Title

Design and Synthesis of C4-modified Group A Streptogramin Analogs

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Design and Synthesis of C4-modified Group A Streptogramin Analogs

by

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DISSERTATION

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This thesis is dedicated to my parents, Chang-Yu Lee and Ai-Chu Cheng, who sacrificed a lot to immigrate to the US and provide a stable life for me. I could not have pursued science and higher education without their constant love and support.

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Contributions

The chapters in this dissertation were performed under the guidance of Dr. Ian Seiple, with the collaboration and feedback of many Seiple lab members. My thesis committee, Dr. Adam Renslo and Dr. James Fraser, provided key scientific guidance that helped made this work successful.

Chapter 1: Unpublished work that has not yet been submitted for publication. This chapter details the design, synthesis, and antibacterial evaluation of C4-modified group A streptogramin analogs. Dr. Qi Li conceptualized the synthetic route and performed the synthesis of the amide linker series. Dr. Nina Perryman optimized the synthetic route of the amide linker left half and right half. Isabel Lee optimized the synthetic route of the alkyl linker series and performed the synthesis of the ester, cyclic amide, and alkyl linker series. The cryoEM structures were obtained in collaboration with Dr. James Fraser and Dr. Jenna Pellegrino.

Chapter 2: Unpublished work that has not yet been submitted for publication. This chapter details the evaluation of the inhibitory activity of streptogramins against *Mycobacterium tuberculosis* (Mtb). Dr. Qi Li and Isabel Lee performed the synthesis of compounds. The biological evaluation of compounds against Mtb was performed in collaboration with Dr. Javid Babak and Adam Fillion.

Isabel Lee provided the experimentation and manuscript for most of the following dissertation, which is a substantive contribution comparable to other dissertations in Chemistry & Chemical Biology. Ian Seiple directed and supervised the research and provided guidance and feedback throughout.

“Life’s a marathon, not a sprint” – *Phillip C. McGraw*

Design and Synthesis of C4-modified Group A Streptogramin Analogs

Isabel Jean Lee

Abstract

Natural products and their derivatives have long served as powerful tools for treating bacterial infections, but the rise of antibiotic resistance threatens their continued effectiveness and has significantly depleted the current antibiotic pipeline. Structural modifications to natural product antibiotics have proven to be effective in overcoming certain resistance mechanisms and extending their clinical utility. The development of C4-modified group A streptogramins that overcome acetyltransferase resistance, a pervasive resistance mechanism to the class, is an example of successful implementation of this strategy. However, the synthetic chemistry to reach these new analogs was inherently limiting, enabled access to only two analogs with modifications at the desired position on the scaffold.

In chapter 1, we report the development of a modified route to group A streptogramins that enables access to a broad diversity of functionality at C4. Using cryo-EM binding data to guide structural modifications, we synthesize several structural series of C4-modified group A streptogramins with sidechains designed to make binding contacts with the exit tunnel of the ribosome. We identify multiple analogs that are active against multidrug-resistant bacteria, including strains that are resistant to macrolides, β-lactams, vancomycin, and prior generations of streptogramins. The three most active analogs (**56a**, **56c**, and **63b**) exhibit MIC values ranging from 0.5-8 μg/mL. We characterize the binding of one analog to the bacterial ribosome, revealing a new pi-stacking interaction between the C4 sidechain and the non-canonical U1782-U2586 base pair.

In chapter 2, we evaluate the inhibitory activity of group A and B streptogramins in *Mycobacterium tuberculosis* (Mtb) as potential next generation antimycobacterial therapeutics. We assess 48 diverse group A streptogramins, along with group B streptogramin **VS1**, and find that nearly 50% of the individual compounds inhibit Mtb over 90%. Notably, flopristin and **47** (**SA1**) emerge as the most potent analogs, maintaining potency at 0.8 μg/mL. Combinations of these two analogs with **VS1** further improve efficacy, inhibiting over 90% of Mtb growth at concentrations as low as 40 ng/mL. This activity is 100-fold more potent than one of the suggested TB treatment options, linezolid.

Collectively, these findings demonstrate how structure-guided drug design can drive the development of next-generation antibiotics and revitalize the therapeutic potential of the streptogramin class.

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List of Abbreviations

Bn – benzyl

BPaLM – bedaquiline, pretomanid, linezolid, and moxifloxacin

Bu – butyl

cryo-EM – cryogenic electron microscopy

Cy – cyclohexyl

dba – dibenzylideneacetone

DBU – 1,8-diazabicyclo[5.4.0]undec-7-ene

DCC – dicyclohexylcarbodiimide

DIBAL – diisobutylaluminum hydride

DIPEA – N,N-diisopropylethylamine

DMAP – 4-dimethylaminopyridine

E. coli – *Escherichia coli*

EPSA – exposed polar surface area

Fmoc - 9-fluorenylmethoxycarbonyl

HATU – 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-*b*]pyridinium-3-oxidhexafluorophosphate

Im – imidazole

MDR-TB – multidrug-resistant tuberculosis

MIC – minimum inhibitory concentration

MLSb – macrolide, lincosamide, group B streptogramin

Mtb; *M. tuberculosis – Mycobacterium tuberculosis*

NMP – N-methyl-2-pyrrolidone

NMR – nuclear magnetic resonance

NPET – nascent peptide exit tunnel

PAMPA – parallel artificial membrane permeability

PTC – peptidyl transferase center

PSA – polar surface area

PyAOP – (7-azabenzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate

RR-TB – rifampicin-resistant tuberculosis

S. aureus – Staphylococcus aureus

SFC – supercritical fluid chromatography

TB – tuberculosis

TBS – *tert*-butyldimethylsilyl

TEA – triethylamine

TES – triethylsilyl

TfO – trifluoromethanesulfonate

TMS – trimethylsilyl

TPSA – total polar surface area

VatA – virginiamycin O-acetyltransferases

Vgb – virginiamycin B

VM1 – virginiamycin M1

VM2 – virginiamycin M2

VREfm – *Enterococcus faecium*

VS1 – virginiamycin S1

XDR-TB – extensively drug-resistant tuberculosis

4C – four-carbon

5C – five-carbon

Chapter 1. Design and Synthesis of C4-modified Group A Streptogramin Analogs

1.1 Introduction

Streptogramin antibiotics are produced by *Streptomyces* spp. and are categorized into two classes: 23-membered macrocycles called group A streptogramins and 17-membered cyclic depsipeptides known as group B streptogramins.[1] A major drawback of natural streptogramins was their relatively low water solubility, preventing formulation for intravenous administration. The rise of vancomycin-resistant *Enterococcus faecium* (VREfm) infections in the 1990s, and the exceptional activity of streptogramin A/B combinations against VREfm, led to a surge of research aimed at optimizing these molecules for clinical use.[1,2] Rhône-Poulenc overcame the solubility limitation by means of addition of tertiary amine-containing sidechains on both streptogramin components, yielding the soluble pair dalfopristin/quinupristin (Synercid™), which was approved by the FDA in 1999 for the treatment of VREfm bacteremia and complicated skin and skin structure infections caused by *Staphylococcus aureus* and *Streptococcus pyogenes.*[3,4] While effective, the intravenous administration and associated side effects, such as venous irritation, have limited its use to a "last resort" combination therapy for vancomycin-resistant infections. The first orally available streptogramin combination, flopristin-linopristin (NXL 103) had improved potency and met clinical endpoints in phase II clinical trials in 2011, but has not progressed further in the clinic due to company turnover and economic considerations.[1]

Streptogramins inhibit bacterial protein synthesis by binding to adjacent sites in the bacterial ribosome: group A streptogramins bind to the peptidyl transferase center (PTC) to prevent peptide bond formation, while group B streptogramins bind to the adjacent nascent peptide exit tunnel (NPET) to prevent polypeptide elongation and release.[5] Notably, group A streptogramins bind first to the PTC, resulting in structural rearrangement of the NPET to facilitate group B streptogramin binding.[1,6] Individually, both groups are bacteriostatic; however, when administered together, they exhibit synergistic activity that can be bactericidal in some bacterial species.[1] This synergy is explained at least in part by their cooperative binding, although it may not account for their pronounced synergistic effects in some species.[6]

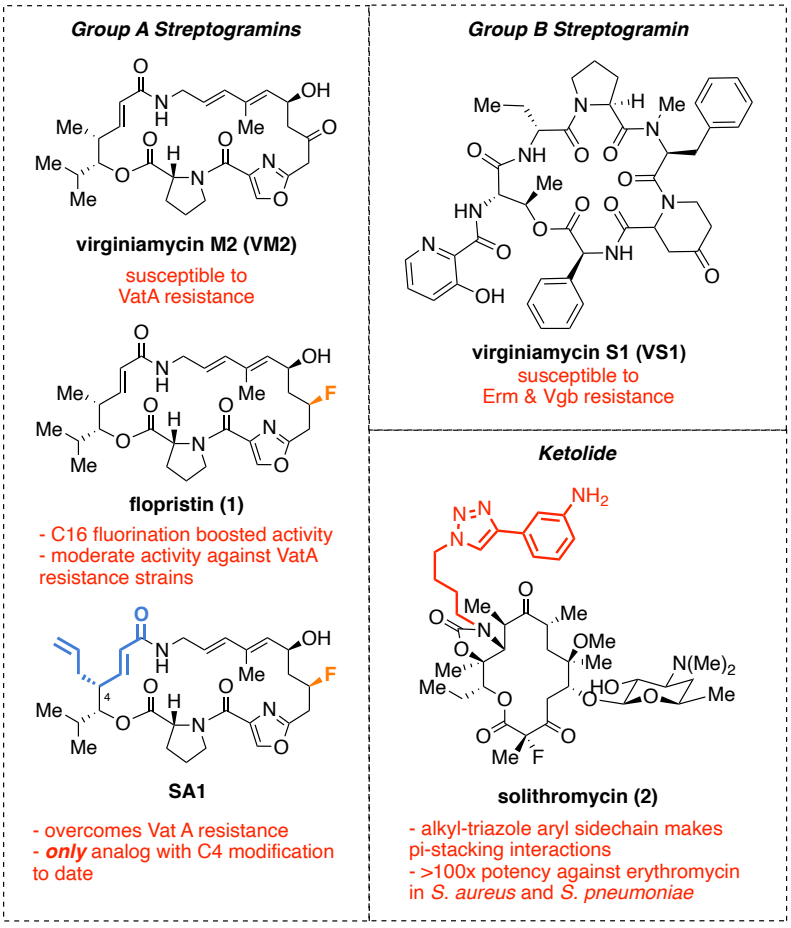


Figure 1.1. Selected natural and synthetic group A and group B streptogramins, along with ketolide antibiotic solithromycin (**2**). Modifications of parent scaffold are highlighted: C16 fluorination of flopristin (**1**) and **SA1** are highlighted in orange, C4-modified sidechain of **SA1** highlighted in blue, and alkyl-triazole-aryl sidechain of solithromycin (**2**) highlighted in red.

Growing antibiotic resistance has rendered many antibiotics, including streptogramins, ineffective. One major resistance mechanism to group A streptogramins involves drug inactivation by virginiamycin O-acetyltransferases (VatA) enzymes, which acetylate the C18 alcohol to disrupt an important hydrogen bond interaction with the phosphodiester backbone of ribosomal RNA at G2505 and decrease their ribosomal binding affinity.[7] Leveraging structural knowledge of VatA resistance and a modular synthesis platform of group A streptogramins, our group synthesized a preliminary library of analogs based on the natural product virginiamycin M2 (**VM2**).[8] Two hit compounds (**SA1** and **SA2**), which also incorporated the C16 fluorine

found in flopristin (**4**), demonstrated potent activity against several bacteria harboring several forms of resistance, including Vat resistance (Figures 1.1, 1.7).

Group B streptogramins have a different resistance profile from group A, and resistance to the B component disrupts synergistic activity, often rendering the combination less effective. For example, ribosome methylation at A2058 caused by *erm* genes, which are widespread due to the pervasive use of macrolide antibiotics, can disrupt group B streptogramin binding (Figure 1.2B).[9] In addition, resistance enzymes such as Vgb lyases that cleave the 17-membered macrocycle can inactivate the B component in bacterial cells.[10] To date, no group B streptogramins have been reported that have overcome these common resistance mechanisms.

Examination of the cryo-EM structure of **SA1** and **VS1** bound to the *E. coli* ribosome reveals that the C4 allyl sidechain of **SA1** points toward **VS1**, nearly coming within van der Waals distance of the C7 aminobutyric acid sidechain (Figure 1.2B). In the absence of a group B streptogramin in the NPET, there is room to extend the C4 sidechain of group A streptogramins to participate in productive binding contacts. In particular, we envisioned addition of an appropriately positioned aryl (or biaryl) group to participate in pi-stacking with the wobble base pair U1782-U2586. A similar strategy has been employed in ketolide antibiotics such as telithromycin and solithromycin (**2**): addition of an aryl–alkyl sidechain (Figure 1.2A) that makes pi-stacking interactions with the A752-U2609 base pair results in substantially enhanced potency over macrolides such as erythromycin.[11,12] Taking inspiration from this precedent, we aimed to synthesize C4-modified group A streptogramins that incorporate aryl-alkyl sidechains of varying lengths and compositions. This would mimic key hydrophobic interactions made by the group B component, enabling a single group A streptogramin analog to achieve high antibacterial activity independently. Notably, the U1782-U2586 wobble base pair is not proximal to A2058 and thus this extension of streptogramin A should not be affected by Erm methylation (Figure 1.2B). Moreover, C4-modified group

A streptogramins would not be substrates for Vgb lyases. Thus, this approach may overcome two resistance mechanisms targeting group B streptogramins.

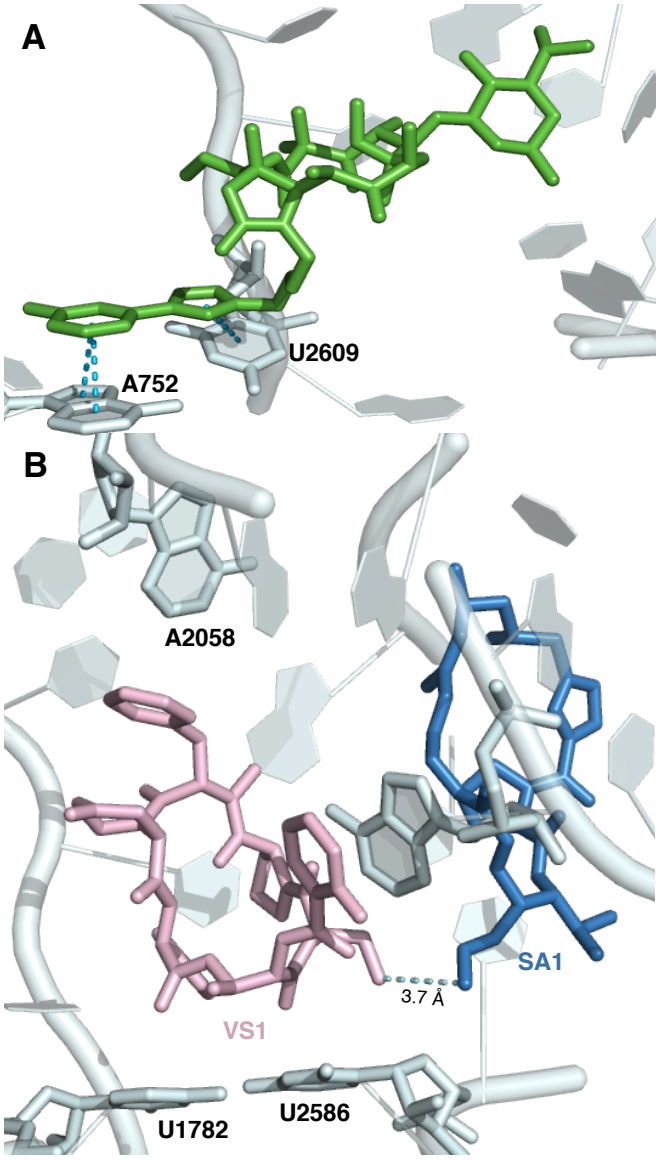


Figure 1.2. A. Cryo-EM structure of ribosome-bound solithromycin (**2**) (green, PDB code 8E3O) reveal the triazole-aryl sidechain picking up pi-stacking interactions with A752-U2609. **B.** Cryo-EM structure of ribosome-bound **SA1** (blue) and **VS1** (pink) (PDB code 6WYV) reveals C4 sidechain pointing towards the binding pocket of VS1.

Herein, we report the design, synthesis, and antibacterial evaluation of novel C4-modified group A streptogramin analogs. We used cryo-EM structures of solithromycin (**2**) and **SA1** bound to the ribosome to guide structure-based drug design. We developed a convergent synthetic route that caters to facile C4

modification and prepared a series of C-4 modified streptogramins in which an aryltriazole was appended with an amide-containing linker. While several analogs in this series inhibited translation in vitro and made the predicted binding contacts as verified by cryo-EM, they showed limited inhibitory activity against bacteria. To address this, we designed a second series with a less polar linker, resulting in reduced topological polar surface area (TPSA). Several members of this series demonstrated potent activity against a broad panel of Gram-positive bacteria, including strains with resistance mechanisms to vancomycin, methicillin, macrolides, and group A and group B streptogramins. This work establishes new structure-activity relationships for group A streptogramins and, more broadly, provides generalizable design principles for antibiotics that bind the catalytic center of the ribosome.

1.2 Results and Discussion

Synthesis of an amide linker series

Our three previous syntheses of group A streptogramins consisted of the assembly of several building blocks into two halves of approximately equal complexity, which could then be coupled, macrocyclized, and deprotected to provide final antibiotic candidates.[8,13–15] In theory, each of the building blocks could be varied, enabling modular replacement of functionality in the final products. In practice, however, the synthesis was not always compatible with additional functionality included in modified building blocks. For example, the vinylogous Mukaiyama aldol reaction that initiated the synthesis of the left half **10** required the preparation of unstable silyl ketene acetals such as **3**, which were purified by distillation and had limited variability (Figure 1.3A). This restricted the ability to modify positions on this building block, resulting in access to only two C4-modified analogs in our previous report.[8] To further explore this portion of the molecule and to test our hypothesis of group B streptogramin replacement, we designed a new synthesis of the left half (**17**) of group A streptogramins that permits expanded functionalization at C4 (Figure 1.3B).

We chose to include an alcohol as a functional handle for late-stage derivatization, shielding it with a benzyl group throughout the synthesis. Our route of the C4-modified left half **17** commenced with an Evans aldol addition between isobutryaldehyde (**4**) and oxazolidinone **11** (available in 3 steps from 1,4-butanediol) to provide aldol product **12** in 97% as a single diastereomer.[16] Hydrolysis of the Evans auxiliary yielded the corresponding carboxylic acid, which was converted to Weinreb amide **13** in 85% yield (2 steps) via coupling with N,O-dimethylhydroxylamine. Reduction with diisobutylaluminum hydride (DIBAL) furnished the crude aldehyde, which was immediately subjected to a Horner-Wadsworth-Emmons (HWE) olefination at 0°C to afford ester **14** in 71% yield (2 steps) as a single diastereomer.[17]

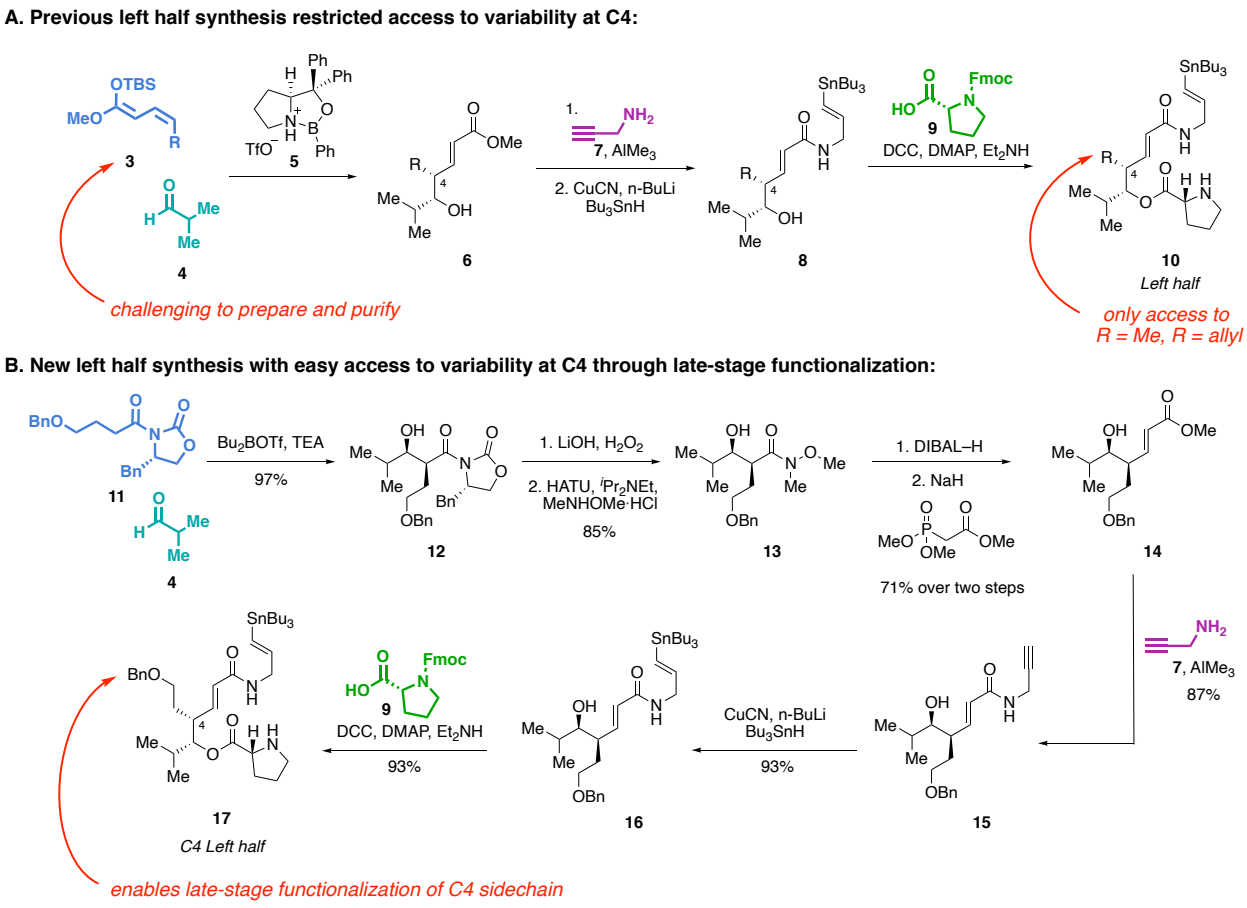


Figure 1.3 A. Previously reported left half synthesis with limited variability at C4 position. **B**. Updated left half synthesis with access to variability at C4 position through late-stage functionalization.

Subsequent transformations followed the previously established synthesis of **VM2** left half.[8,13] Amidation of ester **14** with propargylamine **7** in the presence of trimethylaluminum followed by hydrostannylation of the resulting terminal alkyne in **15** afforded vinyl stannane **16** in 81% yield over two steps. Final esterification of vinyl stannane **16** with Fmoc-protected D-proline **9** using DCC and catalytic DMAP, followed by addition of diethylamine, afforded the complete C4-modified left half **17** in 93% yield. This eight-step sequence proceeded in 44% overall yield from building blocks **4** and **11**, and provided decagrams of **17**.

The previously developed route to the right half was designed to access group A streptogramins with a ketone at the C16 position, such as the natural product **VM2**.[13,14] This enabled the synthesis of over 50 C16-keto analogs. Inspired by flopristin (**1**), which incorporated a fluorine at C16 and displayed enhanced activity over **VM2**, three analogs were fluorinated using a late-stage three-step sequence (Figure 1.4A). These fluorinated analogs had substantially boosted activity compared to their non-fluorinated versions.[8] However, the requirement for three additional steps at the final stages of the synthesis, including a sensitive diastereoselective reduction and reprotection of the C14 alcohol which would later be deprotected again, inherently limited the number of C16-fluorinated analogs that could be generated without impractical expenditure of additional resources. Specifically, for each modified left half that was synthesized, 7 steps were required to reach final fluorinated analogs. This became particularly costly with left halves that were challenging to acquire on large scale.

Since C16-fluorinated derivatives exhibited consistently higher activity in all strains, we sought to design a synthesis of a prefunctionalized right half that would reduce the number of steps required to install the fluorine at the late stage of the synthesis, thus increasing convergency at the cost of a longer right half synthesis. We developed a synthetic route to the right half (**31**), which contains an alcohol that can be deoxyfluorinated in a single step after the halves are coupled and macrocyclization is achieved. This design reduced the late-stage synthetic burden, requiring only 3 steps to reach final compounds after the coupling

of the halves. A first iteration of this strategy was employed in the synthesis of **SA1**, and here we report an adapted version that enabled the generation of a library of fluorinated group A streptogramin analogs (Figure 1.4B).[8] .

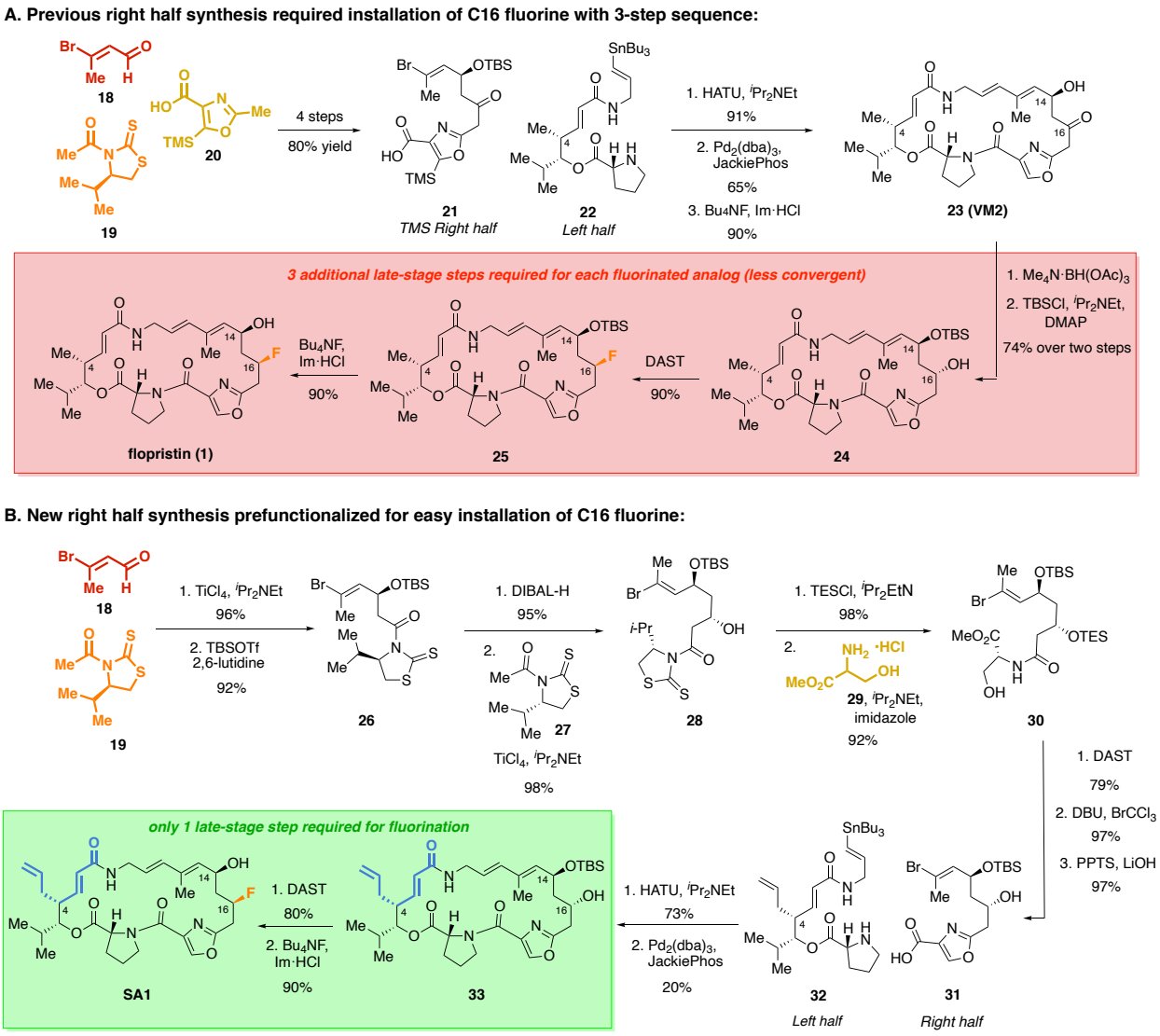


Figure 1.4. A. Previously reported right half synthesis required three late-stage step sequence to C16 fluorination. **B**. Updated right half synthesis prefunctionalized for C16 fluorination in one step.

Our synthesis of right half **31** commenced with the aldol coupling of (*E*)-3-bromobut-2-enal (**18**, available in 3 steps from crotyl alcohol) and acetyl thiazolidinethione **19** (2 steps from (R)-valinol) to afford aldol product in 96% yield as a single diastereomer.[18–21] Subsequent protection of the secondary alcohol with

tert-butyldimethylsilyl trifluoromethanesulfonate (TBSOTf) afforded *tert*-butyldimethylsilyl (TBS)-protected alcohol **26**. Reduction of TBS-protected aldol product **26** with DIBAL furnished aldehyde in 95% yield, which was subjected to another aldol coupling with acetyl thiazolidinethione **27** (2-steps from (S)-valinol) to afford aldol product **28** in 96% yield as a single diastereomer. Protection of the newly formed secondary alcohol using triethylsilylchloride (TESCl), imidazole, and catalytic DMAP afforded triethylsilyl (TES) ether in 98% yield. Displacement of the thiazolidinethione auxiliary with L-serine methyl ester hydrochloride **29** furnished oxazole precursor **30** in 92% yield, which was treated with diethylaminosulfur trifluoride (DAST) and DBU and bromotrichloromethane to afford the cyclized oxazole product in 77% yield over two steps.[22] Global deprotection with pyridinium *p*-toluenesulfonate (PPTS) and lithium hydroxide provided the complete right half **31** in 97% yield. The nine-step sequence proceeded in 54% yield from building blocks **18** and **19**, providing decagrams of **31**.

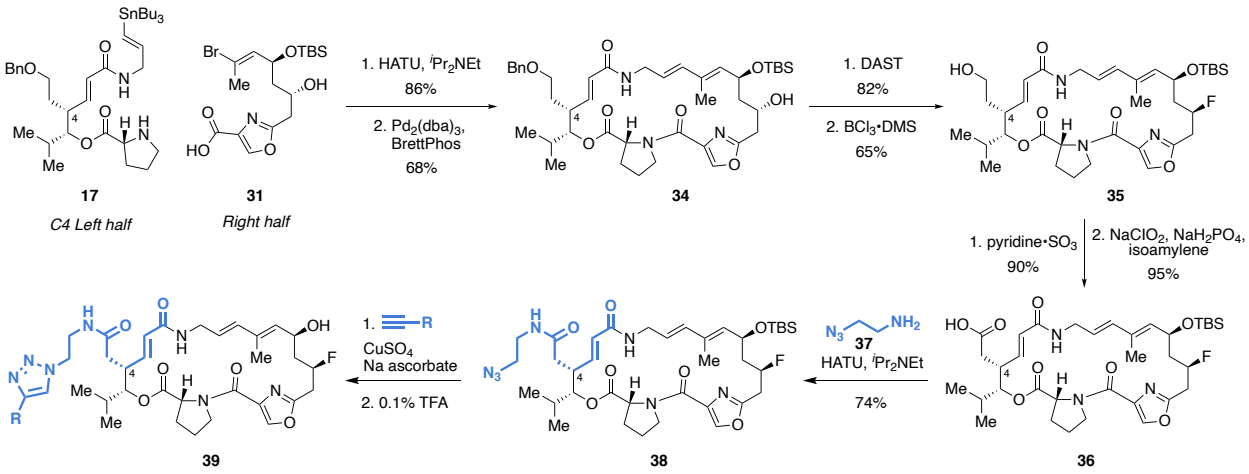


Figure 1.5. Synthesis of C4-modified group A streptogramin analogs. Access to novel analogs **39** with different R substituents.

With an abundant supply of right half **31** and a highly convergent route in hand, we initiated the synthesis of C4-modified, C16-fluorinated group A streptogramins. Coupling of left half **17** with right half **31** using HATU afforded the full linear precursor in 86% yield (Figure 1.5).[8] Macrocyclization via Stille coupling in the presence of tris(dibenzylideneacetone)dipalladium(0) ($Pd_2(dba)_3$) yielded macrocycle **34** in 68% yield.[13]

The C16 fluorine was then installed with DAST, and benzyl deprotection afforded alcohol **35** in 65% yield (2 steps). Sequential oxidation of **35** with Parikh-Doering and Pinnick conditions provided carboxylic acid **36** in 86% yield over two steps.[23,24] Coupling of **36** to 2-azidoethylamine **37** using HATU afforded diversifiable intermediate **38**. We were able to functionalize the azide handle by allowing it to react with various aryl alkynes in the presence of copper(II) sulfate and sodium ascorbate.[25] Final desilylation of the macrocycle yielded a library of novel C4-modified group A streptogramin analogs bearing different aryltriazole sidechains (Figure 1.11).

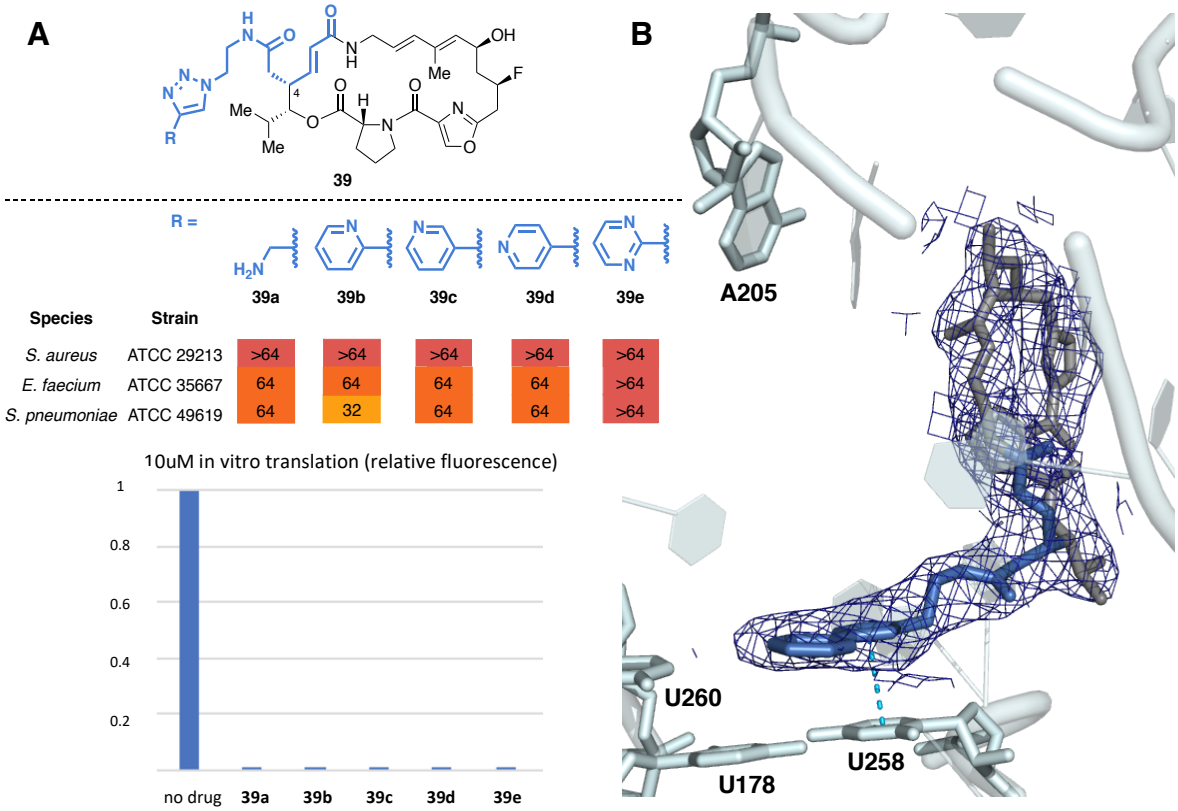


Figure 1.6. **A.** C4 analogs **39** inhibit translation in vitro but are inactive in cells. MIC values (μg/mL) of selected analogs against selected strains. Each MIC was obtained in triplicate. The bottom graph displays in vitro translation that occurs in the presence of 10 μM of each analog (relative to dimethylsulfoxide (DMSO)). **B.** 2.4 Å cryo-EM structure Coulomb potential density map for ribosomes bound to **39d**.

We evaluated a select number of this series for their ability to inhibit protein synthesis in vitro in a transcription-coupled translation assay. Compounds **39a-e** potently inhibited translation at 10 μM,

demonstrating the on-target activity of the analogs (Figure 1.6A). We acquired cryo-EM data that enabled a 2.4-Å model of **39d** bound to the *E. coli* ribosome, revealing the predicted pi-stacking of the triazole-pyridyl group (in blue) with U2586 and U1782, showing the importance of the aryl rings at the C4 extension (Figure 1.6B). Despite the favorable binding and in vitro inhibitory activity of these analogs, only a few analogs were able to inhibit the growth of bacteria at high concentrations (32-64 μg/mL**,** Figure 1.6A, Table 1.1 and 1.2**)**.

The discrepancy between the in vitro and in vivo activities of these analogs suggests that factors affecting cellular accumulation may be hindering the ability of analogs to reach and engage the ribosome within bacterial cells. One key barrier that limits cellular entry is membrane permeability. Although many antibiotics violate Lipinski's Rule of Five – given the distinct nature of bacterial versus eukaryotic cellular envelopes – properties such as high polar surface area (PSA) can still adversely impact permeability and cellular entry, especially for molecules that are too large to enter bacteria through channels such as porins.[26,27] In addition to permeability, active efflux is a major determinant of intracellular drug concentrations and a common antibiotic resistance mechanism.[28] Bacterial efflux pumps can reduce the effectiveness of antibiotics by actively transporting them out of the cell, thereby limiting their activity. Despite designing analogs with favorable binding and high on-target activity, decreased entry or increased efflux can lead to poor cellular activity.

The lack of cellular activity observed in these analogs is likely to arise from two possibilities: (1) the analogs lack cellular permeability, or (2) the analogs are not accumulating due to a combination of efflux and permeability. We hypothesized that the amide functional group connecting the alkyl-triazole sidechain to the macrocycle may be increasing the exposed polar surface area (EPSA) of the analogs, reducing membrane permeability and hindering cellular entry. Unfortunately, PAMPA assays are known to be poor predictors of bacterial cell accumulation, which have different cellular envelopes than eukaryotic cells and

robust mechanisms for efflux of small molecules.[29] Therefore, prediction bacterial permeability often necessitates alternative approaches, like considering PSA of the molecules.

Using TPSA for Analog Design

Calculated PSA is often used as a predictive metric for cell permeability, where higher PSA correlates with poor cell membrane permeability. Two ways of estimating PSA include topological PSA (TPSA) and exposed PSA (EPSA). TPSA is a 2D descriptor calculated from the surface contributions of the polar atoms in the molecule.[30,31] In contrast, EPSA is a 3D descriptor that incorporates conformational effects, such as intramolecular hydrogen bonds, dipole moments, and steric shielding, to measure polarity.[32] While EPSA provides a more accurate representation of a molecule's polarity by including its 3D conformation in the calculation, it is more challenging to calculate and may vary substantially depending on the solvent and other aspects that induce conformational bias, especially in compounds with many rotatable bonds. Computational methods estimate EPSA through TPSA and conformational analysis, and experimental methods require the use of a supercritical fluid chromatography (SFC) technique to measure EPSA, which may not always reflect the conformation of the molecule that is relevant to cellular entry.[33] Since TPSA does not require complex 3D conformational analysis and is readily calculated, TPSA is commonly used as a practical approximation of PSA in drug design.

We calculated the TPSA values for hit compounds **SA1** and **SA2,** as well as for analog **39d**. **SA1** and **SA2,** which exhibit antibacterial activity and thus are assumed to efficiently enter bacteria, have calculated TPSA values of 121 $Å^2$ and 173 $Å^2$. In contrast, analog **39d,** which lacks cellular activity, has a higher TPSA of 195 $Å^2$. (Figure 1.7) These results suggest that there may be a TPSA threshold or cutoff <195 $Å^2$ in which cell penetration is favorable.

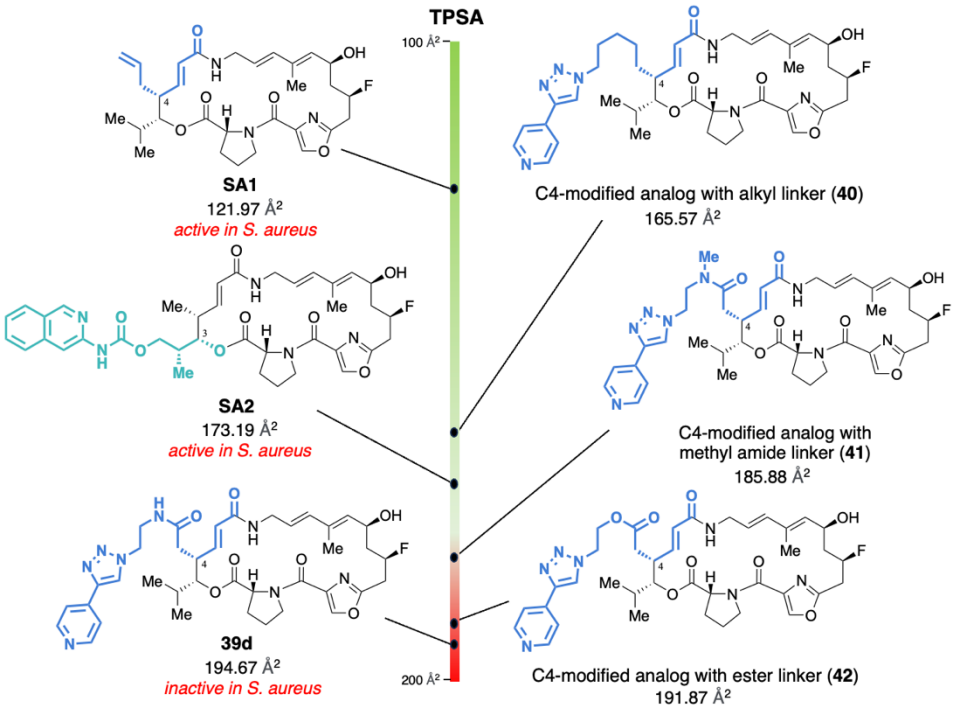


Figure 1.7. Calculated TPSA (Å^2) of **SA1**, **SA2**, **39d**, and amide bioisosteres (**40**, **41**, **42**).

Focusing on our initial hypothesis, we calculated TPSA values for analogs with isosteric replacements for the amide group in the C4 linker. *N*-methylated amide (**41**) and ester substitutions (**42**) have shown to be the simplest and most effective methods in reducing EPSA of amide-containing compounds, reducing EPSA by around 12-15 Å^2.[32] We initially pursued the synthesis of the ester linker series (**43** and **44**) by esterification of alcohol **35** with azide-containing carboxylic acids. In parallel, we explored cyclic amides (**45** and **46**), derived from amidation of alcohol **35** with azide-functionalized N-pyrrolidine and N-piperidine, as a strategy to mask the amide similarly to N-methylation. Additionally, we hypothesized that introducing a nonpolar aryl substituent, such as phenyl, into analogs **39**, **43** – **46** could further reduce TPSA to improve cellular activity. However, consistent with the amide linker series, only a few analogs were able to inhibit the growth of bacteria (*S. aureus*) at high concentrations (64 μg/mL, Figure 1.8).

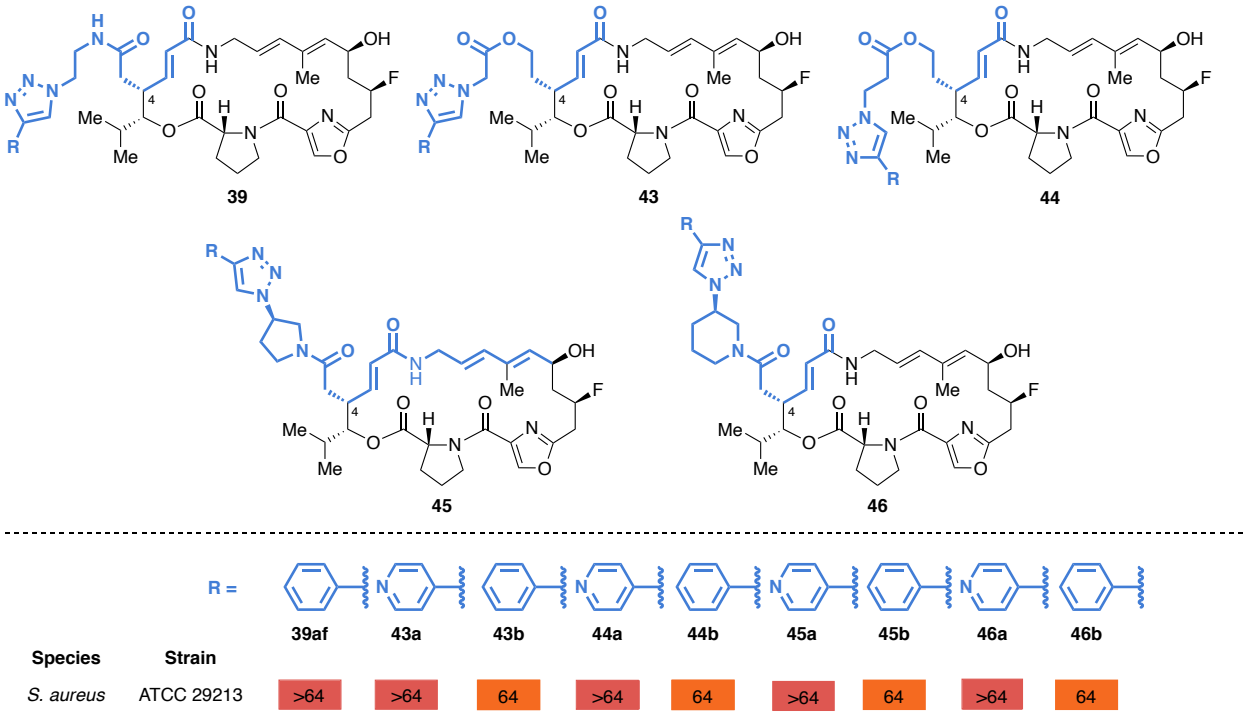


Figure 1.8 MIC values for C4 amide linker analog **39af**, ester linker analogs (**43**, **44**) and cyclic amide linker analogs (**45**, **46)** against *S. aureus* ATCC 29213. Each MIC was obtained in technical triplicate.

Since the cyclic amide and ester substitutions did not improve cellular activity, we explored alternative amide replacements. Given that the initial C4 analog **SA1** —bearing an allyl group— showed enhanced activity, replacing the amide with a full carbon chain (**40**) could also improve both permeability and activity. Of all the different amide bioisostere replacements, we found that replacing the amide with an alkyl chain (**40**) reduced TPSA the most (165 $Å^2$ for a 4-pyridyltriazole sidechain), placing it within the TPSA range of the two active hit compounds **SA1** and **SA2** (Figure 1.7). With that in mind, we decided to synthesize a new series of analogs featuring alkyl chain replacements, including a four-carbon and five-carbon linker.

Synthesis of an alkyl linker series

Our synthesis of the alkyl linker series followed a strategy analogous to that of the amide linker series, with specific adjustments made to accommodate the azide-containing sidechain (Figure 1.9A). The synthesis

commenced with construction of the 5-carbon linker with an Evans aldol addition between oxazolidinone **47** and isobutryaldehyde **4.** $TiCl_4$, N,N-diisopropylethylamine (DIPEA), and NMP were used in place of Bu_2BOTf, as $TiCl_4$ offers a cost-effective alternative without compromising yield (85%).[34] Reduction of the resulting Weinreb amide **49** with DIBAL proved to be ineffective for aldehyde formation. Even with increased loading of DIBAL, extended reaction times, and alternative solvent systems, the reaction resulted in minimal conversion to aldehyde. Switching to lithium aluminum hydride ($LiAlH_4$), a stronger reductant, led to partial conversion to aldehyde and some over-reduction to the alcohol. Unreacted Weinreb amide **49** could be recovered during purification and resubjected to $LiAlH_4$ reduction, which increased aldehyde yields from 67% to 80%.

Amidation of ester **50** with propargylamine (**7**) in the presence of triethylaluminum provided terminal alkyne in **51**. Hydrostannylation of this alkyne initially employed copper catalysis, however this led to a primary amine as a major byproduct, likely due to copper(I)-mediated reduction of the azide function. To preserve the azide, we adopted the palladium-catalyzed protocol developed by Darwish et al., utilizing $Pd_2(dba)_2$, Cy_3PHBF_4, and DIPEA, which enabled regioselective formation of the desired trans-vinyl stannane **52** without azide reduction.[35] Initial efforts to purify vinyl stannane **52** through column chromatography was hindered by its instability on silica.[36,37] The addition of triethylamine to the solvent system mitigated the decomposition and improved yields from 32% to 59%.

Initial coupling of the C4-modified 5C linker left half **53** with right half **31** using HATU resulted in modest yield (47%). Substitution of HATU with PyAOP increased the yield to 59%. Stille macrocyclization with $Pd_2(dba)_3$ in the presence of BrettPhos provided macrocycle **54** in 58% yield.[13,38] Deoxyfluorination at C16 provided fluorinated macrocycle **55** in 80% yield. Importantly, the reduced step count in these late stages of the redesigned synthesis enabled gram quantities of **55** to be synthesized from similar amounts of left half **53**, obviating the need for a decagram synthesis of this half.

Synthesis of the shorter 4-carbon linker-containing left half **60** followed the same route as the 5-carbon linker left half **53**, but starting with Evans addition using oxazolidinone **57**, which was readily obtained in one step from 6-azidohexanoic acid (Figure 1.9B). Left half **60** was carried forward to diversifiable macrocycle **63**.

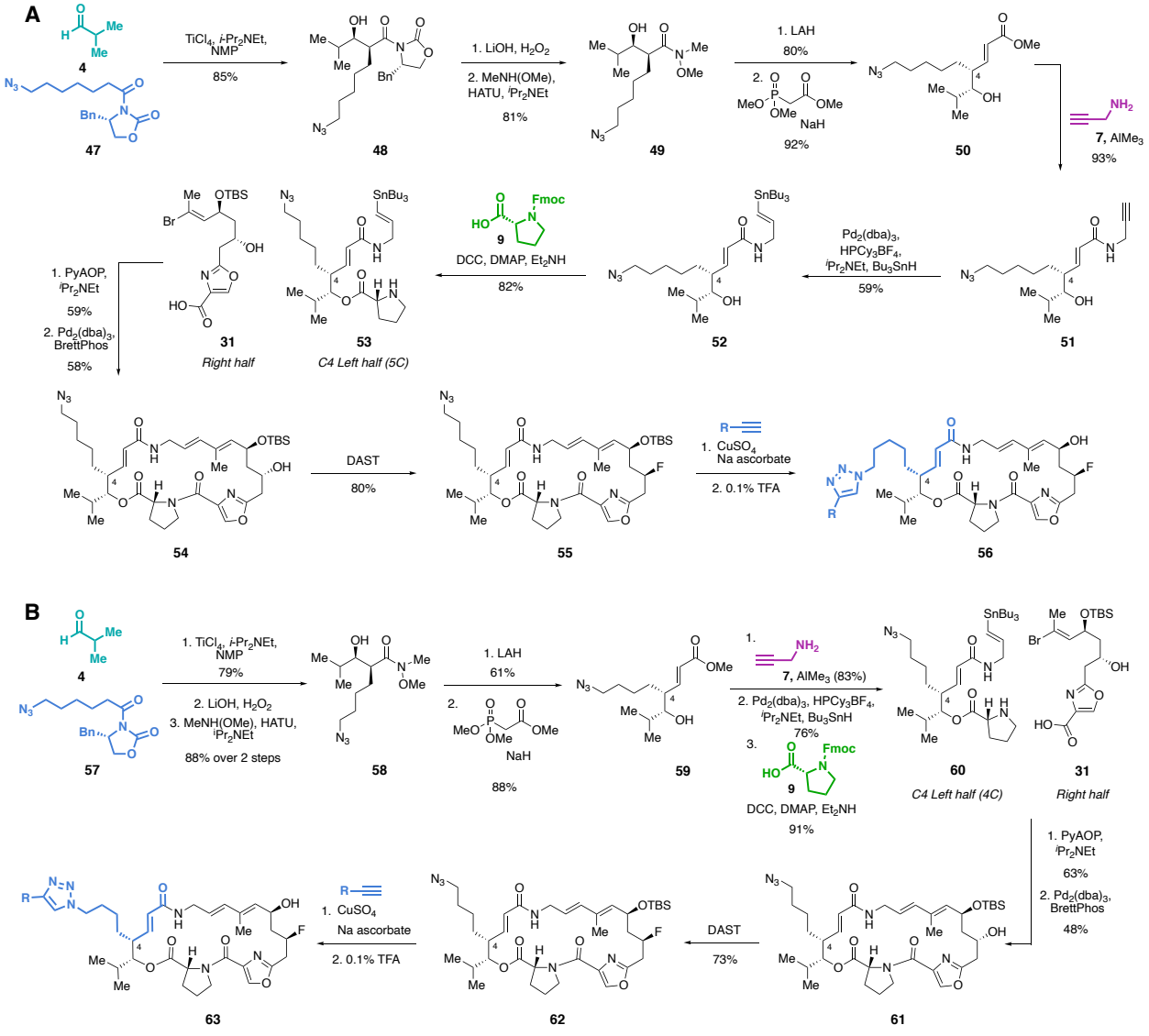
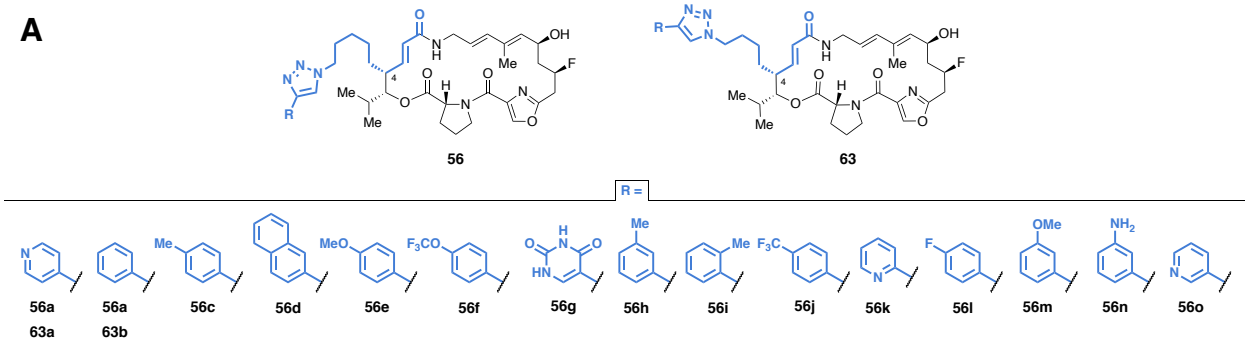


Figure 1.9. A. Synthesis of C4-modified 5C linker analogs, with access to novel analogs **56**. **B.** Synthesis of C4-modified 4C linker analogs, with access to novel analogs **63**.

We subjected azides **55** and **62** to click chemistry conditions to synthesize a library of C4-alkyl-aryl analogs. Specifically, azide **55** was reacted with 15 commercially available aryl alkynes, and azide **62** was reacted with 11 aryl alkynes, resulting in 26 novel streptogramin analogs bearing triazole-aryl sidechains. (Figure 1.12) Compared to the amide linker series, the alkyl linker series incorporated more hydrophobic aryl substituents to reduce overall polarity. Additionally, structural diversity at various positions of the aryl ring was introduced to assess substituent tolerance at each position. (Figure 1.10A)

A



B

Minimal Inhibitory Concentration (MIC) Values (μg/mL)

strain:	*E. faecium* ATCC 35667	*E. faecium* VREfm (vanA) MMX 0752	*S. aureus* CLSI QC ATCC 29213	*S. aureus* Streptogramin A and B resistance (VatA, Vgb) MMX 10227	*S. aureus* MLSb ErmA resistance; inducible MMX 2321	*S. aureus* MLSb ErmA resistance; constitutive MMX 3035	*S. aureus* MLSb ErmC resistance MMX 3037	*S. aureus* MRSA USA 300	*S.pneumoniae* PSSP; CLS QC ATCC 49619	*S. pneumoniae* mef(A) MMX 3032	*S. pneumoniae* erm(B) MMX 3033
Compound ID											
VM2	2	16	16	64	16	8	4	16	16	8	64
flopristin	0.25	1	0.5	2	0.5	0.5	0.25	0.5	1	0.5	2
VS1	8	32	32	>64	32	>64	>64	16	4	4	32
flopristin + VS1	≤0.06	0.12	≤0.06	1	≤0.06	0.12	≤0.06	≤0.06	≤0.06	0.12	≤0.06
56a	2	8	2	8	4	4	1	4	1	1	4
56b	2	8	2	4	4	4	1	4	4	4	16
56c	1	4	4	4	4	2	0.5	2	4	2	8
56d	>64	>64	>64	>64	>64	>64	2	>64	>64	32	>64
56e	4	16	8	16	8	8	2	8	8	8	32
56f	>64	>64	>64	>64	>64	>64	2	>64	>64	>64	>64
56g	16	8	32	64	64	32	8	32	1	1	8
56h	2	>64	4	4	4	2	1	4	4	2	16
56i	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
56j	4	16	>64	>64	>64	>64	2	>64	>64	>64	>64
56k	2	16	8	8	8	4	1	4	4	2	16
56l	2	16	8	8	4	4	0.5	4	8	4	16
56m	4	>64	8	8	8	4	1	4	4	4	16
56n	4	>64	8	16	8	4	1	8	4	4	16
56o	8	>64	32	64	16	16	4	16	8	8	32
63a	2	32	8	32	16	8	4	8	2	2	8
63b	1	8	2	4	4	2	1	2	2	2	8

Figure 1.10. A. Library of C4-modified alkyl linker analogs **56** and **63**. **B.** MIC values (μg/mL) for selected analogs against an expanded panel of pathogens. Each MIC was obtained in technical triplicate.

Antibacterial efficacy

We evaluated the antibacterial activity of 17 C4-alkyl-aryl group A streptogramin analogs, the natural products **VM2** and **VS1**, the semisynthetic group A streptogramin flopristin (**1**), and the combination of flopristin (**1**) with **VS1** against a panel of 18 pathogens (Figure 1.10). These alkyl linker analogs showed an overall improvement in activity compared to the amide linker series (Figure 1.10B). Replacing the polar amide group with a full carbon chain restored antibacterial activity, supporting our hypothesis that reducing TPSA can improve cellular permeability and efficacy.

Although none of the alkyl linker series surpassed activity of flopristin (**1**) or its combination with **VS1**, they exhibited greater potency than the naturally occurring streptogramins **VM2** and **VS1**. The 5-carbon linker series **56** demonstrated enhanced activity compared to the 4-carbon linker series **63** (Table 1.3). This trend is evident when comparing the 5-carbon linker **56a** (4-pyridyl) to its 4-carbon linker counterpart **63a** (4-pyridyl). In contrast, the 5-carbon linker **56b** (phenyl) and 4-carbon linker **63b** (phenyl) exhibited comparable activity. Within the 5-carbon series, subtle modifications to the aryl ring led to significant changes in activity. For instance, analogs **56a** (4-pyridyl) and **56k** (2-pyridyl) showed better activity than **56o** (3-pyridyl). Additionally, **56c** (p-tolyl) and **56h** (m-tolyl) showed excellent activity, whereas **56j** (o-tolyl) completely lost activity, suggesting that para and meta substitutions were better tolerated that ortho substitutions. However, certain substituents at the para position – such as $-OCF_3$ in **56f** and $-CF_3$ in **56j**, and the naphthyl group in **56h** – led to significantly reduced activity across multiple strains, possibly due to steric clashes with U2609 in the binding pocket. Interestingly, the polar uracil ring in **56g** had the potential to pick up an additional interaction with U2609, but this was not borne out in its cellular activity. The increased polarity from the uracil ring may be reducing permeability and cellular entry, thereby diminishing its overall activity.

The most potent analogs – **56a**, **56c**, and **63b** – demonstrated superior activity compared to **VM2** against multiple bacterial strains. These analogs retained activity against strains resistant to both group A and group

B streptogramins, including a 3- to 4-fold increase in potency against VatA/Vgb *S. aureus* relative to **VM2** (64 vs 4-8 μg/mL) and **VS1** (>64 vs 4-8 μg/mL) as well as a 64- to 128-fold increase in potency against ErmC *S. aureus* relative to **VS1** (>64 vs 0.5-1 μg/mL). These results highlight the ability of these group A streptogramins, with extensions that reach into the group B binding site, in overcoming resistance mechanisms that target both group A and group B streptogramins.

While the alkyl linker series addressed limitations in cellular activity, further optimization is required to achieve a highly potent group A streptogramin with C4 derivatization. The conformational flexibility of the alkyl linker could be impacting activity by increasing the entropic penalty of binding, and future iterations could benefit from rigidification of the linker. Given the observed tolerance for ring size and substitution patterns, additional modifications – such as meta-substituted aryl rings or compact 5-membered aromatic rings – could enhance binding. Continued optimization, guided by cryo-EM characterization, may lead to the design of a highly active group A streptogramin capable of independent antibacterial activity without the need for group B synergy.

1.3 Conclusion

Through an integrated approach combining structure-based drug design, modular chemical synthesis, antibacterial evaluation, and high-resolution cryo-EM, we developed a new class of group A streptogramins. Incorporation of an alkyl-aryl sidechain at the C4 position resulted in new binding interactions within the ribosome exit tunnel. Early designs utilized an amide-containing linker to incorporate the sidechain, but increased polarity from the amide impaired cell permeability and therefore limited antibacterial activity. Replacement of the amide linker with a nonpolar, alkyl linker restored activity, and several analogs of the alkyl linker series demonstrated efficacy against resistant strains, including *S. aureus* with VatA and VgB resistance. This proof-of-concept establishes a design framework for developing group A streptogramins as potent monotherapies. Continued design, optimization, and synthesis of group A streptogramins will expand the streptogramin class and contribute to the antibiotic pipeline.

1.4 General experimental procedures, materials, and instrumentation

General experimental procedures: All reactions were performed in oven-dried glassware fitted with rubber septa under a positive pressure of nitrogen or argon, unless otherwise noted. Procedures were conducted at 23ºC unless otherwise noted. All reaction mixtures were stirred throughout the duration of each procedure using Teflon-coated magnetic stir bars. Air- and moisture-sensitive liquids were transferred by means of syringe or stainless-steel cannula. Solutions were concentrated by rotary evaporation at or below 35°C. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25-mm, 60-Å pore size, 230−400 mesh, SILICYCLE INC) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV), and then were stained by submersion in a basic aqueous solution of potassium permanganate or with an acidic ethanolic solution of anisaldehyde, followed by brief heating.

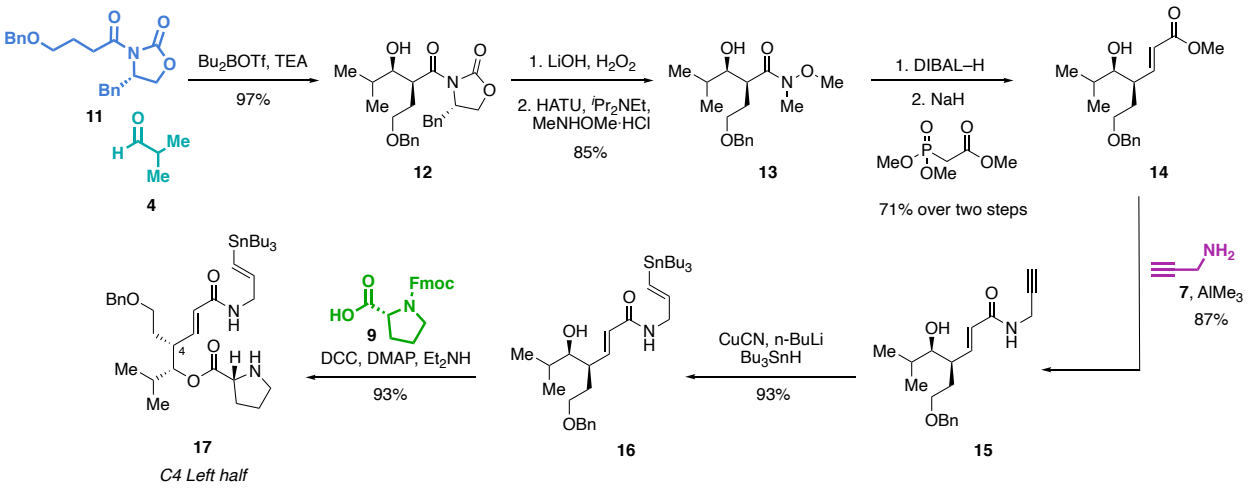
Materials: Dichlorometane (DCM), tetrahydrofuran (THF), and acetonitrile (MeCN) to be used in anhydrous reaction mixtures were dried by passage through activated alumina columns immediately prior to use. Anhydrous toluene, $^{i}Pr_2EtN$, and Et_3N were purchased from Sigma Aldrich in Sure/Seal^{TM} bottles. Hexanes used were ≥85% *n*-hexane. Other commercial solvents and reagents were used as received, unless otherwise noted.

Instrumentation: Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on 300 or 400 MHz Bruker Avance III HD 2-channel instrument NMR spectrometers at 23°C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CHC1_3$: δ 7.26 and CHD_2OD: δ 3.31). Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonance of the NMR solvent ($CDC1_3$: δ 77.2 and CD_3OD: δ 49.0). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet, br = broad, app = apparent), integration, and coupling constant (J) in hertz

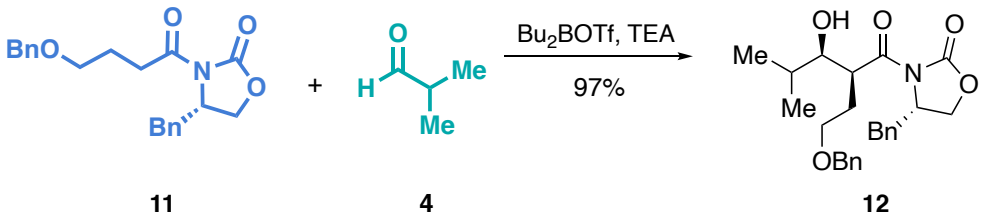
(Hz). High-resolution mass spectra (HRMS) were obtained at the QB3/Chemistry Mass Spectrometry Facility at University of California, Berkeley using a Thermo LTQ-FT mass spectrometer or a Waters Acquity UPLC/Xevo G2-XS QTOF mass spectrometer. HPLC purification was conducted on a Waters Delta Prep 4000 preparative HPLC using a Gemini®-NX (5μm, C18, 110Å, 30.00 mm i.d. x 100 mm) column at a flow rate of 45 mL/min.

1.5 Experimental procedures for synthetic compounds

Scheme I. Preparation for C-4 modified left half 17



Preparation of alcohol **12**



An oven-dried 250-mL round-bottom flask containing oxazolidinone **11** (5.95 g, 16.8 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Then the vessel was sealed with a rubber septum. Dry DCM (84 mL) was added, resulting in a yellow solution, and the vessel was cooled to -78 °C by means of a dry ice-acetone bath. TEA (3.1 mL, 21.9 mmol, 1.3 equiv) was added, followed by a 1 M solution of Bu_2BOTf (20.2 mL, 20.2 mmol, 1.2 equiv) in DCM, resulting in a colorless solution. After 30 min, the reaction mixture was stirred at 0 °C for 90 min. Then the vessel was re-cooled to -78 °C, and isobutyraldehyde (**4**, 3.64 g, 50.5 mmol, 3 equiv) was added via syringe pump over 30 min. After 2 h, the vessel was warmed to 0 °C, the reaction mixture was then cautiously quenched dropwise with pH = 7 phosphate buffer and MeOH (1:3, 15.6 mL), followed by 30% H_2O_2 maintaining the internal temperature between 0-5 °C. After 1 h, the resulting biphasic mixture was transferred to a separatory funnel,

and the layers were separated. The aqueous layer was extracted with DCM (2 × 30 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc: hexanes = 1:10 to 1:2.5) to afford alcohol **12** (6.84 g, 96%) as a colorless oil.

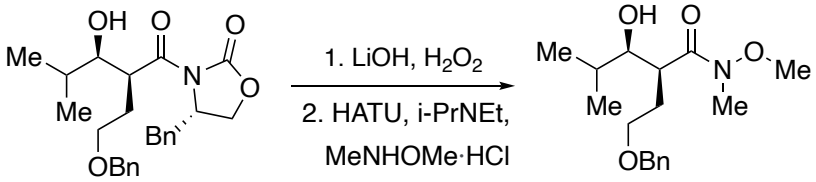
TLC (EtOAc:hexanes = 1:5): R_f = 0.20 (UV).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.23 (m, 8H), 7.16 – 7.09 (m, 2H), 4.58 (ddt, *J* = 10.6, 7.7, 2.9 Hz, 1H), 4.52 (d, *J* = 11.6 Hz, 1H), 4.48 (d, *J* = 11.6 Hz, 1H), 4.33 (ddd, *J* = 9.7, 4.1, 3.0 Hz, 1H), 4.12 (t, *J* = 8.2 Hz, 1H), 4.03 (dd, *J* = 9.1, 2.6 Hz, 1H), 3.72 – 3.59 (m, 2H), 3.56 (dt, *J* = 7.6, 3.9 Hz, 1H), 3.18 (dd, *J* = 13.4, 3.2 Hz, 1H), 2.85 (d, *J* = 3.9 Hz, 1H), 2.29 (dddd, *J* = 14.7, 9.8, 8.0, 5.1 Hz, 1H), 2.12 (dd, *J* = 13.4, 10.5 Hz, 1H), 2.02 (dtd, *J* = 14.5, 4.9, 2.9 Hz, 1H), 1.83 – 1.71 (m, *J* = 6.8 Hz, 1H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.99 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 153.2, 138.2, 135.8, 129.4, 128.9, 128.4, 128.2, 127.8, 127.2, 77.0, 73.4, 69.1, 66.0, 55.8, 43.6, 37.2, 31.4, 27.0, 19.3, 18.6.

HRMS-ESI m/z calcd for $C_{25}H_{32}NO_5^+$ $[M + H]^+$ 426.2275, found 426.2277.

Preparation of Weinreb amide **13**



12 **13**

30% H_2O_2 (7.9 mL, 77.6 mmol, 5 equiv), followed by LiOH (1.95 g, 46.5 mmol, 3 equiv), was added to a solution of alcohol **12** (6.60 g, 15.5 mmol, 1 equiv) in THF-H_2O (120 mL- 40 mL) at 0 ºC. The resulting white suspension was warmed to 23 °C slowly. After 3 h, THF was concentrated under vacuum, and water

(100 mL) and EtOAc (100 mL) were added. The resulting biphasic solution was transferred to a separate funnel, and the layers were separated. The aqueous layer was washed with EtOAc (3 x 50 mL), and the resulting organic layer was discarded. The aqueous layer was then acidified by 2 M HCl to pH = 2. The resulting biphasic solution was transferred to a separate funnel, and the layers were separated. The aqueous layer was abstracted with EtOAc (3 × 50 mL), and the combined organic layer was washed with water (100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4), and the dried solution was concentrated under vacuum. The resulting crude acid was used for the next step without further purification.

Crude acid (3.58 g, 13.4 mmol, 1 equiv), $^{i}Pr_2EtN$ (7.0 mL, 40.3 mmol, 3 equiv), Weinreb amine (2.62 g, 26.9 mmol, 2 equiv), and DCM (134 mL) were added to an oven-dried 250-mL round-bottom flask. HATU (6.39 g, 16.8 mmol, 1.25 equiv) was added in one portion to the resulting colorless solution at 23 ºC. After 3 h, the mixture was transferred to a separatory funnel and washed with water (2 × 50 mL) and brine (50 mL). The washed solution was dried (Na_2SO_4), the dried solution was filtered. The filtrate was concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:6 to 1:4) to Weinreb amide **13** (3.54 g, 85% over two steps) as a colorless oil.

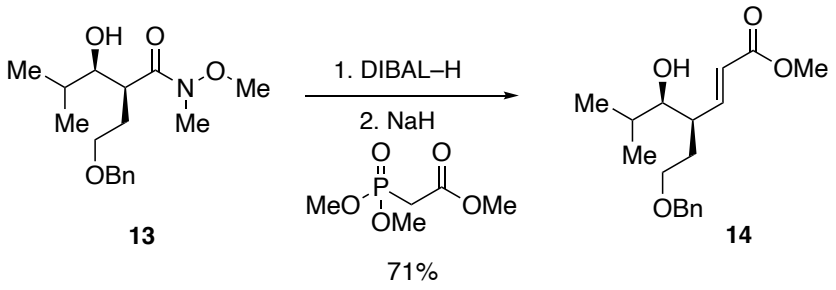
TLC (EtOAc:hexanes = 1:5): R_f = 0.50 (UV).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 – 7.24 (m, 5H), 4.50 (d, *J* = 11.9 Hz, 1H), 4.46 (d, *J* = 11.9 Hz, 1H), 3.64 (s, 3H), 3.59 (dt, *J* = 9.6, 5.5 Hz, 1H), 3.52 (br s, 1H), 3.50 – 3.41 (m, 2H), 3.41 – 3.33 (m, 1H), 3.15 (s, 3H), 2.19 – 2.05 (m, 1H), 2.01 – 1.88 (m, 1H), 1.85 – 1.66 (m, 1H), 1.03 (d, *J* = 6.6 Hz, 3H), 0.92 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.3, 138.4, 128.3, 127.6, 127.5, 77.3, 72.8, 68.6, 61.4, 39.4, 32.1, 30.9, 25.9, 19.2, 18.9.

HRMS-ESI m/z calcd for $C_{17}H_{28}NO_4^+$ $[M + H]^+$ 310.2013, found 310.2015.

Preparation of methyl ester **14**



A 100-mL round-bottom flask containing Weinreb amide **13** (3.00 g, 9.70 mmol, 1 equiv) was evacuated and flushed with nitrogen (the process of nitrogen exchange was repeated a total of 3 times). Dry DCM (97 mL) was added, and the resulting clear solution was cooled to -78 ºC by means of a dry ice/acetone bath. A solution of DIBAL-H in DCM (1.0 M, 29.1 mL, 29.1 mmol, 3 equiv) was added dropwise to this solution. After 1 h, **13** was consumed as indicated by TLC analysis, and MeOH (10 mL) was carefully added (CAUTION: Gas evolution!), followed by saturated aqueous potassium sodium tartrate solution (50 ml). The mixture was allowed to warm to 23 ºC. After 1.5 h, the biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with DCM (2 × 30 mL). The combined organic layers were washed with water (2 × 50 mL) and brine (50 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The crude aldehyde was used for next step immediately without further purification.

A separate oven-dried 1000-mL round-bottom flask containing 60% NaH (1.55 g, 38.8 mmol, 3 equiv) was evacuated and flushed with nitrogen (the process of nitrogen exchange was repeated a total of 3 times). Dry THF (750 mL) was added, and the resulting suspension was cooled to 0 ºC by means of an ice/water bath. Trimethyl phosphonoacetate (6.28 mL, 38.8 mmol, 4 equiv) was added dropwise at 0 ºC. After 1 h, a solution of the above aldehyde in THF (2 mL) was added. After 2 h, saturated aqueous ammonium chloride solution (100 mL) was carefully added, and the resulting biphasic mixture was transferred to a separatory funnel. The layers were separated, and the aqueous layer was extracted with diethyl ether (2 × 30 mL). The combined organic layers were washed with water (2 × 50 mL) and brine (50 mL), and the washed solution

was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:5) to afford methyl ester **14** (2.10 g, 71% yield over 2 steps) as a colorless oil.

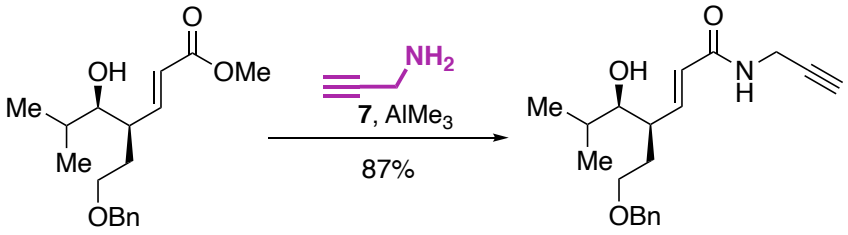
TLC (EtOAc:hexanes = 1:5): R_f = 0.50 (UV).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 – 7.27 (m, 5H), 6.83 (dd, *J* = 15.7, 9.9 Hz, 1H), 5.84 (d, *J* = 15.7 Hz, 1H), 4.53 (d, *J* = 11.9 Hz, 1H), 4.48 (d, *J* = 11.9 Hz, 1H), 3.75 (s, 3H), 3.55 (dt, *J* = 9.4, 5.4 Hz, 1H), 3.43 (td, *J* = 9.0, 4.8 Hz, 1H), 3.34 (ddd, *J* = 7.7, 5.8, 4.2 Hz, 1H), 2.60 – 2.49 (m, 1H), 2.30 (d, *J* = 5.9 Hz, 1H), 2.09 (dddd, *J* = 14.1, 8.9, 5.4, 3.8 Hz, 1H), 1.77 – 1.65 (m, 2H), 0.97 (d, *J* = 6.8 Hz, 3H), 0.88 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 150.0, 138.2, 128.5, 127.9, 127.8, 122.0, 78.1, 73.2, 67.8, 51.6, 44.0, 31.0, 30.3, 20.2, 15.4.

HRMS-ESI m/z calcd for $C_{17}H_{27}O_4^+$ $[M + H]^+$ 307.1904, found 307.1895.

Preparation of alkyne **15**



14 **15**

Propargylamine **7** (1.80 mL, 27.4 mmol, 4 equiv) and dry DCM (115 mL) were added to a 500-mL round-bottom flask under nitrogen. The resulting colorless solution was cooled to 0 ºC by means of an ice/water bath. A solution of $AlMe_3$ in heptane (2.0 M, 9.8 mL, 19.6 mmol, 4 equiv) was added dropwise over 30 min (CAUTION: Gas evolution!). The mixture was allowed to warm to 23 ºC. After 30 min, a solution of **14** (2.10 g, 6.85 mmol, 1 equiv) in DCM (20 mL) was added over 10 min (CAUTION: Gas evolution!).

The vessel was equipped with a reflux condenser, and the solution was brought to reflux by means of a 50 ºC oil bath. After 3 h, the mixture was cooled to 0 °C by means of an ice/water bath, and MeOH (10 mL) was added dropwise (CAUTION: Gas evolution!). Once gas evolution ceased, saturated aqueous potassium sodium tartrate solution (100 mL) was added. After 1 h, the biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with DCM (2 × 30 mL). The combined organic layers were washed with water (100 mL) and brine (100 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:1) to afford amide **15** (2.05 g, 91% yield) as a white solid.

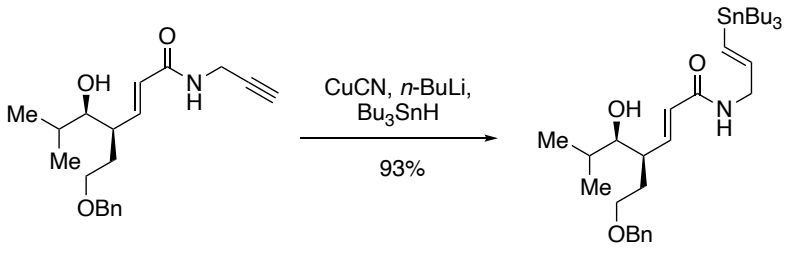
TLC (EtOAc:hexanes = 1:1): R_f = 0.20 (UV).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.26 (m, 5H), 6.66 (dd, *J* = 15.4, 9.8 Hz, 1H), 5.91 (t, *J* = 5.3 Hz, 1H), 5.64 (d, *J* = 15.4 Hz, 1H), 4.51 (d, *J* = 12.0 Hz, 1H), 4.40 (d, *J* = 12.0 Hz, 1H), 4.06 (dd, *J* = 5.3, 2.6 Hz, 2H), 3.50 (dt, *J* = 9.5, 5.3 Hz, 1H), 3.38 (td, *J* = 9.0, 4.7 Hz, 1H), 3.27 (q, *J* = 5.5 Hz, 1H), 2.54 (d, *J* = 5.9 Hz, 1H), 2.48 (tdd, *J* = 9.7, 7.2, 3.6 Hz, 1H), 2.25 (t, *J* = 2.5 Hz, 1H), 2.05 (dddd, *J* = 14.2, 8.9, 5.4, 3.6 Hz, 1H), 1.77 – 1.55 (m, 2H), 0.91 (d, *J* = 6.8 Hz, 3H), 0.84 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.4, 146.1, 138.3, 128.5, 128.1, 127.8, 124.0, 79.6, 78.2, 73.0, 71.8, 67.6, 43.5, 30.9, 29.9, 29.3, 20.1, 15.7.

HRMS-ESI m/z calcd for $C_{17}H_{27}O_4^+$ $[M + H]^+$ 307.2064, found 330.2066.

Preparation of vinyl tin **16**



15 **16**

An oven-dried 500-mL round-bottom flask containing cyanocopper (1.09 g, 12.1 mmol, 2 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Dry THF (120 mL) was added, resulting in a white suspension, and the vessel and its contents were cooled to -78 ºC by means of a dry ice/acetone bath. A solution of *n*-BuLi in hexanes (2.5 M, 10.2 mL, 25.5 mmol, 4.2 equiv) was added dropwise over 10 min, and the resulting light-yellow solution was stirred for 30 min. tributylstannane (6.87 mL, 25.5 mmol, 4.2 equiv) was added dropwise over 5 min. After 30 min, a solution of alkyne **15** (2.00 g, 6.07 mmol, 1 equiv) in THF (15 mL) was added dropwise over 15 min. After 1 h, saturated aqueous ammonium chloride solution (100 mL) was added in one portion. The vessel was removed from the cooling bath, and the system was allowed to warm to 23 ºC while the mixture was rapidly stirred. The biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with EtOAc (2 × 100 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 0:1 to 1:3) to afford vinyl tin **16** (3.49 g, 93% yield) as a colorless oil.

TLC (EtOAc:hexanes = 1:3): R_f = 0.20 (UV).

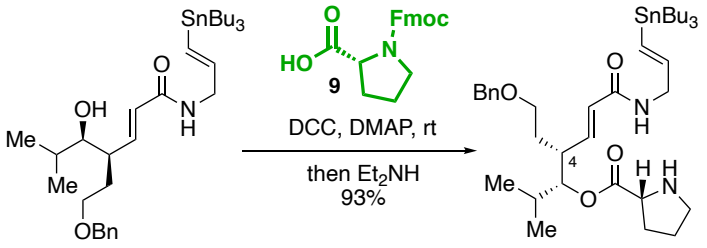
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37 – 7.26 (m, 5H), 6.65 (dd, *J* = 15.3, 9.7 Hz, 1H), 6.12 (dt, *J* = 19.0, 1.5 Hz, 1H), 5.97 (dt, *J* = 19.0, 5.1 Hz, 1H), 5.65 (d, *J* = 15.3 Hz, 1H), 5.43 (t, *J* = 5.8 Hz, 1H), 4.53 (d, *J* = 12.0 Hz, 1H), 4.42 (d, *J* = 12.0 Hz, 1H), 3.97 (td, *J* = 5.4, 1.5 Hz, 2H), 3.53 (dt, *J* = 9.4, 5.3 Hz, 1H), 3.40

(td, J = 9.1, 4.6 Hz, 1H), 3.31 (ddd, J = 7.6, 5.6, 4.1 Hz, 1H), 2.55 – 2.43 (m, 1H), 2.28 (d, J = 5.8 Hz, 1H), 2.06 (ddt, J = 14.2, 8.8, 3.0 Hz, 1H), 1.77 – 1.57 (m, 2H), 1.58 – 1.39 (m, 6H), 1.37 – 1.23 (m, 6H), 1.00 – 0.75 (m, 21H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 145.1, 143.5, 138.4, 130.6, 128.5, 128.1, 127.8, 124.7, 78.1, 73.0, 67.6, 45.1, 43.6, 30.9, 30.2, 29.2, 27.4, 20.2, 15.5, 13.8, 9.6.

HRMS-ESI m/z calcd for $C_{32}H_{56}NO_3Sn^+$ $[M + H]^+$ 622.3277, found 622.3285.

Preparation of proline ester **17**



Fmoc-D-Pro-OH **9** (2.40 g, 7.12 mmol, 1.3 equiv), DMAP (0.13 g, 1.10 mmol, 0.2 equiv) and alcohol **16** (3.40 g, 5.48 mmol, 1 equiv) were added to a 100-mL round-bottom flask. DCM (55 mL) was added, resulting in a colorless solution. DCC (1.70 g, 8.22 mmol, 1.5 equiv) was added in one portion. resulting in a white suspension. After 5 h, alcohol 1**6** was entirely consumed as indicated by TLC analysis (eluent: EtOAc:hexanes = 1:2), and diethylamine (28 mL) was added. After, 3 h, the mixture was filtered through a pad of celite, and the filter cake was washed with DCM (2 × 20 mL). The combined filtrates were concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: NH_4OH:MeOH:DCM = 0.2:1:100 to 0.2:1:50) to afford left half **17** (3.64 g, 93% yield) as light-yellow oil.

TLC (MeOH:DCM = 1:5): R_f = 0.20 (UV).

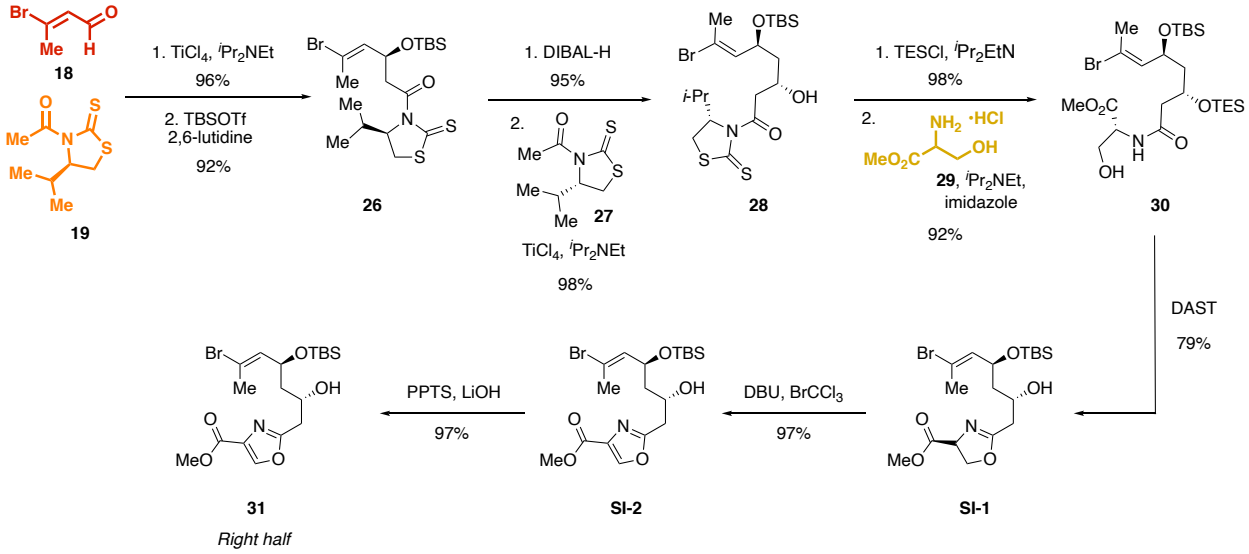
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.26 (m, 5H), 6.49 (dd, J = 15.2, 9.8 Hz, 1H), 6.12 (d, J = 19.0 Hz, 1H), 5.96 (dt, J = 19.0, 5.1 Hz, 1H), 5.62 (d, J = 15.2 Hz, 1H), 5.43 (t, J = 5.8 Hz, 1H), 4.85 (dd, J = 7.7,

4.5 Hz, 1H), 4.51 (d, J = 12.0 Hz, 1H), 4.35 (d, J = 12.0 Hz, 1H), 3.95 (t, J = 5.2 Hz, 2H), 3.79 (dd, J = 8.5, 5.8 Hz, 1H), 3.44 (ddd, J = 9.7, 6.0, 3.9 Hz, 1H), 3.33 (td, J = 9.5, 4.8 Hz, 1H), 3.09 (dt, J = 10.2, 6.8 Hz, 1H), 2.92 (dt, J = 10.3, 6.7 Hz, 1H), 2.70 (tdd, J = 10.6, 7.7, 2.9 Hz, 1H), 2.54 (s, 1H), 2.21 – 2.08 (m, 1H), 1.97 – 1.69 (m, 6H), 1.60 – 1.38 (m, 6H), 1.37 – 1.22 (m, 6H), 0.99 – 0.78 (m, 21H).

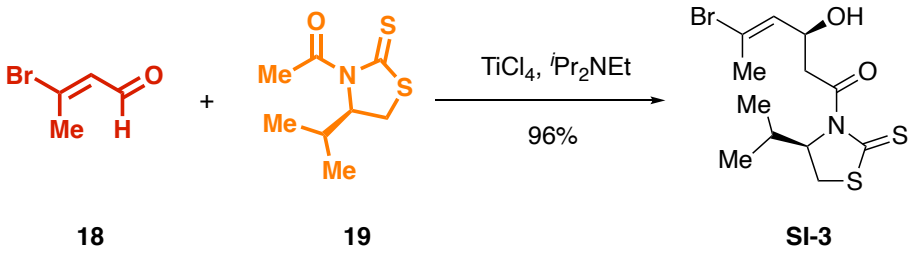
^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 164.9, 143.4, 142.9, 138.7, 130.7, 128.5, 128.1, 127.7, 125.8, 79.8, 72.9, 67.1, 60.0, 47.0, 45.1, 41.2, 30.6, 30.1, 29.6, 29.2, 27.4, 25.6, 19.8, 16.3, 13.8, 9.6.

HRMS-ESI m/z calcd for $C_{37}H_{63}N_2O_4Sn^+$ $[M + H]^+$ 719.3804, found 719.3820.

Scheme II. Preparation for right half 31[8]



Preparation of β-hydroxyl amide **SI-3**[19]



A 250-mL round-bottom flask containing **19**[18] (4.53 g, 22.3 mmol, 1.1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). DCM (41 mL) was added, resulting in a yellow solution, and the vessel and its contents were cooled to -78 ºC by means of a dry ice/acetone bath. A solution of $TiCl_4$ in DCM (1 M, 24.3 mL, 24.3 mmol, 1.2 equiv) was added dropwise, resulting in a deep yellow solution. After 5 min, $^{i}Pr_2EtN$ (4.2 mL, 24.3 mmol, 1.2 equiv) was added over 30 min by means of syringe pump, and the resulting deep red solution was stirred for 2 h at -78 ºC. A solution of aldehyde **18**[20,21] (3.02 g, 20.3 mmol, 1 equiv) in DCM (5 mL) was added by means of syringe pump over 30 min. Stirred for 12 hours at -78 ºC. After 12 hours, water (100 mL) was added. The vessel was removed from the cooling bath, and the system was allowed to warm to 23 ºC while the mixture was rapidly stirred. The biphasic mixture was transferred to a separatory funnel and the layers were separated. The aqueous layer was extracted with DCM (2 × 30 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc: hexanes = 1:10 to 1:2.5) to afford β-hydroxyl amide **SI-3** (6.82 g, 96% yield) as a yellow oil.

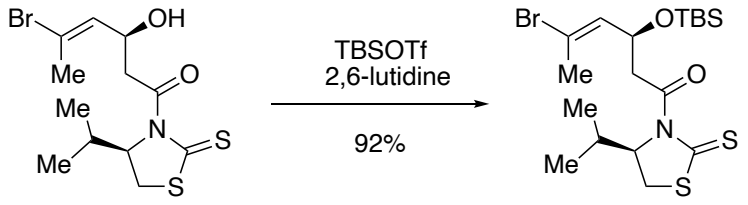
TLC (EtOAc:hexanes = 1:5): R_f = 0.25 (UV and $KMnO_4$).

^{1}H NMR (300 MHz, $CDCl_3$) δ 5.97 (dd, *J* = 8.7, 1.6 Hz, 1H), 5.15 (ddd, *J* = 7.4, 6.3, 1.1 Hz, 1H), 4.81 (td, *J* = 8.6, 3.2 Hz, 1H), 3.62 (dd, *J* = 17.7, 3.2 Hz, 1H), 3.53 (ddd, *J* = 10.8, 7.9, 2.7 Hz, 1H), 3.32 (dd, *J* = 17.7, 8.4 Hz, 1H), 3.08 – 3.02 (m, 1H), 2.60 (s, 1H), 2.38 (dd, *J* = 13.6, 6.7 Hz, 1H), 2.33 (d, *J* = 1.3 Hz, 3H), 1.07 (d, *J* = 6.9 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 202.9, 171.8, 132.4, 124.2, 71.3, 65.7, 44.8, 30.7, 30.6, 24.1, 19.0, 17.7.

HRMS-ESI m/z calcd for $C_{12}H_{17}BrNO_2S_2^-$ $[M - H]^-$ 349.9890, found 349.9886.

Preparation of TBS ether **26**



SI-3 **26**

A 250-mL round-bottom flask containing β-hydroxyl amide **SI-3** (4.71 g, 13.4 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). DCM (134 mL) was added, followed by 2,6-lutidine (3.1 mL, 26.8 mmol, 2 equiv), resulting in a yellow solution. The vessel and its contents were cooled to 0 ºC by means of an ice/water bath, and TBSOTf (3.69 mL, 16.0 mmol, 1.2 equiv) was added dropwise over 10 min. After 30 min, the mixture was transferred to a separatory funnel and washed with water (2 × 100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4). The dried solution was filtrated, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:20) to afford TBS ether **26** (5.76 g, 92% yield) as a light-yellow oil.

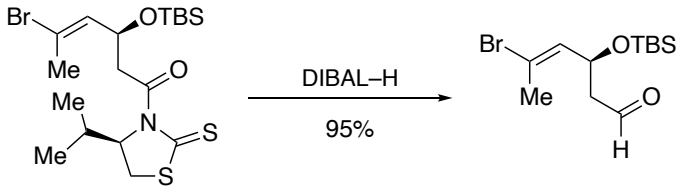
TLC (EtOAc:hexanes = 1:50): R_f= 0.20 (UV)

^{1}H NMR (300 MHz, $CDCl_3$) δ5.87 (dq, *J* = 8.9, 1.3 Hz, 1H), 5.03 (ddd, *J* = 7.6, 6.2, 1.1 Hz, 1H), 4.96 – 4.86 (m, 1H), 3.63 (dd, *J* = 16.5, 8.3 Hz, 1H), 3.47 (dd, *J* = 11.5, 7.9 Hz, 1H), 3.18 (dd, *J* = 16.5, 4.3 Hz, 1H), 3.03 (dd, *J* = 11.4, 1.1 Hz, 1H), 2.36 (dq, *J* = 13.5, 6.8 Hz, 1H), 2.31 (d, *J* = 1.3 Hz, 3H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.97 (d, *J* = 7.0 Hz, 3H), 0.84 (s, 9H), 0.05 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) 202.8, 170.7, 134.5, 121.7, 71.7, 67.2, 45.6, 30.9, 30.8, 25.7, 24.1, 19.1, 18.0, 17.8, -4.5, -5.0.

HRMS-ESI m/z calcd for $C_{18}H_{32}BrNO_2S_2Si^+$ $[M]^+$ 465.0827, found 465.0819.

Preparation of aldehyde **SI-4**



26 **SI-4**

A 250-mL round-bottom flask containing TBS ether **26** (3.00 g, 6.43mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). DCM (65 ml) was added, resulting in a light-yellow solution. The vessel and its contents were cooled to -78 ºC by means of a dry ice/acetone bath, and a solution of DIBAL-H in hexanes (1.0 M, 12.9 mL, 12.9 mmol, 2 equiv) was added dropwise over 10 min. After 1 h, MeOH (5 mL) was carefully added, followed saturated aqueous solution of potassium sodium tartrate (50 mL). The vessel was removed from the cooling bath, and the system was allowed to warm to 23 ºC while the mixture was rapidly stirred. After 1 h, the biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with DCM (2 × 30 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:15) to afford aldehyde **SI-4** (1.87 g, 95% yield) as a colorless oil.

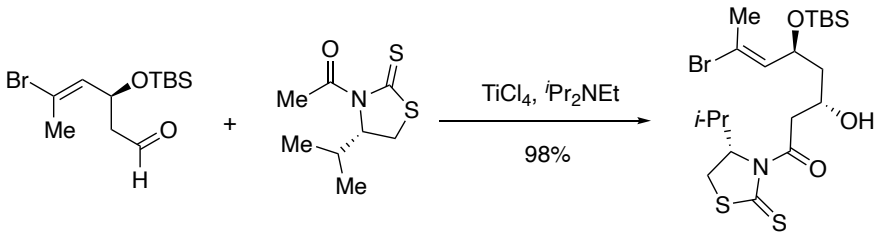
TLC (EtOAc:hexanes = 1:5): R_f = 0.4 ($KMnO_4$).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.76 (t, *J* = 2.1 Hz, 1H), 5.88 (dq, *J* = 9.0, 1.3 Hz, 1H), 4.82 (ddd, *J* = 9.0, 7.7, 4.8 Hz, 1H), 2.69 (ddd, *J* = 16.1, 7.7, 2.3 Hz, 1H), 2.57 – 2.47 (m, 1H), 2.30 (d, *J* = 1.3 Hz, 3H), 0.86 (s, 9H), 0.06 (s, 6H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 200.4, 134.3, 121.6, 65.9, 51.2, 25.6, 24.0, 18.0, -4.4, -5.1.

HRMS-ESI m/z calcd for $C_{12}H_{24}BrO_2Si^+$ $[M + H]^+$ 307.0724, found 307.0720.

Preparation of β-hydroxyl amide **28**



SI-4 **27** **28**

A 500-mL round-bottom flask containing **27** (4.37 g, 21.5 mmol, 1.2 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). DCM (110 mL) was added, resulting in a yellow solution, and the vessel and its contents were cooled to -78 ºC by means of a dry ice/acetone bath. A solution of $TiCl_4$ in DCM (1 M, 23.3 mL, 23.3 mmol, 1.3 equiv) was added dropwise over 5 min, resulting in a deep yellow solution. After 5 min, $^{i}Pr_2EtN$ (4.06 mL, 23.3 mmol, 1.3 equiv) was added over 30 min by means of syringe pump, and the resulting deep red solution was stirred for 2 h at -78 ºC. A solution of aldehyde **SI-4** (5.50 g, 17.9 mmol, 1 equiv) in DCM (18 mL) was added over 30 min by means of syringe pump. Stirred for 12 hours at -78 ºC. After 12 hours, **SI-4** was entirely consumed as indicated by TLC analysis (eluent: EtOAc:hexanes = 1:4), and then water (150 mL) was added. The vessel was removed from the cooling bath, and the system was allowed to warm to 23 ºC while the mixture was rapidly stirred. The biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with DCM (2 × 50 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc: hexanes = 1:10 to 1:2.5) to afford β-hydroxyl amide **28** (9.00 g, 98% yield) as a yellow oil.

TLC (EtOAc:hexanes = 1:3): R_f = 0.25 (UV).

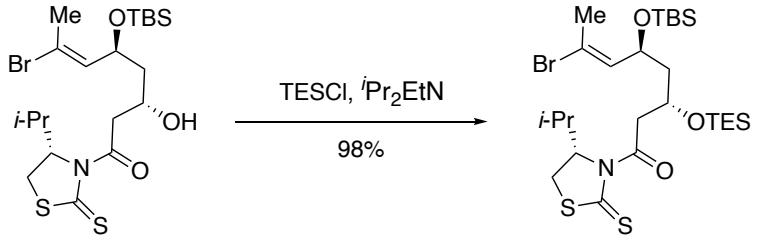
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.92 (dq, *J* = 8.8, 1.3 Hz, 1H), 5.21 – 5.10 (m, 1H), 4.67 – 4.57 (m, 1H), 4.38 (dddd, *J* = 10.7, 5.4, 4.2, 2.8 Hz, 1H), 3.56 – 3.47 (m, 2H), 3.23 (dd, *J* = 17.7, 9.1 Hz, 1H), 3.03 (dd, J

= 11.5, 1.1 Hz, 1H), 2.37 (dq, *J* = 13.5, 6.8 Hz, 1H), 2.26 (d, *J* = 1.3 Hz, 3H), 1.70 – 1.56 (m, 2H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.88 (s, 9H), 0.09 (s, 3H), 0.06 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 202.9, 172.6, 135.6, 120.2, 71.4, 67.6, 64.5, 45.9, 43.5, 30.9, 30.6, 25.8, 23.9, 19.1, 18.07, 17.8, -4.5, -5.1.

HRMS-ESI m/z calcd for $C_{20}H_{37}BrNO_3S_2Si^+$ $[M + H]^+$ 510.1162, found 510.1156.

Preparation of TES ether **SI-5**



28 **SI-5**

A 250-mL round-bottom flask containing β-hydroxyl amide **28** (7.00 g, 13.7 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). DCM (140 mL) was added, followed by $^{i}Pr_2EtN$ (7.20 mL, 41.1 mmol, 3.0 equiv), resulting in a colorless solution. The vessel and its contents were cooled to 0 ºC by means of an ice/water bath. TESCl (3.5 mL, 20.6 mmol, 1.5 equiv) was added dropwise over 10 min, and the mixture was allowed to warm to 23 ºC. After 3 h, the mixture was transferred to a separatory funnel and was washed with water (2 × 100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4), and the dried solution was filtrated. The filtrate was concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:50) to afford TES ether **SI-5** (8.45 g, 98% yield) as a light-yellow oil.

TLC (EtOAc:hexanes = 1:10): R_f = 0.20 (UV).

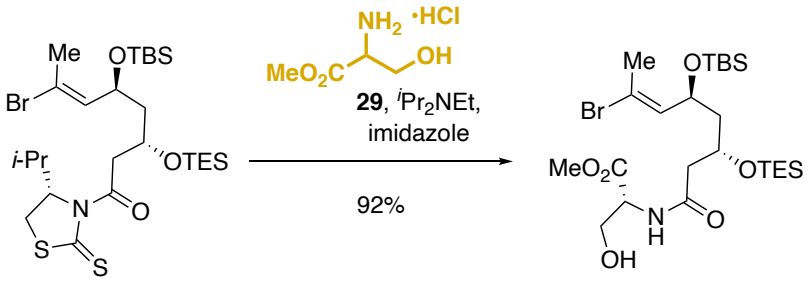
^{1}H NMR (400 MHz, $CDCl_3$) δ 5.83 (d, *J* = 9.2 Hz, 1H), 5.07 (t, *J* = 7.0 Hz, 1H), 4.52 – 4.32 (m, 2H), 3.59 (dd, *J* = 17.2, 7.3 Hz, 1H), 3.46 (dd, *J* = 11.4, 7.8 Hz, 1H), 3.29 (dd, *J* = 17.2, 4.6 Hz, 1H), 3.02 (d, *J* = 11.5

Hz, 1H), 2.37 (dq, *J* = 13.5, 6.8 Hz, 1H), 2.25 (s, 3H), 1.81 (ddd, *J* = 13.7, 7.9, 5.8 Hz, 1H), 1.62 (dt, *J* = 13.9, 5.4 Hz, 1H), 1.06 (d, *J* = 6.8 Hz, 3H), 0.94 (t, *J* = 8.1 Hz, 9H), 0.87 (s, 9H), 0.60 (q, *J* = 7.8 Hz, 6H), 0.05 (s, 3H), 0.05 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 202.6, 171.3, 136.1, 120.7, 71.5, 67.5, 66.2, 46.5, 46.2, 30.8, 30.8, 25.9, 23.86, 19.1, 18.1, 17.9, 7.0, 5.2, -4.0, -4.7.

HRMS-ESI m/z calcd for $C_{26}H_{51}BrNO_3S_2Si_2^+$ $[M + H]^+$ 624.2027, found 624.2022.

Preparation of amide **30**



SI-5 **30**

A 500-mL round-bottom flask containing H-Ser-OMe•HCl **29** (3.36 g, 21.6 mmol, 1.5 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). THF (110 mL) was added, followed by $^{i}Pr_2EtN$ (5.01 mL, 28.8 mmol, 2 equiv). After 30 min, the resulting colorless solution was cooled to 0 ºC by means of an ice/water bath. A solution of **SI-5** (9.00 g, 14.4 mmol, 1 equiv) in THF (15 mL) was added, followed by imidazole (2.94 g, 43.2 mmol, 3.0 equiv), and the vessel and its contents were allowed to warm to 23 ºC. After 12 h, the mixture was concentrated, and the residue was dissolved with DCM (150 mL) and water (150 mL). The resulting biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extract with DCM (50 mL), and the combined layers were washed with water (100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4), and the dried solution was filtered. The filtrate was concentrated, and the resulting crude residue was

purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:10 to 1:1) to afford amide **30** (7.75 g, 92% yield) as a colorless oil.

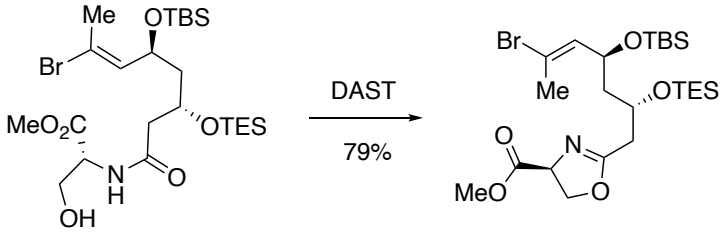
TLC (EtOAc:hexanes = 1:5): R_f = 0.20 (UV)

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.11 (d, *J* = 7.4 Hz, 1H), 5.79 (dt, *J* = 9.0, 1.4 Hz, 1H), 4.62 (dt, *J* = 7.5, 3.8 Hz, 1H), 4.36 (ddd, *J* = 9.4, 7.5, 5.4 Hz, 1H), 4.17 – 4.07 (m, 1H), 3.96 – 3.82 (m, 2H), 3.74 (s, 3H), 3.01 (br s, 1H), 2.51 (dd, *J* = 14.7, 4.8 Hz, 1H), 2.32 (dd, *J* = 14.7, 5.0 Hz, 1H), 2.24 (s, 3H), 1.79 (ddd, *J* = 13.7, 7.5, 6.0 Hz, 1H), 1.64 (dt, *J* = 13.9, 5.7 Hz, 1H), 0.93 (t, *J* = 7.9 Hz, 9H), 0.84 (s, 9H), 0.61 (q, *J* = 8.1 Hz, 6H), 0.02 (s, 3H), 0.01 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.9, 170.7, 135.6, 121.1, 67.3, 66.3, 63.3, 54.7, 52.5, 45.2, 44.3, 25.7, 23.8, 18.0, 6.7, 4.8, -4.0, -4.7.

HRMS-ESI m/z calcd for $C_{24}H_{40}BrNO_6Si_2^+$ $[M + H]^+$ 582.2276, found 582.2268.

Preparation of oxazoline **SI-1**



30 **SI-1**

A 250-mL round-bottom flask containing amide **30** (7.60 g, 13.0 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). DCM (130 mL) was added, and the resulting colorless solution was cooled to -78 ºC by means of a dry ice/acetone bath. DAST (2.15 mL, 16.3 mmol, 1.25 equiv) was added dropwise at -78 ºC under nitrogen. After 3 h, saturated aqueous $NaHCO_3$ solution (50 mL) was added, and the system was allowed to warm to 23 ºC while the mixture was rapidly stirred. The resulting biphasic solution was transferred to a separatory funnel. The organic layer was washed with

water (100 mL) and brine (100 mL), and the washed solution was dried (Na_2SO_4). The dried solution was concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:8) to afford oxazoline **SI-1** (5.78 g, 79% yield) as a colorless oil.

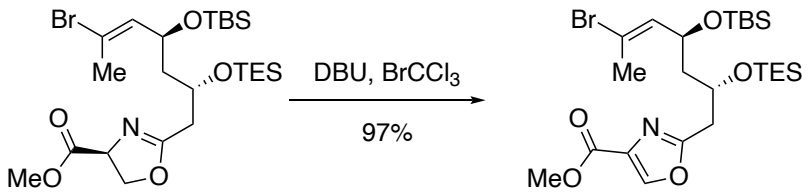
TLC (EtOAc:hexanes = 1:5): R_f = 0.20 (UV).

