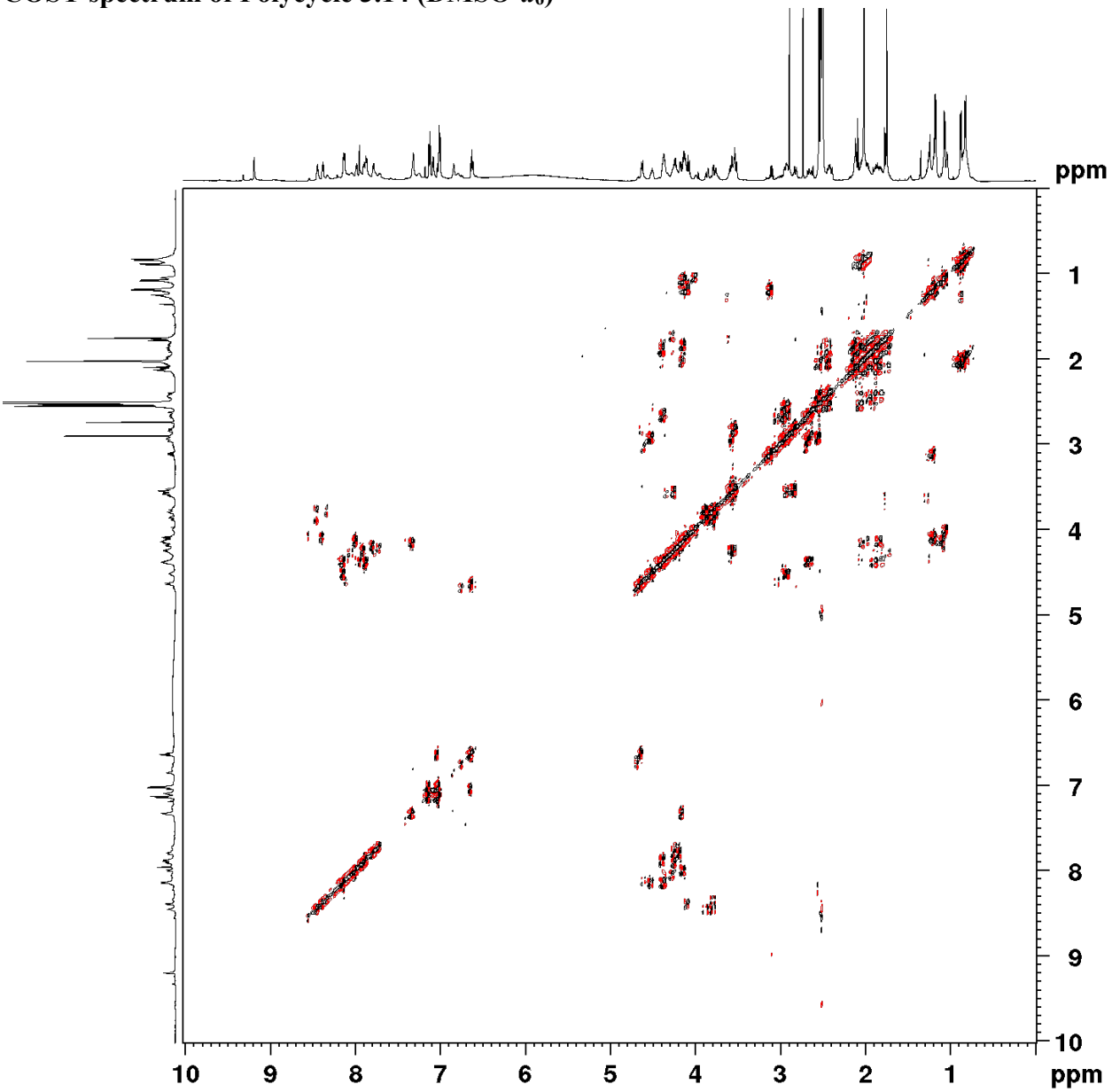
HMBC spectrum of Polycycle 3.14 (DMSO-d_6)



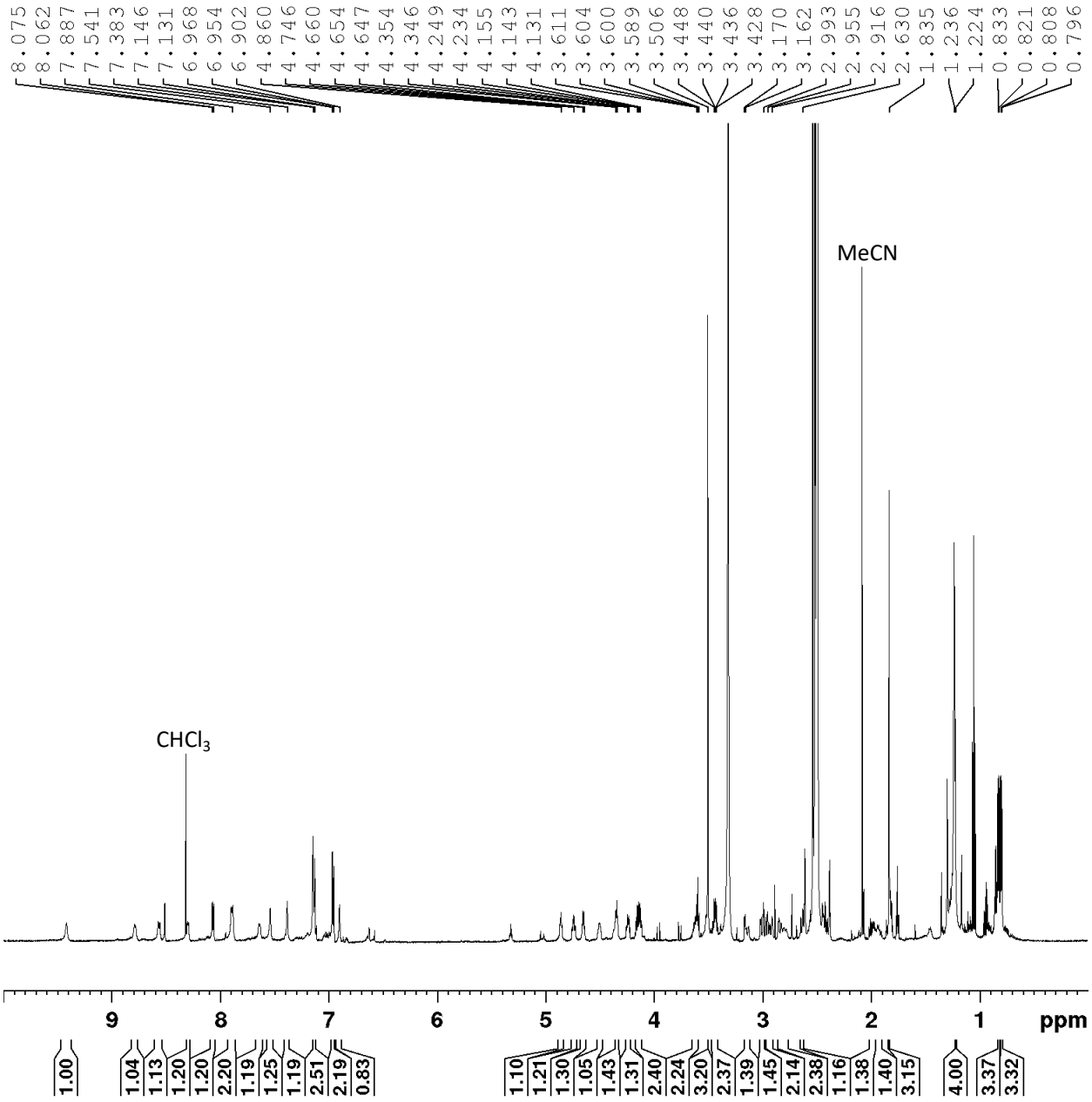
HSQC spectrum of Polycycle 3.14 (DMSO-d_6)



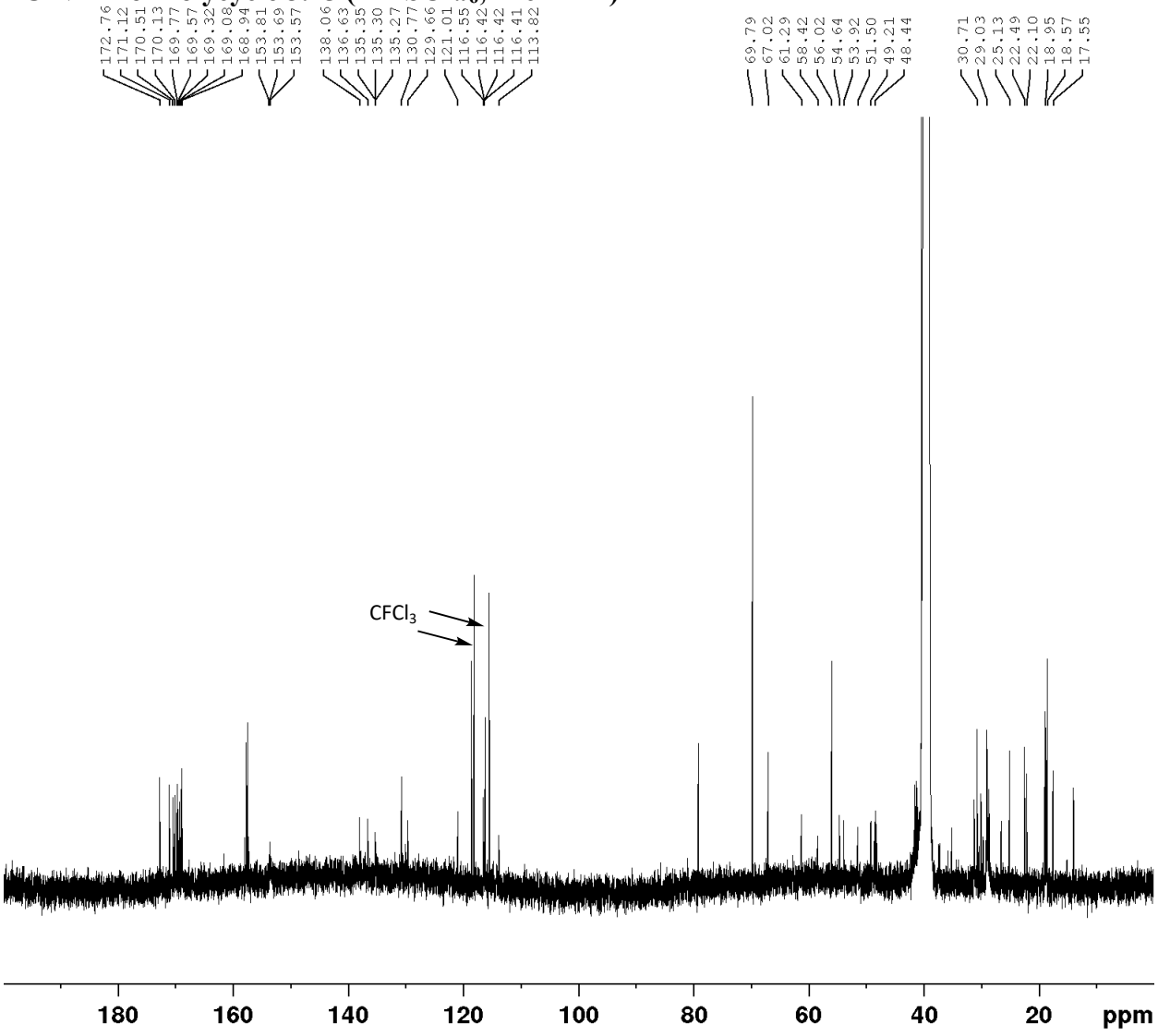
COSY spectrum of Polycycle 3.14 (DMSO-d_6)



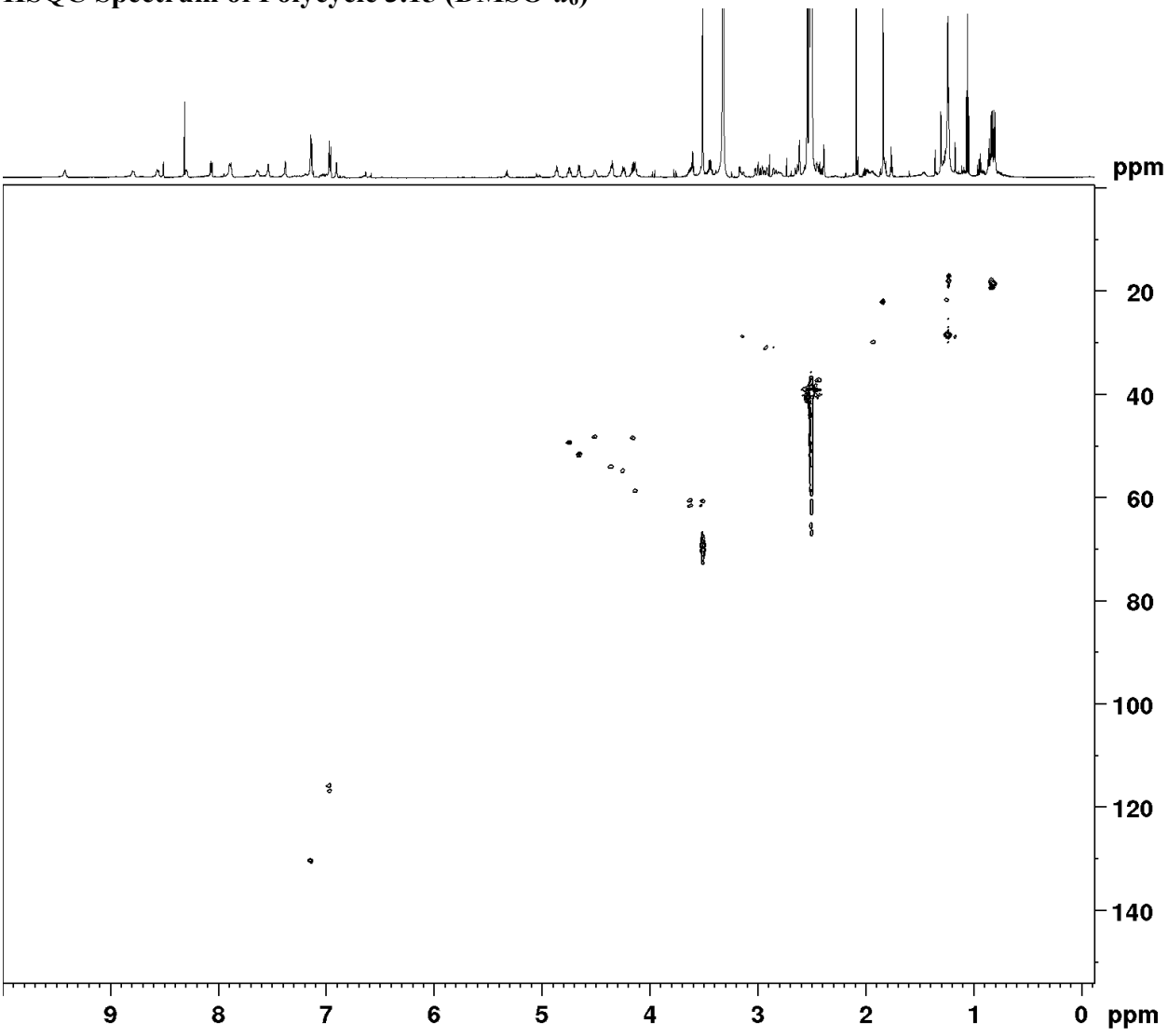
^{1}H NMR of Polycycle 3.15 (DMSO-d_6, 600 MHz)



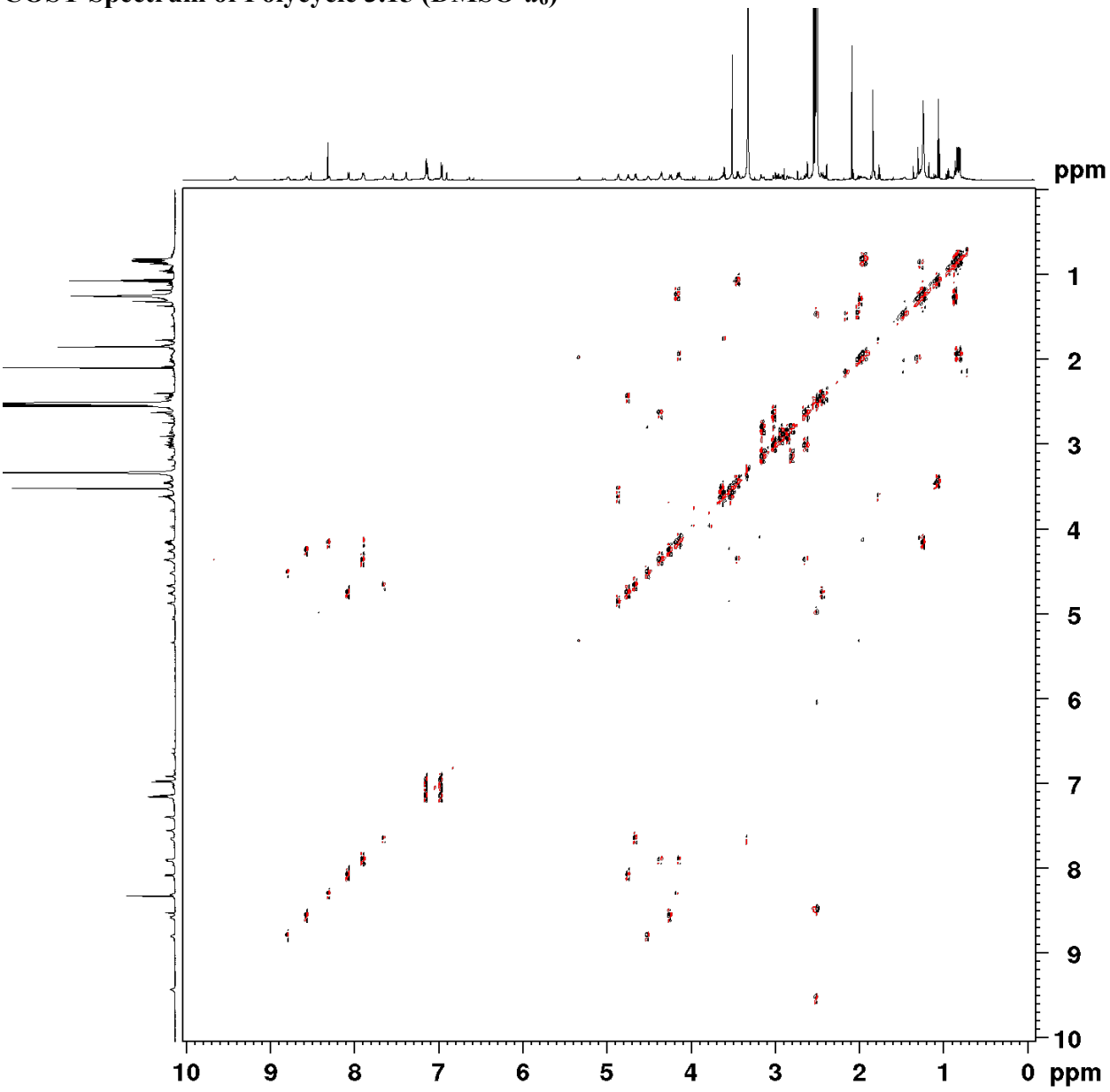
^{13}C NMR of Polycycle 3.15 (DMSO-d_6, 126 MHz)



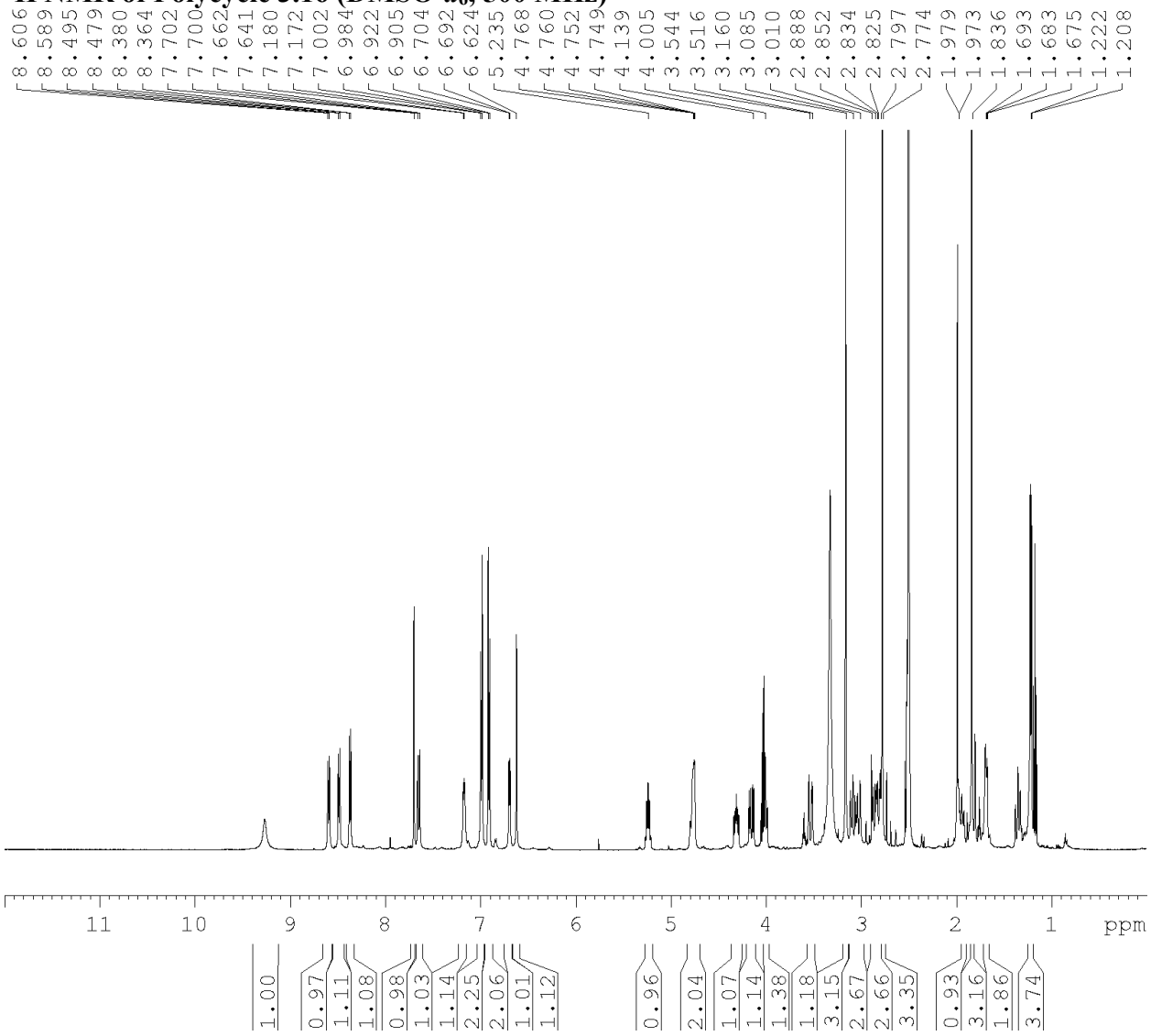
HSQC Spectrum of Polycycle 3.15 (DMSO-d_6)



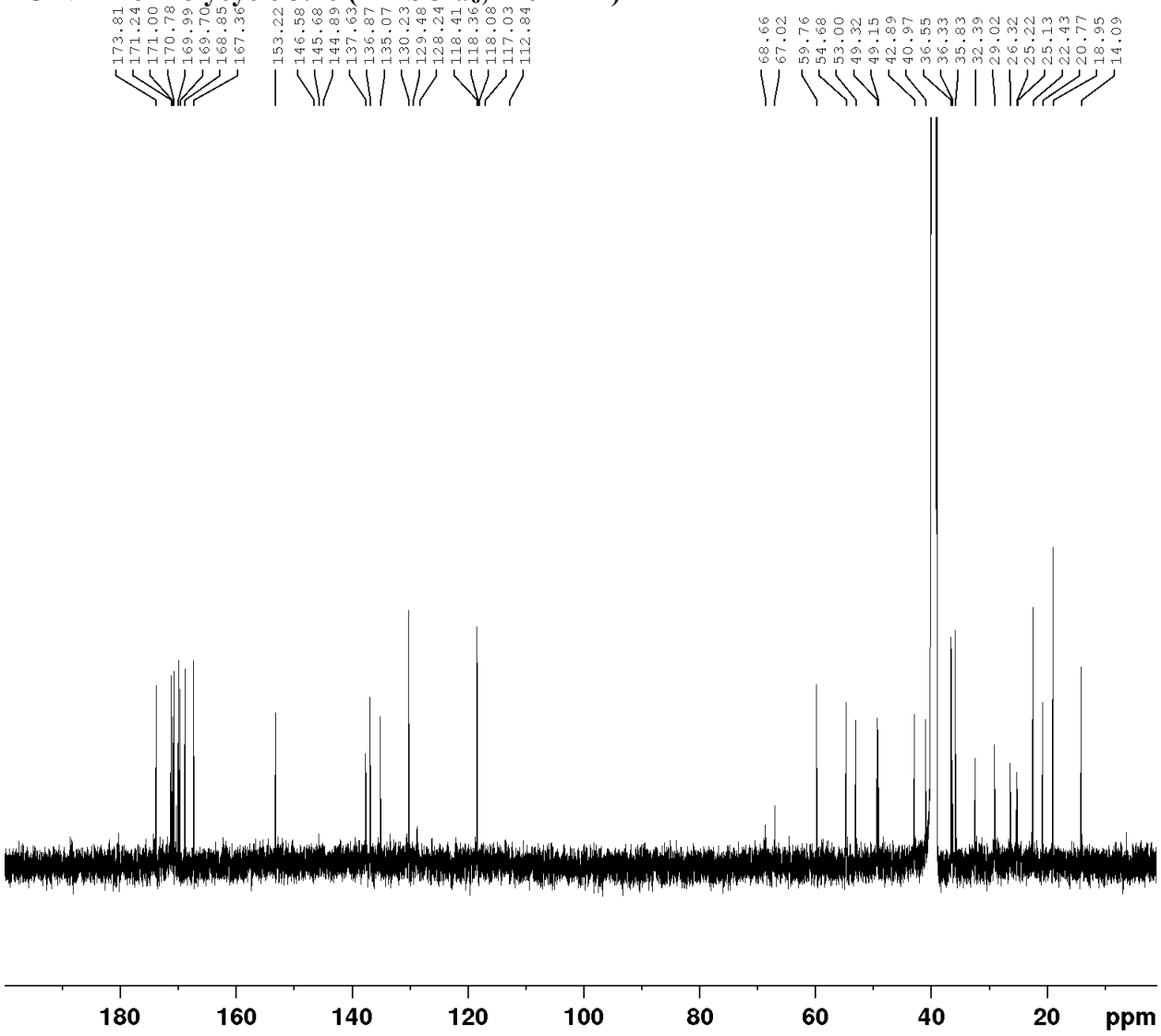
COSY Spectrum of Polycycle 3.15 (DMSO-d_6)



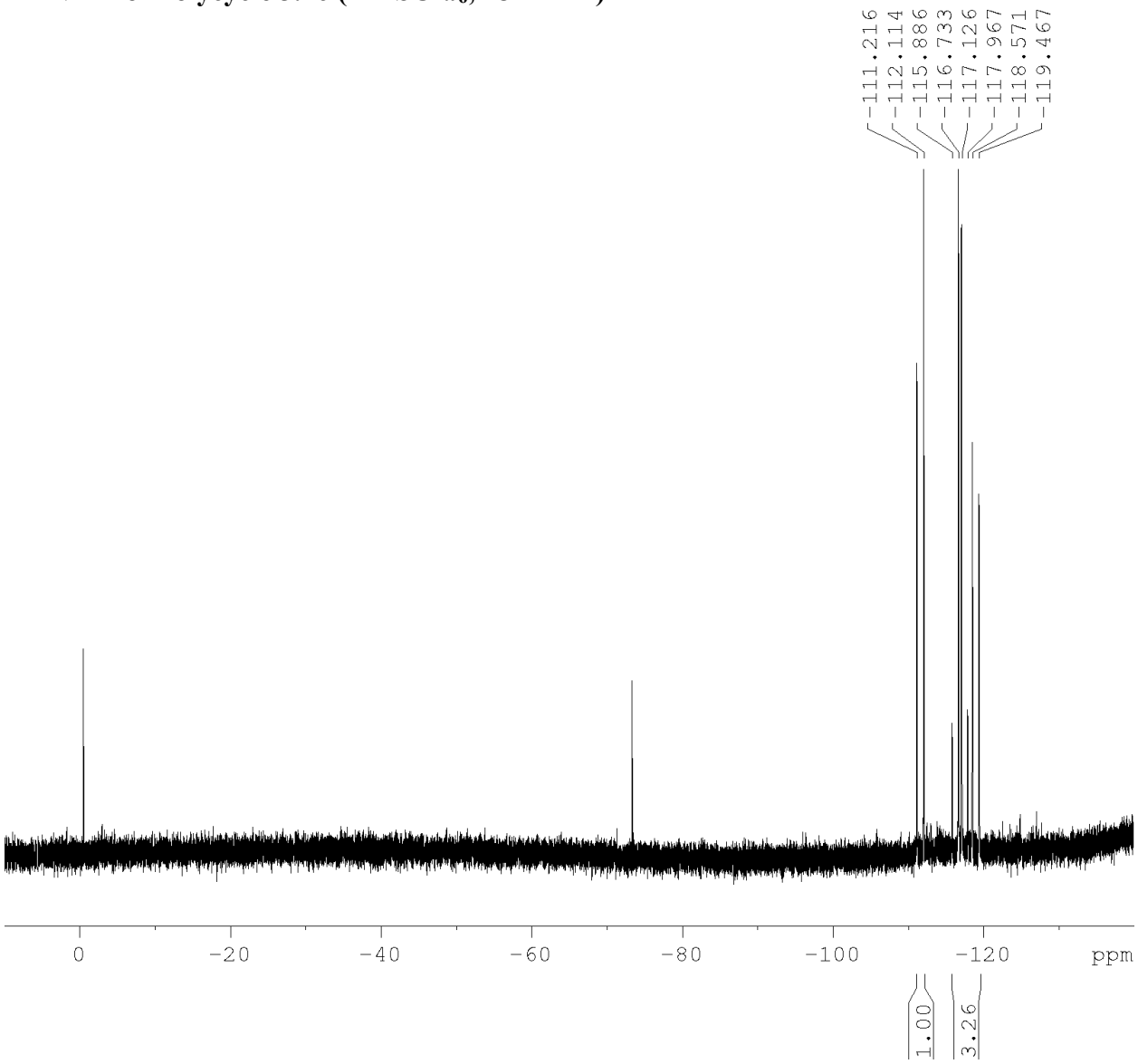
^{1}H NMR of Polycycle 3.16 (DMSO-d_6, 500 MHz)



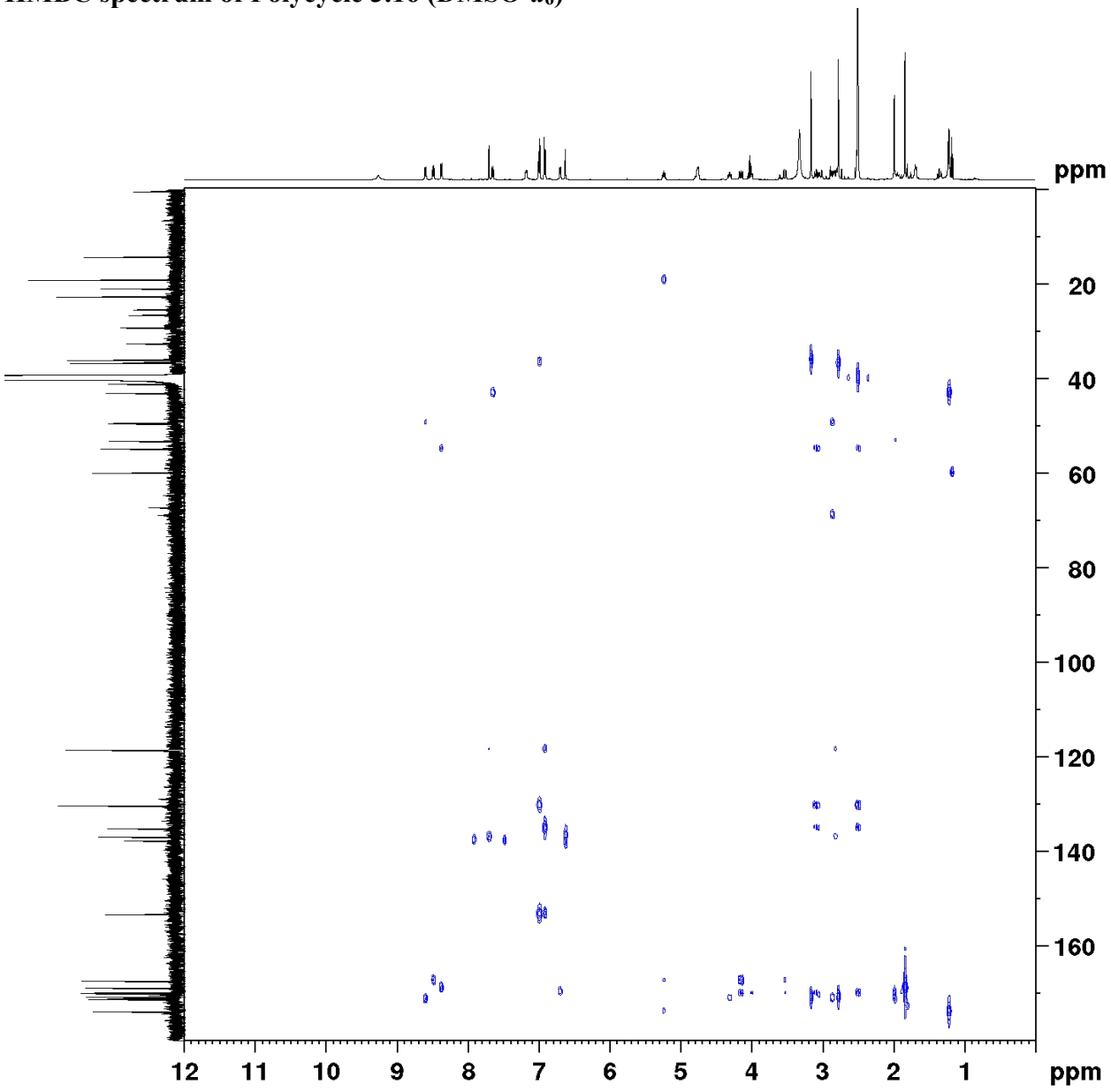
^{13}C NMR of Polycycle 3.16 (DMSO-d_6, 126 MHz)



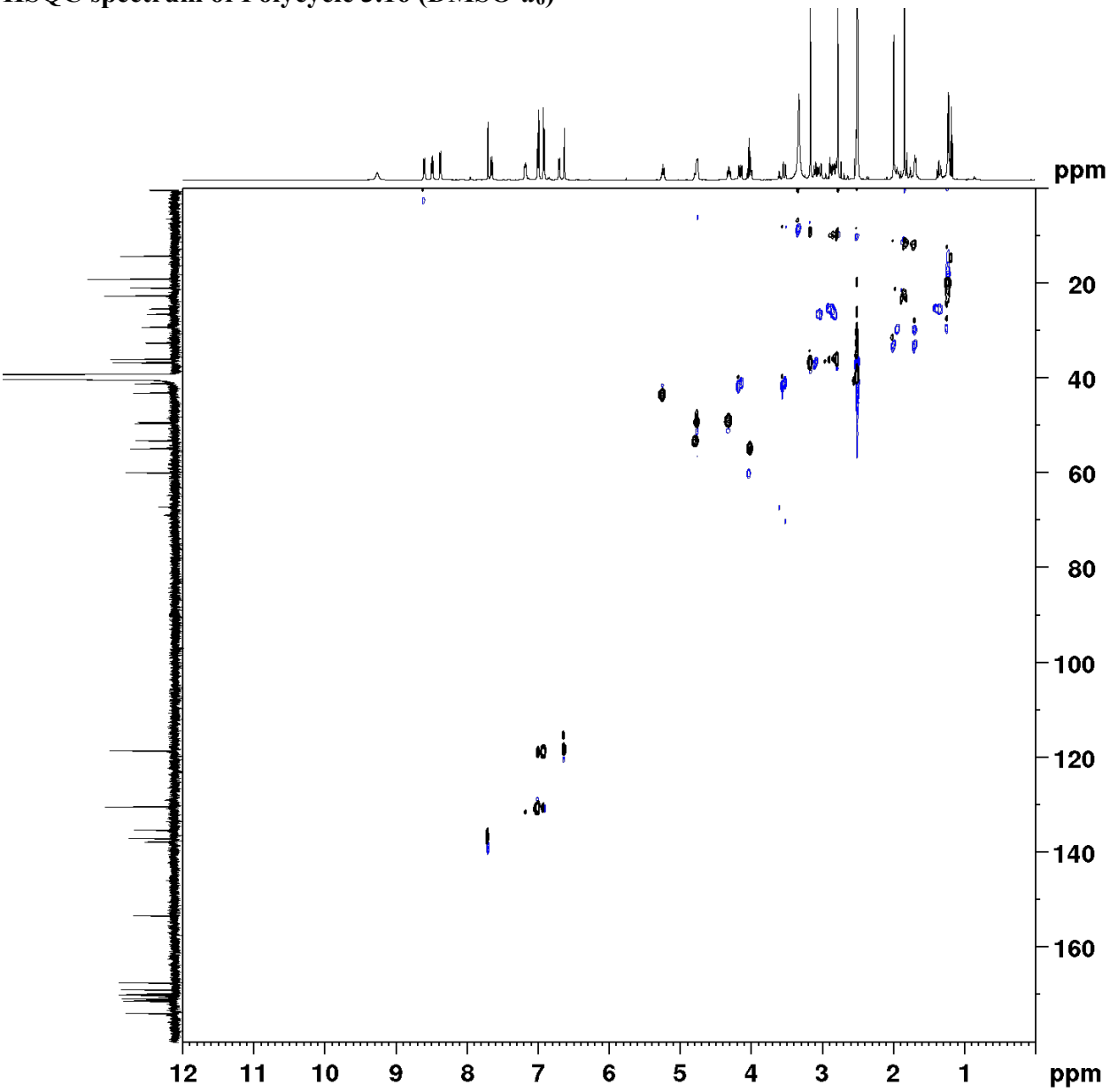
^{19}F NMR of Polycycle 3.16 (DMSO-d_6, 282 MHz)



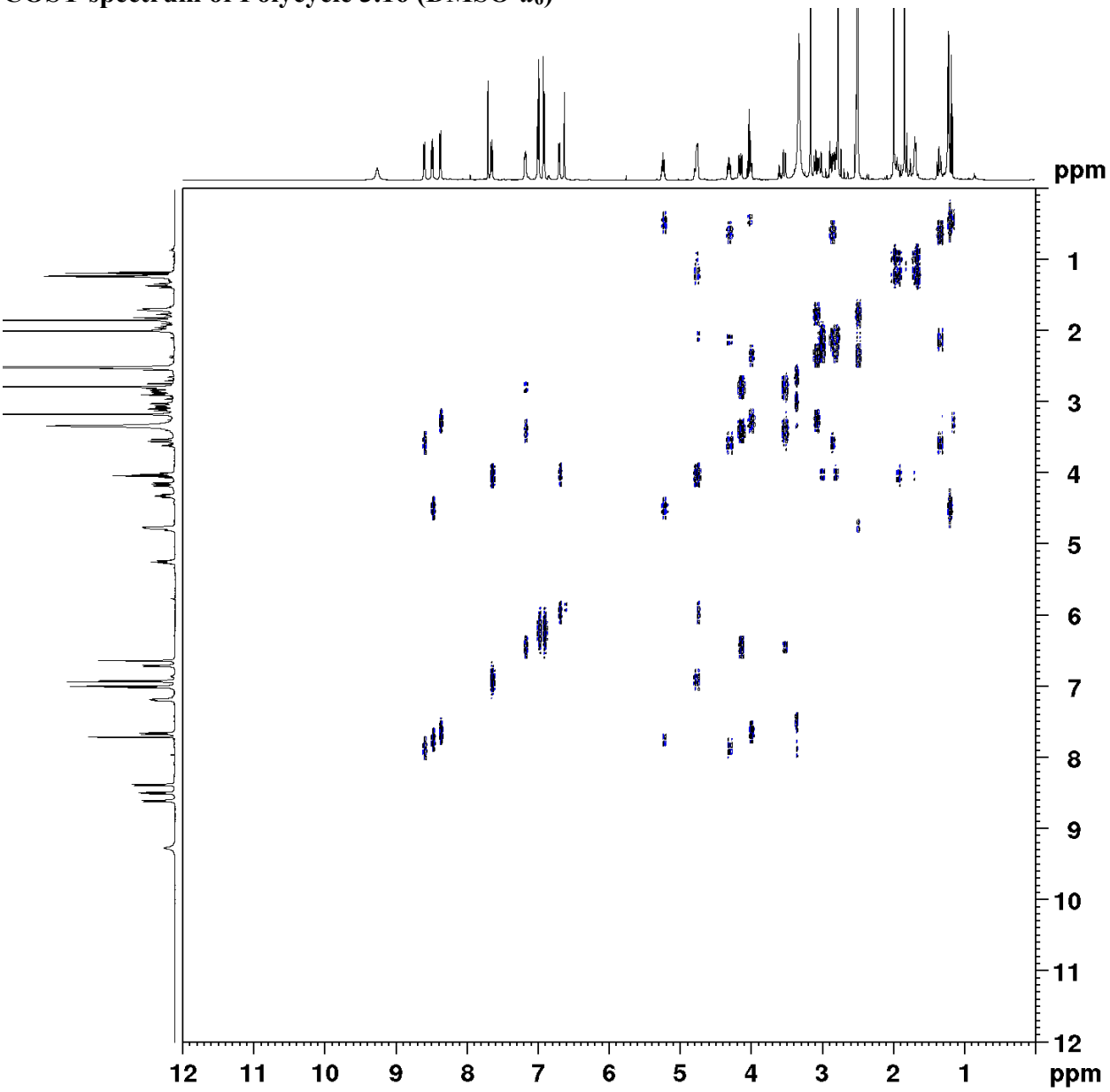
HMBC spectrum of Polycycle 3.16 (DMSO-d_6)



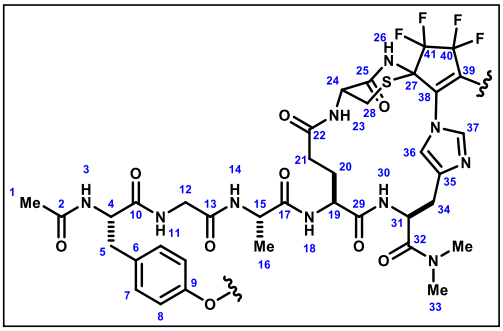
HSQC spectrum of Polycycle 3.16 (DMSO-d_6)



COSY spectrum of Polycycle 3.16 (DMSO-d_6)

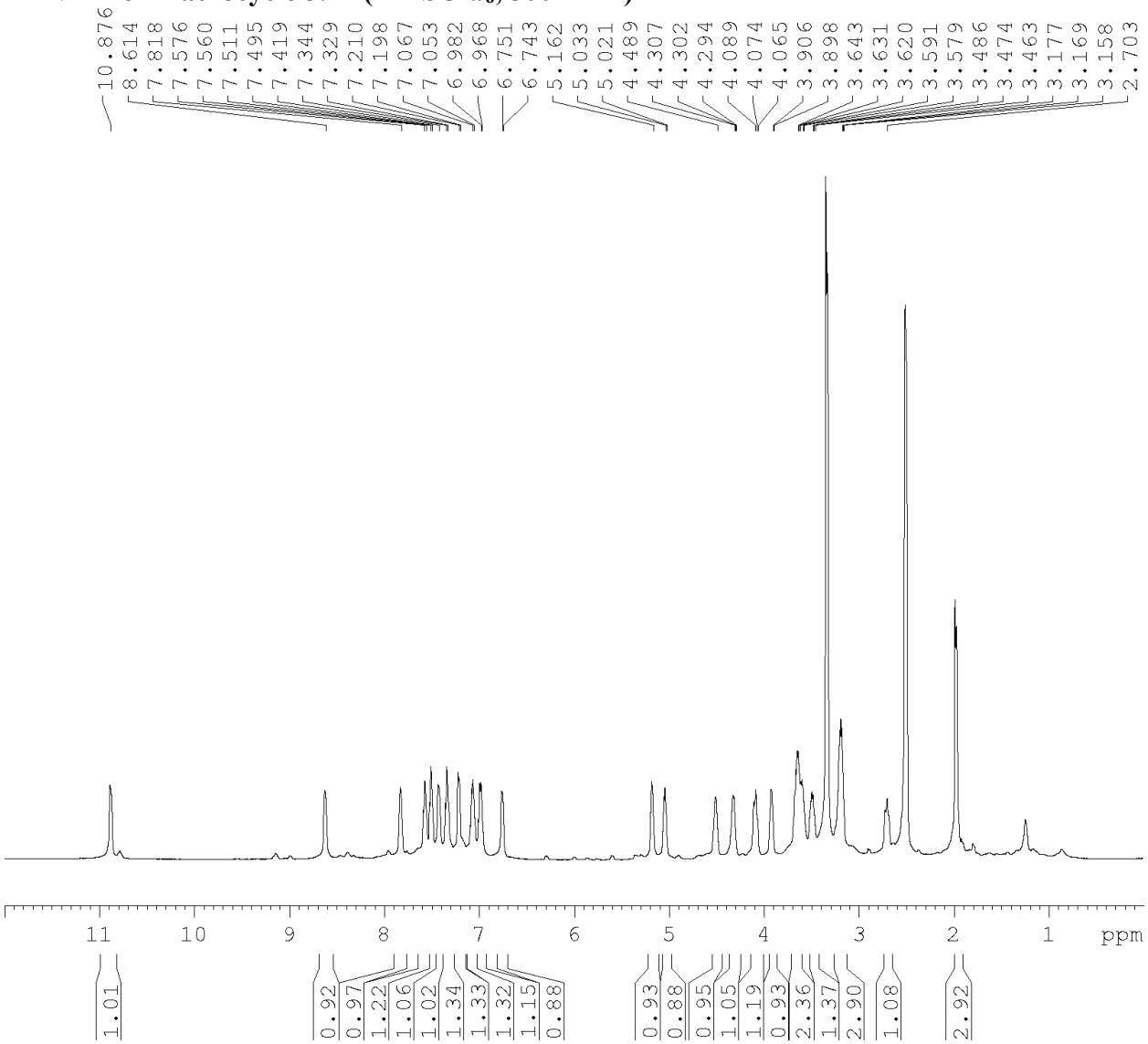


Polycycle **3.16** (DMSO-d_6, 298K)

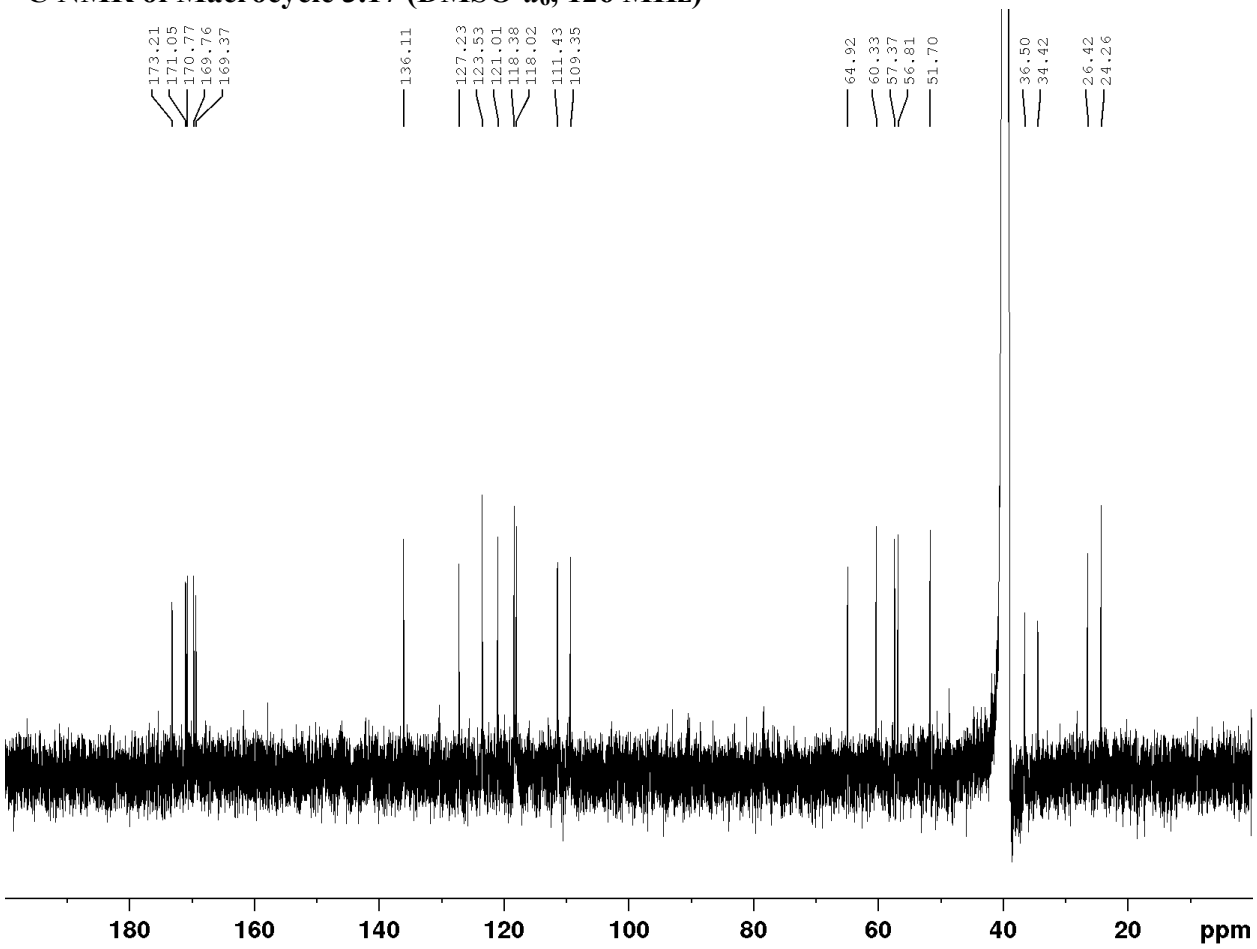


	13C	1H	Key Correlation
1	22.4	1.84 (s, 3H)	1 -> 2 HMBC
2	168.9	-	1 -> 2 HMBC
3	-	8.37 (d, J = 7.7, 1H)	3 -> 2 HMBC
4	54.7	4.03 - 3.97 (m, 1H)	3 -> 4 HMBC
5	36.4	3.12 - 2.98 (m, 1H); 2.53 - 2.47 (m, 1H)	4, 7 -> 5 HMBC
6	135.1	-	4 -> 6 HMBC
7	130.2	6.99 (d, J = 8.7 Hz, 2H); 6.91 (d, J = 8.7, 2H)	8 -> 7 HMBC
8	118.4	6.99 (d, J = 8.7 Hz, 2H); 6.91 (d, J = 8.7, 2H)	7 -> 8 HMBC
9	153.2	-	8 -> 9 HMBC
10	170.0	-	4 -> 10 HMBC
11	-	8.49 (d, J = 8.1 Hz, 1H)	11 -> 12, 13 HMBC
12	41.0	4.15 (dd, J = 17.3, 7.4, 1H); 3.53 (dd, J = 17.3, 3.4, 1H)	12 -> 13, 10 HMBC
13	167.4	-	15 -> 13 HMBC
14	-	7.65 (d, J = 10.1 Hz, 1H)	14 -> 15 HMBC
15	42.9	5.24 (quint, J = 7.1 Hz, 1H)	15 -> 16 HMBC
16	19.0	1.22 (d, J = 6.70, 3H)	16 -> 17 HMBC
17	173.8	-	15, 16 -> 17 HMBC
18	-	7.18 (s, 1H)	18 -> 17 HMBC
19	53.0	4.82 - 4.73 (m, 1H)	18 -> 19 HMBC
20	29.0	1.96 - 1.91 (m, 1H), 1.71 - 1.67 (m, 1H)	20 -> 19, 29 HMBC
21	32.4	2.02 - 1.96 (m, 1H), 1.72 - 1.66 (m, 1H)	21 -> 22, 20 HMBC
22	169.7	-	21 -> 22 HMBC
23	-	6.70 (d, J = 6.2, 1H)	23 -> 22, 24 HMBC
24	49.3	4.36 - 4.26 (m, 1H)	24 -> 28, 25 HMBC
25	171.0	-	24, 28 -> 25 HMBC
26	-	9.26 (s, 1H)	
27	69.5 - 67.8 (m, 1C)	-	28 -> 27 HMBC
28	25.2	3.04 - 2.99 (m, 1H), 2.90 - 2.82 (m, 1H)	28 -> 27 HMBC
29	171.2	-	30 -> 29 HMBC
30	-	8.60 (s, 1H)	30 -> 29 HMBC
31	49.2	4.79 - 4.72 (m, 1H)	31 -> 34 HMBC
32	170.8	-	33 -> 32 HMBC
33	36.5, 35.8	3.16 (s, 3H); 2.77 (s, 3H)	33 -> 32 HMBC
34	26.3	2.85 - 2.79 (m, 2H)	34 -> 35, 36 HMBC
35	136.9	-	36, 37 -> 35 HMBC
36	118.4	6.62 (s, 1H)	36, 37 -> 35 HMBC
37	137.6	7.7 (s, 1H)	36 -> 37 HMBC
38	129.5 - 128.2 (m, 1C)	-	
39	146.5 - 144.6 (m, 1C)	-	
40	118.1 - 117.0 (m, 2C)	-	
41	118.1 - 117.0 (m, 2C)	-	

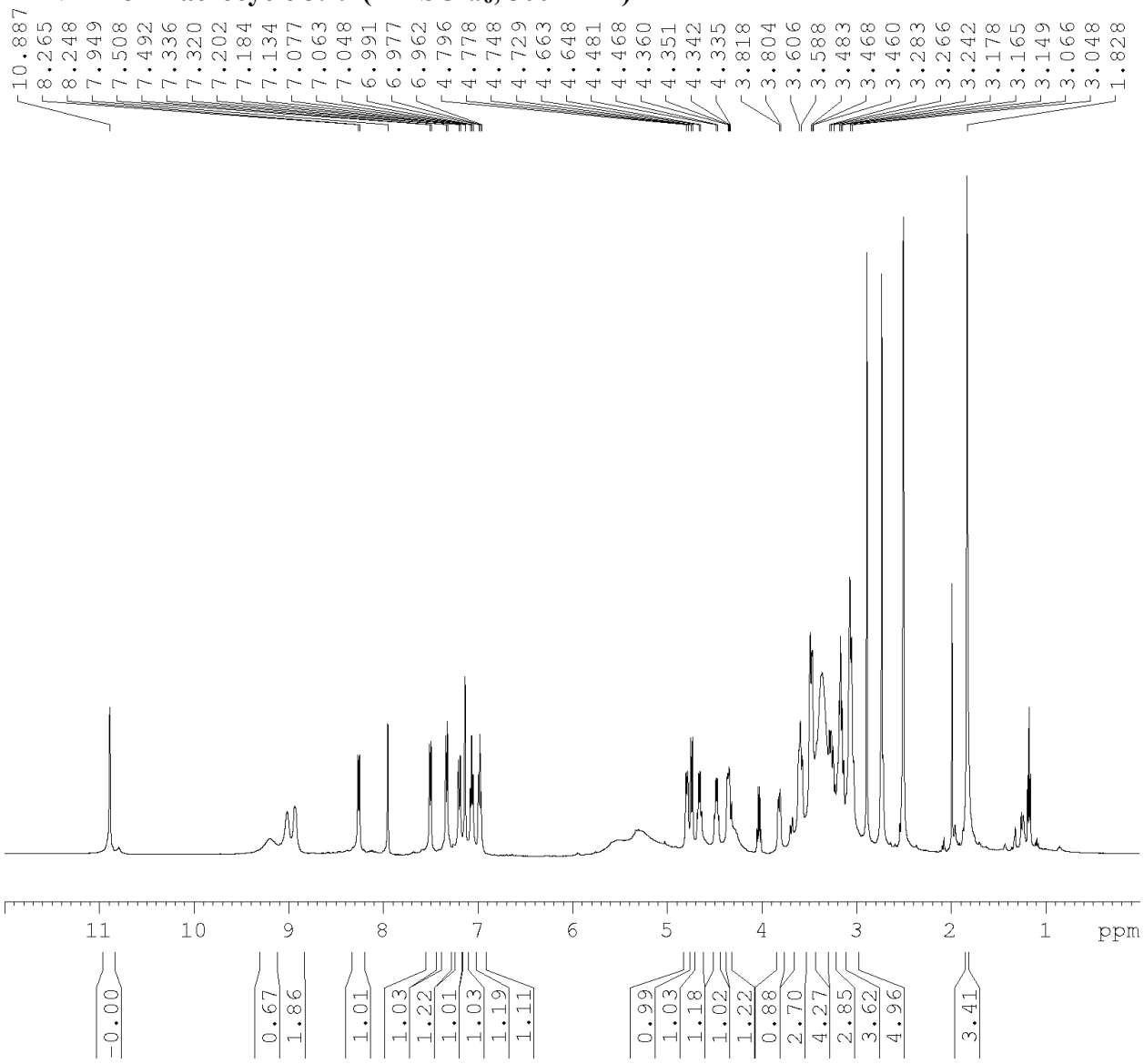
^{1}H NMR of Macrocycle 3.17 (DMSO-d_6, 500 MHz)



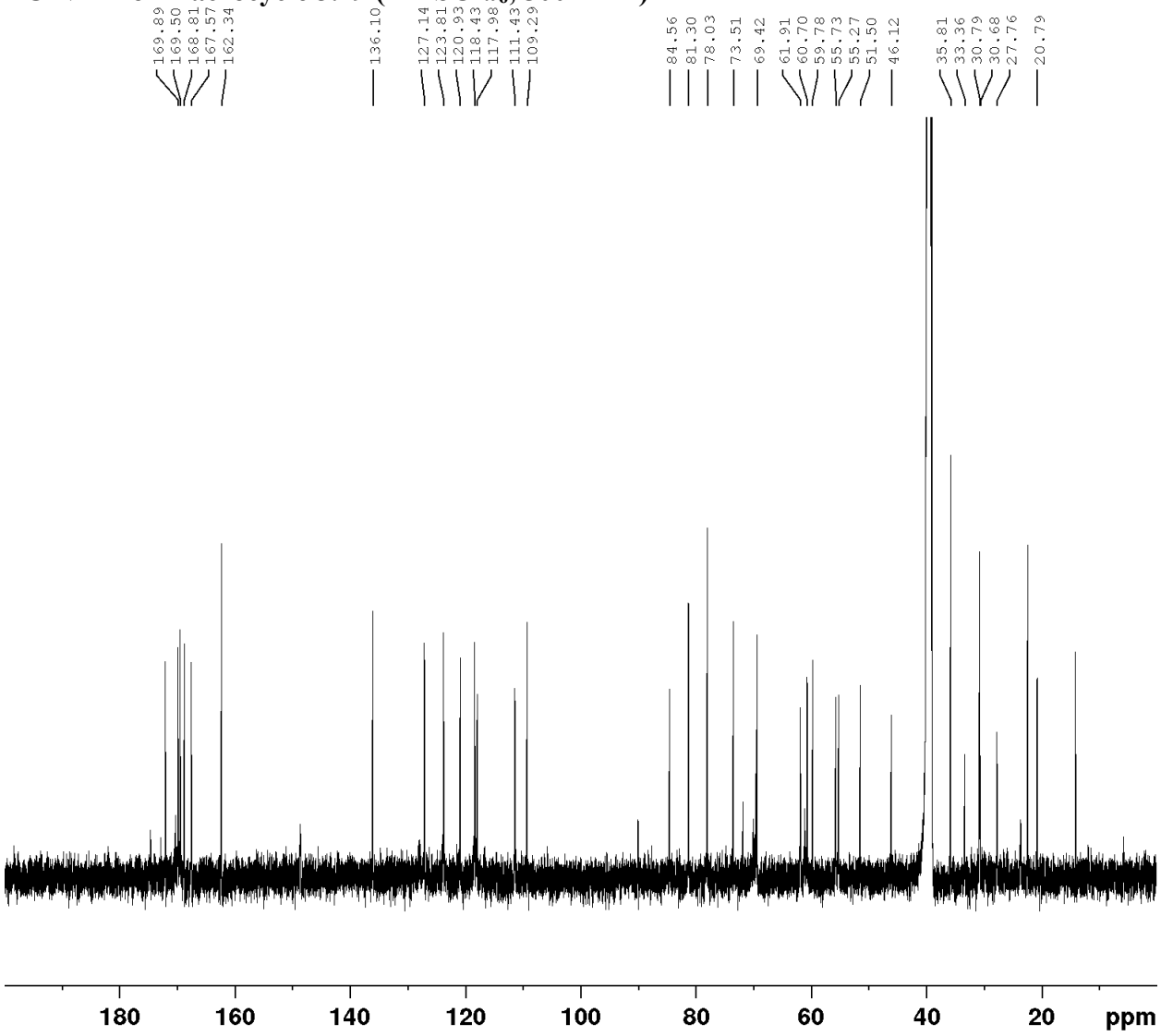
^{13}C NMR of Macrocycle 3.17 (DMSO-d_6, 126 MHz)



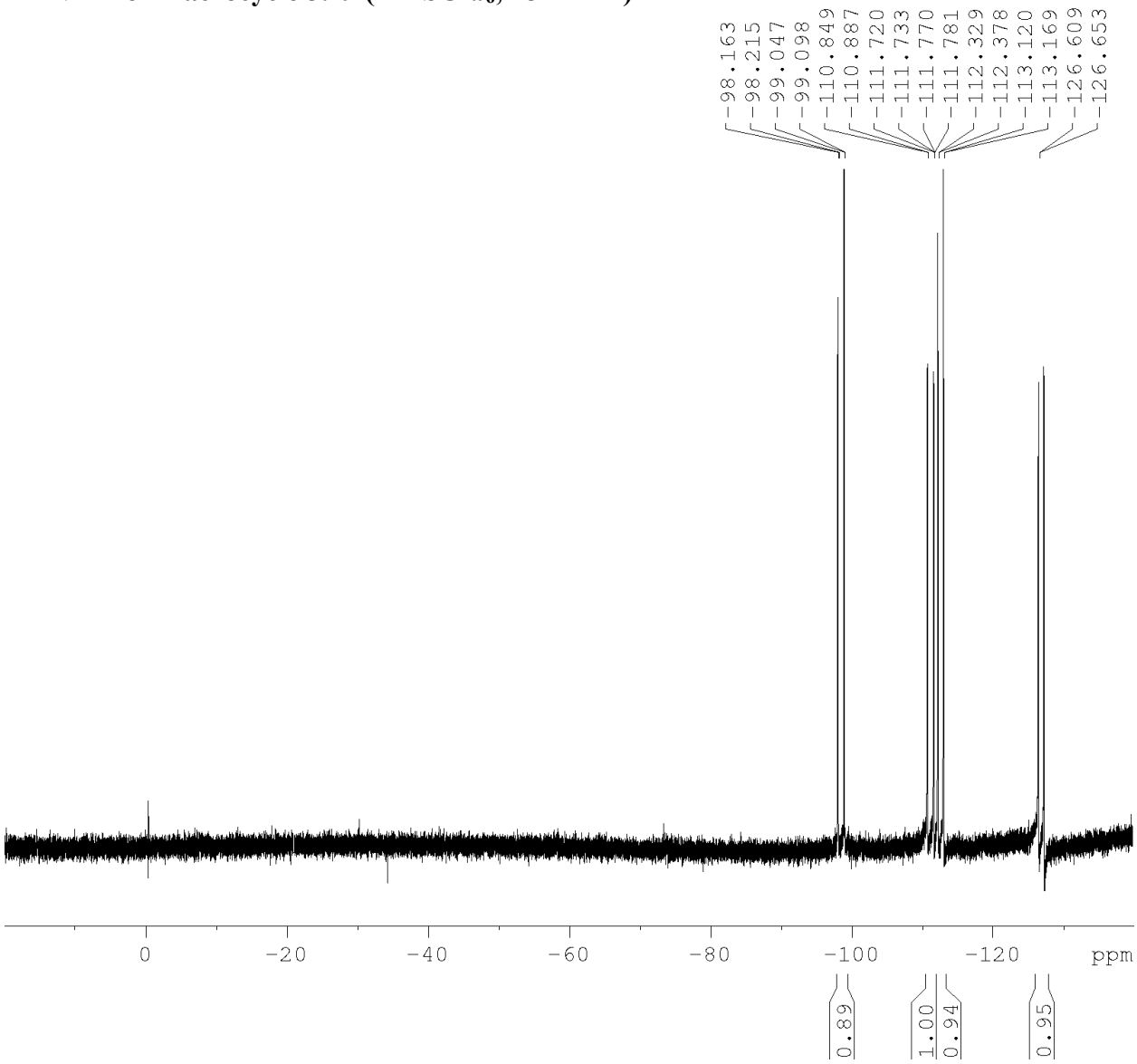
^{1}H NMR of Macrocycle 3.19 (DMSO-d_6, 500 MHz)



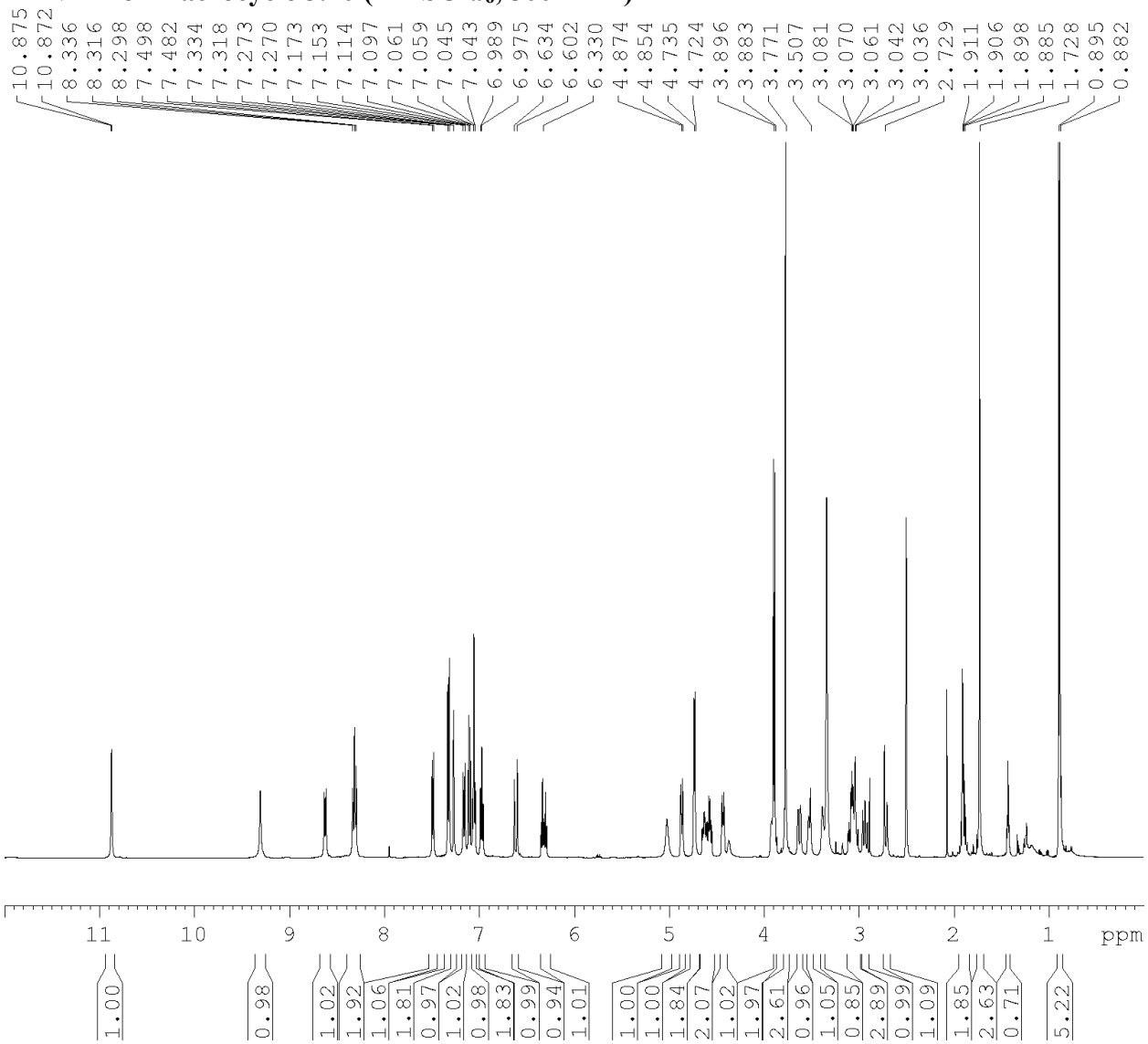
^{13}C NMR of Macrocycle 3.19 (DMSO-d_6, 500 MHz)



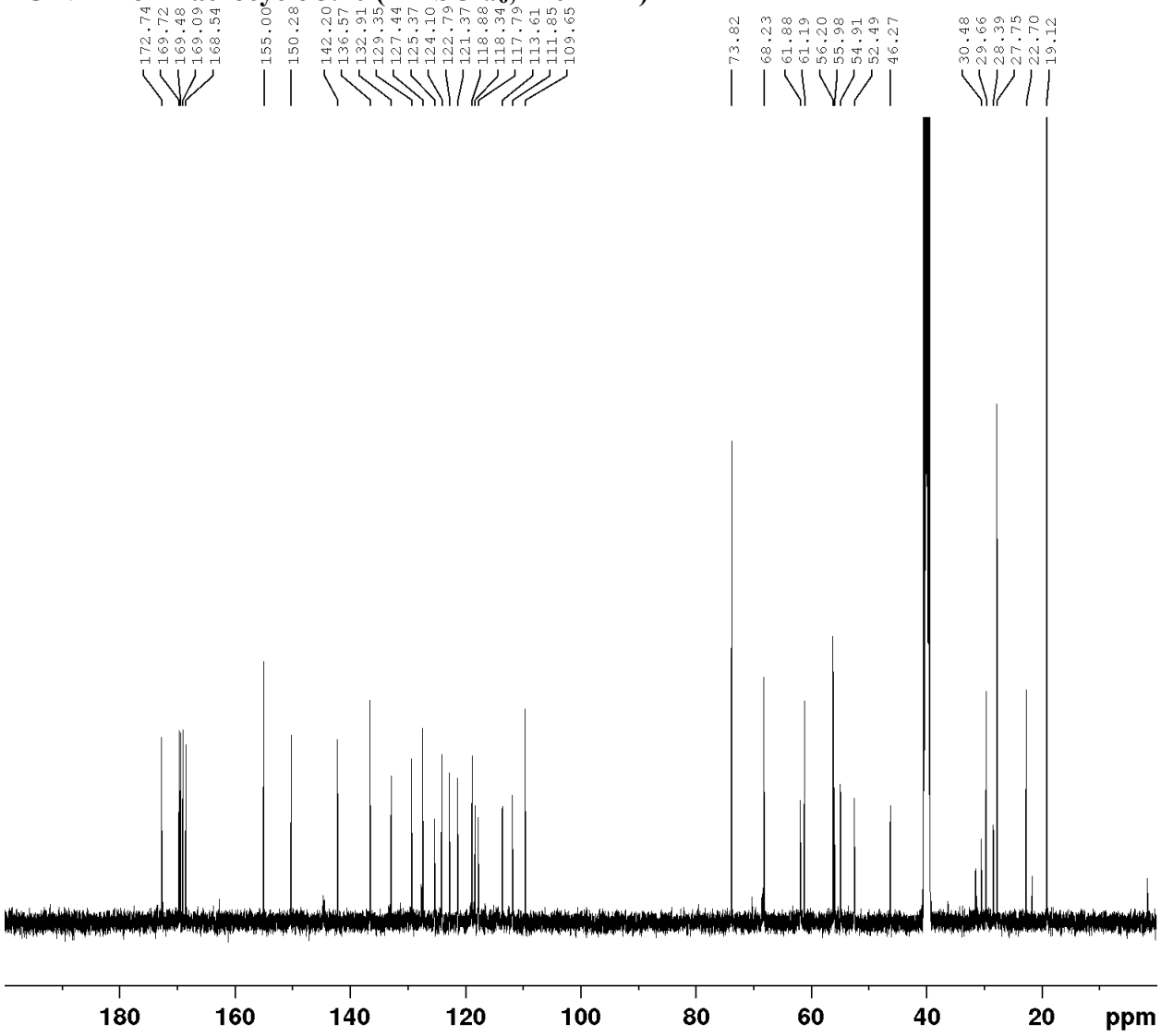
^{19}F NMR of Macrocycle 3.19 (DMSO-d_6, 282 MHz)



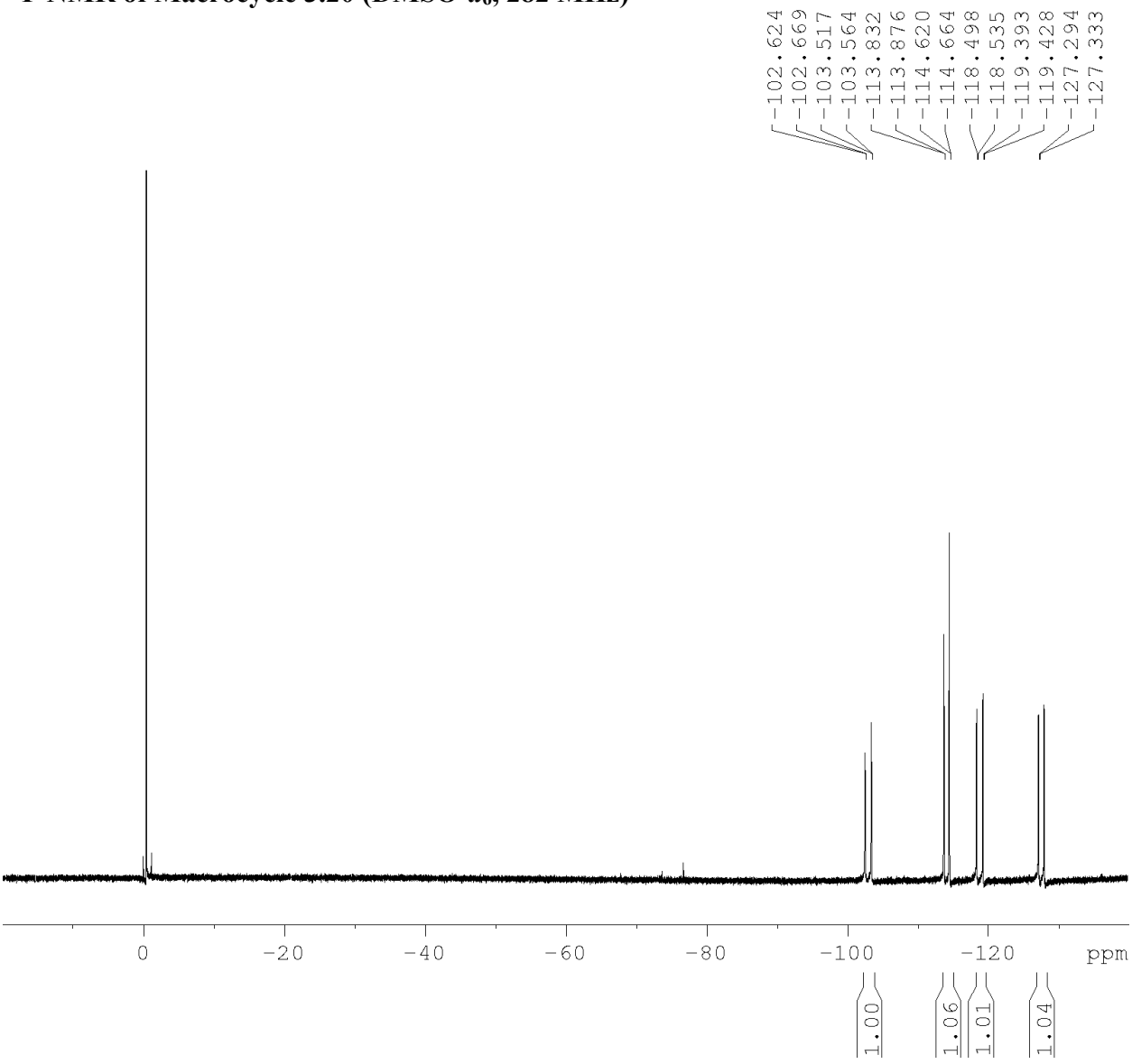
^{1}H NMR of Macrocycle 3.20 (DMSO-d_6, 500 MHz)



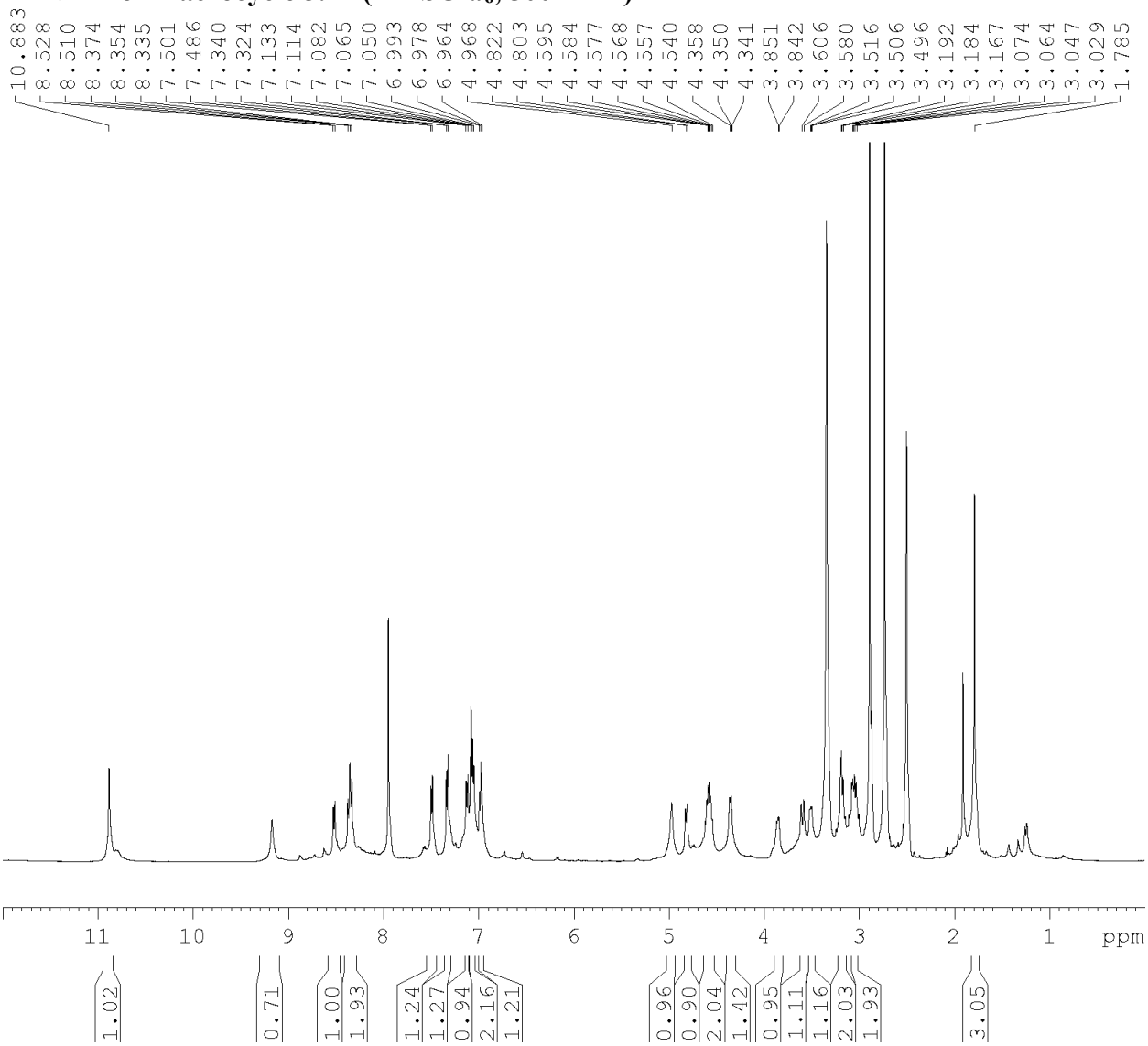
^{13}C NMR of Macrocycle 3.20 (DMSO-d_6, 126 MHz)



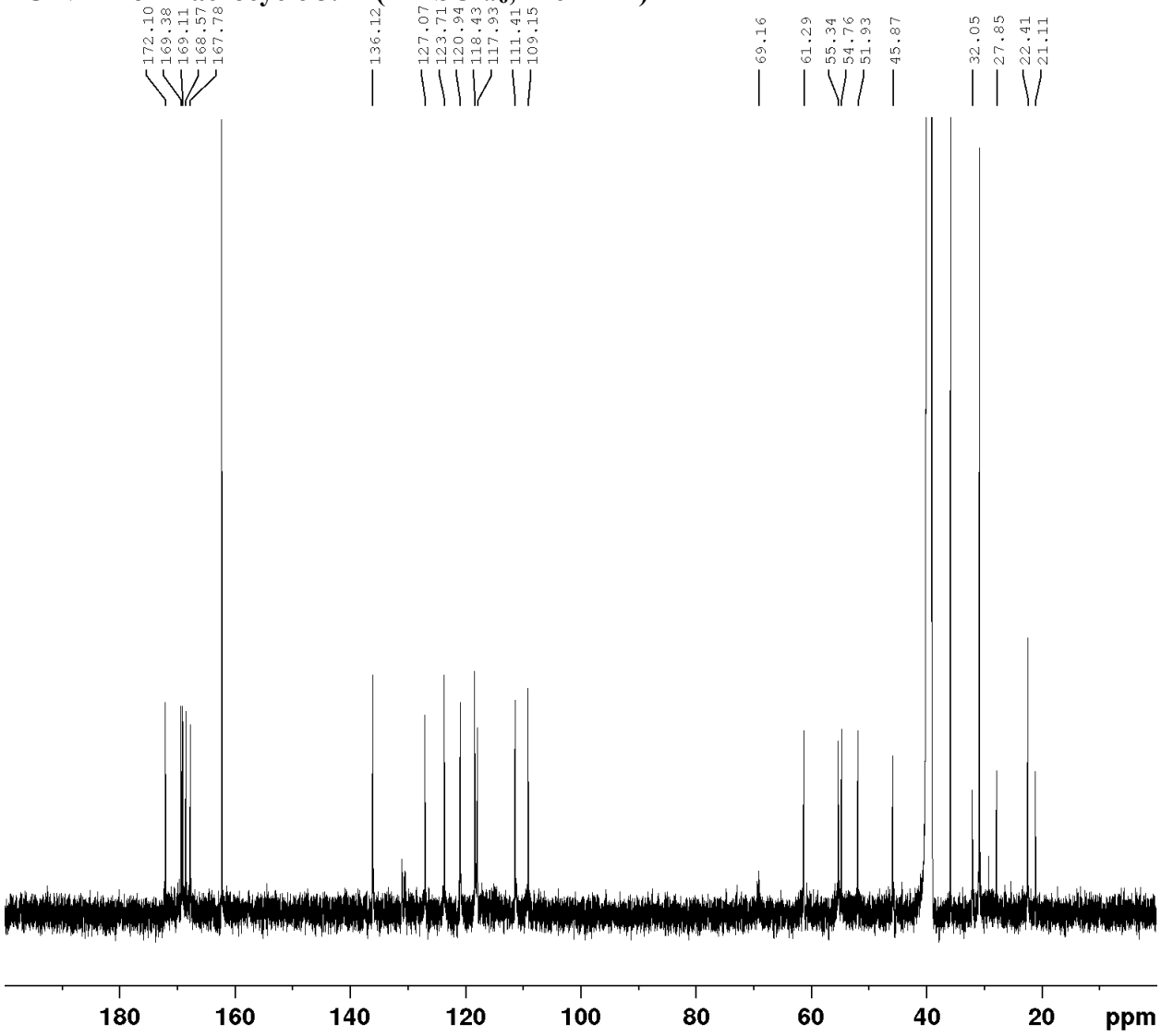
^{19}F NMR of Macrocycle 3.20 (DMSO-d_6, 282 MHz)



