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Celogentin C and Thioviridamide: Synthetic and Structural Studies

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A dissertation submitted to the faculty of
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Doctor of Philosophy

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ABSTRACT

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Project I Celogentin C, isolated by Kobayashi from the seeds of *Celosia argentea* in 2001 exhibits strong inhibitory behavior towards polymerization of tubulin – globular protein, which plays crucial role during the cell division cycle. We have attempted synthesis of the left-hand ring of celogentin C via the intramolecular alkylation and the Knoevenagel condensation strategies. Utilizing synthetic methodologies in the field of nitroacetyl chemistry, developed by Kornblum and Rajappa we have successfully prepared the intermolecular Knoevenagel condensation product – the late-stage precursor to the left-hand ring of celogentin C. Synthesis of this key intermediate subsequently led to the preparation of the left-hand ring and the total synthesis of celogentin C by other members of Castle's group.

Project II Thioviridamide is the potent apoptosis inducer isolated by Hayakawa from the bacterial broth of *Streptomyces olivoviridis*. Unusual structural features of the thioviridamide macrocycle contain two novel amino acids, never before encountered in a natural product - *S*-(2-aminovinyl)cysteine (avCys) and β-hydroxy-N^1,N^3-dimethylhistidinium (hdmHis). No stereochemical information except for the *Z*-configuration of the β-thioenamide linkage was reported in the literature. We have performed a computational study to predict the thioviridamide stereochemistry. Initial populations of conformers for the likely candidate structures were produced using OPLS-AA force field. Prediction of the NMR properties was accomplished at the mPW1PW91/6-311+G(2d,p) level of theory with the polarizable continuum model of salvation. Utilizing Boltzmann averaging and statistical analysis we have determined that the only possible cases of stereochemical inversion occur at the sites of the two novel amino acids.

Project III Model studies towards the synthesis of the β-thioenamide subunit of thioviridamide were performed. The radical addition reaction of thiyl radicals to ynamides produces *Z*- (kinetic products) or *E*- β-thioenamides (thermodynamic products) depending on the reaction conditions. Two distinct sets of reaction conditions allowing kinetic or thermodynamic control of β-thioenamide formation were developed. Synthesis of the model β-thioenamide subunit of thioviridamide was attempted.

Keywords: Celogentin C, Thioviridamide, Knoevenagel, avCys, hdmHys, OPLS-AA, NMR prediction, thyil radical, radical addition, ynamide, thioenamide, triazone

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Brigham Young University

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of a dissertation submitted by

Dmitry Nikolayevich Litvinov

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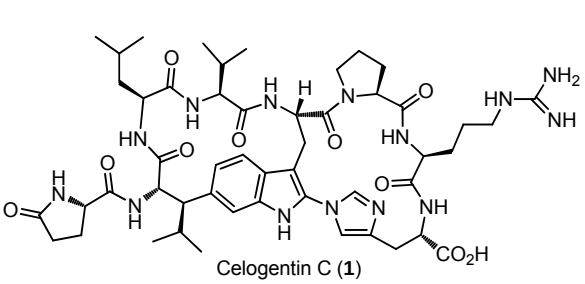
Chapter I. Synthetic Studies of the Left-Hand Ring of Celogentin C

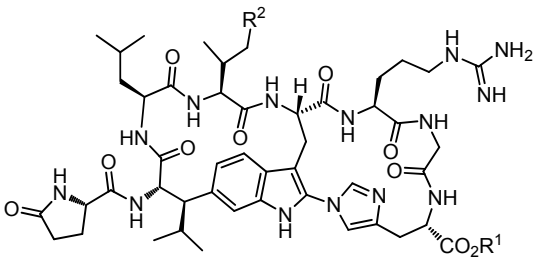
1.1 Biological Activity of Moroidin Family of Natural Products

Microtubules are parts of the cellular structural network and play important roles in cell division and vesicular transport. In addition to structural support, microtubules are involved in many other processes. They are capable of generating force and form *mitotic spindles* used by eukaryotic cells for chromosome segregation during the cell division cycle. These endoskeletal structures are mainly constructed of globular protein designated as α and β tubulin, capable of polymerization and bundling into hollow cylindrical filaments.[1] This polymerization process can be altered by a number of drugs and natural products. For example, taxanes (e. g. paclitaxel or docetaxel) stabilize microtubules, not allowing their dissociation, whereas compounds such as vinblastine dissolve microtubules.[2]

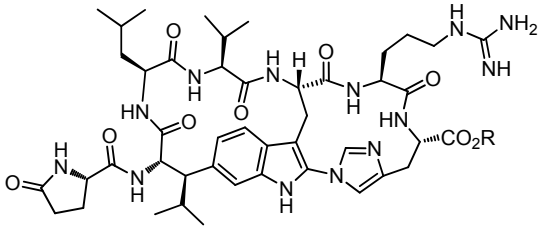
Moroidin, originally discovered in *Laportea moroides*,[3] and isolated by Kobayashi[4] from *Celosia argentea*, was found to be a strong inhibitor of tubulin polymerization,[5] thus preventing formation and stabilization of microtubules. Additionally, nine related compounds were further isolated from *Celosia argentea* (celogentins A-J)[6] and celogentin K was isolated from the same source in 2004 (Figure **1**).[7] Among these compounds, celogentin C has shown the most potent inhibitory activity towards polymerization of tubulin (Table **1**).[8, 9]



Celogentin C (**1**)



Moroidin (**5**): $R^1 = R^2 = H$
Celogentin E (**6**): R^1 = Asp, $R^2 = H$
Celogentin F (**7**): R^1 = Arg, $R^2 = H$
Celogentin G (**8**): $R^1 = H$, R^2 = Me
Celogentin H (**9**): R^1 = Asp, R^2 = Me
Celogentin J (**10**): R^1 = Arg, R^2 = Me



Celogentin A (**2**): R = H
Celogentin B (**3**): R = His
Celogentin D (**4**): R = His–Lys

Compounds		IC_{50} (μM)
Celogentin	A	20
	B	30
	C	0.8
	D	20
	E	2.5
	F	3.0
	G	4.0
	H	2.0
	J	3.0
	K	100
Moroidin		3.0

