TOTAL SYNTHESIS AND STEREOCHEMICAL REVISION OF CILIATAMIDES A-C, TOTAL SYNTHESIS OF 8-*EPI*-LUCENTAMYCIN A, AND DEVELOPMENT OF MICROWAVE METHODOLOGY TO FACILITATE THE SYNTHESIS OF BMP INHIBITORS

By

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To the love of my life,
my wife, Kylynn

ACKNOWLEDGEMENTS

First and foremost I would like to thank my wonderful wife, Kylynn. If it was not for her love, support, and encouragement, none of this would have been possible. During all the times when it seemed like nothing was going right, she was always there to remind me that this is how science works and that it will get better. I could never overstate how important she has been not just to me, but also in keeping me sane during graduate school (which we all know is not easy). She was so much a part of this dissertation that both our names should be on it.

I owe a great deal of thanks to my mentor Dr. Craig Lindsley. I fully realize how lucky I have been to be in this lab. The resources and opportunities that I have been given are amazing. I have been very glad to be able to tell incoming graduate students that while he expects a lot out of his students he is not a demanding dictator. We work hard to keep Craig happy, not because we are afraid that he will yell at us, but because we genuinely like him. I truly appreciate the time that he has allowed me to be in his lab, his guidance and his teaching.

I would also like to thank my committee. Dr. Brian Bachmann has always been willing to talk to me and give advice. I have learned more about synthetic chemistry from Dr. Gary Sulikowski's classes than any other. He also, has certainly encouraged me to work hard throughout graduate school. The latest addition to my committee, Dr. Charles Hong, has always been more than happy

to give his advice and help on science, careers, and life. I have been very appreciative for the opportunity to collaborate with him.

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The members of both the Lindsley group and the synthesis core have not only been a great help to me, but also a lot of fun to be around. Apparently Phil Kennedy and I have had enough fun together that we want to continue that for a few more years. I have enjoyed getting to work closely with Dr. Jana Lewis, and

Olubeminiyi Fadeyi on multiple projects. Nicole Miller, of the Conn laboratory was also a great joy to work with. Although I may not have been able to change his mind about going to med school, I saw it as a privilege to train David Lee. His drive to learn and work hard will serve him well in whatever he pursues. Dr. Bruce Melancon, and Emily Wang were wonderful collaborators, and Bruce was always willing to help with everything from chemistry to presentation skills.

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I certainly could not have gotten this far without the help of many people. I am very grateful to them all!

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CHAPTER I

STATEMENT OF DISSERTATION

This dissertation describes advances in total synthesis, synthetic methodology, and chemical biology. The total synthesis and stereochemical revision of Ciliatamides A-C will be discussed in detail. In addition, the total synthesis of 8-*epi*-Lucentamycin A will be described. Lastly discussed is the development of microwave methodology to improve the synthesis of pyrazolo[1,5-*a*]pyrimidines. This new methodology was used to rapidly optimize a lead pyrazolo[1,5-*a*]pyrimidine screening hit into a highly selective and potent bone morphogenetic protein (BMP) inhibitor as well as selective KDR inhibitors using zebrafish as an *in vivo* screening tool.

Within the Ciliatamide project, the chemistry to synthesize the Ciliatamides A-C was a collaborative effort with Dr. Jana Lewis performing some of the reactions while I performed the others. Dr. Lewis also synthesized the library of analogs, as I worked out the conditions to reduce epimerization in the synthesis of the Ciliatamide C stereoisomers. I also performed all of the spectroscopic analysis and data collection for the NMRs and optical rotations.

The synthesis of the 8-*epi*-Lucentamycin A was a collaborative project with the Sulikowski group. I performed all initial model system studies and developed the route to avoid epimerization while coupling the northern and southern portions of the molecule. A postdoc in the Sulikowski group, Dr. Bruce

Meloncon, with an undergraduate summer student Emily Wang, developed the titanocene chemistry and the route to the proline core. Due to protecting group incompatibilities, I took that basic approach and developed an entirely new sequence which led to the synthesis of 8-*epi*-Lucentamycin A. The biological evaluation of the model system and 8-*epi*-Lucentamycin A was performed by Brenda Crews from the laboratory of Dr. Larry Marnett.

For the project involving the development of microwave methodology and synthesis of BMP inhibitors, I developed all of the microwave chemistry and optimized the conditions to make the first generation libraries which led to the identification of the isopropoxy ether in combination with the pyridine. Further work, by a colleague in the group, elaborated the pyridine to the quinoline, which led to the first selective, potent BMP inhibitor. All of the biological testing was done by our collaborators in the laboratory of Dr. Charles Hong.

CHAPTER II

TOTAL SYNTHESIS AND STEREOCHEMICAL REVISION OF CILIATAMIDES A-C

Background and Retrosynthesis

Leishmaniasis is a group of vector-borne diseases, caused by obligate intra-macrophage parasites of the genus *Leishmania*, which is endemic in the tropics, subtropics, and the Mediterranean basin.[1,2] The disease may manifest itself as a cutaneous, mucosal, dermal, or visceral variant. With visceral leishmaniasis (VL) being the most deadly. Among parasitic diseases leishmaniasis' death toll is second only to malaria, afflicting more than 12 million people in 88 countries with an annual death toll exceeding 50,000. Some severe VL epidemics have been reported. Between 1984 and 1994 in Southern Sudan, VL killed an estimated 100,000 people out of a population of 280,000, though this number was made worse by civil war and famine. VL has its greatest effect on poor communities, generally in remote rural areas, and pushes patients and families further into poverty because of the high direct and indirect costs.

VL is a systemic disease that is deadly if left untreated. Because of the high disease cost and the low standard of living of most infected individuals, symptoms often persist for weeks to months before the patient either seeks medical attention or dies.[3] Untreated VL patients also act as a reservoir for the parasites and contribute to the spread of the disease. The current treatments for leishmaniasis are the pentavalent antimonials Pentostam (**1**) and Glucantime (**2**),

Humatin (**3**), Fungizone (**4**), Pentamidine (**5**) and Impavido (**6**) (**Figure 1**). All of the current drugs have one or more significant drawbacks including cardiotoxic effects, parenteral administration, teratogenic effects, hypokalemia, nephrotoxicity, first-dose anaphylaxis, long treatment regimens, and prohibitively high cost.[1-3] Patient popluations with signs of severe malnutrition and/or advanced disease are at increased rate of death from treatment. Patients under the age of 2 or over the age of 45 are also at increased risk.[3]

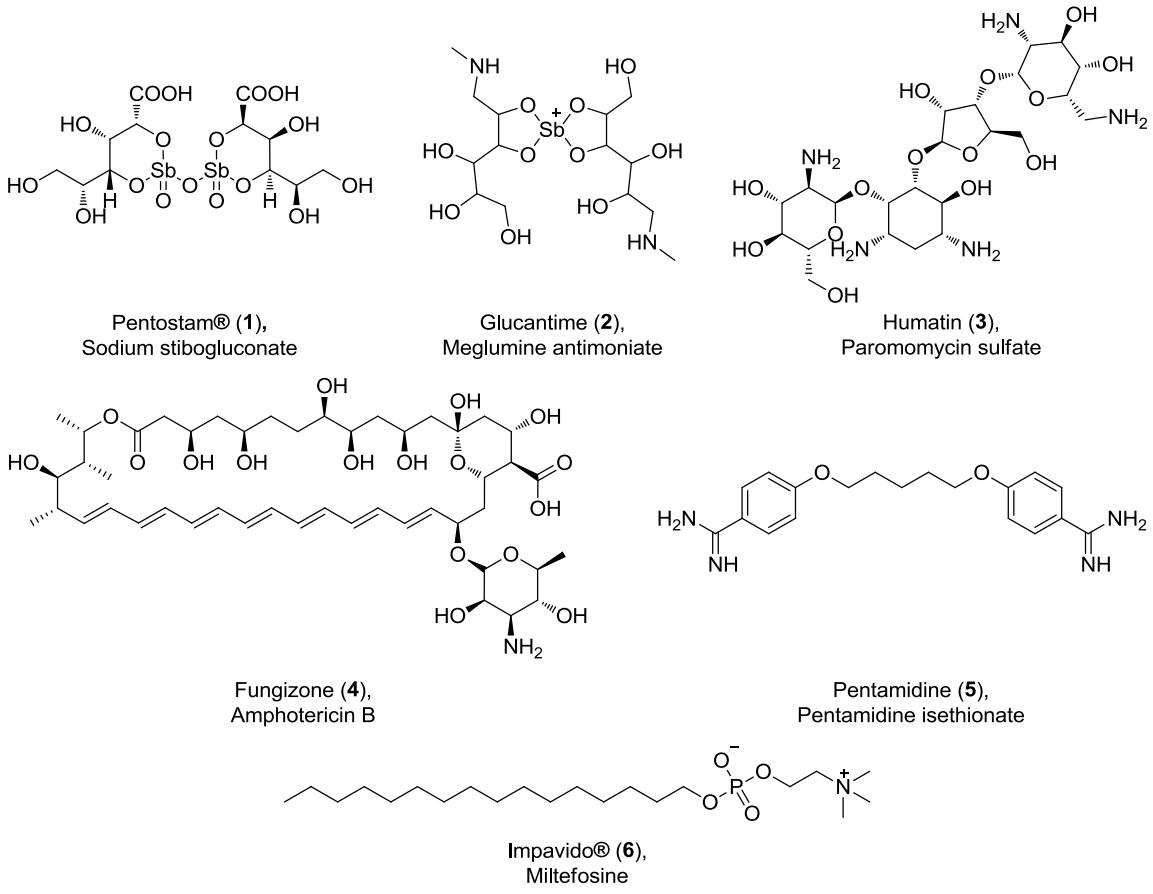


Figure 1. Structures of current antileishmanial treatments

In 2008, Nakao and co-workers reported on the isolation of three lipopeptides, Ciliatamides A (**7**), B (**8**) and C (**9**), from the deep sea sponge *Aaptos ciliata* as the (*S*,*S*)-enantiomers (**Scheme 1**).[4] Their structure were elucidated by a combination of chemical and spectroscopic methods.

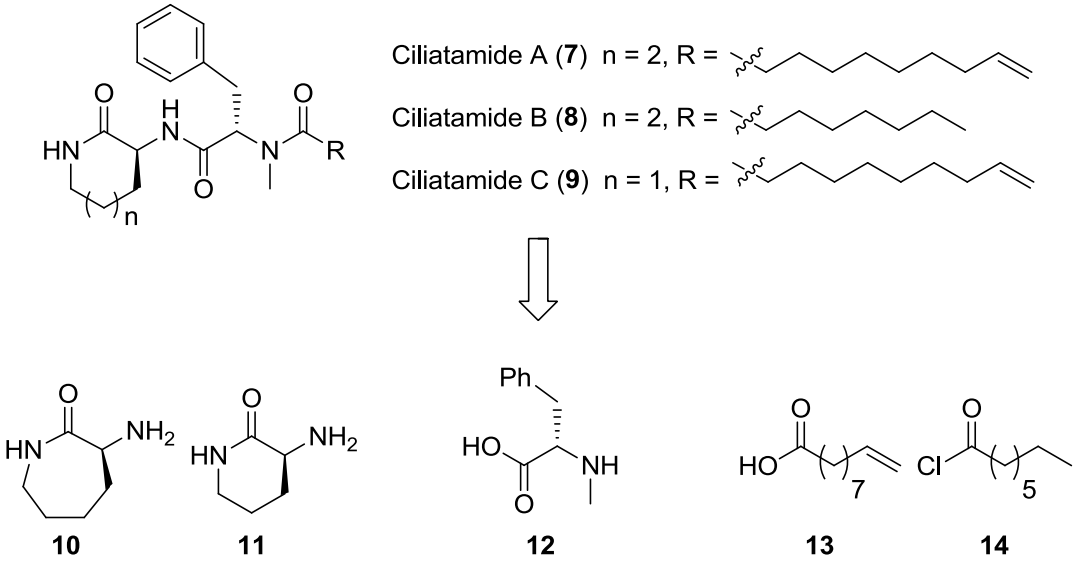
For Ciliatamide A (**7**) interpretation of the data from the ^{1}H NMR, ^{13}C NMR, HSQC, COSY, and HMBC led to the determination of the presence and connectivity of deca-9-enoic acid, *N*-methylphenylalanine, and lysine. From the HMBC correlations it was determined that the lysine was, in fact, an ε-caprolactam moiety. This was confirmed by the comparison of the ^{1}H NMR data of **7** with those of α-acetoamide-ε-caprolactam and acetyl lysine prepared from α-amino-ε-caprolactam and lysine, respectively. Ciliatamide A (**7**) then underwent chemical degradation by being dissolved in 6M hydrochloric acid, which was then heated to 100 ºC overnight. By Marfey's analysis of the acid hydrolysates it was determined that both the *N*-methylphenylalanine and lysine were of the L-configurations. Thus, **7** was assigned the *S*,*S* configuration.

By comparing the NMR spectra of **7** and **8**, as well as, 2D NMR data to determine the fatty acid was octanoic acid for **8** in comparison to deca-9-enoic acid for **7** the planner structure of **8** was assigned. Again the assignment of the *S*,*S* absolute configuration was performed by Marfey's analysis of the acid hydrolysates.

The NMR data of **9** were similar to that of **7** and **8**; however, the fragmentation pattern of **9**, seen on the ESIMS indicated the presence of a cyclized orithine functionality, not the cyclized lysine as seen with **7** and **8**. This

was confirmed by HMBC data. Once more Marfey's analysis was used to assign the absolute stereochemistry as *S*,*S* to give the reported structure of **9**.

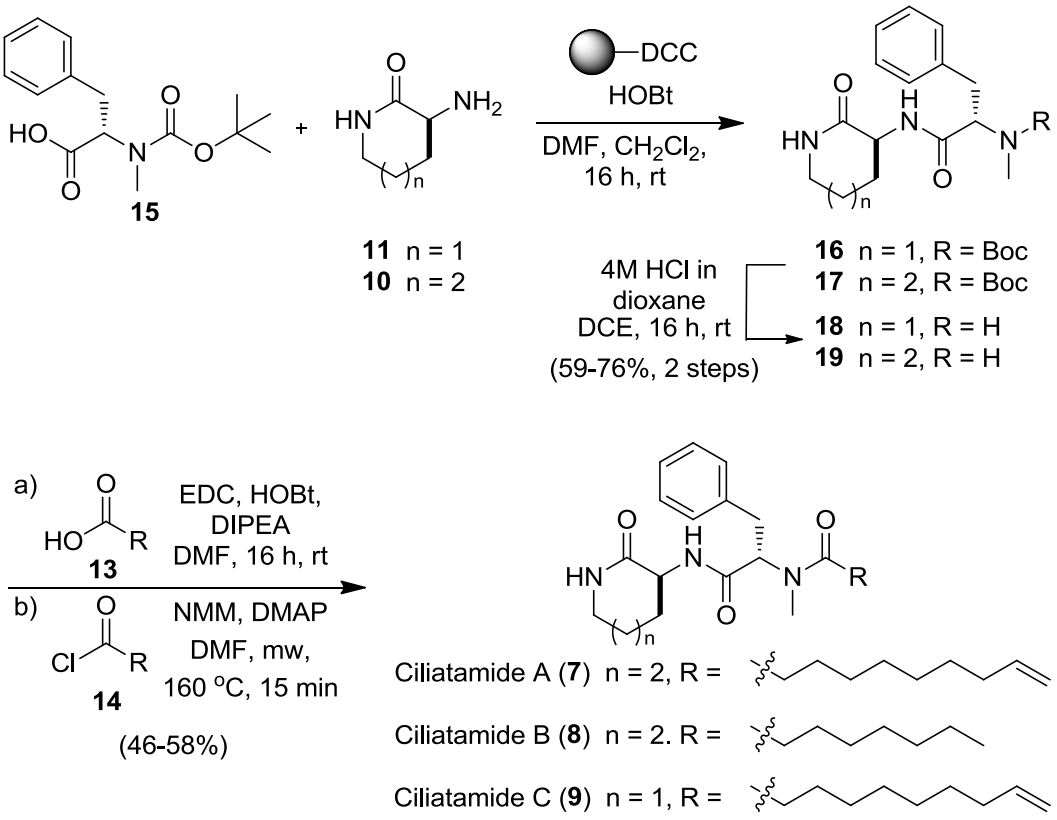
Importantly, Ciliatamides A and B demonstrated significant antileishmanial activity, and thus appeared as ideal targets for total synthesis and further biological evaluation, as **7**-**9** are chemically far less complex than current antileishmanials **1**-**6**.[1-4] The retro-synthesis of **7**-**9** (**Scheme 1**), involved cleavage of the two amide bonds to provide the (S)-3-aminoazepan-2-one (**10**) (for **7** and **8**) or the (S)-3-aminopiperid-2-one (**11**) (for **9**), L-*N*-methyl phenylalanine (**12**) and either decenoic acid (**13**) (for **7** and **9**) or octanoyl chloride (**14**) (for **8**). Fortunately, all of the requisite precursors were commercially available.



Scheme 1. Structures of Ciliatamides A (**7**), B (**8**), C (**9**) and Retrosynthesis

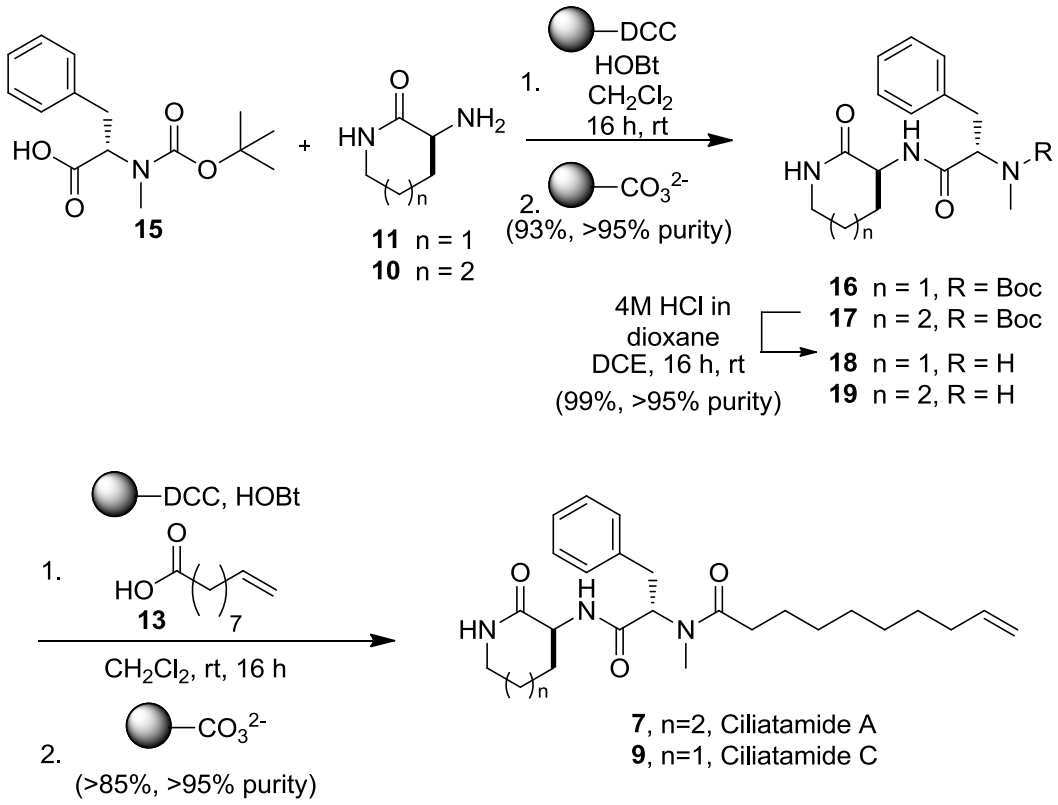
Synthesis of *S*,*S* Isomers of Ciliatamides A-C

To begin the synthesis, Boc protected L-*N*-methyl phenylalanine (**15**) was coupled to **10** with PS-DCC and HOBt, followed by treatment with HCl in dioxane to deliver the free base (**19**) in 59% yield for the two steps after ion-exchange chromatography. Peptide **19** then underwent a second amide coupling with **13** to afford Ciliatamides A (**7**), or coupling with fatty acid **14** to afford Ciliatamide B (**8**) in 56% and 58% yields, respectively. Following the same scheme (**Scheme 2**), but substituting (*S*)-3-aminopiperid-2-one (**11**) for **10**, provided Ciliatamide C (**9**) in 44% over the three steps. The overall yields were lower than anticipated due to the physiochemical properties of **7**-**9**, and poor chromatographic performance of these lipopeptides. Despite these complications, the NMR spectra obtained were in complete accord with those reported by Nakao and co-workers; however, the original spectra were recorded under very dilute conditions to minimize conformer populations.[4]



Scheme 2. Synthesis of Ciliatamides A (**7**), B (**8**), C (**9**)

Based on these results, we then explored a solution phase parallel synthesis approach for the synthesis of Ciliatmides A (**7**) and C (**9**) employing only polymer-supported reagents, scavengers, and ion-exchange chromatography to avoid either normal or reverse-phase chromatography.[5] This strategy afforded improved results, providing **7** and **9** in overall yields in excess of 75% for the three steps with >95% purity (**Scheme 3**).



Scheme 3. Solution Phase Parallel Synthesis of Ciliatamides A (**7**), C (**9**)

Synthesis of Analog Library

Due to the expedited route to **7**-**9**, in parallel, we prepared a 42-member solution phase library of unnatural Ciliatimide analogs **20a**-**n**, **21a**-**n**, and **22a**-**n**. The library employed three scaffolds **18**, **19**, and the unnatural (*R*,*S*) congener of **19**, and a collection of 14 different acid chlorides (**Figure 2**). All final compounds, including additional copies of **7**-**9** were purifed to >98% by mass-directed preparative HPLC and afforded yields ranging from <5% to 60% for the three steps; however, we obtained sufficient quantities for biological evaluation in every case.[6]

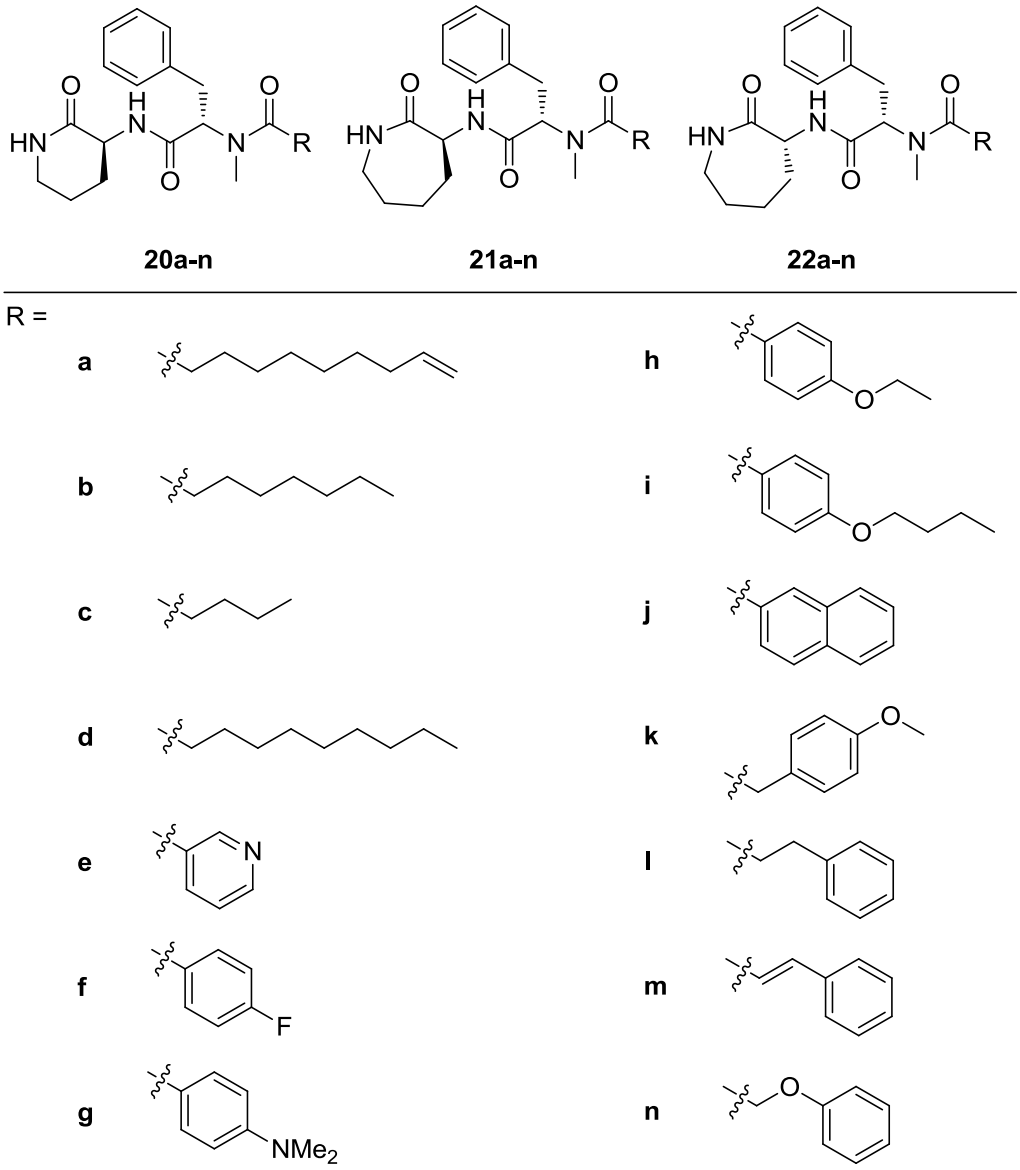


Figure 2. Three by 14 library of unnatural Ciliatamide analogs

Discrepancy of Optical Rotation and Synthesis of all Possible Isomers

As we were compiling the final characterization data for **7**-**9**, a discrepancy was noted with respect to the reported optical rotations, $[\alpha]_D^{20}$ = +40 (c = 0.05, MeOH), $[\alpha]_D^{20}$ = +55 (c = 0.1, MeOH), $[\alpha]_D^{26}$ = +74 (c = 0.1, MeOH) for the natural products **7**-**9**, respectively.[4] While the ^{1}H and ^{13}C NMR spectra of our

synthetic **7**-**9** overlayed with the natural products, the optical rotations were of comparable magnitude, but opposite sign, that is, $[\alpha]_D^{20}$ = -35 (c = 0.05, MeOH), $[\alpha]_D^{20}$ = -44 (c = 0.1, MeOH), $[\alpha]_D^{20}$ = -43 (c = 0.1, MeOH) for synthetic **7**-**9**, respectively. On the basis of these results, we synthesized the four possible stereoisomers ((*S,S*), (*S,R*), (*R,S*) and (*R,R*)) of Ciliatamide A and Ciliatamide B, employing the route depicted in **Scheme 2**, and compared NMR spectra and optical rotations to the reported values (**Figure 3**). For Ciliatamide A (**7**), reported to be the (*S,S*)-enantiomer, the NMRs of diastereomeric pairs **23** (*S,R*) and **24** (*R,S*), as anticipated, did not match **7**; however, the (*R,R*)-enantiomer of Ciliatamide A (**25**) was in complete accord with the published spectral data and possessed an optical rotation ($[\alpha]_D^{20}$ = +42 (c = 0.05, MeOH)) that matched the literature report as well. Similarly, the (*R*,*R*)-enantiomer of Ciliatamide B (**28**) overlayed with the reported NMR spectra of **8** as well as provided optical rotations in agreement with those reported by Nakao and co-workers, ($[\alpha]_D^{20}$ = +49 (c = 0.1, MeOH)) for **28**.

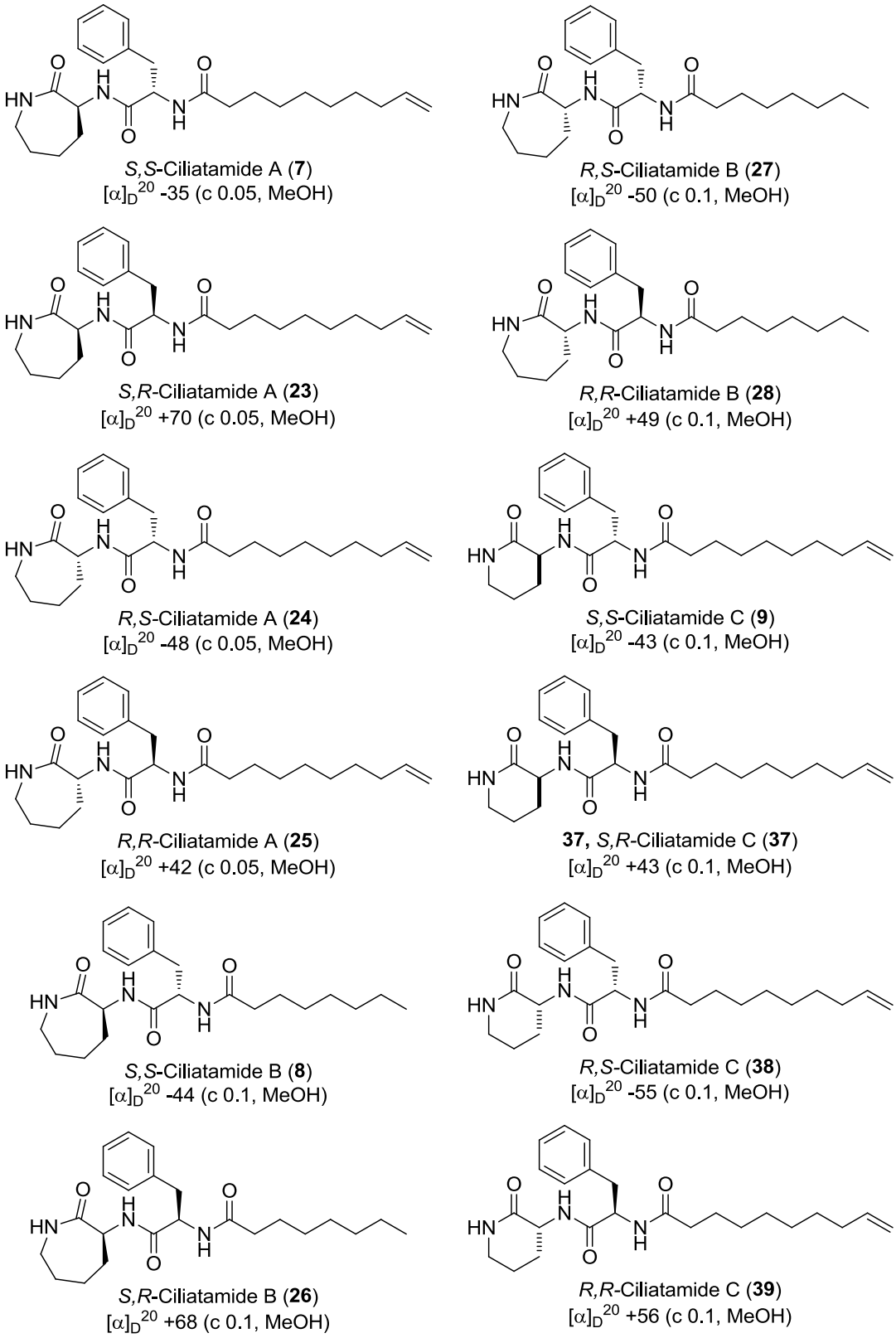
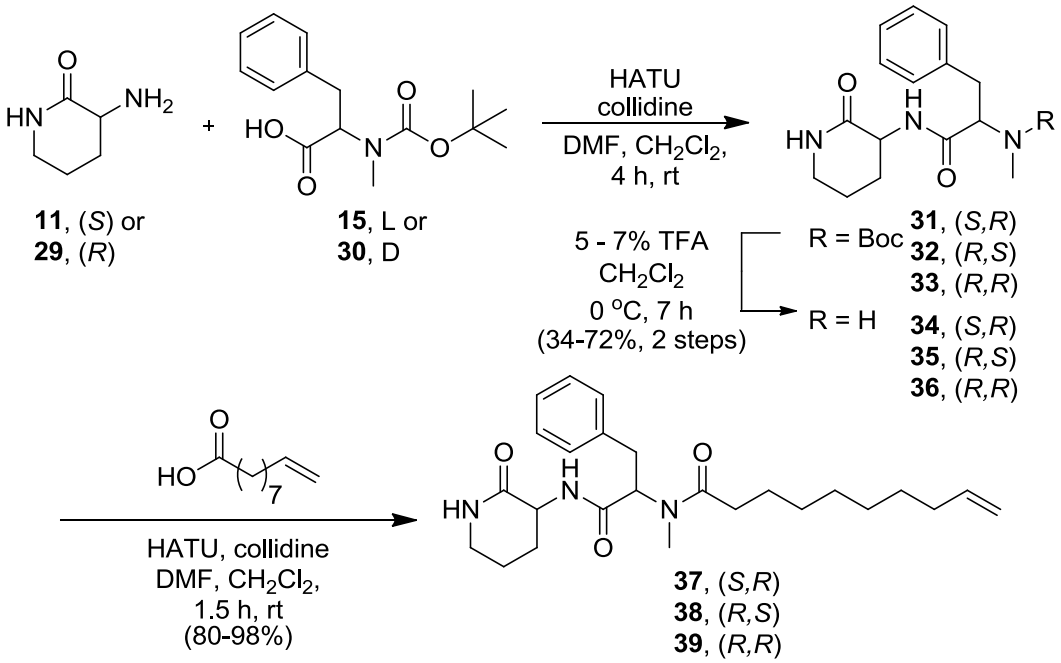


Figure 3. Library of all possible stereoisomers of Ciliatamides A-C and all the corresponding optical rotations

Attempts to prepare the (*S*,*R*), (*R*,*S*) and (*R*,*R*) steroisomers of Ciliatamide C following the route depicted in **Scheme 2** led to significant racemization of the (*R*)-piperidin-2-one, which was not observed within the Ciliatamide A and B series or the (S)-piperdin-2-one (**11**). Therefore, after several approaches, we developed an alternate route that avoided racemization and afforded pure stereoisomers (**Scheme 4**). In this instance, the carbodimide coupling in **Scheme 2** was replaced with a HATU/collidine system for the coupling of pure (*S*)- or (*R*)-piperidin-2-one, (**11**) and (**29**),respectively, with either enantiopure Boc-L-N-MePhe (**15**) or Boc-D-N-MePhe (**30**). The milder acidolysis of the Boc deprotection of **31**-**33** with 5-7% TFA in CH_2Cl_2 in an ice bath instead of 10 equivalents of HCl was used to deliver isomers **34**-**36**. Finally, a second HATU coupling with decenoic acid provided the remaining stereoisomers of Ciliatamide C **37** (*S*,*R*), **38** (*R*,*S*) and **39** (*R*,*R*). As in the case of Ciliatamides A and B, both NMR spectra and optical rotation ($[\alpha]_D^{20}$ = +56 (c = 0.1, MeOH)) for **39** confirm a stereochemical reassignment of the natural product to the (*R*,*R*)-enantiomer.



Scheme 4. Synthesis of the (*S,R*), (*R,S*) and (*S,S*)-stereosiomers of Ciliatamide C, **37**, **38** and **39**, respectively

For Ciliatamides A-C, the optical rotations were positive, and in agreement with the literature report, only when the unnatural D-MePhe was employed. Nakao and co-workers utilized Marfey's analysis[7] to establish the L-configuration of the caprolactams and the MePhe in **7**-**9**; however, the data presented herein suggests that either the reported optical rotations were incorrect, or the stereochemical assignments were incorrect.[4] It is also possible that the natural products, under the forcing acidic conditions of the Marfey's analysis racemized. When Marfey's analysis is employed to determine the absolute stereochemistry of amino acids, harsh acidic conditions are used to degrade the natural product into the amino acid segments. These amino acids then undergo a S_NAr reaction with the L or D enantiomer of fluoro dinitrophenyl-alaninamide (FDAA). The

corresponding products are then eluted through a HPLC column and their retention times are compared to the retention times of standards, which have been prepared from the L or D enantiomers of the corresponding amino acids. The method of chemical degradation used to determine the absolute stereochemistry of the Ciliatamides was to dissolve the natural products in 6M hydrochloric acid, which was then heated to 100 ºC overnight. It is a logical possibility that the amino acids racemizied during acid hydrolosis as our observations showed that the (*R,R*)-analogs are prone to acid catalyzed racemization to the (*S,S*)-enantiomers. Thus, based on our data, we proposed a stereochemical revision for Ciliatimides A-C from the (*S,S*)-**7**, **8**, and **9** to the (*R,R*)-**25**, **28**, and **39** (**Figure 4**).

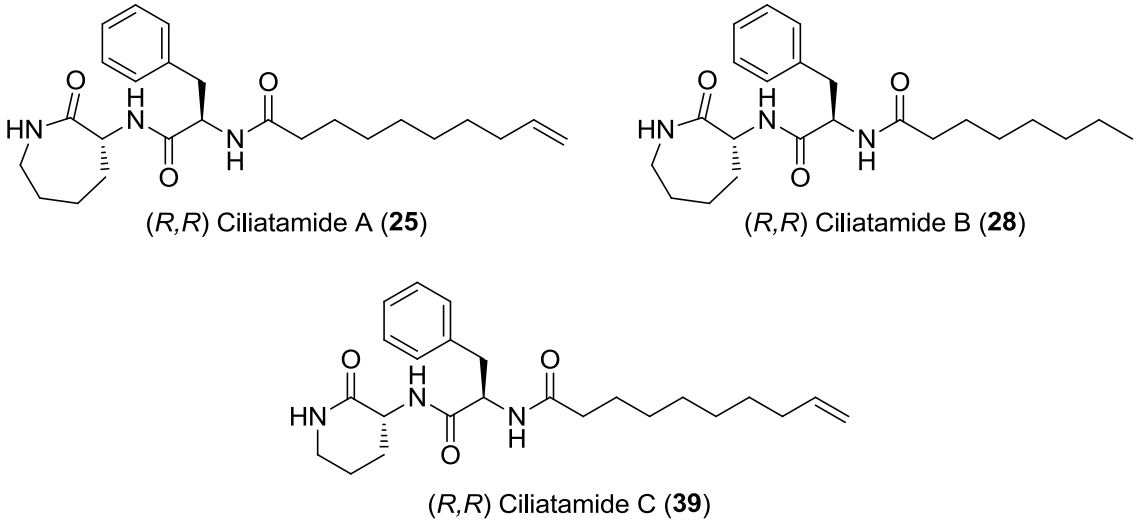


Figure 4. Revised structures of the natural lipopeptides Ciliatimides A-C after stereochemical revision

Conclusions

In summary, we have completed the first total synthesis of Ciliatamides A-C, originally reported as the (*S,S*)-enantiomers **7**-**9**, employing both traditional organic synthesis and solution phase parallel synthesis. Based on spectral and optical rotation data for all the possible stereoisomers of **7**-**9**, we proposed a stereochemical revision for Ciliatamides A-C to the (*R,R*)-enantiomers **25**, **28** and **39,** respectively. Due to the expedited route, we also prepared a library of 42 unnatural Ciliatamide analogs **20a-n**, **21a-n**, and **22a-n**, that when combined with the unnatural stereoisomers, exceeds 50 unnatural analogs.

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Experimental Section

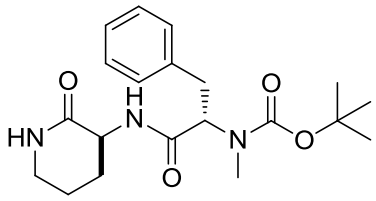
General. Unless otherwise noted, starting materials were obtained from commercial suppliers and used without further purification. $^{1}H/^{13}C$ NMR spectra were recorded on a Bruker 600 MHz AV-NMR spectrometer located in the Small Molecule NMR Facility at Vanderbilt University. Minor conformer signals attributed by Nakao and co-workers to conformers around the *N*-methyl amide bond between MePhe residues and fatty acids were also observed[4] however, since chemical shifts for both sets of conformers were not observed for all shifts, only major conformer signals are reported below. Electrospray atmospheric pressure ionization (ES-API) liquid chromatography/mass spectrometry (LCMS) was performed on an Agilent Technologies 6130 Quadrupole, and the data were analyzed using LC/MSD ChemStation software. As described by Nakao and co-workers, a fragment ion peak was observed in the ES-MS corresponding to cleavage at the amide bond closest to the lactam. The compounds were purified by preparative LCMS to >98% purity unless otherwise noted. A Micromass Q-TOF API-US mass spectrometer was used to acquire high-resolution mass spectrometry (HRMS) data. The HRMS results were obtained with ES as the ion source and leucine enkephalin as the reference.

General procedure for amide coupling A. Carboxylic acid (1.5 equiv), 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC, 2.0 equiv), 1-hydroxybenzotriazole (HOBt, 3.0 equiv), and DIPEA (2.0 equiv) were added to a solution of amine (1.0 equiv) in anhydrous DMF (23 mM). The mixture was stirred at room temperature overnight.

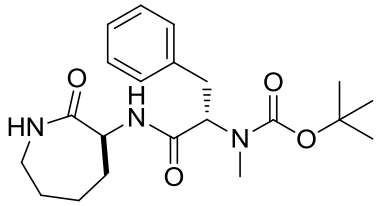
General procedure for amide coupling B. To a solution of amine (1.0 equiv) in anhydrous DMF (23 mM) in a 5 mL microwave reaction vial were added acid chloride (3.0 equiv), NMM (3.6 equiv), and a catalytic amount of DMAP. The vial was heated in a microwave synthesizer to 160 °C for 15 min.

General procedure for amide coupling C. Carboxylic acid (1.5 equiv), *N*-cyclohexylcarbodiimide-*N'*-propyloxymethyl polystyrene (PS-DCC, 2.0 equiv), and HOBt (1.7 equiv) were suspended in CH_2Cl_2 (65 mM amine) and mixed for at least 10 min prior to the addition of amine (1.0 equiv). The mixture was agitated at room temperature overnight. After the reaction, the HOBt was scavenged using macroporous triethylammonium methylpolystyrene carbonate (MP-carbonate, 5 equiv) for at least 2 h at room temperature prior to filtration.

General procedure for amide coupling D. *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 1.5 equiv) was added to a flame dried flask under argon and evacuated and filled with argon (3x). Solutions of amine (1.0 equiv) and carboxylic acid (1.0 equiv) and in anhydrous DMF (0.3 M) were then added. An equal volume of anhydrous CH_2Cl_2 was added to the flask before adding collidine (2.0 equiv). The mixture was stirred at room temperature for 4 h. HCl (1 N) was added to neutralize the reaction, diluted with CH_2Cl_2 and washed with water (3x). The solution was then concentrated *in vacuo* to yield the crude product.

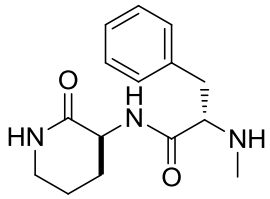


***tert*-butyl methyl((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)carbamate (16).** Boc-*N*-methyl-L-phenylalanine (**15**, 668 mg, 2.39 mmol) was dissolved in about 3 mL of DMF. 30 mL of dry CH_2Cl_2 was added to the mixture along with PS-DCC (3.187 g, 3.98 mmol), and the resin was allowed to swell for 10 min at room temperature with agitation. HOBt (404 mg, 2.99 mmol) was added with agitation for 10 min followed by the addition of a suspension of (*S*)-3-aminopiperid-2-one HCl (**11**, 300 mg, 1.99 mmol) in a mixture of 1 mL DMF, 1 mL MeOH, and 9 mL CH_2Cl_2. The mixture was agitated overnight at room temperature. The resin was removed by filtration through a pad of Celite followed by rinsing with CH_2Cl_2. The organics layer was washed with 2 × 25 mL of 1 N HCl, dried over sodium sulfate, and concentrated by rotary evaporation to yield pale yellow oil. LCMS, single peak, 2.782 min, ES-API: *m/z* 376.2 $[M+H]^+$.



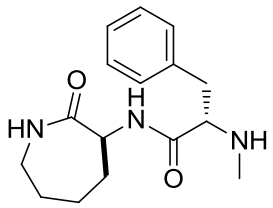
***tert*-butyl methyl((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)carbamate (17).** **17** was synthesized by the procedure used for **16** starting

from Boc-*N*-methyl-L-phenylalanine (**15**, 1.339 g, 4.79 mmol) and (*S*)-3-aminoazepan-2-one (**10**, 512 mg, 3.99 mmol) to yield a pale yellow oil. LCMS, single peak, 2.998 min, ES-API: *m/z* 390.3 $[M+H]^+$.

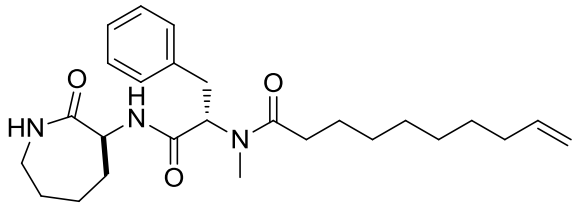


***N*-(*S*)-piperid-2-one-3-yl-(*S*)-2-methylamino-3-phenylpropanamide (18).** **16** (748 mg, 1.99 mmol) was dissolved in 43 mL of dichloroethane, and 5 mL of 4 M HCl in dioxane was added to the stirring solution. One mL of MeOH was added to the mixture to keep reagents and products solubilized. The mixture was stirred overnight at room temperature. The reaction mixture was concentrated by rotary evaporation to yield a yellow solution, which was taken up in CH_2Cl_2 and loaded onto a Varian Bond Elut 10 g SCX cartridge. The cartridge was washed with 25 mL each of CH_2Cl_2 then MeOH and was then eluted with 2 M NH_3 in MeOH, and these elution fractions were concentrated by rotary evaporation to a yellow oil. 2-step yield: 76% (418 mg). $[\alpha]_D^{20}$ +12 (*c* 0.25, MeOH); R_f 0.16 (9:1 DCM/MeOH); IR (neat) 3281, 3225, 2921, 2866, 1644, 1552, 1492, 1325 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz, 25 °C): δ 7.25 (m, 5H), 4.11 (dd, *J* = 10.6, 5.9 Hz, 1H), 3.51 (dd, *J* = 7.6, 5.9 Hz, 1H), 3.44 (t, *J* = 6.8 Hz, 1H), 3.27 (m, 2H), 3.04 (dd, *J* = 13.7, 6.5 Hz, 1H), 2.91 (dd, *J* = 13.7, 7.0 Hz, 1H), 2.39 (s, 3H), 1.98 (m, 1H), 1.89 (m, 1H), 1.82 (m, 1H), 1.76 (qd, *J* = 11.7, 3.4 Hz, 1H); ^{13}C NMR

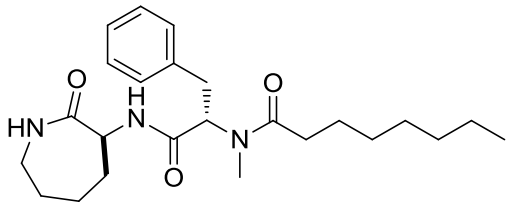
(CD_3OD, 150 MHz, 25 °C): δ 173.8, 172.4, 137.8, 130.5, 129.6, 128.0, 66.0, 51.2, 42.7, 39.7, 34.3, 28.5, 22.4; LCMS, single peak, 1.743 min, ES-API: *m/z* 276.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{15}H_{21}N_3O_2Na$ $[M+Na]^+$ calc'd 298.1531, found 298.1526.



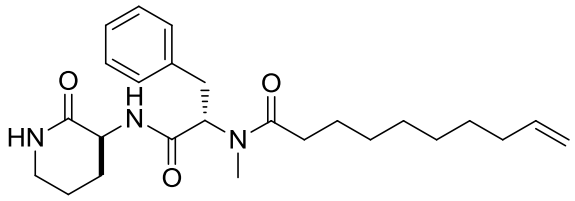
***N*-(*S*)-azepan-2-one-3-yl-(*S*)-2-methylamino-3-phenylpropanamide (19).** **19** was synthesized by the procedure used for **18** starting from **17** (1.556 g, 3.99 mmol) to yield a colorless oil. 2-step yield: 59% (685 mg). $[\alpha]_D^{20}$ -18 (*c* 0.25, MeOH); R_f 0.32 (9:1 DCM/MeOH); IR (neat) 3318, 2931, 2855, 1653, 1508, 1478, 1435 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz, 25 °C): δ 7.27 (t, *J* = 7.4 Hz, 2H), 7.21 (m, 3H), 4.49 (dd, *J* = 11.2, 1.6 Hz, 1H), 3.27 (m, 2H), 3.19 (bdd, *J* = 15.2, 4.2 Hz, 1H), 3.01 (dd, *J* = 13.7, 5.6 Hz, 1H), 2.80 (dd, *J* = 11.4, 7.8 Hz, 1H), 2.29 (s, 3H), 1.97 (dt, *J* = 14.0, 3.5 Hz, 1H), 1.84 (m, 3H), 1.45 (qd, *J* = 12.8, 2.8 Hz, 1H), 1.35 (m, 1H); ^{13}C NMR (CD_3OD, 150 MHz, 25 °C): δ 176.8, 174.9, 138.7, 130.3, 129.5, 127.8, 67.0, 53.0, 42.5, 40.4, 35.1, 32.7, 29.9, 29.0; LCMS, single peak, 1.735 min, ES-API: *m/z* 290.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{16}H_{23}N_3O_2Na$ $[M+Na]^+$ calc'd 312.1688, found 312.1693.



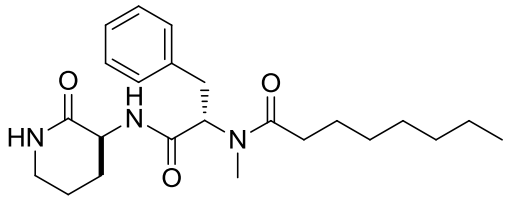
***N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)dec-9-enamide, (*S,S*)-Ciliatamide A (7 or 21a).** Amide coupling according to general procedure A starting from amine **19** (100 mg, 0.35 mmol), 9-decenoic acid (**13**, 88 mg, 96 µL, 0.52 mmol), EDC (132 mg, 0.69 mmol), HOBt (140 mg, 1.04 mmol), and DIPEA (89 mg, 120 µL, 0.69 mmol) led to the formation of the desired product **7** as a pale yellow oil. Yield: 56%. $[\alpha]_D^{20}$ –35 (*c* 0.05, MeOH); R_f 0.46 (9:1 DCM/MeOH); IR (neat) 3286, 2926, 2854, 1655, 1479, 1289 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz, 25 °C): *δ* 7.72 (bd, *J* = 8.3 Hz, 1H, NH), 7.45 (t, *J* = 7.4 Hz, 1H, NH), 7.22 (m, 4H), 7.17 (t, *J* = 7.0 Hz, 1H), 5.80 (ddt, *J* = 17.1, 10.2, 6.7 Hz, 1H), 5.37 (dd, *J* = 11.1, 5.4 Hz, 1H), 4.97 (dd, *J* = 17.1, 1.2 Hz, 1H), 4.91 (dt, *J* = 10.2, 1.2 Hz, 1H), 4.53 (dd, *J* = 11.2, 1.6 Hz, 1H), 3.33 (dd, *J* = 14.4, 5.4 Hz, 1H), 3.22 (bdd, *J* = 14.1, 2.9 Hz, 2H), 3.04 (dd, *J* = 14.4, 11.1 Hz, 1H), 2.90 (s, 3H), 2.23 (t, *J* = 7.4 Hz, 2H), 2.03 (q, *J* = 6.7 Hz, 2H), 2.00 (m, 1H), 1.93 (m, 1H), 1.82 (m, 1H), 1.79 (m, 1H), 1.62 (m, 1H), 1.51 (qd, *J* = 14.1, 2.9 Hz, 1H), 1.37 (m, 2H), 1.34 (m, 2H), 1.28 (m, 2H), 1.24 (m, 2H), 1.16 (m, 2H); ^{13}C NMR (CD_3OD, 150 MHz, 25 °C): *δ* 176.9, 176.7, 171.6, 140.1, 138.8, 130.0, 129.5, 127.7, 114.7, 59.7, 53.5, 42.5, 35.1, 34.9, 34.4, 33.1, 32.2, 30.8, 30.3, 30.2, 30.0, 29.9, 29.1, 26.1; LCMS, single peak, 3.527 min, ES-API: *m/z* 905.5 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{26}H_{39}N_3O_3Na$ $[M+Na]^+$ calc'd 464.2889, found 464.2906.



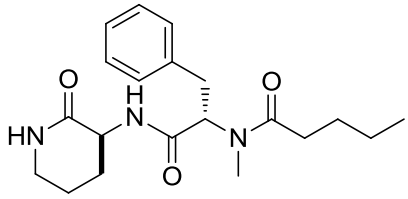
***N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)octanamide, (*S,S*)-Ciliatamide B (8 or 21b).** **8** was synthesized according to the general procedure for amide coupling B starting from amine **19** (100 mg, 0.35 mmol), octanoyl chloride (**14**, 169 mg, 178 µL, 1.04 mmol), NMM (126 mg, 137 µL, 1.24 mmol), and a catalytic amount of DMAP to yield a pale yellow oil. Yield: 58% (83.6 mg). $[\alpha]_D^{20}$ –44 (*c* 0.10, MeOH); R_f 0.44 (9:1 DCM/MeOH); IR (neat) 3286, 2928, 2855, 1656, 1479, 1288 cm^{-1}; ^1H NMR (CD_3CN, 600 MHz, 25 °C): *δ* 7.20 (m, 5H), 7.10 (bd, *J* = 5.8 Hz, 1H, NH), 6.45 (bs, 1H, NH), 5.37 (dd, *J* = 10.5, 5.5 Hz, 1H), 4.40 (ddd, *J* = 11.0, 6.2, 1.4 Hz, 1H), 3.22 (dd, *J* = 14.5, 5.5 Hz, 1H), 3.16 (m, 2H), 2.91 (dd, *J* = 14.5, 10.5 Hz, 1H), 2.82 (s, 3H), 2.18 (m, 2H), 1.97 (m, 1H), 1.89 (m, 2H), 1.77 (m, 1H), 1.70 (m, 1H), 1.37 (quintet, *J* = 7.4 Hz, 2H), 1.32 (m, 1H), 1.26 (m, 2H), 1.21 (m, 4H), 1.14 (m, 2H), 0.87 (t, *J* = 7.2, 3H); ^{13}C NMR (CD_3CN, 150 MHz, 25 °C): *δ* 175.5, 174.9, 169.9, 139.2, 129.9, 129.2, 127.3, 58.2, 53.0, 42.1, 34.2, 33.9, 32.45, 32.39, 31.9, 29.80, 29.78, 29.7, 28.8, 25.8, 23.3, 14.4; LCMS, single peak, 3.380 min, ES-API: *m/z* 853.5 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{24}H_{37}N_3O_3Na$ $[M+Na]^+$ calc'd 438.2733, found 438.2716.