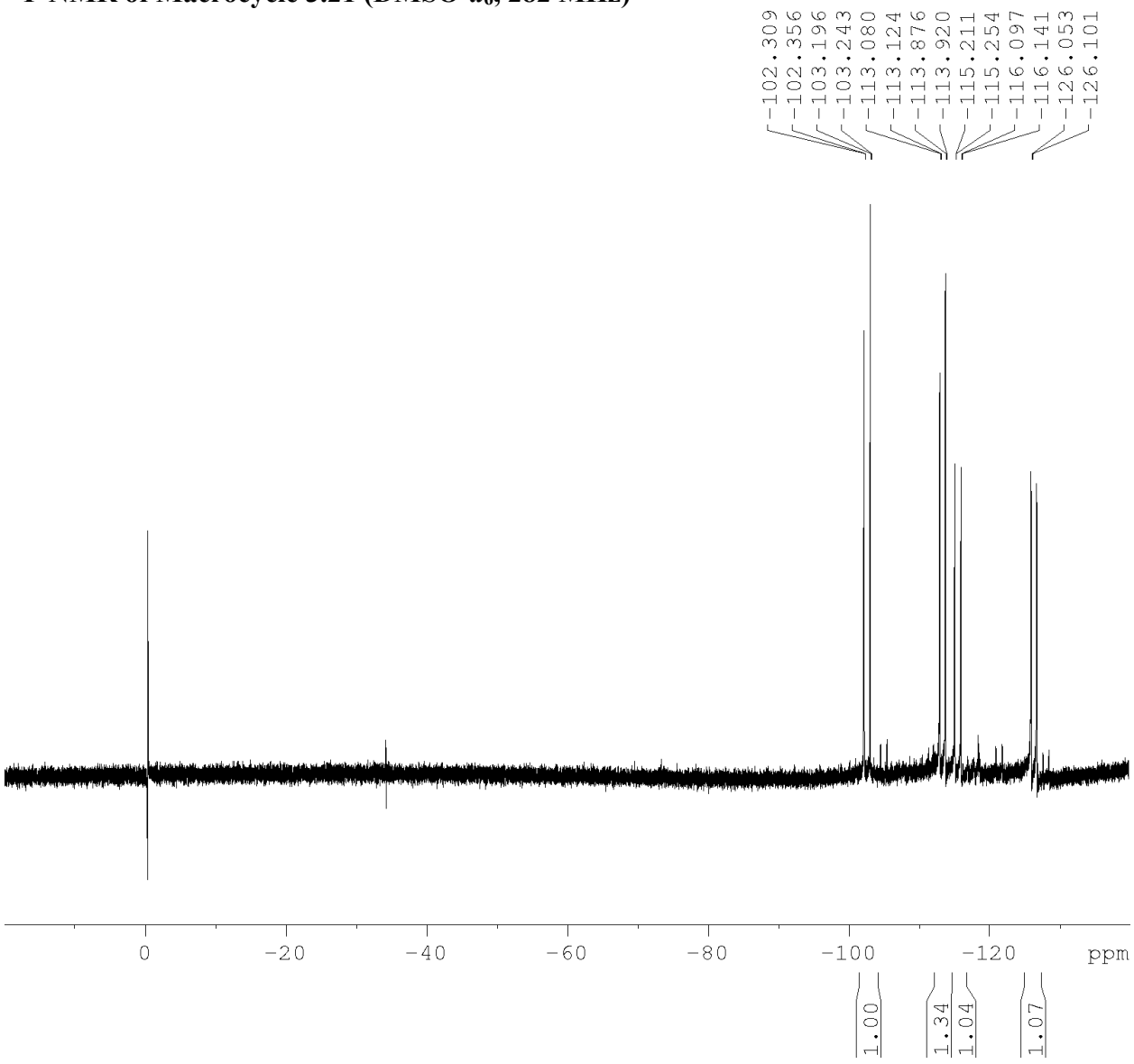
^{1}H NMR of Macrocycle 3.21 (DMSO-d_6, 500 MHz)



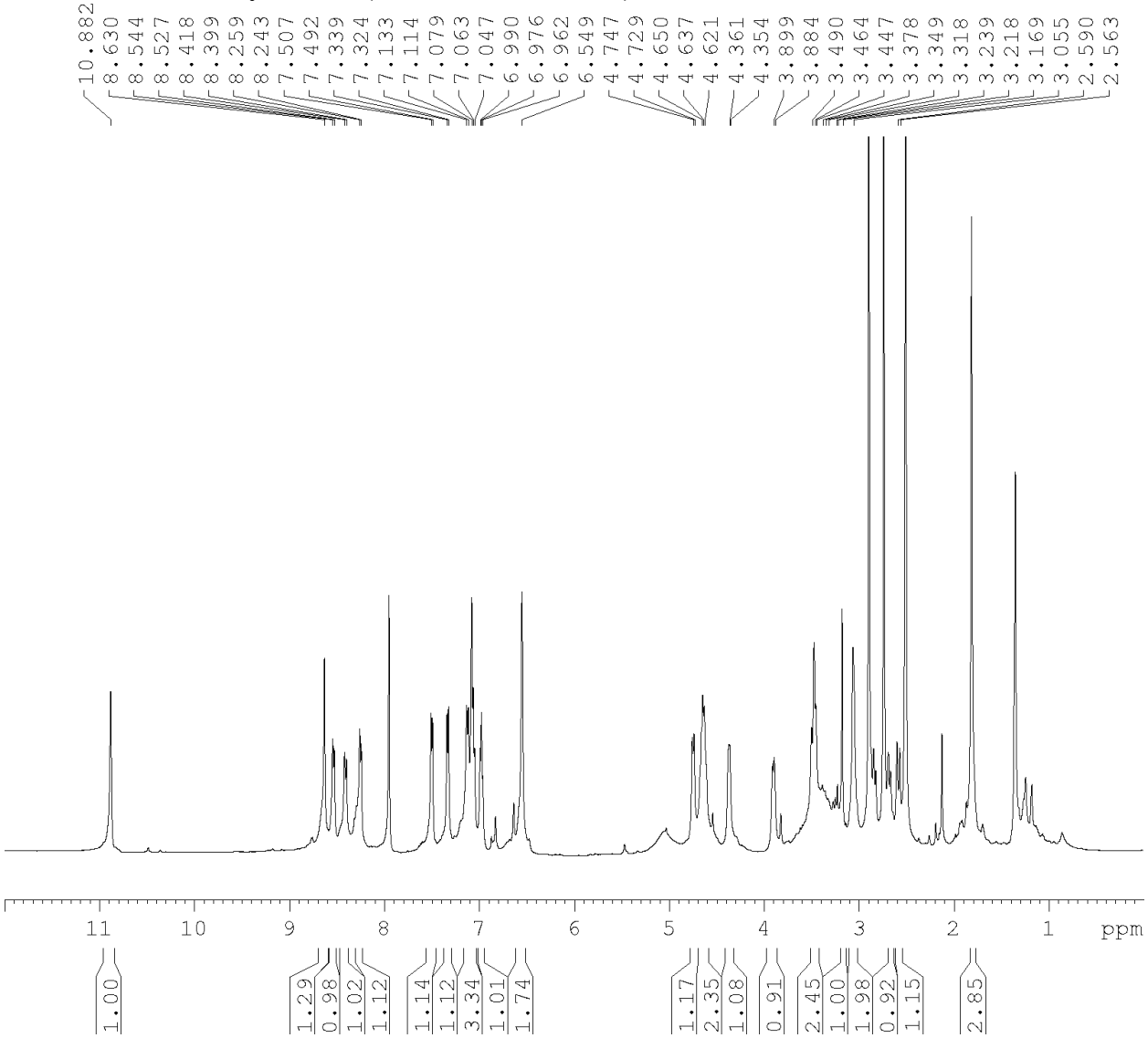
^{13}C NMR of Macrocycle 3.21 (DMSO-d_6, 126 MHz)



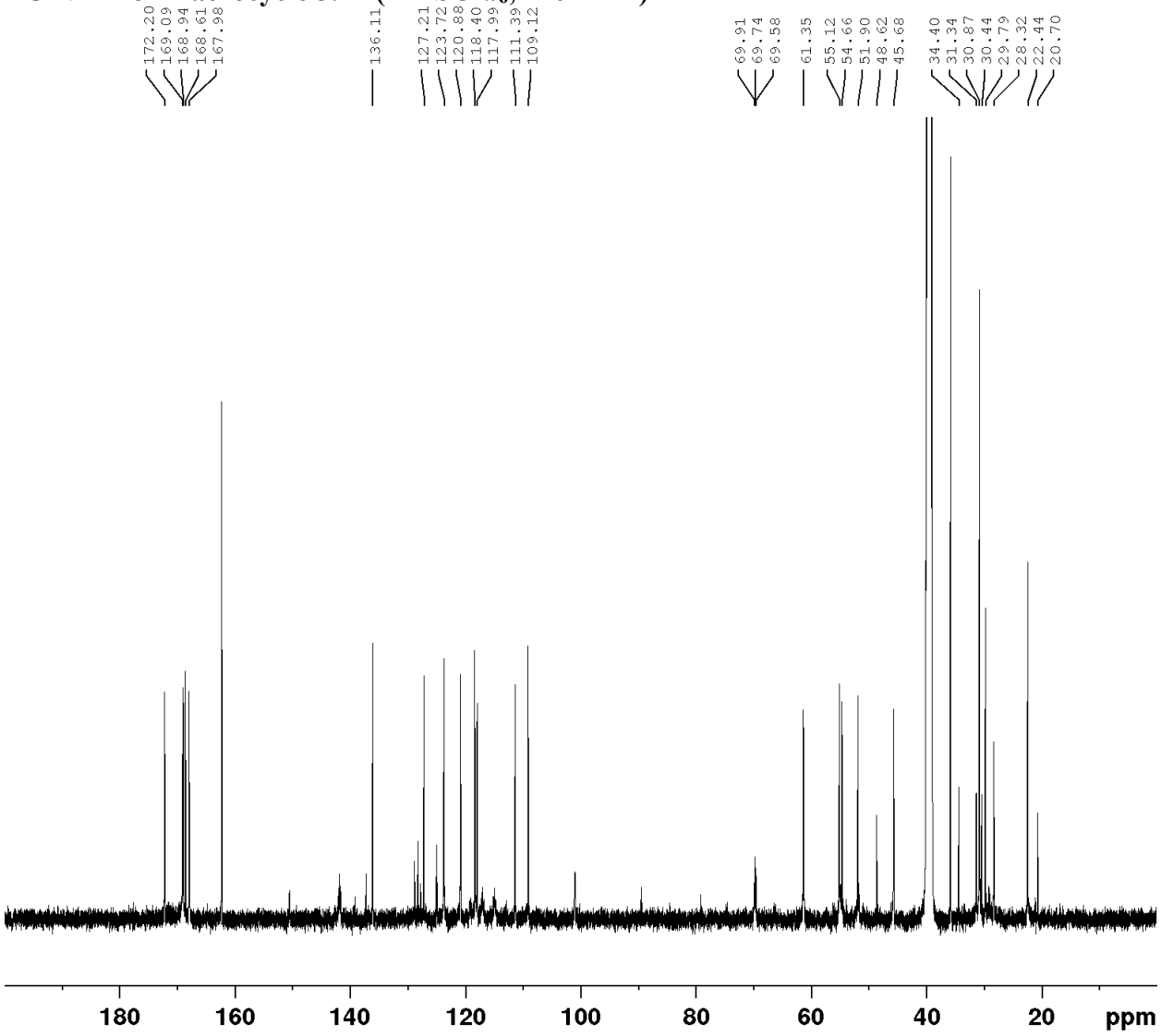
^{19}F NMR of Macrocycle 3.21 (DMSO-d_6, 282 MHz)



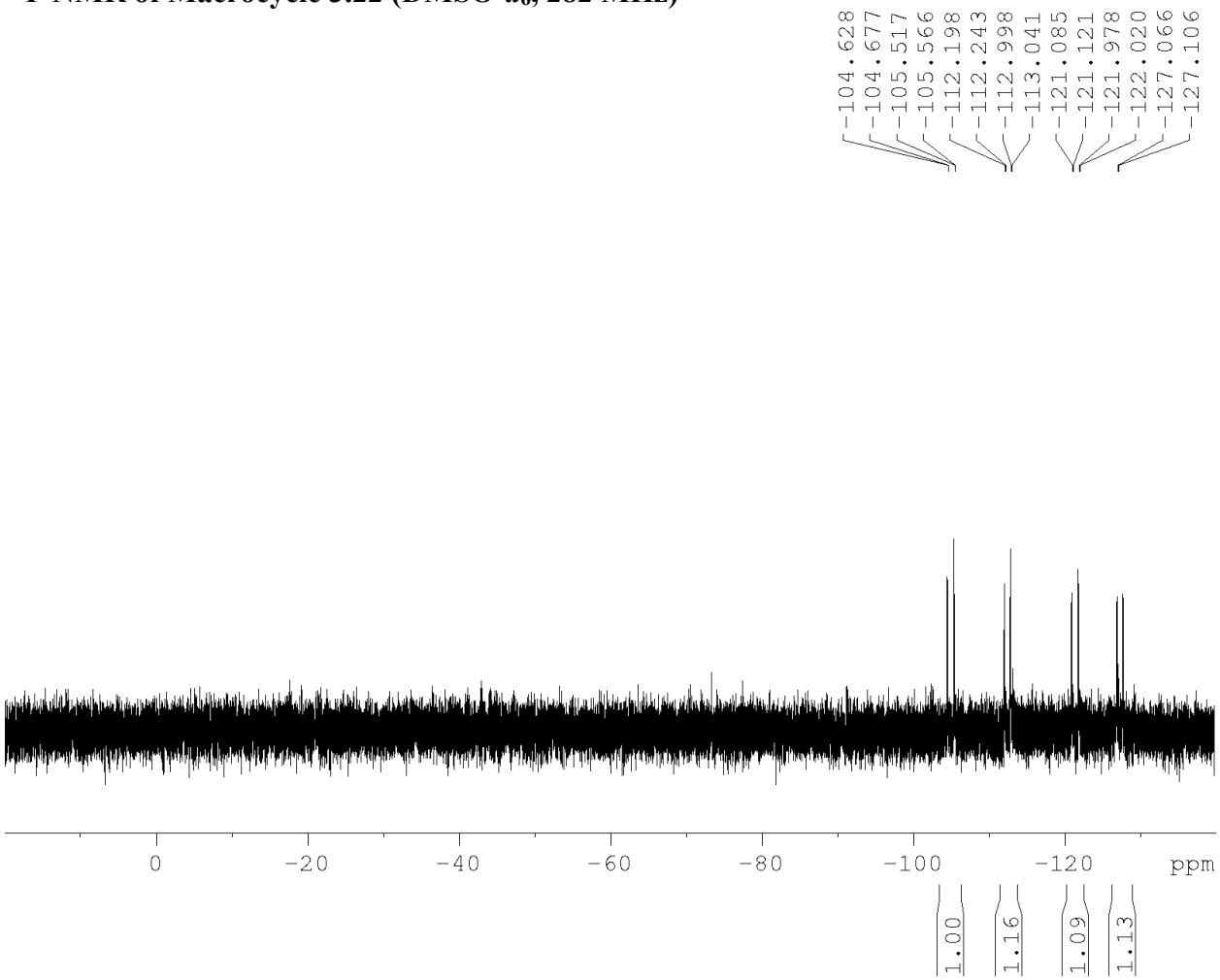
^{1}H NMR of Macrocycle 3.22 (DMSO-d_6, 500 MHz)



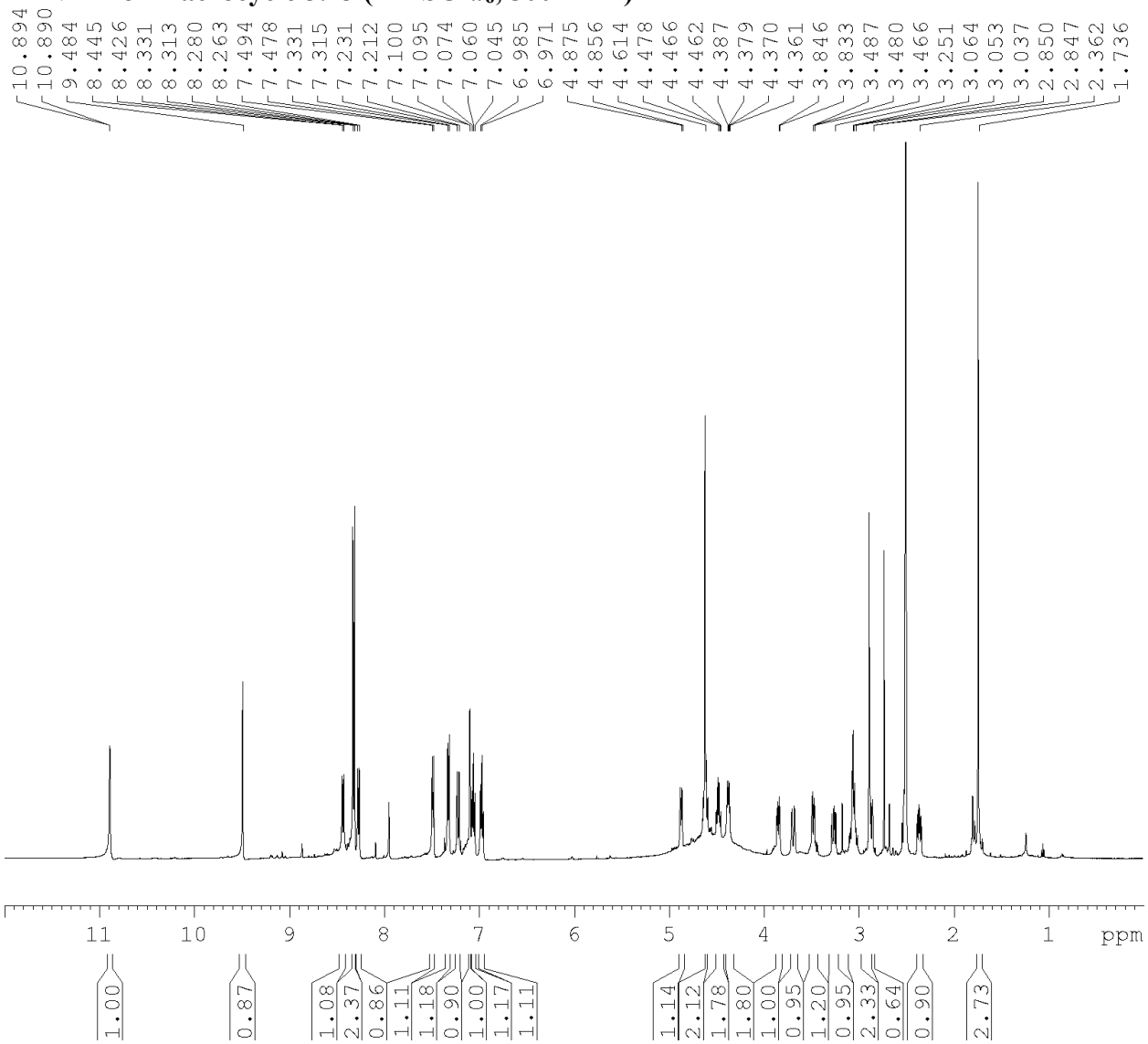
^{13}C NMR of Macrocycle 3.22 (DMSO-d_6, 126 MHz)



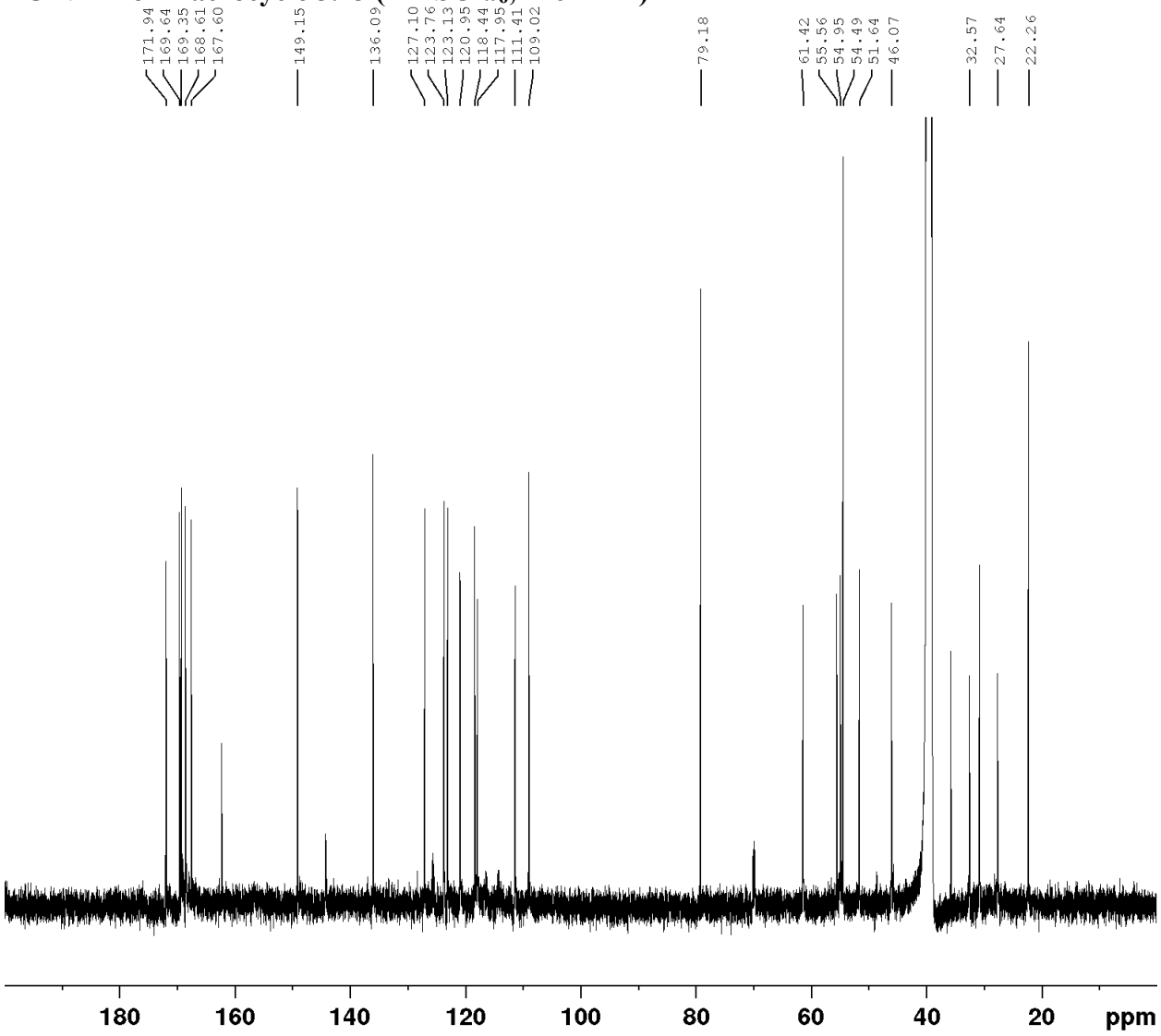
^{19}F NMR of Macrocycle 3.22 (DMSO-d_6, 282 MHz)



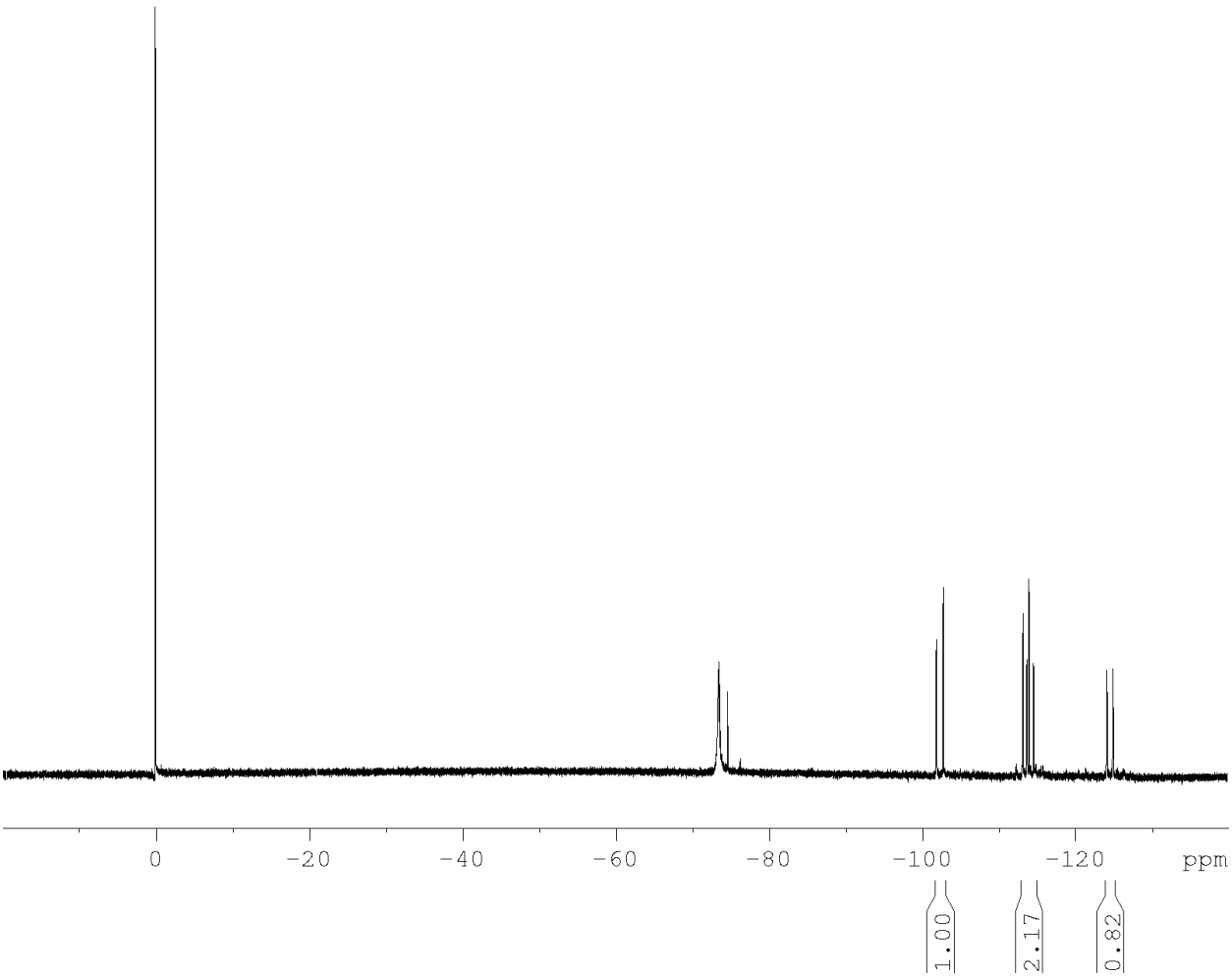
^{1}H NMR of Macrocycle 3.23 (DMSO-d_6, 500 MHz)



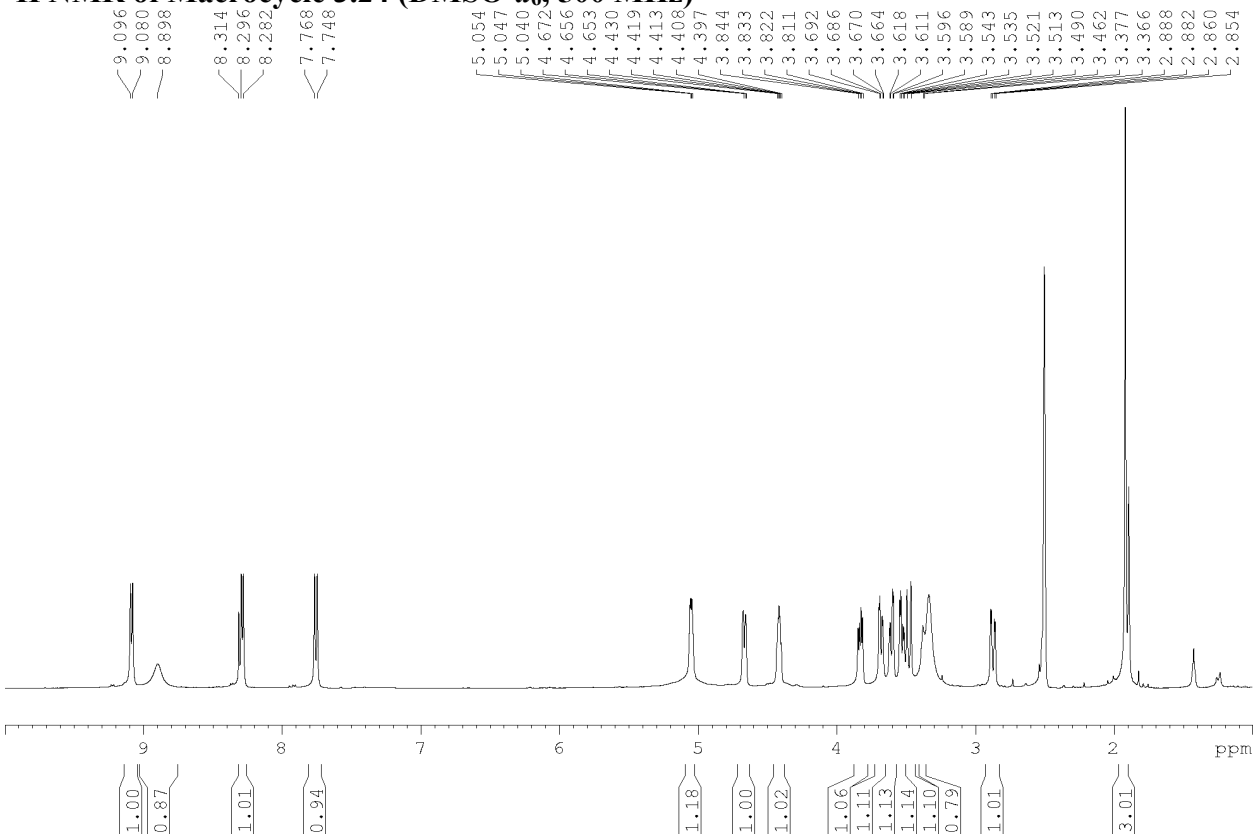
^{13}C NMR of Macrocycle 3.23 (DMSO-d_6, 126 MHz)



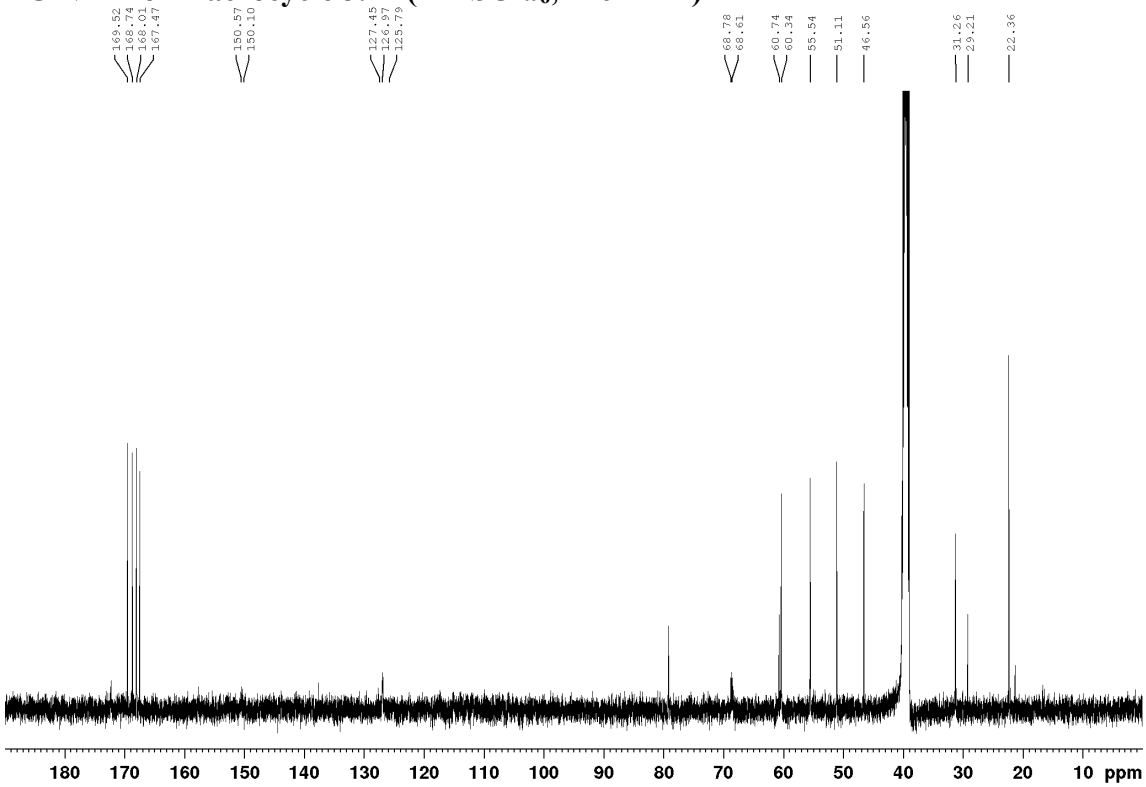
^{19}F NMR of Macrocycle 3.23 (DMSO-d_6, 282 MHz)



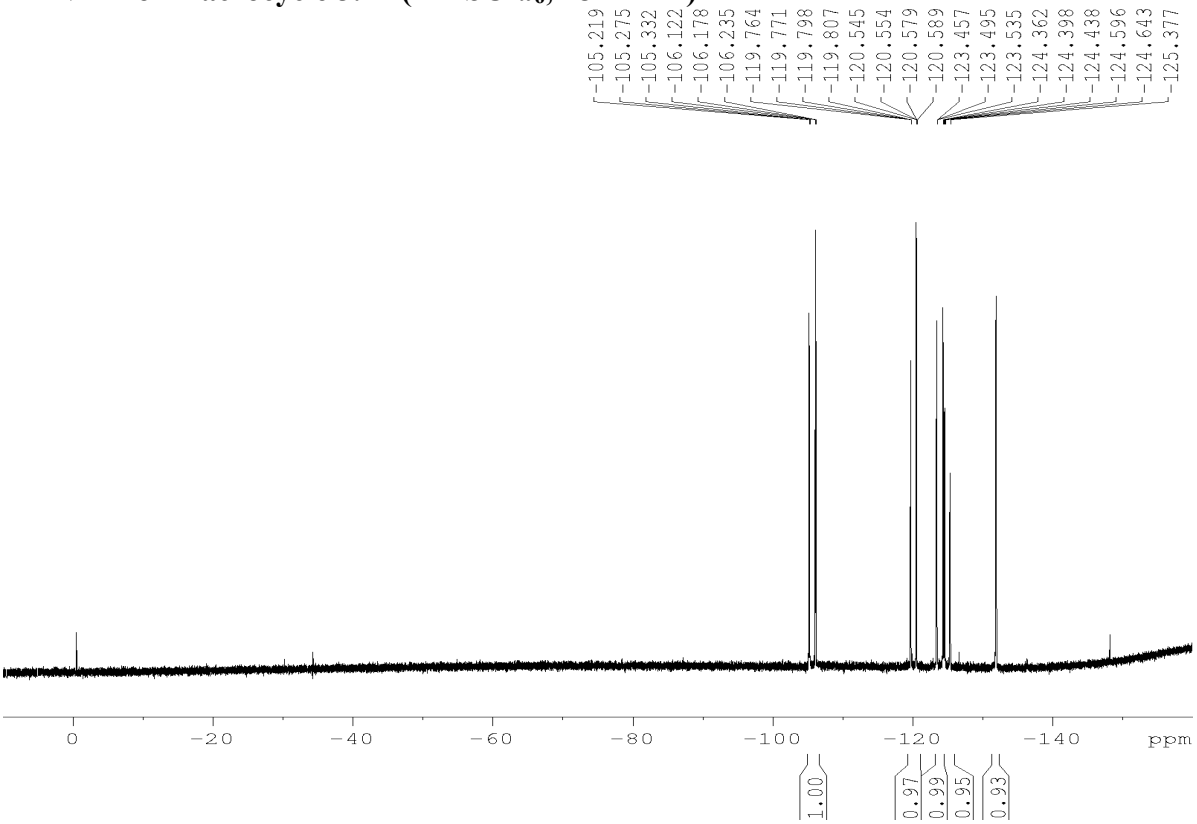
^{1}H NMR of Macrocycle 3.24 (DMSO-d_6, 500 MHz)



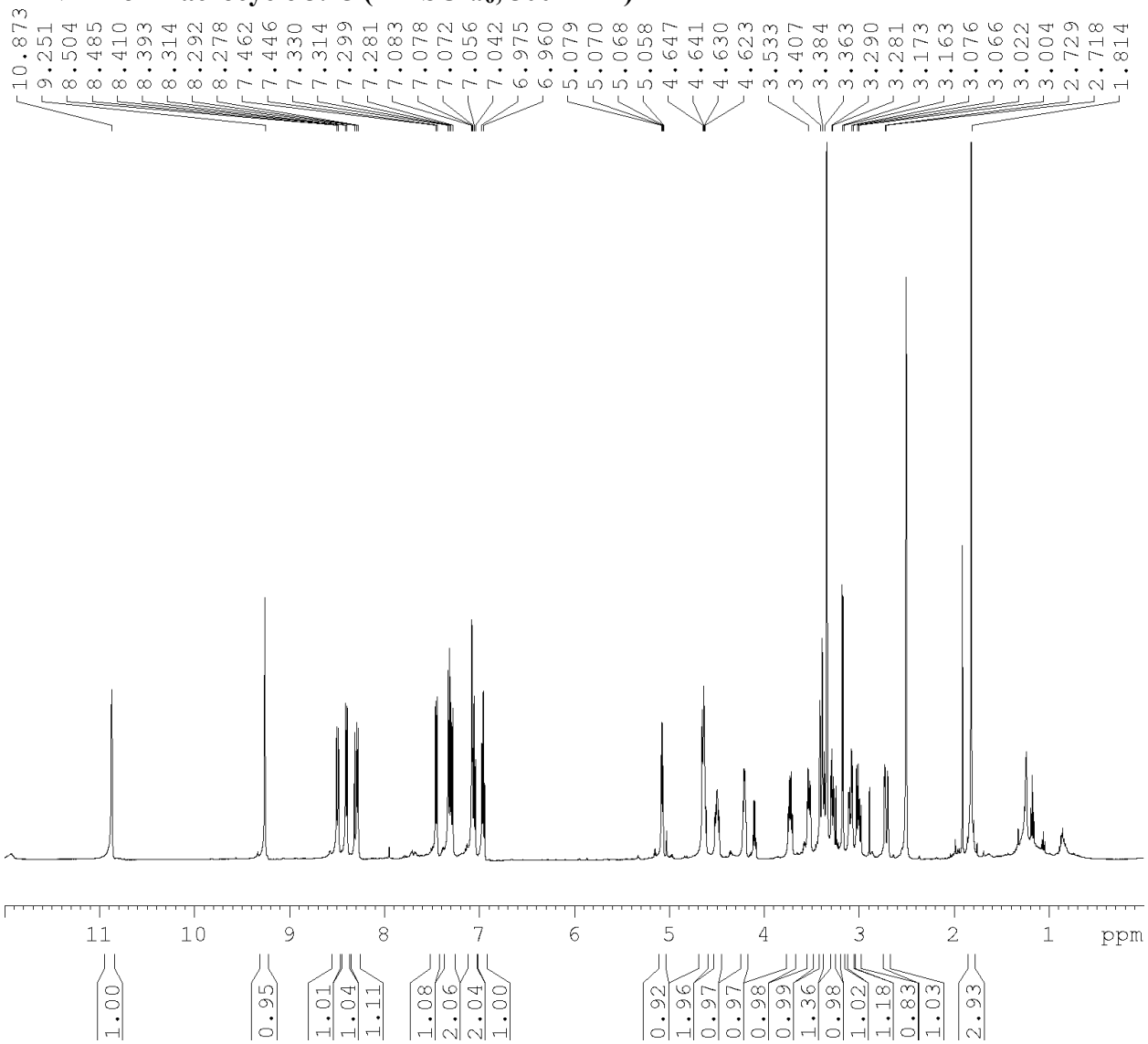
^{13}C NMR of Macrocycle 3.24 (DMSO-d_6, 126 MHz)



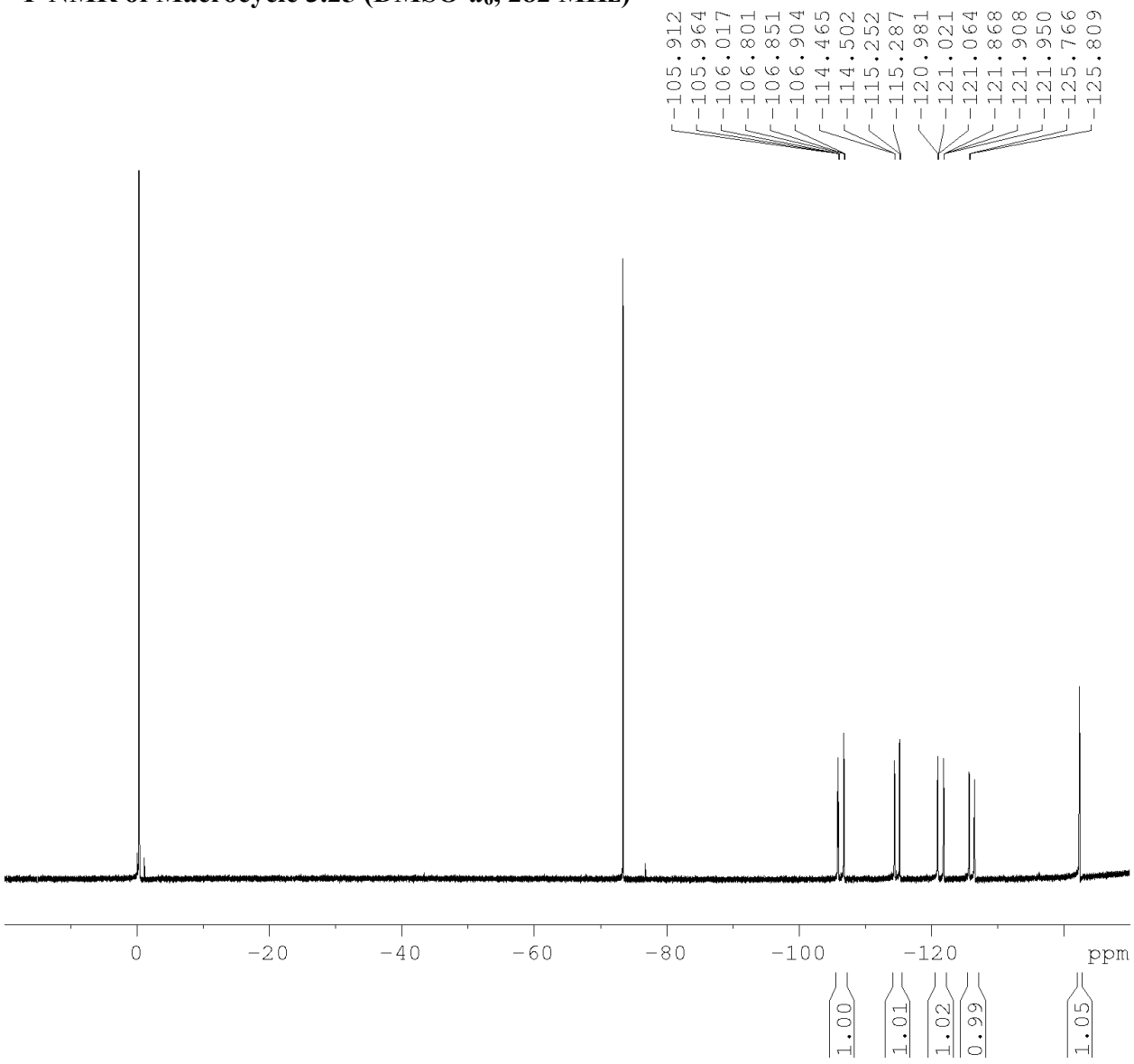
^{19}F NMR of Macrocycle 3.24 (DMSO-d_6, 282 MHz)



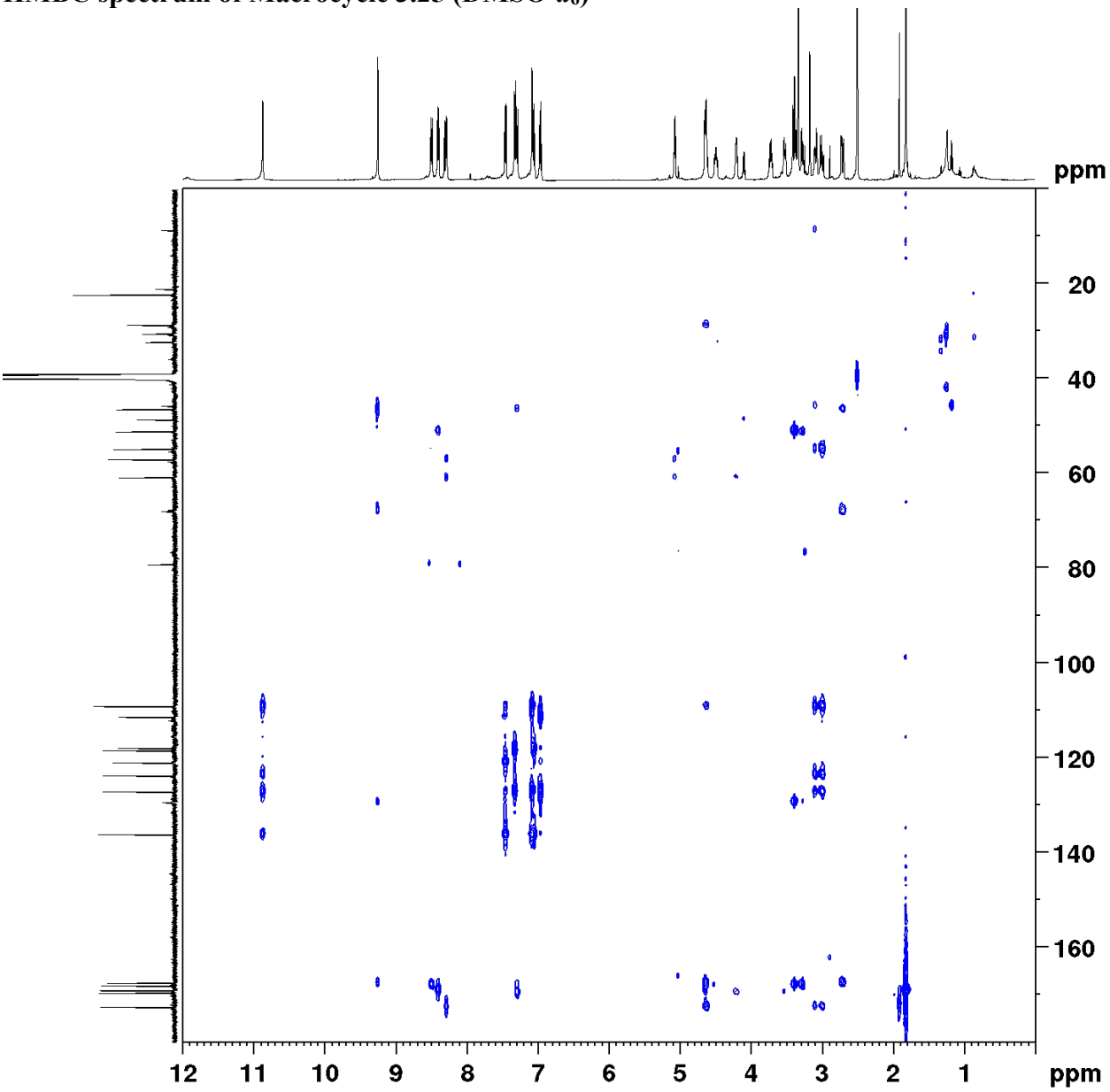
^{1}H NMR of Macrocycle 3.25 (DMSO-d_6, 500 MHz)



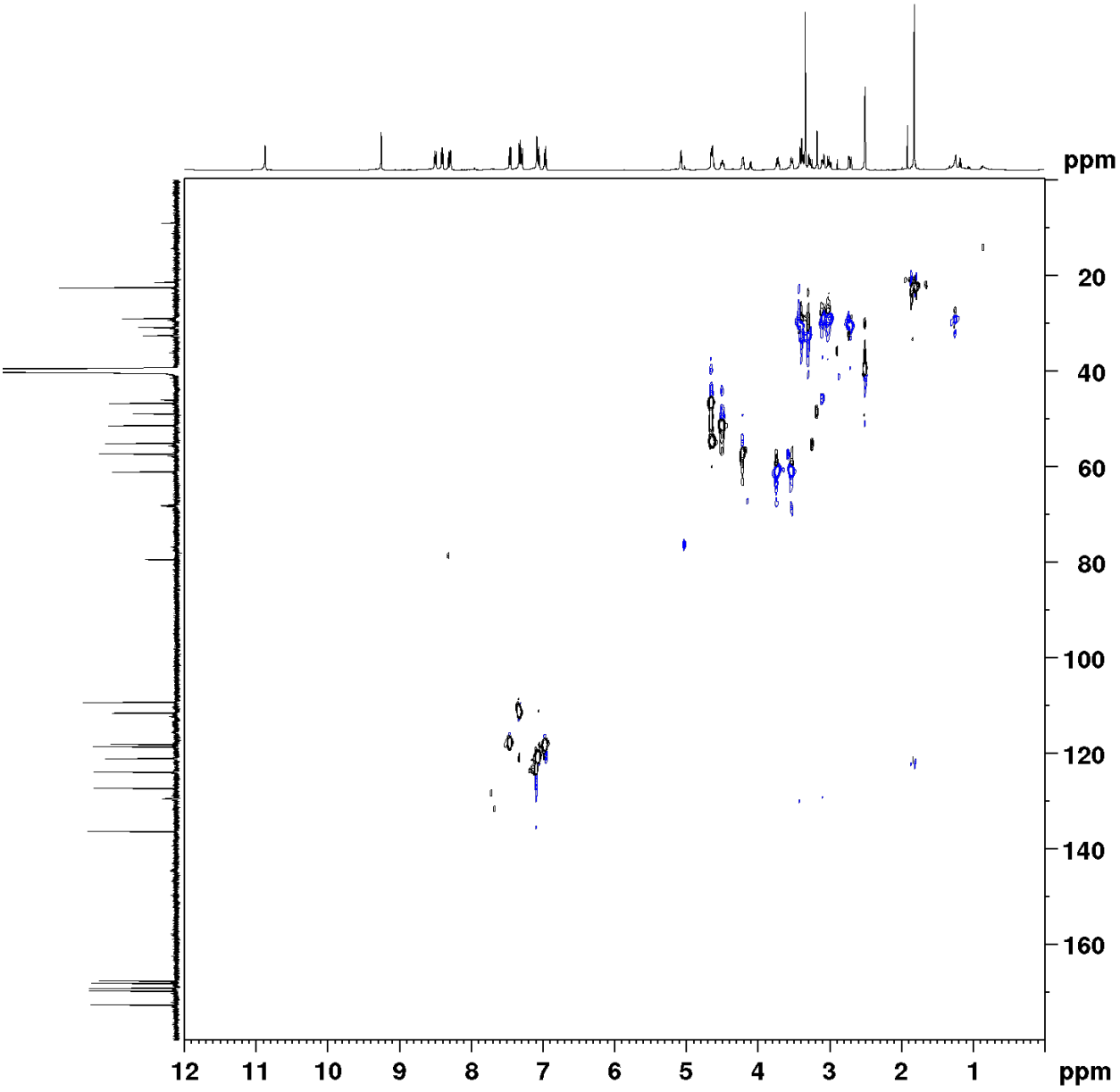
^{19}F NMR of Macrocycle 3.25 (DMSO-d_6, 282 MHz)



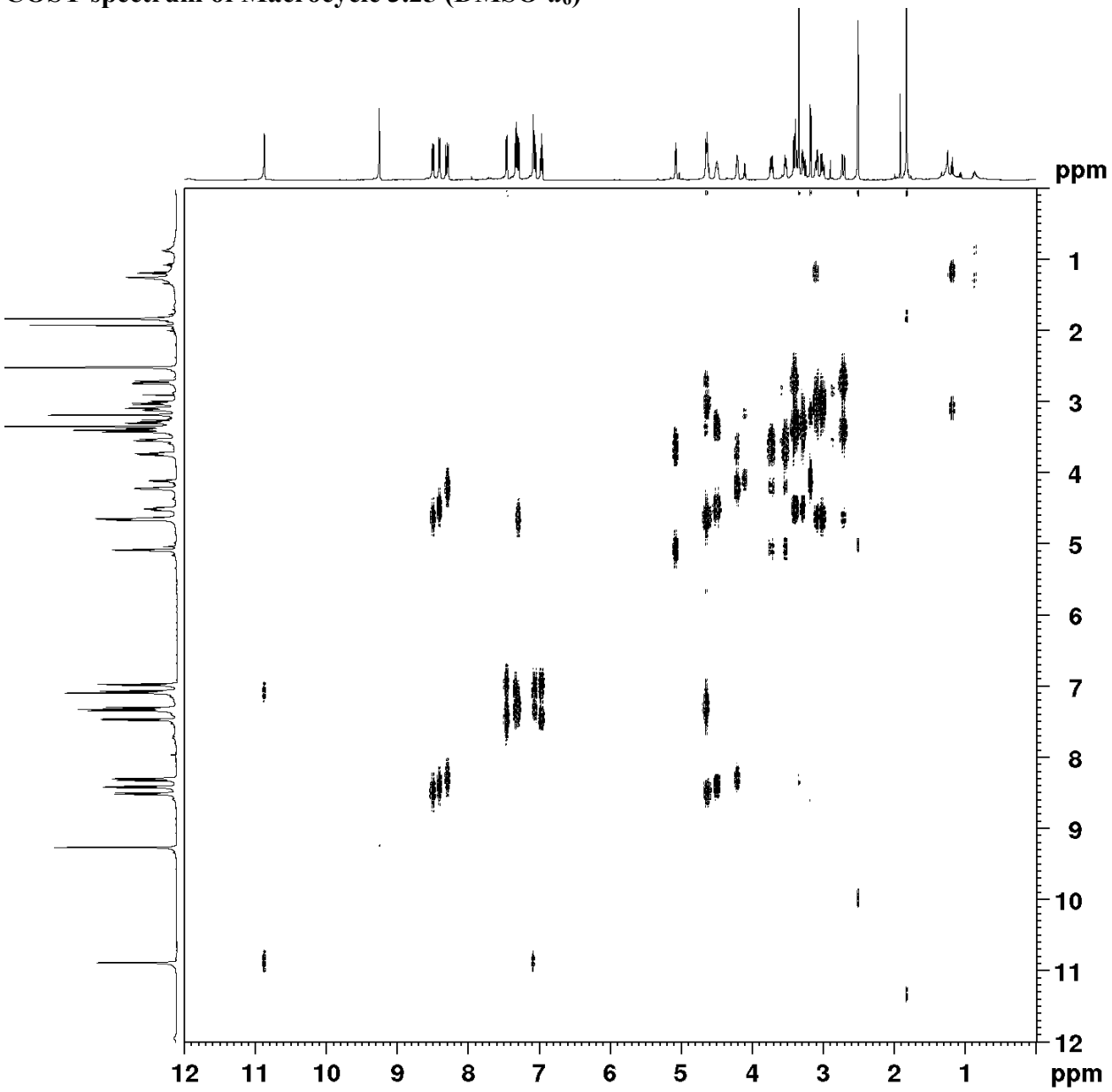
HMBC spectrum of Macrocycle 3.25 (DMSO-d_6)



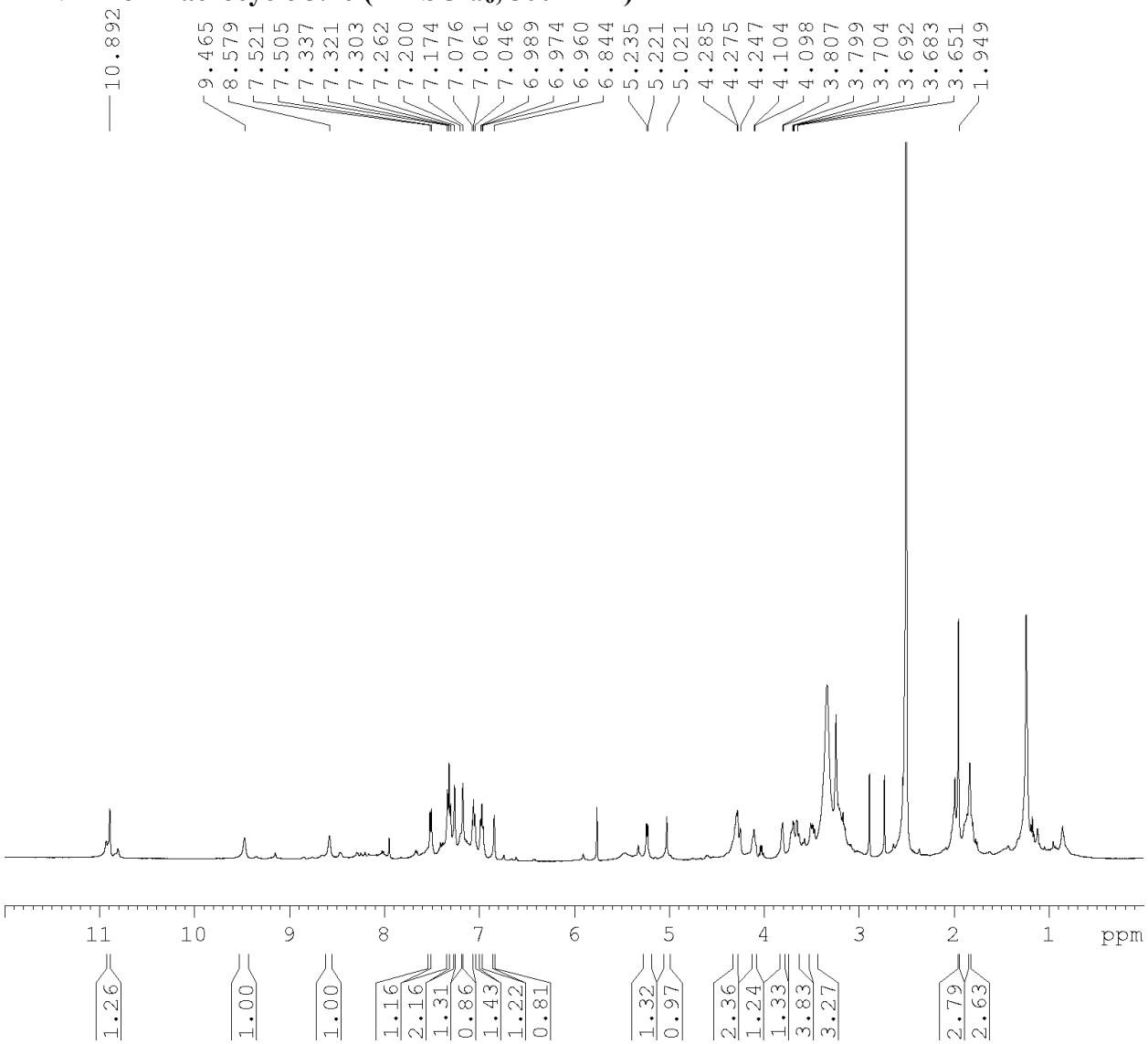
HSQC spectrum of Macrocycle 3.25 (DMSO-d_6)



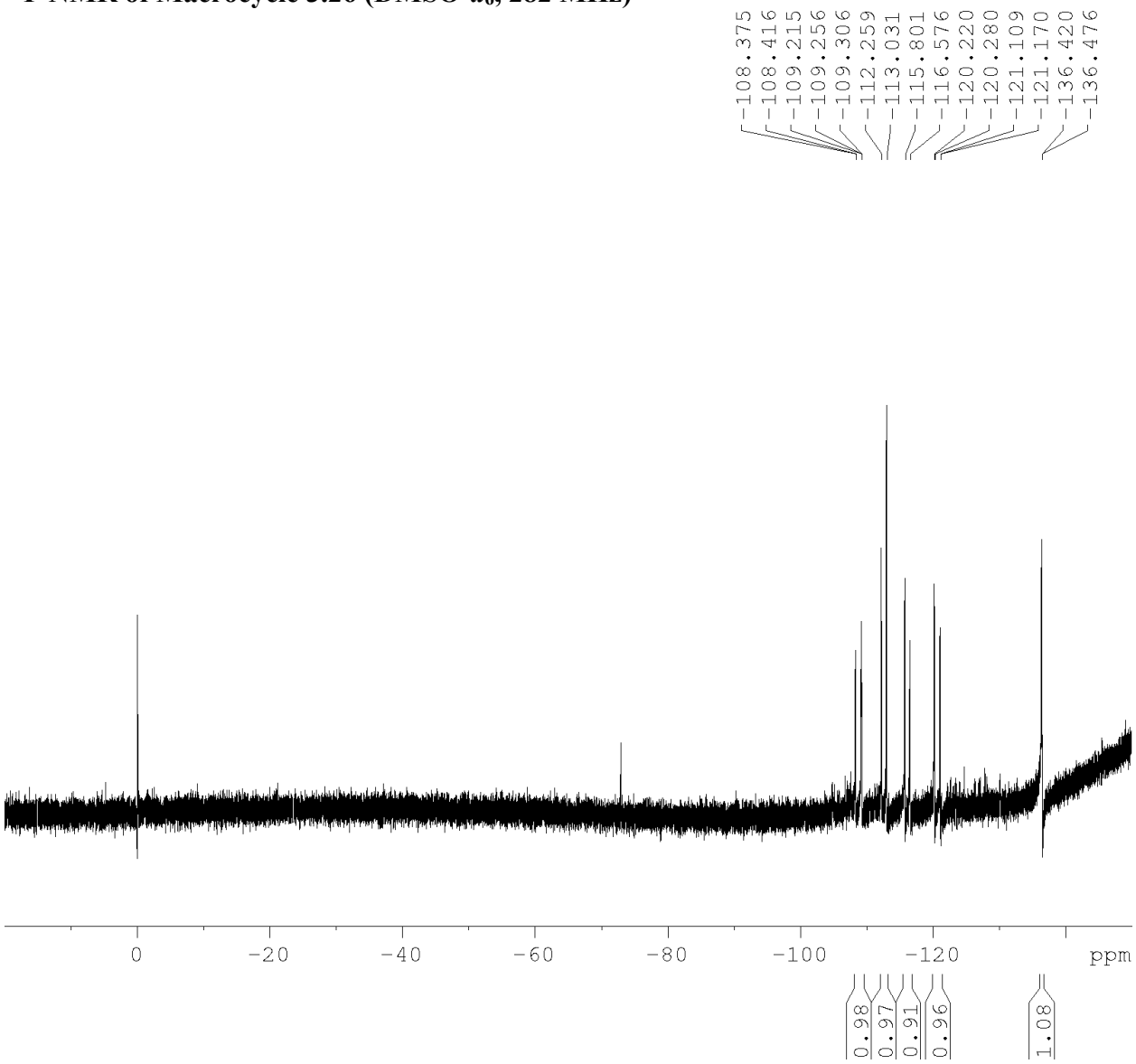
COSY spectrum of Macrocycle 3.25 (DMSO-d_6)



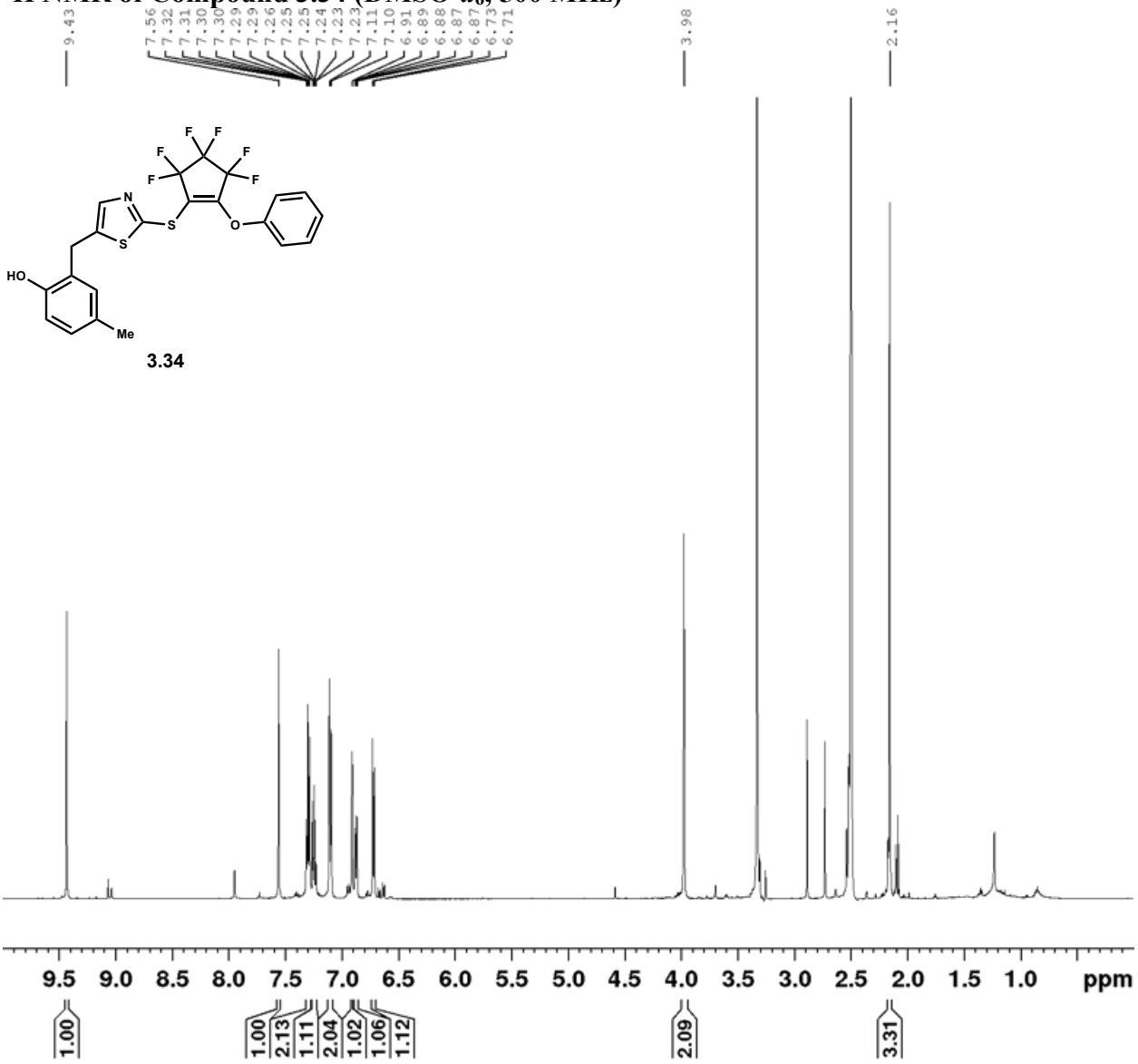
^{1}H NMR of Macrocycle 3.26 (DMSO-d_6, 500 MHz)



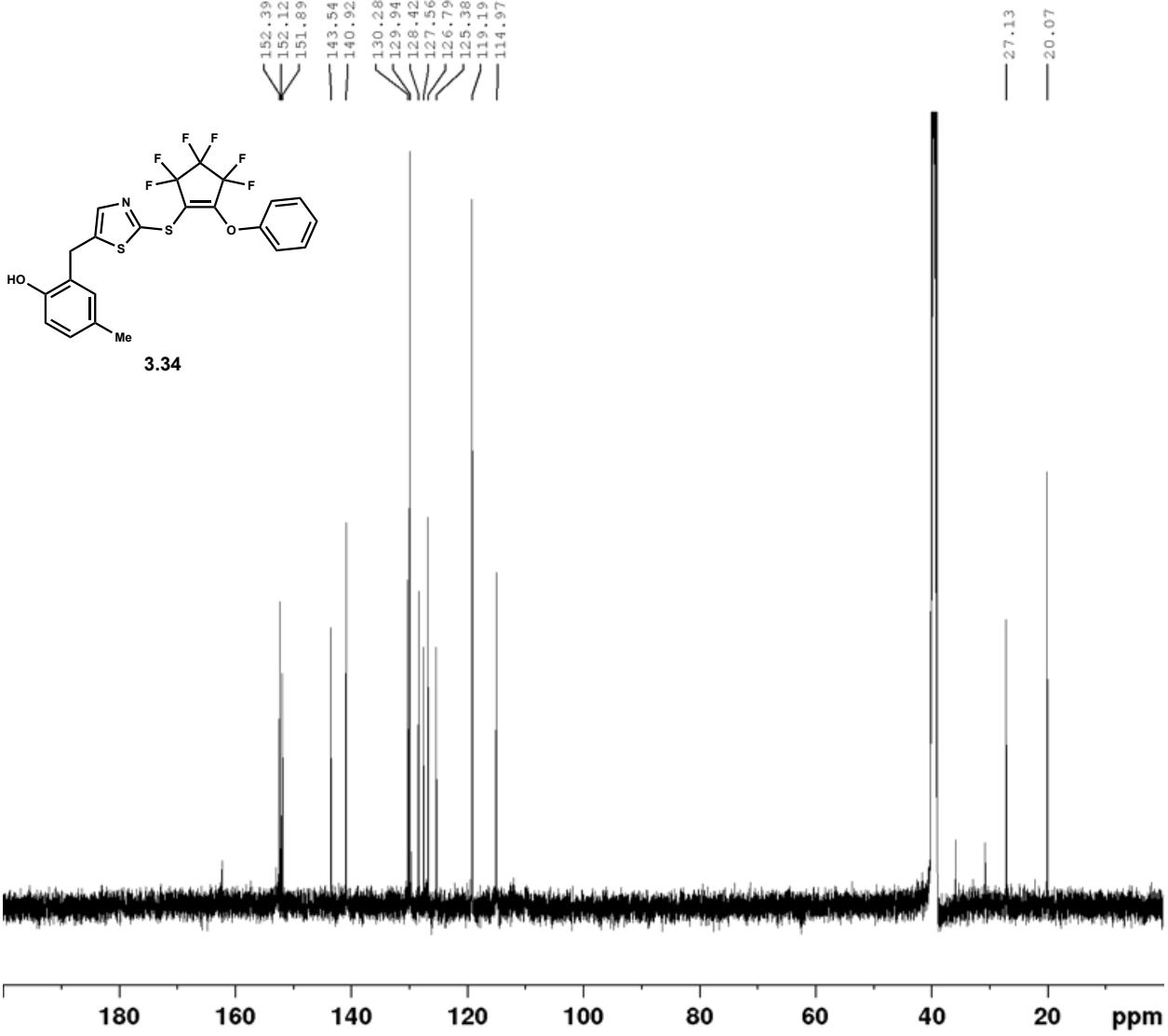
^{19}F NMR of Macrocycle 3.26 (DMSO-d_6, 282 MHz)



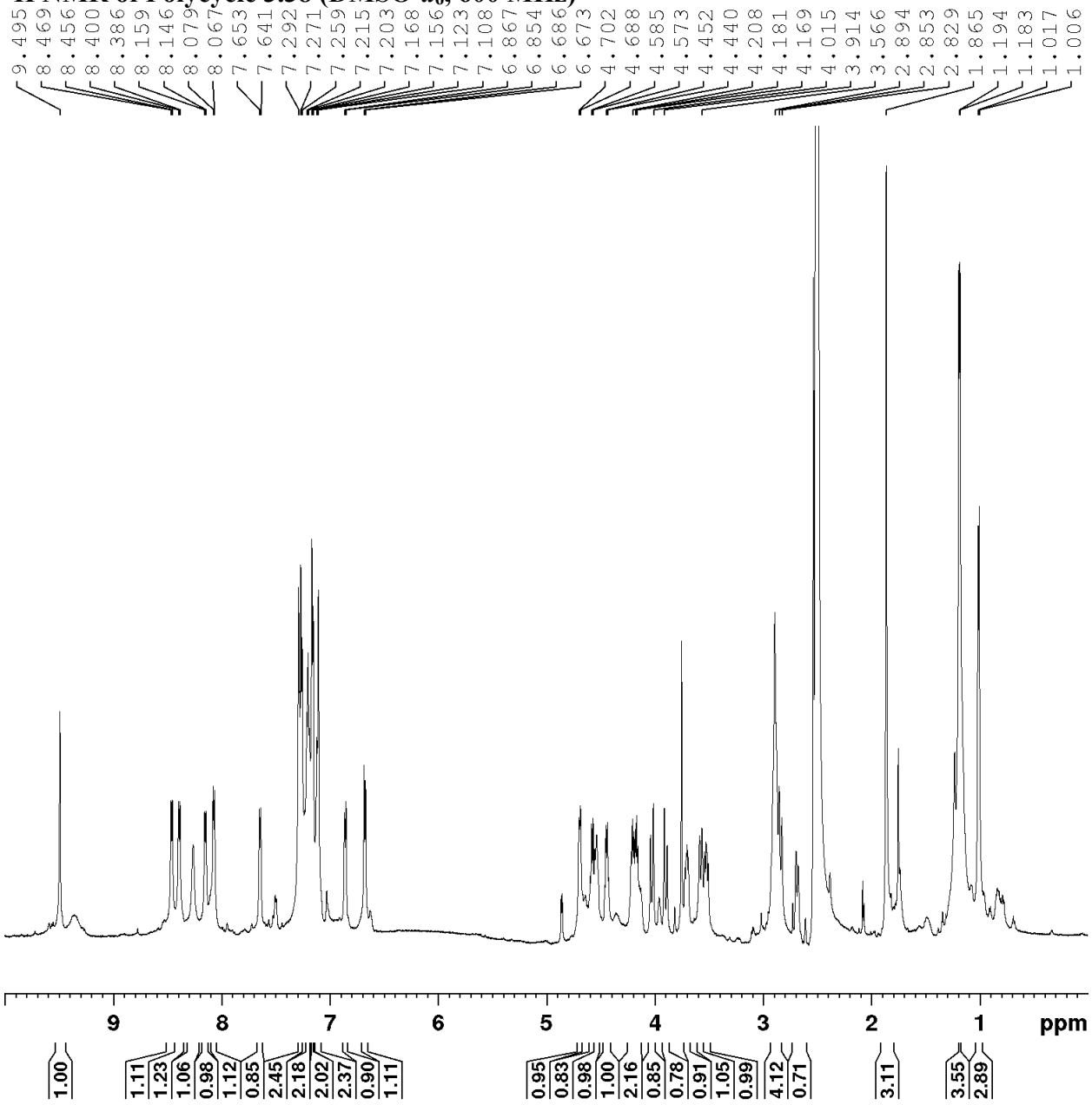
^{1}H NMR of Compound 3.34 (DMSO-d_6, 500 MHz)



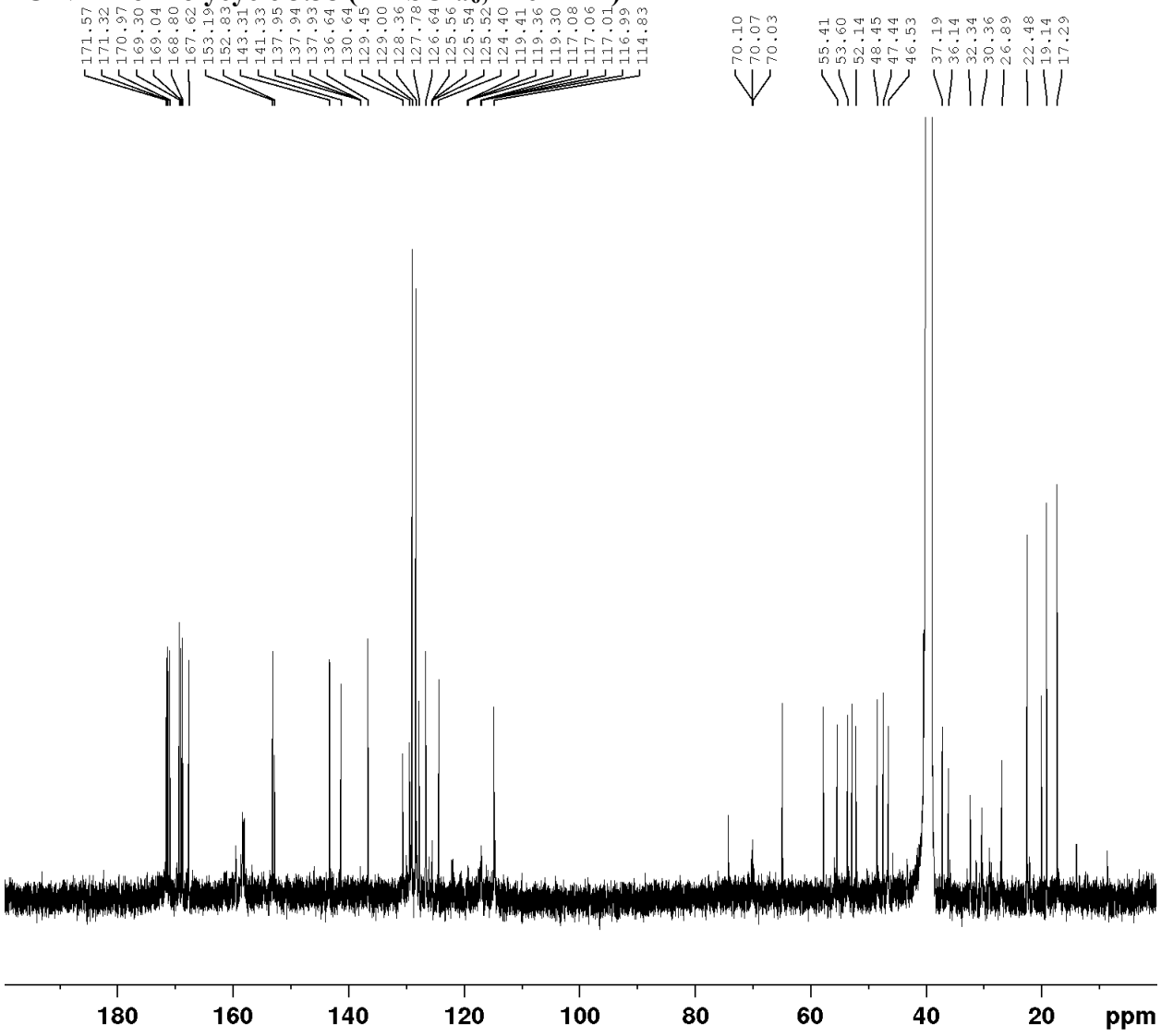
^{13}C NMR of Compound 3.34 (DMSO-d_6, 126 MHz)



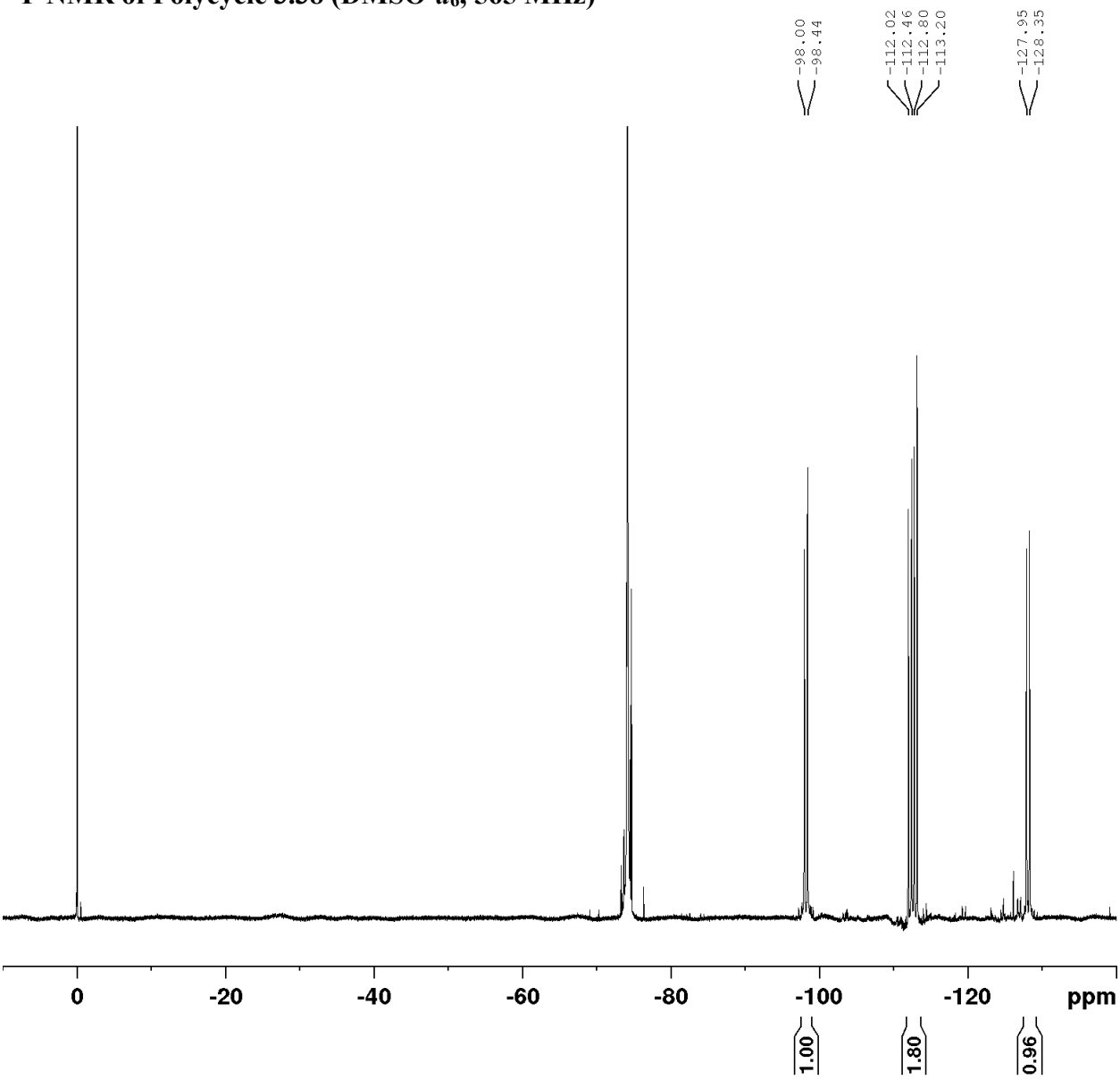
^{1}H NMR of Polycycle 3.38 (DMSO-d_6, 600 MHz)



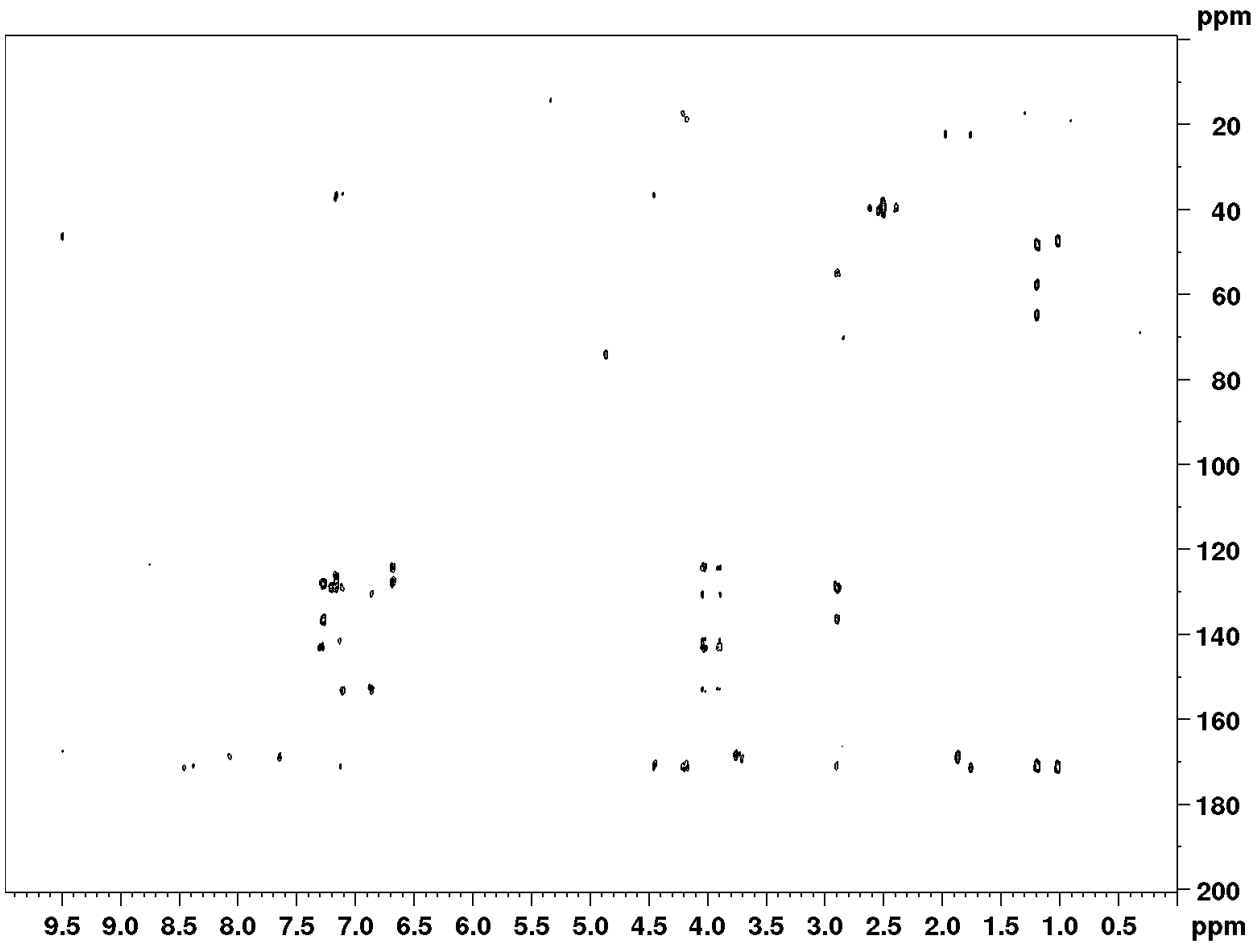
^{13}C NMR of Polycycle 3.38 (DMSO-d_6, 126 MHz)