Table 1. Inhibitory effects to the polymerization of tubulin

Figure 1. Structure of moroidin and celogentins A-J

1.2 Studies towards the Synthesis of Celogentin C

Due to its potent bioactivity, the moroidin family of natural products has drawn significant attention from the synthetic community. Their unusual structural features such as the cross-links between the β-C of leucine and the C-6 of indole, and between the C-2 of indole and N-1 of imidazole make these natural products quite challenging for total synthesis. Moody reported[10] the synthesis of stephanotic acid methyl ester (Figure **2**), a cyclic pentapeptide closely related to the left-hand ring of moroidin and isolated from the stem of *Stephanotis floribunda*.[11]

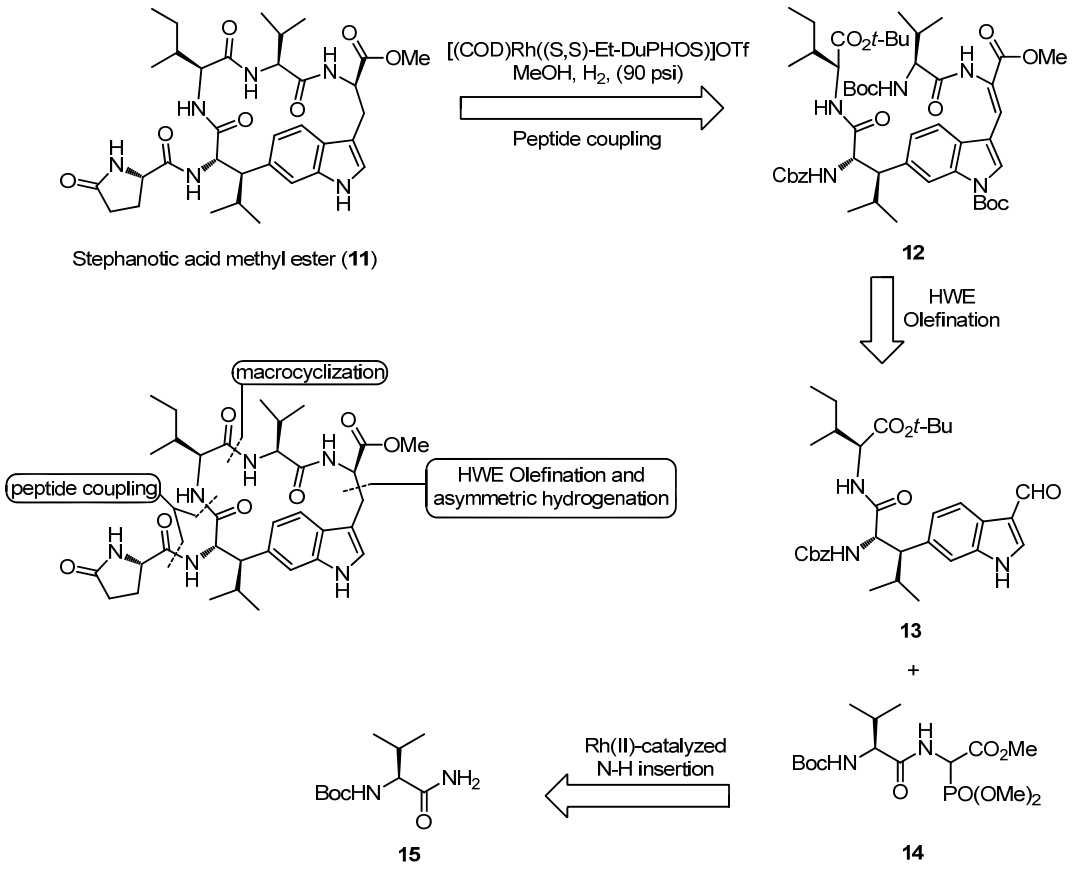


Figure 2. Moody's synthesis of stephanotic acid methyl ester

The macrocycle was formed through macrolactamization between the valine – isoluecine moieties, and another important step was formation of the tryptophan side chain through the Horner-Wadsworth-Emmons olefination. The phosphonovaline **14** was obtained via a dirhodium(II)-catalyzed N-H insertion of the carbene intermediate into the NH group of N-Boc-valinamide **15**. According to Moody the key step in the synthesis is the installation of the appropriate stereochemistry at the leucine-tryptophan junction via Hoppe methodology previously used for the formation of hindered dehydroaminoacids (Figure **3**).[12]

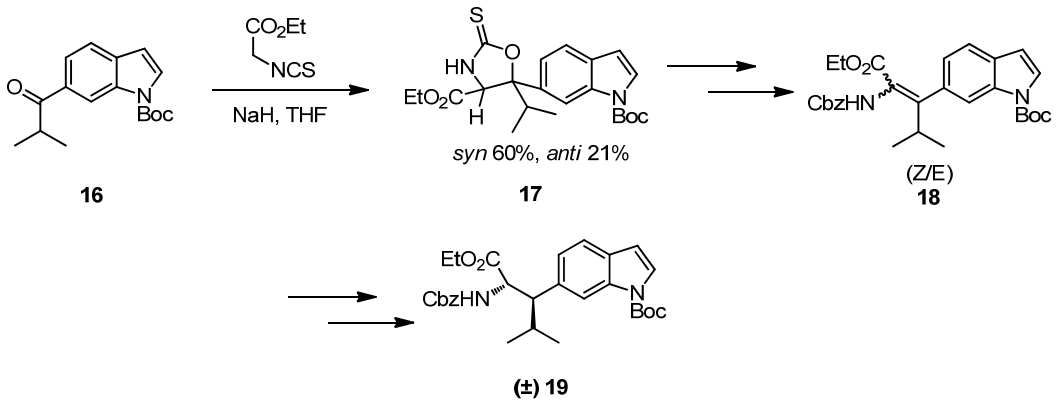


Figure 3. Assembly of the β-substituted leucine residue

Three years prior to that Moody and coworkers reported synthesis of the right-hand ring of moroidin (Figure **4**).[8]

