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Molecular Amalgamations that Generate Constrained Peptidomimetic Macrocycles

Author

Bernardino, Salvador Jurado

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Molecular Amalgamations that Generate Constrained Peptidomimetic Macrocycles

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy in Chemistry

by

Salvador Jurado Bernardino

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ABSTRACT OF THE DISSERTATION

Molecular Amalgamations that Generate Constrained

Peptidomimetic Macrocycles

by

Salvador Jurado Bernardino

Doctor of Philosophy in Chemistry

University of California, Los Angeles, 2022

Professor Patrick G. Harran, Chair

Peptidomimetic macrocycles are increasingly growing as attractive scaffolds for the development of therapeutics targeting protein-protein interactions. Compounds of this type have been traditionally underrepresented in drug discovery platforms due to their structural characteristics not conforming to typical guidelines for ‘drug-likeness’. We have developed methods to simultaneously investigate new bioactive chemotypes and their pharmacological properties in a systematic manner. Several libraries of peptidyl macrocyclic and polycyclic compounds are examined here based on previously developed template methodology and novel discoveries using a highly reactive fluorinated reagent. Both methods allow us to rapidly build

complexity from machine made, protecting-group-free bioactive starting materials. In this way, we can efficiently and broadly survey the chemical space of macrocycles through a diversity-oriented strategy.

In Chapter 2, we show that unprotected peptides react with commercial octafluorocyclopentene (OFCP) to afford hexafluorinated macrocycles via successive ipso substitutions of vinyl fluoride. Sequence variants having combinations of cysteine, tyrosine, histidine, and serine residues 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 details a broad scope of polycyclic compounds provided directly through one-pot methods. Macrocycle and spirocycle variations are also examined. We found the vinyl fluoride position to be amenable to linker systems that provide access to polycycles incorporating previously unused amino acid residues. Non-cell-based permeability assays are performed to evaluate the properties of these structures.

In Chapter 4, using cell-based permeability assays we study the pharmacological properties of macrocycles incorporating various template generations derived from a core cinnamyl alcohol motif. Structure-permeability relationships are explored with these compounds systematically via a small library.

The thesis of Salvador Jurado Bernardino is approved.

Jose A. Rodriguez

Hosea M. Nelson

Craig A. Merlic

Patrick G. Harran, Committee Chair

University of California, Los Angeles

2022

This dissertation is dedicated to my parents, Pablo and Maria Bernardino.

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VITA

FORMAL EDUCATION

University of California, Los Angeles, Los Angeles, CA
Doctor of Philosophy in Chemistry — Expected: December 2022
Master of Science in Chemistry — May 2019

University of California, Davis, Davis, CA
Bachelor of Science in Pharmaceutical Chemistry — June 2017

San Joaquin Delta Community College, Stockton, CA — May 2015
Associate of Science in Mathematics and Science
Associate of Art in Interdisciplinary Studies - Honors

PUBLICATIONS

S.J. Bernardino, A. Mendoza, M.J. Dweck, Declan, P.G. Harran, Cascade synthesis of fluorinated spiroheterocyclic scaffolding for peptidyl turns, loops, and macrobicyclic structures, *Manuscript in Preparation*.

T. Tsunemi, **S.J. Bernardino**, A. Mendoza, C. G. Jones, P. G. Harran, Syntheses of atypically fluorinated peptidyl macrocycles via sequential vinylic substitutions**,** *Angew. Chem.* **2020**, *132*, 684-688.

PRESENTATIONS

Bernardino, S.J., "Molecular Amalgamations that Generate Constrained Peptidomimetic Macrocycles" 2022 Organic Graduate Symposium, UCLA, Los Angeles, CA, June 10, 2022.

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HONORS - AWARDS

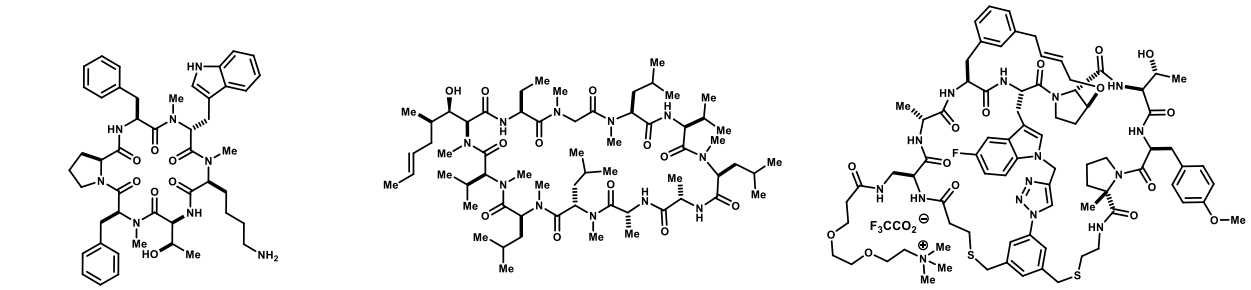
2022 Majeti-Alepati Dissertation Award
2021 Merck Research Award for Underrepresented Chemists of Color (UCC)
2017 – 2021 Eugene V. Cota-Robles Fellow
2017 American Chemical Society Scholar
2016 NSF-UF US/France REU Fellow
2015 Cal Aggie Alumni Association Leadership Scholar

Chapter 1 – Introduction

1.1 Background and Rationale

The use of small molecules at conventional protein targets such as enzymes, receptors and ion channels has historically been highly effective in treating many types of diseases.[1,2] However, these compounds are generally limited to targeting well-defined binding pockets. A majority of the human proteome include proteins involved in intracellular signaling events through so-called protein-protein interactions (PPIs).[3-5] In that regard, they provide the largest class of potential drug targets. However, the pockets and grooves present at the surfaces of proteins that bind to small molecules differ substantially from the shallow extended solvent-exposed surfaces of proteins involved in PPIs.[6] For this reason the classic small molecule drug discovery approach is not amenable to targeting PPIs and have been conventionally considered as “undruggable”. Most contact surfaces in protein-protein interfaces involve noncontiguous amino acid residues within the polymer chain. Although these interfaces are large, only a small subset of residues contribute to most of the binding-free energy known as ‘hotspots’.[7-12] Traditional high-throughput screening libraries, tend to represent a relatively small number of chemical scaffolds geared towards typical enzyme targets, which are not suitable for the particularity of these PPI interfaces.[13-14] Historically, strict adherence to chemotypes following ‘drug-likeness’ has hampered progress in this area.[15] Thus, creative construction of new chemical libraries through diversity-oriented methods is needed to more appropriately sample the chemical space required to target PPIs.

Peptides serve as a logical starting point for developing inhibitors of PPIs as they mimic natural protein-protein contacts. Macrocyclic peptides, however, retain the active structural components of linear peptides, while also improving key pharmacological properties. Their cyclic nature imparts on them several notable advantages over their linear counterparts including: (1) improved transport properties across biological membranes, achieved via the masking of polar



Tri-*N*-methylated Veber-Hirschmann peptide
somatostatin receptor (SRIF) ligand

Cyclosporine A
immunosuppressor

"Compound 44"
PCSK9 Inhibitor

Figure 1.1. Notable examples of modified/naturally occurring cyclic peptides and peptidomimetic macrocycles.

functionality through internal hydrogen bonding[16-17], (2) increased structural rigidity, in turn improving target binding affinity[18-20] and (3) their increased resistance to proteolytic degradation.[21-23] Various conventional macrocyclization techniques currently exist along with numerous contemporary methods recently published with increased interest in the field.[24-28] Over the years, peptidomimetic macrocycle design strategies have focused on mimicking secondary structures such as β- and γ-turns, β-strands, β-sheets and α-helices.[29-34] Simple chemical modifications to further improve properties have also been developed including the use of D-amino acids,[35] retro-inverse peptides,[36] N-methylation,[37] and side chain lipidation.[38] Cyclosporine A (CSA), a cyclic nonribosomal peptide natural product, is a well-known example of a permeable and orally bioavailable drug that violates conventional rules-of-thumb for drug-likeness.[39] CSA's capacity to passively diffuse across cell membranes has inspired many mechanistic studies with the intention of gaining insights to guide cell-permeable cyclic peptide design.[40-41] For example, N-methylations and D-amino acid incorporation of the Veber-Hirschman peptide (Figure 1), a somatostatin derivative with reported sst2 and sst5 somatostatin receptor selectivity, demonstrated improved bioavailability in Caco-2 cell monolayers, degradation resistance in plasma, and greater oral bioavailability in rats (F = 10%).[42] Regardless of these modifications, the conformation of the bioactive non-N-methylated parent peptide was preserved with the presence of dual β-turn structures. Recently, Merck has developed a potent tricyclic peptidomimetic PCSK9 inhibitor (termed "Compound 44") currently at the preclinical stage for the treatment of high LDL cholesterol

levels.[43] The structure has a molecular weight of 1612 g/mol and contains 9 H-bond donors yet demonstrates sufficient oral bioavailability to maintain therapeutic levels in animals. Despite these advances, efficient methods to incorporate unique biologically active lead structures with favorable properties are in demand.

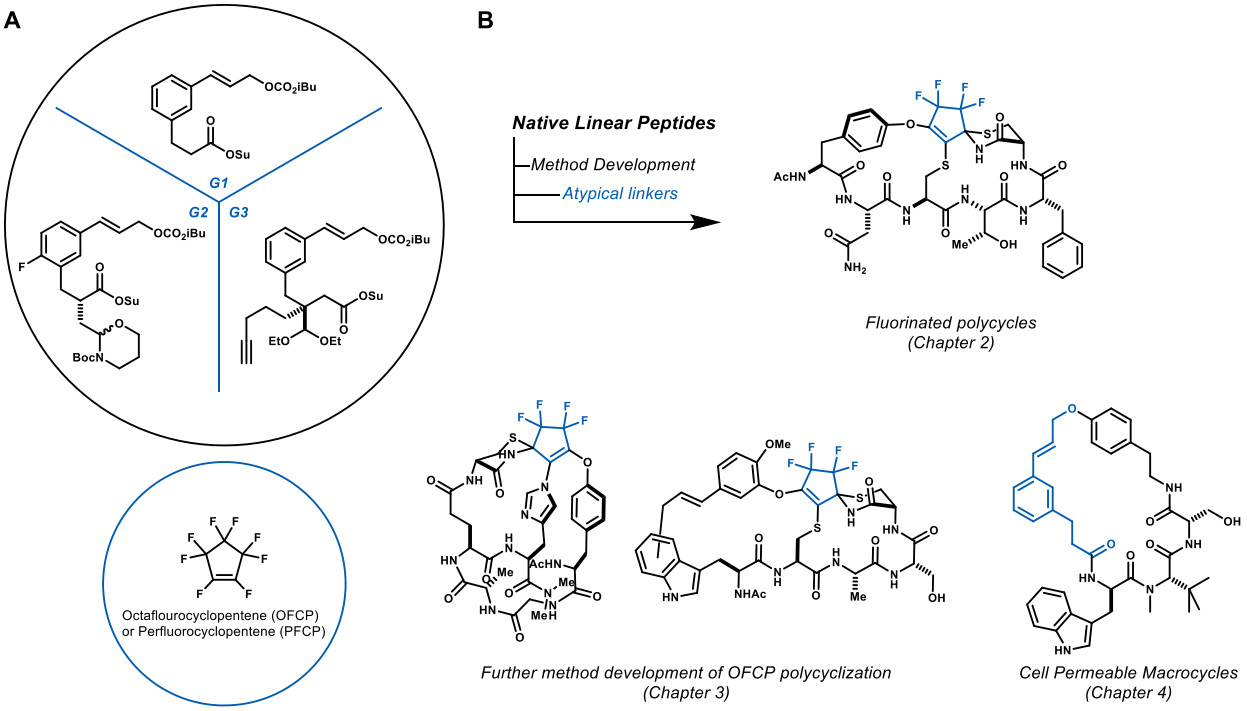


Figure 1.2. Outline of research detailed in this dissertation. **A.** Previously developed template and new commercially available linker systems that are incorporated in peptidyl macrocycles. **B.** Peptidomimetic macrocycles and polycycles synthesized using linkers.

For more than a decade, our lab has been interested in developing efficient, protecting-group-free methods for synthesizing macrocyclic peptides utilizing synthetic templates for facile macrocyclization.[44-49] By incorporating novel functionality into subsequent generations of our templates, we've been able to develop increasingly complex macrocycles while fine-tuning their physical properties and subsequent transformations. Discovery- and target-oriented methods to survey the therapeutic potential of these hybrid macrocycles are needed, and the efficient assembly-line like protocols we have developed directly address this issue while expanding a

previously narrow chemical space. This dissertation details the synthesis of peptidomimetic macrocycles/polycycles and subsequent studies of their pharmacological properties.

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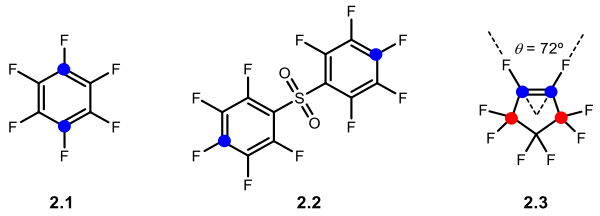
Chapter 2 – Synthesis of atypically fluorinated peptidyl macrocycles via sequential vinylic substitutions

2.1 Introduction

Ipso substitution of halogen to form large rings was first demonstrated during synthetic studies on vancomycin class antibiotics. In those experiments, biarylether motifs were formed by unimolecular S_NAr reactions involving phenoxide addition to fluoro nitro arenes.[1] More recently, exploiting reactivity of polyfluorinated aromatics, Spokoyny and Pentelute showed 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 decafluorodiphenyl sulfone **2.2** would react with diamine bearing peptides to afford 4,4'-diamino octafluorodiphenyl sulfone bridged macrocycles.[3-5]



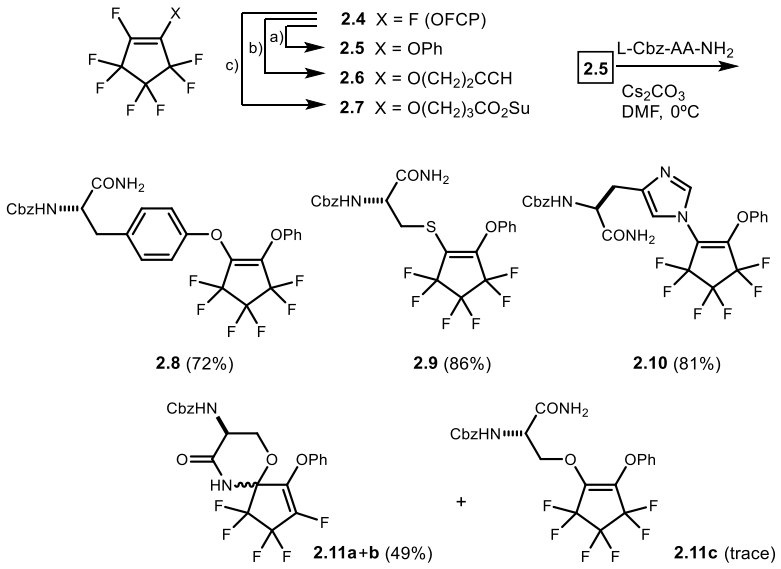
2.1 **2.2** **2.3**

Figure 2.1. Polyfluorinated arylation and vinylation reagents. Octafluorocyclopentene (OFCP) (**2.3**) has the potential to react multiple times as an electrophile, at both primary (blue) and secondary (red) positions.

In our own studies on constrained peptidomimetics, we were considering ways to build bridged bicyclic structures via 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 more compatible with smaller polyheterocyclic structures. Rather than aromatic substitutions, we choose to examine vinylic substitutions of fluoride from octafluorocyclopentene (OFCP, **2.3**, Figure 2.1).[7-16] Simple phenols[7], amines[10], mercaptans[14,15] and alcohols[14-16] were known to displace fluoride from this commercial reagent, and the extent of substitution could be varied. Herein we report that successive, dissymmetric substitutions[17] of fluoride on OFCP by functionalized peptides occurs readily, and provides flexible means to generate constrained fluorinated macrocycles.

2.2 Results and Discussion

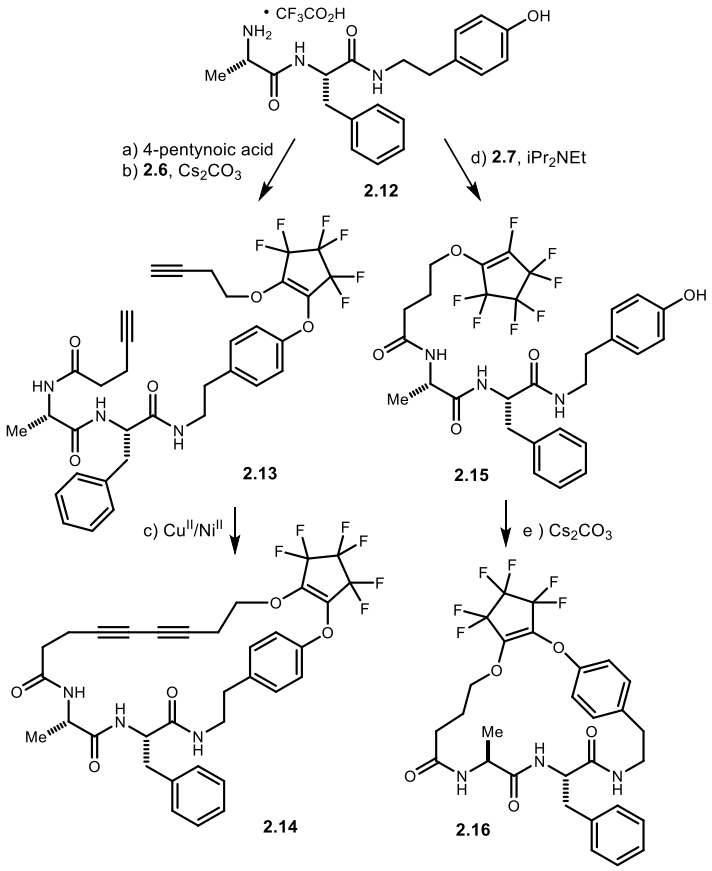
To model ring closure steps in projected macrocyclizations involving amino acid side chains, we first converted OFCP to mono phenoxy derivative **2.5** (0.9 eq PhOH, Et_3N, 74%). This molecule was found to react (Cs_2CO_3, DMF, 0 ˚C) individually with tyrosine, cysteine and histidine monomers to afford hexafluorocyclopentenylated adducts **2.8**, **2.9** and **2.10**, respectively, in high yields (Scheme 2.1). The reaction to give **2.9** was near instantaneous (< 15 sec) and those affording **2.8** and **2.10** were complete within minutes (see Appendix). L-Cbz-Ser-NH_2 reacted more slowly, and further. The carboxamide in initially formed substitution product **2.11c** internally displaced an allylic fluoride via 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 Appendix).



Scheme 2.1. Bimolecular amino acid derivatizations using OFCP. (a) OFCP, PhOH, Et_3N, DMF, 0 °C, 1 h, 74%. (b) OFCP, 3-butyn-1-ol, DMF, 0 °C to rt, 3 h, 57%. (c) i) OFCP, $HO(CH_2)_3CO_2Bn$, Et_3N, DMF, 0 °C to rt, 1 h, 61%. ii) H_2, 10% Pd/C, EtOH. iii) HOSu, EDC·HCl, DCM, rt, 3 h, 83% (2 steps).

To examine if single vinyl fluoride substitutions on OFCP could be used to form peptidyl macrocycles, we prepared butynyloxy (**2.6**) and carboxypropyloxy derivatives (**2.7**) of OFCP as shown in Scheme 2.1. Both structures were stable oils that could be purified by chromatography. Starting with the tyramide derivative of L-AF (**2.12**, Scheme 2.2), 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[18,19] afforded aryloxy hexafluorocyclopentene containing macrocyclic diyne **2.14**. We then tested if vinylic substitution could be made the ring closure event. Acylation of **2.12** with **2.7** and treating the product with Cs_2CO_3 (1.5 eq, DMF, 0 °C -> 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.



Scheme 2.2. 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) $Cu(OAc)_2 \cdot H_2O$, $NiCl_2$, Et_3N, pyridine, EtOH, 50 °C, 3 h, 52%. (d) **2.7**, iPr_2EtN, DMF, 0 °C, 1 h. (e) Cs_2CO_3, DMF, 10 mM, 0 °C to rt, 2 h, 47% from **2.12**.

We next tested if 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 (Scheme 2.3). Treatment of product **2.19** with Cs_2CO_3 in DMF selectively formed imidazole

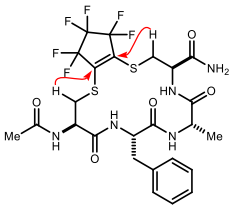
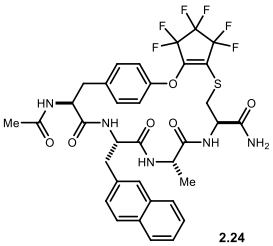
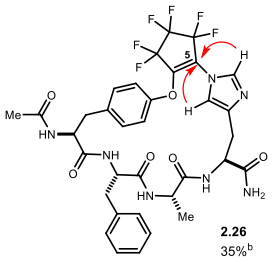
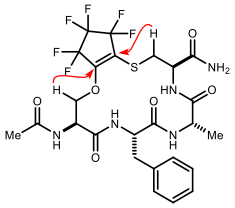
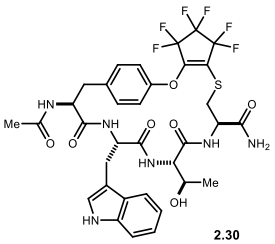
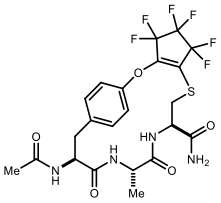
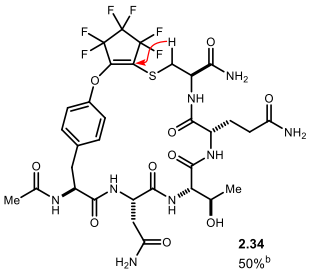
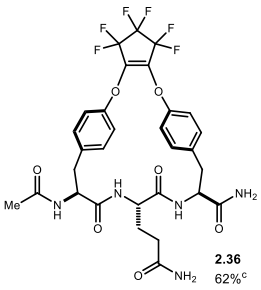
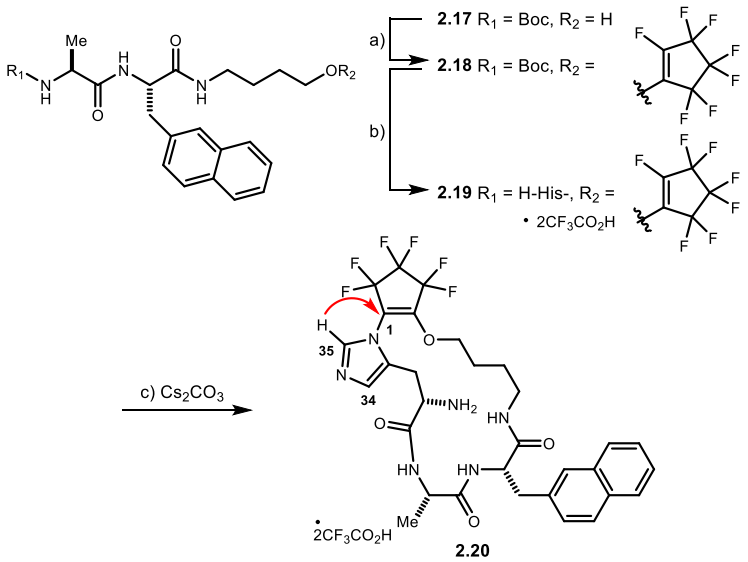
Ac-CFAC-NH_2 (**2.21**)	Ac-Y(2-Nal)AC-NH_2 (**2.23**)	Ac-YFAH-NH_2•TFA (**2.25**)	Ac-SFAC-NH_2 (**2.27**)
			
2.22 51%[a]	**2.24** 58%[b]	**2.26** 35%[b]	**2.28** 56%[b]
Ac-YWTC-NH_2 (**2.29**)	Ac-YAC-NH_2 (**2.31**)	Ac-YNTQC-NH_2 (**2.33**)	Ac-YQY-NH_2 (**2.35**)
			
2.30 42%[b]	**2.32** 46%[b]	**2.34** 50%[b]	**2.36** 62%[c]

Table 2.1. Direct macrocyclizations mediated by OFCP. Reagents and conditions: (a) OFCP, TEA, DMF, 0 °C, 2 h. (b) OFCP (5 eq), TEA, DMF, 0 °C, 30 min, then Cs_2CO_3, 0 °C, 30 min - 1 h. (c) OFCP, Cs_2CO_3, rt, 1h. — HMBC correlations.

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**, Table 2.1). Taken together, data from biomolecular models and incremental ring syntheses suggested linear peptides harboring combinations of cysteine, tyrosine, histidine and serine residues could participate in direct macrocyclizations mediated by OFCP. This proved facile for sequences containing two cysteine residues. Stirring Ac-CFAC-NH_2 (**2.21**) with 1.5 eq OFCP in the presence of Et_3N (DMF, 0 ˚C) provided macrocycle **2.22** in 51% isolated yield. In mixed nucleophile systems, a two-stage, one-pot procedure proved most effective. Tetrapeptide **2.23** contained cysteine and tyrosine residues. Exposure of **2.23** to 5.0 eq OFCP in the presence of Et_3N (DMF, 0 ˚C) selectively derivatized the thiol within minutes. Subsequent removal of excess OFCP (b.p. 27 ˚C, 1 atm) via rotary evaporation and adding solid

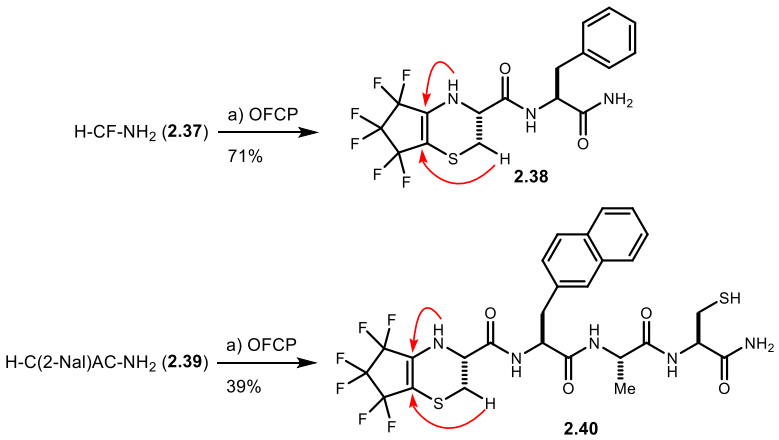
Cs_2CO_3 to the solution gave unique cyclophane **2.24**. The same protocol executed on Ac-YFAH-NH_2 engaged the tyrosine and histidine side chains to afford macrocycle **2.26**. The histidine reacted with OFCP first, vinylating its distal nitrogen, which after tyrosine capture produced a regioisomeric variant of the imidazole linkage observed in macrocycle **2.20** (Scheme 2.3). HMBC correlations were observed between C5 and both imidazole protons in **2.26**. Table 1 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 ˚C 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[20] except for serine linked macrocycle **2.28**. This molecule gradually ringed opened via net hydrolysis upon standing in solution at room temperature.[21]



Scheme 2.3 Free amine embedded OFCP macrocyclic products. (a) OFCP, Et_3N, DMF, rt, 3 h, 40%. (b) i) TFA, DCM, rt, 1 h. ii) Boc-His-OH, EDC·HCl, K-Oxyma, iPr_2EtN, DMF, rt, 3 h. iii) TFA, DCM, rt, 2 h, 45% from **2.18**. (c) Cs_2CO_3, DMF, 0 °C to rt, 2h, 39%. — HMBC correlation.

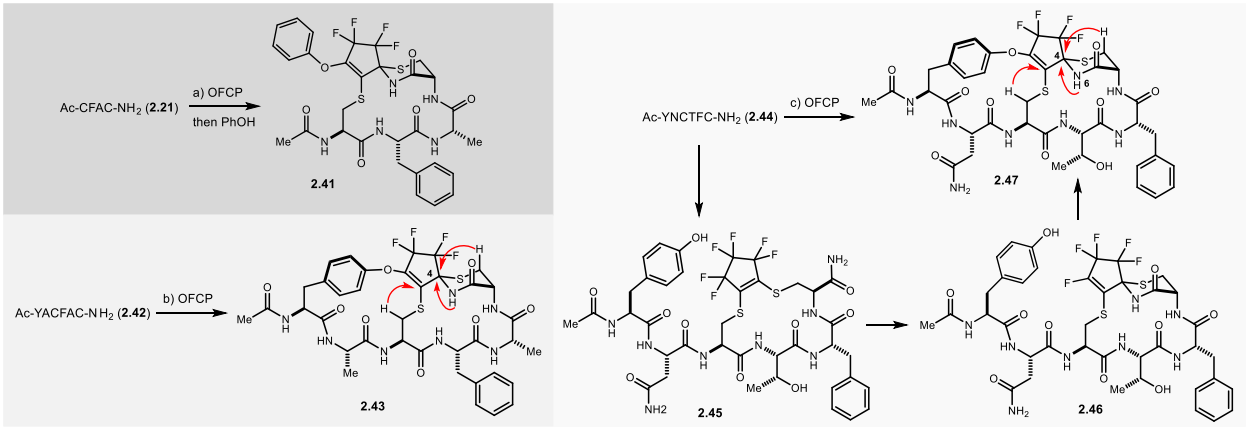
In free base form, the amino side chains of lysine and ornithine would react with OFCP, but the resultant substitution products were generally not stable. In contrast, OFCP reacted with free N-terminal cysteine residues to form isolable hexafluoro cyclopentathiazine derivatives

(Scheme 2.4). The reactions examined were complete within minutes. The ability to rapidly hexafluorocylopentenylate the N-terminus of peptides and peptidomimetics may have utility, for example, in probe syntheses for positron emission tomography.[22]



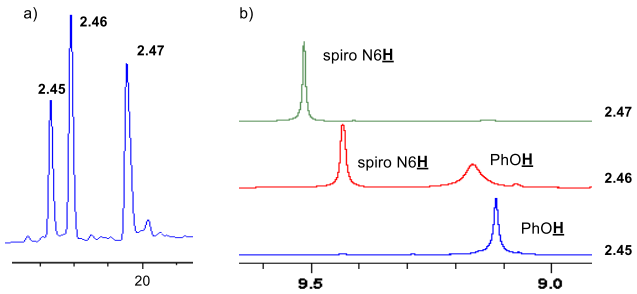
Scheme 2.4. Hexafluoro cyclopentathiazinone derivatives formed from N-terminal capture of OFCP. Reagents and conditions: (a) OFCP, TEA, DMF (10 mM), 0 °C, 30 min. — HMBC correlations.

Substituting the two vinyl positions on OFCP with heteroatoms gave products potentially susceptible to further internal reactions, as was observed during conversion of **2.11c** to **2.11a/b** (Scheme 2.1). Experimenting initially with **2.22**, we found that treatment with PhOH/Cs_2CO_3 in DMF gave spirocyclic thiazinone-containing phenyl ether **2.41**, wherein two additional fluorine atoms had been displaced from the cyclopentene core. This result implied that phenoxide could be captured intramolecularly. In fact, we discovered that hexapeptides of consensus sequence YXCXXC would undergo remarkable polysubstitution reactions with OFCP. Stirring Ac-YACFAC-NH_2 with 1.5 eq OFCP in DMF at 0 ˚C 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.



Scheme 2.5. Initial discovery of cascade polysubstitutions of OFCP. Reagents and conditions: (a) OFCP, TEA, DMF (5 mM), 0 °C, 30 min, then Cs_2CO_3, rt, 6 h, 21% (unoptimized). (b) OFCP, TEA, DMF (5 mM), 0 °C, 30 min, then Cs_2CO_3, rt, 4 h, 28%. (c) OFCP, TEA, DMF (5 mM), 0 °C, 10 min, then Cs_2CO_3, rt, 6 h, 41%.[23] — HMBC correlations.

This gave macrobicyclic structure **2.43**, wherein the peptide had displaced four fluorine atoms from OFCP, forming three new rings (Scheme 2.5) in the process. The outcome was rationalized in terms of the C-terminal carboxamide cyclizing at C4 in a first 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** (vide infra). Similar results were obtained starting with Ac-YNCTFC-NH_2 (**2.44**) wherein 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 (Figure 2.2A). Data obtained on purified samples of those compounds (Figure 2.2B) suggested they were cyclic bis-thioether **2.45** and spirocycle **2.46**, fully consistent with the progression of events proposed above. 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-CNYTFC-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, Figure S7). The YXCXXC consensus was required to form macrobicyclic structures via four substitutions.



Scheme 2.6. NMR investigations supporting polycyclizations. a) Analytical HPLC trace (C18, 50 → 100% MeCN + 0.1% TFA) 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** and **2.47**, having m/z = 963.3, 943.3, and 923.2 respectively. See SI for details.

After much effort, the structure of **2.47** was firmly established by X-ray analysis of a single crystal grown using the vapor diffusion method (DCM into DMSO/PhH at 25 ˚C). 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 (Figure 2.3).

2.3 Conclusions

The above reaction cascades convert linear, unprotected peptides into conformationally defined polyheterocycles having 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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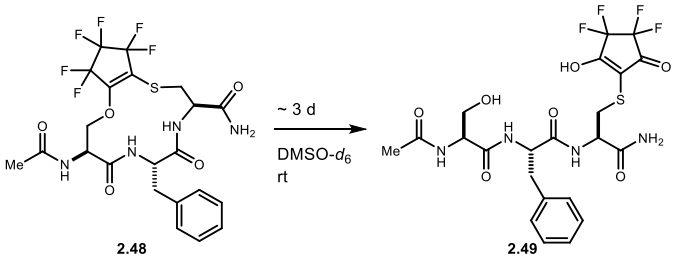
70. For example, macrocycle **2.22** was stable in 1M aq. HCl / MeOH (1:9) solution for 24 hours; whereas it decomposed within 1 hr in basic media (1M aq. NaOH in MeOH (1:9)). See Figures S5 and S6. In general, reactions promoted with Cs_2CO_3 were quenched with AcOH to avoid degradations during aqueous workup.

71. Macrocycle **2.28** was stable as a solid, but upon standing in DMSO-d_6, gradually (several weeks) ring opened via hydrolysis (+ $2H_2O$ –HF net). Related structure **2.48**, having a smaller macrocycle size, hydrolyzed more rapidly. See Appendix for details.



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73. Adding up to 20 vol % water to the reaction of **2.44** with OFCP in the presence of Et_3N had no discernible impact on the formation of **2.45** (see SI, Figure S4).

74. CCDC 1946197 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

Chapter 3 – Studies on shape and property altering polysubstitution cascades

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 aim is to build molecular complexity quickly. 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 and related oligomers in successive ring forming reactions to afford polycyclic derivatives.[3] In the example shown in Fig. 3.1B,[3] three successive 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. Other methods to cyclize unprotected peptides using native functional groups (e.g. Fig. 3.1A)[4-6] achieve less bonding with the scaffold and install fewer conformational constraints. 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.

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 transannular hydrogen bonding. We recently discovered polysubstitution cascades that achieve this outcome (Figure 3.1C).[7] The reactions modify linear, unprotected peptides in a single flask at room temperature. They require no catalysts or heavy metals and generate a wealth of previously unknown heterocyclic ring systems with tunable properties.

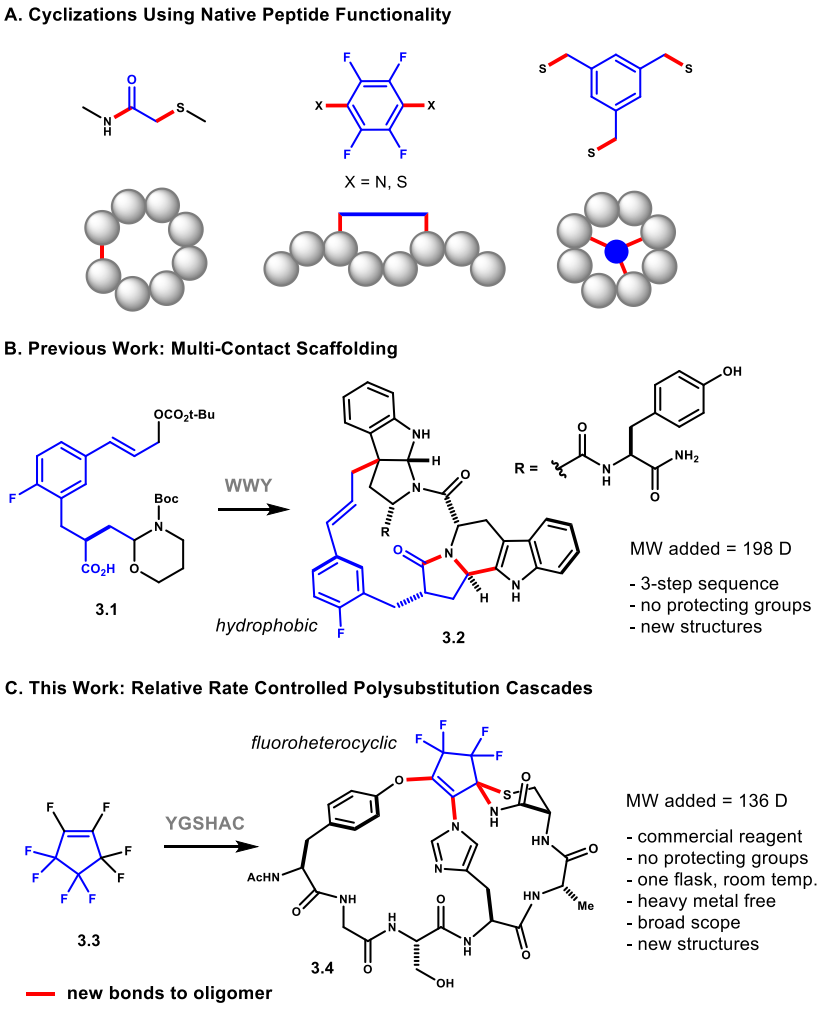

Figure 3.1. Background and rationale. **A**) Halo acetamides, perfluoroarenes and 1,3,5-tris-halomethyl benzenes engage nucleophilic peptide side chains in ring forming reactions. **B**) Reagent **3.1** sheds polar functionality while reacting with peptides to form composites with hydrophobic scaffolding (blue) bonded multiple times (red) to the oligomer. **C**) We have discovered that commercial octafluorocyclopentene can react with unprotected peptides in polysubstitution cascades that form multiple rings in a single flask.

Electron deficient aryl fluorides participate in nucleophilic ipso substitution (SNAr) reactions. Sanger's method for N-terminal peptide sequencing using 2,4-dinitrofluoro benzene is a seminal example.[8] More heavily fluorinated aromatics such as hexafluorobenzene can engage two nucleophiles successively at ring positions 1 and 4 (Figure 3.1A). The reaction is efficient with sulfur nucleophiles and has been used to generate macrocyclic peptides via cysteine 'stapling'. Poly substitutions become possible when using perfluorinated cycloalkenes. Commercial octafluorocyclopentene (OFCP) reacts with nucleophiles rapidly at both its vinyl positions, and

subsequently at its allylic positions to afford adducts having up to six fluorine atoms replaced. Previously we showed peptide side chain nucleophile to form fluorocyclopentenylated macrocycles via cross-linked cysteine, tyrosine, histidine, and serine residues in various combinations. Here we describe OFCP initiated reaction cascades that convert initially formed peptidyl macrocycles into conformationally rigidified polycyclic derivatives via additional internal fluoride displacements. We show that spirothiazinone intermediates can be intercepted to afford diverse functionalization and conjugation products. We examine the molecular properties of products and computationally evaluate if fluorospirocyclic scaffolding can create new structural mimics of major loop types observed in the Protein Data Bank.

3.2 Results and Discussion

Linear peptides having three proximal nucleophilic residues react with OFCP (25 °C, Et_3N, DMF) according to the progression shown in Figure 3.2. Two successive vinylic substitutions occur rapidly (k_{rel} for Ser:Tyr:His:Cys ~ 1:30:45:1000) to afford macrocycles **3.6**. Excess OFCP is removed in vacuo and the residue is redissolved in a DMF/THF solution containing 3 equiv. of $KOSiMe_3$. This initiates spirocyclization via Sn2' displacement of a third fluorine atom (when Nu3 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. AcYNCTCF-NH_2 reacts with OFCP at its two cysteine residues within minutes at 25 °C. Subsequent $KOSiMe_3$ treatment installs the spirothiazinone motif while generating a new vinyl fluoride that then captures the N-terminal tyrosine residue to afford **9**. By initiating reaction cascades at a C-terminal cysteine amide, it is possible to synthesize a range of macrobyclic 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 **3.13**, bridge position epimer **3.14** can be prepared readily. Diastereoselectivity at the newly

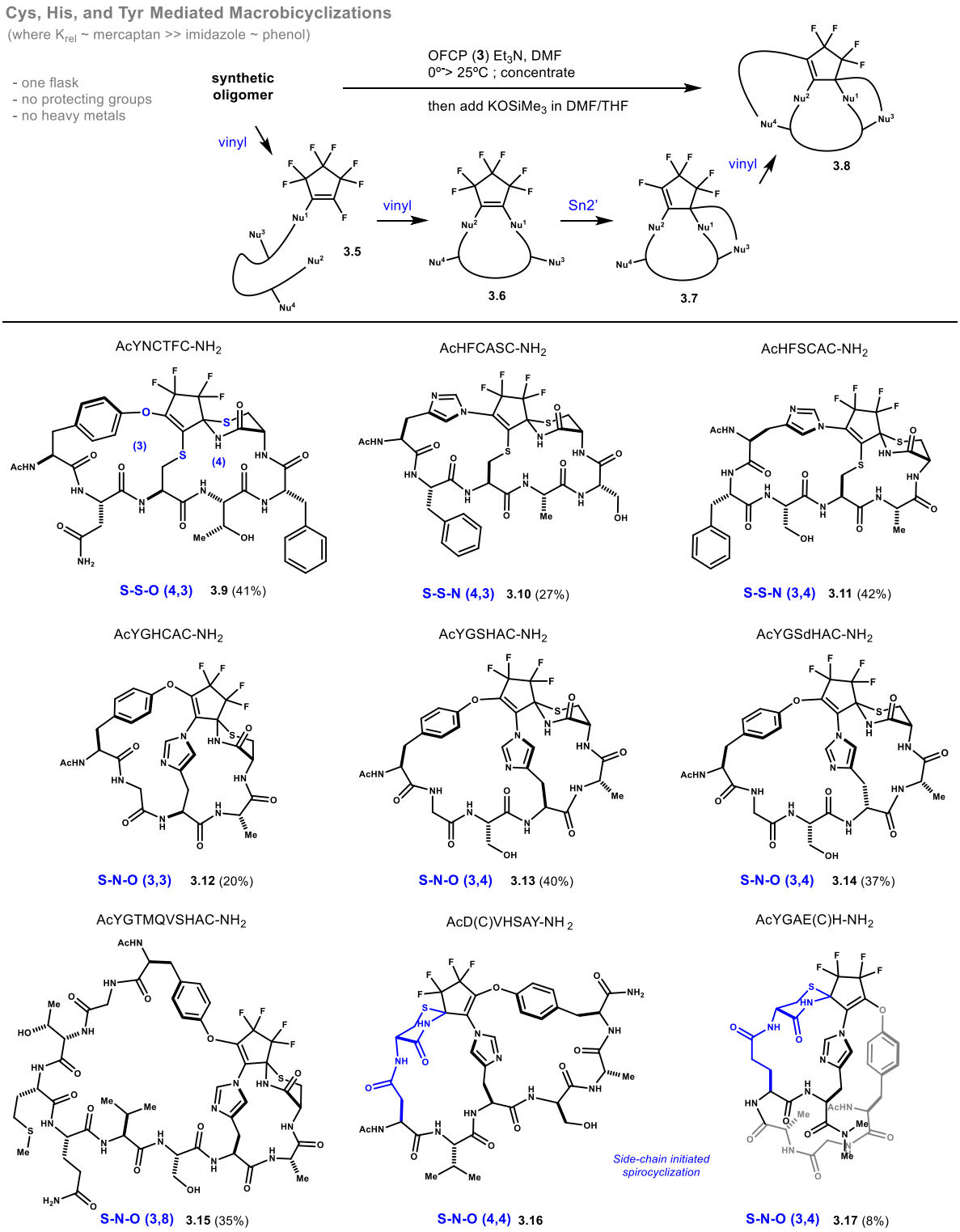

Figure 3.2. Direct one-pot polycyclizations of peptides with OFCP

formed spiro center is high (>10:1) across the series. Products have well resolved ^{1}H 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 peptides AcD(C)VHSAC-NH_2 AcYGAE(C)H-NH_2 affords the striking double looped polycycles **3.16** and **3.17**, respectively when reacted successively with OFCP and $KOSiMe_3$. To our knowledge, each of the structural types shown in Figure 3.2 are without precedent in the literature.

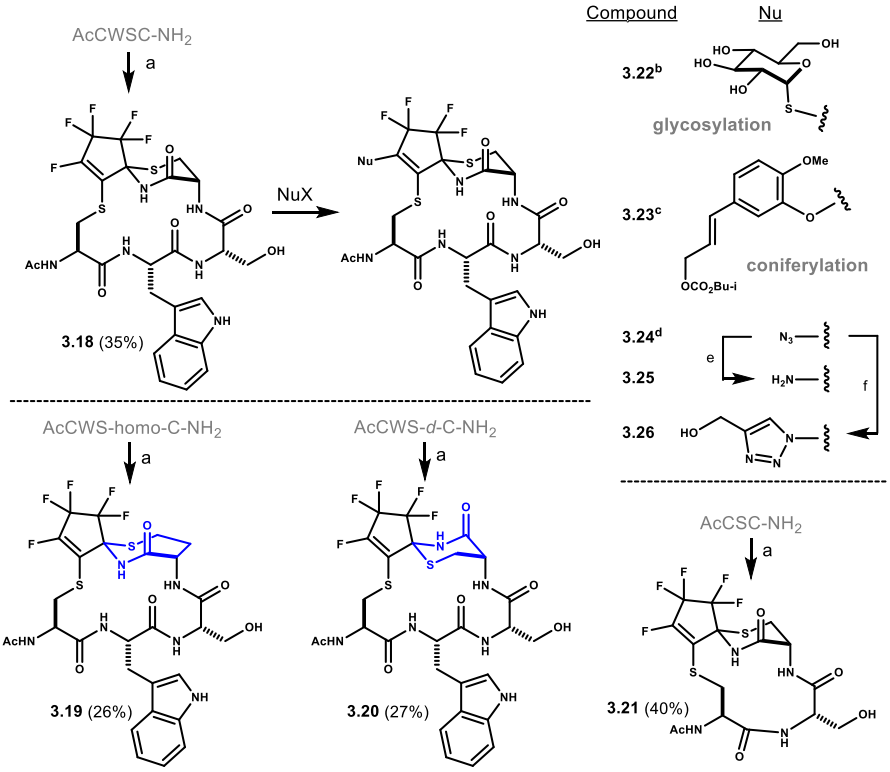

Figure 3.3. Examining spirocyclic variations and coupling partners at vinyl fluoride position. Reagents and conditions: a) OFCP (1.5 equiv), Et_3N (2.5 equiv), DMF (5 mM), 0 °C, 30 min; conc. then $KOSiMe_3$ (2 equiv), 1:4 DMF/THF (5 mM), 0 °C -> RT, 1 h, (>10:1 d.r.). b) beta-D-Thioglucose sodium salt (1 equiv), DMF (50 mM), 0 °C, 1 h, >95%. c) Coniferyl Carbonate **3.27** (2 equiv), $KOSiMe_3$ (2 equiv), DMF (50 mM), 0 °C -> RT, 12 h, 53%. d) NaN_3 (1 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 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%.

It is also possible to isolate and characterize vinyl fluorides. Treatments of AcCWSC-NH_2 with OFCP followed by $KOSiMe_3$ affords spiro tricyclic compound **3.18**. Under identical conditions, the same sequence containing D-cysteine affords epimeric macrocycle **3.20**, while its homo cysteine variant affords spiro thiazepinone **3.19**. Smaller analogs are also accessible. Omitting the tryptophan residue results in the formation of spiro tricyclic substance **3.21**.

The vinyl fluoride in **3.18** 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 **3.22** in near quantitative yield. Variations in thioglycoside and peptide sequence could be used to generate a new class of glycosylated macrocycles. Compound **3.18** also reacts with a coniferyl alcohol derivative to afford **3.23**. The cinnamyl carbonate in **3.23** provides means to form an additional large ring via electrophilic capture of amines, carboxylates, imidazoles and pi basic aromatic yield. Azidation of **3.18** with NaN_3 in DMF provides vinyl azide **3.24** in >95% yield This molecule participates in Sharpless/Huisgen 'click' cycloadditions with terminal alkynes. Stirring **3.24** with 1 equiv. of propargyl alcohol in the presence of a phenylenediamine ligated Cu^{II} catalyst affords triazole **3.26** in 75% yield. Azide **3.24** can also be hydrogenated to afford β-thio enamine **3.25**. We note also the possibility that electrophiles of type **3.18** could be used to form novel antibody / macrocycle conjugates via reactions at solvent exposed cysteine or tyrosine residues.

Capture of spirocyclic vinyl fluorides with insert molecules allows syntheses of macrobicyclic structures from peptide sequences that would otherwise give mono macrocyclic products (Figure 3.4). These insert molecules can be coupled to the vinyl fluoride position of spirocyclic intermediates in an efficient one-pot manner (**3.28**). Various linker systems can be used to further expand the scope of polycycles by interacting with side chain residues that wouldn't normally react with OFCP directly either based on its nucleophilicity or strained configuration (**3.29**). For example, thiazole **3.30** rapidly reacts at the vinyl fluoride position the provide **3.31**. Ionization of the alcohol under acidic conditions gives Friedel-Crafts products (see **3.32** and **3.33)**, albeit after long reaction times while heated (12 h, 80 °C). Direct polycyclizations of these peptides (AcYACFAC-NH_2 and ACYCAC-NH_2) with OFCP would not be possible due to constrained nature required for the final ring closure. Reminiscent of our template work discussed earlier, we were inspired to develop a similar system arriving at the phenolic insert **3.27**. This

molecule couples slowly but effectively to provide stable and isolable carbonate linkers. Thus far, we have demonstrated their utility with tyrosine (**3.35**) and tryptophan (**3.36**) residues, most

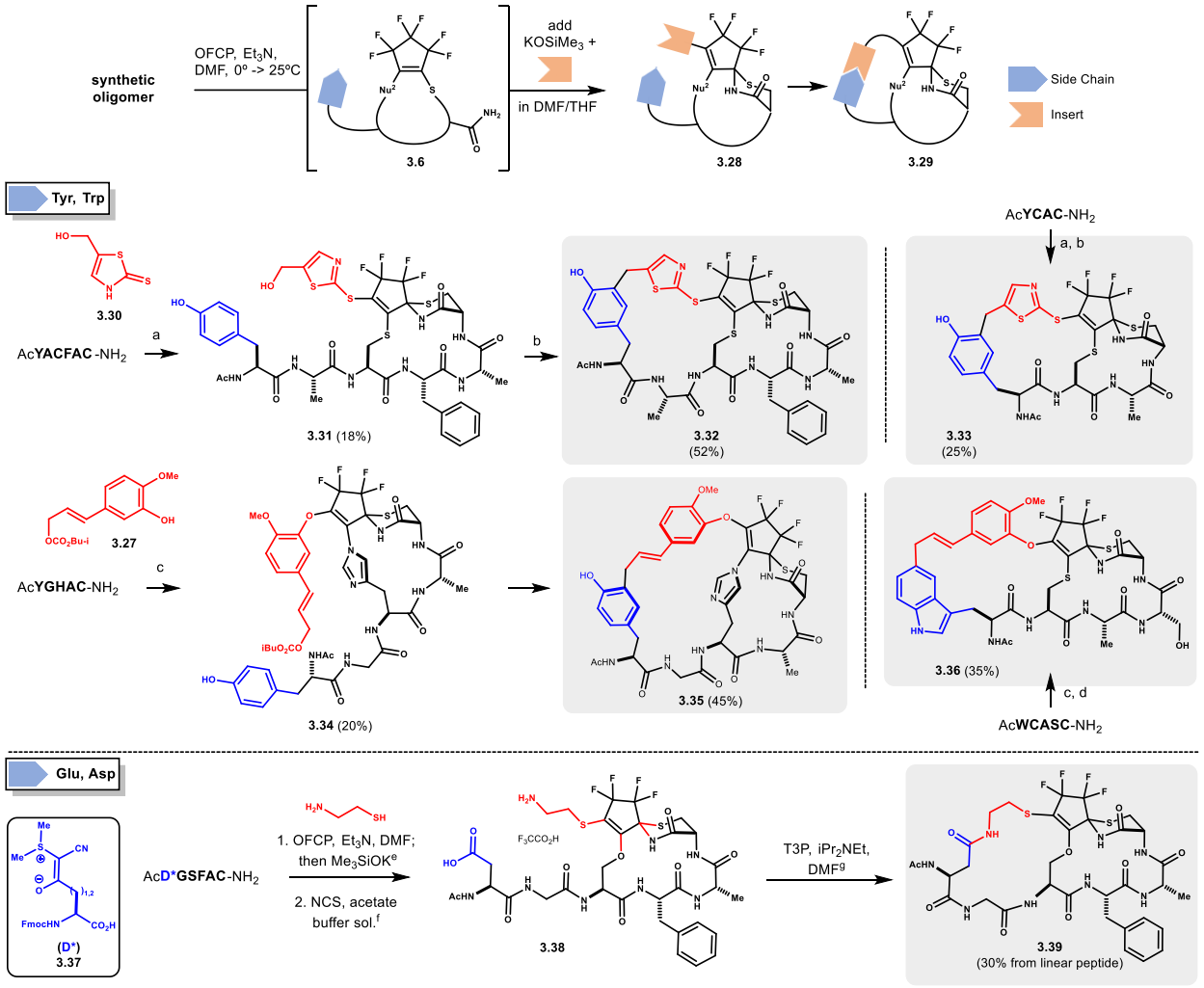

Figure 3.4. Polycyclizations via various linker insert methods. Reagents and conditions: a) OFCP (1.5 equiv), Et_3N (3 equiv), DMF (50 mM), 0 °C, 30 min; conc. then $KOSiMe_3$ (3.5 equiv), thiazole **3.30** (1.1 equiv), 1:4 DMF/THF (50 mM), 0 °C -> RT b) 5% TFA, $MeNO_2$ (5 mM), 80 °C, 12 h. c) OFCP (1.5 equiv), Et_3N (2.5 equiv), DMF (5 mM), 0 °C, 30 min; conc. then $KOSiMe_3$ (4 equiv), **3.27** (2 equiv), 1:4 DMF/THF (50 mM), 0 °C -> RT, 12 h. d) $ScOTf_3$ (1 equiv), $MeNO_2$ (5 mM), RT, 2 h. e) OFCP (1.5 equiv), Et_3N (3 equiv), DMF (10 mM), 0 °C, 30 min; conc. then Cs_2CO_3 (4 equiv), Cysteamine (1.1 equiv), 0 °C, 1:4 DMF/THF (5 mM) f) NCS (1.5 equiv), 2:8 MeCN:NaOAc/AcOH (aq.) (1 mM), RT 45 min g) T3P (1.5 equiv), iPr_2NEt (3.5 equiv), DMF (5mM), 0 °C.

importantly. Under lewis acidic conditions, tryptophan reacts rapidly to give three major regioisomers with **3.35** being the major product isolated. We've also recently obtained macrocycle products with carboxylic acid functionality. This was made possible with the help of masking Asn

or Gln residues as cyanosulfurylides (**3.37**), chemistry originally developed by the Bode group as a means to prevent aspartimide formation during peptide synthesis.[9] This has provided the potential for macrolactone and macrolactam polycyclic products. For example, AcD*GSFAC-NH_2 can be reacted with OFCP followed by capture of the vinyl fluoride position using cysteamine and subsequently revealing the carboxylic acid using NCS as seen in **3.38**. Macrolactamization can then be achieved by subjecting **3.38** with T3P coupling conditions to give **3.39** in 30% yield from the linear peptide. We are currently working to expand the scope of polycycles with these methods including varying both the participating and spectating functionality of the peptide backbone and alternative insert molecules.

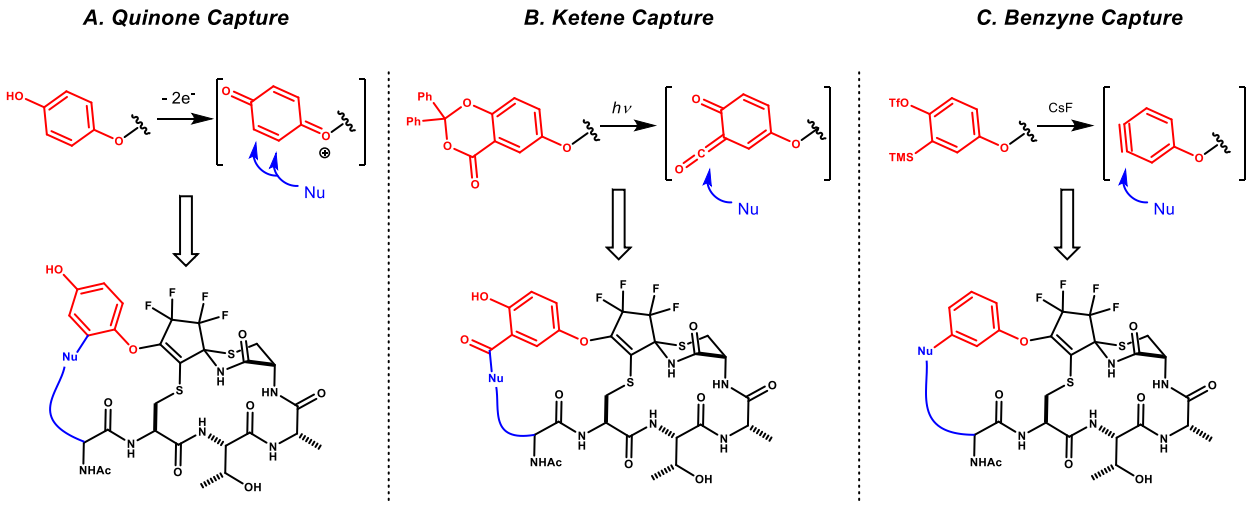

Figure 3.5. Studies on several linker systems with alternative modes of capture.

Considering other linker system types, we continued to focus our strategies on methods which were both general and amenable to conditions compatible with the polar functionality present within our peptide substrates. We have since begun to examine three other systems that we believe have the potential to tolerate these guidelines (Figure 3.5). After coupling of hydroquinone to the vinyl fluoride position, electrochemical oxidation to the quinone intermediate may promote nucleophilic capture of neighboring amino acid residues (Figure 3.5A). However, one-pot methods to couple hydroquinone to form the acyclic starting material proved difficult. For

example, when reacting AcWCASC-NH_2 with standard macrocyclization conditions (1.5 equiv. OFCP, 3 equiv. Et_3N) followed by hydroquinone and Cs_2CO_3, we observed slow reaction times (overnight) and incomplete conversion (Figure 3.6A). Reverse-phase HPLC purification of these quenched reaction mixtures was required due to increased polarity from the free phenol but did not lead to product isolation possibly due to instability. 4-methoxyphenol was then used to probe the reactivity and stability compared to hydroquinone. At small scales, we found its reactivity comparable to hydroxyquinone with incomplete conversion over long reaction times. Its increased lipophilic nature may allow normal flash column chromatography isolation techniques, which we will be examining in future experiments.

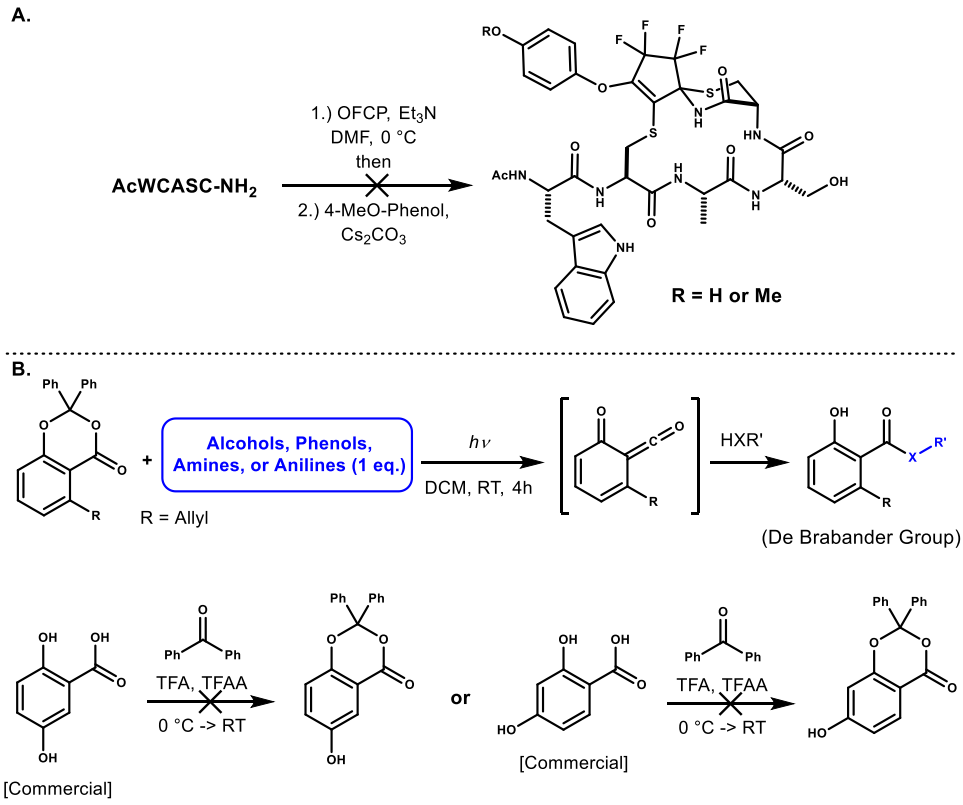

Figure 3.6. Attempts to synthesize hydroquinone incorporated structures and benzodioxinone substrates.

De Brabander, and coworkers have developed a method to synthesize *ortho*-substituted salicylic esters and amides through the photolysis of *ortho*-substituted benzodioxinones (Figure 3.6B).[10] Photochemical fragmentation of benzodioxinones to quinoketenes allowed the capture

alcohols, phenols, amines, and anilines using mild reaction conditions (neutral, RT). This general method could prove incredibly useful if successfully incorporated as a linker molecule (Figure 3.5B). The reported method includes the use of 5-hydroxybenzodioxinone, however we chose to synthesize the unreported 6-hydroxy regiosiomer, from 2,5-dihydroxy benzoic acid and benzophenone, to avoid steric complications during macrocyclizations. Unfortunately, numerous attempts to access this ketene precursor were unsuccessful due to hydrolytic instability. Future attempts will focus on synthesizing regioisomeric analogs of this substrate and their potential as a method to form macrolactam/lactone products.

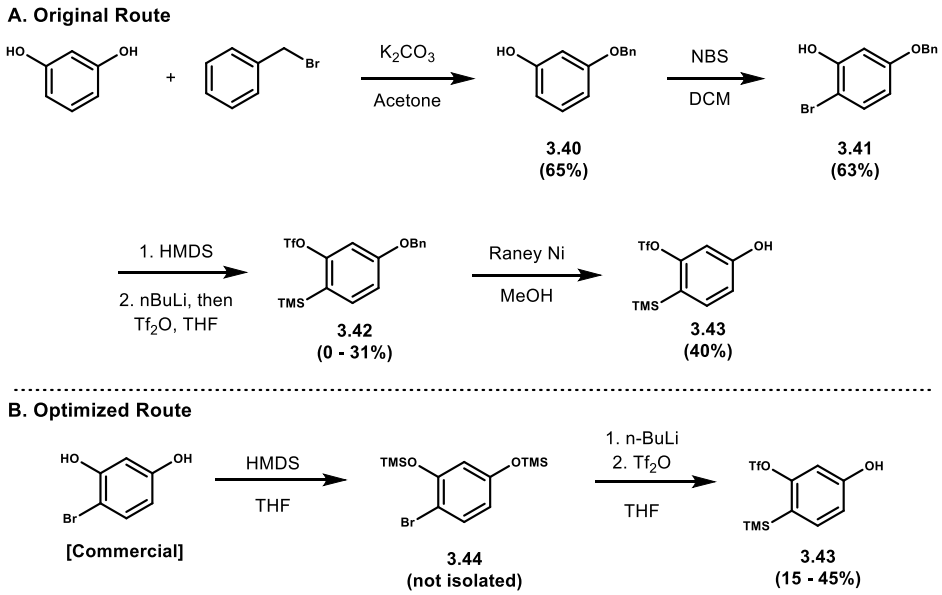

Figure 3.7. Our original and optimized routes to benzyne precursor substrate **3.42**.

We believe benzyne-type intermediates have the potential to engage a broad range of amino acid residues (Figure 3.5C). It is well known that these intermediates can capture a variety of nucleophiles including alcohols, amines, carboxylates, carboxamides, and others.[11] With this substrate our goal is to develop methods towards direct synthesis of ansa -bridged macrobicyclic structures from unprotected peptide precursors. Silyl aryl triflates are one of the most common benzyne intermediate precursors known, so we developed several routes to phenoxy silyl triflate **3.43** (Figure 3.7). Our initial route begins with the monobenzylation of resorcinol followed by

monobromination to give **3.41** (Figure 3.7A). Initial silylation then lithiation promotes a Brooke-like rearrangement to ultimately capture Tf_2O from the resulting phenoxide. Finally, Raney Ni hydrogenolysis of **3.42** provided the desired substrate **3.43**. We later adopted a more efficient strategy through a one-pot method from 4-bromoresorcinol.[12] We found both **3.42** and **3.43** to be highly unstable, especially when neat for short periods of time, making both isolation and subsequent coupling reactions with this substrate very challenging. Loss of the silyl group was found to be the major form of decomposition as observed by NMR.

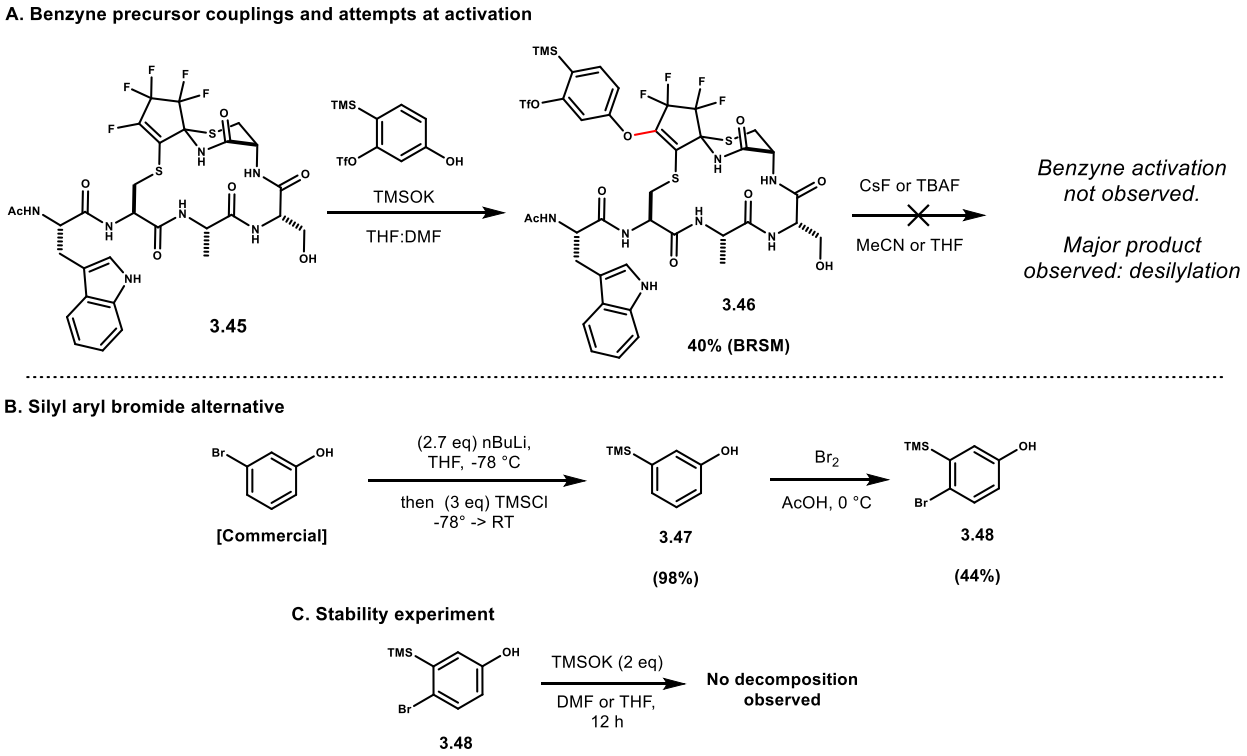

Figure 3.8. Initial challenges when studying benzyne precursor substrate reactivity and stable alternatives.

Couplings of **3.43** to the vinyl fluoride position of **3.45** was found to be sluggish, but most importantly unstable under these conditions (Figure 3.8). We found the presence of DMF under basic conditions promoted decomposition. DMF is necessary for the solubility of these substrates, leading to solvent mixtures of 10 – 20% DMF/THF to be ideal albeit with modest yield. Regardless, after numerous attempts to activate the silyl aryl triflate of **3.46** under various conditions we arrived at the same outcome with the major product being desilylation. In our efforts to find a more stable

alternative, we found that the Daugulis group has used silyl aryl bromides towards aryne reactivity, however with most of their examples using dimethyl silanes rather than the traditional TMS group.[13] We synthesized the silyl aryl bromide **3.48** as an alternative substrate in two steps from 3-bromophenol (Figure 3.8B). We found this substrate to be considerably more stable compared to the previous silyl aryl triflate in basic conditions using either DMF or THF for long reaction times (Figure 3.8C). This silyl aryl bromide coupled to compound **3.45** with modest yields (41 – 56%)

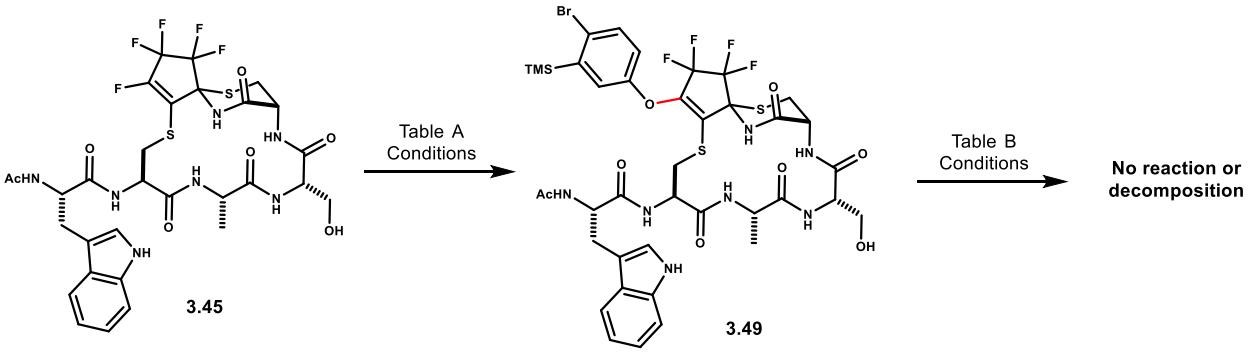

Table A

Entry	3.48 (# eq.)	Base	Time	Solvent	Result
1	2 eq.	Me_3SiOK (2 eq.)	36 h	10% DMF/THF	56%
2	5 eq.	Me_3SiOK (3 eq.)	8 h	DMF	41%

Table B

Entry	Fluoride Source	Temperature	Time	Solvent	Result
1	CsF	RT	6 h	THF	N.R.
2	CsF	50 °C	12 h	THF	N.R.
3	TBAF	RT	6 h	THF	Partial desilylation
4	TMAF	RT	6 h	THF	N.R.
5	TMAF	40 °C	4 h	THF	Partial desilylation
6	TMAF	40 °C	4 h	THF/MeCN	Complete desilylation
7	CsF, AgF	RT	7 h	THF/MeCN	Partial desilylation

Table 3.1. Silyl aryl bromide coupling and activation conditions attempted.

with no observed decomposition (Table 3.1A). Various conditions were used to examine benzyne activation which is outlined in Table 3.1B. CsF led to no reaction even at elevated temperatures over 12 h. TBAF and TMAF resulted in desilylation in varying amounts depending on the reaction

temperature. Once again, this substrate demonstrated instability in a similar manner to other cases, however mostly under forcing conditions in this case. The Harrity group reported a similar issue of protodesilylation when attempting activation of silyl aryl iodides for benzyne reactivity.[14]

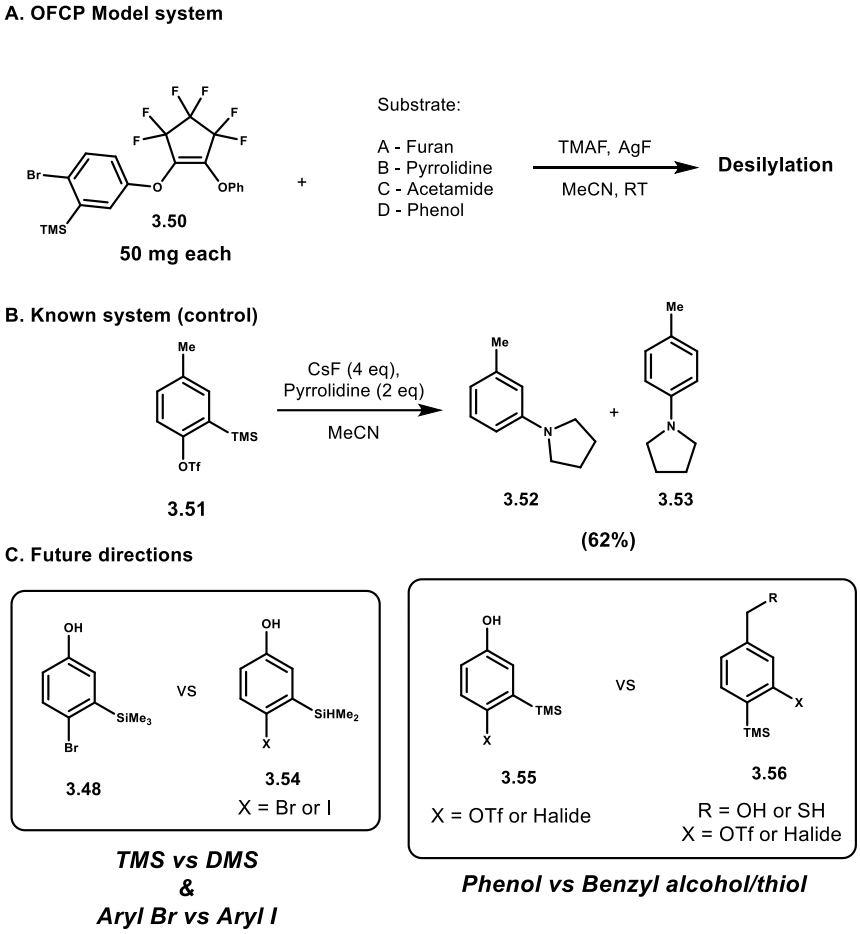

Figure 3.9. Examining model systems for reactivity and considering future directions to promote activation over protodesilylation.

They found that by using AgF as an additive in the presence of CsF, ratios of protodesilylation to elimination were reduced. In our case, the use of AgF as an additive did not reduce undesired decomposition. We further tested this substrate in a simpler model system and continued to observe decomposition in the presence of various known coupling partners (Figure 3.9A). Finally as a control we successfully replicated known reaction of **3.51** to give regioisomeric products **3.52** and **3.53** using CsF and pyrrolidine (Figure 3.9B). Considering the various instances of

protodesilylation we experienced, there are several approaches we could still examine (Figure 3.9C). The Daugulis and Harrity groups both reported successful benzyne reactivity with the use of either dimethylsilane or iodide functionality, respectively. Also, incorporating a methylene unit to the substrate as the benzyl alcohol/thiol analog may alter its electronic properties in a way that reduces its propensity to decompose readily. Future experiments should focus on these substrates to further probe reactivity.

As a compound class, cyclic peptides and peptidomimetics are often developed to target extracellular proteins because their passive membrane permeability is limited. Developing strategies to improve membrane permeability is critical for the targeting of intracellular PPIs. There has been a massive effort to understand factors dictating passive permeability in hopes that cell permeant analogs could be designed.[15] The fluorospiroheterocycle-scaffolded structures described here have not previously been evaluated for passive permeability in any format. A 42 compound library including OFCP macrocycles and polycycles with varying characteristics was evaluated for permeability through a parallel artificial membrane permeability assay (PAMPA) kit. Select examples from this library are shown in Figure 3.6 (See Appendix for full data set). More than half of this set exhibited permeability greater than the low permeability standard theophylline. Interestingly, three compounds performed better than the high permeability standard, which included two polycycles with structural characteristics not typically associated with permeable compounds. Compared to compound **3.13**, its epimeric analog (**3.14**) displayed a notably greater permeability rate. This may suggest significant conformational differences wherein possible intramolecular hydrogen bonding (IMHB) present in **3.14** leads to the observed differences in outcomes between the epimers. A computational model of **3.14** was generated demonstrating this possibility with IMHB interactions from the bridging histidine and the peptide backbone. Overall, these results are promising for our OFCP-embedded macrocycles and polycycles and we are working to further evaluate additional analogs of similar structure types.

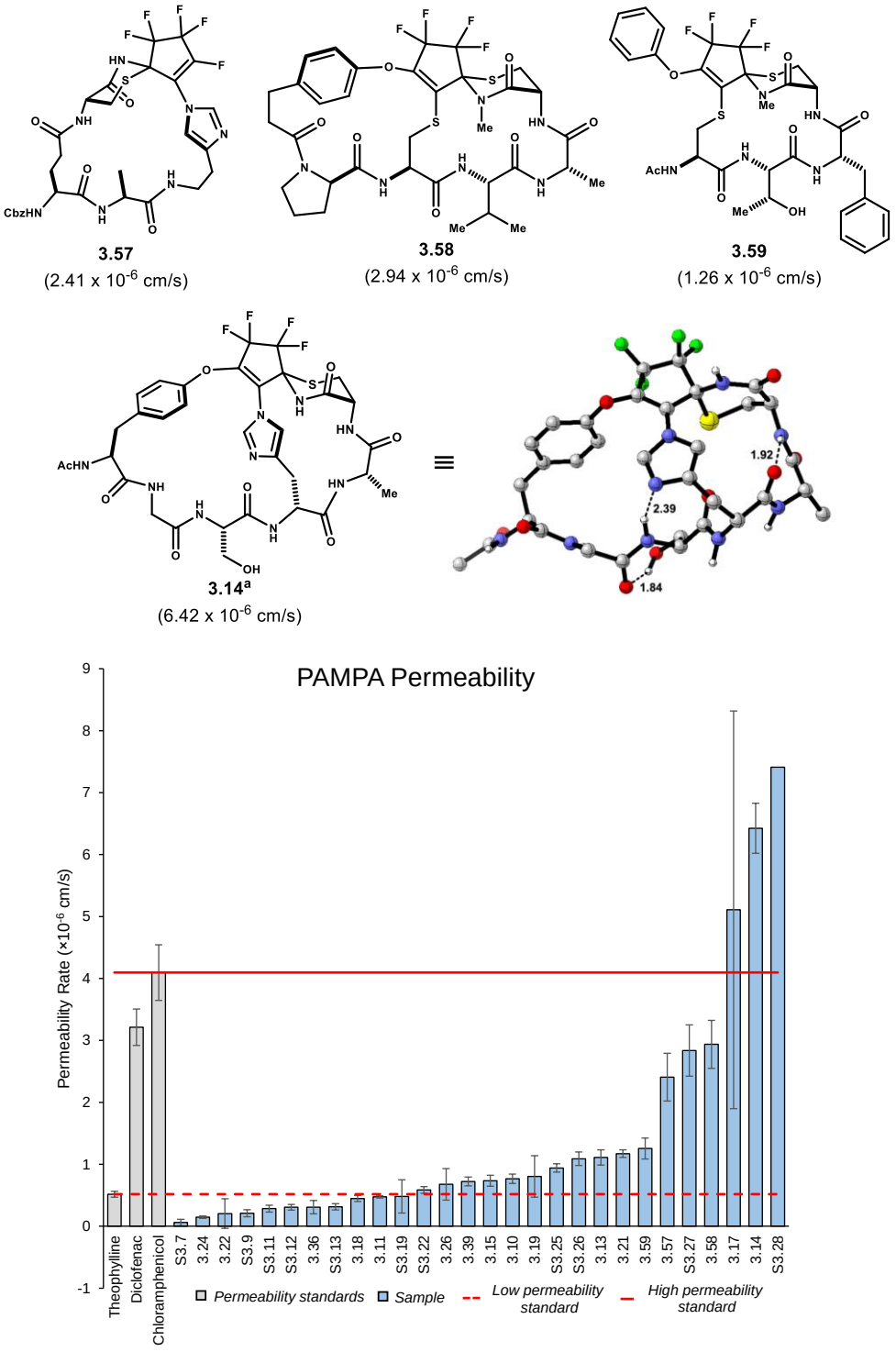

Figure 3.10. PAMPA permeability of select structures. Average permeability rate from assay performed in duplicate; error bars: standard deviation (see Appendix for more information). Gray = low (theophylline), medium (diclofenac), and high (chloramphenicol) permeability standards. (a) Structure was determined via geometry minimization of **3.14** carried out on Schrödinger's Maestro Macromodel® using AMBER* force field in water as the solvent. Hydrogens bonds were determined via identification of polar contact surface area using literature reported donor-acceptor distances and bond angle ranges.

3.3 Conclusions

Relative rate controlled polysubstitution cascades using perfluorocycloalkenes is a new, simple means to rapidly generate fluorinated composite macrocycles. Cyclic peptides and peptidomimetics serve as leads in drug discovery, as probe compounds in chemical biology and as building blocks in materials science. This application describes synthetic tools to generate a previously unknown class of fluorinated, conformationally constrained composite peptidomimetics also enabling the study of their novel stability, reactivity, and properties. In the future, this methods platform will enable us to test whether fluorospirocyclic scaffolding can create membrane permeant structural mimics of the major loop types observed in the Protein Data Bank.[16]

3.4 References

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Chapter 4 – Cell-permeable peptidomimetic macrocycles from template precursors

4.1 Introduction

Macrocyclic peptide-based therapeutics have gained increasing attention for their potential to target clinically important protein-protein interactions (PPIs).[1-4] Their structural characteristics, however, often place them outside most classical definitions of 'drug-likeness' leading to underrepresentation in HTS drug discovery programs. These restrictions define property boundaries for hydrogen bond donor/acceptor groups, rotatable bonds, molecular weight, and polar surface area as predictors of oral bioavailability and cellular permeability.[5,6] Widespread adoption of these guidelines by industry leaders and scientists has persisted for decades since its introduction, leading to a uniform chemical space represented in therapeutics.[7-9] Recently, many have begun to reconsider how we assess 'drug-like' properties and the overall hypothesis itself. For example, Schultz has reported that since 1997 the threshold for both molecular weight and hydrogen bond acceptors has increased substantially based on hundreds of small molecules approved by the FDA over the last two decades.[10] This evolving understanding is now being considered especially with the emergence of new chemical modalities in drug development.

Macrocyclic peptides often exhibit structural elements outside of desired ranges defined by these guidelines, yet there are many notable exceptions. Today, there exist many macrocyclic drugs in clinical use such as cyclosporine A (CSA), rapamycin, and vancomycin, however, they still only represent a fraction of the market. Considering their structural features, their surprising bioactivity and bioavailability has inspired extensive mechanistic studies that may help facilitate design of cell-permeable compounds. CSA's conformational flexibility for example, enables it to exhibit "chameleonic" behavior with its ability shift from displaying a more polar surface in aqueous environments to presenting a less polar surface when crossing lipophilic cell membranes through its ability to transiently form intramolecular hydrogen bonds (IMHBs).[11-12] Recently, the Baker

group successfully used computational-aided design of membrane permeable and orally bioavailable peptide macrocycles incorporating this conformational switching characteristic in their design models.[13] Backbone N-methylation is also widely known to be correlated with increased membrane permeability.[14,15] Interestingly, N-methylations have been found to do so not just by simply masking H-bond donors but by decreasing flexibility in the peptide backbone, thereby stabilizing hydrogen-bonded conformations.[16] Computational estimations of topological polar surface area (TPSA) of a given molecule is commonly used for determining overall polarity when assessing bioavailability.[17] Calculating TPSA is both rapid and convenient, however it is conformationally independent and does not account for potential intramolecular hydrogen bonding. Goetz and coworkers have developed a method using supercritical fluid chromatography which correlates chromatographic retention with the exposed polar surface area (EPSA) of a molecule.[18,19] This technique relies on the idea that molecules that can form IMHBs are better able to hide their polarity than those that cannot, leading to lower retention times. EPSA has been used to determine unknown IMHB patterns in cyclic peptides via systematic N-methylations. For example, N-methylations resulting in a decrease or increase in EPSA would indicate a previously exposed N-H group or a significant conformational change now exposing polar functionality, respectively. Methylations leading to unchanged an EPSA value may indicate a hidden N-H group, likely due to intramolecular hydrogen bonding. Most importantly, correlations between EPSA and cellular permeability have been described using Ralph Russ Canine Kidney (RRCK) cells. With its ability to accurately identify IMHBs, independent of molecular weight and lipophilicity, this method acts as a valuable tool in drug design programs as a predictor of efficient membrane permeability. Combining these advancements with synthetic methods to develop unique structures with favorable properties are needed.

Our group has long-sought facile methods to convert linear peptide oligomeric sequences to diverse macrocyclic structures with favorable pharmacokinetic properties. Designed synthetic

templates **G1**-**G3** have provided access to increasingly complex peptidomimetic macrocycles (Figure 4.1).[20-25] Each template generation contains a succinimidyl ester for efficient acylation and a cinnamyl carbonate core motif amenable to reaction conditions that selectively provide C-O, C-N, and C-C bonded products. Templates **G2** and **G3** contain additional functionality in the form of hidden aldehyde moieties allowing subsequent Pictet-Spengler transformations giving polycyclic structures. The alkyne in **G3** acts as a handle for further late-stage functionalization. This versatile system allows us to study the pharmacological properties of these structures in a systematic manner. Through collaborative efforts with Pfizer Inc., we have begun to explore structure-permeability relationships of peptidomimetic macrocycles in detail described here.

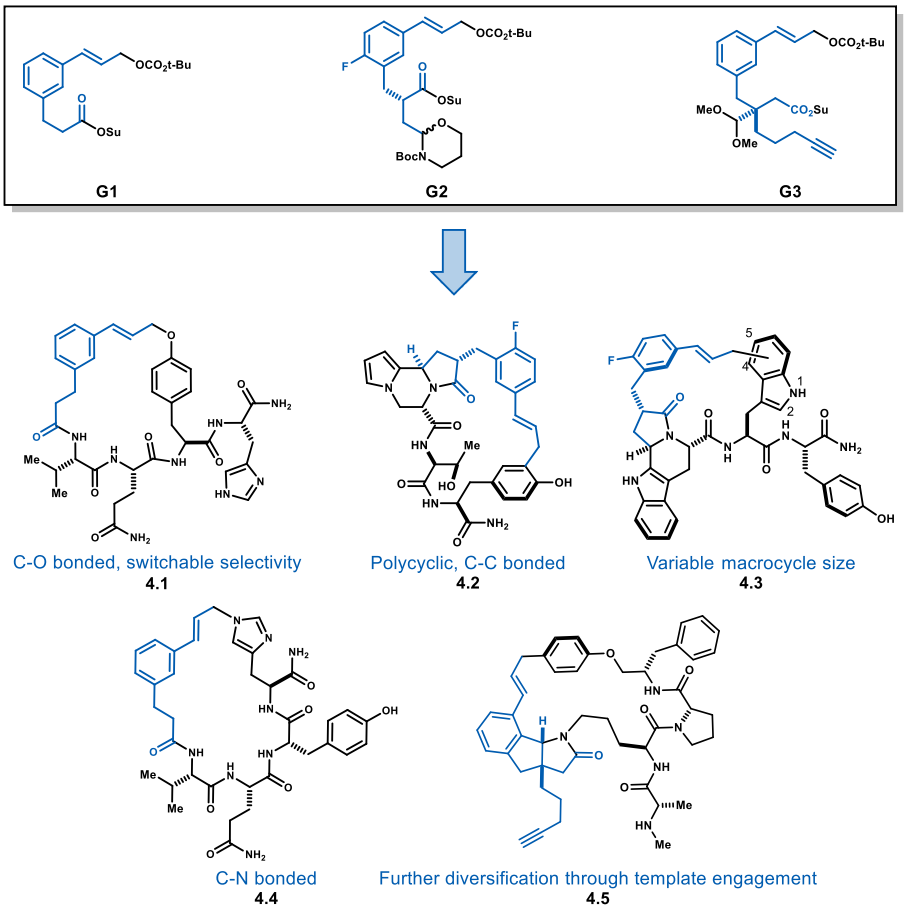


Figure 4.1. Previously developed synthetic templates **G1** – **G3** and representative outcomes for diverse macrocyclization.

4.2 Results and Discussion

Over several rounds, we synthesized a small library of 39 peptidomimetic macrocycles using our template methodology to evaluate cellular permeability (RRCK assays). This compound set consisted of compounds that surveyed a diverse array of functionality including systematic N-methylations, nonproteinogenic amino acids, and incorporation of the **G1**-**G3** template systems. Overall, most compounds were found to exhibit at least moderate permeability, with nine falling in the "not permeable" range. Seven of these macrocycles displayed good permeability with RRCK apparent permeability (P_{app}) values greater than 5×10^{-6} cm/s. Notably, when plotting cellular permeability as a function of molecular weight (Figure 4.2A), compounds throughout the 500 – 800 MW range exhibited moderate permeability. EPSA values were also determined for the entire set providing values ranging from 69 – 119 (Figure 4.2B). According to Goetz and coworkers, compounds with EPSA values below 100 are more likely to exhibit passive permeability compared to those above that cutoff. In our case, this criterion seems to hold true with most of our significantly permeable macrocycles occupying the space below this EPSA threshold (gray line, Figure 4.2B).

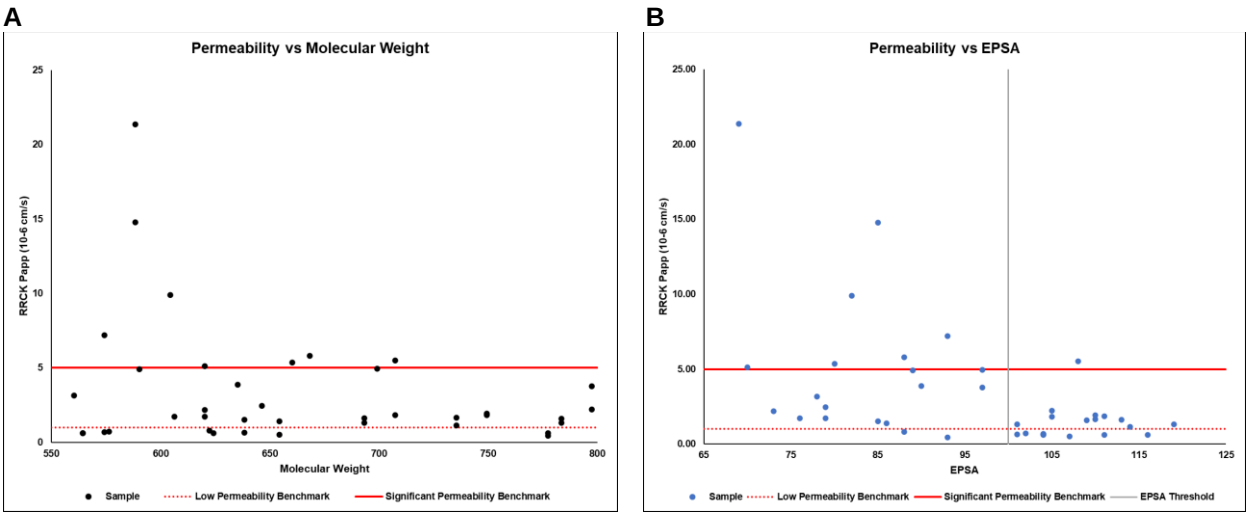


Figure 4.2. Correlation between RRCK apparent permeability and molecular weight or exposed polar surface area (EPSA) of macrocycle library. (Note: EPSA values are determined based on retention times directly compared with a set of calibration standards. See appendix for more information).

As mentioned previously, our template methodology enables facile synthesis of macrocycles with switchable selectivity. Following initial acylation of a tyrosine embedded peptide to **G1**, acyclic structures can undergo Friedel-Crafts reactivity (5-10% TFA in $MeNO_2$) or Tsuji-Trost type reaction conditions ($Pd(PPh_3)_4$ in DMF) arriving at C-C or C-O macrocycles, respectively. Using these methods, five pairs of compounds were synthesized using template **G1** to examine direct differences in permeability between these two macrocycle types (Figure 4.3). Most C-O linked macrocycles (**4.11** – **4.13**) exhibited greater passive permeability compared to their C-C linked counterparts (**4.6** – **4.8**) as predicted by EPSA. In two examples, we observed either a decrease in permeability (**4.9** & **4.14**) or no significant change (**4.10** & **4.15**). Based on these results, major conformational differences leading to favorable IMHB interactions, rather than simply masking H-bond donors, may explain the improved permeabilities.

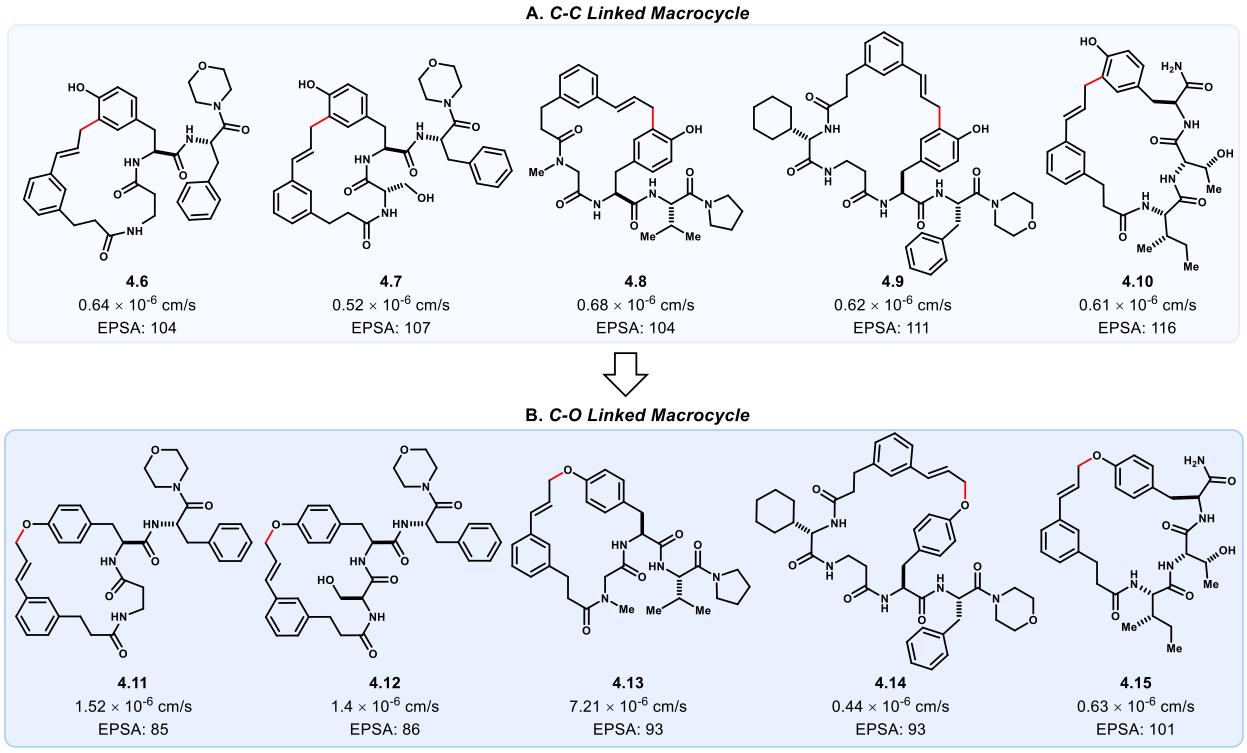


Figure 4.3. Direct comparison of C-C and C-O linked macrocycles with their experimentally determined permeabilities.

We then explored various systematic modifications from parent structures **4.16** (Figure 4.4A) and **4.20** (Figure 4.4B) and their impact on permeability. Analogs were synthesized with changes at each amino acid position of the peptide backbone (P1 – P3). N-methyl amino acid incorporation at P1 for **4.16** significantly improved permeability with compounds **4.13** and **4.17**, whereas substituting valine with a more polar threonine (**4.18**) at P3 had a negative effect, unsurprisingly. In the case of compounds **4.13** and **4.17**, EPSA was not a reliable predictor of the observed permeability. Direct methyl capping of the tyrosine phenol in compound **4.8** (Figure 4.3) provided compound **4.19** with a substantial increase in permeability efficiency. Both macrocycles **4.13** and **4.19** positively affect RRCK P_{app} by masking the tyrosine phenol, however the significant

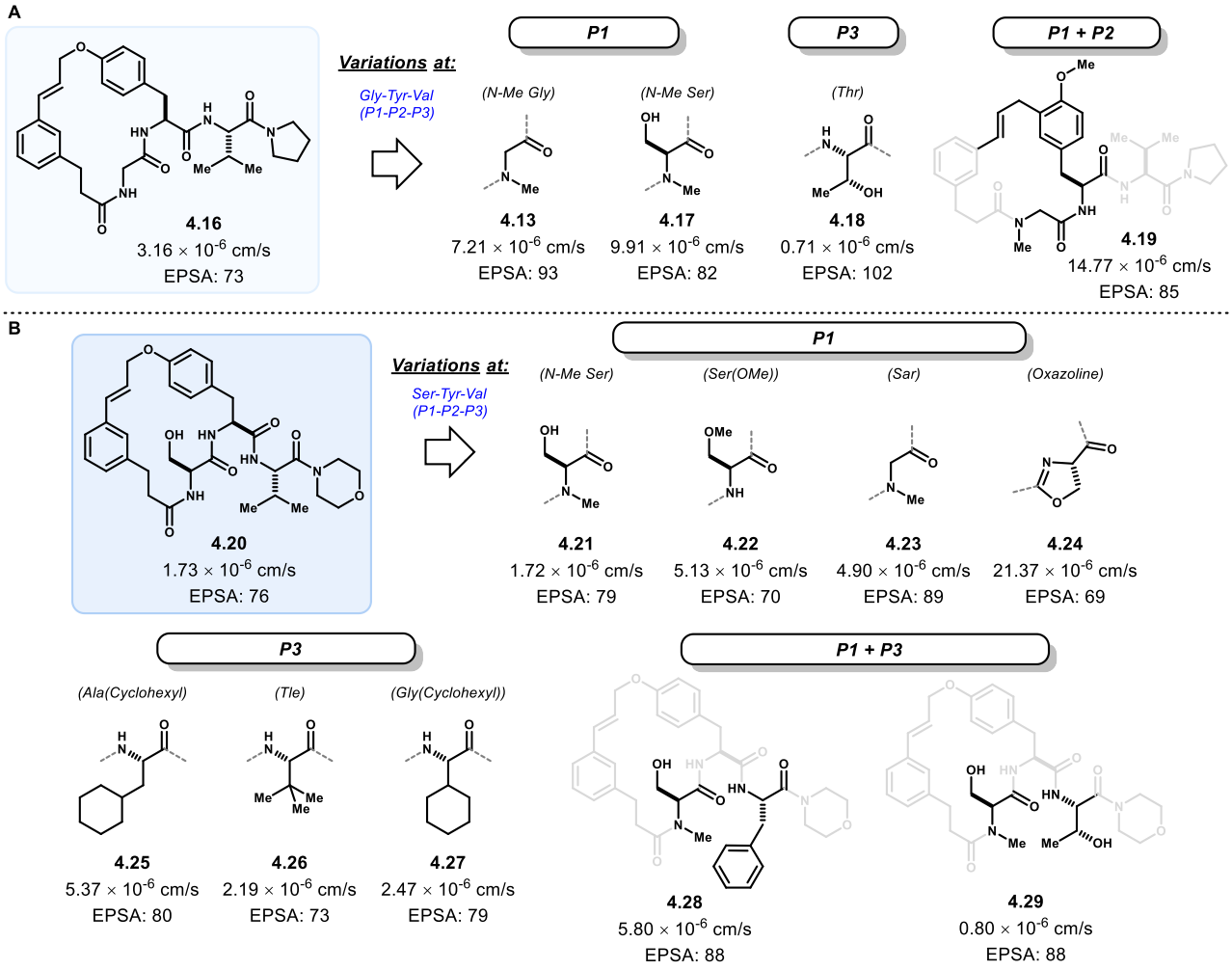
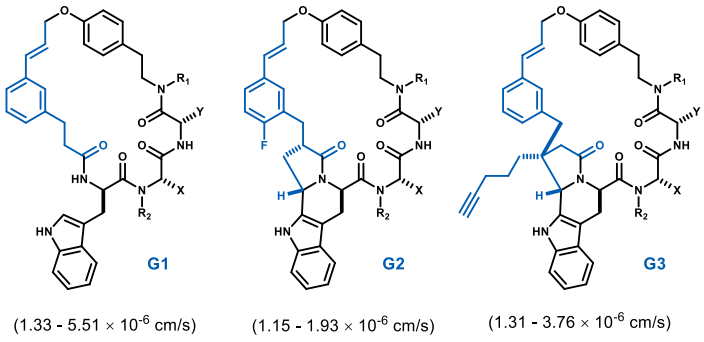


Figure 4.4. Evaluating pharmacological properties of macrocycles through various systematic changes.

disparity between the two suggests the presence of favorable conformational arrangements not easily predicted by such a minor modification. N-methylation of **4.20** at P1 lead to no noteworthy change in both permeability rate and EPSA retention (compound **4.13**). This likely indicates that the N-H of serine is involved in an IMHB in macrocycle **4.20**. Other P1 substitutions with Ser(OMe) and sarcosine greatly improved their permeability compared to the parent structure. Interestingly, cyclodehydration of **4.20** to give oxazoline **4.24** provided our most permeable macrocycle in this library (RRCK P_{app} 21.37×10^{-6} cm/s). Unfortunately, attempts at assaying similar compounds with this functionality proved challenging due to its inherent instability. Use of nonproteinogenic lipophilic amino acids Ala(Cyclohexyl), Tle, and Gly(Cyclohexyl) at P3 had the expected desired effect. Compared to **4.21**, substituting the amino acid at P3 with either phenylalanine (**4.28**) or threonine (**4.29**) gave a notable increase and minor decrease in permeability, respectively.



(1.33 - 5.51 × 10^{-6} cm/s) (1.15 - 1.93 × 10^{-6} cm/s) (1.31 - 3.76 × 10^{-6} cm/s)

Compound	Permeability	EPSA	R_1/R_2	X/Y	Template Gen.
4.30	1.33	119	H/H	Tle/Ser	G1
4.31	1.15	114	H/H	Tle/Ser	G2
4.32	1.60	109	H/H	Tle/Ser	G3
4.33	1.63	113	H/H	Ser/Tle	G1
4.34	1.65	110	H/H	Ser/Tle	G2
4.35	1.31	101	H/H	Ser/Tle	G3
4.36	1.84	111	H/Me	Tle/Ser	G1
4.37	1.93	110	H/Me	Tle/Ser	G2
4.38	2.22	105	H/Me	Tle/Ser	G3
4.39	5.51	108	Me/H	Tle/Ser	G1
4.40	1.82	105	Me/H	Tle/Ser	G2
4.41	3.76	97	Me/H	Tle/Ser	G3

Figure 4.5. Sub library of twelve macrocycles incorporating **G1**, **G2**, and **G3** templates and their corresponding permeability rates.

Subsequent studies focused on examining permeability differences among macrocycles bearing either the **G1**, **G2**, or **G3** template. A set of 12 compounds were synthesized using four peptides derived from the parent sequence H_2N-Trp-Tle-Ser-Tyramine (Figure 4.5). The peptides contain tryptophan at P1 for additional reactivity with the **G2**/**G3** templates and tyrosine at P4 for efficient Tsuji-Trost reactivity giving C-O linked macrocycles. The remainder of the sequence

contains amphiphilic residues and individual N-methylations to study the potential for hybrid compounds of this type to carry polar side chains across a cell membrane. All 12 macrocycles exhibited apparent permeability ranging from 1.15 - 5.51×10^{-6} cm/s. No change was observed between parent (**4.30** – **4.32**) and transposed (**4.33** – **4.35**) sequences, whereas N-methylations (**4.36** – **4.41**) led to the greatest increase in observed permeability. When comparing macrocycles across all three template systems, cellular permeability did not significantly vary. It's possible that structures bearing templates **G2**/**G3** benefit from increased lipophilicity, while those containing template **G1** are more flexible and can more effectively participate in IMHBs.

4.3 Conclusions

The work described here details various systematic modifications to examine structure-permeability relationships of a library of macrocycles. With peptidyl-based macrocycle inhibitors of PPIs increasingly growing in interest, studies on their pharmacological properties are greatly needed. The methodologies our group has developed to efficiently synthesize peptidomimetic macrocycles are uniquely amenable to this demand. Overall, we found most compounds exhibited at lease moderate permeability in RRCK cell lines with observed improvements based on site-selective modifications (e.g. N-methylations). In all cases the linear peptide starting materials were not permeable compared to their cyclic counterparts. This is a strong early starting point for us to understand important properties of these types of structures. Similar studies on analogous peptidomimetic macrocyclic systems are ongoing.

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Experimental Appendices

Chapter 2 Appendix Material

General 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, Waters LCT Premier, and Agilent 6545 LC-QTOF. NMR spectra were recorded on Bruker Avance (300, 400 or 500 MHz) spectrometers. HSQC, COSY, HMBC NMR experiments were used to aid assignment of NMR peaks when required.

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.

Experimental Procedures

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

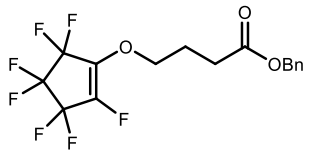
A solution of phenol (1.09 g, 11.6 mmol) in DMF (60 ml) was cooled to 0 °C and added octafluorocyclopentene (1.70 ml, 12.7 mmol) and Et_3N (2.66 ml, 19.1 mmol). The resulting solution was allowed to stir for 1 hr at 0 °C. The reaction mixture was added H_2O, extracted with EtOAc, washed with brine (x5), 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. ^{1}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 (DMSO-d_6, 282 MHz) δ -113.5 (d, J = 14 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-d_6, 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 NMR (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 (S2.1)



A solution of benzyl 4-hydroxybutanoate[S1] (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 (qui, 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 $C_{16}H_{13}F_7O_3$ 386.0747, found 386.0748.

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

A solution of **S2.1** (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 $CHCl_3$. 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 33-50% EtOAc/hexane) 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, 2H), 2.82 (brs, 4H), 2.09 (qui, *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* = 1.3 Hz). ^{19}F NMR (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-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 compound (108 mg, 0.186 mmol, 72%) as a white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 7.46 (brs, 1H), 7.39 (d, *J* = 8.8 Hz, 1H), 7.32-7.24 (m, 5H), 7.20 (t, *J* = 8.5, 2H), 7.11 (d, *J* = 8.6 Hz), 7.06-7.03 (m, 2H), 6.75 (d, *J* = 8.0 Hz, 2H), 6.72 (d, *J* = 8.7 Hz), 4.95 and 4.93 (AB quartet, *J* = 12.8 Hz, 2H), 4.11-4.06 (m, 1H), 2.89 (dd, *J* = 13.7, 3.9 Hz), 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 $C_{28}H_{22}F_6N_2O_5H$ 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. ^{1}H NMR (DMSO-d_6, 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

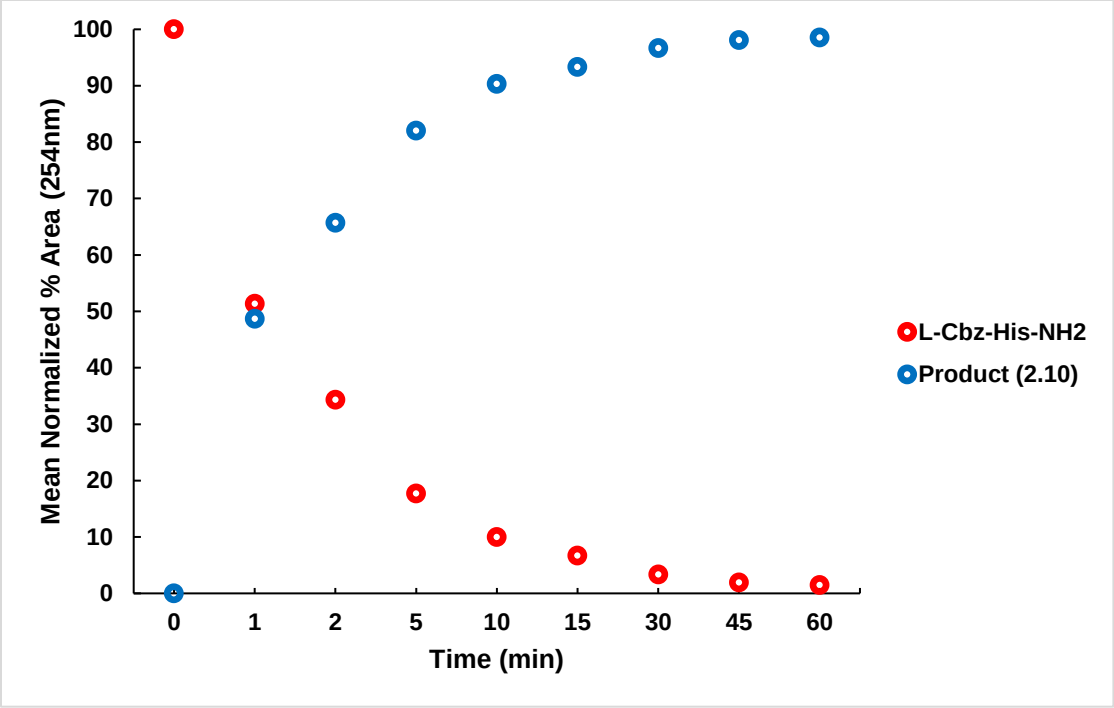
organic layer was washed with brine (5 times), dried over Na_2SO_4, filtered and the solvent was removed by rotary evaporation to give a crude oil. Purification by column chromatography (SiO_2, gradient 0-5% MeOH/$CHCl_3$) afforded the title compound (120 mg, 0.216 mmol, 81%) as a white solid. ^{1}H 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), 2.81 (dd, *J* = 15, 4.2 Hz), 2.68 (dd, *J* = 15, 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 ((8*R*)-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 **2.5** (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 **2.11a** provided a single compound, confirming the presence the initial rotamer mixture. **2.11a**: ^{1}H NMR (DMSO-d_6, 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-d_6, 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 – (-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. **2.11b**: ^{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-d_6, 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 (dd, *J* = 243.6, *J* = 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.

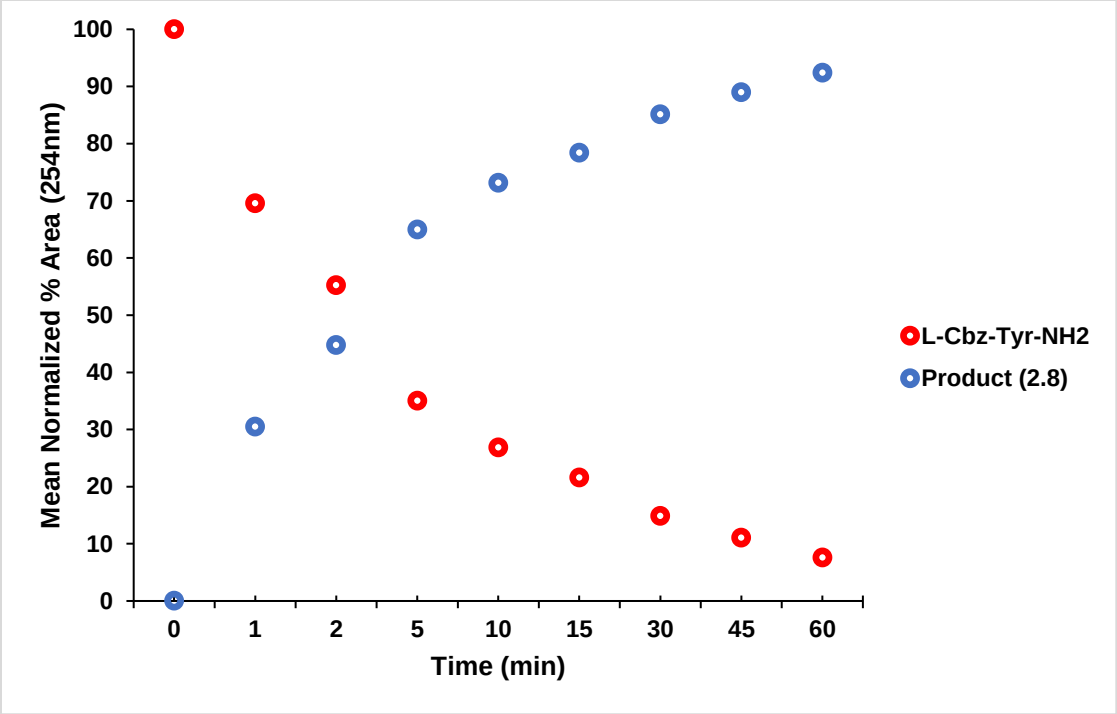
*Rate determination for individual reactions of L-Cbz-His-NH_2, L-Cbz-Tyr-NH_2, L-Cbz-Ser-NH_2, and L-Cbz-Cys-NH_2 with mono phenoxy derivative **2.5***

Amino acids (0.05 mmol) L-Cbz-Ser-NH_2, L-Cbz-Tyr-NH_2, L-Cbz-His-NH_2, and L-Cbz-Cys-NH_2 were individually subjected (as triplicates) to reactions with mono phenoxy derivative **5** (0.065 mmol, 1.3 eq) and Cs_2CO_3 (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-NH_2 was observed to be fully consumed within 15 seconds.



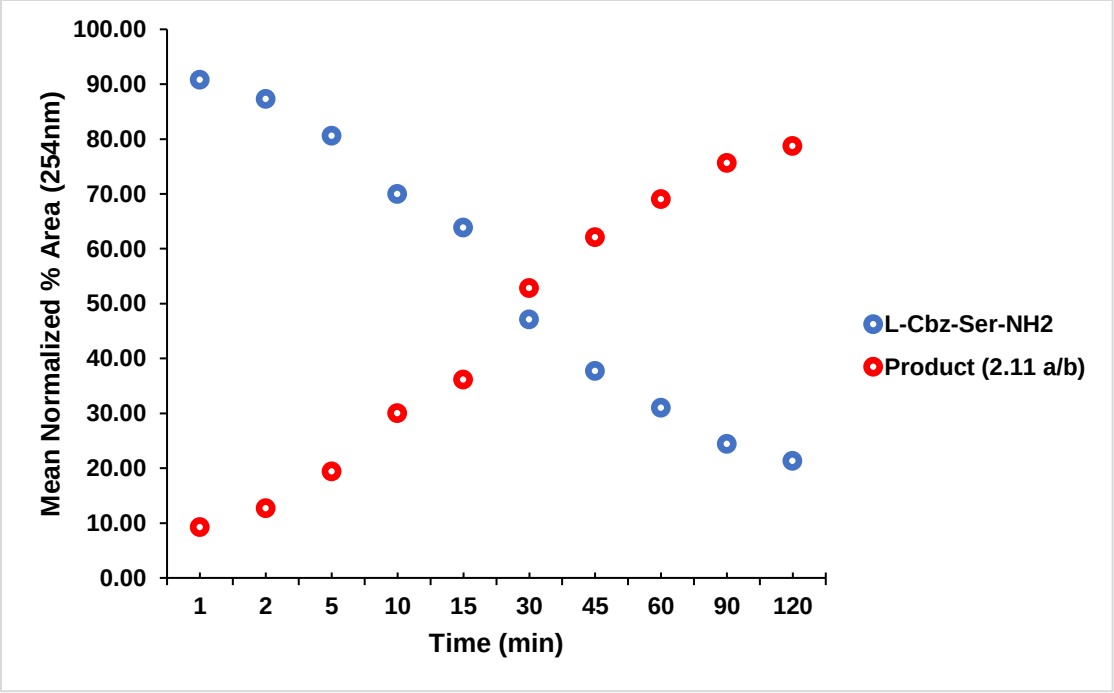
	Mean (n = 3) Normalized % Area (254 nm)	
Time (min)	L-Cbz-His-NH_2	Product (**2.10**)
0	100	0
1	51.3	48.7
2	34.3	65.7
5	17.7	82.0
10	10.0	90.3
15	6.7	93.3
30	3.3	96.7
45	1.9	98.1
60	1.5	98.5

Figure S1. Rate determination for the reaction of L-Cbz-His-NH_2 with mono phenoxy derivative **2.5**.



	Mean (n = 3) Normalized % Area (254 nm)	
Time (min)	L-Cbz-Tyr-NH_2	Product (**2.8**)
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

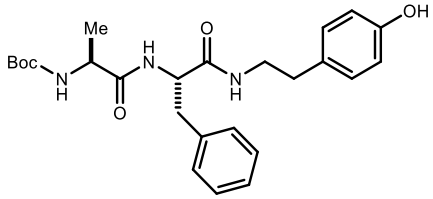
Figure S2. Rate determination for the reaction of L-Cbz-Tyr-NH_2 with mono phenoxy derivative **2.5**.



	Mean (n = 3) Normalized % Area (254 nm)	
Time (min)	L-Cbz-Ser-NH_2	Product (**2.11 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

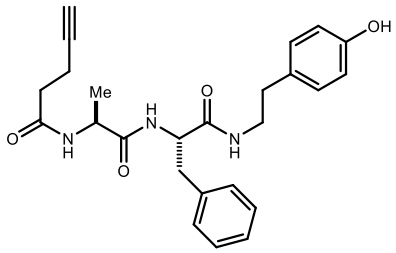
Figure S3. Rate determination for the reaction of L-Cbz-Ser-NH_2 with mono phenoxy derivative **2.5**.

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



Tyramine·HCl (1.70 g, 9.80 mmol), iPr_2EtN (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 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 (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), iPr_2EtN (2.17 ml, 12.5 mmol) and then by HBTU (2.05 g, 5.41 mmol). After stirring 1 hr, added H_2O and extracted with EtOAc. The combined organic phase was washed sequentially with 1N HCl, sat. $NaHCO_3$ and brine. Dried over Na_2SO_4 and concentrated. Purification by column chromatography (SiO_2, 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 (S2.3)**



TFA (10 ml) was added to a solution of **S2.2** (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 **2.12** (1.80 g, 3.84 mmol). The residue **2.12** (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), iPr_2EtN (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 $NaHCO_3$ and brine, then dried over Na_2SO_4 and concentrated. The residue was triturated with Et_2O, 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)**

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. ^{1}H 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/hexane) 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 **S2.2** (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 **2.12** (1.80 g, 3.84 mmol). The residue **2.12** (62 mg, 0.131 mmol, analytical) was dissolved in DMF (1.3 ml) and added **2.7** (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 **2.15**. The residue **2.15** 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) δ172.6, 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), iPr_2EtN (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-d_6, 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) δ172.3, 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), 1.54-1.15 (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-(1*H*-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 **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* = 6.1 Hz, 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, 2CF3**C**O2H), 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 2**C**F_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 **2.19** (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, CF3**C**O2H), 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, **C**F_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-d_6, 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 **2.21** (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), 4.51-4.45 (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 171.9, 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-Nal)AC-NH_2 (2.23)

Synthesized according to General Procedure A. ^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.13 (br s, 1H), 8.25 (d, *J* = 6.9 Hz, 1H), 8.09 (d, *J* = 8.0, 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-d_6, 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, 126 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/H_2O) 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, 126 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-d_6, 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 **2.29** (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. ^{1}H 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. ^{1}H 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. ^{1}H 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. ^{1}H 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 white solid. ^{1}H NMR (DMSO-d_6, 500 MHz) 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 **2.35** (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. ^{1}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. ^{19}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)

37[S2] (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) -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-Nal)AC-NH_2·TFA (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, 125.5, 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), -111.3 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, 3H), 1.20 (d, *J* = 7.1 Hz, 3H). ^{13}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, 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), 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. ^{1}H 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-d_6, 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-d_6, 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-d_6, 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-d_6, 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-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 $[M + Na]^+$ calc'd for $C_{39}H_{42}F_4N_8O_{10}S_2Na$ 945.2299, found 945.2327.

Ac-SFC-NH_2 (S2.4)

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.

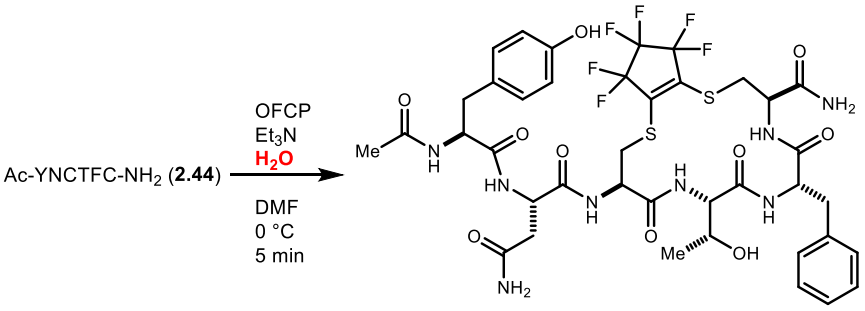
Macrocyclic Product (2.48)

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

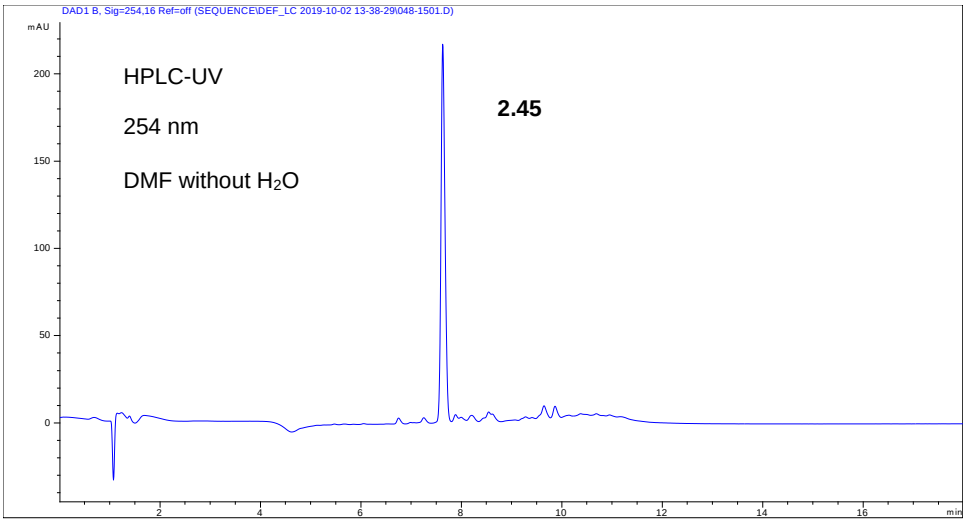
^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.68 (d, J = 7.9 Hz, 1H), 8.45 (d, J = 7.6 Hz, 1H), 8.28-8.18 (m, 3H), 7.42 (brs, 1H), 7.33-7.16 (m, 6H), 4.66-4.57 (m, 1H), 4.38-4.24 (m, 2H), 4.19 (dd, J = 7.0, 14.5 Hz, 1H), 4.11-4.02 (m, 1H), 3.12 (dd, J = 14.0, 4.3 Hz, 1H), 2.89-2.76 (m, 3H), 2.00 (s, 3H). ^{13}C 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.

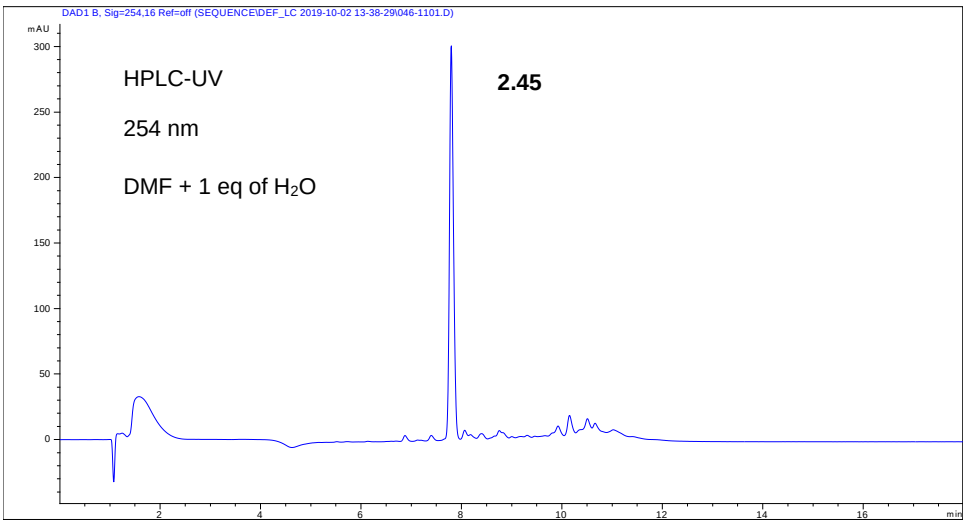
Water Sensitivity Experiments

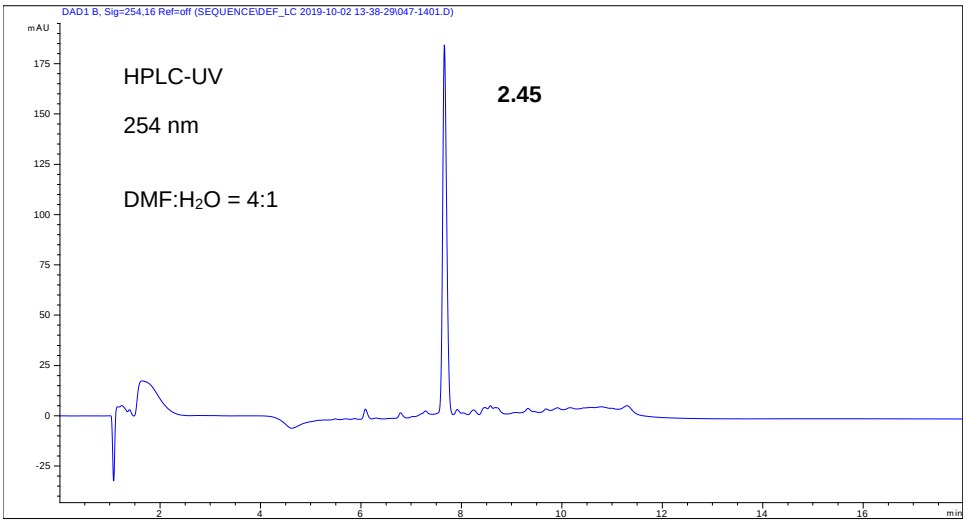


2.45

A flask was charged with linear precursor **2.44** (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

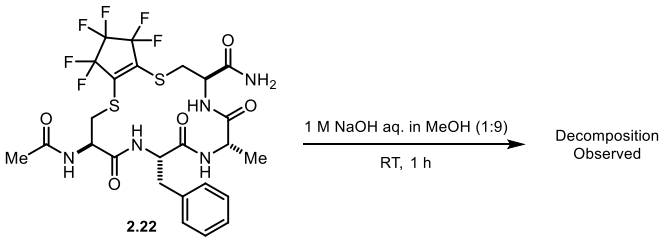
Column: Agilent Zorbax SB-Aq 4.6 x 50 mm, 1.8 µm
Solvent A: H_2O + 0.1% TFA
Solvent B: MeCN + 0.1% TFA
Flow rate: 1.00 ml/min

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

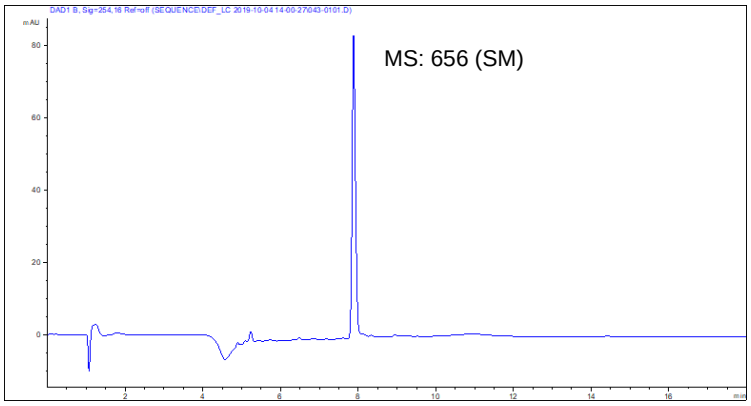
Figure S2.4. Method for the determination of aqueous stability in the reaction of **44** to **45**.

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 hours. These results were determined based on HPLC traces obtained at various time points as outlined below.



T = 0 h



T = 1 h (NaOH)

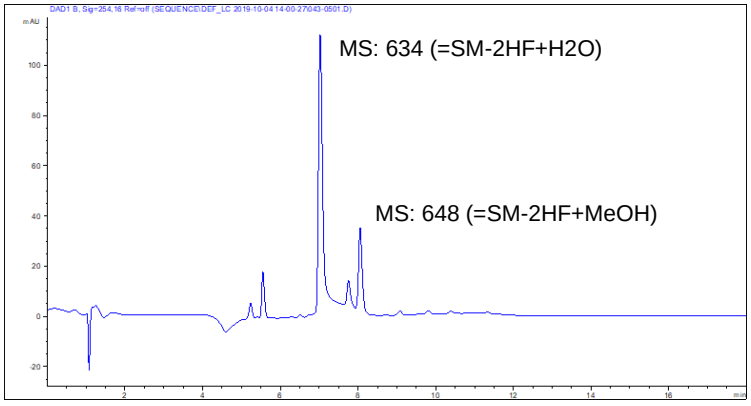
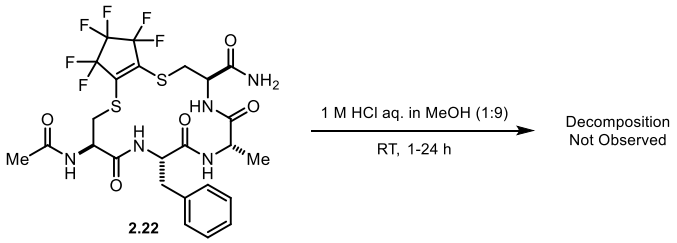


Figure S2.5. Determination of basic pH stability of **2.22** by HPLC over 1 hour.



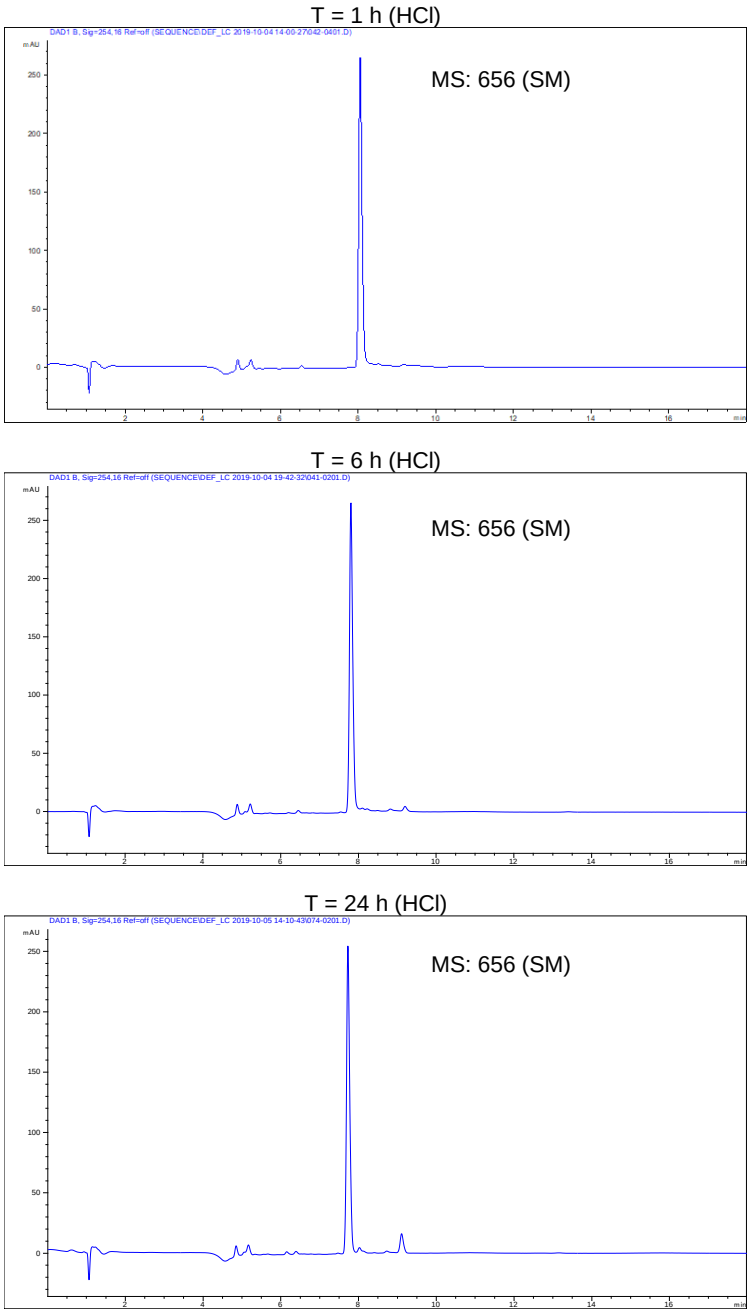
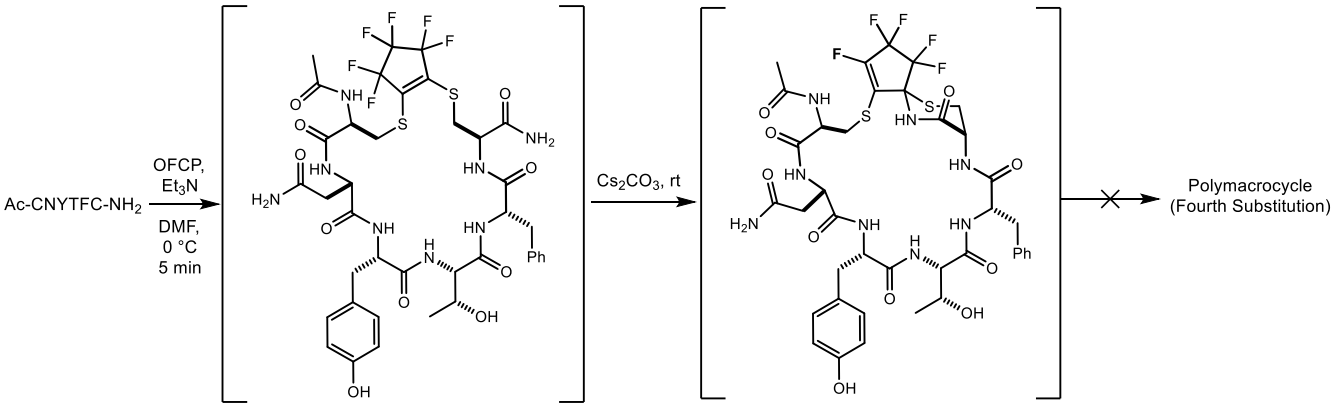
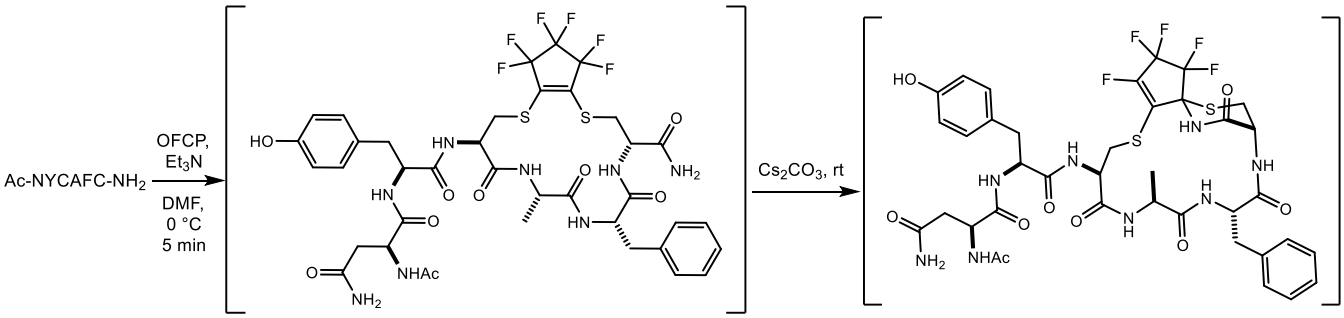


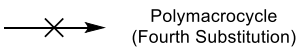
Figure S2.6. Rate determination for the reaction of L-Cbz-His-NH_2 with mono phenoxy derivative **5**.

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 **2.43** and **2.47**, 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.







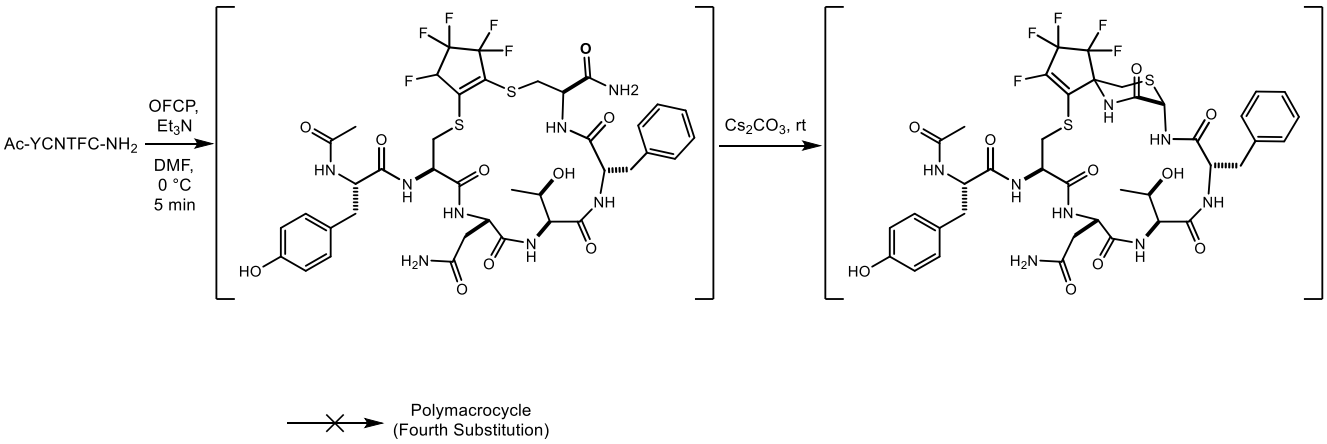


Figure S2.7. Probing the YXCXXC consensus sequence for macrobicycle syntheses using scrambled variations of Ac-YNCTFC-NH_2

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.

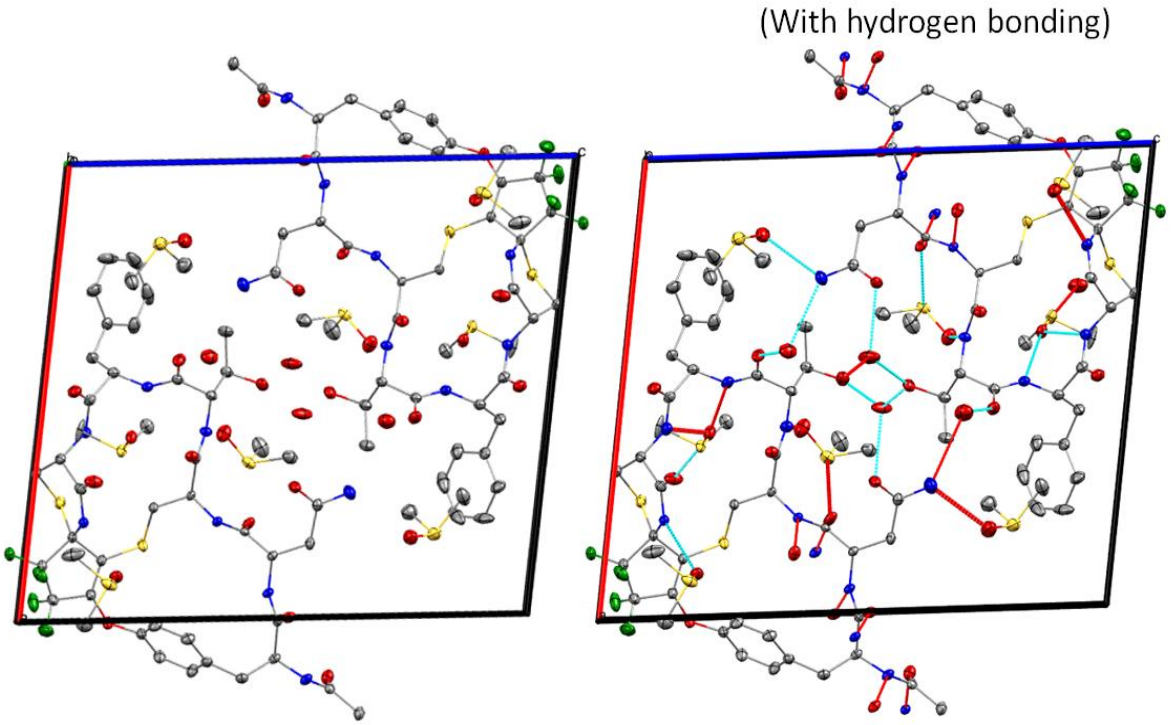
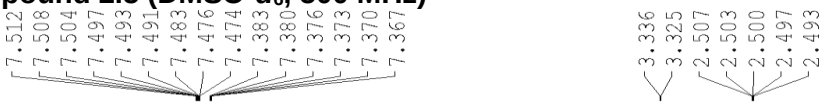
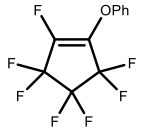


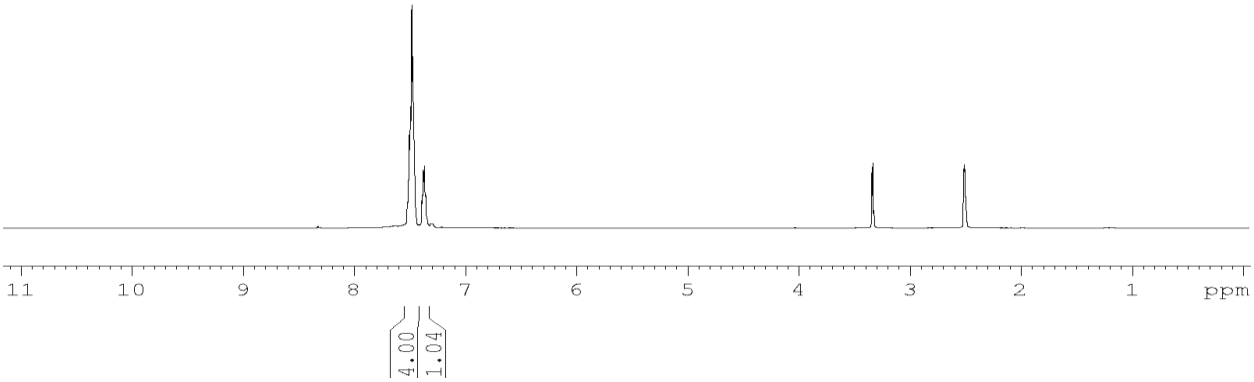
Figure S2.8. Structure was solved in space group P2(1) with ORTEP showing 50% probability. Both residual DMSO and water molecules can be observed within the unit cell. Water atoms can be seen localized between the two asymetric units of the of the unit cell and may be providing additional stabilization effects through hydrogen bonding.