^{1}H NMR (400 MHz, $CDCl_3$) δ 5.82 (dq, *J* = 9.3, 1.4 Hz, 1H), 4.71 (dd, *J* = 10.6, 8.0 Hz, 1H), 4.48 – 4.34 (m, 3H), 4.18 (tdd, *J* = 7.0, 5.9, 4.1 Hz, 1H), 3.78 (s, 3H), 2.52 (ddd, *J* = 8.1, 6.5, 1.0 Hz, 1H), 2.25 (d, *J* = 1.4 Hz, 3H), 1.76 (ddd, *J* = 14.0, 8.5, 4.0 Hz, 1H), 1.60 – 1.53 (m, 1H), 0.95 (t, *J* = 7.9 Hz, 9H), 0.86 (s, 9H), 0.59 (q, *J* = 8.0 Hz, 6H), 0.03 (s, 3H), 0.03 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 167.9, 136.0, 120.8, 69.2, 68.1, 67.1, 66.5, 52.6, 46.1, 37.2, 25.8, 23.8, 18.0, 6.8, 5.1, -3.8, -4.7.

HRMS-ESI m/z calcd for $C_{24}H_{47}BrNO_5Si_2^+$ $[M + H]^+$ 564.2171, found 564.2162.

Preparation of oxazole **SI-2**



SI-1 **SI-2**

A 250-mL round-bottom flask containing oxazoline **SI-1** (5.78 g, 10.2 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). DCM (130 mL) and $BrCCl_3$ (5.04 mL, 51.2 mmol, 5 equiv) were added, and the resulting colorless solution was cooled to 0 ºC by means of an ice/water bath. DBU (7.71 mL, 51.2 mmol, 5 equiv) was added dropwise at 0 ºC. After 24 h, saturated aqueous ammonium chloride solution (100 mL) was added, and the biphasic mixture was transferred to a separatory funnel. The layers were separated, and the aqueous layer was extracted with DCM (50 mL). The

combined organic layers were washed with water (2 × 100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4), and the dried solution was concentrated. The residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:10) to afford oxazole **SI-2** (5.57 g, 97% yield) as a colorless oil.

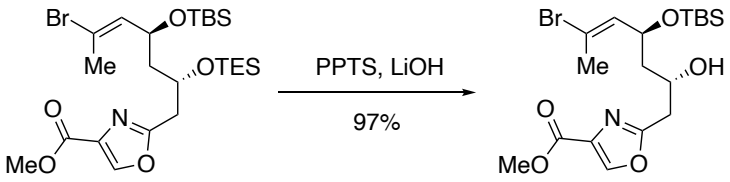
TLC (EtOAc:hexanes = 1:5): R_f = 0.20 (UV).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s, 1H), 5.81 (dt, *J* = 9.3, 1.5 Hz, 1H), 4.44 (td, *J* = 8.9, 4.0 Hz, 1H), 4.29 (qd, *J* = 6.5, 4.1 Hz, 1H), 3.91 (d, *J* = 0.7 Hz, 3H), 2.99 (d, *J* = 6.3 Hz, 2H), 2.26 (d, *J* = 1.3 Hz, 3H), 1.78 – 1.66 (m, 1H), 1.63 – 1.53 (m, 1H), 0.93 (t, *J* = 8.0 Hz, 9H), 0.85 (s, 9H), 0.57 (q, *J* = 8.0 Hz, 6H), 0.04 (s, 3H), 0.02 (s, 3H).

^{13}C NMR (100 MHz, CDCl3) δ 163.1, 161.7, 143.9, 135.9, 133.3, 120.9, 67.3, 67.1, 52.1, 46.0, 37.2, 25.8, 23.8, 18.0, 6.8, 5.0, -3.7, -4.7.

HRMS-ESI m/z calcd for $C_{24}H_{45}BrNO_5Si_2^+$ $[M + H]^+$ 562.2014, found 562.2009.

Preparation of acid **31**



SI-2 **31**

Right half

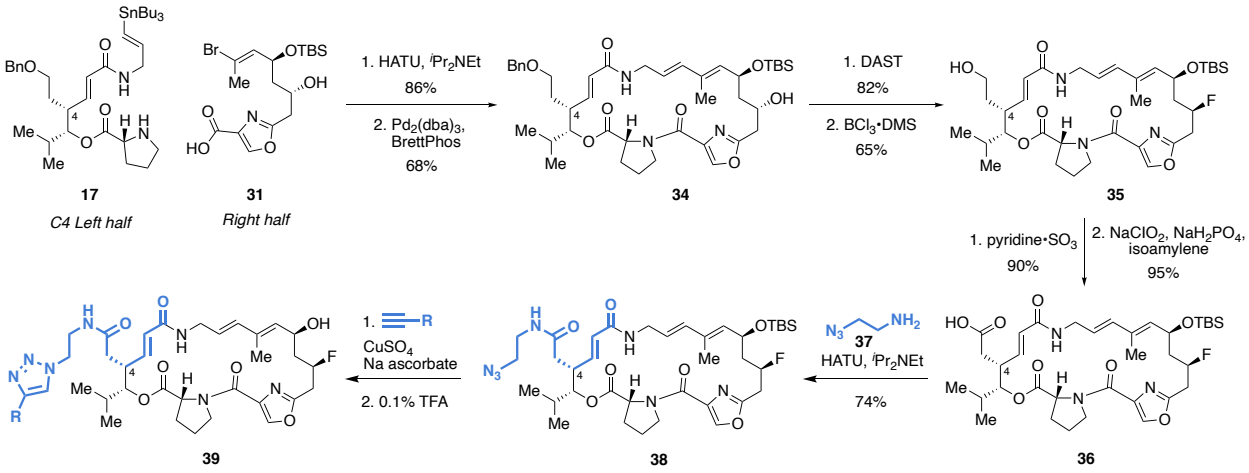
To a solution of oxazole **SI-2** (1.20 g, 2.13 mmol, 1 equiv) in MeOH (21 mL) was added PPTS (53.6 mg, 0.21 mmol, 0.1 equiv). After 1 h, **SI-2** was entirely consumed as indicated by TLC analysis (EtOAc:hexanes = 1:3), and then a solution of LiOH in water (1 M, 6.40 mL, 6.40 mmol, 3 equiv) was added. After 12 h, the mixture was concentrated, and then water (100 mL) and EtOAc (100 mL) were added, followed by aqueous 1.0 N HCl solution (20 mL) to adjust the pH to 3. The resulting biphasic mixture was transferred

to a separatory funnel, and the layers were separated. The organic layer was washed with water (100 mL) and brine (100 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue **31** (897 mg, 97% yield) was used for next step without further purification.

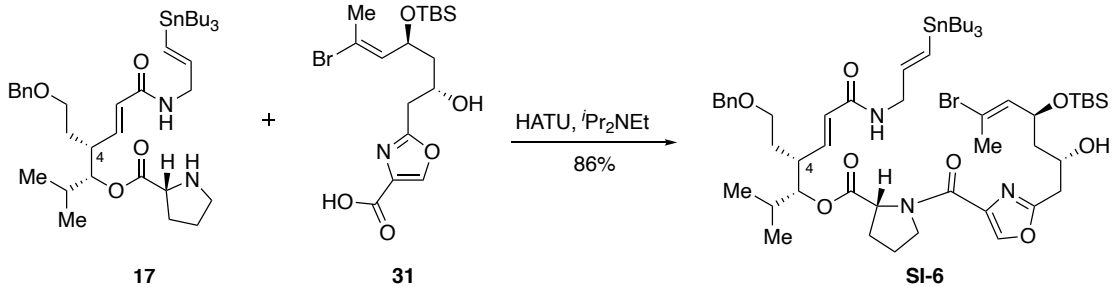
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.26 (s, 1H), 5.95 (dd, J = 8.9, 1.4 Hz, 1H), 4.67 (dt, J = 9.4, 5.3 Hz, 1H), 4.46 – 4.34 (m, 1H), 3.01 (d, J = 6.3 Hz, 2H), 2.31 – 2.24 (m, 3H), 1.71 (t, J = 5.6 Hz, 2H), 0.88 (s, 9H), 0.08 (d, J = 1.9 Hz, 3H), 0.06 (s, 3H).

HRMS-ESI m/z calcd for $C_{17}H_{28}BrNNaO_5Si^+$ $[M + Na]^+$ 456.0812, found 456.0811.

Scheme III. Preparation for C-4 modified amide analogs 39



Preparation of Stille precursor **SI-6**



Acid **31** (6.50 g, 15.0 mmol 1 equiv), $^{i}Pr_2EtN$ (5.23 mL, 30.0 mmol, 2 equiv), amine **17** (10.7 g, 15.0 mmol, 1 equiv) and DCM (150 mL) was added to a 500-mL round-bottom flask, resulting in a clear, colorless solution. HATU (7.11 g, 18.7 mmol, 1.25 equiv) was added in one portion. After 5 h, the mixture was diluted with DCM (30 mL), and the solution was transferred to a separatory funnel and was washed with water (2 × 150 mL) and brine (150 mL). The washed solution was dried (Na_2SO_4), and the dried solution was filtered. The filtrate was concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:6 to 1:3) to afford Stille coupling precursor **SI-6** (14.5 g, 86% yield) as a light-yellow foam.

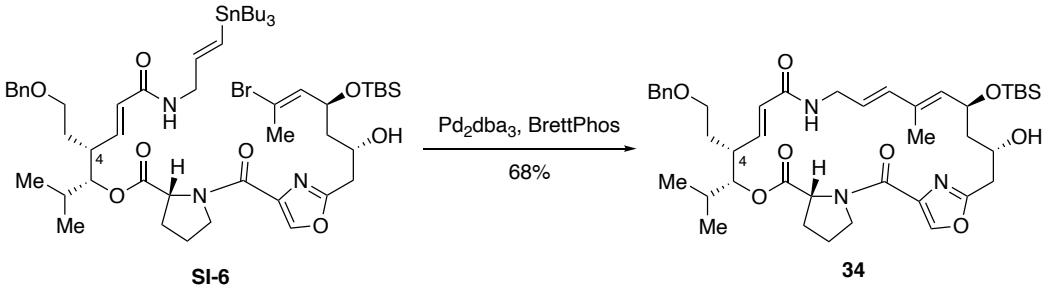
TLC (EtOAc:hexanes = 1:2): R_f = 0.3 (UV)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.13 (s, 0.5H), 8.12 (s, 0.5H), 7.38 – 7.23 (m, 5H), 6.51 – 6.33 (m, 1H), 6.13 (d, *J* = 19.0 Hz, 1H), 6.03 – 5.86 (m, 2H), 5.81 (t, *J* = 5.7 Hz, 0.5H), 5.66 (d, *J* = 15.5 Hz, 0.5H), 5.60 – 5.45 (m, 1H), 4.89 – 4.73 (m, 1H), 4.72 – 4.58 (m, 1H), 4.50 – 4.17 (m, 3H), 4.08 (t, *J* = 6.6 Hz, 1H), 4.02 – 3.89 (m, 2H), 3.83 – 3.68 (m, 1H), 3.71 – 3.58 (m, 1H), 3.41 (ddd, *J* = 10.0, 6.2, 4.0 Hz, 1H), 3.35 – 3.23 (m, 1H), 2.91 – 2.84 (m, 1H), 2.79 (dd, *J* = 6.0, 3.2 Hz, 1H), 2.66 (ddt, *J* = 9.9, 6.6, 3.1 Hz, 1H), 2.35 – 2.21 (m, 5H), 2.18 – 1.82 (m, 5H), 1.71 – 1.55 (m, 3H), 1.56 – 1.37 (m, 6H), 1.30 (dqd, *J* = 14.1, 7.2, 2.7 Hz, 6H), 1.00 – 0.77 (m, 30H), 0.15 – -0.01 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$, mixtures of rotamers) δ 172.88, 172.02, 165.37, 164.87, 162.36, 162.24, 160.53, 160.31, 143.69, 143.66, 143.45, 143.41, 142.92, 142.62, 138.68, 138.59, 136.81, 135.84, 135.44, 130.72, 130.46, 128.49, 128.47, 128.04, 128.01, 127.68, 126.15, 125.87, 120.58, 120.36, 80.65, 80.38, 72.89, 67.82, 67.69, 67.36, 67.34, 65.66, 65.25, 61.12, 60.37, 48.94, 47.40, 45.14, 45.07, 44.21, 43.64, 41.40, 40.93, 36.35, 35.96, 31.83, 30.24, 30.20, 29.18, 27.40, 25.97, 25.89, 25.55, 24.03, 24.00, 21.83, 19.97, 19.68, 18.21, 17.02, 16.98, 13.83, 9.59, 9.57, -4.31, -4.34, -4.77, -5.00.

HRMS-ESI m/z calcd for $C_{54}H_{88}BrN_3O_8SiSn^+$ $[M + H]^+$ 1134.4619, found 1134.4633.

Preparation of Stille product **34**



SI-6 **34**

An oven-dried 3000-mL round-bottom flask containing BrettPhos (1.37 g, 2.56 mmol, 0.2 equiv), $Pd_2(dba)_3$ (1.17 g, 1.28 mmol, 0.1 equiv) and Stille coupling precursor **SI-6** (14.5 g, 12.79 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Dry Toluene (2500 mL) was added, resulting in a red suspension. A stream of argon was passed through the solution by a stainless-steel needle for 30 min. The mixture was heated by means of a 50 ºC pre-heated oil bath. After 12 h, **SI-6** was entirely consumed as indicated by TLC analysis (eluent: EtOAc:hexanes = 1:1), and the mixture was allowed to cool to 23 ºC. The mixture was concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes =1:3 to 1:1) to afford Stille coupling product **34** (5.56 g, 57% yield) as a light-yellow solid.

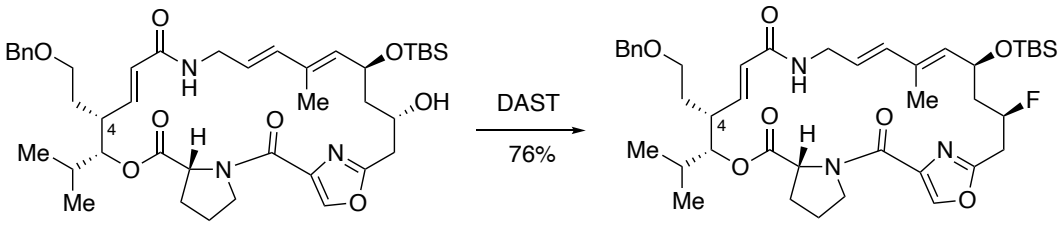
TLC (EtOAc:hexanes = 1:1): R_f = 0.1 (UV)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 7.38 – 7.25 (m, 5H), 6.24 – 6.14 (m, 2H), 5.95 (dd, *J* = 9.4, 3.1 Hz, 1H), 5.82 (dd, *J* = 16.3, 1.5 Hz, 1H), 5.76 (d, *J* = 9.1 Hz, 1H), 5.66 (ddd, *J* = 15.1, 10.3, 4.3 Hz, 1H), 4.98 (ddd, *J* = 9.2, 4.8, 2.2 Hz, 1H), 4.75 – 4.64 (m, 2H), 4.57 – 4.39 (m, 5H), 3.80 (t, *J* = 6.5 Hz, 2H), 3.58 (ddd, *J* = 9.2, 6.7, 4.2 Hz, 1H), 3.44 (td, *J* = 9.0, 5.7 Hz, 1H), 3.30 (ddd, *J* = 13.7, 10.4, 3.0 Hz, 1H), 3.05 (dd, *J* = 16.7, 2.4 Hz, 1H), 2.85 – 2.72 (m, 2H), 2.32 (d, *J* = 14.0 Hz, 1H), 2.12 (tdd, *J* = 12.9, 8.8, 5.4 Hz, 1H), 2.05 – 1.76 (m, 6H), 1.71 (s, 3H), 1.62 (ddt, *J* = 15.0, 10.4, 5.1 Hz, 1H), 0.99 (d, *J* = 6.4 Hz, 3H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.90 (s, 9H), 0.09 (s, 3H), 0.04 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 167.3, 161.3, 160.5, 143.4, 142.1, 138.4, 137.7, 136.8, 134.3, 131.8, 128.5, 127.7, 127.6, 125.3, 124.8, 82.6, 73.2, 70.0, 68.0, 66.8, 59.4, 48.3, 43.0, 41.8, 39.6, 35.2, 29.4, 28.4, 25.8, 25.8, 25.6, 20.1, 18.6, 18.1, 12.6, -4.3, -5.1.

HRMS-ESI m/z calcd for $C_{42}H_{62}N_3O_8Si^+$ $[M + H]^+$ 764.4301, found 764.4311.

Preparation of fluorinated compound **SI-7**



An oven-dried 1000-mL round-bottom flask containing Stille product **34** (5.50 g, 7.20 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Dry DCM (360 mL) was added, and the resulting colorless solution was cooled to -78 ºC by means of a dry ice/acetone bath. DAST (2.38 mL, 18.0 mmol, 2.5 equiv) was added dropwise, and the vessel and its contents were warmed to 0 ºC by means of an ice/water bath. After 3 h, saturated aqueous $NaHCO_3$ solution (200 mL) was added. After stirring for 30 min, the biphasic mixture was transferred to a separatory funnel, the layers were separated. The organic layer was washed with water (250 mL) and brine (250 mL), and the washed solution was dried (Na_2SO_4). The dried solution was concentrated, and the residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:2) to afford fluorinated product **SI-7** (4.18 g, 76% yield) as a white solid.

TLC (EtOAc:hexanes = 1:1): R_f = 0.30 (UV, *p*-anisaldehyde).

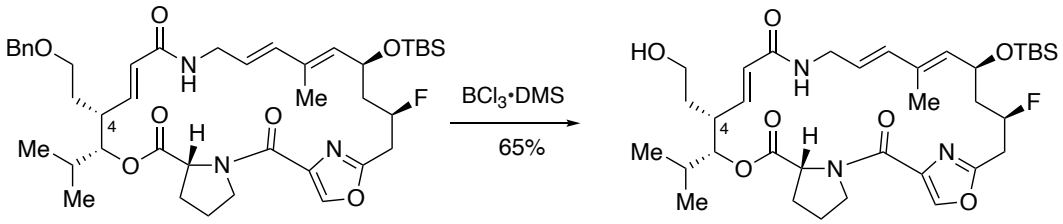
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 7.37 – 7.27 (m, 5H), 6.42 (dd, *J* = 16.2, 6.0 Hz, 1H), 6.17 (d, *J* = 15.6 Hz, 1H), 6.01 (dd, *J* = 8.8, 3.2 Hz, 1H), 5.86 (dd, *J* = 16.2, 1.5 Hz, 1H), 5.66 (ddd, *J* = 15.6, 8.5, 4.3 Hz, 1H), 5.30 (d, *J* = 8.9 Hz, 1H), 5.04 (dm, *J* = 48.7 Hz, 1H), 4.89 – 4.76 (m, 2H), 4.76 – 4.68 (m, 1H),

4.59 – 4.44 (m, 3H), 4.07 (ddd, *J* = 11.2, 8.1, 5.0 Hz, 1H), 3.85 (dt, *J* = 11.3, 7.0 Hz, 1H), 3.59 (ddd, *J* = 9.3, 6.7, 4.2 Hz, 1H), 3.54 – 3.41 (m, 2H), 3.15 (td, *J* = 16.8, 6.4 Hz, 1H), 2.91 (ddd, *J* = 20.7, 16.4, 5.6 Hz, 1H), 2.83 – 2.70 (m, 1H), 2.23 – 2.06 (m, 2H), 2.07 – 1.88 (m, 5H), 1.78 (s, 3H), 1.70 – 1.50 (m, 2H), 0.96 (d, *J* = 6.1 Hz, 3H), 0.94 (d, *J* = 6.2 Hz, 3H), 0.87 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 166.4, 160.7, 160.2, 160.1, 143.3, 142.9, 138.5, 136.8, 136.7, 134.8, 133.5, 128.5, 127.7, 127.7, 125.2, 124.6, 89.2 (d, *J* = 170.3 Hz), 82.1, 73.2, 68.0, 66.7, 59.2, 48.7, 43.7 (d, *J* = 20.6 Hz), 41.4, 39.0, 34.0 (d, *J* = 25.2 Hz), 29.6, 28.4, 26.5, 25.9, 25.1, 20.0, 18.7, 18.3, 13.1, -4.3, -4.8.

HRMS-ESI m/z calcd for $C_{42}H_{61}FN_3O_7Si^+$ $[M + H]^+$ 766.4257, found 766.4268.

Preparation of primary alcohol **35**



SI-7 **35**

An oven-dried 200-mL round-bottom containing compound **SI-7** (1.60 g, 2.09 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Dry DCM (108 mL) and DMS (36 mL) were added, followed by a solution of BCl_3•DMS in DCM (2.0 M, 5.22 mL, 10.4 mmol, 5 equiv). After stirring for 5 hours at 23 °C, the reaction mixture was quenched with methanol (10 mL) and saturated aqueous $NaHCO_3$ solution (50 mL). The resulting biphasic mixture was stirred for 1 h at 23 °C, and was transferred to a separatory funnel. The organic layer was washed with water (2 × 100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: acetone:hexanes = 1:3.5 to 1:2.5) to afford primary alcohol **35** (0.92 g, 65% yield) as a light-yellow solid.

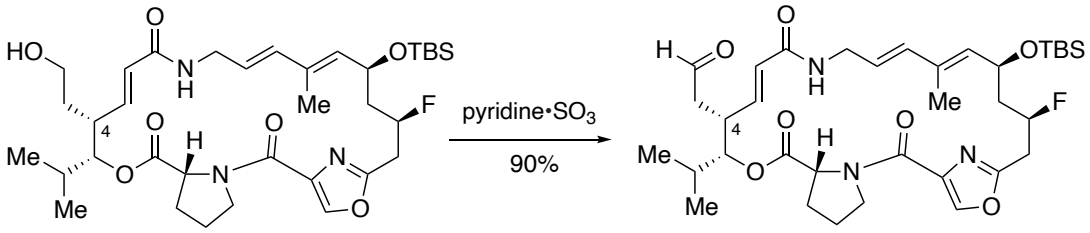
TLC (acetone:hexanes = 1:2.5): R_f = 0.20 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 6.43 (dd, *J* = 16.2, 6.1 Hz, 1H), 6.21 – 6.12 (m, 2H), 5.88 (dd, *J* = 16.1, 1.5 Hz, 1H), 5.65 (ddd, *J* = 15.7, 8.5, 4.3 Hz, 1H), 5.29 (d, *J* = 9.0 Hz, 1H), 5.03 (dm, *J* = 48.7 Hz, 1H), 4.89 – 4.77 (m, 2H), 4.77 – 4.66 (m, 1H), 4.49 (ddd, *J* = 15.0, 6.8, 2.8 Hz, 1H), 4.06 (ddd, *J* = 11.3, 8.2, 5.0 Hz, 1H), 3.83 (dt, *J* = 11.3, 7.1 Hz, 1H), 3.79 – 3.69 (m, 1H), 3.66 – 3.53 (m, 1H), 3.45 (ddd, *J* = 15.4, 8.5, 3.2 Hz, 1H), 3.14 (td, *J* = 16.7, 6.4 Hz, 1H), 2.91 (ddd, *J* = 21.6, 16.4, 5.6 Hz, 1H), 2.85 – 2.74 (m, 1H), 2.29 (br s, 1H), 2.23 – 2.08 (m, 2H), 1.94 (dddd, *J* = 19.2, 11.6, 9.7, 6.1 Hz, 5H), 1.77 (s, 3H), 1.71 – 1.52 (m, 2H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.86 (s, 9H), 0.04 (s, 3H), 0.00 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 166.4, 160.7, 160.2, 160.2, 143.2, 142.9, 136.8, 136.6, 134.8, 133.5, 125.3, 124.6, 89.2 (d, *J* = 170.3 Hz), 82.1, 66.6, 60.1, 59.2, 48.7, 43.7 (d, *J* = 20.7 Hz), 41.3, 38.7, 34.0 (d, *J* = 25.1 Hz), 29.5, 29.0, 28.4, 25.9, 25.0, 19.9, 18.7, 18.2, 13.1, -4.3, -4.8.

HRMS-ESI m/z calcd for $C_{35}H_{55}FN_3O_7Si^+$ $[M + H]^+$ 676.3788, found 676.3796.

Preparation of aldehyde **SI-8**



35 **SI-8**

DMSO (2.05 mL, 28.8 mmol, 10 equiv) and TEA (2.4 mL, 17.3 mmol, 6 equiv) were added to a stirred solution of alcohol **35** (1.95 g, 2.88 mmol, 1 equiv) in DCM (100 mL), followed by SO_3•py (1.38g, 8.65 mmol, 3 equiv) at 23 ºC. The resulting mixture was stirred for 1 h before it was quenched with H_2O (50 mL) and extracted with EtOAc (3× 50 mL). The combined organic layers were dried (Na_2SO_4), the dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash

chromatography (silica gel, eluent: acetone:hexanes = 1:3.5 to 1:2.5) to afford aldehyde **SI-8** (1.74 g, 90% yield) as a light-yellow solid.

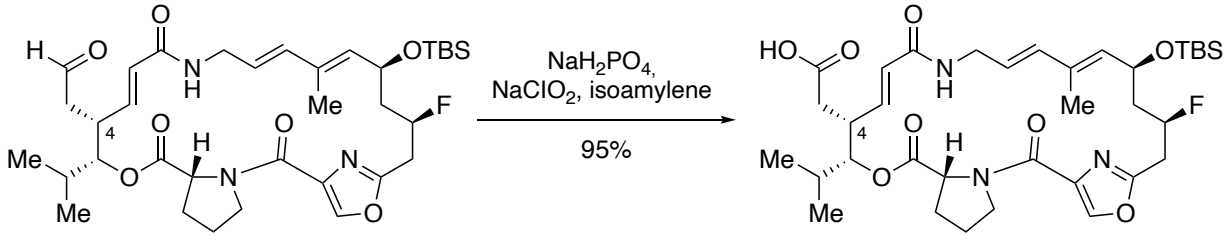
TLC (acetone:hexanes = 1:2.5): R_f = 0.30 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.80 (d, *J* = 1.5 Hz, 1H), 8.06 (s, 1H), 6.48 (dd, *J* = 16.2, 4.9 Hz, 1H), 6.16 (d, *J* = 15.6 Hz, 1H), 6.11 (dd, *J* = 9.1, 3.2 Hz, 1H), 5.82 (dd, *J* = 16.2, 1.8 Hz, 1H), 5.63 (ddd, *J* = 15.6, 8.7, 4.3 Hz, 1H), 5.20 (d, *J* = 48.4 Hz, 1H), 4.91 (dd, *J* = 10.3, 2.0 Hz, 1H), 4.81 (dd, *J* = 8.6, 3.6 Hz, 1H), 4.72 (td, *J* = 9.7, 3.9 Hz, 1H), 4.50 (ddd, *J* = 13.7, 8.6, 4.2 Hz, 1H), 4.05 (ddd, *J* = 11.2, 7.6, 5.3 Hz, 1H), 3.83 (dt, *J* = 11.3, 6.9 Hz, 1H), 3.51 – 3.36 (m, 1H), 3.28 (ddq, *J* = 9.0, 4.5, 2.2 Hz, 1H), 3.13 (td, *J* = 16.9, 6.7 Hz, 1H), 2.89 (ddd, *J* = 21.9, 16.6, 5.2 Hz, 1H), 2.74 – 2.58 (m, 2H), 2.27 – 2.06 (m, 3H), 1.87 (dddd, *J* = 23.4, 13.0, 10.1, 6.0 Hz, 4H), 1.76 (s, 3H), 1.61 (dddd, *J* = 40.3, 14.3, 10.3, 2.0 Hz, 1H), 0.96 (d, *J* = 6.4 Hz, 3H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.86 (s, 9H), 0.04 (s, 3H), 0.00 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 199.9, 171.4, 165.4, 160.6, 160.2, 160.2, 143.1, 141.8, 136.6, 136.6, 134.7, 133.5, 125.2, 124.5, 89.1 (d, *J* = 169.8 Hz), 80.5, 66.5, 58.9, 48.6, 43.6 (d, *J* = 20.2 Hz), 41.2, 40.8, 36.4, 33.9 (d, *J* = 25.2 Hz), 29.7, 28.3, 25.8, 25.0, 19.6, 18.6, 18.1, 12.9, -4.4, -4.9.

HRMS-ESI m/z calcd for $C_{35}H_{53}FN_3O_7Si^+$ $[M + H]^+$ 674.3631, found 674.3634.

Preparation of key intermediate acid **36**



SI-8 **36**

Aldehyde **SI-8** (1.74 g, 2.58 mmol, 1 equiv) and isoamylene (1.81 g, 25.8 mmol, 10 equiv) were dissolved in *t*-BuOH (46 mL). An aqueous solution (23 ml) of NaH_2PO_4 (1.55g, 12.9 mmol, 5 equiv) and $NaClO_2$

(1.17 g, 10.3 mmol, 4 equiv) was added to the resulting reaction mixture at 0 °C. After 2 h, the reaction mixture was acidified to pH = 3 with 1 M HCl and extracted with EtOAc (3× 50 mL). The combined organic layers were dried (Na_2SO4), the dried solution was filtered, and the filtrate was concentrated. The resulting crude acid **36** (1.70 g, 95% yield) was used without further purification.

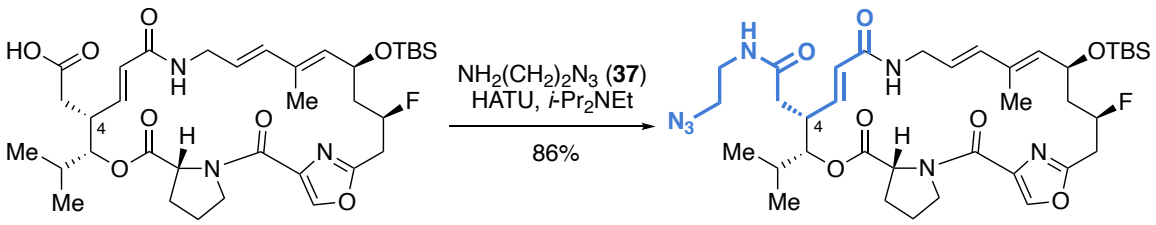
TLC (MeOH:DCM = 1:25): R_f = 0.30 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 6.55 (dd, *J* = 16.4, 4.8 Hz, 1H), 6.30 (dd, *J* = 8.9, 3.2 Hz, 1H), 6.17 (d, *J* = 15.6 Hz, 1H), 6.00 (dd, *J* = 16.3, 1.7 Hz, 1H), 5.62 (ddd, *J* = 15.7, 9.0, 4.3 Hz, 1H), 5.31 (d, *J* = 8.9 Hz, 1H), 5.09 (dm, *J* = 47.6 Hz, 1H), 4.83 (ddd, *J* = 17.8, 9.4, 2.7 Hz, 2H), 4.72 (td, *J* = 9.6, 3.7 Hz, 1H), 4.54 (ddd, *J* = 14.9, 6.7, 2.8 Hz, 1H), 4.06 (ddd, *J* = 12.2, 7.7, 5.3 Hz, 1H), 3.84 (dt, *J* = 11.6, 7.0 Hz, 1H), 3.42 (ddd, *J* = 15.2, 9.0, 3.2 Hz, 1H), 3.25 – 3.18 (m, 1H), 3.13 (dd, *J* = 17.2, 6.5 Hz, 1H), 2.90 (ddd, *J* = 21.7, 16.6, 5.2 Hz, 1H), 2.65 (dd, *J* = 16.5, 2.7 Hz, 1H), 2.42 (dd, *J* = 16.3, 11.3 Hz, 1H), 2.17 (ddd, *J* = 12.8, 9.3, 5.7 Hz, 2H), 1.98 – 1.84 (m, 4H), 1.77 (s, 3H), 1.61 (dddd, *J* = 40.3, 14.3, 10.3, 2.0 Hz, 1H), 0.98 (d, *J* = 6.4 Hz, 3H), 0.98 (d, *J* = 6.6 Hz, 3H), 0.87 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 171.9, 167.5, 160.8, 160.3, 160.3, 143.6, 143.4, 137.1, 136.7, 135.1, 133.4, 124.2, 123.9, 89.3 (d, *J* = 170.3 Hz), 80.9, 66.7, 59.2, 48.8, 43.8 (d, *J* = 20.4 Hz), 43.7, 41.7, 39.1, 34.0 (d, *J* = 25.0 Hz), 31.7, 31.2, 29.8, 29.7, 28.4, 25.9, 25.1, 22.8, 19.9, 18.6, 18.3, 14.3, 13.0, -4.3, -4.8.

HRMS-ESI m/z calcd for $C_{35}H_{53}FN_3O_8Si^+$ $[M + H]^+$ 690.3581, found 690.3585.

Preparation of click chemistry precursor **38**



36 **38**

$^{i}Pr_2EtN$ (0.12 mL, 0.70 mmol, 2 equiv), 2-azidoethan-1-amine **37** (45.3 mg, 0.53 mmol, 1.5 equiv), and acid **36** (0.24 g, 0.35 mmol, 1 equiv) were added to a 50-mL round bottom flask. DCM (12 mL) was added, resulting in a colorless solution. HATU (0.17 g, 0.44 mmol, 1.25 equiv) was added in one portion. After 5 h, the mixture was diluted with DCM (30 mL). The resulting solution was transferred to a separatory funnel and was washed with water (2 × 25 mL) and brine (25 mL). The washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: acetone:hexanes = 1:6 to 1:2) to afford click chemistry precursor **38** (0.22 g, 84% yield) as a white solid.

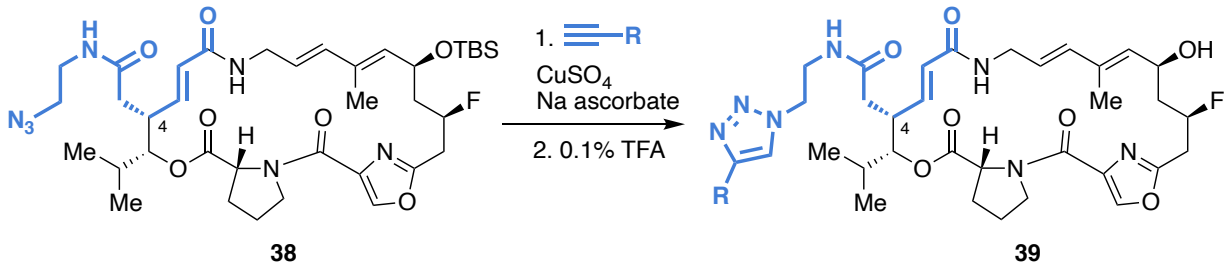
TLC (acetone:hexanes = 1:2): R_f = 0.30 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 6.49 (dd, *J* = 16.2, 5.4 Hz, 1H), 6.24 – 6.13 (m, 2H), 6.07 (dd, *J* = 9.1, 3.2 Hz, 1H), 5.87 (dd, *J* = 16.2, 1.7 Hz, 1H), 5.66 (ddd, *J* = 15.6, 8.6, 4.4 Hz, 1H), 5.30 (d, *J* = 9.0 Hz, 1H), 5.02 (dm, *J* = 48.6 Hz, 1H), 4.91 (dd, *J* = 10.2, 2.0 Hz, 1H), 4.83 (dd, *J* = 9.0, 3.2 Hz, 1H), 4.73 (td, *J* = 9.6, 3.9 Hz, 1H), 4.52 (ddd, *J* = 14.2, 8.9, 4.3 Hz, 1H), 4.08 (ddd, *J* = 12.2, 8.1, 4.7 Hz, 1H), 3.82 (dt, *J* = 11.3, 7.2 Hz, 1H), 3.54 – 3.31 (m, 6H), 3.32 – 3.23 (m, 1H), 3.24 – 3.14 (m, 1H), 2.93 (ddd, *J* = 20.5, 16.4, 5.6 Hz, 1H), 2.54 (dd, *J* = 14.9, 3.5 Hz, 1H), 2.17 (dddd, *J* = 15.7, 13.3, 8.1, 4.7 Hz, 3H), 2.01 – 1.91 (m, 2H), 1.91 – 1.83 (m, 1H), 1.77 (s, 3H), 1.71 – 1.52 (m, 1H), 0.99 (d, *J* = 6.7 Hz, 3H), 0.96 (d, *J* = 6.5 Hz, 3H), 0.87 (s, 9H), 0.05 (s, 3H), 0.01 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 171.1, 165.9, 160.6, 160.5, 160.5, 143.0, 142.3, 136.7, 136.6, 134.8, 133.7, 125.0, 124.8, 89.3 (d, *J* = 170.2 Hz), 81.1, 66.6, 59.2, 50.9, 48.7, 43.7 (d, *J* = 20.5 Hz), 41.2, 39.2, 39.1, 34.0 (d, *J* = 25.2 Hz), 33.8, 29.8, 28.6, 25.9, 25.1, 19.9, 18.7, 18.3, 13.0, -4.3, -4.8.

HRMS-ESI m/z calcd for $C_{37}H_{57}FN_7O_7Si^+$ $[M + H]^+$ 758.4067, found 758.4076.

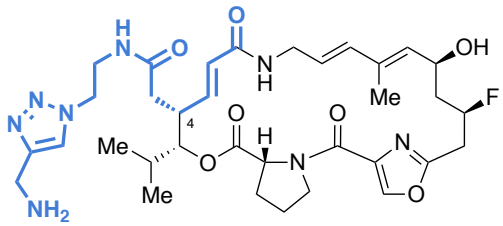
General procedure for preparation of C-4 analogs **39**



38 **39**

An aqueous solution of sodium ascorbate (0.05M, 0.2 equiv) and an aqueous solution of copper(II) sulfate (0.05 M, 0.05 equiv) were added to a suspension of azide compound **38** (1 equiv) and alkyne (3 equiv) in *t*-BuOH-H_2O (3mL-1mL) at 23°C. After stirring at 23°C for 12 h, the solvent was removed under vacuum. The residue was purified by flash chromatography over silica gel (5→10% methanol in dichloromethane) to afford the product as an off white solid. Then the white solid was dissolved with 0.1% TFA in MeCN-H_2O (95/5 v/v), and the solution was stirred for 12 hours at 23°C. The reaction mixture was concentrated, and the resulting crude residue was purified by preparative HPLC (eluent: 0.1% TFA in H_2O: 0.1% TFA in acetonitrile = 95:5 to 5:95 over 15 min) to afford C-4 modified analog **39** TFA salt as a white solid.

Analog **39a** (SA0113142)



Prepared according to general procedure of C-4 analogs 39. Analog **39a** (8 mg, 55% yield over 2 steps) was obtained as a white solid.

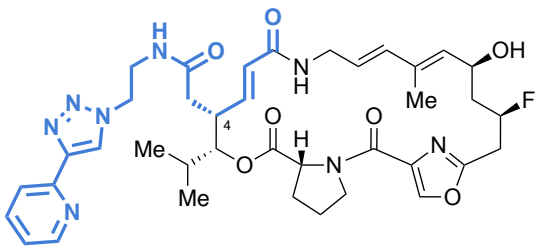
TLC (MeOH:DCM = 1:5): R_f = 0.12 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, MeOD) δ 8.26 (s, 1H), 8.05 (s, 1H), 6.56 (dd, *J* = 16.0, 5.0 Hz, 1H), 6.27 (d, *J* = 15.7 Hz, 1H), 5.93 (dd, *J* = 16.0, 1.7 Hz, 1H), 5.77 (ddd, *J* = 15.6, 8.0, 4.1 Hz, 1H), 5.40 (d, *J* = 9.1 Hz, 1H), 5.07 (dm, *J* = 47.7Hz, 1H), 4.96 (dd, *J* = 10.3, 2.2 Hz, 1H), 4.81 (dd, *J* = 8.7, 3.2 Hz, 1H), 4.72 (td, *J* = 9.1, 4.9 Hz, 1H), 4.55 (t, *J* = 5.7 Hz, 2H), 4.25 (s, 2H), 4.21 – 4.05 (m, 2H), 3.84 – 3.66 (m, 3H), 3.62 (dt, *J* = 14.3, 5.7 Hz, 1H), 3.28 – 3.02 (m, 3H), 2.55 (dd, *J* = 15.2, 3.5 Hz, 1H), 2.39 (dd, *J* = 15.1, 11.1 Hz, 1H), 2.25 –1.98 (m, 4H), 1.98 – 1.87 (m, 2H), 1.84 (s, 3H), 1.78 – 1.69 (m, 1H), 1.00 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, MeOD) δ 174.4, 171.7, 167.5, 162.6, 162.1, 145.1, 144.9, 141.1, 137.8, 136.9, 136.7, 134.7, 126.1, 125.9, 125.6, 90.4 (d, *J* = 170.4 Hz), 82.4, 66.0, 60.5, 50.8, 50.1, 43.1 (d, *J* = 20.1 Hz), 41.7, 40.6, 39.9, 35.5, 34.1 (d, *J* = 24.9 Hz), 33.5, 30.7, 29.2, 25.9, 20.1, 18.7, 13.2.

HRMS-ESI m/z calcd for $C_{34}H_{48}FN_8O_7^+$ $[M + H]^+$ 699.3625, found 699.3630.

Analog **39b** (SA0113143)



Prepared according to general procedure of C-4 analogs 39. Analog **39b** (6.5 mg, 33% yield over 2 steps) was obtained as a white solid.

TLC (MeOH:DCM = 1:20): R_f = 0.13 (UV, *p*-anisaldehyde).

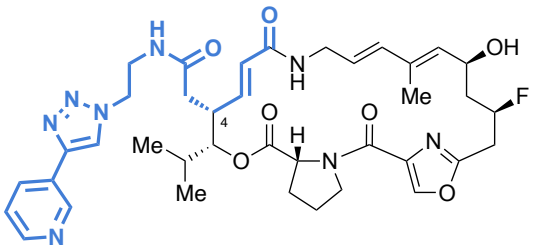
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.57 (s, 1H), 8.23 (s, 1H), 8.17 – 8.05 (m, 2H), 7.81 (t, *J* = 7.7 Hz, 1H), 6.61 (s, 1H), 6.52 (dd, *J* = 16.2, 5.5 Hz, 1H), 6.50 (s,1H), 6.15 (d, *J* = 15.6 Hz, 1H), 5.90 (d, *J* = 16.0 Hz, 1H), 5.68 (ddd, *J* = 15.7, 8.1, 4.1 Hz, 1H), 5.34 (d, *J* = 8.9 Hz, 1H), 5.05 (dm, *J* = 48.7 Hz, 1H), 4.86 (d, *J* = 9.9 Hz, 1H), 4.77 (td, *J* = 9.0, 4.0 Hz, 2H), 4.55 (t, *J* = 5.6 Hz, 2H), 4.31 (s, 1H), 4.04 (ddd, *J* = 12.9, 8.0, 4.8

Hz, 1H), 3.91 – 3.70 (m, 3H), 3.60 – 3.45 (m, 1H), 3.20 (td, J = 16.9, 16.4, 5.4 Hz, 2H), 2.99 (td, J = 16.3, 6.8 Hz, 1H), 2.52 (dd, J = 15.2, 3.4 Hz, 1H), 2.35 – 2.21 (m, 2H), 2.21 – 2.06 (m, 3H), 1.96 – 1.84 (m, 4H), 1.79 (s, 3H), 1.69 – 1.50 (m, 1H), 1.02 – 0.95 (d, J = 6.6 Hz, 3H), 0.93 (d, J = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 171.2, 165.9, 160.6, 160.1, 160.0, 149.4, 143.4, 142.8, 137.4, 136.8, 136.2, 135.4, 133.2, 125.6, 124.9, 123.4, 120.6, 89.4 (d, J = 170.6 Hz), 81.0, 65.8, 59.2, 50.1, 48.7, 42.4 (d, J = 20.5 Hz), 40.9, 39.6, 39.1, 33.8 (d, J = 25.5 Hz), 33.3, 29.8, 29.7, 28.5, 25.0, 19.8, 18.7, 14.3, 13.1.

HRMS-ESI m/z calcd for $C_{38}H_{48}FN_8O_7^+$ $[M + H]^+$ 747.3625, found 747.3629.

Analog **39c** (SA0113144)



Prepared according to general procedure of C-4 analogs 39. Analog **39c** (11.5 mg, 58% yield over 2 steps) was obtained as a white solid.

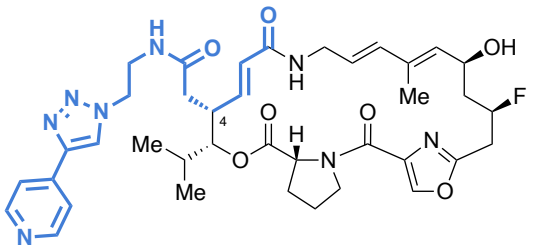
TLC (MeOH:DCM = 1:20): R_f = 0.10 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.98 (s, 1H), 8.49 (d, J = 4.7 Hz, 1H), 8.28 (s, 1H), 8.17 (dd, J = 8.0, 1.9 Hz, 1H), 8.07 (s, 1H), 7.32 (dd, J = 8.0, 4.8 Hz, 1H), 7.03 (t, J = 6.0 Hz, 1H), 6.58 – 6.42 (m, 2H), 6.17 (d, J = 15.7 Hz, 1H), 5.90 (dd, J = 16.1, 1.6 Hz, 1H), 5.72 (ddd, J = 15.7, 7.7, 4.1 Hz, 1H), 5.35 (d, J = 9.0 Hz, 1H), 5.05 (dm, J = 47.4 Hz, 1H), 4.87 (dd, J = 10.1, 2.1 Hz, 1H), 4.78 (dt, J = 9.2, 4.6 Hz, 1H), 4.75 – 4.65 (m, 1H), 4.52 (q, J = 4.5 Hz, 2H), 4.39 (dd, J = 16.9, 7.8 Hz, 1H), 3.98 (ddq, J = 20.6, 10.8, 4.8 Hz, 2H), 3.73 (ddt, J = 14.3, 11.1, 4.8 Hz, 2H), 3.50 (ddd, J = 16.4, 8.0, 3.5 Hz, 1H), 3.28 – 3.11 (m, 2H), 2.97 (td, J = 16.7, 6.6 Hz, 1H), 2.53 (dd, J = 15.0, 3.5 Hz, 1H), 2.29 – 2.10 (m, 3H), 1.98 – 1.90 (m, 2H), 1.80 (s, 3H), 1.76 – 1.51 (m, 4H), 0.95 (d, J = 6.7 Hz, 3H), 0.92 (d, J = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.7, 171.3, 165.9, 160.6, 160.2, 160.1, 149.1, 147.0, 144.7, 143.3, 142.5, 136.7, 136.1, 135.5, 133.4, 133.2, 125.5, 125.0, 123.9, 122.3, 89.3 (d, *J* = 170.6 Hz), 81.0, 65.6, 59.1, 50.2, 48.7, 42.4 (d, *J* = 20.5 Hz), 40.9, 39.7, 39.0, 33.8 (d, *J* = 25.1 Hz), 33.4, 29.6, 28.4, 24.9, 19.8, 18.6, 13.1.

HRMS-ESI m/z calcd for $C_{38}H_{48}FN_8O_7^+$ $[M + H]^+$ 747.3625, found 747.3632.

Analog **39d** (SA0113146)



Prepared according to general procedure of C-4 analogs 39. Analog **39d** (11.5 mg, 58% yield over 2 steps) was obtained as a white solid.

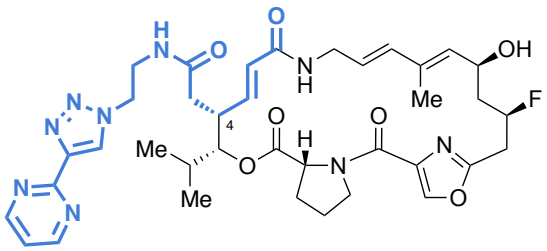
TLC (MeOH:DCM = 1:20): R_f = 0.08 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.60 (d, *J* = 6.2 Hz, 2H), 8.36 (s, 1H), 8.07 (s, 1H), 7.80 (d, *J* = 6.1 Hz, 2H), 6.64 (t, *J* = 6.0 Hz, 1H), 6.46 (dd, *J* = 16.1, 5.4 Hz, 1H), 6.34 (dd, *J* = 8.6, 3.4 Hz, 1H), 6.16 (d, *J* = 15.7 Hz, 1H), 5.88 (dd, *J* = 16.1, 1.6 Hz, 1H), 5.69 (ddd, *J* = 15.8, 7.8, 4.2 Hz, 1H), 5.34 (d, *J* = 8.9 Hz, 1H), 5.16 – 4.93 (m, 1H), 4.88 (dd, *J* = 10.1, 2.2 Hz, 1H), 4.79 (dt, *J* = 9.2, 4.6 Hz, 1H), 4.73 (dd, *J* = 9.0, 3.4 Hz, 1H), 4.59 – 4.40 (m, 3H), 4.00 (ddq, *J* = 19.6, 11.0, 4.9 Hz, 2H), 3.74 (dq, *J* = 12.3, 6.1, 4.8 Hz, 2H), 3.48 (ddd, *J* = 16.0, 7.8, 3.3 Hz, 1H), 3.29 – 3.13 (m, 2H), 2.99 (td, *J* = 16.7, 6.5 Hz, 1H), 2.52 (dd, *J* = 14.9, 3.5 Hz, 1H), 2.25 – 2.15 (m, 2H), 2.11 – 1.93 (m, 4H), 1.80 (s, 3H), 1.72 – 1.52 (m, 3H), 0.96 (d, *J* = 6.7 Hz, 3H), 0.93 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.4, 171.2, 165.8, 160.4, 160.1, 160.0, 150.2, 145.1, 143.1, 142.2, 138.1, 136.5, 135.8, 135.5, 133.3, 125.1, 124.9, 123.4, 120.0, 89.1 (d, *J* = 169.8 Hz), 80.8, 65.5, 59.0, 50.1, 48.6, 42.2 (d, *J* = 21.2 Hz), 40.7, 39.5, 38.9, 33.6 (d, *J* = 26.1 Hz), 33.3, 29.5, 28.3, 24.8, 19.6, 18.5, 13.0.

HRMS-ESI m/z calcd for $C_{38}H_{48}FN_8O_7^+$ $[M + H]^+$ 747.3625, found 747.3630.

Analog **39e** (SA0113147)



Prepared according to general procedure of C-4 analogs 39. Analog **39e** (13 mg, 66% yield over 2 steps) was obtained as a white solid.

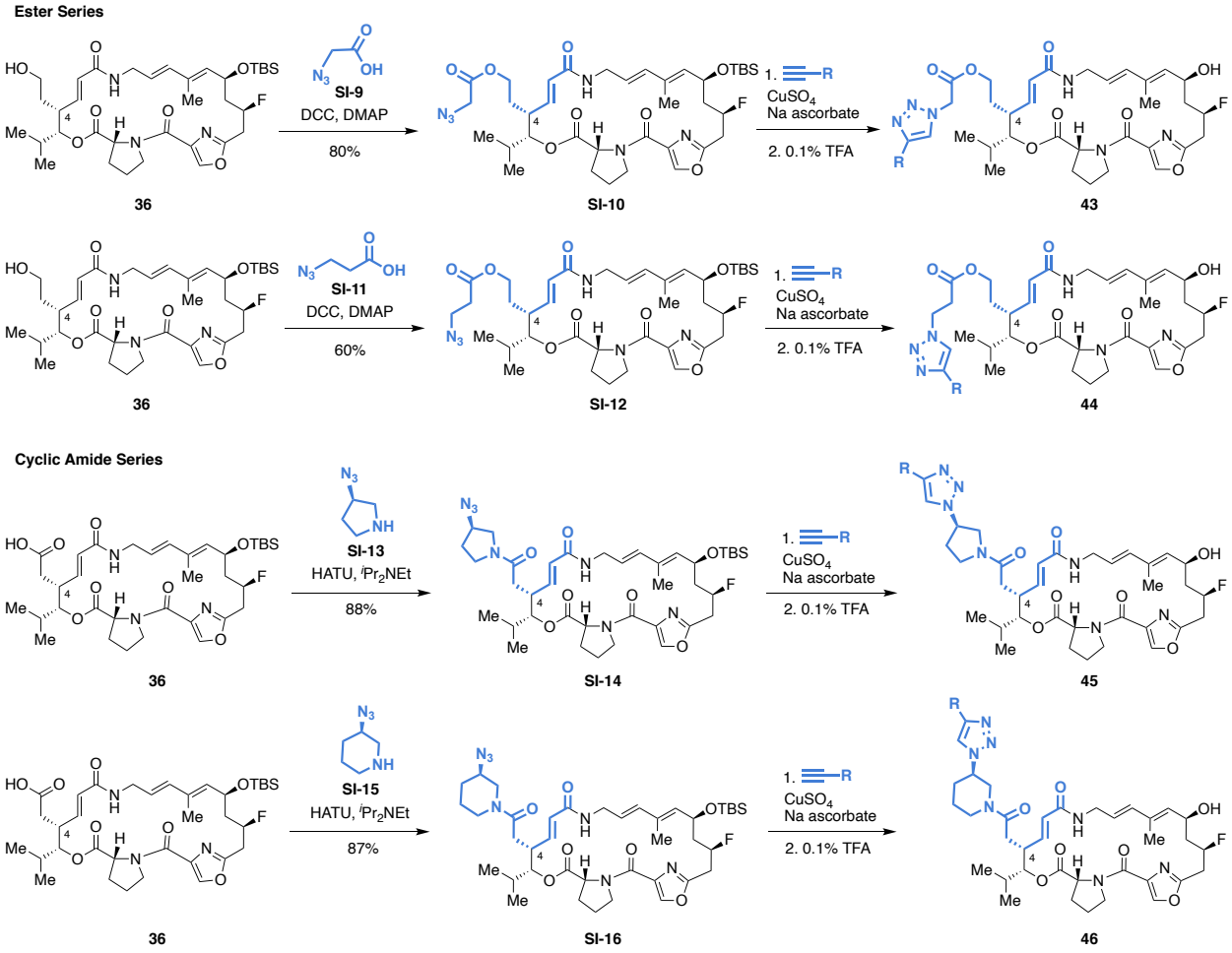
TLC (MeOH:DCM = 1:20): R_f = 0.08 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, *J* = 4.8 Hz, 2H), 8.27 (s, 1H), 8.09 (s, 1H), 7.22 (t, *J* = 4.6 Hz, 1H), 6.69 – 6.50 (m, 2H), 6.15 (d, *J* = 15.6 Hz, 1H), 5.93 (d, *J* = 15.9 Hz, 1H), 5.66 (ddd, *J* = 15.7, 7.8, 4.2 Hz, 1H), 5.34 (d, *J* = 8.9 Hz, 1H), 5.04 (dm, *J* = 47.4 Hz, 1H), 4.85 (dd, *J* = 10.0, 2.0 Hz, 1H), 4.74 (dd, *J* = 8.7, 3.6 Hz, 2H), 4.55 (t, *J* = 5.8 Hz, 3H), 4.22 (dd, *J* = 14.0, 8.6 Hz, 1H), 4.00 (ddd, *J* = 12.2, 8.1, 4.6 Hz, 1H), 3.90 – 3.65 (m, 4H), 3.65 – 3.55 (m, 1H), 3.29 (d, *J* = 11.4 Hz, 1H), 3.19 (td, *J* = 17.0, 5.3 Hz, 1H), 2.98 (td, *J* = 16.6, 6.6 Hz, 1H), 2.54 (td, *J* = 21.5, 18.3, 8.8 Hz, 1H), 2.41 – 2.29 (m, 1H), 2.21 – 1.95 (m, 5H), 1.87 (ddd, *J* = 12.9, 6.7, 3.4 Hz, 2H), 1.77 (s, 3H), 0.94 (d, *J* = 7.0 Hz, 3H), 0.91 (d, *J* = 8.3 Hz, 3H).

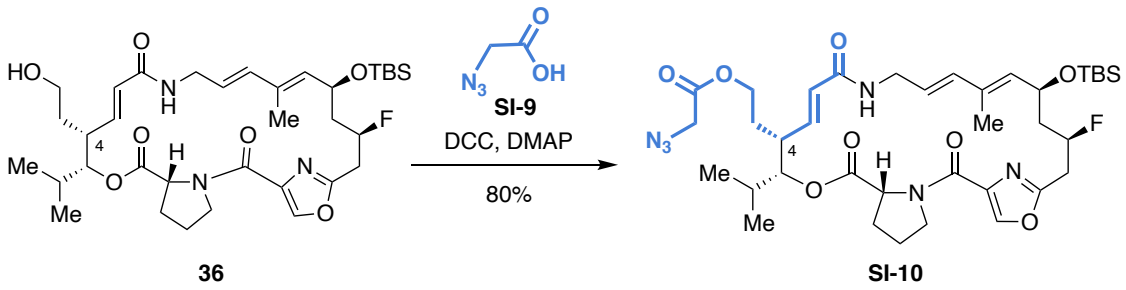
^{13}C NMR (100 MHz, $CDCl_3$) δ 171.9, 171.1, 165.9, 160.6, 160.0, 158.9, 157.6, 147.1, 143.3, 143.1, 136.8, 136.0, 135.5, 133.2, 126.2, 125.6, 124.7, 120.0, 89.4 (d, *J* = 170.8 Hz), 81.1, 65.7, 59.1, 50.2, 48.7, 42.4 (d, *J* = 19.8 Hz), 40.9, 39.6, 39.1, 33.7 (d, *J* = 25.3 Hz), 33.2, 29.8, 29.7, 28.4, 25.0, 19.8, 18.7, 13.1.

HRMS-ESI m/z calcd for $C_{37}H_{47}FN_9O_7^+$ $[M + H]^+$ 748.3577, found 748.3583.

Scheme IV. Preparation for C-4 modified analogs 43, 44, 45, and 46



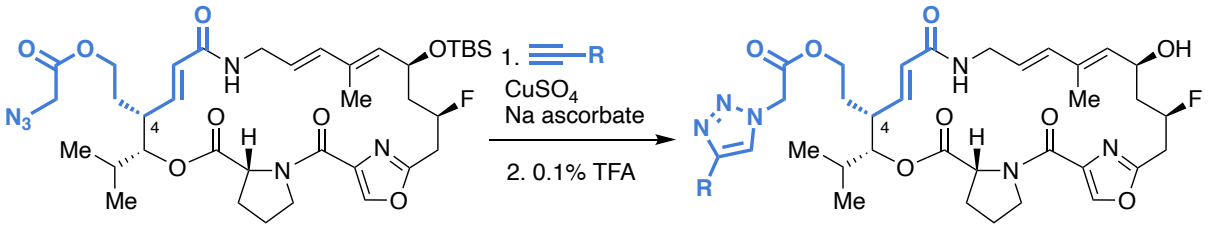
Preparation of click chemistry precursor **SI-10**



2-azidoacetic acid **SI-9** (4.59 µL, 61.3 µmol, 1.35 equiv), DMAP (1.11 mg, 9.08 µmol, 0.2 equiv) and acid **36** (30.7 mg, 45.4 µmol, 1 equiv) were added to a 5-mL round-bottom flask. DCM (0.45 mL) was added,

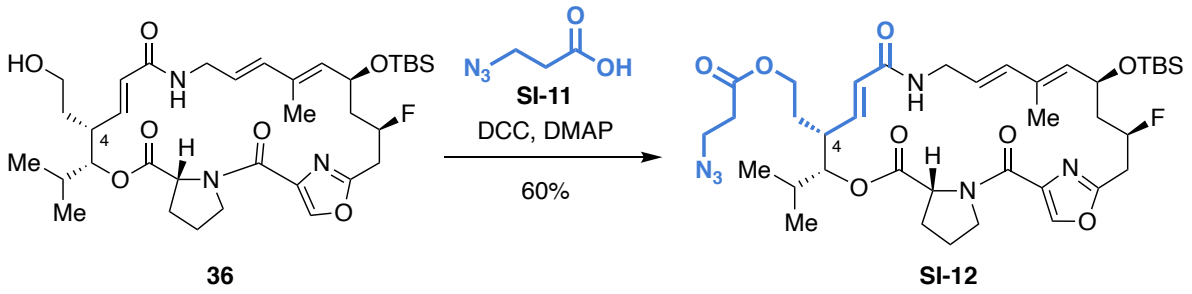
resulting in a colorless solution. DCC (17.4 µL, 68.1 µmol, 1.5 equiv) was added in one portion. resulting in a white suspension. After 5 h, alcohol **36** was entirely consumed as indicated by TLC analysis (eluent: EtOAc:hexanes = 1:1). The mixture was filtered through a pad of celite, and the filter cake was washed with DCM (2 × 20 mL). The combined filtrates were concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes =1:1) to afford click chemistry precursor **SI-10** (27.7 mg, 80% yield) as a white solid.

General procedure for preparation of C-4 analogs **43**



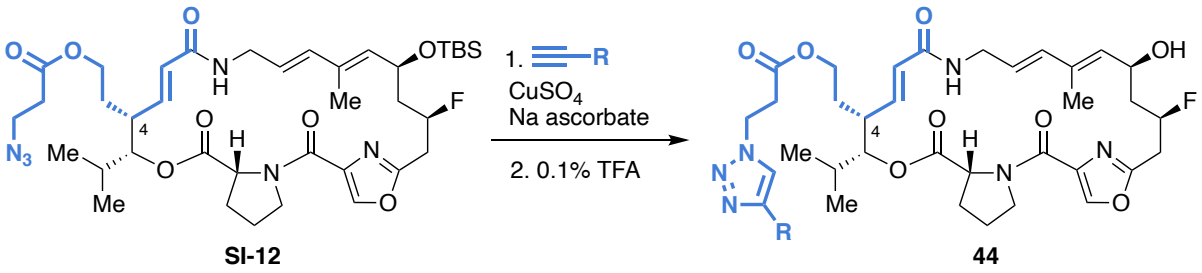
An aqueous solution of sodium ascorbate (0.05M, 0.2 equiv) and an aqueous solution of copper(II) sulfate (0.05 M, 0.05 equiv) were added to a suspension of azide compound **SI-10** (1 equiv) and alkyne (3 equiv) in *t*-BuOH-H_2O (3mL-1mL) at 23°C. After stirring at 23°C for 12 h, the solvent was removed under vacuum. The residue was purified by flash chromatography over silica gel (5→10% methanol in dichloromethane) to afford the product as an off white solid. Then the white solid was dissolved with 0.1% TFA in MeCN-H_2O (95/5 v/v), and the solution was stirred for 12 hours at 23°C. The reaction mixture was concentrated, and the resulting crude residue was purified by flash chromatography over silica gel (0→10% methanol in dichloromethane) to afford C-4 modified analog **43** TFA salt as a white solid.

Preparation of click chemistry precursor **SI-12**



2-azidoacetic acid **SI-11** (10.6 µL, 0.11 mmol, 1.35 equiv), DMAP (2.06 mg, 16.9 µmol, 0.2 equiv) and acid **36** (57.1 mg, 84.5 µmol, 1 equiv) were added to a 5-mL round-bottom flask. DCM (0.85 mL) was added, resulting in a colorless solution. DCC (32.4 µL, 0.13 mmol, 1.5 equiv) was added in one portion. resulting in a white suspension. After 5 h, alcohol **36** was entirely consumed as indicated by TLC analysis (eluent: EtOAc:hexanes = 1:1). The mixture was filtered through a pad of celite, and the filter cake was washed with DCM (2 × 20 mL). The combined filtrates were concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes =1:1) to afford click chemistry precursor **SI-12** (39.1 mg, 60% yield) as a white solid.

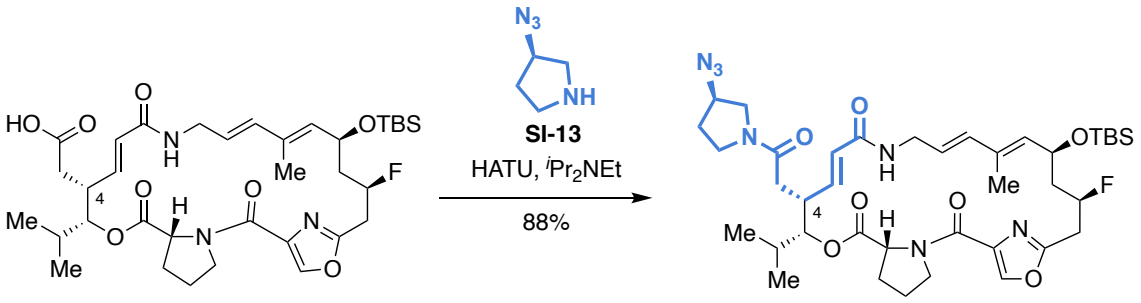
General procedure for preparation of C-4 analogs **44**



An aqueous solution of sodium ascorbate (0.05M, 0.2 equiv) and an aqueous solution of copper(II) sulfate (0.05 M, 0.05 equiv) were added to a suspension of azide compound **SI-12** (1 equiv) and alkyne (3 equiv) in *t*-BuOH-H_2O (3mL-1mL) at 23°C. After stirring at 23°C for 12 h, the solvent was removed under vacuum. The residue was purified by flash chromatography over silica gel (5→10% methanol in

dichloromethane) to afford the product as an off white solid. Then the white solid was dissolved with 0.1% TFA in MeCN-H_2O (95/5 v/v), and the solution was stirred for 12 hours at 23°C. The reaction mixture was concentrated, and the resulting crude residue was purified by flash chromatography over silica gel (0→10% methanol in dichloromethane) to afford C-4 modified analog **44** TFA salt as a white solid.

Preparation of click chemistry precursor **SI-14**



$^{i}Pr_2EtN$ (0.21 mL, 1.16 mmol, 2 equiv), (R)-3-azidopyrrolidine **SI-13** (131 mg, 0.58 mmol, 2 equiv), and acid **36** (0.20 g, 0.29 mmol, 1 equiv) were added to a 50-mL round bottom flask. DCM (12 mL) was added, resulting in a colorless solution. HATU (0.14 g, 0.36 mmol, 1.25 equiv) was added in one portion. After 5 h, the mixture was diluted with DCM (30 mL). The resulting solution was transferred to a separatory funnel and was washed with water (2 × 25 mL) and brine (25 mL). The washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: acetone:hexanes = 1:6 to 1:2) to afford click chemistry precursor **SI-14** (0.19 g, 88% yield) as a white solid.

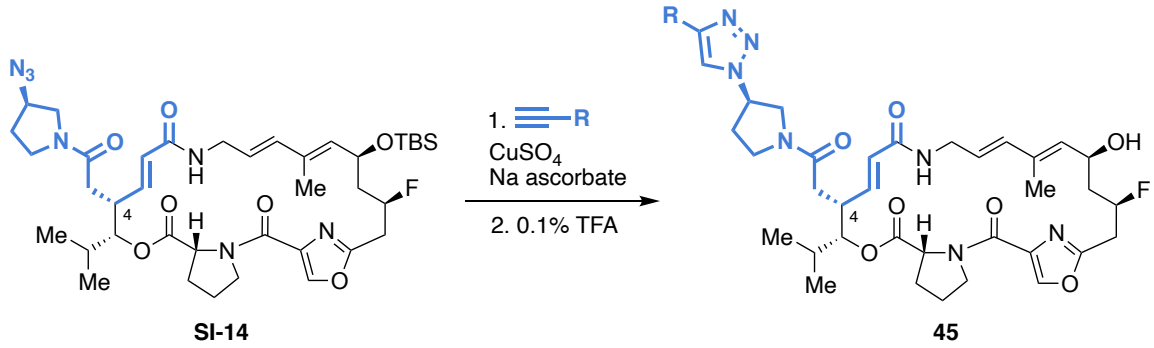
TLC (acetone:hexanes = 1:2): R_f = 0.30 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (d, *J* = 3.3 Hz, 1H), 6.53 (ddd, *J* = 15.6, 10.0, 5.4 Hz, 1H), 6.24 (dd, *J* = 18.5, 10.5 Hz, 2H), 5.80 (t, *J* = 14.9 Hz, 1H), 5.59 (ddt, *J* = 14.7, 9.2, 4.5 Hz, 1H), 5.37 (d, *J* = 9.0 Hz, 1H), 5.08 (s, 1H), 4.96 – 4.87 (m, 1H), 4.76 (ddt, *J* = 23.0, 9.6, 3.7 Hz, 2H), 4.35 – 4.13 (m, 2H), 4.02 (dt, *J* = 11.6, 6.8 Hz, 1H), 3.81 (s, 3H), 3.57 (qt, *J* = 10.0, 4.1 Hz, 4H), 3.38 – 3.31 (m, 1H), 3.14 (td, *J* = 14.2,

8.4 Hz, 1H), 2.95 (ddd, *J* = 26.3, 16.6, 4.2 Hz, 1H), 2.55 – 2.44 (m, 1H), 2.43 – 2.31 (m, 1H), 2.21 – 2.12 (m, 4H), 2.05 (d, *J* = 10.6 Hz, 1H), 1.98 – 1.93 (m, 1H), 1.86 (d, *J* = 8.1 Hz, 1H), 1.75 (d, *J* = 4.4 Hz, 3H), 1.40 (dd, *J* = 10.5, 6.7 Hz, 1H), 0.95 (t, *J* = 7.6 Hz, 6H), 0.86 (s, 9H), 0.05 (s, 3H), 0.01 (s, 3H).

^{13}C NMR (100 MHz, MeOD) δ 172.0, 171.8, 171.6, 167.3, 162.6, 145.9, 144.6, 137.7, 137.1, 135.6, 135.1, 126.1, 125.0, 90.5 (d, *J* = 163.6 Hz), 82.5, 67.9, 62.0, 60.7, 60.4, 55.9 (d, *J* = 17.4 Hz), 52.8, 52.0, 50.1, 45.9, 45.2, 41.9, 39.6 (d, *J* = 25.8 Hz), 34.1, 32.3, 30.7, 29.3, 26.3, 26.3, 25.9, 20.1, 19.0, 18.9, 13.3, -4.2, -4.7.

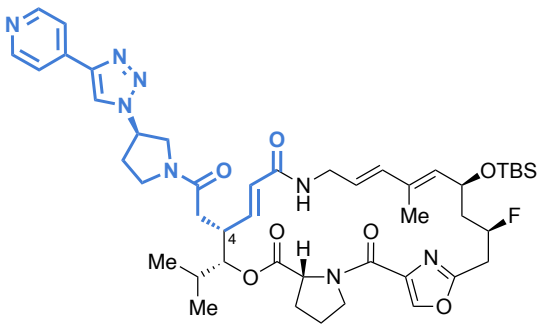
General procedure for preparation of C-4 analogs **45**



SI-14 **45**

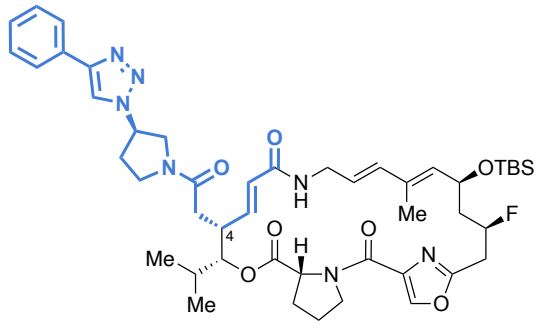
An aqueous solution of sodium ascorbate (0.05M, 0.2 equiv) and an aqueous solution of copper(II) sulfate (0.05 M, 0.05 equiv) were added to a suspension of azide compound **SI-14** (1 equiv) and alkyne (3 equiv) in *t*-BuOH-H_2O (3mL-1mL) at 23°C. After stirring at 23°C for 12 h, the solvent was removed under vacuum. The residue was purified by flash chromatography over silica gel (5→10% methanol in dichloromethane) to afford the product as an off white solid. Then the white solid was dissolved with 0.1% TFA in MeCN-H_2O (95/5 v/v), and the solution was stirred for 12 hours at 23°C. The reaction mixture was concentrated, and the resulting crude residue was purified by flash chromatography over silica gel (0→10% methanol in dichloromethane) to afford C-4 modified analog **45** TFA salt as a white solid.

Analog **45a** - TBS (SA1101149)



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 2H), 8.44 – 8.00 (m, 2H), 7.90 (d, *J* = 31.0 Hz, 2H), 6.71 – 6.44 (m, 1H), 6.18 (d, *J* = 14.3 Hz, 1H), 5.86 (t, *J* = 15.3 Hz, 1H), 5.73 – 5.53 (m, 1H), 5.46 – 5.17 (m, 2H), 5.06 (d, *J* = 48.3 Hz, 1H), 4.94 (d, *J* = 10.2 Hz, 1H), 4.84 (ddd, *J* = 18.7, 8.7, 3.5 Hz, 1H), 4.72 (dd, *J* = 9.4, 4.4 Hz, 1H), 4.43 (s, 1H), 4.16 (d, *J* = 6.0 Hz, 1H), 4.09 – 3.97 (m, 2H), 3.90 – 3.72 (m, 3H), 3.53 (s, 1H), 3.39 (s, 1H), 3.12 (dd, *J* = 17.3, 6.4 Hz, 1H), 2.97 – 2.85 (m, 1H), 2.77 – 2.68 (m, 1H), 2.62 – 2.51 (m, 2H), 2.23 – 2.10 (m, 2H), 2.08 (s, 1H), 1.91 (s, 2H), 1.77 (s, 2H), 1.74 (s, 1H), 1.70 – 1.50 (m, 2H), 1.49 – 1.34 (m, 2H), 0.97 (t, *J* = 6.6 Hz, 6H), 0.87 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H).

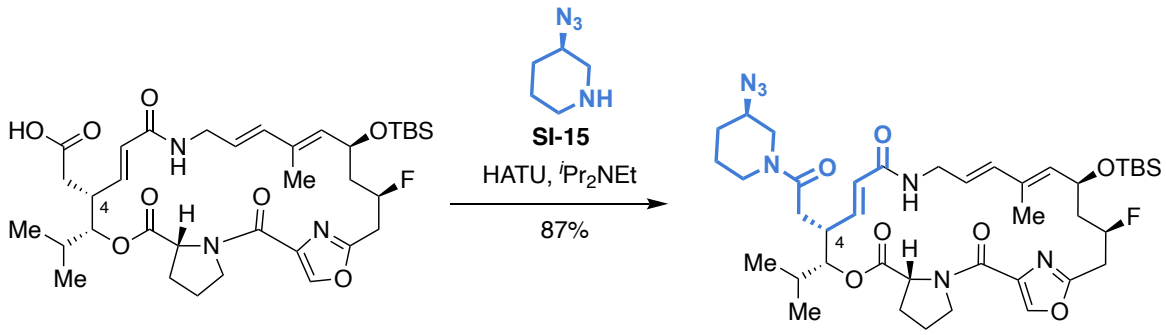
Analog **45b** - TBS (SA1101150)



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 6.1 Hz, 2H), 7.92 – 7.70 (m, 2H), 7.50 – 7.29 (m, 2H), 6.66 (dd, *J* = 15.9, 4.4 Hz, 1H), 6.55 – 6.47 (m, 1H), 6.22 – 6.10 (m, 1H), 6.05 – 5.88 (m, 1H), 5.86 – 5.75 (m, 1H), 5.64 (ddd, *J* = 15.8, 8.6, 4.2 Hz, 1H), 5.33 (d, *J* = 8.4 Hz, 1H), 5.15 (s, 1H), 4.96 (dt, *J* = 10.2, 2.4 Hz, 2H), 4.86 (dt, *J* = 8.7, 3.2 Hz, 1H), 4.73 (dt, *J* = 9.6, 5.0 Hz, 1H), 4.55 (q, *J* = 6.0 Hz, 1H), 4.30 – 4.20 (m, 1H), 4.12 (dq, *J* = 13.4, 6.0 Hz, 2H), 4.00 (dd, *J* = 12.5, 6.8 Hz, 1H), 3.86 – 3.77 (m, 2H), 3.70 – 3.65 (m, 1H),

3.63 – 3.55 (m, 2H), 3.41 (dd, J = 11.1, 4.6 Hz, 2H), 3.14 (td, J = 17.0, 6.7 Hz, 1H), 2.94 – 2.83 (m, 1H), 2.54 (d, J = 6.2 Hz, 1H), 2.42 – 2.31 (m, 1H), 2.17 – 2.12 (m, 2H), 2.05 (s, 1H), 1.89 (dd, J = 8.0, 3.4 Hz, 2H), 1.76 (s, 3H), 1.68 – 1.61 (m, 1H), 1.53 (dd, J = 23.3, 11.6 Hz, 1H), 0.97 (td, J = 4.8, 2.5 Hz, 6H), 0.87 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H).

Preparation of click chemistry precursor **SI-16**



36 **SI-16**

$^{i}Pr_2EtN$ (0.21 mL, 1.16 mmol, 2 equiv), (R)-3-azidopiperidine **SI-15** (139 mg, 0.58 mmol, 2 equiv), and acid **36** (0.20 g, 0.29 mmol, 1 equiv) were added to a 50-mL round bottom flask. DCM (12 mL) was added, resulting in a colorless solution. HATU (0.14 g, 0.36 mmol, 1.25 equiv) was added in one portion. After 5 h, the mixture was diluted with DCM (30 mL). The resulting solution was transferred to a separatory funnel and was washed with water (2 × 25 mL) and brine (25 mL). The washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: acetone:hexanes = 1:6 to 1:2) to afford click chemistry precursor **SI-16** (0.20 g, 87% yield) as a white solid.

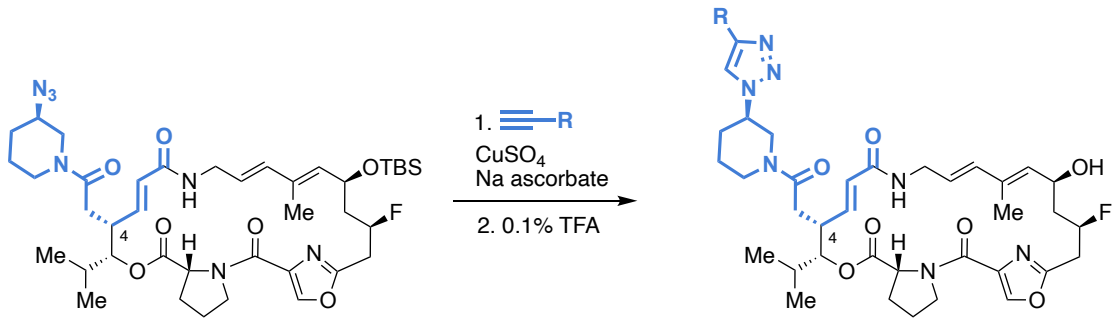
TLC (acetone:hexanes = 1:2): R_f = 0.40 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (s, 1H), 6.49 (dd, J = 16.0, 4.8 Hz, 1H), 6.17 (d, J = 15.7 Hz, 1H), 6.07 – 5.95 (m, 1H), 5.81 (t, J = 17.2 Hz, 1H), 5.64 (ddd, J = 15.7, 8.7, 4.1 Hz, 1H), 5.32 (d, J = 9.0 Hz, 1H), 5.12 (d, J = 5.3 Hz, 1H), 4.94 (d, J = 10.2 Hz, 1H), 4.83 (dd, J = 8.5, 4.0 Hz, 1H), 4.73 (td, J = 9.6, 3.9 Hz,

1H), 4.48 (s, 1H), 4.14 – 3.99 (m, 2H), 3.85 – 3.77 (m, 1H), 3.62 – 3.48 (m, 3H), 3.37 (q, J = 7.1 Hz, 2H), 3.26 (dt, J = 17.0, 6.9 Hz, 1H), 3.13 (td, J = 17.0, 6.9 Hz, 1H), 2.98 – 2.83 (m, 1H), 2.60 – 2.43 (m, 2H), 2.19 – 2.11 (m, 2H), 1.95 – 1.89 (m, 3H), 1.85 – 1.80 (m, 2H), 1.76 (s, 3H), 1.61 (dt, J = 40.6, 12.2 Hz, 3H), 1.45 – 1.38 (m, 1H), 0.99 – 0.92 (m, 6H), 0.86 (s, 9H), 0.04 (s, 3H), 0.01 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 169.4, 165.7, 160.7, 160.4, 143.7, 143.2, 136.3, 134.7, 133.7, 124.9, 124.3, , 89.3 (d, J = 169.7 Hz), 81.2, 66.6, 59.1, 56.9, 56.4, 45.8 (d, J = 13.9 Hz), 45.7, 41.3, 38.7, 38.6, 34.0 (d, J = 25.4 Hz), 30.0, 29.8, 29.4, 29.1, 28.4, 25.9, 25.0, 23.3, 22.1, 19.8, 18.8, 18.2, 13.0, -4.3, -4.8.

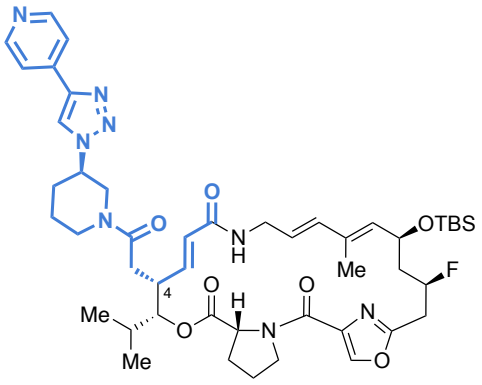
General procedure for preparation of C-4 analogs **46**



SI-16 **46**

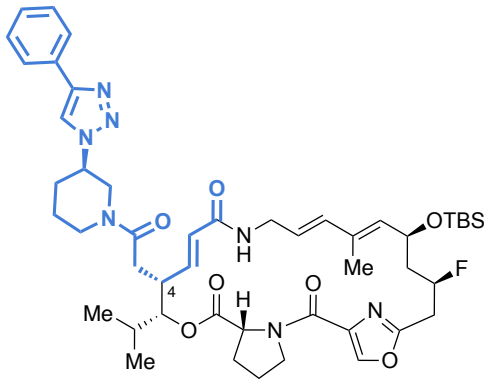
An aqueous solution of sodium ascorbate (0.05M, 0.2 equiv) and an aqueous solution of copper(II) sulfate (0.05 M, 0.05 equiv) were added to a suspension of azide compound **SI-16** (1 equiv) and alkyne (3 equiv) in *t*-BuOH-H_2O (3mL-1mL) at 23°C. After stirring at 23°C for 12 h, the solvent was removed under vacuum. The residue was purified by flash chromatography over silica gel (5→10% methanol in dichloromethane) to afford the product as an off white solid. Then the white solid was dissolved with 0.1% TFA in MeCN-H_2O (95/5 v/v), and the solution was stirred for 12 hours at 23°C. The reaction mixture was concentrated, and the resulting crude residue was purified by flash chromatography over silica gel (0→10% methanol in dichloromethane) to afford C-4 modified analog **46** TFA salt as a white solid.

Analog **46a** - TBS (SA1101151)



^{1}H NMR (400 MHz, $CDCl_3$) δ 8.67 (s, 2H), 8.19 (d, *J* = 10.4 Hz, 1H), 8.08 (d, *J* = 11.3 Hz, 1H), 7.81 (s, 2H), 6.64 – 6.50 (m, 1H), 6.19 (d, *J* = 16.3 Hz, 1H), 5.84 (d, *J* = 16.0 Hz, 1H), 5.63 (d, *J* = 15.3 Hz, 1H), 5.34 (d, *J* = 8.9 Hz, 1H), 5.11 (s, 1H), 5.03 – 4.86 (m, 2H), 4.83 – 4.69 (m, 2H), 4.54 (d, *J* = 11.3 Hz, 1H), 4.11 (dd, *J* = 16.9, 9.7 Hz, 2H), 3.94 (d, *J* = 34.7 Hz, 1H), 3.87 – 3.74 (m, 2H), 3.64 (d, *J* = 11.7 Hz, 1H), 3.49 (d, *J* = 10.6 Hz, 1H), 3.36 (s, 2H), 3.12 (dd, *J* = 16.0, 6.6 Hz, 1H), 2.94 (d, *J* = 23.1 Hz, 1H), 2.55 (d, *J* = 6.2 Hz, 1H), 2.35 (s, 1H), 2.23 – 2.13 (m, 2H), 2.09 (s, 2H), 2.04 (s, 1H), 1.87 (s, 4H), 1.76 – 1.71 (m, 3H), 1.57 (t, *J* = 11.8 Hz, 1H), 1.44 – 1.39 (m, 1H), 0.97 – 0.90 (m, 6H), 0.87 (s, 9H), 0.05 (s, 3H), 0.02 (s, 3H).

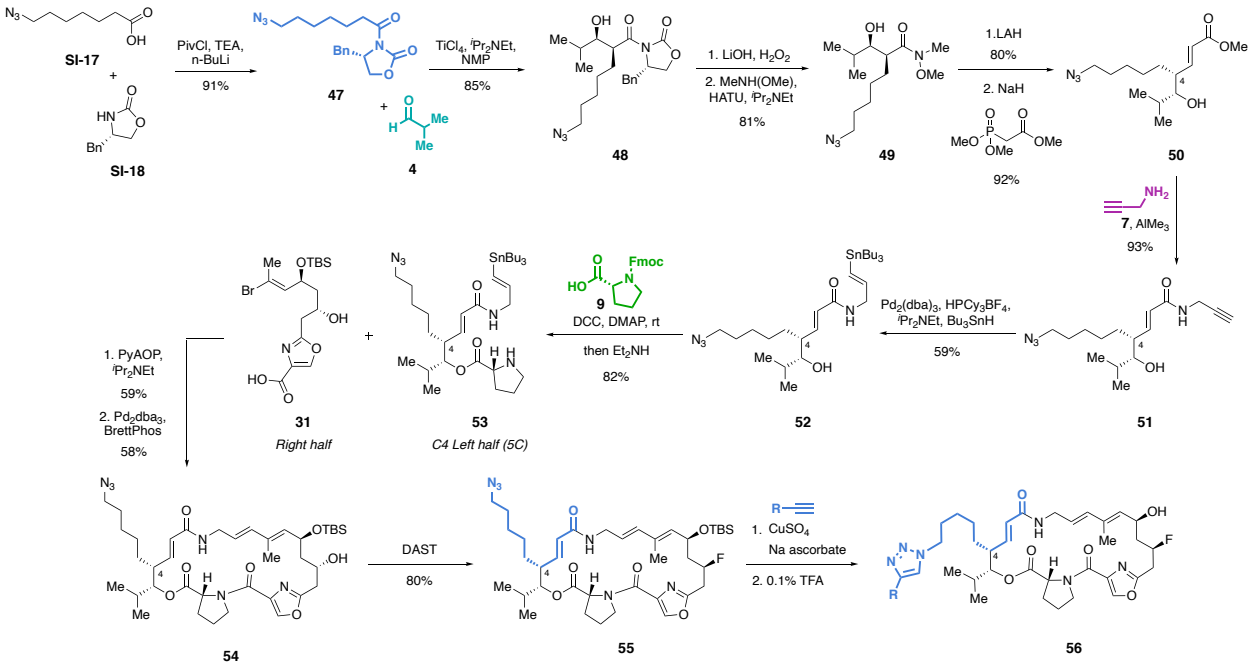
Analog **46b** - TBS (SA1101152)



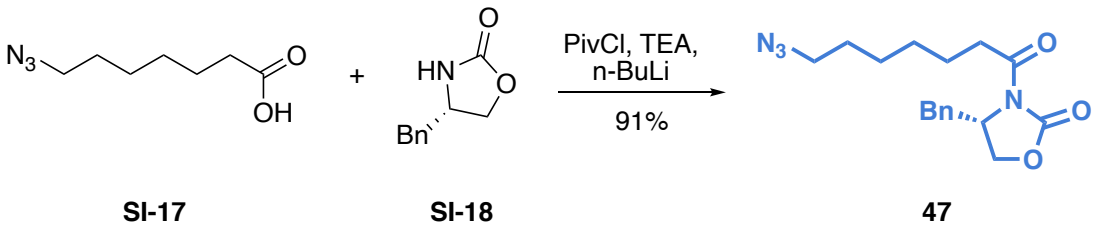
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09 (d, *J* = 5.1 Hz, 1H), 7.91 (d, *J* = 3.3 Hz, 1H), 7.85 – 7.79 (m, 2H), 7.50 – 7.38 (m, 2H), 6.73 – 6.58 (m, 1H), 6.52 (dd, *J* = 16.2, 4.9 Hz, 1H), 6.20 (dd, *J* = 19.7, 15.5 Hz, 1H), 5.97

– 5.76 (m, 2H), 5.67 (dd, *J* = 10.5, 5.3 Hz, 1H), 5.62 – 5.51 (m, 1H), 5.34 (t, *J* = 8.3 Hz, 1H), 5.13 (d, *J* = 15.1 Hz, 1H), 4.96 (dd, *J* = 15.6, 10.2 Hz, 1H), 4.84 (ddd, *J* = 14.5, 8.5, 3.4 Hz, 1H), 4.73 (tt, *J* = 12.2, 5.8 Hz, 2H), 4.60 – 4.50 (m, 1H), 4.38 (d, *J* = 9.7 Hz, 1H), 4.12 (q, *J* = 7.2 Hz, 1H), 4.07 – 3.98 (m, 1H), 3.94 – 3.79 (m, 3H), 3.63 (dd, *J* = 13.0, 8.6 Hz, 1H), 3.53 (s, 1H), 3.41 (d, *J* = 8.8 Hz, 1H), 3.11 (td, *J* = 16.5, 6.8 Hz, 1H), 2.96 – 2.82 (m, 1H), 2.59 – 2.47 (m, 2H), 2.35 (d, *J* = 24.7 Hz, 2H), 2.20 – 2.10 (m, 2H), 2.05 (d, *J* = 8.0 Hz, 1H), 1.92 – 1.84 (m, 3H), 1.77 (d, *J* = 3.9 Hz, 3H), 1.60 (dd, *J* = 28.9, 12.9 Hz, 1H), 0.95 (td, *J* = 7.8, 4.4 Hz, 6H), 0.87 (d, *J* = 1.5 Hz, 9H), 0.05 (d, *J* = 2.0 Hz, 3H), 0.02 (d, *J* = 2.1 Hz, 3H).

Scheme V. Preparation of C4 alkyl analogs (5C linker) 56



Preparation of oxazolidinone **47**



An oven-dried 25-mL round-bottom flask containing acid **SI-17** (164 mg, 0.96 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Then the vessel was sealed with a rubber septum. Dry THF (2.1 mL) was added, resulting in a colorless solution, and the vessel was cooled to 0 °C by means of ice/water bath. TEA (0.15 mL, 1.05 mmol, 1.1 equiv) and PivCl (0.13 mL, 1.05 mmol, 1.1 equiv) were added, resulting in a white suspension. The reaction mixture was stirred at 0 °C for 3 h. In a separate oven-dried 10-mL round-bottom vessel, oxazolidinone **SI-18** (203 mg, 1.15 mmol, 1.2 equiv) was added, which was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Then the vessel was sealed with a rubber septum. Dry THF (2.1 mL) was added, resulting in a clear solution, and the vessel was cooled to -78 °C by means of dry ice/acetone bath. A solution of *n*-BuLi in hexanes butyllithium (2.3 M, 0.50 mL, 1.15 mmol, 1.2 equiv) was added dropwise to this solution. After the reaction mixture was stirred at -78 °C for 3 h, this mixture was added via cannula to the 25-mL round-bottom flask which was cooled to -78 °C in advance by means of dry ice/acetone bath. After 2 h, saturated aqueous ammonium chloride solution (10 mL) was added in one portion. The resulting biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with EtOAc (4 × 10 mL). The combined organic layers were washed with brine (10 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc: hexanes = 1:10) to afford oxazolidinone **47** (287 mg, 91%) as a white solid.

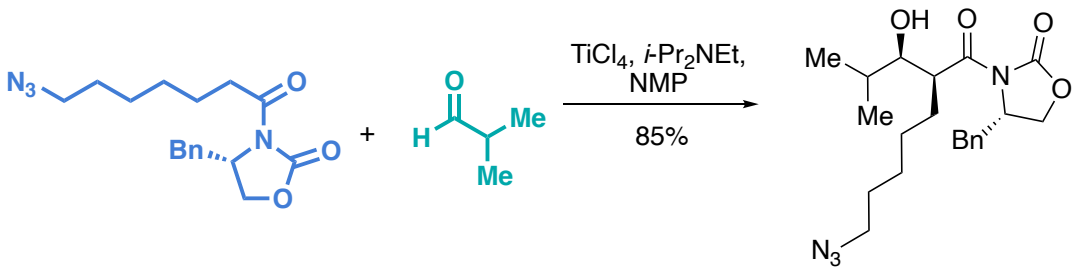
TLC (EtOAc:hexanes = 1:4): R_f = 0.33 (UV, p-anisaldehyde).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.43 – 7.27 (m, 3H), 7.26 – 7.17 (m, 2H), 4.75 – 4.62 (m, 1H), 4.28 – 4.13 (m, 2H), 3.37 – 3.22 (m, 3H), 3.08 – 2.83 (m, 2H), 2.77 (dd, *J* = 13.3, 9.7 Hz, 1H), 1.80 – 1.55 (m, 4H), 1.43 (td, *J* = 5.7, 3.3 Hz, 4H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.3, 153.6, 135.4, 129.5, 129.1, 127.5, 66.3, 55.2, 51.5, 38.0, 35.5, 28.8, 28.7, 26.6, 24.1.

HRMS-ESI m/z calcd for $C_{17}H_{22}N_4NaO_3^+$ $[M + Na]^+$ 353.1590, found 353.1586.

Preparation of alcohol **48**



47 **4** **48**

An oven-dried 25-mL round-bottom flask containing oxazolidinone **47** (172 mg, 0.52 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Then the vessel was sealed with a rubber septum. Dry DCM (5.2 mL) was added, resulting in a colorless solution, and the vessel was cooled to -78 ºC by means of a dry ice-acetone bath. A solution of titanium tetrachloride in DCM (1.0 M, 0.54 mL, 0.55 mmol, 1.05 equiv) was added dropwise, resulting in a yellow solution. After stirring for 5 min, ^{i}Pr$_2$EtN (98.1 µL, 0.57 mmol, 1.1 equiv) was added dropwise to the mixture, followed by NMP (50.0 µL, 0.52 mmol, 1 equiv), resulting in a dark red solution. After 1 h, freshly distilled isobutyraldehyde (**4**, 0.52 µL, 0.57 mmol, 1.1 equiv) was added dropwise. After 12 h, half-saturated aqueous ammonium chloride solution (10 mL) was carefully added, and the mixture was allowed to warm to 23 °C. The resulting biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with DCM (2 × 20 mL). The combined organic layers were washed with water (20 mL) and brine (20 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc: hexanes = 1:10 to 1:5) to afford alcohol **48** (178 mg, 85%) as a colorless oil.

TLC (EtOAc:hexanes = 1:2): R_f = 0.29 (UV, p-anisaldehyde).

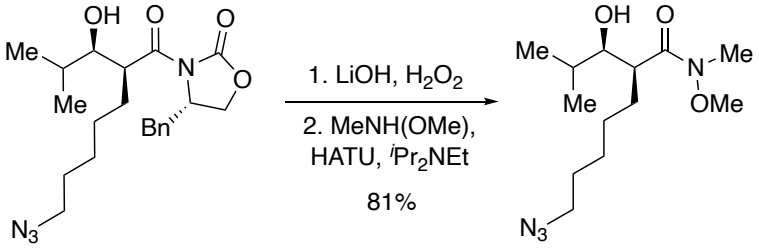
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.39 – 7.25 (m, 4H), 7.27 – 7.19 (m, 2H), 4.70 (ddt, *J* = 10.1, 6.7, 3.6 Hz, 1H), 4.25 – 4.13 (m, 3H), 3.49 (dd, *J* = 7.7, 3.5 Hz, 1H), 3.36 (dd, *J* = 13.2, 3.4 Hz, 1H), 3.26 (t, *J* = 6.9

Hz, 2H), 2.71 (dd, *J* = 13.2, 10.1 Hz, 1H), 1.98 – 1.82 (m, 1H), 1.81 – 1.55 (m, 4H), 1.49 – 1.32 (m, 4H), 1.01 (d, *J* = 6.6 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 176.9, 153.2, 135.3, 129.5, 129.2, 127.6, 77.4, 66.1, 55.8, 51.5, 45.0, 38.1, 31.5, 28.8, 27.1, 27.1, 26.0, 19.3, 19.0.

HRMS-ESI m/z calcd for $C_{21}H_{30}N_4NaO_4^+$ $[M + Na]^+$ 425.2159, found 425.2156.

Preparation of Weinreb amide **49**



48 **49**

30% H_2O_2 (2.59 mL, 25.3 mmol, 5 equiv), followed by LiOH (638 mg, 15.2 mmol, 3 equiv), was added to a solution of alcohol **48** (2.03 g, 5.06 mmol, 1 equiv) in THF-H_2O (3.8 mL – 1.3 mL) at 0 °C. The resulting white suspension was warmed to 23 °C slowly. After 3 h, THF was concentrated under vacuum, and water (100 mL) and EtOAc (100 mL) were added. The resulting biphasic solution was transferred to a separate funnel, and the layers were separated. The aqueous layer was washed with EtOAc (3 x 50 mL), and the resulting organic layer was discarded. The aqueous layer was then acidified by 2 M HCl to pH = 2. The resulting biphasic solution was transferred to a separate funnel, and the layers were separated. The aqueous layer was abstracted with EtOAc (3 × 50 mL), and the combined organic layer was washed with water (100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4), and the dried solution was concentrated under vacuum. The resulting crude acid was used for the next step without further purification.

Crude acid (727 mg, 2.99 mmol, 1 equiv), ^{i}Pr$_2$EtN (2.1 mL, 12.0 mmol, 4 equiv), Weinreb amine (583 mg, 5.98 mmol, 2 equiv), and DCM (30 mL) were added to an oven-dried 250-mL round-bottom flask. HATU

(1.36 g, 3.58 mmol, 1.2 equiv) was added in one portion to the resulting colorless solution at 23 °C. After 3 h, the mixture was transferred to a separatory funnel and washed with water (2 × 50 mL) and brine (50 mL). The washed solution was dried (Na_2SO_4), the dried solution was filtered. The filtrate was concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:4) to Weinreb amide **49** (756 mg, 81% over two steps) as a colorless oil.

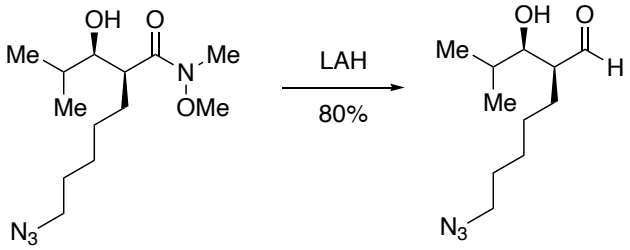
TLC (EtOAc:hexanes = 1:2): R_f = 0.49 (UV, p-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 3.69 (s, 3H), 3.35 (dd, *J* = 8.3, 2.8 Hz, 1H), 3.26 – 3.19 (m, 5H), 3.16 (dd, *J* = 14.6, 8.3 Hz, 1H), 1.87 – 1.69 (m, 2H), 1.64 – 1.50 (m, 3H), 1.46 – 1.32 (m, 2H), 1.32 – 1.20 (m, 2H), 1.02 (d, *J* = 6.5 Hz, 3H), 0.88 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.8, 77.6, 61.7, 51.5, 41.6, 32.1, 30.9, 28.8, 27.5, 27.1, 25.3, 19.3, 19.3.

HRMS-ESI m/z calcd for $C_{13}H_{27}N_4O_3^+$ $[M + H]^+$ 287.2077, found 287.2080.

Preparation of aldehyde **SI-19**



49 **SI-19**

A 50-mL round-bottom flask containing Weinreb amide **49** (756 mg, 2.64 mmol, 1 equiv) was evacuated and flushed with nitrogen (the process of nitrogen exchange was repeated a total of 3 times). Dry THF (18 mL) was added, and the resulting clear solution was cooled to -78 ºC by means of a dry ice/acetone bath. A solution of LAH in THF (2.0 M, 6.6 mL, 13.2 mmol, 5 equiv) was added dropwise to this solution. After 5 h, EtOAc (10 mL) was carefully added (CAUTION: Gas evolution!), followed by dropwise addition of saturated aqueous potassium sodium tartrate solution (50 ml). The mixture was allowed to warm to 23 ºC.

After 1 h, the biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with water (2 × 50 mL) and brine (50 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:10 to 1:3) to afford aldehyde **SI-19** (402 mg, 67%) as a light yellow oil. The reaction does not go to completion, so Weinreb amide **49** (232 mg, 31%) was recovered during purification and resubmitted to afford an overall yield of aldehyde **SI-19** (478 mg, 80%).

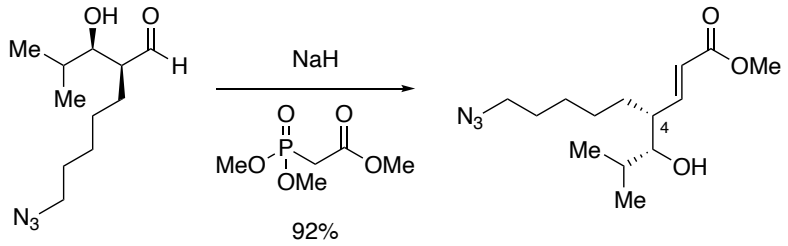
TLC (EtOAc:hexanes = 1:4): R_f = 0.28 (p-anisaldehyde)

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.78 – 9.69 (m, 1H), 3.73 – 3.62 (m, 1H), 3.31 – 3.20 (m, 3H), 2.46 (dtt, *J* = 9.7, 4.1, 1.9 Hz, 1H), 1.75 (dtd, *J* = 12.5, 6.4, 1.6 Hz, 2H), 1.63 – 1.58 (m, 3H), 1.42 (dtt, *J* = 15.5, 9.9, 4.0 Hz, 4H), 0.99 (dd, *J* = 6.6, 1.4 Hz, 3H), 0.92 (dd, *J* = 6.8, 1.4 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 205.3, 75.8, 55.0, 51.4, 31.4, 28.7, 27.4, 27.1, 23.7, 19.5, 17.9.

HRMS-ESI m/z calcd for $C_{11}H_{21}N_3NaO_2^+$ $[M + Na]^+$ 250.1526, found 250.1526.

Preparation of methyl ester **50**



SI-19 **50**

An oven-dried 500-mL round-bottom flask containing 60% NaH (822 mg, 20.5 mmol, 4 equiv) was evacuated and flushed with nitrogen (the process of nitrogen exchange was repeated a total of 3 times). Dry THF (347 mL) was added, and the resulting suspension was cooled to 0 ºC by means of an ice/water bath. Trimethyl phosphonoacetate (3.33 mL, 20.5 mmol, 4 equiv) was added dropwise at 0 ºC. After 1 h, a

solution of aldehyde **SI-19** in THF (1.5 mL) was added. After 2 h, the saturated aqueous ammonium chloride solution (150 mL) was carefully added, and the resulting biphasic mixture was transferred to a separatory funnel. The layers were separated, and the aqueous layer was extracted with diethyl ether (2 × 50 mL). The combined organic layers were washed with water (2 × 100 mL) and brine (100 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:15) to afford methyl ester **50** (1.34 g, 92% yield) as a colorless oil.

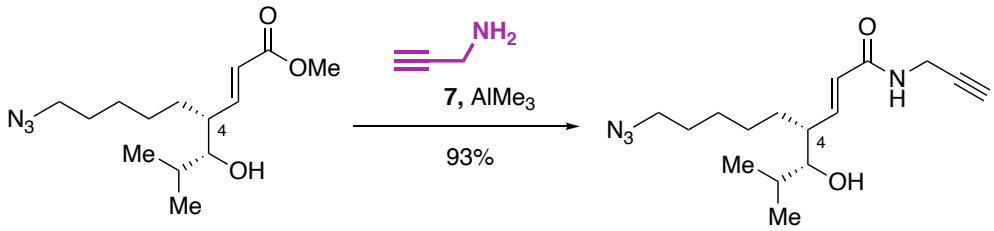
TLC (EtOAc:hexanes = 1:4): R_f = 0.26 (UV, p-anisaldehyde).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.74 (dd, *J* = 15.7, 10.0 Hz, 1H), 5.84 (d, *J* = 15.7 Hz, 1H), 3.74 (s, 3H), 3.36 – 3.26 (m, 2H), 3.31 – 3.19 (m, 3H), 2.28 (dt, *J* = 10.0, 7.2 Hz, 1H), 1.88 – 1.63 (m, 3H), 1.66 – 1.51 (m, 3H), 1.45 – 1.28 (m, 6H), 1.24 – 1.11 (m, 1H), 0.94 (d, *J* = 6.9 Hz, 4H), 0.85 (d, *J* = 6.7 Hz, 4H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 150.4, 122.1, 78.5, 51.6, 51.6, 51.4, 46.9, 31.0, 29.5, 28.8, 26.8, 20.2, 15.2.

HRMS-ESI m/z calcd for $C_{14}H_{26}N_3O_3^+$ $[M + H]^+$ 250.1968, found 284.1971.

Preparation of alkyne **51**



Propargylamine **7** (1.2 mL, 18.9 mmol, 4 equiv) and dry DCM (31 mL) were added to a 100-mL round-bottom flask under nitrogen. The resulting colorless solution was cooled to 0 ºC by means of an ice/water bath. A solution of $AlMe_3$ in heptane (2 M, 9.4 mL, 18.9 mmol, 4 equiv) was added dropwise over 30 min

(CAUTION: Gas evolution!). The mixture was allowed to warm to 23 ºC. After 30 min, a solution of methyl ester **50** (1.34 g, 4.72 mmol, 1 equiv) in DCM (4.5 mL) was added over 10 min (CAUTION: Gas evolution!). The vessel was equipped with a reflux condenser, and the solution was brought to reflux by means of a 50 ºC oil bath. After 3 h, the mixture was cooled to 0 °C by means of an ice/water bath, and MeOH (10 mL) was added dropwise (CAUTION: Gas evolution!). Once gas evolution ceased, saturated aqueous potassium sodium tartrate solution (50 mL) was added. After 1 h, the biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with DCM (2 × 30 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:2) to afford amide **51** (1.34 g, 93% yield) as a yellow oil.

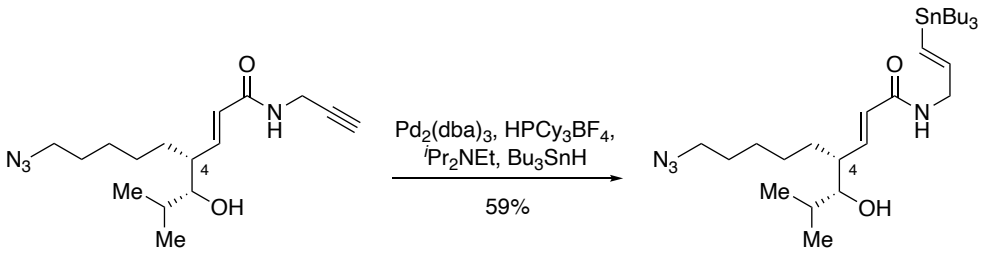
TLC (EtOAc:hexanes = 1:1): R_f = 0.24 (UV, p-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.66 (dd, *J* = 15.3, 10.0 Hz, 1H), 5.90 – 5.68 (m, 2H), 4.12 (dd, *J* = 5.3, 2.6 Hz, 2H), 3.29 (dd, *J* = 7.4, 4.1 Hz, 1H), 3.24 (t, *J* = 6.9 Hz, 2H), 2.31 – 2.20 (m, 2H), 2.03 (s, 1H), 1.73 (ttd, *J* = 13.6, 6.6, 4.0 Hz, 2H), 1.64 – 1.51 (m, 2H), 1.33 (tdt, *J* = 12.5, 8.5, 4.1 Hz, 4H), 1.21 – 1.09 (m, 1H), 0.95 – 0.89 (m, 3H), 0.84 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 146.8, 124.0, 79.5, 78.6, 71.9, 51.5, 46.7, 30.9, 29.5, 29.4, 28.8, 27.0, 26.9, 20.2, 15.4.

HRMS-ESI m/z calcd for $C_{16}H_{27}N_4O_2^+$ $[M + H]^+$ 307.2129, found 307.2131.

Preparation of vinyl tin **52**



51 **52**

A 250-mL round-bottom flask containing $Pd_2(dba)_3$ (90.0 mg, 98.2 μmol, 0.01 equiv), tricyclohexylphosphonium tetrafluoroborate (145 mg, 0.39 mmol, 0.04 equiv), and alkyne **51** (2.61 g, 8.51 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Dry DCM (81 mL) and $^{i}Pr_2EtN$ (0.34 mL, 2.0 mmol, 0.2 equiv) were added, resulting in a red solution. After 10 min, the vessel and its contents were cooled to 0 ºC by means of an ice/water bath. Tributylstannane (3.2 mL, 11.8 mmol, 1.2 equiv) was added dropwise via syringe pump over 15 min. After 4 h, the vessel was removed from the cooling bath and DCM was concentrated under vacuum. Diethyl ether (100 mL) was added, and the mixture was filtered through a pad of celite. The filter cake was washed with diethyl ether (2 × 50 mL). The combined filtrates were concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: TEA:EtOAc:hexanes = 0.01:0:1 to 0.1:1:10) to afford vinyl tin **52** (3.45 g, 59% yield) as a light-yellow oil.