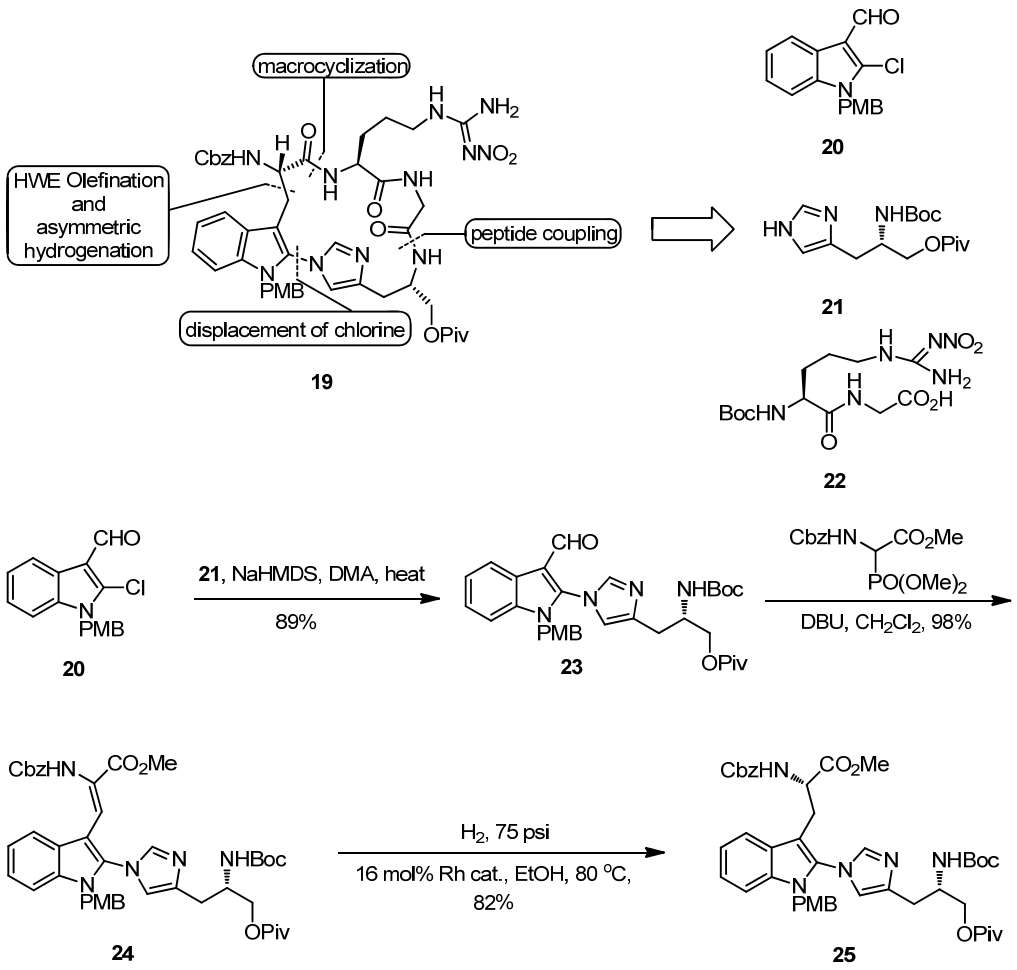


Figure 4. Synthesis of the protected right-hand ring of moroidin

Assembly of the tryptophan-histidine junction in the intermediate **23** was accomplished via a nucleophilic displacement of chlorine in chloroindole **20** with the histidine-derived nucleophile. This can be seen as an interesting alternative to the oxidative coupling described below.

Another synthesis of stephanotic acid methyl ester was reported by Jia and coworkers (Figure **5**).[13] Methodology developed by Jia was used in their lab to complete efficient and stereocontrolled synthesis of (-)-Celogentin C by 2010.[14] Jia and coworkers used the oxidative coupling approach developed in our lab in order to assemble the Trp-His junction and an asymmetric Michael addition to install the Leu-Trp bond.