NMR Spectra

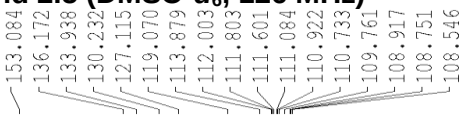
^{1}H NMR of compound 2.5 (DMSO-d_6, 500 MHz)

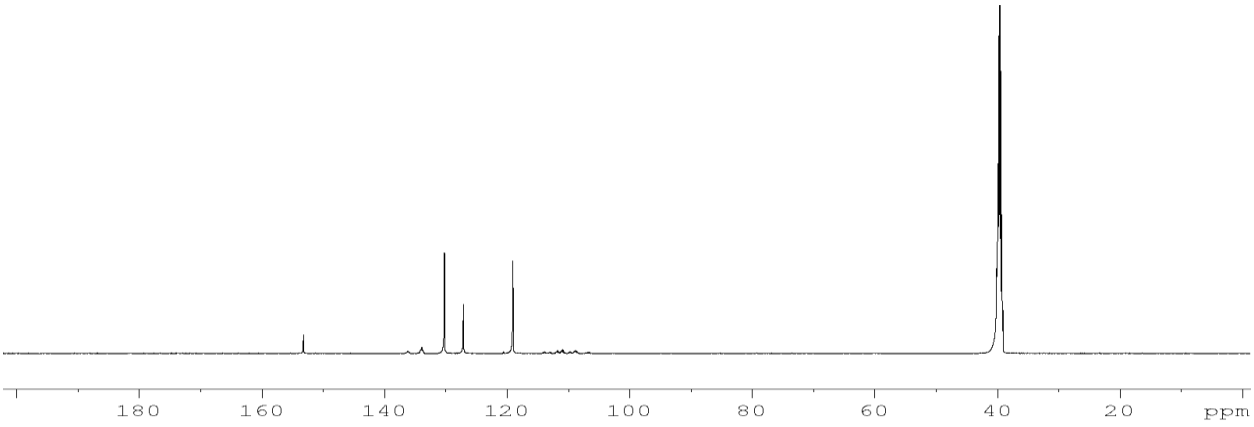




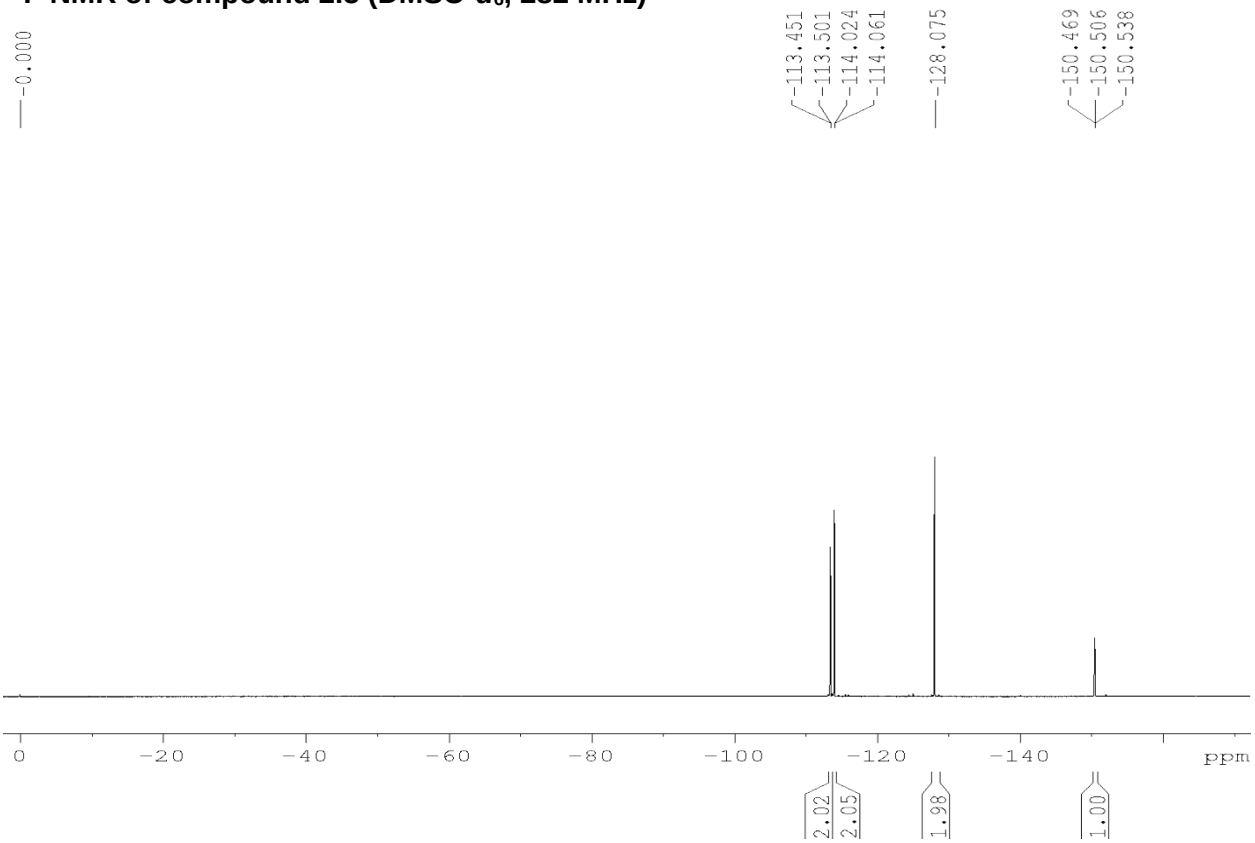


^{13}C NMR of compound 2.5 (DMSO-d_6, 126 MHz)

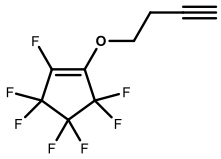




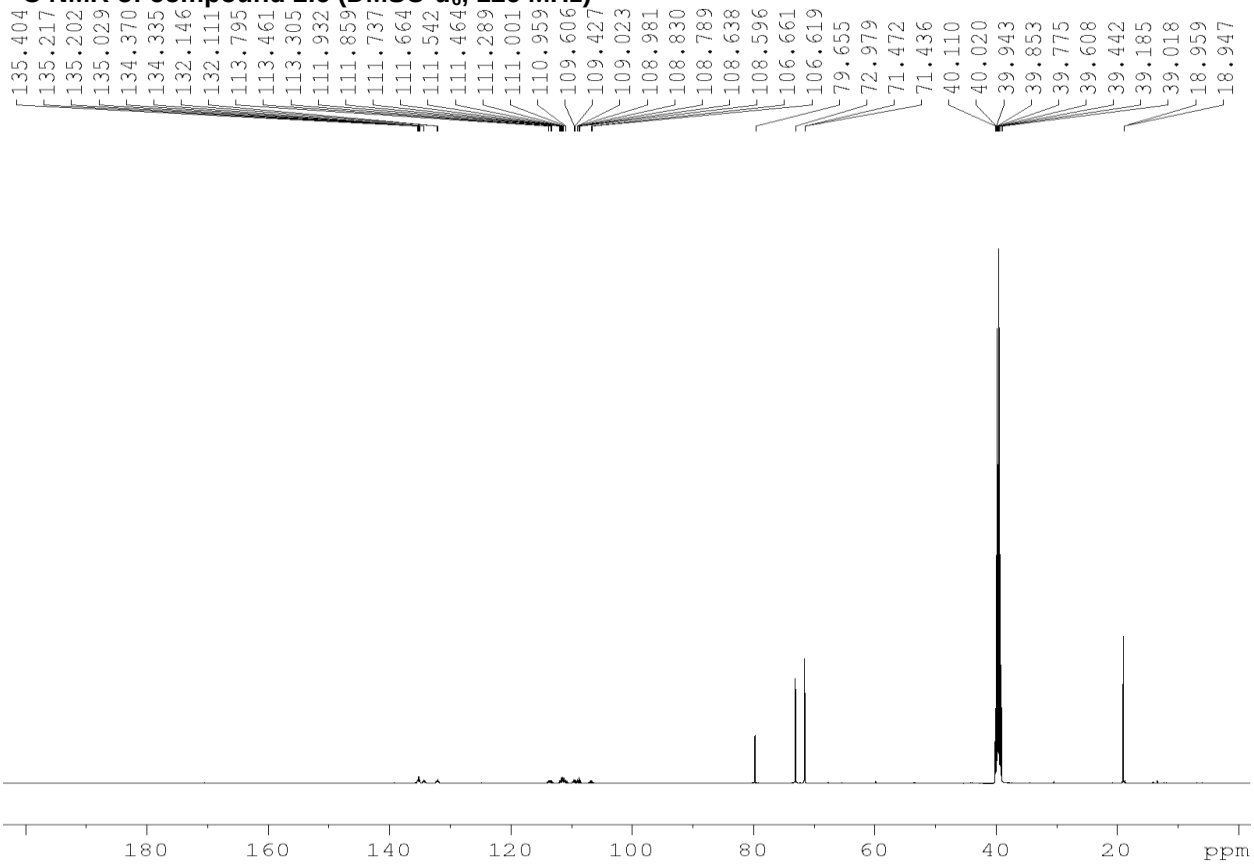
^{19}F NMR of compound 2.5 (DMSO-d_6, 282 MHz)



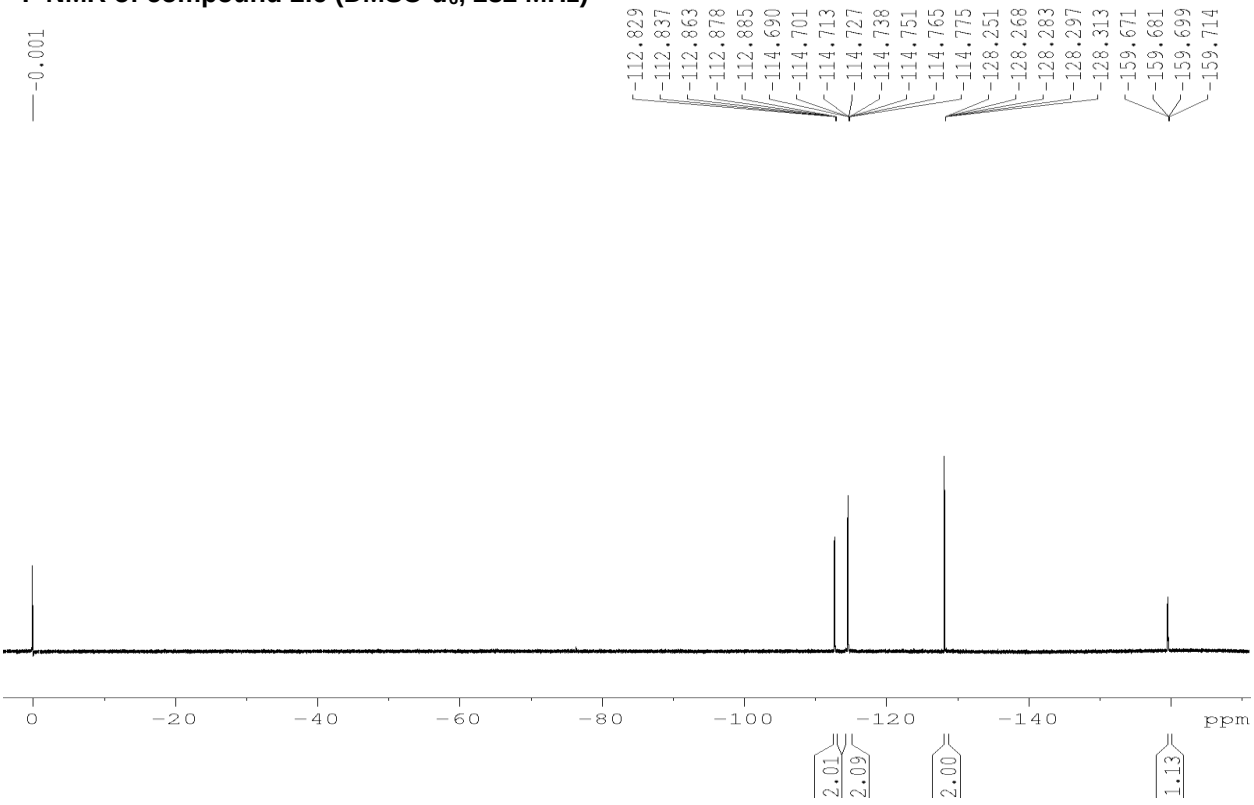
^{1}H NMR of compound 2.6 (DMSO-d_6, 500 MHz)



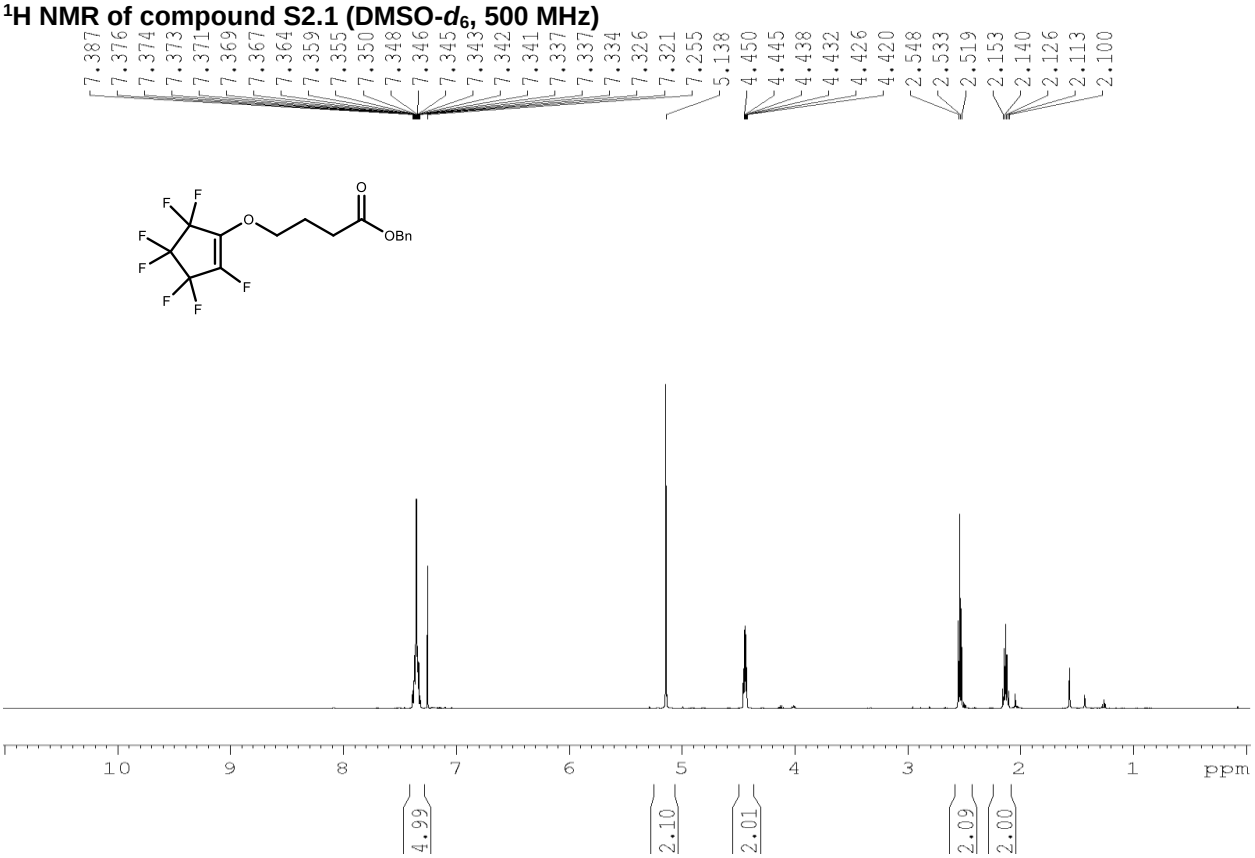
^{13}C NMR of compound 2.6 (DMSO-d_6, 126 MHz)



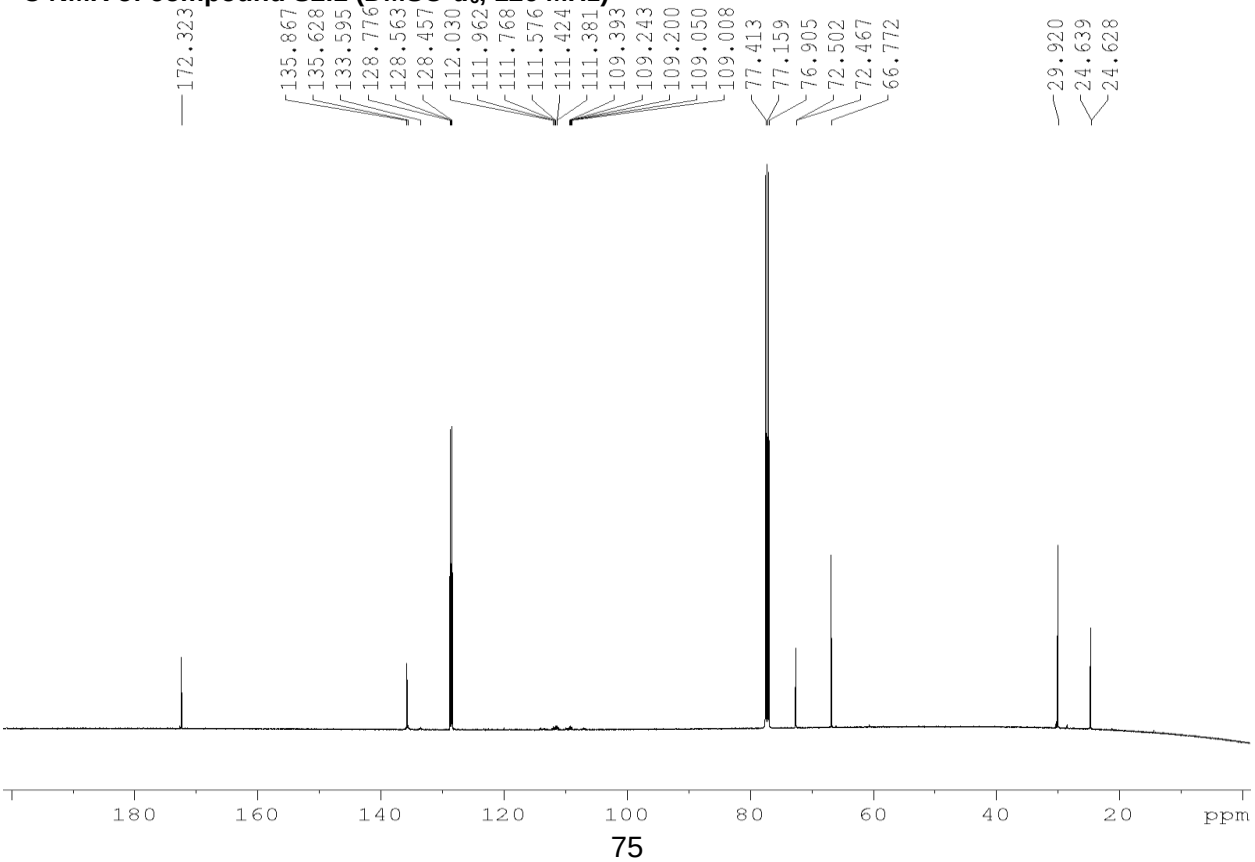
^{19}F NMR of compound 2.6 (DMSO-d_6, 282 MHz)



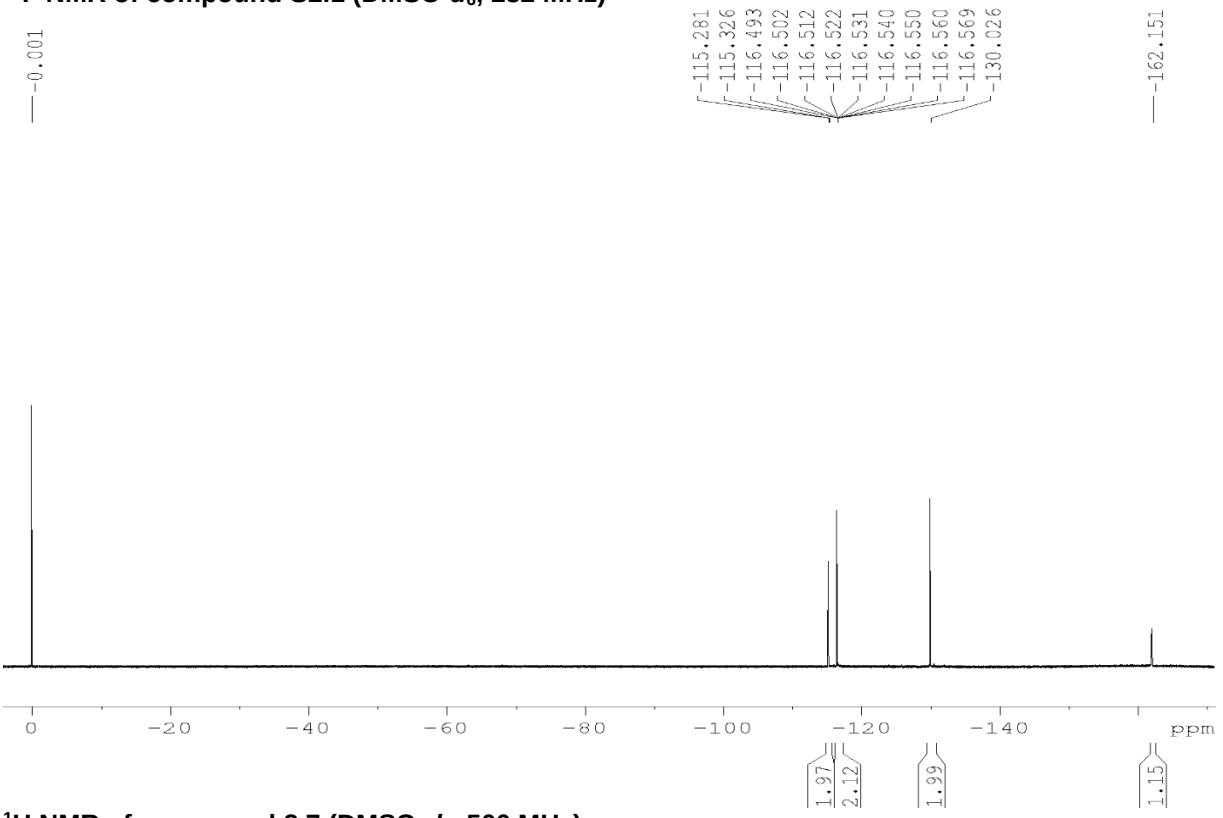
^{1}H NMR of compound S2.1 (DMSO-d_6, 500 MHz)



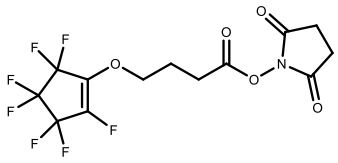
^{13}C NMR of compound S2.1 (DMSO-d_6, 126 MHz)



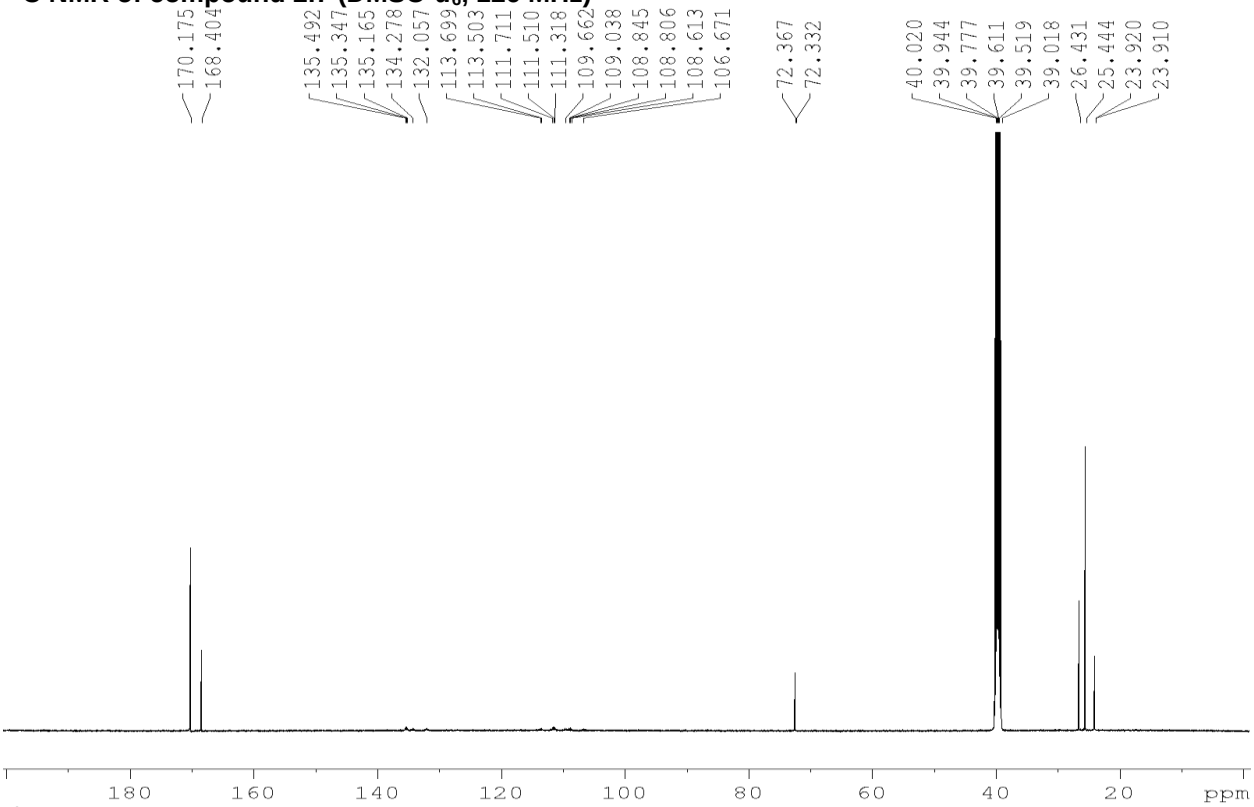
^{19}F NMR of compound S2.1 (DMSO-d_6, 282 MHz)



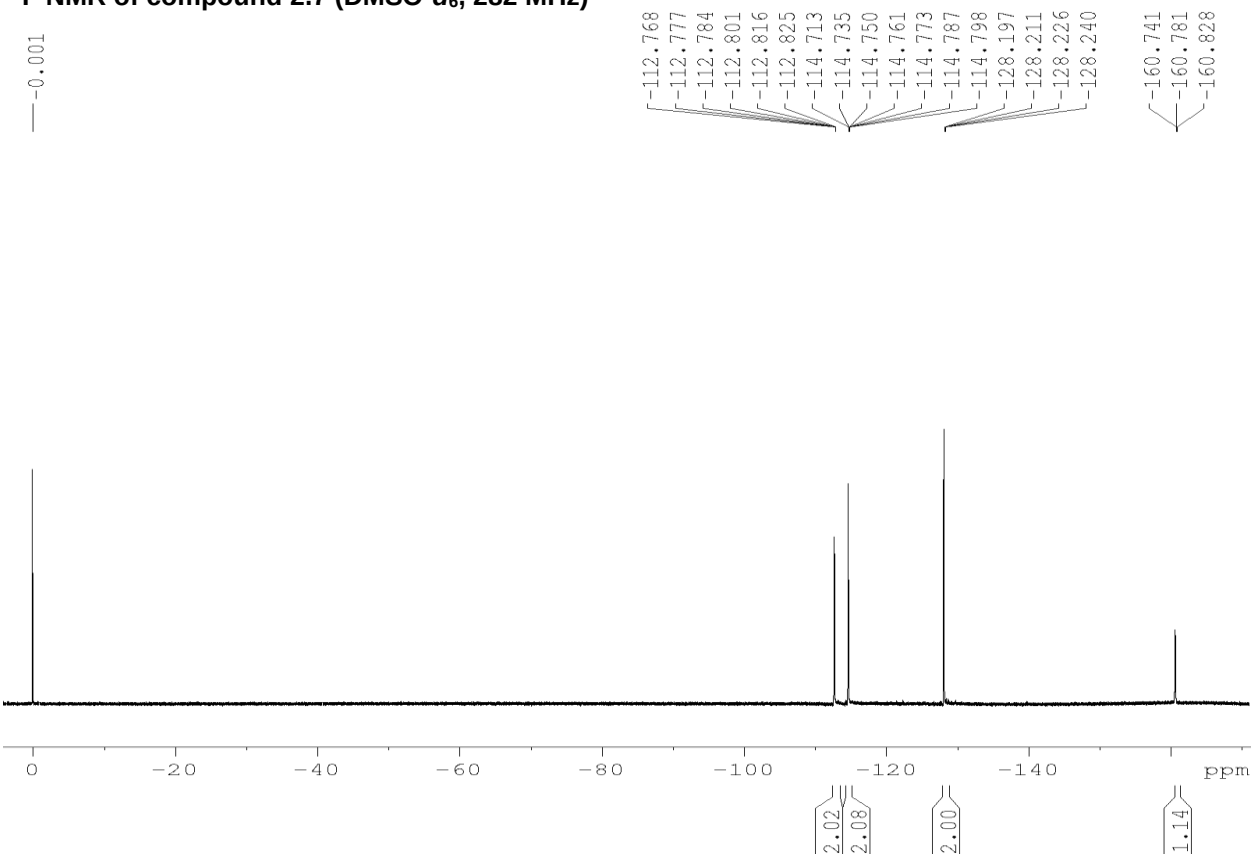
^{1}H NMR of compound 2.7 (DMSO-d_6, 500 MHz)



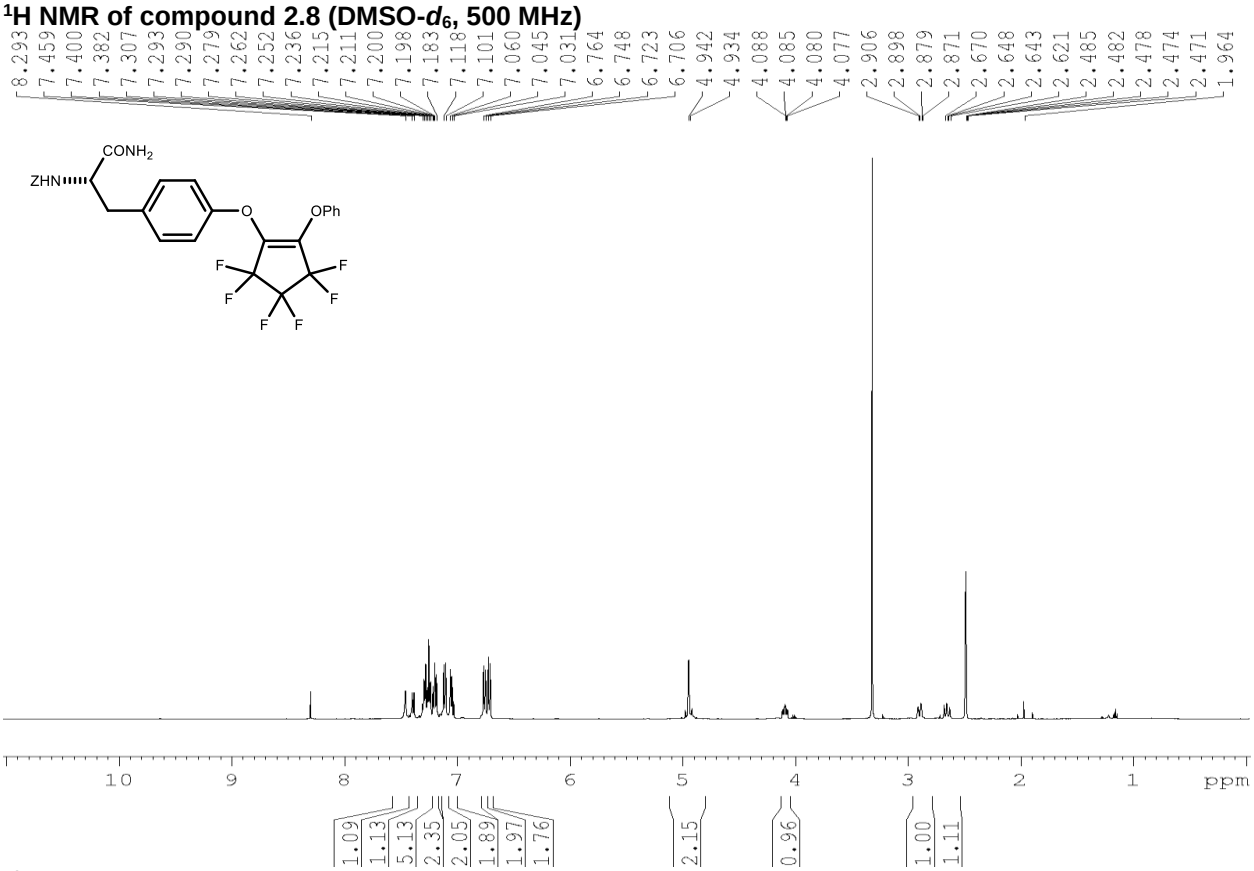
^{13}C NMR of compound 2.7 (DMSO-d_6, 126 MHz)



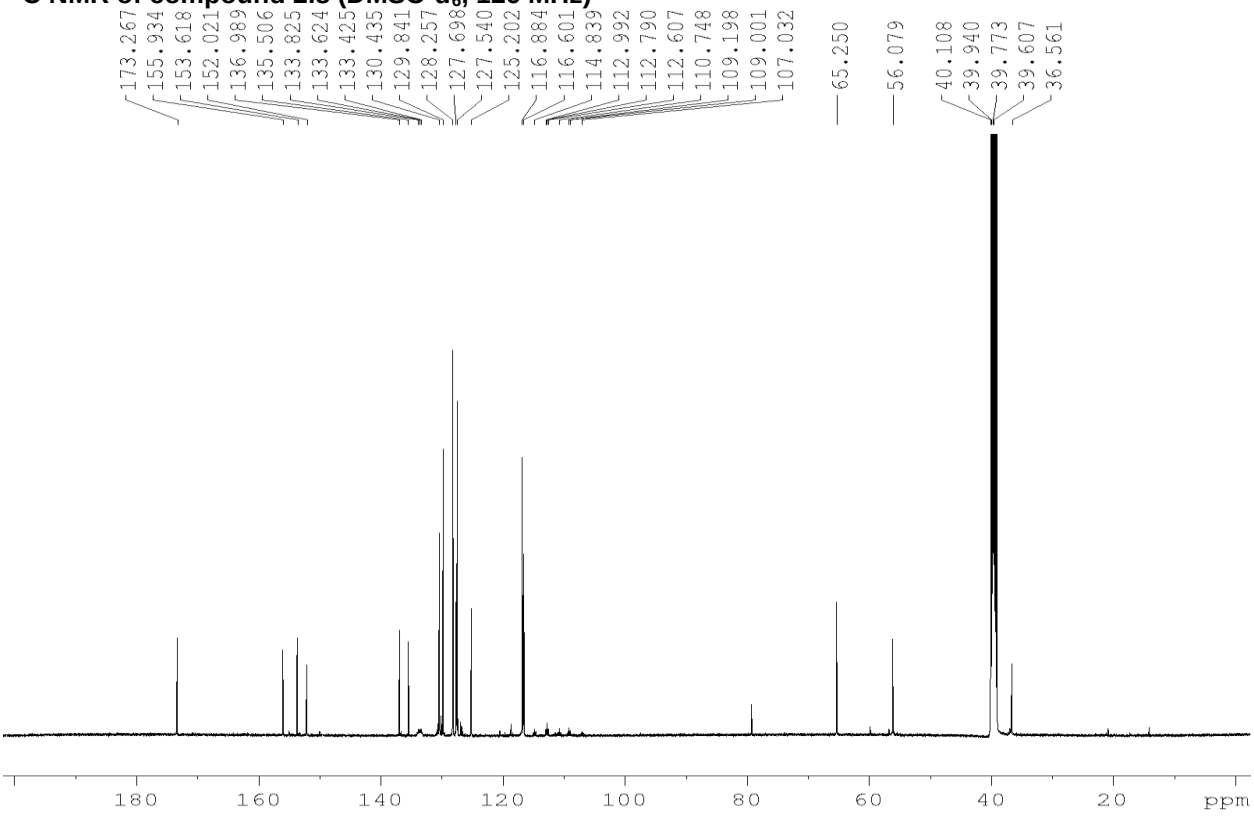
^{19}F NMR of compound 2.7 (DMSO-d_6, 282 MHz)



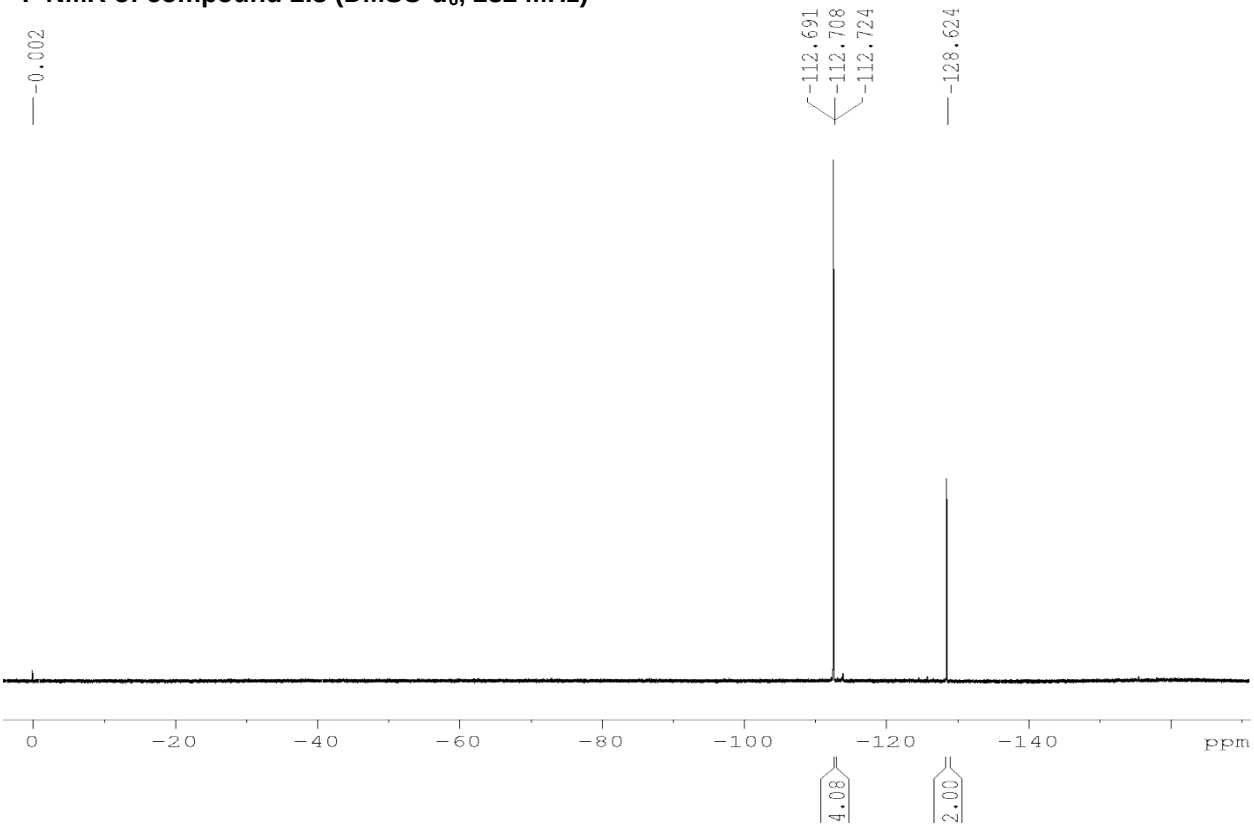
^{1}H NMR of compound 2.8 (DMSO-d_6, 500 MHz)



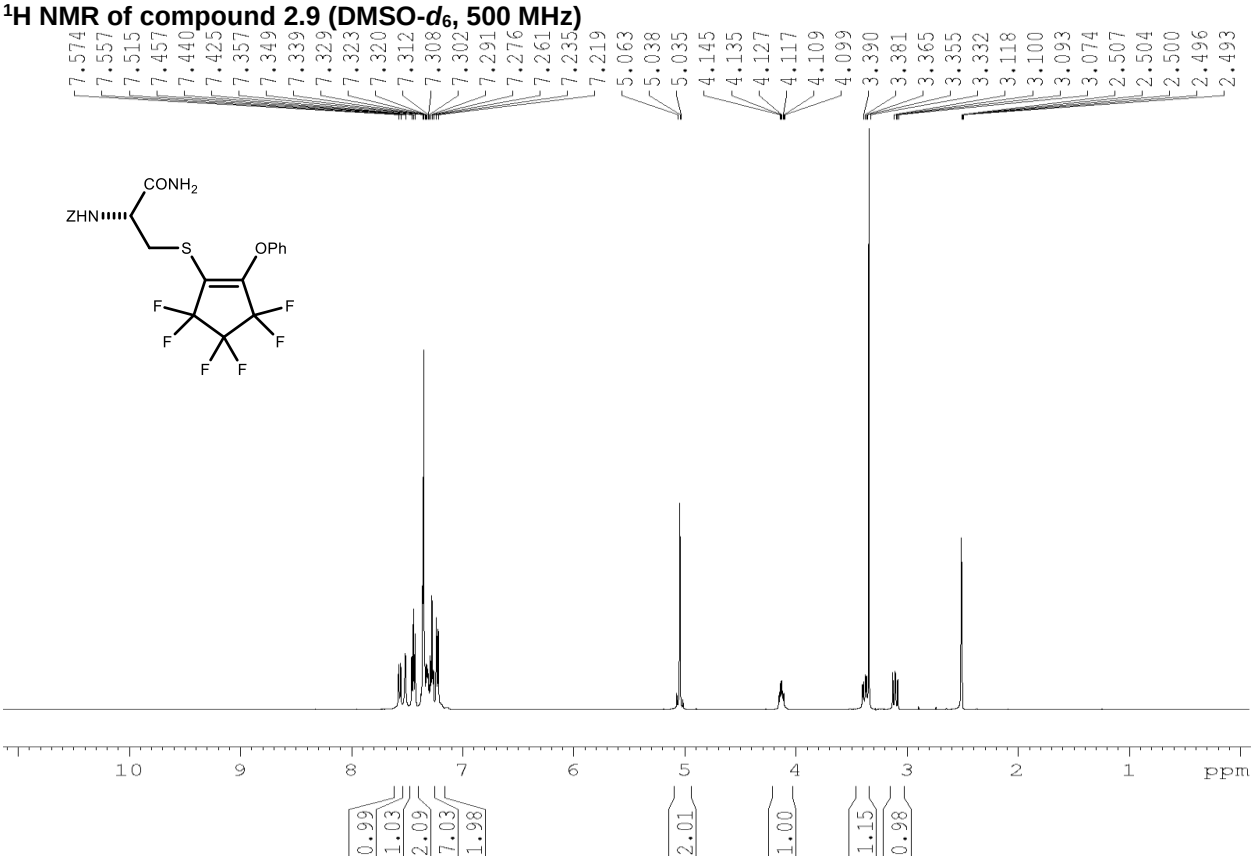
^{13}C NMR of compound 2.8 (DMSO-d_6, 126 MHz)



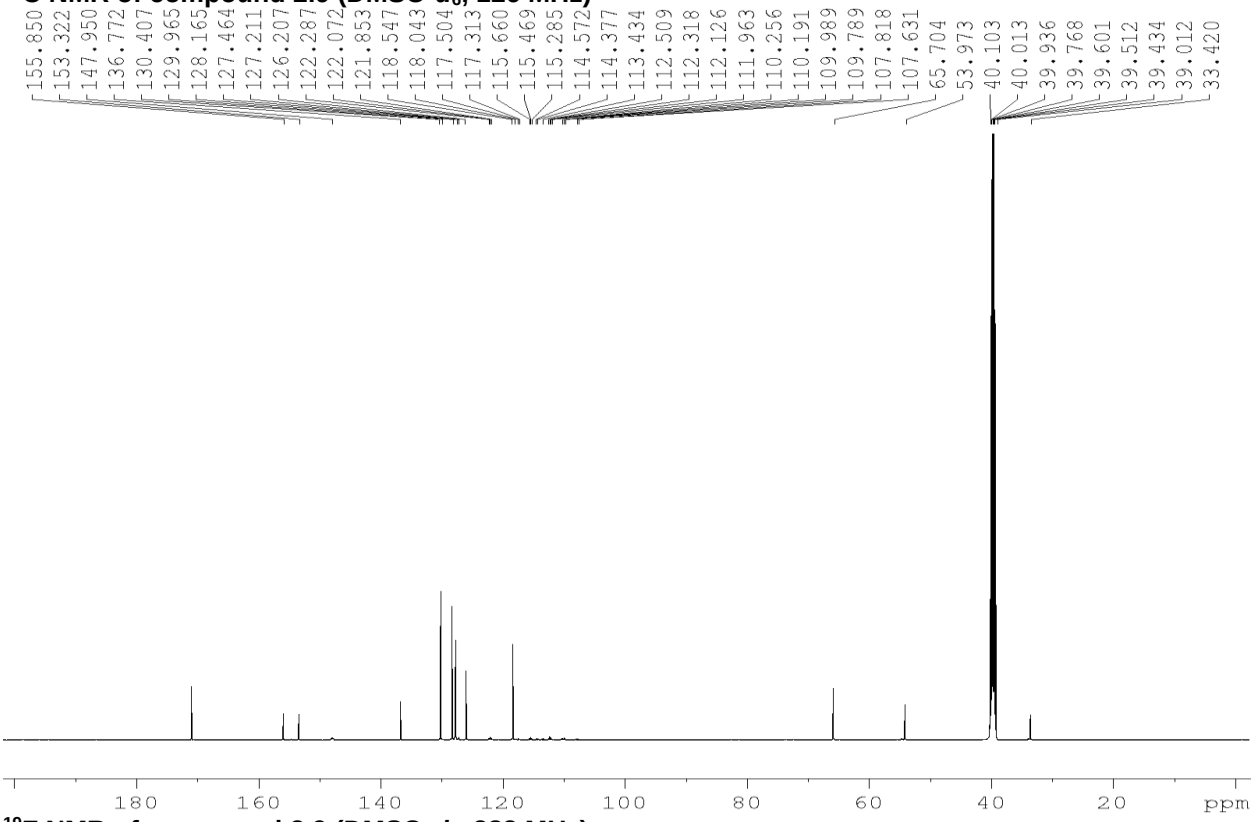
^{19}F NMR of compound 2.8 (DMSO-d_6, 282 MHz)



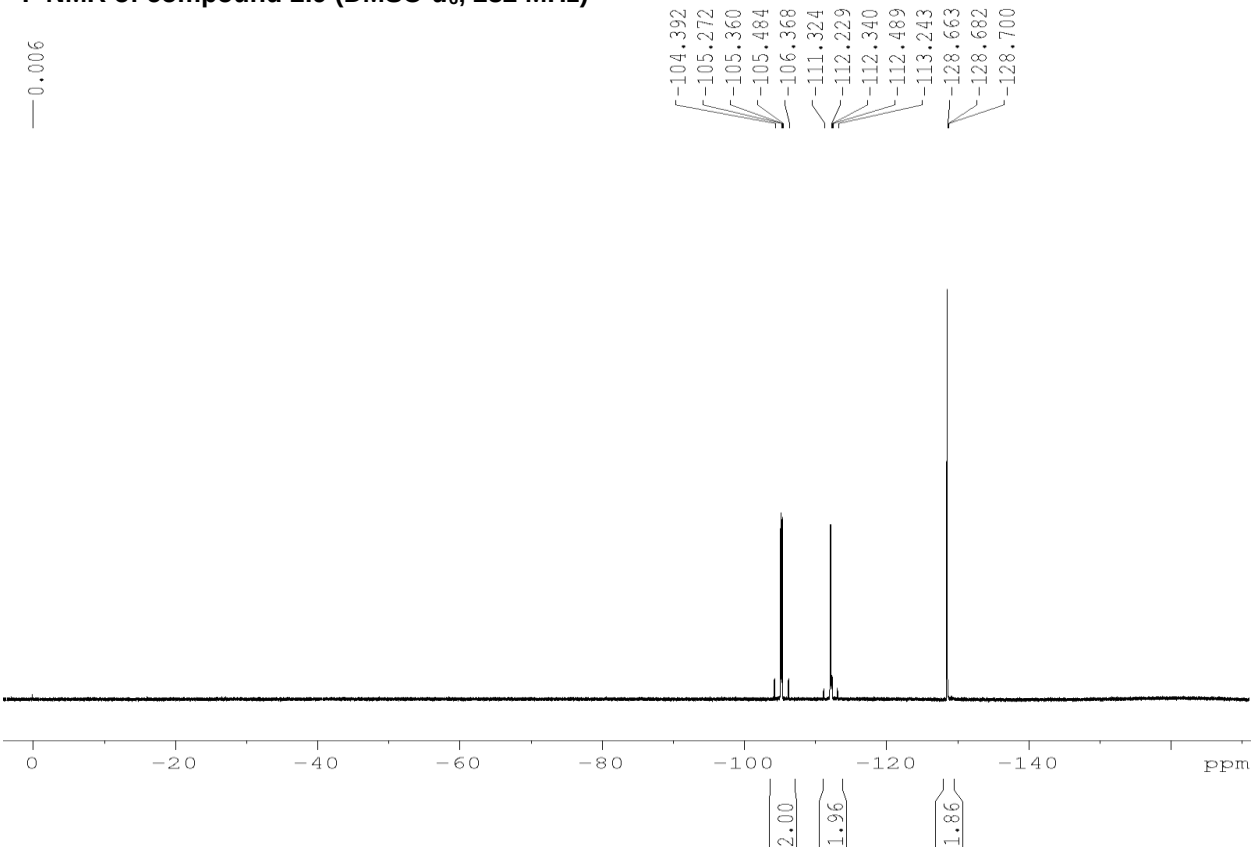
^{1}H NMR of compound 2.9 (DMSO-d_6, 500 MHz)



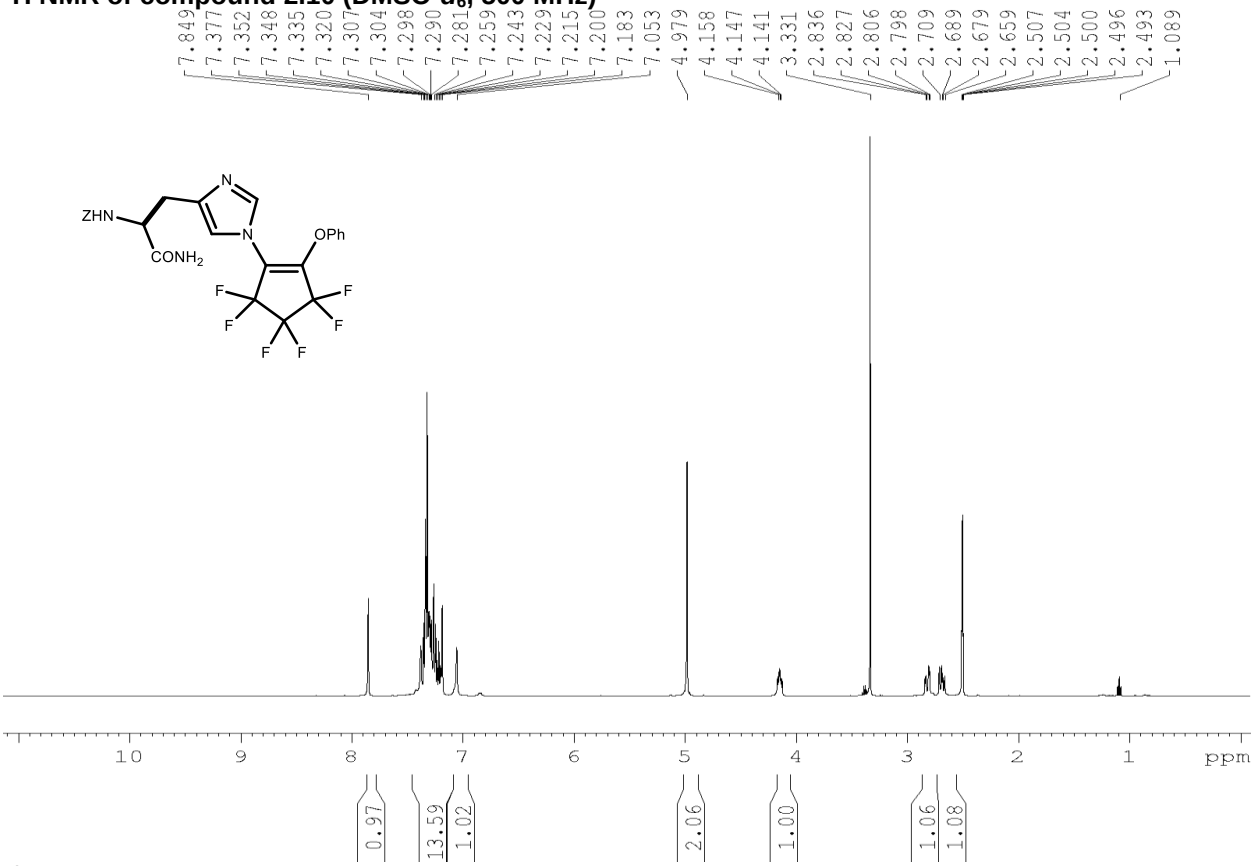
^{13}C NMR of compound 2.9 (DMSO-d_6, 126 MHz)



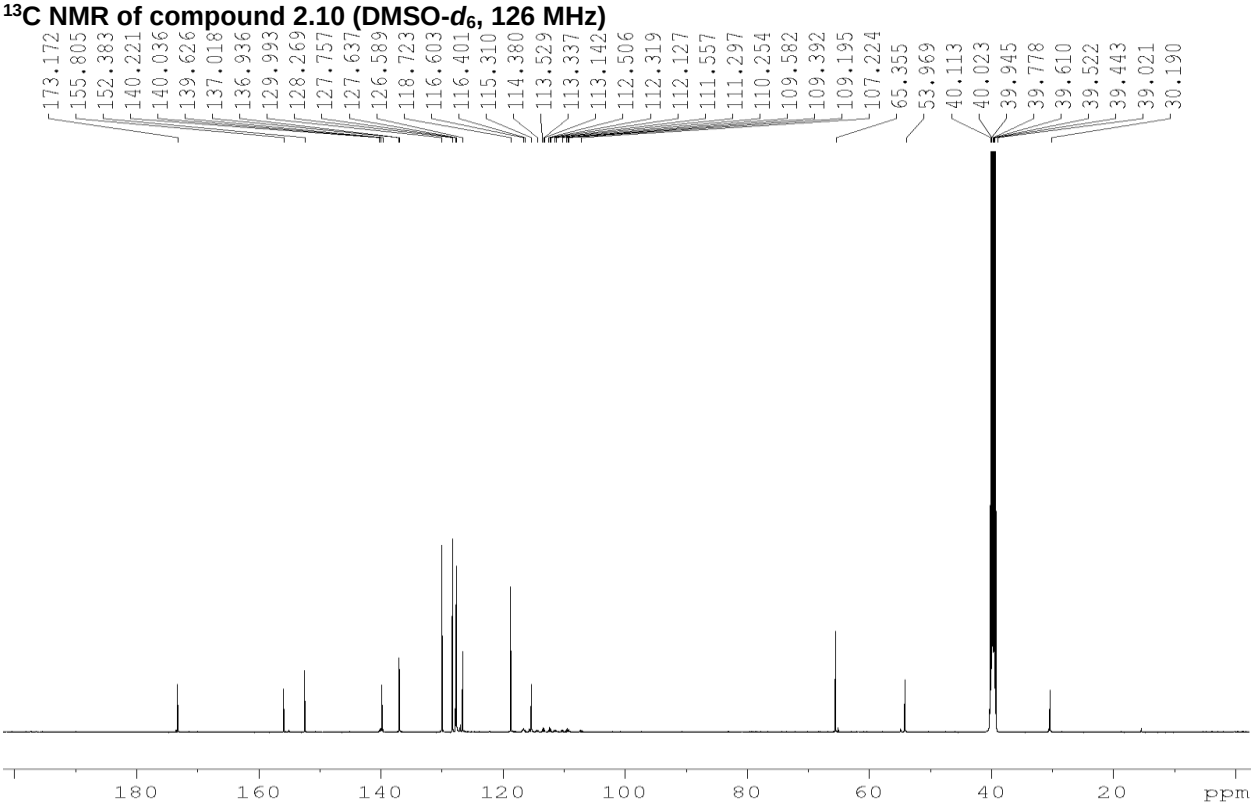
^{19}F NMR of compound 2.9 (DMSO-d_6, 282 MHz)



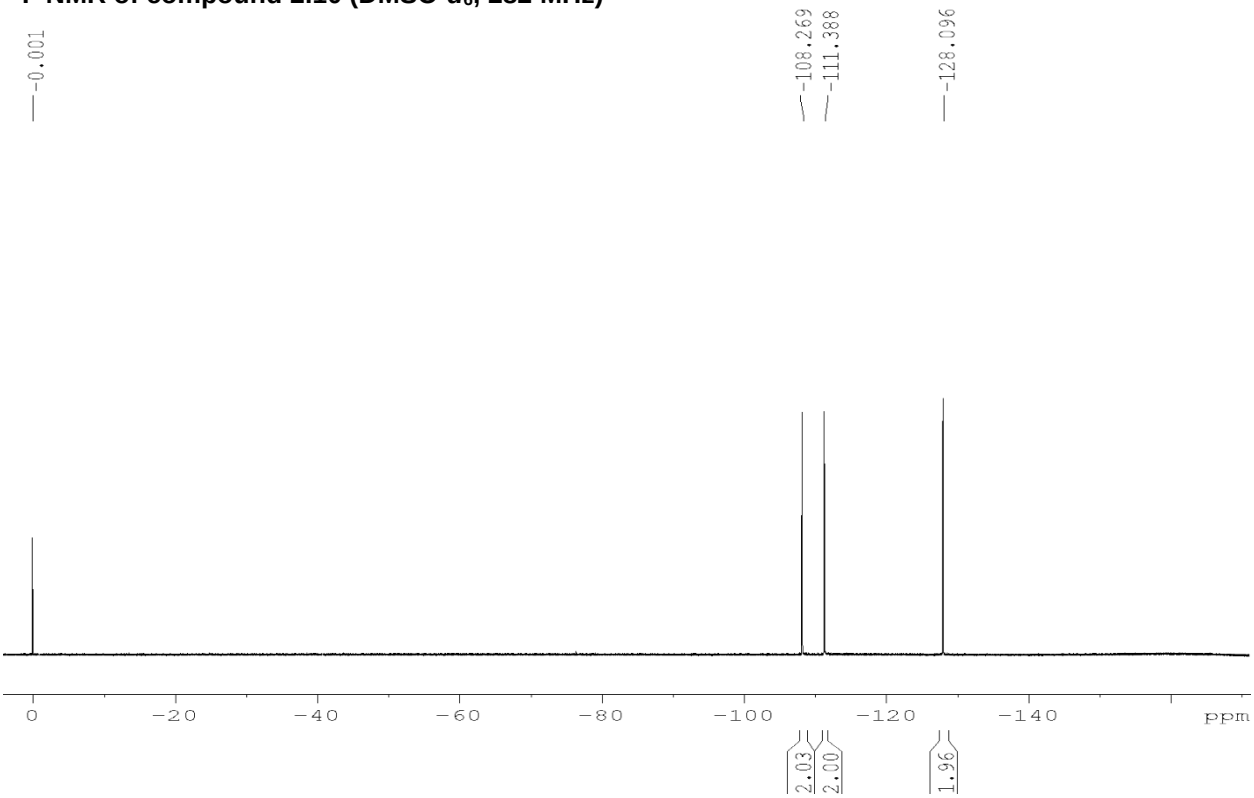
^{1}H NMR of compound 2.10 (DMSO-d_6, 500 MHz)



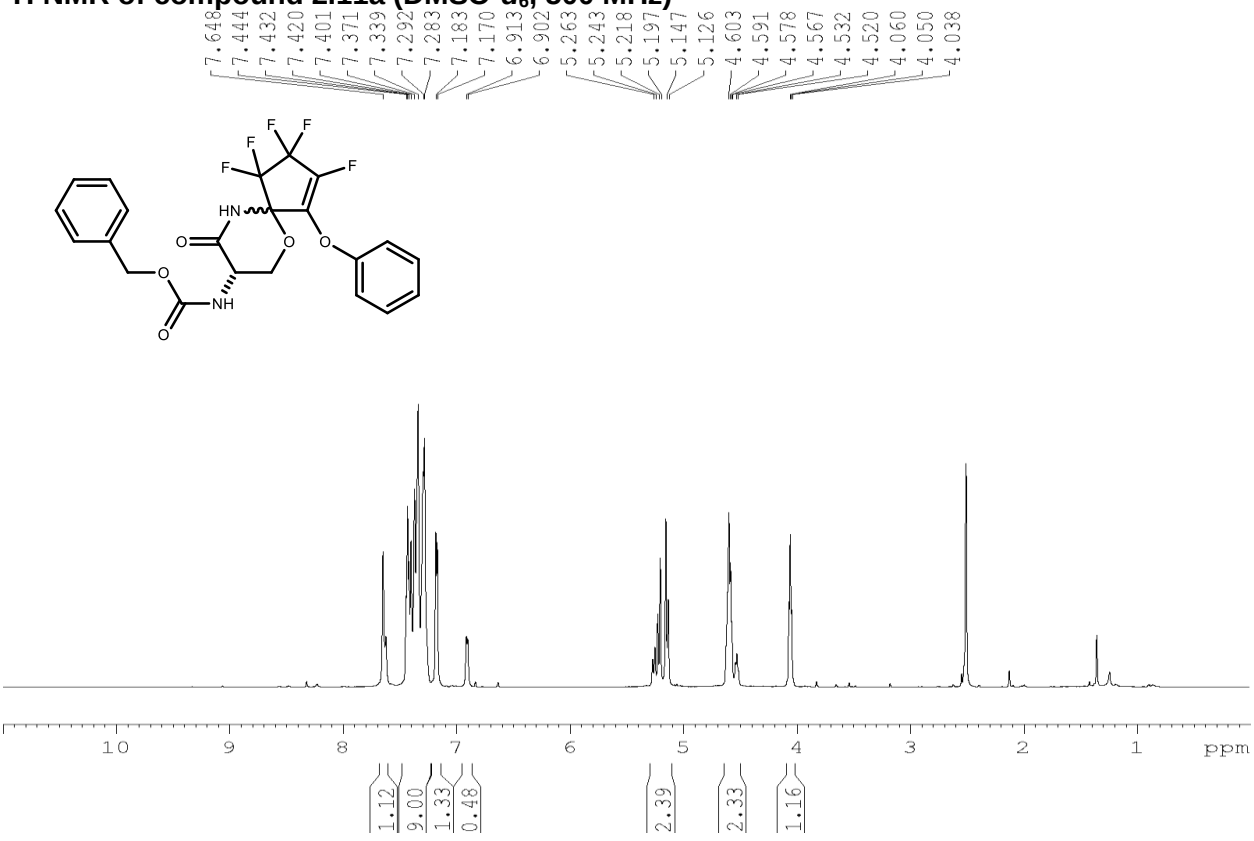
^{13}C NMR of compound 2.10 (DMSO-d_6, 126 MHz)



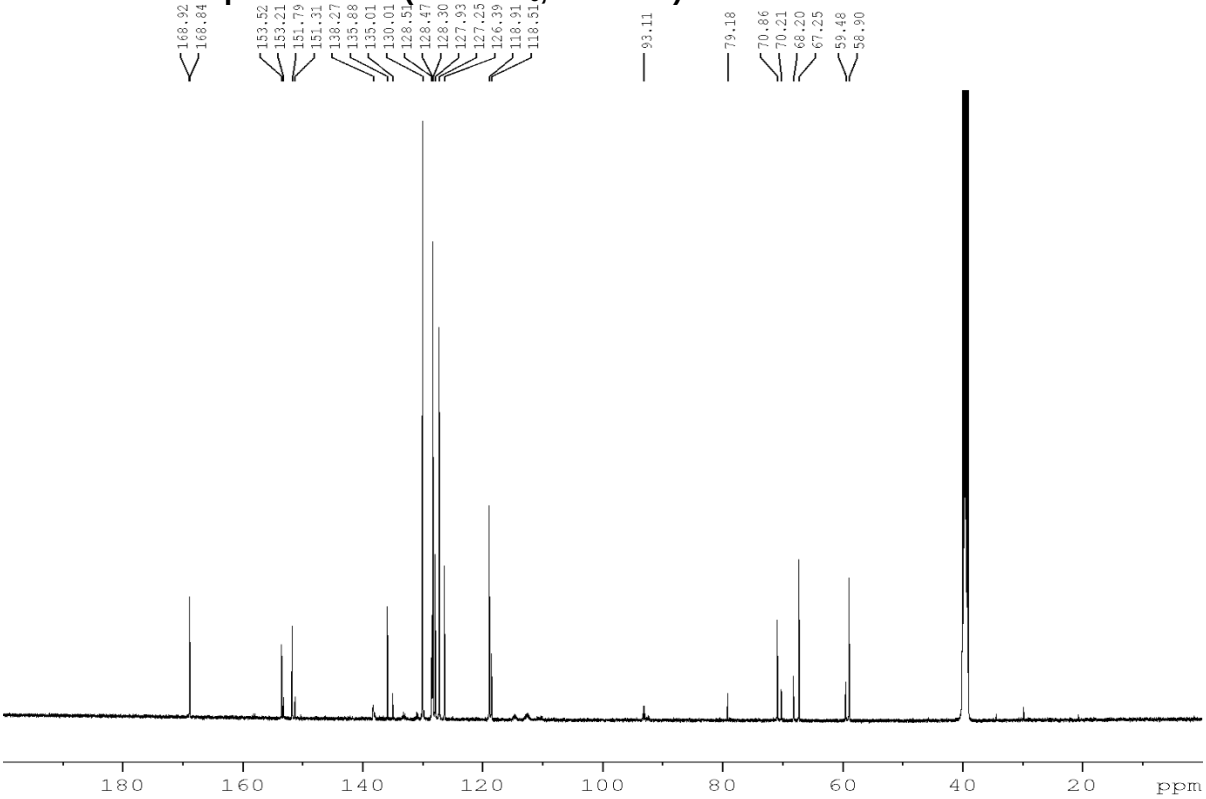
^{19}F NMR of compound 2.10 (DMSO-d_6, 282 MHz)



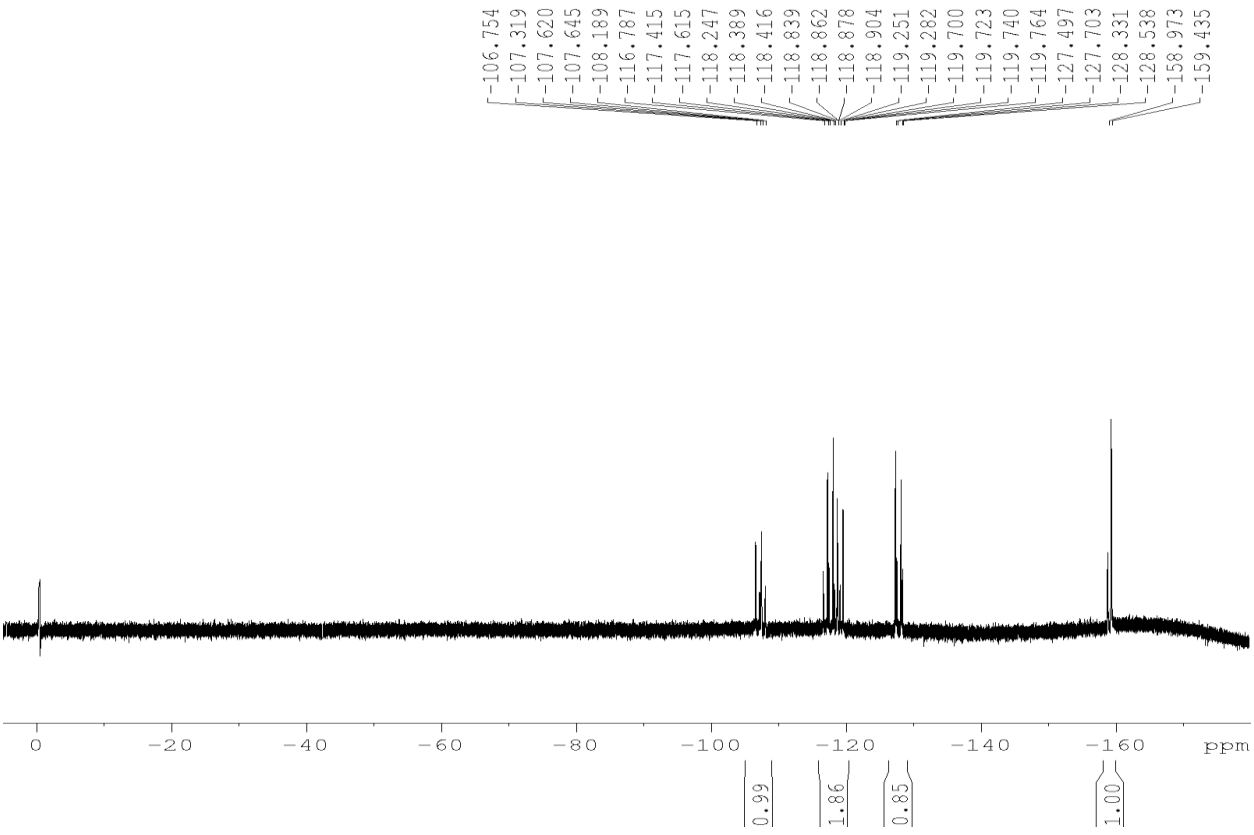
^{1}H NMR of compound 2.11a (DMSO-d_6, 500 MHz)



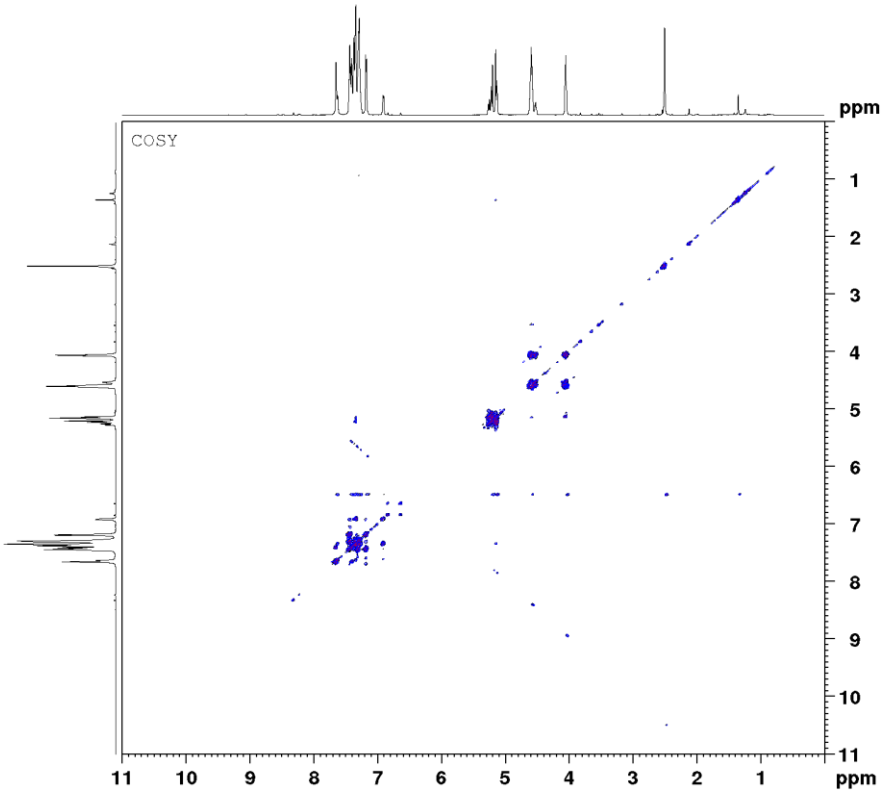
^{13}C NMR of compound 2.11a (DMSO-d_6, 126 MHz)



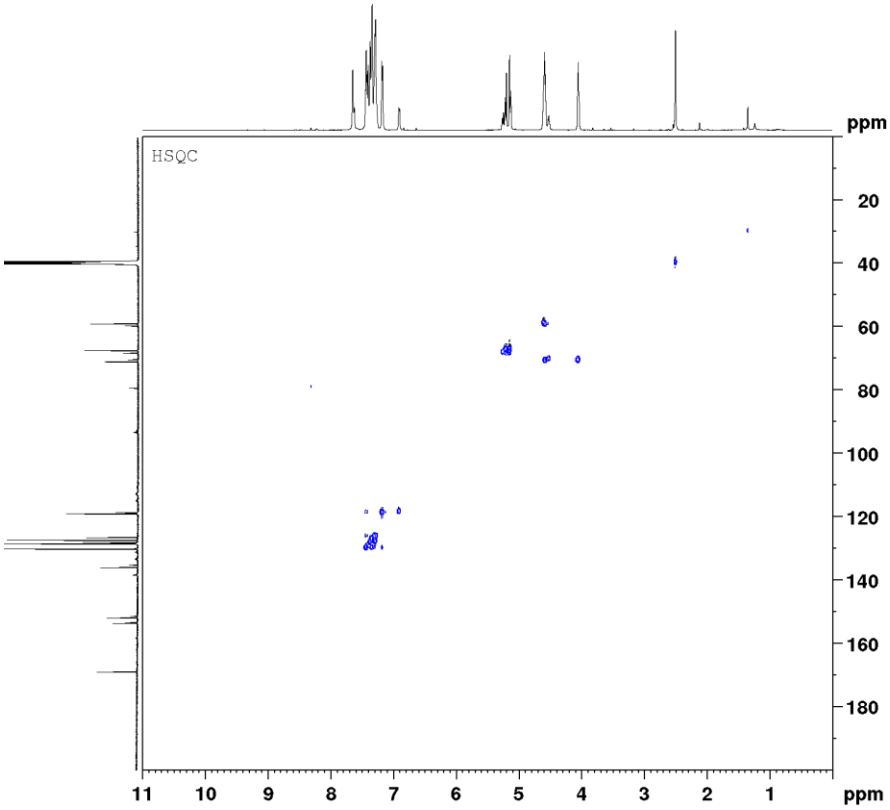
^{19}F NMR of compound 2.11a (DMSO-d_6, 282 MHz)



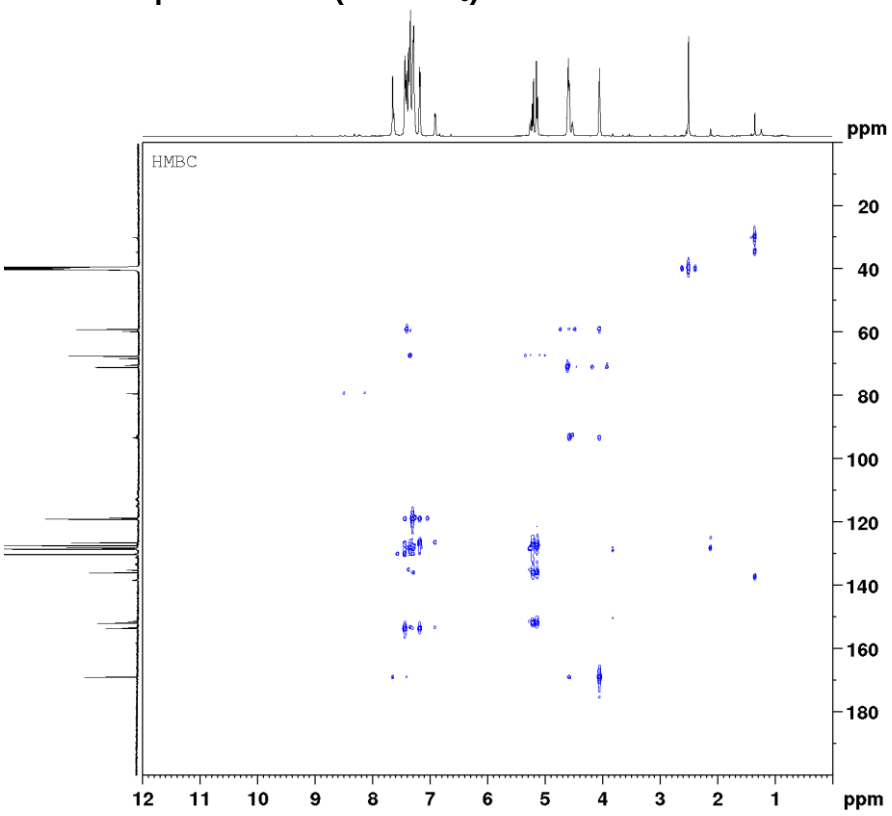
COSY spectrum of compound 2.11a (DMSO-d_6)



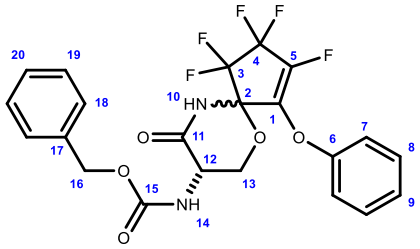
HSQC spectrum of compound 2.11a (DMSO-d_6)



HMBC spectrum of compound 2.11a (DMSO-d_6)

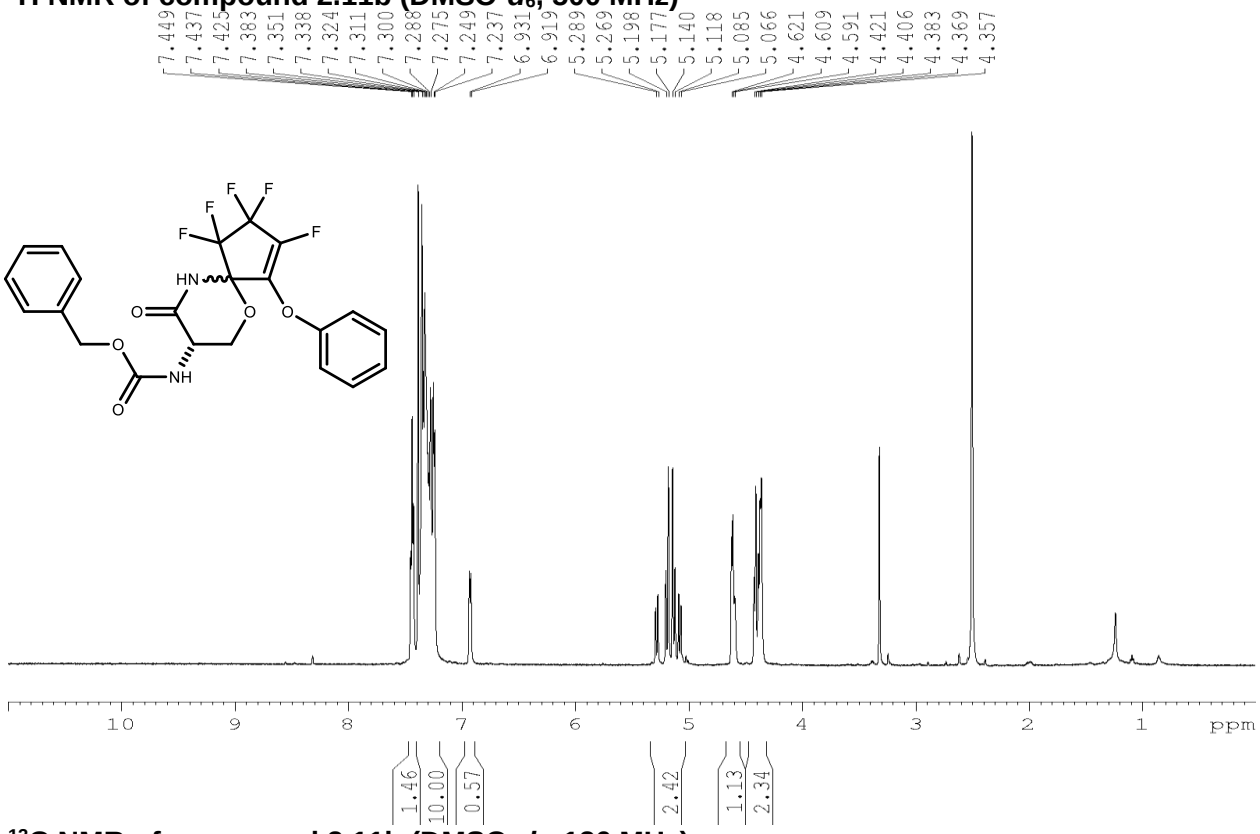


Compound 2.11a (DMSO-d_6)

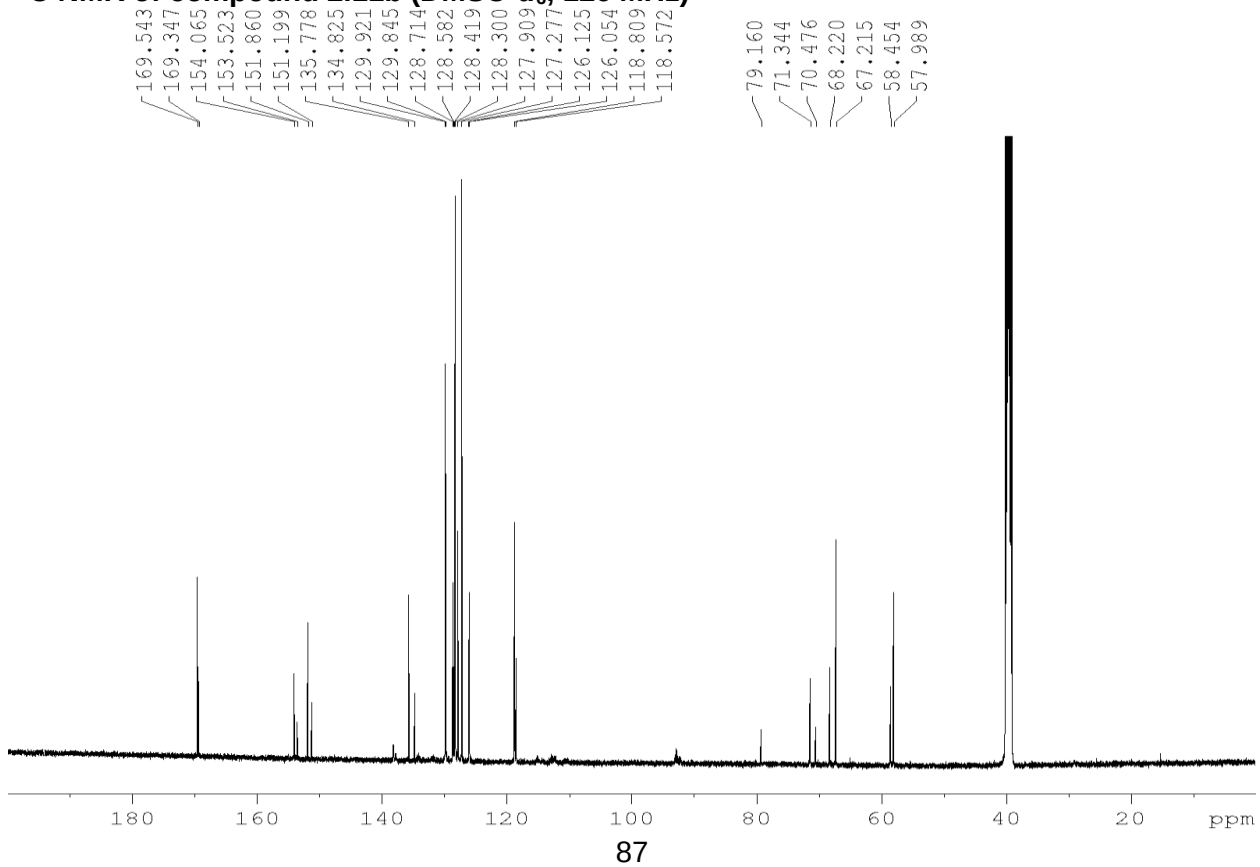


	13C	1H	Key Correlation
1	138.5 – 137.9 (m)	-	-
2	93.5 – 92.8 (m)	-	HMBC 13 -> 2
3	115.2 – 109.8 (m)	-	-
4	115.2 – 109.8 (m)	-	-
5	133.7 – 130.5 (m)	-	-
6	153.5	-	HMBC 7 -> 6
7	118.9, 118.5	7.18 (d, J = 7.5 Hz, 1.5H), 6.90 (J = 6.9 Hz, 0.5H)	HMBC 7 -> 6
8	130.0	7.50 -7.22 (m, 2H) overlap	HMBC 7 -> 8
9	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

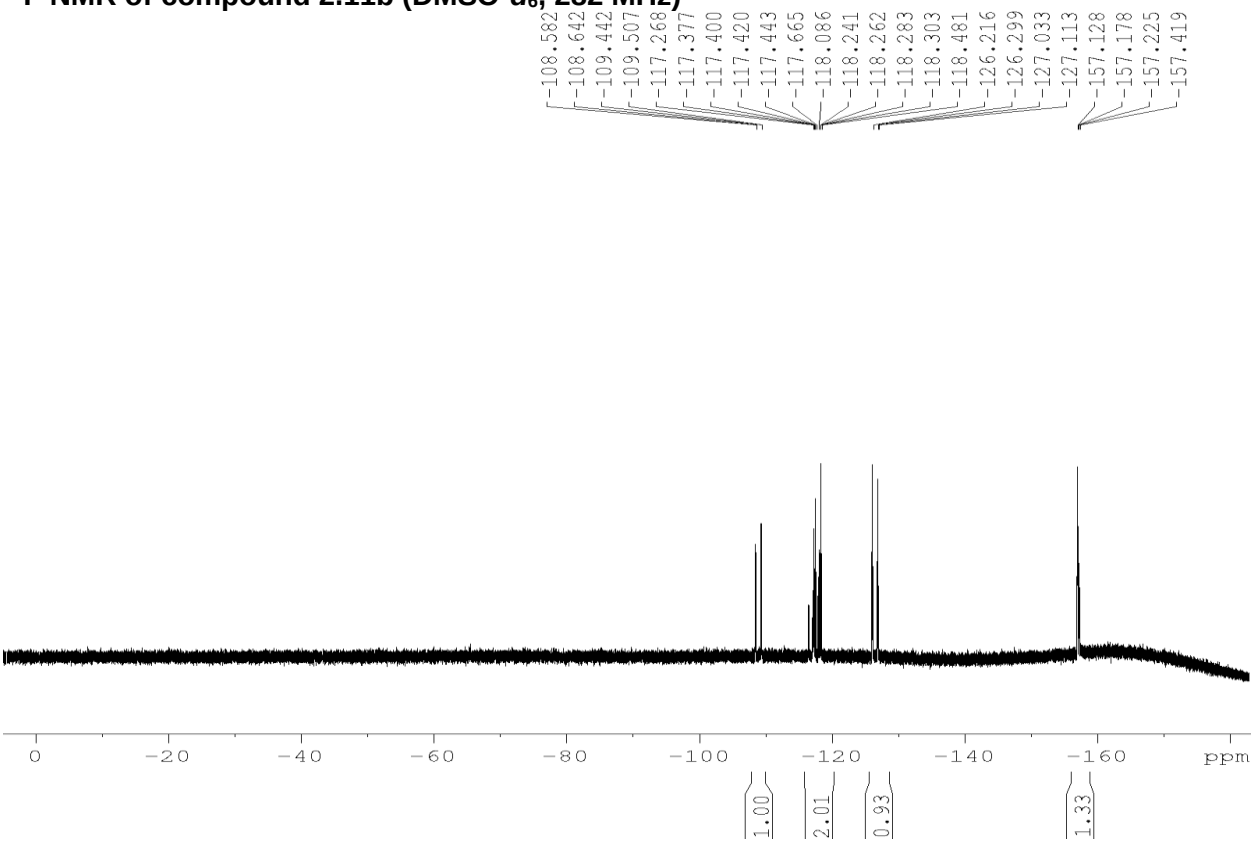
^{1}H NMR of compound 2.11b (DMSO-d_6, 500 MHz)



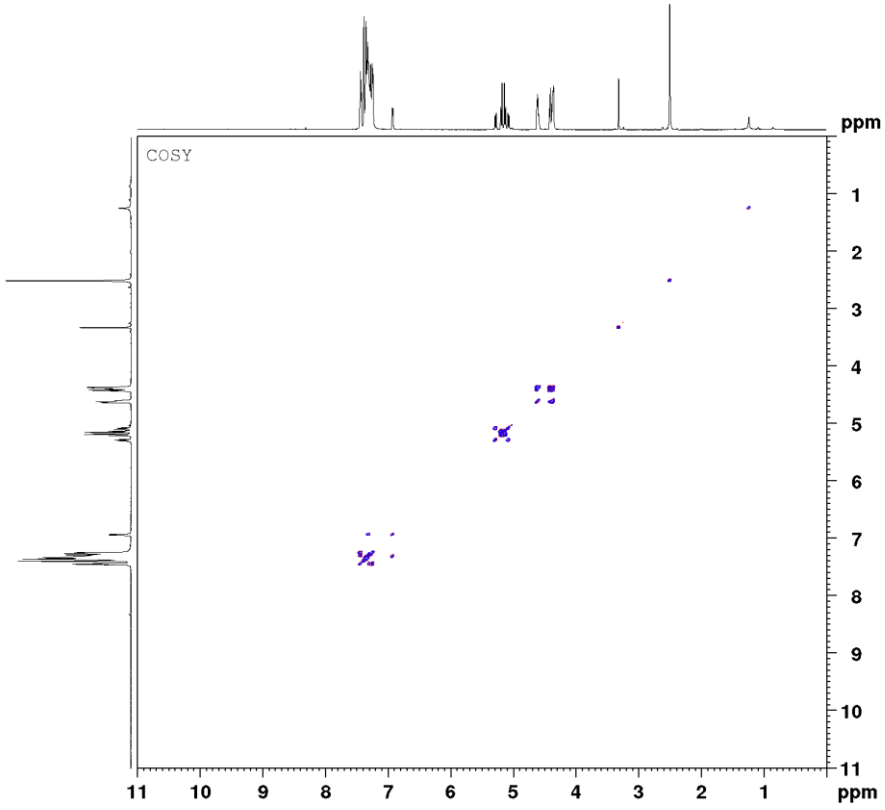
^{13}C NMR of compound 2.11b (DMSO-d_6, 126 MHz)



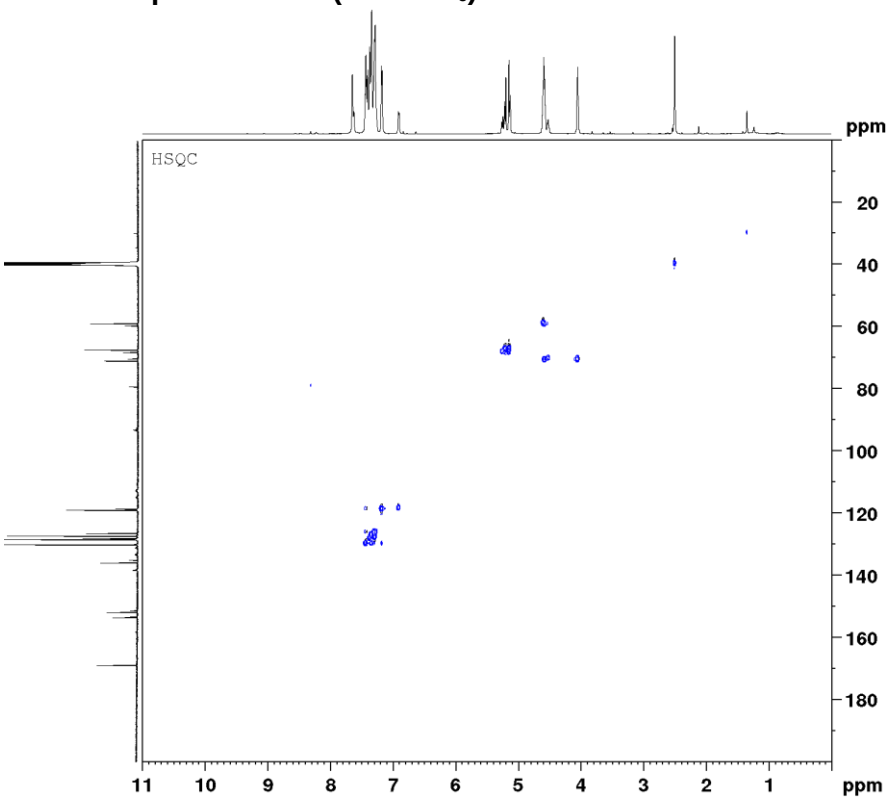
^{19}F NMR of compound 2.11b (DMSO-d_6, 282 MHz)



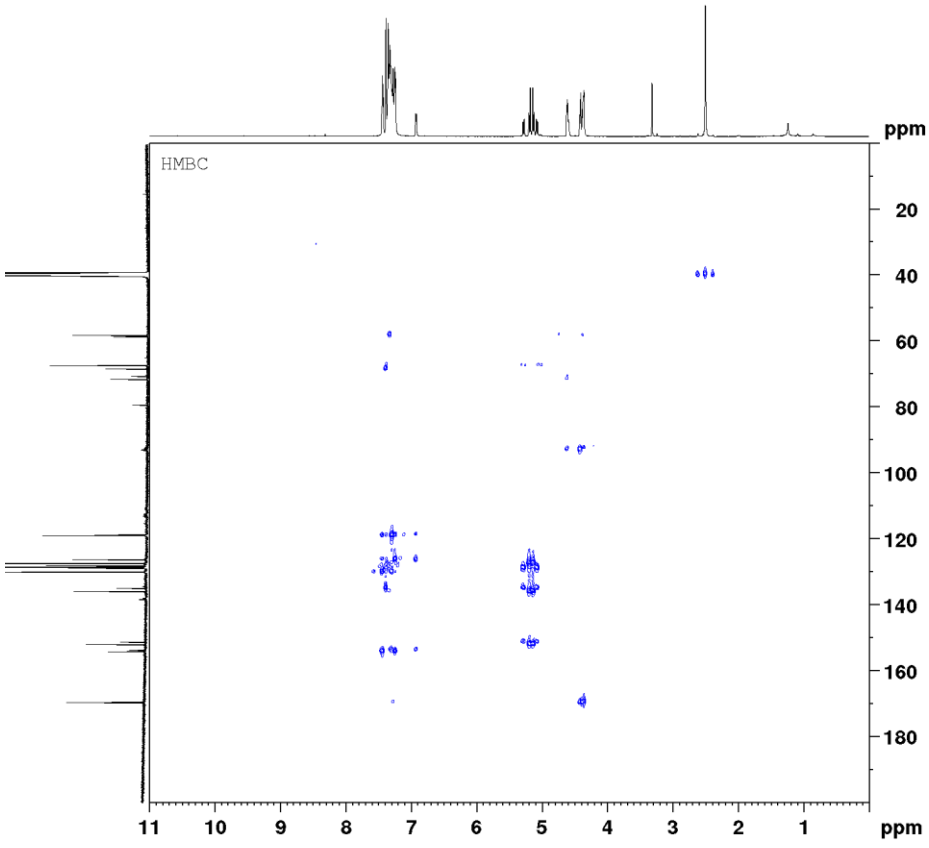
COSY spectrum of compound 2.11b (DMSO-d_6)



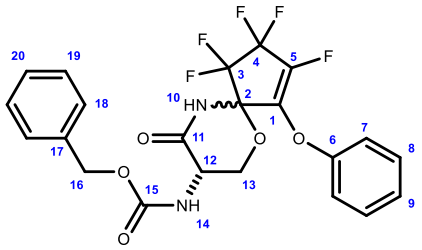
HSQC spectrum of compound 2.11b (DMSO-d_6)



HMBC spectrum of compound 2.11b (DMSO-d_6)



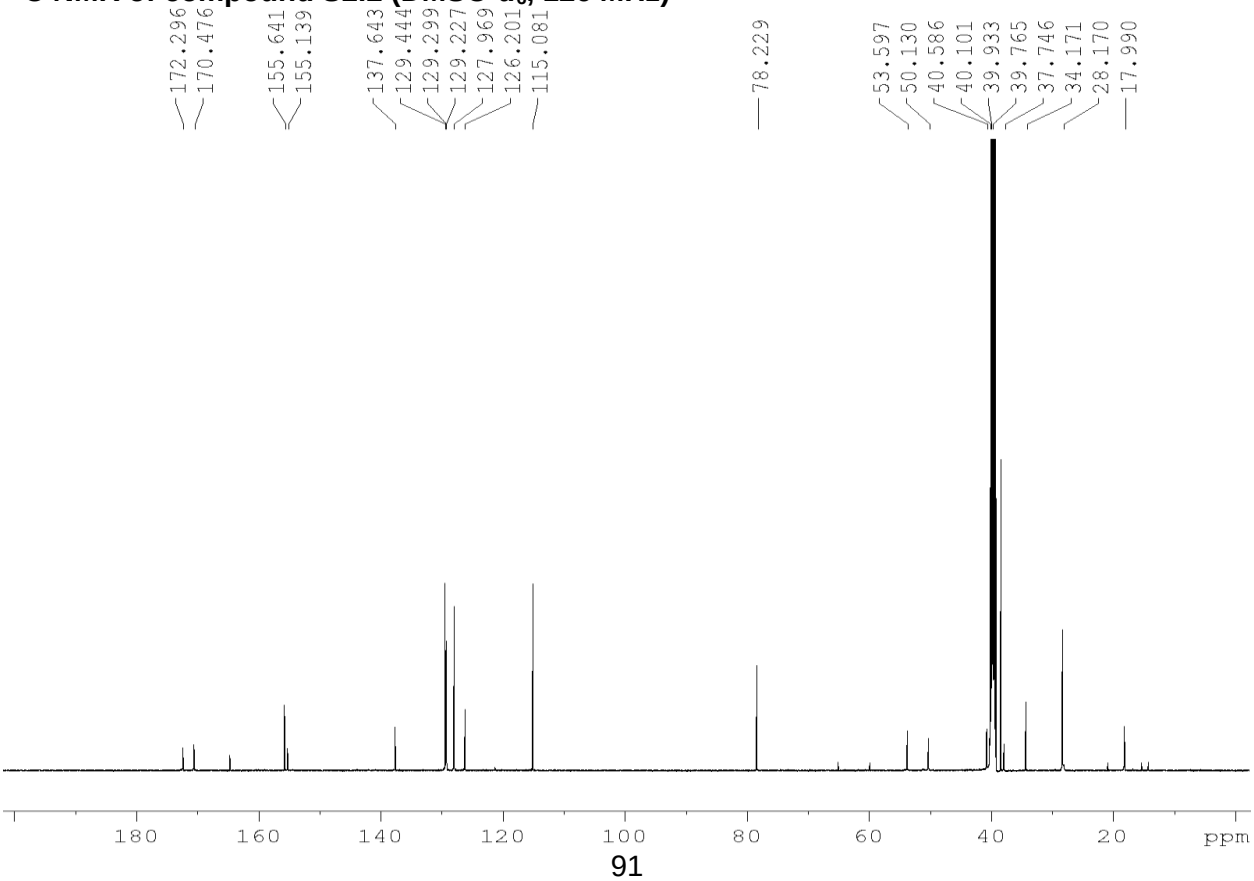
Compound 2.11b (DMSO-d_6)



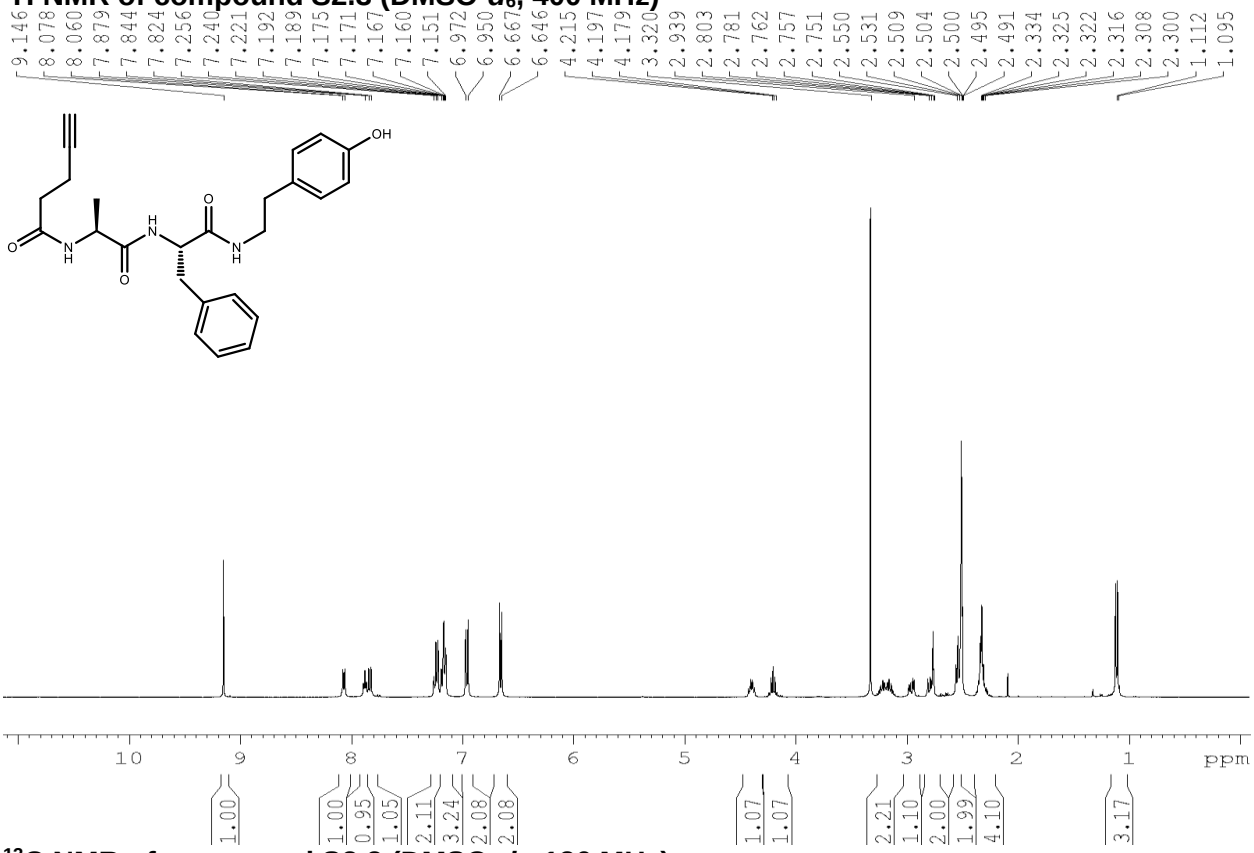
	13C	1H	Key Correlation
1	138.4 - 137.8 (m)	-	-
2	93.1 – 91.7 (m)	-	HMBC 13 -> 2
3	115.7 – 109.8 (m)	-	-
4	115.7 – 109.8 (m)	-	-
5	134.7 – 131.3 (m)	-	-
6	154.1 (major), 153.5 (minor)	-	HMBC 7 -> 6
7	118.8, 118.6	7.40 – 7.20 (m, 1.5H) overlap, 6.92 (d, J = 7.5, 0.5H)	HMBC 7 -> 6
8	129.9, 129.8	7.40 – 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 S2.2 (DMSO-d_6, 500 MHz)

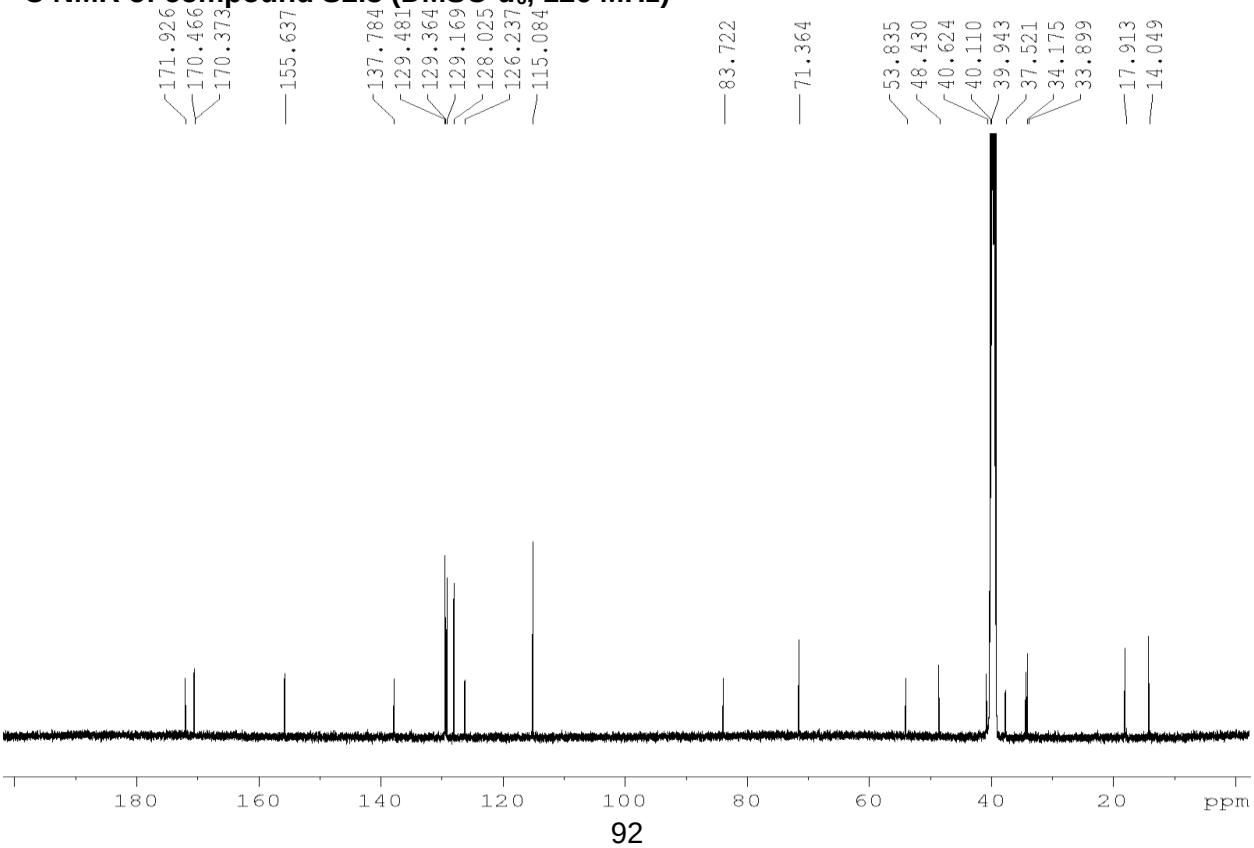
^{13}C NMR of compound S2.2 (DMSO-d_6, 126 MHz)



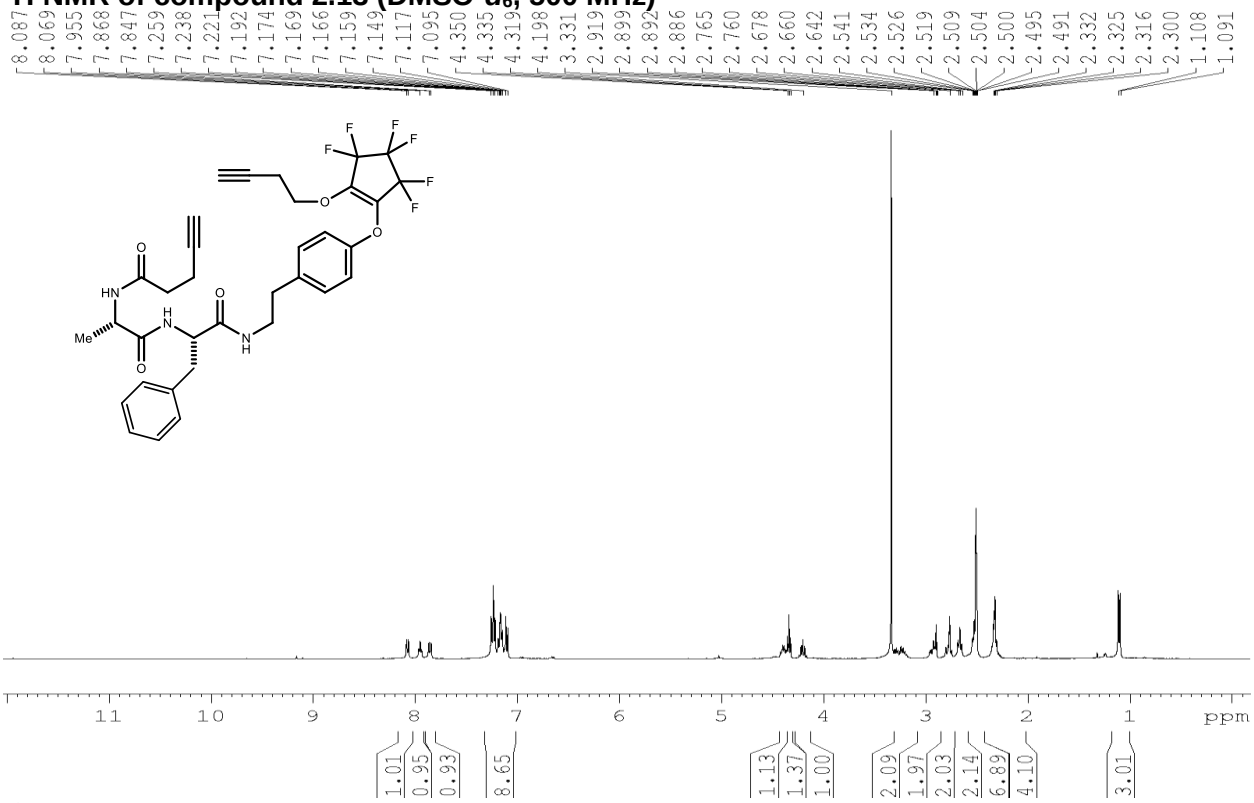
^{1}H NMR of compound S2.3 (DMSO-d_6, 400 MHz)



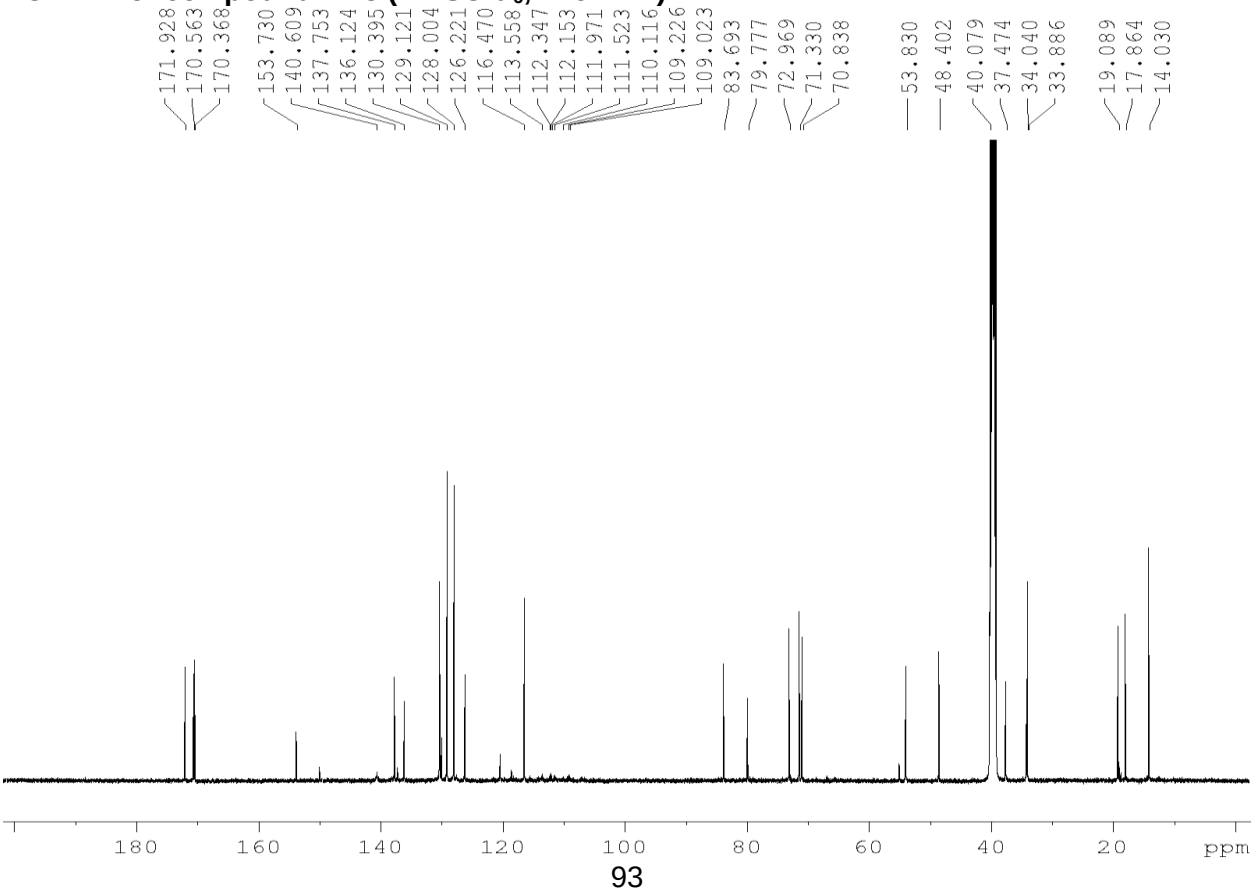
^{13}C NMR of compound S2.3 (DMSO-d_6, 126 MHz)



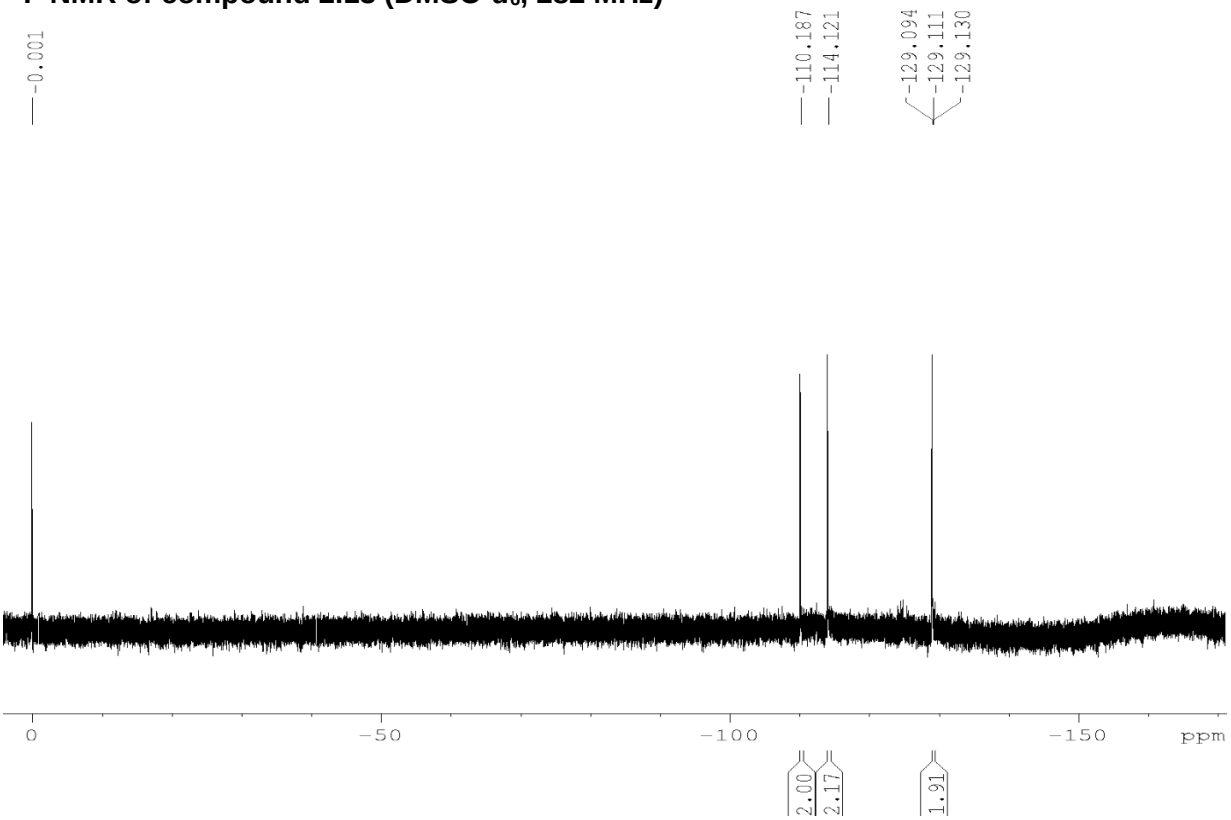
^{1}H NMR of compound 2.13 (DMSO-d_6, 500 MHz)



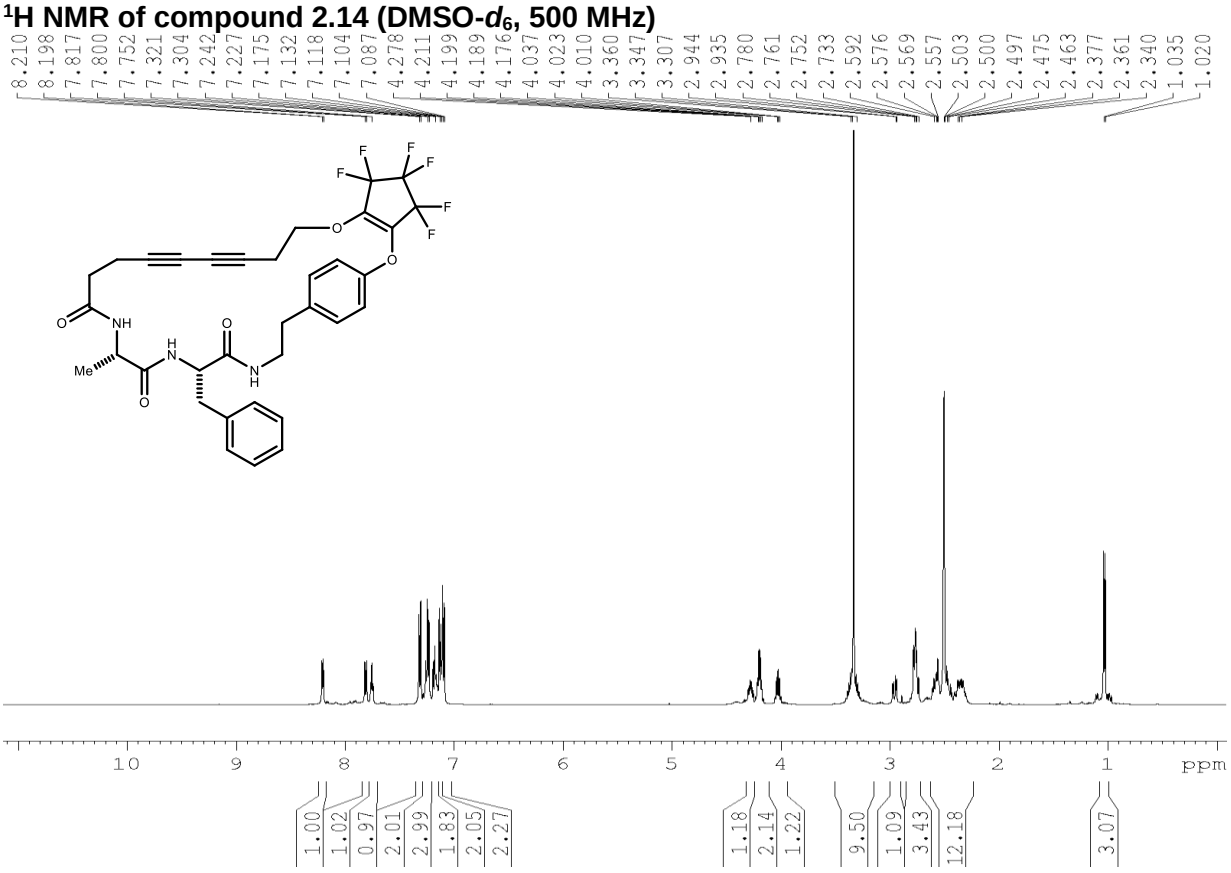
^{13}C NMR of compound 2.13 (DMSO-d_6, 126 MHz)



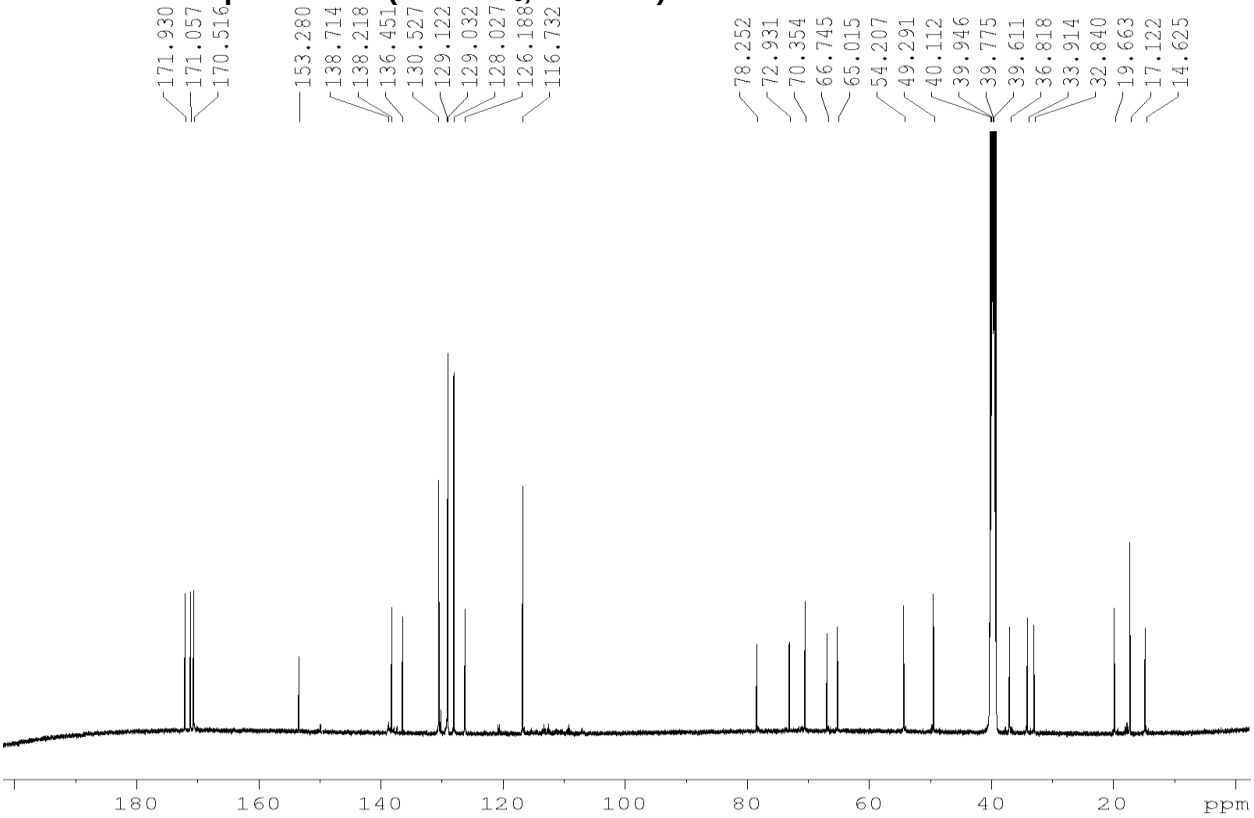
^{19}F NMR of compound 2.13 (DMSO-d_6, 282 MHz)



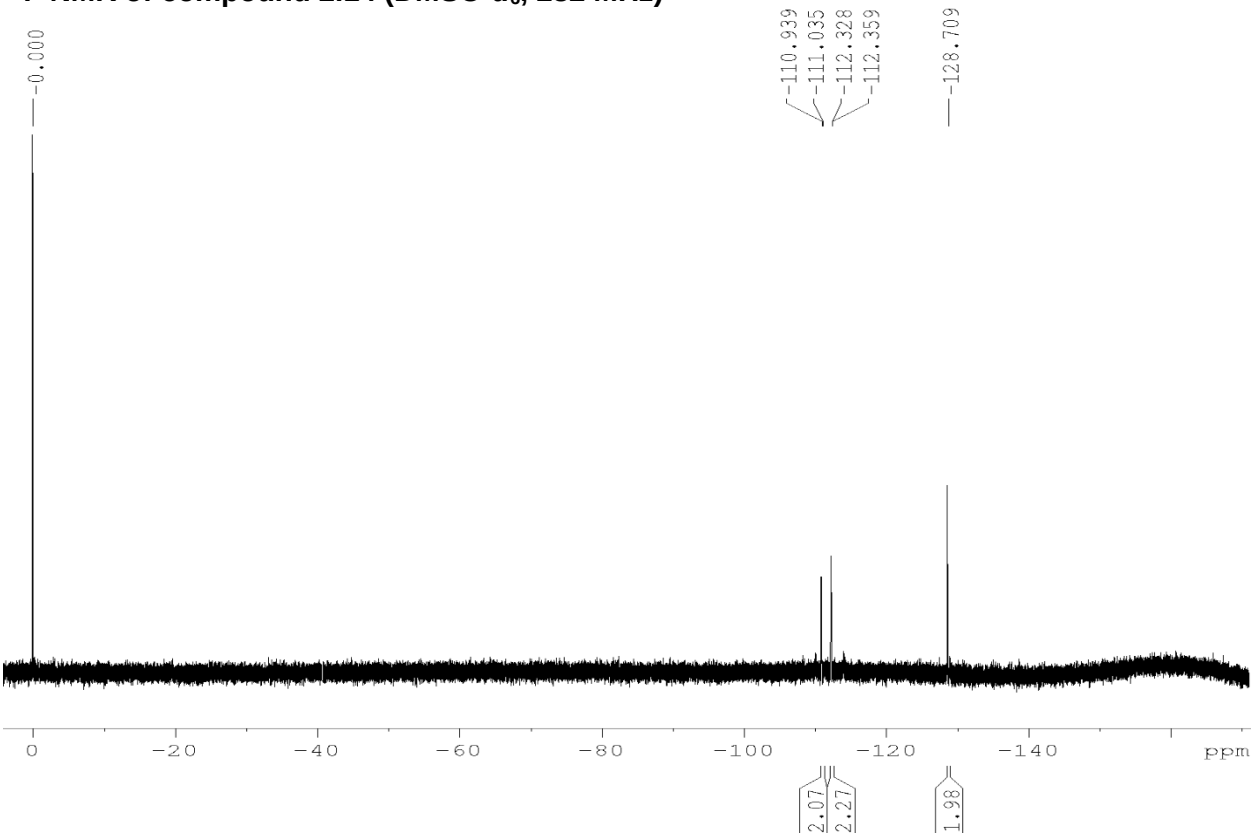
^{1}H NMR of compound 2.14 (DMSO-d_6, 500 MHz)



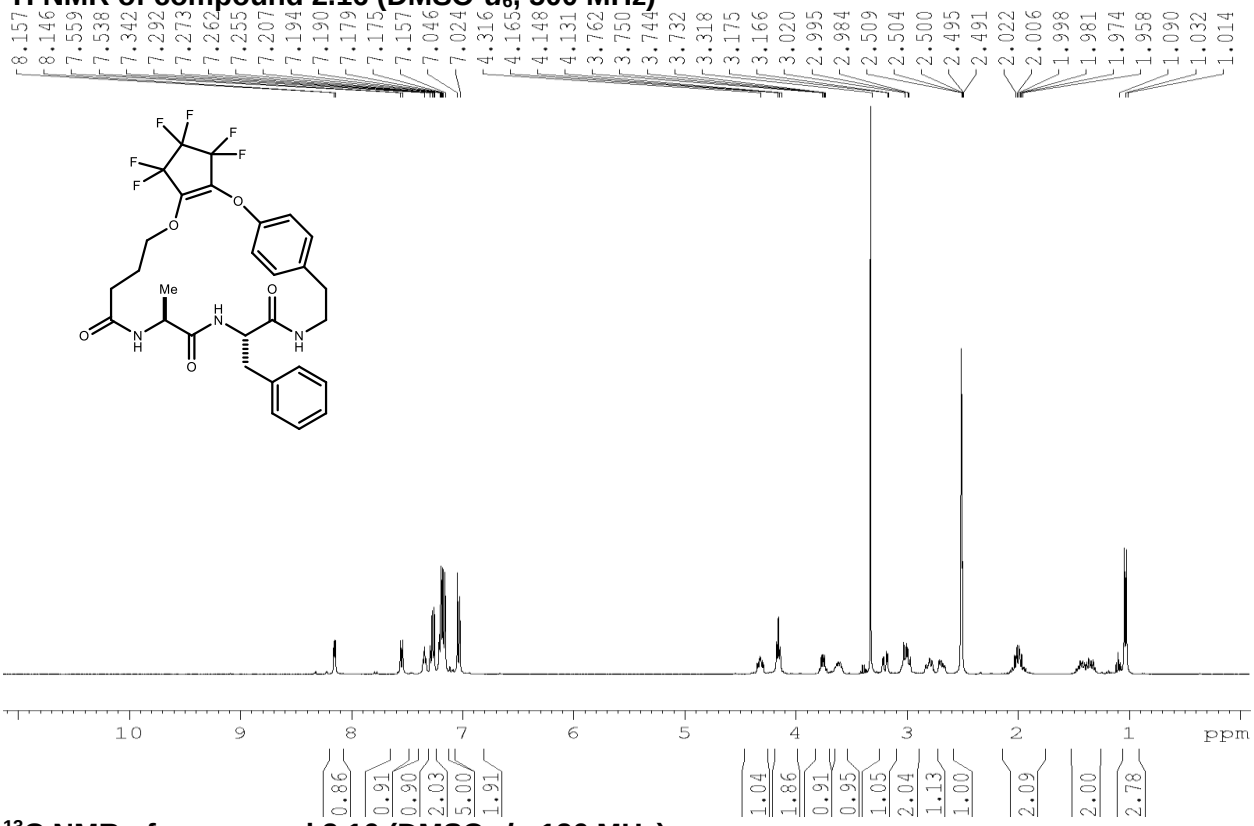
^{13}C NMR of compound 2.14 (DMSO-d_6, 126 MHz)



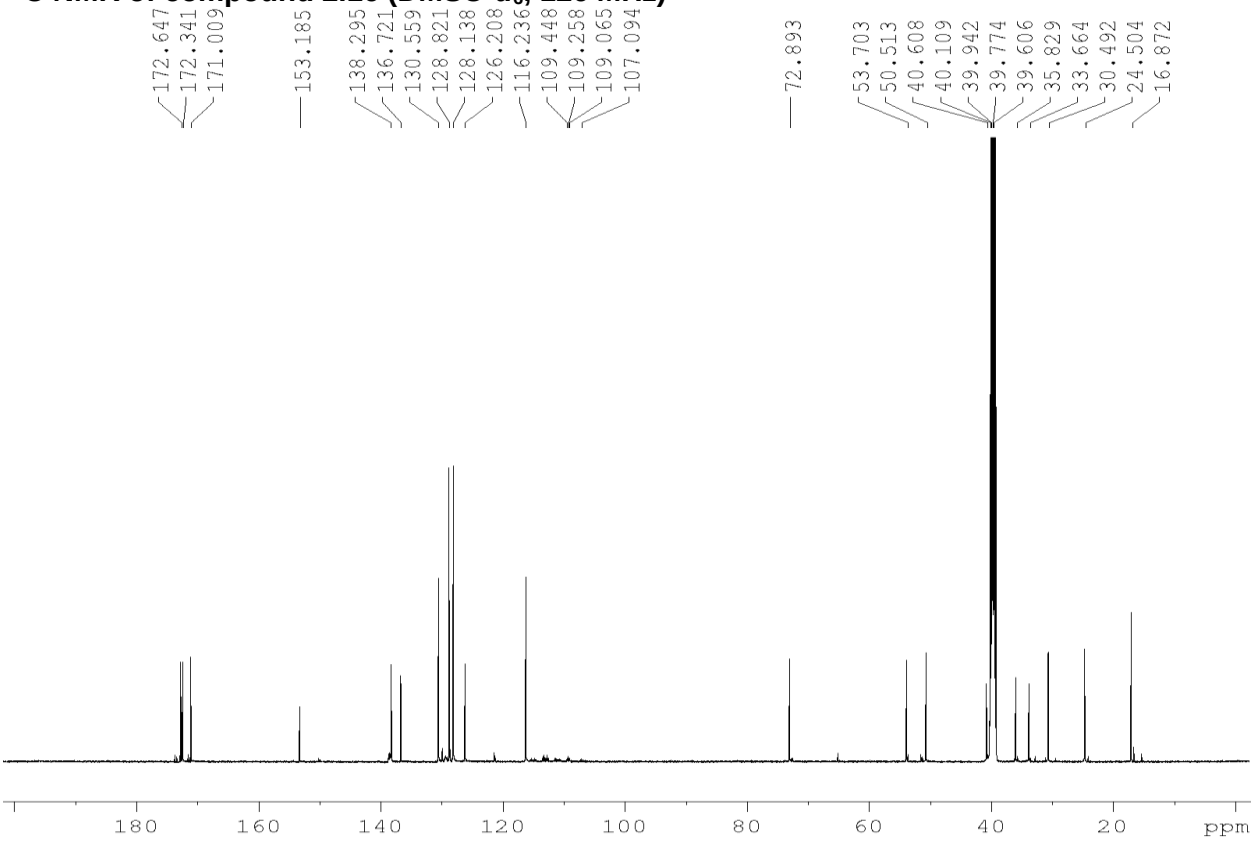
^{19}F NMR of compound 2.14 (DMSO-d_6, 282 MHz)



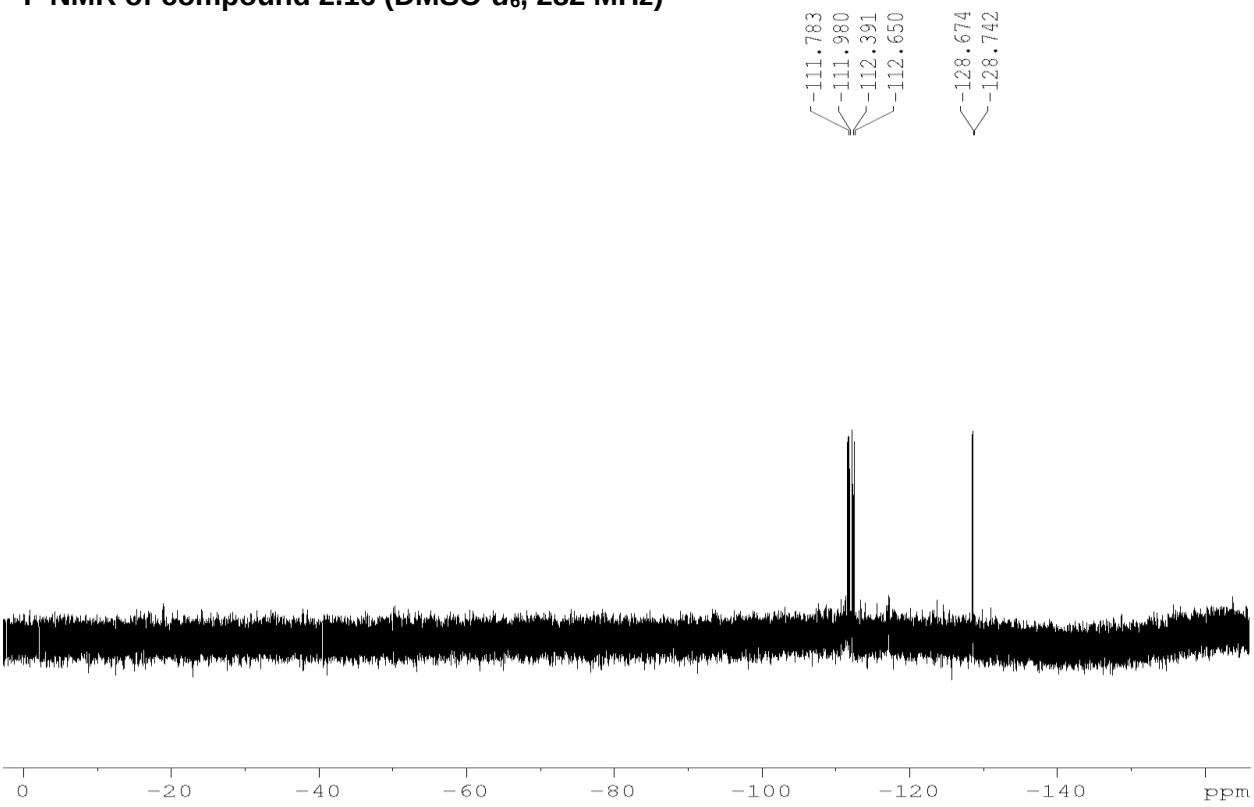
^{1}H NMR of compound 2.16 (DMSO-d_6, 500 MHz)



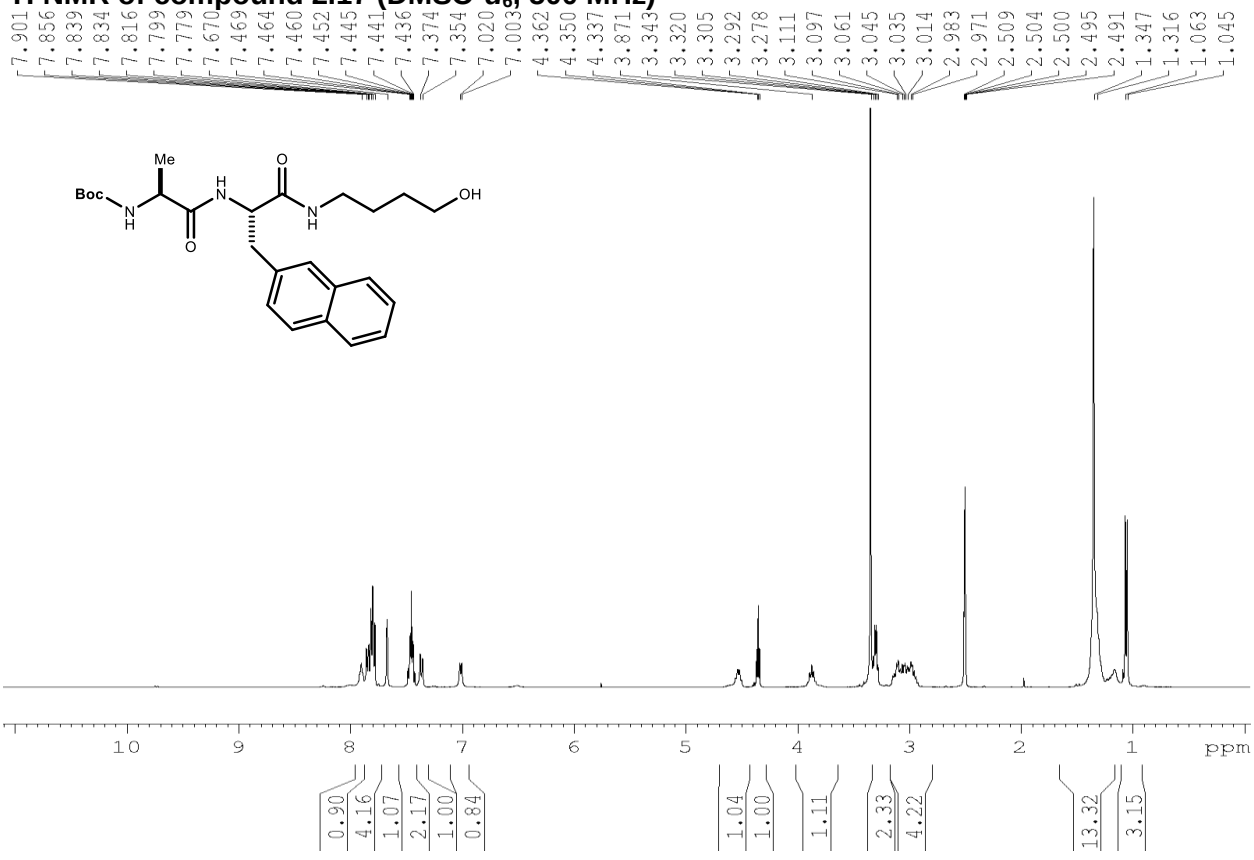
^{13}C NMR of compound 2.16 (DMSO-d_6, 126 MHz)



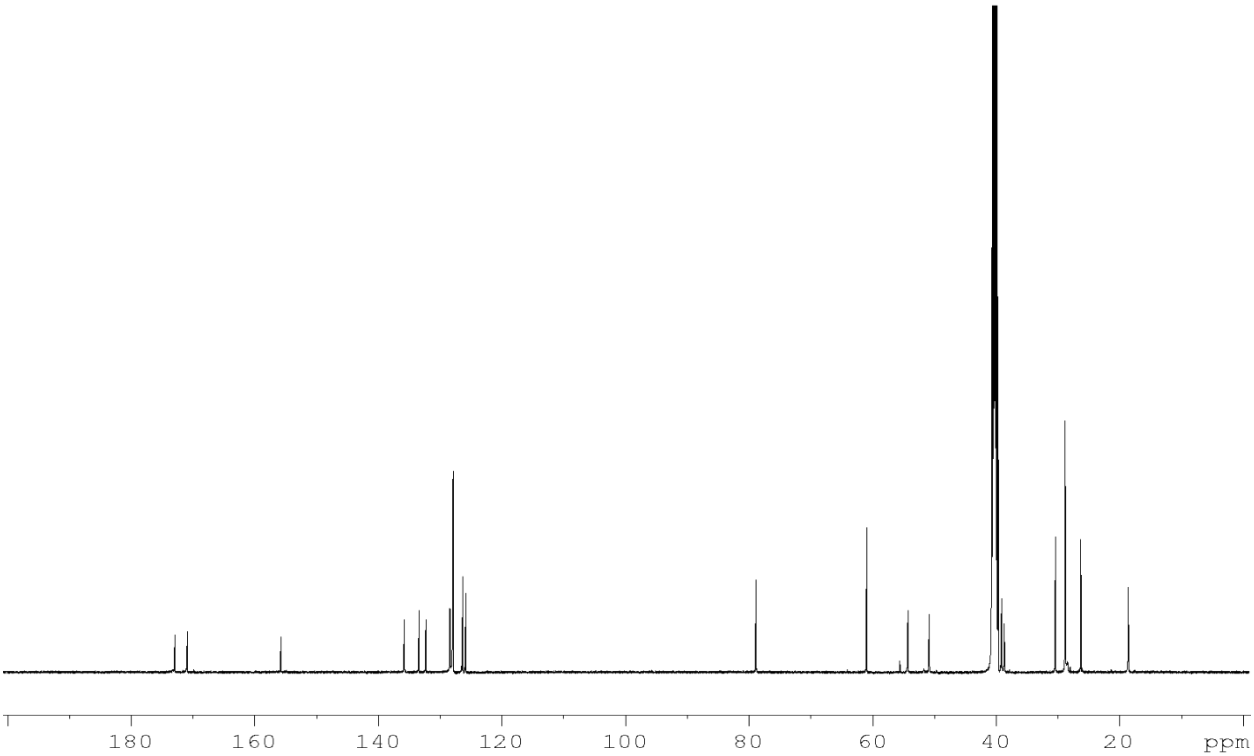
^{19}F NMR of compound 2.16 (DMSO-d_6, 282 MHz)



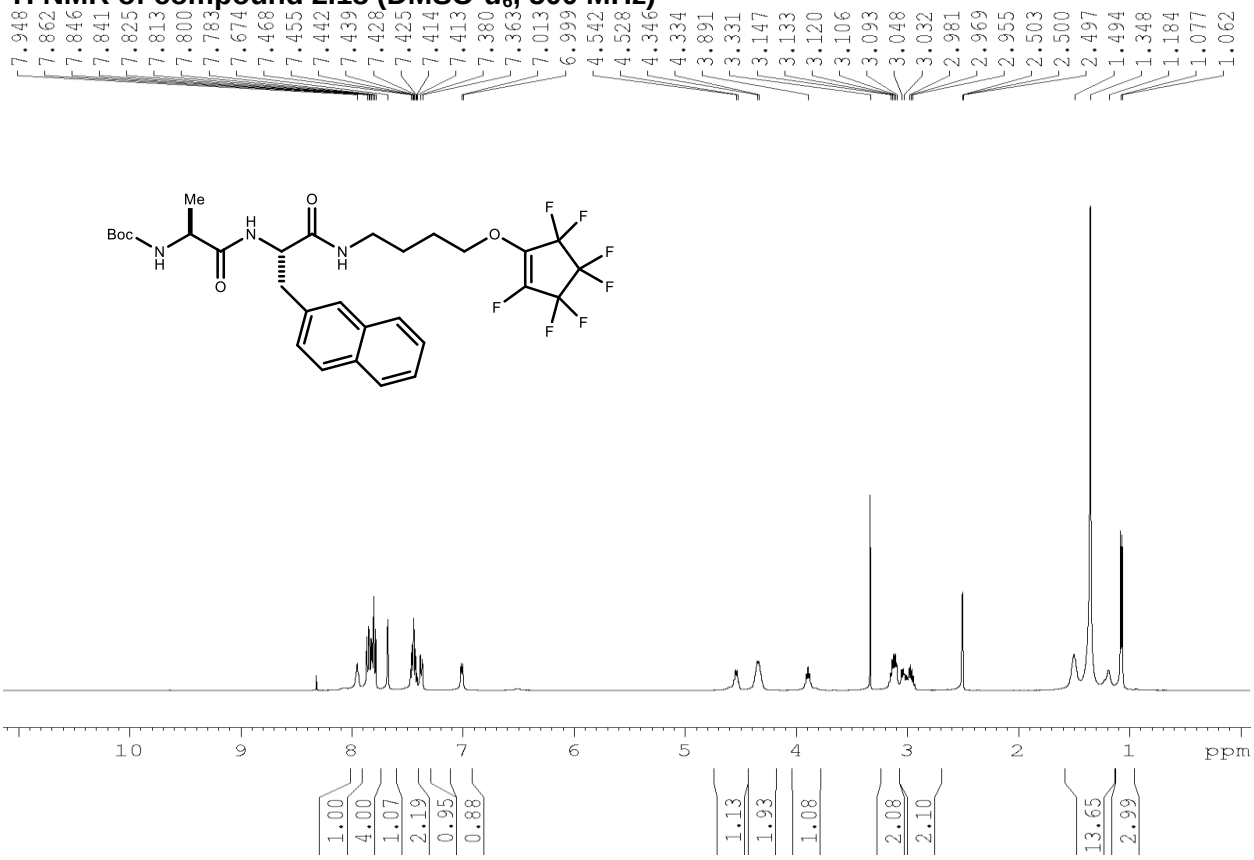
^{1}H NMR of compound 2.17 (DMSO-d_6, 500 MHz)



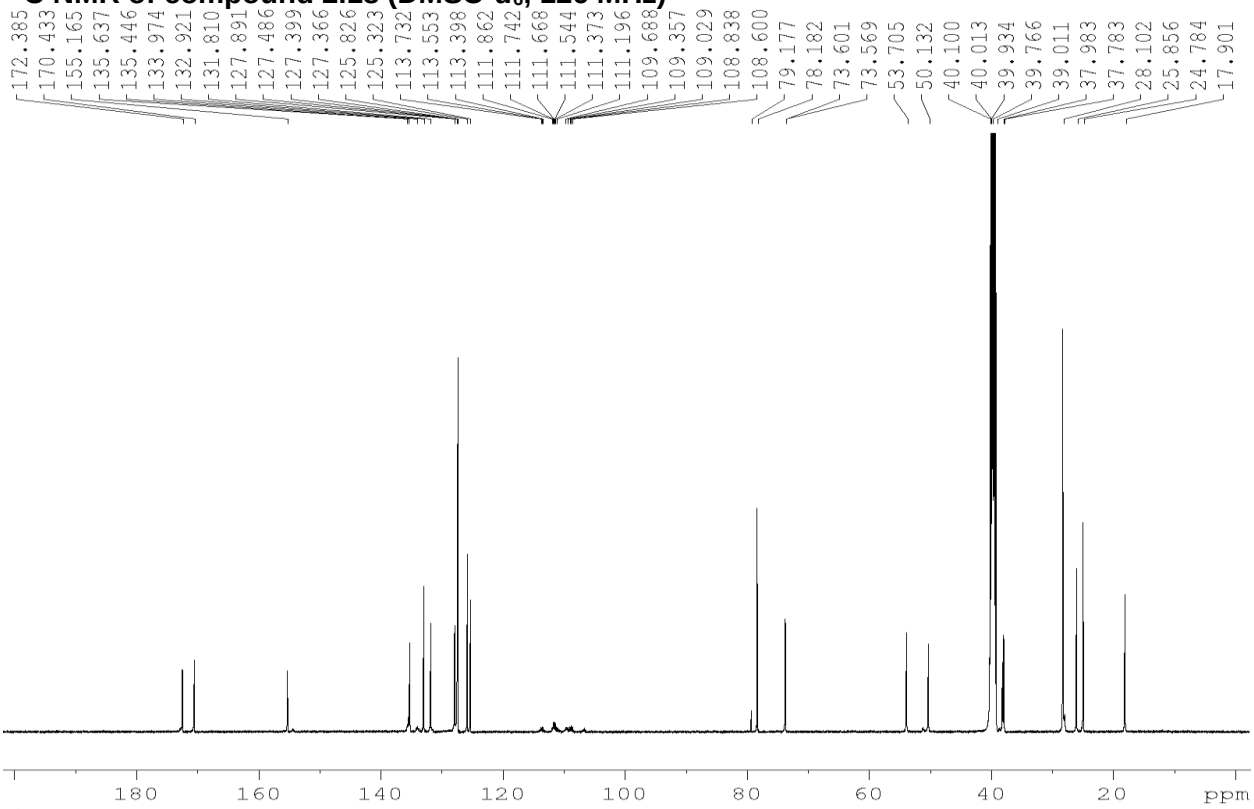
^{13}C NMR of compound 2.17 (DMSO-d_6, 126 MHz)



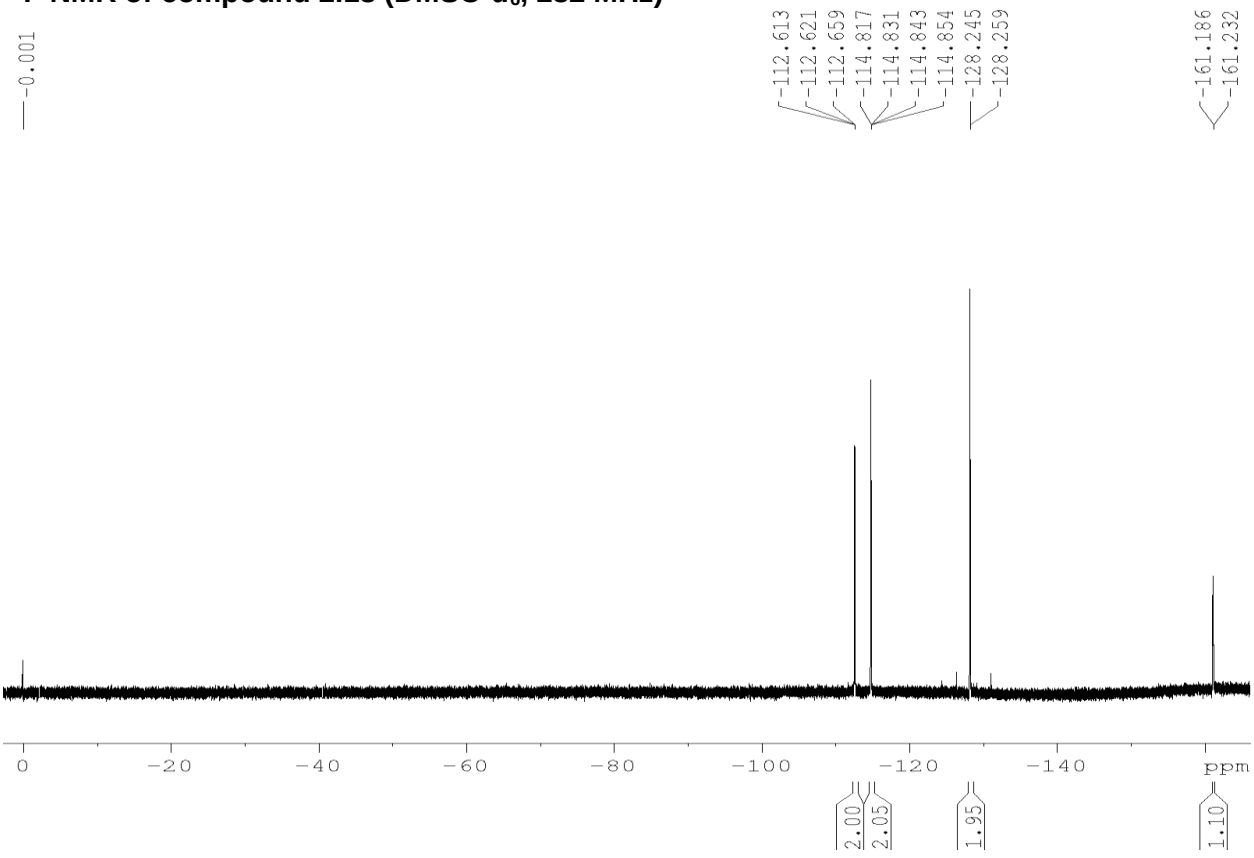
^{1}H NMR of compound 2.18 (DMSO-d_6, 500 MHz)



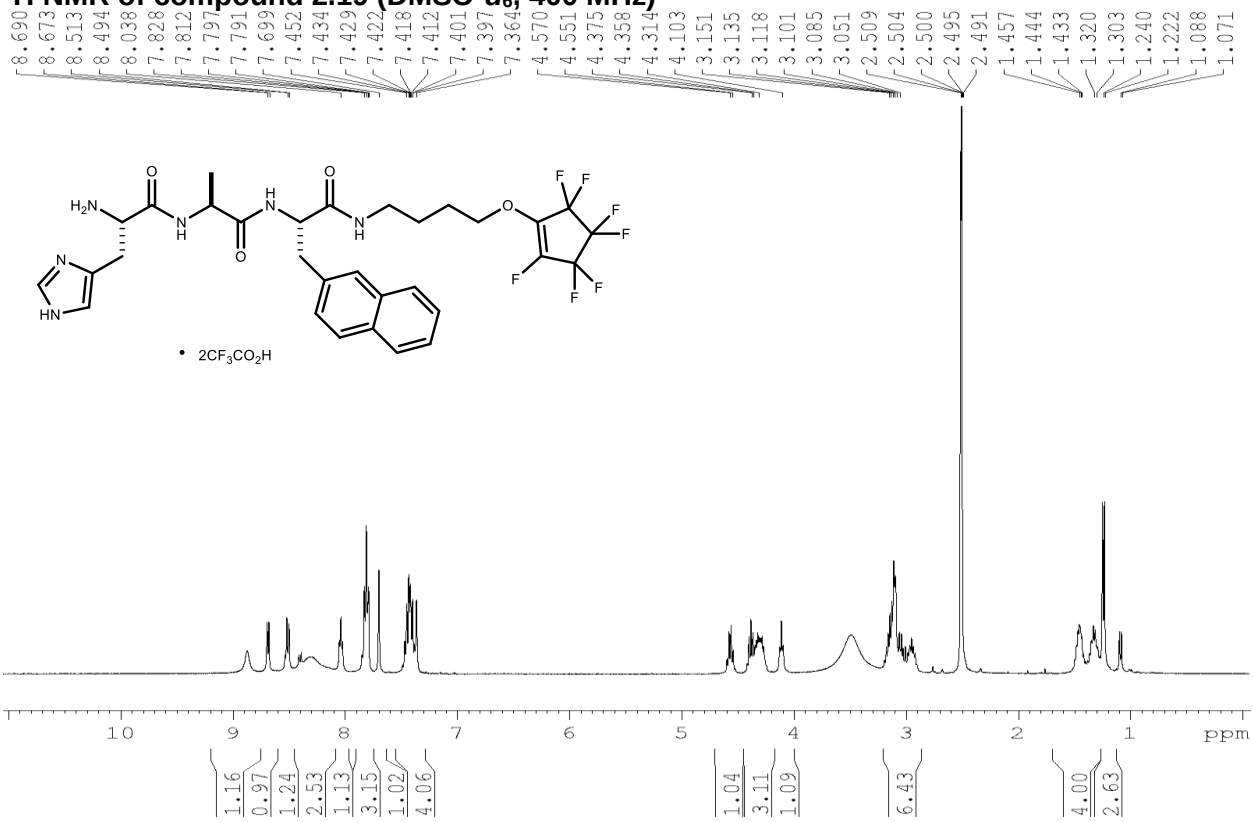
^{13}C NMR of compound 2.18 (DMSO-d_6, 126 MHz)



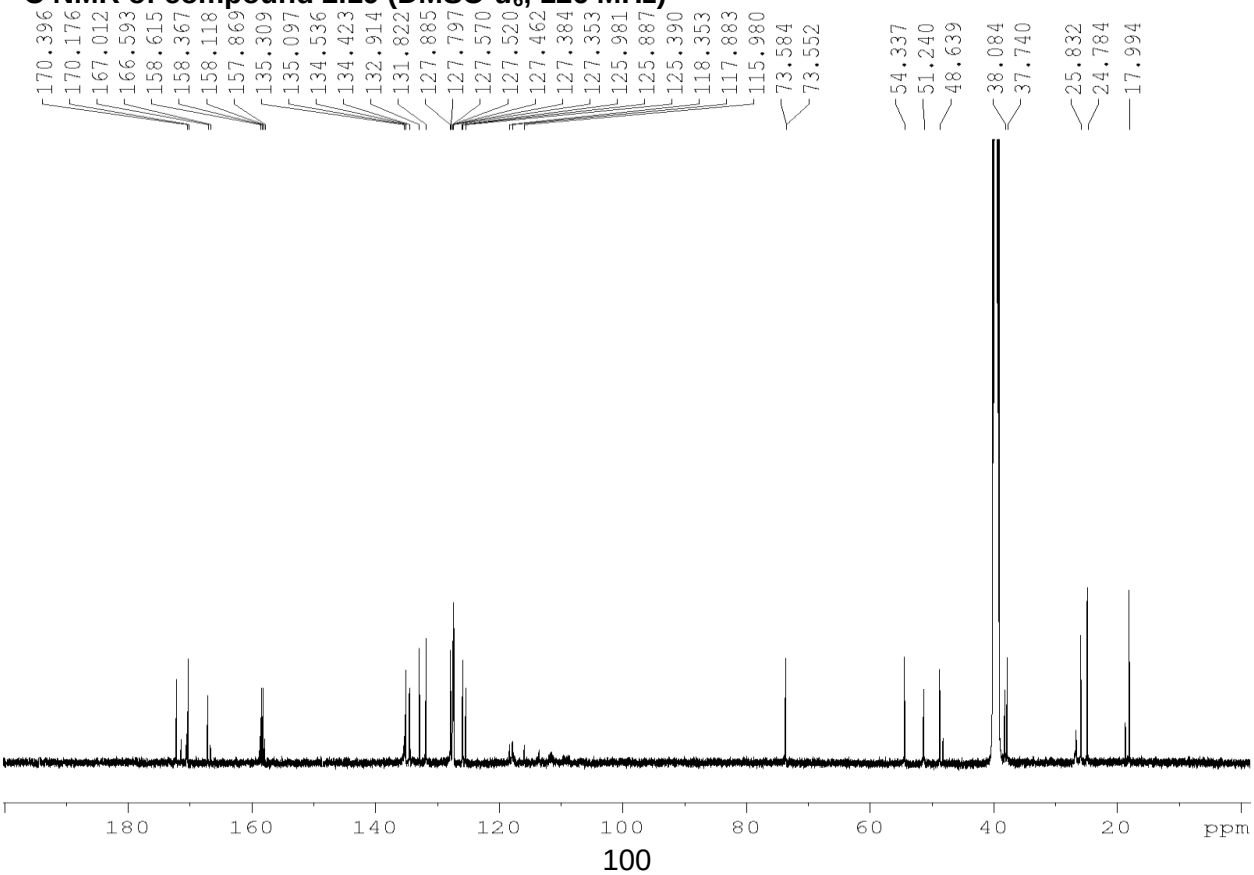
^{19}F NMR of compound 2.18 (DMSO-d_6, 282 MHz)



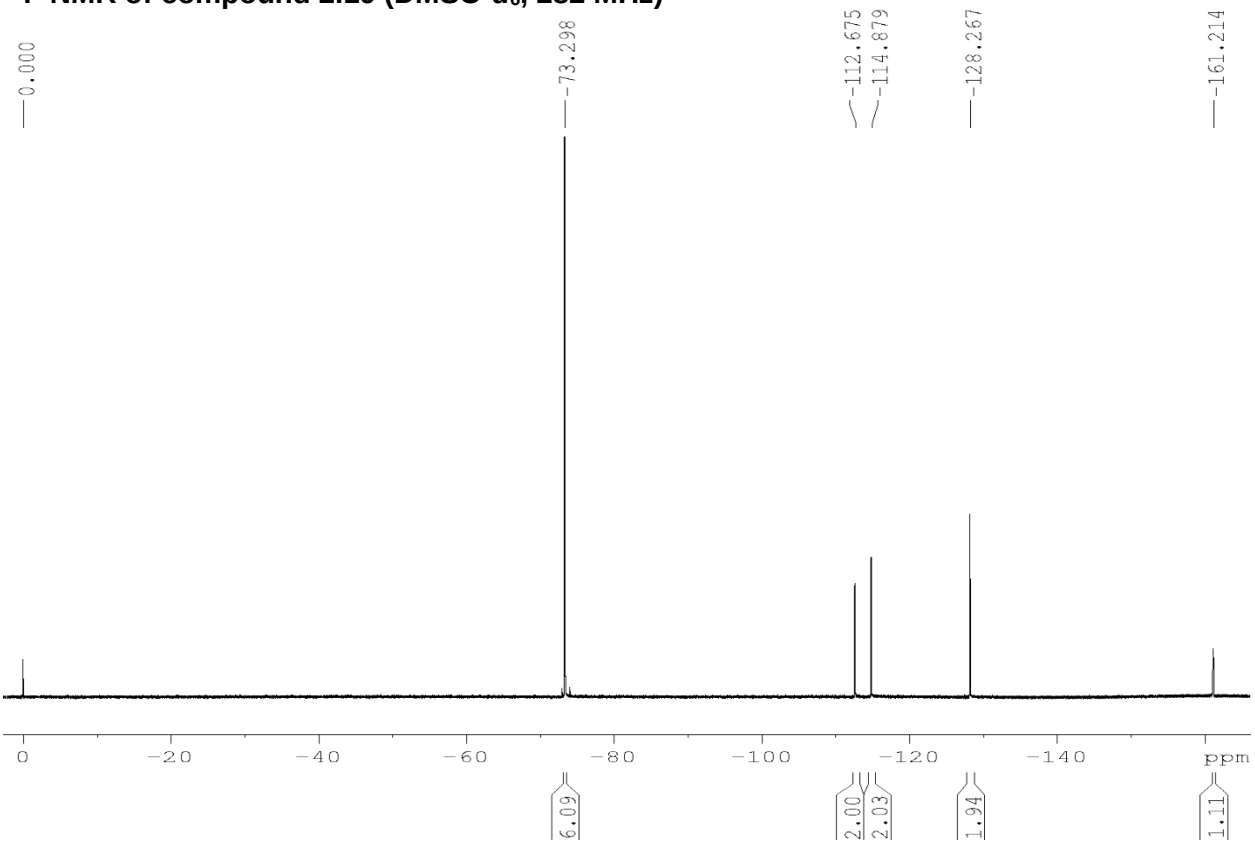
^{1}H NMR of compound 2.19 (DMSO-d_6, 400 MHz)



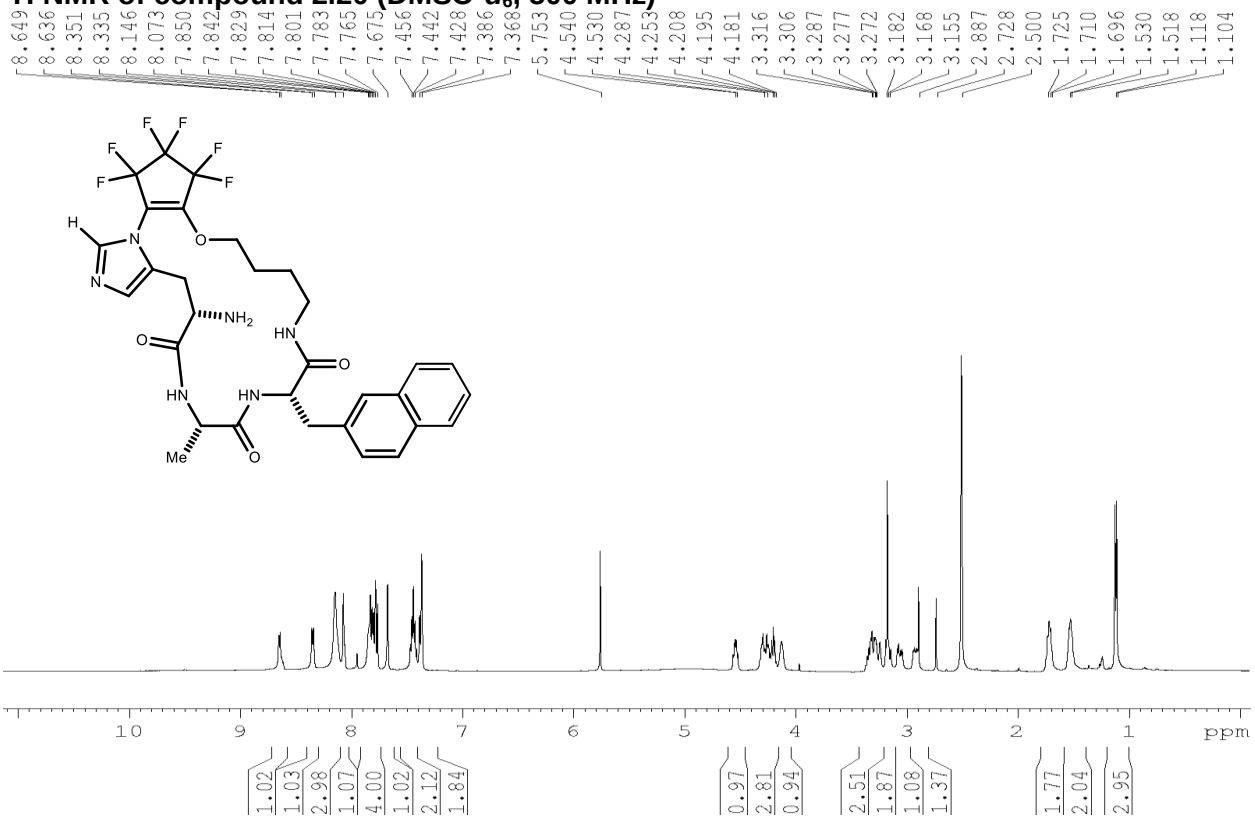
^{13}C NMR of compound 2.19 (DMSO-d_6, 126 MHz)



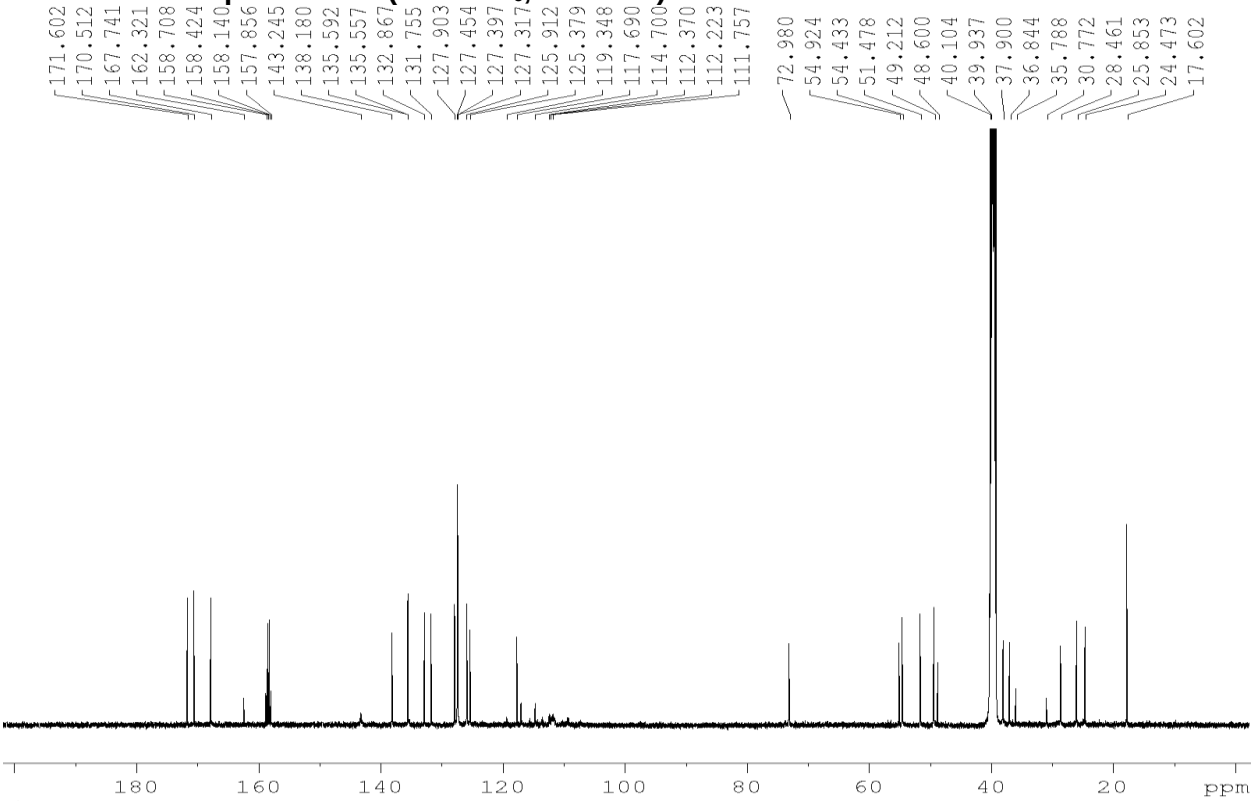
^{19}F NMR of compound 2.19 (DMSO-d_6, 282 MHz)



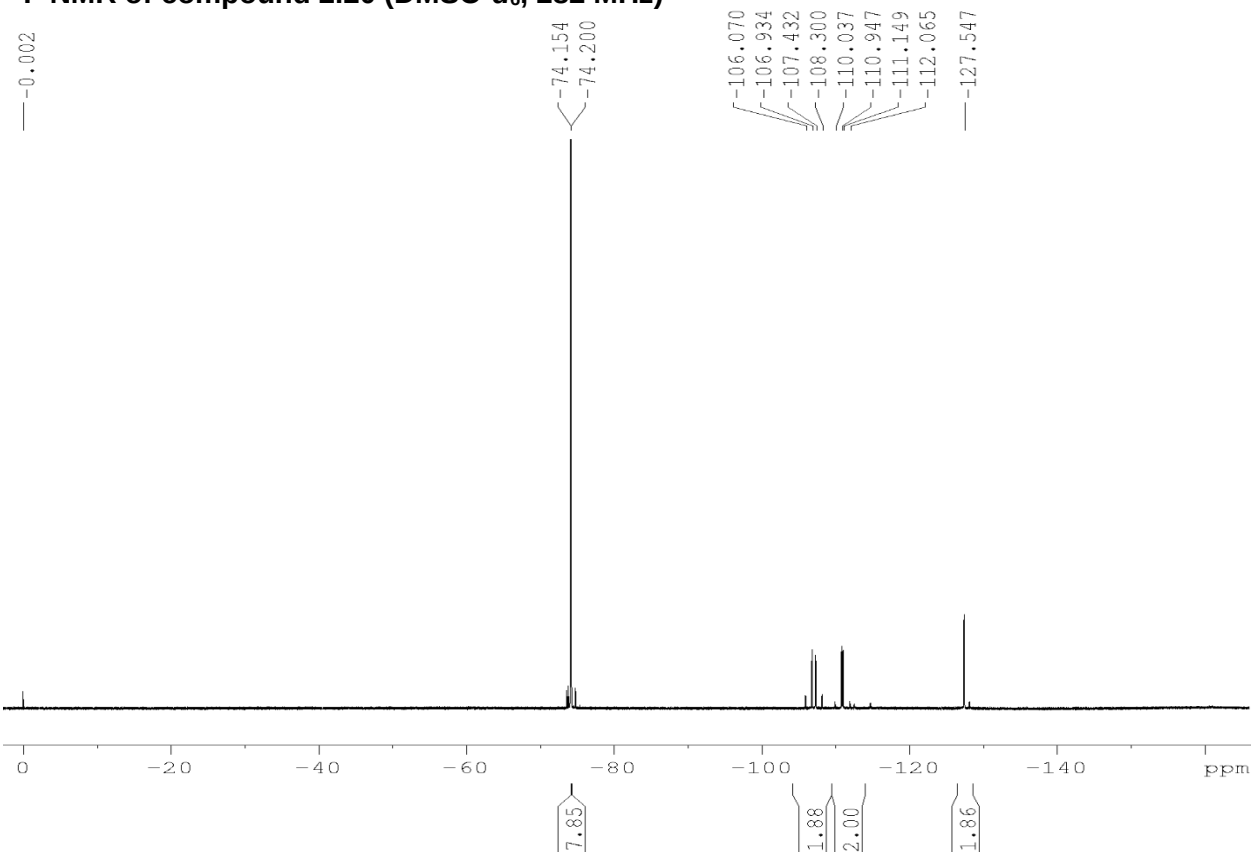
^{1}H NMR of compound 2.20 (DMSO-d_6, 500 MHz)



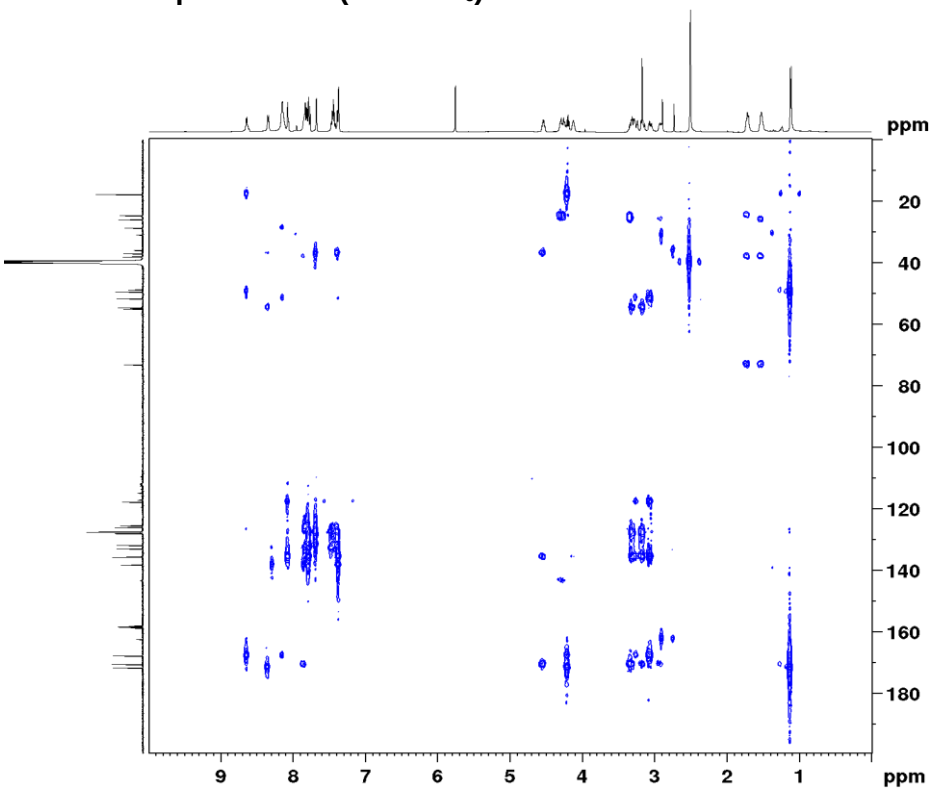
^{13}C NMR of compound 2.20 (DMSO-d_6, 126 MHz)



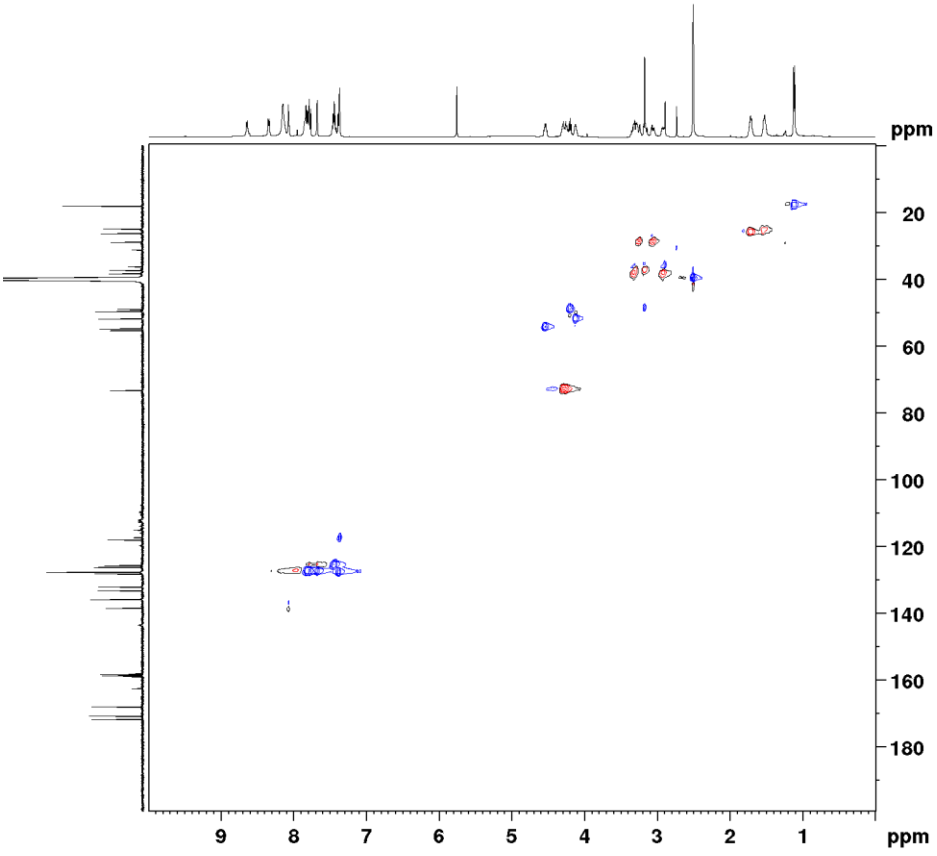
^{19}F NMR of compound 2.20 (DMSO-d_6, 282 MHz)



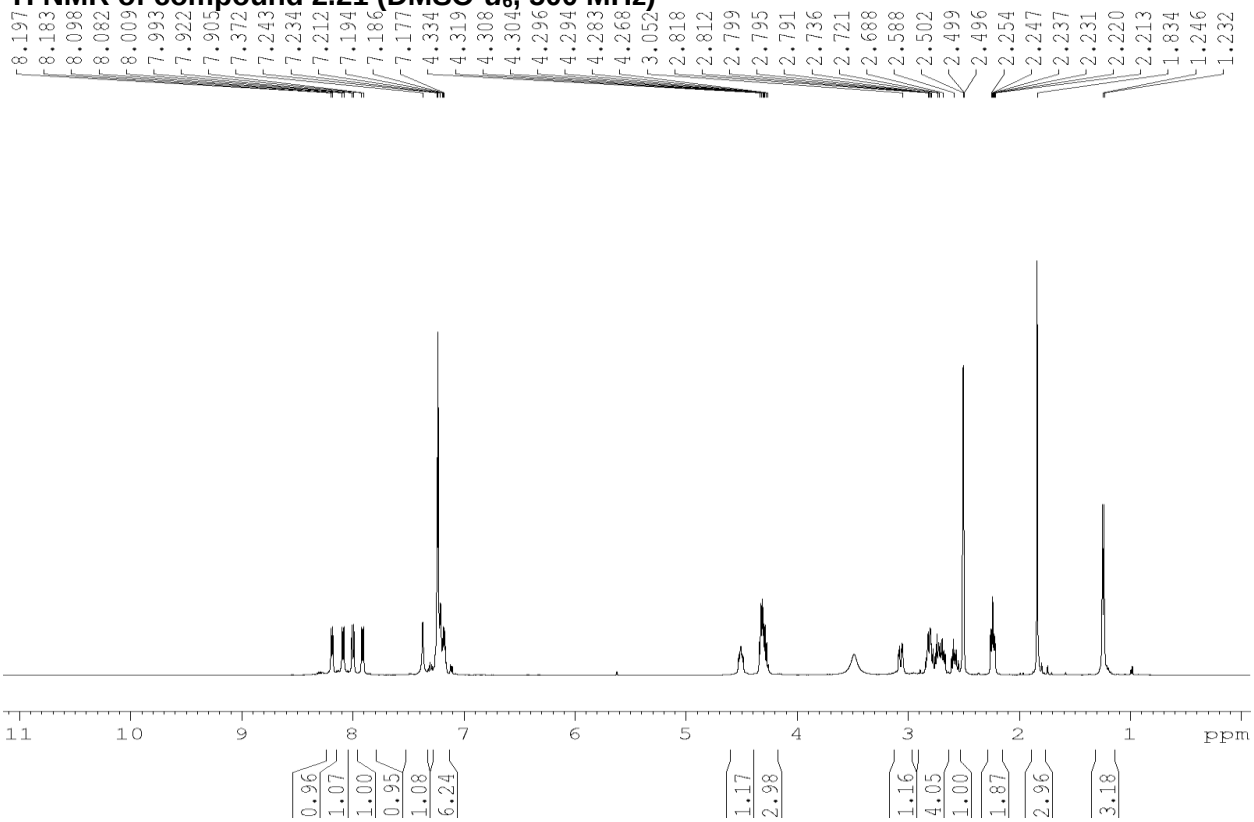
HMBC spectrum of compound 2.20 (DMSO-d_6)