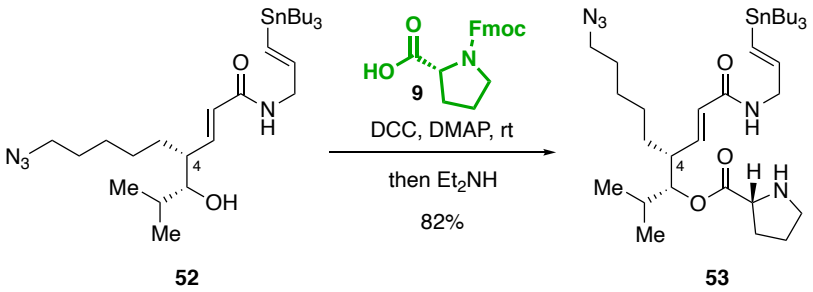
TLC (EtOAc:hexanes = 1:2): R_f = 0.31 (UV, p-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.64 (dd, *J* = 15.2, 10.0 Hz, 1H), 6.13 (dt, *J* = 19.0, 1.4 Hz, 1H), 5.98 (dt, *J* = 19.0, 5.2 Hz, 1H), 5.80 (d, *J* = 15.3 Hz, 1H), 5.56 – 5.45 (m, 1H), 4.00 (td, *J* = 5.5, 1.5 Hz, 2H), 3.31 (ddd, *J* = 7.6, 5.6, 4.0 Hz, 1H), 3.24 (t, *J* = 6.9 Hz, 2H), 2.25 (tdd, *J* = 10.3, 7.4, 3.0 Hz, 1H), 1.75 (dddt, *J* = 13.8, 10.9, 6.9, 3.7 Hz, 2H), 1.64 – 1.53 (m, 3H), 1.52 – 1.44 (m, 6H), 1.37 – 1.25 (m, 10H), 1.18 (dtd, *J* = 10.5, 6.3, 3.1 Hz, 1H), 0.95 – 0.83 (m, 21H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.3, 145.7, 143.5, 130.8, 124.8, 78.6, 51.5, 46.7, 45.2, 30.8, 29.7, 29.2, 28.9, 27.4, 27.0, 26.9, 20.3, 15.3, 13.8, 9.6.

HRMS-ESI m/z calcd for $C_{28}H_{55}N_4O_2Sn^+$ $[M + H]^+$ 599.3342, found 599.3347.

Preparation of proline ester **53**



Fmoc-D-Pro-OH **9** (332 mg, 0.98 mmol, 1.35 equiv), DMAP (17.8 mg, 0.15 mmol, 0.2 equiv) and alcohol **52** (436 mg, 0.73 mmol, 1 equiv) were added to a 25-mL round-bottom flask. DCM (7.3 mL) was added, resulting in a colorless solution. DCC (0.28 mL, 1.09 mmol, 1.5 equiv) was added in one portion. Resulting in a white suspension. After 5 h, alcohol **52** was entirely consumed as indicated by TLC analysis (eluent: EtOAc:hexanes = 1:2), and diethylamine (3.7 mL) was added. After 3 h, the mixture was filtered through a pad of celite, and the filter cake was washed with DCM (2 × 20 mL). The combined filtrates were concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: NH_4OH:MeOH:DCM = 0.2:1:100) to afford left half **53** (416 mg, 82% yield) as a yellow oil.

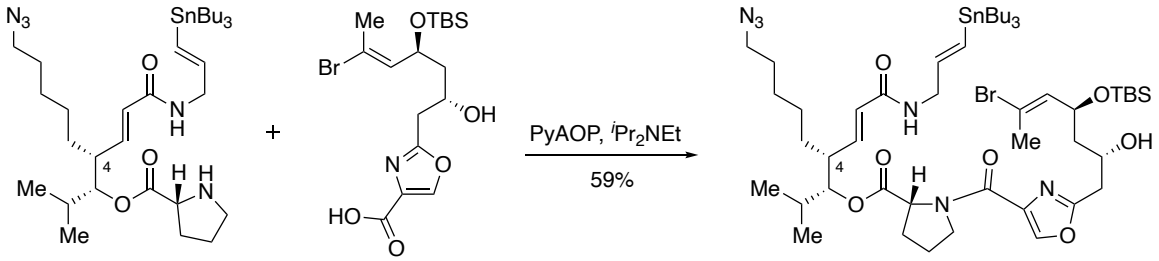
TLC (MeOH:DCM = 1:6): R_f = 0.46 (UV, p-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.56 (dd, *J* = 15.2, 9.9 Hz, 1H), 6.13 (dt, *J* = 19.0, 1.4 Hz, 1H), 5.97 (dt, *J* = 19.0, 5.1 Hz, 1H), 5.82 (dd, *J* = 15.3, 7.2 Hz, 1H), 5.58 (t, *J* = 5.8 Hz, 1H), 4.86 (dd, *J* = 8.0, 4.2 Hz, 1H), 4.00 (td, *J* = 5.5, 1.5 Hz, 2H), 3.86 (dd, *J* = 8.3, 5.6 Hz, 1H), 3.23 (t, *J* = 6.8 Hz, 2H), 3.14 (dt, *J* = 9.9, 6.5 Hz, 1H), 2.99 (dt, *J* = 10.2, 6.6 Hz, 1H), 2.51 – 2.41 (m, 1H), 2.25 – 2.15 (m, 1H), 1.97 – 1.74 (m, 4H), 1.61 – 1.51 (m, 3H), 1.51 – 1.39 (m, 6H), 1.36 – 1.24 (m, 10H), 1.22 – 1.01 (m, 2H), 0.98 – 0.78 (m, 21H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 175.2, 164.9, 143.8, 143.4, 130.8, 125.6, 79.8, 60.0, 51.4, 47.0, 45.1, 44.7, 30.6, 30.0, 29.7, 29.2, 28.8, 27.4, 26.8, 26.7, 25.6, 20.0, 16.0, 13.8, 9.6.

HRMS-ESI m/z calcd for $C_{33}H_{62}N_5O_3Sn^+$ $[M + H]^+$ 696.3869, found 696.3883.

Preparation of Stille precursor **SI-20**



53 **31** **SI-20**

Acid **31** (1.23 g, 2.79 mmol 1 equiv), $^{i}Pr_2EtN$ (1.46 mL, 8.38 mmol, 3 equiv), and amine **53** (1.94 g, 2.79 mmol, 1 equiv) were added to a 100-mL round-bottom flask. DCM (28 mL) was added, resulting in a clear, colorless solution. PyAOP (2.18 g, 4.19 mmol, 1.5 equiv) was then added in one portion. After 12 h, the mixture was diluted with DCM (30 mL), and the solution was transferred to a separatory funnel and was washed with water (2 × 100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4), and the dried solution was filtered. The filtrate was concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: TEA:EtOAc:hexanes = 0.003:1:2) to afford Stille coupling precursor **SI-20** (1.84 g, 59% yield) as a light-yellow oil.

TLC (EtOAc:hexanes = 1:1): R_f = 0.44 (UV, p-anisaldehyde)

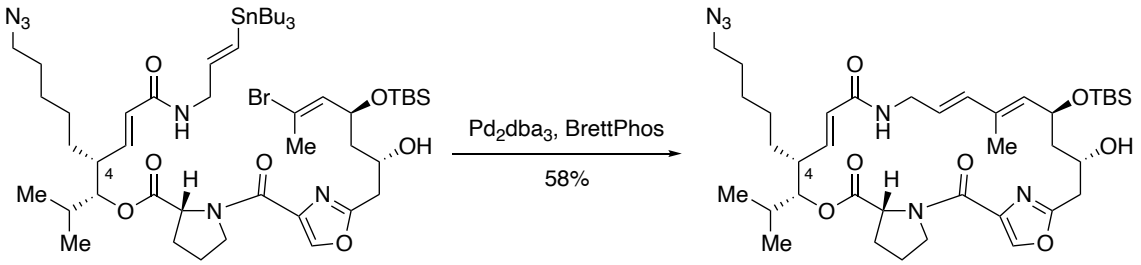
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.22 – 8.08 (m, 1H), 6.49 (td, *J* = 15.6, 9.7 Hz, 1H), 6.28 – 6.03 (m, 1H), 6.03 – 5.88 (m, 2H), 5.85 – 5.66 (m, 2H), 4.82 (dt, *J* = 6.9, 4.7 Hz, 1H), 4.73 – 4.60 (m, 2H), 4.31 (d, *J* = 47.6 Hz, 1H), 4.09 (t, *J* = 6.4 Hz, 1H), 4.03 – 3.87 (m, 2H), 3.80 – 3.63 (m, 2H), 3.21 (dt, *J* = 9.6, 6.8 Hz, 2H), 2.89 (dtd, *J* = 12.6, 7.5, 5.1 Hz, 2H), 2.48 – 2.35 (m, 1H), 2.31 – 2.21 (m, 4H), 2.17 – 2.08 (m, 1H), 2.06 – 1.95 (m, 2H), 1.95 – 1.83 (m, 2H), 1.74 (d, *J* = 4.7 Hz, 1H), 1.66 (tt, *J* = 7.5, 2.5 Hz, 2H), 1.47 (dddd,

J = 13.0, 6.0, 4.8, 3.6 Hz, 7H), 1.29 (dddd, *J* = 13.9, 8.6, 7.1, 4.1 Hz, 10H), 1.11 (td, *J* = 10.7, 7.0 Hz, 2H), 0.95 – 0.80 (m, 30H), 0.12 – 0.05 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$, mixture of rotamers) δ 172.97, 172.01, 165.39, 164.93, 162.33, 162.26, 160.39, 160.25, 143.68, 143.61, 143.41, 136.90, 136.83, 135.78, 135.40, 130.68, 130.53, 125.88, 125.75, 120.61, 120.41, 80.64, 80.29, 67.83, 67.69, 65.68, 65.23, 61.15, 60.37, 51.44, 51.40, 49.25, 48.95, 47.45, 45.20, 45.11, 45.01, 44.33, 44.18, 43.61, 36.28, 35.98, 34.09, 31.76, 30.13, 29.48, 29.27, 29.17, 29.07, 28.97, 28.81, 27.97, 27.67, 27.40, 27.13, 26.98, 26.92, 26.79, 26.71, 26.67, 25.97, 25.93, 25.89, 25.74, 25.57, 25.08, 24.03, 24.01, 21.82, 20.12, 19.86, 18.21, 17.66, 16.90, 16.72, 13.83, 13.75, 9.57, 9.56, -4.30, -4.36, -4.76, -5.01.

HRMS-ESI m/z calcd for $C_{50}H_{88}BrN_6O_7SiSn^+$ $[M + H]^+$ 1111.4684, found 1111.4706.

Preparation of Stille product **54**



SI-20 **54**

An oven-dried 100-mL round-bottom flask containing BrettPhos (19.7 mg, 36.7 μmol, 0.2 equiv), $Pd_2(dba)_3$ (16.8 mg, 18.4 μmol, 0.1 equiv) and Stille coupling precursor **SI-20** (204 mg, 184 μmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Dry toluene (37 mL) was added, resulting in a red suspension. A stream of argon was passed through the solution by a stainless-steel needle for 30 min. The mixture was heated by means of a 50 °C pre-heated oil bath. After 12 h, **SI-20** was entirely consumed as indicated by TLC analysis (eluent: EtOAc:hexanes = 1:1), and the mixture was allowed to cool to 23 ºC. The mixture was concentrated, and the resulting crude residue was purified by

flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:1) to afford Stille coupling product **54** (68.1 mg, 50% yield) as a light-yellow solid.

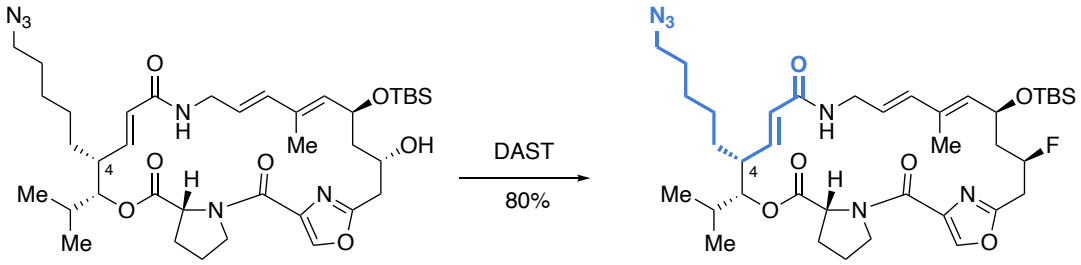
TLC (EtOAc:hexanes = 1:1): R_f = 0.15 (UV, p-anisaldehyde)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 6.25 – 6.12 (m, 2H), 6.00 – 5.91 (m, 1H), 5.88 – 5.74 (m, 2H), 5.68 (ddd, *J* = 15.1, 10.2, 4.3 Hz, 1H), 4.99 (d, *J* = 2.6 Hz, 1H), 4.69 (ddd, *J* = 23.4, 9.5, 2.7 Hz, 2H), 4.57 – 4.38 (m, 3H), 3.82 (t, *J* = 6.6 Hz, 2H), 3.33 – 3.22 (m, 3H), 3.06 (dd, *J* = 16.7, 2.5 Hz, 1H), 2.80 (dd, *J* = 16.8, 10.4 Hz, 1H), 2.52 (s, 1H), 2.33 (d, *J* = 13.8 Hz, 1H), 2.24 – 2.13 (m, 1H), 2.05 – 1.85 (m, 5H), 1.72 (d, *J* = 1.2 Hz, 3H), 1.63 – 1.57 (m, 4H), 1.28 – 1.23 (m, 4H), 1.00 (d, *J* = 6.4 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H), 0.90 (s, 9H), 0.09 (d, *J* = 2.0 Hz, 3H), 0.05 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.3, 167.3, 161.2, 160.4, 143.3, 142.3, 137.7, 136.6, 134.2, 131.6, 125.2, 124.7, 82.5, 69.8, 66.7, 59.3, 51.4, 48.2, 42.9, 42.7, 41.8, 29.3, 28.8, 28.3, 27.2, 27.0, 25.7, 25.7, 25.7, 24.9, 20.0, 18.7, 18.0, 14.2, 12.5, -3.6, -4.4, -5.3.

HRMS-ESI m/z calcd for $C_{38}H_{79}N_6NaO_7Si^+$ $[M + Na]^+$ 763.4185, found 763.4197.

Preparation of fluorinated compound **55**



54 **55**

An oven-dried 100-mL round-bottom flask containing Stille product **54** (520 mg, 7.20 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Dry DCM (35 mL) was added, and the resulting colorless solution was cooled to -78 ºC by means of a dry ice/acetone bath. DAST (0.23 mL, 1.75 mmol, 2.5 equiv) was added dropwise, and the vessel and its contents were warmed to 0 ºC

by means of an ice/water bath. After 3 h, saturated aqueous $NaHCO_3$ solution (25 mL) was added. After stirring for 30 min, the biphasic mixture was transferred to a separatory funnel, the layers were separated. The organic layer was washed with water (25 mL) and brine (25 mL), and the washed solution was dried (Na_2SO_4). The dried solution was concentrated, and the residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:2) to afford fluorinated product **55** (414 mg, 80% yield) as a light-yellow solid.

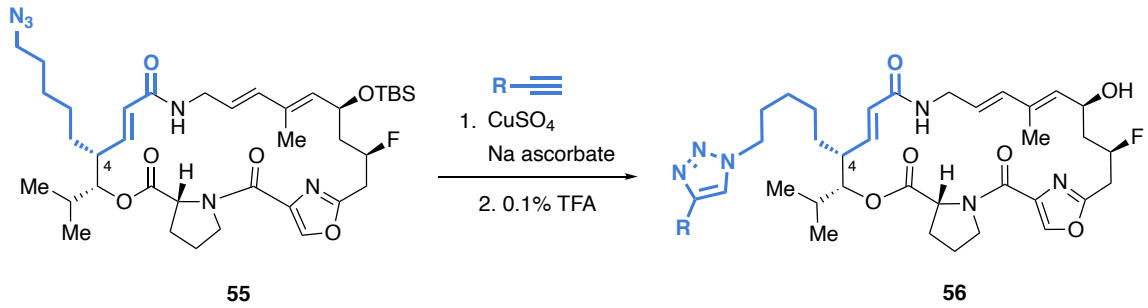
TLC (EtOAc:hexanes = 1:2): R_f = 0.15 (UV, p-anisaldehyde)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 6.41 (dd, *J* = 16.2, 6.0 Hz, 1H), 6.20 – 6.13 (m, 1H), 6.01 (d, *J* = 8.1 Hz, 1H), 5.87 (dd, *J* = 16.1, 1.5 Hz, 1H), 5.66 (ddd, *J* = 15.6, 8.5, 4.3 Hz, 1H), 5.32 – 5.26 (m, 1H), 5.17 – 4.91 (m, 1H), 4.81 (ddd, *J* = 12.2, 9.4, 2.8 Hz, 2H), 4.72 (ddd, *J* = 10.2, 9.0, 3.9 Hz, 1H), 4.57 – 4.47 (m, 1H), 4.14 – 3.99 (m, 1H), 3.84 (dt, *J* = 11.4, 7.1 Hz, 1H), 3.46 (ddd, *J* = 15.7, 8.5, 3.2 Hz, 1H), 3.26 (t, *J* = 6.8 Hz, 2H), 3.15 (td, *J* = 16.8, 6.4 Hz, 1H), 2.90 (ddd, *J* = 20.8, 16.4, 5.6 Hz, 1H), 2.55 – 2.48 (m, 1H), 2.20 – 2.11 (m, 2H), 1.96 (ddt, *J* = 12.4, 9.6, 5.4 Hz, 3H), 1.77 (d, *J* = 1.2 Hz, 3H), 1.59 (dddd, *J* = 13.0, 8.5, 5.1, 2.1 Hz, 4H), 1.45 – 1.32 (m, 4H), 1.28 – 1.21 (m, 2H), 0.95 (d, *J* = 6.5 Hz, 3H), 0.92 (d, *J* = 6.7 Hz, 3H), 0.86 (s, 9H), 0.04 (s, 3H), 0.01 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.4, 160.7, 160.2, 160.1, 143.3, 143.3, 136.8, 136.5, 134.8, 133.5, 125.2, 124.6, 89.2 (d, *J* = 170.2 Hz), 82.1, 66.6, 59.2, 51.5, 48.7, 43.7 (d, *J* = 20.5 Hz), 42.1, 41.3, 36.8, 34.0 (d, *J* = 25.1 Hz), 29.5, 28.9, 28.4, 27.3, 27.1, 26.0, 25.9, 25.0, 19.9, 18.9, 18.3, 13.1, -4.3, -4.8.

HRMS-ESI m/z calcd for $C_{38}H_{79}FN_6O_7Si^+$ $[M + H]^+$ 743.4322, found 743.4341.

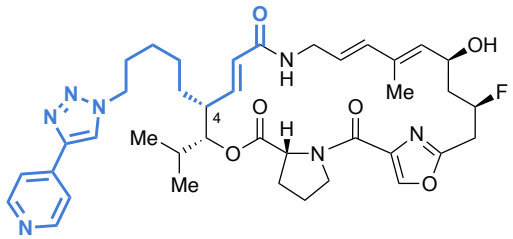
General procedure for preparation of C-4 analogs **56**



55 **56**

An aqueous solution of sodium ascorbate (0.05M, 0.2 equiv) and an aqueous solution of copper(II) sulfate (0.05 M, 0.05 equiv) were added to a suspension of azide compound **55** (1 equiv) and alkyne (3 equiv) in *t*-BuOH-H_2O (3mL-1mL) at 23°C. After stirring at 23°C for 12 h, the solvent was removed under vacuum. The residue was purified by flash chromatography over silica gel (0→100% hexanes in ethyl acetate) to afford the product as an off white solid. Then the white solid was dissolved with 0.1% TFA in MeCN-H_2O (95/5 v/v), and the solution was stirred for 12 hours at 23°C. The reaction mixture was concentrated, and the resulting crude residue was purified by flash chromatography over silica gel (0→10% methanol in dichloromethane) to afford C-4 modified analog **56**.

Analog **56a** (SA1103064)



Prepared according to general procedure of C-4 analogs 56. Analog **56a** (12.7 mg, 54% yield over 2 steps) was obtained as a light yellow solid.

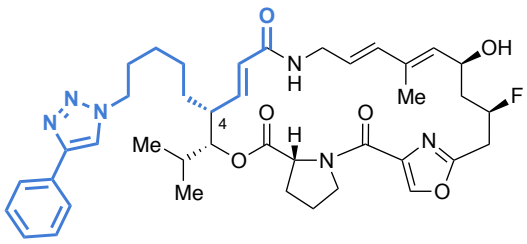
TLC (MeOH:DCM = 1:40): R_f = 0.11 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.69 (s, 2H), 8.11 (s, 1H), 7.96 (s, 1H), 7.78 (d, *J* = 5.0 Hz, 2H), 6.44 (dd, *J* = 16.1, 6.2 Hz, 1H), 6.20 (d, *J* = 15.7 Hz, 1H), 6.06 (d, *J* = 7.9 Hz, 1H), 5.89 (d, *J* = 16.0 Hz, 1H), 5.77 (ddd, *J* = 15.9, 7.9, 4.2 Hz, 1H), 5.37 (d, *J* = 8.8 Hz, 1H), 5.14 (s, 1H), 5.03 (s, 1H), 4.82 (td, *J* = 8.5, 4.3 Hz, 3H), 4.46 (t, *J* = 7.0 Hz, 2H), 4.08 (s, 1H), 3.86 (dt, *J* = 13.8, 7.0 Hz, 1H), 3.67 (d, *J* = 5.4 Hz, 1H), 3.57 – 3.49 (m, 1H), 3.37 (s, 1H), 3.21 (dd, *J* = 17.0, 5.5 Hz, 1H), 3.01 (td, *J* = 16.5, 6.6 Hz, 1H), 2.53 (s, 1H), 2.37 (t, *J* = 7.5 Hz, 1H), 2.21 (dd, *J* = 18.5, 7.9 Hz, 3H), 1.99 (d, *J* = 8.0 Hz, 4H), 1.85 (s, 3H), 1.65 (d, *J* = 8.1 Hz, 4H), 1.44 (s, 2H), 0.97 (d, *J* = 6.4 Hz, 3H), 0.94 (s, 3H).

^{13}C NMR (100 MHz, MeOD) δ 171.7, 167.7, 162.5, 162.1, 147.2, 146.4, 144.7, 137.9, 136.8, 136.6, 134.5, 133.1, 126.3, 125.9, 125.8, 122.4, 90.4 (d, *J* = 170.4 Hz), 83.6, 66.0, 60.5, 51.7, 50.1, 43.1 (d, *J* = 19.0 Hz), 41.6, 34.1 (d, *J* = 24.9 Hz), 31.1, 30.6, 29.2, 28.0, 27.6, 27.3, 26.8, 25.9, 24.4, 21.3, 20.2, 19.0, 17.9, 13.2.

HRMS-ESI m/z calcd for $C_{39}H_{51}FN_7O_6^+$ $[M + H]^+$ 732.3879, found 732.3883.

Analog **56b** (SA1103065)



Prepared according to general procedure of C-4 analogs 56. Analog **56b** (6.4 mg, 49% yield over 2 steps) was obtained as a white solid.

TLC (MeOH:DCM = 1:40): R_f = 0.17 (UV, *p*-anisaldehyde).

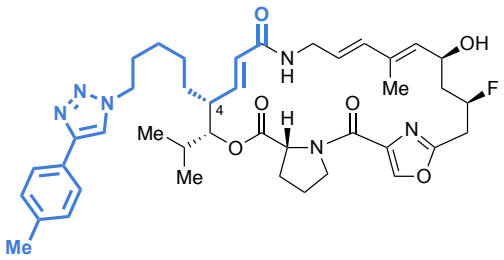
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.88 – 7.83 (m, 2H), 7.78 (s, 1H), 7.44 (t, *J* = 7.5 Hz, 2H), 7.34 (t, *J* = 7.4 Hz, 1H), 6.42 (dd, *J* = 16.1, 6.3 Hz, 1H), 6.17 (d, *J* = 15.8 Hz, 1H), 6.09 (s, 1H), 5.92 – 5.82 (m, 1H), 5.75 (ddd, *J* = 15.9, 8.0, 4.3 Hz, 1H), 5.34 (d, *J* = 8.7 Hz, 1H), 5.12 (s, 1H), 5.00 (s, 1H), 4.83 – 4.74 (m, 2H), 4.41 (t, *J* = 6.9 Hz, 2H), 4.10 – 3.99 (m, 1H), 3.89 – 3.80 (m, 1H), 3.66 (d, *J* = 9.6 Hz, 1H), 3.58

– 3.47 (m, 1H), 3.35 (s, 1H), 3.21 (td, J = 16.9, 5.4 Hz, 1H), 2.99 (td, J = 16.2, 6.7 Hz, 1H), 2.50 (s, 1H), 2.34 (t, J = 7.5 Hz, 1H), 2.25 – 2.12 (m, 3H), 1.93 (d, J = 6.6 Hz, 4H), 1.82 (d, J = 1.2 Hz, 3H), 1.60 (dd, J = 15.5, 6.4 Hz, 4H), 1.46 – 1.36 (m, 3H), 0.95 (d, J = 6.5 Hz, 3H), 0.91 (d, J = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.4, 160.6, 160.0, 148.0, 143.4, 143.4, 136.9, 136.1, 135.8, 133.1, 130.8, 129.0, 128.3, 125.8, 125.5, 125.3, 119.6, 89.3 (d, J = 171.3 Hz), 82.2, 66.0, 60.5, 59.3, 50.4, 48.7, 42.4 (d, J = 29.4 Hz), 41.1, 36.8, 33.9 (d, J = 26.0 Hz), 30.3, 29.5, 28.5, 27.1, 26.8, 25.9, 25.1, 24.8, 19.9, 19.0, 13.2.

HRMS-ESI m/z calcd for $C_{40}H_{52}FN_7O_6^+$ $[M + H]^+$ 731.3927, found 731.3932.

Analog **56c** (SA1103105)



Prepared according to general procedure of C-4 analogs 56. Analog **56c** (6.6 mg, 74% yield over 2 steps) was obtained as a light yellow solid.

TLC (MeOH:DCM = 1:40): R_f = 0.23 (UV, *p*-anisaldehyde).

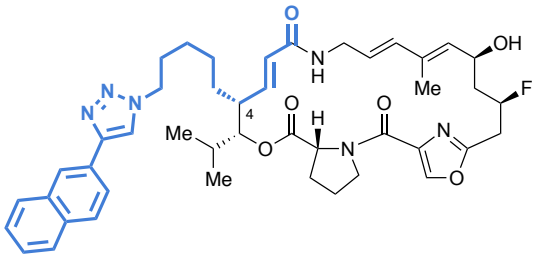
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.80 – 7.66 (m, 4H), 7.23 (s, 1H), 6.41 (dd, J = 16.1, 6.2 Hz, 1H), 6.17 (d, J = 15.7 Hz, 1H), 6.07 (d, J = 5.8 Hz, 1H), 5.86 (dd, J = 16.1, 1.4 Hz, 1H), 5.74 (ddd, J = 15.6, 7.9, 4.3 Hz, 1H), 5.34 (d, J = 9.0 Hz, 1H), 5.13 (d, J = 8.5 Hz, 1H), 4.98 (d, J = 7.5 Hz, 1H), 4.79 (td, J = 9.6, 3.0 Hz, 3H), 4.39 (t, J = 7.0 Hz, 2H), 4.10 – 3.99 (m, 1H), 3.90 – 3.76 (m, 2H), 3.58 – 3.46 (m, 1H), 3.21 (td, J = 16.9, 5.5 Hz, 2H), 2.98 (td, J = 16.4, 6.7 Hz, 1H), 2.50 (s, 1H), 2.38 (s, 4H), 2.27 – 2.12

(m, 3H), 1.98 – 1.92 (m, 4H), 1.82 (d, J = 1.1 Hz, 3H), 1.58 (d, J = 14.3 Hz, 4H), 1.39 (s, 2H), 0.94 (d, J = 6.5 Hz, 3H), 0.91 (t, J = 2.9 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.4, 160.6, 159.9, 159.8, 148.0, 143.5, 143.4, 138.2, 136.9, 136.1, 135.8, 133.1, 129.7, 125.8, 125.5, 125.3, 119.3, 89.3 (d, J = 170.9 Hz), 82.2, 66.0, 59.3, 50.4, 48.7, 42.5, 42.4 (d, J = 20.5 Hz), 41.1, 36.8, 33.9 (d, J = 25.8 Hz), 30.3, 29.5, 28.5, 27.1, 26.7, 25.9, 25.1, 24.8, 21.4, 19.9, 19.0, 13.2.

HRMS-ESI m/z calcd for $C_{41}H_{54}FN_6O_6^+$ $[M + H]^+$ 745.4083, found 745.4091.

Analog **56d** (SA1103106)



Prepared according to general procedure of C-4 analogs 56. Analog **56d** (7.6 mg, 85% yield over 2 steps) was obtained as a light yellow solid.

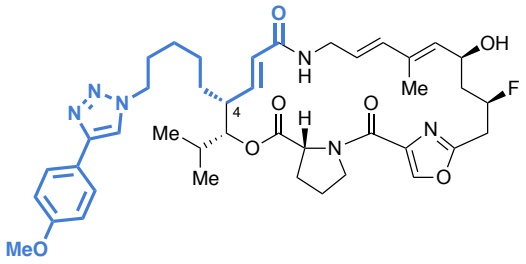
TLC (MeOH:DCM = 1:40): R_f = 0.21 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.39 (s, 1H), 8.10 (s, 1H), 7.99 – 7.92 (m, 4H), 7.87 (dd, J = 6.7, 2.6 Hz, 1H), 7.54 – 7.50 (m, 2H), 6.45 (dd, J = 16.1, 6.3 Hz, 1H), 6.19 (d, J = 15.6 Hz, 1H), 6.13 (s, 1H), 5.90 (d, J = 16.1 Hz, 1H), 5.76 (ddd, J = 15.6, 7.7, 4.2 Hz, 1H), 5.36 (d, J = 9.0 Hz, 1H), 5.12 (s, 1H), 5.01 (s, 1H), 4.81 (td, J = 9.1, 4.1 Hz, 2H), 4.47 (t, J = 7.0 Hz, 3H), 4.07 (d, J = 5.7 Hz, 1H), 3.90 – 3.81 (m, 1H), 3.73 (d, J = 32.8 Hz, 1H), 3.61 – 3.49 (m, 1H), 3.23 (td, J = 17.1, 5.6 Hz, 1H), 3.01 (td, J = 16.2, 6.6 Hz, 1H), 2.54 (d, J = 9.1 Hz, 1H), 2.36 (t, J = 7.6 Hz, 2H), 2.27 – 2.14 (m, 3H), 1.97 (d, J = 16.8 Hz, 4H), 1.84 (s, 3H), 1.62 (d, J = 13.9 Hz, 4H), 1.46 – 1.41 (m, 2H), 0.97 (d, J = 6.5 Hz, 3H), 0.93 (d, J = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ δ 171.6, 166.4, 160.6, 159.9, 159.8, 148.0, 143.4, 136.9, 136.1, 135.8, 133.7, 133.3, 133.1, 128.7, 128.3, 127.9, 126.6, 126.3, 125.5, 125.3, 124.6, 124.0, 120.0, 89.3 (d, J = 171.3 Hz), 82.2, 66.0, 59.3, 50.5, 48.7, 42.4 (d, J = 28.3 Hz), 41.1, 36.8, 33.8 (d, J = 25.8 Hz), 30.4, 29.5, 28.5, 27.1, 26.8, 25.9, 25.1, 24.8, 19.9, 19.0, 13.2.

HRMS-ESI m/z calcd for $C_{44}H_{54}FN_6O_6^+$ $[M + H]^+$ 781.4083, found 781.4094.

Analog **56e** (SA1103112)



Prepared according to general procedure of C-4 analogs 56. Analog **56e** (7 mg, 50% yield over 2 steps) was obtained as a light yellow solid.

TLC (MeOH:DCM = 1:40): R_f = 0.26 (UV, *p*-anisaldehyde).

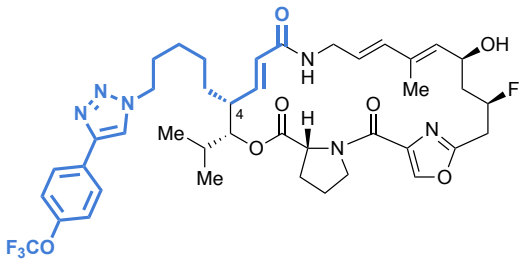
^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 (s, 1H), 7.77 (d, J = 8.4 Hz, 2H), 7.69 (s, 1H), 6.97 (d, J = 8.2 Hz, 2H), 6.42 (dd, J = 16.1, 6.1 Hz, 1H), 6.17 (d, J = 15.7 Hz, 1H), 5.86 (d, J = 16.1 Hz, 1H), 5.80 – 5.67 (m, 1H), 5.44 – 5.32 (m, 1H), 5.05 (d, J = 40.5 Hz, 2H), 4.86 – 4.66 (m, 3H), 4.38 (d, J = 8.6 Hz, 2H), 4.05 (s, 1H), 3.84 (s, 6H), 3.49 (s, 1H), 3.34 – 3.12 (m, 2H), 2.99 (d, J = 6.6 Hz, 1H), 2.48 (s, 1H), 2.23 (d, J = 7.0 Hz, 4H), 1.92 (s, 4H), 1.82 (s, 3H), 1.72 – 1.48 (m, 4H), 1.33 (s, 3H), 0.97 – 0.90 (m, 3H), 0.88 (d, J = 4.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.4, 160.6, 159.8, 147.8, 143.4, 143.4, 142.1, 136.9, 136.1, 135.8, 133.1, 127.2, 125.5, 125.3, 123.4, 118.9, 114.4, 99.8, 89.3 (d, J = 170.9 Hz), 82.2, 66.0, 59.3, 55.5, 50.4,

48.7, 42.4 (d, J = 28.7 Hz), 41.0, 33.8 (d, J = 25.7 Hz), 30.3, 29.7, 29.5, 28.5, 27.1, 26.8, 25.9, 25.1, 19.9, 19.0, 17.6, 13.2.

HRMS-ESI m/z calcd for $C_{41}H_{54}FN_6O_7^+$ $[M + H]^+$ 761.4033, found 761.4046.

Analog **56f** (SA1103113)



Prepared according to general procedure of C-4 analogs 56. Analog **56f** (6.8 mg, 52% yield over 2 steps) was obtained as a white solid.

TLC (MeOH:DCM = 1:40): R_f = 0.24 (UV, *p*-anisaldehyde).

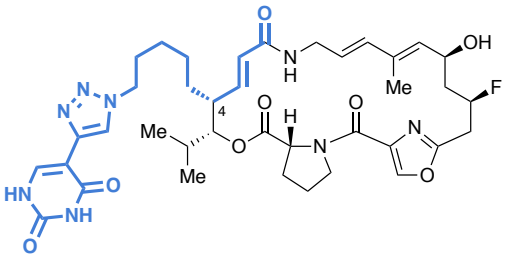
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.92 – 7.88 (m, 2H), 7.81 (d, J = 2.1 Hz, 1H), 7.30 (d, J = 8.1 Hz, 2H), 6.44 (dd, J = 16.1, 6.2 Hz, 1H), 6.20 (d, J = 15.6 Hz, 1H), 6.10 (s, 1H), 5.89 (dd, J = 15.9, 7.4 Hz, 1H), 5.76 (ddd, J = 15.5, 7.7, 4.1 Hz, 1H), 5.38 (dd, J = 15.5, 8.5 Hz, 1H), 5.13 (s, 1H), 5.02 (s, 1H), 4.81 (ddd, J = 12.1, 8.3, 3.0 Hz, 2H), 4.42 (q, J = 8.5 Hz, 2H), 4.12 – 4.02 (m, 1H), 3.86 (dt, J = 11.4, 7.1 Hz, 1H), 3.77 (t, J = 5.4 Hz, 1H), 3.59 – 3.49 (m, 1H), 3.36 (d, J = 14.5 Hz, 1H), 3.21 (dd, J = 17.3, 5.5 Hz, 1H), 3.01 (d, J = 6.7 Hz, 1H), 2.52 (d, J = 9.0 Hz, 1H), 2.30 (s, 2H), 2.22 – 2.10 (m, 3H), 2.01 – 1.93 (m, 4H), 1.87 – 1.82 (m, 3H), 1.75 (d, J = 4.4 Hz, 2H), 1.62 (dt, J = 22.0, 8.2 Hz, 3H), 1.46 – 1.39 (m, 2H), 0.96 (t, J = 6.3 Hz, 3H), 0.93 (d, J = 6.9 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.4, 160.6, 149.1, 146.7, 143.4, 136.9, 136.1, 135.8, 133.2, 129.6, 127.2, 125.5, 125.4, 121.5, 119.8, 99.8, 89.3 (d, J = 170.9 Hz), 82.2, 66.0, 59.3, 50.5, 48.7, 42.4 (d, J = 20.9

Hz), 42.2, 41.1, 36.8, 33.9 (d, J = 24.9 Hz), 30.4, 29.8, 29.5, 28.5, 26.8, 25.9, 25.1, 24.8, 23.5, 19.9, 19.0, 17.6, 13.2.

HRMS-ESI m/z calcd for $C_{41}H_{51}F_4N_6O_7^+$ $[M + H]^+$ 815.3750, found 815.3762.

Analog **56g** (SA1103114)



Prepared according to general procedure of C-4 analogs 56. Analog **56g** (7.8 mg, 49% yield over 2 steps) was obtained as a white solid.

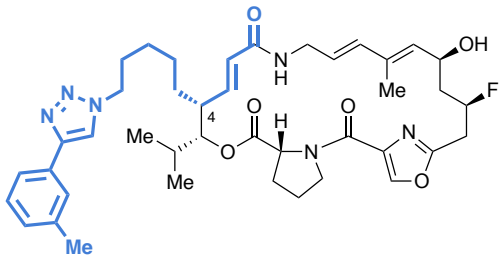
TLC (MeOH:DCM = 1:10): R_f = 0.29 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, MeOD) δ 8.31 (d, J = 3.5 Hz, 1H), 8.25 (s, 1H), 8.14 – 8.10 (m, 1H), 7.71 (s, 1H), 6.63 (dd, J = 15.8, 6.2 Hz, 1H), 6.24 (d, J = 15.7 Hz, 1H), 5.96 (d, J = 16.4 Hz, 1H), 5.85 – 5.71 (m, 1H), 5.38 (q, J = 10.9 Hz, 1H), 5.04 (dd, J = 47.7, 6.1 Hz, 1H), 4.80 (dd, J = 8.7, 3.4 Hz, 1H), 4.70 (dd, J = 9.2, 5.0 Hz, 1H), 4.58 (d, J = 7.6 Hz, 1H), 4.43 (q, J = 7.0 Hz, 3H), 4.13 – 4.05 (m, 1H), 3.82 – 3.63 (m, 3H), 3.53 (s, 1H), 3.25 – 3.11 (m, 2H), 3.11 – 2.97 (m, 1H), 2.60 (s, 1H), 2.32 (dd, J = 21.9, 12.6 Hz, 2H), 2.22 – 2.16 (m, 1H), 2.07 – 2.01 (m, 2H), 1.94 (s, 3H), 1.83 (s, 2H), 1.80 (d, J = 10.8 Hz, 2H), 1.64 (d, J = 21.0 Hz, 2H), 1.50 (t, J = 9.3 Hz, 1H), 1.17 (s, 1H), 0.95 (d, J = 7.0 Hz, 3H), 0.91 (d, J = 6.0 Hz, 3H).

^{13}C NMR (100 MHz, MeOD) δ 171.7, 167.7, 164.1, 162.5, 162.0, 152.8, 147.3, 146.4, 144.7, 139.0, 136.8, 136.6, 134.4, 126.3, 125.8, 123.7, 106.1, 98.9, 90.4 (d, J = 169.8 Hz), 83.6, 82.7, 75.9, 66.0, 60.6, 51.3, 43.1 (d, J = 18.0 Hz), 41.6, 34.1 (d, J = 25.6 Hz), 31.2, 30.6, 29.2, 28.0, 27.6, 26.7, 25.9, 20.2, 19.0, 13.2.

HRMS-ESI m/z calcd for $C_{38}H_{50}FN_8O_8^+$ $[M + H]^+$ 765.3730, found 765.3733.

Analog **56h** (SA1103115)



Prepared according to general procedure of C-4 analogs 56. Analog **56h** (10.5 mg, 78% yield over 2 steps) was obtained as a light yellow solid.

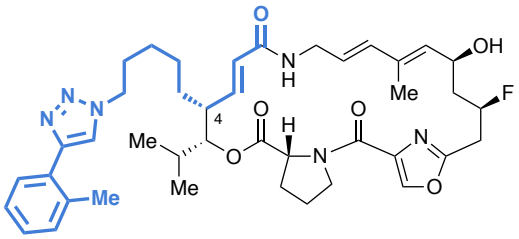
TLC (MeOH:DCM = 1:40): R_f = 0.23 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.10 (s, 1H), 7.77 (s, 1H), 7.72 (s, 1H), 7.63 (d, *J* = 7.7 Hz, 1H), 7.34 (t, *J* = 7.6 Hz, 1H), 7.17 (d, *J* = 7.6 Hz, 1H), 6.45 (dd, *J* = 16.1, 6.2 Hz, 1H), 6.20 (d, *J* = 15.8 Hz, 1H), 6.14 (dd, *J* = 8.4, 3.5 Hz, 1H), 5.93 – 5.85 (m, 1H), 5.76 (ddd, *J* = 15.6, 7.9, 4.3 Hz, 1H), 5.43 – 5.30 (m, 1H), 5.07 (dd, *J* = 47.3, 7.7 Hz, 1H), 4.81 (ddt, *J* = 13.7, 9.2, 4.0 Hz, 2H), 4.76 – 4.65 (m, 1H), 4.46 – 4.37 (m, 3H), 4.07 (ddd, *J* = 12.0, 8.1, 4.7 Hz, 1H), 3.86 (dt, *J* = 11.7, 7.2 Hz, 1H), 3.54 (ddd, *J* = 15.9, 8.0, 3.4 Hz, 1H), 3.32 – 3.11 (m, 2H), 3.00 (td, *J* = 16.5, 6.7 Hz, 1H), 2.51 (s, 1H), 2.43 (s, 5H), 2.26 – 2.12 (m, 2H), 2.03 (d, *J* = 6.9 Hz, 1H), 1.96 (q, *J* = 5.8 Hz, 4H), 1.84 (s, 3H), 1.73 (s, 1H), 1.64 (s, 1H), 1.61 – 1.52 (m, 2H), 1.41 (d, *J* = 9.1 Hz, 1H), 1.23 (s, 1H), 1.18 (s, 1H), 0.97 (d, *J* = 6.3 Hz, 3H), 0.93 (d, *J* = 5.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.3, 160.6, 159.9, 159.8, 148.1, 143.4, 138.7, 136.9, 136.0, 135.8, 133.2, 130.6, 129.1, 128.9, 126.5, 125.5, 125.3, 122.9, 119.6, 89.3 (d, *J* = 170.9 Hz), 82.2, 66.0, 59.3, 50.4, 48.7, 42.4 (d, *J* = 30.4 Hz), 41.0, 33.9 (d, *J* = 25.8 Hz), 30.3, 29.5, 28.5, 27.1, 26.7, 25.9, 25.1, 24.0, 21.6, 19.9, 19.0, 13.2.

HRMS-ESI m/z calcd for $C_{41}H_{54}FN_6O_6^+$ $[M + H]^+$ 745.4083, found 745.4093.

Analog **56i** (SA1103116)



Prepared according to general procedure of C-4 analogs 56. Analog **56i** (5.6 mg, 43% yield over 2 steps) was obtained as a light yellow solid.

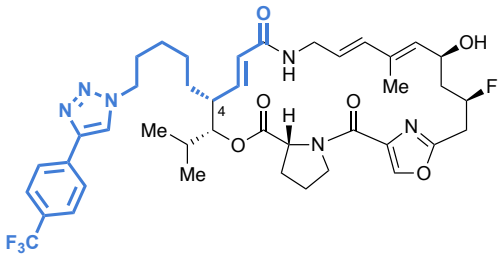
TLC (MeOH:DCM = 1:40): R_f = 0.19 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (d, *J* = 6.5 Hz, 1H), 7.88 – 7.69 (m, 2H), 7.65 (s, 1H), 7.23 – 7.13 (m, 1H), 6.43 (dd, *J* = 16.0, 6.2 Hz, 1H), 6.31 – 6.13 (m, 1H), 6.10 (s, 1H), 5.87 (d, *J* = 16.3 Hz, 1H), 5.74 (ddd, *J* = 15.5, 7.9, 4.1 Hz, 1H), 5.36 (dd, *J* = 16.5, 8.2 Hz, 1H), 5.10 (s, 1H), 5.00 (s, 1H), 4.85 – 4.69 (m, 2H), 4.41 (dt, *J* = 12.7, 6.9 Hz, 3H), 4.14 – 3.99 (m, 1H), 3.90 – 3.78 (m, 1H), 3.74 (s, 1H), 3.66 (d, *J* = 1.6 Hz, 1H), 3.48 (s, 1H), 3.28 – 3.12 (m, 2H), 2.99 (td, *J* = 16.3, 6.7 Hz, 1H), 2.48 (d, *J* = 2.4 Hz, 5H), 2.33 (s, 1H), 2.24 – 2.19 (m, 1H), 2.17 (s, 1H), 2.13 (d, *J* = 6.3 Hz, 1H), 2.08 (s, 1H), 2.02 (s, 1H), 1.96 (q, *J* = 6.6 Hz, 4H), 1.82 (d, *J* = 1.2 Hz, 2H), 1.71 (s, 1H), 1.65 – 1.51 (m, 3H), 1.42 (d, *J* = 5.7 Hz, 1H), 0.97 – 0.91 (m, 3H), 0.91 – 0.87 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.3, 160.7, 147.2, 143.4, 142.1, 136.9, 136.1, 135.6, 133.1, 131.1, 129.0, 128.3, 126.3, 125.5, 125.3, 121.8, 103.9, 90.3 (d, *J* = 178.1 Hz), 82.5, 82.2, 66.3, 60.3, 59.3, 50.3, 48.7, 45.3, 42.38 (d, *J* = 28.4 Hz), 41.0, 33.0 (d, *J* = 27.7 Hz), 30.4, 29.7, 29.5, 27.1, 26.8, 25.9, 25.1, 21.6, 19.9, 19.6, 13.2.

HRMS-ESI m/z calcd for $C_{41}H_{54}FN_6O_6^+$ $[M + H]^+$ 745.4083, found 745.4093.

Analog **56j** (SA1103117)



Prepared according to general procedure of C-4 analogs 56. Analog **56j** (6.3 mg, 43% yield over 2 steps) was obtained as a light yellow solid.

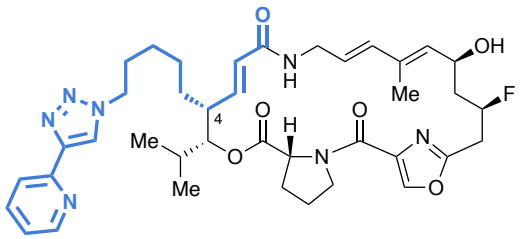
TLC (MeOH:DCM = 1:40): R_f = 0.19 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.97 (d, *J* = 8.1 Hz, 2H), 7.86 (s, 1H), 7.68 (d, *J* = 8.0 Hz, 2H), 6.42 (dd, *J* = 16.1, 6.2 Hz, 1H), 6.17 (d, *J* = 15.6 Hz, 1H), 6.07 (s, 1H), 5.87 (d, *J* = 15.9 Hz, 1H), 5.78 – 5.66 (m, 1H), 5.48 (s, 1H), 5.34 (d, *J* = 8.6 Hz, 1H), 5.05 (d, *J* = 43.8 Hz, 1H), 4.83 – 4.54 (m, 3H), 4.41 (dd, *J* = 14.7, 7.9 Hz, 3H), 4.05 (s, 1H), 3.85 (dd, *J* = 12.6, 5.9 Hz, 1H), 3.75 (s, 1H), 3.50 (d, *J* = 14.1 Hz, 1H), 3.29 – 3.15 (m, 1H), 2.99 (td, *J* = 16.3, 6.6 Hz, 1H), 2.51 (d, *J* = 15.5 Hz, 2H), 2.36 – 2.11 (m, 4H), 2.07 – 1.87 (m, 4H), 1.86 – 1.78 (m, 3H), 1.74 – 1.46 (m, 4H), 1.45 – 1.36 (m, 2H), 0.97 – 0.91 (m, 3H), 0.91 – 0.87 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.4, 160.6, 146.6, 143.4, 136.9, 136.0, 135.8, 134.2, 133.2, 130.6, 126.0, 125.5, 120.4, 99.8, 89.3 (d, *J* = 170.9 Hz), 82.2, 65.9, 60.5, 59.3, 50.5, 48.7, 42.4 (d, *J* = 17.6 Hz), 42.2, 41.1, 36.8, 33.9 (d, *J* = 24.6 Hz), 31.7, 30.3, 29.8, 29.5, 28.5, 27.1, 27.1, 26.8, 25.9, 25.1, 19.9, 19.6, 19.0, 13.2.

HRMS-ESI m/z calcd for $C_{41}H_{51}F_4N_6O_6^+$ $[M + H]^+$ 799.3801, found 799.3816.

Analog **56k** (SA1103118)



Prepared according to general procedure of C-4 analogs 56. Analog **56k** (12.6 mg, 66% yield over 2 steps) was obtained as a light yellow solid.

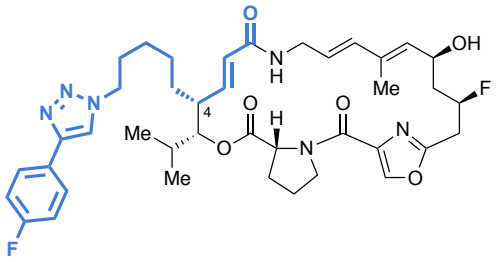
TLC (MeOH:DCM = 1:40): R_f = 0.11 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, MeOD) δ 8.62 (s, 1H), 8.52 (d, *J* = 6.5 Hz, 1H), 8.37 – 8.22 (m, 1H), 8.14 (d, *J* = 8.1 Hz, 1H), 8.04 (t, *J* = 7.9 Hz, 1H), 7.47 (s, 1H), 6.63 (dd, *J* = 15.9, 6.0 Hz, 1H), 6.24 (d, *J* = 15.6 Hz, 1H), 6.06 – 5.89 (m, 1H), 5.78 (ddt, *J* = 15.4, 7.9, 3.5 Hz, 1H), 5.37 (p, *J* = 8.7 Hz, 1H), 5.16 – 4.94 (m, 1H), 4.80 (dd, *J* = 8.7, 3.4 Hz, 1H), 4.73 – 4.57 (m, 2H), 4.55 – 4.42 (m, 3H), 4.08 (td, *J* = 13.0, 6.6 Hz, 1H), 3.82 – 3.63 (m, 2H), 3.27 – 3.20 (m, 1H), 3.20 – 3.03 (m, 2H), 2.61 (s, 1H), 2.45 – 2.26 (m, 2H), 2.18 (t, *J* = 7.1 Hz, 1H), 2.10 – 1.96 (m, 4H), 1.94 (s, 1H), 1.90 (d, *J* = 4.1 Hz, 1H), 1.83 (s, 3H), 1.79 (d, *J* = 7.5 Hz, 1H), 1.67 (s, 1H), 1.61 (s, 1H), 1.53 (d, *J* = 9.7 Hz, 1H), 1.45 – 1.34 (m, 2H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.91 (d, *J* = 6.6 Hz, 3H).

^{13}C NMR (100 MHz, MeOD) δ 171.7, 167.7, 162.5, 162.1, 162.0, 149.2, 146.4, 144.7, 140.5, 137.9, 136.8, 136.6, 134.5, 126.3, 125.8, 124.7, 122.2, 90.4 (d, *J* = 170.1 Hz), 83.6, 66.0, 60.6, 57.5, 51.6, 50.1, 43.1 (d, *J* = 14.4 Hz), 41.6, 34.1 (d, *J* = 25.0 Hz), 31.2, 30.7, 30.6, 29.2, 28.0, 27.6, 27.3, 26.7, 25.9, 20.2, 19.0, 13.2.

HRMS-ESI m/z calcd for $C_{39}H_{51}FN_7O_6^+$ $[M + H]^+$ 732.3879, found 732.3887.

Analog **56l** (SA1103119)



Prepared according to general procedure of C-4 analogs 56. Analog **56l** (18.5 mg, 82% yield over 2 steps) was obtained as a light yellow solid.

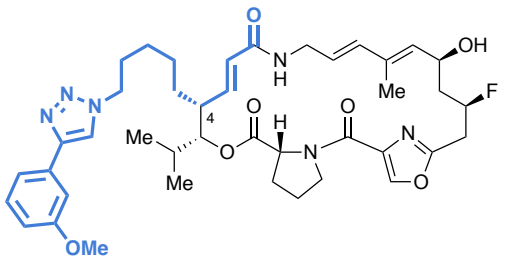
TLC (MeOH:DCM = 1:40): R_f = 0.15 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.81 (dd, *J* = 8.6, 5.4 Hz, 2H), 7.75 (s, 1H), 7.12 (d, *J* = 8.6 Hz, 2H), 6.42 (dd, *J* = 16.0, 6.2 Hz, 1H), 6.20 (d, *J* = 12.4 Hz, 1H), 6.14 (s, 1H), 5.91 – 5.83 (m, 1H), 5.72 (ddd, *J* = 15.7, 7.8, 4.2 Hz, 1H), 5.35 (dd, *J* = 17.9, 8.8 Hz, 1H), 5.10 (d, *J* = 7.6 Hz, 1H), 4.98 (d, *J* = 9.0 Hz, 1H), 4.78 (ddd, *J* = 10.1, 7.4, 2.9 Hz, 2H), 4.71 (d, *J* = 9.2 Hz, 1H), 4.38 (q, *J* = 8.0 Hz, 3H), 4.04 (td, *J* = 9.7, 4.9 Hz, 1H), 3.82 (dt, *J* = 11.6, 7.2 Hz, 1H), 3.75 (s, 1H), 3.57 – 3.45 (m, 1H), 3.19 (td, *J* = 16.7, 5.5 Hz, 2H), 3.00 (dd, *J* = 16.6, 6.7 Hz, 1H), 2.49 (s, 1H), 2.23 – 2.12 (m, 2H), 1.97 – 1.90 (m, 4H), 1.81 (s, 3H), 1.65 – 1.55 (m, 2H), 1.51 (dt, *J* = 10.6, 6.9 Hz, 1H), 1.35 (dd, *J* = 15.3, 8.0 Hz, 3H), 1.20 (s, 1H), 1.15 (s, 1H), 0.93 (d, *J* = 6.1 Hz, 3H), 0.89 (d, *J* = 6.2 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.3, 164.0, 161.6, 160.6, 160.0, 147.0, 143.5, 143.3, 136.8, 136.0, 135.7, 133.2, 127.6, 127.5, 125.4, 125.3, 119.5, 116.1, 115.9, 89.3 (d, *J* = 170.9 Hz), 82.2, 65.9, 59.3, 50.4, 48.7, 42.4 (d, *J* = 21.3 Hz), 42.2, 41.0, 33.9 (d, *J* = 26.8 Hz), 30.3, 29.5, 28.5, 27.1, 26.7, 25.9, 25.0, 19.8, 18.9, 13.2.

HRMS-ESI m/z calcd for $C_{40}H_{51}F_2N_6O_6^+$ $[M + H]^+$ 749.3833, found 749.3844.

Analog **56m** (SA1103120)



Prepared according to general procedure of C-4 analogs 56. Analog **56m** (10.2 mg, 73% yield over 2 steps) was obtained as a light yellow solid.

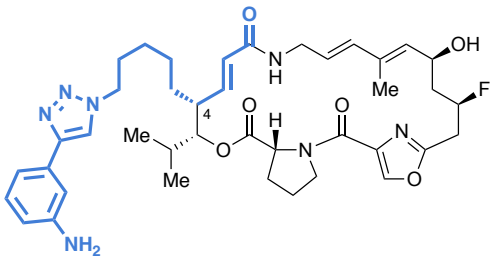
TLC (MeOH:DCM = 1:40): R_f = 0.28 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.07 (s, 1H), 7.77 (d, *J* = 2.7 Hz, 1H), 7.53 – 7.43 (m, 1H), 7.39 – 7.31 (m, 2H), 6.89 (ddd, *J* = 7.8, 2.7, 1.4 Hz, 1H), 6.42 (dd, *J* = 16.1, 6.3 Hz, 1H), 6.32 – 6.04 (m, 2H), 5.94 – 5.78 (m, 1H), 5.74 (ddd, *J* = 15.7, 7.9, 4.2 Hz, 1H), 5.36 (dd, *J* = 16.7, 8.2 Hz, 1H), 5.10 (s, 1H), 5.00 (s, 1H), 4.83 – 4.60 (m, 3H), 4.39 (dt, *J* = 11.2, 6.8 Hz, 3H), 4.03 (d, *J* = 12.1 Hz, 1H), 3.87 (d, *J* = 2.0 Hz, 5H), 3.74 (s, 1H), 3.48 (s, 1H), 3.40 – 3.16 (m, 2H), 3.08 – 2.91 (m, 1H), 2.50 (d, *J* = 17.4 Hz, 1H), 2.33 (t, *J* = 7.5 Hz, 1H), 2.25 – 2.05 (m, 4H), 2.01 – 1.89 (m, 5H), 1.85 – 1.79 (m, 3H), 1.71 (s, 1H), 1.66 – 1.58 (m, 2H), 1.44 – 1.35 (m, 2H), 0.96 – 0.91 (m, 3H), 0.89 (t, *J* = 3.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.4, 160.2, 147.8, 143.5, 143.4, 136.9, 136.1, 135.7, 133.1, 132.0, 130.0, 125.5, 125.3, 119.8, 118.2, 114.4, 110.9, 99.8, 89.3 (d, *J* = 170.9 Hz), 82.2, 66.0, 59.3, 55.5, 50.4, 48.7, 42.4 (d, *J* = 29.5 Hz), 41.0, 33.9 (d, *J* = 25.7 Hz), 30.3, 29.5, 28.5, 27.1, 26.7, 25.9, 25.1, 19.9, 19.0, 17.6, 13.2.

HRMS-ESI m/z calcd for $C_{41}H_{54}FN_6O_7^+$ $[M + H]^+$ 761.4033, found 761.4044.

Analog **56n** (SA1103121)



Prepared according to general procedure of C-4 analogs 56. Analog **56n** (12 mg, 76% yield over 2 steps) was obtained as a light brown solid.

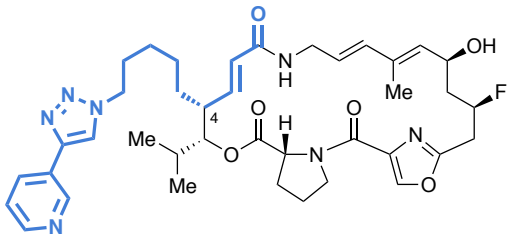
TLC (MeOH:DCM = 1:20): R_f = 0.09 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, MeOD) δ 8.35 (q, *J* = 6.5 Hz, 1H), 8.24 (d, *J* = 5.2 Hz, 1H), 7.62 – 7.50 (m, 2H), 7.42 – 7.38 (m, 1H), 7.07 (d, *J* = 5.8 Hz, 1H), 6.63 (dd, *J* = 15.9, 6.0 Hz, 1H), 6.23 (dd, *J* = 15.4, 7.6 Hz, 1H), 5.96 (dd, *J* = 15.9, 1.5 Hz, 1H), 5.77 (ddd, *J* = 15.8, 7.8, 4.0 Hz, 1H), 5.38 (d, *J* = 8.8 Hz, 1H), 5.09 (d, *J* = 6.1 Hz, 1H), 4.98 (d, *J* = 6.0 Hz, 1H), 4.71 (td, *J* = 9.1, 5.2 Hz, 2H), 4.46 (q, *J* = 8.5 Hz, 3H), 4.14 – 4.01 (m, 2H), 3.82 – 3.62 (m, 3H), 3.24 (dt, *J* = 17.2, 4.7 Hz, 1H), 3.20 – 3.07 (m, 2H), 3.07 – 3.00 (m, 1H), 2.60 (s, 1H), 2.35 (s, 1H), 2.19 – 2.11 (m, 1H), 2.05 – 1.95 (m, 4H), 1.94 (s, 2H), 1.83 (d, *J* = 1.2 Hz, 3H), 1.69 – 1.60 (m, 2H), 1.52 (s, 1H), 1.14 (d, *J* = 17.9 Hz, 1H), 0.94 (d, *J* = 6.8 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 3H).

^{13}C NMR (100 MHz, MeOD) δ 171.7, 167.7, 163.2, 162.5, 148.0, 146.4, 144.7, 137.9, 136.8, 136.6, 134.5, 133.5, 131.5, 130.6, 126.3, 125.8, 122.7, 117.9, 90.4 (d, *J* = 170.4 Hz), 83.6, 66.0, 60.6, 57.5, 57.3, 51.4, 50.1, 43.1 (d, *J* = 14.9 Hz), 43.0, 41.6, 34.1 (d, *J* = 24.6 Hz), 31.2, 30.6, 29.2, 28.0, 27.6, 26.8, 25.9, 20.2, 19.0, 13.2.

HRMS-ESI m/z calcd for $C_{40}H_{53}FN_7O_6^+$ $[M + H]^+$ 746.4036, found 746.4043.

Analog **56o** (SA1103122)



Prepared according to general procedure of C-4 analogs 56. Analog **56o** (13.9 mg, 67% yield over 2 steps) was obtained as a light yellow solid.

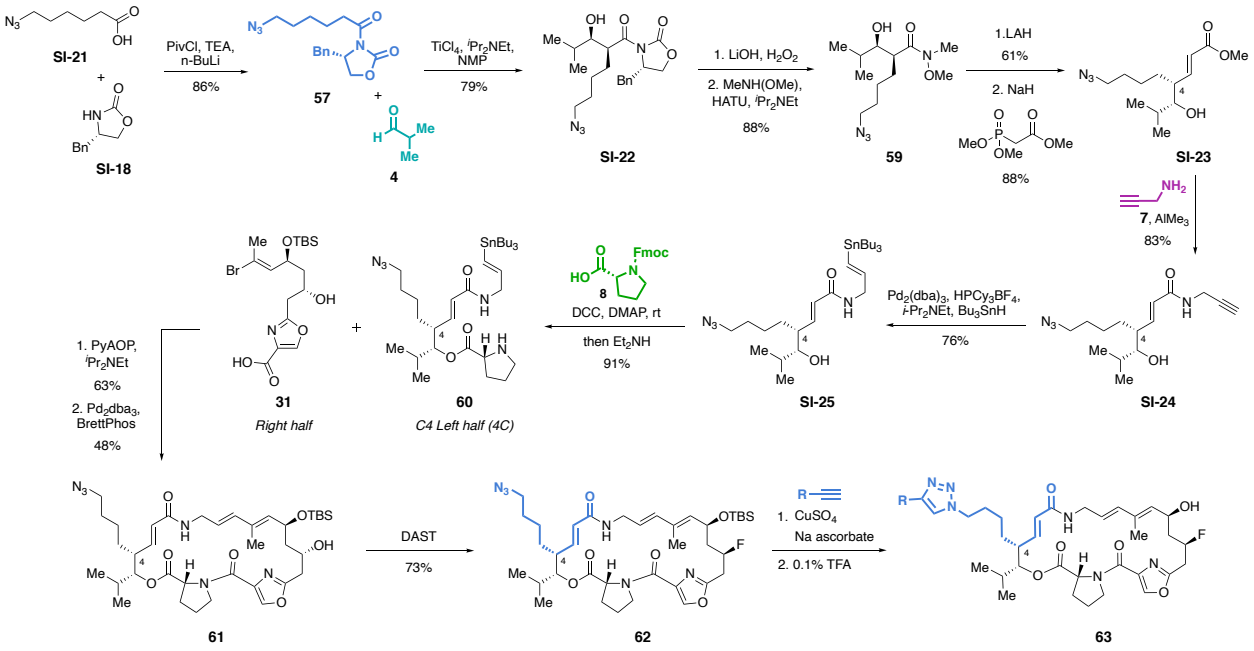
TLC (MeOH:DCM = 1:40): R_f = 0.12 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, MeOD) δ 9.17 (s, 1H), 8.65 – 8.58 (m, 3H), 8.34 – 8.23 (m, 1H), 7.81 (d, *J* = 8.4 Hz, 1H), 6.63 (dd, *J* = 15.8, 5.9 Hz, 1H), 6.35 – 6.17 (m, 1H), 5.97 (dd, *J* = 15.8, 1.5 Hz, 1H), 5.89 – 5.72 (m, 2H), 5.38 (d, *J* = 9.3 Hz, 1H), 5.09 (d, *J* = 6.8 Hz, 1H), 4.74 – 4.68 (m, 2H), 4.60 (d, *J* = 6.3 Hz, 1H), 4.54 – 4.45 (m, 3H), 4.12 – 4.04 (m, 1H), 3.82 – 3.62 (m, 3H), 3.25 – 3.20 (m, 1H), 3.13 (dd, *J* = 35.1, 5.7 Hz, 2H), 3.03 (d, *J* = 7.7 Hz, 1H), 2.61 (s, 1H), 2.41 – 2.27 (m, 2H), 2.17 (d, *J* = 11.1 Hz, 1H), 2.00 (s, 4H), 1.94 (s, 1H), 1.83 (s, 3H), 1.69 – 1.64 (m, 1H), 1.61 (s, 1H), 1.53 (d, *J* = 9.3 Hz, 1H), 1.38 (d, *J* = 10.4 Hz, 1H), 0.95 (d, *J* = 6.8 Hz, 3H), 0.92 – 0.89 (m, 3H).

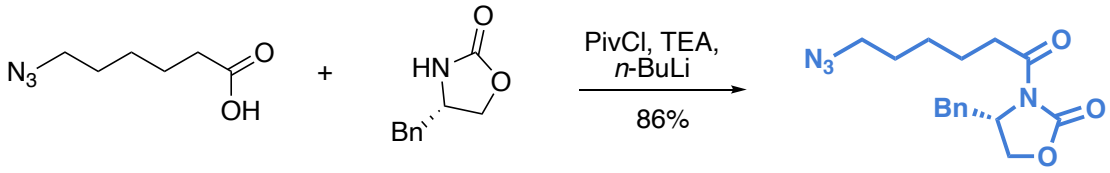
^{13}C NMR (100 MHz, MeOD) δ 171.7, 167.7, 162.5, 146.4, 145.5, 144.7, 143.8, 143.4, 139.2, 137.8, 136.8, 136.6, 134.5, 127.3, 126.3, 125.8, 124.1, 90.4 (d, *J* = 169.7 Hz), 83.6, 66.0, 62.0, 60.6, 51.6, 50.1, 43.1 (d, *J* = 17.5 Hz), 41.6, 34.1 (d, *J* = 25.4 Hz), 31.1, 30.6, 29.2, 28.0, 27.6, 27.3, 26.8, 25.9, 20.2, 19.4, 19.0, 13.2.

HRMS-ESI m/z calcd for $C_{39}H_{51}FN_7O_6^+$ $[M + H]^+$ 732.3879, found 732.3888.

VI. Preparation for C4-modified alkyl analogs (4C linker) 63



Preparation of oxazolidinone **57**



SI-21 **SI-18** **57**

An oven-dried 500-mL round-bottom flask containing acid **SI-21** (9.84 g, 62.6 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Then the vessel was sealed with a rubber septum. Dry THF (139 mL) was added, resulting in a colorless solution, and the vessel was cooled to 0 °C by means of ice/water bath. TEA (9.6 mL, 68.9 mmol, 1.1 equiv) and PivCl (8.5 mL, 68.9 mmol, 1.1 equiv) were added, resulting in a white suspension. The reaction mixture stirred at 0 °C for 3 h. In a separate oven-dried 250-mL round-bottom vessel, oxazolidinone **SI-18** (13.3 g, 75.1 mmol, 1.2 equiv) was added, which was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Then the vessel was sealed with a rubber septum. Dry THF (139 mL) was added, resulting in a clear

solution, and the vessel was cooled to -78 °C by means of dry ice/acetone bath. A solution of *n*-BuLi in hexanes butyllithium (2.48 M, 30.3 mL, 75.1 mmol, 1.2 equiv) was added dropwise to this solution. After the reaction mixture stirred at -78 °C for 3 h, this mixture was added via cannula to the 500-mL round-bottom flask which was cooled to -78 °C in advance by means of dry ice/acetone bath. After 2 h, saturated aqueous ammonium chloride solution (100 mL) was added in one portion. The resulting biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with EtOAc (4 × 50 mL). The combined organic layers were washed with brine (100 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc: hexanes = 1:10) to afford oxazolidinone **57** (17.1 g, 86%) as a white solid.

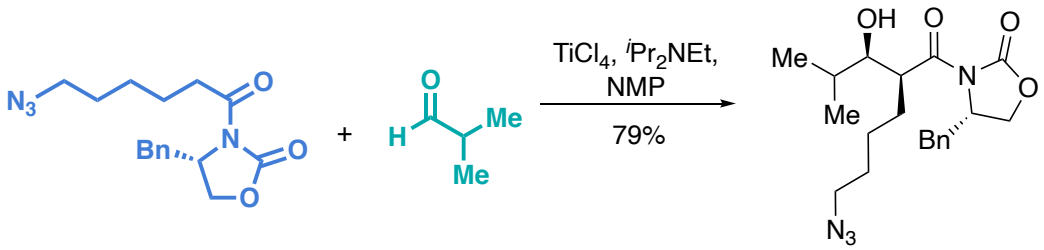
TLC (EtOAc:hexanes = 1:3): R_f = 0.31 (UV, p-anisaldehyde).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32 (ddd, *J* = 11.6, 7.7, 5.9 Hz, 3H), 7.24 – 7.18 (m, 2H), 4.68 (td, *J* = 6.8, 3.5 Hz, 1H), 4.26 – 4.12 (m, 2H), 3.36 – 3.23 (m, 3H), 3.06 – 2.84 (m, 2H), 2.77 (dd, *J* = 13.3, 9.6 Hz, 1H), 1.70 (dp, *J* = 21.5, 7.1 Hz, 4H), 1.54 – 1.41 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 173.1, 153.6, 135.4, 129.5, 129.1, 127.5, 66.4, 55.3, 51.4, 38.1, 35.5, 28.8, 26.3, 23.9.

HRMS-ESI m/z calcd for $C_{16}H_{20}N_4NaO_3^+$ $[M + Na]^+$ 339.1428, found 339.1429.

Preparation of alcohol **SI-22**



57 **4** **SI-22**

An oven-dried 100-mL round bottom flask containing oxazolidinone **57** (17.1 g, 54.1 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Then the vessel was sealed with a rubber septum. Dry DCM (541 mL) was added, resulting in a colorless solution, and the vessel was cooled to -78 ºC by means of a dry ice-acetone bath. A solution of titanium tetrachloride in DCM (1.0 M, 56.8 mL, 56.8 mmol, 1.05 equiv) was added dropwise, resulting in a yellow solution. After stirring for 5 min, $^{i}Pr_2EtN$ (10.2 mL, 59.5 mmol, 1.1 equiv) was added dropwise to the mixture, followed by NMP (5.21 mL, 54.1 mmol, 1 equiv), resulting in a dark red solution. After 1 h, freshly distilled isobutyraldehyde (**4**, 5.92 mL, 64.9 mmol, 1.1 equiv) was added dropwise. After 12 h, half-saturated aqueous ammonium chloride solution (100 mL) was carefully added, and the mixture was allowed to warm to 23 °C. The resulting biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with DCM (2 × 200 mL). The combined organic layers were washed with water (200 mL) and brine (200 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc: hexanes = 1:10 to 1:5) to afford alcohol **SI-22** (16.6 g, 79%) as a colorless oil.

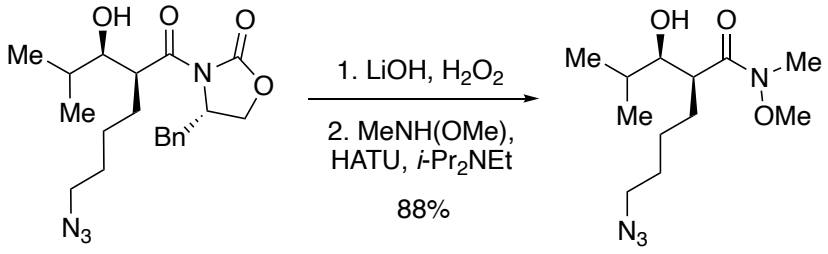
TLC (EtOAc:hexanes = 1:2): R_f = 0.26 (UV, p-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.38 – 7.33 (m, 2H), 7.32 – 7.27 (m, 1H), 7.26 – 7.22 (m, 2H), 4.72 (ddt, *J* = 10.0, 6.6, 3.6 Hz, 1H), 4.27 – 4.15 (m, 3H), 3.52 (dd, *J* = 7.6, 3.7 Hz, 1H), 3.39 (dd, *J* = 13.2, 3.3 Hz, 1H), 3.30 (td, *J* = 6.8, 2.4 Hz, 2H), 2.74 (dd, *J* = 13.2, 10.1 Hz, 1H), 2.44 (s, 1H), 1.95 (ddt, *J* = 13.5, 10.1, 7.8 Hz, 1H), 1.81 – 1.57 (m, 4H), 1.50 – 1.40 (m, 2H), 1.02 (d, *J* = 6.6 Hz, 3H), 0.96 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 176.6, 153.2, 135.2, 129.4, 129.0, 127.5, 77.2, 66.1, 55.7, 51.2, 44.9, 38.0, 31.4, 29.2, 25.7, 24.6, 19.2, 18.7.

HRMS-ESI m/z calcd for $C_{20}H_{28}N_4NaO_4^+$ $[M + Na]^+$ 411.2003, found 411.2005.

Preparation of Weinreb amide **58**



SI-22 **58**

30% H_2O_2 (5.5 mL, 53.4 mmol, 5 equiv), followed by LiOH (1.34 g, 32.0 mmol, 3 equiv), was added to a solution of alcohol **SI-22** (4.15 g, 10.7 mmol, 1 equiv) in THF-H_2O (8.0 mL- 2.6 mL) at 0 ºC. The resulting white suspension was warmed to 23 °C slowly. After 3 h, THF was concentrated under vacuum, and water (100 mL) and EtOAc (100 mL) were added. The resulting biphasic solution was transferred to a separate funnel, and the layers were separated. The aqueous layer was washed with EtOAc (3 x 50 mL), and the resulting organic layer was discarded. The aqueous layer was then acidified by 2 M HCl to pH = 2. The resulting biphasic solution was transferred to a separate funnel, and the layers were separated. The aqueous layer was abstracted with EtOAc (3 × 50 mL), and the combined organic layer was washed with water (100 mL) and brine (100 mL). The washed solution was dried (Na_2SO_4), and the dried solution was concentrated under vacuum. The resulting crude acid was used for the next step without further purification.

Crude acid (2.01 g, 8.77 mmol, 1 equiv), $^{i}Pr_2EtN$ (6.1 mL, 35.1 mmol, 4 equiv), Weinreb amine (1.71 g, 17.5 mmol, 2 equiv), and DCM (88 mL) were added to an oven-dried 250-mL round-bottom flask. HATU (4.00 g, 10.5 mmol, 1.2 equiv) was added in one portion to the resulting colorless solution at 23 ºC. After 3 h, the mixture was transferred to a separatory funnel and washed with water (2 × 50 mL) and brine (50 mL). The washed solution was dried (Na_2SO_4), the dried solution was filtered. The filtrate was concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:4) to Weinreb amide **58** (2.22 g, 88% over two steps) as a colorless oil.

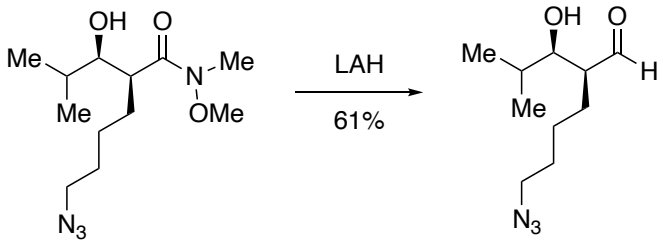
TLC (EtOAc:hexanes = 1:2): R_f = 0.40 (UV).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.66 (dd, *J* = 15.3, 10.0 Hz, 1H), 5.88 – 5.74 (m, 2H), 4.12 (dd, *J* = 5.3, 2.6 Hz, 2H), 3.33 – 3.16 (m, 3H), 2.25 (h, *J* = 2.9 Hz, 2H), 2.06 (s, 1H), 1.76 (ddtd, *J* = 33.9, 13.5, 7.3, 3.4 Hz, 2H), 1.65 – 1.44 (m, 2H), 1.44 – 1.28 (m, 2H), 1.28 – 1.13 (m, 1H), 0.92 (d, *J* = 6.9 Hz, 3H), 0.84 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 177.6, 77.6, 77.4, 61.7, 51.4, 41.7, 32.1, 30.9, 29.3, 25.1, 19.3, 19.3.

HRMS-ESI m/z calcd for $C_{12}H_{25}N_4O_3^+$ $[M + H]^+$ 273.1921, found 273.1922.

Preparation of aldehyde **SI-26**



58 **SI-26**

A 250-mL round-bottom flask containing Weinreb amide **58** (2.22 g, 8.15 mmol, 1 equiv) was evacuated and flushed with nitrogen (the process of nitrogen exchange was repeated a total of 3 times). Dry THF (54 mL) was added, and the resulting clear solution was cooled to -78 ºC by means of a dry ice/acetone bath. A solution of LAH in THF (2.0 M, 20.4 mL, 40.7 mmol, 5 equiv) was added dropwise to this solution. After 4 h, EtOAc (10 mL) was carefully added (CAUTION: Gas evolution!), followed by dropwise addition of saturated aqueous potassium sodium tartrate solution (50 ml). The mixture was allowed to warm to 23 ºC. After 1 h, the biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with water (2 × 50 mL) and brine (50 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:10 to 1:3) to afford aldehyde **SI-26** (852 mg, 49%) as a light yellow

oil. The reaction does not go to completion, so Weinreb amide **58** (410.9 mg, 19%) was recovered during purification and resubmitted to afford an overall yield of aldehyde **SI-26** (1.06 g, 61%).

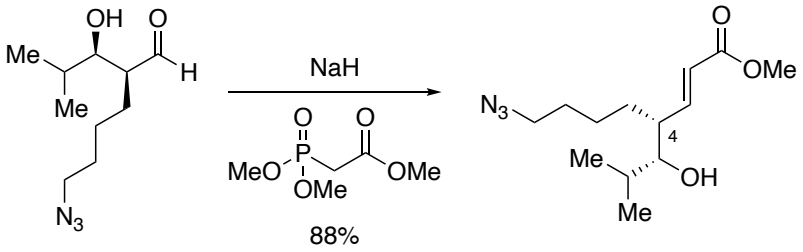
TLC (EtOAc:hexanes = 1:4): R_f = 0.24 (p-anisaldehyde).

^{1}H NMR (300 MHz, $CDCl_3$) δ 9.74 (s, 1H), 3.70 (dt, *J* = 7.2, 4.7 Hz, 1H), 3.29 (td, *J* = 6.3, 2.5 Hz, 3H), 2.52 – 2.41 (m, 1H), 1.75 (q, *J* = 4.4 Hz, 2H), 1.64 – 1.58 (m, 3H), 1.46 – 1.34 (m, 2H), 0.99 (d, *J* = 6.6 Hz, 3H), 0.93 (dd, *J* = 6.8, 3.0 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 204.9, 75.8, 54.9, 51.3, 31.4, 29.3, 25.2, 23.5, 19.5, 17.9.

HRMS-ESI m/z calcd for $C_{10}H_{19}N_3NaO_2^+$ $[M + Na]^+$ 236.1370, found 236.1370.

Preparation of methyl ester **SI-23**



SI-26 **SI-23**

An oven-dried 500-mL round-bottom flask containing 60% NaH (663 mg, 16.6 mmol, 4 equiv) was evacuated and flushed with nitrogen (the process of nitrogen exchange was repeated a total of 3 times). Dry THF (280 mL) was added, and the resulting suspension was cooled to 0 ºC by means of an ice/water bath. Trimethyl phosphonoacetate (2.68 mL, 16.6 mmol, 4 equiv) was added dropwise at 0 ºC. After 1 h, a solution of aldehyde **SI-26** in THF (2 mL) was added. After 2 h, saturated aqueous ammonium chloride solution (100 mL) was carefully added, and the resulting biphasic mixture was transferred to a separatory funnel. The layers were separated, and the aqueous layer was extracted with diethyl ether (2 × 50 mL). The combined organic layers were washed with water (2 × 50 mL) and brine (100 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting residue

was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:15) to afford methyl ester **SI-23** (984 mg, 88% yield) as a colorless oil.

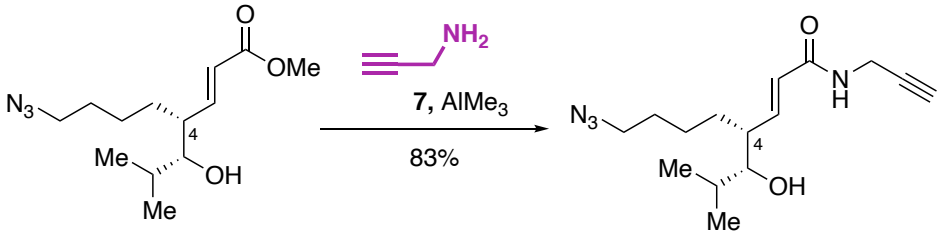
TLC (EtOAc:hexanes = 1:4): R_f = 0.32 (UV, p-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.74 (dd, *J* = 15.7, 10.1 Hz, 1H), 5.85 (dd, *J* = 15.7, 0.7 Hz, 1H), 3.74 (s, 3H), 3.30 (dd, *J* = 7.6, 4.1 Hz, 1H), 3.25 (td, *J* = 6.9, 3.4 Hz, 2H), 2.28 (tdd, *J* = 10.1, 7.4, 3.0 Hz, 1H), 1.82 (dddt, *J* = 13.2, 10.3, 6.8, 3.0 Hz, 1H), 1.70 (dqd, *J* = 9.3, 6.7, 3.3 Hz, 1H), 1.62 – 1.51 (m, 3H), 1.42 – 1.30 (m, 2H), 1.28 – 1.18 (m, 1H), 0.94 (d, *J* = 6.9 Hz, 3H), 0.85 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.9, 150.0, 122.4, 78.4, 51.7, 51.4, 46.9, 31.0, 29.3, 29.0, 24.6, 20.2, 15.2.

HRMS-ESI m/z calcd for $C_{13}H_{24}N_3O_3^+$ $[M + H]^+$ 270.1812, found 270.1813.

Preparation of alkyne **SI-24**



SI-23 **SI-24**

Propargylamine **7** (0.82 mL, 12.7 mmol, 4 equiv) and dry DCM (21 mL) were added to a 100-mL round-bottom flask under nitrogen. The resulting colorless solution was cooled to 0 ºC by means of an ice/water bath. A solution of $AlMe_3$ in heptane (2 M, 6.4 mL, 12.7 mmol, 4 equiv) was added dropwise over 30 min (CAUTION: Gas evolution!). The mixture was allowed to warm to 23 ºC. After 30 min, a solution of methyl ester **SI-23** (860 mg, 2.19 mmol, 1 equiv) in DCM (4 mL) was added over 10 min (CAUTION: Gas evolution!). The vessel was equipped with a reflux condenser, and the solution was brought to reflux by means of a 50 ºC oil bath. After 3 h, the mixture was cooled to 0 °C by means of an ice/water bath, and

MeOH (10 mL) was added dropwise (CAUTION: Gas evolution!). Once gas evolution ceased, saturated aqueous potassium sodium tartrate solution (50 mL) was added. After 1 h, the biphasic mixture was transferred to a separatory funnel, and the layers were separated. The aqueous layer was extracted with DCM (2 × 30 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), and the washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:2) to afford amide **SI-24** (776 mg, 83% yield) as a yellow oil.

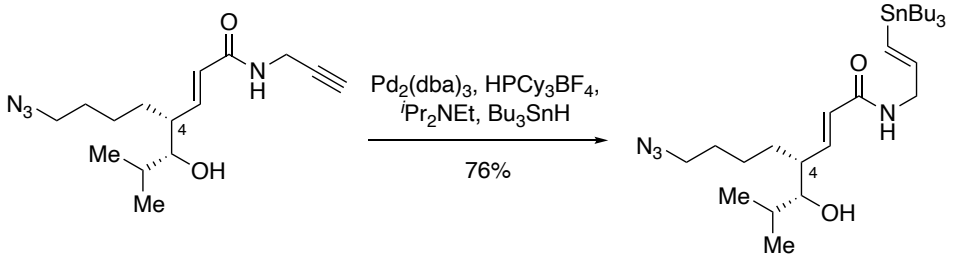
TLC (EtOAc:hexanes = 1:1): R_f = 0.18 (UV, p-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.68 (dd, *J* = 15.3, 10.0 Hz, 1H), 5.91 – 5.77 (m, 2H), 4.14 (dd, *J* = 5.3, 2.6 Hz, 2H), 3.36 – 3.19 (m, 3H), 2.27 (h, *J* = 2.9 Hz, 2H), 2.09 (s, 1H), 1.78 (ddtd, *J* = 33.9, 13.5, 7.3, 3.4 Hz, 2H), 1.67 – 1.47 (m, 2H), 1.47 – 1.31 (m, 2H), 1.31 – 1.15 (m, 1H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.86 (d, *J* = 6.7 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 146.5, 124.1, 79.5, 78.5, 71.9, 51.4, 46.7, 30.9, 29.4, 29.2, 29.0, 24.6, 20.2, 15.4.

HRMS-ESI m/z calcd for $C_{15}H_{24}N_4O_3^+$ $[M + H]^+$ 293.1972, found 293.1976.

Preparation of vinyl tin **SI-25**



SI-24 **SI-25**

An oven-dried 100-mL round-bottom flask containing $Pd_2(dba)_3$ (19.7 mg, 21.5 μmol, 0.01 equiv), tricyclohexylphosphonium tetrafluoroborate (31.7 mg, 86.2 μmol, 0.04 equiv), and alkyne **SI-24** (0.63 g,

2.15 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Dry DCM (18 mL) and $^{i}Pr_2EtN$ (75 µL, 0.43 mmol, 0.2 equiv) were added, resulting in a red solution. After 10 min, the vessel and its contents were cooled to 0 ºC by means of an ice/water bath. tributylstannane (0.70 mL, 2.59 mmol, 1.2 equiv) was added dropwise via syringe pump over 15 min. After 4 h, the vessel was removed from the cooling bath and DCM was concentrated under vacuum. Diethyl ether (50 mL) was added, and the mixture was filtered through a pad of celite. The filter cake was washed with diethyl ether (2 × 20 mL). The combined filtrates were concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: TEA:EtOAc:hexanes = 0.01:0:1 to 0.01:1:8) to afford vinyl tin **SI-25** (0.96 g, 76% yield) as a light-yellow oil.

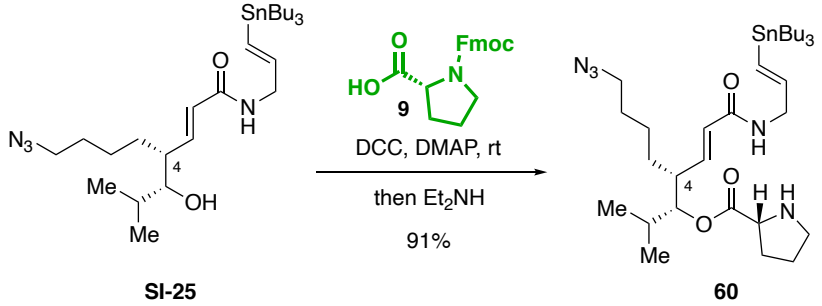
TLC (EtOAc:hexanes = 1:2): R_f = 0.18 (UV, p-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.64 (dd, *J* = 15.3, 10.0 Hz, 1H), 6.13 (dt, *J* = 19.0, 1.5 Hz, 1H), 5.98 (dt, *J* = 19.0, 5.1 Hz, 1H), 5.81 (d, *J* = 15.3 Hz, 1H), 5.51 (t, *J* = 5.7 Hz, 1H), 4.01 (ddd, *J* = 6.5, 5.3, 1.5 Hz, 2H), 3.35 – 3.16 (m, 3H), 2.26 (tdd, *J* = 10.4, 7.6, 3.1 Hz, 1H), 1.86 – 1.69 (m, 2H), 1.62 – 1.55 (m, 3H), 1.52 – 1.43 (m, 6H), 1.36 – 1.24 (m, 9H), 0.95 – 0.83 (m, 21H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 165.2, 145.5, 143.4, 130.8, 124.9, 78.6, 51.5, 46.7, 45.1, 30.8, 29.4, 29.2, 29.1, 27.4, 24.7, 20.2, 15.2, 13.8, 9.6.

HRMS-ESI m/z calcd for $C_{27}H_{53}N_4O_2Sn^+$ $[M + H]^+$ 585.3185, found 585.3191.

Preparation of proline ester **60**



Fmoc-D-Pro-OH **9** (251 mg, 0.75 mmol, 1.35 equiv), DMAP (13.5 mg, 0.11 mmol, 0.2 equiv) and alcohol **SI-25** (322 mg, 0.55 mmol, 1 equiv) were added to a 25-mL round-bottom flask. DCM (5.5 mL) was added, resulting in a colorless solution. DCC (0.21 mL, 0.83 mmol, 1.5 equiv) was added in one portion. resulting in a white suspension. After 5 h, alcohol **SI-25** was entirely consumed as indicated by TLC analysis (eluent: EtOAc:hexanes = 1:2), and diethylamine (2.8 mL) was added. After, 3 h, the mixture was filtered through a pad of celite, and the filter cake was washed with DCM (2 × 20 mL). The combined filtrates were concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: NH_4OH:MeOH:DCM = 0.2:1:100) to afford left half **60** (341 mg, 91% yield) as a yellow oil.

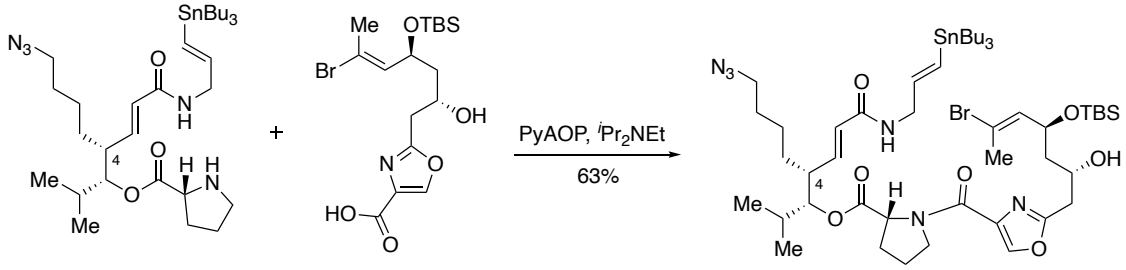
TLC (MeOH:DCM = 1:6): R_f = 0.46 (UV, p-anisaldehyde).

^{1}H NMR (300 MHz, $CDCl_3$) δ 6.56 (dd, *J* = 15.2, 9.8 Hz, 1H), 6.13 (d, *J* = 18.8 Hz, 1H), 5.97 (dt, *J* = 19.2, 5.0 Hz, 1H), 5.84 (d, *J* = 15.3 Hz, 1H), 5.57 (t, *J* = 5.8 Hz, 1H), 4.86 (dd, *J* = 8.2, 4.1 Hz, 1H), 3.99 (t, *J* = 5.3 Hz, 2H), 3.83 (dd, *J* = 8.4, 5.4 Hz, 1H), 3.23 (dt, *J* = 8.5, 6.6 Hz, 2H), 3.12 (dt, *J* = 10.0, 6.5 Hz, 1H), 2.96 (dt, *J* = 10.1, 6.5 Hz, 1H), 2.72 (s, 1H), 2.53 – 2.33 (m, 1H), 2.18 (dq, *J* = 12.0, 7.8 Hz, 1H), 1.94 – 1.72 (m, 4H), 1.62 – 1.41 (m, 9H), 1.28 (dt, *J* = 14.4, 7.3 Hz, 9H), 0.95 – 0.79 (m, 21H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 174.9, 164.8, 143.4, 143.2, 130.7, 125.6, 79.7, 59.9, 51.2, 46.9, 45.1, 44.7, 30.5, 30.0, 29.3, 29.1, 28.9, 27.3, 25.5, 24.3, 19.9, 15.8, 13.7, 9.5.

HRMS-ESI m/z calcd for $C_{32}H_{60}N_5O_3Sn^+$ $[M + H]^+$ 682.3713, found 682.3719.

Preparation of Stille precursor **SI-27**



60 **31** **SI-27**

Acid **31** (1.12 g, 2.59 mmol 1 equiv), $^{i}Pr_2EtN$ (1.36 mL, 7.76 mmol, 3 equiv), and amine **60** (1.76 g, 2.59 mmol, 1 equiv) were added to a 100-mL round-bottom flask. DCM (26 mL) was added, resulting in a clear, colorless solution. PyAOP (2.02 g, 18.7 mmol, 1.5 equiv) was then added in one portion. After 12 h, the mixture was diluted with DCM (30 mL), and the solution was transferred to a separatory funnel and was washed with water (2 × 50 mL) and brine (50 mL). The washed solution was dried (Na_2SO_4), and the dried solution was filtered. The filtrate was concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: TEA:EtOAc:hexanes = 0.003:1:2) to afford Stille coupling precursor **SI-27** (1.79 g, 63% yield) as a light-yellow oil.

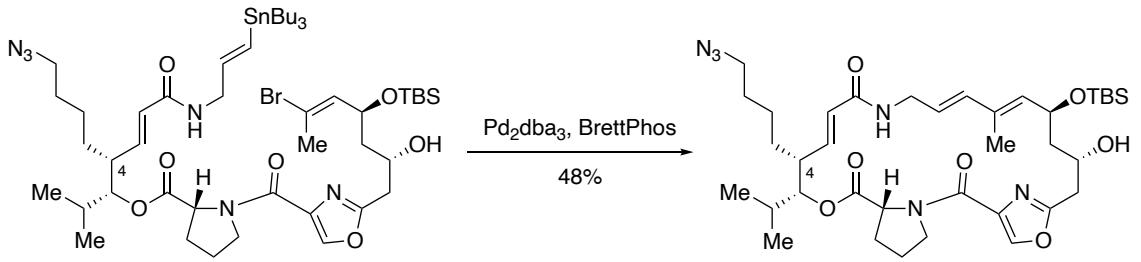
TLC (EtOAc:hexanes = 1:1): R_f = 0.30 (UV, p-anisaldehyde)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.21 – 8.11 (m, 1H), 6.56 – 6.39 (m, 1H), 6.13 (dt, *J* = 19.0, 1.5 Hz, 1H), 6.04 – 5.87 (m, 2H), 5.87 – 5.62 (m, 2H), 4.82 (td, *J* = 6.9, 4.8 Hz, 1H), 4.67 (dtd, *J* = 12.2, 5.2, 3.5 Hz, 1H), 4.43 – 4.21 (m, 1H), 4.09 (t, *J* = 6.2 Hz, 1H), 4.03 – 3.87 (m, 2H), 3.81 – 3.63 (m, 2H), 3.30 – 3.06 (m, 2H), 2.94 – 2.82 (m, 2H), 2.45 – 2.35 (m, 1H), 2.35 – 2.18 (m, 4H), 2.16 – 2.06 (m, 1H), 2.06 – 1.93 (m, 2H), 1.93 – 1.80 (m, 1H), 1.76 – 1.58 (m, 4H), 1.57 – 1.41 (m, 7H), 1.36 – 1.23 (m, 10H), 1.02 – 0.71 (m, 30H), 0.16 – 0.02 (m, 6H).

^{13}C NMR (100 MHz, $CDCl_3$, mixtures of rotamers) δ 172.97, 172.02, 165.30, 164.88, 162.36, 162.29, 160.39, 160.26, 143.71, 143.68, 143.57, 143.40, 143.36, 143.15, 136.89, 136.82, 135.79, 135.40, 130.73, 130.58, 126.04, 125.90, 120.61, 120.41, 80.59, 80.19, 67.85, 67.70, 65.70, 65.26, 61.15, 60.42, 51.30, 51.27, 48.95, 47.45, 45.20, 45.12, 45.03, 44.41, 44.17, 43.61, 36.29, 35.98, 31.76, 30.17, 30.15, 29.83, 29.34, 29.28, 29.22, 29.18, 29.07, 28.98, 28.90, 28.86, 28.80, 27.97, 27.68, 27.52, 27.47, 27.40, 27.13, 26.98, 25.97, 25.94, 25.89, 25.58, 24.59, 24.38, 24.03, 24.01, 21.82, 20.10, 19.87, 18.22, 17.66, 16.93, 16.61, 13.84, 13.75, 9.58, 9.56, 8.85, -4.30, -4.35, -4.76, -5.01.

HRMS-ESI m/z calcd for $C_{49}H_{86}BrN_6O_7SiSn^+$ $[M + H]^+$ 1097.4527 found 1097.4528.

Preparation of Stille product **61**



SI-27 **61**

An oven-dried 100-mL round-bottom flask containing BrettPhos (23.5 mg, 43.8 μmol, 0.2 equiv), $Pd_2(dba)_3$ (20.0 mg, 21.9 μmol, 0.1 equiv) and Stille coupling precursor **SI-27** (240 mg, 0.22 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Dry toluene (44 mL) was added, resulting in a red suspension. A stream of argon was passed through the solution by a stainless-steel needle for 30 min. The mixture was heated by means of a 50 ºC oil bath. After 12 h, **SI-27** was entirely consumed as indicated by TLC analysis (eluent: EtOAc:hexanes = 1:1), and the mixture was allowed to cool to 23 ºC. The mixture was concentrated, and the resulting crude residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes =1:1) to afford Stille coupling product **61** (75.5 mg, 48% yield) as a brown solid.

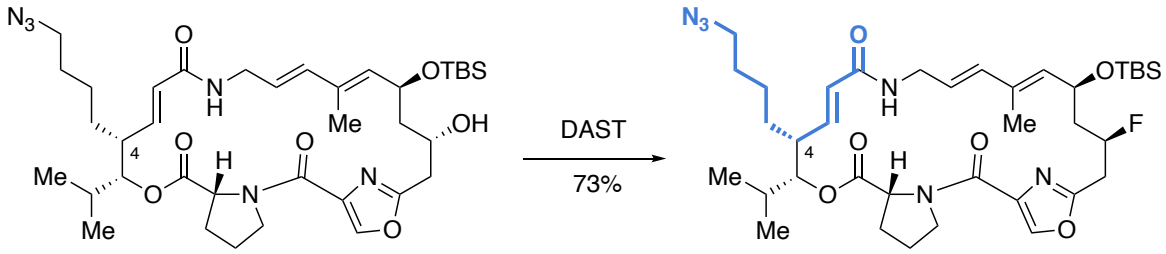
TLC (EtOAc:hexanes = 3:2): R_f = 0.23 (UV, p-anisaldehyde)

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s, 1H), 6.25 – 6.11 (m, 2H), 5.95 (s, 1H), 5.89 – 5.74 (m, 2H), 5.69 (td, *J* = 10.7, 5.2 Hz, 1H), 5.02 – 4.92 (m, 1H), 4.70 (ddd, *J* = 20.9, 9.4, 2.7 Hz, 2H), 4.48 (dt, *J* = 33.0, 9.7 Hz, 3H), 3.82 (t, *J* = 6.3 Hz, 2H), 3.29 (t, *J* = 6.7 Hz, 3H), 3.06 (dd, *J* = 16.7, 2.5 Hz, 1H), 2.81 (dd, *J* = 16.7, 10.4 Hz, 1H), 2.53 (s, 1H), 2.33 (d, *J* = 14.0 Hz, 1H), 2.19 – 2.12 (m, 1H), 2.04 – 1.88 (m, 4H), 1.87 – 1.81 (m, 2H), 1.72 (d, *J* = 1.2 Hz, 2H), 1.60 (s, 4H), 1.40 (s, 1H), 1.26 (s, 1H), 1.00 (d, *J* = 6.4 Hz, 2H), 0.94 (d, *J* = 6.7 Hz, 4H), 0.90 (s, 9H), 0.10 (d, *J* = 2.2 Hz, 3H), 0.06 (d, *J* = 7.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 172.4, 167.4, 161.4, 160.5, 143.4, 142.2, 137.8, 136.8, 134.4, 131.8, 125.5, 124.8, 82.6, 70.0, 66.9, 59.4, 51.4, 48.3, 43.0, 42.8, 41.9, 35.2, 29.4, 29.2, 28.4, 25.9, 25.8, 25.8, 25.0, 24.9, 20.1, 18.9, 18.1, 12.6, -3.4, -4.3, -5.1.

HRMS-ESI m/z calcd for $C_{37}H_{59}N_6O_7Si^+$ $[M + H]^+$ 727.4209, found 727.4211.

Preparation of fluorinated compound **62**



61 **62**

An oven-dried 100-mL round-bottom flask containing Stille product **61** (490 mg, 0.68 mmol, 1 equiv) was evacuated and flushed with nitrogen (this process was repeated a total of 3 times). Dry DCM (34 mL) was added, and the resulting colorless solution was cooled to -78 ºC by means of a dry ice/acetone bath. DAST (0.23 mL, 1.69 mmol, 2.5 equiv) was added dropwise, and the vessel and its contents were warmed to 0 ºC by means of an ice/water bath. After 3 h, saturated aqueous $NaHCO_3$ solution (20 mL) was added. After stirring for 30 min, the biphasic mixture was transferred to a separatory funnel, the layers were separated. The organic layer was washed with water (25 mL) and brine (25 mL), and the washed solution was dried (Na_2SO_4). The dried solution was concentrated, and the residue was purified by flash chromatography (silica gel, eluent: EtOAc:hexanes = 1:2) to afford fluorinated product **62** (360 mg, 73% yield) as a white solid.

TLC (EtOAc:hexanes = 1:1): R_f = 0.28 (UV, *p*-anisaldehyde).

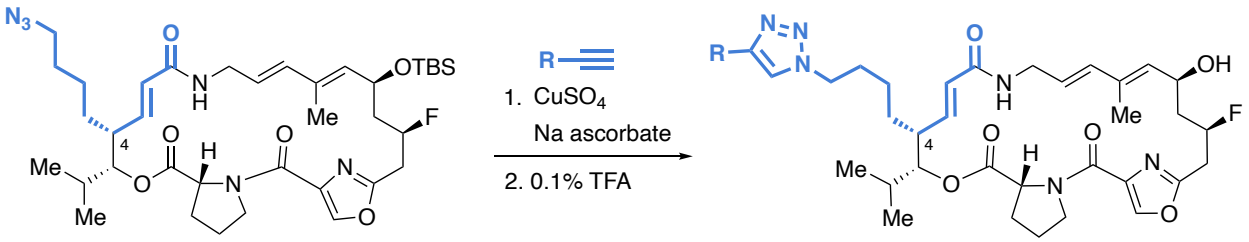
^{1}H NMR (400 MHz, $CDCl_3$) δ 8.06 (s, 1H), 6.41 (dd, J = 16.1, 6.1 Hz, 1H), 6.16 (d, J = 15.7 Hz, 1H), 6.03 (dd, J = 8.8, 3.2 Hz, 1H), 5.87 (dd, J = 16.1, 1.5 Hz, 1H), 5.67 (ddd, J = 15.6, 8.4, 4.3 Hz, 1H), 5.29 (d, J =

9.0 Hz, 1H), 5.17 – 4.89 (m, 1H), 4.82 (td, *J* = 9.5, 2.8 Hz, 2H), 4.72 (ddd, *J* = 10.2, 8.9, 3.8 Hz, 1H), 4.57 – 4.48 (m, 1H), 4.07 (dddd, *J* = 11.9, 8.7, 5.5, 2.4 Hz, 1H), 3.85 (dt, *J* = 11.3, 7.1 Hz, 1H), 3.46 (ddd, *J* = 15.5, 8.4, 3.2 Hz, 1H), 3.27 (td, *J* = 6.6, 1.4 Hz, 2H), 3.15 (td, *J* = 16.7, 6.3 Hz, 1H), 2.91 (ddd, *J* = 20.3, 16.4, 5.7 Hz, 1H), 2.53 (t, *J* = 8.8 Hz, 1H), 2.21 – 2.11 (m, 2H), 1.99 – 1.91 (m, 3H), 1.77 (d, *J* = 1.2 Hz, 3H), 1.64 – 1.53 (m, 5H), 1.38 (s, 1H), 1.29 – 1.24 (m, 2H), 0.94 (dd, *J* = 11.3, 6.6 Hz, 6H), 0.86 (s, 9H), 0.04 (s, 3H), 0.01 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.6, 166.3, 160.7, 160.2, 160.1, 143.3, 143.1, 136.8, 136.5, 134.8, 133.5, 125.4, 124.6, 89.2 (d, *J* = 169.9 Hz), 82.1, 66.7, 59.2, 51.4, 48.7, 43.7 (d, *J* = 20.4 Hz), 42.1, 41.3, 36.8, 34.0 (d, *J* = 25.1 Hz), 29.5, 29.2, 28.4, 25.9, 25.0, 24.9, 23.5, 19.9, 18.9, 18.2, 13.1, -4.3, -4.8.

HRMS-ESI m/z calcd for $C_{37}H_{57}FN_6NaO_6Si^+$ $[M + Na]^+$ 751.3985, found 751.3986.

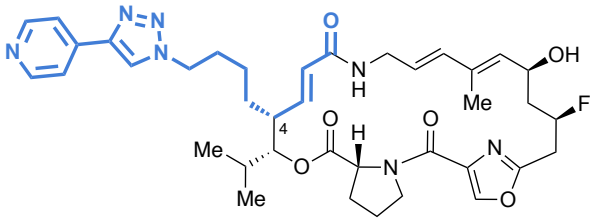
General procedure for preparation of C-4 analogs **63**



62 **63**

An aqueous solution of sodium ascorbate (0.05M, 0.2 equiv) and an aqueous solution of copper(II) sulfate (0.05 M, 0.05 equiv) were added to a suspension of azide compound **62** (1 equiv) and alkyne (3 equiv) in *t*-BuOH-H_2O (3mL-1mL) at 23°C. After stirring at 23°C for 12 h, the solvent was removed under vacuum. The residue was purified by flash chromatography over silica gel (0→100% hexanes in ethyl acetate) to afford the product as an off white solid. Then the white solid was dissolved with 0.1% TFA in MeCN-H_2O (95/5 v/v), and the solution was stirred for 12 hours at 23°C. The reaction mixture was concentrated, and the resulting crude residue was purified by flash chromatography over silica gel (0→10% methanol in dichloromethane) to afford C-4 modified analog **63**.

Analog **63a** (SA1103066)



Prepared according to general procedure of C-4 analogs 63. Analog **63a** (5.8 mg, 69% yield over 2 steps) was obtained as a light yellow solid.

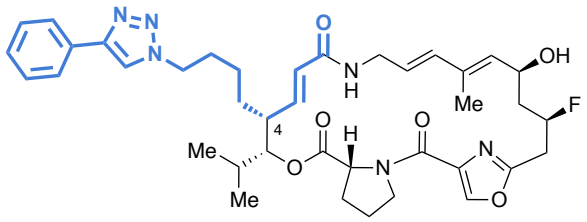
TLC (MeOH:DCM = 1:40): R_f = 0.10 (UV, *p*-anisaldehyde).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.67 (d, *J* = 5.3 Hz, 2H), 8.08 (s, 1H), 8.00 (s, 1H), 7.88 – 7.73 (m, 2H), 6.42 (dd, *J* = 16.0, 6.2 Hz, 1H), 6.27 – 6.08 (m, 1H), 5.88 (dd, *J* = 16.1, 1.3 Hz, 1H), 5.72 (ddd, *J* = 15.7, 7.8, 4.1 Hz, 1H), 5.39 – 5.21 (m, 1H), 5.12 (d, *J* = 6.7 Hz, 1H), 4.96 (d, *J* = 7.3 Hz, 1H), 4.79 (tt, *J* = 8.9, 4.7 Hz, 2H), 4.45 (t, *J* = 7.0 Hz, 3H), 4.12 – 3.97 (m, 1H), 3.92 – 3.74 (m, 1H), 3.61 – 3.45 (m, 1H), 3.21 (td, *J* = 17.0, 5.6 Hz, 1H), 2.98 (td, *J* = 16.5, 6.7 Hz, 1H), 2.52 (s, 1H), 2.34 (t, *J* = 7.5 Hz, 1H), 2.27 – 2.08 (m, 3H), 2.03 – 1.89 (m, 4H), 1.81 (d, *J* = 1.1 Hz, 3H), 1.73 (d, *J* = 5.6 Hz, 1H), 1.69 – 1.57 (m, 2H), 1.56 – 1.42 (m, 3H), 1.39 (s, 1H), 0.98 – 0.92 (m, 3H), 0.89 (d, *J* = 7.6 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 166.2, 160.6, 160.0, 159.9, 150.5, 145.5, 143.3, 143.0, 138.2, 136.9, 136.0, 135.7, 133.2, 125.5, 125.4, 121.3, 120.1, 89.3 (d, *J* = 171.2 Hz), 82.0, 65.9, 59.3, 50.4, 48.7, 42.4 (d, *J* = 20.8 Hz), 42.2, 33.9 (d, *J* = 26.0 Hz), 33.7, 30.4, 29.8, 29.5, 28.5, 25.4, 25.1, 24.5, 19.9, 19.0, 13.2.