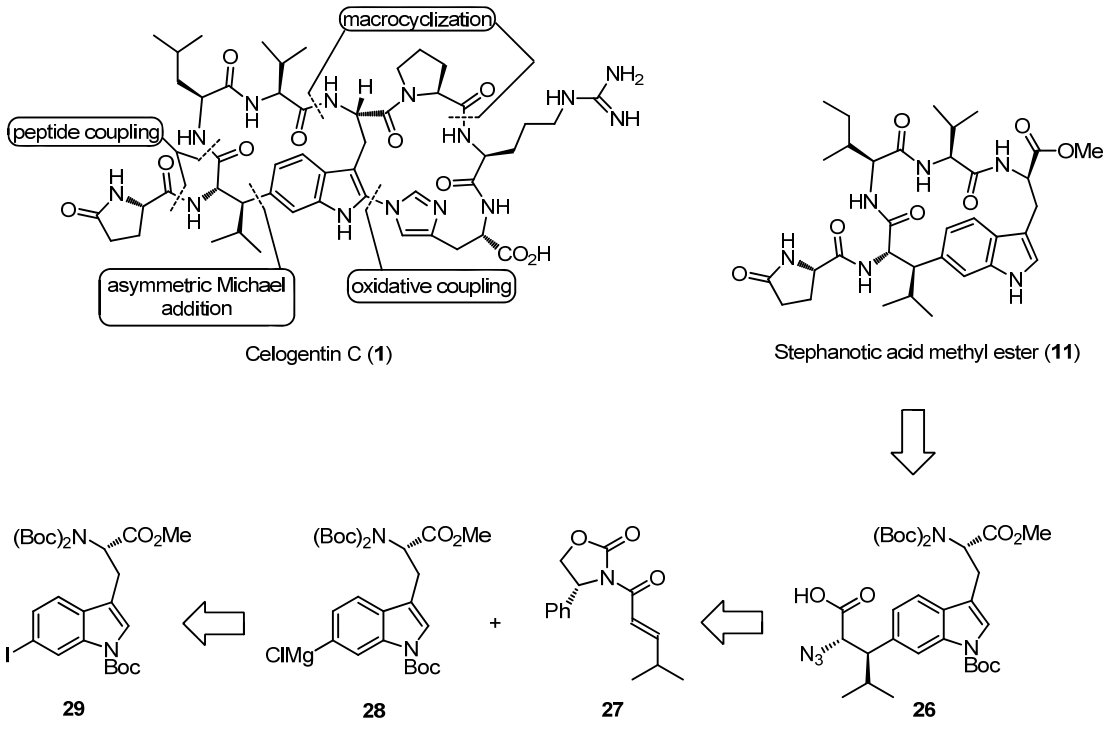


Figure 5. Jia's synthesis of stephanotic acid methyl ester and celogentin C

Another total synthesis of celogentin C has recently been reported by Chen and coworkers.[14] The key Leu-Trp motif was assembled via a novel palladium-catalyzed C-H bond activation strategy (Figure **6**). The Trp-His junction was assembled through oxidative coupling methodology developed in our lab.

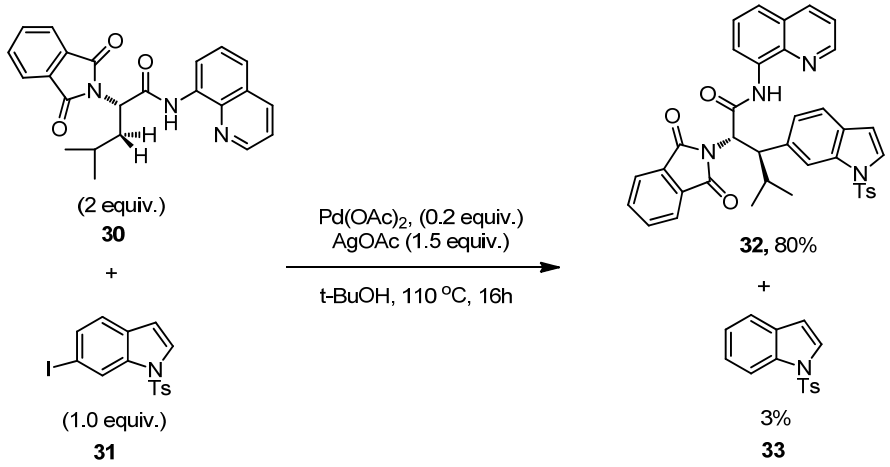


Figure 6. Chen's assembly of the Leu-Trp junction

Much effort concentrated on construction of the central tryptophan residue of Celogentin C, and progress was reported by several research groups.[9, 15, 16] The most significant contribution to the field was made by the Castle group when the first catalytic asymmetric synthesis of the tryptophan residue was reported in 2003,[17] with Pd-catalyzed Cook-type heteroannulation[18] as the key step. Phase-transfer catalyzed alkylation was used to establish correct stereochemistry at the chiral center of tryptophan (Figure **7**).

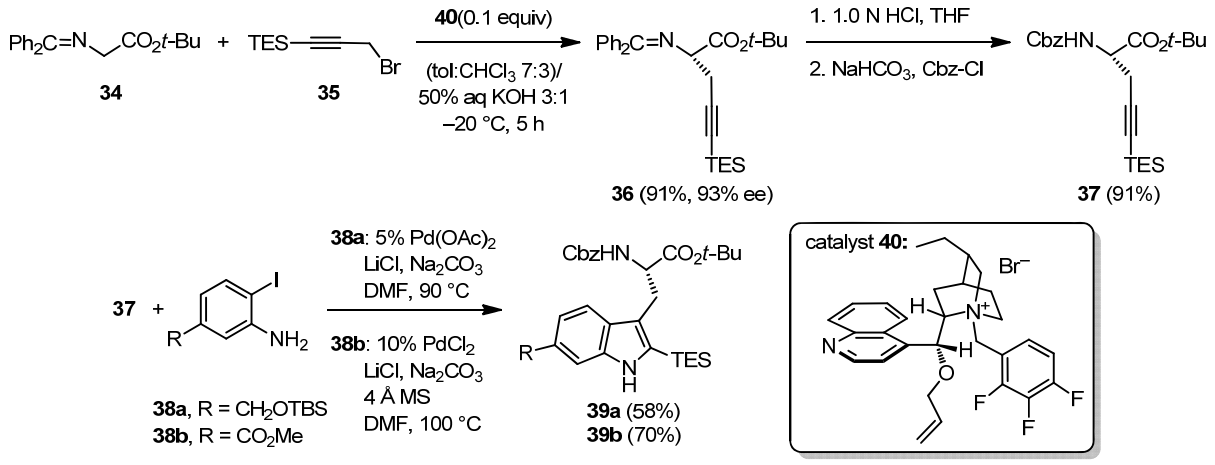


Figure 7. Synthesis of the central tryptophan residue of Celogentin C

The synthesis of the right-hand ring of celogentin C was accomplished by the Castle's group in 2006.[19] (Figure **8**).