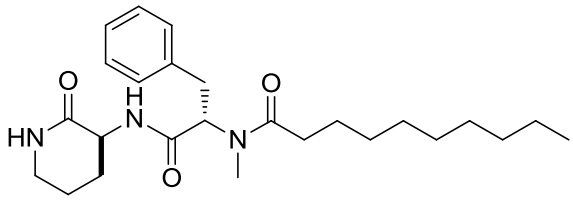
***N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)dec-9-enamide, (*S,S*)-Ciliatamide C (9 or 20a).** **9** was synthesized by the general procedure for amide coupling A starting from **18** (70 mg, 0.25 mmol) and 9-decenoic acid (**13**, 65 mg, 71 µL, 0.38 mmol) to yield a yellow oil. Yield: 47% (51.4 mg). $[\alpha]_D^{20}$ –43 (*c* 0.10, MeOH); R_f 0.44 (9:1 DCM/MeOH); IR (neat) 3274, 2926, 2854, 1655, 1527, 1492 cm^{-1}; ^1H NMR (CD_3CN, 600 MHz, 25 °C): δ 7.20 (m, 5H), 6.76 (bd, *J* = 6.6 Hz, 1H, NH), 6.15 (bs, 1H, NH), 5.82 (ddt, *J* = 17.0, 10.3, 6.5 Hz, 1H), 5.37 (dd, *J* = 10.8, 5.2 Hz, 1H), 4.99 (dd, *J* = 17.0, 1.5 Hz, 1H), 4.92 (dt, *J* = 10.3, 1.5 Hz, 1H), 4.15 (dt, *J* = 11.7, 6.6 Hz, 1H), 3.26 (dd, *J* = 14.6, 5.2 Hz, 1H), 3.21 (m, 2H), 2.88 (dd, *J* = 14.6, 10.8 Hz, 1H), 2.80 (s, 3H), 2.15 (m, 3H), 2.02 (m, 2H), 1.82 (m, 2H), 1.64 (qd, *J* = 11.7, 4.9 Hz, 1H), 1.33 (m, 4H), 1.26 (m, 2H), 1.21 (m, 2H), 1.10 (m, 2H); ^{13}C NMR (CD_3CN, 150 MHz, 25 °C): δ 174.8, 171.1, 140.2, 139.3, 129.9, 129.2, 127.2, 114.7, 58.4, 50.9, 42.4, 34.43, 34.36, 33.9, 32.0, 29.9, 29.8, 29.7, 29.6, 28.4, 25.6, 22.1; LCMS, single peak, 3.358 min, ES-API: *m/z* 877.5 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{25}H_{37}N_3O_3Na$ $[M+Na]^+$ calc'd 450.2733, found 450.2729.



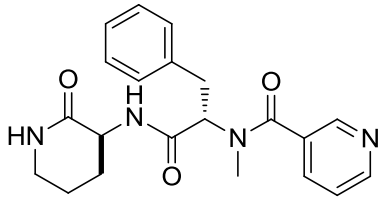
***N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)octanamide (20b).** Coupling **18** (20 mg, 0.073 mmol) with octanoyl chloride (**14**, 35 mg, 0.22 mmol) using method B afforded the amide **20b** as a yellow oil. Yield: 47% (13.7 mg). LCMS, single peak, 3.192 min, ES-API: *m/z* 825.5 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{23}H_{35}N_3O_3Na$ $[M+Na]^+$ calc'd 424.2576, found 424.2565.



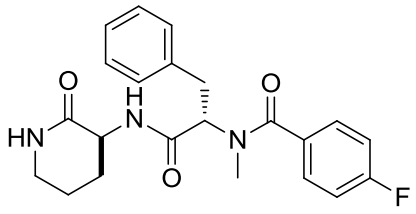
***N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)pentanamide (20c).** Coupling **18** (20 mg, 0.073 mmol) with valeroyl chloride (26 mg, 26 µL, 0.22 mmol) using method B afforded the amide **20c** as a pale yellow oil. Yield: 31% (8.1 mg). LCMS, single peak, 2.641 min, ES-API: *m/z* 741.4 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{20}H_{29}N_3O_3Na$ $[M+Na]^+$ calc'd 382.2107, found 382.2092.



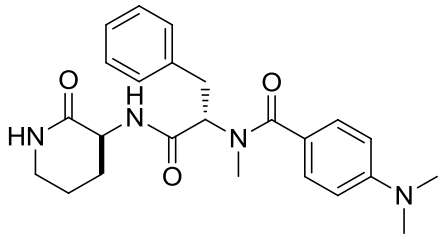
***N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)decanamide (20d).** Coupling **18** (20 mg, 0.073 mmol) with decanoyl chloride (42 mg, 45 µL, 0.22 mmol) using method B afforded the amide **20d** as a yellow oil. Yield: 8% (2.5 mg). LCMS, single peak, 3.562 min, ES-API: *m/z* 881.5 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{25}H_{39}N_3O_3Na$ $[M+Na]^+$ calc'd 452.2889, found 452.2884.



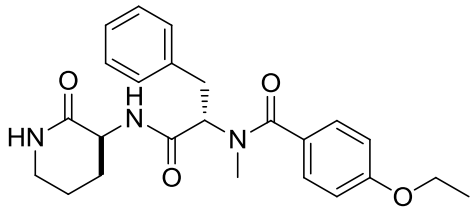
***N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)nicotinamide (20e).** Coupling **18** (20 mg, 0.073 mmol) with nicotinic acid (13 mg, 0.110 mmol) using method A afforded the amide **20e** as a yellow oil. Yield: 46% (16.5 mg). LCMS, single peak, 1.961 min, ES-API: *m/z* 381.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{21}H_{24}N_4O_3Na$ $[M+Na]^+$ calc'd 403.1746, found 403.1752.



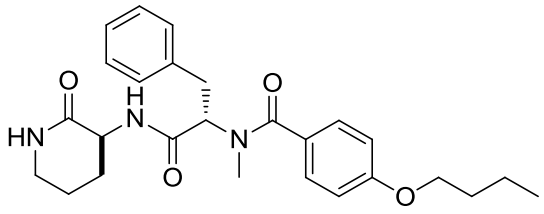
4-Fluoro-*N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (20f). Coupling **18** (20 mg, 0.073 mmol) with 4-fluorobenzoyl chloride (35 mg, 26 µL, 0.22 mmol) using method B afforded the amide **20f** as a pale yellow oil. Yield: 29% (8.3 mg). LCMS, single peak, 2.621 min, ES-API: *m/z* 398.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{22}H_{24}FN_3O_3Na$ $[M+Na]^+$ calc'd 420.1699, found 420.1692.



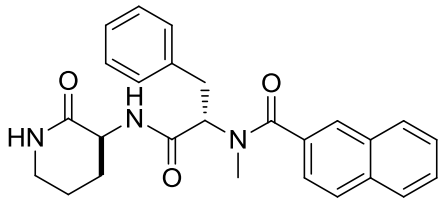
4-(Dimethylamino)-*N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (20g). Coupling **18** (20 mg, 0.073 mmol) with 4-(dimethylamino)benzoyl chloride (40 mg, 0.22 mmol) using method B afforded the amide **20g** as a brown oil. Yield: 30% (11.7 mg). LCMS, single peak, 2.265 min, ES-API: *m/z* 423.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{24}H_{30}N_4O_3Na$ $[M+Na]^+$ calc'd 445.2216, found 445.2224.



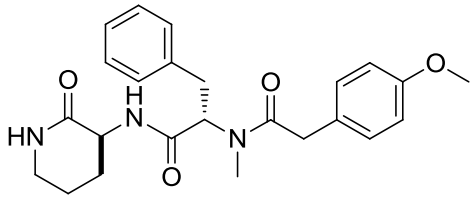
4-Ethoxy-*N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (20h). Coupling **18** (20 mg, 0.073 mmol) with 4-ethoxybenzoyl chloride (40 mg, 40 µL, 0.22 mmol) using method B afforded the amide **20h** as a yellow oil. Yield: 31% (9.5 mg). LCMS, single peak, 2.747 min, ES-API: *m/z* 424.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{24}H_{29}N_3O_4Na$ $[M+Na]^+$ calc'd 446.2056, found 446.2072.



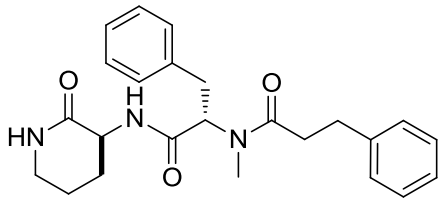
4-Butoxy-*N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (20i). Coupling **18** (20 mg, 0.073 mmol) with 4-butoxybenzoyl chloride (46 mg, 41 µL, 0.22 mmol) using method B afforded the amide **20i** as a yellow oil. Yield: 58% (19.0 mg). LCMS, single peak, 3.117 min, ES-API: *m/z* 926.4 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{26}H_{33}N_3O_4Na$ $[M+Na]^+$ calc'd 474.2369, found 474.2380.



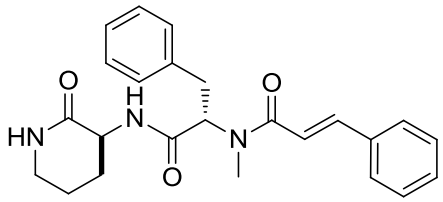
***N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)-2-naphthamide (20j).** Coupling **18** (20 mg, 0.073 mmol) with 2-naphthoyl chloride (42 mg, 0.22 mmol) using method B afforded the amide **20j** as a yellow oil. Yield: 39% (12.2 mg). LCMS, single peak, 2.867 min, ES-API: *m/z* 430.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{26}H_{27}N_3O_3Na$ $[M+Na]^+$ calc'd 452.1950, found 452.1944.



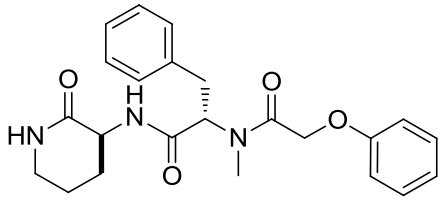
4-Methoxy-*N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (20k). Coupling **18** (20 mg, 0.073 mmol) with 4-methoxyphenylacetyl chloride (40 mg, 33 µL, 0.22 mmol) using method B afforded the amide **20k** as a yellow oil. Yield: 1% (0.2 mg). LCMS, single peak, 2.643 min, ES-API: *m/z* 424.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{24}H_{29}N_3O_4Na$ $[M+Na]^+$ calc'd 446.2056, found 446.2054.



***N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)-3-phenylpropanamide (20l).** Coupling **18** (20 mg, 0.073 mmol) with hydrocinnamoyl chloride (37 mg, 32 µL, 0.22 mmol) using method B afforded the amide **20l** as a colorless oil. Yield: 53% (15.6 mg). ^{1}H NMR (DMSO-d_6, 600 MHz, 25 °C): δ 7.84 (t, J = 5.9 Hz, 1H, NH), 7.66 (d, J = 6.3 Hz, 1H, NH), 7.58 (d, J = 6.9 Hz, 1H), 7.37 (m, 3H), 7.25 (m, 3H), 7.18 (m, 1H), 7.09 (t, J = 7.2 Hz, 1H), 7.05 (d, J = 15.2 Hz, 1H), 5.46 (dd, J = 10.3, 6.3 Hz, 1H), 4.37 (dd, J = 10.3, 6.4 Hz, 1H), 3.23 (dd, J = 14.4, 5.8 Hz, 1H), 3.16 (m, 2H), 3.05 (s, 3H), 2.94 (q, J = 12.1 Hz, 1H), 2.87 (s, 2H), 1.86 (m, 1H), 1.78 (m, 1H), 1.73 (m, 1H), 1.63 (quintet, J = 12.7 Hz, 1H), 1.35 (m, 1H), 1.18 (quintet, J = 11.5 Hz, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz, 25 °C): δ 173.9, 168.8, 166.3, 142.0, 138.0, 134.9, 129.2, 128.7, 128.1, 126.2, 118.2, 60.1, 57.1, 51.5, 40.6, 33.9, 31.4, 30.9, 28.8, 27.6; LCMS, single peak, 2.805 min, ES-API: *m/z* 408.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{24}H_{29}N_3O_3Na$ $[M+Na]^+$ calc'd 430.2107, found 430.2107.

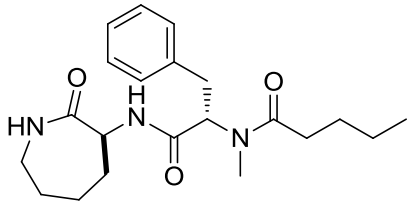


***N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)cinnamamide (20m).** Coupling **18** (20 mg, 0.073 mmol) with cinnamoyl chloride (36 mg, 0.22 mmol) using method B afforded the amide **20m** as a pale yellow oil. Yield: 18% (5.4 mg). LCMS, single peak, 2.768 min, ES-API: *m/z* 833.3 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{24}H_{27}N_3O_3Na$ $[M+Na]^+$ calc'd 428.1950, found 428.1959.

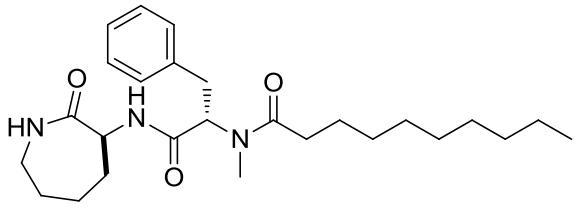


***N*–methyl-((*S*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)phenoxyacetamide (20n).** Coupling **18** (20 mg, 0.073 mmol) with 2-phenoxyacetyl chloride (37 mg, 30 µL, 0.22 mmol) using method B afforded the amide **20n** as a colorless oil. Yield: 53% (15.8 mg). ^{1}H NMR (DMSO-d_6, 600 MHz, 25 °C): δ 8.05 (bd, J = 7.6 Hz, 1H, NH), 7.60 (m, 1H, NH), 7.33 (m, 1H), 7.23 (m, 4H), 7.16 (t, J = 7.6 Hz, 2H), 6.87 (t, J = 7.6 Hz, 1H), 6.52 (d, J = 7.6 Hz, 2H), 5.27 (dd, J = 11.0, 4.6 Hz, 1H), 4.71 (d, J = 15.7 Hz, 1H), 4.52 (d, J = 15.7 Hz, 1H), 4.16 (m, 1H), 3.26 (dd, J = 14.2, 4.6 Hz, 1H), 3.13 (m, 2H), 2.96 (dd, J =

14.2, 11.0 Hz, 1H), 2.86 (s, 3H), 1.91 (m, 1H), 1.78 (m, 1H), 1.72 (m, 2H); ^{13}C NMR (DMSO-d_6, 150 MHz, 25 °C): δ 169.5, 169.1, 167.9, 157.9, 138.0, 129.1, 128.9, 128.1, 126.1, 120.5, 114.3, 65.2, 57.3, 49.1, 41.1, 33.7, 29.9, 28.7, 27.6; LCMS, single peak, 2.648 min, ES-API: *m/z* 410.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{23}H_{27}N_3O_4Na$ $[M+Na]^+$ calc'd 432.1889, found 432.1898.

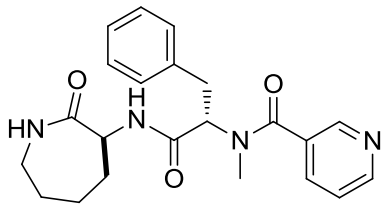


***N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)pentanamide (21c).** Coupling **19** (21 mg, 0.073 mmol) with valeroyl chloride (26 mg, 26 µL, 0.22 mmol) using method B afforded the amide **21c** as a colorless oil. Yield: 39% (10.7 mg). LCMS, single peak, 2.846 min, ES-API: *m/z* 769.4 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{21}H_{31}N_3O_3Na$ $[M+Na]^+$ calc'd 396.2263, found 396.2260.

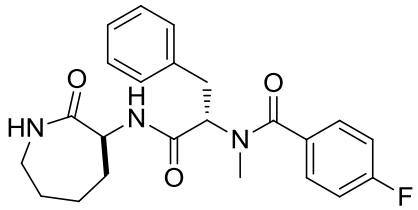


***N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)decanamide (21d).** Coupling **19** (21 mg, 0.073 mmol) with decanoyl chloride

(42 mg, 45 µL, 0.22 mmol) using method B afforded the amide **21d** as a colorless oil. Yield: 43% (13.7 mg). LCMS, single peak, 3.732 min, ES-API: *m/z* 909.6 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{26}H_{41}N_3O_3Na$ $[M+Na]^+$ calc'd 466.3046, found 466.3052.

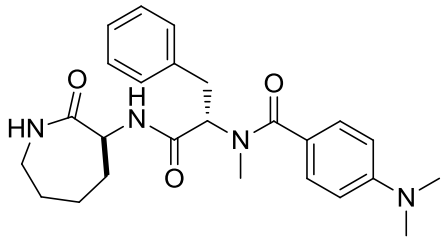


***N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)nicotinamide (21e).** Coupling **19** (21 mg, 0.073 mmol) with nicotinic acid (13 mg, 0.110 mmol) using method A afforded the amide **21e** as a colorless oil. Yield: 33% (12.2 mg). LCMS, single peak, 2.123 min, ES-API: *m/z* 395.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{22}H_{26}N_4O_3Na$ $[M+Na]^+$ calc'd 417.1903, found 417.1894.

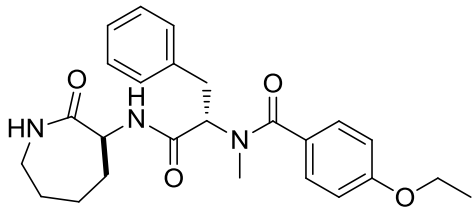


4-Fluoro-*N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (21f). Coupling **19** (21 mg, 0.073 mmol) with 4-fluorobenzoyl chloride (35 mg, 26 µL, 0.22 mmol) using method B afforded the amide **21f** as a yellow oil. Yield: 32% (9.7 mg). LCMS, single peak, 2.821 min, ES-API: *m/z*

412.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{23}H_{26}FN_3O_3Na$ $[M+Na]^+$ calc'd 434.1856, found 434.1853.

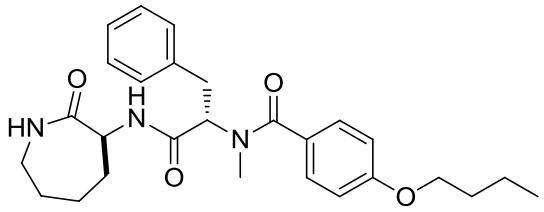


4-(Dimethylamino)-*N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (21g). Coupling **19** (21 mg, 0.073 mmol) with 4-(dimethylamino)benzoyl chloride (40 mg, 0.22 mmol) using method B afforded the amide **21g** as a brown oil. Yield: 33% (13.2 mg). LCMS, single peak, 2.489 min, ES-API: *m/z* 437.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{25}H_{32}N_4O_3Na$ $[M+Na]^+$ calc'd 459.2372, found 459.2383.

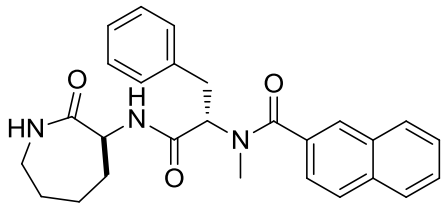


4-Ethoxy-*N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (21h). Coupling **19** (21 mg, 0.073 mmol) with 4-ethoxybenzoyl chloride (40 mg, 40 µL, 0.22 mmol) using method B afforded the amide **21h** as a yellow oil. Yield: 36% (11.4 mg). LCMS, single peak, 2.950 min, ES-API: *m/z*

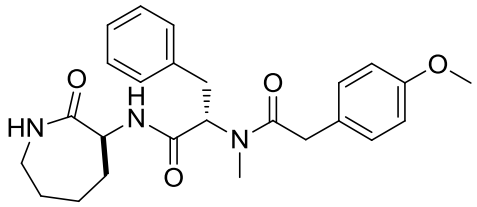
897.4 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{25}H_{31}N_3O_4Na$ $[M+Na]^+$ calc'd 460.2212, found 460.2223.



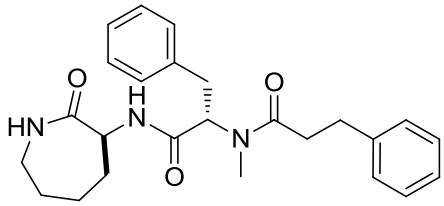
4-Butoxy-*N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (21i). Coupling **19** (21 mg, 0.073 mmol) with 4-butoxybenzoyl chloride (46 mg, 41 µL, 0.22 mmol) using method B afforded the amide **21i** as a pale yellow oil. Yield: 43% (14.4 mg). LCMS, single peak, 3.322 min, ES-API: *m/z* 953.4 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{27}H_{35}N_3O_4Na$ $[M+Na]^+$ calc'd 488.2525, found 488.2541.



***N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)-2-naphthamide (21j).** Coupling **19** (21 mg, 0.073 mmol) with 2-naphthoyl chloride (42 mg, 0.22 mmol) using method B afforded the amide **21j** as a pale yellow oil. Yield: 37% (12.0 mg). LCMS, single peak, 3.062 min, ES-API: *m/z* 444.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{27}H_{29}N_3O_3Na$ $[M+Na]^+$ calc'd 466.2107, found 466.2111

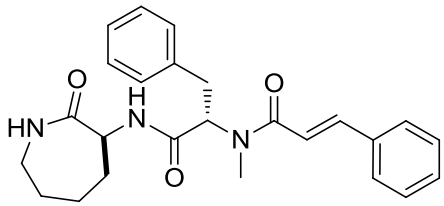


4-Methoxy-*N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (21k). Coupling **19** (21 mg, 0.073 mmol) with 4-methoxyphenylacetyl chloride (40 mg, 33 µL, 0.22 mmol) using method B afforded the amide **21k** as a yellow oil. Yield: 36% (11.3 mg). LCMS, single peak, 2.830 min, ES-API: *m/z* 897.4 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{25}H_{31}N_3O_4Na$ $[M+Na]^+$ calc'd 460.2212, found 460.2209.

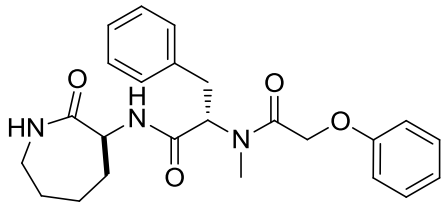


***N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)-3-phenylpropanamide (21l).** Coupling **19** (21 mg, 0.073 mmol) with hydrocinnamoyl chloride (37 mg, 32 µL, 0.22 mmol) using method B afforded the amide **21l** as a pale yellow oil. Yield: 50% (15.4 mg). ^{1}H NMR (DMSO-d_6, 600 MHz, 25 °C): δ 7.86 (m, 1H, NH), 7.60 (d, J = 6.7 Hz, 1H, NH), 7.22 (m, 6H), 7.16 (m, 2H), 7.11 (d, J = 7.3 Hz, 1H), 7.03 (d, J = 7.3 Hz, 1H), 5.33 (dd, J = 10.4, 5.4 Hz, 1H), 4.37 (dd, J = 10.9, 7.6 Hz, 1H), 3.18 (dd, J = 14.8, 5.4 Hz, 1H), 3.05 (m, 2H), 2.86 (dd, J = 14.8, 10.4 Hz, 1H), 2.82 (s, 3H), 2.65 (m, 2H), 2.53 (m, 1H),

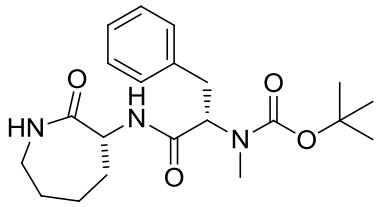
2.43 (dd, *J* = 9.2, 6.6 Hz, 1H), 1.87 (m, 1H), 1.75 (m, 2H), 1.64 (m, 1H), 1.37 (quintet, *J* = 12.6 Hz, 1H), 1.19 (m, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz, 25 °C): δ 173.9, 172.3, 168.8, 141.3, 138.0, 129.0, 128.8, 128.2, 128.1, 126.1, 125.7, 60.2, 56.9, 51.5, 40.6, 34.3, 33.8, 30.9, 30.2, 28.8, 27.6; LCMS, single peak, 2.997 min, ES-API: *m/z* 444.2 $[M+Na]^+$; HRMS (TOF, ES+) $C_{25}H_{31}N_3O_3Na$ $[M+Na]^+$ calc'd 444.2263, found 444.2250.



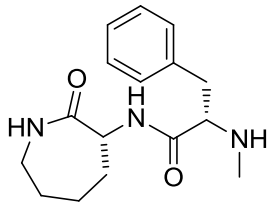
***N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)cinnamamide (21m).** Coupling **19** (21 mg, 0.073 mmol) with cinnamoyl chloride (36 mg, 0.22 mmol) using method B afforded the amide **21m** as a pale yellow oil. Yield: 41% (12.6 mg). LCMS, single peak, 2.947 min, ES-API: *m/z* 861.4 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{25}H_{29}N_3O_3Na$ $[M+Na]^+$ calc'd 442.2107, found 442.2114.



***N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)phenoxyacetamide (21n).** Coupling **19** (21 mg, 0.073 mmol) with 2-phenoxyacetyl chloride (37 mg, 30 µL, 0.22 mmol) using method B afforded the amide **21n** as a colorless oil. Yield: 43% (13.1 mg). ^{1}H NMR (DMSO-d_6, 600 MHz, 25 °C): δ 7.84 (t, J = 6.0 Hz, 1H, NH), 7.74 (d, J = 7.2 Hz, 1H, NH), 7.33 (m, 1H), 7.24 (m, 4H), 7.17 (t, J = 7.7 Hz, 2H), 6.88 (t, J = 7.7 Hz, 1H), 6.57 (d, J = 7.7 Hz, 2H), 5.29 (dd, J = 10.7, 5.0 Hz, 1H), 4.75 (d, J = 15.2 Hz, 1H), 4.56 (d, J = 15.2 Hz, 1H), 4.40 (dd, J = 9.7, 7.2 Hz, 1H), 3.22 (dd, J = 14.2, 5.0 Hz, 1H), 3.17 (m, 1H), 3.06 (m, 1H), 2.99 (dd, J = 14.2, 10.7 Hz, 1H), 2.89 (s, 3H), 1.88 (m, 1H), 1.82 (m, 1H), 1.75 (m, 1H), 1.64 (m, 1H), 1.41 (quintet, J = 12.4 Hz, 1H), 1.20 (quintet, J = 12.4 Hz, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz, 25 °C): δ 173.9, 168.4, 168.1, 157.9, 137.9, 129.2, 128.8, 128.2, 126.2, 120.5, 114.3, 65.2, 57.0, 51.5, 40.6, 33.6, 30.9, 29.9, 28.8, 27.6; LCMS, single peak, 2.834 min, ES-API: *m/z* 424.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{24}H_{29}N_3O_4Na$ $[M+Na]^+$ calc'd 446.2056, found 446.2041.

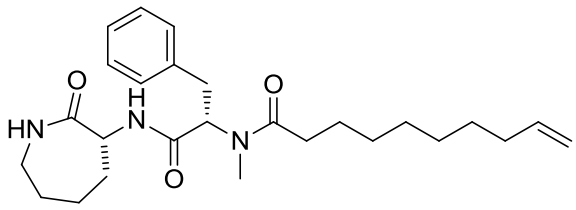


***tert*-butyl methyl((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)carbamate (40).** **40** was synthesized by the procedure used for **16** starting from Boc-*N*-methyl-L-phenylalanine (**15**, 668 mg, 2.39 mmol) and (*R*)-3-aminoazepan-2-one (255 mg, 1.99 mmol) to yield a pale yellow oil. LCMS, single peak, 3.019 min, ES-API: *m/z* 390.3 $[M+H]^+$.



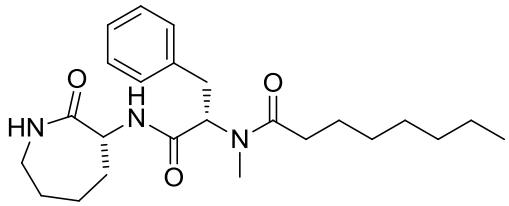
***N*-(*R*)-azepan-2-one-3-yl-(*S*)-2-methylamino-3-phenylpropanamide (41).** **41** was synthesized by the procedure used for **18** starting from **40** (776 mg, 1.99 mmol) to yield a pale yellow oil. 2-step yield: 85% (491 mg). $[\alpha]_D^{20}$ +27 (*c* 0.25, MeOH); R_f 0.32 (9:1 DCM/MeOH); IR (neat) 3318, 2931, 2855, 1653, 1508, 1478, 1435 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz, 25 °C): δ 7.26 (t, *J* = 7.5 Hz, 2H), 7.20 (m, 3H), 4.46 (dd, *J* = 11.5, 1.4 Hz, 1H), 3.32 (m, 1H), 3.25 (dd, *J* = 14.8, 11.5 Hz, 1H), 3.18 (bdd, *J* = 14.2, 4.4 Hz, 1H), 2.88 (m, 2H), 2.29 (s, 3H), 1.91 (dt, *J* = 14.0, 3.3 Hz, 1H), 1.80 (dd, *J* = 14.2, 4.1 Hz, 1H), 1.72 (qt, *J* = 13.2, 3.5 Hz, 1H), 1.58 (d, *J* = 13.7 Hz, 1H), 1.32 (m, 2H); ^{13}C NMR (CD_3OD, 150 MHz, 25

°C): δ 176.8, 174.6, 138.8, 130.3, 129.5, 127.7, 66.9, 53.0, 42.4, 40.5, 34.7, 32.2, 29.9, 29.0; LCMS, single peak, 1.886 min, ES-API: *m/z* 290.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{16}H_{23}N_3O_2Na$ $[M+Na]^+$ calc'd 312.1688, found 312.1691.

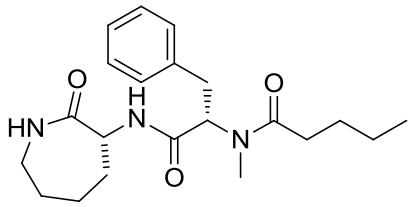


***N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)dec-9-enamide, (*R,S*)-Ciliatamide A, (22a or 24).** **24** was synthesized by the general procedure for amide coupling A starting from **41** (33 mg, 0.12 mmol) and 9-decenoic acid (**13**, 29 mg, 32 µL, 0.17 mmol) to yield a pale brown oil. Yield: 19% (9.5 mg). $[\alpha]_D^{20}$ –48 (*c* 0.05, MeOH); R_f 0.46 (9:1 DCM/MeOH); IR (neat) 3286, 2926, 2854, 1655, 1479, 1289 cm^{-1}; ^{1}H NMR (DMSO-d_6, 600 MHz, 25 °C): δ 7.83 (m, 1H, NH), 7.56 (bd, *J* = 6.2 Hz, 1H, NH), 7.22 (m, 4H), 7.14 (t, *J* = 6.8 Hz, 1 H), 5.78 (ddt, *J* = 17.2, 10.3, 6.9 Hz, 1H), 5.36 (dd, *J* = 10.5, 5.5 Hz, 1H), 4.99 (dd, *J* = 17.2, 1.4 Hz, 1H), 4.93 (dt, *J* = 10.3, 1.4 Hz, 1H), 4.34 (dd, *J* = 9.9, 4.3 Hz, 1H), 3.16 (dd, *J* = 14.1, 5.5 Hz, 1H), 3.04 (bdd, *J* = 13.8, 4.9 Hz, 2H), 2.88 (dd, *J* = 14.1, 10.5 Hz, 1H), 2.83 (s, 3H), 2.12 (t, *J* = 7.1 Hz, 2H), 1.99 (q, *J* = 6.9 Hz, 2H), 1.86 (m, 1H), 1.77 (m, 1H), 1.74 (m, 1H), 1.71 (m, 1H), 1.61 (m, 1H), 1.38 (qd, *J* = 13.8, 4.9 Hz, 1H), 1.29 (m, 4H), 1.17 (m, 4H), 1.07 (m, 2H); ^{13}C NMR (DMSO-d_6, 150 MHz, 25 °C): δ 173.9, 172.9, 168.9, 138.8, 137.9, 128.8, 128.0, 126.1, 114.6, 56.5, 51.5, 40.6, 33.8, 33.2, 32.6, 31.2, 30.9, 28.8, 28.6,

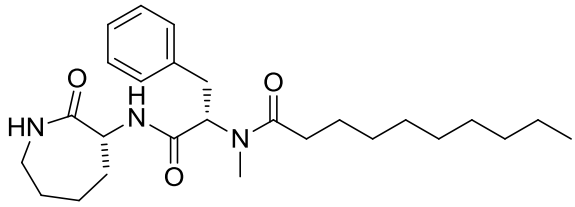
28.4, 28.2, 27.6, 24.4; LCMS, single peak, 3.532 min, ES-API: *m/z* 442.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{26}H_{39}N_3O_3Na$ $[M+Na]^+$ calc'd 464.2889, found 464.2905.



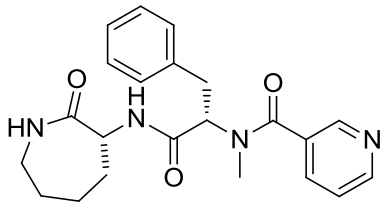
***N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)octanamide, (*R,S*)-Ciliatamide B (22b or 27).** **27** was synthesized according to the general procedure for amide coupling B starting from amine **41** (33 mg, 0.12 mmol) and octanoyl chloride (56 mg, 59 µL, 0.35 mmol) to yield a pale yellow oil. Yield: 24% (11.3 mg). $[\alpha]_D^{20}$ –50 (*c* 0.10, MeOH); R_f 0.44 (9:1 DCM/MeOH); IR (neat) 3286, 2928, 2855, 1656, 1479, 1288 cm^{-1}; ^1H NMR (DMSO-d_6, 600 MHz, 25 °C): δ 7.87 (m, 1H, NH), 7.60 (bd, *J* = 6.5 Hz, 1H, NH), 7.22 (m, 5H), 7.14 (t, *J* = 6.8 Hz, 1H), 5.37 (dd, *J* = 10.5, 5.6 Hz, 1H), 4.34 (dd, *J* = 10.0, 6.4 Hz, 1H), 3.16 (m, 2H), 3.04 (m, 1H), 2.88 (dd, *J* = 14.5, 10.5 Hz, 1H), 2.83 (s, 3H), 2.12 (dt, *J* = 7.4, 2.3 Hz, 2H), 1.86 (m, 1H), 1.74 (m, 2H), 1.62 (m, 1H), 1.38 (m, 1H), 1.28 (quintet, *J* = 7.4 Hz, 2H), 1.22 (m, 3H), 1.15 (m, 4H), 1.06 (m, 1H), 1.02 (m, 1H), 0.84 (t, *J* = 7.2, 3H); ^{13}C NMR (DMSO-d_6, 150 MHz, 25 °C): δ 174.0, 172.9, 168.9, 137.9, 128.8, 128.0, 126.1, 60.5, 56.5, 51.5, 33.8, 32.6, 31.2, 31.1, 30.9, 28.8, 28.5, 28.4, 27.6, 24.4, 22.0, 13.9; LCMS, single peak, 3.384 min, ES-API: *m/z* 416.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{24}H_{37}N_3O_3Na$ $[M+Na]^+$ calc'd 438.2733, found 438.2731.



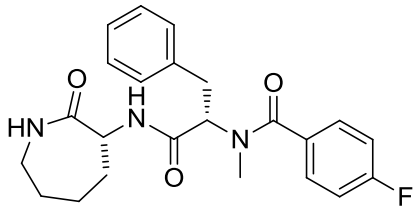
***N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)pentanamide (22c).** Coupling **41** (21 mg, 0.073 mmol) with valeroyl chloride (26 mg, 26 µL, 0.22 mmol) using method B afforded the amide **22c** as a yellow oil. Yield: 13% (3.3 mg). LCMS, single peak, 2.842 min, ES-API: *m/z* 374.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{21}H_{31}N_3O_3Na$ $[M+Na]^+$ calc'd 396.2263, found 396.2261.



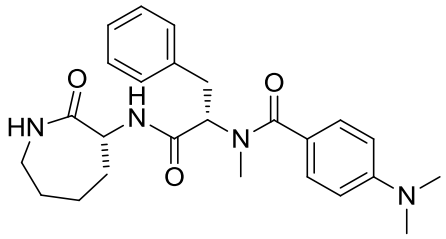
***N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)decanamide (22d).** Coupling **41** (21 mg, 0.073 mmol) with decanoyl chloride (42 mg, 45 µL, 0.22 mmol) using method B afforded the amide **22d** as a pale yellow oil. Yield: 56% (17.1 mg). LCMS, single peak, 3.731 min, ES-API: *m/z* 444.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{26}H_{41}N_3O_3Na$ $[M+Na]^+$ calc'd 466.3046, found 466.3047.



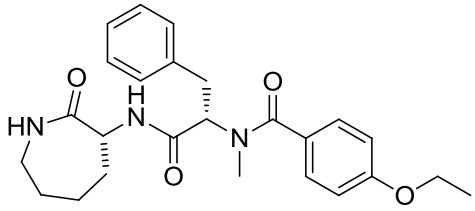
***N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)nicotinamide (22e).** Coupling **41** (21 mg, 0.073 mmol) with nicotinic acid (13 mg, 0.11 mmol) using method A afforded the amide **22e** as a pale yellow oil. Yield: 23% (8.1 mg). LCMS, single peak, 2.163 min, ES-API: *m/z* 395.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{22}H_{26}N_4O_3Na$ $[M+Na]^+$ calc'd 417.1903, found 417.1902.



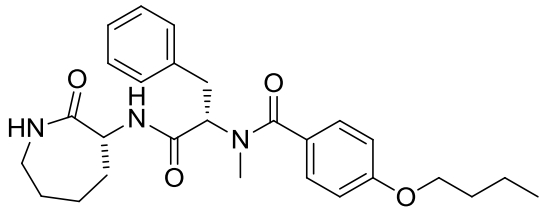
4-Fluoro-*N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (22f). Coupling **41** (21 mg, 0.073 mmol) with 4-fluorobenzoyl chloride (35 mg, 26 µL, 0.22 mmol) using method B afforded the amide **22f** as a pale yellow oil. Yield: 29% (8.6 mg). LCMS, single peak, 2.851 min, ES-API: *m/z* 412.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{23}H_{26}FN_3O_3Na$ $[M+Na]^+$ calc'd 434.1856, found 434.1852.



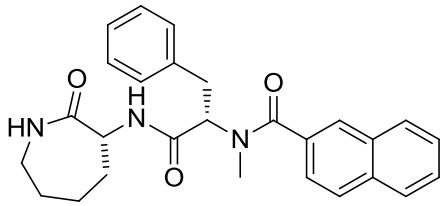
4-(Dimethylamino)-*N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (22g). Coupling **41** (21 mg, 0.073 mmol) with 4-(dimethylamino)benzoyl chloride (40 mg, 0.22 mmol) using method B afforded the amide **22g** as a brown oil. Yield: 35% (14.0 mg). LCMS, single peak, 2.492 min, ES-API: *m/z* 437.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{25}H_{32}N_4O_3Na$ $[M+Na]^+$ calc'd 459.2372, found 459.2368.



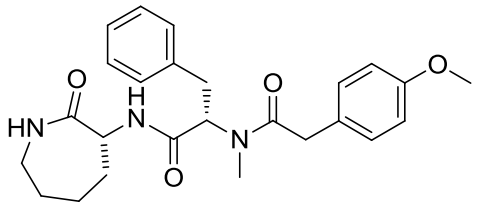
4-Ethoxy-*N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (22h). Coupling **41** (21 mg, 0.073 mmol) with 4-ethoxybenzoyl chloride (40 mg, 40 µL, 0.22 mmol) using method B afforded the amide **22h** as a pale yellow oil. Yield: 39% (12.5 mg). LCMS, single peak, 2.970 min, ES-API: *m/z* 438.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{25}H_{31}N_3O_4Na$ $[M+Na]^+$ calc'd 460.2212, found 460.2228.



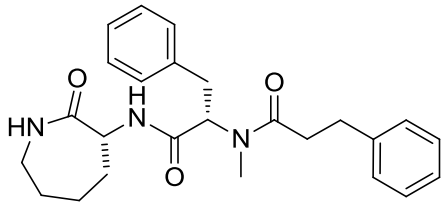
4-Butoxy-*N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (22i). Coupling **41** (21 mg, 0.073 mmol) with 4-butoxybenzoyl chloride (46 mg, 41 µL, 0.22 mmol) using method B afforded the amide **22i** as a yellow oil. Yield: 25% (8.0 mg). LCMS, single peak, 3.325 min, ES-API: *m/z* 466.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{27}H_{35}N_3O_4Na$ $[M+Na]^+$ calc'd 488.2525, found 488.2542.



***N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)-2-naphthamide (22j).** Coupling **41** (21 mg, 0.073 mmol) with 2-naphthoyl chloride (42 mg, 0.22 mmol) using method B afforded the amide **22j** as a yellow oil. Yield: 28% (8.9 mg). LCMS, single peak, 3.089 min, ES-API: *m/z* 444.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{27}H_{29}N_3O_3Na$ $[M+Na]^+$ calc'd 466.2107, found 466.2120.

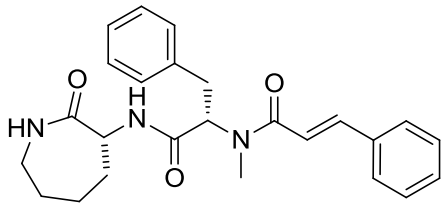


4-Methoxy-*N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)benzamide (22k). Coupling **41** (21 mg, 0.073 mmol) with 4-methoxyphenylacetyl chloride (40 mg, 33 µL, 0.22 mmol) using method B afforded the amide **22k** as a pale yellow oil. Yield: 20% (6.1 mg). LCMS, single peak, 2.840 min, ES-API: *m/z* 438.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{25}H_{31}N_3O_4Na$ $[M+Na]^+$ calc'd 460.2212, found 460.2213.

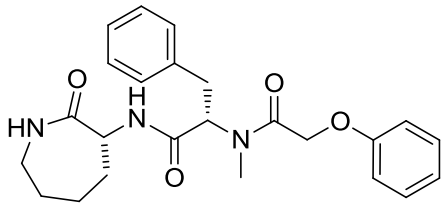


***N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)-3-phenylpropanamide (22l).** Coupling **41** (21 mg, 0.073 mmol) with hydrocinnamoyl chloride (37 mg, 32 µL, 0.22 mmol) using method B afforded the amide **22l** as a colorless oil. Yield: 47% (13.6 mg). ^{1}H NMR (DMSO-d_6, 600 MHz, 25 °C): δ 7.85 (t, J = 6.1 Hz, 1H, NH), 7.61 (d, J = 6.2 Hz, 1H, NH), 7.23 (m, 6H), 7.14 (m, 3H), 7.03 (d, J = 6.9 Hz, 1H), 4.82 (dd, J = 10.5, 4.6 Hz, 1H), 4.35 (dd, J = 10.0, 6.4 Hz, 1H), 3.17 (m, 2H), 3.09 (dd, J = 14.1, 4.6 Hz, 1H), 2.90 (dd, J = 14.1, 10.5 Hz, 1H), 2.85 (s, 3H), 2.64 (m, 2H), 2.53 (dd, J = 15.6,

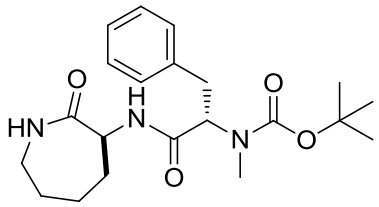
6.5 Hz, 1H), 2.43 (dd, *J* = 9.2, 6.5 Hz, 1H), 1.86 (m, 1H), 1.73 (m, 2H), 1.62 (m, 1H), 1.32 (quintet, *J* = 12.6 Hz, 1H), 1.18 (m, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz, 25 °C): δ 173.9, 172.2, 168.8, 141.2, 137.9, 129.2, 128.8, 128.2, 128.1, 126.1, 125.8, 60.2, 56.8, 51.5, 40.6, 34.4, 33.9, 30.9, 30.3, 28.8, 27.6; LCMS, single peak, 3.003 min, ES-API: *m/z* 422.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{25}H_{31}N_3O_3Na$ $[M+Na]^+$ calc'd 444.2263, found 444.2247.



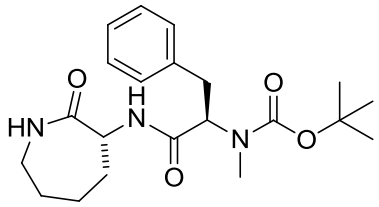
***N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)cinnamamide (22m).** Coupling **41** (21 mg, 0.073 mmol) with cinnamoyl chloride (36 mg, 0.22 mmol) using method B afforded the amide **22m** as a pale yellow oil. Yield: 43% (12.4 mg). LCMS, single peak, 2.959 min, ES-API: *m/z* 421.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{25}H_{29}N_3O_3Na$ $[M+Na]^+$ calc'd 442.2107, found 442.2120.



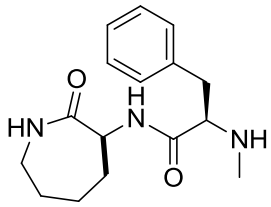
***N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)phenoxyacetamide (22n).** Coupling **41** (21 mg, 0.073 mmol) with 2-phenoxyacetyl chloride (37 mg, 30 µL, 0.22 mmol) using method B afforded the amide **22n** as a yellow oil. Yield: 57% (16.6 mg). ^{1}H NMR (DMSO-d_6, 600 MHz, 25 °C): δ 7.84 (m, 1H, NH), 7.73 (bd, J = 6.6 Hz, 1H, NH), 7.33 (m, 1H), 7.25 (m, 4H), 7.17 (t, J = 7.9 Hz, 2H), 6.88 (t, J = 7.1 Hz, 1H), 6.58 (d, J = 8.1 Hz, 1H), 6.49 (d, J = 7.9 Hz, 1H), 5.33 (dd, J = 10.6, 5.4 Hz, 1H), 4.74 (d, J = 15.6 Hz, 1H), 4.56 (d, J = 15.6 Hz, 1H), 4.39 (dd, J = 10.2, 6.8 Hz, 1H), 3.19 (dd, J = 13.9, 5.4 Hz, 1H), 3.15 (m, 1H), 3.06 (m, 1H), 2.97 (dd, J = 13.9, 10.6 Hz, 1H), 2.93 (s, 3H), 1.90 (m, 1H), 1.74 (m, 2H), 1.63 (m, 1H), 1.39 (quintet, J = 12.5 Hz, 1H), 1.20 (m, 1H); ^{13}C NMR (DMSO-d_6, 150 MHz, 25 °C): δ 173.9, 168.6, 168.0, 157.9, 137.7, 129.2, 128.9, 128.1, 126.2, 120.5, 114.3, 65.2, 56.8, 51.5, 40.6, 33.7, 30.8, 29.9, 28.8, 27.6; LCMS, single peak, 2.858 min, ES-API: *m/z* 424.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{24}H_{29}N_3O_4Na$ $[M+Na]^+$ calc'd 446.2056, found 446.2073.



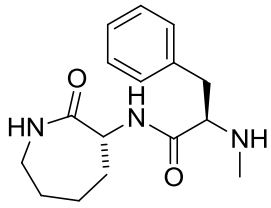
***tert*-butyl methyl((*S*)-azepan-2-one-3-ylamino-(*R*)-oxo-3-phenylpropan-2-yl)carbamate (42).** **42** was synthesized using the general procedure for amide coupling C starting from Boc-*N*-methyl-D-phenylalanine (**30**, 409 mg, 1.46 mmol) and (*S*)-3-aminoazepan-2-one (**10**, 125 mg, 0.98 mmol) to yield a yellow oil. LCMS, single peak, 3.000 min, ES-API: *m/z* 390.2 $[M+H]^+$.



***tert*-butyl methyl((*R*)-azepan-2-one-3-ylamino-(*R*)-oxo-3-phenylpropan-2-yl)carbamate (43).** **43** was synthesized using the general procedure for amide coupling C starting from Boc-*N*-methyl-D-phenylalanine (**30**, 409 mg, 1.46 mmol) and (*R*)-3-aminoazepan-2-one (125 mg, 0.98 mmol) to yield a pale yellow oil. LCMS, single peak, 2.982 min, ES-API: *m/z* 390.3 $[M+H]^+$.

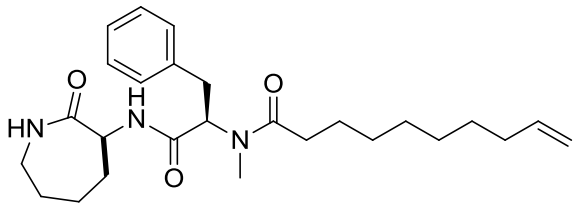


***N*-(*S*)-azepan-2-one-3-yl-(*R*)-2-methylamino-3-phenylpropanamide (44).** **44** was synthesized by the procedure used for **18** starting from **42** (380 mg, 0.98 mmol) to yield a colorless oil. 2-step yield: 38% (107 mg). $[\alpha]_D^{20}$ -25 (*c* 0.10, MeOH); R_f 0.32 (9:1 DCM/MeOH); IR (neat) 3318, 2931, 2855, 1653, 1508, 1478, 1435 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz, 25 °C): δ 7.26 (t, *J* = 7.4 Hz, 2H), 7.20 (m, 3H), 4.46 (dd, *J* = 11.3, 1.8 Hz, 1H), 3.31 (m, 1H), 3.25 (dd, *J* = 14.6, 11.5 Hz, 1H), 3.18 (bdd, *J* = 13.5, 3.8 Hz, 1H), 2.88 (m, 2H), 2.29 (s, 3H), 1.91 (dt, *J* = 14.8, 4.1 Hz, 1H), 1.80 (dd, *J* = 14.3, 4.5 Hz, 1H), 1.72 (qt, *J* = 13.5, 3.8 Hz, 1H), 1.58 (d, *J* = 13.8 Hz, 1H), 1.32 (m, 2H); ^{13}C NMR (CD_3OD, 150 MHz, 25 °C): δ 176.8, 174.7, 138.8, 130.3, 129.5, 127.7, 66.9, 53.0, 42.4, 40.5, 34.7, 32.2, 29.9, 29.0; LCMS, single peak, 1.878 min, ES-API: *m/z* 290.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{16}H_{23}N_3O_2Na$ $[M+Na]^+$ calc'd 312.1688, found 312.1691.



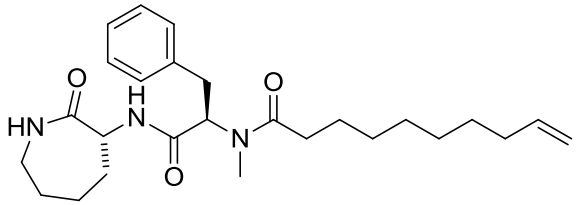
***N*-(*R*)-azepan-2-one-3-yl-(*R*)-2-methylamino-3-phenylpropanamide (45).** **45** was synthesized by the procedure used for **18** starting from **43** (380 mg, 0.98

mmol) to yield a pale yellow oil. 2-step yield: 86% (242 mg). $[\alpha]_D^{20}$ +6 (*c* 0.10, MeOH); R_f 0.32 (9:1 DCM/MeOH); IR (neat) 3318, 2931, 2855, 1653, 1508, 1478, 1435 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz, 25 °C): δ 7.27 (t, *J* = 7.4 Hz, 2H), 7.21 (m, 3H), 4.46 (dd, *J* = 11.3, 1.6 Hz, 1H), 3.26 (m, 2H), 3.20 (dd, *J* = 15.1, 5.0 Hz, 1H), 3.01 (dd, *J* = 13.7, 5.5 Hz, 1H), 2.80 (dd, *J* = 13.7, 7.7 Hz, 2H), 2.28 (s, 3H), 1.97 (dt, *J* = 14.4, 3.5 Hz, 1H), 1.83 (m, 3H), 1.45 (qd, *J* = 12.3, 2.9 Hz, 1H), 1.35 (m, 1H); ^{13}C NMR (CD_3OD, 150 MHz, 25 °C): δ 176.8, 174.9, 138.7, 130.3, 129.5, 127.8, 67.0, 53.0, 42.5, 40.3, 35.1, 32.7, 29.9, 29.0; LCMS, single peak, 1.664 min, ES-API: *m/z* 290.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{16}H_{23}N_3O_2Na$ $[M+Na]^+$ calc'd 312.1688, found 312.1688.



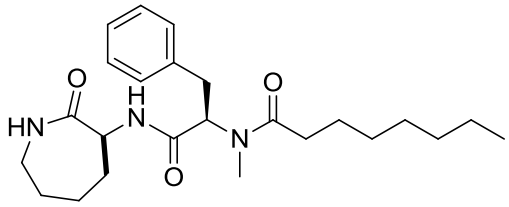
***N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*R*)-oxo-3-phenylpropan-2-yl)dec-9-enamide, (*S,R*)-Ciliatamide A (23).** **23** was synthesized by the general procedure for amide coupling A starting from **44** (50 mg, 0.17 mmol) and 9-decenoic acid (**13**, 43 mg, 47 µL, 0.25 mmol) to yield a colorless oil. Yield: 16% (12.0 mg). $[\alpha]_D^{20}$ +70 (*c* 0.05, MeOH); R_f 0.46 (9:1 DCM/MeOH); IR (neat) 3286, 2926, 2854, 1655, 1479, 1289 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz, 25 °C): δ 7.23 (m, 4H), 7.17 (t, *J* = 7.0 Hz, 1 H), 5.80 (ddt, *J* = 17.0, 10.2, 6.9 Hz, 1H), 5.42 (dd, *J* = 10.3, 5.6 Hz, 1H), 4.97 (dd, *J* = 17.0, 1.7 Hz, 1H), 4.91 (dt, *J* = 10.2, 1.7 Hz,

1H), 4.52 (dd, *J* = 11.3, 1.6 Hz, 1H), 3.33 (dd, *J* = 14.5, 5.6 Hz, 1H), 3.21 (bdd, *J* = 12.9, 2.4 Hz, 2H), 2.97 (dd, *J* = 14.5, 10.3 Hz, 1H), 2.91 (s, 3H), 2.25 (t, *J* = 7.5 Hz, 2H), 2.03 (q, *J* = 6.9 Hz, 2H), 1.99 (m, 1H), 1.89 (m, 1H), 1.83 (m, 1H), 1.77 (m, 1H), 1.58 (m, 1H), 1.49 (qd, *J* = 12.9, 2.4 Hz, 1H), 1.37 (m, 4H), 1.28 (m, 2H), 1.24 (m, 2H), 1.16 (m, 2H); ^{13}C NMR (CD_3OD, 150 MHz, 25 °C): *δ* 176.9, 176.8, 171.3, 140.1, 138.8, 130.1, 129.4, 127.6, 114.7, 59.5, 53.4, 42.5, 35.0, 34.9, 34.4, 32.8, 32.2, 30.3, 30.2, 30.1, 30.0, 29.9, 29.1, 26.0; LCMS, single peak, 3.508 min, ES-API: *m/z* 442.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{26}H_{39}N_3O_3Na$ $[M+Na]^+$ calc'd 464.2889, found 464.2903.