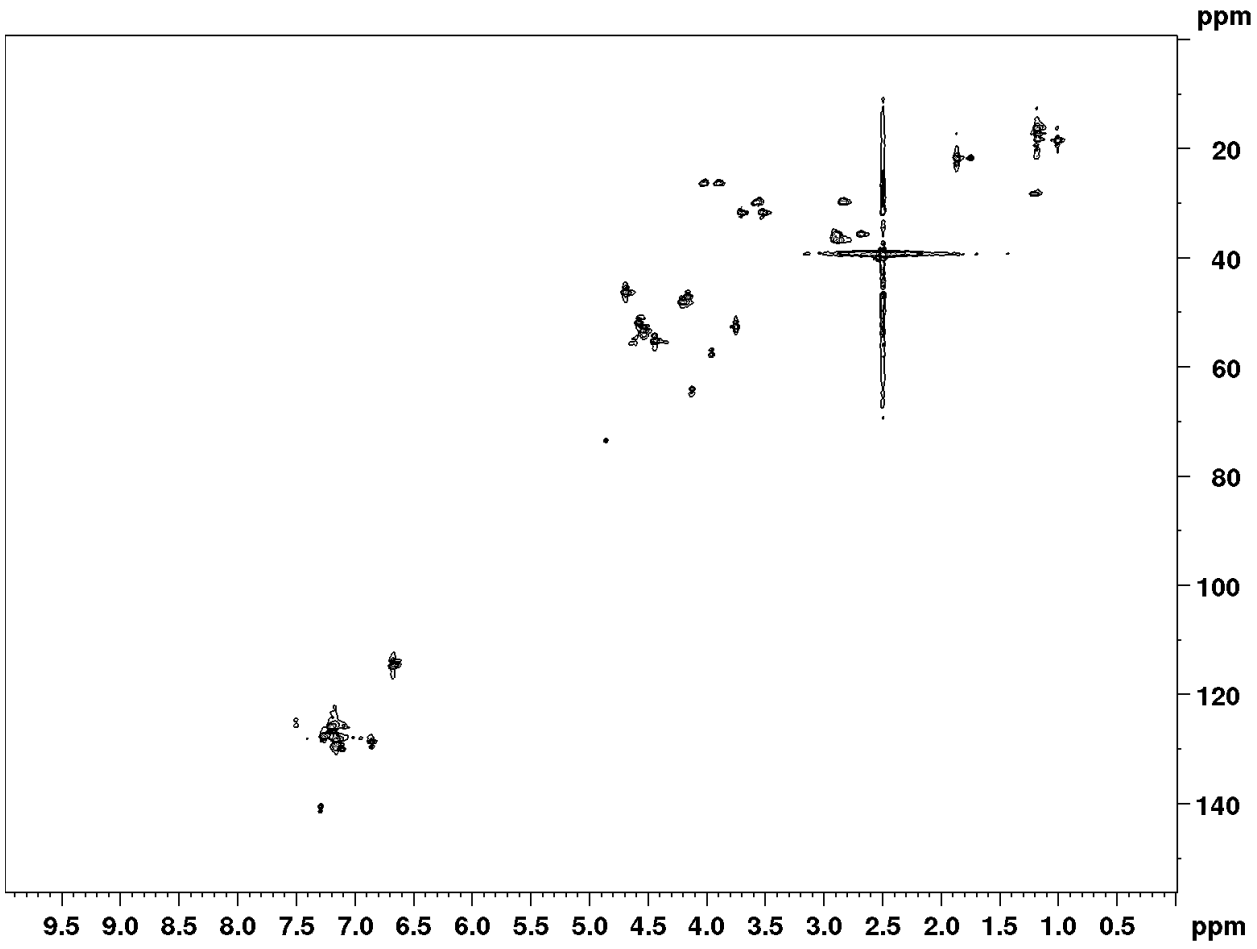
^{19}F NMR of Polycycle 3.38 (DMSO-d_6, 565 MHz)



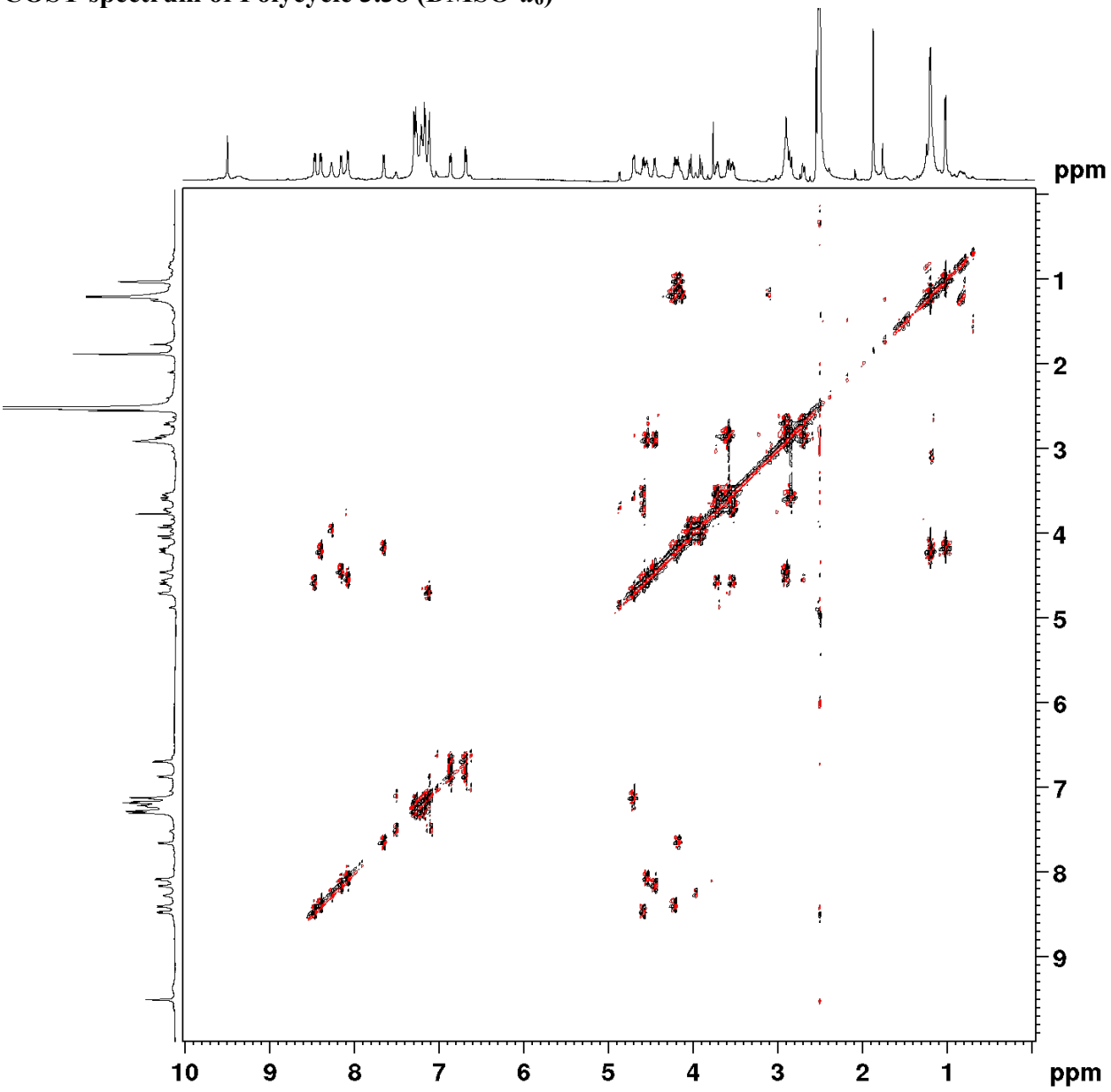
HMBC spectrum of Polycycle 3.38 (DMSO-d_6)



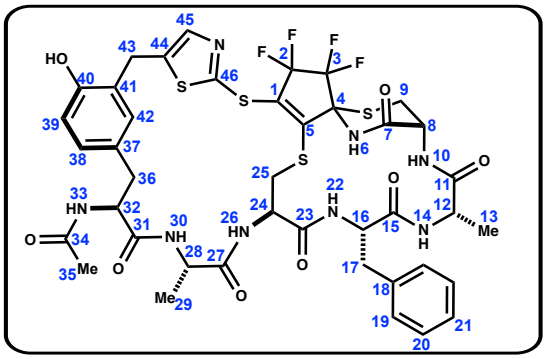
HSQC spectrum of Polycycle 3.38 (DMSO-d_6)



COSY spectrum of Polycycle 3.38 (DMSO-d_6)

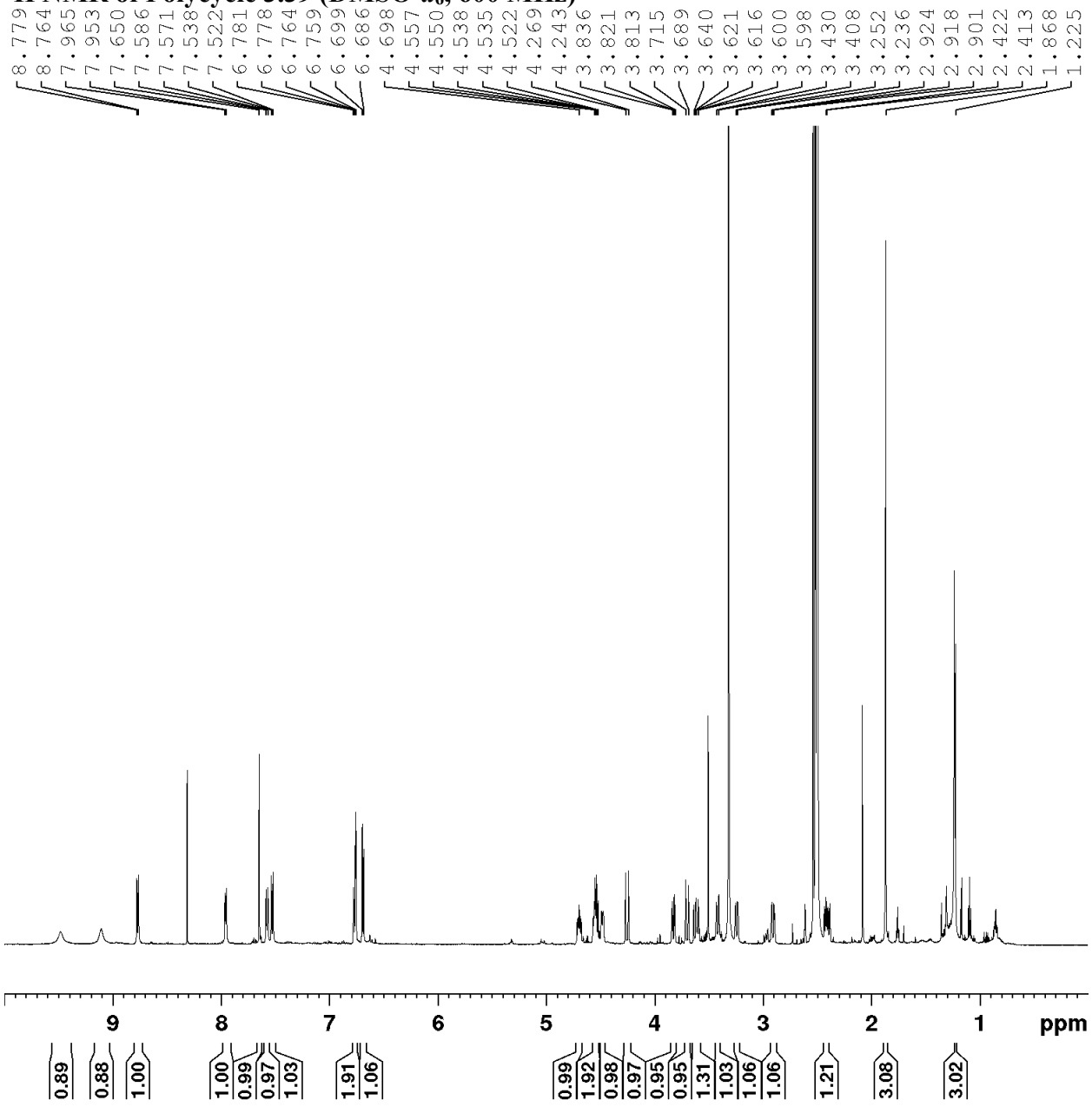


Polycycle 3.38 (DMSO-d_6)

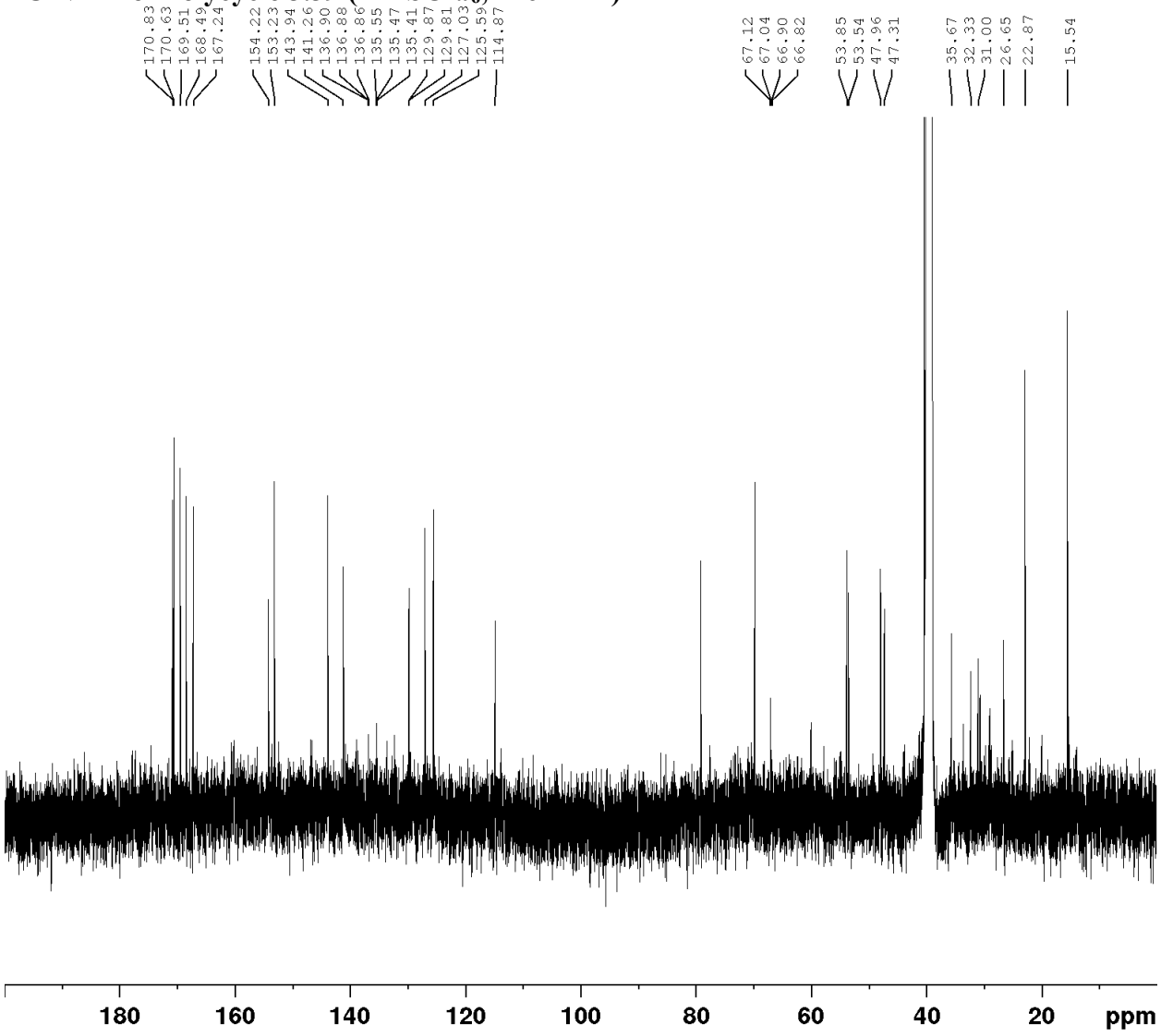


	13C	1H	Key Correlation
1	137.95-137.93 (m, 1C)	–	–
2	117.08-117.00 (m ,1C)	–	–
3	119.41-119.30 (m, 1C)	–	–
4	70.10-70.03 (m, 1C)	–	9->4 HMBC
5	125.56-125.52 (m, 1C)	–	25->5 HMBC
6	–	9.49 (s,1H)	6->7 HMBC
7	167.62	–	6->7 HMBC
8	46.53	4.70 (d, J = 8.2 Hz, 1H)	8->7 HMBC, HSQC
9	30.35	3.72-3.69 (dd, J = 12.2, 7.1 Hz, 1H) 3.53 (dd, J = 12.4, 8.1 Hz, 1H)	9->8 HMBC, HSQC
10	–	7.11 (d, 1H)	10->11 HMBC
11	171.57	–	10->11 HMBC
12	48.45	4.26-4.15 (m, 1H) overlap	12->13 HMBC, 12->13 COSY, HSQC
13	17.29	1.18 (d, J = 6.6 Hz, 3H)	12->13 HMBC, 12->13 COSY, HSQC
14	–	8.46 (d, J = 7.5 Hz, 1H)	14->12 HMBC
15	171.32	–	14->15 HMBC
16	53.59	4.56-4.50 (m, 1H)	16->15 HMBC, HSQC
17	37.19	2.95-2.85 (m, 2H) overlap	16->17 HMBC, HSQC
18	136.64	–	17->18 HMBC
19	129.00	7.15 (m, 2H)	19->20 HMBC, HSQC
20	128.35	7.20 (m, 2H)	20->19 HMBC, HSQC
21	126.64	7.30-7.25 (m, 1H)	20->21 HMBC, HSQC
22	–	8.39 (d, J = 8.3 Hz, 1H)	16->22 HMBC
23	168.70	–	22->23, 24->23 HMBC
24	52.14	4.44 (dd, J = 14.1, 7.2 Hz, 1H)	24->23 HMBC
25	32.34	3.57 (d, J = 13.1 Hz, 1H), 2.72–2.61 (m, 1H)	24->25, 25->27 HMBC, HSQC
26	–	8.07 (d, J = 7.5 Hz, 1H)	26->24 HMBC
27	168.80	–	26->27 HMBC
28	47.44	4.19 (m, 1H) overlap	29->28 HMBC, HSQC
29	19.14	1.00 (d, J = 6.5 Hz, 3H)	28->29 HMBC, HSQC
30	–	8.15 (d, J = 7.7 Hz, 1H)	30->28 HMBC
31	169.30	–	30->31 HMBC
32	55.41	4.61-4.56 (m, 1H) overlap	33->32 HMBC, HSQC
33	–	7.65 (d, J = 8.0 Hz, 1H)	34->33 HMBC
34	169.04	–	35-> 34 HMBC
35	22.48	1.86 (s, 3H)	HSQC
36	36.14	2.97-2.83 (m, 2H) overlap	32->36 HMBC, HSQC
37	130.63	–	36->37 HMBC
38	129.45	6.86 (d, J = 7.3 Hz, 1H)	38->37, 39->38 HMBC
39	114.83	6.68 (d, J = 7.9 Hz, 1H)	38->39 HMBC
40	127.78	–	39->40 HMBC
41	124.40	–	43->41 HMBC
42	129.45	7.16 (d, 1H)	42->41 HMBC
43	26.88	4.03 (d, J = 15.6 Hz, 1H), 3.90 (d, J = 15.6 Hz, 1H)	43->44, 43->41 HMBC
44	143.31	–	45->44 HMBC
45	141.33	7.29 (s, 1H)	45->44 HMBC
46	153.19	–	45->46 HMBC

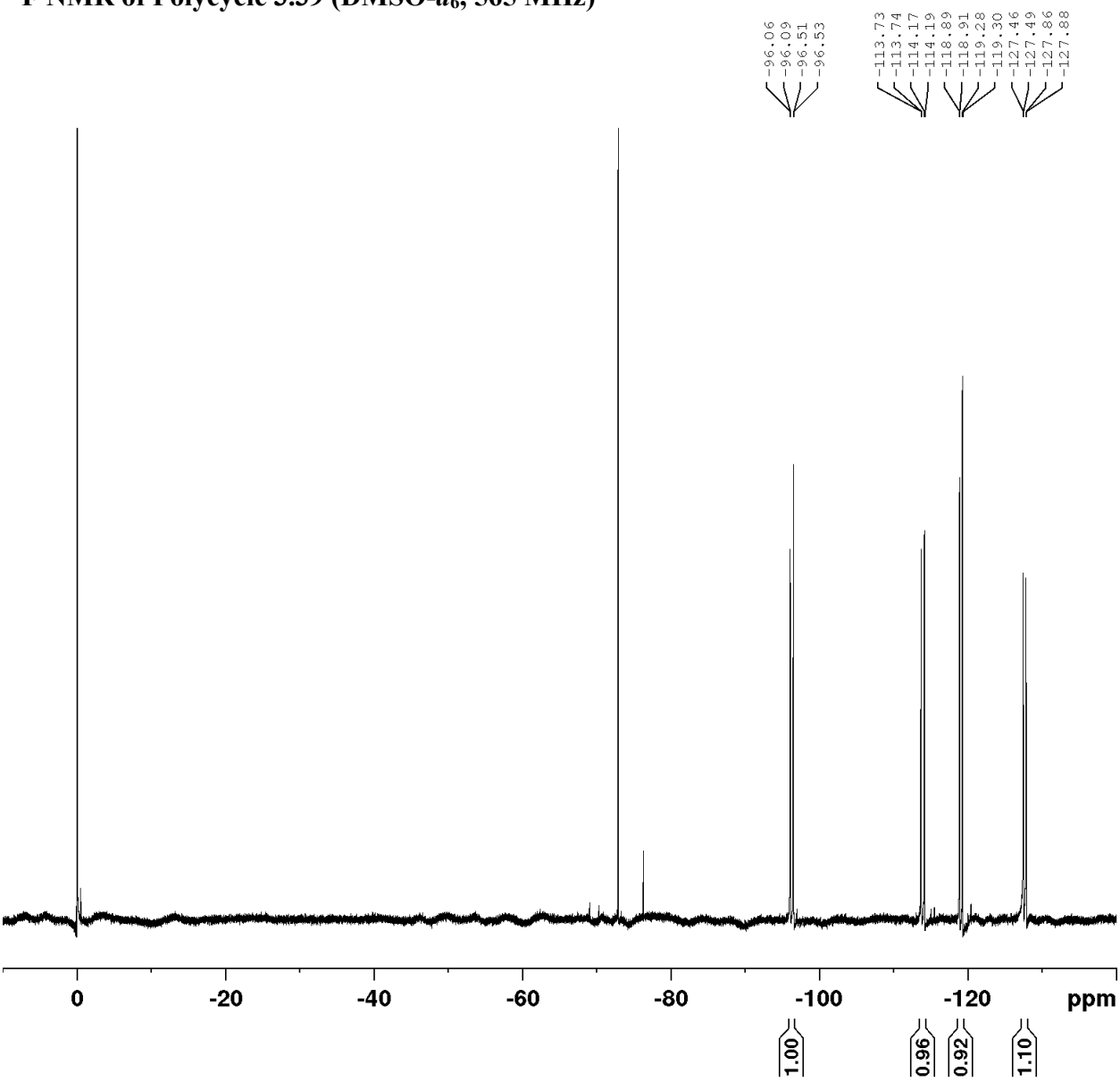
^{1}H NMR of Polycycle 3.39 (DMSO-d_6, 600 MHz)



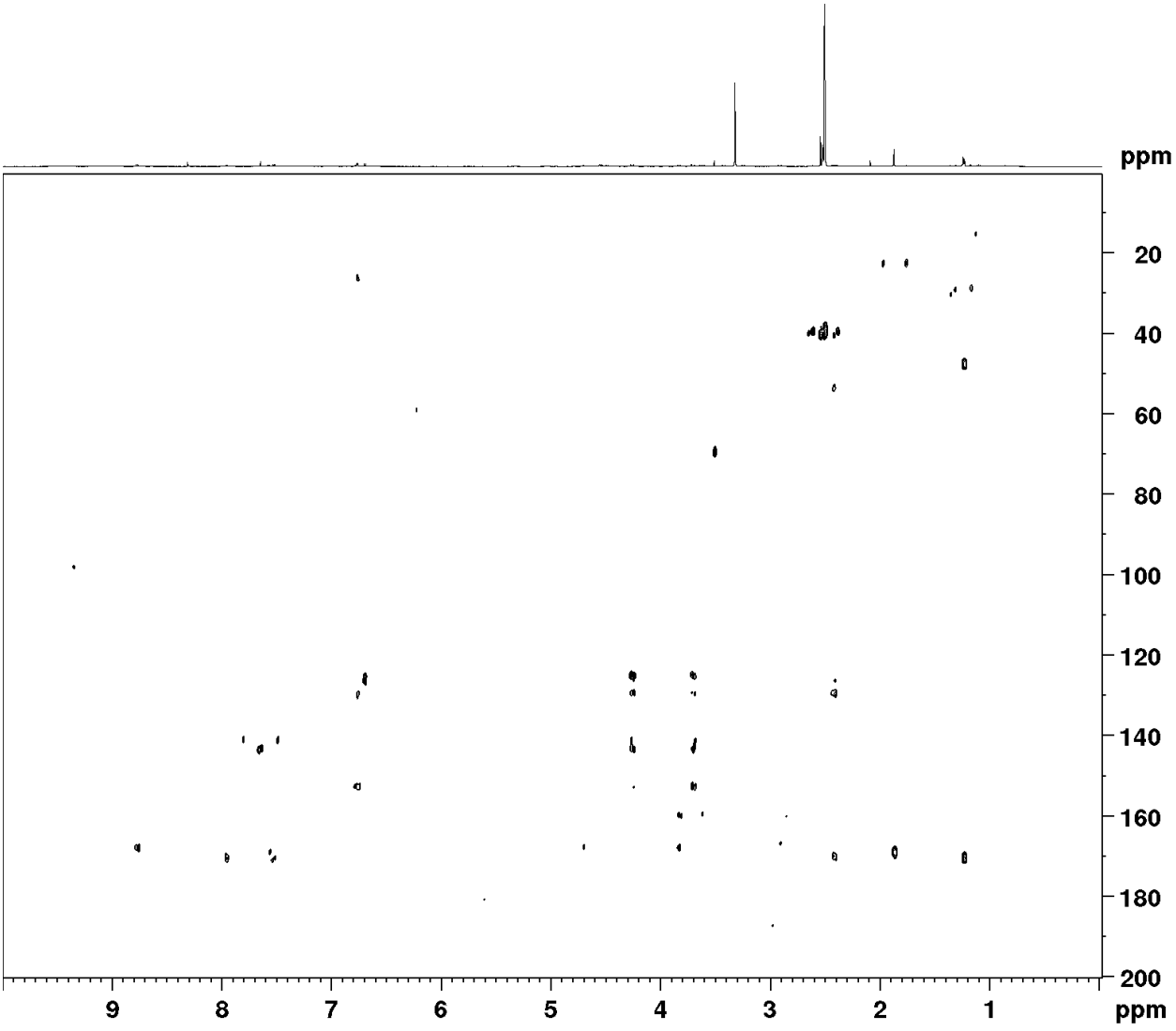
^{13}C NMR of Polycycle 3.39 (DMSO-d_6, 126 MHz)



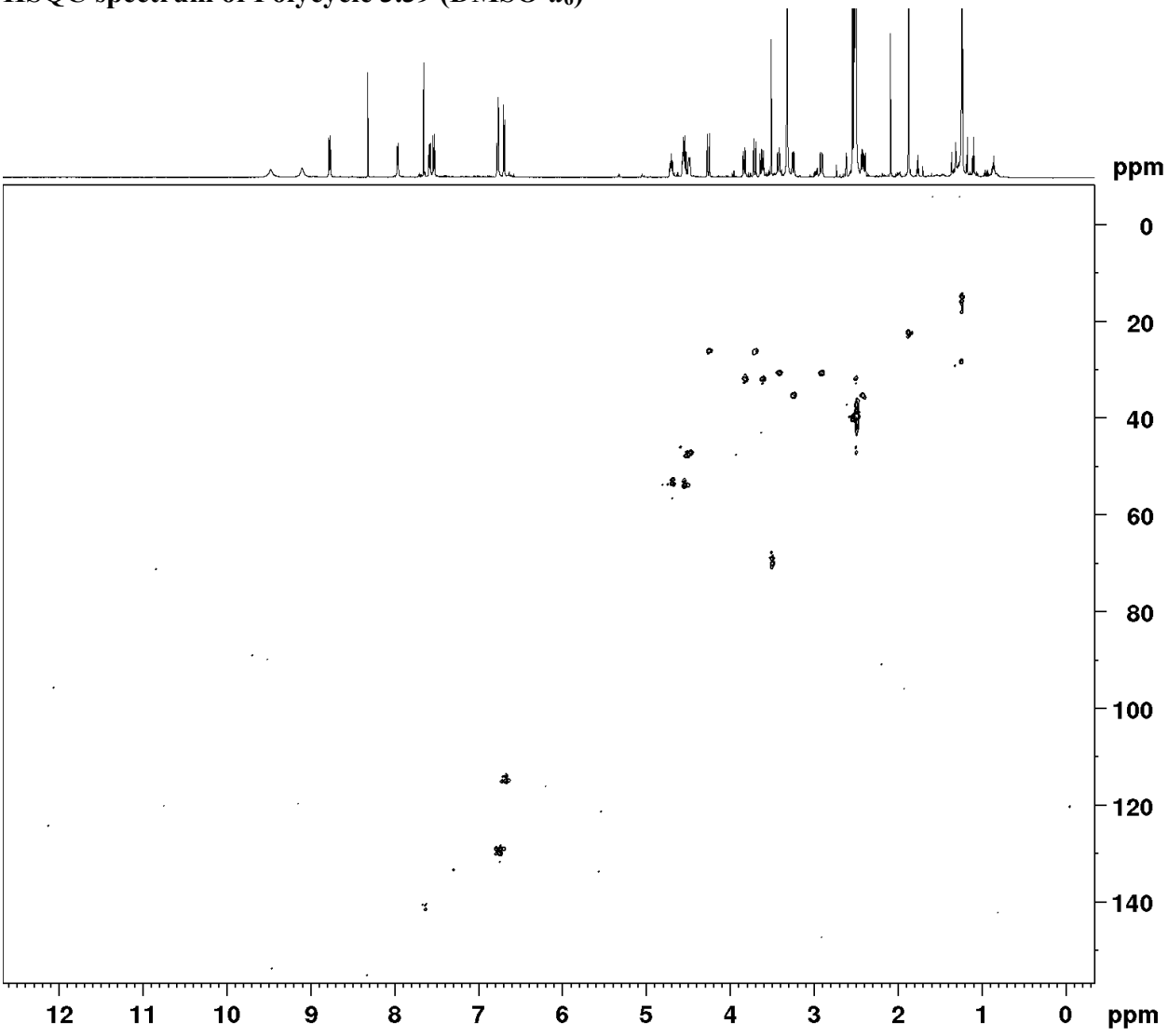
^{19}F NMR of Polycycle 3.39 (DMSO-d_6, 565 MHz)



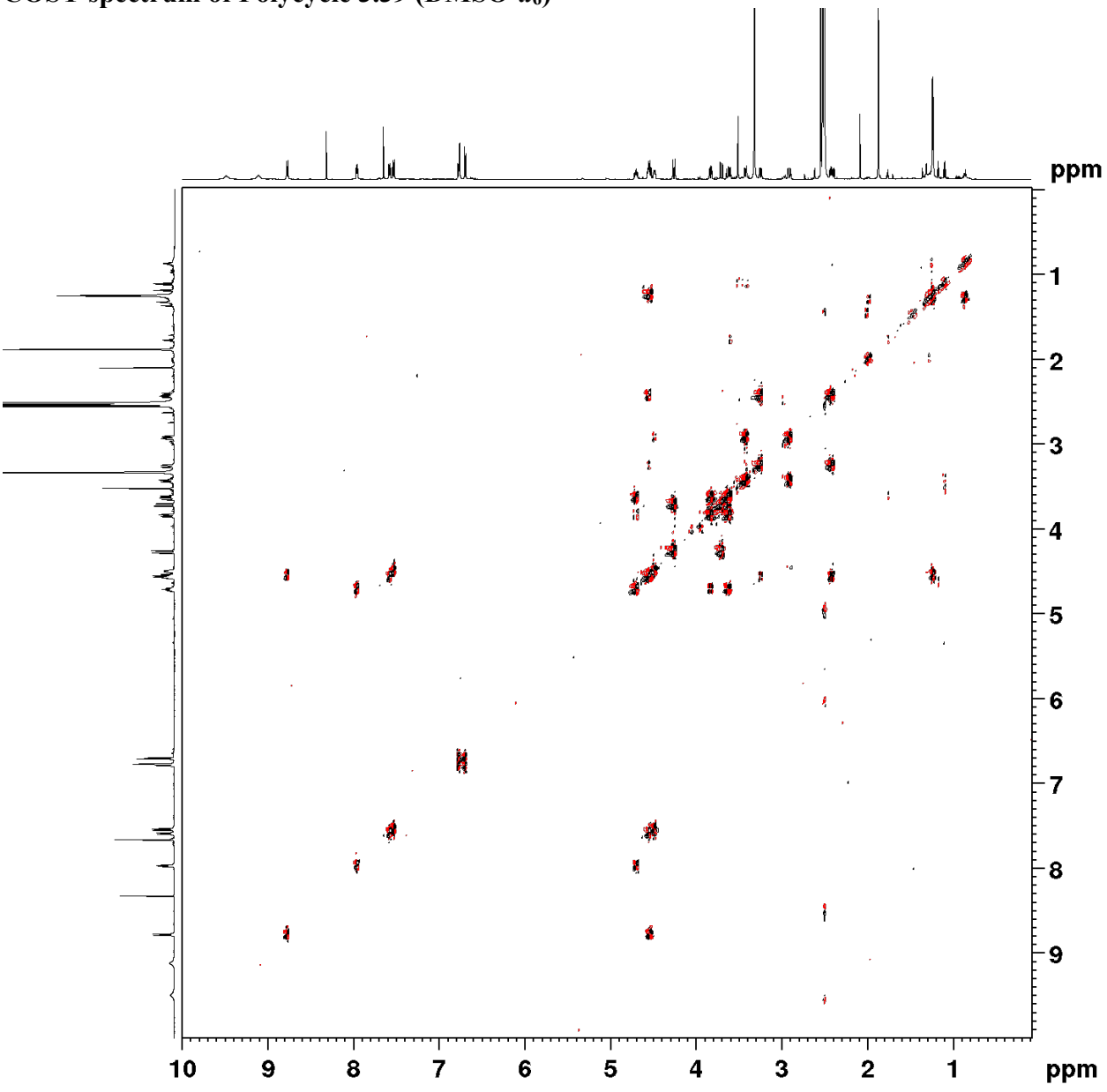
HMBC spectrum of Polycycle 3.39 (DMSO-d_6)



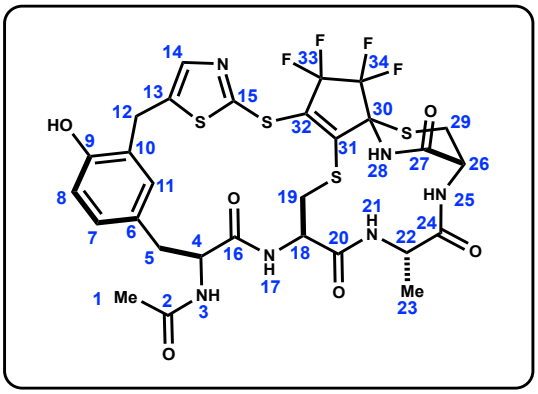
HSQC spectrum of Polycycle 3.39 (DMSO-d_6)



COSY spectrum of Polycycle 3.39 (DMSO-d_6)

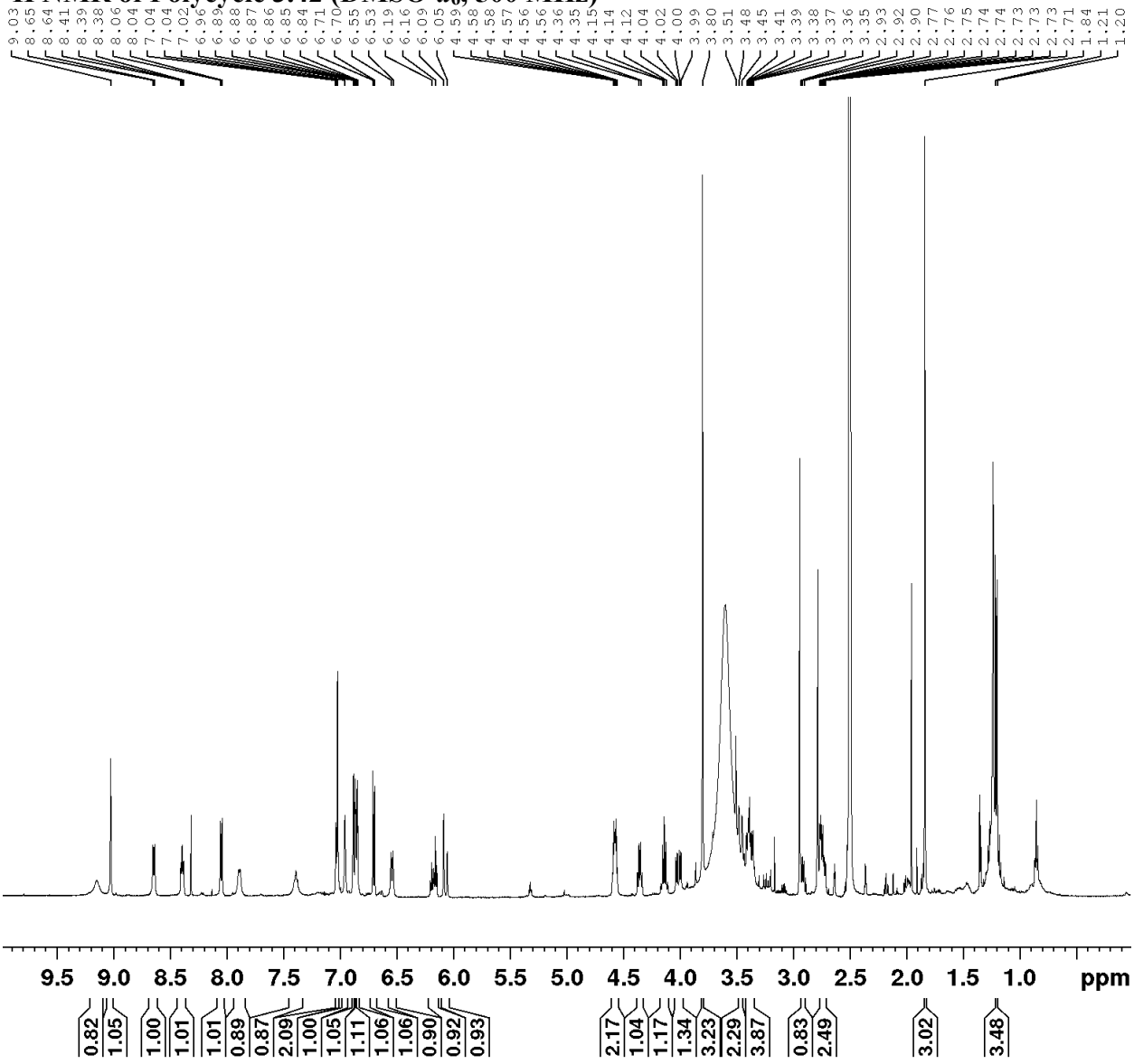


Polycycle **3.39** (DMSO-d_6, 298K)

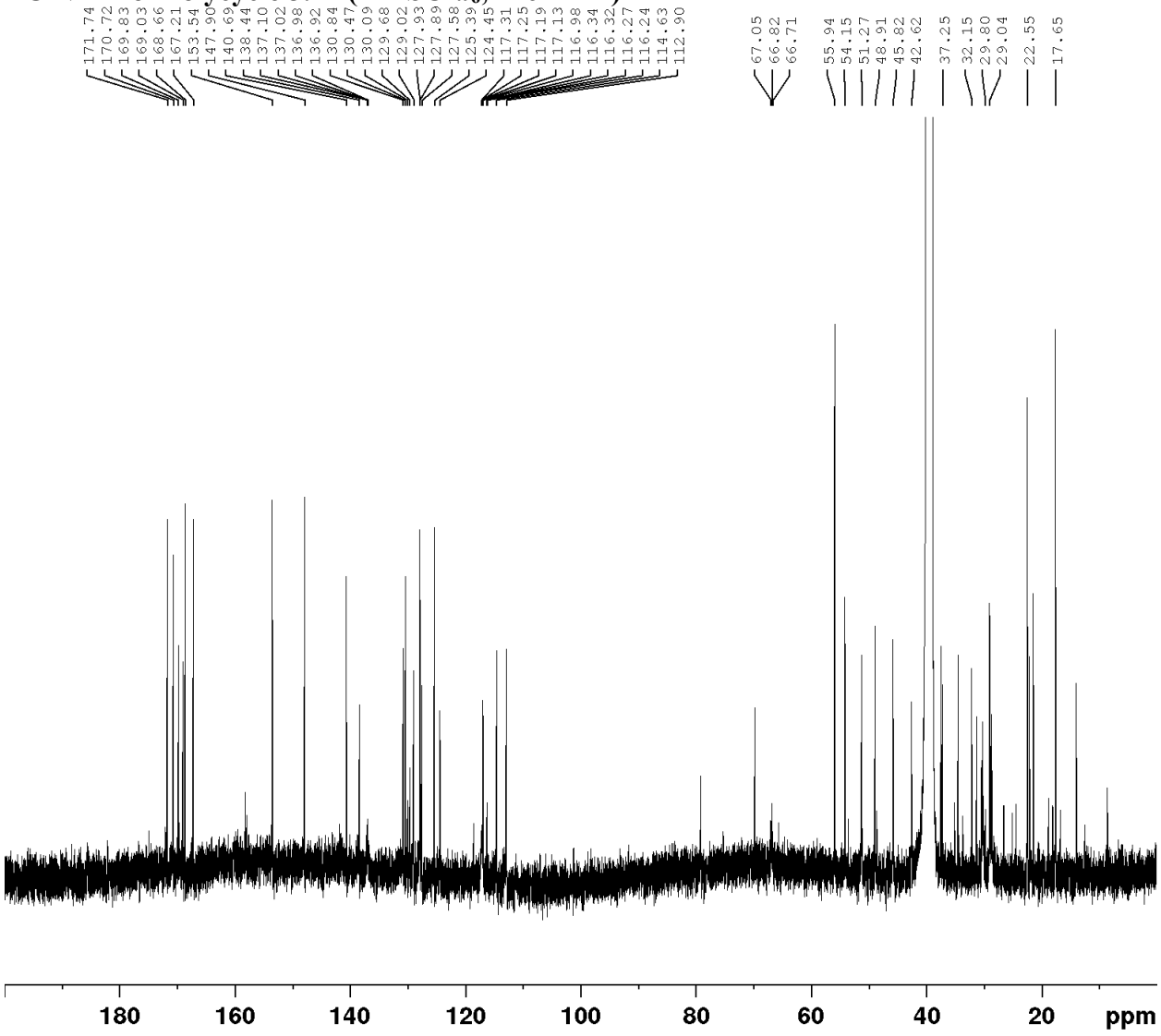


	13C	1H	Key Correlation
1	22.87	1.87 (s, 3H)	HMBC, HSQC
2	169.51	-	1->2 HMBC
3	-	7.58 (d, 1H)	2->3 HMBC
4	53.86	4.55 (m, 1H) overlap	3->4 COSY, HSQC
5	35.67	3.24 (dd, J = 13.1, 3.2 Hz, 1H), 2.42 (dd, J = 13.1, 7.9 Hz, 1H)	HSQC
6	125.59	-	5->4 HMBC
7	127.03	6.76 (m, 1H) overlap	8->7 HMBC
8	114.9	6.69 (d, J = 8.0 Hz, 1H)	HSQC
9	153.22	-	8->7 HMBC
10	129.82	-	12->10 HMBC
11	129.89	6.76 (m, 1H) overlap	10->11 HMBC, HSQC
12	26.65	4.26 (d, J = 15.6 Hz, 1H), 3.70 (d, J = 15.5 Hz, 1H)	13->12 HMBC, HSQC
13	143.94	-	13->12 HMBC, HSQC
14	141.26	7.65 (s, 1H)	HSQC
15	154.22	-	-
16	170.83	-	17->16 HMBC
17	-	7.96 (d, J = 7.3 Hz, 1H)	18->17 COSY
18	53.54	4.70 (m, 1H)	20->18 HMBC, HSQC
19	32.33	3.83 (dd, J = 14.0, 4.8 Hz, 1H), 3.62 (m, 1H)	18->19 COSY, HSQC
20	168.49	-	21->20 HMBC
21	-	8.77 (d, J = 9.2 Hz, 1H)	22->21 COSY
22	47.96	4.54 (m, 1H) overlap	23->22 COSY, HSQC
23	15.54	1.23 (d, J = 5.3 Hz, 1 H)	HSQC
24	170.63	-	22->24 HMBC
25	-	7.53 (d, J = 9.6 Hz, 1H)	
26	47.31	4.49 (m, 1H)	25->26 COSY, HSQC
27	167.24	-	26->27 HMBC
28	-	9.11 or 9.48	-
29	31.00	3.41 (m, 1H), 2.91 (m, 1H)	26->29 HMBC, HSQC
30	66.98 (m, 1C)	-	-
31	135.47 (m, 1C)	-	-
32	136.88 (m, 1C)	-	-
33	-	-	-
34	-	-	-

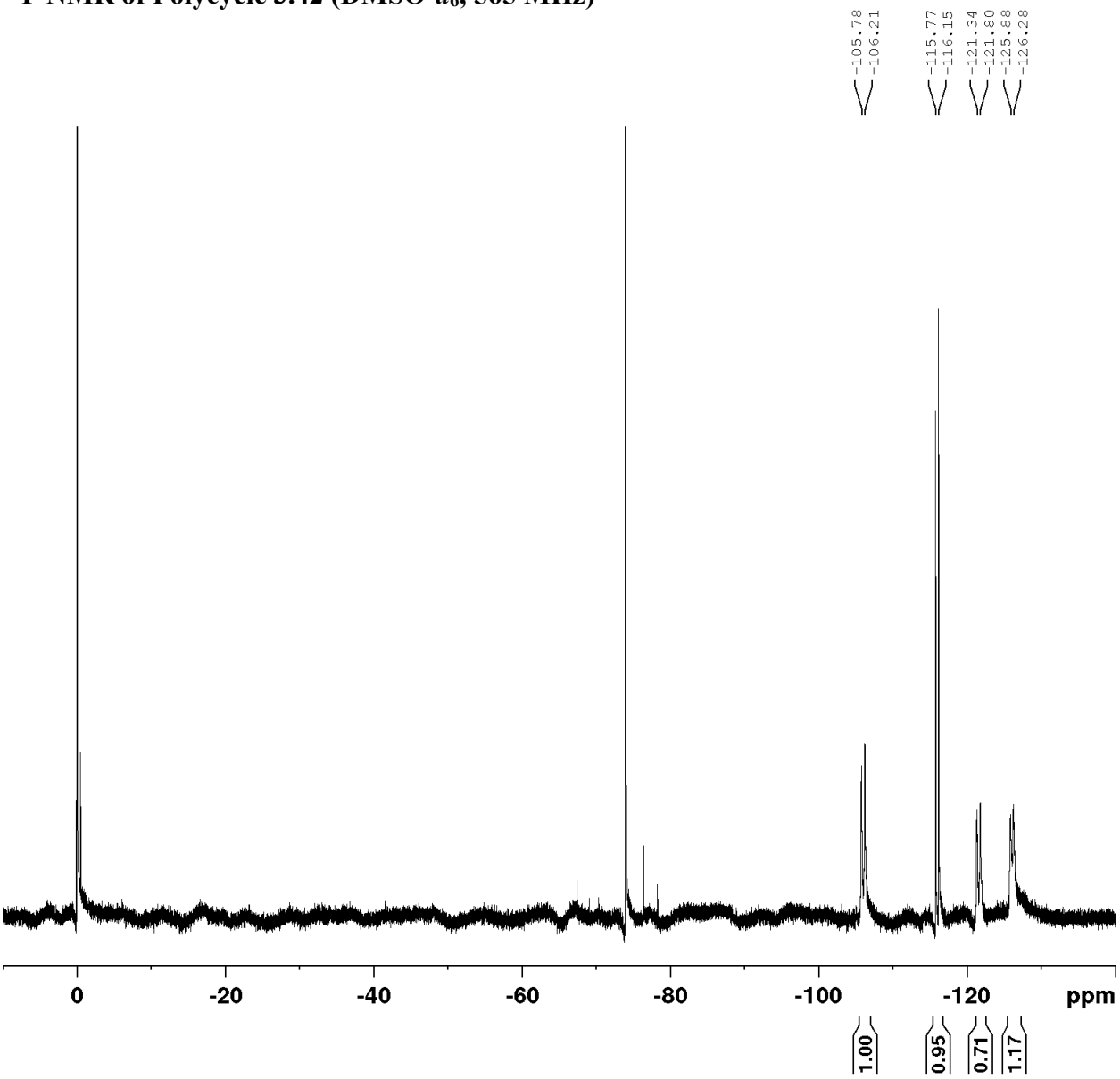
^{1}H NMR of Polycycle 3.42 (DMSO-d_6, 500 MHz)



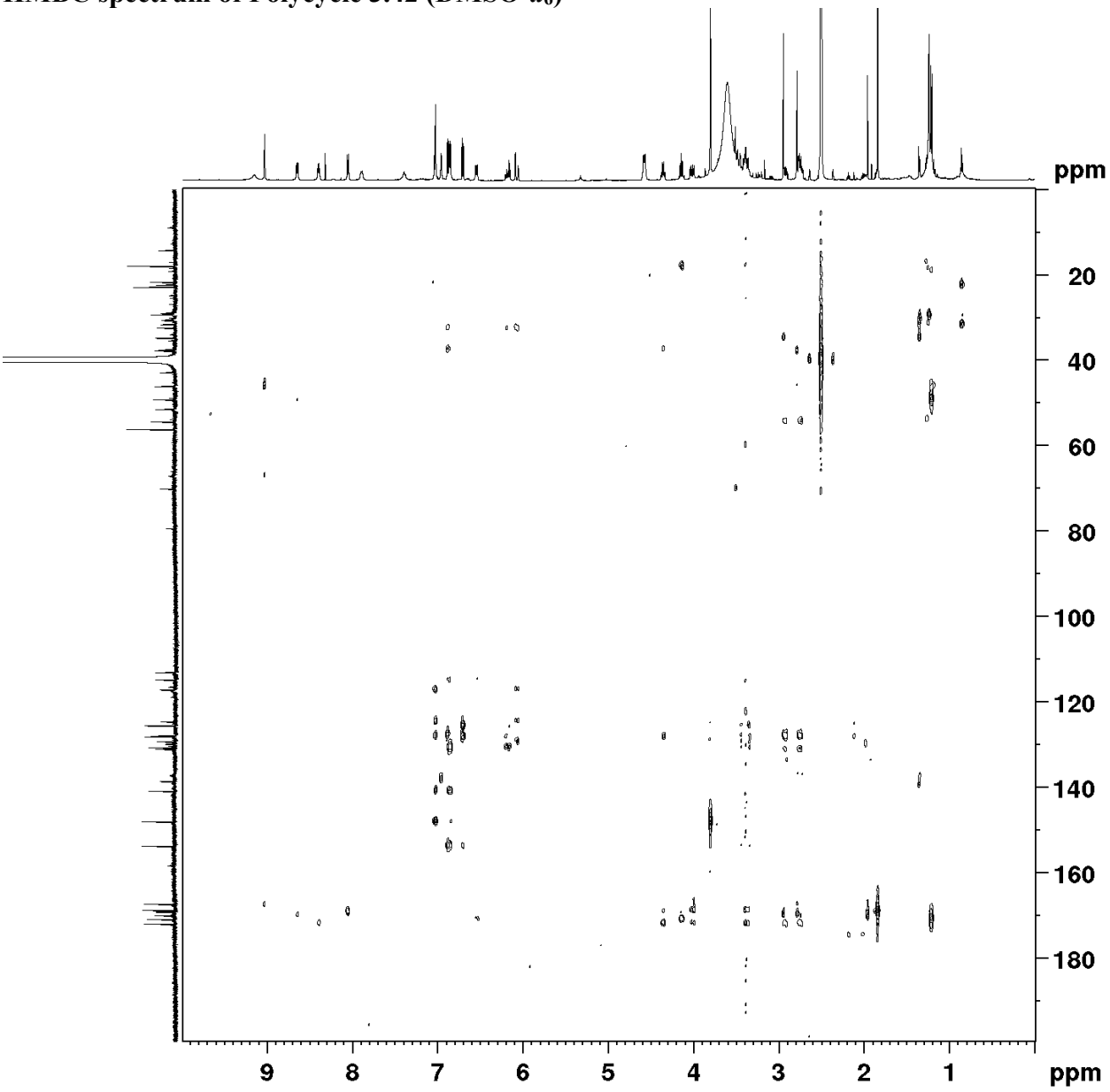
^{13}C NMR of Polycycle 3.42 (DMSO-d_6, 126 MHz)



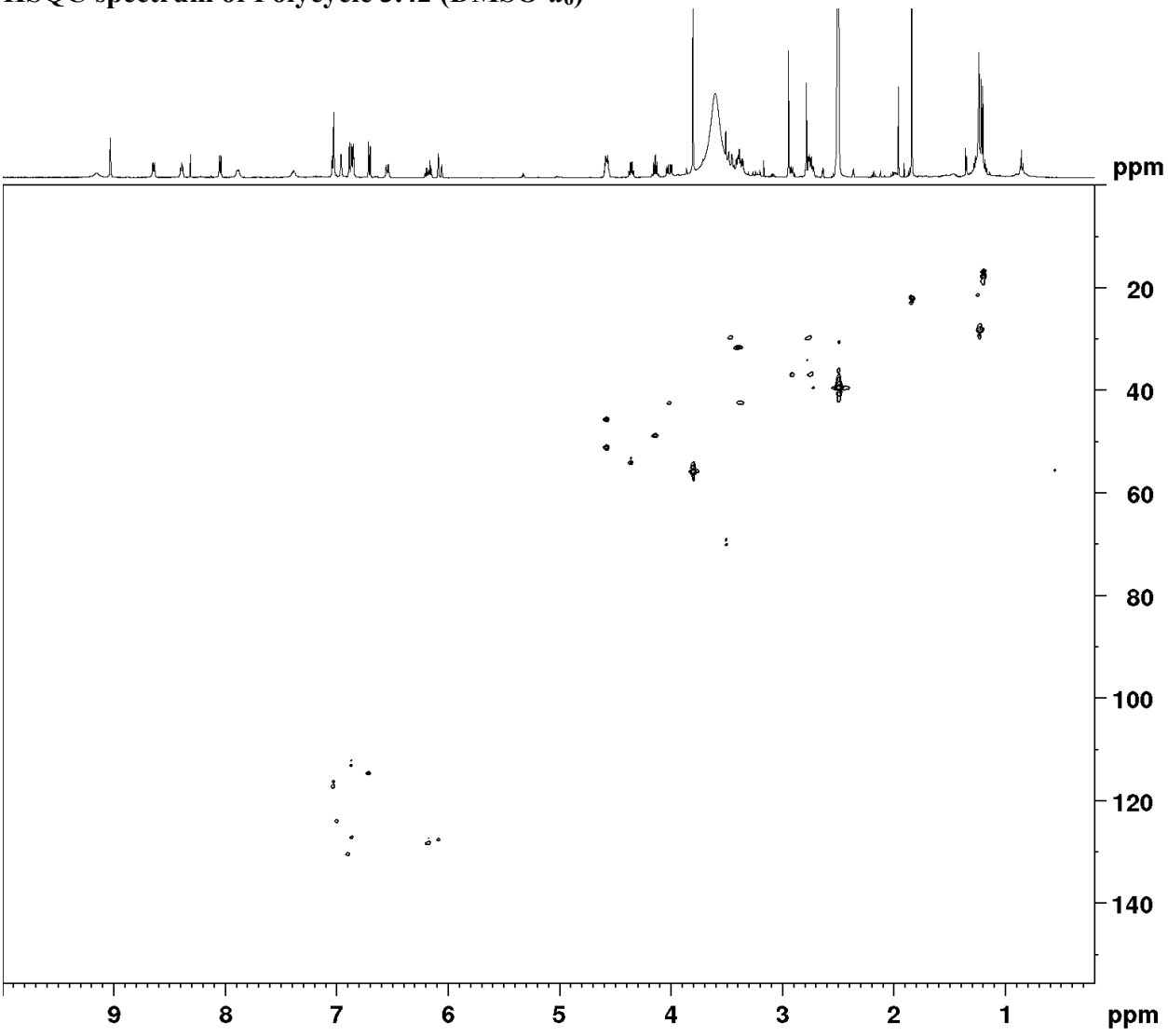
^{19}F NMR of Polycycle 3.42 (DMSO-d_6, 565 MHz)



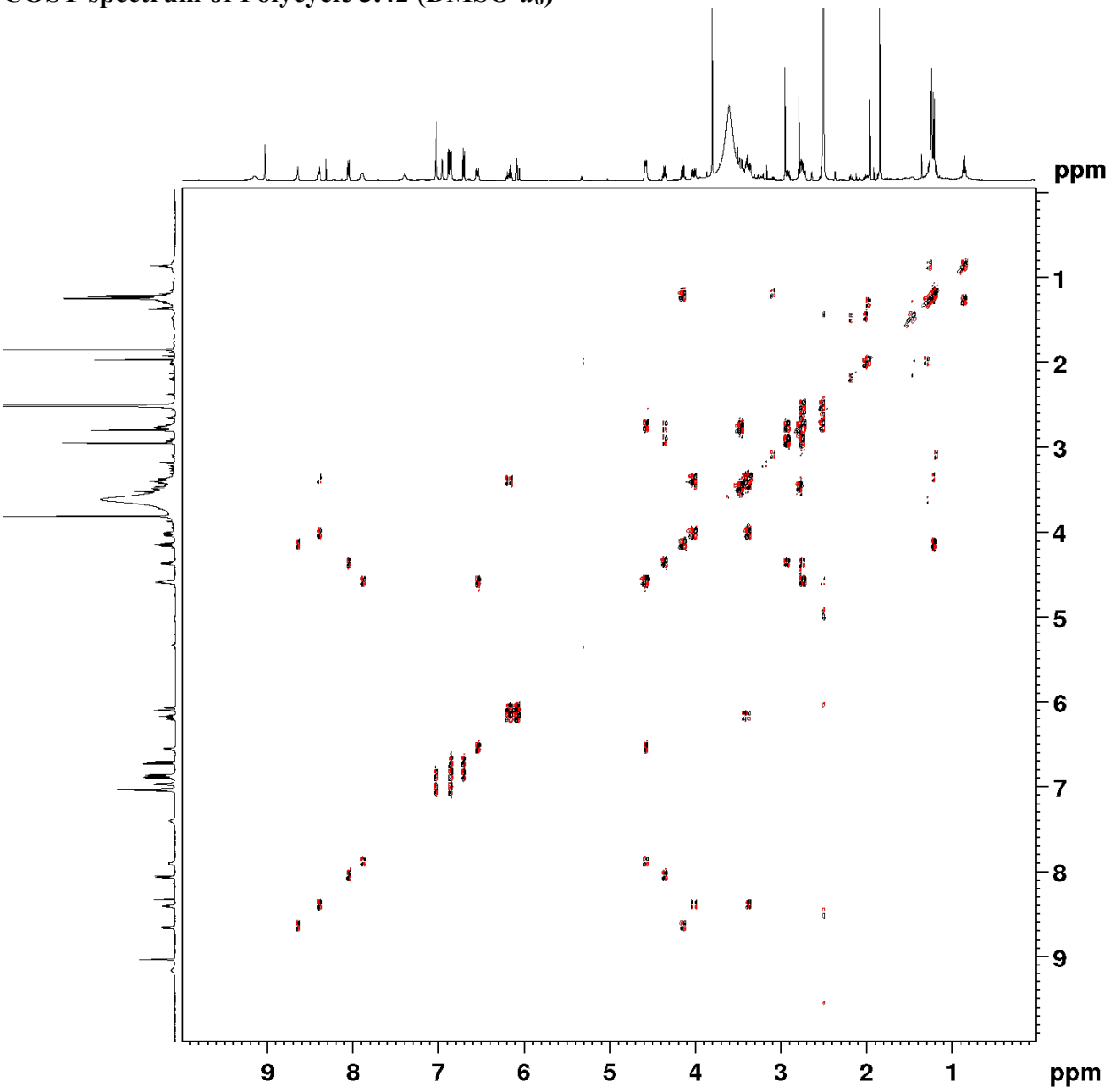
HMBC spectrum of Polycycle 3.42 (DMSO-d_6)



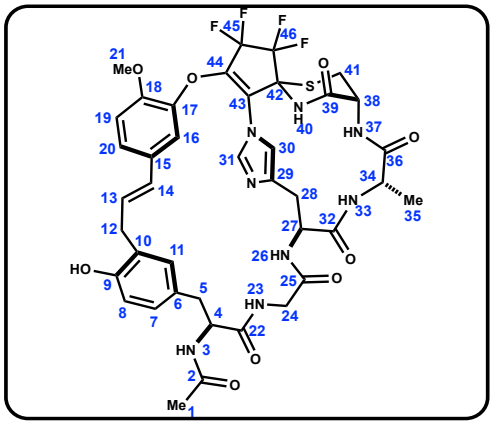
HSQC spectrum of Polycycle 3.42 (DMSO-d_6)



COSY spectrum of Polycycle 3.42 (DMSO-d_6)

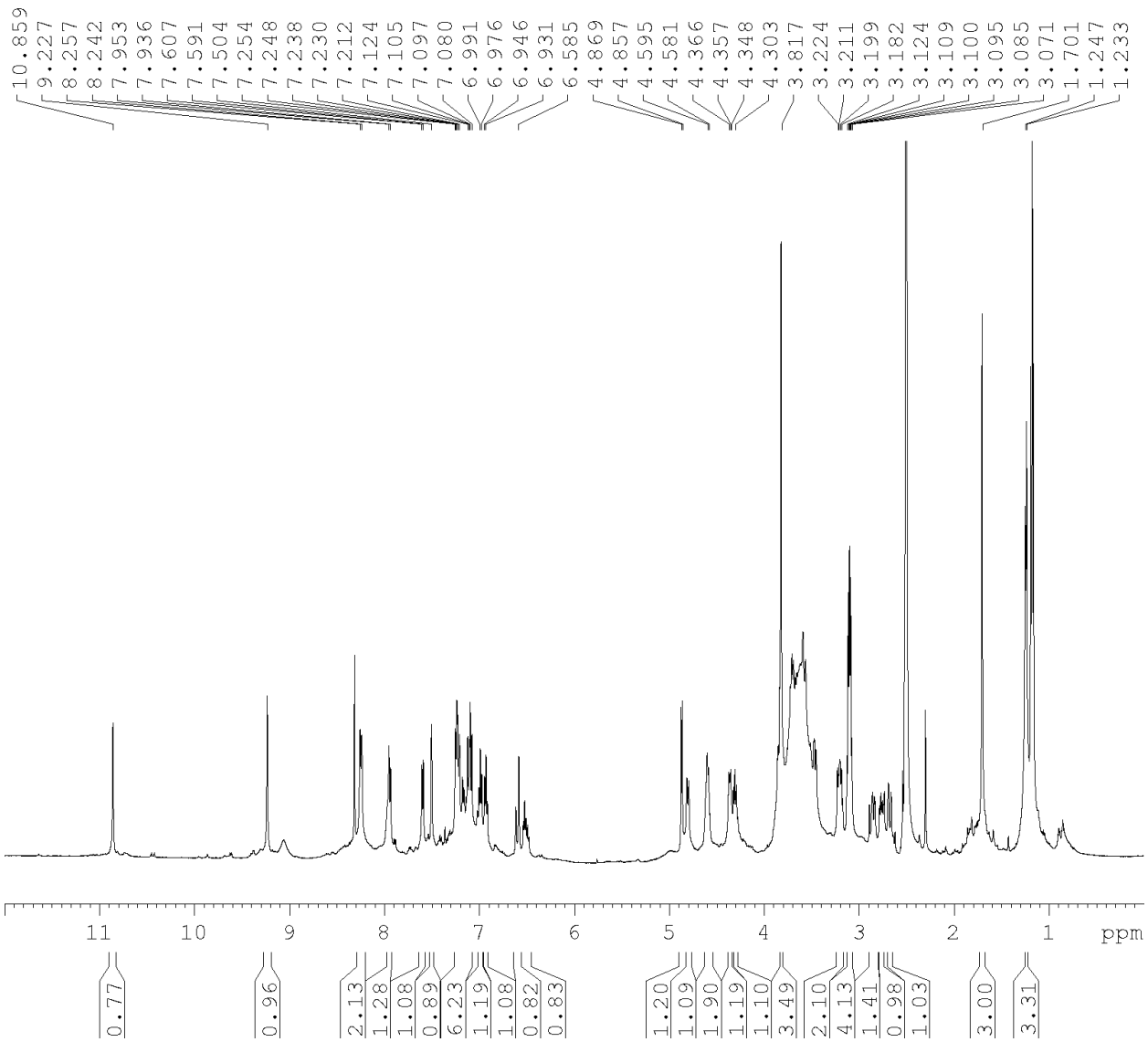


Polycycle **3.42** (DMSO-d_6, 298K)

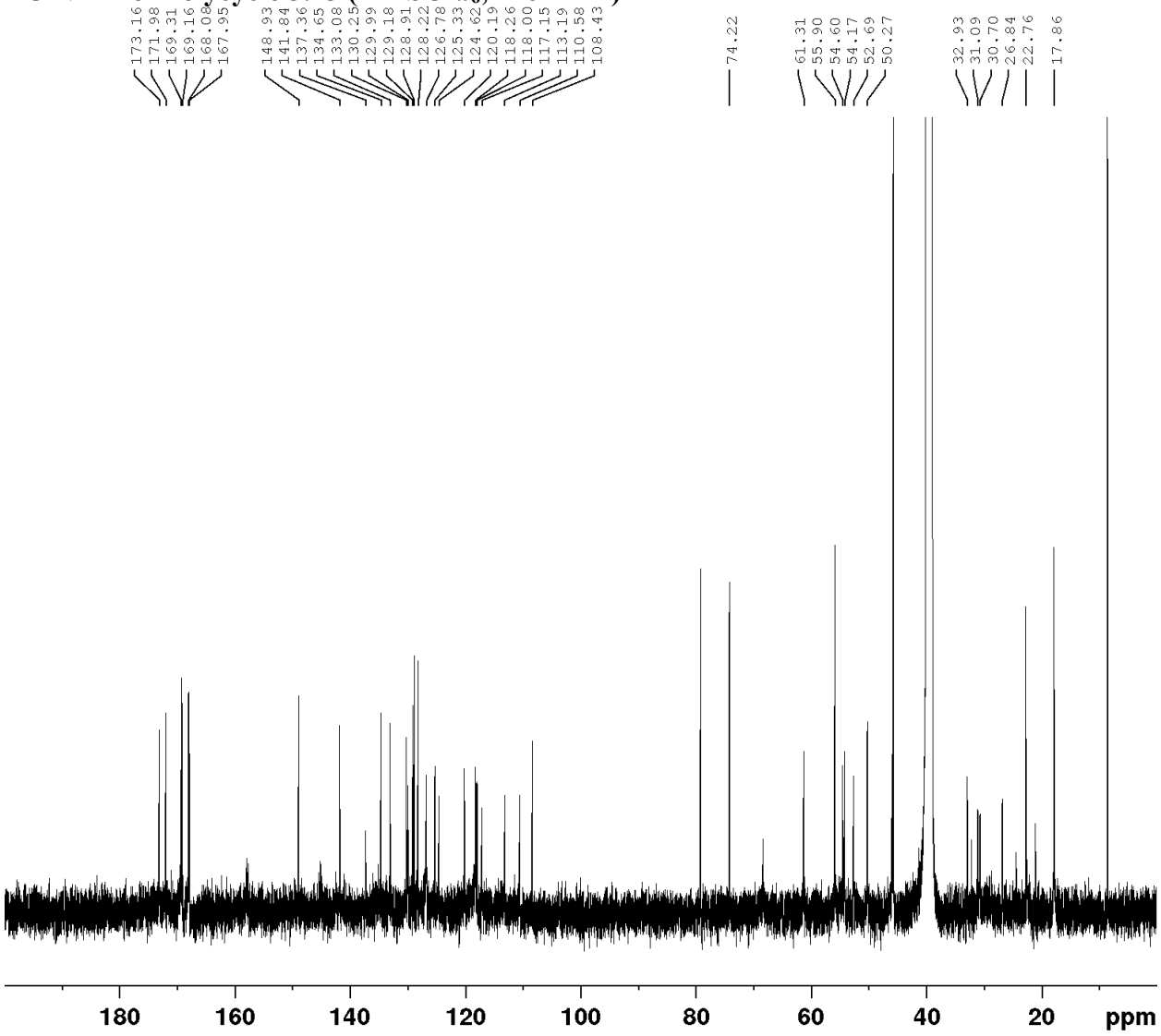


	13C	1H	Key Correlation
1	22.55	1.84 (s, 3H)	2->1 HMBC, HSQC
2	169.03	-	1->2 HMBC
3	-	8.05 (d, 2H)	2->3 HMBC
4	54.15	4.35 (q, 1H)	3->4 COSY, HSQC
5	37.25	2.94–2.89 (m, 1H), 2.77–2.71 (m, 1H)	4->5 COSY, HSQC
6	130.02	-	5->4 HMBC
7	127.58	6.87–6.85 (m, 1H)	8->7 COSY, HSQC
8	114.63	6.70 (d, 1 H) J = 8.2 Hz	7->8 COSY, HSQC, HMBC
9	153.54	-	8->7 COSY, HSQC, HMBC
10	129.02	-	12->10 HMBC
11	124.48	7.02 (m, 1H) overlap	6->11 HMBC, HSQC
12	32.15	3.42–3.35 (m, 2H)	13->12 COSY, HSQC
13	127.9	6.20–6.14 (dt, 1H) J = 15.9, 6.2 Hz	15->14 HMBC, HSQC
14	127.59	6.07 (d, 1H) J = 15.9 Hz	14->13 COSY, HSQC
15	116.98	-	14->15 HMBC, HSQC
16	130.49	6.89 (overlap, 1H)	15->16 HMBC, HSQC
17	147.89	-	16->17 HMBC
18	140.69	-	19->18 HMBC
19	112.94	6.84 (m, 1H)	20->19 COSY, HMBC, HSQC
20	125.41	7.04–7.02 (m, 1H) overlap	19->20 COSY, HMBC, HSQC
21	55.94	3.80 (s, 3H)	2->1 HMBC, HSQC
22	171.76	-	4->22 HMBC
23	-	8.39 (t, 1H)	22->23 HMBC
24	42.62	4.01 (dd, 1H) J = 15.9, 7.0 Hz 3.37–3.33 (m, 1H) overlap	23–>24 COSY, HSQC
25	168.67	-	24->25 HMBC
26	-	7.89 (d, 1H)	27->27 HMBC
27	45.82	4.57 (m, 1H) overlap	32->27 HMBC, HSQC
28	29.48	3.39 (m, 2H)	26->28 COSY, HSQC
29	138.44	-	30->29 HMBC, HSQC
30	116.98	6.52 (m, 1H)	29->30 HMBC, HSQC
31	130.83	6.96 (s, 1H)	HSQC
32	169.64	-	33->32 HMBC
33	-	8.65 (d, 1H)	34->33 COSY
34	48.91	4.14 (m, 1H)	35->34 COSY
35	17.65	1.20 (d, 3 H) J = 7.39 Hz	34->35 COSY, HSQC
36	170.73	-	34->36 HMBC
37	-	7.39 (m, 1H)	36->37 HMBC
38	51.27	4.57 (m, 1H) overlap	37->38 COSY, HMBC, HSQC
39	167.22	-	40->39 HMBC
40	-	9.03 (s, 1H)	39->40 HMBC
41	29.04	3.47 (m, 1H), 2.75 (m, 1H)	38->41 COSY, HMBC, HSQC
42	66.82 (m, 1C)	-	40->42 HMBC
43	117.18 (m, 1C)	-	-
44	137.02 (m, 1C)	-	-
45	116.29 (m, 1C)	-	-
46	116.29 (m, 1C)	-	-

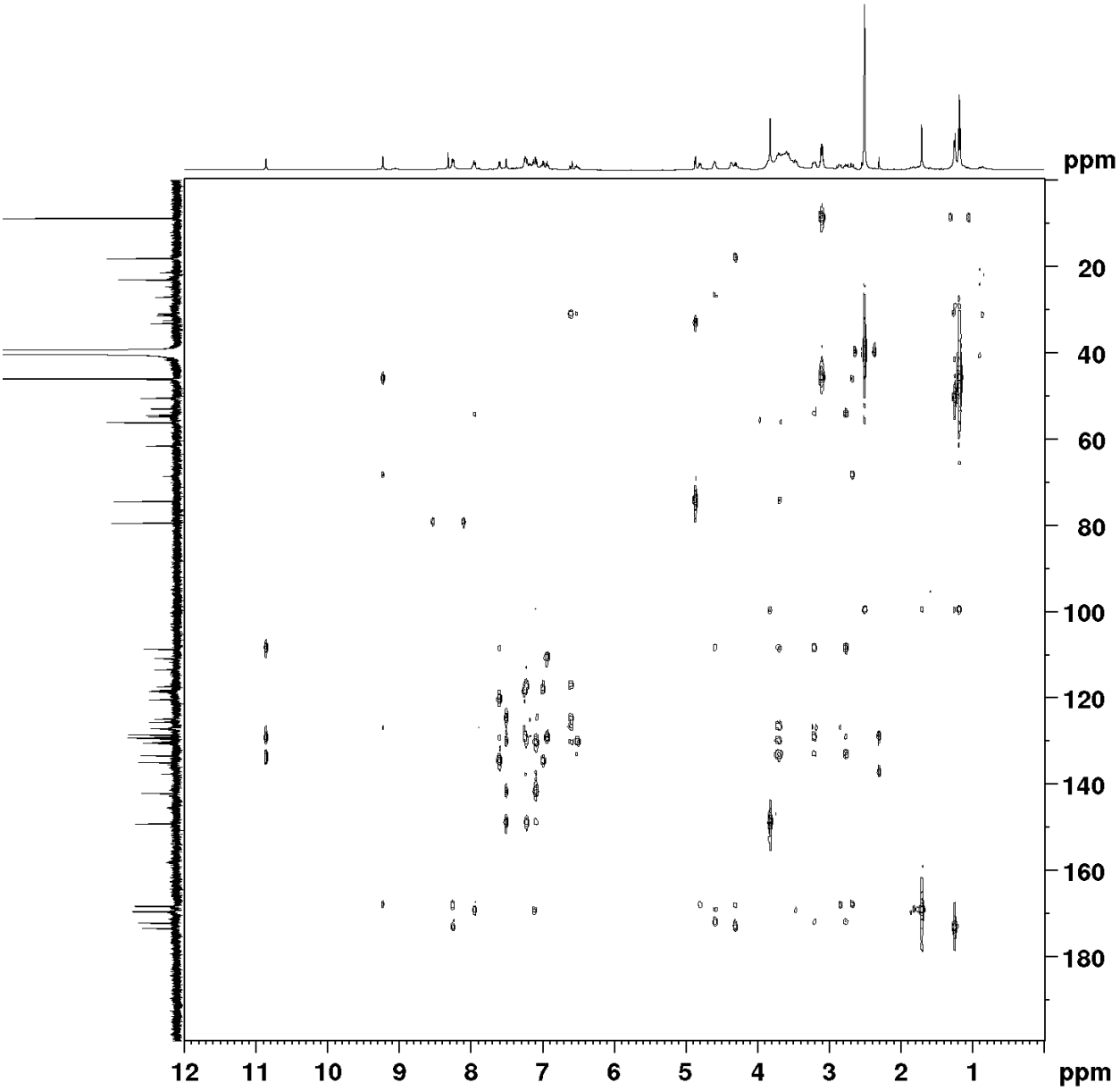
^{1}H NMR of Polycycle 3.43 (DMSO-d_6, 500 MHz)



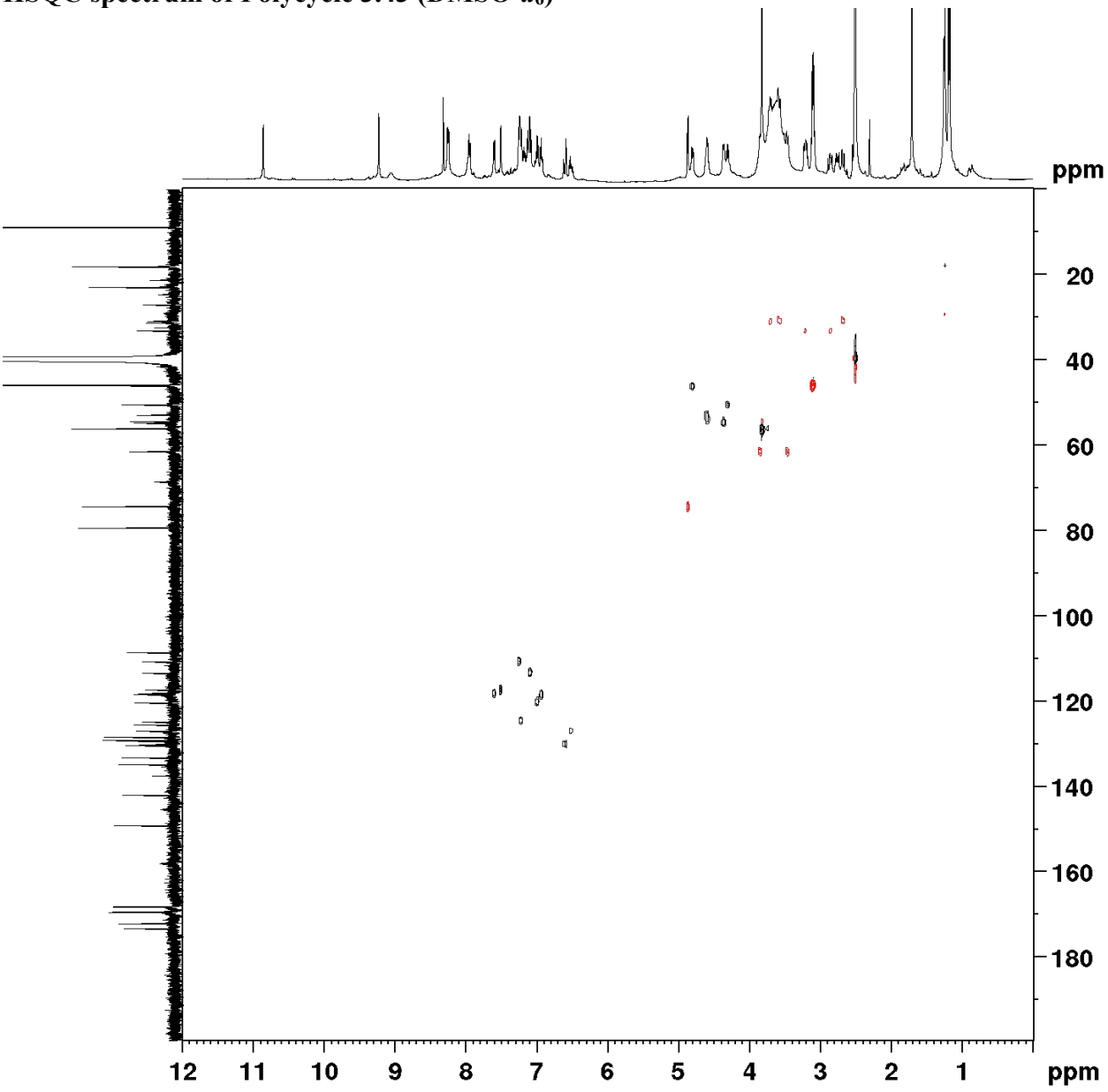
^{13}C NMR of Polycycle 3.43 (DMSO-d_6, 126 MHz)



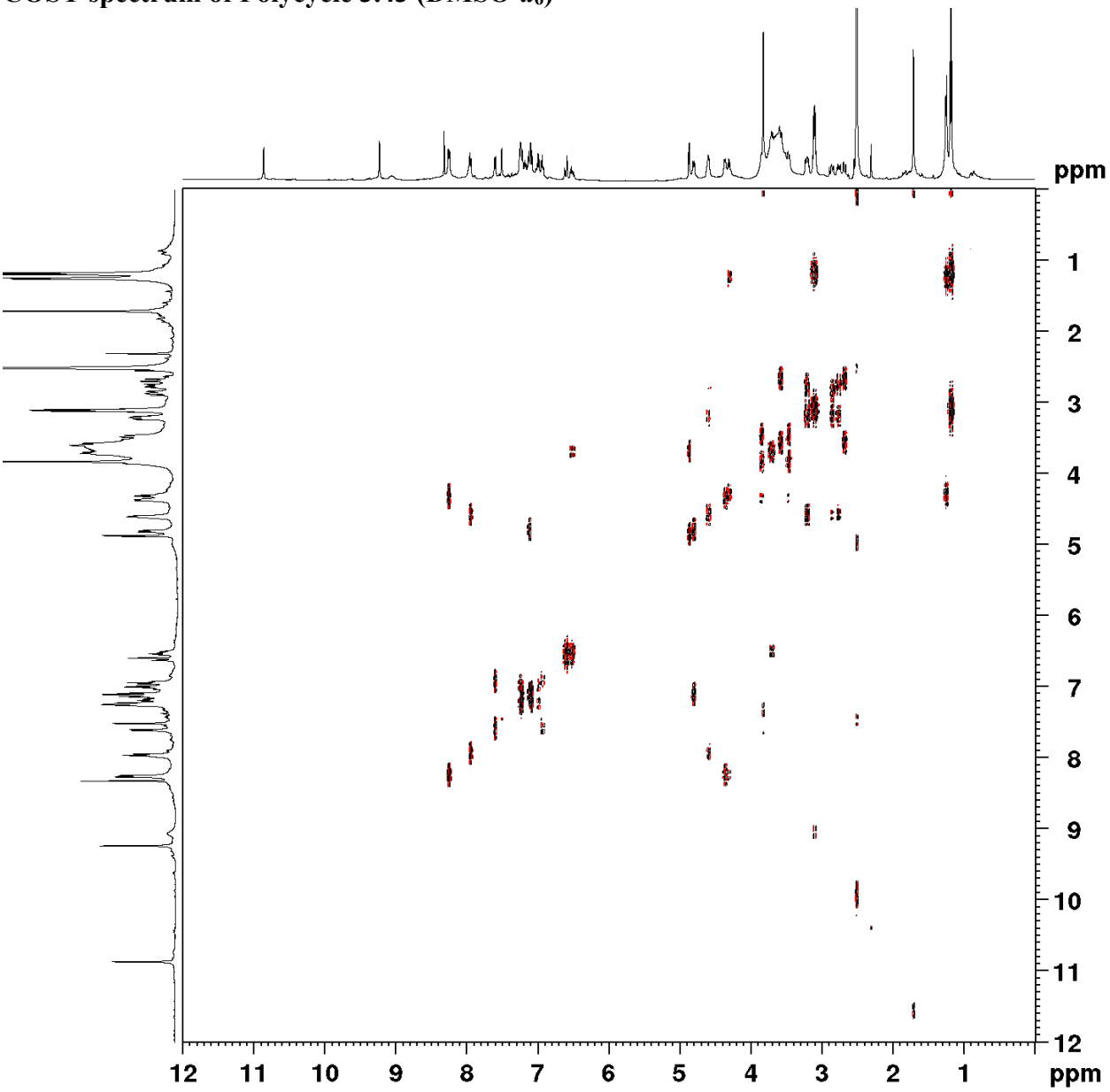
HMBC spectrum of Polycycle 3.43 (DMSO-d_6)