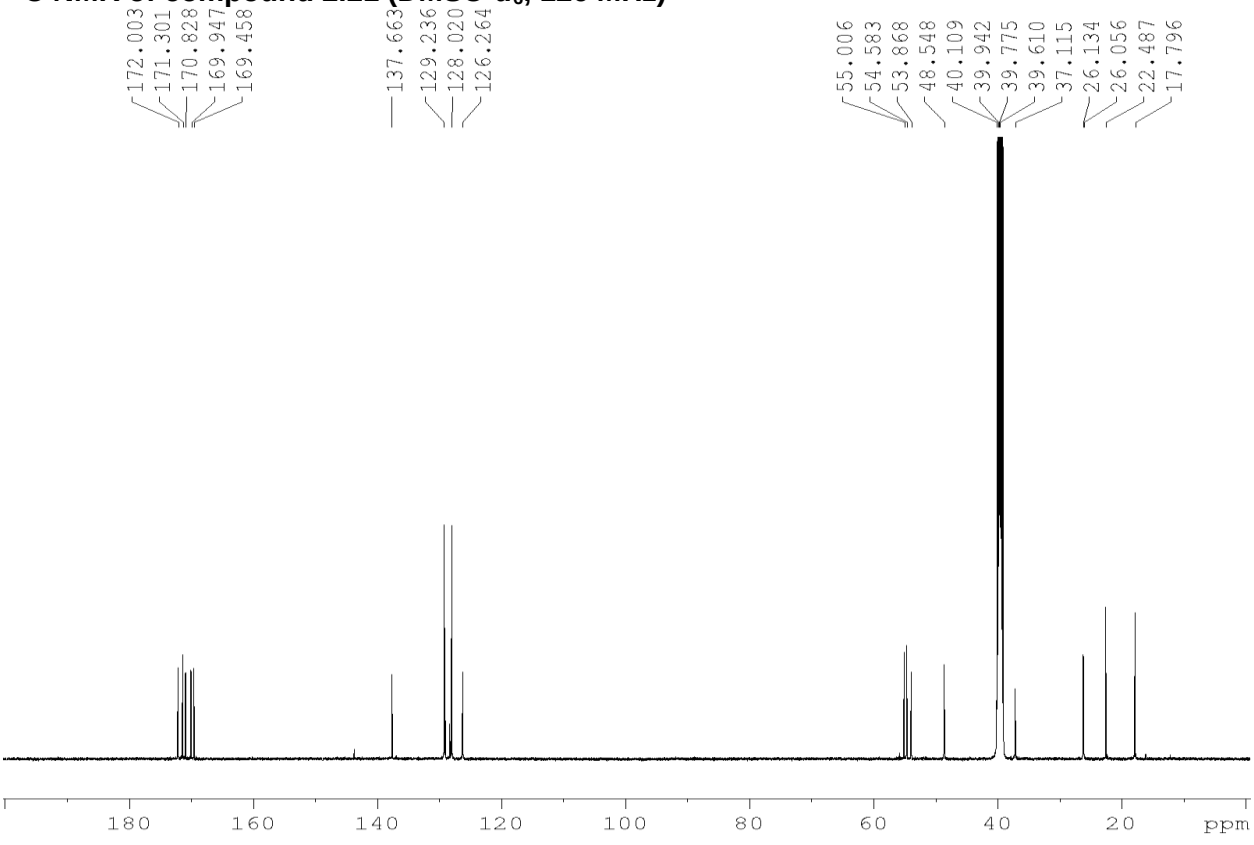
HSQC spectrum of compound 2.20 (DMSO-d_6)



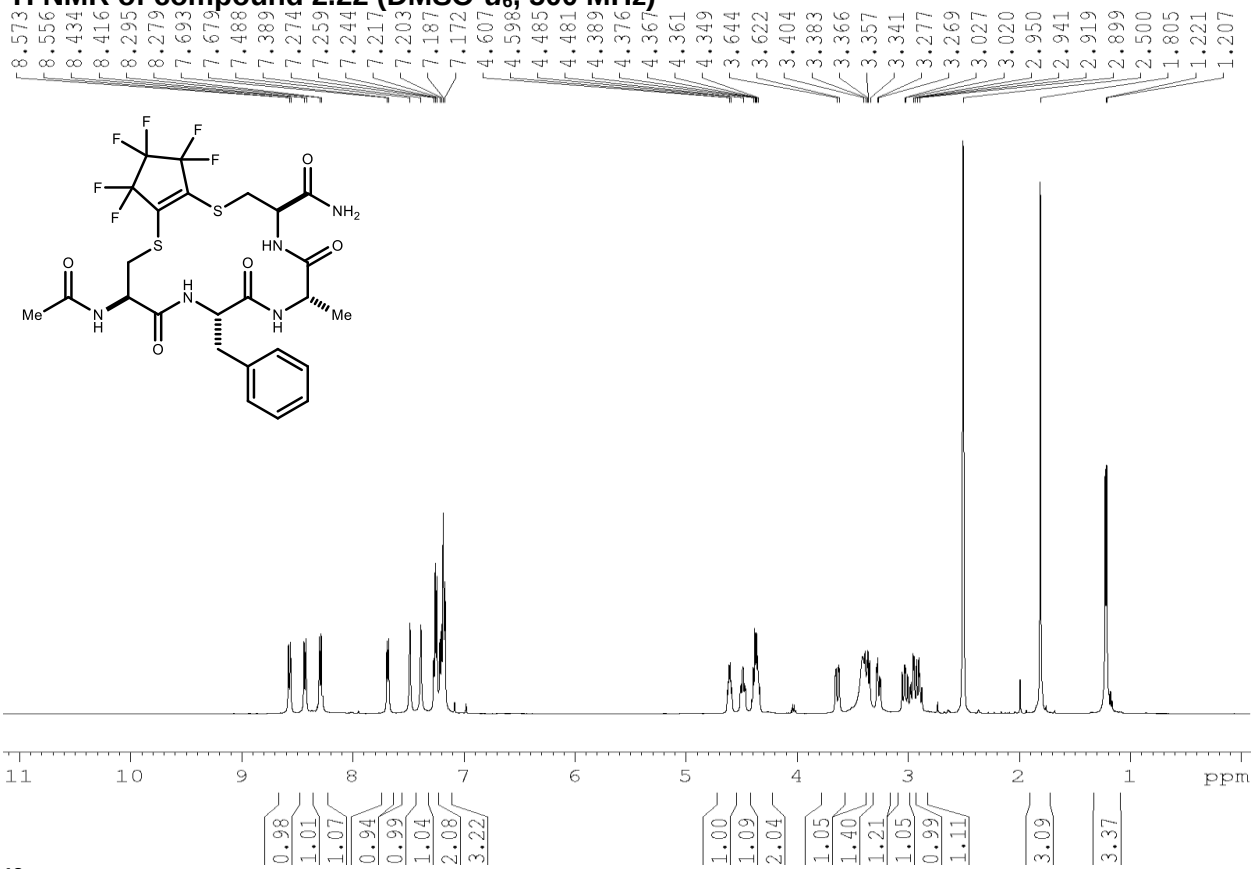
^{1}H NMR of compound 2.21 (DMSO-d_6, 500 MHz)



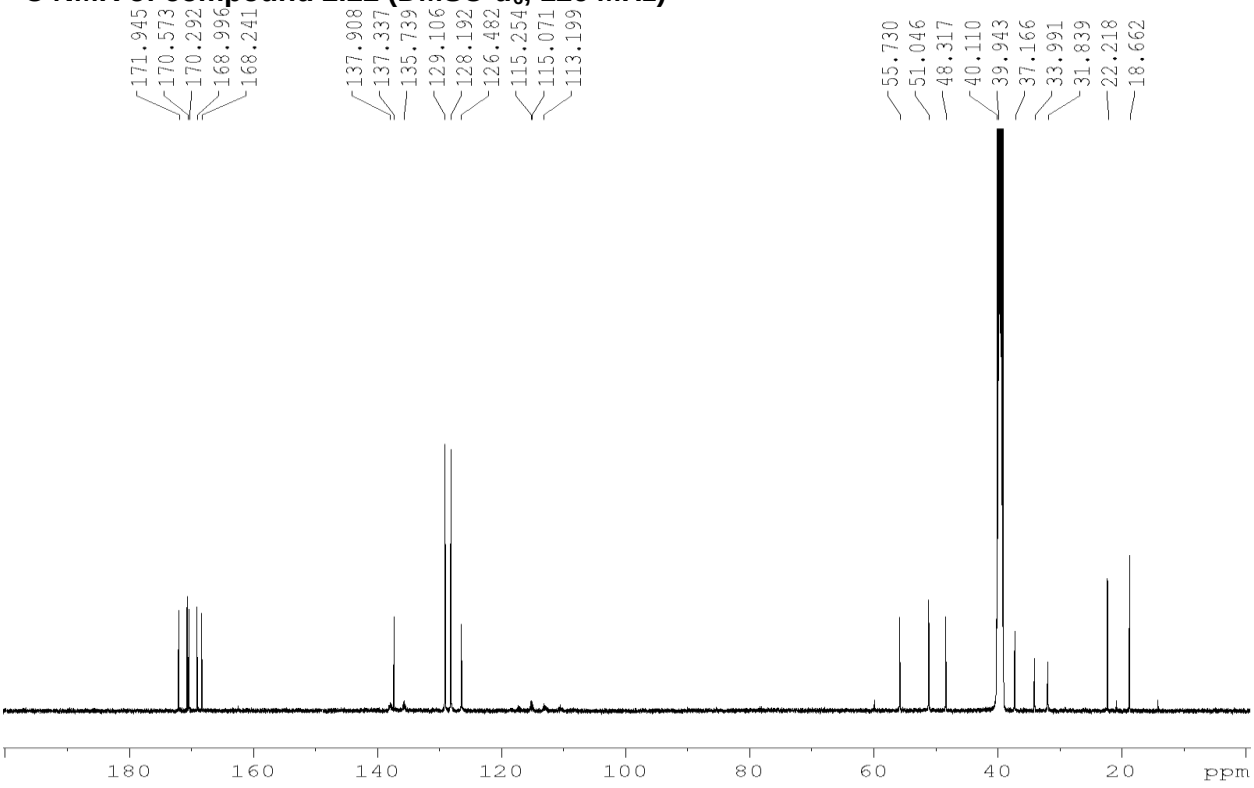
^{13}C NMR of compound 2.21 (DMSO-d_6, 126 MHz)



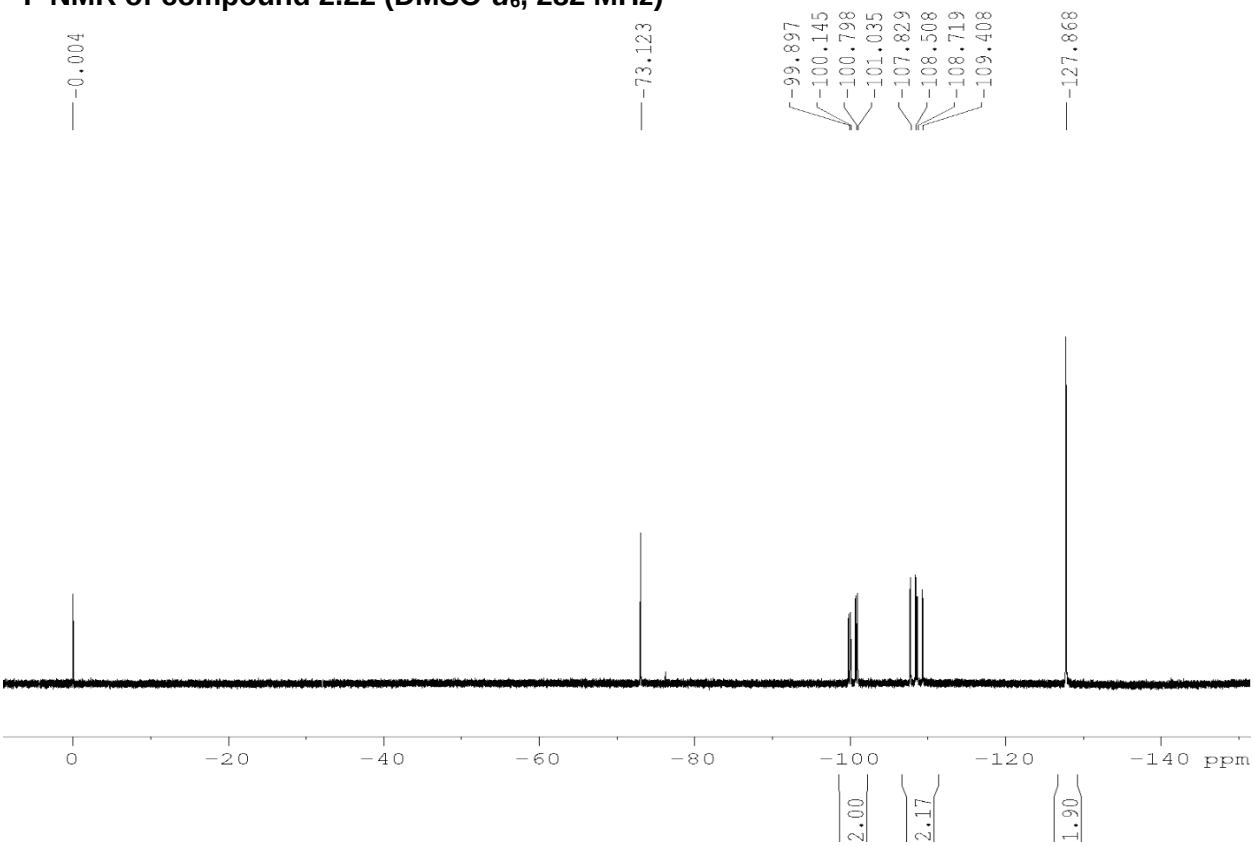
^{1}H NMR of compound 2.22 (DMSO-d_6, 500 MHz)



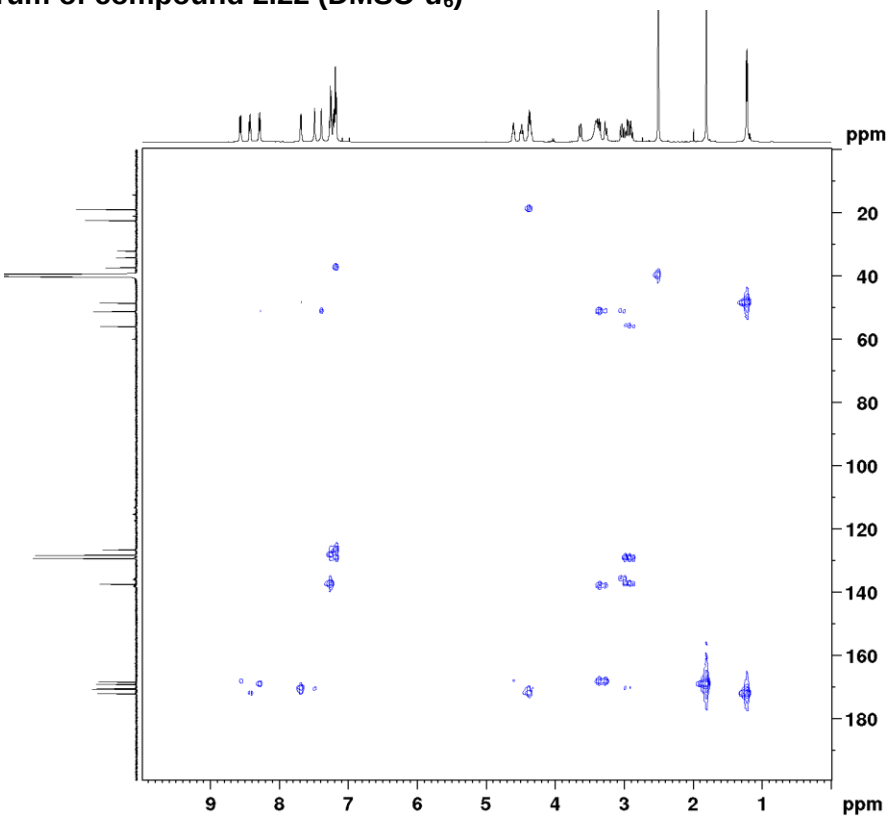
^{13}C NMR of compound 2.22 (DMSO-d_6, 126 MHz)



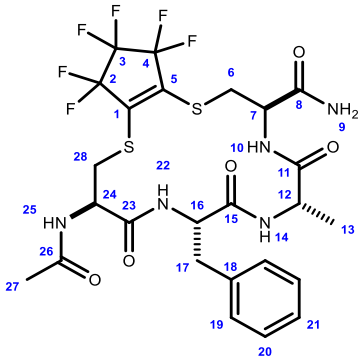
^{19}F NMR of compound 2.22 (DMSO-d_6, 282 MHz)



HMBC spectrum of compound 2.22 (DMSO-d_6)

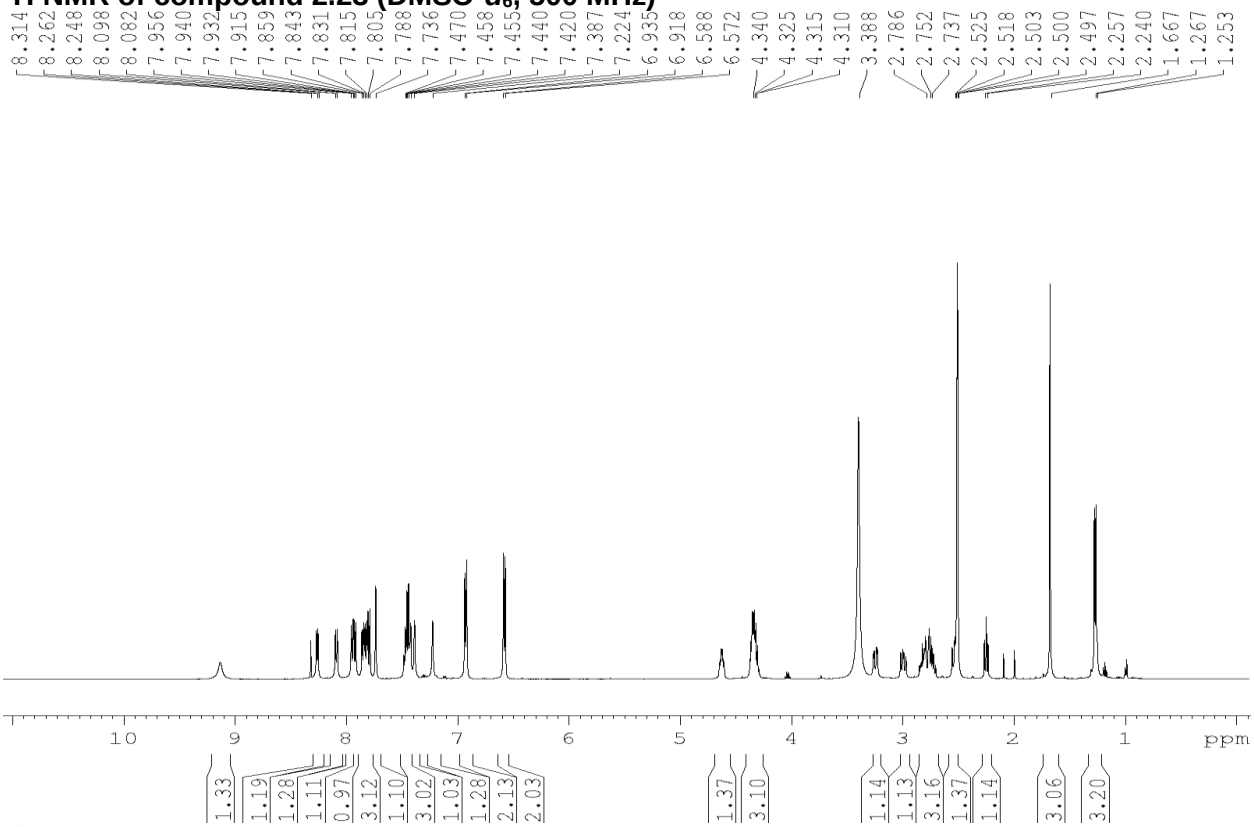


Compound 2.22 (DMSO-d_6)

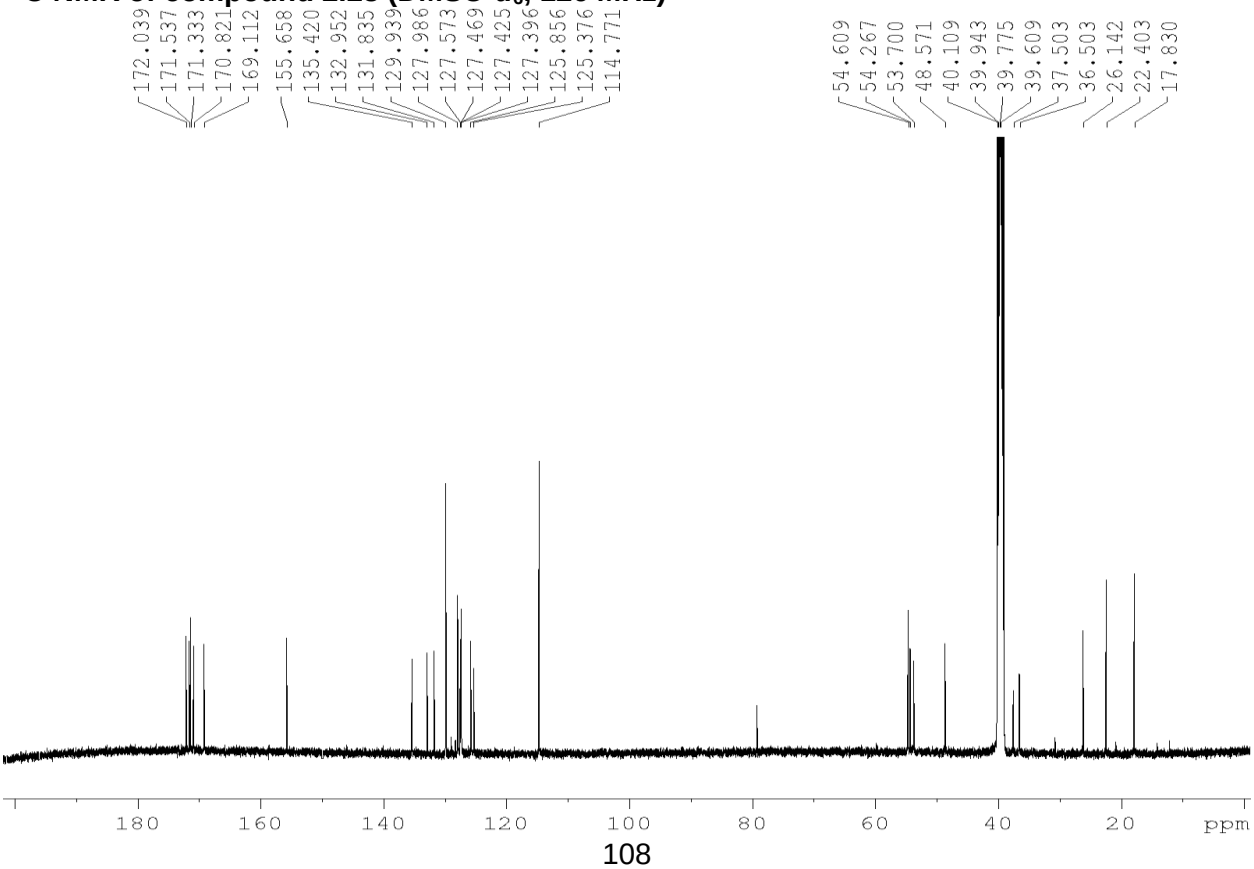


	13C	1H	Key Correlation
1	138.2–137.7 (m)	–	HMBC 28 -> 1
2	118.6–108.2 (m)	–	
3	118.6–108.2 (m)	–	
4	118.6–108.2 (m)	–	
5	136.0–135.4 (m)	–	HMBC 6 -> 5
6	34	3.63 (dd, J = 13.9, 3.3 Hz, 1H), 3.02 (dd, J = 13.9, 10.7 Hz, 1H)	HMBC 6 -> 5,8
7	51	4.51–4.45 (m, 1H)	HMBC 9 -> 7, HMBC 7 -> 6
8	170.3	–	HMBC 10 -> 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

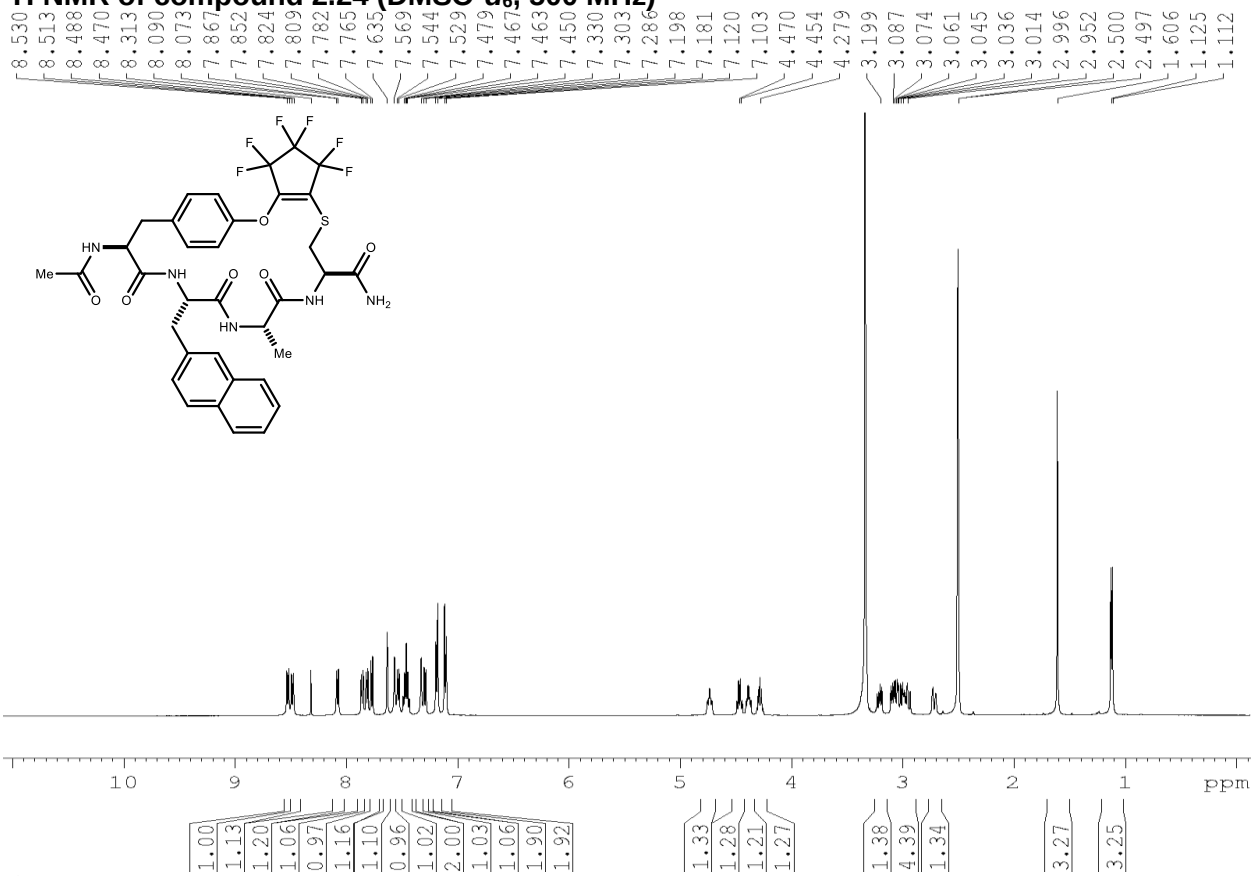
^{1}H NMR of compound 2.23 (DMSO-d_6, 500 MHz)



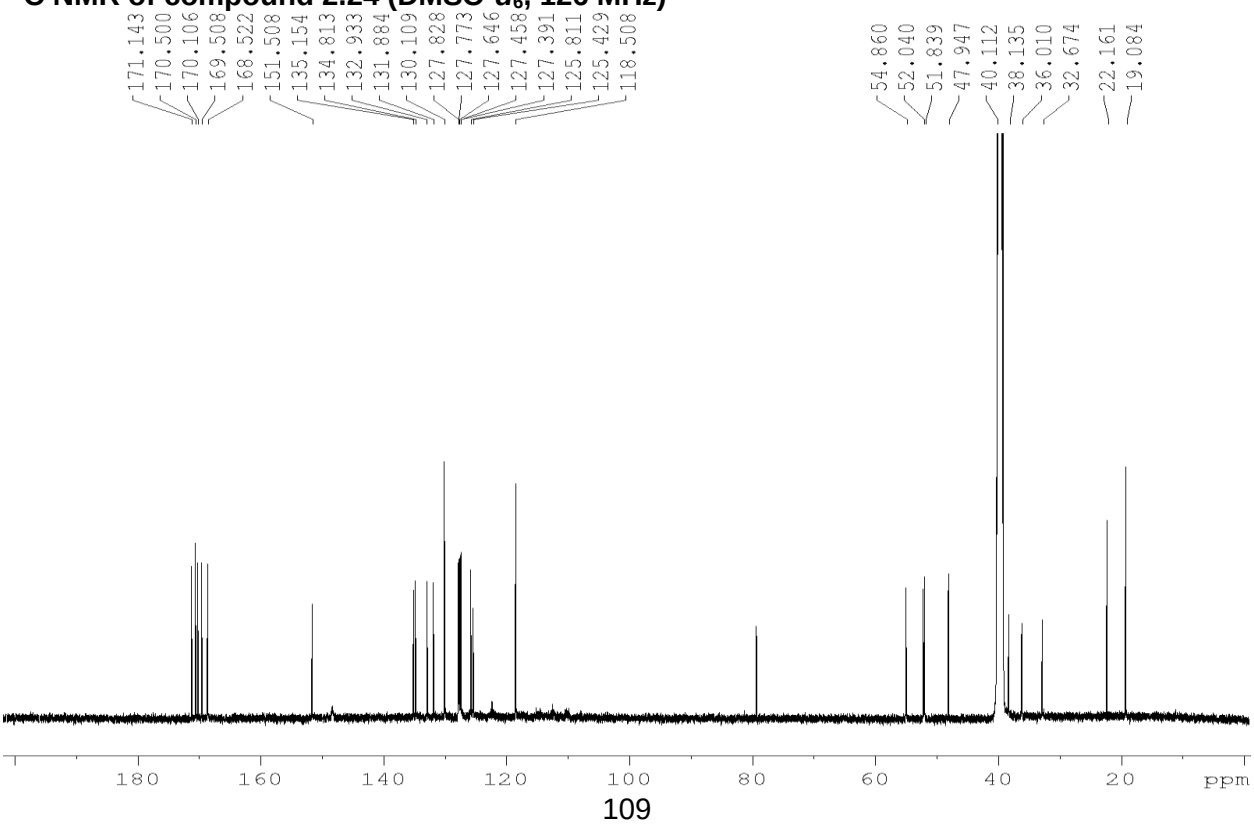
^{13}C NMR of compound 2.23 (DMSO-d_6, 126 MHz)



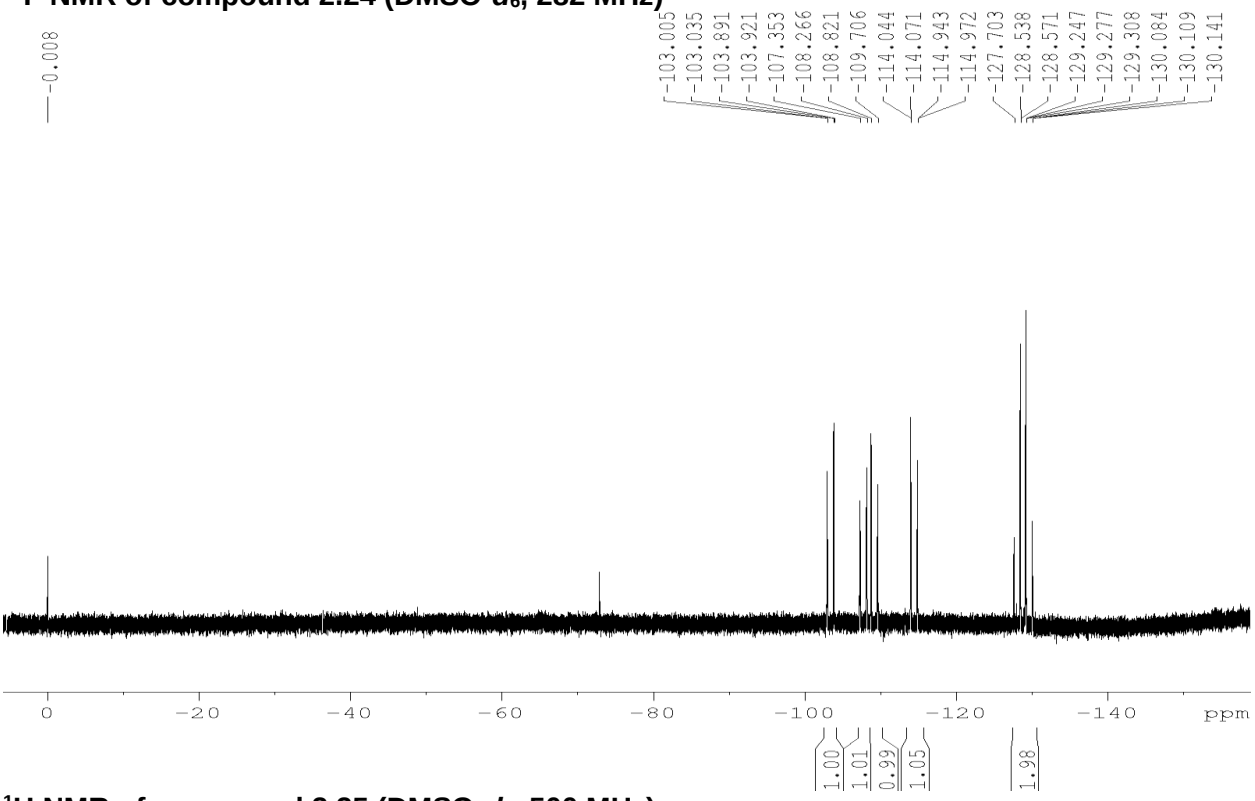
^{1}H NMR of compound 2.24 (DMSO-d_6, 500 MHz)



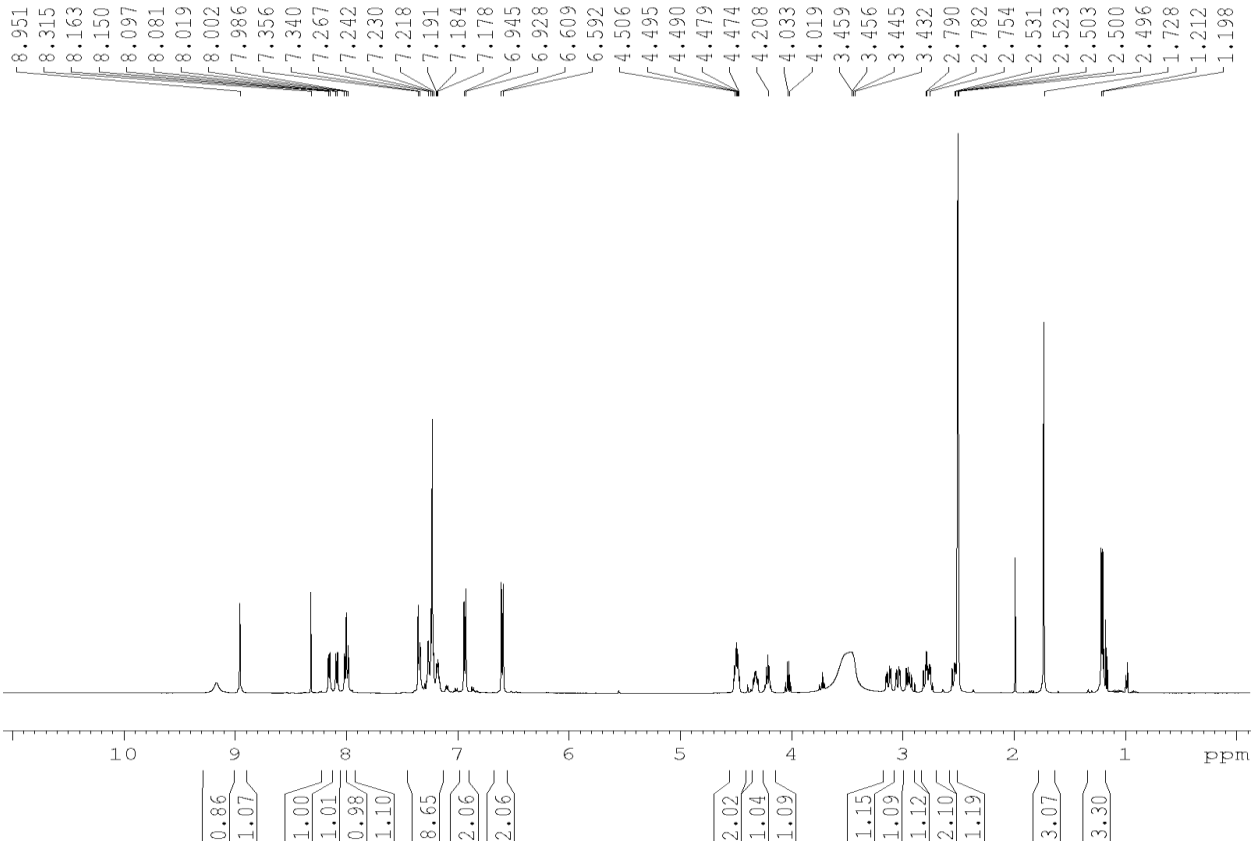
^{13}C NMR of compound 2.24 (DMSO-d_6, 126 MHz)



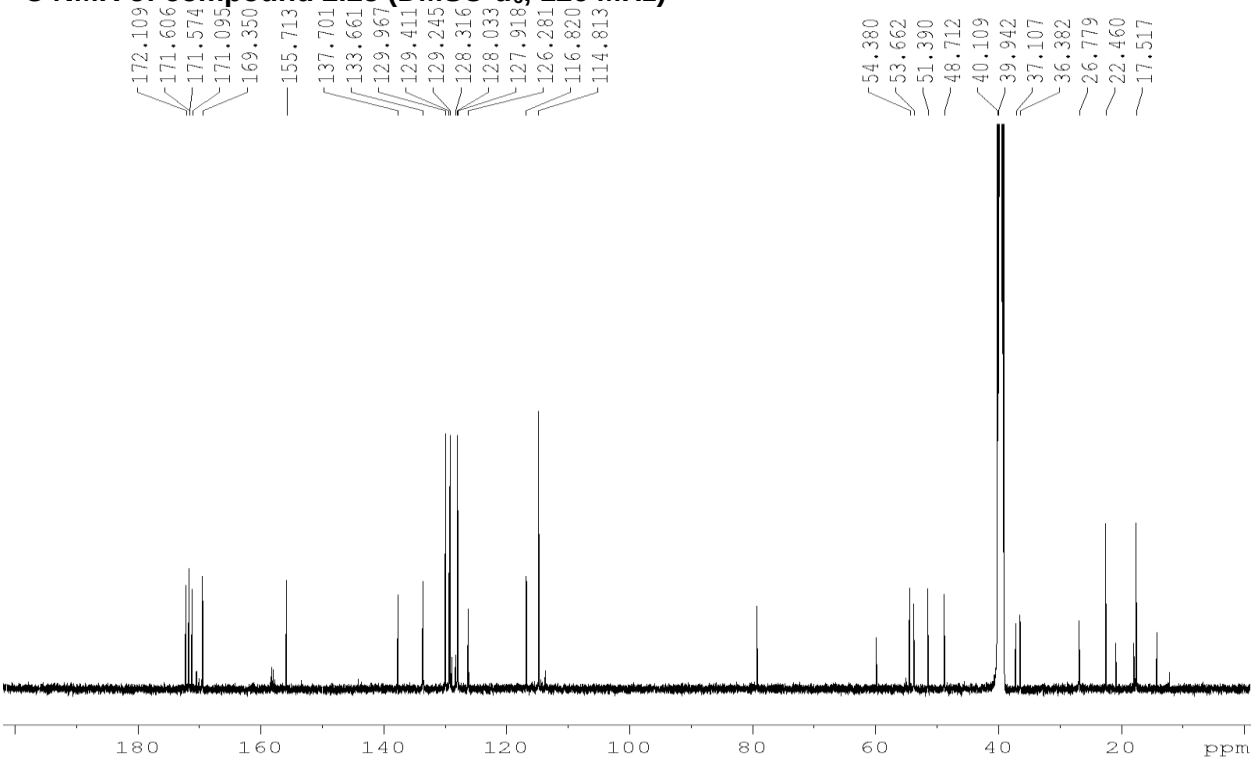
^{19}F NMR of compound 2.24 (DMSO-d_6, 282 MHz)



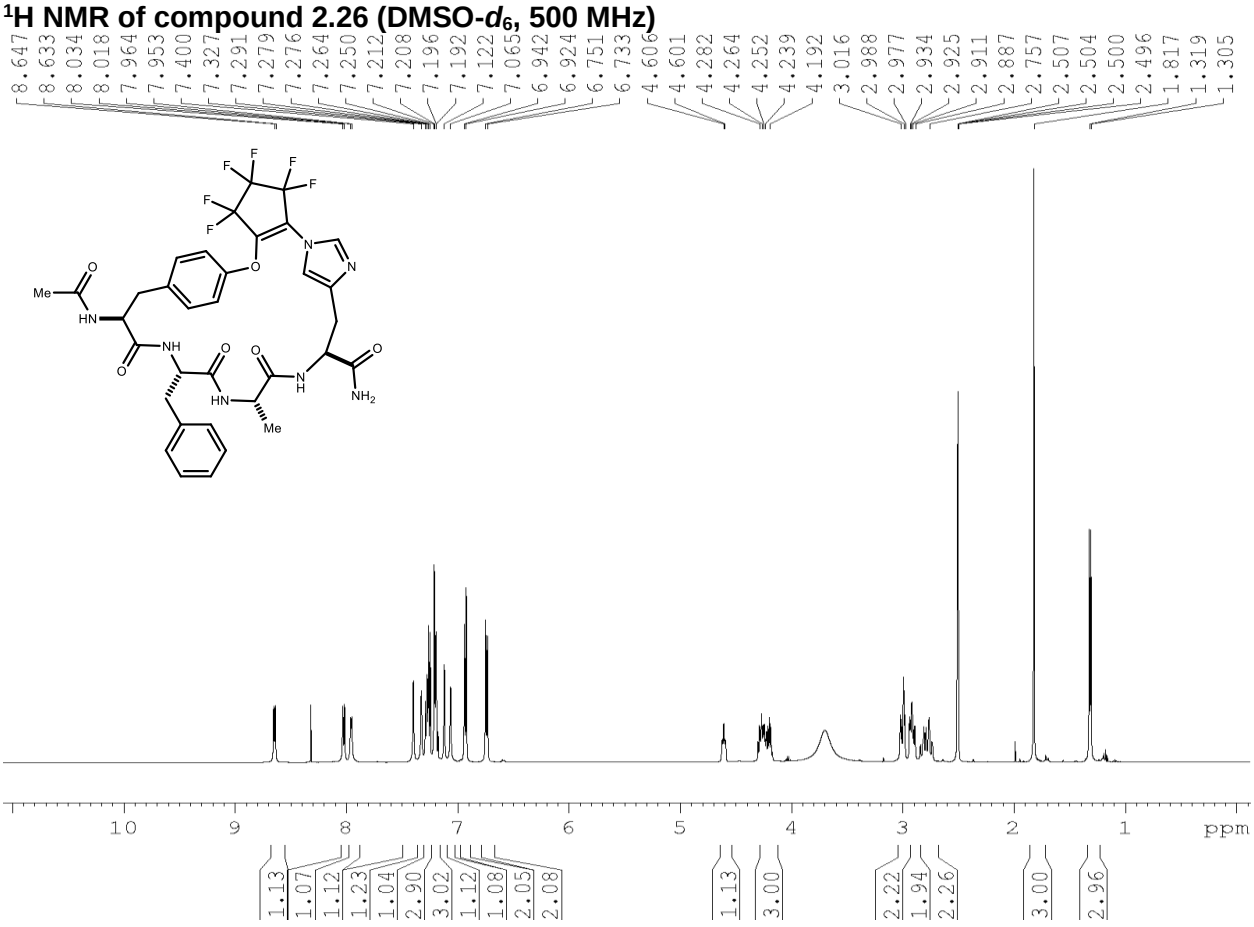
^{1}H NMR of compound 2.25 (DMSO-d_6, 500 MHz)



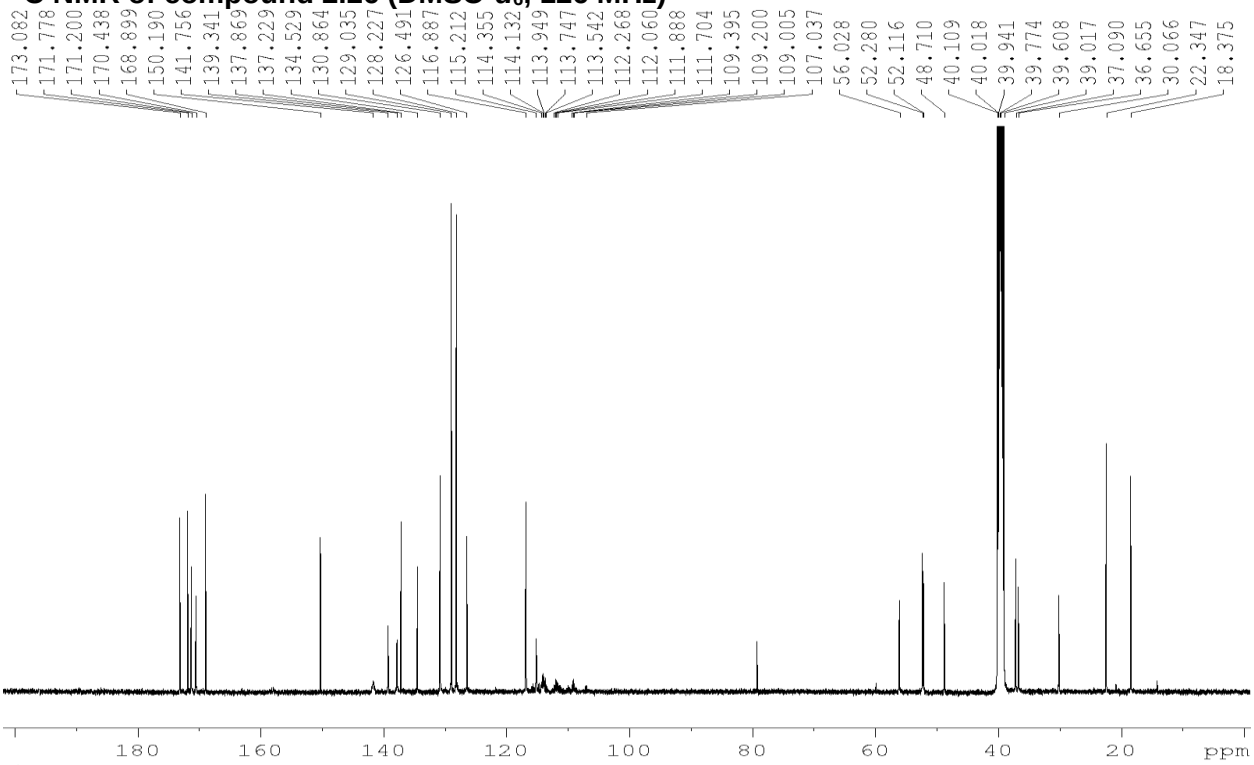
^{13}C NMR of compound 2.25 (DMSO-d_6, 126 MHz)



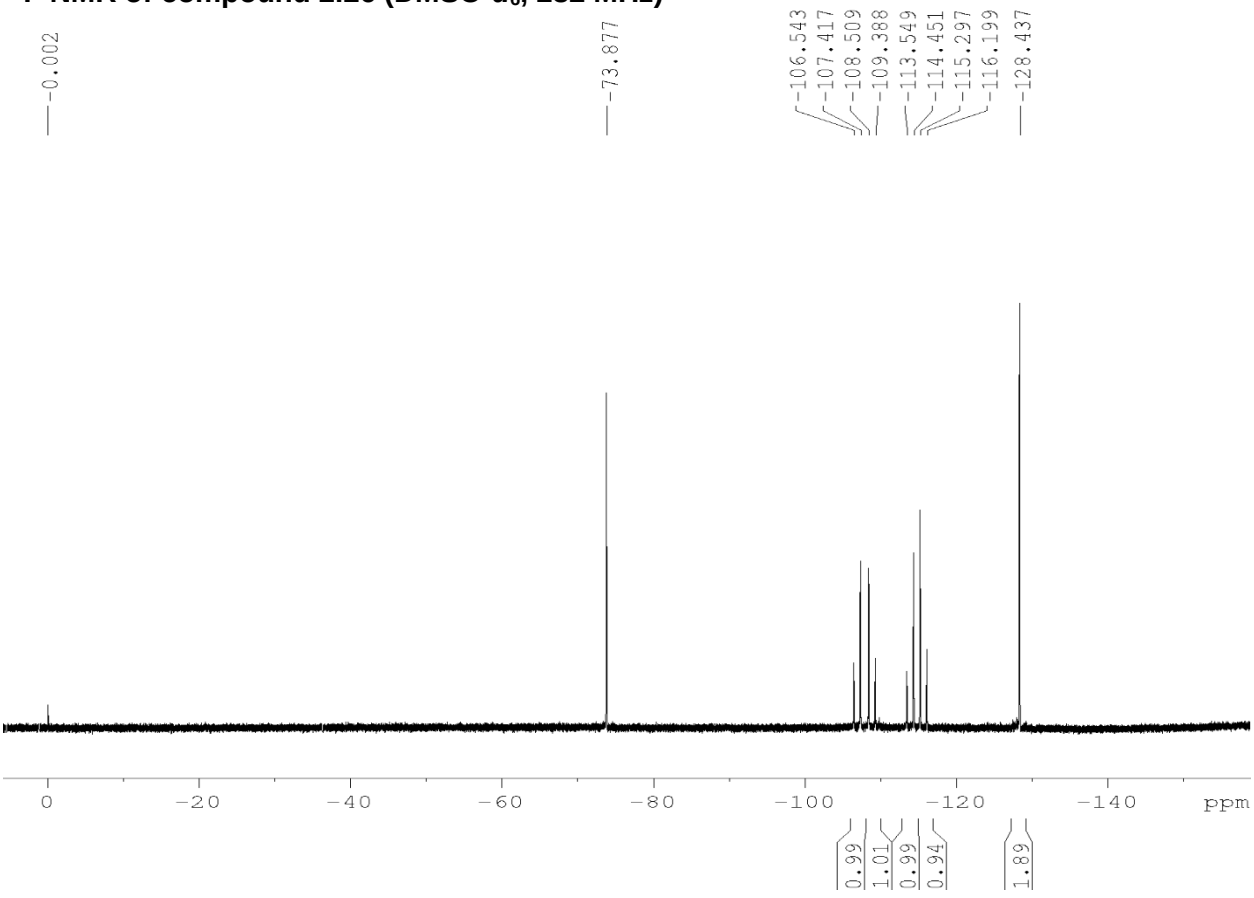
^{1}H NMR of compound 2.26 (DMSO-d_6, 500 MHz)



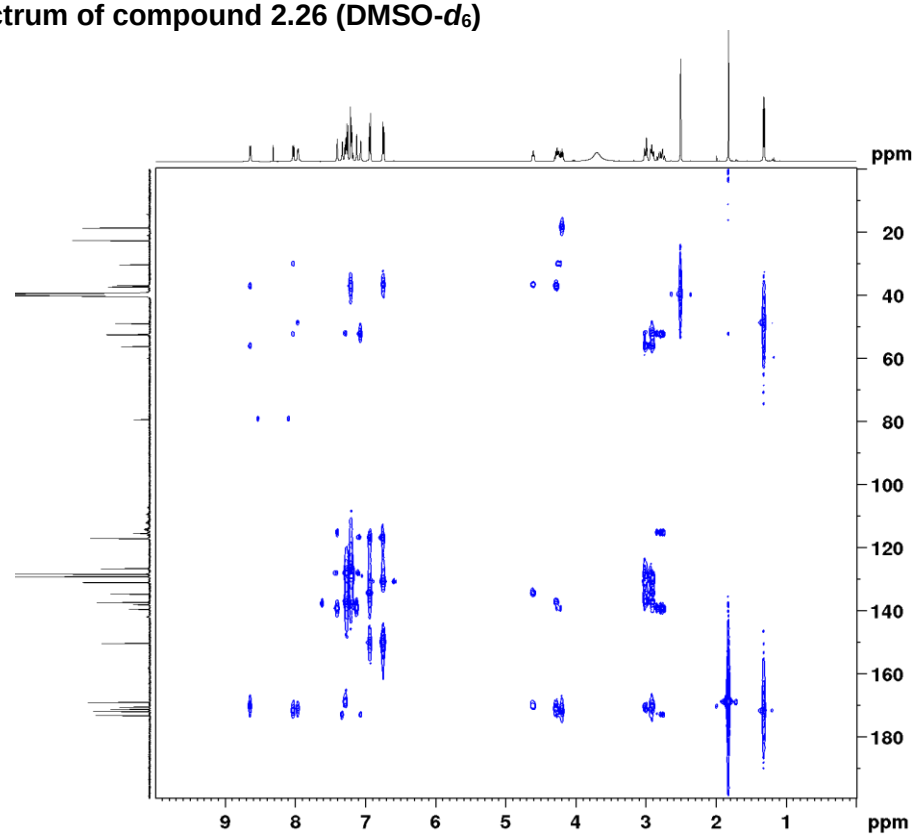
^{13}C NMR of compound 2.26 (DMSO-d_6, 126 MHz)



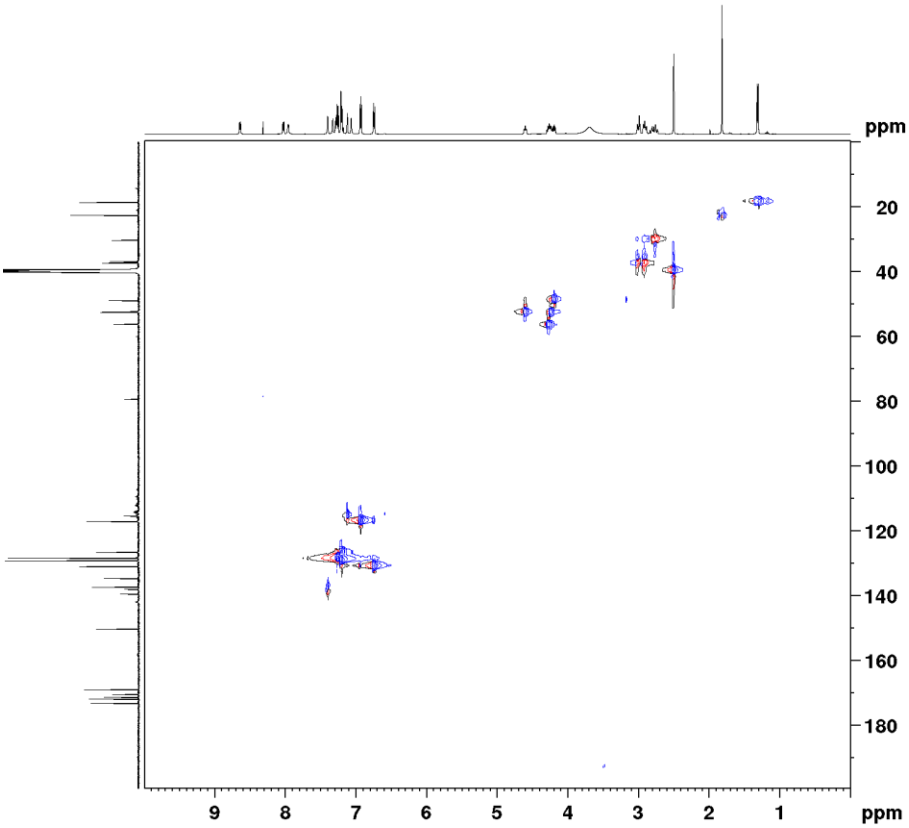
^{19}F NMR of compound 2.26 (DMSO-d_6, 282 MHz)



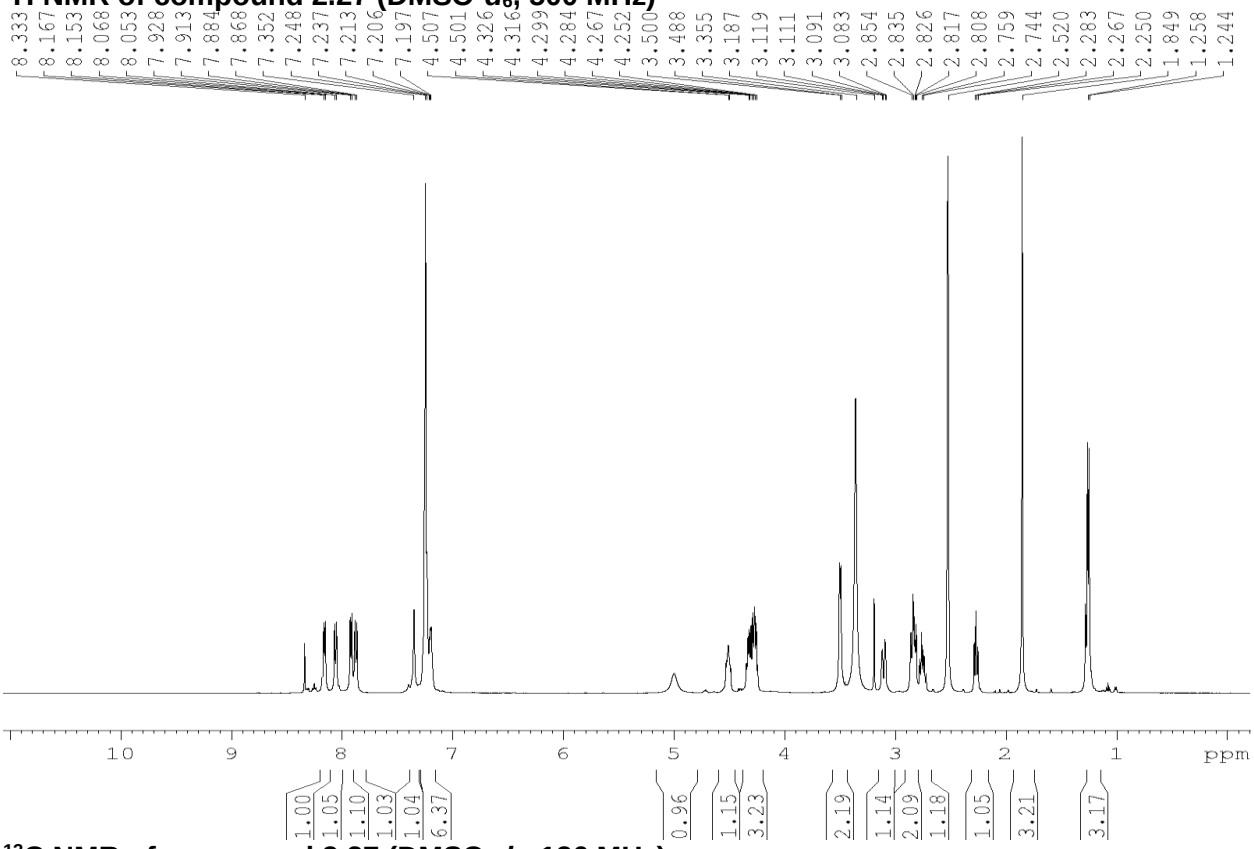
HMBC spectrum of compound 2.26 (DMSO-d_6)



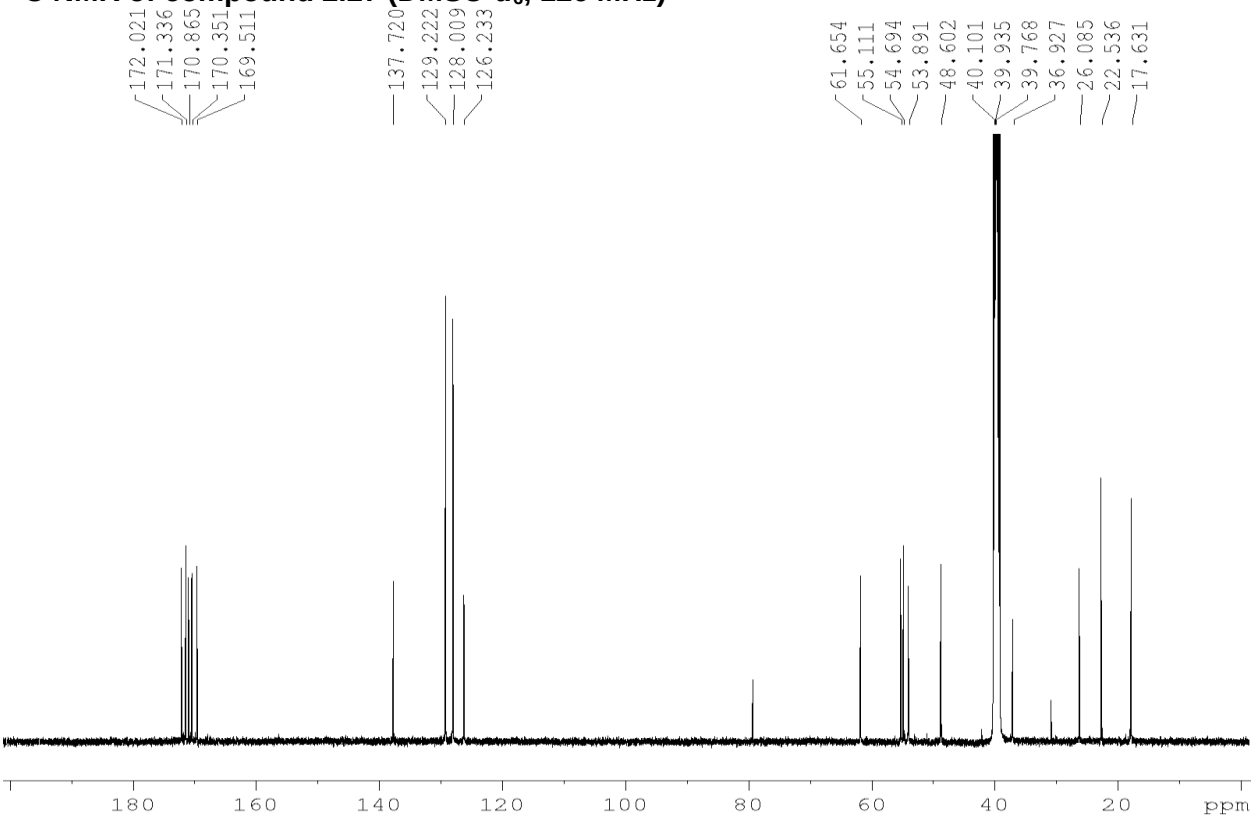
HSQC spectrum of compound 2.26 (DMSO-d_6)



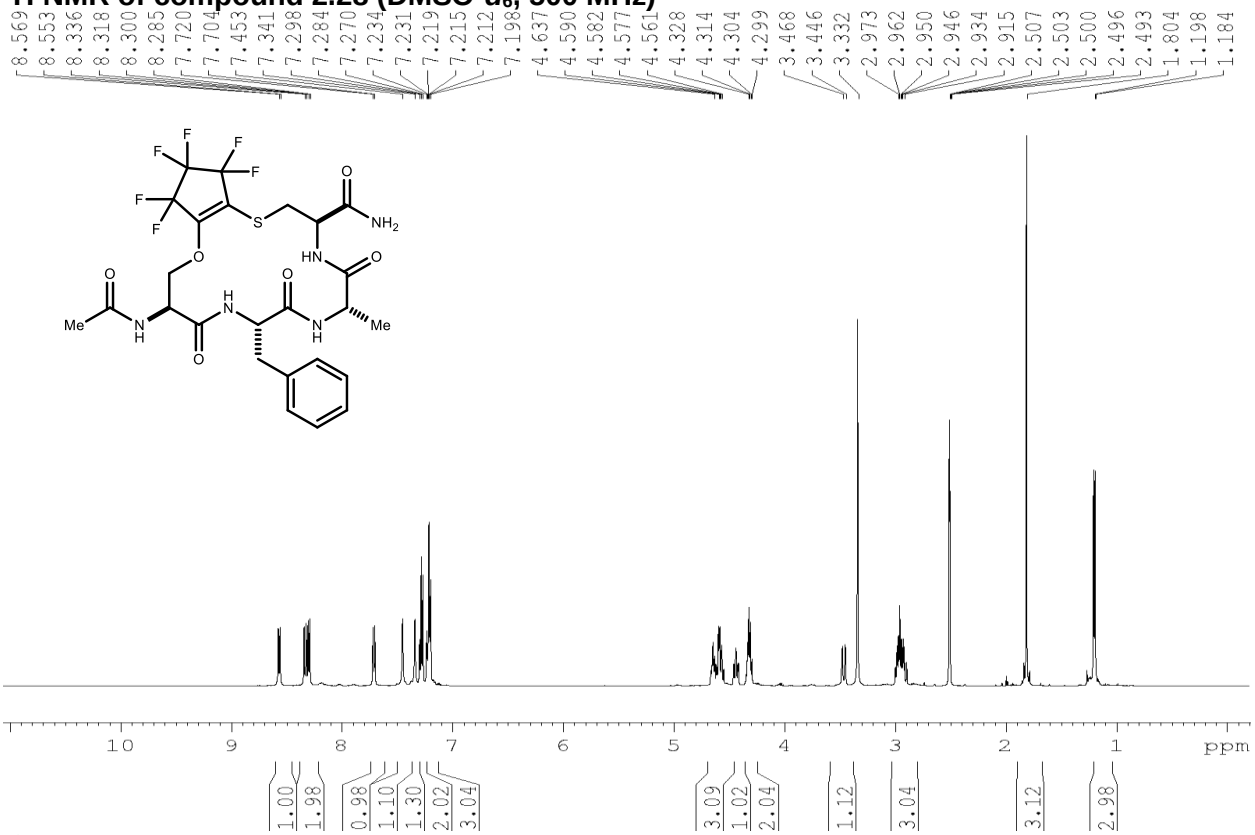
^{1}H NMR of compound 2.27 (DMSO-d_6, 500 MHz)



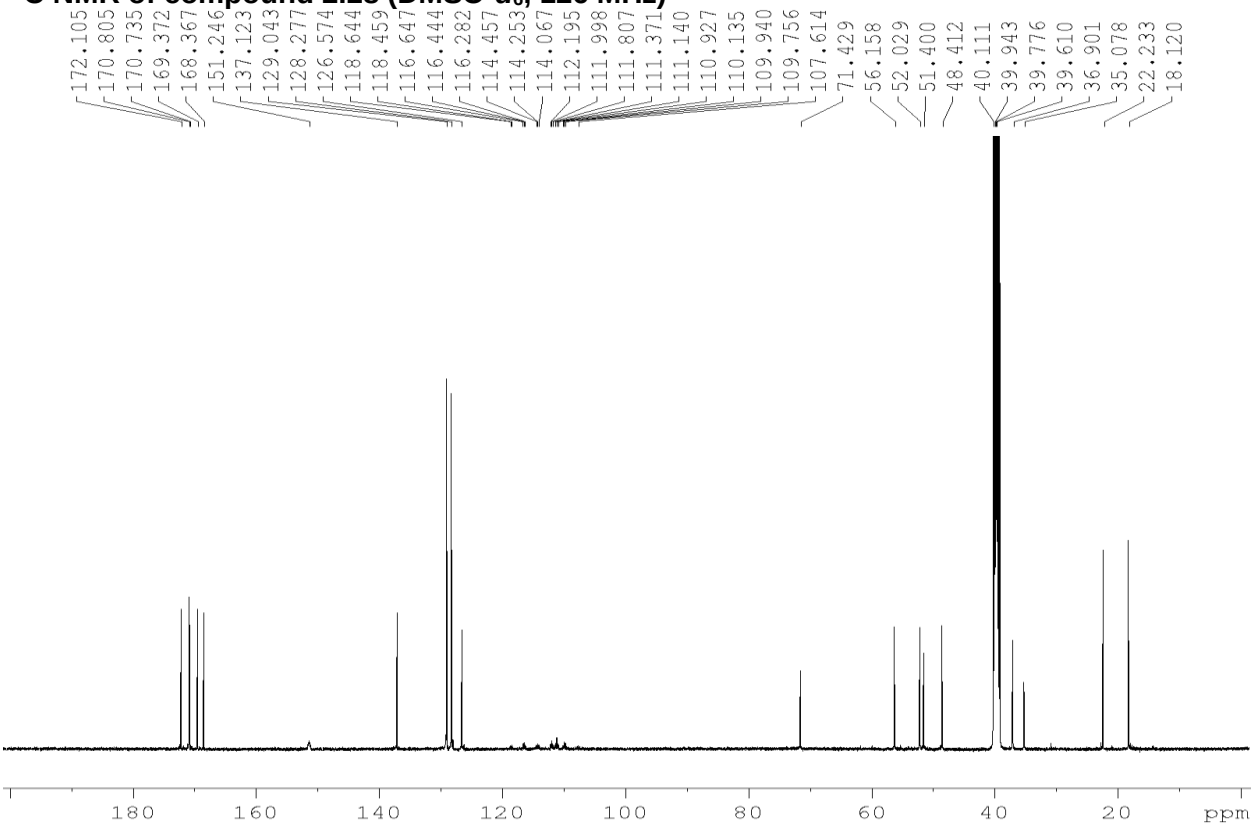
^{13}C NMR of compound 2.27 (DMSO-d_6, 126 MHz)



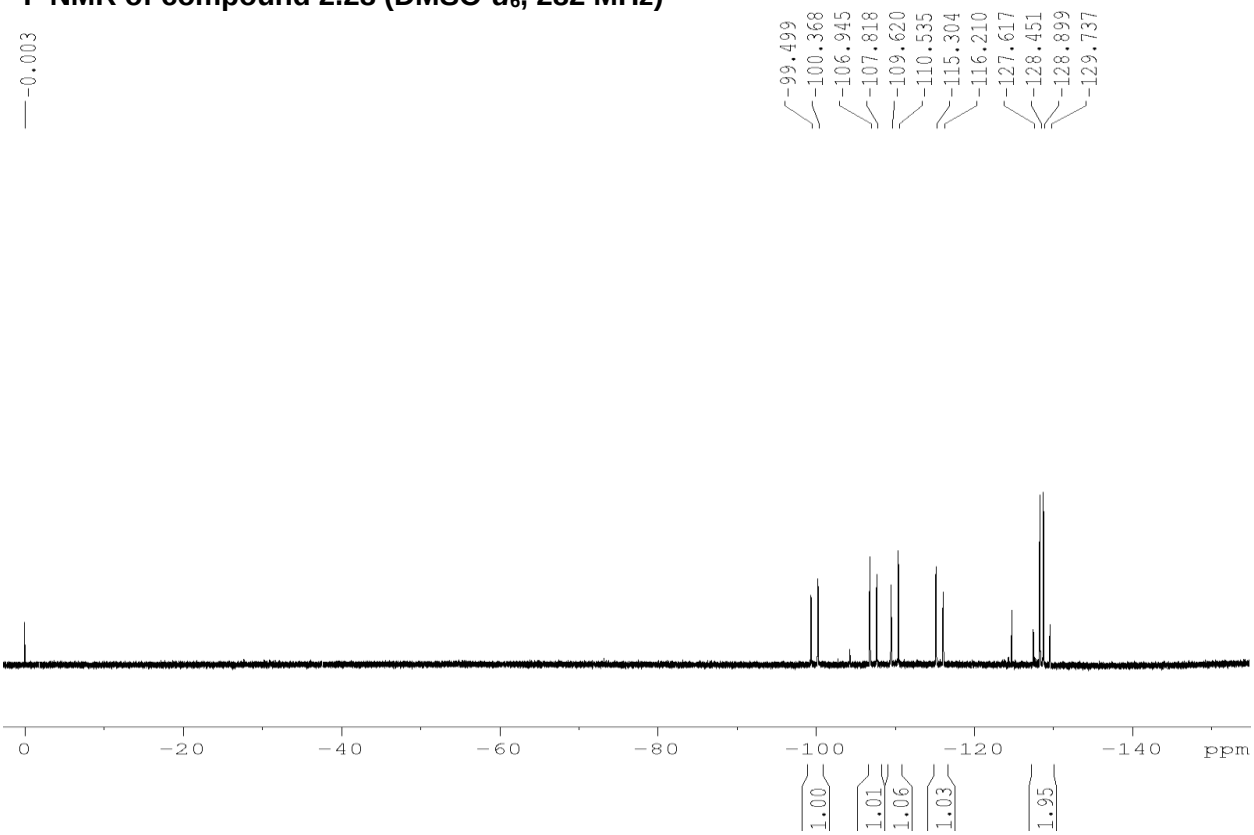
^{1}H NMR of compound 2.28 (DMSO-d_6, 500 MHz)



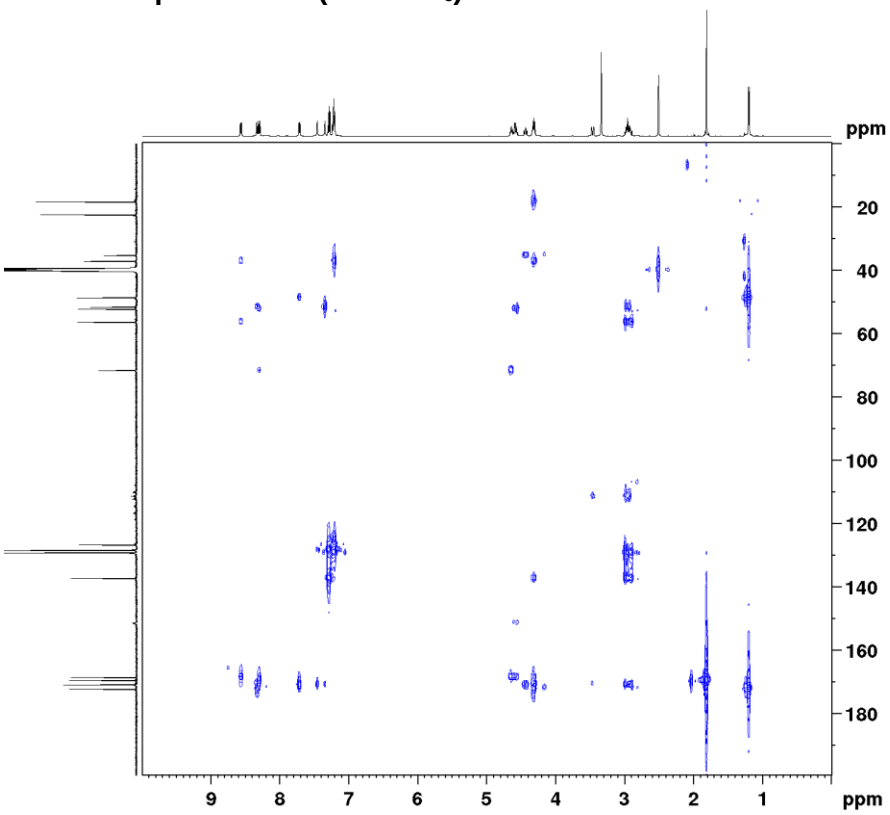
^{13}C NMR of compound 2.28 (DMSO-d_6, 126 MHz)



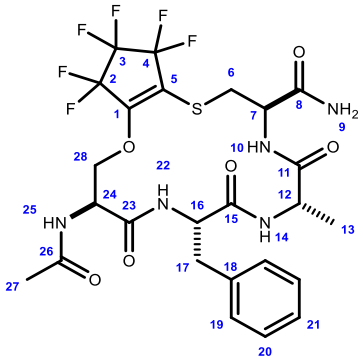
^{19}F NMR of compound 2.28 (DMSO-d_6, 282 MHz)



HMBC spectrum of compound 2.28 (DMSO-d_6)

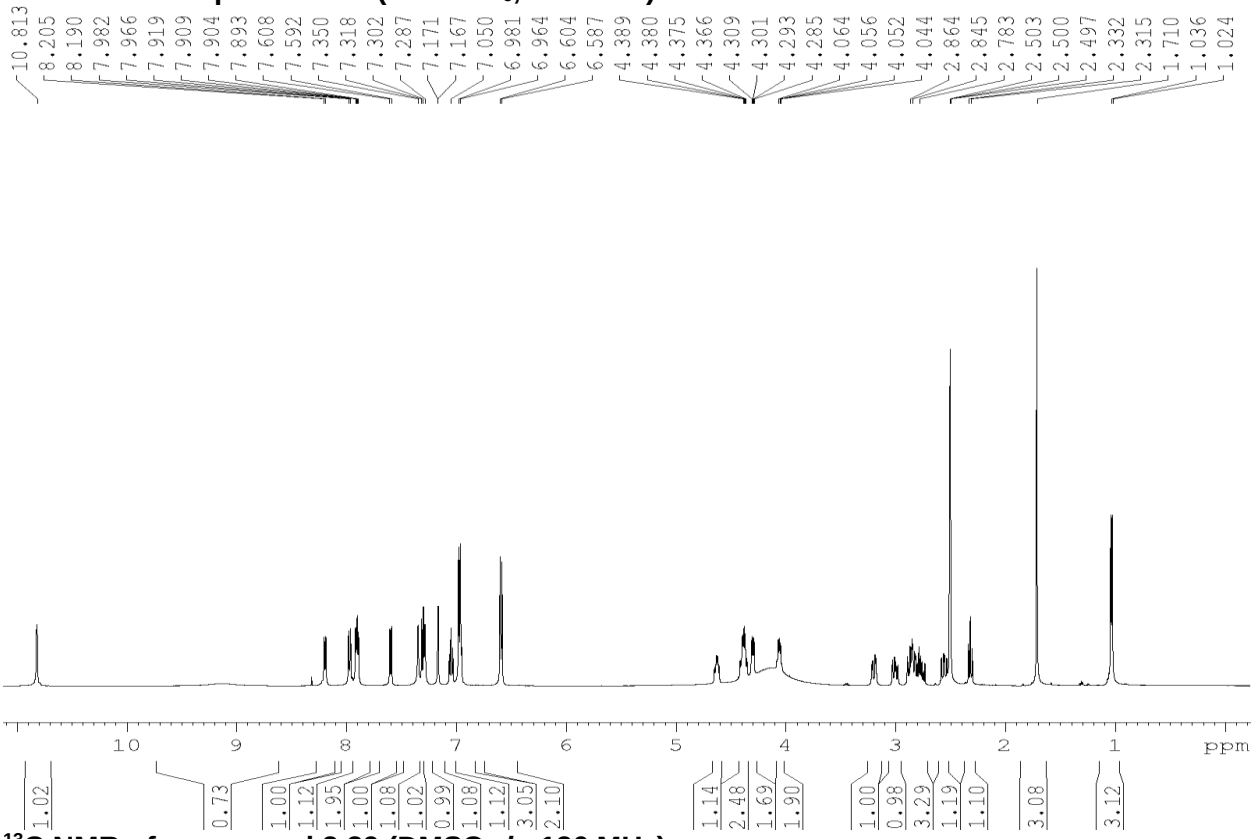


Compound 2.28 (DMSO-d_6)

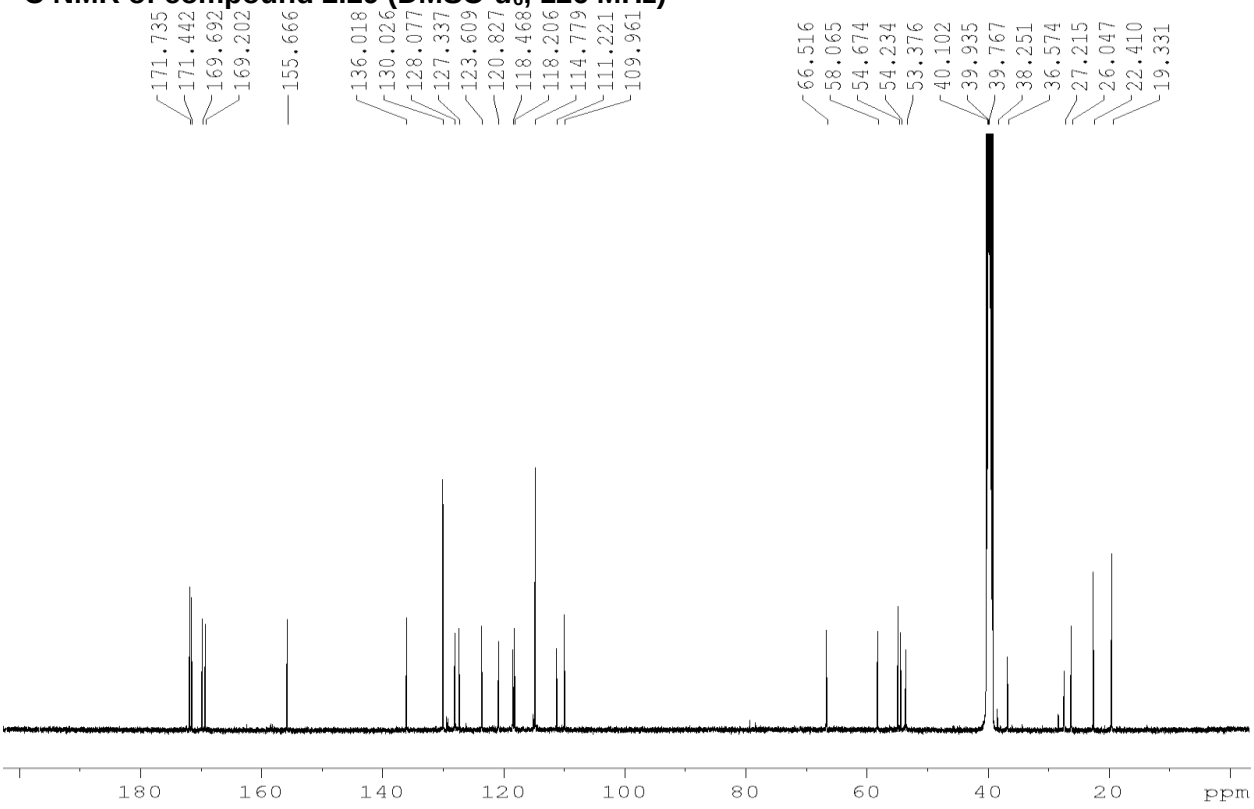


	13C	1H	Key Correlation
1	151.5-151.1 (m, 1C)	-	HMBC 28 -> 1
2	126.6, 118.6-107.6 (m, 3C)	-	
3	126.6, 118.6-107.6 (m, 3C)	-	
4	126.6, 118.6-107.6 (m, 3C)	-	
5	111.4-110.8 (m, 1C)	-	HMBC 6 -> 5
6	35.1	3.46 (dd, J = 13.9, 3.0 Hz, 1H), 3.00-2.90 (m, 3H)	HMBC 6 -> 5,7; HMBC 7,10 -> 6
7	51.4	4.46-4.41 (m, 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

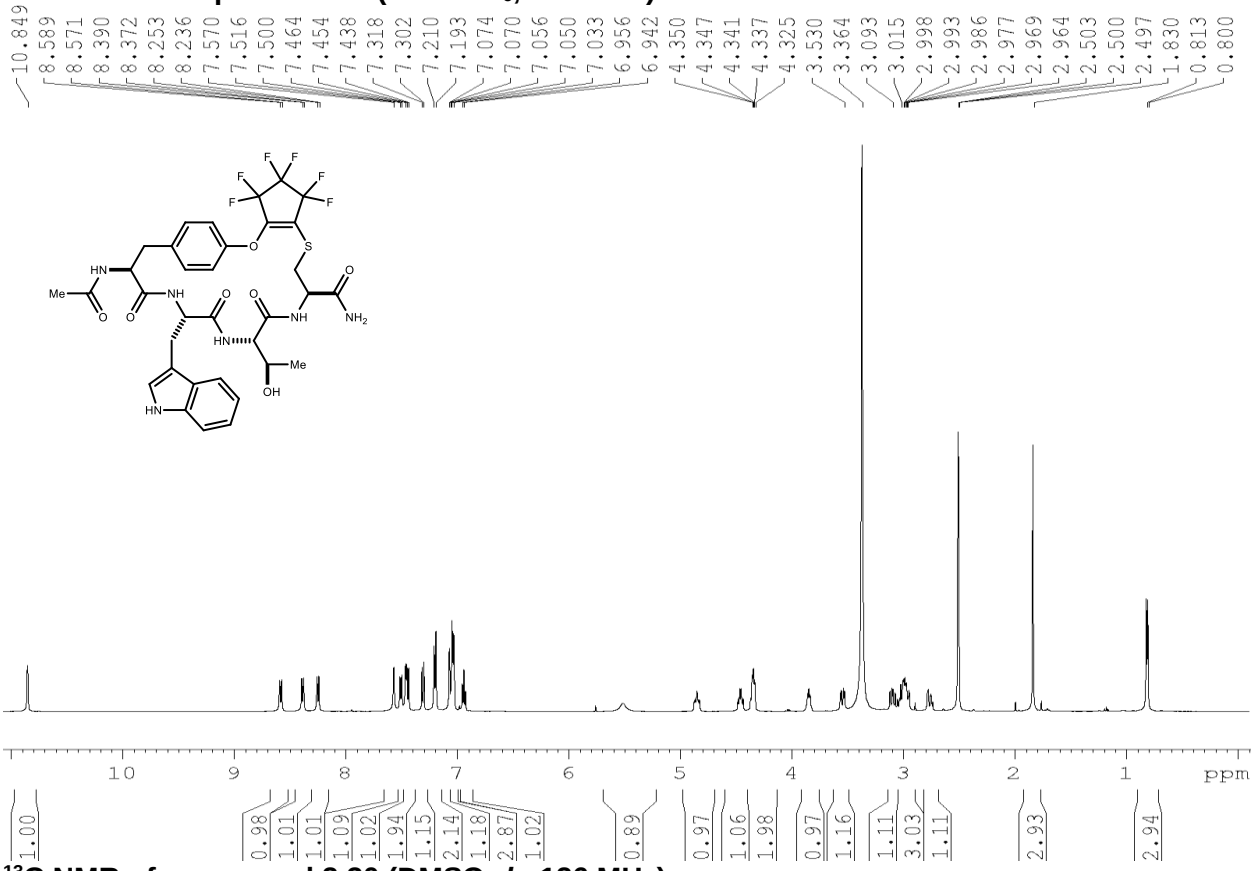
^{1}H NMR of compound 2.29 (DMSO-d_6, 500 MHz)



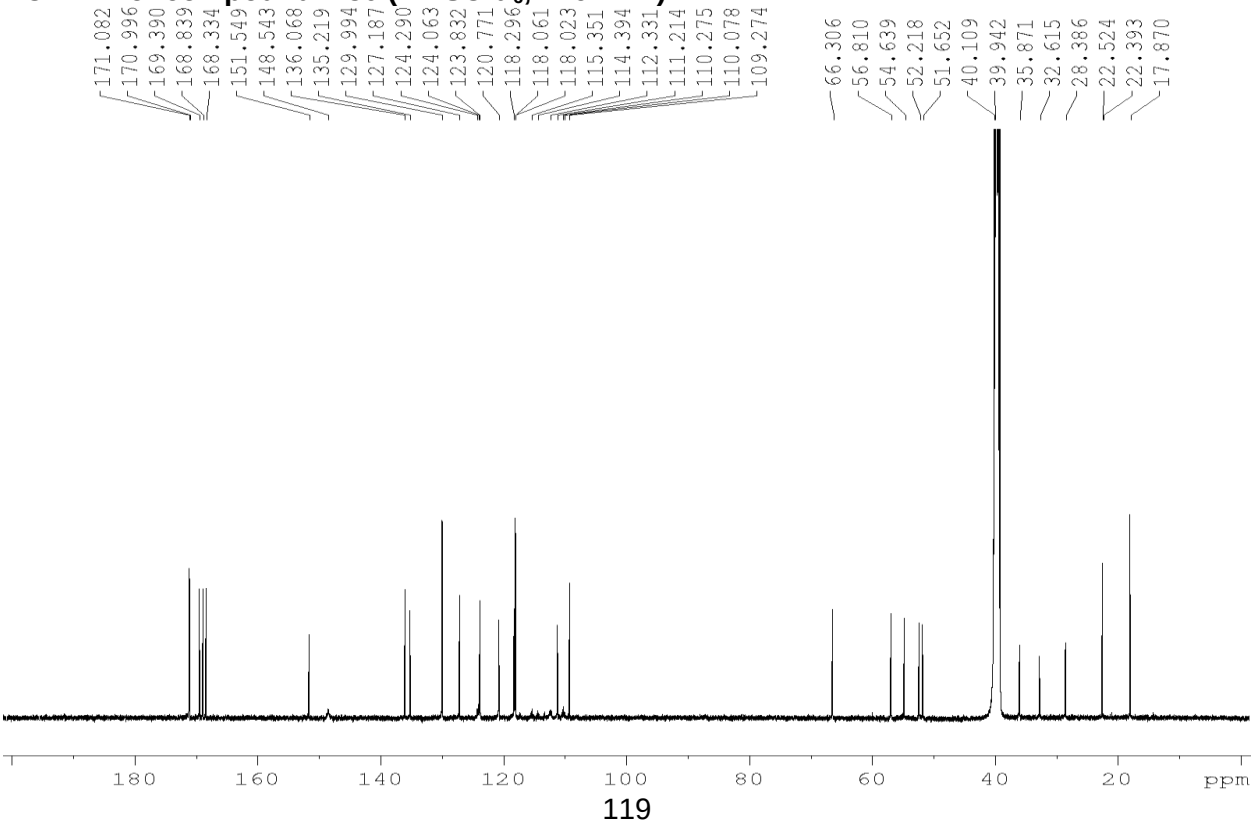
^{13}C NMR of compound 2.29 (DMSO-d_6, 126 MHz)



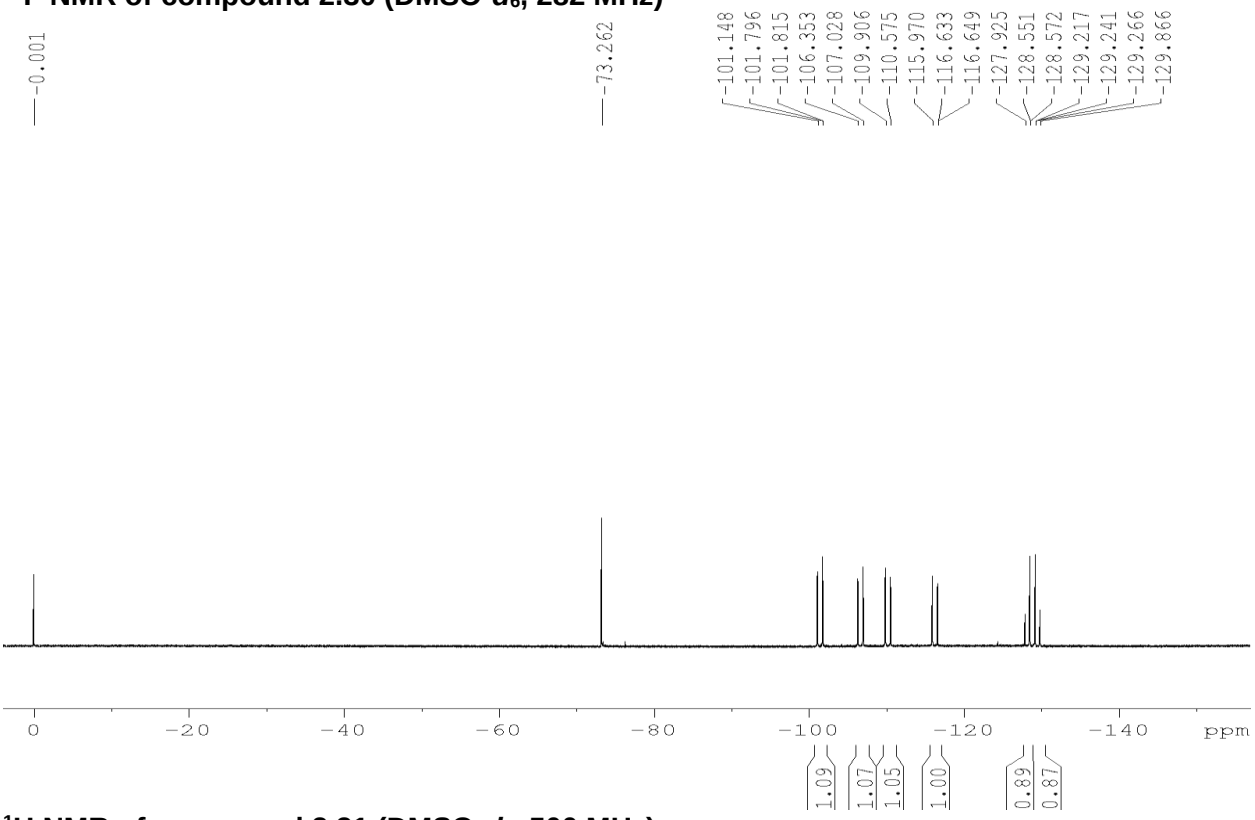
^{1}H NMR of compound 2.30 (DMSO-d_6, 500 MHz)



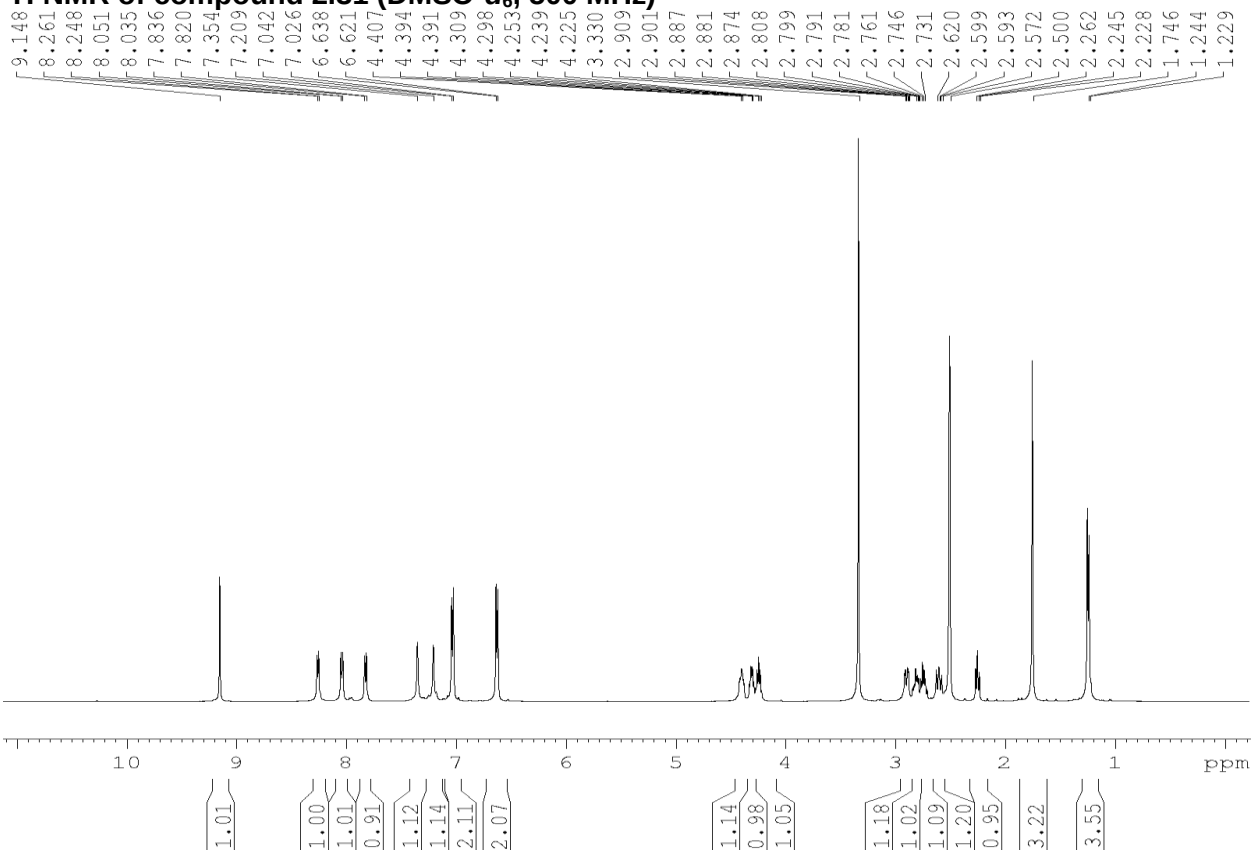
^{13}C NMR of compound 2.30 (DMSO-d_6, 126 MHz)



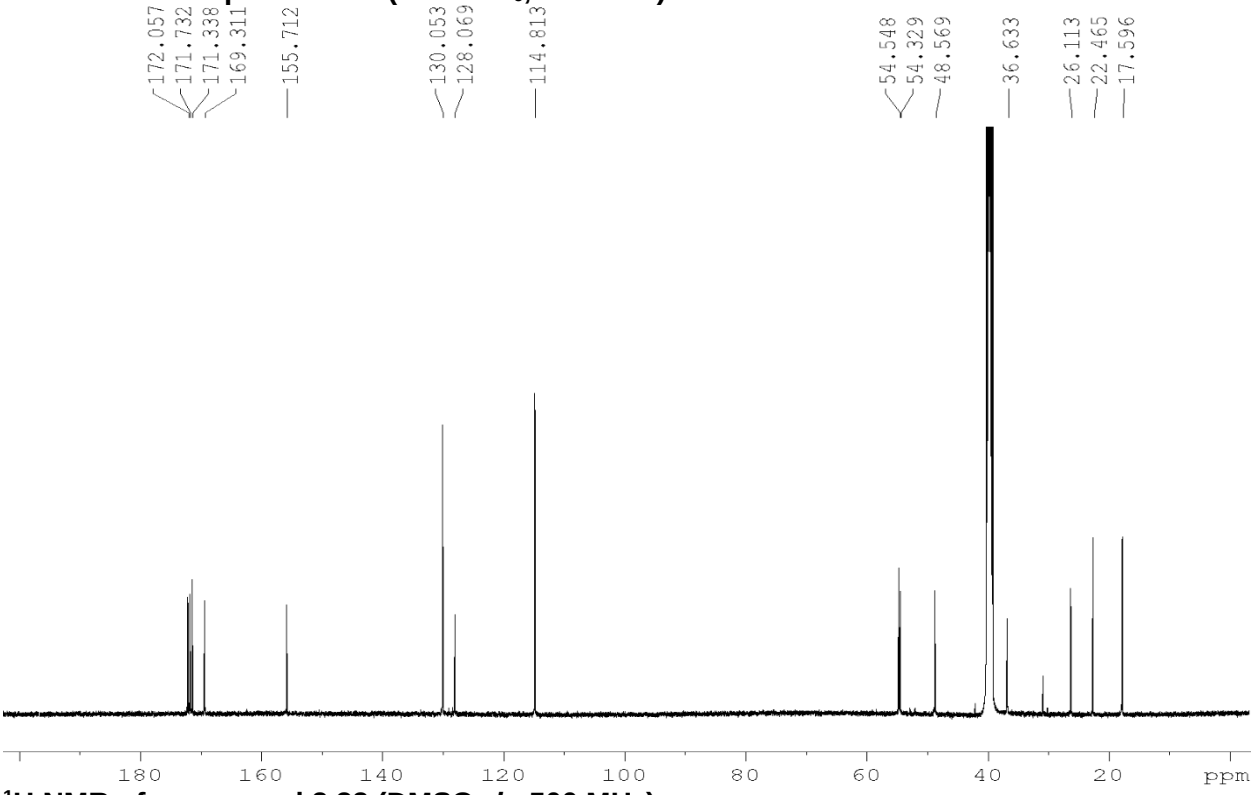
^{19}F NMR of compound 2.30 (DMSO-d_6, 282 MHz)



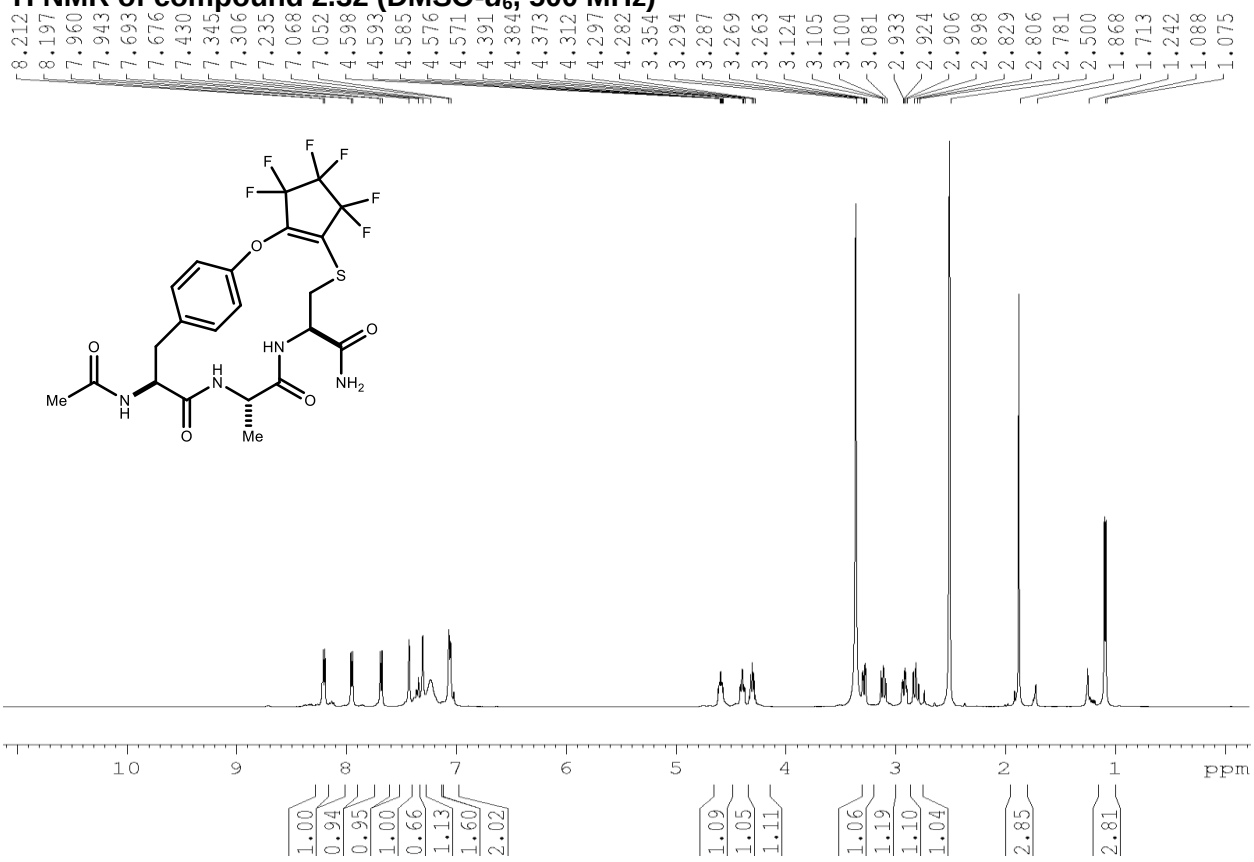
^{1}H NMR of compound 2.31 (DMSO-d_6, 500 MHz)



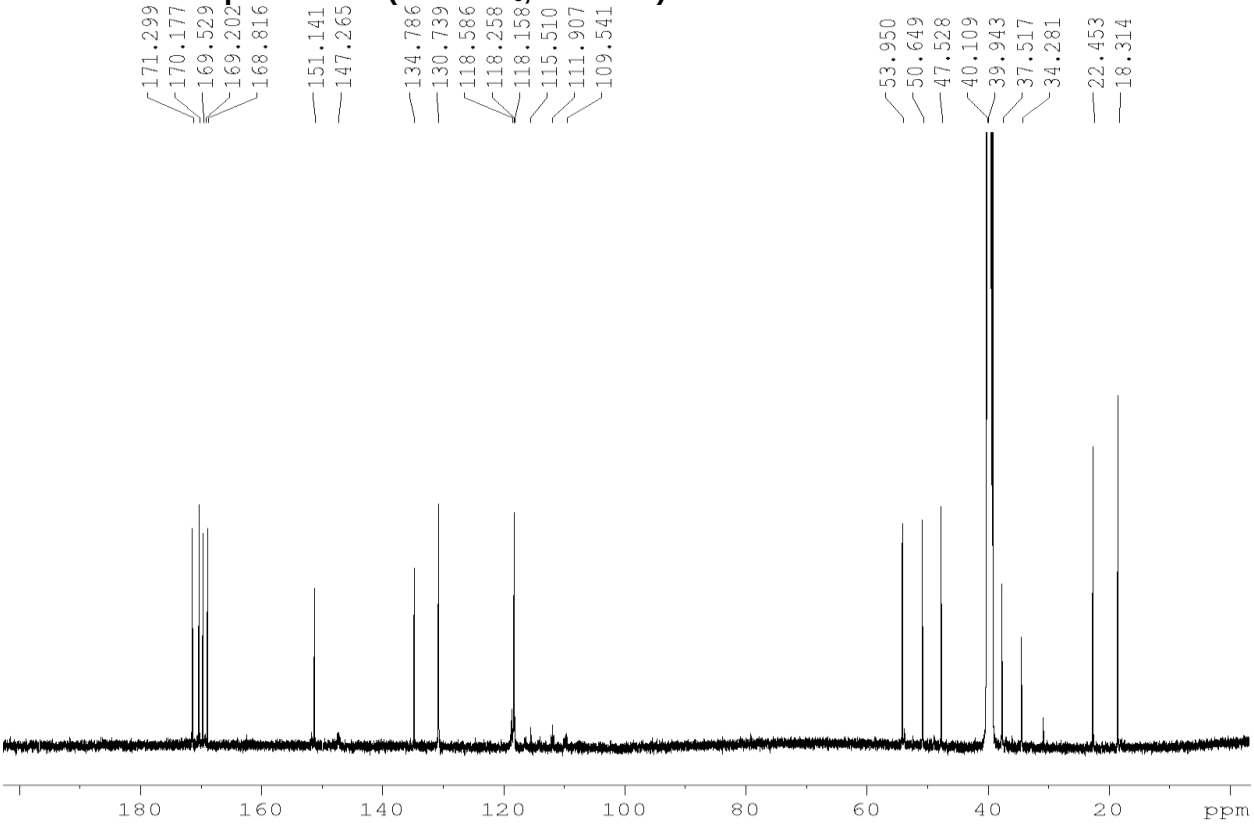
^{13}C NMR of compound 2.31 (DMSO-d_6, 126 MHz)



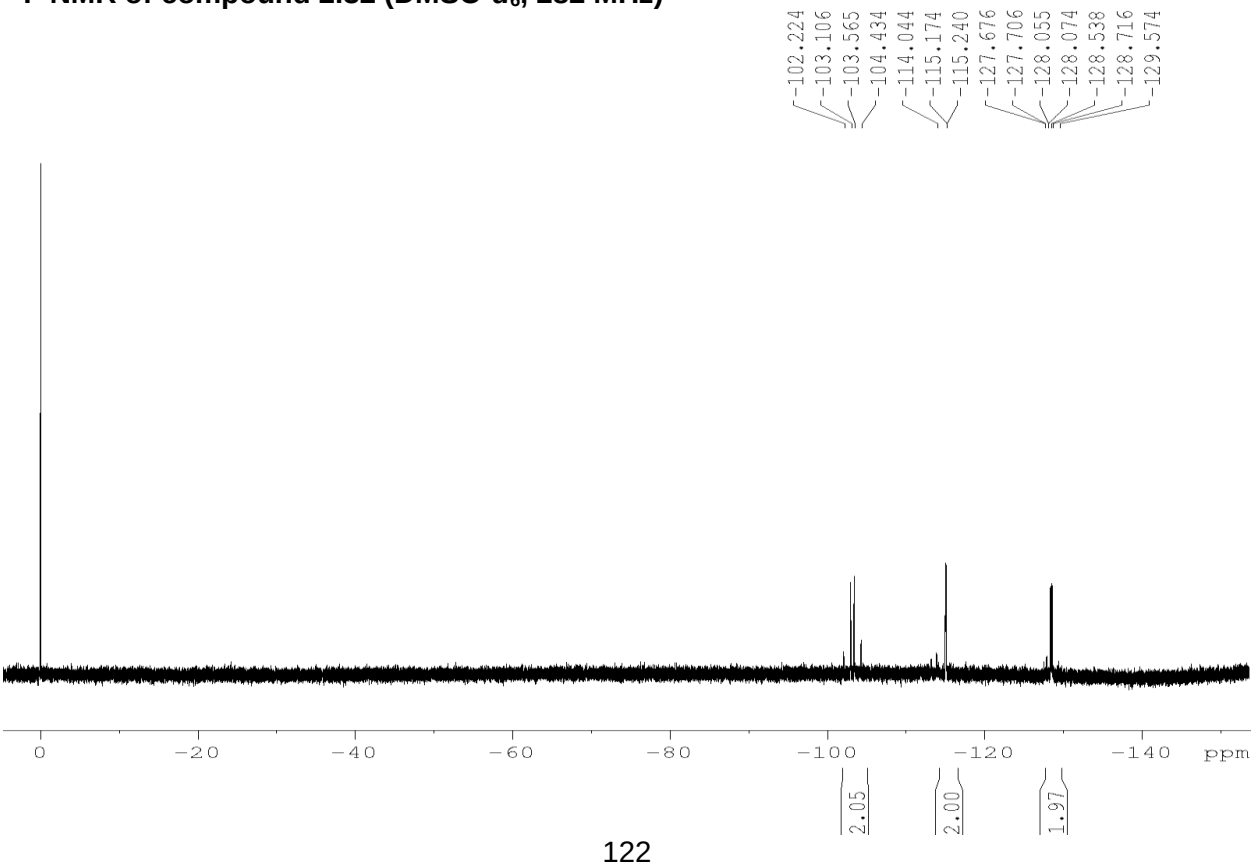
^{1}H NMR of compound 2.32 (DMSO-d_6, 500 MHz)



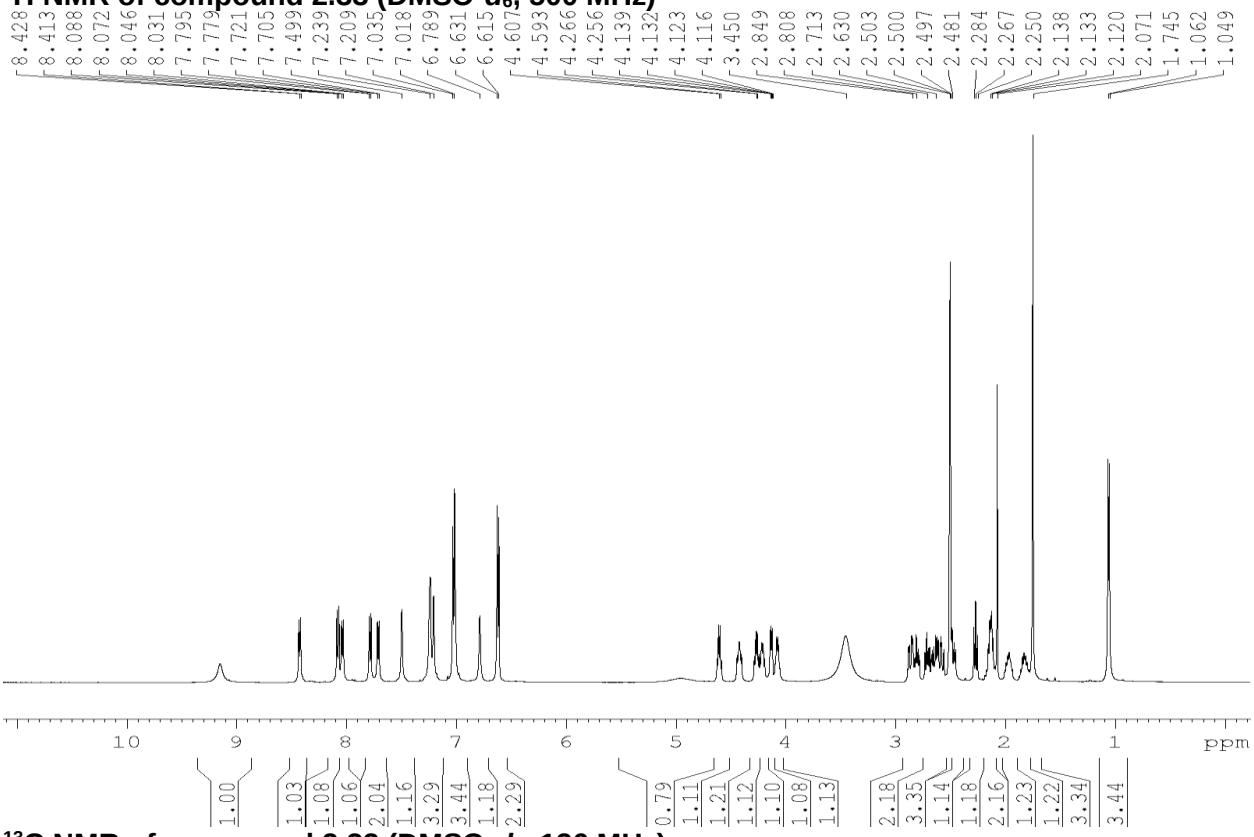
^{13}C NMR of compound 2.32 (DMSO-d_6, 126 MHz)



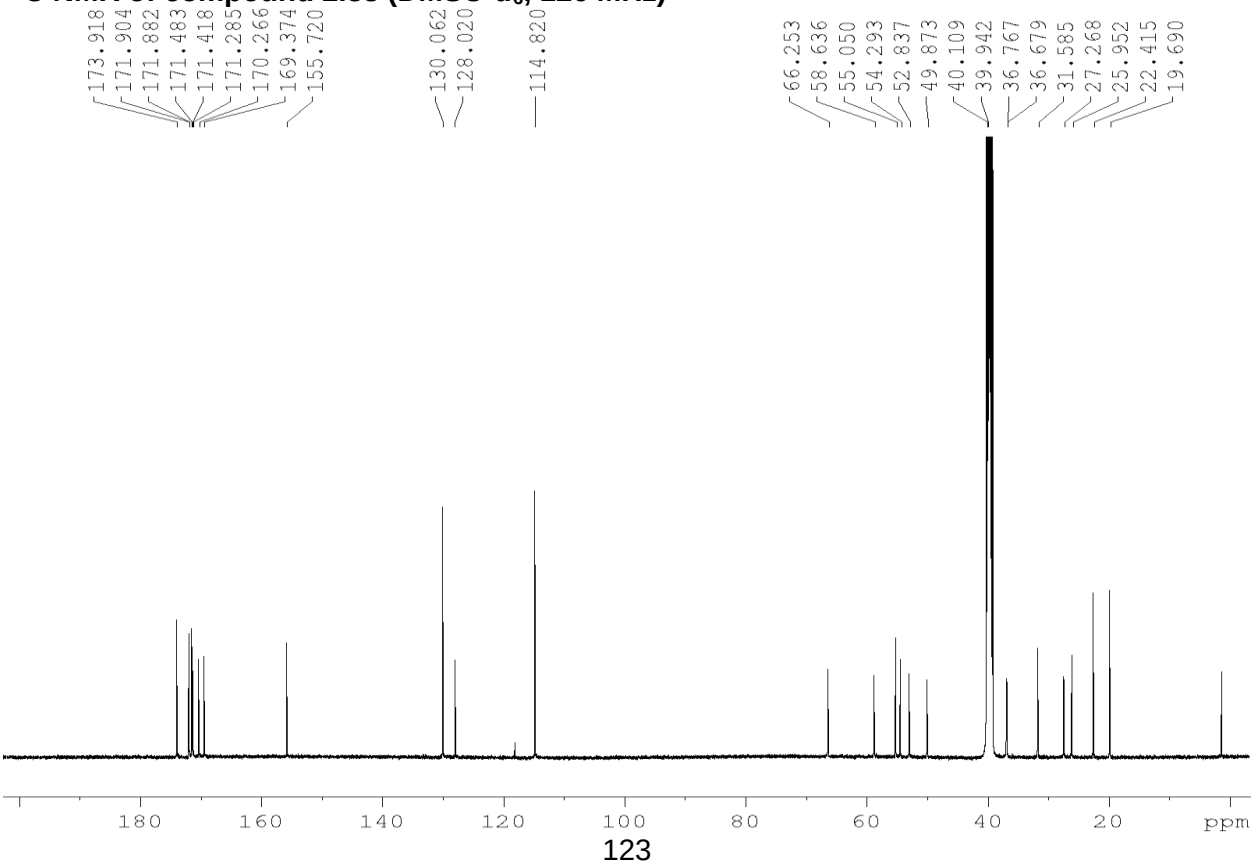
^{19}F NMR of compound 2.32 (DMSO-d_6, 282 MHz)



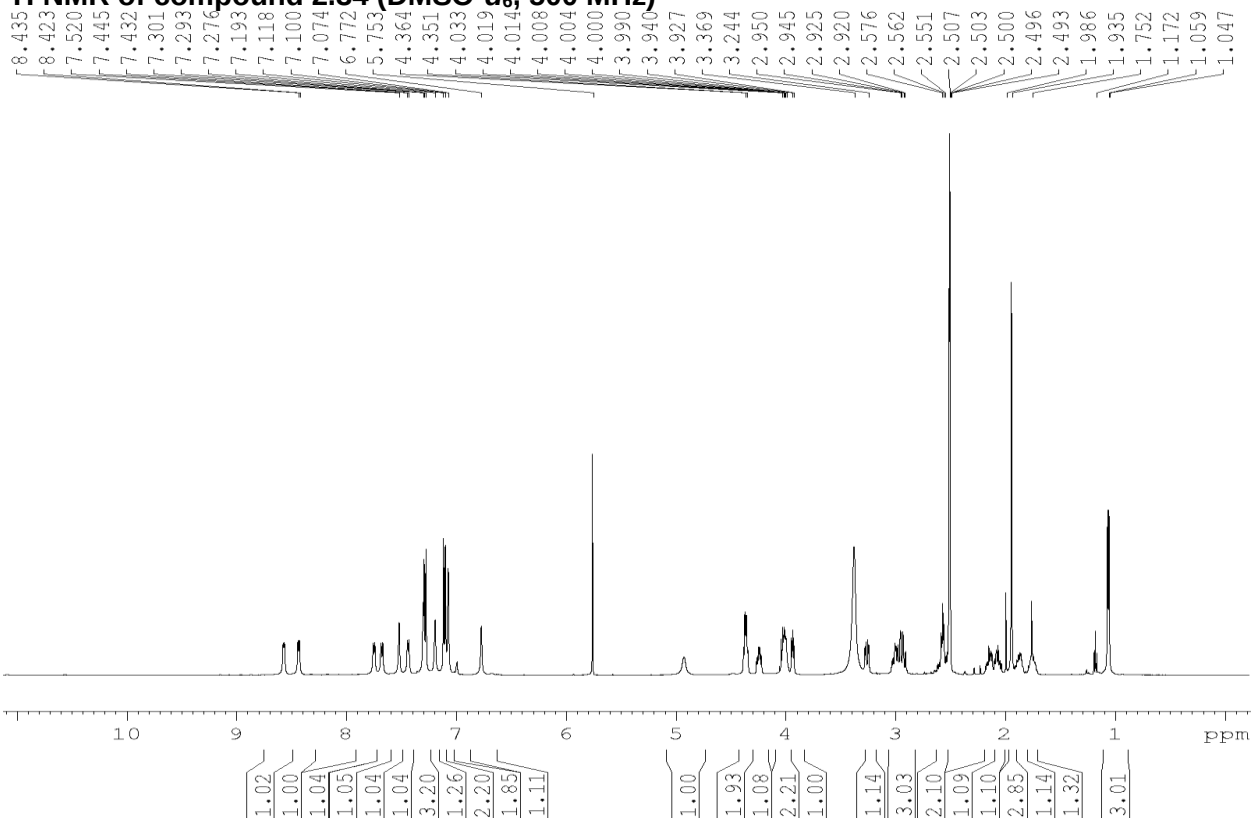
^{1}H NMR of compound 2.33 (DMSO-d_6, 500 MHz)



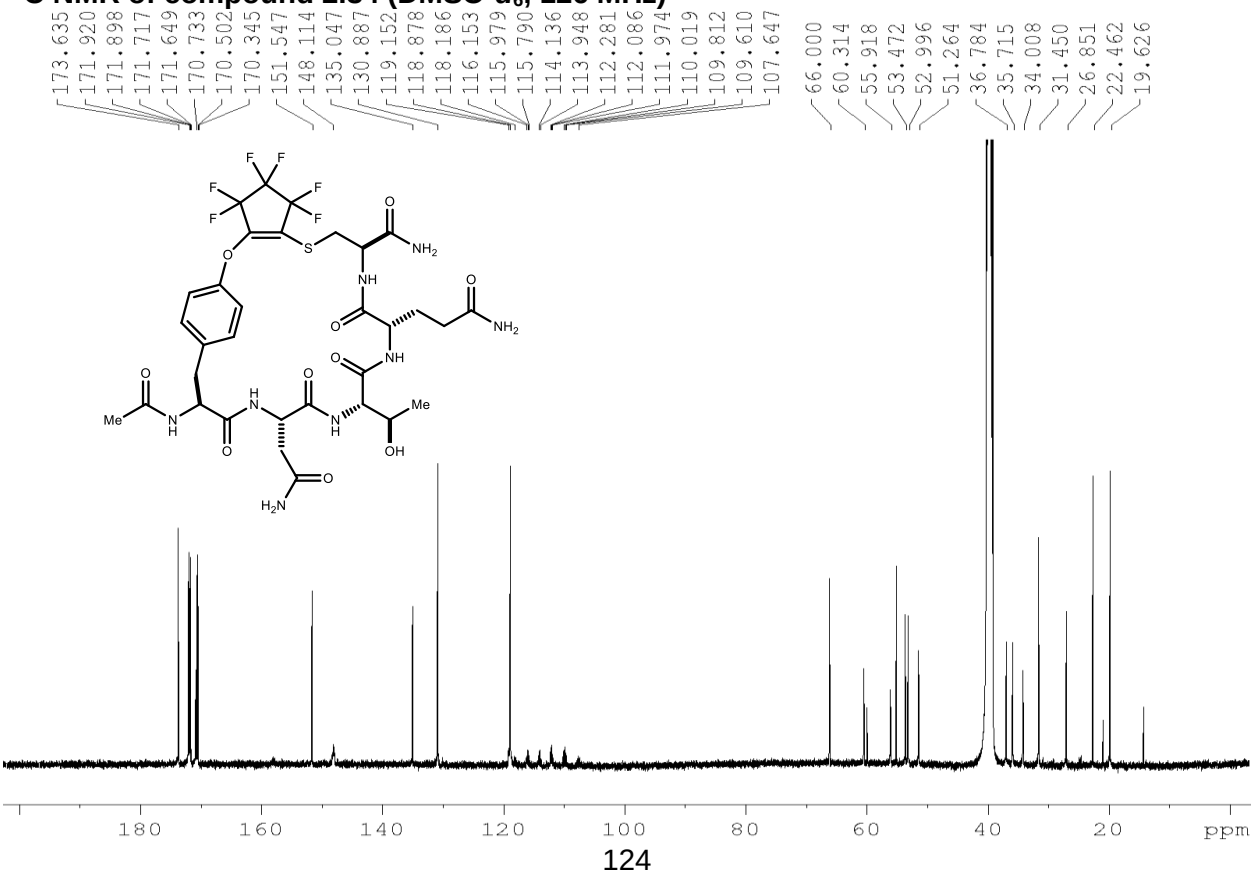
^{13}C NMR of compound 2.33 (DMSO-d_6, 126 MHz)



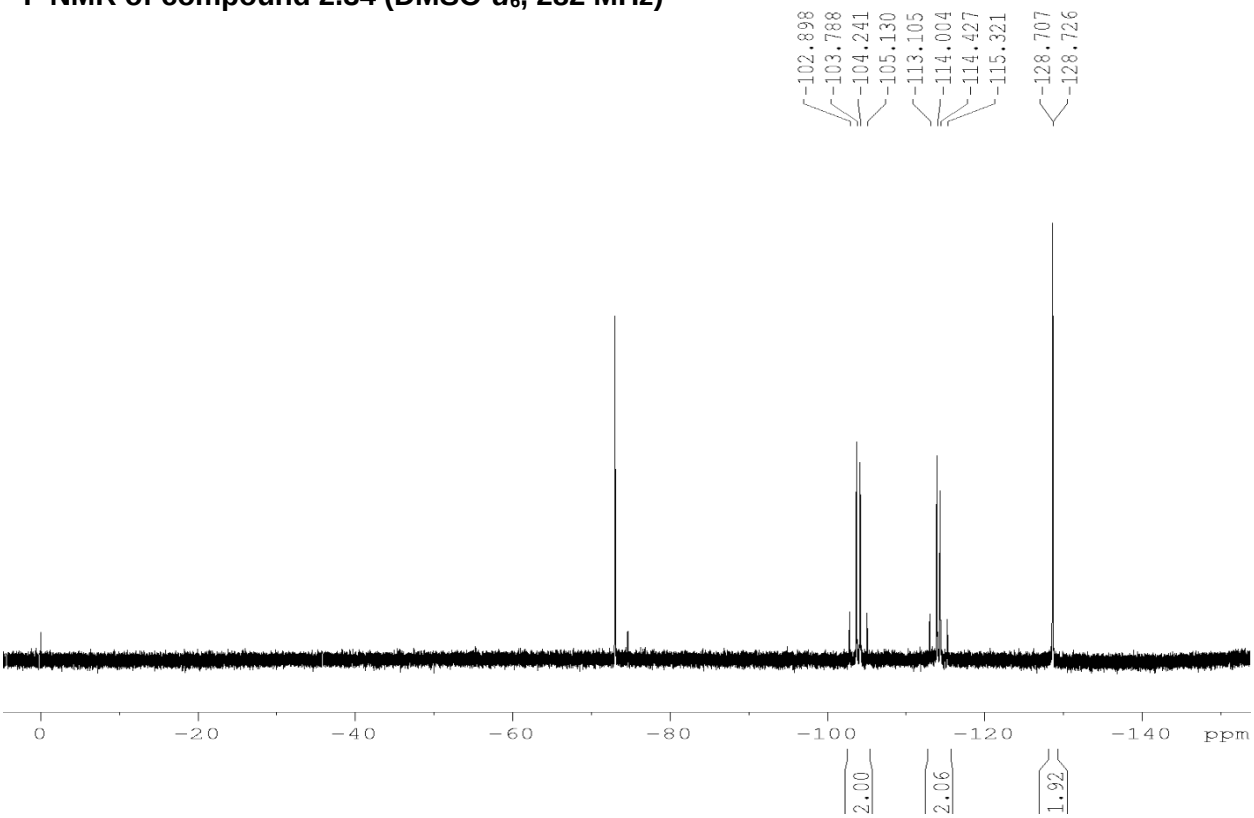
^{1}H NMR of compound 2.34 (DMSO-d_6, 500 MHz)



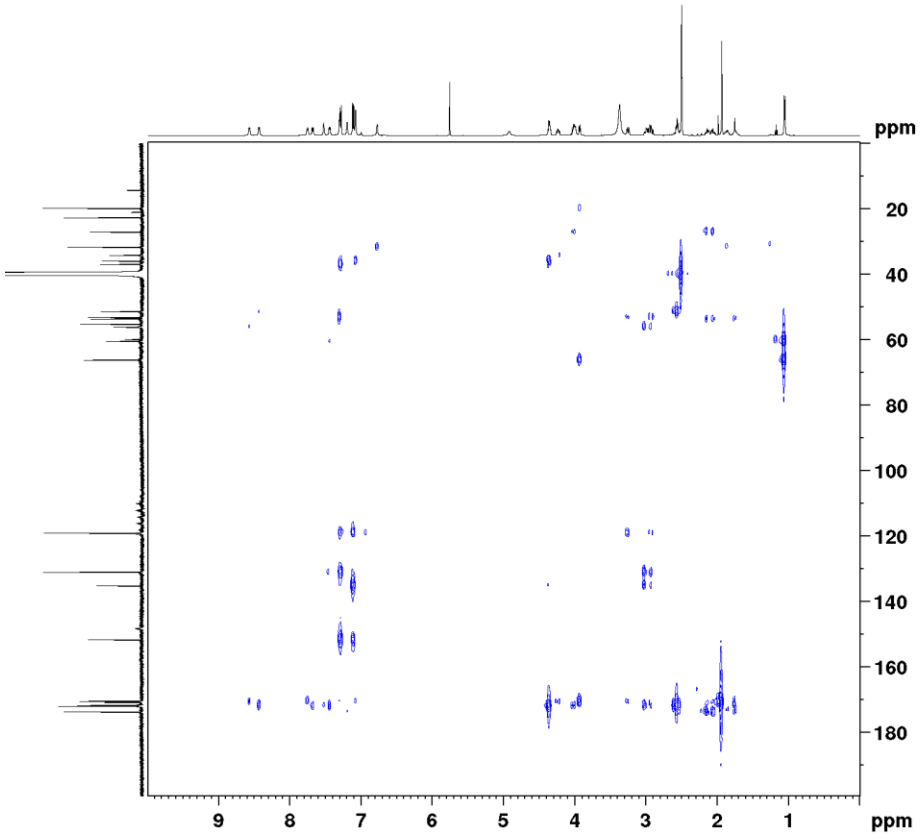
^{13}C NMR of compound 2.34 (DMSO-d_6, 126 MHz)



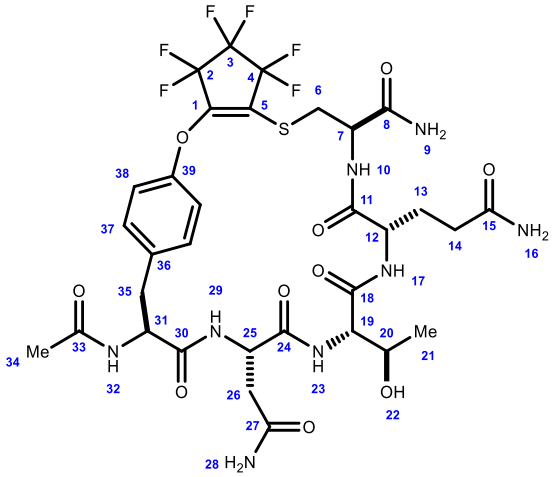
^{19}F NMR of compound 2.34 (DMSO-d_6, 282 MHz)



HMBC spectrum of compound 2.34 (DMSO-d_6)

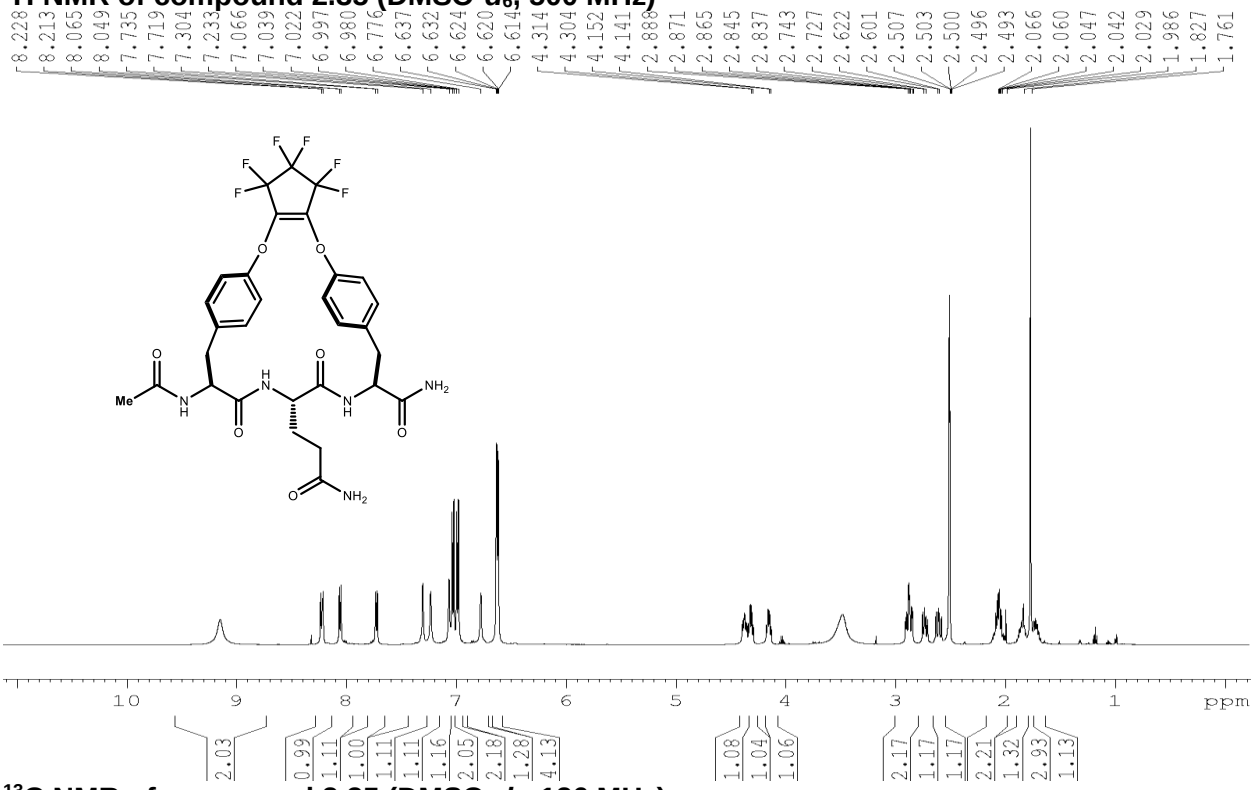


Compound 2.34 (DMSO-d_6)

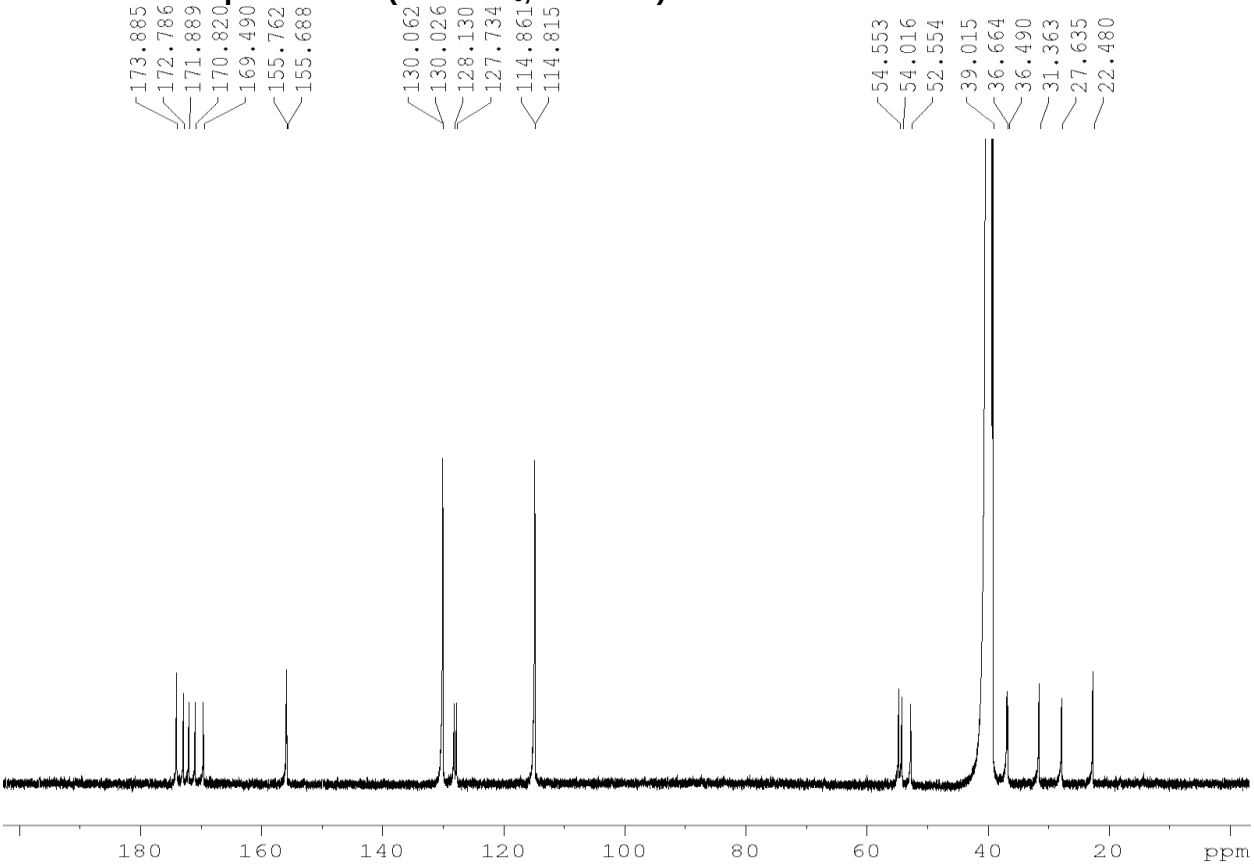


	13C	1H	Key Correlation
1	148.5–147.9 (m, 1C)	–	
2	118.6–106.7 (m, 3C)	–	
3	118.6–106.7 (m, 3C)	–	
4	118.6–106.7 (m, 3C)	–	
5	119.2–118.6 (m, 1C)	–	HMBC 6 -> 5
6	34.0	3.25 (dd, J = 12.7 4.3 Hz, 1H), 3.02–2.89 (m, 1H) overlap	HMBC 6 -> 5; HMBC 7 -> 6, HSQC
7	53.0	4.27–4.21 (m, 1H)	HMBC 6 -> 7; HMBC 7 -> 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

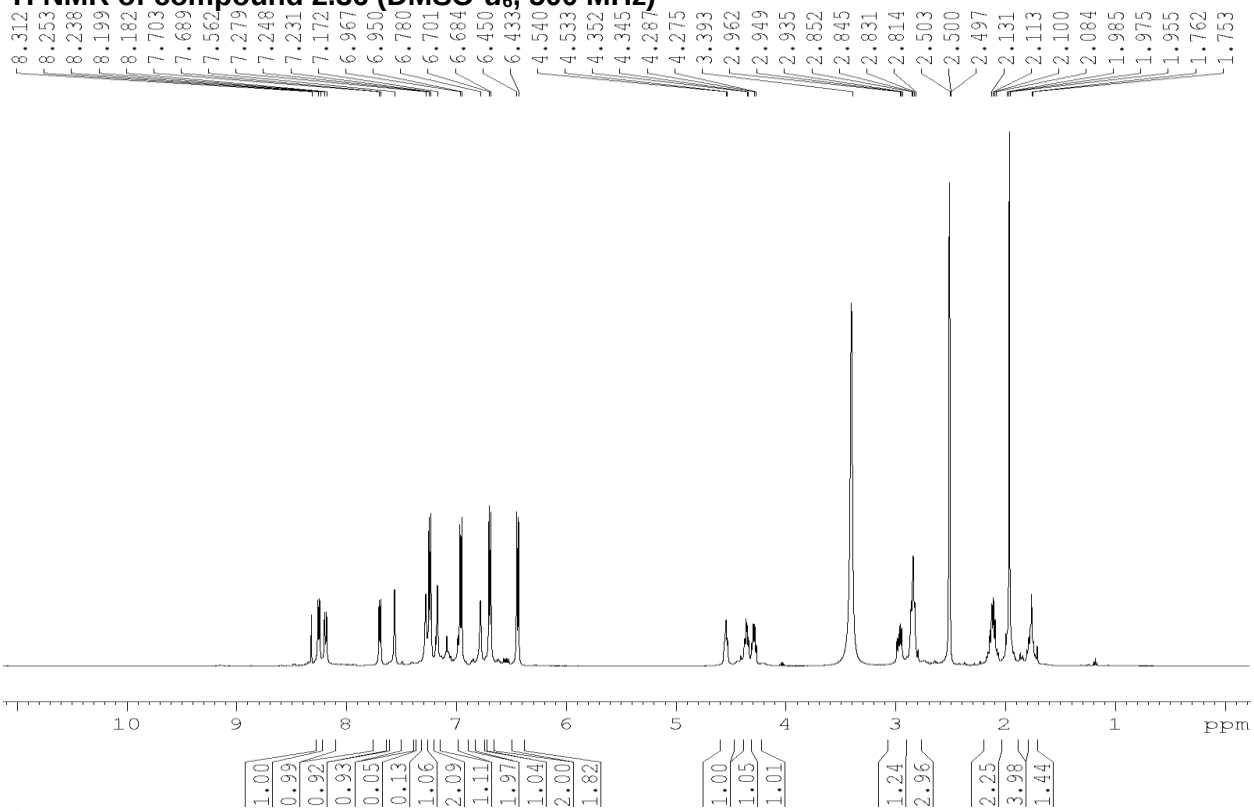
^{1}H NMR of compound 2.35 (DMSO-d_6, 500 MHz)



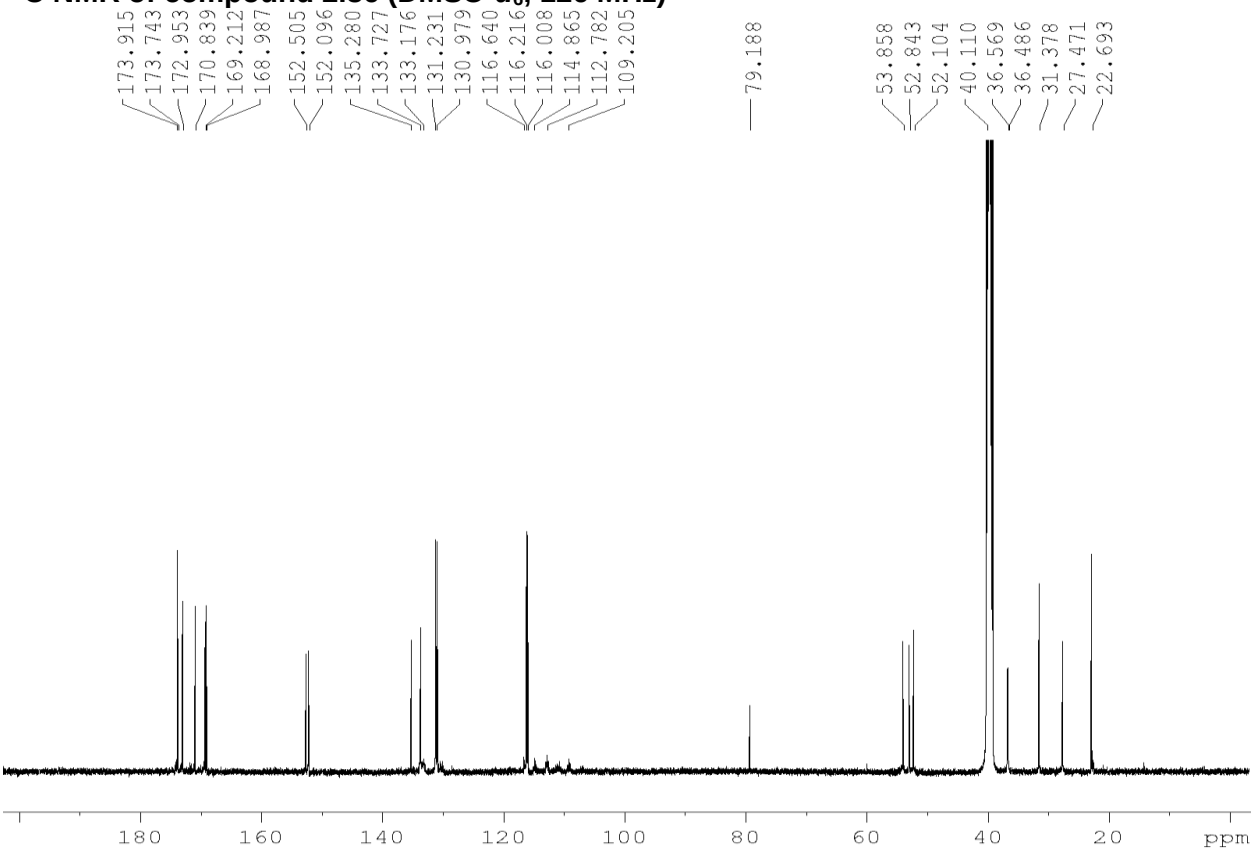
^{13}C NMR of compound 2.35 (DMSO-d_6, 126 MHz)



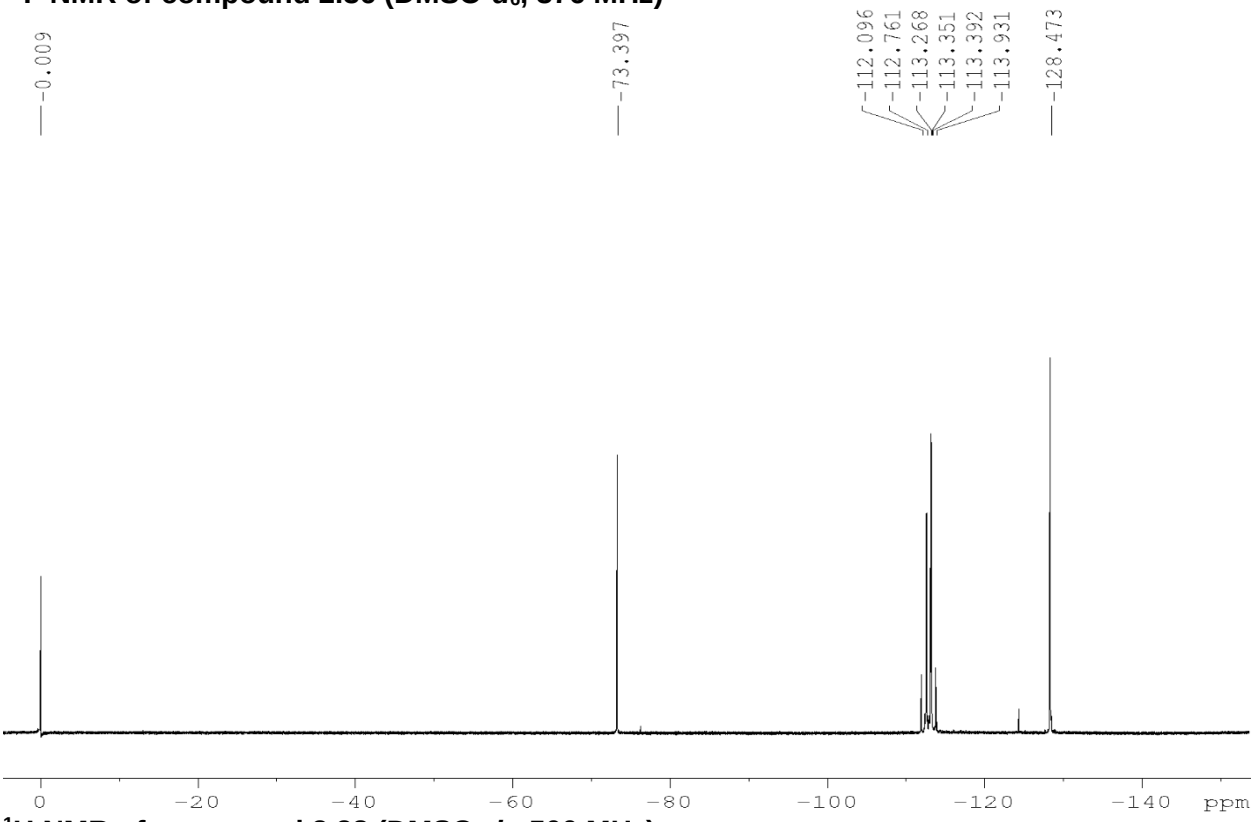
^{1}H NMR of compound 2.36 (DMSO-d_6, 500 MHz)



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

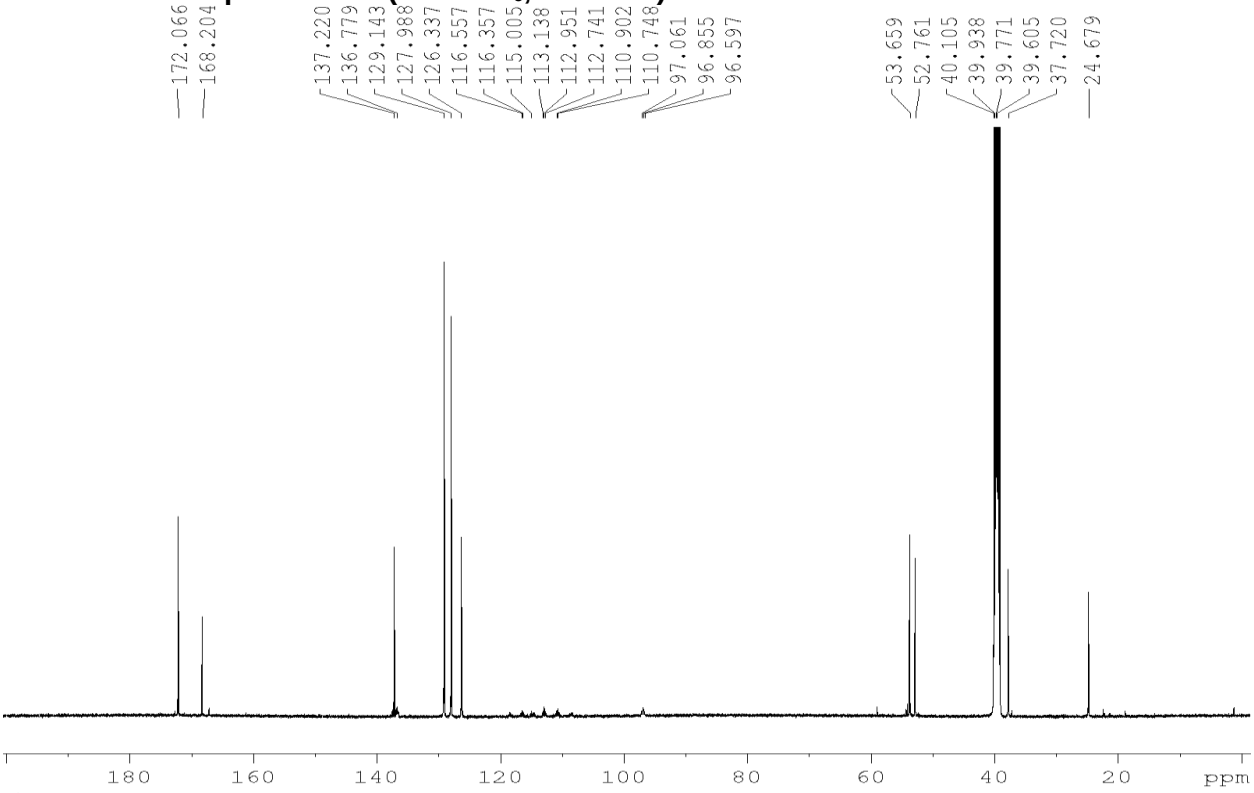


^{19}F NMR of compound 2.36 (DMSO-d_6, 376 MHz)

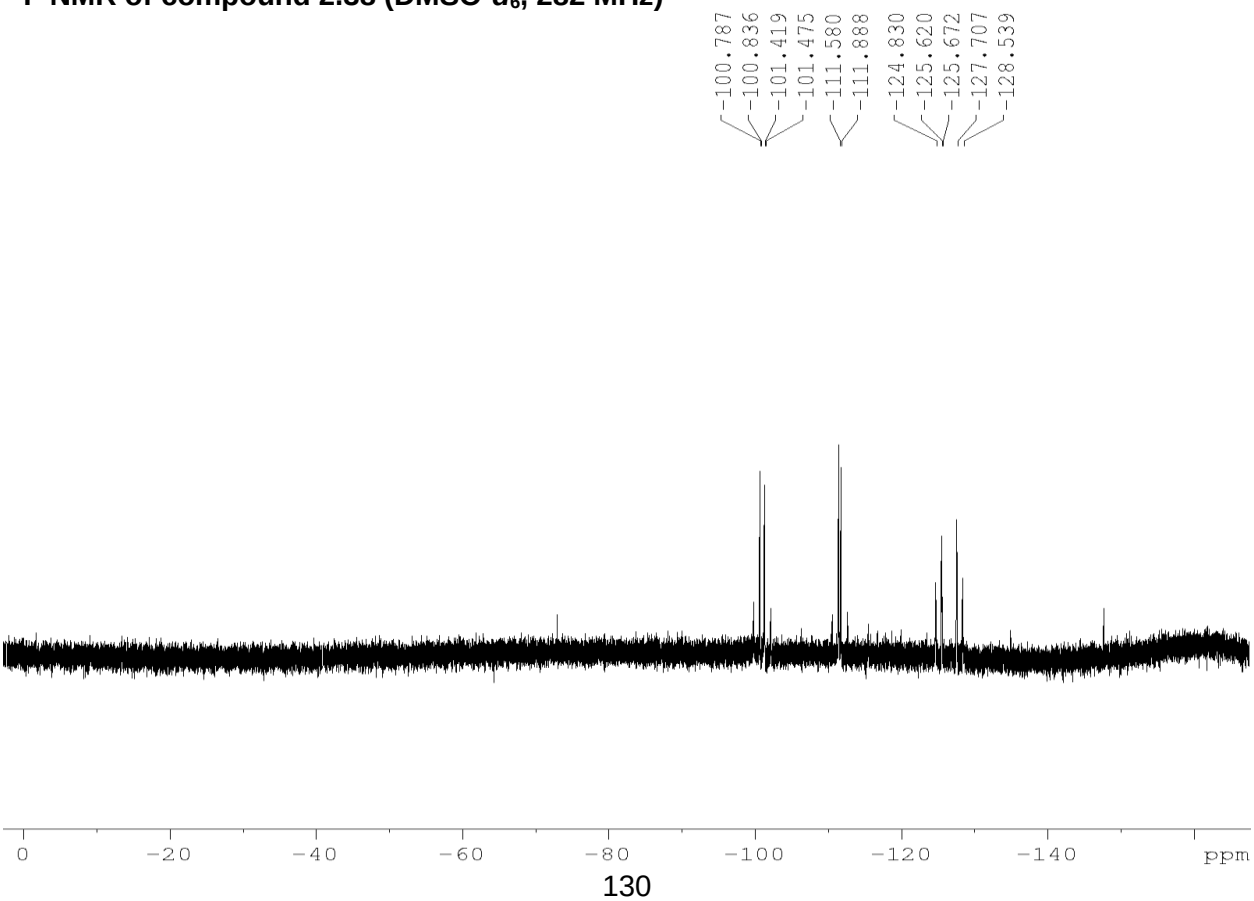


^{1}H NMR of compound 2.38 (DMSO-d_6, 500 MHz)

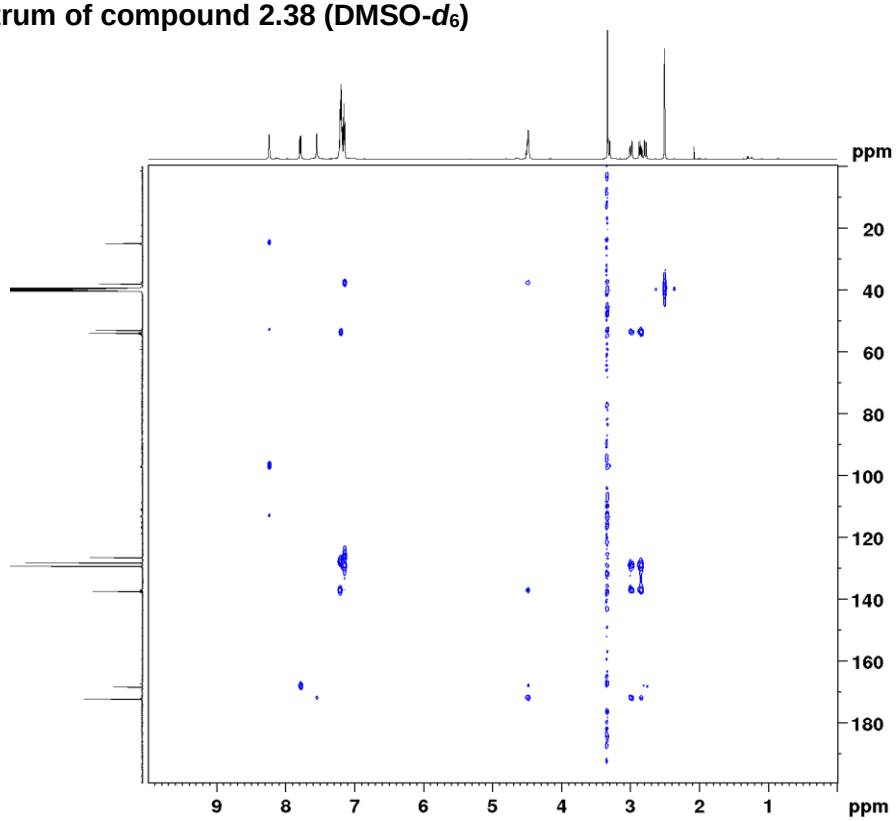
^{13}C NMR of compound 2.38 (DMSO-d_6, 126 MHz)



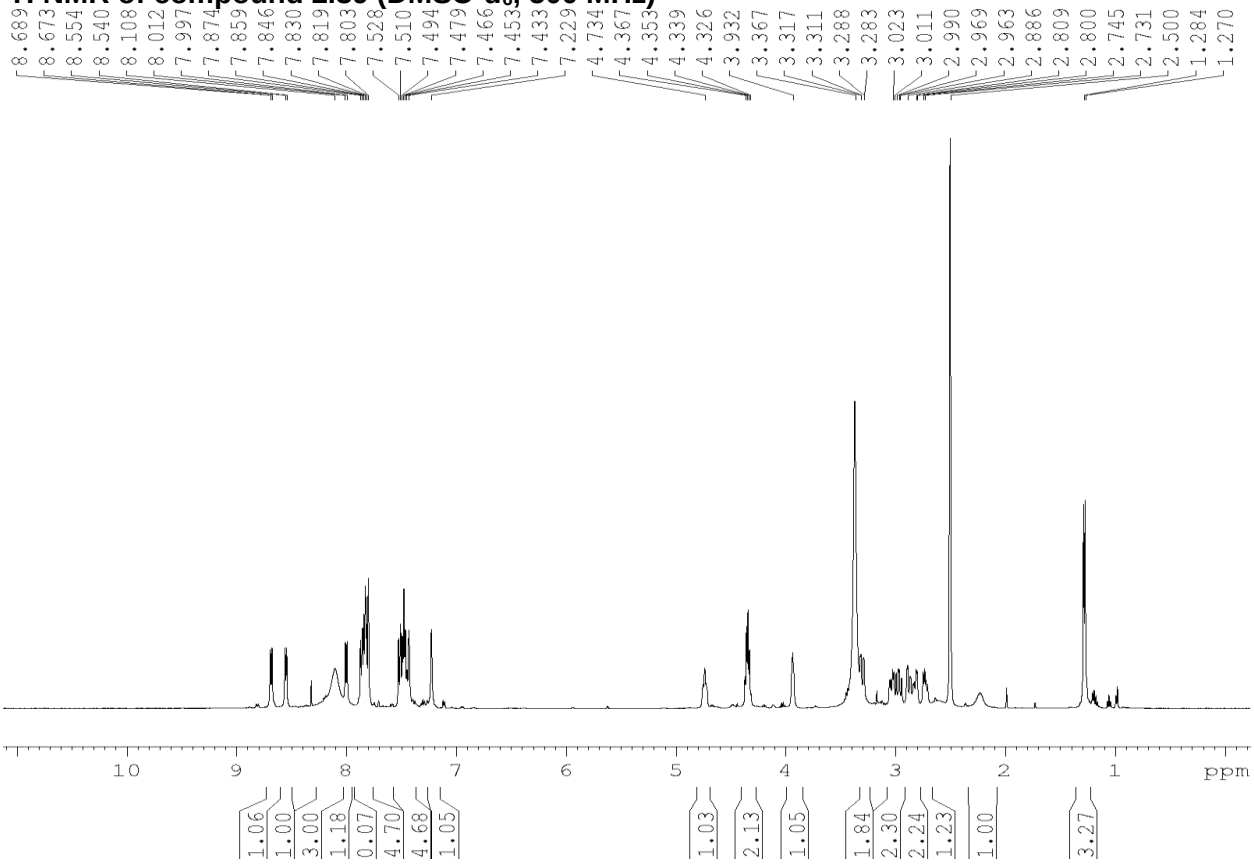
^{19}F NMR of compound 2.38 (DMSO-d_6, 282 MHz)