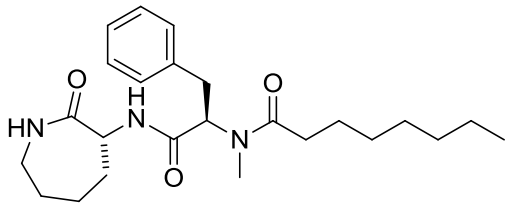
***N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*R*)-oxo-3-phenylpropan-2-yl)dec-9-enamide, (*R,R*)-Ciliatamide A (25).** **25** was synthesized by the general procedure for amide coupling A starting from **45** (50 mg, 0.17 mmol) and 9-decenoic acid (**13**, 43 mg, 47 µL, 0.25 mmol) to yield a colorless oil. Yield: 20% (15.6 mg). $[\alpha]_D^{20}$ +42 (*c* 0.05, MeOH); R_f 0.46 (9:1 DCM/MeOH); IR (neat) 3286, 2926, 2854, 1655, 1479, 1289 cm^{-1}; ^{1}H NMR (CD_3OD, 600 MHz, 25 °C): *δ* 7.23 (m, 4H), 7.17 (t, *J* = 7.1 Hz, 1 H), 5.80 (ddt, *J* = 17.0, 10.2, 6.7 Hz, 1H), 5.37 (dd, *J* = 11.0, 5.5 Hz, 1H), 4.97 (dd, *J* = 17.0, 1.2 Hz, 1H), 4.91 (dt, *J* = 10.2, 1.2 Hz, 1H), 4.53 (dd, *J* = 11.2, 1.5 Hz, 1H), 3.27 (dd, *J* = 14.6, 5.5 Hz, 1H), 3.21 (bdd, *J*

= 14.1, 2.7 Hz, 2H), 3.04 (dd, *J* = 14.6, 11.0 Hz, 1H), 2.90 (s, 3H), 2.23 (t, *J* = 7.4 Hz, 2H), 2.03 (q, *J* = 6.7 Hz, 2H), 1.93 (m, 1H), 1.99 (m, 1H), 1.83 (m, 1H), 1.78 (m, 1H), 1.60 (m, 1H), 1.51 (qd, *J* = 14.1, 2.7 Hz, 1H), 1.38 (m, 2H), 1.34 (m, 2H), 1.28 (m, 2H), 1.24 (m, 2H), 1.16 (m, 2H); ^{13}C NMR (CD_3OD, 150 MHz, 25 °C): *δ* 176.9, 176.7, 171.6, 140.1, 138.8, 130.1, 129.5, 127.7, 114.7, 59.7, 53.5, 42.5, 35.1, 34.9, 34.4, 33.1, 32.2, 30.4, 30.3, 30.2, 30.0, 29.9, 29.1, 26.1; LCMS, single peak, 3.508 min, ES-API: *m/z* 905.5 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{26}H_{39}N_3O_3Na$ $[M+Na]^+$ calc'd 464.2889, found 464.2888.



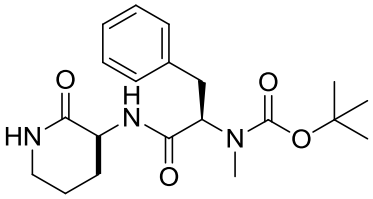
***N*-methyl-((*S*)-azepan-2-one-3-ylamino-(*R*)-oxo-3-phenylpropan-2-yl)octanamide, (*S,R*)-Ciliatamide B (26).** **26** was synthesized according to the general procedure for amide coupling B starting from amine **44** (80 mg, 0.28 mmol) and octanoyl chloride (**14**, 135 mg, 142 µL, 0.83 mmol) to yield a pale yellow oil. Yield: 33% (37.5 mg). $[\alpha]_D^{20}$ +68 (*c* 0.10, MeOH); R_f 0.44 (9:1 DCM/MeOH); IR (neat) 3286, 2928, 2855, 1656, 1479, 1288 cm^{-1}; ^{1}H NMR (CD_3CN, 600 MHz, 25 °C): *δ* 7.20 (m, 5H), 7.09 (bd, *J* = 4.1 Hz, 1H, NH), 6.41 (bs, 1H, NH), 5.37 (dd, *J* = 10.3, 5.4 Hz, 1H), 4.36 (ddd, *J* = 11.1, 5.7, 1.2 Hz, 1H), 3.24 (dd, *J* = 14.6, 5.7 Hz, 1H), 3.15 (m, 2H), 2.89 (dd, *J* = 14.6, 10.3 Hz, 1H), 2.83 (s, 3H), 2.17 (m, 2H), 1.95 (m, 1H), 1.89 (m, 2H), 1.77 (m, 1H), 1.70

(m, 1H), 1.38 (quintet, *J* = 7.5 Hz, 2H), 1.33 (m, 1H), 1.26 (m, 2H), 1.21 (m, 4H), 1.14 (m, 2H), 0.87 (t, *J* = 7.2, 3H); ^{13}C NMR (CD_3CN, 150 MHz, 25 °C): *δ* 175.6, 174.8, 170.1, 139.3, 129.9, 129.2, 127.2, 58.3, 53.0, 42.1, 34.3, 34.0, 32.5, 32.2, 32.0, 29.83, 29.79, 29.7, 28.8, 25.7, 23.3, 14.4; LCMS, single peak, 3.353 min, ES-API: *m/z* 416.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{24}H_{37}N_3O_3Na$ $[M+Na]^+$ calc'd 438.2733, found 438.2724.

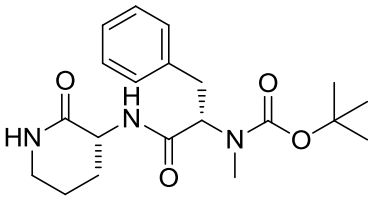


***N*-methyl-((*R*)-azepan-2-one-3-ylamino-(*R*)-oxo-3-phenylpropan-2-yl)octanamide, (*R,R*)-Ciliatamide B (28).** **28** was synthesized according to the general procedure for amide coupling B starting from amine **45** (82 mg, 0.28 mmol) and octanoyl chloride (**14**, 138 mg, 146 µL, 0.85 mmol) to yield a colorless oil. Yield: 44% (50.9 mg). $[\alpha]_D^{20}$ +49 (*c* 0.10, MeOH); R_f 0.44 (9:1 DCM/MeOH); IR (neat) 3286, 2928, 2855, 1656, 1479, 1288 cm^{-1}; ^{1}H NMR (CD_3CN, 600 MHz, 25 °C): *δ* 7.22 (m, 5H), 7.09 (bd, *J* = 4.8 Hz, 1 H, NH), 6.41 (bs, 1H, NH), 5.37 (dd, *J* = 10.4, 5.6 Hz, 1H), 4.39 (ddd, *J* = 11.3, 5.9, 1.5 Hz, 1H), 3.32 (dd, *J* = 14.7, 5.6 Hz, 1H), 3.15 (m, 2H), 2.91 (dd, *J* = 14.7, 10.4 Hz, 1H), 2.82 (s, 3H), 2.18 (m, 2H), 1.97 (m, 1H), 1.89 (m, 2H), 1.77 (m, 1H), 1.71 (m, 1H), 1.38 (quintet, *J* = 7.5 Hz, 2H), 1.31 (m, 1H), 1.27 (m, 2H), 1.21 (m, 4H), 1.14 (m, 2H), 0.87 (t, *J* = 7.0, 3H); ^{13}C NMR (CD_3CN, 150 MHz, 25 °C): *δ* 175.4, 174.8, 169.9,

139.3, 129.9, 129.2, 127.2, 58.2, 53.0, 42.1, 34.2, 33.9, 32.45, 32.43, 31.8, 29.81, 29.79, 29.7, 28.8, 25.8, 23.3, 14.4; LCMS, single peak, 3.351 min, ES-API: *m/z* 853.5 $[M_2+Na]^+$; HRMS (TOF, ES+) $C_{24}H_{37}N_3O_3Na$ $[M+Na]^+$ calc'd 438.2733, found 438.2719.

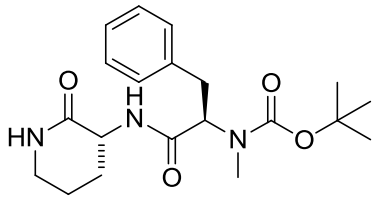


***tert*-butyl methyl((*S*)-piperid-2-one-3-ylamino-(*R*)-oxo-3-phenylpropan-2-yl)carbamate (31).** **31** was synthesized by amide coupling procedure D starting from Boc-*N*-methyl-D-phenylalanine (**30**, 30 mg, 0.11 mmol) and (*S*)-3-aminopiperid-2-one (**11**, 16 mg, 0.11 mmol) to yield a pale yellow oil. LCMS, single peak, 2.747 min, ES-API: *m/z* 376.3 $[M+H]^+$.

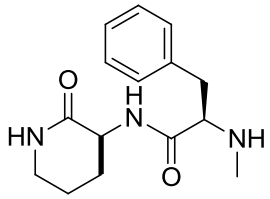


***tert*-butyl methyl((*R*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)carbamate (32).** **32** was synthesized using amide coupling procedure D starting from Boc-*N*-methyl-L-phenylalanine (**15**, 419 mg, 1.50 mmol) and (*R*)-3-

aminopiperid-2-one (**29**, 226 mg, 1.50 mmol) to yield a pale yellow oil. LCMS, single peak, 2.742 min, ES-API: *m/z* 376.3 $[M+H]^+$.

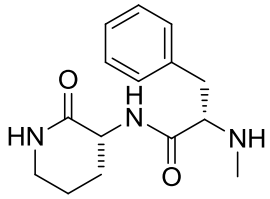


***tert*-butyl methyl((*R*)-piperid-2-one-3-ylamino-(*R*)-oxo-3-phenylpropan-2-yl)carbamate (33).** **33** was synthesized using amide coupling procedure D starting from **30** (336 mg, 1.20 mmol) and **29** (181 mg, 1.20 mmol) to yield a pale yellow oil. LCMS, single peak, 2.740 min, ES-API: *m/z* 376.3 $[M+H]^+$.



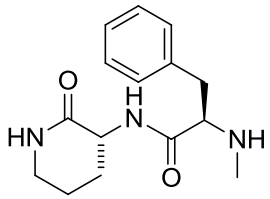
***N*-(*S*)-piperid-2-one-3-yl-(*R*)-2-methylamino-3-phenylpropanamide (34).** A solution of **31** (40 mg, 0.11 mmol) in anhydrous CH_2Cl_2 (30 mM) was cooled in an ice bath before slowly adding a solution of TFA for a final concentration of 5% TFA (v/v). The solution was stirred for 7 hours while cooling in an ice bath. The mixture was co-evaporated with MeOH (3 × 10 mL) before loading onto a Varian Bond Elut 10 g SCX cartridge in minimal CH_2Cl_2. The cartridge was washed with 25 mL each of CH_2Cl_2 then MeOH and was then eluted with 2 M NH_3 in MeOH,

and these elution fractions were concentrated by rotary evaporation to a colorless oil. 2-step yield: 73% (21.4 mg). $[\alpha]_D^{20}$ -36 (*c* 0.25, MeOH); R_f 0.17 (9:1 DCM/MeOH); IR (neat) 3281, 3225, 2921, 2866, 1644, 1552, 1492, 1325 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz, 25 °C): δ 7.27 (t, *J* = 7.7 Hz, 2H), 7.21 (m, 3H), 4.30 (dd, *J* = 10.6, 5.9 Hz, 1H), 3.37 (t, *J* = 7.4 Hz, 1H), 3.21 (m, 2H), 2.93 (m, 2H), 2.40 (s, 3H), 1.82 (m, 1H), 1.74 (m, 2H), 1.40 (m, 1H); ^{13}C NMR (CD_3OD, 150 MHz, 25 °C): δ 174.1, 172.4, 138.1, 130.5, 129.6, 127.9, 66.5, 50.4, 42.7, 40.0, 34.2, 28.6, 21.9; LCMS, single peak, 1.732 min, ES-API: *m/z* 276.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{15}H_{21}N_3O_2Na$ $[M+Na]^+$ calc'd 298.1531, found 298.1534.

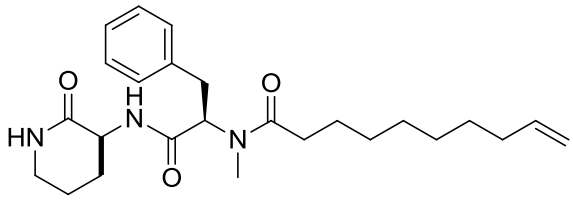


***N*-(*R*)-piperid-2-one-3-yl-(*S*)-2-methylamino-3-phenylpropanamide (35).** **35** was synthesized by the procedure used for **34** starting from **32** (563 mg, 1.50 mmol) to yield a colorless oil. 2-step yield: 43% (177 mg). $[\alpha]_D^{20}$ +51 (*c* 0.25, MeOH); R_f 0.17 (9:1 DCM/MeOH); IR (neat) 3281, 3225, 2921, 2866, 1644, 1552, 1492, 1325 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz, 25 °C): δ 7.26 (t, *J* = 7.5 Hz, 2H), 7.21 (m, 3H), 4.28 (dd, *J* = 10.5, 6.0 Hz, 1H), 3.25 (t, *J* = 7.4 Hz, 1H), 3.21 (dd, *J* = 9.4, 5.8 Hz, 2H), 2.88 (d, *J* = 7.4 Hz, 2H), 2.33 (s, 3H), 1.83 (m, 1H), 1.74 (m, 2H), 1.41 (m, 1H); ^{13}C NMR (CD_3OD, 150 MHz, 25 °C): δ 175.5, 172.5, 138.8, 130.4, 129.5, 127.7, 67.0, 50.4, 42.7, 40.5, 34.6, 28.6, 21.9; LCMS, single

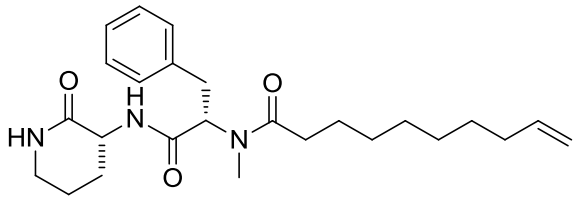
peak, 1.733 min, ES-API: *m/z* 276.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{15}H_{21}N_3O_2Na$ $[M+Na]^+$ calc'd 298.1531, found 298.1528.



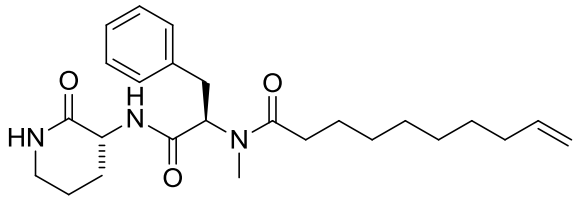
***N*-(*R*)-piperid-2-one-3-yl-(*R*)-2-methylamino-3-phenylpropanamide (36).** **36** was synthesized starting from **33** (451 mg, 1.20 mmol) in anhydrous CH_2Cl_2 (30 mM) cooled in an ice bath followed by a slow addition of a solution of TFA for a final concentration of 7% TFA (v/v) with continued stirring in an ice bath for 7 h. Compound **36** was purified by the same procedure used for **34** to yield a colorless oil. 2-step yield: 67% (222 mg). $[\alpha]_D^{20}$ -1 (*c* 0.25, MeOH); R_f 0.16 (9:1 DCM/MeOH); IR (neat) 3281, 3225, 2921, 2866, 1644, 1552, 1492, 1325 cm^{-1}; ^1H NMR (CD_3OD, 600 MHz, 25 °C): δ 7.27 (t, *J* = 7.4 Hz, 2H), 7.22 (m, 3H), 4.14 (dd, *J* = 11.1, 6.2 Hz, 1H), 3.32 (m, 1H), 3.26 (m, 2H), 3.00 (dd, *J* = 13.6, 6.2 Hz, 2H), 2.85 (dd, *J* = 13.6, 7.0 Hz, 2H), 2.32 (s, 3H), 1.99 (m, 1H), 1.88 (m, 1H), 1.81 (m, 1H), 1.72 (qd, *J* = 11.8, 3.5 Hz, 1H); ^{13}C NMR (CD_3OD, 150 MHz, 25 °C): δ 175.2, 172.5, 138.5, 130.5, 129.5, 127.8, 66.6, 51.0, 42.7, 40.1, 34.7, 28.6, 22.3; LCMS, single peak, 1.706 min, ES-API: *m/z* 276.2 $[M+H]^+$; HRMS (TOF, ES+) $C_{15}H_{21}N_3O_2Na$ $[M+Na]^+$ calc'd 298.1531, found 298.1538.



***N*-methyl-((*S*)-piperid-2-one-3-ylamino-(*R*)-oxo-3-phenylpropan-2-yl)dec-9-enamide, (*S,R*)-Ciliatamide C (37).** **37** was synthesized by the general procedure for amide coupling D starting from **34** (16 mg, 0.057 mmol) and 9-decenoic acid (**13**, 10.6 µL, 0.06 mmol) to yield a pale yellow oil. Yield: 81% (20.1 mg). $[\alpha]_D^{20}$ +43 (*c* 0.10, MeOH); R_f 0.44 (9:1 DCM/MeOH); IR (neat) 3274, 2926, 2854, 1655, 1527, 1492 cm^{-1}; ^1H NMR (CD_3CN, 600 MHz, 25 °C): δ 7.20 (m, 5H), 6.70 (bd, *J* = 7.1 Hz, 1H, NH), 6.10 (bs, 1H, NH), 5.83 (ddt, *J* = 17.1, 10.3, 6.8 Hz, 1H), 5.40 (dd, *J* = 10.5, 5.5 Hz, 1H), 5.00 (dd, *J* = 17.1, 1.6 Hz, 1H), 4.92 (dt, *J* = 10.3, 1.6 Hz, 1H), 4.21 (dt, *J* = 12.1, 6.8 Hz, 1H), 3.27 (dd, *J* = 14.7, 5.5 Hz, 1H), 3.20 (m, 2H), 2.88 (m, 1H), 2.87 (s, 3H), 2.17 (td, *J* = 7.4, 4.4 Hz, 2H), 2.11 (m, 1H), 2.03 (m, 2H), 1.83 (m, 2H), 1.60 (qd, *J* = 12.1, 5.7 Hz, 1H), 1.33 (m, 4H), 1.26 (m, 2H), 1.22 (m, 2H), 1.11 (m, 2H); ^{13}C NMR (CD_3CN, 150 MHz, 25 °C): δ 175.5, 171.9, 171.8, 140.9, 140.0, 130.6, 129.8, 127.8, 115.3, 58.8, 51.2, 43.0, 35.2, 35.1, 34.6, 32.5, 30.6, 30.4, 30.31, 30.28, 29.1, 26.2, 22.9; LCMS, single peak, 3.300 min, ES-API: *m/z* 428.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{25}H_{37}N_3O_3Na$ $[M+Na]^+$ calc'd 450.2733, found 450.2729.



***N*-methyl-((*R*)-piperid-2-one-3-ylamino-(*S*)-oxo-3-phenylpropan-2-yl)dec-9-enamide, (*R,S*)-Ciliatamide C (38).** **38** was synthesized by the general procedure for amide coupling D starting from **35** (156 mg, 0.57 mmol) and 9-decenoic acid (**13**, 105 µL, 0.56 mmol) to yield a colorless oil. Yield: 80% (193 mg). $[\alpha]_D^{20}$ –55 (*c* 0.10, MeOH); R_f 0.44 (9:1 DCM/MeOH); IR (neat) 3274, 2926, 2854, 1655, 1527, 1492 cm^{-1}; ^1H NMR (CD_3CN, 600 MHz, 25 °C): δ 7.20 (m, 5H), 6.70 (bd, *J* = 6.9 Hz, 1H, NH), 6.11 (bs, 1H, NH), 5.83 (ddt, *J* = 17.1, 10.3, 6.7 Hz, 1H), 5.40 (dd, *J* = 10.5, 5.5 Hz, 1H), 5.00 (dd, *J* = 17.1, 1.5 Hz, 1H), 4.92 (dt, *J* = 10.3, 1.5 Hz, 1H), 4.21 (dt, *J* = 11.8, 6.8 Hz, 1H), 3.27 (dd, *J* = 14.6, 5.5 Hz, 1H), 3.20 (m, 2H), 2.89 (m, 1H), 2.87 (s, 3H), 2.17 (td, *J* = 7.4, 4.0 Hz, 2H), 2.10 (m, 1H), 2.03 (m, 2H), 1.83 (m, 2H), 1.60 (qd, *J* = 11.8, 5.3 Hz, 1H), 1.33 (m, 4H), 1.26 (m, 2H), 1.22 (m, 2H), 1.11 (m, 2H); ^{13}C NMR (CD_3CN, 150 MHz, 25 °C): δ 174.8, 171.3, 171.2, 140.2, 139.3, 130.0, 129.1, 127.2, 114.7, 58.2, 50.6, 42.4, 34.5, 34.4, 33.9, 31.9, 29.9, 29.8, 29.7, 29.6, 28.5, 25.6, 22.2; LCMS, single peak, 3.304 min, ES-API: *m/z* 428.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{25}H_{37}N_3O_3Na$ $[M+Na]^+$ calc'd 450.2733, found 450.2737.



***N*-methyl-((*R*)-piperid-2-one-3-ylamino-(*R*)-oxo-3-phenylpropan-2-yl)dec-9-enamide, (*R,R*)-Ciliatamide C (39).** **39** was synthesized by the general procedure for amide coupling D starting from **36** (185 mg, 0.67 mmol) and 9-decenoic acid (**13**, 124 µL, 0.67 mmol) to yield a pale yellow oil. Yield: 98% (283 mg). $[\alpha]_D^{20}$ +56 (*c* 0.10, MeOH); R_f 0.44 (9:1 DCM/MeOH); IR (neat) 3274, 2926, 2854, 1655, 1527, 1492 cm^{-1}; ^1H NMR (CD_3CN, 600 MHz, 25 °C): *δ* 7.20 (m, 5H), 6.77 (bs, 1H, NH), 6.18 (bs, 1H, NH), 5.83 (ddt, *J* = 17.0, 10.3, 6.7 Hz, 1H), 5.38 (dd, *J* = 10.7, 5.3 Hz, 1H), 4.99 (dd, *J* = 17.0, 1.7 Hz, 1H), 4.92 (dt, *J* = 10.3, 1.7 Hz, 1H), 4.16 (dt, *J* = 11.7, 6.6 Hz, 1H), 3.26 (dd, *J* = 14.7, 5.3 Hz, 1H), 3.21 (m, 2H), 2.88 (dd, *J* = 14.7, 10.7 Hz, 1H), 2.81 (s, 3H), 2.17 (m, 2H), 2.13 (m, 1H), 2.02 (m, 2H), 1.83 (m, 2H), 1.64 (qd, *J* = 11.7, 5.1 Hz, 1H), 1.33 (m, 4H), 1.26 (m, 2H), 1.22 (m, 2H), 1.11 (m, 2H); ^{13}C NMR (CD_3CN, 150 MHz, 25 °C): *δ* 174.8, 171.13, 171.10, 140.2, 139.4, 129.9, 129.2, 127.2, 114.7, 58.4, 50.9, 42.4, 34.43, 34.39, 33.9, 32.0, 29.9, 29.8, 29.7, 29.6, 28.4, 25.6, 22.1; LCMS, single peak, 3.301 min, ES-API: *m/z* 428.3 $[M+H]^+$; HRMS (TOF, ES+) $C_{25}H_{37}N_3O_3Na$ $[M+Na]^+$ calc'd 450.2733, found 450.2733.

CHAPTER III

TOTAL SYNTHESIS AND BIOLOGICAL EVALUATION OF 8-*EPI*-LUCENTAMYCIN A AND ANALOGS

Background

Recently, Fenical and co-workers reported on the isolation and characterization of four novel 3-methyl-4-ethylideneproline-containing peptides, Lucentamycins A-D (**46-49**), from the fermentation broth of a marine-derived actinomycete identified as *Nocardiopsis lucentensis* (**Figure 5**).[8] Importantly, Lucentamycins A (**46**) and B (**47**) displayed significant in vitro cytotoxicity, with IC_{50} values of 0.2 μM and 11 μM, respectively, against HCT-116 human colon carcinoma.[8]

The structures of **46-49** were determined by a combination of 1D and 2D NMR techniques in addition to an advanced Marfey's analysis. For **46**, interpretation of the data from the ^{1}H NMR, ^{13}C NMR, HSQC, COSY, TOCSY, and HMBC led to the determination of the presence and connectivity of leucine, homoarginine, benzoic acid, and the unprecedented 3-methyl-4-ethylideneproline moieties. ROESY techniquies were employed to determine the relative stereochemistry of the substitutions on the proline unit. ROESY correlations were used to determine the *Z* geometry of the olefin. The correlations from the methyl proton to H-9 and from H-9 to the α-proton were used to determine the *cis* configuration of the proline residue. Marfey's analysis of the acid hydrolysates

was then used to determine the absolute stereochemistry as (2*S*,3*R*)-3-methyl-4-ethylideneproline.

Based on the biological activity of Lucentamycin A and the broad-spectrum of biological activity (antibiotic, antifungal, kinase inhibition) of other agents derived from Nocardiopsis,[8-11] a total synthesis campaign targeting Lucentamycin A (**46**) to provide sufficient material for biological evaluation seemed warranted.[12] Moreover, the Lucentamycins were attractive as a target for our program in the synthesis of unnatural analogs coupled with biological evaluation and target elucidation.[13-17]

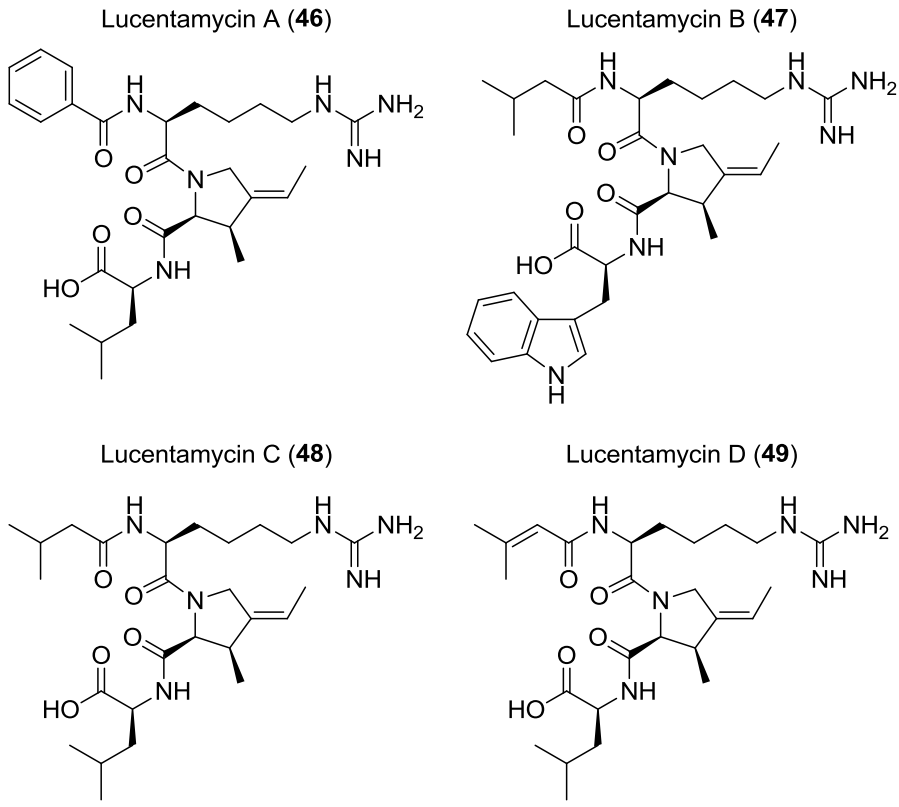
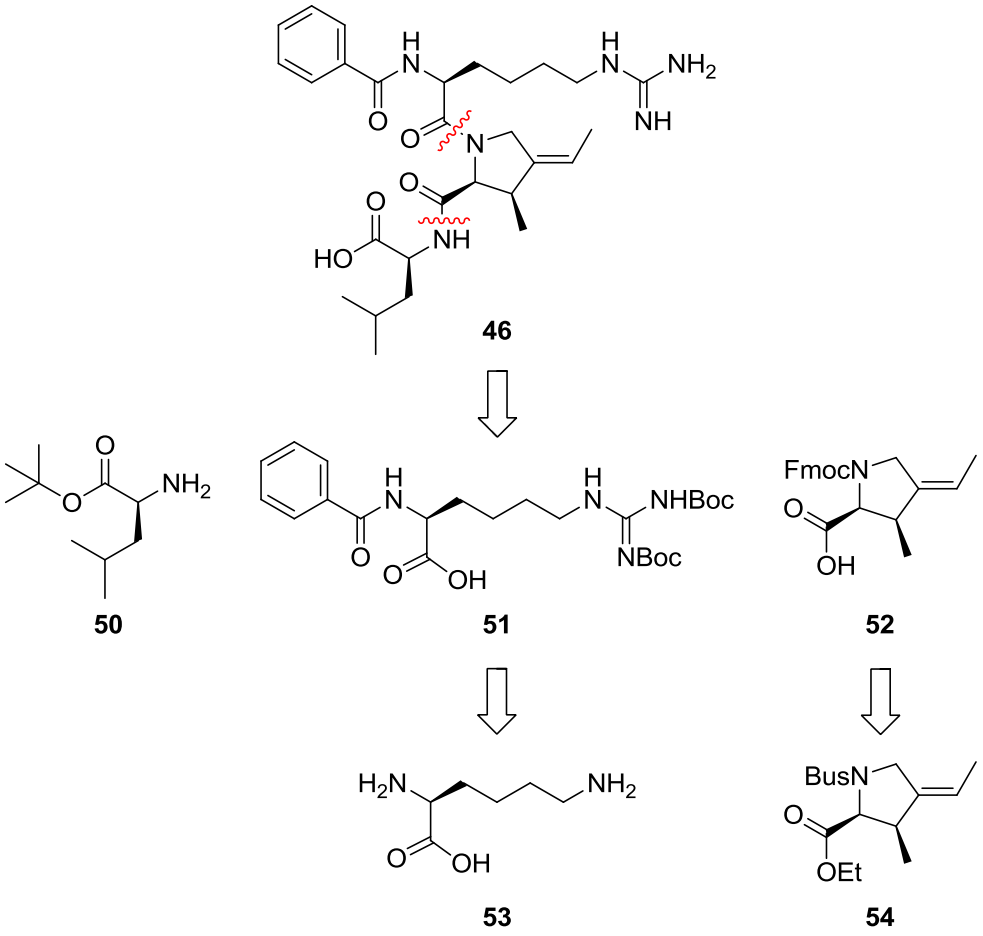


Figure 5. Structures of Lucentamycins A-D (**46**-**49**)

Retrosynthesis

The retro-synthesis of Lucentamycin A (**46**) involved cleavage of the two amide bonds of the non-proteogenic amino acid, 3-methyl-4-ethylideneproline nucleus to afford L-leucine *tert*-butyl ester (**50**), the functionalized lysine (**51**), and unnatural proline (**52**) (**Scheme 5**). The functionalized lysine was envisioned to arise by acylation and guanidation of L-lysine (**53**). The key non-proteogenic 3-methyl-4-ethylideneproline (**54**) would be accessed through chiral vinyl aminosulfoxonium salt chemistry as reported by Gais, for which a single X-ray crystal of **54** was disclosed.[18]

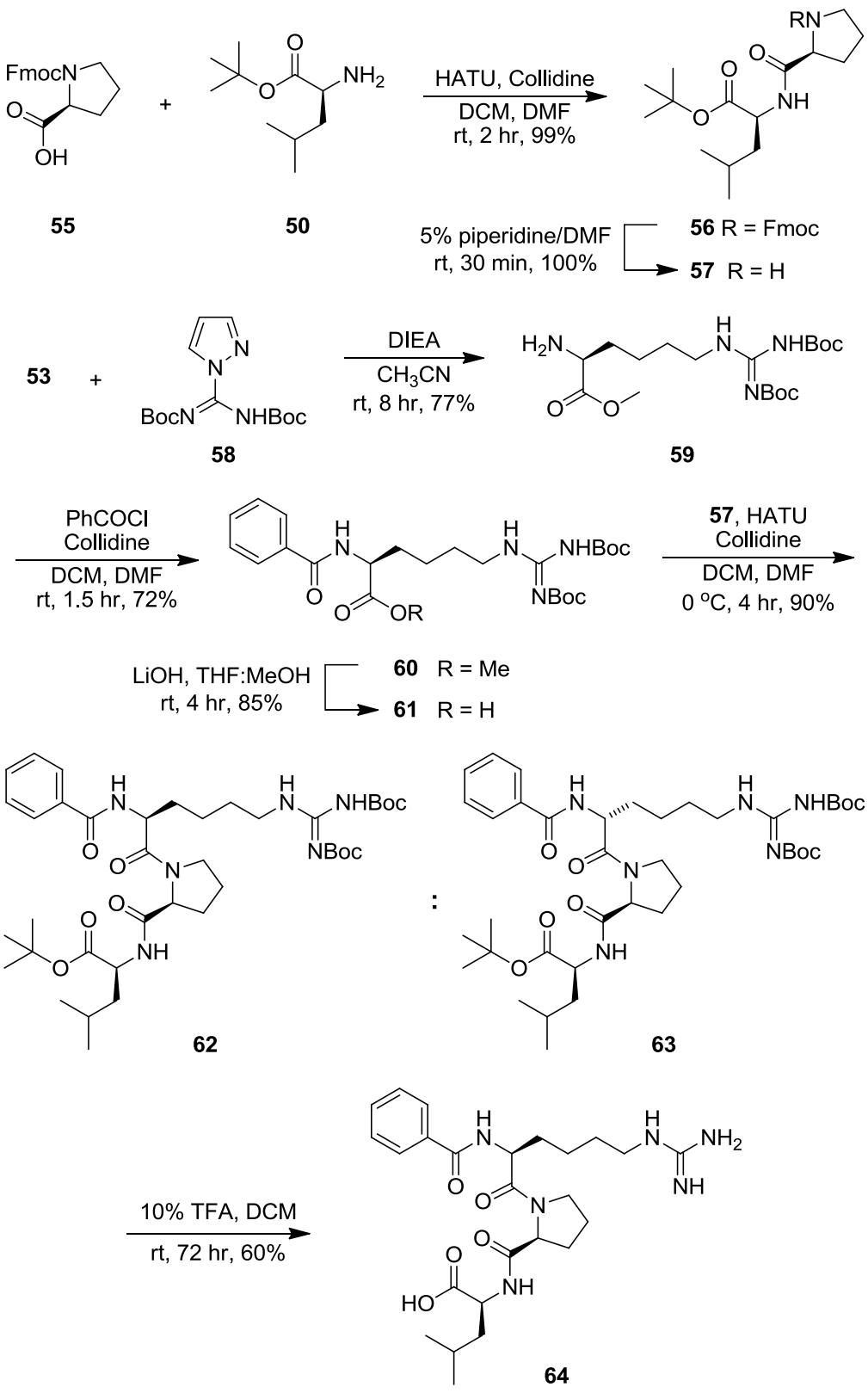


Scheme 5. Retrosynthesis of Lucentamycin A (**46**)

Model System Study

While synthetic effort was focused on the synthesis of **54**, we initiated a model study en route to an unnatural congener (**64**) of Lucentamycin A, wherein the non-proteogenic 3-methyl-4-ethylideneproline **54** was replaced with natural L-proline (**Scheme 6**) in order to evaluate potential racemization problems and to develop structure-activity-relationships for the cytotoxicity of **46** against HCT-116 cells. To begin the synthesis, Fmoc-protected L-proline (**55**) was coupled under HATU conditions with leucine *tert*-butyl ester **50** to provide the dipeptide (**56**) in 99% yield. Standard Fmoc deprotection with 5% piperidine in DMF delivered the key lower peptide (**57**) in quantitative yield. L-lysine **53** was treated with N,N'-Di-Boc-1H-pyrazole-1-carboxamidine (**58**) to provide the bis-*N*-Boc-protected arginine derivative (**59**) in 77% yield.[19] Acylation with benzoyl chloride and hydrolysis employing LiOH afforded the northern fragment (**61**) in 62% yield over the two steps. The coupling of peptide **57** to peptide **61** justified the model study, as our initial EDCI/HOBt/collidine coupling conditions generated >90% chemical yield, but a 39:61 ratio of the desired coupled product (**62**) to the epimerized coupled product (**63**). The degree of racemization was determined by analytical LCMS and confirmed by ^{1}H NMR. At this point, we evaluated a variety of coupling reagents, additives and solvent/temperature conditions. These conditions included EDCI/HOBt/collidine, as discussed, TFFH/HOAt/collidine, and HATU/collidine with or without HOAt as an additive, at various temperatures. Ultimately, the conditions of HATU and collidine without HOAt proved optimal, generating a 92:8 ratio of **62**:**63** in yields exceeding 90% and readily separable

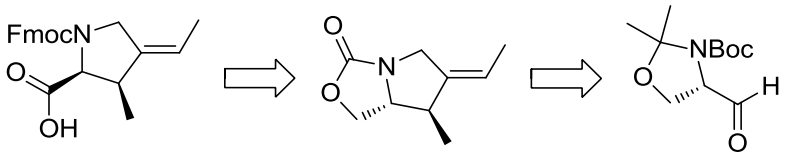
by column chromatography. A final global deprotection with 10% TFA in DCM afforded the unnatural analog (**64**) of Lucentamycin A in 60% yield. We then evaluated its affect on an HCT-116 cell line in order to determine if the stereochemistry and functionality of the non-proteogenic 3-methyl-4-ethylideneproline nucelus was critical for biological activity. Thus, a standard 48 hour cell viability assay was performed with **64** at five concentrations (0, 0.025 μM, 0.1 μM, 0.4 μM, 2.0 μM and 10 μM) relative to podophyllotoxin as a positive control.[20] The control performed as expected, providing an IC_{50} value of 0.03 μM. However, the unnatural, Lucentamycin A analog (**64**) had no affect on HCT-116 cell viability up to 10 μM. These data suggest that the the topology afforded by the natural product is essential for potent *in vitro* cytotoxicity (IC_{50} = 0.2 μM) of Lucentamycin A (**46**) against HCT-116 cells.



Scheme 6. Lucentamycin A model study to deliver model system (**64**)

Synthesis of Non-Proteogenic Core

Attention now turned to the construction of the key non-proteogenic 3-methyl-4-ethylideneproline (**52**). Application of the Gais protocol proved arduous, with difficult E/Z mixtures at multiple points along the 11 step sequence, ultimately resulting in complex chromatographic separations and even sophisticated reverse phase systems failed to deliver key intermediates in yields satisfactory for carrying forward en route to a total synthesis of **46**. At this point, we revised our retrosynthesis for **52**, and we envisioned access to **52** by a titanium-mediated cycloisomerization reaction[21] (**Scheme 7**) to afford bicycle (**65**), which would be derived from Garner's aldehyde (**66**). Key to the success of this route would be the ability to epimerize the α-carbon; however, this route would also enable the synthesis of the C8 epimer of Lucentamycin A, 8-*epi*-lucentamycin A, and further construct SAR.

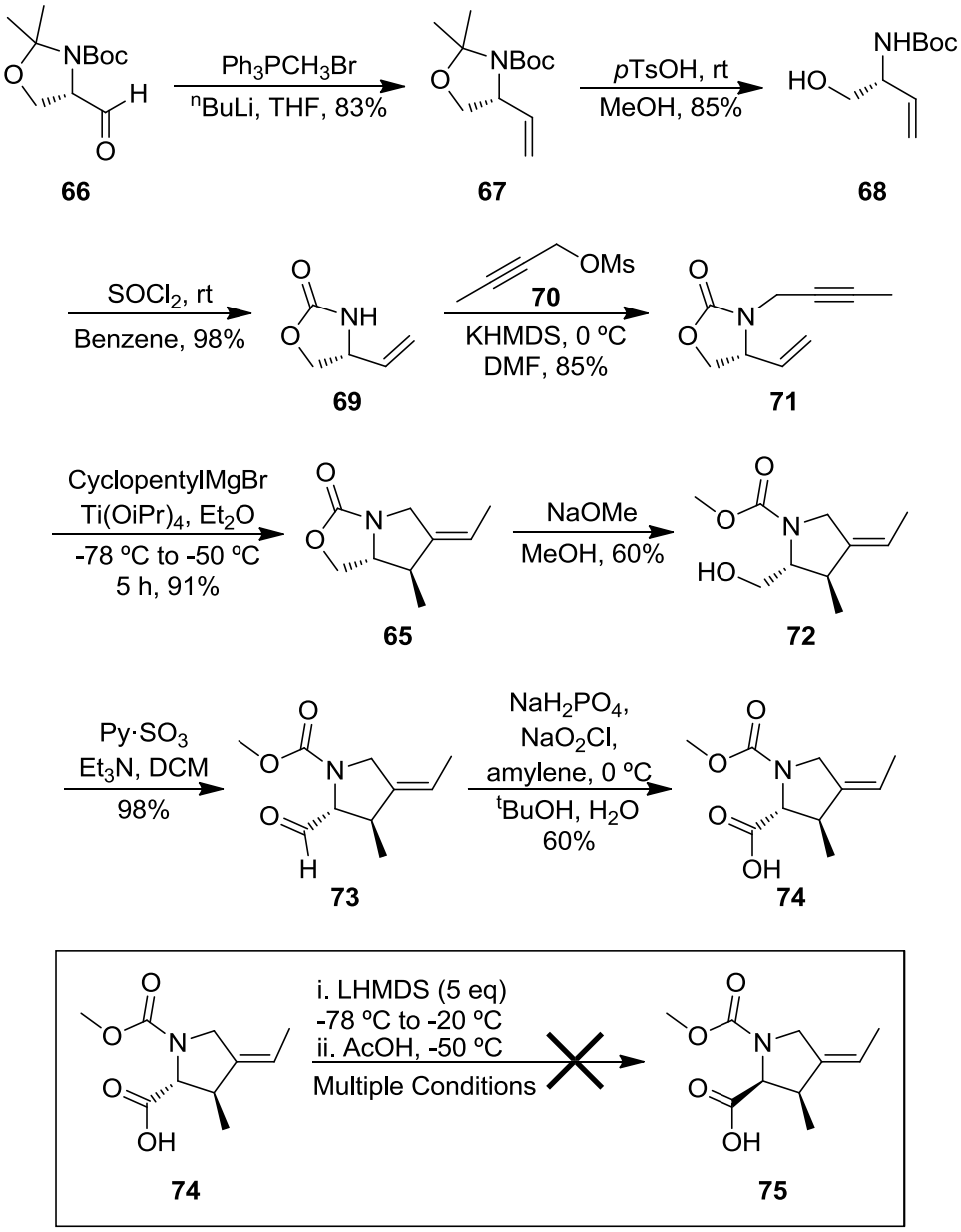


52 **65** **66**

Scheme 7. Revised retrosynthesis of non-proteogenic core (**52**)

To begin the synthesis of proline **52**, Garner's aldehyde (**66**) smoothly undergoes a Wittig reaction providing olefin (**67**) in 83% yield (**Scheme 8**).[22] Deprotection with *p*-TsOH in MeOH affords alcohol (**68**), which is then converted to the corresponding oxazolidin-2-one (**69**) in 83% yield for the two steps.

Deprotonation with KHMDS and alkylation with propargyl mesylate (**70**) delivers ene-yne (**71**). A titanium-mediated cycloisomerization reaction delivers bicycle (**65**), which sets the methyl stereocenter and the relative regiochemistry of the 3-methyl-4-ethylideneproline nucleus in 91% yield.[21] Detailed nOe studies and historical accounts confirmed the stereochemical assignment. Opening of the cyclic carbamate with methoxide generates methyl carbamate (**72**), which is oxidized to the aldehyde (**73**) in 98% yield. Finally, a buffered bleach oxidation of **73** leads to the carboxylic acid (**74**), the epimer of the key non-proteogenic 3-methyl-4-ethylideneproline (**52**) in 60% yield. A double deprotonation/kinetic quench with AcOH failed to provide the desired carboxylic acid (**75**), affording only starting material **73** with no evidence of any epimerization. Alternative approaches to epimerize **73** met with similar unproductive results.[23] Thus, our strategy adjusted to target the synthesis and biological evaluation of 8-*epi*-Lucentamycin A employing **73**. However, removal of the methyl carbamate in **73** proved equally challenging, and we were unable to affect this key transformation under a variety of reaction conditions. These conditions included TMSI/DCM, TMSI/MP·Carbonate/DCM, sulphur anion nucleophiles in THF with different concentrations of HMPA. Other conditions tested were, different bases ($Ba(OH)_2$, LiOH, ^{n}BuLi), as well as different acids (TFA, HCl). All of these conditions led either to decomposition or no reaction.

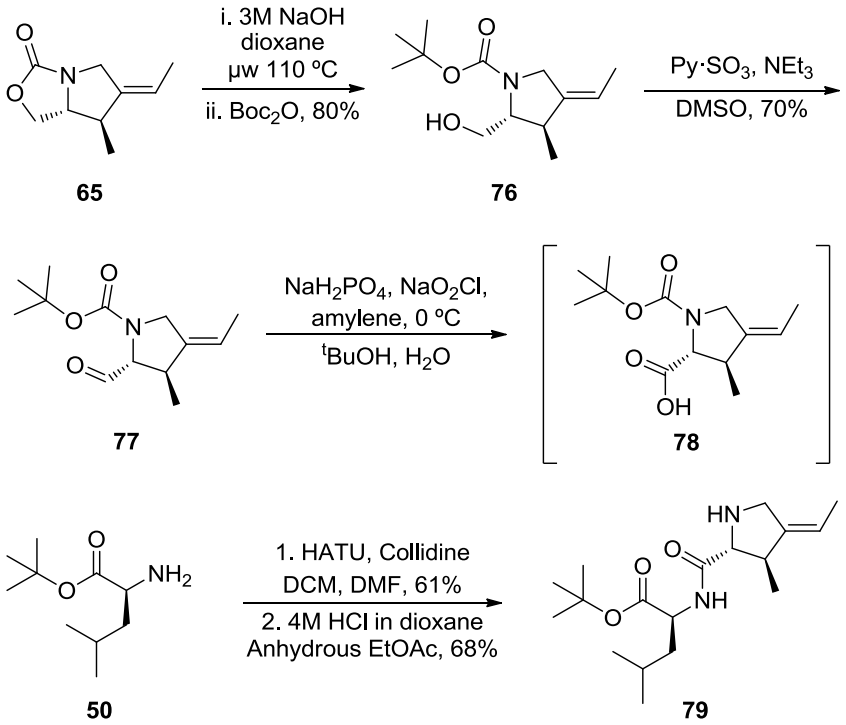


SCHEME 8. Synthesis of an epimeric non-proteogenic 3-methyl-4-ethylideneproline (**74**) and attempts to epimerize to deliver desired carboxylic acid (**75**)

Total Synthesis of 8-*epi*-Lucentamycin A

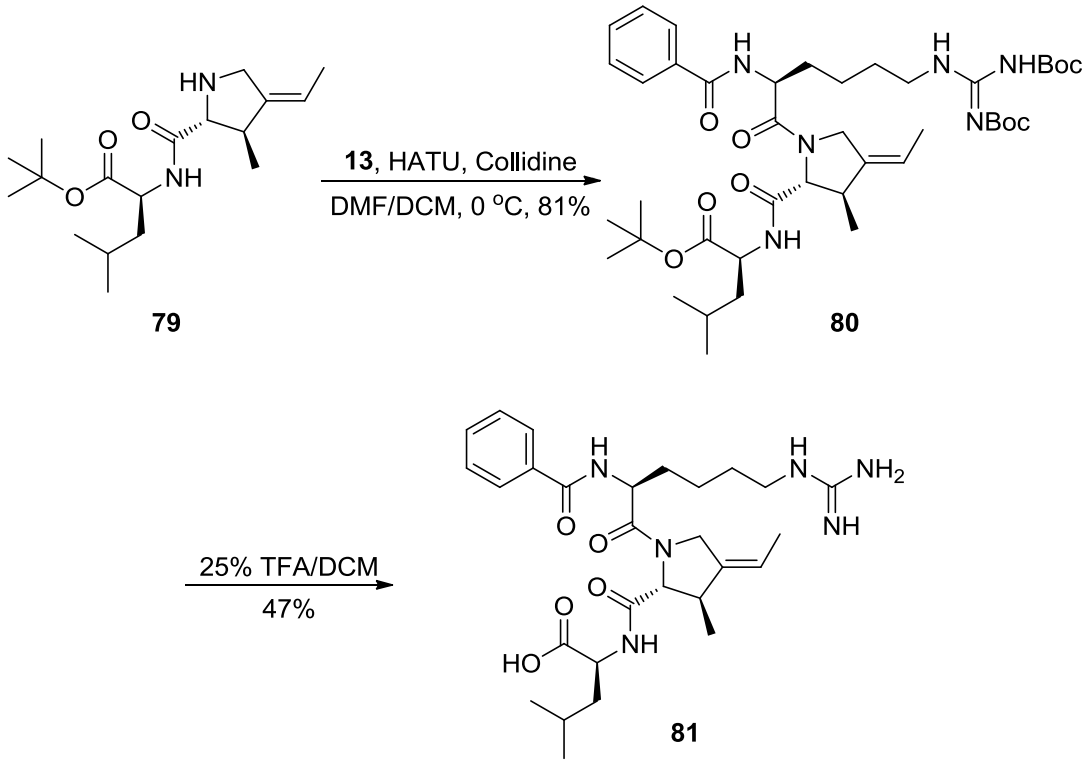
Based on these results, we again modified our approach for the total synthesis of 8-epi-Lucentamycin A. As shown in (**Scheme 9**), bicycle (**65**) was

treated in a single pot with 3.0 M NaOH under microwave irradiation, followed by an *in situ* protection of the secondary amine to deliver Boc-protected pyrrolidine (**76**) in 80% yield (**Scheme 9**).[24] Alcohol **76** smoothly underwent oxidation to the corresponding aldehyde (**77**) in 70% yield. Repetition of the buffered bleach oxidation of **77** provided carboxyclic acid (**78**), the epimer of the key non-proteogenic 3-methyl-4- ethylideneproline (**52**). The reaction delivered acid **78** proceeded cleanly, therefore crude material (>95% pure) was carried forward into the coupling step. The crude **78** was directly coupled to L-leucine *tert*-butyl ester (**50**) under HATU conditions (61% for two steps), followed by a chemoselective deprotection of the Boc group under anhydrous acidic conditions to deliver southern dipetide fragment (**79**) in 68% yield.[25]



Scheme 9. Synthesis of the key non-proteogenic 3-methyl-4-ethylideneproline (**78**) and southern dipeptide fragment (**79**)

Dipeptides **79** and **61** were treated with our optimal coupling system of HATU and collidine in DMF/DCM at 0 ^{o}C to deliver protected tetrapeptide (**80**) in 81% yield, as a 92:8 ratio of diastereomers. After separation of the racemized material, a global deprotection employing 25% TFA in DCM provided 8-*epi*-Lucentamycin A (**81**) in an unoptimized 47% yield (**Scheme 10**). NMR data of the epimeric Lucentamycin A **81** agreed well with the natural product **46**, with the expected exceptions due to the epimerization of C8.



Scheme 10. Total synthesis of 8-*epi*-Lucentamycin A (**81**)

Biological Data

Lucentamycin A (**46**) displayed significant *in vitro* cytotoxicity, IC_{50} value of 0.2 μM, against HCT-116 human colon carcinoma. With 8-*epi*-lucentamycin A

(**81**) in hand, we evaluated its affect on an HCT-116 cell line in order to determine if the stereochemistry of the non-proteogenic 3-methyl-4-ethylideneproline nucelus was critical for biological activity. Thus, a standard 48 hour cell viability assay was again performed with **64** and **81** at five concentrations (0, 0.025 μM, 0.1 μM, 0.4 μM, 2.0 μM and 10 μM) relative to podophyllotoxin as a positive control.[20] The control performed as expected, providing an IC_{50} value of 0.03 μM. However, the unnatural, epimeric Lucentamycin A (**81**) had no affect on HCT-116 cell viability up to 10 μM. The cytotoxicity data generated with both **81** and **64** suggest that the stereochemistry at C8, and the topology afforded by the natural product, is essential for potent *in vitro* cytotoxicity (IC_{50} = 0.2 μM) of Lucentamycin A (**46**) against HCT-116 cells (**Figure 6**).