HRMS-ESI m/z calcd for $C_{38}H_{49}FN_7O_6^+$ $[M + H]^+$ 718.3723, found 718.3733.

Analog **63b** (SA1103067)



Prepared according to general procedure of C-4 analogs 63. Analog **63b** (6.4 mg, 80% yield over 2 steps) was obtained as a white solid.

TLC (MeOH:DCM = 1:40): R_f = 0.15 (UV, *p*-anisaldehyde).

^{1}H NMR (300 MHz, $CDCl_3$) δ8.08 (s, 1H), 7.83 (dt, *J* = 6.2, 1.4 Hz, 2H), 7.77 (s, 1H), 7.47 – 7.39 (m, 2H), 7.37 – 7.30 (m, 1H), 6.43 (dd, *J* = 16.0, 6.2 Hz, 1H), 6.27 – 6.08 (m, 2H), 5.91 – 5.76 (m, 1H), 5.76 – 5.63 (m, 1H), 5.35 (d, *J* = 8.8 Hz, 1H), 5.11 (s, 1H), 4.98 (d, *J* = 6.8 Hz, 1H), 4.79 (ddd, *J* = 11.8, 6.8, 2.7 Hz, 2H), 4.43 (t, *J* = 7.0 Hz, 2H), 4.38 – 4.27 (m, 1H), 4.04 (ddd, *J* = 12.6, 7.8, 4.6 Hz, 1H), 3.83 (dt, *J* = 11.3, 7.3 Hz, 1H), 3.59 (ddd, *J* = 15.7, 8.1, 4.2 Hz, 1H), 3.21 (td, *J* = 17.1, 5.5 Hz, 1H), 2.99 (td, *J* = 16.5, 6.6 Hz, 1H), 2.51 (s, 1H), 2.33 (t, *J* = 7.5 Hz, 1H), 2.18 (ddd, *J* = 21.0, 10.1, 6.7 Hz, 3H), 1.99 – 1.91 (m, 4H), 1.82 (d, *J* = 1.2 Hz, 3H), 1.64 – 1.57 (m, 3H), 1.53 – 1.47 (m, 2H), 1.39 (s, 1H), 0.98 – 0.92 (m, 3H), 0.92 – 0.89 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 166.1, 160.6, 159.8, 148.1, 143.4, 143.1, 136.9, 136.1, 135.8, 133.1, 130.7, 129.0, 128.4, 125.8, 125.5, 125.4, 119.7, 89.4 (d, *J* = 170.9 Hz), 81.9, 66.0, 59.3, 56.1, 50.1, 48.7, 42.4 (d, *J* = 26.8 Hz), 41.0, 36.8, 33.8 (d, *J* = 26.0 Hz), 30.3, 29.5, 28.5, 25.1, 24.8, 24.3, 23.5, 19.9, 19.0, 13.2.

HRMS-ESI m/z calcd for $C_{38}H_{50}FN_6O_6^+$ $[M + H]^+$ 717.3770, found 717.3779.

1.6 Methods for measuring minimum inhibitory concentrations (MICs)

For Figures 1.6A and 1.10B, and Tables 1.1-1.3, compounds were evaluated by Microbiologics got Minimum Inhibitory Concentration (MIC) activity using the broth microdilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI).[39,40] Pre-weighed vials of the test agents were stored at -20°C until testing. On the day of the assay, the compounds were dissolved in 100% DMSO (Sigma; St. Louis, MO; Lot No. SHBR5703) to a stock concentration of 6,464 μg/mL. All stock solutions were held for at least 1 hour to auto-sterilize. The concentration range tested for each compound was 64-0.064 μg/mL. For more details on test organisms, media, and methods, see below.

Test Organisms

Test organisms were either reference strains from the American Type Culture Collection (ATCC; Manassas, VA) or clinical isolates from the Microbiologics repository (MMX; Kalamazoo, MI). Upon receipt at Microbiologics the isolates were streaked under suitable conditions onto agar medium appropriate to each organism. The bacterial isolates were incubated for 18 to 48 hr at 35°C. Colonies harvested from these growth plates were resuspended in the appropriate medium containing a cryoprotectant. Aliquots of each suspension were then frozen at -80°C.

Prior to testing, the isolates were streaked from frozen vials onto Trypticase soy agar (TSA) with 5% sheep blood (Remel; Lenexa, KS; Lot No. 218206) and were incubated for 18 to 24 hr at 35°C. *Haemophilus influenzae* was streaked onto Chocolate Agar (Remel; Lot No. 222918). Additionally, *Streptococcus spp.* and *H. influenzae* were incubated in 5% carbon dioxide.

Test Media

Organisms were tested in the appropriate media according to CLSI guidelines (1, 2). Cation-adjusted Mueller Hinton broth (CAMHB; Becton Dickinson [BD]; Franklin Lakes, NJ; Lot No. 4102223) was used for broth microdilution testing of aerobic isolates except for specialized media described below. CAMHB

was supplemented with 3% laked horse blood (LHB; Hemostat; Dixon, CA; Lot No. 788089) for testing of *Streptococcus* spp. MIC testing of *H. influenzae* was performed in Haemophilus Test Medium Broth (HTM) which was made by supplementing MHB (BD; Lot No. 3150802) with 15 μg/mL nicotinamide adenine dinucleotide (NAD; Sigma; Lot. No. SLBX4629), 15 μg/mL porcine hematin (Sigma; Lot. No. SLCL9885), 5 g/L yeast extract (Sigma; Lot. No. 7179576).

Broth microdilution MIC procedure

MIC values were determined using the broth microdilution procedure described by CLSI (1, 2). Automated liquid handlers (Multidrop 384, Labsystems, Helsinki, Finland; Biomek 3000 and Biomek FX, Beckman Coulter Fullerton CA) were used to conduct serial dilutions and liquid transfers.

All wells in columns 2 through 12 of a standard 96-well microdilution plate (Costar 3795) were filled with 150 μL of the appropriate diluent. Then, 300 μL of the tested agents were added to the wells of column 1 of the plates at 101X, the highest final concentration to be tested. Serial two-fold dilutions were made across the rows through column 11 using the

Biomek 3000. The wells of column 12 contained no drug and served as growth control wells. This plate served as the "mother plate" from which MIC assay plates or "daughter plates" were made.

The daughter plates were loaded with 190 μL of appropriate medium using the Multidrop 384. The daughter plates were then created using the Biomek FX which transferred 2 μL of drug solution from each well of a mother plate to each corresponding well of the daughter plate in a single step.

A standardized inoculum of each test organism was prepared in appropriate medium per CLSI methods (1, 2). The plates were then inoculated with 10 μL of the diluted inoculum using the Biomek 3000 from low to high drug concentration, resulting in a final concentration of approximately 5 x 10^5CFU/mL (bacteria). An un-inoculated plate was incubated for the purpose of assessing solubility of the drug in the test medium.

The plates were stacked 3 to 4 high, covered with a sterile lid on the top plate, and incubated aerobically at 35ºC for 20 hrs. Following incubation, the microplates were removed from the incubator and viewed from the bottom using a plate viewer. For each of the test media and drugs, an un-inoculated solubility control plate was observed for evidence of drug precipitation. The MIC was recorded as the lowest concentration of drug that completely inhibited visible growth of the organism.

Methods for in vitro translation assay 10-μM screen

The ability of group A streptogramin analogs to inhibit the 70S E. coli ribosome was first screened using the PURExpress® In Vitro Protein Synthesis Kit (E6800, NEB), murine RNAse inhibitor (M0314, NEB), and 6.66 ng/μl of template DNA encoding the fluorescent protein mEGFP (extracted by using E.N.Z.A. Plasmid DNA Mini Kit I, omega BIOTEK). The volume of the reaction mixture was scaled down 5-fold from the NEB protocol for a final reaction volume of 5 μL. Analogs were screened at a final concentration of 10 μM in 10% DMSO. Translation reactions were carried out in triplicate at 37 °C for 1 hour, then transferred to a 0 °C metal block. To assist in the transfer of reactions to 96-well half-area Non-Binding Surface (NBS) microplates (Corning 3993) for final measurements, the reaction volume was increased to 50 μL by adding buffer (20 mM Tris-HCl pH 7.5, 60 mM NH_4Cl, 6 mM $MgCl_2$, 0.5 mM EDTA). Using a Cytation 5 plate reader (BioTek), translated mEGFP was excited at 485 nm; its emission was recorded at 535 nm. For comparison of analog activities across multiple initial screens, fluorescence readouts were normalized to the blank. Data were analyzed using Microsoft Excel.

1.7 Methods for CryoEM sample preparation and image reconstruction[8]

Cryo-EM sample preparation: For cryo-EM analysis, purified 50S ribosomes from *E. coli* strain MRE600[41] were prepared in 50 mM HEPES pH 7.5, 150 mM potassium acetate, 6 mM magnesium acetate, and 7 mM fresh β-mercaptoethanol (BME). Inhibitor was added, mixed gently, and incubated on ice for 1 h. The final concentration of ribosomes was 100 nM; the final concentration of each inhibitor was 60 μM. For samples prepared with two inhibitors, both were added in a 1:1 ratio. For each grid (Quantifoil holey carbon grids, C2-C14nCu30-01 or N1-C14nCu40-01, Quantifoil Micro Tools Gmbh), 3.5 μl of sample was deposited onto a freshly glow-discharged (EMS-100 Glow Discharge System, Electron Microscopy Sciences, 30 s at 15 mA) grid and incubated for 30 s at 25 °C and 100% humidity. Grids were vitrified by plunge-freezing into liquid ethane[42] using a FEI Vitrobot Mark IV (ThermoFisher). To achieve optimal ice quality for collection, liquid was blotted from the grid using Whatman #1 filter paper and multiple grids for each sample were frozen with a range of different blotting times. Grids were screened using a FEI Talos Arctica electron microscope (ThermoFisher, operating at 200 kV, located at UCSF) to check ice quality and identify the optimum grids for data collection.

Cryo-EM data collection: All datasets were collected on FEI Titan Krios electron microscopes (ThermoFisher, operating at 300 kV, located at UCSF or NCCAT). Automated data collection at UCSF was facilitated by SerialEM (v3.6)[43]; collection at NCCAT was via Leginon (v.3.4) Data sets were collected on a K3 (Gatan) Direct Electron Detector (DED) with a Gatan Imaging Filter (Gatan, 20 eV slit) using a nine-shot beam-image shift approach with coma compensation[44]. Pixel sizes, number of images in dose-fractionated micrographs, dose rates, and defocus ranges varied slightly. All image stacks were collected in super-resolution mode.

Cryo-EM image and data processing: Super-resolution image stacks were binned by a factor of 2, corrected for beam-induced motion, and dose-weighted using MotionCor2 (v.1.2.1)[45]. All Coulomb potential density maps were reconstructed in cisTEM (1.0.0-beta)[46] using dose-weighted micrographs. Initial CTF parameters were determined using CTFFIND4, included as part of the cisTEM package, with

the resolution range between 30 and 4 Å included in the fitting. Bad micrographs (crystalline ice, poor CTF fits) were excluded from processing through visual inspection. Particles were picked in cisTEM by matching to a soft-edged disk template with a maximum particle radius of 110 Å and a characteristic particle radius of 90 Å. CisTEM refinement packages were made using a particle molecular weight of 1,800 kDa. Particles were 2D-classified into 50 classes with a mask radius of 150 Å. Classes containing the 50S ribosome were carried forward into single-class auto refinement with an outer mask radius of 125 Å and a default starting resolution of 20 Å. A filtered volume was used to make a binary mask; the volume eraser tool from UCSF Chimera (v.1.12)[47] was used to exclude the mobile L1 stalk from the mask. This mask was used in single-class manual refinement with a final high-resolution limit of either 3.50 or 3.00 Å. Unsharpened maps were used in model refinement and for all figures.

Cryo-EM model building and refinement with OPLS3e: We used UCSF Chimera (v.1.12) to rigid body align a high-resolution X-ray structure of the *E. coli* ribosome (PDB code 4YBB[48]) into our maps. Principle versions of the PHENIX suite used for cryo-EM model building were 1.17, and 1.19. Initially, the ligand restraints files (CIF files) were generated with phenix.eLBOW[49] using the analog's SMILES string and a 'final geometry' reference PDB of the analogue that was derived from the pose of flopristin bound to the *E. coli* ribosome (PDB code 4U20[6]). These ligands were superimposed into 4YBB based on the binding pose of flopristin in 4U20, and manual edits to the surrounding structure were performed in Coot (v.0.8.9.2).

After constructing these initial models, structures were refined using phenix.real_space_refine with the default protocol, initially with CIF restraints files from phenix.eLBOW. These resulted, however, in non-physical high energy conformations of the ligands. To improve the models of the ligands, we used a new version of phenix.real_space_refine interfaced with the OPLS3e/VSGB2.1 force field, a high quality force field for ligands[50]. This approach allows obtaining physics-based energies and gradients for either the whole or part of the structure without resorting to accurate manual CIF restraint generation. Standard PHENIX restraints were used for the macromolecule, while the ligand was governed by the OPLS3e/VSGB2.1 force field. Precisely, the unliganded complex and ligand were individually prepped using phenix.ready_set and

prepwizard, respectively, and subsequently recombined. The recombined complex served as input for refinement using the additional Schrödinger-dependent options use_schrodinger = True maestro_file = ligand.mae schrodinger.selection = “resname LIG”, in which ‘ligand.mae’ describes the ligand structure in Maestro format and LIG is the residue three-letter code, and otherwise default parameters.

The PHENIX-OPLS3e/VSGB2.1 interface works as follows: the PHENIX refinement engine spawns an external process serving as an energy server, initialized with the ligand structure present in the provided maestro_file option. When the refinement engine requests energies and gradients, the ligand’s internal coordinates are written to file and read in by the external server. After updating ligand coordinates on the server side, the energy and gradients are calculated and exchanged with the refinement engine. The refinement engine on its side updates the ligand energy and gradients contribution in its energy function using a default weight factor of 10 for the OPLS3e/VSGB2.1 energies. Refinement with the OPLS3e/VSGB2.1 force field reduced the energy for all ligands compared to the conformations refined using CIF based restraints calculated by phenix.eLBOW.

For all cryo-EM figures, the full, unsharpened density maps and full PDB models were boxed using phenix.map_box with a selection radius of 20 Å around the ligand(s). Boxed map and model were loaded into PyMol (incentive v.2.4.1) with set normalize_ccp4_maps, off. Maps were contoured at 4σ for tight density (dark blue) and 1σ for loose density (light grey), both centered around the ligand with a carve of 1.4.

1.8 Extended library of C4-modified amide linker analogs

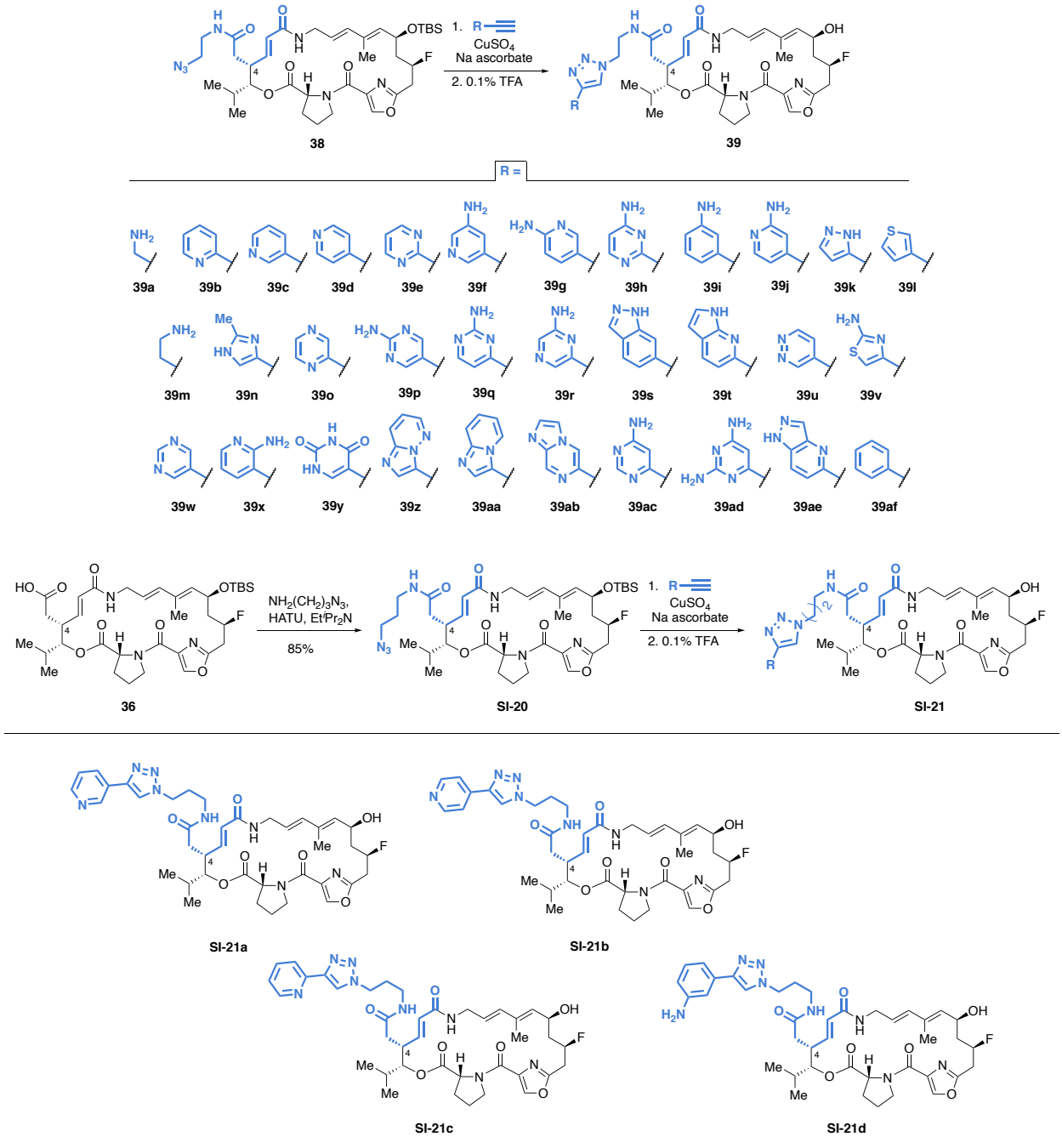


Figure 1.11. Structures of all C-4 modified amide linker analogs synthesized.

1.9 Inhibitory activity of amide linker series against Gram-positive organisms

Table 1.1. Extended MIC table of all synthesized C-4 amide linker analogs against an expanded panel of Gram-positive pathogens.

Minimal Inhibitory Concentration (MIC) Values (μg/mL)

Compound ID	SA Barcode	*E. faecalis* CLSI QC, *In vivo* strain ATCC 29212	*E. faecium* ATCC isolate ATCC 35667	*E. faecium* VREfm (vanA) MMX 0752	*S. aureus* CLSI QC ATCC 29213	*S. aureus* Streptogramin A and B resistance (VatA, Vgb) MMX 10227	*S. aureus* MRSA MMX 2001; ATCC 33591	*S. aureus* MLSb ErmA resistance; inducible MMX 2321	*S. aureus* MLSb ErmA resistance; constitutive MMX 3035	*S. aureus* Linezolid-resistant; cfr MMX 3067	*S. pneumoniae* PSSP; CLS QC ATCC 49619
VM2	SA0106120	>64	4	8a	16	>64	16	32	8	>64	8
flopristin	SA0110272	>64	0.5	2a	0.5a	8	0.5a	1a	0.5	>64	2
39a	SA0113142	>64	64	>64	>64	64	>64	>64	64	>64	64
39b	SA0113143	>64	32	>64	>64	>64	>64	>64	>64	>64	64
39c	SA0113144	>64	64	>64	>64	>64	>64	>64	>64	>64	64
39d	SA0113146	>64	64	>64	>64	>64	>64	>64	>64	>64	64
39e	SA0113147	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
39f	SA0113149	>64	>64	>64	>64	>64	>64	>64	>64	>64	64
39g	SA0113150	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
39h	SA0113152	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
39i	SA0113153	>64	>64	>64	>64	>64	>64	>64	>64	>64	64
39j	SA0113154	>64	>64	>64	>64	>64	>64	>64	>64	>64	64
39k	SA0113156	>64	64	>64	>64	>64	>64	>64	>64	>64	>64
39l	SA0113157	>64	>64	>64	>64	>64	>64	>64	>64	>64	16
39m	SA0113162	>64	64	>64	>64	>64	>64	>64	>64	>64	>64
39n	SA0113165	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
39o	SA0113166	>64	64	>64	>64	>64	>64	>64	>64	>64	64
39p	SA0113167	>64	64	>64	>64	>64	>64	>64	>64	>64	>64
39q	SA0113169	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
39r	SA0113170	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
39s	SA0113171	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
39t	SA0113173	>64	16	>64	>64	>64	>64	>64	>64	>64	>64
39u	SA0113174	>64	64	>64	>64	>64	>64	>64	>64	>64	32
39v	SA0113176	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
39w	SA0113177	>64	64	>64	>64	>64	>64	>64	>64	>64	>64
39x	SA0113178	>64	>64	>64	>64	>64	>64	>64	>64	>64	64
39y	SA0113179	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
39z	SA0113180	>64	>64	>64	>64	>64	>64	>64	>64	>64	64
39aa	SA0113181	>64	>64	>64	>64	>64	>64	>64	>64	>64	64
39ab	SA0113183	>64	>64	>64	>64	>64	>64	>64	>64	>64	64
39ac	SA0113184	>64	16	>64	>64	>64	>64	>64	>64	>64	>64
39ad	SA0113185	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
39ae	SA0113186	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
SI-21a	SA0113193	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
SI-21b	SA0113194	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
SI-21c	SA0113195	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
SI-21d	SA0113197	>64	64	>64	>64	>64	>64	>64	>64	>64	>64

1.10 Inhibitory activity of amide linker series against Gram-negative organisms

Table 1.2. Extended MIC table of all synthesized C-4 amide linker analogs against an expanded panel of Gram-negative pathogens.

Minimal Inhibitory Concentration (MIC) Values (μg/mL)

Compound ID	SA Barcode	*A. baumannii* *In vivo* strain ATCC1 19606	*E. coli* CLSI QC, *In vivo* strain ATCC 25922	*E. coli* TolC efflux defective MMX 121	*E. coli* permeability mutant BAS1314::IMP4213ZAB::TN10 MMX 206	*E. coli* permeability mutant BAS2006, OMP C, OMP F MMX 207	E. coli ΔompC768 KanR insert MMX 9658	*K. pneumoniae* ATCC isolate ATCC 43816	*K. pneumoniae* MMX 8438	*P. aeruginosa* CLSI QC, *In vivo* strain ATCC 27853	*P. aeruginosa* MDR clinical isolate MMX 8799	*H. influenzae* *In vivo* ATCC 49247
VM2	SA0106120	>64	>64	2	2	2	nt	>64	>64	>64	>64	16
flopristin	SA0110272	>64	32	1	≤0.06	0.25	64	>64	>64	>64	>64	0.25
39a	SA0113142	>64	>64	8	8	16	>64	>64	>64	>64	>64	>64
39b	SA0113143	>64	>64	8	32	16	>64	>64	>64	>64	>64	64
39c	SA0113144	>64	>64	8	32	32	>64	>64	>64	>64	>64	>64
39d	SA0113146	>64	>64	8	32	32	>64	>64	>64	>64	>64	>64
39e	SA0113147	>64	>64	16	64	32	>64	>64	>64	>64	>64	>64
39f	SA0113149	>64	>64	16	32	64	>64	>64	>64	>64	>64	>64
39g	SA0113150	>64	>64	16	32	32	>64	>64	>64	>64	>64	>64
39h	SA0113152	>64	>64	16	32	64	>64	>64	>64	>64	>64	>64
39i	SA0113153	>64	>64	8	32	16	>64	64	>64	>64	>64	32
39j	SA0113154	>64	>64	16	64	64	>64	>64	>64	>64	>64	>64
39k	SA0113156	>64	>64	16	32	64	>64	>64	>64	>64	>64	>64
39l	SA0113157	>64	>64	4	8	4	>64	>64	>64	>64	>64	16
39m	SA0113162	>64	>64	16	16	16	>64	>64	>64	>64	>64	>64
39n	SA0113165	>64	>64	32	64	64	>64	>64	>64	>64	>64	>64
39o	SA0113166	>64	>64	16	32	32	>64	>64	>64	>64	>64	>64
39p	SA0113167	>64	>64	32	32	64	>64	>64	>64	>64	>64	>64
39q	SA0113169	>64	>64	16	32	32	>64	>64	>64	>64	>64	>64
39r	SA0113170	>64	>64	32	32	64	>64	>64	>64	>64	>64	>64
39s	SA0113171	>64	>64	32	16	64	>64	>64	>64	>64	>64	>64
39t	SA0113173	>64	>64	16	32	>64	>64	>64	>64	>64	>64	>64
39u	SA0113174	>64	>64	8	16	32	>64	>64	>64	>64	>64	64
39v	SA0113176	>64	>64	32	32	64	>64	>64	>64	>64	>64	>64
39w	SA0113177	>64	>64	32	16	64	>64	>64	>64	>64	>64	>64
39x	SA0113178	>64	>64	16	32	64	>64	>64	>64	>64	>64	64
39y	SA0113179	>64	>64	32	64	>64	>64	>64	>64	>64	>64	>64
39z	SA0113180	>64	>64	8	32	16	>64	>64	>64	>64	>64	>64
39aa	SA0113181	>64	>64	8	32	32	>64	>64	>64	>64	>64	>64
39ab	SA0113183	>64	>64	16	32	64	>64	>64	>64	>64	>64	>64
39ac	SA0113184	>64	>64	32	64	>64	>64	>64	>64	>64	>64	>64
39ad	SA0113185	>64	>64	32	32	64	>64	>64	>64	>64	>64	>64
39ae	SA0113186	>64	>64	16	32	32	>64	>64	>64	>64	>64	>64
flopristin + VS1	SA0113191	>64	32	1	1	≤0.06	32	>64	>64	64	>64	0.5
SI-21a	SA0113193	>64	>64	16	32	64	>64	>64	>64	>64	>64	>64
SI-21b	SA0113194	>64	>64	16	32	32	>64	>64	>64	>64	>64	>64
SI-21c	SA0113195	>64	>64	16	32	>64	>64	>64	>64	>64	>64	>64
SI-21d	SA0113197	>64	>64	8	32	16	>64	>64	>64	>64	>64	64
39a + VS1	SA0113224	>64	>64	16	16	16	>64	>64	>64	>64	>64	>64
39d + VS1	SA0113225	>64	>64	16	16	32	>64	>64	>64	>64	>64	>64

1.11 Extended library of C4-modified alkyl linker analogs

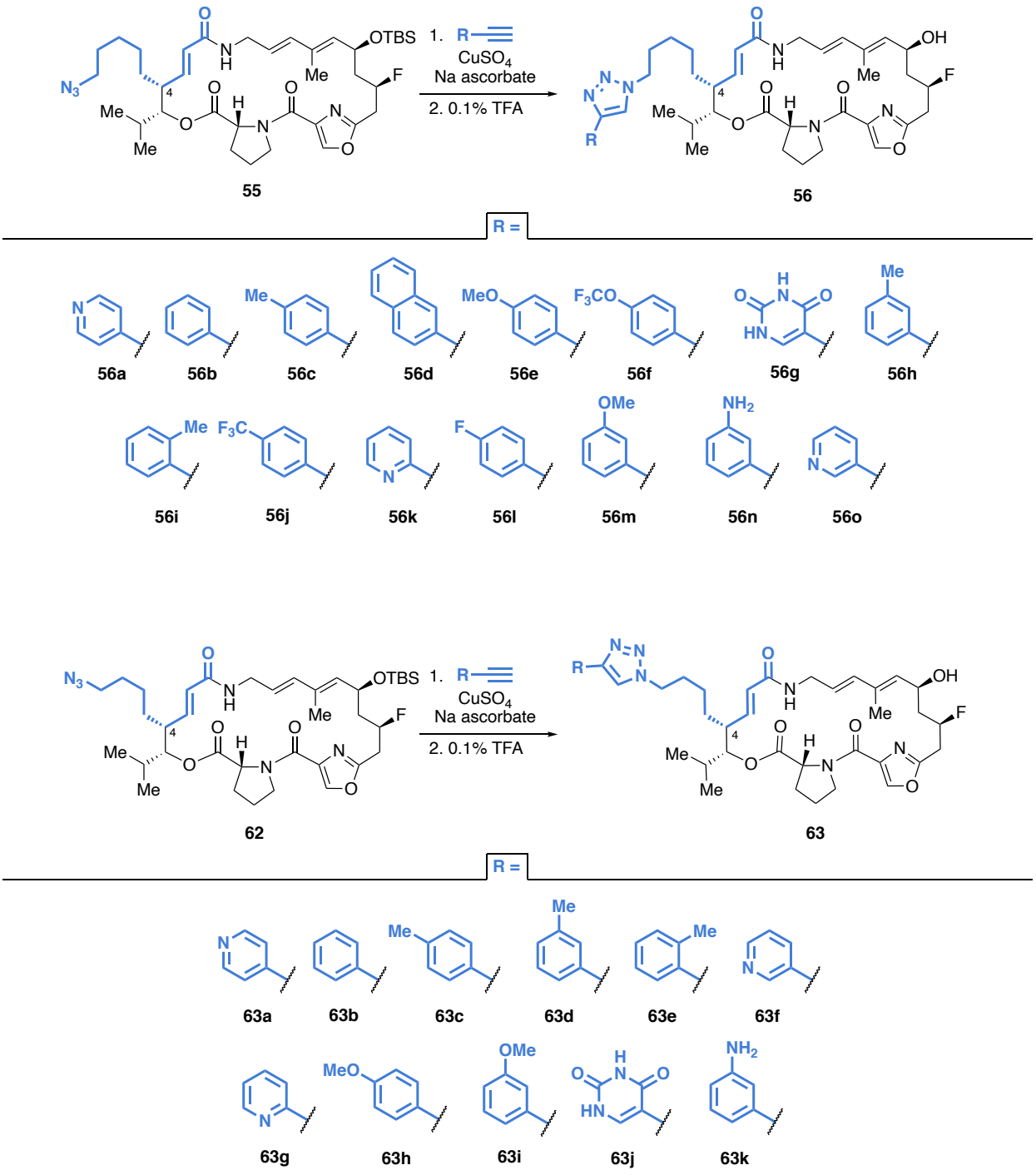
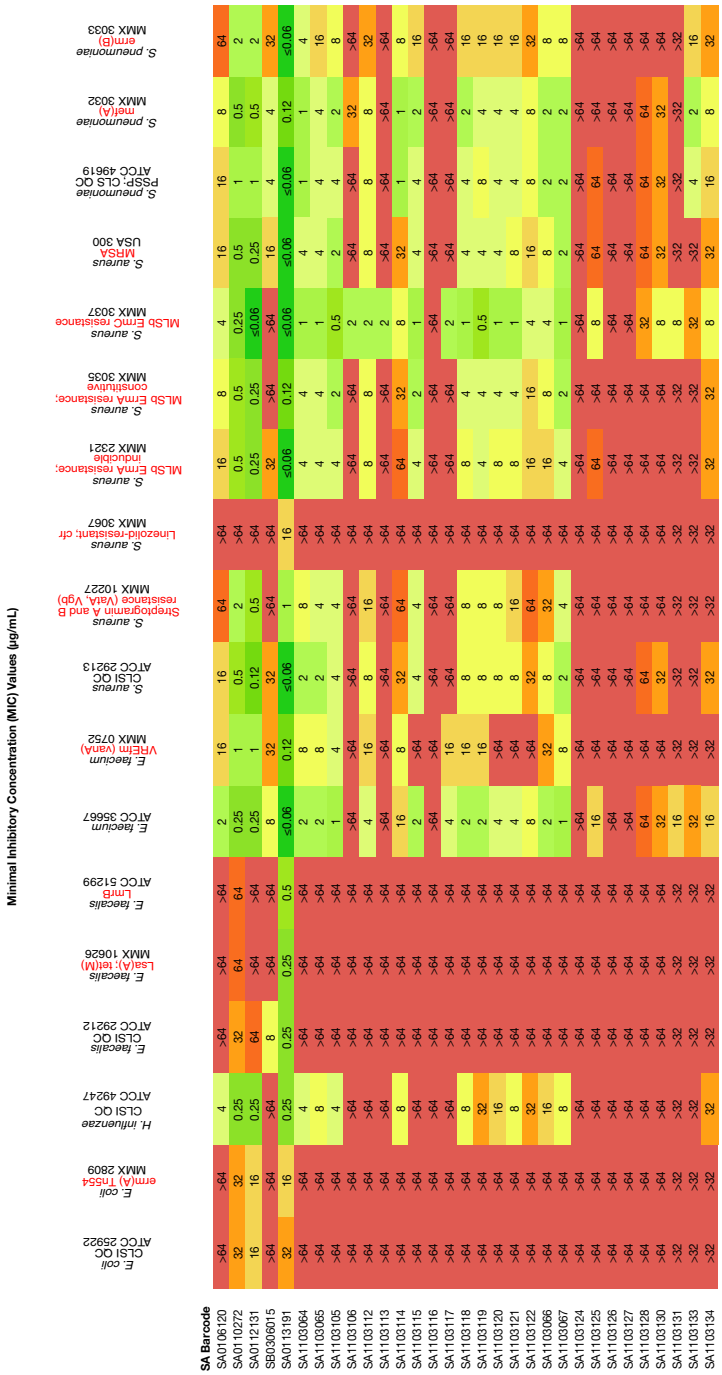


Figure 1.12. Structures of all C-4 modified alkyl linker analogs synthesized.

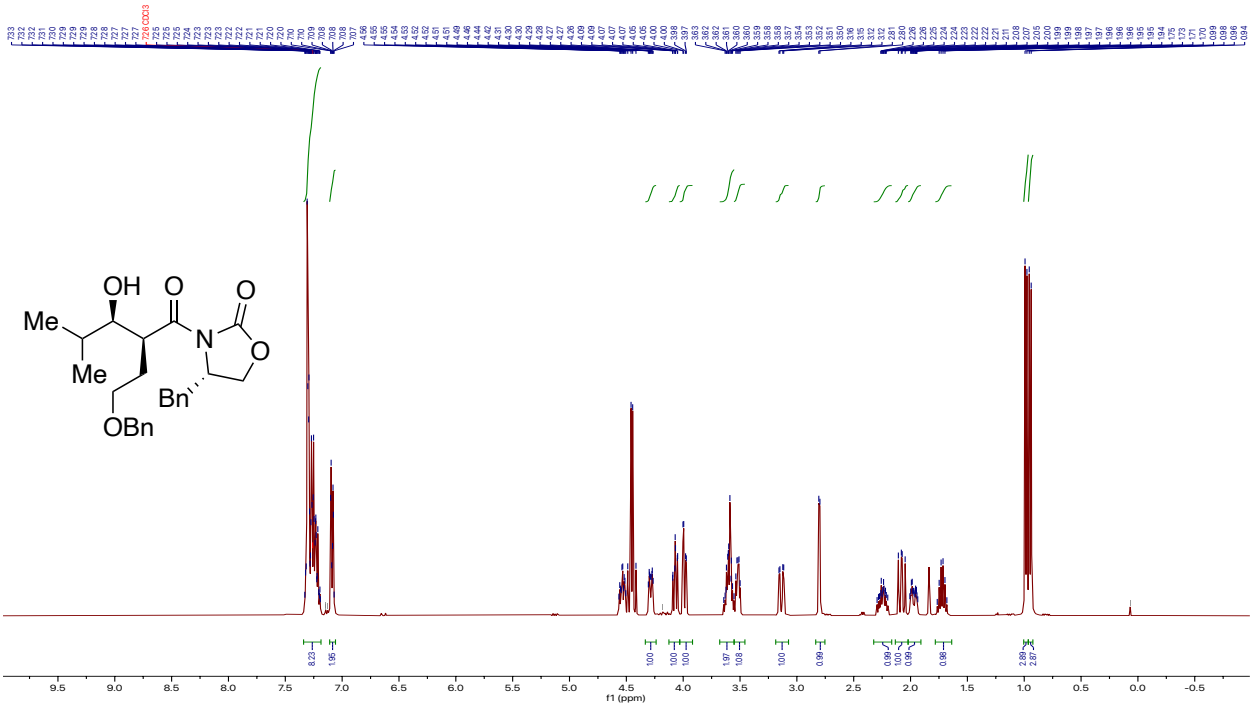
1.12 Inhibitory activity of alkyl linker series against Gram-positive and Gram-negative organisms

Table 1.3. Extended MIC table of all synthesized C-4 alkyl linker analogs against an expanded panel of Gram-positive and Gram-negative pathogens.

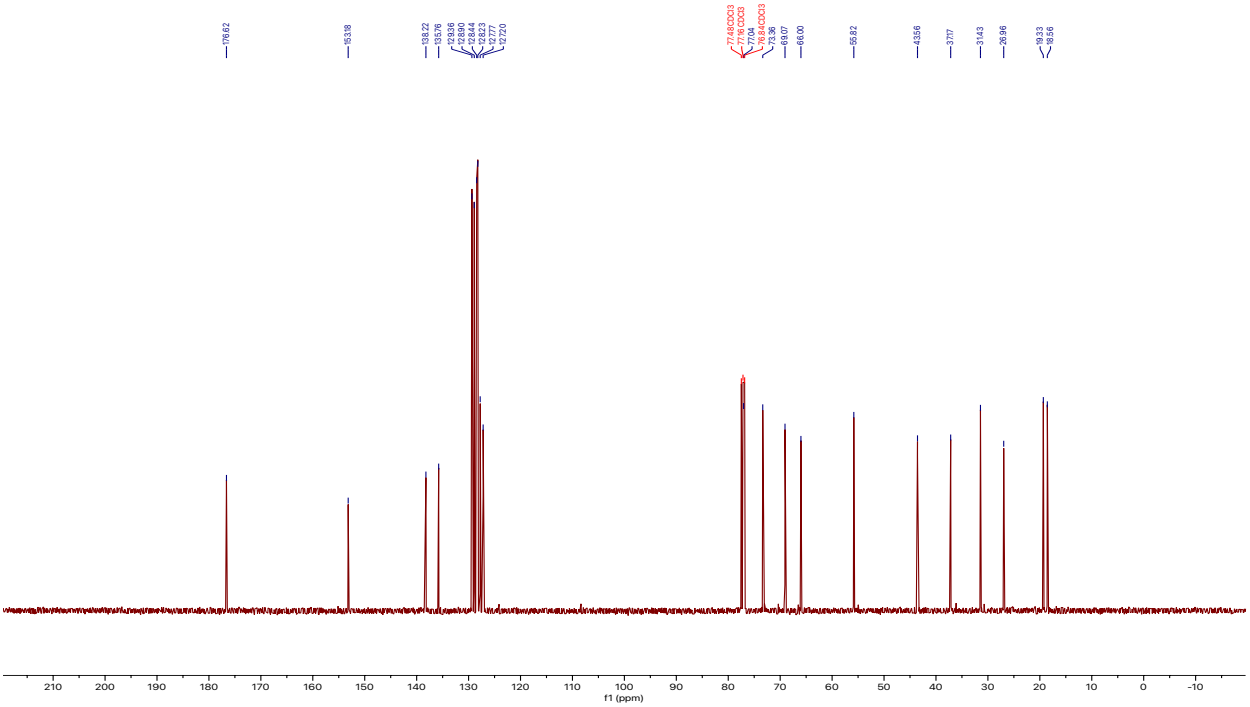


1.13 ^{1}H NMR and ^{13}C NMR spectra

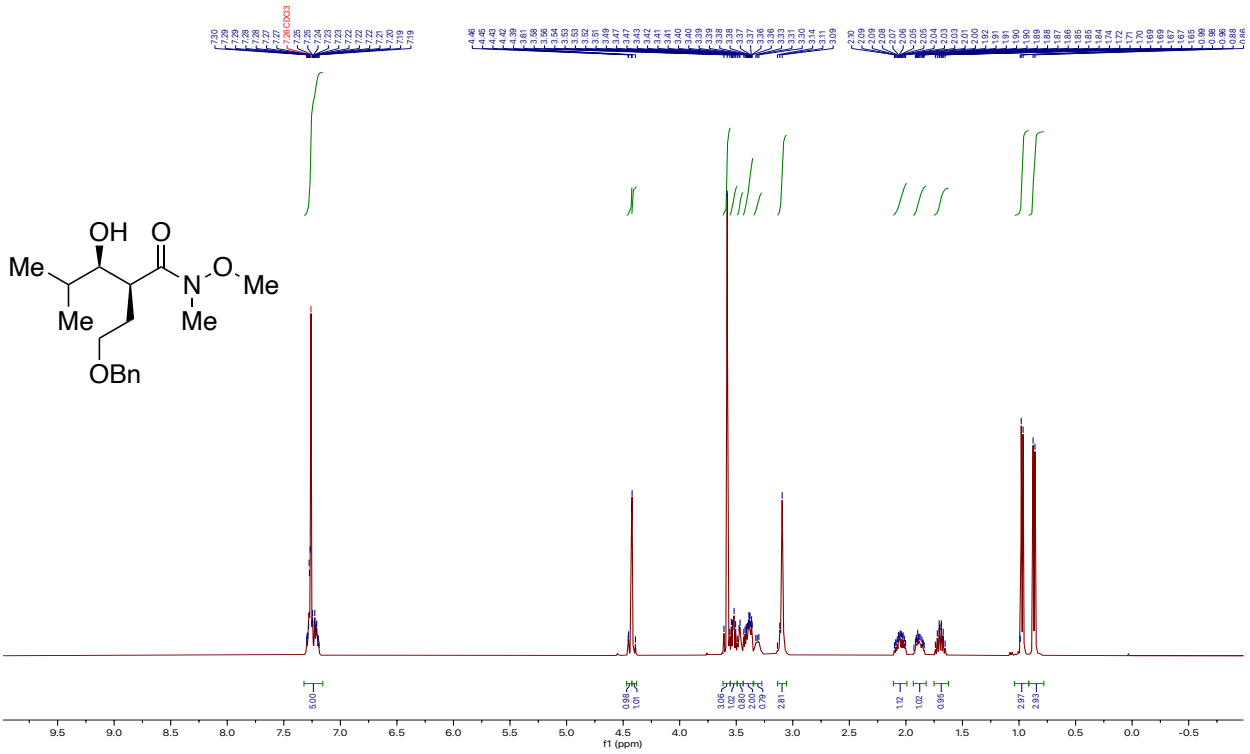
Compound **12**: ^{1}H NMR (400 MHz, $CDCl_3$)



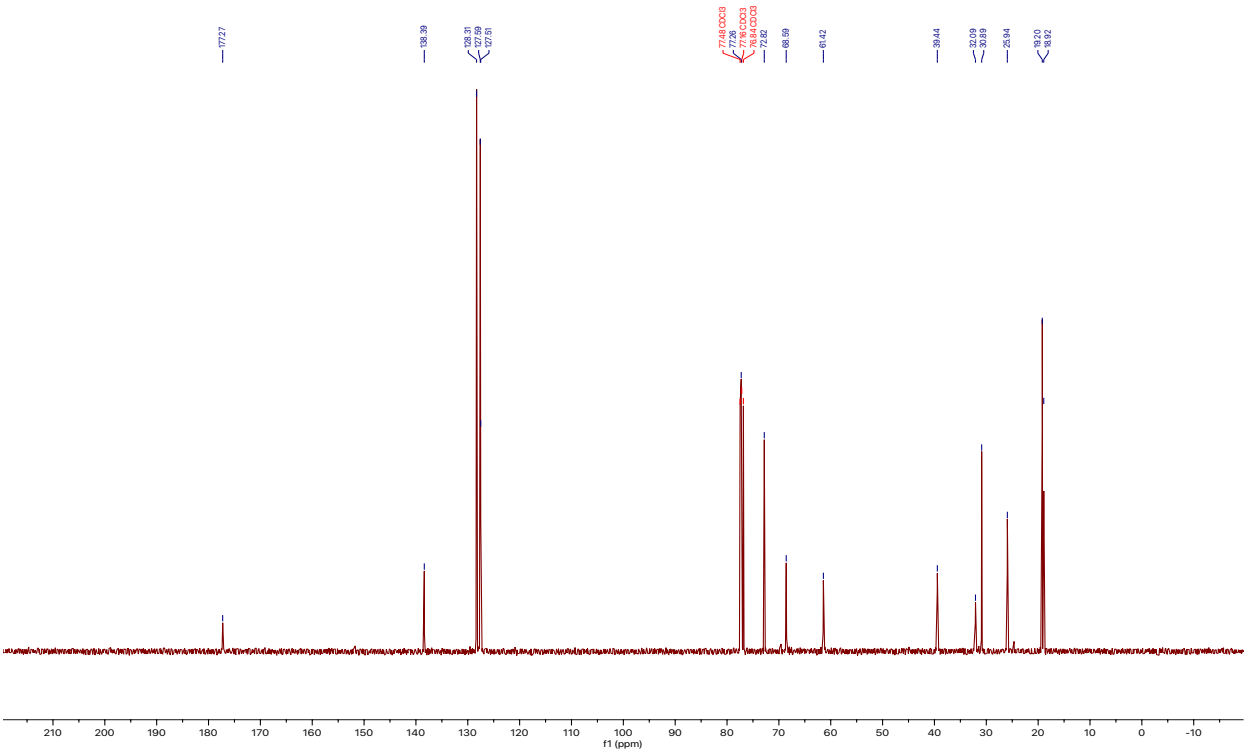
Compound **12**: ^{13}C NMR (100 MHz, $CDCl_3$)



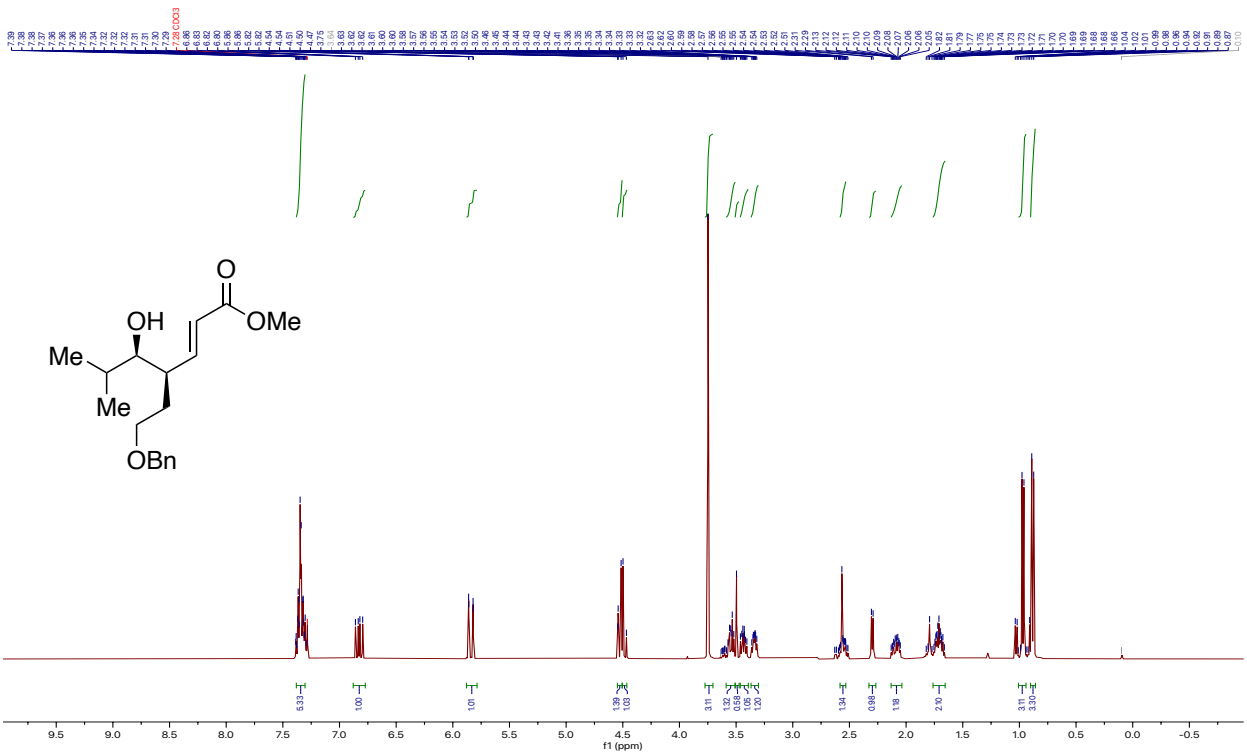
Compound **13**: ^{1}H NMR (400 MHz, $CDCl_3$)



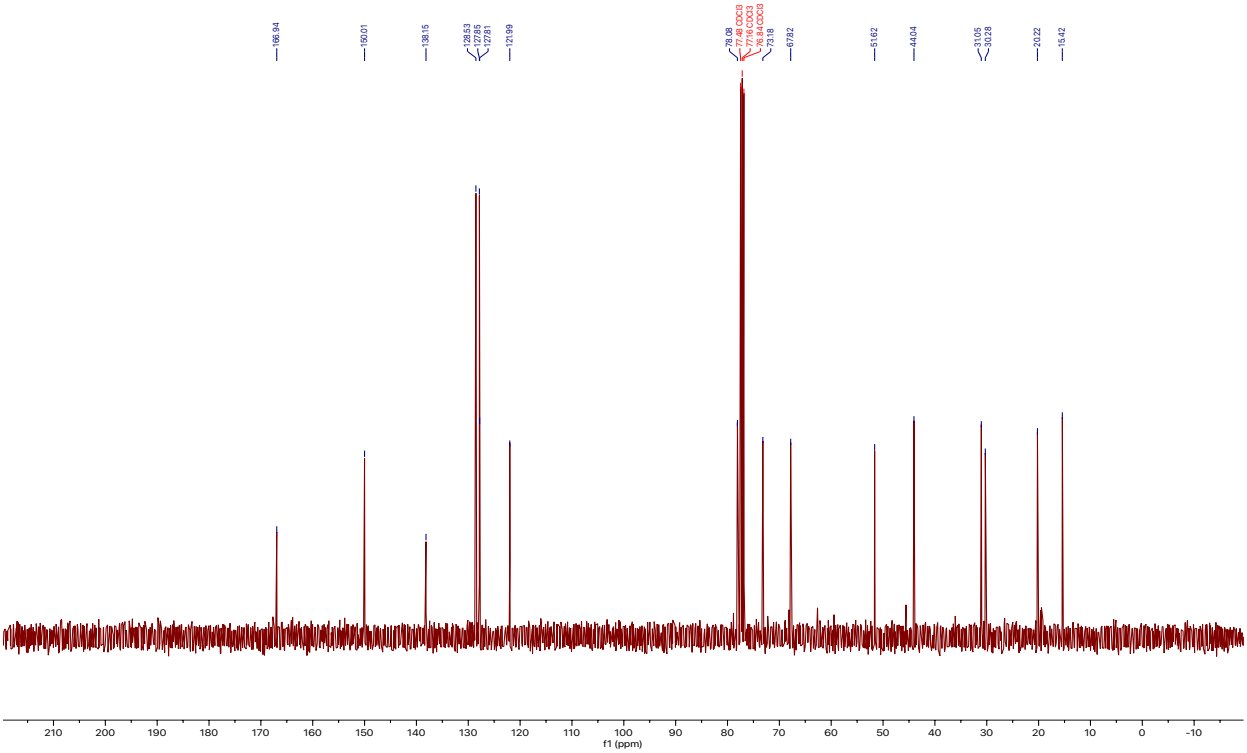
Compound **13**: ^{13}C NMR (100 MHz, $CDCl_3$)



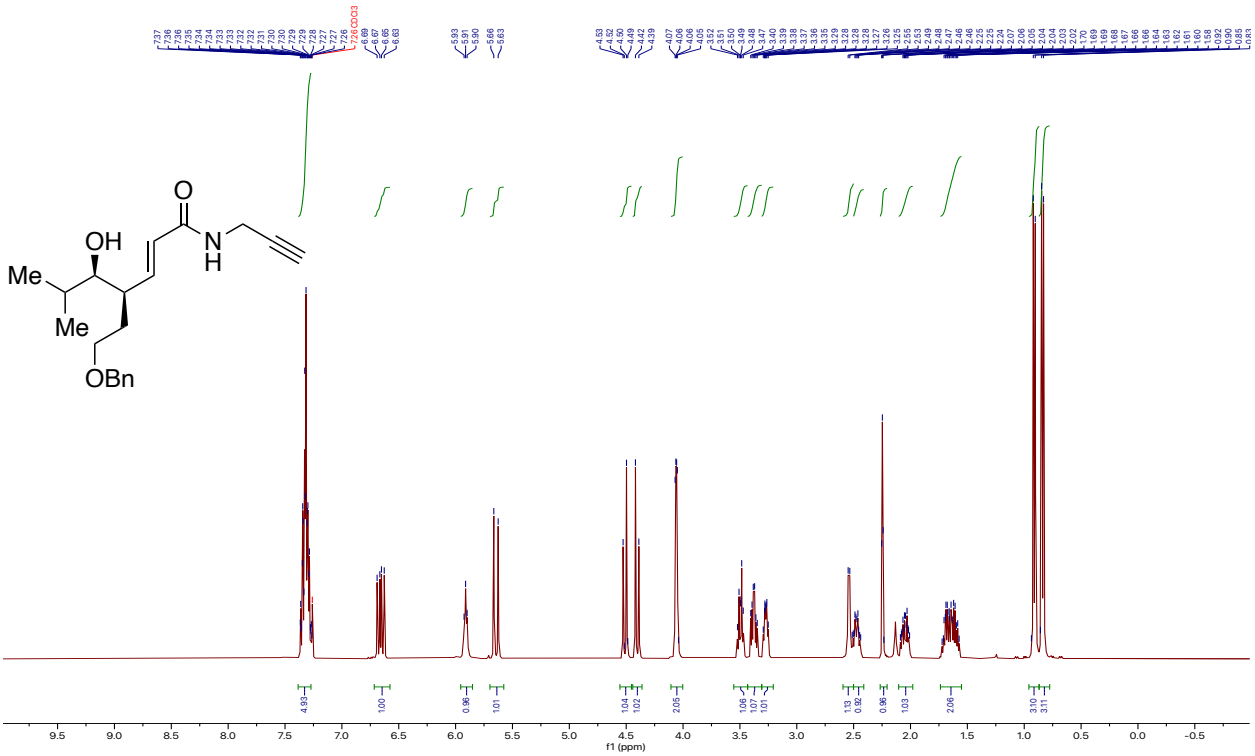
Compound **14**: ^{1}H NMR (400 MHz, $CDCl_3$)



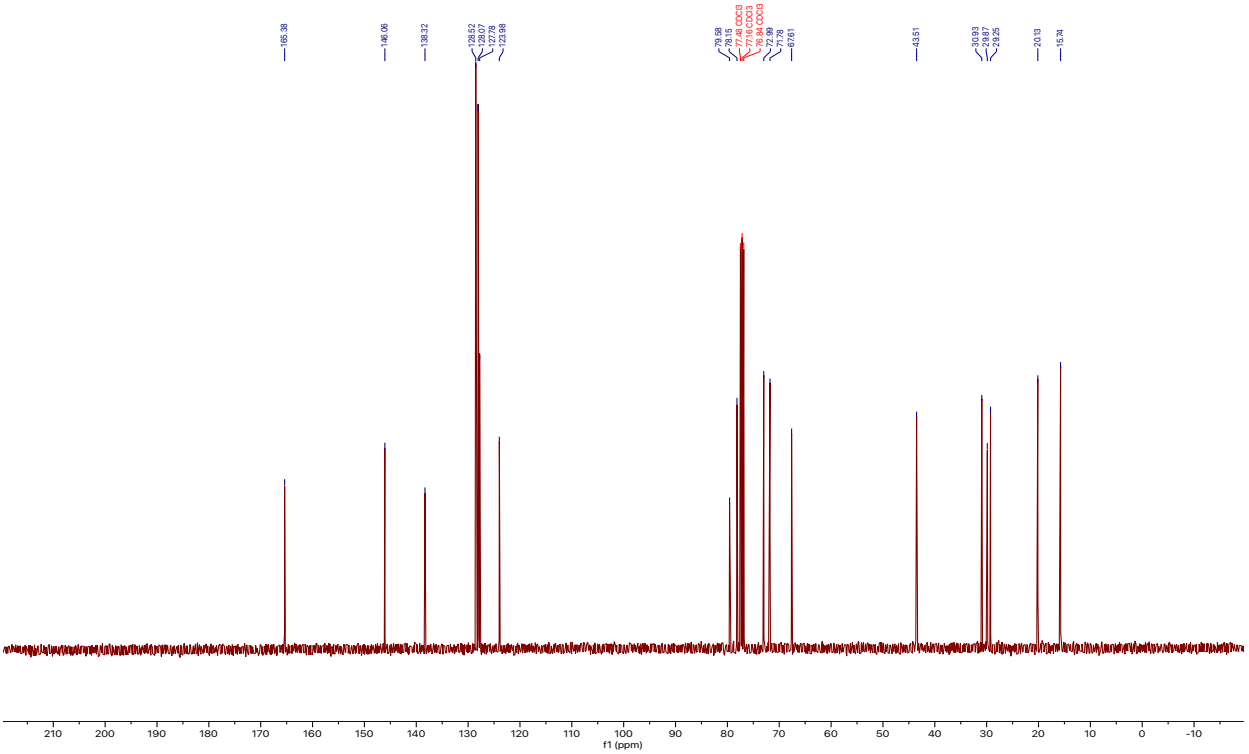
Compound **14**: ^{13}C NMR (100 MHz, $CDCl_3$)



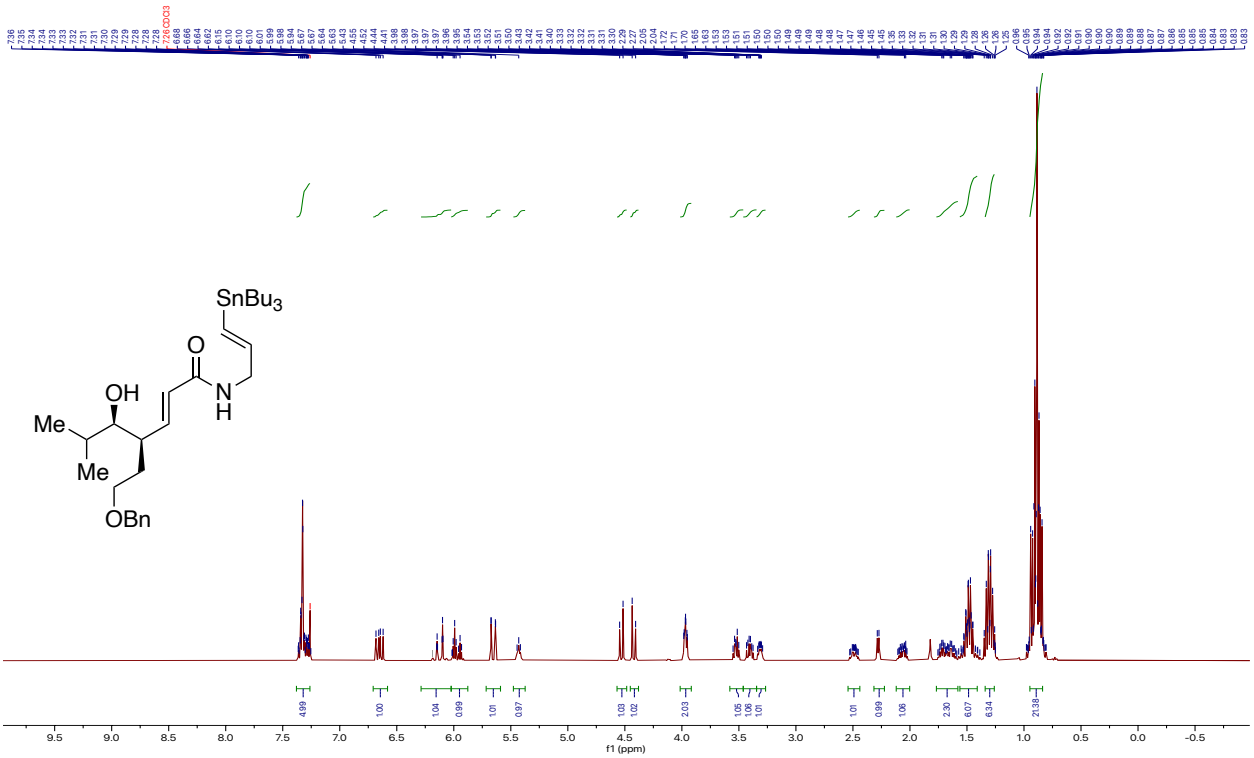
Compound **15**: ^{1}H NMR (400 MHz, $CDCl_3$)



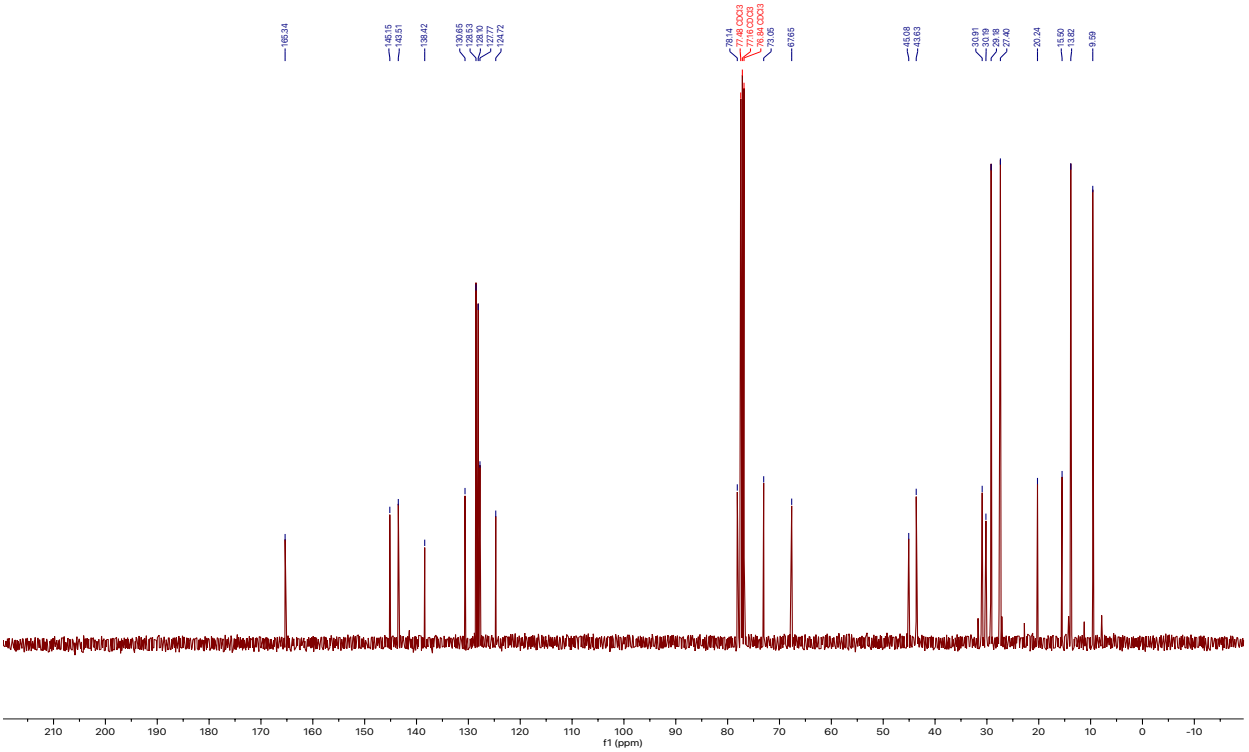
Compound **15**: ^{13}C NMR (100 MHz, $CDCl_3$)



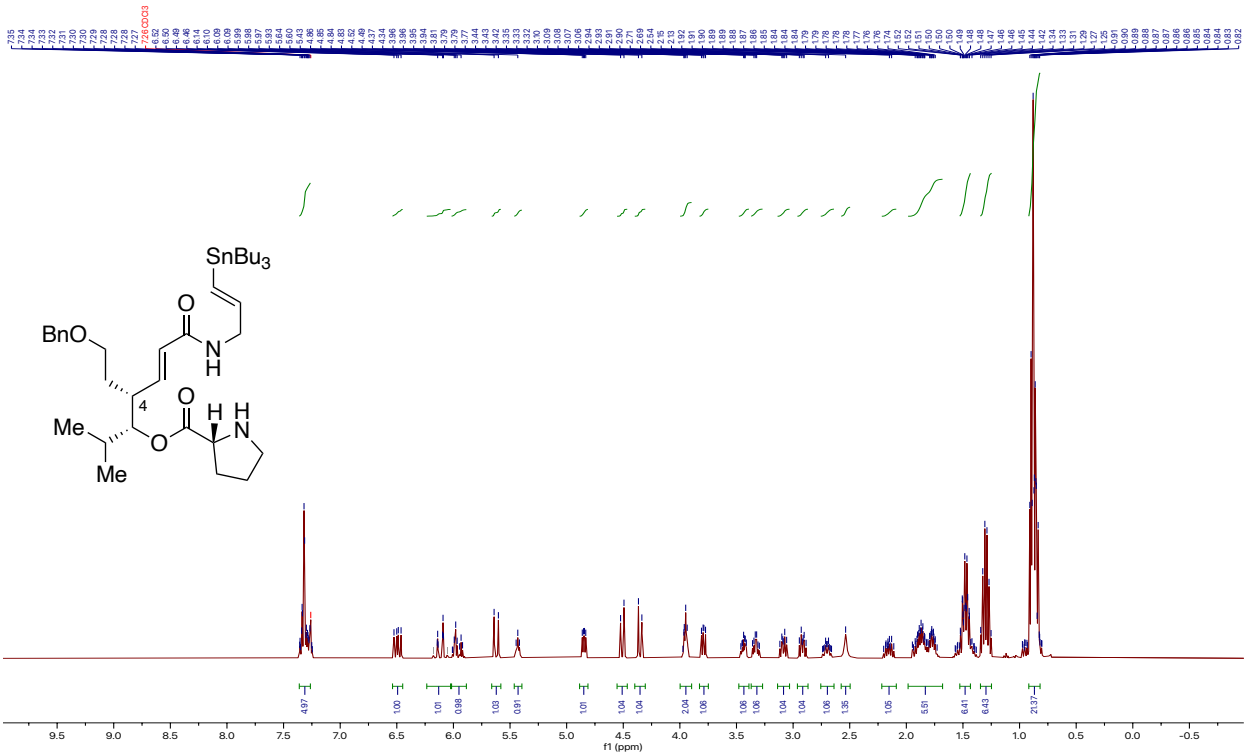
Compound **16**: ^{1}H NMR (400 MHz, $CDCl_3$)



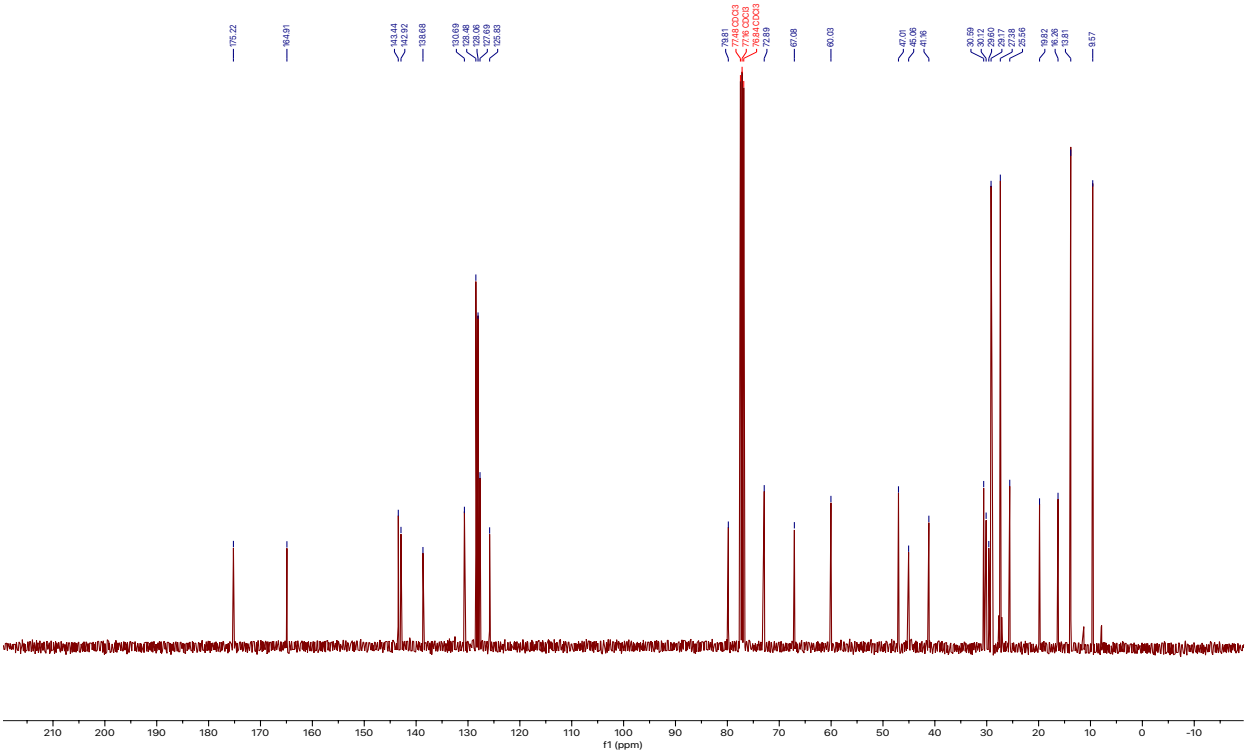
Compound **16**: ^{13}C NMR (100 MHz, $CDCl_3$)



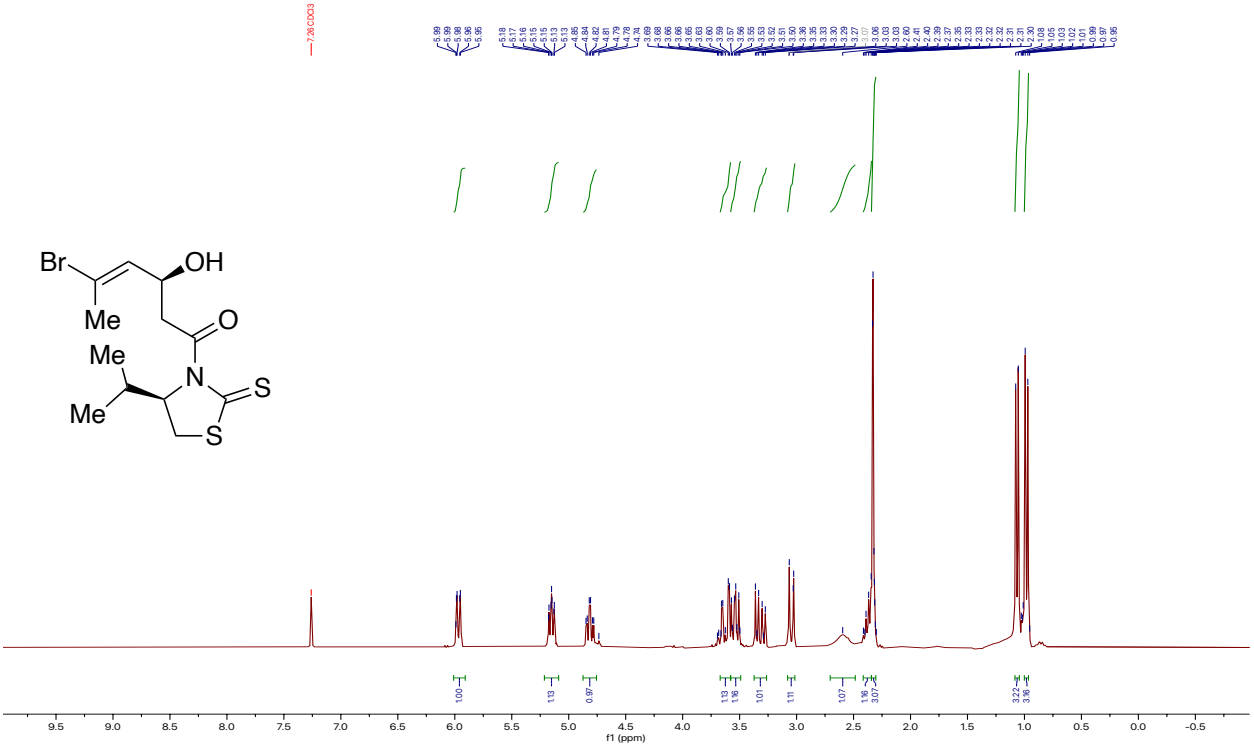
Compound **17**: ^{1}H NMR (400 MHz, $CDCl_3$)



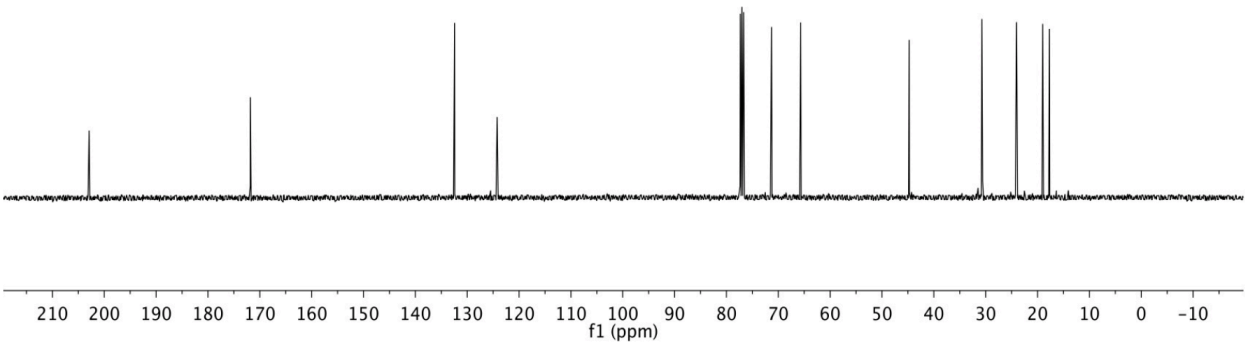
Compound **17**: ^{13}C NMR (100 MHz, $CDCl_3$)



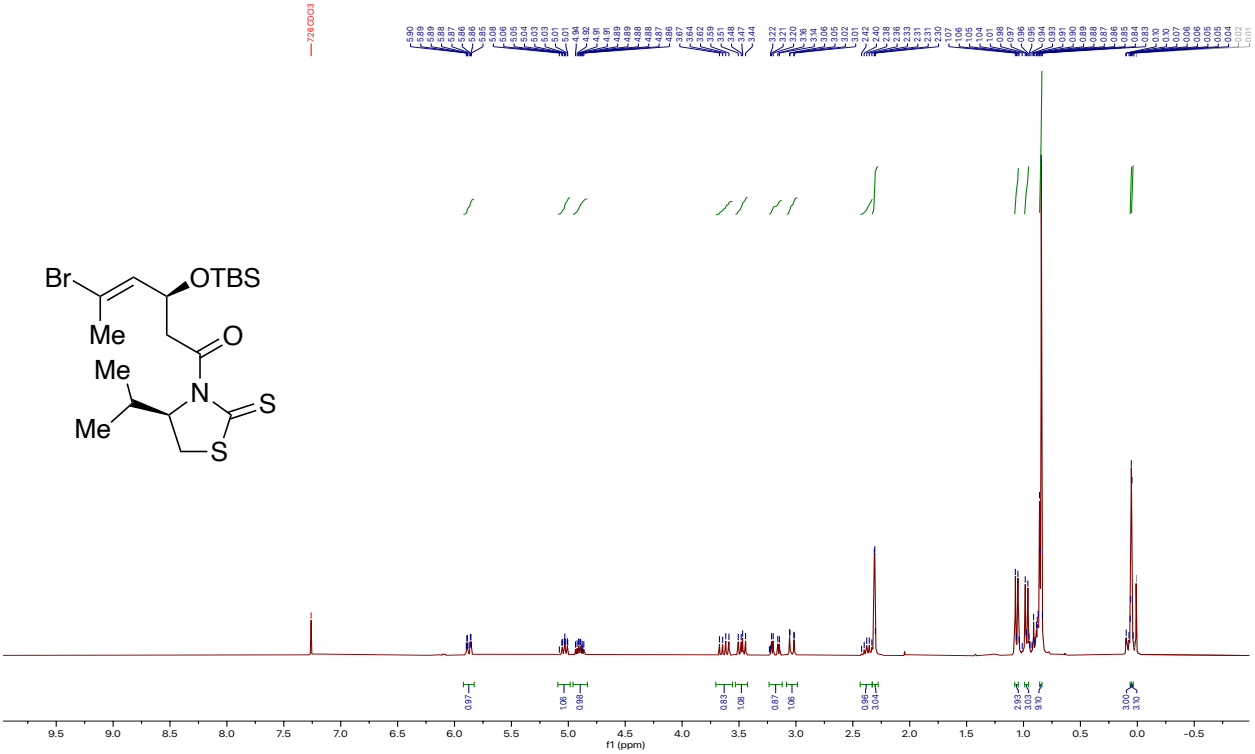
Compound **SI-3**: ^{1}H NMR (300 MHz, $CDCl_3$)



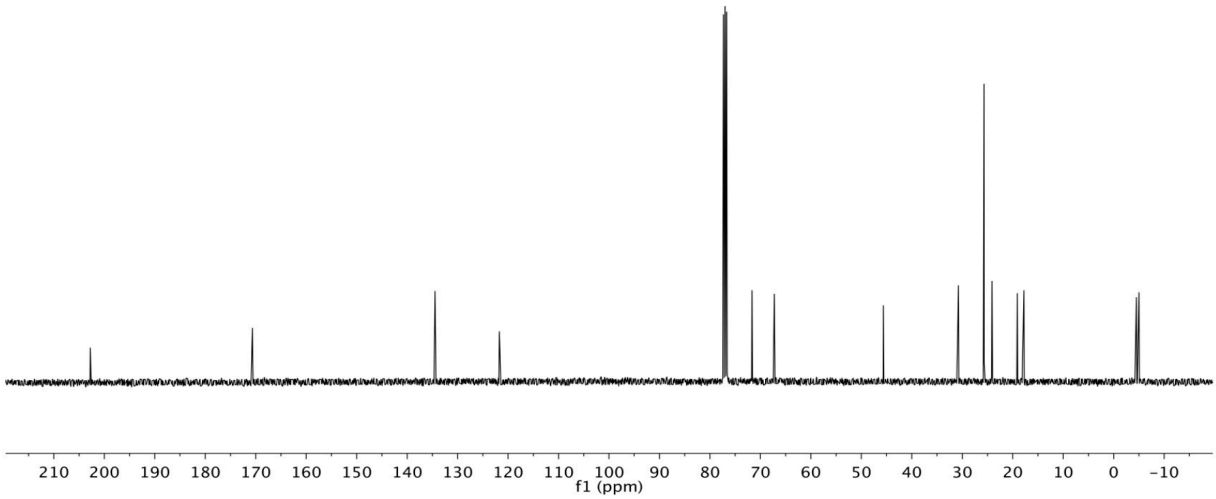
Compound **SI-3**: ^{13}C NMR (100 MHz, $CDCl_3$)



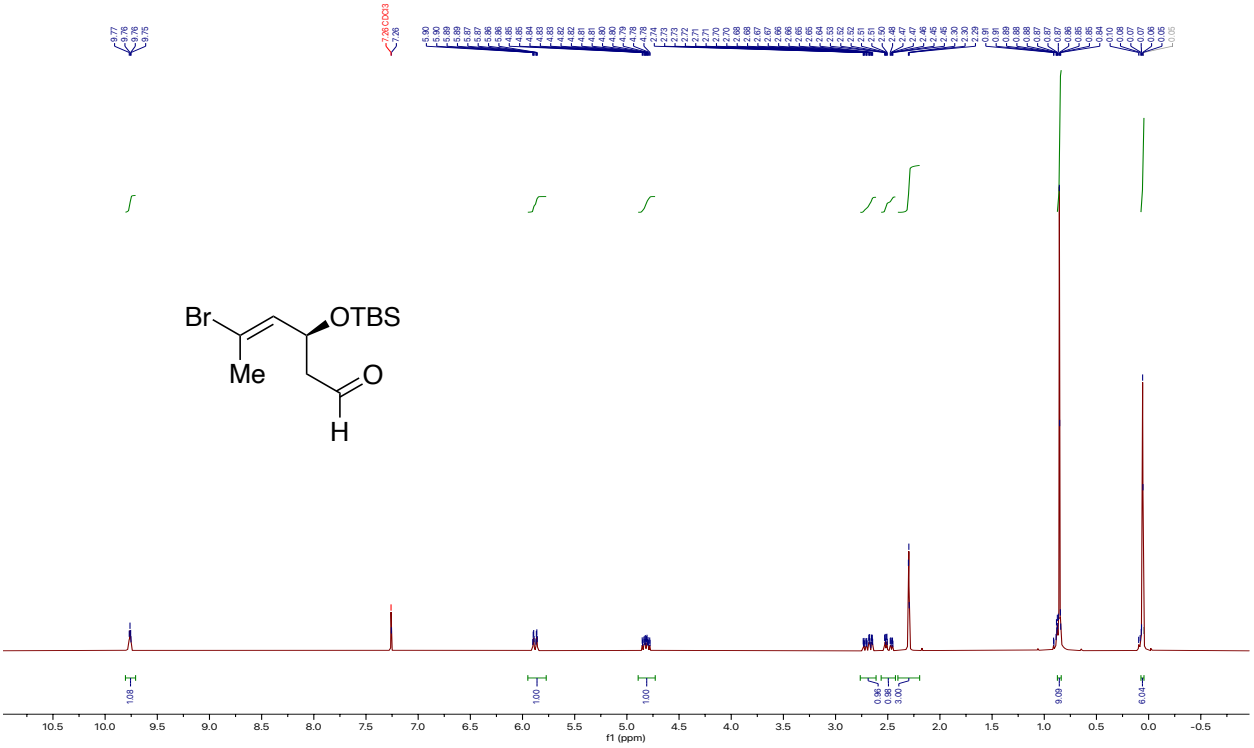
Compound **26**: ^{1}H NMR (300 MHz, $CDCl_3$)



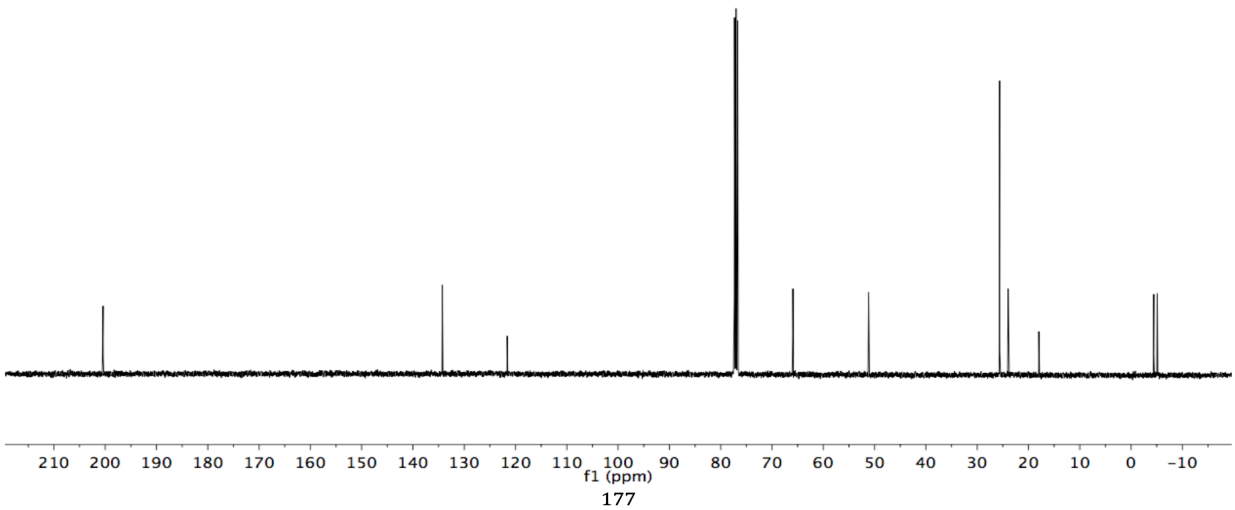
Compound **26**: ^{13}C NMR (100 MHz, $CDCl_3$)



Compound **SI-4**: ^{1}H NMR (300 MHz, $CDCl_3$)

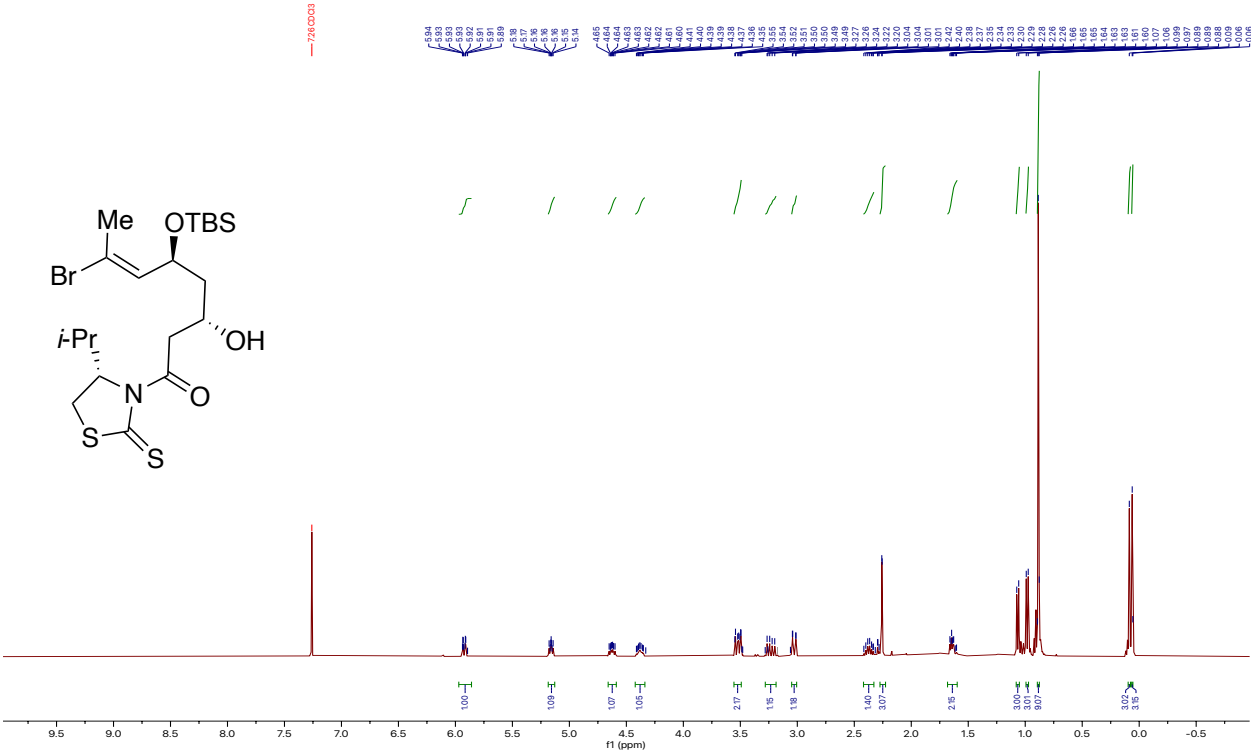


Compound **SI-4**: ^{13}C NMR (100 MHz, $CDCl_3$)

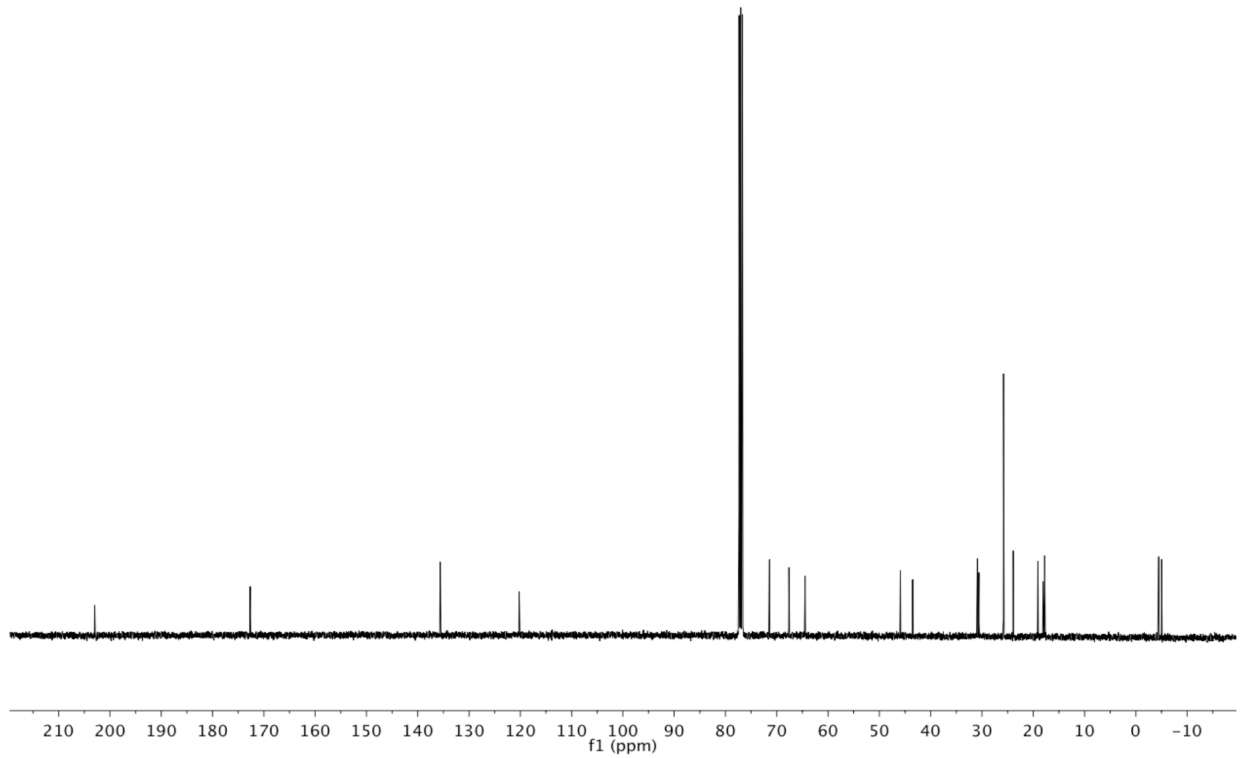


177

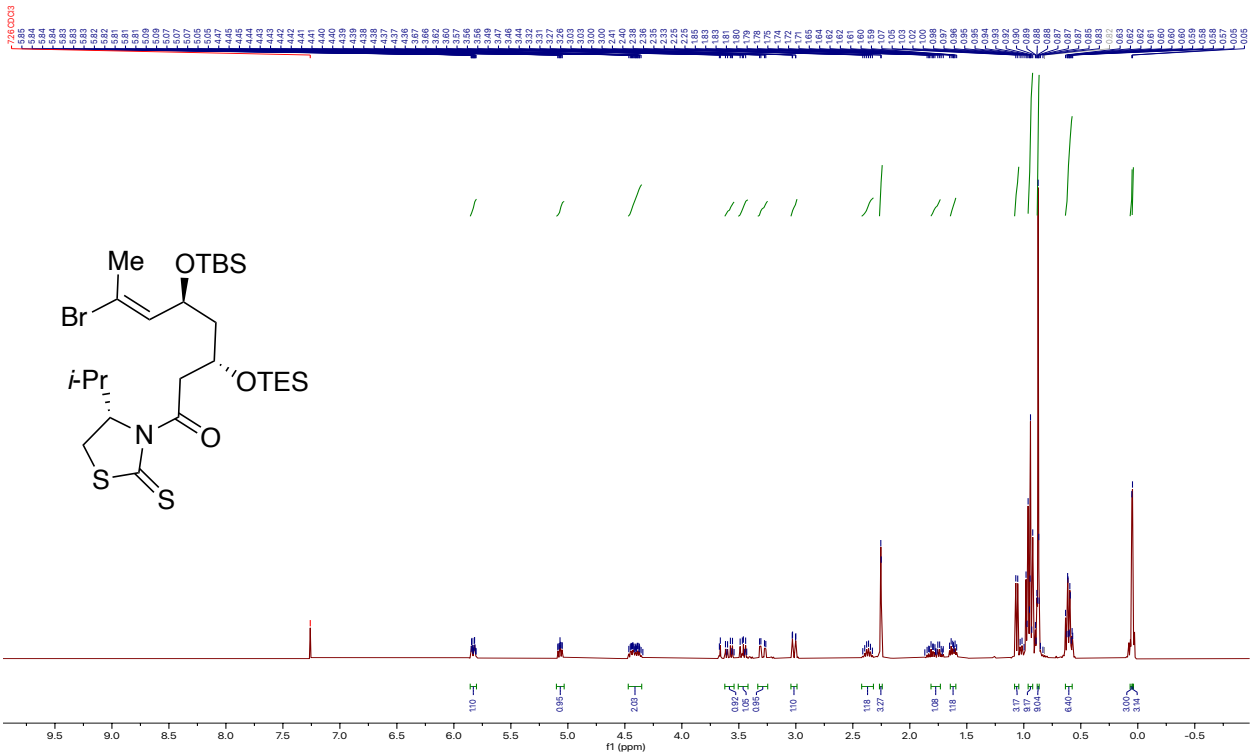
Compound **28**: ^{1}H NMR (400 MHz, $CDCl_3$)



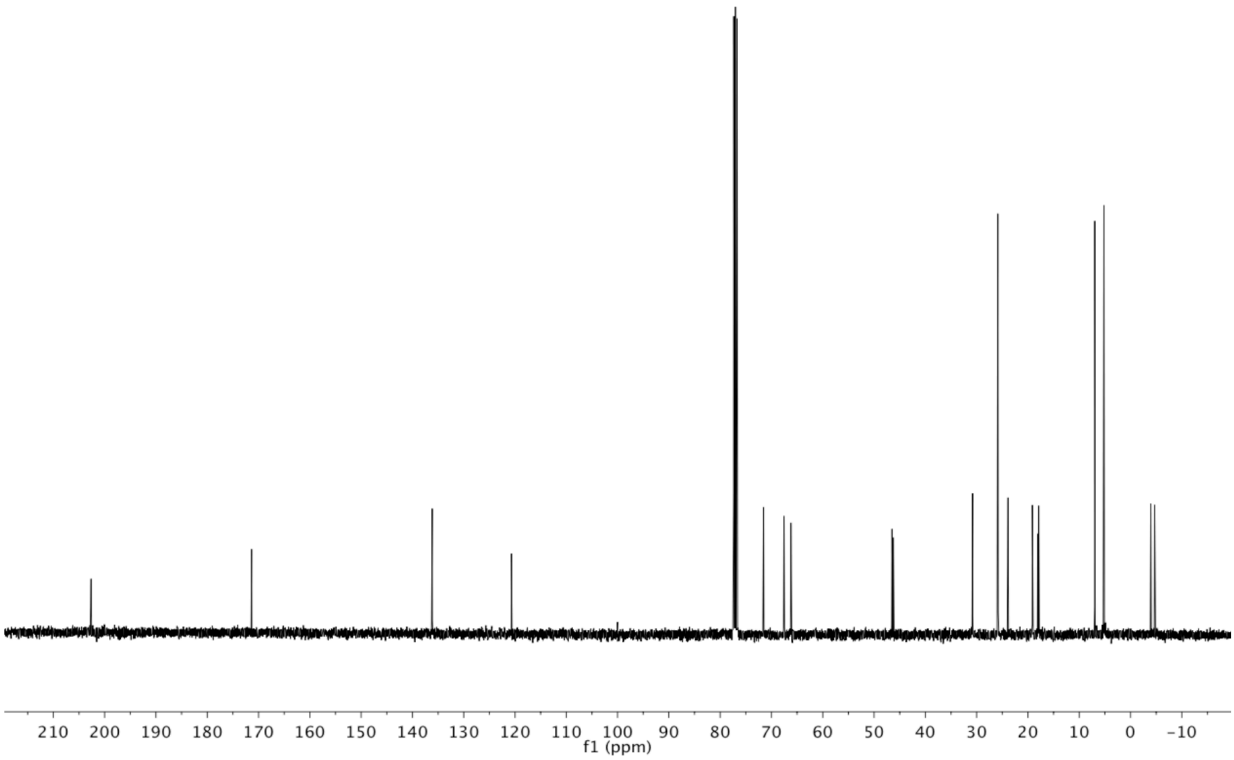
Compound **28**: ^{13}C NMR (100 MHz, $CDCl_3$)



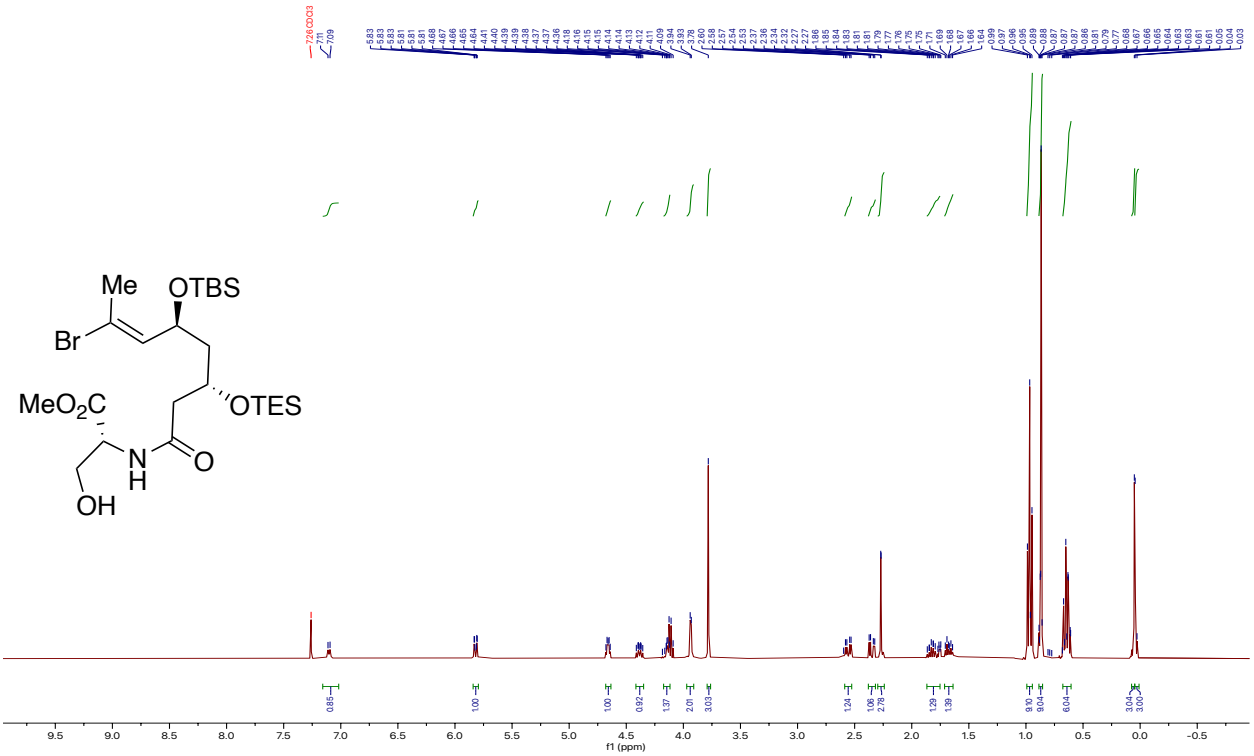
Compound **SI-5**: ^{1}H NMR (400 MHz, $CDCl_3$)



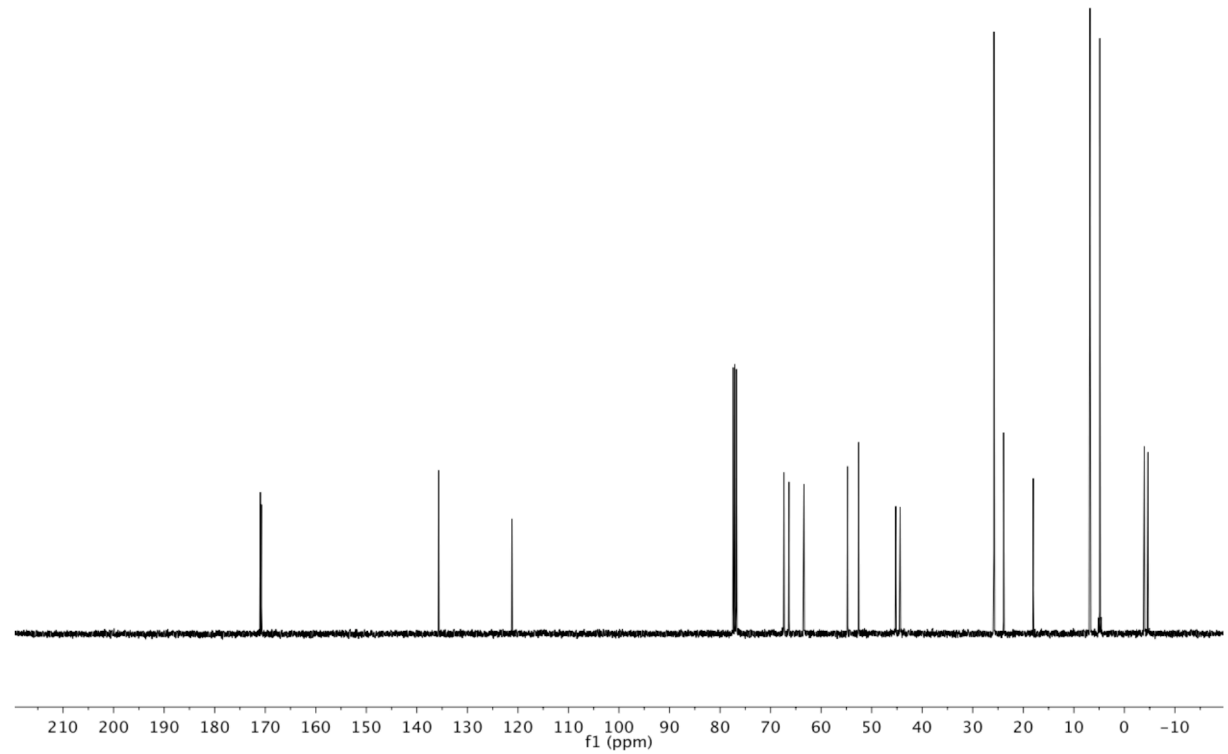
Compound **SI-5**: ^{13}C NMR (100 MHz, $CDCl_3$)



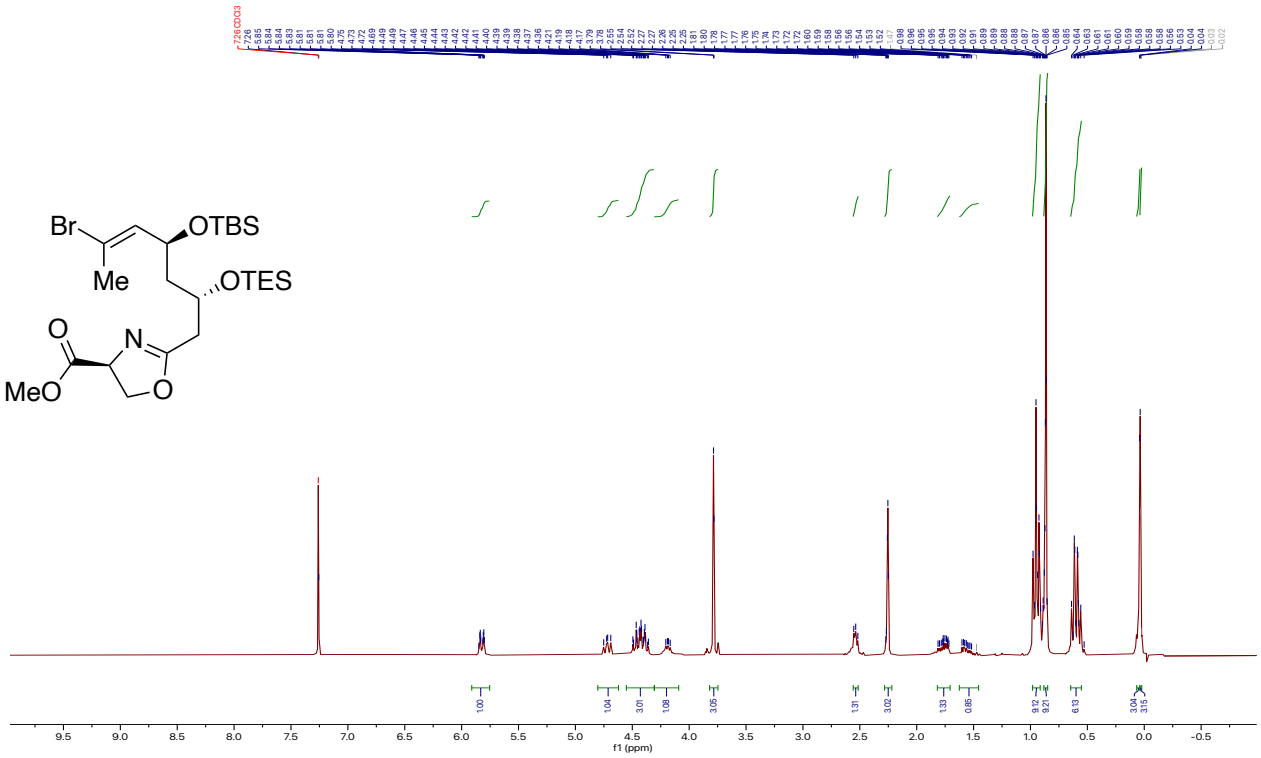
Compound **30**: ^{1}H NMR (400 MHz, $CDCl_3$)



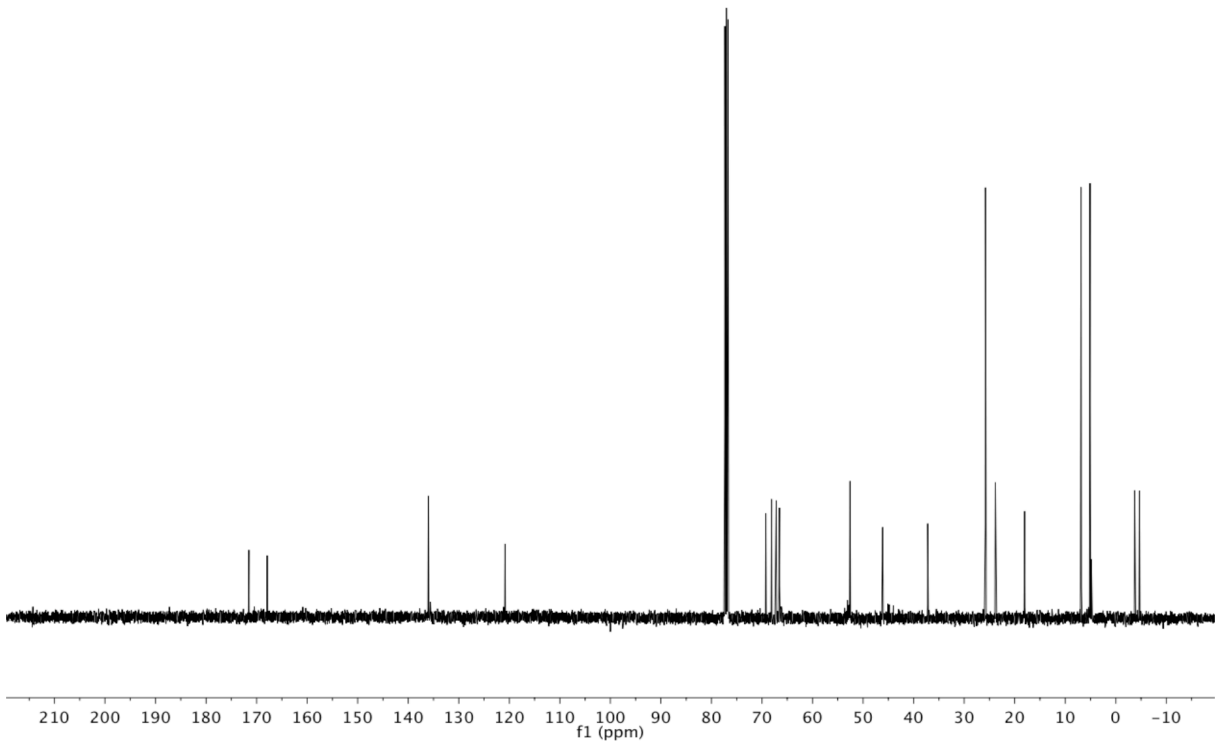
Compound **30**: ^{13}C NMR (100 MHz, $CDCl_3$)