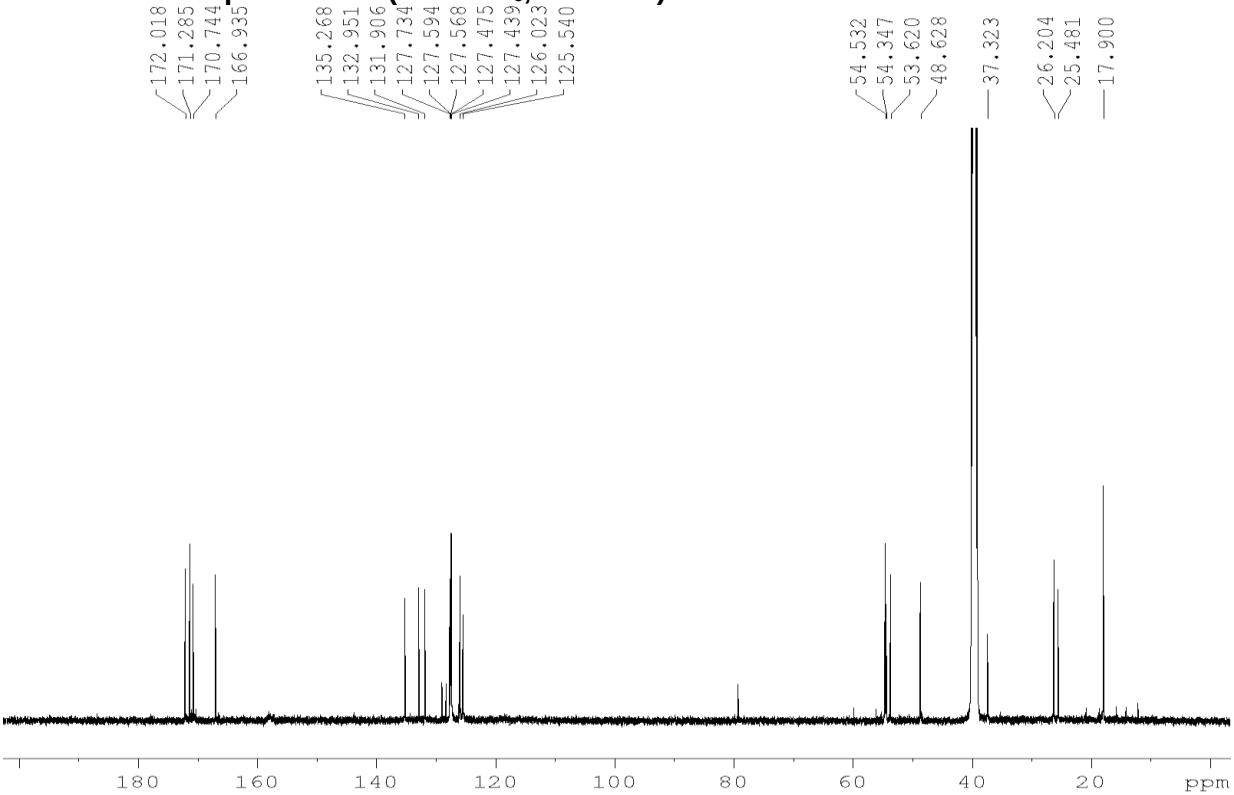
HMBC spectrum of compound 2.38 (DMSO-d_6)



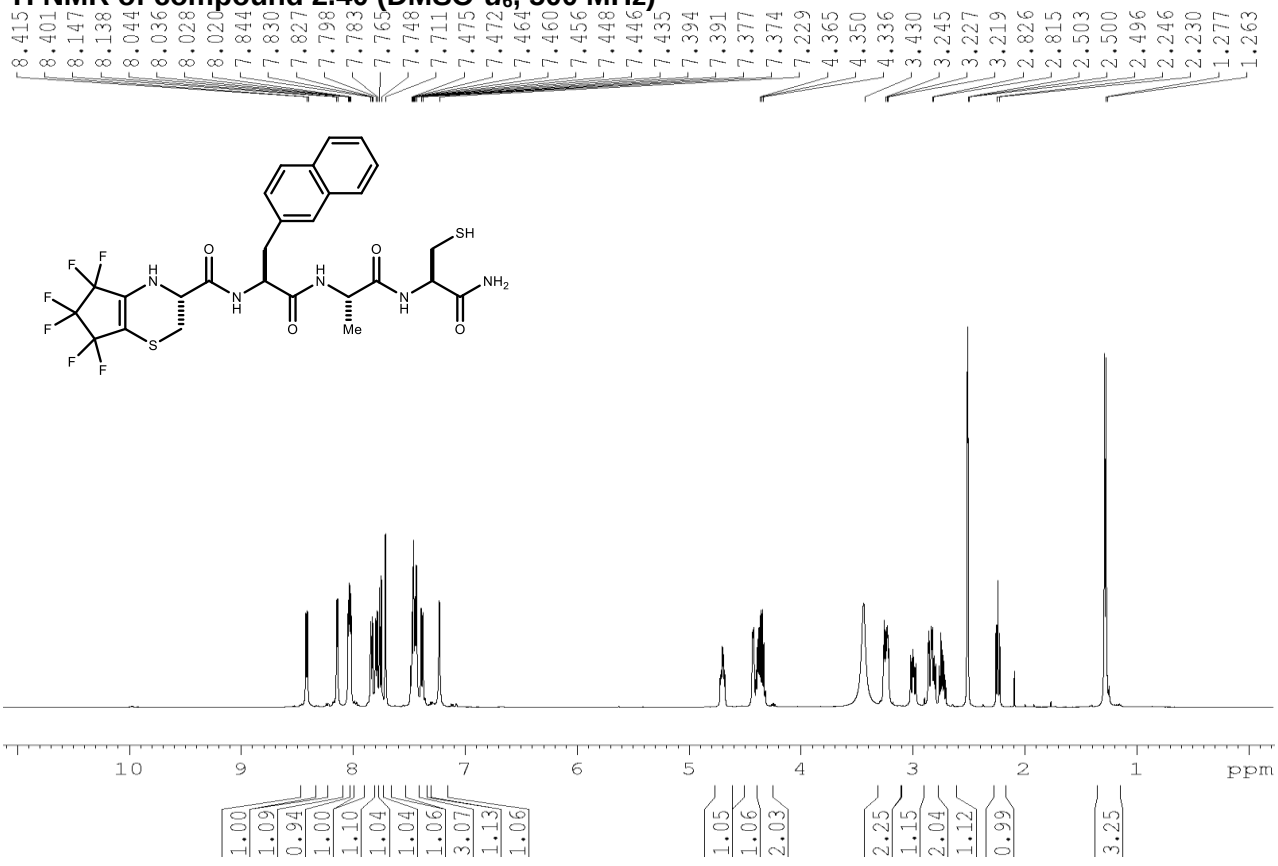
^{1}H NMR of compound 2.39 (DMSO-d_6, 500 MHz)



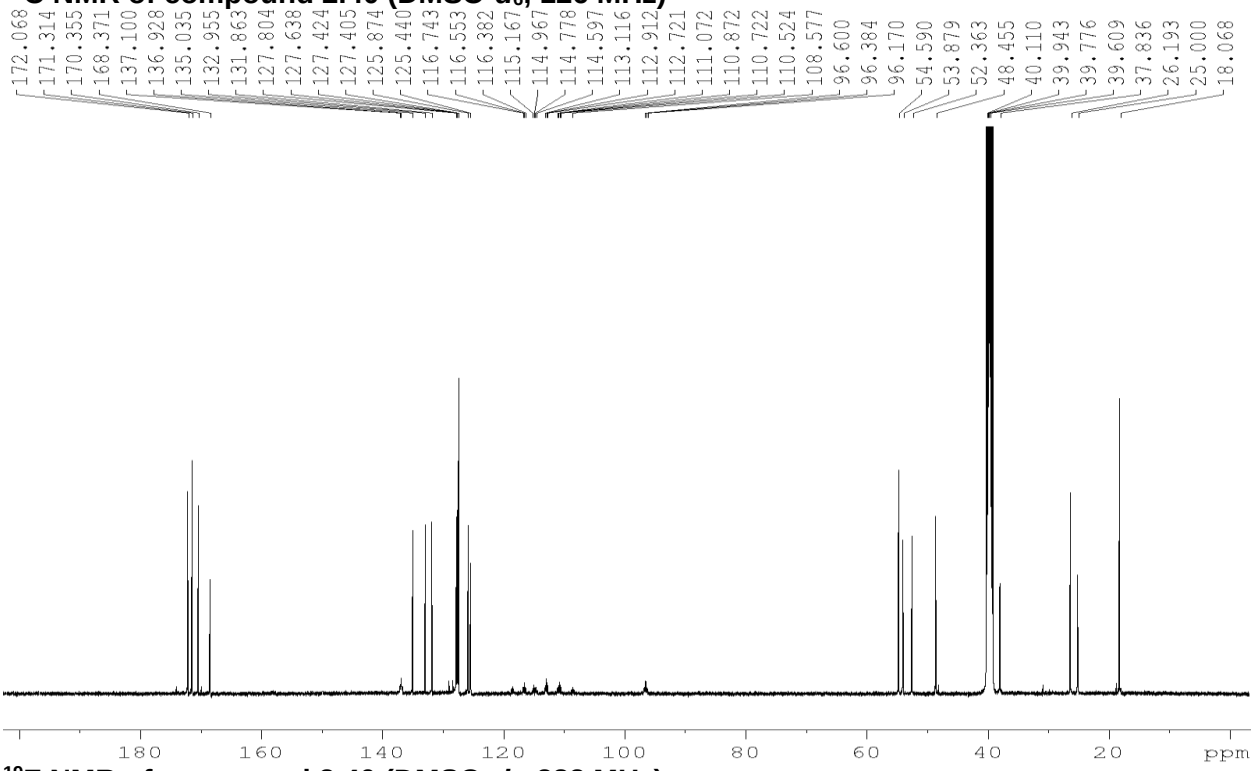
^{13}C NMR of compound 2.39 (DMSO-d_6, 126 MHz)



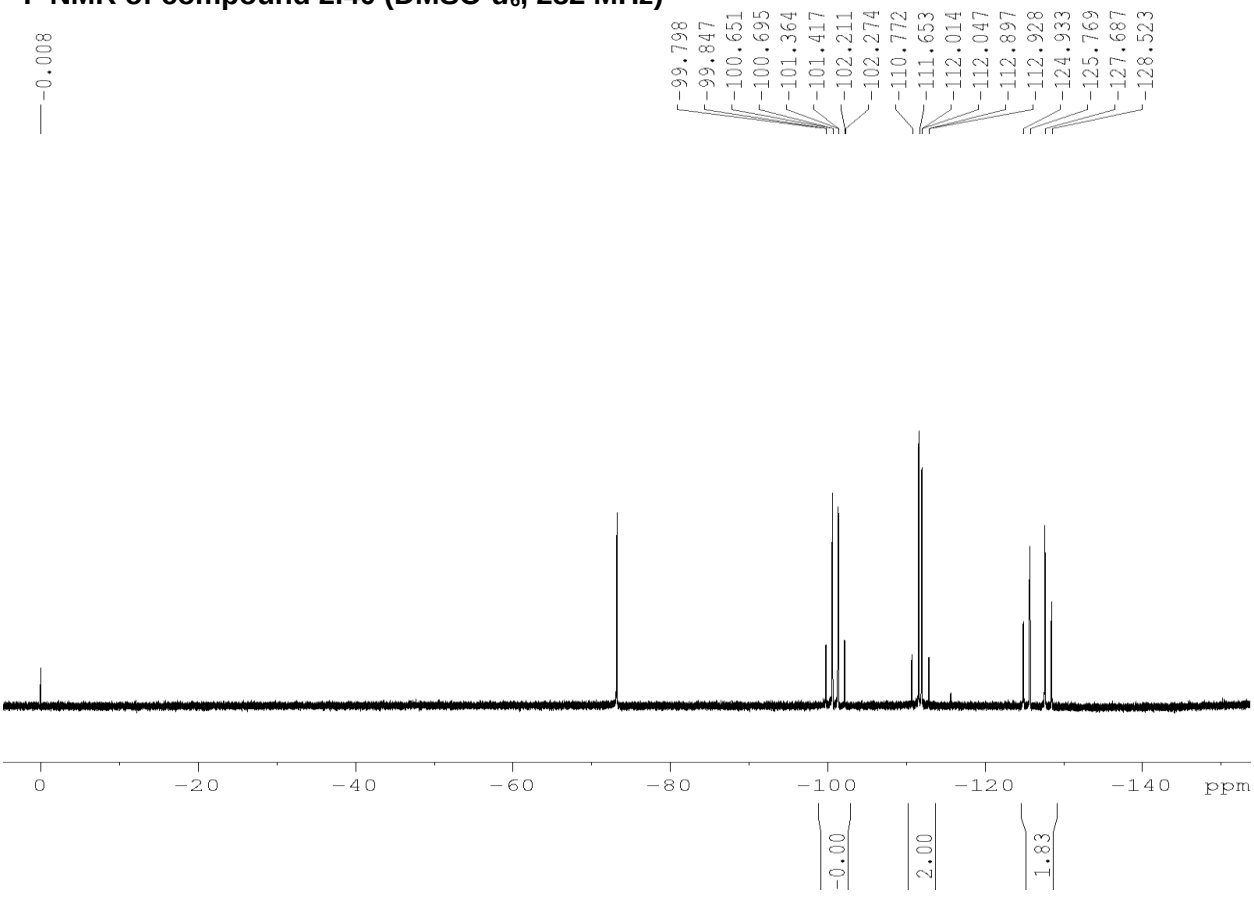
^{1}H NMR of compound 2.40 (DMSO-d_6, 500 MHz)



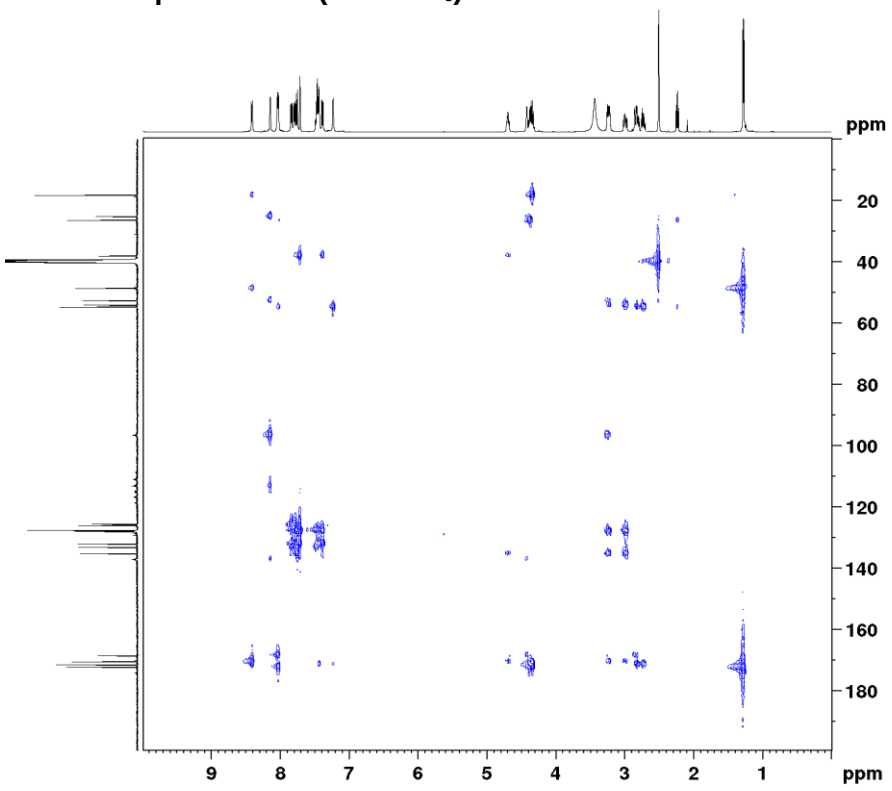
^{13}C NMR of compound 2.40 (DMSO-d_6, 126 MHz)



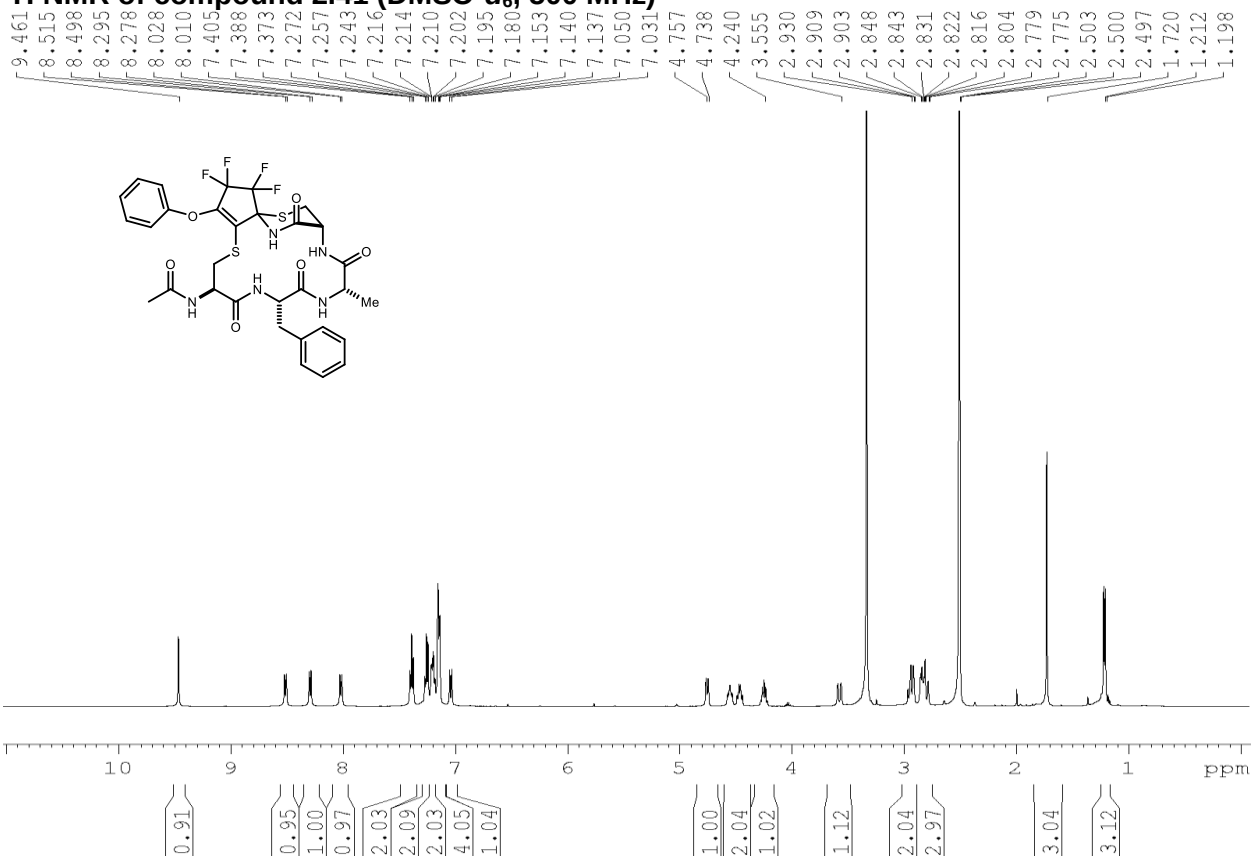
^{19}F NMR of compound 2.40 (DMSO-d_6, 282 MHz)



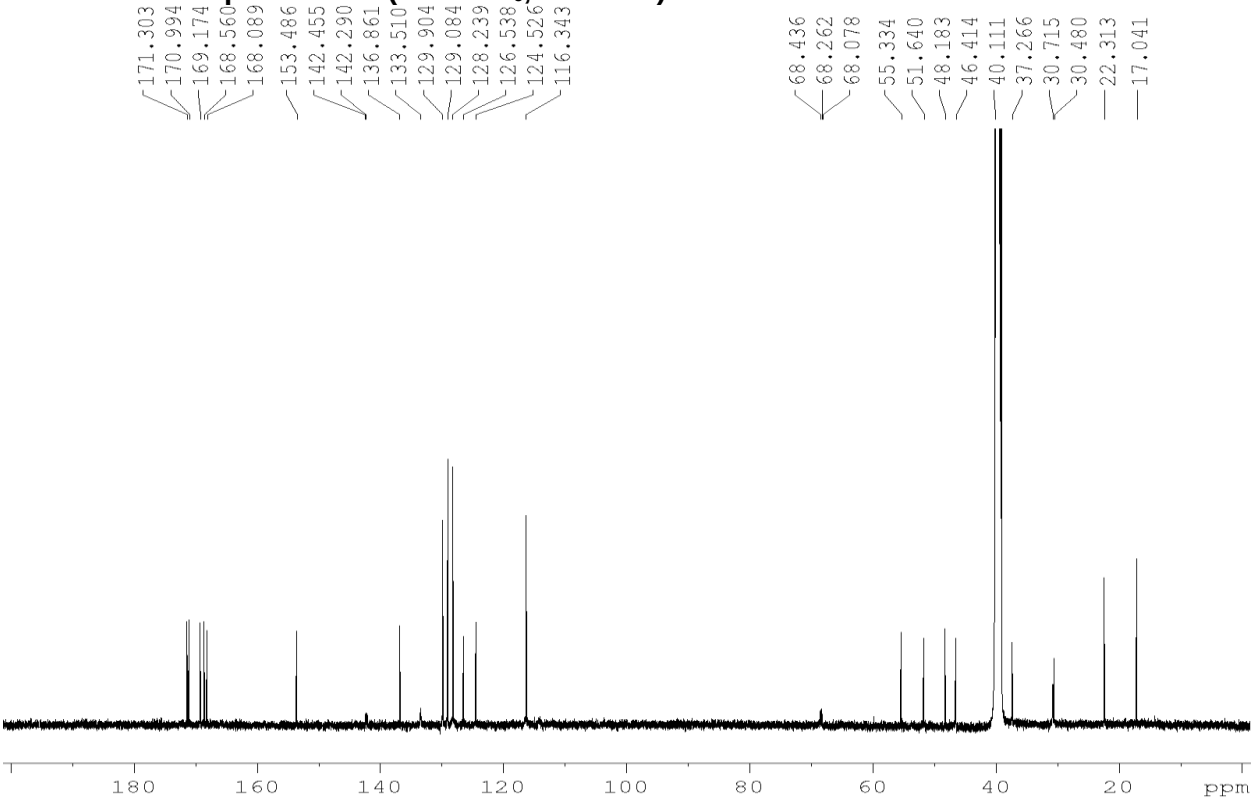
HMBC spectrum of compound 2.40 (DMSO-d_6)



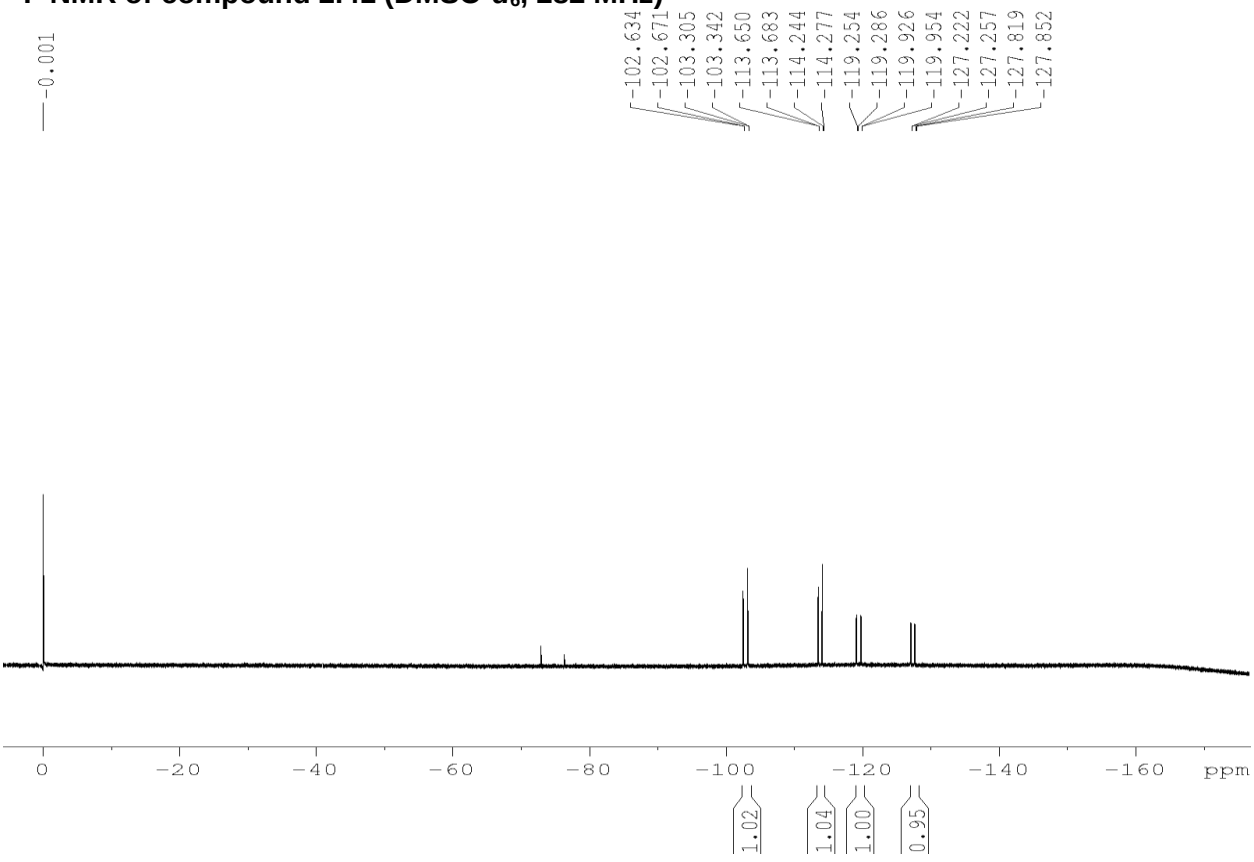
^{1}H NMR of compound 2.41 (DMSO-d_6, 500 MHz)



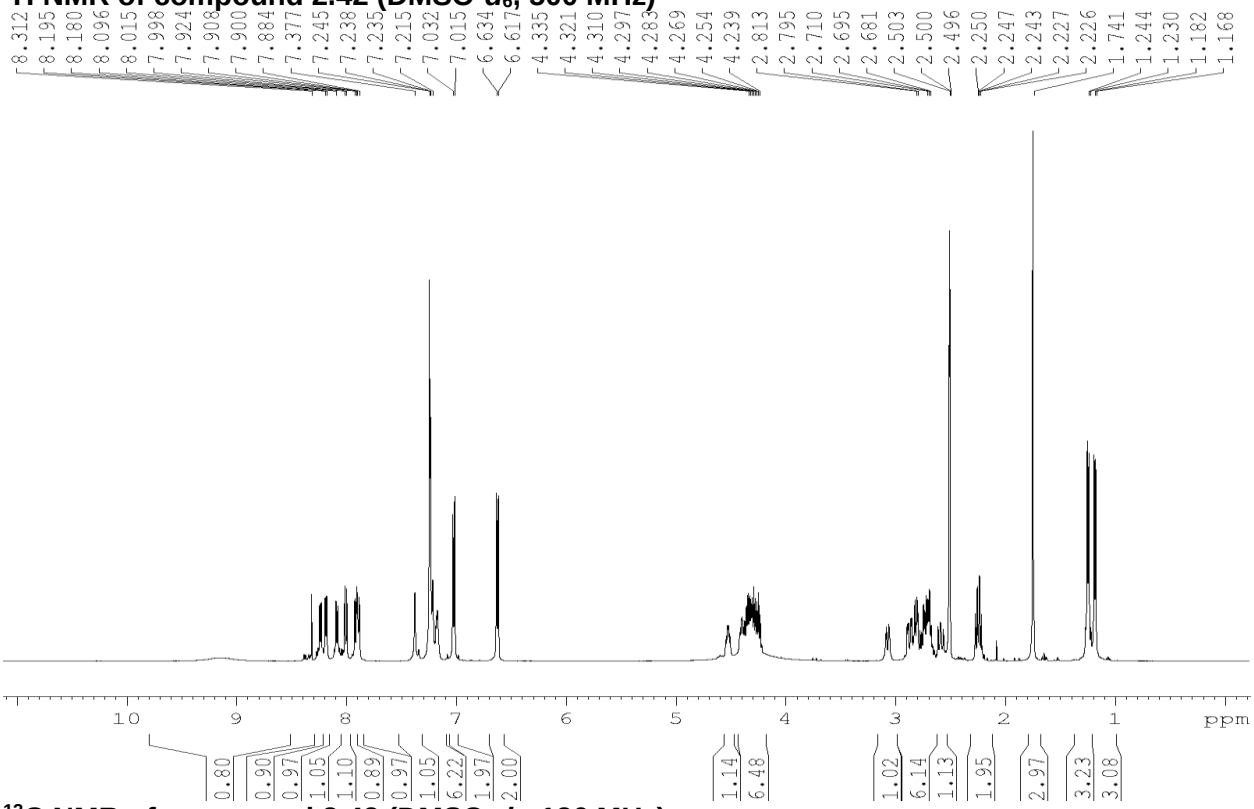
^{13}C NMR of compound 2.41 (DMSO-d_6, 126 MHz)



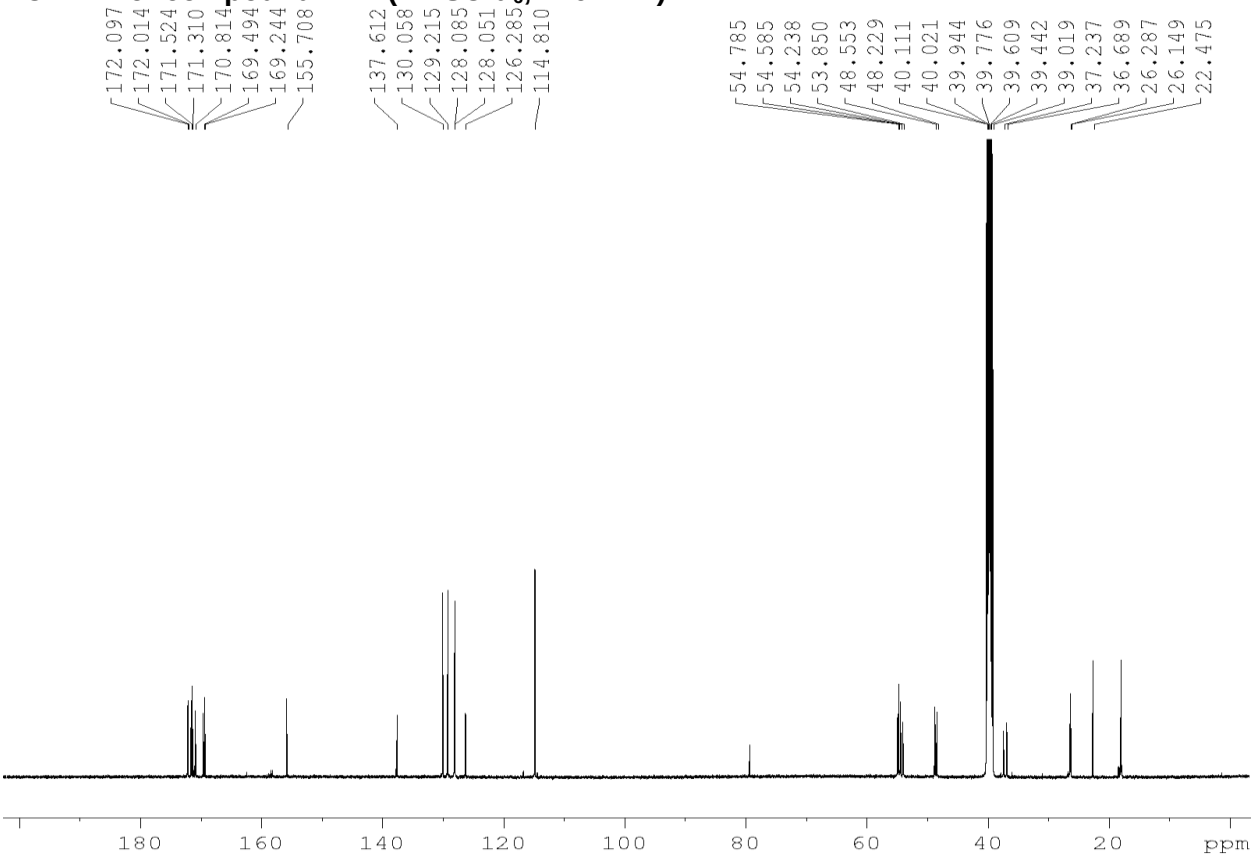
^{19}F NMR of compound 2.41 (DMSO-d_6, 282 MHz)



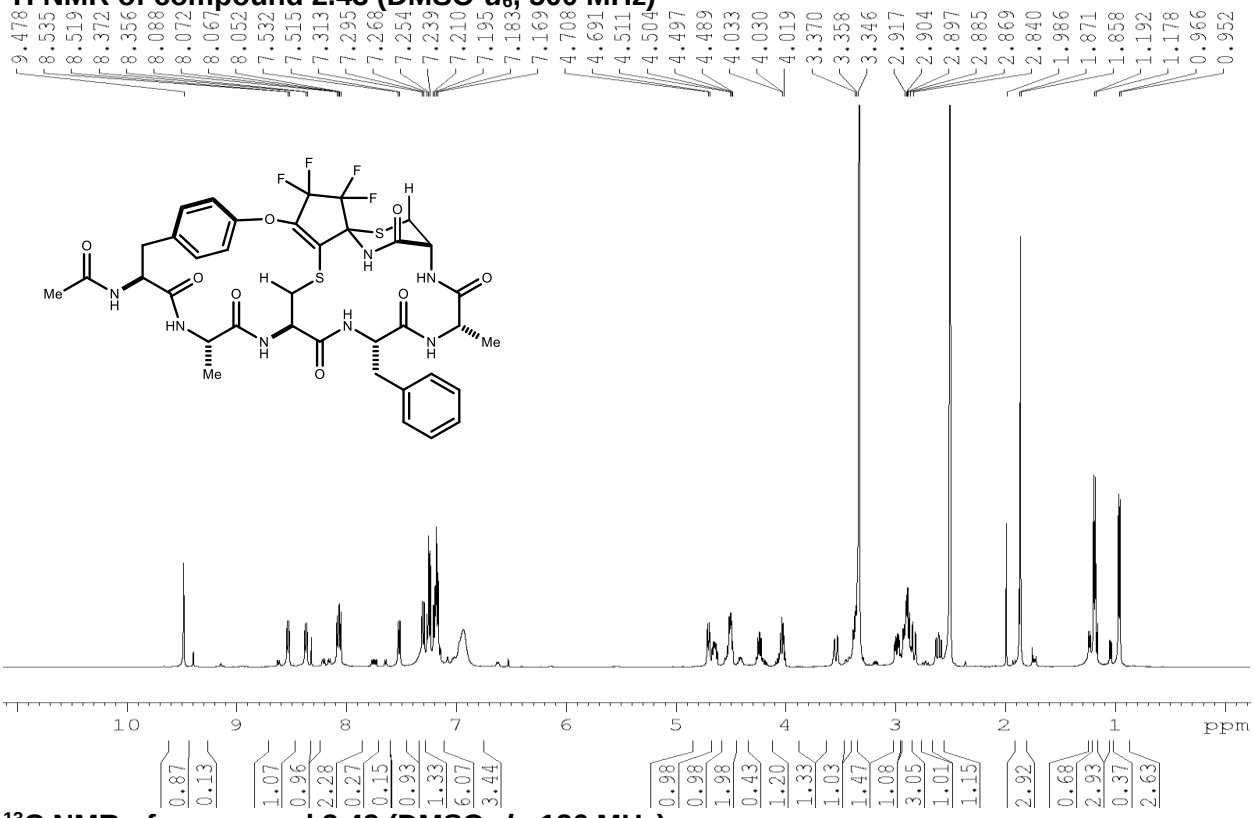
^{1}H NMR of compound 2.42 (DMSO-d_6, 500 MHz)



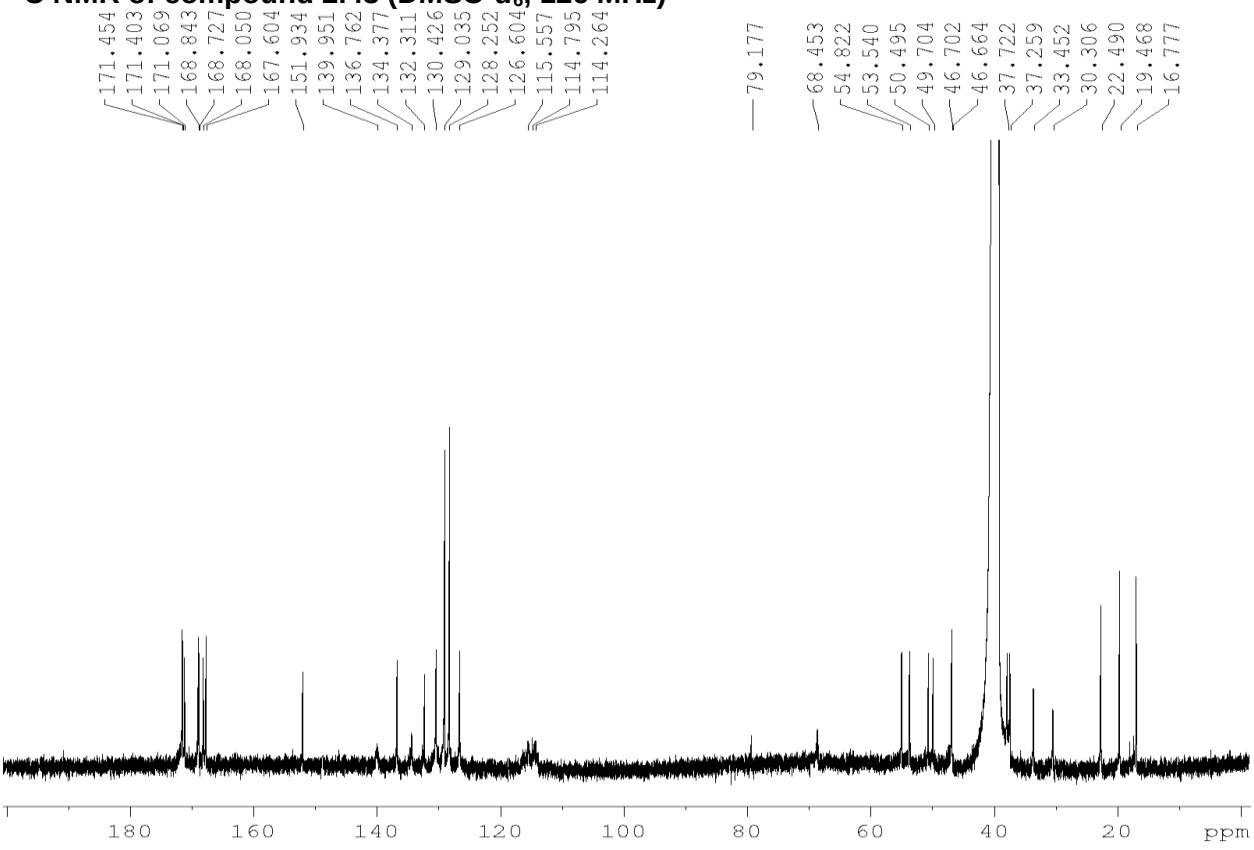
^{13}C NMR of compound 2.42 (DMSO-d_6, 126 MHz)



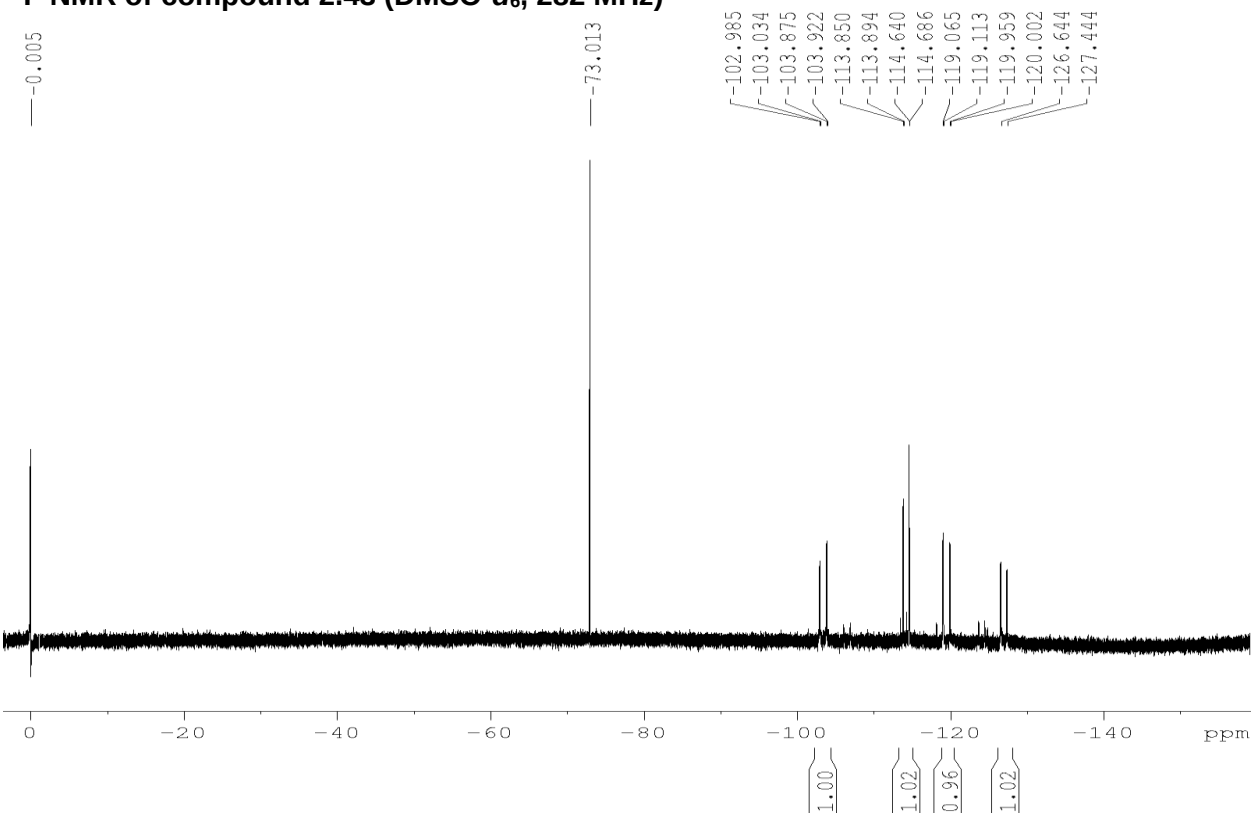
^{1}H NMR of compound 2.43 (DMSO-d_6, 500 MHz)



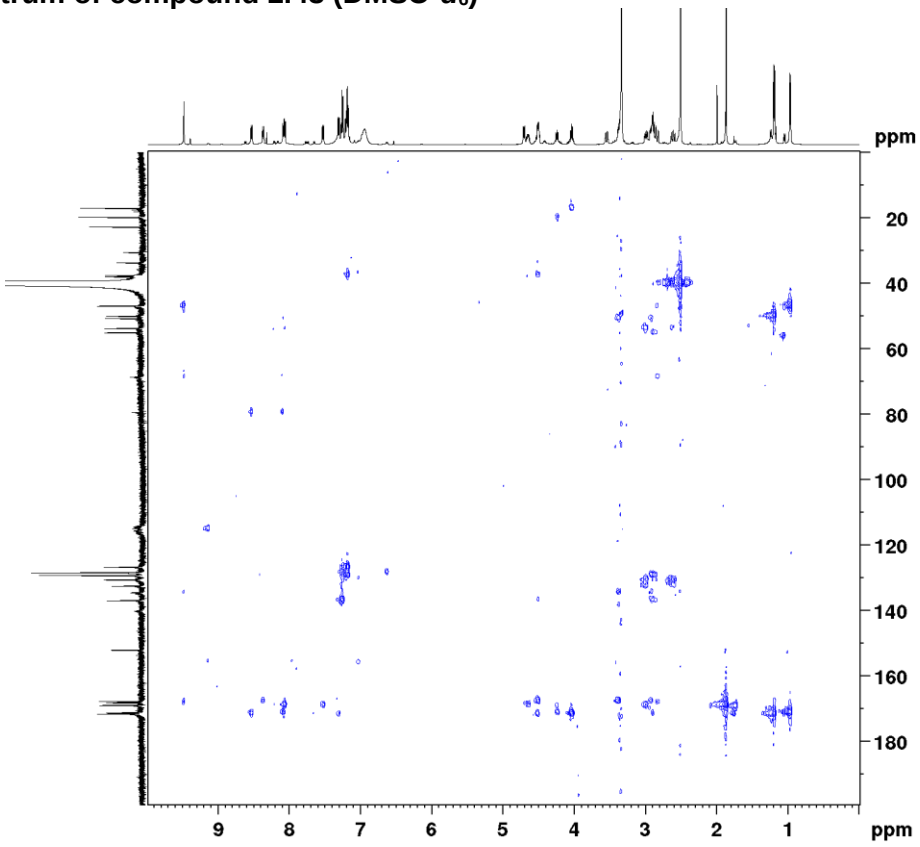
^{13}C NMR of compound 2.43 (DMSO-d_6, 126 MHz)



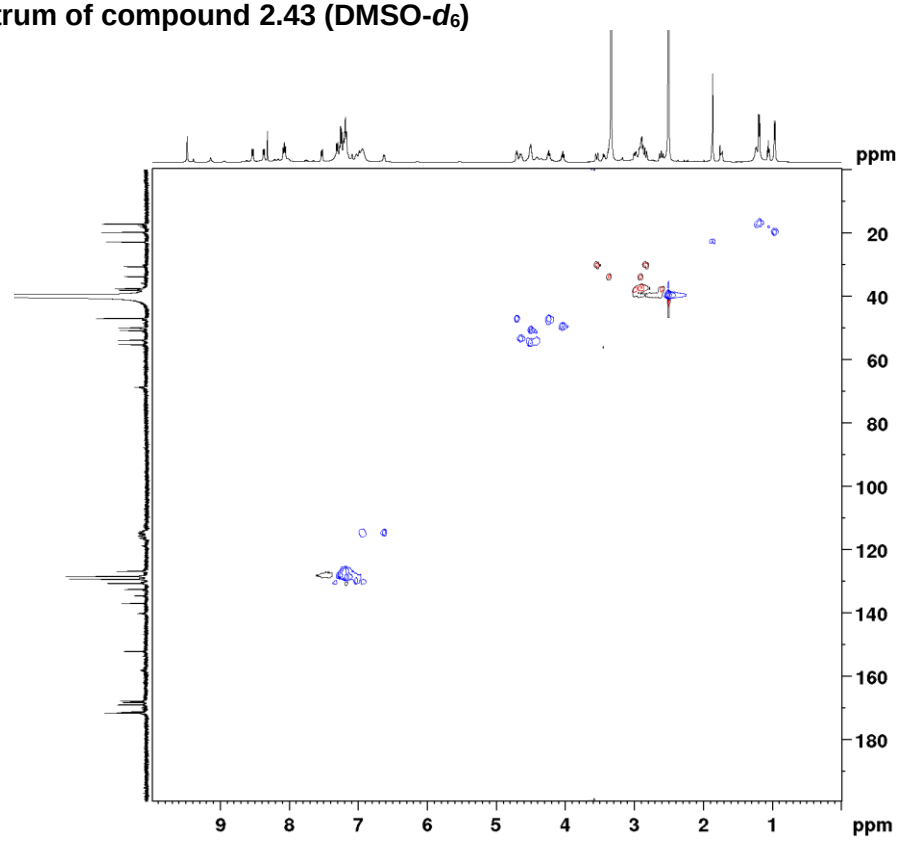
^{19}F NMR of compound 2.43 (DMSO-d_6, 282 MHz)



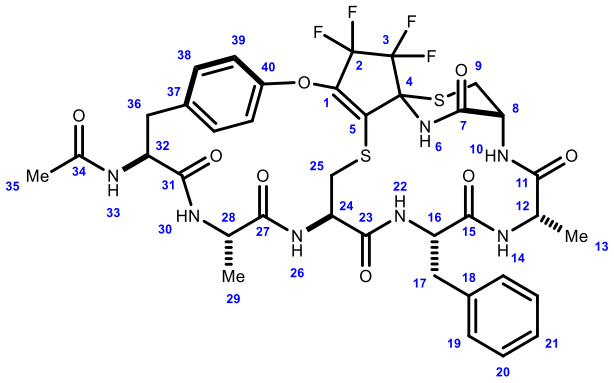
HMBC spectrum of compound 2.43 (DMSO-d_6)



HSQC spectrum of compound 2.43 (DMSO-d_6)

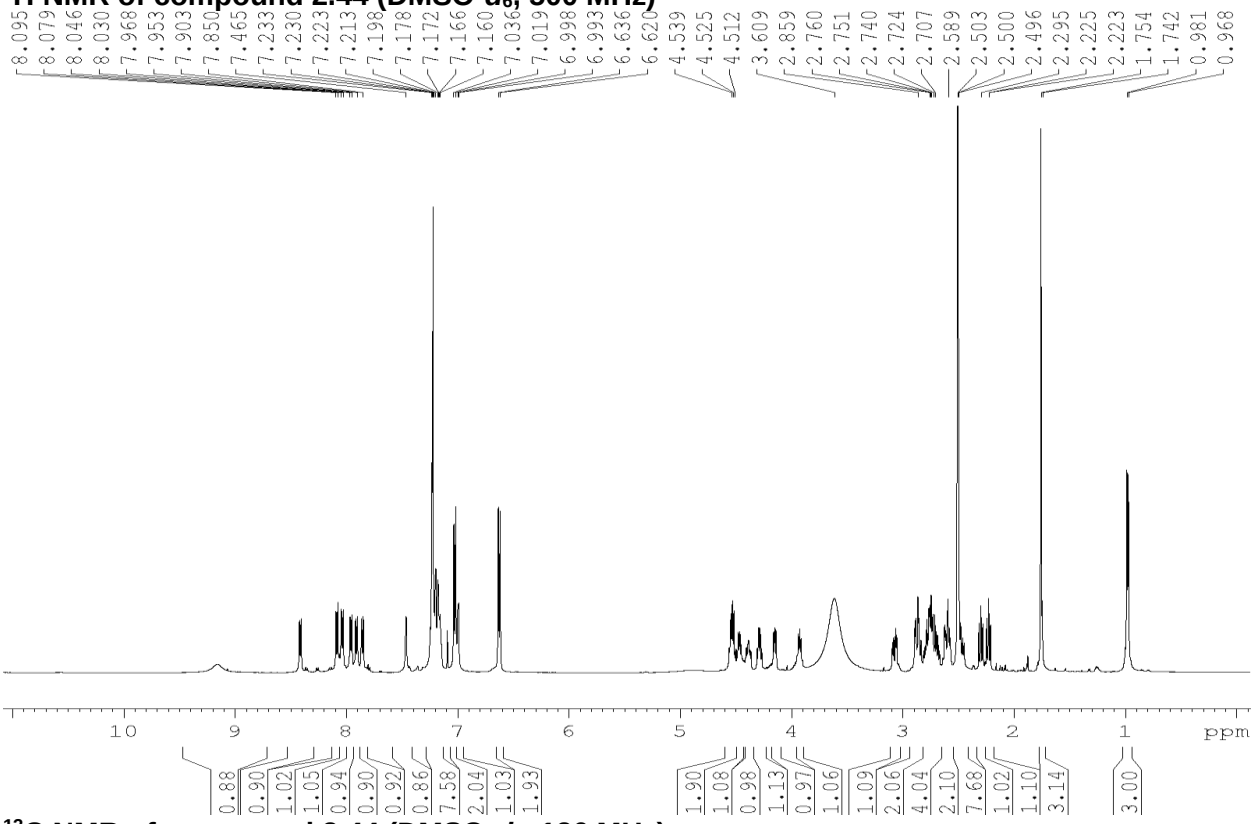


Compound 2.43 (DMSO-d_6)

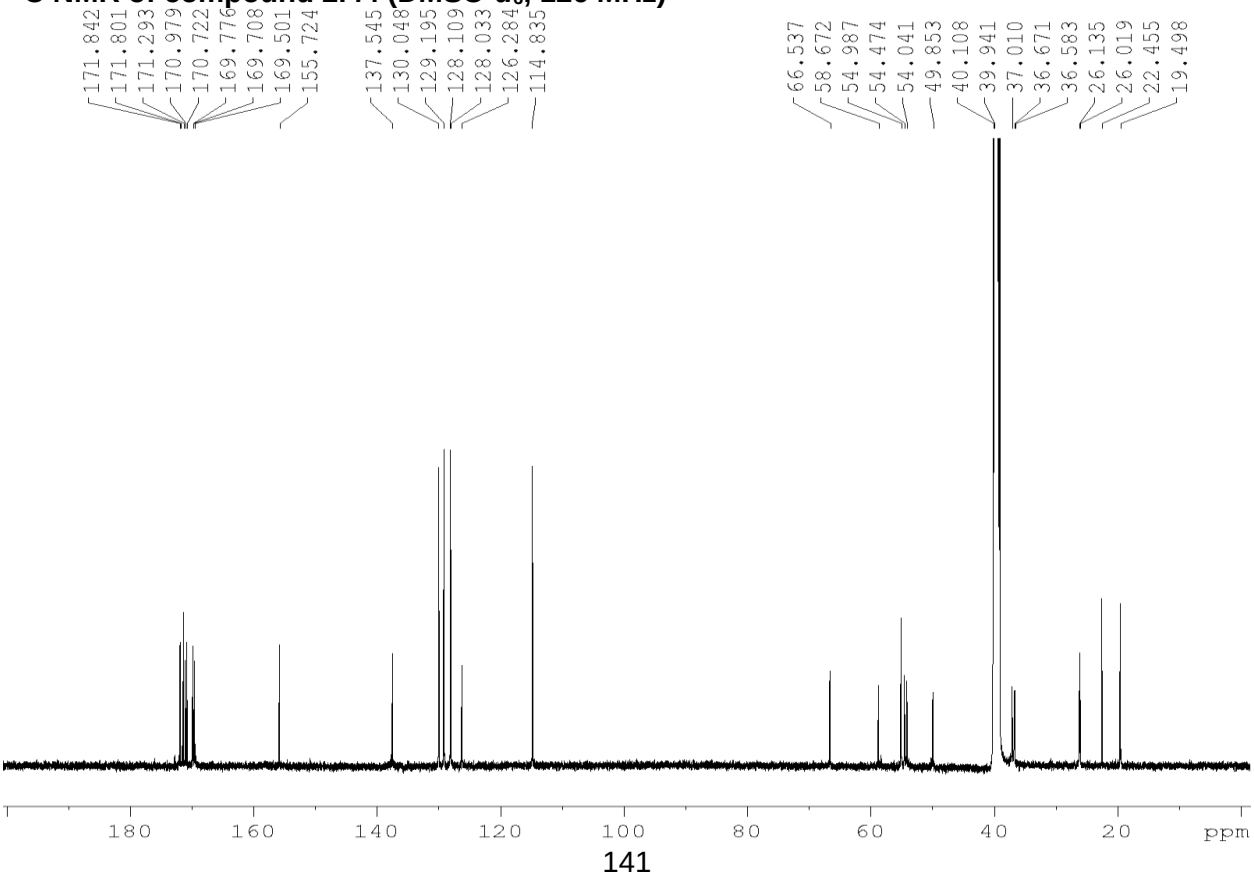


	13C	1H	Key Correlation
1	140.3–139.8 (m, 1C)	–	
2	116.6–113.8 (m, 1C)	–	
3	116.6–113.8 (m, 1C)	–	
4	69.0–68.2 (m, 1C)	–	HMBC 6, 9 -> 4
5	134.6–134.3 (m, 1C)	–	HMBC 6, 25 -> 5
6	–	9.48 (s, 1H)	HMBC 6 -> 7
7	168.1	–	HMBC 6 -> 7
8	46.7	4.70 (dt, J = 8.9, 2.7 Hz, 1H)	HMBC 8 -> 7, HSQC
9	30.3	3.54 (dd, J = 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

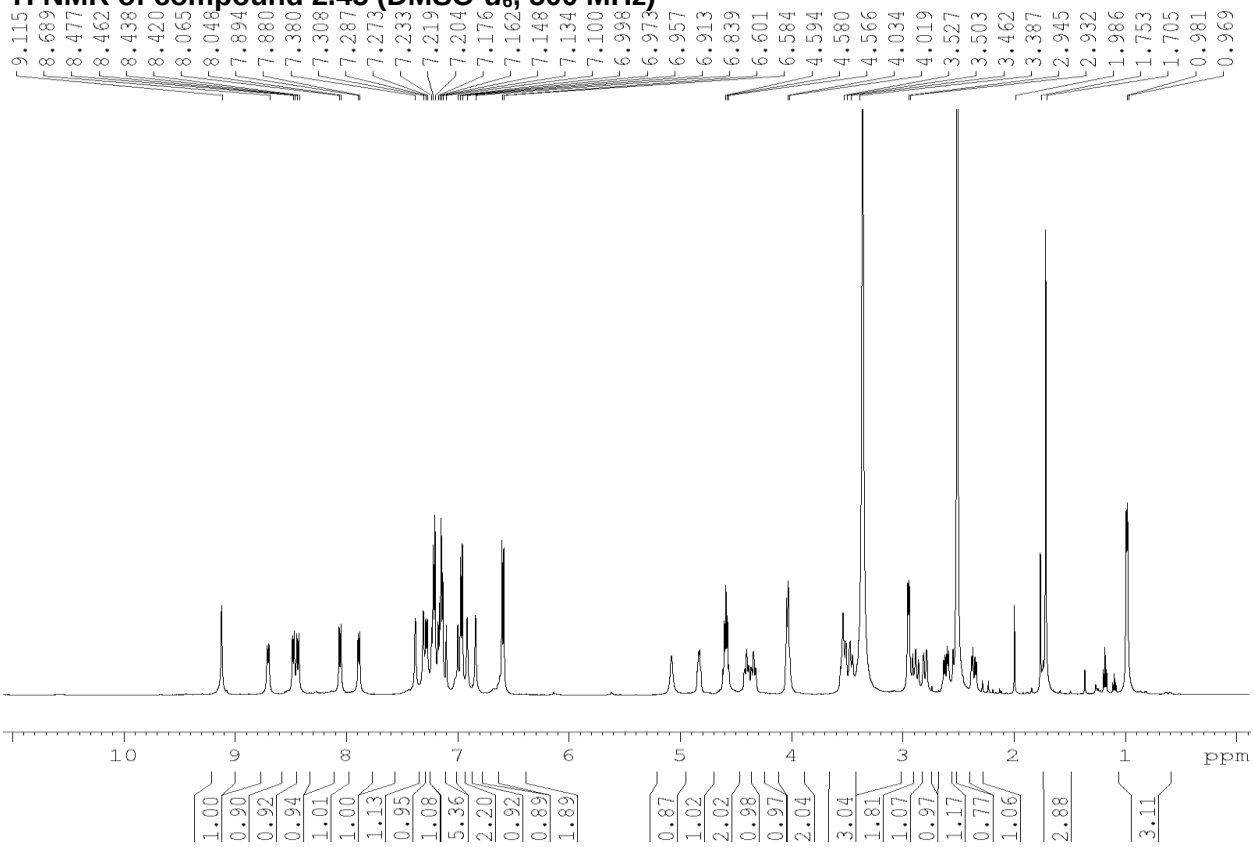
^{1}H NMR of compound 2.44 (DMSO-d_6, 500 MHz)



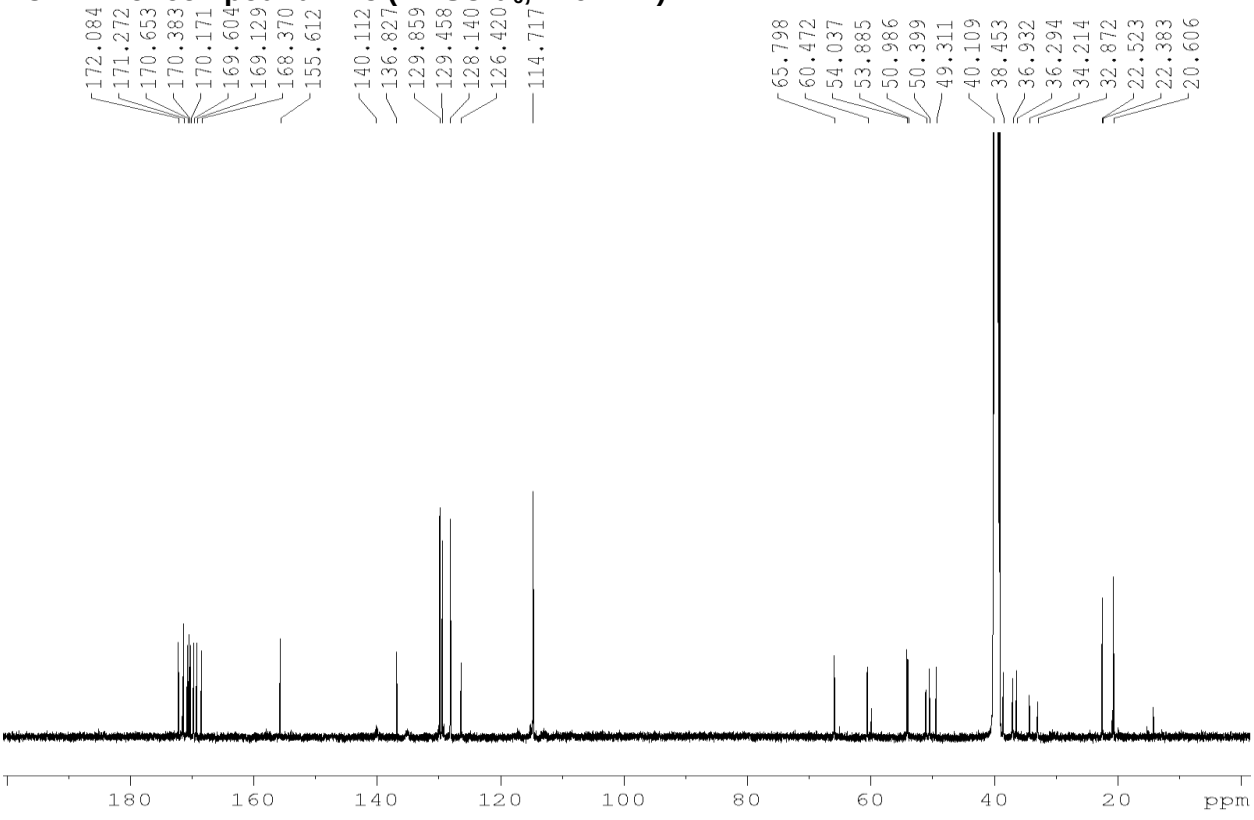
^{13}C NMR of compound 2.44 (DMSO-d_6, 126 MHz)



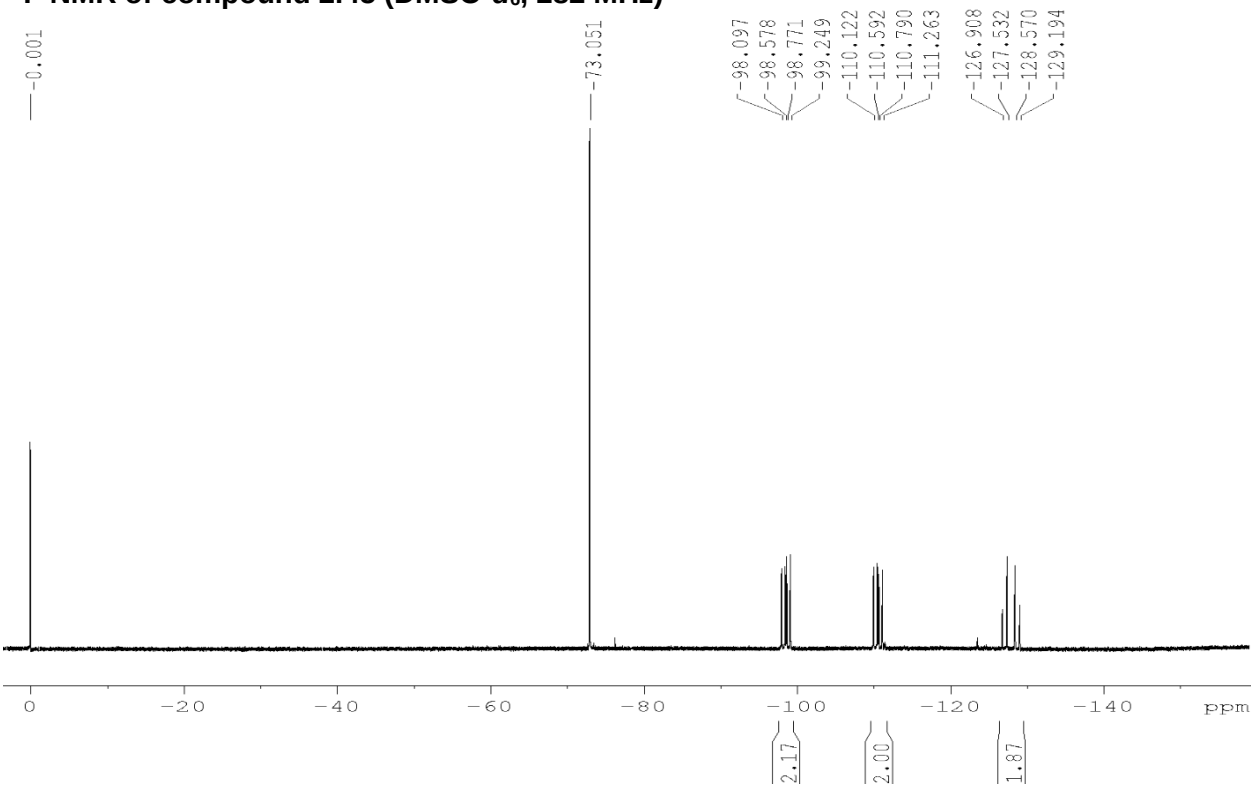
^{1}H NMR of compound 2.45 (DMSO-d_6, 500 MHz)



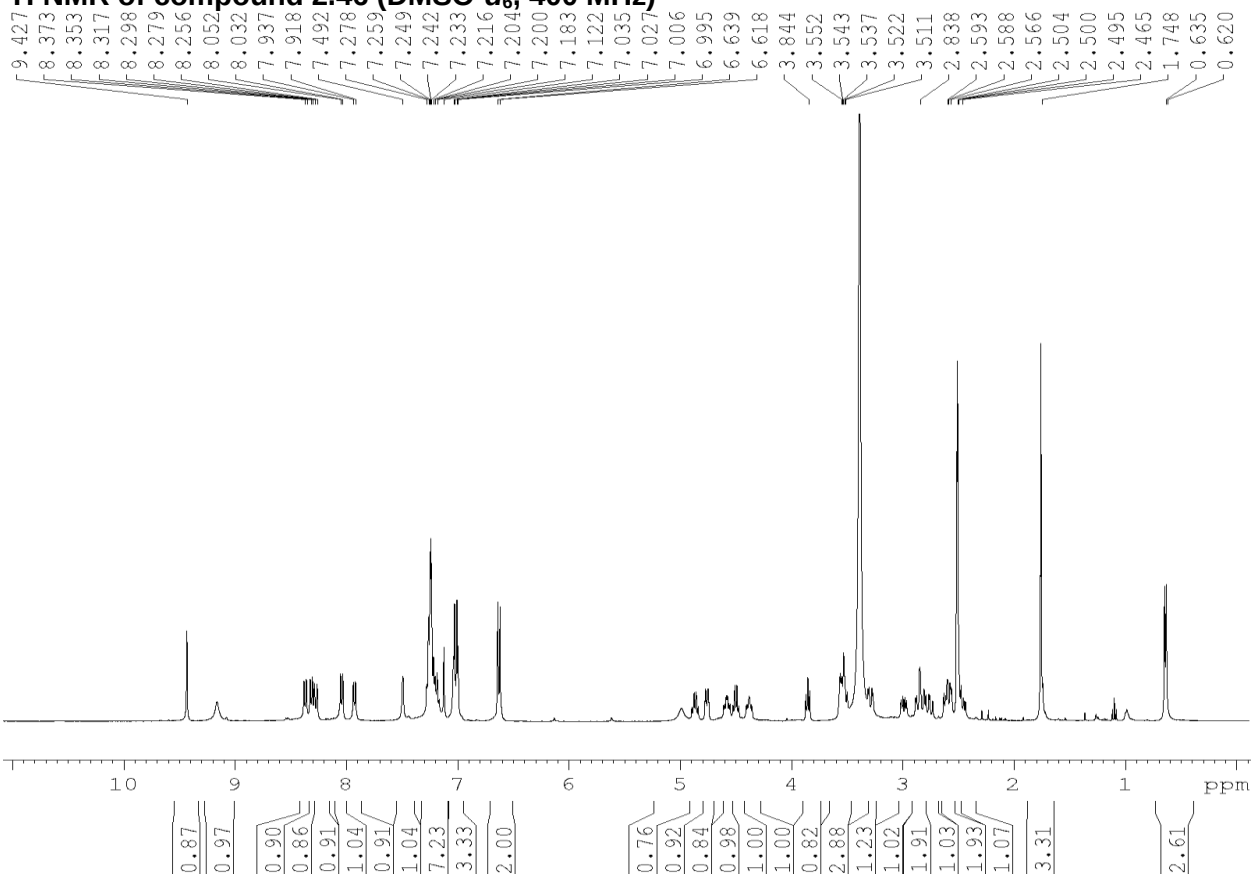
^{13}C NMR of compound 2.45 (DMSO-d_6, 126 MHz)



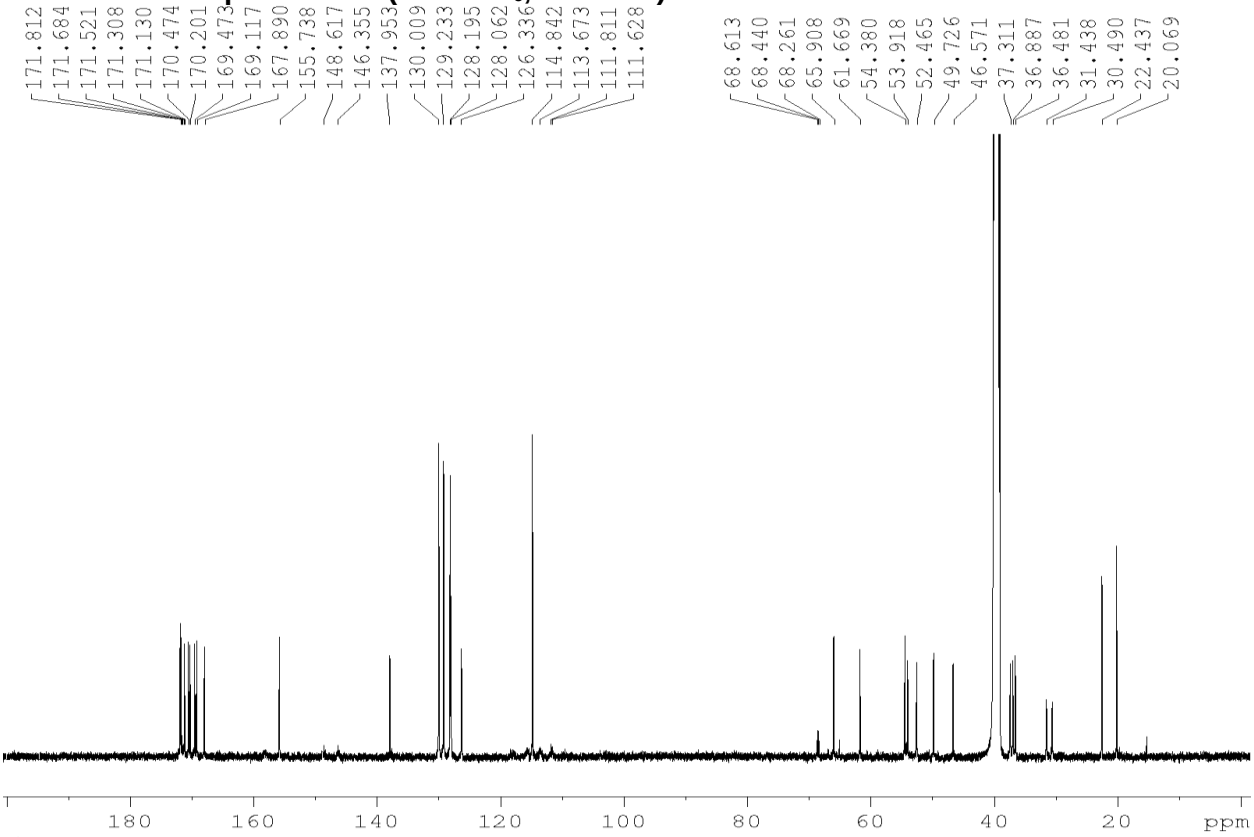
^{19}F NMR of compound 2.45 (DMSO-d_6, 282 MHz)



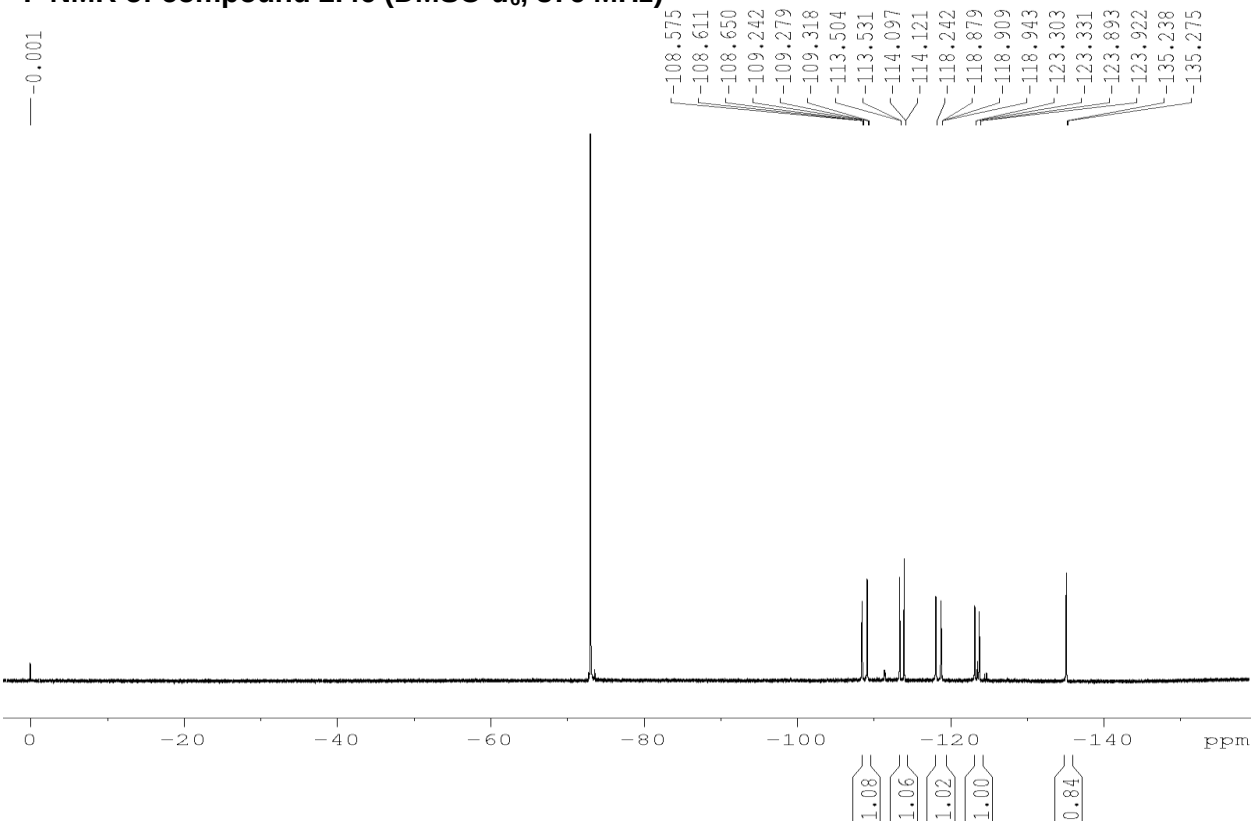
^{1}H NMR of compound 2.46 (DMSO-d_6, 400 MHz)



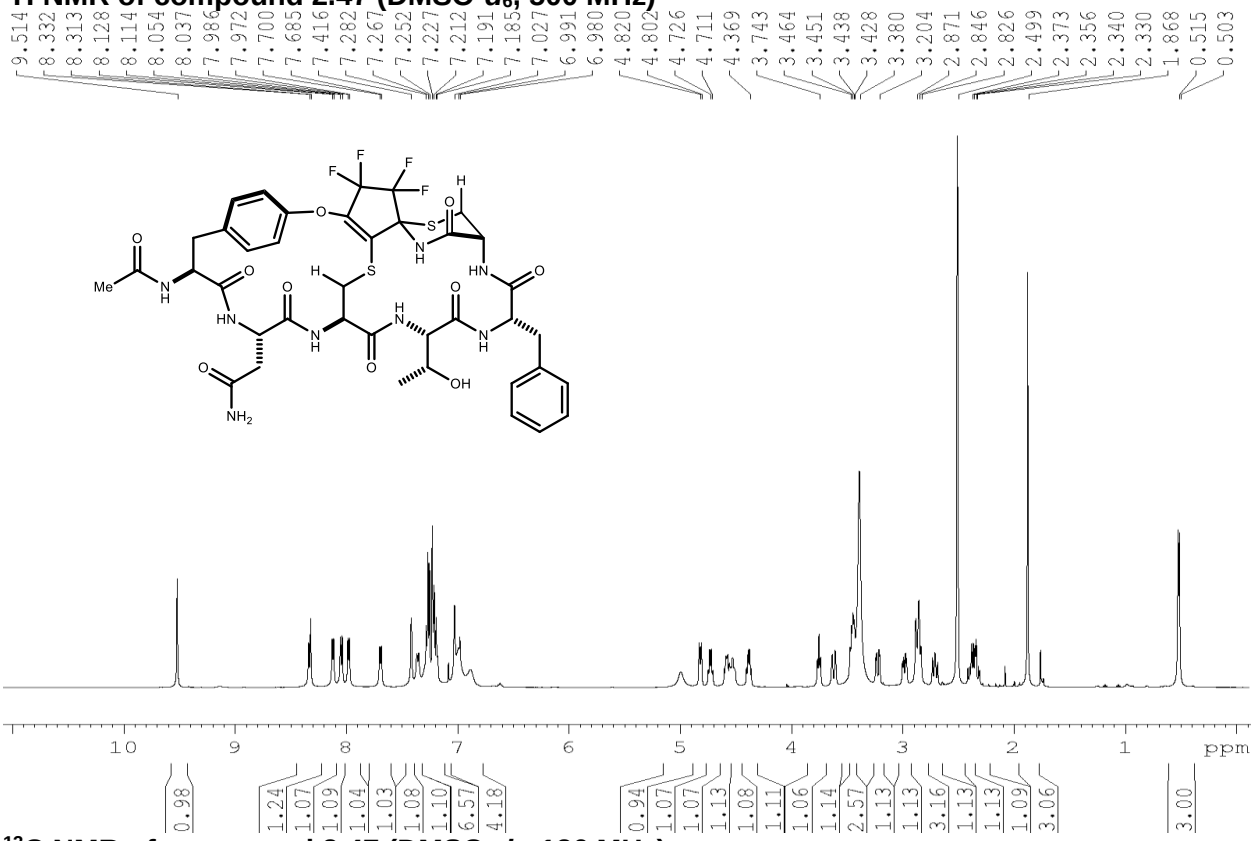
^{13}C NMR of compound 2.46 (DMSO-d_6, 126 MHz)



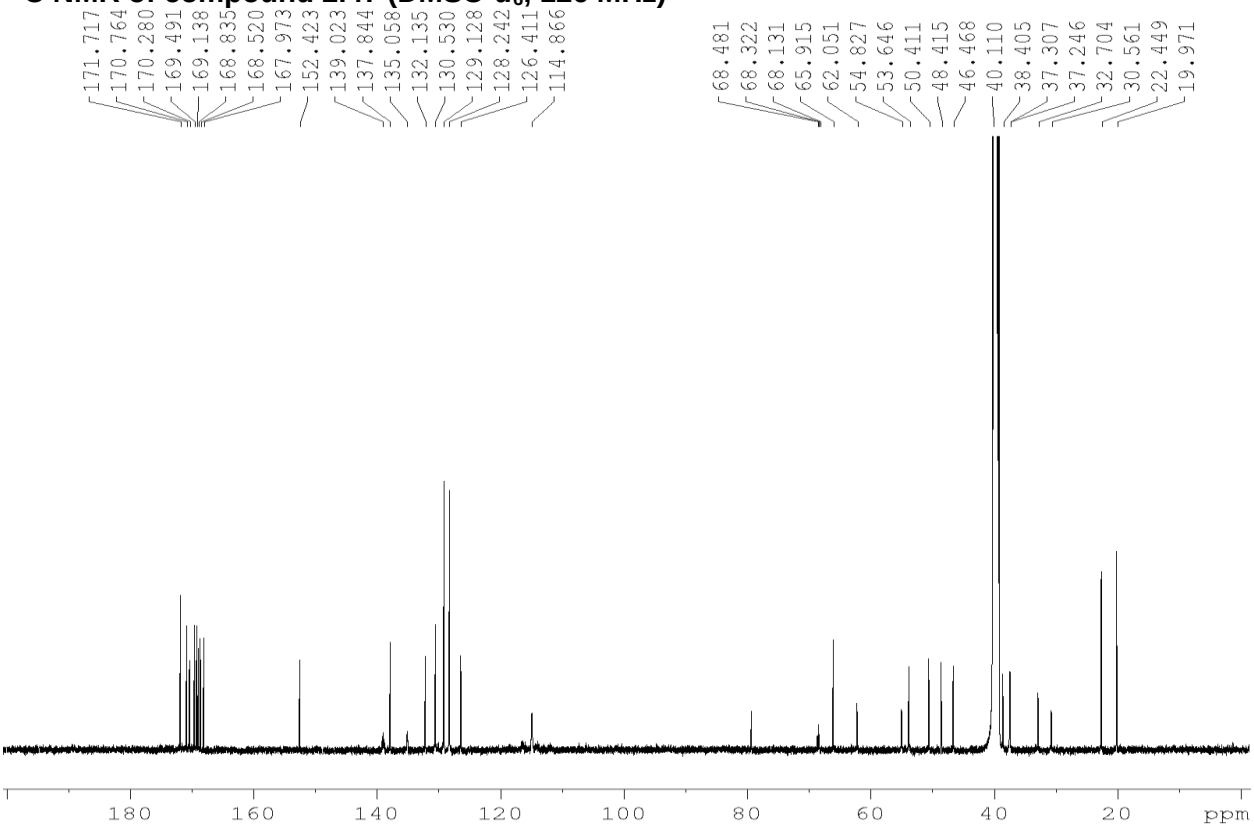
^{19}F NMR of compound 2.46 (DMSO-d_6, 376 MHz)



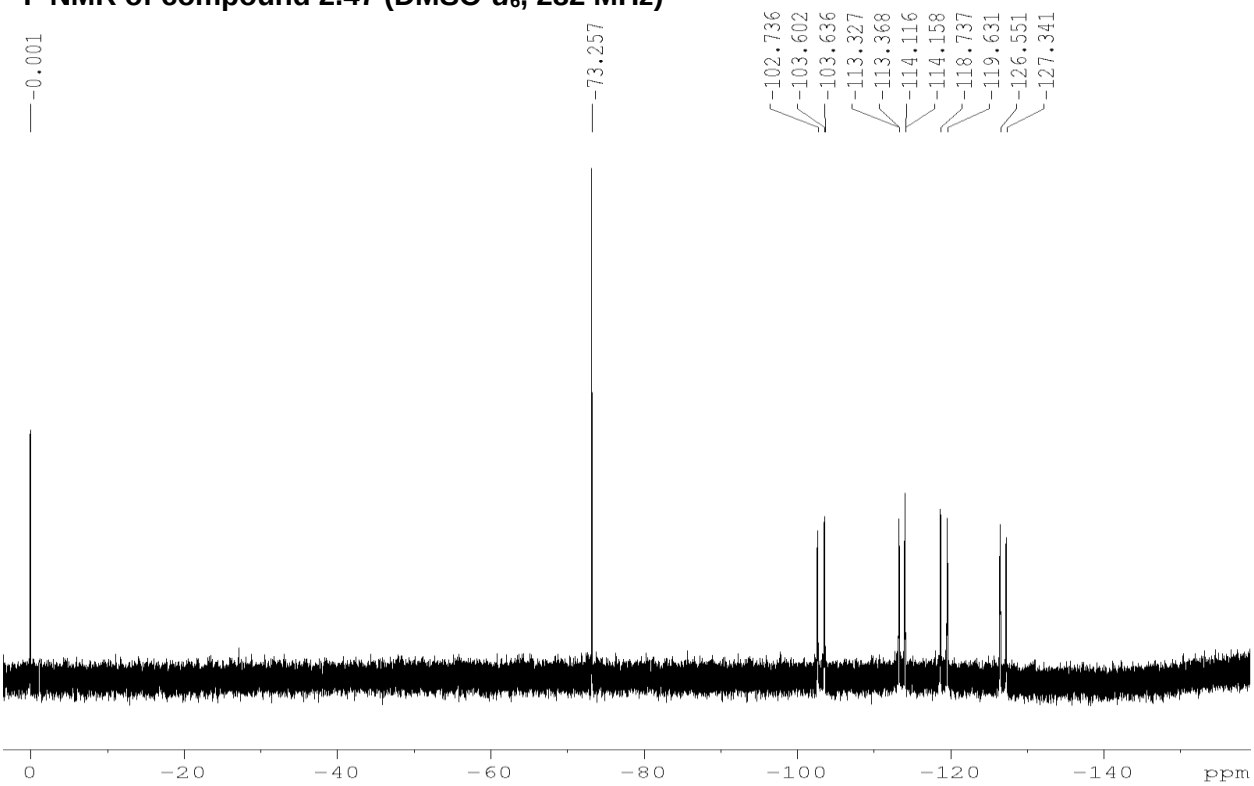
^{1}H NMR of compound 2.47 (DMSO-d_6, 500 MHz)



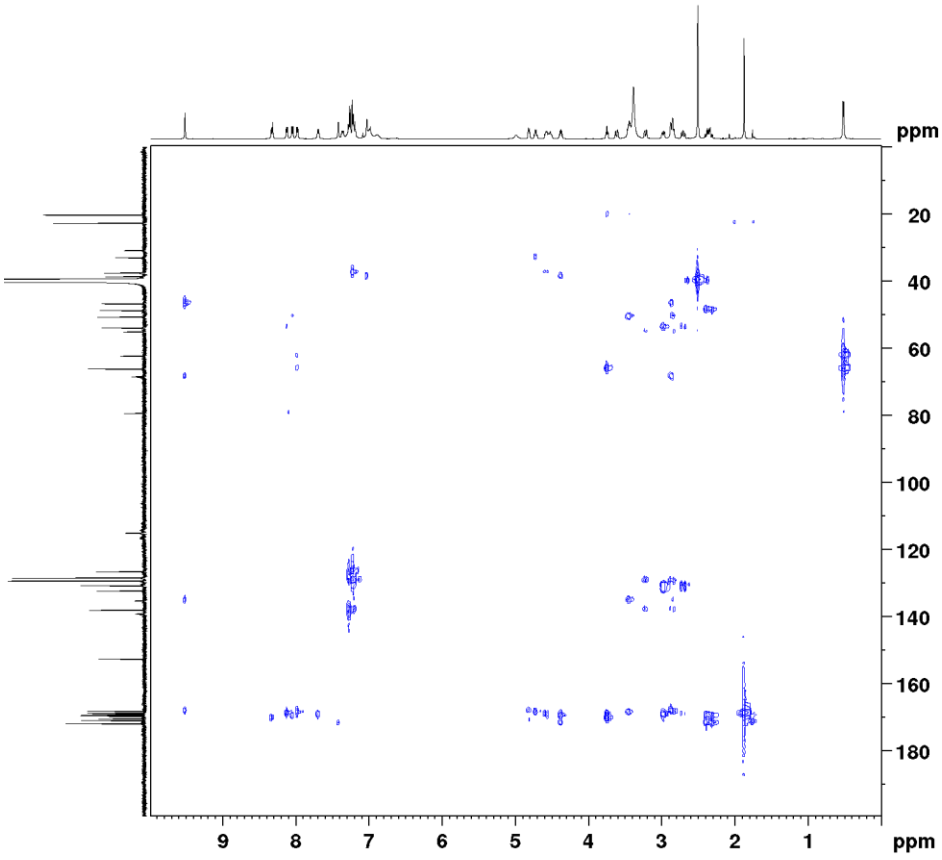
^{13}C NMR of compound 2.47 (DMSO-d_6, 126 MHz)



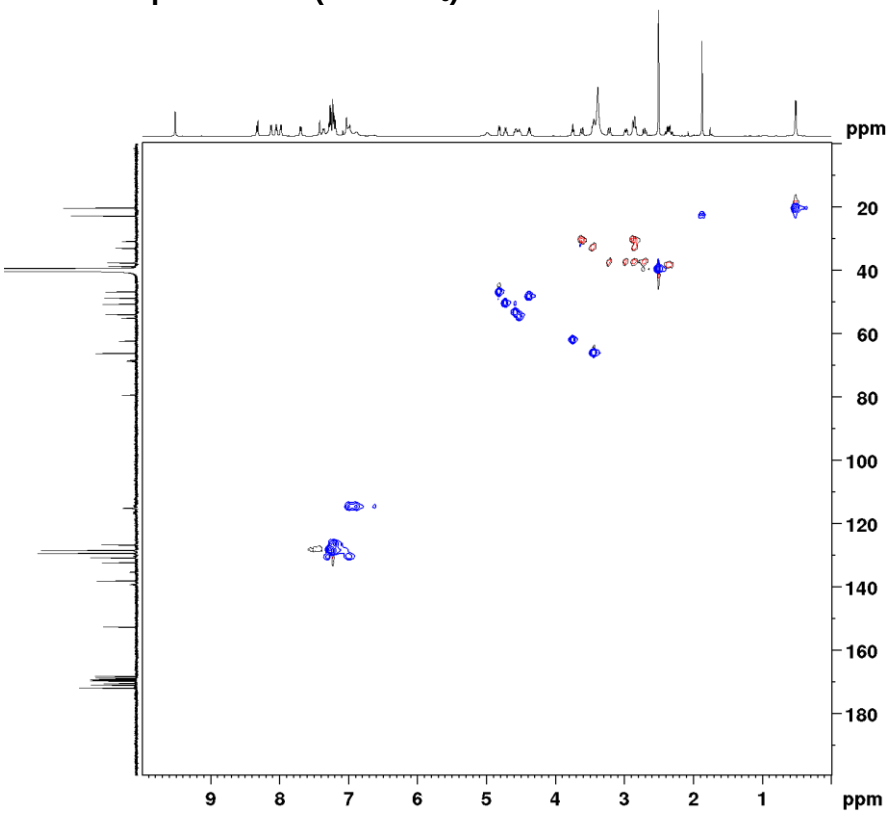
^{19}F NMR of compound 2.47 (DMSO-d_6, 282 MHz)



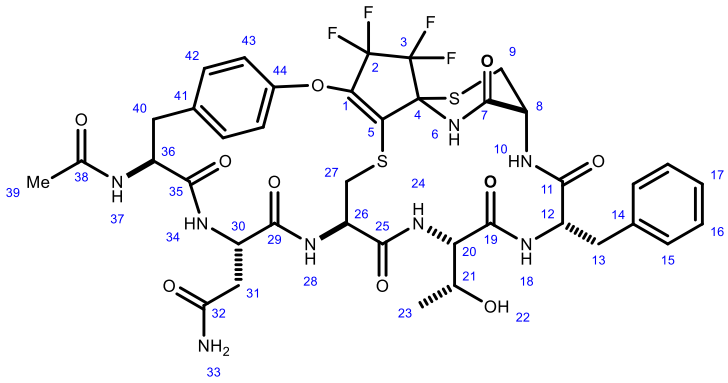
HMBC spectrum of compound 2.47 (DMSO-d_6)



HSQC spectrum of compound 2.47 (DMSO-d_6)

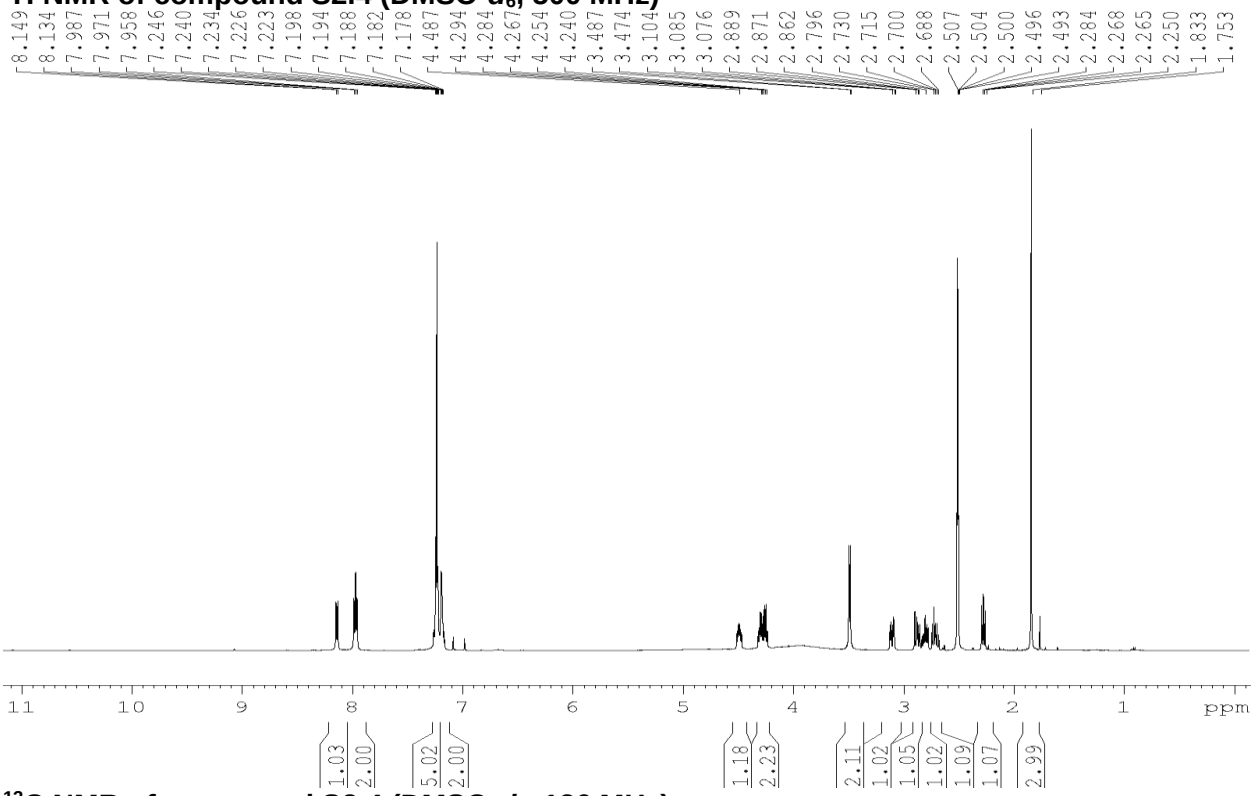


Compound 2.47 (DMSO-d_6)

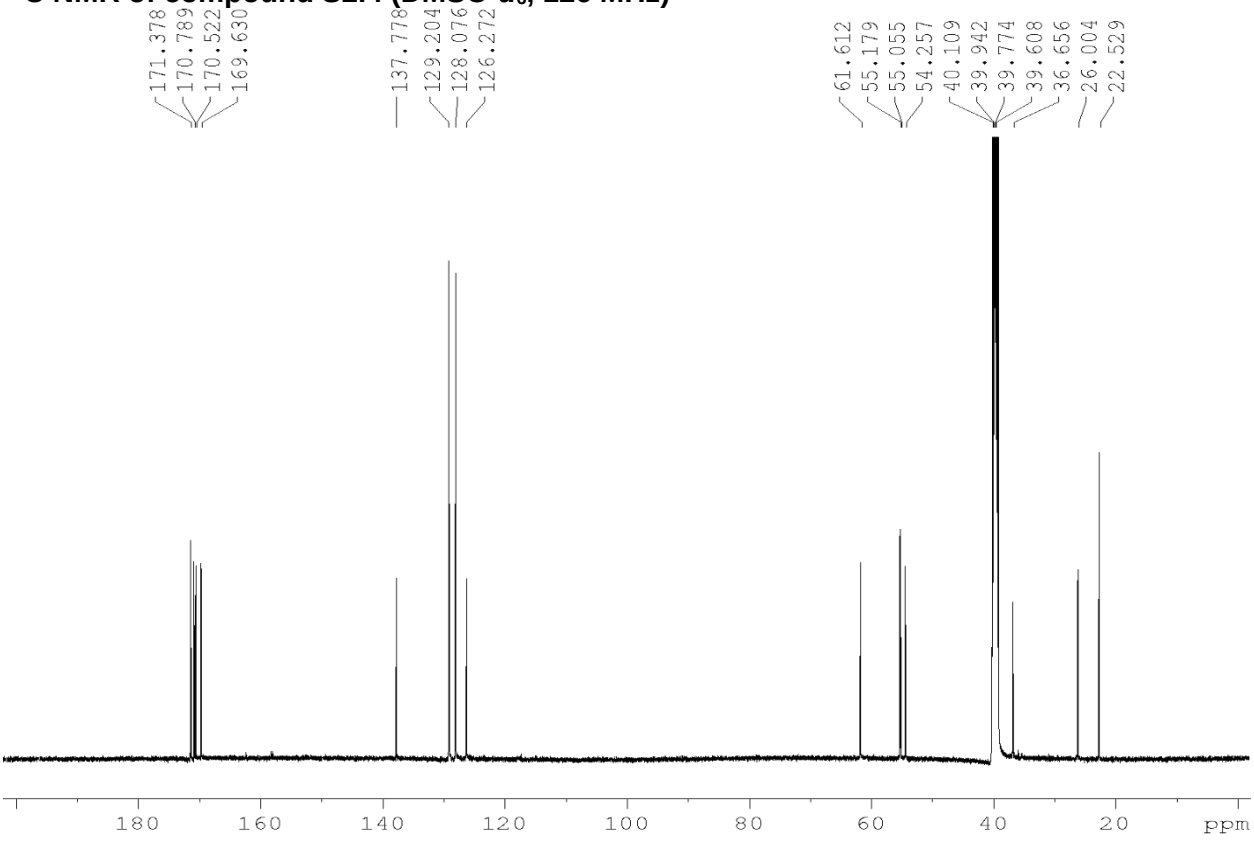


	13C	1H	Key Correlation
1	139.2–138.8 (m, 1C)	–	
2	118.7–111.5 (m, 2C)	–	
3	118.7–111.5 (m, 2C)	–	
4	68.6–68.1 (m, 1C)	–	HMBC 6, 9 -> 4
5	135.2–134.9 (m, 1C)	–	HMBC 6, 9, 27 -> 5
6	–	9.51 (s, 1H)	HMBC 6 -> 7
7	168.0	–	HMBC 6 -> 7
8	46.5	4.84–4.79 (m, 1H)	HMBC 8 -> 7, HSQC
9	30.6	3.61 (d, J = 14.0 Hz, 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

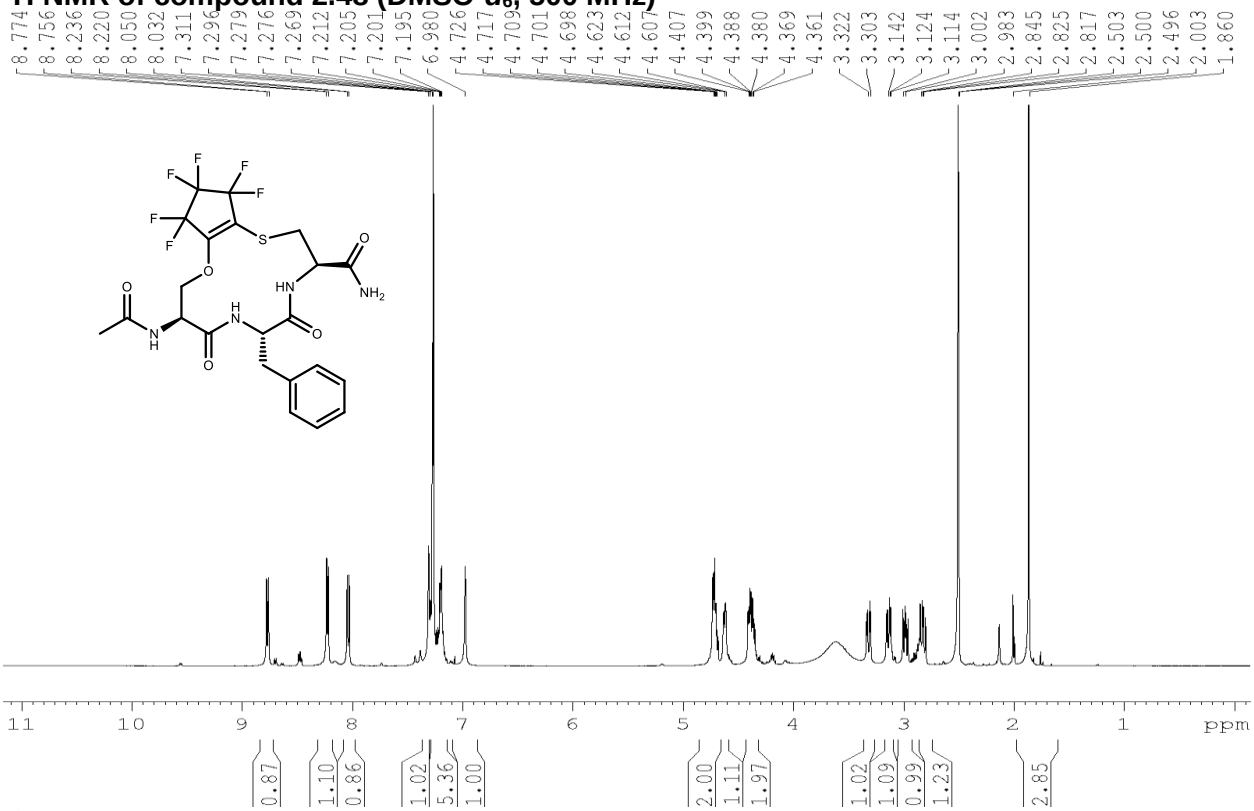
^{1}H NMR of compound S2.4 (DMSO-d_6, 500 MHz)



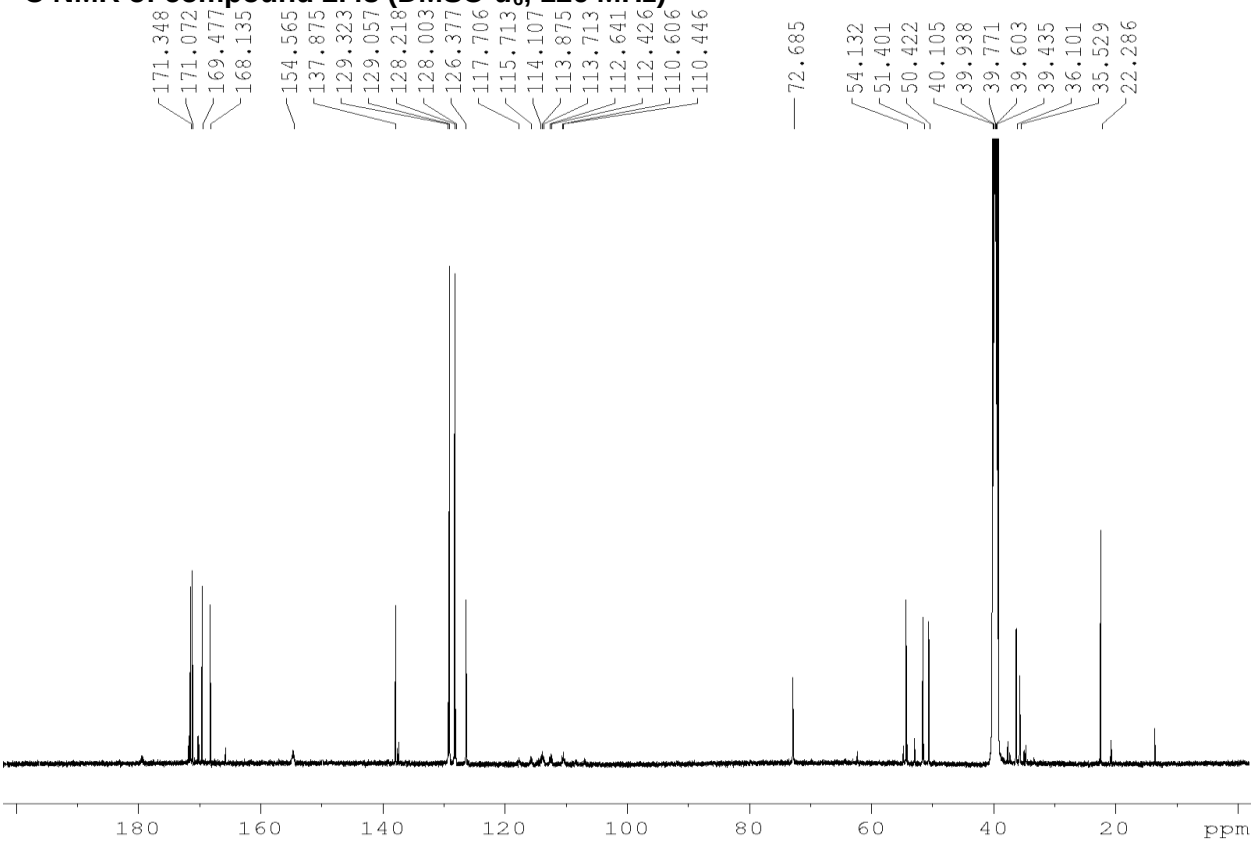
^{13}C NMR of compound S2.4 (DMSO-d_6, 126 MHz)



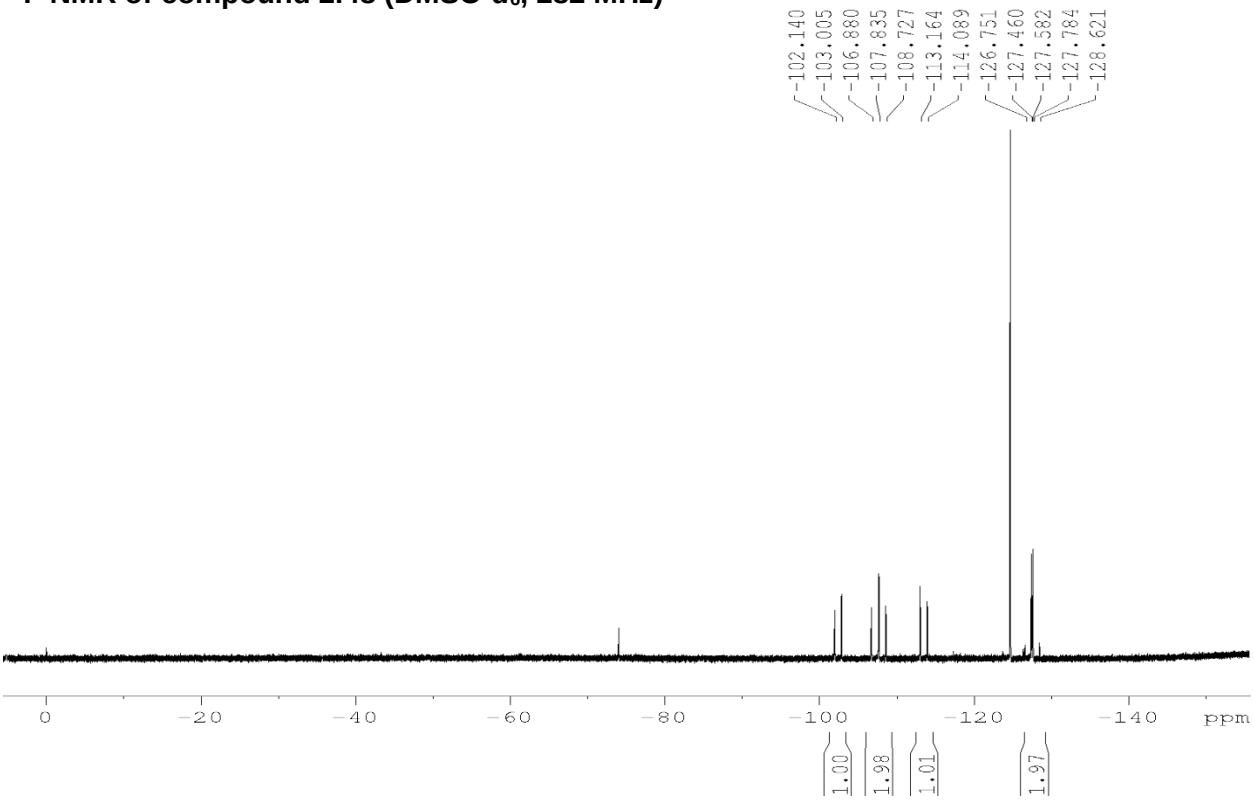
^{1}H NMR of compound 2.48 (DMSO-d_6, 500 MHz)



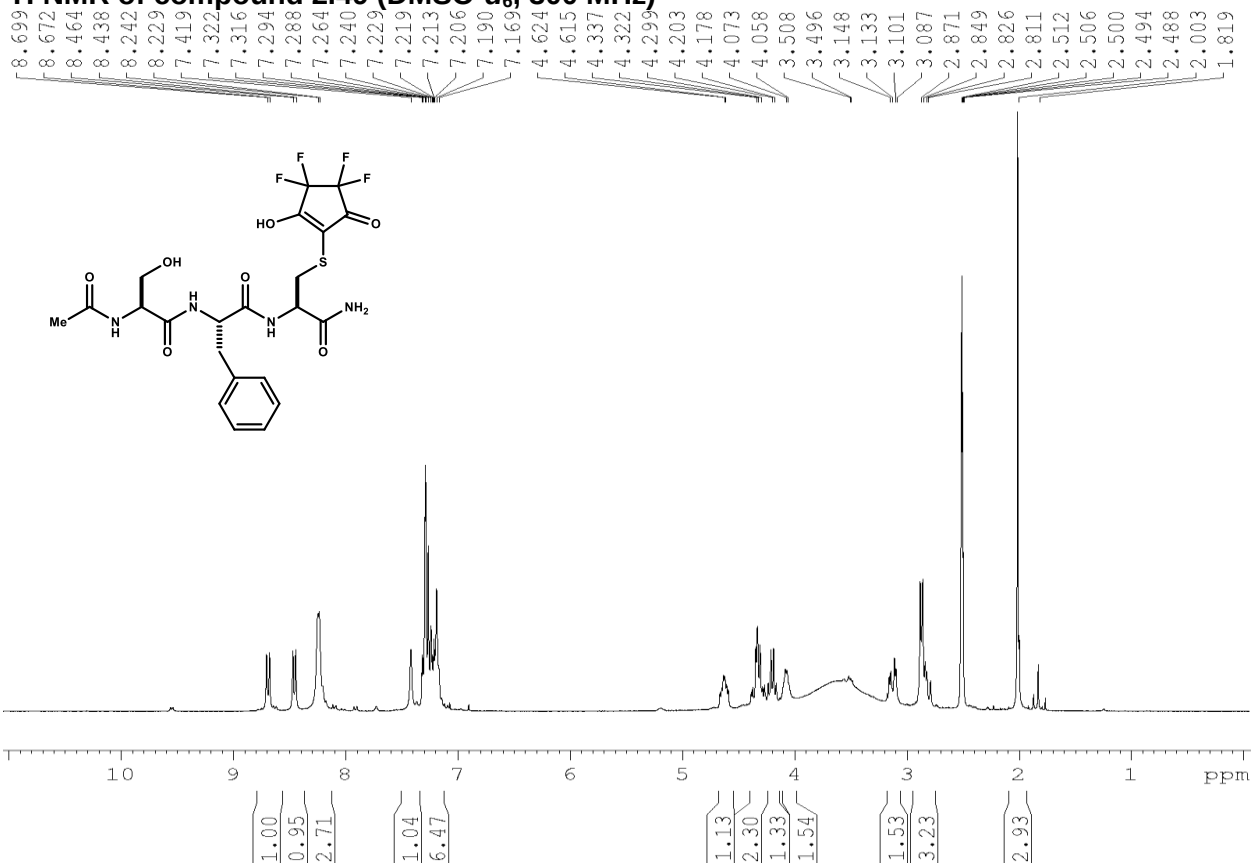
^{13}C NMR of compound 2.48 (DMSO-d_6, 126 MHz)



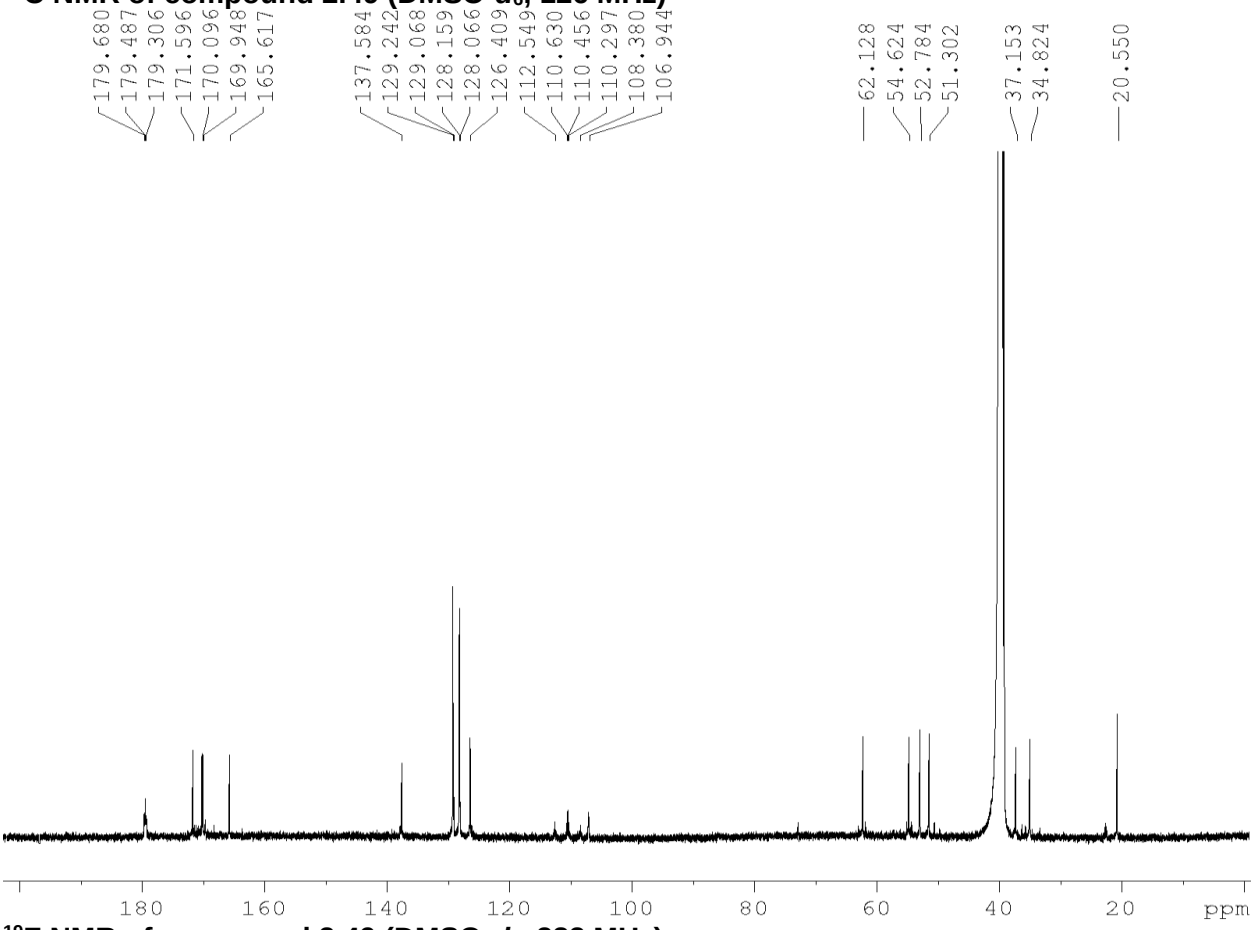
^{19}F NMR of compound 2.48 (DMSO-d_6, 282 MHz)



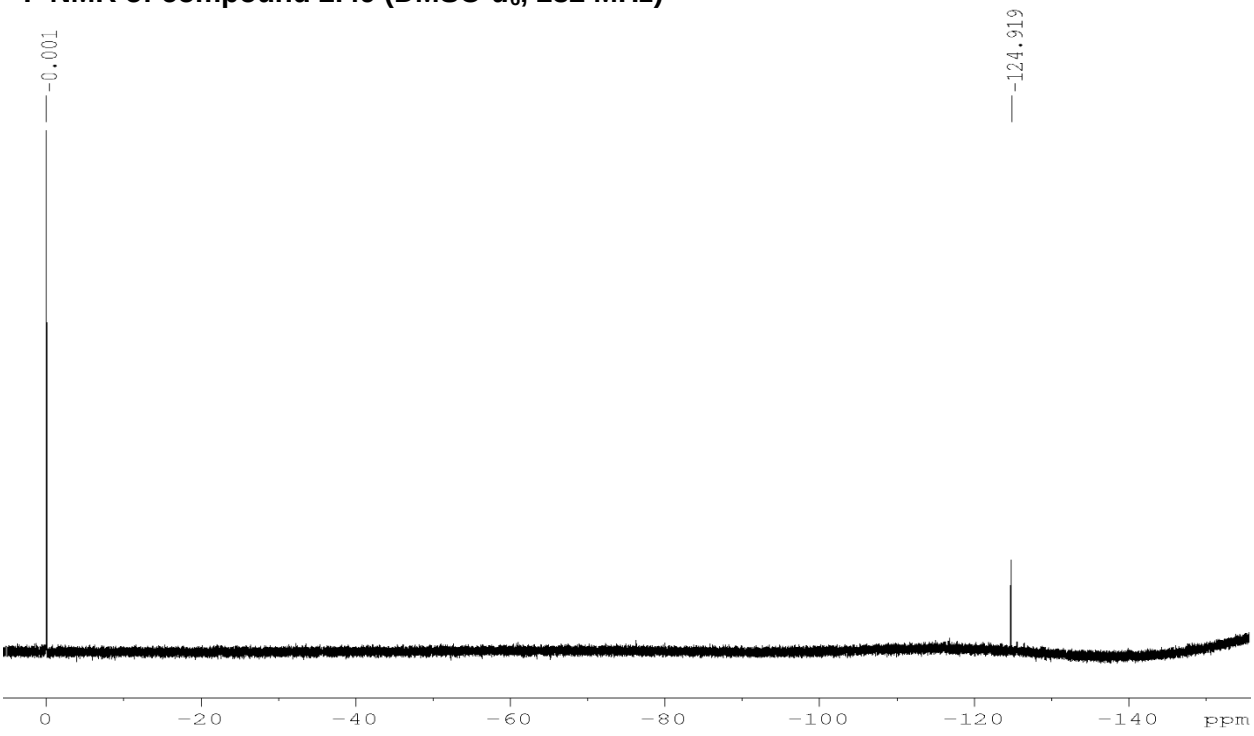
^{1}H NMR of compound 2.49 (DMSO-d_6, 300 MHz)



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



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



References

[S1] N. Hada, Y. Shida, N. Negishi, F. Schweizer, T. Takeda, *Chem. Pharm. Bull*., **2009**, *57*, 1081-1088.
[S2] H. Albrich, H. Vahrenkamp, *Chem. Ber*. **1994**, *127*, 1223- 1233

Chapter 3 Appendix Material

General Methods

See Chapter 2 Appendix Material

General procedure A – Peptide Synthesis:

Peptides were prepared using standard solution phase techniques and the Boc protection strategy, or standard solid phase peptide synthesis (SPPS) using the Fmoc protection strategy. Boc deprotection was performed using 4 M HCl in dioxane or 1:1 TFA in DCM. Cbz deprotection was performed with palladium on carbon in methanol under an atmosphere of hydrogen. Prior to removal of the final protecting group, peptides were purified by column chromatography on SiO_2 eluted with 2-12% MeOH in $CHCl_3$. Peptide identities were verified by HPLC-ESI-MS.Using SPPS 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 0.50 – 1.50 mmol scale using a fritted glass reaction vessel. Fmoc-deprotection was achieved with 5% piperazine and 2% DBU in DMF (2 x 15 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 (3 eq.) and HBTU (3 eq.) followed by DMF (10-20 ml) and iPr_2NEt (10 eq). The resin was agitated for 45 min, 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 into cold Et_2O. The peptide was precipitated with Et_2O and isolated by centrifugation. The peptide pellet was repeatedly triturated with Et_2O and isolated by centrifugation to ensure complete removal of cleavage reagents. Peptides were typically used as crude moving forward, but purified by preparative RP-HPLC if necessary.

General Procedure B – One-pot polycylization

A flask was charged with linear precursor (1 eq) and DMF (5 mM in substrate). The mixture was added OFCP (1M in MeCN, 1.5 eq) and Et_3N (2.5 eq) at 0 °C. The mixture was stirred for 30 minutes (until HPLC indicated completion) and then excess amount of OFCP and MeCN were removed by rotary evaporation. The mixture was then cooled again to 0 °C then added Cs_2CO_3 (4 eq) or Me_3SiOK (4 eq) and at let stir at rt until completion observed by HPLC. After completion the mixture was quenched with AcOH (10 eq) and concentrated under reduced pressure to afford crude product. Purification performed by RP-HPLC to give desired product.

Experimental Procedures

Compound 3.10

Synthesized according to General Procedure B using crude peptide AcHFCASC-NH_2 to give **3.10** (27%). ^{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 NMR (DMSO-d6, 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 NMR (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 $[M + H]^+$ calc'd for $C_{34}H_{37}F_4N_9O_8S_2H$ 840.2221, found 840.2233.

Compound 3.11

Synthesized according to General Procedure B using crude peptide AcHFCASC-NH_2 to give **3.11** (42%). ^{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-d6, 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 $[M + H]^+$ calc'd for $C_{44}H_{37}F_4N_9O_8S_2H$ 840.2221, found 840.2213.

Compound 3.12

Synthesized according to General Procedure B using crude peptide AcYGHCAC-NH_2 to give **3.12** (20%).

Compound 3.13

Synthesized according to General Procedure B using crude peptide AcYGSHAC-NH_2 to give **3.13** (40%). ^{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-d6, 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 $[M + H]^+$ calc'd for $C_{33}H_{35}F_4N_9O_9SH$ 810.2293, found 810.2294.

Compound 3.14

Synthesized according to General Procedure B using crude peptide AcYGSdHAC-NH_2 to give **3.14** (37%). ^{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, 3H), 1.93 (s, 3H), 1.13 (d, J = 7.3 Hz, 3H). ^{13}C NMR (DMSO-d6, 126 MHz) 171.2, 170.7, 170.6, 170.1, 169.4, 167.0, 153.8, 139.6, 134.0, 131.2, 128.3, 127.6, 116.8, 115.3, 67.0, 61.5, 56.3, 52.9, 52.5, 50.0, 46.0, 43.0, 37.8, 30.2, 28.1, 25.1, 22.7, 16.7. HRMS m/z $[M + H]^+$ calc'd for $C_{33}H_{35}F_4N_9O_9SH$ 810.2293, found 810.2293.

Compound 3.15

Synthesized according to general procedure B using crude peptide AcYGTMQVSHAC-NH_2 to give **3.15** (35%). See NMR Spectra Appendix section below for crude NMR spectra of this compound. HRMS m/z $[M + H]^+$ calc'd for $C_{52}H_{69}F_4N_{14}O_{15}S_2H$ 1269.4444, found 1269.4454.

Compound 3.16

Synthesized according to general procedure B using crude peptide AcD(C)VHSAY-NH_2 to give **3.16**.

Compound 3.17

Synthesized according to general procedure B using crude peptide AcYGAE(C)H-NH_2 to give **3.17** (8%). ^{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-d6, 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 $[M + H]^+$ calc'd for $C_{37}H_{42}F_4N_{10}O_9SH$ 879.2871, found 879.2868.

Compound 3.18

Synthesized according to general procedure B using crude peptide Ac-CWSC-NH_2 to give **3.18** (35%). ^{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-d6, 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 $[M + H]^+$ calc'd for $C_{27}H_{28}F_5N_6O_6S_2H$ 691.1432, found 691.1431.

Compoud 3.19

Synthesized according to general procedure B using crude peptide Ac-CWS-homo-C-NH_2 to give **3.18** (35%). ^{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 $[M + H]^+$ calc'd for $C_{28}H_{29}F_5N_6O_6S_2H$ 705.1588, found 705.1590.

Compound 3.20

Synthesized according to general procedure B using crude peptide AcCWS-*d*-C-NH_2 to give **3.20** (27%).^{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-d6, 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 [M + H]$^+$ calc'd for $C_{27}H_{27}F_5N_6O_6S_2H$ 691.1432 found 691.1432.

Compound 3.21

Synthesized according to general procedure B using crude peptide AcCSC-NH_2 to give **3.21** (40%). ^{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-d6, 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 [M + H]$^+$ calc'd for $C_{16}H_{17}F_5N_4O_5S_2H$ 505.0639 found 505.0640.

Compound 3.22

A flask was charged with compound **3.18**, diluted with DMF (50 mM), and cooled to 0 °C. To the flask was added beta-D-thioglucose sodium salt (1 equiv) and stirred for 1 h. After completion the reaction was concentrated under reduced pressured and purified by RP-HPLC to give **3.22** (>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-d6, 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 [M + H]$^+$ calc'd for $C_{33}H_{38}F_4N_6O_{11}S_3H$ 867.1775 found 867.1776.

Compound 3.23

A flask was charged with compound **3.18**, diluted with DMF (50 mM), and cooled to 0 °C. To the flask was added coniferyl carbonate **3.27** (2 equiv), $KOSiMe_3$ (2 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 RP-HPLC to give **3.22** (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-d6, 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 $[M + H]^+$ calc'd for $C_{42}H_{46}F_4N_6O_{11}S_2H$ 951.2680 found 951.2686.

Compound 3.24

A flask was charged with compound **3.18**, diluted with DMF (50 mM), and cooled to 0 °C. To the flask was added NaN_3 (1 equiv) and stirred for 1 h. After completion the reaction was concentrated under reduced pressured and purified by RP-HPLC to give **3.24** (>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-d6, 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 $[M + H]^+$ calc'd for $C_{27}H_{27}F_4N_9O_6S_2H$ 714.1540 found 714.1542.

Compound 3.25

A flask was charged with compound **3.24**, 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. After completion the reaction was filtered with celite and concentrated under reduced pressure. Purification by RP-HPLC gave **3.25** (87%). ^{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-d6, 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 $[M + H]^+$ calc'd for $C_{27}H_{29}F_4N_7O_6S_2H$ 688.1635 found 688.1637.

Compound 3.26

A flask was charged with compound **3.24**, propargyl alcohol (1 equiv), phenylenediamine (15 mol %), sodium ascorbate (10 mol%), $CuSO_4$•5H2O (5 mol%) in 2:3 H_2O:tBuOH (0.2 M). The reaction was let stir for 12 h until observed completion by HPLC. After completion the reaction was concentrated under reduced pressure and purified by RP-HPLC gave **3.26** (75%). ^{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-d6, 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 $[M + H]^+$ calc'd for $C_{30}H_{31}F_4N_9O_7S_2H$ 770.1802 found 770.1806.

3-((tert-butyldimethylsilyl)oxy)-4-methoxybenzaldehyde (S3.1)

To a flame-dried flask with 3-hydroxy-4-methoxybenzaldehyde was added DMF (1 M) and iPr_2EtN (1.2 equiv). The reaction was cooled to 0 °C and added TBDMS-Cl (1.1 equiv) slowly then stirred for 18 h at rt. The reaction was diluted with H_2O then extracted with EtOAc. The combined organic phases were washed with brine (x3), dried over $NaSO_4$ concentrated in vacuo. The residue was purified by flash column chromatography (0-15% EtOAc/Hexanes) to give **S3.1** (97%) as a clear oil. ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.81 (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) δ 191.0, 156.7, 145.7, 130.3, 126.4, 120.1, 111.3, 55.6, 25.7, 18.5, -4.6 HRMS m/z $[M + H]^+$ calc'd for $C_{14}H_{22}O_3SiH$ 267.1416, found 267.1424.

ethyl (E)-3-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)acrylate (S3.2)

To a stirred solution of **S3.1** in DCM (30 mM) was added ethyl 2-(triphenyl-l5-phosphaneylidene)acetate (1.5 equiv) at room temperature under inert atmosphere. The reaction mixture was stirred at room temperature for 24 h. Solvent was removed under reduced pressure and residue was purified by flash column chromatography (0-15% EtOAc/Hexanes) to give **S.3.2** (70%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.58 (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) δ 167.5, 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 $[M + H]^+$ calc'd for $C_{18}H_{28}O_4SiH$ 337.1835, found 337.1836.

(E)-3-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)prop-2-en-1-ol (S3.3)

To a stirred solution of **S3.2** in DCM (0.2 M) was added DIBAL-H (3 equiv) at RT under inert atmosphere. The reaction mixture was stirred at RT for 24 h. After completion the reaction was concentrated under reduced pressure and the resulting residue was purified by flash column chromatography (0 – 20% EtOAc/Hex) to give **S3.3** (62%).^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.95 – 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) δ 151.0, 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 $[M + H]^+$ calc'd for $C_{16}H_{27}O_3Si$ 295.1729, found 295.1735.

(E)-3-(3-((tert-butyldimethylsilyl)oxy)-4-methoxyphenyl)allyl isobutyl carbonate (S3.4)

To a stirred solution of **S3.3** and N-methylmorpholine (4.5 equiv) in DCM (0.5 M) was cooled to -5 °C under argon. Isobutyl chloroformate (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, washed with $NaHCO_3$ (x2), brine, dried with $NaSO_4$, and concentrated under reduced pressure. 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 (3.27)

To a stirred solution of **S3.4** in DMF/H_2O (10:1) was added Cs_2CO_3 (0.5 equiv) and allowed to stir at RT until complete by TLC. The reaction was then diluted with EtOAc, washed with bring (x2), dried by $NaSO_4$, and concentrated under reduced pressure. The crude product was purified by flash column chromatography (0 – 30% EtOAc/Hex) to give **3.27** as a clear oil (75%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.01 (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 $[M + H]^+$ calc'd for $C_{15}H_{20}O_5H$ 281.1389, found 281.1394.

Compound S3.5

To a flask was added ethyl 2-aminothiazole-5-carboxylate, $NaNO_2$ (1.1 equiv), diluted with MeCN (1 equiv) and H_2O (1 equiv), and allowed to stir at 0 °C. Then CuBr (1.1equiv) in solution of HBr (1 equiv 1.23 M) was added dropwise at 0 °C. The reaction mixture was then allowed to gradually warm up to 23 ºC and allowed to stir for 16 h. The mixture was then diluted with ice/DCM and then transferred to an erlenmeyer flask. The solution was kept cold and then gradually quenched with solid $NaHCO_3$ and then transferred to a separatory funnel. The aqueous layer was then extracted with DCM (3 times), organic layers combined, washed with brine, dried over Na_2SO_4, filtered, and concentrated to afford crude product. Purification by FCC (0 – 2.5% EtOAc/Hex) afforded desired product **S3.5** as a faint yellow oil (62%). ^{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-d6) δ 159.47, 148.17, 142.19, 132.75, 61.94, 14.04. HRMS [M+1] calc'd for $C_6H_6BrNO_2SH$ 236.9375383, found 236.93706.

Compound S3.6

To a flask equipped with a reflux condenser was added **S3.5**, NaSH (2 equiv, 60% wt), then diluted with EtOH (1 equiv, 0.45 M). The reaction was left overnight, then allowed to cool to 23 °C and poured onto an iced solution of aq. 1N HCl. The mixture was then extracted with EtOAc (x4), organic layers combined, dried over $MgSO_4$, filtered, and concentrated to afford crude product as a white solid. Purification by FCC (15 – 30% EtOAc/Hex) afforded desired product **S3.6** as a white-solid (99%). ^{1}H-NMR (500 MHz, DMSO-d6) δ 13.80 (s, 1H), 8.08 (s, 1H), 4.24 (q, 2H), 1.25 (t, 3H). ^{13}C-NMR (500 MHz, DMSO-d6) δ 190.93, 158.87, 136.83, 118.59, 61.43, 14.08. HRMS [M+1] calc'd for $C_6H_6NO_2S_2H$ 189.9996, found 189.9998.

Compound 3.30

To a flask was added **S3.6** and diluted with anhydrous THF (0.14 M) and cooled to -78 °C. DIBAL-H (3 equiv, 1M) was then added dropwise at allowed to stir at -78 °C for 20 min then warmed to 0 °C and stirred for an additional 2 min. After 2 h, Rochelle's salt was added and allowed to stir at RT for 1 h. The reaction mixture was then filtered through celite and $NaSO_4$ and concentrated under reduced pressure to afford crude product. Purification by FCC (35 – 75% EtOAc/Hex) afforded desired product **3.30** as a white solid (90%). ^{1}H-NMR (500 MHz, DMSO-d6) δ 12.97 (s, 1H), 7.16 (s, 1H), 5.46 (s, 1H), 4.37 (s, 2H) ^{13}C-NMR (500 MHz, DMSO-d6) δ 188.59, 132.59, 125.19, 55.88. HRMS [M+1] calc'd for $C_4H_5NOS_2H$ 147.9891, found 147.9894.

Compound 3.31

To a stirred solution of crude peptide AcYACFAC-NH_2 in DMF (50 mM) was added Et_3N (3 equiv) and OFCP (1.5 equiv) at 0 °C. After stirring for 30 minutes the reaction was concentrated under reduced pressure. The crude product was then diluted with 16% DMF in THF (50 mM) and added compound **3.30** (1.1 equiv) followed by Me_3SiOK (3.5 equiv) at 0 °C. The mixture was then allowed to stir at RT until HPLC indicated full conversion. The reaction mixture was then cooled to 0 °C and quenched with AcOH (10 equiv), concentrated under reduced pressure, and purified by FCC 10 – 15% MeOH/$CHCl_3$ to give **3.31** (18%).

Compound 3.32

To a stirred solution of **3.31** in $MeNO_2$ (5 mM) was added TFA (1 equiv) dropwise. The reaction was then heated to 80 °C for 12 h. After completion the reaction was quenched with $NaHCO_3$ (xs), concentrated under reduced pressure then purified by RP-HPLC to afford **3.32** (52%). ^{1}H-

NMR (500 MHz, DMSO-d6) δ 9.5 (s, 1H), 8.47 (d, J = 8.2 Hz, 1H), 8.40 (d, J = 8.5 Hz, 1H), 8.17 (d, J = 7.9 Hz, 1H), 8.08 (d, J = 7.6 Hz, 1H), 7.95 (s, 2H), 7.65 (d, J = 7.6 Hz, 1H), 7.31 – 7.25 (m, 3H), 7.21 (q, J = 7.9 Hz, 3H), 7.16 (d, J = 7.5 Hz, 2H), 7.12 (d, J = 12.1 Hz, 2H), 6.86 (d, J = 7.2 Hz, 2H), 6.68 (d, J = 8.1 Hz, 2H), 4.86 (d, J = 5.7 Hz, 1H), 4.70 (d, J = 8.0 Hz, 1H), 4.62 – 4.50 (m, 2H), 4.44 (q, J = 7.5 Hz, 1H), 4.25 – 4.14 (m, 2H), 4.03 (d, J = 15.7 Hz, 1H), 3.90 (d, J = 15.7 Hz, 1H), 3.76 – 3.66 (m, 1H), 3.61 – 3.49 (m, 2H), 1.86 (s, 3H), 1.25 – 1.22 (m, 3H). 1.01 (d, J = 6.8 Hz, 3H). ^{13}C-NMR (500 MHz, DMSO-d6) δ 171.6, 171.3, 171.0, 169.3, 169.0, 168.8, 167.6, 153.2, 152.8, 143.3, 141.3, 136.6, 130.6, 129.4, 129.0, 128.4, 127.8, 126.6, 124.4, 124.4, 114.8, 55.4, 53.6, 53.6, 52.1, 48.4, 47.4, 46.5, 37.2, 36.1, 34.3, 32.9, 32.3, 22.5, 19.1, 17.3. HRMS [M+1] calc'd for $C_{41}H_{42}F_4N_8O_8S_4H$ 979.2023, found 979.2025.

Compound 3.33

3.33 was made based on procedures for compounds **3.31** and **3.32** to give a 25% yield over 2 steps. See NMR Spectra Appendix section below for crude NMR spectra of this compound. HRMS [M+1] calc'd for $C_{29}H_{28}F_4N_6O_6S_4H$ 761.0968, found 761.0970.

Compound 3.34

A flask was charged with compound crude peptide AcYGHAC-NH_2 diluted with DMF (10 mM), Et_3N (1.5 equiv), and cooled to 0 °C. To the flask was added OFCP (3 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 **3.27** (4 equiv), Cs_2CO_3 (6 equiv), and let stir until completion observed by HPLC. After completion the reaction was quenched with AcOH (15 equiv), concentrated under reduced pressured and purified by RP-HPLC to give **3.34** (20%).

Compound 3.35

To a flask was added **3.34** and diluted with dry $MeNO_2$ (5 mM). Tf_2NH (5 equiv) in dry $MeNO_2$ (5 mM) was then added under argon. The reaction mixture was then allowed to stir at RT for 1 h or until completion observed by HPLC. Afterwards, the reaction mixture was quenched with iPr_2Net (10 equiv) and concentrated under reduced pressure to afford crude product. Purification by RP-HPLC afforded pure **3.35** (45%) as a TFA salt.^{1}H NMR (DMSO-d_6, 500 MHz) δ 9.15 (bs, 1H), 9.02 (s, 1H), 8.65 (d, J = 7.5 Hz, 1H), 8.40 (t, J = 6.2 Hz, 1H), 8.05 (d, J = 7.7 Hz, 1H), 7.89 (d, J = 6.6 Hz, 1H), 7.45 – 7.33 (m, 1H), 7.06 – 7.0 (m, 2H), 6.96 (s, 1H), 6.90 – 6.83 (m, 3H), 6.70 (d, J = 8.1 Hz, 1H), 6.54 (d, J = 9.3 Hz, 1H), 6.18 (dt, J = 15.8, 6.3 Hz), 6.07 (d, J = 15.8 Hz, 1H), 4.61 – 4.53 (m, 2H), 4.35 (q, J = 7.0, 1H), 4.14 (quint, J = 7.4 Hz, 1H), 4.01 (dd, J = 15.9, 7.0 Hz), 3.80 (s, 3H), 3.42 – 3.33 (m, 4H), 2.94 – 2.89 (m, 1H), 2.77 – 2.71 (m, 3H), 1.84 (s, 3H), 1.21 (d, J = 7.3 Hz, 3H). HRMS [M+1] calc'd for $C_{40}H_{40}F_4N_8OSH$ 885.2653, found 885.2684.

Compound 3.36

A flask was charged with compound crude peptide AcWCASC-NH_2, diluted with DMF (10 mM), added Et_3N (1.5 equiv), and cooled to 0 °C. To the flask was added OFCP (3 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 **3.27** (4 equiv), Cs_2CO_3 (6 equiv), and let stir until completion observed by HPLC. After completion the reaction was quenched with AcOH (15 equiv), concentrated under reduced pressured and purified by RP-HPLC. The acyclic intermediate was then diluted with dry $MeNO_2$ (5 mM). To the flask was added $ScOTf_3$ (2 equiv) and let stir until completion observed by HPLC. Upon completion, the mixture was concentrated under reduced pressure and purified by RP-HPLC to obtain **3.36** (35%) and two other regioisomeric products for a 70% combined yield.

^{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-d6) 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] calc'd for $C_{40}H_{41}F_4N_7O_9SH$ 904.2422, found 904.2423.

Compound 3.38

To a stirred solution of crude peptide AcAsp(CSY)-Gly-Ser-Ala-Cys-NH_2 in dry DMF (10 mM) was added Et_3N (3 equiv), then OFCP (1.5 equiv) dropwise at 0 °C. The reaction was allowed to stir at 0 °C for 30 min until completion of macrocycle intermediate formation. The reaction was then concentrated under reduced pressure to remove excess Et_3N, OFCP, and MeCN. The mixture was then cooled to 0 °C and added Me_3SiOK (2 equiv) and allowed to stir at RT for 30 min. Then cysteamine (1.1 equiv) was added and let stir for 15 minutes. After completion as observed by HPLC, the reaction was quenched with AcOH (10 equiv) and concentrated under reduced pressure to afford crude product. Purification by FCC (5 -10% MeOH/CHCl3.) afforded acyclic intermediate macrocycle. This material was added to a flask diluted with 2:8 MeCN:NaOAc/AcOH (aq) (1 mM) then added NCS in MeCN (1 equiv, 10 mM) in two 0.5 equiv portions. After completion as observed by HPLC the reaction was concentrated under reduced pressure. Purification by RP-HPLC afforded **3.38**. Material was pushed forward.

Compound 3.39

To a stirred solution of **3.38** in dry DMF (5 mM) was added iPr_2Net (3.5 equiv) and allowed to cool to 0 °C. Then T3P (1.5 equiv) was added and the reaction mixture was allowed to stir at 0 °C for 30 minutes. Upon completion as observed by HPLC, the mixture was quenched with AcOH (5 equiv). The reaction was then concentrated under reduced pressure to afford an orange oil as crude product. Purification by FCC (5 – 10% MeOH/$CHCl_3$) afforded **3.39** (50%; 30% from linear peptide SM). ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.6 (J = 4.1 Hz, 1H), 8.06 (d, J = 7.7 Hz, 1H), 7.92 (d, J = 7.7 Hz, 1H), 7.81 – 7.73 (m, 1H), 7.56 – 7.52 (s, 1H), 7.50 (d, J = 7.6 Hz, 1H), 7.41 (s, 1H), 7.31 – 7.27 (m, 2H), 7.27 – 7.19 (m, 4H), 4.95 (d, J = 7.3 Hz, 1H), 4.83 (d, J = 9.8 Hz, 1H), 4.56 - 4.48 (m, 1H), 4.26 – 4.14 (m, 5H), 4.10 – 4.04 (m, 2H), 3.37 – 3.26 (m, 3H), 3.18 – 3.07 (m, 2H), 3.04 – 2.97 (m, 2H), 1.80 (s, 3H), 1.16 (d, J = 6.7 Hz, 3H). ^{13}C-NMR (500 MHz, DMSO-d6) δ 171.4, 171.2, 170.9, 168.9, 168.6, 168.3, 167.8, 162.3, 136.9, 129.0, 128.3, 126.6, 57.3, 56.4, 52.6, 50.4, 48.5, 41.6, 41.1, 36.1, 35.8, 32.0, 30.8, 30.3, 22.5, 16.0. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -102.7 – (104.2) (m, 1F), -107.0 – (-108.3) (m, 1F), -114.6 – (-115.9) (m, 1F), -114.4 – (-120.8) (m, 1F). HRMS [M+1] calc'd for $C_{33}H_{38}F_4N_8O_9S_2H$ 831.2218, found 831.2220.

Compound 3.40

To a stirred solution of resorcinol (2 equiv) in acetone (0.5 M) was added benzyl chloride (1 equiv) and K_2CO_3 (1 equiv) then allowed to reflux overnight. After completion as observed by TLC the reaction was filtered and concentrated under reduced pressure then purified by FCC (10 – 40% EtOAc/Hex) to afford **3.40** (65%). ^{1}H and ^{13}C NMR were identical to literature reported spectra.

Compound 3.41

To a stirred solution of **3.40** in DCM (25 mM) was added NBS (1 equiv) at 0 °C. The reaction was stirred at RT for 2 h, then washed with water, separated the organic layer, dried over Na_2SO_4 and

concentrated under reduced pressure. Purification by FCC (2 – 10% EtOAc/Hex) afforded **3.41**. ^{1}H and ^{13}C NMR were identical to literature reported spectra.

Compound 3.42[S1]

A stirred solution of **3.41** and HMDS (2 equiv) in THF (2.9 M) was refluxed for 90 min. The solvent was evaporated under reduced pressure, and the residue was subjected to vacuum to remove excess NH_3 and unreacted HMDS. The crude product was then dissolved in THF (0.78 M), cooled to -100 °C and n-BuLi (2 equiv) was added dropwise. The mixture was stirred for 20 min while the temperature reached -80 °C. Then the mixture was cooled again to -100 °C, and added Tf_2O (2 equiv) dropwise while continuing to stir for 20 min while the temperature reached -80 °C. Cold sat. aq. $NaHCO_3$ was added, the phases separated and extracted with Et_2O. The combined organic layers were dried with Na_2SO_4, filtered and concentrated under reduced pressure. Purification by FCC afforded **3.42** (0 – 31%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.47 – 7.32 (m, 6H), 7.02 – 6.93 (m, 2H), 5.08 (s, 2H), 0.34 (s, 9H). ^{13}C-NMR (500 MHz, $CDCl_3$) δ 161.1, 155.7, 136.9, 136.1, 128.9, 127.7, 123.4, 114.0, 107.2, 70.6, -0.7.

Compound 3.43

Original Route

3.42 was dissolved in MeOH (40 mM). Raney Ni slurry was weighed out (~1g), added to the reaction mixture, flushed with argon and allowed to stir at RT. After 1 h the reaction showed completion. The mixture was then filtered with celite and concentrated under reduced pressure. Purification by FCC (100% DCM) to give **3.43** as a clear oil (40%).

Optimized Route[S2]

To a stirred solution of 4-bromoresorcinol in THF (0.53 M) under inert atmosphere was added HMDS (2.2 equiv). The resulting solution was heated to reflux for 12 h, then concentrated under reduced pressure. The crude material was dissolved in anhydrous THF (0.4 M) and cooled to -78 °C under inert atmosphere. nBuLi (1.1 equiv) was slowly added dropwise, then Tf_2O (1.5 equiv) was added and stirred for 10 min. The reaction was then quenched with 5% aq. HCl. The resulting mixture was extracted with EtOAc, organic layers combined washed with sat. $NaHCO_3$, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by FCC (5% EtOAc/Hex) afforded **3.43** as a clear oil (15 – 45%). ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37 (d, J = 8.2 Hz, 1H), 6.87 (d, J = 2.16 hz, 1H), 6.82 (dd, J = 8.2, 2.2 Hz, 1H), 0.33 (s, 9H).

Compound 3.46

To a stirred solution of **3.45** in 20% DMF/THF (50 mM) was added **3.43** (10 equiv) and Me_3SiOK (2 equiv) at 0 °C. The reaction was allowed to stir until complete as observed by HPLC. Upon completion the reaction was quenched with AcOH (5 equiv) and concentrated under reduced pressure. Purification by FCC (0 – 10% MeOH/$CHCl_3$) afforded **3.46**. ^{1}H NMR (DMSO-d_6, 300 MHz) δ 10.81 (s, 1H), 8.57 (d, J = 8.2 Hz, 1H), 8.38 (d, J = 9.0 Hz, 1H), 8.29 (d, J = 8.1 Hz, 1H), 8.01 (d, J = 7.6 Hz, 1H), 7.64 (d, J = 8.4 Hz, 1H), 7.53 (d, J = 7.8 Hz, 1H), 7.36 (dd, J = 8.3, 2.2 Hz, 1H), 7.31 (d, J = 8.0 Hz, 1H), 7.22 – 7.13 (m, 2H), 7.13 – 6.91 (m, 3H), 5.06 – 4.94 (m, 1H), 4.89 – 4.79 (m, 1H), 4.68 – 4.56 (m, 1H), 4.52 – 4.42 (m, 1H), 4.41 – 4.32 (m, 1H), 4.32 – 4.19 (m, 1H), 3.92 – 3.80 (m, 1H), 3.68 – 3.56 (m, 1H), 3.56 – 3.45 (m, 1H), 3.23 – 2.93 (m, 1H), 2.92 – 2.71 (m, 1H), 1.74 (s, 3H), 1.23 (d, J = 7.3 Hz, 3H), 0.31 (s, 9H).

Compound 3.47[S3]

To a solution of 3-bromophenol in THF (0.25 M) was added nBuLi (2.7 eq.) at -78 °C dropwise. The reaction mixture was stirred at this temperature for 30 min, then added TMSCl (3 equiv) and the solution was allowed to reach room temperature. The reaction was quenched with sat. aq. NH_4Cl, extracted with EtOAc (x3), dried combined organic layers over Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by FCC (100% Hex) afforded **3.47** (98%). ^1H and ^{13}C NMR were identical to literature reported spectra.[S4]

Compound 3.48[S5]

To a solution of **3.47** in AcOH (1.19 M) was added a solution of Br_2 in AcOH (6.2 M) at 0 °C. After 30 min the reaction mixture was transferred to a separatory funnel and washed with water (x5), extracted with EtOAc, dried over Na_2SO_4 and concentrated under reduced pressure. Purification by FCC (0-5% EtOAc/Hex) afforded **3.48** (44%). ^1H NMR ($CDCl_3$, 500 MHz) δ 7.37 (d, J = 8.52 Hz, 1H), 6.91 (d, J = 3.12 Hz, 1H), 6.69 (dd, J = 8.5, 3.2 Hz, 1H), 4.91 (bs, 1H), 0.34 (s, 9H). ^{13}C NMR ($CDCl_3$) δ 154.3, 142.7, 133.9, 123.0, 120.8, 117.9, -0.57.

Compound 3.50

To a stirred solution of **2.5** in DMF (0.1 M) was added **3.48** (1.1 equiv) and Cs_2CO_3 (1.1 equiv) at 0 °C and allowed to stir until completion as observed by TLC. Upon completion, the reaction mixture was quenched with AcOH (5 equiv) concentrated under reduced pressure. Purification by FCC (100% Hex) afforded **3.50** (57%). ^1H NMR ($CDCl_3$, 500 MHz) δ 7.31 (d, J = 8.6 Hz, 1H), 7.32 – 7.12 (m, 2H), 7.08 – 7.0 (m, 1H), 6.69 – 6.60 (m, 3H), 6.60 – 6.53 (m, 1H), 0.31 (s, 1H).

Compound 3.51

A stirred solution of 2-Bromo-4-methylphenol and HMDS (1.1 equiv) in THF (2.9 M) was refluxed for 90 min. The solvent was evaporated under reduced pressure, and the residue was subjected to vacuum to remove excess NH_3 and unreacted HMDS. The crude product was then dissolved in THF (0.78 M), cooled to -100 °C and n-BuLi (1.1 equiv) was added dropwise. The mixture was stirred for 20 min while the temperature reached -80 °C. Then the mixture was cooled again to -100 °C, and added Tf_2O (1.5 equiv) dropwise while continuing to stir for 20 min while the temperature reached -80 °C. Cold sat. aq. $NaHCO_3$ was added, the phases separated and extracted with Et_2O. The combined organic layers were dried with Na_2SO_4, filtered and concentrated under reduced pressure. Purification by FCC (0 – 5% EtOAc/Hex) afforded **3.51** (65%).^1H and ^{13}C NMR were identical to literature reported spectra.

Compound 3.52 and 3.53

To a stirred solution of **3.51** in MeCN (0.1 M) was added pyrrolidine (2 equiv) and CsF (2 equiv) at 0 °C. The reaction was stirred until completion as observed by TLC. Upon completion the mixture was washed with sat. aq. $NaHCO_3$, extracted with EtOAc, dried over Na_2SO_4, and concentrated under reduced pressure. Purification by (0 – 10% EtOAc/Hex) afforded regioisomers **3.52** and **3.53** (62% combined). ^1H and ^{13}C NMR were identical to literature reported spectra.

Compound 3.57

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 equiv) and let stir at RT. After reaction completion as observed by HPLC the mixtue was quenched with AcOH (10 equiv) and concentrated under reduced pressure. Purification by FCC

(0 - 10% MeOH/$CHCl_3$) afforded **3.57** (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-d6) 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 3.58

To a stirred solution of crude peptide des-aminoTyr-Pro-Cys-Val-Ala-Cys-NH_2 in dry DMF (5 mM) was added Et_3N (3 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 Cs_2CO_3 (8 equiv) and let stir at RT. After reaction completion as observed by HPLC the mixture was quenched with AcOH (20 equiv) and concentrated under reduced pressure. Purification by FCC (0 - 10% MeOH/$CHCl_3$) afforded **3.58** (26%).[Note: rotational isomers present] ^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.31 (d, J = 9.5 Hz, 1H), 7.90 (d, J = 7.66 Hz, 1H), 7.70 (d, J = 6.9 Hz, 1H), 7.56 (d, J = 9.0 Hz, 1H), 7.42 (d, J = 8.4, 1H), 7.35 (bs, 1H), 7.30 (d, J = 8.3, 1H), 7.22 – 7.14 (m, 1H), 6.97 – 6.90 (m, 1H), 4.81 (d, J= 7.8 Hz, 1H), 4.71 (d, J = 6.6 Hz, 1H), 4.40 (d, J = 6.7 Hz, 1H), 4.34 – 4.28 (m, 1H), 4.25 – 4.18 (m, 1H), 3.94 – 3.88 (m, 1H), 3.64 – 3.53 (m, 2H), 3.51 – 3.41 (m, 1H), 3.07 – 2.97 (m, 1H), 2.86 (d, J = 4.8 Hz, 3H), 2.82 (d, J = 3.8 Hz, 1H), 2.78 (d, J = 14.5 Hz, 1H), 2.75 – 2.68 (m, 1H), 2.22 – 2.15 (m, 1H), 2.08 – 1.93 (m, 2H), 1.88 – 1.82 (m, 1H), 1.70 – 1.63 (m, 2H), 1.31 (d, J = 7.4 Hz, 3H), 0.80 (dd, J = 15.9 , 6.5 Hz, 6H). ^{13}C-NMR (500 MHz, DMSO-d6) δ 172. 2, 171.9, 171.4, 170.4, 168.5, 167.6, 152.8, 137.8, 131.5, 128.9, 128.4, 127.8, 118.6, 115.6, 60.1, 58.8, 55.1, 52.1, 49.5, 48.4, 47.3, 35.5, 35.3, 35.2, 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 -106.2 – (-107.4) (m, 1F), -113.0 – (-114.0) (m, 1F), -119.1 – (-120.8) (m, 2F). HRMS [M+1] calc'd for $C_{34}H_{40}F_4N_6O_7S_2H$ 785.2414, found 785.2414.

Compound 3.59

To a stirred solution of Ac-Cys-Thr-Phe-Cys-NHMe in DMF (5 mM) was added Et_3N (2.5 equiv) and OFCP (1.5 equiv) dropwise 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 Cs_2CO_3 (4 equiv), phenol (1.5 equiv) and let stir at RT. After reaction completion as observed by HPLC the mixture was quenched with AcOH (10 equiv) and concentrated under reduced pressure. Purification by FCC (0 - 10% MeOH/$CHCl_3$) afforded **3.59**.

Compound S3.7

To a stirred solution of **3.18** 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 equiv) and concentrated under reduced pressure. Purification by RP-HPLC afforded the dimerized product **S3.7** (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-d6) 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 S3.11

To a stirred solution of crude peptide AcCysSerCys-NH_2 in DMF (5 mM) was added Et_3N (2.5 equiv) and OFCP (1.5 equiv) at 0 °C. The reaction was allowed to stir until completion as observed by HPLC. Upon completion, the reaction was concentrated under reduced pressure and purified by FCC (0 – 10% MeOH/$CHCl_3$) to give **S3.11** (55%). 1H NMR (DMSO-d_6, 500 MHz) δ 8.63 (d, J = 9.2 Hz, 1H), 8.22 (d, J = 7.4 Hz, 1H), 8.12 (d, J = 8.9 Hz, 1H), 7.41 (s, 1H), 7.23 (s, 1H), 4.99 (t, J = 5.4 Hz, 1H), 4.75 (q, J = 6.2 Hz, 1H), 4.63 – 4.48 (m, 2H), 3.67 – 3.50 (m, 2H), 3.45 (dd, J = 14.0, 4.1 Hz, 1H), 3.36 – 3.34 (m, 1H), 3.24 (dd, J = 13.9, 8.7 Hz, 1H), 1.89 (s, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.0, 170.5, 169.2, 168.9, 143.2 – 142.2 (m, 1C), 138.2 – 137.3 (m, 1C), 119.0 – 110.3 (m, 3C), 60.9, 54.5, 51.9, 50.6, 35.5, 34.8, 22.4. ^{19}F NMR (DMSO-d_6, 282 MHz) δ -104.7 – -108.3 (m, 4F), -128.8 (q, J = 260 Hz, 2F). HRMS m/z $[M + H]^+$ calc'd for $C_{16}H_{18}F_6N_4O_5S_2H$ 525.0701, found 525.0712.

Compound S3.12

Synthesized according to General Procedure B using crude peptide AcCysValTrpSerCys-NH_2 and purified by FCC (0 – 10% MeOH/$CHCl_3$) to give **S3.12** (25%).^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.86 (s, 1H), 8.40 (d, J = 8.4 Hz, 1H), 8.29 (d, J = 8.4 Hz, 1H), 8.10 (d, J = 8.5 hz, 1H), 7.95 (d, J = 6.0 Hz, 1H), 7.84 (d, J = 6.4 Hz, 1H), 7.56 (d, J = 7.9 Hz, 1H), 7.33 (d, J = 8.1 Hz, 2H), 7.20 (s, J = 8.1 Hz, 1H), 7.06 (t, J = 7.5 Hz, 1H), 7.0 (t, J = 7.3 Hz, 2H), 4.88 (s, 1H), 4.74 (q, J = 7.7 Hz, 1H), 4.45 – 4.43 (m, 2H), 4.22 – 3.98 (m, 3H), 3.77 – 3.68 (m, 1H), 3.57 – 3.45 (m, 2H), 3.31 – 3.22 (m, 2H), 3.22 – 3.13 (m, 4H), 3.07 – 2.99 (m, 1H), 1.85 (s, 3H), 0.67 (dd, 6H).^{13}C NMR (DMSO-d_6, 126 MHz) δ 171.4, 171.3, 169.6, 169.4, 169.3, 168.5, 136.1, 127.3, 123.6, 121.0, 118.3, 111.3, 109.6, 60.7, 60.1, 58.8, 55.4, 54.9, 54.4, 52.0, 48.6, 48.4, 33.3, 30.7, 30.3, 29.2, 29.1, 27.6, 22.3, 19.2, 17.3.

Compound S3.13

To a stirred solution of crude peptide AcTyr(OMe)CysAlaSerCys-NH_2 in DMF (5 mM) was added Et_3N (2.5 equiv) and OFCP (1.5 equiv) at 0 °C. The reaction was allowed to stir until completion as observed by HPLC. Upon completion, the reaction was concentrated under reduced pressure and purified by FCC (0 – 10% MeOH/$CHCl_3$) to give **S3.13** (42%).^{1}H NMR (DMSO-d_6, 500 MHz) δ 8.70 (d, J = 9.4 Hz, 1H), 8.63 (d, J = 7.3 Hz, 1H), 8.25 (d, J = 7.4 Hz, 1H), 8.11 (d, J = 8.32 Hz, 1H), 7.51 (s, 1H), 7.25 (d, J = 7.2 Hz, 1H), 7.24 (s, 1H), 7.14 (d, 2H), 6.80 (d, J = 8.7 Hz, 2H), 5.48 (bs, 1H), 4.73 (q, J = 5.9 Hz, 1H), 4.53 – 4.34 (m, 3H), 4.32 – 4.24 (m, 1H), 4.10 (quint, J = 7.3 Hz, 1H), 3.78 (dd, J = 14.7, 3.0 Hz, 1H), 3.70 (s, 3H), 3.69 – 3.63 (m, 1H), 3.53 – 3.42 (m, 2H), 2.91 – 2.81 (m, 2H), 2.65 (dd, J = 13.8, 9.7 Hz, 1H), 1.73 (s, 3H), 1.27 (d, J = 7.4 Hz, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.1, 171.4, 171.0, 169.2, 167.9, 157.8, 130.1, 129.6, 113.4, 61.8, 54.9, 54.0, 50.7, 50.5, 50.3, 36.4, 34.0, 32.2, 30.7, 22.4, 17.3.

Compound S3.19

Synthesized according to General Procedure B using crude peptide AcTrpHisAlaSerCys-NH_2 and purified by FCC (0 – 10% MeOH/$CHCl_3$) to give **S3.13** (23%).^{1}H NMR (DMSO-d_6, 500 MHz) δ 10.90 (s, 1H), 9.50 (s, 1H), 8.54 (d, J = 7.4 Hz, 1H), 8.26 (d, J = 6.5 Hz, 1H), 8.03 (s, 1H), 7.99 (d, J = 7.0 Hz, 1H), 7.59 (d, J = 7.6 Hz, 1H), 7.50 (s, 1H), 7.40 – 7.27 (m, 3H), 7.18 (s, 1H), 7.09 – 6.93 (m, 3H), 4.97 (s, 1H), 4.58 – 4.45 (m, 2H), 4.45 – 4.35 (m, 1H), 4.13 – 4.04 (m, 1H), 3.98 – 3.90 (m, 1H), 3.77 – 3.65 (m, 2H), 3.22 – 3.12 (m, 3H), 3.01 – 2.92 (m, 1H), 1.84 (s, 3H), 1.23 (d, J = 6.5, 3H). ^{13}C NMR (DMSO-d_6, 126 MHz) δ 172.7, 172.1, 171.5, 170.2, 167.9, 140.2, 137.9, 136.1, 127.2, 123.6, 120.9, 118.3, 116.1, 111.4, 111.1, 110.1, 60.4, 56.2, 54.3, 51.6, 50.1, 48.0, 45.6, 31.1, 28.9, 27.1, 22.6, 17.3.

Parallel Artificial Membrane Permeability Assay (PAMPA) Experiment

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 hours. 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 x 10^{-6}.