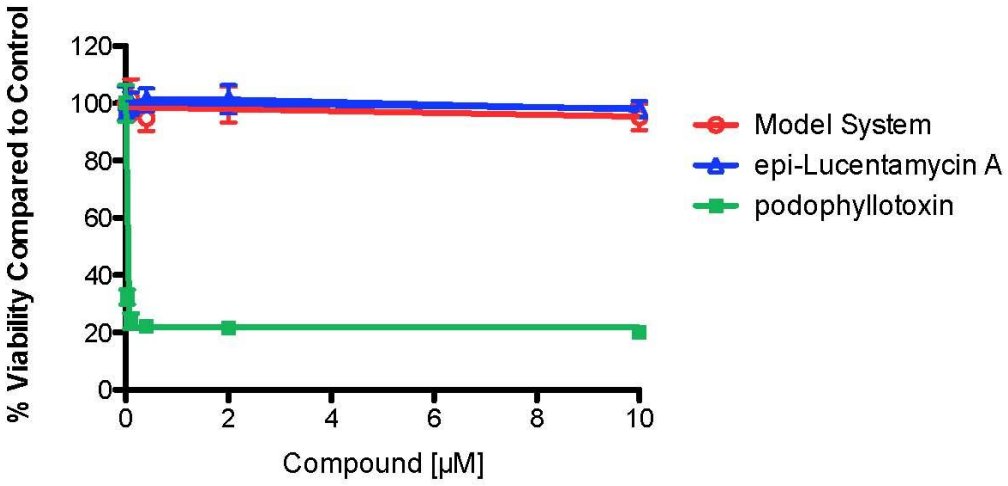


Figure 6. Cytotoxicity of Model System (**64**) and 8-*epi*-Lucentamycin A (**81**) against HCT-116 human colon carcinoma

Conclusions

Thus, the first synthetic efforts towards the Lucentamycins A-D, **46**-**49**, have been reported culminating in the total synthesis of 8-*epi*-Lucentamycin A (**81**). The synthesis features a titanium-mediated cycloisomerization reaction to construct the key, epimeric, non-proteogenic 3-methyl-4-ethylideneproline **78**. The convergent synthetic route afforded 8-*epi*-Lucentamycin A (**81**) in 15 steps, with 10 steps longest linear sequence, and an overall yield of 2.2%. Biological evaluation of **81** and another unnatural congener **64** indicated that both were inactive relative to natural **46**, with an IC_{50} value of >10 μM in a HCT-116 human carcinoma cell line. Interestingly, these studies suggest that the natural configuration of the non-proteogenic 3-methyl-4-ethylideneproline (**52**) is essential for bioactivity.

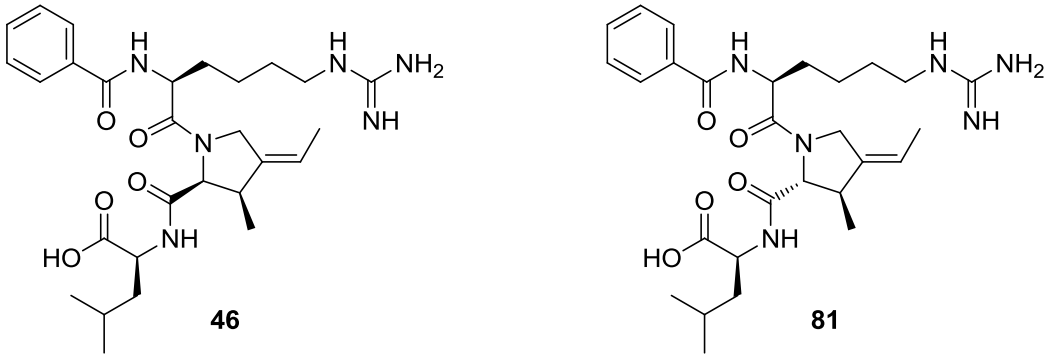


Figure 7. Compounds synthesized that did not match natural product spectral data

Within a day of our synthesis being published online a total synthesis of Lucentamycin A was also published.[26] However the spectroscopic data from the synthetic Lucentamycin A did not match the natural product suggesting that the

natural product is actually an epimer of the reported structure. Our labratory synthesized **81** while Del Valle, et. al. synthesized **46** (**Figure 7**). Upon comparison of the NMR what was reported at the natural product did not match either synthetic compound and the molecules synthesized were found to have no biological activitiy. Considering the facts that both **81** and **46** were synthesized, both were biologically inactive, and did not match the NMRs of the natural product, we determined that the natural product had been assigned the incorrect stereochemistry. It is possible that the correct stereochemistry could be the opposite absolute stereochemistry at C-8 and C-9 than reported, the proline could have a trans configuration with the opposite configuration than **81**, the olefin may possibly have an E configuration instead of Z, the homoarginene moiety could be assigned incorrectly, or some combination of all of the above. However, ROESY correlations supporting both the Z alkene and 2,3-*cis* substitution were observed for the natural Lucentamycin A. These data suggest that the relative stereochemistry on the proline is correct and that it is only the absolute stereochemistry that was incorrectly reported.

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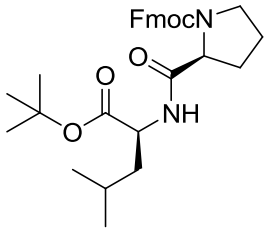
Experimental Section

General Experimental

All ^{1}H and ^{13}C NMR spectra were recorded on 300 MHz**,** 400 MHz or 500 MHz instruments. Chemical shifts are reported in ppmrelative to residual solvent peaks as an internal standard set to δ 7.26 and δ 77.0 (CDCl3). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q =quartet, br = broad, m = multiplet), integration, coupling constant (Hz). 2-Dimensional NMR spectra (NOESY and COSY) were obtained on a 500 spectrometer (operating at 500.134 MHz for ^{1}H and 124.996 MHz for ^{13}C). IR spectra were recorded as thin films and are reported in wavenumbers (cm-1). Low resolution mass spectra were obtained on an analytical LCMS with electrospray ionization. High resolution mass spectra were recorded on a Qtof-API-US system. The HRMS results were obtained with ES as the ion source and leucine enkephalin as the reference. Analytical thin layer chromatography was performed on 250 μM silica gel 60 F254 plates. Visualization was accomplished with UV light, and/or the use of ninhydrin, anisaldehyde and ceric ammonium molybdate solutions followed by charring on a hot-plate. Chromatography on silica gel was performed using Silica Gel 60 (230- 400 mesh) from Sorbent Technologies. Analytical HPLC was performed on an analytical LCMS with UV detection at 214 nm and 254 nm along with ELSD detection. Chiral HPLC was performed on an analytical HPLC utilizing a Chiracel OD, OJ or Chiralpak AD columns (4.6 mm x 25 cm). Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased and were used without

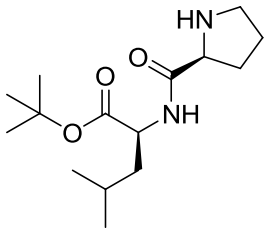
purification. All polymer-supported reagents were purchased. Flame-dried (under vacuum) glassware was used for all reactions. All reagents and solvents were commercial grade and purified prior to use when necessary. Mass spectra were obtained on a Q-TOF API-US mass spectrometer to acquire high-resolution mass spectrometry (HRMS) data.

Experimental:



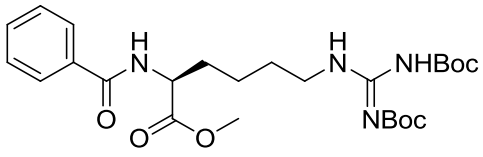
(*S*)-(9H-fluoren-9-yl)methyl 2-((*S*)-1-*tert*-butoxy-4-methyl-1-oxopentan-2-ylcarbamoyl)pyrrolidine-1-carboxylate (56). N-Fmoc-L-Proline (101 mg, 0.3 mmol), L-Leucine tert-butyl ester hydrochloride (67 mg, 0.3 mmol) and O-(7-Azabenzotriazol-1-yl)-N,N,N,N-tetramethyluronium hexafluorophosphate (171 mg, 0.45 mmol) were added to a flame dried flask under argon. The flask was evacuated and backfilled with argon three times. Anhydrous DCM (1 mL), anhydrous DMF (0.5 mL), and collidine (79 µL, 0.6 mmol) were added in that order and stirred at room temperature for 3hr. HCl (1 N, 1 mL) was added to quench the reaction. The reaction mixture was diluted with DCM (3 mL) and washed with H_2O (3 x 1.5 mL) and filtered through a phase separator. The crude mixture was concentrated *in vacuo* and then purified by automated flash

chromatography (1:0 to 1:1 Hex:EtOAc) to yield the product as a white solid (151 mg, 0.3 mmol) in 99% yield. mp 154.9 °C; $[\alpha]_D^{20}$ -56 (c = 0.2, $CHCl_3$); R_f 0.4 (1:1, EtOAc:Hex); IR (thin film) 3329, 2956, 2928, 1733, 1685, 1450, 1418, 1149 cm^{-1}; ^1H NMR (500.1 MHz, MeOD) δ (ppm): 7.80 (d, *J* = 7.5 Hz, 2H), 7.63 (d, *J* = 7.5 Hz, 1H), 7.62 (dd, *J* = 35.2, 7.5 Hz, 1H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 4.29 (m, 5H), 3.52 (m, 2H), 2.27 (m, 1H), 2.00 (m, 3H), 1.61 (m, 3H), 1.44(d, *J* = 11.1 Hz, 9H), 0.93 (dd, *J* = 22.7, 6.6 Hz, 3H), 0.77 (dd, *J* = 30.6, 6.5 Hz, 3H); ^{13}C NMR (100.6 MHz, MeOD) δ (ppm): 175.2, 174.9, 173.3, 173.2, 156.7, 156.5, 145.52, 145.47, 145.2, 144.9, 142.7, 142.6, 142.54, 142.46, 128.90, 128.85, 128.82, 128.3, 128.20, 128.18, 126.4, 126.2, 121.0, 82.63, 82.59, 69.2, 68.7, 61.4, 61.2, 53.1, 48.1, 41.5, 41.4, 32.7, 31.4, 28.2, 26.00, 25..96, 25.3, 24.5, 23.3, 23.2, 22.0, 21.8; HRMS (TOF, ES+) $C_{30}H_{38}N_2O_5Na$ $[M+Na]^+$ calc'd 529.2678, found 529.2695



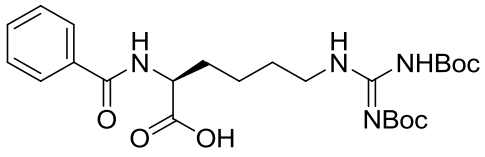
(*S*)-*tert*-butyl 4-methyl-2-((*S*)-pyrrolidine-2-carboxamido)pentanoate (57). **56** (101 mg, 0.2 mmol) was added to a flask and dissolved in a solution of 5% piperidine in DMF. The mixture was stirred at room temperature for 0.5 hr. The crude mixture was concentrated *in vacuo* and then purified by automated flash chromatography (1:0 to 9:1 DCM:MeOH) to yield the product as a white solid (57

mg, 0.2 mmol) in 100% yield. R_f 0.17 (9:1, DCM:MeOH); IR (thin film) 3330, 2958, 2871, 1735, 1671, 1508, 1368, 1150 cm^{-1}; ^1H NMR (500.1 MHz, MeOD) δ (ppm): 4.32 (dd, *J* = 8.2, 6.7 Hz, 1H), 3.67 (dd, *J* = 8.3, 5.7 Hz, 1H), 3.00 (m, 1H), 2.90 (m, 1H), 2.13 (m, 1H), 1.77 (m, 3H), 1.65 (m, 1H), 1.59 (m, 2H), 1.45 (s, 9H), 0.95 (d, *J* = 6.4 Hz, 3H), 0.91 (d, *J* = 6.4 Hz, 3H); ^{13}C NMR (100.6 MHz, MeOD) δ (ppm): 176.7, 173.2, 82.7, 61.4, 52.7, 48.1, 41.7, 32.2, 28.2, 26.9, 26.1, 23.3, 21.9; HRMS (TOF, ES+) $C_{15}H_{29}N_2O_3$ $[M+H]^+$ calc'd 285.2178, found 285.2169



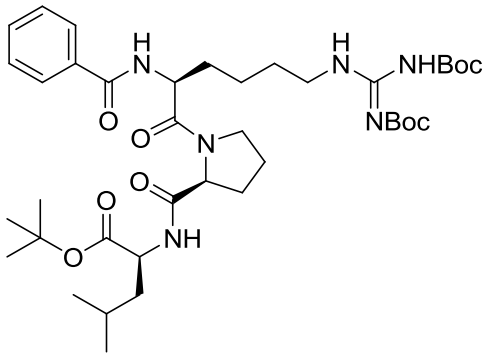
(*S*)-methyl 2-benzamido-6-(2,3-bis(*tert*-butoxycarbonyl)guanidino)hexanoate (60). To a solution of (2*S*)-2-Amino-6-[N,N'-bis(tert-butoxycarbonyl)guanidino]hexanoic Acid Methyl Ester (99 mg, 0.25 mmol) in anhydrous DCM (1 mL) and anhydrous DMF (0.5 mL) in a flame dried flask. Collidine (35.8 µL, 0.27 mmol) and benzoyl chloride (27 µL, 0.23 mmol) were added in that order and stirred at room temperature for 1.5 hr. HCl (1 N, 1 mL) was added to neutralize the reaction. The reaction mixture was diluted with DCM (3 mL) and washed with H_2O (3 x 2 mL) and filtered through a phase separator. The crude mixture was *in vacuo* and then purified by automated flash chromatography (1:0 to 0:1 Hex:EtOAc) to yield the product as a crusty foam (90 mg, 0.18 mmol) in 72% yield. mp 51.7 °C; $[\alpha]_D^{20}$ 26 (c = 0.15, $CHCl_3$); R_f 0.34

(1:1, EtOAc:Hex); IR (thin film) 3331, 2926, 2854, 1722, 1642, 1156, 1135, 738 cm^{-1}; ^{1}H NMR (600.1 MHz, MeOD) δ (ppm): 7.84 (m, 2H), 7.54 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.5 Hz, 2H), 4.60 (dd, *J* = 9.5, 5.1 Hz, 1H), 3.74 (s, 3H), 3.37 (t, *J* = 7.0 Hz, 2H), 1.98 (m, 1H), 1.87 (m, 1H), 1.63 (m, 2H), 1.53 (m, 2H), 1.49 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (150.9 MHz, MeOD) δ (ppm): 174.3, 170.5, 164.6, 157.6, 154.2, 135.2, 132.9, 129.6, 128.5, 84.4, 80.4, 54.3, 52.7, 41.4, 31.9, 29.6, 28.6, 28.2, 24.4; HRMS (TOF, ES+) $C_{25}H_{38}N_4O_7Na$ $[M+Na]^+$ calc'd 529.2638, found 529.2663



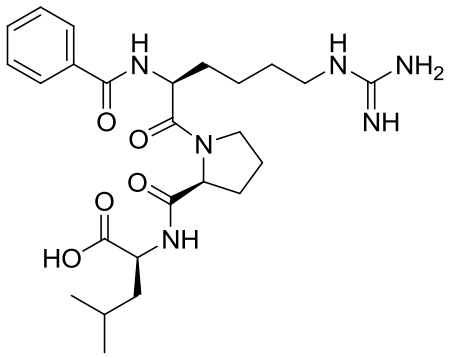
(*S*)-2-benzamido-6-(2,3-bis(*tert*-butoxycarbonyl)guanidino)hexanoic acid (61). 60 (90 mg, 0.18 mmol) was added to a flask and dissolved in THF (1.6 mL), and MeOH (0.4 mL). A solution of LiOH (17 mg, 0.71 mmol) in H_2O (0.4 mL) was then added to the reaction, which was then stirred at room temperature for 4 hrs. HCl (0.5 N) was added to bring the mixture to pH 4. The aqueous layer was extracted with DCM (3 x 3 mL), the combined organic layers were filtered through a phase separator. The crude mixture was concentrated *in vacuo* and then purified by automated flash chromatography (1:0 to 9:1 DCM:MeOH) to yield the product as a crusty foam (76 mg, 0.15 mmol) in 85% yield. $[\alpha]_D^{20}$ 31.7 (c = 0.2, $CHCl_3$); R_f 0.23 (9:1, DCM:MeOH); IR (thin film) 3330, 2979, 2935, 1722, 1641, 1615, 1368, 1332, 1154, 1135 cm^{-1}; ^{1}H NMR (600.1

MHz, MeOD) δ (ppm): 7.85 (m, 2H), 7.52 (t, *J* = 7.4 Hz, 1H), 7.46 (t, *J* = 7.7 Hz, 2H), 4.91 (brs, 1H), 4.60 (dd, *J* = 9.5, 4.6 Hz, 1H), 3.37 (t, *J* = 7.1 Hz, 2H), 2.01 (m, 1H), 1.88 (m, 1H), 1.64 (m, 2H), 1.53 (m, 2H), 1.48 (s, 9H), 1.45 (s, 9H); ^{13}C NMR (150.9 MHz, MeOD) δ (ppm): 175.6, 170.4, 164.2, 157.5, 154.1, 135.3, 132.8, 129.5, 128.5, 84.5, 80.5, 54.2, 41.6, 32.1, 29.7, 28.6, 28.2, 24.5; HRMS (TOF, ES+) $C_{24}H_{37}N_4O_7$ $[M+H]^+$ calc'd 493.2662, found 493.2664



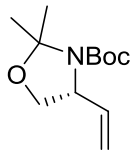
(*S*)-*tert*-butyl 2-((*S*)-1-((S)-2-benzamido-6-(2,3-bis(tert-butoxycarbonyl)guanidino)hexanoyl)pyrrolidine-2-carboxamido)-4-methylpentanoate (62). **57** (14.2 mg, 0.05 mmol) and **61** (24.6 mg, 0.05 mmol), in anhydrous DCM (0.2 mL each), were added to a flame dried flask under argon via syringe. Anhydrous DMF (0.2 mL) was added and the solution was cooled to 0 °C. O-(7-Azabenzotriazol-1-yl)-N,N,N,N-tetramethyluronium hexafluorophosphate (28.5 mg, 0.075 mmol) was added followed by collidine (7.3 µL, 0.055 mmol). The reaction was stirred at 0 °C for 4.5 hr and then diluted with DCM (3 mL) and washed with H_2O (3 x 1.5 mL) and filtered through a phase separator. The crude mixture was concentrated *in vacuo* and then purified by automated flash chromatography (1:0 to 0:1 Hex:EtOAc) to yield the product as a

crusty foam (34.2 mg, 0.045 mmol) in 90% yield. $[\alpha]_D^{20}$ -57 (c = 0.2, $CHCl_3$); R_f 0.53 (EtOAc); IR (thin film) 3330, 2976, 1720, 1637, 1418, 1368, 1331, 1155, 1134 cm^{-1}; ^1H NMR (600.1 MHz, MeOD) δ (ppm): 7.85 (m, 2H), 7.53 (m, 1H), 7.45 (m, 2H), 4.80 (dd, *J* = 9.5, 4.8 Hz, 1H), 4.49 (dd, *J* = 8.5, 4.3 Hz, 1H), 4.60 (dd, *J* = 9.5, 4.6 Hz, 1H), 4.27 (t, *J* = 7.6 Hz, 1H), 3.94 (m, 1H), 3.74 (m, 1H), 3.39 (m, 2H), 2.22 (m, 1H), 2.10 (m, 1H), 2.03 (m, 2H), 1.94 (m, 1H), 1.84 (m, 1H), 1.76 (m, 1H), 1.66 (m, 2H), 1.56 (m, 4H), 1.49 (s, 9H), 1.450 (s, 9H), 1.447 (s, 9H), 0.95 (d, *J* = 6.6 Hz, 3H), 0.90 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (100.6 MHz, MeOD) δ (ppm): 174.2, 173.4, 173.1, 170.3, 164.6, 157.6, 154.2, 135.1, 132.8, 129.5, 128.6, 84.4, 82.5, 80.3, 61.2, 53.3, 53.1, 48.7, 41.6, 41.5, 31.8, 30.5, 29.8, 28.6, 28.3, 28.2, 26.0, 25.9, 24.2, 23.3, 22.1; HRMS (TOF, ES+) $C_{39}H_{63}N_6O_9$ $[M+H]^+$ calc'd 759.4657, found 759.4659



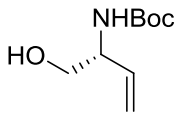
(*S*)-2-((*S*)-1-((*S*)-2-benzamido-6-guanidinohexanoyl)pyrrolidine-2-carboxamido)-4-methylpentanoic acid (64). 63 (36.9 mg, 0.05 mmol) was added to a flask and a stock solution of 20 % TFA in DCM and stirred under argon for 18 h. MeOH was added to the reaction mixture which was then

concentrated *in vacuo*. More MeOH was added and the reaction concentrated again. Because of the high polarity of the compound, the product was purified by reverse phase preparatory HPLC to yield the product as a light yellow oil (14.7 mg, 0.03 mmol) in 60 % yield. R_f 0.025 (9:1, DCM:MeOH); ^{1}H NMR (600.1 MHz, DMSO-d_6) δ (ppm): 10.47 (m, 1H), 8.42 (d, *J* = 7.2 Hz, 1H), 7.87 (m, 2H), 7.52 (t, *J* = 7.4 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 6.98 (m, 3H), 4.81 (m, 1H), 4.20 (dd, *J* = 8.9, 2.5 Hz, 1H), 3.83 (q, *J* = 6.6 Hz, 1H), 3.75 (dd, *J* = 7.8, 5.5 Hz, 2H), 3.32 (brs, 2H), 3.12 (m, 1H), 3.03 (m, 1H), 1.98 (m, 3H), 1.84 (m, 2H), 1.66 (m, 2H), 1.58 (m, 3H), 1.50 (m, 1H), 1.44 (m, 2H), 1.37 (m, 1H), 0.86 (d, *J* = 6.6 Hz, 3H), 0.85 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ (ppm): 175.9, 170.8, 169.7, 165.8, 157.2, 133.7, 131.4, 128.2, 127.5, 61.3, 52.4, 51.0, 47.0, 42.8, 41.2, 31.5, 29.4, 28.6, 24.6, 24.3, 23.0, 22.9, 22.8; HRMS (TOF, ES+) $C_{25}H_{39}N_6O_5$ $[M+H]^+$ calc'd 503.2982, found 503.2976



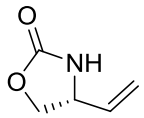
(*R*)-*tert*-butyl 2,2-dimethyl-4-vinyloxazolidine-3-carboxylate (67). To a suspension of $Ph_3P^+CH_3Br^-$ (11.5 g, 32.189 mmol) in THF (268 mL) was added n-BuLi (2 M, 17.4 mL, 34.872 mmol). The deep red solution was stirred at 0 °C for 1 h. Then, a solution of Garner's aldehyde **66** (6.15 g, 26.824 mmol) in THF (15 ml) was added dropwise at 0 °C. The red solution was warmed to room temperature and stirred for 12 h. After complete consumption of starting material

by TLC analysis ($KMnO_4$, R_f = 0.61; 3:1 hexanes:EtOAc), hexanes was added to the reaction mixture in 4:1 (v:v) ratio. The suspension was filtered through Celite and concentrated. Purification by flash chromatography (10:1 hexanes-ethyl acetate on silica gel) gave the desired vinyloxazolidine **67** (4.86 g, 83%) as a pale yellow oil. $[\alpha]_D^{20}$ +16.29 (c = 0.032, $CHCl_3$); IR 2980, 2935, 1699, 1384, 1091, 860 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ (ppm)): 5.79 (ddd; *J*=17.2, 10.2, 5.2 Hz; C*H*=CH_2; 1H), 5.08 (d; *J*=16.8 Hz; CH=C*H*$_2$; 1H), 4.97 (d; *J*=10.0 Hz; CH=C*H*$_2$; 1H), 4.14 (bs; C*H*-N; 1H), 3.72 (dd; *J*=8.8, 2.0 Hz; C*H*$_2$-O-; 1H), 3.50 (dd; *J*=8.4, 2.0 Hz; CH_2-O-; 1H), 1.66 (s; *CH*$_3$; 3H), 1.53 (s; *CH*$_3$; 3H), 1.40 (s, C(C*H*$_3$)$_3$; 9H); ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 152.40, 138.68, 115.69, 94.57, 79.67, 68.66, 60.37, 28.89, 27.48, 24.82; HRMS (TOF, ES+) $C_{12}H_{22}NO_3Na$ $[M+Na]^+$ calc'd 228.1600, found 228.1602.



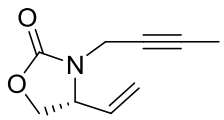
(*R*)-*tert*-butyl 1-hydroxybut-3-en-2-ylcarbamate (68). To a solution of vinyloxazolidine **67** (1.50 g, 6.599 mmol) in MeOH (66.0 mL) was added *p*-toluenesulfonic acid monohydrate (0.628 g, 3.300 mmol) as a solid addition at 0 °C. The mixture was slowly warmed to room temperature and stirred for 12 h. After complete consumption of starting material by TLC analysis ($KMnO_4$, R_f = 0.35; 1:1 hexanes:EtOAc), the reaction mixture was concentrated and the residue was diluted with ethyl acetate (50 mL). The organic layer was

transferred to a separatory funnel and washed with saturated sodium bicarbonate solution (3 X 20 mL). The organic phase was dried over $MgSO_4$, filtered, and concentrated to give the desired amino alcohol **68** (0.93 g, 75%) as a pale yellow oil. $[\alpha]_D^{20}$ +24.05 (c = 0.017, $CHCl_3$); IR 3344, 2998, 2932, 1692, 1522, 1366, 1171, 1053 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ (ppm)): 5.79 (ddd; *J*=17.2, 10.2, 5.2 Hz; C*H*=CH_2; 1H), 5.25 (dd; *J*=18.8, 1.2 Hz; CH=C*H*$_2$; 1H), 5.21 (dd; *J*=11.6, 1.2 Hz; CH=C*H*$_2$; 1H), 4.99 (d; *J*=7.6 Hz; N*H*; 1H), 4.21 (bs; C*H*-N; 1H), 3.68 (d; *J*=11.2, 4.4 Hz; C*H*$_2$-OH; 1H), 3.60 (d; *J*=11.2, 5.6 Hz; CH_2-OH; 1H), 2.53 (bs; OH; 1H), 1.43 (s, C(C*H*$_3$)$_3$; 9H); ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 156.26, 135.69, 116.65, 80.05, 65.29, 54.90, 50.86, 28.55; HRMS (TOF, ES+) $C_9H_{17}NO_3Na$ $[M+Na]^+$ calc'd 210.1106, found 210.1108.



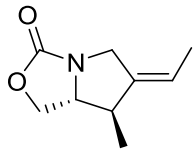
(*R*)-4-vinyloxazolidin-2-one (69). To a solution of amino alcohol **68** (2.29 g, 12.230 mmol) in THF (245 mL) was added thionyl chloride (7.1 mL, 97.842 mmol) dropwise at 0 °C. The resulting mixture was slowly warmed to room temperature and stirred for 12 h. After complete consumption of starting material by TLC analysis ($KMnO_4$, R_f = 0.17; 1:1 hexanes:EtOAc), the solvent was removed under reduced pressure. The remaining brown residue was purified by Biotage chromatography to give the desired vinyloxazolidin-2-one **69 (**1.07 g, 78%) as a yellow oil. $[\alpha]_D^{20}$ +20.32 (c = 0.017, $CHCl_3$); IR 3281, 2918, 2850,

1745, 1397, 1232, 931 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm)): 6.04 (bs; N*H*; 1H), 5.82 (ddd; *J*=17.2, 10.4, 7.2 Hz; C*H*=CH_2; 1H), 5.32 (d; *J*=16.8 Hz; CH=CH_2; 1H), 5.25 (d; *J*=10.0 Hz; CH=CH_2; 1H), 4.53 (dd; *J*=8.8, 8.4 Hz; CH_2-O; 1H), 4.38 (ddd; *J*=7.6, 7.6, 7.6, Hz; C*H*-NH; 1H), 4.06 (dd; *J*=8.8, 8.4 Hz; CH_2-O; 1H); ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 159.90, 135.90, 18.89, 70.17, 55.45; HRMS (TOF, ES+) $C_5H_8NO_2$ $[M+H]^+$ calc'd 114.0555, found 114.0558.



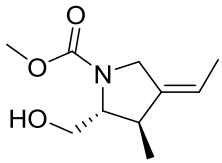
(*R*)-3-(but-2-ynyl)-4-vinyloxazolidin-2-one (71). To a solution of vinyloxazolidin-2-one **69** (0.250 g, 2.210 mmol) in DMF (22.1 mL) was added potassium bis(trimethylsilyl)amide (KHMDS, 0.5 M, 5.3 mL, 2.652 mmol) dropwise at 0 °C. After stirring for 10 min at 0 °C, propargyl mesylate **70** (0.491 g, 3.315 mmol) in DMF (2 mL) was added at 0 °C. The resulting mixture was slowly warmed to room temperature and stirred for 4 h. The reaction mixture was quenched with water (5:1 water/DMF (v:v)) and diluted with ethyl acetate. The aqueous layer was extracted with ethyl acetate (3 X 25 mL). The combined organic phases were washed with water (3 X 10 mL), dried over $MgSO_4$, filtered, and concentrated. Purification by Biotage chromatography gave the desired 3-(but-2-ynyl)-4-vinyloxazolidin-2-one **71** (0.310 g (85%) as a colorless oil. $[\alpha]_D^{20}$ -18.63 (c = 0.03, $CHCl_3$); IR 3085, 2985, 2921, 2292, 2236, 1447, 1439 1352, 1248, 1177, 939 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, δ (ppm)): 5.69 (ddd; *J*=16.8, 10.0, 8.4

Hz; C*H*=CH_2; 1H), 5.45 (d; *J*=16.8 Hz; CH=C*H*$_2$; 1H), 5.40 (d; *J*=10.6 Hz; CH=C*H*$_2$; 1H), 4.47 (dd; *J*=8.8, 8.0 Hz; C*H*$_2$-O; 1H), 4.39 (ddd; *J*=8.4, 8.0, 8.0, Hz; C*H*-N; 1H), 4.29 (dq, *J*=15.2, 2.4 Hz; N-C*H*$_2$-C≡C; 1H) 3.97 (dd; *J*=8.0, 7.6 Hz; C*H*$_2$-O; 1H). 3.59 (dq; *J*=17.2, 2.4 Hz; N-C*H*$_2$-C≡C; 1H), 1.81 (t; *J*=2.0 Hz; C≡C-C*H*$_3$; 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 157.73, 134.04, 121.93, 80.79, 72.28, 67.29, 58.30, 32.32, 3.56; HRMS (TOF, ES+) $C_9H_{11}NO_2Na$ $[M+Na]^+$ calc'd 188.0687, found 188.0689.



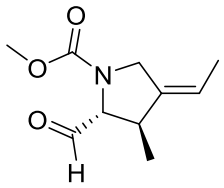
(7*R*, 7a*R*, *Z*)-6-ethylidene-7-methyltetrahydropyrrolo[1,2-*c*]oxazol-3(1*H*)-one (65). A 125 mL, three-neck, round bottom flask was fitted with an internal thermometer and a 50 mL addition funnel. The flask was charged with a solution of Ti(O*i*Pr)$_4$ (2.4 mL, 8.257 mmol) in Et_2O (23.6 mL). The addition funnel was charged with a solution of 3-(but-2-ynyl)-4-vinyloxazolidin-2-one **71** (0.310 g, 1.877 mmol) in Et_2O (37.5 mL). The entire apparatus was placed in a CyroCool bath set to -78 °C and the solution was chilled until the internal temperature reached -78 °C. Cyclopentyl magnesium bromide (2.0 M, 8.3 mL, 16.514 mmol) was added slowly dropwise via syringe to the titanium solution, ensuring the internal temperature never rose above -70 °C. After 30 min, the solution of 3-(but-2-ynyl)-4-vinyloxazolidin-2-one **71** was added via addition funnel over 20 minutes, keeping the internal temperature below -70 °C during the addition. The

mixture was stirred for 30 min at -70 °C, then warmed to -50 °C over 30 min and stirred for 5 h at -50 °C. The reaction mixture was quenched with saturated NH_4Cl solution (10 mL) and diluted with EtOAc (20 mL). The aqueous layer was extracted with EtOAc and the combined organic phases were washed with water (2 X 10 mL), dried over $MgSO_4$, filtered, and concentrated. Purification by Biotage chromatography gave the desired ethylideneproline **65** (0.220 g, 91%) as a colorless oil. $[\alpha]_D^{20}$ +78.06 (c = 0.05, $CHCl_3$); IR 2923, 2872, 1754, 1456, 1394, 1203, 977, 775 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ (ppm)): 5.26-5.18 (m; C*H*=CR_2; 1H), 4.34 (dd; *J*=8.8, 7.6 Hz; C*H*$_2$-O-; 1H), 4.13 (d; *J*=15.6 Hz; N-C*H*$_2$; 1H), 4.07 (dd; *J*=9.2, 2.4 Hz; C*H*$_2$-O; 1H), 3.70 (d; *J*=15.2 Hz; N-C*H*$_2$; 1H), 3.30 (ddd; *J*=10.0, 7.2, 2.4 Hz; C*H*-NR_2; 1H), 2.21-2.12 (m; C*H*-CH_3; 1H), 1.52 (d; *J*=6.8 Hz; C*H*$_3$-CH=CR_2; 3H), 0.96 (d; *J*=6.4 Hz; CH-C*H*$_3$; 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 161.50, 140.99, 116.80, 66.51, 65.35, 48.48, 42.24, 13.93, 13.10; HRMS (TOF, ES+) $C_9H_{14}NO_2$ $[M+H]^+$ calc'd 168.1025, found 168.1026.

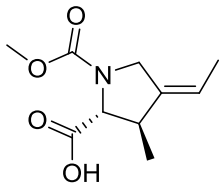


(2*R*, 3*R*, *Z*)-methyl 4-ethylidene-2-(hydroxymethyl)-3-methylpyrrolidine-1-carboxylate (72). In a 250 mL round bottom flask under argon fitted with a stirbar, sodium metal (Na°, 2.8 g, 122.60 mmol) was treated at 0 °C with dry MeOH (120 mL). The solvent became cloudy and was stirred for 1 h at 0 °C until

the entire mass of sodium metal was dissolved. A solution of ethylideneproline **65** (410 mg, 2.45 mmol) in MeOH (5 mL) was added dropwise to the solution. The resulting pale yellow solution was stirred at room temperature for 12-16 h. The solvent was concentrated and the residue was diluted with EtOAc (100 mL). The organic phase was washed with saturated NH_4Cl solution (2 X 25 mL) and with brine (1 X 25 mL), dried over $MgSO_4$, filtered and concentrated to give a pale yellow residue. The residue was purified by silica gel chromatography (5:1 hexanes/EtOAc then 3:1 hexanes/EtOAc) to provide the desired ethylidene-2-(hydroxymethyl)-3-methylpyrrolidine-1-carboxylate **72** (422 mg, 86%) as a colorless oil. $[\alpha]_D^{20}$ +28.83 (c = 0.04, $CHCl_3$); IR 3431, 2958, 2869, 1698, 1454, 1393, 1062 cm^{-1}; ^1H NMR (400 MHz @ 342K C_6D_6, δ (ppm)): 5.06-5.00 (m; CH_3-C*H*=CR_2; 1H), 4.02 (app d; *J*=14.8 Hz; C*H*$_2$-OH; 1H), 3.81 (app d; *J*=15.2 Hz; C*H*$_2$-OH; 1H), 3.63-3.57 (m; N-C*H*$_2$; N-C*H*-CH_2; 2H), 3.53 (dd; *J*=10.4, 5.2 Hz; N-C*H*$_2$; 1H), 3.49 (s; CO_2C*H*$_3$; 3H), 2.34-2.16 (m; CH_3-C*H*; 1H), 1.32 (dd; *J*=6.8, 1.2 Hz; C*H*$_3$-CH=CR_2; 3H), 0.89 (d; *J*=7.2 Hz; C*H*$_3$-CH; 3H); ^{13}C NMR (100 MHz @ 342K, C_6D_6, δ (ppm)): 156.47, 140.69, 115.40, 67.67, 64.83, 51.76, 48.04, 40.26, 29.69, 13.57; HRMS (TOF, ES+) $C_{10}H_{18}NO_3$ $[M+H]^+$ calc'd 200.1287, found 200.1284.

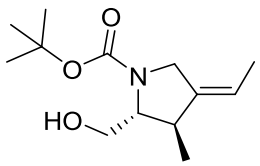


(2*R*, 3*R*, *Z*)-methyl 4-ethylidene-2-formyl-3-methylpyrrolidine-1-carboxylate (73). To a solution of ethylidene-2-(hydroxymethyl)-3-methylpyrrolidine-1-carboxylate **72** (73 mg, 0.37 mmol) in DMSO (dry, 0.92 mL) was added NEt_3 (0.32 mL, 1.21 mmol) immediately followed by SO_3·pyridine (192 mg, 2.31 mmol) in DMSO (0.93 mL). The resulting yellow-orange solution was stirred at room temperature for 30 min. The reaction was quenched with 2 N HCl and extracted three times with EtOAc (3 X 15 ml). The organic layer was washed with 2 N HCl (1 X 10 ml), saturated $NaHCO_3$ solution (3 X 15 mL), and brine solution (1 X 10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated. The residue was purifed by column chromatography (5:1 hexanes/EtOAc then 3:1 hexanes/EtOAc on silica gel) to provide the desired aldehyde **73** (70.1 mg, 98%) as a pale yellow oil. IR 2961, 2870, 1703, 1698, 1451, 1070, 771 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ (ppm)): 9.56-9.43 (m; C*H*O; 1H), 5.36-5.29 (m; CH_3-C*H*=CR_2; 1H), 4.25-4.04 (m; C*H*-CHO and N-CH_2; 3H), 3.81-3.68 (m; CO_2CH_3; 3H), 2.78-2.66 (m; CH_3-C*H*; 1H), 1.65-1.56 (m; CH_3-CH=CR_2; 3H), 1.18 (d; *J*=6.8 Hz; CH_3-CH; 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ (ppm)): 198.69, 156.18, 139.07, 117.47, 66.31, 52.79, 48.06, 39.40, 20.94, 14.44; HRMS (TOF, ES+) $C_{10}H_{16}NO_3$ $[M+H]^+$ calc'd 198.1130, found 198.1130.



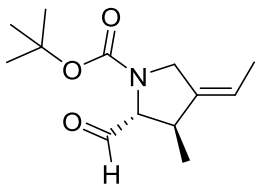
(2*R*, 3*R*, *Z*)-4-ethylidene-1-(methoxycarbonyl)-3-methylpyrrolidine-2-carboxylic acid (74). To a stirring solution of aldehyde **73** (70 mg, 0.36 mmol) in *t*BuOH (2 mL) was added 2-methyl-2-butene (1 mL). In a separate vial, NaH_2PO_4 (460 mg, 3.34 mmol) and NaO_2Cl (212 mg, 2.34 mmol) were added and dissolved in water (2 mL). The *t*BuOH solution was cooled to 0 °C and the bleach solution was added dropwise. The resulting yellow solution was stirred at 0 °C for 2 h then quenched with saturated $Na_2S_2O_3$ solution (5 mL) and acidified to pH 2. The aqueous solution was extracted with EtOAc (3 X 25 mL) and dried over $MgSO_4$, filtered and concentrated. The remaining residue was dissolved in methanolic ammonia (pH 9) and passed through a strong cation exchange (SCX) column. Elution of the carboxylic acid **74** was performed using methanolic HCl (pH 1, *Note:* See General Methods section for exact procedure). The methanolic solution was concentrated and redissolved in EtOAc (5 mL) and washed with water (2 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated to provide the desired carboxylic acid **74** (59 mg, 78%) as a yellow foam. IR 3136, 3043, 1709, 1403 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$, δ (ppm)): 10.53 (bs; CO_2H; 1H), 5.35-5.33 (m; CH_3-C*H*=CR_2; 1H), 4.24-3.98 (m; C*H*-CO_2H and N-CH_2; 3H), 3.74-3.70 (m; CO_2CH_3; 3H), 2.94-2.82 (m; CH_3-C*H*; 1H), 1.59-1.57 (m; CH_3-CH=CR_2; 3H), 1.23-1.21 (m; CH_3-CH; 3H); ^{13}C NMR (100 MHz, $CDCl_3$, δ

(ppm)): 176.89, 156.60, 139.38, 117.57, 66.11, 53.23, 48.56, 29.84, 20.90, 14.32; HRMS (TOF, ES+) $C_{10}H_{16}NO_4$ $[M+H]^+$ calc'd 214.1079, found 214.1078.

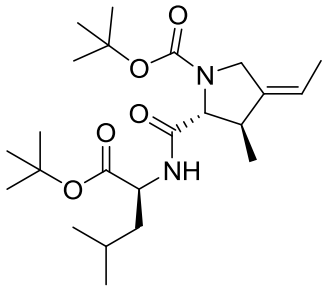


(2*R*,3*R*,*Z*)-*tert*-butyl 4-ethylidene-2-(hydroxymethyl)-3-methylpyrrolidine-1-carboxylate (76). 65 (98.3 mg, 0.59 mmol), was added to a microwave vial and dissolved in dioxane (3.75 mL). NaOH (3N, 1.47 mL, 4.41 mmol) was then added. The vial was then irradiated for 1.5 hrs to a temperature of 110 °C. The vial was then cooled to 0 °C and Boc_2O (307.9 mg, 1.41 mmol) dissolved in dioxane (3.75 mL) was then added dropwise. The reaction was stirred at 0 °C for 2 hr and then neutralized with citric acid (10%). H_2O (10 mL) was added and the mixture was extracted with EtOAc (3 x 20 mL). The combined organic layers were then dried ($MgSO_4$), filtered, and concentrated *in vacuo* and then purified by flash chromatography (1:0 to 2:1 Hex:EtOAc) to yield the product as an oil (112.8 mg, 0.48 mmol) in 79.5 % yield. $[\alpha]_D^{20}$ 22.4 (c = 0.2, $CHCl_3$); R_f 0.40 (2:1, Hex:EtOAc); IR (thin film) 3417, 2967, 2924, 2869, 1699, 1673, 1407, 1366, 1172, 1120 cm^{-1}; ^1H NMR (600.1 MHz, $CDCl_3$, 40 °C) δ (ppm): 5.26 (m, 1H), 4.03 (m, 1H), 3.85 (d, *J* = 15.4 Hz, 1H), 3.57 (m, 2H), 3.48 (m, 1H), 2.29 (brs, 1H), 1.56 (m, 3H), 1.44 (d, *J* = 3.1 Hz, 9H), 1.09 (m, 3H); ^{13}C NMR (150.9 MHz, $CDCl_3$, 40 °C) δ (ppm): 156.6, 140.2, 115.8, 80.2, 67.3, 66.0, 48.4, 40.3, 28.4,

17.8, 14.1; HRMS (TOF, ES+) $C_{13}H_{24}NO_3$ $[M+H]^+$ calc'd 242.1756, found 242.1757

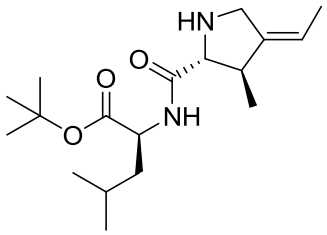


(2*R*,3*R*,*Z*)-tert-butyl 4-ethylidene-2-formyl-3-methylpyrrolidine-1-carboxylate (77). To a solution of **76** (102 mg, 0.42 mmol) in anhydrous DMSO (1.06 mL) was added NEt_3 (0.37 mL, 2.66 mmol) immediately followed by SO_3·pyridine (222 mg, 1.39 mmol) in anhydrous DMSO (1.07 mL). The resulting yellow-orange solution was stirred at room temperature for 30 min. The reaction was neutralized with 2 N HCl and extracted with EtOAc (3 X 15 ml). The organic layer was washed with brine solution (1 X 10 mL), dried over $MgSO_4$, filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (1:0 to 4:1 Hex:EtOAc) to yield the product as an oil (70.6 mg, 0.30 mmol) in 69.7 % yield. $[\alpha]_D^{20}$ 92.4 (c = 0.2, $CHCl_3$); R_f 0.72 (2:1, Hex:EtOAc); IR (thin film) 2974, 2928, 2869, 1737, 1702, 1396, 1368, 1172, 1124 cm^{-1}; ^1H NMR (600.1 MHz, $CDCl_3$, 50 °C) δ (ppm): 9.42 (m, 1H), 5.31 (m, 1H), 4.07 (m, 2H), 3.63 (m, 1H), 2.67 (m, 1H), 1.60 (d, *J* = 7.0 Hz, 3H), 1.43 (m, 9H), 1.14 (d, *J* = 6.8 Hz, 3H); ^{13}C NMR (150.9 MHz, $CDCl_3$, 50 °C) δ (ppm): 198.8, 154.2, 138.4, 116.9, 81.0, 71.9, 48.3, 39.3, 28.2, 15.9, 14.3; HRMS (TOF, ES+) $C_{13}H_{22}NO_3$ $[M+H]^+$ calc'd 240.1600, found 240.1599.



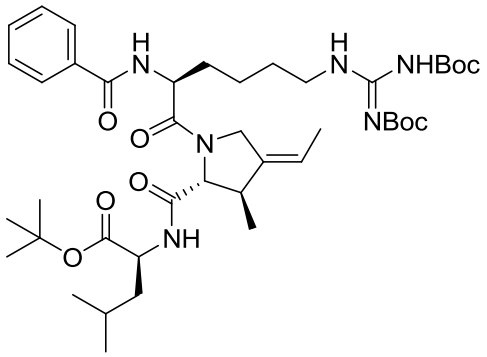
(2*R*,3*R*,*Z*)-tert-butyl 2-((*S*)-1-tert-butoxy-4-methyl-1-oxopentan-2-ylcarbamoyl)-4-ethylidene-3-methylpyrrolidine-1-carboxylate (82): To a stirring solution of **77** (49.2 mg, 0.21 mmol) in *t*BuOH (1.5 mL) was added 2-methyl-2-butene (0.75 mL). In a separate vial, NaH_2PO_4 (267 mg, 1.93 mmol) and NaO_2Cl (123 mg, 1.36 mmol) were added and dissolved in water (1.5 mL). The *t*BuOH solution was cooled to 0 °C and the bleach solution was added dropwise. The resulting yellow solution was stirred at 0 °C for 2 hr then quenched with saturated $Na_2S_2O_3$ solution until cloudy and acidified to pH 4. The aqueous solution was extracted with EtOAc (3 X 25 mL) and dried over $MgSO_4$, filtered and concentrated *in vacuo*. The crude product was then dissolved in anhydrous DCM (1 mL) and DMF (1 mL) under argon. L-Leucine tert-butyl ester hydrochloride (46.0 mg, 0.21 mmol), O-(7-Azabenzotriazol-1-yl)-N,N,N,N-tetramethyluronium hexafluorophosphate (117 mg, 0.31 mmol), and collidine (54.6 µL, 0.41 mmol) were added in that order and stirred at room temperature for 3hr. HCl (1N) was added to neutralize the reaction. The reaction mixture was diluted with DCM (10 mL) and washed with H_2O (3 x 5 mL) and filtered through a phase separator. The crude mixture was concentrated *in vacuo* and then purified by automated flash chromatography (1:0 to 4:1 Hex:EtOAc) to yield the product

as a crusty foam (52.9 mg, 0.12 mmol) in 61 % yield. $[\alpha]_D^{20}$ 36.8 (c = 0.2, $CHCl_3$); R_f 0.48 (4:1, Hex:EtOAc); IR (thin film) 3279, 2963, 2929, 1743, 1704, 1656, 1385, 1371, 1145 cm^{-1}; ^1H NMR (600.1 MHz, $CDCl_3$, 35 °C) δ (ppm): 6.37 (m, 1H), 5.30 (m, 1H), 4.46 (m, 1H), 4.20 (brs, 1H), 3.99 (d, *J* = 14.6 Hz, 1H), 3.84 (brs, 1H), 2.88 (brs, 1H), 1.66 (m, 1H), 1.58 (m, 4H), 1.44 (m, 9H), 1.41 (m, 9H), 1.17 (d, *J* = 7.0 Hz, 3H), 0.93 (d, *J* = 6.7 Hz, 3H) , 0.91 (d, *J* = 6.7 Hz, 3H); ^{13}C NMR (150.9 MHz, $CDCl_3$, 35 °C) δ (ppm): 171.8, 171.5, 155.0, 139.0, 116.6, 81.6, 80.6, 68.3, 51.1, 47.9, 43.6, 42.1, 28.3, 27.9, 24.8, 22.8, 22.0, 19.6, 14.4; HRMS (TOF, ES+) $C_{23}H_{41}N_2O_5$ $[M+H]^+$ calc'd 425.3015, found 425.3015



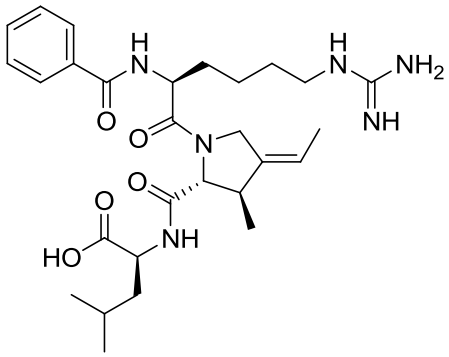
(*S*)-*tert*-butyl 2-((2*R*,3*R*,*Z*)-4-ethylidene-3-methylpyrrolidine-2-carboxamido)-4-methylpentanoate (79). 82 (50.8 mg, 0.12 mmol) was added to a small vial and placed under argon. Anhydrous EtOAc (0.45 mL) and anhydrous HCl in dioxane (4 M, 0.15 mL, 0.6 mmol) were then added and the reaction was stirred for 1 hr. Additional anhydrous EtOAc (0.45 mL) and anhydrous HCl in dioxane (4 M, 0.15 mL, 0.6 mmol) were then added at 1 hr, 2 hr, and 4 hr total reaction time for a total of four injections of each EtOAc and HCl in dioxane. NaOH (1 N) was added to neutralize the reaction. The aqueous layer was separated an extracted with EtOAc (3 x 5 mL). The combined organic layers were dried over $MgSO_4$,

filtered and concentrated *in vacuo*. The residue was purified by flash chromatography (1:0 to 19:1 DCM:MeOH) to yield the product as an oil (26.2 mg, 0.081 mmol) in 67.5 % yield. $[\alpha]_D^{20}$ 29.7 (c = 0.2, $CHCl_3$); R_f 0.52 (9:1, DCM:MeOH); IR (thin film) 3331, 2960, 2929, 2870, 1735, 1673, 1509, 1368, 1151 cm^{-1}; ^1H NMR (600.1 MHz, $CDCl_3$) δ (ppm): 7.58 (d, *J* = 8.5 Hz, 1H), 5.24 (m, 1H), 4.47 (td, *J* = 8.9, 5.0 Hz, 1H), 3.64 (s, 2H), 3.25 (d, *J* = 7.5 Hz, 1H), 2.63 (m, 1H), 2.36 (brs, 1H), 1.62 (m, 1H), 1.56 (d, *J* = 6.5 Hz, 3H), 1.52 (m, 1H), 1.43 (s, 9H), 1.19 (d, *J* = 6.8 Hz, 3H), 0.93 (d, *J* = 6.2 Hz, 6H); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ (ppm): 173.1, 172.1, 143.8, 114.3, 81.5, 68.3, 50.8, 47.8, 42.7, 41.7, 27.9, 25.0, 22.9, 21.9, 17.7, 14.5; HRMS (TOF, ES+) $C_{18}H_{33}N_2O_3$ $[M+H]^+$ calc'd 325.2491, found 325.2491



(*S*)-*tert*-butyl 2-((2*R*,3*R*,4*Z*)-1-((*S*)-2-benzamido-6-(2,3-bis(*tert*-butoxycarbonyl)guanidino)hexanoyl)-4-ethylidene-3-methylpyrrolidine-2-carboxamido)-4-methylpentanoate (80). **79** (23.7 mg, 0.073 mmol) and **61** (36.0 mg, 0.073 mmol), in anhydrous DCM (0.3 mL each), were added to a flame dried flask under argon via syringe. Anhydrous DMF (0.3 mL) was added and

the solution was cooled to 0 °C. O-(7-Azabenzotriazol-1-yl)-N,N,N,N-tetramethyluronium hexafluorophosphate (41.7 mg, 0.111 mmol) was added immediately followed by collidine (10.6 µL, 0.080 mmol). The reaction was stirred at 0 °C for 6 hr and then diluted with DCM (4 mL) and washed with H_2O (3 x 2 mL) and filtered through a phase separator. The crude mixture was concentrated *in vacuo* and then purified by flash chromatography (1:0 to 1:1 Hex:EtOAc) to yield the product as a crusty foam (47.0 mg, 0.059 mmol) in 80.5 % yield. $[\alpha]_D^{20}$ 23.9 (c = 0.2, $CHCl_3$); IR (thin film) 3330, 2964, 2929, 2929, 1722, 1639, 1154, 1135 cm^{-1}; ^1H NMR (600.1 MHz, $CDCl_3$) δ (ppm): 11.47 (brs, 1H), 8.35 (brs, 1H), 7.82 (m, 2H), 7.48 (t, *J* = 7.4 Hz, 1H), 7.40 (t, *J* = 7.7 Hz, 2H), 7.11 (d, *J* = 8.5 Hz, 1H), 6.94 (brs, 1H), 5.44 (m, 1H), 4.83 (q, *J* = 6.8 Hz, 1H), 4.49 (d, *J* = 14.3 Hz, 1H), 4.42 (m, 1H), 4.37 (d, *J* = 1.4 Hz, 1H), 4.30 (d, *J* = 14.3 Hz, 1H), 3.43 (m, 2H), 3.14 (q, *J* = 7.0 Hz, 1H), 1.93 (m, 1H), 1.86 (m, 1H), 1.67 (m, 2H), 1.62 (d, *J* = 6.8 Hz, 3H), 1.59 (m, 1H), 1.52 (m, 4H), 1.48 (s, 9H), 1.47 (s, 9H), 1.36 (s, 9H), 1.16 (d, *J* = 7.2 Hz, 3H), 0.82 (d, *J* = 6.1 Hz, 3H), 0.70 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (150.9 MHz, $CDCl_3$) δ (ppm): 171.81, 171.76, 169.8, 167.5, 163.3, 156.1, 153.2, 139.0, 133.3, 131.8, 128.5, 127.3, 118.1, 83.2, 81.2, 79.5, 66.6, 51.7, 51.2, 47.8, 41.2, 40.9, 40.6, 31.7, 28.9, 28.2, 28.0, 27.9, 24.6, 22.9, 22.6, 21.8, 21.2, 14.7; HRMS (TOF, ES+) $C_{42}H_{67}N_6O_9$ $[M+H]^+$ calc'd 799.4970, found 799.4970



8-*epi*-Lucentamycin A (81). **80** (38.3 mg, 0.048 mmol) was dissolved in anhydrous DCM (0.38 mL) and cooled to 0 °C. TFA (0.12 mL) was added dropwise and the solution was allowed to warm to room temperature over 3 hrs and stirred overnight under an argon atmosphere. MeOH was added to the reaction mixture which was then concentrated *in vacuo*. More MeOH was added and the reaction concentrated again. Because of the high polarity of the compound, the product was purified by reverse phase preparatory HPLC to yield the product as a white solid (12.3 mg, 0.023 mmol) in 47.3 % yield. $[\alpha]_D^{20}$ 28.5 (c = 0.2, MeOH); ^{1}H NMR (600.1 MHz, DMSO-d_6) δ (ppm): 10.19 (m, 1H), 8.63 (d, *J* = 7.1 Hz, 1H) 8.22 (d, *J* = 9.0 Hz, 1H), 7.90 (m, 2H), 7.51 (t, *J* = 7.3 Hz, 1H), 7.44 (t, *J* = 7.6 Hz, 2H), 6.95 (m, 2H), 5.26 (m, 1H), 4.69 (d, *J* = 1.7 Hz, 1H), 4.45 (m, 1H), 4.16 (d, *J* = 16.2 Hz, 1H), 4.00 (m, 1H), 3.89 (d, *J* = 16.5 Hz, 1H), 3.32 (brs, 2H), 3.05 (m, 1H), 2.93 (m, 1H), 2.62 (m, 1H), 1.65 (m, 1H), 1.55 (m, 7H), 1.47 (m, 1H), 1.39 (m, 1H), 1.32 (m, 2H) 1.16 (d, *J* = 7.0 Hz, 3H), 0.86 (d, *J* = 6.1 Hz, 3H), 0.80 (d, *J* = 6.1 Hz, 3H); ^{13}C NMR (150.9 MHz, DMSO-d_6) δ (ppm): 177.0, 171.3, 170.5, 166.3, 157.6, 140.4, 133.8, 131.3, 128.1, 127.6, 115.0, 66.5, 52.3,

51.4, 47.9, 45.1, 41.3, 40.6, 40.0, 30.1, 28.1, 24.9, 23.2, 21.6, 21.2, 14.3; HRMS (TOF, ES+) $C_{28}H_{43}N_6O_5$ $[M+H]^+$ calc'd 543.3295, found 543.3295.