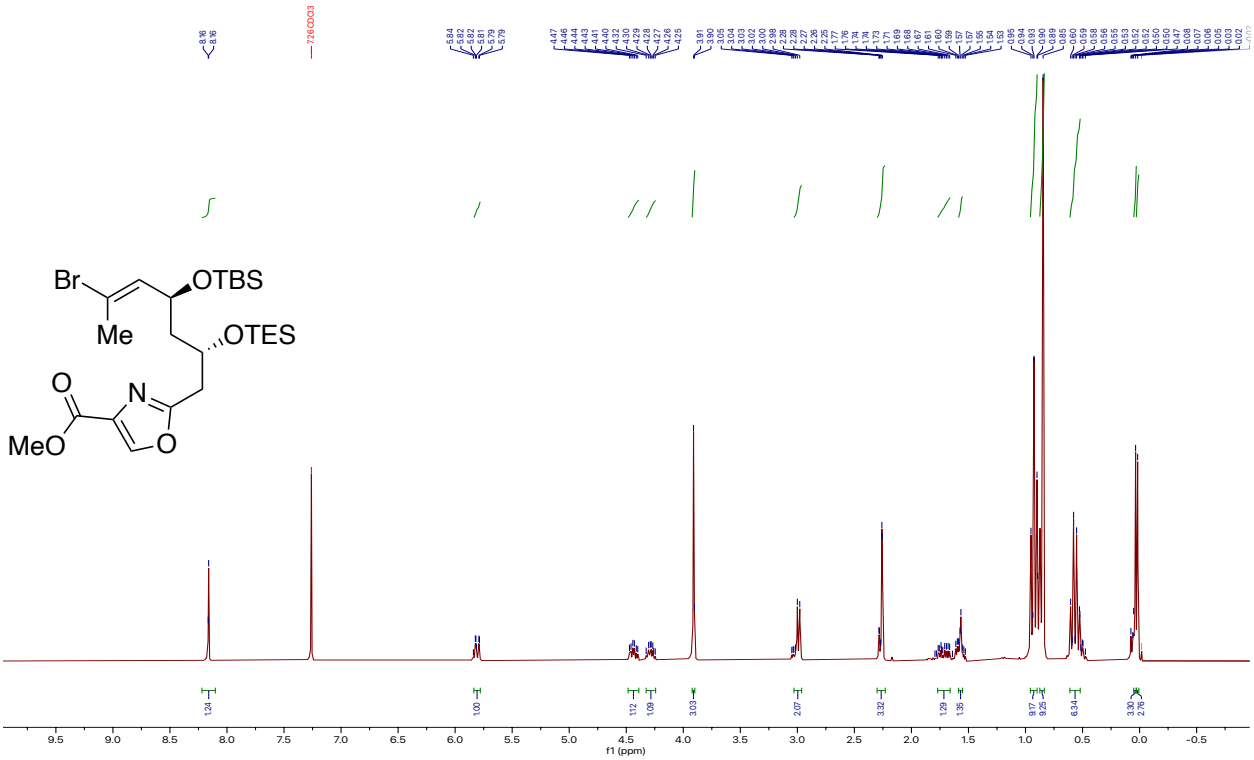
Compound **SI-1**: ^{1}H NMR (400 MHz, $CDCl_3$)



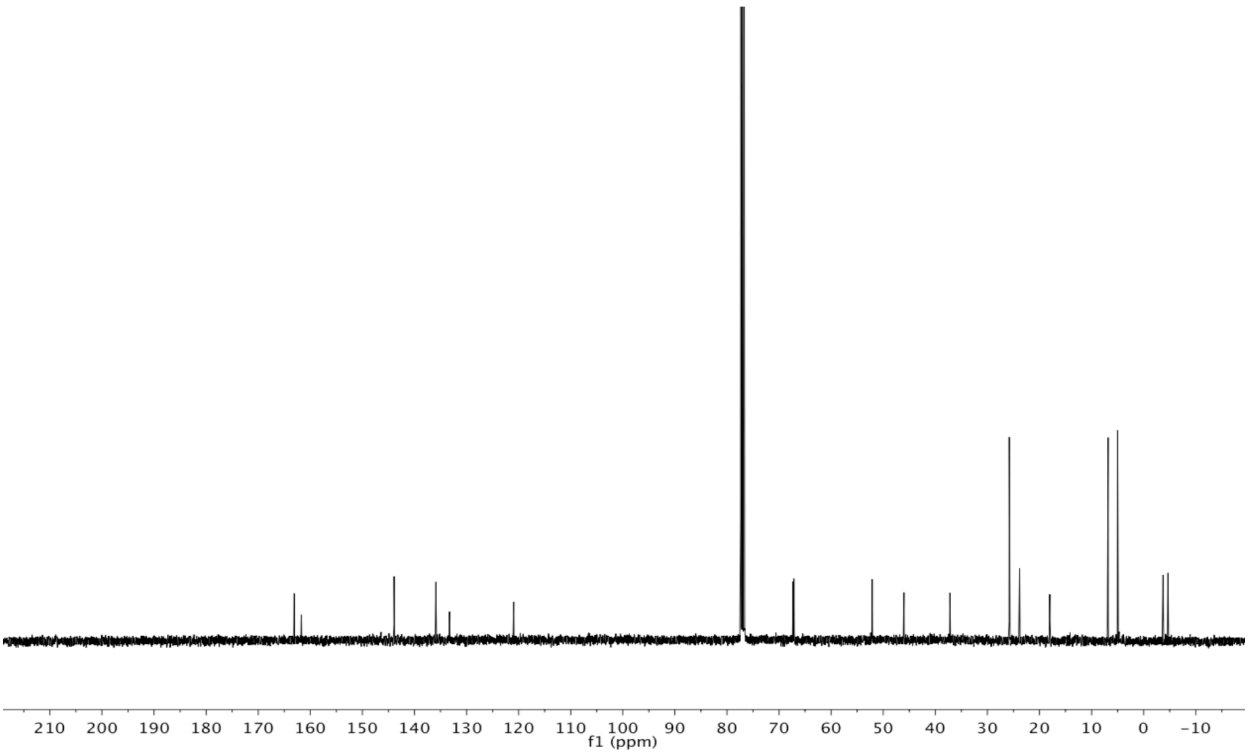
Compound **SI-I**: ^{13}C NMR (100 MHz, $CDCl_3$)



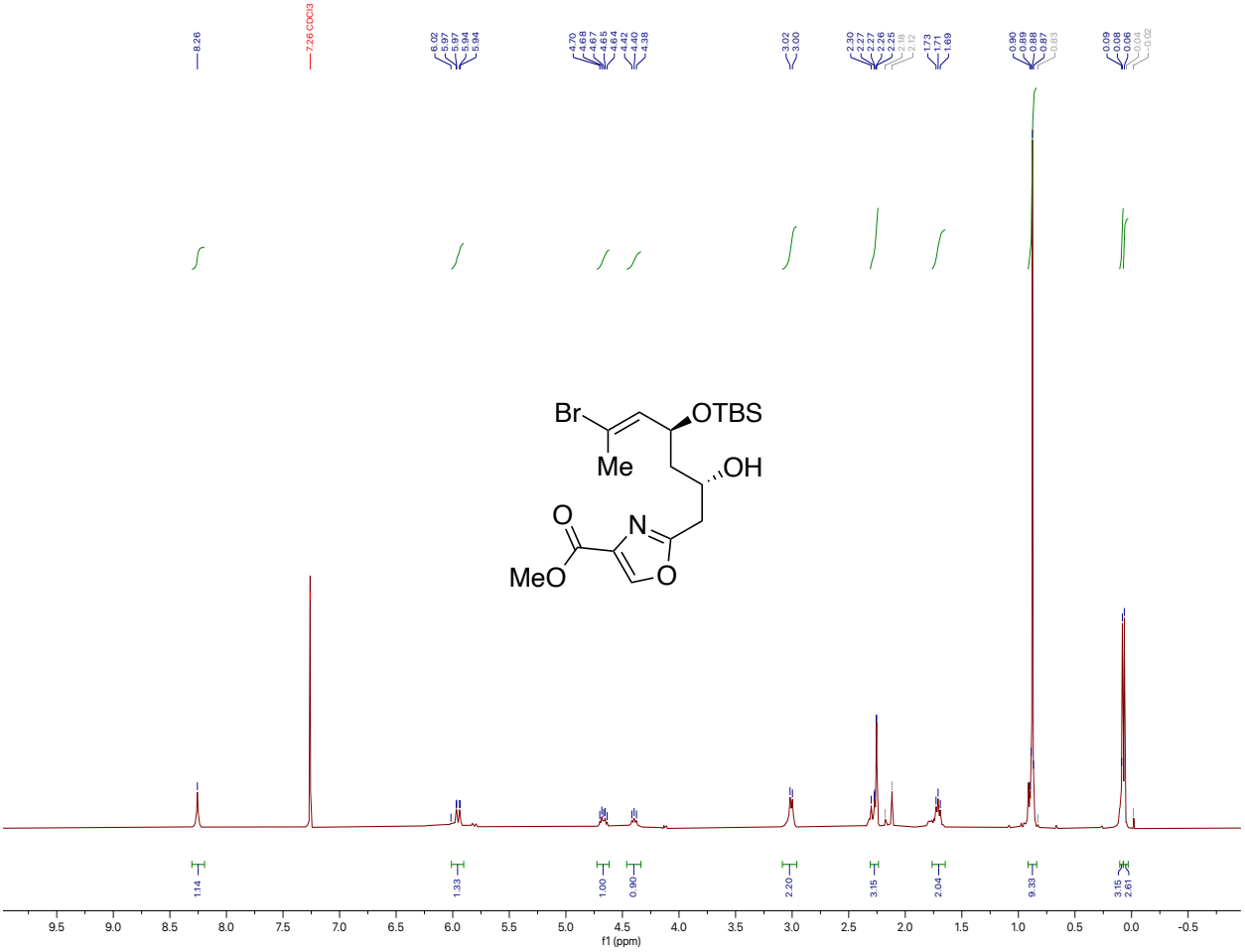
Compound **SI-2**: ^{1}H NMR (400 MHz, $CDCl_3$)



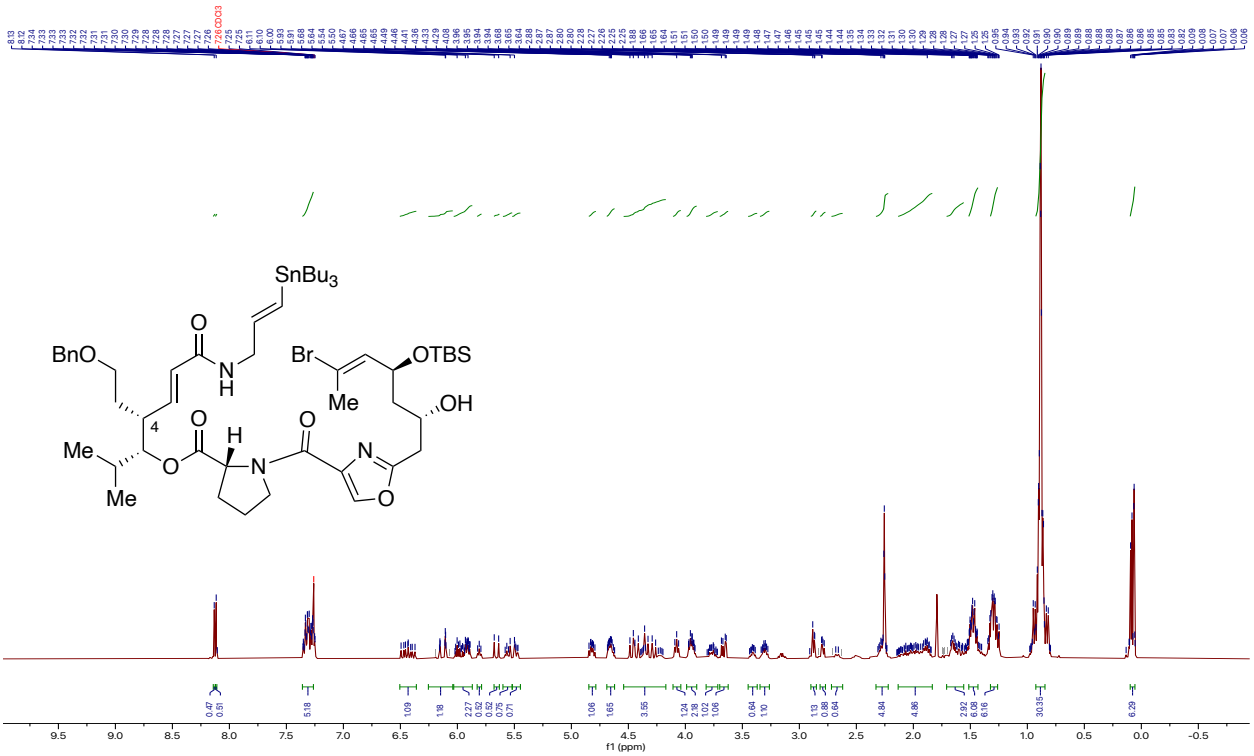
Compound **SI-2**: ^{13}C NMR (100 MHz, $CDCl_3$)



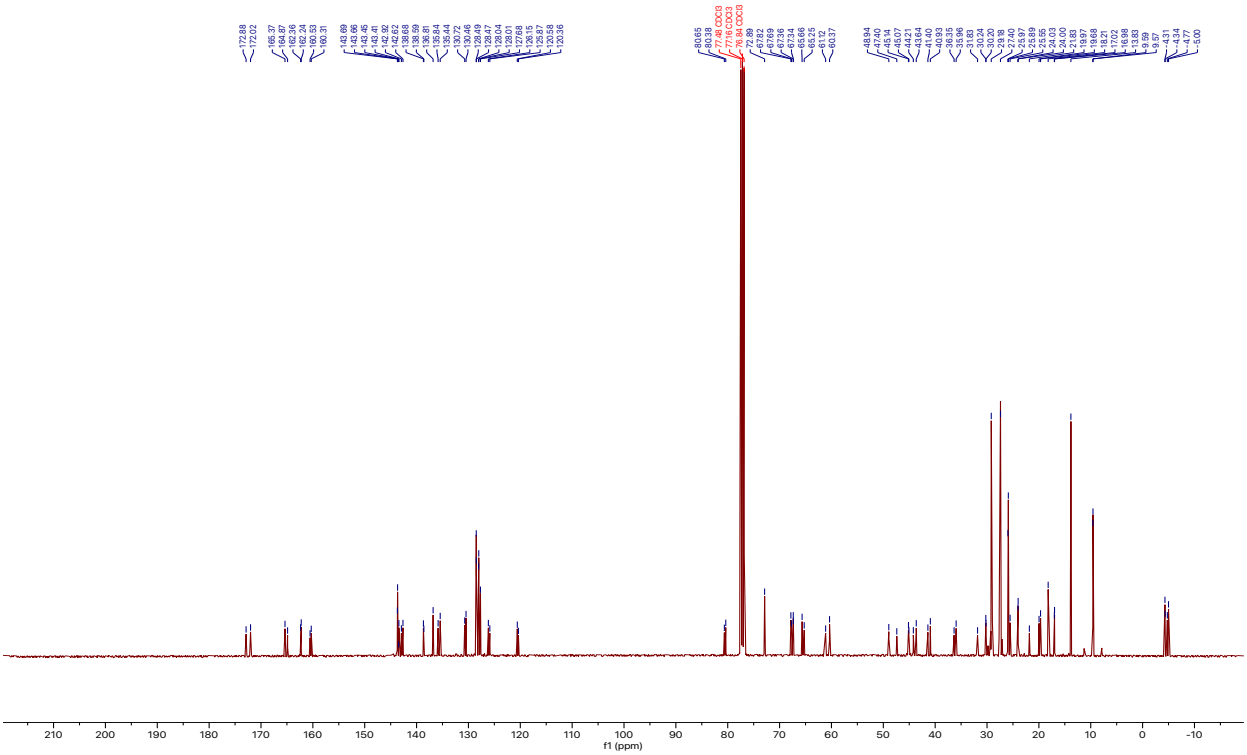
Compound **31**: ^{1}H NMR (300 MHz, $CDCl_3$)



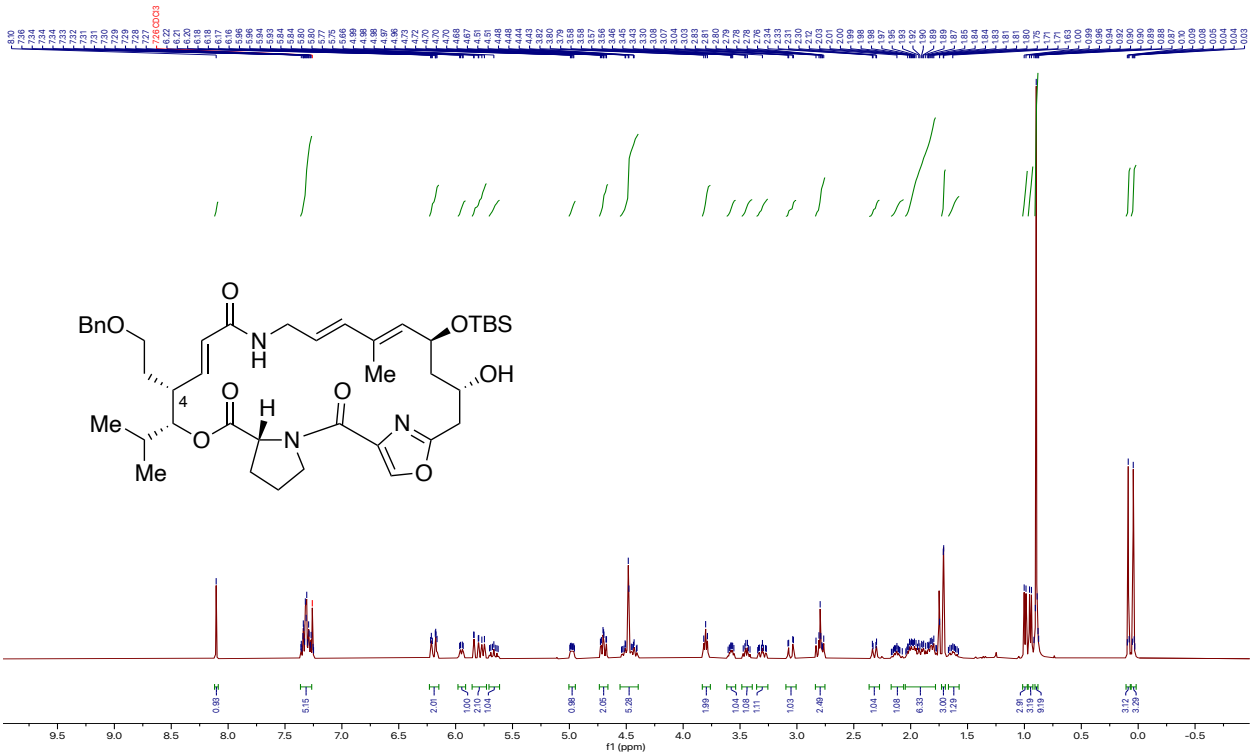
Compound **SI-6**: ^{1}H NMR (400 MHz, $CDCl_3$)



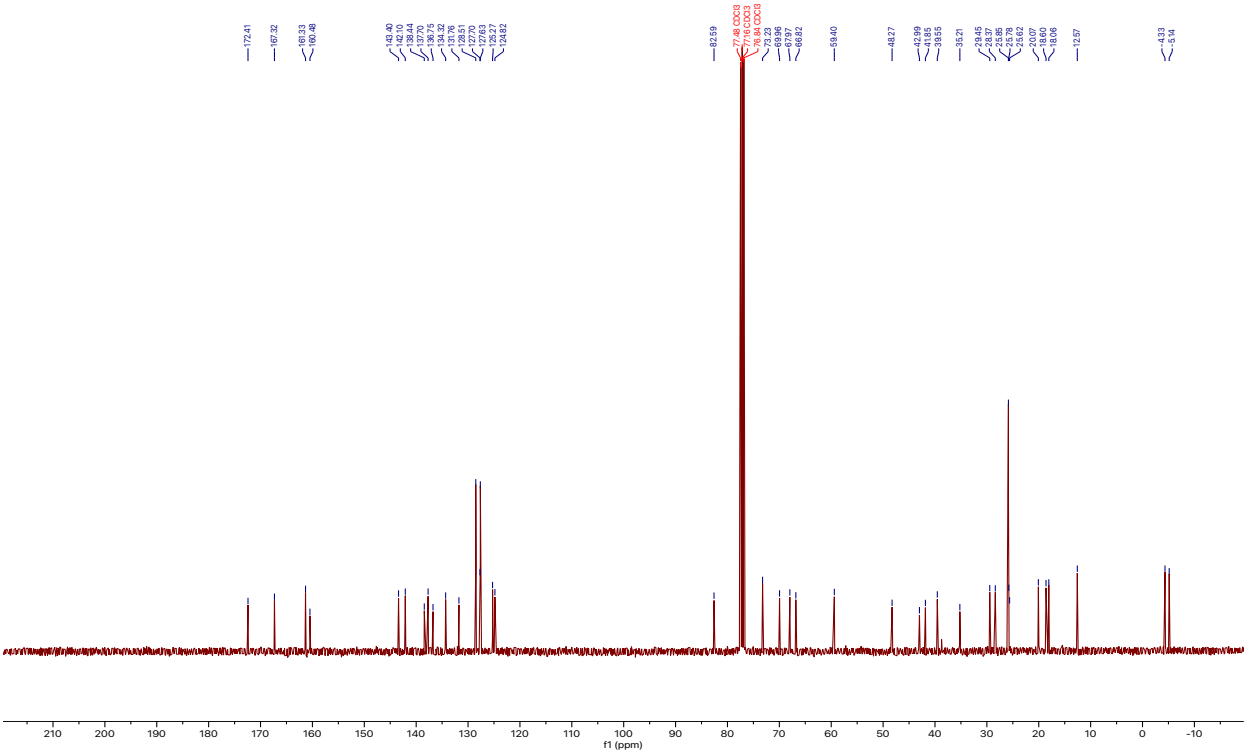
Compound **SI-6**: ^{13}C NMR (100 MHz, $CDCl_3$)



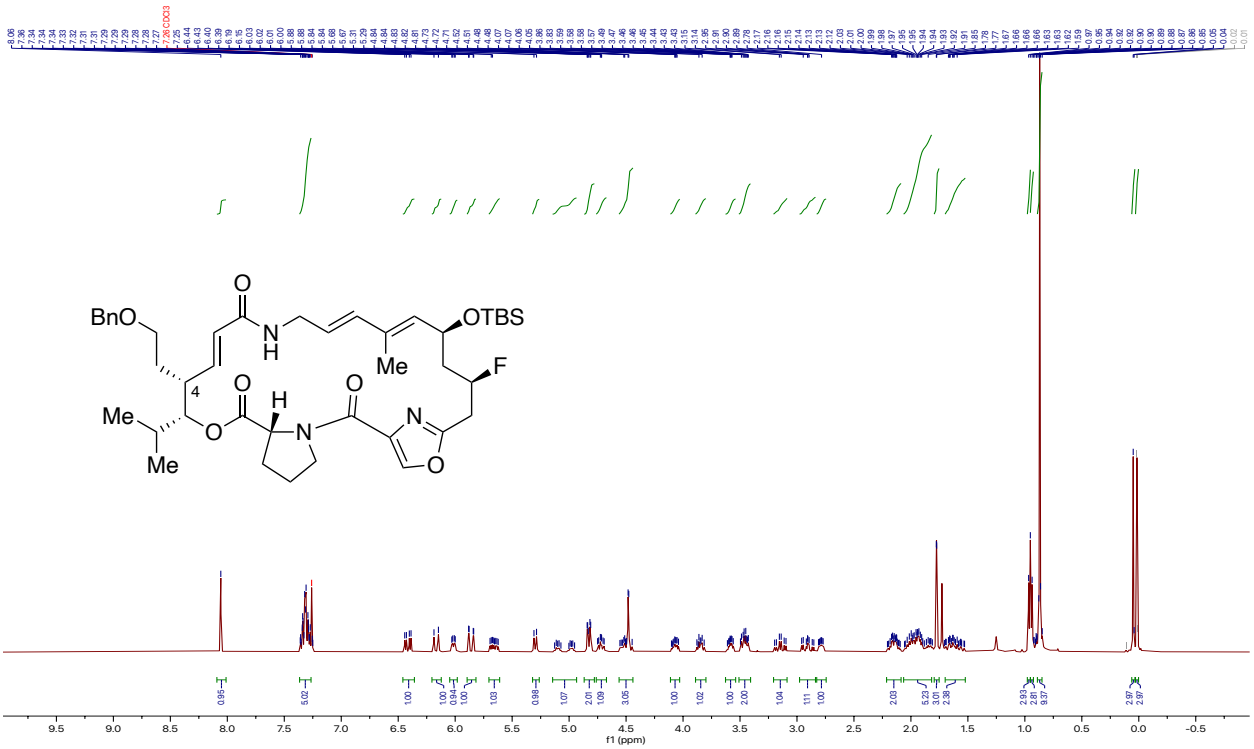
Compound **34**: ^{1}H NMR (400 MHz, $CDCl_3$)



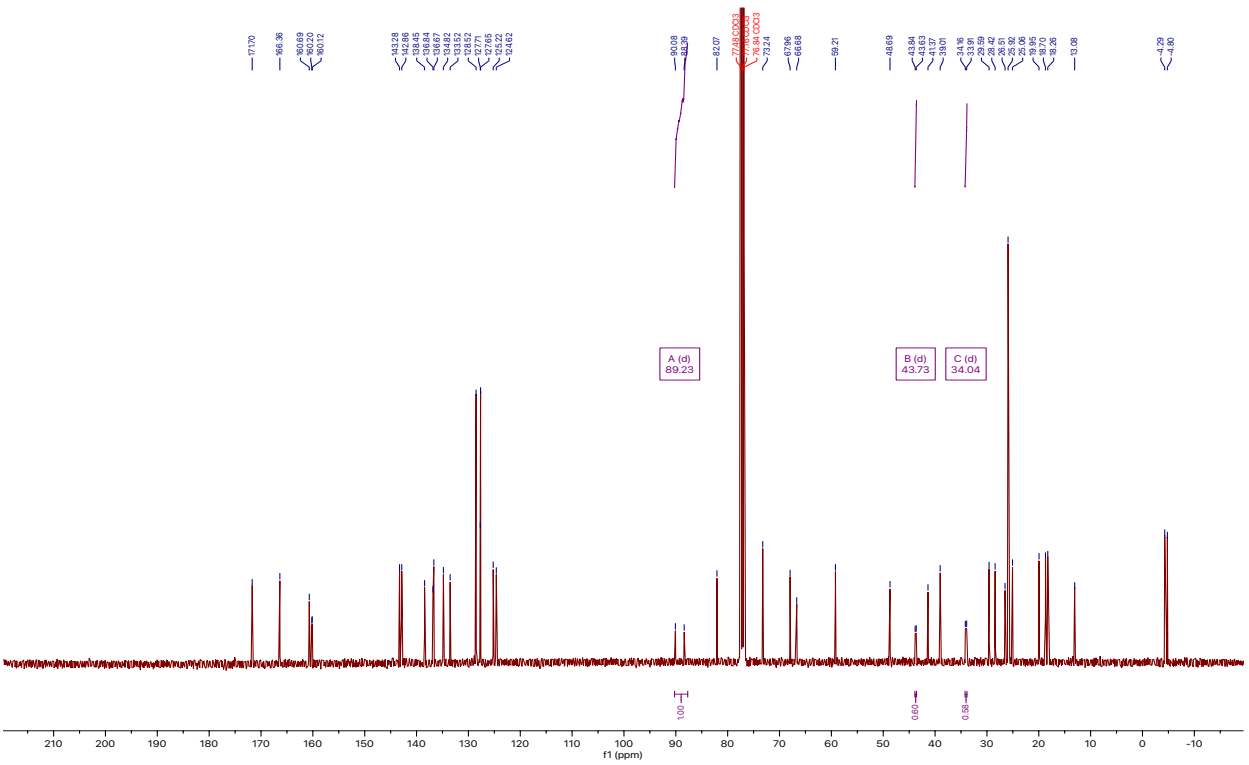
Compound **34**: ^{13}C NMR (100 MHz, $CDCl_3$)



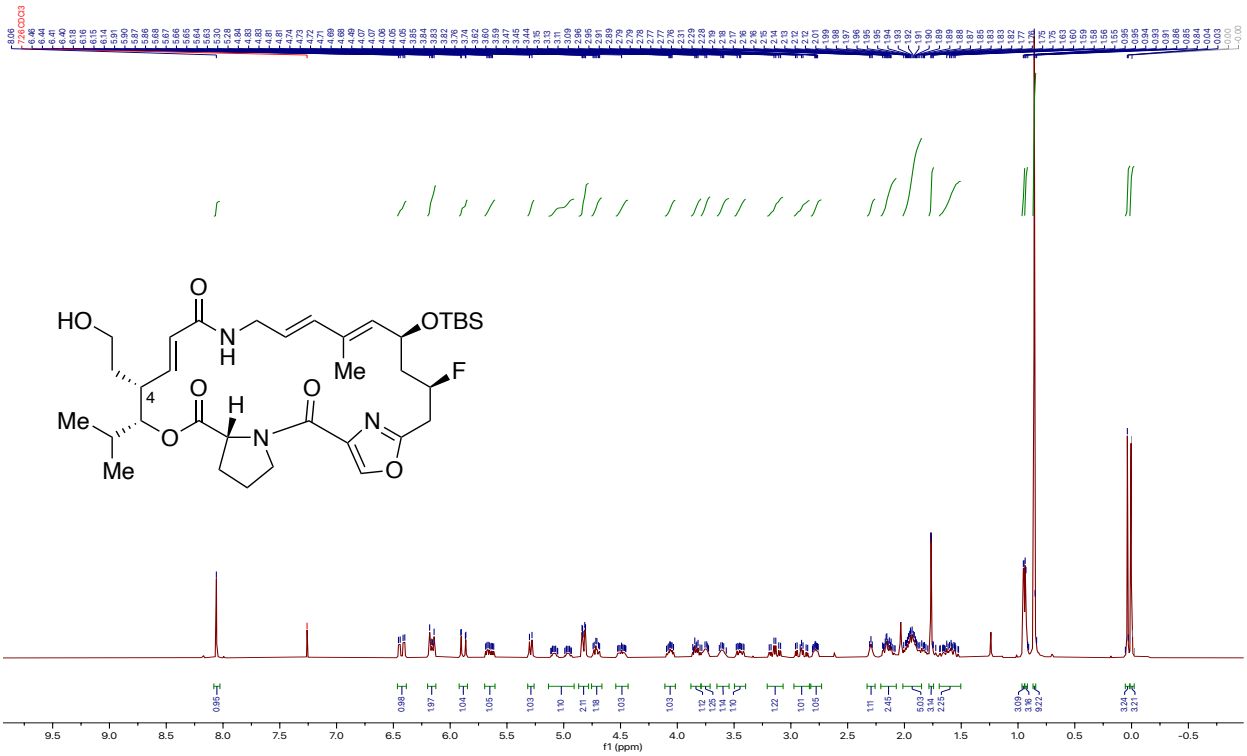
Compound **SI-7**: ^{1}H NMR (400 MHz, $CDCl_3$)



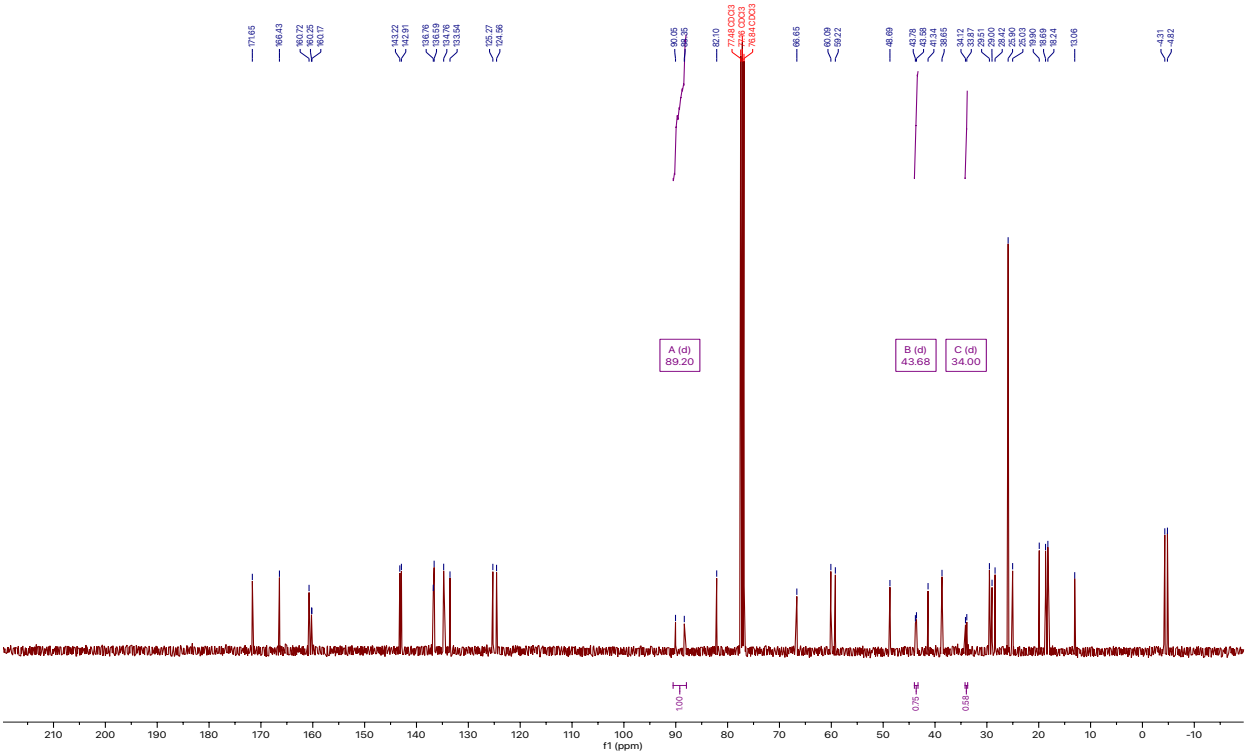
Compound **SI-7**: ^{13}C NMR (100 MHz, $CDCl_3$)



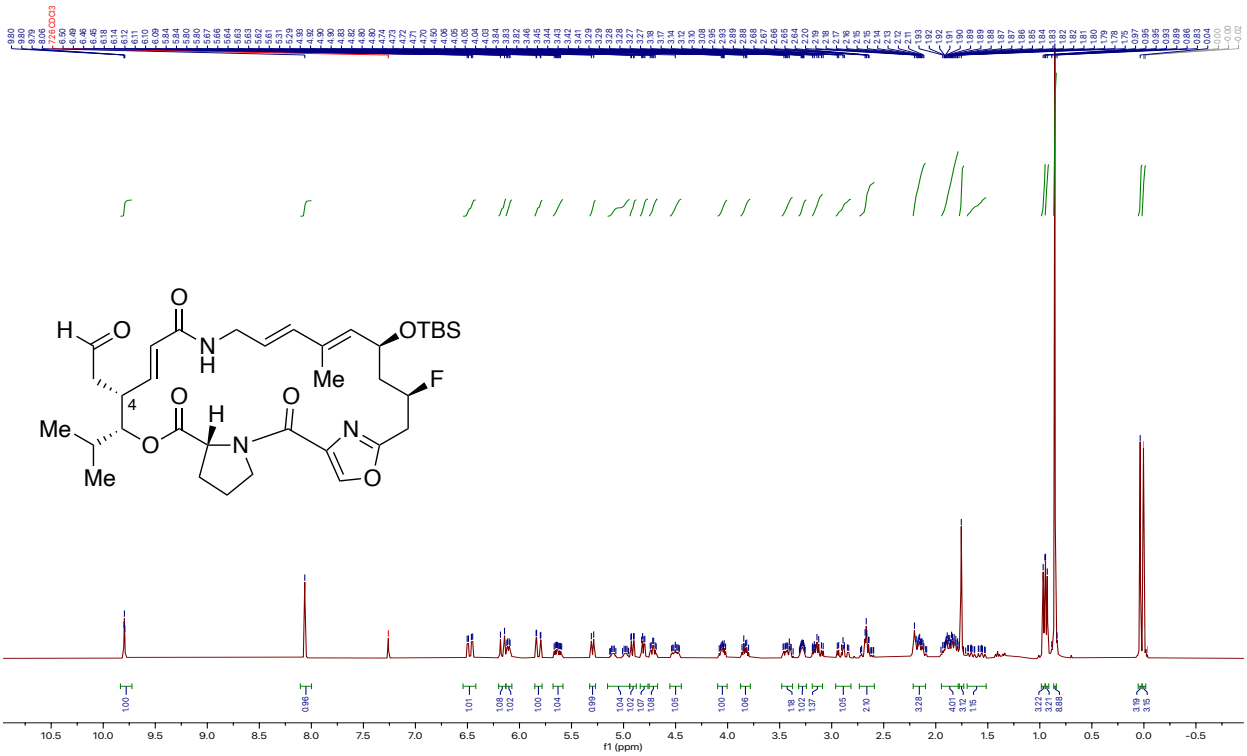
Compound **35**: ^{1}H NMR (400 MHz, $CDCl_3$)



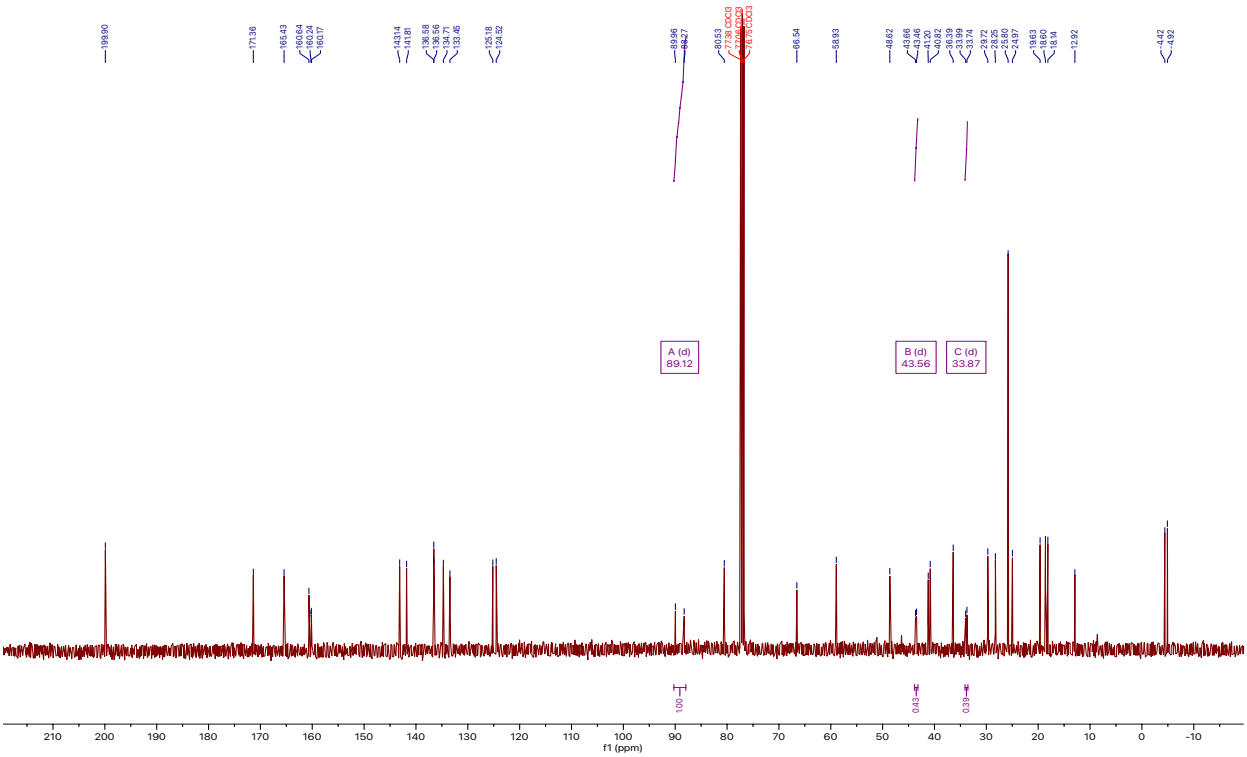
Compound **35**: ^{13}C NMR (100 MHz, $CDCl_3$)



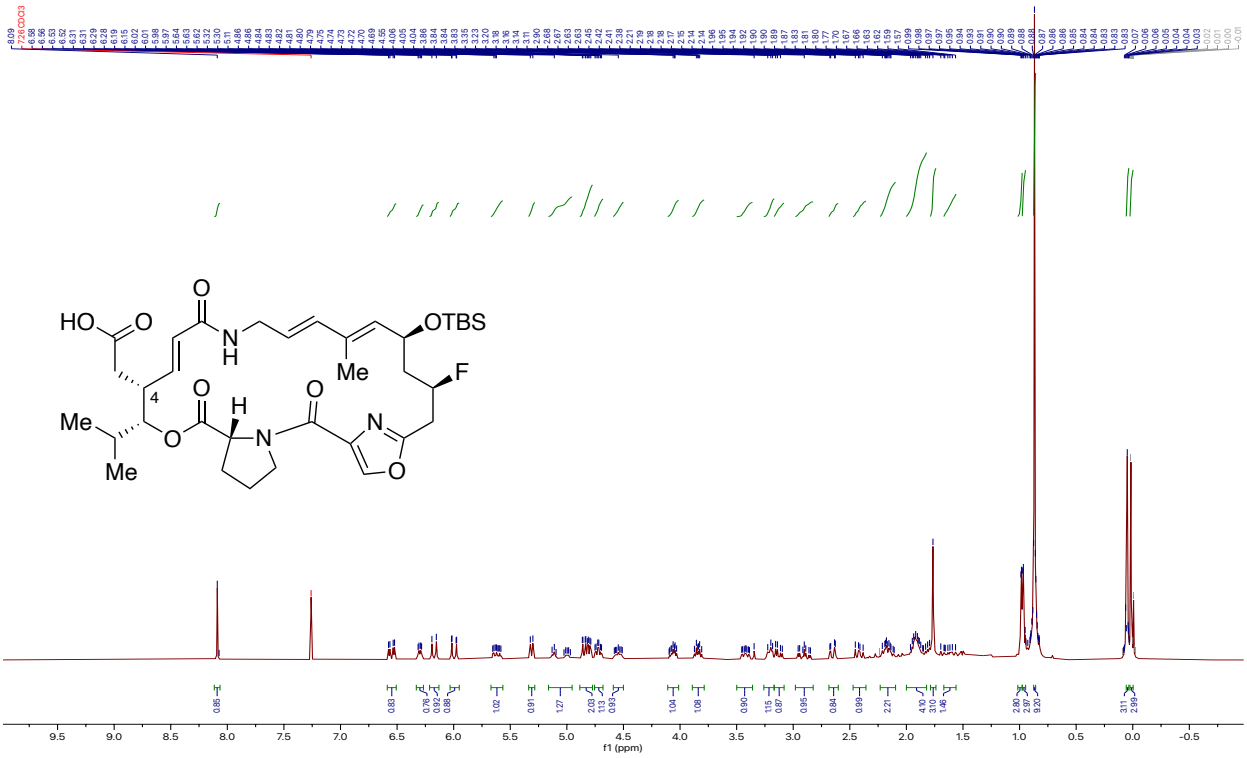
Compound **SI-8**: ^{1}H NMR (400 MHz, $CDCl_3$)



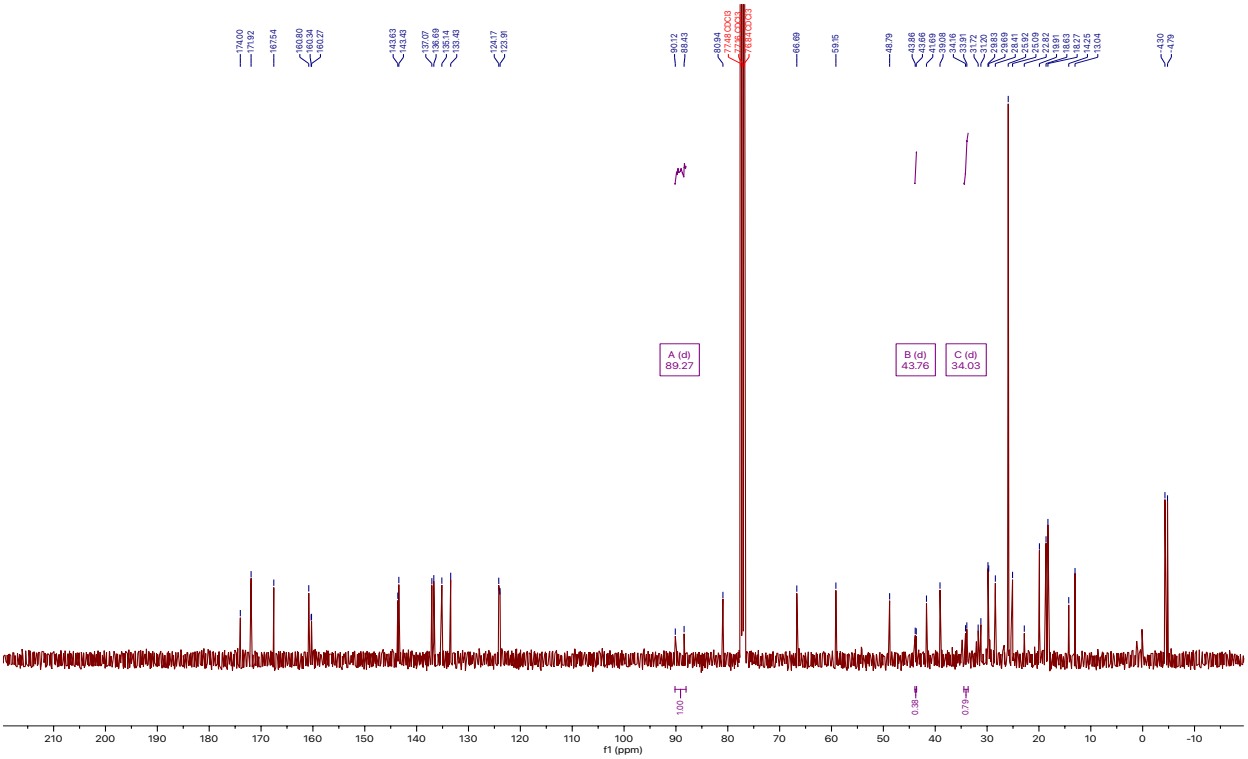
Compound **SI-8**: ^{13}C NMR (100 MHz, $CDCl_3$)



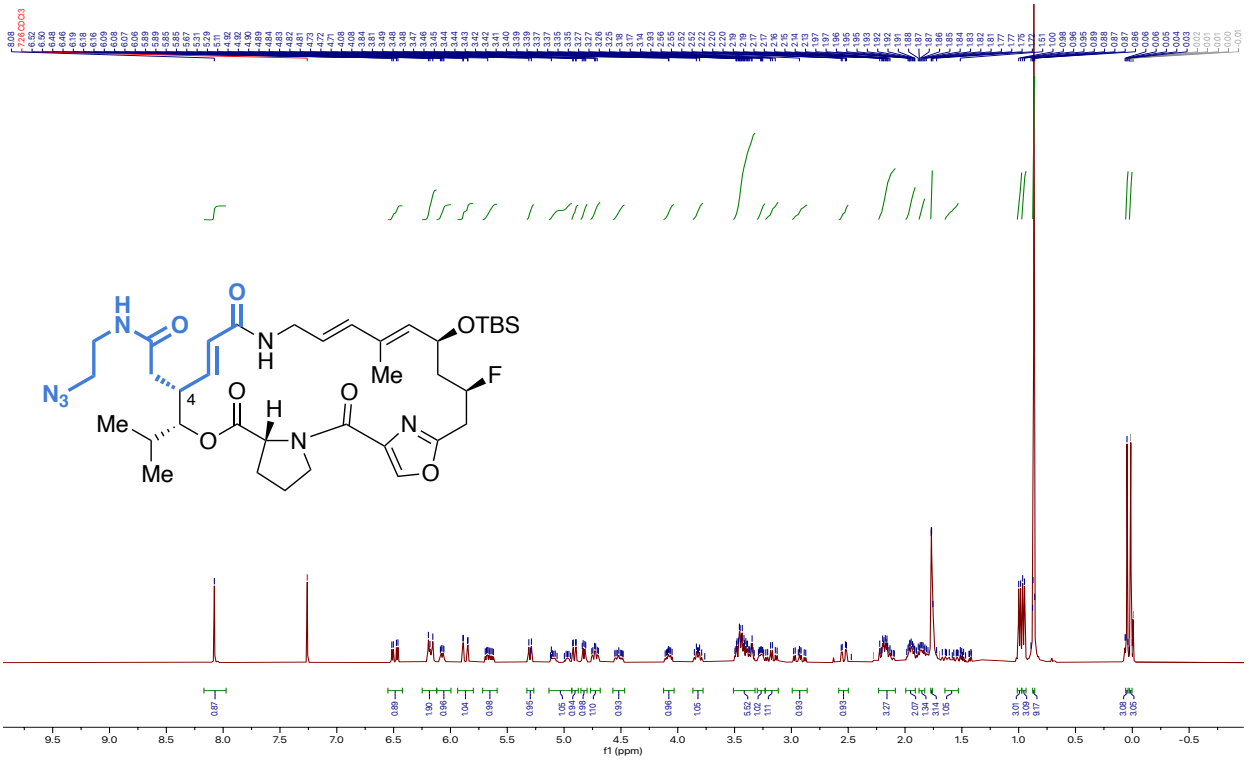
Compound **36**: ^{1}H NMR (400 MHz, $CDCl_3$)



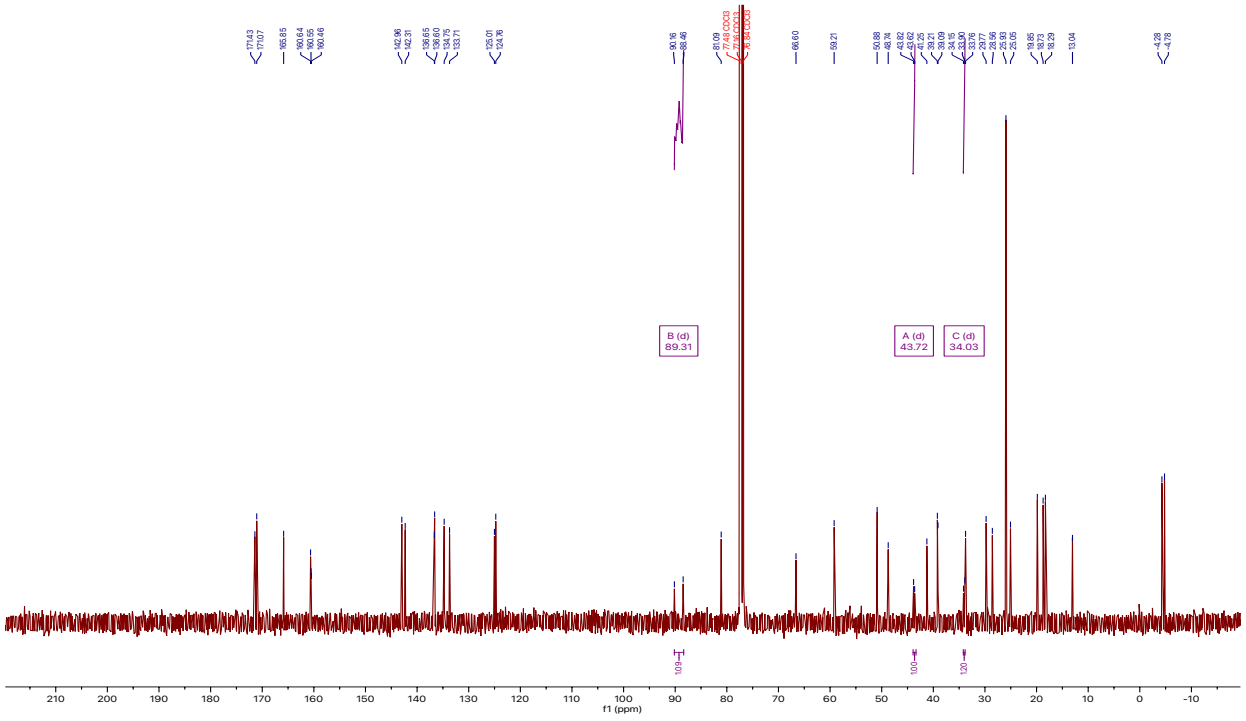
Compound **36**: ^{13}C NMR (100 MHz, $CDCl_3$)



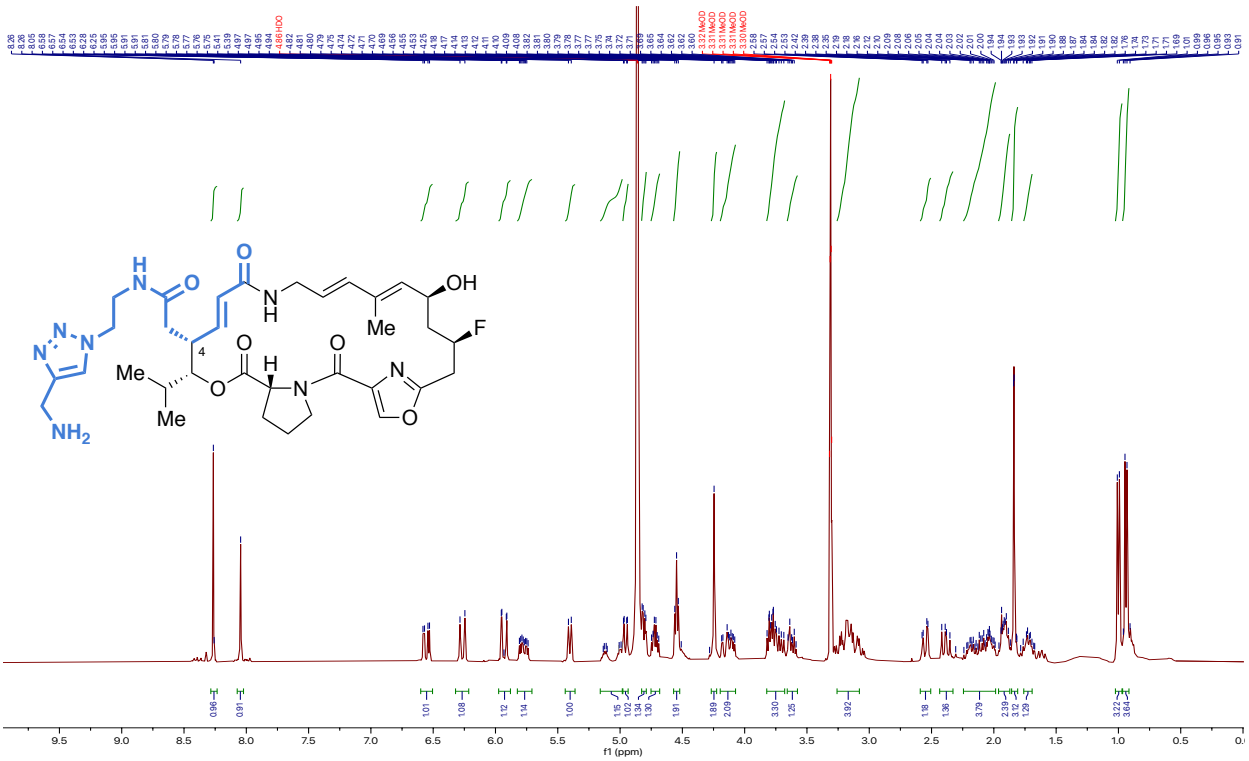
Compound **38**: ^{1}H NMR (400 MHz, $CDCl_3$)



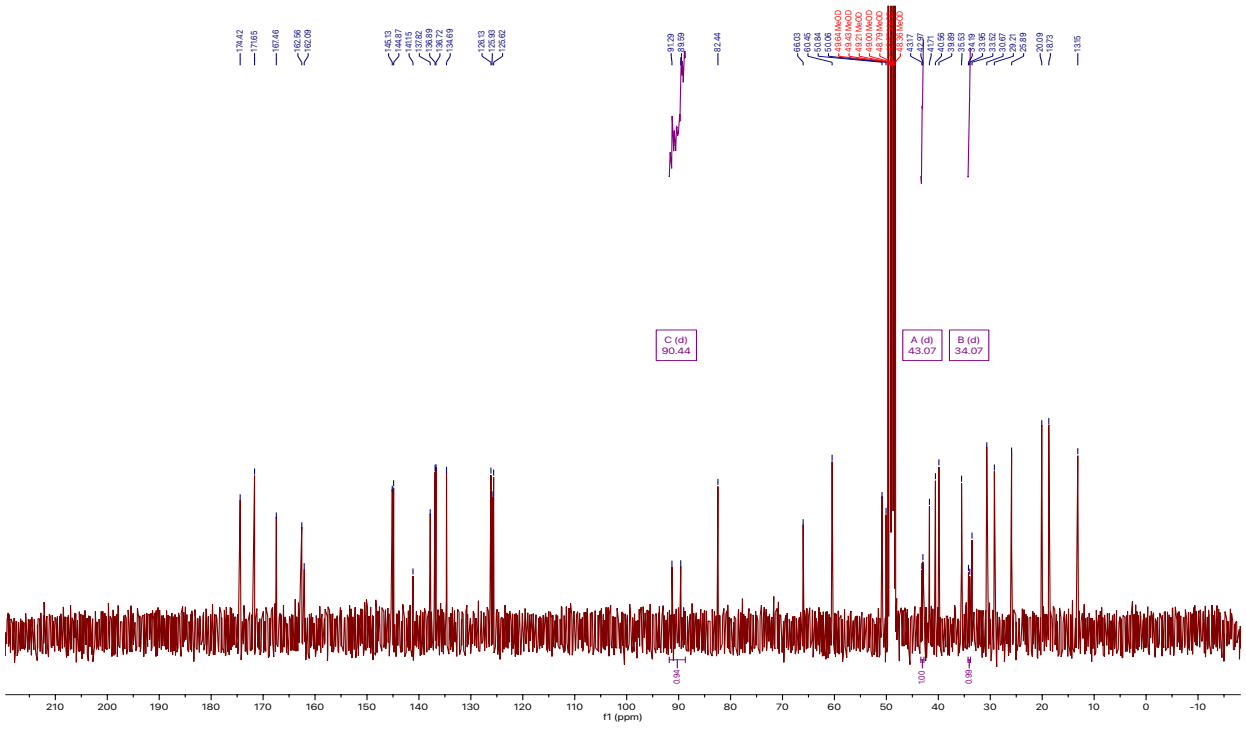
Compound **38**: ^{13}C NMR (100 MHz, $CDCl_3$)



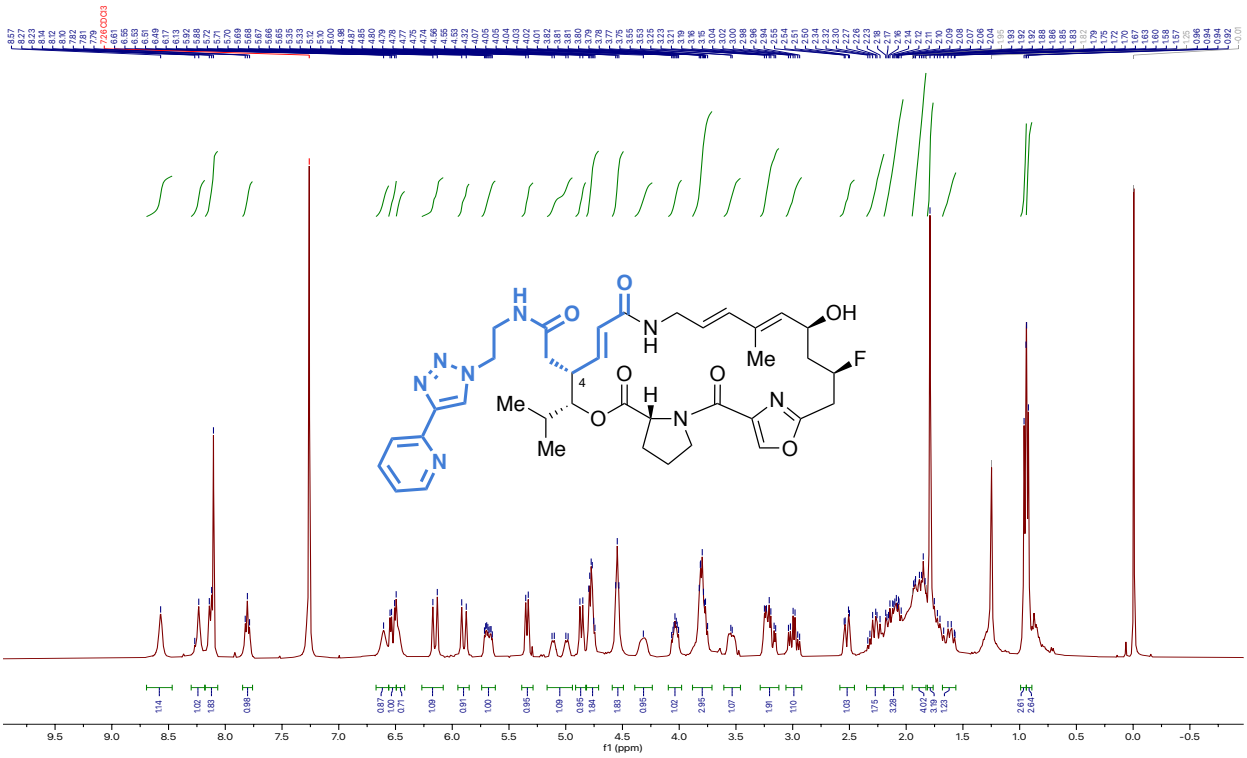
Compound **39a**: ^{1}H NMR (400 MHz, MeOD)



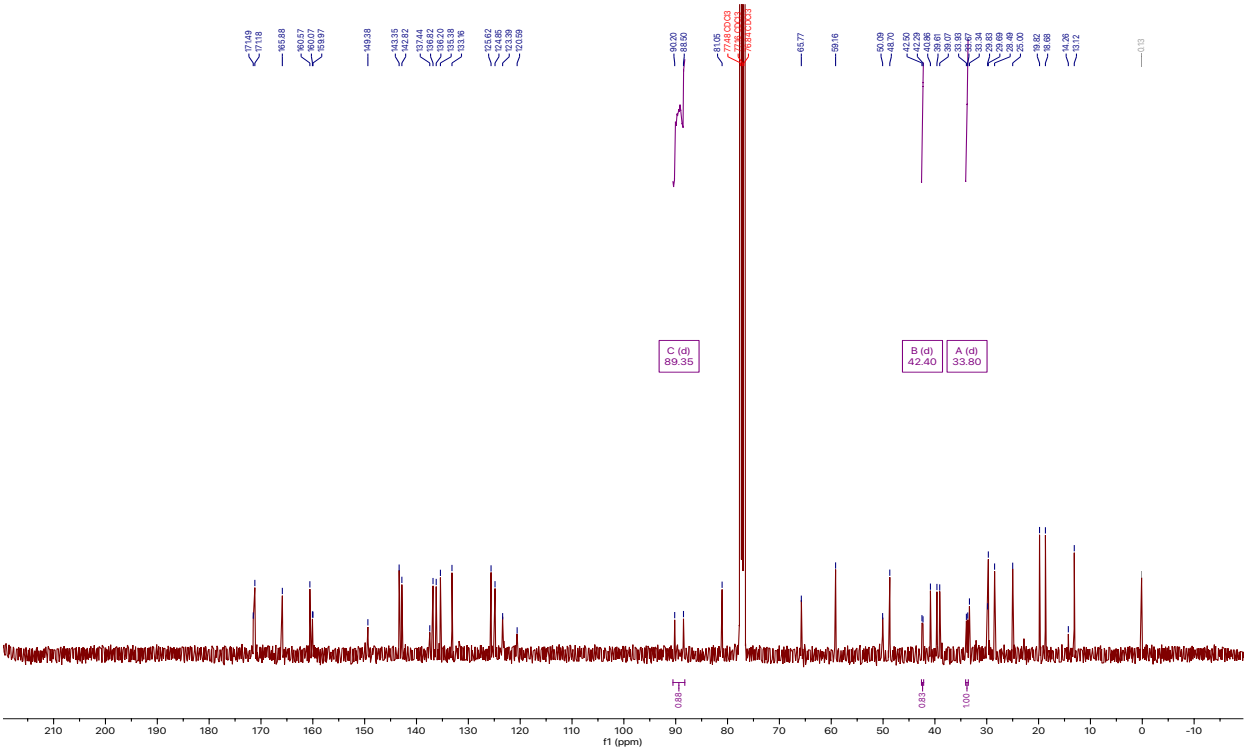
Compound **39a**: ^{13}C NMR (100 MHz, MeOD)



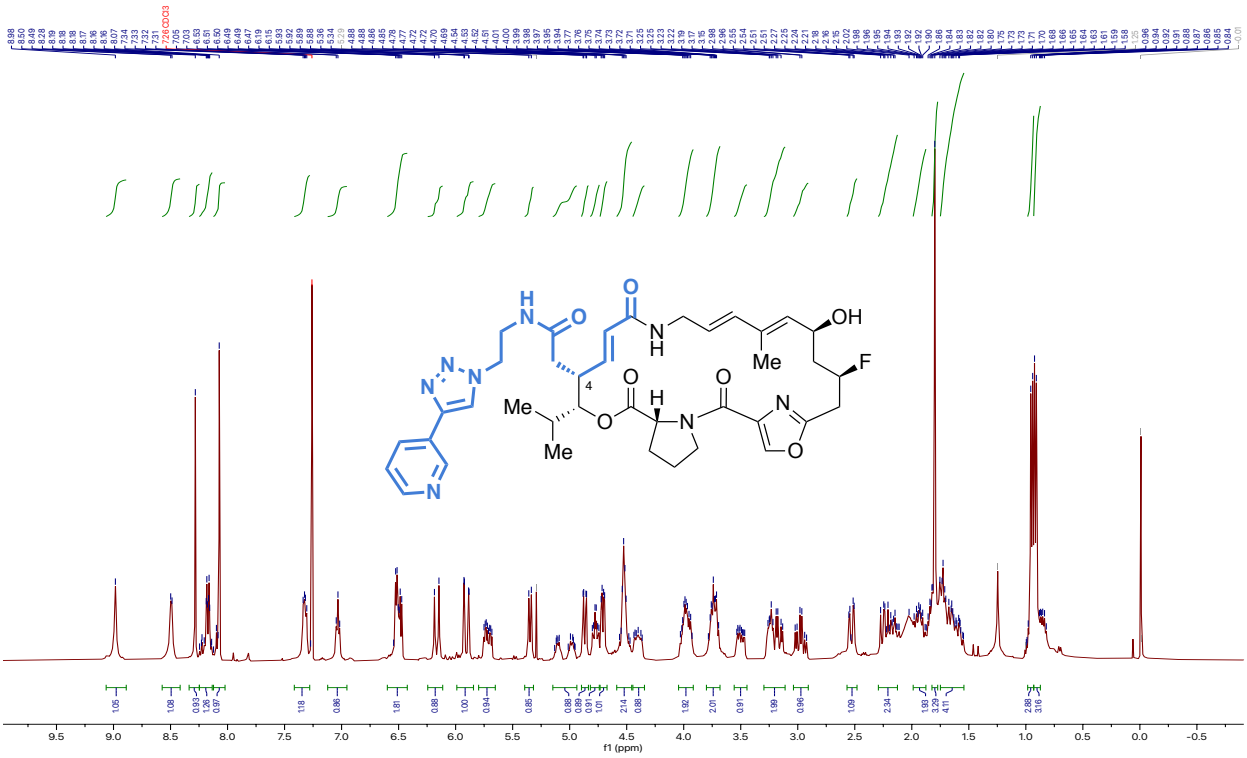
Compound **39b**: ^{1}H NMR (400 MHz, $CDCl_3$)



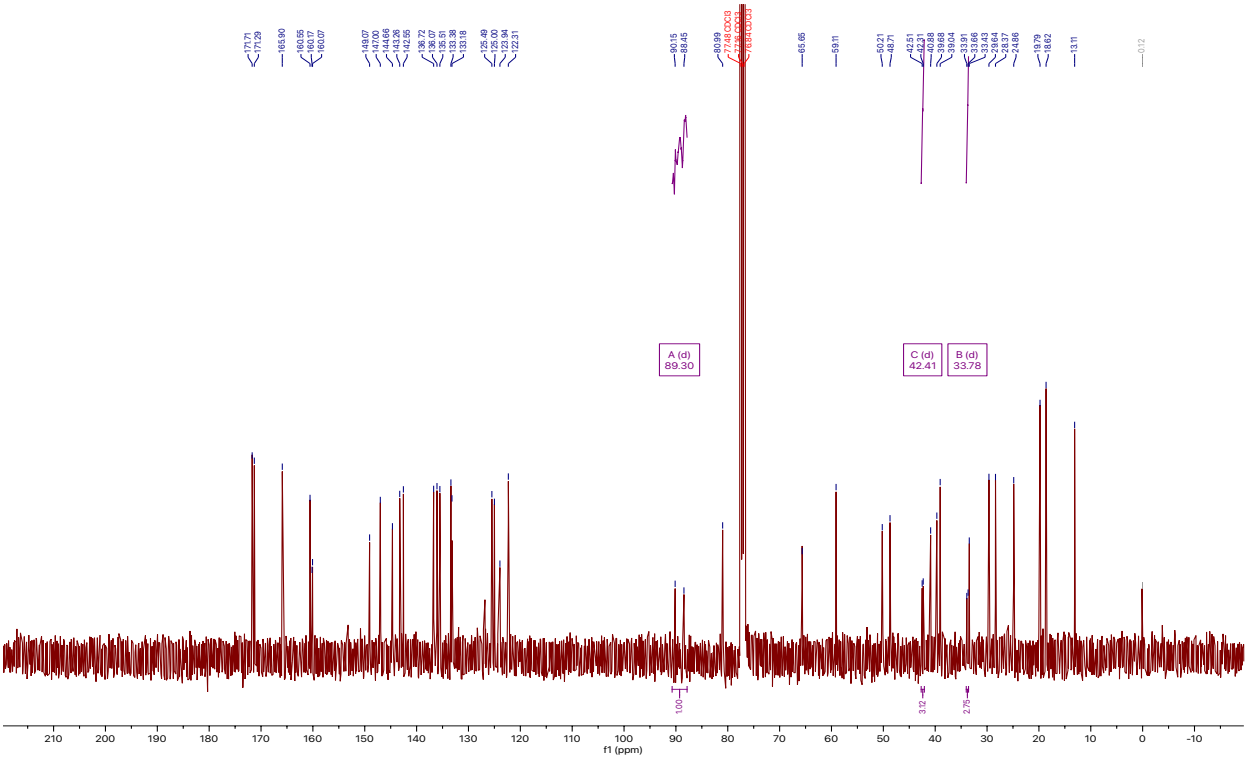
Compound **39b**: ^{13}C NMR (100 MHz, $CDCl_3$)



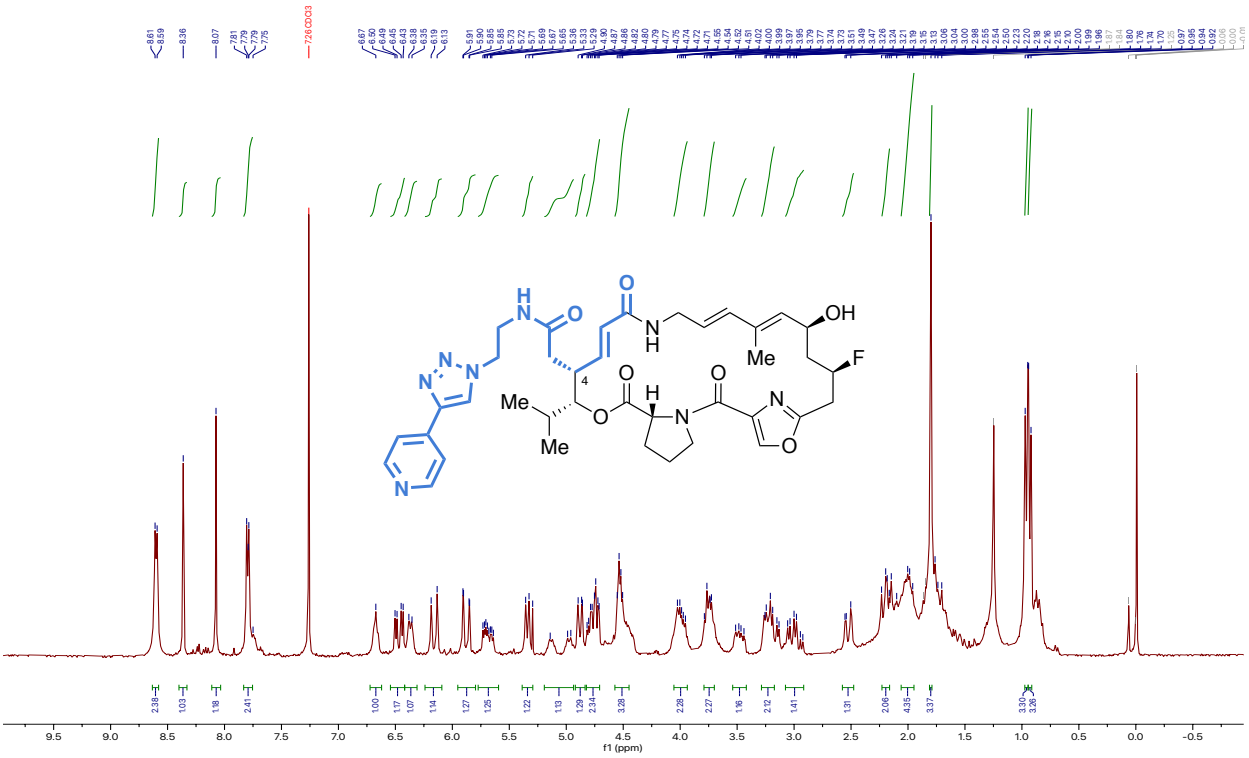
Compound **39c**: ^{1}H NMR (400 MHz, $CDCl_3$)



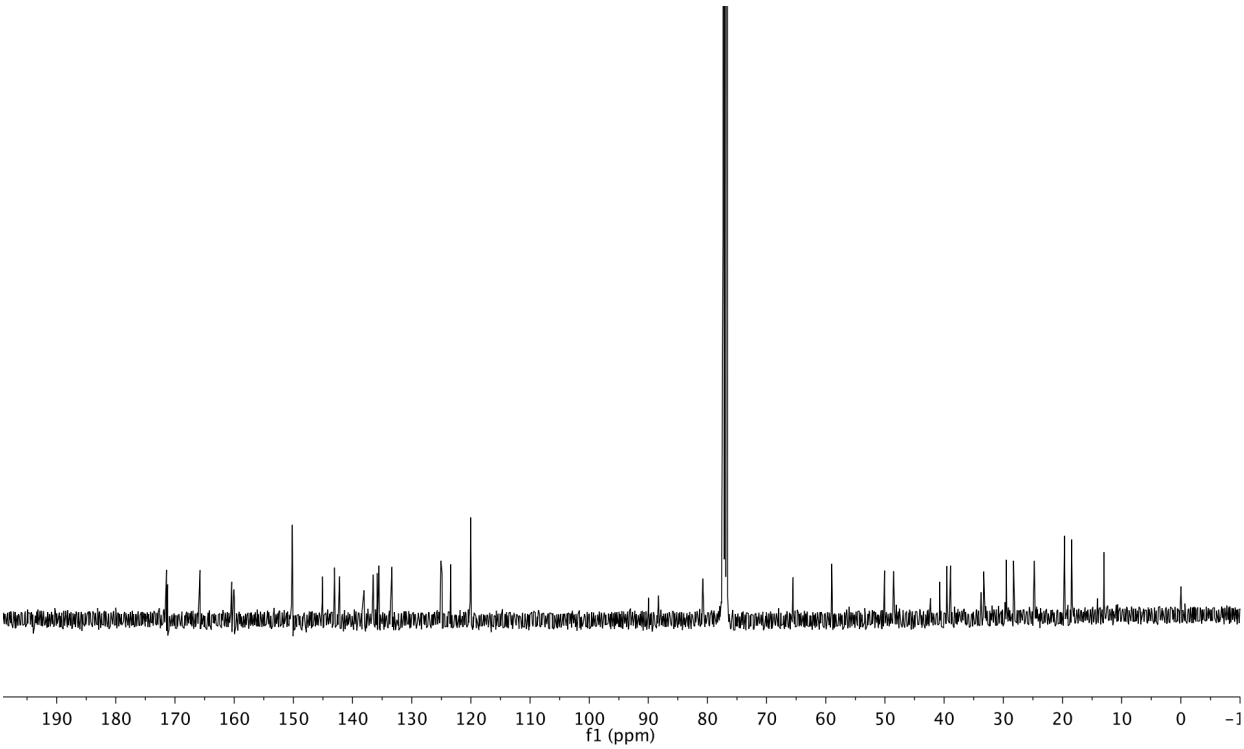
Compound **39c**: ^{13}C NMR (100 MHz, $CDCl_3$)



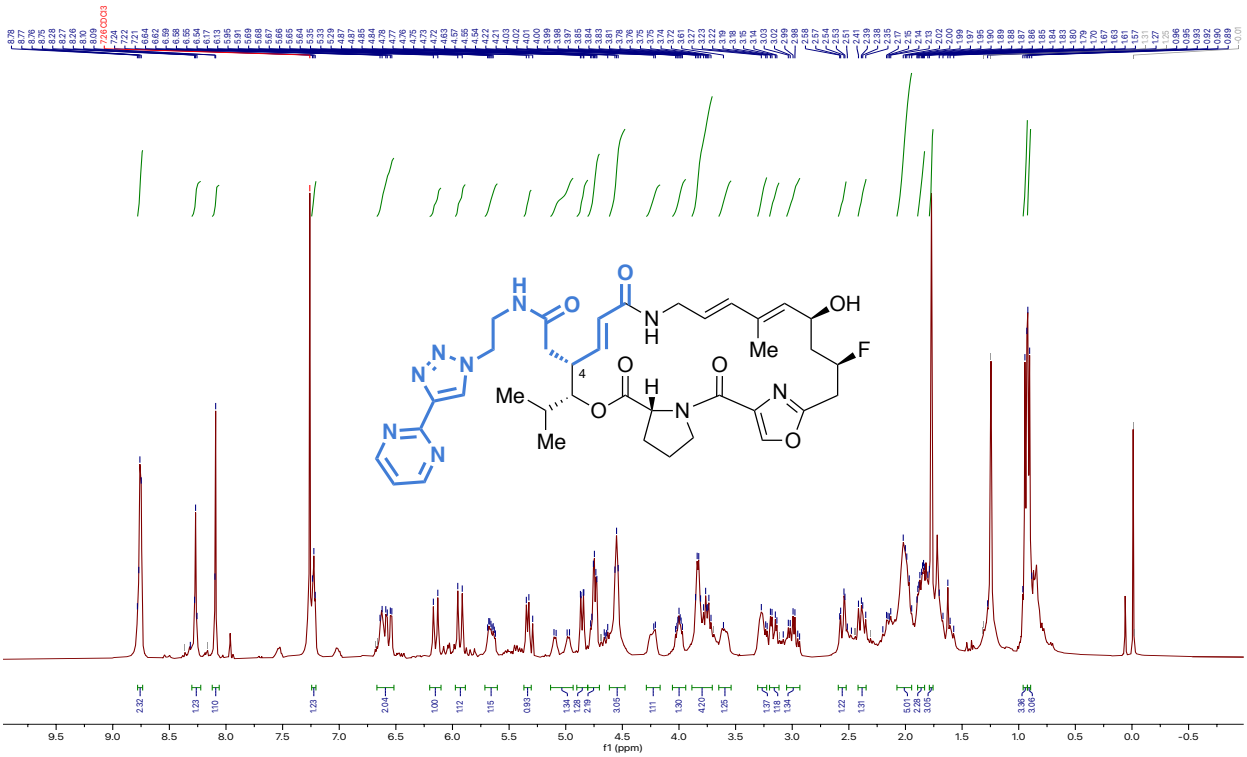
Compound **39d**: ^{1}H NMR (400 MHz, $CDCl_3$)



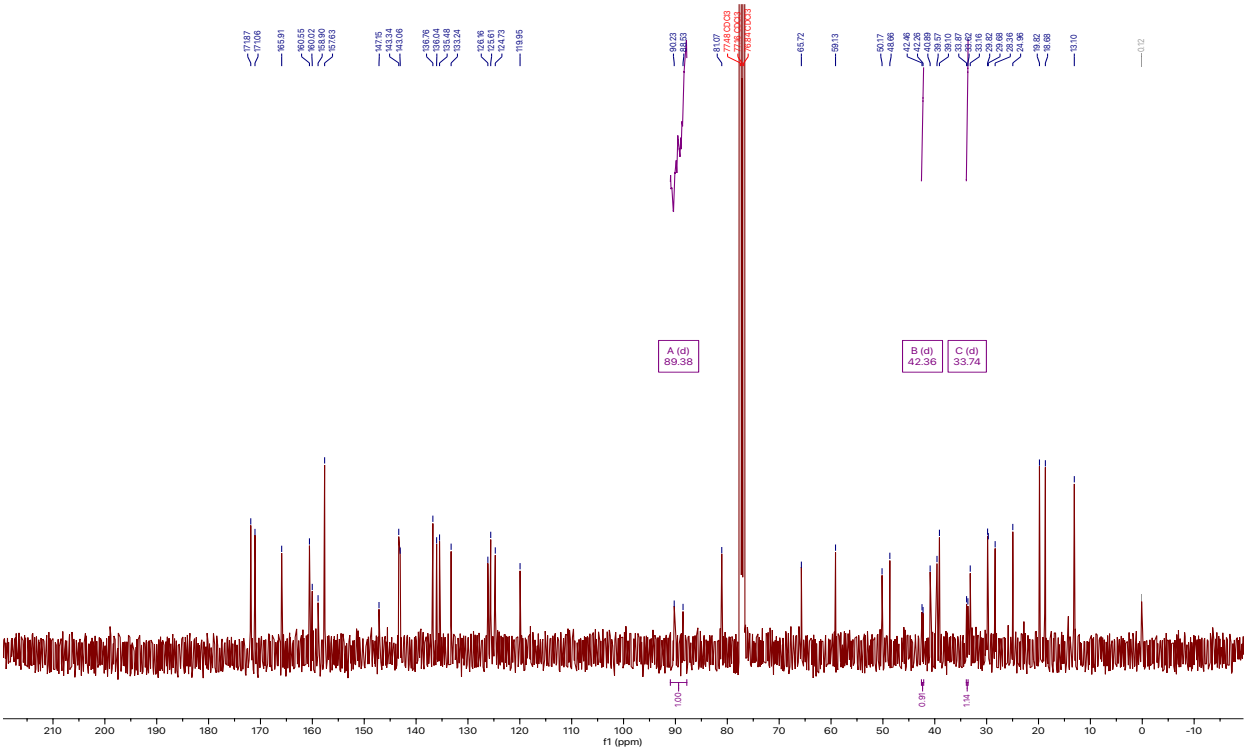
Compound **39d**: ^{13}C NMR (100 MHz, $CDCl_3$)



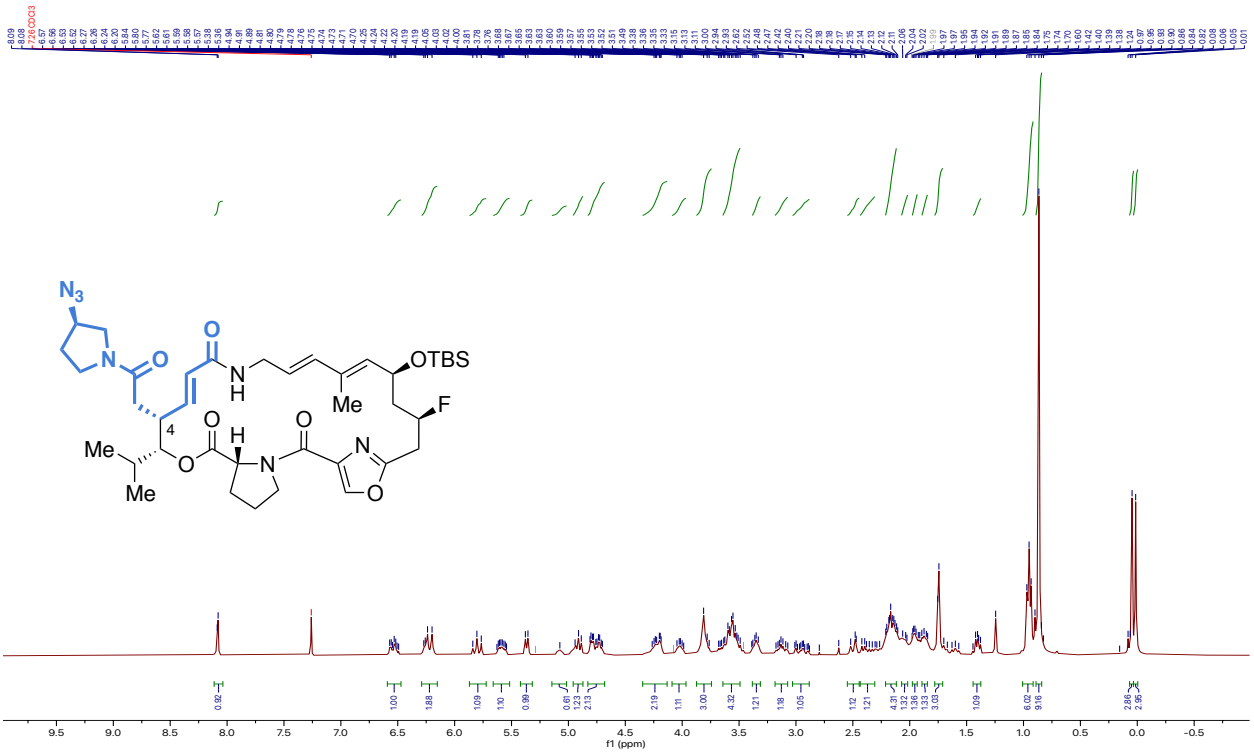
Compound **39e**: ^{1}H NMR (400 MHz, $CDCl_3$)



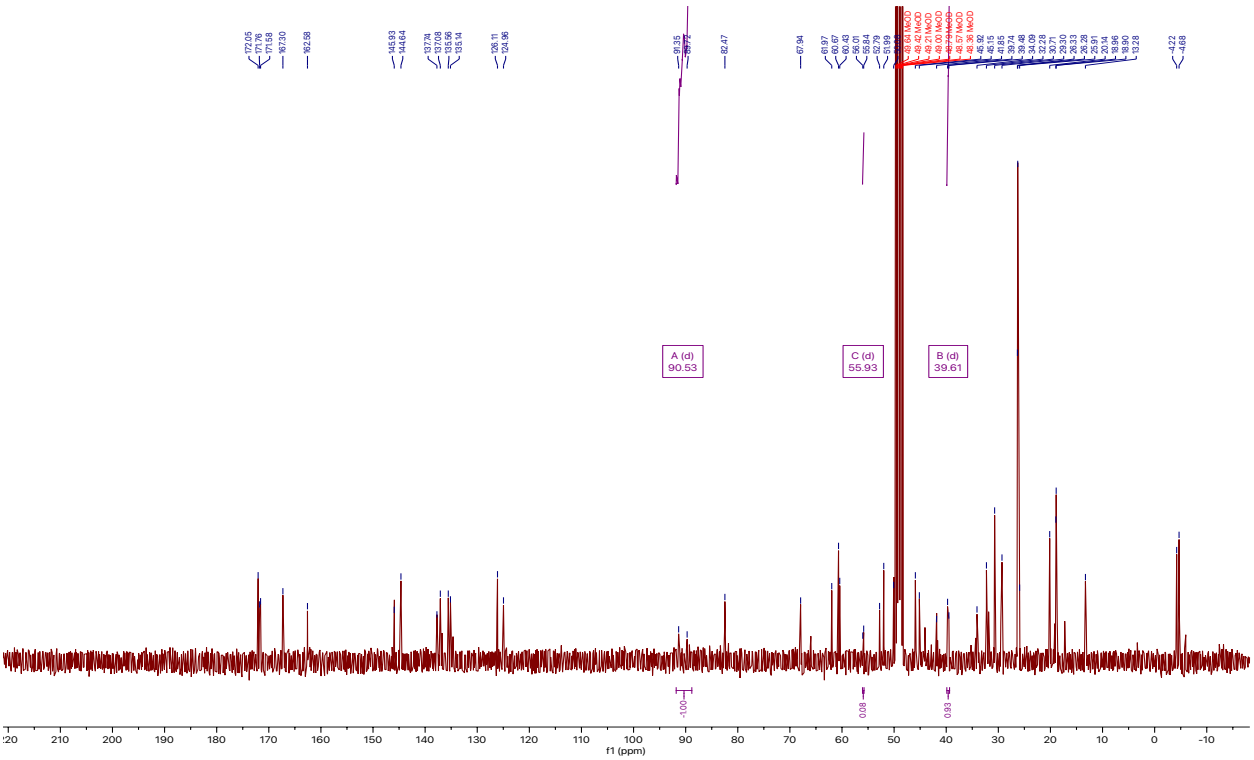
Compound **39e**: ^{13}C NMR (100 MHz, $CDCl_3$)



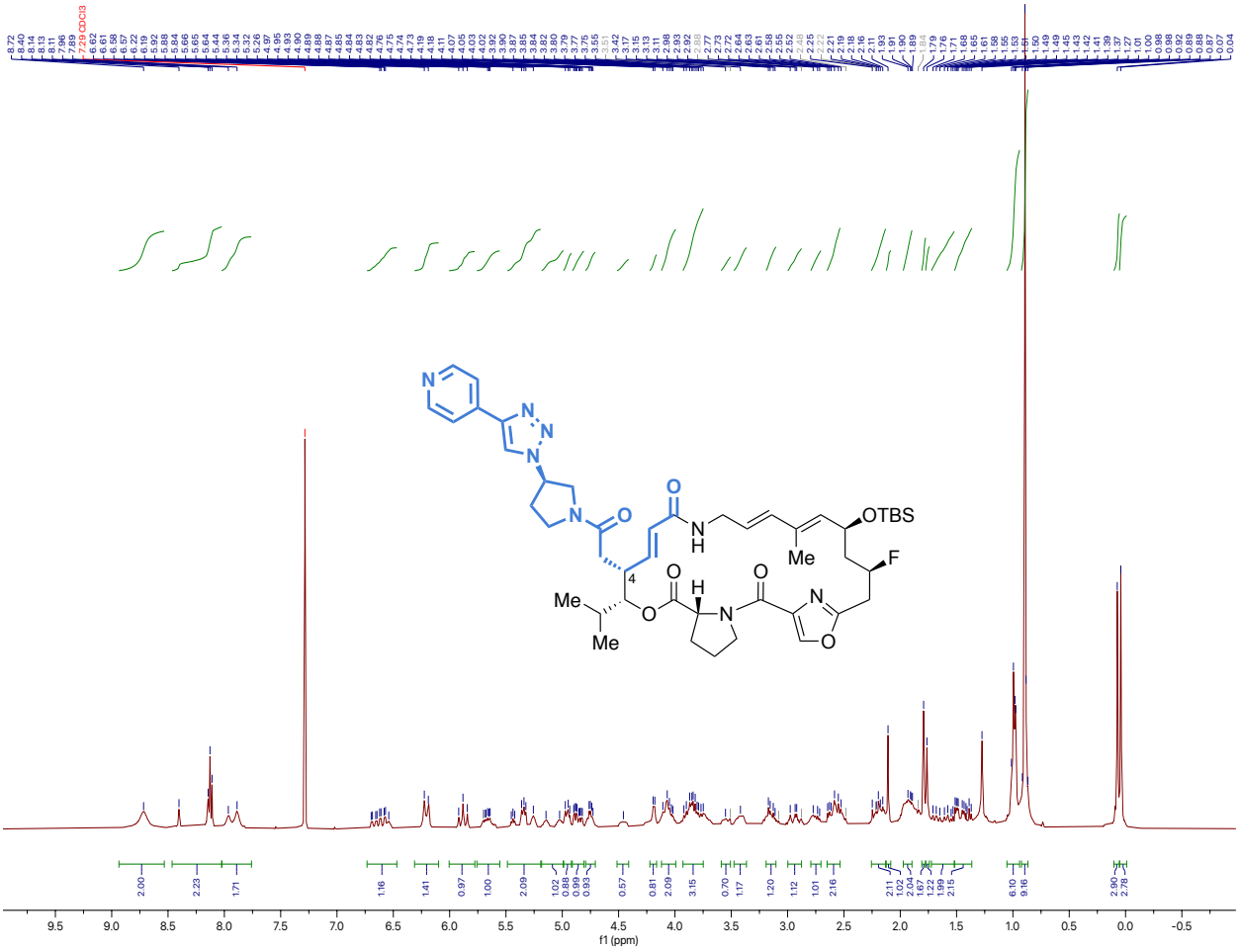
Compound **SI-14**: ^{1}H NMR (400 MHz, $CDCl_3$)



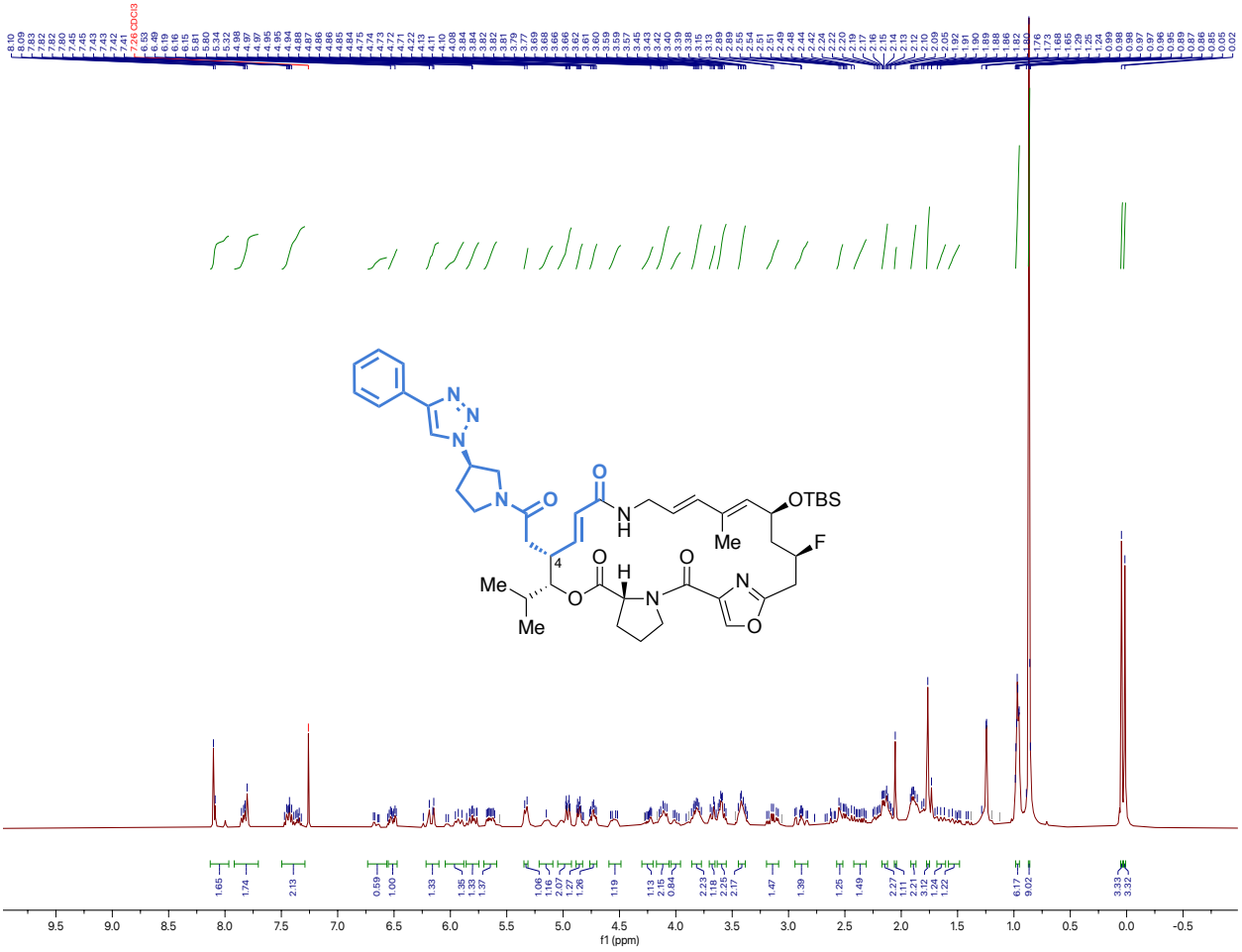
Compound **SI-14**: ^{13}C NMR (100 MHz, MeOD)



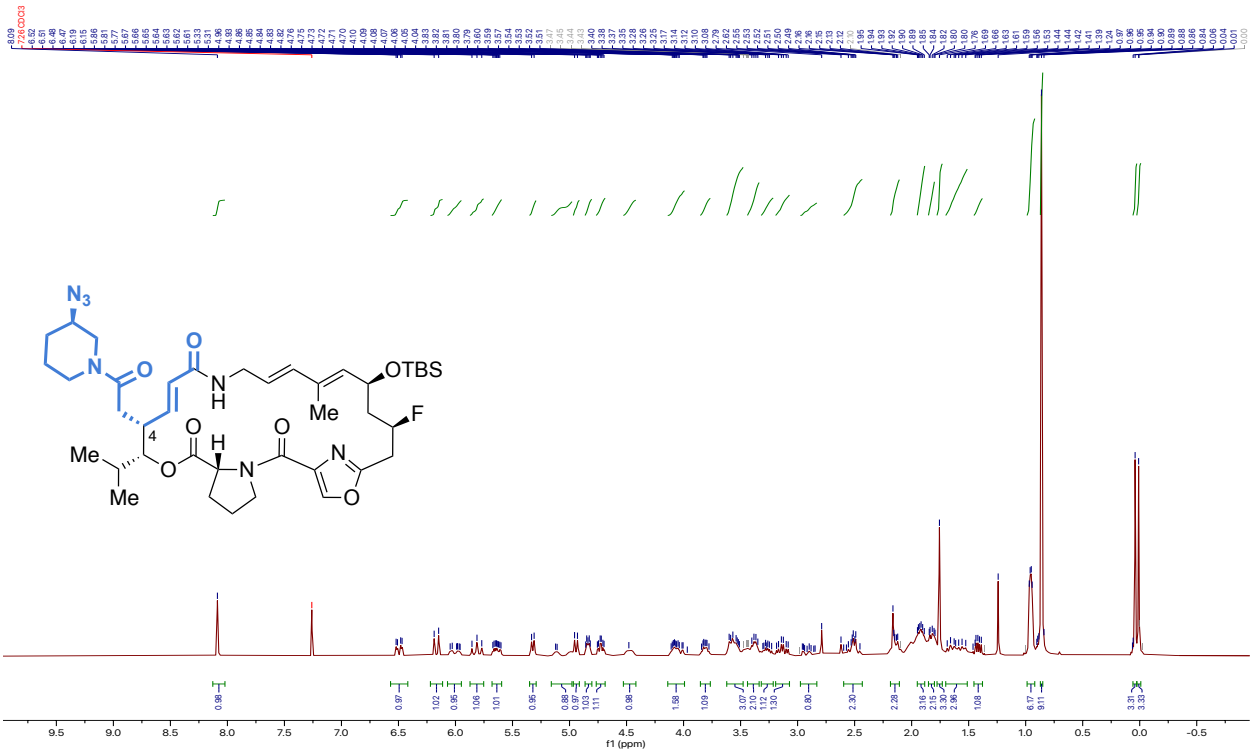
Compound **45a TBS**: ^{1}H NMR (400 MHz, $CDCl_3$)



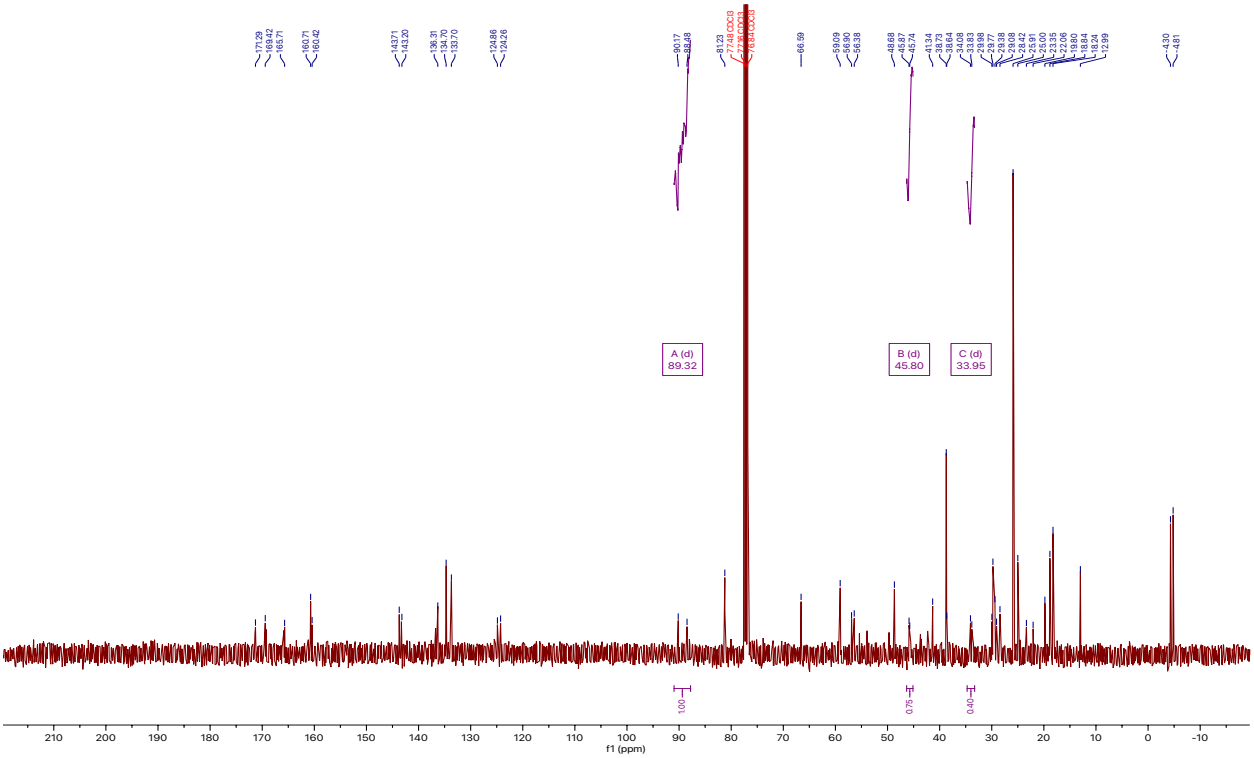
Compound **45b TBS**: ^{1}H NMR (400 MHz, $CDCl_3$)



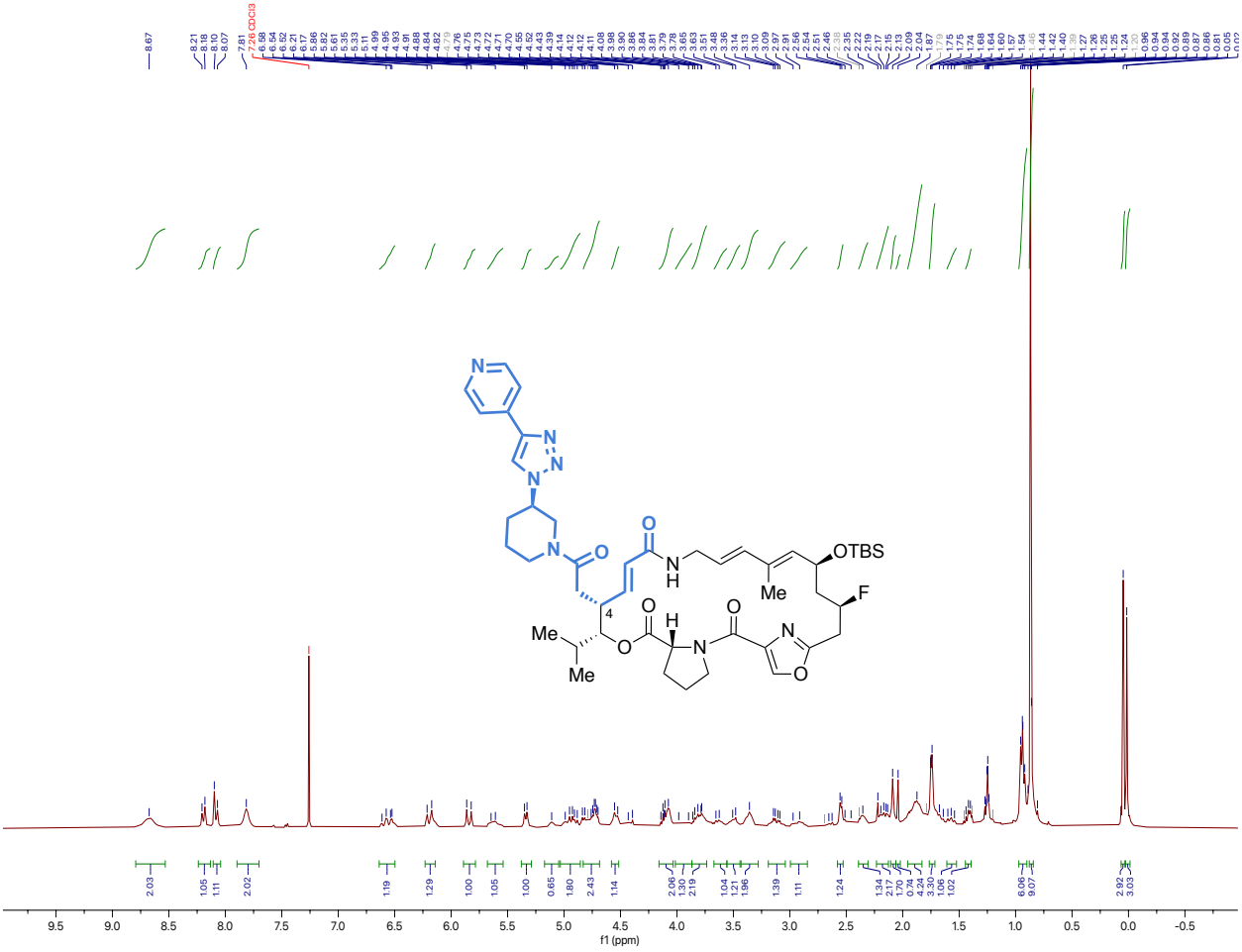
Compound **SI-16**: ^{1}H NMR (400 MHz, $CDCl_3$)