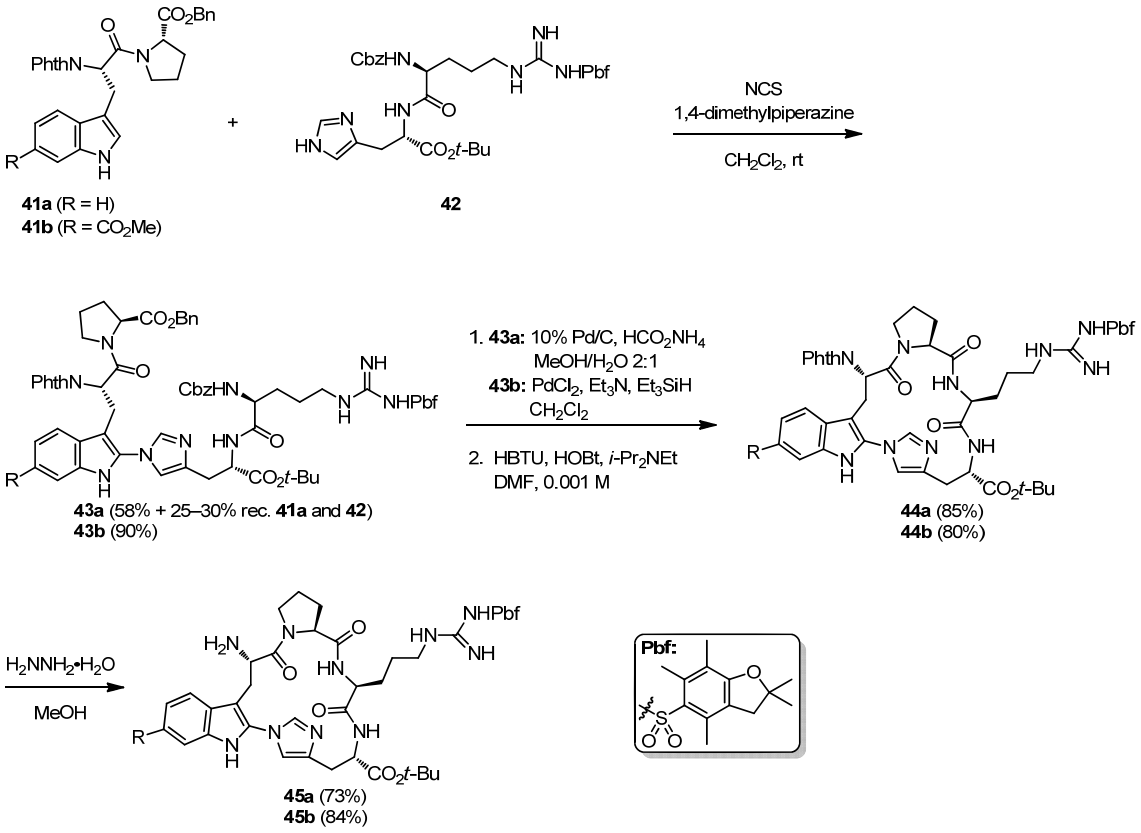


Figure 8. Synthesis of Celogentin C Right-Hand Ring via Oxidative Coupling and Macrolactamization

The critical indole-imidazole linkage was assembled via the oxidative coupling reaction of the two highly functionalized dipeptides. This was followed by the macrolactamization and subsequent deprotection of the N-terminus of the right-hand ring .

1.3 Initial Approach to the Synthesis of the Left-Hand Ring

When I joined the project, I was assigned the task of synthesis of the left-hand ring of celogentin C. Our retrosynthetic analysis of the celogentin C left-hand ring (**46**) is depicted in Figure **9**.

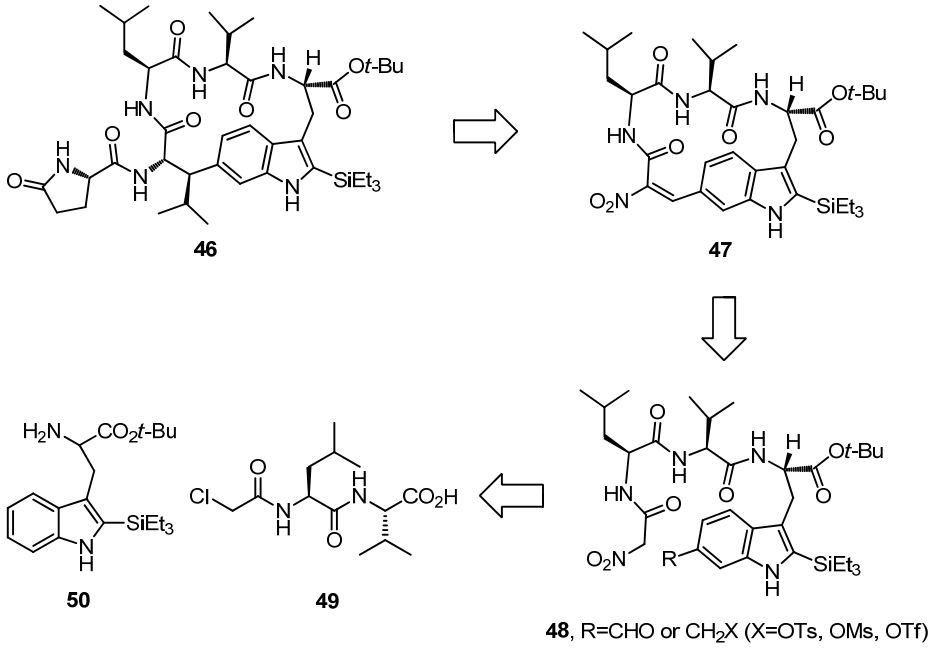


Figure 9. Initial approach to the synthesis of the Left-hand ring: retrosynthesis

The bond between the indole C-6 of tryptophan and the leucine β-carbon creates a β-substituted α-amino acid. This structural motif can be prepared using our recently developed

radical conjugate addition chemistry,[20, 21] and simplification of **46** along these lines affords cyclic peptide **47**. This intermediate contains an α, β-unsaturated α-nitro amide moiety, which can be disconnected at the C–C double bond to provide an acyclic compound **48**. In the synthetic direction, either intramolecular Knoevenagel-type condensation[22] of an aldehyde or alkylation of a substrate containing a suitable leaving group[23] followed by dehydrogenation[24] would conceivably transform **48** into **47**. To the best of our knowledge, neither approach has been previously employed in the synthesis of cyclic peptides. Finally, amide bond scission reveals N-chloroacetyl dipeptide **49** and modified tryptophan **50** as precursors of the Left-hand ring. The N-protected version of **50** has been synthesized in our laboratory,[17] and **49** should be available via straightforward chemistry. Initial research suggested that performing peptide couplings with N-nitroacetyl-containing substrates would be difficult, as the α-nitroamide methylene protons can be abstracted by the amine coupling partner to form a salt, which could occur to the exclusion of amide bond formation.[25] Accordingly, we planned to introduce the nitro group subsequent to coupling of **49** and **50** via displacement of a primary iodide with $AgNO_2$. To our knowledge, no examples of this chemistry applied to peptide substrates exist in the literature, so we were anxious to determine the viability of this approach.