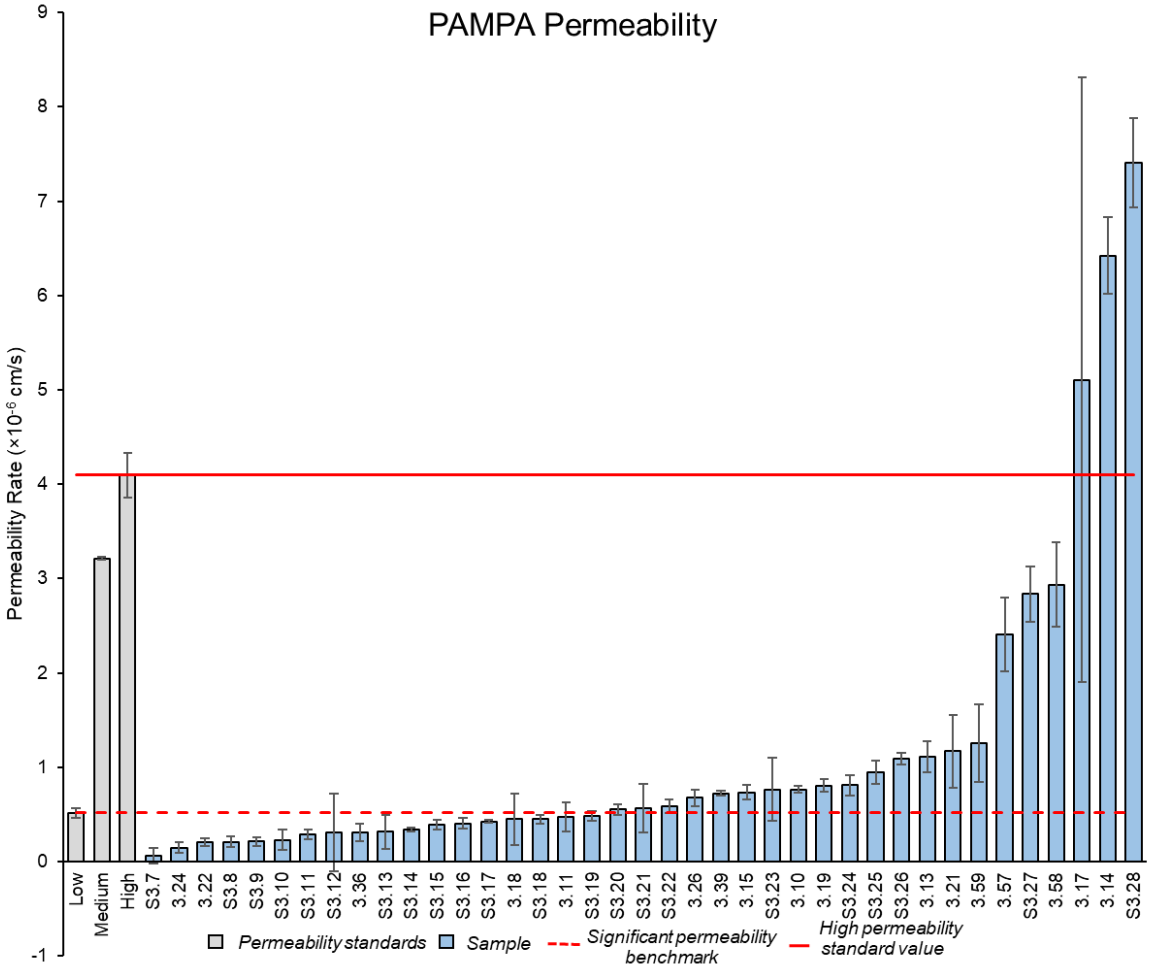


Figure S3.1. Permeability rates of full library including permeability standards.

Compound #	Mean Pe	Stdev
Low	5.17E-07	4.73E-08
Medium	3.21E-06	2.94E-07
High	4.09E-06	4.48E-07
S3.7	5.99E-08	5.21E-08
3.24	1.47E-07	1.82E-08
3.22	2.04E-07	2.39E-07
S3.8	2.09E-07	8.56E-08
S3.9	2.10E-07	5.65E-08
S3.10	2.26E-07	3.89E-08
S3.11	2.84E-07	5.46E-08
S3.12	3.06E-07	4.65E-08
3.36	3.07E-07	1.08E-07
S3.13	3.15E-07	5.05E-08
S3.14	3.40E-07	4.15E-07
S3.15	3.90E-07	9.41E-08
S3.16	4.05E-07	1.80E-07
S3.17	4.25E-07	1.87E-08
3.18	4.48E-07	5.02E-08
S3.18	4.49E-07	5.60E-08
3.11	4.73E-07	1.87E-08
S3.19	4.82E-07	2.69E-07
S3.20	5.51E-07	1.53E-07
S3.21	5.67E-07	4.90E-08
S3.22	5.86E-07	5.38E-08
3.26	6.77E-07	2.55E-07
3.39	7.22E-07	7.14E-08
3.15	7.34E-07	8.83E-08
S3.23	7.61E-07	2.63E-08
3.10	7.65E-07	7.58E-08
3.19	8.03E-07	3.34E-07
S3.24	8.08E-07	3.65E-08
S3.25	9.41E-07	6.74E-08
S3.26	1.09E-06	1.10E-07
3.13	1.11E-06	1.24E-07
3.21	1.17E-06	6.17E-08
3.59	1.26E-06	1.69E-07
3.57	2.41E-06	3.85E-07
S3.27	2.84E-06	4.15E-07
3.58	2.94E-06	3.88E-07
3.17	5.11E-06	3.21E-06
3.14	6.42E-06	4.05E-07
S3.28	7.41E-06	4.75E-07

Table S3.1. Permeability rates and standard deviations of full library including permeability standards.

Stock Solution Library Vial	NB/P	Compound #	PAMPA Donor Plate Well	Duplicate
A1	SB-IV-60	S3.12	A1	D10
A2	SB-IV-37	S3.11	A2	D11
A3	SB-IV-39	3.21	A3	D12
A4	SB-IV-20	S3.19	A4	E1
A5	SB-IV-102	3.19	A5	E2
A6	SB-IV-56	3.22	A6	E3
A7	SB-IV-282	S3.13	A7	E4
A8	SB-IV-121	S3.15	A8	E5
A9	SB-V-54	3.57	A9	E6
A10	SB-V-90	3.18	A10	E7
B1	SB-V-87	S3.7	A11	E8
B2	SB-V-79	3.17	A12	E9
B3	SB-IV-303	3.13	B1	E10
B4	SB-V-8	3.11	B2	E11
B5	SB-V-9	3.10	B3	E12
B6	SB-V-24	3.15	B4	F1
B7	IV-I-20	3.14	B5	F2
B8	IV-I-21	S3.18	B6	F3
B9	SB-V-96A	3.36	B7	F4
B10	SB-V-99A	3.26	B8	F5
C1	AM-2022-207	S3.9	B9	F6
C2	AM-2022-218	3.58	B10	F7
C3	AM-2022-228	3.39	B11	F8
C4	AM-2022-234	S3.22	B12	F9
C5	SB-IV-148	3.24	C1	F10
C6	TT-I-122	S3.8	C2	F11
C7	TT-I-145	S3.10	C3	F12
C8	TT-II-11	S3.23	C4	G1
C9	TT-II-19	S3.17	C5	G2
C10	TT-II-59	S3.26	C6	G3
D1	TT-II-60	S3.24	C7	G4
D2	TT-II-78-1	S3.14	C8	G5
D3	TT-II-78-2	S3.16	C9	G6
D4	TT-II-89	S3.21	C10	G7
D5	TT-II-114-2	3.59	C11	G8
D6	TT-II-120	S3.27	C12	G9
D7	TT-II-123	S3.20	D1	G10
D8	TT-I-74	S3.28	D2	G11
D9	TT-I-81	S3.25	D3	G12
D10	SB-I-34	4.30	D4	H1
E1	TR-7-85	4.13	D5	H2
E2	BC-V-251	4.19	D6	H3
E3	Low Perm. Stnd.	Theophylline	D7	H4
E4	Med. Perm. Stnd.	Diclofenac	D8	H5
E5	High Perm. Stnd.	Chloraphenicol	D9	H6

Table S3.2. Compound numbers and notebook pages corresponding to wells of 96-well plate

Raw Experimental Absorbance Data

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 Standard Absorbances

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

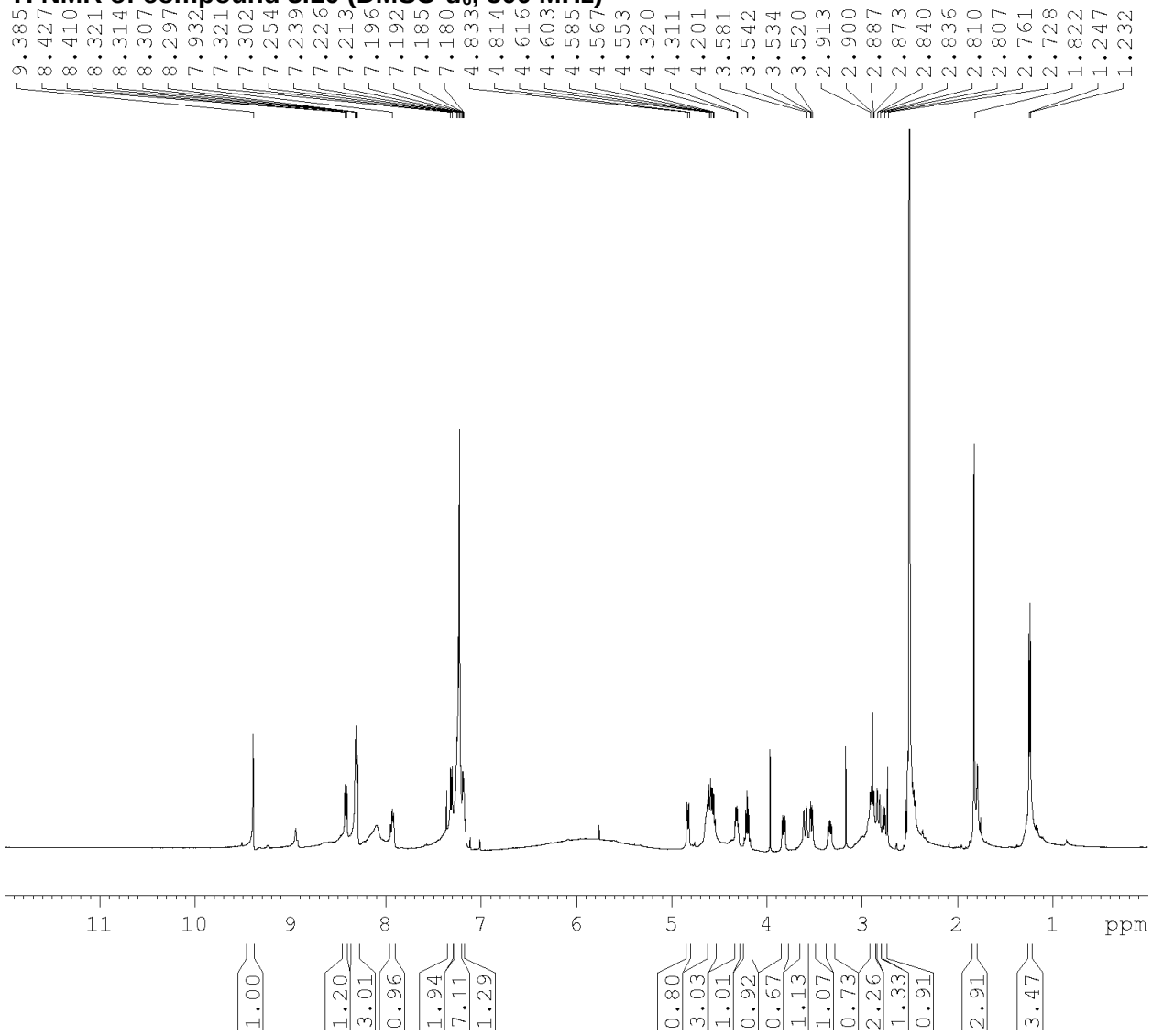
Wavelength/Well	**C9**	**C10**	**C11**	**C12**	**D1**	**D2**	**D3**	**D4**
230	1.413	1.837	1.418	1.349	1.628	1.572	1.637	2.542
240	0.334	0.481	0.299	0.15	0.481	0.144	0.332	1.433
250	0.28	0.331	0.241	0.095	0.418	0.077	0.26	1.386
260	0.292	0.285	0.236	0.085	0.412	0.064	0.24	1.417
270	0.287	0.264	0.222	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

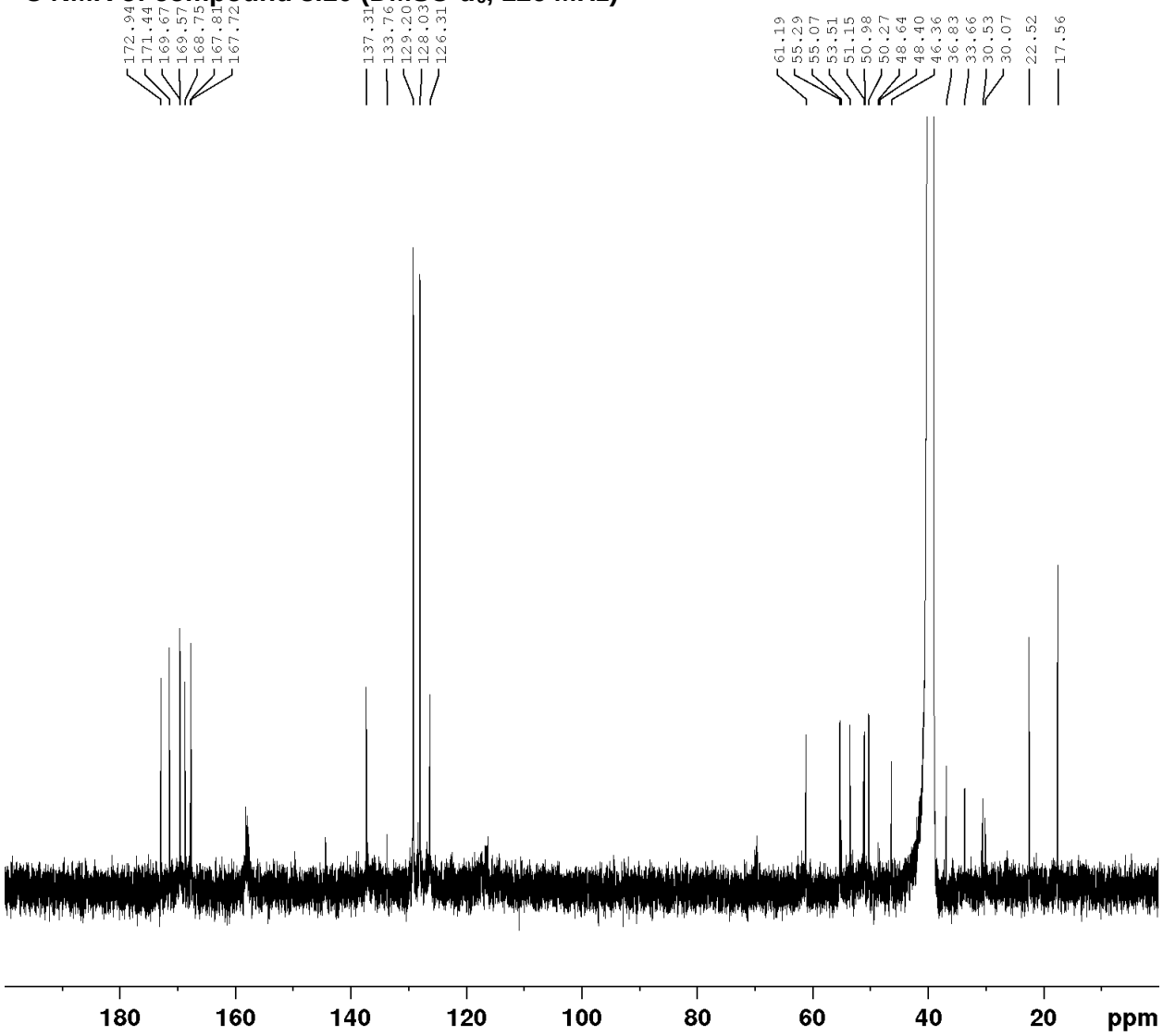
Wavelength/Well	**D5**	**D6**	**D7**	**D8**	**D9**	**D10**
230	1.603	1.828	1.936	2.232	1.801	1.493
240	0.393	0.713	0.361	0.586	0.368	0.122
250	0.42	0.715	0.343	0.411	0.373	0.06
260	0.402	0.732	0.568	0.525	0.519	0.042
270	0.26	0.639	0.773	0.676	0.668	0.032
280	0.135	0.467	0.649	0.704	0.718	0.028
290	0.09	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

NMR Spectra

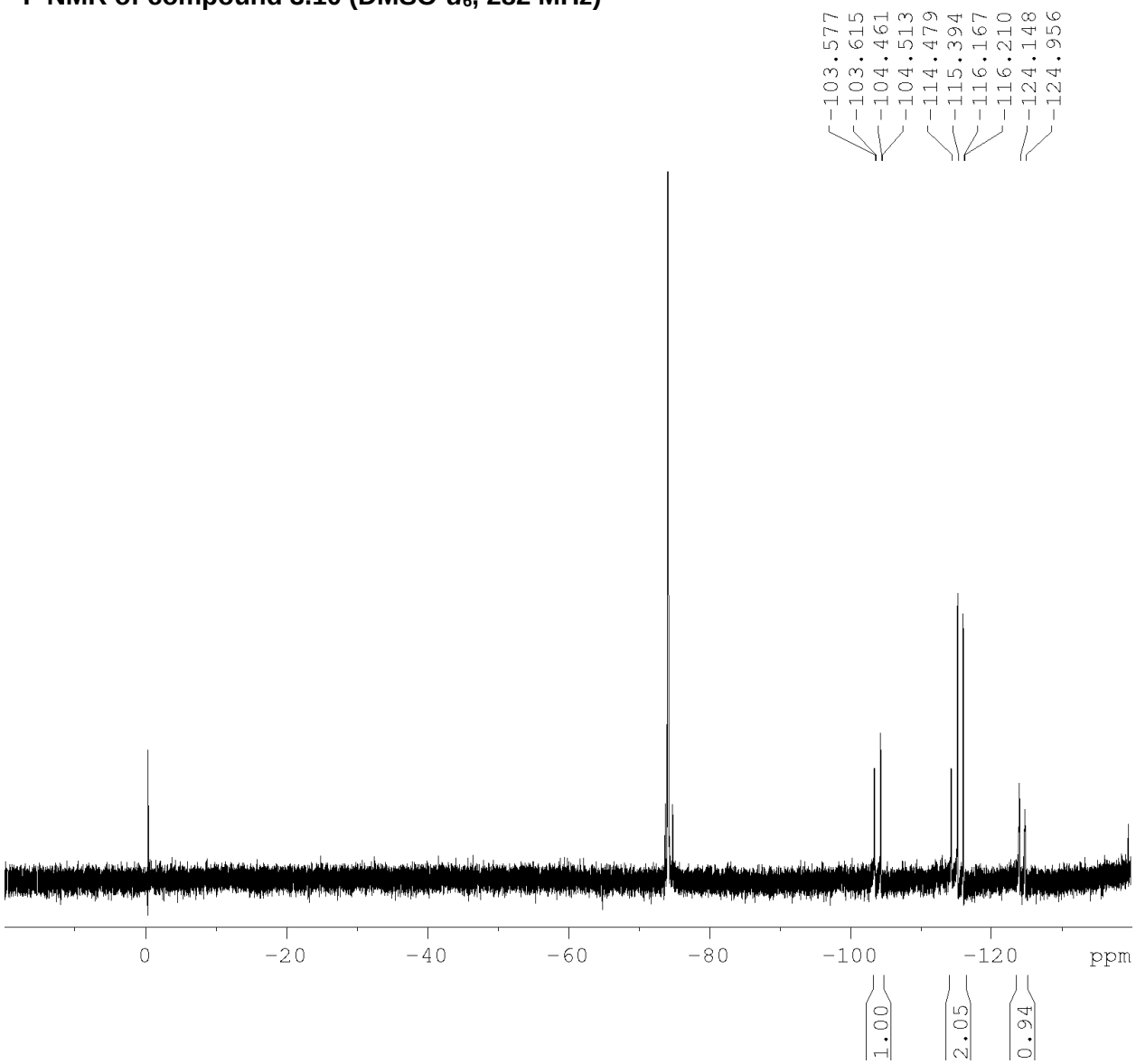
^{1}H NMR of compound 3.10 (DMSO-d_6, 500 MHz)



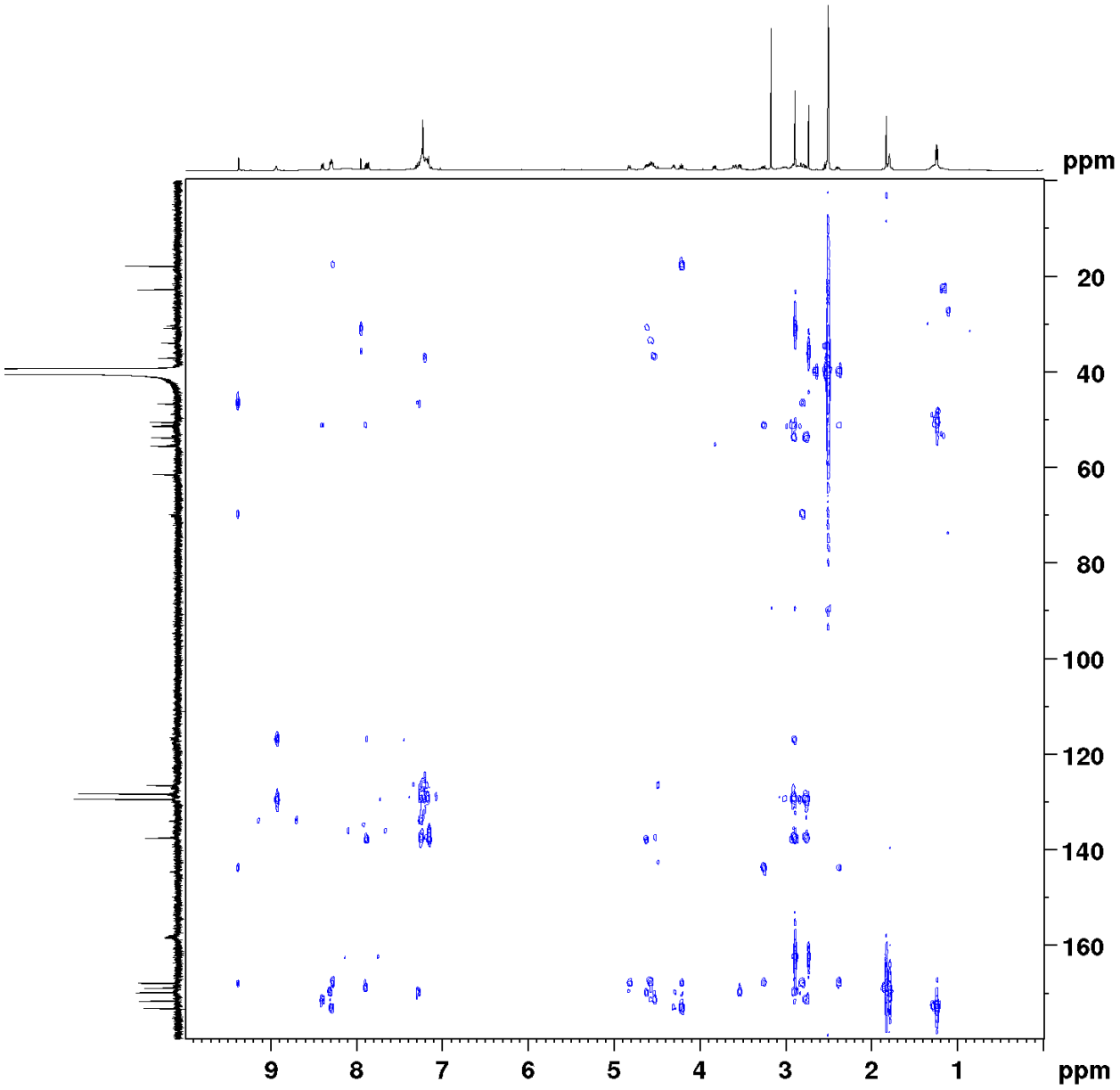
^{13}C NMR of compound 3.10 (DMSO-d_6, 126 MHz)



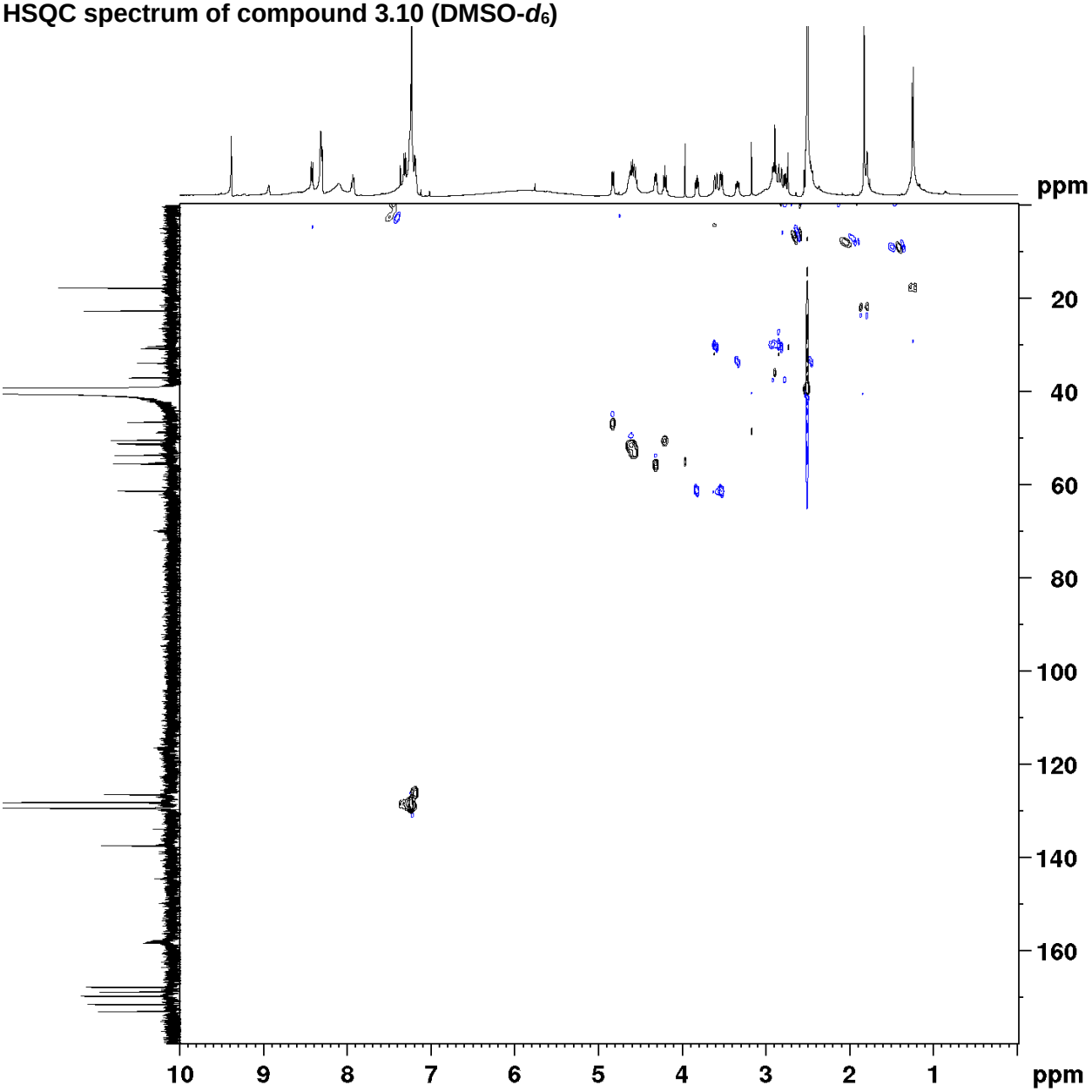
^{19}F NMR of compound 3.10 (DMSO-d_6, 282 MHz)



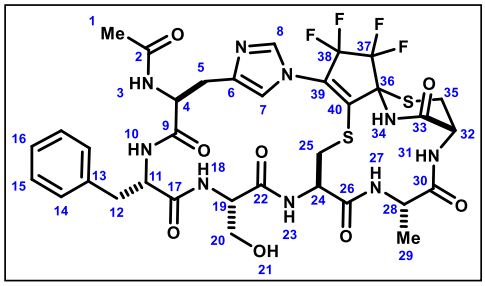
HMBC spectrum of compound 3.10 (DMSO-d_6)



HSQC spectrum of compound 3.10 (DMSO-d_6)

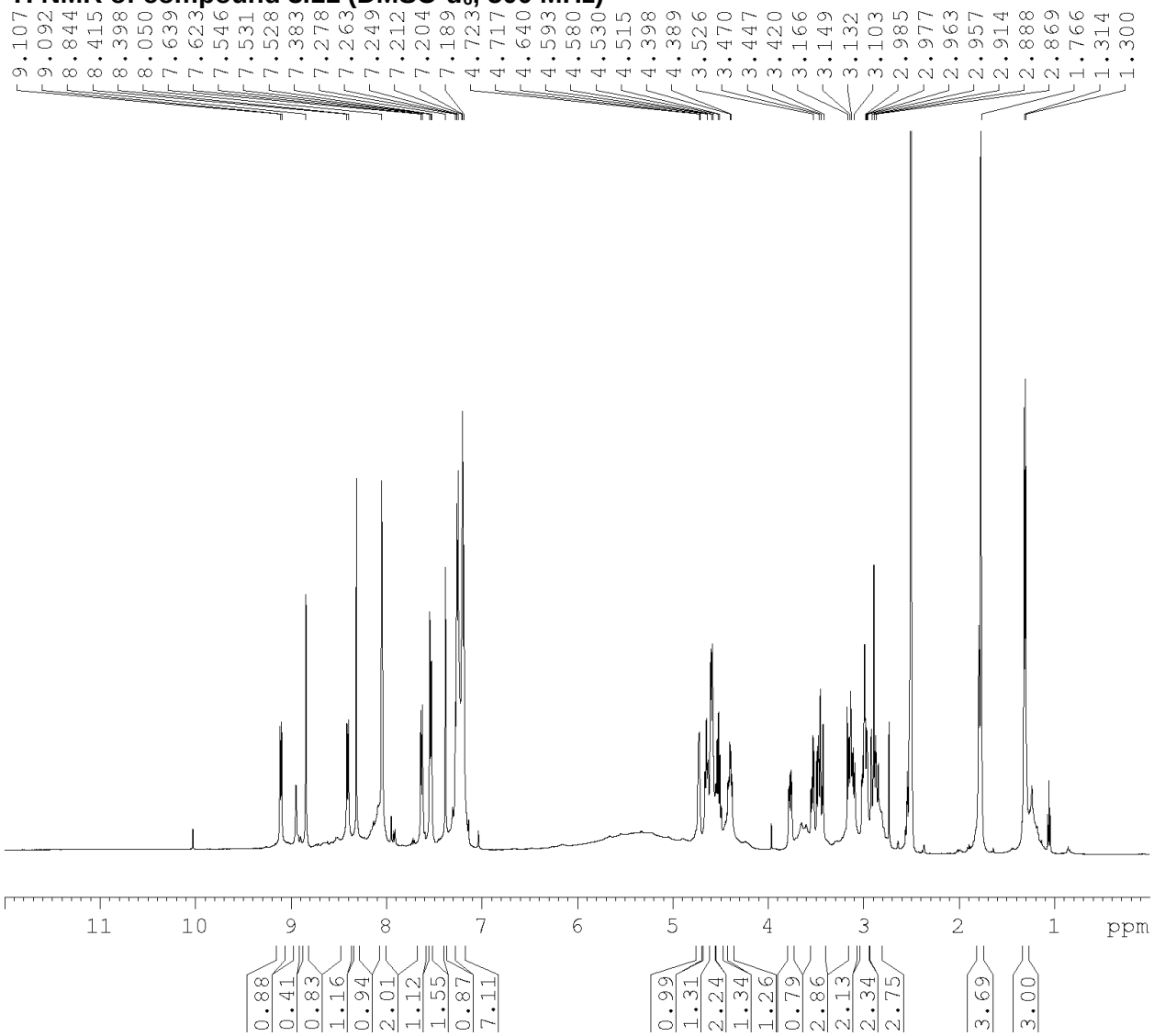


Polycycle **3.10** (600 MHz, DMSO-d6, 298K)

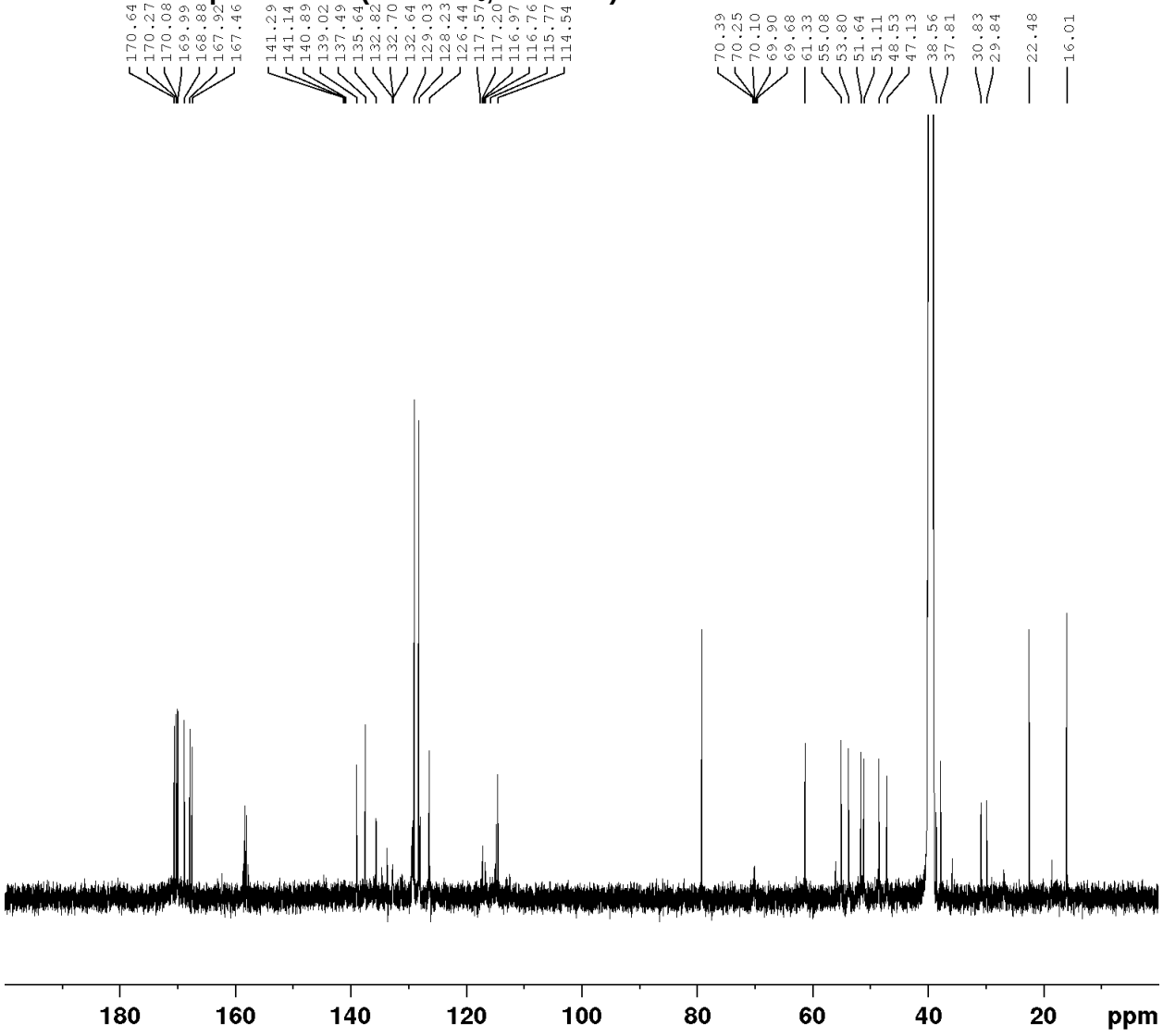


	13C	1H	Key Correlation
1	22.5	1.77 (s, 3H)	1 -> 2 HMBC
2	168.9	-	1 -> 2 HMBC
3	-	7.63 (d, J = 8.1 Hz, 1H)	3 -> 2, 4 HMBC
4	51.1	4.68 - 4.62 (m, 1H)	3 -> 4 HMBC
5	29.8	3.16 - 3.05 (m, 2H); 2.94 - 2.81 (m, 3H)	4 -> 5 HMBC
6	139.0	-	4, 5 -> 6 HMBC
7	114.5	7.38 (s, 1H)	8 -> 7 HMBC
8	135.6	8.07 - 8.01 (m, 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)	-	

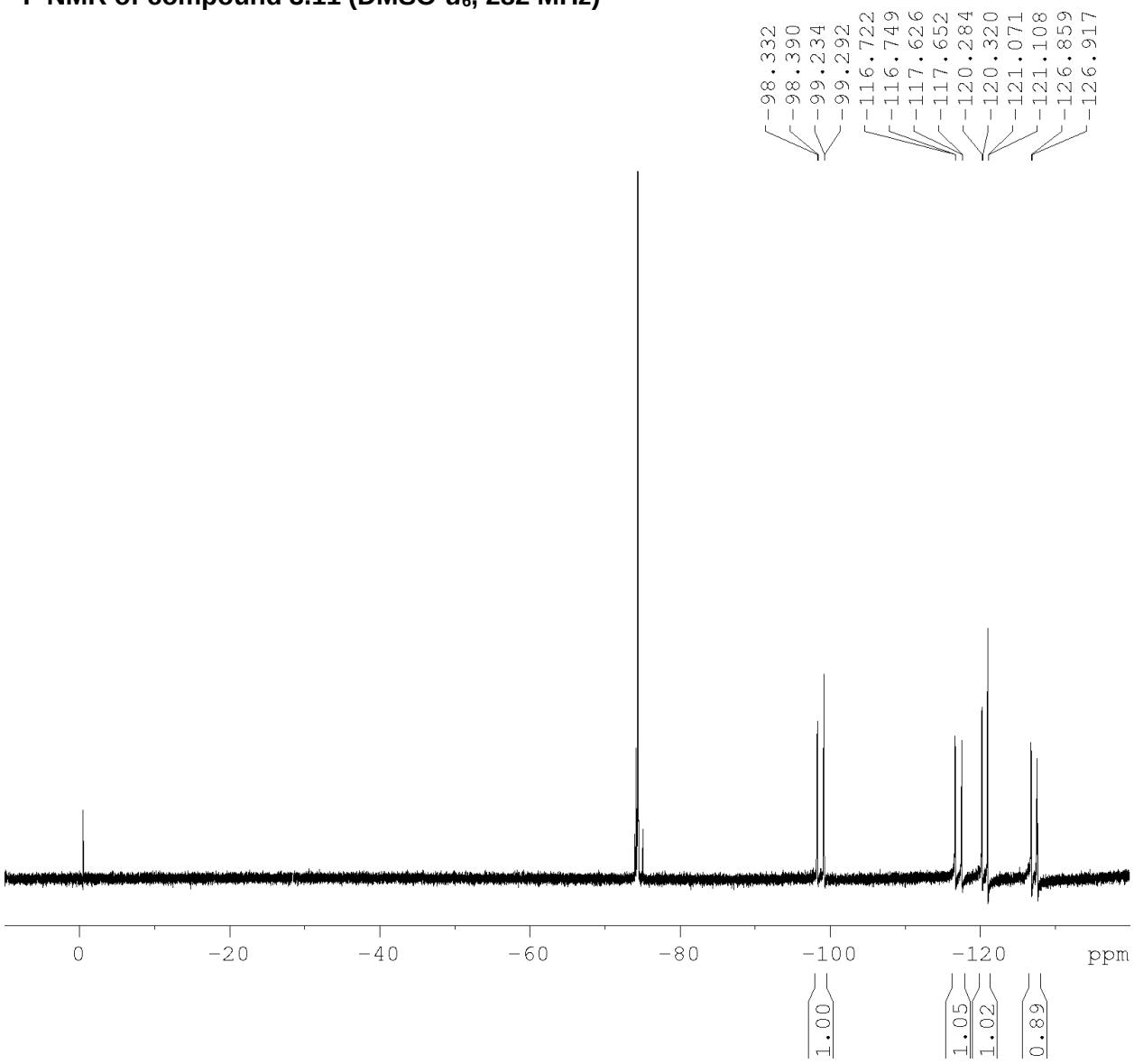
^{1}H NMR of compound 3.11 (DMSO-d_6, 500 MHz)



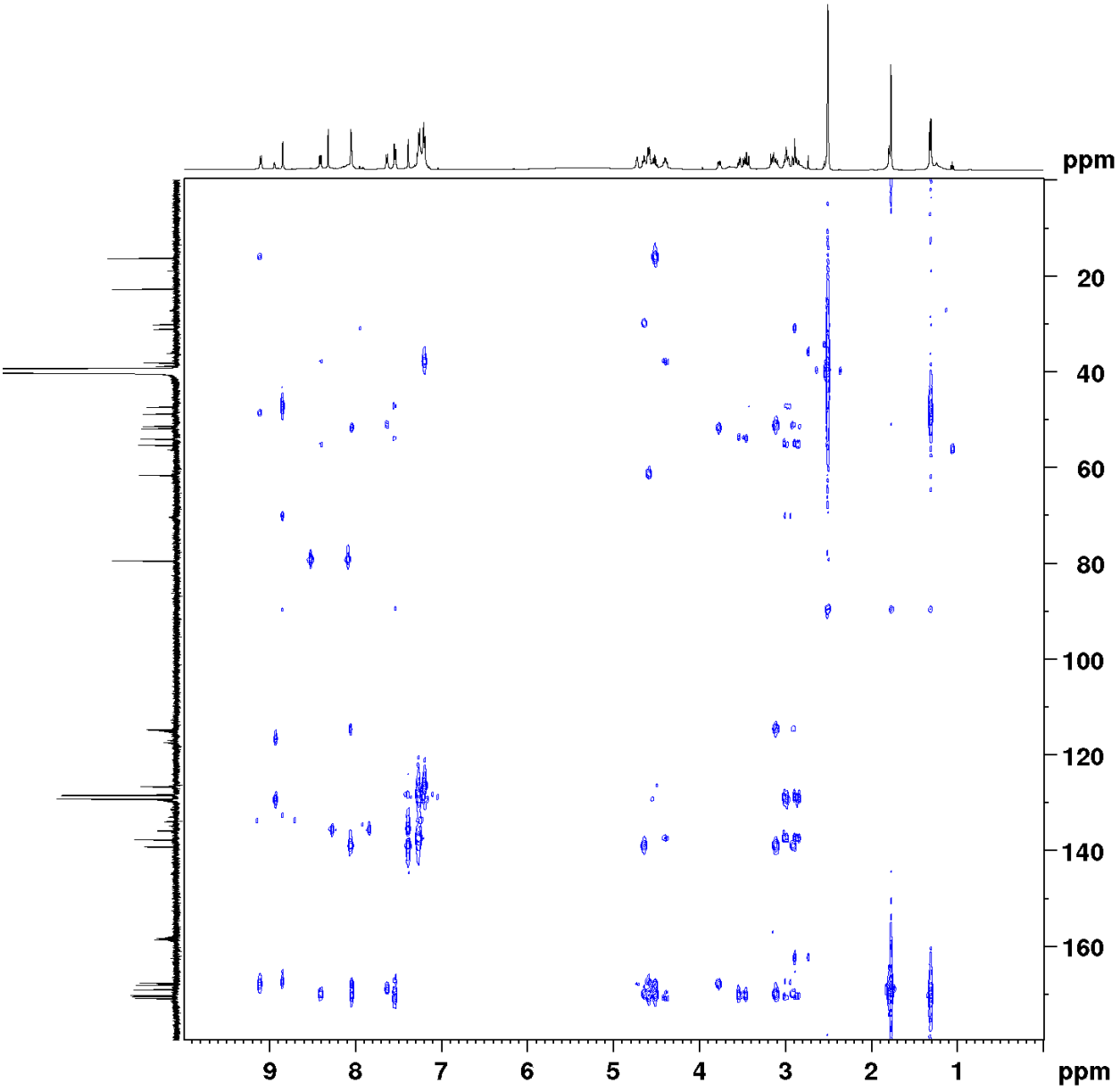
^{13}C NMR of compound 3.11 (DMSO-d_6, 126 MHz)



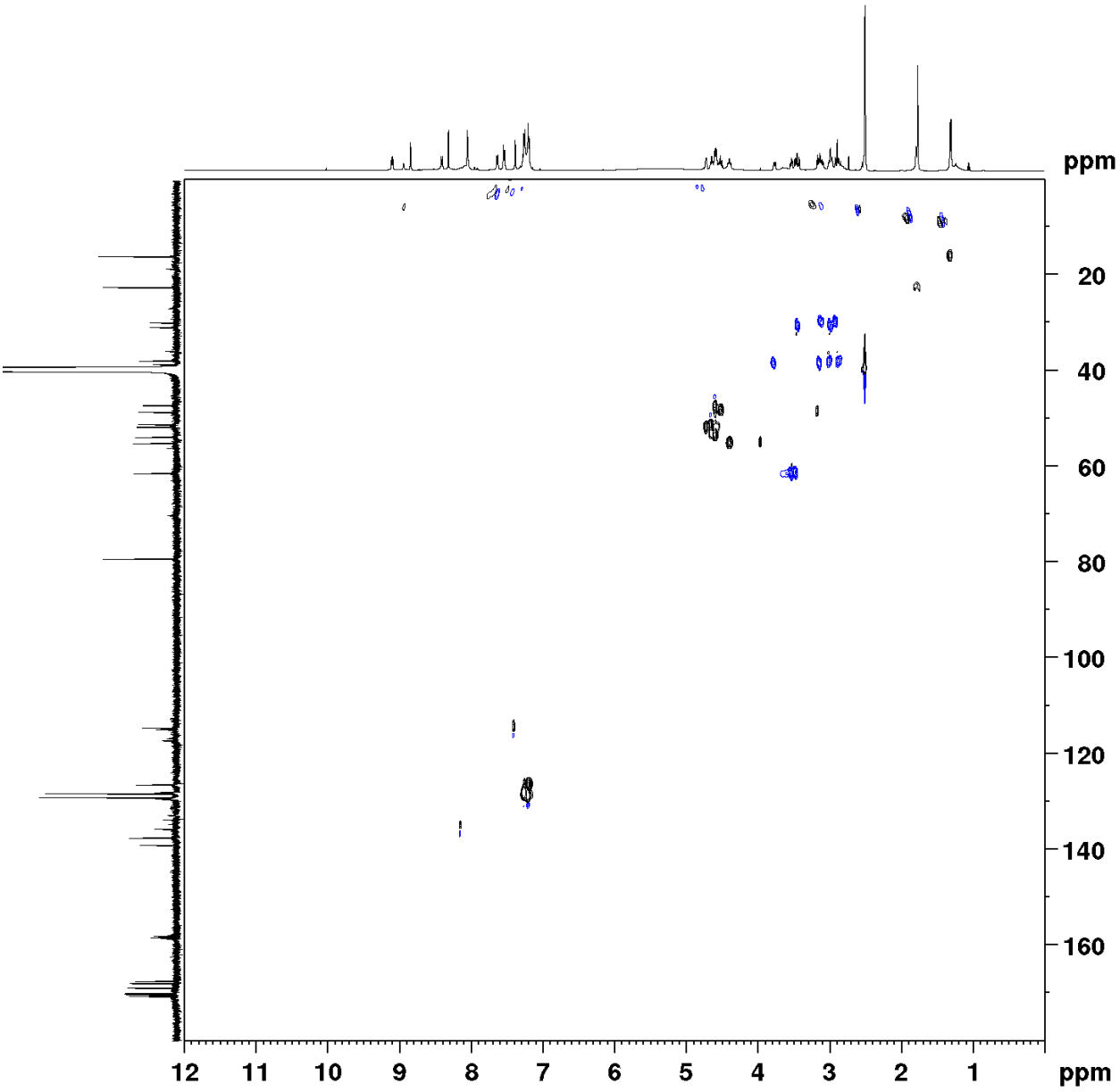
^{19}F NMR of compound 3.11 (DMSO-d_6, 282 MHz)



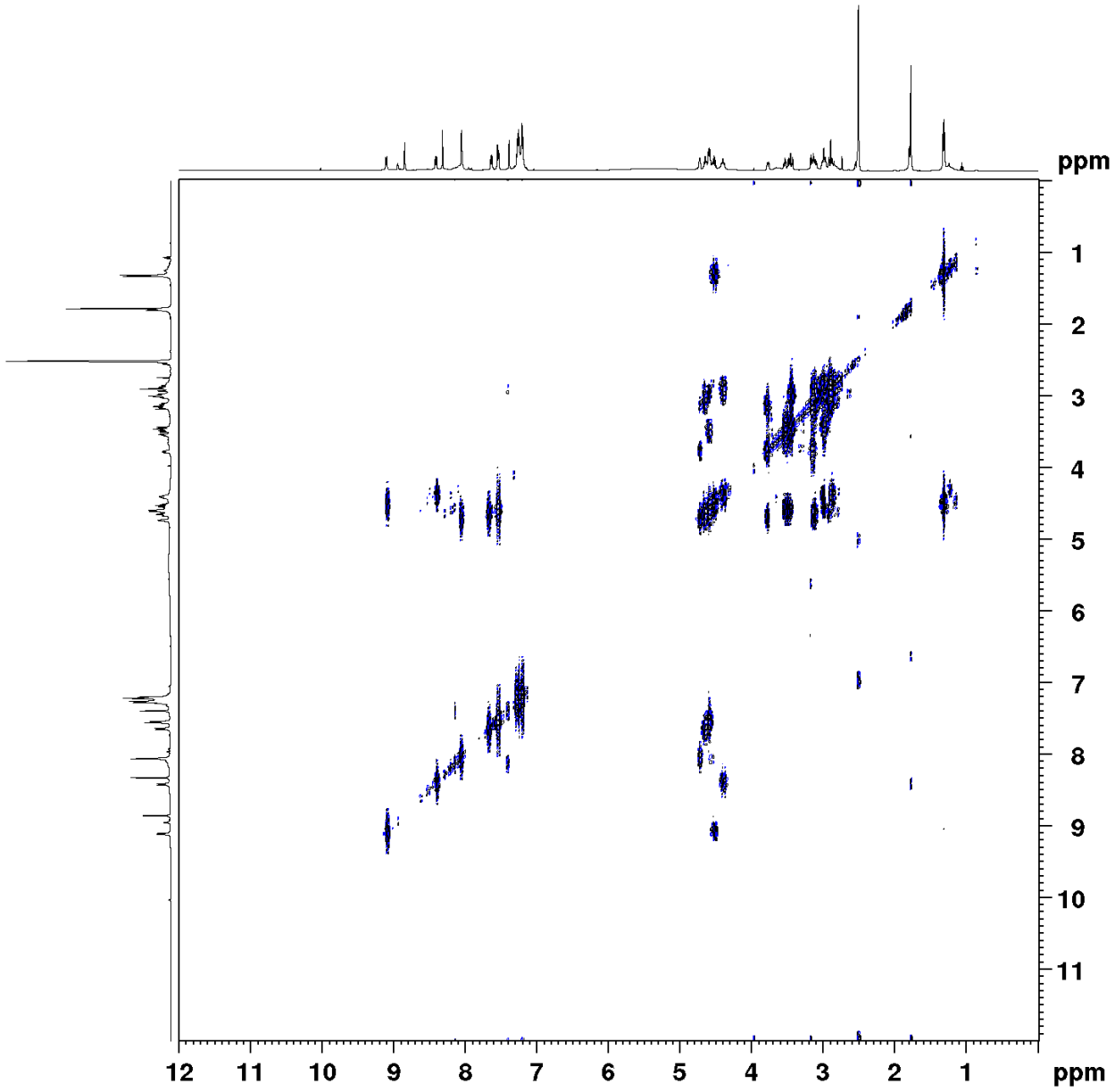
HMBC spectrum of compound 3.11 (DMSO-d_6)



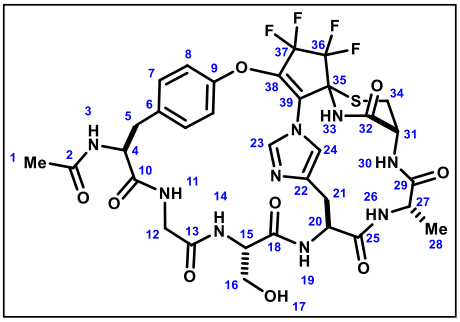
HSQC spectrum of compound 3.11 (DMSO-d_6)



COSY spectrum of compound 3.11 (DMSO-d_6)

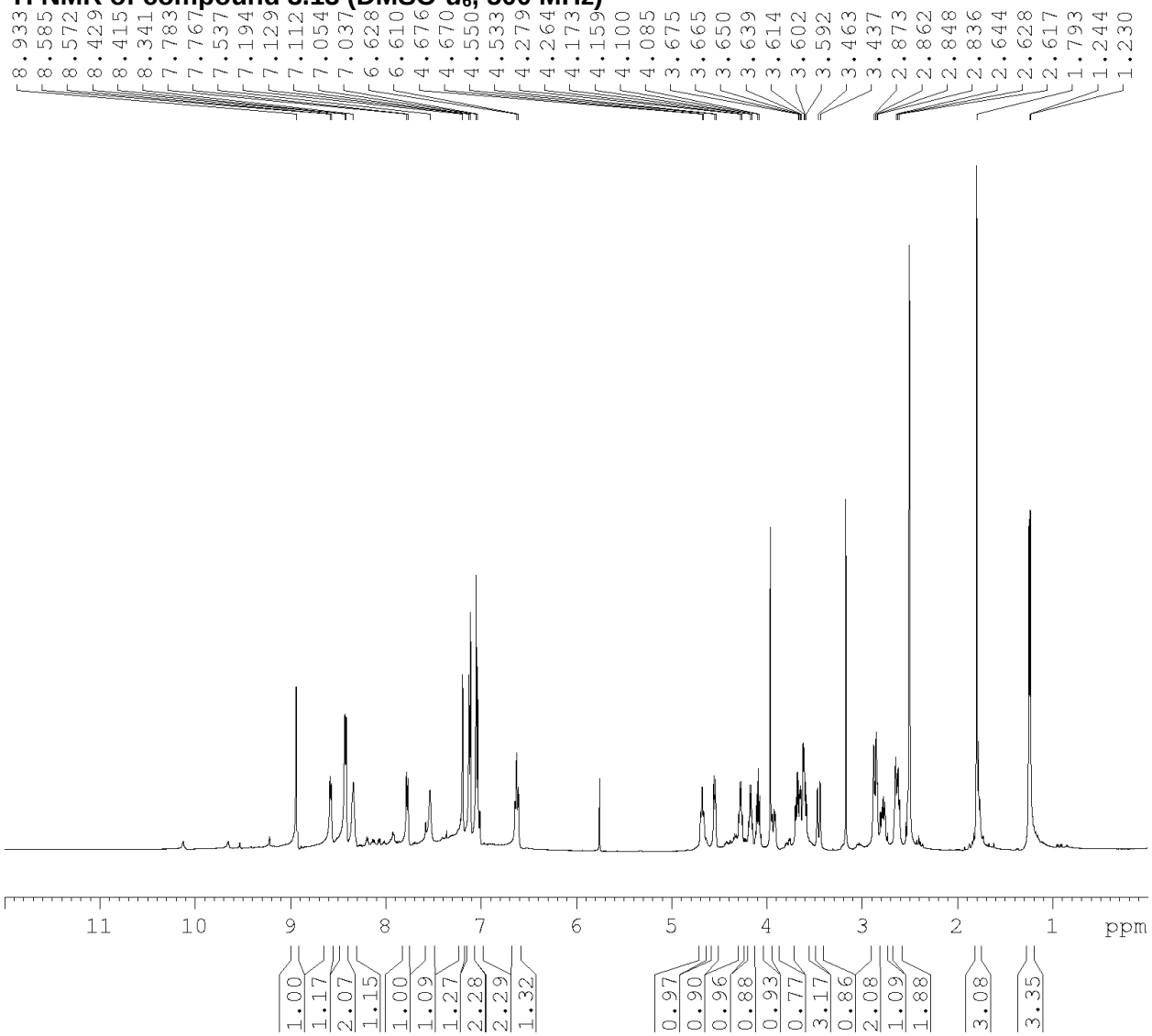


Polycycle **3.13** (600 MHz, DMSO-d6, 298K)

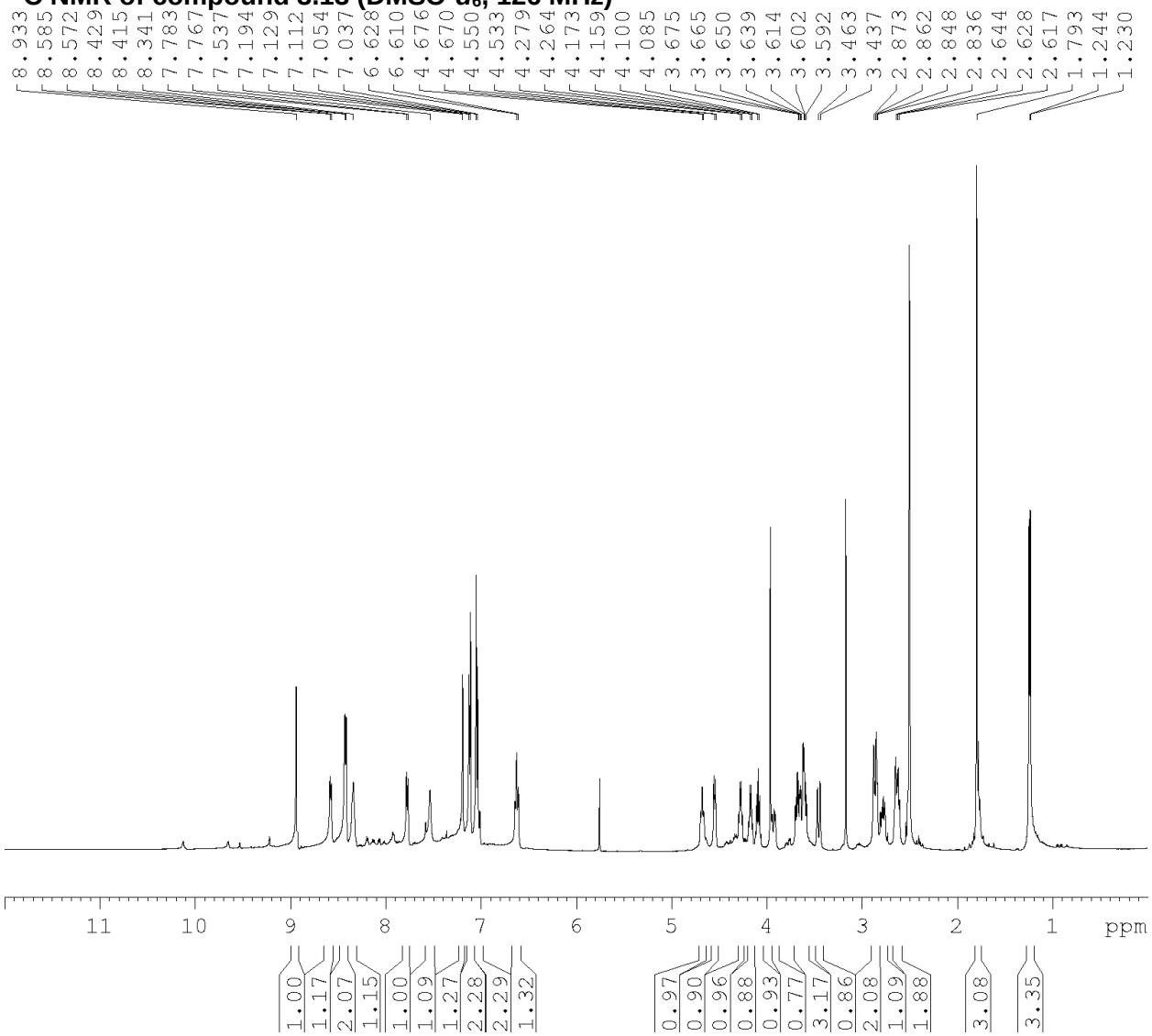


	13C	1H	Key Correlation
1	22.3	1.79 (s, 1H)	1 -> 2 HMBC
2	169.7	-	1, 3 -> 2 HMBC
3	-	8.42 (d, J = 6.6 Hz, 2H)	3 -> 2, 4, 5 HMBC
4	55.8	4.17 (q, J = 7.0 Hz, 1H)	3, 5 -> 4
5	36.7	2.90 - 2.83 (m, 2H); 2.68 - 2.58 (m, 2H)	5 -> 4, 6 HMBC
6	136.8	-	4, 5 -> 6 HMBC
7	130.8	7.15 - 7.10 (m, 2H)	5 -> 7 HMBC
8	119.2	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)	-	

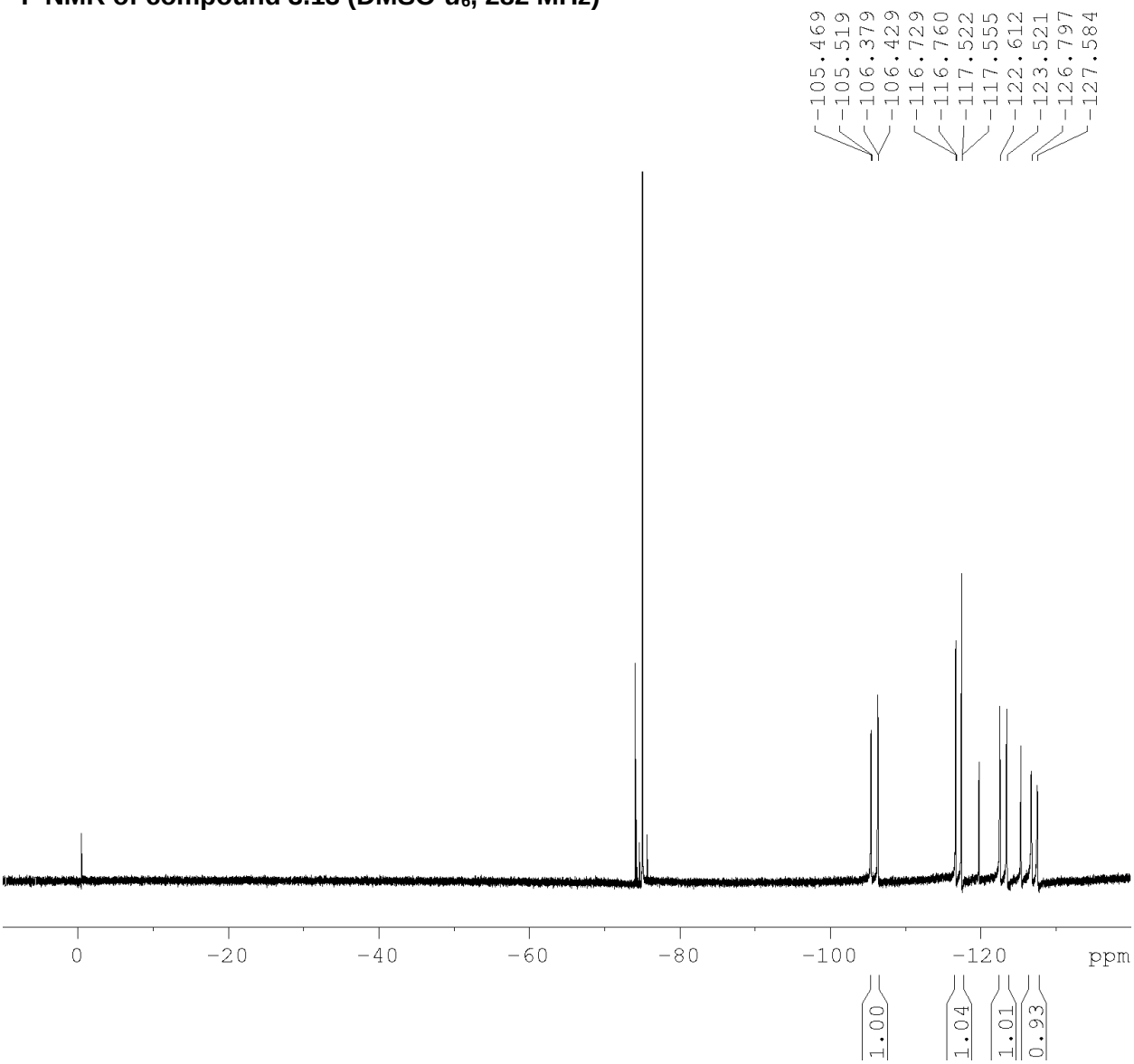
^{1}H NMR of compound 3.13 (DMSO-d_6, 500 MHz)



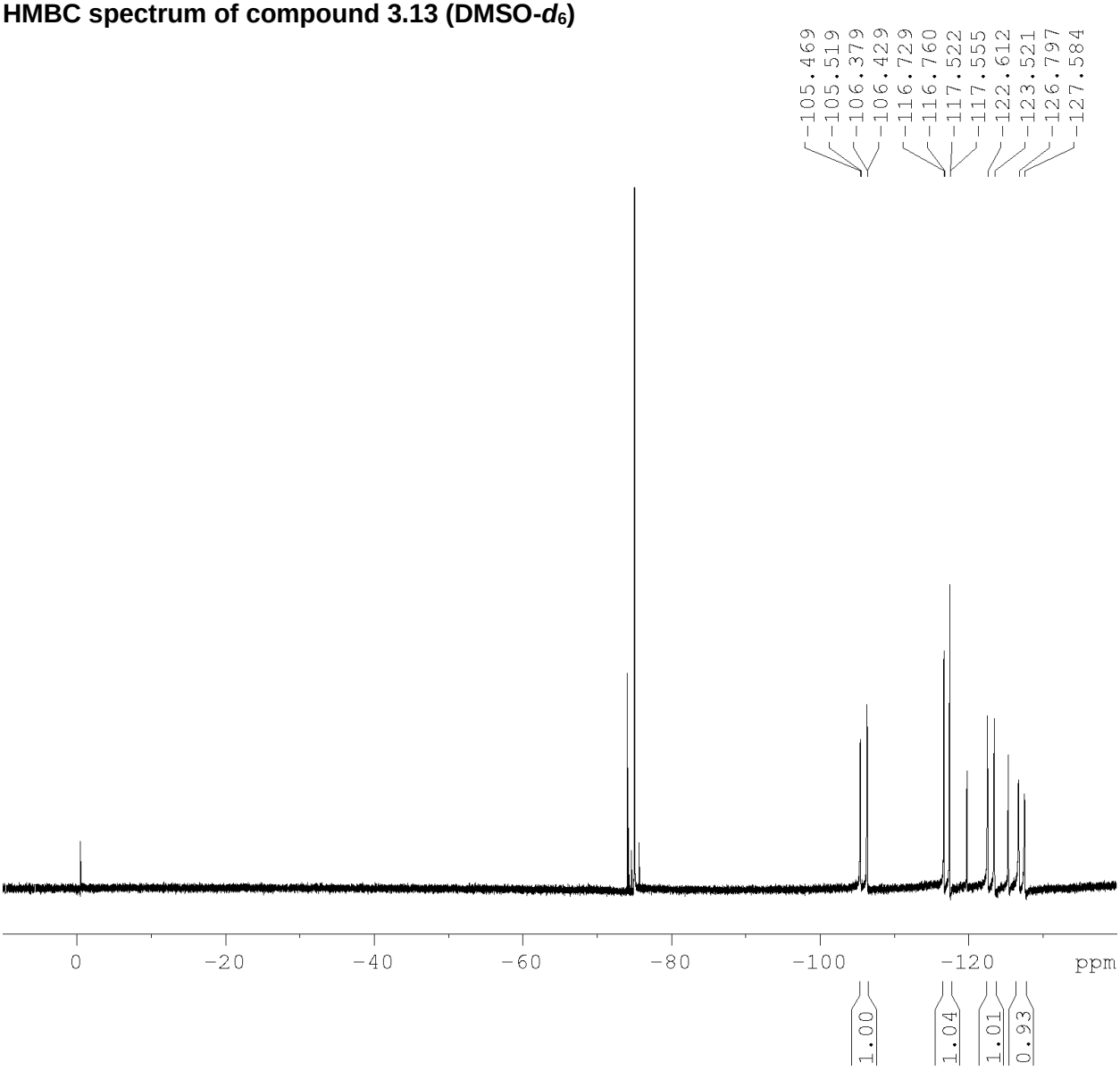
^{13}C NMR of compound 3.13 (DMSO-d_6, 126 MHz)



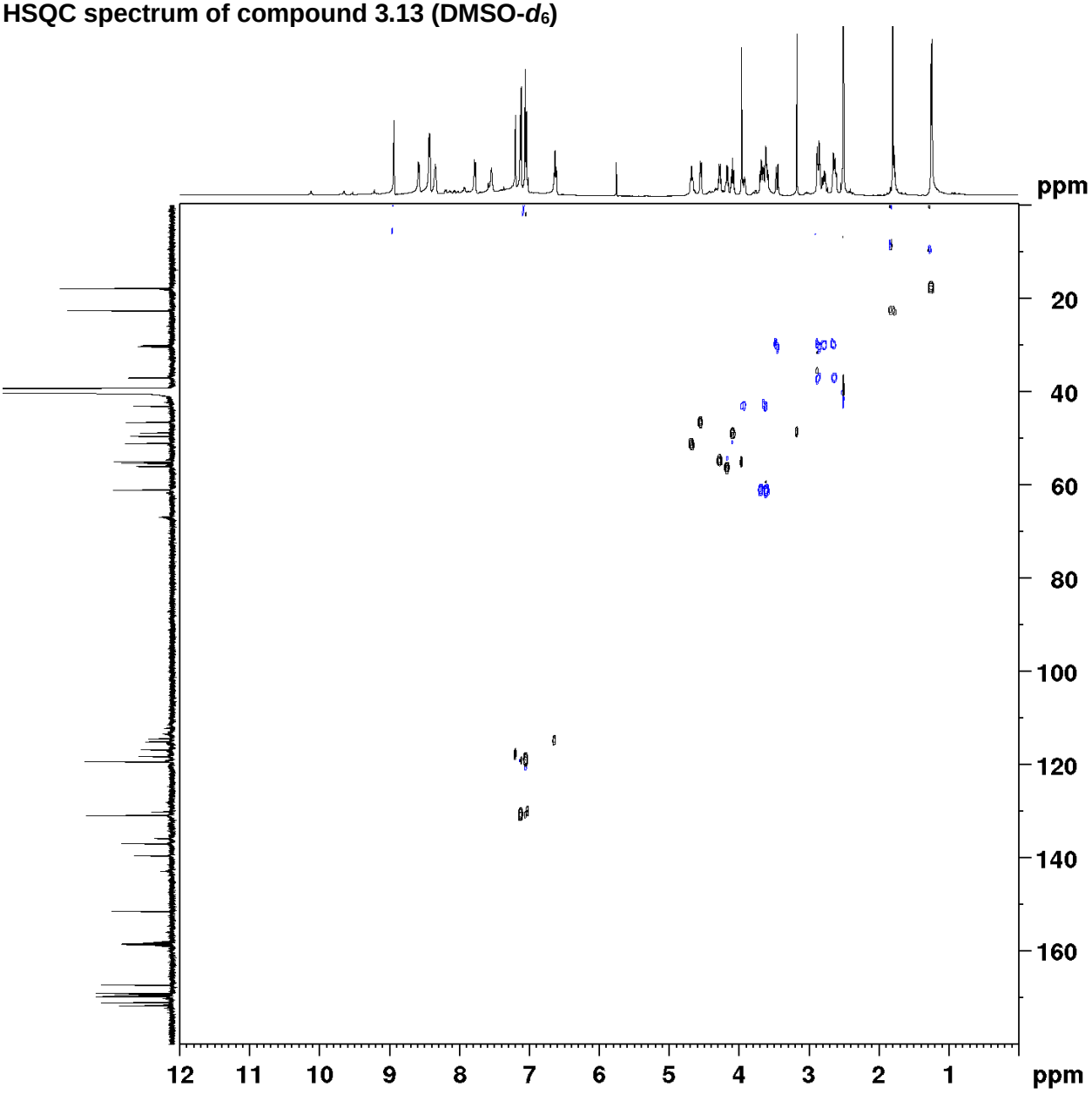
^{19}F NMR of compound 3.13 (DMSO-d_6, 282 MHz)



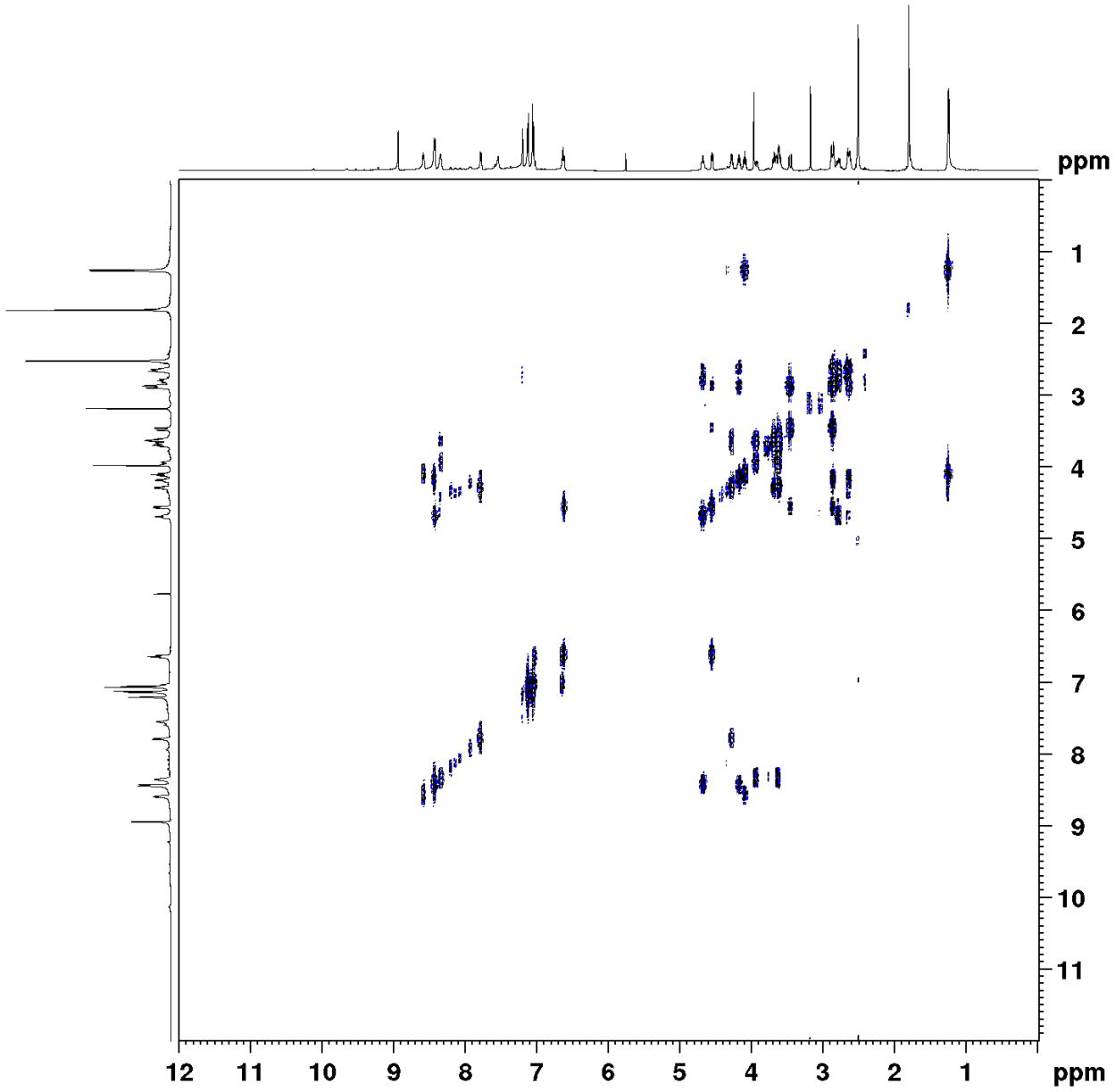
HMBC spectrum of compound 3.13 (DMSO-d_6)



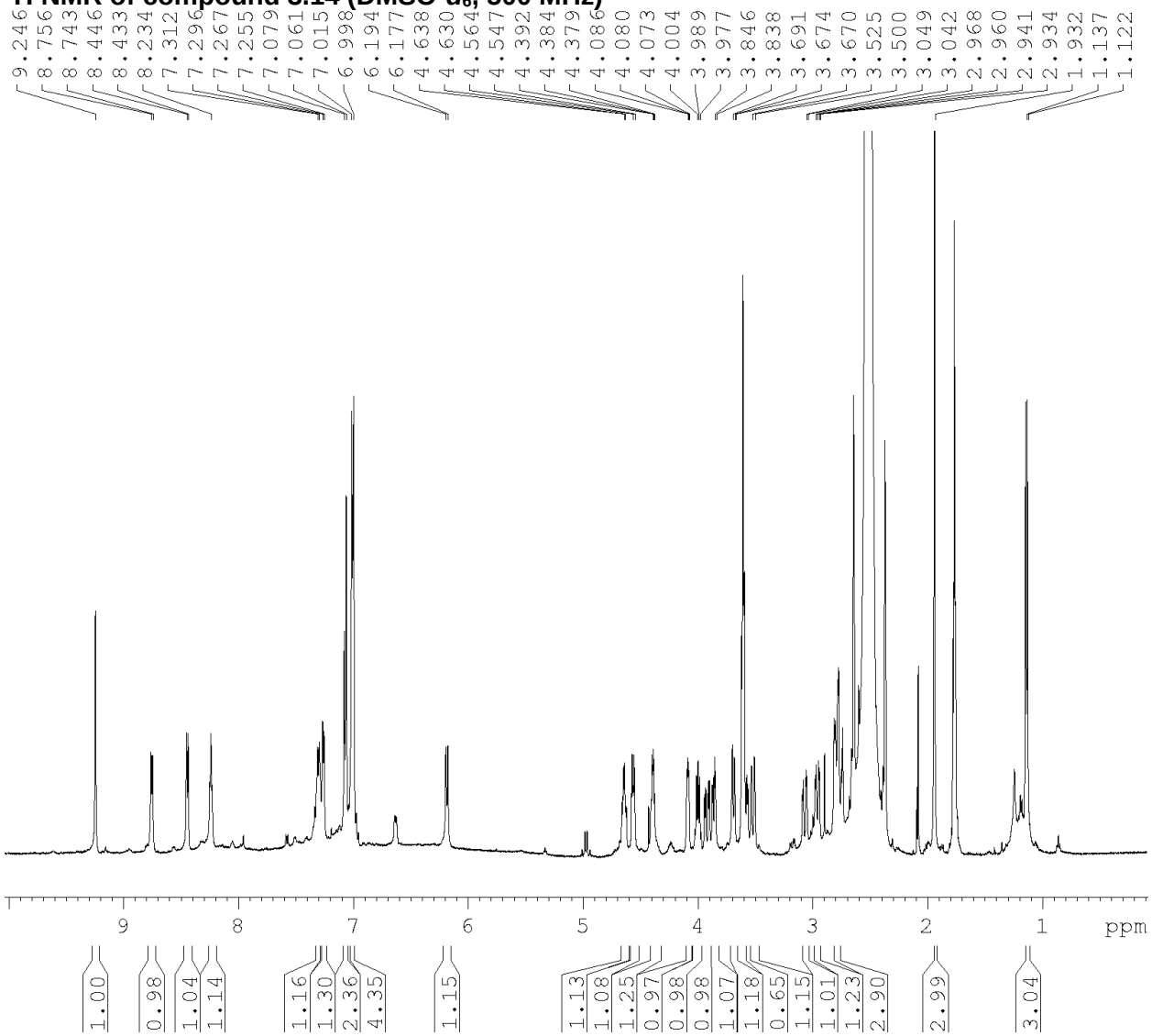
HSQC spectrum of compound 3.13 (DMSO-d_6)



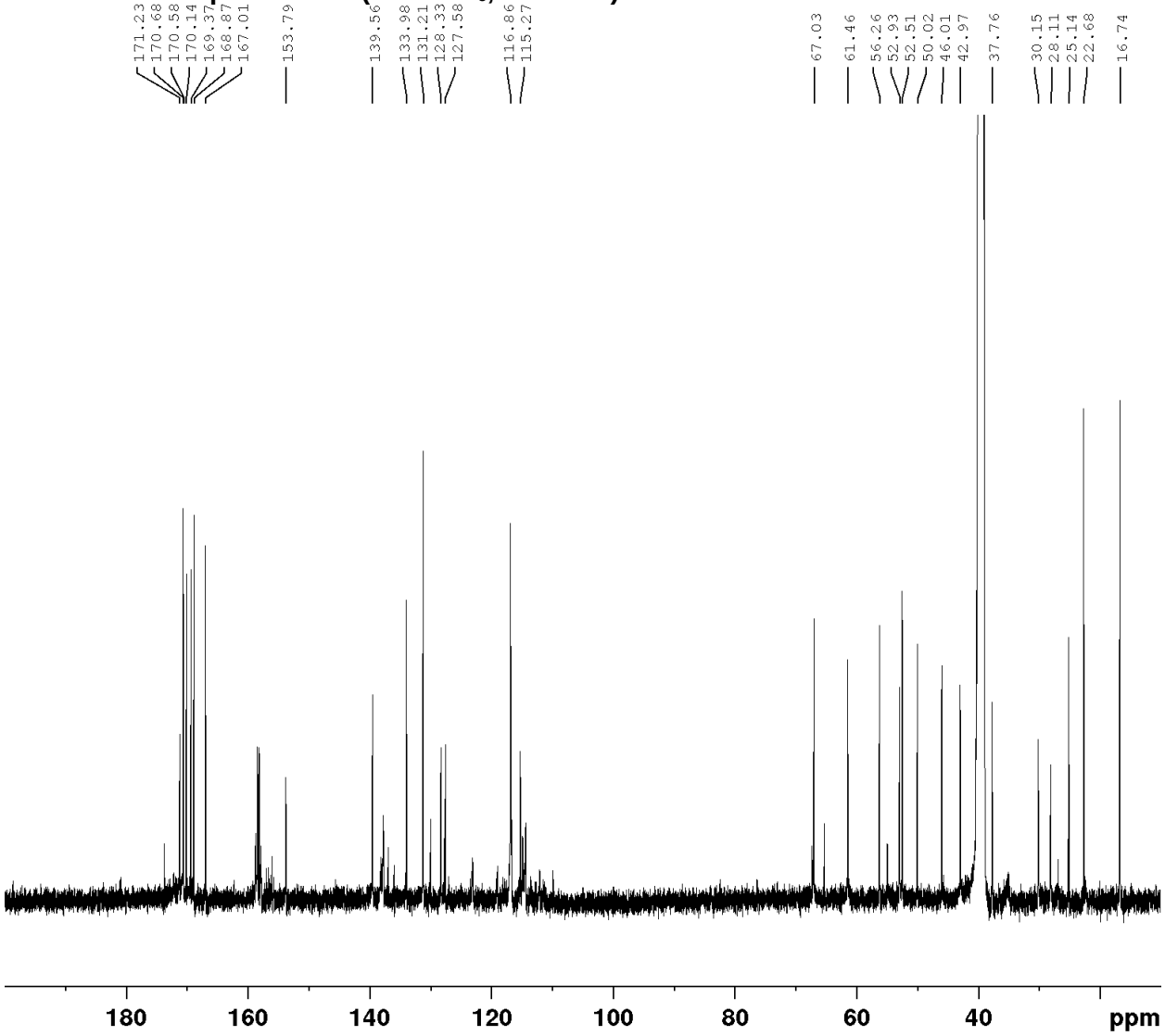
COSY spectrum of compound 3.13 (DMSO-d_6)



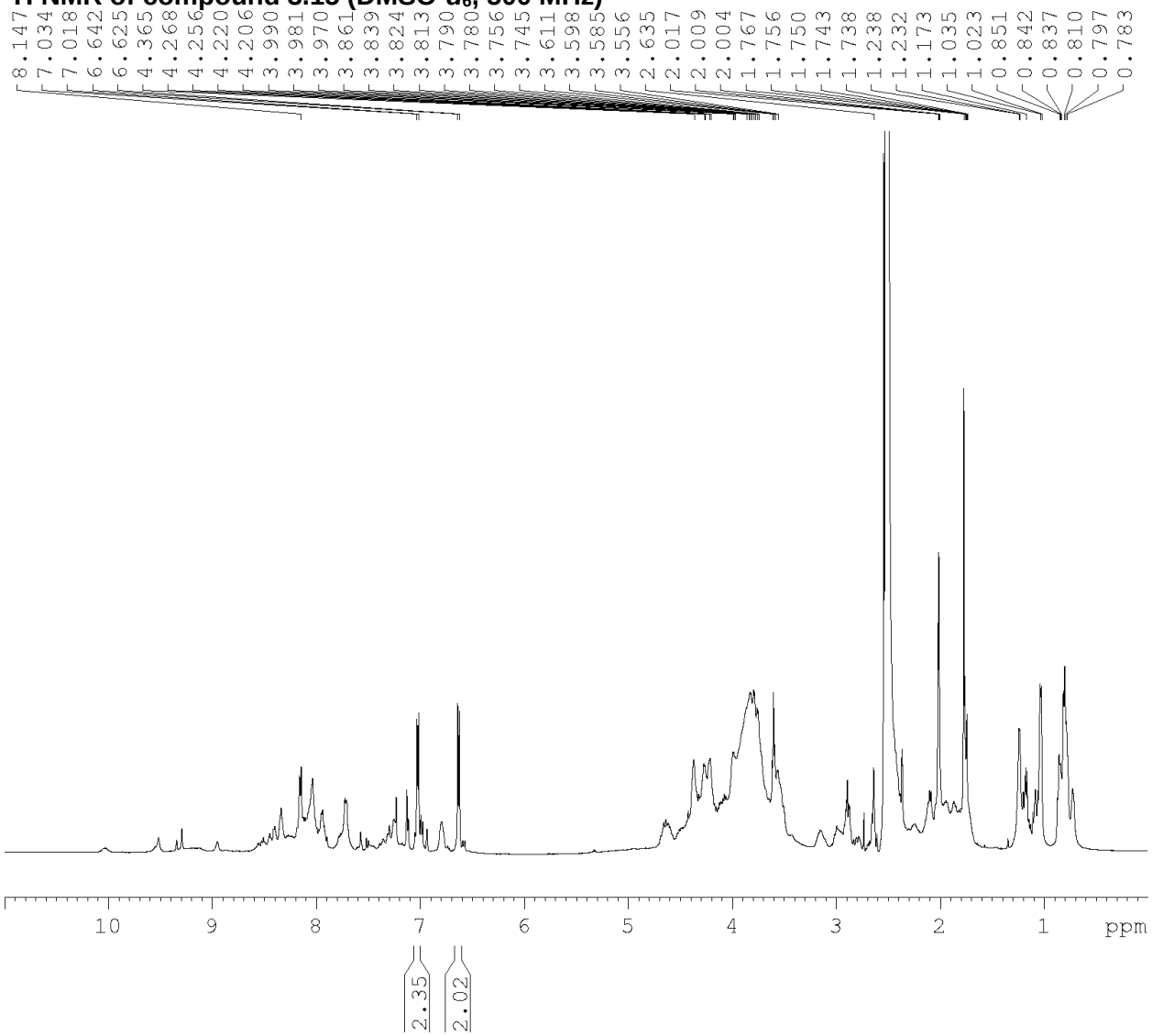
^{1}H NMR of compound 3.14 (DMSO-d_6, 500 MHz)



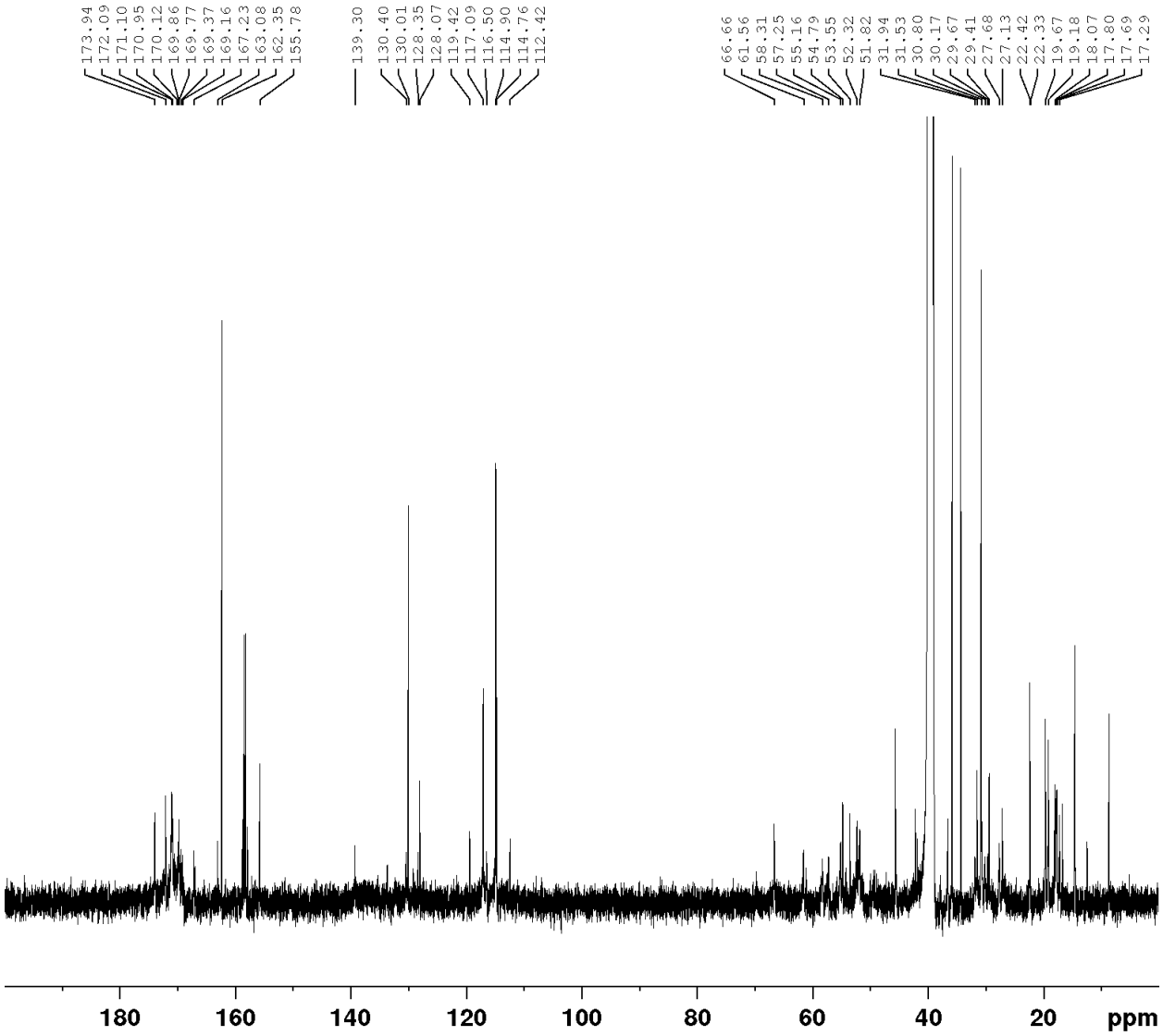
^{13}C NMR of compound 3.14 (DMSO-d_6, 126 MHz)



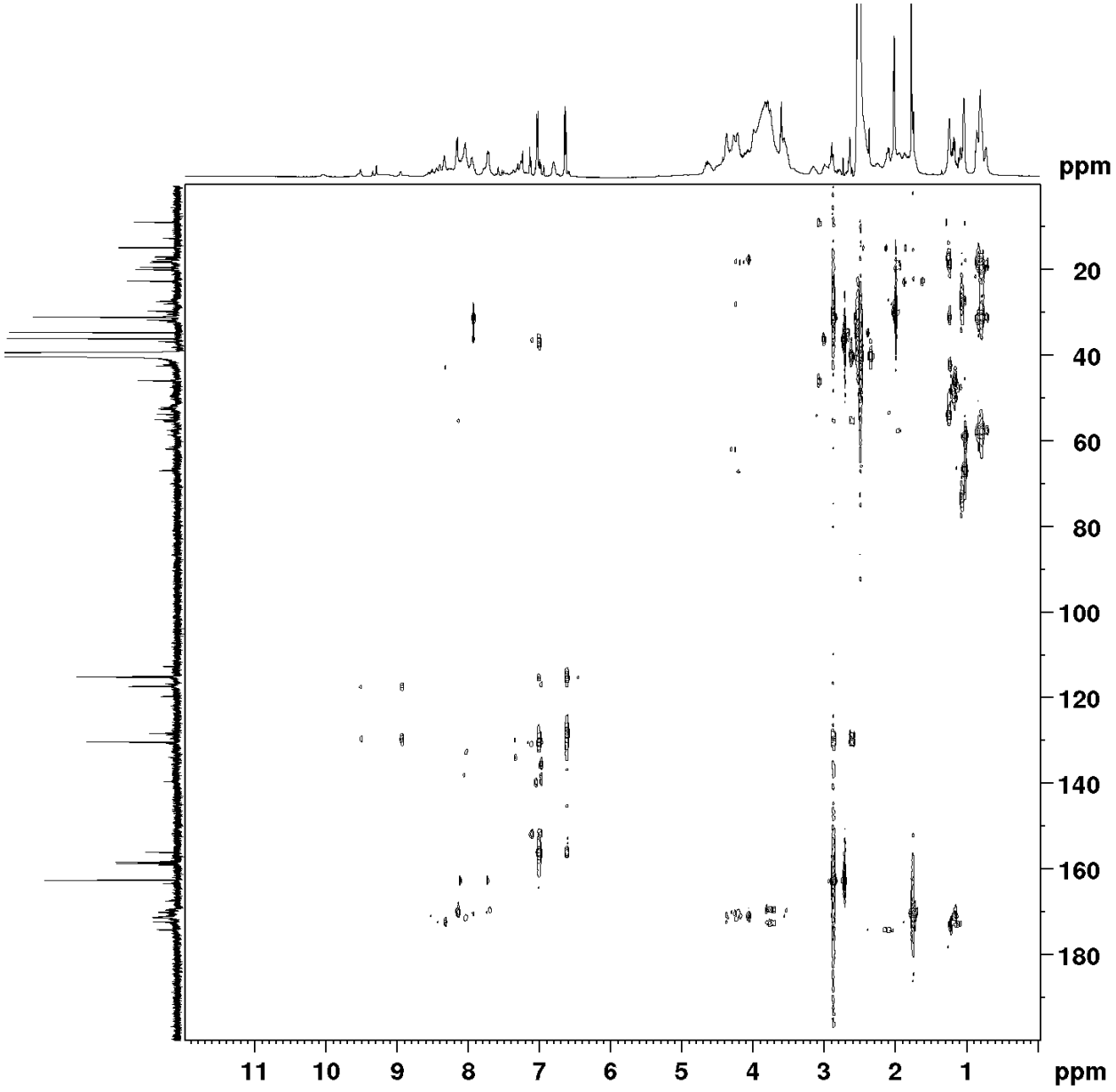
^{1}H NMR of compound 3.15 (DMSO-d_6, 500 MHz)



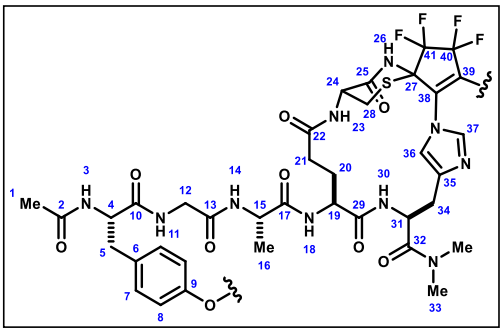
^{13}C NMR of compound 3.15 (DMSO-d_6, 126 MHz)



HMBC spectrum of compound 3.13 (DMSO-d_6)

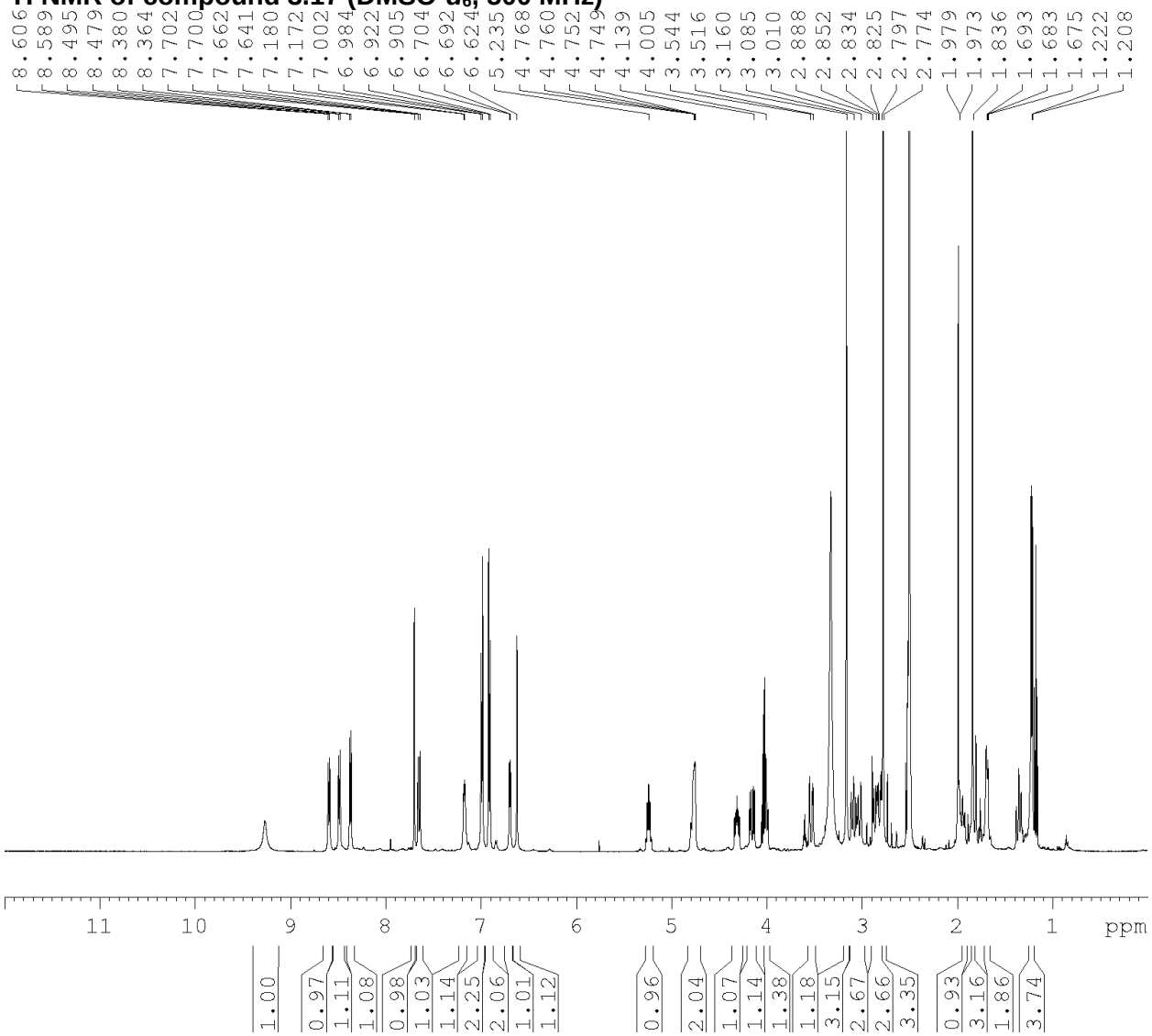


Polycycle **3.17** (600 MHz, DMSO-d6, 298K)

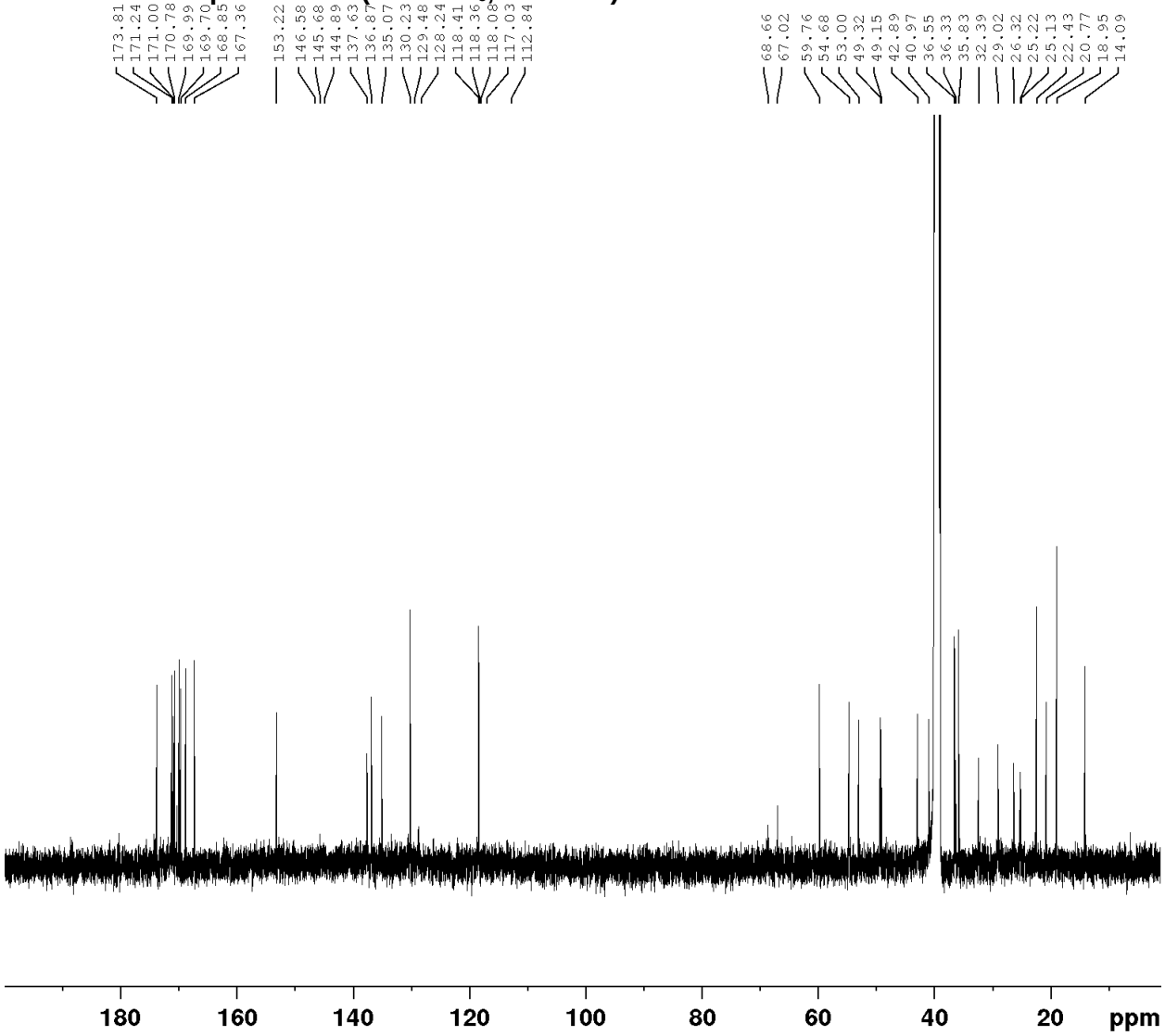


	13C	1H	Key Correlation
1	22.4	1.84 (s, 3H)	1 -> 2 HMBC
2	168.9	-	1 -> 2 HMBC
3	-	8.37 (d, J = 7.7, 1H)	3 -> 2 HMBC
4	54.7	4.03 - 3.97 (m, 1H)	3 -> 4 HMBC
5	36.4	3.12 - 2.98 (m, 1H); 2.53 - 2.47 (m, 1H)	4, 7 -> 5 HMBC
6	135.1	-	4 -> 6 HMBC
7	130.2	6.99 (d, J = 8.7 Hz, 2H); 6.91 (d, J = 8.7, 2H)	8 -> 7 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)	-	

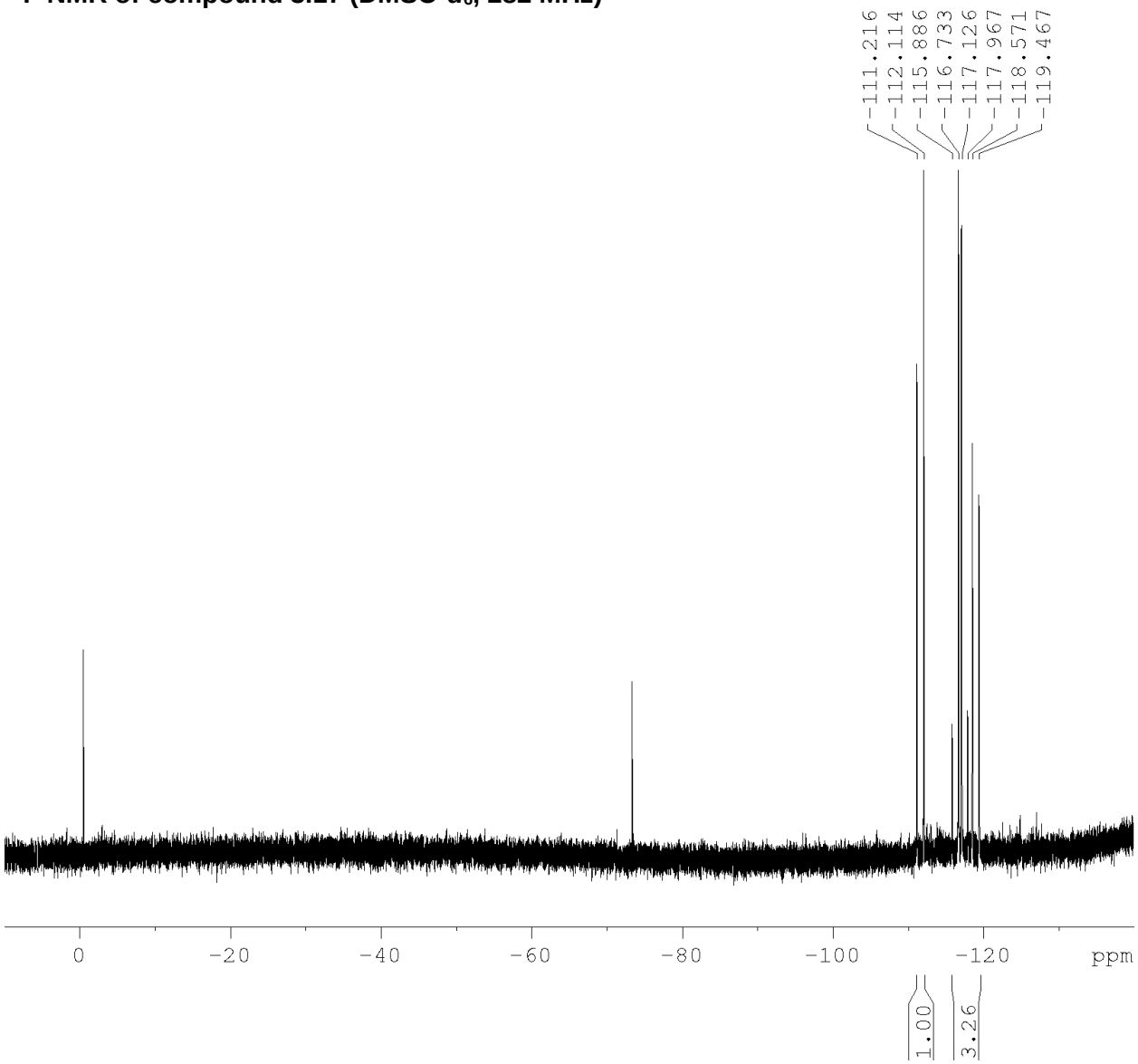
^{1}H NMR of compound 3.17 (DMSO-d_6, 500 MHz)



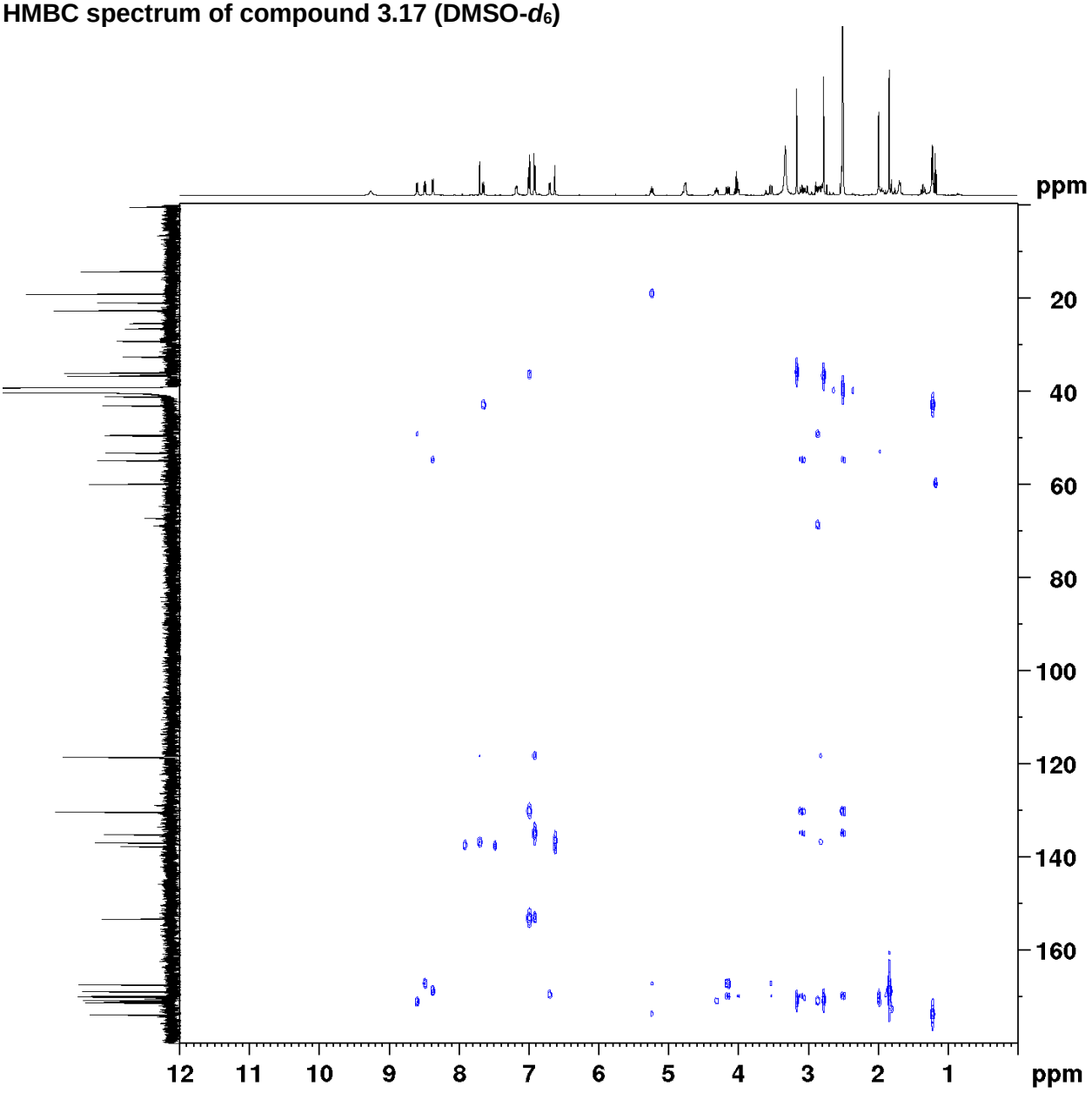
^{13}C NMR of compound 3.17 (DMSO-d_6, 126 MHz)



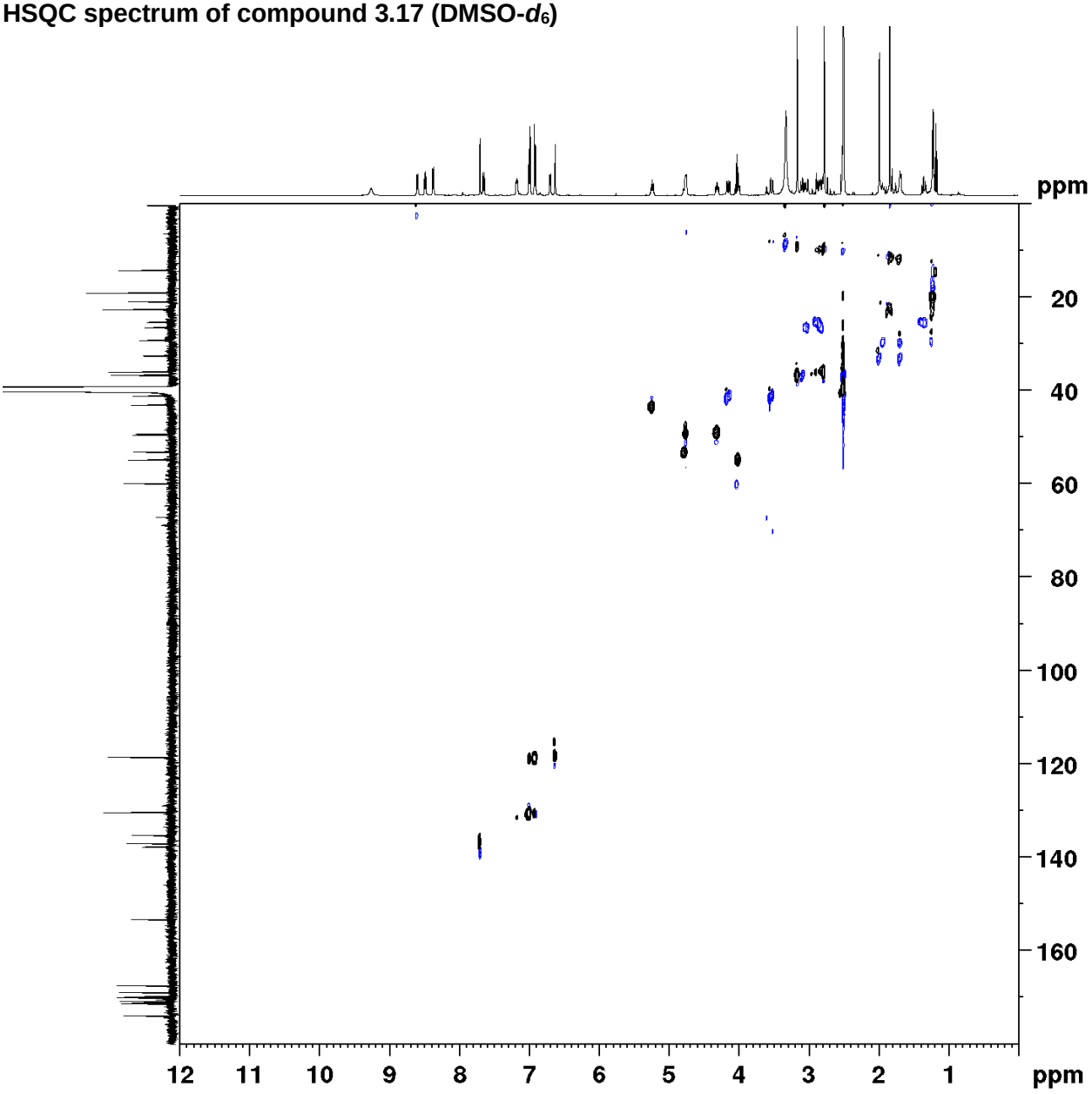
^{19}F NMR of compound 3.17 (DMSO-d_6, 282 MHz)



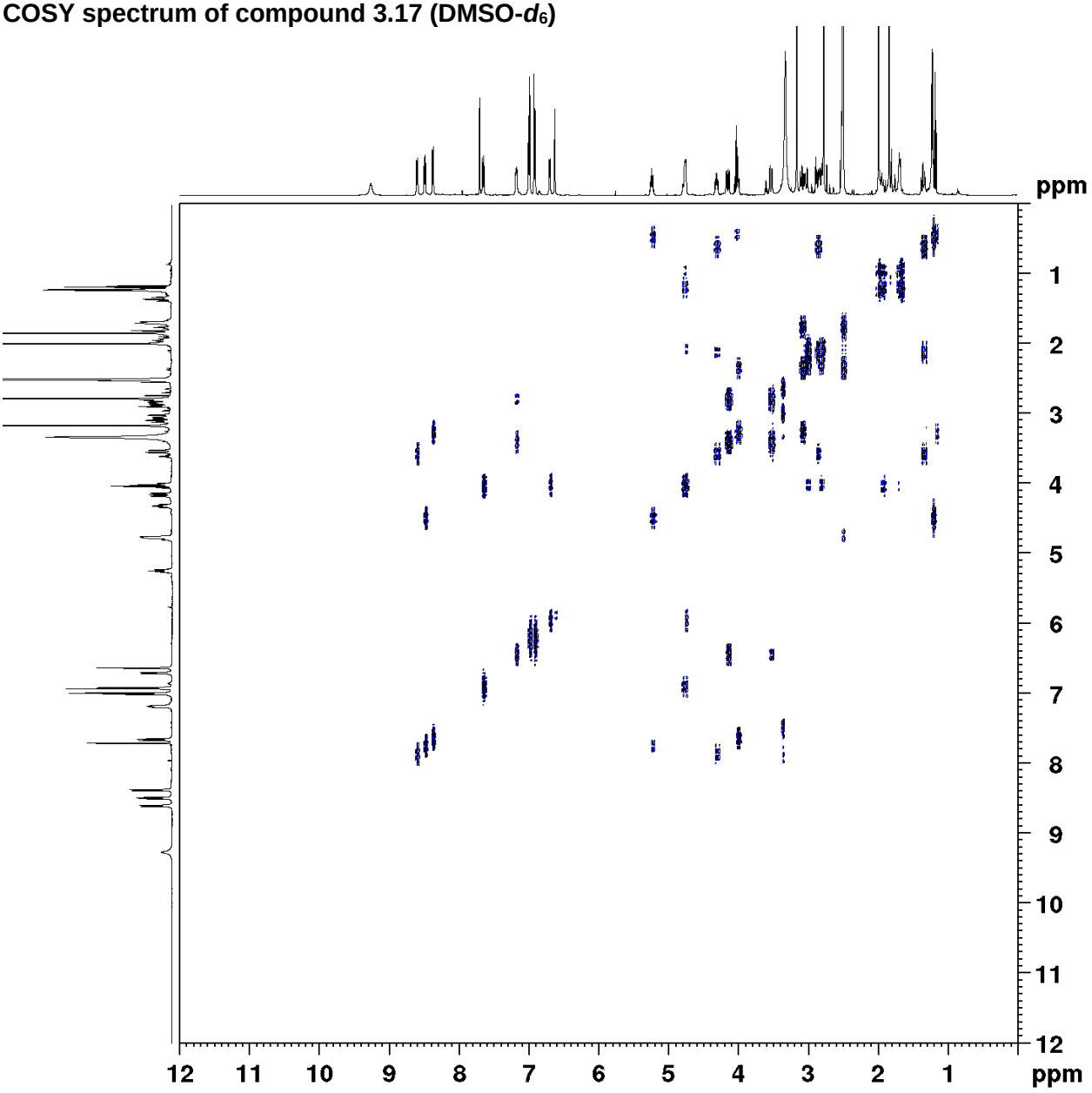
HMBC spectrum of compound 3.17 (DMSO-d_6)



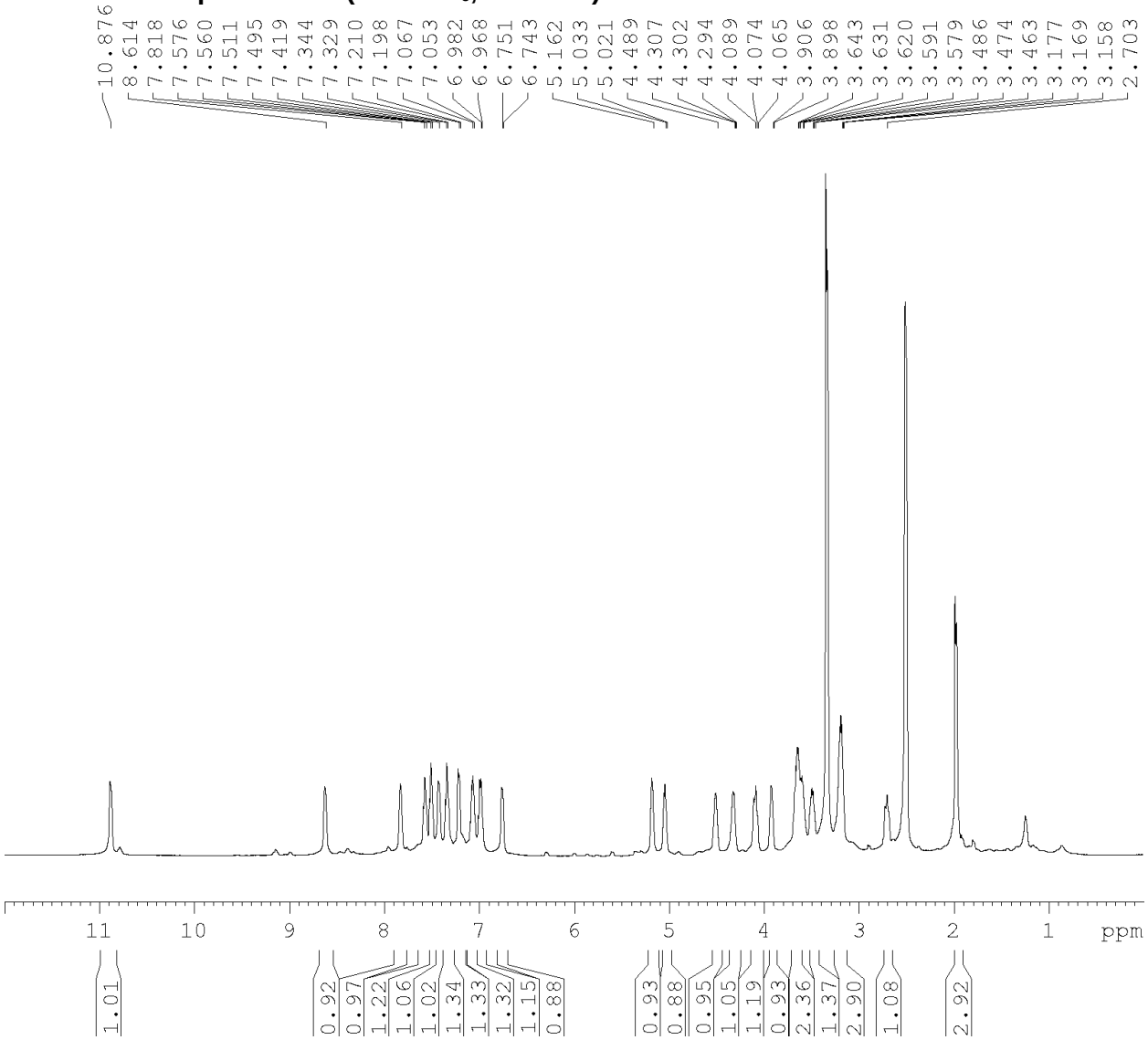
HSQC spectrum of compound 3.17 (DMSO-d_6)



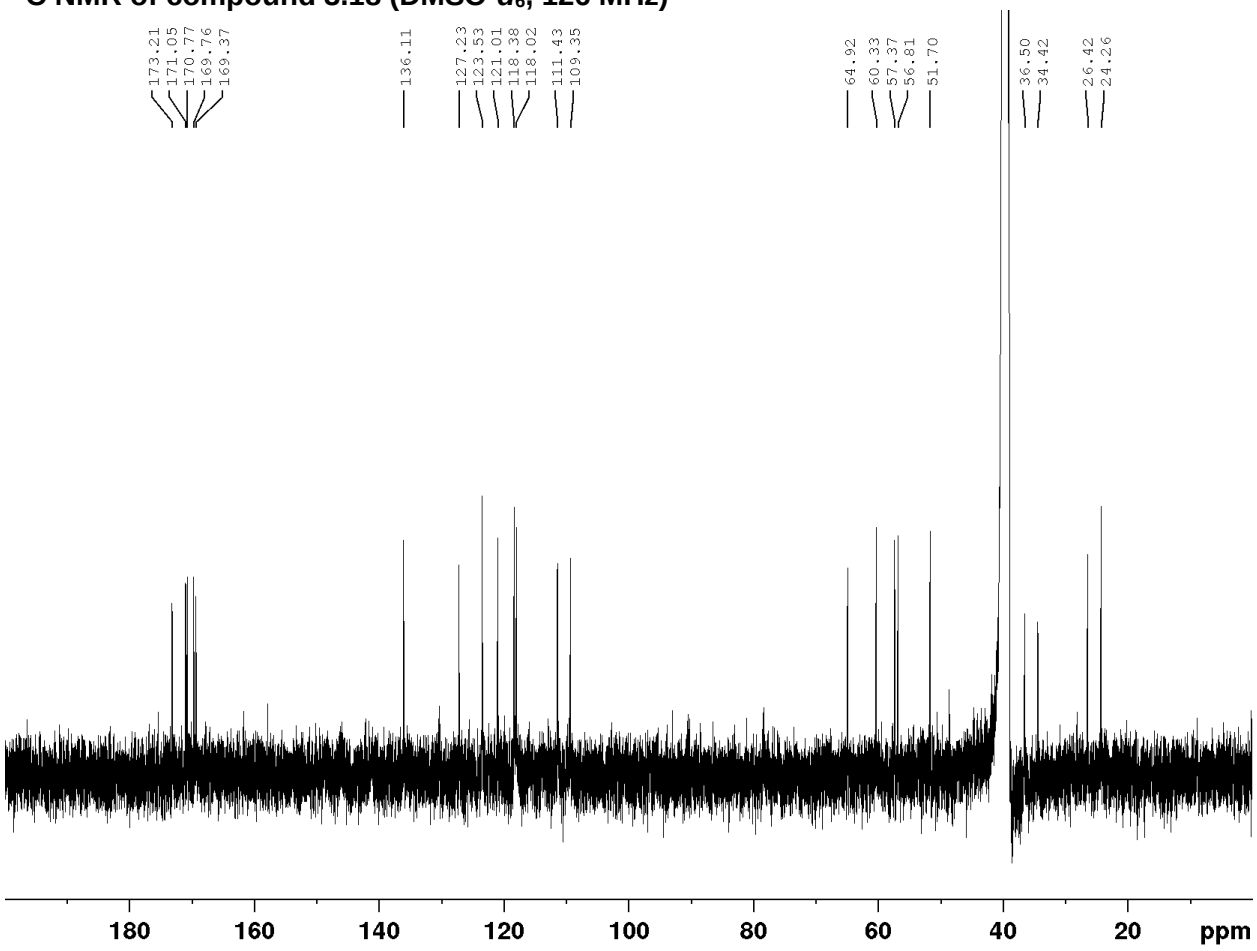
COSY spectrum of compound 3.17 (DMSO-d_6)



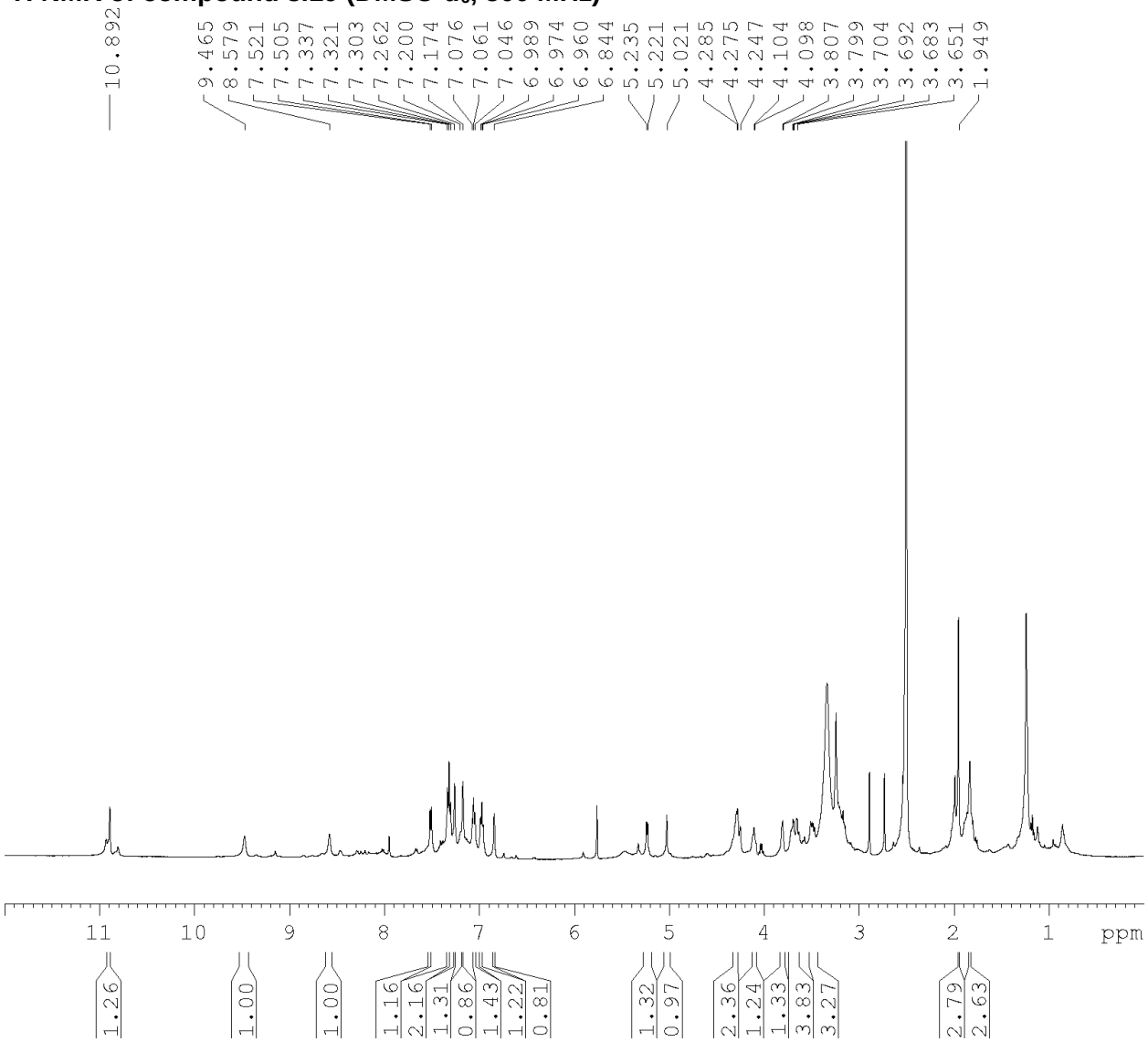
^{1}H NMR of compound 3.18 (DMSO-d_6, 500 MHz)



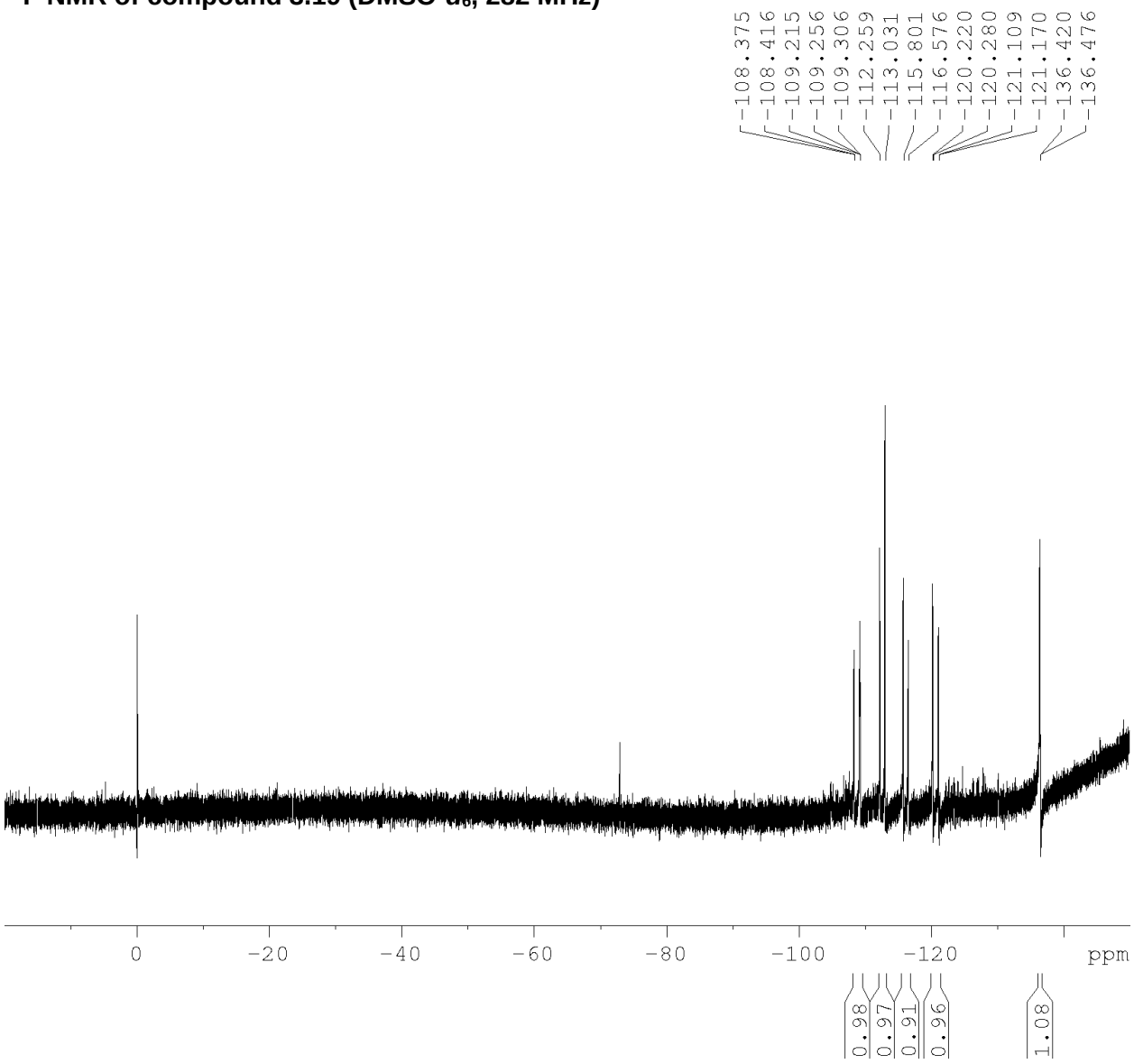
^{13}C NMR of compound 3.18 (DMSO-d_6, 126 MHz)



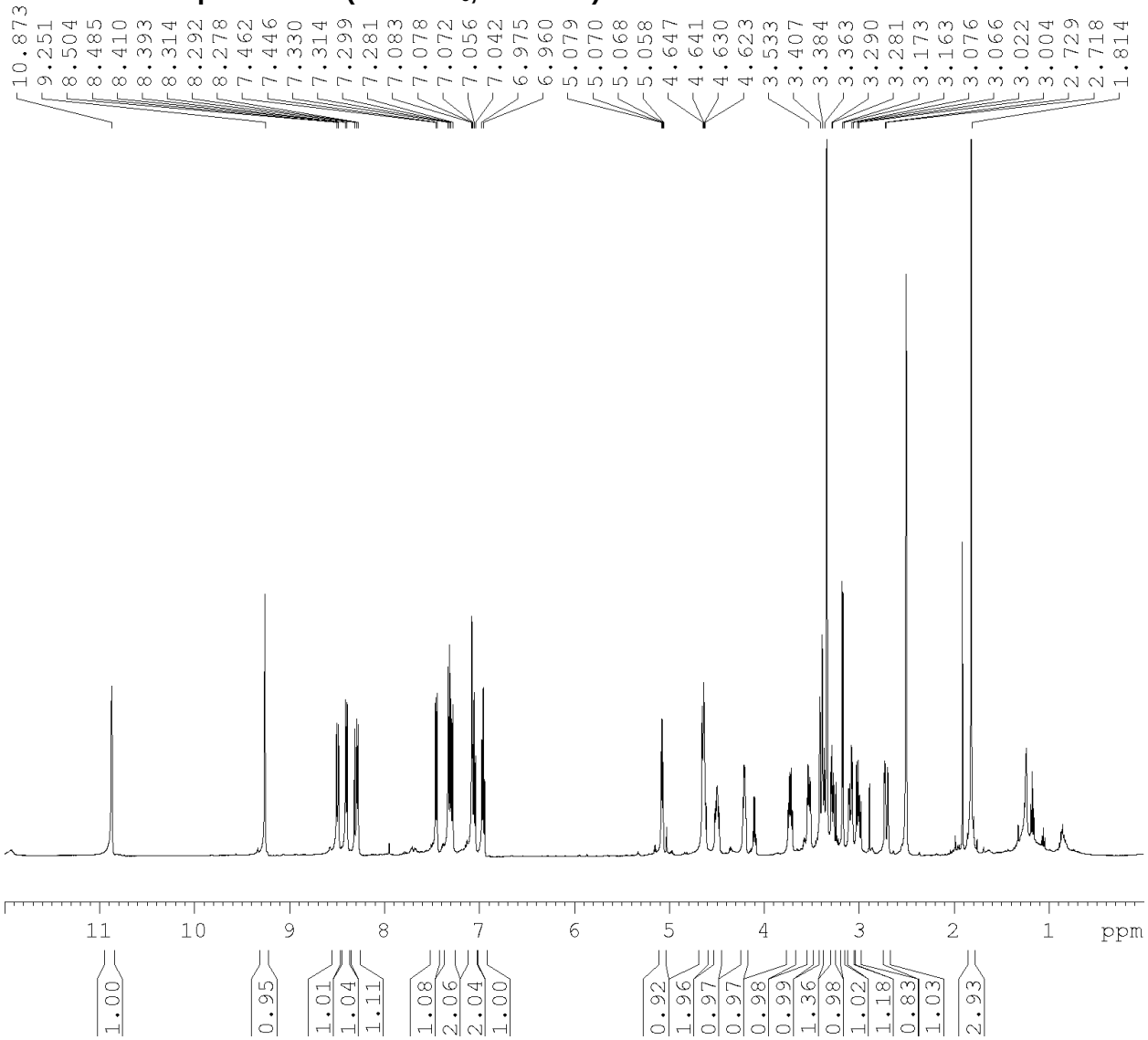
^{1}H NMR of compound 3.19 (DMSO-d_6, 500 MHz)



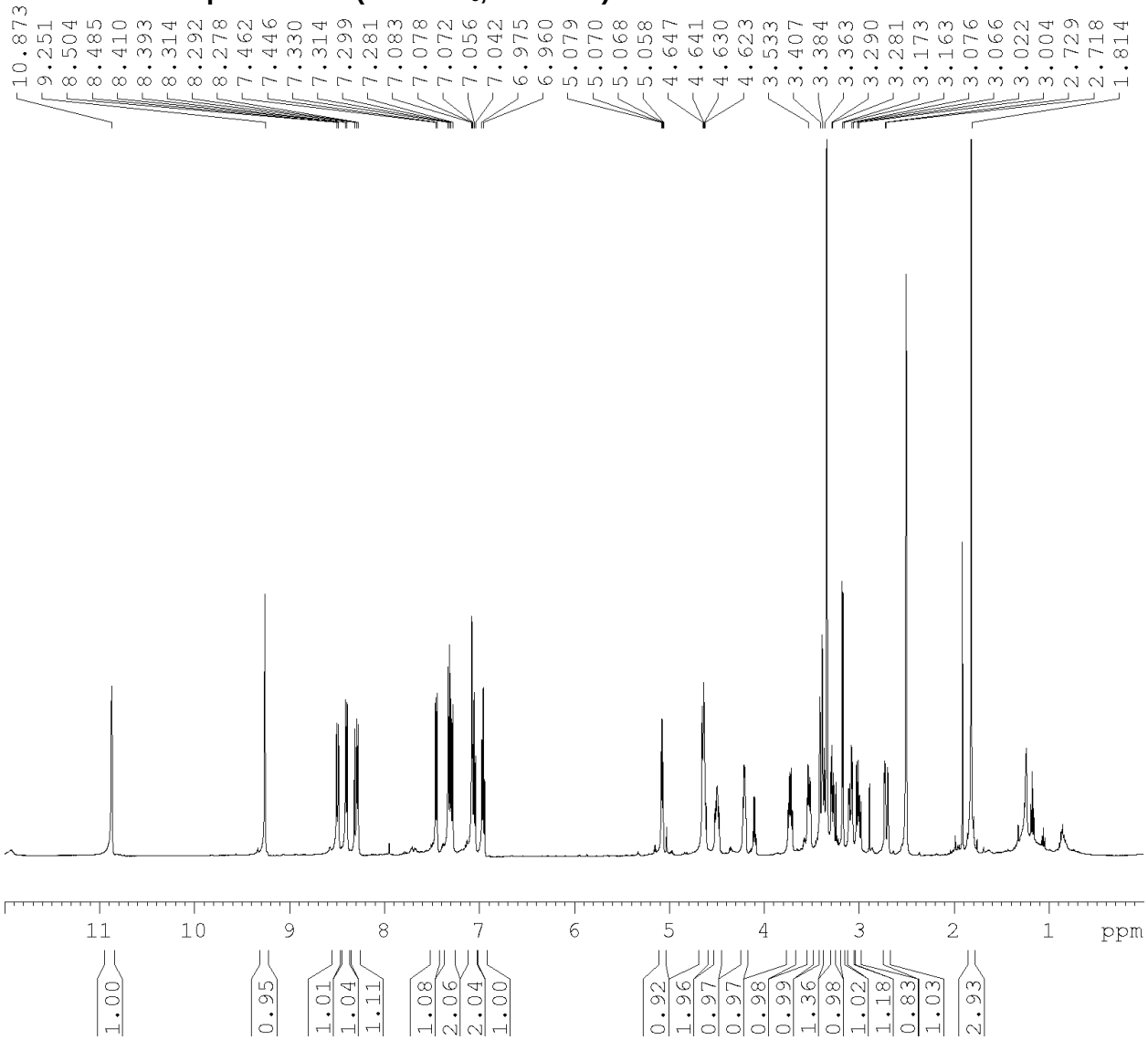
^{19}F NMR of compound 3.19 (DMSO-d_6, 282 MHz)



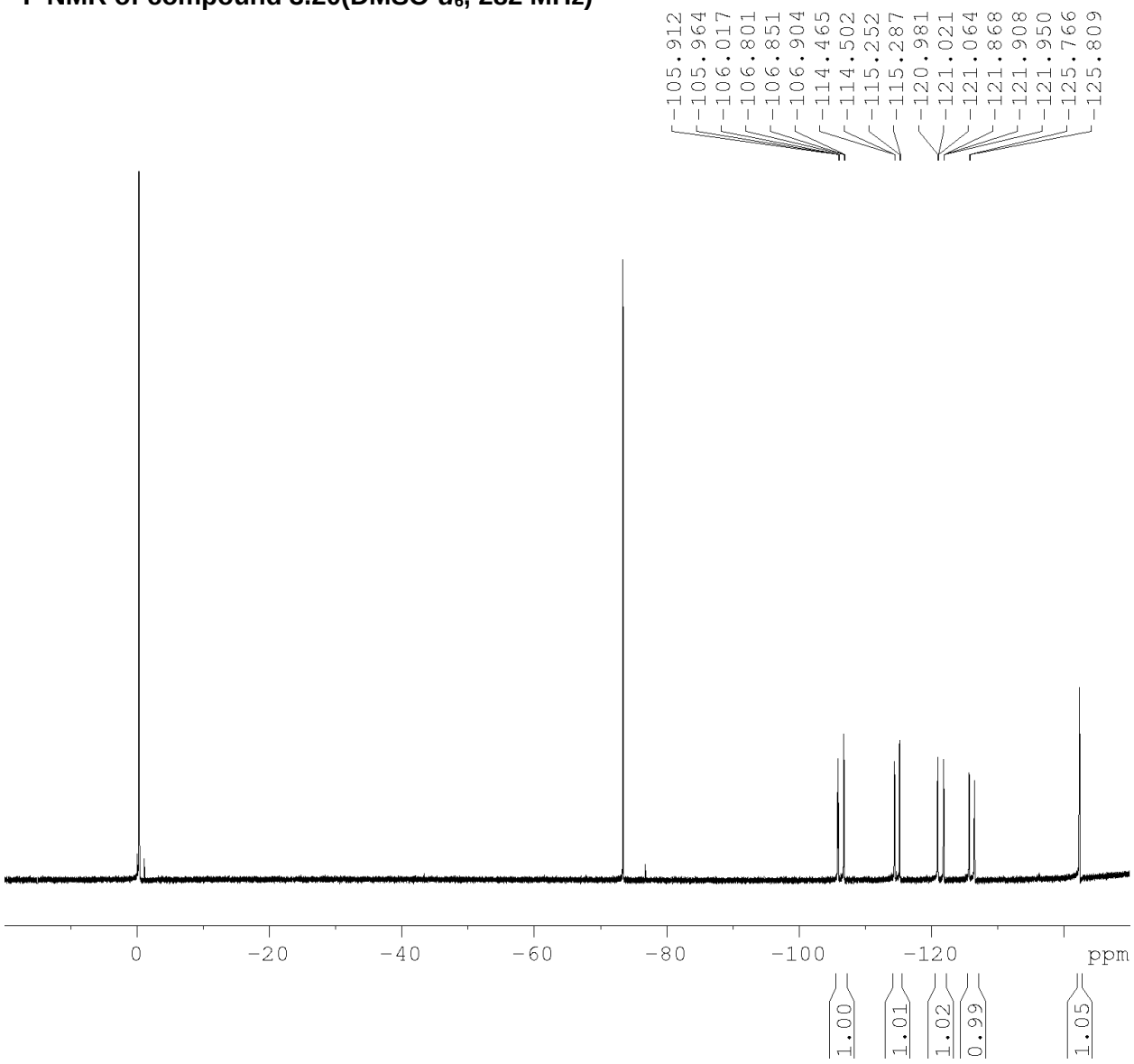
^{1}H NMR of compound 3.20 (DMSO-d_6, 500 MHz)



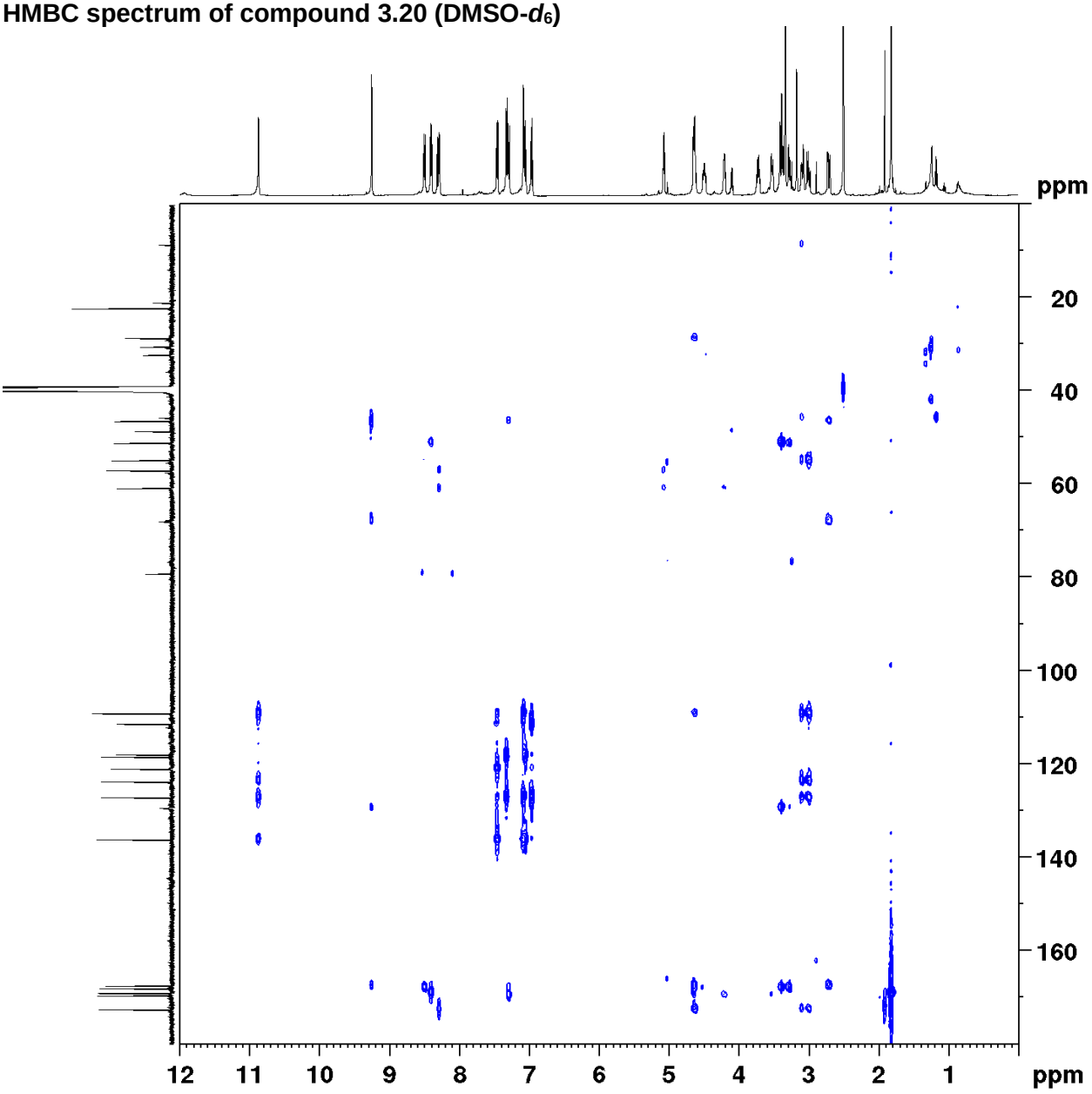
^{13}C NMR of compound 3.20 (DMSO-d_6, 126 MHz)



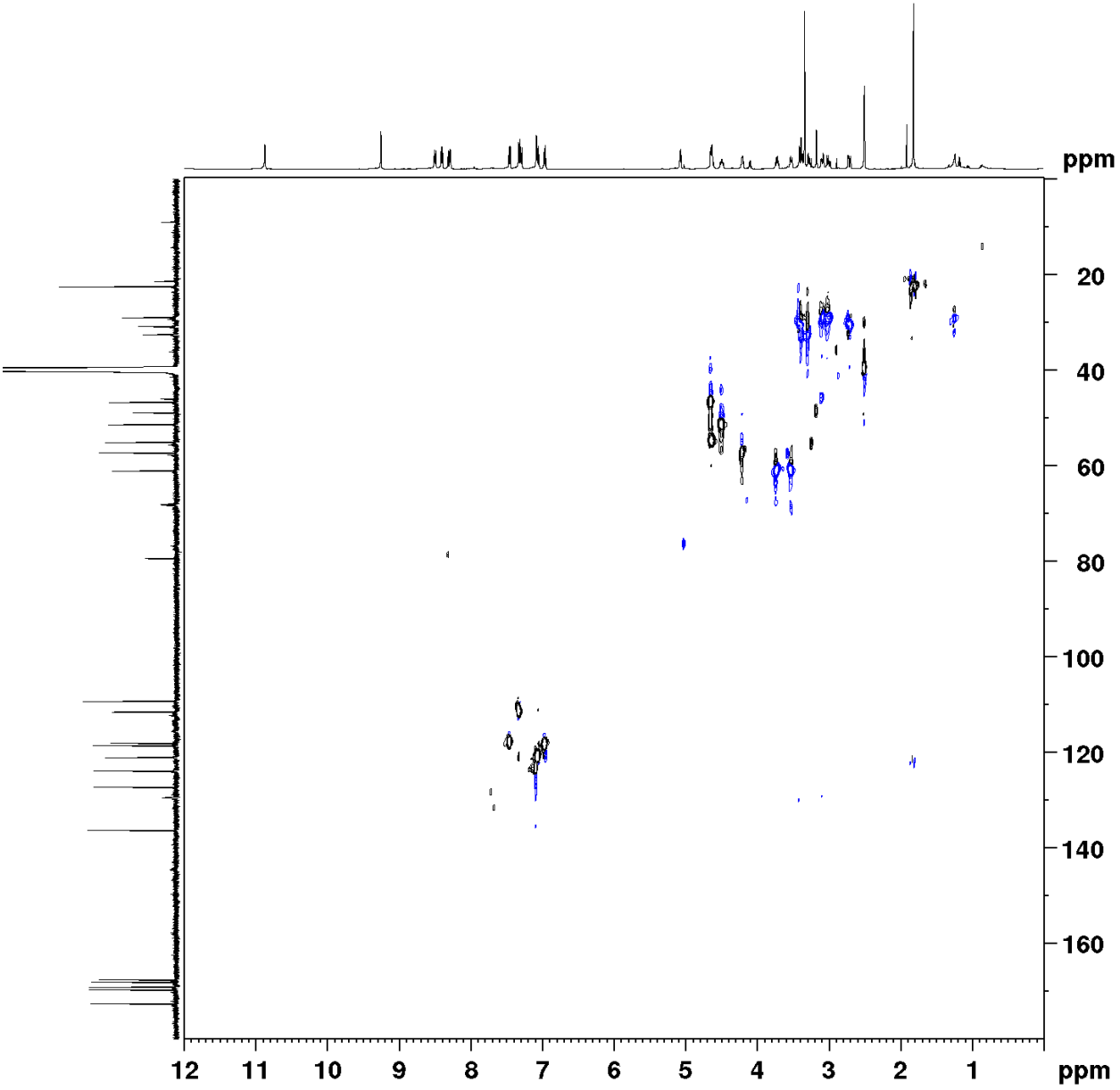
^{19}F NMR of compound 3.20(DMSO-d_6, 282 MHz)



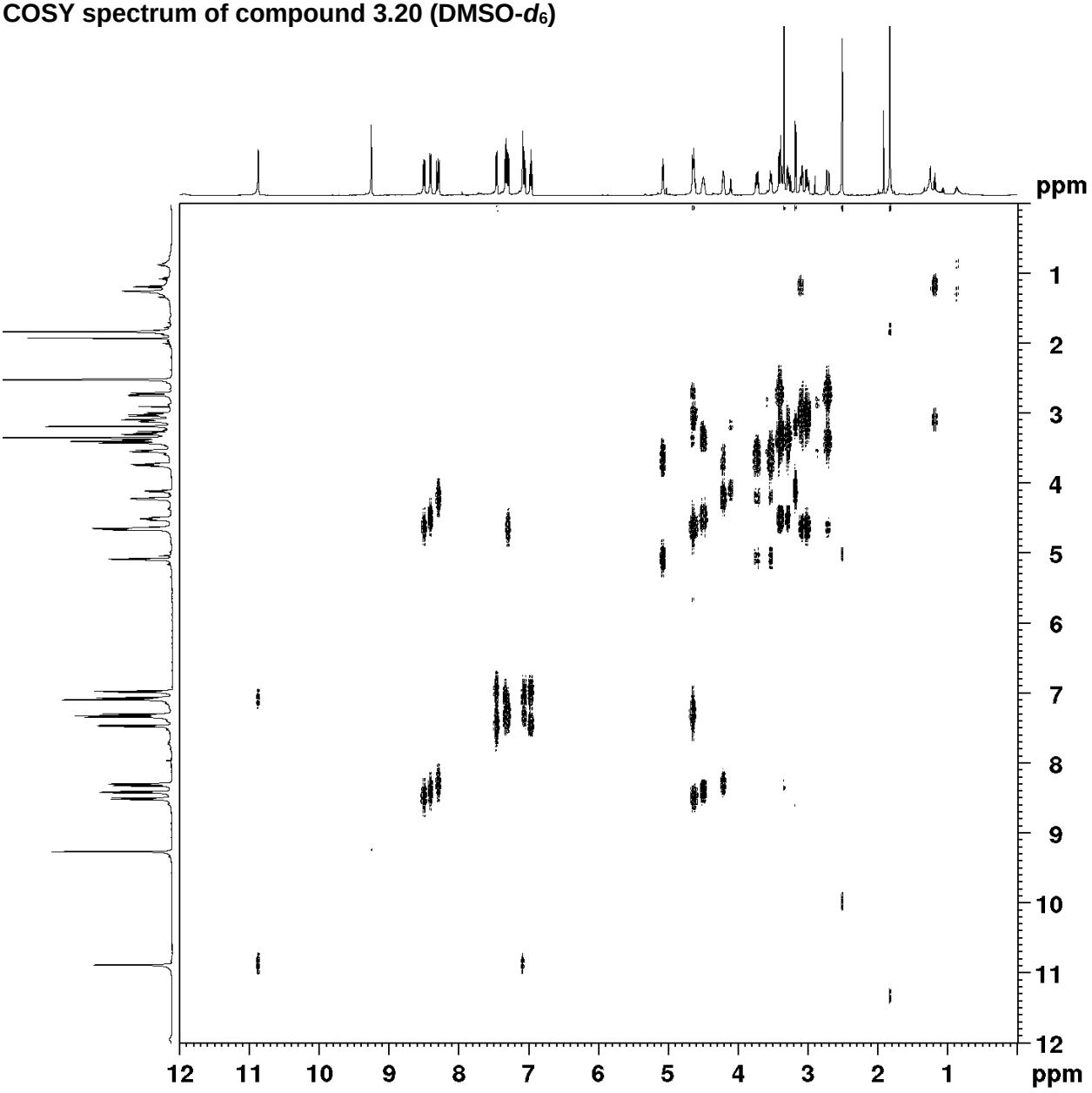
HMBC spectrum of compound 3.20 (DMSO-d_6)



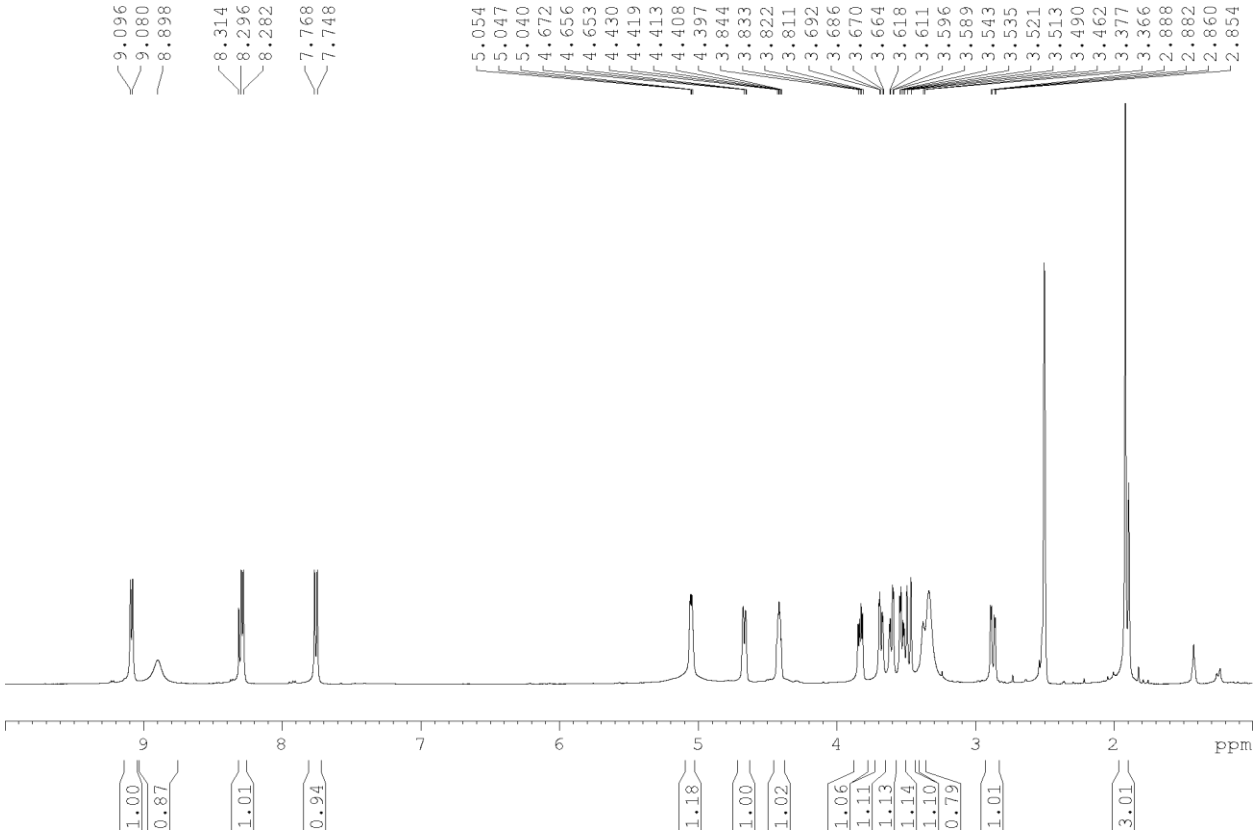
HSQC spectrum of compound 3.20 (DMSO-d_6)



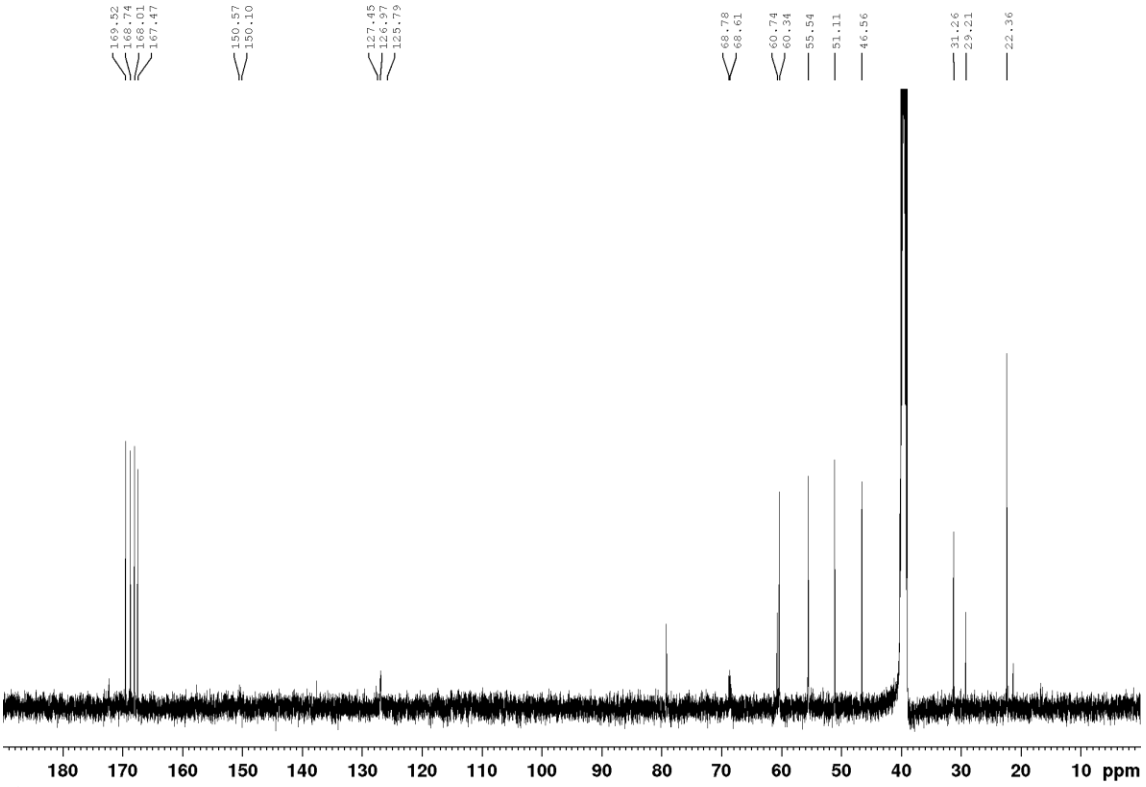
COSY spectrum of compound 3.20 (DMSO-d_6)