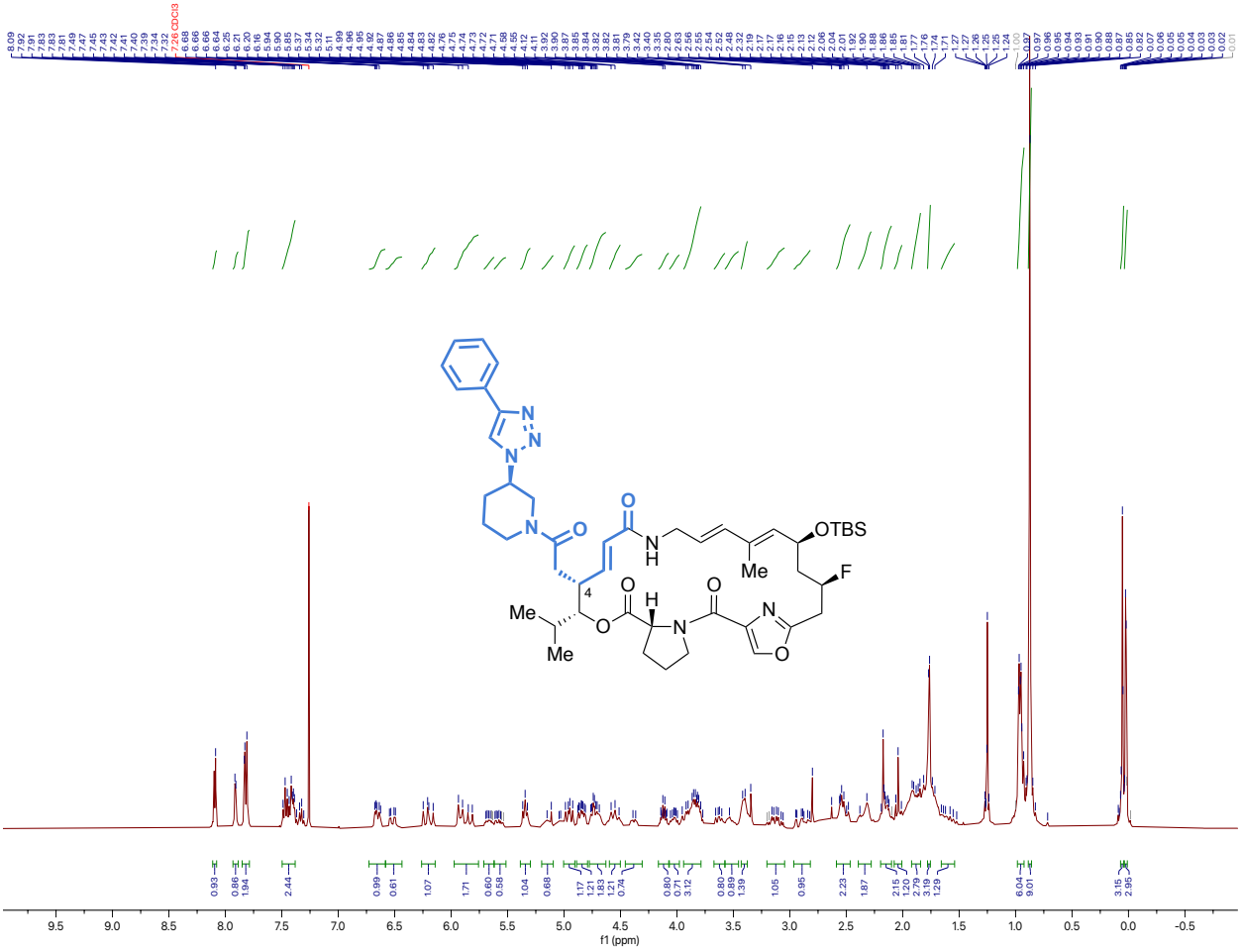
Compound **SI-16**: ^{13}C NMR (100 MHz, $CDCl_3$)



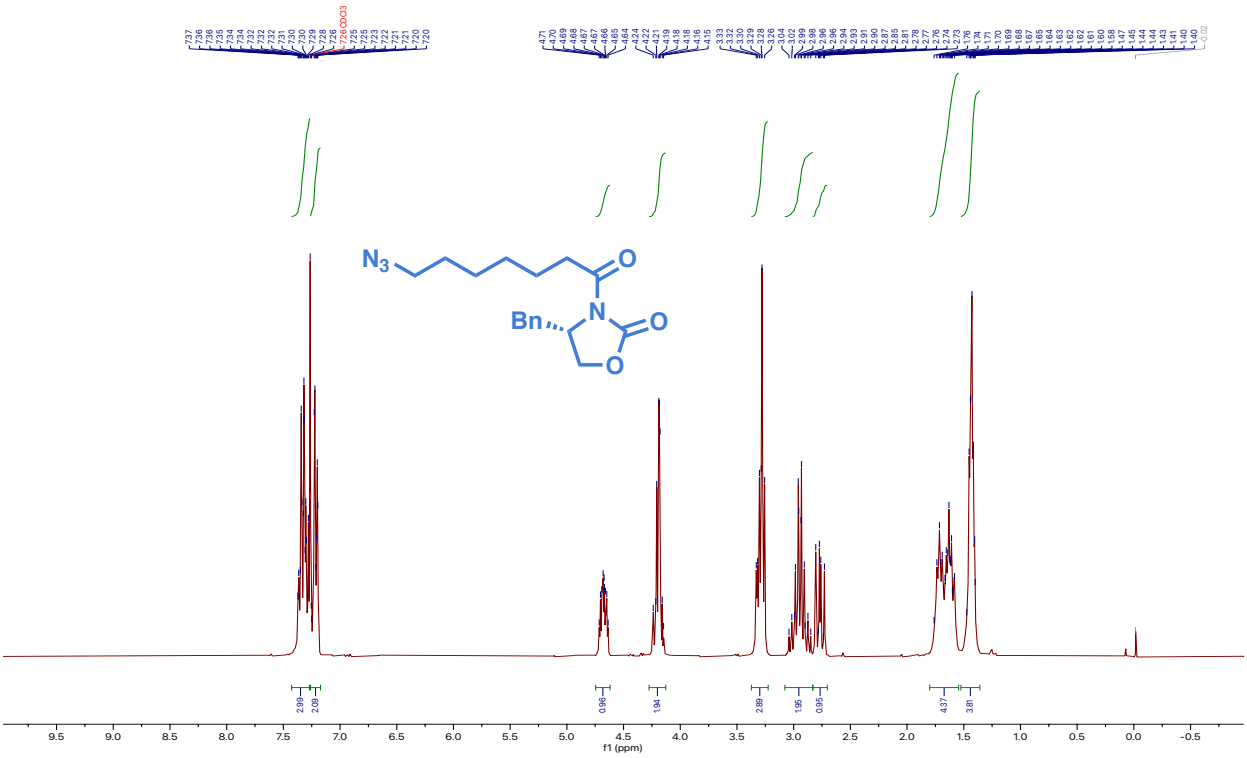
Compound **46a TBS**: ^{1}H NMR (400 MHz, $CDCl_3$)



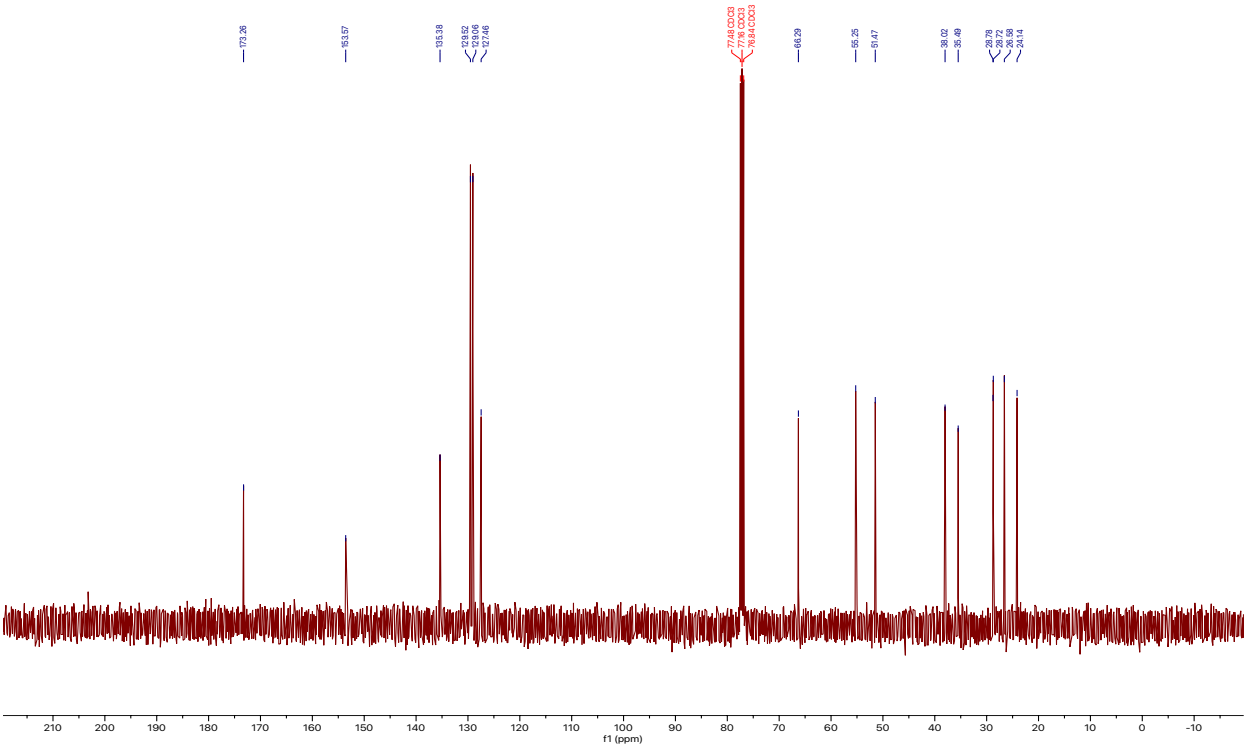
Compound **46b TBS**: ^{1}H NMR (400 MHz, $CDCl_3$)



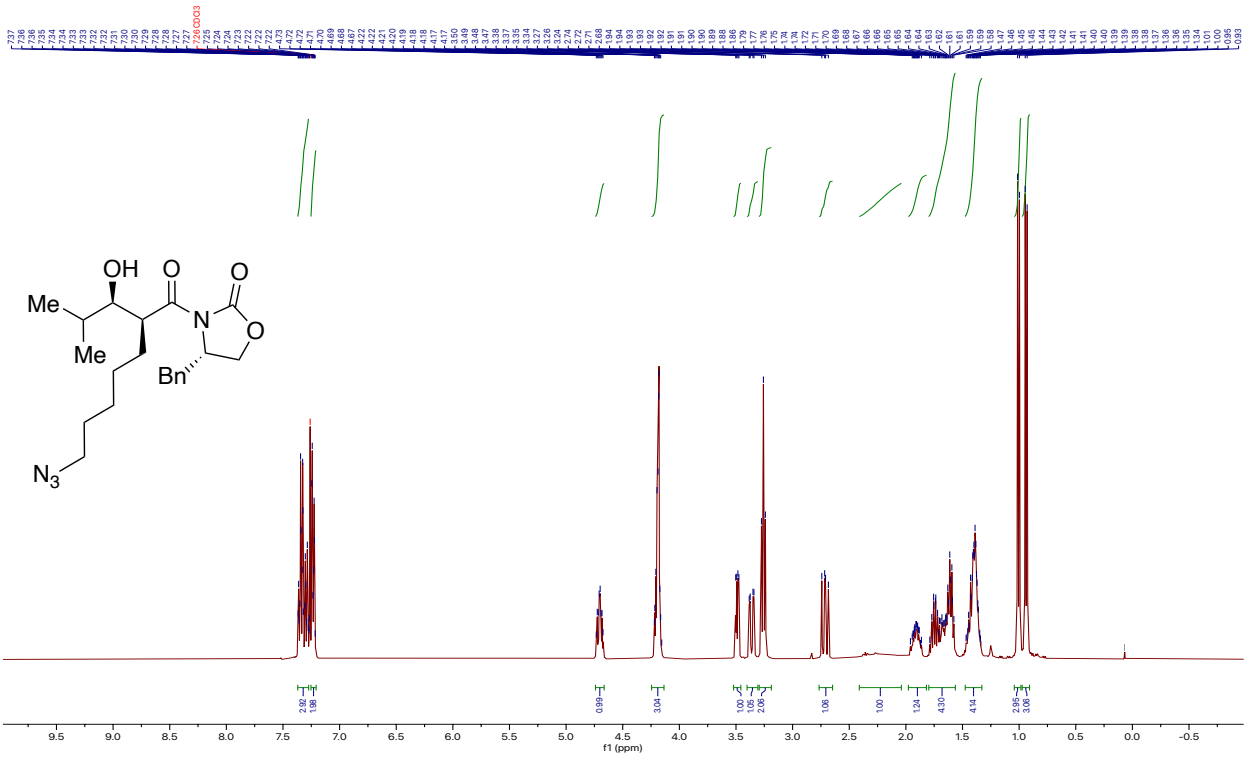
Compound **47**: ^{1}H NMR (300 MHz, $CDCl_3$)



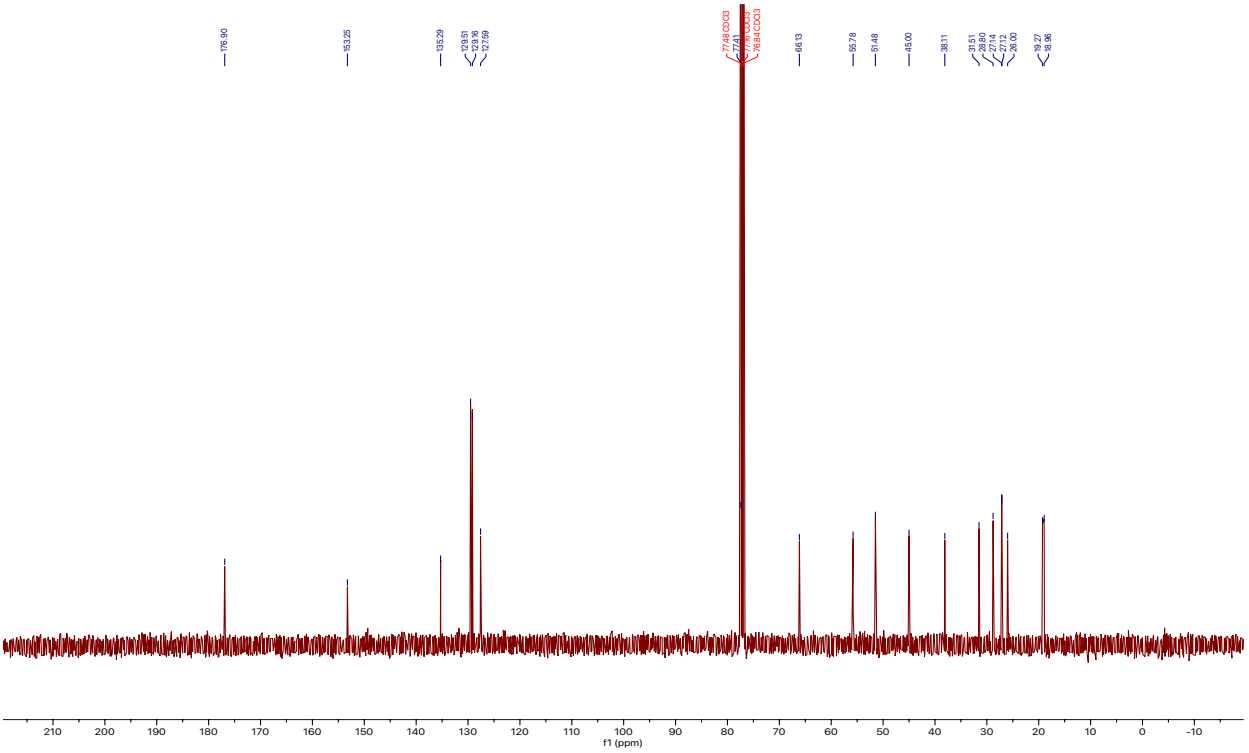
Compound **47**: ^{13}C NMR (100 MHz, $CDCl_3$)



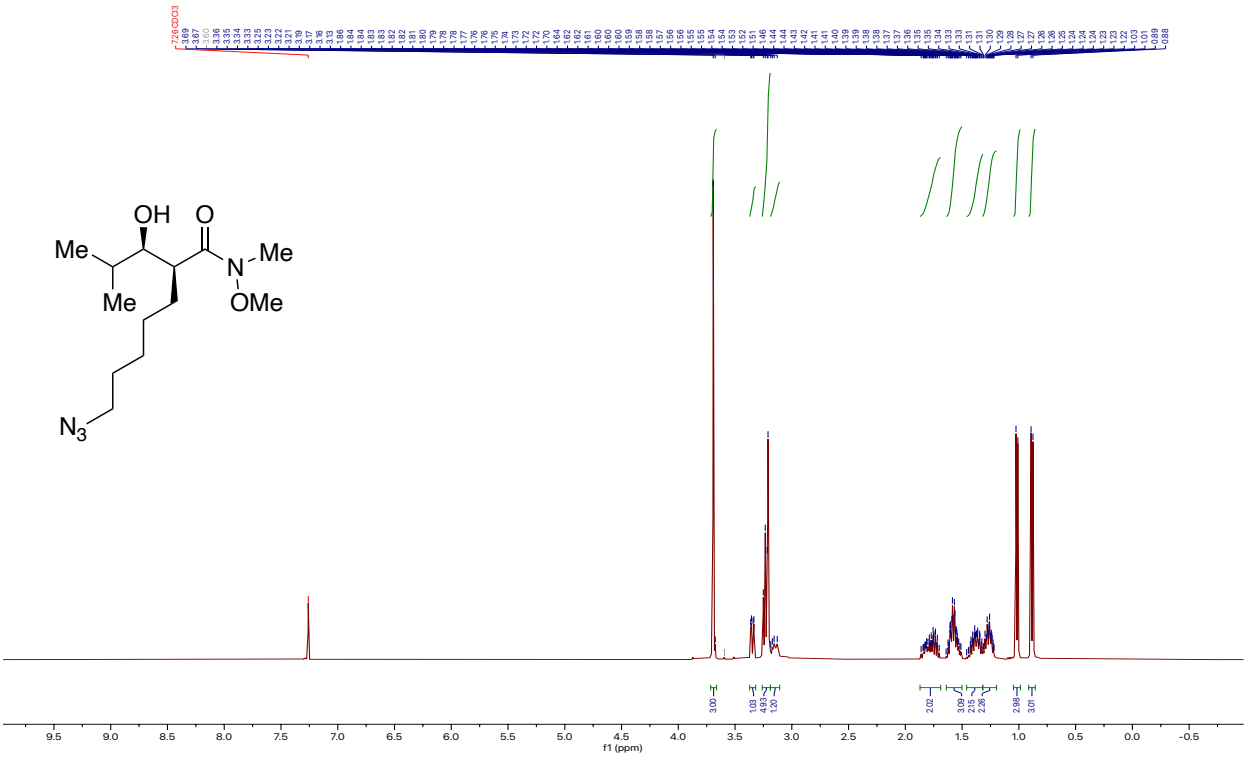
Compound **48**: ^{1}H NMR (400 MHz, $CDCl_3$)



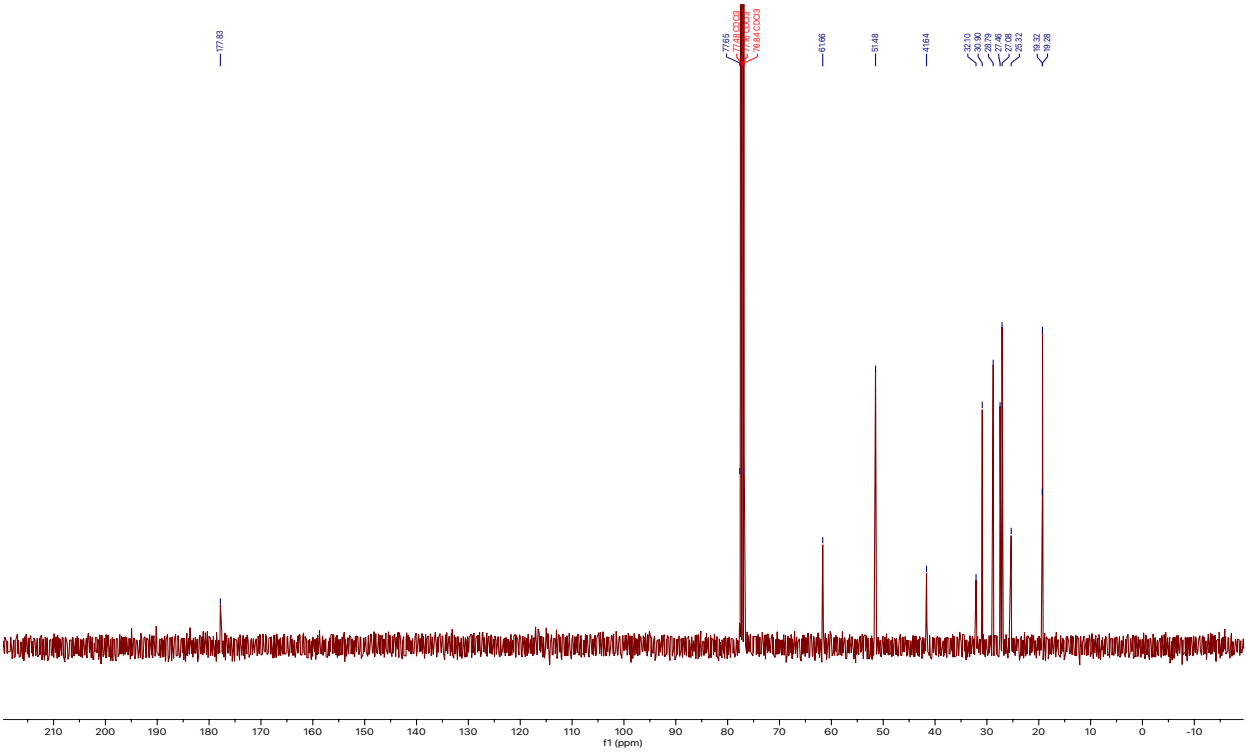
Compound **48**: ^{13}C NMR (100 MHz, $CDCl_3$)



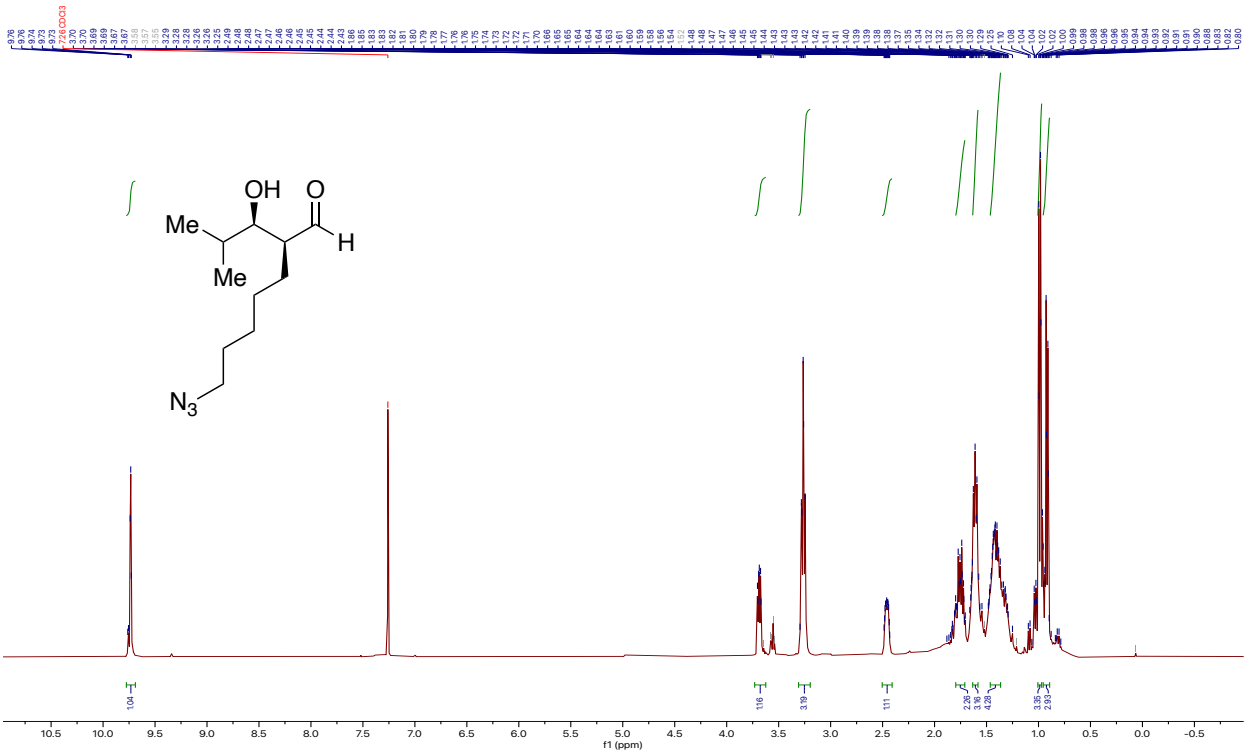
Compound **49**: ^{1}H NMR (400 MHz, $CDCl_3$)



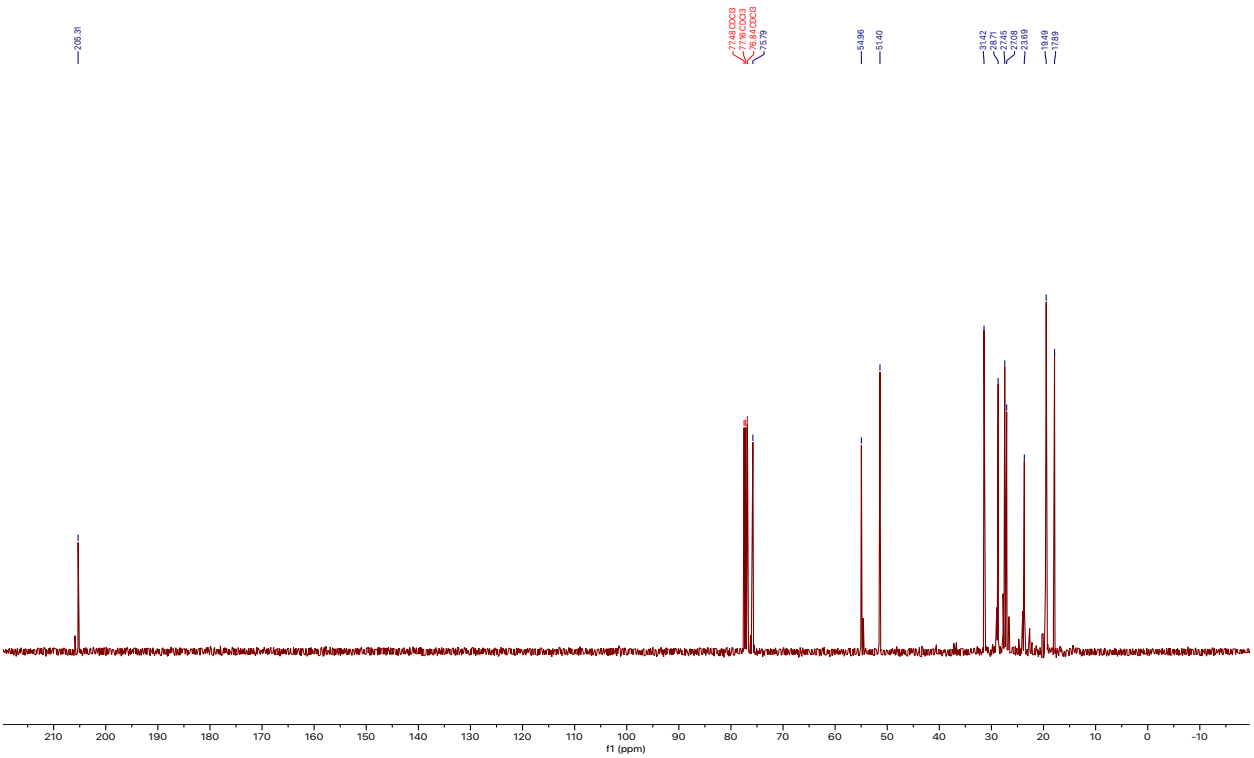
Compound **49**: ^{13}C NMR (100 MHz, $CDCl_3$)



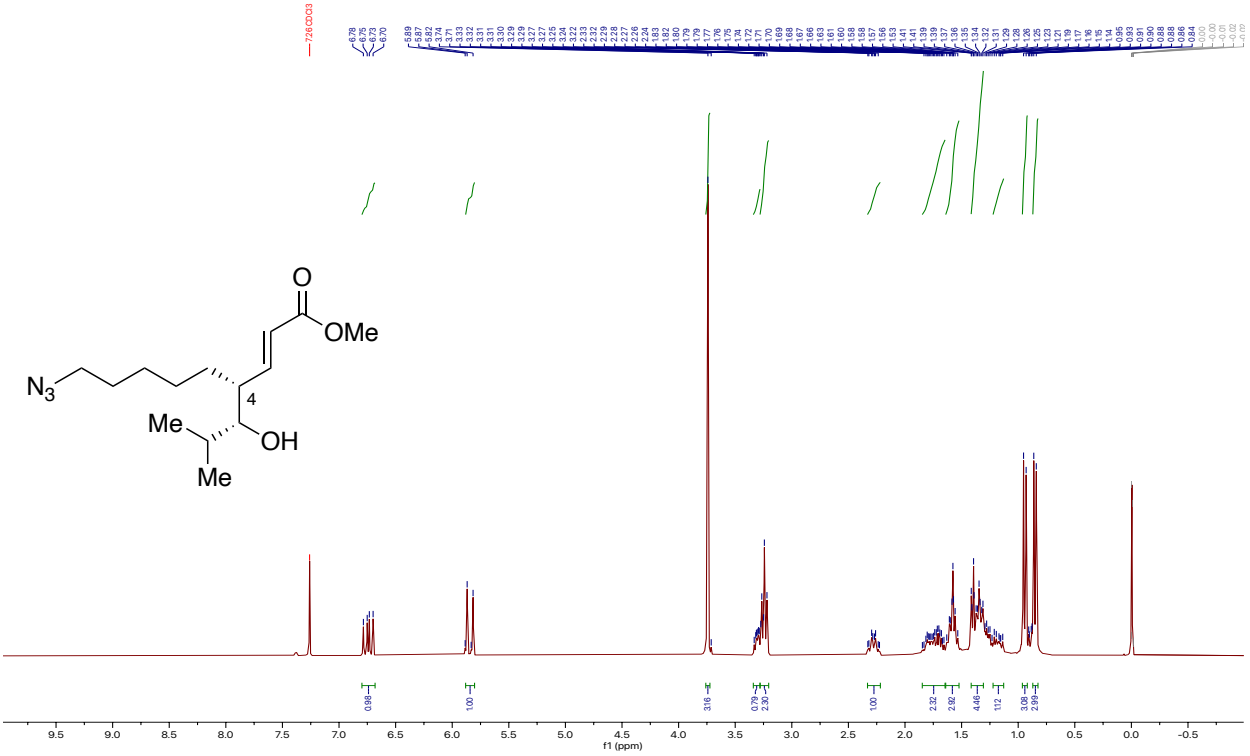
Compound **SI-19**: ^{1}H NMR (300 MHz, $CDCl_3$)



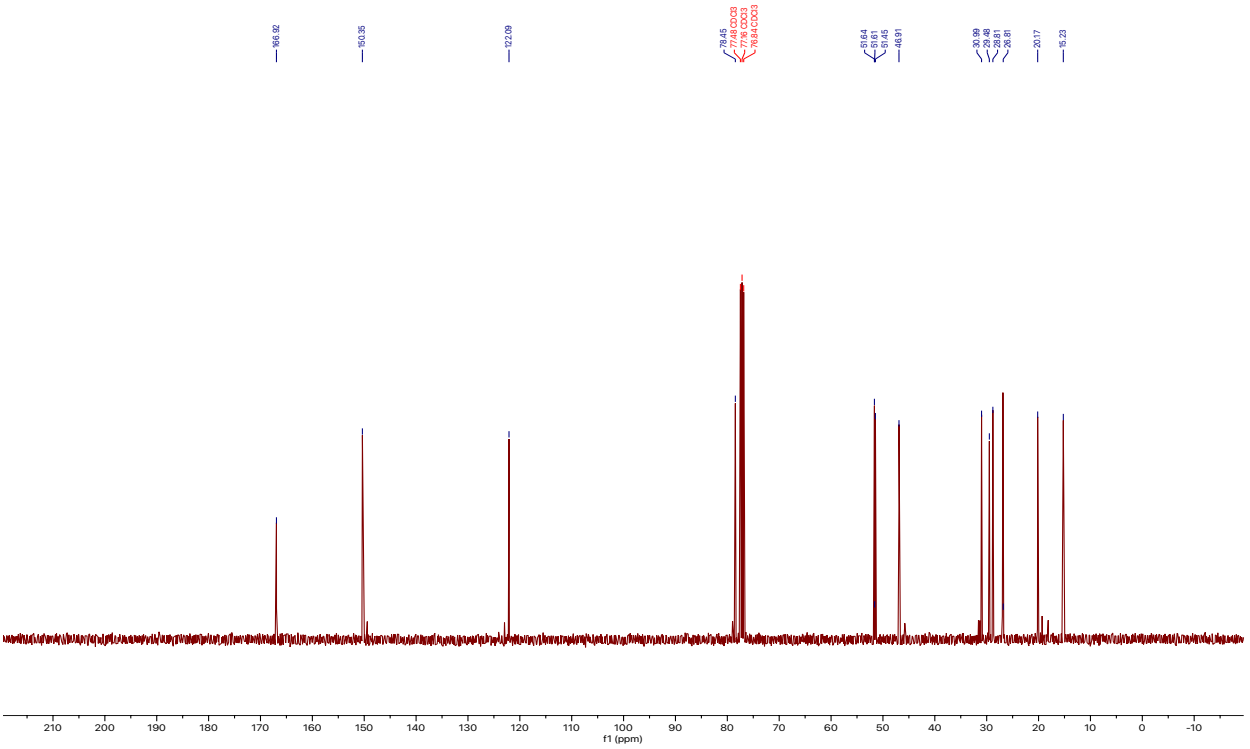
Compound **SI-19**: ^{13}C NMR (100 MHz, $CDCl_3$)



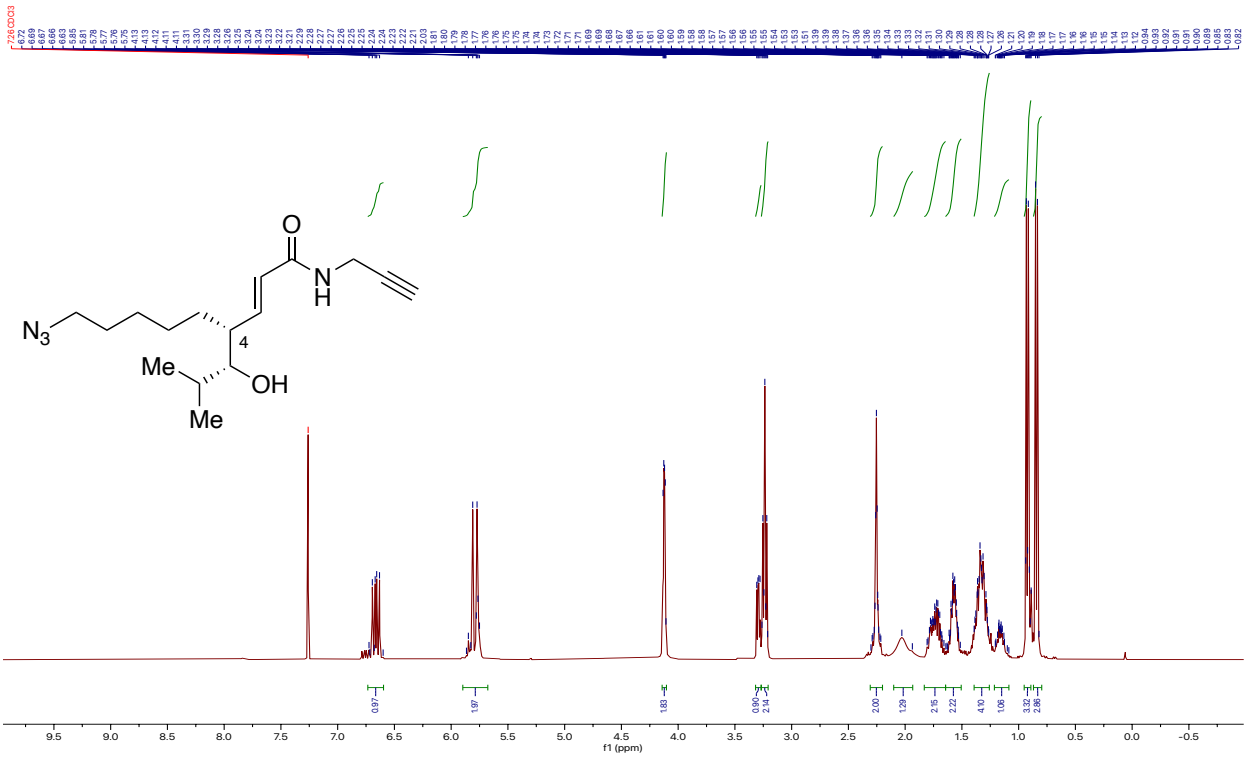
Compound **50**: ^{1}H NMR (400 MHz, $CDCl_3$)



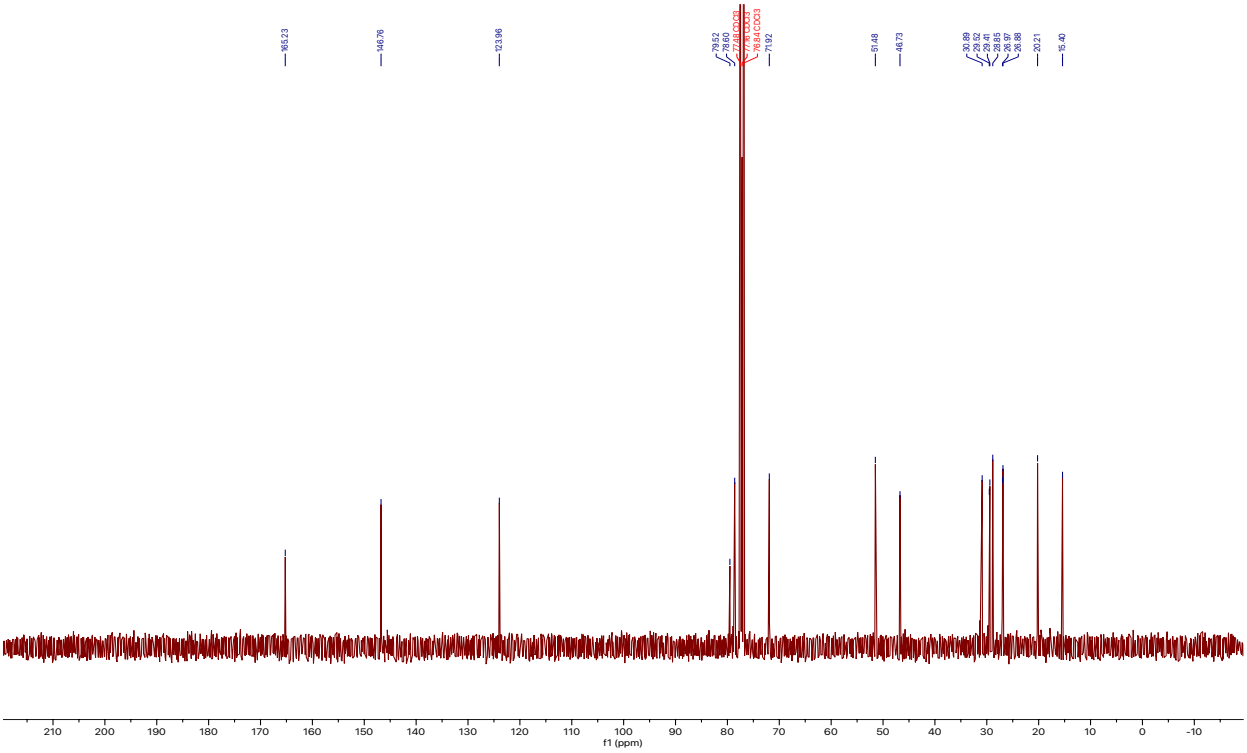
Compound **50**: ^{13}C NMR (100 MHz, $CDCl_3$)



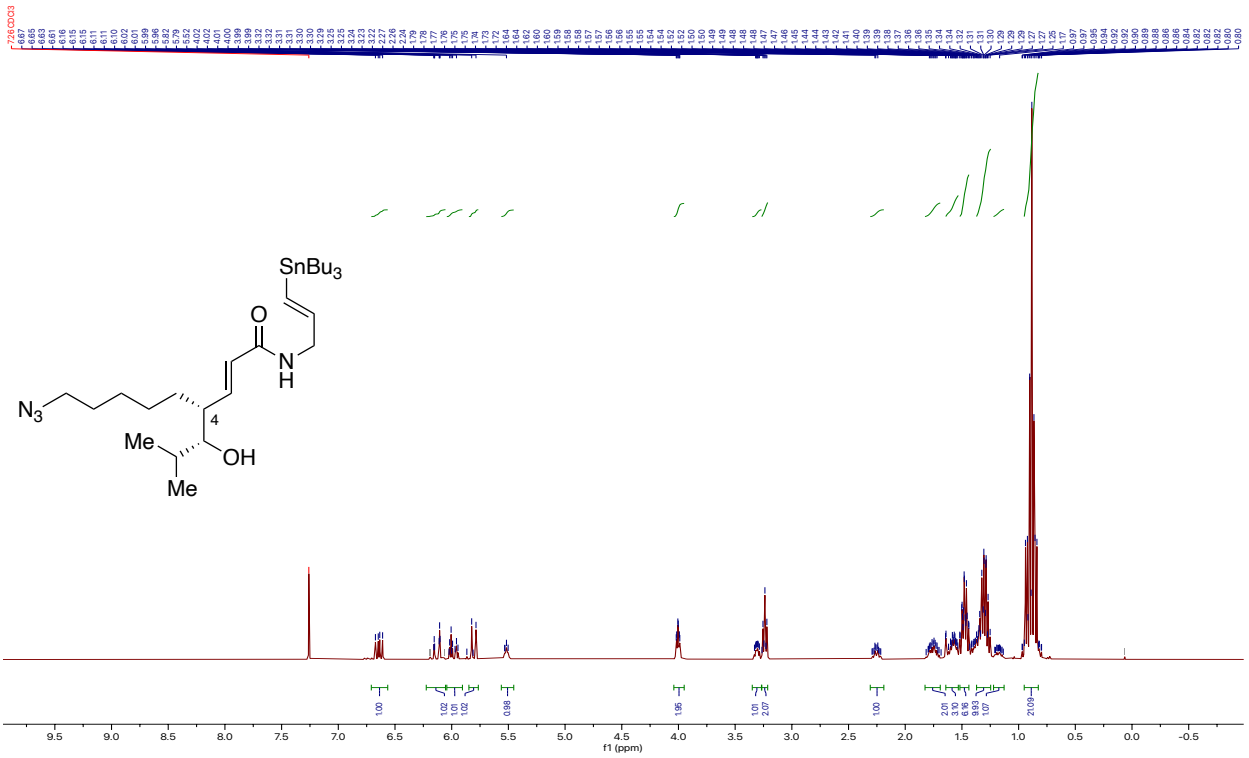
Compound **51**: ^{1}H NMR (400 MHz, $CDCl_3$)



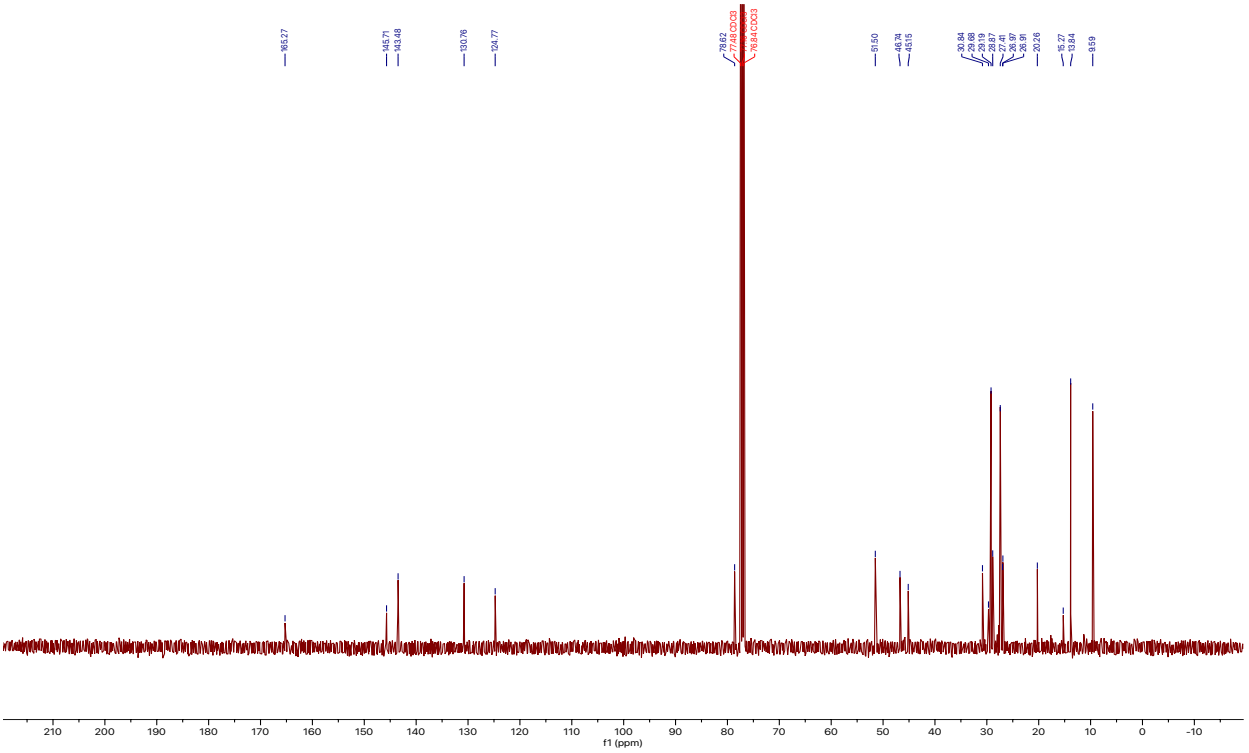
Compound **51**: ^{13}C NMR (100 MHz, $CDCl_3$)



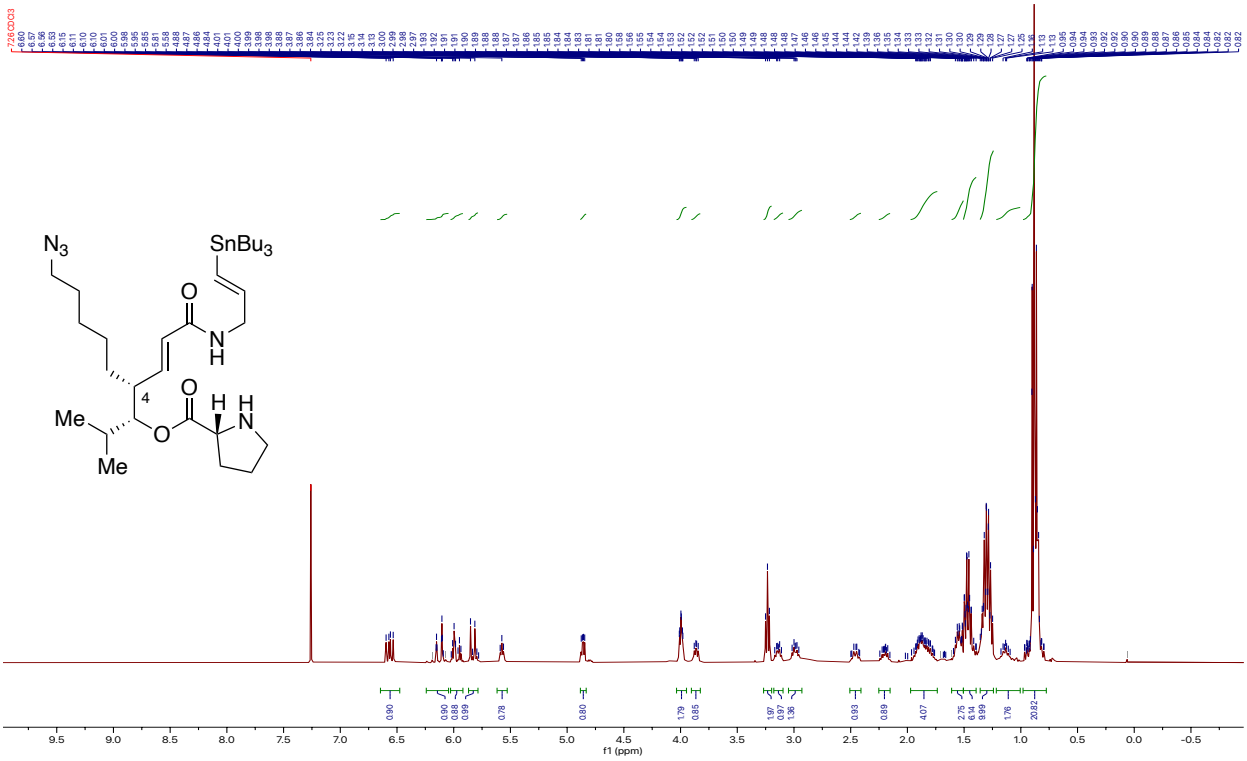
Compound **52**: ^{1}H NMR (400 MHz, $CDCl_3$)



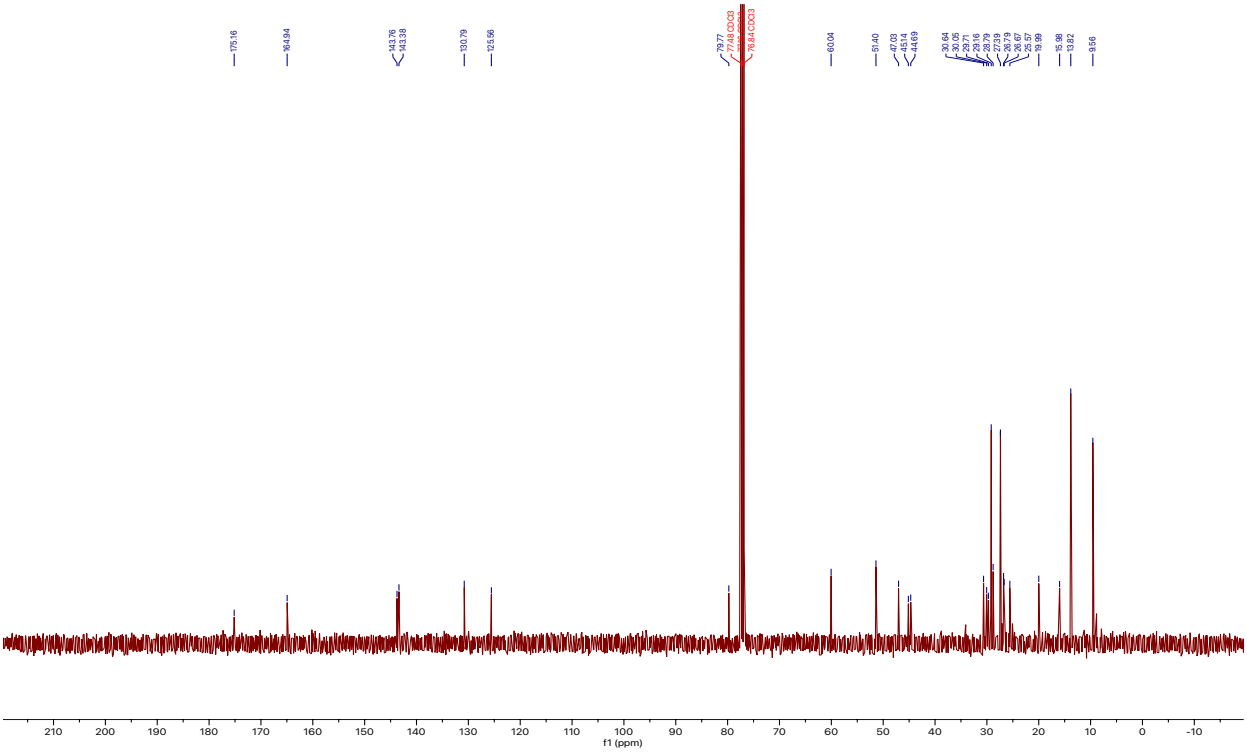
Compound **52**: ^{13}C NMR (100 MHz, $CDCl_3$)



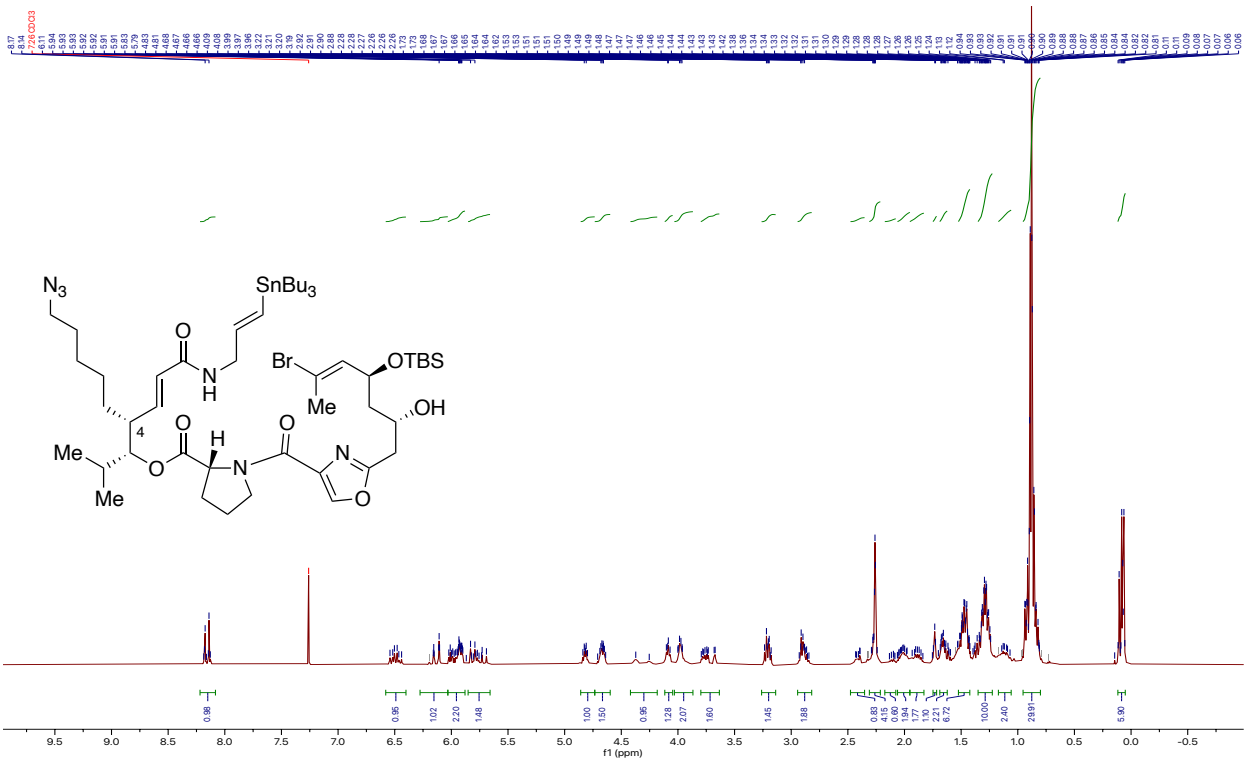
Compound **53**: ^{1}H NMR (300 MHz, $CDCl_3$)



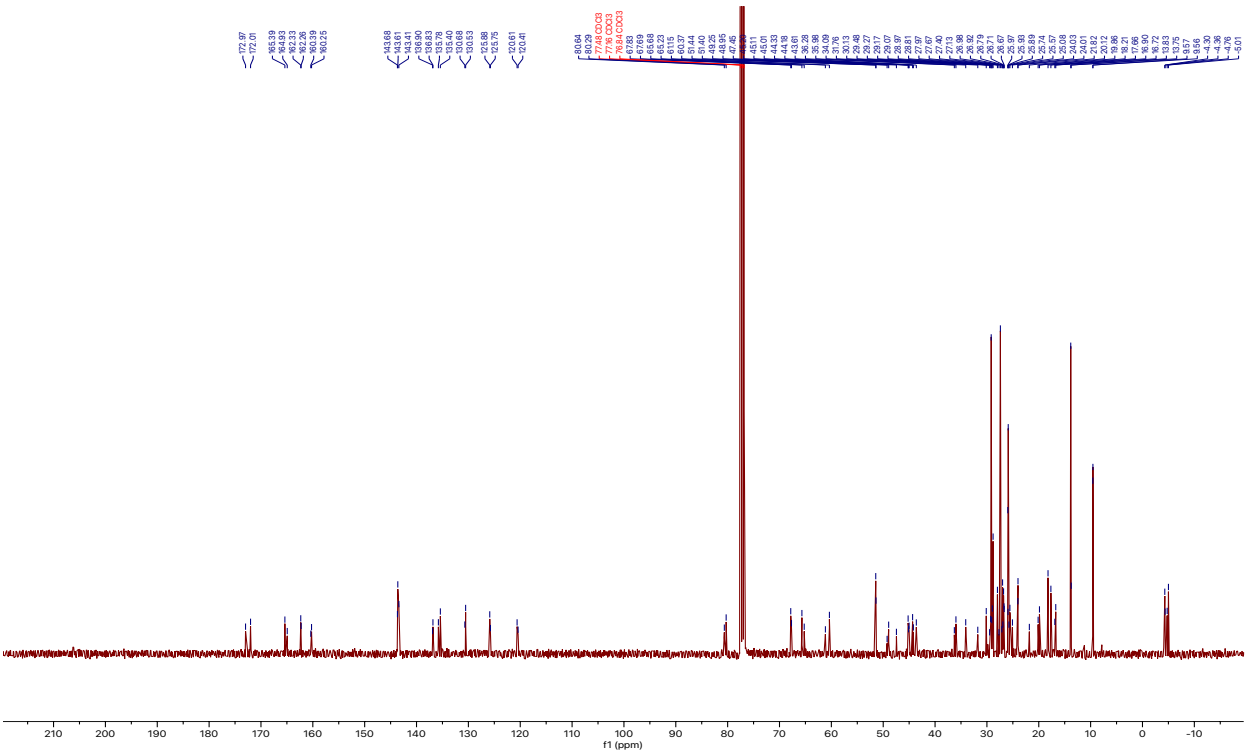
Compound **53**: ^{13}C NMR (100 MHz, $CDCl_3$)



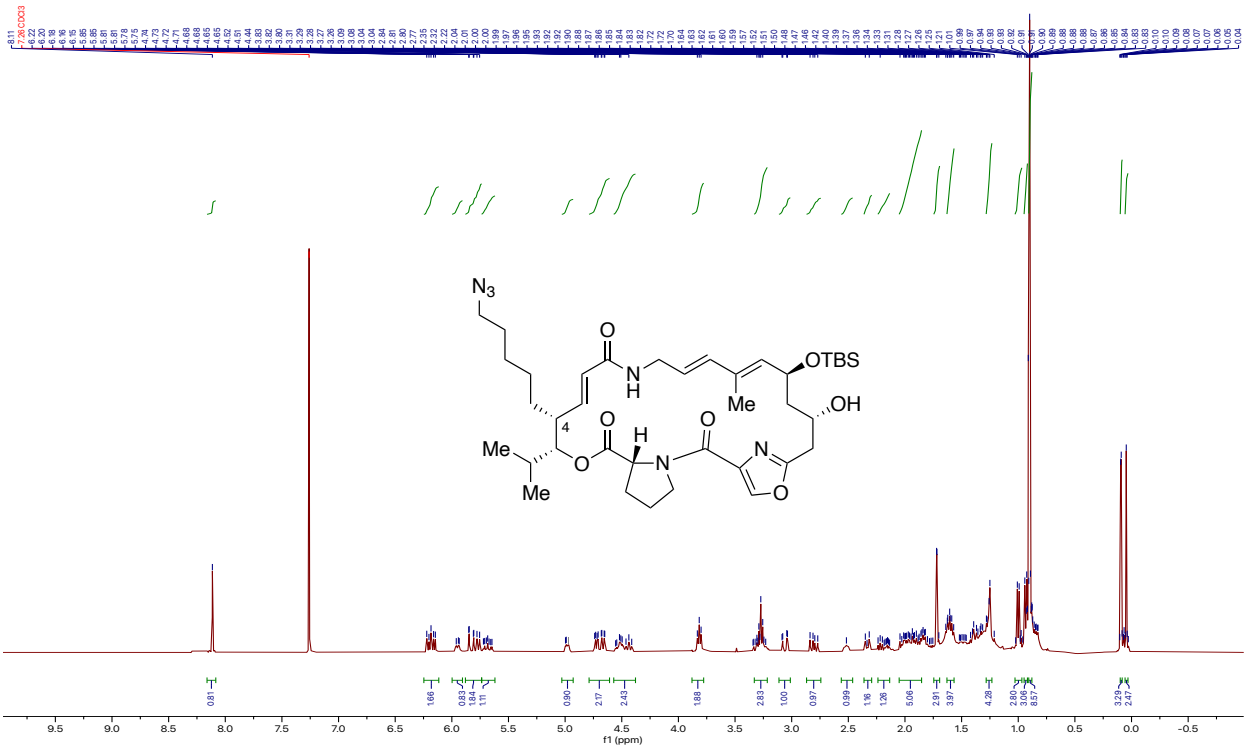
Compound **SI-20**: ^{1}H NMR (400 MHz, $CDCl_3$)



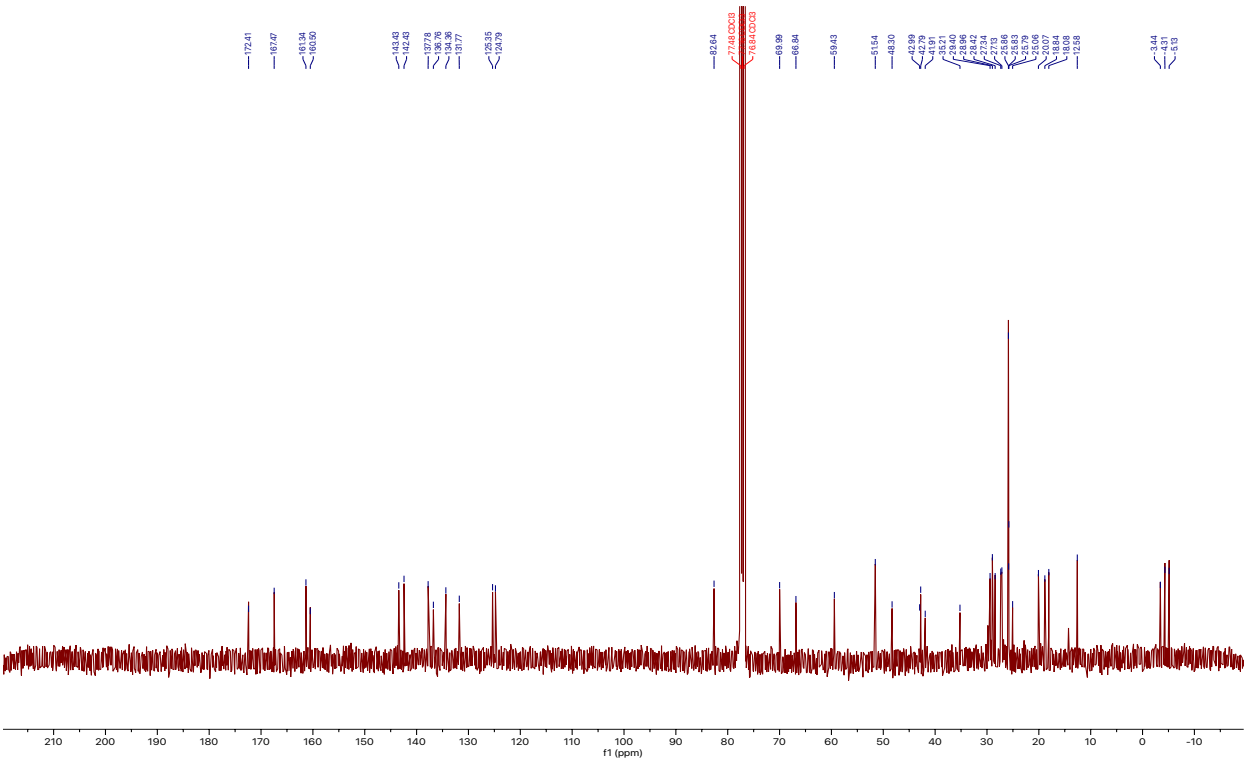
Compound **SI-20**: ^{13}C NMR (100 MHz, $CDCl_3$)



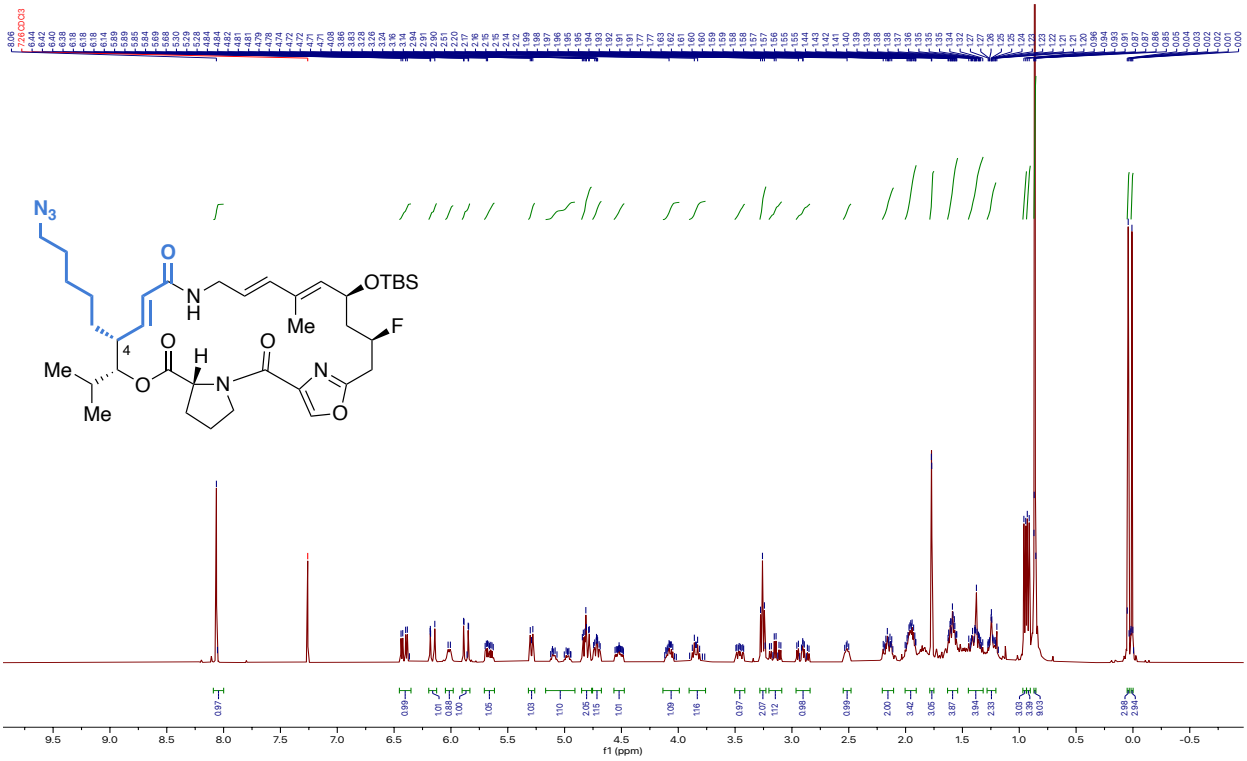
Compound **54**: ^{1}H NMR (400 MHz, $CDCl_3$)



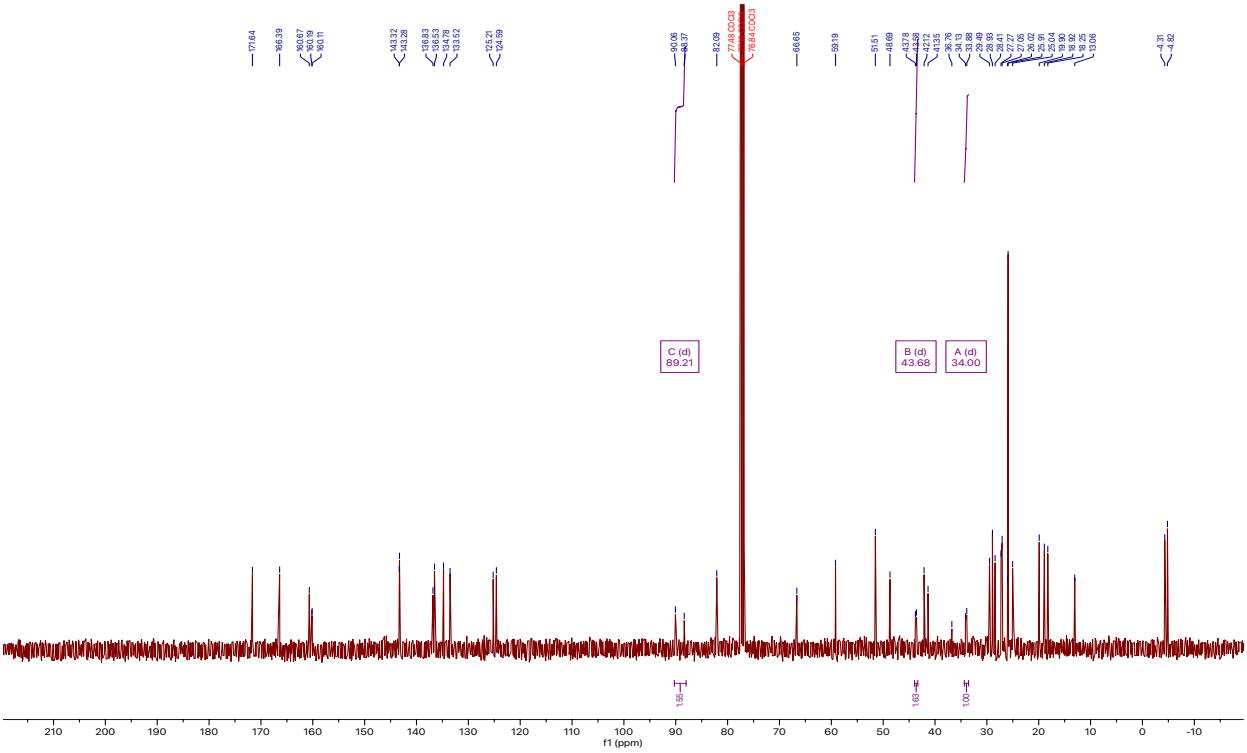
Compound **54**: ^{13}C NMR (100 MHz, $CDCl_3$)



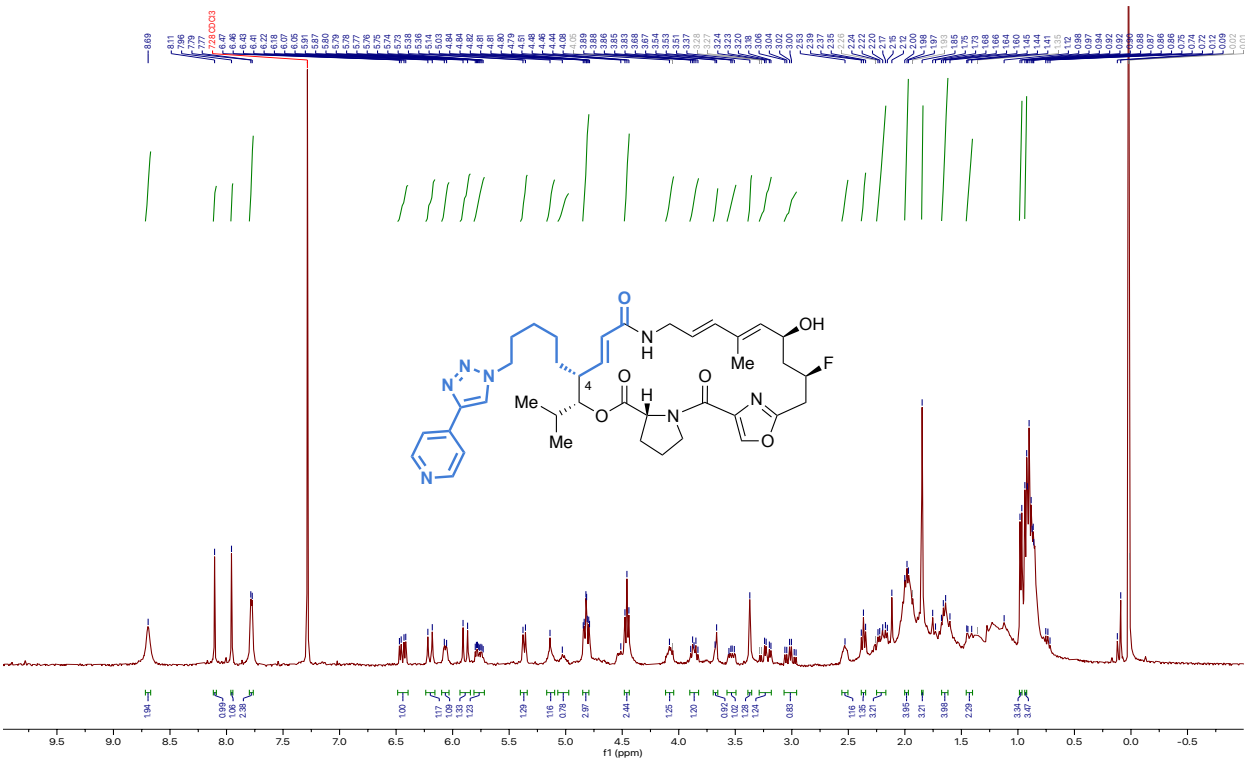
Compound **55**: ^{1}H NMR (400 MHz, $CDCl_3$)



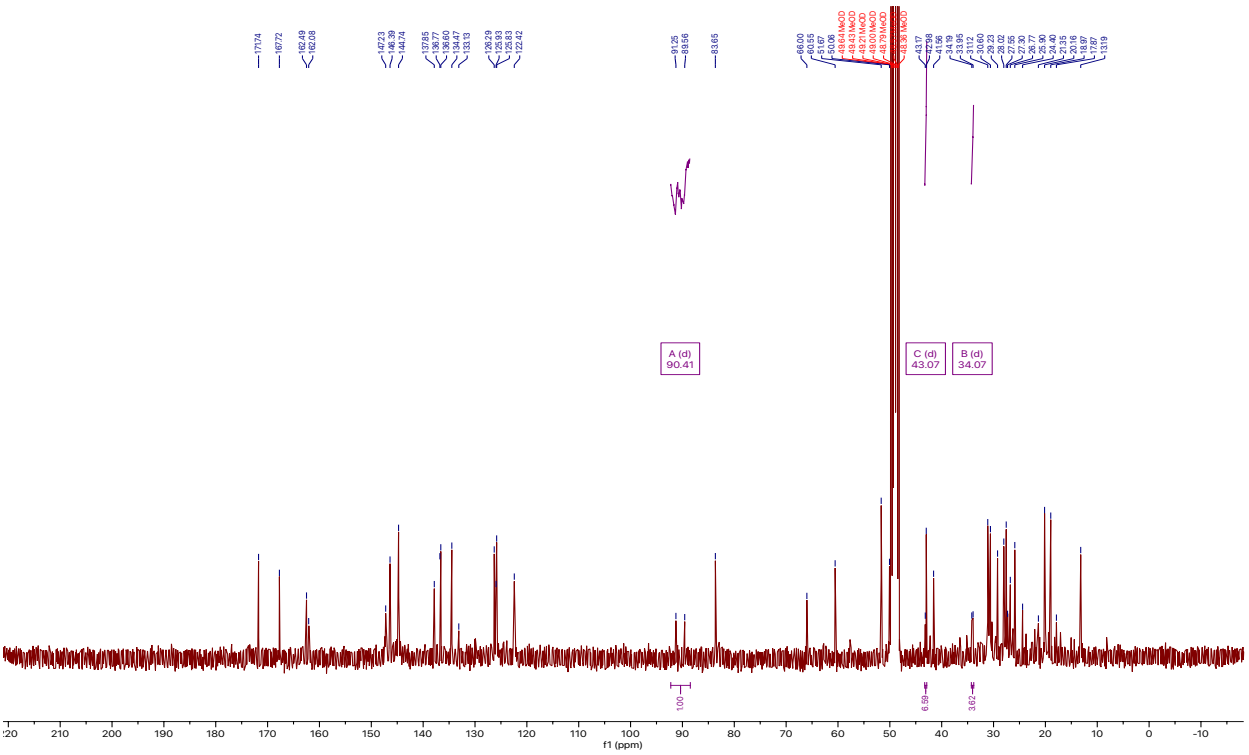
Compound **55**: ^{13}C NMR (100 MHz, $CDCl_3$)



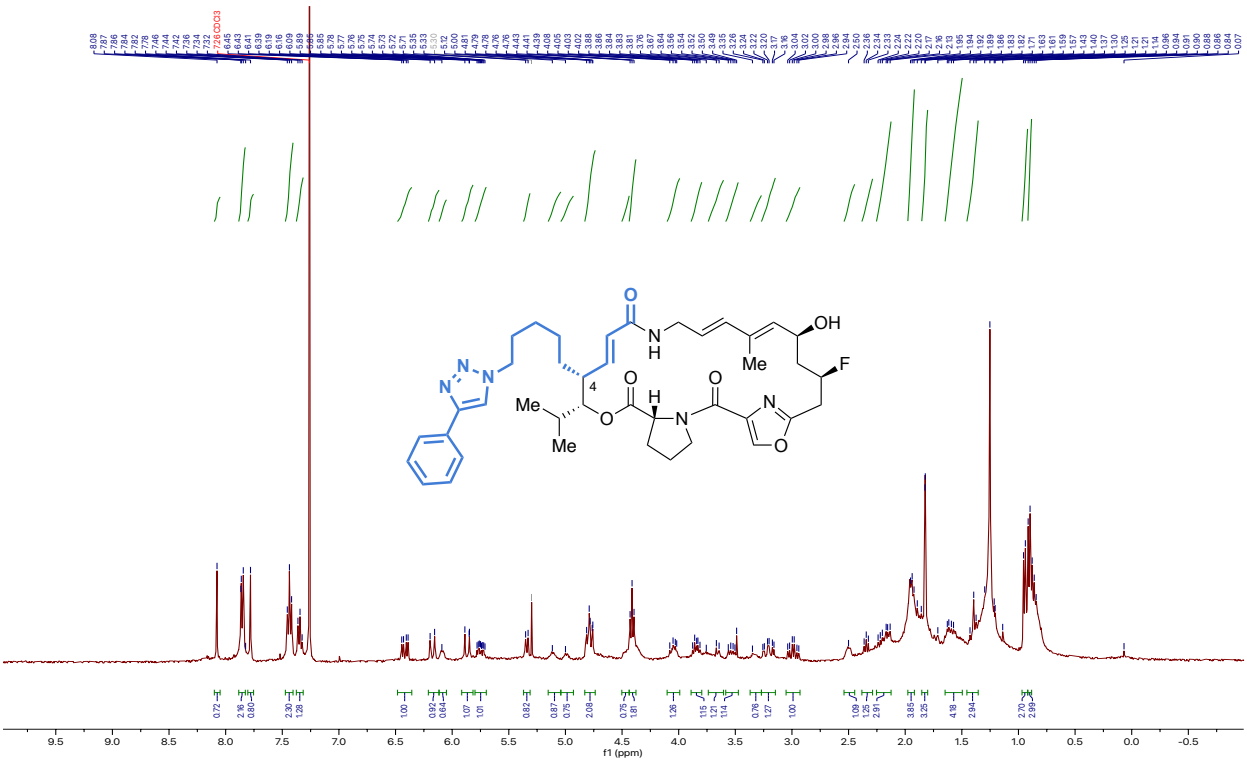
Compound **56a**: ^{1}H NMR (400 MHz, $CDCl_3$)



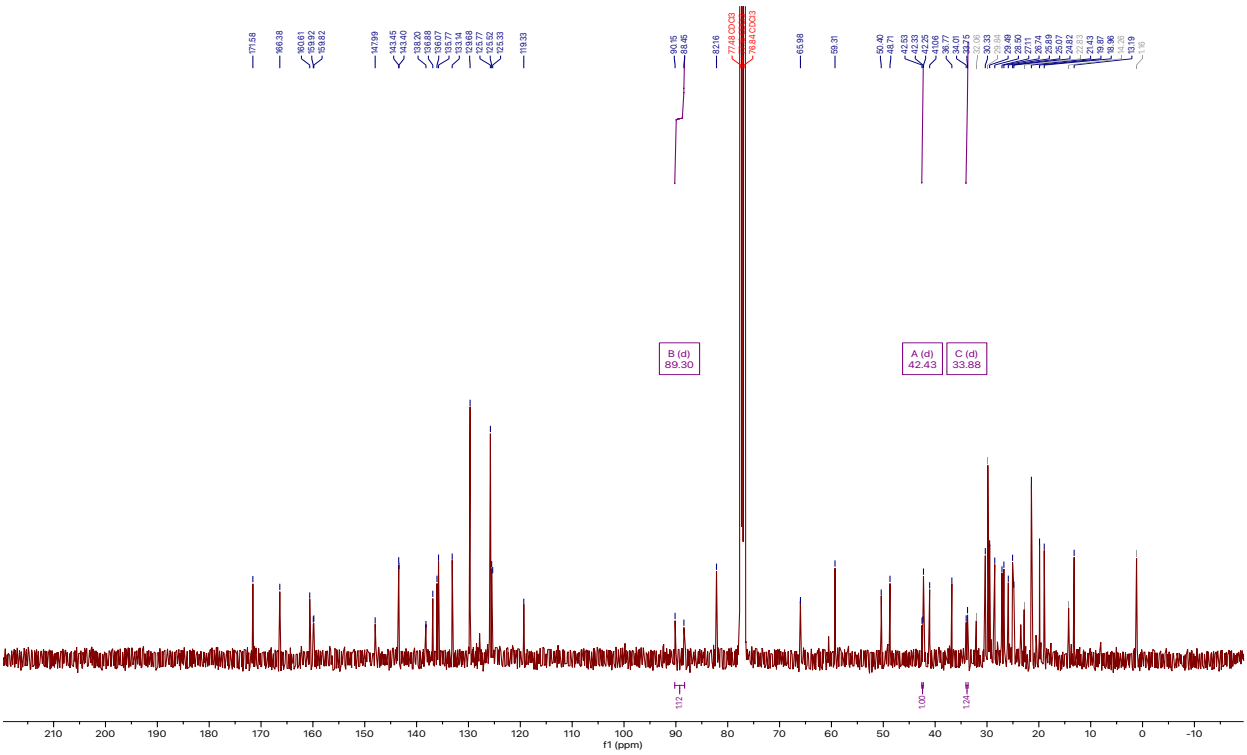
Compound **56a**: ^{13}C NMR (100 MHz, MeOD)



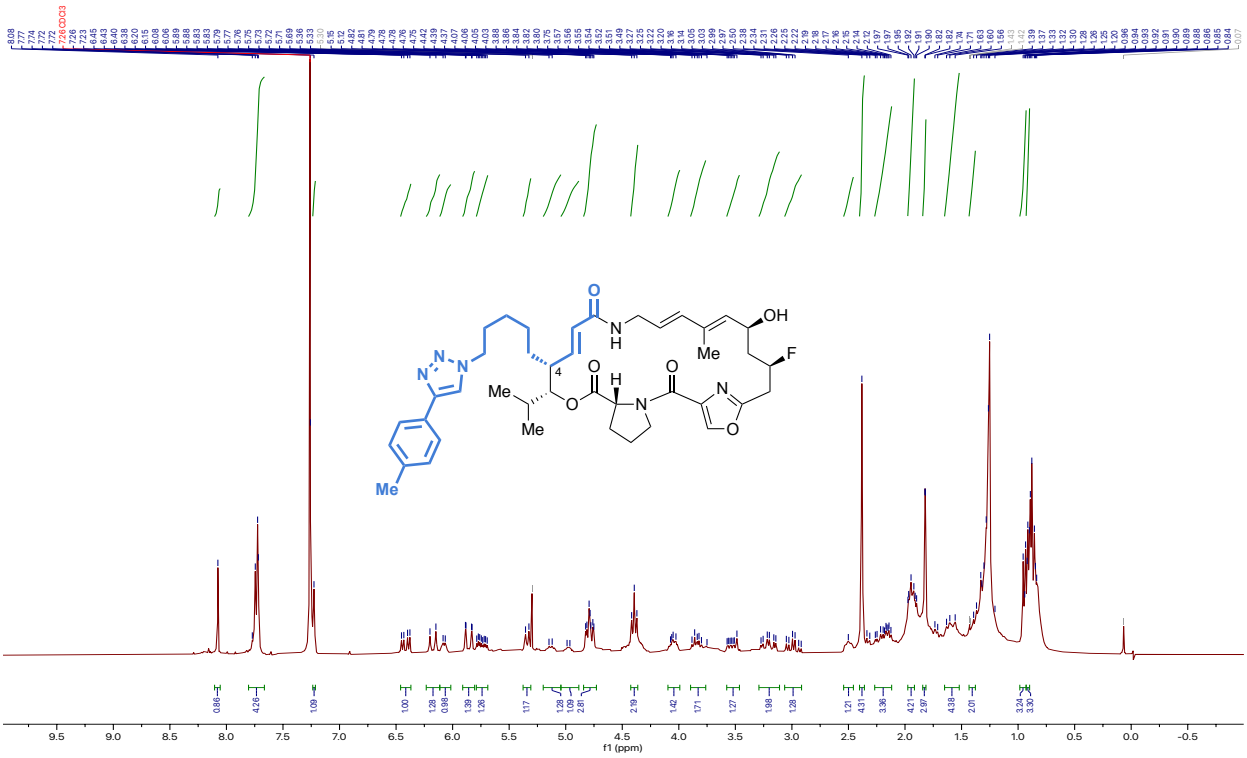
Compound **56b**: ^{1}H NMR (400 MHz, $CDCl_3$)



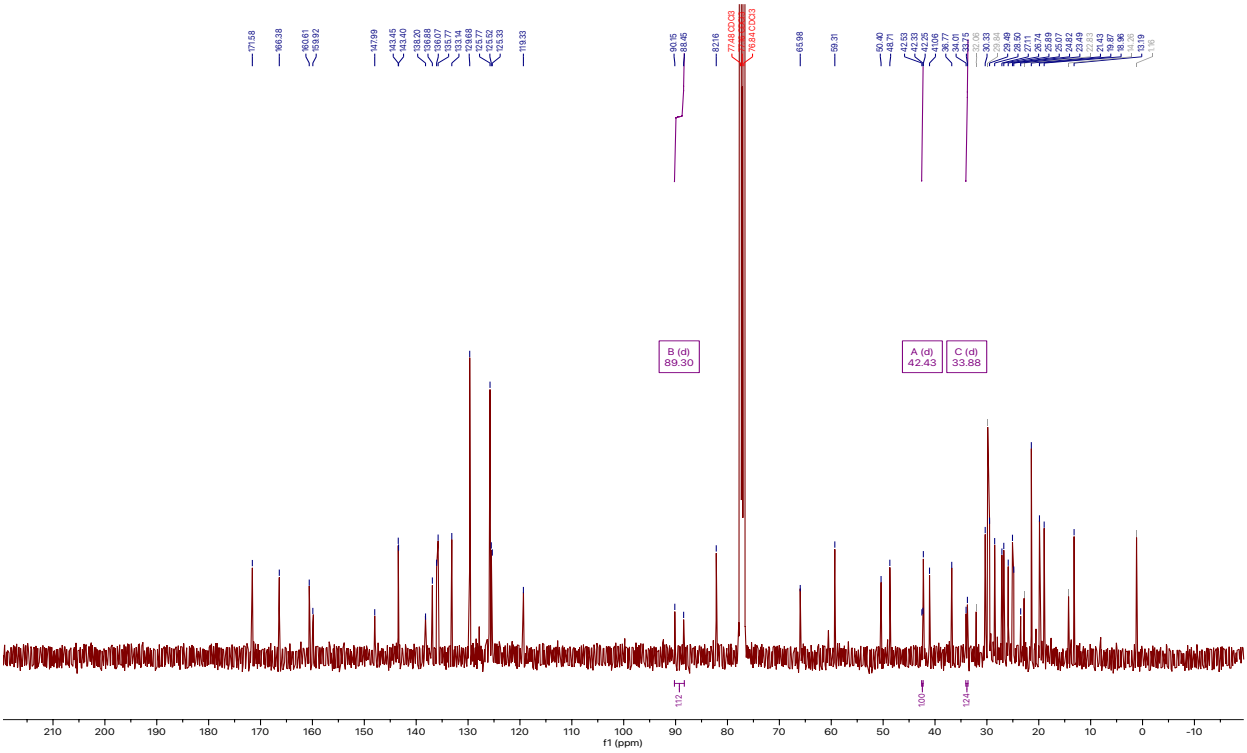
Compound **56b**: ^{13}C NMR (100 MHz, $CDCl_3$)



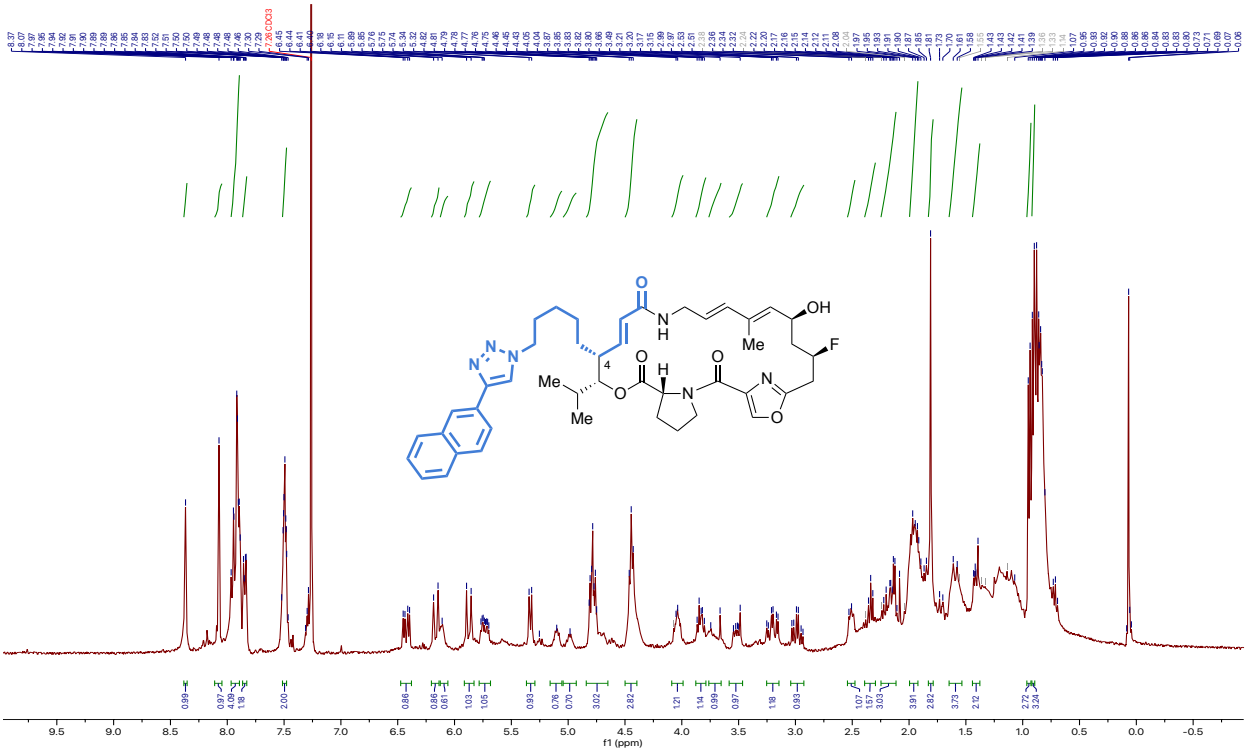
Compound **56c**: ^{1}H NMR (400 MHz, $CDCl_3$)



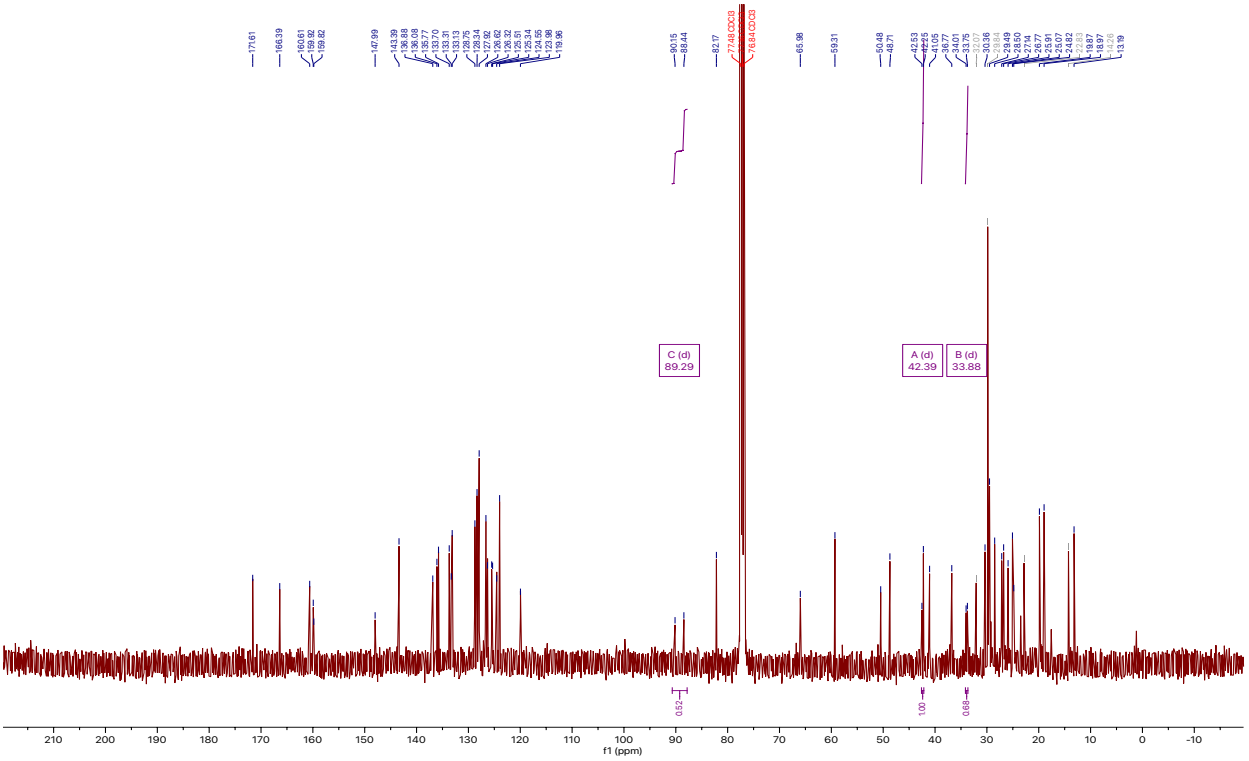
Compound **56c**: ^{13}C NMR (100 MHz, $CDCl_3$)



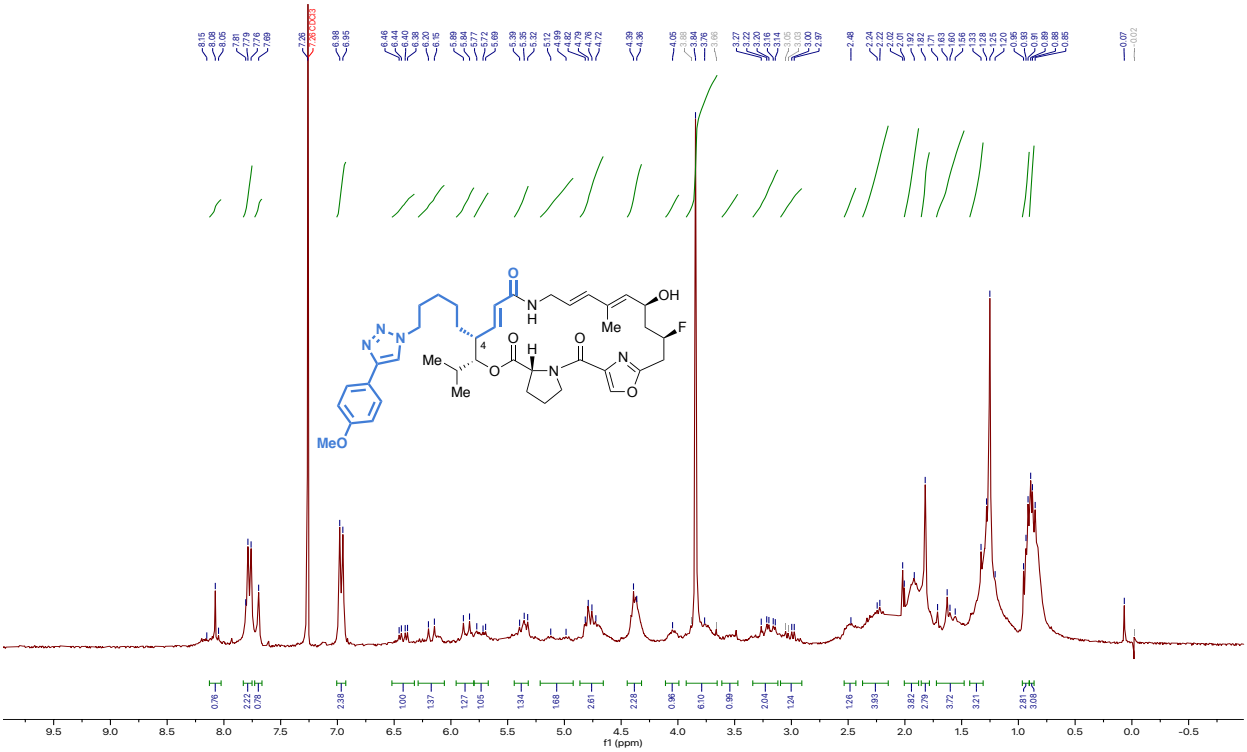
Compound **56d**: ^{1}H NMR (400 MHz, $CDCl_3$)



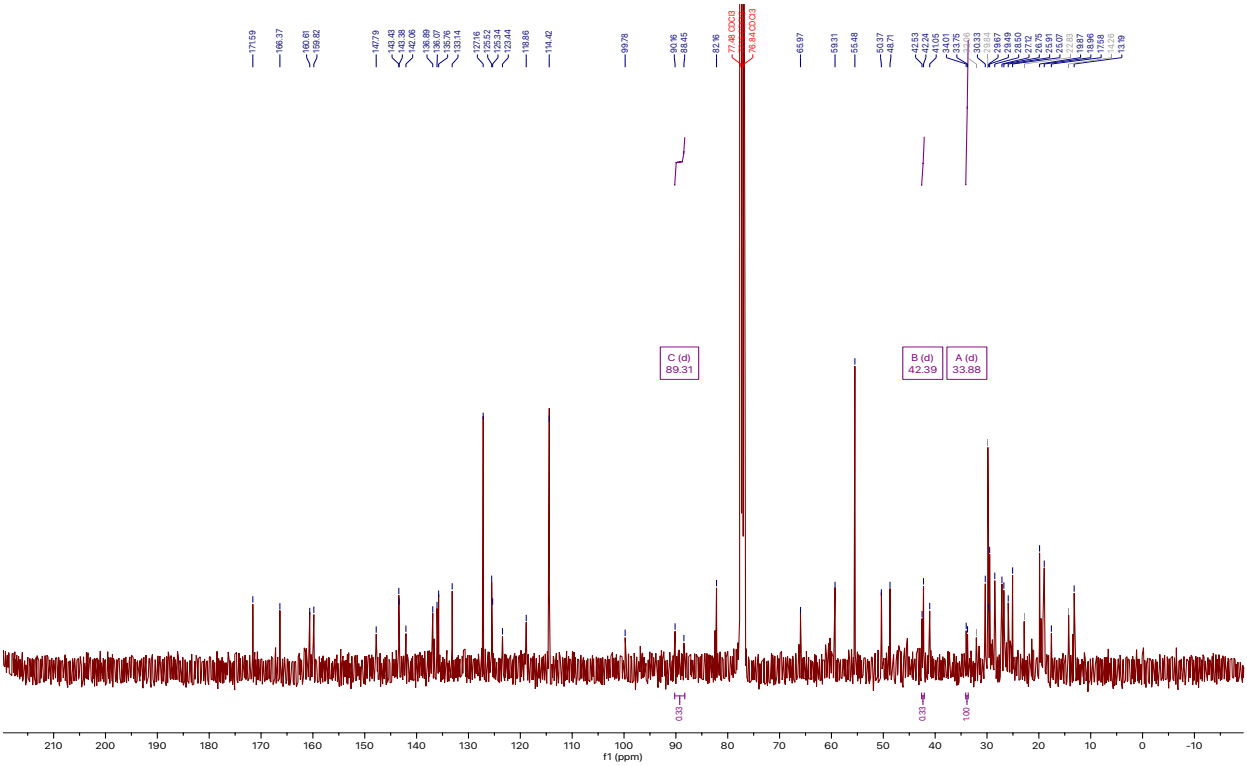
Compound **56d**: ^{13}C NMR (100 MHz, $CDCl_3$)



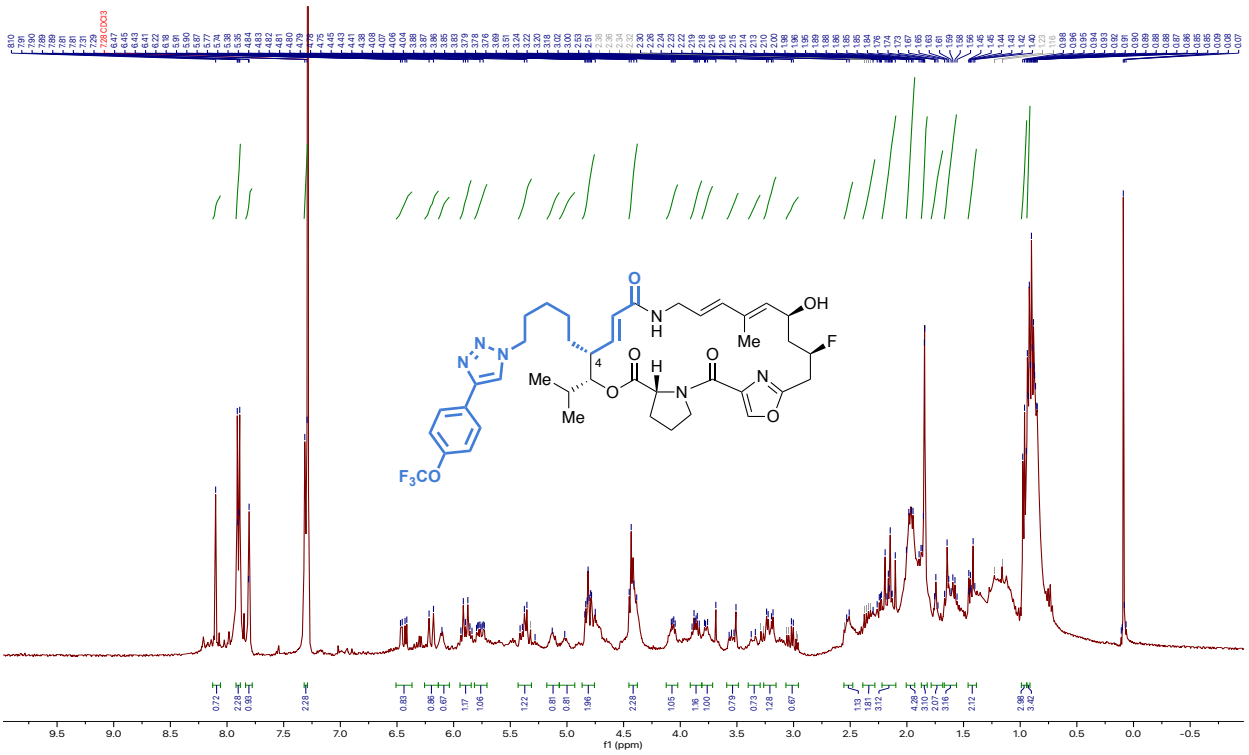
Compound **56e**: ^{1}H NMR (400 MHz, $CDCl_3$)



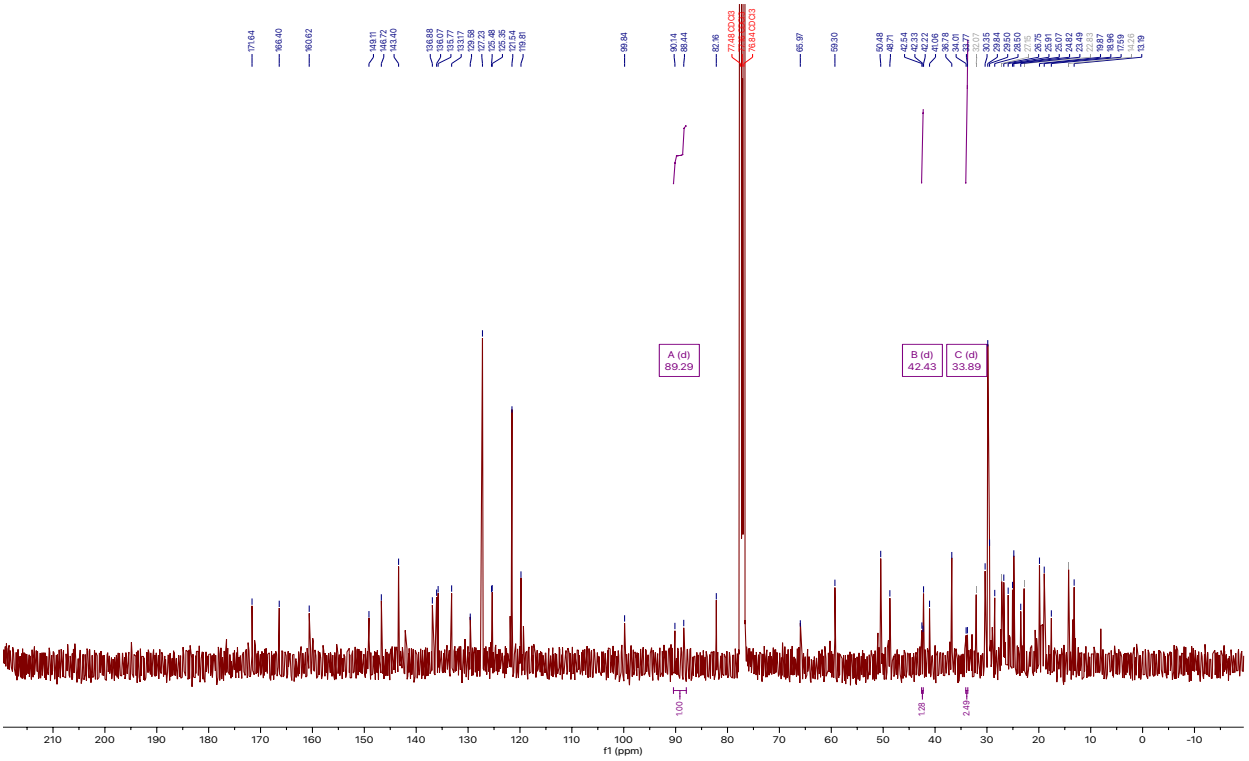
Compound **56e**: ^{13}C NMR (100 MHz, $CDCl_3$)



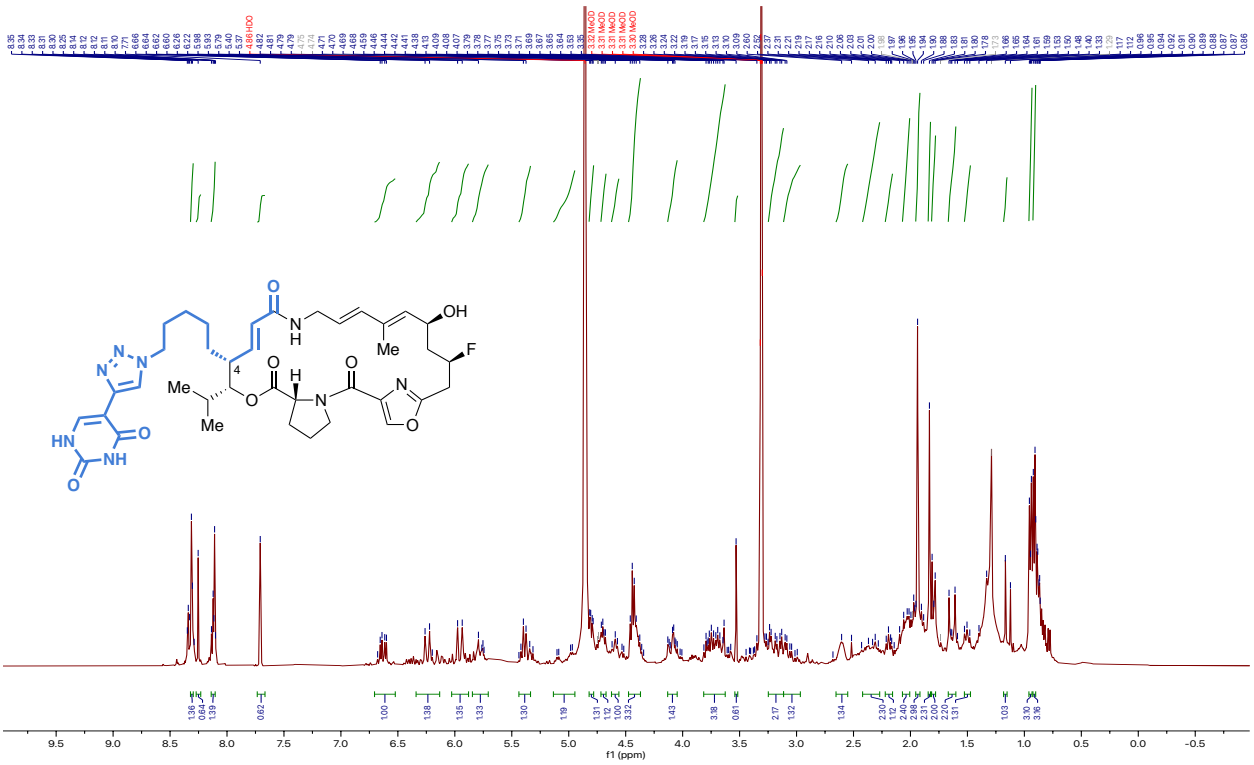
Compound **56f**: ^{1}H NMR (400 MHz, $CDCl_3$)