We initiated the synthesis with assembly of the chloropeptide **49**. The commercially available L-leucine (**51**) was refluxed overnight with chloroacetyl chloride, the product **52** was coupled with the valine *tert*-butyl ester, and the resulting chlorodipeptide *tert*-butyl ester **53** was deprotected with boron trichloride (Figure **10**).

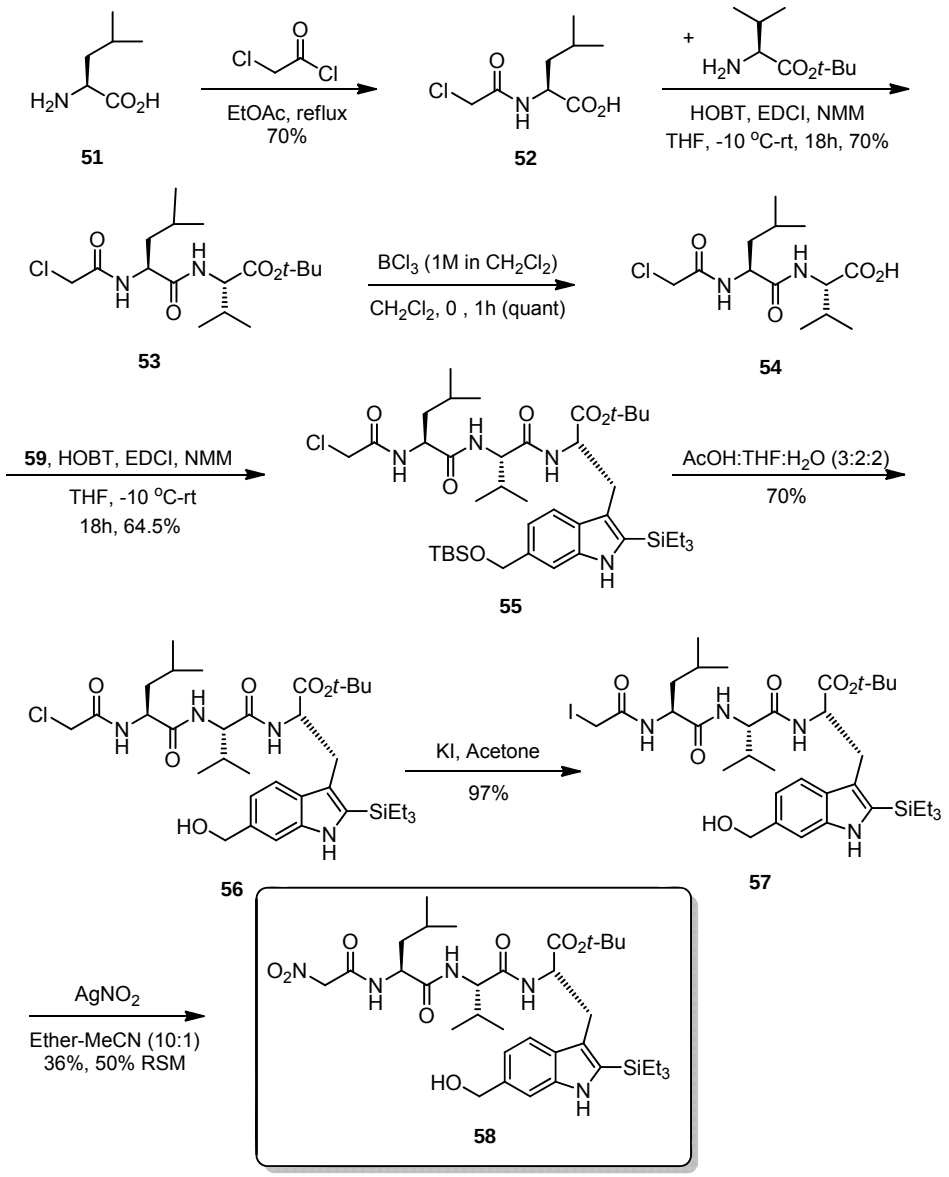


Figure 10. Initial approach to the synthesis of the Left-hand ring

The chlorodipeptide acid **54** was coupled with the Cbz-deprotected tryptophan amine **59**, derived from **39a**, synthesized previously (Figure **11**), giving the tripeptide **55**, which was obtained in reasonable yield and successfully purified by column chromatography without loss of chlorine. The *tert*-butyldimethylsilyl group of this tripeptide was selectively deprotected under mildly acidic conditions using Paquette's methodology[26] without cleavage of either the *tert*-butyl or the triethylsilyl groups or loss of the chlorine moiety. The use of stronger Brønsted acids

resulted in loss of the primary chloride, presumably via solvolysis. The alcohol **56** was treated with potassium iodide in acetone, according to the Finkelstein conditions,[27] yielding the iodoalcohol **57** in nearly quantitative yield. This alcohol could not be purified due to loss of iodine, so it was subjected to the action of silver (I) nitrate in non-polar media, producing the nitro compound **58** in low yields. This reaction was further investigated in our lab.[28]

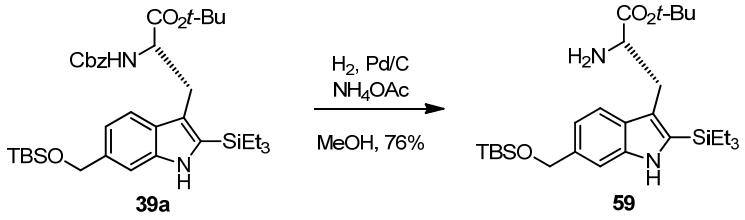


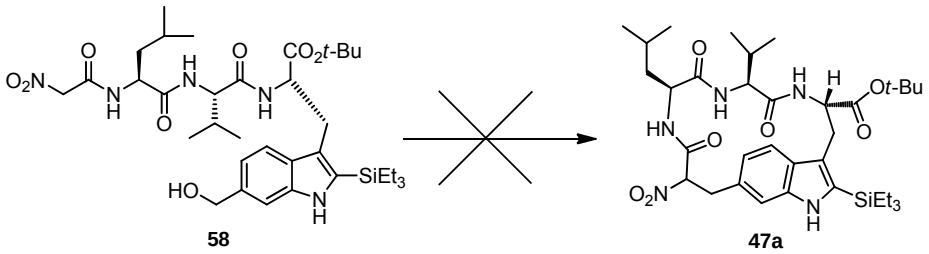
Figure 11. Synthesis of the chiral tryptophan amine **59**