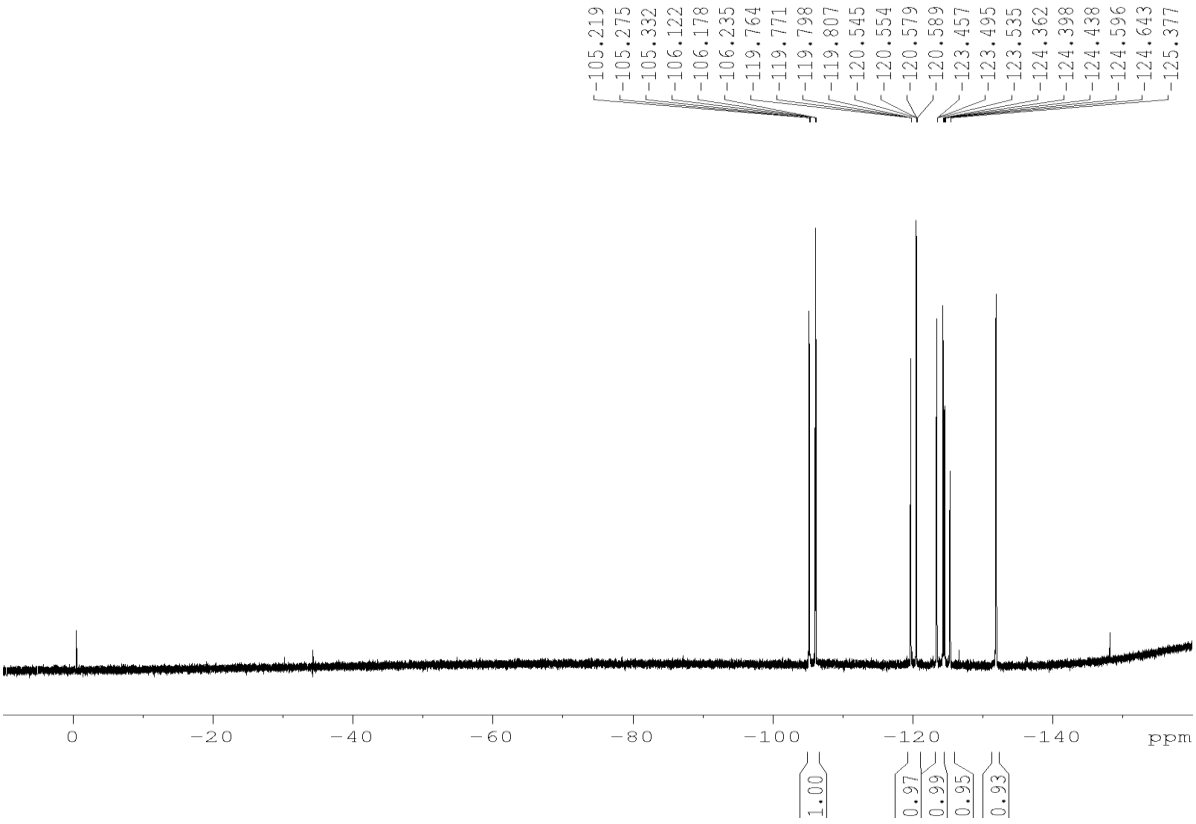
^{1}H NMR of compound 3.21 (DMSO-d_6, 500 MHz)



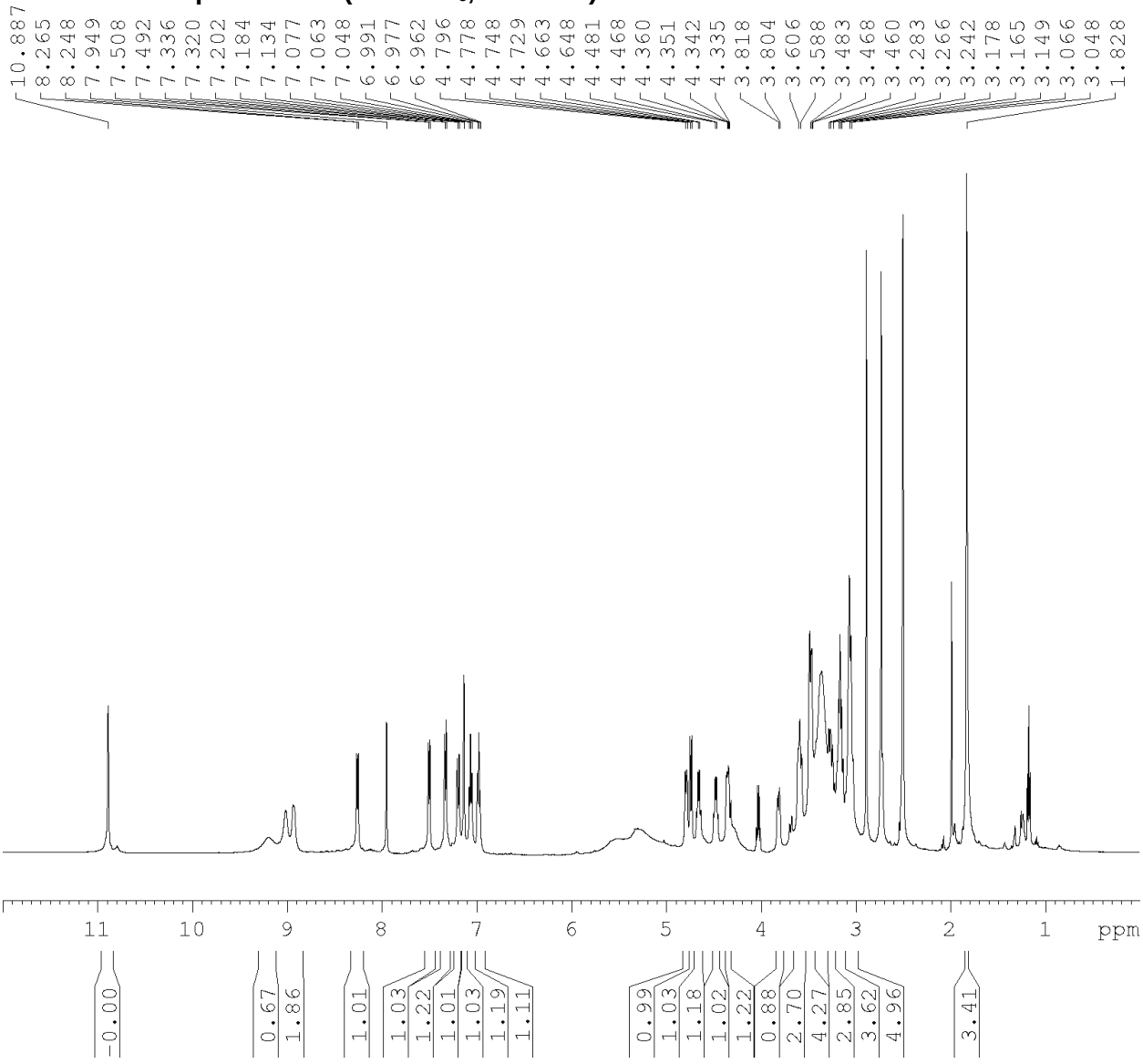
^{13}C NMR of compound 3.21 (DMSO-d_6, 126 MHz)



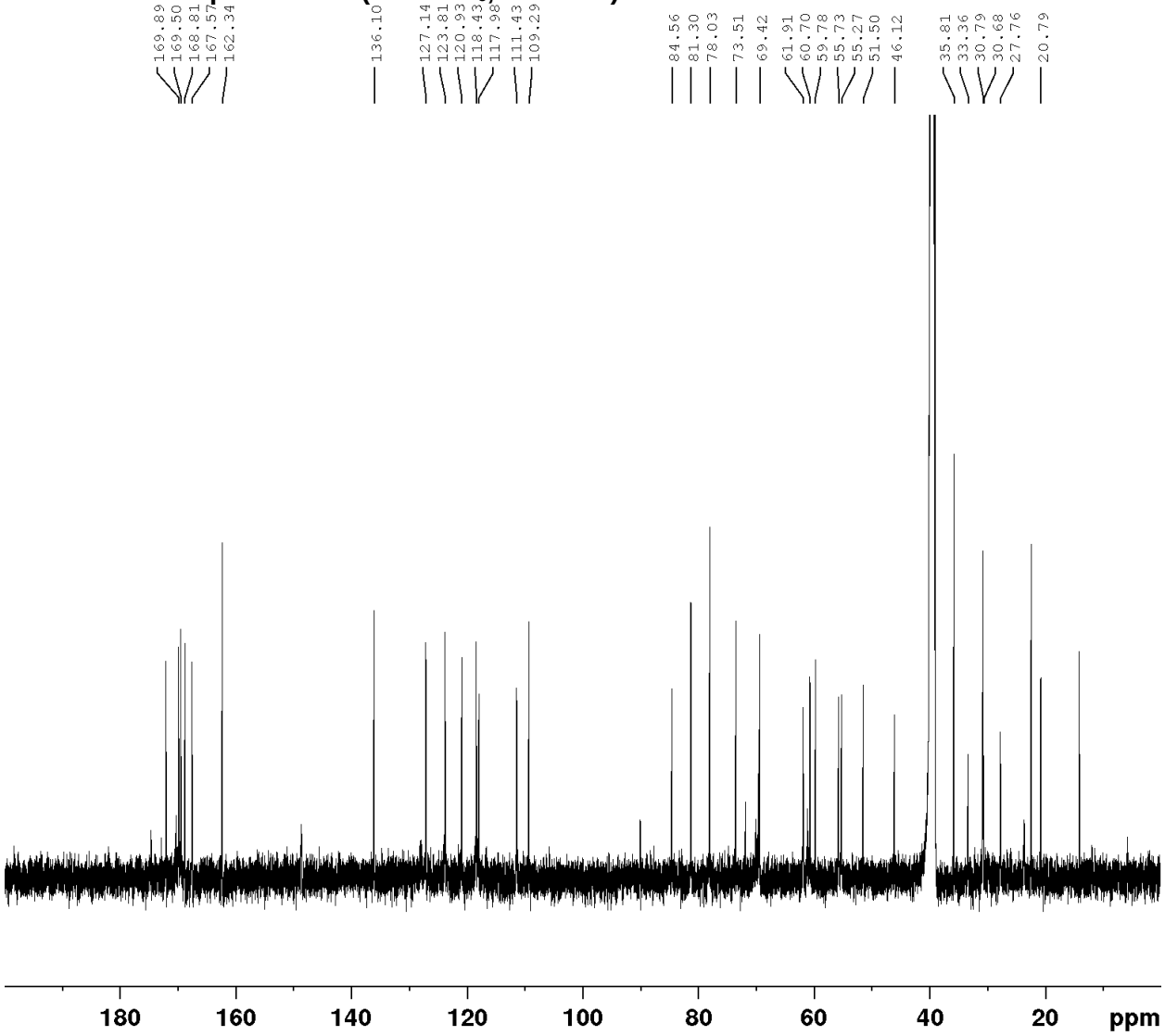
^{19}F NMR of compound 3.21 (DMSO-d_6, 282 MHz)



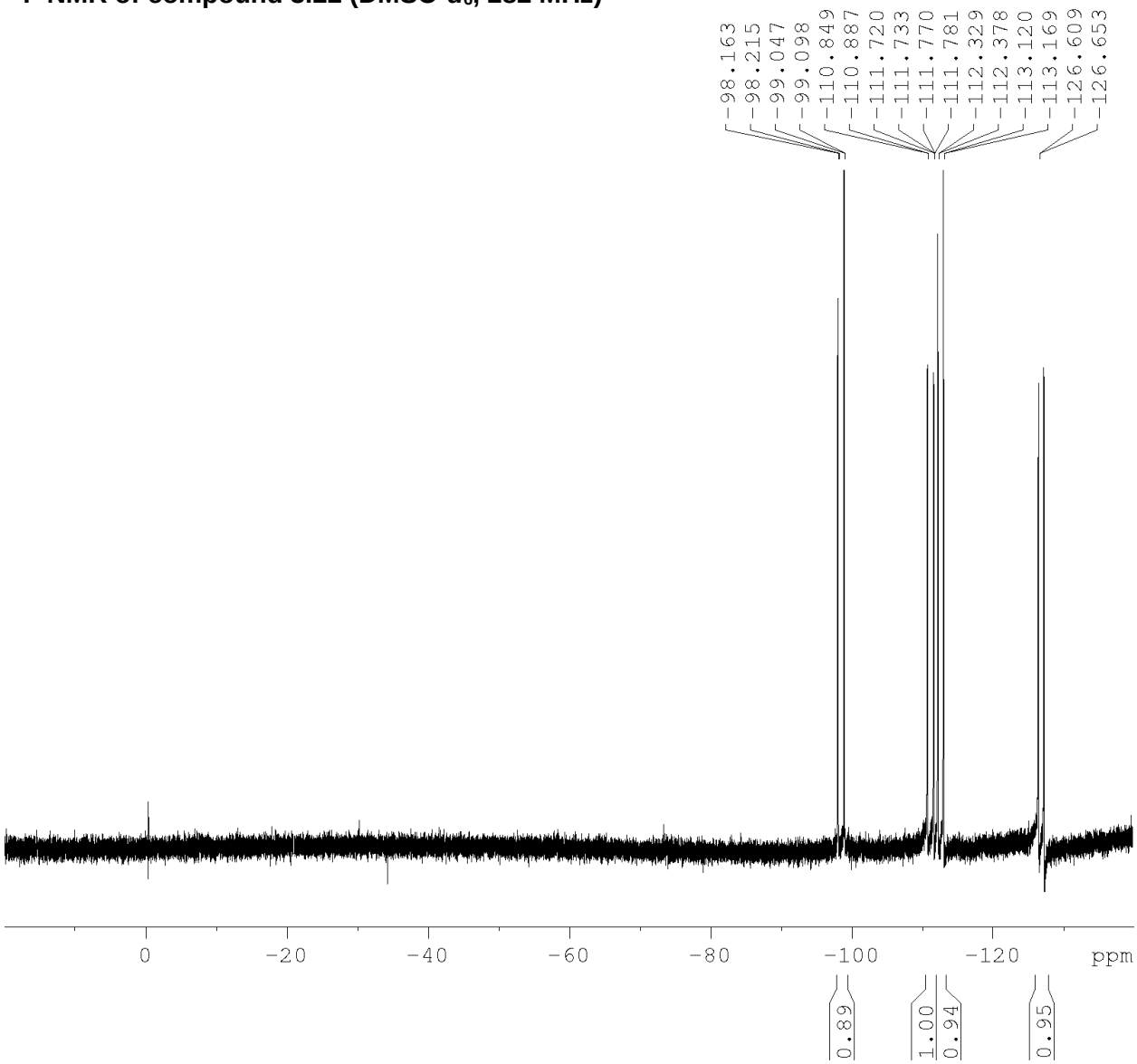
^{1}H NMR of compound 3.22 (DMSO-d_6, 500 MHz)



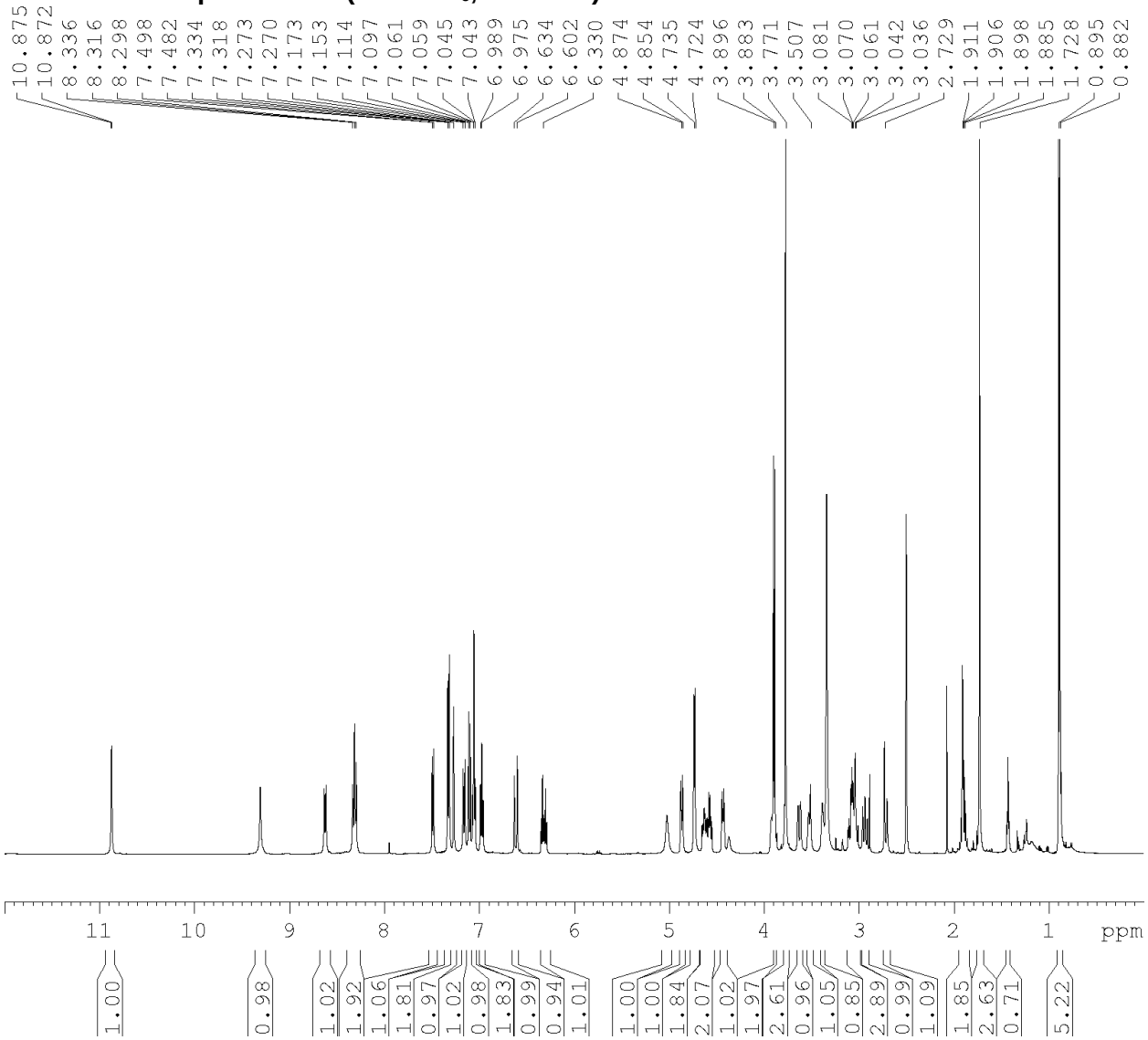
^{13}C NMR of compound 3.22 (DMSO-d_6, 126 MHz)



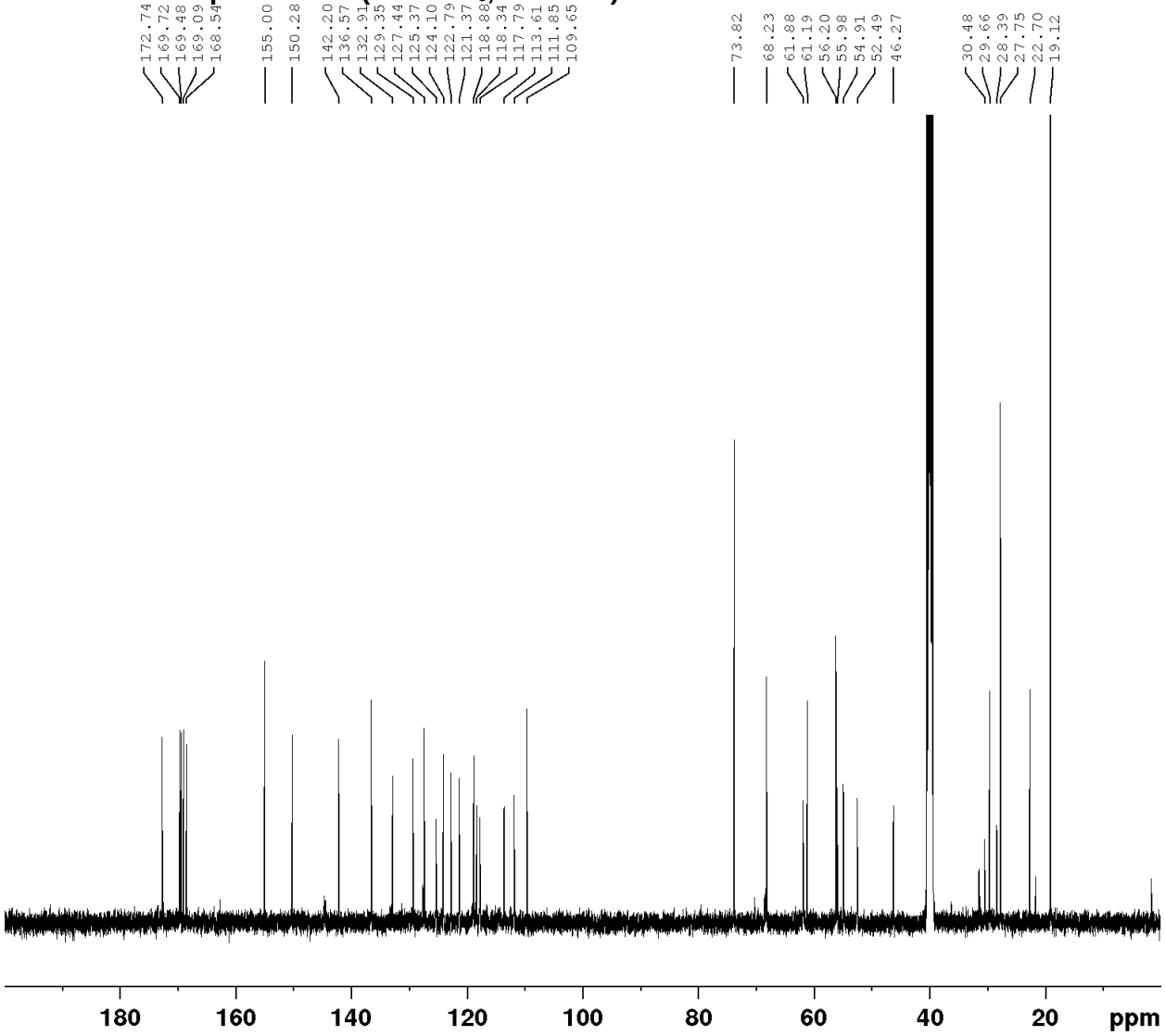
^{19}F NMR of compound 3.22 (DMSO-d_6, 282 MHz)



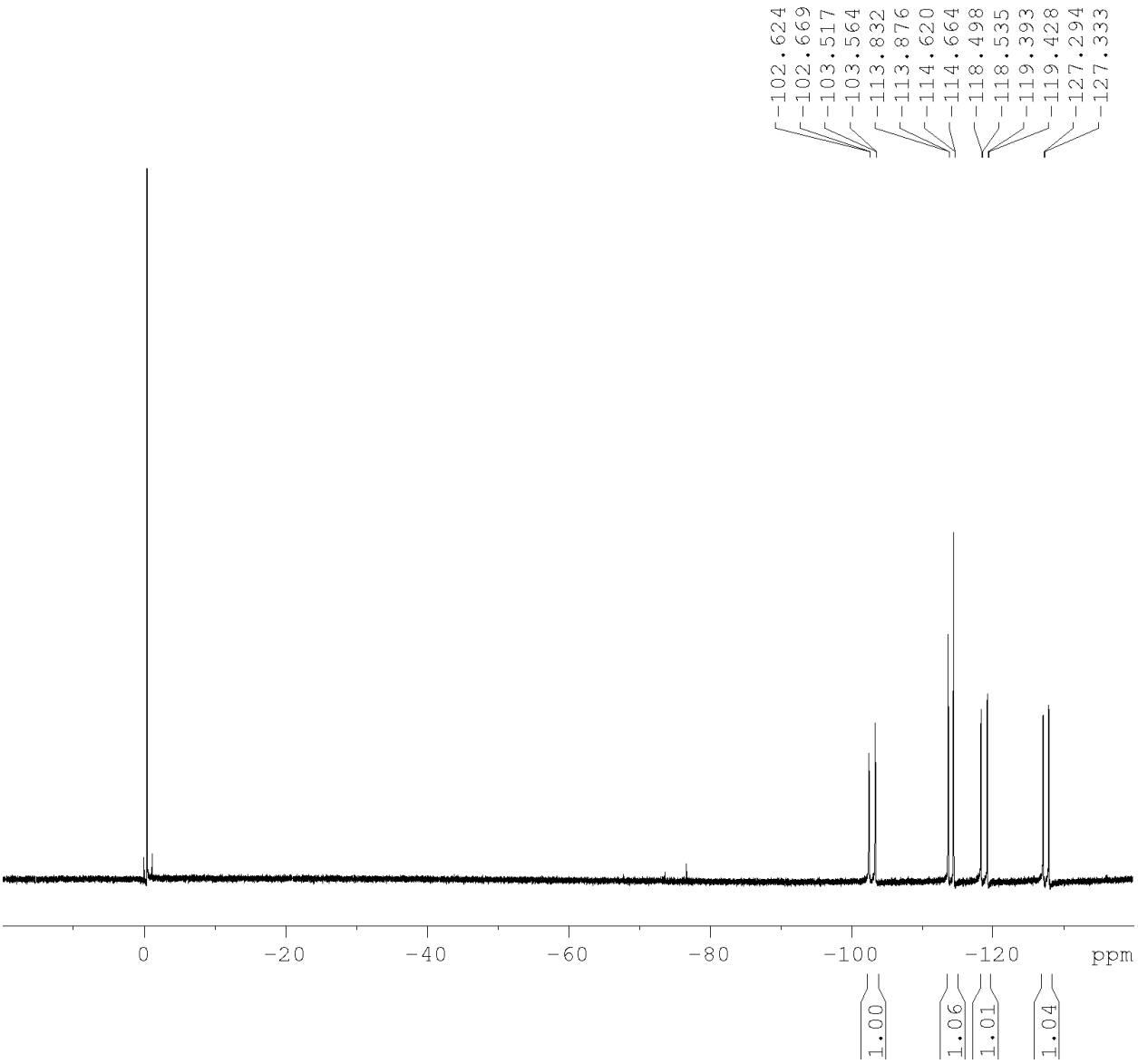
^{1}H NMR of compound 3.23 (DMSO-d_6, 500 MHz)



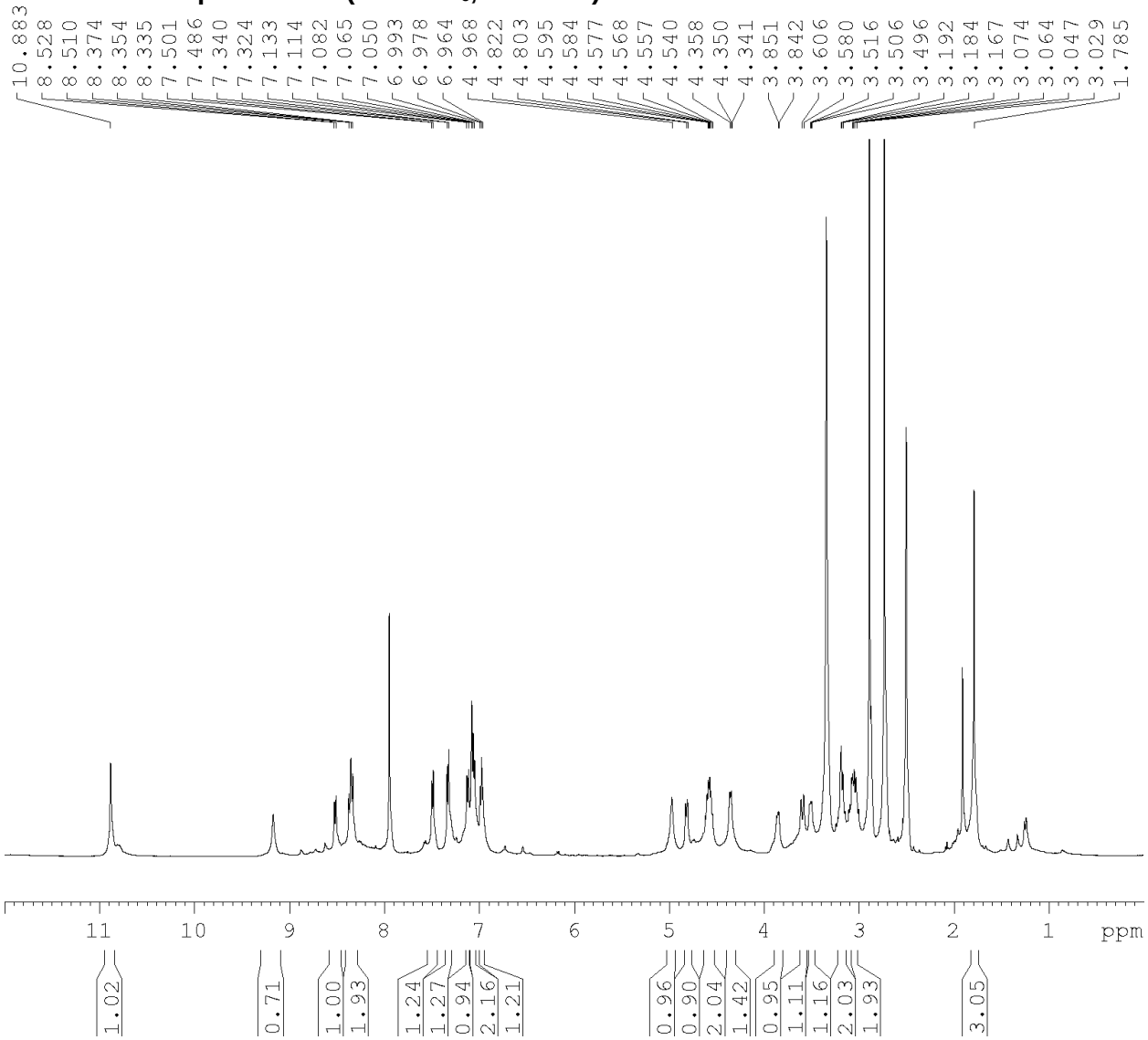
^{13}C NMR of compound 3.23 (DMSO-d_6, 126 MHz)



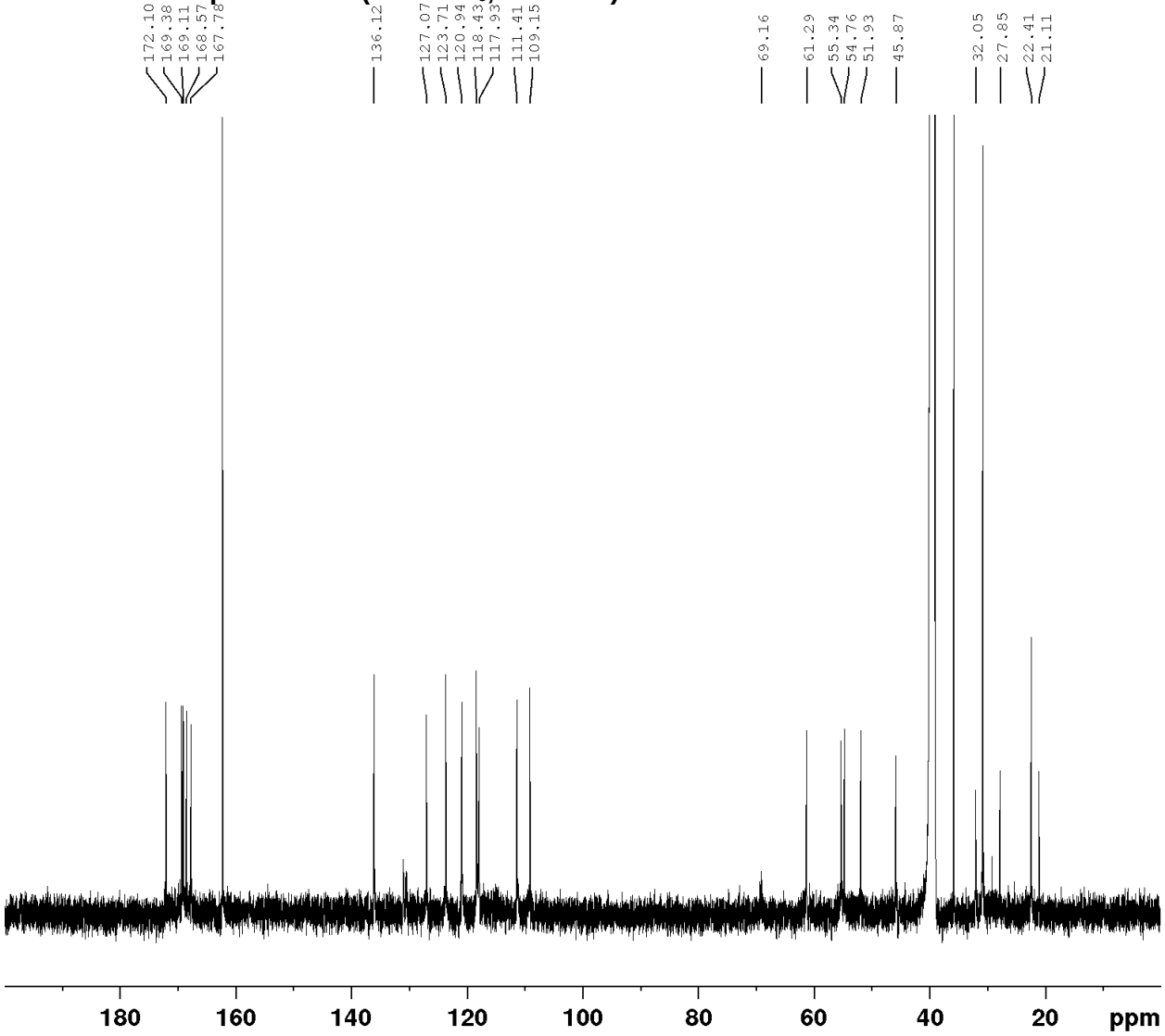
^{19}F NMR of compound 3.23 (DMSO-d_6, 282 MHz)



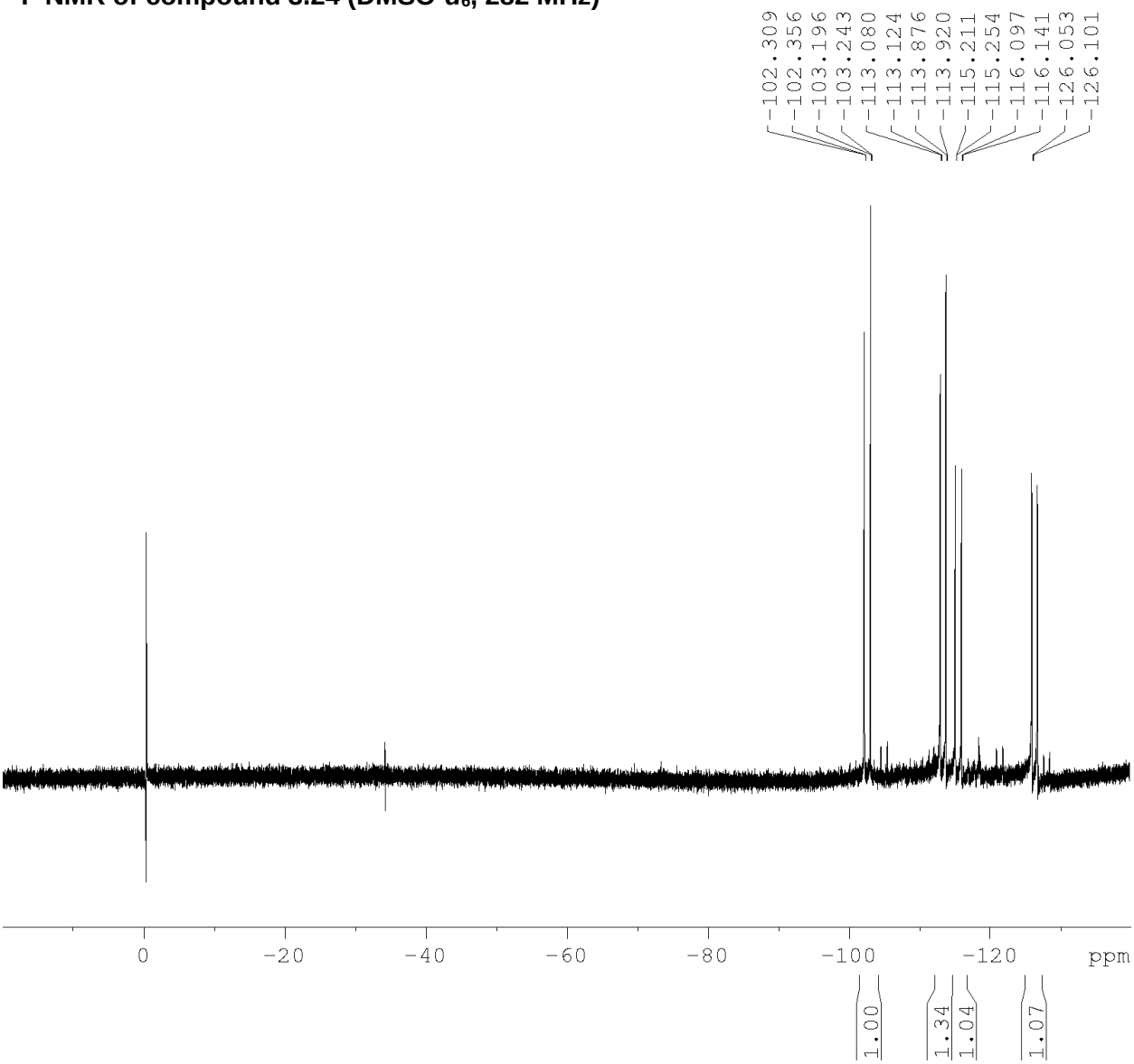
^{1}H NMR of compound 3.24 (DMSO-d_6, 500 MHz)



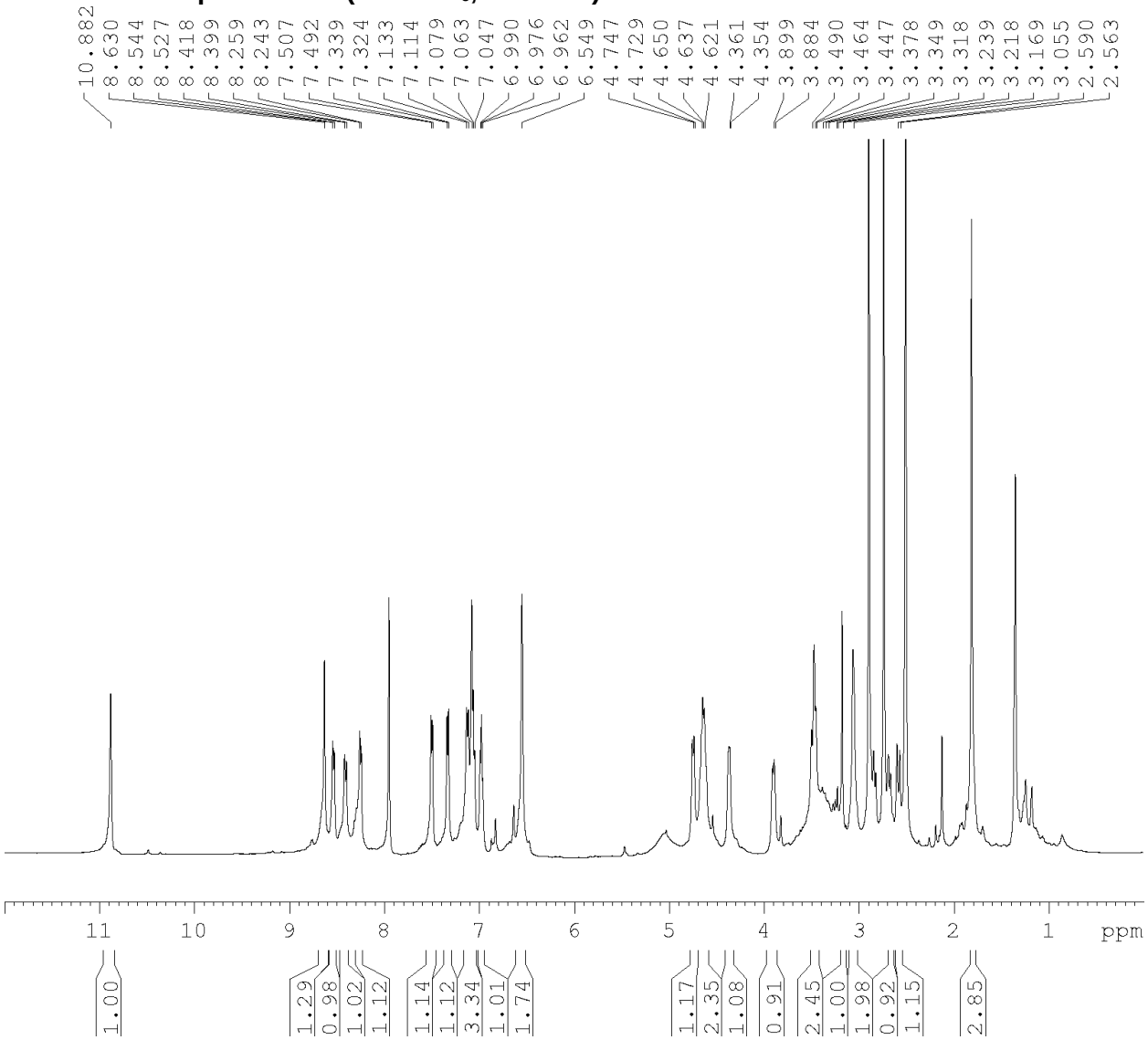
^{13}C NMR of compound 3.24 (DMSO-d_6, 126 MHz)



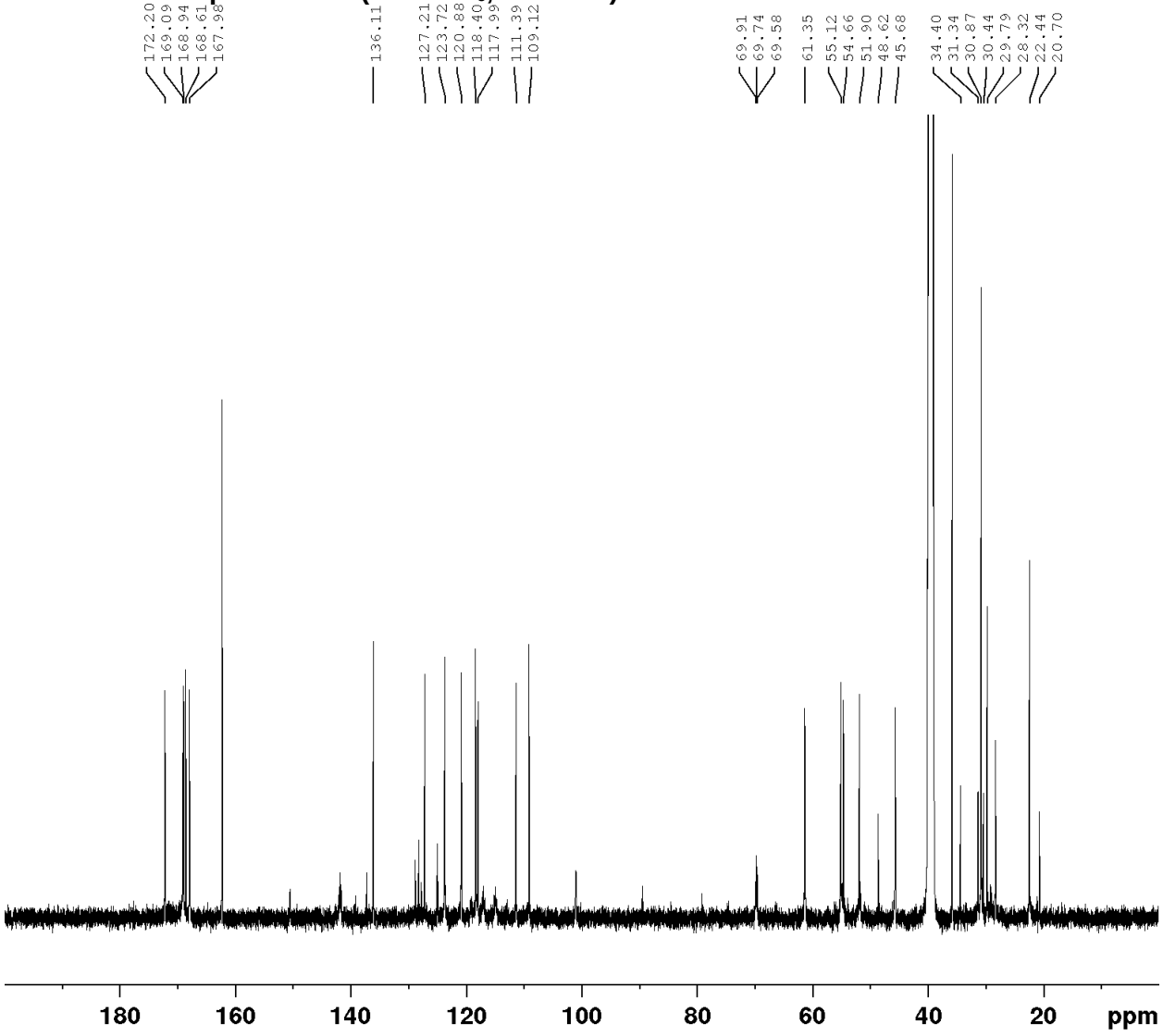
^{19}F NMR of compound 3.24 (DMSO-d_6, 282 MHz)



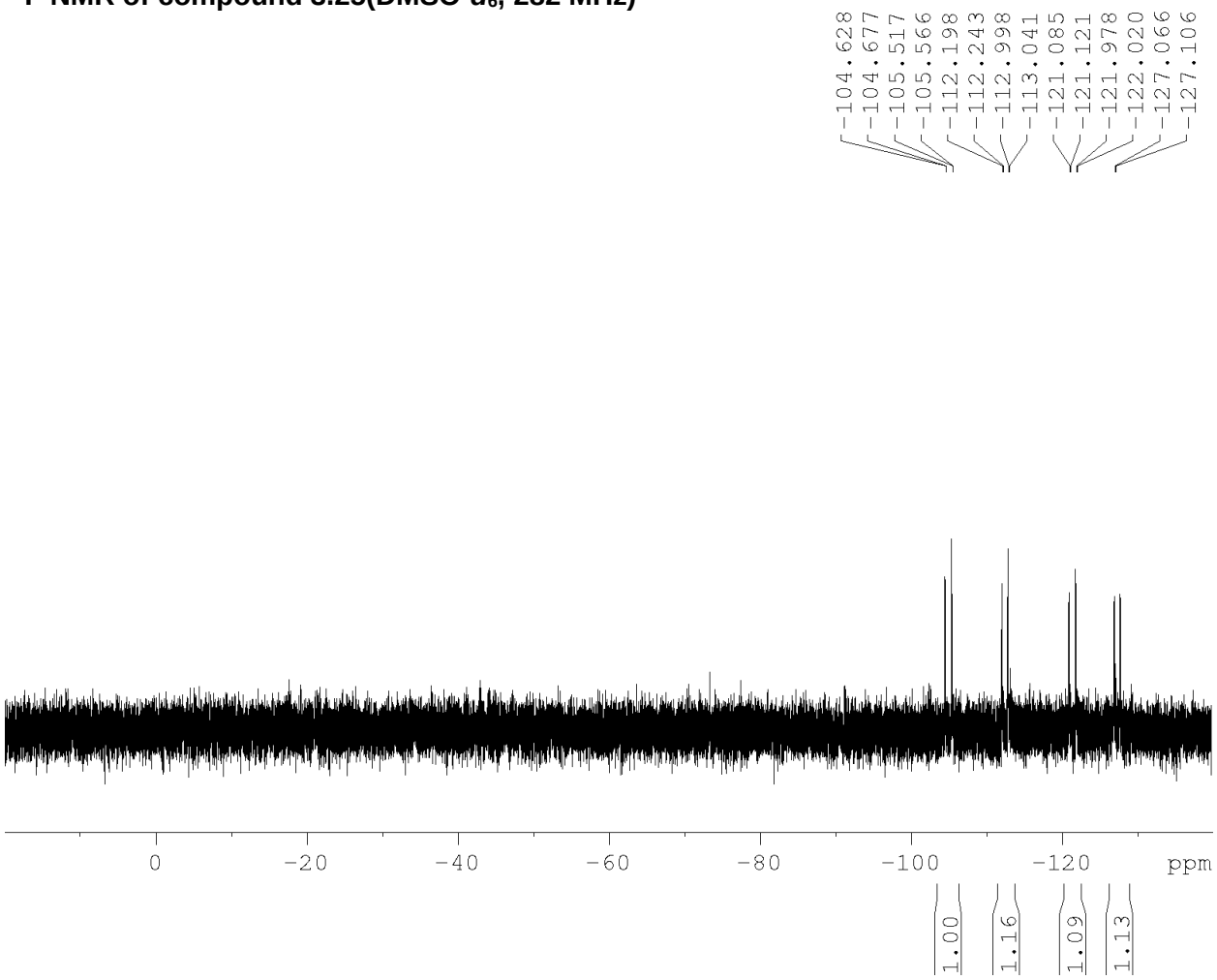
^{1}H NMR of compound 3.25 (DMSO-d_6, 500 MHz)



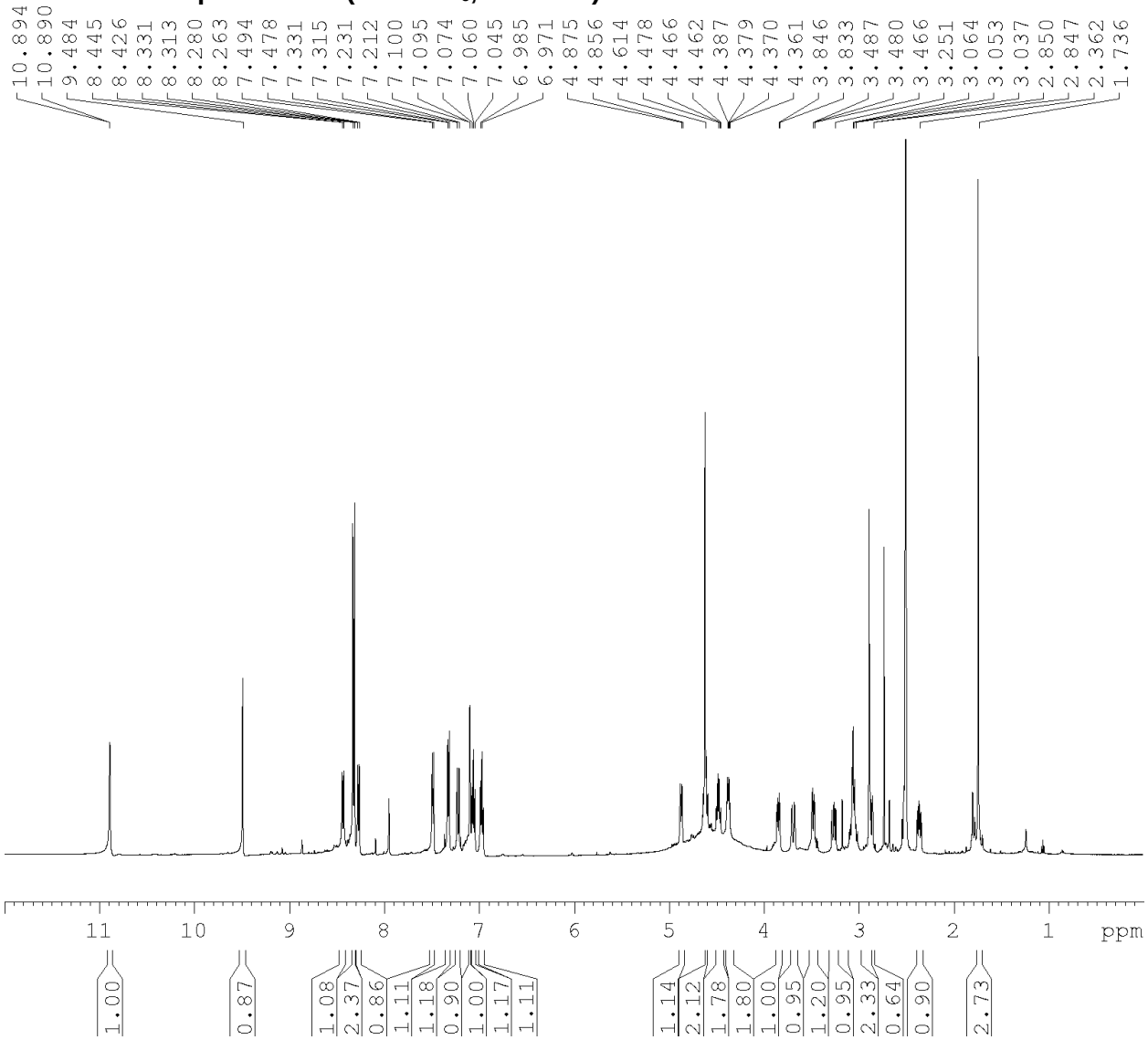
^{13}C NMR of compound 3.25 (DMSO-d_6, 126 MHz)



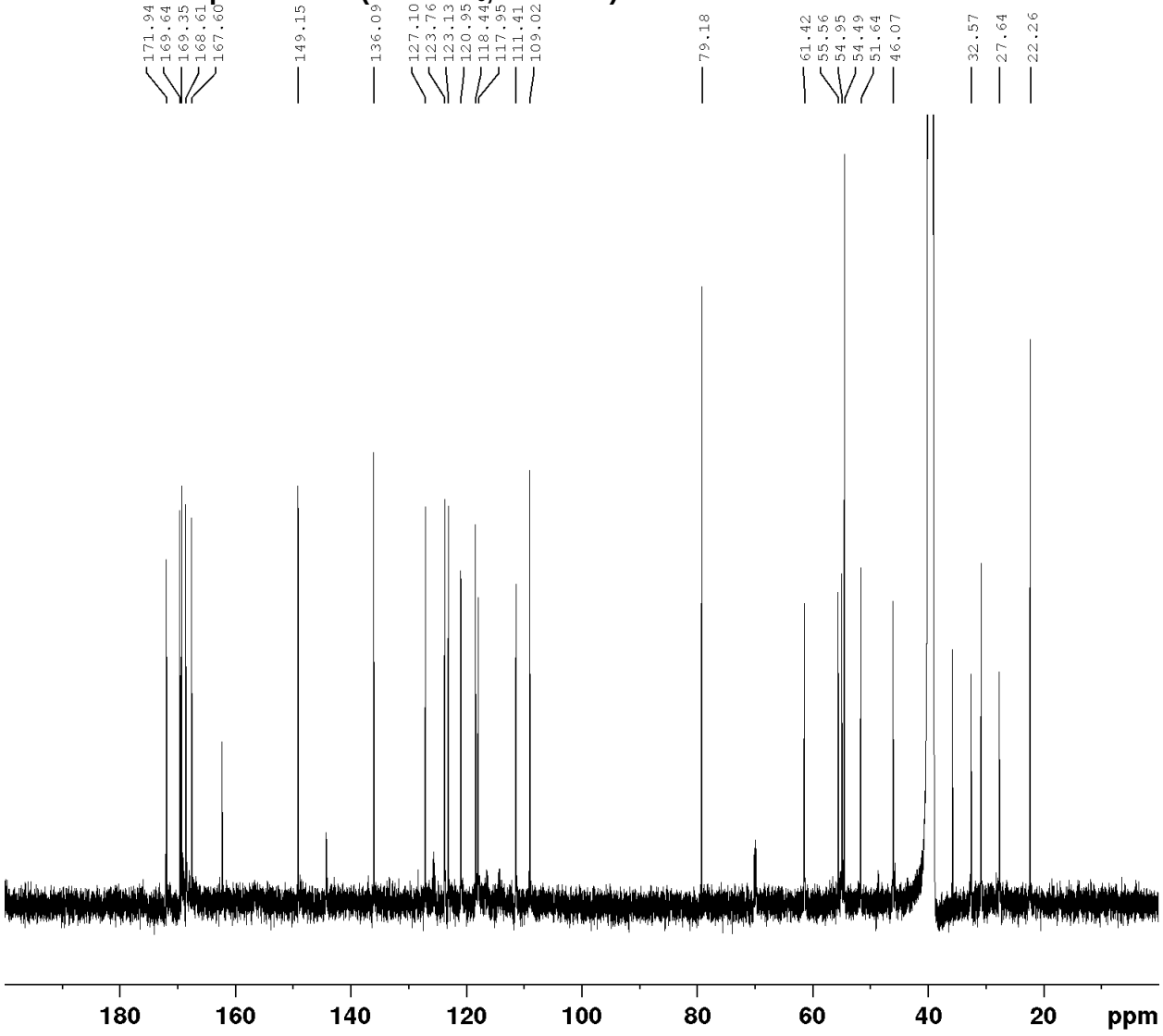
^{19}F NMR of compound 3.25(DMSO-d_6, 282 MHz)



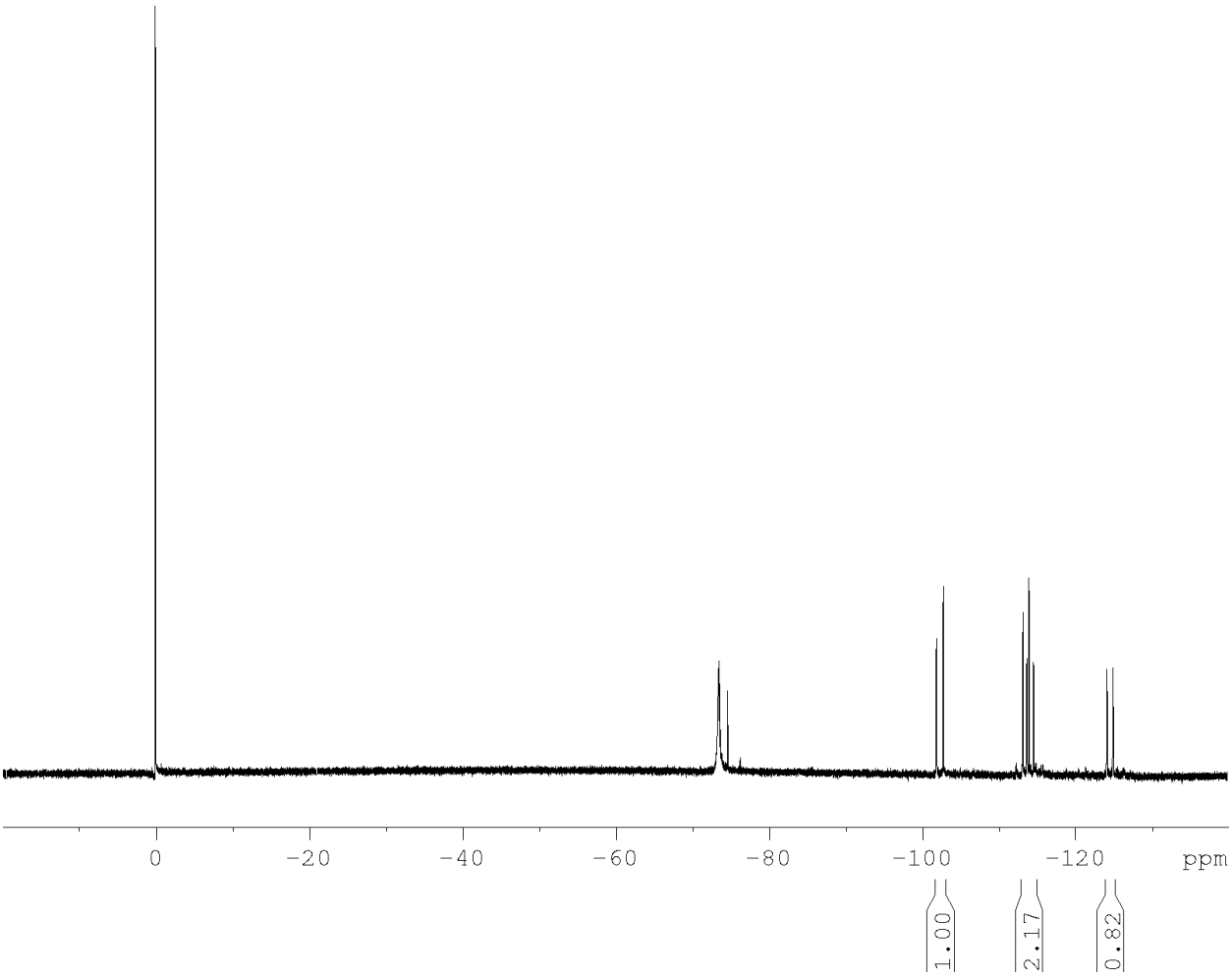
^{1}H NMR of compound 3.26 (DMSO-d_6, 500 MHz)



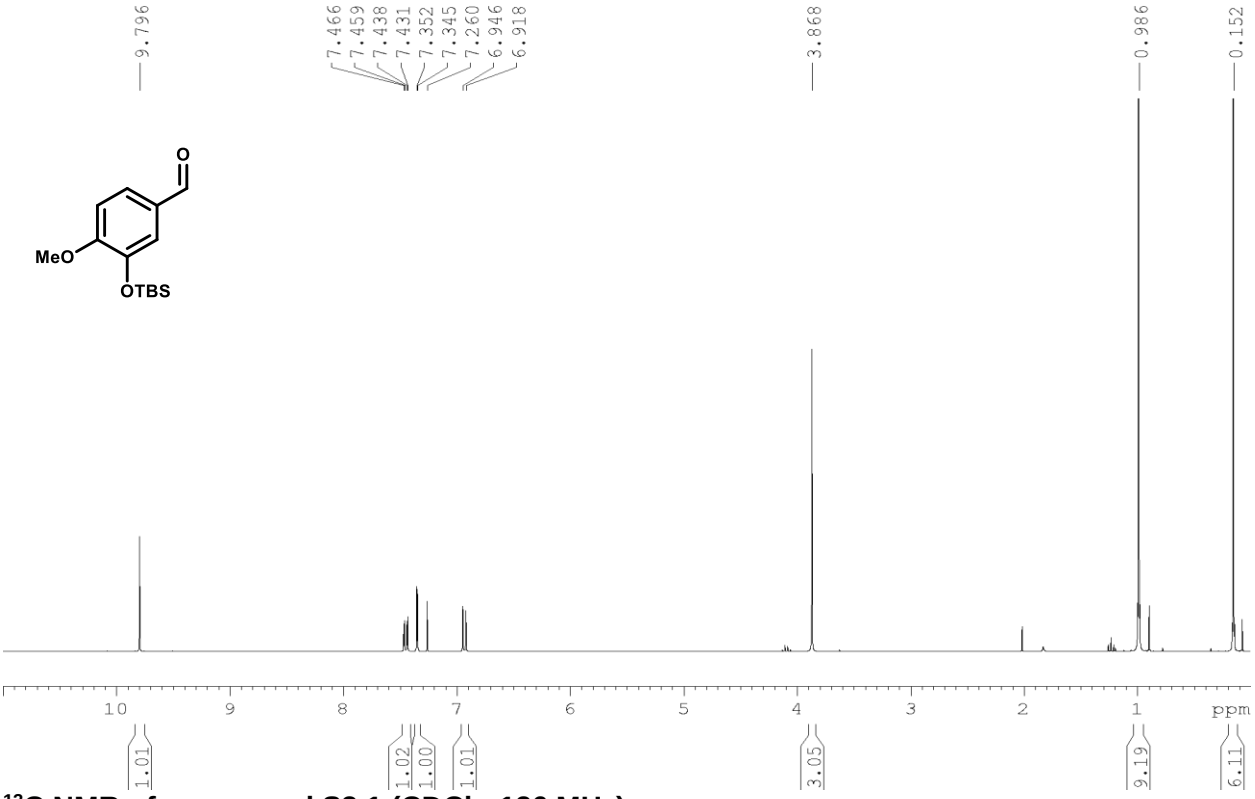
^{13}C NMR of compound 3.26 (DMSO-d_6, 126 MHz)



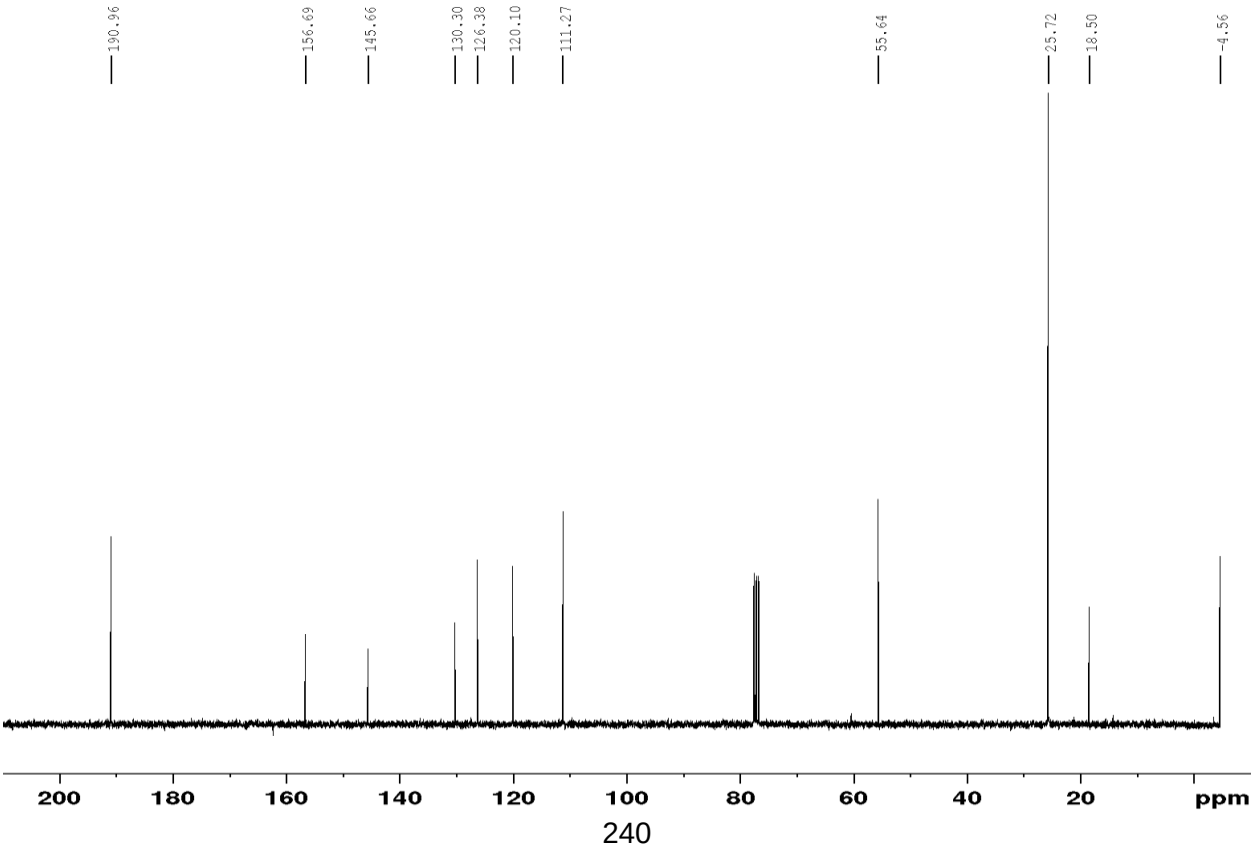
^{19}F NMR of compound 3.26 (DMSO-d_6, 282 MHz)



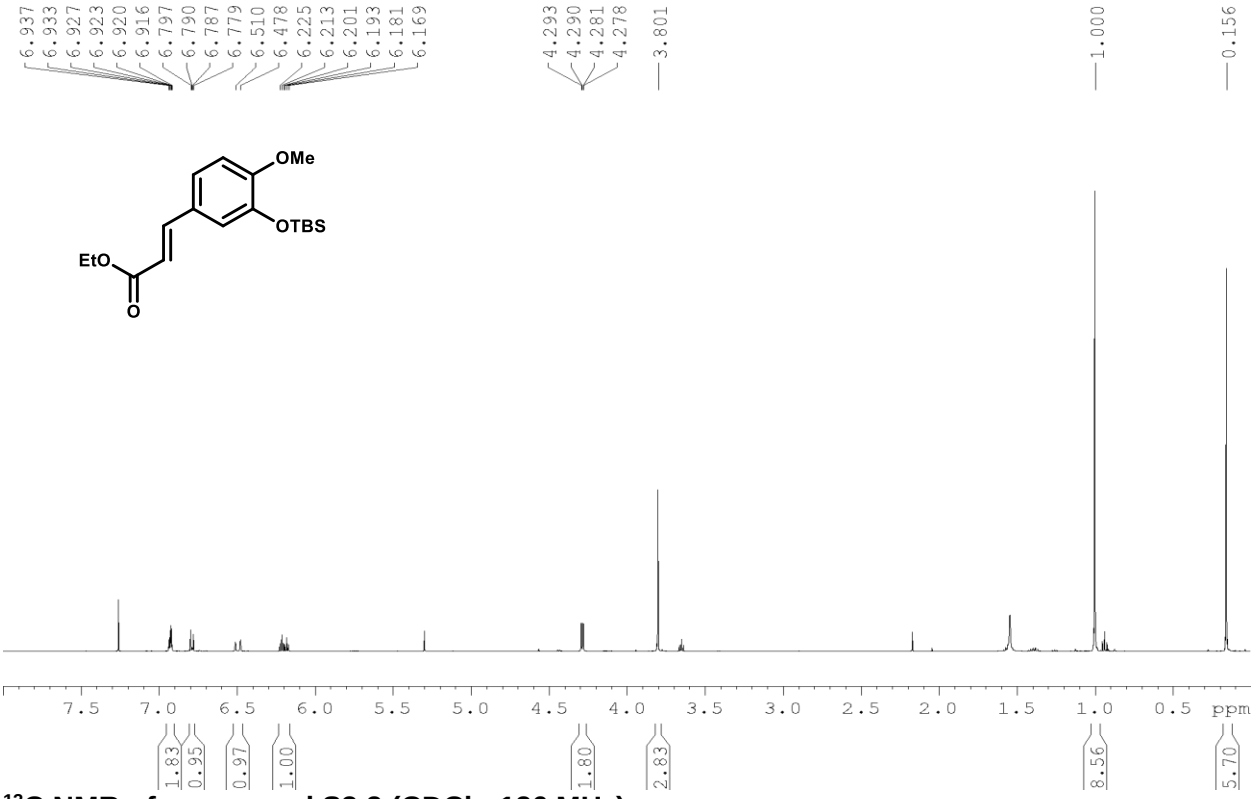
^{1}H NMR of compound S3.1 ($CDCl_3$, 300 MHz)



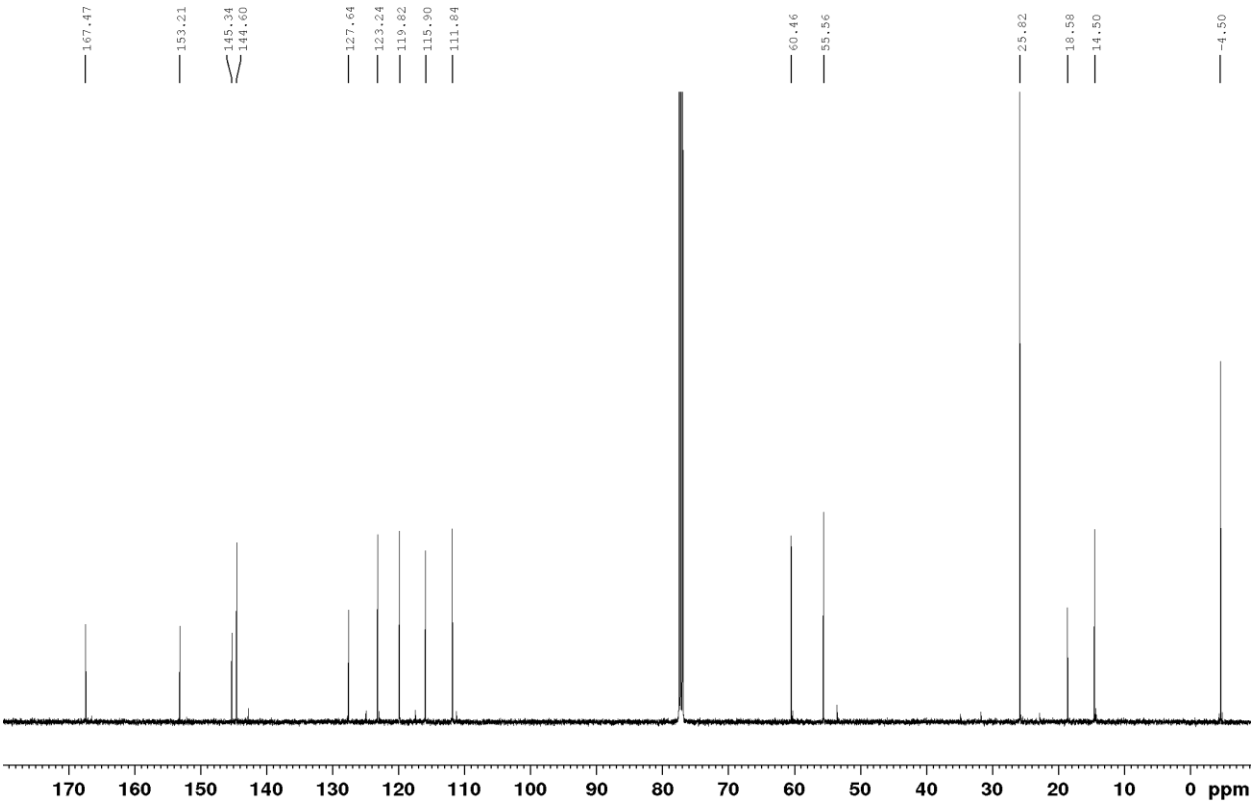
^{13}C NMR of compound S3.1 ($CDCl_3$, 126 MHz)



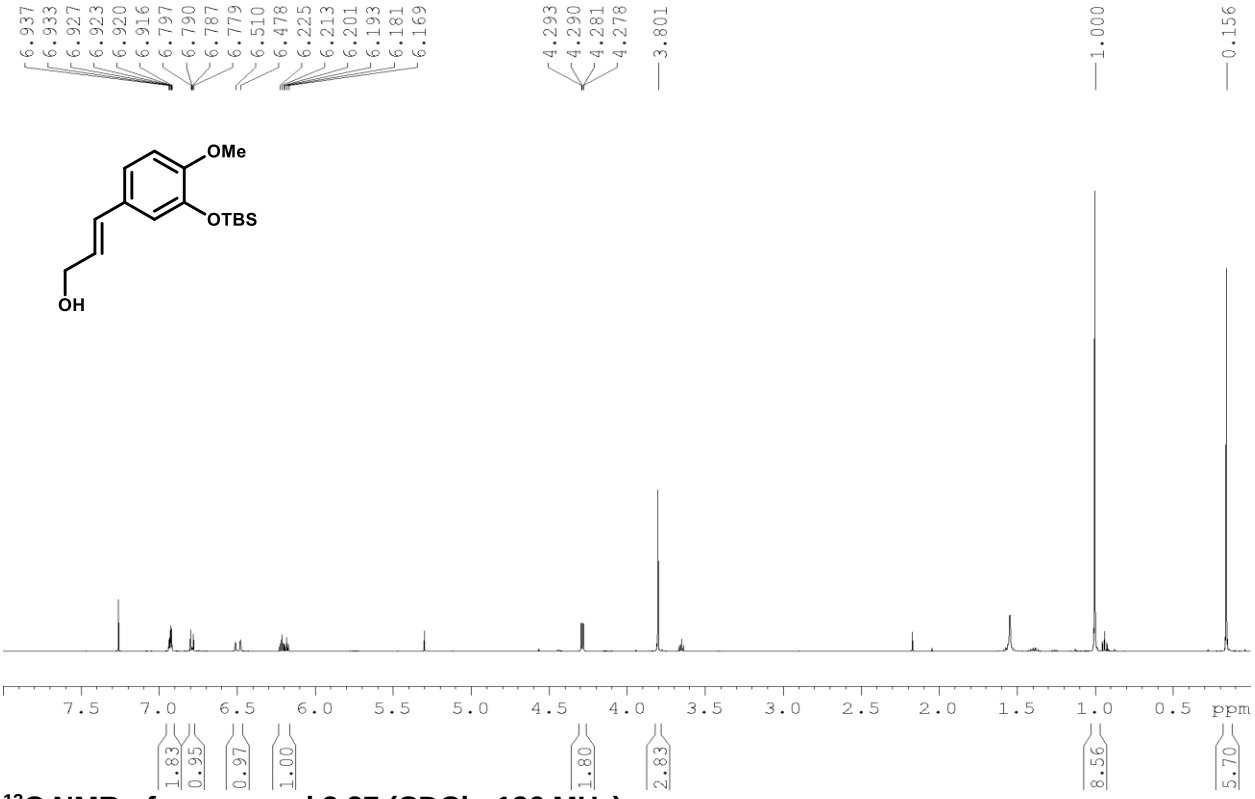
^{1}H NMR of compound S3.2 ($CDCl_3$, 500 MHz)



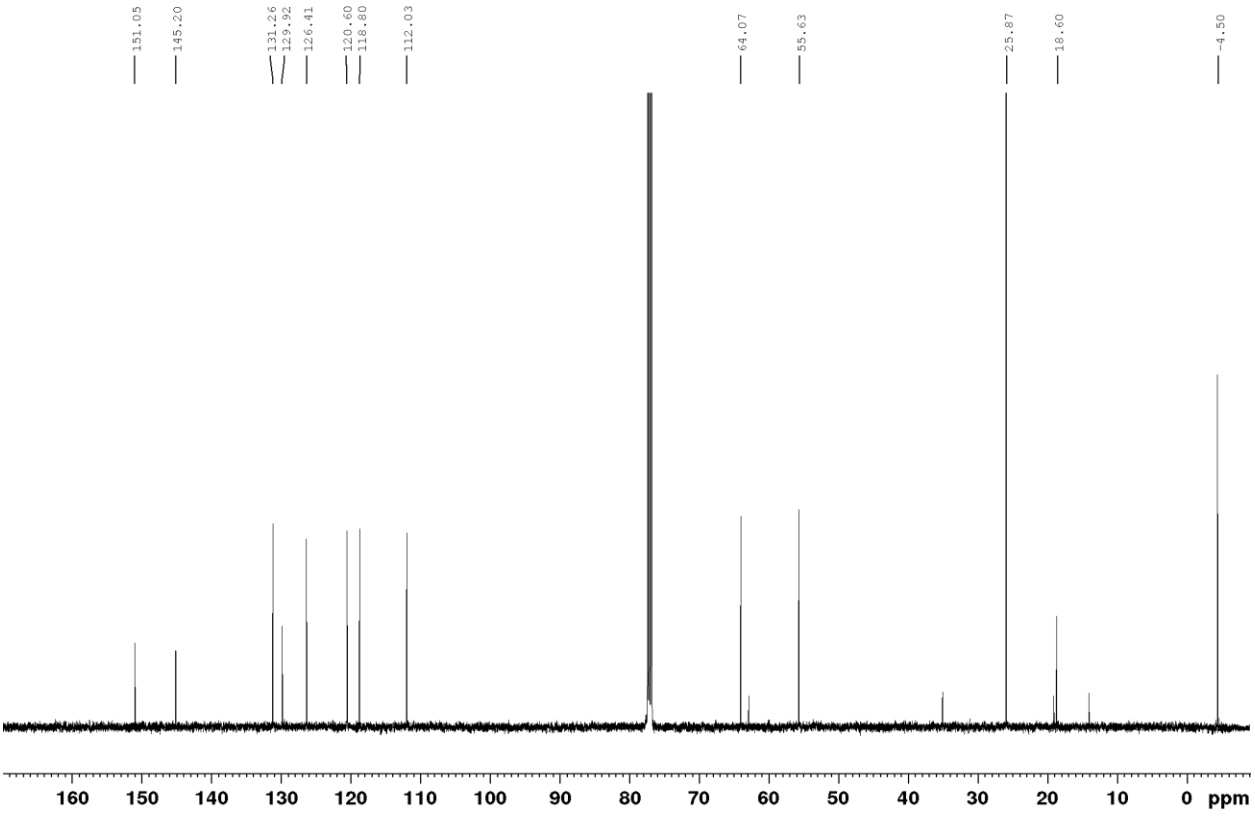
^{13}C NMR of compound S3.2 ($CDCl_3$, 126 MHz)



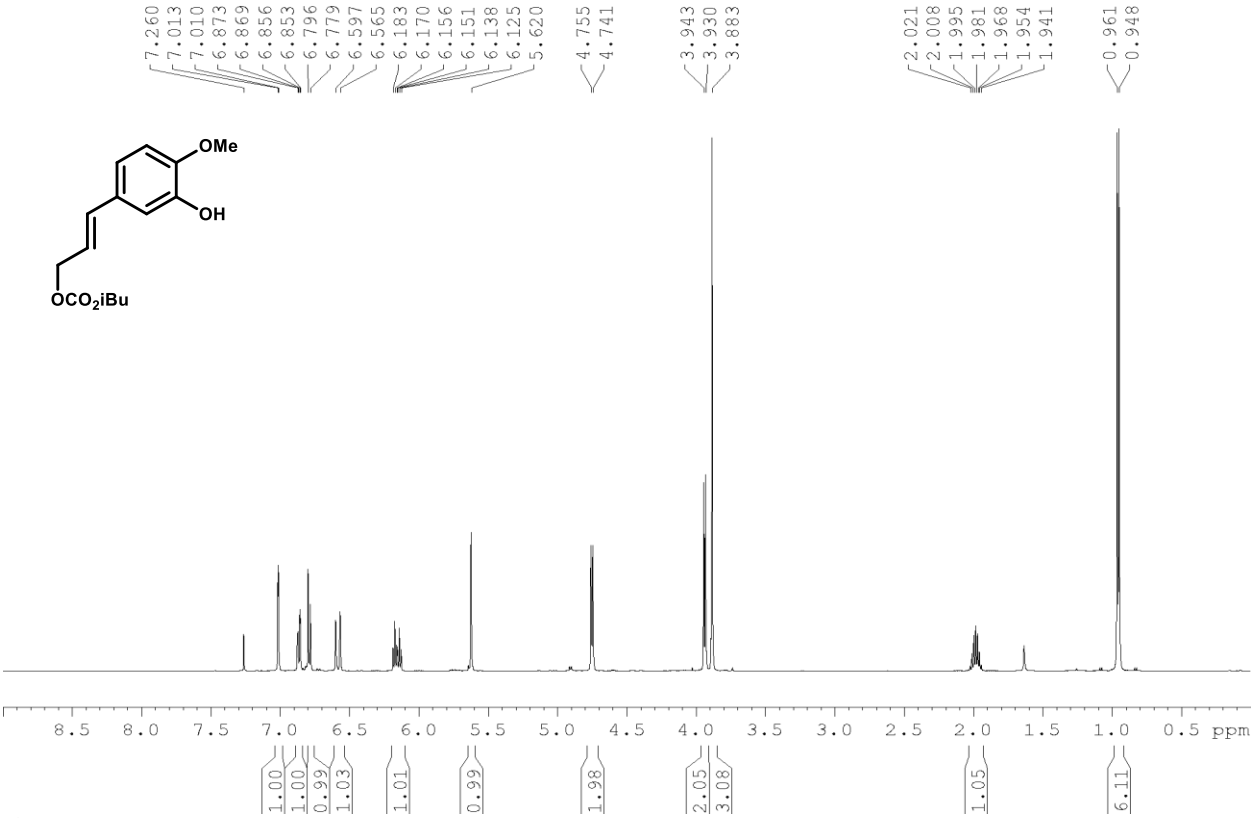
^{1}H NMR of compound S3.3 ($CDCl_3$, 500 MHz)



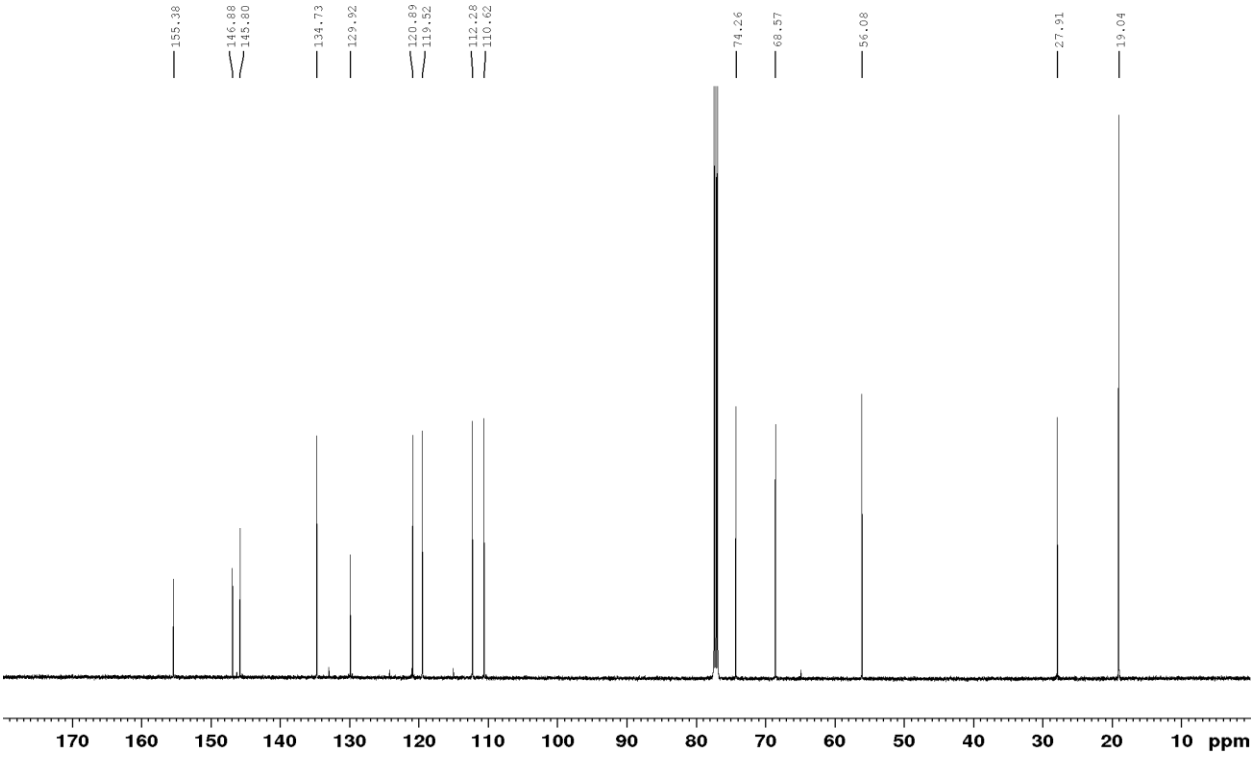
^{13}C NMR of compound 3.27 ($CDCl_3$, 126 MHz)



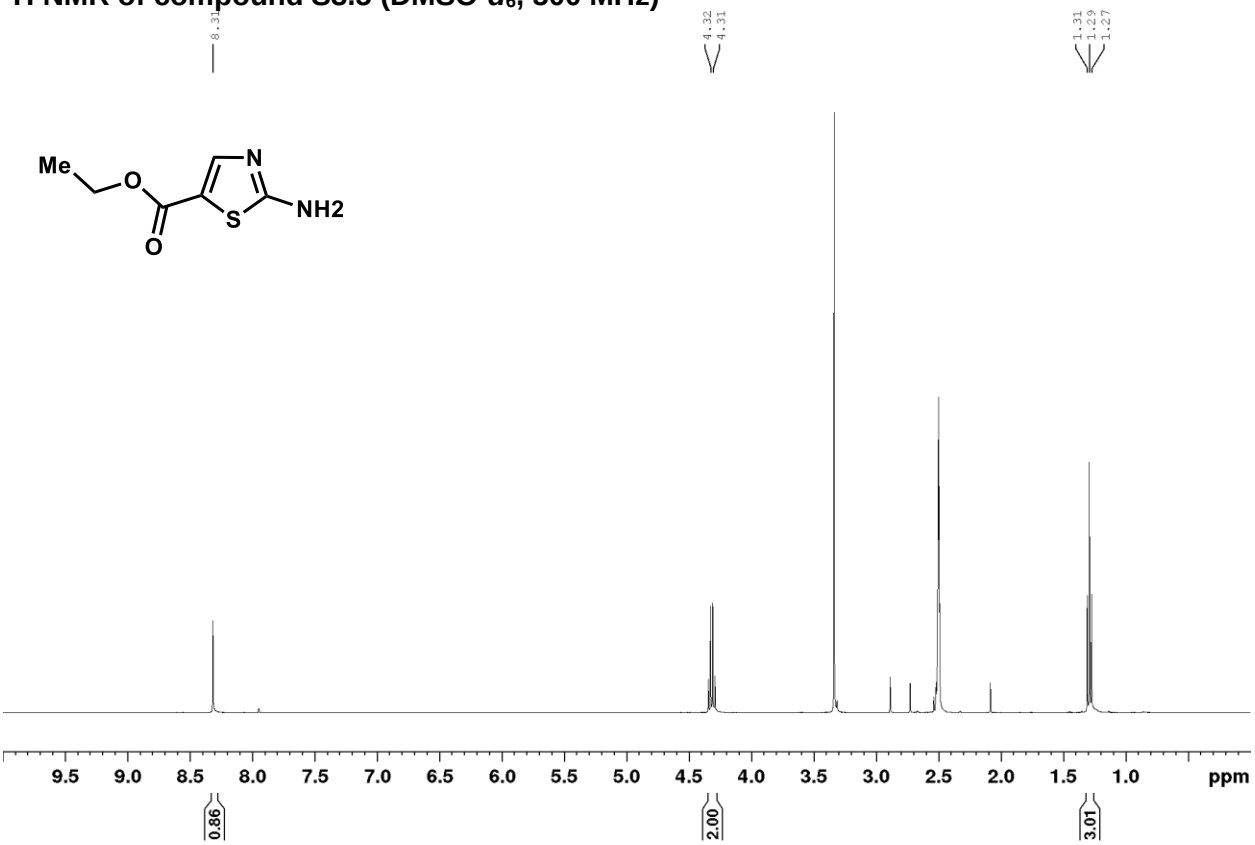
^{1}H NMR of compound 3.27 ($CDCl_3$, 500 MHz)



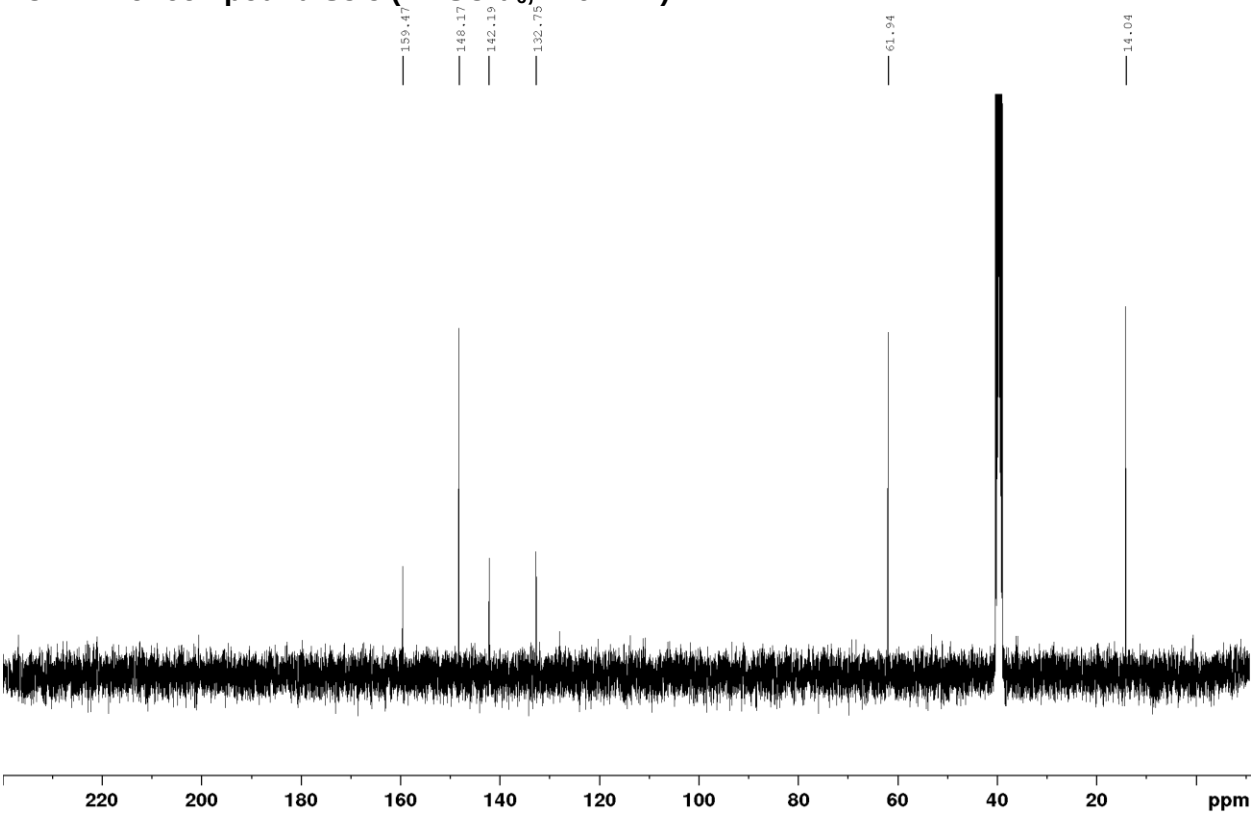
^{13}C NMR of compound 3.27 ($CDCl_3$, 126 MHz)



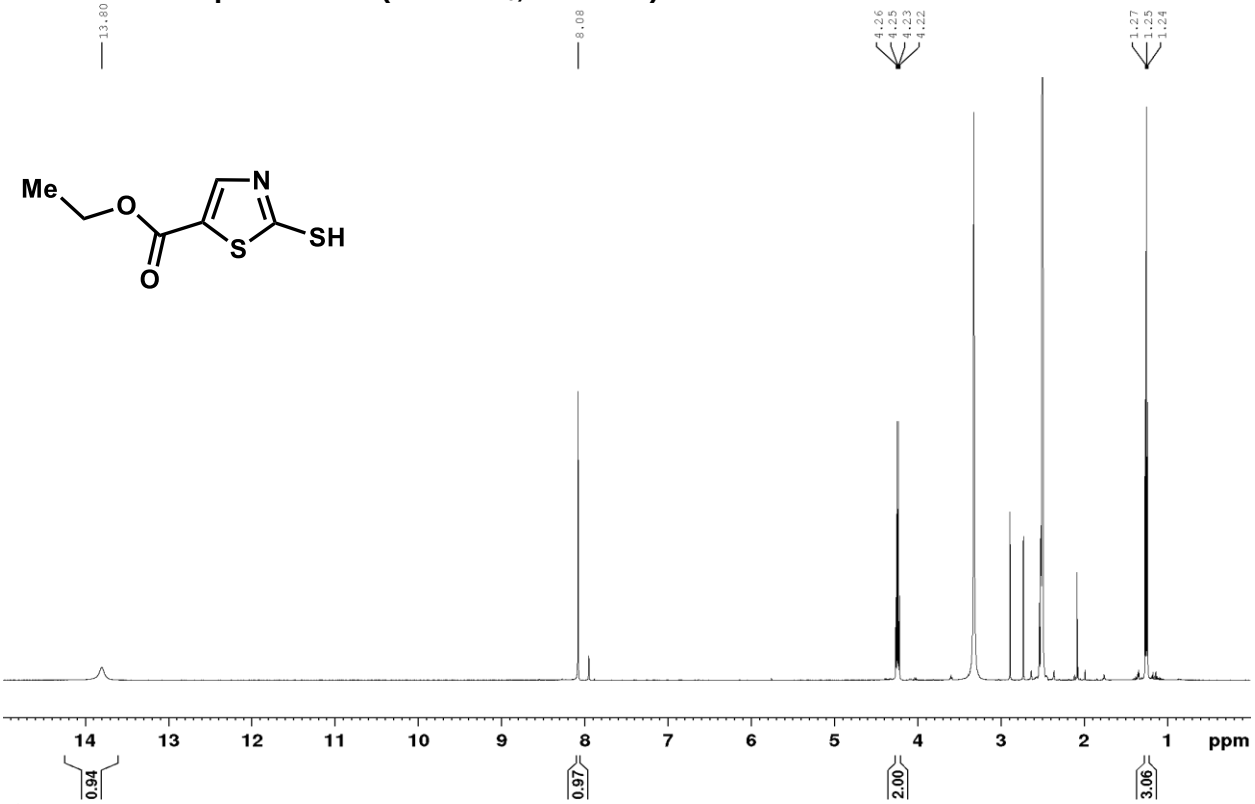
^{1}H NMR of compound S3.5 (DMSO-d_6, 500 MHz)



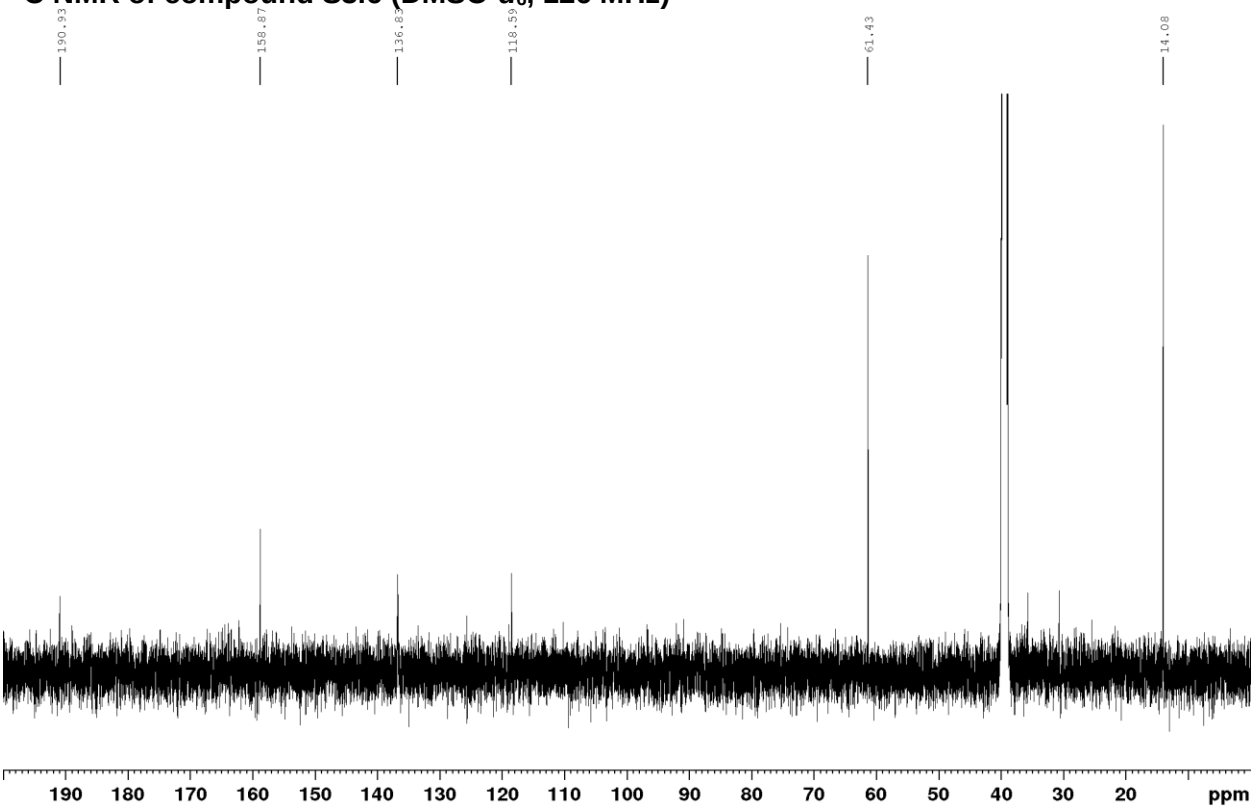
^{13}C NMR of compound S3.5 (DMSO-d_6, 126 MHz)



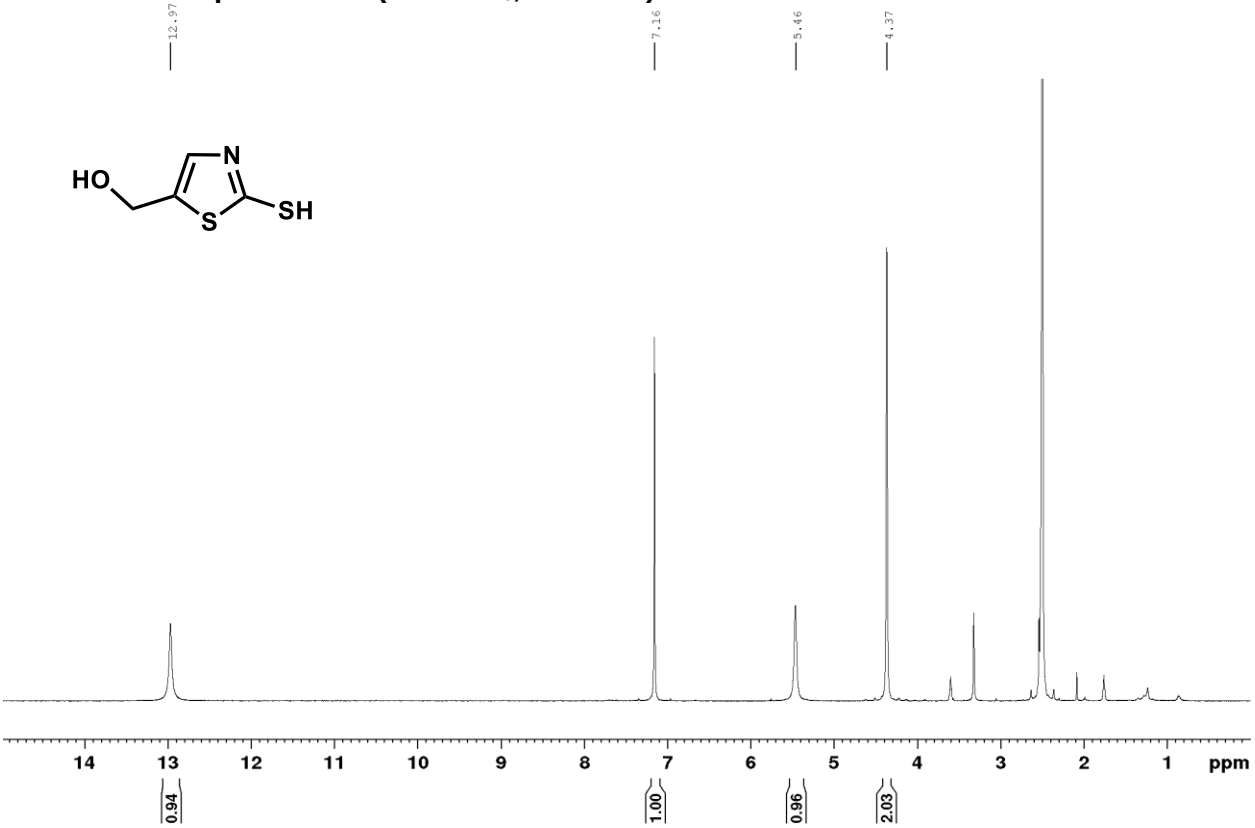
^{1}H NMR of compound S3.6 (DMSO-d_6, 500 MHz)



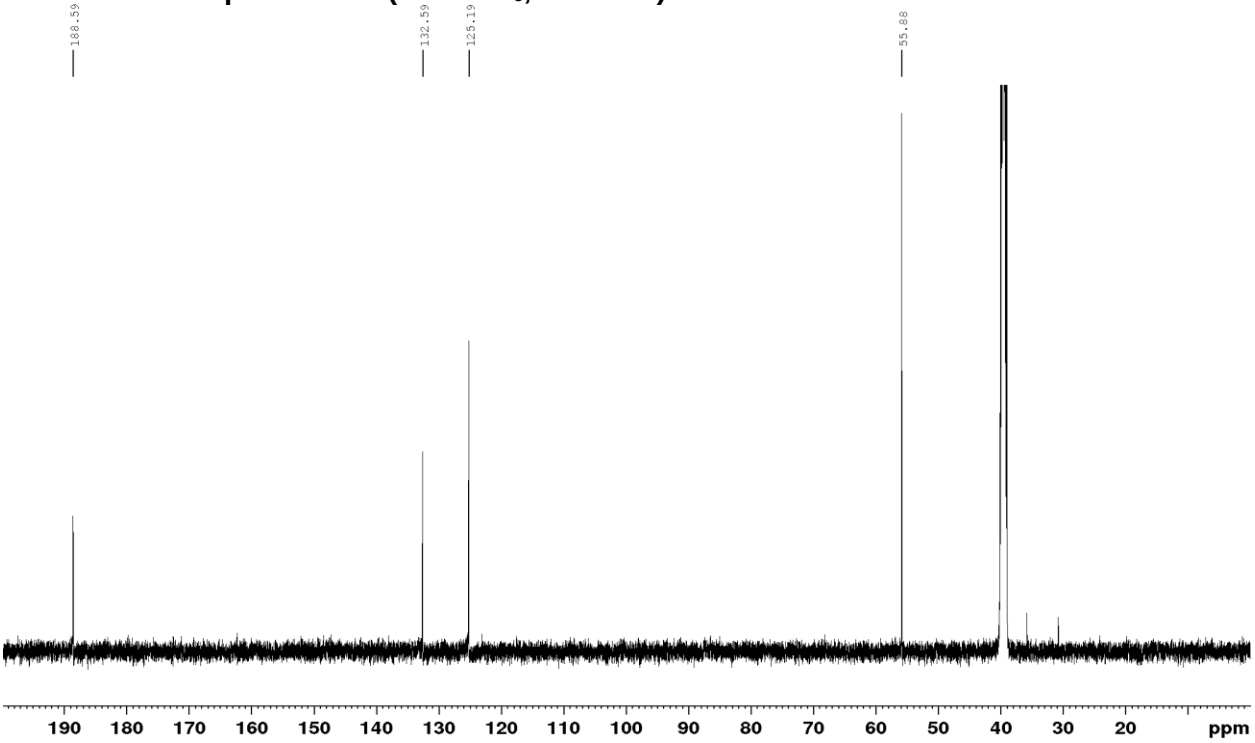
^{13}C NMR of compound S3.6 (DMSO-d_6, 126 MHz)



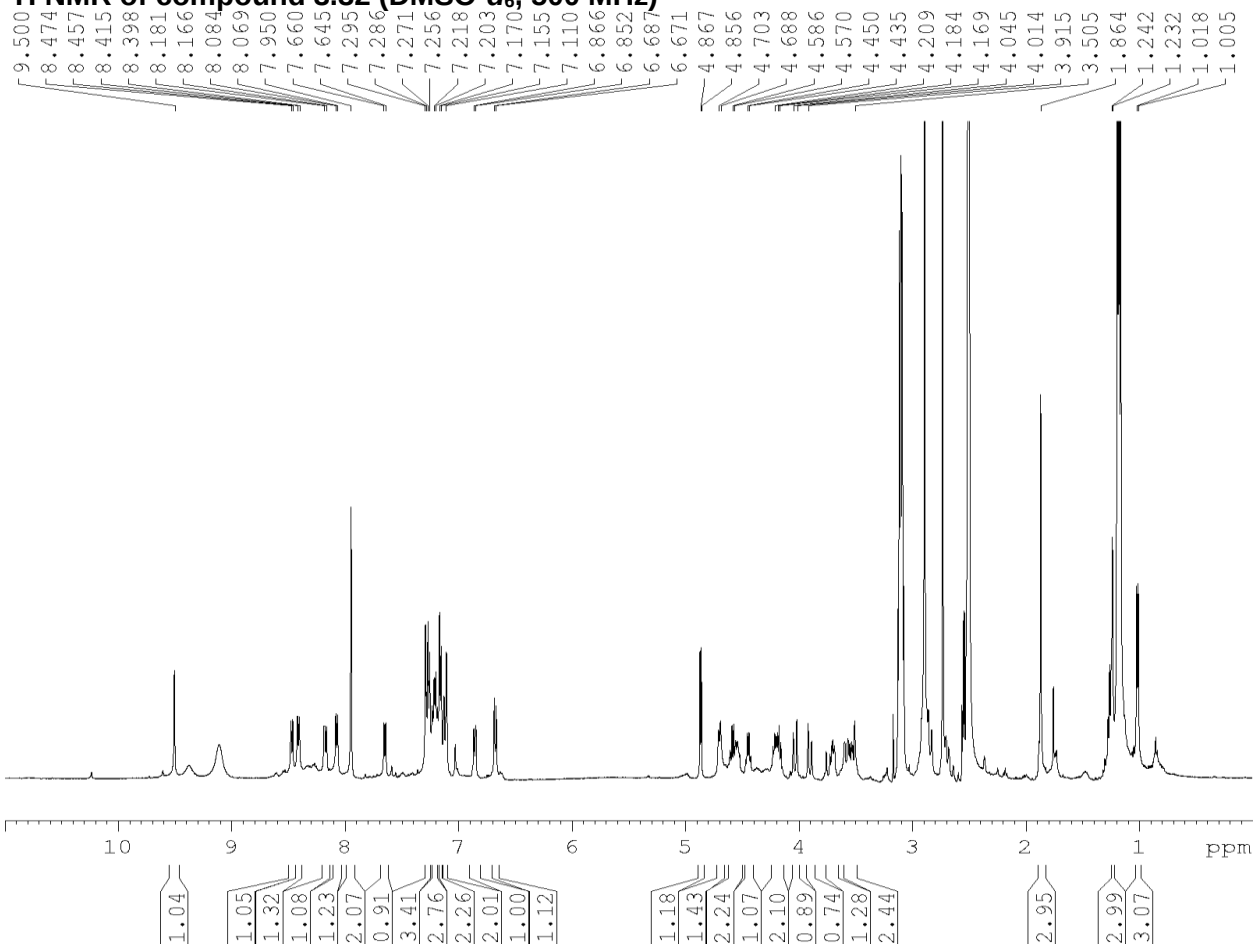
^{1}H NMR of compound 3.30 (DMSO-d_6, 500 MHz)



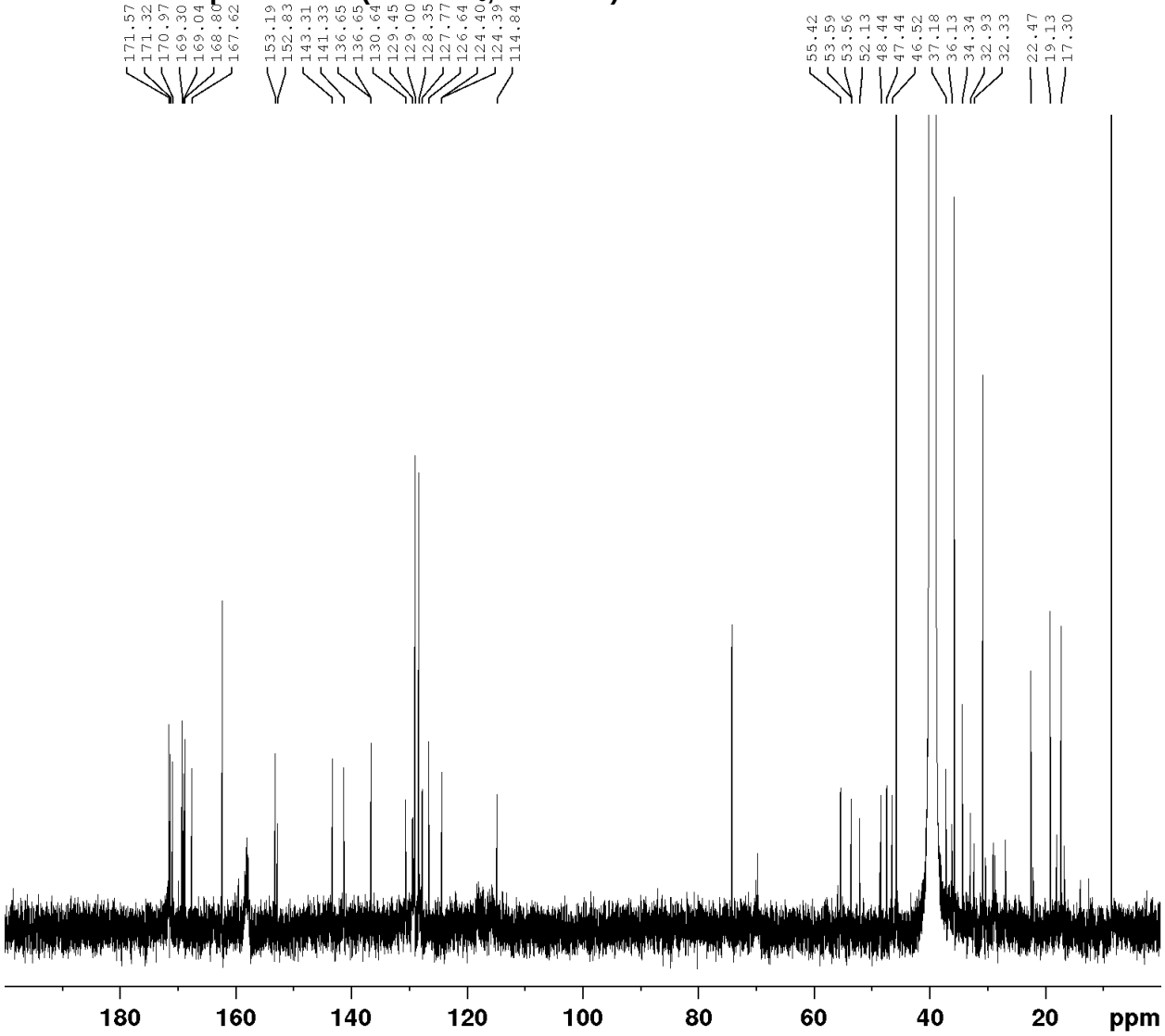
^{13}C NMR of compound 3.30 (DMSO-d_6, 126 MHz)



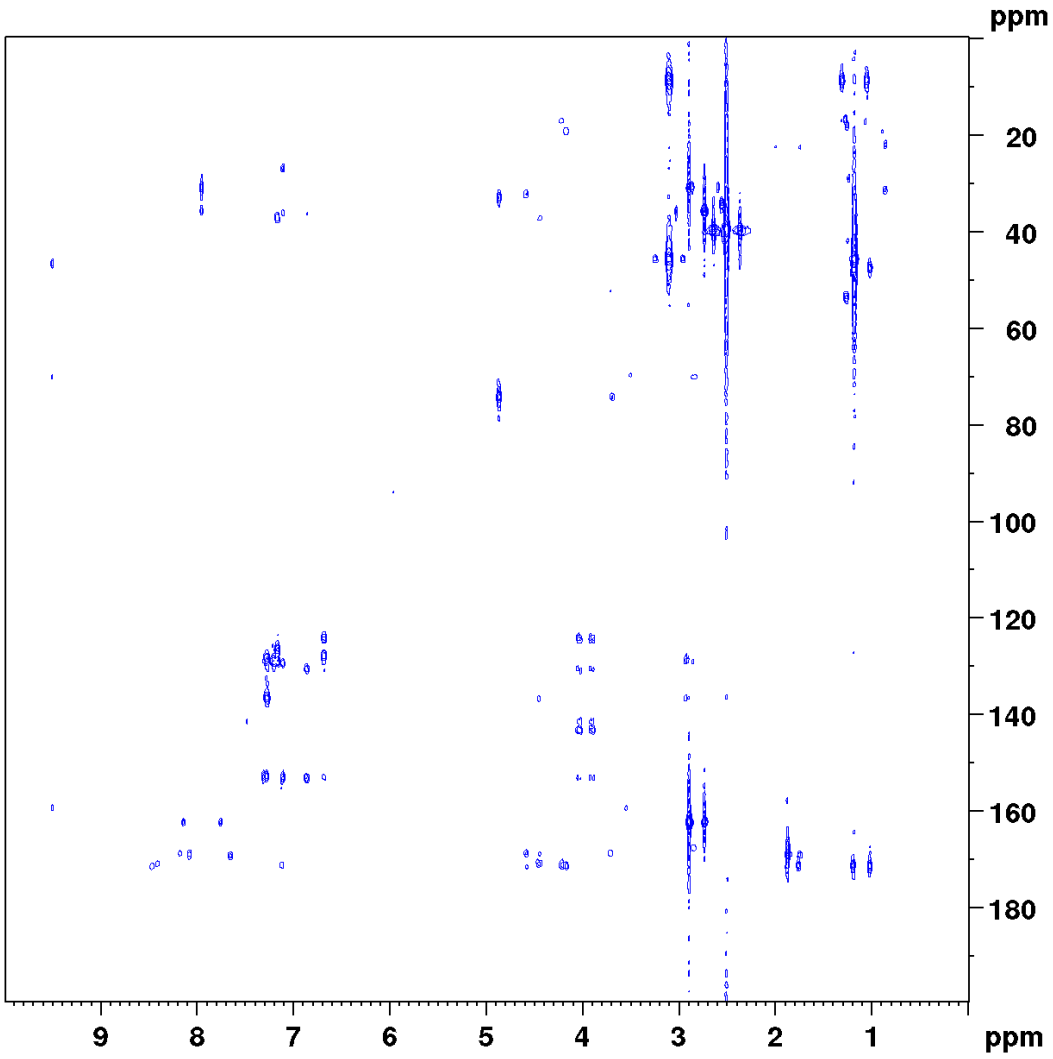
^{1}H NMR of compound 3.32 (DMSO-d_6, 500 MHz)



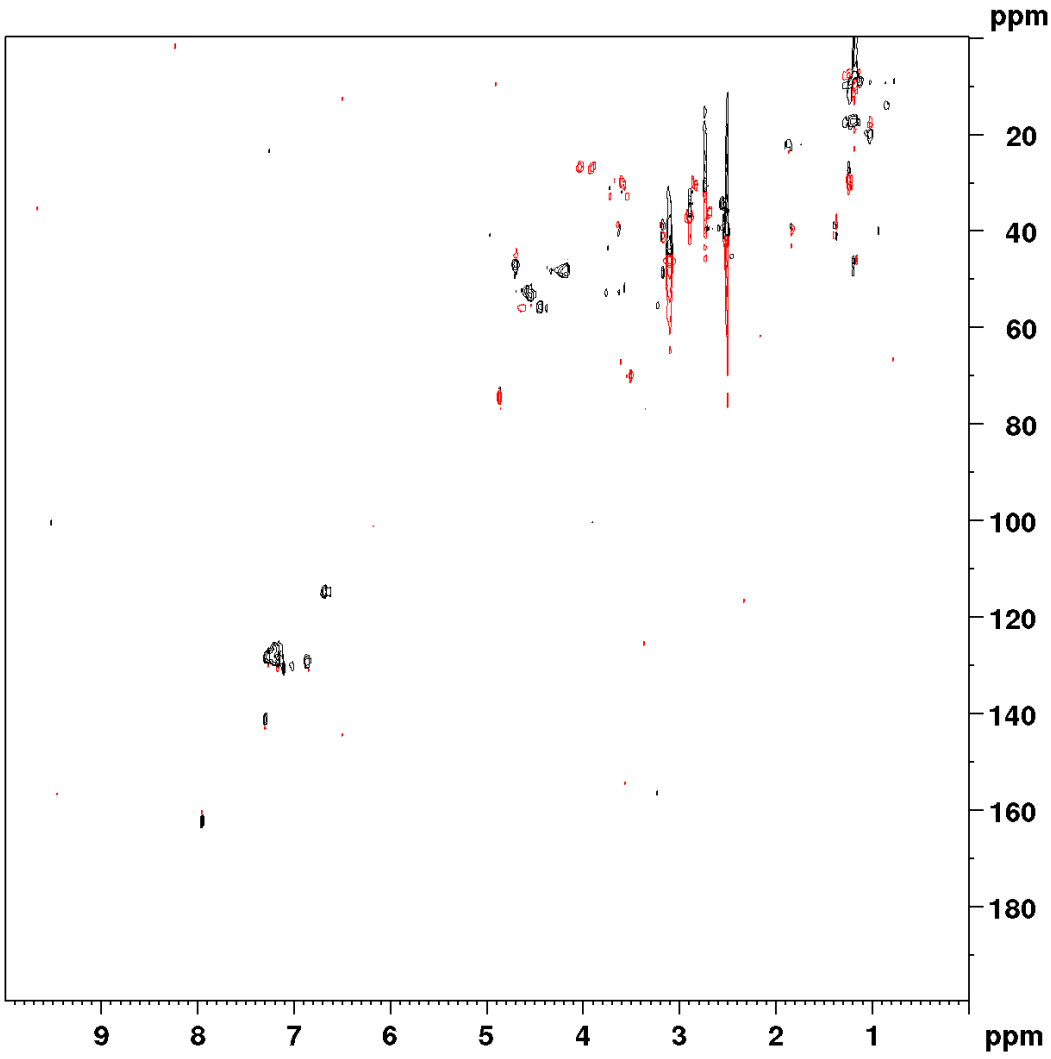
^{13}C NMR of compound 3.32 (DMSO-d_6, 126 MHz)



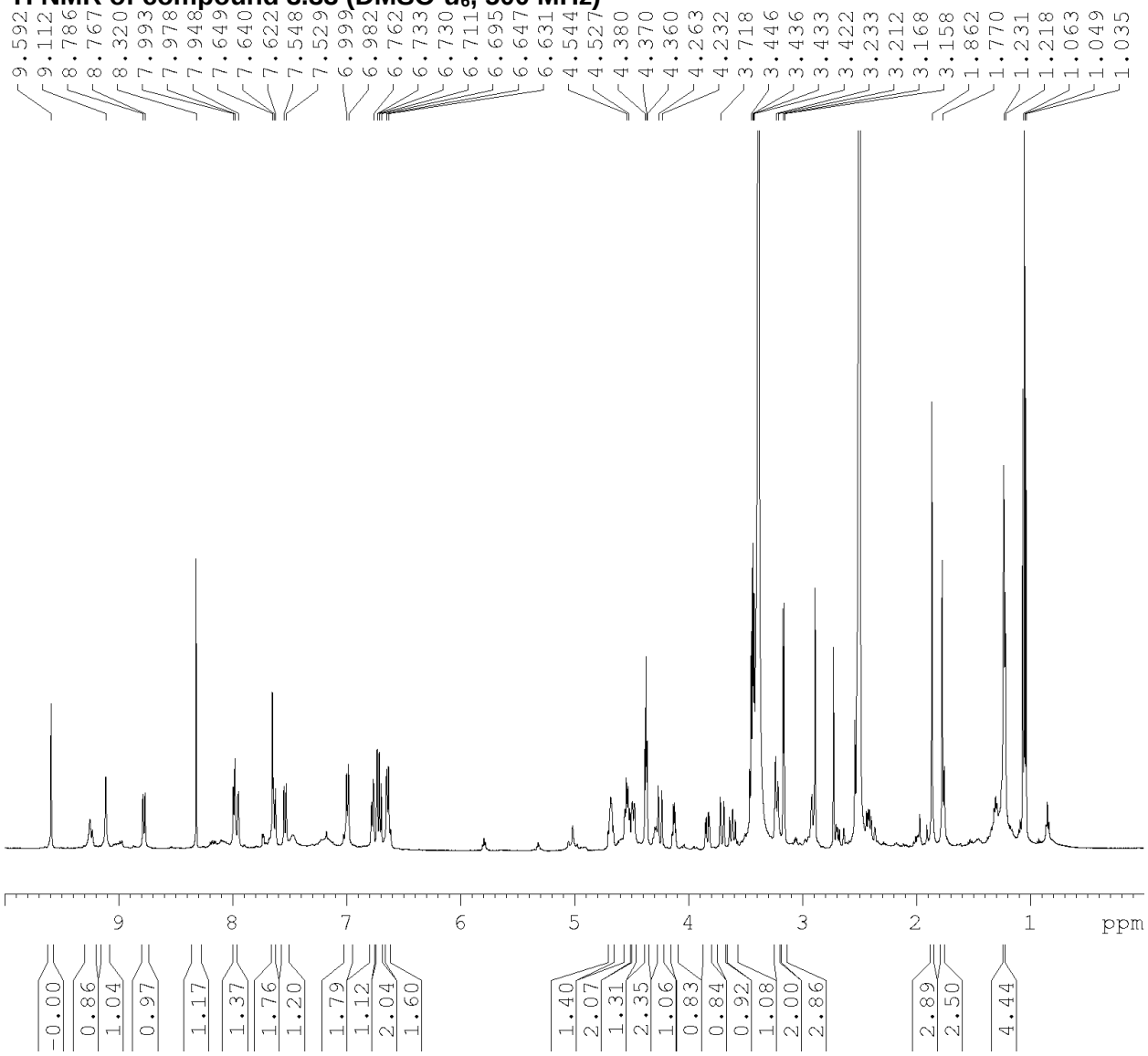
HMBC spectrum of compound 3.32 (DMSO-d_6)



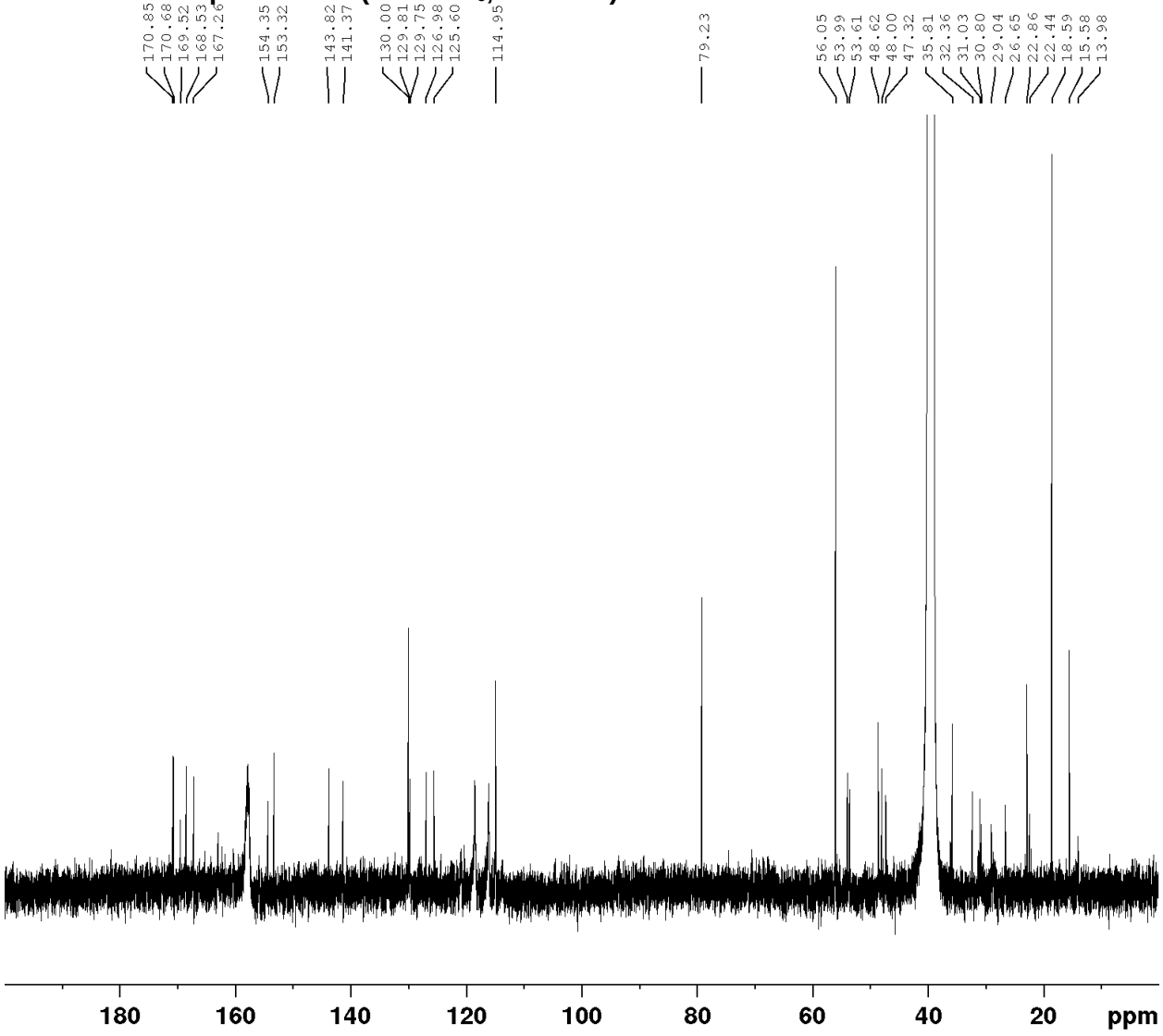
HSQC spectrum of compound 3.32 (DMSO-d_6)



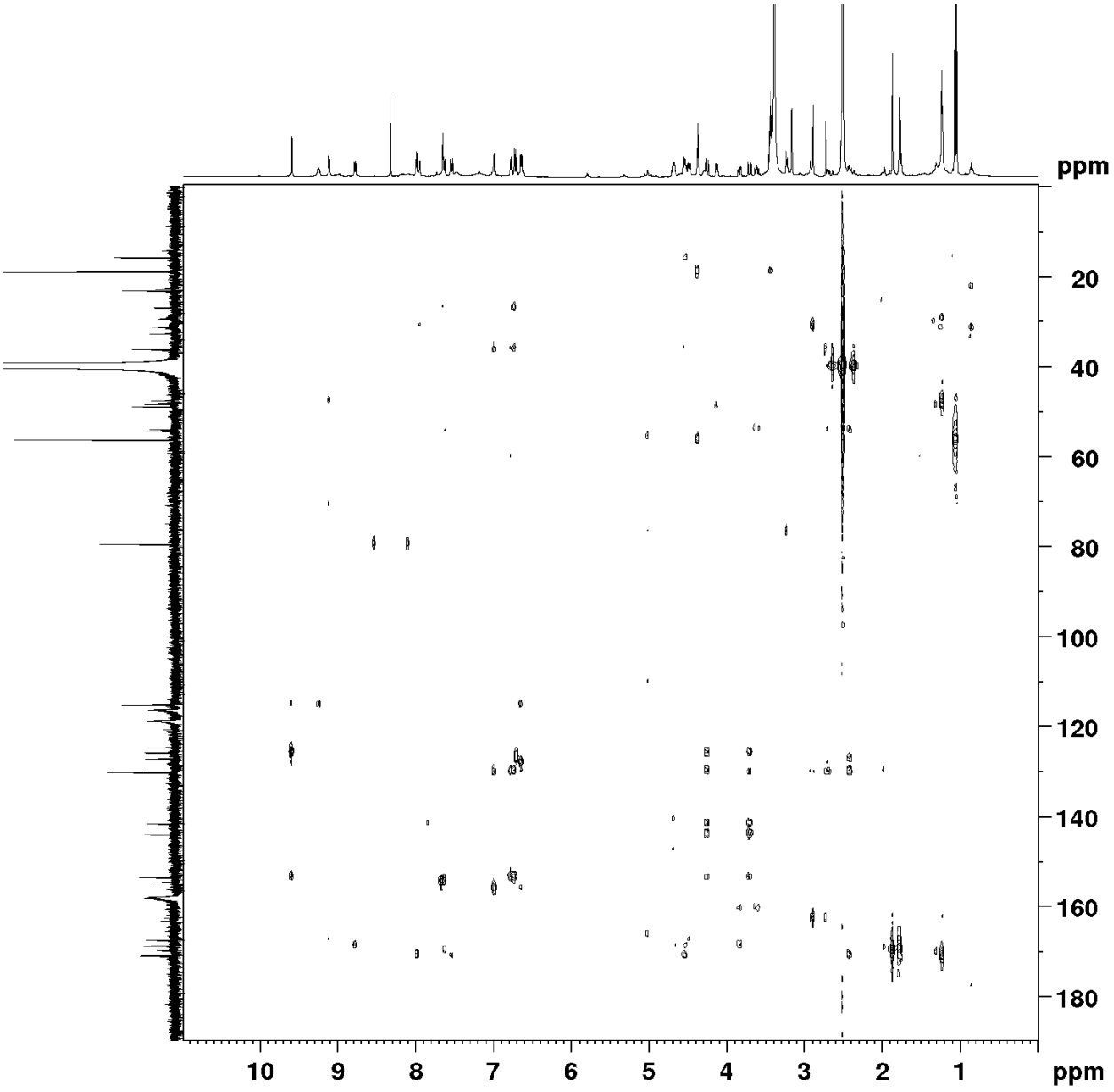
^{1}H NMR of compound 3.33 (DMSO-d_6, 500 MHz)



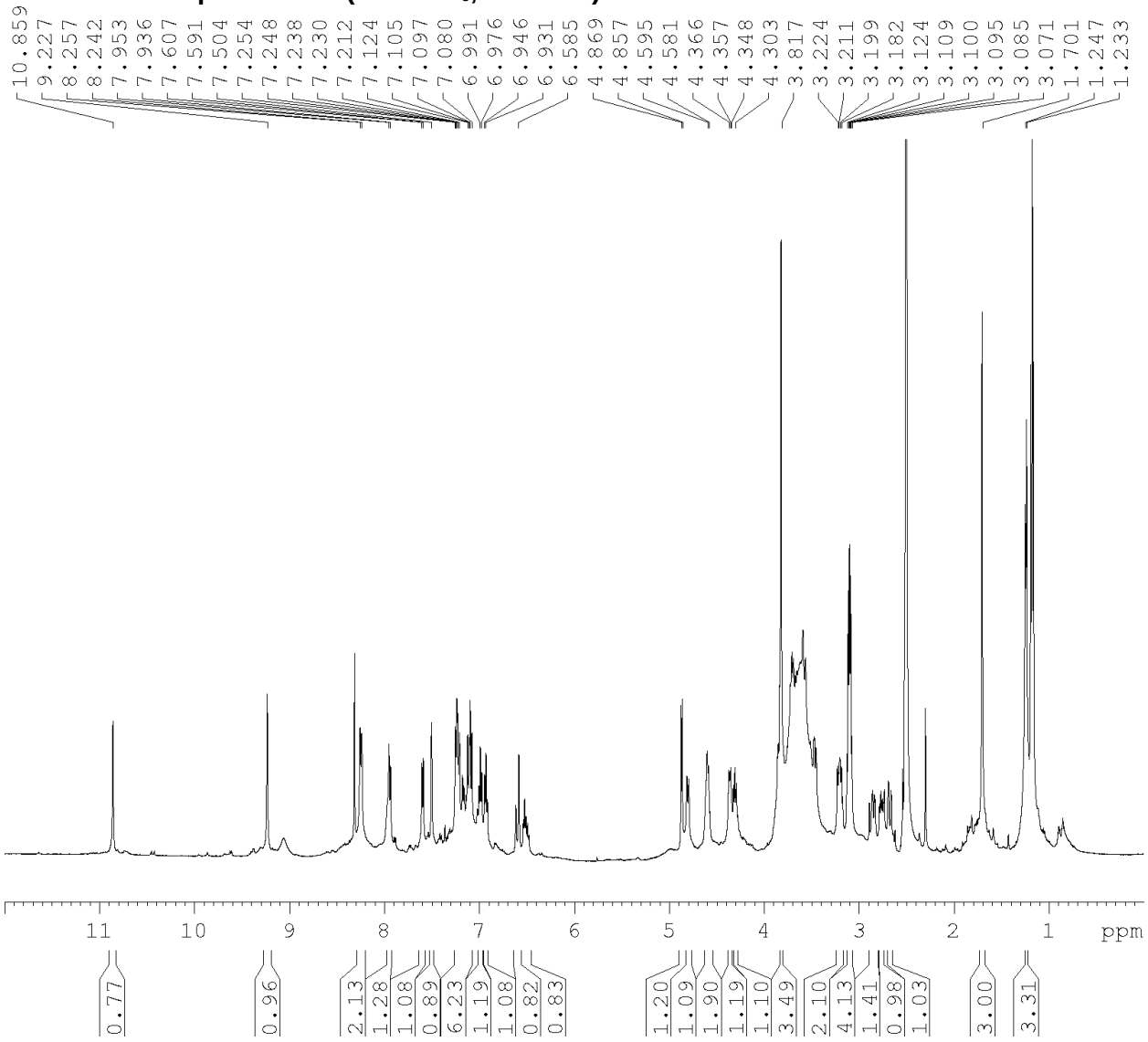
^{13}C NMR of compound 3.33 (DMSO-d_6, 126 MHz)



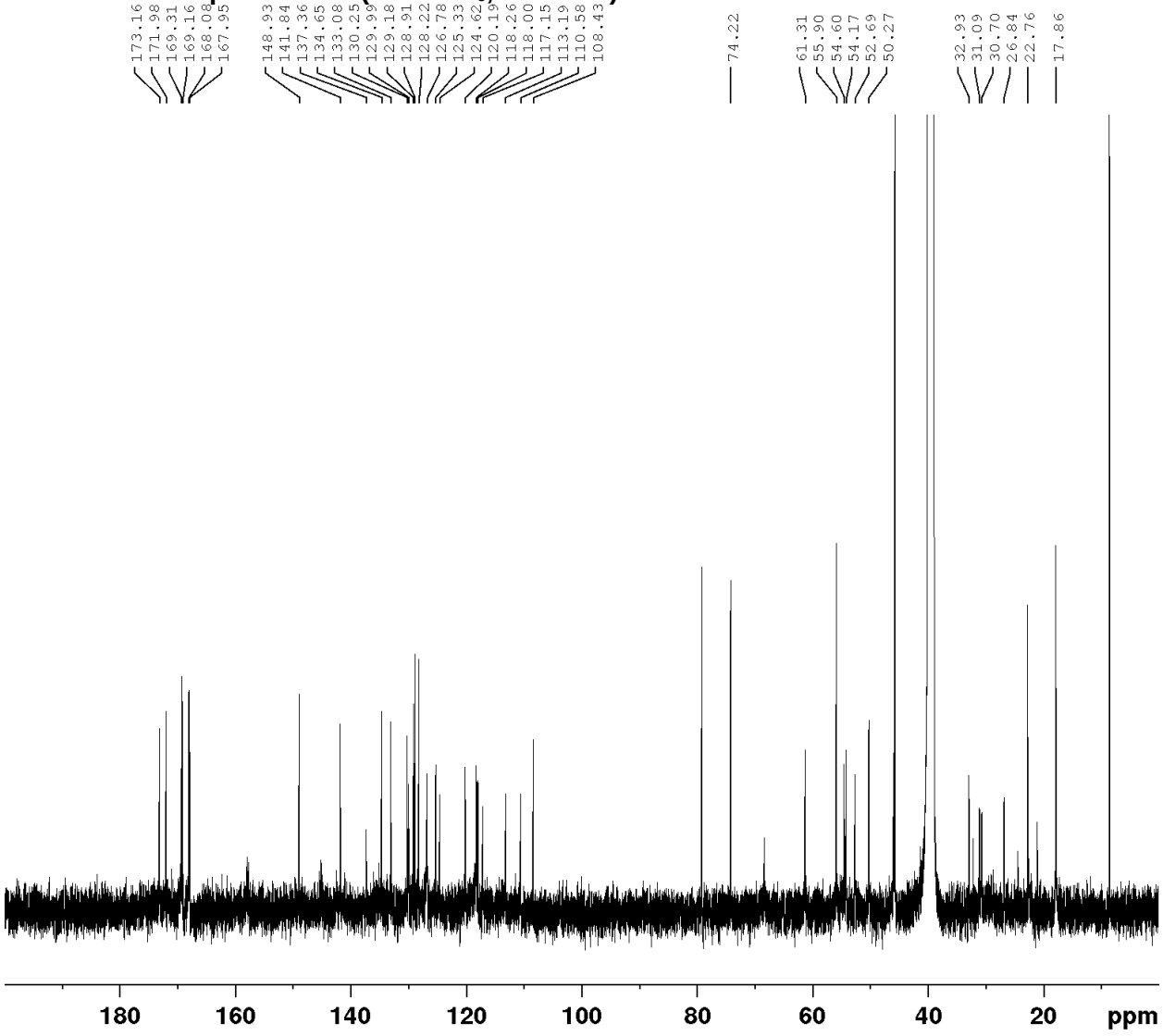
HMBC spectrum of compound 3.33 (DMSO-d_6)



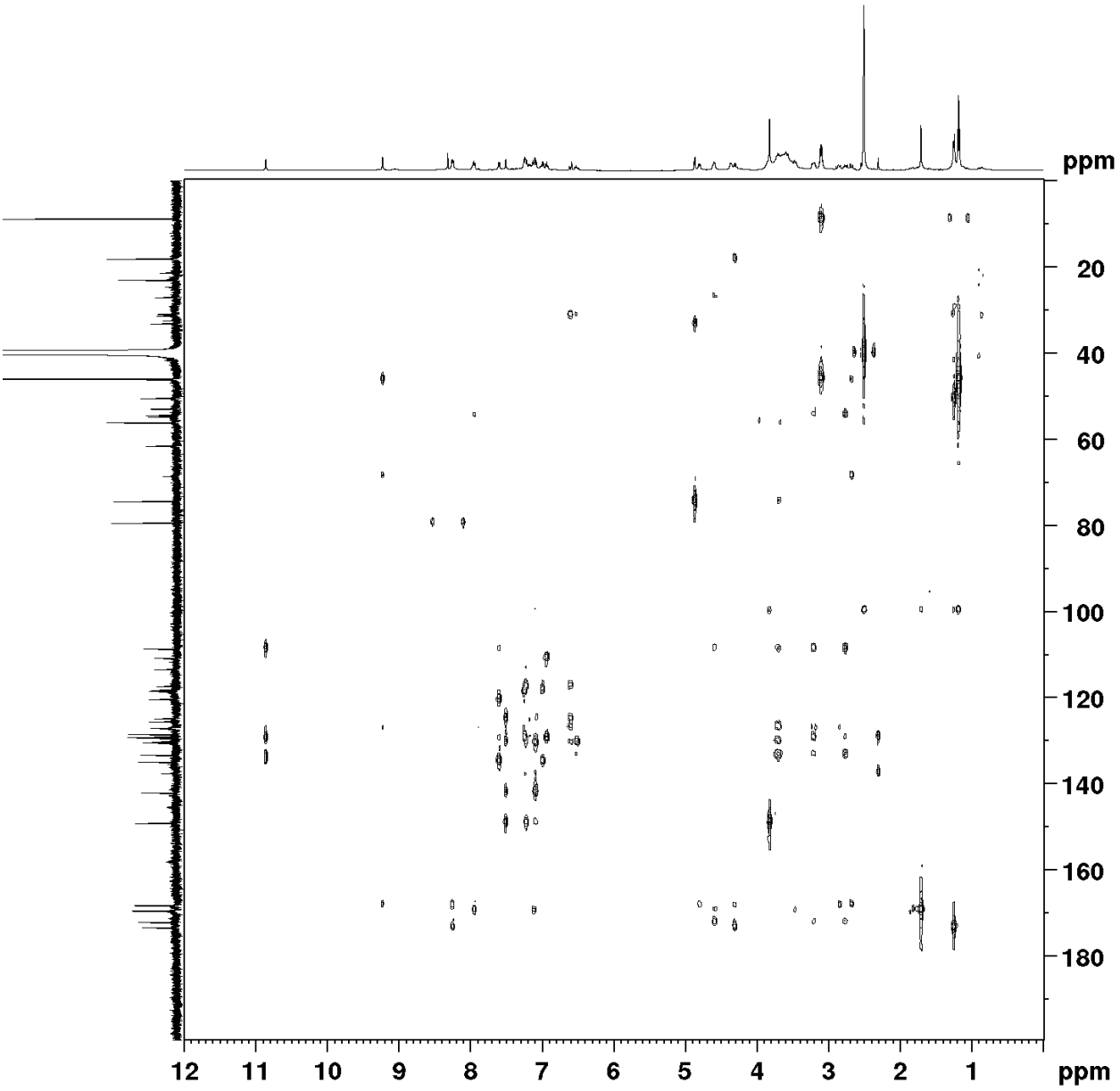
^{1}H NMR of compound 3.36 (DMSO-d_6, 500 MHz)



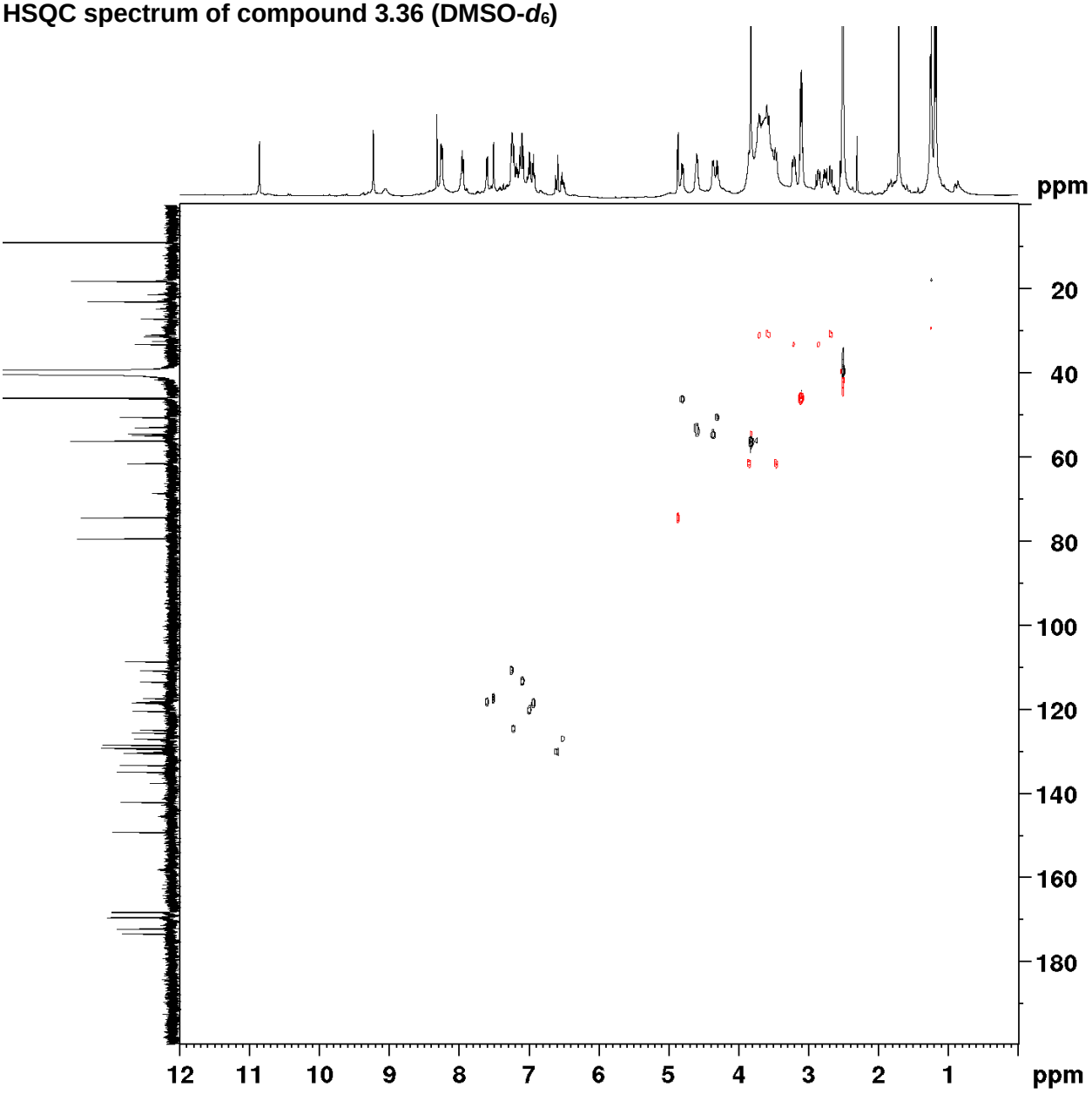
^{13}C NMR of compound 3.36 (DMSO-d_6, 126 MHz)



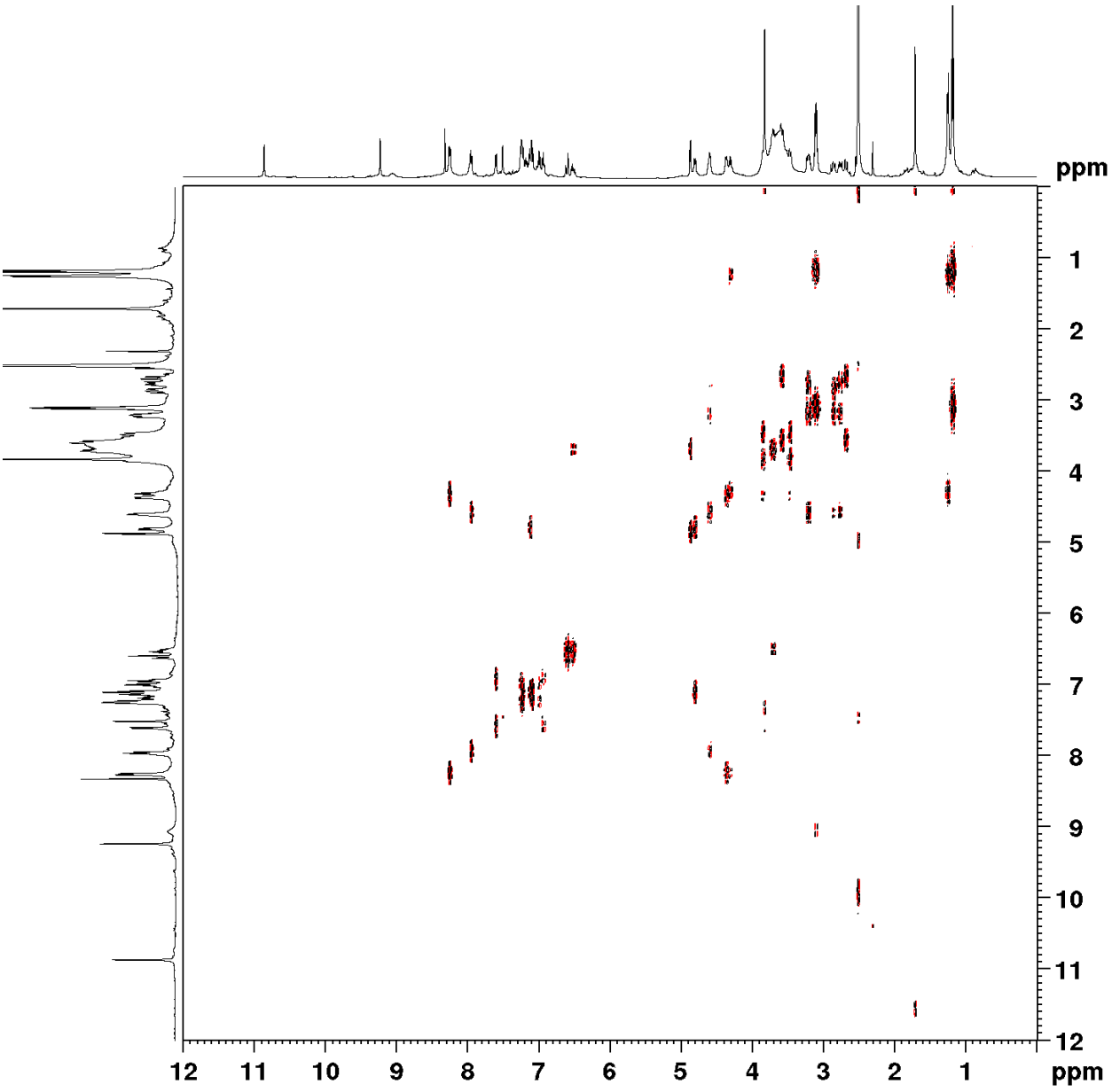
HMBC spectrum of compound 3.36 (DMSO-d_6)



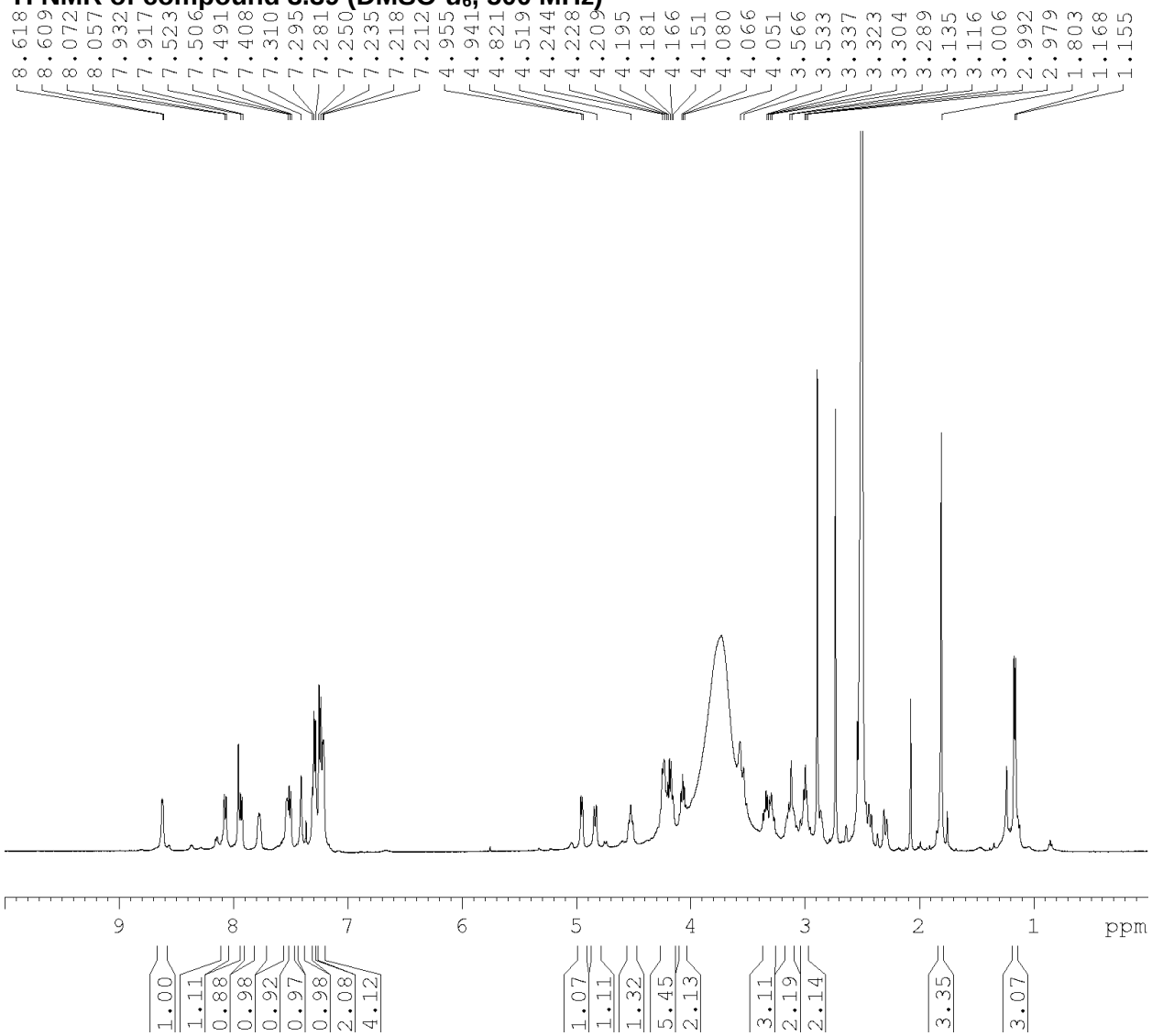
HSQC spectrum of compound 3.36 (DMSO-d_6)



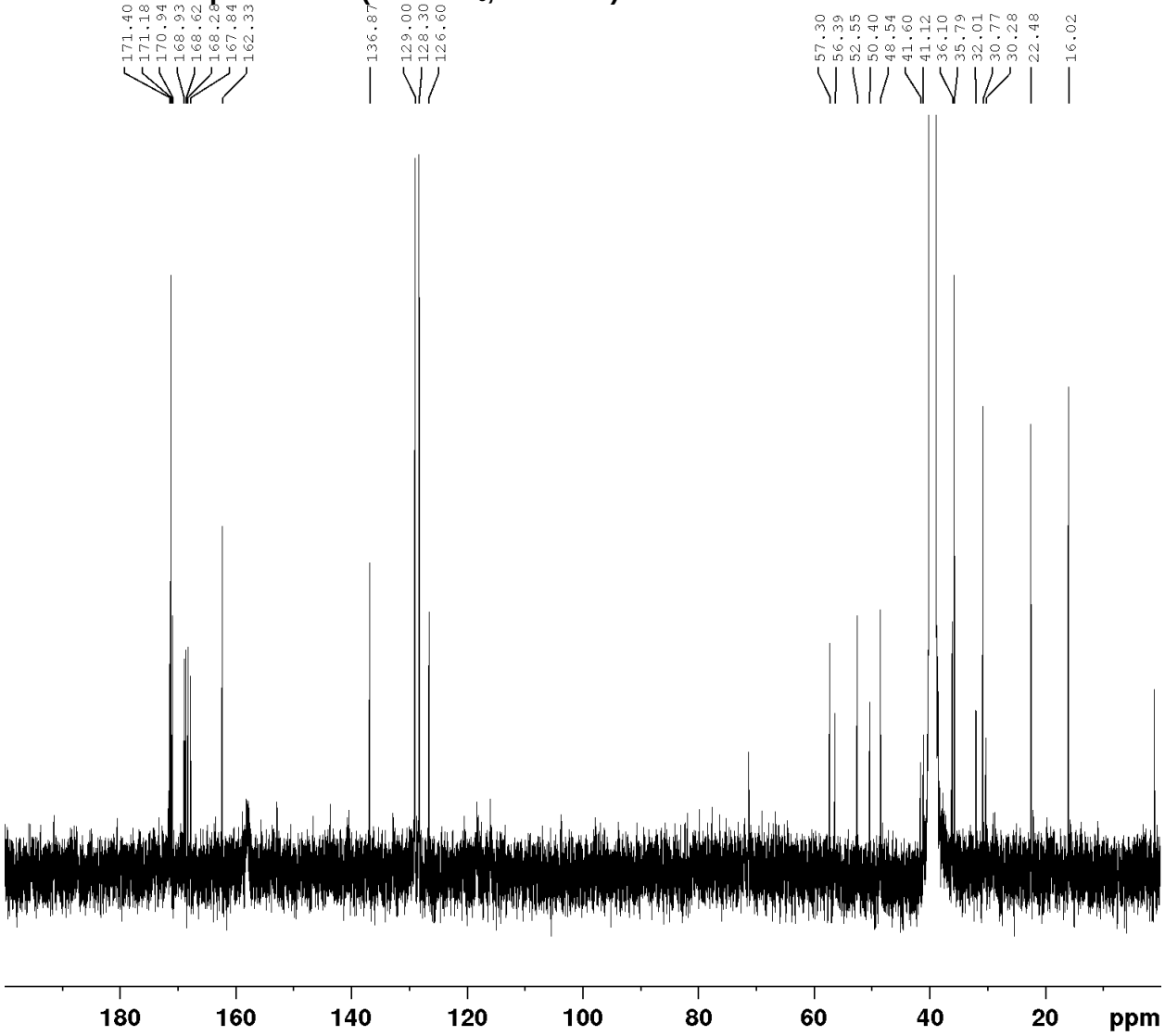
COSY spectrum of compound 3.36 (DMSO-d_6)



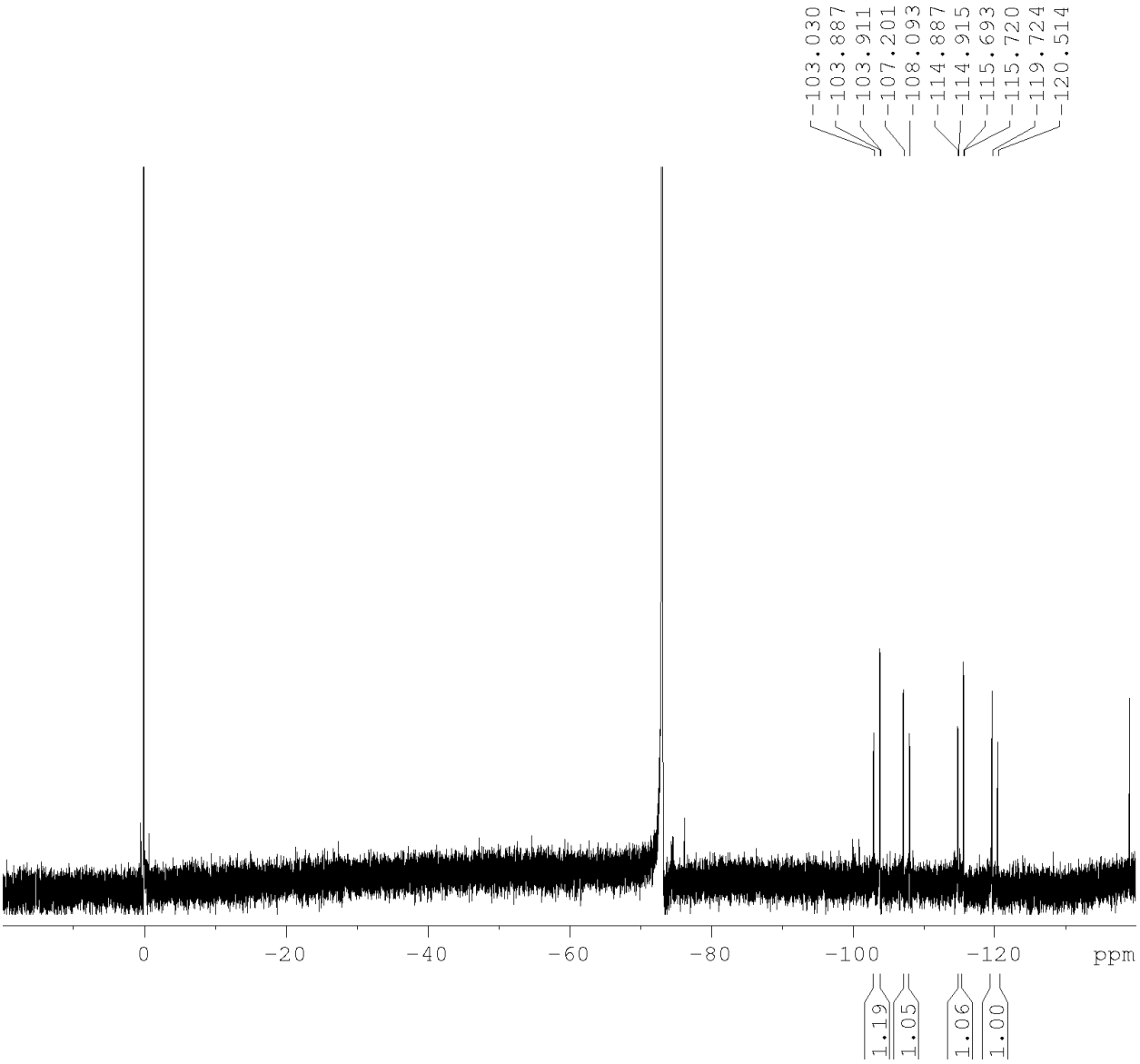
^{1}H NMR of compound 3.39 (DMSO-d_6, 500 MHz)