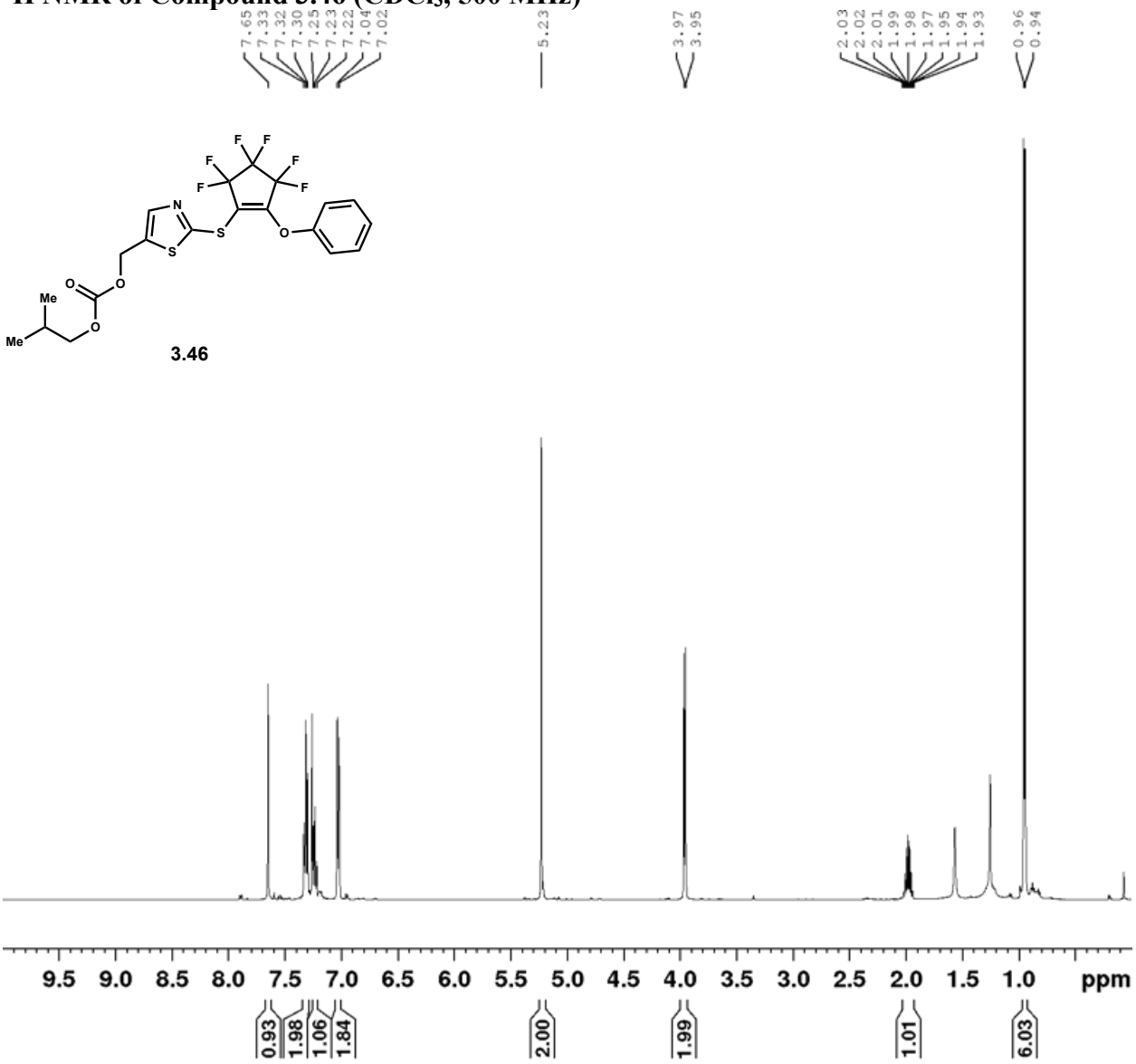
HSQC spectrum of Polycycle 3.43 (DMSO-d_6)



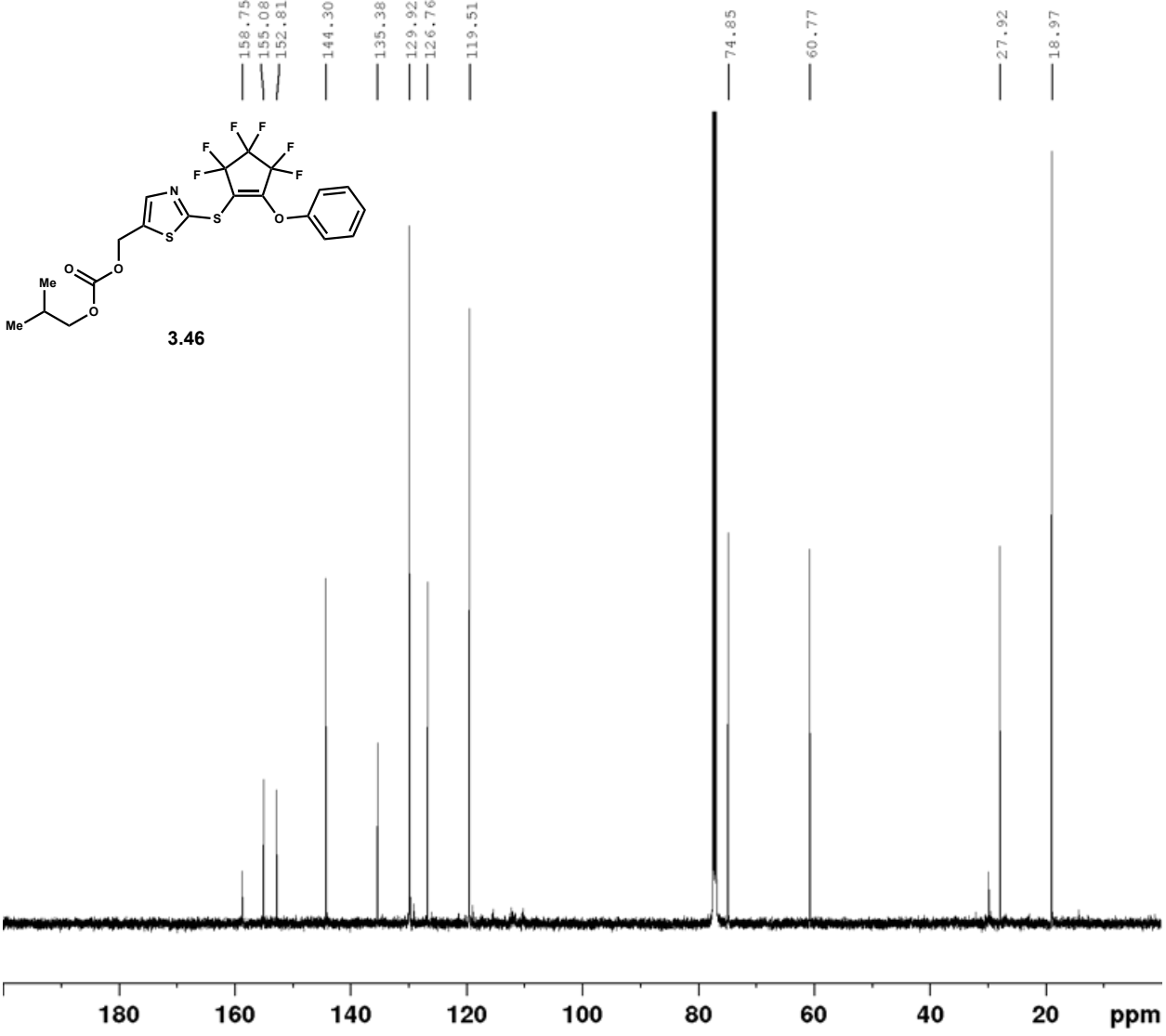
COSY spectrum of Polycycle 3.43 (DMSO-d_6)



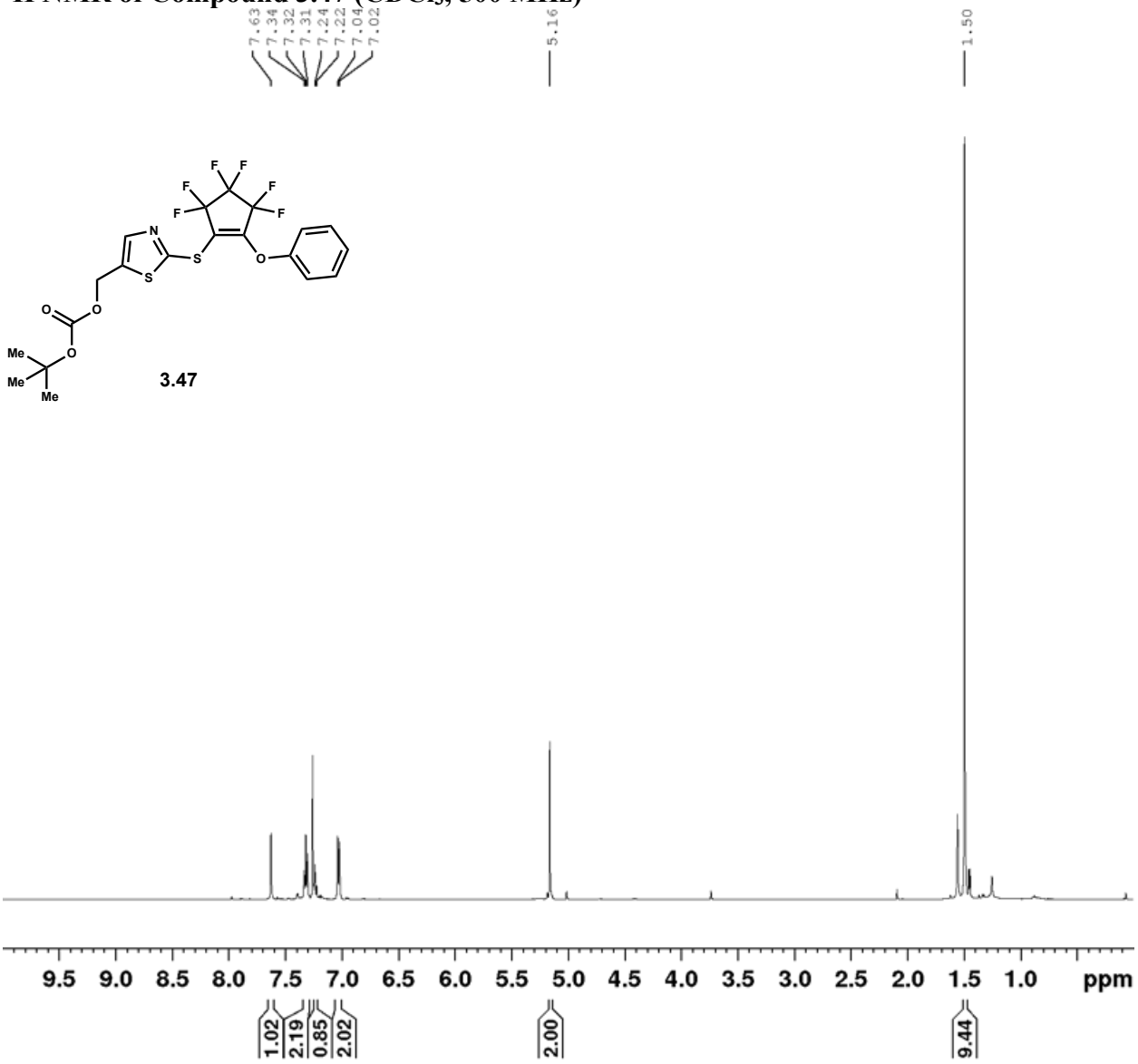
^{1}H NMR of Compound 3.46 ($CDCl_3$, 500 MHz)



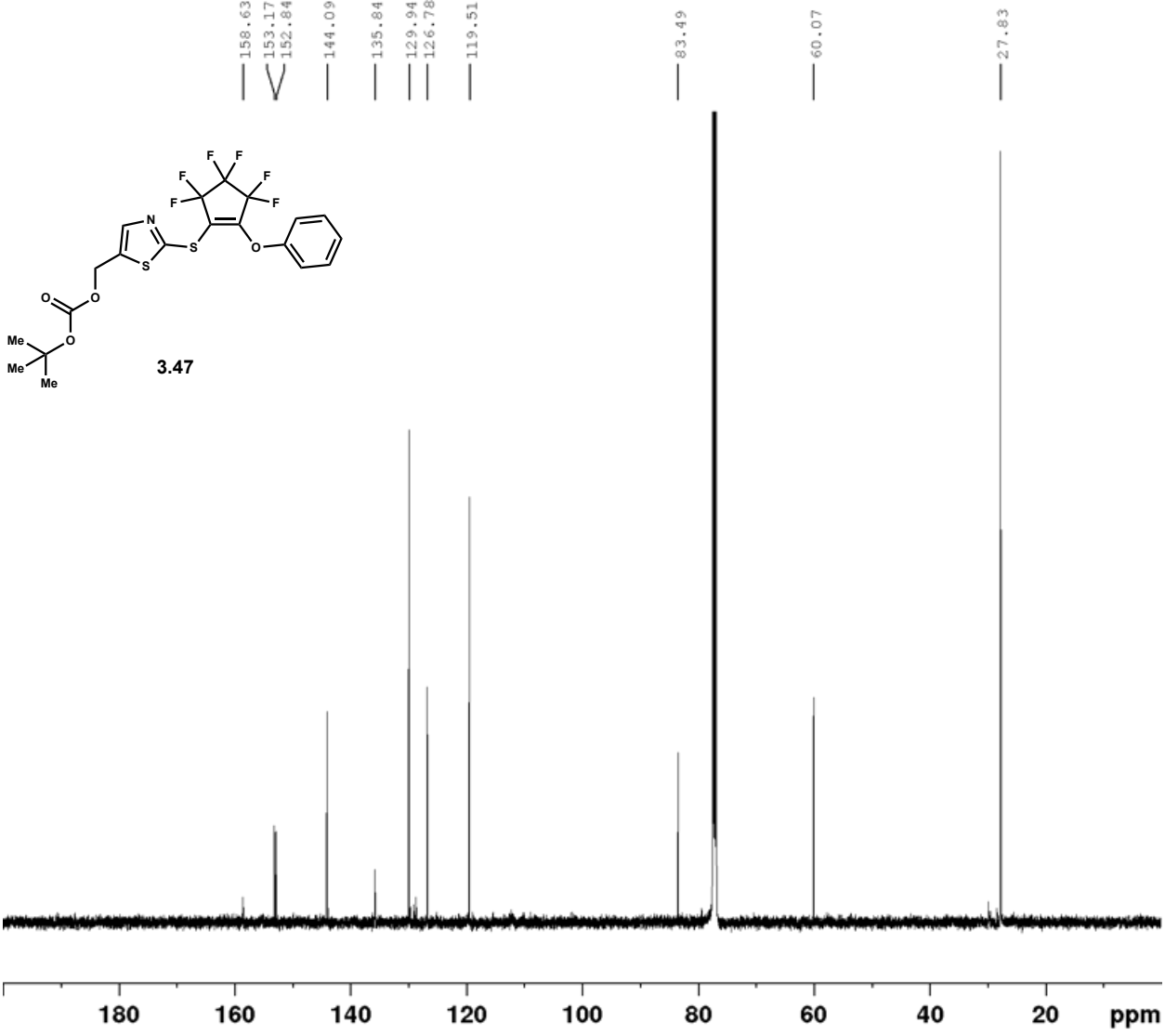
^{13}C NMR of Compound 3.46 ($CDCl_3$, 126 MHz)



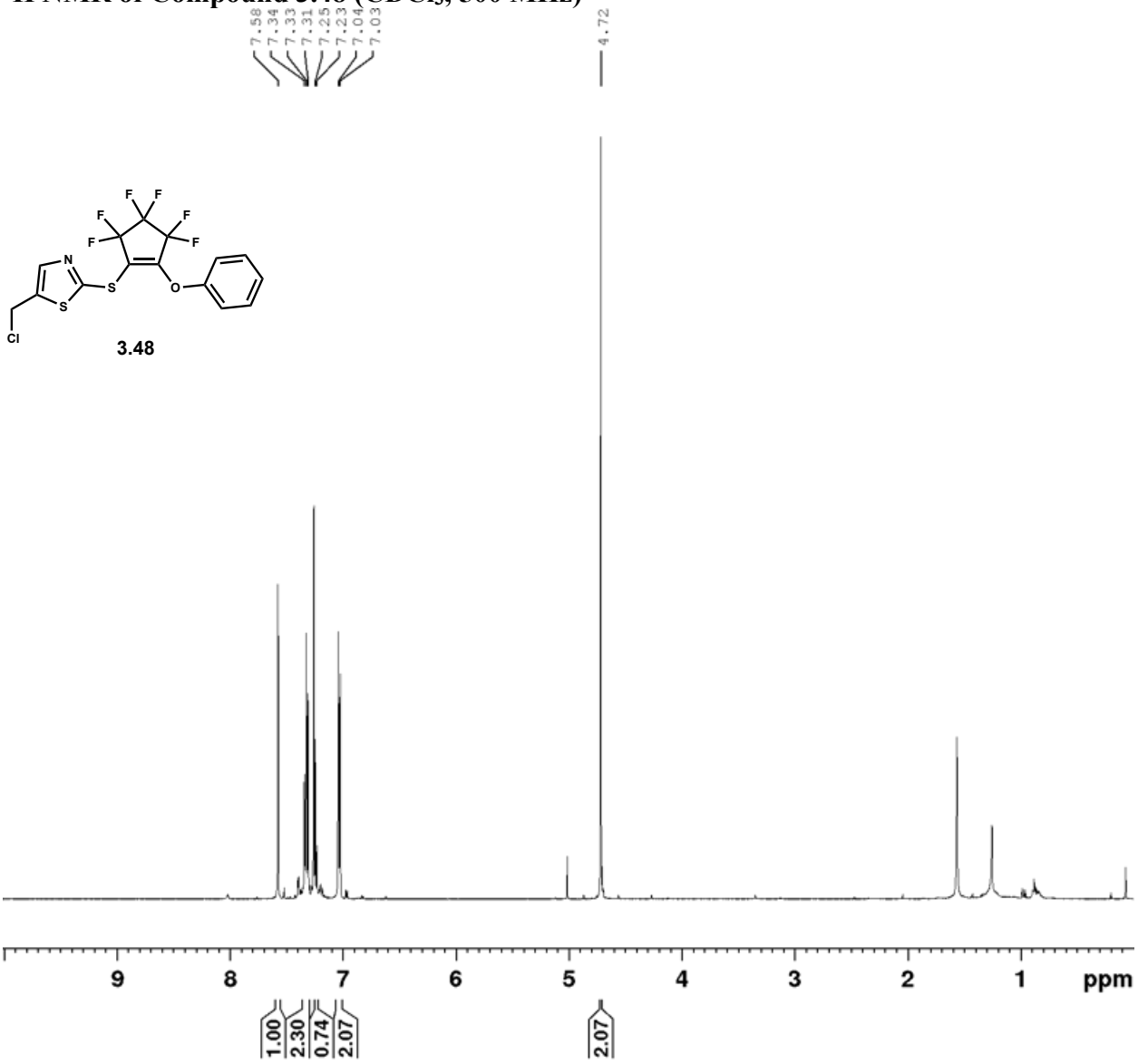
^{1}H NMR of Compound 3.47 ($CDCl_3$, 500 MHz)



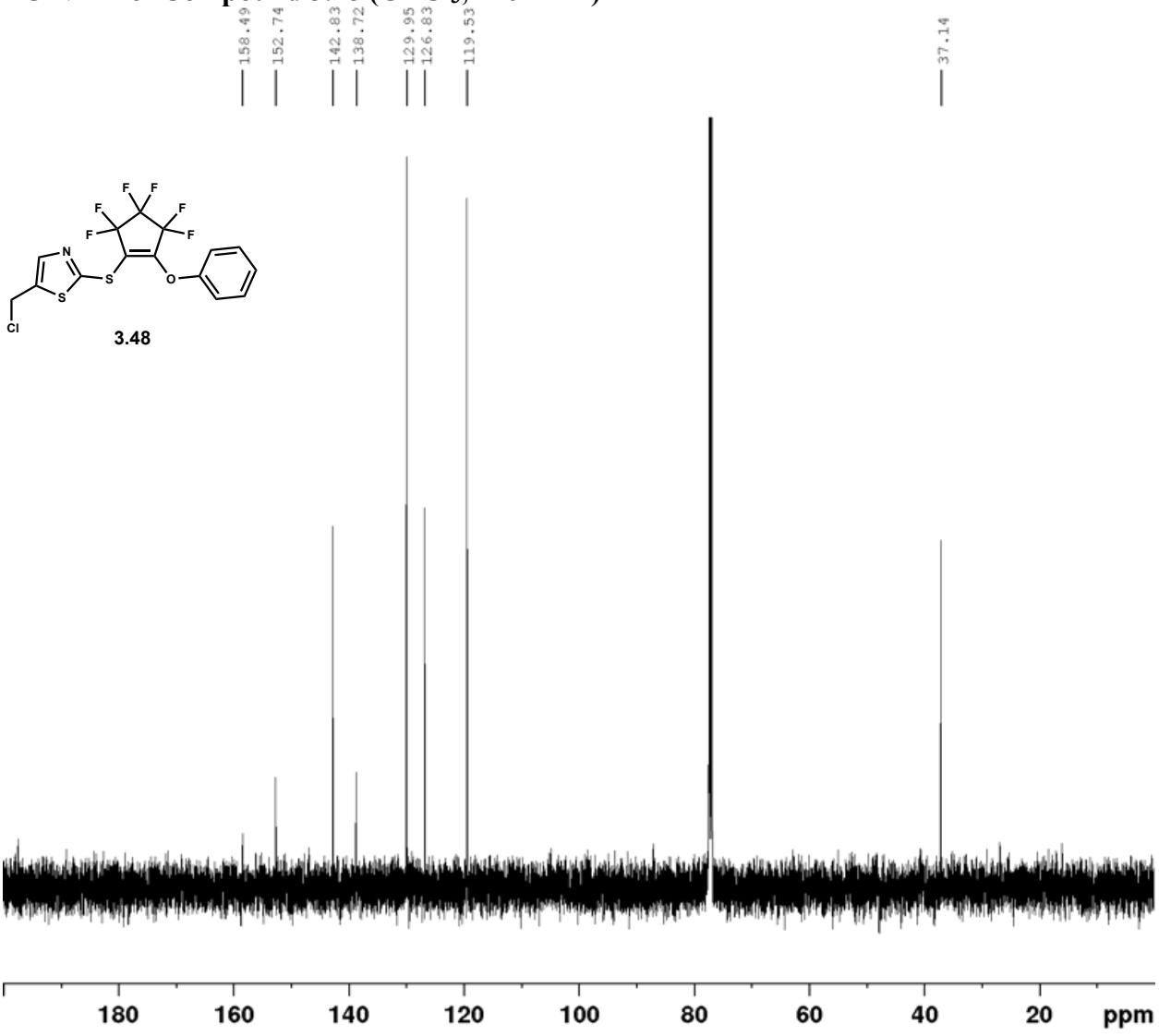
^{13}C NMR of Compound 3.47 ($CDCl_3$, 126 MHz)



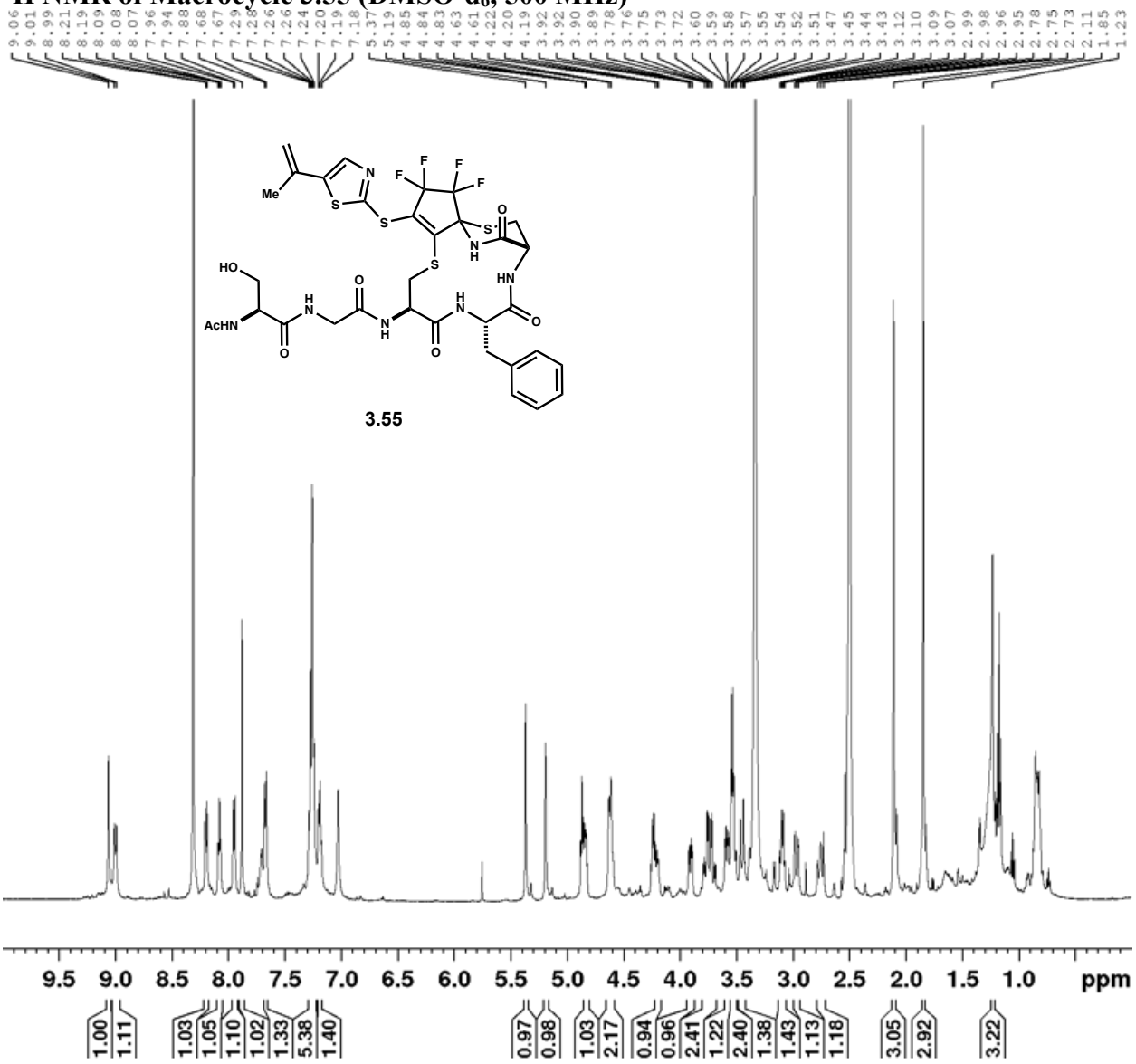
^{1}H NMR of Compound 3.48 ($CDCl_3$, 500 MHz)



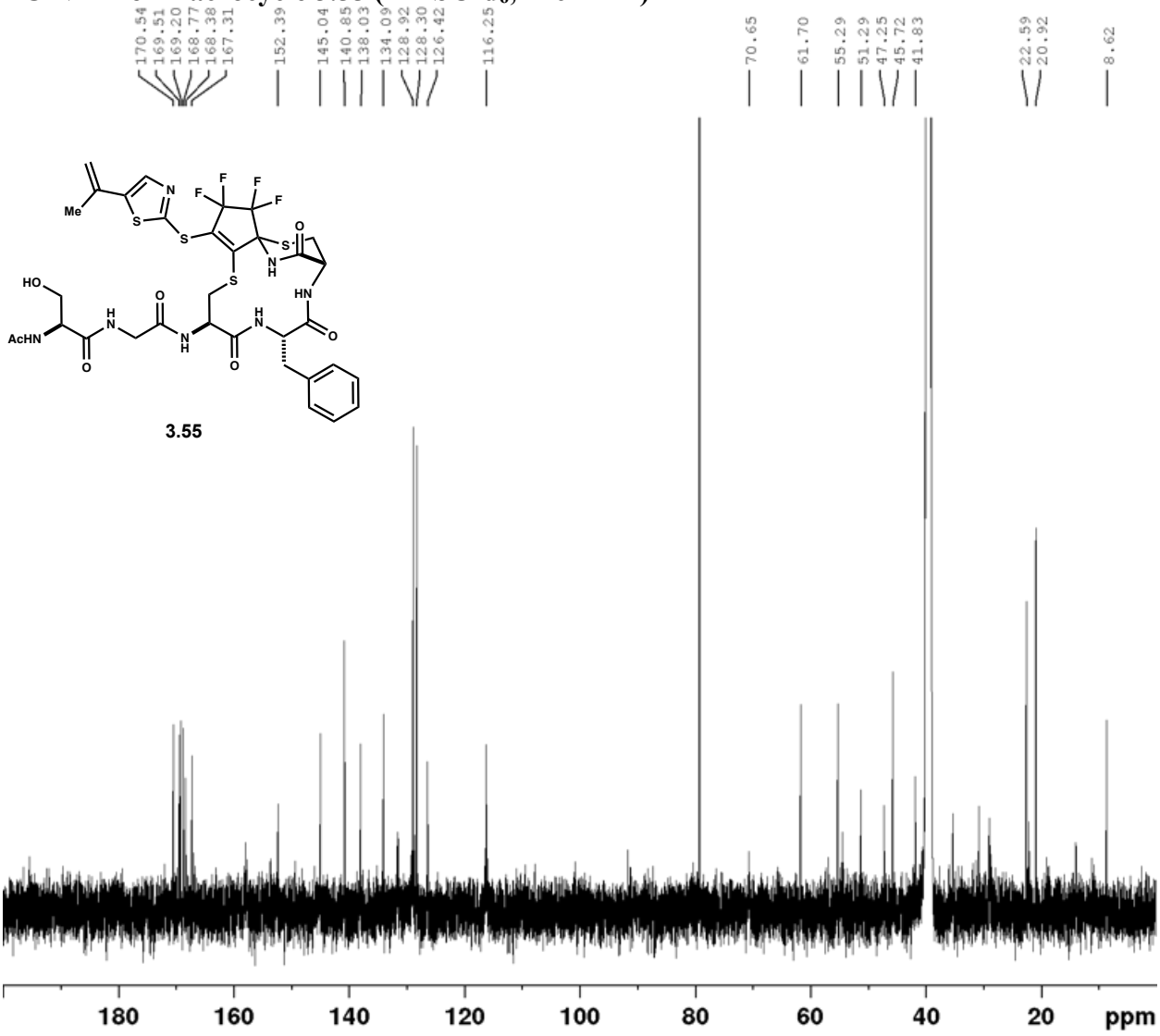
^{13}C NMR of Compound 3.48 ($CDCl_3$, 126 MHz)



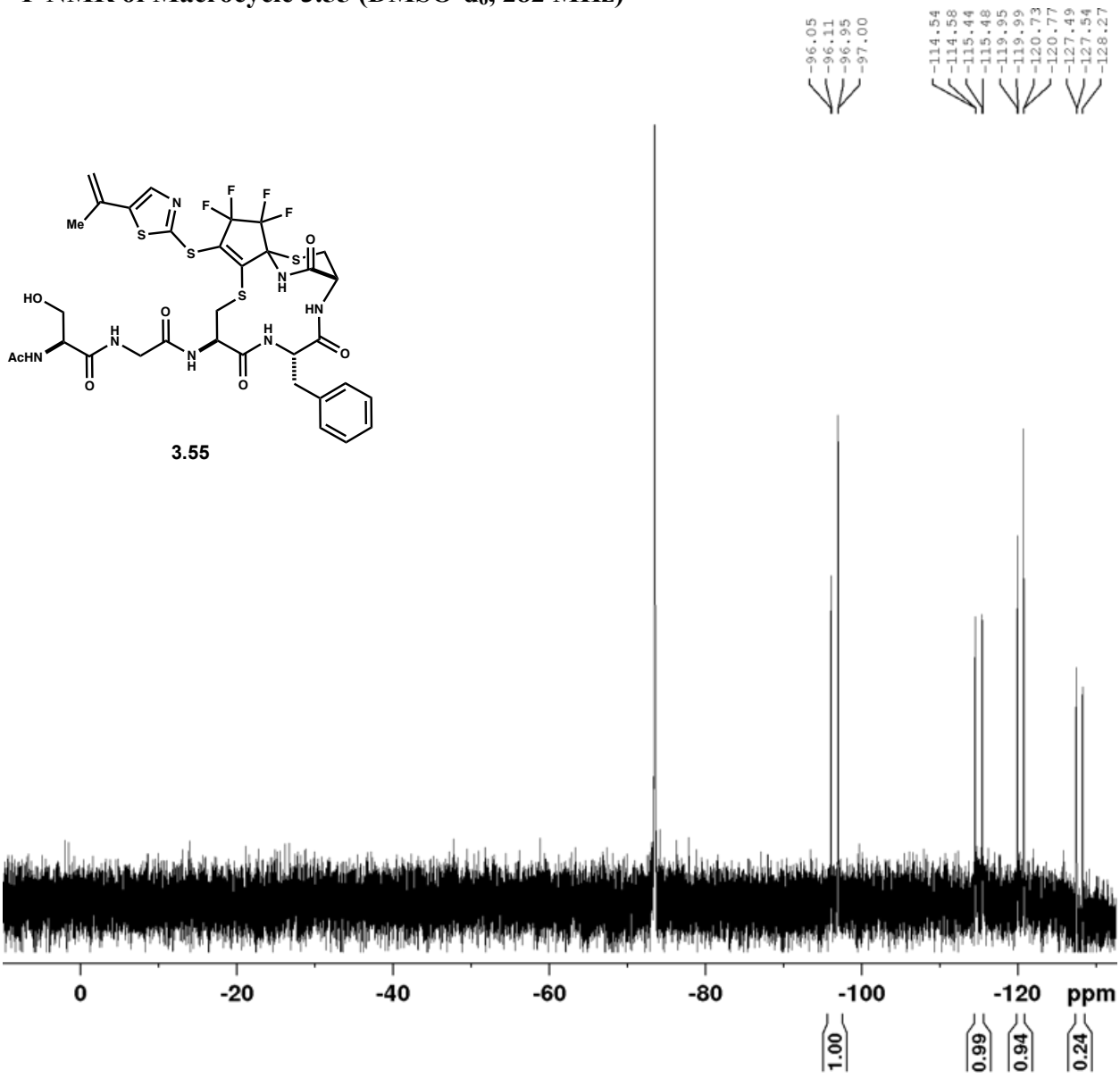
^{1}H NMR of Macrocycle 3.55 (DMSO-d_6, 500 MHz)



^{13}C NMR of Macrocycle 3.55 (DMSO-d_6, 126 MHz)

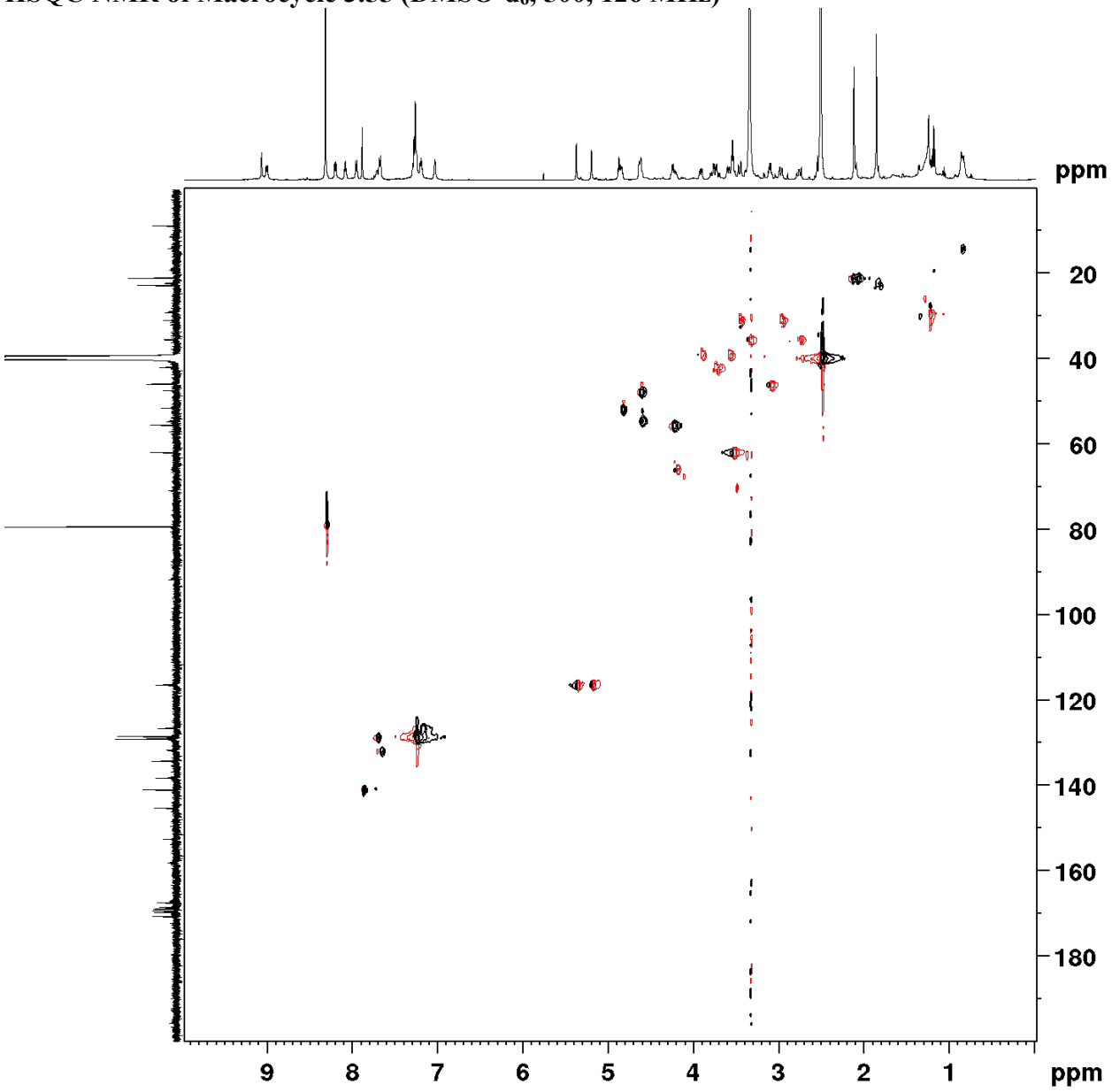


^{19}F NMR of Macrocycle 3.55 (DMSO-d_6, 282 MHz)

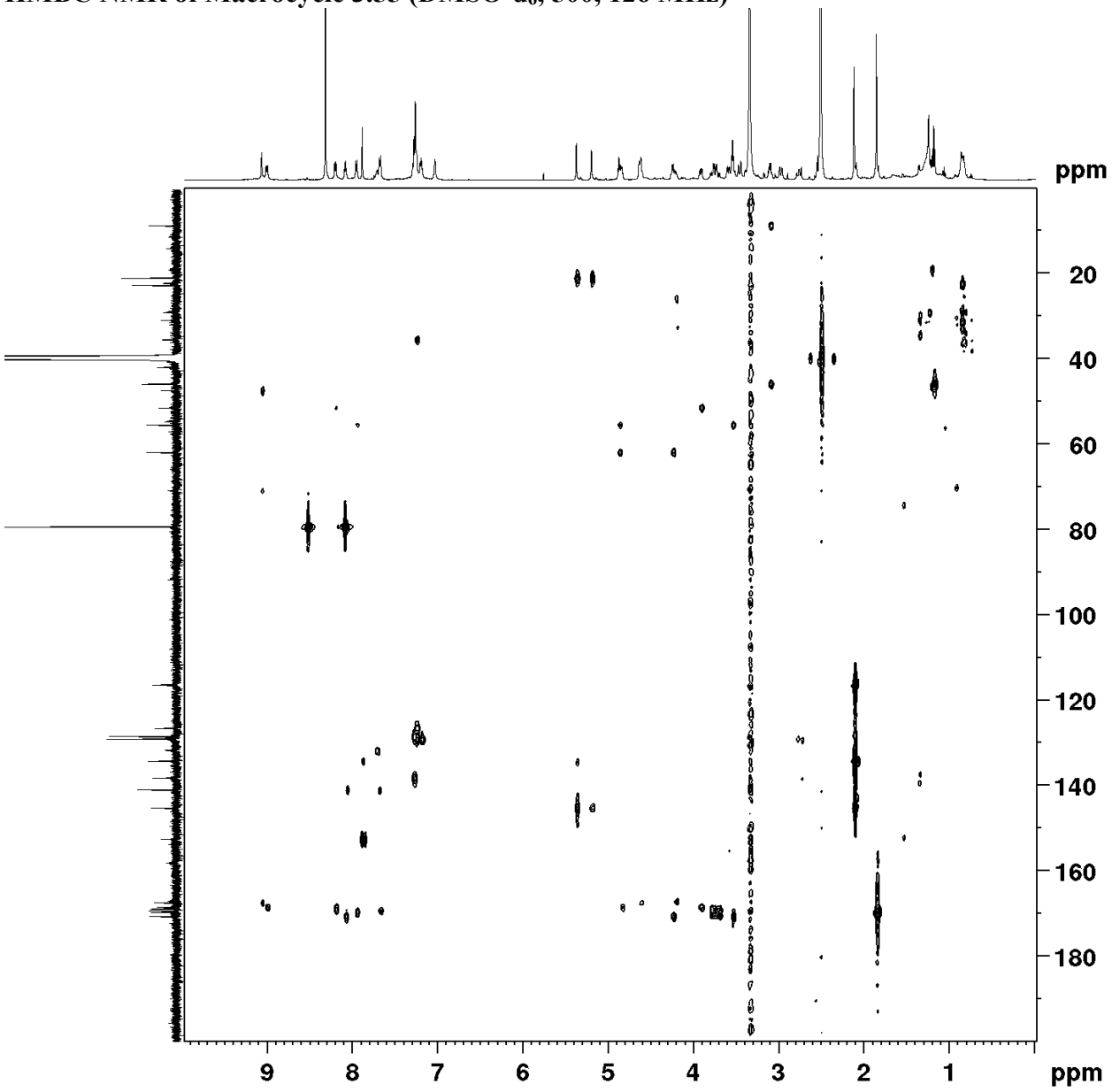


3.55

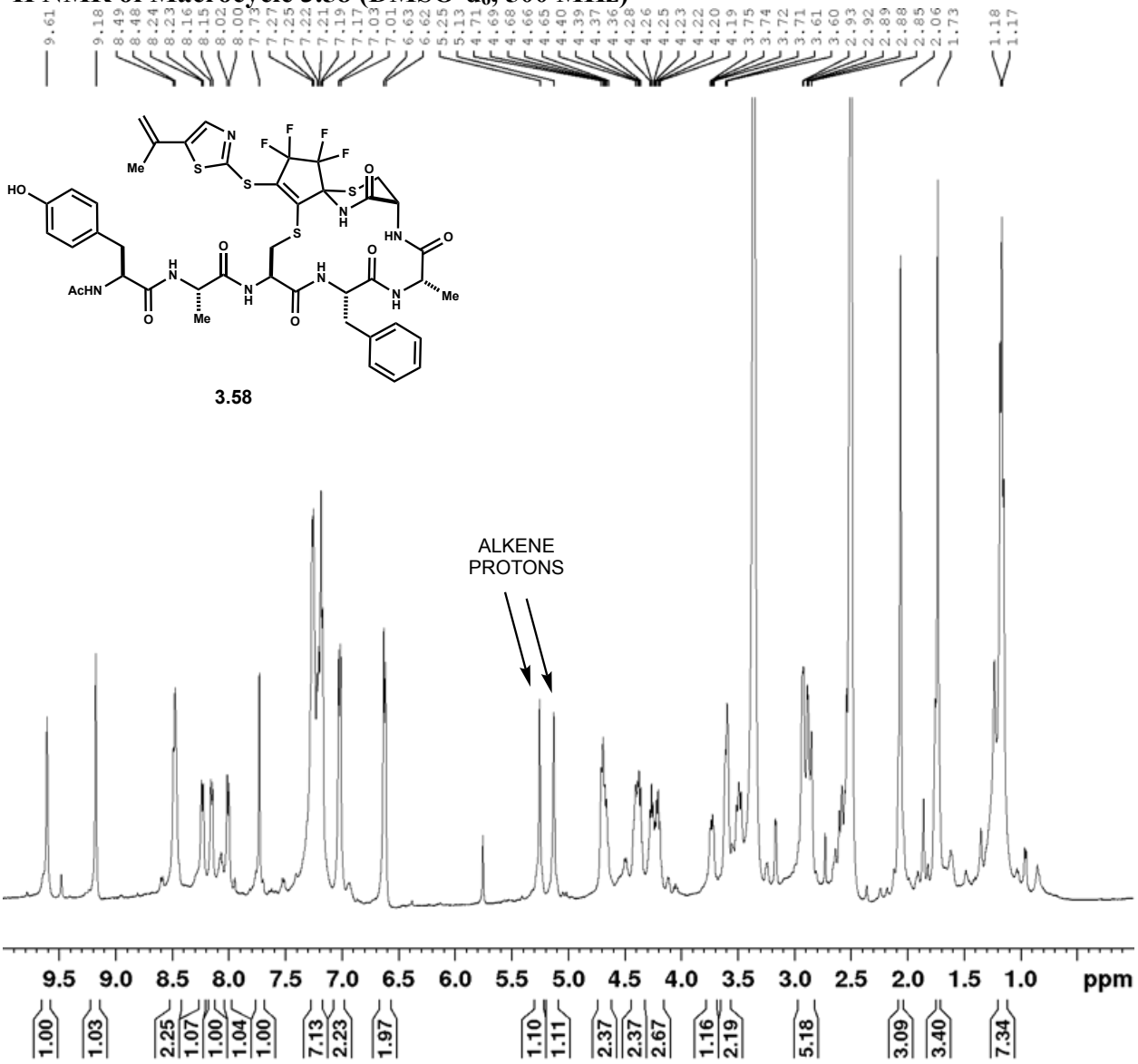
HSQC NMR of Macrocycle 3.55 (DMSO-d_6, 500, 126 MHz)



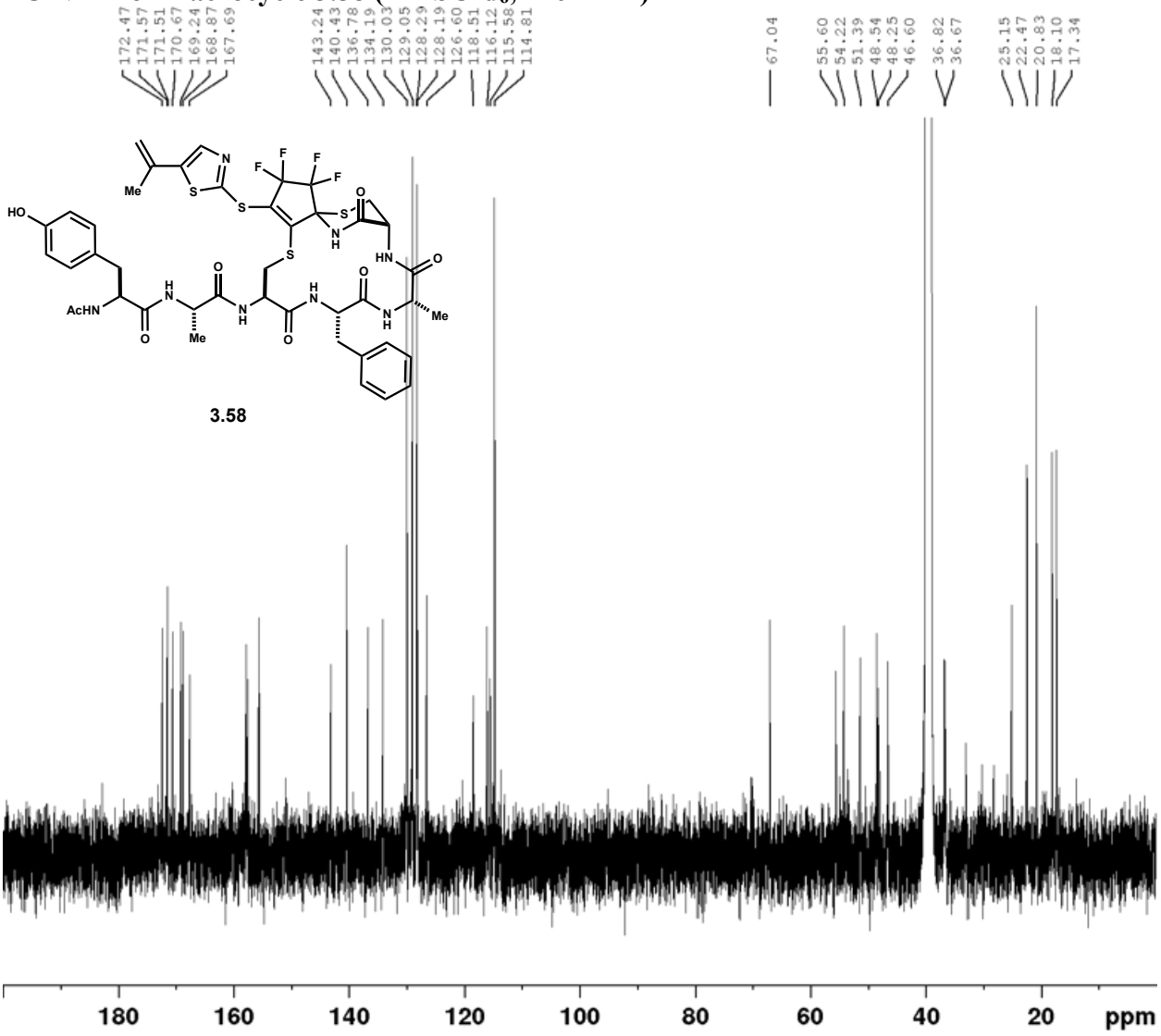
HMBC NMR of Macrocycle 3.55 (DMSO-d_6, 500, 126 MHz)



^{1}H NMR of Macrocycle 3.58 (DMSO-d_6, 500 MHz)

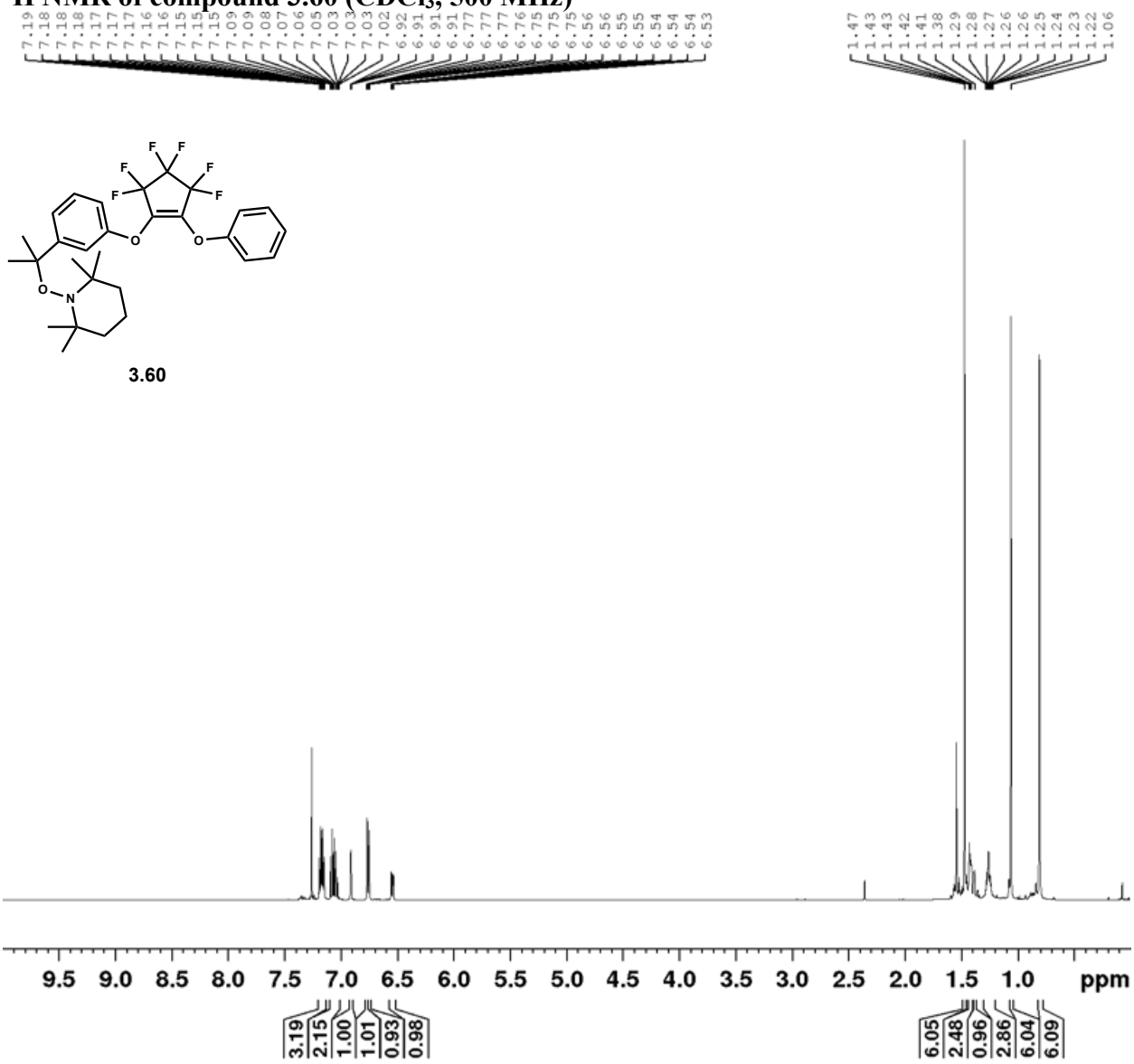


^{13}C NMR of Macrocycle 3.58 (DMSO-d_6, 126 MHz)

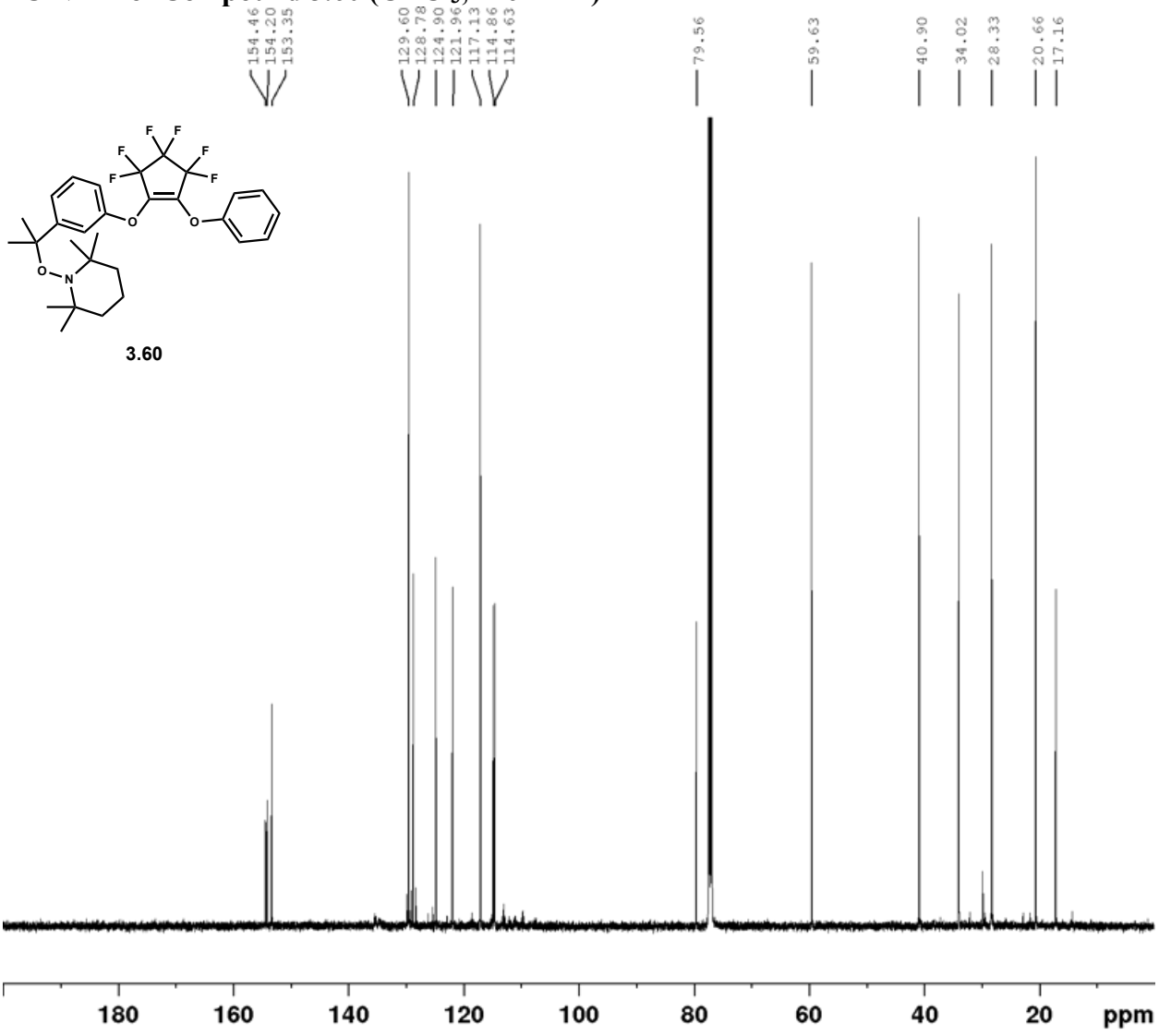


3.58

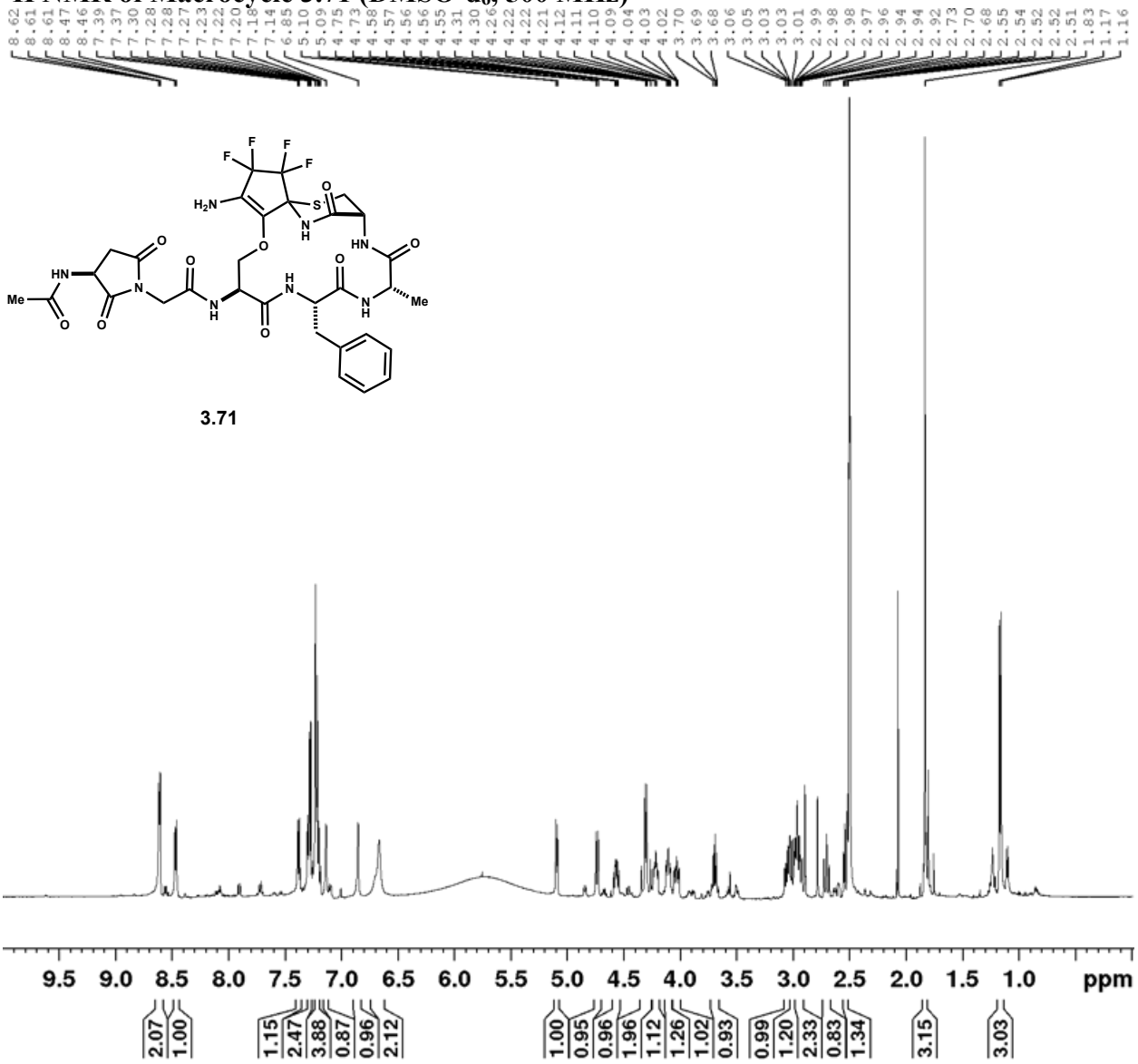
^{1}H NMR of compound 3.60 ($CDCl_3$, 500 MHz)



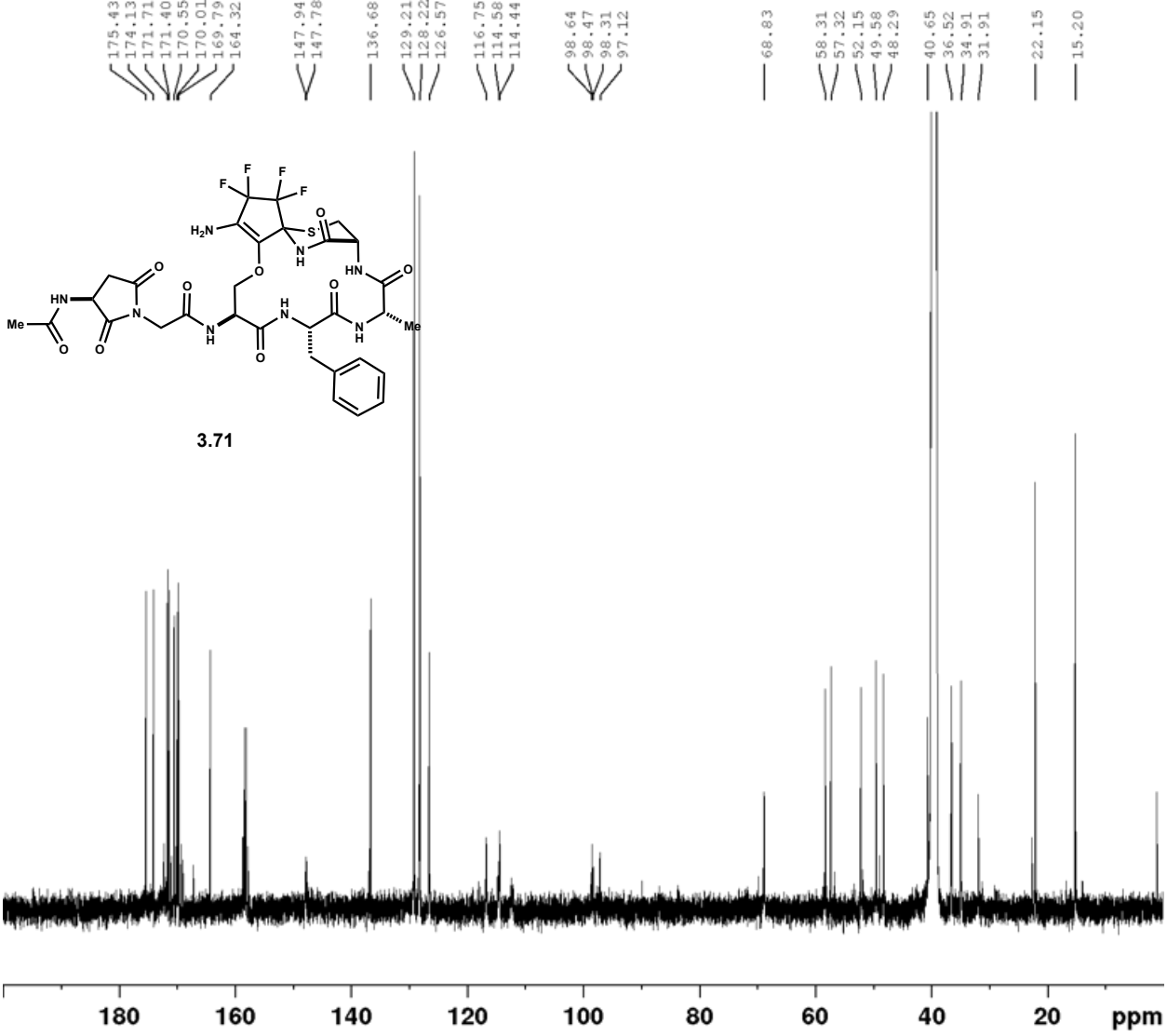
^{13}C NMR of Compound 3.60 ($CDCl_3$, 126 MHz)



^{1}H NMR of Macrocycle 3.71 (DMSO-d_6, 500 MHz)

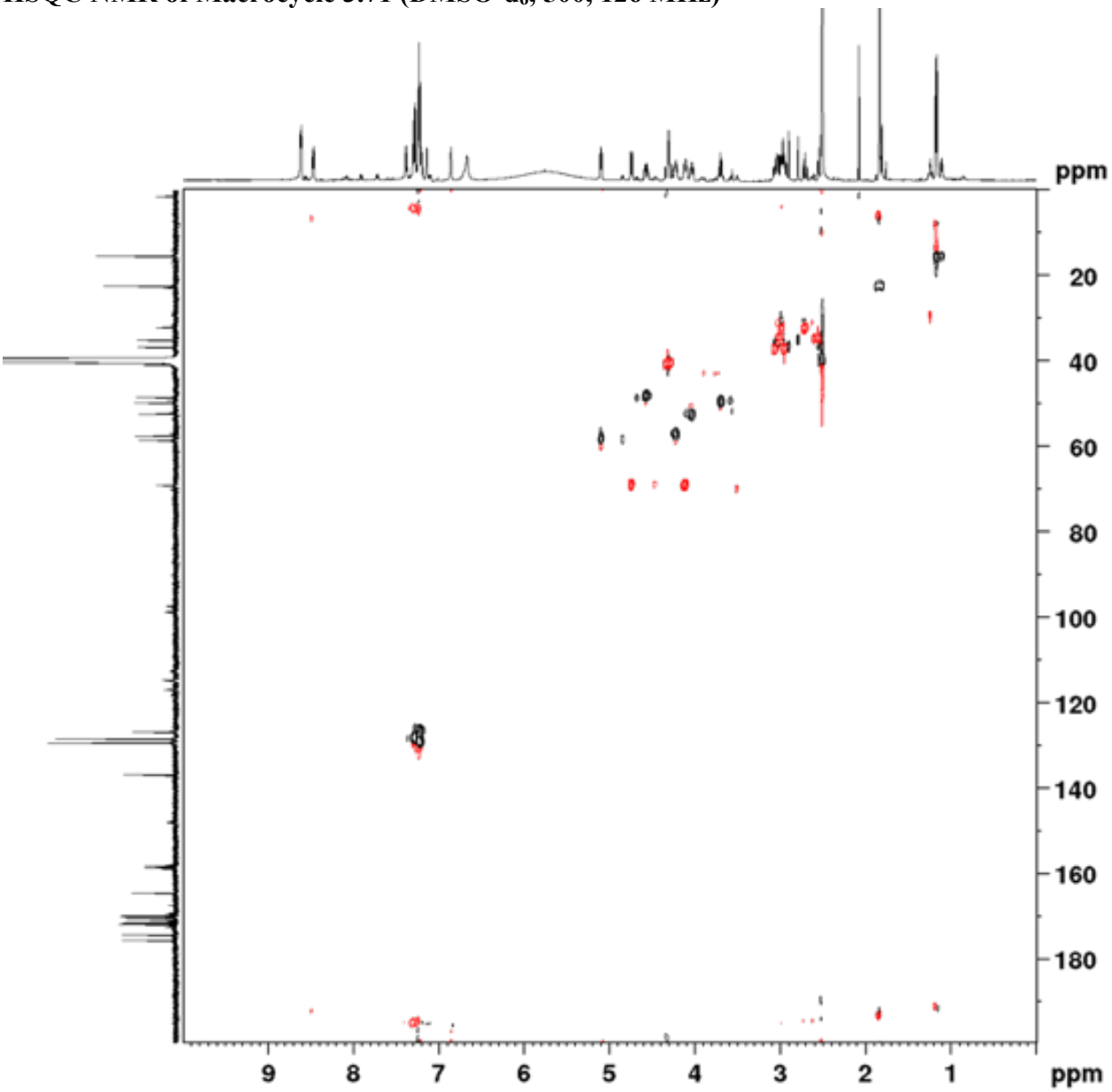


^{13}C NMR of Macrocycle 3.71 (DMSO-d_6, 126 MHz)

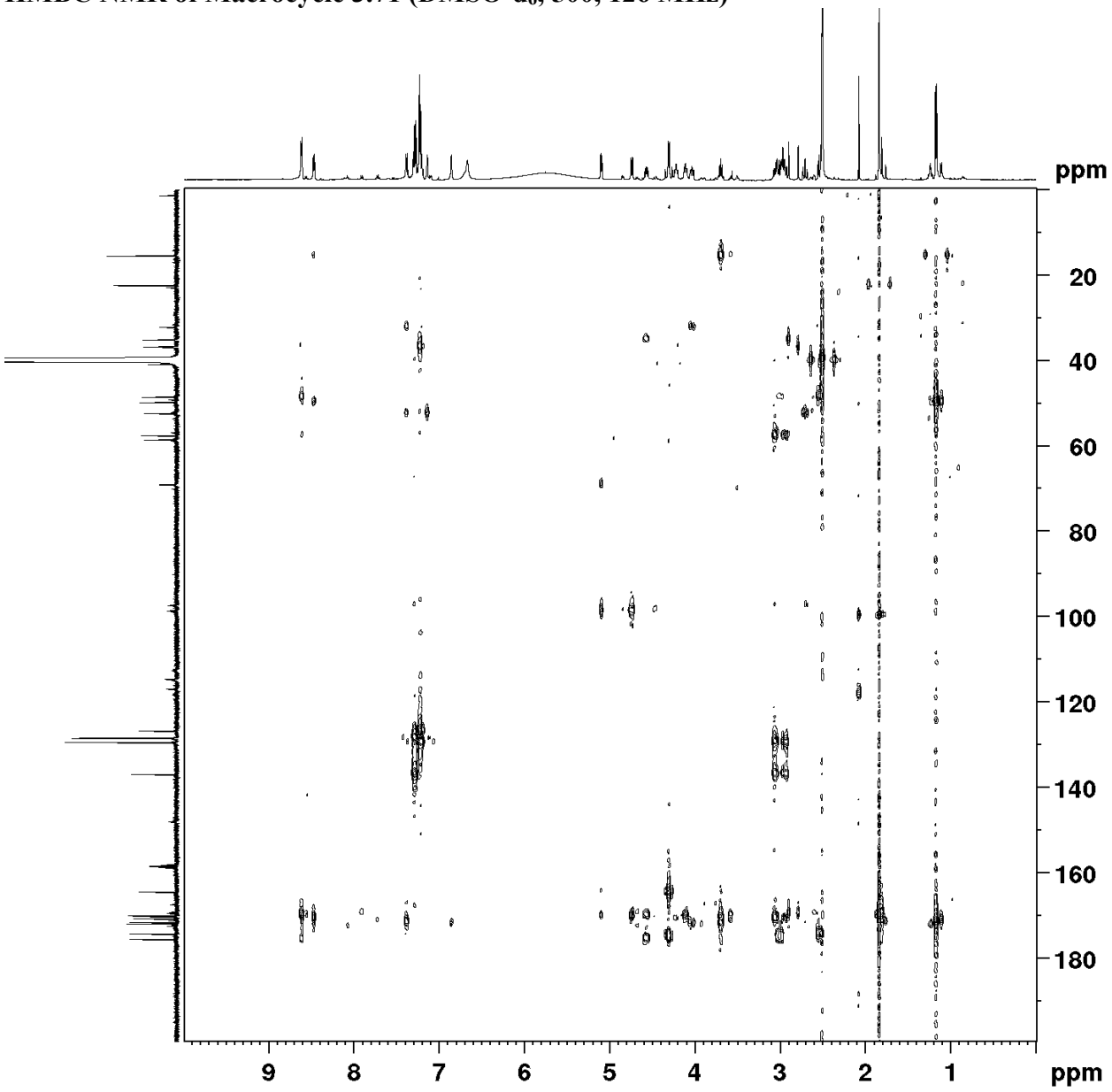


3.71

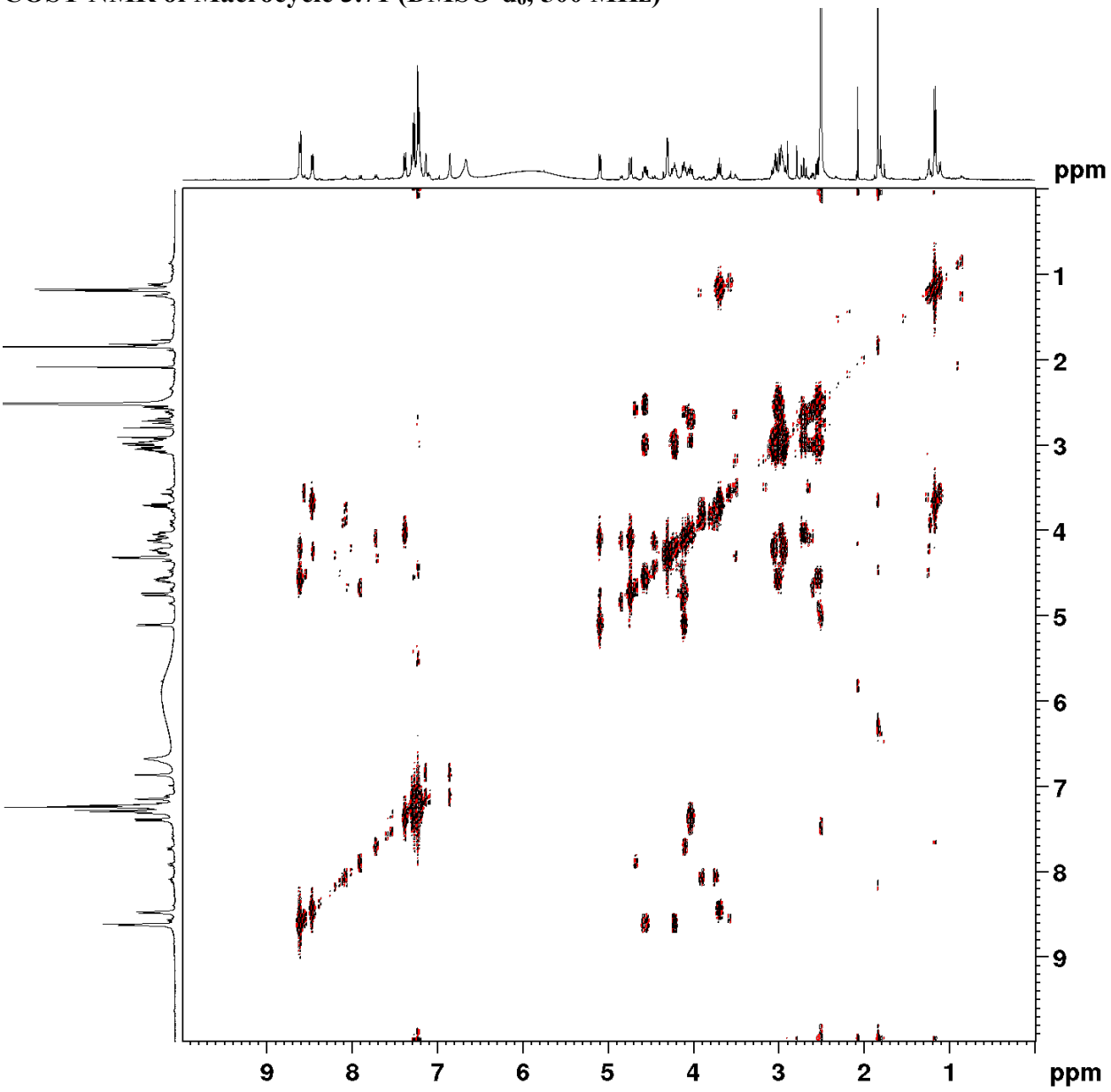
HSQC NMR of Macrocycle 3.71 (DMSO-d_6, 500, 126 MHz)



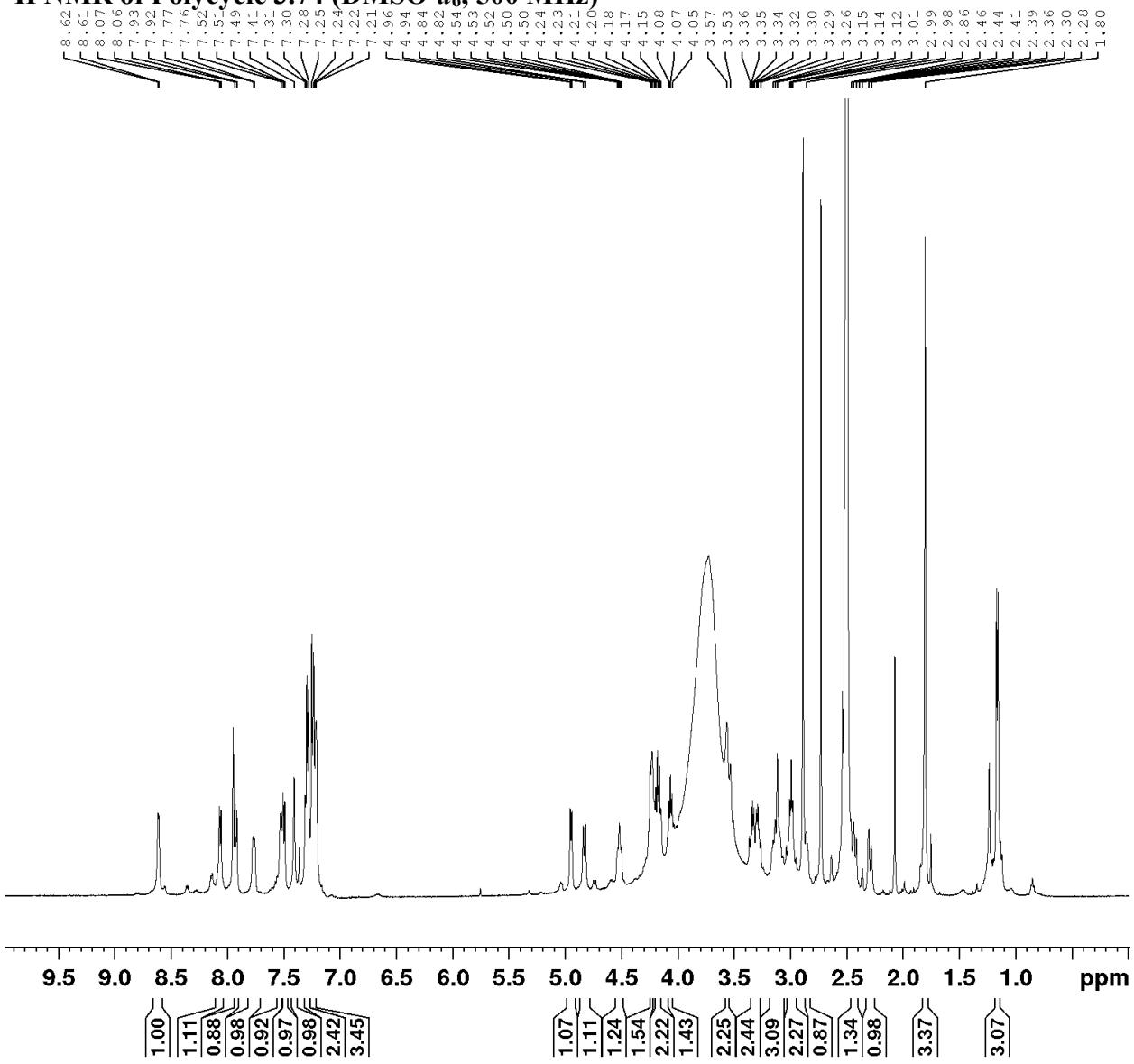
HMBC NMR of Macrocycle 3.71 (DMSO-d_6, 500, 126 MHz)



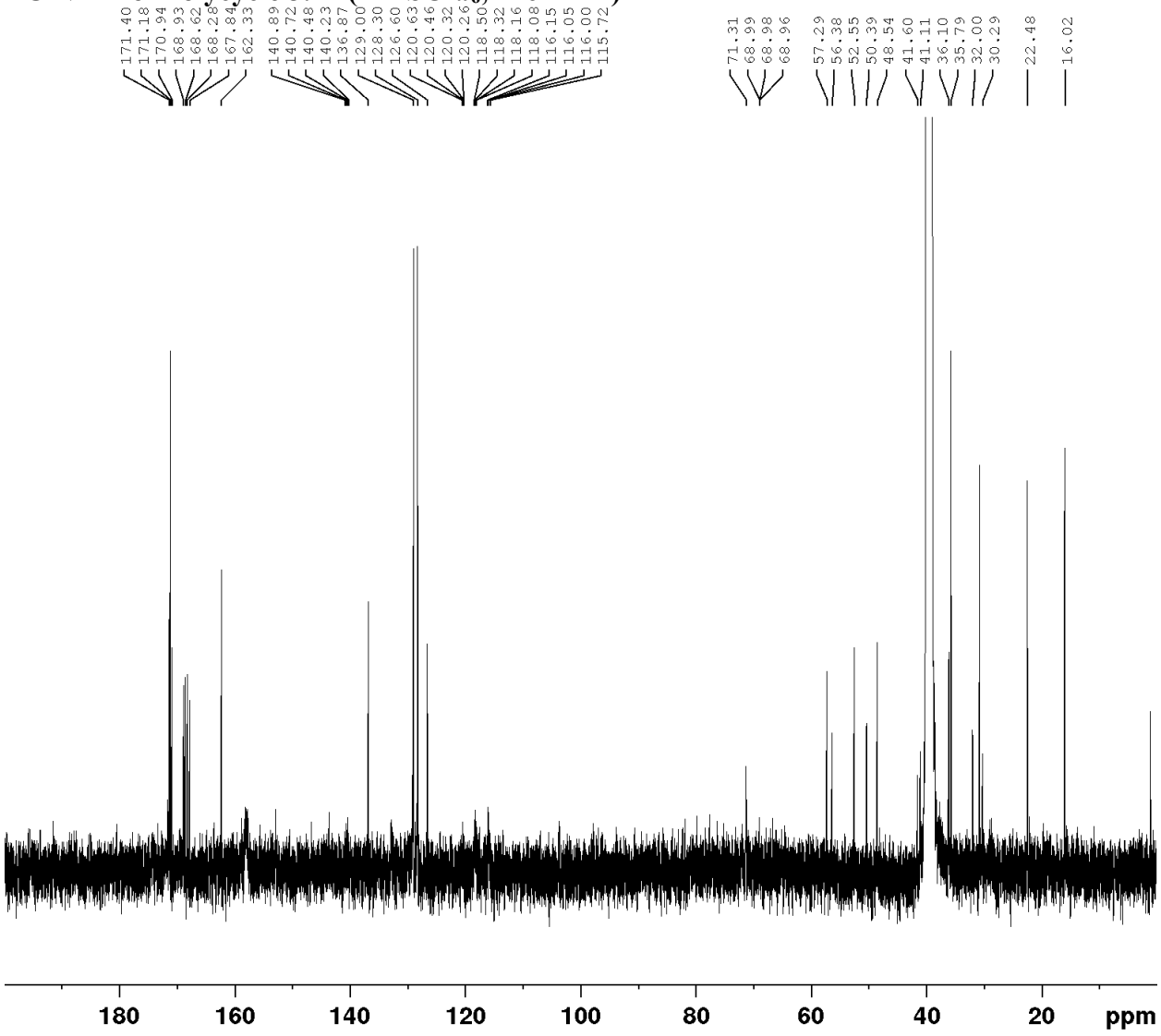
COSY NMR of Macrocycle 3.71 (DMSO-d_6, 500 MHz)