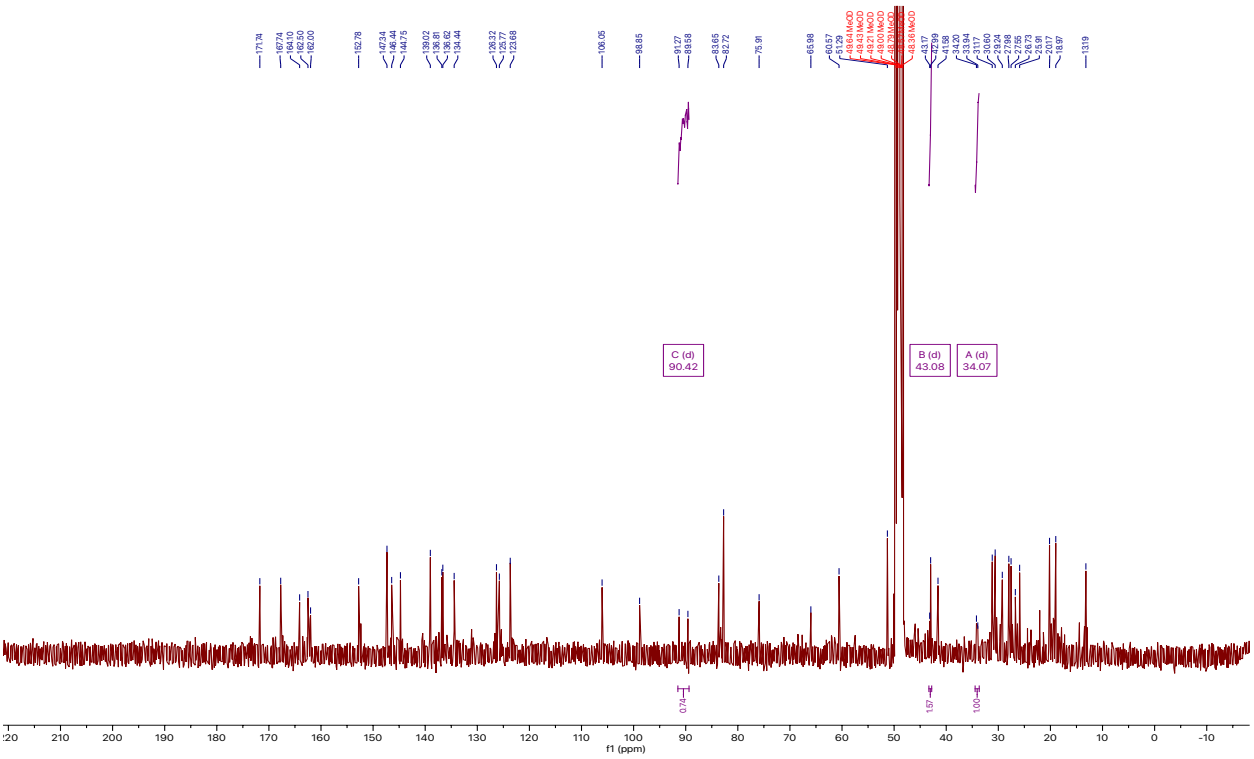
Compound **56f**: ^{13}C NMR (100 MHz, $CDCl_3$)



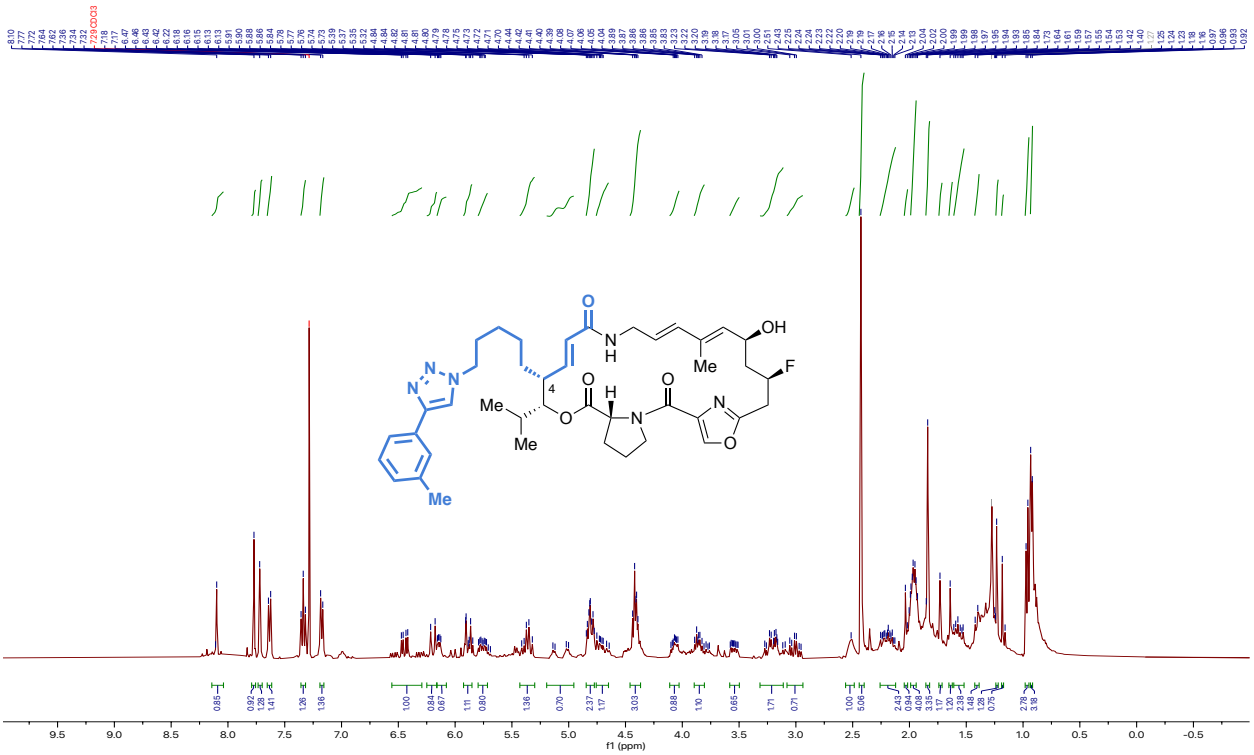
Compound **56g**: ^{1}H NMR (400 MHz, MeOD)



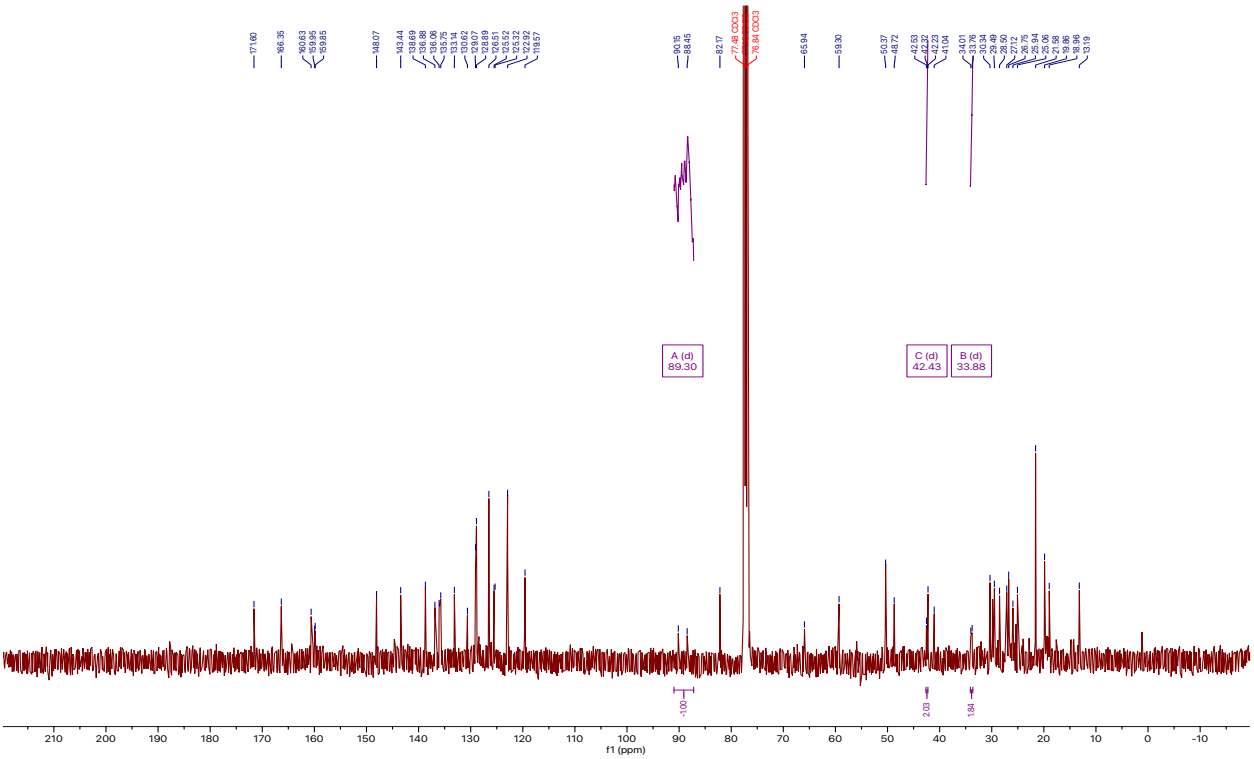
Compound **56g**: ^{13}C NMR (100 MHz, MeOD)



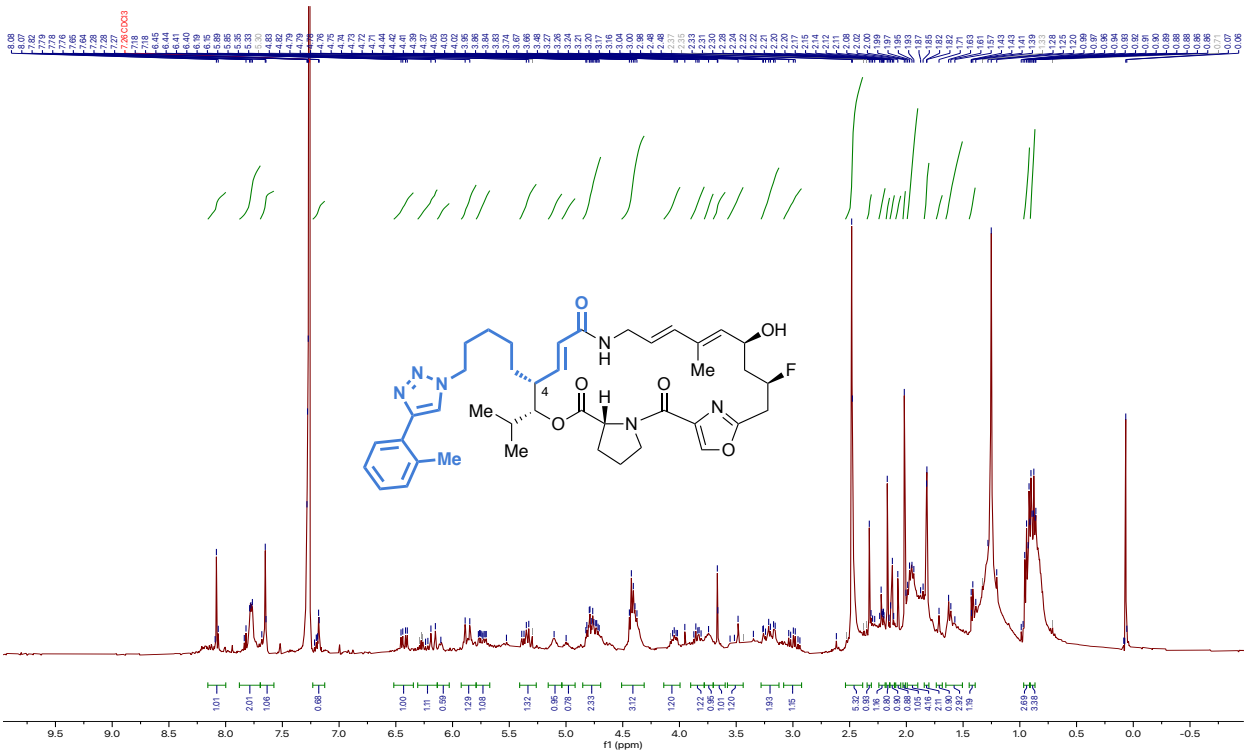
Compound **56h**: ^{1}H NMR (400 MHz, $CDCl_3$)



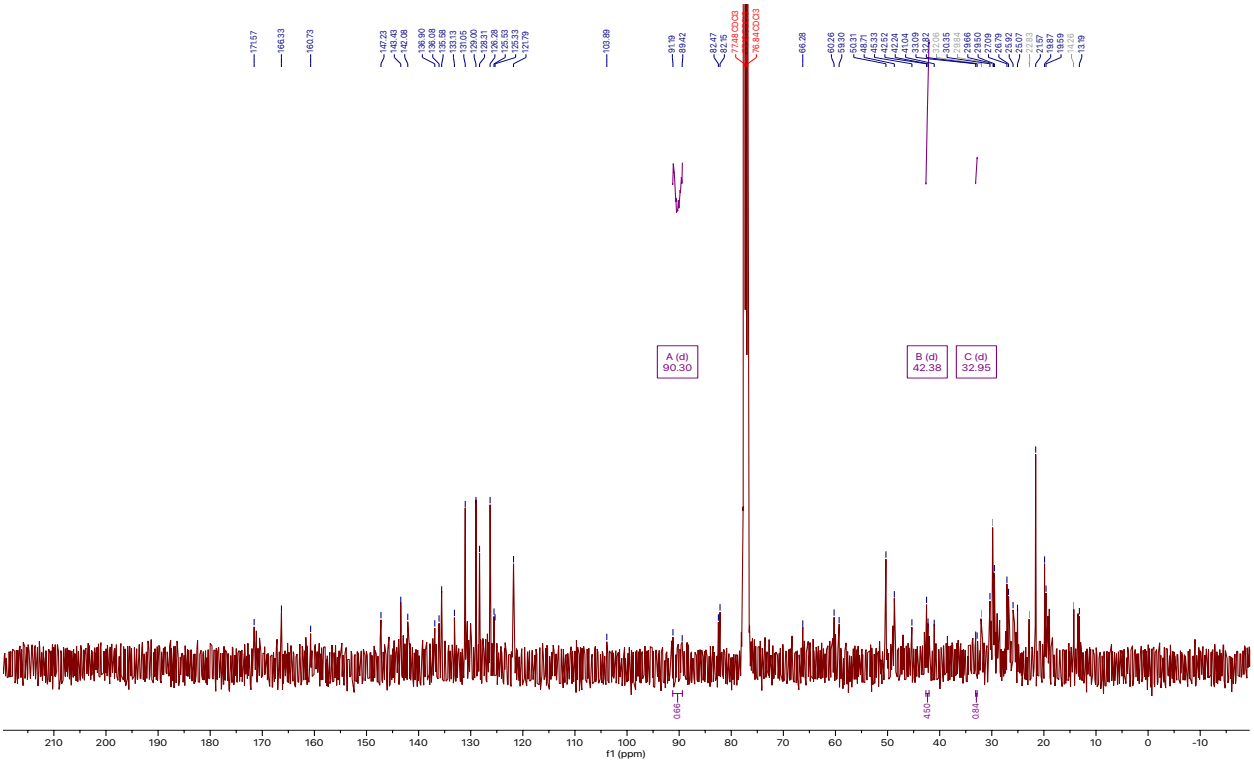
Compound **56h**: ^{13}C NMR (100 MHz, $CDCl_3$)



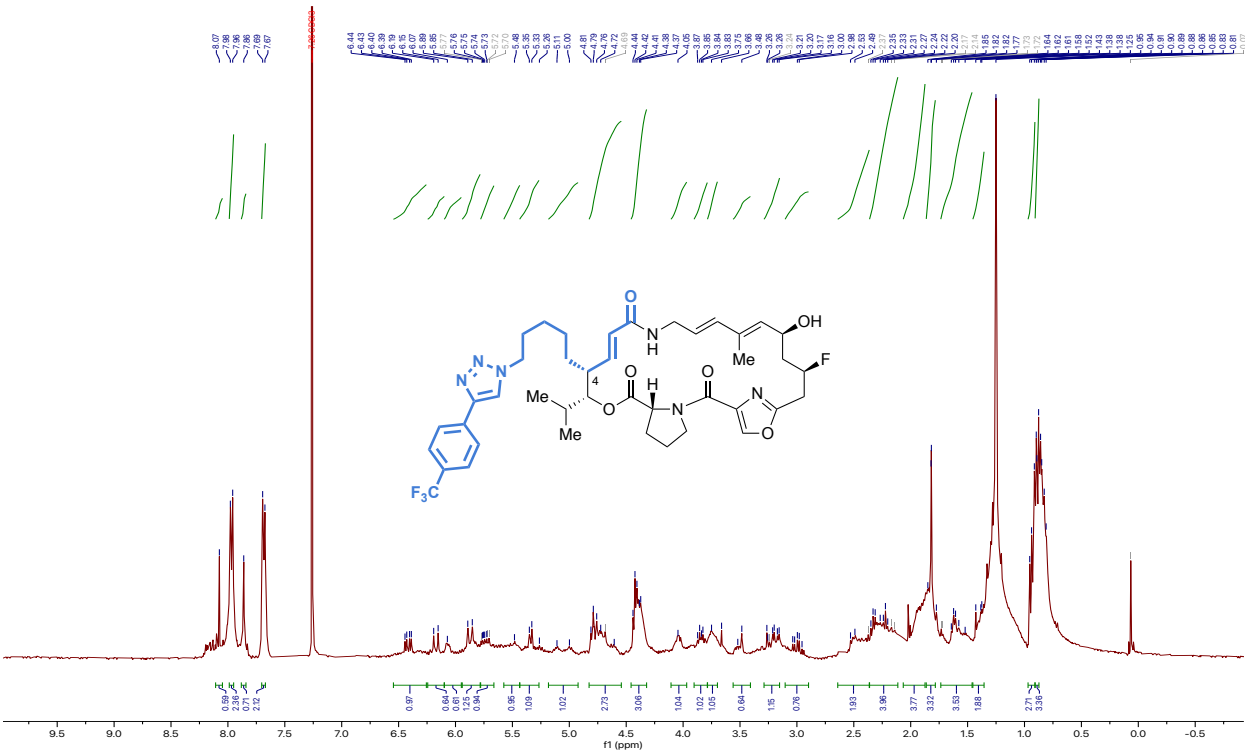
Compound **56i**: ^{1}H NMR (400 MHz, $CDCl_3$)



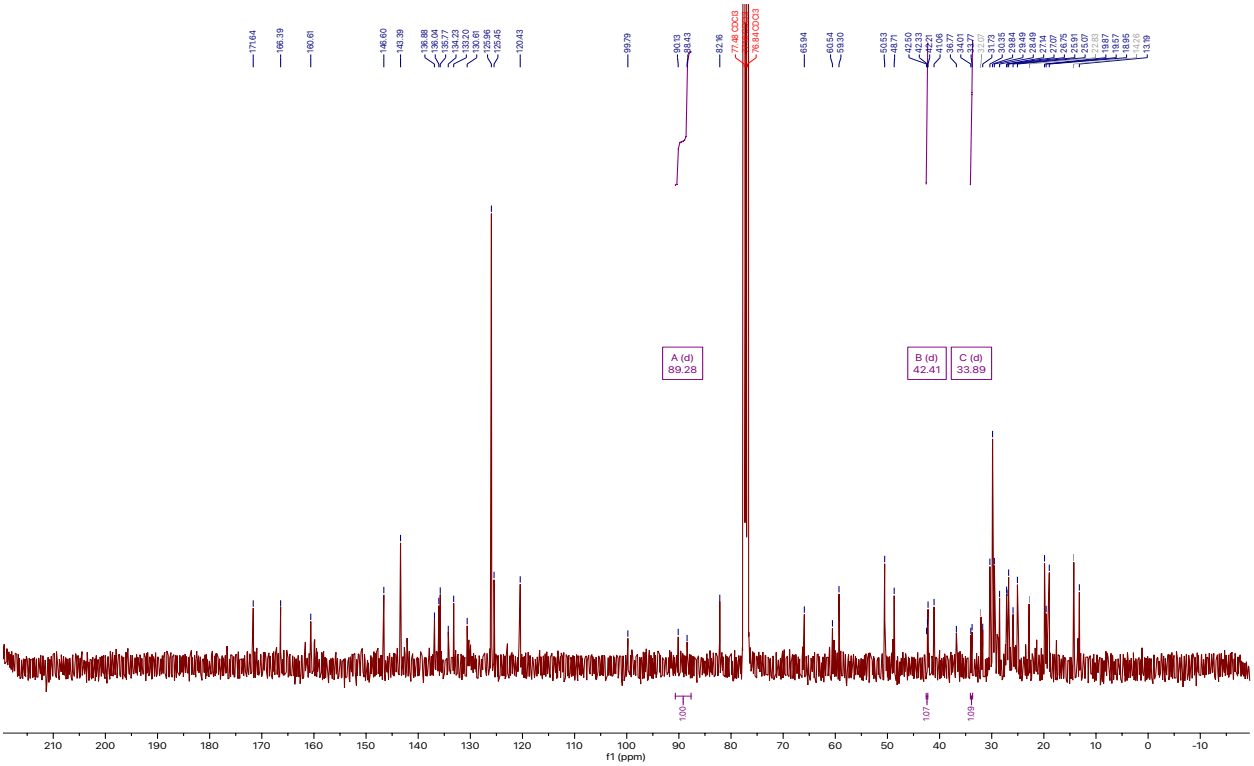
Compound **56i**: ^{13}C NMR (100 MHz, $CDCl_3$)



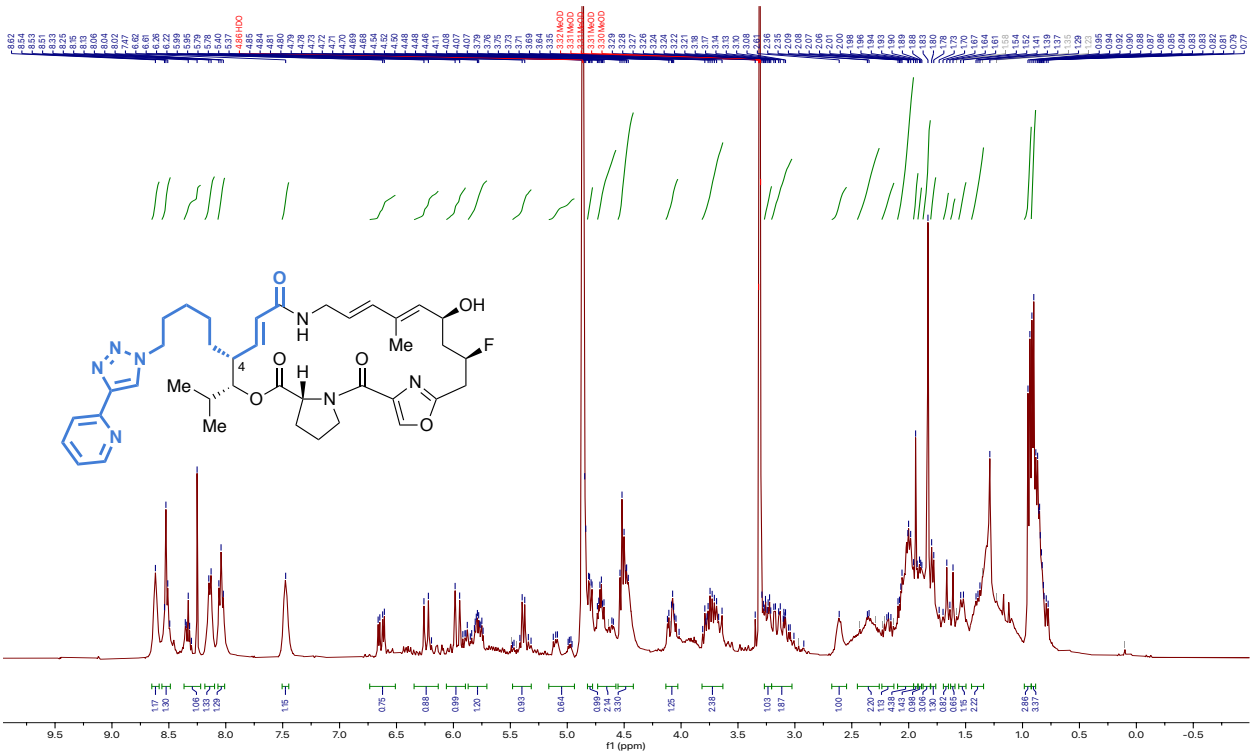
Compound **56j**: ^{1}H NMR (400 MHz, $CDCl_3$)



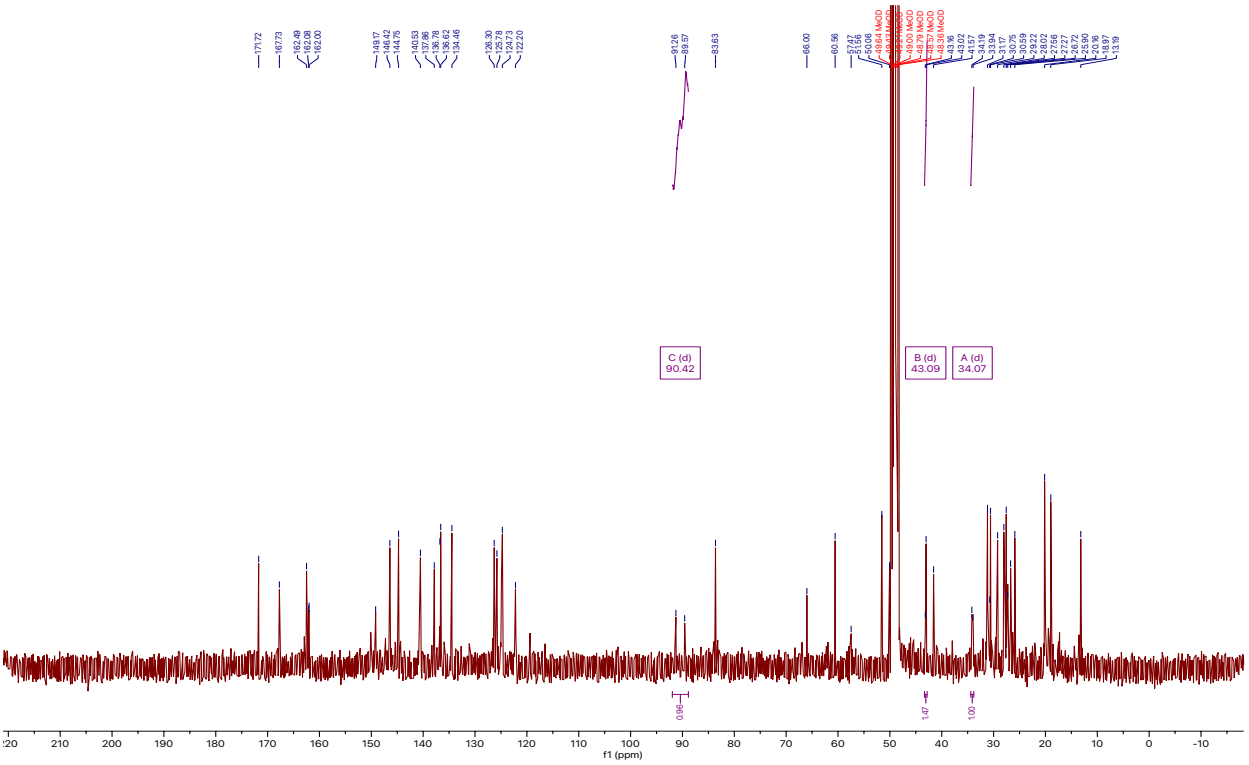
Compound **56j**: ^{13}C NMR (100 MHz, $CDCl_3$)



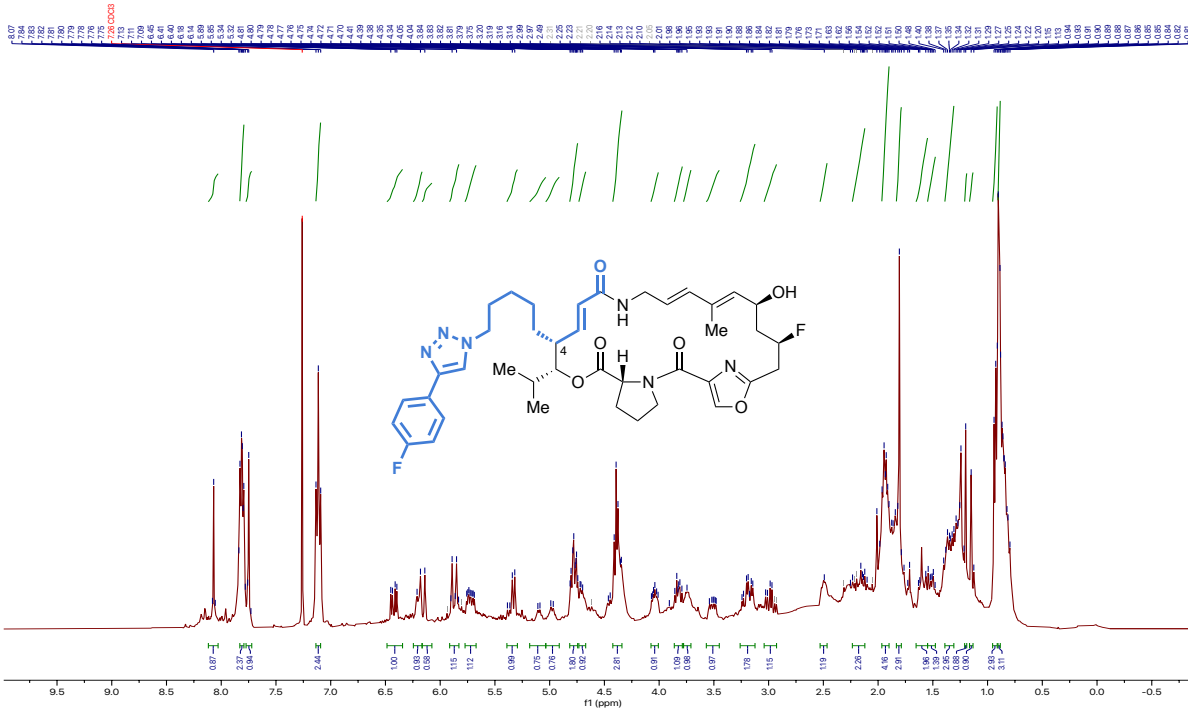
Compound **56k**: ^{1}H NMR (400 MHz, $CDCl_3$)



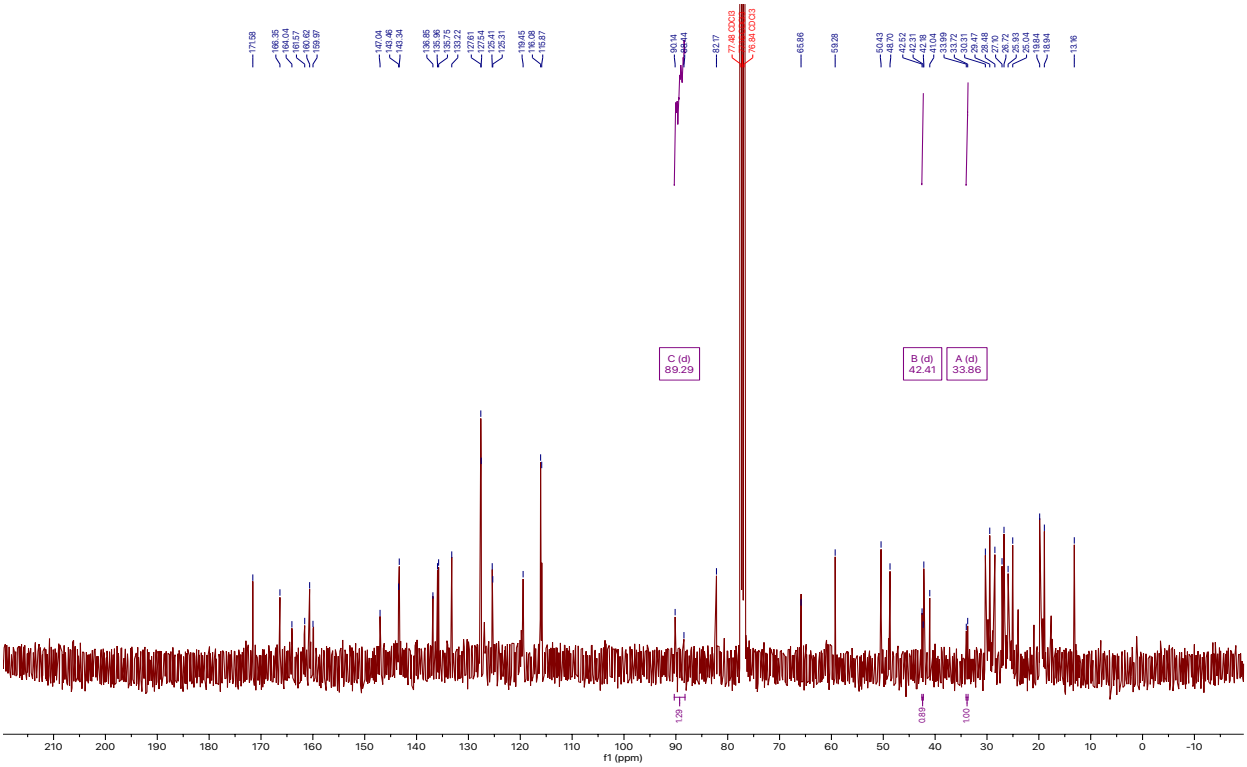
Compound **56k**: ^{13}C NMR (100 MHz, MeOD)



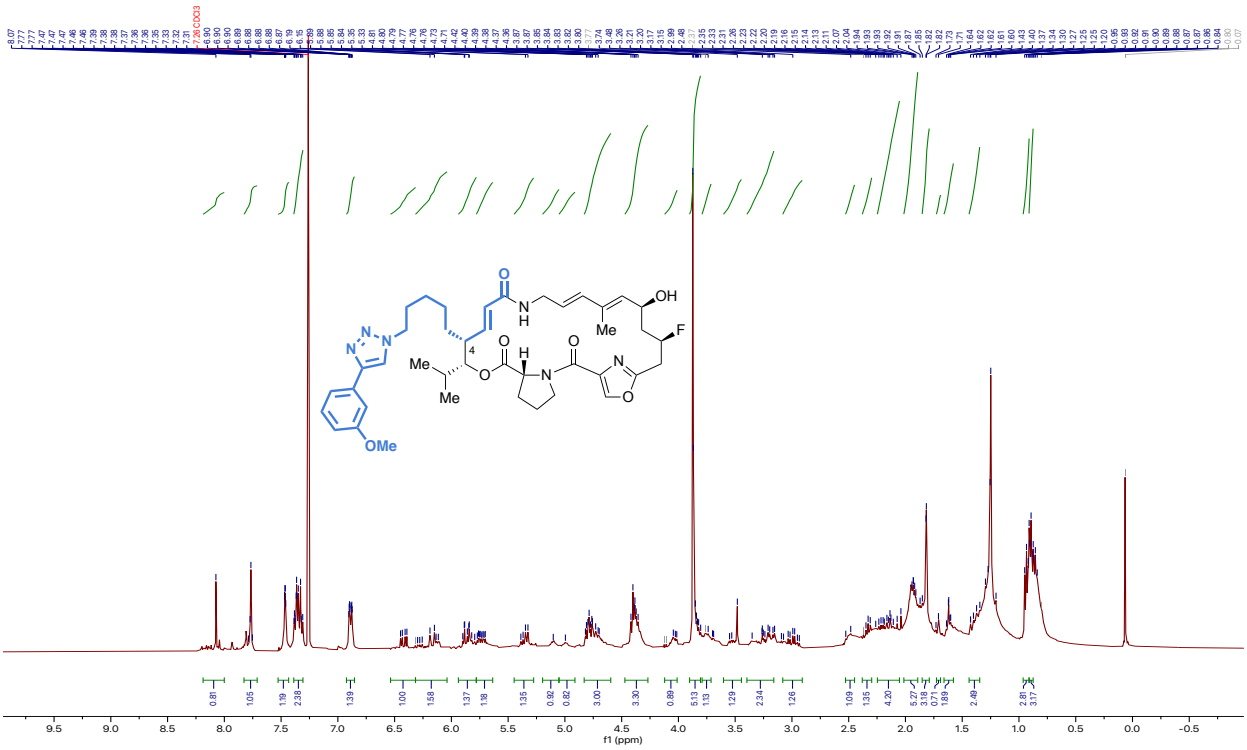
Compound **56l**: ^{1}H NMR (400 MHz, $CDCl_3$)



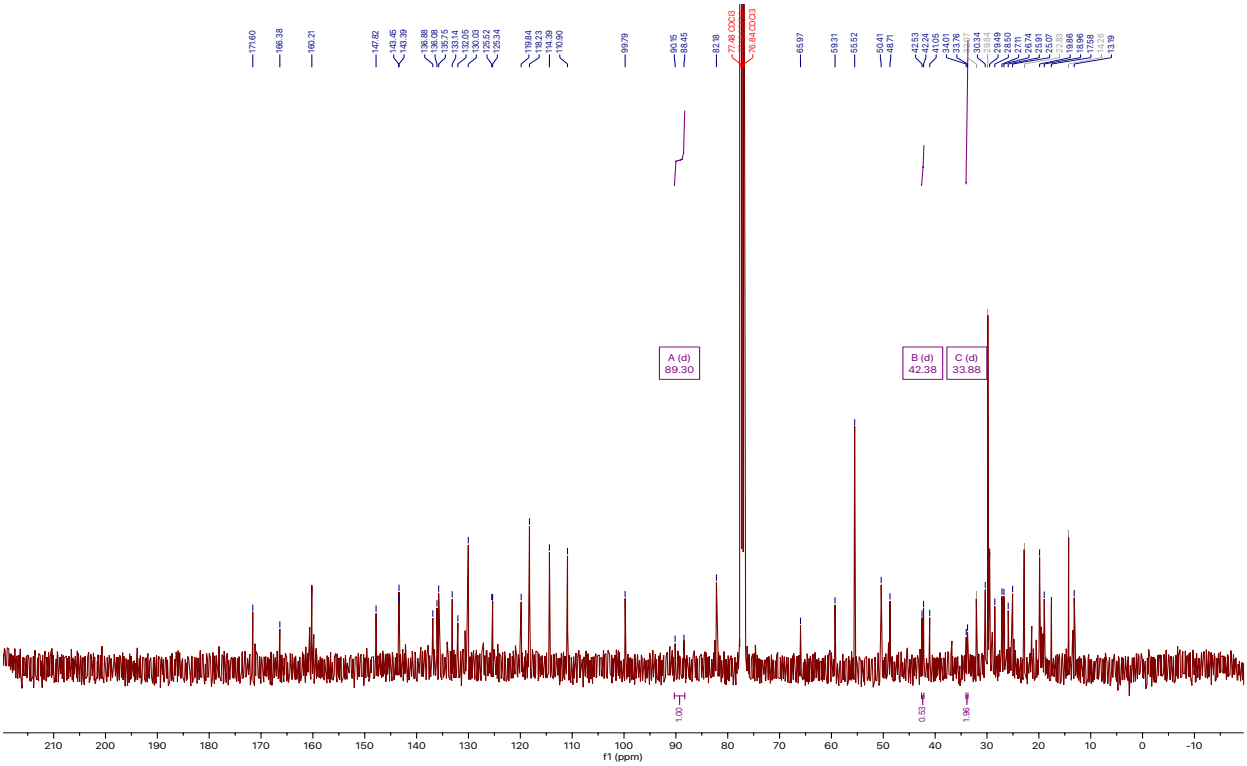
Compound **56l**: ^{13}C NMR (100 MHz, $CDCl_3$)



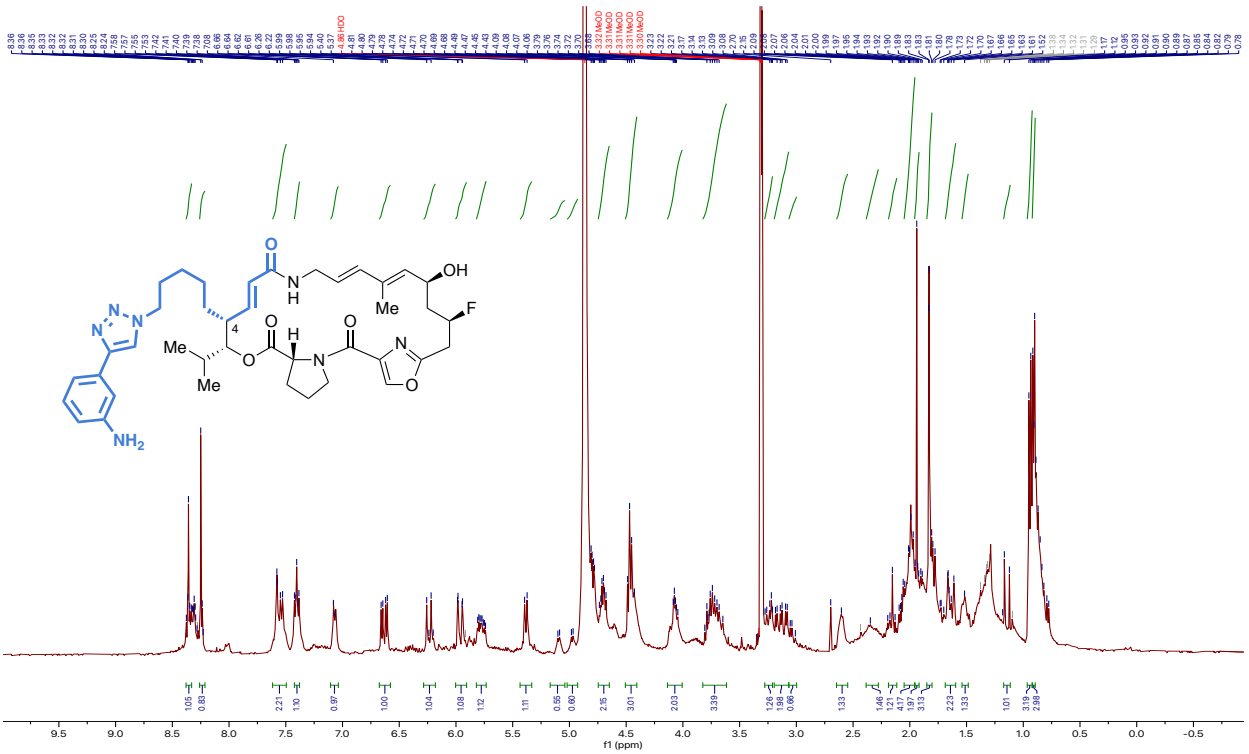
Compound **56m**: ^{1}H NMR (400 MHz, $CDCl_3$)



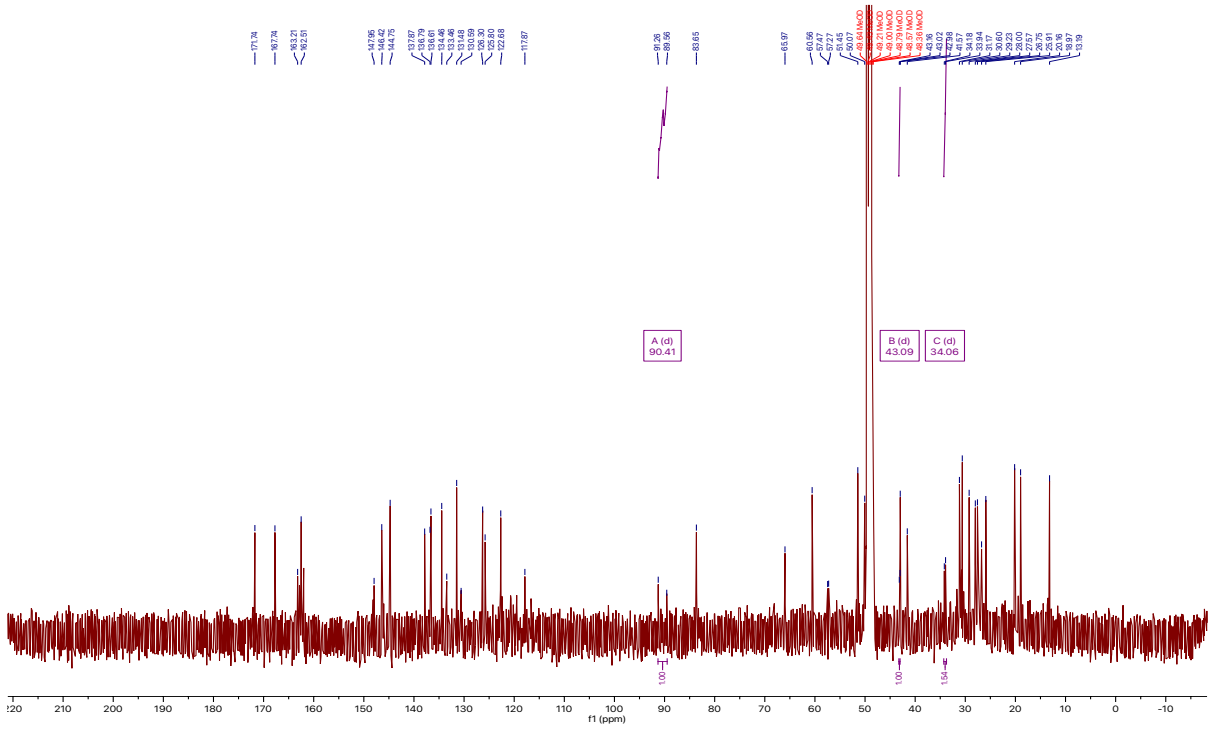
Compound **56m**: ^{13}C NMR (100 MHz, $CDCl_3$)



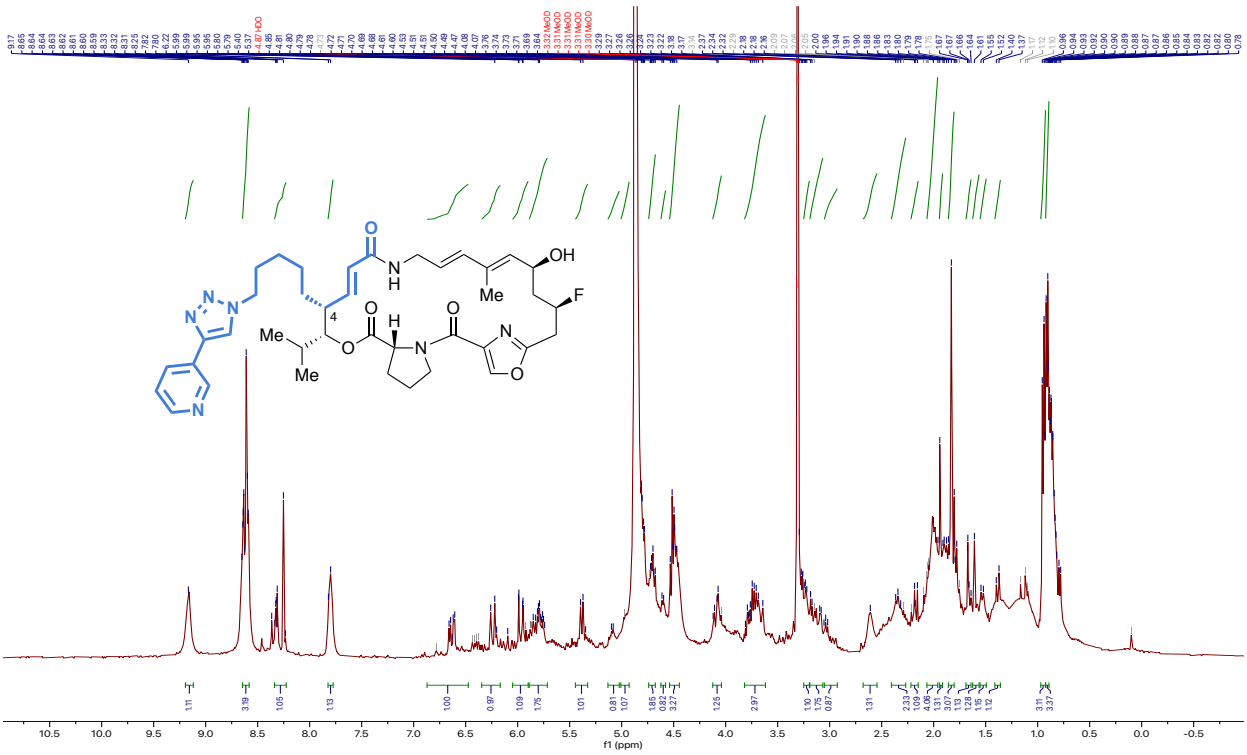
Compound **56n**: ^{1}H NMR (400 MHz, MeOD)



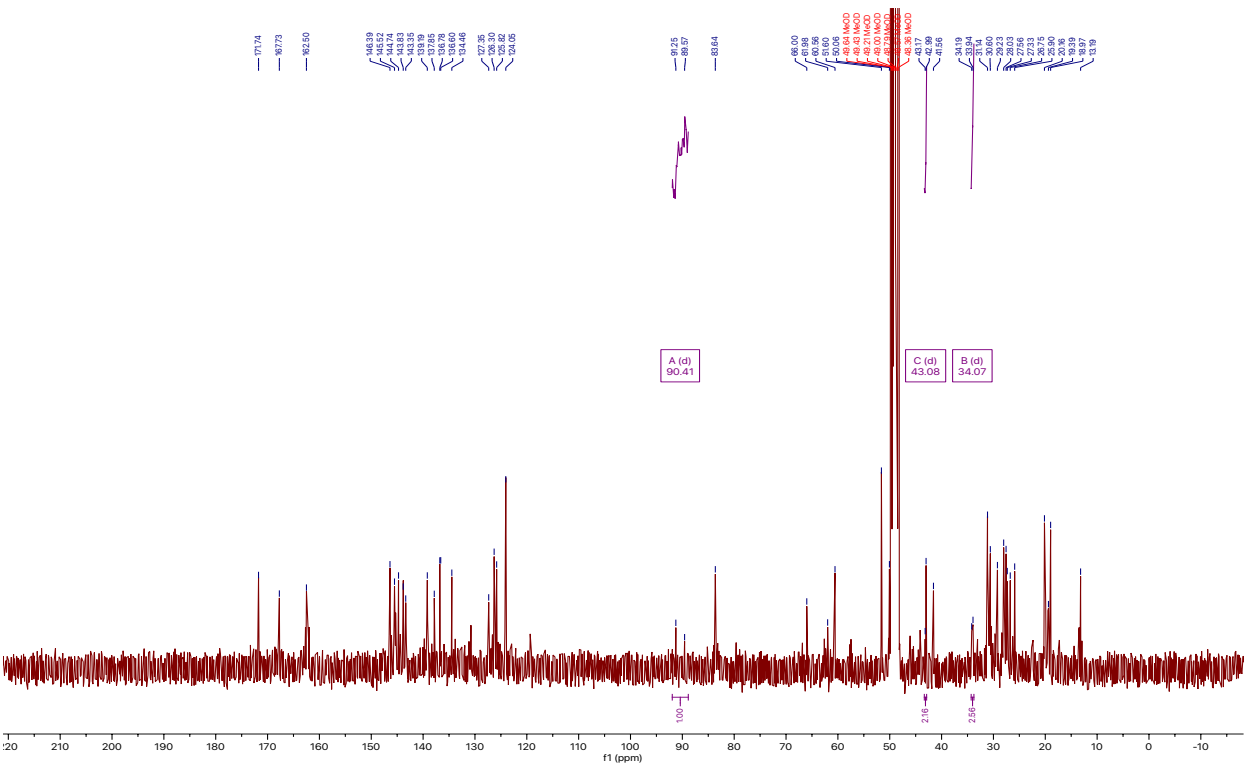
Compound **56n**: ^{13}C NMR (100 MHz, MeOD)



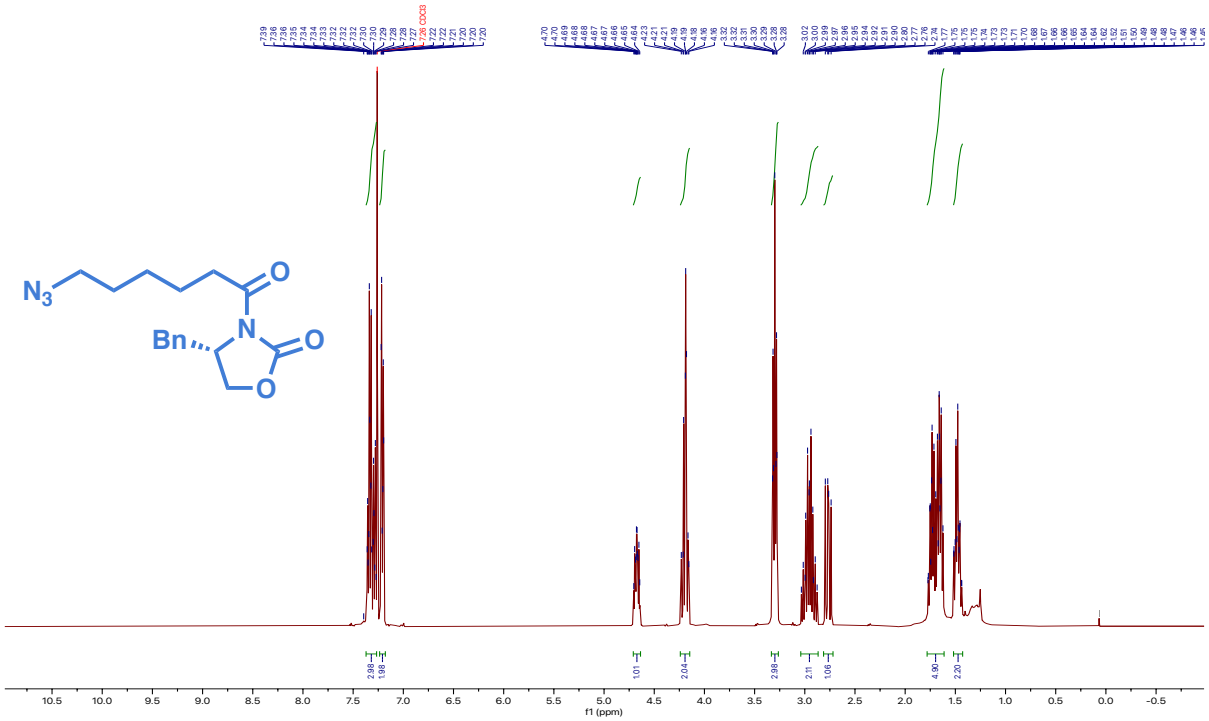
Compound **56o**: ^{1}H NMR (400 MHz, MeOD)



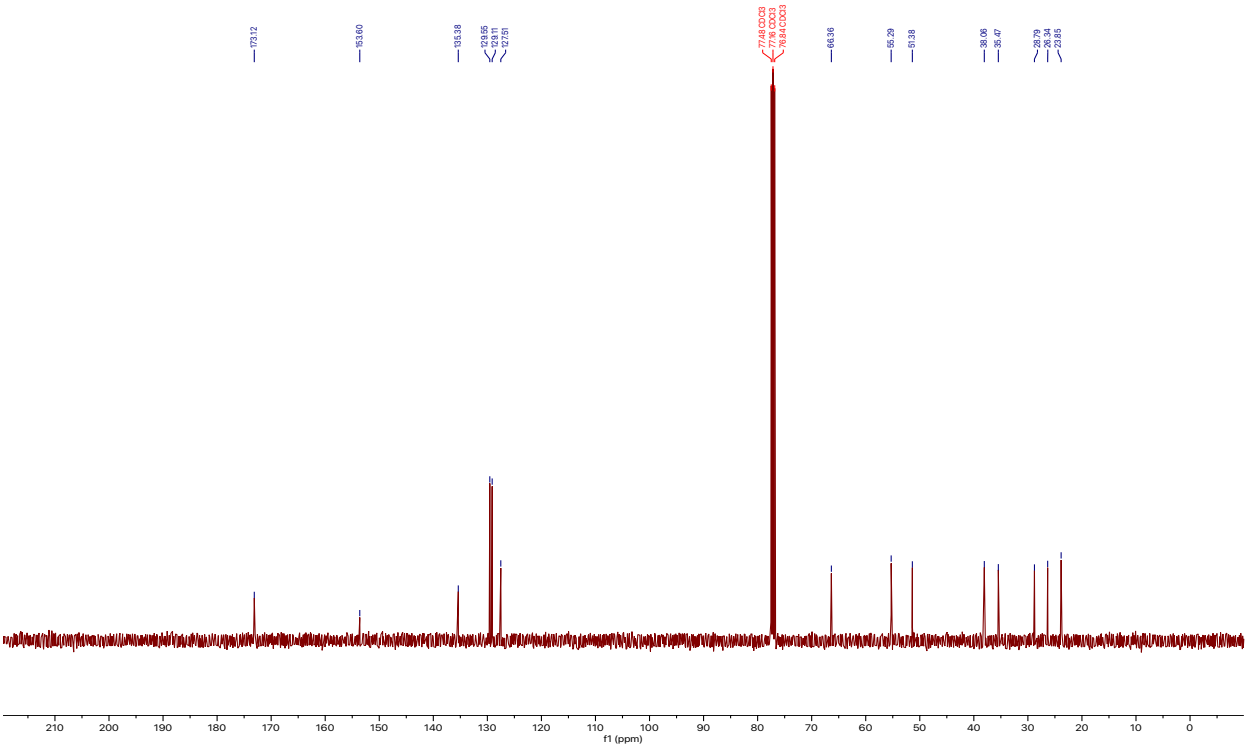
Compound **56o**: ^{13}C NMR (100 MHz, MeOD)



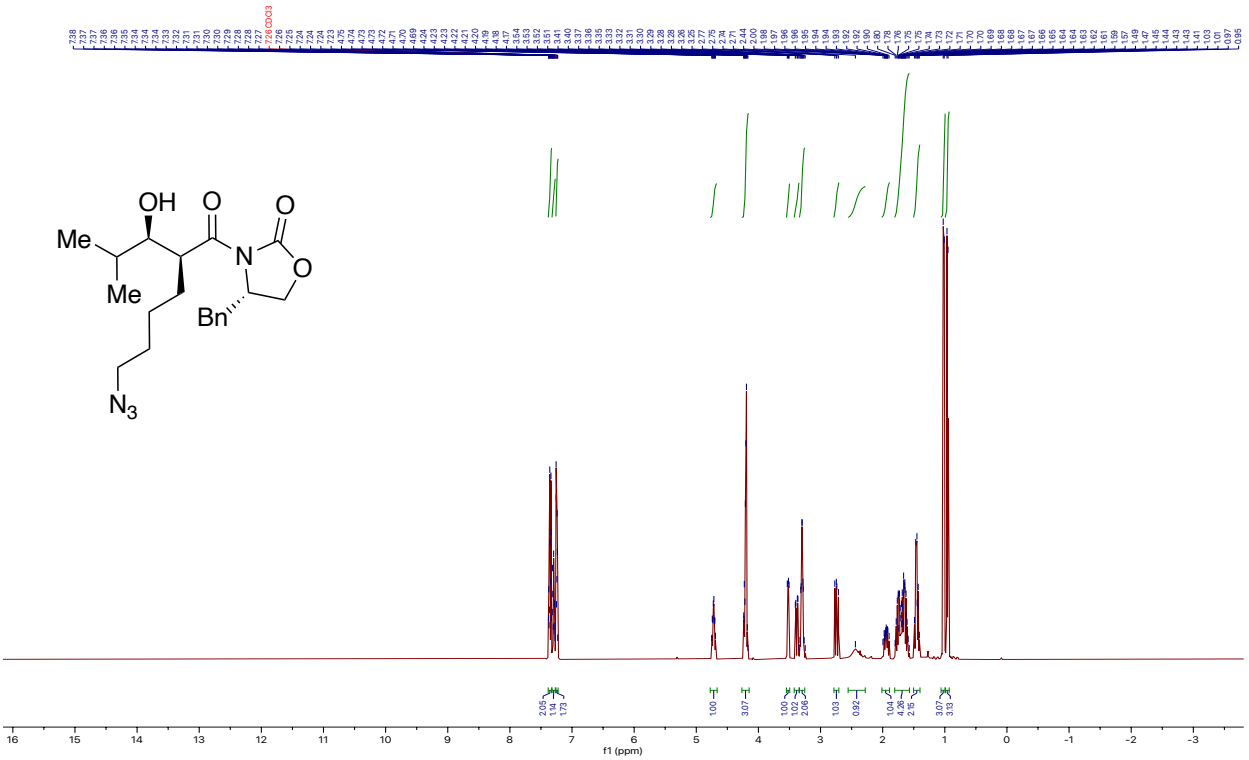
Compound **57**: ^{1}H NMR (300 MHz, $CDCl_3$)



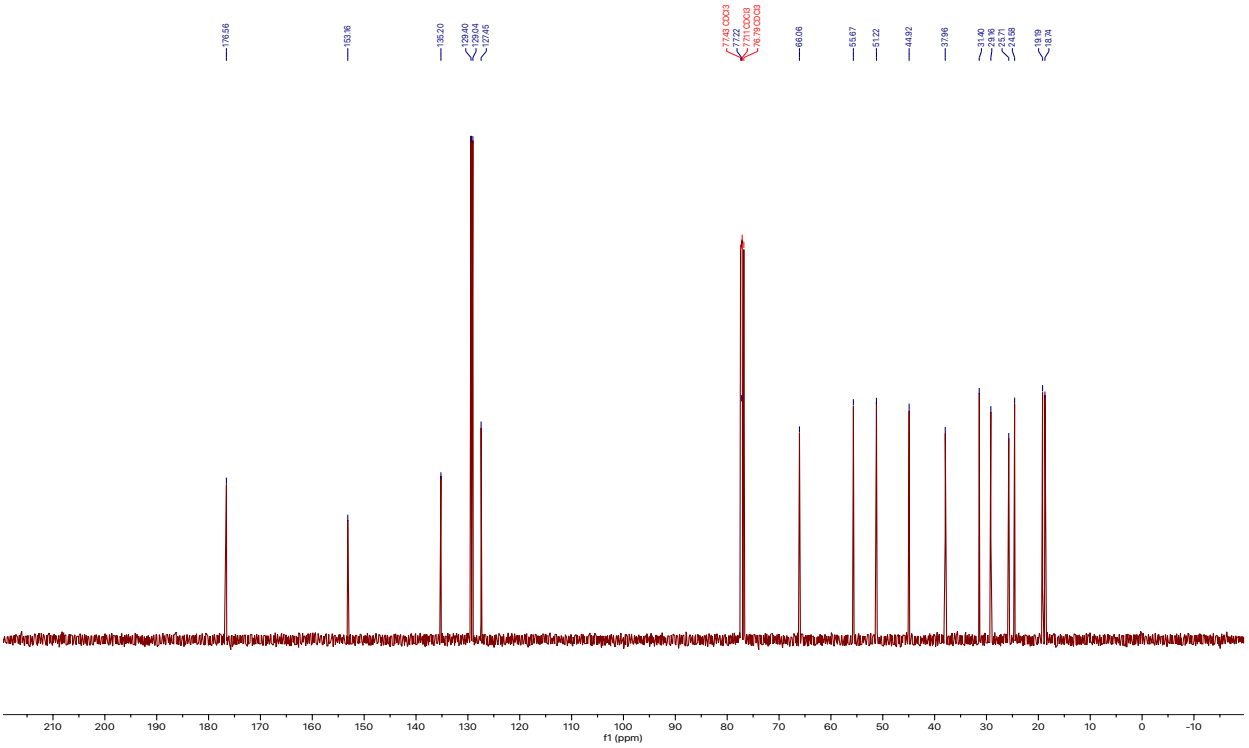
Compound **57**: ^{13}C NMR (100 MHz, $CDCl_3$)



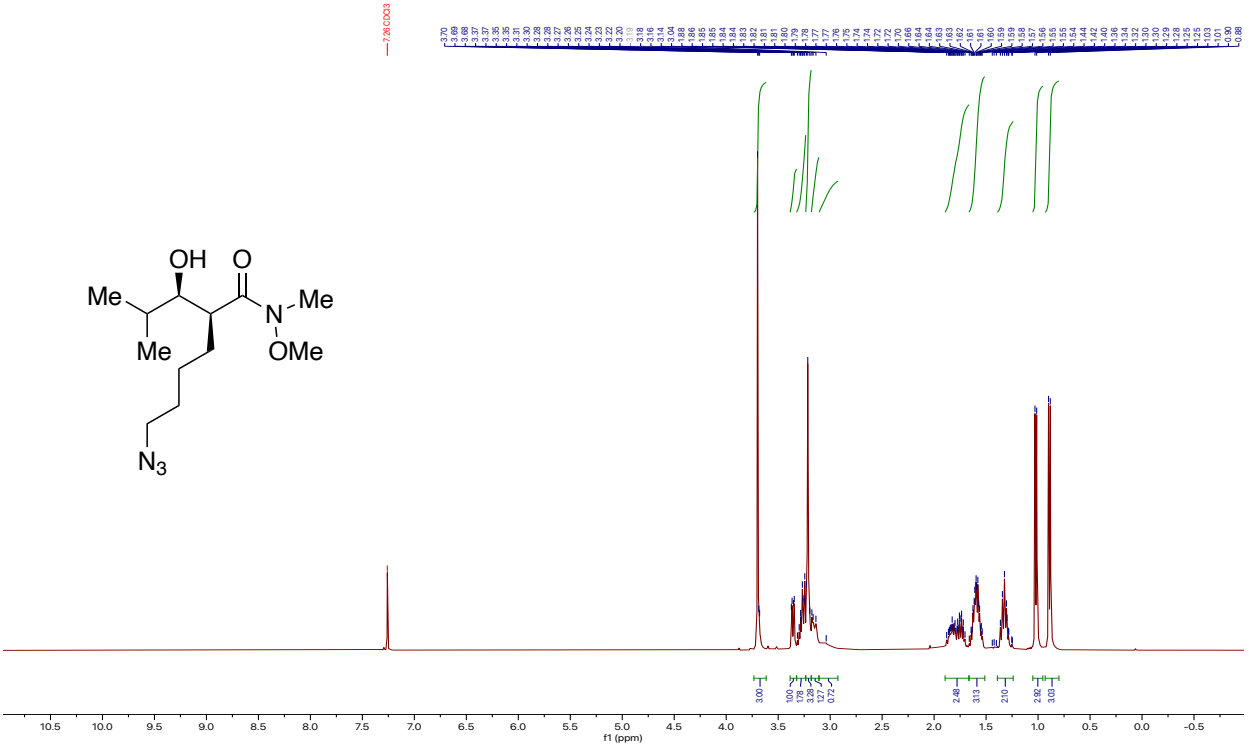
Compound **SI-22**: ^{1}H NMR (400 MHz, $CDCl_3$)



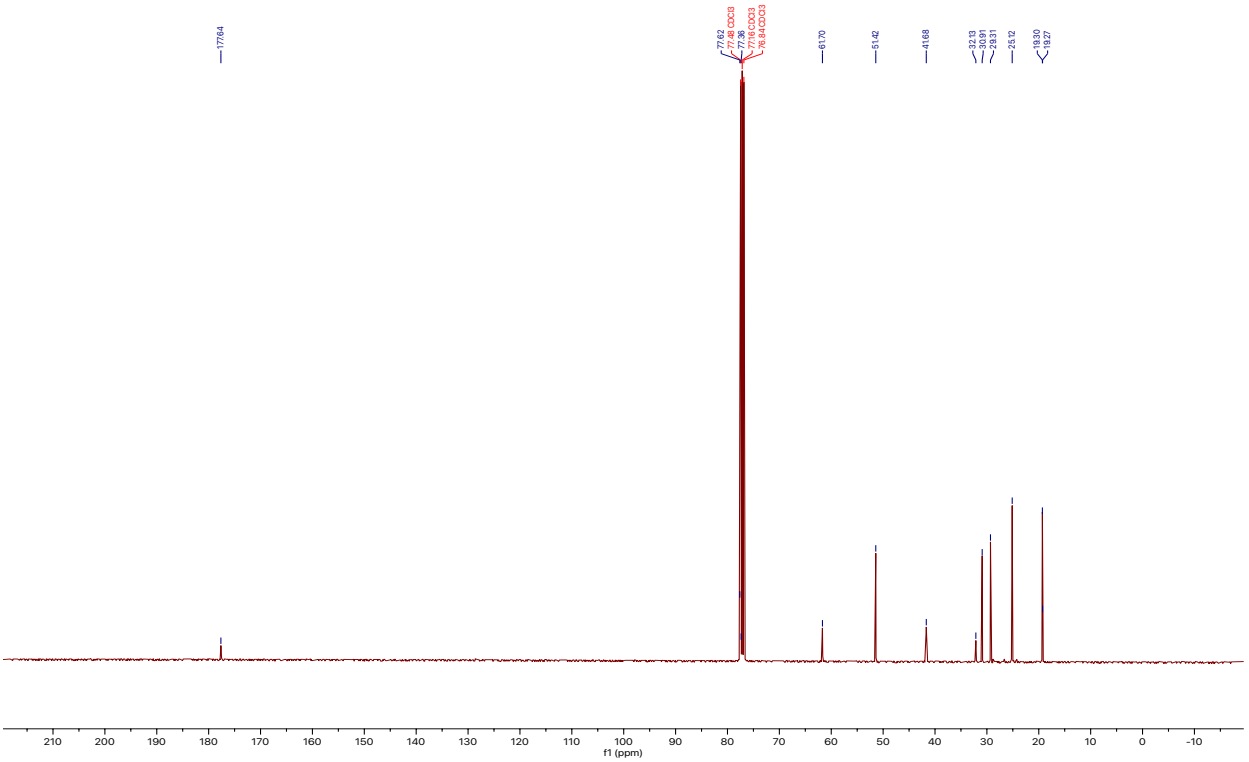
Compound **SI-22**: ^{13}C NMR (100 MHz, $CDCl_3$)



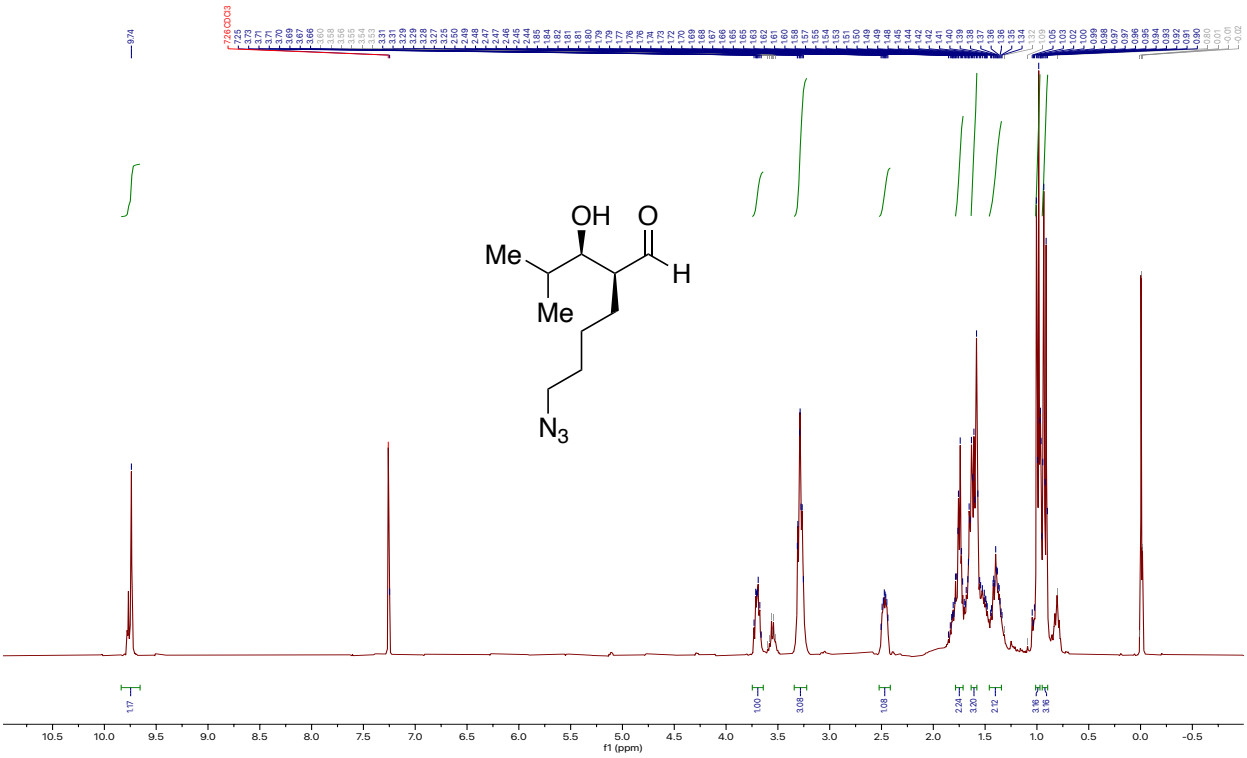
Compound **58**: ^{1}H NMR (400 MHz, $CDCl_3$)



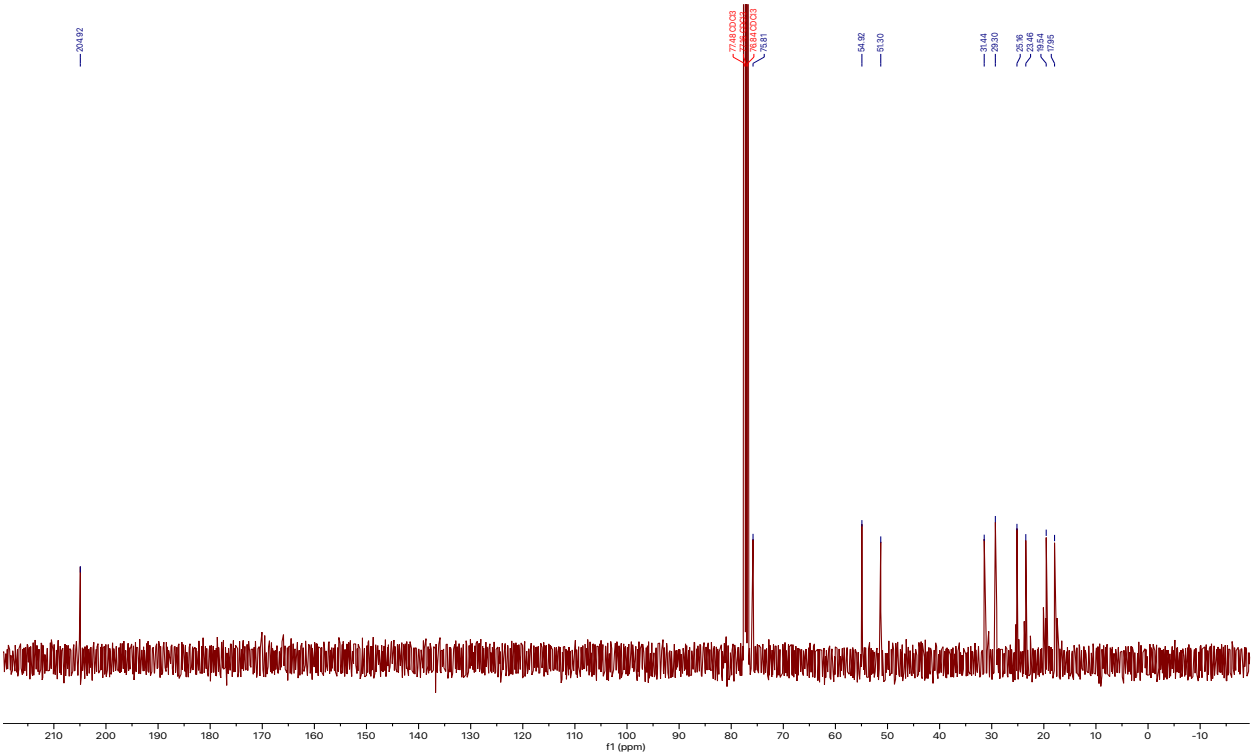
Compound **58**: ^{13}C NMR (100 MHz, $CDCl_3$)



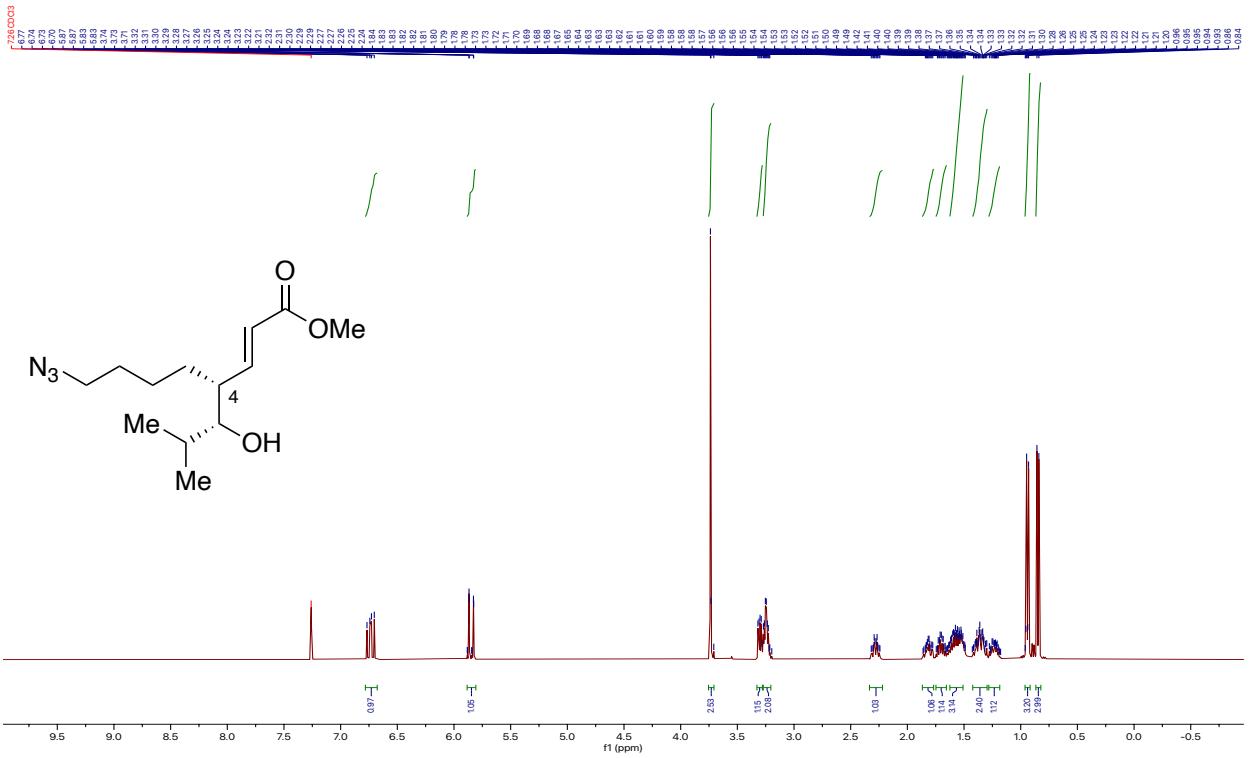
Compound **SI-26**: ^{1}H NMR (300 MHz, $CDCl_3$)



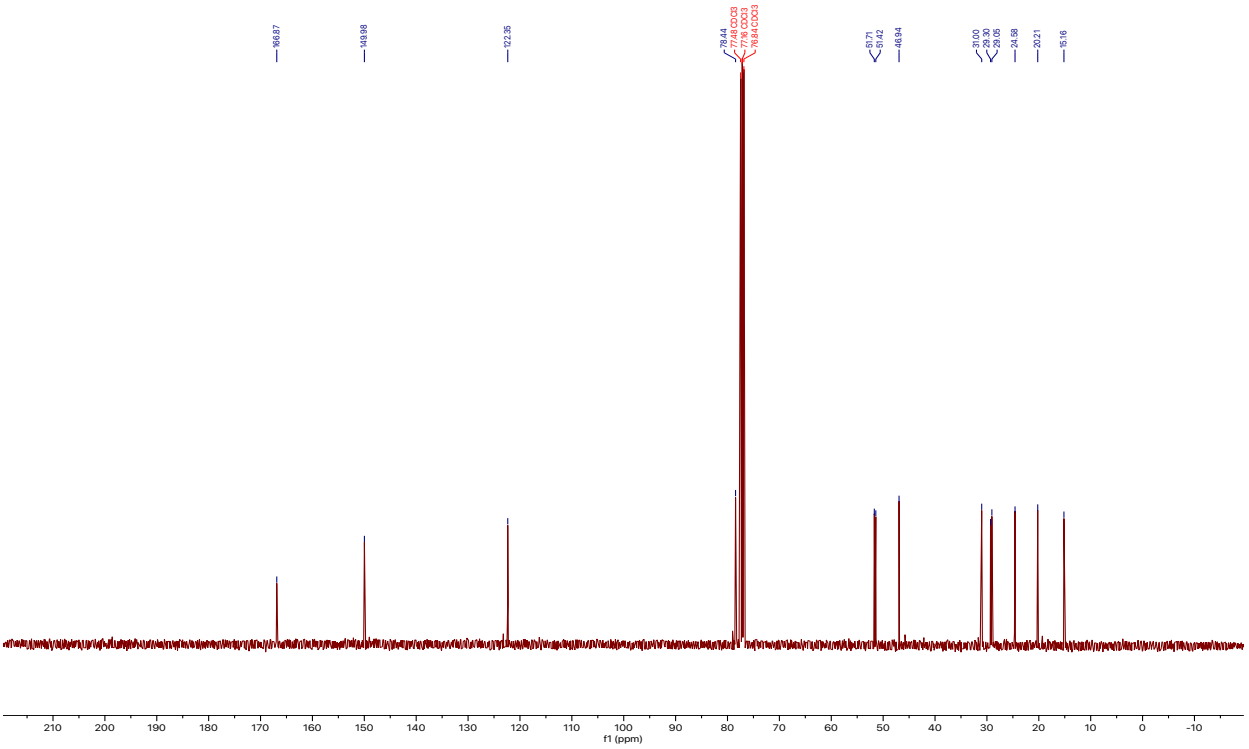
Compound **SI-26**: ^{13}C NMR (100 MHz, $CDCl_3$)



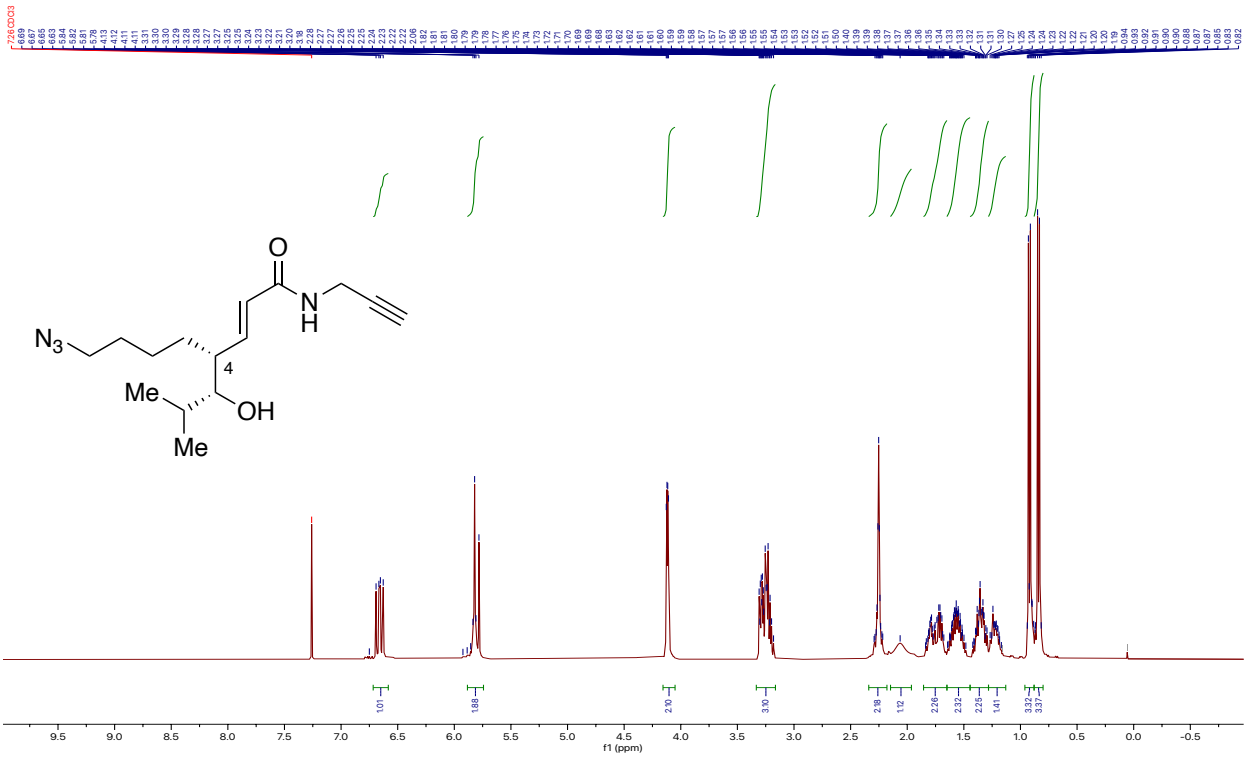
Compound **SI-23**: ^{1}H NMR (400 MHz, $CDCl_3$)



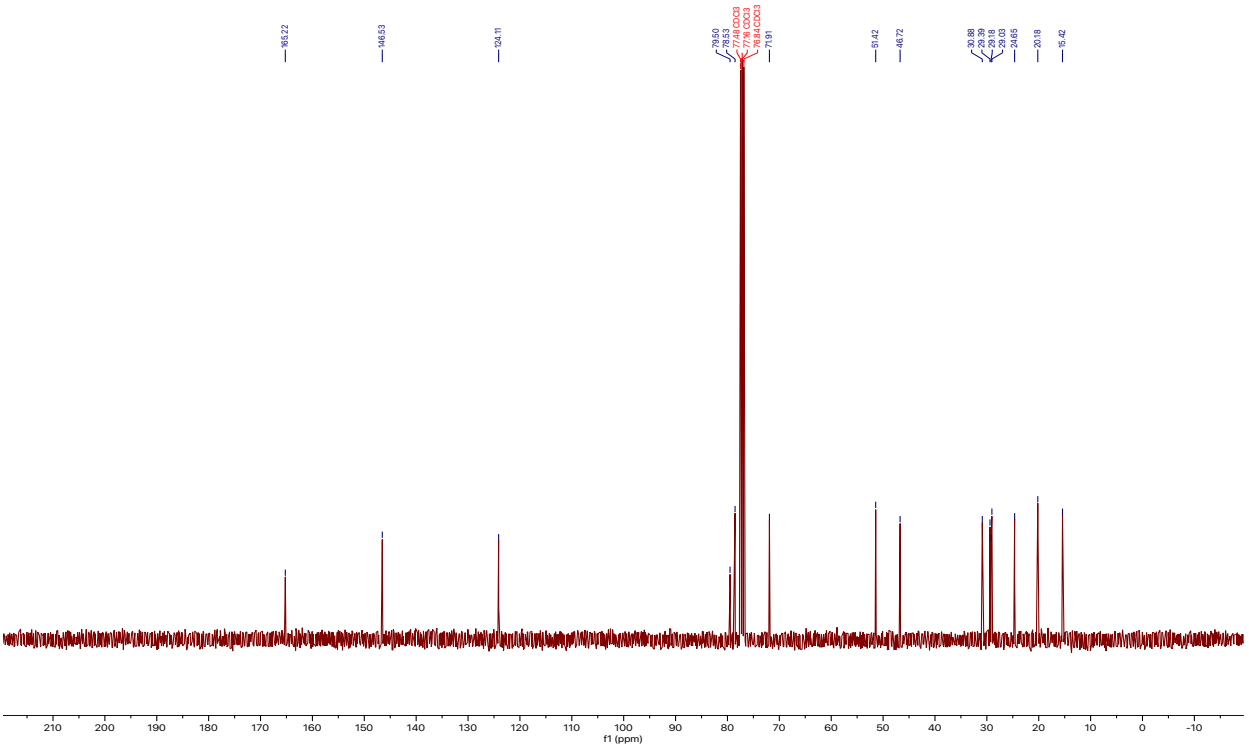
Compound **SI-23**: ^{13}C NMR (100 MHz, $CDCl_3$)



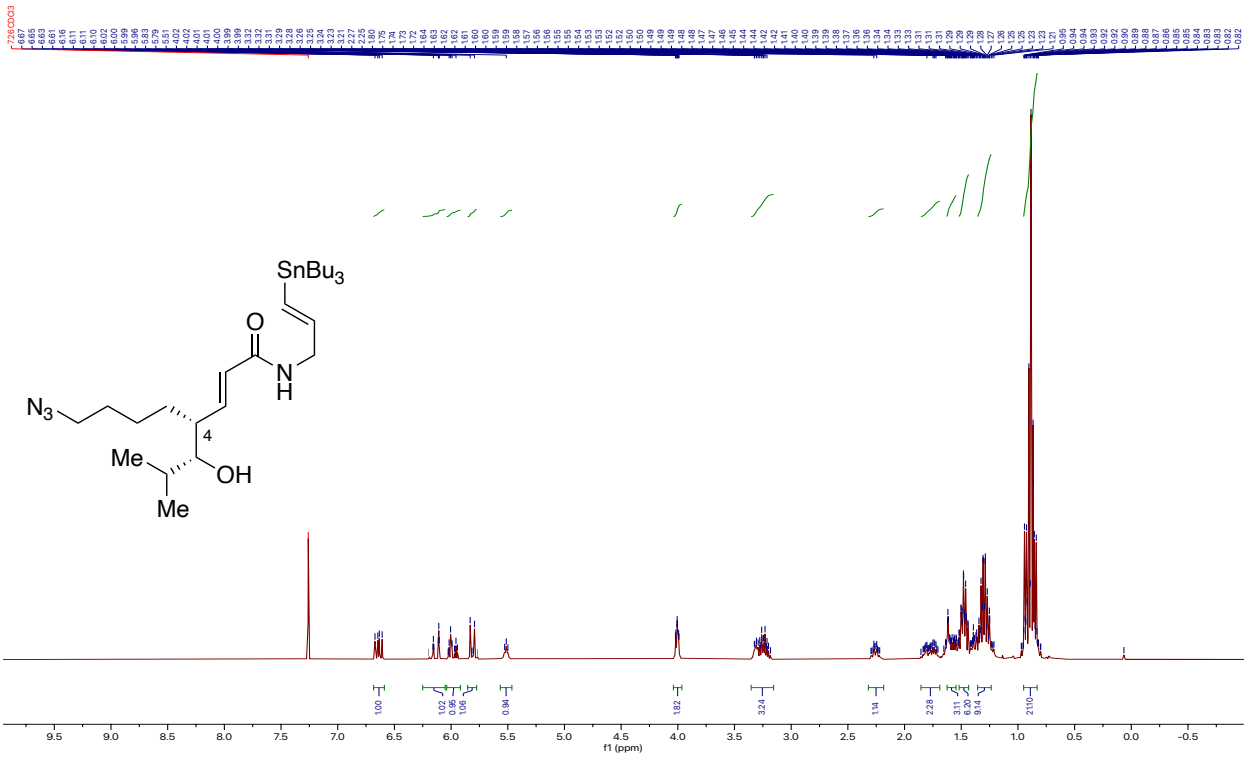
Compound **SI-24**: ^{1}H NMR (400 MHz, $CDCl_3$)



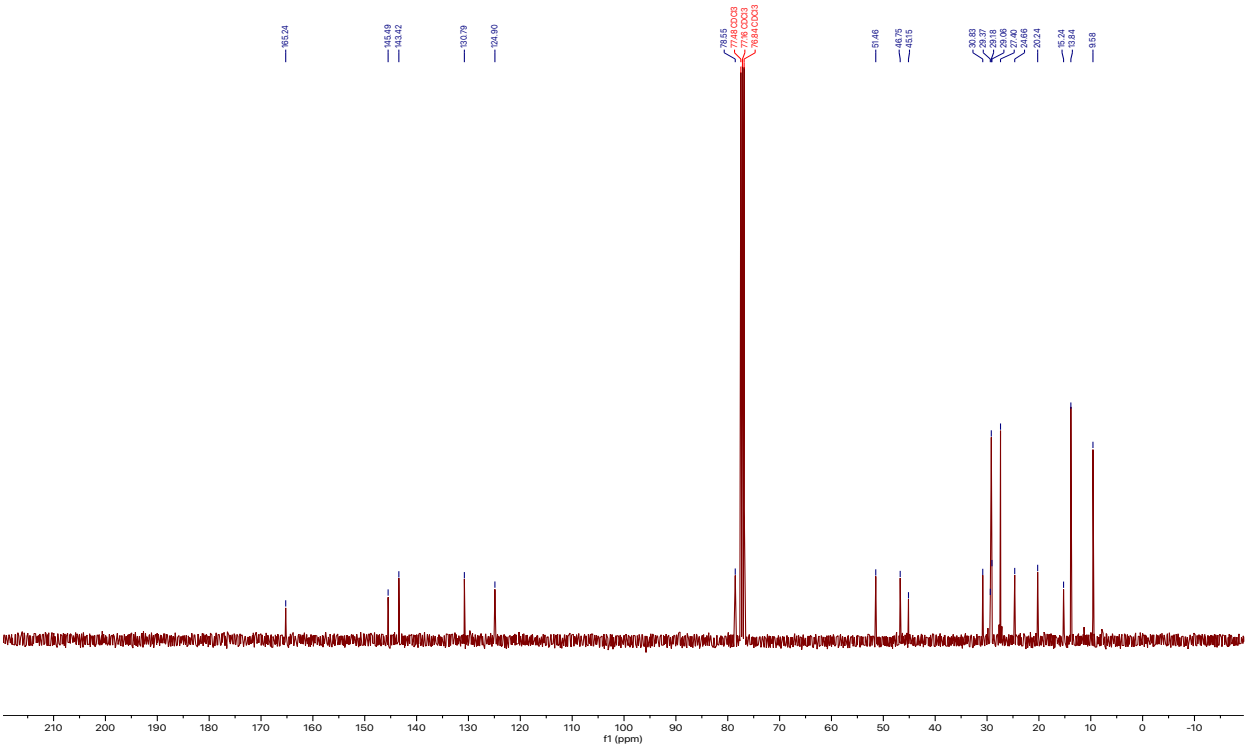
Compound **SI-24**: ^{13}C NMR (100 MHz, $CDCl_3$)



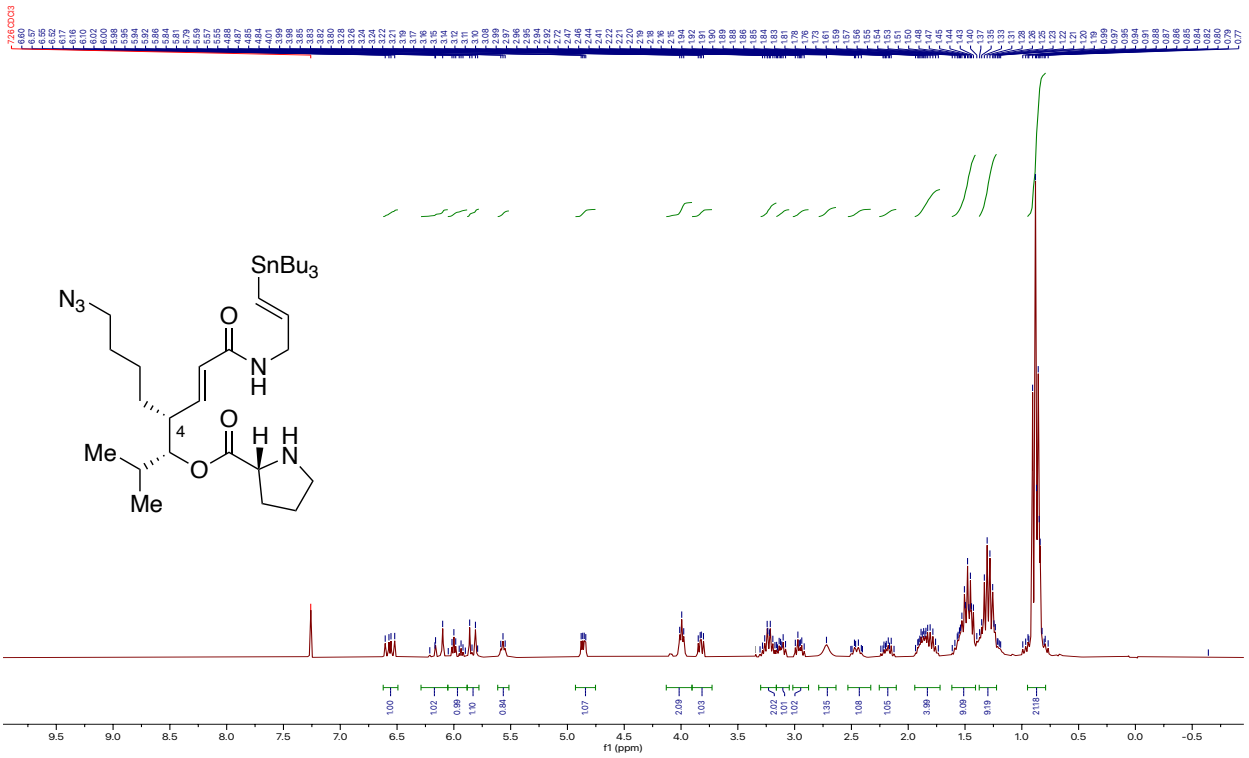
Compound **SI-25**: ^{1}H NMR (400 MHz, $CDCl_3$)



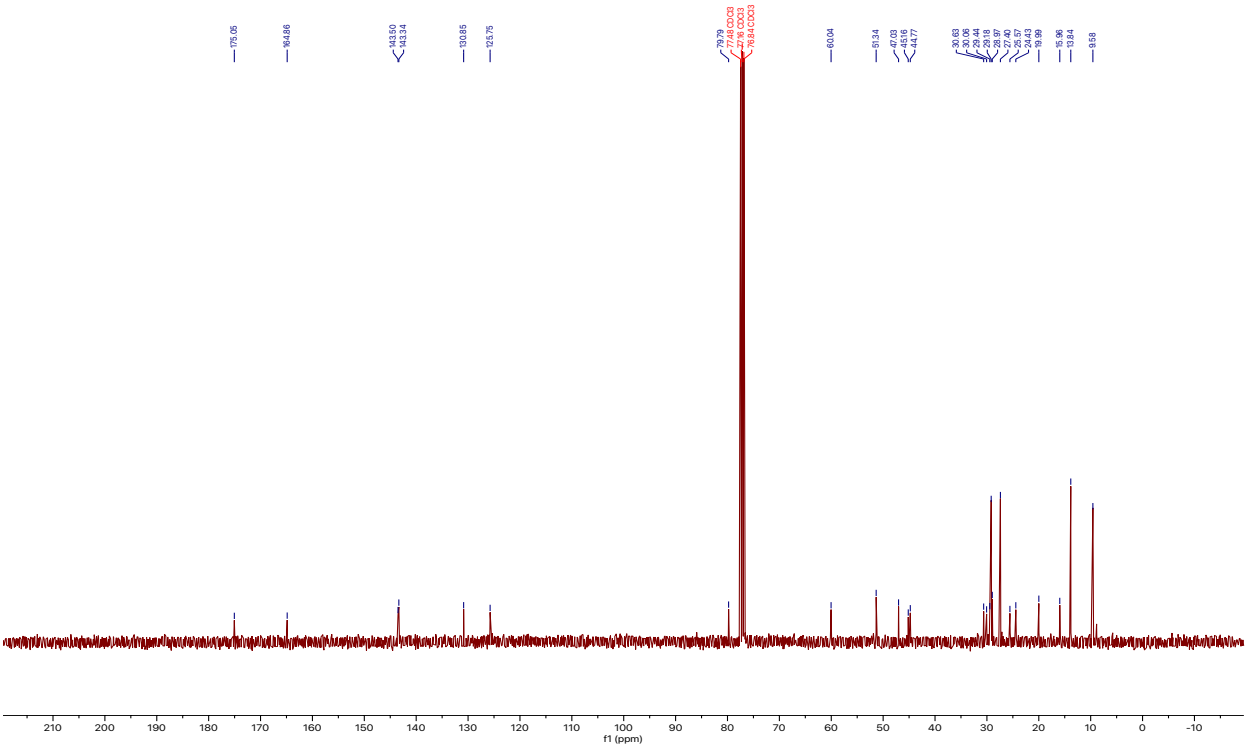
Compound **SI-25**: ^{13}C NMR (100 MHz, $CDCl_3$)



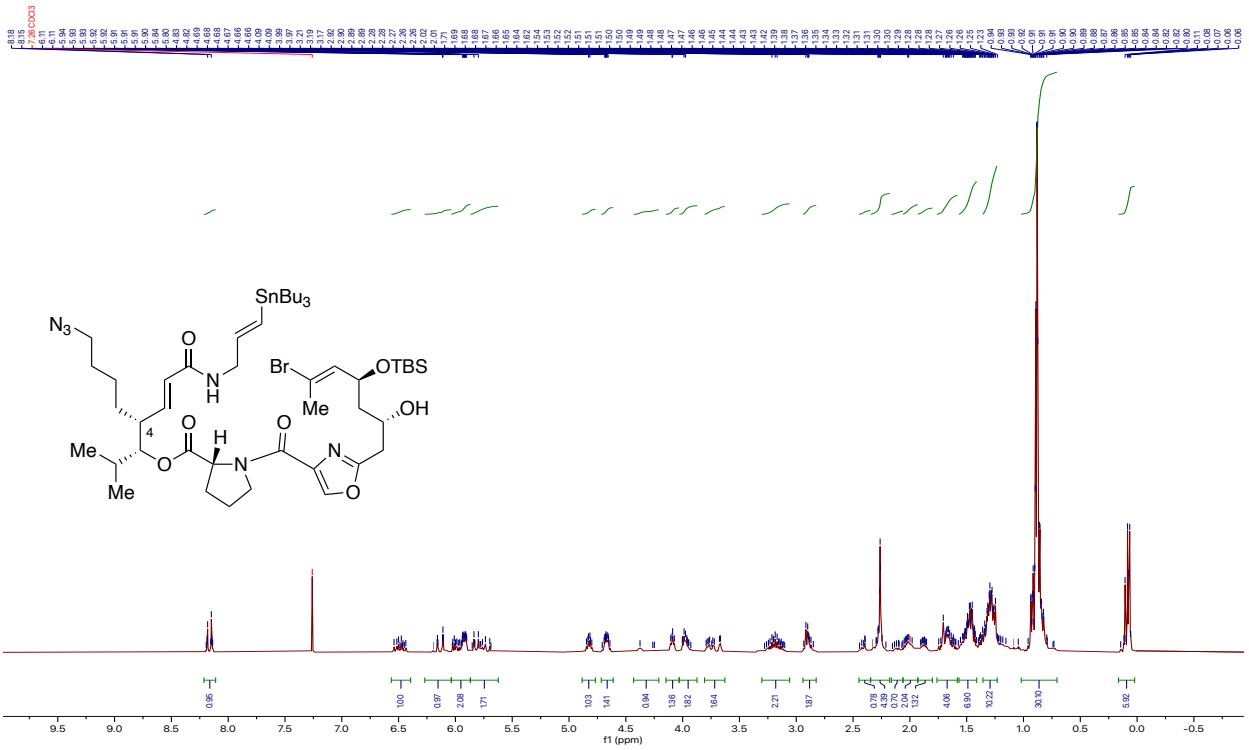
Compound **60**: ^{1}H NMR (300 MHz, $CDCl_3$)



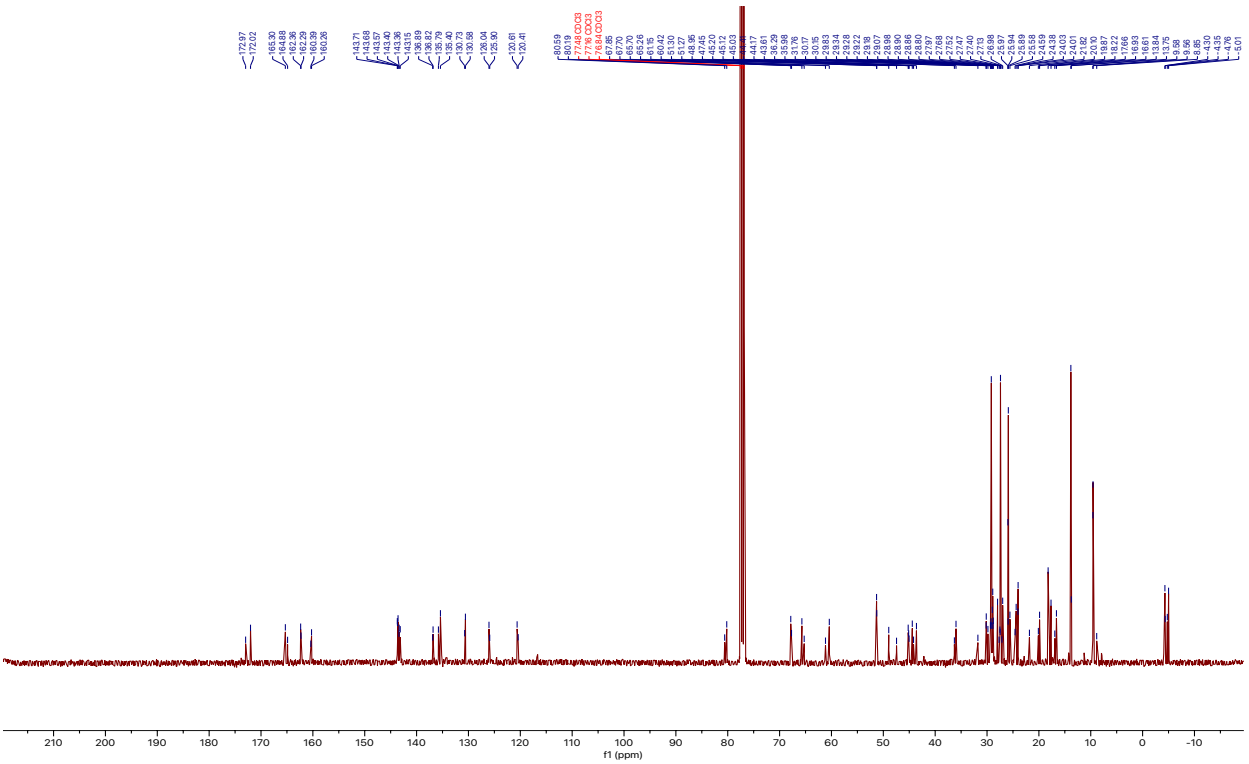
Compound **60**: ^{13}C NMR (100 MHz, $CDCl_3$)