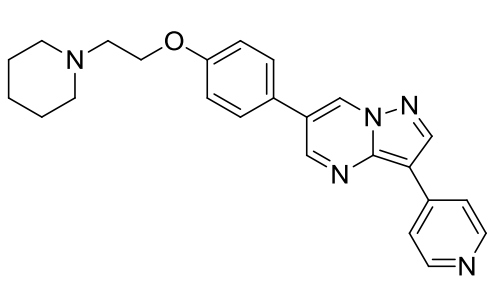
CHAPTER IV

DEVELOPMENT OF MICROWAVE METHODOLOGY TO FACILITATE THE SYNTHESIS OF BMP INHIBITORS

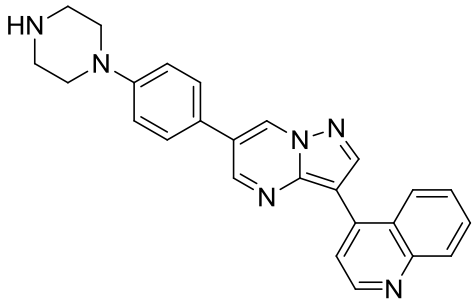
Background

Bone morphogenetic proteins (BMPs) are soluble proteins that are part of the transforming growth factor-β (TGF-β) superfamily.[27] They were originally discovered for their ability to induce endochondral bone formation,[28] and are now understood to be a group of morphogenetic signals which coordinate tissue architecture throughout the body.[29]

Activating mutations in ALK2 (ACVR1), a BMP type-I receptor, have been shown to be the cause of the debilitating and heretofore incurable fibrodysplasia ossificans progressiva (FOP).[30-32] FOP causes fibrous tissues such as muscles, ligaments, and tendons, when damaged, to be become ossified. This progressive heterotopic ossification immobilizes the host and causes death.[33] The discovery of the pyrazolo[1,5-*a*]pyrimidine, Dorsomorphin (**83**), has immediate therapeutic implications for FOP. Not only does Dorsomorphin have implications for FOP, but Dorsomorphin and its analogue LDN-193189 (**84**) (**Figure 8**) have also been used to direct differentiation of stem cells and to demonstrate the therapeutic potential of targeting BMP signals for anemia and hyperossification syndromes in general.[34-36]



Dorsomorphin (**83**)



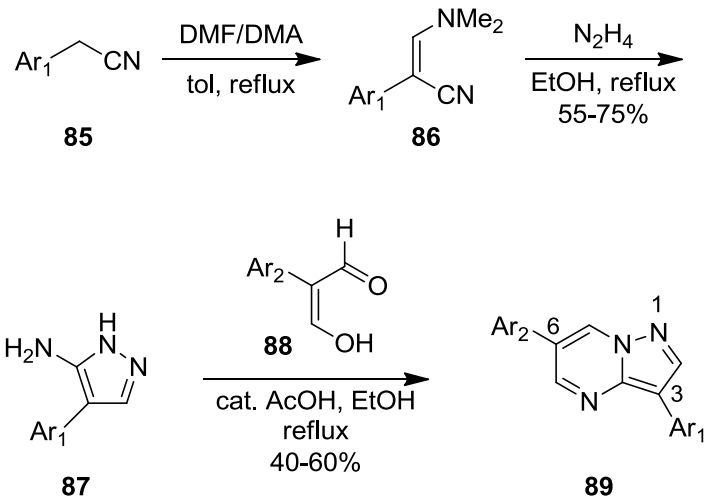
LDN-193189 (**84**)

Figure 8. Dorsomorphin (**83**) and analog LDN-193189 (**84**)

Pyrazolo[1,5-*a*]pyrimidines represent an important biologically active class of nitrogen-containing heterocycles. Pyrazolo[1,5-*a*]pyrimidines with aryl and/or heteroaryl substituents in the 3- and 6-positions are known ATP-competitive kinase inhibitors with nanomolar potency for a variety of kinases, such as KDR, and can be considered a kinase "privileged structure".[37,38]

Our laboratory employs an iterative analog library synthesis approach to rapidly develop structure-activity-relationships (SAR) and proof of concept compounds; however, the known synthetic routes to pyrazolo[1,5-*a*]pyrimidines, such as **83**, were inefficient and not ammenable to iterative library synthesis (**Scheme 11**). Classical conditions involve refluxing a 5-amino-4-arylpyrazole (**87**) with a commercially available 2-arylmalondialdehyde (**88**) in ethanol with catalytic acetic acid for 24 hours to deliver pyrazolo[1,5-*a*]pyrimidine (**89**) in 40-60% yield. The starting 5-amino-4-arylpyrazoles (**87**) were prepared in two steps from the corresponding acetonitriles (**85**) by refluxing in DMF/dimethylacetamide (DMA) to afford acrylonitrile (**86**), followed by treatment with hydrazine in

refluxing ethanol to afford **87** in 55-75% yield. The overall sequence required three overnight (72 hours total) reflux reactions.[37,38]



Scheme 11. Classical synthesis of 3,6-disubstituted pyrazolo[1,5-*a*]pyrimidines (**89**)

As many of the leads identified from HTS campaigns are small heterocyclic compounds, our laboratory has devoted significant effort to develop efficient protocols for the preparation of diverse heterocyclic templates employing microwave-assisted organic synthesis (MAOS). In recent reports, we have described general, high-yielding MAOS protocols for the expedient synthesis of 1,2,4-triazines (**90**), imidazoles (**91**), quinoxalines (**92**), pyrazinone (**93**), 5-amniooxazoles (**94**), and quinoxalinones (**95**) from simple starting materials (**Figure 9**).[39] Therefore, application of MAOS to develop a general, high-yielding and expedient synthesis of pyrazolo[1,5-*a*]pyrimidines (**89**) seemed warranted.

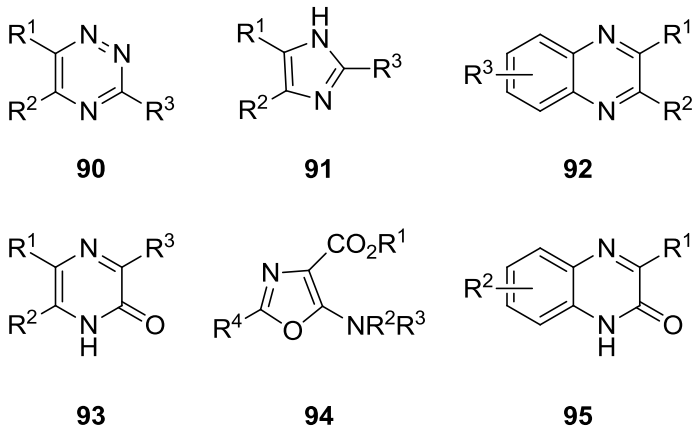
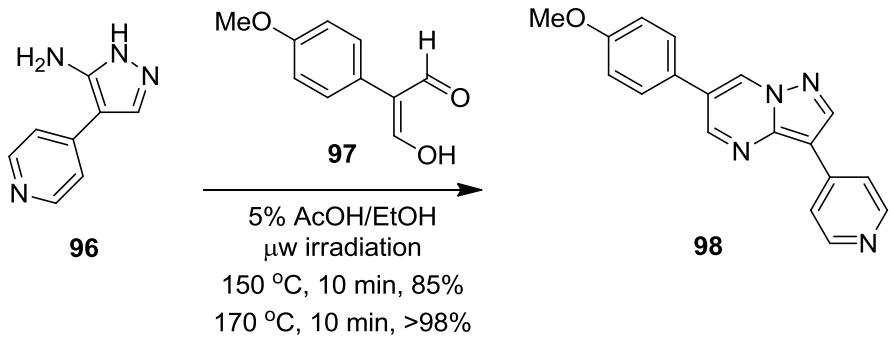


Figure 9. Heterocyclic templates accessed by microwave-assisted organic synthesis

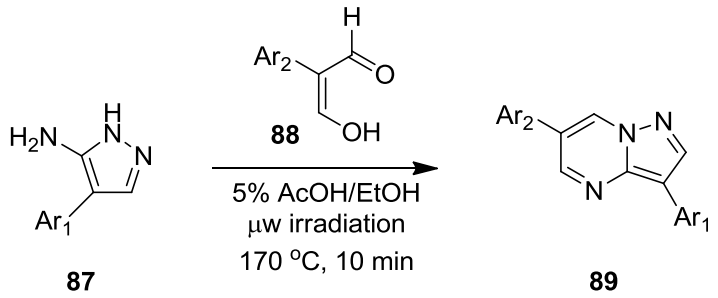
Methodology Development

Conventional thermal conditions were quickly adapted and optimized on a single-mode microwave synthesizer. Our initial optimization work was aimed at preparing the pyrazolo[1,5-*a*]pyrimidine (**98**), which is an intermediate in the synthesis of the analogs of Dorsomorphin. In the snythesis, exposing aminopyrazole (**96**) and malondialdehyde (**97**) in 5% AcOH/EtOH at 150^{o}C for 10 minutes afforded pyrazolo[1,5-*a*]pyrimidine (**98**) in 85% isolated yield (**Scheme 12**). The product **98** precipitated from solution upon the end-of-run rapid cooling to 40^{o}C in the microwave synthesizer, providing analytically pure material by filtration. Further optimization of time and temperature identified 10 minute microwave irradiation at 170^{o}C as the optimal reaction conditions to deliver **98** in >98% yield on either a 50 mg or 1 g scale.

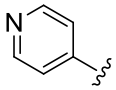
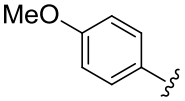
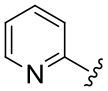
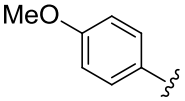
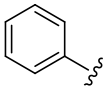
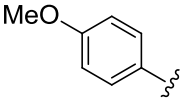
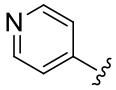
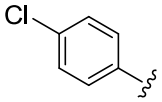
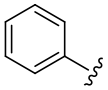
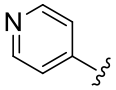
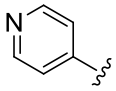
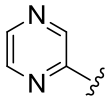


Scheme 12. Microwave-assisted organic synthesis of pyrazolo[1,5-*a*]pyrimidine (**98**)

As shown in Table 1, the MAOS conditions proved to be general for the reacting malondialdehyde providing C-6 functionalized pyrazolo[1,5-*a*]pyrimidines (**89**) in excellent isolated yields by simple filtration to afford the acetate salts. Neutral products could be obtained by neutralization of the crude reaction with concentrated NH_4OH and filtration. Pyridine heterocycles (entries **a**, **b**, **d** and **f**) were tolerated in the pyrazole component **87**, as were unsubstituted phenyl congeners (entries 3 and 6). A 5-amino-4-bromopyrazole derivative **87** (where Ar_1=Br) afforded the desired product **89**, but in low yield. This was unfortunate as the bromo analogue offered opportunities for further analogue libraries through MAOS-Suzuki couplings. With respect to the malonoaldehyde component **88**, both aryl and heteroaryl congeners provided uniformly good results. Thus, our new MAOS protocol (10 minutes, 170^{o}C) afforded the desired products **89** in **>**90% conversion.



87 89

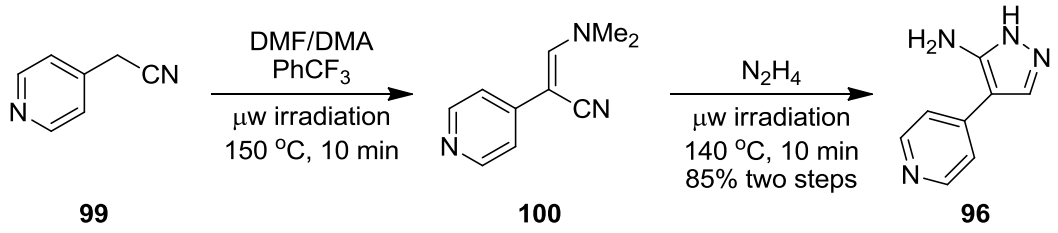
entry	Ar_1	Ar_2	conversion (%)[a]
89a			**>90**
89b			**>90**
89c			**>90**
89d			**>90**
89e			**>90**
89f			**>90**

[a]Percent conversion as determined by LCMS.

Table 1. Representative pyrazolo[1,5-*a*]pyrimidines (**89a-89f**)

With a general MAOS protocol for the expedient synthesis of 3,6-disubstituted pyrazolo[1,5-*a*]pyrimidines **89**, attention now focused on improving the synthesis of the requisite starting 5-amino-4-arylpyrazoles **87**. Few of these analogs are commercially available, and those that can be purchased are expensive (~$100/g). In order to improve the synthesis, we again employed MAOS. Nitrile (**99**) was heated at 150°C in DMF/DMA to afford complete

conversion to acrylonitrile (**100**) in 10 minutes, as judged by analytical LC/MS. To the same microwave vial, was added hydrazine via syringe, and the vial was heated again under microwave irradiation to 140^{o}C for 10 minutes to deliver the desired 5-amino-4-pyridylpyrazole (**96**) in 85% isolated yield (**Scheme 13**). This represents a significant improvement over the conventional thermal protocol that required 48 hours of reaction time with only a 55-75% yield. Thus, a reaction sequence that required >72 hours and provided 40-60% yield has been optimized to require only 30 minutes total reaction time (for three steps) with overall yields in excess of 80%.

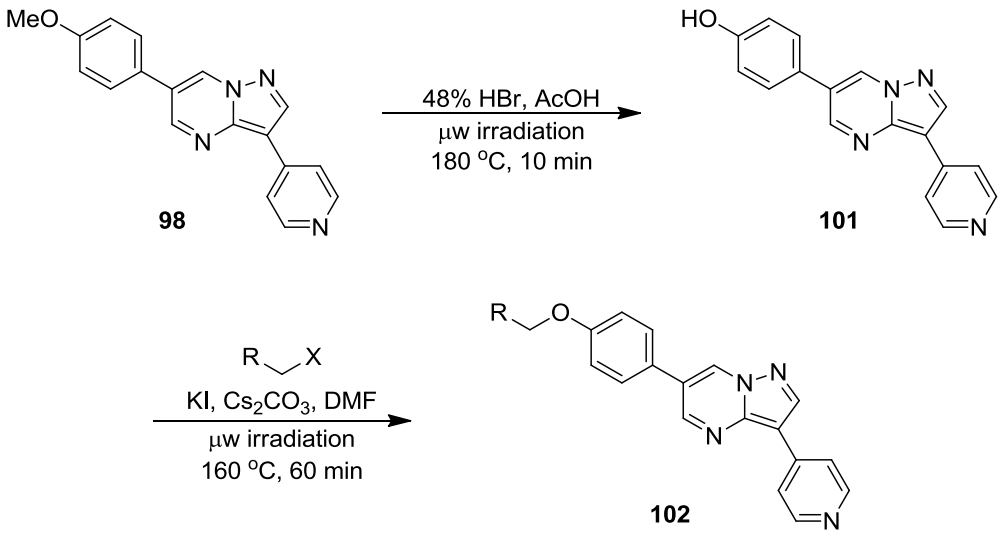


Scheme 13. Microwave-assisted organic synthesis protocol for the synthesis of 5-amino-4-pyridylpyrazole (**96**)

Synthesis of Analog Library

The desire for selective potent BMP inhibitors and the pyrazolo[1,5-*a*]pyrimidine lead compound Dorsomorphin (**83**) led us to initiate a medicinal chemistry program using our methodology. Employing MAOS, a small library of 14 compounds was afforded by first deprotecting the methyl ether of **98** by adapting thermal conditions for use on a single-mode microwave synthesizer to yield the phenol (**101**) (**Scheme 14**). Phenol **101** was then alkylated using

microwave procedures developed in our lab to yield pyrazolo[1,5-*a*]pyrimidine (**102**).



Scheme 14. Synthesis of Dorsomorphin analog library

Fourteeen different halides were used in the alkylations consisting of alkyl chlorides, alkyl bromides, α-bromo ketones, and α-bromo esters (**Figure 10**). Use of the microwave for the deprotection of the methyl ether and the alkylations allowed for a significant decrease in the reaction times and an increase in the yield for the alkylations as compared to conventional heating.[38]

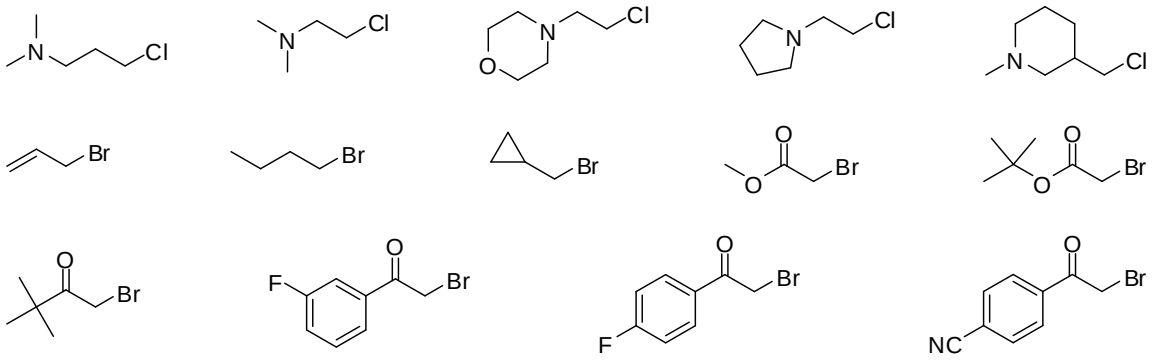


Figure 10. Library of halides used for diversification of Dorsomorphin

Biological Evaluation of Analogs

When BMP signaling is interrupted in zebrafish embryos a dorsalization of the embryonic axis occurs, as seen below, compared to normal zebrafish embryonic axis development (**Figure 11**). Dr. Charles Hong tests for BMP inhibition, and KDR inhibition on zebrafish.

Figure 11. Zebrafish embryo with normal axis and dorsalized axis

Inhibition of intersomitic (IS) angiogenic sprouting in zebrafish can be used as an *in vivo* assay for KDR inhibitory activity. This is performed by dosing transgenic fish expressing green fluorescent protein in endothelial cells two days after fertilization (**Figure 12**).

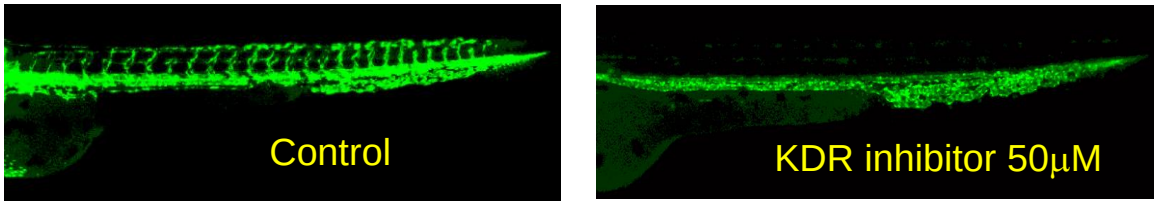
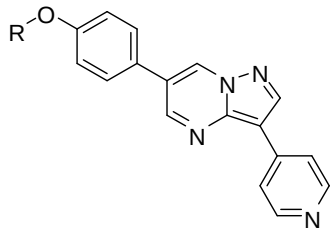


Figure 12. Inhibition of IS sprouting in zebrafish expressing green fluorescent protein in endothelial cells by inhibiting KDR

The compounds given to Dr. Hong were first tested in the zebrafish to determine if they were BMP inhibitors. The compounds that inhibited BMP, and were not lethal to the zebrafish after 48 hours, were then tested to determine if they inhibited KDR (**Table 2**).



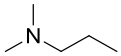
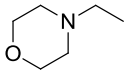
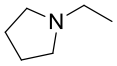
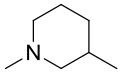
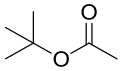
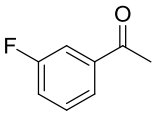
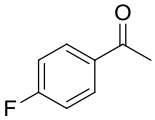
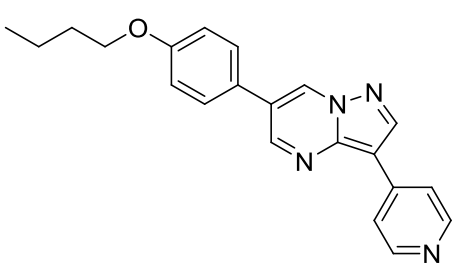
R =	Efficacy for BMP Inhibition	Lethal	IS Angiogenic Sprouting
H	2 μM	yes	not determined
CH3	>50 μM	no	not determined
	5-10 μM	no	somewhat abnormal at 20 μM
	>50 μM	no	not determined
	2 μM	yes	not determined
	>50 μM	no	not determined
	5-10 μM	no	somewhat abnormal at 20 μM
	<5 μM	yes	not determined
	10 μM	no	normal at 50 μM
	5 μM	no	somewhat abnormal at 50 μM
	<5 μM	yes	not determined
	5-10 μM	no	somewhat abnormal at 50 μM
	5-10 μM	no	somewhat abnormal at 20 μM
	2-5 μM	no	all abnormal at 20 μM

Table 2. Biological evaluation of Dorsomorphin analogs

Compounds of special interest are entries 9 and 10 of **Table 2**; the butyl ether (**103**) and cyclopropylmethyl (**104**) ether, respectively (**Figure 13**). Compound **103** inhibited BMP at 10 µM, was not lethal, and showed no effect on KDR at 50 µM, while **104** inhibited BMP at 5 µM, was not lethal, and showed a mild inhibition of KDR as evident by somewhat abnormal IS angiogenic sprouting in zebrafish at 50 µM. Both of these compounds are improvements on the original lead of Dorsomorphin **83**, which inhibited BMP at 5 µM, was not lethal, and showed a mild inhibition of KDR as evident by somewhat abnormal IS angiogenic sprouting in zebrafish at 20 µM. This led to the determination that an aliphatic group in the 6-position is important for BMP selectivity.



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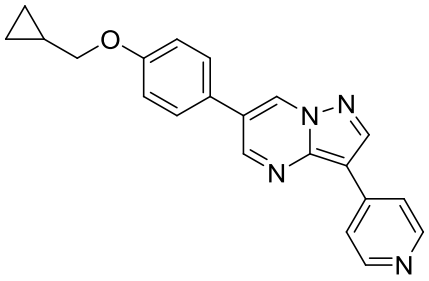
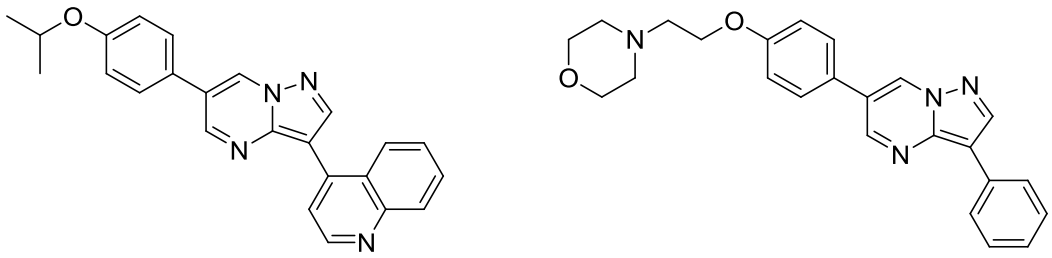


104

Figure 13. Compounds of special interest

This work was carried on by others and eventually both a potent and very selective inhibitor of BMP signaling, DMH1 (**105**), and a potent, selective VEGFR inhibitor, DMH4 (**106**) were developed (**Figure 14**)[40]. Using *in vitro* kinase assays, compound **105** had an IC_{50} of 107.9 nM at ALK2 (BMPR-I) and showed no inhibition of the closely related ALK5 (TGFβR-I), AMPK, KDR (VEGFR2), or

PDGFRβ. When **106** was tested in the same assays it showed selectivity for VEGFR with an IC_{50} of 161 nM at KDR (VEGFR2), IC_{50}s of 3.6 μM and 8.0 μM at ALK2 (BMPR-I) and AMPK, respectively, and no inhibition of ALK5 (TGFβR-I), while not tested on PDGFRβ. DMH1 (**105**) is not only potent, but also much selective than the lead compound Dorsomorphin (**83**) and other reported BMP inhibitors such as LDN-193189 (**84**) (**Table 3**).



DMH1 (**105**) DMH4 (**106**)

Figure 14. DMH1 (**105**) and DMH4 (**106**) selective and potent inhibitors of BMP and VEGF respectively

	I_C50 (nM)				
	ALK2 (BMPR-I)	ALK5 (TGFβR-I)	AMPK	KDR (VEGFR2)	PDGFRβ
DMH1 (**105**)	107.9	>30000	>30000	>30000	>30000
DMH4 (**106**)	3558.0	>30000	8038.0	161.0	n.t.
DM (**83**)	148.1	>30000	234.6	25.1	n.t.
LDN-193189 (**84**)	40.7	565.0	1122.0	214.7	n.t.

Table 3. Effects of Dorsomorphin and analogues on *in vitro* kinase assays

Conclusions

Thus, microwave assisted organic chemistry was applied to the synthesis of pyrazolo[1,5-*a*]pyrimidines. By doing this, a reaction sequence that required

>72 hours and provided 40-60% yield has been optimized to require only 30 minutes total reaction time (for three steps) with overall yields in excess of 80%. This new methodology was then used in the synthesis of a small library of Dorsomorphin analogs which were evaluated for their activity against BMP and VEGF. With the first library, a compound was found that was more selective and potent than the lead compound **83**. This work was continued on to develop the first selective potent BMP inhibitor, DMH1 (**105**). With this compound in hand other heterocycles can be explored that keep the substitutions in the 3 and 6 positions of the pyrazolo[1,5-*a*]pyrimidines in the same physical space while varying the heterocycle core. This strategy could provide some room in the intellectual property space, which in turn would allow the development of a BMP inhibitor into a drug. A drug that inhibits BMP could be a life altering and even a life saving therapy for patients with FOP.

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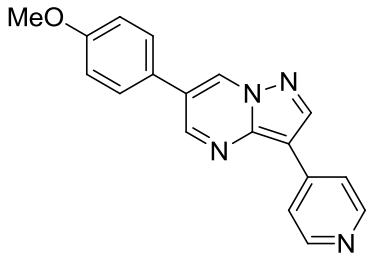
Experimental Section

General Experimental

All ^{1}H and ^{13}C NMR spectra were recorded on 300 MHz**,** 400 MHz or 500 MHz instruments. Chemical shifts are reported in ppmrelative to residual solvent peaks as an internal standard set to δ 7.26 and δ 77.0 (CDCl3). Data are reported as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q =quartet, br = broad, m = multiplet), integration, coupling constant (Hz). 2-Dimensional NMR spectra (NOESY and COSY) were obtained on a 500 spectrometer (operating at 500.134 MHz for ^{1}H and 124.996 MHz for ^{13}C). IR spectra were recorded as thin films and are reported in wavenumbers (cm-1). Low resolution mass spectra were obtained on an analytical LCMS with electrospray ionization. High resolution mass spectra were recorded on a Qtof-API-US system. The HRMS results were obtained with ES as the ion source and leucine enkephalin as the reference. Analytical thin layer chromatography was performed on 250 μM silica gel 60 F254 plates. Visualization was accomplished with UV light, and/or the use of ninhydrin, anisaldehyde and ceric ammonium molybdate solutions followed by charring on a hot-plate. Chromatography on silica gel was performed using Silica Gel 60 (230- 400 mesh) from Sorbent Technologies. Analytical HPLC was performed on an analytical LCMS with UV detection at 214 nm and 254 nm along with ELSD detection. Chiral HPLC was performed on an analytical HPLC utilizing a Chiracel OD, OJ or Chiralpak AD columns (4.6 mm x 25 cm). Solvents for extraction, washing and chromatography were HPLC grade. All reagents were purchased and were used without

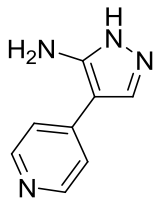
purification. All polymer-supported reagents were purchased. Flame-dried (under vacuum) glassware was used for all reactions. All reagents and solvents were commercial grade and purified prior to use when necessary. Mass spectra were obtained on a Q-TOF API-US mass spectrometer to acquire high-resolution mass spectrometry (HRMS) data.

Experimental:

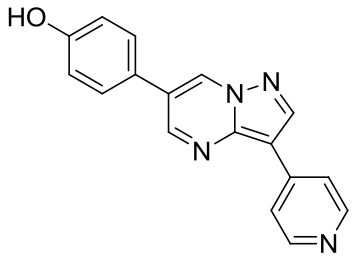


6-(4-methoxy)-3-(pyridin-4-yl)pyrazolo[1,5-*a*]pyrimidine (98). The experimental for **98** is the general procedure for pyrazolo[1,5-*a*]pyrimidines (**89**). Pyrazole **96** (664 mg, 4mmol) is dissolved in 3.5 mL of 5% AcOH/EtOH and then 2-(4-methoxyphenyl)malondialdehyde (**97**) (712 mg, 4 mmol) was added. The microwave reaction vessel was sealed and irradiated with microwaves to 170^{o}C for 10 minutes. Upon rapid cooling to 40^{o}C, the product precipitated from solution. NH_4OH was added to neutralize the solution, and the product collected by filtration and washed with water to afford **98** as a white solid (1.18 g, 98%). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 8.87 (d, *J*=2 Hz, 1H), 8.82 (d, *J*=2 Hz, 1H), 8.64 (d, *J*=5.6 Hz, 2H), 8.53 (s, 1H), 8.01 (d, *J*=6 Hz, 2H), 7.54 (d, *J*=8.8 Hz, 2H),

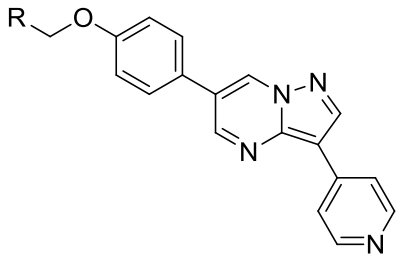
7.07 (d, *J*=8.8 Hz, 2H), 3.88 (s, 3H);); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 160, 150.3, 150.1, 144.4, 143.2, 139.6, 131.6, 128, 125.7, 123.2, 120.2, 115, 107.7, 55.4; LCMS, single peak, 2.27 min, m/e, 303.1 (M+1).



4-(pyridin-4-yl)-1*H*-pyrazol-5-amine (96). The experimental for **96** is the general procedure for the aminopyrazoles **87**. 2-(pyridin-4-yl)acetonitrile **99** (565 mg, 5 mmol) was disolved in a 3 mL solution of DMA:DMF:$PhCF_3$ (1:1:2). The 5 mL microwave reaction vessel was heated to 150^{o}C for 10 minutes by microwave irradiation. LCMS (single peak, 1.1 min, m/e, 174.1 (M+1)) indicated that all **99** was consumed affording acrylonitrile **100**. Hydrazine (160 µL, 5.1 mmol) was then added via syringe and the vial heated to 140^{o}C for 10 minutes. Aqueous work-up, followed by preparartive LCMS afforded 680 mg (85%) of 4-(pyridin-4-yl)-1*H*-pyrazol-5-amine, **96** as a purple solid. ^{1}H NMR (d_6-DMSO, 400 MHz): δ (ppm): 8.38 (d, *J*=6 Hz, 2H), 7.84 (s, 1H), 7.47 (d, *J*=6 Hz, 2H), 5.14 (bs, 2H); ^{13}C NMR (d_6-DMSO, 100 MHz): δ (ppm) 154, 149.5, 141.4, 132, 124, 119; LCMS, single peak, 0.39 min, m/e, 161.1 (M+1).



6-(4-phenol)-3-(pyridin-4-yl)pyrazolo[1,5-*a*]pyrimidine (101). **98** (1.5g, 5mmol) was disolved in a 20 mL solution of 48% HBr:Acetic acid (1:1) in a 20 mL microwave reaction vessel which was then heated to 180^{o}C for 10 minutes by microwave irradiation. Upon cooling the phenol **101** precipatated out of soultion and was filtered to yield the HBr salt as a yellow solid (1.83g, quanitative yield). %). ^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm): 9.84 (brs, 1H), 9.61 (d, *J*=2.4 Hz, 1H), 9.25 (s, 1H), 8.83 (d, *J*=6.8 Hz, 2H), 8.71 (d, *J*=6.8 Hz, 2H), 7.74 (d, *J*=8.8 Hz, 2H), 6.93 (d, *J*=8.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ (ppm): 158.4, 152.7, 148.4, 145.8, 144.9, 141.6, 133.3, 128.5, 124.1, 123.0, 120.6, 116.1, 104.2; LCMS, single peak, 1.95 min, m/e, 289.1 (M+1).

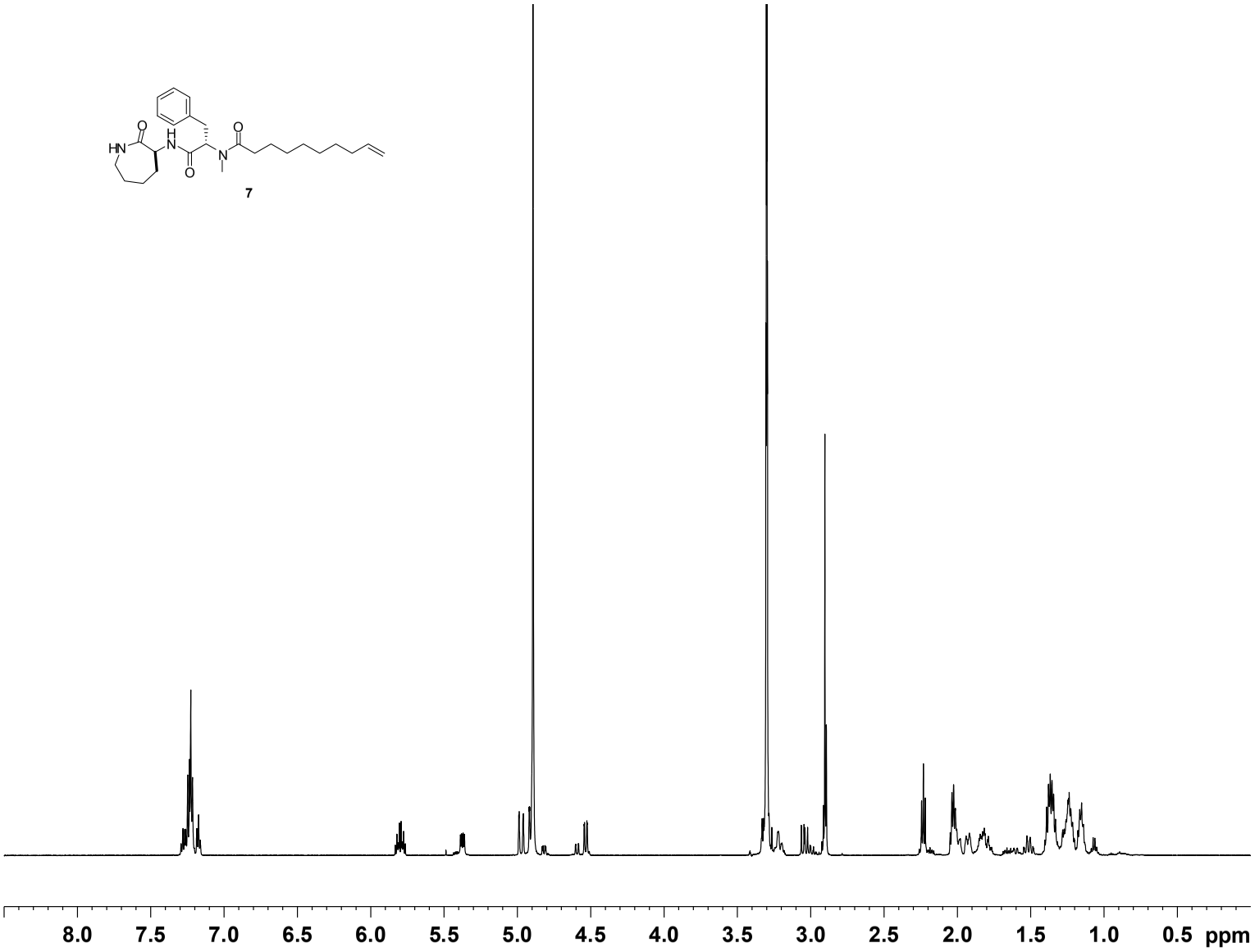


Alkyl ethers (102). General procedure: **101** (50mg, 0.14mmol) was dissloved in 5 mL of DMF in a 5 mL microwave reaction vessel. KI (69.7mg, 0.42mmol),

Cs_2CO_3 (182mg, 0.56mmol) and the alkyl halide (0.42mmol) were added the vial sealed and irradiated with microwaves to 160^{o}C for 60 minutes. Upon cooling the mixture was filtered, the eluent dried down on the sample concentrater, and purified by preparartive LCMS to afford **102** in >90% purity.

APPENDIX

NMR SPECTRA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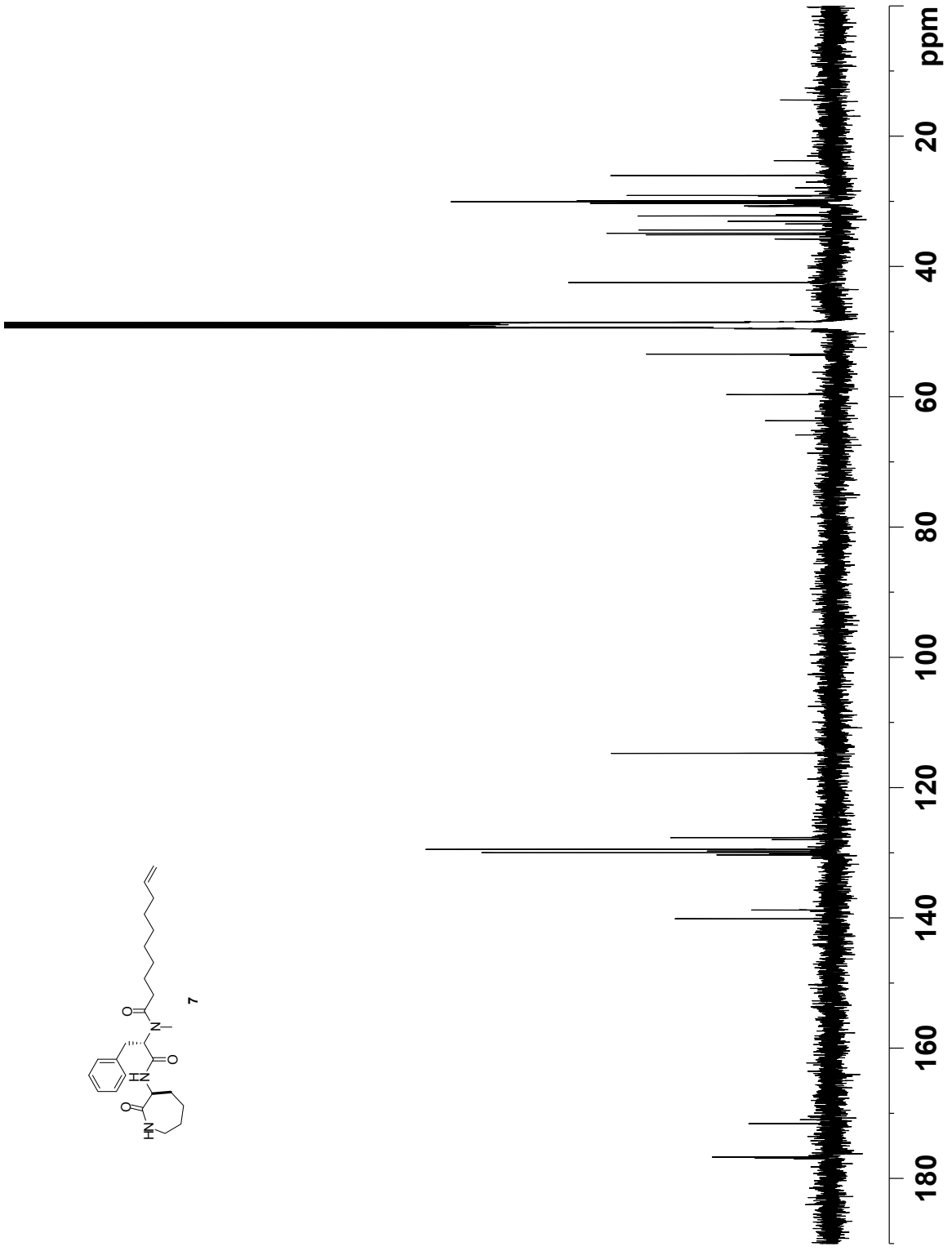
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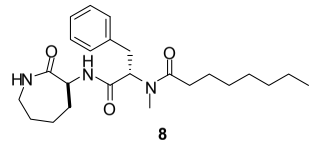

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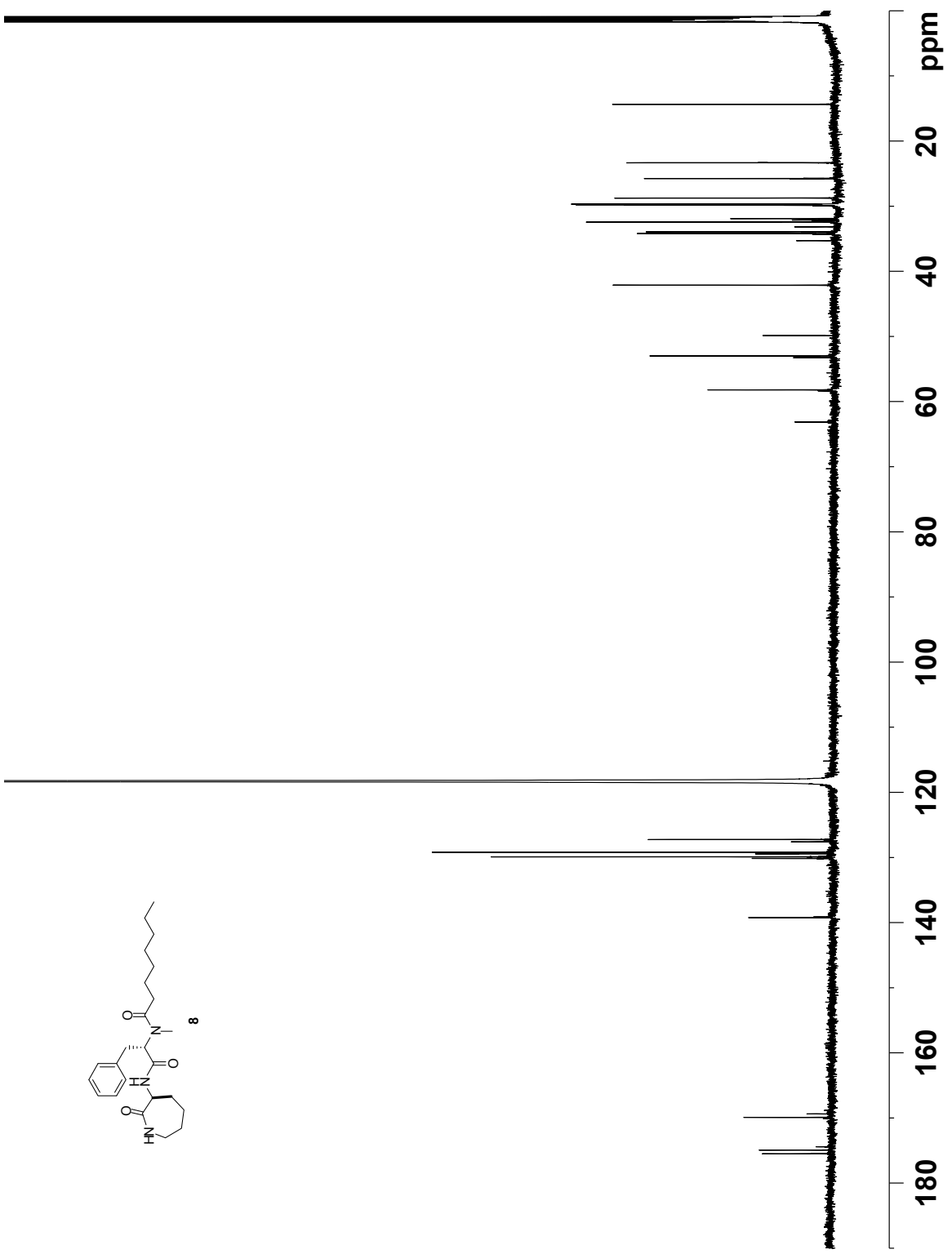
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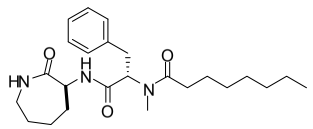

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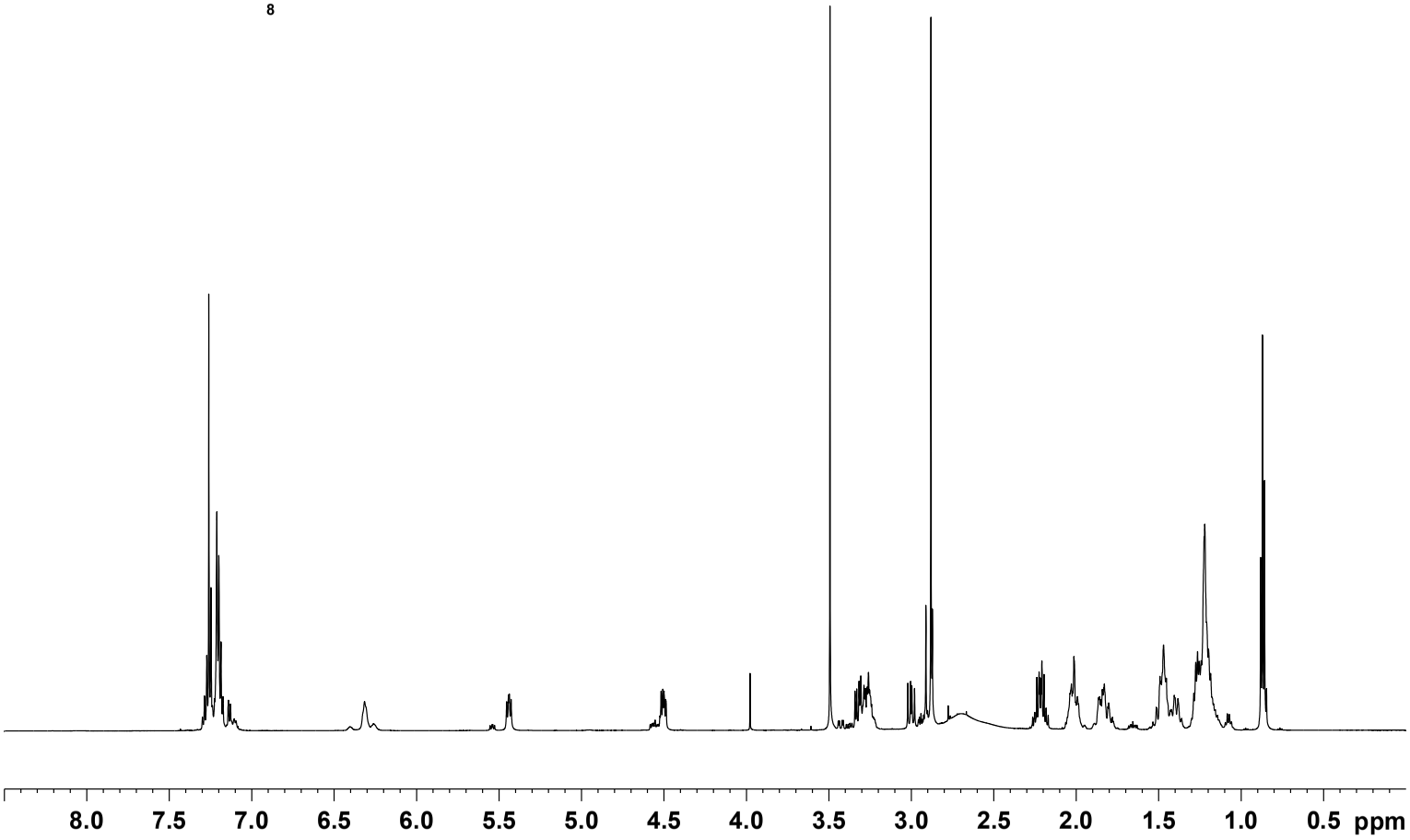

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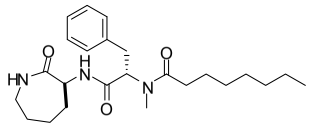

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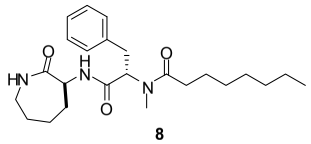

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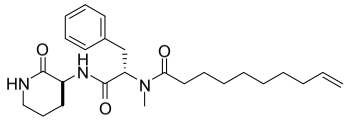

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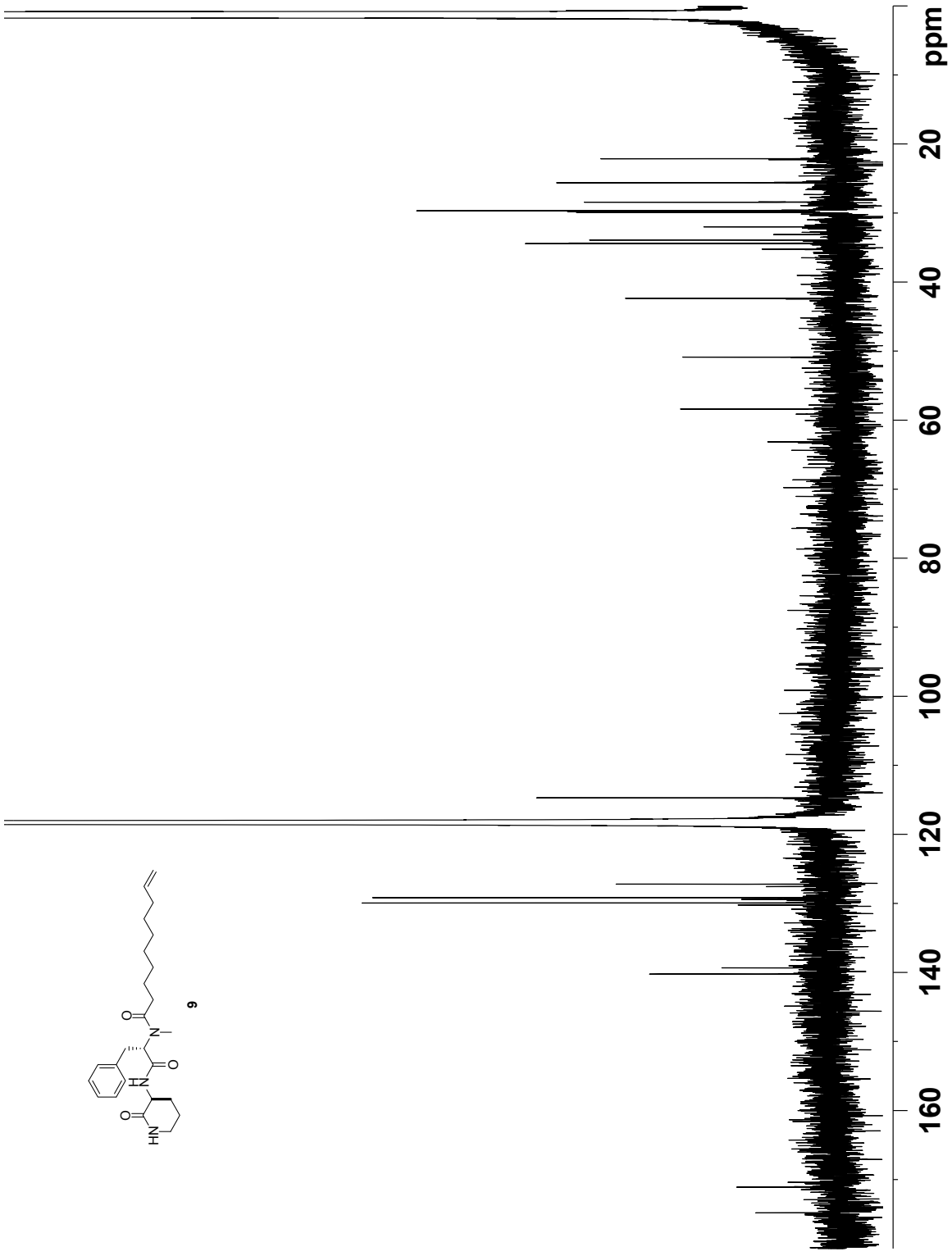
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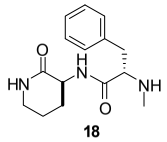