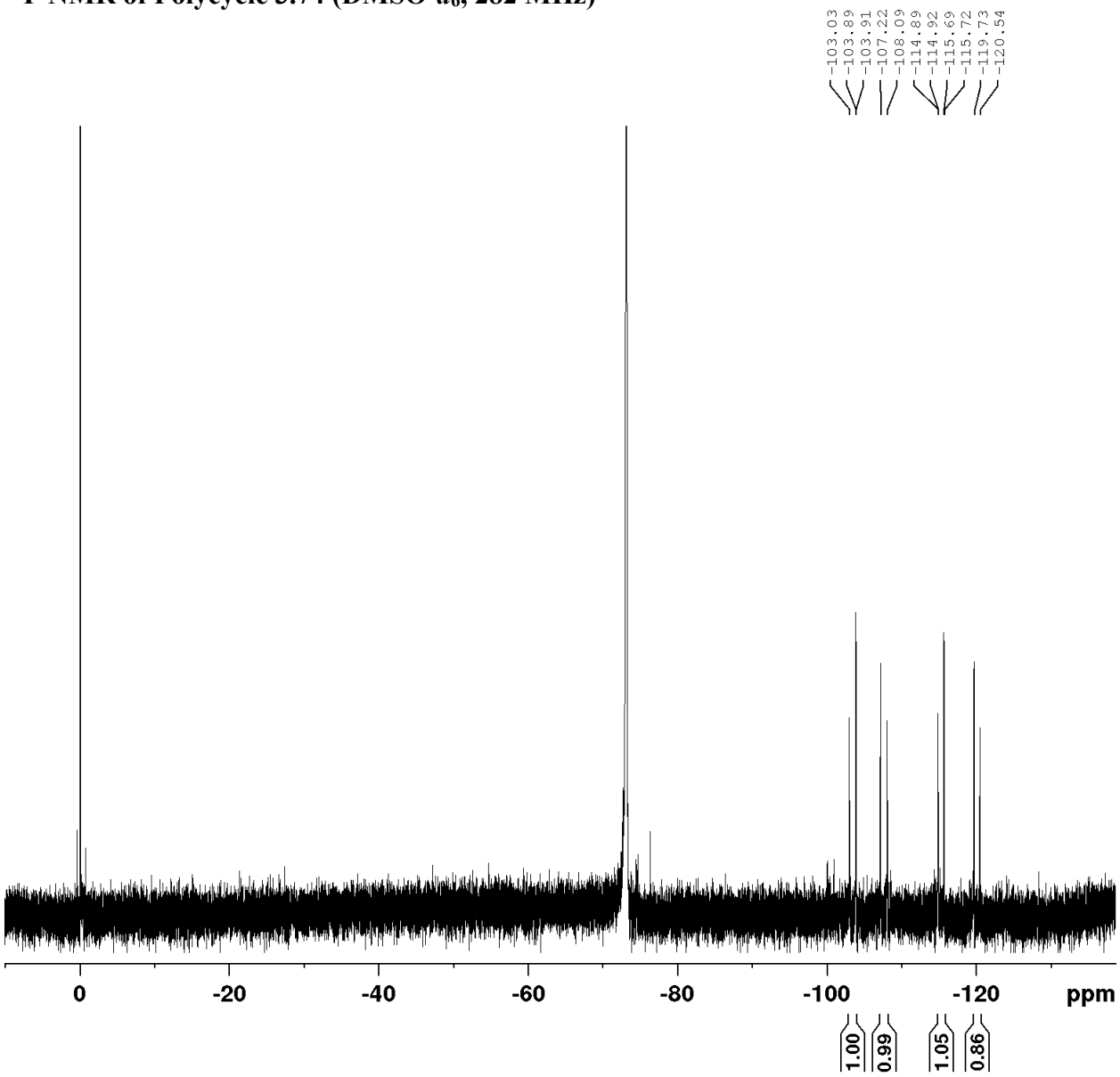
^{1}H NMR of Polycycle 3.74 (DMSO-d_6, 500 MHz)



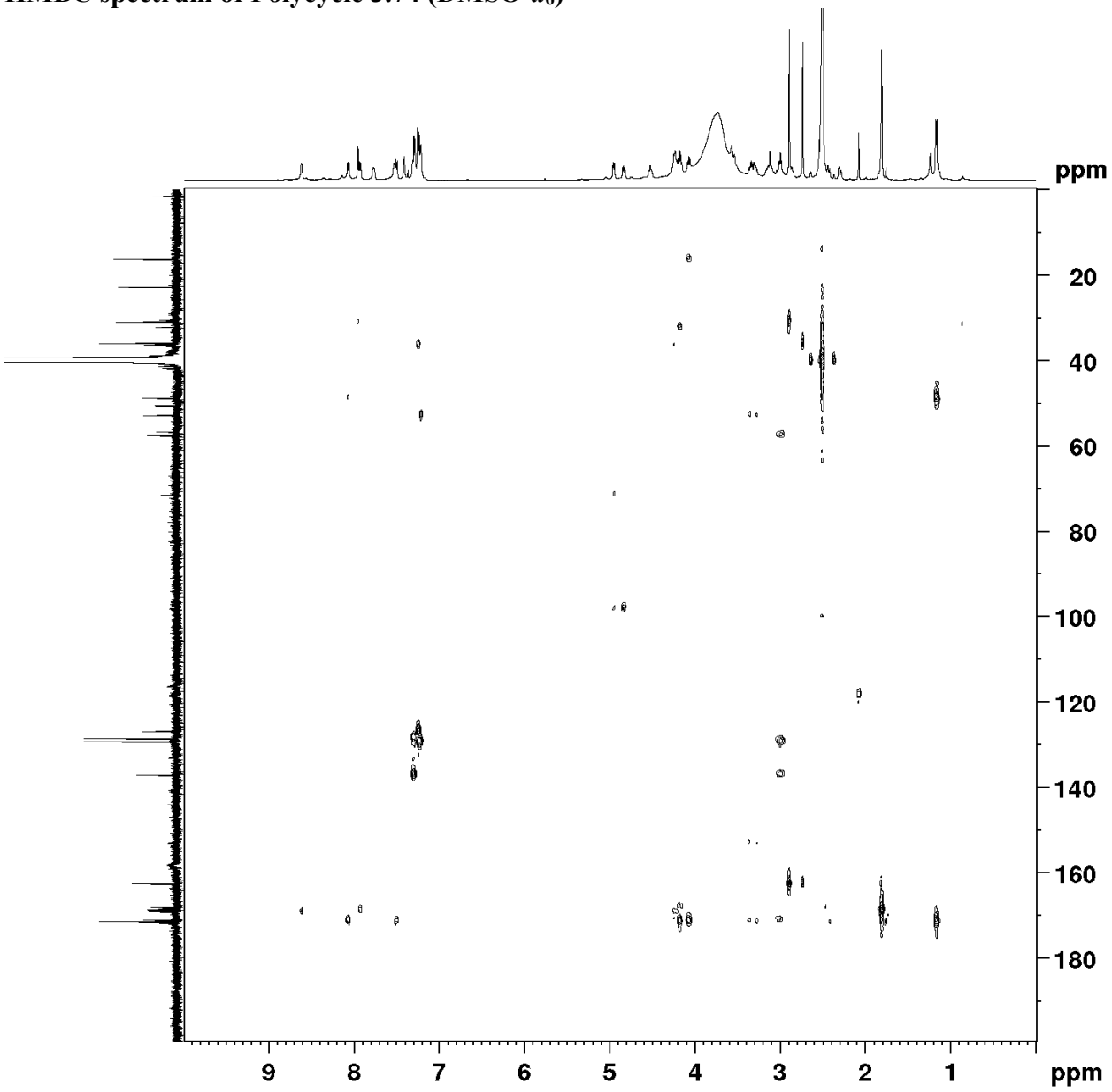
^{13}C NMR of Polycycle 3.74 (DMSO-d_6, 126 MHz)



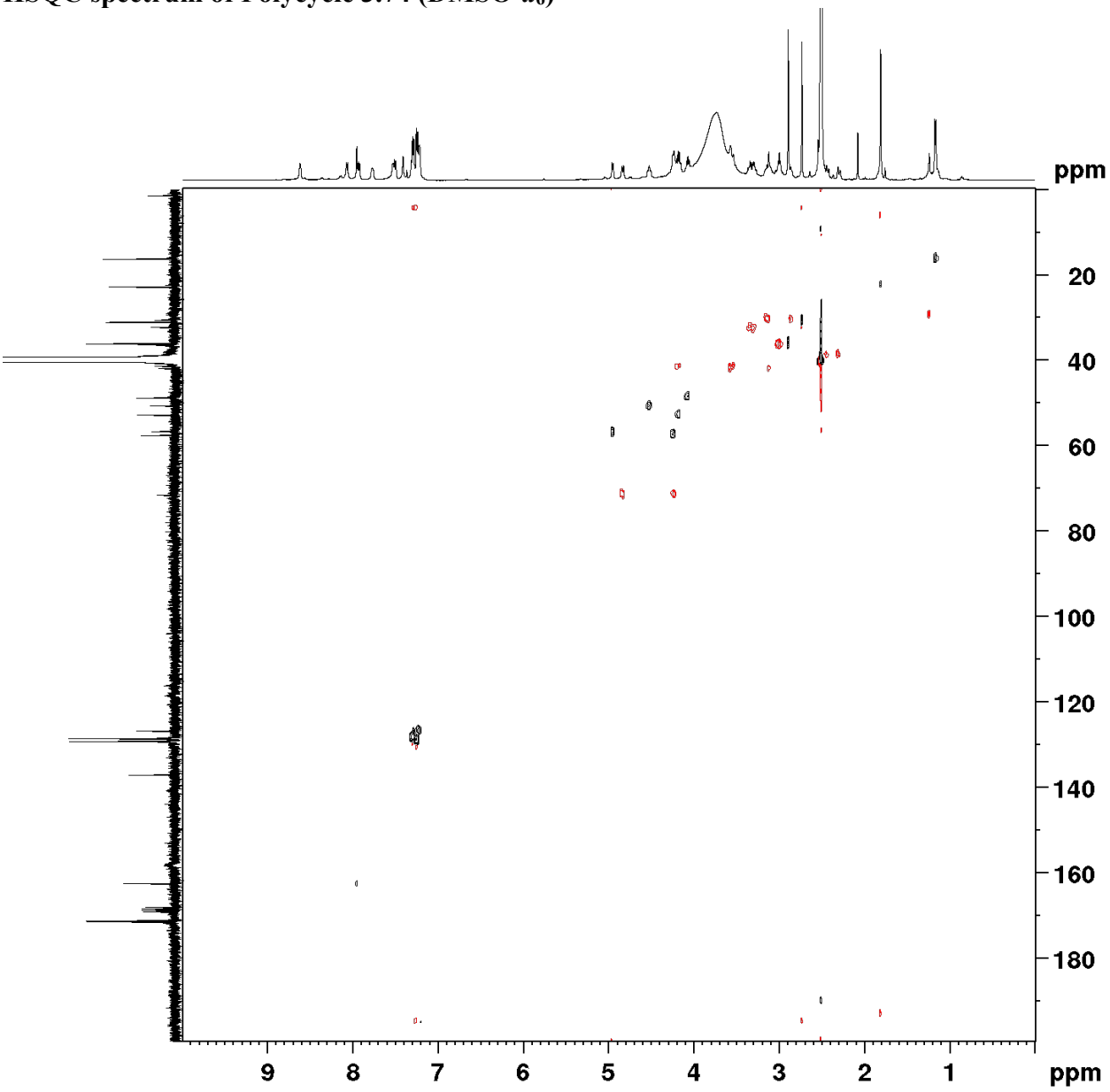
^{19}F NMR of Polycycle 3.74 (DMSO-d_6, 282 MHz)



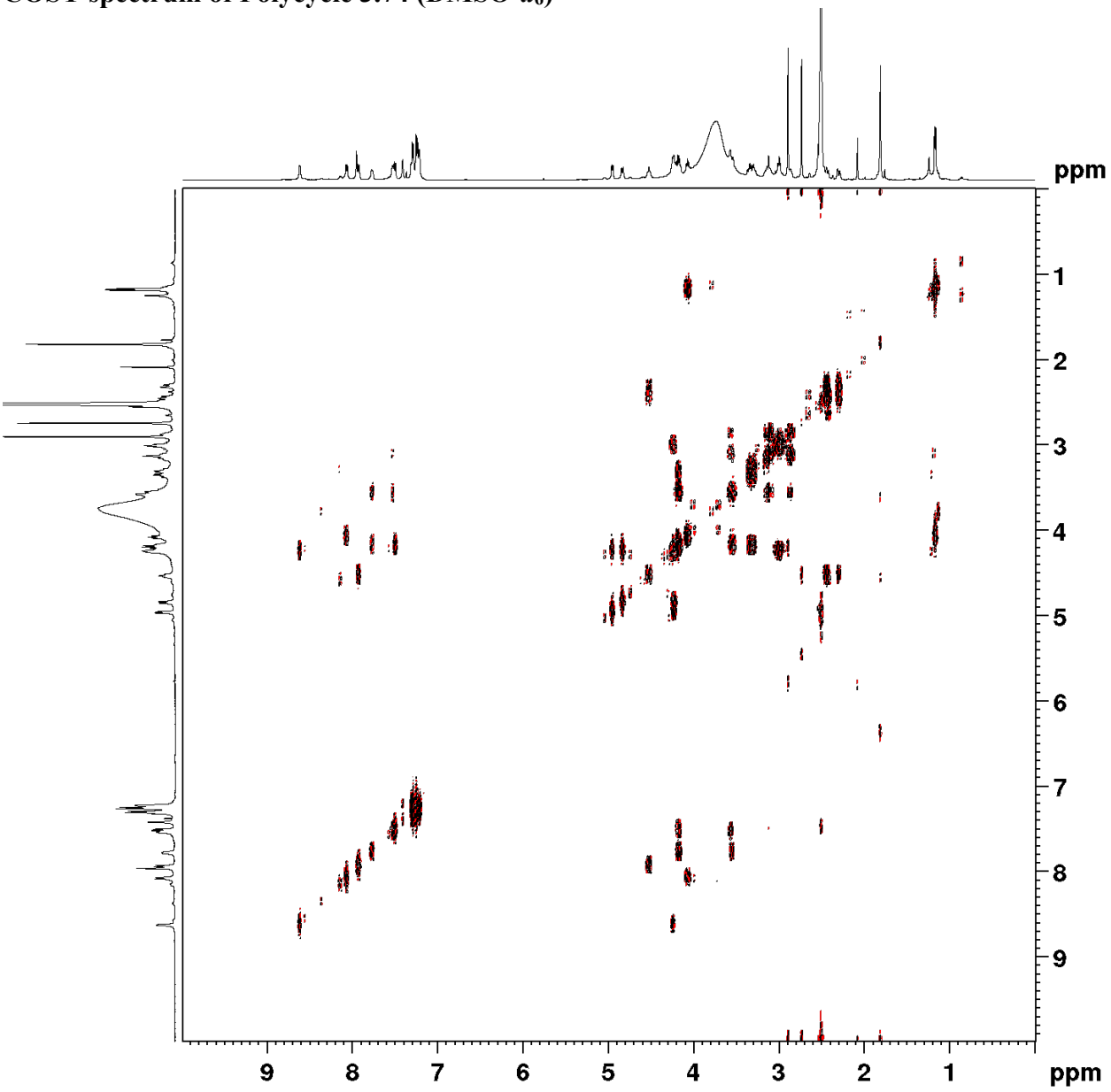
HMBC spectrum of Polycycle 3.74 (DMSO-d_6)



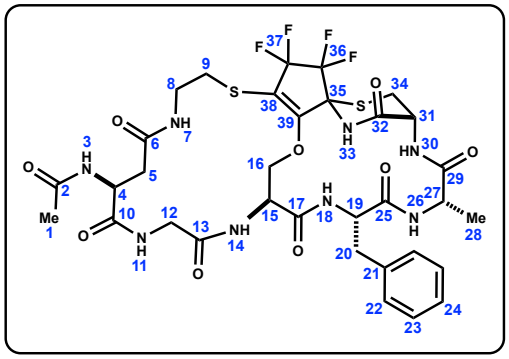
HSQC spectrum of Polycycle 3.74 (DMSO-d_6)



COSY spectrum of Polycycle 3.74 (DMSO-d_6)

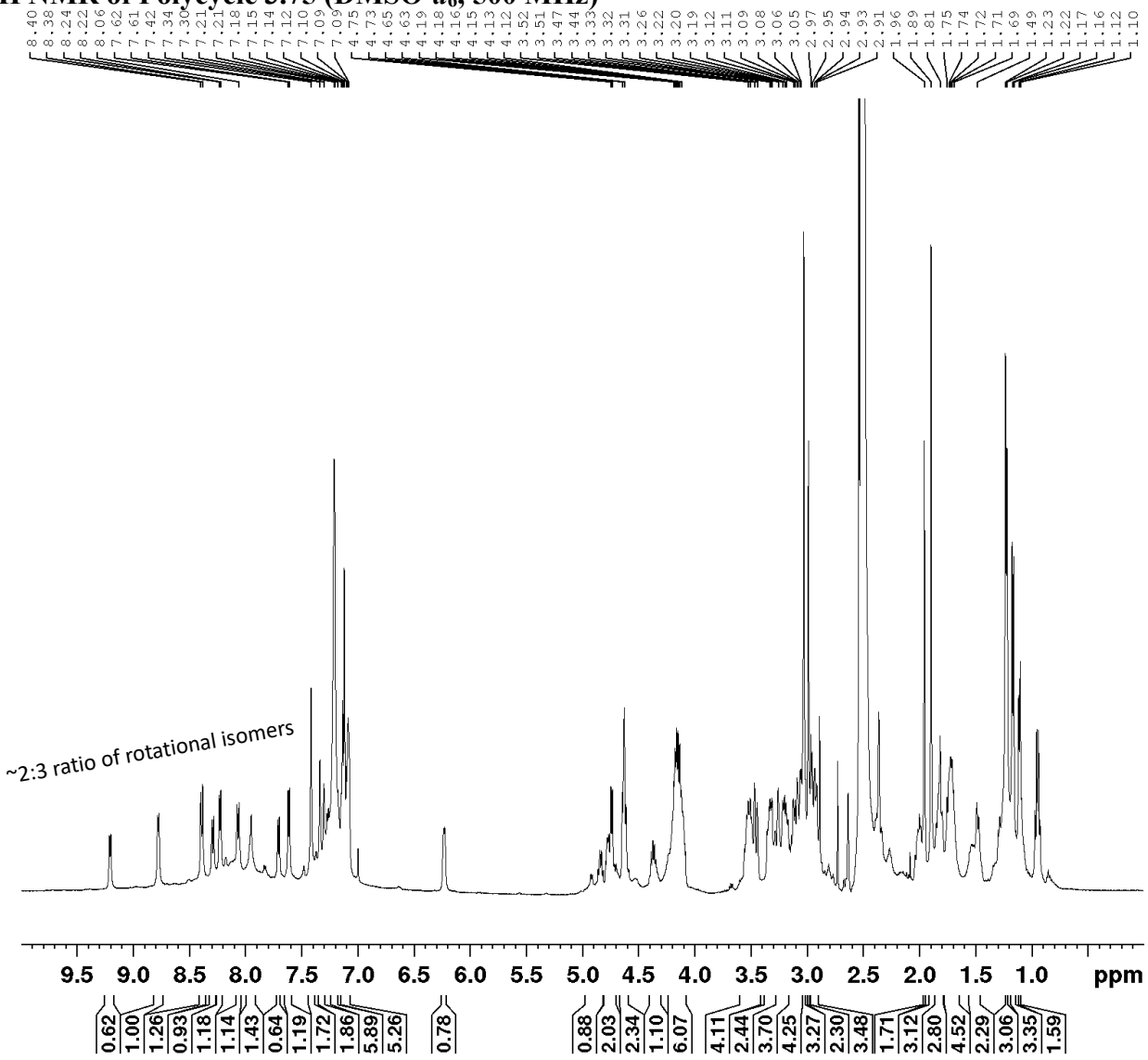


Polycycle **3.74** (DMSO-d_6, 298K)

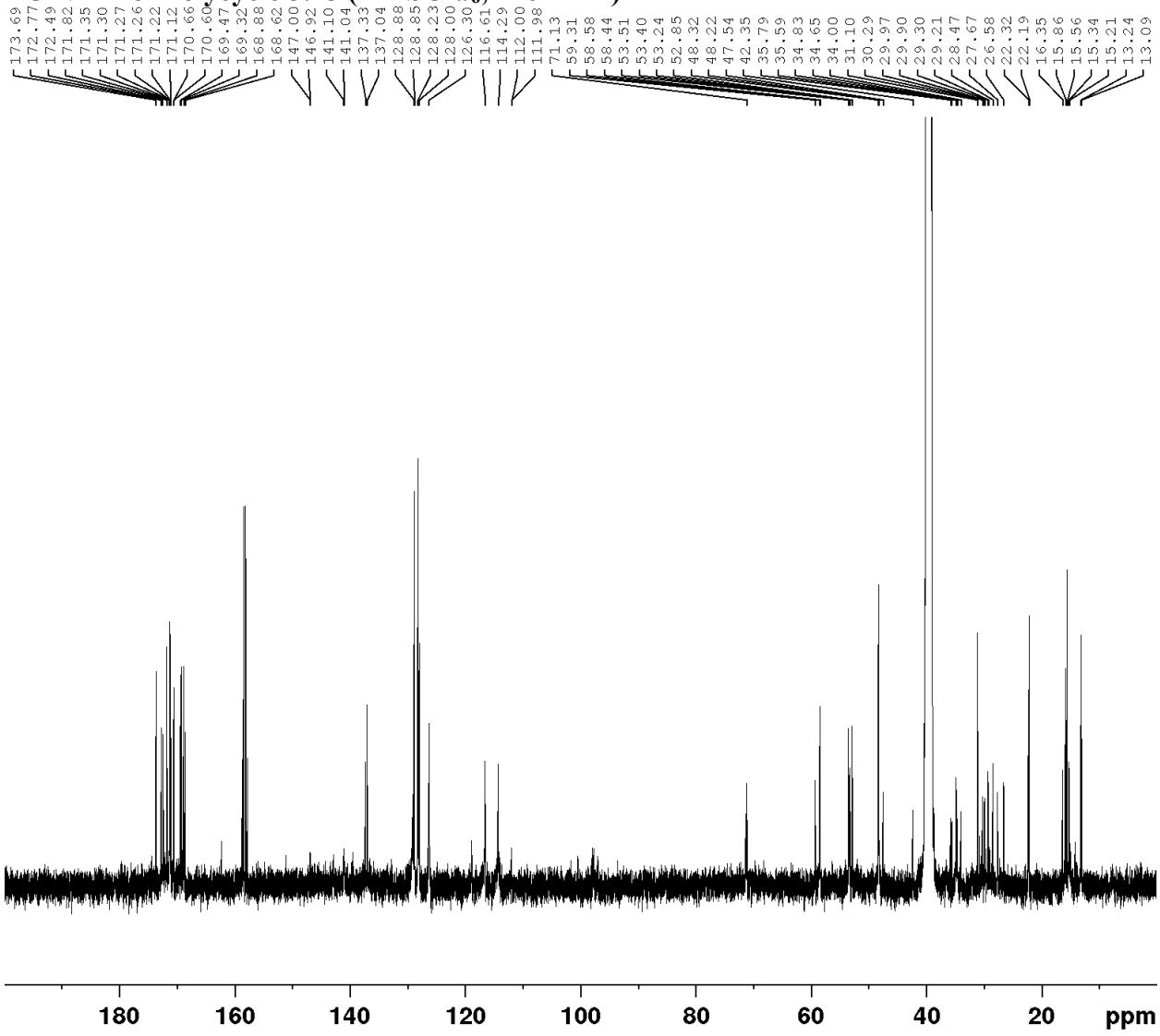


	13C	1H	Key Correlation
1	22.48	1.80 (s, 3H)	27->28 COSY, HMBC, HSQC
2	168.62	-	1->2 HMBC
3	-	7.92 (d, J = 7.7 Hz, 1H)	2->3 HMBC
4	50.39	4.52 (m, 1H)	3->4 COSY, HMBC, HSQC
5	35.79	2.42 (m, 1H), 2.29 (m, 1H)	4->5 COSY, HSQC
6	171.40	-	5->6 HMBC, COSY, HMBC
7	-	7.77 (m, 1H)	6->7 HMBC
8	41.11	4.17 (m, 1H), 3.55 (m, 1H) overlap	7->8 COSY, HSQC
9	52.58	3.14 (m, 2H) overlap	8->9 COSY, HSQC
10	167.23	-	11->10 HMBC
11	-	7.53 (m, 1H)	10->11 HMBC
12	41.59	4.16 (m, 1H) overlap, 3.55 (m, 1H)	11->12 COSY, HSQC
13	162.33	-	12->13 HMBC
14	-	7.50 (d, J = 7.6 Hz, 1H)	15->14 COSY
15	71.32	4.83 (m, 1H)	17->15 HMBC, HSQC
16	32.00	3.31 (m,2H)	15->16 COSY, HSQC
17	168.94	-	18->17 HMBC
18	-	8.61 (d, J = 4.1 Hz, 1H)	19->20 COSY
19	57.29	4.24 (m, 1H)	20->19 COSY
20	36.01	2.99 (m, 2H)	21->20 HMBC, HSQC
21	136.87	-	20->21 HMBC
22	129.00	7.32–7.21 (m, 2H)	COSY, HMBC, HSQC
23	128.30	7.32-7.21 (m, 2H)	COSY, HMBC, HSQC
24	126.60	7.32–7.21 (m,1H)	COSY, HMBC, HSQC
25	168.23	-	19->25 HMBC
26	-	8.07 (d, J = 7.5 Hz, 1H)	27->26 COSY, HSQC
27	48.54	4.07 (m, 1H)	28->27 COSY
28	16.02	1.16 (d, J = , 3H)	27->28 HMBC, COSY, HSQC
29	170.94	-	27->29 HMBC
30	-	-	-
31	56.38	4.95 (m 1H)	32->31 HMBC, HSQC
32	171.19	-	33->32 HMBC
33	-	7.40 (s, 1H)	-
34	30.29	3.11 (m, 1H), 2.86 (m, 1H) overlap	31->34 HMBC, COSY, HSQC
35	69.0–68.9 (m, 1C)	-	-
36	114.7–116.1 (m, 1C)	-	-
37	118.1–118.5 (m, 1C)	-	-
38	120.3–120.6 (m, 1C)	-	-
39	140.2–140.9(m, 1C)	-	-

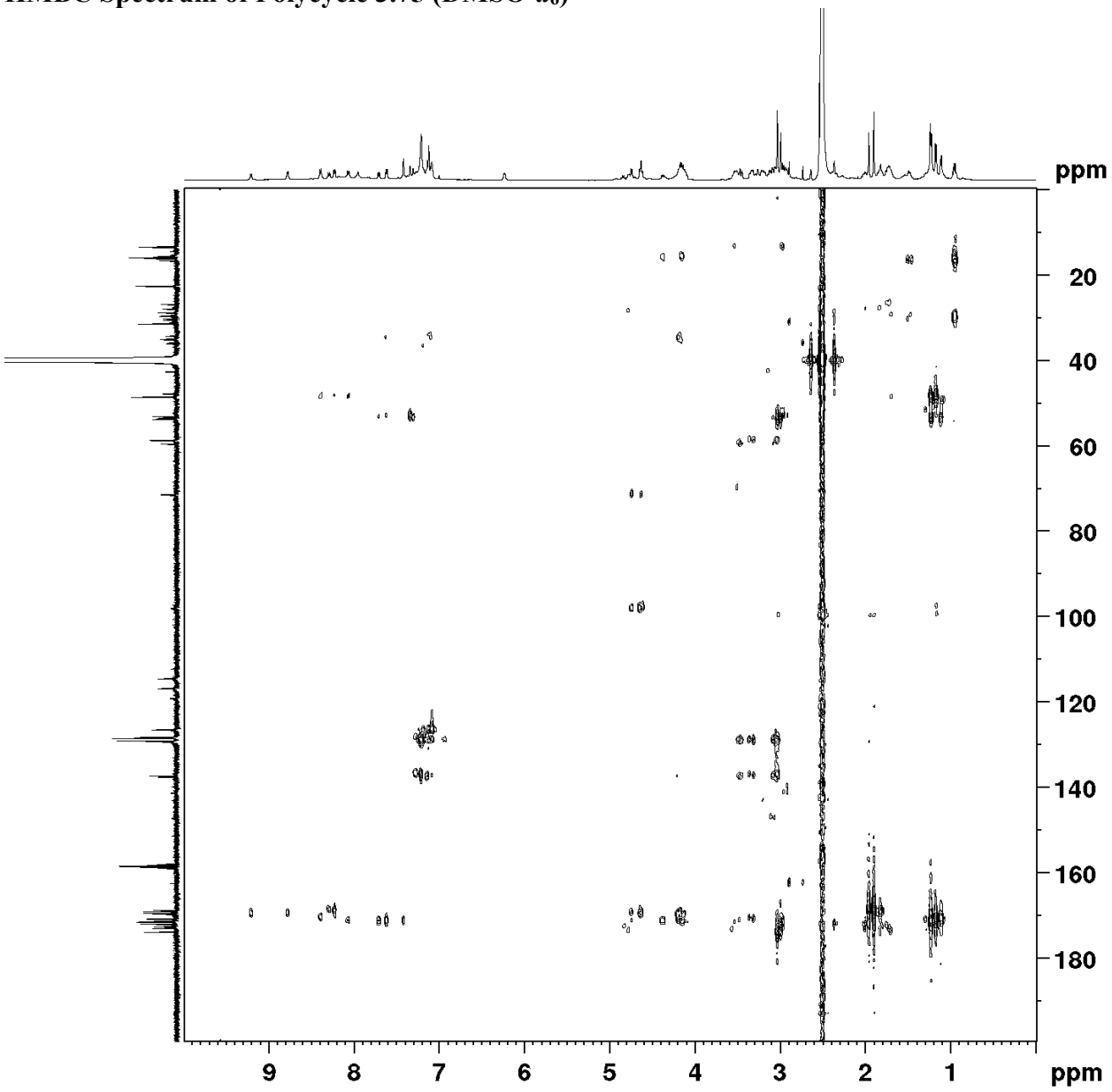
^{1}H NMR of Polycycle 3.75 (DMSO-d_6, 500 MHz)



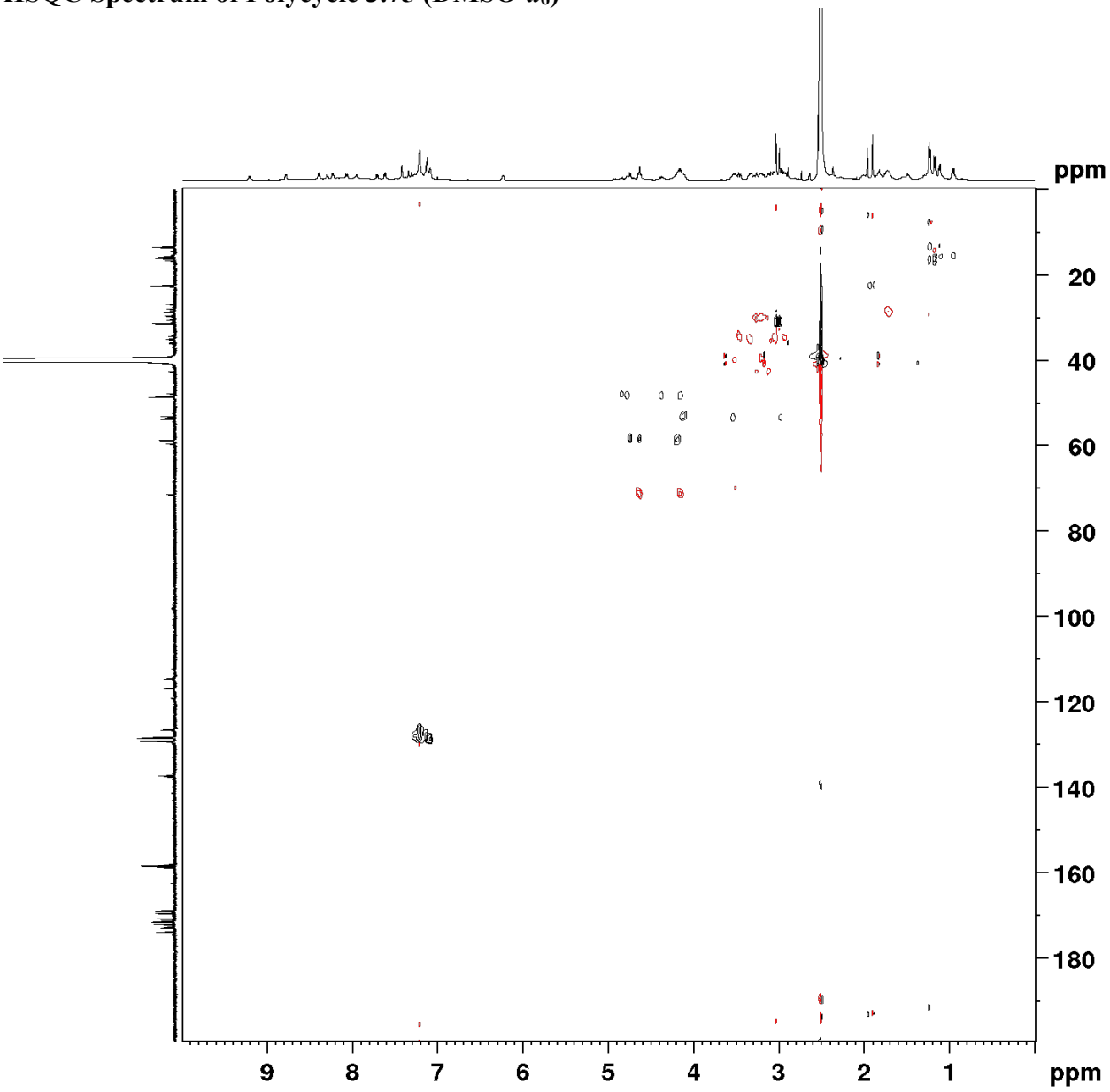
^{13}C NMR of Polycycle 3.75 (DMSO-d_6, 126 MHz)



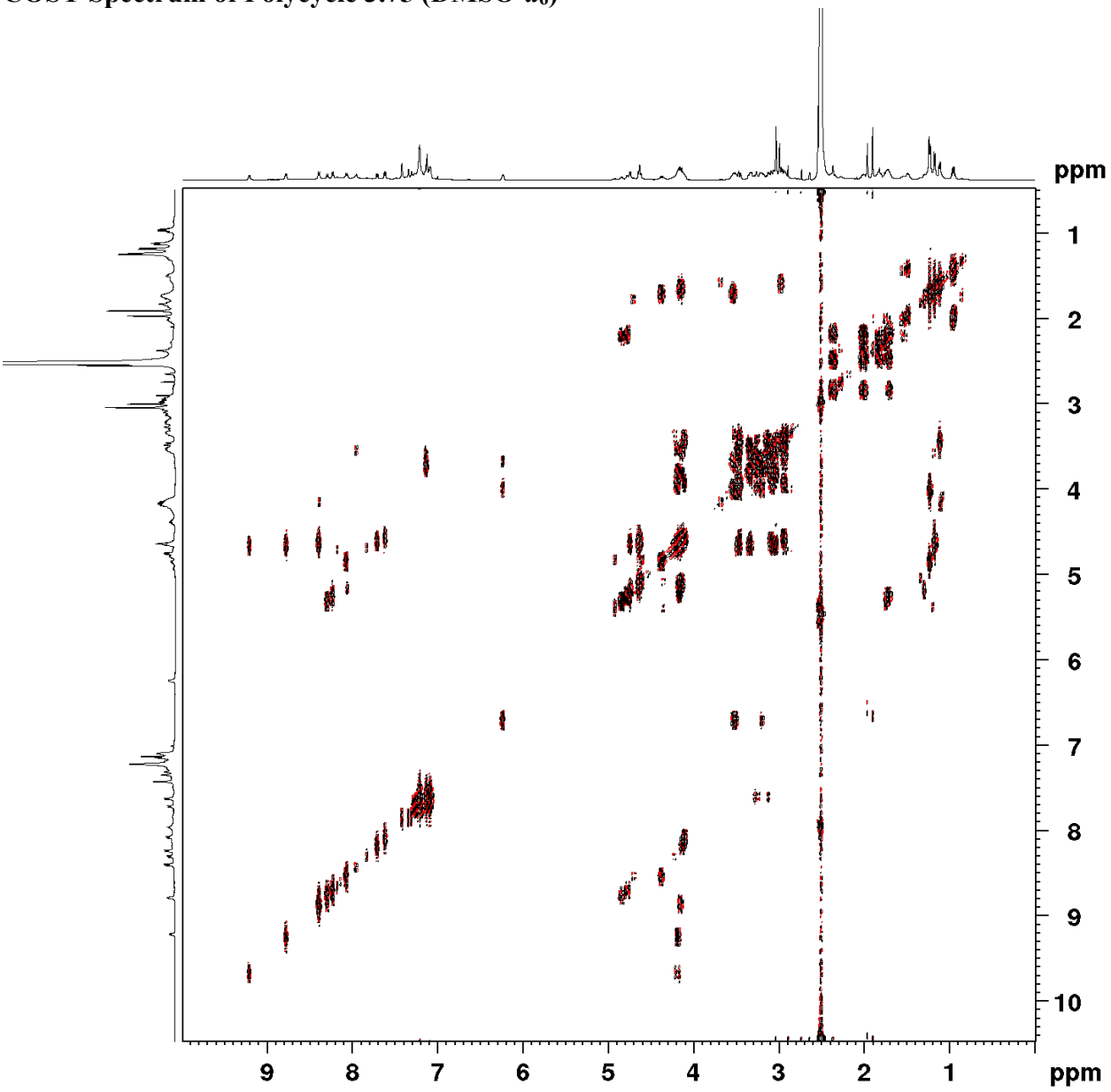
HMBC Spectrum of Polycycle 3.75 (DMSO-d_6)



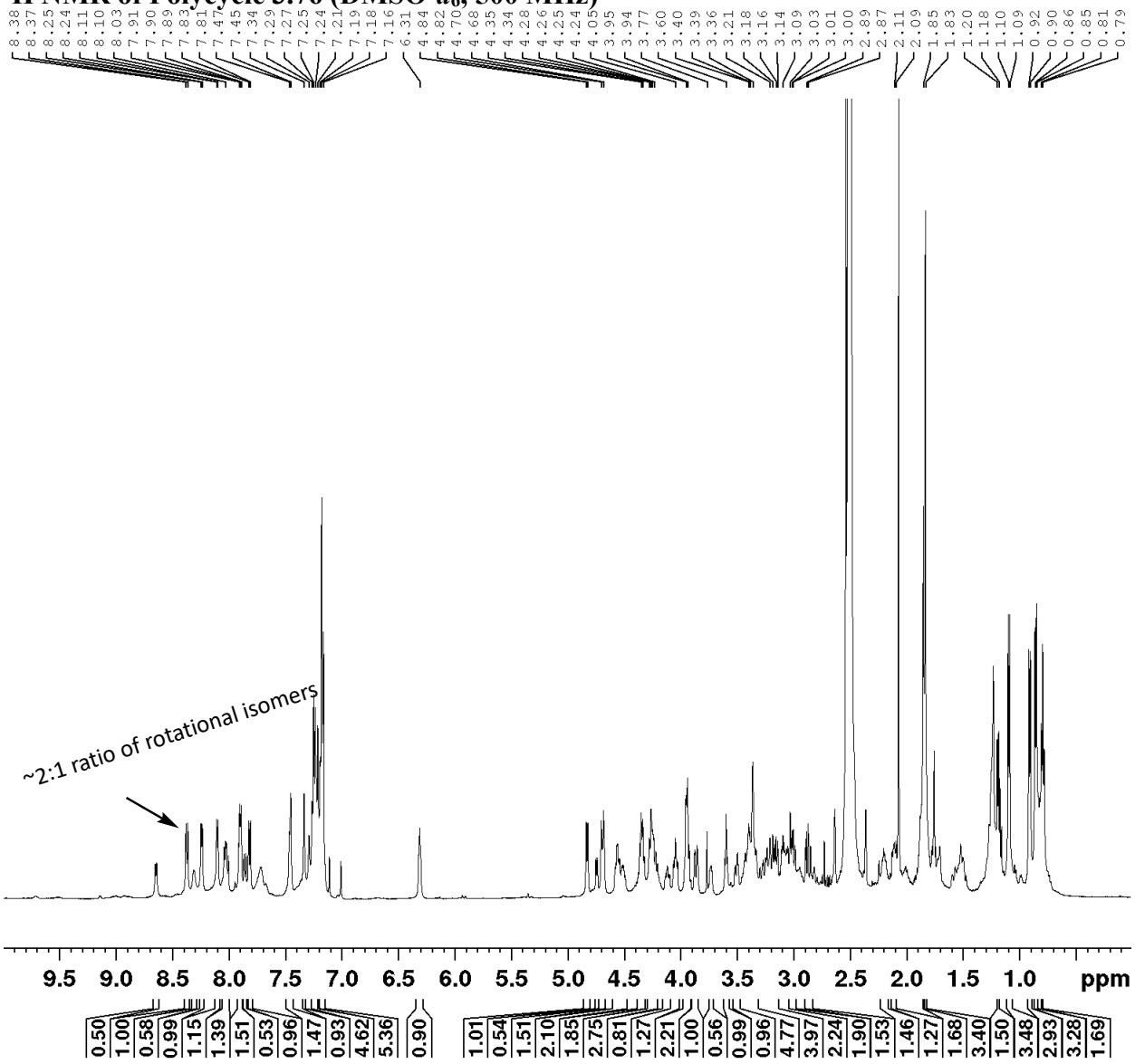
HSQC Spectrum of Polycycle 3.75 (DMSO-d_6)



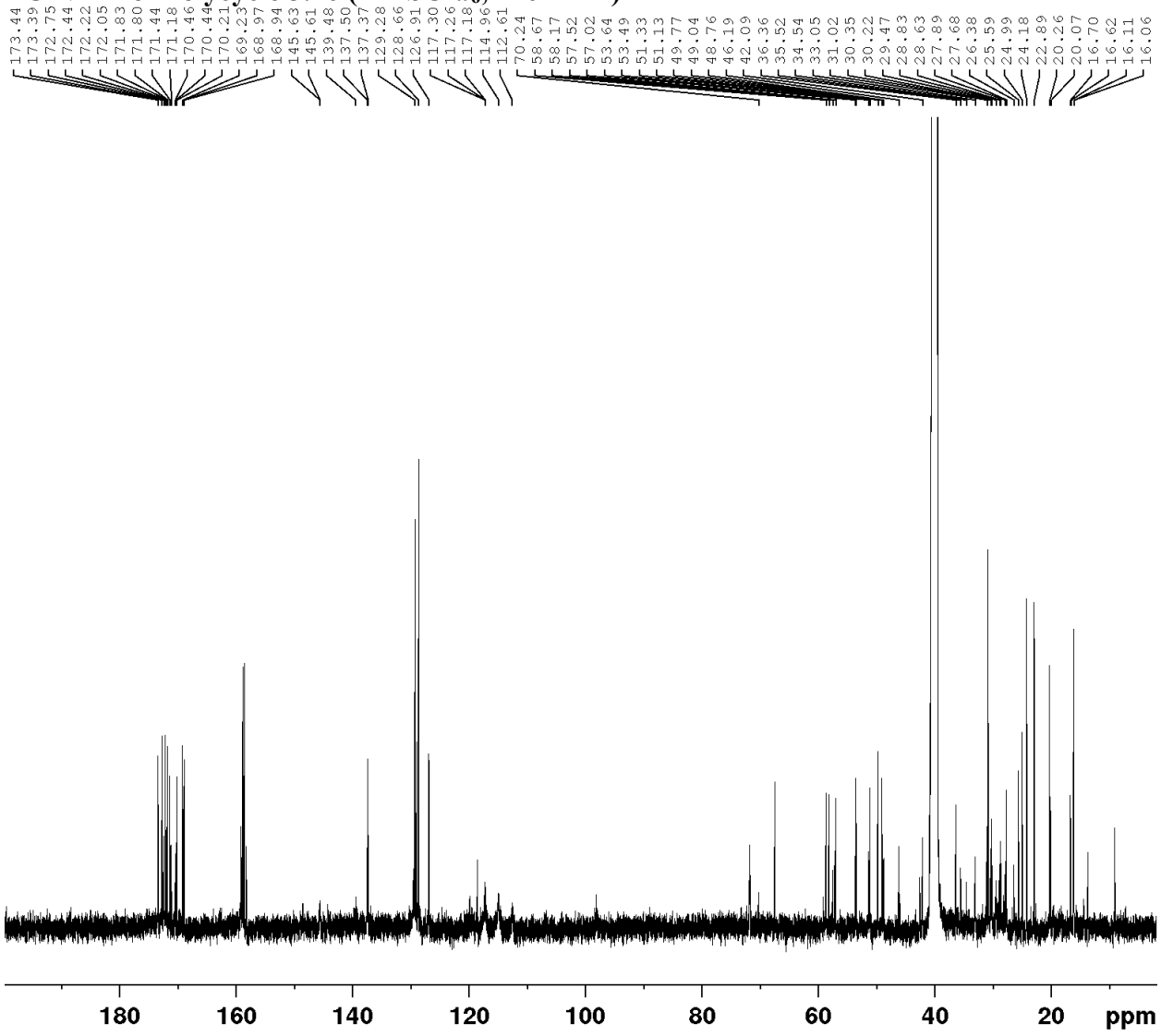
COSY Spectrum of Polycycle 3.75 (DMSO-d_6)



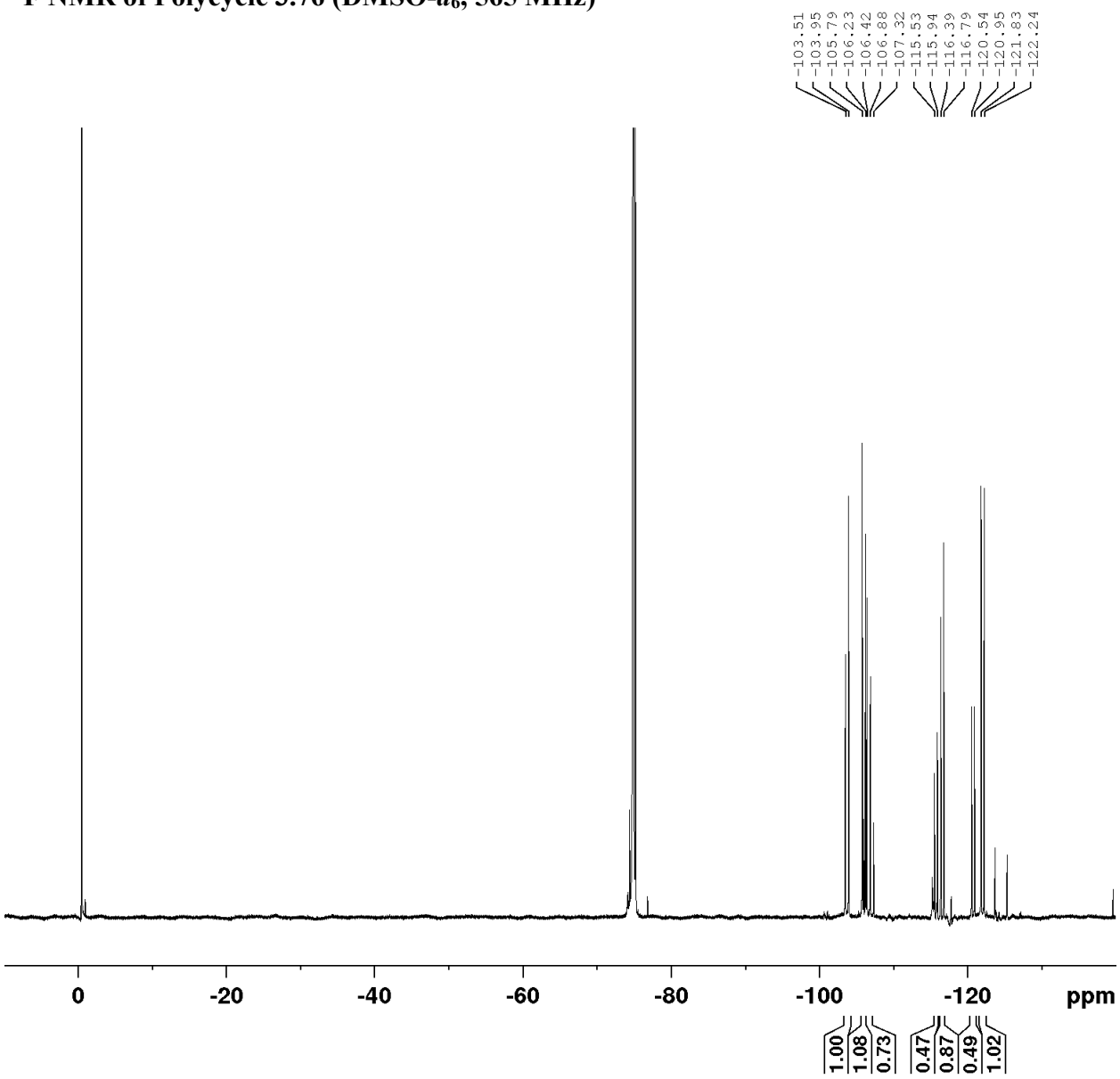
^{1}H NMR of Polycycle 3.76 (DMSO-d_6, 500 MHz)



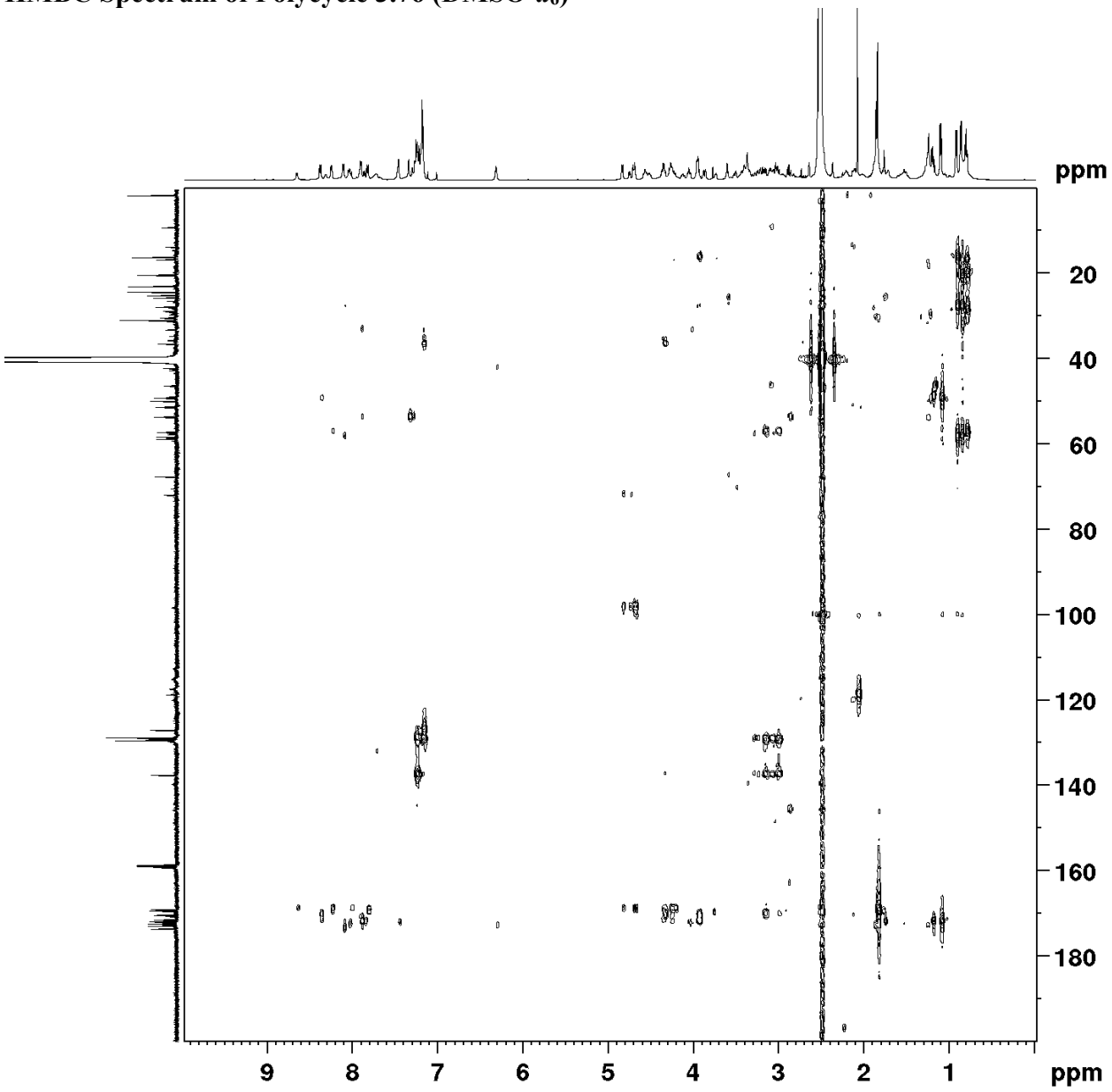
^{13}C NMR of Polycycle 3.76 (DMSO-d_6, 126 MHz)



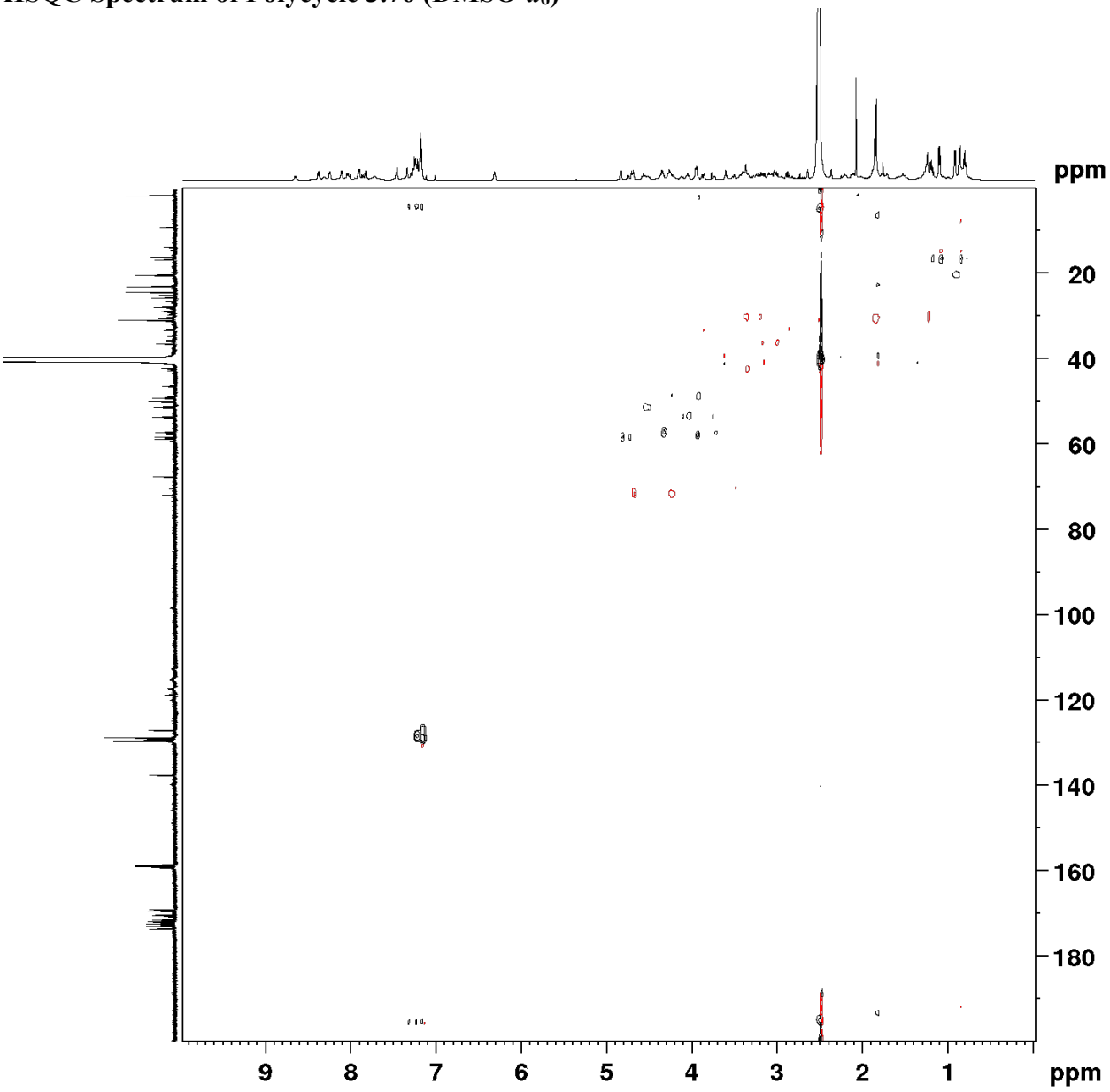
^{19}F NMR of Polycycle 3.76 (DMSO-d_6, 565 MHz)



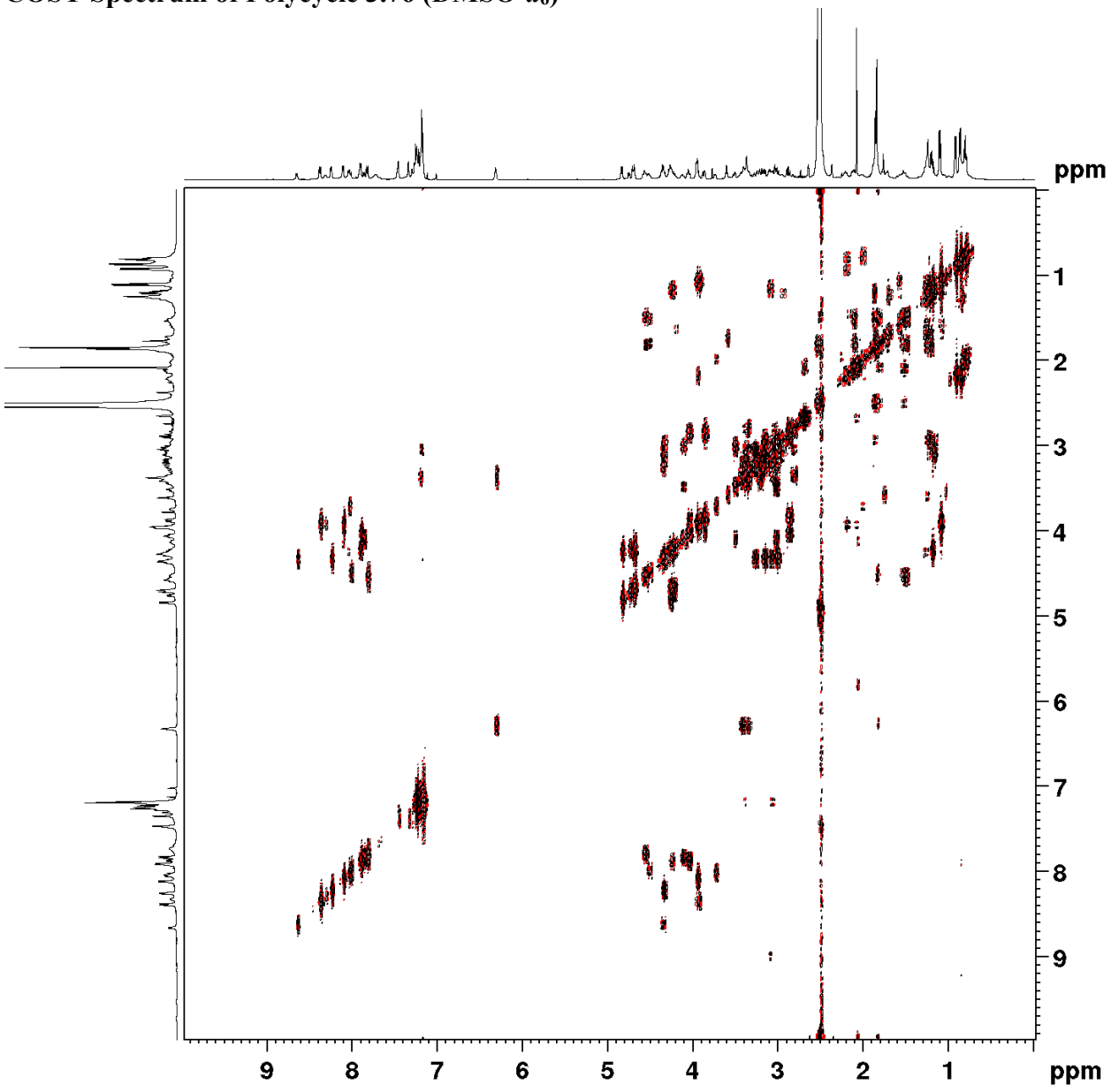
HMBC Spectrum of Polycycle 3.76 (DMSO-d_6)



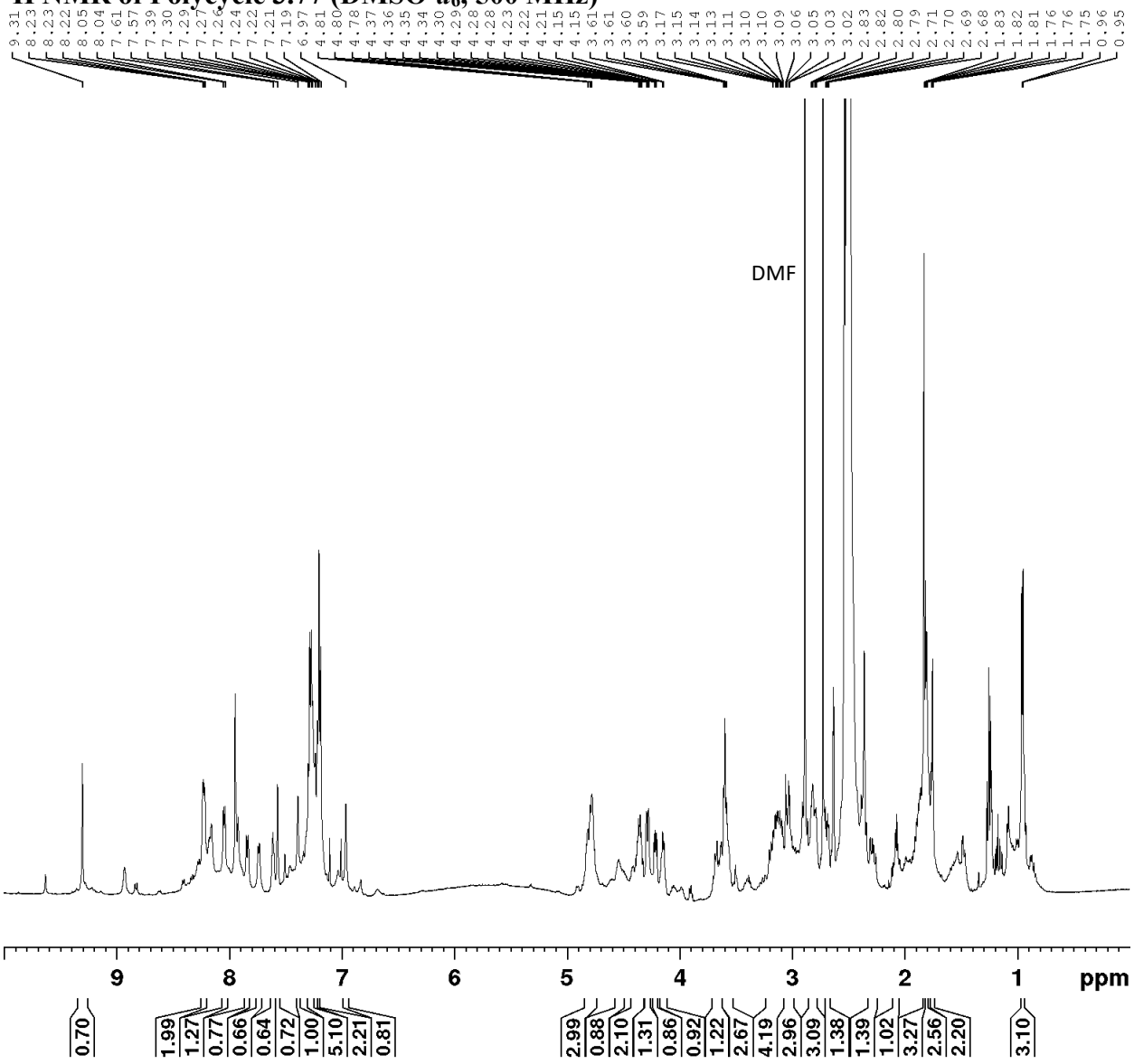
HSQC Spectrum of Polycycle 3.76 (DMSO-d_6)



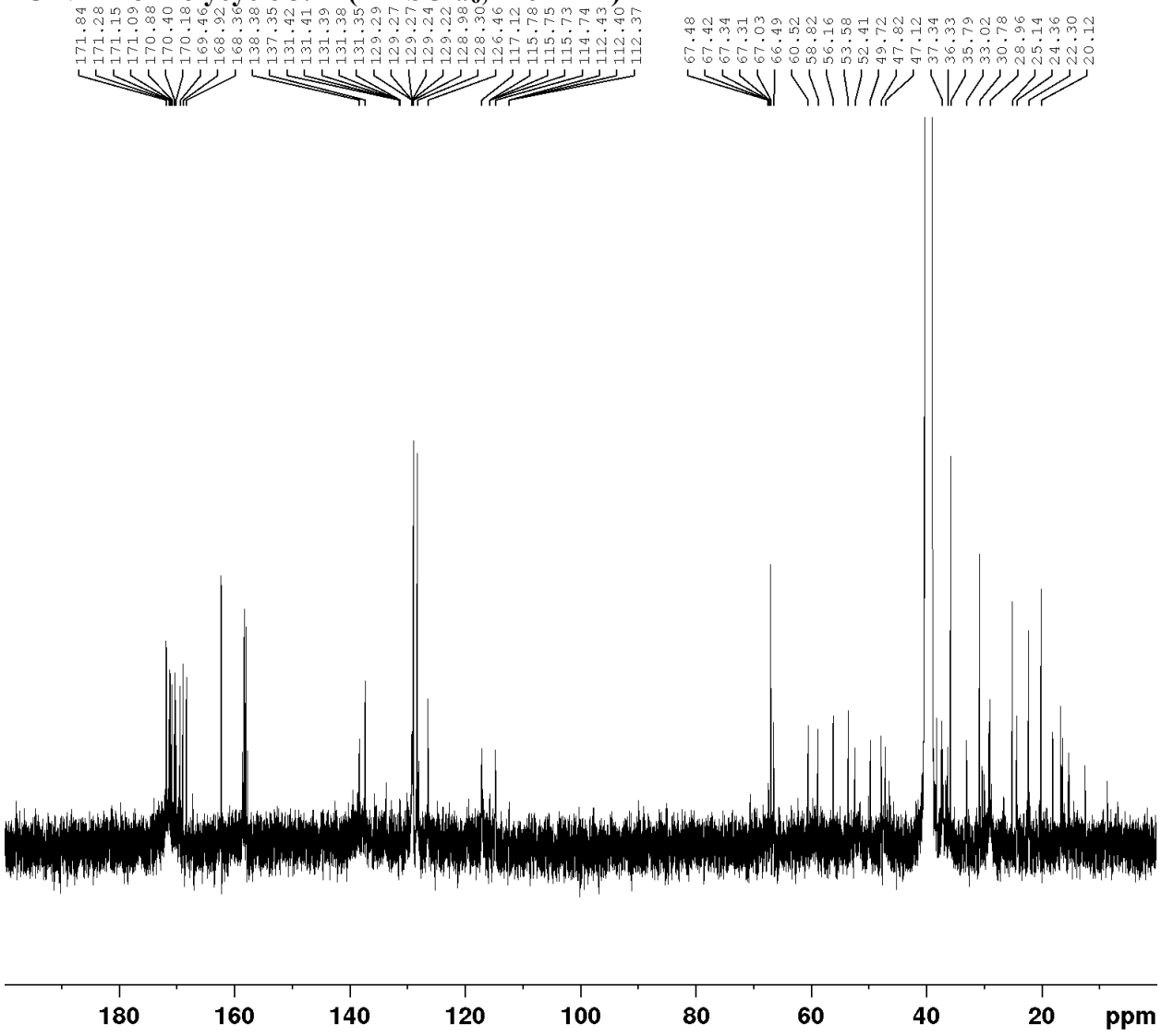
COSY Spectrum of Polycycle 3.76 (DMSO-d_6)



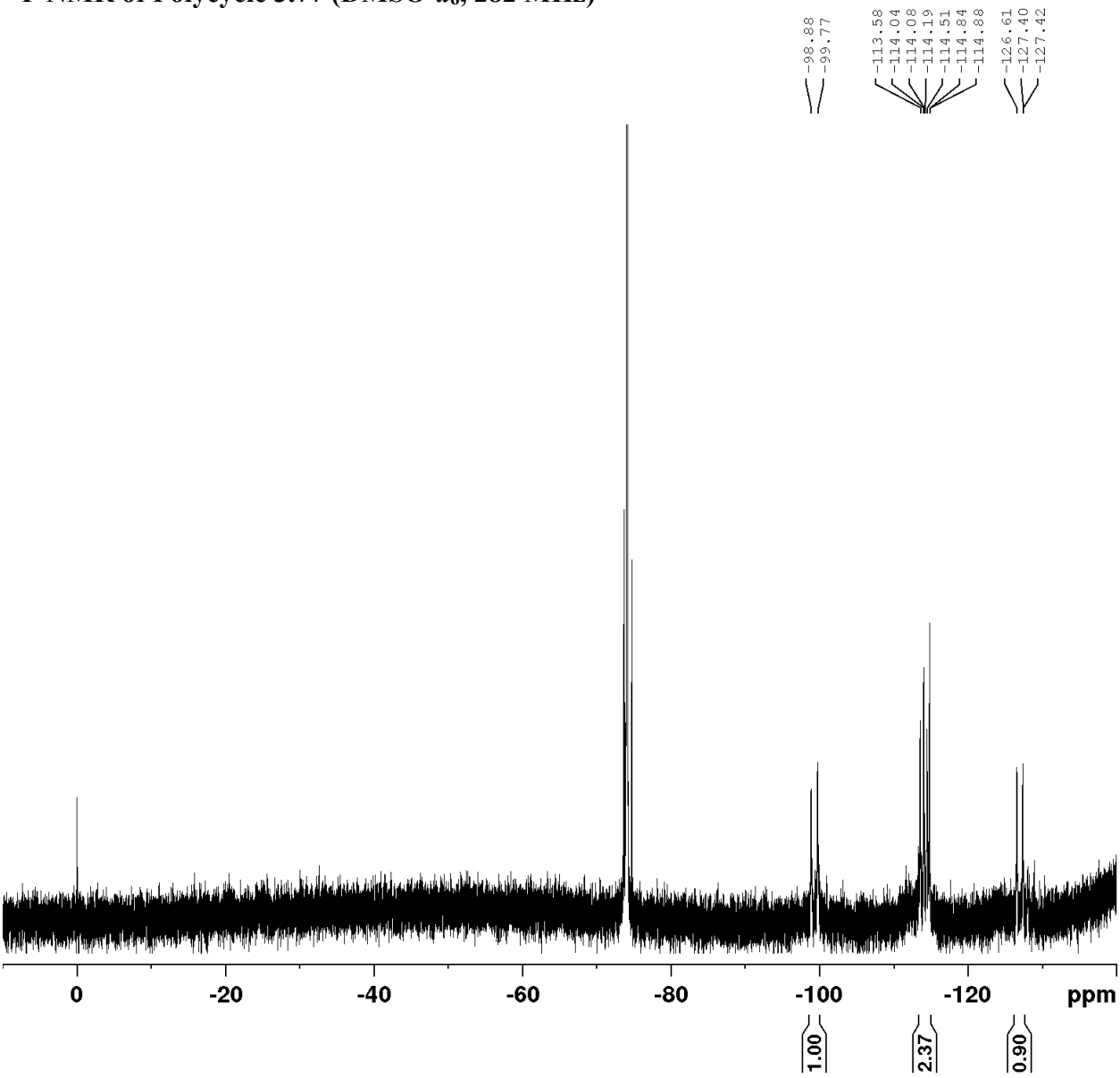
^{1}H NMR of Polycycle 3.77 (DMSO-d_6, 500 MHz)



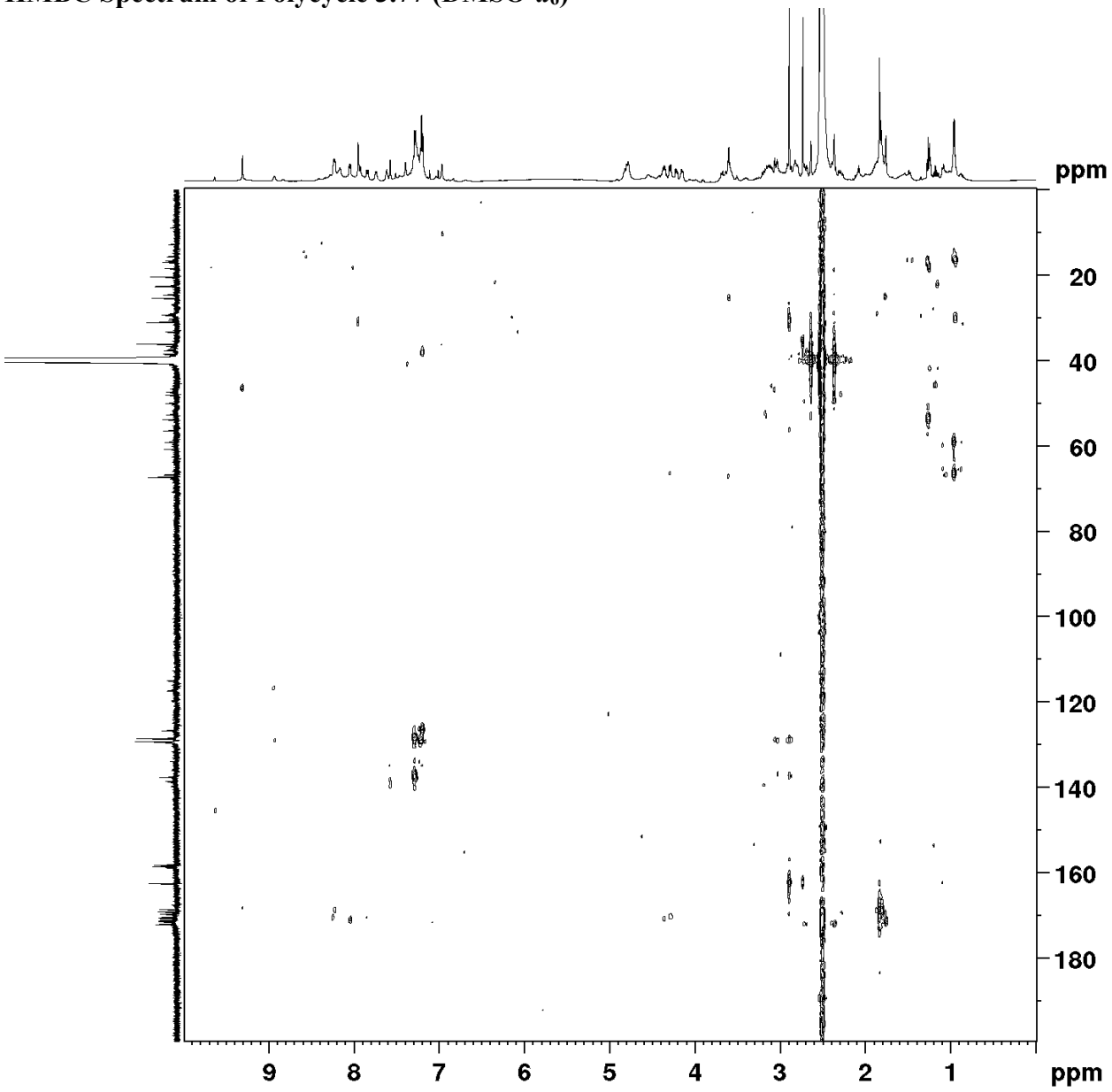
^{13}C NMR of Polycycle 3.77 (DMSO-d_6, 126 MHz)



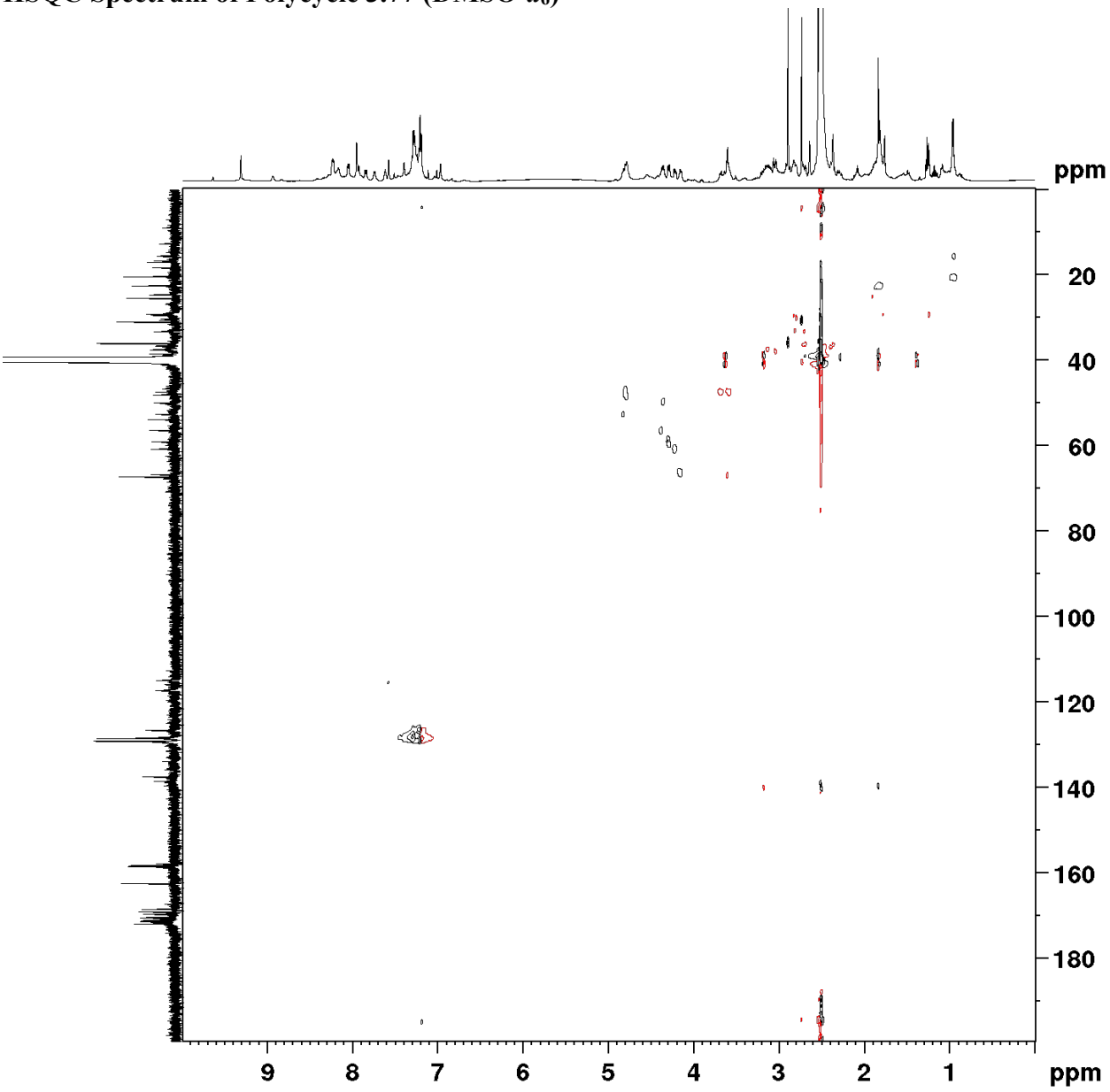
^{19}F NMR of Polycycle 3.77 (DMSO-d_6, 282 MHz)



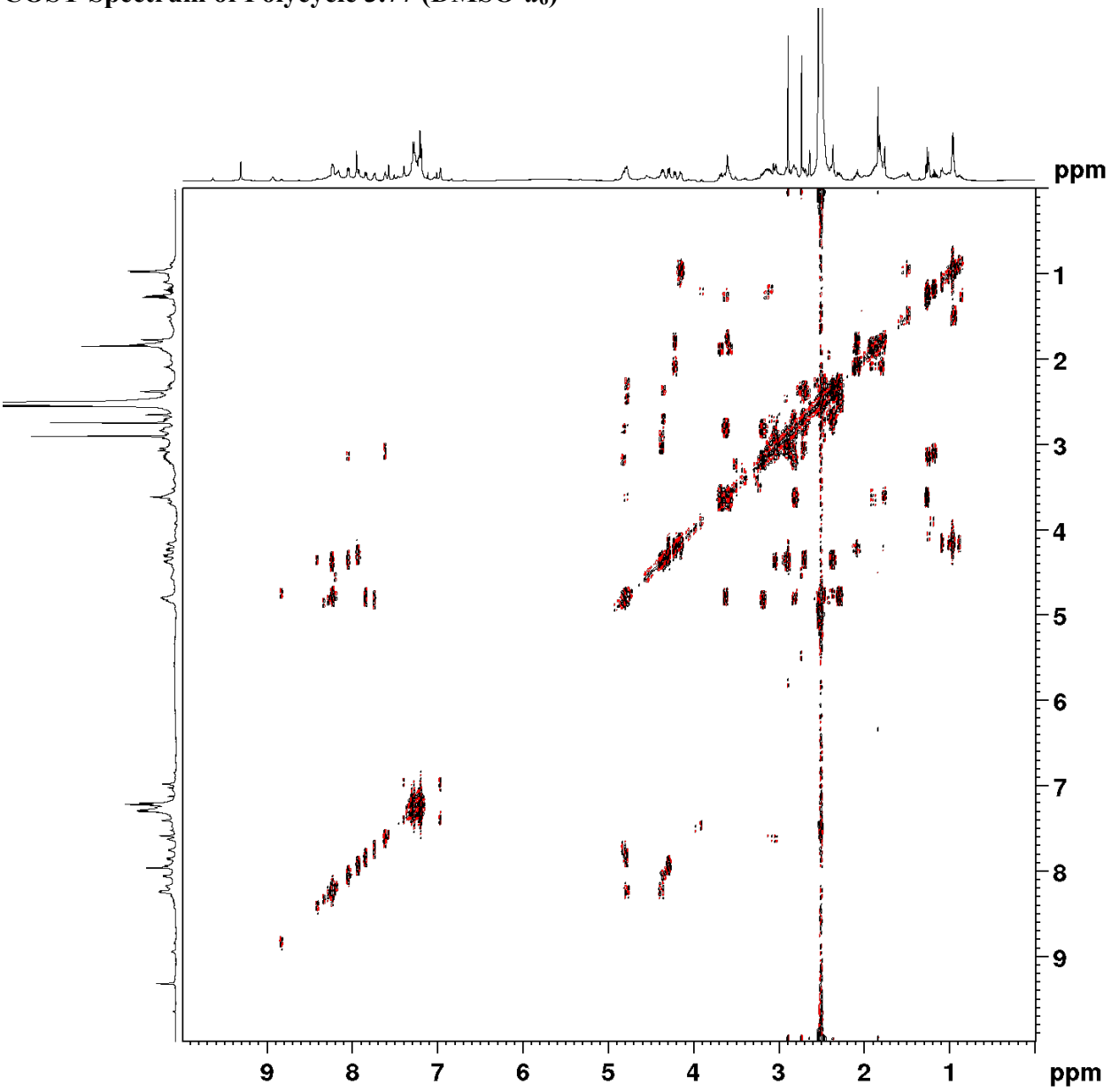
HMBC Spectrum of Polycycle 3.77 (DMSO-d_6)



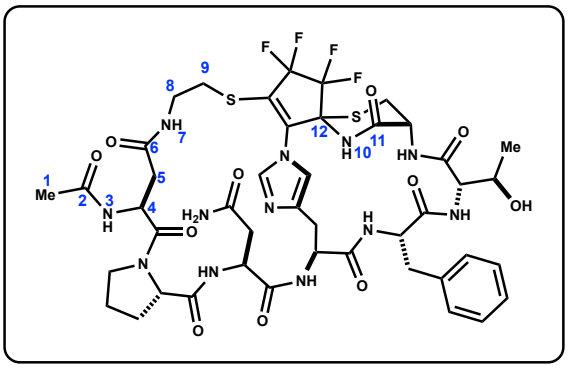
HSQC Spectrum of Polycycle 3.77 (DMSO-d_6)



COSY Spectrum of Polycycle 3.77 (DMSO-d_6)

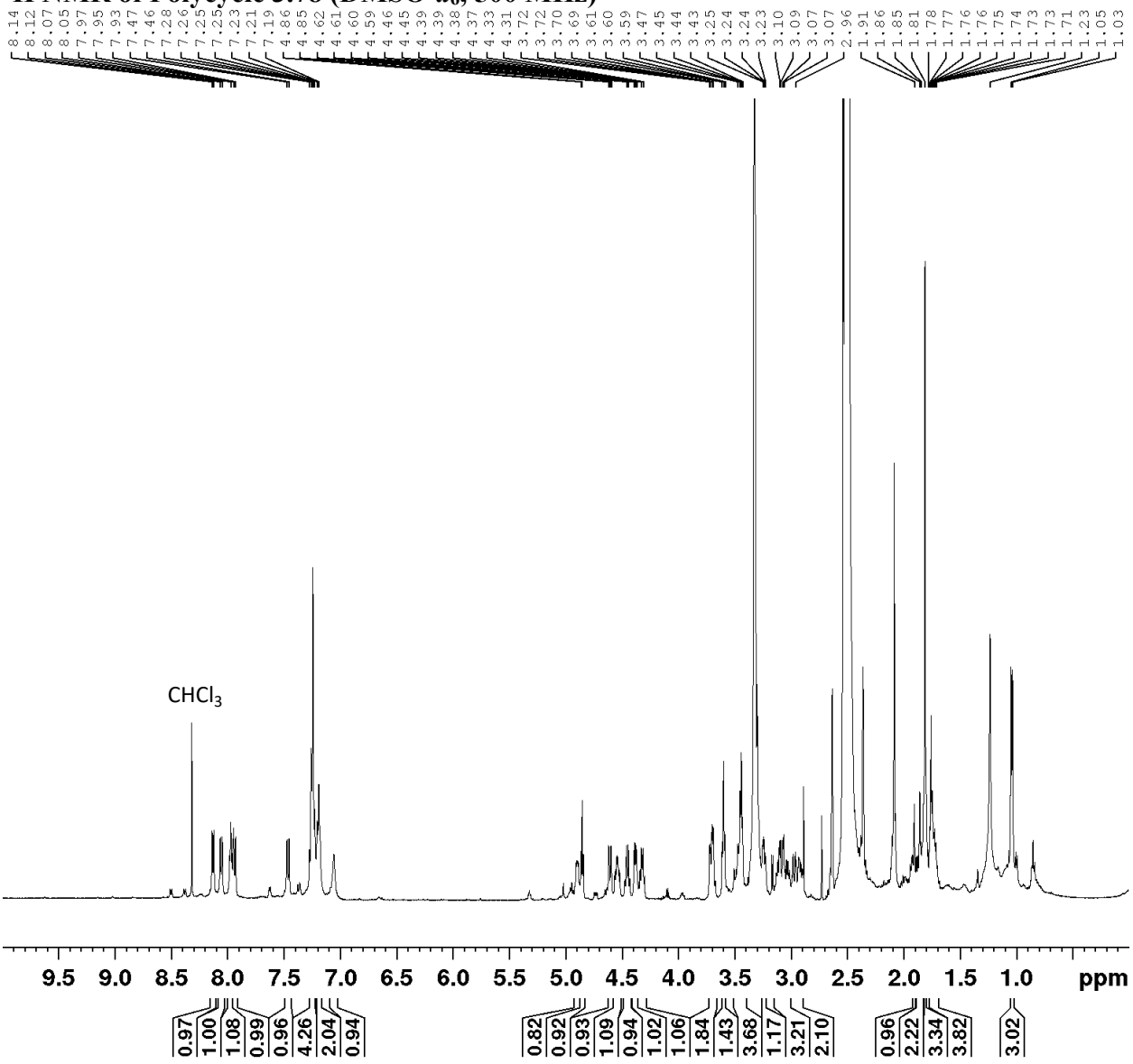


Polycycle 3.77 (DMSO-d_6)