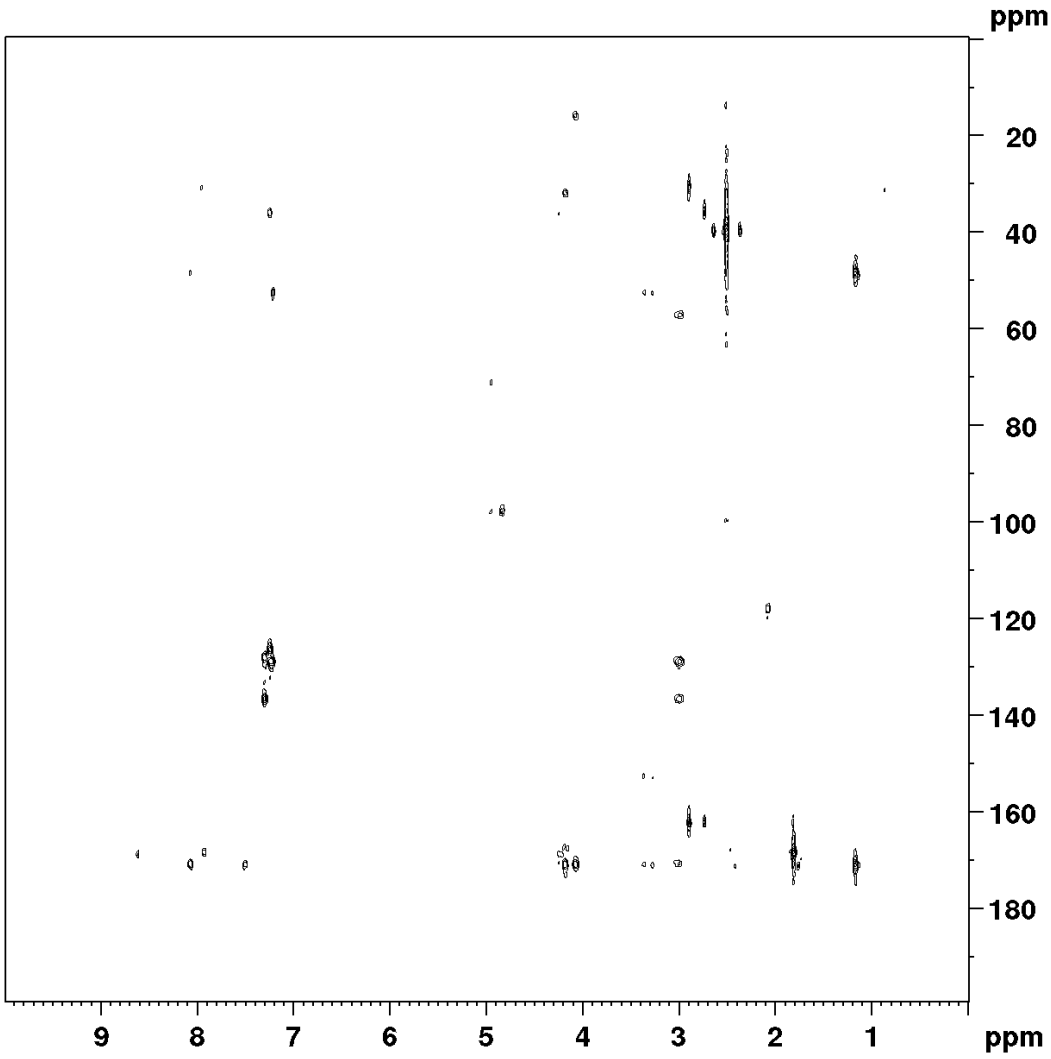
^{13}C NMR of compound 3.39 (DMSO-d_6, 126 MHz)



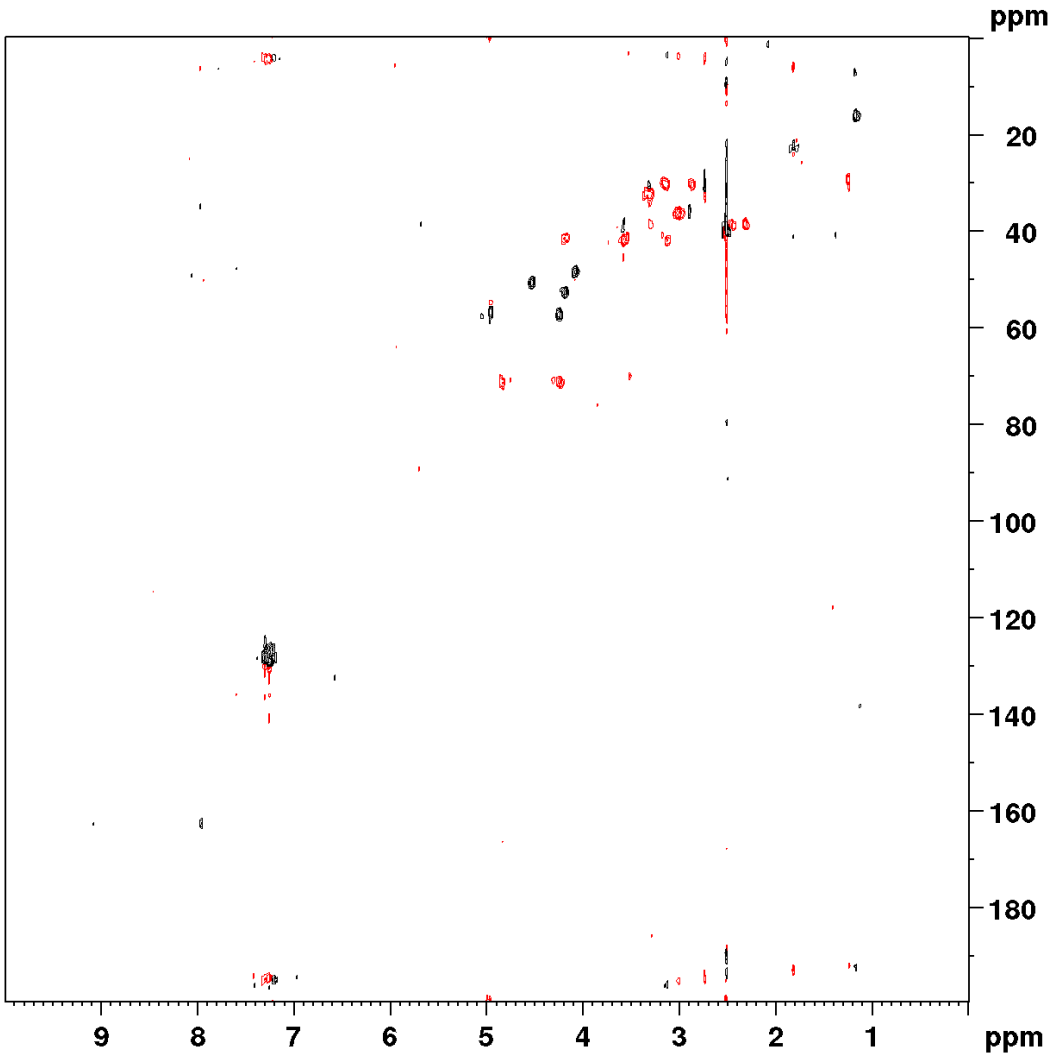
^{19}F NMR of compound 3.39 (DMSO-d_6, 282 MHz)



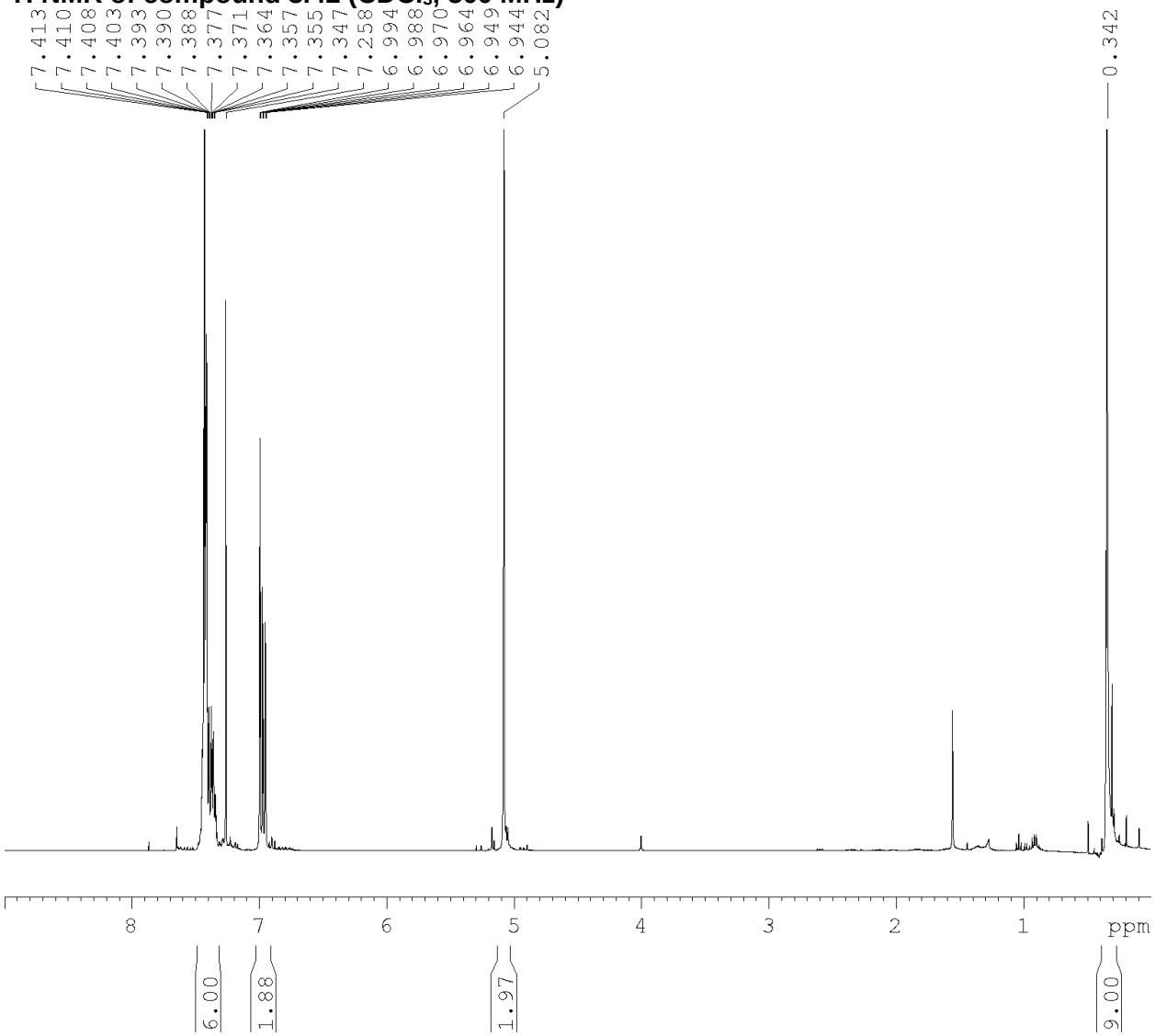
HMBC spectrum of compound 3.39 (DMSO-d_6)



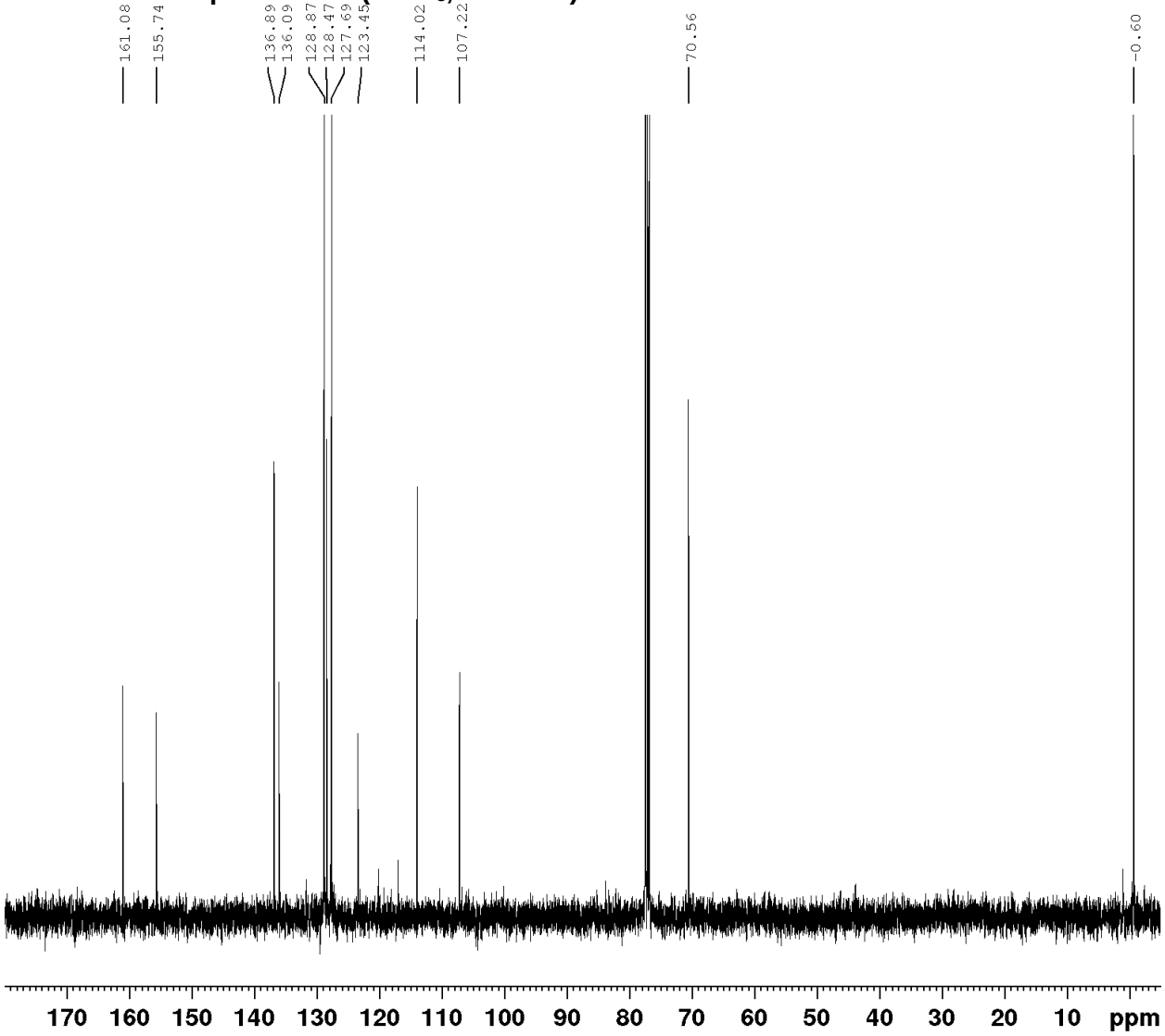
HSQC spectrum of compound 3.39 (DMSO-d_6)



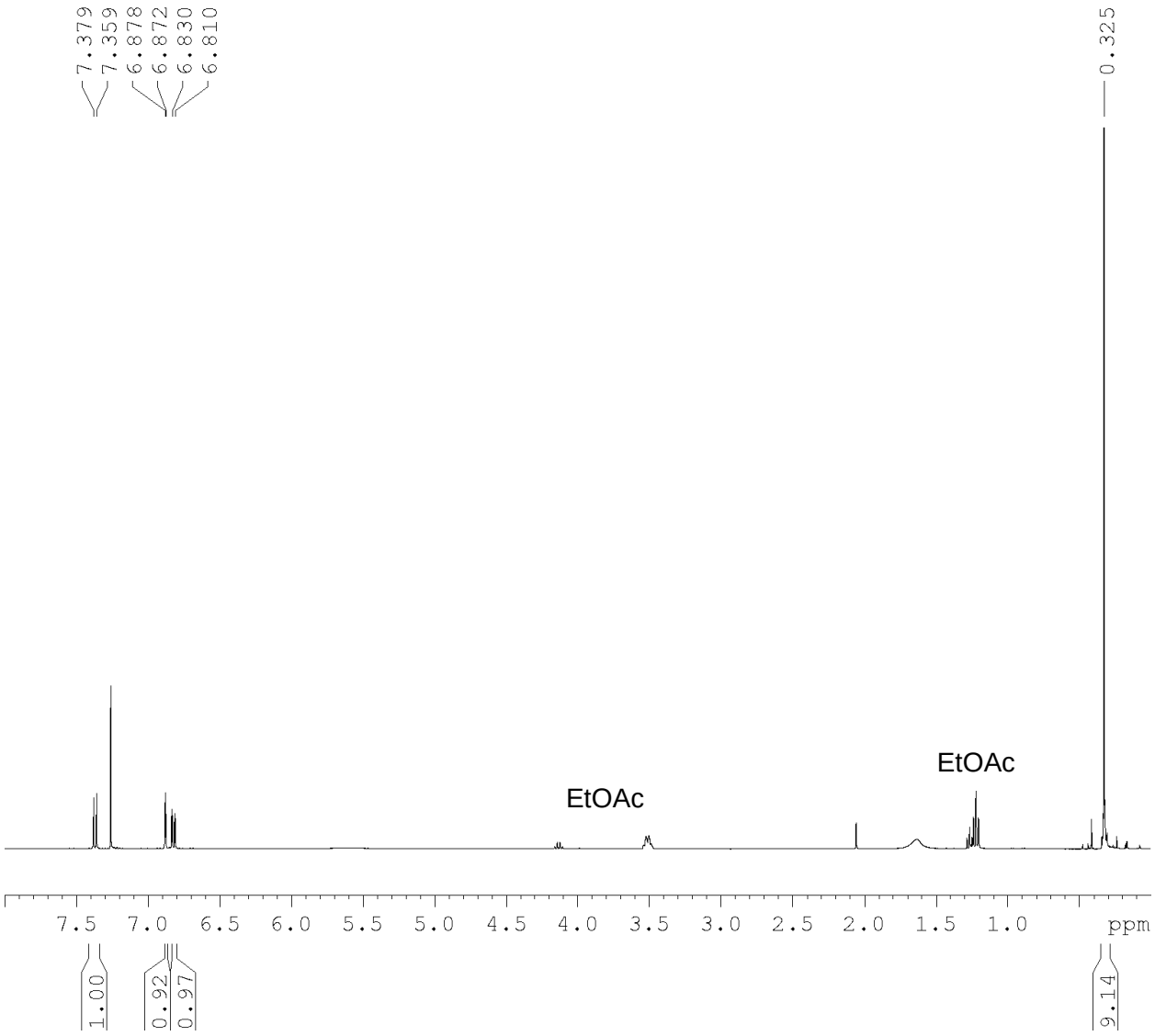
^{1}H NMR of compound 3.42 ($CDCl_3$, 500 MHz)



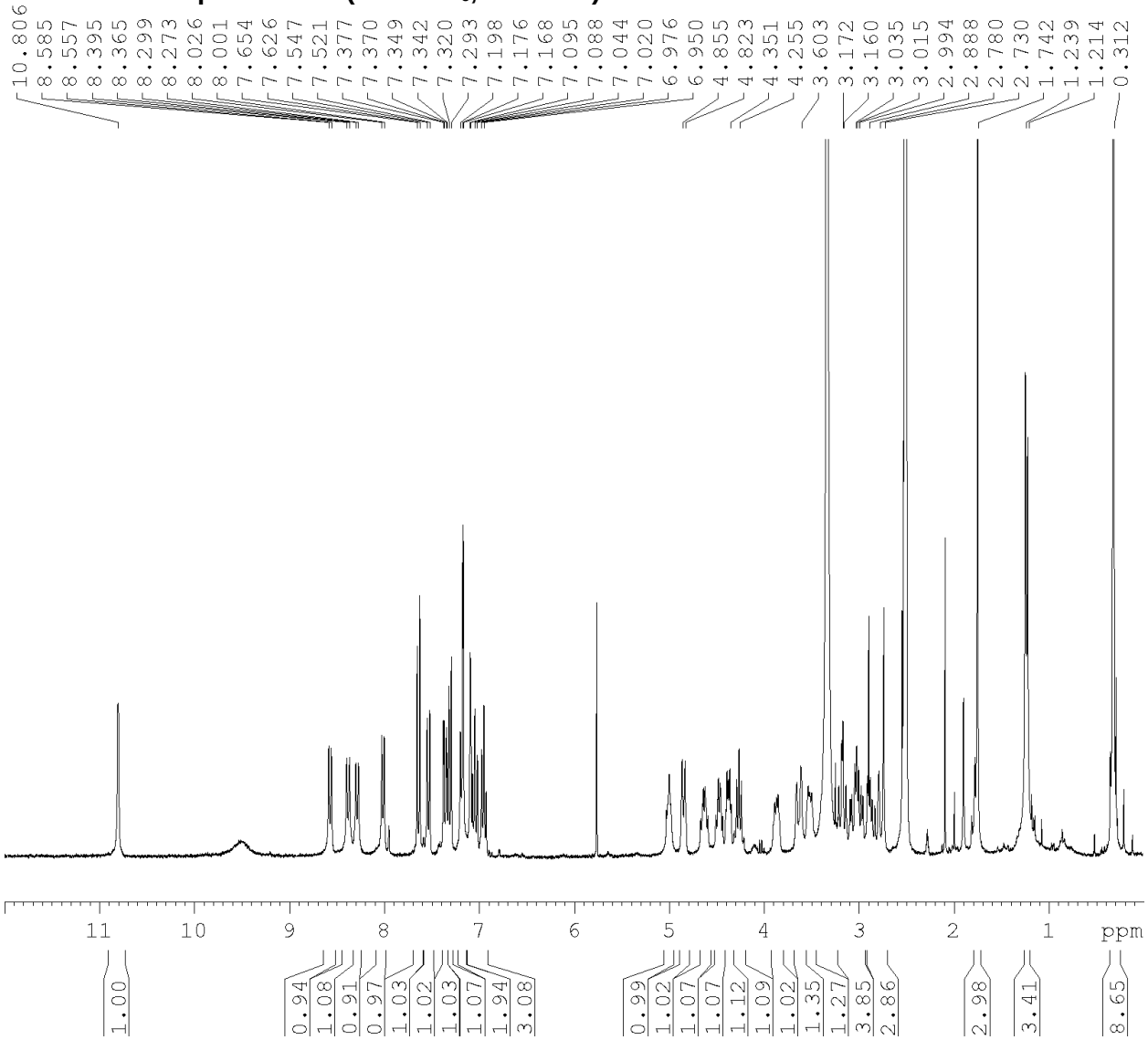
^{13}C NMR of compound 3.42 ($CDCl_3$, 126 MHz)



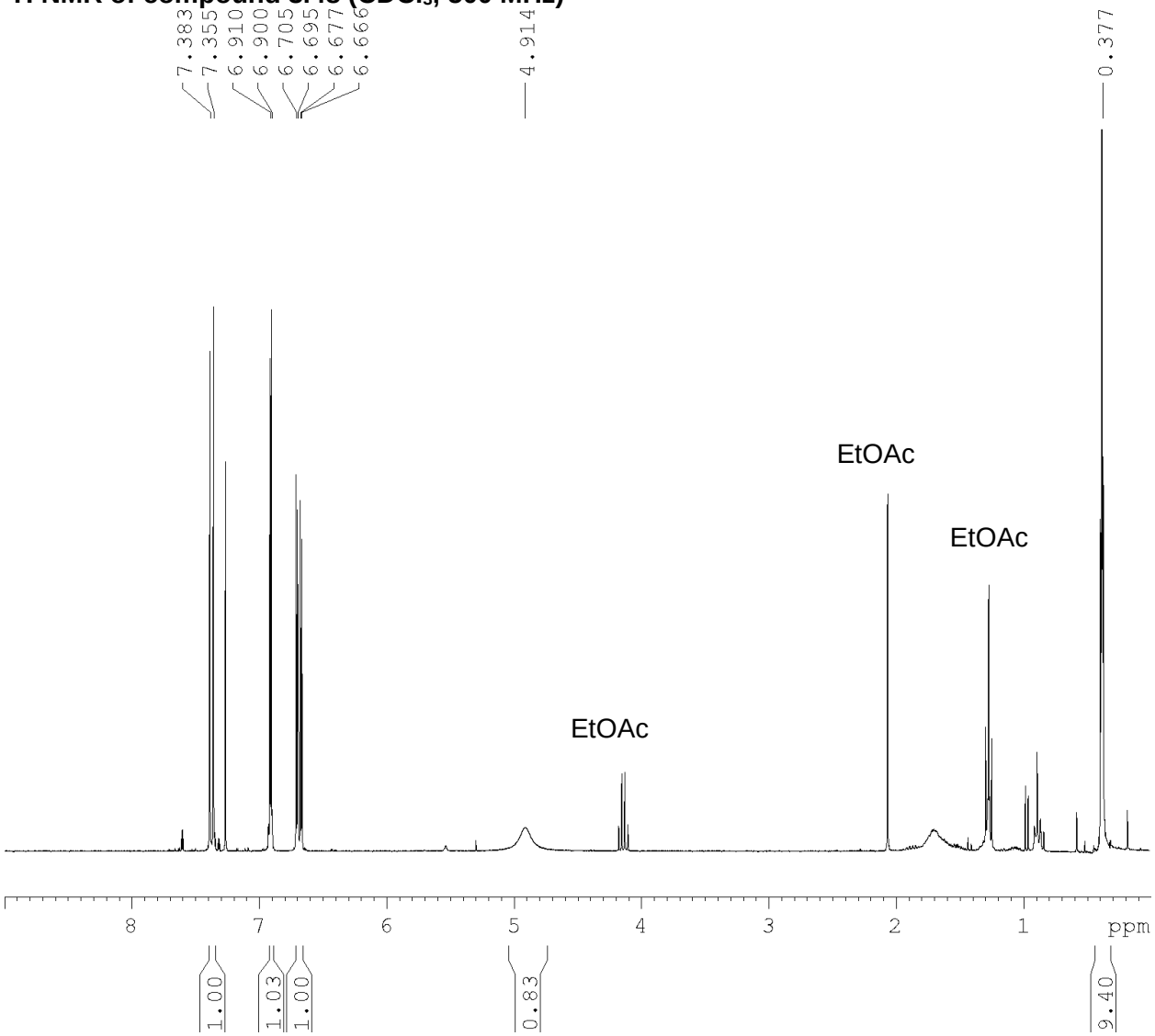
^{1}H NMR of compound 3.43 ($CDCl_3$, 500 MHz)



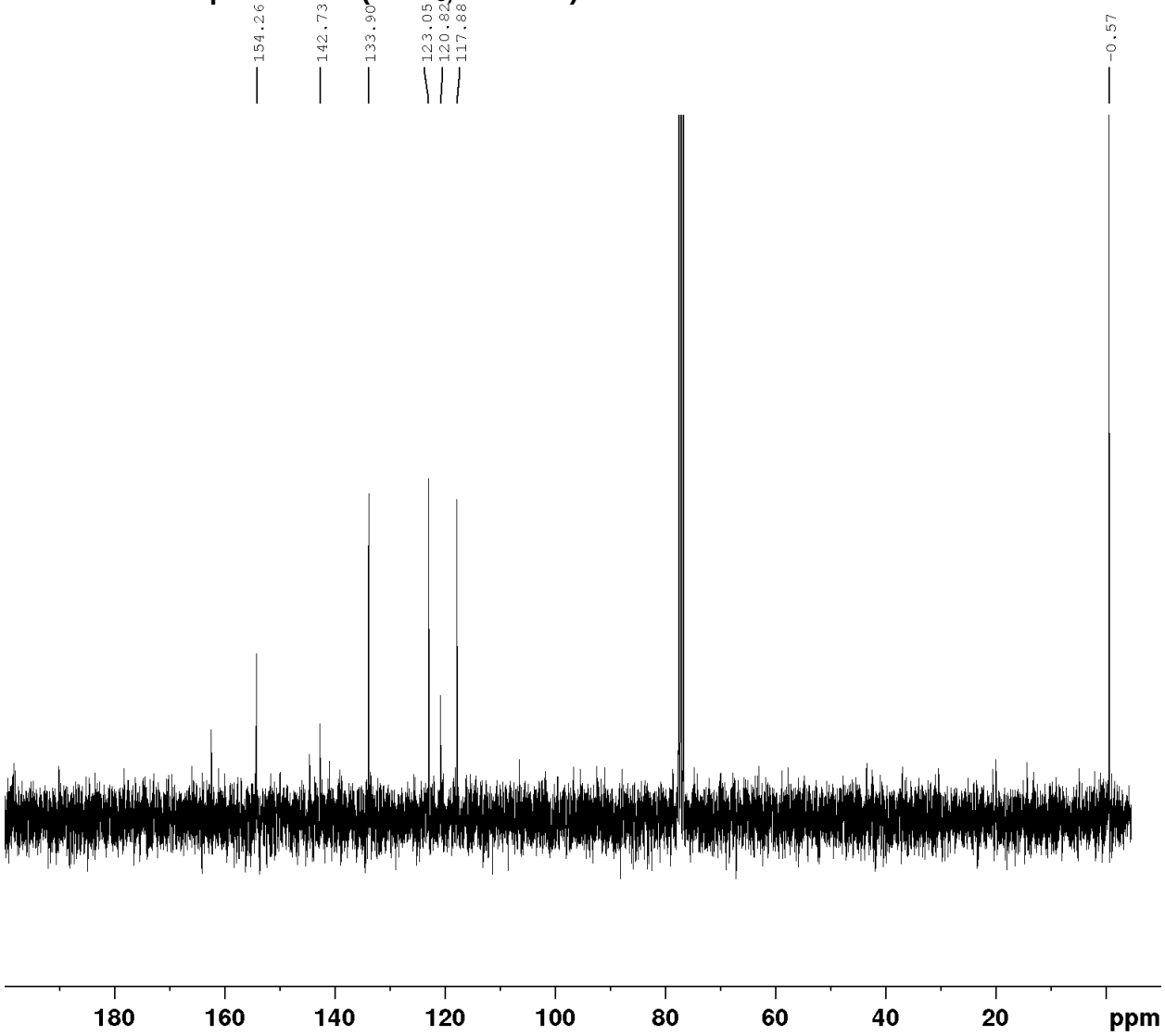
^{1}H NMR of compound 3.46 (DMSO-d_6, 500 MHz)



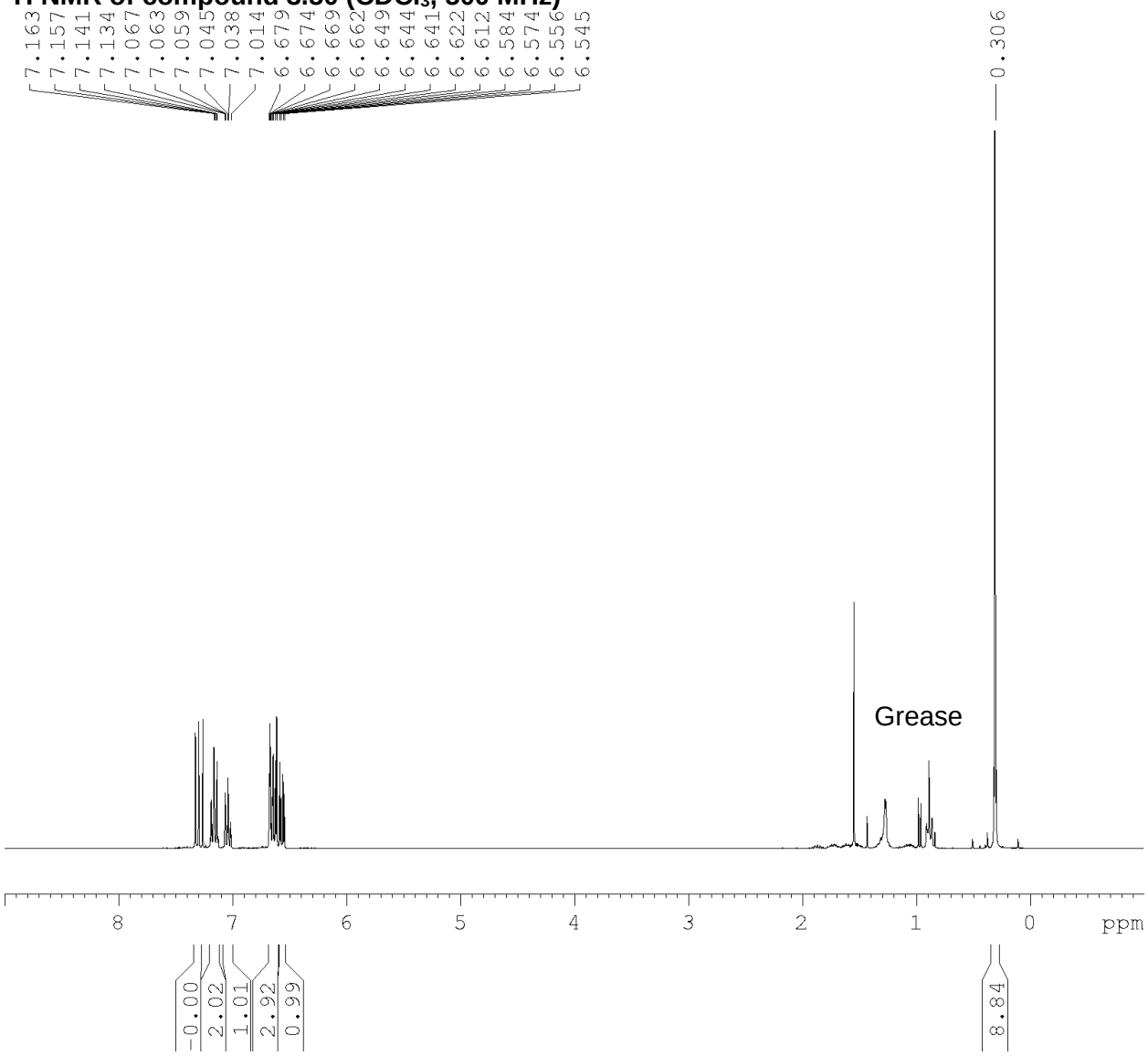
^{1}H NMR of compound 3.48 ($CDCl_3$, 500 MHz)



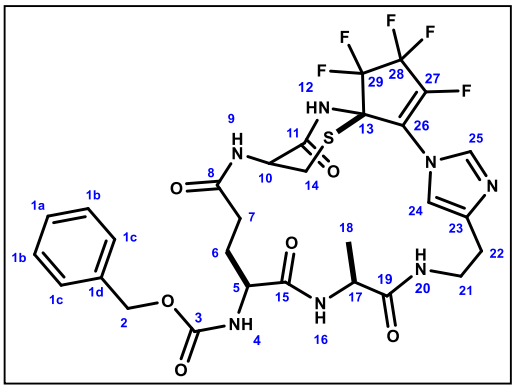
^{13}C NMR of compound 3.48 ($CDCl_3$, 126 MHz)



^{1}H NMR of compound 3.50 ($CDCl_3$, 500 MHz)

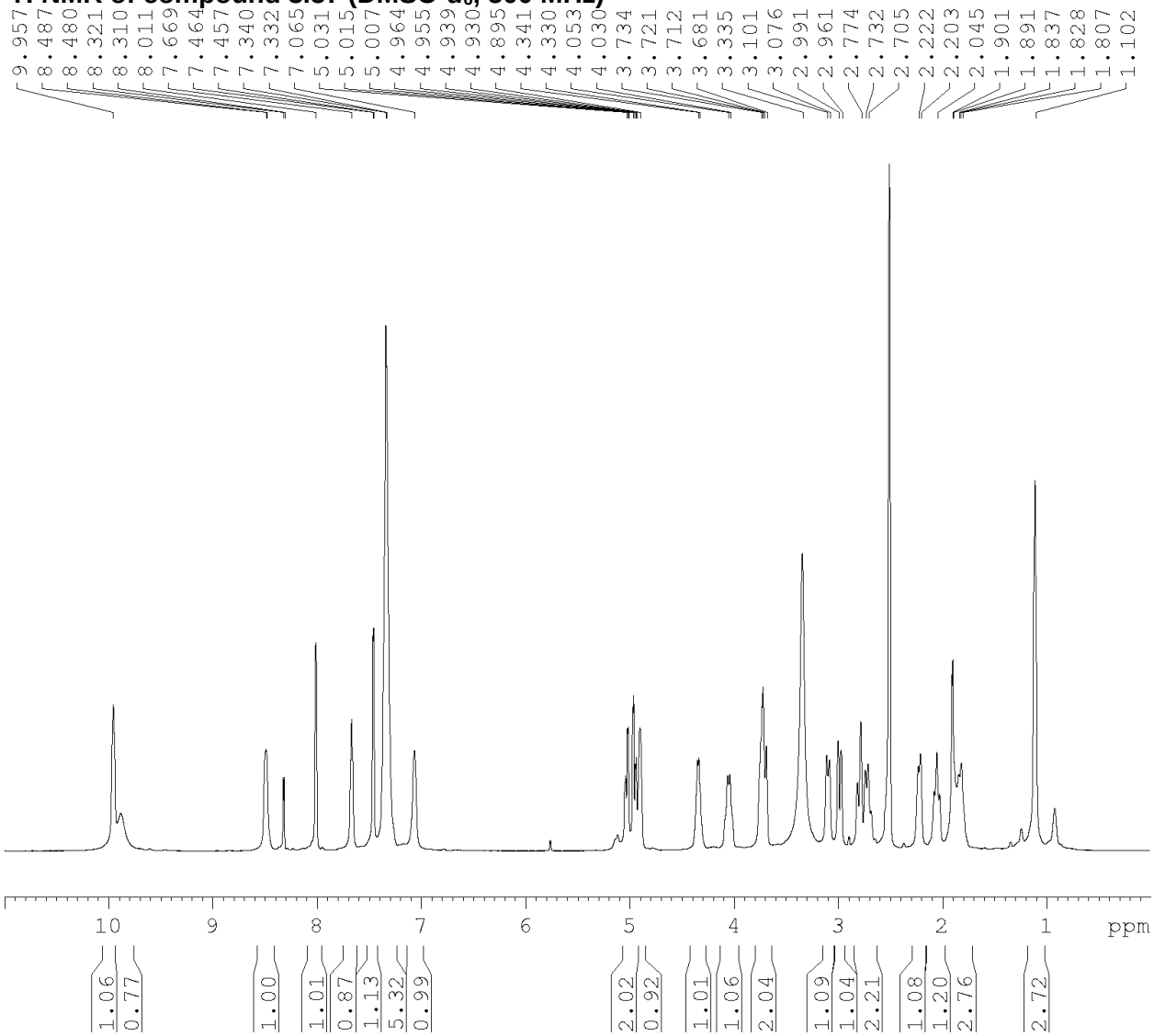


Compound **3.57** (500 MHz, DMSO-d6, 298K)

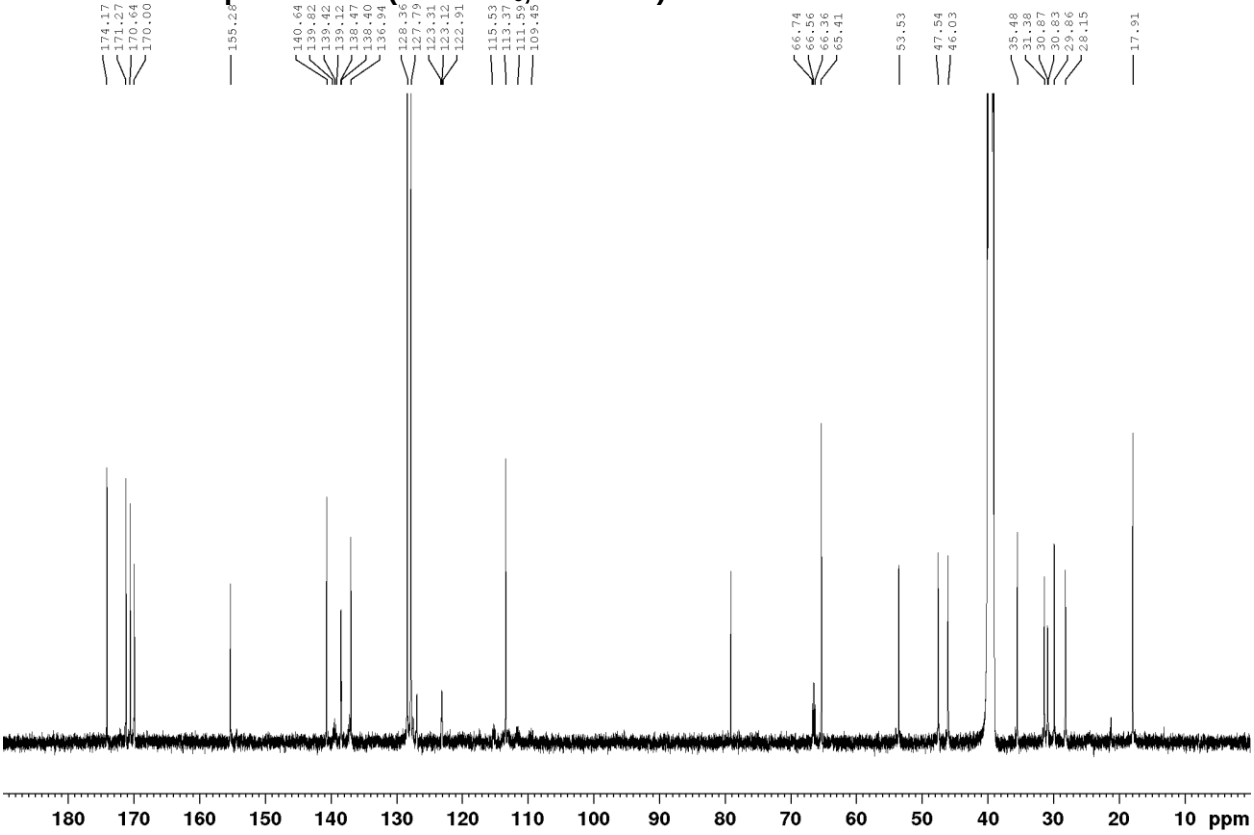


	13C	1H	Key Correlation
1a	127.8	7.42 - 7.24 (m, 5H)	HMBC 2 -> 1d, 1c, 3
1b	128.4	7.42 - 7.24 (m, 5H)	HMBC 1b -> 1c, 1d
1c	127.9	7.42 - 7.24 (m, 5H)	HMBC 1c -> 2
1d	137	-	HMBC 1d -> 2
2	65.4	5.07 - 4.92 (m, 2H)	HMBC 2 -> 1d, 1c, 3
3	155.3	-	HMBC 2,4 -> 3
4	-	7.67 (s, 1H)	HMBC 4 -> 3,5
5	53.5	3.8 - 3.64 (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)	-	

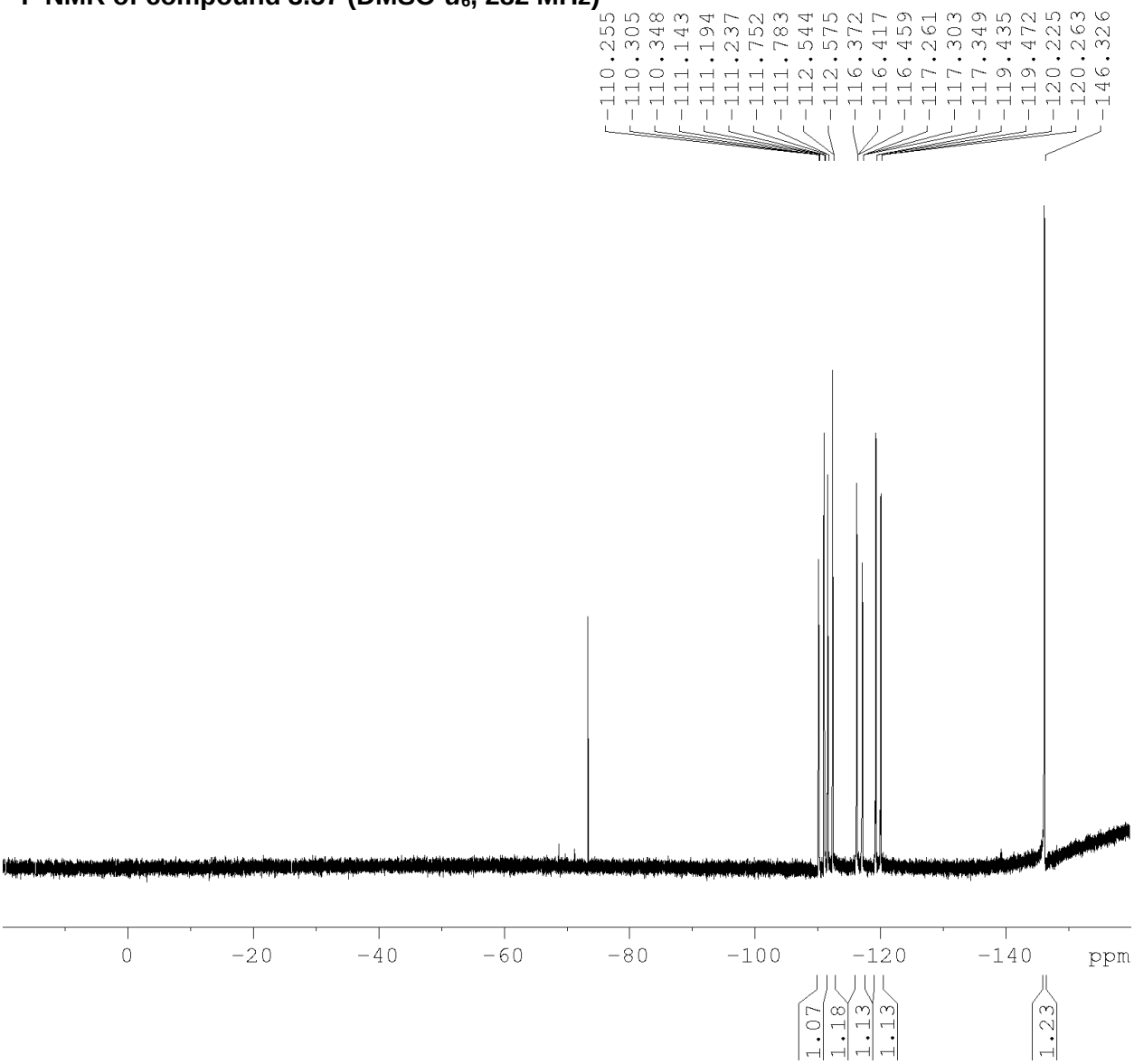
^{1}H NMR of compound 3.57 (DMSO-d_6, 500 MHz)



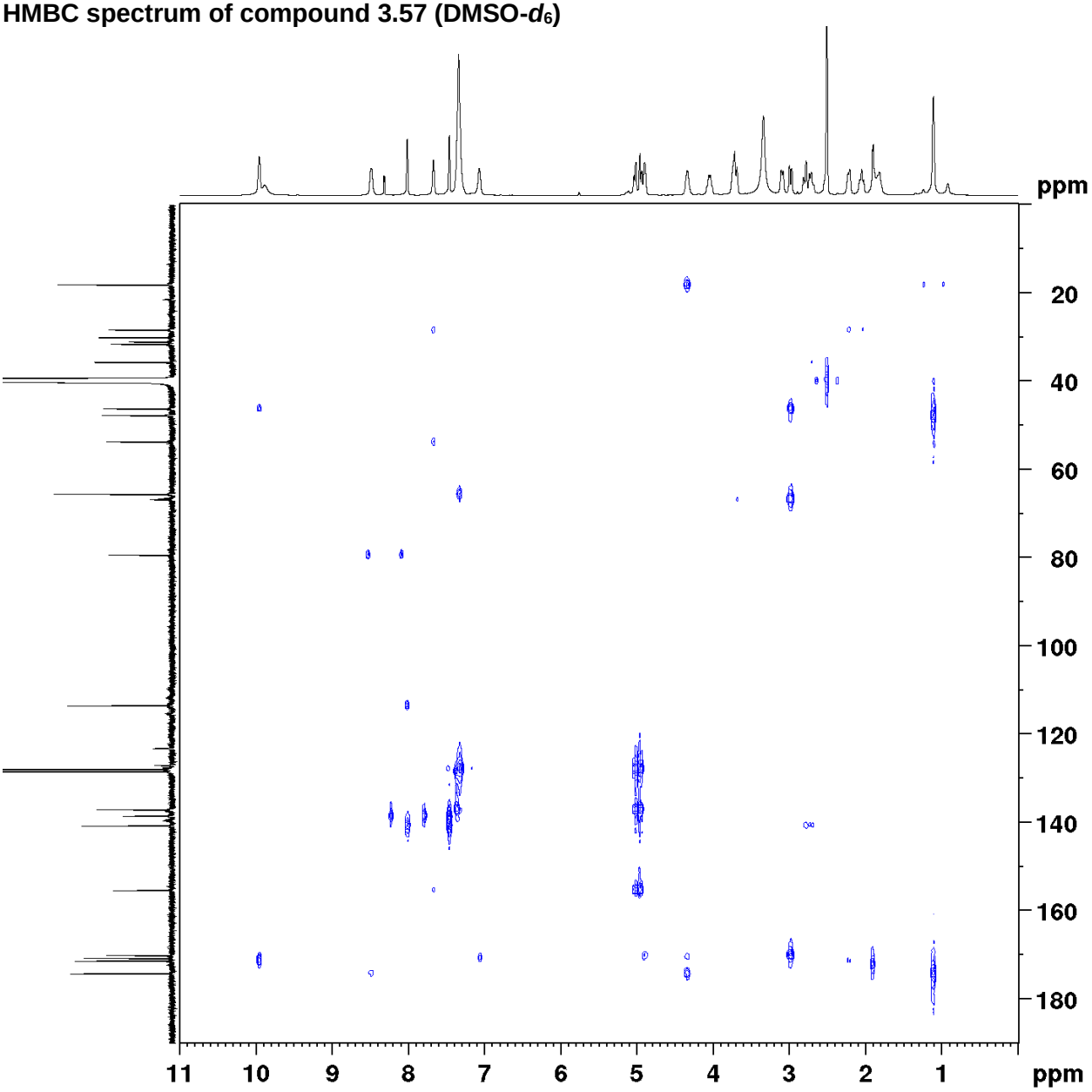
^{13}C NMR of compound 3.57 (DMSO-d_6, 126 MHz)



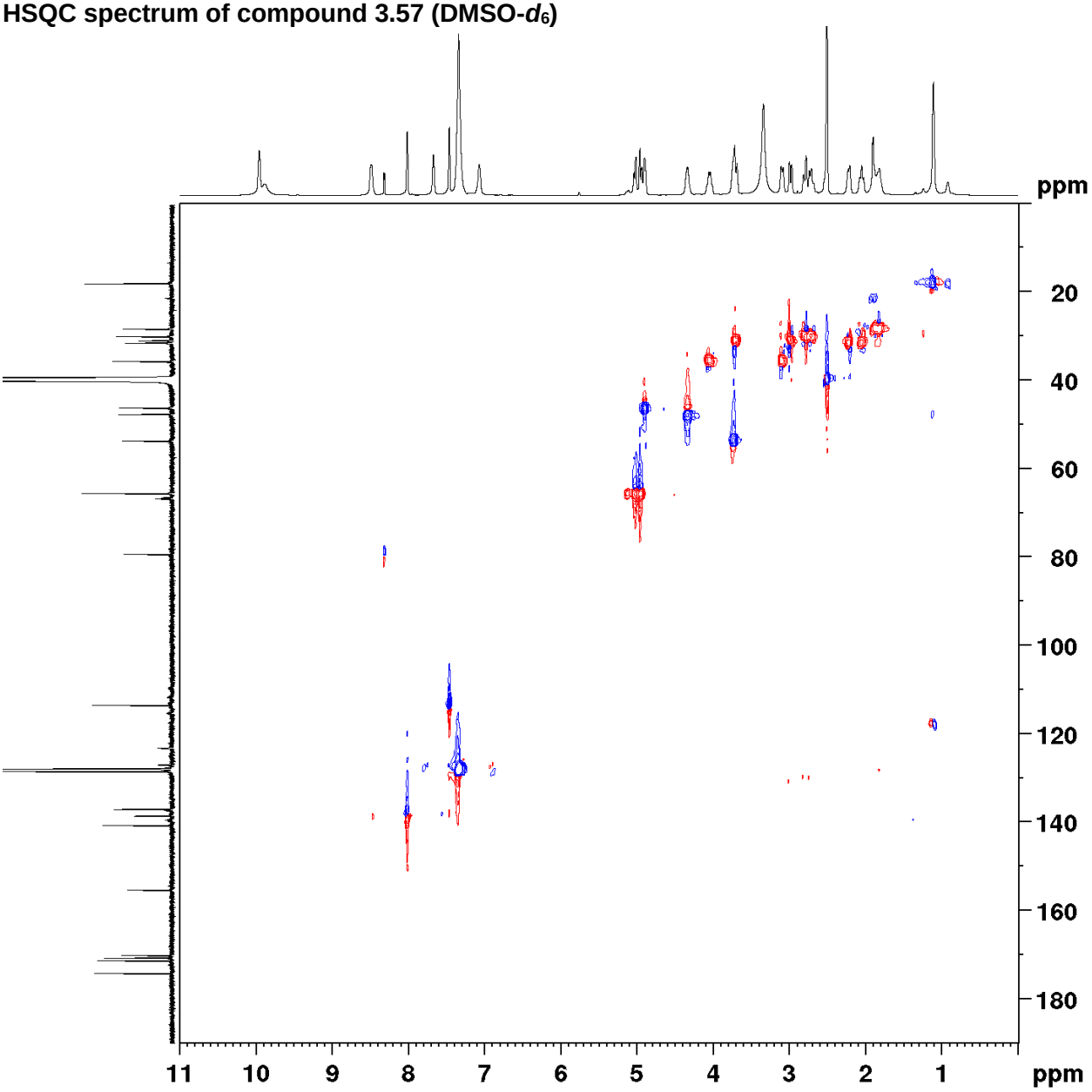
^{19}F NMR of compound 3.57 (DMSO-d_6, 282 MHz)



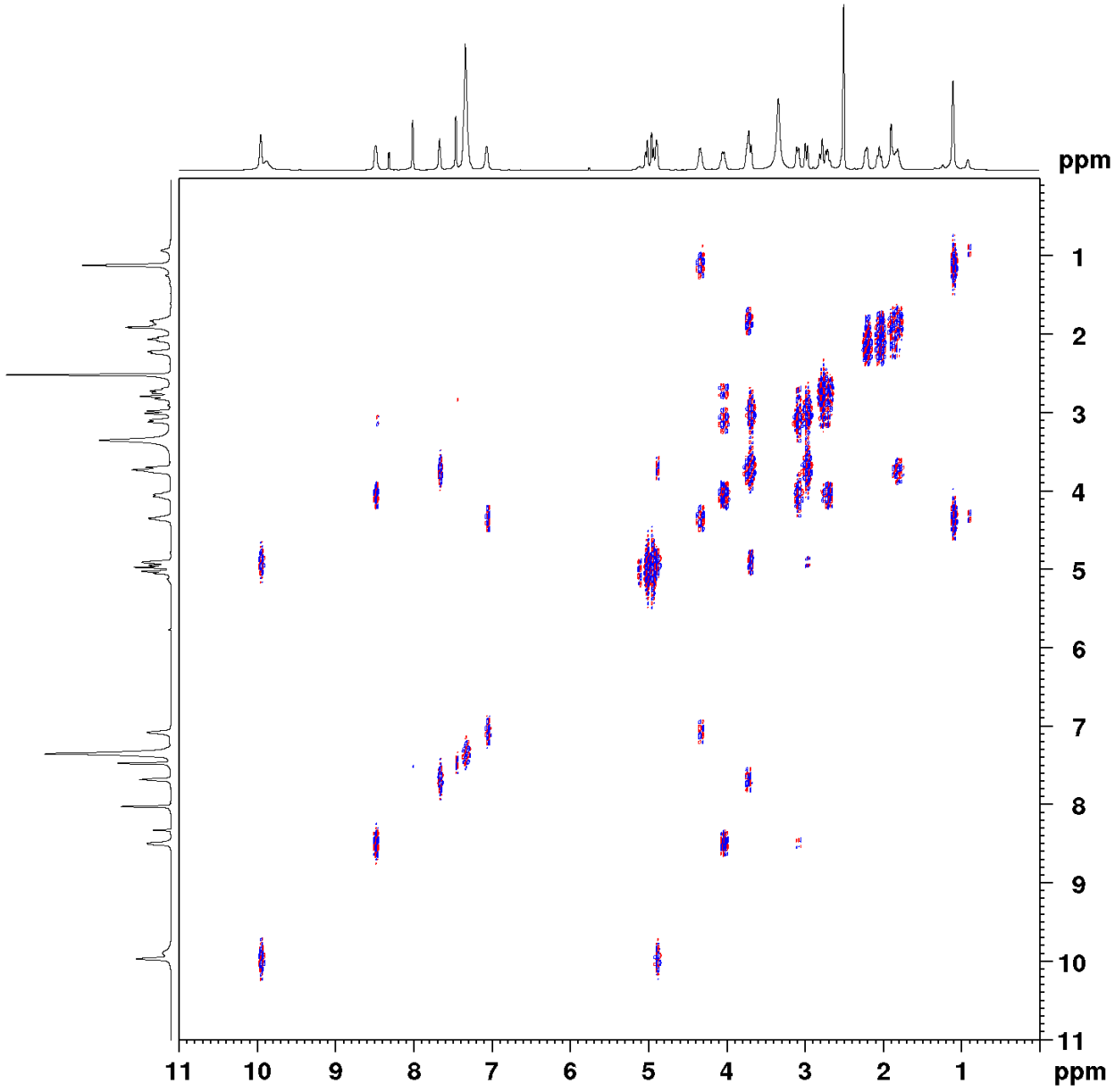
HMBC spectrum of compound 3.57 (DMSO-d_6)



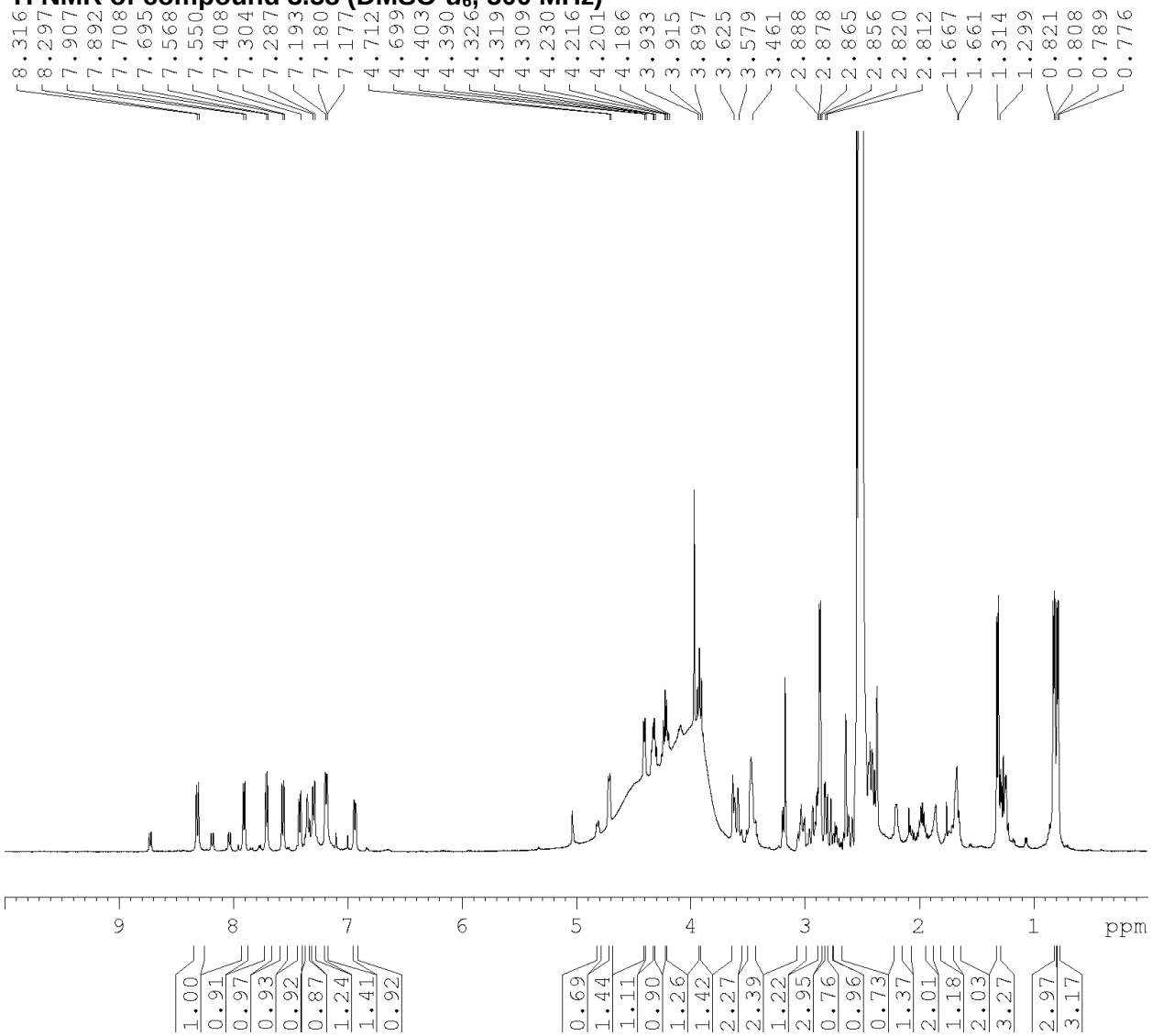
HSQC spectrum of compound 3.57 (DMSO-d_6)



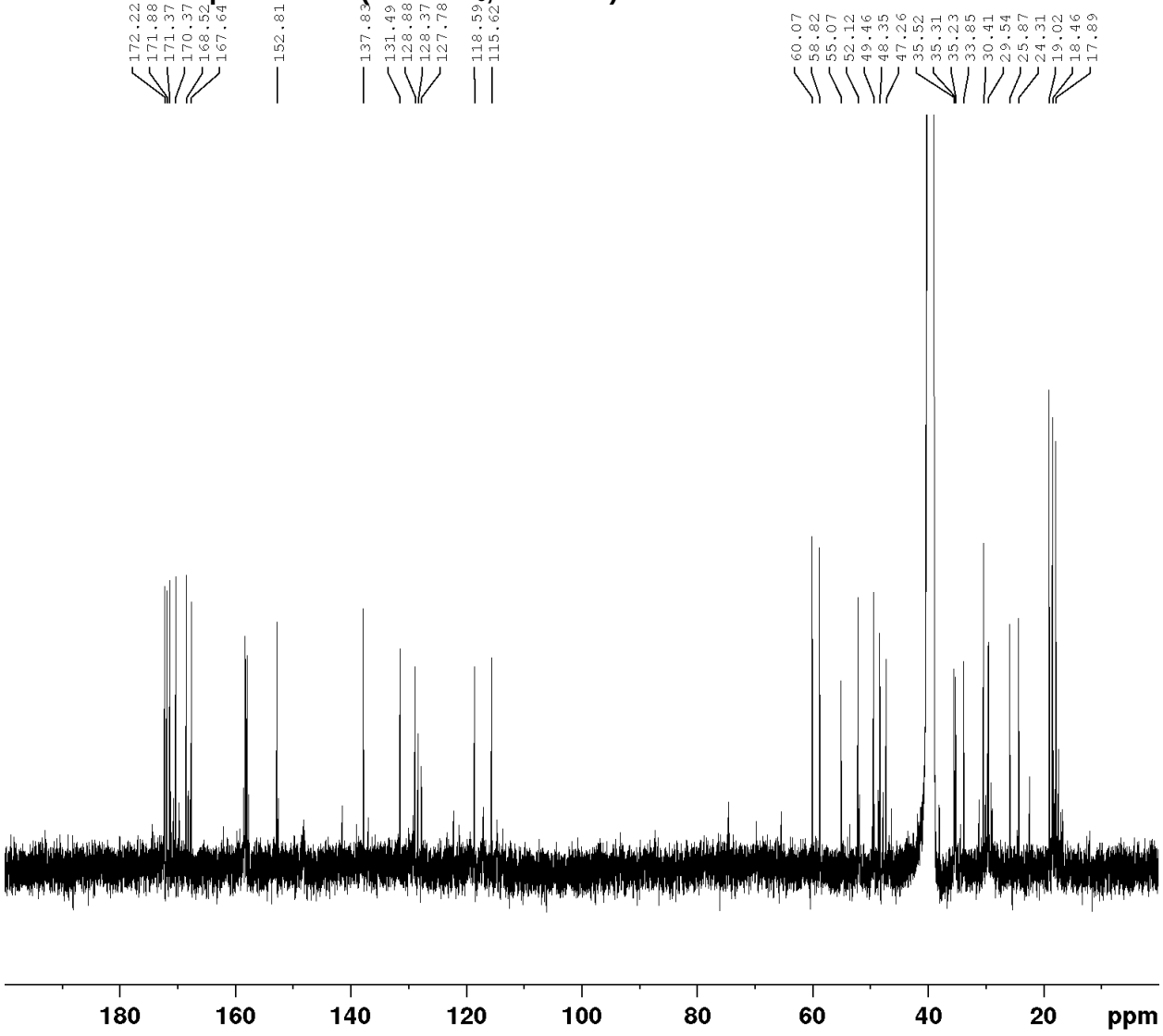
COSY spectrum of compound 3.57 (DMSO-d_6)



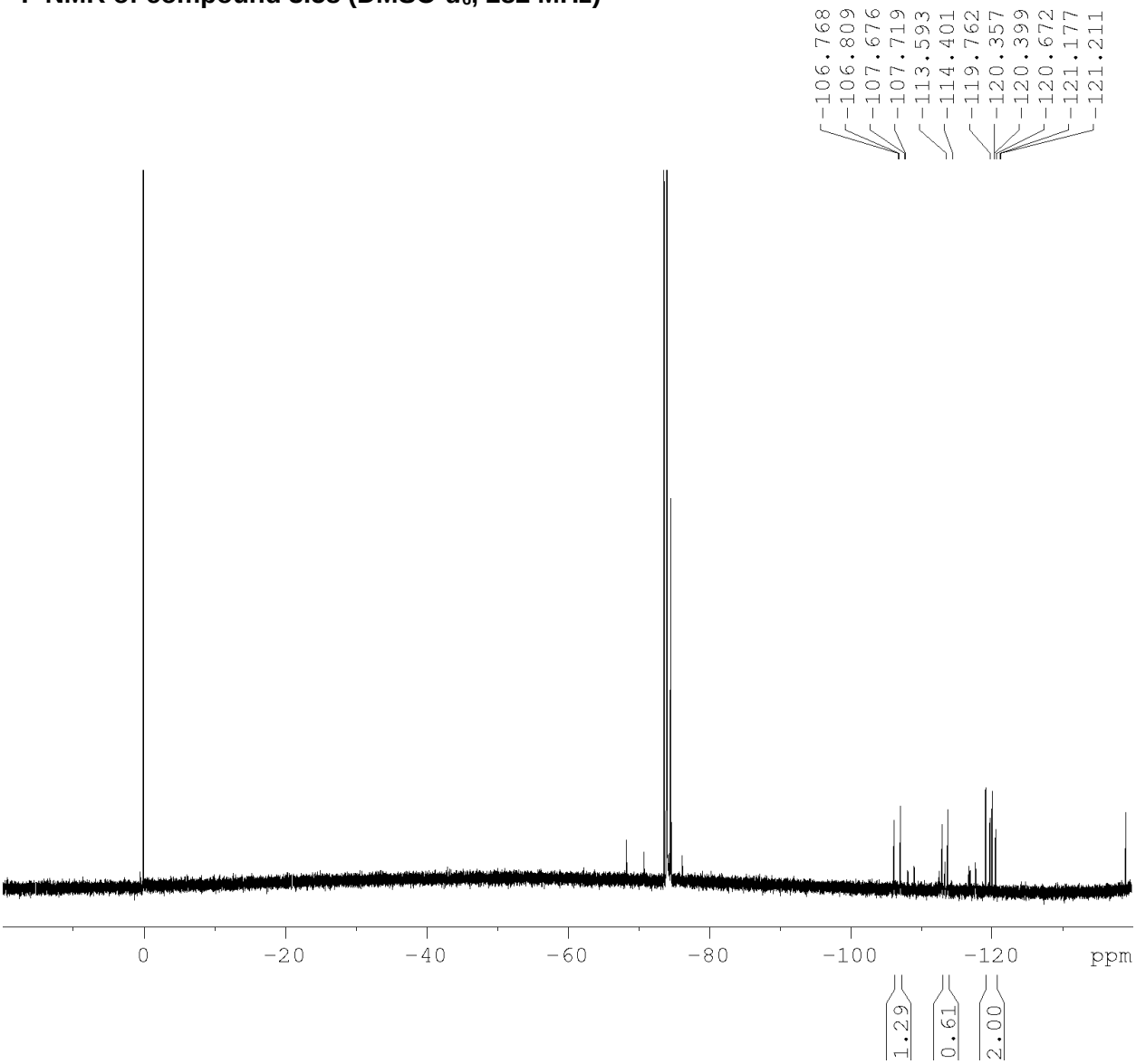
^{1}H NMR of compound 3.58 (DMSO-d_6, 500 MHz)



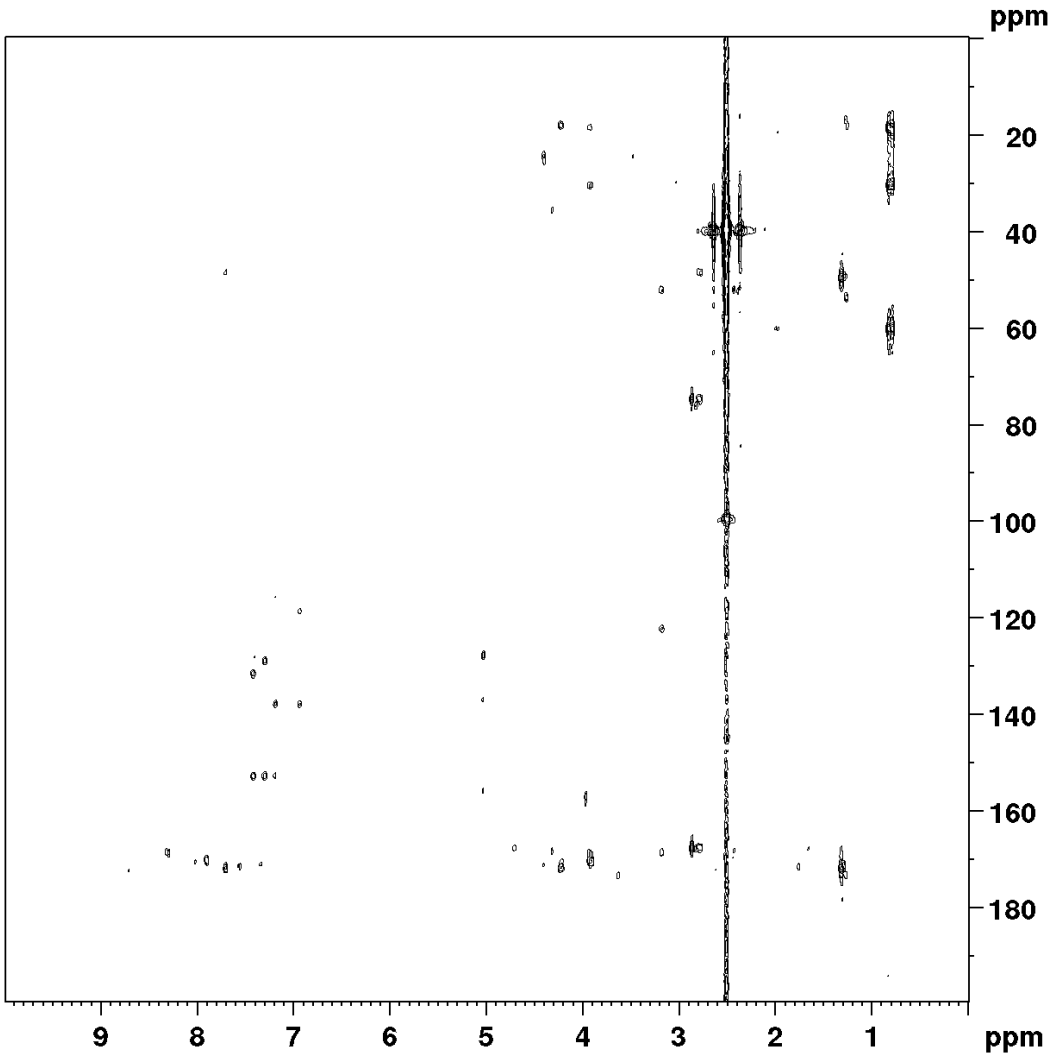
^{13}C NMR of compound 3.58 (DMSO-d_6, 126 MHz)



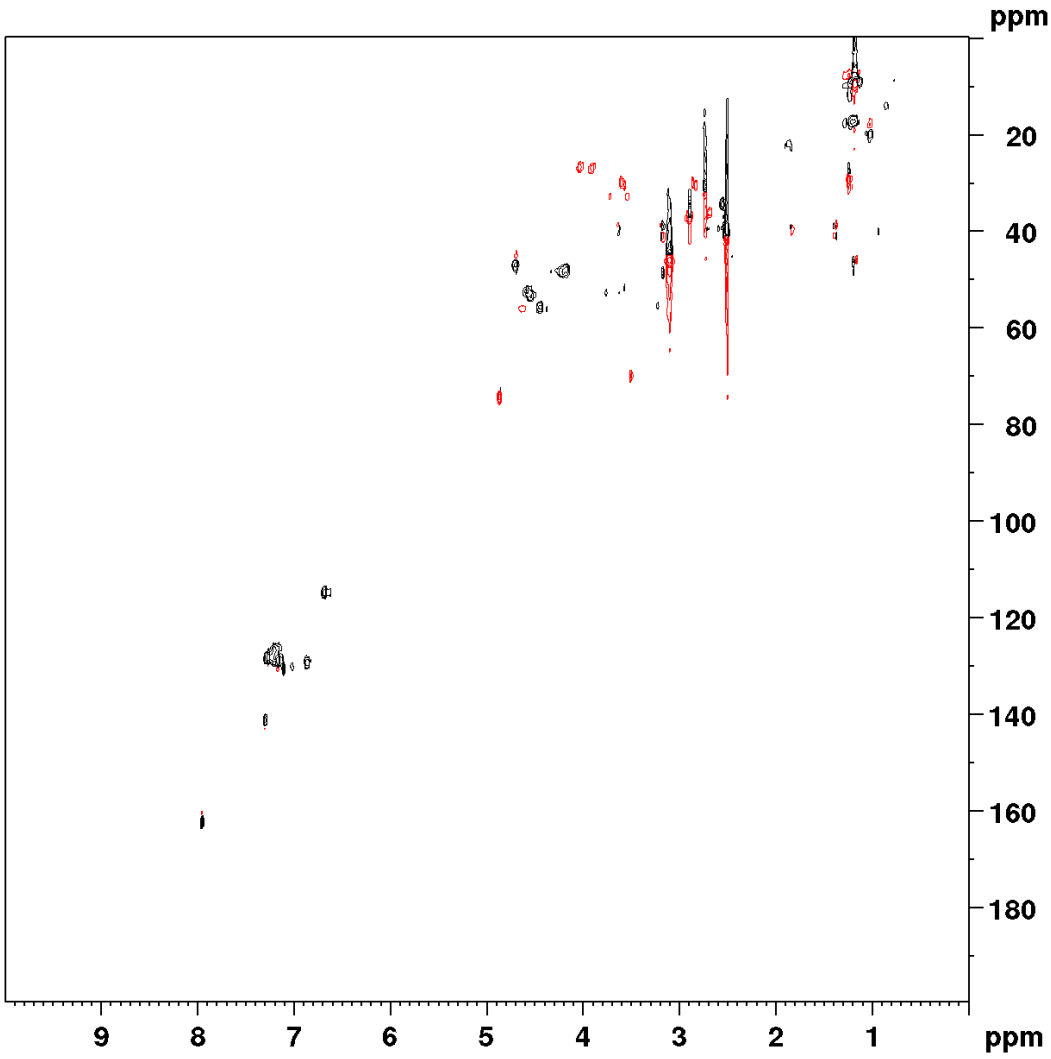
^{19}F NMR of compound 3.58 (DMSO-d_6, 282 MHz)



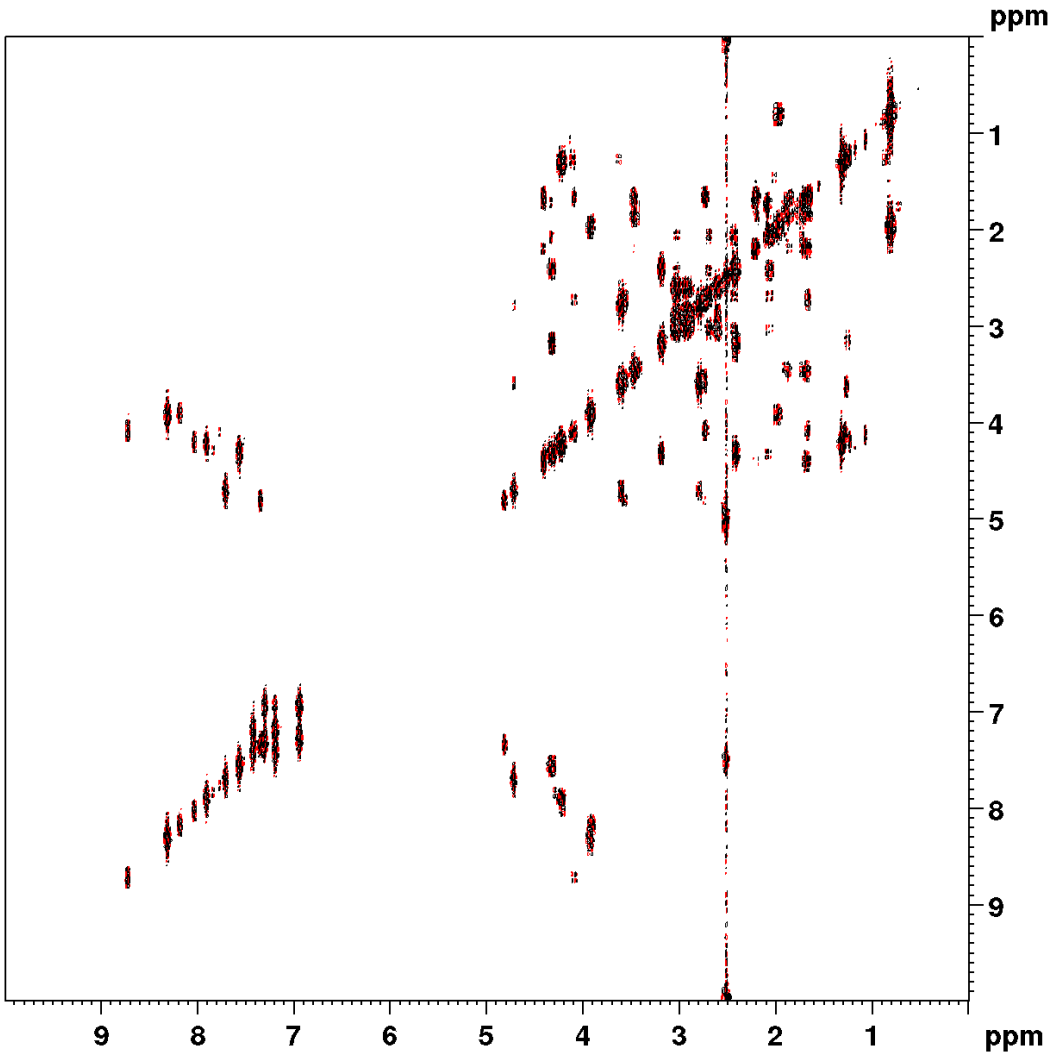
HMBC spectrum of compound 3.58 (DMSO-d_6)



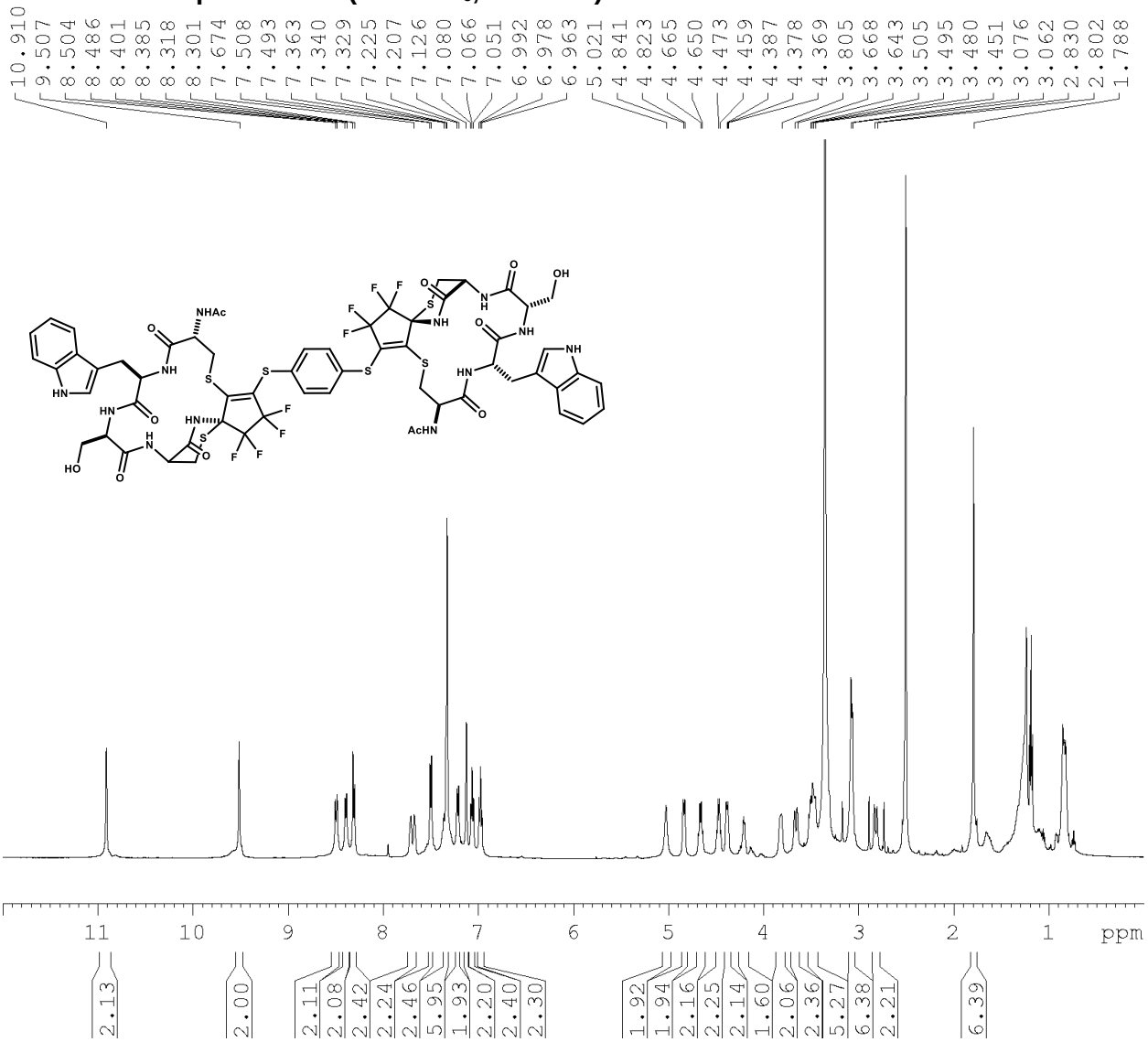
HSQC spectrum of compound 3.58 (DMSO-d_6)



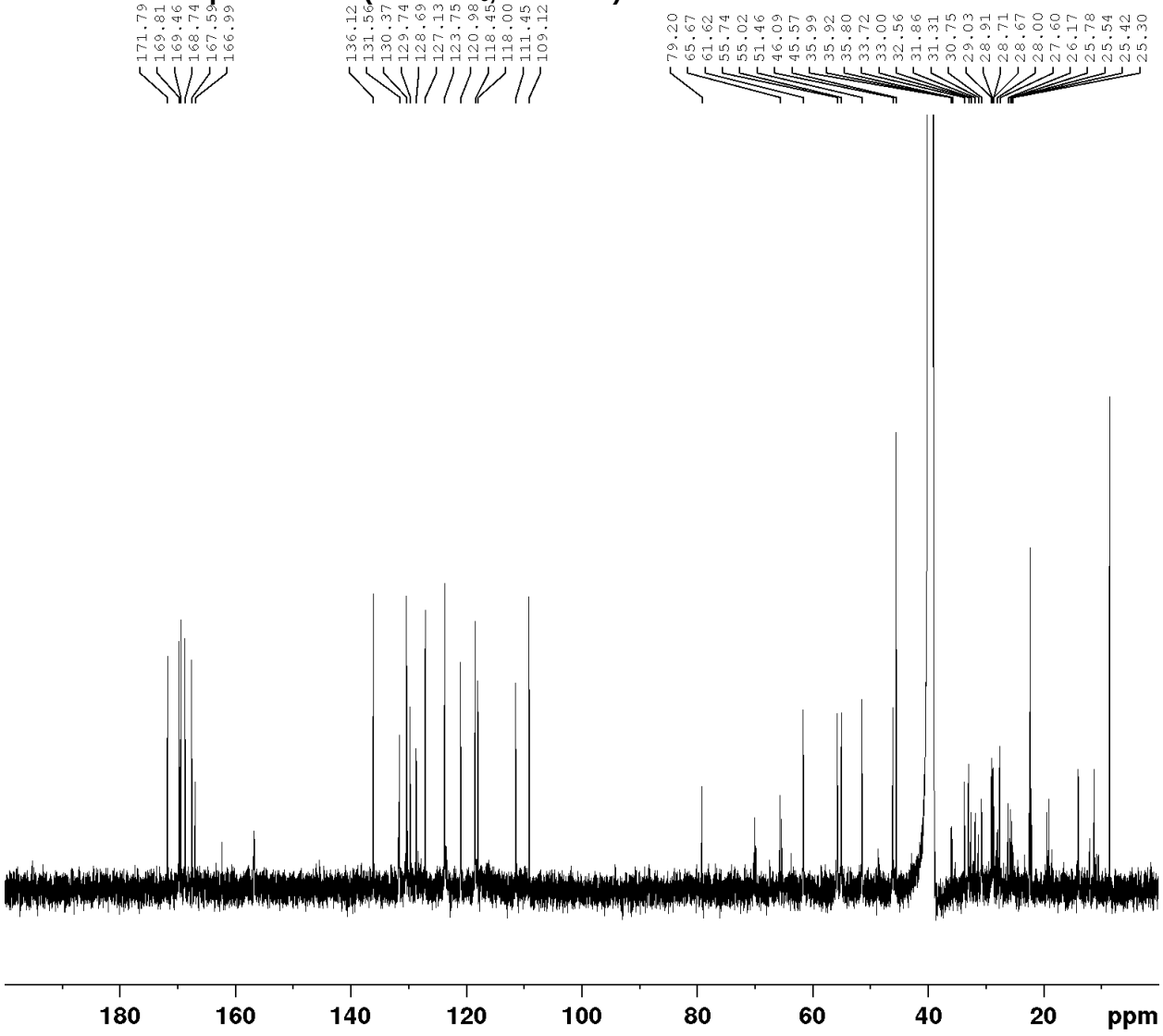
COSY spectrum of compound 3.58 (DMSO-d_6)



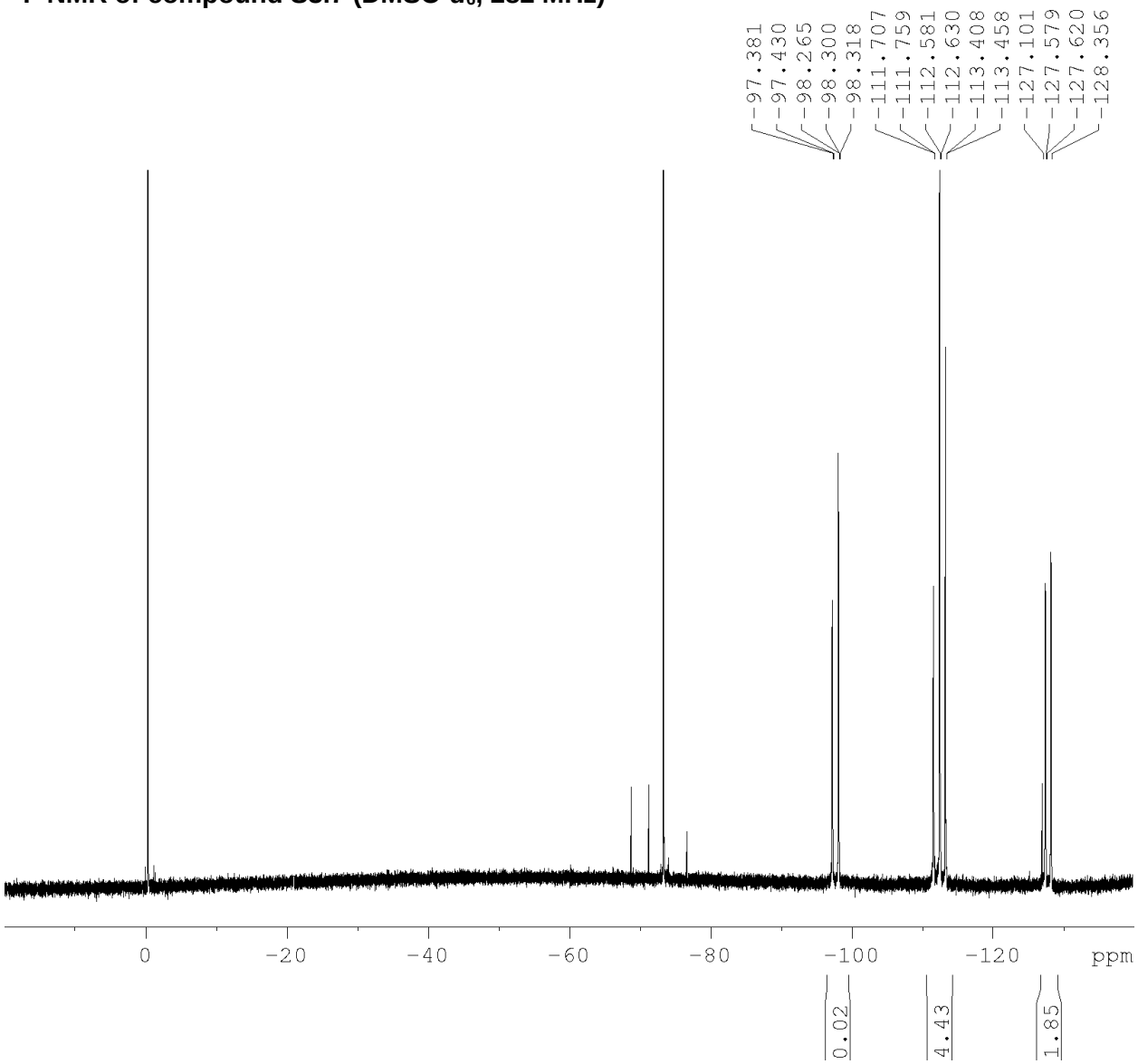
^{1}H NMR of compound S3.7 (DMSO-d_6, 500 MHz)



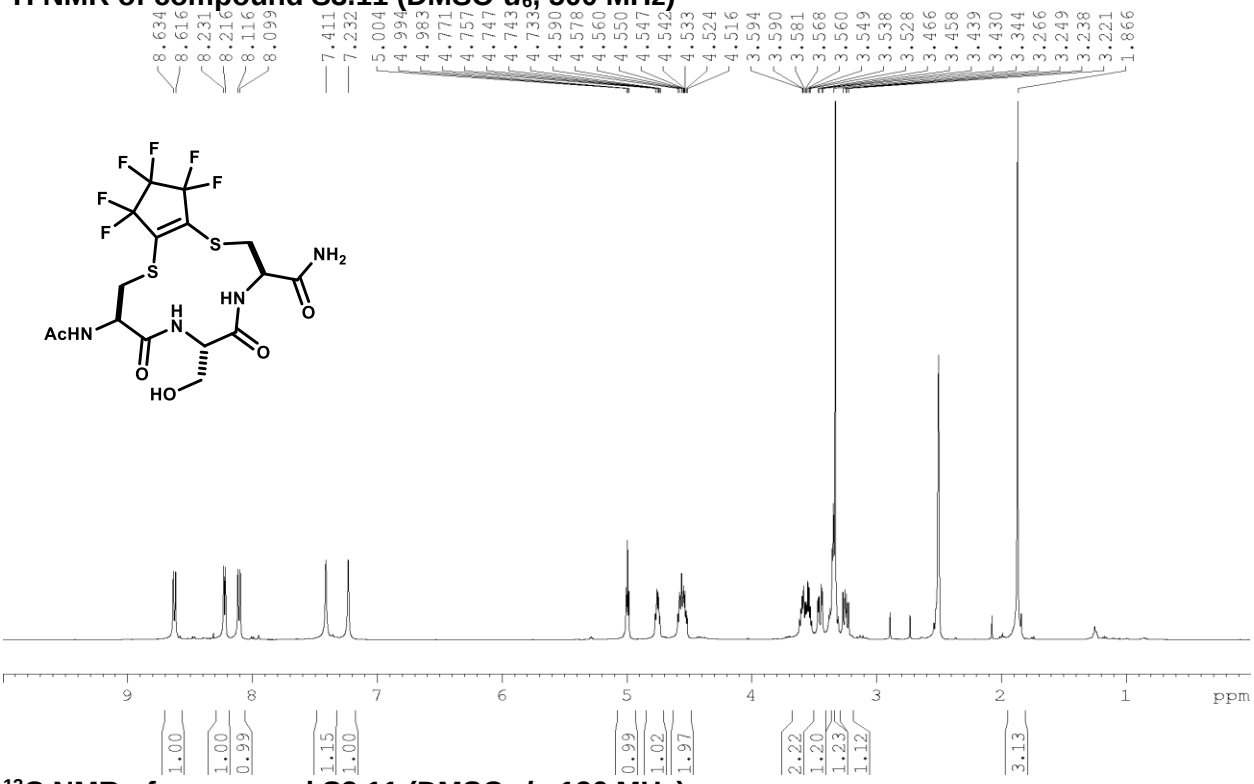
^{13}C NMR of compound S3.7 (DMSO-d_6, 126 MHz)



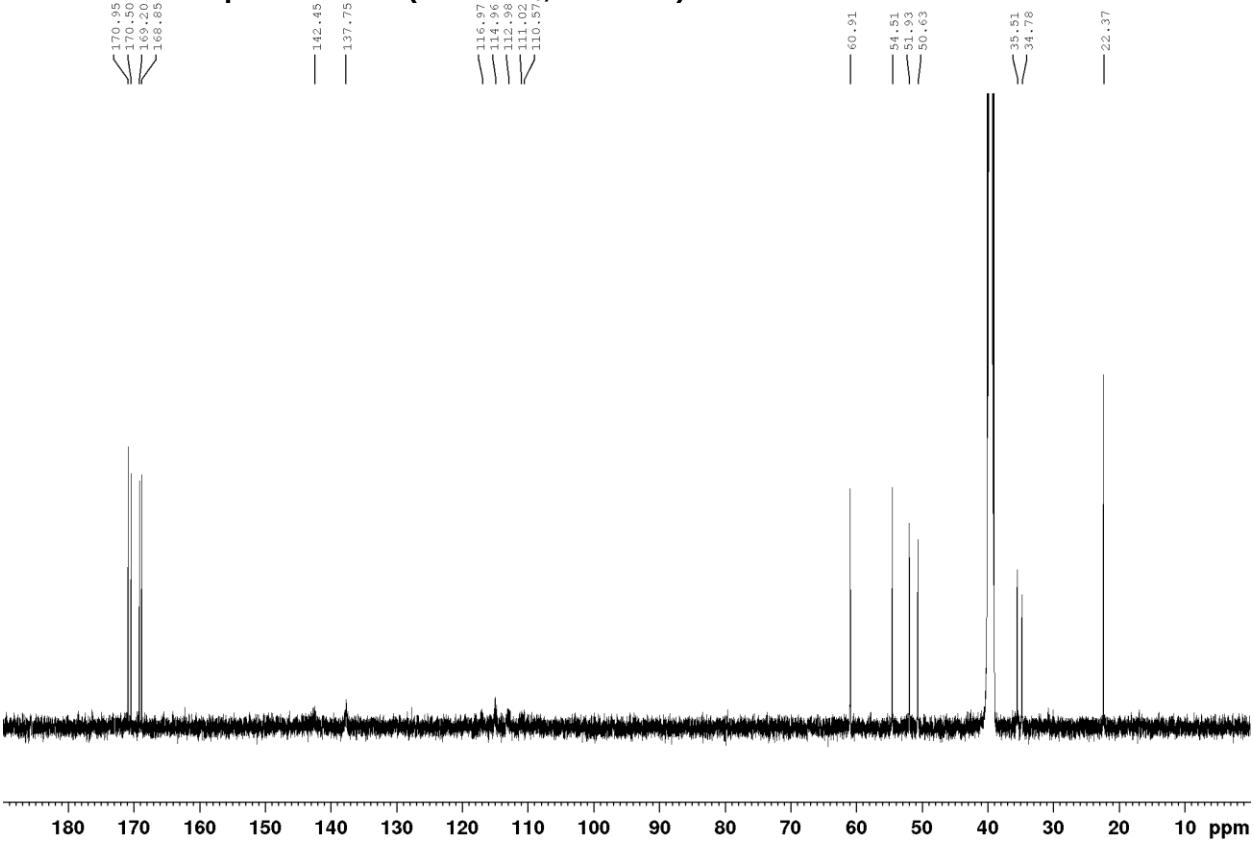
^{19}F NMR of compound S3.7 (DMSO-d_6, 282 MHz)



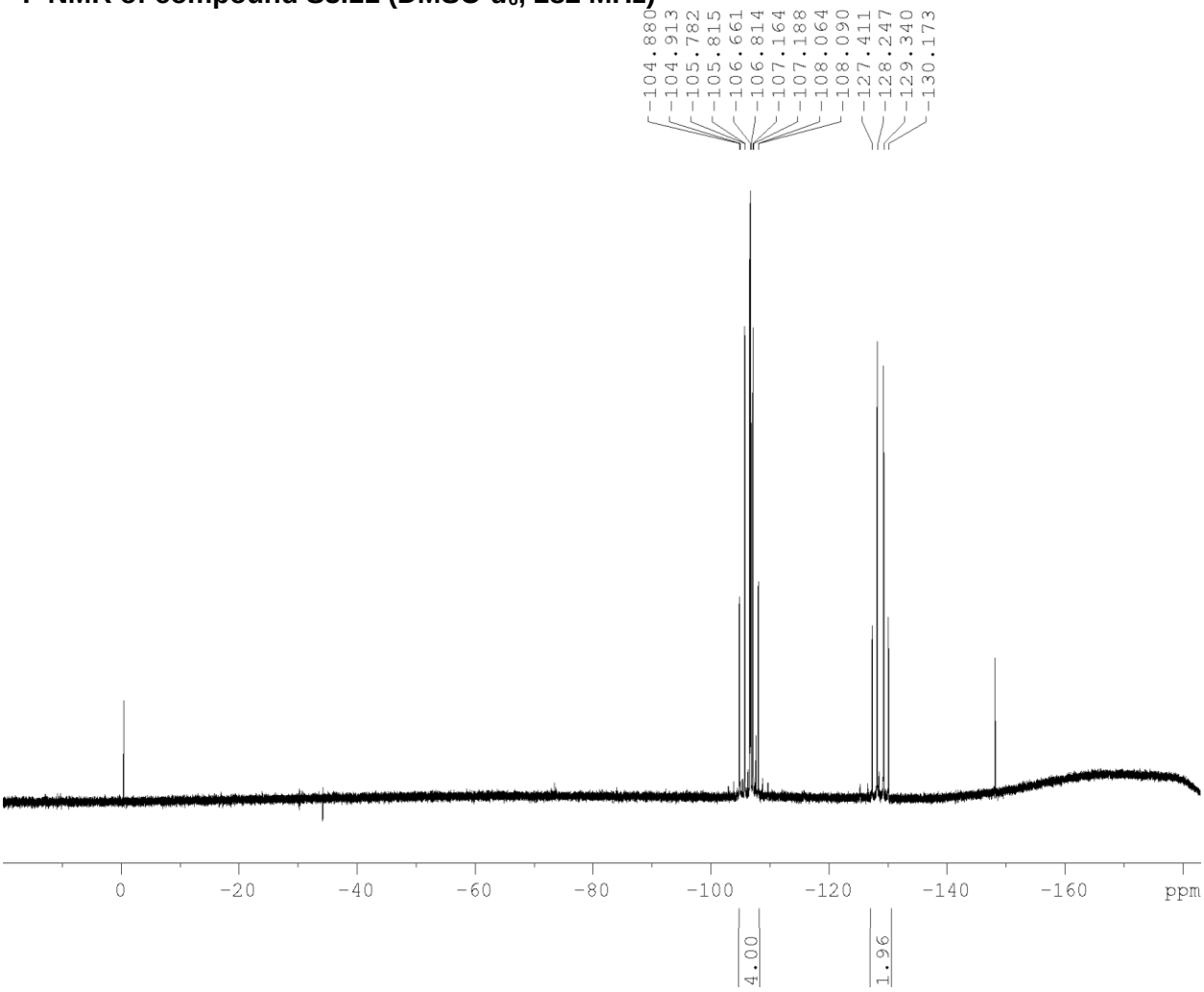
^{1}H NMR of compound S3.11 (DMSO-d_6, 500 MHz)



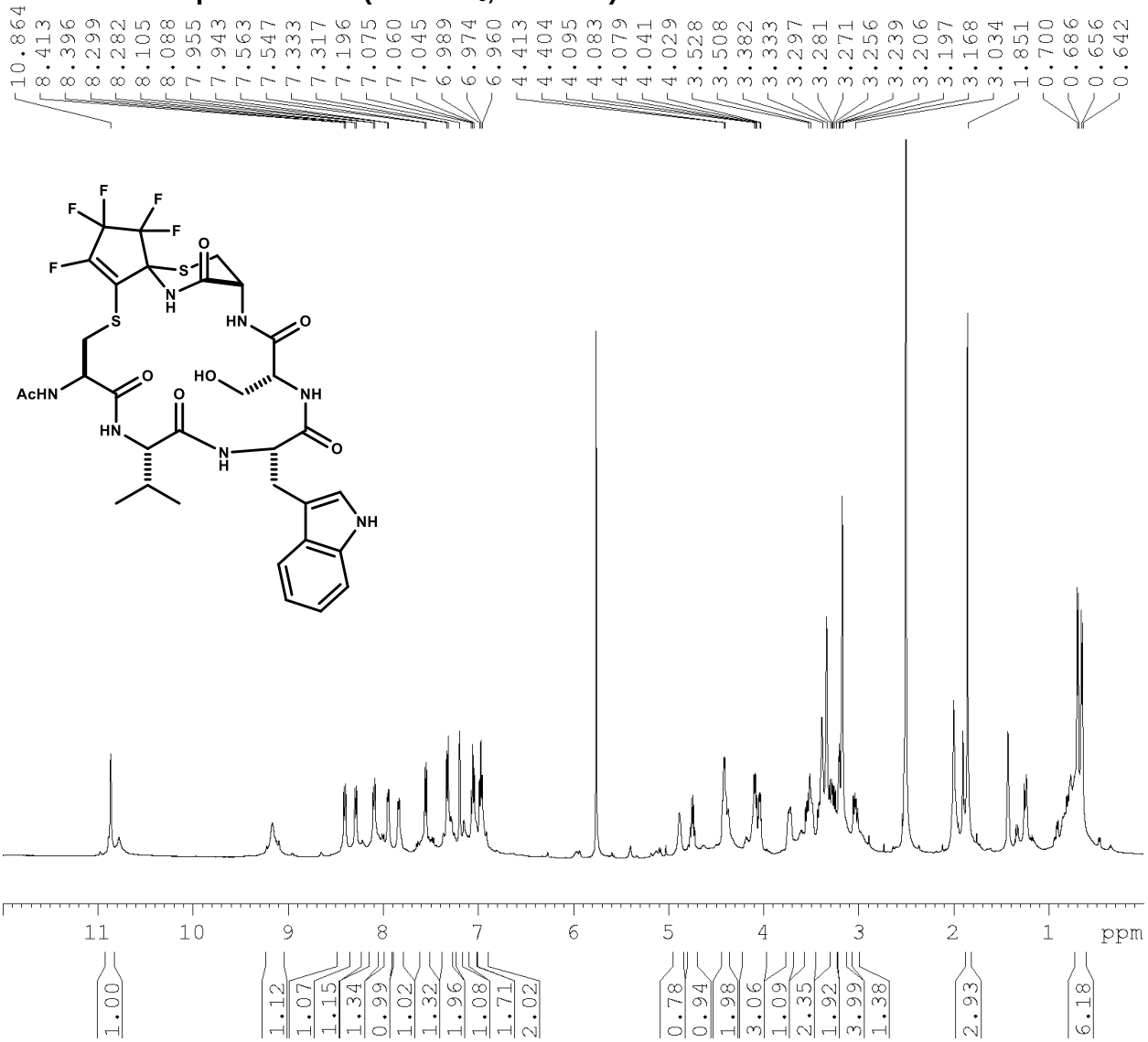
^{13}C NMR of compound S3.11 (DMSO-d_6, 126 MHz)



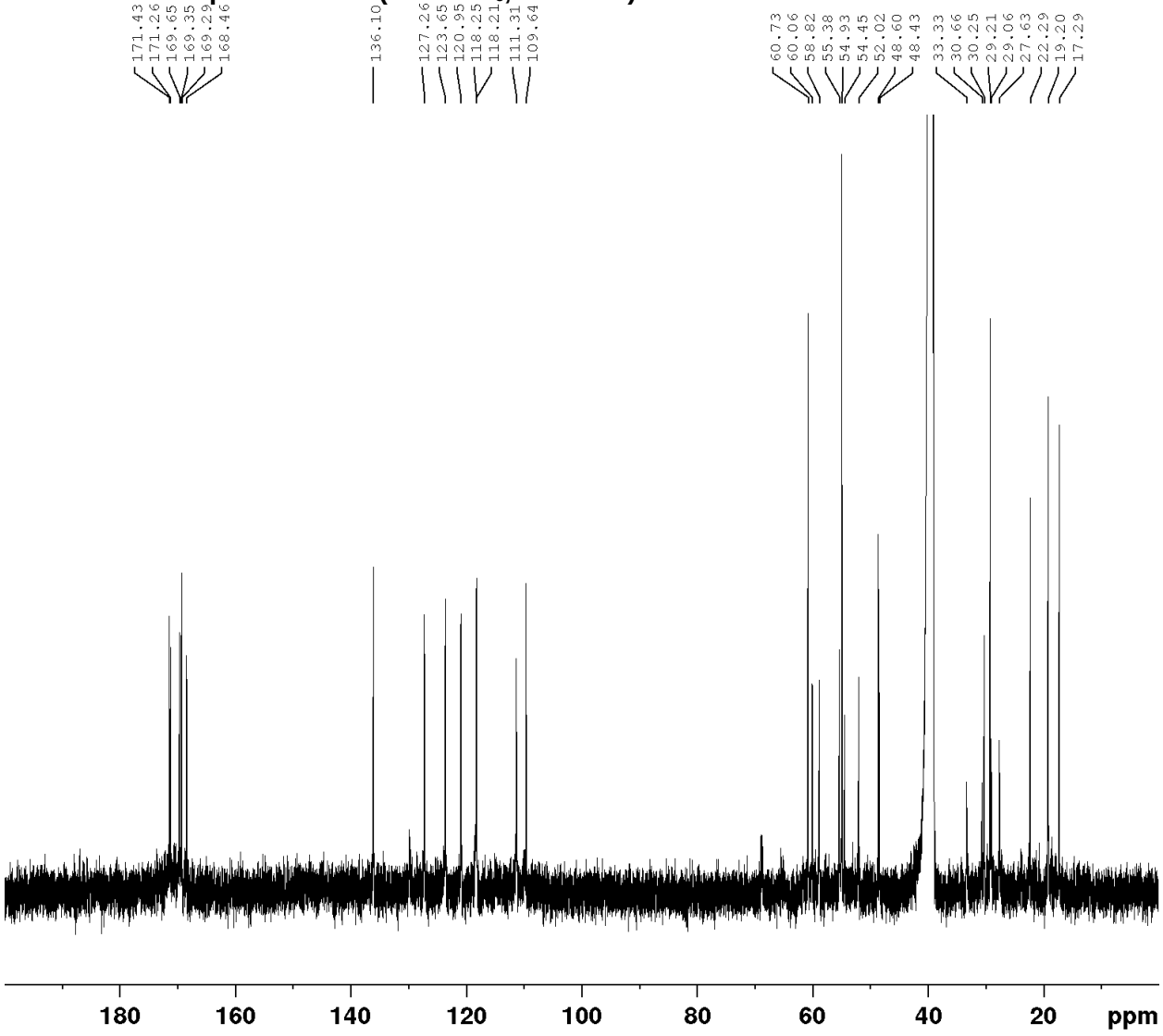
^{19}F NMR of compound S3.11 (DMSO-d_6, 282 MHz)



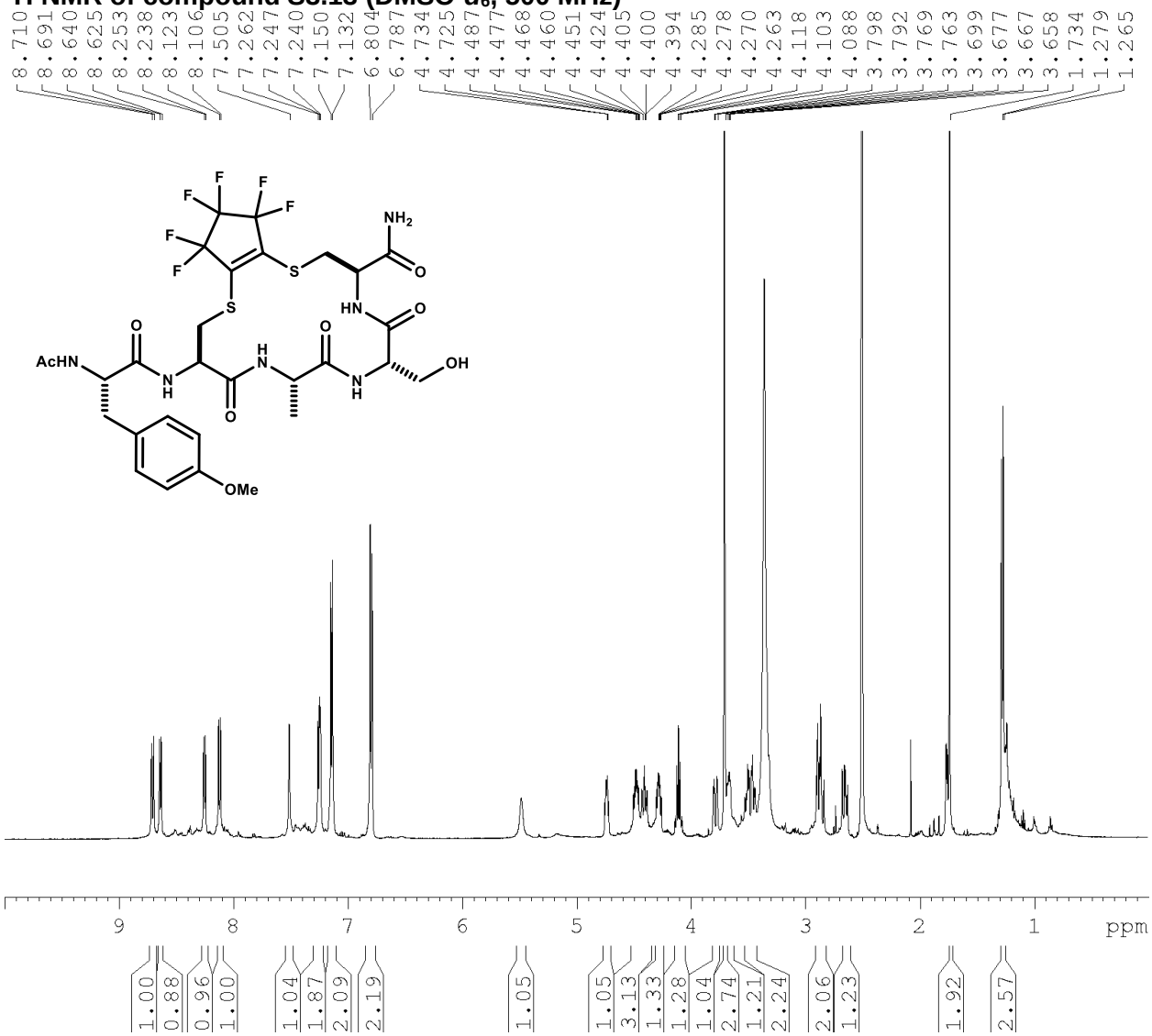
^{1}H NMR of compound S3.12 (DMSO-d_6, 500 MHz)



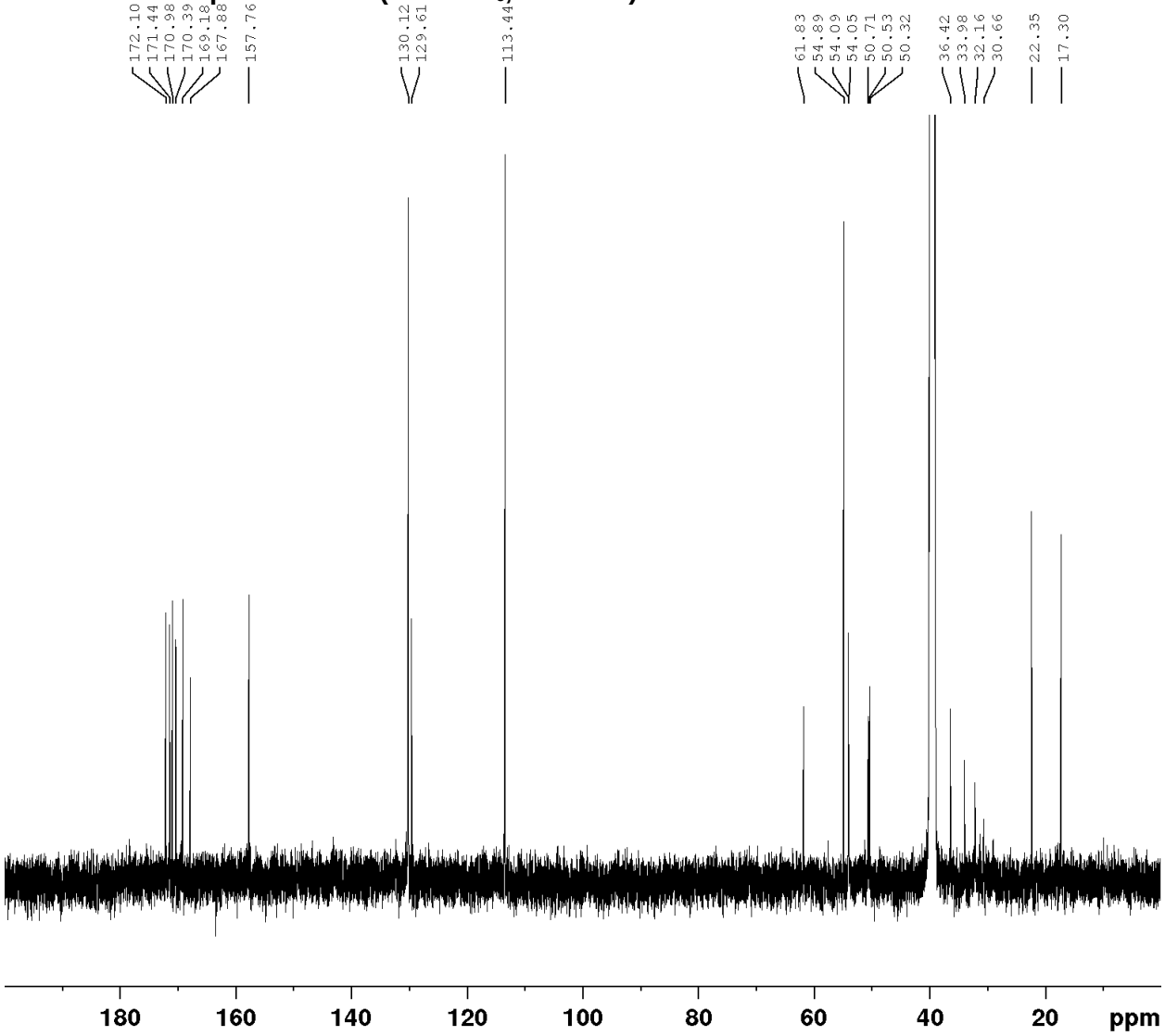
^{13}C NMR of compound S3.12 (DMSO-d_6, 126 MHz)



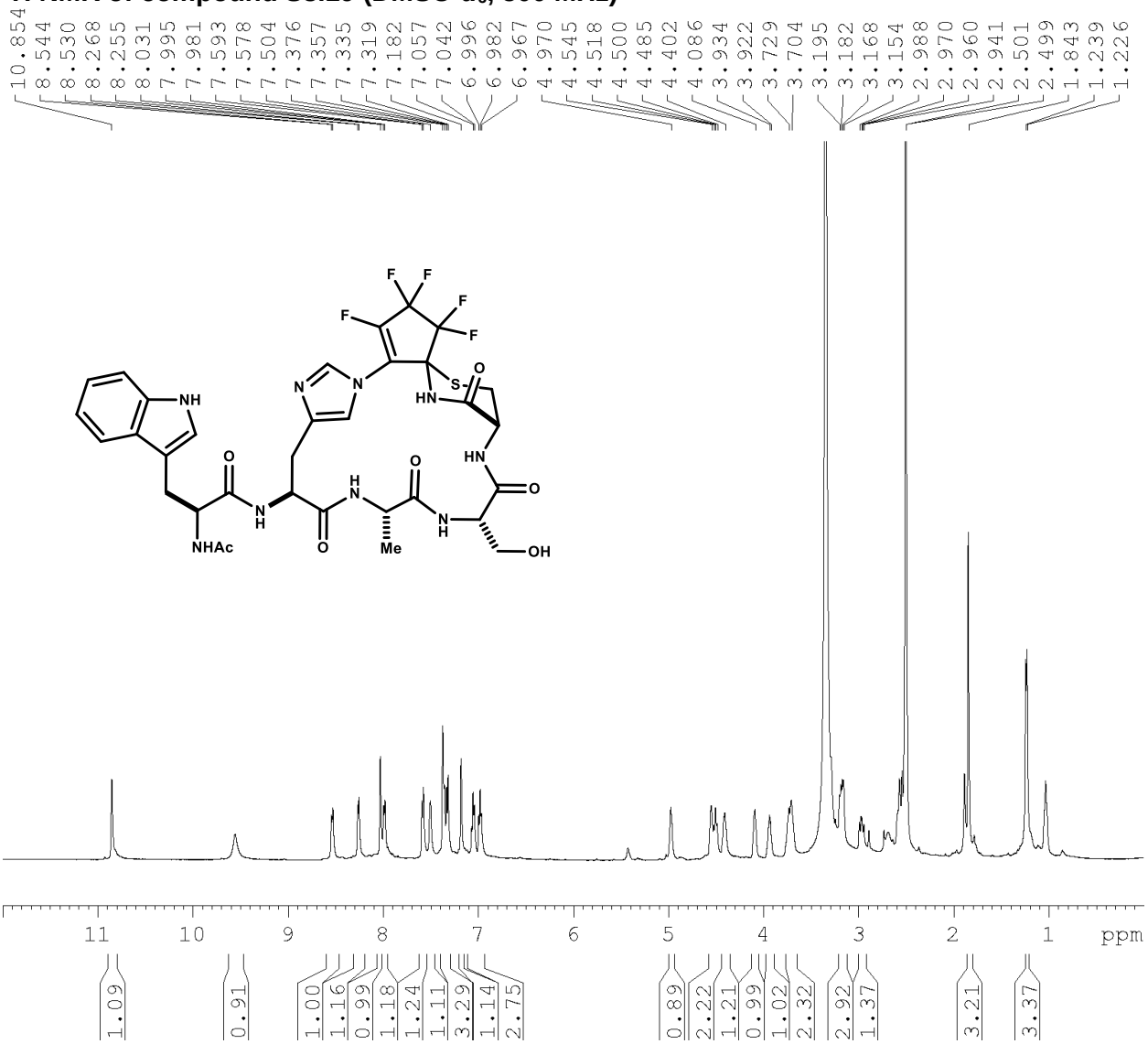
^{1}H NMR of compound S3.13 (DMSO-d_6, 500 MHz)



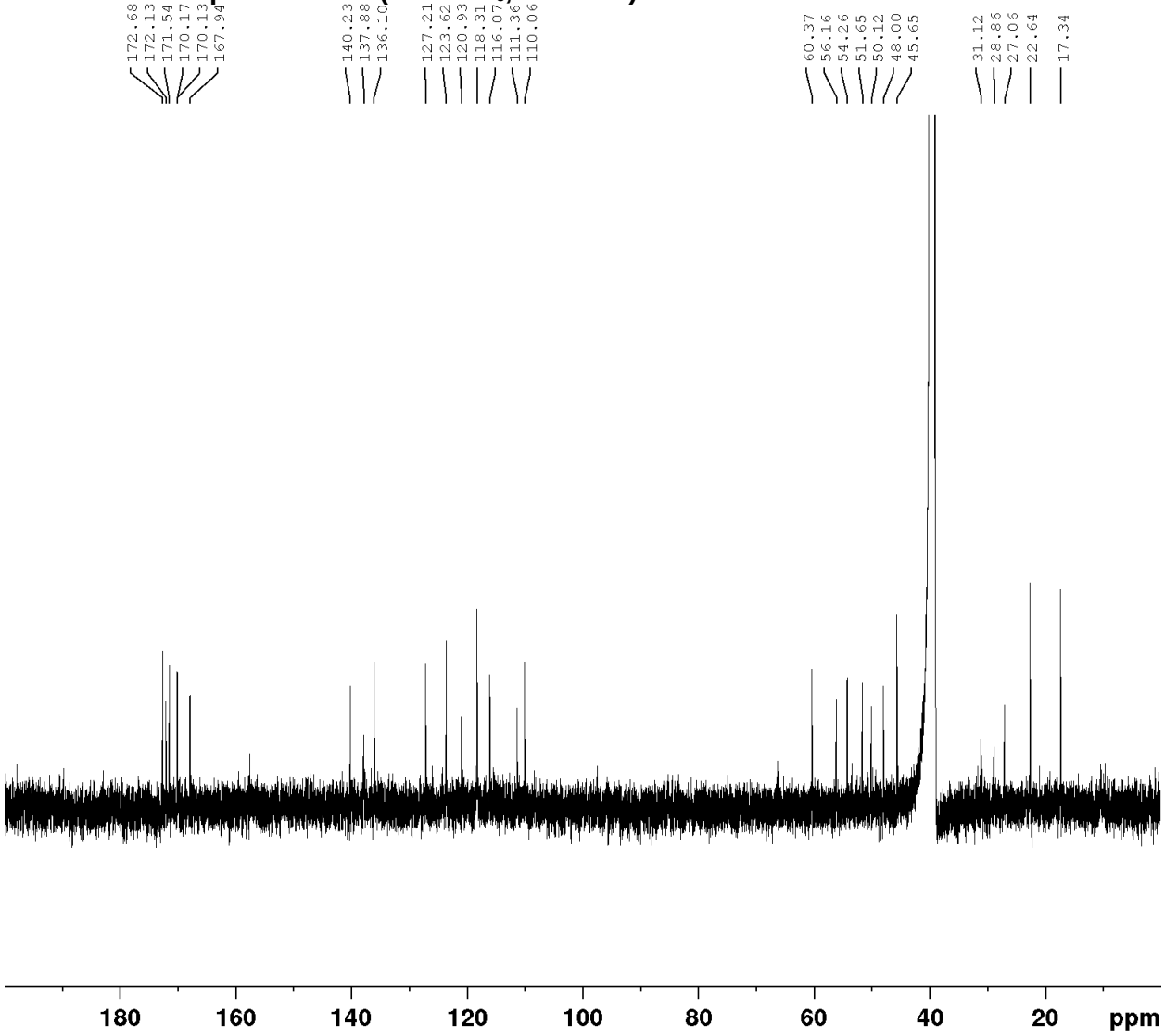
^{13}C NMR of compound S3.13 (DMSO-d_6, 126 MHz)



^{1}H NMR of compound S3.19 (DMSO-d_6, 500 MHz)



^{13}C NMR of compound S3.19 (DMSO-d_6, 126 MHz)



References

[S1] Lakshmi, B. V.; Wefelscheid, U. K.; Kazmaier, U. *Synlett*, **2011** *2011*(03), 345-348.

[S2] Xu, H.; He, J.; Shi, J.; Tan, L.; Qiu, D.; Luo, X.; Li, Y. *J. Am. Chem. Soc.* **2018**, *140*(10), 3555-3559.

[S3] Guo, P.; Wang, K.; Jin, W. J.; Xie, H.; Qi, L.; Liu, X.Y.; Shu, X. Z. *J. Am. Chem. Soc.* **2020** *143*(1), 513-523.

[S4] Dichiarante, V.; Salvaneschi, A.; Protti, S.; Dondi, D.; Fagnoni, M.; Albini, A. *J. Am. Chem. Soc.* **2007**, *129*(51), 15919-15926.

[S5] Tadashi, H. *Yakugaku Zasshi* **1960**, 80, 1399 – 1404.

Chapter 4 Appendix Material

General Methods

See Chapter 2 Appendix Material.

General Procedure A – Peptide synthesis

Peptides were prepared using standard solution phase techniques and the Boc protection strategy, or standard solid phase synthesis using the Fmoc protection strategy. Couplings were performed with either O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate (HBTU) or O-(benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate (TBTU). Boc deprotection was performed using 4 M HCl in dioxane or 1:1 TFA in DCM. Cbz deprotection was performed with palladium on carbon in methanol under an atmosphere of hydrogen. Fmoc deprotection was performed as described using DBU in the presence of octyl mercaptan. N-Acetylation was performed under coupling conditions with acetic acid. Prior to removal of the final protecting group, peptides were purified by column chromatography on SiO_2 eluted with 2-12% MeOH in $CHCl_3$. Peptide identities were verified by HPLC-ESI-MS.

General Procedure B – Acylation of peptides with G1

The peptide (1.5 eq) was dissolved in N,N-DMF (0.2M), neutralized with Hünig's base (1.5 eq) to free the ammonium salt, where necessary, and treated with **G1**. The reaction was stirred at room temperature until complete by TLC or HPLC, and was worked up by partitioning between EtOAc and saturated $NaHCO_3$. The organic phase was washed with saturated $NaHCO_3$ (x2) and brine, then dried over $MgSO_4$ and concentrated. Purification was accomplished by flash chromatography on SiO_2 eluted with 0-12% MeOH in $CHCl_3$.

General Procedure C – Acylation of peptides with G2/G3 then Pictet Spengler Annulation

The peptide (1.5 eq) was dissolved in N,N-DMF (0.2M), neutralized with Hünig's base (1.5 eq) to free the ammonium salt, where necessary, and treated with **G1**. The reaction was stirred at room temperature until complete by TLC or HPLC, and was worked up by partitioning between EtOAc and saturated $NaHCO_3$. The organic phase was washed with saturated $NaHCO_3$ (x2) and brine, then dried over $MgSO_4$ and concentrated. Crude linear precursor was dissolved in a 4:1 mixture of AcOH/H_2O (0.2M) and sturred until HPLC analysis confirmed reaction completion – typicall 12 hours. The volatiles were removed and the residue was rotovapped from acetonitrile (x3) followed by $CHCl_3$ (x3) to remove residual AcOH. Purification was accomplished by flash chromatography on SiO_2 eluted with 0-12% MeOH in $CHCl_3$.

General Procedure D – Palladium catalyzed macrocyclization

For G1 and G2 Macrocycles – $Pd(PPh_3)_4$ as catalyst

The acyclic cinnamyl carbonate was dissolved in dry N,N-DMF (5 mM) and sparged with argon for approximately 15 min. The reaction vessel was briefly opened to introduce $Pd(PPh_3)_4$ (5 mol%) as a solid or stock solution in N,N-DMF, and sparging was continued for approximately 5 min. All reactions proceeded to completion within 60 min at rt, and were halted by passing air through the reaction for several minutes, causing the yellow color of the catalyst to fade. The reaction mixture was then concentrated to dryness, reconstituted in N,N-DMF and purified by preparative RP-HPLC unless otherwise noted.

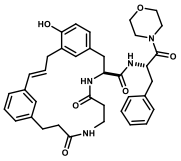
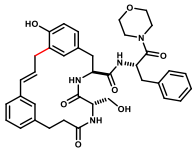
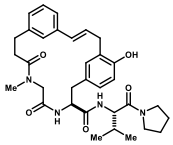
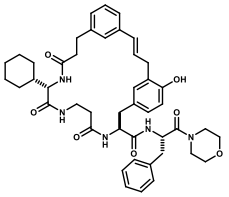
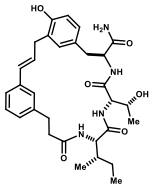
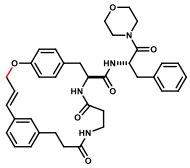
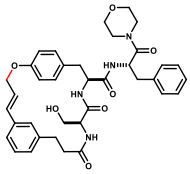
For G3 Macrocycles – $[PdCl(C_3H_5)]_2$ as catalyst

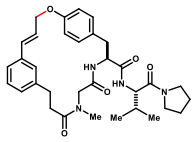
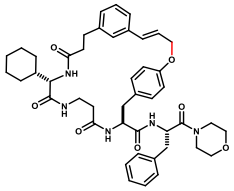
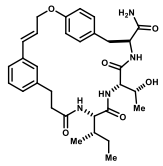
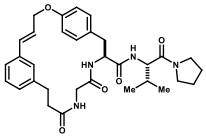
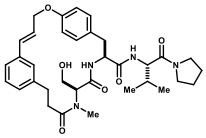
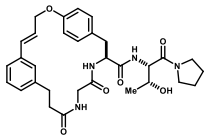
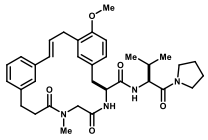
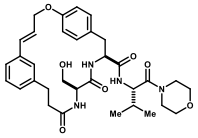
A flask was charged with Pictet-Spengler product (1 eq.), Cs_2CO_3 (2 eq.), and DMF (5 mM in substrate) and sparged for 30 minutes. In a glove bag, a flame-dried Schlenk tube was charged with $[PdCl(C_3H_5)]_2$ (9 mg) and Xantphos (37 mg). Outside of the glovebag, the Schlenk tube was charged with 9 mL of 1:1 THF/DMF, which had been separately sparged for 1 hour. The catalyst solution was stirred for 5 minutes under Ar and 4 mol% Pd was added to the reaction flask via syringe. Reaction progress was monitored by analytical HPLC-MS. After reaction completion, the reaction was diluted with EtOAc and washed with 3x NH4Cl and 1x brine. The organic layer was dried with MgSO4 and concentrated in vacuo. Purificaiton by preparative HPLCby first dissolving the crude in ~1 mL DMSO.

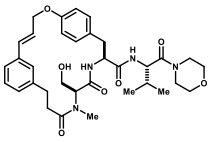
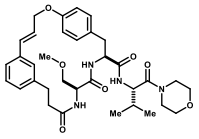
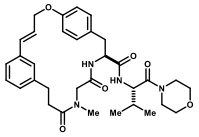
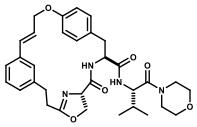
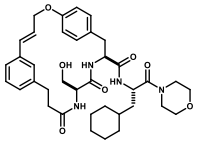
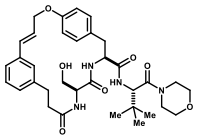
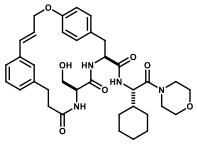
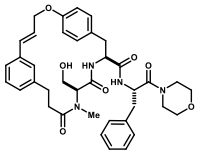
General Procedure E – Acid promoted macrocyclization

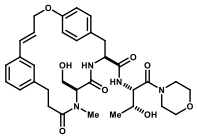
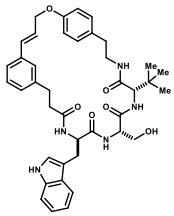
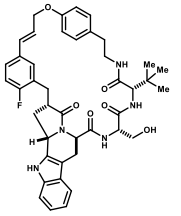
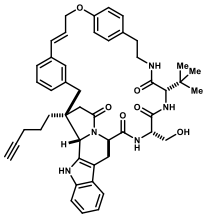
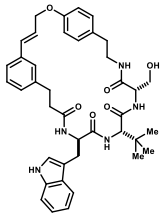
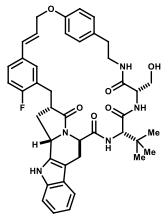
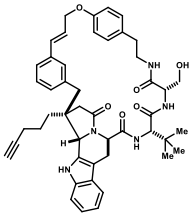
(*Note: Commercial nitromethane should be aged over activated 3 Å molecular sieves for approximately one week. Alternatively, nitromethane may be treated with oven dried Brockmann type 1 neutral alumina overnight.)* The acyclic tert-butyl/iso-butyl carbonate was dissolved or suspended in dry nitromethane (5 mM) under ambient atmosphere, and treated with TFA (5-10%) t room temperature. After consumption of SM the reaction was worked up by partitioning between EtOAc and saturated $NaHCO_3$. The organic phase was washed with saturated $NaHCO_3$ (x2) and brine, then dried over $MgSO_4$ and concentrated. Alternatively, reactions were quenched with Hünig's base (15 eq.) and concentrated. The product mixture was reconstituted in N,N-DMF and purified by preparative RP-HPLC (see individual examples, below). Samples were analyzed by RP-HPLC-UV with detection at 254nm (see individual methods, chromatograms, below).

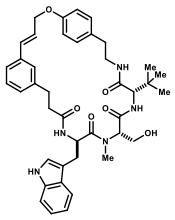
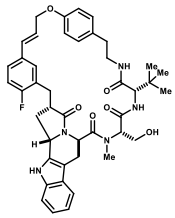
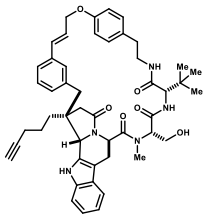
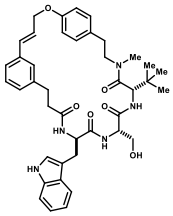
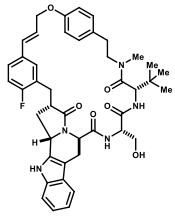
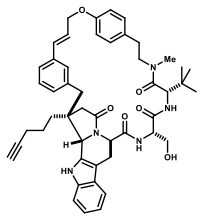
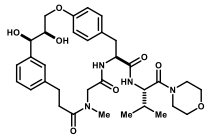
Full Library Data Set

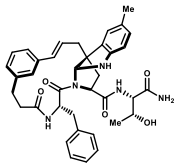
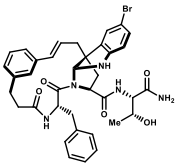
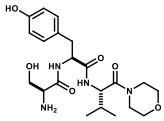
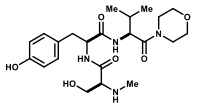
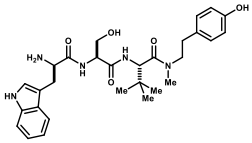
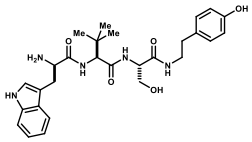
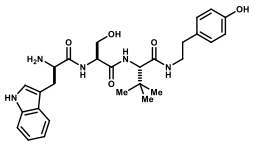
Compound	Structure	RRCK P_{app} ($\times 10^{-6}$ cm/s)	% RRCK Recovery	EPSA Mean	MW	% UV Purity	% ELSD Purity	Observed Mass Ionization
4.6		0.64	98	104	638.3	100	100	M+1, M+Na
4.7		0.52	93	107	654.3	100	100	M+1, M+Na
4.8		0.68	95	104	574.3	73	100	M+1, M+Na, (impurities: 590, 386, 609 m/z)
4.9		0.62	90	111	777.4	30	100	M+1, M+Na, (impurities: 776, 798 m/z)
4.10		0.61	90	116	564.3	100	100	M+1, M+Na, (impurities: 548 m/z)
4.11		1.52	90	85	638.3	100	100	M+1, M+Na
4.12		1.40	81	86	654.3	95	100	M+1, M+Na, (impurities: 695 m/z)

Compound	Structure	RRCK P_{app} ($\times 10^{-6}$ cm/s)	% RRCK Recovery	EPSA Mean	MW	% UV Purity	% ELSD Purity	Observed Mass Ionization
4.13		7.21	88	93	574.3	90	100	M+1, M+Na, (impurities: 279, 615 m/z)
4.14		0.44	86	93	777.4	100	100	M+1, M+Na
4.15		0.63	65	101	564.3	84	21	M+1, M+Na, (impurities: 548, 386, 309, 178 m/z)
4.16		3.16	71	78	560.3	100	100	M+1, Dimer, $M+H_2O$
4.17		9.91	59	82	604.3	100	100	Low concentration, M+1
4.18		0.71	79	102	576.3	100	N/A	M+1, Very low concentration, too low to detect ELSD
4.19		14.77	76	85	588.3	100	100	M+1
4.20		1.73	87	76	606.3	100	100	M+1, Dimer, $M+H_2O$, M-87 m/z

Compound	Structure	RRCK P_{app} ($\times 10^{-6}$ cm/s)	% RRCK Recovery	EPSA Mean	MW	% UV Purity	% ELSD Purity	Observed Mass Ionization
4.21		1.72	101	79	620.3	85	100	M+1, (impurity: 809 m/z)
4.22		5.13	70	70	620.3	100	100	M+1, M+H_2O, (impurities: 279, 534 m/z
4.23		4.90	79	89	590.3	88	100	M+1, (impurities: 368, 450, 1182 m/z)
4.24		21.37	70	69	588.3	53	81	M+1, Dimer, (impurities: 279, 533, 607, 770 m/z)
4.25		5.37	103	80	660.4	100	100	M+1, M-87, (impurity: 467 m/z)
4.26		2.19	74	73	620.3	100	100	M+1, Dimer, (impurity: 378 m/z)
4.27		2.47	61	79	646.3	76	100	M+1, Dimer, M+Na, (impurities: 293, 243, 380, 533 m/z)
4.28		5.80	90	88	668.3	100	100	M+1, M+H_2O, (impurities: 293, 533 m/z)

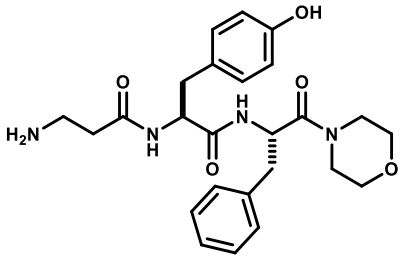
Compound	Structure	RRCK P_{app} ($\times 10^{-6}$ cm/s)	% RRCK Recovery	EPSA Mean	MW	% UV Purity	% ELSD Purity	Observed Mass Ionization
4.29		0.80	73	88	622.3	98	100	M+1, (impurity: 463 m/z)
4.30		1.33	119	119	693.4	100	100	M+1, Dimer
4.31		1.15	80	114	735.3	70	94	M+1, Dimer, (impurities: 751, 753, 767 m/z)
4.32		1.60	110	109	783.4	100	100	M+1, Dimer
4.33		1.63	124	113	693.4	100	100	M+1, Dimer
4.34		1.65	95	110	735.3	77	100	M+1, Dimer, (impurities: 610, 753, 767 m/z)
4.35		1.31	100	101	783.4	100	100	M+1

Compound	Structure	RRCK P_{app} ($\times 10^{-6}$ cm/s)	% RRCK Recovery	EPSA Mean	MW	% UV Purity	% ELSD Purity	Observed Mass Ionization
4.36		1.84	111	111	707.4	73	94	Three M+1 peaks, possibly falling apart
4.37		1.93	100	110	749.4	90	100	M+1, Dimer
4.38		2.22	115	105	797.4	87	100	M+1, Dimer, (impurities: 811, 829 m/z)
4.39		5.51	108	108	707.4	77	97	M+1, (impurities: 729 m/z) sample appears to be falling apart/ streaking
4.40		1.82	87	105	749.4	97	100	M+1, Dimer
4.41		3.76	99	97	797.4	89	100	M+1, Dimer
S4.1		0.63	88	104	624.3	100	100	M+1, Trimer (weak)

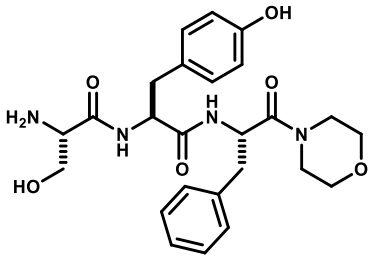
Compound	Structure	RRCK P_{app} ($\times 10^{-6}$ cm/s)	% RRCK Recovery	EPSA Mean	MW	% UV Purity	% ELSD Purity	Observed Mass Ionization
S4.2		3.86	84	90	635.3	100	100	M+1, Dimer
S4.3		4.94	110	97	699.2	93	100	M+1, Dimer, (impurities: 741 m/z)
S4.9		0.25	76	115	436.5	100	100	M+1, dimer
S4.10		0.36	87	92	451	100	100	M+1, sodium salt of dimer observed, low concentration
S4.21		0.30	75	130	537.7	78	100	M+1, dimer, (impurities: 463, 594 m/z)
S4.19		0.21	89	144	523.6	85	100	M+1, dimer, (impurities: 387, 580 m/z)
S4.18		0.17	60	140	523.6	100	Transparent	M+1, sodium salt of dimer observed, low concentration

Experimental Procedures

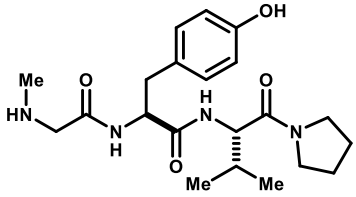
Peptide S4.1
Synthesized according to general procedure A (solution phase).



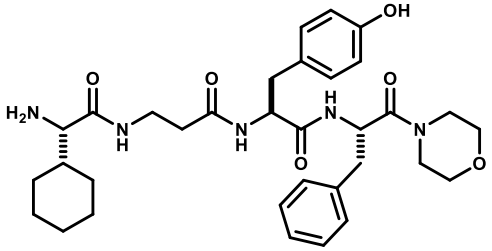
Peptide S4.2
Synthesized according to general prodedure A (solution phase).



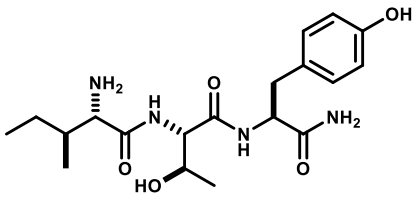
Peptide S4.3
Synthesized according to general procedure A (solution phase).



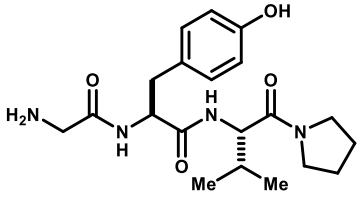
Peptide S4.4
Synthesized according to general procedure A (solution phase).



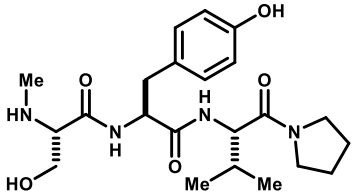
Peptide S4.5
Synthesized according to general procedure A (solid phase).



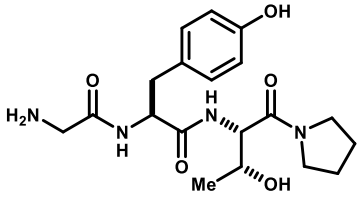
Peptide S4.6
Synthesized according to general procedure A (solution phase).



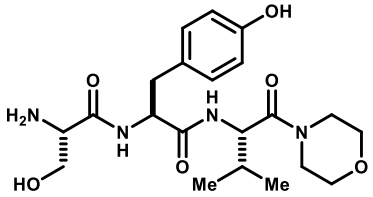
Peptide S4.7
Synthesized according to general procedure A (solution phase).



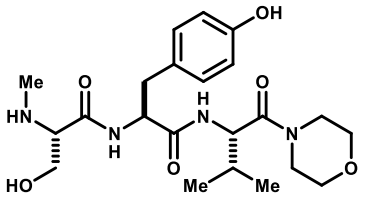
Peptide S4.8
Synthesized according to general procedure A (solution phase).



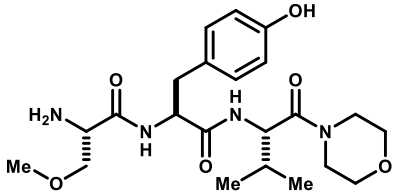
Peptide S4.9
Synthesized according to general procedure A (solution phase).



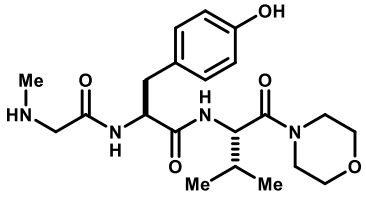
Peptide S4.10
Synthesized according to general procedure A (solution phase).



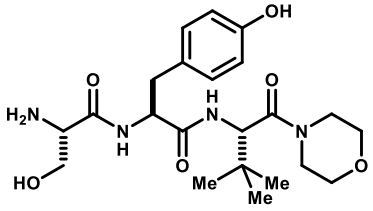
Peptide S4.11
Synthesized according to general procedure A (solution phase).



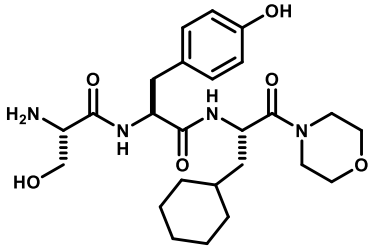
Peptide S4.12
Synthesized according to general procedure A (solution phase).



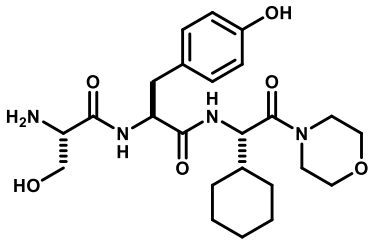
Peptide S4.13
Synthesized according to general procedure A (solution phase).



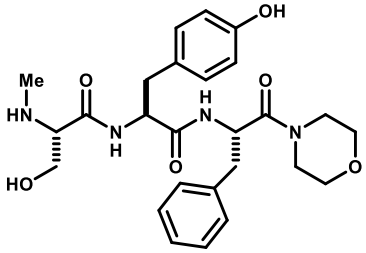
Peptide S4.14
Synthesized according to general procedure A (solution phase).



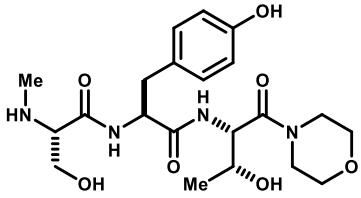
Peptide S4.15
Synthesized according to general procedure A (solution phase).



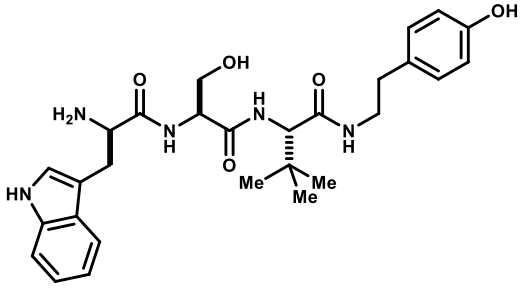
Peptide S4.16
Synthesized according to general procedure A (solution phase).



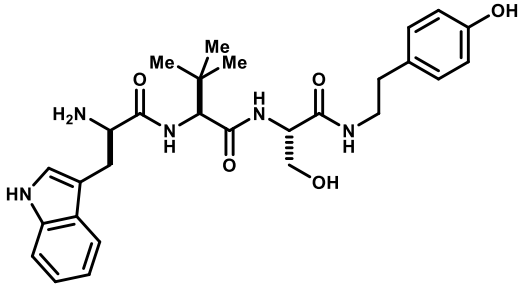
Peptide S4.17
Synthesized according to general procedure A (solution phase).



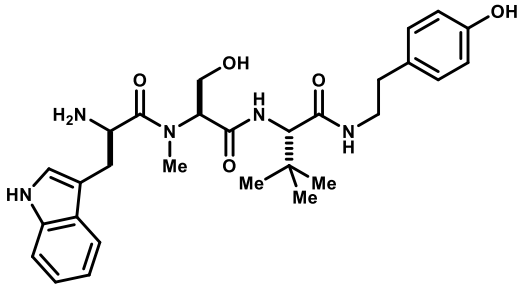
Peptide S4.18
Synthesized according to general procedure A (solution phase).



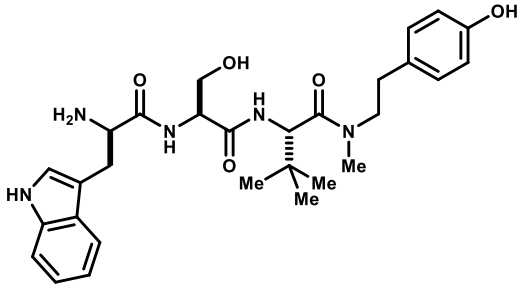
Peptide S4.19
Synthesized according to general procedure A (solution phase).



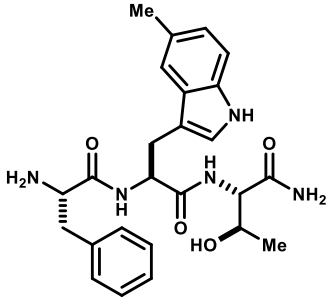
Peptide S4.20
Synthesized according to general procedure A (solution phase).



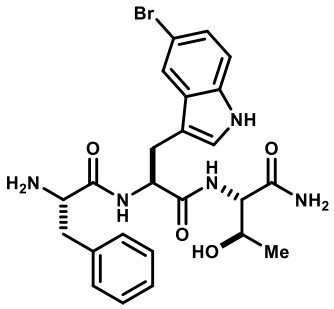
Peptide S4.21
Synthesized according to general procedure A (solution phase).



Peptide S4.22
Synthesized according to general procedure A (solid phase).

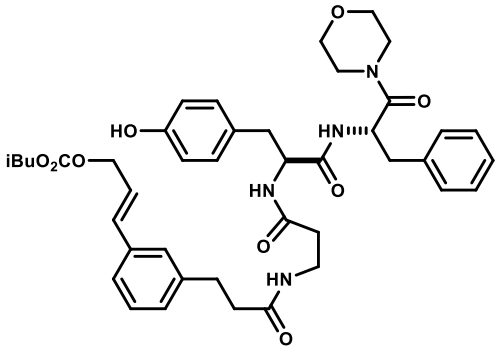


Peptide S4.23
Synthesized according to general procedure A (solid phase).

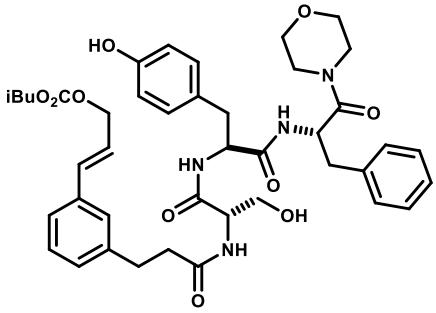


Acyclic Product S4.24

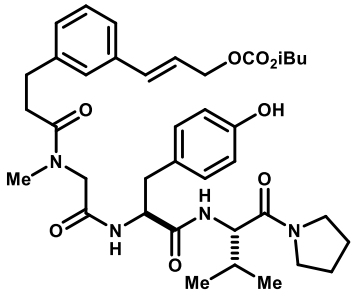
Synthesized according to general procedure B and **G1** using **S4.1**.



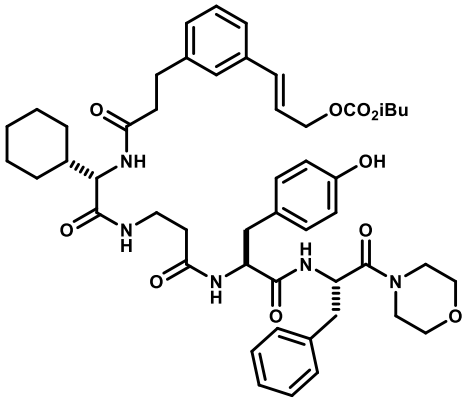
Acyclic Product S4.25
Synthesized according to general procedure B and **G1** using **S4.2**.



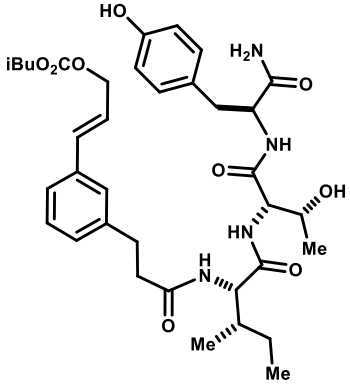
Acyclic Product S4.26
Synthesized according to general procedure B and **G1** using **S4.3**.



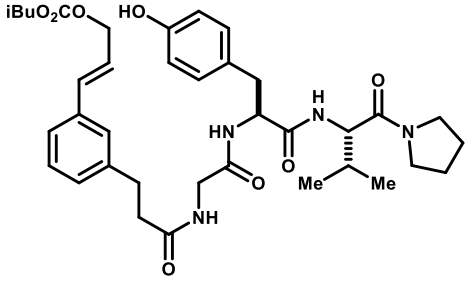
Acyclic Product S4.27
Synthesized according to general procedure B and **G1** using **S4.4**.



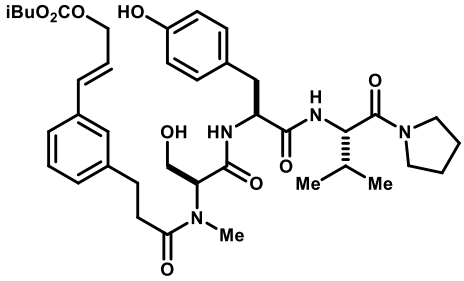
Acyclic Product S4.28
Synthesized according to general procedure B and **G1** using **S4.5**.



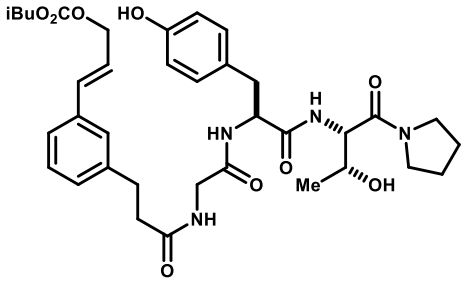
Acyclic Product S4.29
Synthesized according to general procedure B and **G1** using **S4.6**.



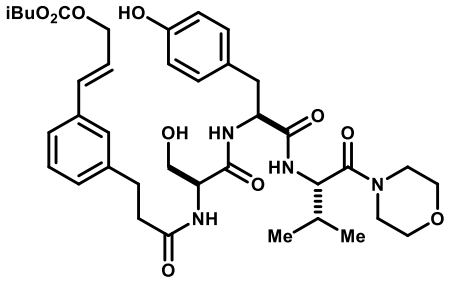
Acyclic Product S4.30
Synthesized according to general procedure B and **G1** using **S4.7**.



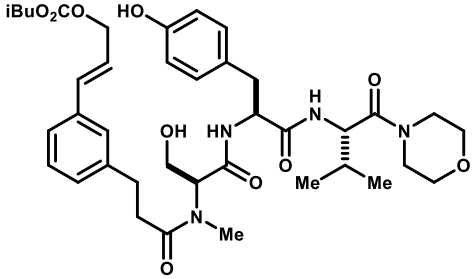
Acyclic Product S4.31
Synthesized according to general procedure B and **G1** using **S4.8**.



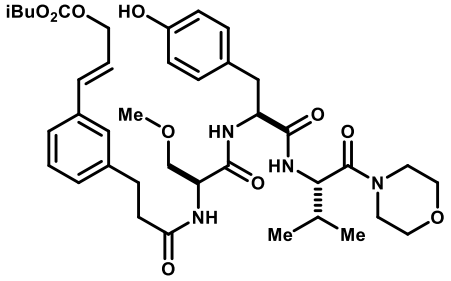
Acyclic Product S4.32
Synthesized according to general procedure B and **G1** using **S4.9**.



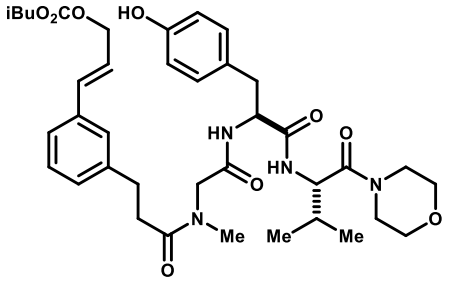
Acyclic Product S4.33
Synthesized according to general procedure B and **G1** using **S4.10**.



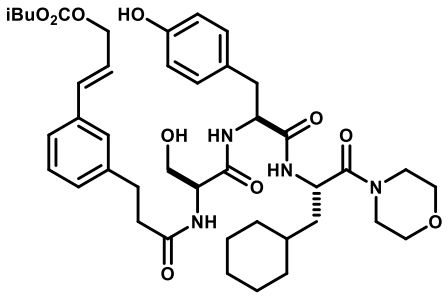
Acyclic Product S4.34
Synthesized according to general procedure B and **G1** using **S4.11**.



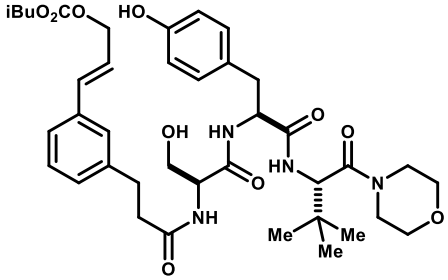
Acyclic Product S4.35
Synthesized according to general procedure B and **G1** using **S4.12**



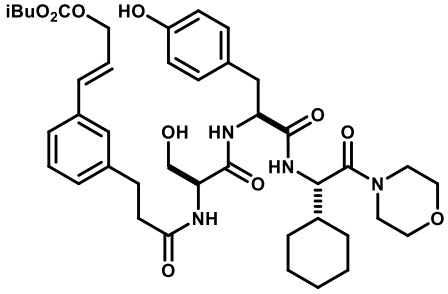
Acyclic Product S4.36
Synthesized according to general procedure B and **G1** using **S4.13**.



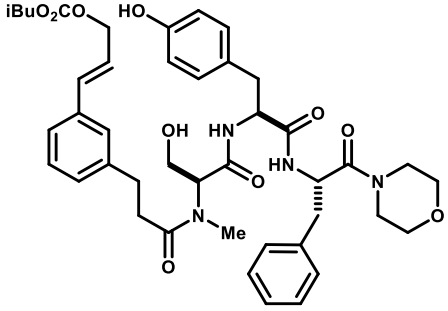
Acyclic Product S4.37
Synthesized according to general procedure B and **G1** using **S4.14**.



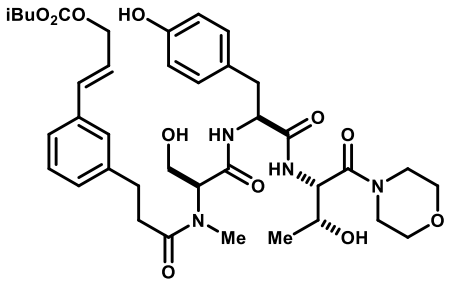
Acyclic Product S4.38
Synthesized according to general procedure B and **G1** using **S4.15**



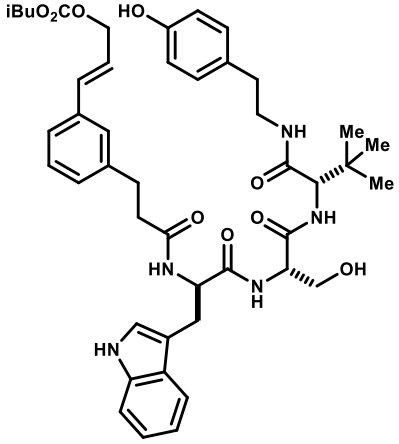
Acyclic Product S4.39
Synthesized according to general procedure B and **G1** using **S4.16**.



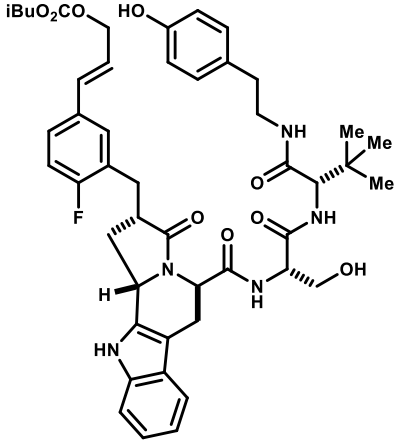
Acyclic Product S4.40
Synthesized according to general procedure B and **G1** using **S4.17**.



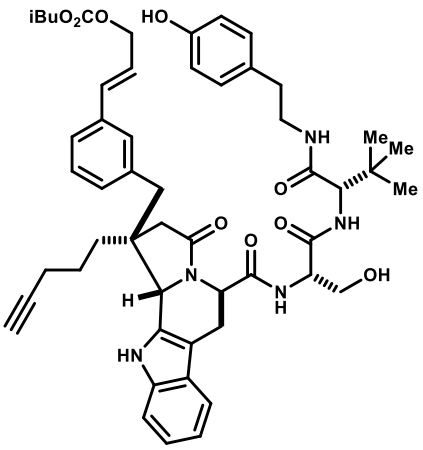
Acyclic Product S4.41
Synthesized according to general procedure B and **G1** using **S4.18**.



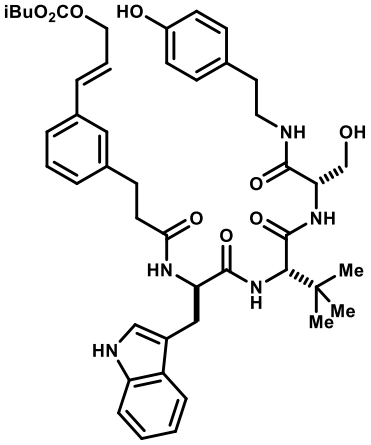
Acyclic Product S4.42
Synthesized according to general procedure C and **G2** using **S4.18** (54%, over 2 steps).