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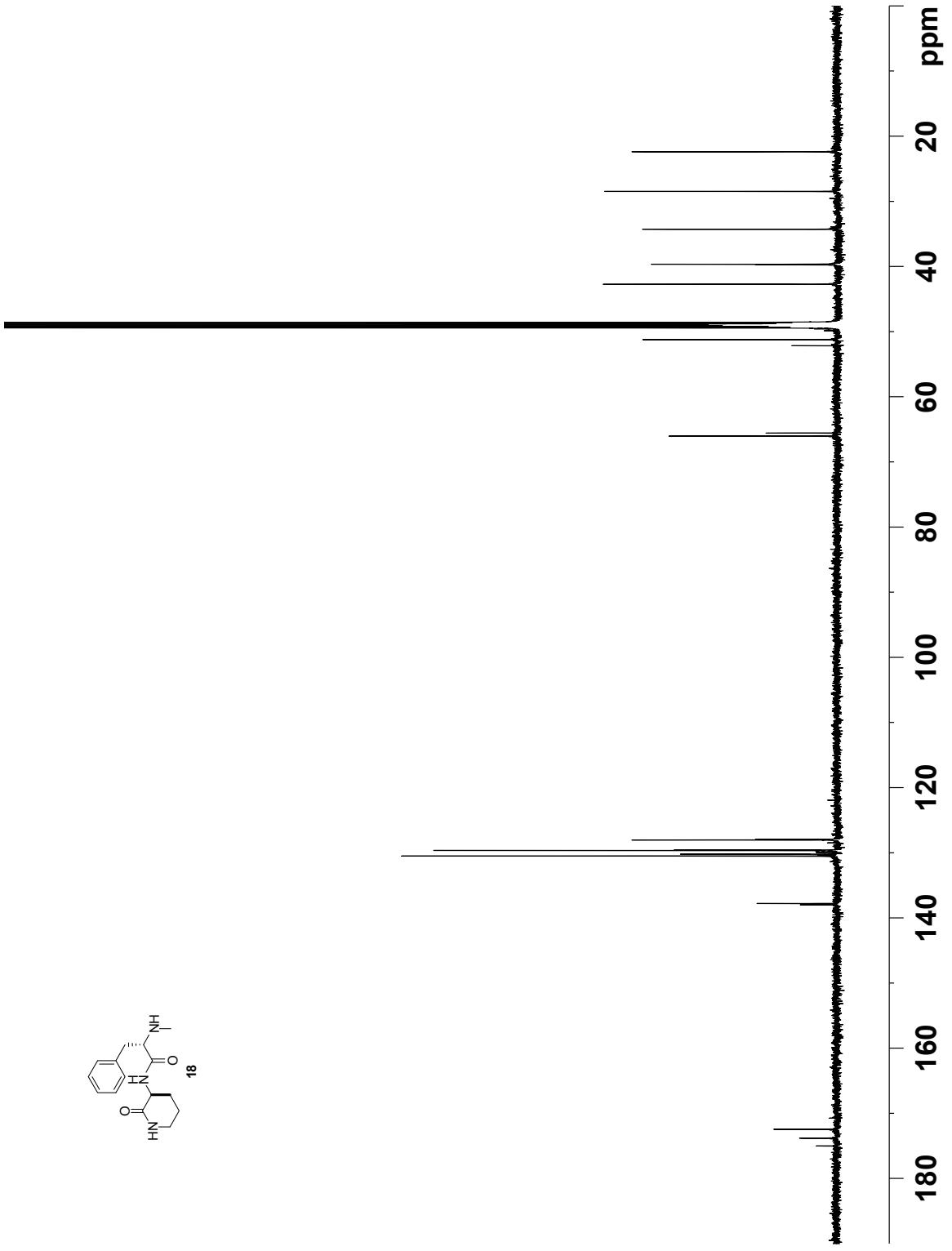

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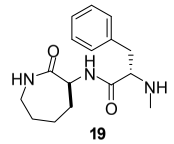

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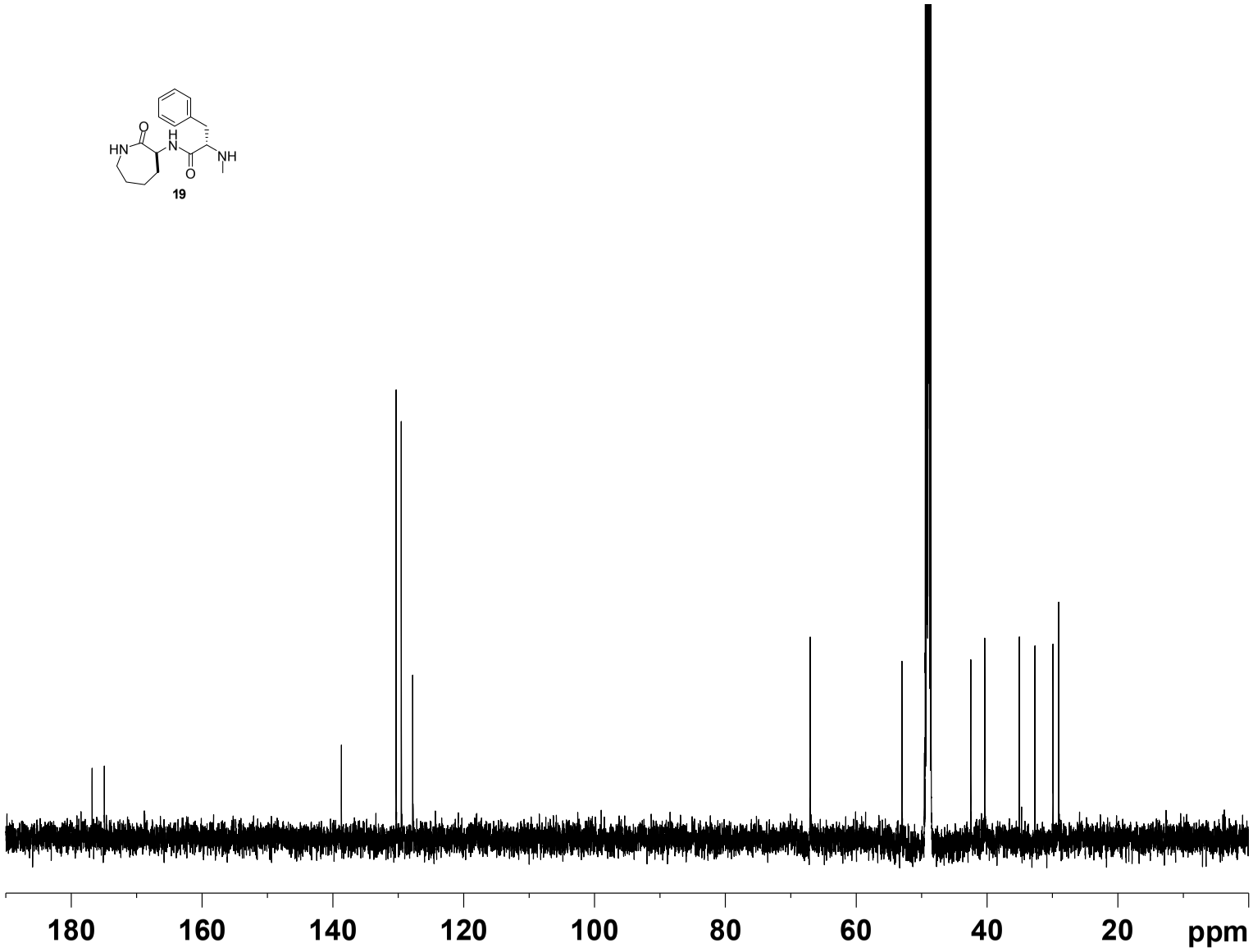
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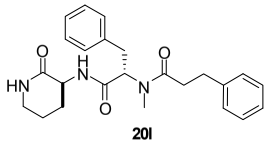

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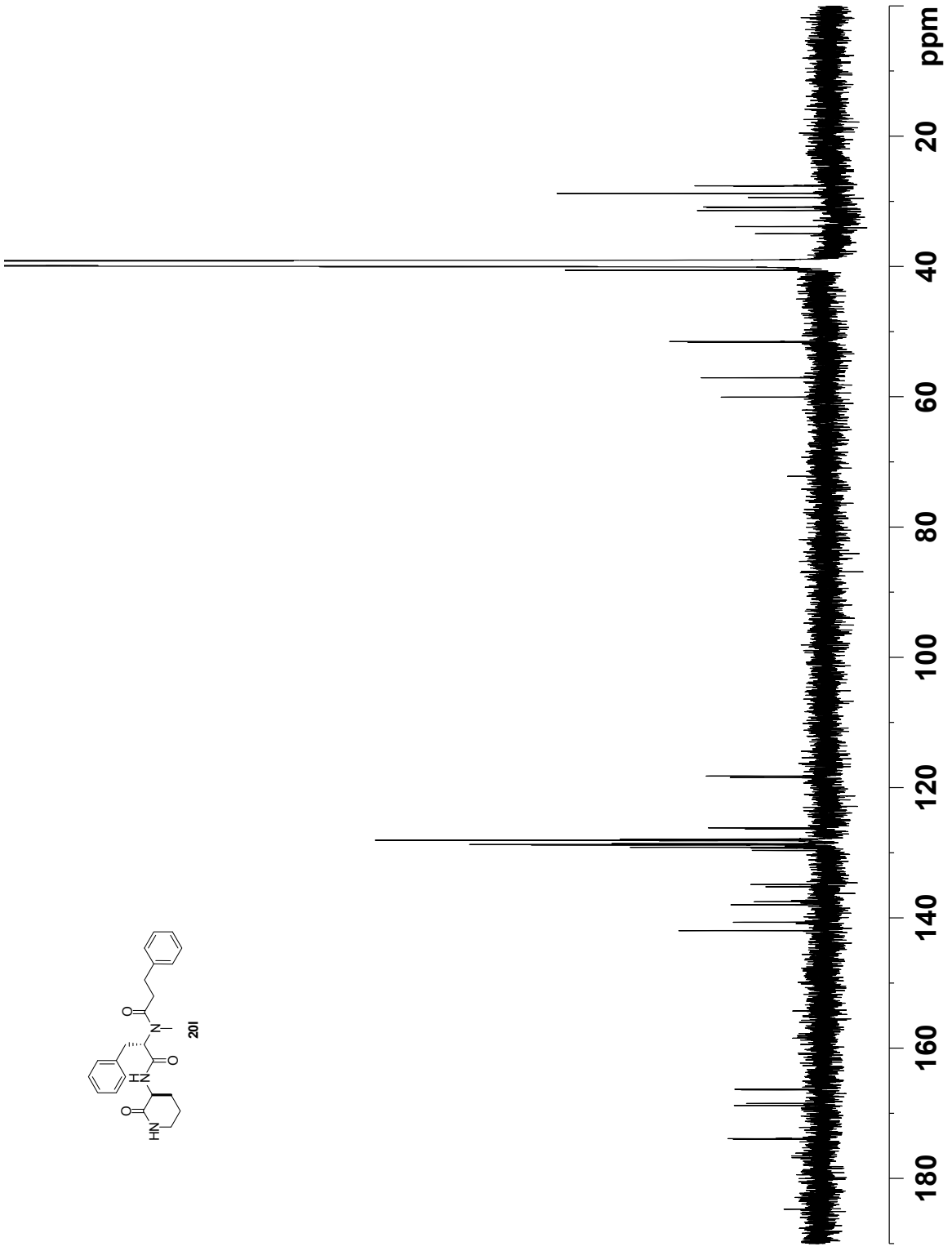
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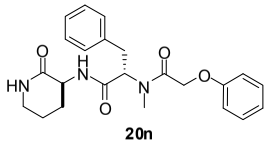

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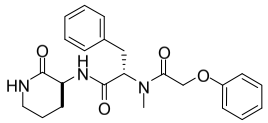

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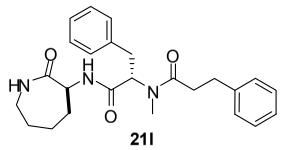

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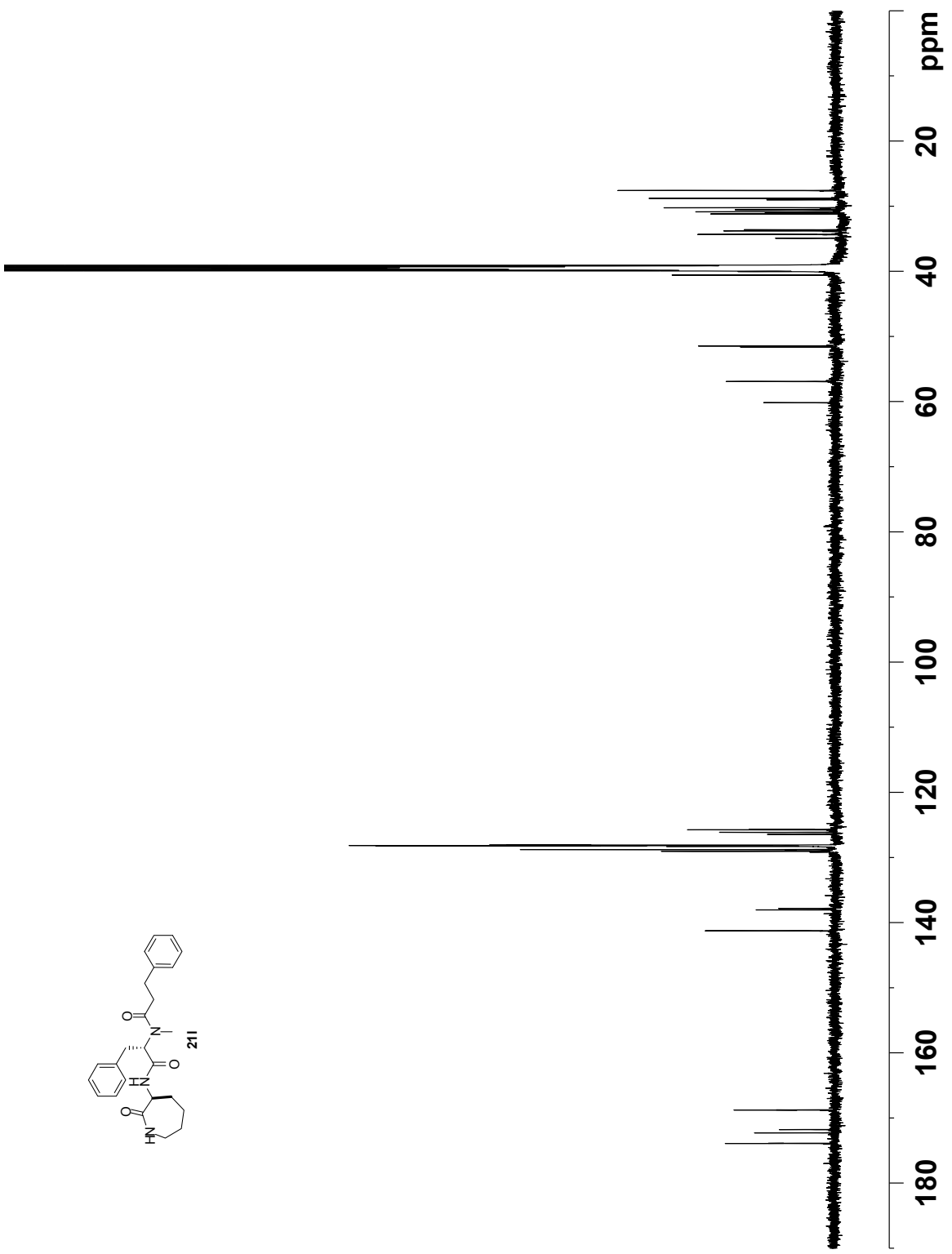
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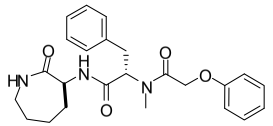

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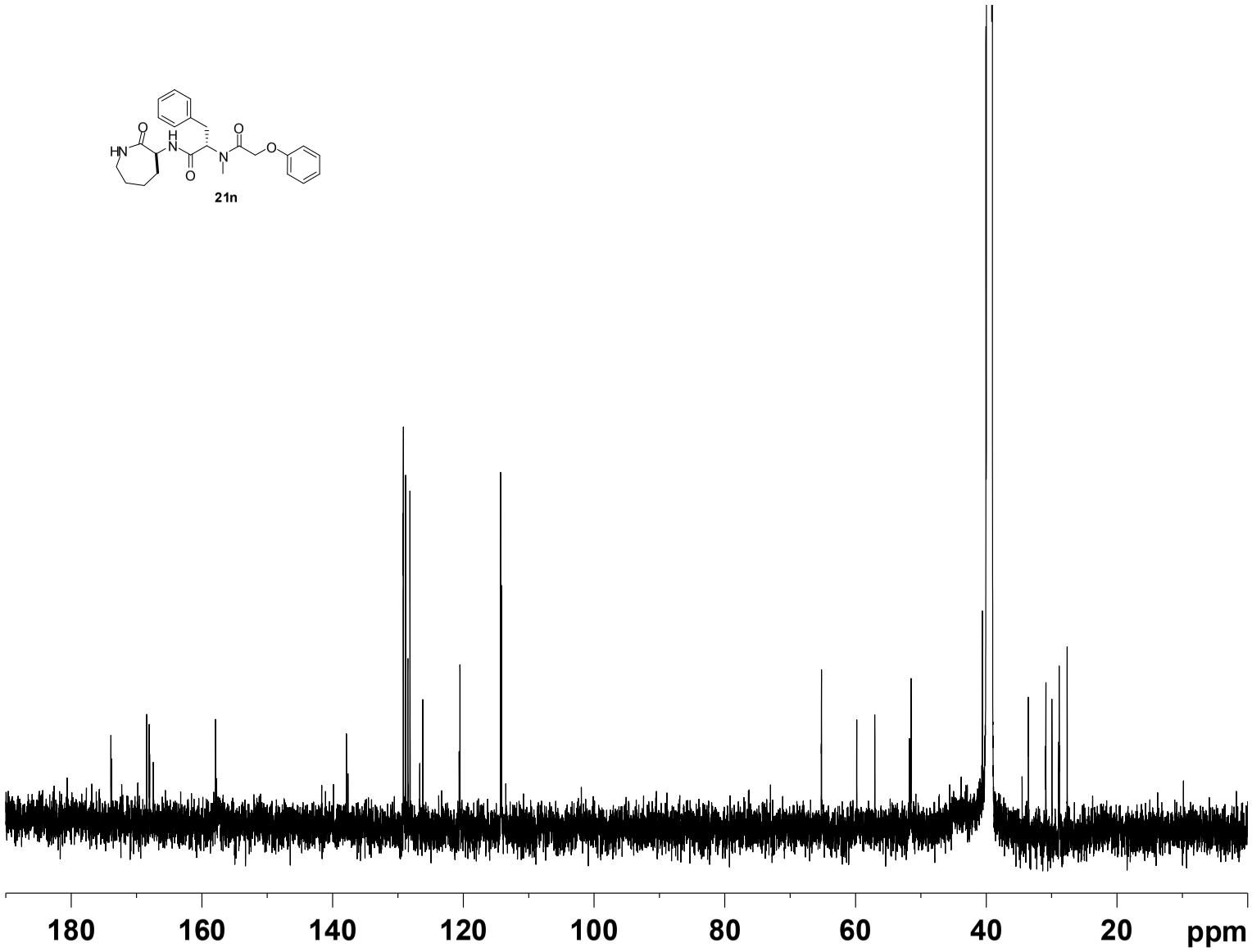
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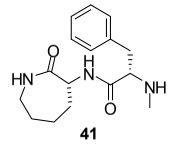

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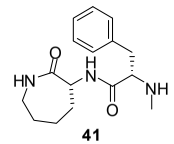

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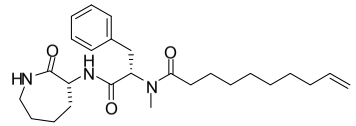

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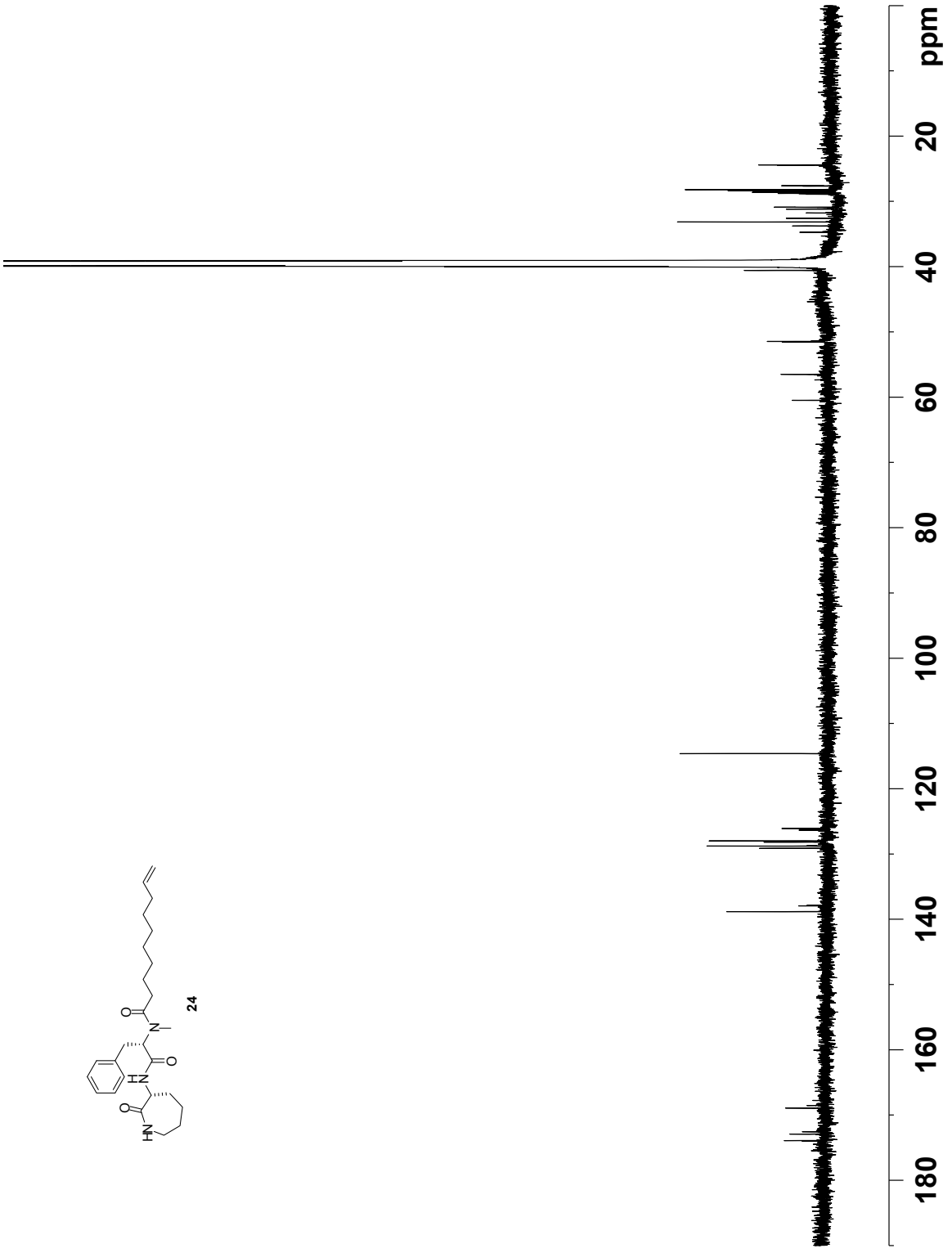
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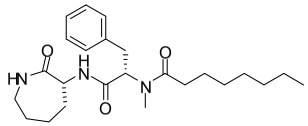

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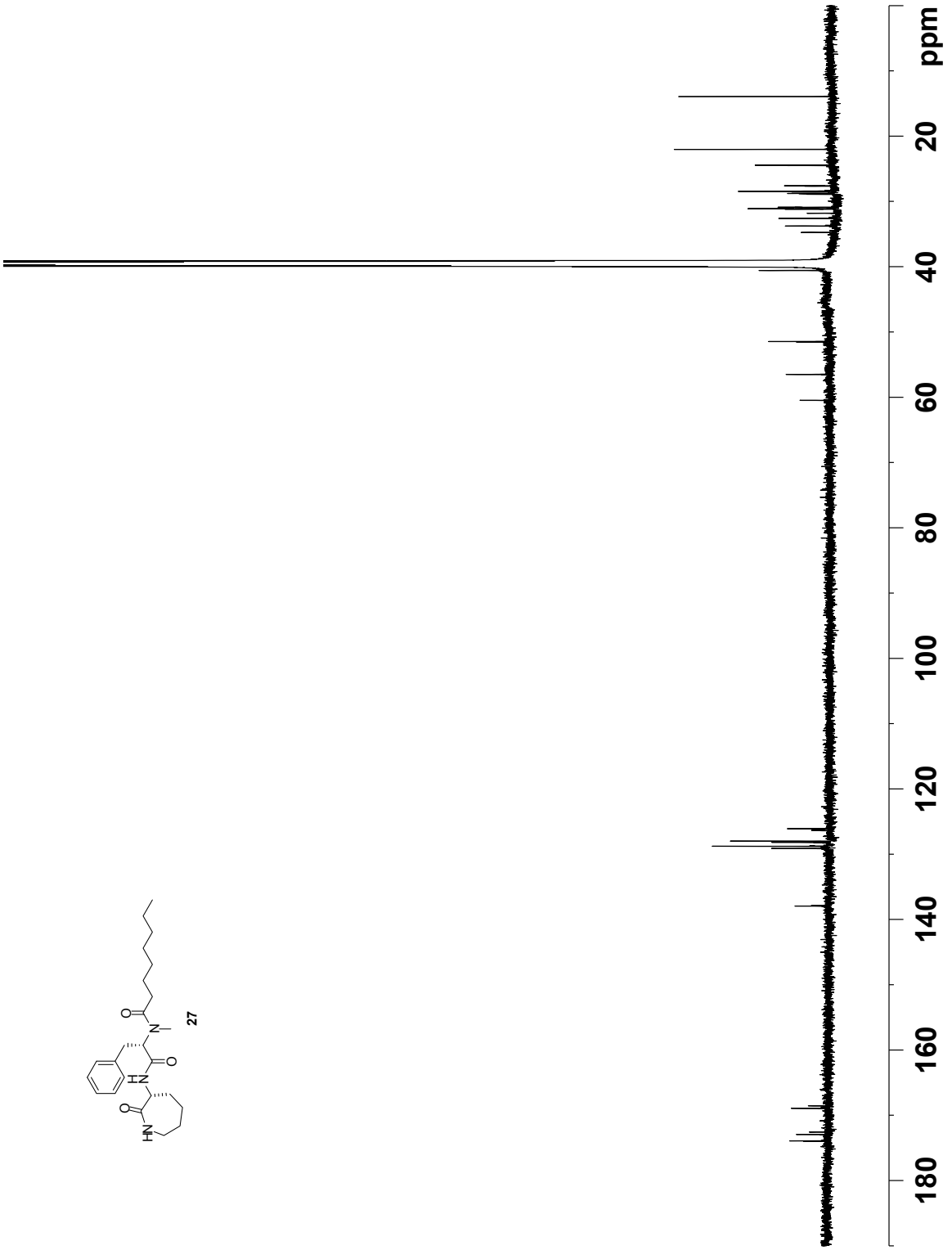
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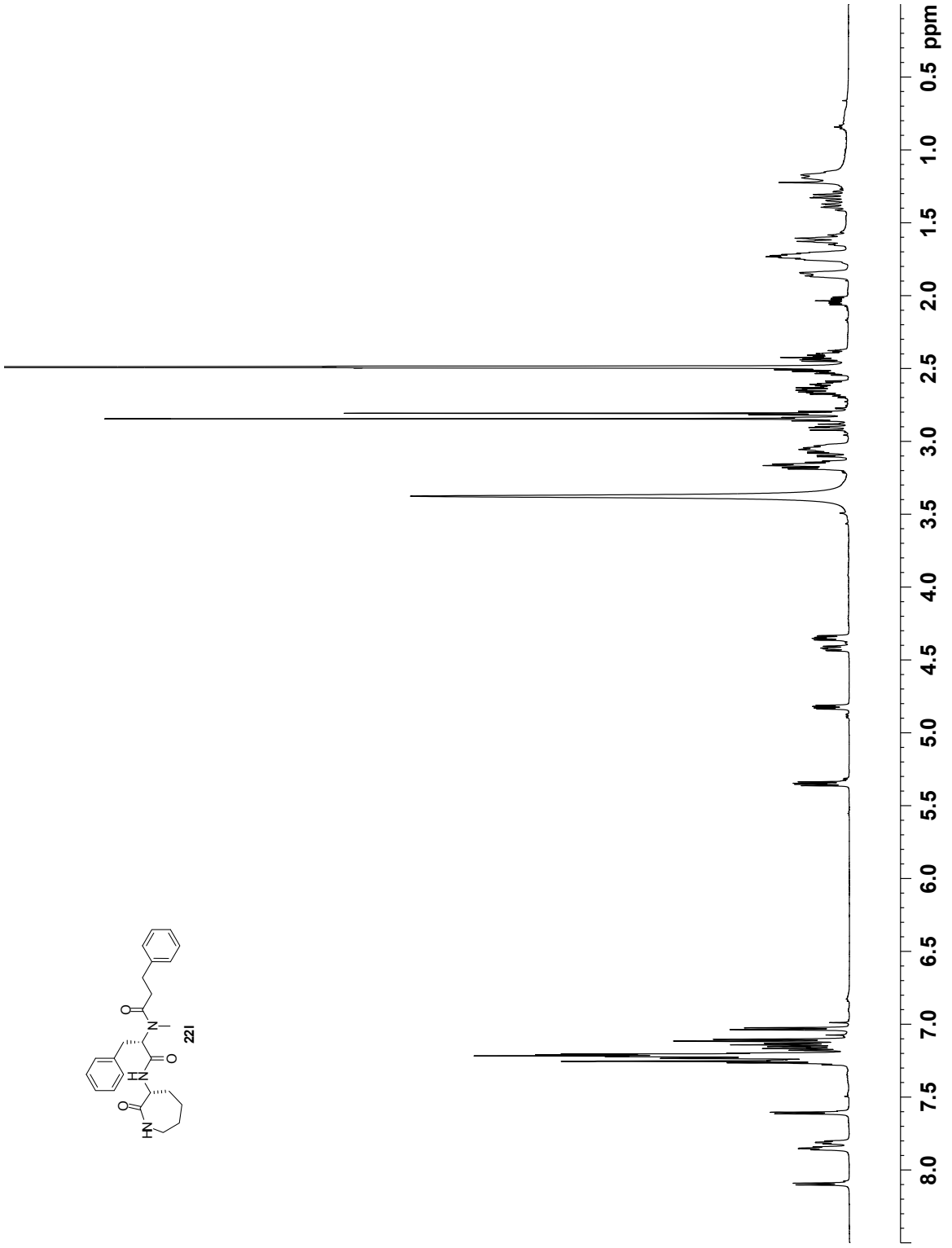
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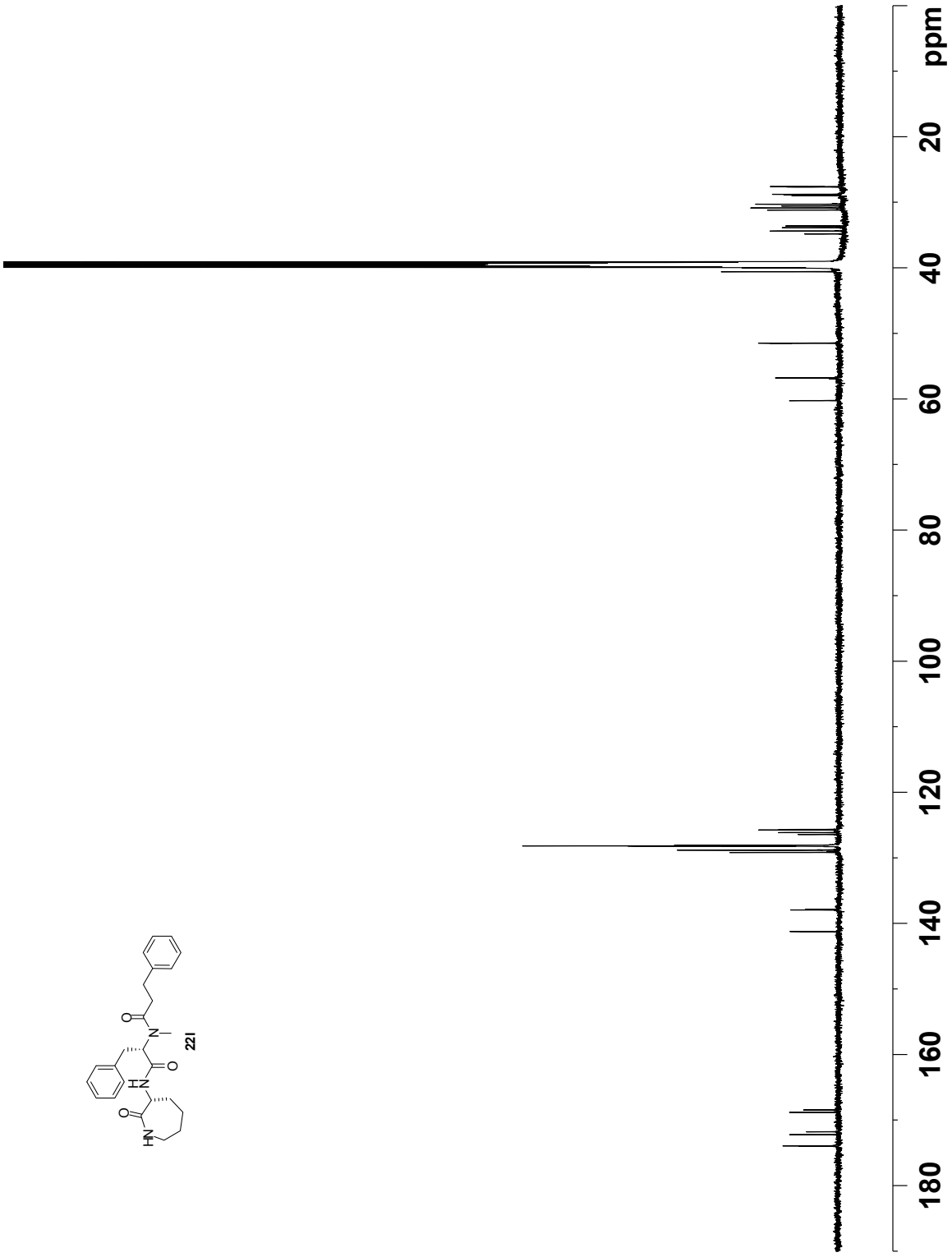
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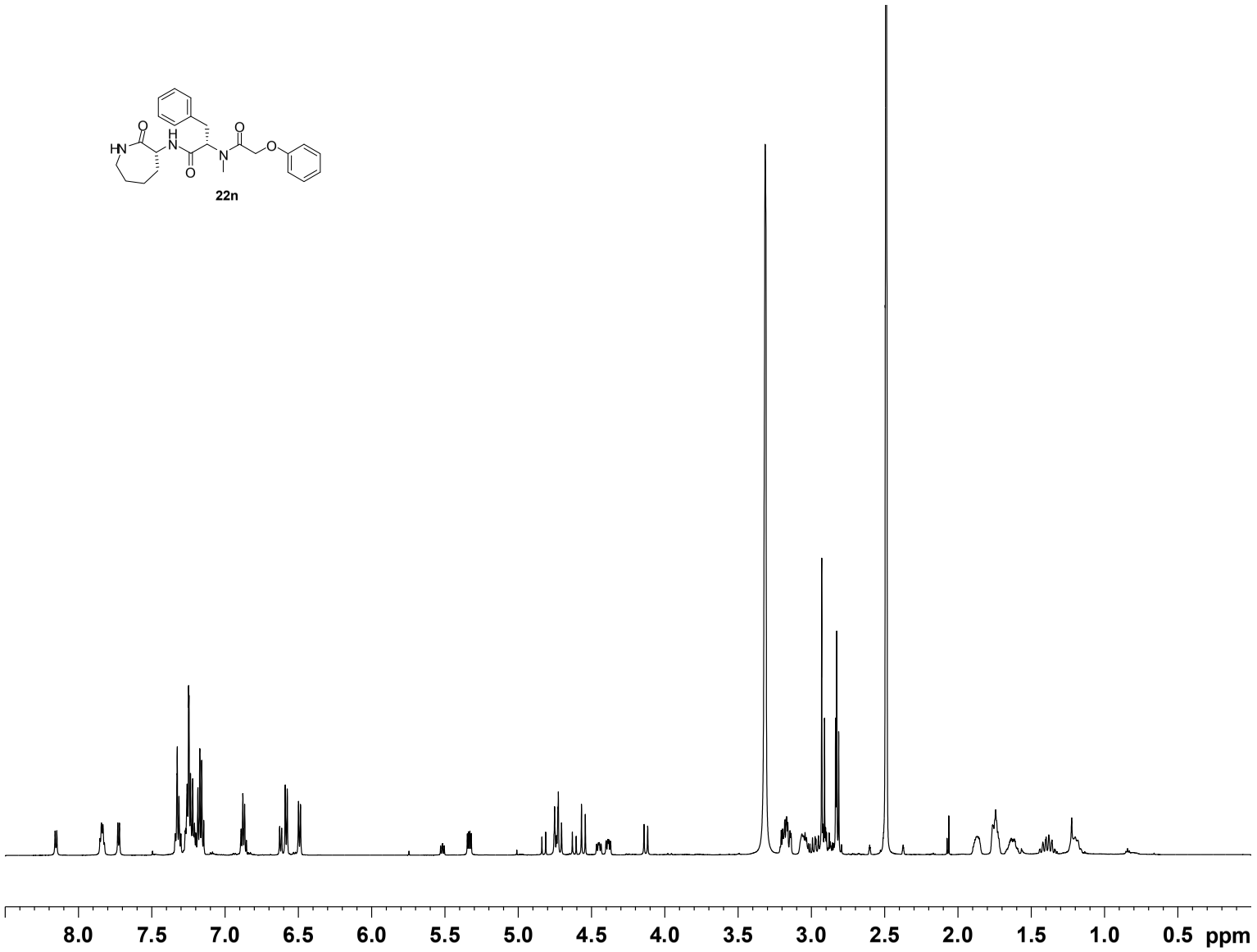
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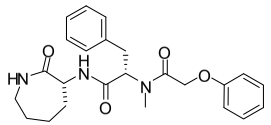

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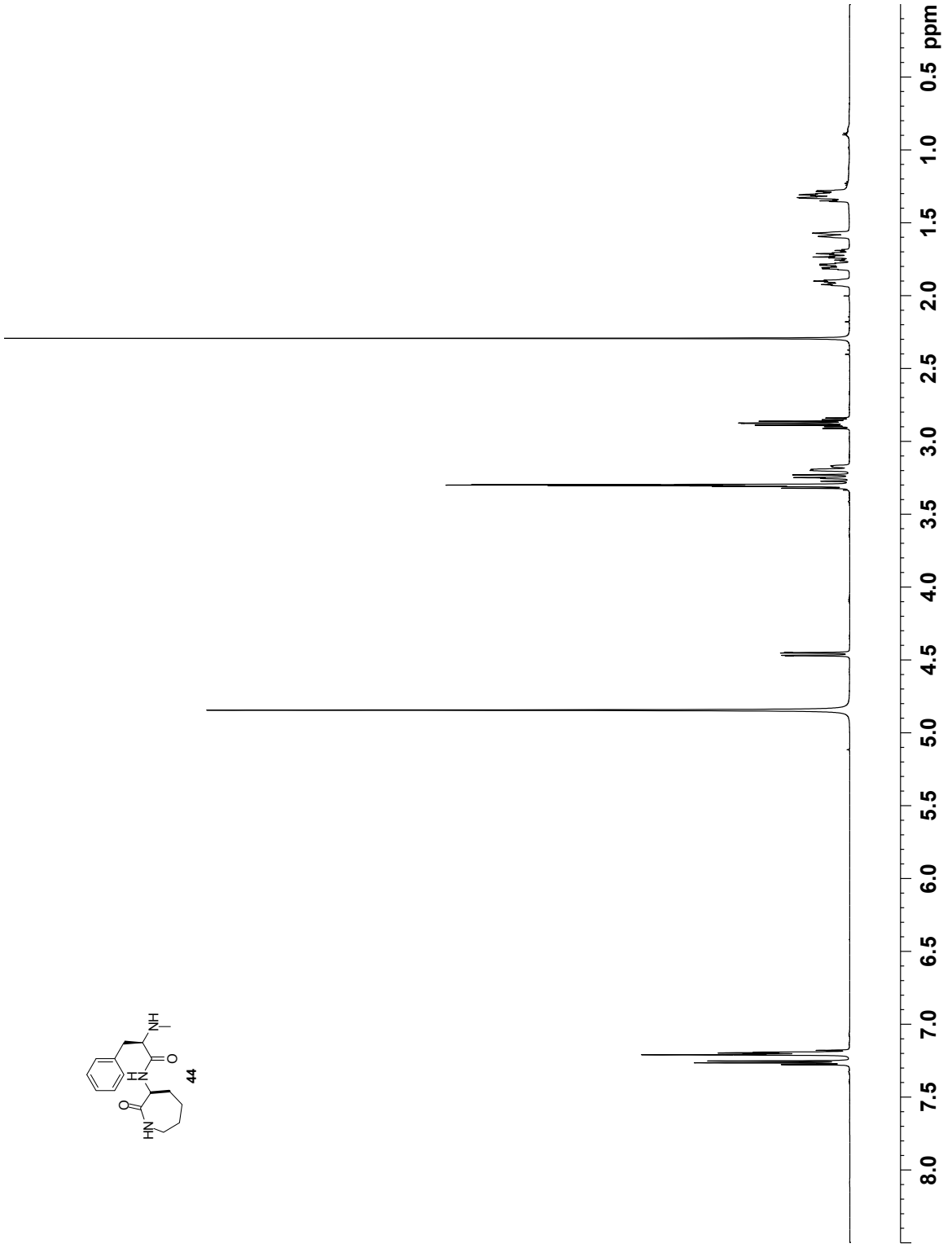
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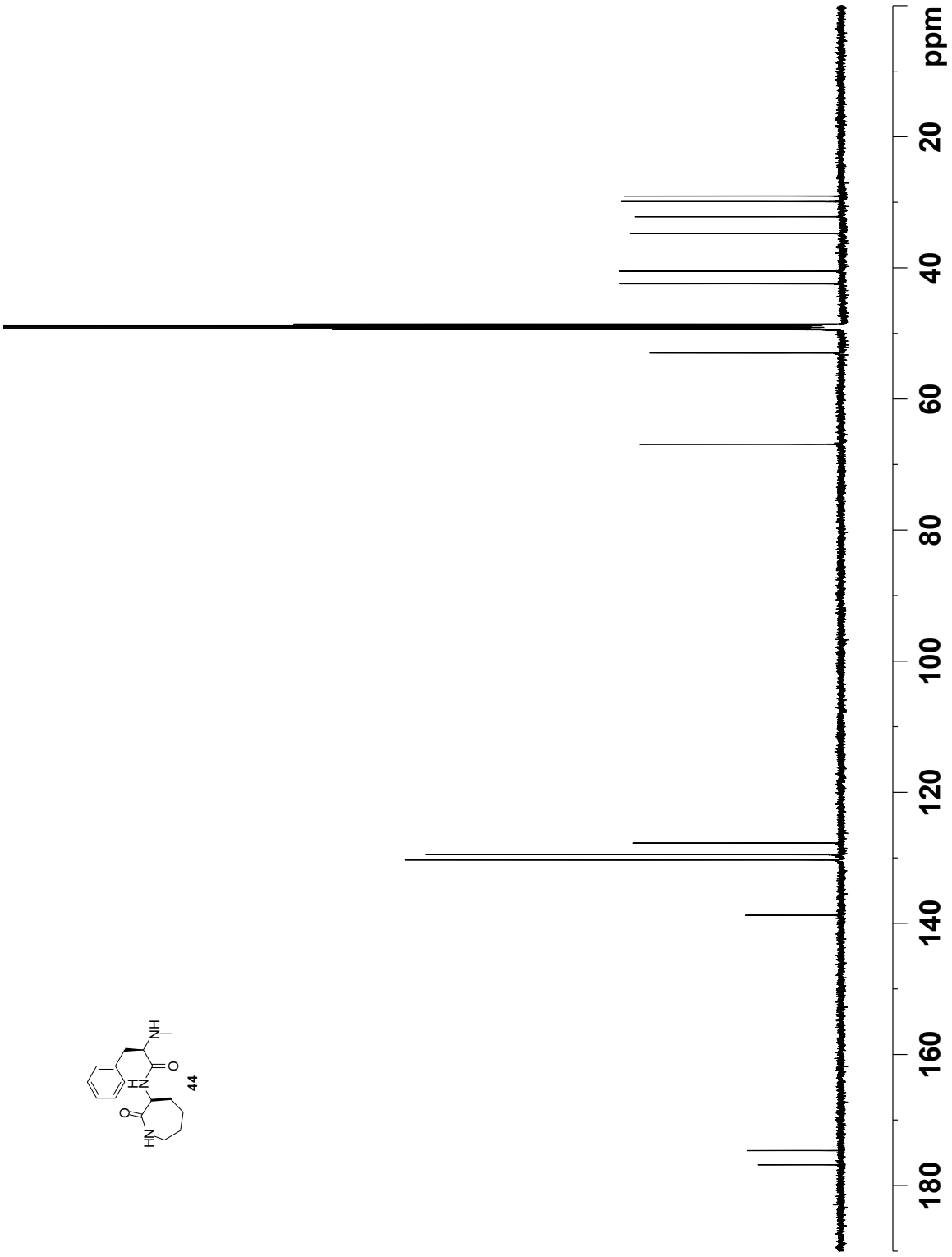

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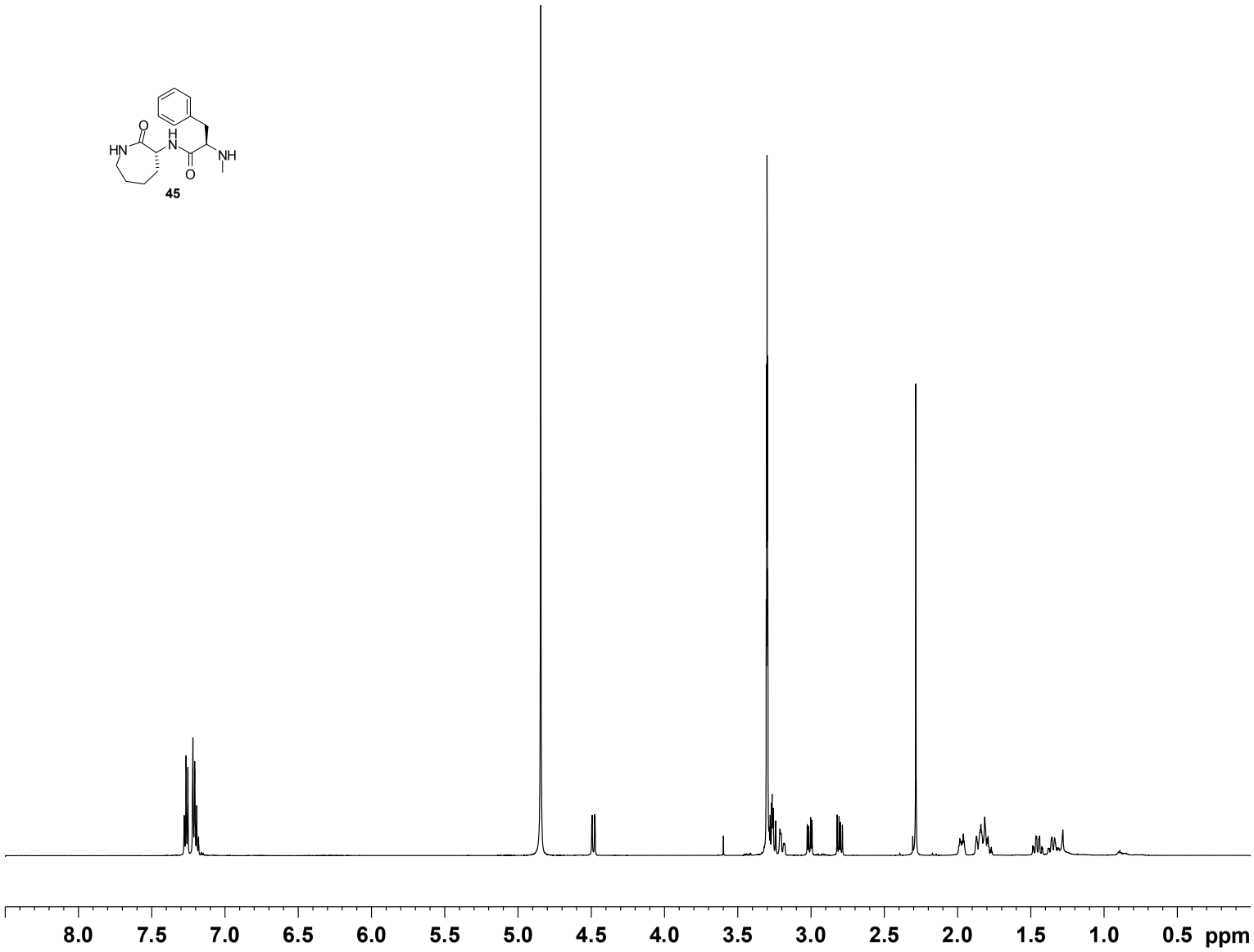
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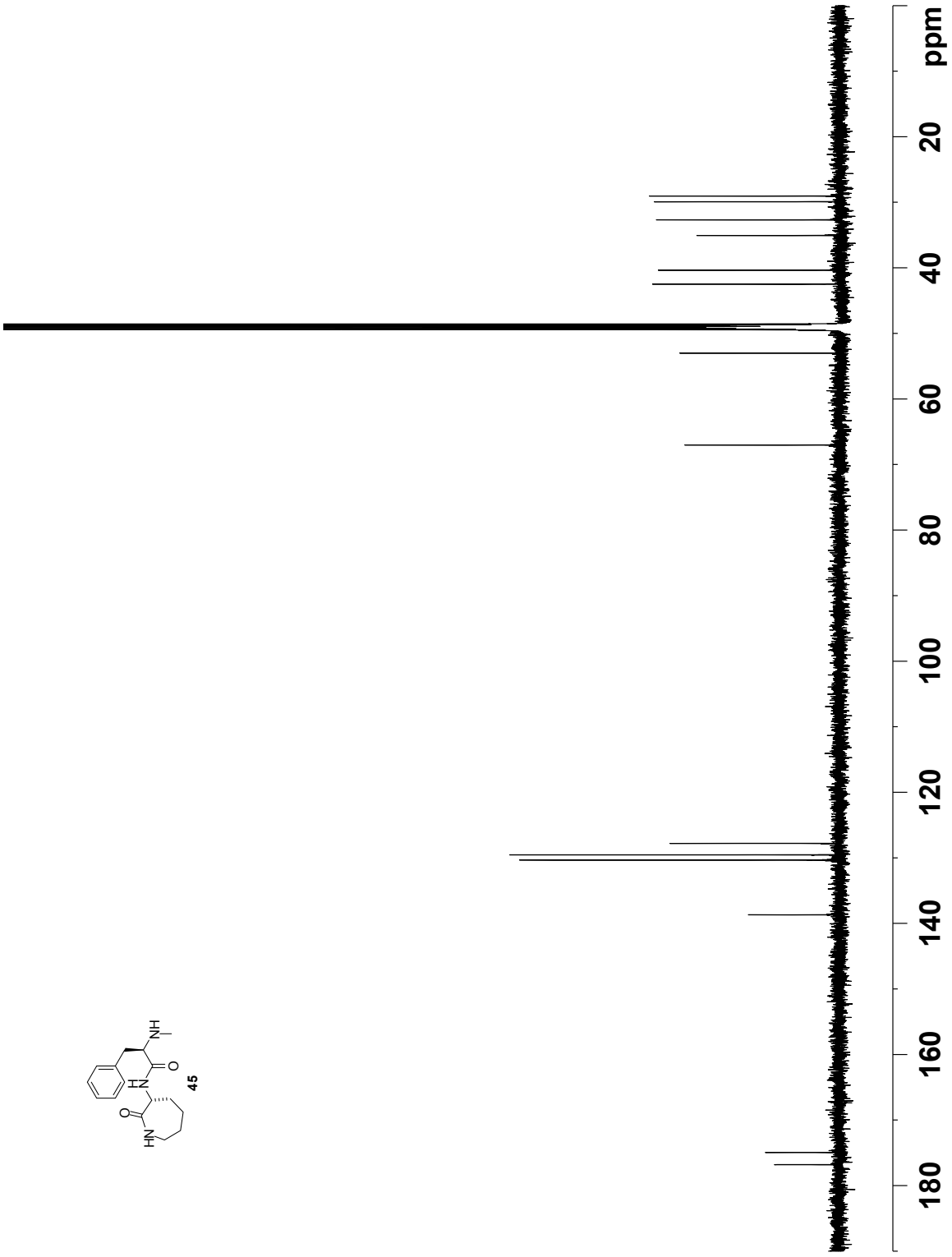

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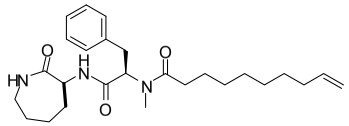

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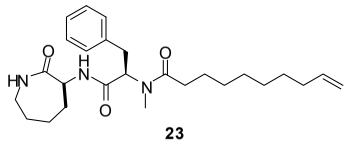