Initially the conversion of iodide **57** was attempted under Kornblum conditions ($AgNO_2$, anhydrous Et_2O, 0 °C),[29] the process was found to be extremely sluggish and unreliable. Increased reaction times led to consumption of starting material without product **58** being produced, , and a reaction conducted in a sealed tube at 35 °C provided the corresponding alcohol side product as the main compound. Nitrite esters are known to hydrolyze easily to the corresponding alcohols.[29] Formation of the alcohol indicated that the undesirable nitrite ester was forming in the reaction mixture. Literature suggests[30] that this nitrite ester can consume desirable nitro compound **58** in the presence of nitrite ions. Hence, we searched for the means to eliminate production of this nitrite ester. However, each alternative examined delivered poorer results than the original protocol. For example, use of $NaNO_2$ in DMSO[31] returned only the alcohol. Employing KNO_2/18-crown-6 in DMSO[32] or ether led to complex mixtures in which **58** could not be detected. We were intrigued by a recent report detailing efficient reactions of polymer-bound nitrite with bromoacetic

esters and bromoacetamides.[33] Unfortunately, application of this protocol to the bromide corresponding to **58** yielded a complex mixture. Finally, reaction of $AgNO_2$ with this bromide produced the alcohol as the only product we could isolate.

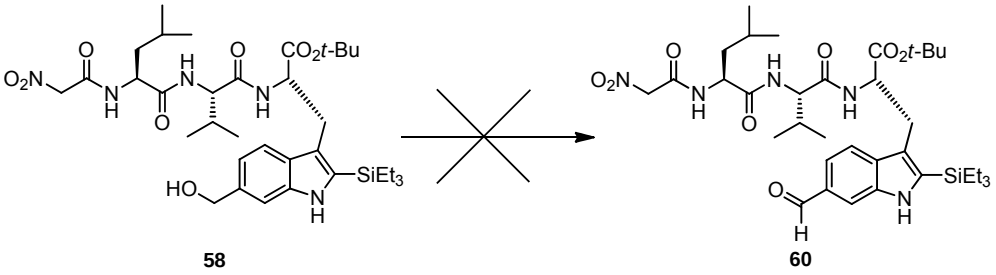
Since $AgNO_2$ is virtually insoluble in ether, we reasoned that a certain amount of moisture was required to dissolve sufficient amounts of the reagent for the reaction to occur at a useful rate. However, according to Kornblum, use of water or another polar solvent should lead to increased levels of nitrite ester formation.[34] In an effort to find a balance between these counteracting factors, we decided to use a small amount of acetonitrile as a co-solvent. Fortunately, conducting the reaction with 6 equivalents of $AgNO_2$ in 10:1 ether–acetonitrile mixture afforded the desired nitro compound **58** as the major product (36%). We were unable to push the reaction to completion, as longer reaction times or increased amounts of $AgNO_2$ led to formation of greater amounts of the alcohol or other unidentified byproducts. Our best results came from terminating the reaction at 50% conversion; this protocol resulted in a 71% yield of **58** based on recovered **57**. Some alcohol was also obtained (10%); nevertheless, the desired product and the recyclable starting material made up the bulk of the mass balance.

However, any attempts on intramolecular alkylation of **58** or oxidation into the corresponding aldehyde **60** proved futile (Figure **12**).



58 **47a**

Entry	Conditions	Results
1.	MsCl, Et_3N (2.3x), DCM, 0 °C to rt	No product **47a** detected
2.	TsCl, Pyridine, DCM, rt	No product **47a** detected
3.	Tf_2O, DIPEA, DCM, 0 °C to rt	No product **47a** detected



58 **60**

Entry	Conditions	Results
1.	TPAP, NMO, DCM, 0 °C - rt	No product **60** detected
2.	DDQ, DCM, 0 °C	No product **60** detected
3.	MnO_2, DCM, 72h	Traces of **60** detected by mass spec.
4.	Oxalyl chloride, DMSO, Et_3N, DCM	No product **60** detected

Figure 12. Attempted intramolecular alkylation and oxidation of the nitro alcohol **58**

1.4 The Second Generation Approach to the Synthesis of the Left-Hand Ring

Being unable to convert alcohol **58** into the unsaturated left-hand ring intermediate **47** or to produce the nitroaldehyde **60** by oxidation, we moved on to an alternative approach to this key intermediate **60**, projecting installation of the nitroacetyl moiety at the later stages of the synthesis following introduction of the aldehyde (Figure **13**).