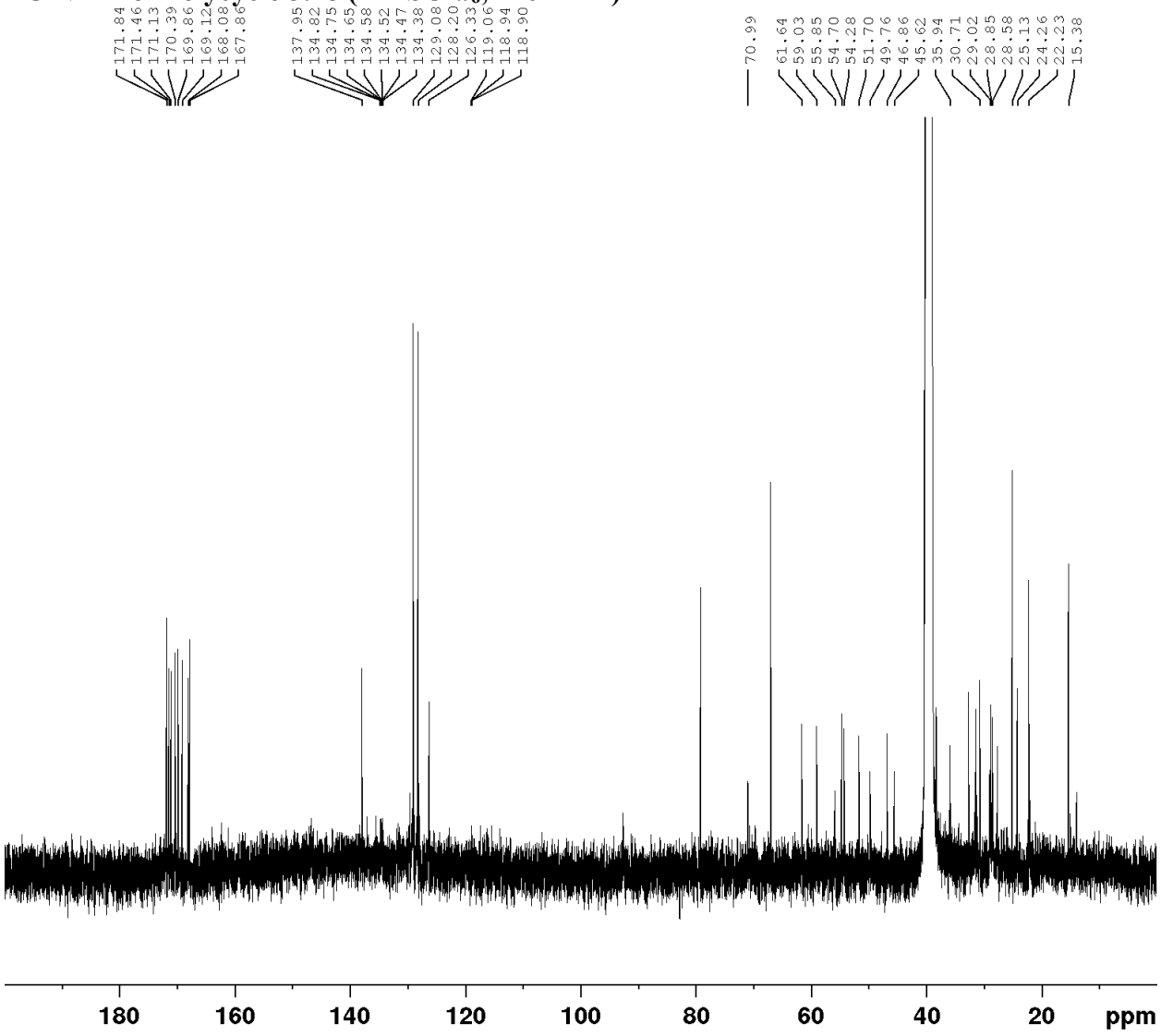


	13C	1H	Key Correlation
1		1.83 (s, 3H)	HSQC
2	168.93	-	1->2 HMBC
3	-	8.22 (m, 1H) overlap	2->3 HMBC
4	47.82	4.78 (m, 1H) overlap	3->4 COSY, HSQC
5	38.24	2.69 (dd, J = 10.3, 4.6 Hz, 1H), 2.29 (dd, J = 15.2, 9.0 Hz, 1H)	4->5 COSY, HSQC
6	171.85	-	5->6 HMBC
7	-	7.61 (t, J = 4.8 Hz, 1H)	6->7 HMBC
8	37.35	3.10 (m, 1H), 3.04 (m, 1H) overlap	7->8 COSY, HSQC
9	28.97	2.07 (m, 1H), 1.77 (m, 1H) overlap	8->9 HMBC, HSQC
10	-	9.31 (s, 1H)	11->10 HMBC
11	168.36	-	10->11 HMBC
12	67.3–67.5 (m, 1C)	-	10->12 HMBC

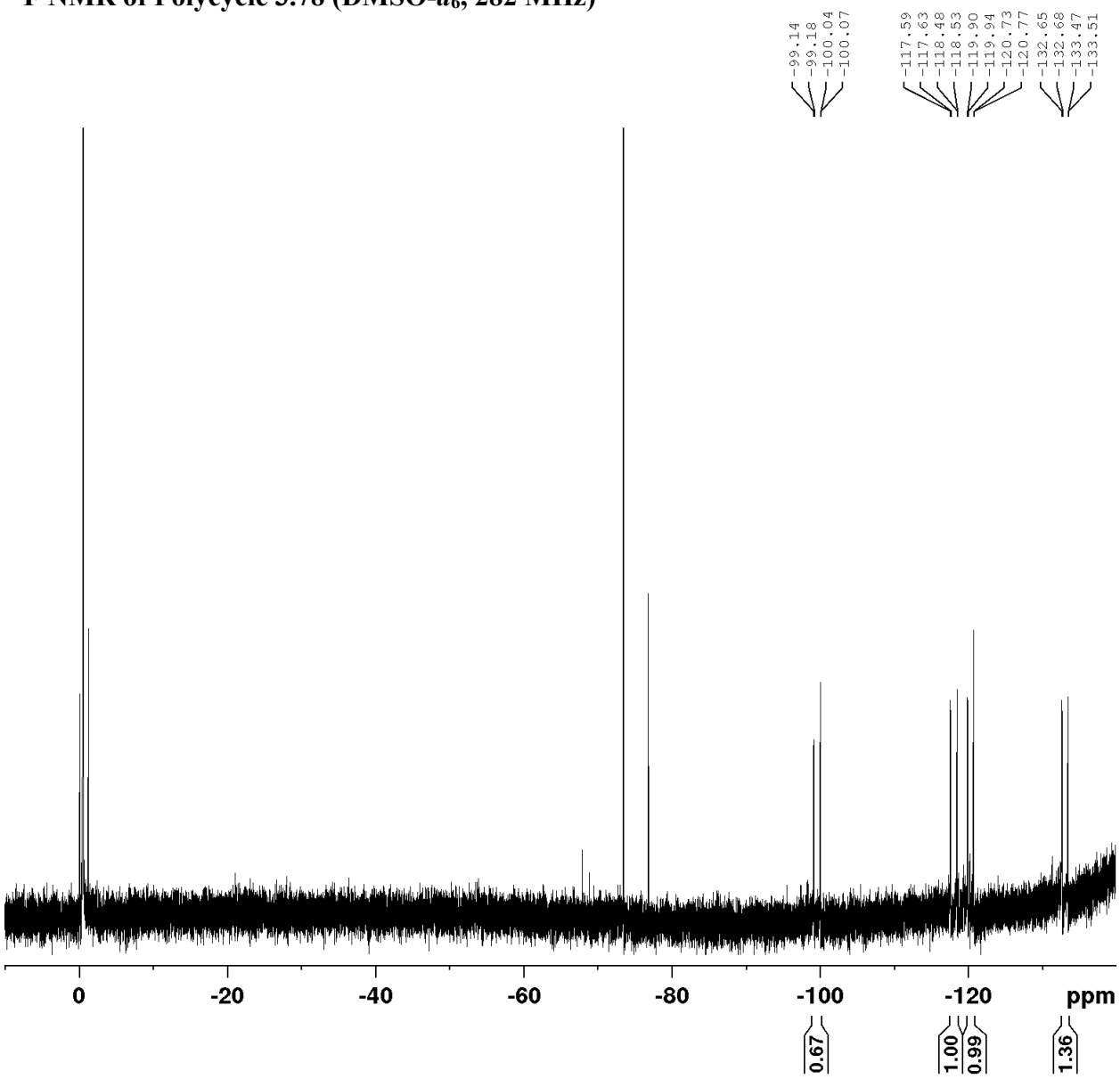
^{1}H NMR of Polycycle 3.78 (DMSO-d_6, 500 MHz)



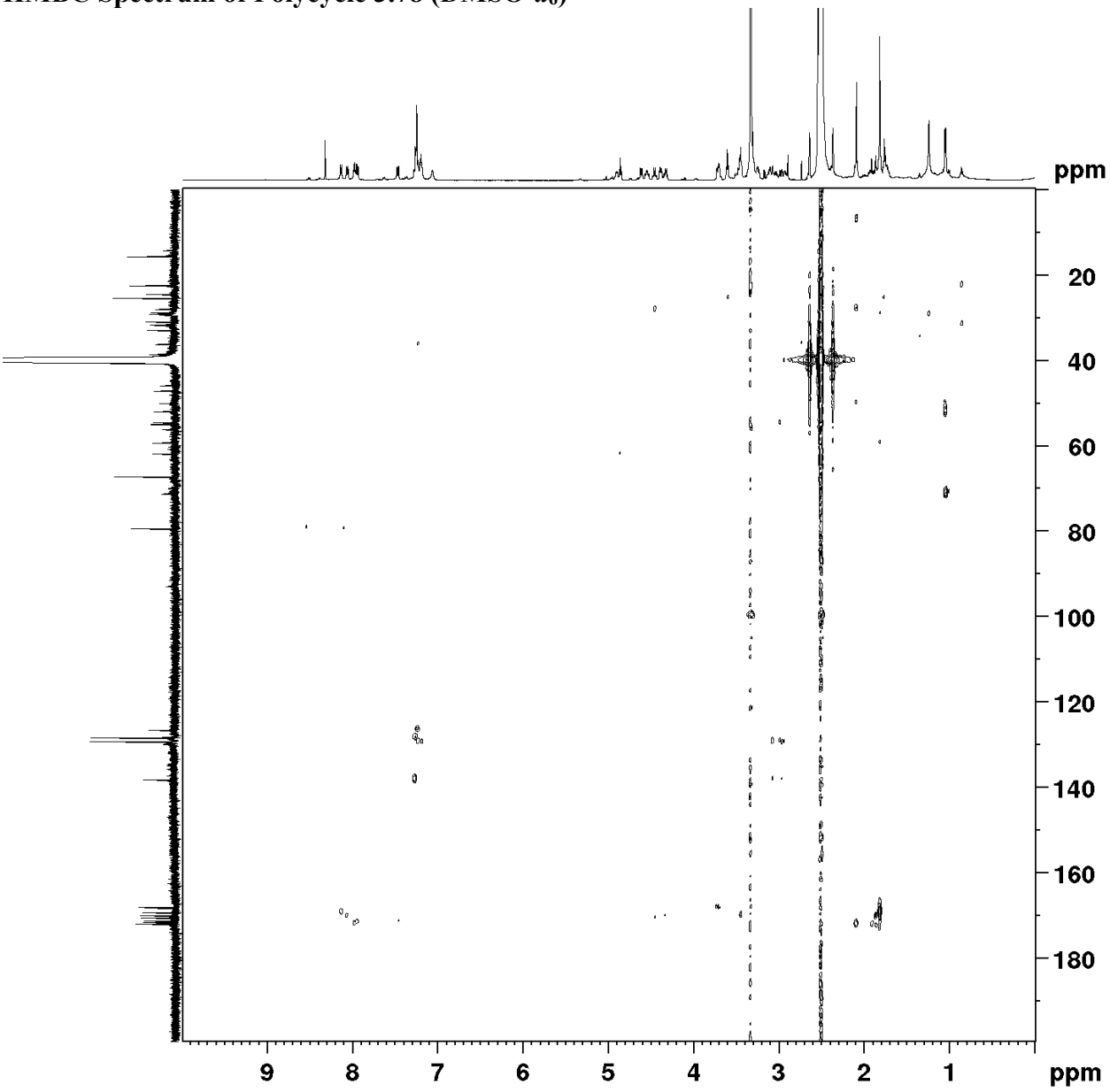
^{13}C NMR of Polycycle 3.78 (DMSO-d_6, 126 MHz)



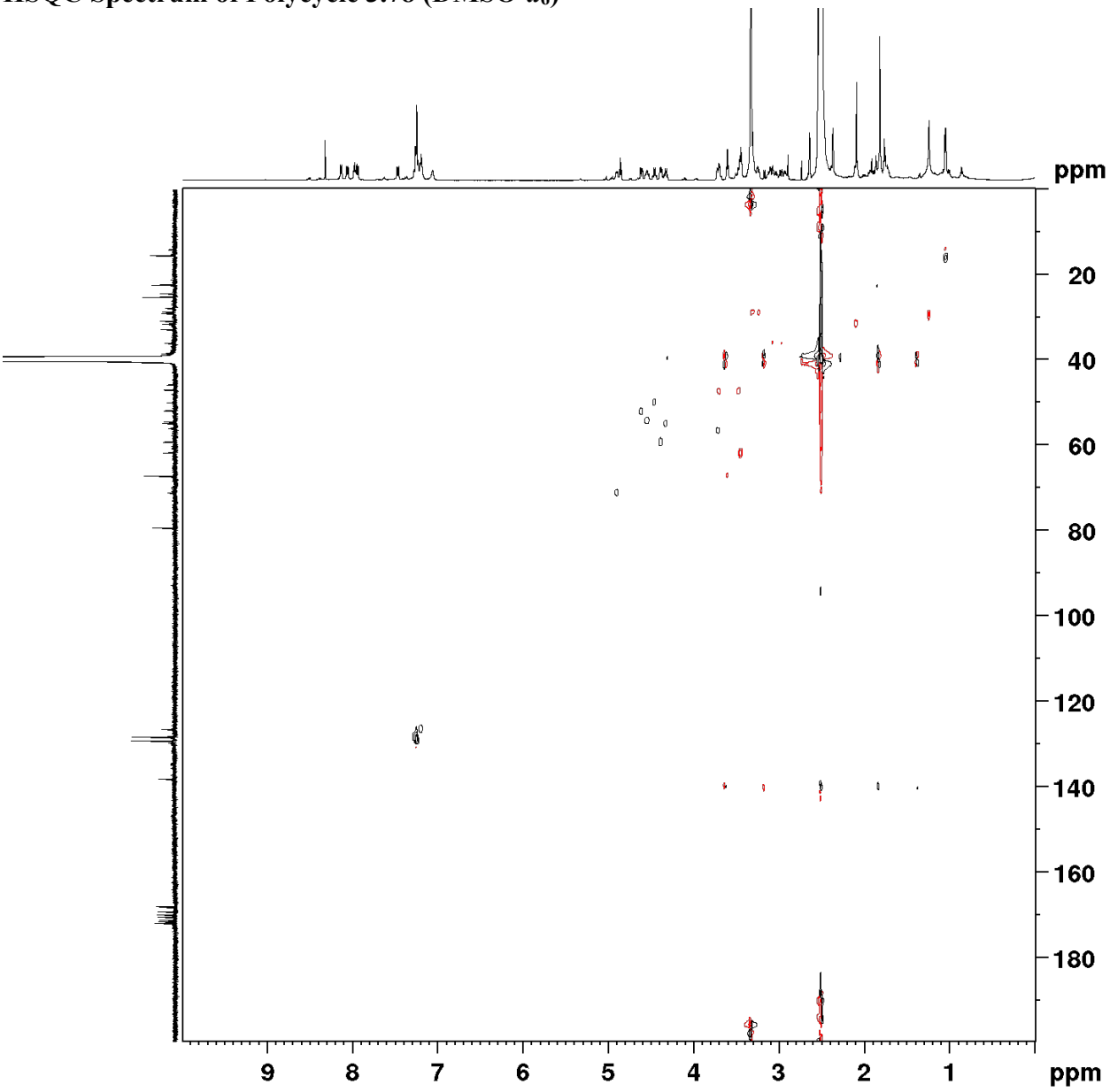
^{19}F NMR of Polycycle 3.78 (DMSO-d_6, 282 MHz)



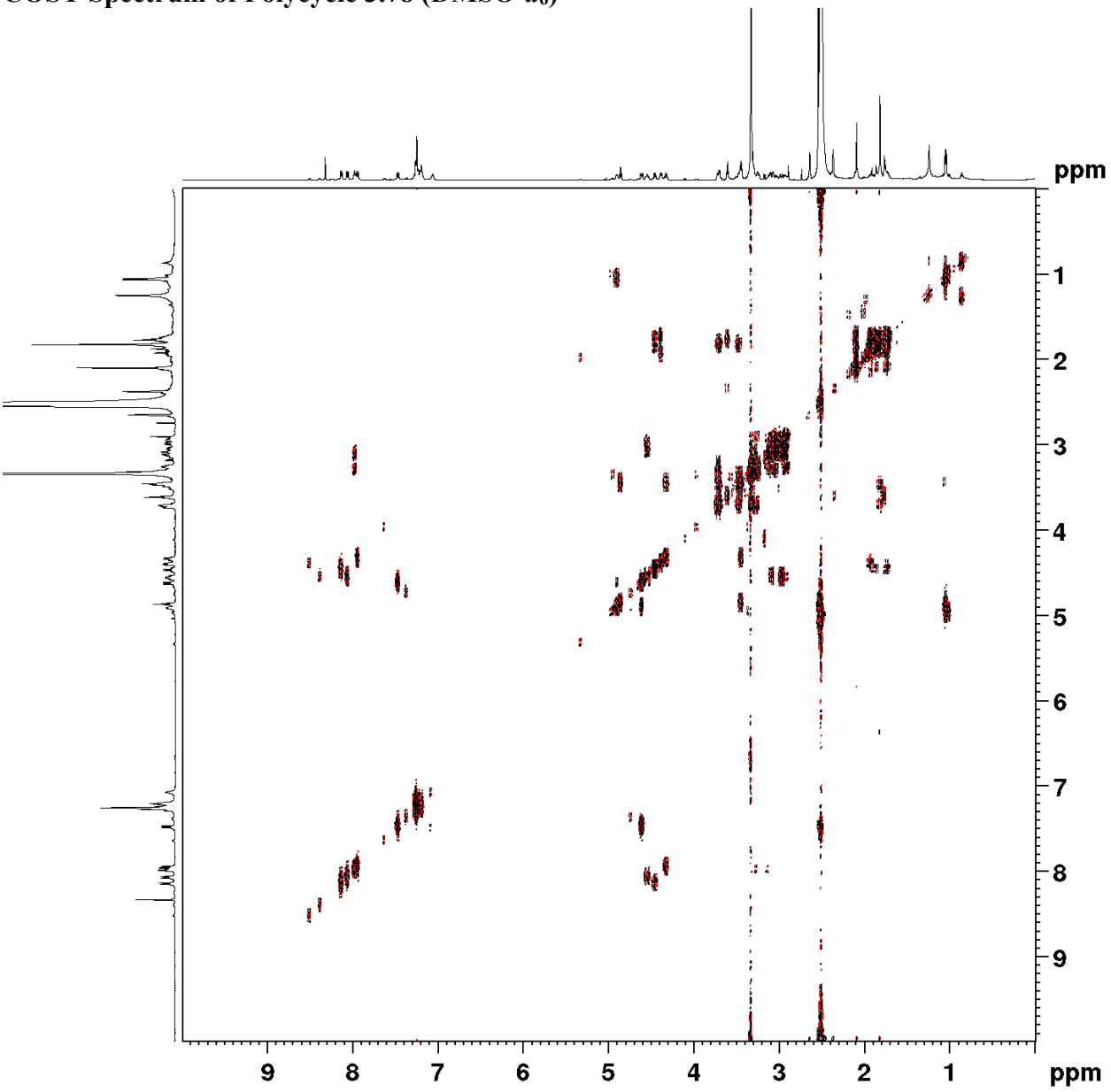
HMBC Spectrum of Polycycle 3.78 (DMSO-d_6)



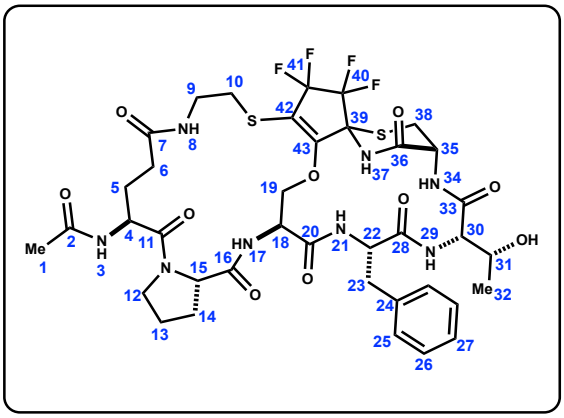
HSQC Spectrum of Polycycle 3.78 (DMSO-d_6)



COSY Spectrum of Polycycle 3.78 (DMSO-d_6)

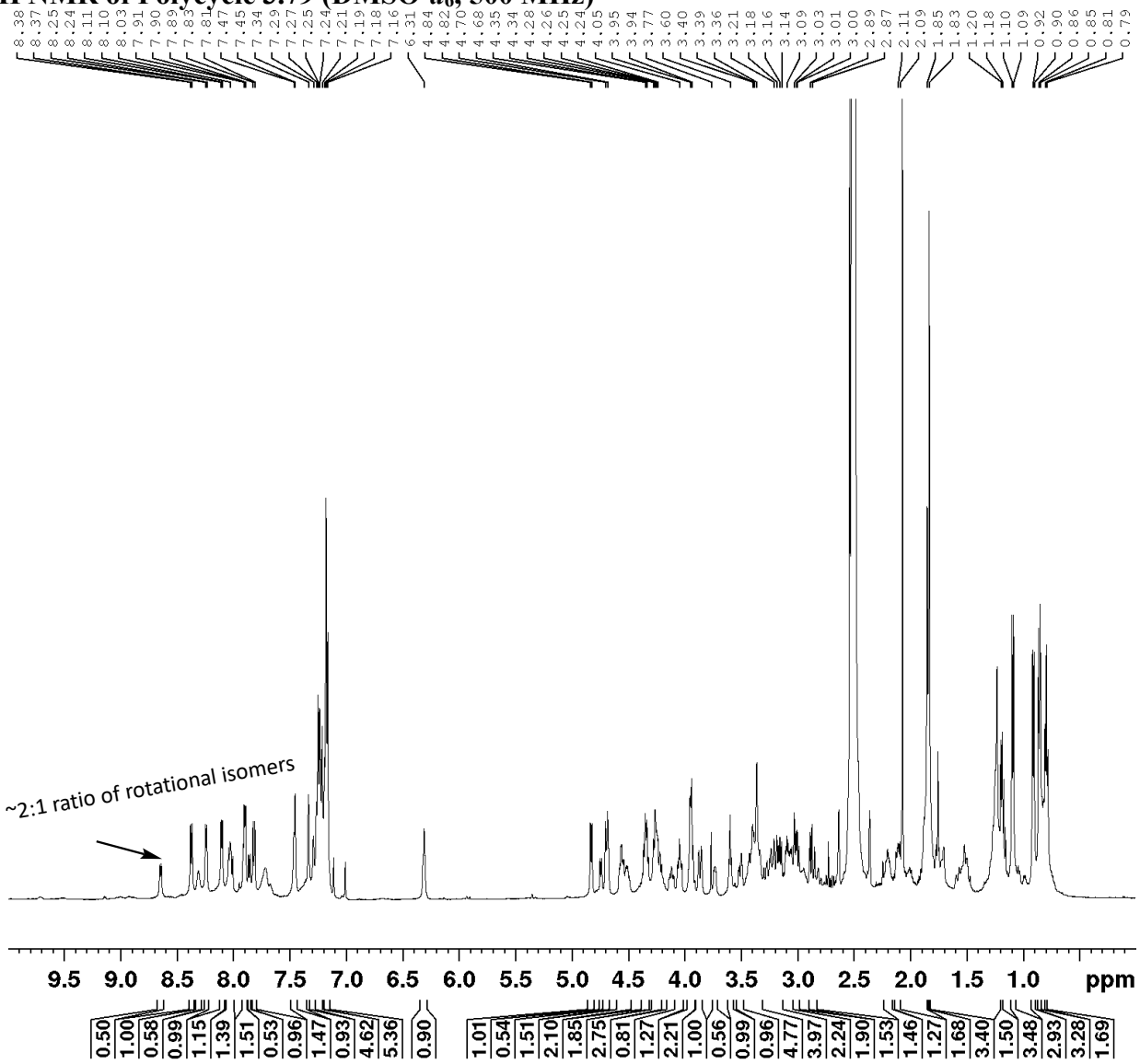


Polycycle 3.78 (DMSO-d_6)

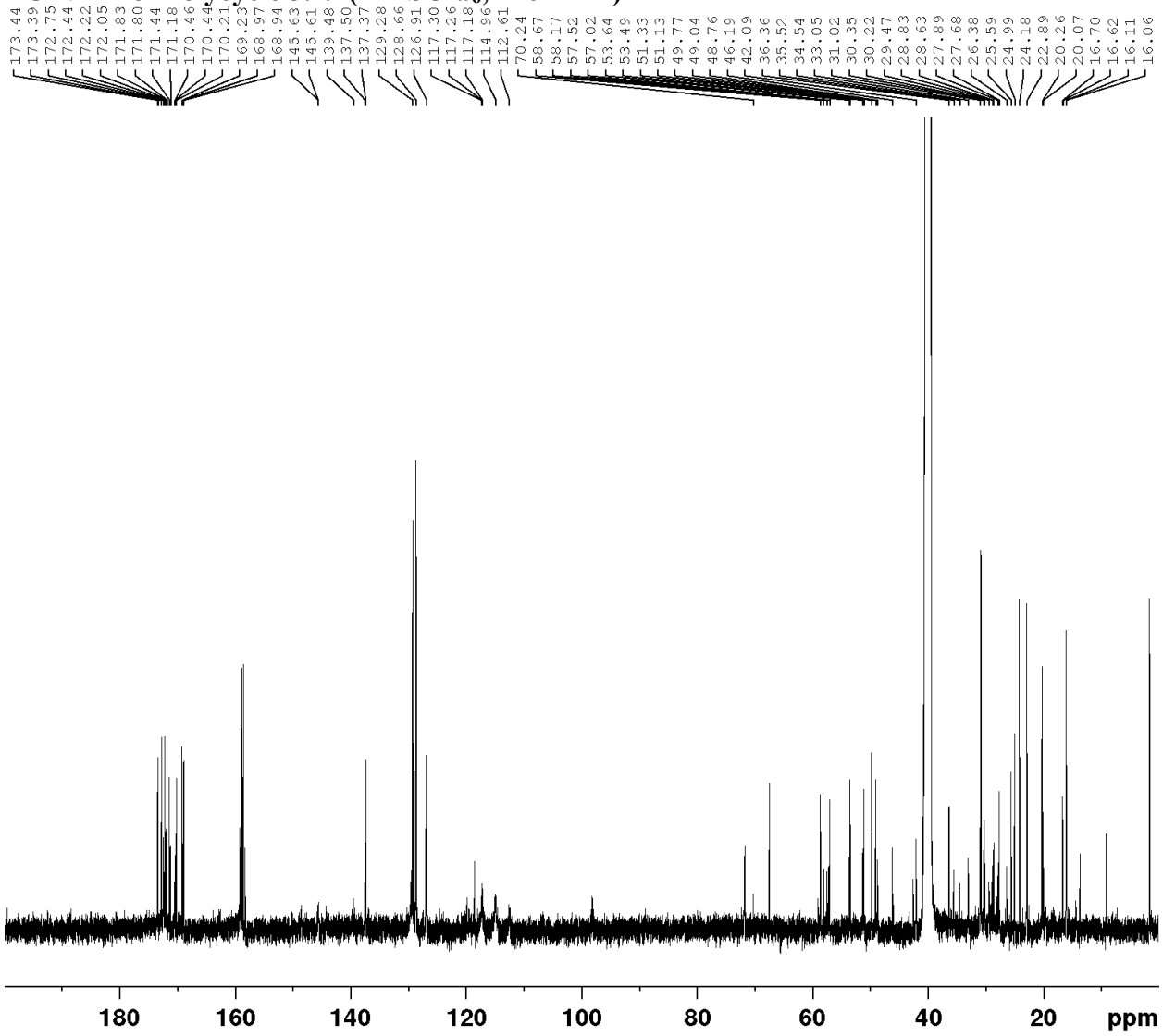


	13C	1H	Key Correlation
1	25.13	1.81 (s, 3H)	HSQC
2	169.13	-	1->2 HMBC
3	-	8.13 (d, J = 7.5 Hz, 1H)	2->3 HMBC
4	49.77	4.45 (q, J = 7.3 Hz , 1H)	3->4 COSY, HSQC
5	29.02	1.86 (m, 1H), 1.72 (overlap, 1H), overlap	4->5 COSY, HSQC
6	30.71	3.09 (m, 1H), 2.97 (m, 1H) overlap	7->6 HMBC, HSQC
7	171.86	-	8->7 HMBC
8	-	7.93 (t, J = 5.6 Hz, 1H)	7->8 HMBC
9	28.58	3.10 (m, 1H), 3.25 (m, 1H) overlap	8->9 COSY, HSQC
10	28.86	1.86 (m, 1H), 1.71 (m, 1H) overlap	8->9 HMBC, HSQC
11	170.39	-	4->11 HMBC
12	46.86	3.69 (m, 1H), 3.47 (m, 1H) overlap	14->12 HMBC, HSQC
13	22.23	1.84 (m, 1H), 1.76 (m, 1H) overlap	14->13 COSY, HSQC
14	45.61	3.70 (m, 1H), 3.45 (m, 1H) overlap	15->14 HMBC, HSQC
15	55.86	4.85 (t, J = 5.5 Hz, 1H)	16->15 HMBC, HSQC
16	171.47	-	17->16 HMBC
17	-	7.94 (d, J = 8.2 Hz,1H)	18->17 HMBC
18	54.70	4.32 (m, 1H)	20->18 HMBC
19	61.64	3.44 (m, 2H)	18->19 COSY, HSQC
20	169.87	-	21->20 HMBC
21	-	8.06 (d, J = 8.4 Hz, 1H)	22->21 COSY
22	54.28	4.54 (td, J = 8.8, 3.3 Hz, 1H)	23->22 COSY, HSQC
23	35.94	3.08 (m, 1H), 2.96 (m, 1H)	24->23 HMBC, HSQC
24	137.96	-	25, 26 ->24 HMBC
25	129.08	7.28–7.23 (m, 2H)	HSQC
26	128.20	7.28–7.23(m, 2H)	HSQC
27	126.33	7.21–7.19 (m, 1H)	HSQC
28	171.13	-	22->28 HMBC
29	-	7.47 (d, J = 9.3 Hz, 1H)	30->31 COSY
30	51.70	4.61 (dd, J = 9.4, 3.1 Hz, 1H)	31->30 COSY, HSQC
31	70.99	4.90 (dd, J = 6.3, 3.2 Hz, 1H)	32->31 COSY, HSQC
32	15.36	1.04 (d, J = 6.4 Hz, 3H)	HSQC
33	168.08	-	30->33 HMBC
34	-	-	-
35	59.03	4.38 (dd, J = 8.2, 3.4 Hz, 1H)	36->35 COSY, HSQC
36	167.08	-	35->36 HMBC
37	-	7.06 (s, 1H)	-
38	24.26	1.90 (overlap, 1H), 1.73 (overlap, 1H)	35->38 COSY, HMBC, HSQC
39	70.7–70.6 (m, 1C)	-	-
40	119.1–118.9 (m, 1C)	-	-
41	119.1–118.9 (m, 1C)	-	-
42	134.9–134.6 (m, 1C)	-	-
43	134.6–131.4 (m, 1C)	-	-

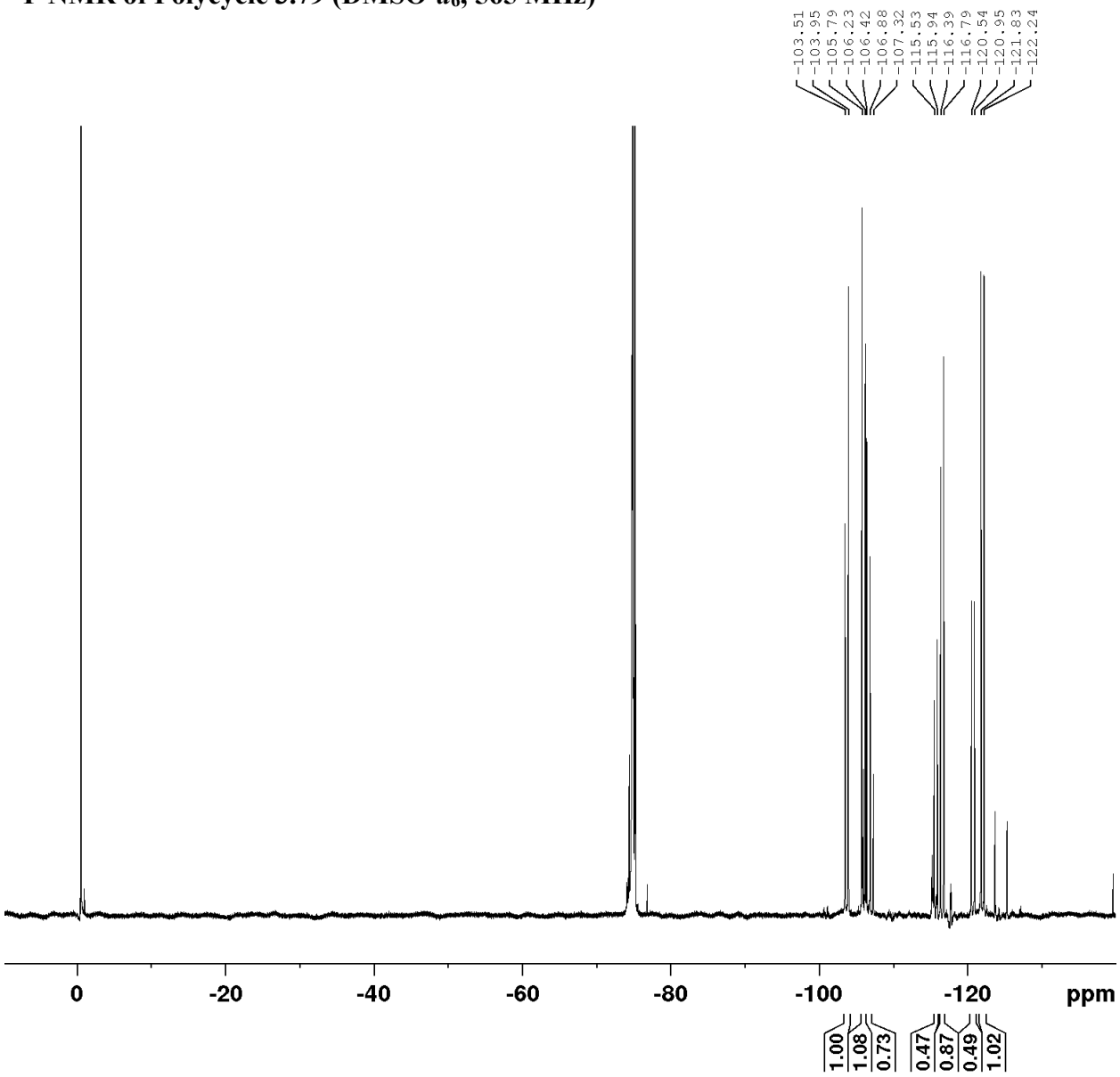
^{1}H NMR of Polycycle 3.79 (DMSO-d_6, 500 MHz)



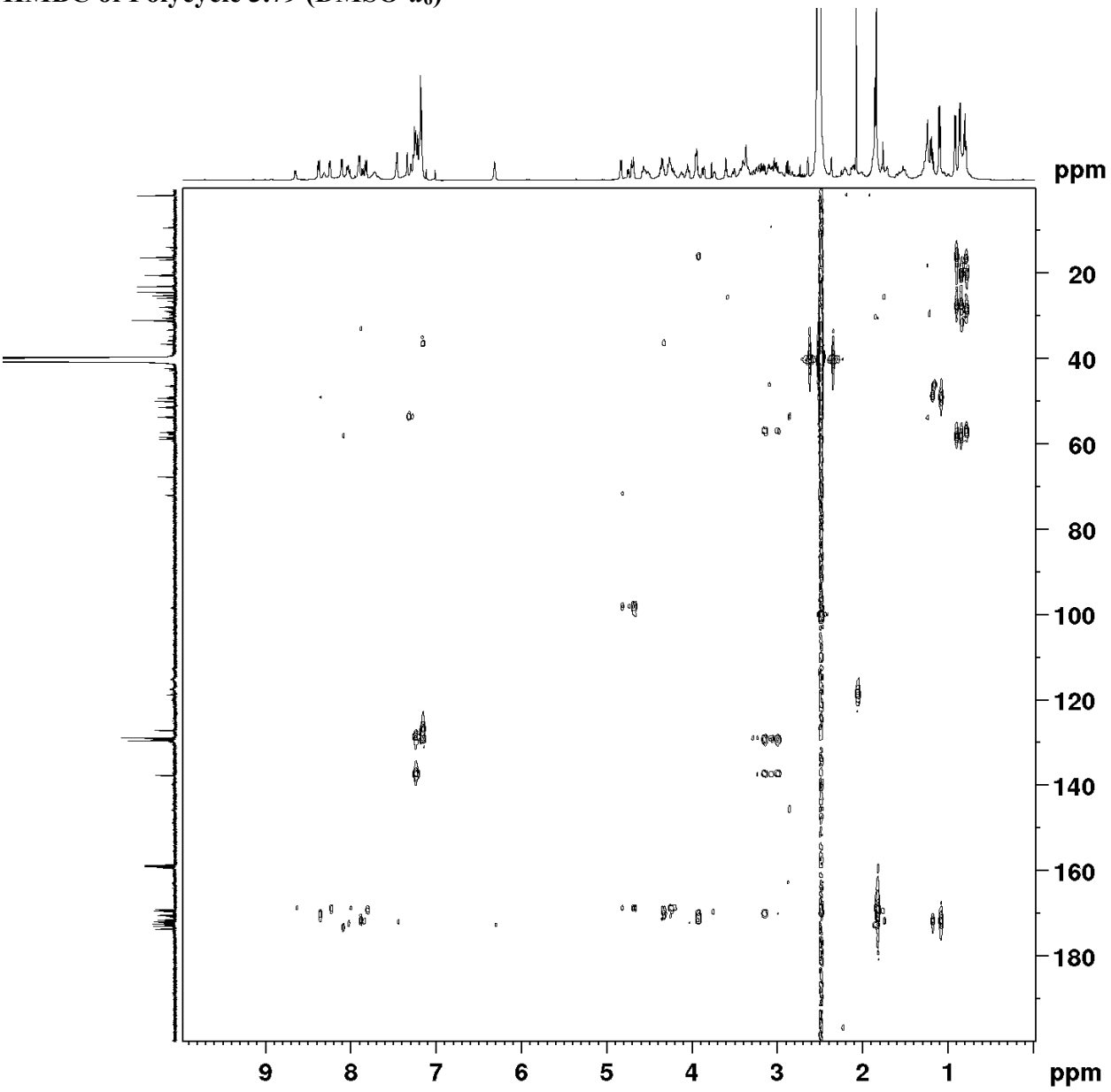
^{13}C NMR of Polycycle 3.79 (DMSO-d_6, 126 MHz)



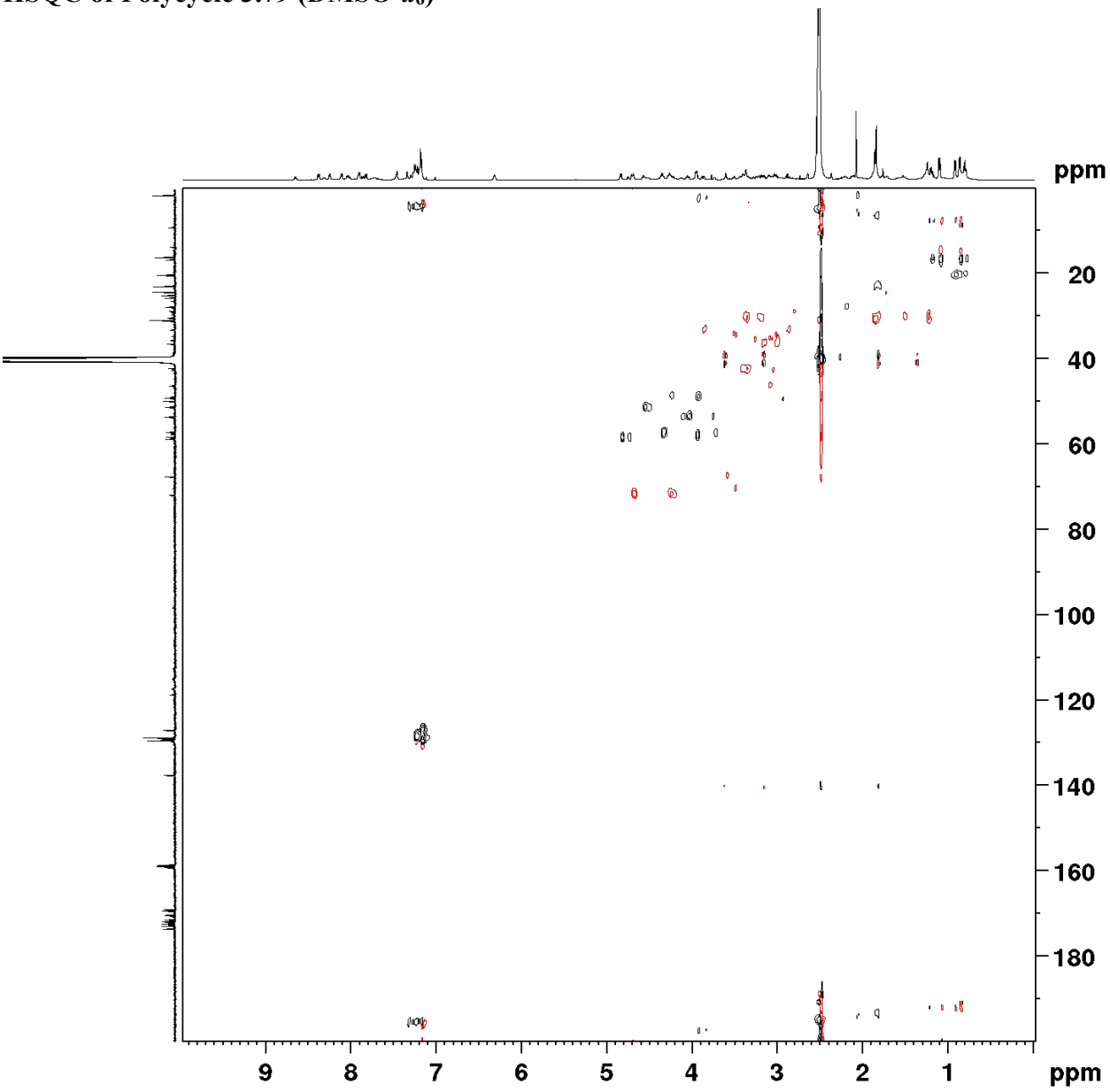
^{19}F NMR of Polycycle 3.79 (DMSO-d_6, 565 MHz)



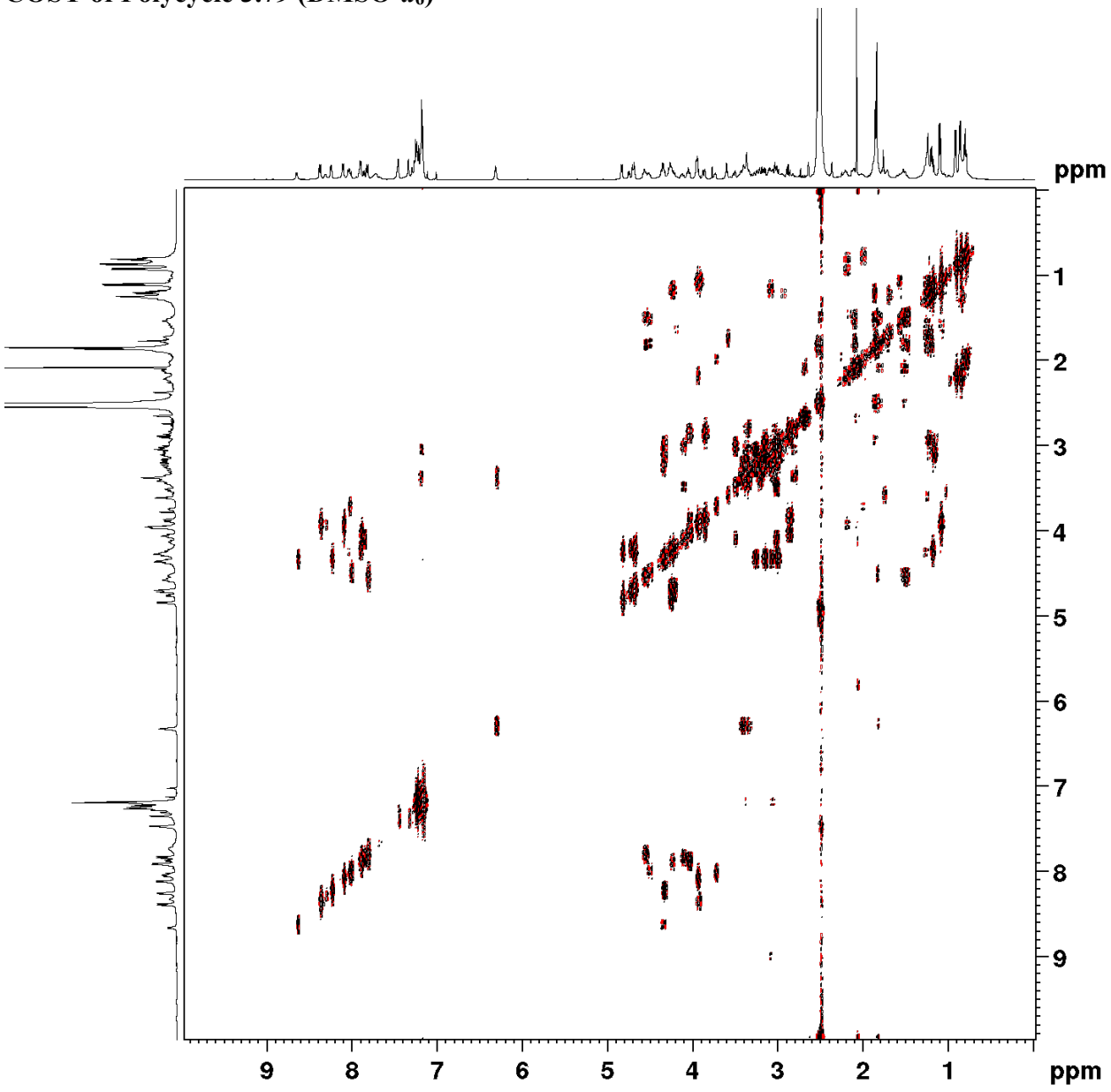
HMBC of Polycycle 3.79 (DMSO-d_6)



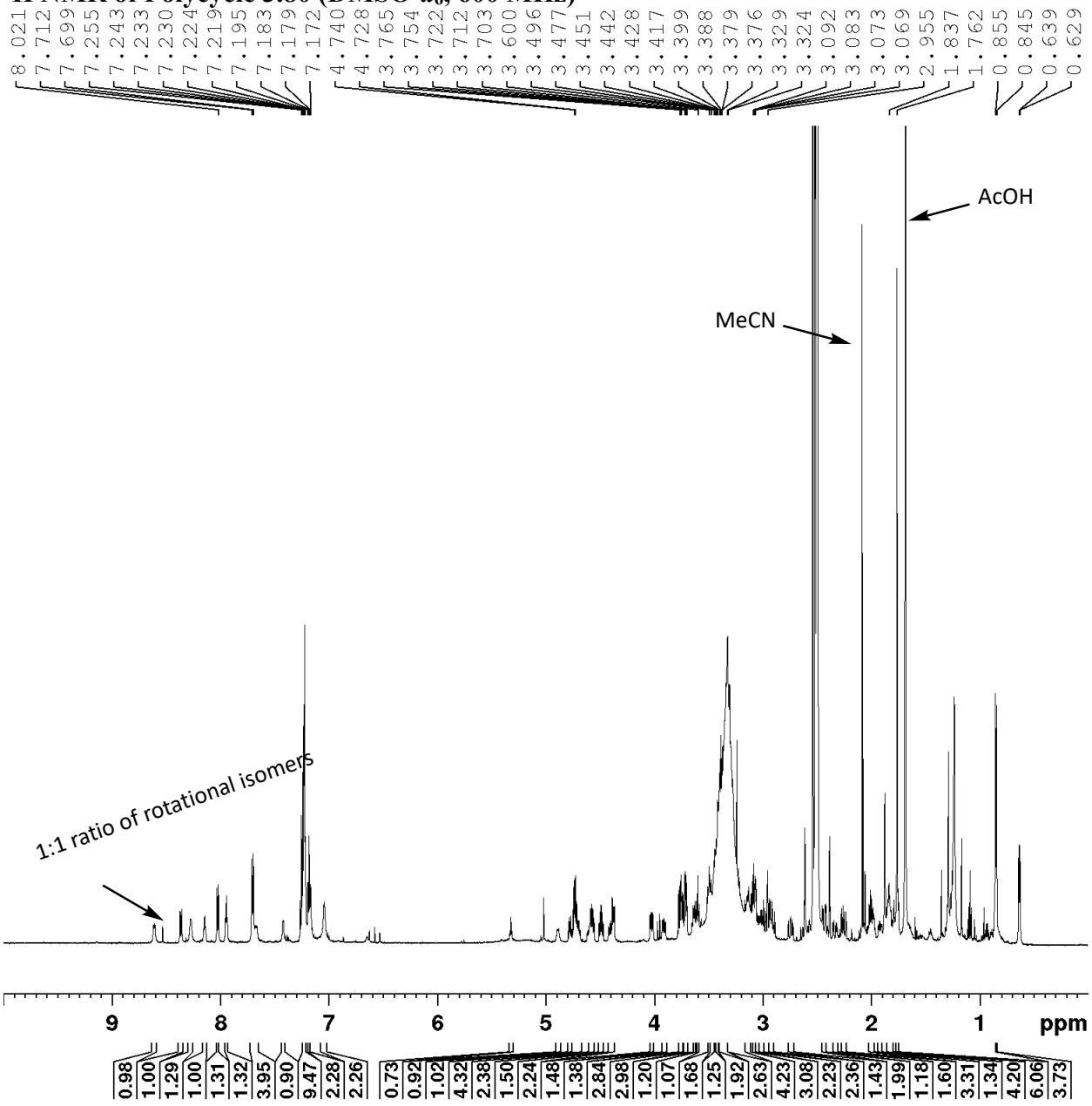
HSQC of Polycycle 3.79 (DMSO-d_6)



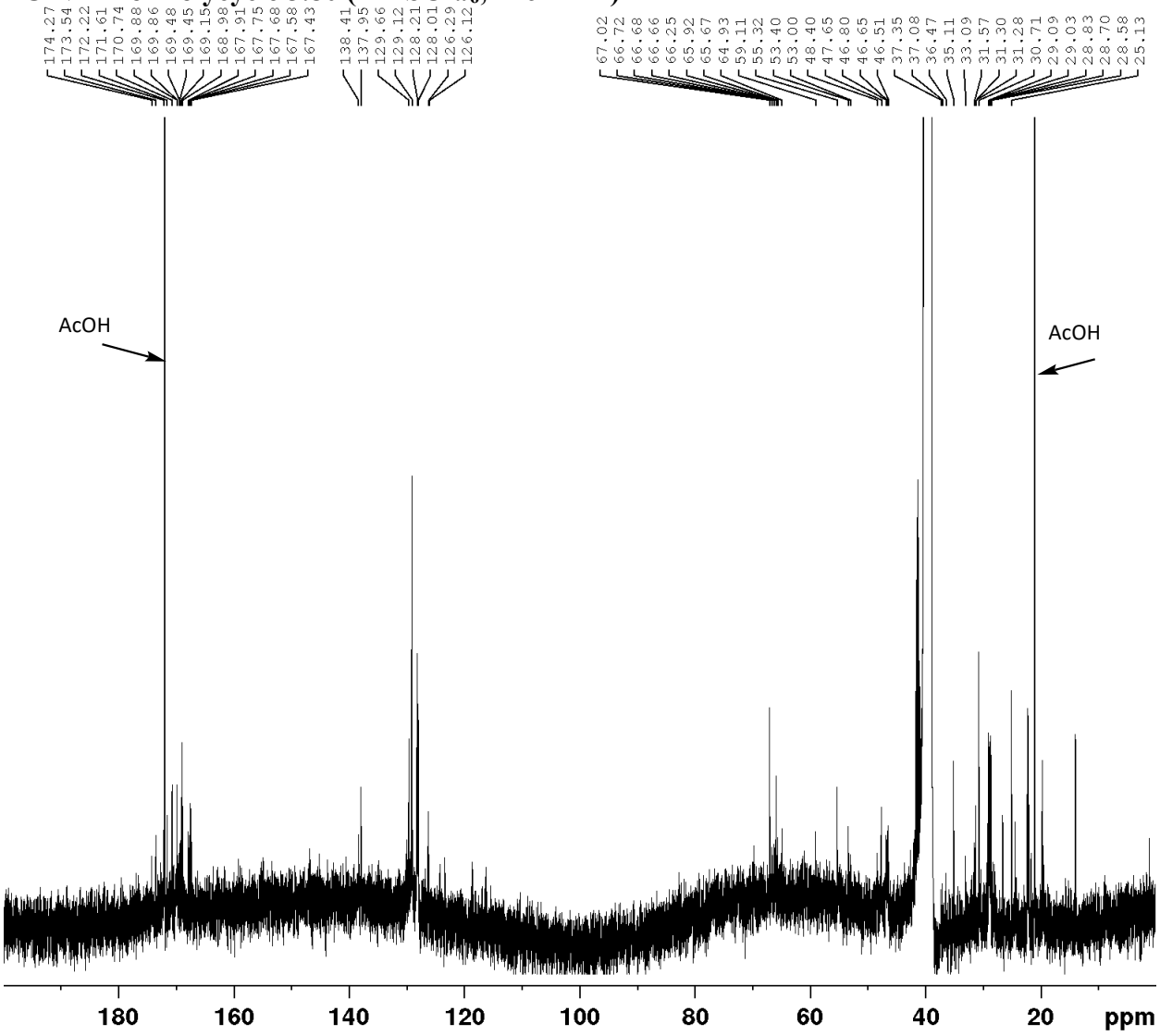
COSY of Polycycle 3.79 (DMSO-d_6)



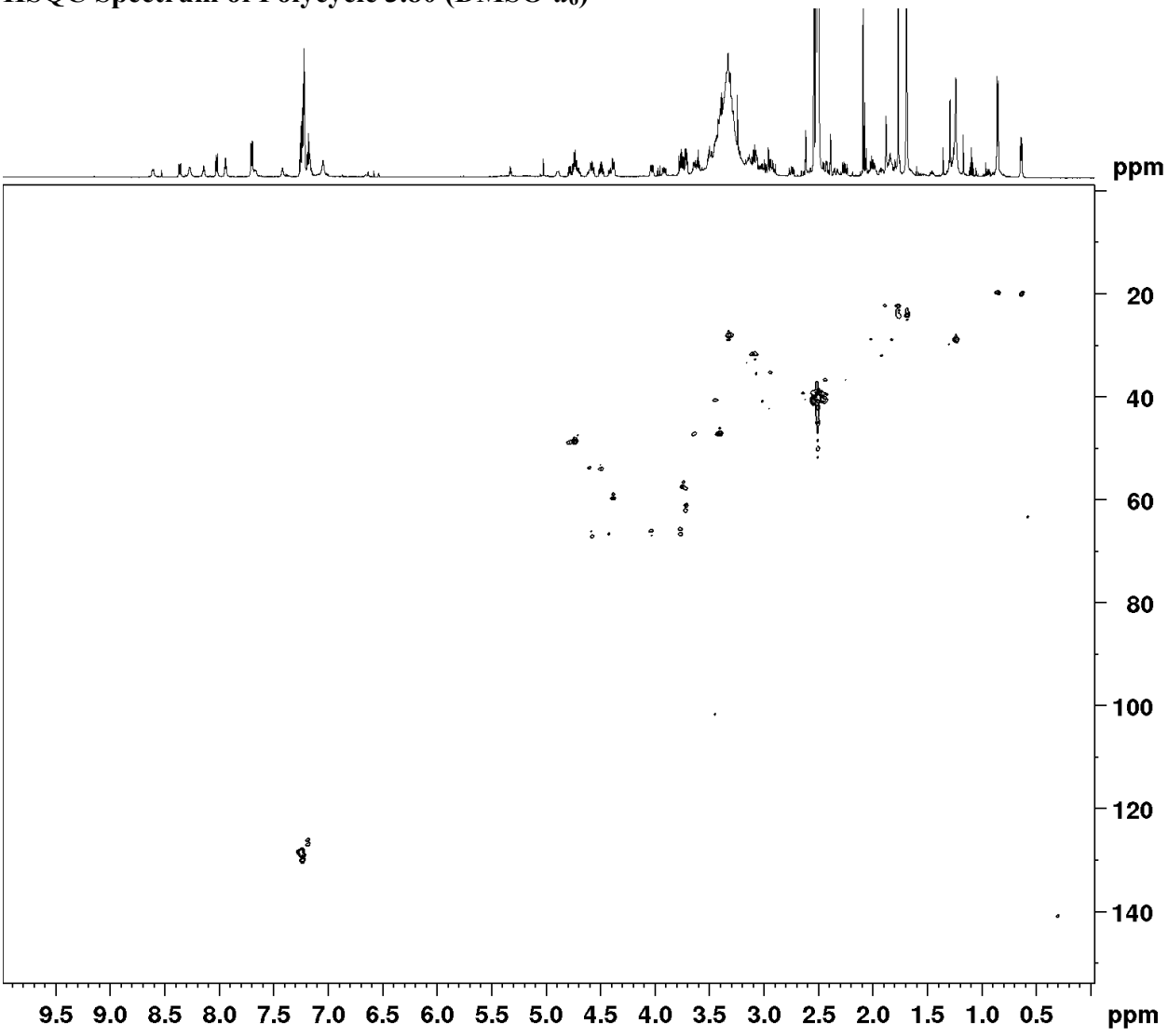
^{1}H NMR of Polycycle 3.80 (DMSO-d_6, 600 MHz)



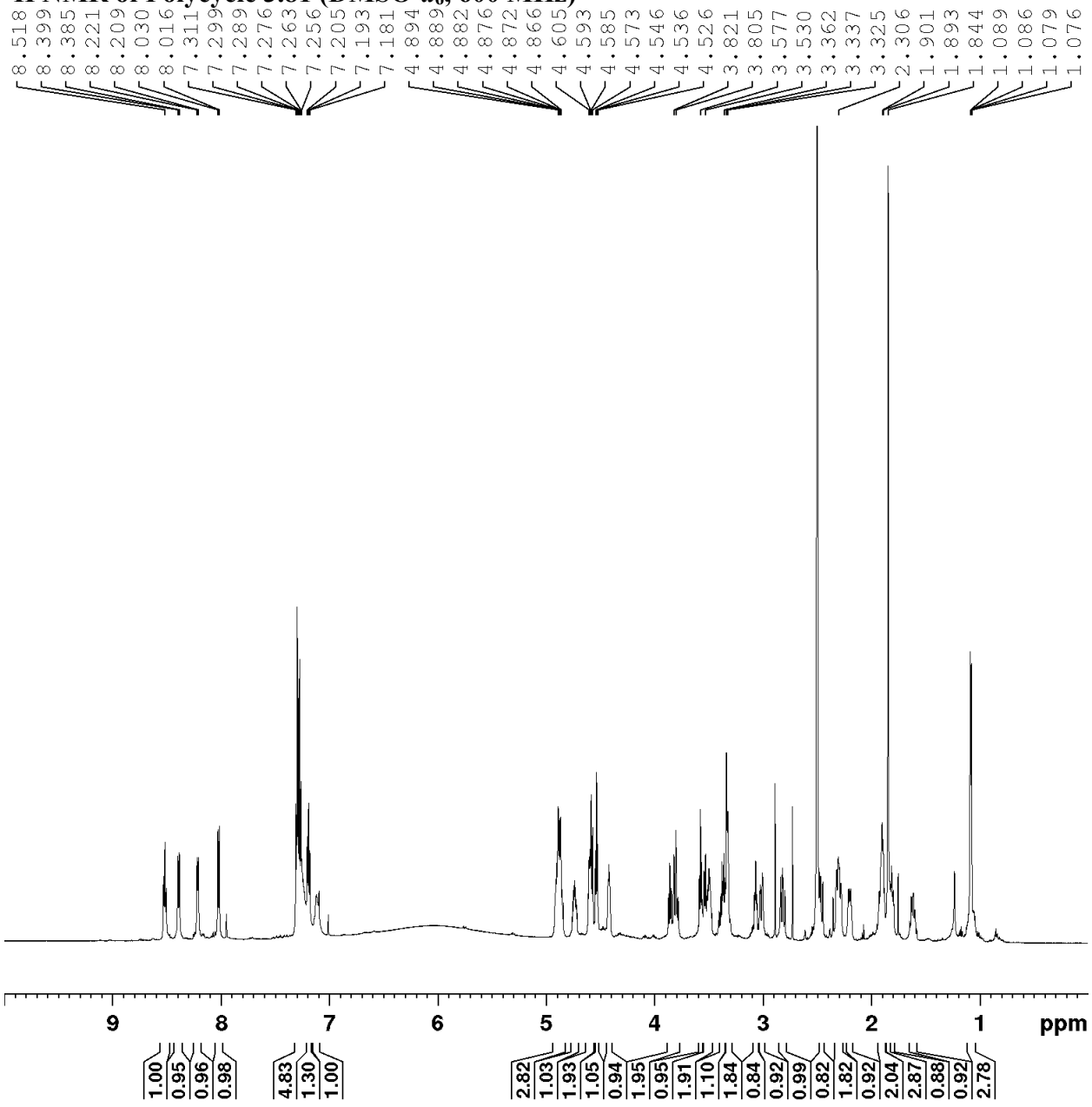
^{13}C NMR of Polycycle 3.80 (DMSO-d_6, 126 MHz)



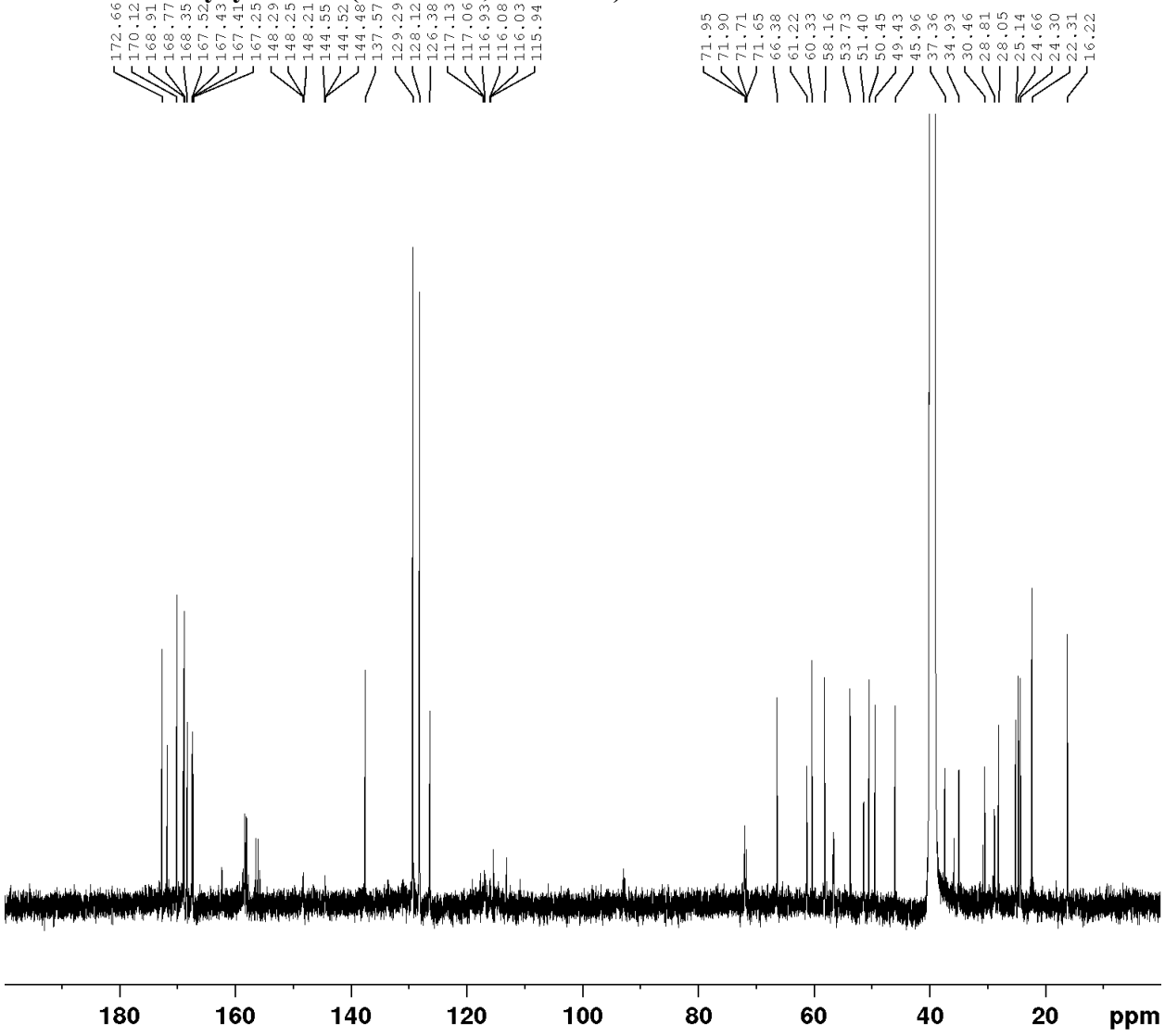
HSQC Spectrum of Polycycle 3.80 (DMSO-d_6)



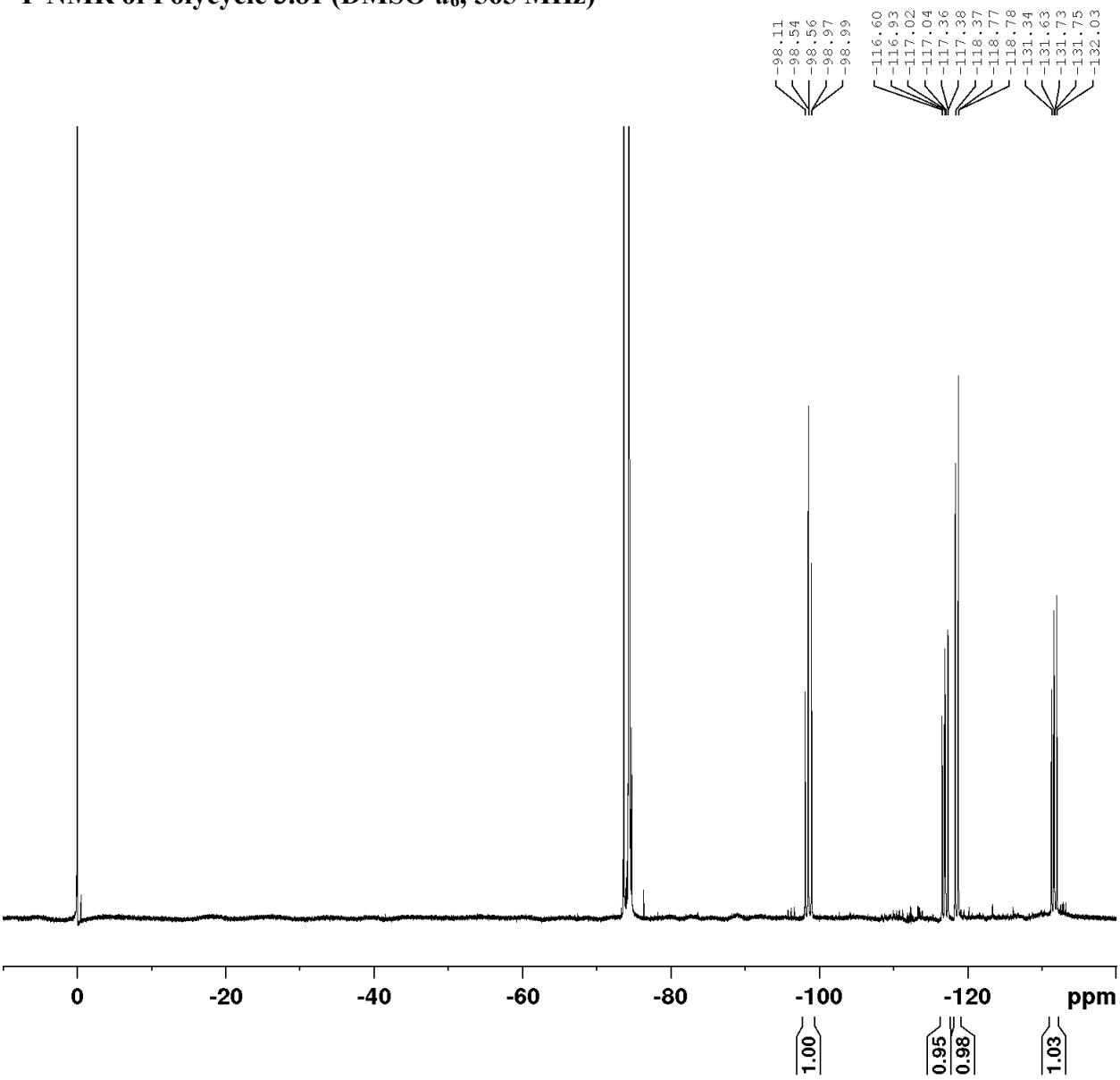
^{1}H NMR of Polycycle 3.81 (DMSO-d_6, 600 MHz)



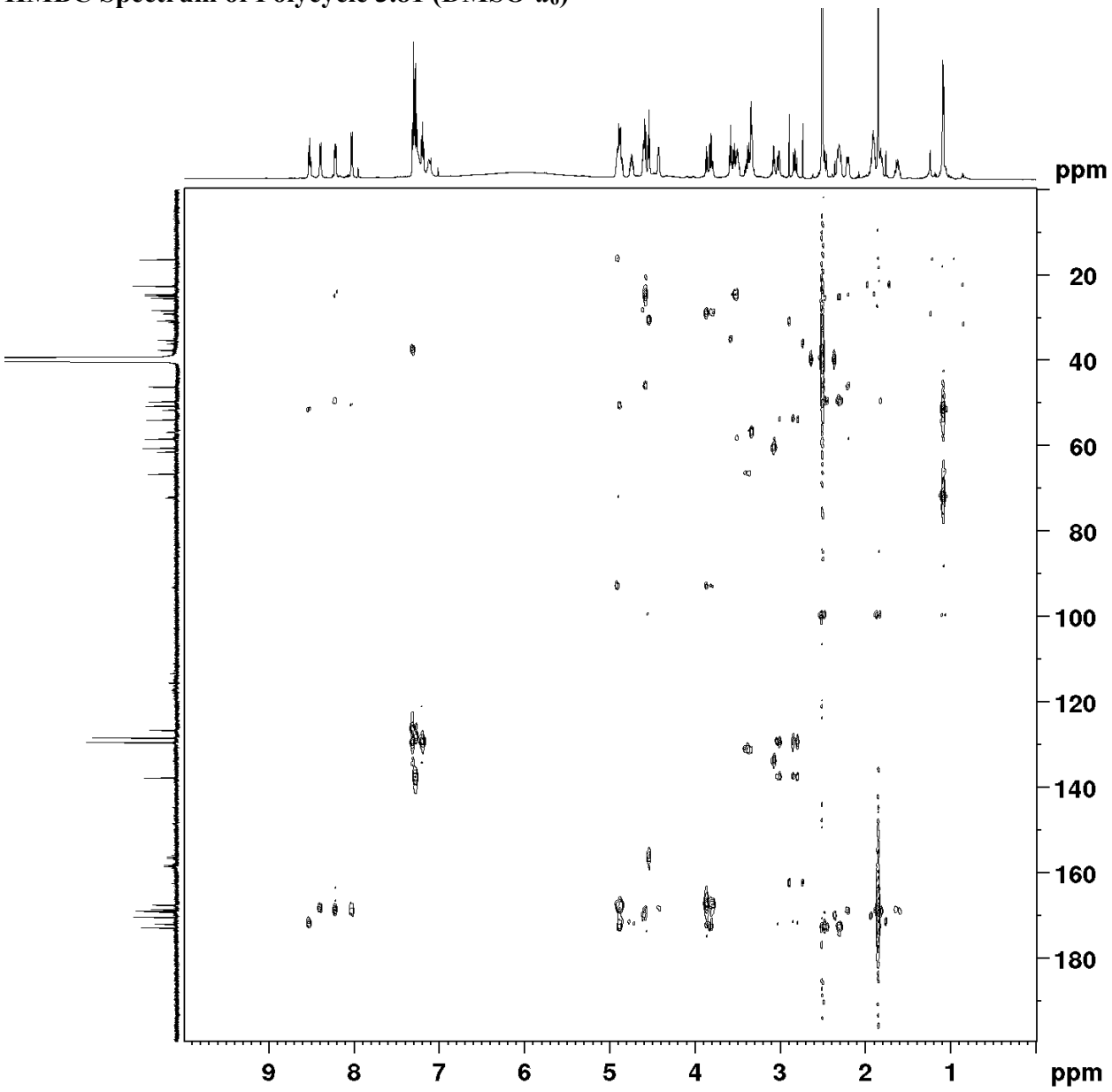
^{13}C NMR of Polycycle 3.81 (DMSO-d_6, 600 MHz)



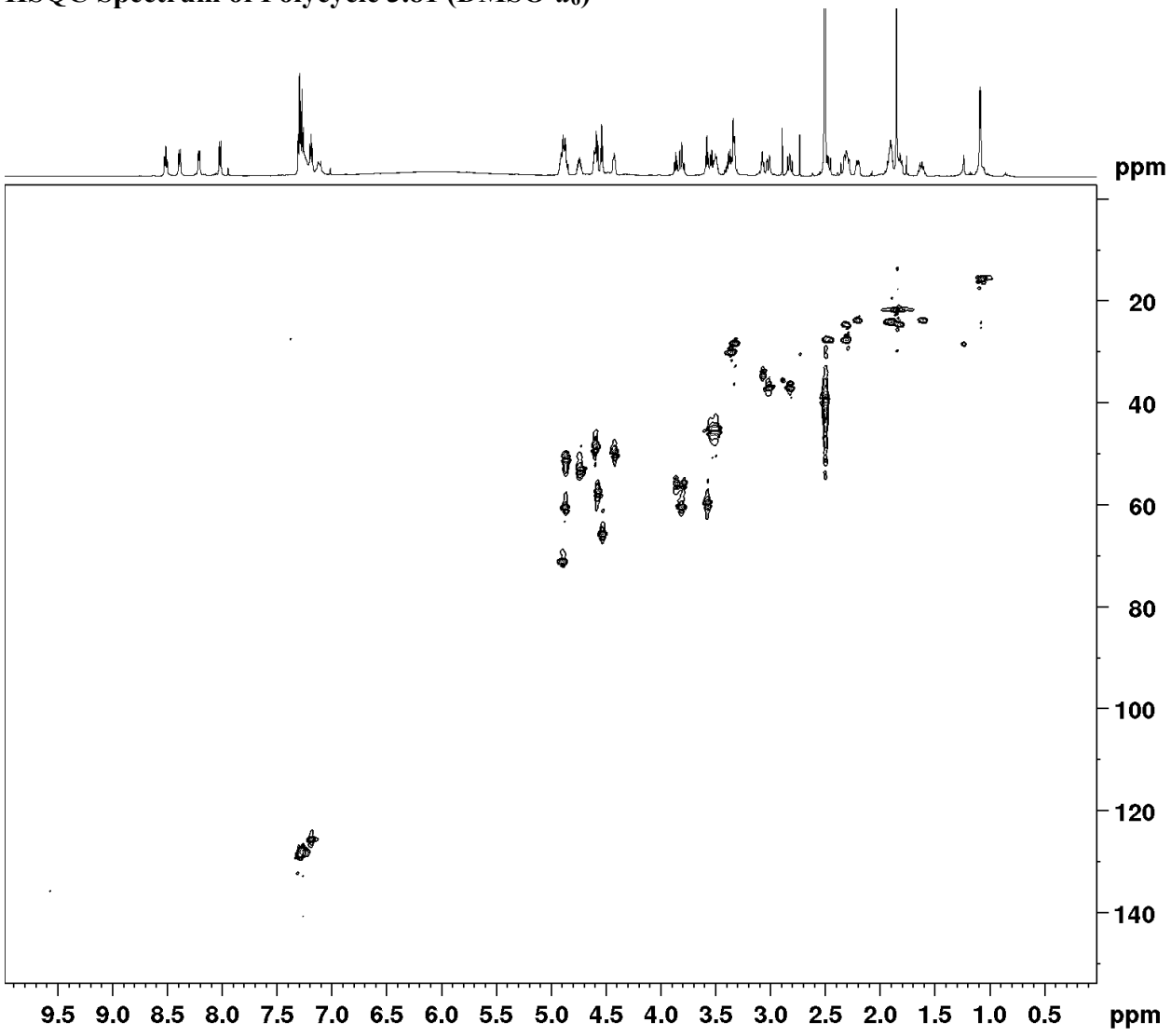
^{19}F NMR of Polycycle 3.81 (DMSO-d_6, 565 MHz)



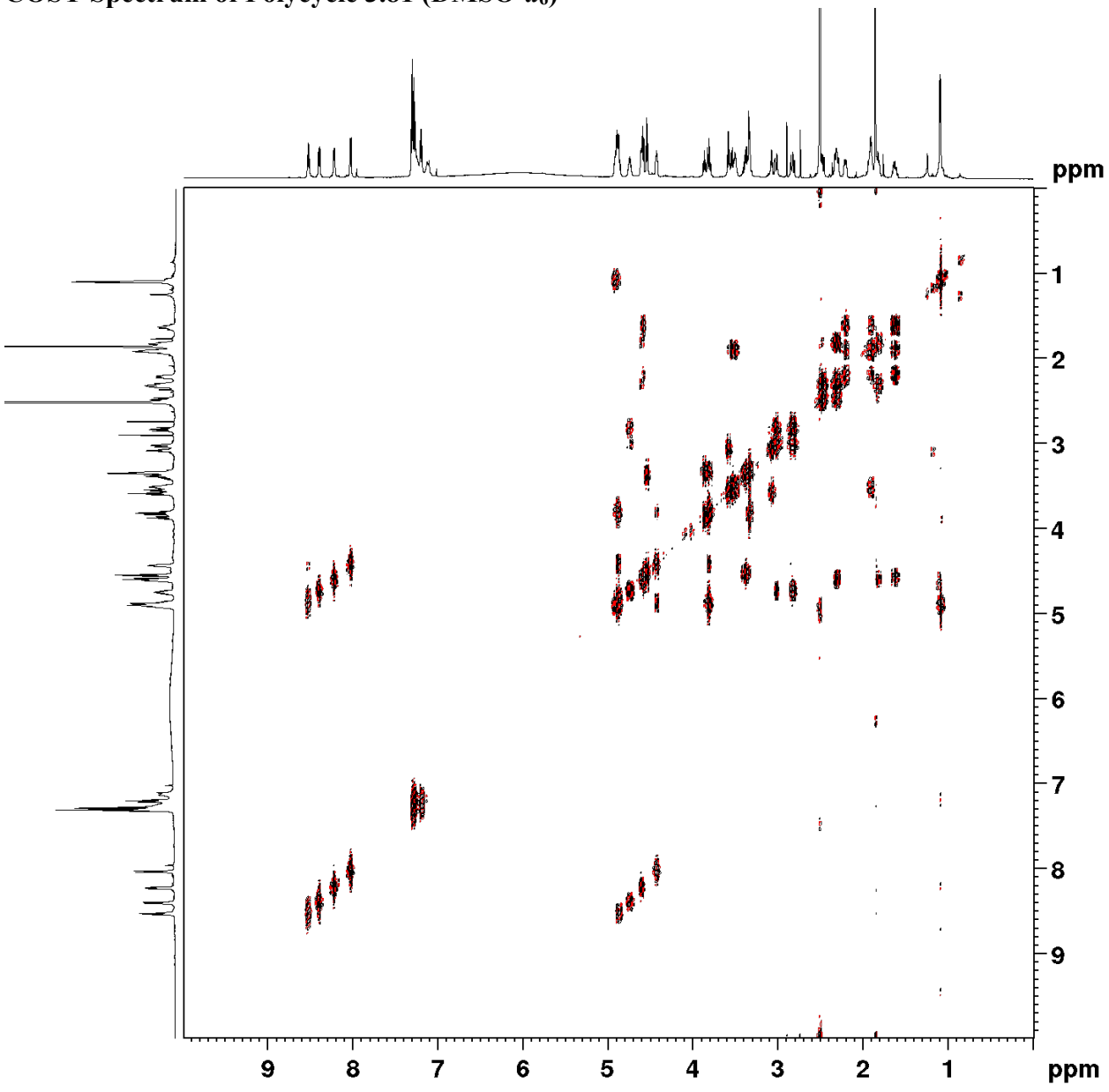
HMBC Spectrum of Polycycle 3.81 (DMSO-d_6)