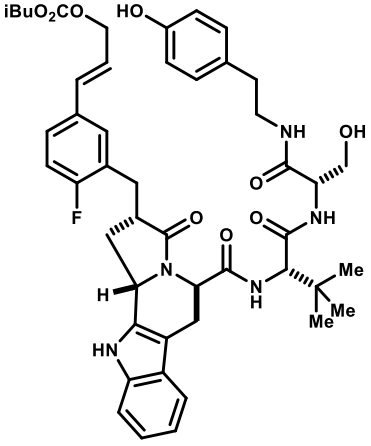
Acyclic Product S4.43
Synthesized according to general procedure C and **G3** using **S4.18** (60%, over 2 steps).



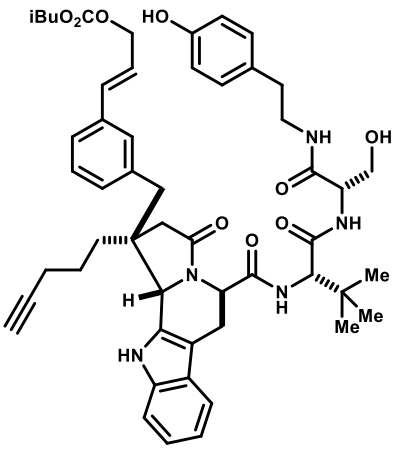
Acyclic Product S4.44
Synthesized according to general procedure B and **G1** using **S4.19**.



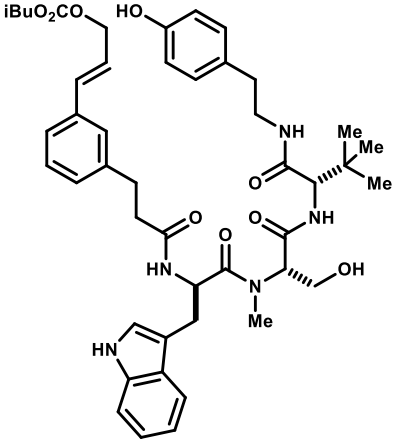
Acyclic Product S4.45
Synthesized according to general procedure C and **G2** using **S4.19** (45%, over 2 steps).



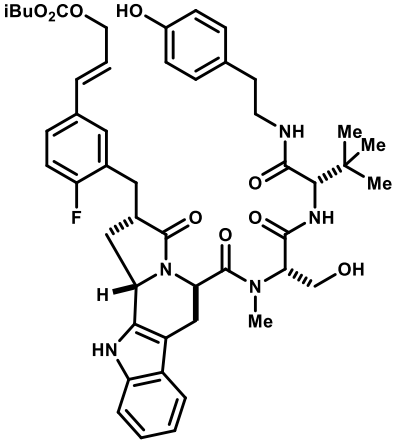
Acyclic Product S4.46
Synthesized according to general procedure C and **G3** using **S4.19** (54%, over 2 steps).



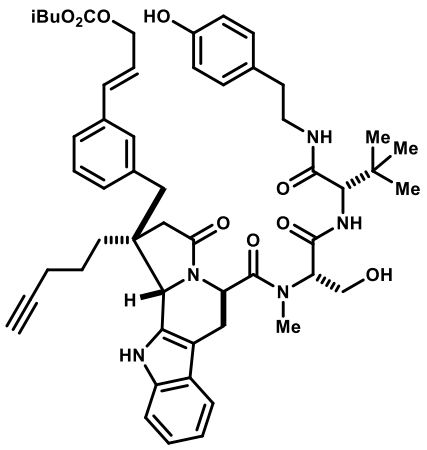
Acyclic Product S4.47
Synthesized according to general procedure B and **G1** using **S4.20**.



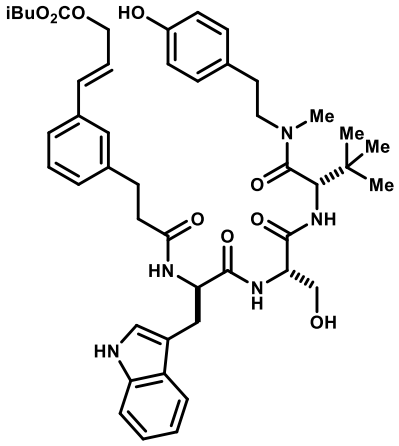
Acyclic Product S4.48
Synthesized according to general procedure C and **G2** using **S4.20** (34%, over 2 steps).



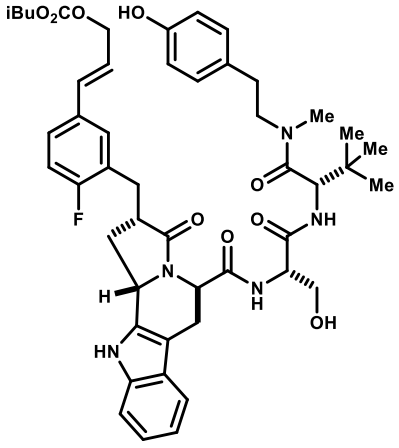
Acyclic Product S4.49
Synthesized according to general procedure C and **G3** using **S4.20** (38%, over 2 steps).



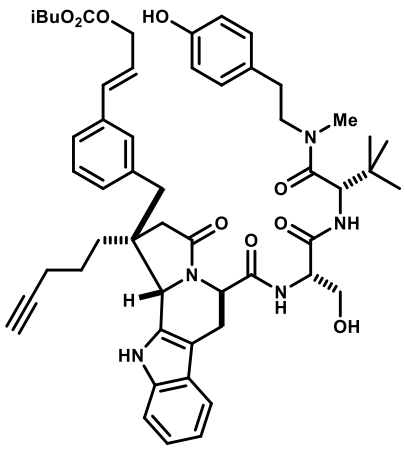
Acyclic Product S4.50
Synthesized according to general procedure B and **G1** using **S4.21**.



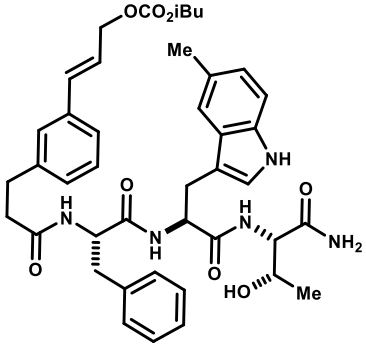
Acyclic Product S4.51
Synthesized according to general procedure C and **G2** using **S4.21** (75%, over 2 steps).



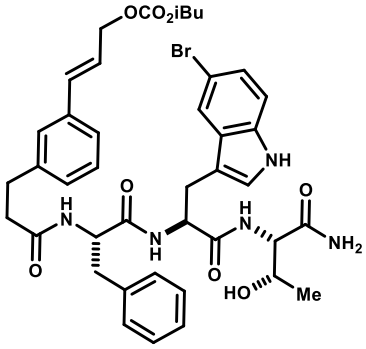
Acyclic Product S4.52
Synthesized according to general procedure C.



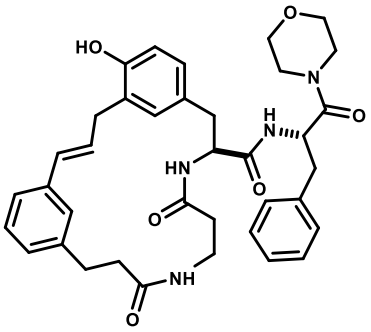
Acyclic Product S4.53
Synthesized according to general procedure B and **G1** using **S4.22**.

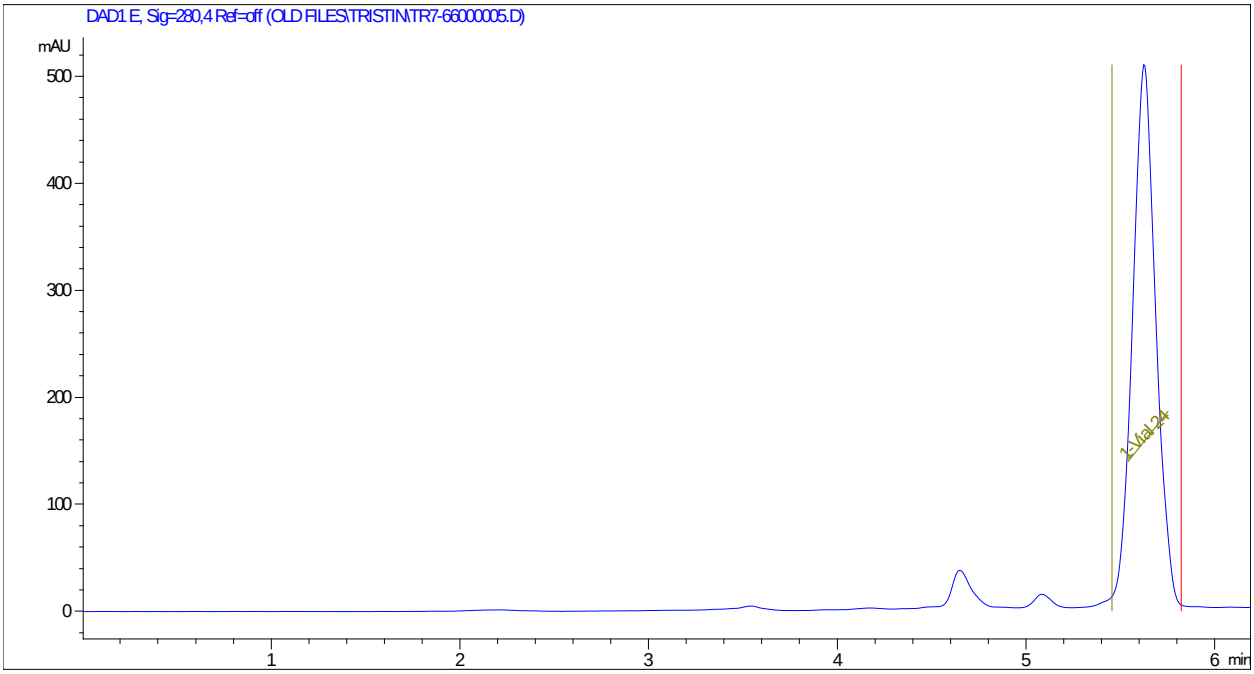


Acyclic Product S4.54
Synthesized according to general procedure B and **G2** using **S4.23**.



Macrocycle 4.6
Synthesized according to general procedure **E** using **S4.24**.

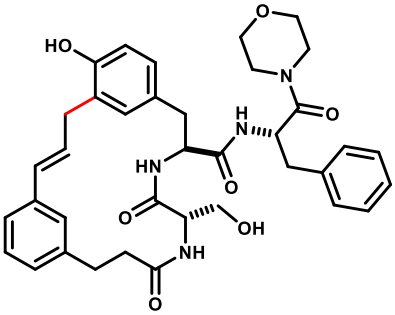


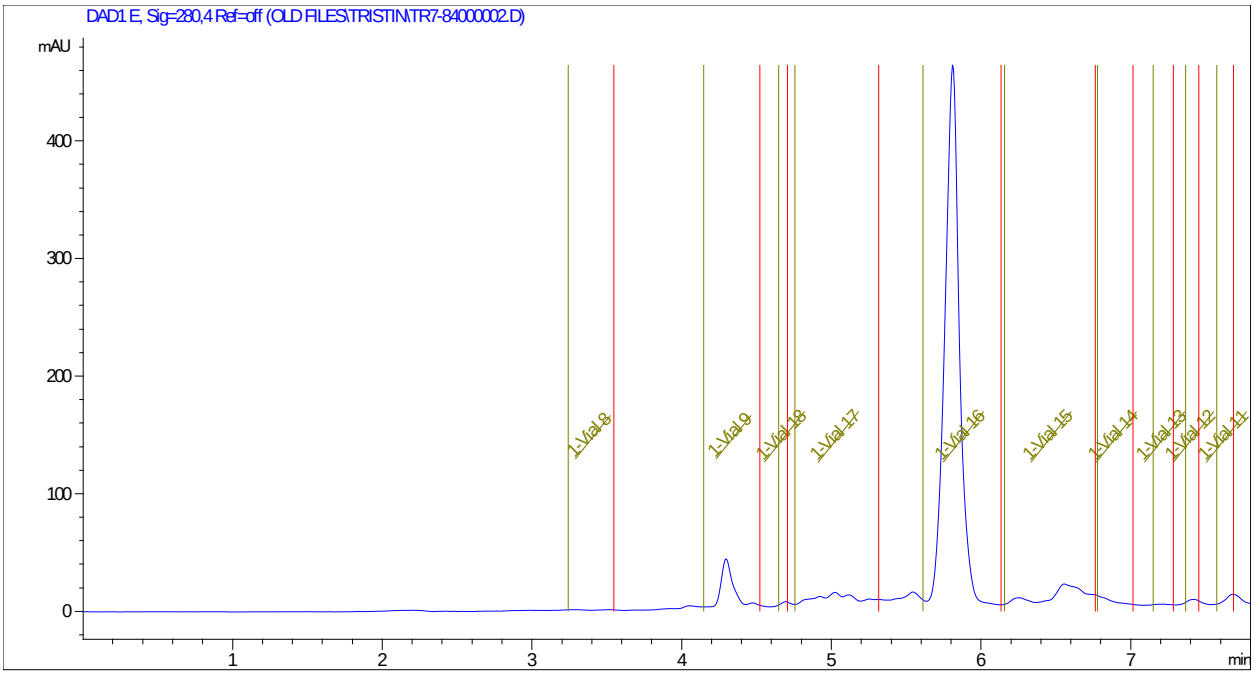


Peparative HPLC method
Column: Waters Sunfire™ C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.7
Synthesized according to general procedure **E** using **S4.25**.

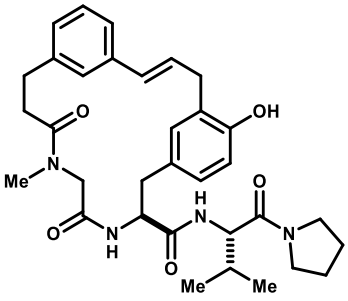


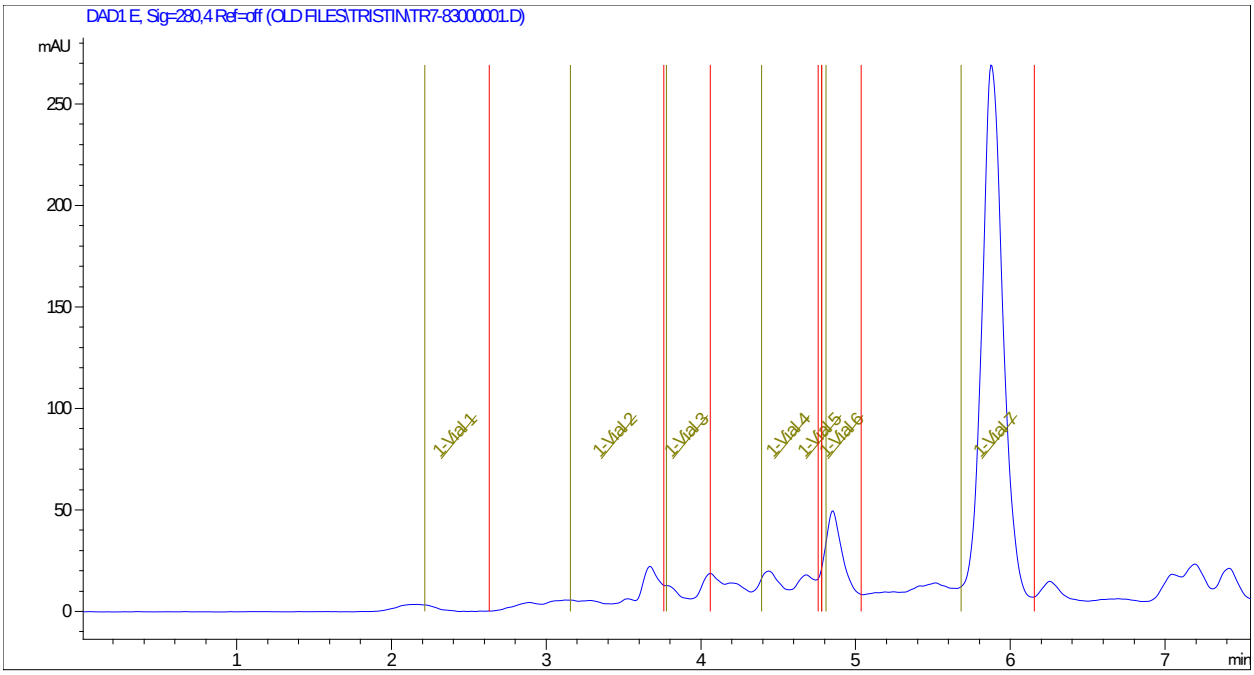


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	50
14	70

Macrocycle 4.8
Synthesized according to general procedure **E** using **S4.26**.

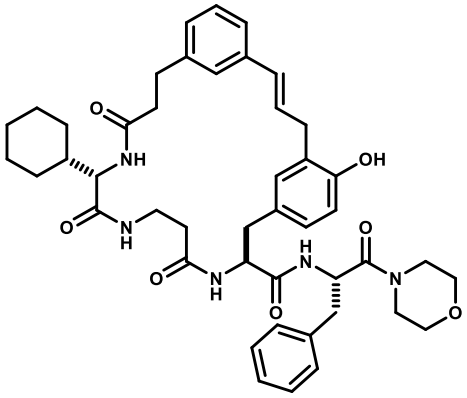


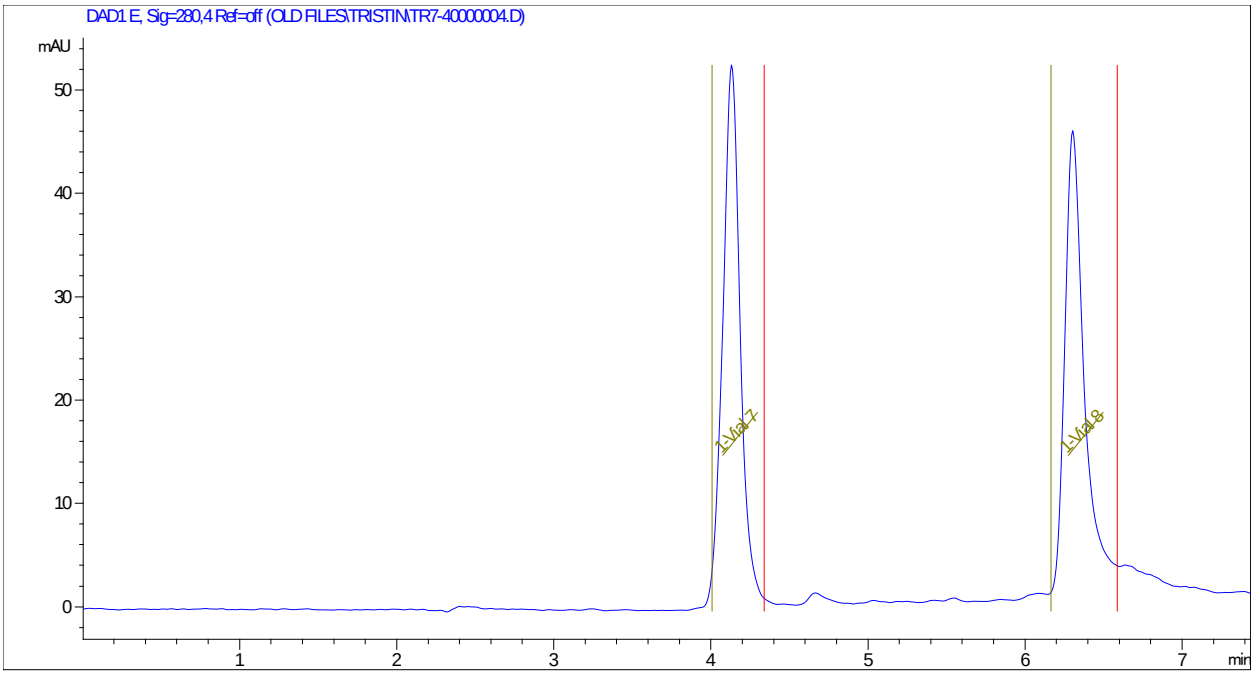


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	50
14	70

Macrocycle 4.9
Synthesized according to general procedure **E** using **S4.27**.

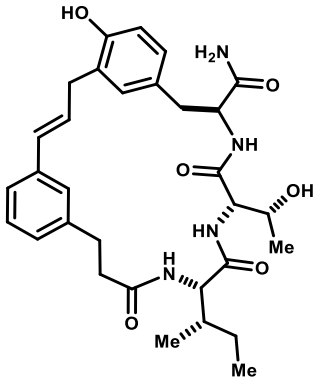




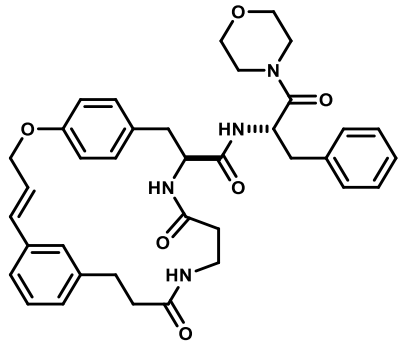
Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

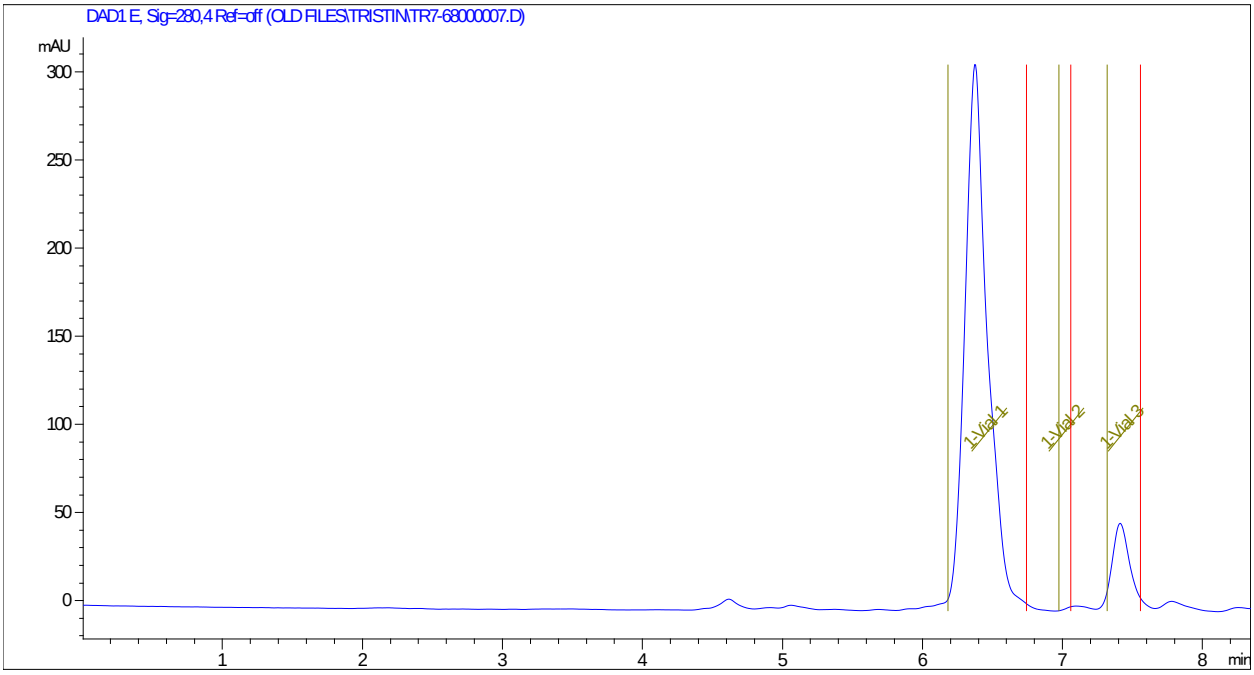
Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.10
Synthesized according to general procedure **E** using **S4.28**.



Macrocycle 4.11
Synthesized according to general procedure **D** using **S4.24**

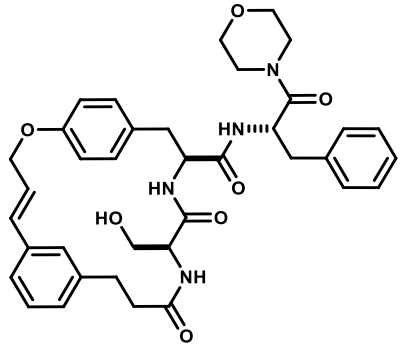


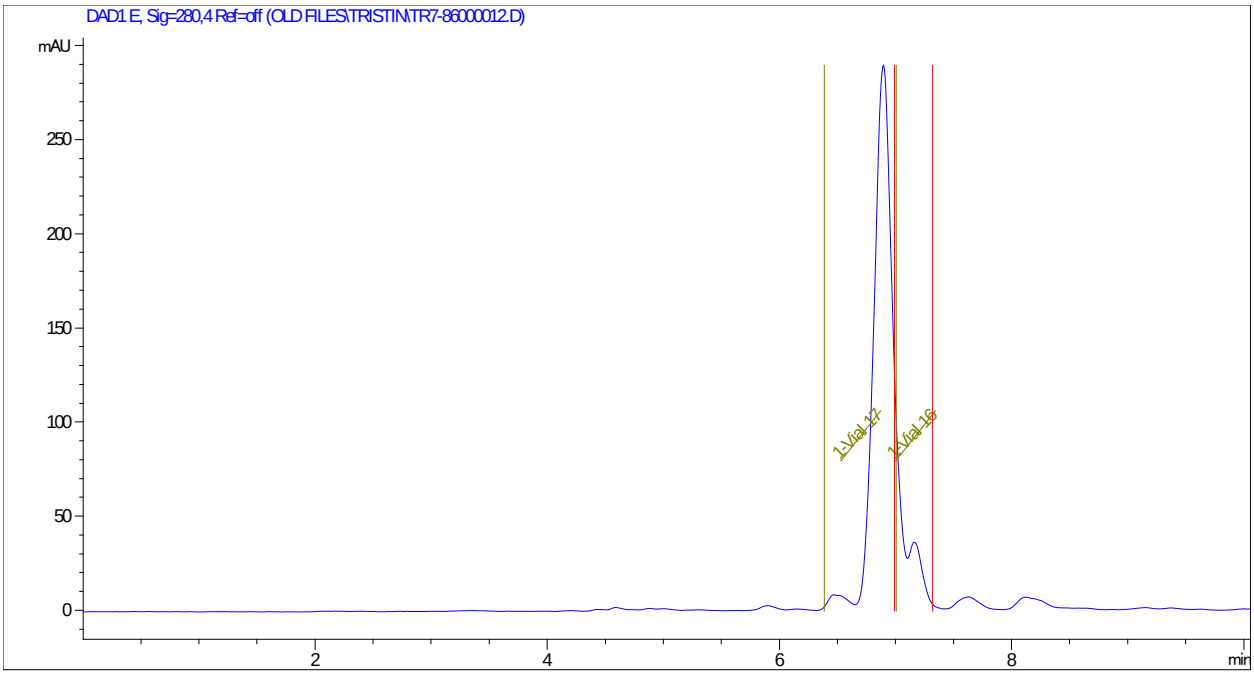


Peparative HPLC method
Column: Waters Sunfire™ C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.12
Synthesized according to general procedure **D** using **S4.25**.

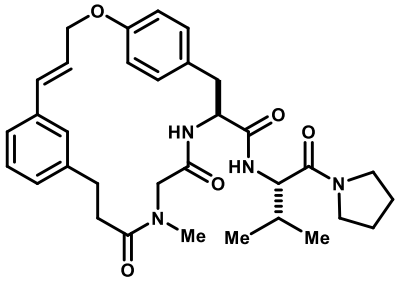


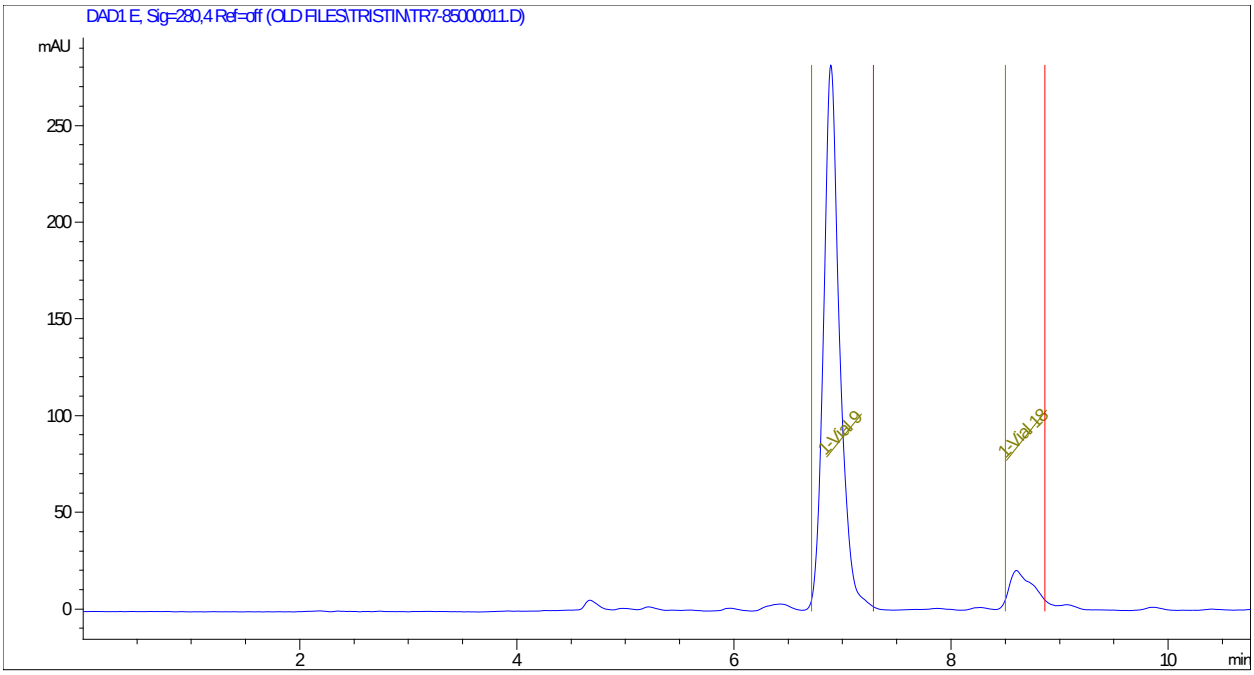


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.13
Synthesized according to general procedure **D** using **S4.26**.

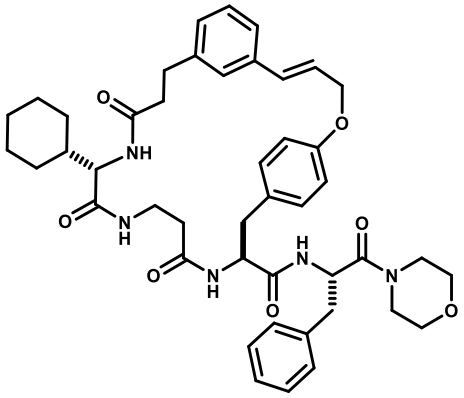




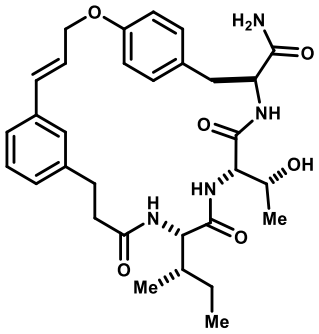
Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

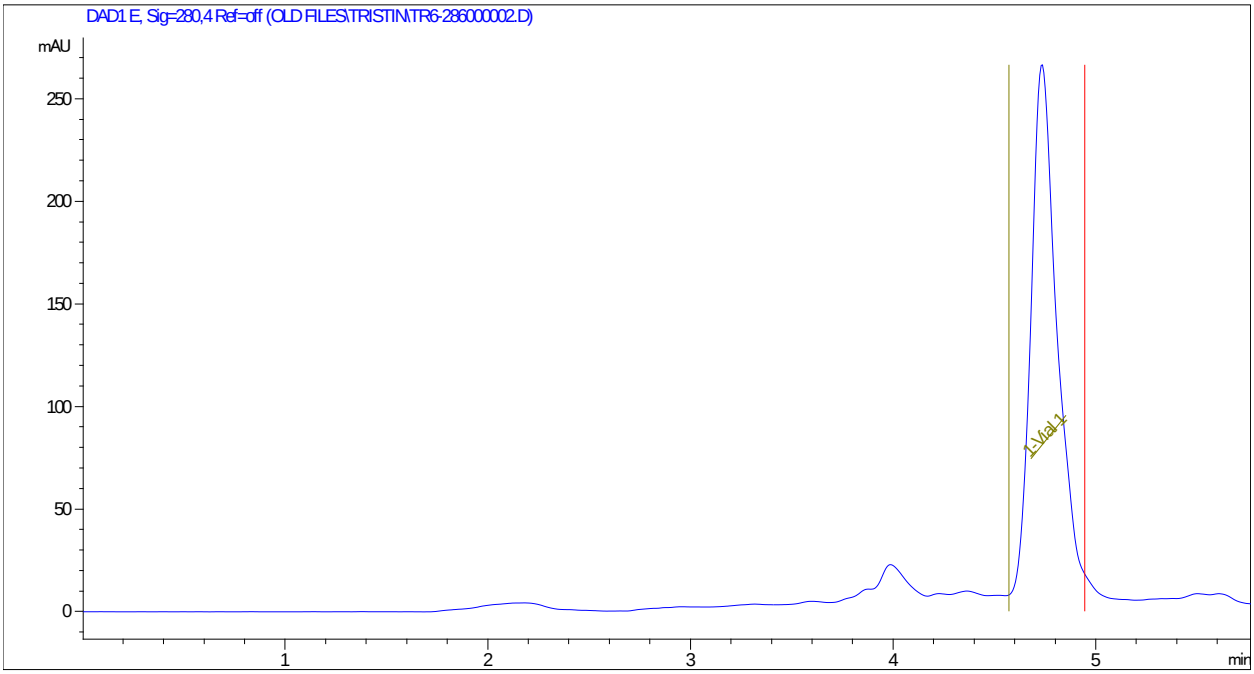
Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.14
Synthesized according to general procedure **D** using **S4.27**



Macrocycle 4.15
Synthesized according to general procedure **D** using **S4.28**.

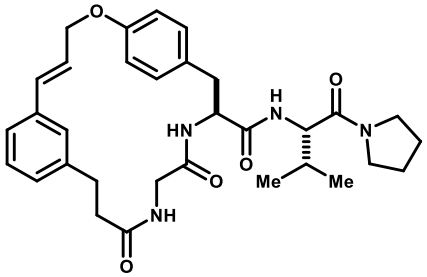


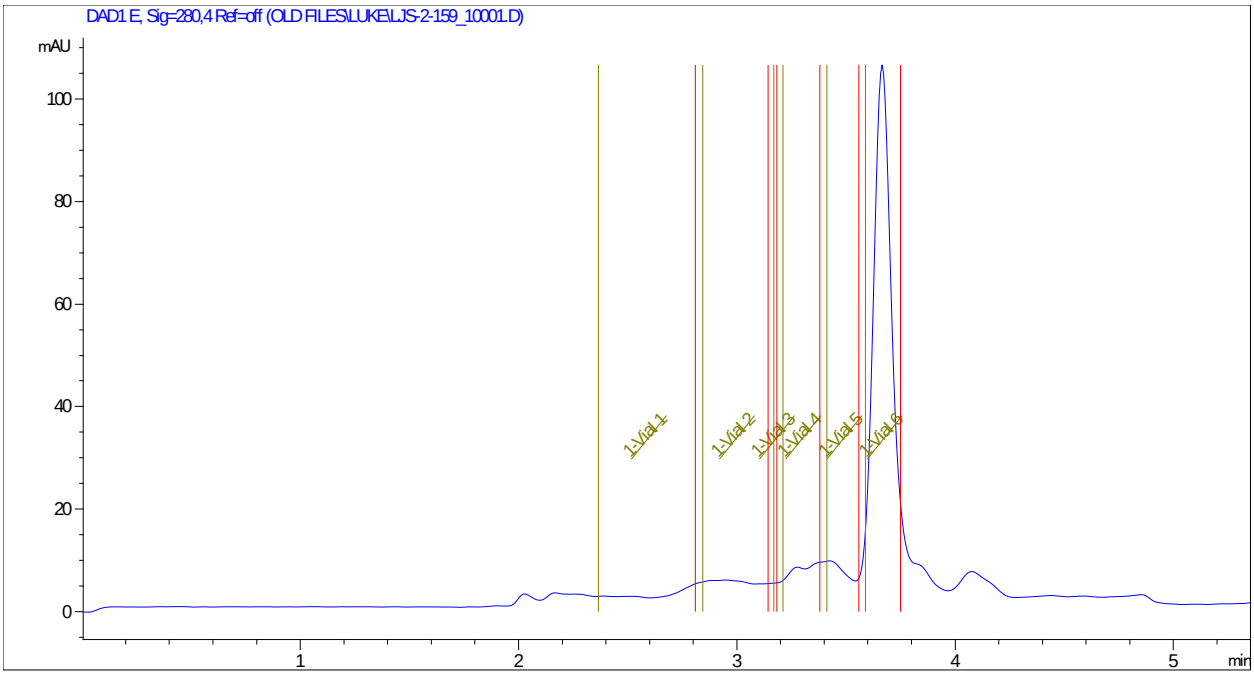


Peparative HPLC method
Column: Waters Sunfire™ C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.16
Synthesized according to general procedure **D S4.29**.

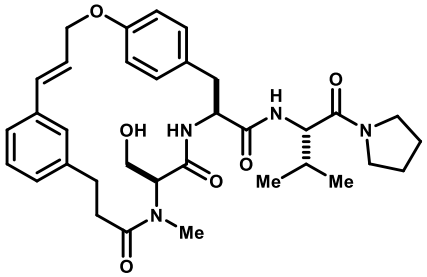


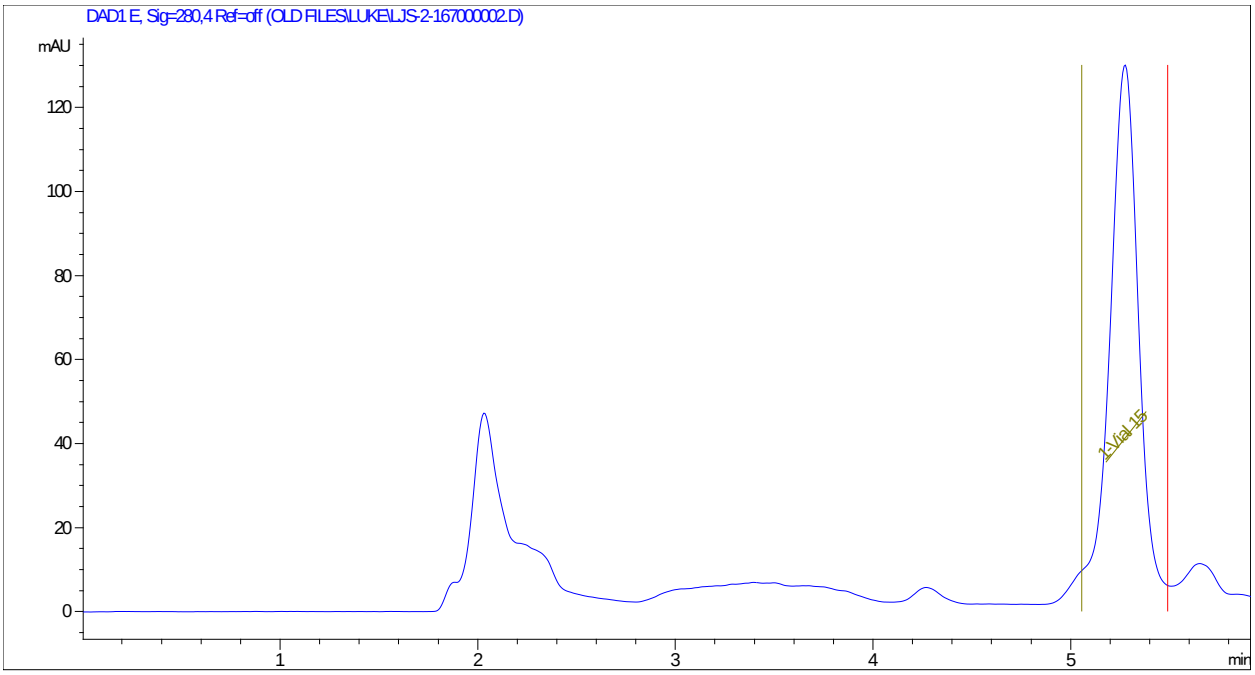


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	55
14	70

Macrocycle 4.17
Synthesized according to general procedure **D** using **S4.30**.

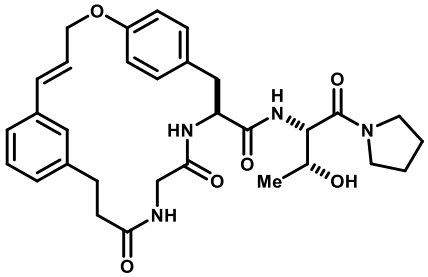


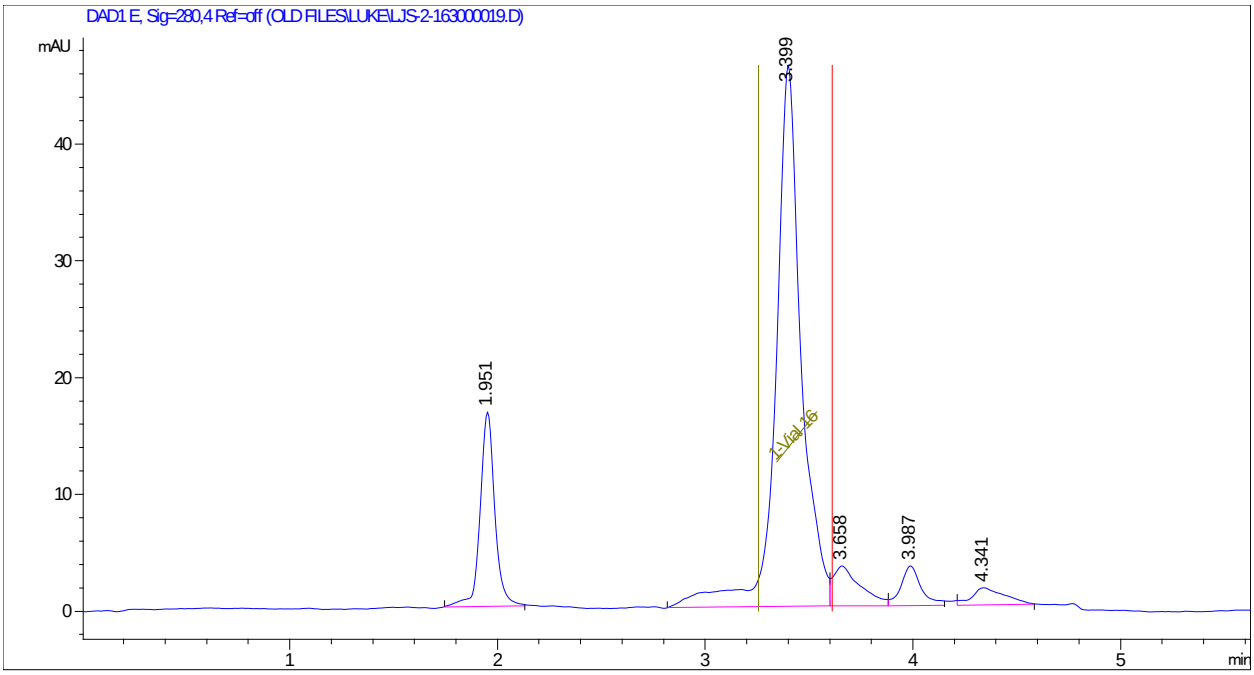


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	55
14	70

Macrocycle 4.18
Synthesized according to general procedure **D** using **S4.31**.

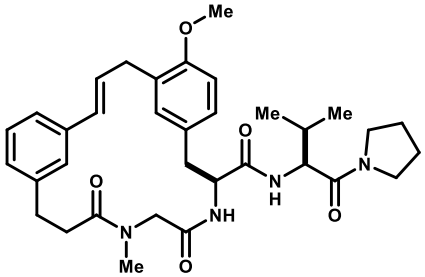


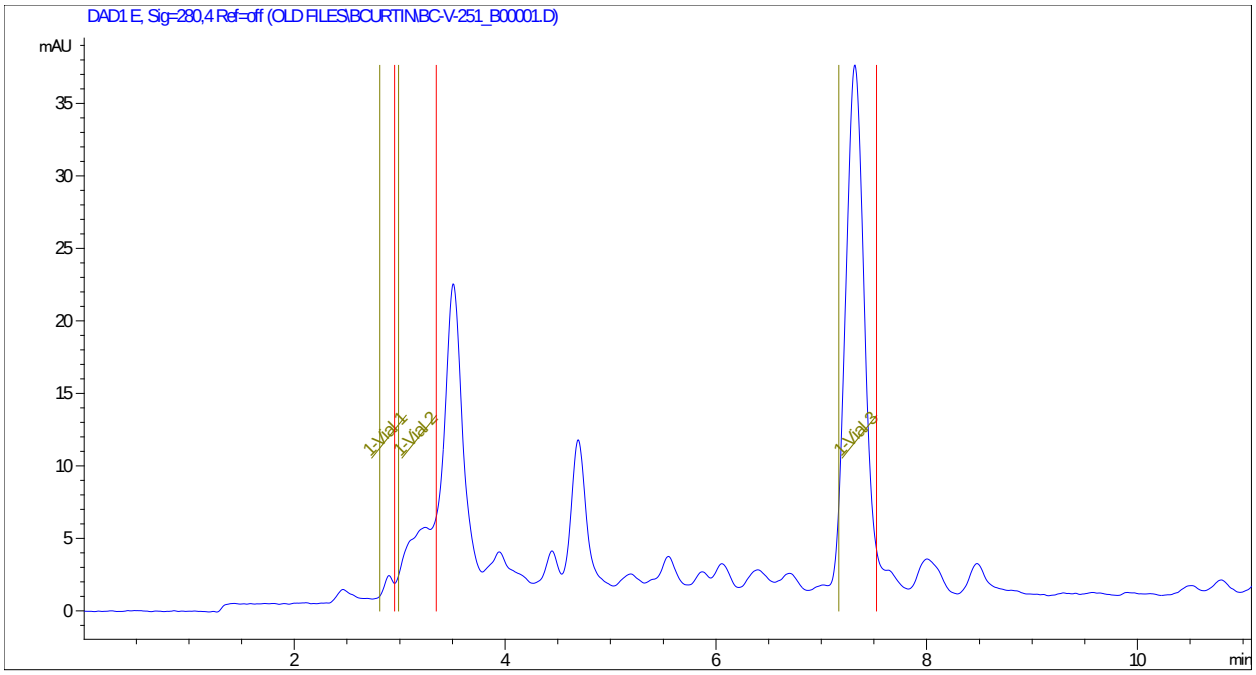


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	55
14	70

Macrocycle 4.19
Synthesized according to general procedure **D** using macrocycle **4.8**.

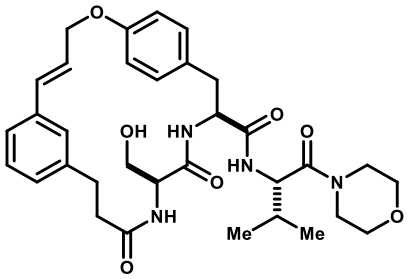




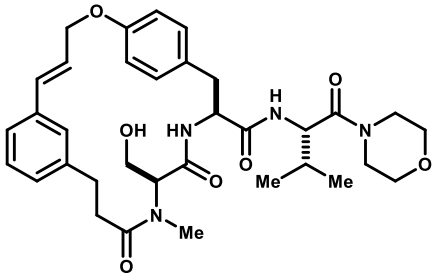
Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.20
Synthesized according to general procedure **D** using **S4.32**.

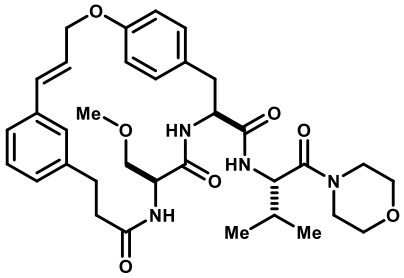


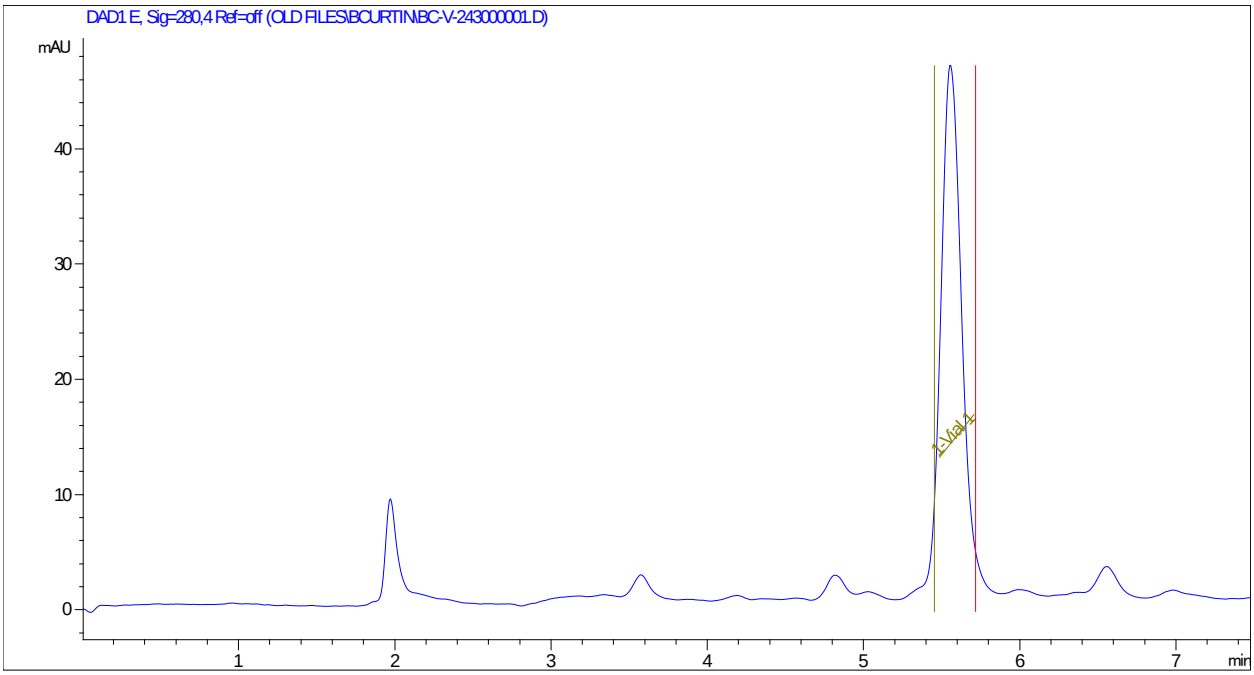
Macrocycle 4.21
Synthesized according to general procedure **D** using **S4.33**.



Macrocycle 4.22

Synthesized according to general procedure **D** using **S4.34**.

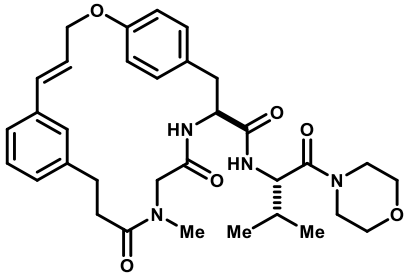




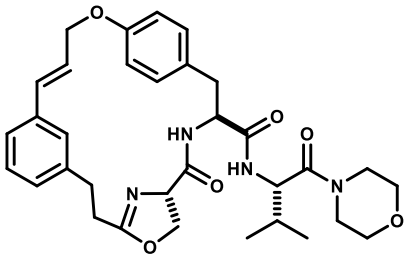
Peparative HPLC method
Column: Waters Sunfire™ C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	55
14	70

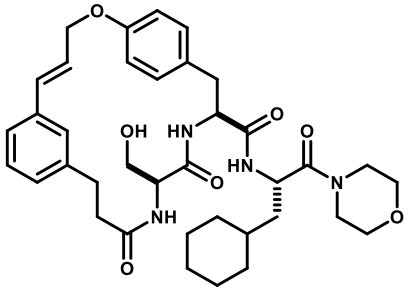
Macrocycle 4.23
Synthesized according to general procedure **D** using **S4.35**.

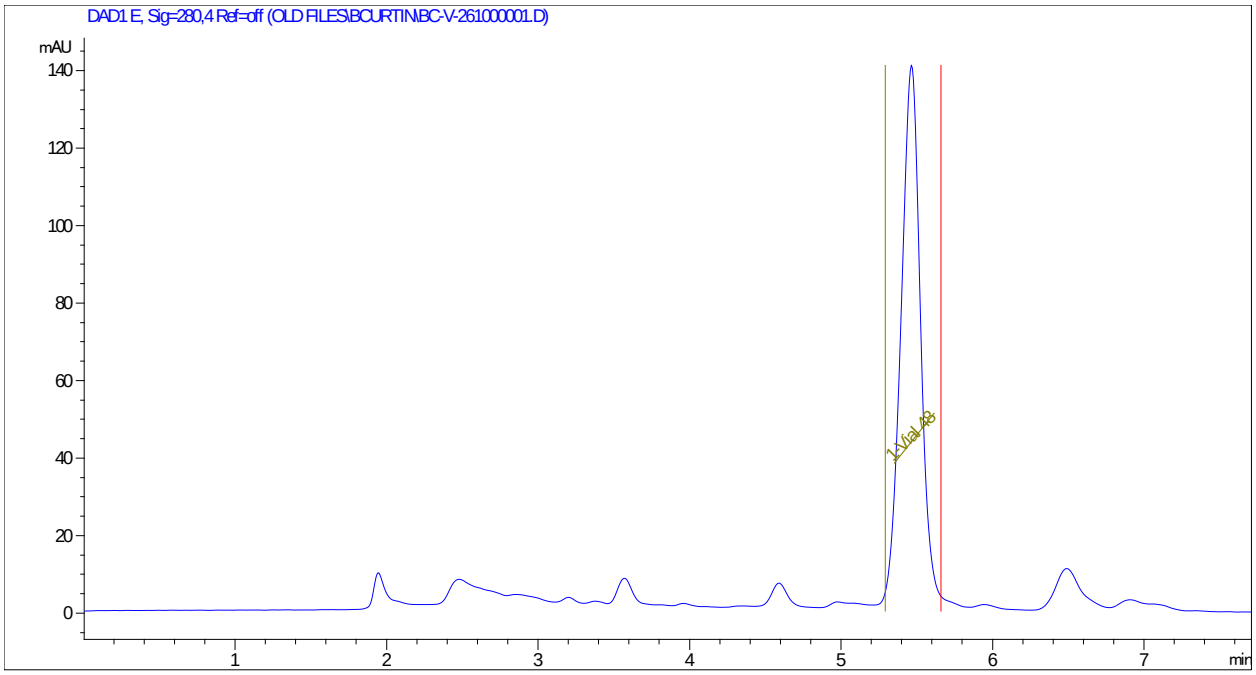


Macrocycle 4.24



Macrocycle 4.25
Synthesized according to general procedure **D** using **S4.36**.

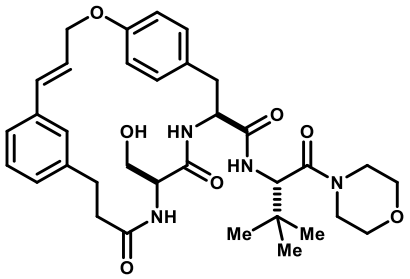


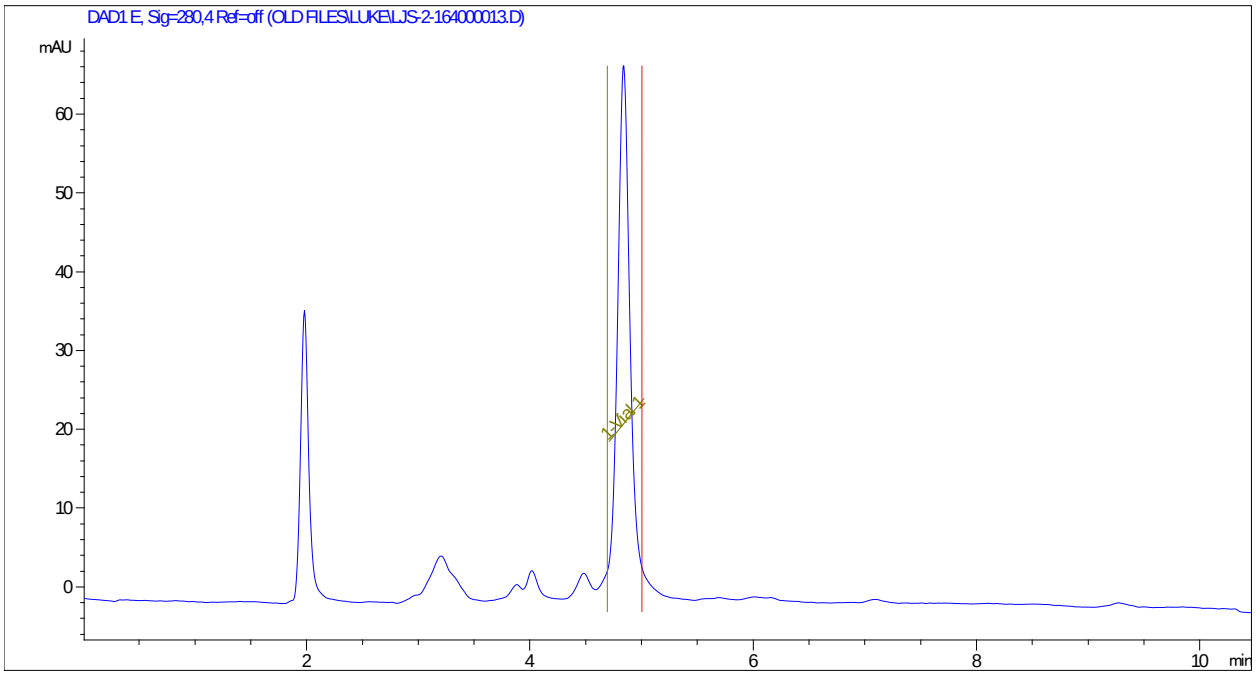


Peparative HPLC method
Column: Waters Sunfire™ C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	65
14	70

Macrocycle 4.26
Synthesized according to general procedure **D** using **S4.37**.

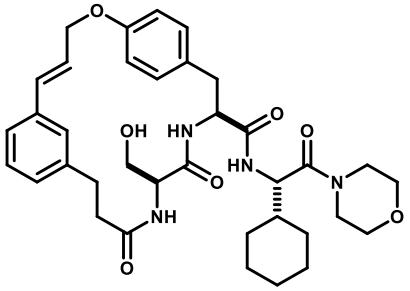


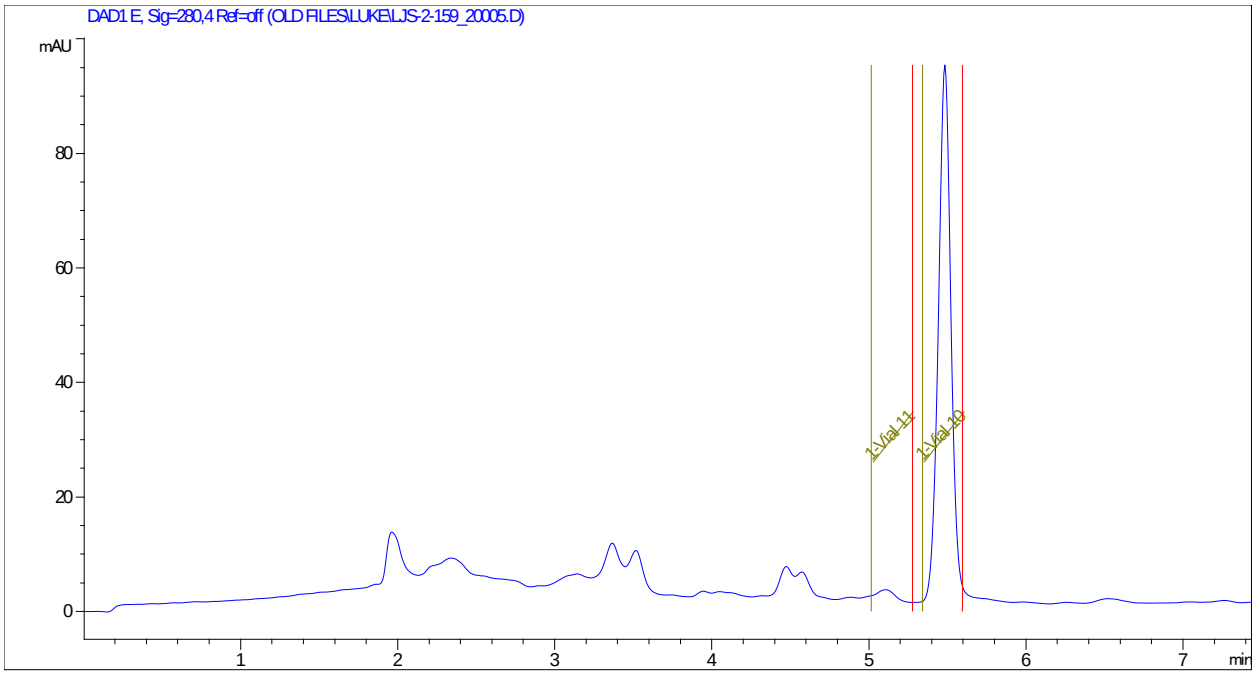


Peparative HPLC method
Column: Waters Sunfire™ C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	70

Macrocycle 4.27
Synthesized according to general procedure **D** using **S4.38**.

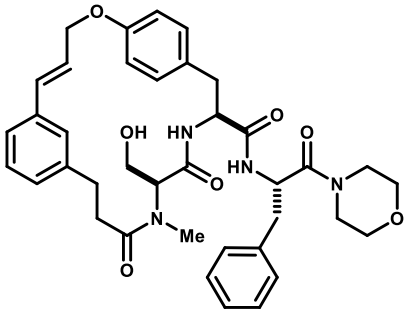


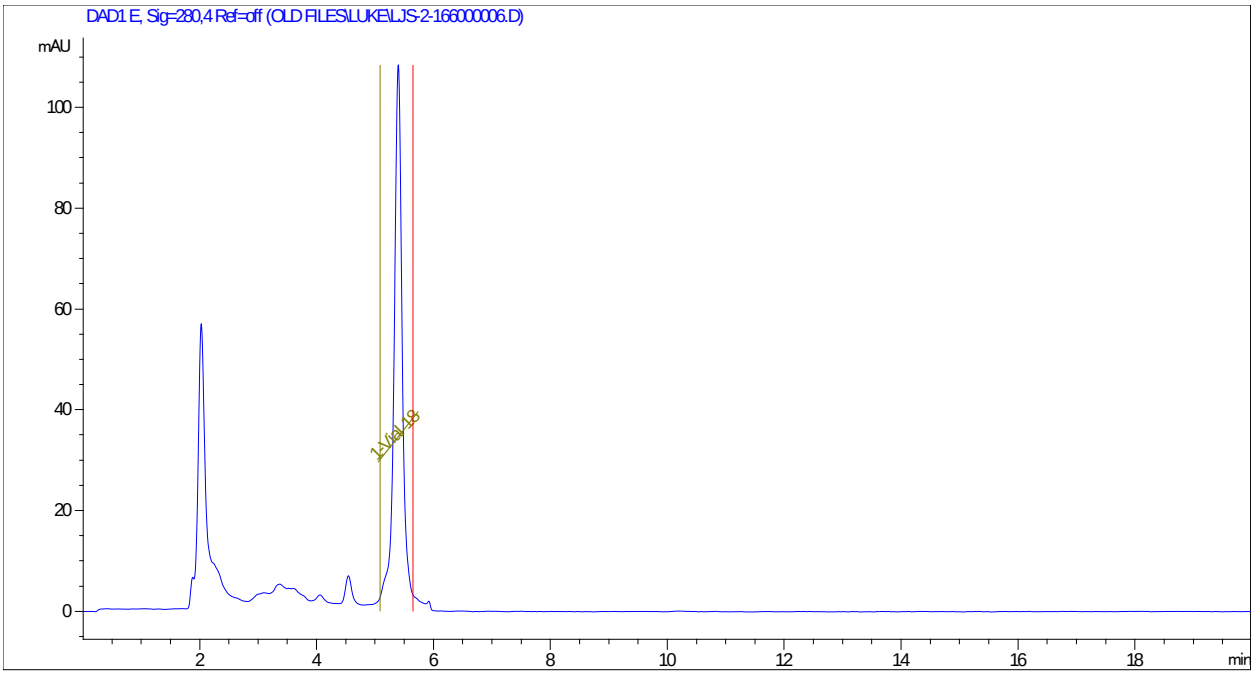


Peparative HPLC method
Column: Waters Sunfire™ C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	55
14	65

Macrocycle 4.28
Synthesized according to general procedure **D** using **S4.39**.

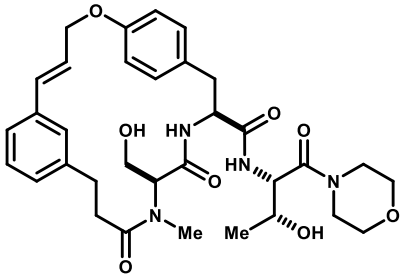


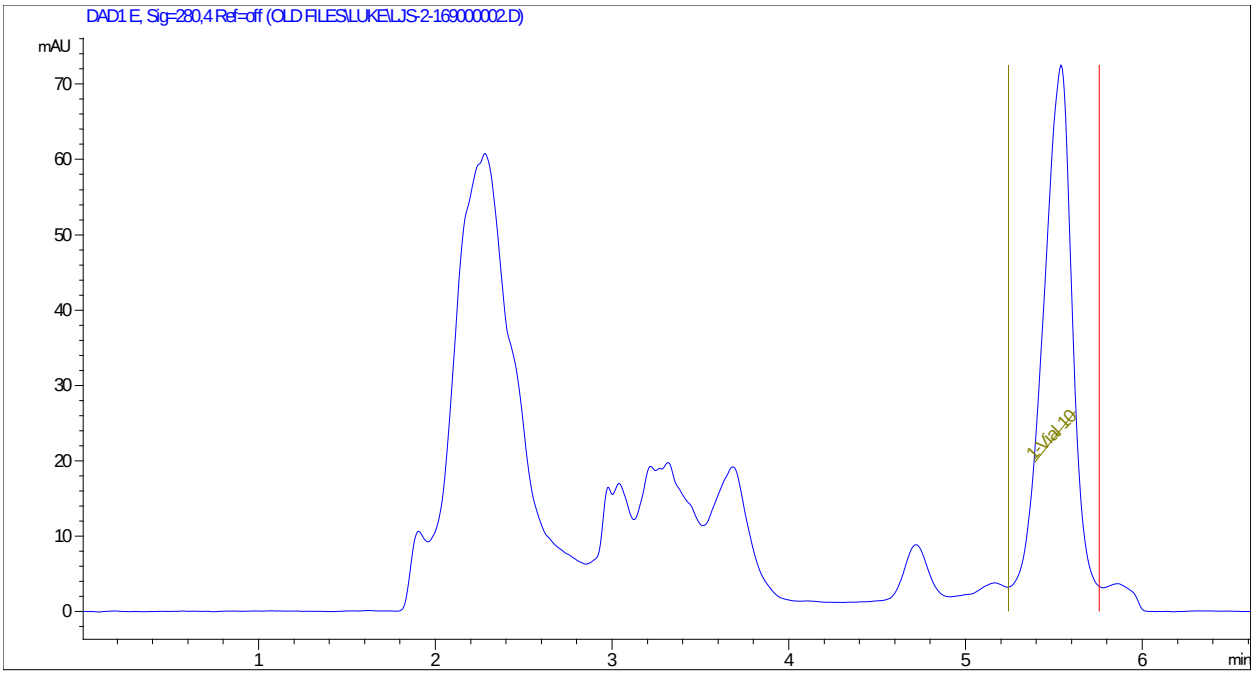


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.29
Synthesized according to general procedure **D** using **S4.40**.

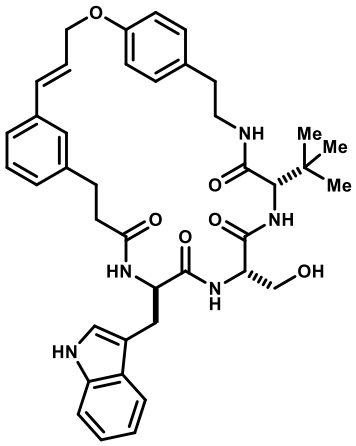


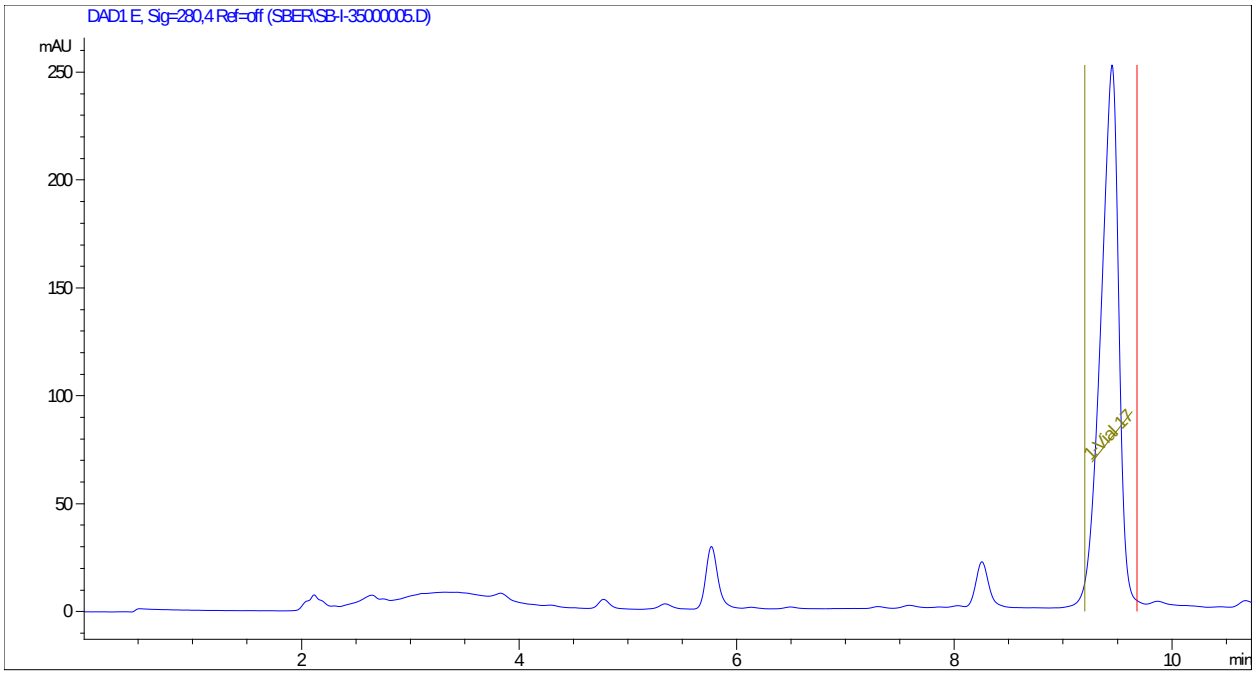


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.30
Synthesized according to general procedure **D** using **S4.41** (26%, over 2 steps).

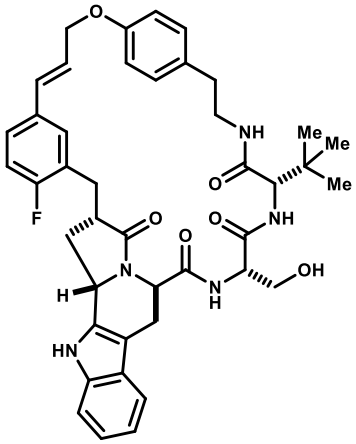


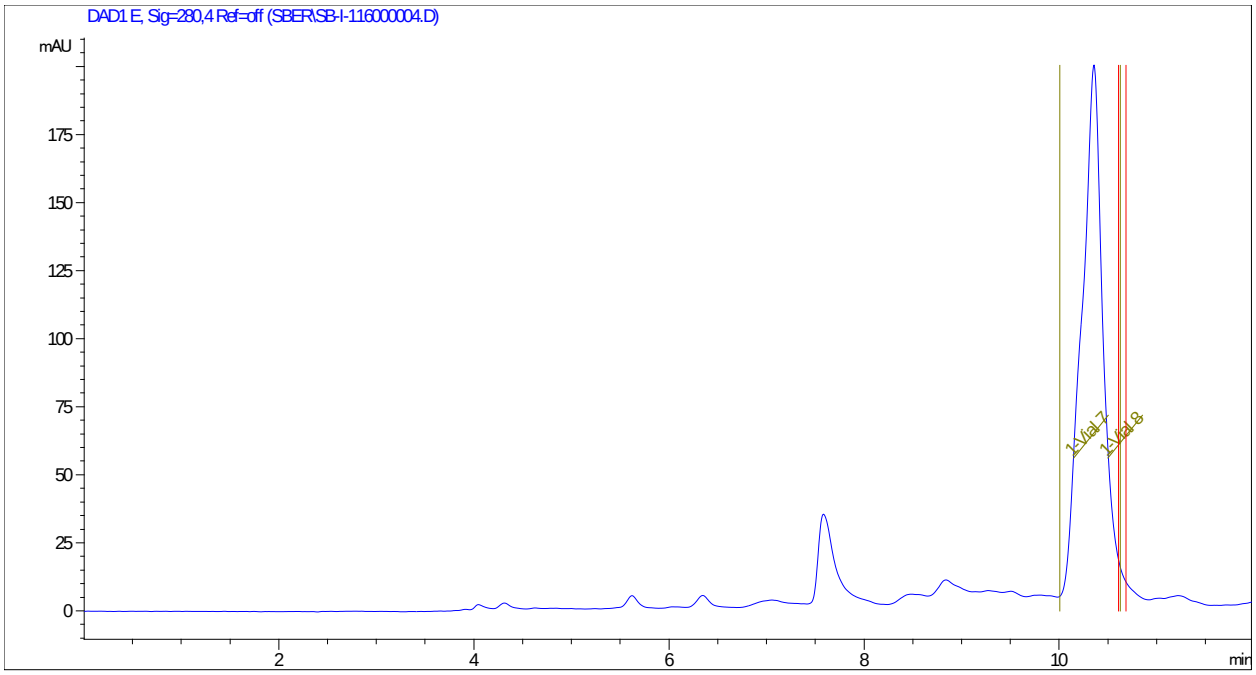


Peparative HPLC method
Column: Waters Sunfire™ C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	55
14	75

Macrocycle 4.31
Synthesized according to general procedure **D** using **S4.42** (55% yield).

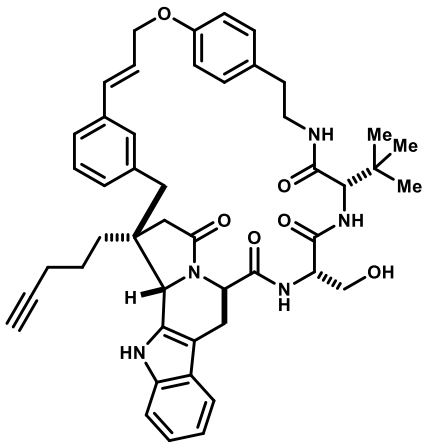


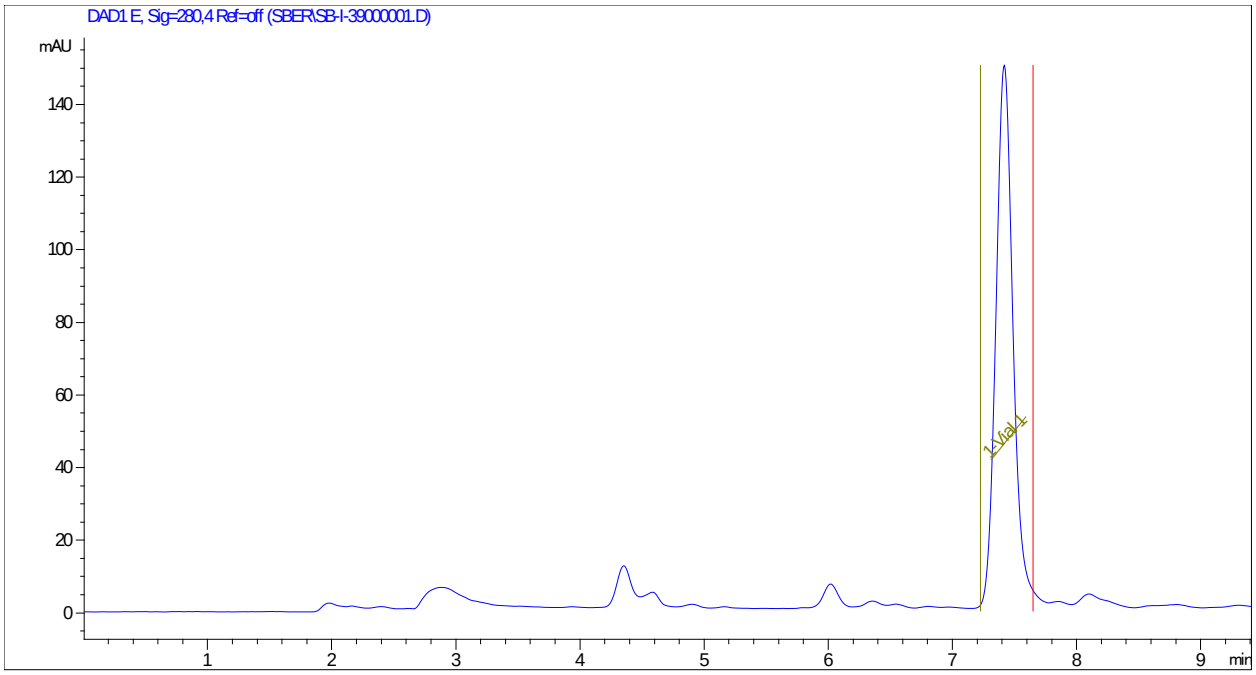


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.32
Synthesized according to general procedure **D** using using **S4.43** (30% yield).

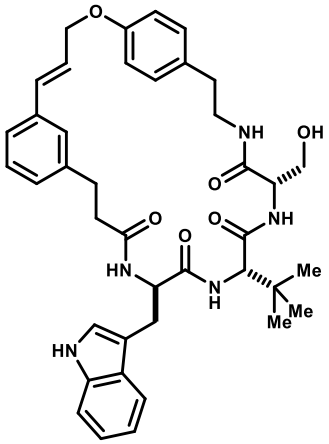


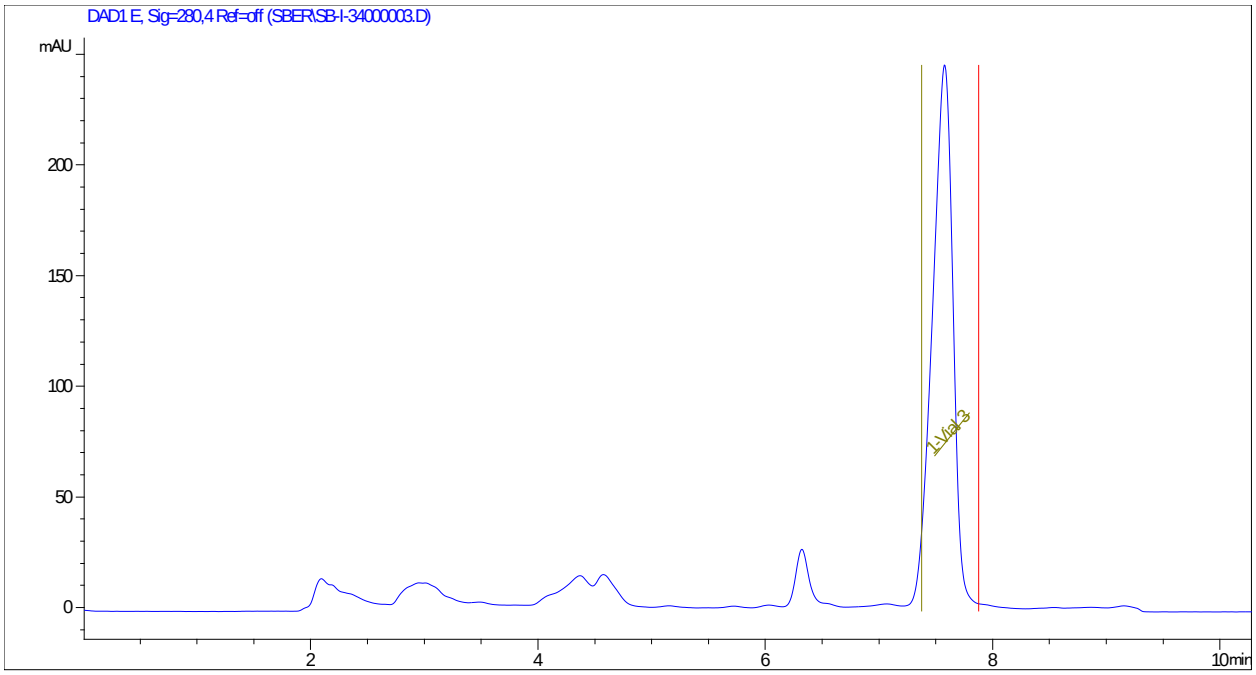


Peparative HPLC method
Column: Waters Sunfire™ C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	65
14	75

Macrocycle 4.33
Synthesized according to general procedure **D** using **S4.44** (25%, over 2 steps).

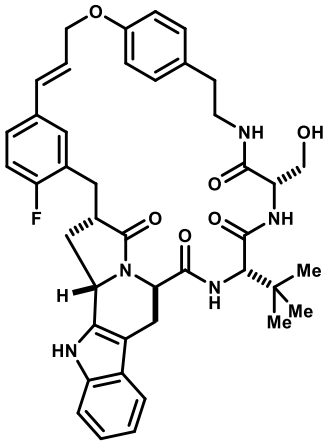


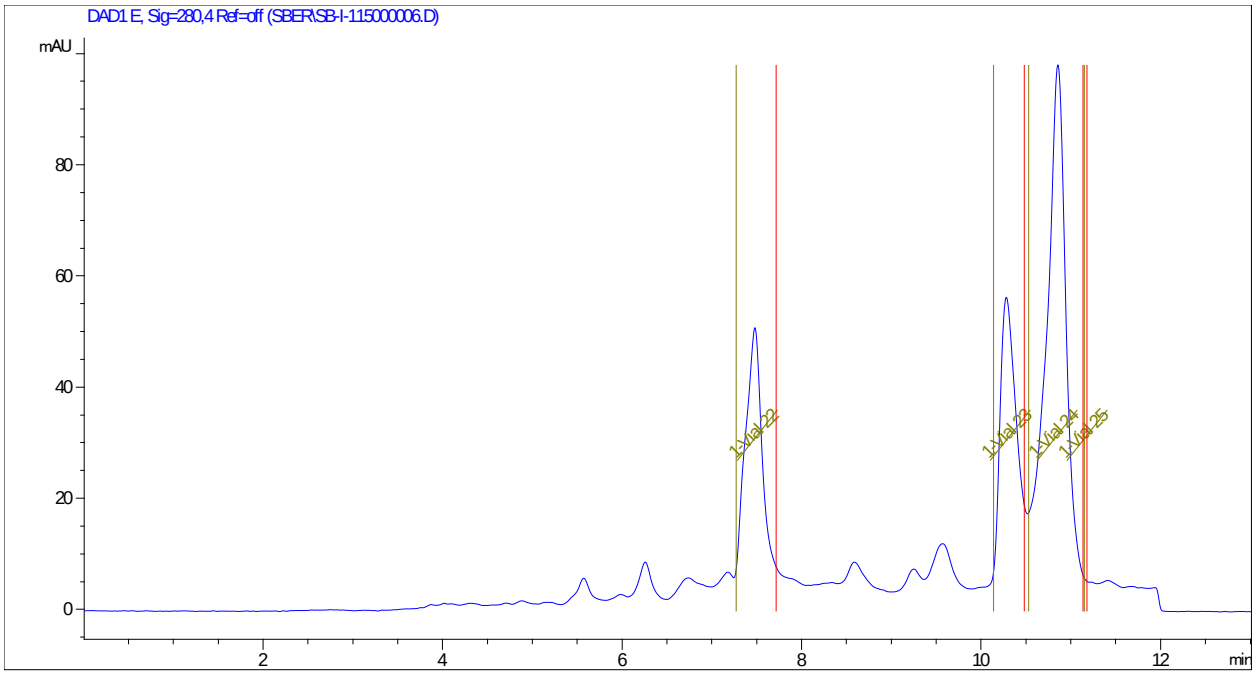


Peparative HPLC method
Column: Waters Sunfire™ C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.34
Synthesized according to general procedure **D** using **S4.45** (32% yield).

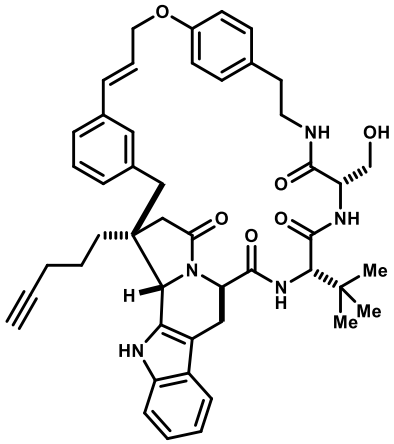




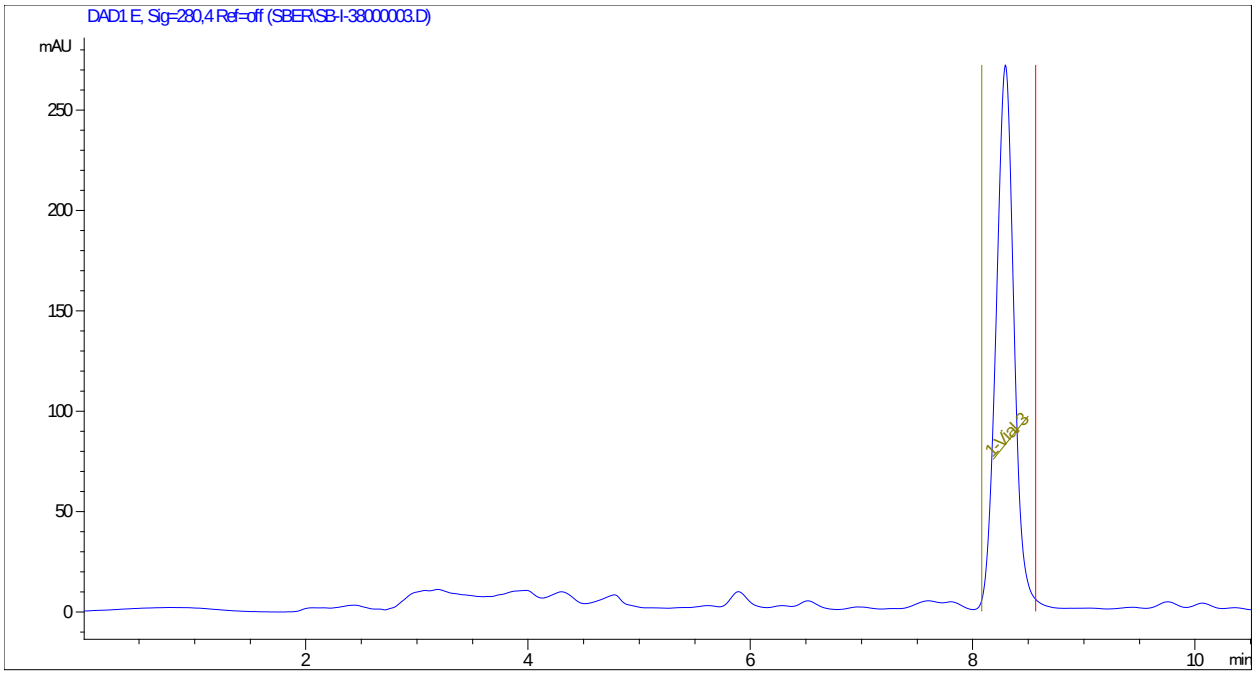
Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.35
Synthesized according to general procedure **D** using **S4.46** (28% yield).



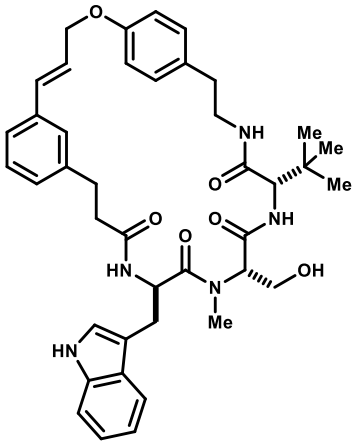
SB-I-38 (65-75%)

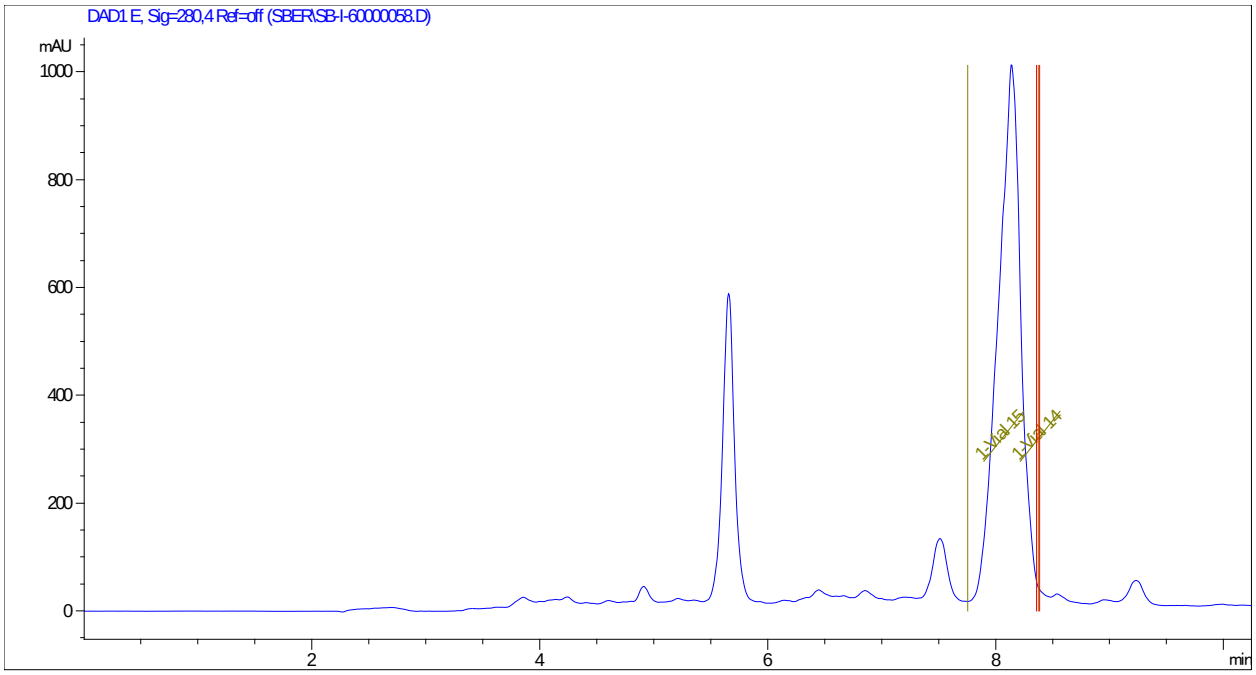


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	65
14	75

Macrocycle 4.36
Synthesized according to general procedure **D** using **S4.47** (40%, over 2 steps).

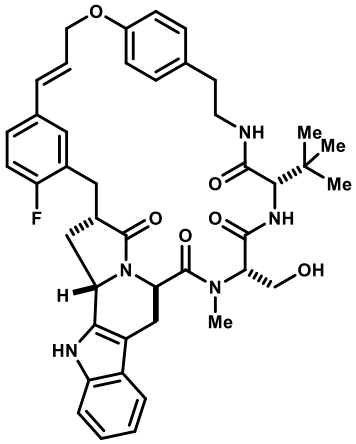


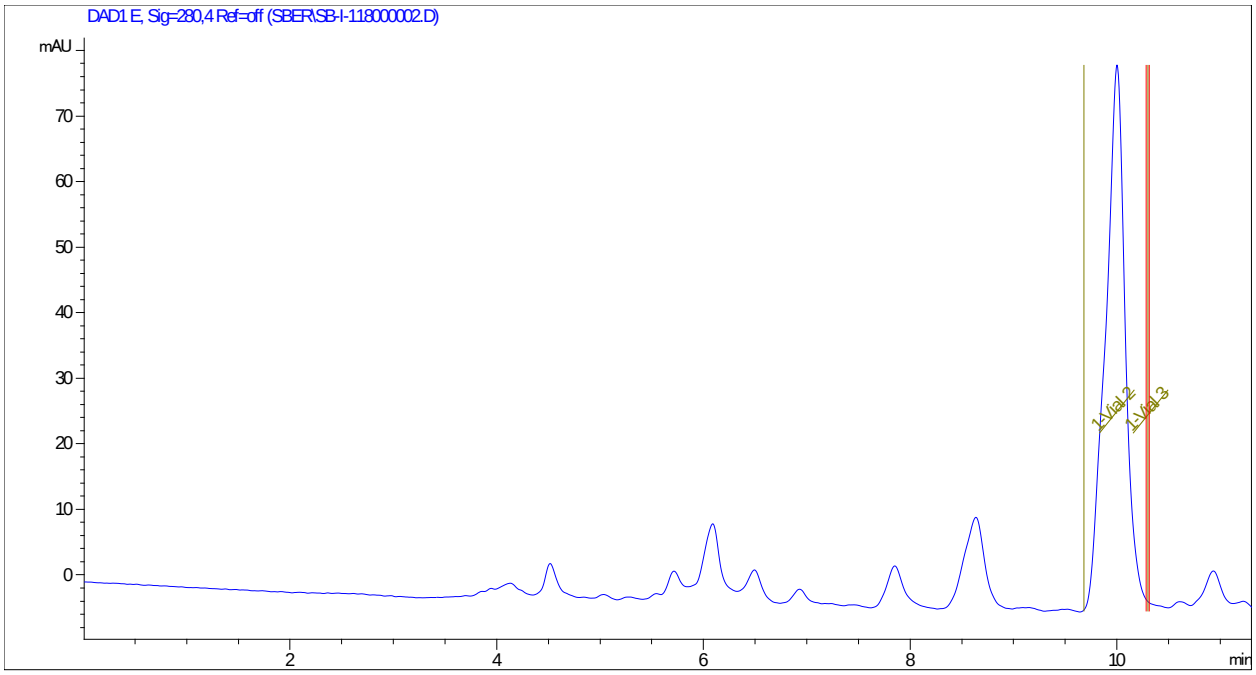


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.37
Synthesized according to general procedure **D** using **S4.48** (18% yield).

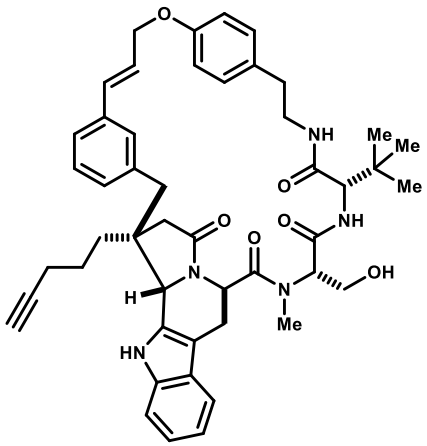


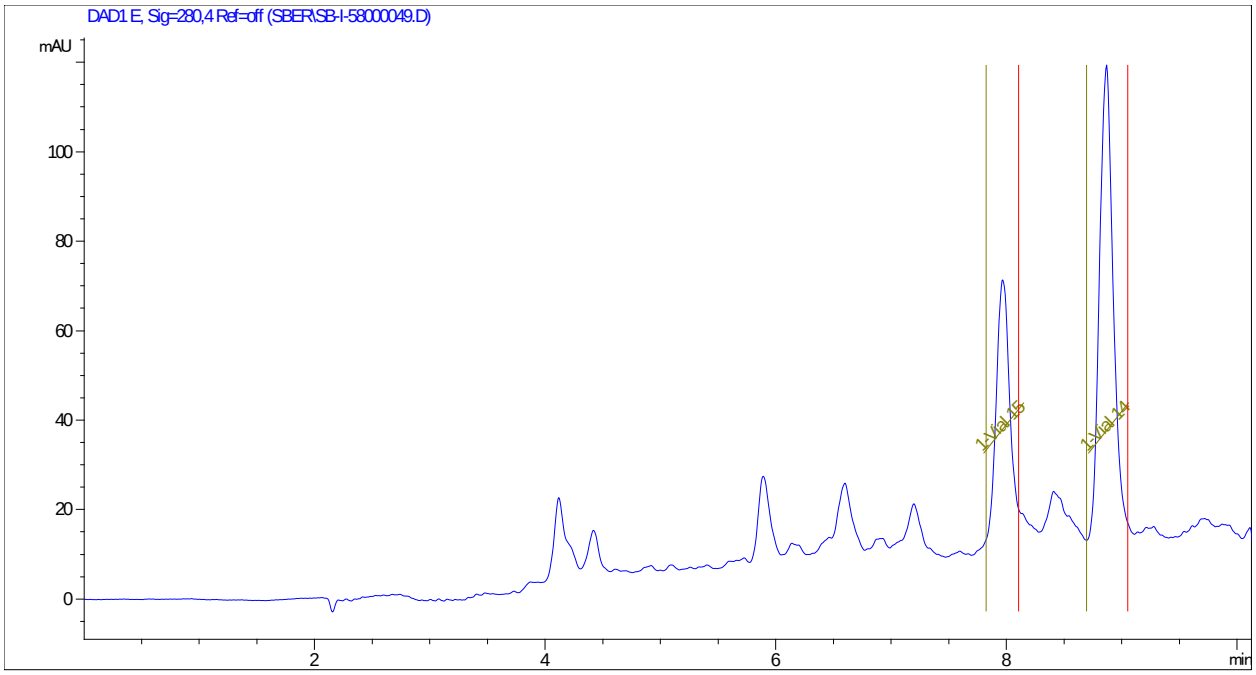


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.38
Synthesized according to general procedure **D** using **S4.49** (11% yield).

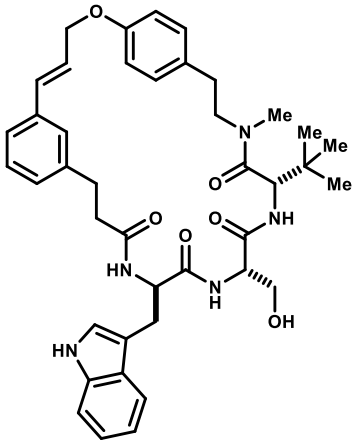


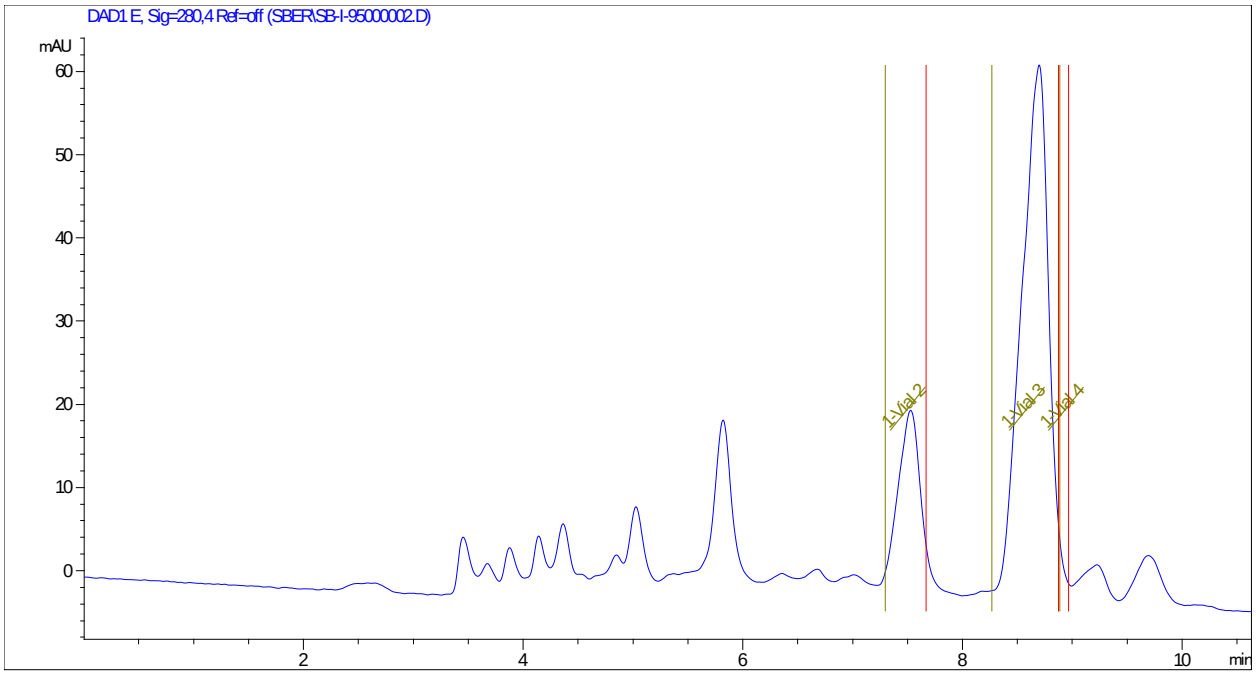


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.39
Synthesized according to general procedure **D** using **S4.50** (21%, over 2 steps).

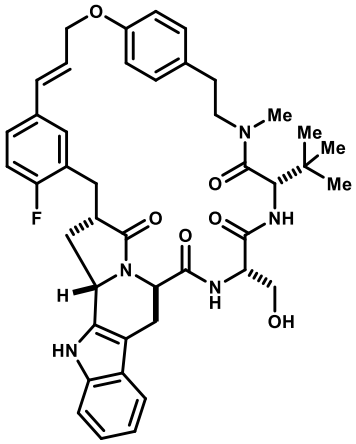


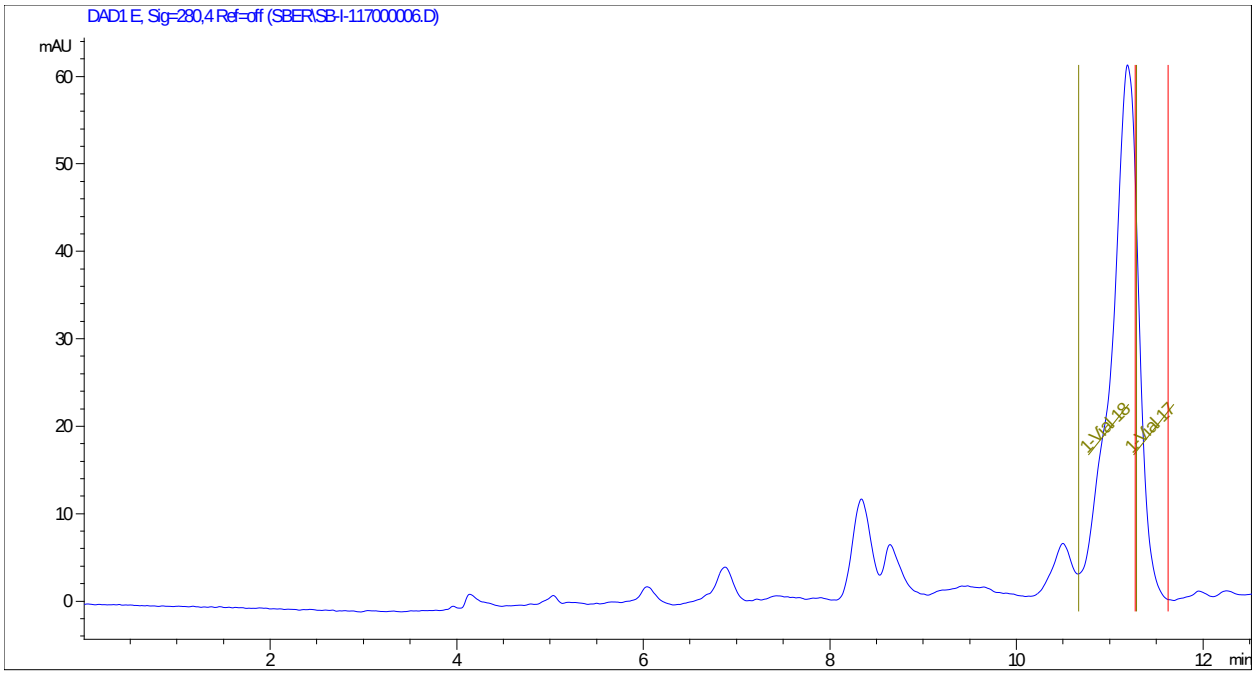


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.40
Synthesized according to general procedure **D** using **S4.51** (24% yield).

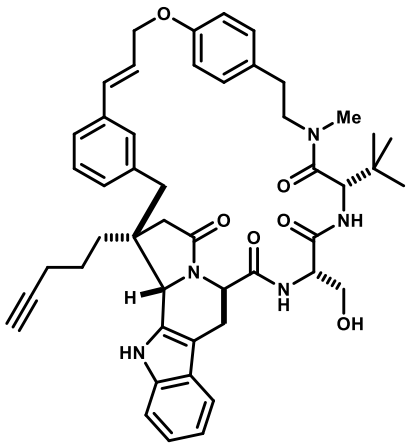


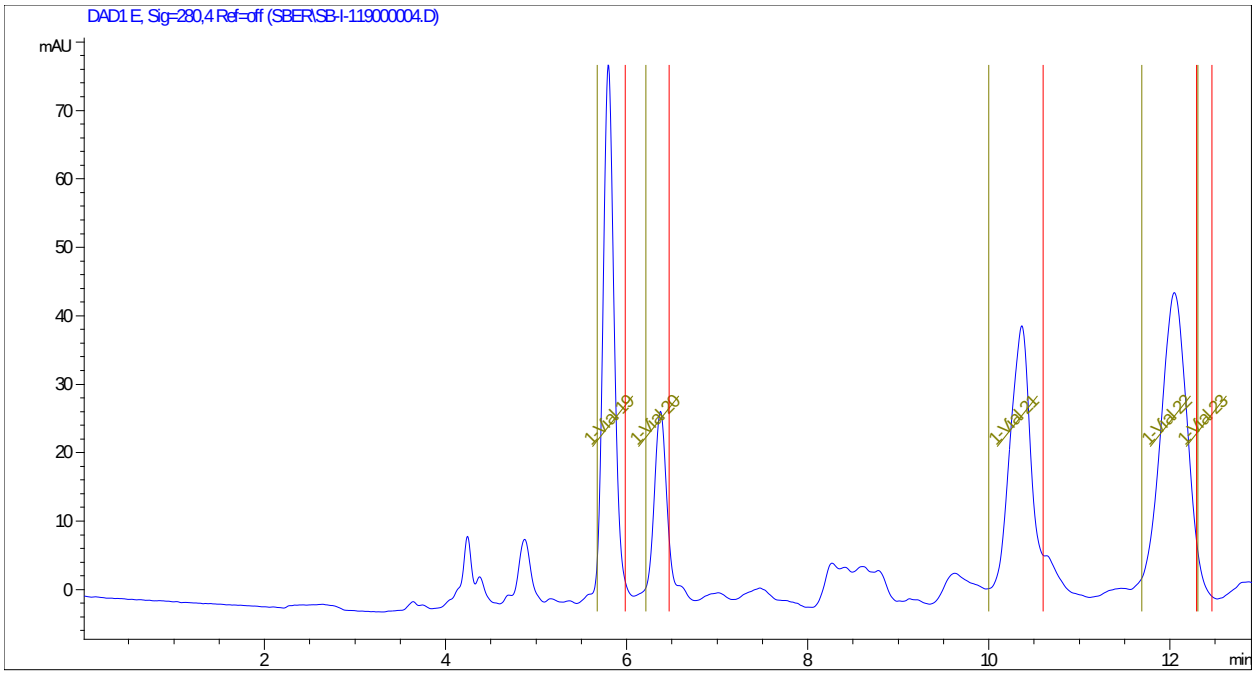


Peparative HPLC method
Column: Waters Sunfire™ C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

Macrocycle 4.41
Synthesized according to general procedure **D** using **S4.52** (15% yield).

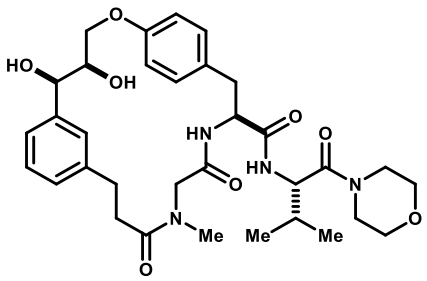




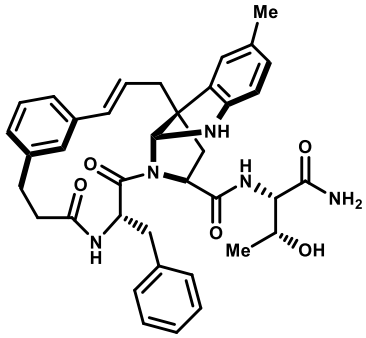
Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	60
14	80

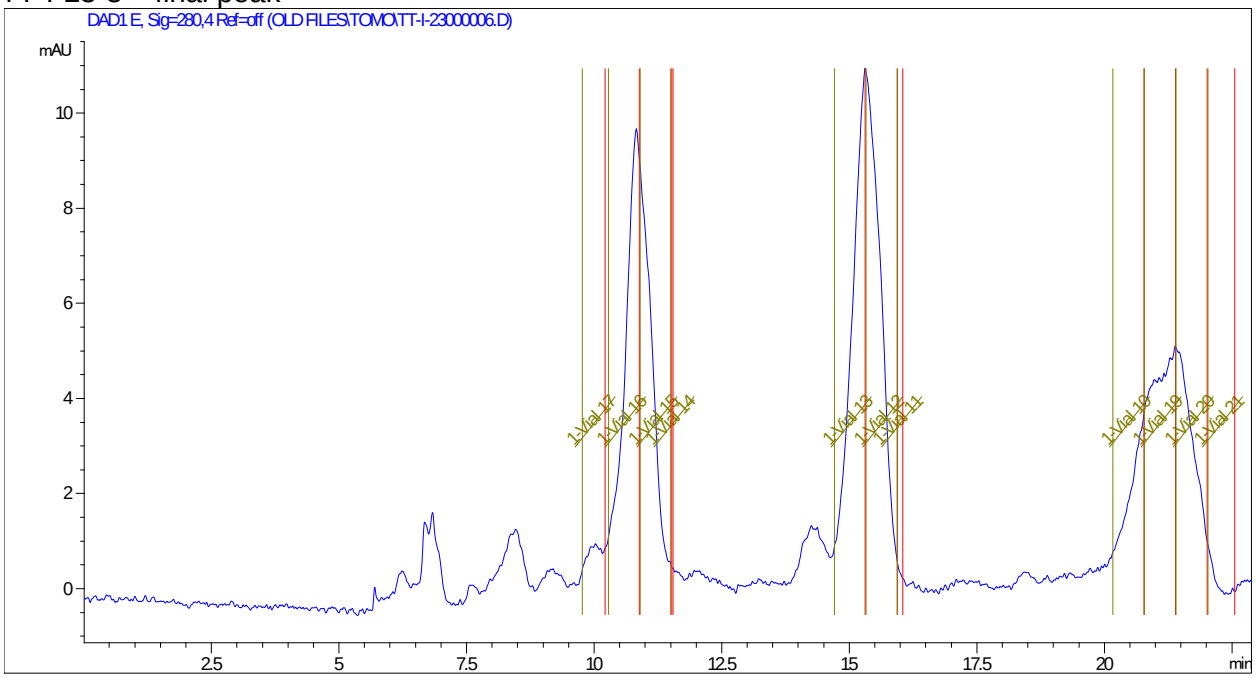
Macrocycle 4.42



Macrocycle 4.43
Synthesized according to general procedure **E** using **S4.53**.



TT-I-23-3 – final peak

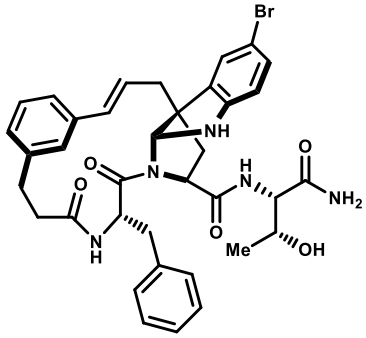


Peparative HPLC method
Column: Waters Sunfire^{TM} C_{18}, 19x250mm, 5µm
Solvent A: H_2O + 0.1%v TFA
Solvent B: ACN + 0.1%v TFA
Flow rate: 18.00 ml/min

Time (min)	%B
0	10
2	55
14	75

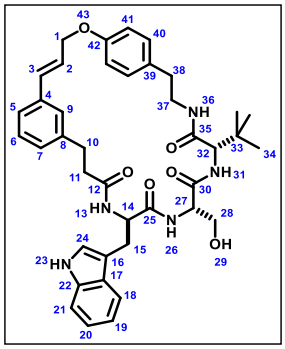
Macrocycle 4.44

Synthesized according to general procedure **E** using **S4.54**.



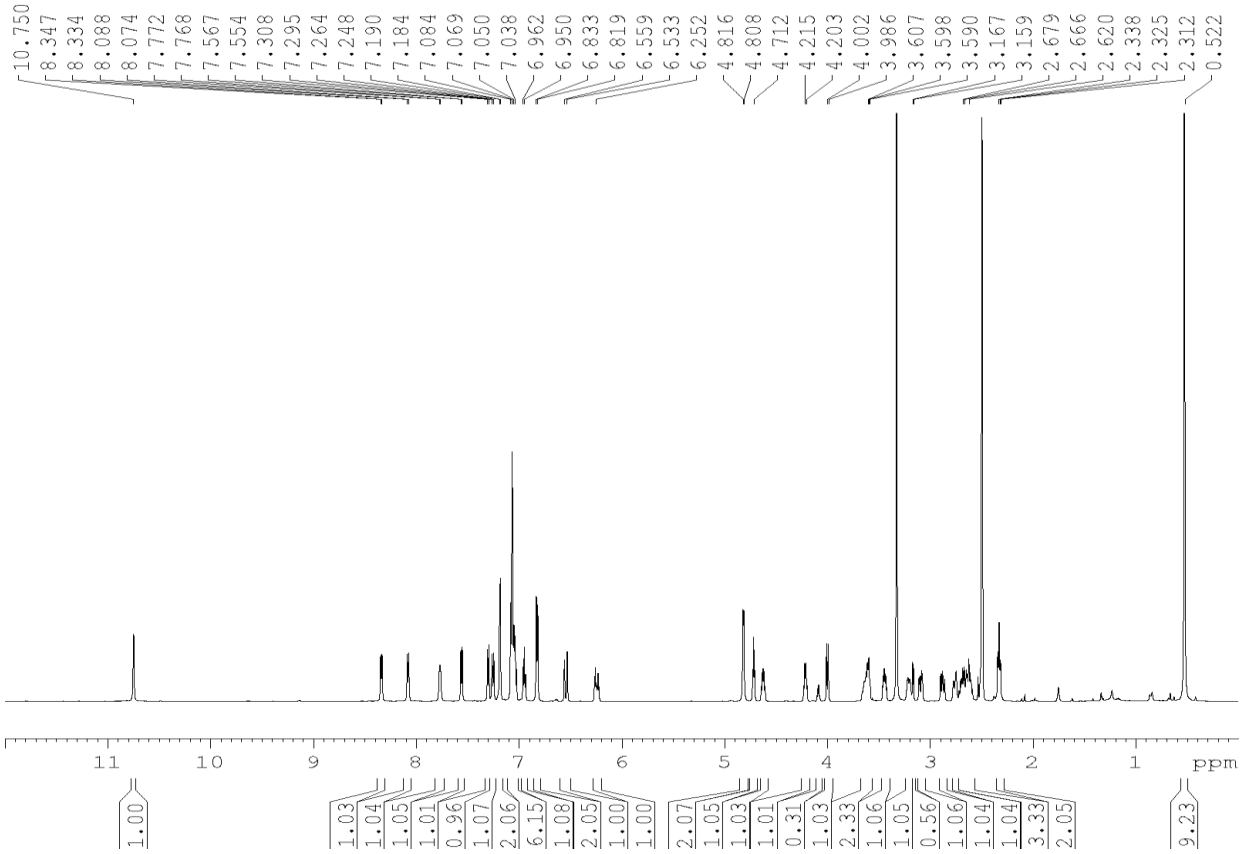
NMR Spectra

Macrocycle **4.30** (600 MHz, DMSO-d6, 298K)



	13C	1H	Key Correlation
1	66.6	4.82 (d) J = 5 Hz (2H)	
2	124.6	6.25 (ddd) J = 16.0, 5.7, 5.7 Hz (1H)	COSY 1 -> 2
3	132.1	6.55 (d) J = 16 Hz (1H)	COSY 2 -> 3
4	135.9	-	HMBC 2 -> 4
5	124.3	7.22 - 7.18 (m) (1H)	HMBC 3-> 5
6	128.3	7.22 - 7.18 (m) (1H)	COSY 5 -> 6
7	127.8	7.09 - 7.03 (m) (1H)	COSY 6 ->7
8	141.7	-	HMBC 11 -> 8
9	125.3	7.1 - 7.07 (m) (1H)	HMBC 9 -> 3,10
10	30.1	2.73 - 2.58 (m) (1H)	HMBC 10 -> 9
11	38.6	3.68 - 3.57 (m) (1H)	COSY 10 -> 11
12	170.8	-	HMBC 10 -> 12
13	-	8.09 (d) J = 8.3 Hz (1H)	HMBC 13 -> 25
14	52.6	4.65 - 4.59 (m) (1H)	HMBC 14 -> 12
15	27.2	3.1 (dd) J = 14.5, 5.3 Hz; 2.88 (dd) J = 14.5, 8.9 Hz (2H)	HMBC 24 ->15
16	109.9	-	HMBC 14 -> 16
17	127.1	-	HMBC 15, 19, 21 -> 17
18	118.2	7.57 (d) J = 7.7 (1H)	HMBC 18 -> 17
19	117.9	6.96 (dd) J = 7.4, 7.4 Hz (1H)	HMBC 19 -> 17
20	120.5	7.07 - 7.05 (m) (1H)	HMBC 18 -> 20
21	111	7.31 (d) J = 8.1 Hz (1H)	HMBC 21 -> 17
22	135.9	-	HMBC 18 -> 22
23	-	10.76 (s) (1H)	HMBC 23 -> 16, 17, 21
24	123.2	7.09 - 7.07 (m) (1H)	COSY 23 -> 24
25	172.1	-	HMBC 15 -> 25
26	-	8.35 (dd) J = 7.7 (1H)	HMBC 27 -> 26
27	54.2	4.22 (dd) J = 13.4, 6.0 Hz (1H)	HMBC 29 -> 27
28	59.8	3.67 - 3.57 (m), 3.47 - 3.41 (m) (2H)	HMBC 29 -> 28
29	-	4.72 (dd) J = 5.3 (1H)	HMBC 29 -> 27
30	169	-	HMBC 32 -> 30
31	-	7.26 (d) J = 9.8 (1H)	HMBC 31 -> 35
32	59.5	4.00 (d) J = 9.6 (1H)	HMBC 32 -> 34
33	34	-	HMBC 34 -> 33
34	26	0.53 (s) (9H)	HMBC 32 -> 34
35	168.9	-	HMBC 31 -> 35
36	-	7.78 (dd) J = 6.3, 3.5 (2H)	HMBC 36 -> 32, 38
37	36.7	2.33 (dd) J = 7.8 (2H)	COSY 38 -> 37
38	33.2	2.78 - 2.74 (m), 2.7 - 2.64 (m) (2H)	HMBC 40 -> 38
39	131	-	HMBC 41 -> 39
40	129.6	7.11 - 7.07 (m) (2H)	HMBC 42 -> 40
41	114.4	6.83 (d) (2H)	COSY 41 -> 40
42	155.7	-	HMBC 1 -> 42
43	-	-	-

^{1}H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7.295
7.264
7.248
7.190
7.184
7.084
7.069
7.050
7.038
6.962
6.950
6.833
6.819
6.559
6.533
6.252
4.816
4.808
4.712
4.215
4.203
4.002
3.986
3.607
3.598
3.590
3.167
3.159
2.679
2.666
2.620
2.338
2.325
2.312
0.522
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.03
1.04
1.05
1.01
0.96
1.07
2.06
6.15
1.08
2.05
1.00
1.00
2.07
1.05
1.03
1.01
0.31
1.03
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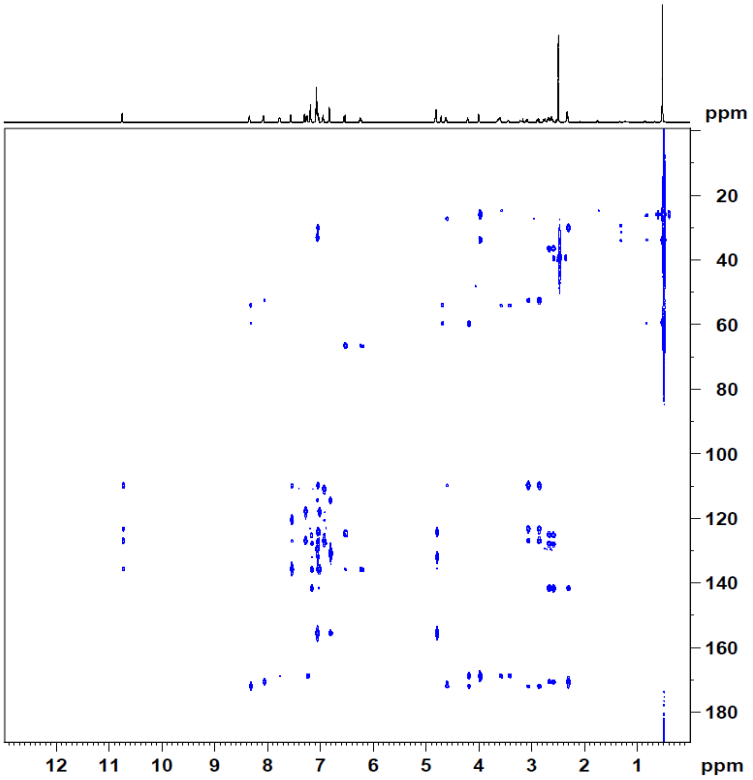
1.06
1.05
0.56
1.06
1.04
1.04
3.33
2.05
9.23

HMBC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60
80
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120
140
160
180
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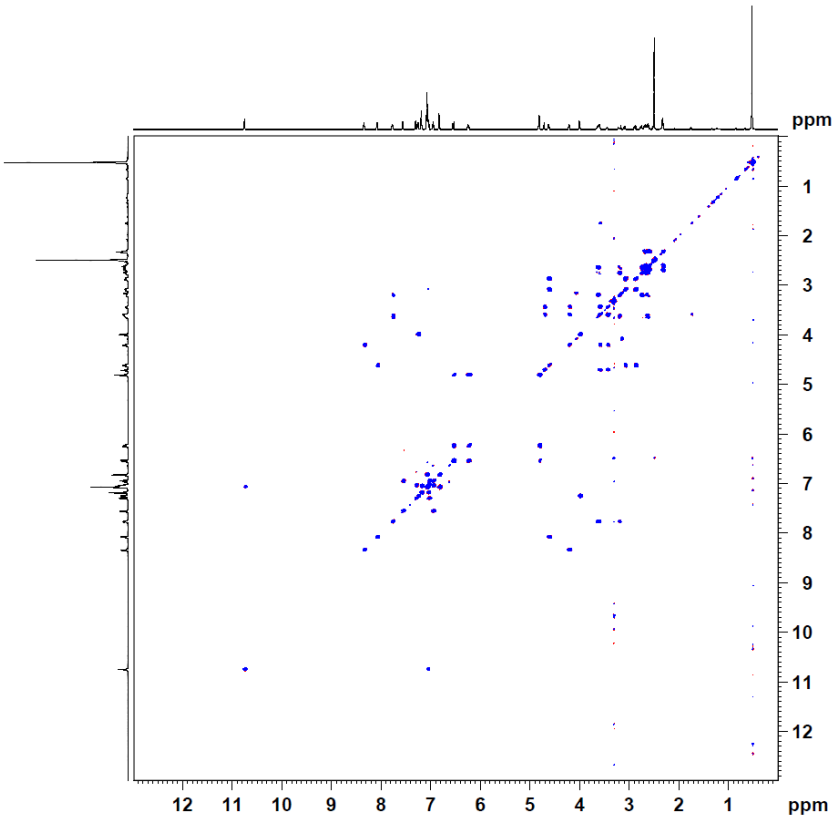
ppm

COSY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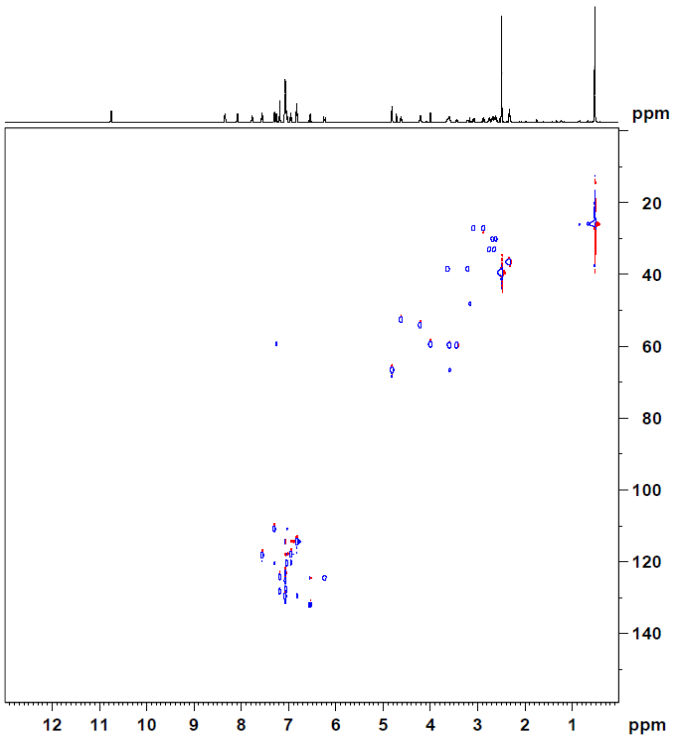
12 11 10 9 8 7 6 5 4 3 2 1
ppm

HSQC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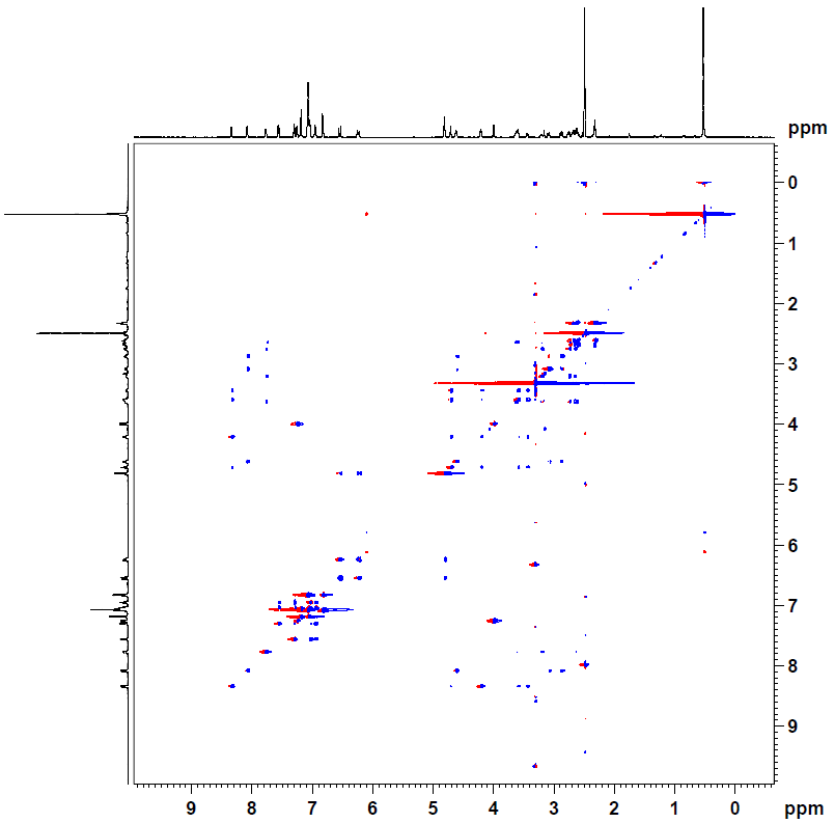
80
100
120
140
12 11 10 9 8 7 6 5 4 3 2 1
ppm

TOCSY


ppm
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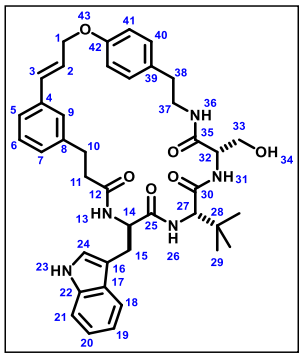
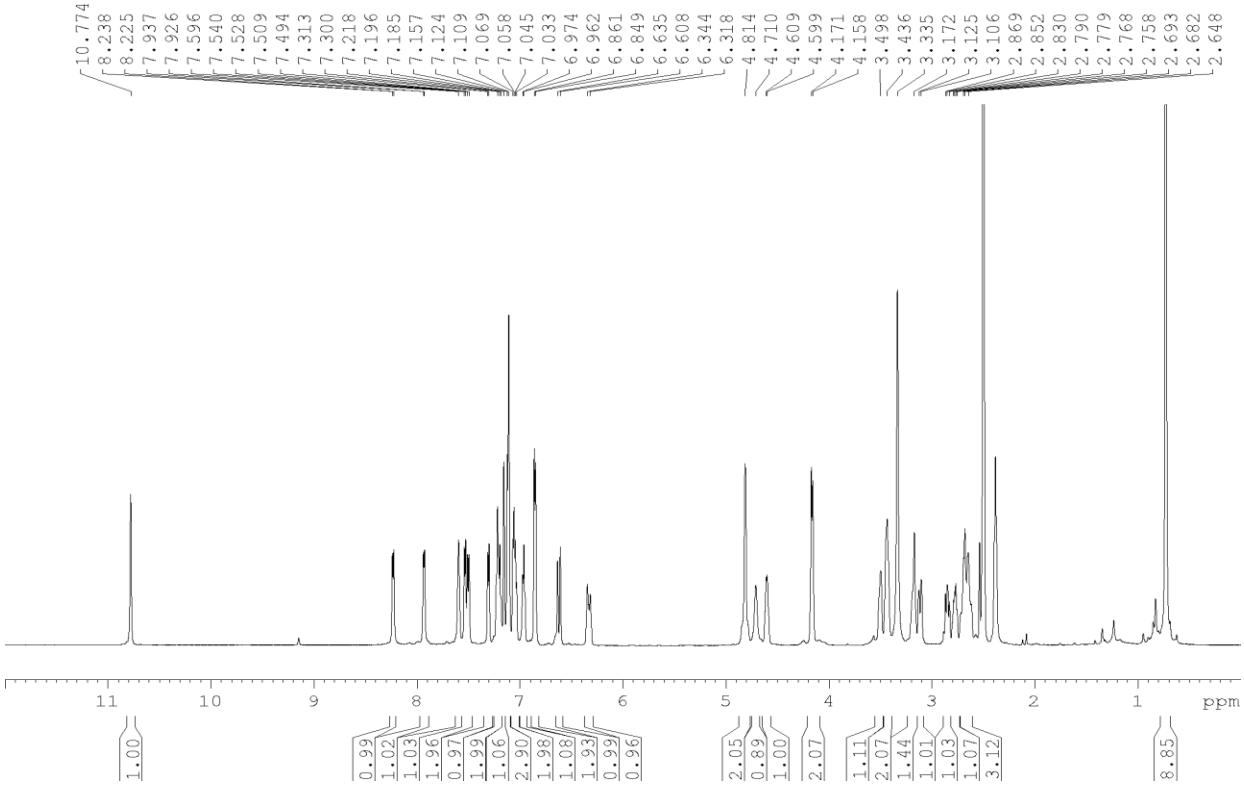
6
7
8
9
9 8 7 6 5 4 3 2 1 0
ppm

Macrocycle **4.33** (600MHz, DMSO-d6, 298K)



	13C	1H	Key Correlation
1	67.5	4.83 (dd) J = 14.3, 5.0 Hz; 4.8 (dd) J = 14.2, 5.0 Hz (2H)	
2	125.3	6.37 - 6.29 (m) (1H)	COSY 2 -> 1
3	132.2	6.62 (d) J= 16.0 (1H)	COSY 3 -> 2
4	135.8	-	HMBC 2 -> 4
5	124	7.25 - 7.2 (m) (1H)	HMBC 3 -> 5
6	128.3	7.23 - 7.16 (m) (1H)	COSY 6 -> 7, HMBC 6 -> 4
7	127.8	7.08 - 7.04 (m) (1H)	TOCSY 5 -> 7, COSY 6 -> 7
8	141.7	-	HMBC 6 -> 8
9	126	7.16 (s) (1H)	TOCSY 5 -> 9
10	30.3	2.81 - 2.74 (m); 2.7 - 2.64 (m) (2H)	HMBC 9,7 -> 10
11	36.4	2.38 (t) J = 6.7, 6.7 Hz (2H)	HMBC 11 -> 8
12	171.3	-	HMBC 10 -> 12
13	-	8.23 (d) J = 7.6 Hz	COSY 14 -> 13
14	53.8	4.6 (dd) J = 14.0, 7.3 Hz (1H)	COSY 15 -> 14
15	26.9	3.12 (dd) J = 14.1, 3.3 Hz; 2.85, J = 14.0, 9.8 Hz (2H)	HMBC 24 -> 15
16	110.1	-	HMBC 23, 14 -> 16
17	126.9	-	23,15,21,19 -> 17
18	118.1	7.53 (d) J = 7.8 Hz (1H)	COSY 19 ->18
19	118.1	6.96 (dd) J = 7.1, 7.1 Hz (1H)	HMBC 19 -> 17
20	120.6	7.05 (dd) J = 7.2, 7.2 Hz (1H)	COSY 21 -> 20
21	111	7.31 (d) J= 7.6 Hz (1H)	HMBC 21 ->20, TOCSY 19 -> 21
22	135.8	-	HMBC 20 -> 22
23	-	10.77 (s) (1H)	HMBC 23 -> 16
24	123.1	7.11 (s) (1H)	COSY 23 -> 24
25	170.9	-	HMBC 15 -> 25
26	-	7.5 (d) J = 9.1 Hz (1H)	TOCSY 27 -> 26
27	59	4.16 (d) J = 7.6 Hz (1H)	HMBC 29 -> 27
28	34.2	-	HMBC 29 -> 28
29	26.1	0.73 (s) (9H)	HMBC 27 -> 29
30	169.5	-	HMBC 26 -> 30
31	-	7.93 (d) J = 6.4 Hz (1H)	HMBC 31 -> 33
32	55	4.16 (d) J = 7.6 Hz (1H)	TOCSY 31 -> 32
33	39.2	3.44 (dd) J = 6.8, 6.8 Hz; 3.17 (dd) J = 6.4, 6.4 Hz (2H)	HMBC 31 -> 33
34	-	4.71 (s) (1H)	TOCSY 34 -> 33
35	169.6	-	HMBC 37,31 -> 35
36	-	7.6 (dd), J = 5.3, 5.3 Hz (1H)	TOCSY 36 -> 37
37	39.2	3.47 - 3.41 (m) (2H)	HMBC 39 -> 37
38	32.9	2.73 - 2.6 (m) (2H)	HMBC 40 -> 38, 38 -> 40
39	131.4	-	HMBC 41 -> 39
40	129.6	7.12 (s) (2H)	HMBC 42 -> 40
41	114.7	6.86 (d) J = 7.4 (1H)	COSY 41 -> 40
42	156.3	-	HMBC 1 -> 42
43	-	-	

^{1}H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8.225
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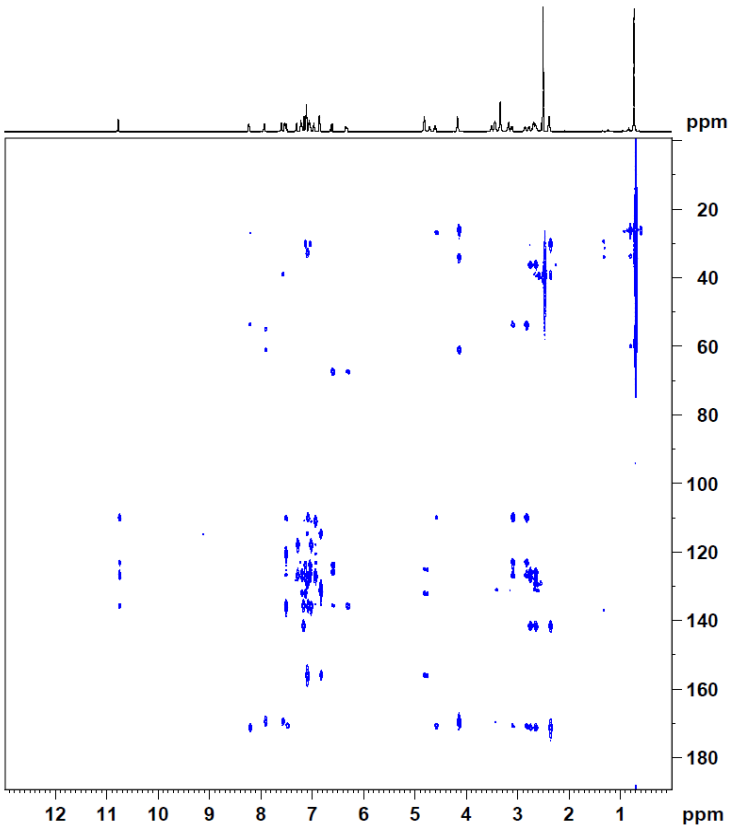
7.926
7.596
7.540
7.528
7.509
7.494
7.313
7.300
7.218
7.196
7.185
7.157
7.124
7.109
7.069
7.058
7.045
7.033
6.974
6.962
6.861
6.849
6.635
6.608
6.344
6.318
4.814
4.710
4.609
4.599
4.171
4.158
3.498
3.436
3.335
3.172
3.125
3.106
2.869
2.852
2.830
2.790
2.779
2.768
2.758
2.693
2.682
2.648
11
10
9
8
7
6
5
4
3
2
1
ppm
1.00
0.99
1.02
1.03
1.96
0.97
1.99
1.06
2.90
1.98
1.08
1.93
0.99
0.96
2.05
0.89
1.00
2.07
1.11
2.07
1.44
1.01
1.03
1.07
3.12
8.85

HMBC


ppm
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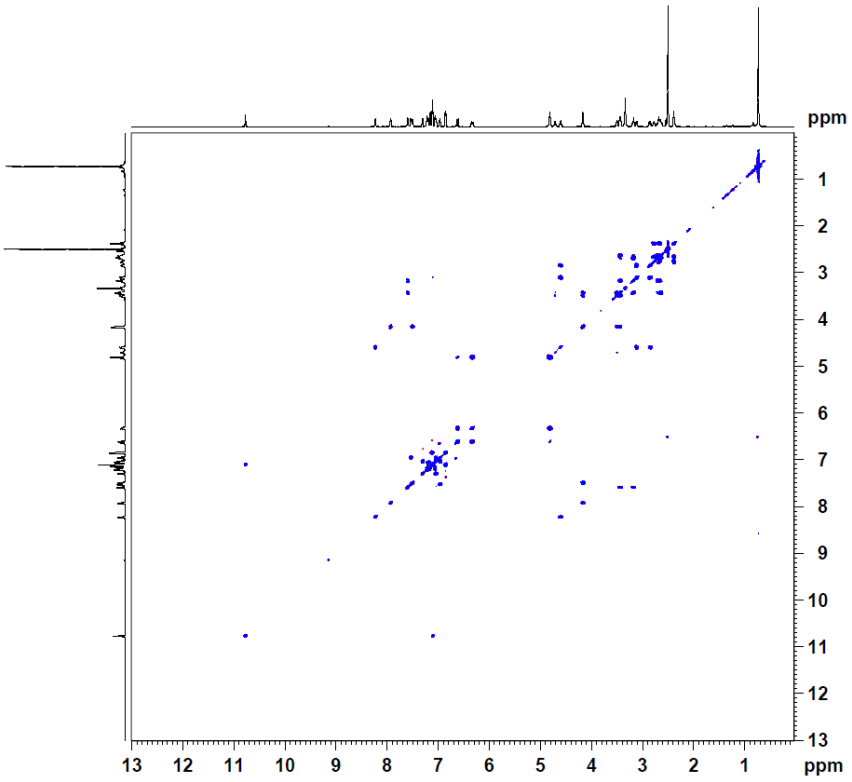
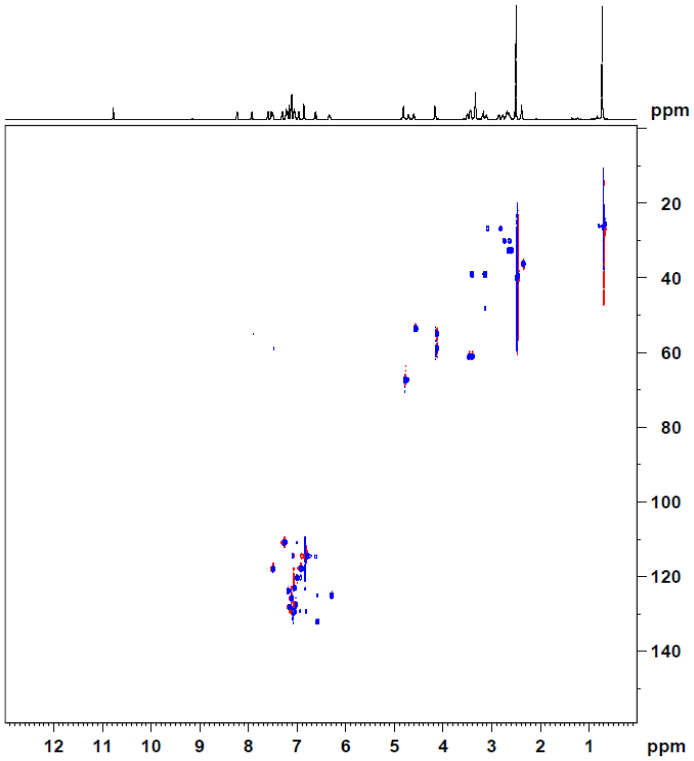
60
80
100
120
140
160
180
12 11 10 9 8 7 6 5 4 3 2 1
ppm

COSY


ppm
1
2
3
4
5
6
7
8
9
10
11
12
13
13 12 11 10 9 8 7 6 5 4 3 2 1
ppm

HSQC


ppm
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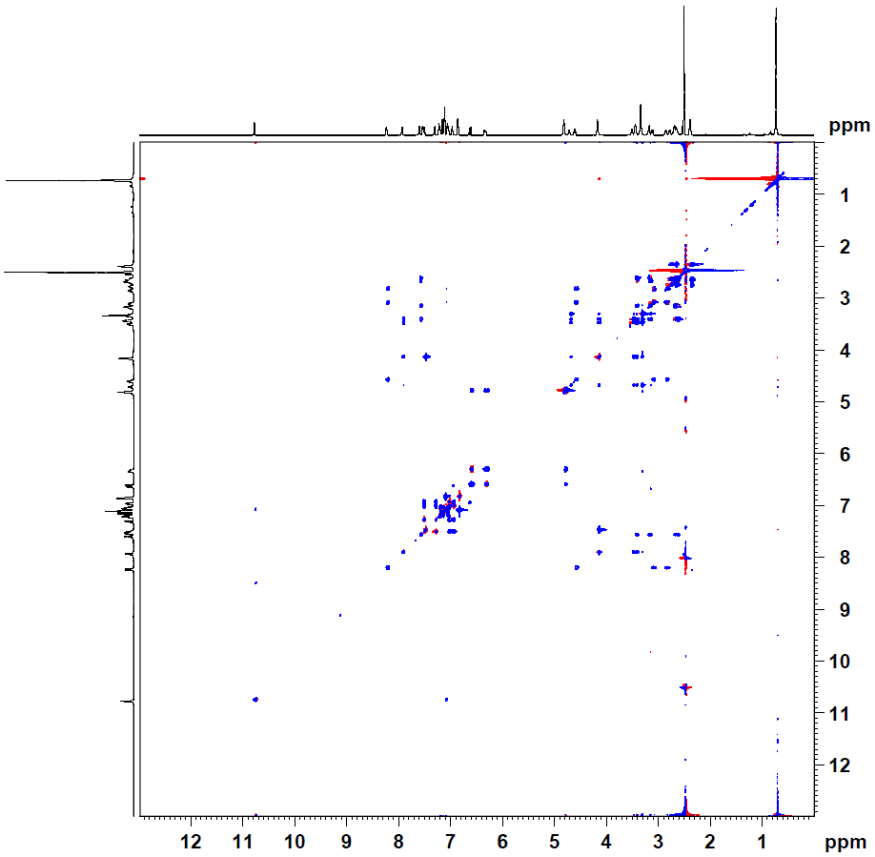
60
80
100
120
140
12 11 10 9 8 7 6 5 4 3 2 1
ppm

TOCSY


ppm
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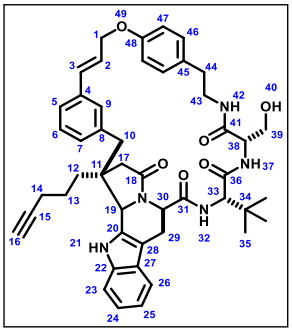
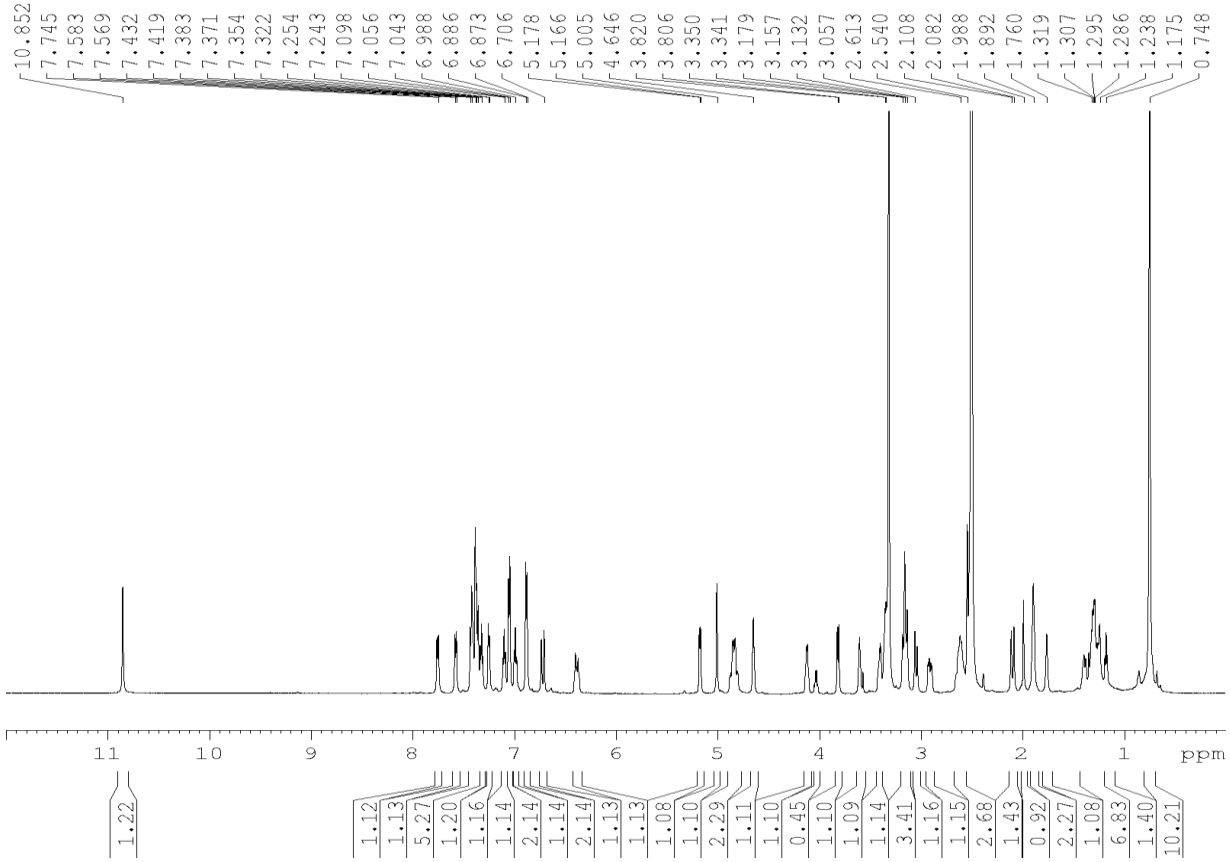
4
5
6
7
8
9
10
11
12
12 11 10 9 8 7 6 5 4 3 2 1
ppm

Macrocycle **4.35** (600MHz, DMSO-d6, 298K)



	13C	1H	Key Correlation
1	67.2	4.84 (ddd) J = 27.0, 14.7, 5.5 Hz (2H)	
2	126	6.42 - 6.34 (m) (1H)	COSY 1 -> 2
3	132	6.72 (d) (1H)	COSY 2 -> 3
4	136.2	-	HMBC 3 -> 4
5	124.4	7.39 - 7.35 (m) (2H)	HMBC 3 -> 5
6	128.4	7.32 (dd), J = 7.4, 7.4 Hz (1H)	COSY 5, 7 -> 6
7	130.5	7.25 (d) J = 7.0 Hz (1H)	TOCSY 5 -> 7
8	137.6	-	HMBC 6, 10 -> 8
9	128.7	7.39 - 7.37 (m), (2H)	HMBC 3, 5, 7 -> 9
10	40.5	3.16 (dd) J = 14.5, 14.0; 3.05 (d), J = 13.8 (2H)	HMBC 9 -> 10
11	44.3	-	HMBC 13 -> 11
12	34.5	1.43 - 1.28 (m) (2H)	HMBC 19 -> 12, COSY 12 -> 13
13	22.5	1.42 - 1.29 (m) (2H)	COSY 12 -> 14
14	18.1	1.89 (dd) J = 6.6, 6.6 Hz (2H)	COSY/TOCSY 13 -> 14
15	83.8	-	HMBC 13 -> 15
16	70.9	2.57 - 2.54 (m) (1H)	TOCSY 16 -> 14
17	38.5	2.10 (d) J = 15.7 Hz (1H)	HMBC 12 -> 17, 17 -> 12
18	170.3	-	HMBC 30 -> 18
19	58.4	5.00 (s) (1H)	HMBC 10 -> 19, 19 -> 28, 10, 11
20	105.9	-	HMBC 29 -> 20
21	-	10.85 (s) (1H)	HMBC 21 -> 22, 27, 28
22	136.6	-	HMBC 21 -> 22
23	111.1	7.45 -7.42 (m) (1H)	COSY 24 -> 23
24	120.9	7.10 (t) J = 7.4, 7.4 Hz (1H)	HMBC 24 -> 22, 26
25	118.5	6.99 (t) J = 7.4 Hz (1H)	COSY 26 -> 25
26	117.2	7.41 - 7.36 (m) (1H)	HMBC 26 -> 24, 22, 28
27	129.5	-	HMBC 21, 29 -> 27
28	105.9	-	HMBC 26, 21, 19 -> 28
29	23.7	3.16 (dd) J = 14.5, 14.0; 2.91 (dd), J = 15.4, 6.9 (2H)	COSY 29 -> 30
30	48.6	5.17 (d) J = 7.2 (1H)	HMBC 30 -> 28, 19
31	169.5	-	HMBC 33 -> 31
32	-	7.76 (d) J = 8.5 Hz (1H)	COSY 33 -> 32
33	60	3.81 (d) J = 8.9 Hz (1H)	HMBC 35 -> 33
34	33.3	-	HMBC 33 -> 34
35	26.1	0.75 (s) (9H)	HMBC 33 -> 33
36	170.3	-	HMBC 32 -> 36
37	-	7.58 (d) J = 8.3 Hz (1H)	HMBC 37 -> 38, 41; COSY 37 -> 38
38	55.1	4.12 (dd) J = 12.6, 5.2 Hz (1H)	HMBC 40 -> 38; TOCSY 40 -> 38
39	61.2	3.43 - 3.38 (m), 3.37 - 3.32 (m) (2H)	COSY 40 -> 39; HMBC 39 -> 41
40	-	4.65 (t) J = 4.6, 4.6 Hz (1H)	HMBC 40 -> 38, 39; COSY 40 -> 39
41	169.3	-	HMBC 43 -> 41
42	-	7.42 - 7.39 (m) (1H)	COSY 43 -> 42; HMBC 43 -> 42
43	39.5	3.38 - 3.33 (m) (2H)	COSY 44 -> 43; HMBC 43 -> 45
44	33.1	2.67 - 2.58 (m) (2H)	HMBC 46 -> 44
45	131.4	-	HMBC 47 -> 45
46	129.7	7.05 (d) J = 8.1 Hz (2H)	HMBC 46 -> 48
47	115.1	6.88 (d) J = 8.1 HZ (2H)	HMBC 47 -> 48
48	155.9	-	HMBC 1 -> 48
49	-	-	-

^{1}H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5.166
5.005
4.646
3.820
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3.179
3.157
3.132
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1.319
1.307
1.295
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10
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8
7
6
5
4
3
2
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ppm
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2.14
1.14
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1.13
1.13
1.08
1.10
2.29
1.11
1.10
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1.10
1.09
1.14
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1.43
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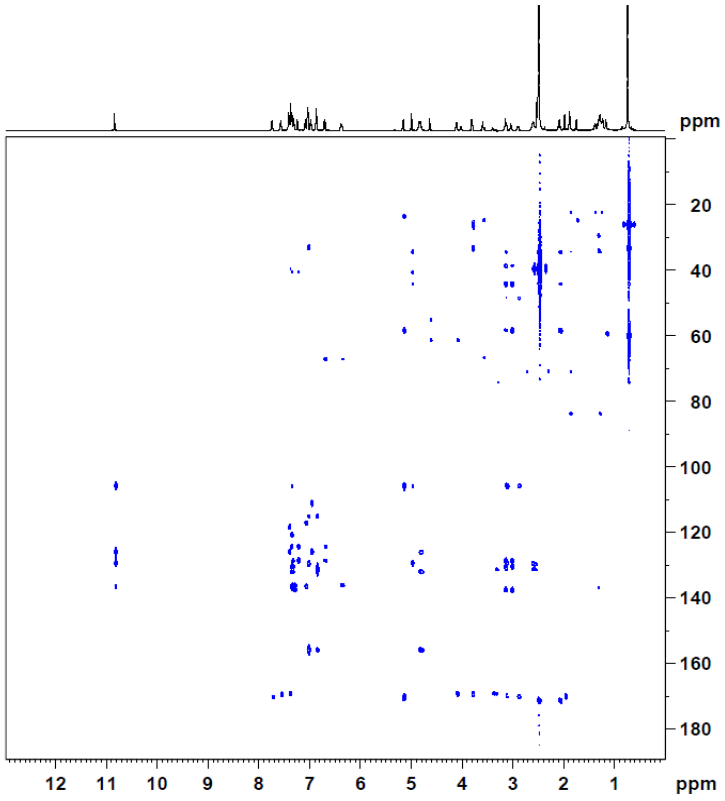
2.27
1.08
6.83
1.40
10.21

HMBC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80
100
120
140
160
180
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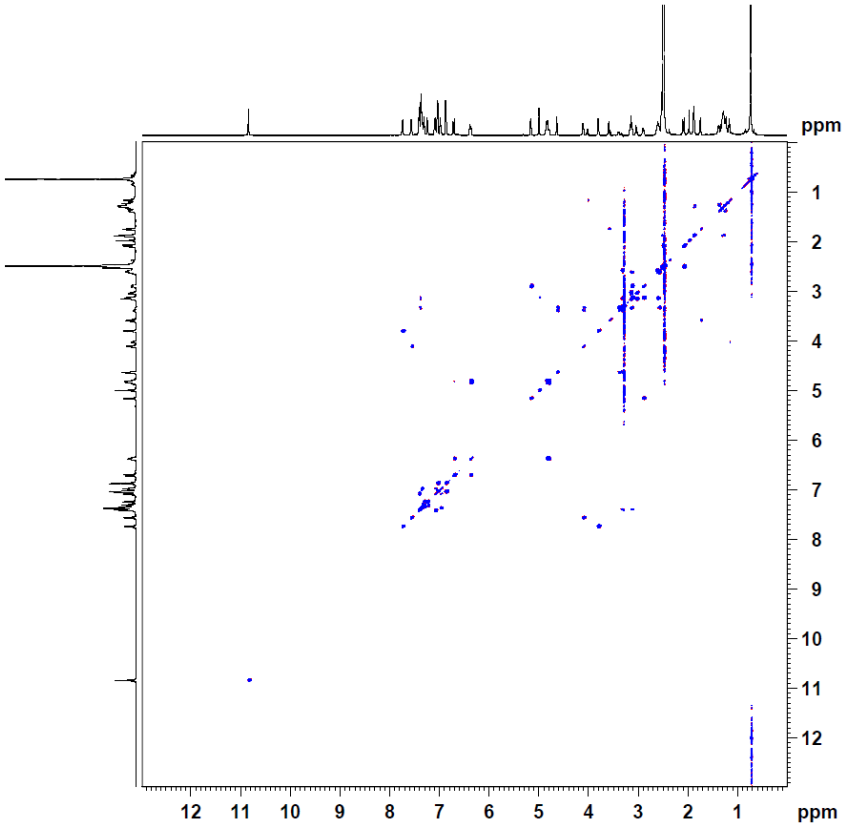
ppm

COSY


ppm
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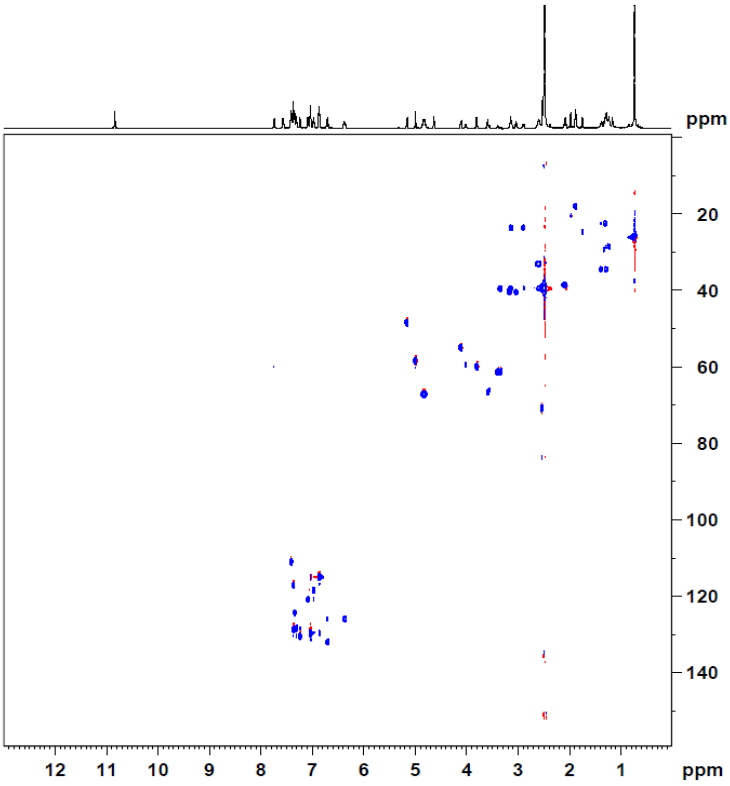
ppm

HSQC


ppm
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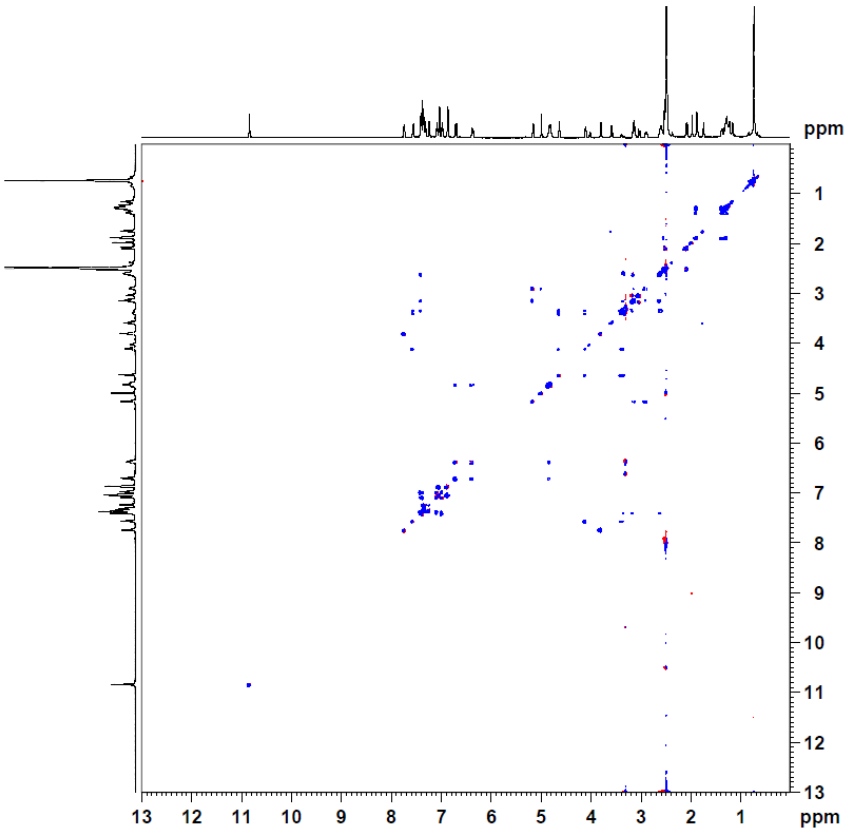
60
80
100
120
140
12 11 10 9 8 7 6 5 4 3 2 1
ppm

TOCSY


ppm
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8
9
10
11
12
13
13 12 11 10 9 8 7 6 5 4 3 2 1
ppm

NOESY