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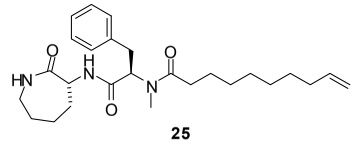

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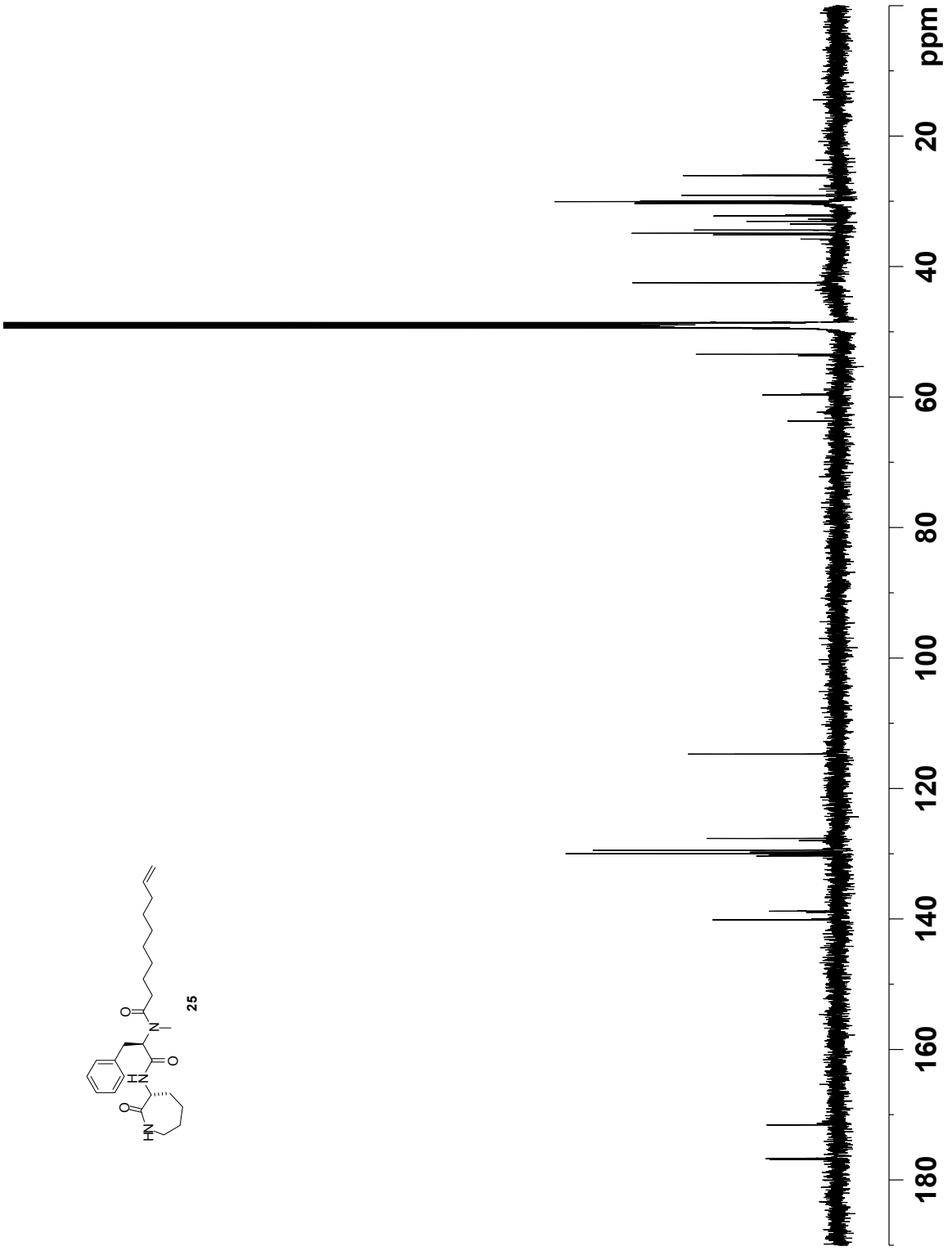
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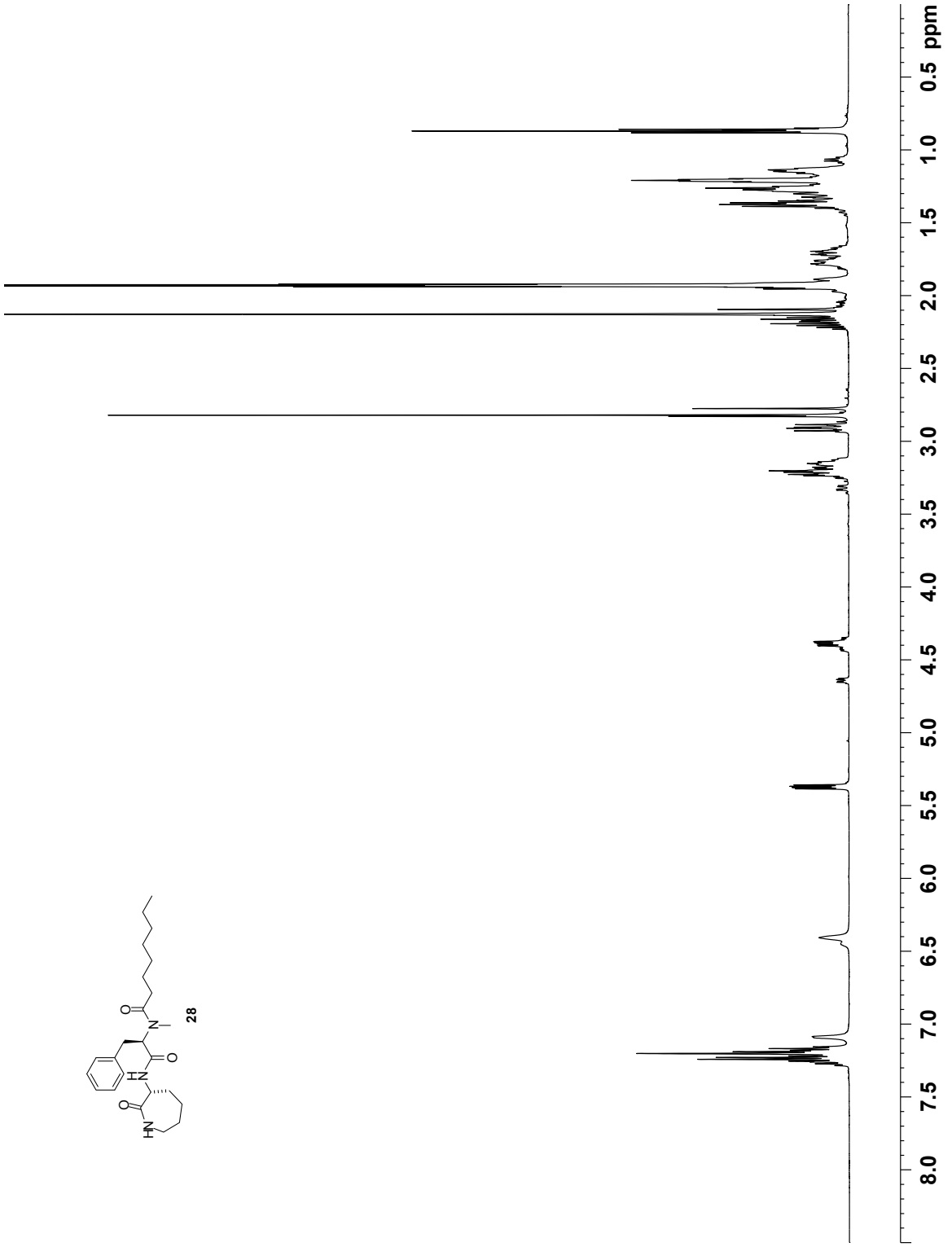
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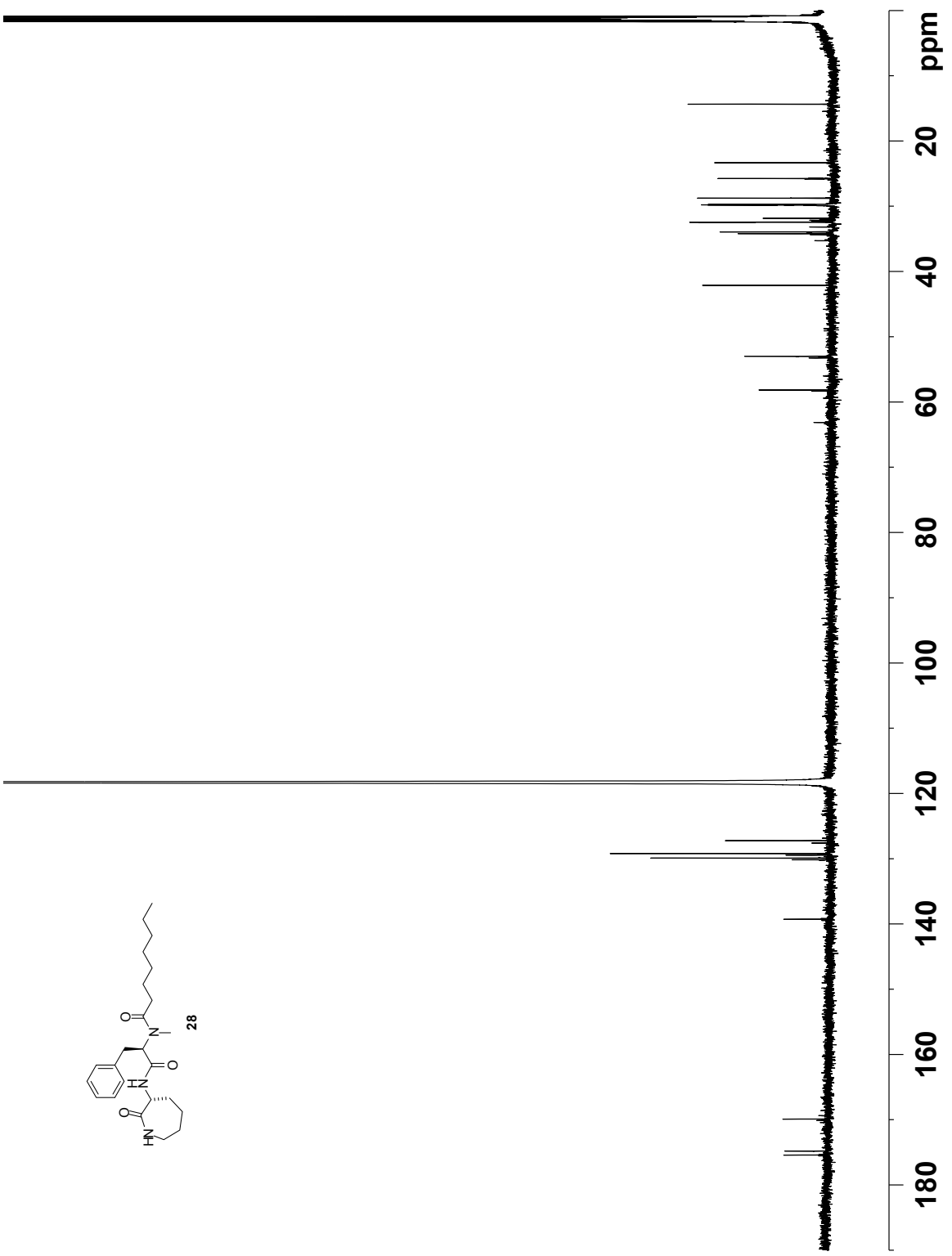
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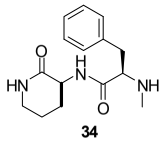

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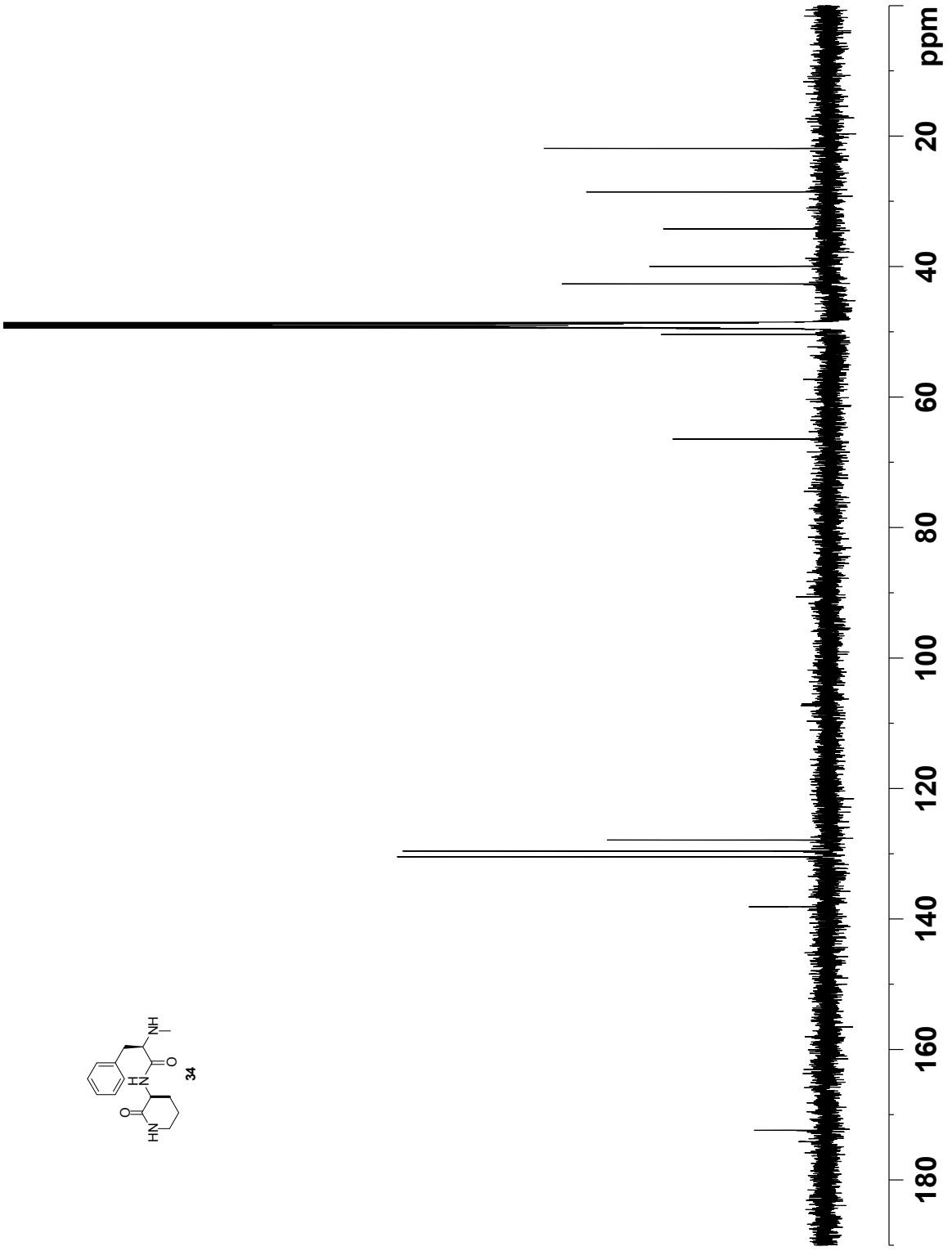

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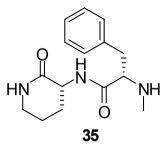

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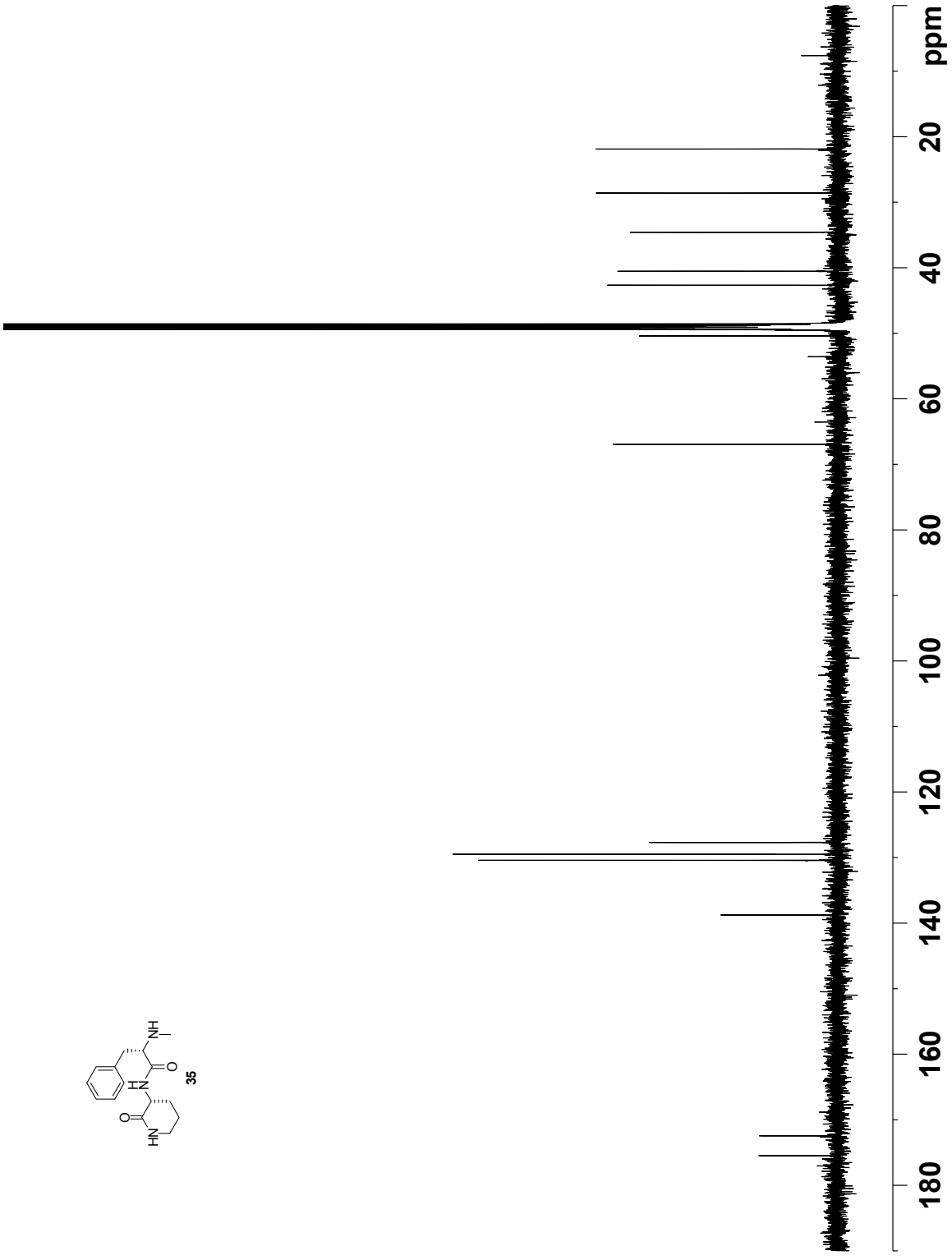

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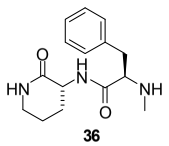

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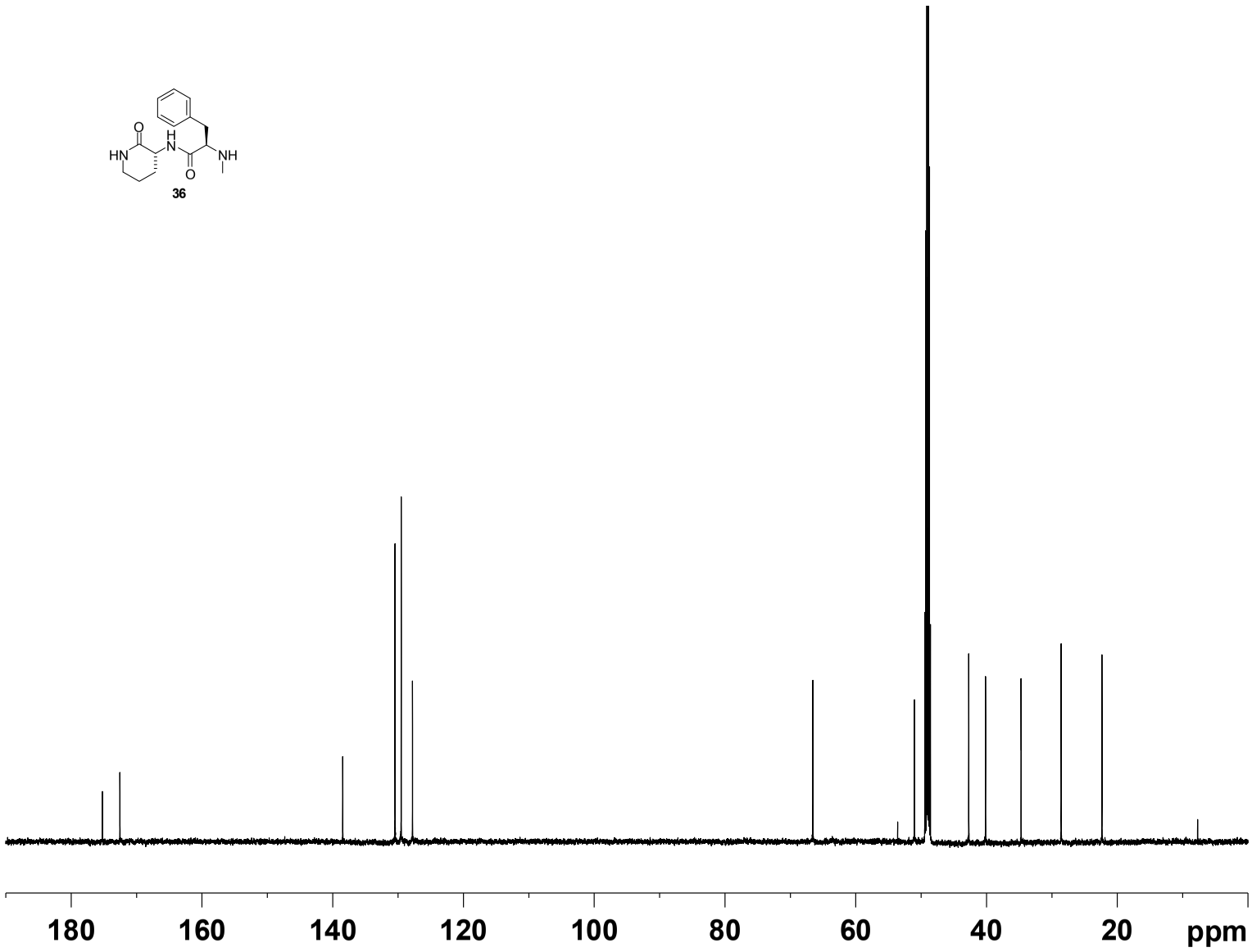
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NH
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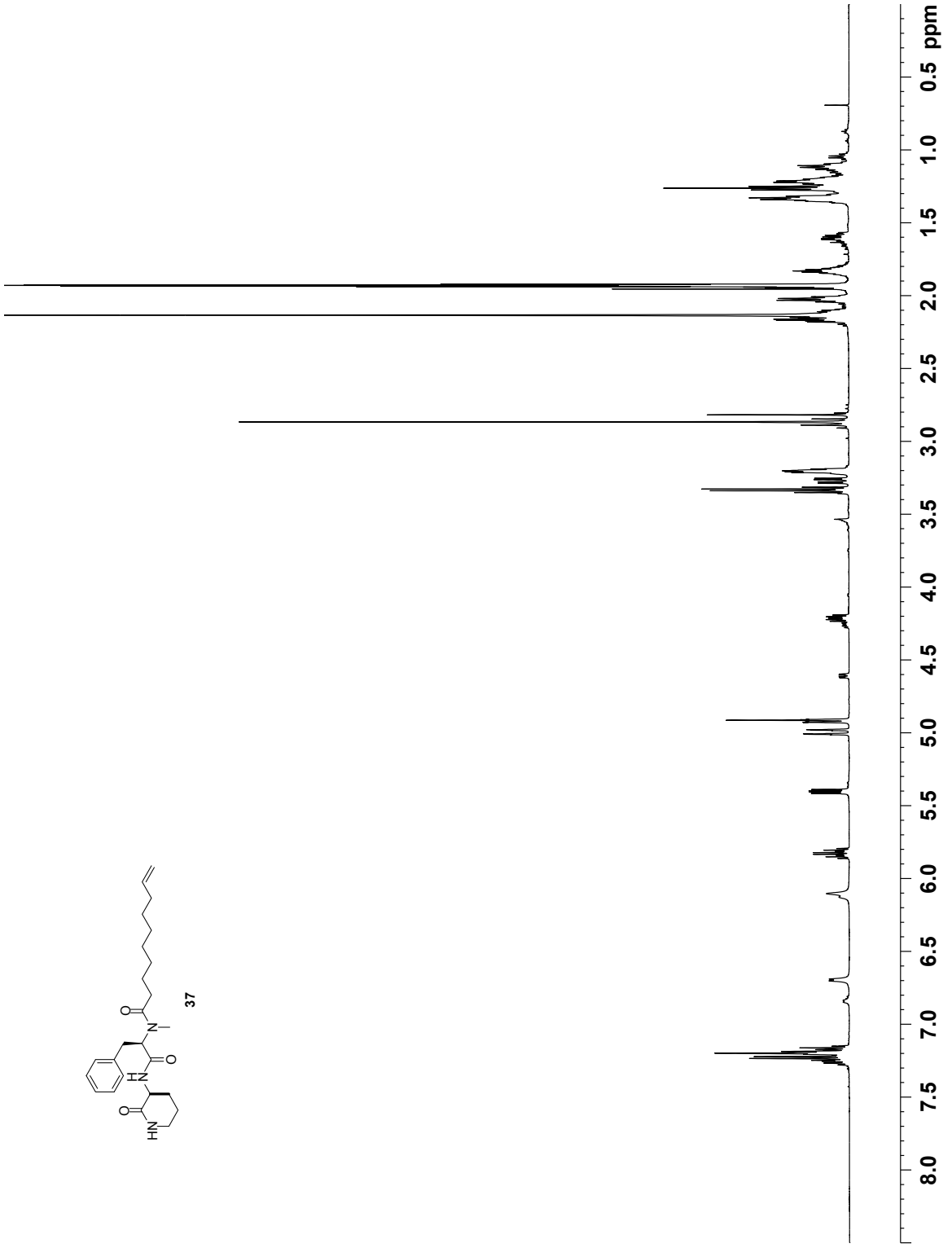
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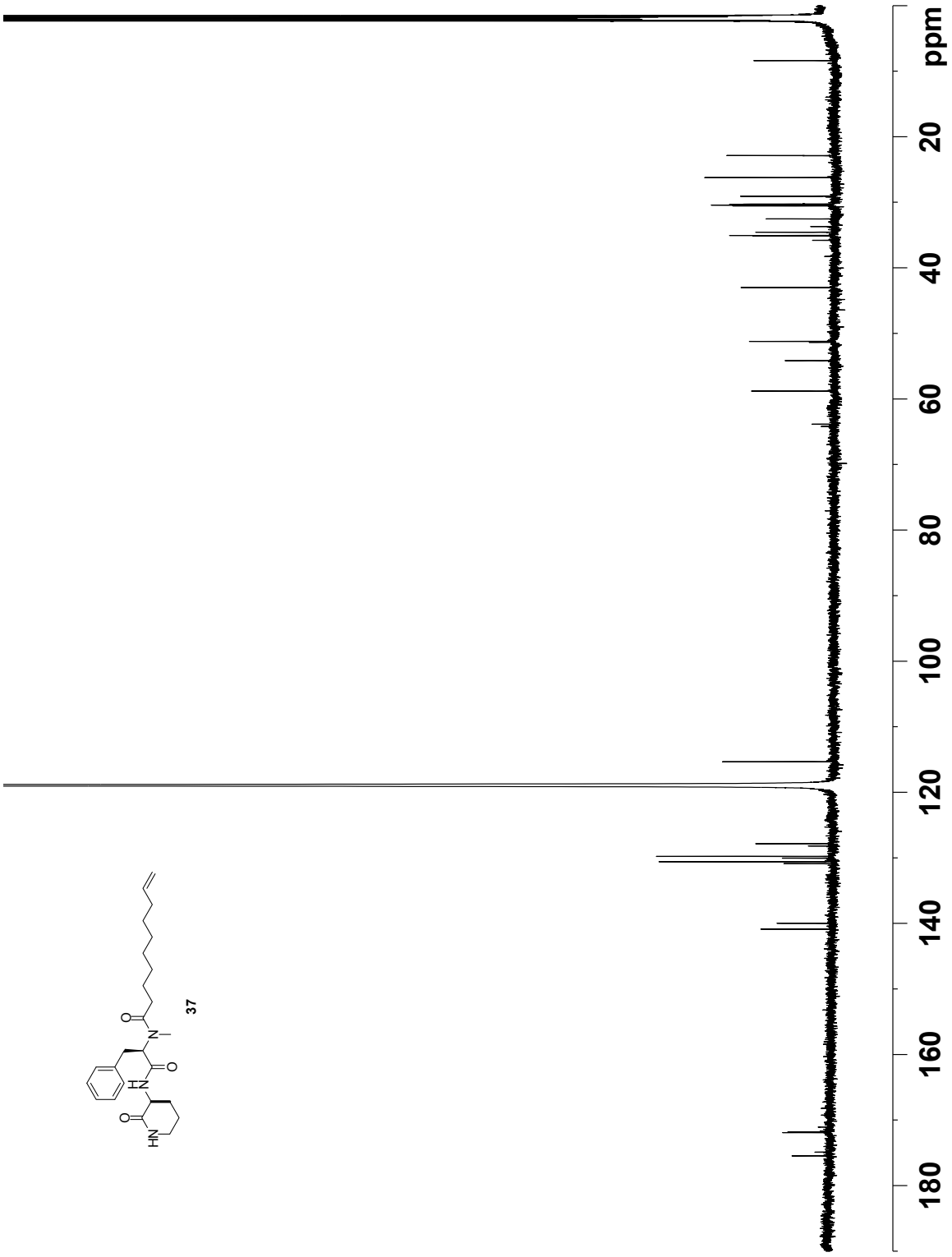
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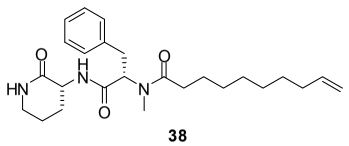

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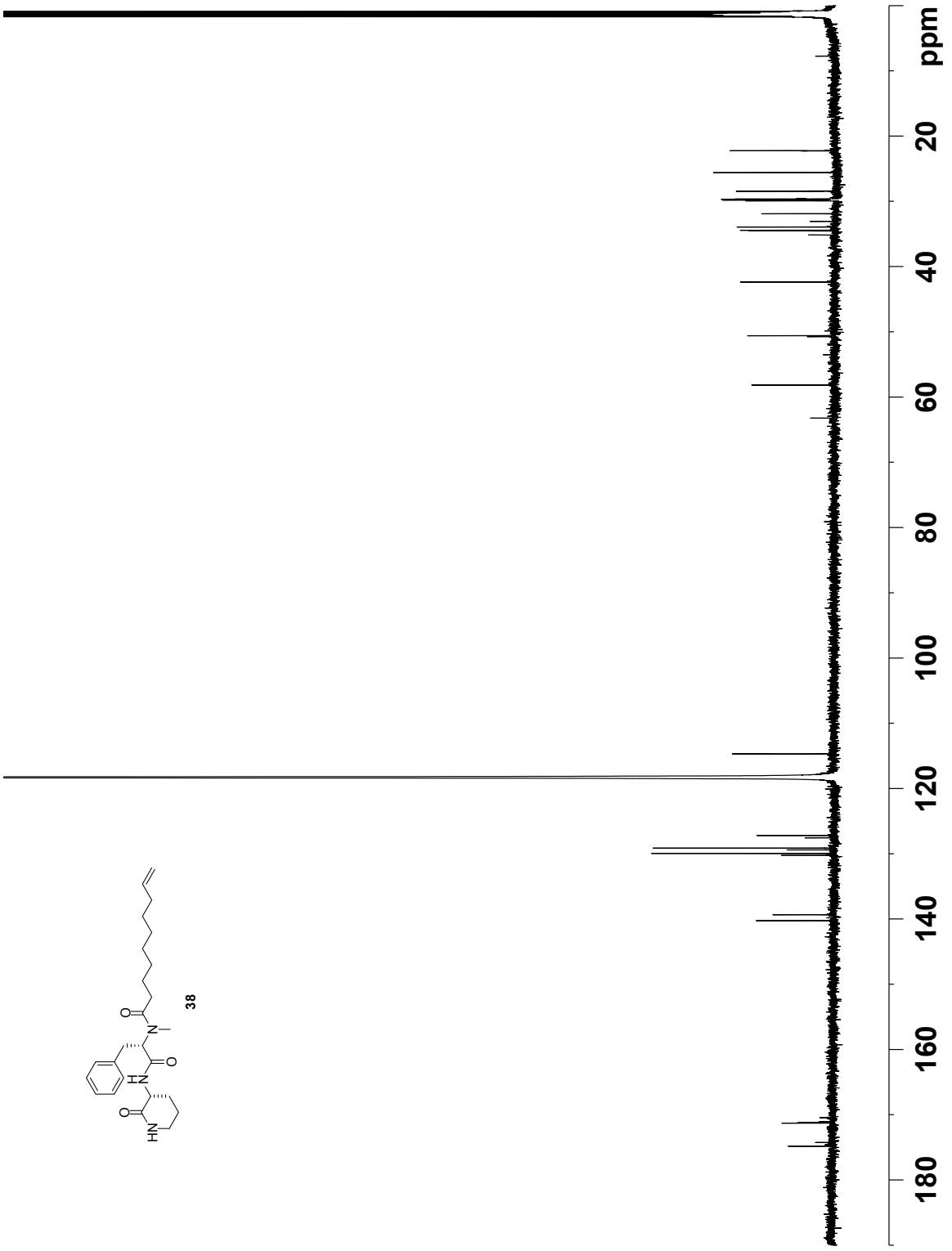
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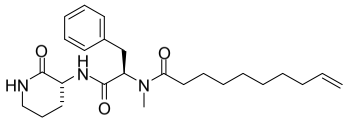

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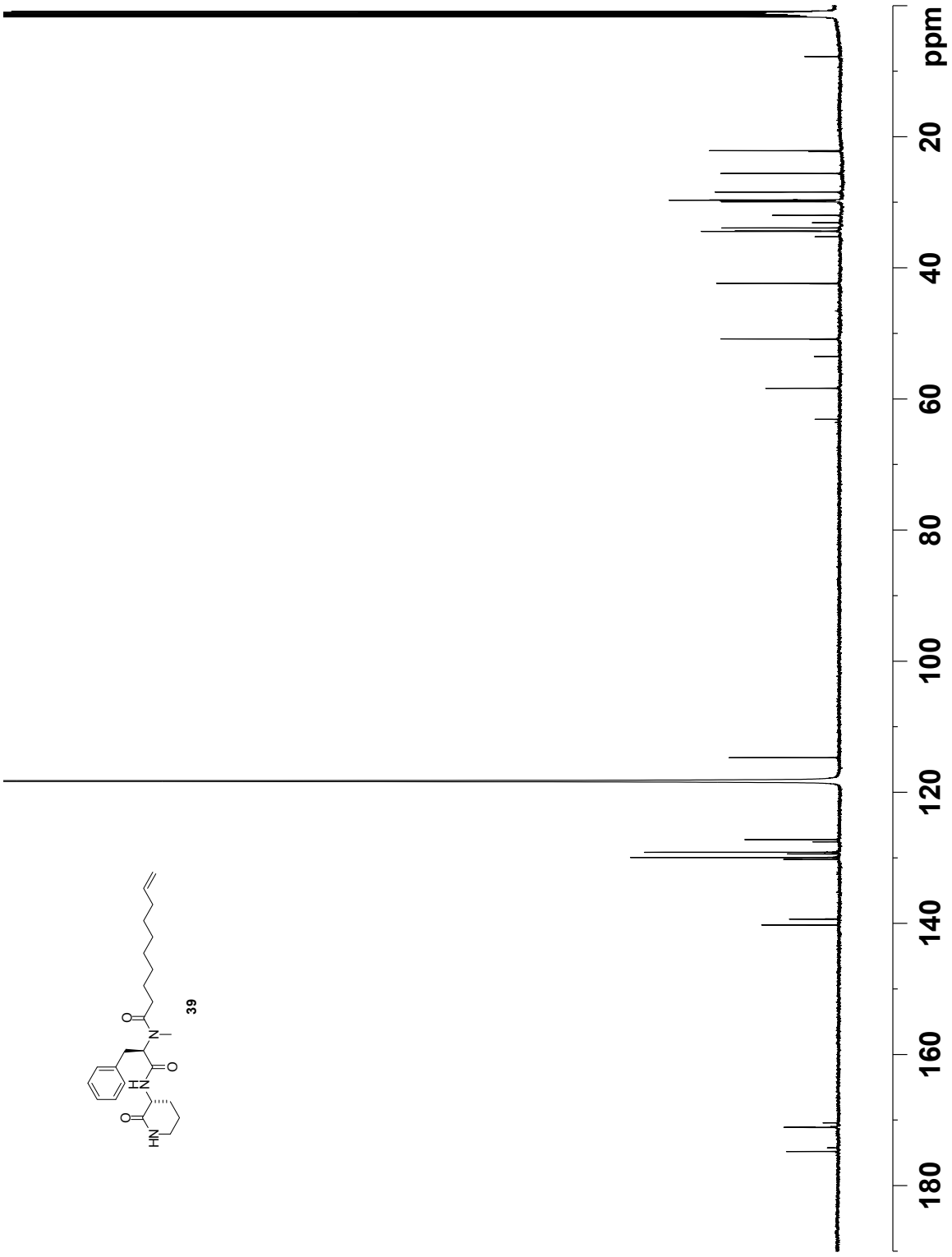
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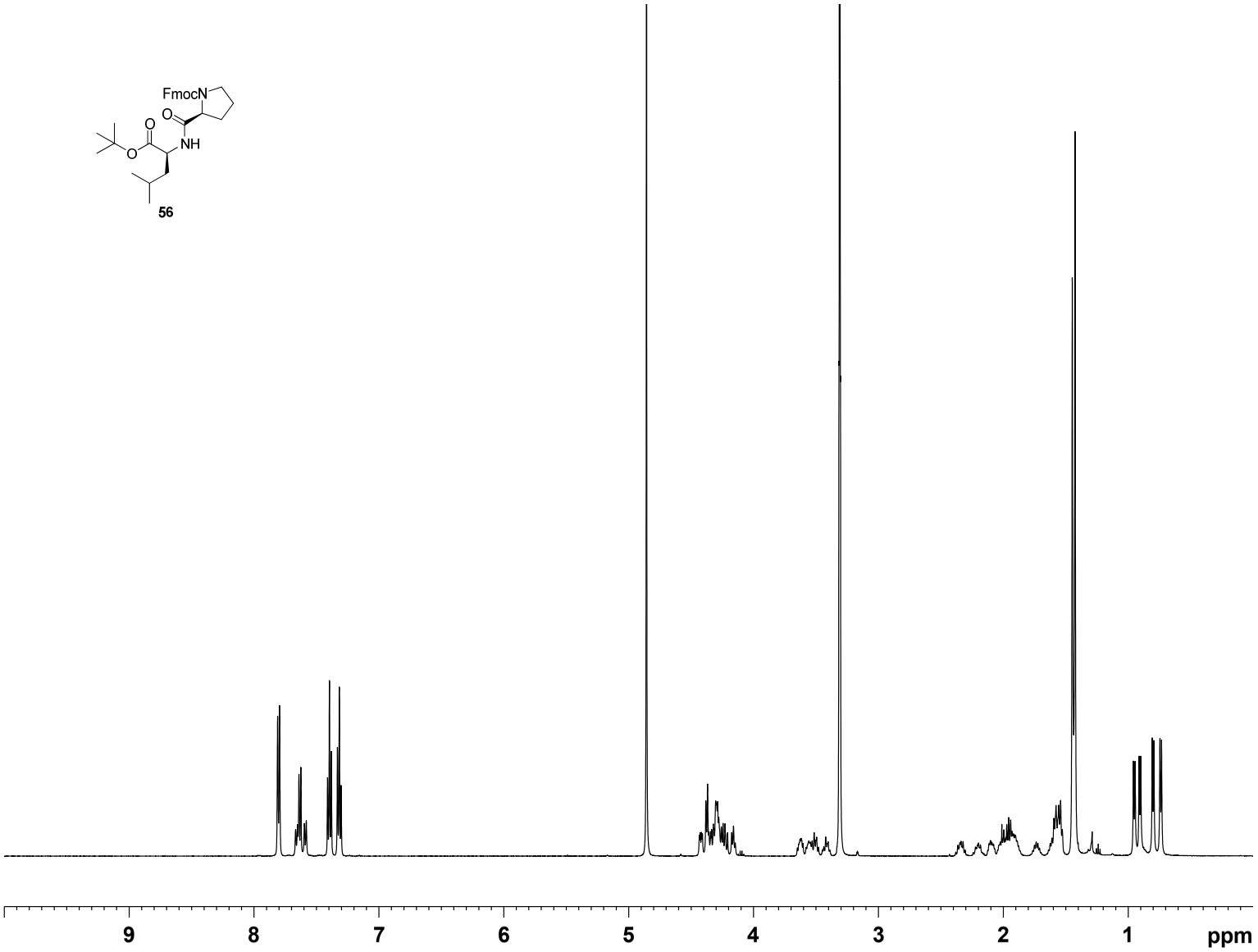
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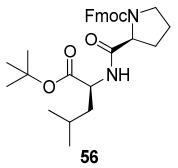

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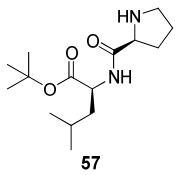

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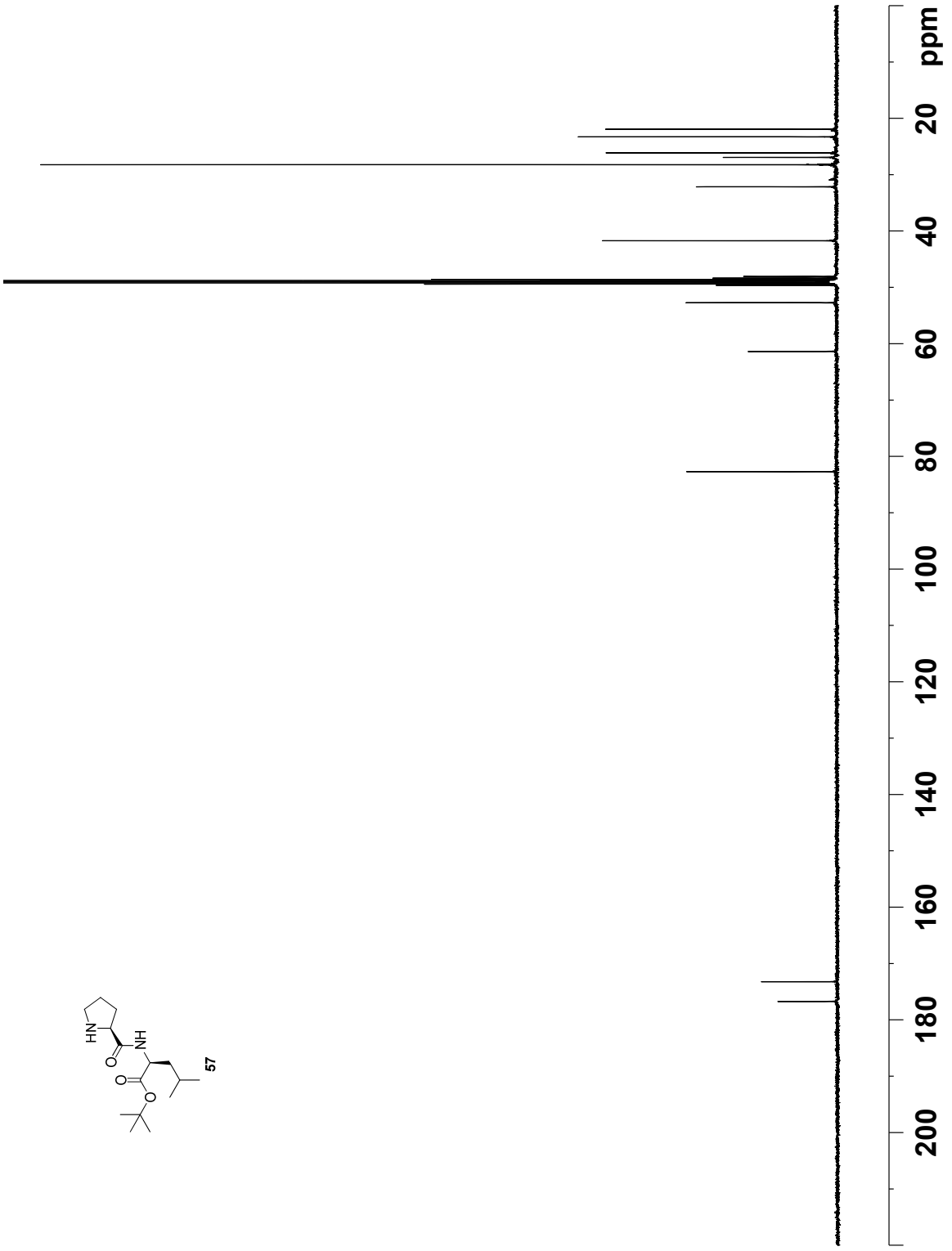
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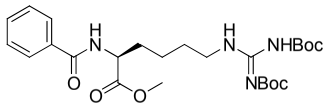

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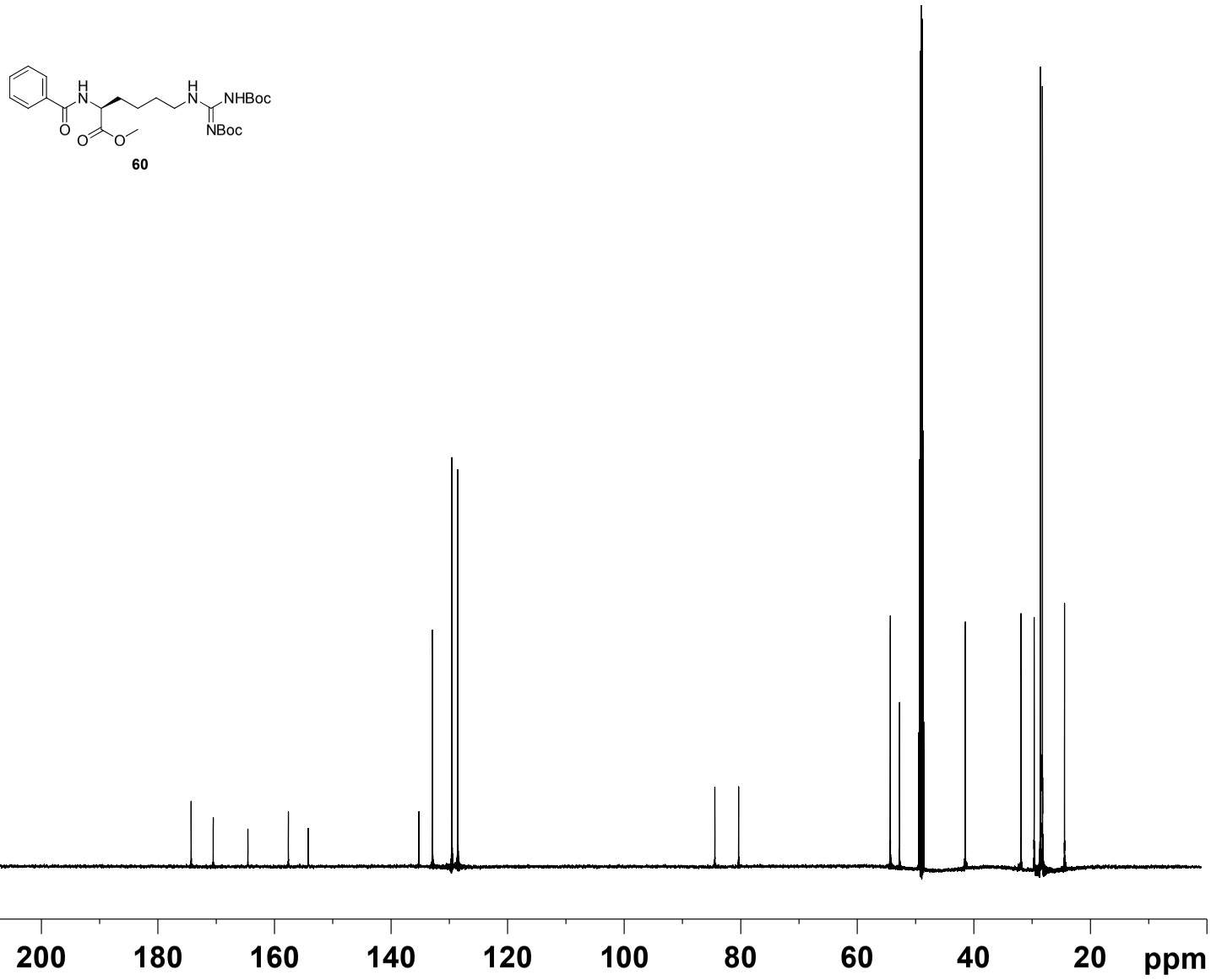
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NBoc
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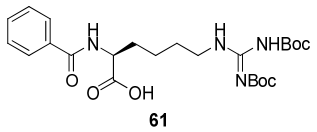
60
40
20
ppm



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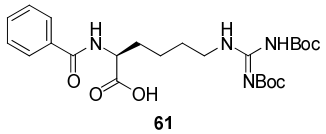

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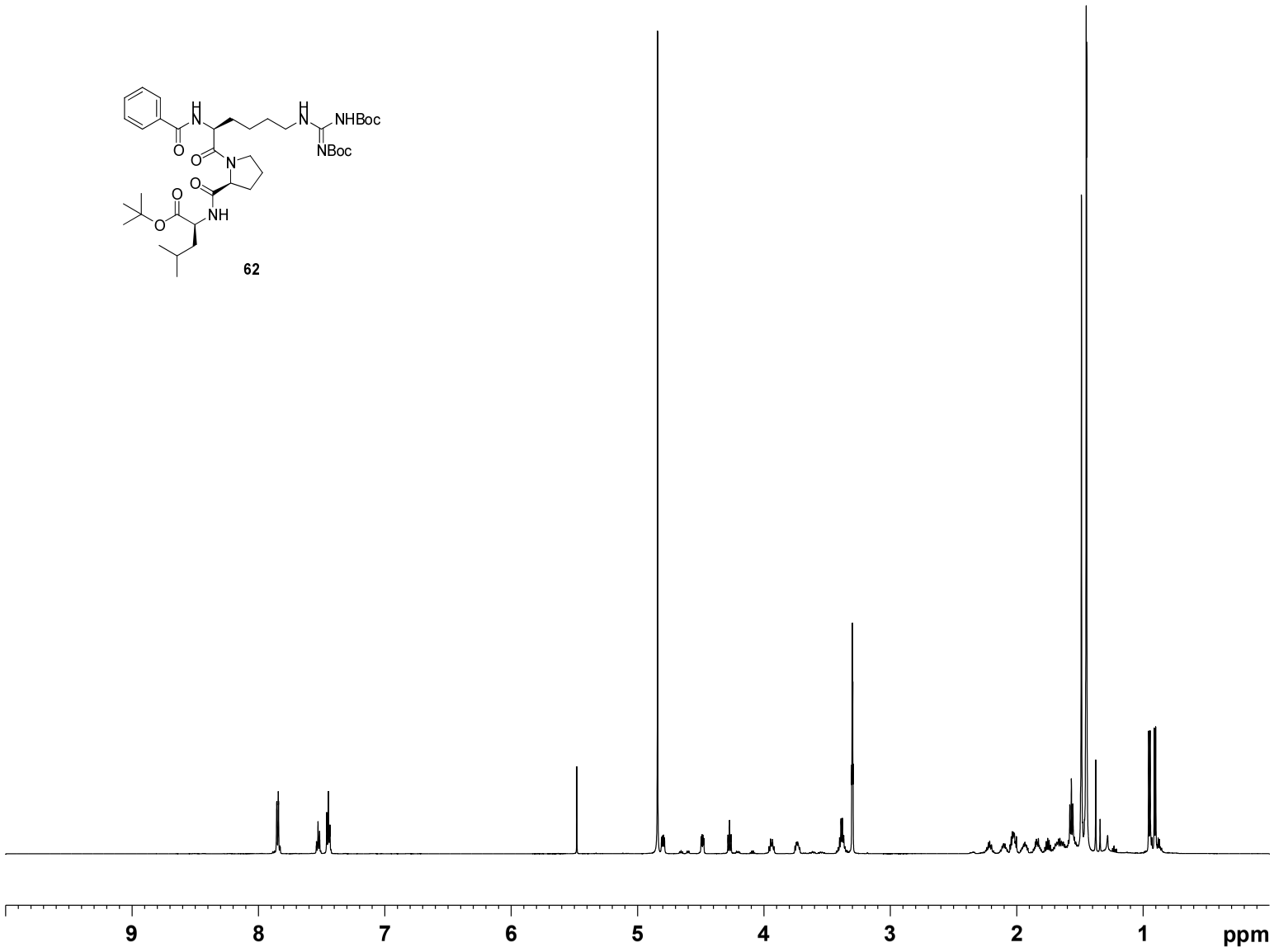
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NBoc
NH
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5
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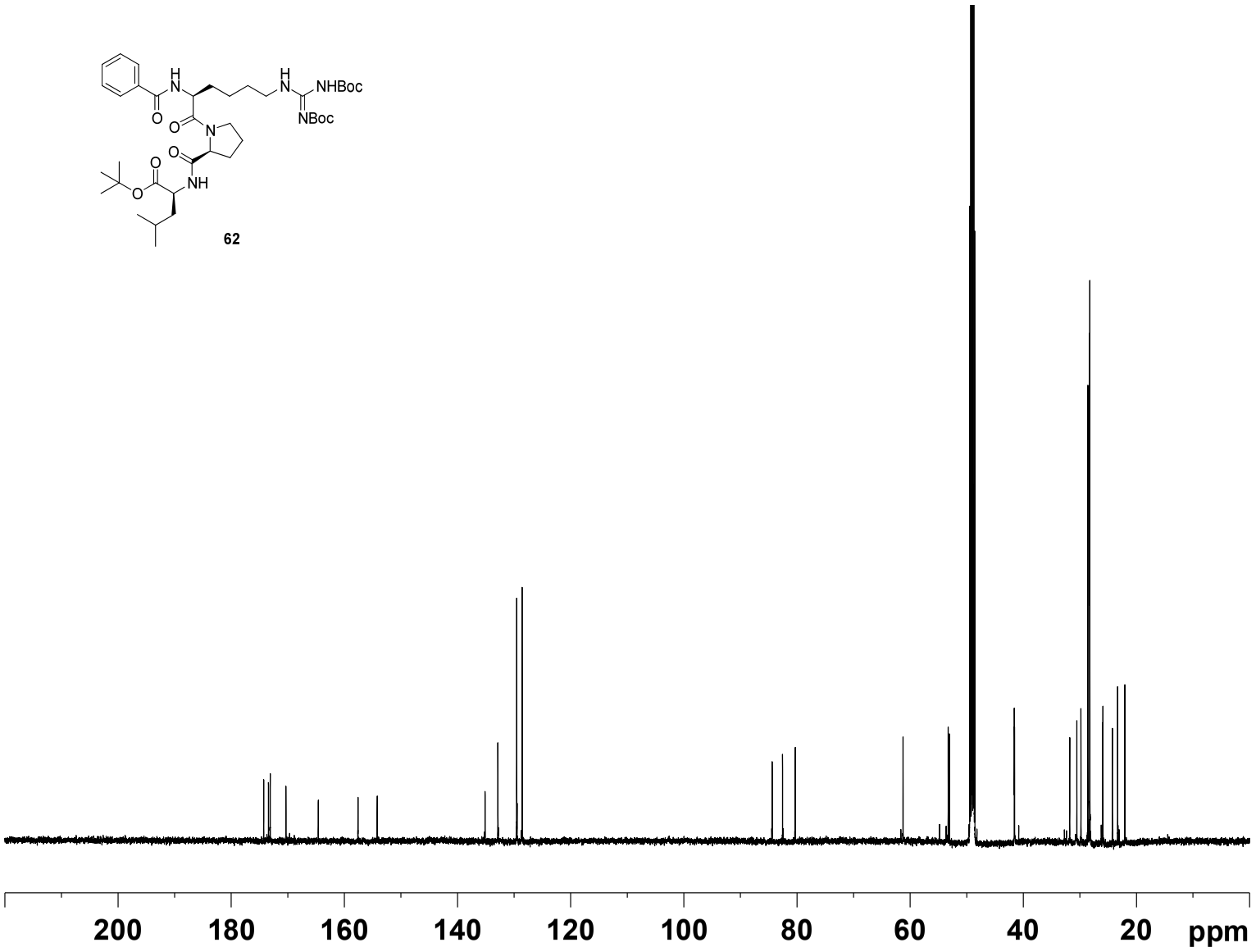
3
2
1
ppm