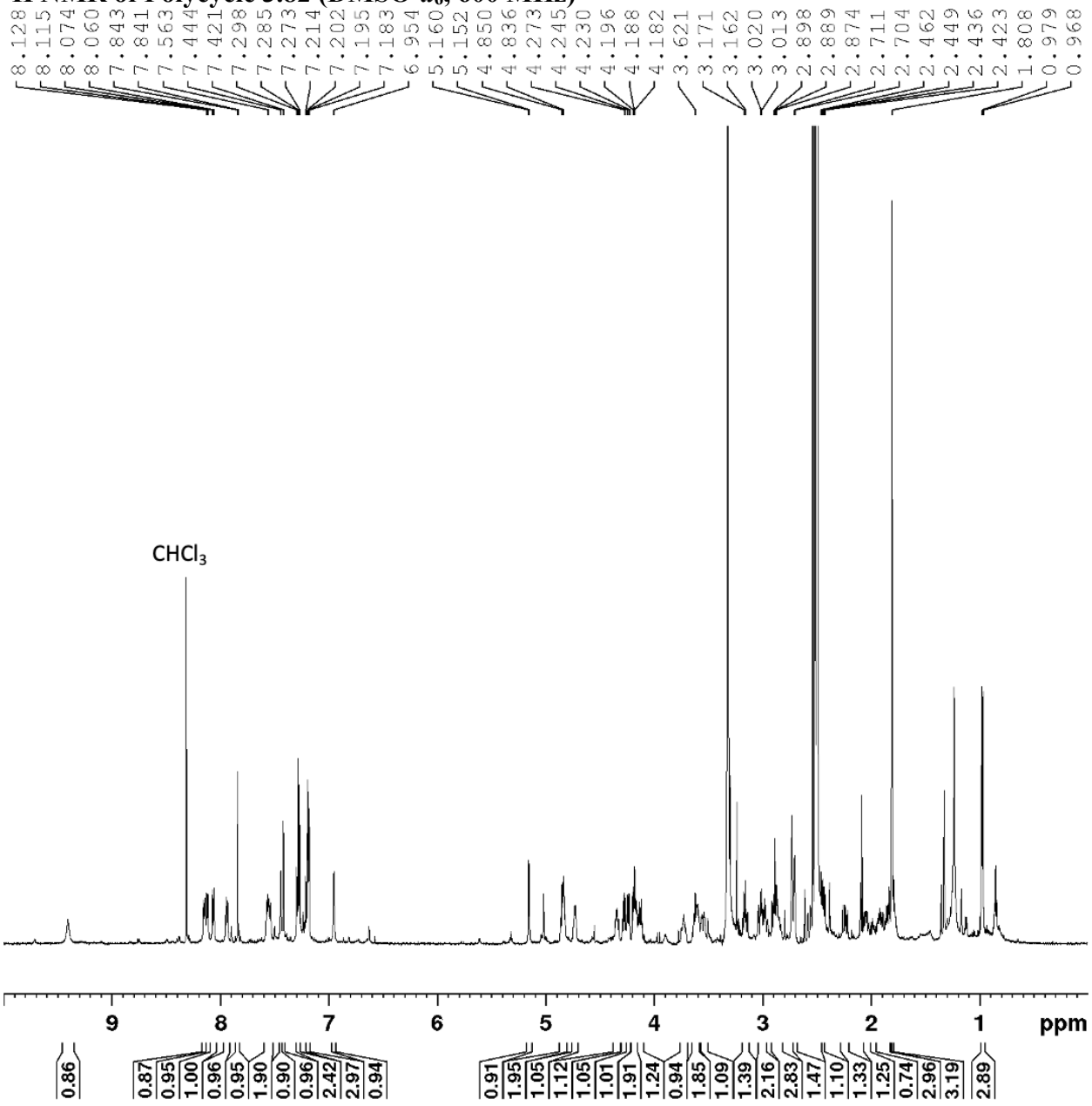
HSQC Spectrum of Polycycle 3.81 (DMSO-d_6)



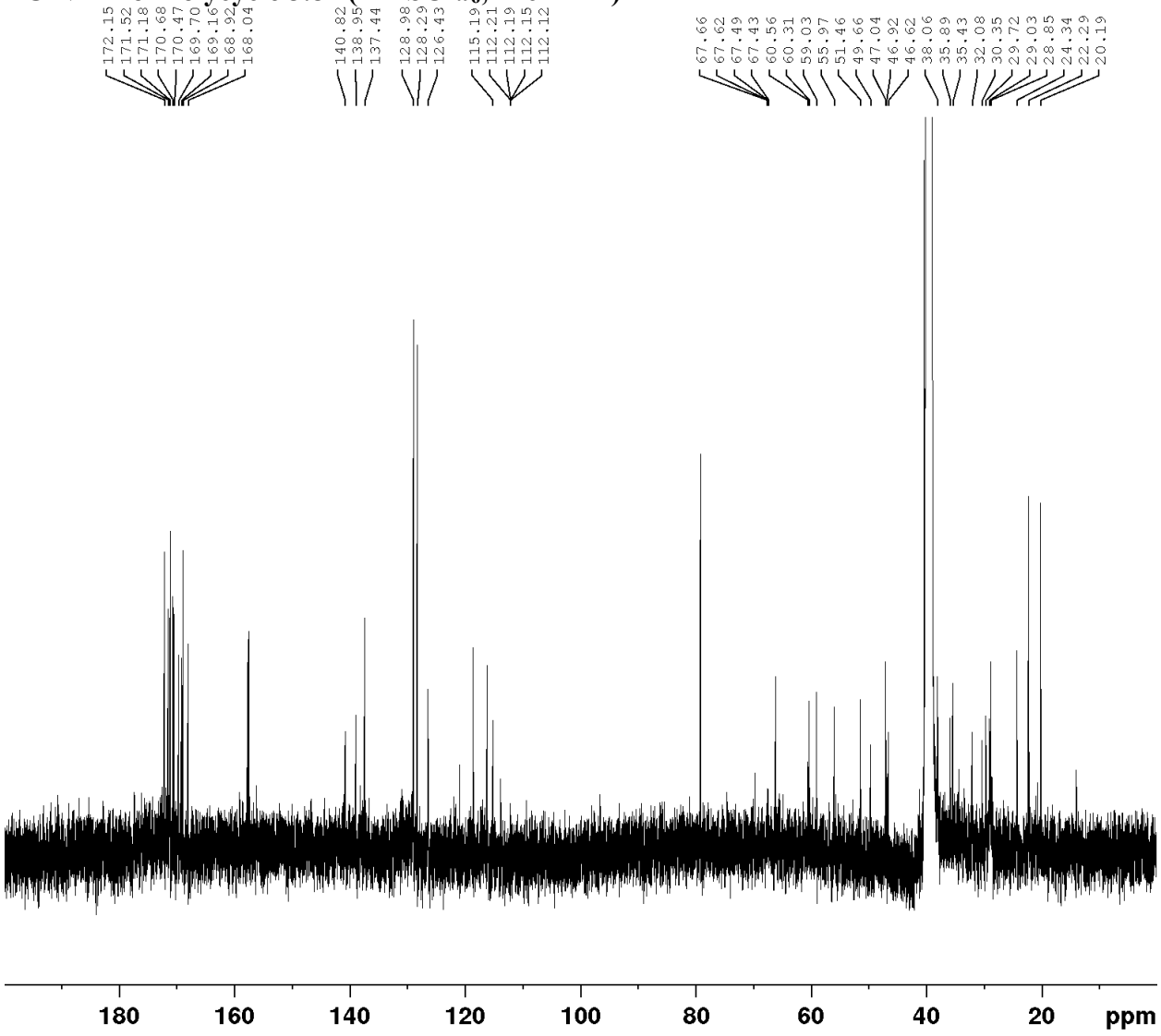
COSY Spectrum of Polycycle 3.81 (DMSO-d_6)



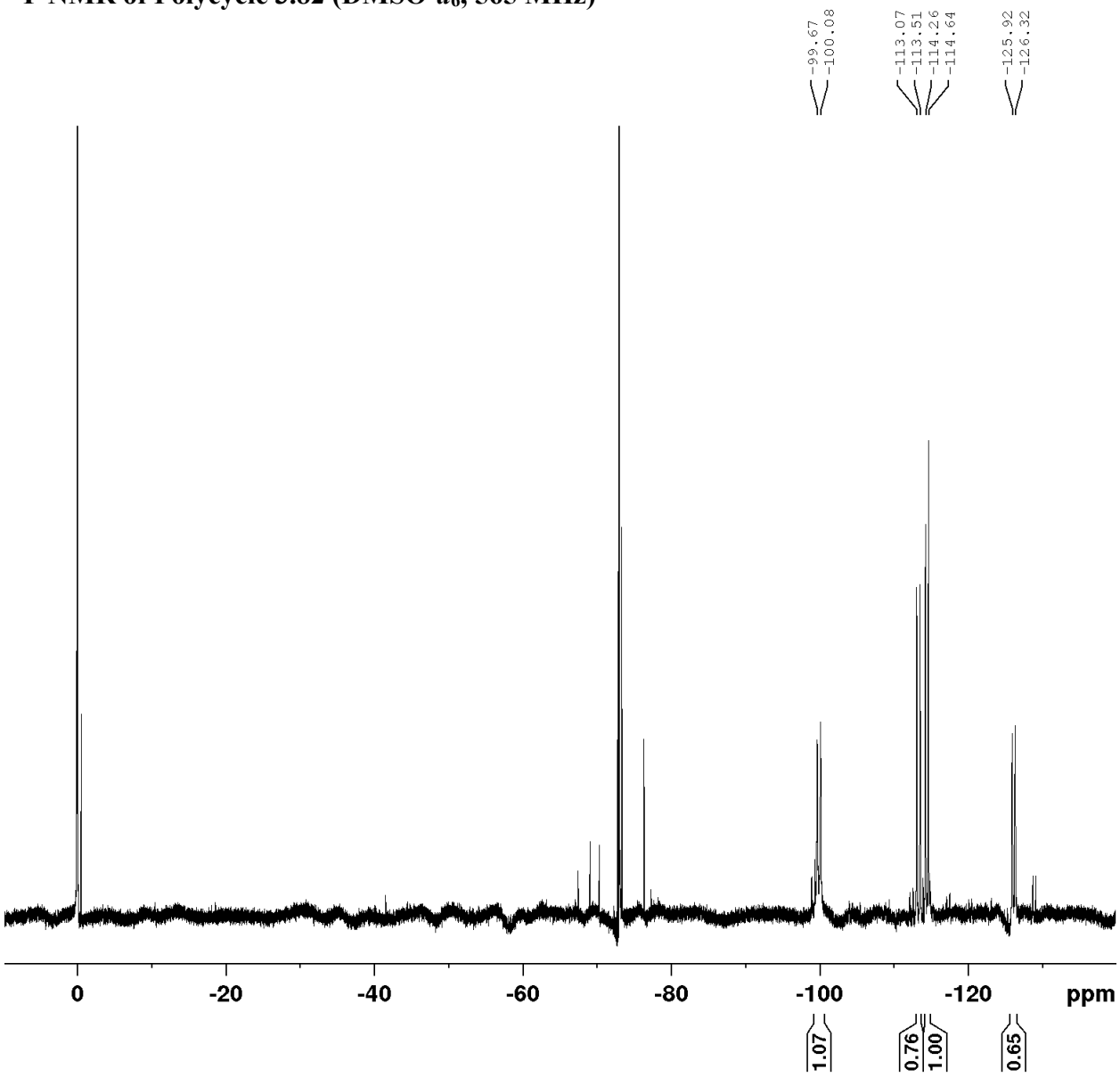
^{1}H NMR of Polycycle 3.82 (DMSO-d_6, 600 MHz)



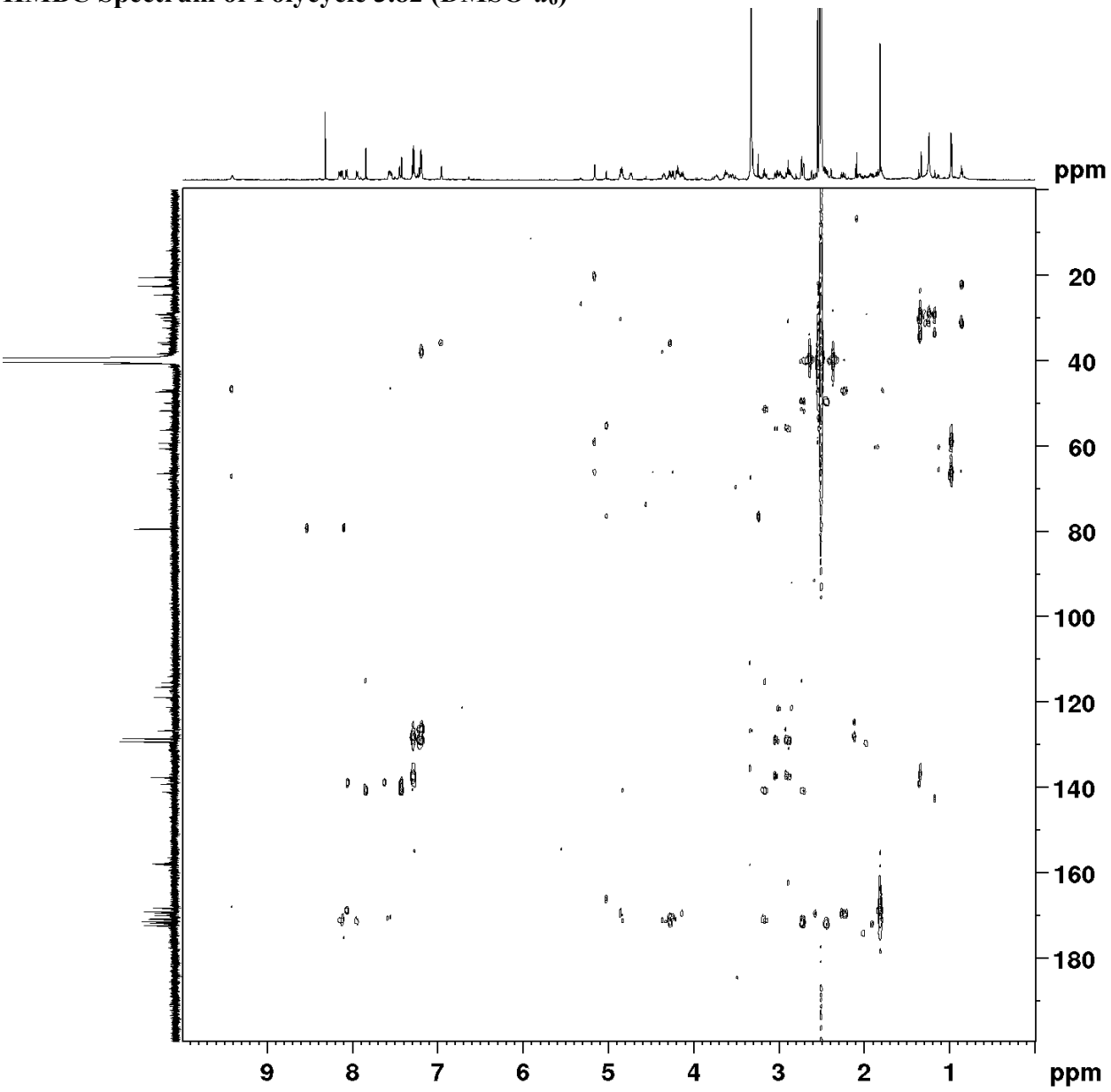
^{13}C NMR of Polycycle 3.82 (DMSO-d_6, 126 MHz)



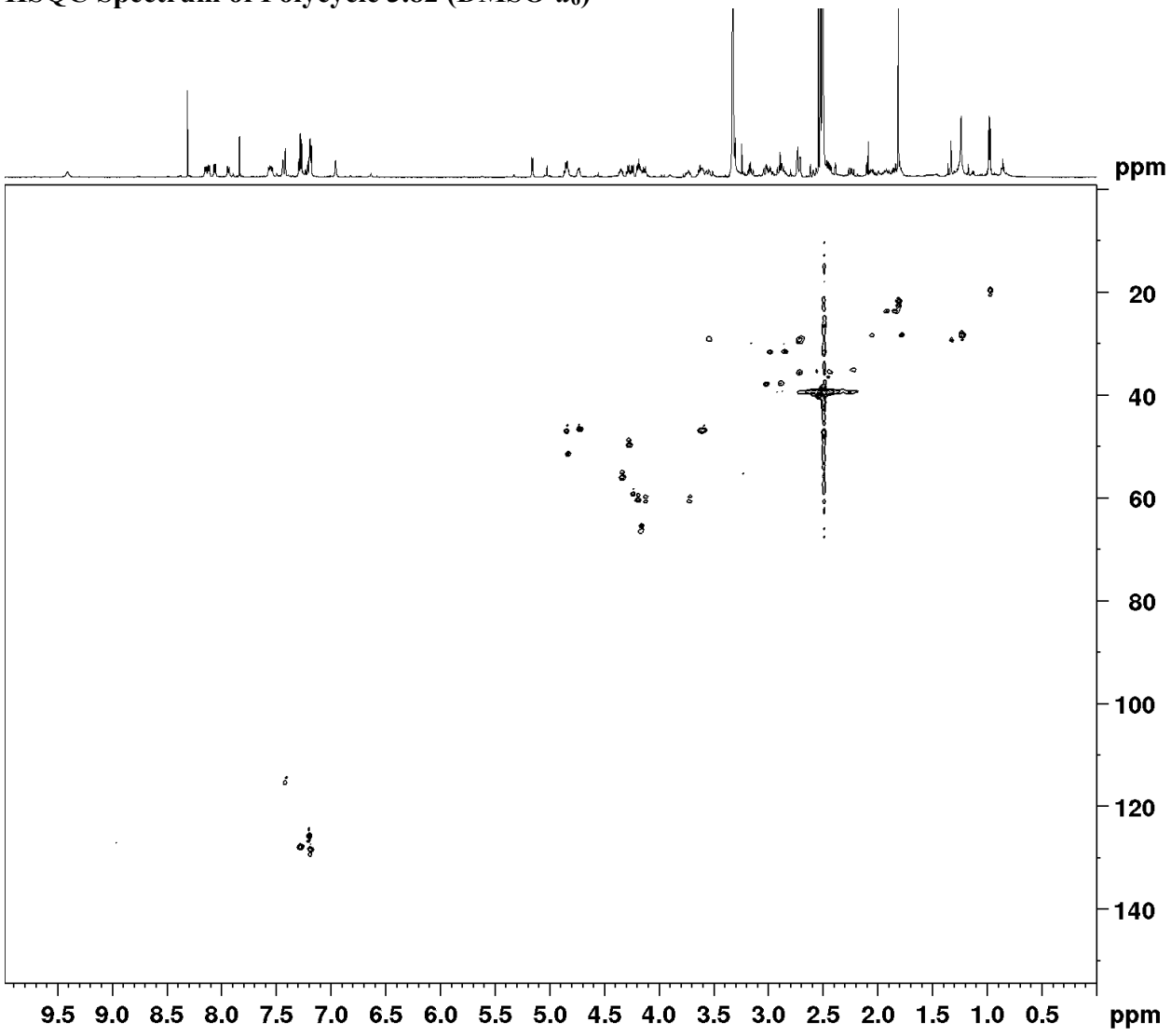
^{19}F NMR of Polycycle 3.82 (DMSO-d_6, 565 MHz)



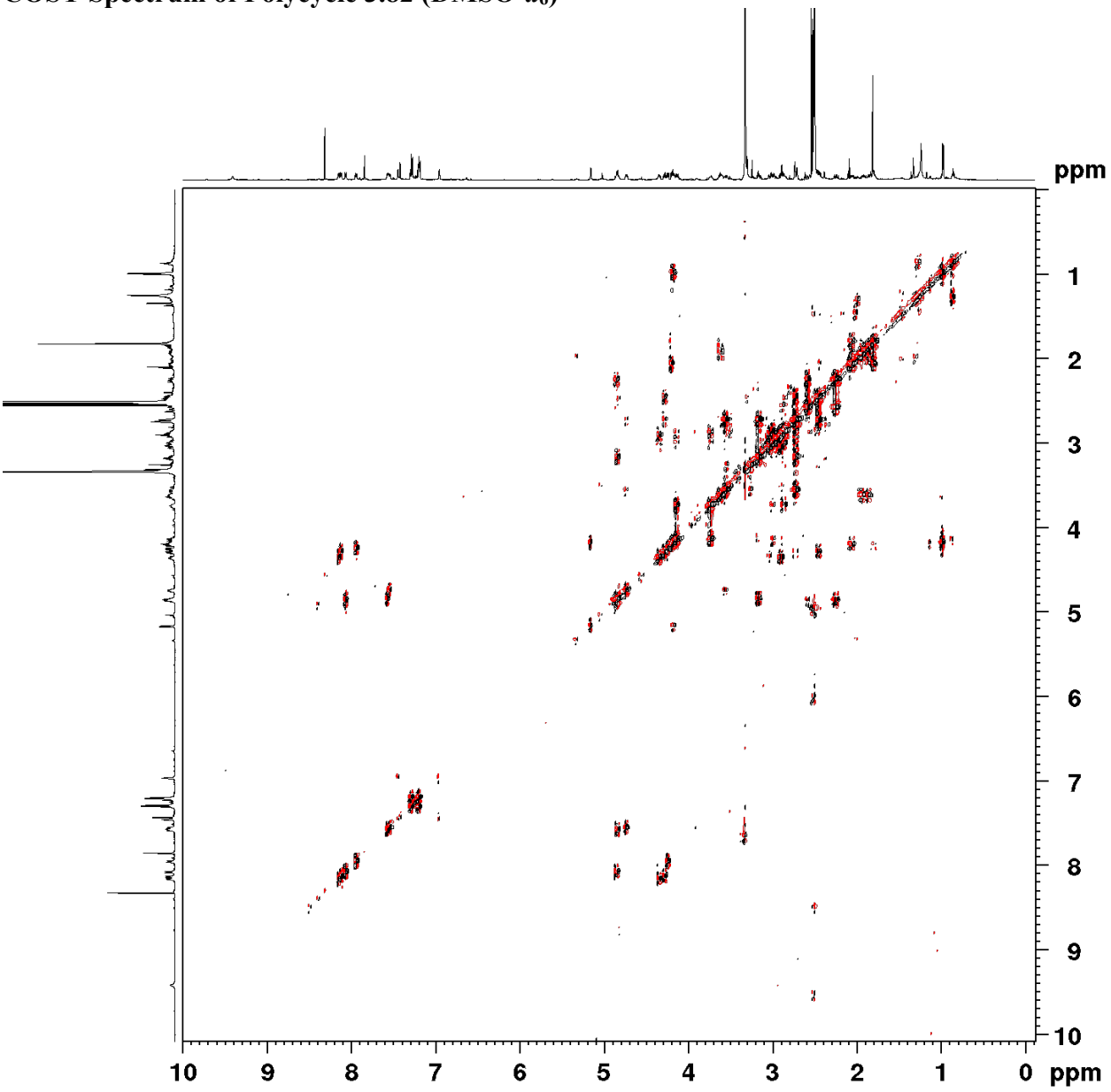
HMBC Spectrum of Polycycle 3.82 (DMSO-d_6)



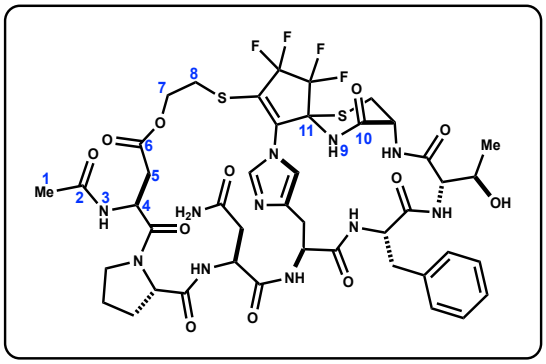
HSQC Spectrum of Polycycle 3.82 (DMSO-d_6)



COSY Spectrum of Polycycle 3.82 (DMSO-d_6)

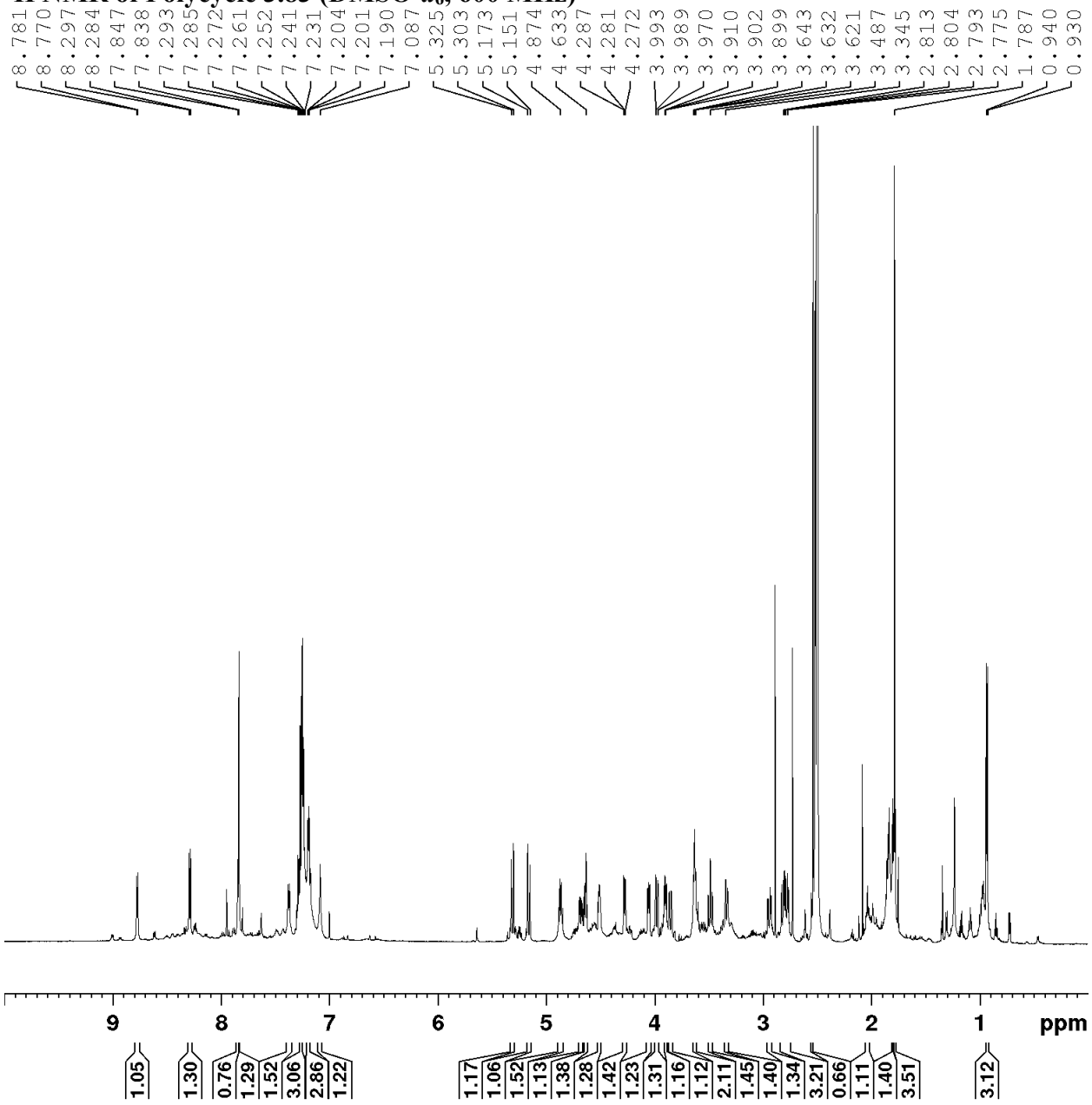


Polycycle 3.82 (DMSO-d_6)

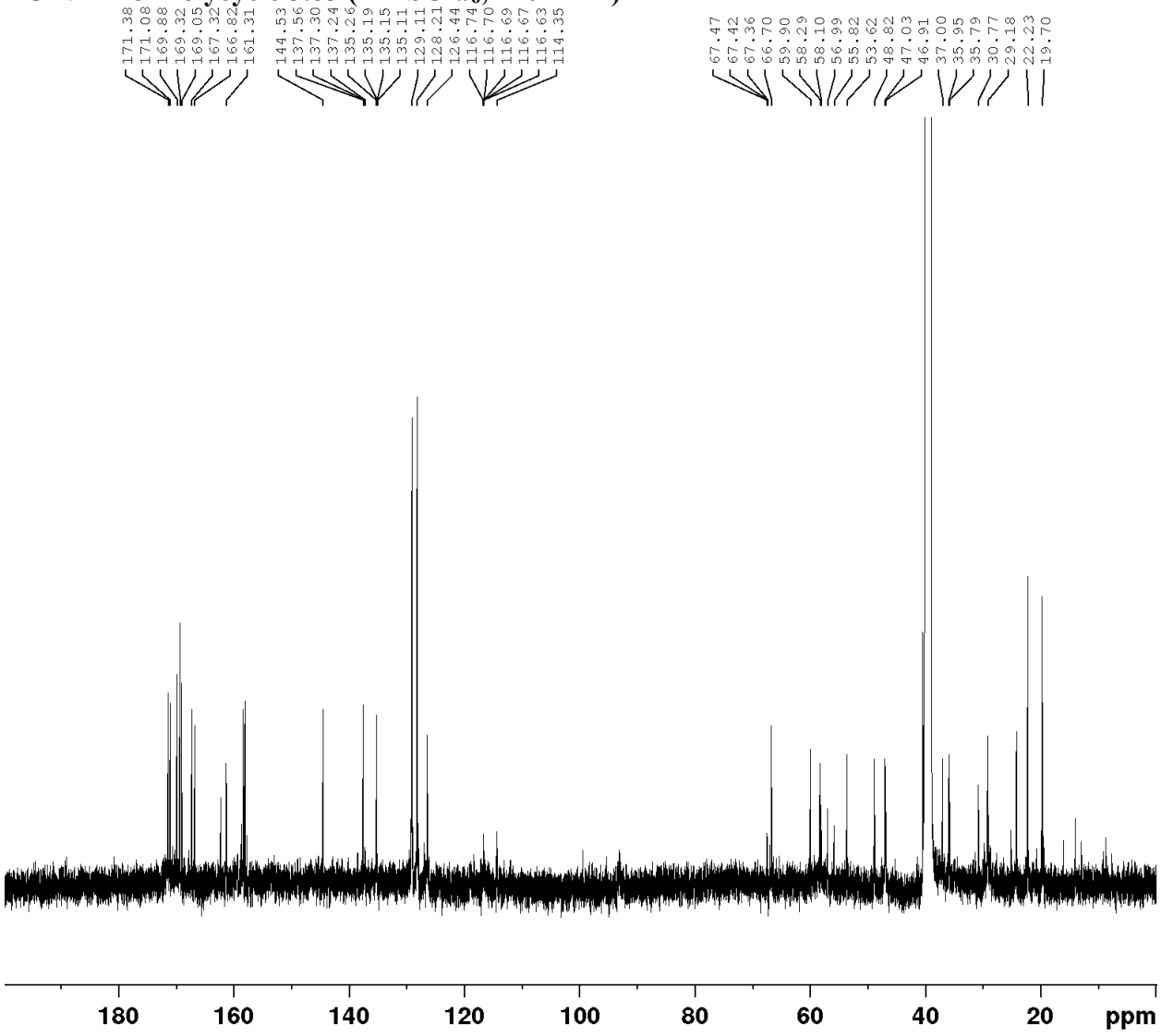


	13C	1H	Key Correlation
1	22.29	1.81 (s, 3H)	HSQC
2	168.93	-	1->2 HMBC
3	-	8.07 (d, J = 8.3 Hz, 1H)	2->3 HMBC
4	46.62	4.87-4.81 (m, 1H) overlap	3->4 COSY, HSQC
5	35.43	2.24 (dd, J = 15.4, 10.4 Hz, 1H), 2.44 (dd, J = 15.4, 7.8 Hz, 1H)	4->5 COSY, HSQC
6	169.71	-	5->6 HMBC
7	60.31	4.15–4.10 (m, 1H), 3.76–3.69 (m, 1H)	7->6 HMBC, HSQC
8	38.06	2.89 (m, 2H) overlap	7->8 COSY, HMBC, HSQC
9	-	9.41 (s, 1H)	11->9 HMBC
10	168.05	-	9->10 HMBC
11	67.7–66.4 (m, 1C)	-	9->11 HMBC

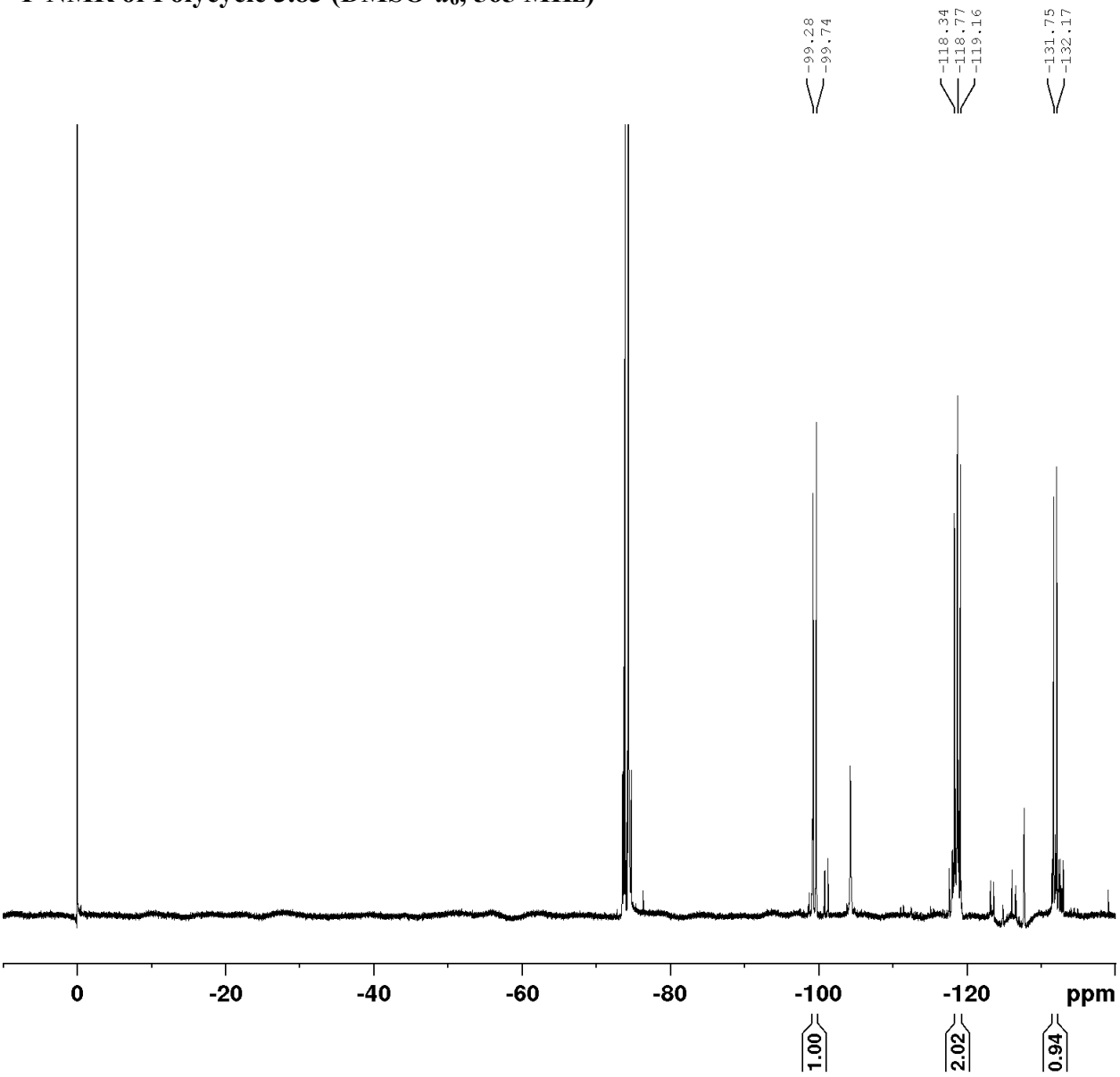
^{1}H NMR of Polycycle 3.83 (DMSO-d_6, 600 MHz)



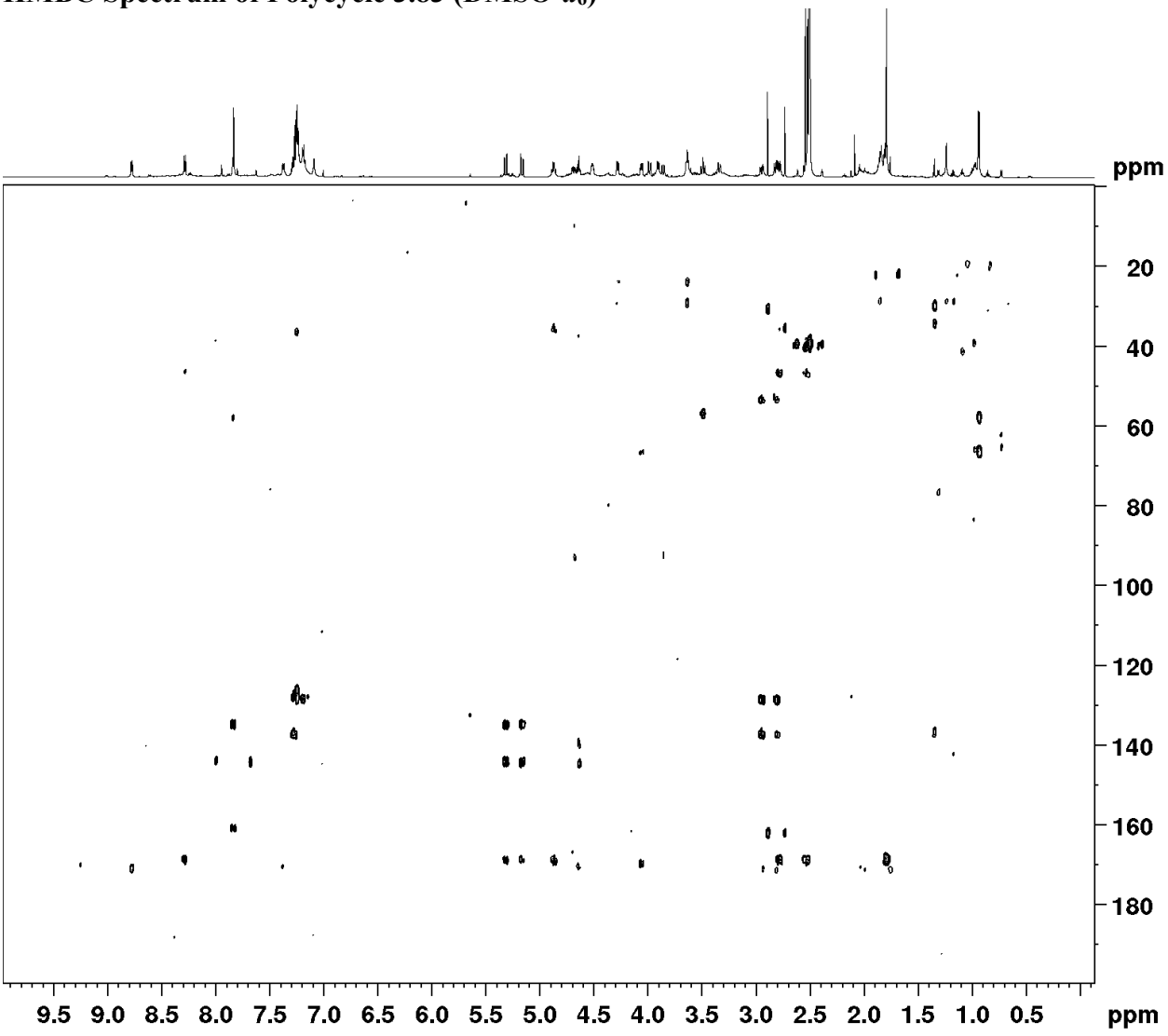
^{13}C NMR of Polycycle 3.83 (DMSO-d_6, 126 MHz)



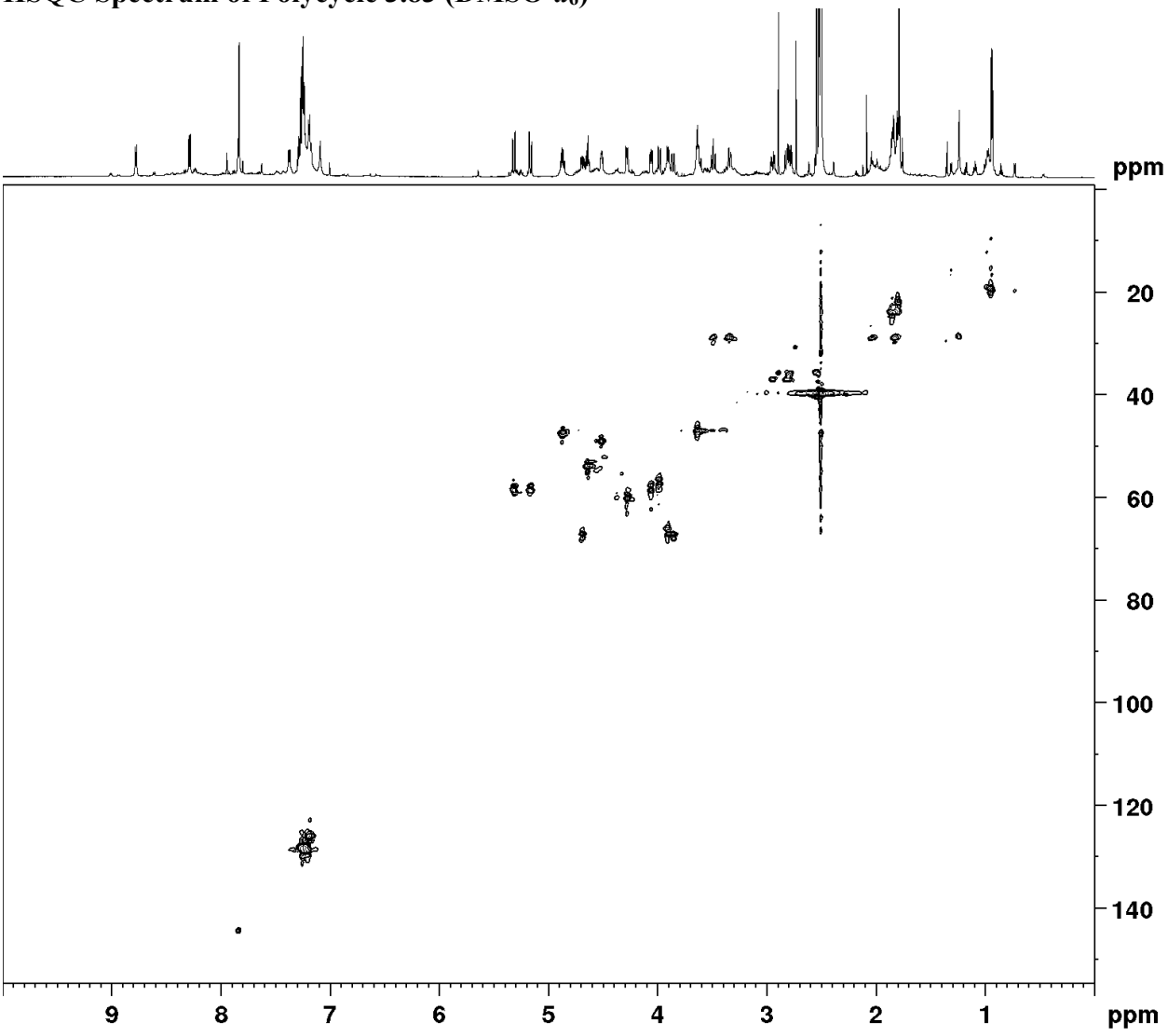
^{19}F NMR of Polycycle 3.83 (DMSO-d_6, 565 MHz)



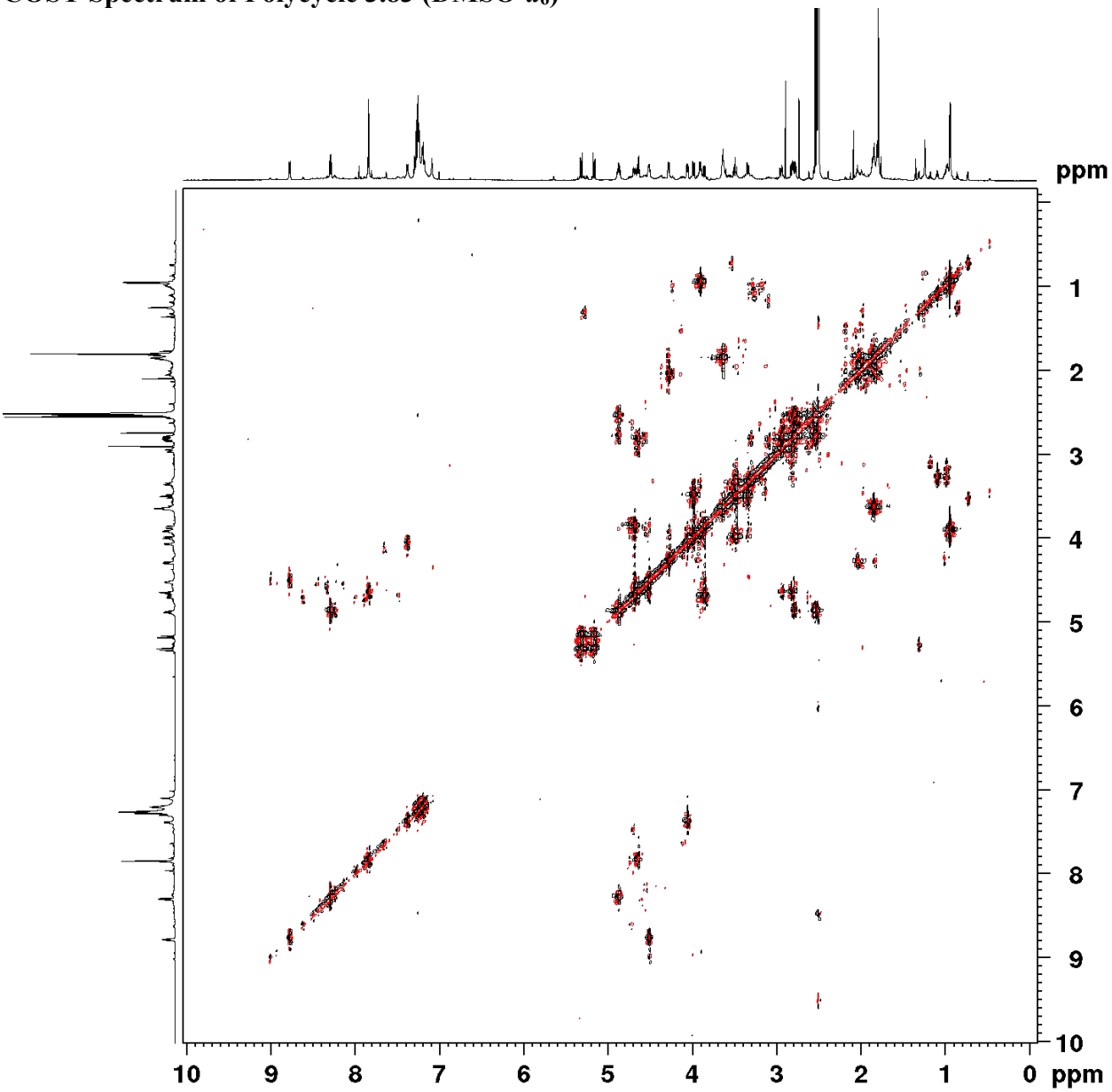
HMBC Spectrum of Polycycle 3.83 (DMSO-d_6)



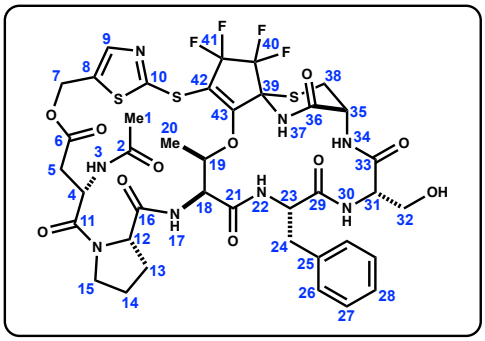
HSQC Spectrum of Polycycle 3.83 (DMSO-d_6)



COSY Spectrum of Polycycle 3.83 (DMSO-d_6)

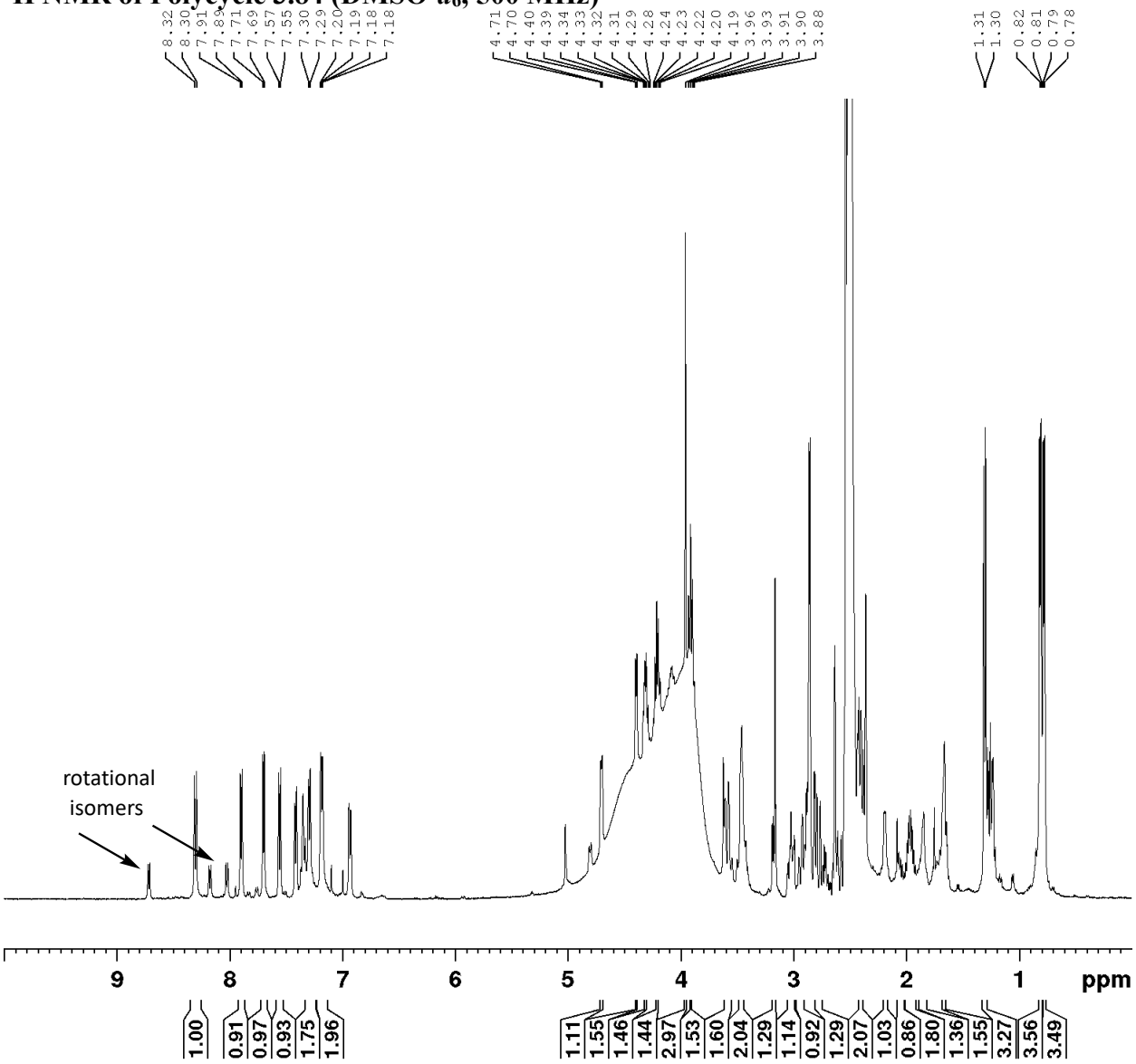


Polycycle 3.83 (DMSO-d_6)

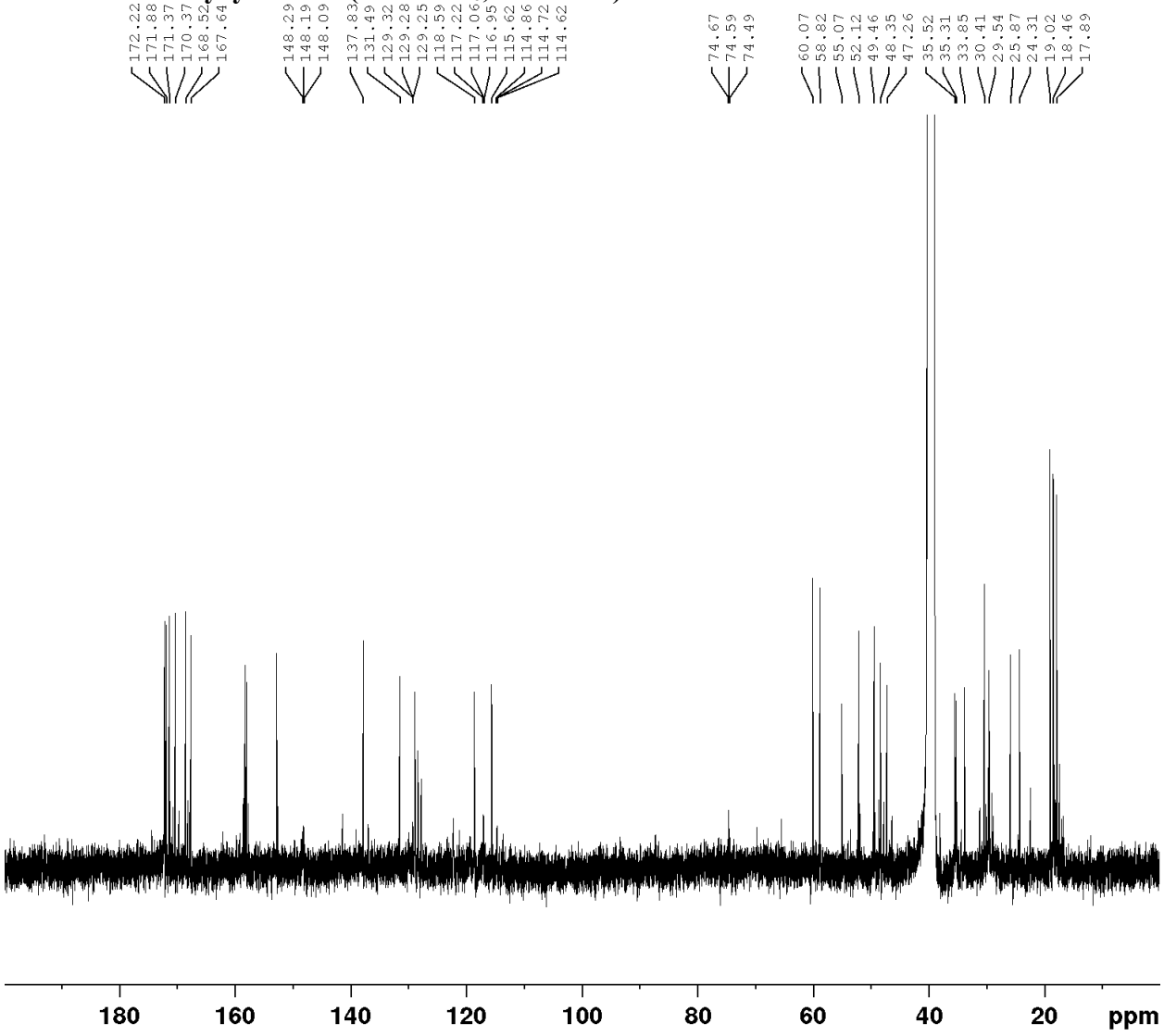


	13C	1H	Key Correlation
1	22.23	1.79 (s, 3H)	HSQC
2	169.05	-	1->2 HMBC
3	-	8.29 (d, J = 8.0 Hz, 1H)	2->3 HMBC
4	47.03	4.87 (q, J = 5.9 Hz, 1H)	2->3 HMBC, HSQC
5	35.79	2.78 (m, 1H), 2.52 (m, 1H)	4->5 COSY, HSQC
6	169.32	-	5->6 HMBC and 7->6 HMBC
7	58.29	5.31 (d, J = 13.0 Hz, 1H), 5.16 (d, J = 13.0 Hz, 1H)	6->7, 8-7, 9->7 HMBC, HSQC
8	135.26	-	9->8 HMBC
9	144.53	7.74 (s, 1H)	HSQC, 7->9 HMBC, HSQC
10	114.44	-	-
11	169.88	-	4->11 HMBC
12	55.82	4.68 (m, 1H) overlap	16->12 HMBC
13	35.95	2.80 (overlap, 1H), 2.53 (overlap, 1H)	12->13 HMBC, HSQC
14	29.18	2.03 (m, 1H), 1.84 (m, 1H) overlap	13->14 HMBC, HSQC
15	46.92	3.64 (m, 2H)	14->15 HMBC, HSQC
16	171.38	-	17->16 HMBC
17	-	7.38 (d, J = 7.8 Hz, 1H)	18->17 COSY
18	58.10	4.06 (dd, J = 7.9, 4.2 Hz , 1H)	20->18 HMBC, HSQC
19	66.74	3.90 (m, 1H)	20->19 COSY
20	19.71	0.94 (d, J = 6.3 Hz, 3H)	HSQC
21	161.32	-	22->21 HMBC
22	-	7.85 (m, 1H) overlap	23->22 COSY
23	53.62	4.63 (m, 1H) overlap	24->23 COSY, HSQC
24	37.00	2.94 (dd, J = , 1H), 2.80 (overlap, 1H)	25->24 HMBC, 26->24 HMBC, HSQC
25	137.56	-	24->25 HMBC
26	129.12	7.27–7.23, 7.21–7.17 (m, 2)	HSQC
27	128.22	7.27–7.23, 7.21–7.17 (m, 2)	HSQC
28	126.45	7.27–7.23, 7.21–7.17 (m, 1)	HSQC
29	171.08	-	23->29 HMBC
30	-	8.77 (d, J = 7.0 Hz, 1H)	31->30 HMBC, HSQC
31	48.82	4.51 (m, 1H)	30->31 COSY, HSQC
32	56.99	3.98 (dd, J =, 1H), 3.86 (dd, J=, 1H)	31->32 HMBC, HSQC
33	167.32	-	31->30 HMBC
34	-	-	-
35	59.90	4.28 (m, 1H)	38->35 HMBC, HSQC
36	166.82	-	35->36 HMBC
37	-	7.09	-
38	30.77	3.49 (m, 1H), 3.33 (m, 1H)	38->35 HMBC
39	67.3–67.5 (m, 1C)	-	-
40	116.6–116.7 (m, 1C)	-	-
41	116.6–116.7 (m, 1C)	-	-
42	137.2–137.3 (m, 1C)	-	-
43	135.1–135.2 (m, 1C)	-	-

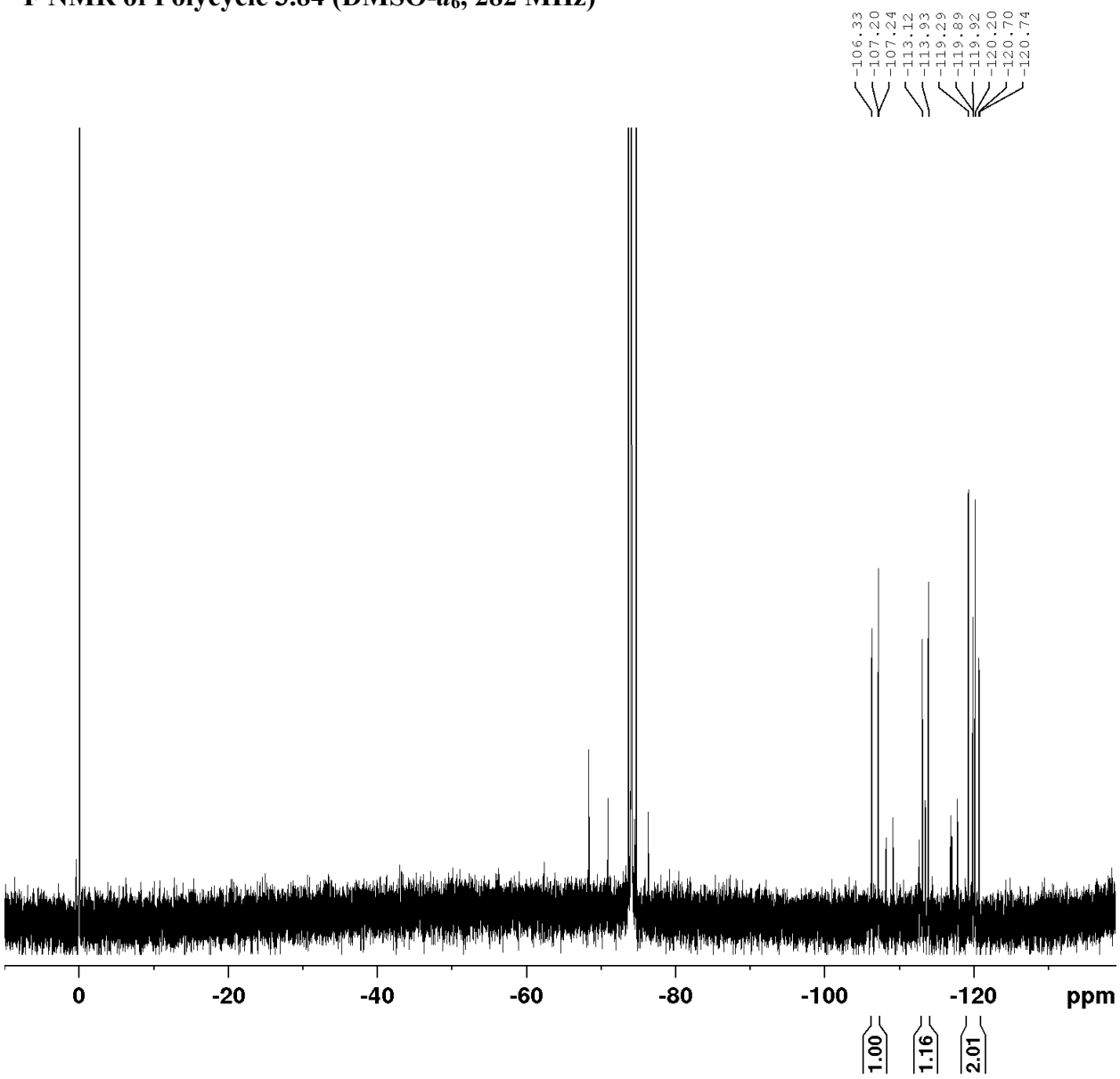
^{1}H NMR of Polycycle 3.84 (DMSO-d_6, 500 MHz)



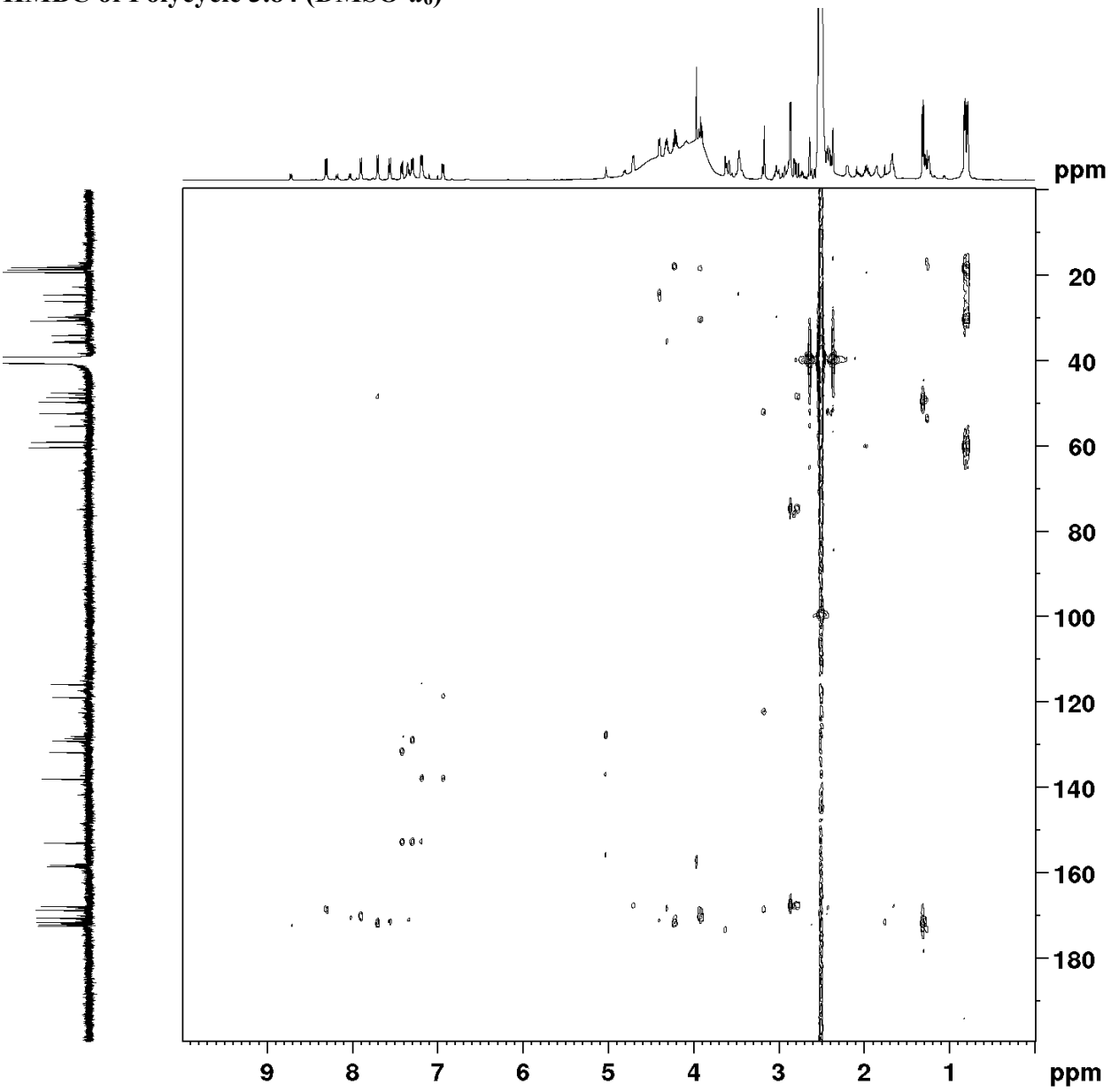
^{13}C NMR of Polycycle 3.84 (DMSO-d_6, 126 MHz)



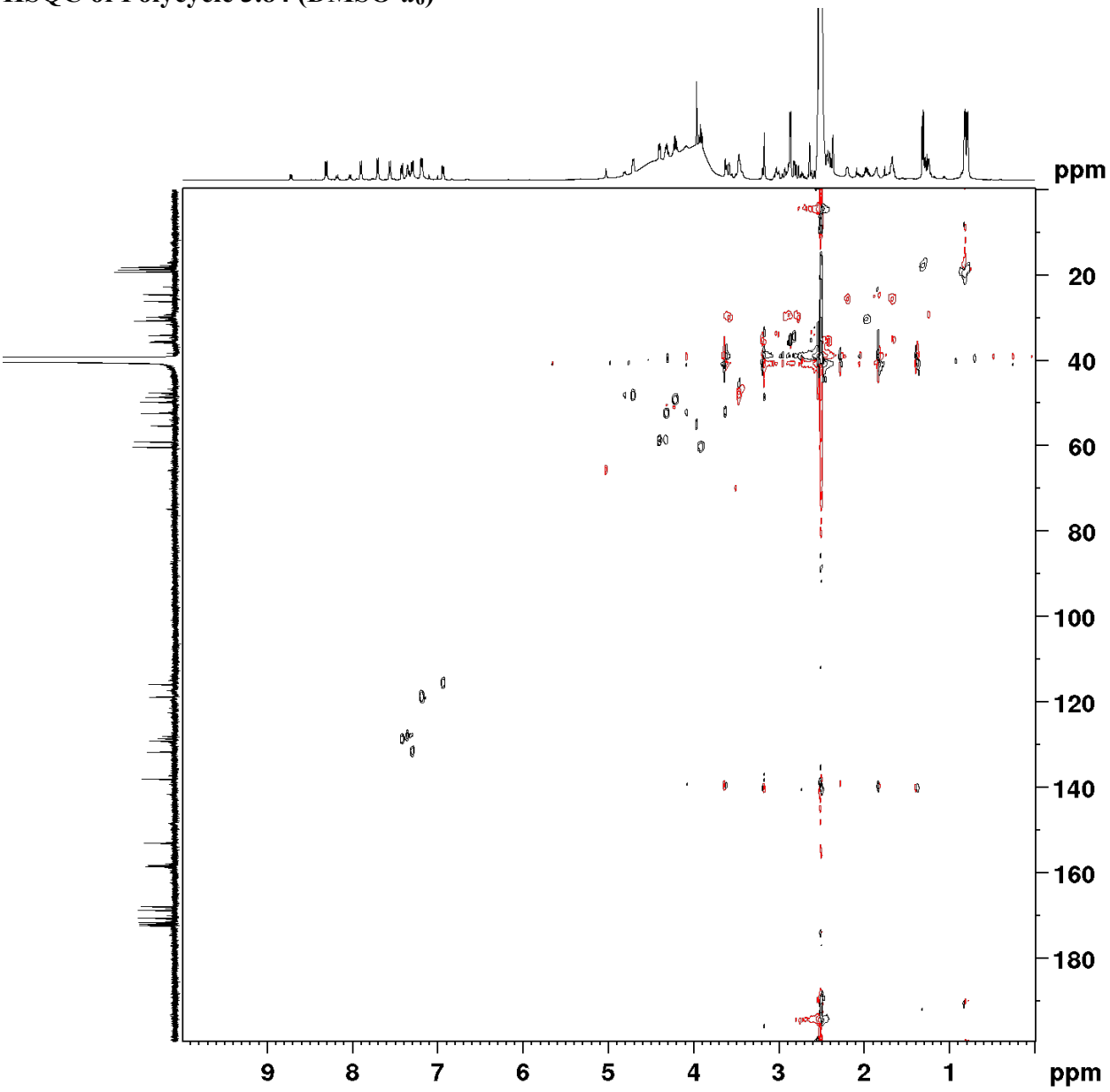
^{19}F NMR of Polycycle 3.84 (DMSO-d_6, 282 MHz)



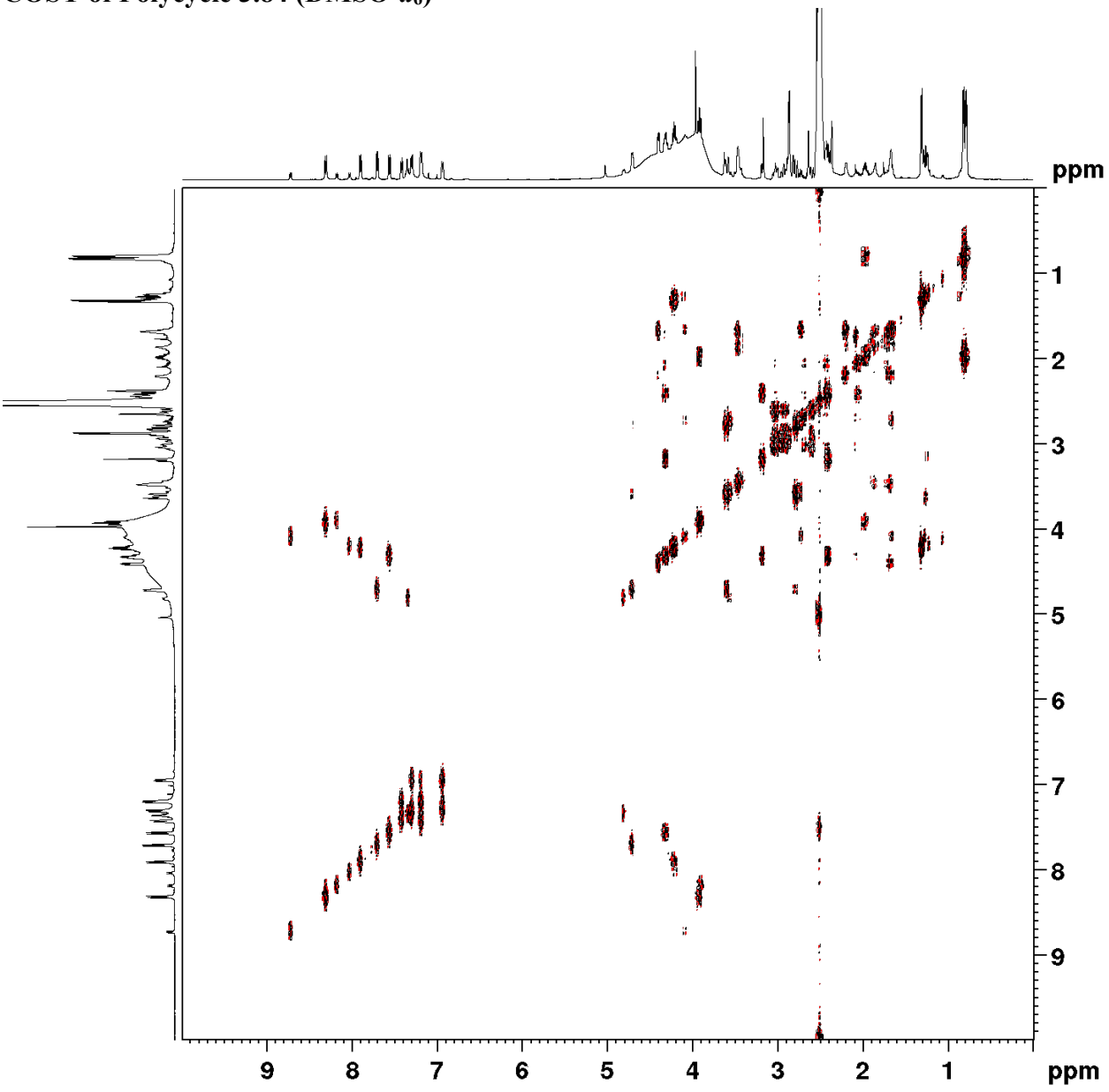
HMBC of Polycycle 3.84 (DMSO-d_6)



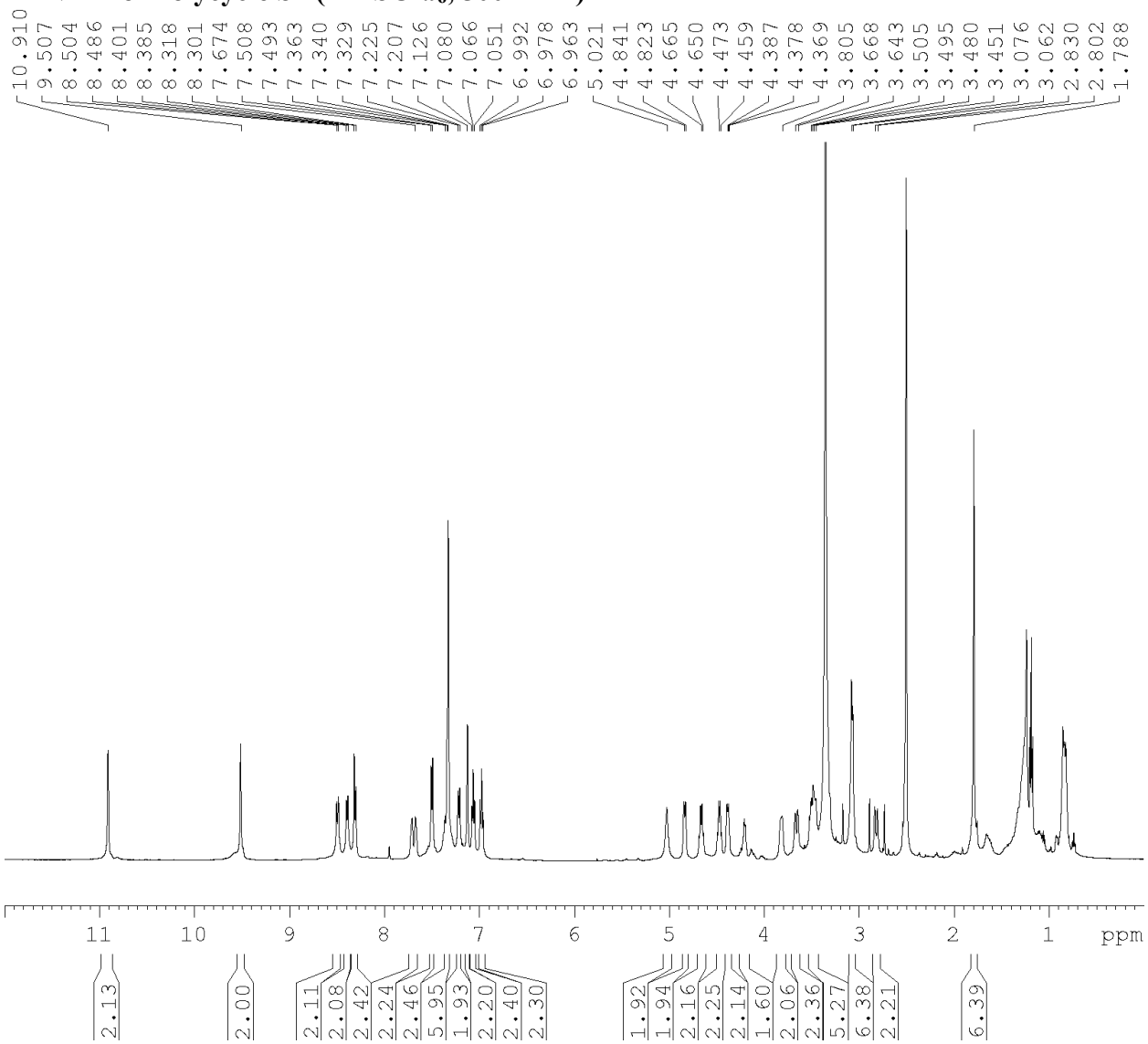
HSQC of Polycycle 3.84 (DMSO-d_6)



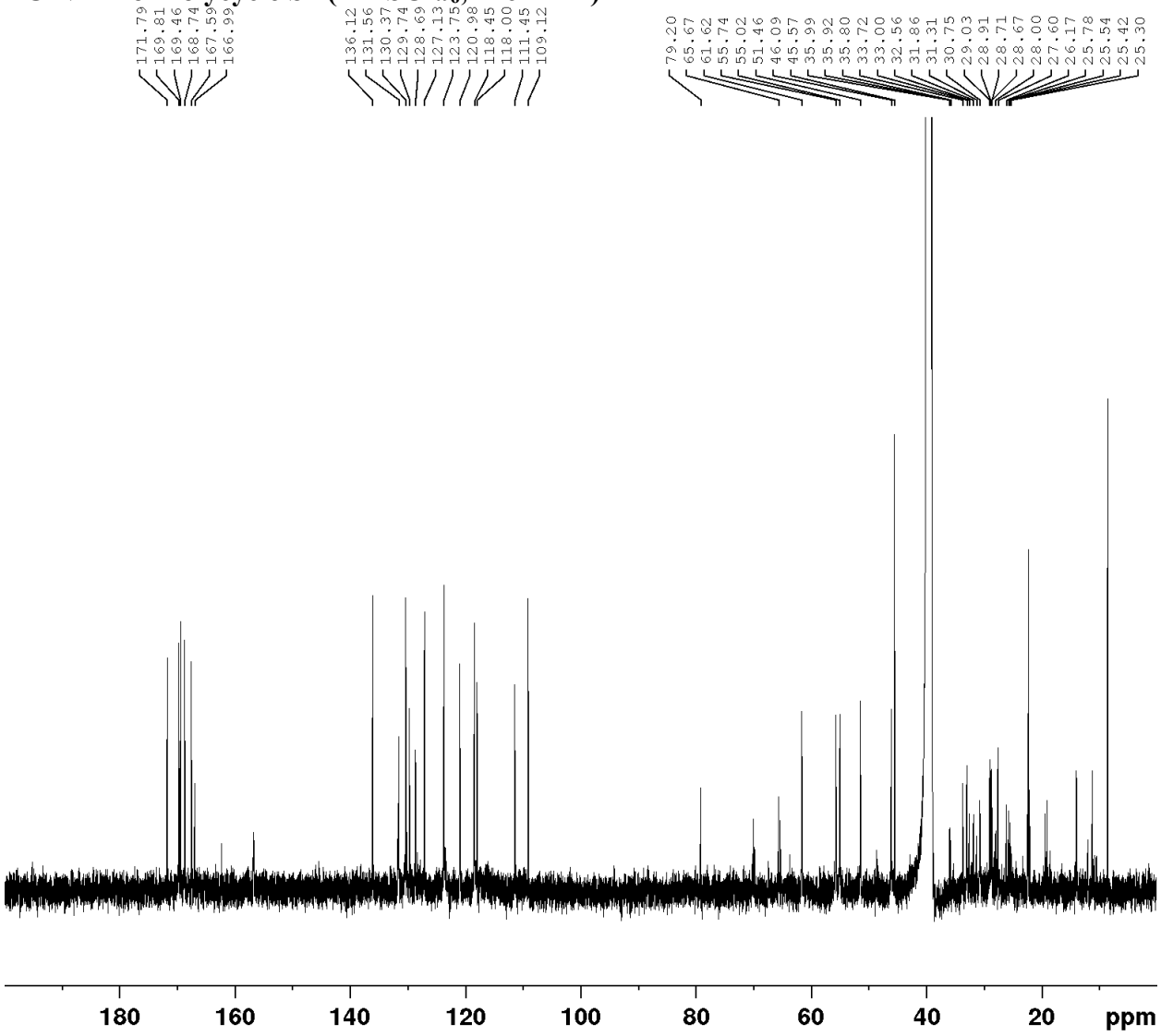
COSY of Polycycle 3.84 (DMSO-d_6)



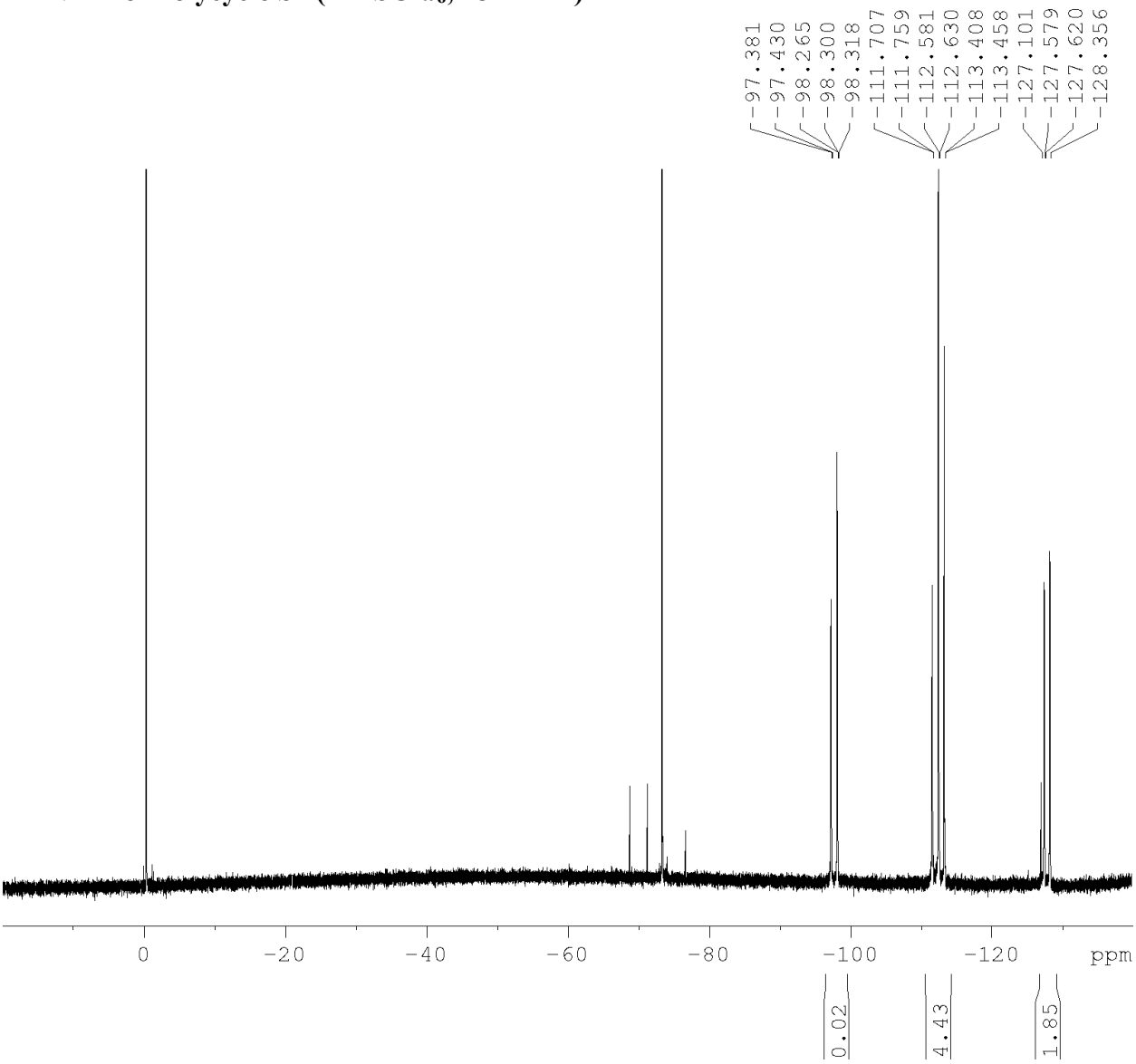
^{1}H NMR of Polycycle S1 (DMSO-d_6, 500 MHz)