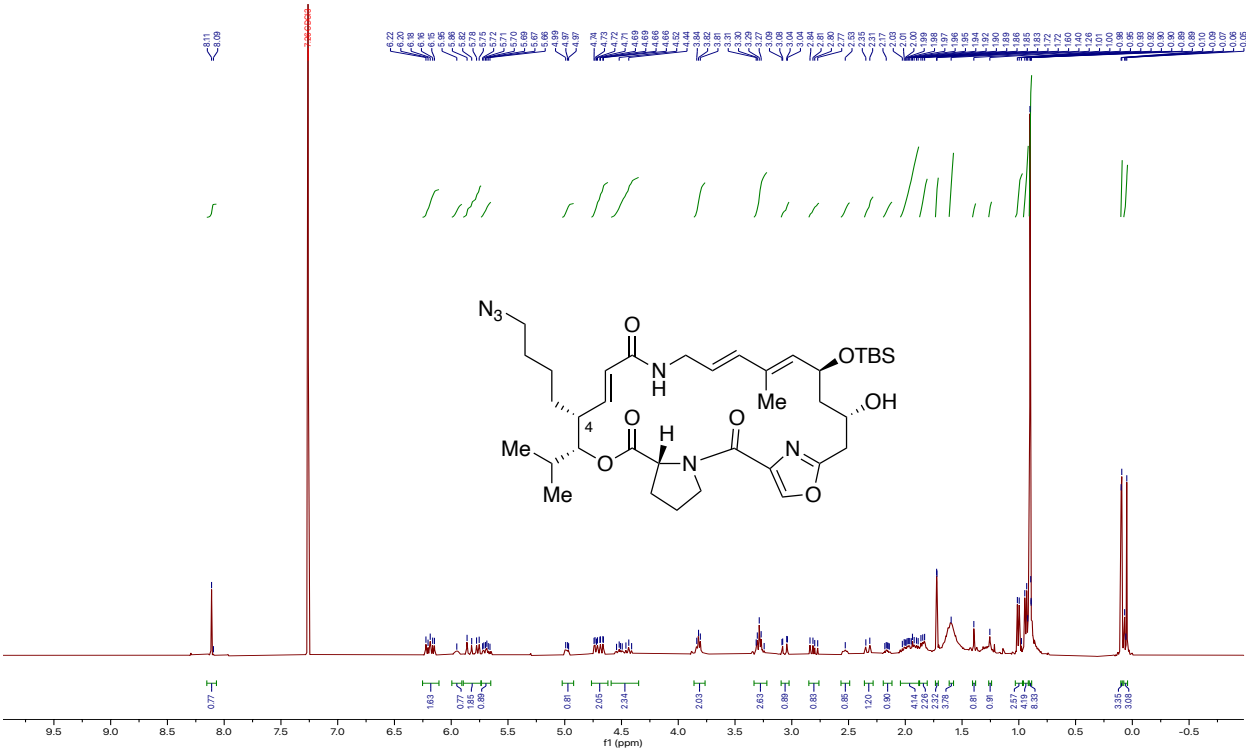
Compound **SI-27**: ^{1}H NMR (400 MHz, $CDCl_3$)



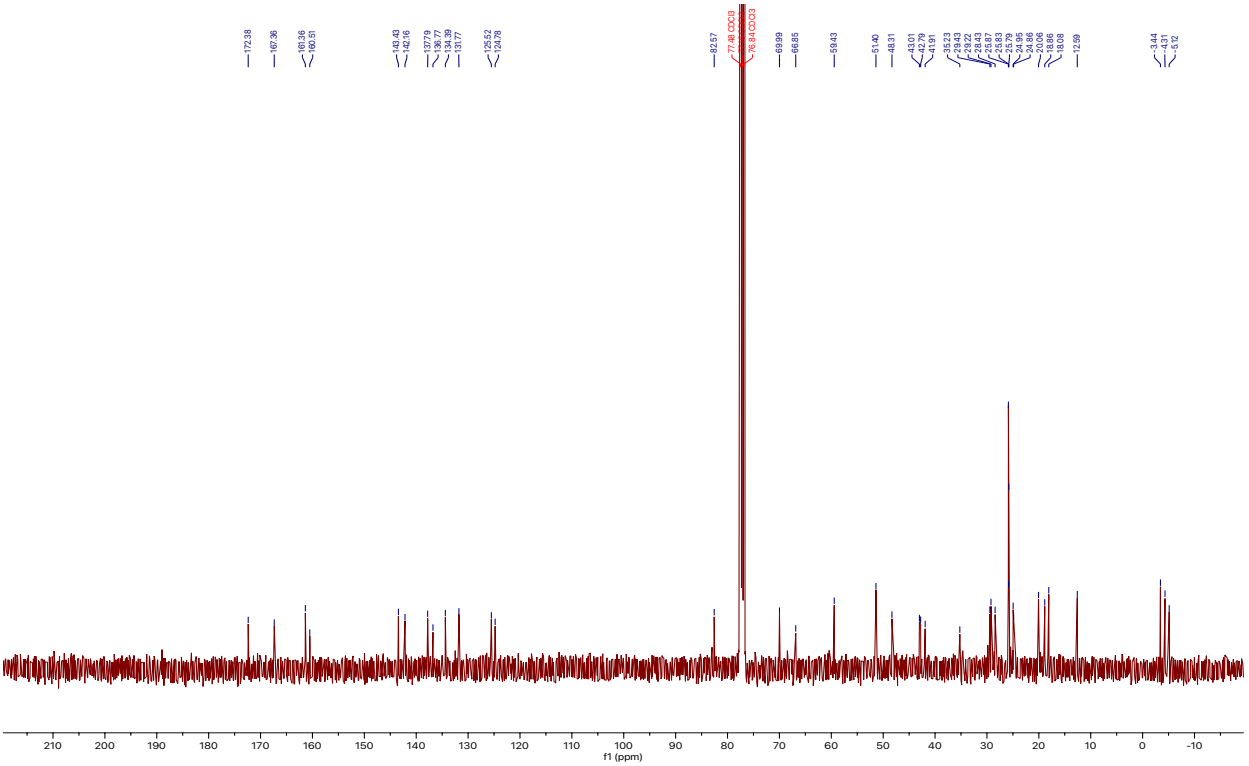
Compound **SI-27**: ^{13}C NMR (100 MHz, $CDCl_3$)



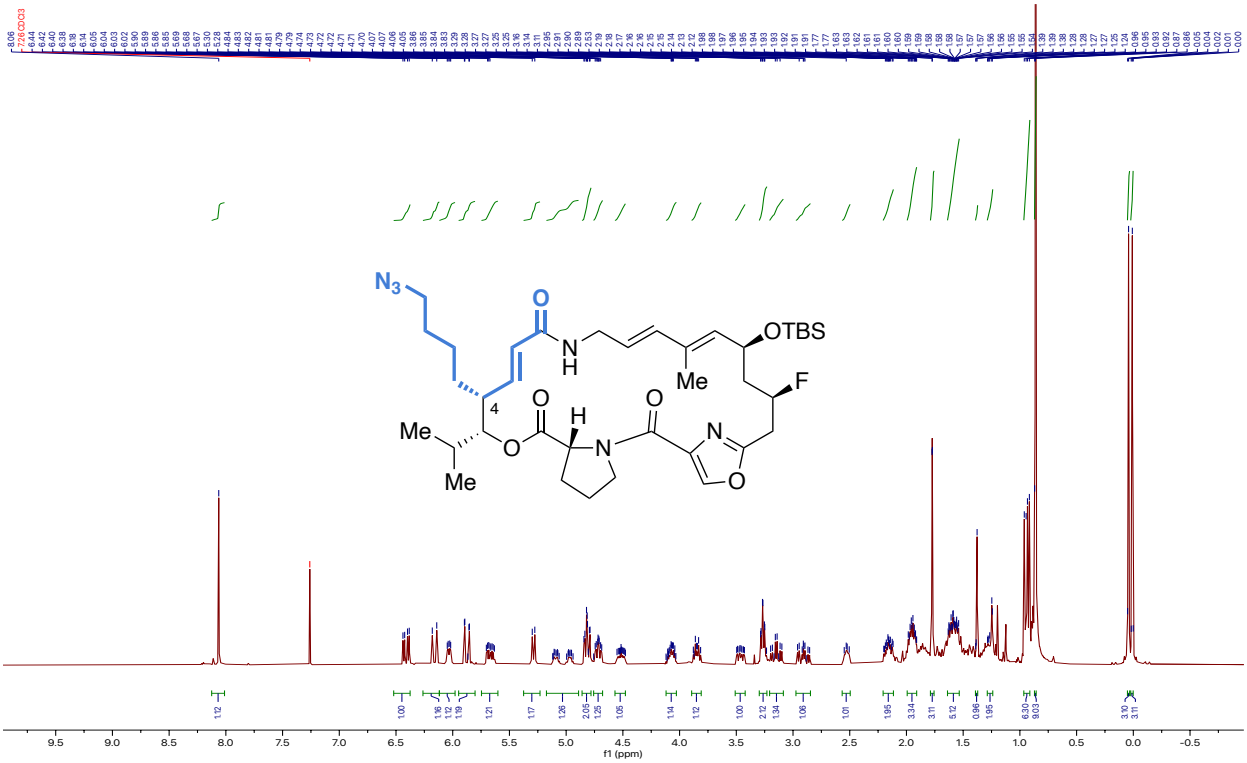
Compound **61**: ^{1}H NMR (400 MHz, $CDCl_3$)



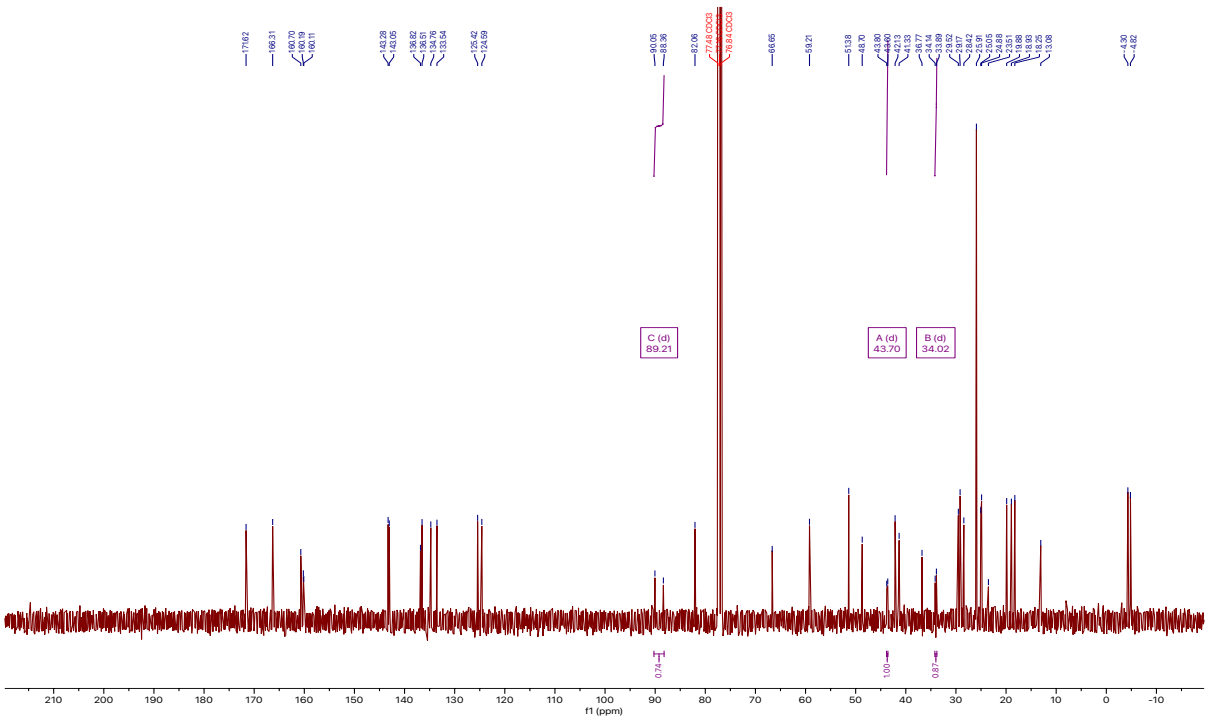
Compound **61**: ^{13}C NMR (100 MHz, $CDCl_3$)



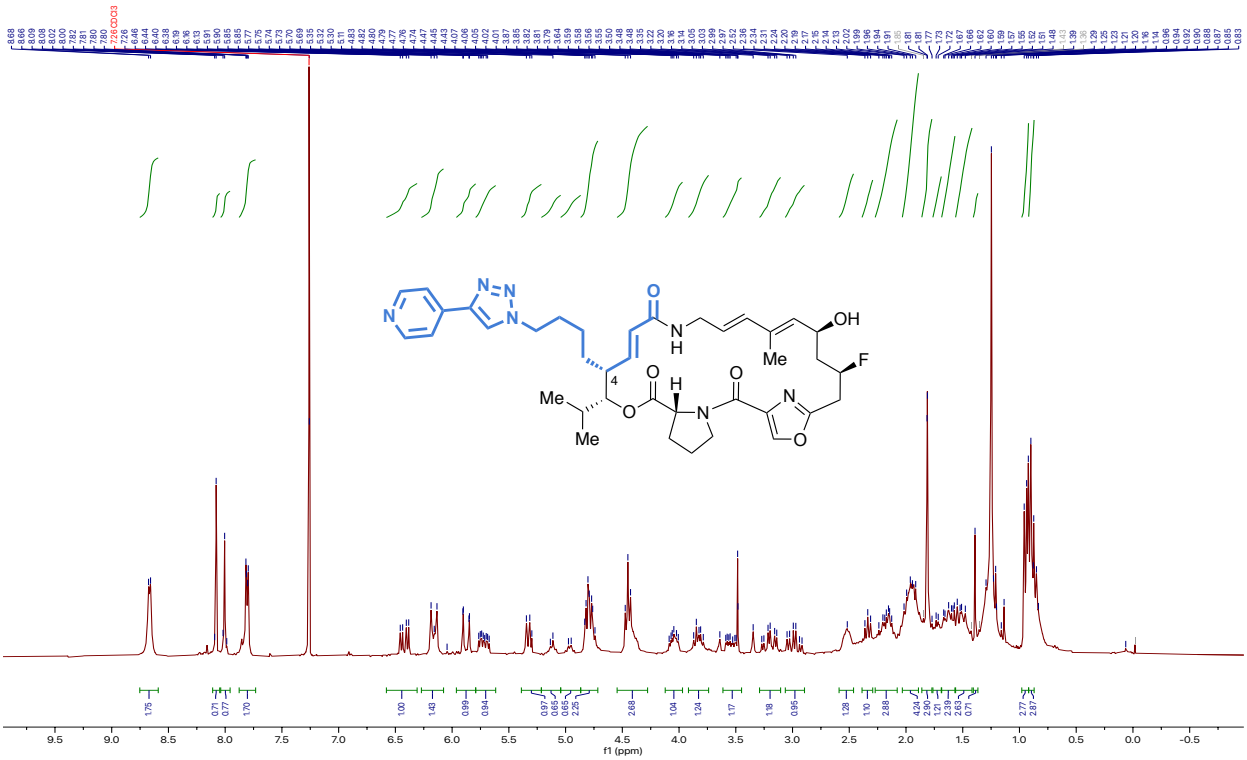
Compound **62**: ^{1}H NMR (400 MHz, $CDCl_3$)



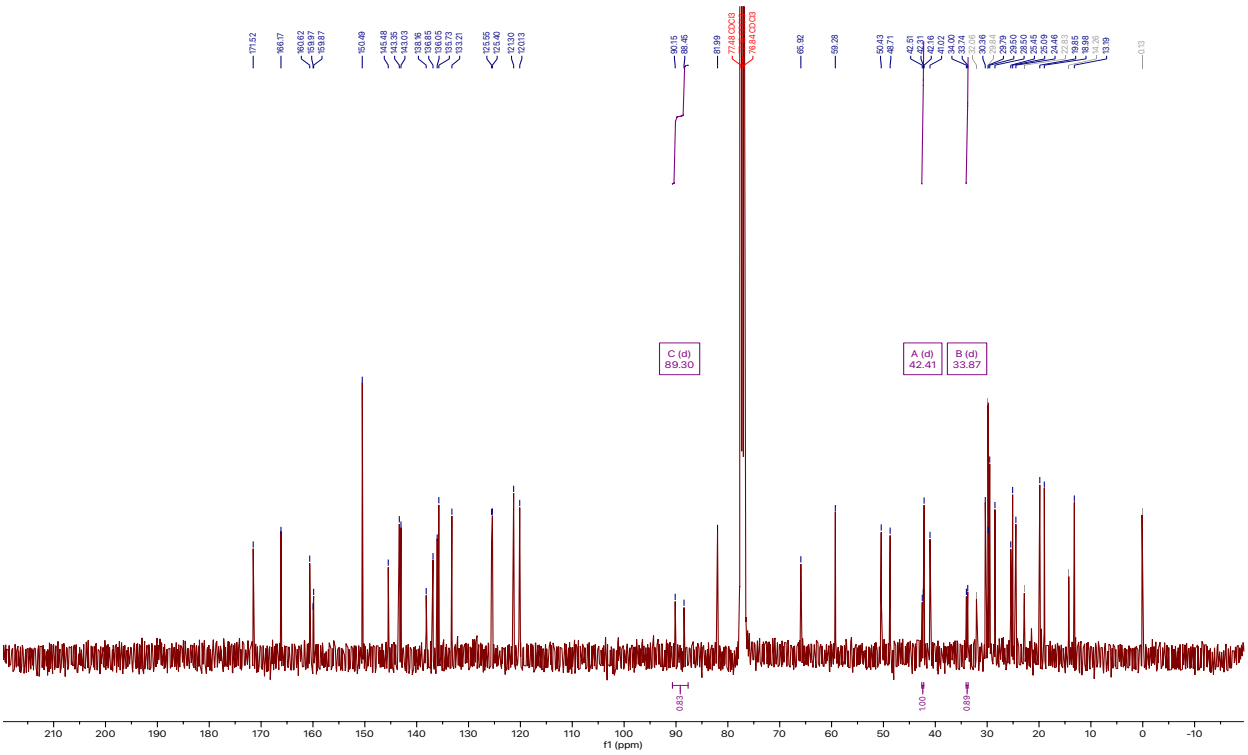
Compound **62**: ^{13}C NMR (100 MHz, $CDCl_3$)



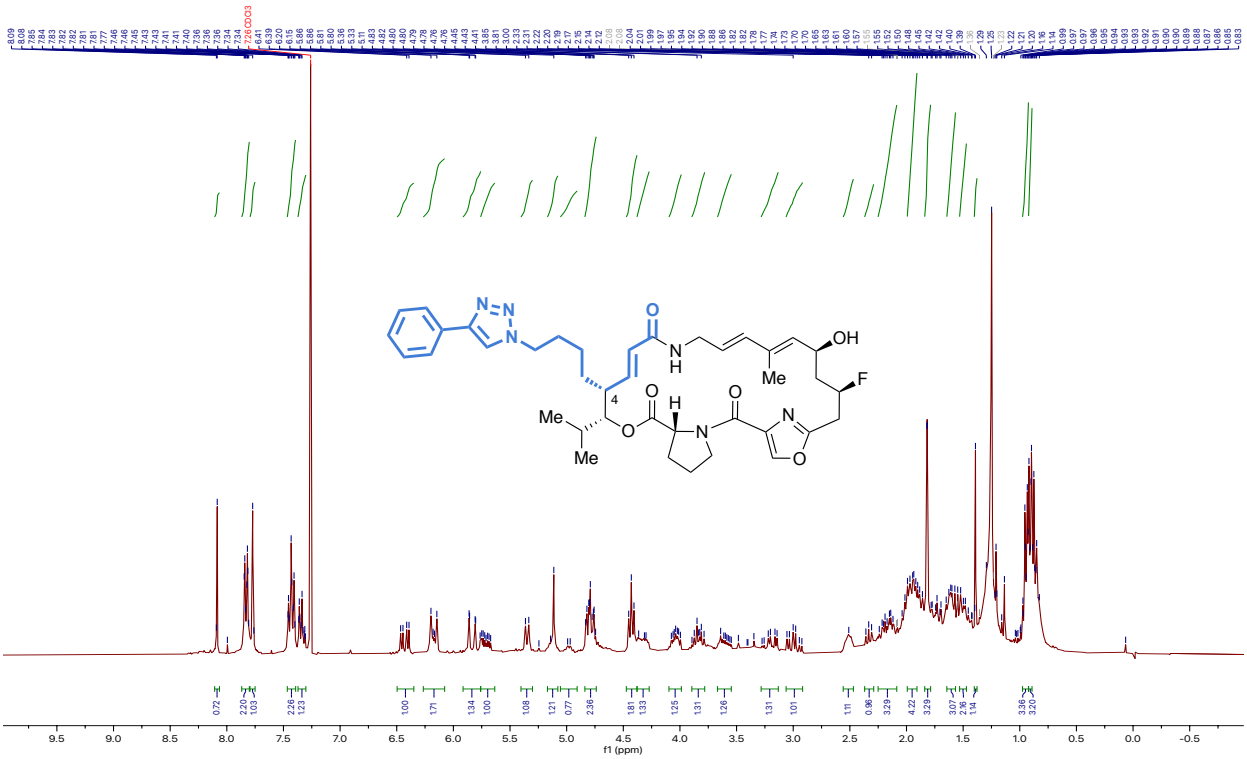
Compound **63a**: ^{1}H NMR (300 MHz, $CDCl_3$)



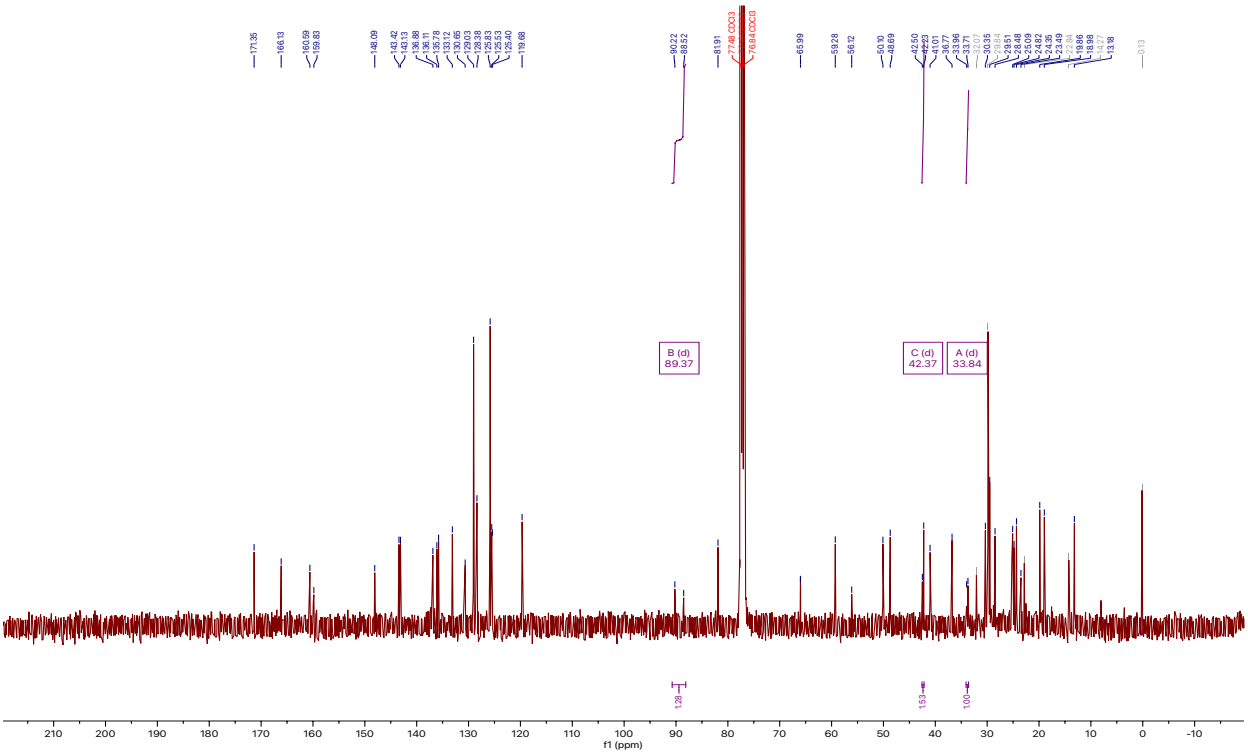
Compound **63a**: ^{13}C NMR (100 MHz, $CDCl_3$)



Compound **63b**: ^{1}H NMR (300 MHz, $CDCl_3$)



Compound **63b**: ^{13}C NMR (100 MHz, $CDCl_3$)



Chapter 2. Streptogramins as New TB Therapeutics

2.1 Introduction

Tuberculosis (TB) remains one of the deadliest infections globally, caused by the single pathogen *Mycobacterium tuberculosis* (Mtb). It is also the leading cause of death attributed to antimicrobial resistance.[51] Resistance to first-line antibiotics, specifically rifampicin and isoniazid, poses significant treatment challenges, leading to limited treatment plans that are often expensive and associated with severe toxicity. In 2022, The World Health Organization (WHO) endorsed the use of BPaLM (bedaquiline, pretomanid, linezolid, and moxifloxacin) to treat drug-resistant TB in a 6-month treatment regimen.[52] However, treating rifampicin-resistant tuberculosis (RR-TB) and multidrug-resistant tuberculosis (MDR-TB)—defined as resistance to both rifampicin and isoniazid— still requires prolonged regimens with significant adverse effects. Notably, linezolid (**1**), a key component of many TB regimens, is associated with substantial risks such as bone marrow suppression and peripheral neuropathy.[53,54] (Figure 2.1B) These toxicities, along with the long duration of the treatment, contribute to high patient deaths before treatment completion and low cure rates.[51]

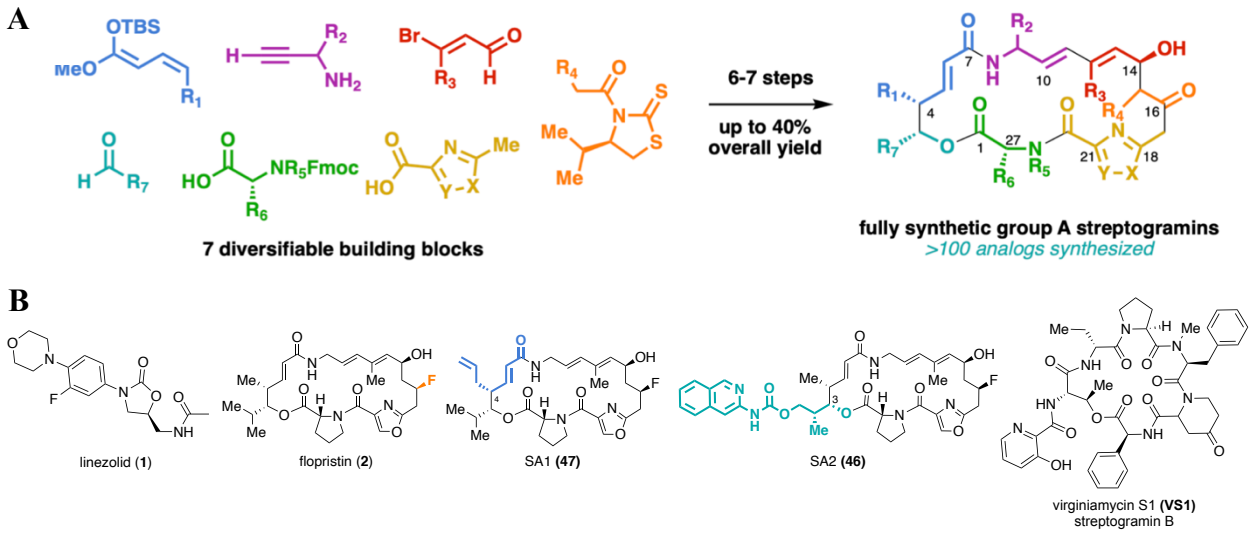
Moreover, linezolid (**1**) exhibits only bacteriostatic activity against latent TB and must be combined with bactericidal agents for efficacy.[55] A less toxic alternative to linezolid (**1**) would be particularly beneficial for patients with immunodeficiencies or other risk factors. Alarmingly, resistance is also emerging for these newer core drugs, giving rise to extensively drug-resistant TB (XDR-TB) which has resistance to rifampicin, any fluoroquinolone, and either bedaquiline or linezolid (**1**). There is a dire need in discovering novel therapeutics that can combat growing Mtb resistance with less toxic side effects.

Streptogramin antibiotics, such as quinupristin-dalfopristin (Synercid), have been used for decades to treat resistant Gram-positive infections. However, their intravenous administration and associated side effects, including venous irritation, have limited their clinical use to a “last resort” combination therapy for vancomycin-resistant infections. In contrast, pristinamycin – an orally bioavailable streptogramin combination, has been extensively used in France for over 50 years, with minimal reported resistance.[1]

Streptogramins offer several advantages over linezolid (**1**): they avoid health risks associated with linezolid (**1**), such as bone marrow suppression and neuropathies, and are bactericidal (rather than bacteriostatic) in a majority of Gram-positive organisms when group A and group B components are used in combination.[1,6,56] Additionally, the dual-component nature of streptogramins make it difficult to confer resistance to both components.[57] Despite these advantages, there is currently no published susceptibility data for streptogramin antibiotics against Mtb.

Compared to Gram-positive organisms that are susceptible to streptogramins, Mtb contains resistance systems that include low permeability to its waxy cell wall, drug exporters, and drug-modifying systems, which are activated by the transcriptional regulator encoded by *whiB7*.[58] Additionally, macrolide resistance caused by *ermMT* (*erm37*) gene in Mtb may also cause resistance towards group B streptogramins.[59] While there is possibility of conferring resistance to streptogramins, the extent in which these Mtb resistance mechanisms impact streptogramin activity can only be confirmed experimentally.

Our group developed a modular synthesis of group A streptogramins from seven modifiable building blocks.[8,13,14] (Figure 2.1A) This synthetic strategy enabled the generation of a diverse library of fully synthetic group A streptogramin analogs, specifically modifying the scaffold of virginiamycin M2 (**VM2**) and flopristin (**2**). From this library, we obtained two hit compounds (**46** and **47**). These analogs overcame the most common streptogramin resistance mechanism, VatA-mediated resistance, and maintained or exhibited enhanced potency against both Gram-positive and Gram-negative bacteria compared to their parent molecule, flopristin (**2**).[8] (Figure 2.1B and 2.1C).



C

Minimal Inhibitory Concentration (MIC) Values (μg/mL)

	S. aureus ATCC 29213	*S. aureus* VatA streptogramin acetyltransferase-mediated resistance	*S. aureus* MRSA ATCC 33591	*S. aureus* Linezolid-resistant; cfr	*E. faecalis* ATCC1 29212	*E. faecium* ATCC 35667	*E. faecium* vancomycin resistant (VREF)	*S. pneumoniae* ATCC 49619	*H. influenzae* ATCC 49247	*M. tuberculosis*
linezolid	2	nd	1	16	2	0.25	8	1	8	0.5
flopristin	0.5	2	0.5	>64	32	>64	1	1	0.25	??
SA1	0.25	0.5	0.25	>64	32	0.125	0.5	1	0.25	??
SA2	2	1	0.5	>64	>64	2	2	2	1	??
flopristin+VS1	≤0.06	1	≤0.06	16	0.25	≤0.06	0.125	≤0.06	0.25	??
SA1+VS1	≤0.06	1	0.5	4	0.25	≤0.06	≤0.06	≤0.06	0.25	??
SA2+VS1	0.25	1	0.25	4	0.5	≤0.06	0.125	≤0.06	0.25	??

Figure 2.1. A. Our modular synthesis to group A streptogramin antibiotics. **B.** Structures of linezolid (**1**), group A streptogramins (flopristin (**1**), **46**, **47**), and group B streptogramin (**VS1**). **C.** MIC activity of linezolid vs group A streptogramins and group A/B combinations against a panel of resistant bacteria.

Additionally, these novel group A streptogramins, along with flopristin (**2**), are much more potent than linezolid (**1**) among both Gram-positive and Gram-negative organisms. When combined with streptogramin B (**VS1**), the synergistic interaction between group A and group B streptogramins produced >100-fold more potency than linezolid (**1**) in some bacterial strains.[60] Based on this activity, we hypothesize that some of the group A streptogramins will be active against *M. tuberculosis* and be strong candidates in replacing linezolid (**1**) in combination TB therapy.

2.2 Results and Discussion

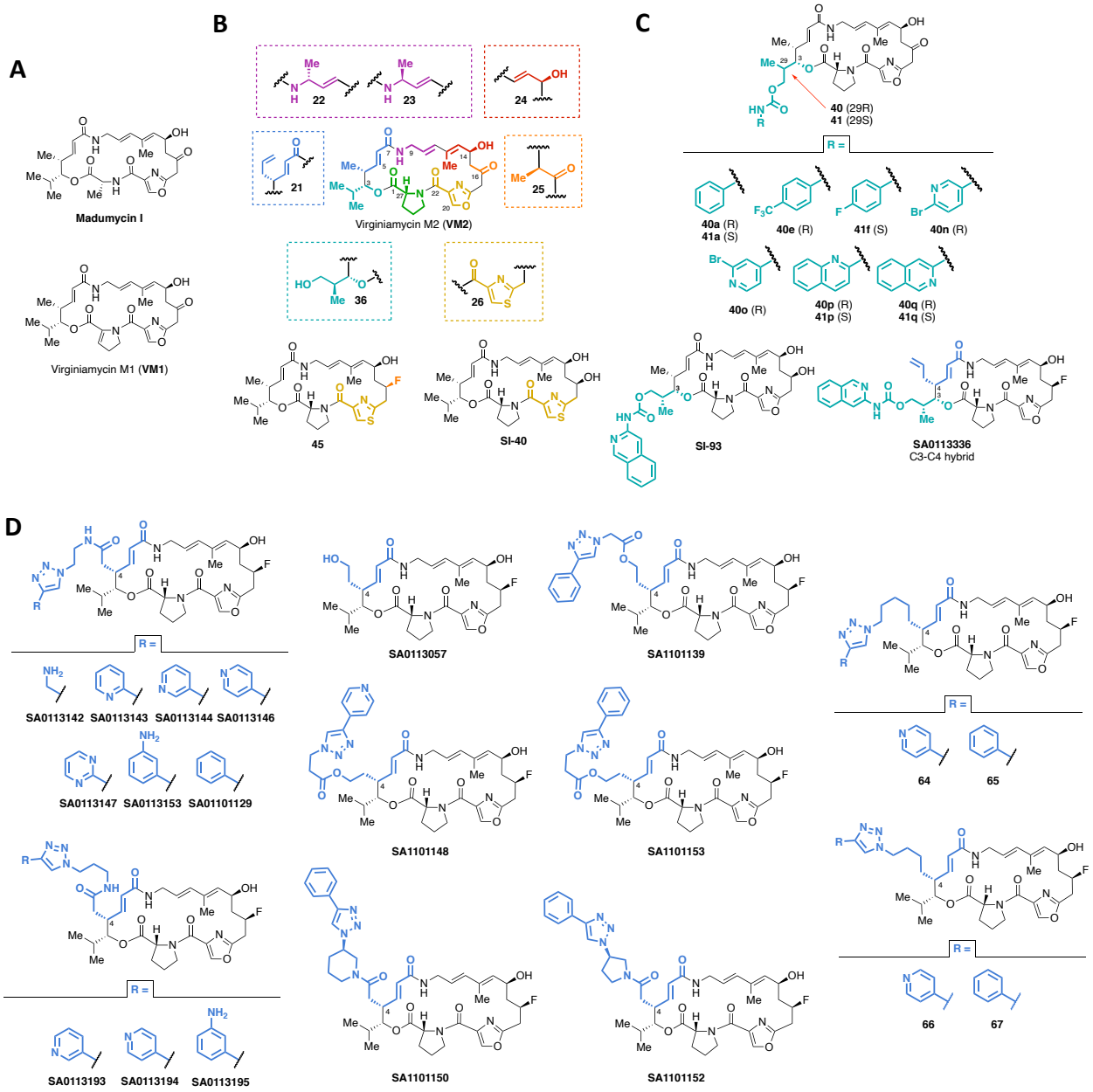


Figure 2.2. A. Structures of group A streptogramin natural products madumycin I and **VM1**. **B.** Nine group A streptogramins accessed by modifying parent scaffold (**VM2**). The fragments displayed in the dashed boxes represent the structural variability compared to **VM2**. **C.** Structures of C3-modified group A streptogramin analogs, including a C3-C4 hybrid molecule. **D.** Structures of C4-modified group A streptogramin analogs with C16 fluorinated.

We evaluated the inhibitory activity of 49 group A streptogramin analogs, as well as their group A/B combinations against Mtb. This library included natural products **VM1**, **VM2**, madumycin I and **VS1** as well as a variety of fully synthetic group A streptogramins.[8,13] Some of these analogs feature structural modifications made around the core scaffold or specific modifications made to the C3 and C4 site. (Figure 2.1A and 2.2)

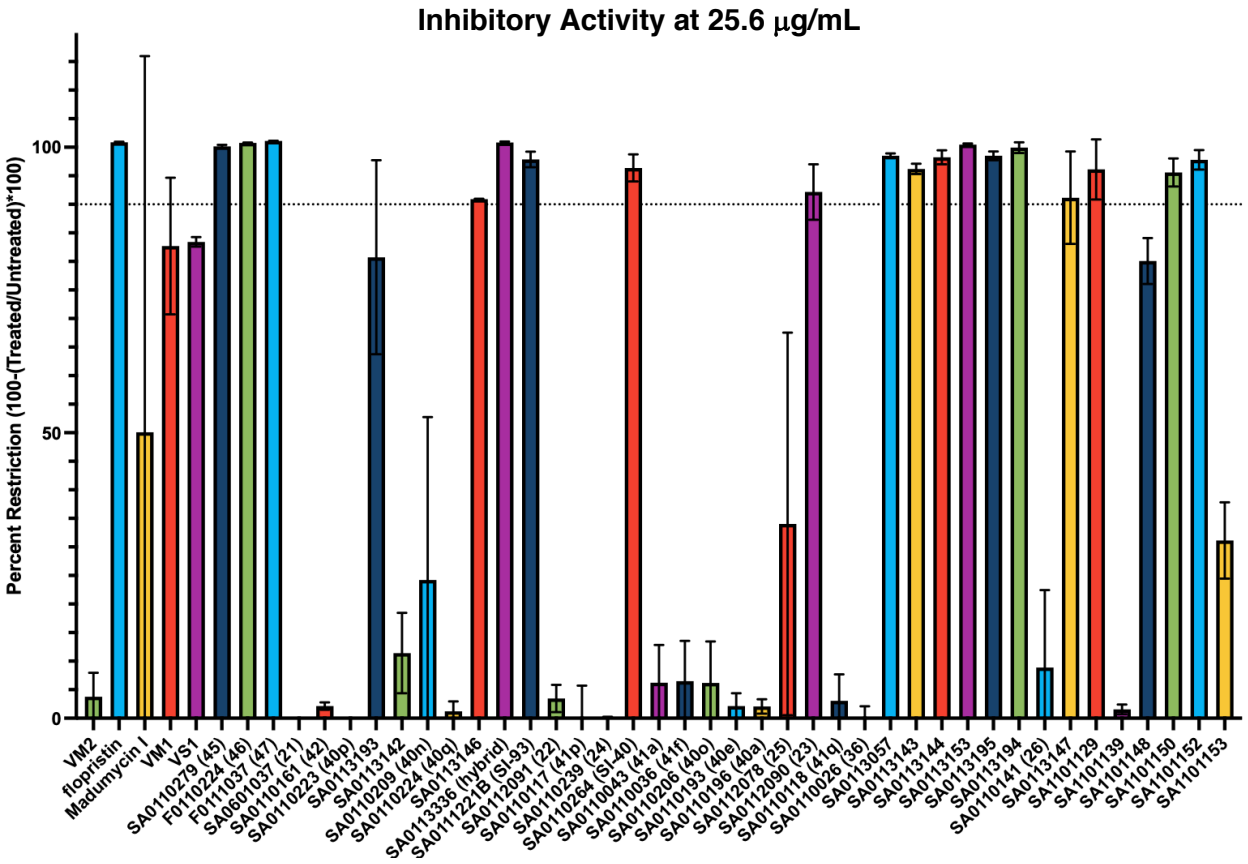


Figure 2.3. Percent restriction of Mtb measured in the presence of 25.6 μg/mL of each analog. The dotted line represents 90% restriction of Mtb.

An initial screen of 44 group A streptogramins and one group B streptogramin (**VS1**) was conducted against Mtb. Among the 45 individual compounds tested, 19 compounds inhibited Mtb growth by more than 90%. (Figure 2.3) Notably, this group included flopristin (**2**) and fully synthetic group A streptogramins **45**, **46**,

and **47**. The C3-C4 hybrid molecule, incorporating the C3 sidechain from hit analog **46** and the C4 sidechain from **47**, also showed high activity. Interestingly, several analogs (**SA0113143**, **SA0113144**, **SA0113146**, **SA0113194**, **SA0113195**, **SA0113153**, **SA1101129**, **SA1101150**, **SA1101152**) that were inactive (or only active at only high concentrations) in *S. aureus* and other Gram-positive pathogens were active in Mtb.

Evaluation of the group A/B combinations – each group A analog paired with **VS1** – demonstrated their strong synergistic effect against Mtb. Specifically, 31 out of the 44 combinations restricted Mtb growth by more than 90%. (Figure 2.4) Seeing how potent the group A/B combinations were in the presence of Mtb, the streptogramin combinations show potential as effective therapeutics in treating Mtb.

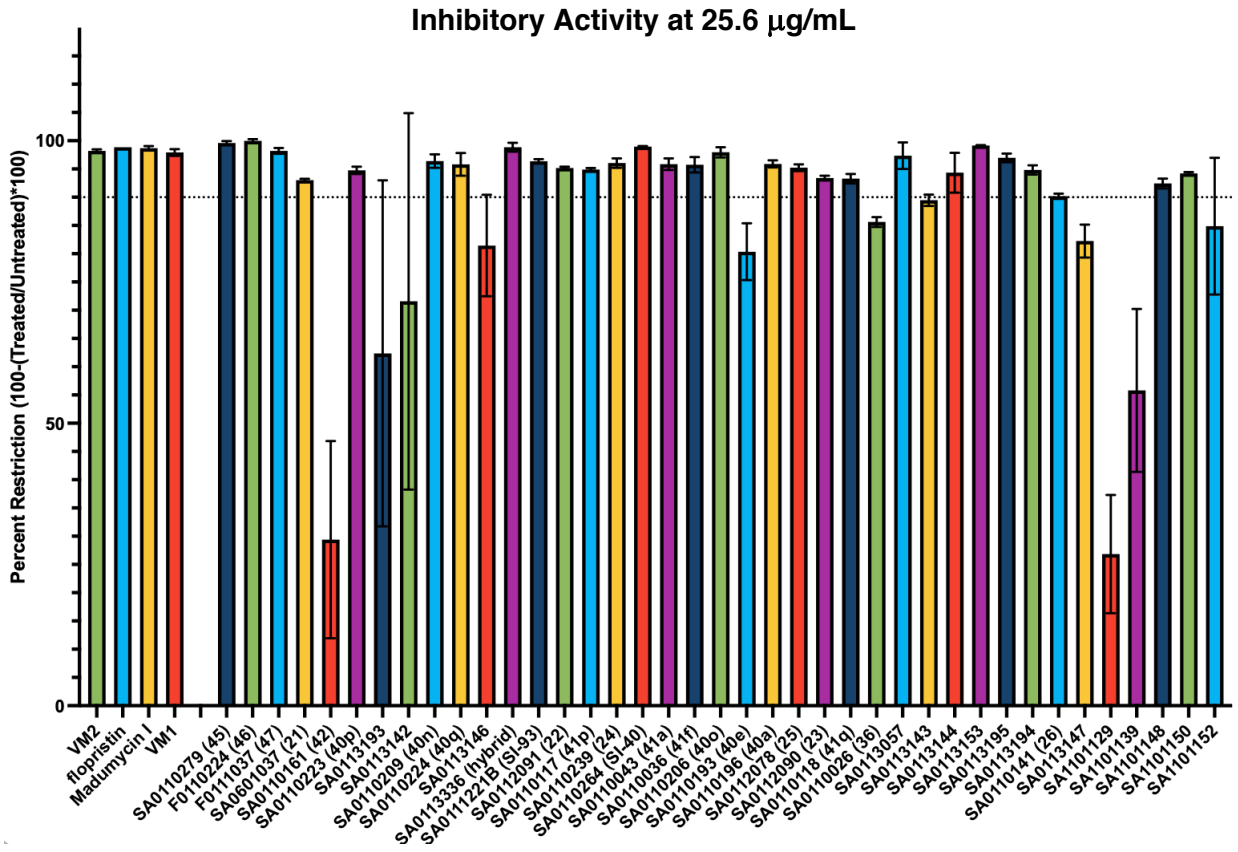


Figure 2.4. Percent restriction of Mtb measured in the presence of 25.6 μg/mL of each group A/B streptogramin combination. Each combination mixture was 70:30 group A streptogramin/VS1. The dotted line represents 90% restriction of Mtb.

For the 19 compounds that showed over 90% inhibition, we measured the IC_{90} values of six lead analogs via a dilution series ranging from 25.6 μg/mL to 0.4 μg/mL. (Figure 2.5A) Most of the compounds showed excellent potency, with flopristin (**2**) and **47** achieving sub-micromolar IC_{90} values. In contrast, **SA0113153** rapidly lost activity at concentrations below 12.8 μg/mL, similar in activity to **VS1** which is normally less potent than group A streptogramins.

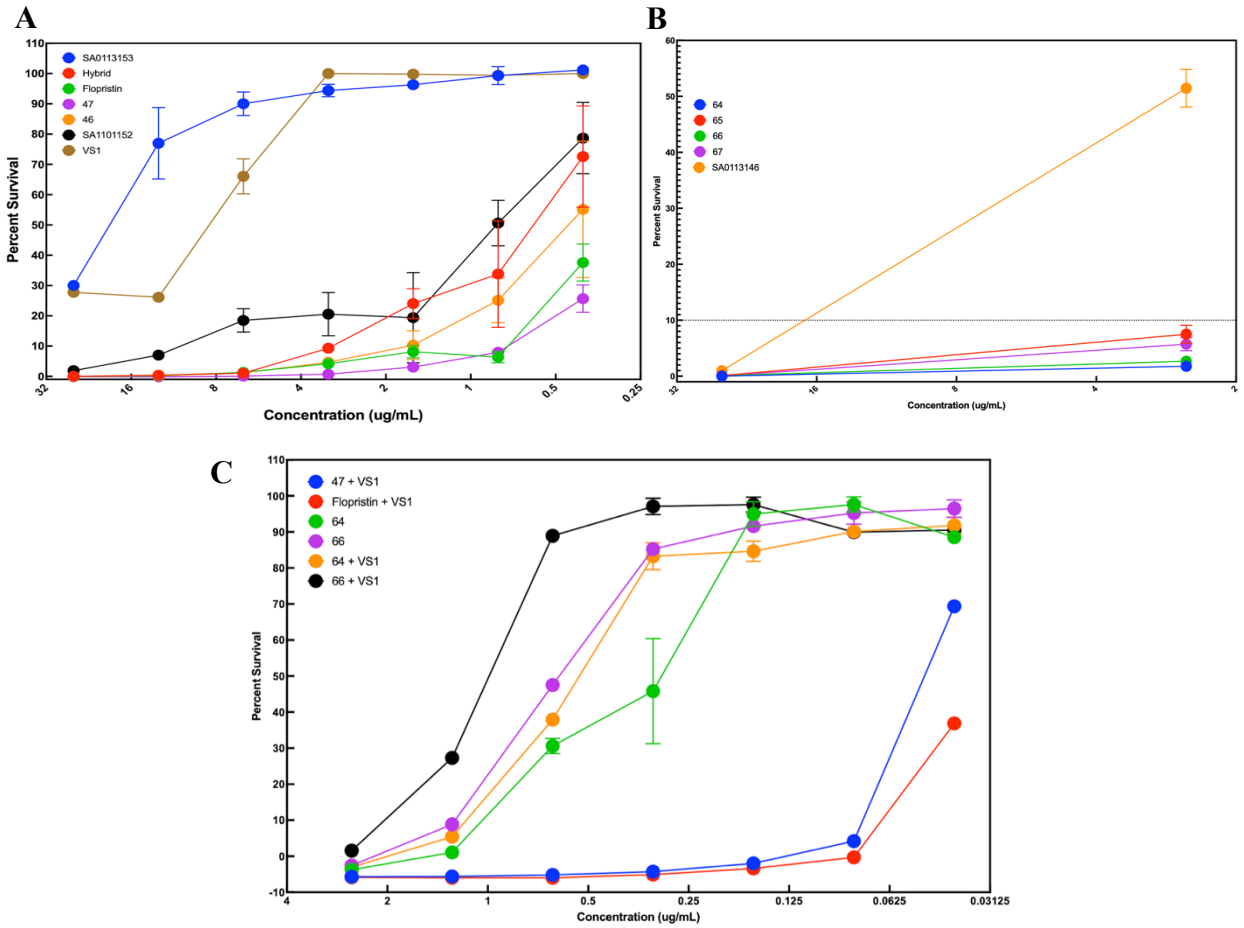


Figure 2.5. A. IC90 curves measured in a dilution series from 25.6 μg/mL to 0.4 μg/mL for selected group A streptogramins and group B streptogramin **VS1**. **B.** Percent restriction of Mtb measured at concentrations 25.6 μg/mL and 2.56 μg/mL for selected group A streptogramins. The dotted line represents 90% restriction of Mtb. **C.** IC90 curves measured in a dilution series from 2.56 μg/mL to 40 ng/mL for selected group A streptogramins and their combinations with **VS1**. Each combination mixture was 70:30 group A streptogramin/**VS1**.

Additional analogs of the C4-modified alkyl linker series – compounds **64** and **65** (5-carbon linker) and **66** and **67** (4-carbon linker) – as well as the C4-modified amide linker analog **SA0113146,** were tested at two concentrations (25.6 μg/mL and 2.56 μg/mL) to compare their activity with the initial six hit compounds. (Figures 2.2D and 2.5B) All four C4-modified alkyl linker analogs effectively inhibited Mtb at lower concentrations, surpassing the activity of the hybrid and **SA1101152**. (Figure 2.5A) Compounds **64** and **66** retained potency at 2.56 μg/mL.

To further assess their efficacy, we obtained IC_{90} curves of **64** and **66** at even lower concentrations ranging from 2.56 μg/mL to 40 ng/mL, both as single agents and in combination with **VS1**. We generated similar IC_{90} curves for flopristin (**2**) + **VS1** and **47** + **VS1** to evaluate their combinations. While flopristin (**2**) and **47** showed slightly higher activity than **64** and **66**, their combinations with **VS1** exhibited >90% restriction of Mtb at 80 ng/mL. In contrast, the combinations of **64** and **66** with **VS1** did not exhibit enhanced activity, suggesting a lack of synergy (Figure 2.5C).

The lack of synergy supports our hypothesis that the extended C4 sidechain in the alkyl linker analogs interfere with **VS1** binding. A cryo-EM structure of **SA0113146** (39d), with the only difference having an amide linker instead of an alkyl linker, bound to the *E. coli* ribosome reveals that the C4 sidechain occupies the NPET where a group B streptogramin would bind. (Figure 1.6B) As a result, the C4-modified alkyl linker analogs **64** and **66** were unable to synergize with **VS1** and did not exhibit the enhanced activity observed in other combinations such as flopristin (**2**) + **VS1** or **47** + **VS1**.

2.3 Conclusion

We evaluated 48 total group A streptogramin analogs, along with group B streptogramin **VS1**, for their inhibitory activity against Mtb. Of these, 23 individual compounds inhibited more than 90% of Mtb growth. When paired with **VS1**, group A streptogramins exhibited significantly enhanced activity due to synergistic

interactions – 31 out of 44 combinations achieved greater than 90% inhibition. This high success rate highlights the therapeutic potential of streptogramins as candidates for replacing linezolid in Mtb treatment.

IC_{90} values were determined for seven lead compounds, with flopristin (**2**) and **47** emerging as the most potent and both maintaining sub-micromolar activity. Combinations of these two analogs with **VS1** further improved efficacy, inhibiting over 90% of Mtb growth at concentrations as low as 40 ng/mL. These streptogramin combinations demonstrated >100-fold more potency than linezolid, underscoring their promise as next-generation antimycobacterial agents.

Future work will focus on determining the frequency of resistance against Mtb for both flopristin (**2**) and **47**, as well as their respective combinations with **VS1**. Having established strong in vitro efficacy, the next step is to evaluate these combinations in vivo using Mtb-infected mice models to assess their therapeutic potential in a physiological context.

2.4 General experimental procedures, materials, and instrumentation

General experimental procedures: All reactions were performed in oven-dried glassware fitted with rubber septa under a positive pressure of nitrogen or argon, unless otherwise noted. Procedures were conducted at 23ºC unless otherwise noted. All reaction mixtures were stirred throughout the duration of each procedure using Teflon-coated magnetic stir bars. Air- and moisture-sensitive liquids were transferred by means of syringe or stainless-steel cannula. Solutions were concentrated by rotary evaporation at or below 35°C. Analytical thin-layer chromatography (TLC) was performed using glass plates pre-coated with silica gel (0.25-mm, 60-Å pore size, 230−400 mesh, SILICYCLE INC) impregnated with a fluorescent indicator (254 nm). TLC plates were visualized by exposure to ultraviolet light (UV), and then were stained by submersion in a basic aqueous solution of potassium permanganate or with an acidic ethanolic solution of anisaldehyde, followed by brief heating.

Materials: Dichlorometane (DCM), tetrahydrofuran (THF), and acetonitrile (MeCN) to be used in anhydrous reaction mixtures were dried by passage through activated alumina columns immediately prior to use. Anhydrous toluene, $^{i}Pr_2EtN$, and Et_3N were purchased from Sigma Aldrich in Sure/Seal^{TM} bottles. Hexanes used were ≥85% *n*-hexane. Other commercial solvents and reagents were used as received, unless otherwise noted.

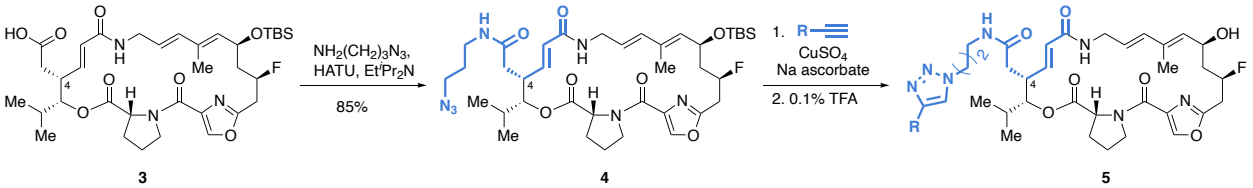
Instrumentation: Proton nuclear magnetic resonance (^{1}H NMR) spectra and carbon nuclear magnetic resonance (^{13}C NMR) spectra were recorded on 300 or 400 MHz Bruker Avance III HD 2-channel instrument NMR spectrometers at 23°C. Proton chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to residual protium in the NMR solvent ($CHC1_3$: δ 7.26 and CHD_2OD: δ 3.31). Carbon chemical shifts are expressed in parts per million (ppm, δ scale) and are referenced to the carbon resonance of the NMR solvent ($CDC1_3$: δ 77.2 and CD_3OD: δ 49.0). Data are represented as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, dd = doublet of doublets, dt = doublet of triplets, m = multiplet, br = broad, app = apparent), integration, and coupling constant (J) in hertz

(Hz). High-resolution mass spectra (HRMS) were obtained at the QB3/Chemistry Mass Spectrometry Facility at University of California, Berkeley using a Thermo LTQ-FT mass spectrometer or a Waters Acquity UPLC/Xevo G2-XS QTOF mass spectrometer. HPLC purification was conducted on a Waters Delta Prep 4000 preparative HPLC using a Gemini®-NX (5μm, C18, 110Å, 30.00 mm i.d. x 100 mm) column at a flow rate of 45 mL/min.

2.5 Experimental procedure for synthetic compounds

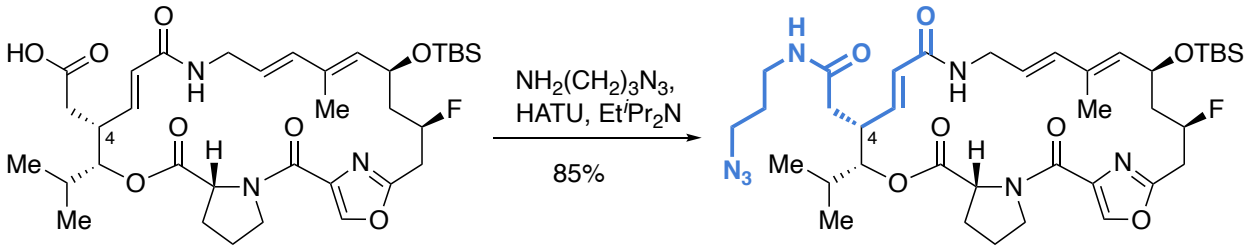
Fully synthetic analogs **21-26**, **36**, **35**, **SI-40**, and C3-modified analogs **40a**, **40e**, **40n**, **40o**, **40p**, **40q**, **41a**, **41f**, **41p**, **41q**, and **SI-93** were synthesized as reported.[8] General procedures of C4-modified analogs (**SA0113057**, **SA0113142, SA0113143**, **SA0113144**, **SA0113146, SA0113147, SA1103153, SA1101129, SA1101139**, **SA1101148**, **SA1101150**, **SA1101152**, **SA1101153**, **64, 65, 66, 67**) are found in Chapter 1.5.

Scheme I. Preparation of C-4 modified amide (longer) linker analogs **5**



Intermediate **3** (36) was prepared by procedures found in Chapter 1.5.

Preparation of click chemistry precursor **4**



A 50-mL round-bottom flask was charged with $^{i}Pr_2EtN$ (0.18 mL, 1.04 mmol, 2 equiv), 2-azidopropan-1-amine (78.4 mg, 0.78 mmol, 1.5 equiv), and acid **3** (0.36 g, 0.52 mmol, 1 equiv). DCM (12 mL) was added, resulting in a colorless solution. HATU (0.25 g, 0.65 mmol, 1.25 equiv) was added in one portion. After 5 h, the mixture was diluted with DCM (30 mL). The resulting solution was transferred to a separatory funnel and was washed with water (2 × 25 mL) and brine (25 mL). The washed solution was dried (Na_2SO_4). The dried solution was filtered, and the filtrate was concentrated. The resulting crude residue was purified by

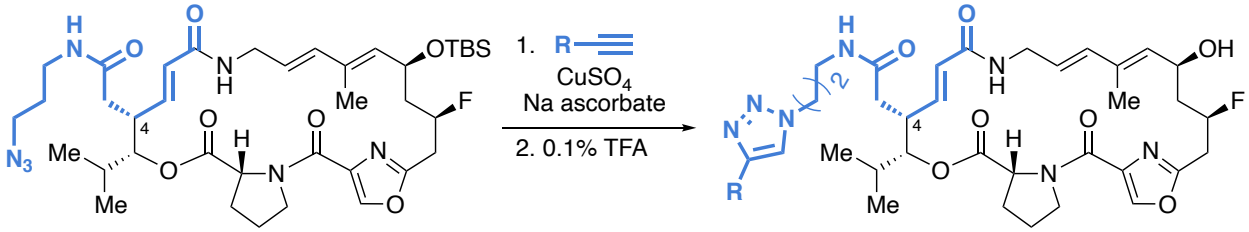
flash chromatography (silica gel, eluent: acetone:hexanes = 1:6 to 1:2) to afford click chemistry precursor **4** (0.34 g, 85% yield) as a white solid.

TLC (acetone:hexanes = 1:2): R_f = 0.30 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 8.08 (s, 1H), 6.48 (dd, *J* = 16.2, 5.4 Hz, 1H), 6.32 (t, *J* = 5.9 Hz, 1H), 6.24 – 6.10 (m, 2H), 5.86 (dd, *J* = 16.2, 1.7 Hz, 1H), 5.65 (ddd, *J* = 15.6, 8.6, 4.3 Hz, 1H), 5.29 (d, *J* = 9.0 Hz, 1H), 5.04 (dm, *J* = 48.1 Hz, 1H), 4.89 (dd, *J* = 10.2, 2.0 Hz, 1H), 4.81 (dd, *J* = 8.9, 3.3 Hz, 1H), 4.71 (td, *J* = 9.9, 3.8 Hz, 1H), 4.51 (ddd, *J* = 13.8, 8.6, 4.1 Hz, 1H), 4.07 (ddd, *J* = 11.2, 8.1, 4.6 Hz, 1H), 3.80 (dt, *J* = 11.2, 7.2 Hz, 1H), 3.46 – 3.22 (m, 7H), 3.14 (td, *J* = 16.7, 6.5 Hz, 1H), 2.92 (ddd, *J* = 21.9, 16.5, 5.6 Hz, 1H), 2.50 (dd, *J* = 14.7, 3.4 Hz, 1H), 2.23 – 2.10 (m, 5H), 1.99 – 1.77 (m, 3H), 1.75 (s, 3H), 1.57 (dddd, *J* = 40.5, 14.2, 10.2, 1.8 Hz, 1H), 0.98 (d, *J* = 6.7 Hz, 3H), 0.93 (d, *J* = 6.4 Hz, 3H), 0.85 (s, 9H), 0.03 (s, 3H), -0.00 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.3, 171.0, 165.8, 160.6, 160.5, 160.4, 142.9, 142.5, 136.6, 136.5, 134.7, 133.7, 124.9, 124.7, 89.3 (d, *J* = 170.4 Hz), 81.1, 66.5, 59.1, 49.4, 48.7, 43.6 (d, *J* = 20.3 Hz), 41.2, 39.2, 37.4, 34.0 (d, *J* = 25.3 Hz), 33.8, 29.7, 28.8, 28.5, 25.9, 25.0, 19.8, 18.6, 18.2, 13.0, -4.3, -4.8.

General procedure for preparation of C-4 analogs **5**

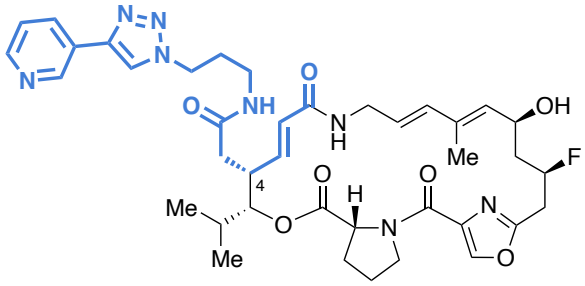


4 **5**

An aqueous solution of sodium ascorbate (0.05M, 0.2 equiv) and an aqueous solution of copper(II) sulfate (0.05 M, 0.05 equiv) were added to a suspension of azide compound **4** (1 equiv) and alkyne (3 equiv) in *t*-BuOH-H_2O (3mL-1mL) at 23°C. After stirring at 23°C for 12 h, the solvent was removed under vacuum.

The residue was purified by flash chromatography over silica gel (5→10% methanol in dichloromethane) to afford the product as an off white solid. Then the white solid was dissolved with 0.1% TFA in MeCN-H_2O (95/5 v/v), and the solution was stirred for 12 hours at 23°C. The reaction mixture was concentrated, and the resulting crude residue was purified by preparative HPLC (eluent: 0.1% TFA in H_2O: 0.1% TFA in acetonitrile = 95:5 to 5:95 over 15 min) to afford C-4 modified analog **5** TFA salt as a white solid.

Analog 5a (**SA0113193**)

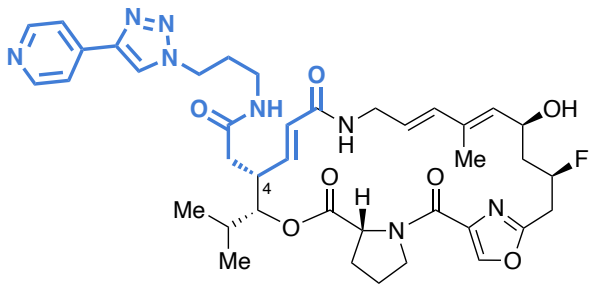


Prepared according to general procedure for C-4 modified analogs 5. Analog 5a (11.5 mg, 47% yield over 2 steps) was obtained as a white solid.

TLC (MeOH:DCM = 1:20): R_f = 0.08 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, MeOD) δ 9.30 (s, 1H), 8.95 (d, *J* = 8.1 Hz, 1H), 8.78 (d, *J* = 5.6 Hz, 1H), 8.69 (s, 1H), 8.26 (s, 1H), 8.10 (ddd, *J* = 8.3, 5.5, 2.6 Hz, 1H), 6.74 – 6.64 (m, 1H), 6.25 (d, *J* = 15.6 Hz, 1H), 6.00 (dd, *J* = 15.9, 1.7 Hz, 1H), 5.75 (ddd, *J* = 15.6, 8.2, 4.1 Hz, 1H), 5.40 (d, *J* = 9.0 Hz, 1H), 5.23 – 5.00 (m, 1H), 5.00 – 4.94 (m, 1H), 4.83 (dd, *J* = 8.7, 3.4 Hz, 1H), 4.71 (td, *J* = 9.0, 5.1 Hz, 1H), 4.54 (t, *J* = 7.0 Hz, 2H), 4.09 (qd, *J* = 12.3, 10.4, 6.3 Hz, 2H), 3.78 (dt, *J* = 11.5, 7.6 Hz, 1H), 3.74 – 3.60 (m, 1H), 3.28 – 3.02 (m, 5H), 2.60 (dd, *J* = 15.0, 3.7 Hz, 1H), 2.49 (dd, *J* = 14.2, 10.9 Hz, 1H), 2.20 (pt, *J* = 6.6, 4.0 Hz, 3H), 2.10 (td, *J* = 9.4, 8.3, 3.9 Hz, 2H), 1.98 – 1.88 (m, 2H), 1.82 (s, 3H), 1.78 – 1.70 (m, 1H), 1.02 (d, *J* = 6.7 Hz, 3H), 0.95 (d, *J* = 6.4 Hz, 3H).

Analog 5b (**SA0113194**)



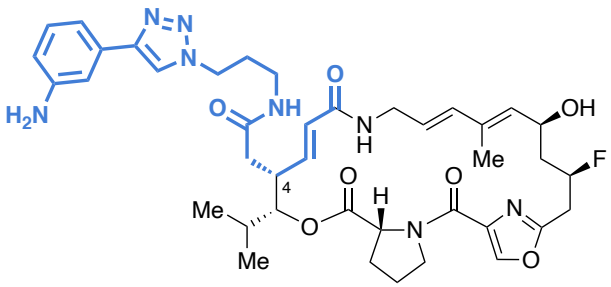
Prepared according to general procedure for C-4 modified analogs 5. Analog 5b (11 mg, 45% yield over 2 steps) was obtained as a white solid.

TLC (MeOH:DCM = 1:20): R_f = 0.06 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, MeOD) δ 8.93 (s, 1H), 8.84 (d, *J* = 6.9 Hz, 2H), 8.48 (d, *J* = 6.9 Hz, 2H), 8.26 (s, 1H), 6.68 (dd, *J* = 15.9, 5.2 Hz, 1H), 6.25 (d, *J* = 15.6 Hz, 1H), 6.00 (dd, *J* = 16.0, 1.8 Hz, 1H), 5.75 (ddd, *J* = 15.7, 8.1, 4.1 Hz, 1H), 5.40 (d, *J* = 9.2 Hz, 1H), 5.13 – 5.00 (m, 1H), 4.98 (dd, *J* = 10.2, 2.3 Hz, 1H), 4.83 (dd, *J* = 8.9, 2.7 Hz, 1H), 4.71 (td, *J* = 9.0, 5.0 Hz, 1H), 4.56 (t, *J* = 7.0 Hz, 2H), 4.10 (ddt, *J* = 12.0, 8.4, 4.1 Hz, 2H), 3.84 – 3.76 (m, 1H), 3.66 (dd, *J* = 15.5, 8.1 Hz, 1H), 3.29 – 3.01 (m, 5H), 2.59 (dd, *J* = 15.0, 3.7 Hz, 1H), 2.47 (dd, *J* = 14.9, 11.0 Hz, 1H), 2.24 – 2.16 (m, 3H), 2.13 – 2.06 (m, 2H), 1.99 – 1.88 (m, 2H), 1.82 (s, 3H), 1.78 – 1.67 (m, 1H), 1.02 (d, *J* = 6.7 Hz, 3H), 0.95 (d, *J* = 6.5 Hz, 3H).

^{13}C NMR (100 MHz, MeOD) δ 174.2, 171.7, 167.3, 162.6, 162.1, 162.1, 149.1, 145.2, 144.9, 143.6, 143.4, 137.8, 136.8, 136.7, 134.6, 127.8, 126.2, 125.7, 123.4, 90.4 (d, *J* = 170.2 Hz), 82.5, 66.0, 60.5, 50.1, 49.3, 43.0 (d, *J* = 20.0 Hz), 41.6, 40.2, 37.3, 34.0 (d, *J* = 24.6 Hz), 33.7, 31.0, 30.7, 29.2, 25.9, 20.1, 18.7, 13.1.

Analog 5c (**SA0113195**)



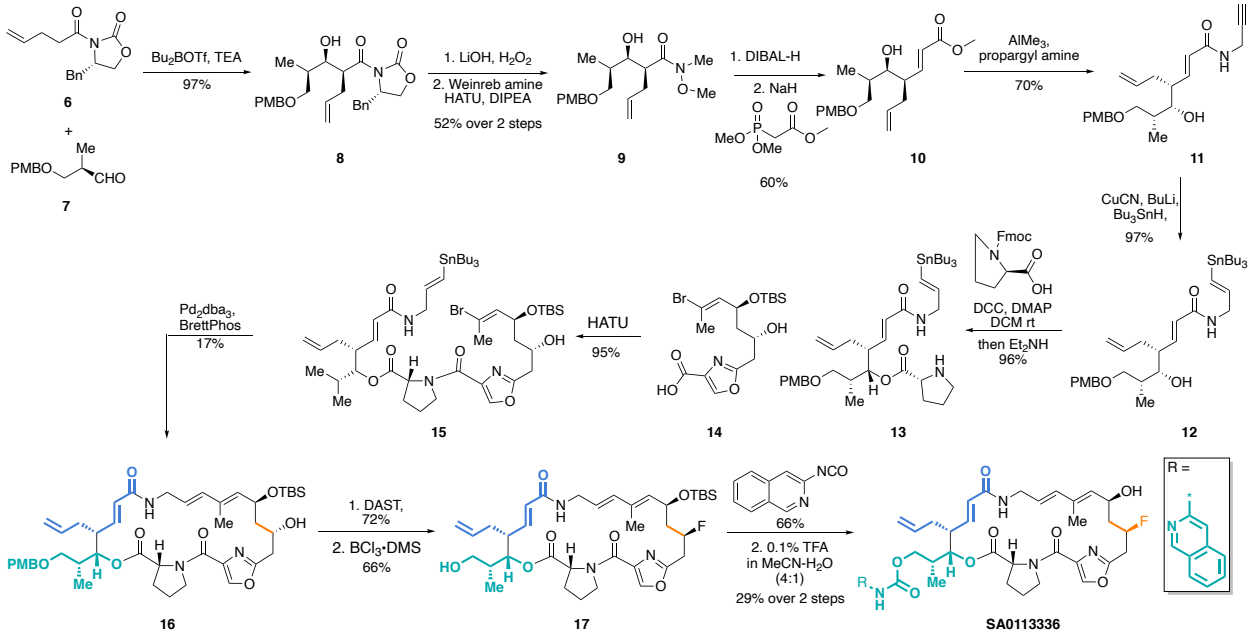
Prepared according to general procedure for C-4 modified analogs 5. Analog 5c (12 mg, 48% yield over 2 steps) was obtained as a white solid.

TLC (MeOH:DCM = 1:20): R_f = 0.10 (UV, *p*-anisaldehyde).

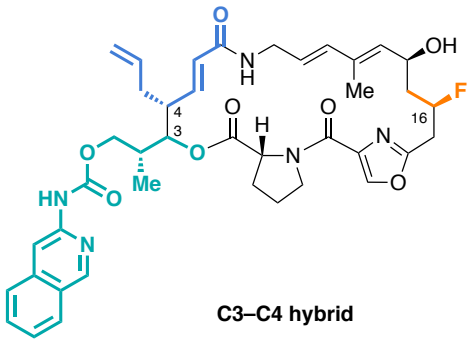
^{1}H NMR (400 MHz, MeOD) δ 8.45 (s, 1H), 8.26 (s, 1H), 7.90 (dd, *J* = 4.1, 2.2 Hz, 2H), 7.61 (t, *J* = 8.1 Hz, 1H), 7.38 (dd, *J* = 8.0, 2.2 Hz, 1H), 6.68 (dd, *J* = 15.9, 5.1 Hz, 1H), 6.24 (d, *J* = 15.7 Hz, 1H), 6.00 (dd, *J* = 16.0, 1.7 Hz, 1H), 5.77 (dd, *J* = 8.1, 4.2 Hz, 1H), 5.39 (d, *J* = 9.0 Hz, 1H), 5.23 – 5.06 (m, 2H), 4.82 (dd, *J* = 8.7, 3.5 Hz, 1H), 4.71 (td, *J* = 8.9, 5.1 Hz, 1H), 4.50 (t, *J* = 6.9 Hz, 2H), 4.17 – 3.97 (m, 2H), 3.78 (dt, *J* = 11.5, 7.6 Hz, 1H), 3.68 (dd, *J* = 15.3, 7.9 Hz, 1H), 3.28 – 3.01 (m, 5H), 2.58 (dd, *J* = 14.9, 3.8 Hz, 1H), 2.46 (dd, *J* = 14.8, 11.0 Hz, 1H), 2.27 – 2.14 (m, 3H), 2.14 – 2.02(m, 2H), 1.99 – 1.85 (m, 2H), 1.82 (s, 3H), 1.79 – 1.67 (m, 1H), 1.01 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.5 Hz, 3H).

^{13}C NMR (100 MHz, MeOD) δ 174.2, 171.7, 167.3, 162.6, 162.1, 161.3, 147.0, 145.3, 144.9, 137.8, 136.9, 136.7, 134.6, 134.1, 132.0, 127.1, 126.2, 123.5, 123.4, 120.8, 115.7, 90.5 (d, *J* = 168.8 Hz), 82.5, 66.0, 60.5, 50.1, 43.0 (d, *J* = 20.5 Hz), 41.6, 40.2, 37.5, 34.0 (d, *J* = 24.4 Hz), 33.6, 31.0, 30.7, 29.2, 25.9, 20.1, 18.7, 13.1

Scheme II. Preparation of C3-C4 hybrid analog (**SA0113336**)



All the intermediates were prepared by procedures in Chapter 1.5.



TLC (MeOH:DCM = 1:20): R_f = 0.29 (UV, *p*-anisaldehyde).

^{1}H NMR (400 MHz, $CDCl_3$) δ 9.24 (s, 1H), 8.95 (s, 1H), 8.26 (s, 1H), 8.24 (s, 1H), 7.88 – 7.84 (m, 1H), 7.75 (d, *J* = 8.2 Hz, 1H), 7.59 (ddd, *J* = 8.2, 6.8, 1.2 Hz, 1H), 7.41 (ddd, *J* = 8.1, 6.8, 1.1 Hz, 1H), 6.56 (dd, *J* = 16.1, 5.0 Hz, 1H), 6.17 (d, *J* = 15.7 Hz, 1H), 6.05 (dd, *J* = 8.8, 3.2 Hz, 1H), 5.93 (dd, *J* = 16.1, 1.8 Hz, 1H), 5.85 – 5.61 (m, 2H), 5.37 (d, *J* = 9.0 Hz, 1H), 5.25 (dd, *J* = 10.0, 2.2 Hz, 1H), 5.17 – 4.97 (m, 3H), 4.91 (dd, *J* = 8.8, 3.3 Hz, 1H), 4.78 (td, *J* = 9.2, 4.3 Hz, 1H), 4.59 – 4.45 (m, 1H), 4.41 (dd, *J* = 11.4, 4.0

Hz, 1H), 4.13 (ddd, *J* = 11.6, 8.3, 4.8 Hz, 1H), 4.03 (dd, *J* = 11.4, 7.3 Hz, 1H), 3.84 (dt, *J* = 11.5, 7.2 Hz, 1H), 3.45 (ddd, *J* = 16.0, 8.0, 3.1 Hz, 1H), 3.19 (ddd, *J* = 17.9, 16.4, 5.7 Hz, 1H), 2.95 (td, *J* = 16.9, 6.4 Hz, 1H), 2.65 (ddt, *J* = 10.5, 5.1, 2.5 Hz, 1H), 2.48 – 2.34 (m, 2H), 2.30 – 2.06 (m, 6H), 1.98 – 1.88 (m, 1H), 1.80 (s, 3H), 1.73 – 1.51 (m, 1H), 1.02 (d, *J* = 7.0 Hz, 3H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 171.5, 165.7, 160.8, 159.9, 159.8, 153.9, 151.3, 147.4, 144.3, 142.9, 138.1, 136.8, 135.9, 135.7, 135.5, 133.4, 130.7, 127.5, 126.6, 126.0, 125.4, 125.4, 125.2, 117.5, 106.5, 89.3 (d, *J* = 170.7 Hz), 78.9, 68.5, 65.8, 59.6, 48.9, 42.4 (d, *J* = 20.1 Hz), 41.6, 40.9, 34.3, 33.8 (d, *J* = 25.4 Hz), 30.8, 28.4, 25.0, 13.9, 13.1.

HRMS-ESI m/z calcd for $C_{40}H_{47}FN_5O_8^+$ $[M + H]^+$ 744.3403, found 744.3417.

2.6 Methods for measuring minimum inhibitory concentrations in *Mycobacterium tuberculosis* H37Rv

Test Media

10x ADC (Albumin, Dextrose, Catalase) Supplement media was prepared by dissolving 50 g/L bovine serum albumin, fraction V (Millipore, 12659) in milliQ water. Once completely dissolved at room temperature, 0.03 g/L catalase (Sigma, SRE0041), 20 g/L dexatrose (Sigma, D9434), and 8.5 g/L NaCl (Sigma, S9888) were added to the solution and dissolved with continuous stirring. Media was sterile filtered through a 0.22 μm PES membrane and stored at 4 °C.

Mycobacterium tuberculosis (Mtb) lab strain H37Rv was grown in 7H9, typically used for slow growing mycobacterial species. 7H9 was prepared from Difco Middlebrook 7H9 broth (BD biosciences, 271310) by weighing out 4.7 g and dissolving in 900 mL of milliQ water. To this solution, 2 mL of glycerol (Sigma, G7757) was added to bring the final concentration to 0.2% (v/v). The solution was thoroughly mixed before being autoclaved at 121 °C for 15 minutes. After the cycle was completed, the media was allowed to cool to 60 °C, and then 100 mL of 10x ADC and 0.05% (v/v) Tween-80 (Sigma, 8.22187) was added and thoroughly mixed before being filtered through a 0.22 μm PES membrane. The media was stored at 4 °C until the time of the experiment.

Test Organism

Prior to the experiment, frozen glycerol stocks of H37Rv were rapidly thawed, and 200 μL of stock was added to 5 mL of 7H9 warmed to 37 °C. The culture was incubated with constant shaking at 37 °C for 5 days. Once the culture reached an OD_{600} of 0.8 – 1.0, the culture was diluted back to 0.025 and incubated for another 5 days to allow for a single passage, at which point it was ready to be used for the experiment.

Evaluating MIC

To prepare dilutions of test compounds suspended in DMSO, a Beckman Coulter 650 Echo acoustic liquid handler transferred the appropriate amount of compound to be diluted to a final volume of 200 µL. Compounds were added to a black-walled, transparent flat-bottom 96-well plate. Wells 2–11 in rows B–G were used for testing, with the remaining wells filled with PBS to avoid any potential evaporation at the edges of the plate. Three wells on each plate were reserved for our negative control, media supplemented with 0.5% DMSO in place of the antibiotic; five wells on each plate were reserved for our positive control, media supplemented with 10 ng/mL rifampicin; two wells on each plate received 200 uL of plain media which would be used to assess the baseline fluorescence of resazurin in the absence of living organisms. All wells were brought to a volume of 100 µL with 7H9.

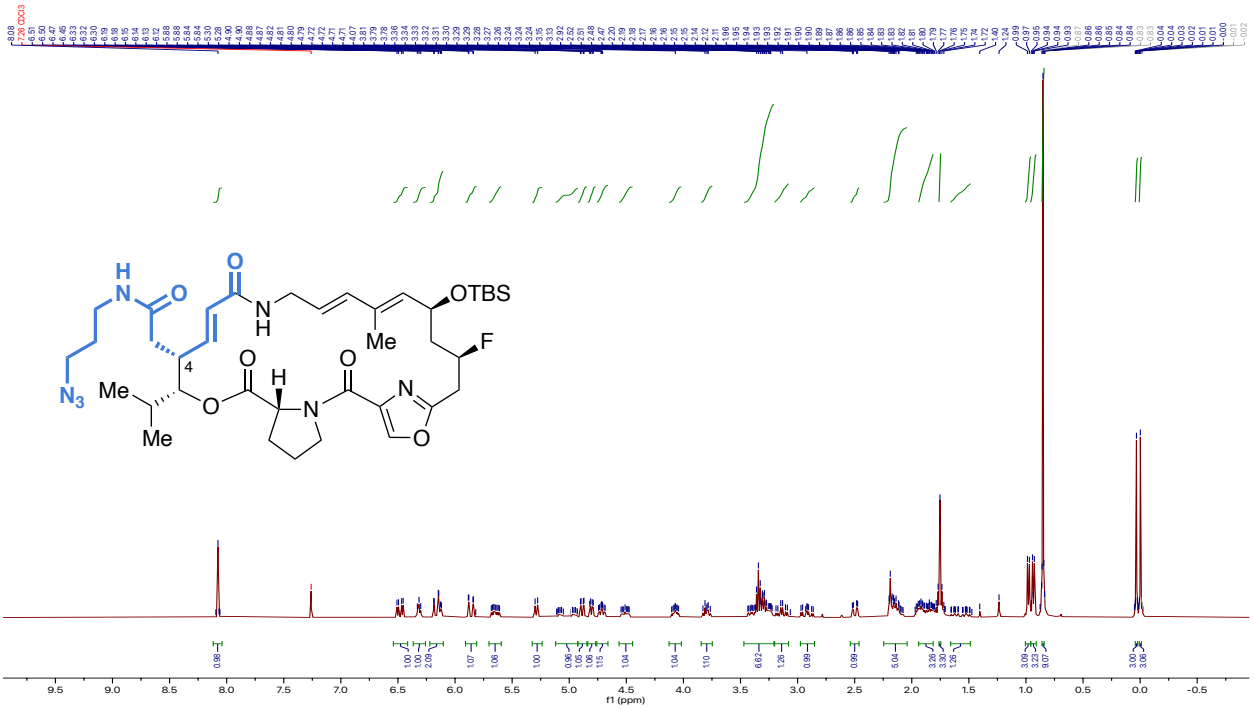
The culture of H37Rv at an OD_{600} of 0.8 – 1.0 was diluted to 100,000 bacilli/mL. 100 µL of diluted H37Rv was added to each of the antibiotic-containing wells, bringing the final concentration to 50,000 bacilli/mL with a total volume of 200 µL in each well. The plates were placed in a secondary container and then incubated at 37 °C for 7 days, undisturbed.

After 7 days, 30 µL of 0.02% resazurin (Fisher, S25783) was added to each well, bringing the volume to 230 µL. Each well was mixed to resuspend any settled bacteria, and then the plates were returned to the 37 °C incubator and left undisturbed for another 24 hrs. After 24 hrs, the supernatants were transferred to a 96-well 0.22 µm filter and centrifuged at 1000 xg for 10 min into a clean 96-well plate. The sterile supernatant was sealed and placed in a secondary container that was then decontaminated and removed from the BSL3 facility so that the resazurin could be measured using a plate reader.

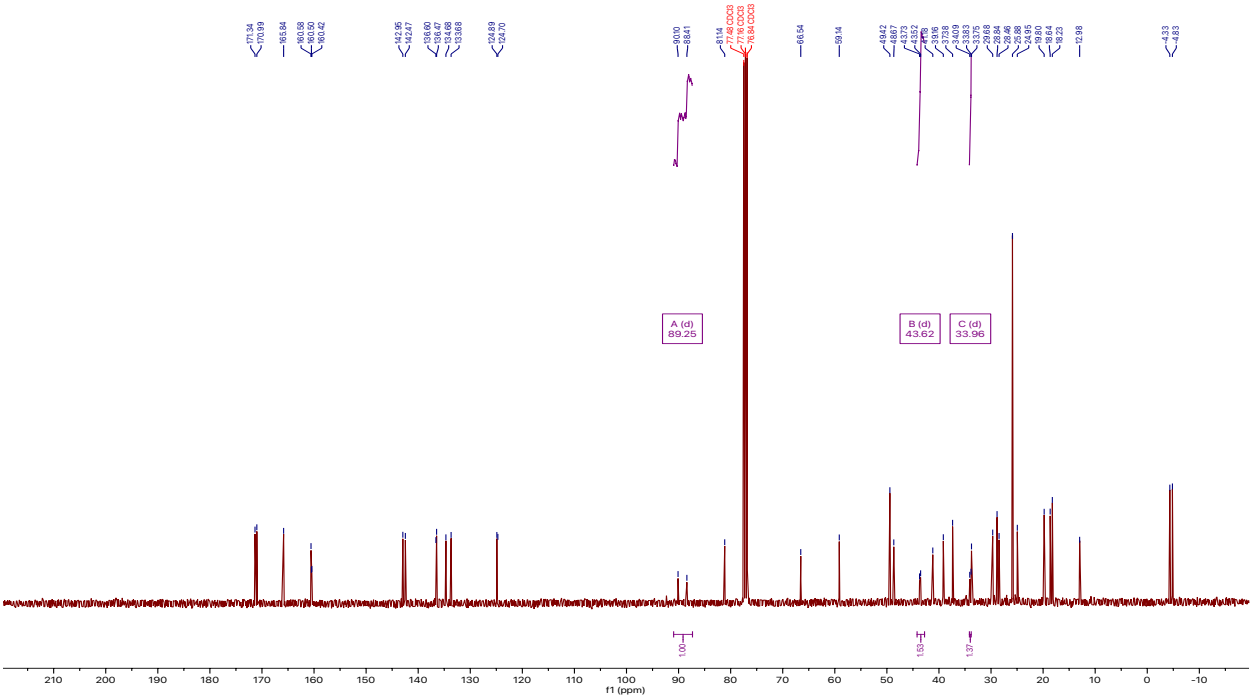
Using a BioTek Synergy H1 plate reader, the resazurin in the 96-well plates was measured for changes in fluorescence using an excitation wavelength of 530 nm and an emission wavelength of 590 nm. Values were normalized to wells that received plain media with resazurin.

2.7 ^{1}H NMR and ^{13}C NMR spectra

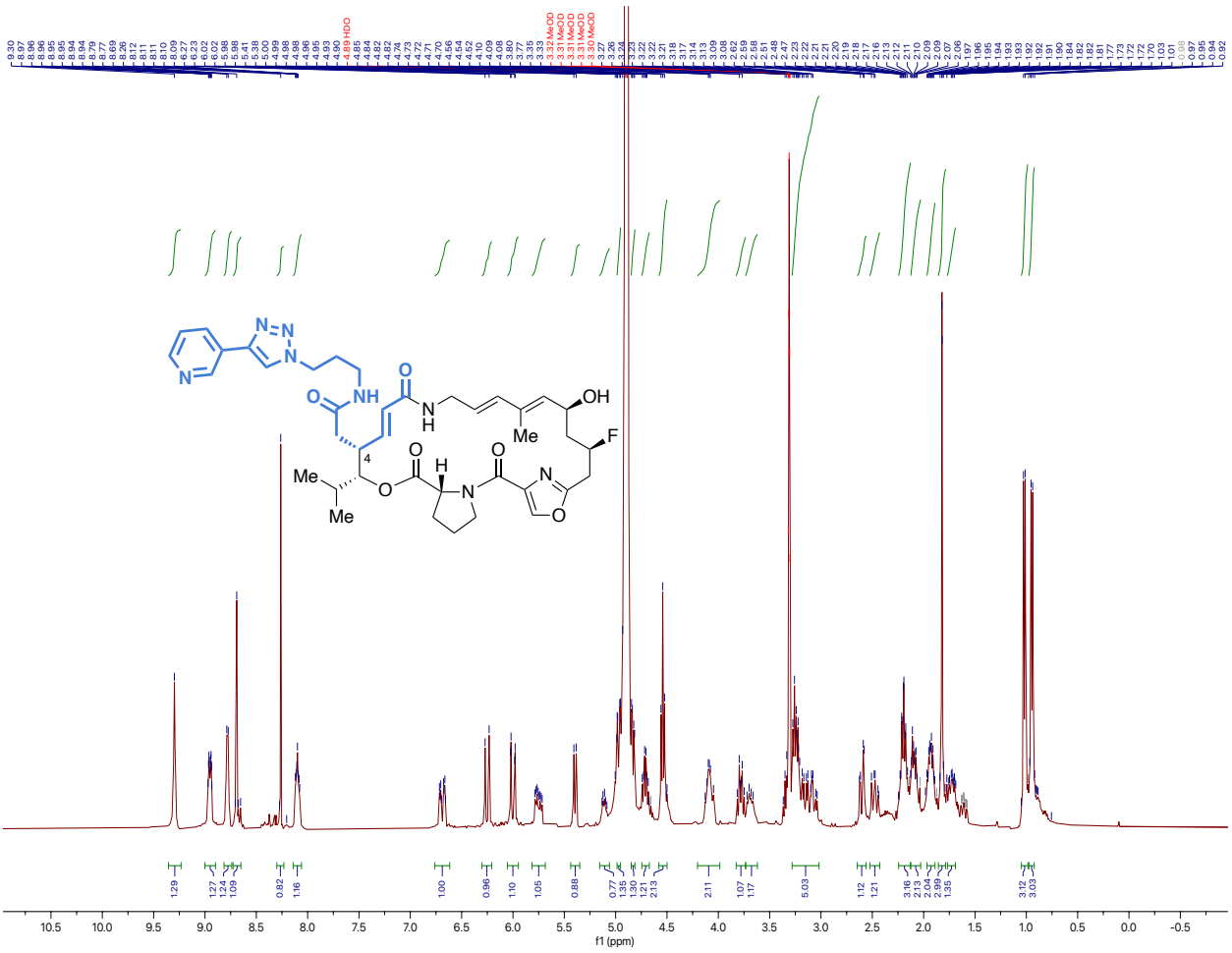
Compound **4**: ^{1}H NMR (400 MHz, $CDCl_3$)



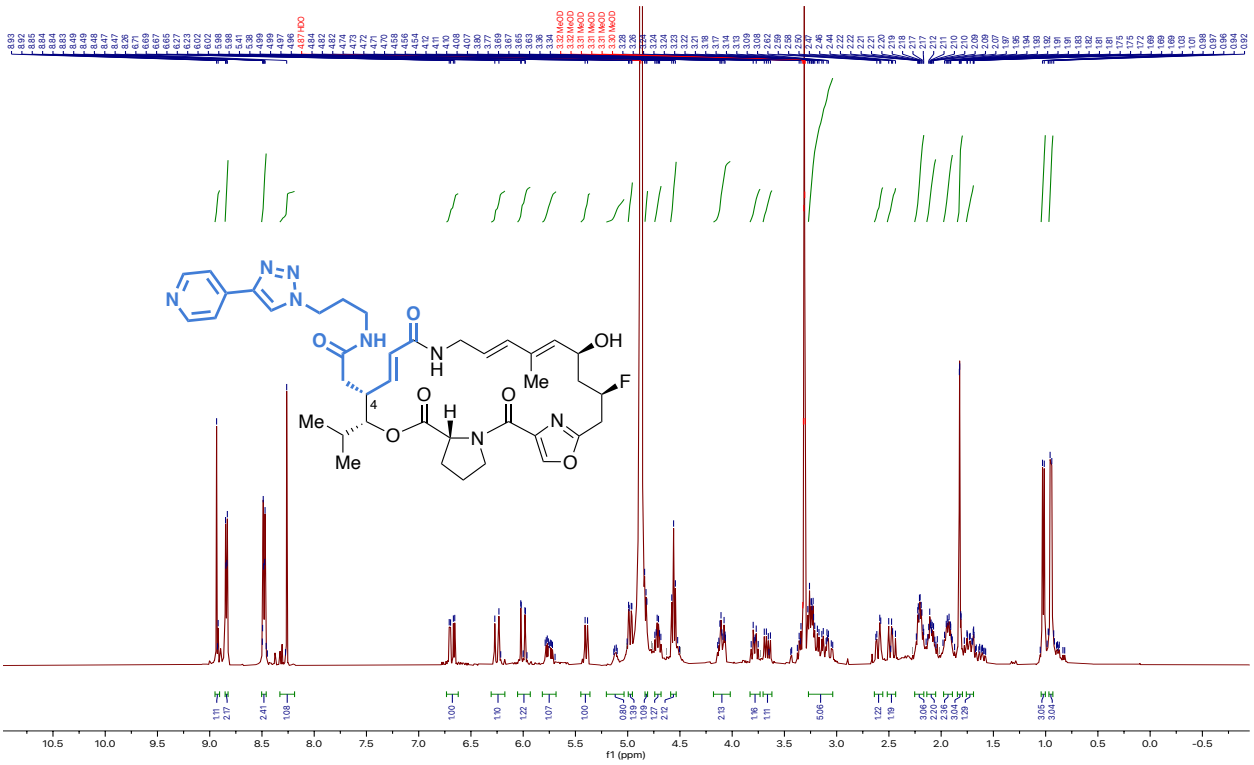
Compound **4**: ^{13}C NMR (100 MHz, $CDCl_3$)



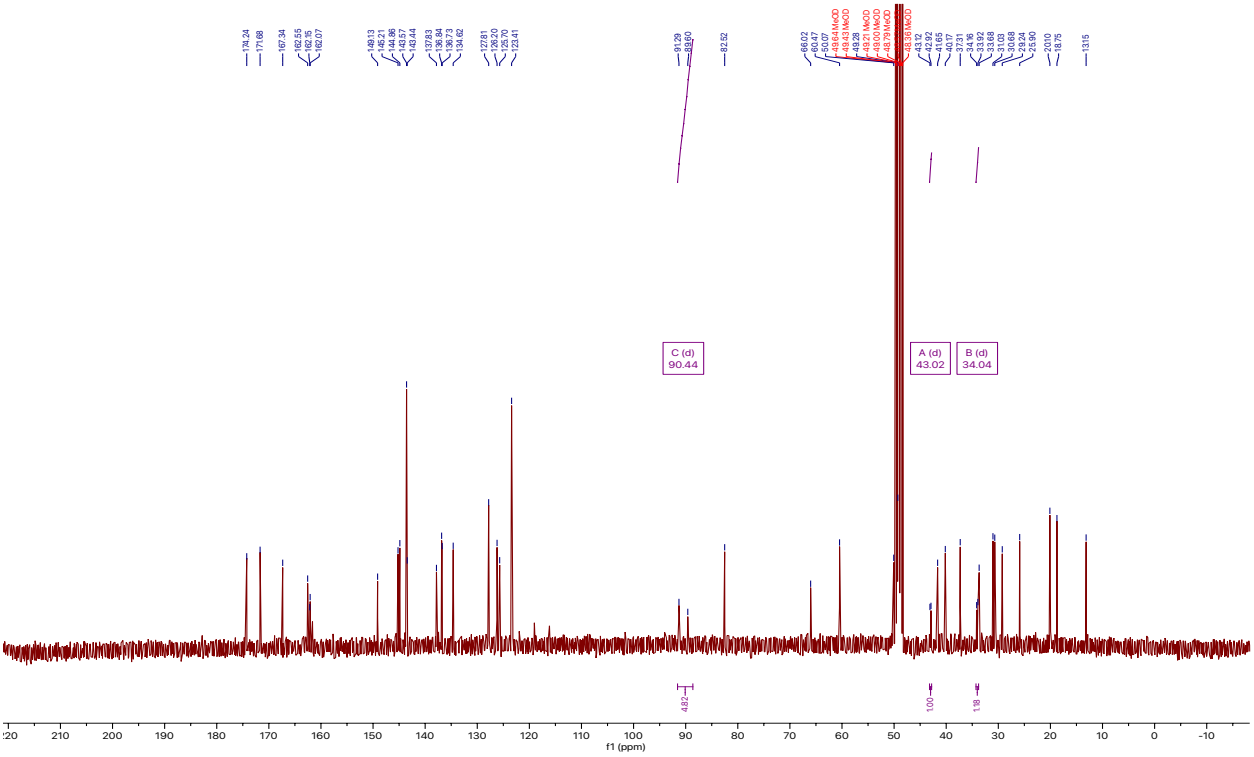
SA0113193: ^{1}H NMR (400 MHz, MeOD)



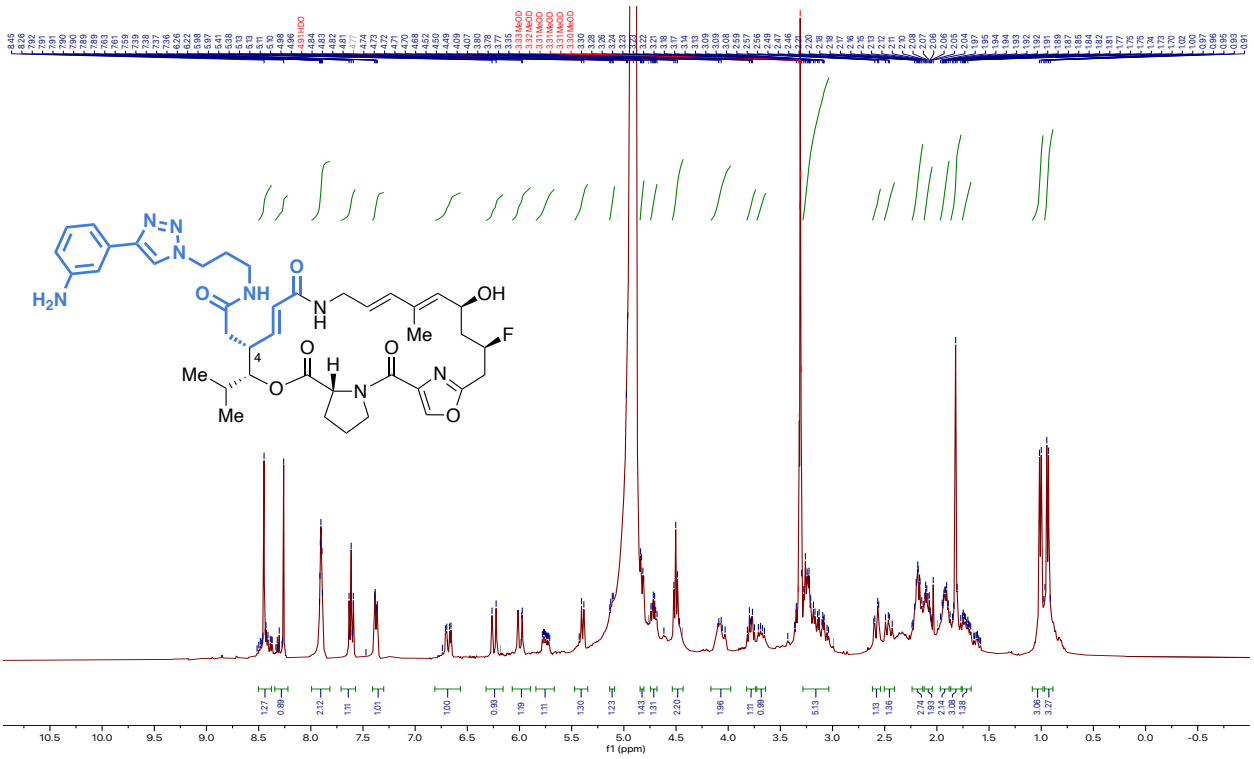
SA0113194: ^{1}H NMR (400 MHz, MeOD)



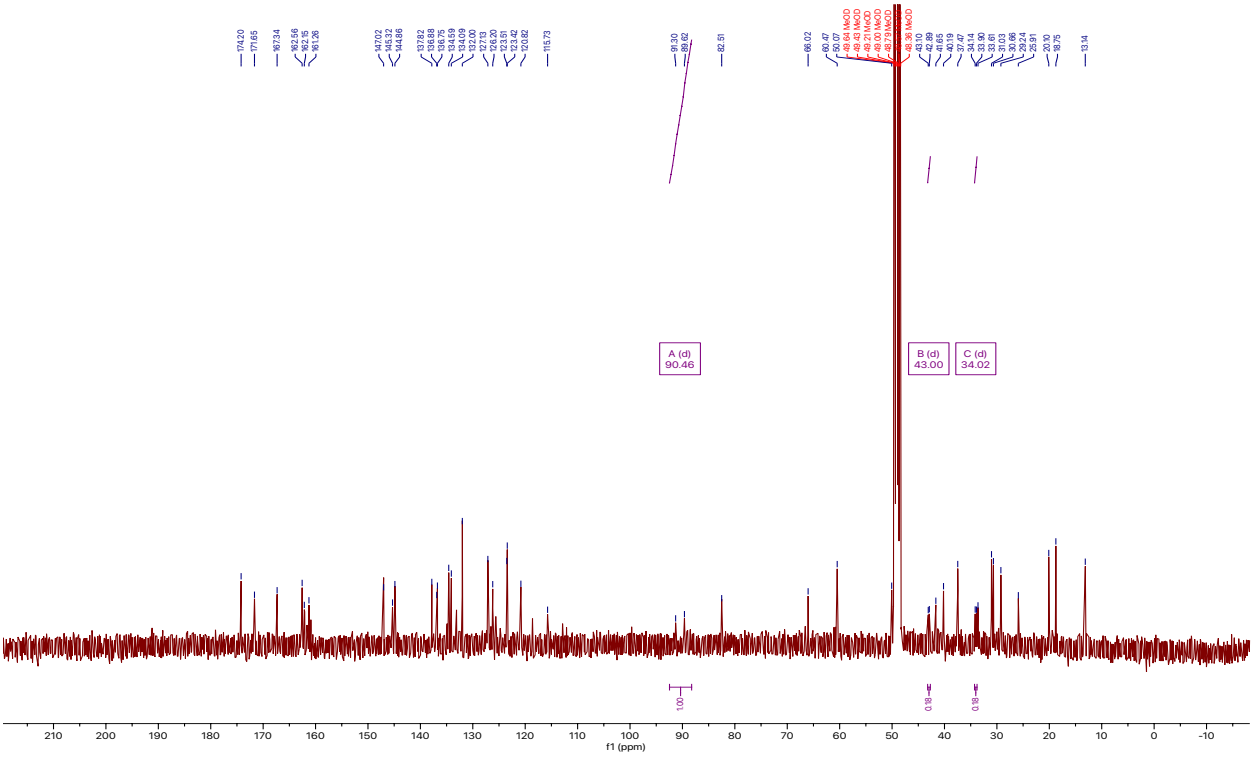
SA0113194: ^{13}C NMR (100 MHz, MeOD)



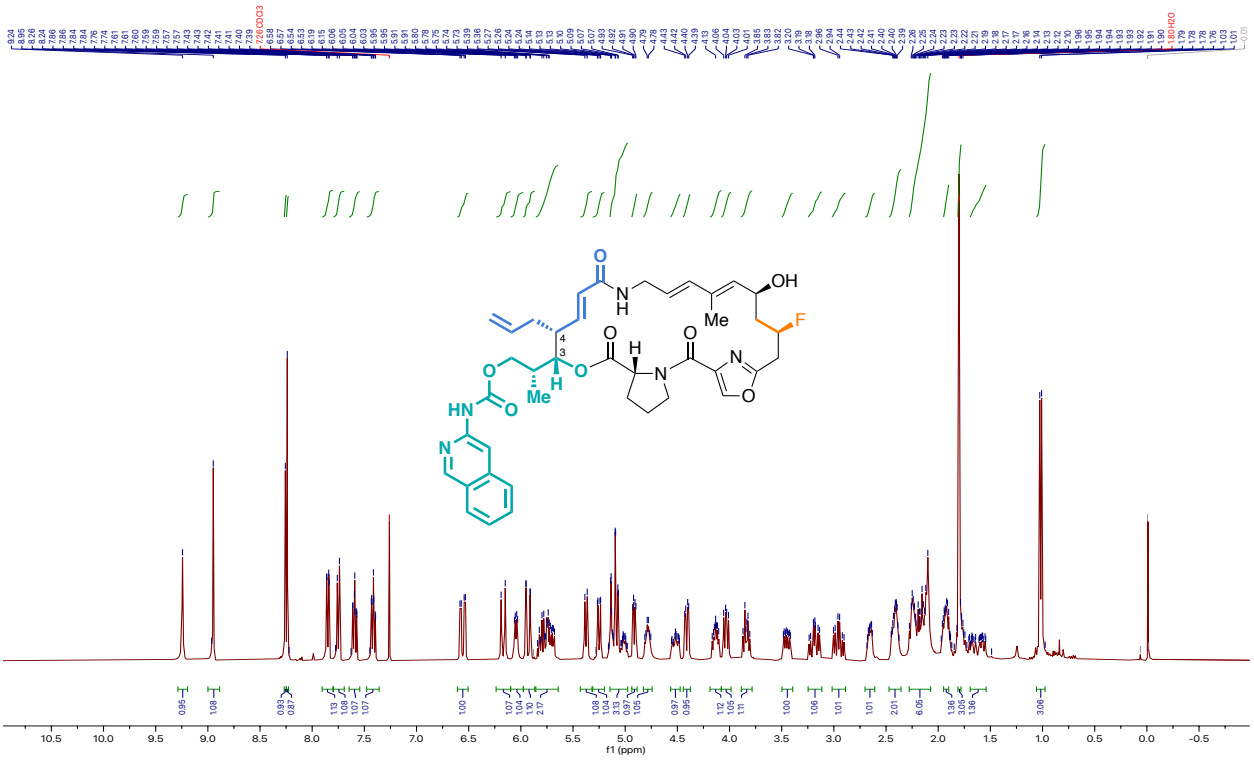
SA0113195: ^{1}H NMR (400 MHz, MeOD)



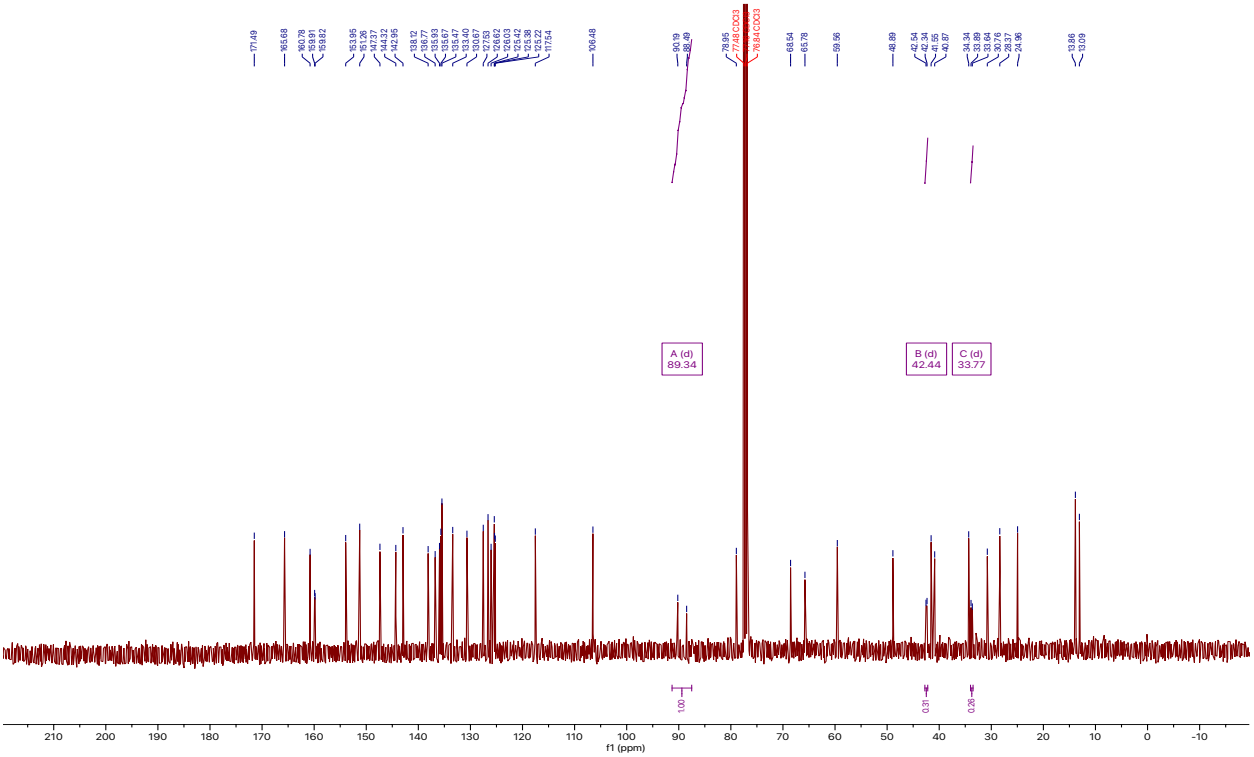
SA0113195: ^{13}C NMR (100 MHz, MeOD)



SA1013336: ^{1}H NMR (400 MHz, $CDCl_3$)



SA1013336: ^{13}C NMR (100 MHz, $CDCl_3$)



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