NHBoc
NBoc
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200
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100
80
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40
20
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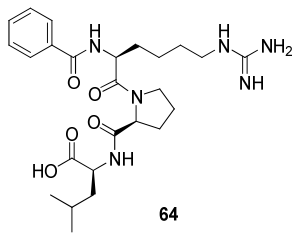
64

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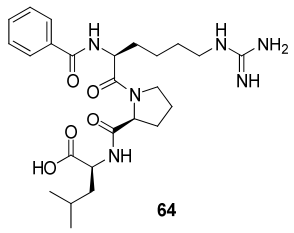

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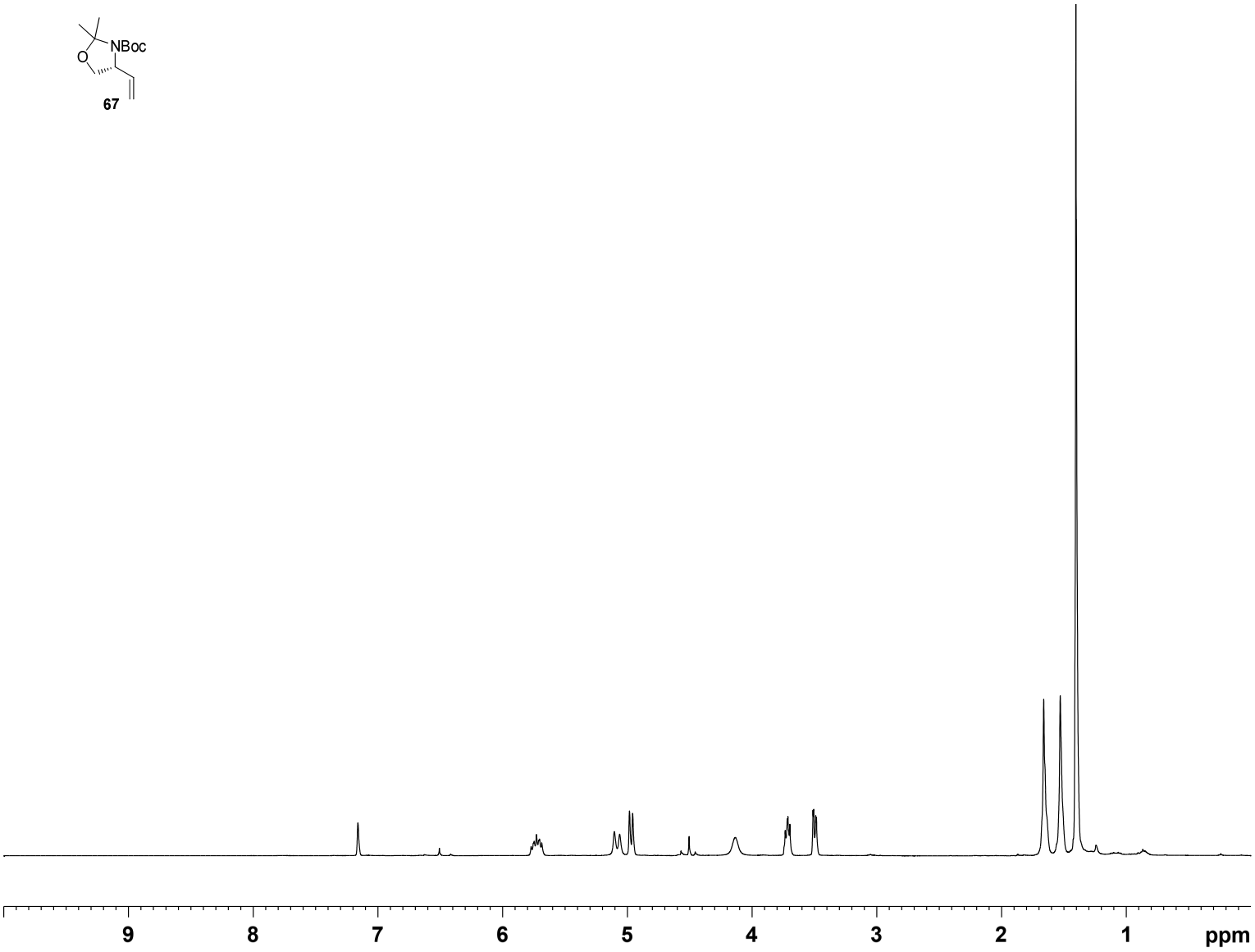
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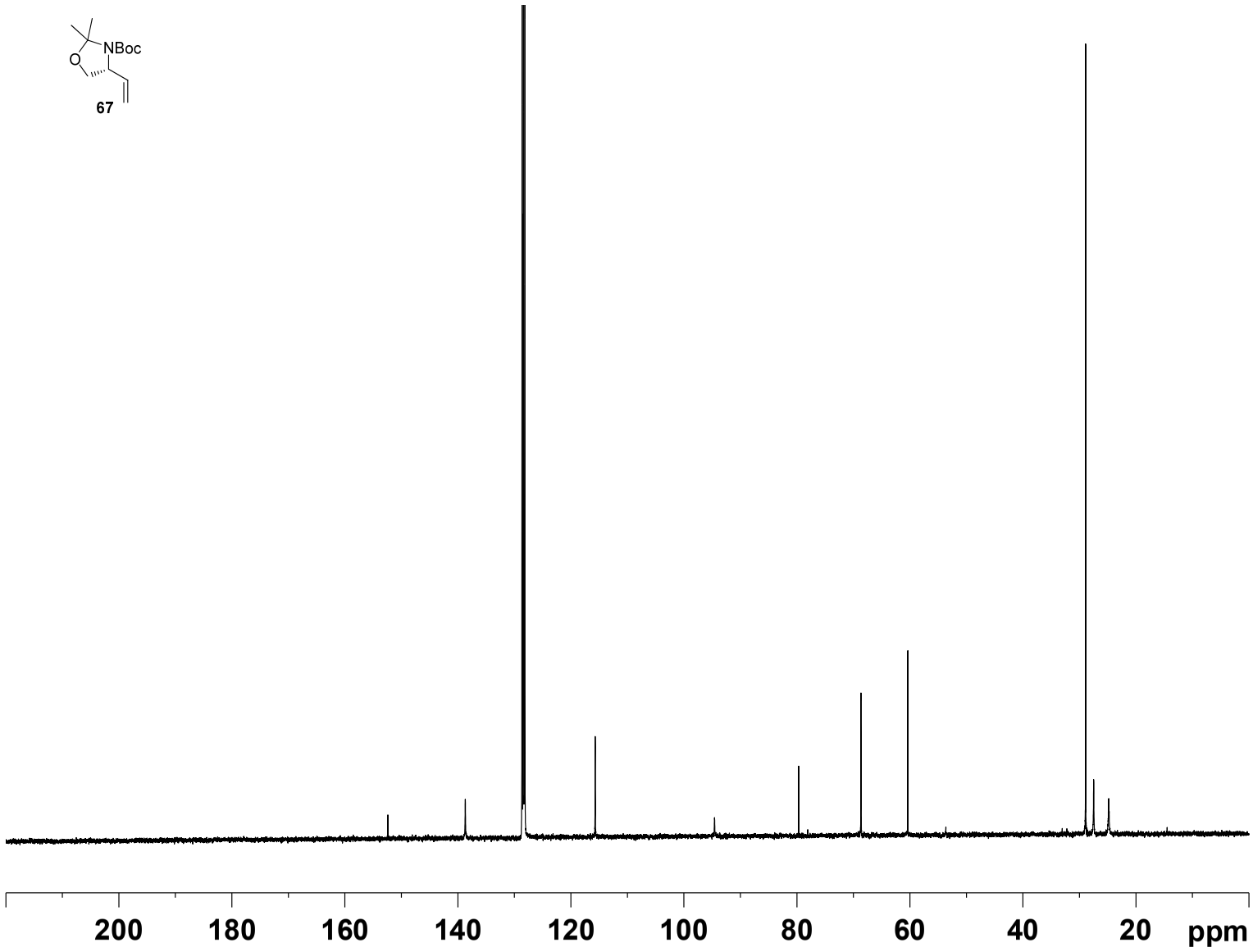
2
1
ppm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O
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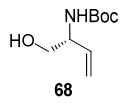
20
ppm



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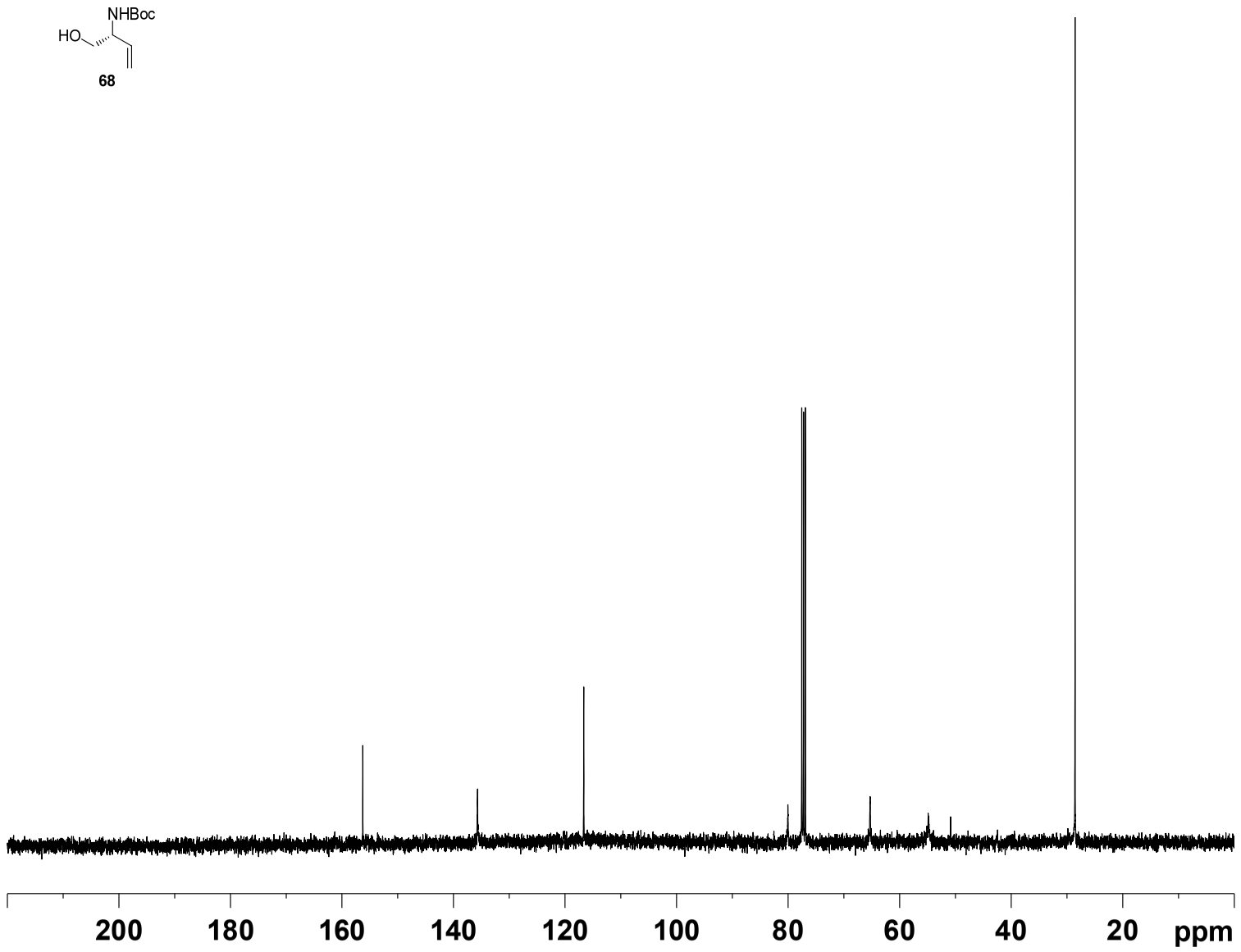
NHBoc
HO
68
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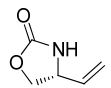

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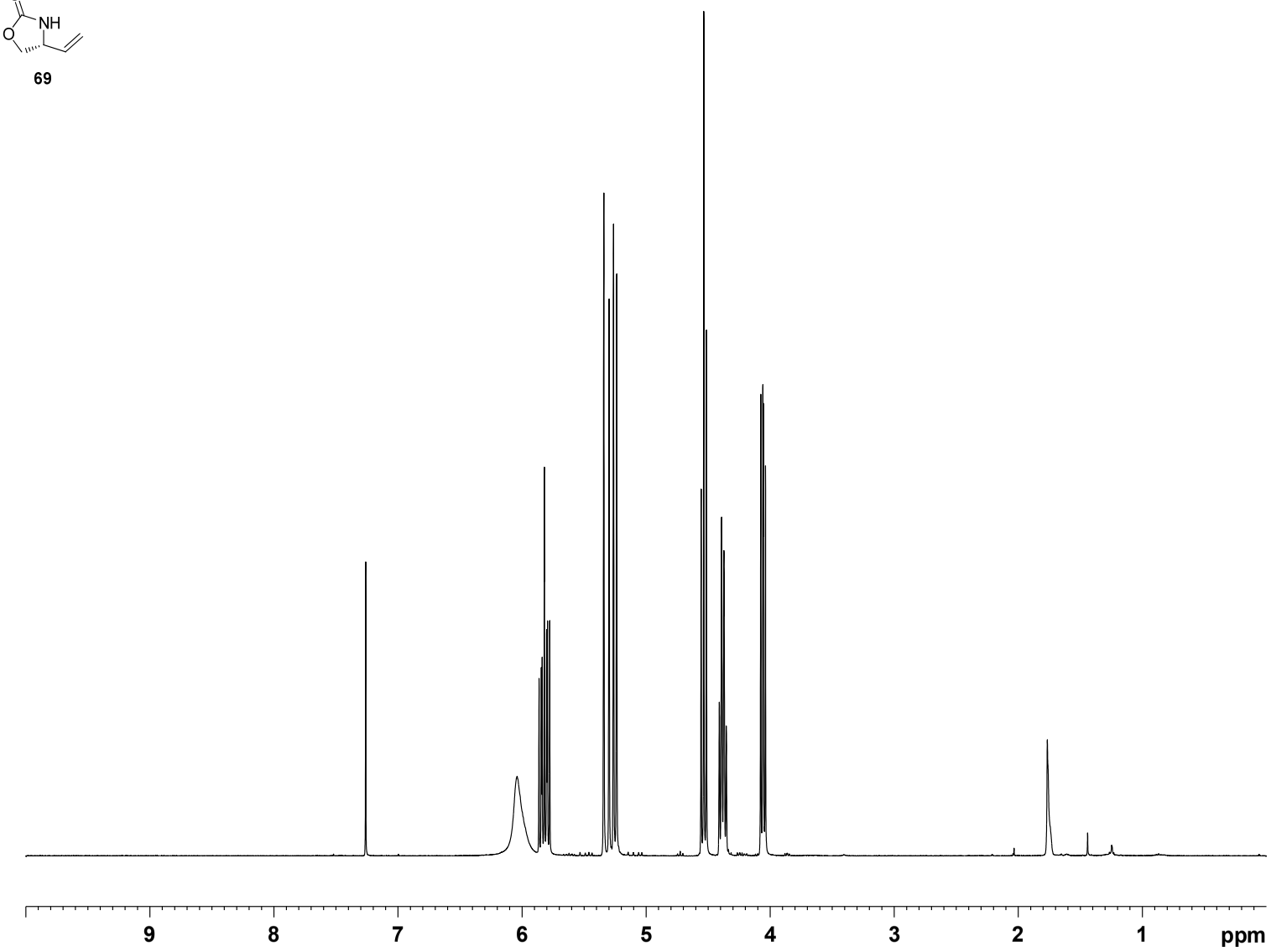

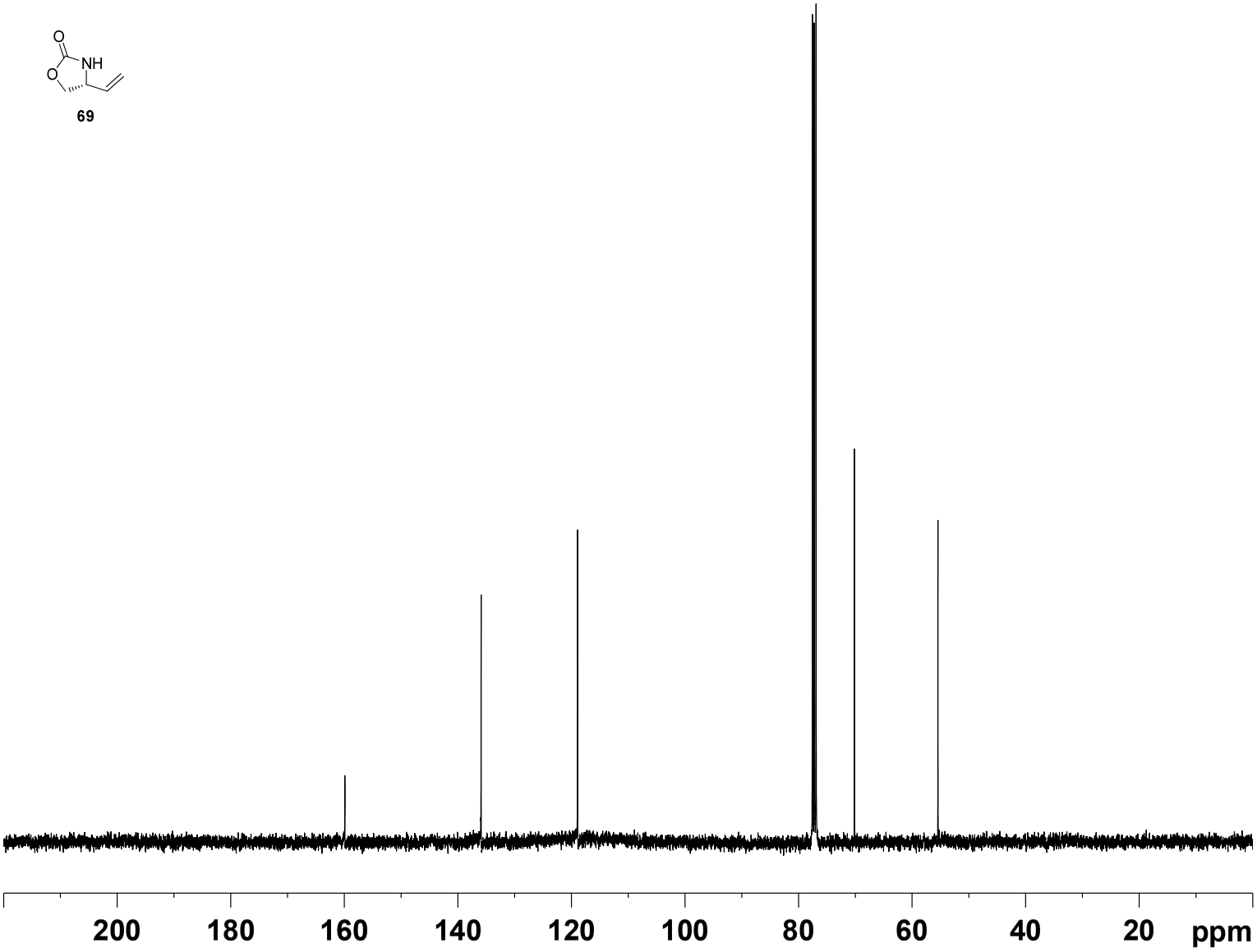
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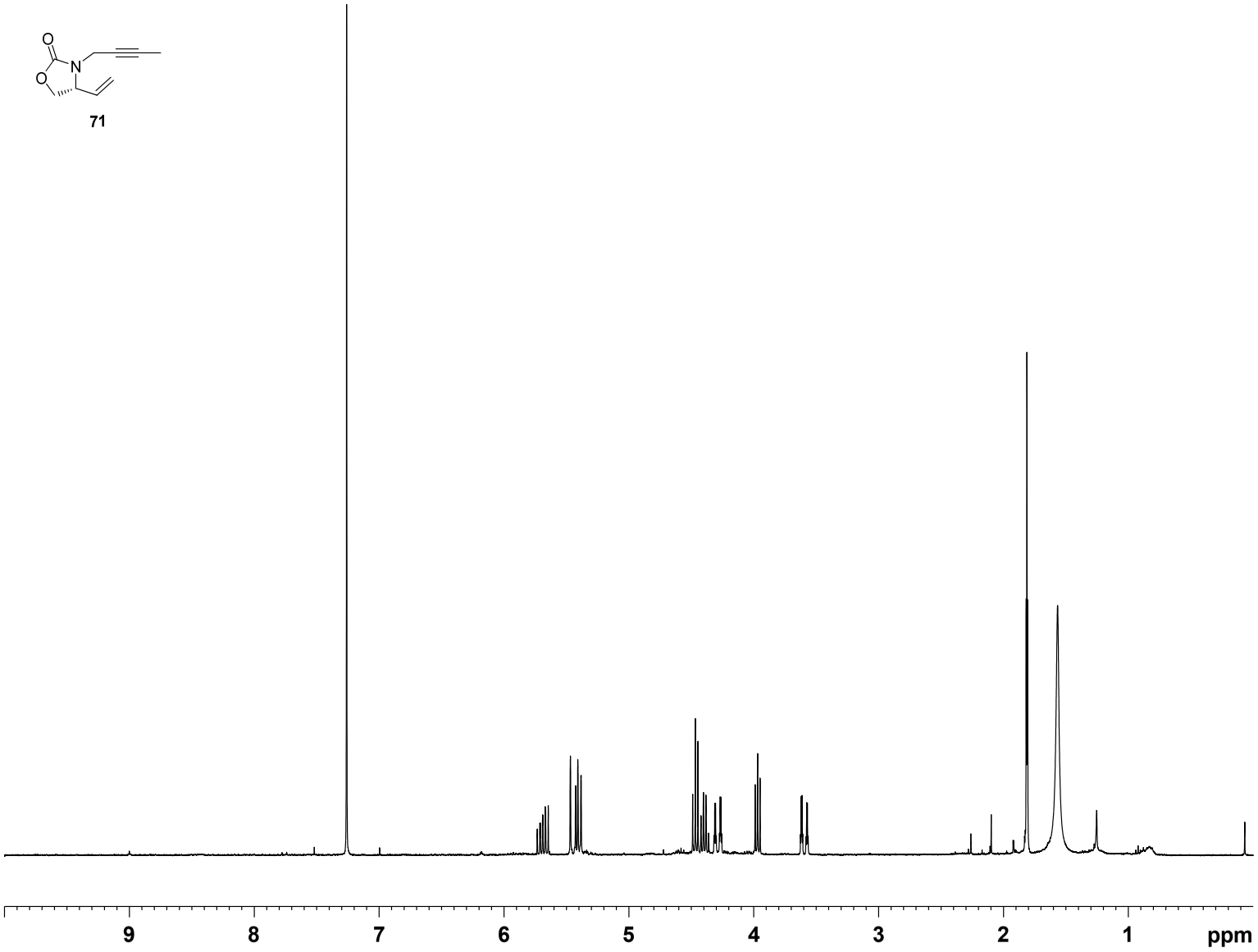
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9
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ppm

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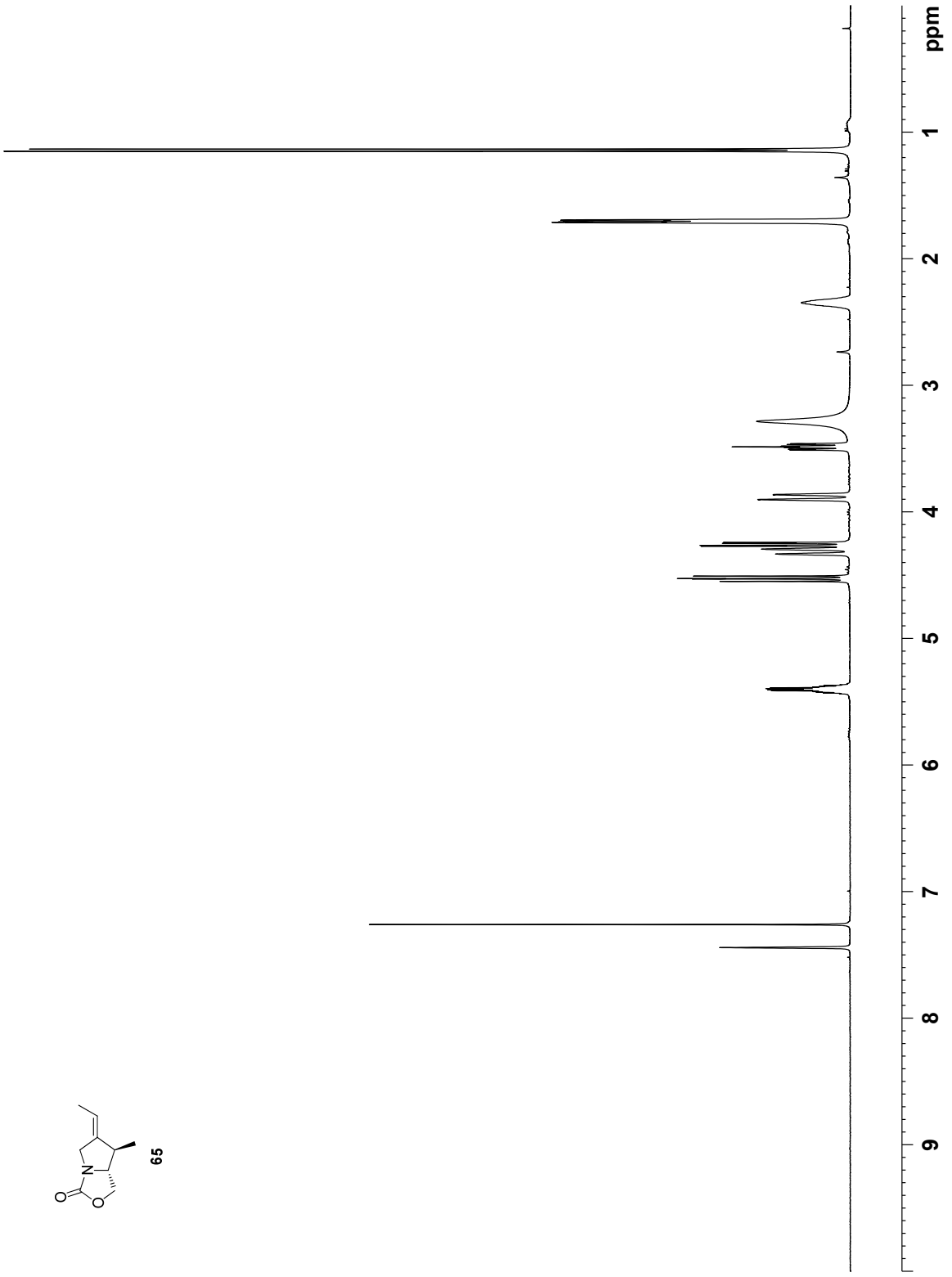
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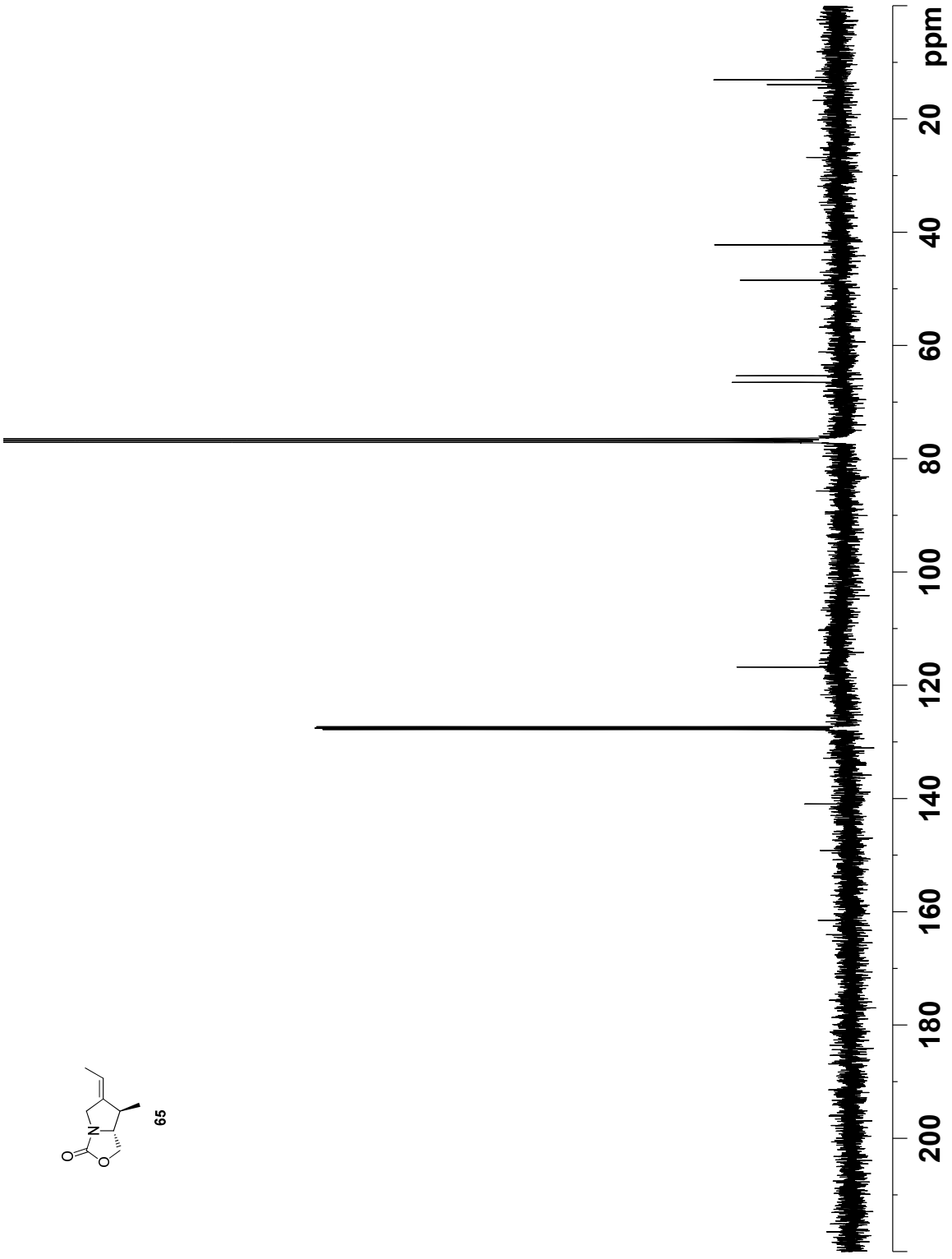
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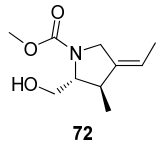

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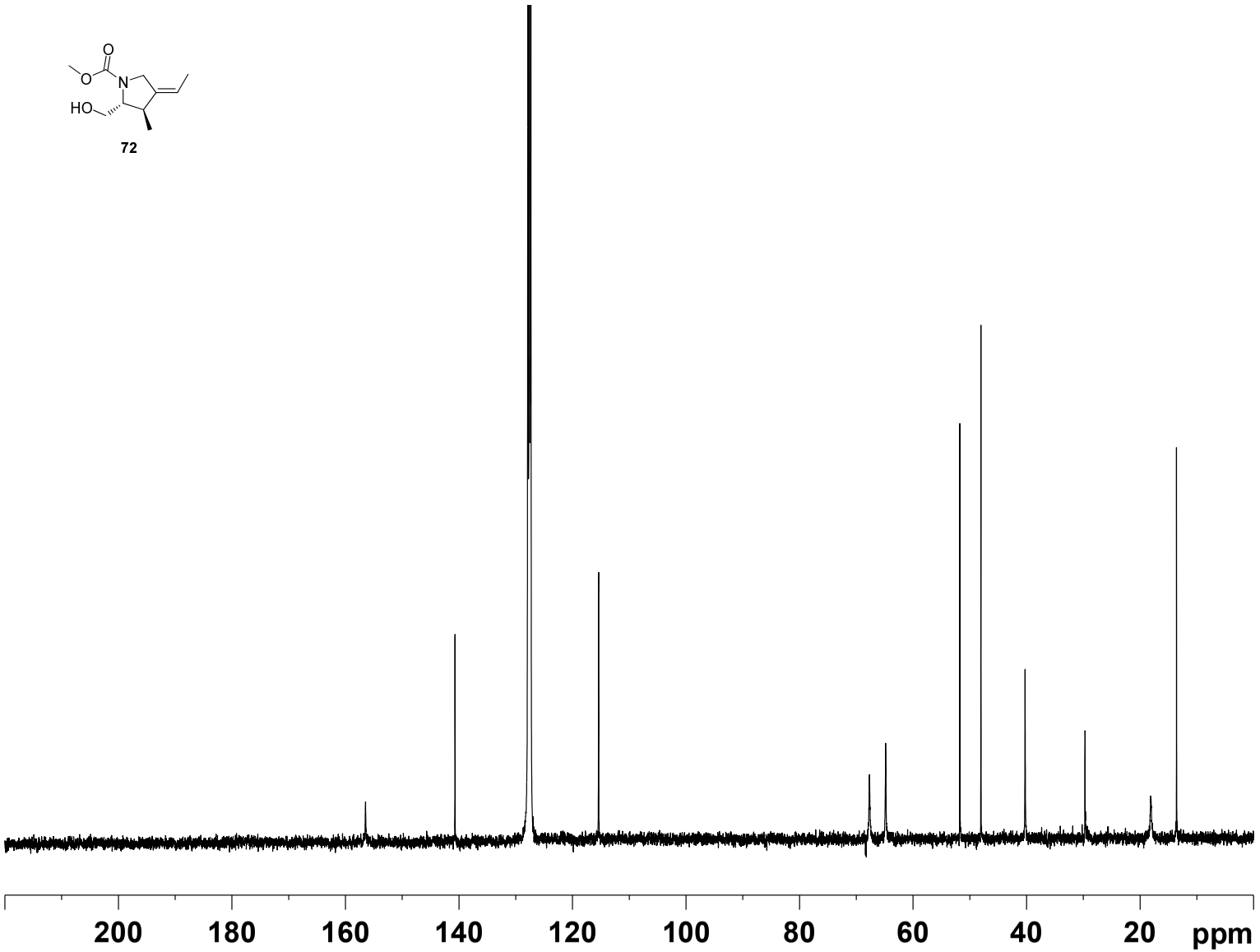
O
O
N
HO
72
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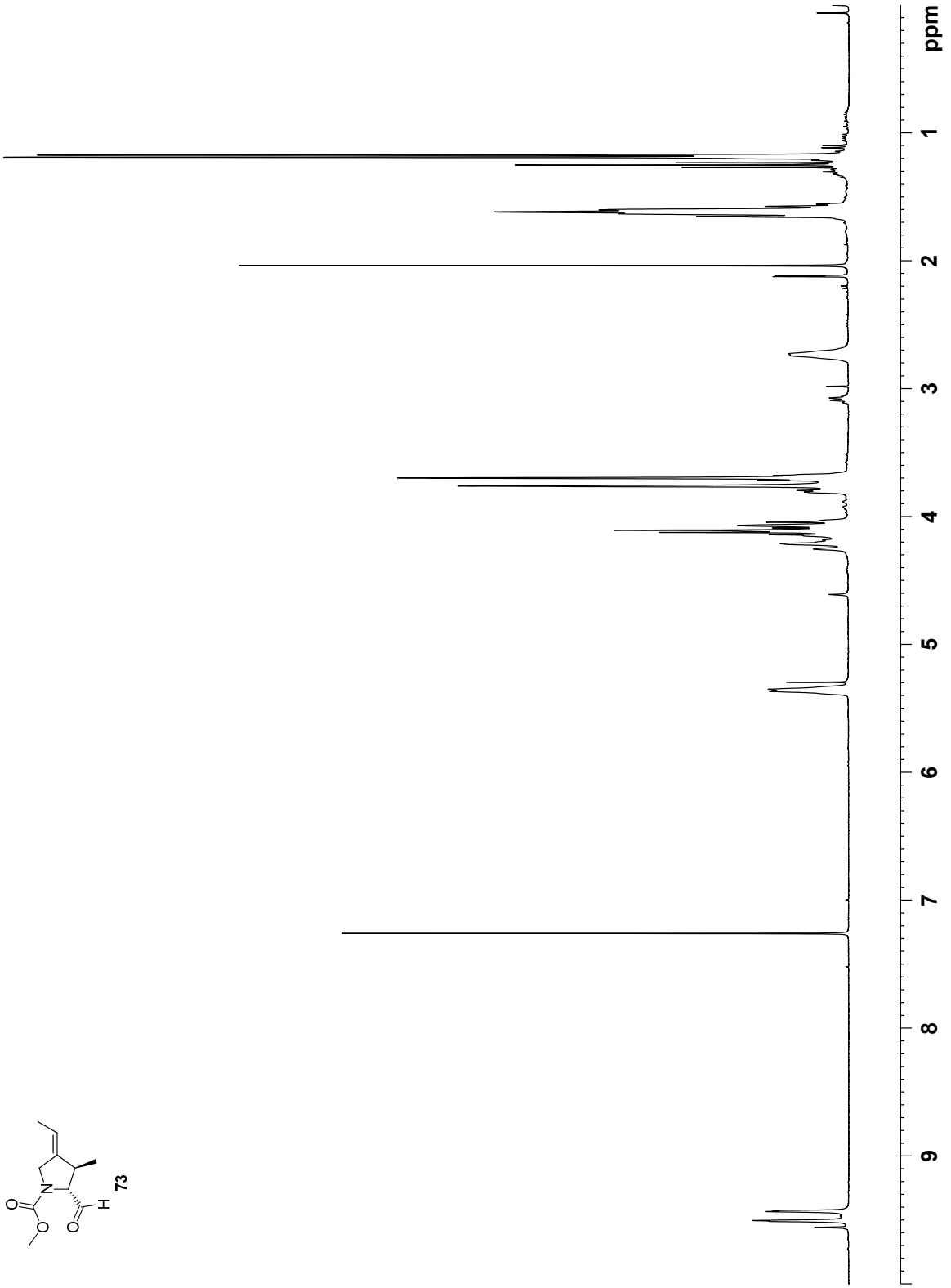
ppm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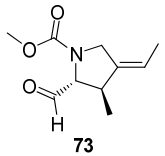

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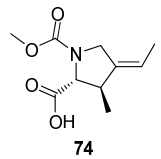

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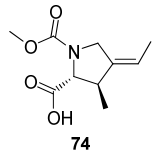

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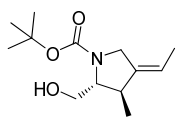

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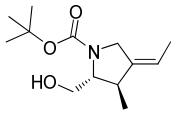

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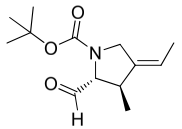

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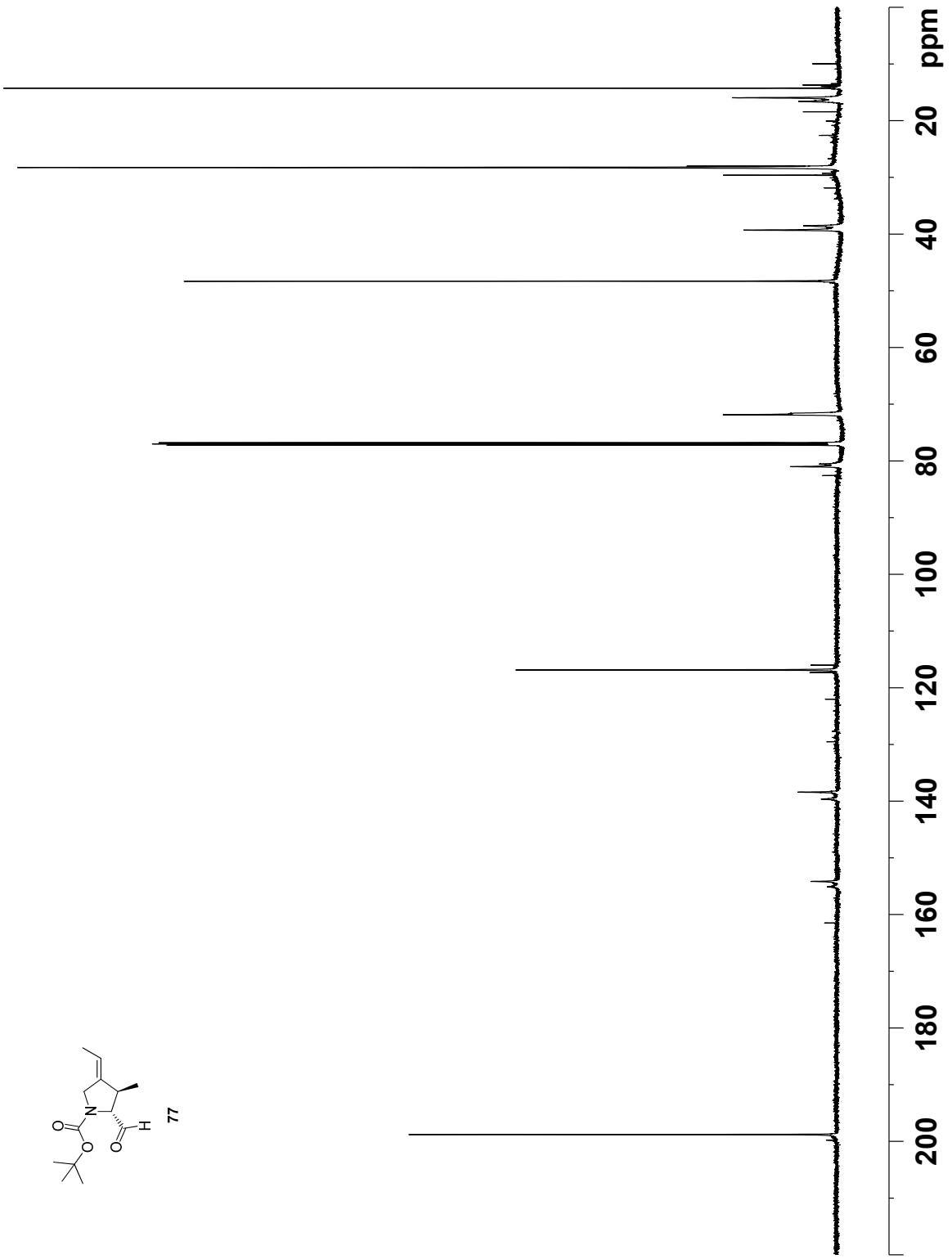
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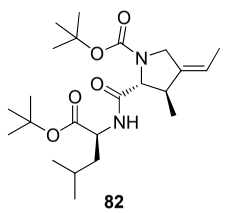

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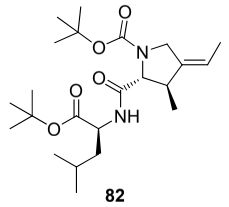

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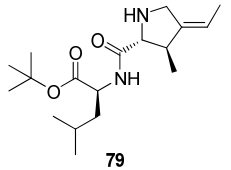

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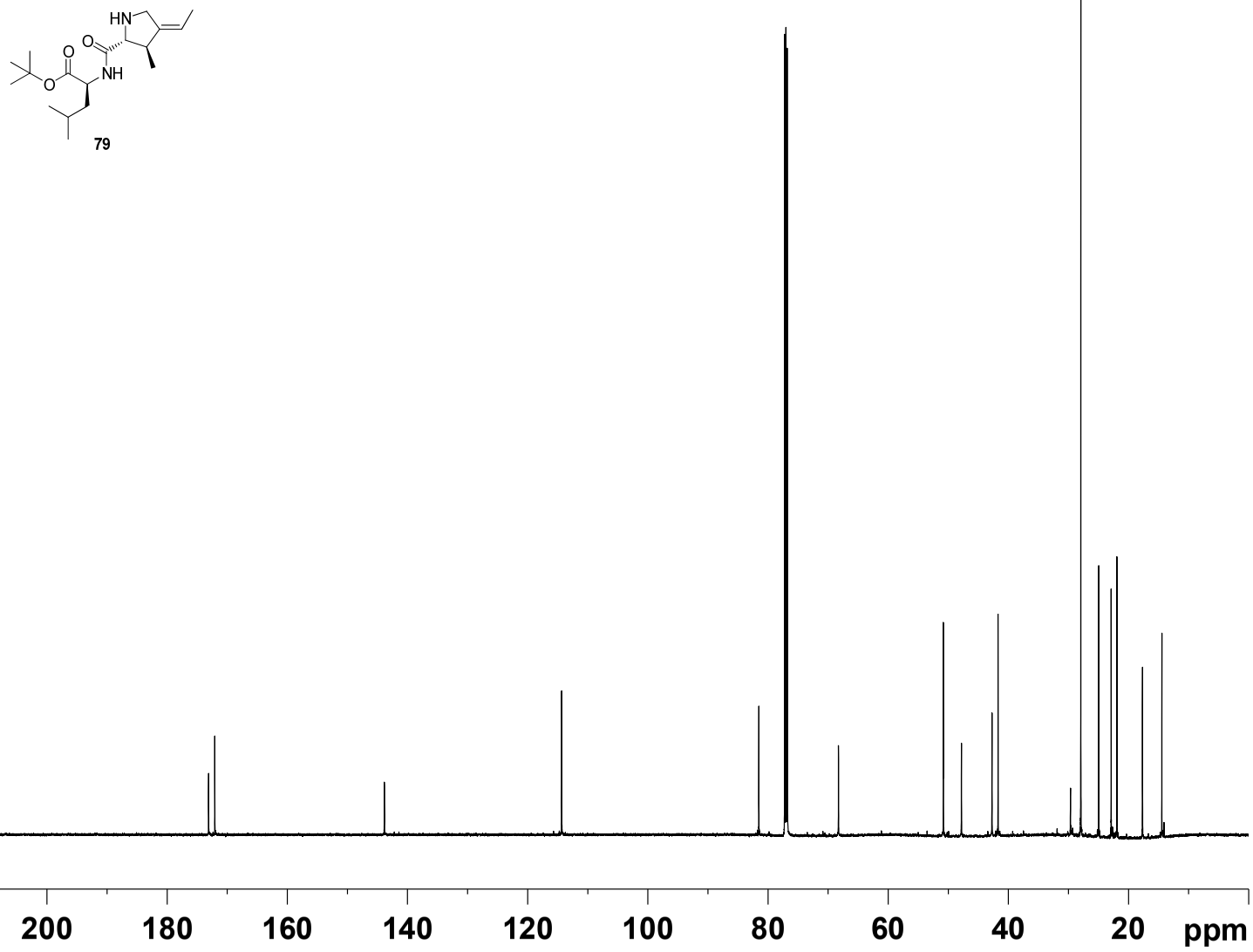
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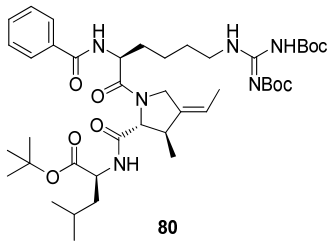
ppm



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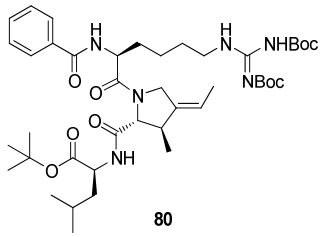
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NH
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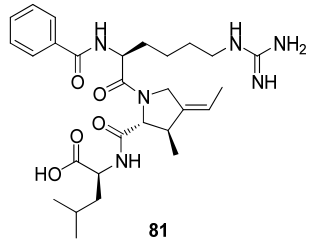

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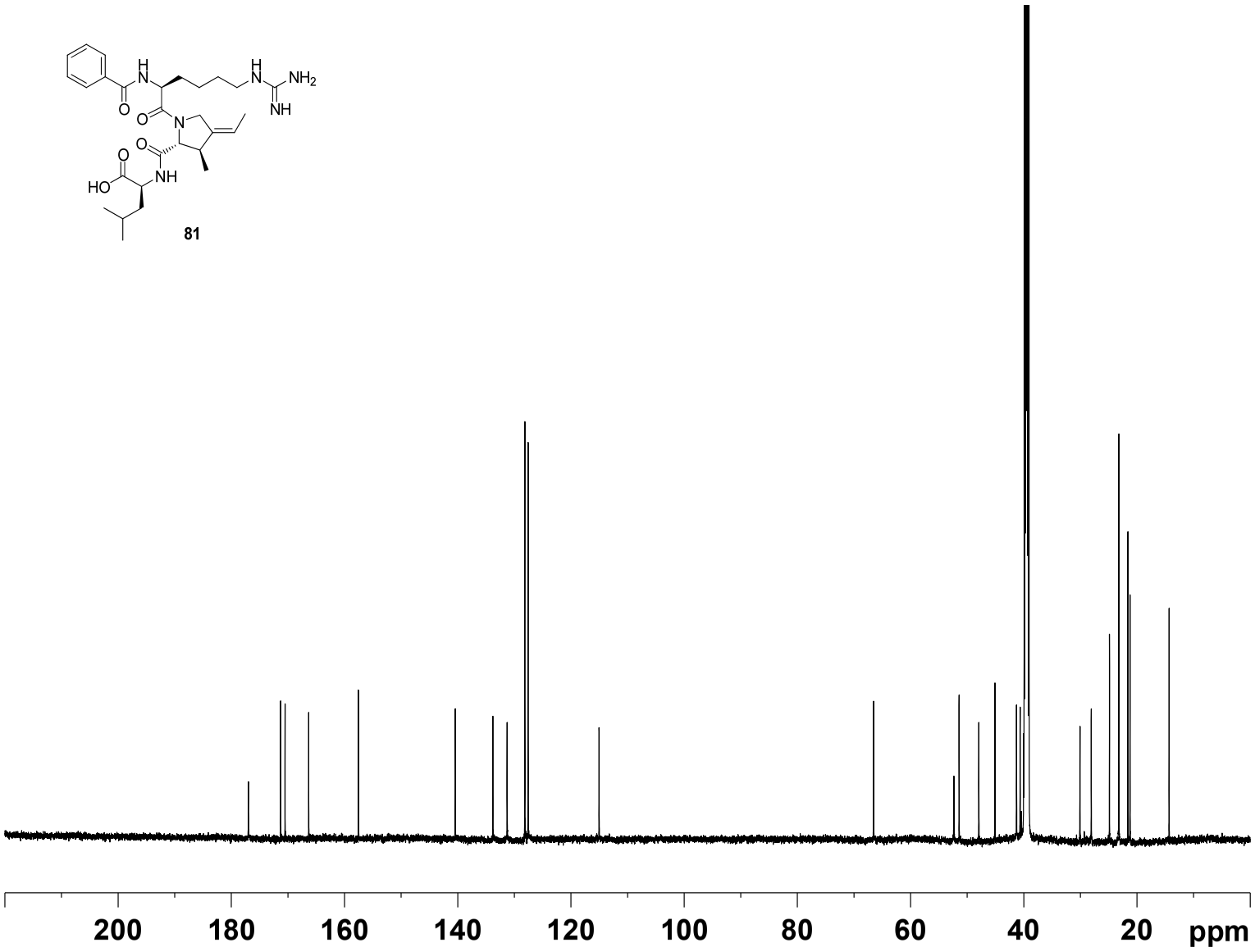
81
200
180
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140
120
100
80
60
40
20
ppm

nOe Observed with bicycle **65**.