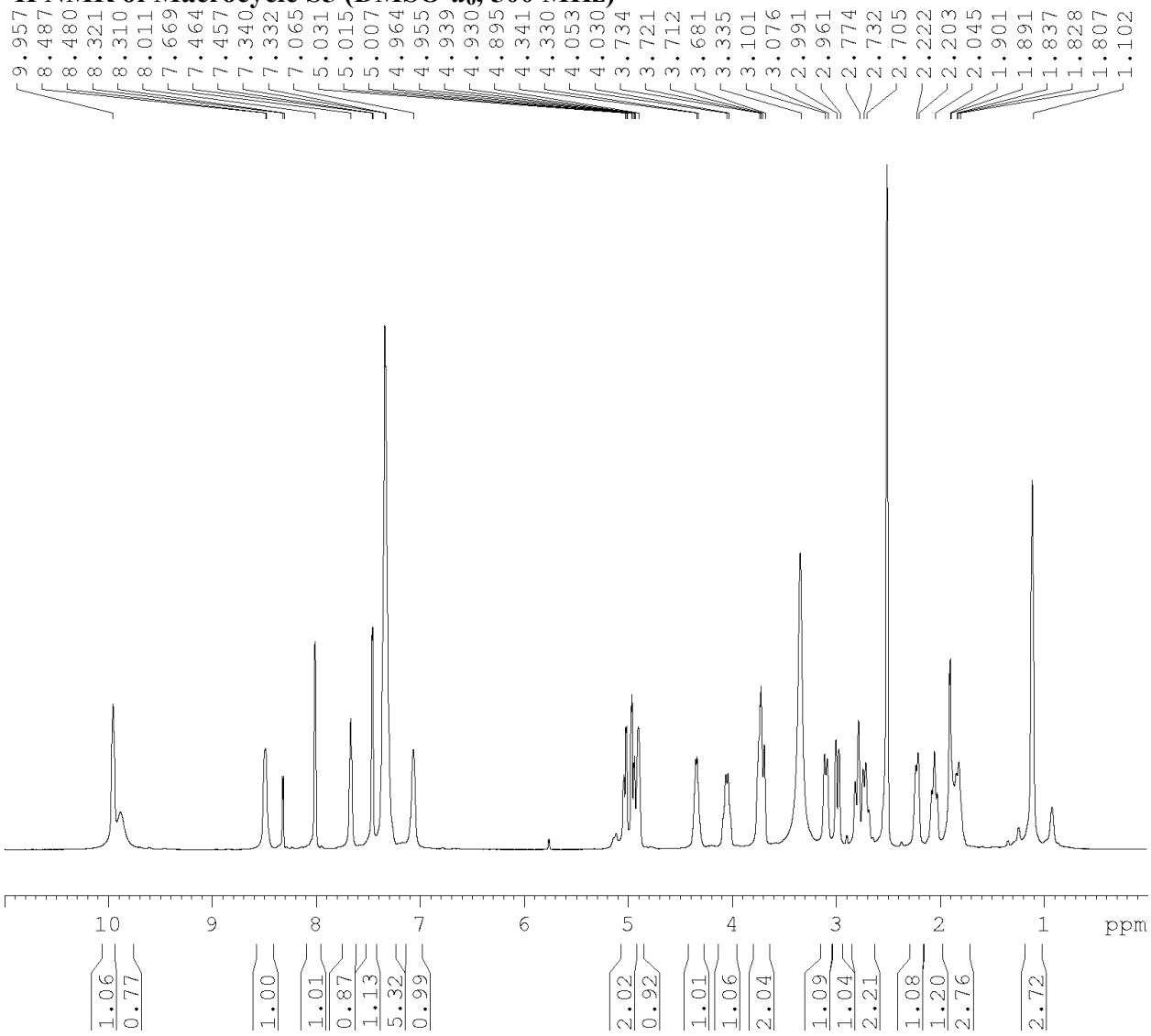
^{13}C NMR of Polycycle S1 (DMSO-d_6, 126 MHz)



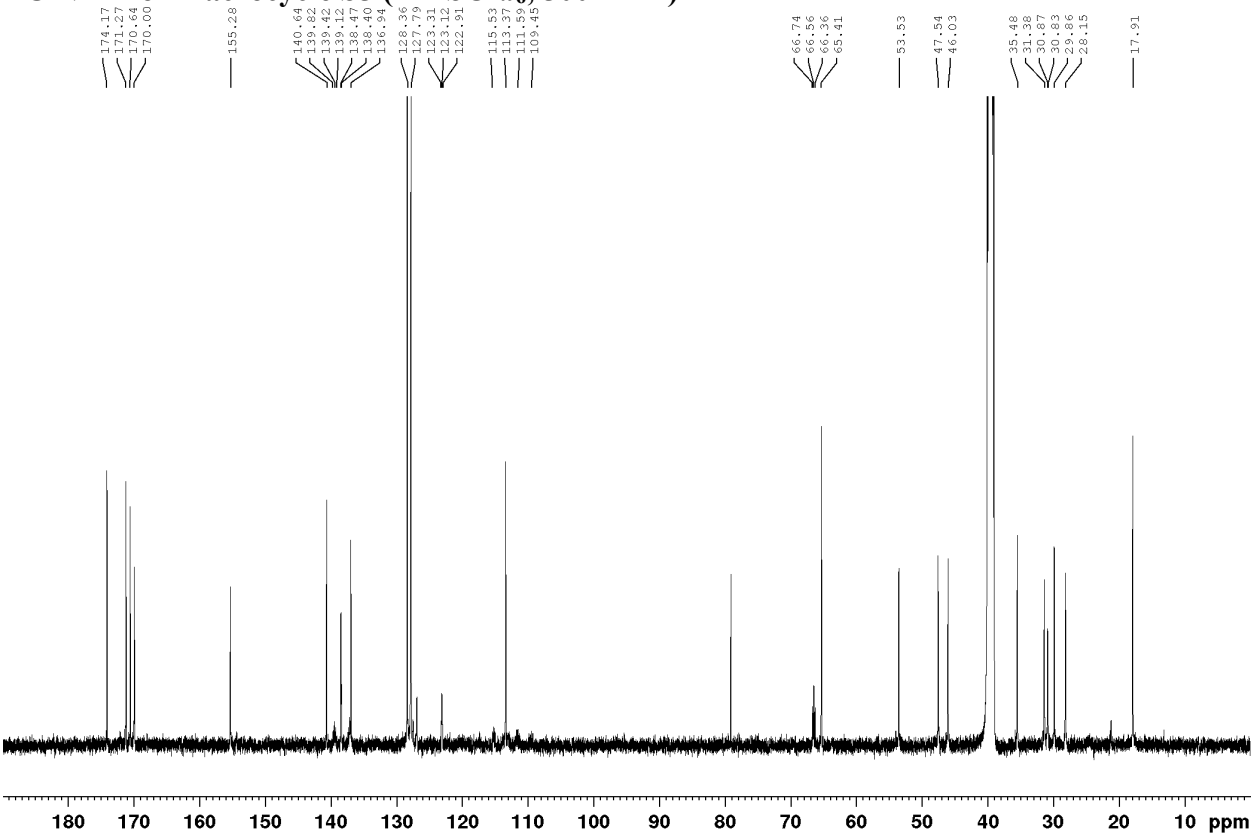
^{19}F NMR of Polycycle S1 (DMSO-d_6, 282 MHz)



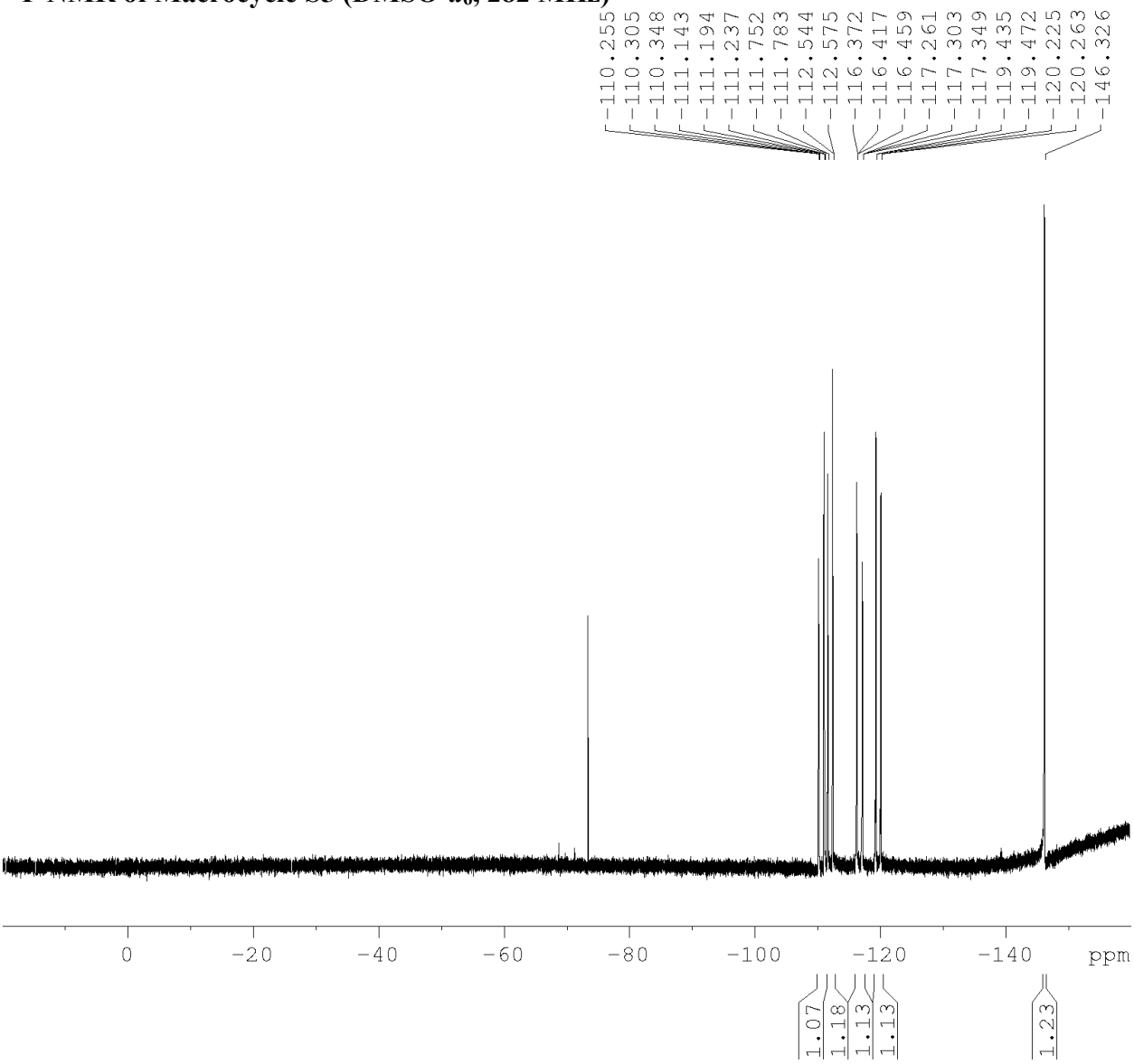
^{1}H NMR of Macrocycle S5 (DMSO-d_6, 500 MHz)



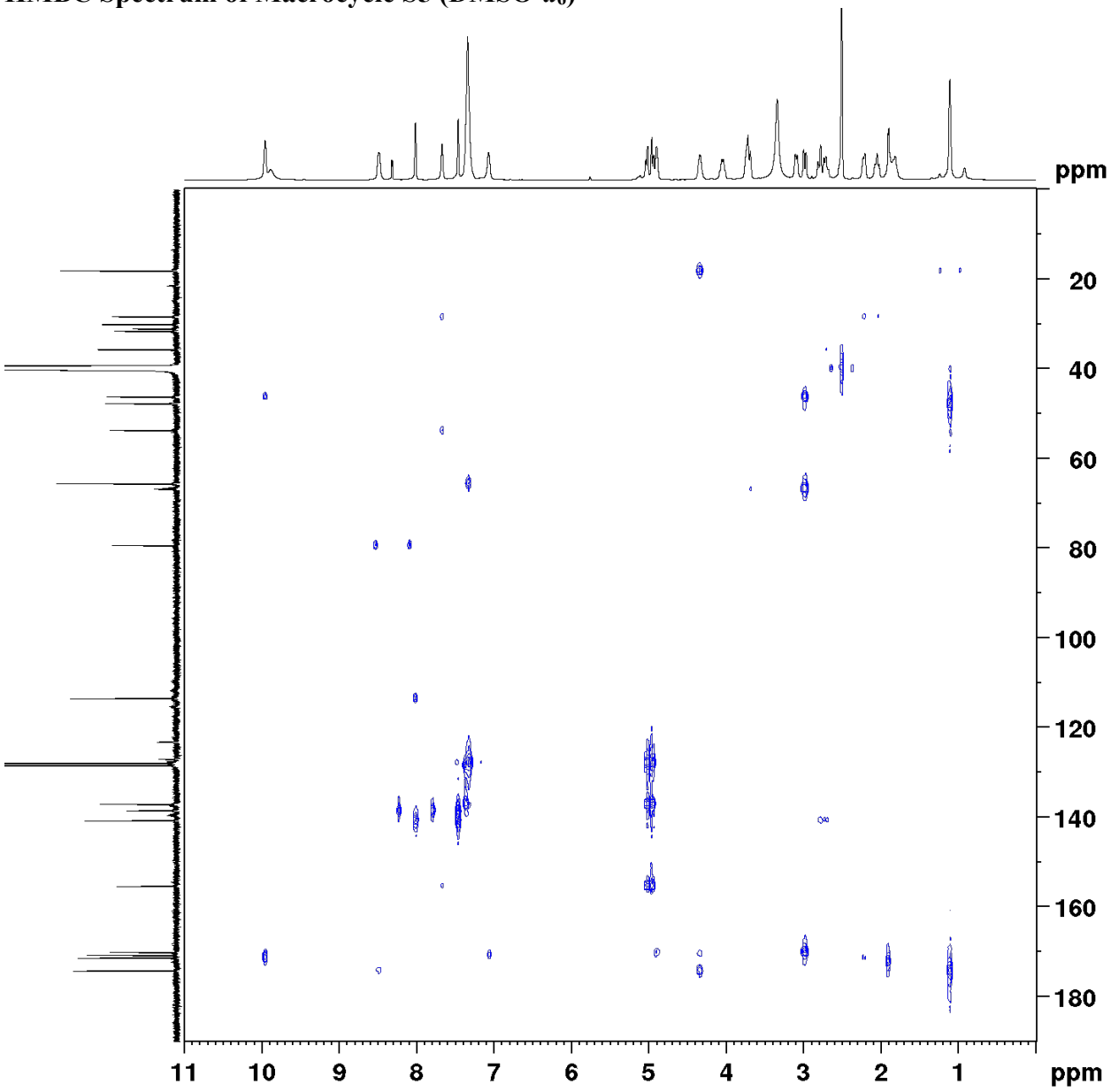
^{13}C NMR of Macrocycle S5 (DMSO-d_6, 500 MHz)



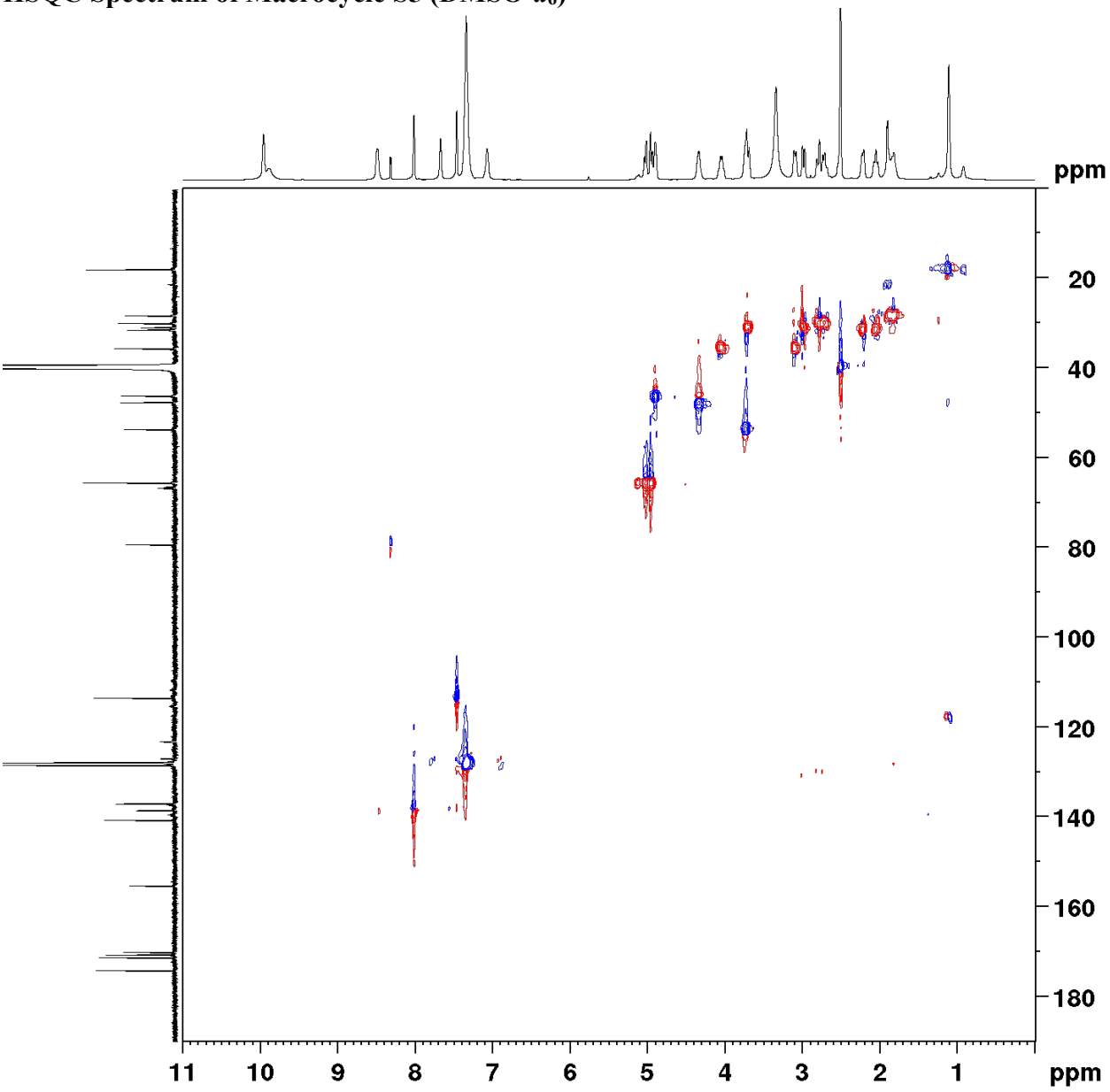
^{19}F NMR of Macrocycle S5 (DMSO-d_6, 282 MHz)



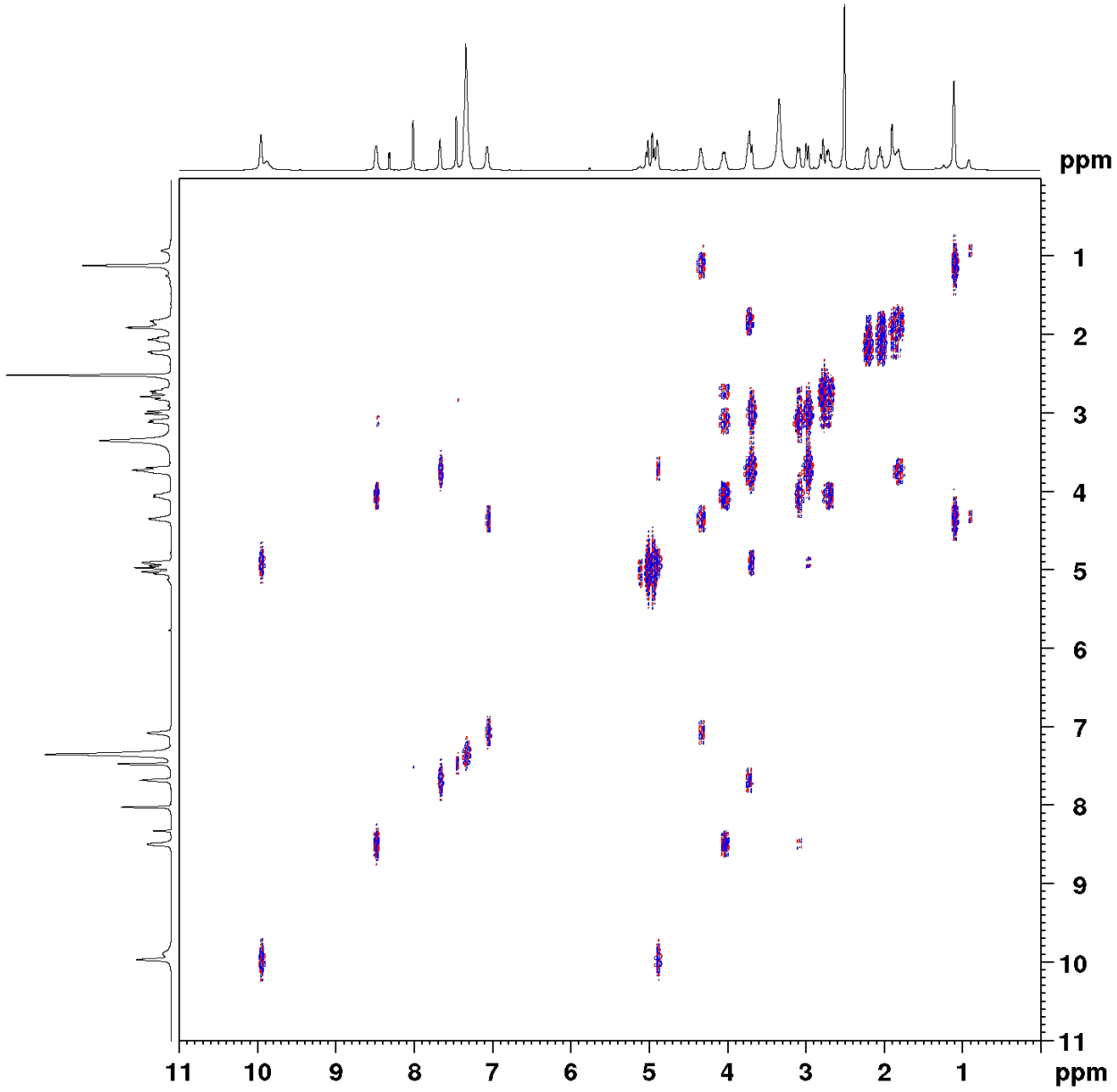
HMBC Spectrum of Macrocycle S5 (DMSO-d_6)



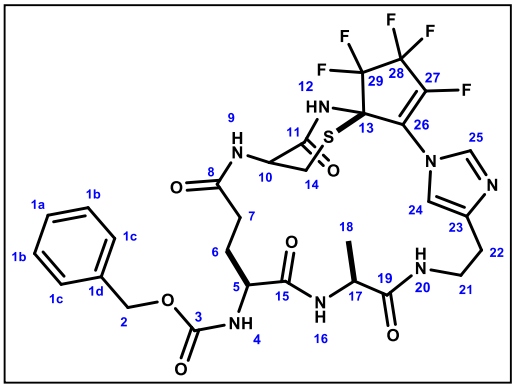
HSQC Spectrum of Macrocycle S5 (DMSO-d_6)



COSY Spectrum of Macrocycle S5 (DMSO-d_6)

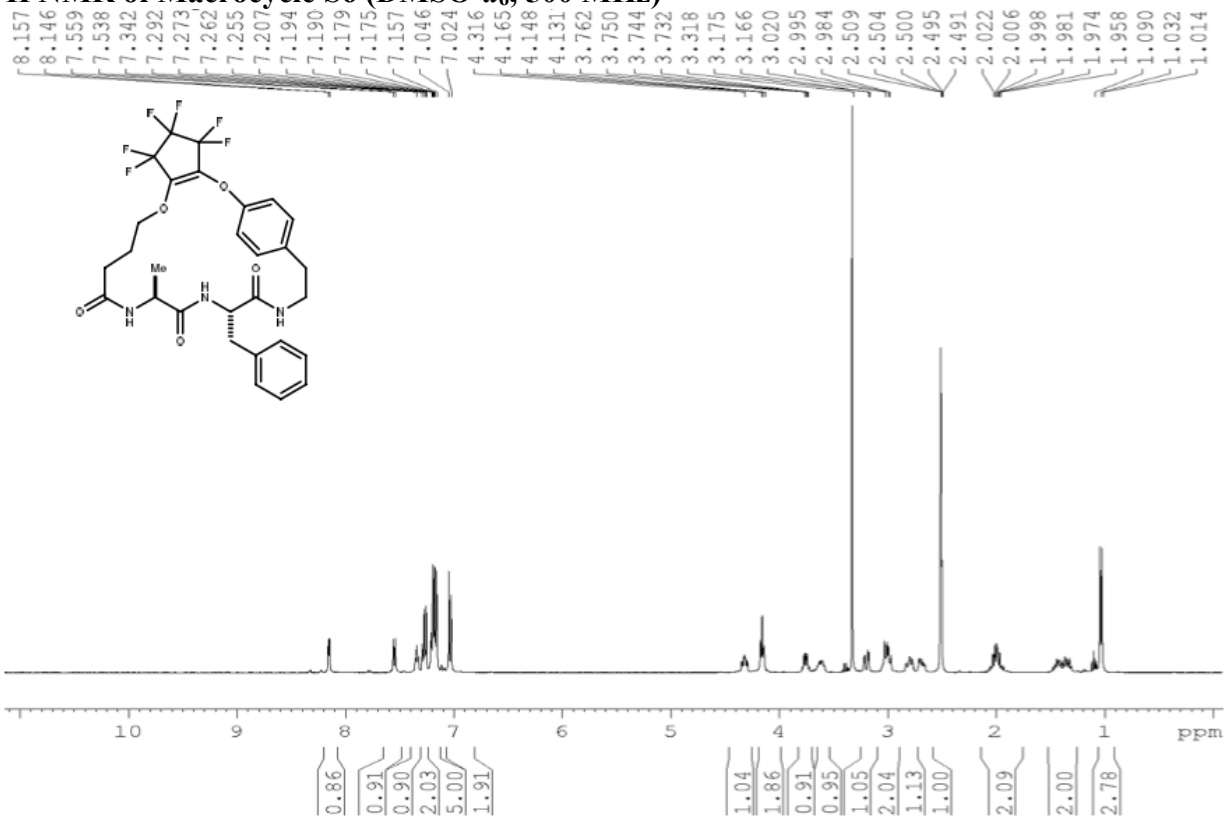


Macrocycle S5 (DMSO-d_6)

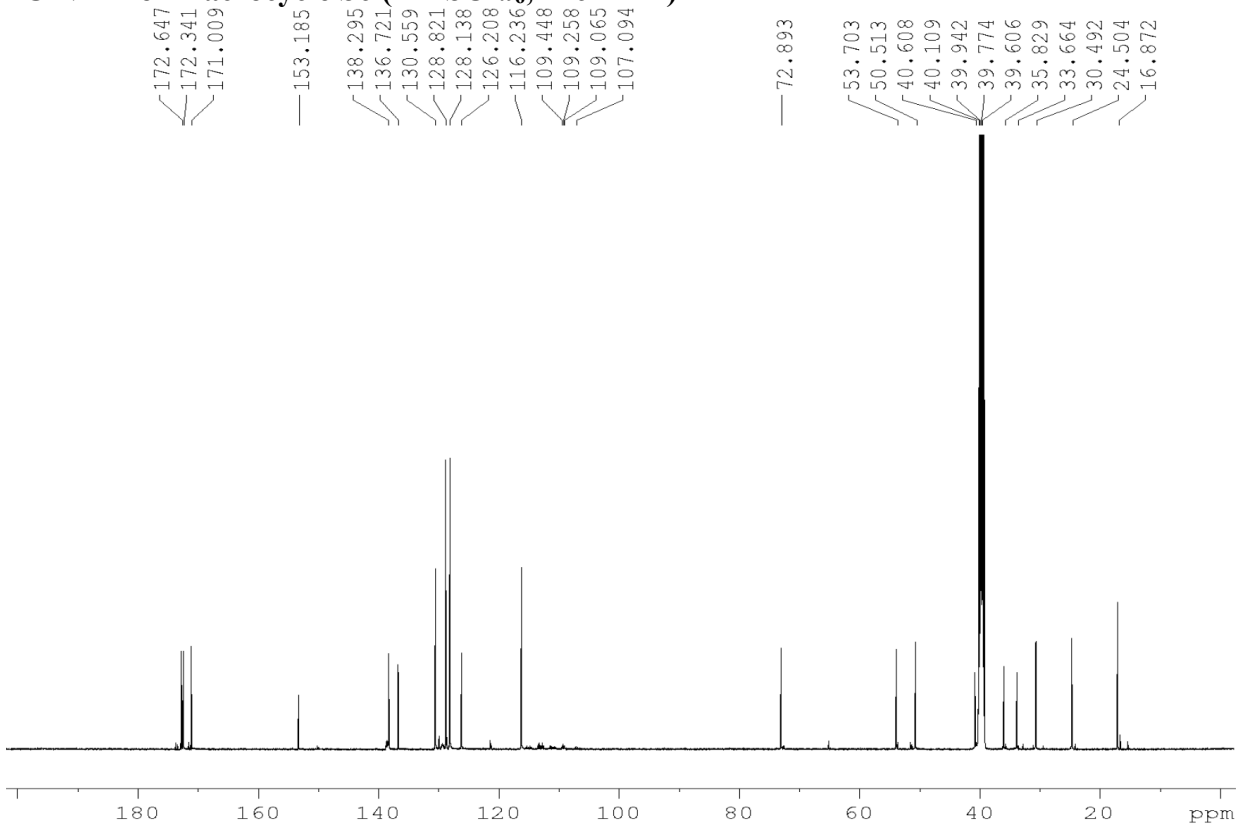


	13C	1H	Key Correlation
1a	127.8	7.42 - 7.24 (m, 5H)	HMBC 2 -> 1d, 1c, 3
1b	128.4	7.42 - 7.24 (m, 5H)	HMBC 1b -> 1c, 1d
1c	127.9	7.42 - 7.24 (m, 5H)	HMBC 1c -> 2
1d	137	-	HMBC 1d -> 2
2	65.4	5.07 - 4.92 (m, 2H)	HMBC 2 -> 1d, 1c, 3
3	155.3	-	HMBC 2,4 -> 3
4	-	7.67 (s, 1H)	HMBC 4 -> 3,5
5	53.5	3.8 - 3.64 (m, 1H)	HMBC 5 -> 6, 15
6	28.2	1.97 - 1.72 (m, 2H)	HMBC 6 -> 5,7
7	31.4	2.29 - 2.16 (m, 1H); 2.13 - 1.98 (m, 1H)	HMBC 7 -> 6,8
8	171.3	-	HMBC 7 -> 8
9	-	9.96 (bs, 1H)	COSY 9 -> 10
10	46	4.89 (s, 1H)	HMBC 10 -> 14, 11
11	170	-	HMBC 12, 14 -> 11
12	-	9.88 (bs, 1H)	HMBC 12 -> 10
13	66.6 (m, 1C)	-	HMBC 14 -> 13
14	30.9	2.98 (d, J = 15.0 Hz, 1H); 3.8 - 3.65 (m) (1H)	HMBC 14 -> 13, 10
15	170.6	-	HMBC 5, 6 -> 15
16	-	7.07 (s, 1H)	HMBC 16 -> 15, 17
17	47.5	4.4 - 4.25 (m, 1H)	HMBC 17 -> 18
18	17.9	1.18 - 1.02 (m, 3H)	HMBC 18 -> 17
19	174.2	-	HMBC 17, 20 -> 19
20	-	8.48 (s, 1H)	HMBC 20 -> 19
21	35.5	4.04 (q, J = 12.3 Hz 1H); 3.09 (d, J = 13.0 Hz, 1H)	HMBC 21 -> 22
22	29.9	2.85 - 2.63 (m, 2H)	HMBC 22 -> 23
23	140.6	-	HMBC 22, 24 -> 23
24	113.4	7.46 (s, 1H)	HMBC 24 -> 23, 26
25	138.4	8.01 (s, 1H)	HMBC 24 -> 25
26	123.3 - 122.9 (m, 1C)	-	HMBC 24 -> 26
27	139.8 - 139.1 (m, 1C)	-	
28	115.5 - 109.5 (m, 2C)	-	
29	115.5 - 109.5 (m, 2C)	-	

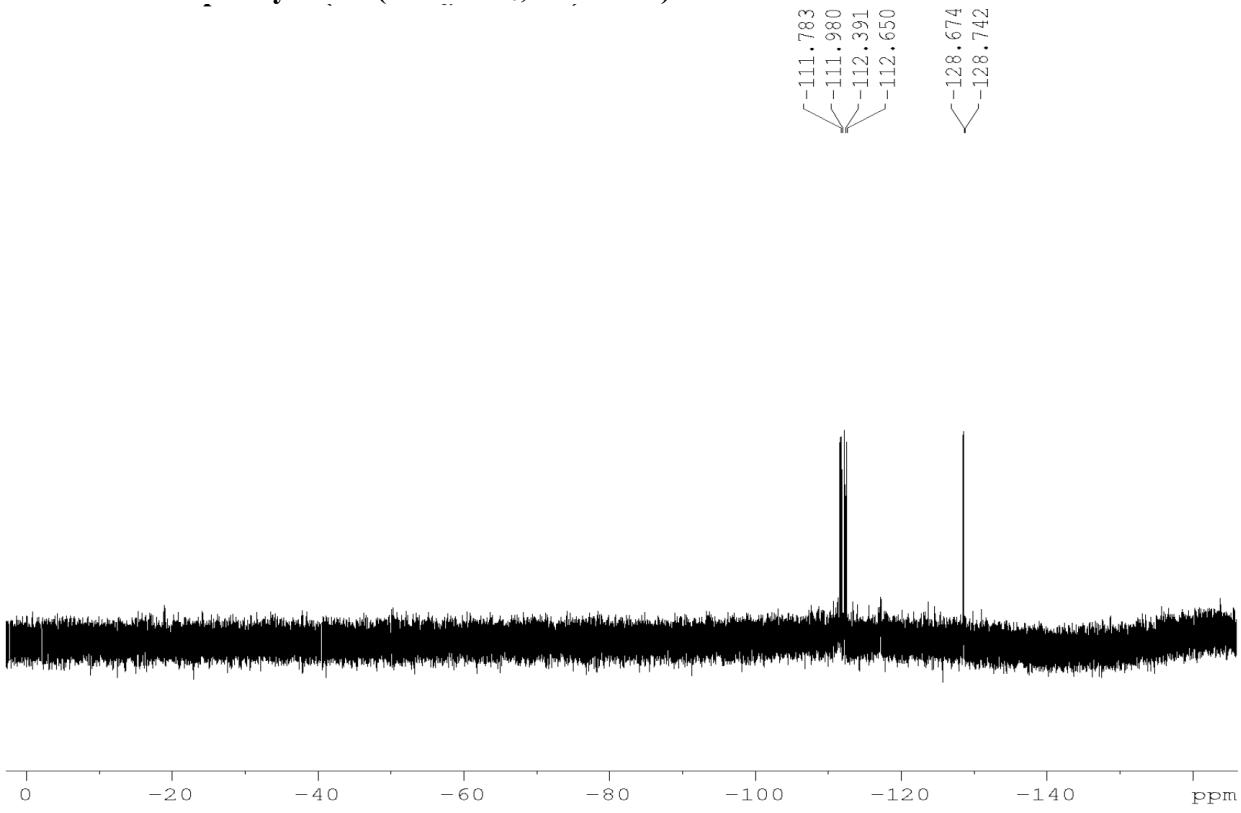
^{1}H NMR of Macrocycle S6 (DMSO-d_6, 500 MHz)



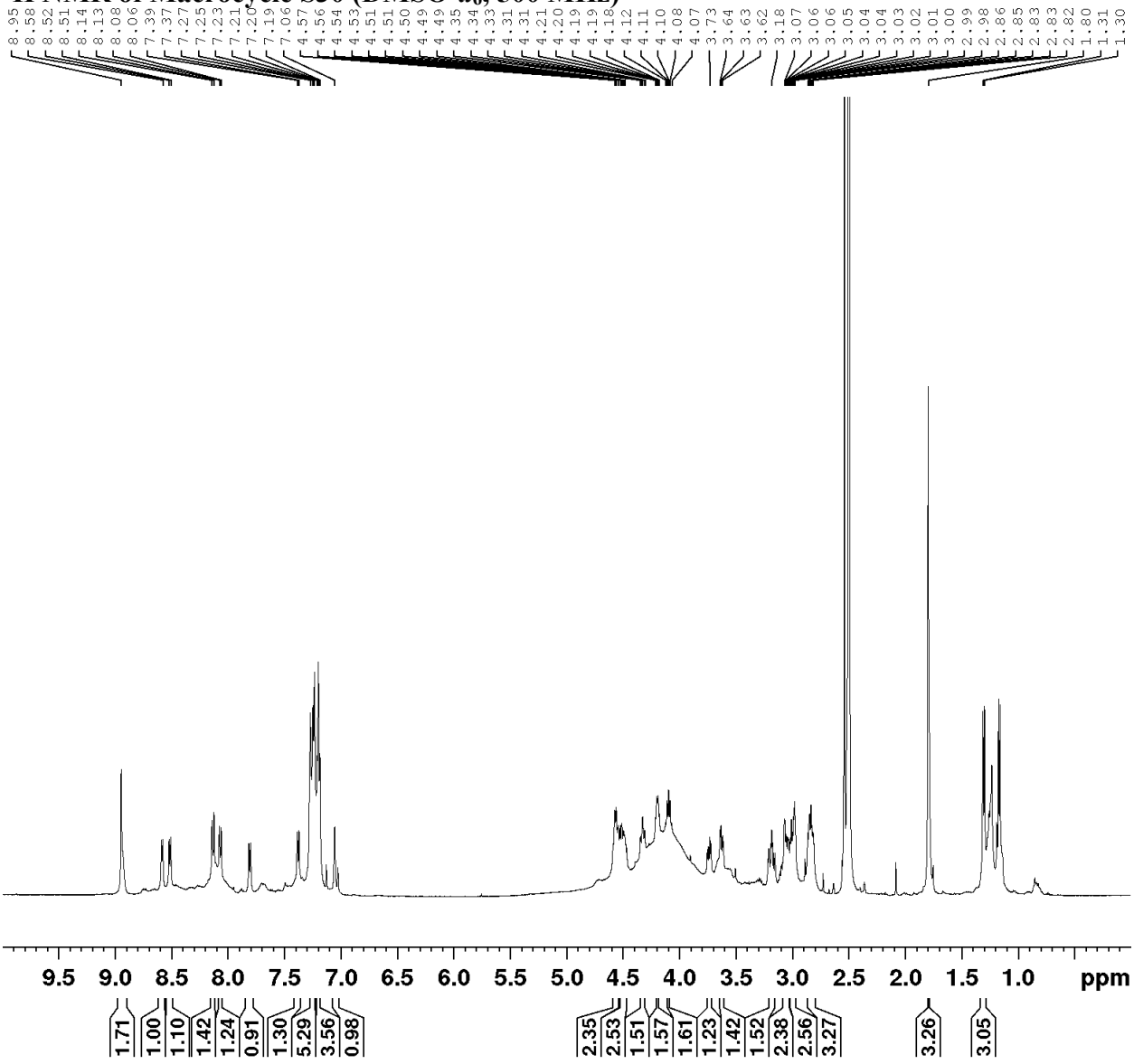
^{13}C NMR of Macrocycle S6 (DMSO-d_6, 126 MHz)



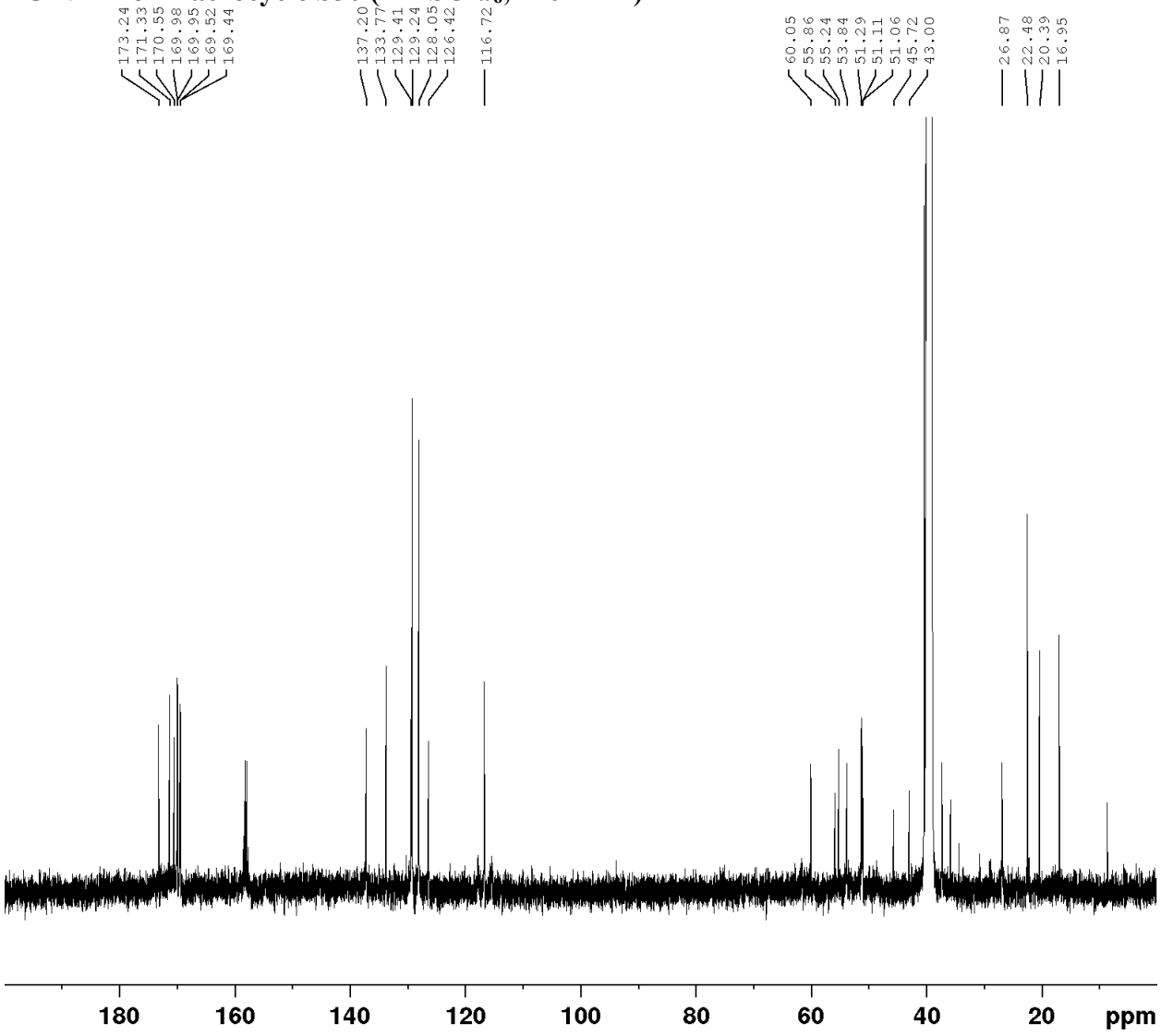
^{19}F NMR of Macrocycle S6 (DMSO-d_6, 500 MHz)



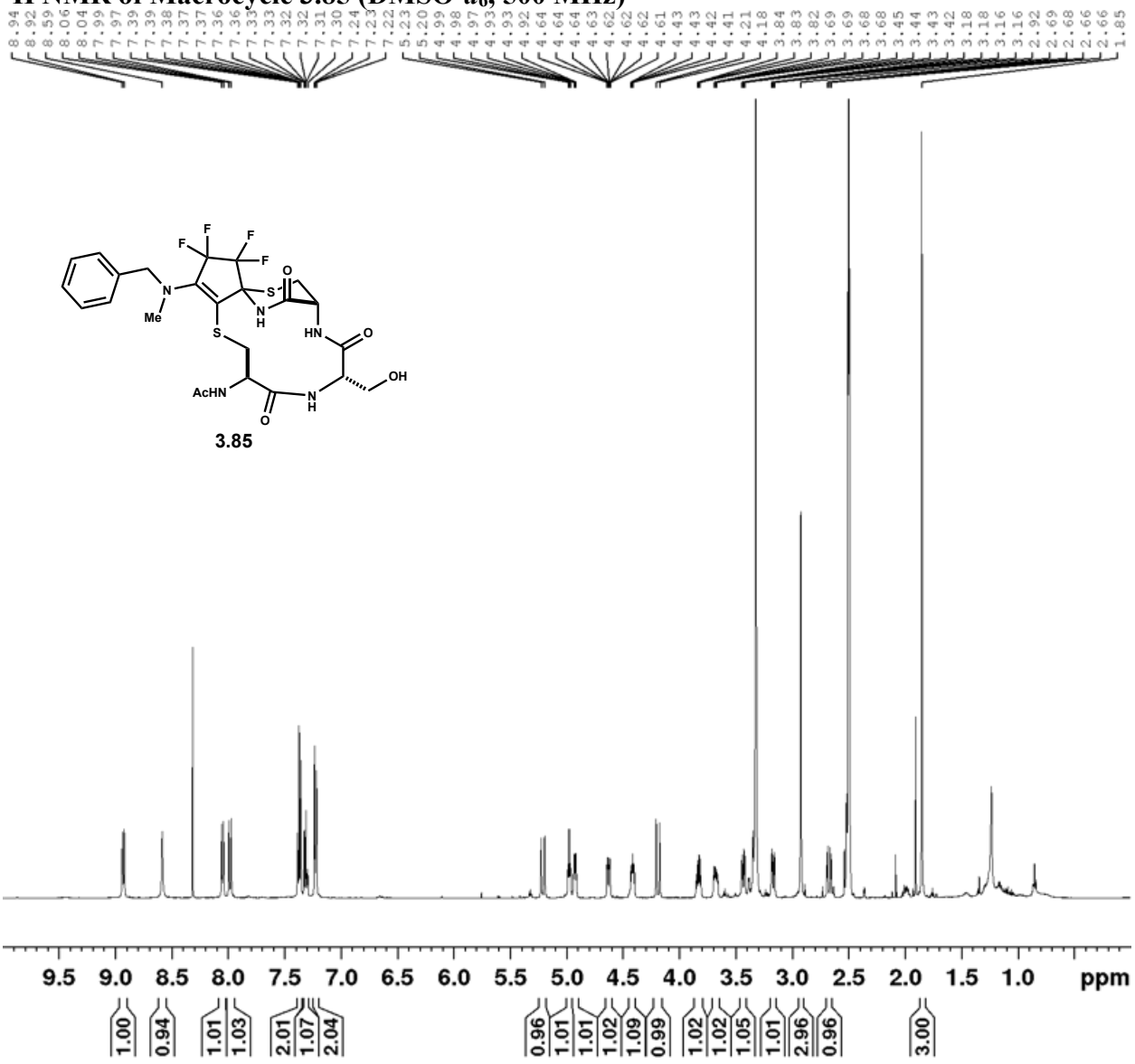
^{1}H NMR of Macrocycle S30 (DMSO-d_6, 500 MHz)



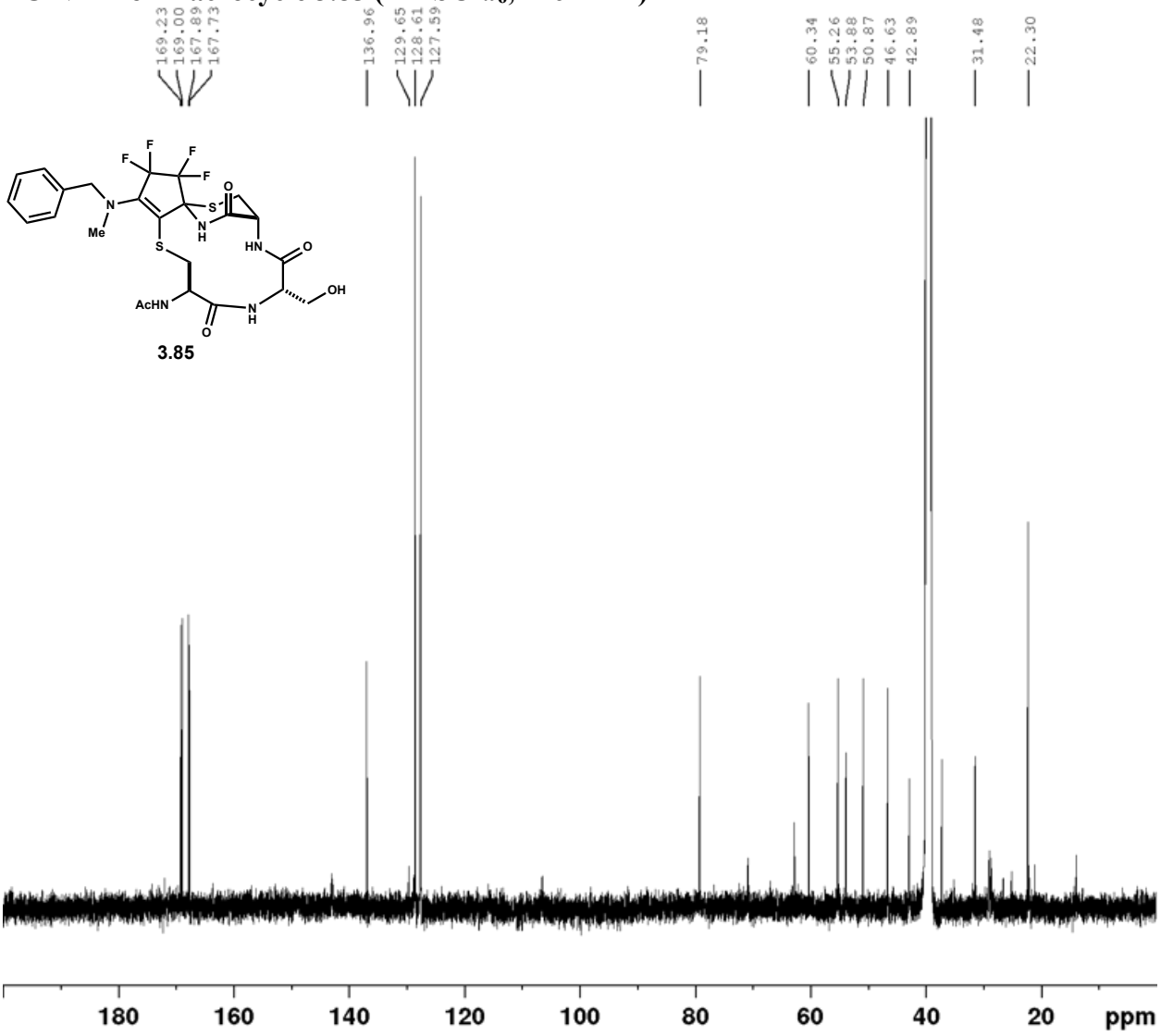
^{13}C NMR of Macrocycle S30 (DMSO-d_6, 126 MHz)



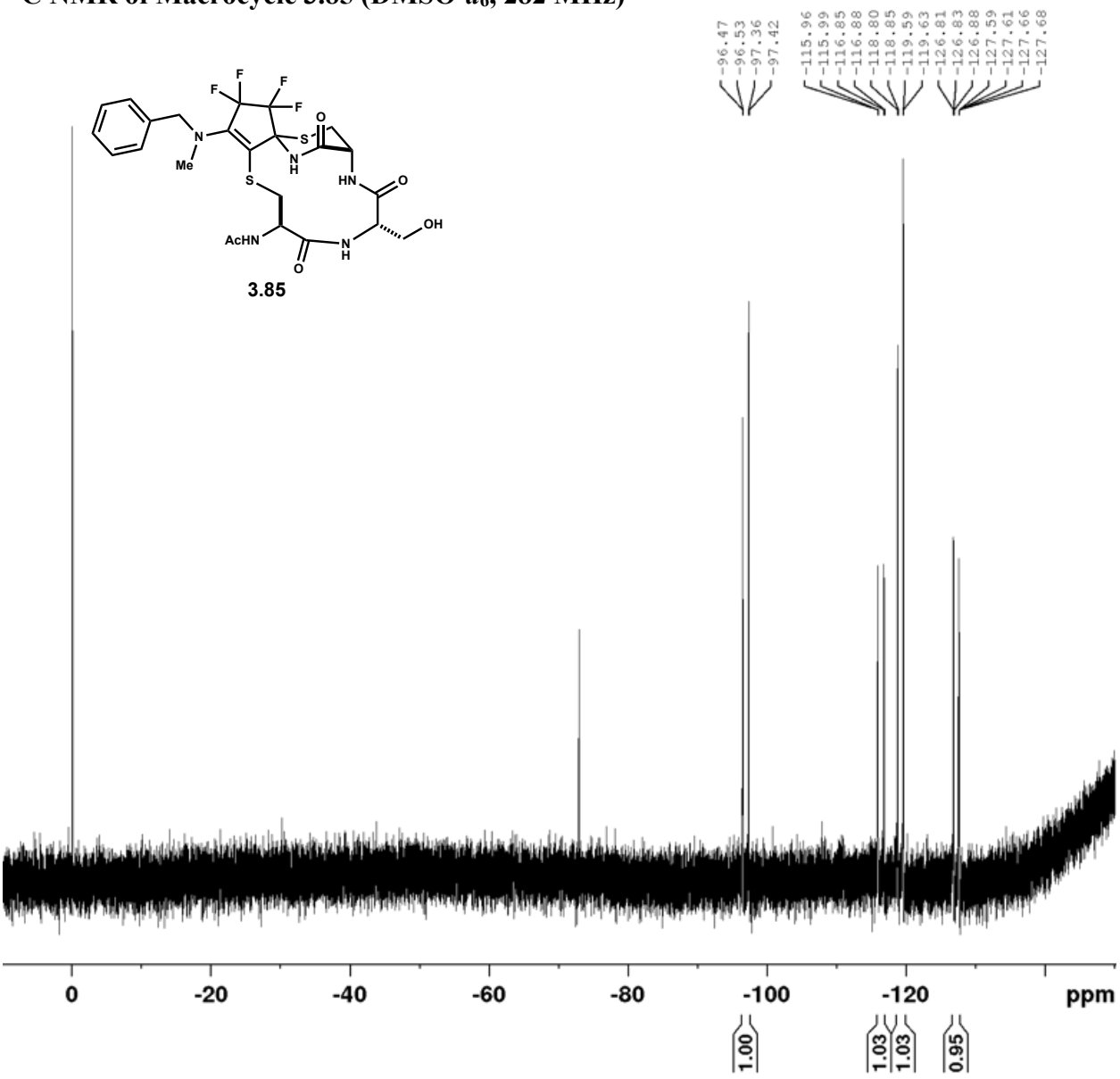
^{1}H NMR of Macrocycle 3.85 (DMSO-d_6, 500 MHz)



^{13}C NMR of Macrocycle 3.85 (DMSO-d_6, 126 MHz)

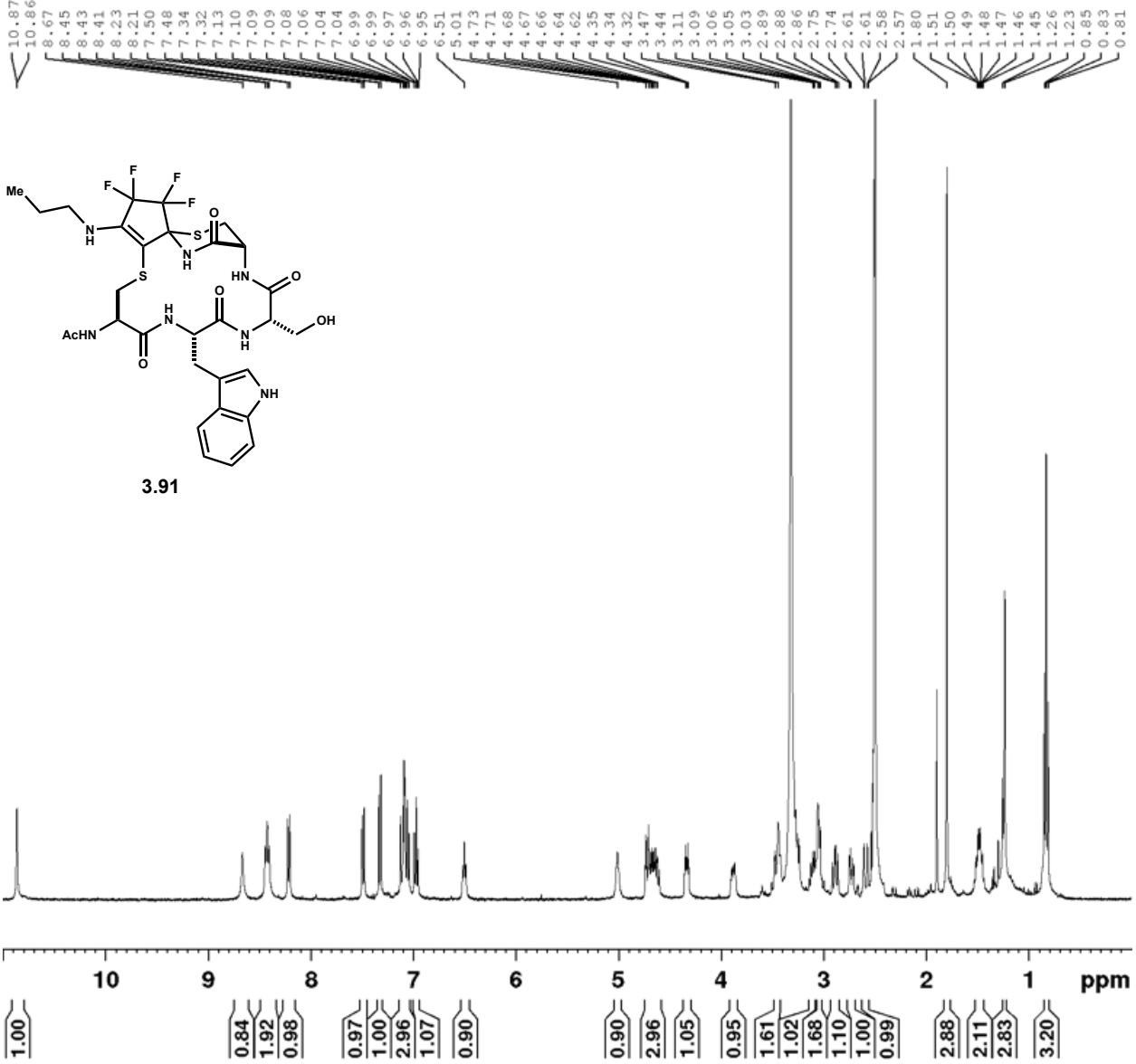


^{19}C NMR of Macrocycle 3.85 (DMSO-d_6, 282 MHz)



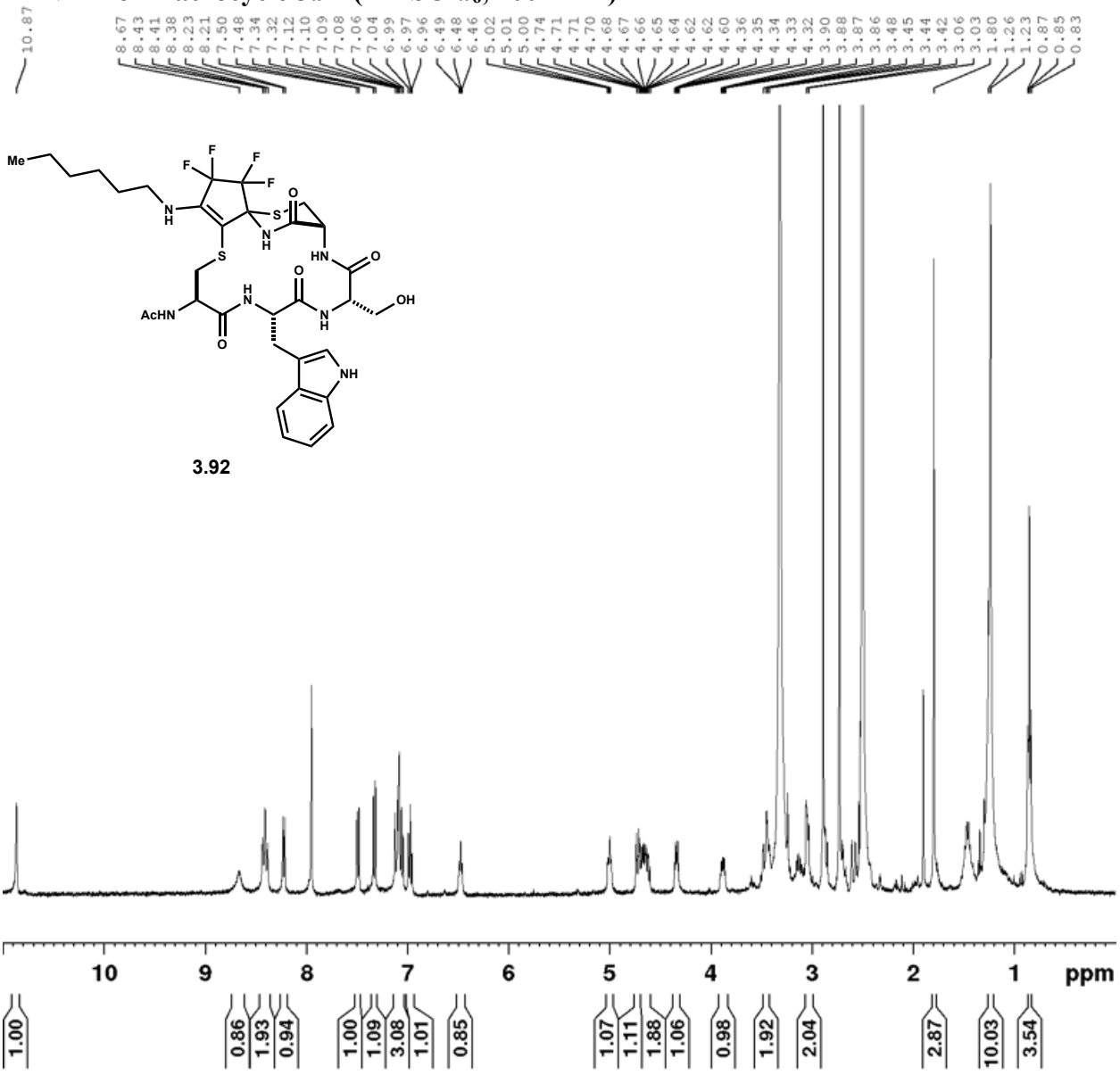
3.85

^{1}H NMR of Macrocycle 3.91 (DMSO-d_6, 400 MHz)



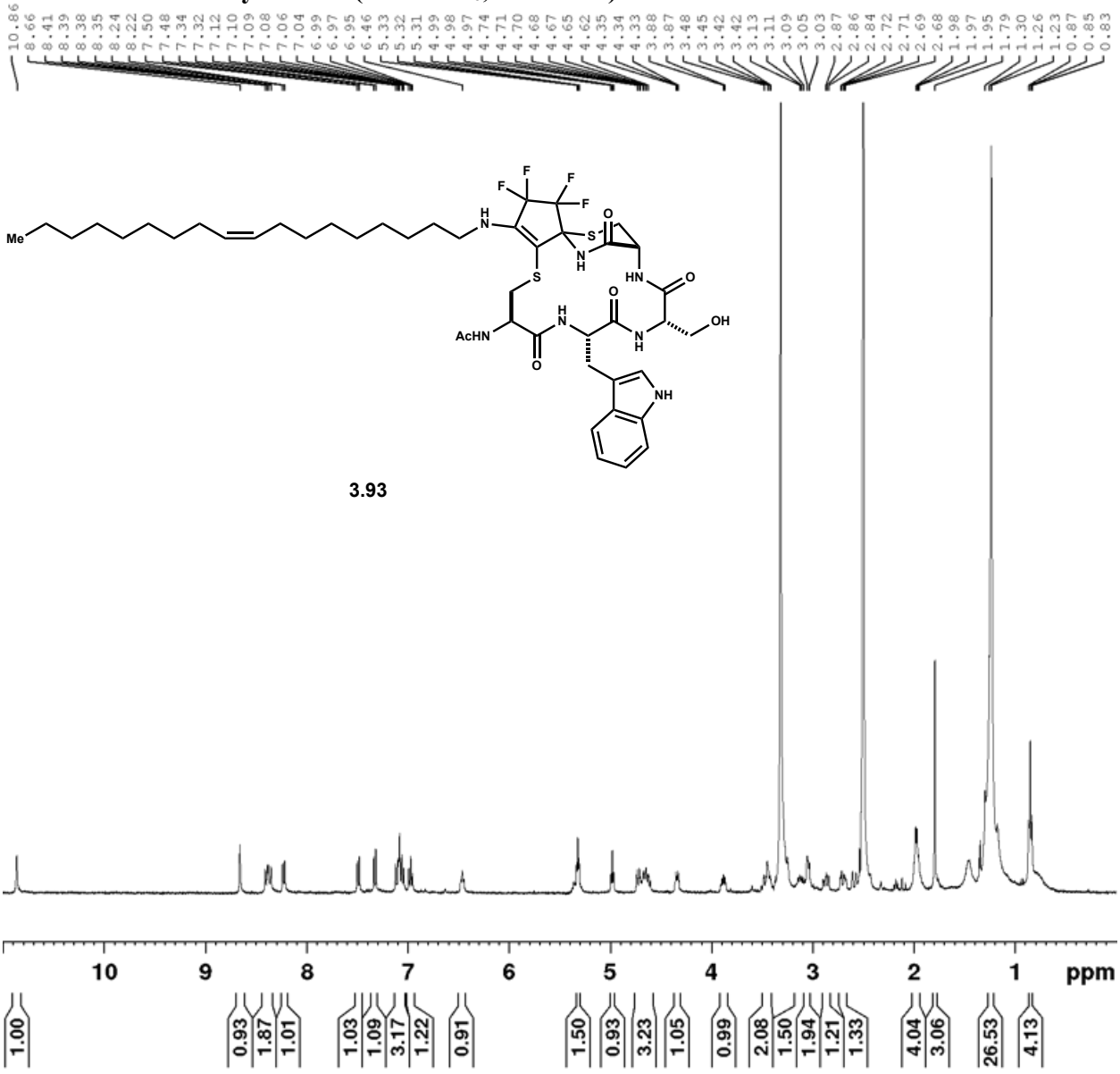
3.91

^{1}H NMR of Macrocycle 3.92 (DMSO-d_6, 400 MHz)

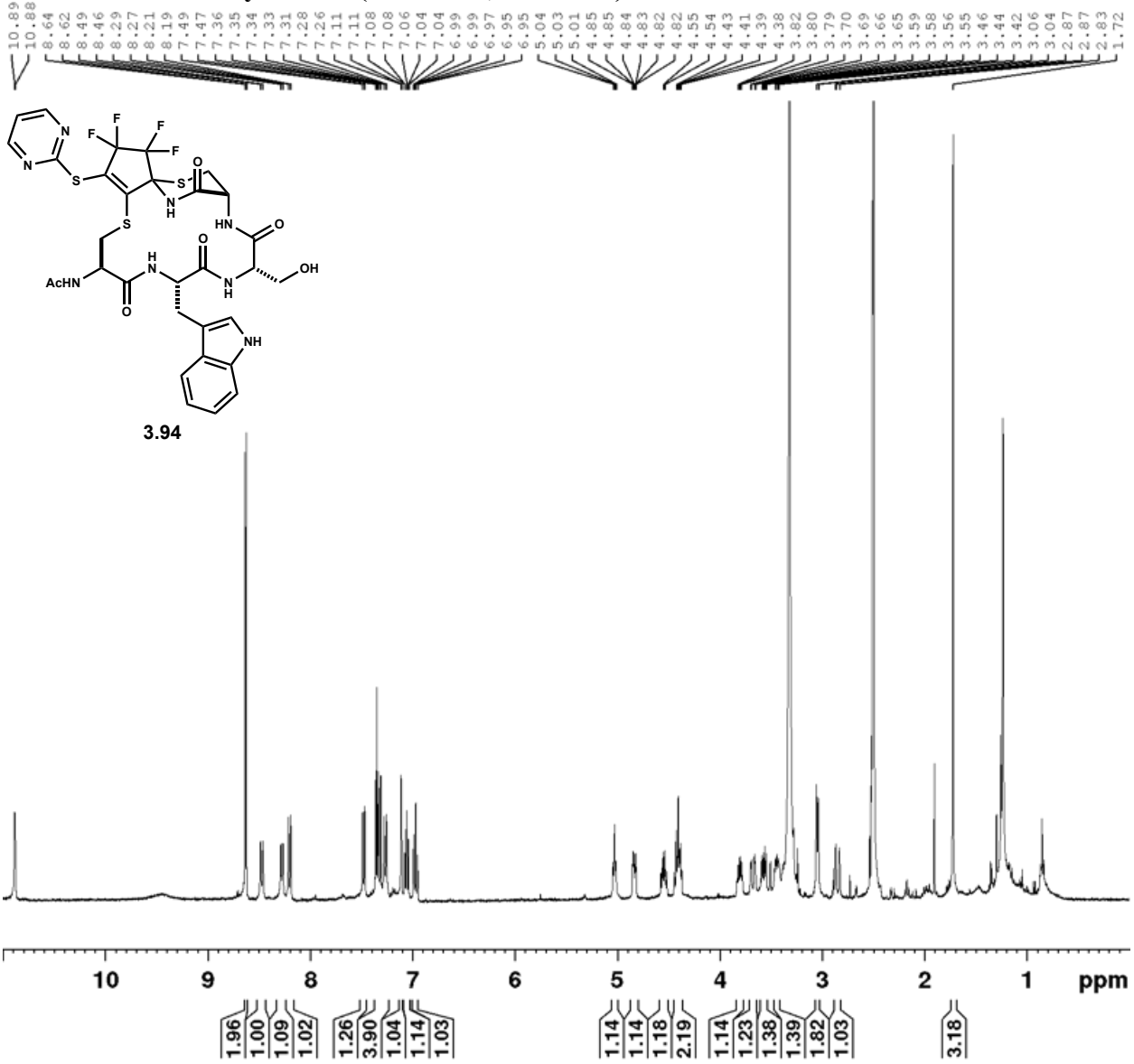


3.92

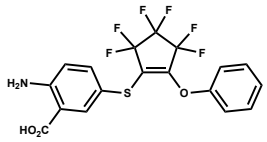
^{1}H NMR of Macrocycle 3.93 (DMSO-d_6, 400 MHz)



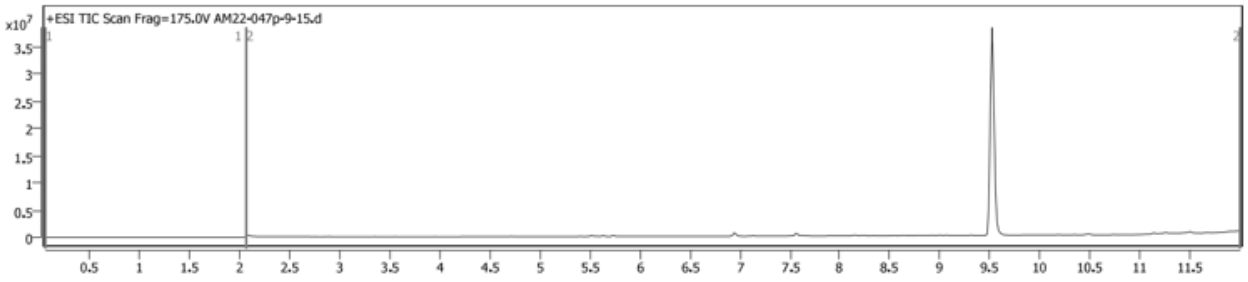
^{1}H NMR of Macrocycle 3.94 (DMSO-d_6, 400 MHz)



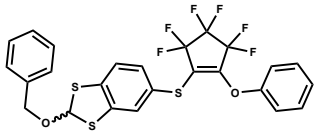
HPLC Mass Trace of **3.114**



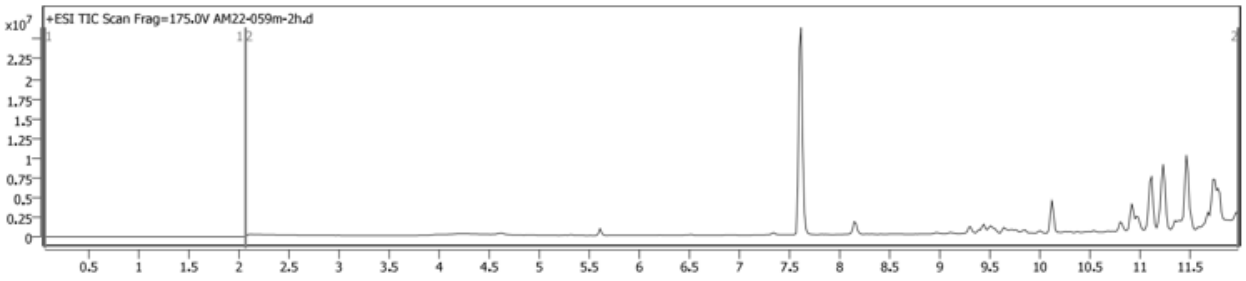
3.114



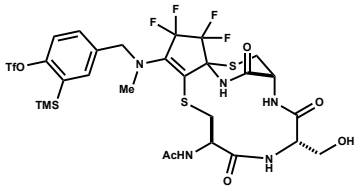
HPLC Mass Trace of crude **3.115**



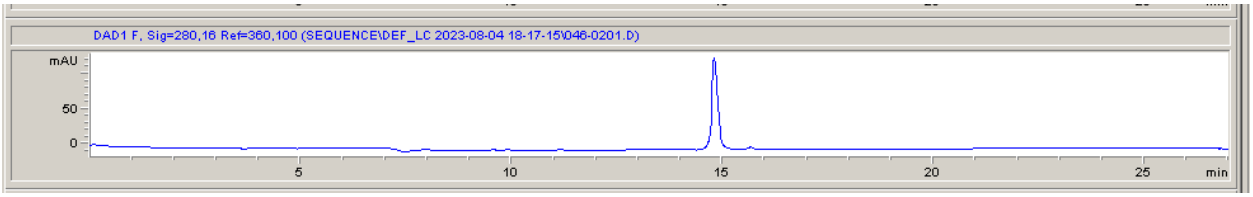
3.115



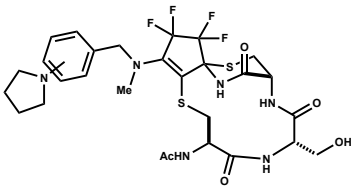
HPLC UV Trace (280 nm) of **3.118**



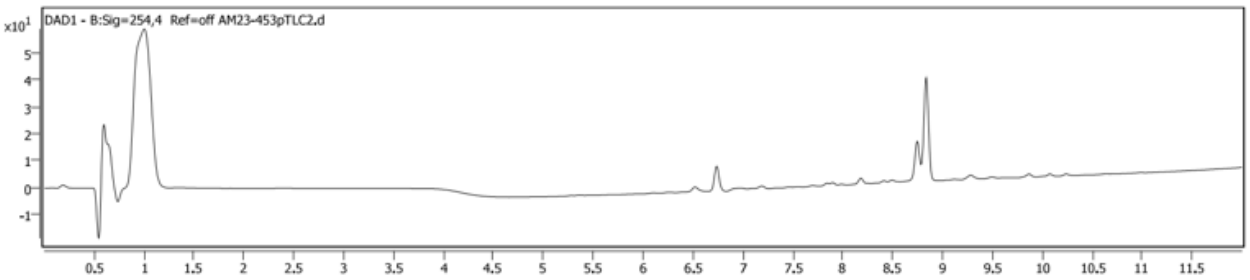
3.118



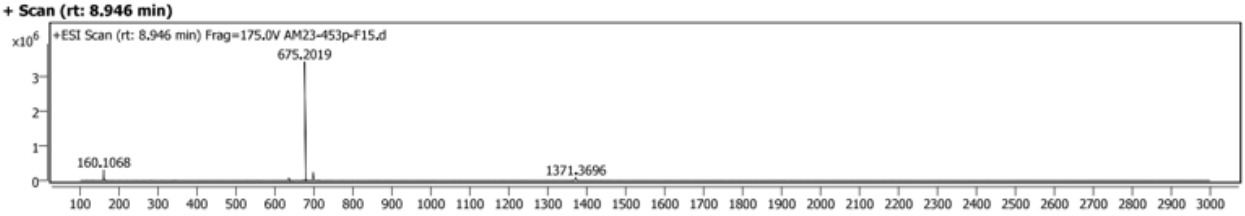
HPLC UV Trace (254 nm) and HRMS trace of **3.118**



3.119



+ Scan (rt: 8.946 min)



7.2 Variable Temperature NMR Experiments

^{1}H NMR of Polycycle 42 (DMSO-d_6, 500 MHz)

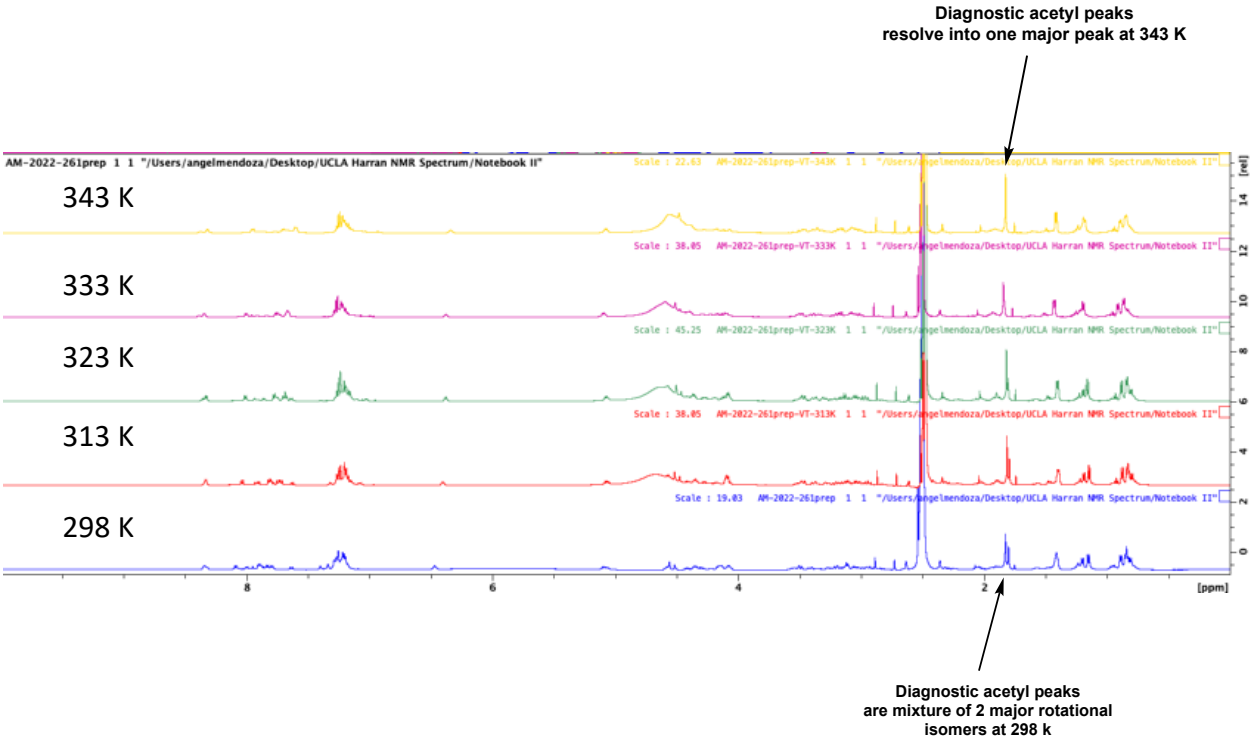


Fig. S25. Variable temperature NMR experiment of polycycle **42**. Temperature range from 298 K – 343 K. At 298 K, diagnostic acetyl peaks are at a ratio of ~3:2 due to rotational isomers. Acetyl peak resolves into one major peak at 343 K. ~4.5 ppm is water hump. NMR sample was stored in the freezer from between taking of 298 K spectrum and 313 K – 343 K VT NMR experiment. 298 K proton spectrum was taken 2 weeks before VT NMR experiment.

^{1}H NMR of Polycycle 45 (DMSO-d_6, 500 MHz)

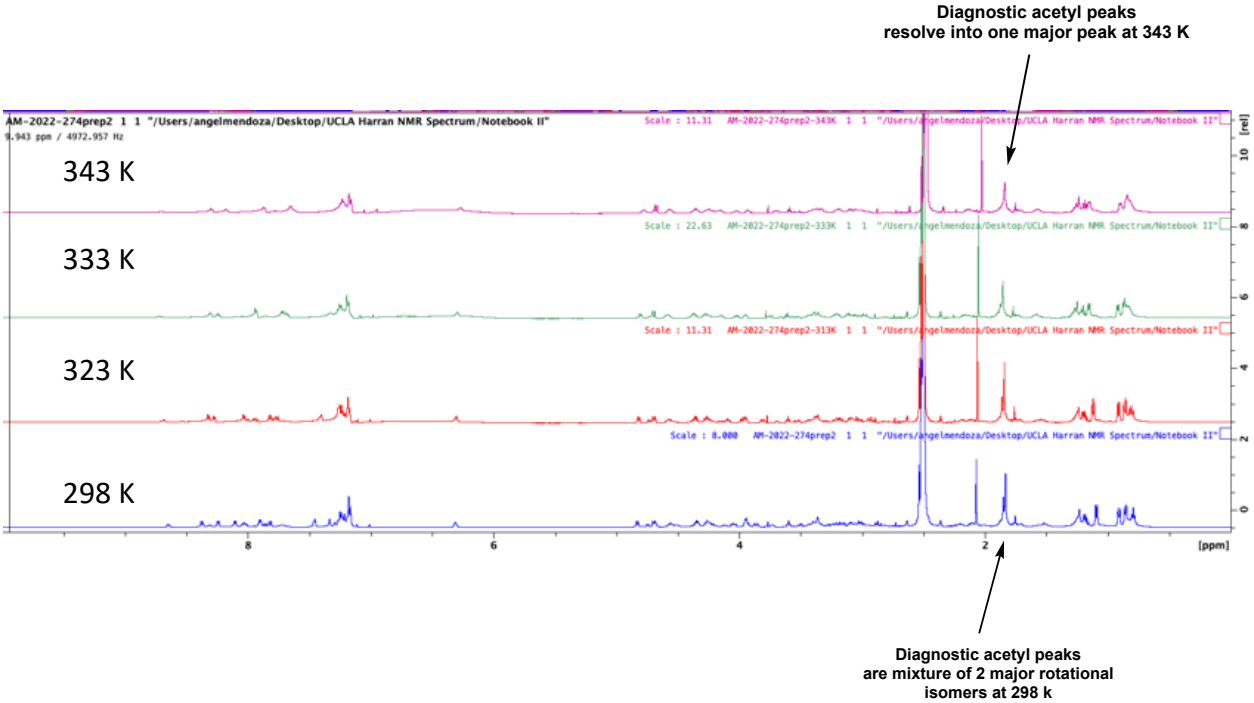


Fig. S26. Variable temperature NMR experiment of polycycle **45**. Temperature range from 298 K – 343 K. At 298 K, diagnostic acetyl peaks are at a ratio of ~2:1 due to rotational isomers. Acetyl peak resolves into one major peak at 343 K.

7.3 Crystal Structure Analysis

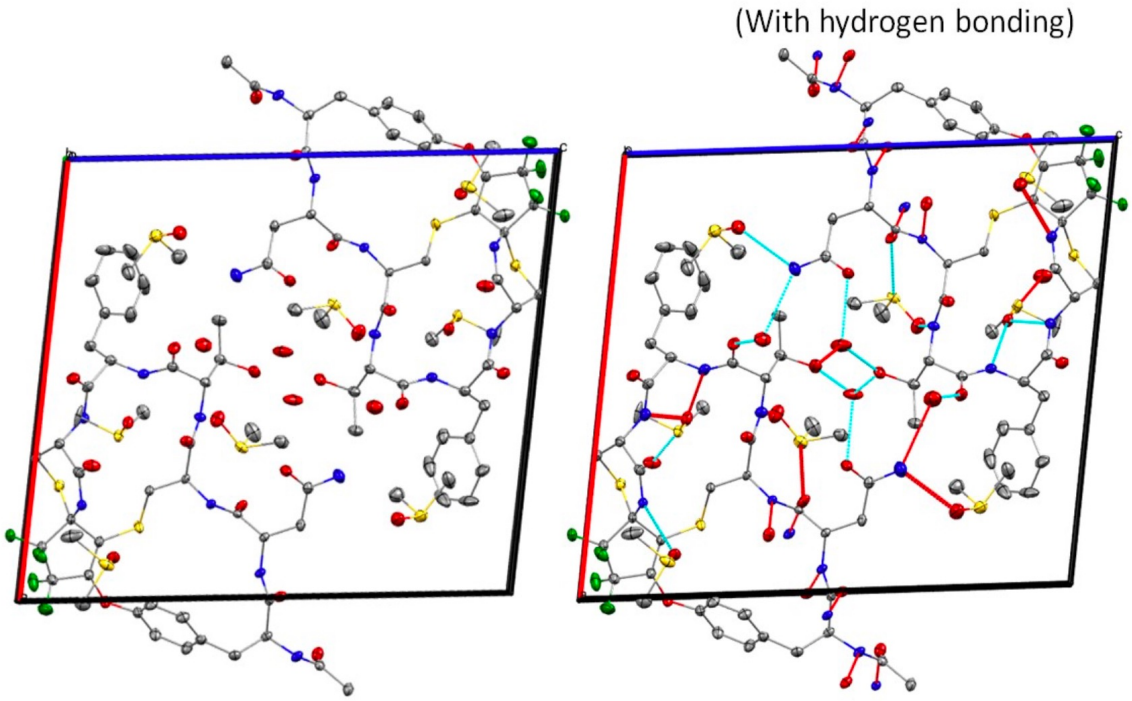


Fig. S27. Crystal Structure of polycycle **9**. Structure was solved in space group P2 with ORtEP showing 50% probability. Both residual DMSO and water molecules can be observed within the unit cell. Water atoms can be seen localized between the two asymmetric units of the unit cell and may be providing additional stabilization effects through hydrogen bonding.

8. Supplemental References

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