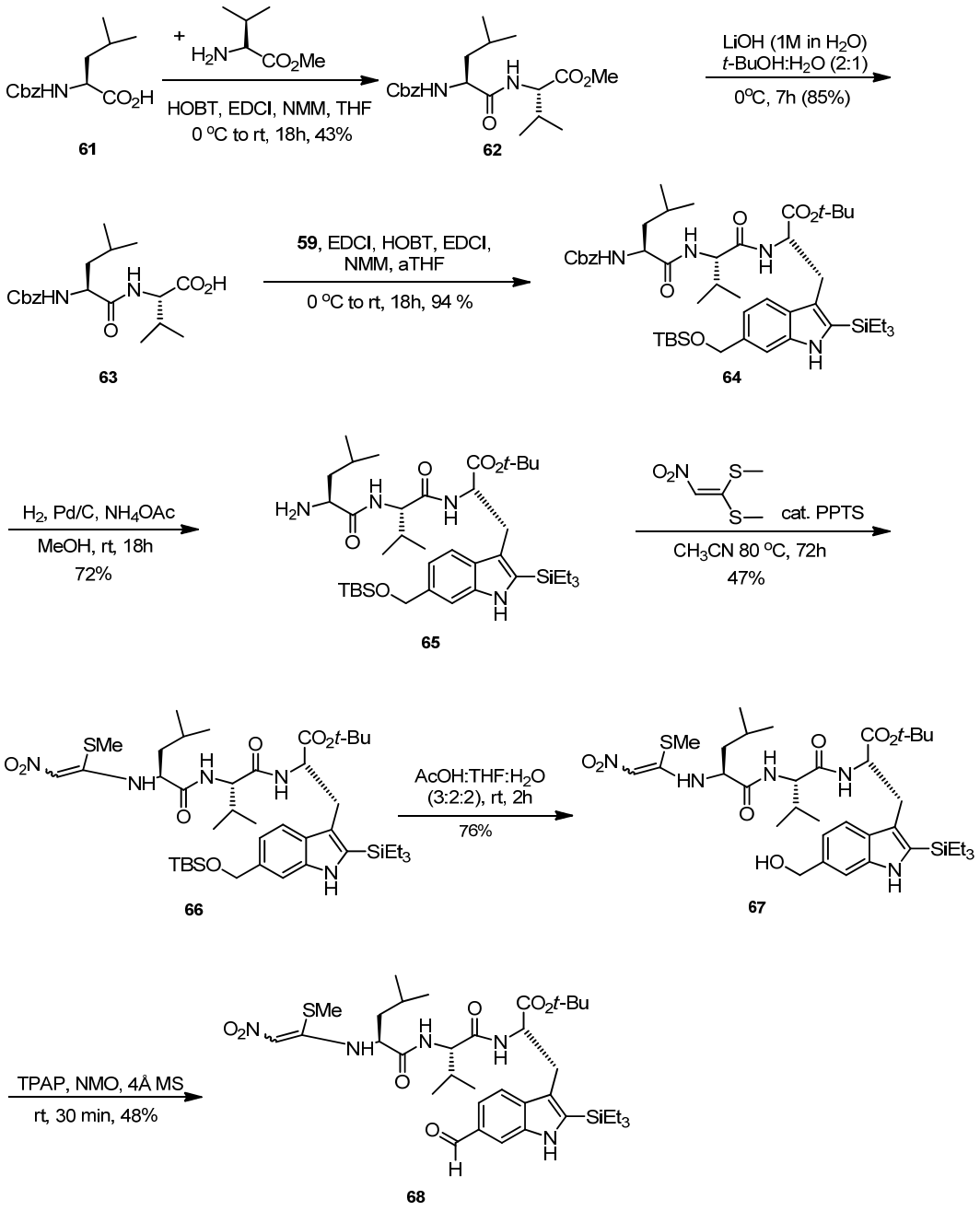
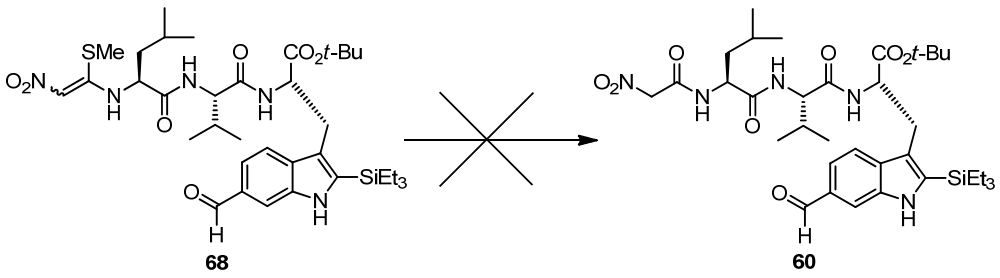


Figure 13. The 2nd generation approach to the synthesis of the Left-hand ring

The synthesis commenced with the peptide coupling of commercially available Cbz-protected L-leucine **61** with the free base of L-valine methyl ester, obtained in a single step from the amino acid. The methyl ester group in the coupling product **62** was cleanly deprotected with lithium hydroxide in *tert*-butanol-water solvent system. The acid **63** was coupled with the free amine **59**,

derived from the compound **39a**, previously synthesized, and the Cbz group removed from the protected tripeptide **64** by Pd/C-catalyzed hydrogenation at balloon pressure. It was observed that presence of NH_4OAc was crucial for this reaction, as no amine **65** had been detected in the absence of ammonium acetate. Utilizing Rajappa's methodology,[35] protected nitroacetamide equivalent **66** was obtained in somewhat mediocre yields, with pyridinium *p*-toluenesulfonate (PPTS) acting as a mildly acidic catalyst, as the use of camphorsulfonic acid (CSA) lead only to the decomposition of the starting material. Despite the known sensitivity of the vinyl thioether to acidic media, selective deprotection of compound **66** produced alcohol **67** in good yields, which was in turn oxidized with TPAP/NMO or DDQ providing protected aldehyde **68** with moderate (TPAP/NMO) or excellent (DDQ) yields. Unfortunately, all our attempts to deprotect the nitroacetyl terminus in this compound were unsuccessful. Treatment with $HgCl_2$ in water–acetonitrile led to a complex mixture of products, and treatment with mild to strong acids (PPTS, CSA, TFA) had only decomposed valuable compound **68** or returned the starting material (Figure **14**).



Entry	Conditions	Results
1.	$HgCl_2$, H_2O-CH_3CN (3:1) varying temps.	No product **60** detected
2.	PPTS, H_2O-CH_3CN (3:1)	Returned SM
3.	CSA, THF-H_2O (10:1), 0 ^oC to rt	No product **60** detected
4.	TFA, DCM, - 78 ^oC to rt	No product **60** detected

Figure 14. Attempted deprotection of the thioether **68**

1.5 Intermolecular Knoevenagel Condensation Studies

At this point, we abandoned the idea of intramolecular Knoevenagel condensation and moved on to alternative approaches. As the thought of using Knoevenagel condensation to assemble the Leu-Trp junction still appealed to us, we envisioned the alternative approach to left-hand ring of celogentin C (Figure **15**).

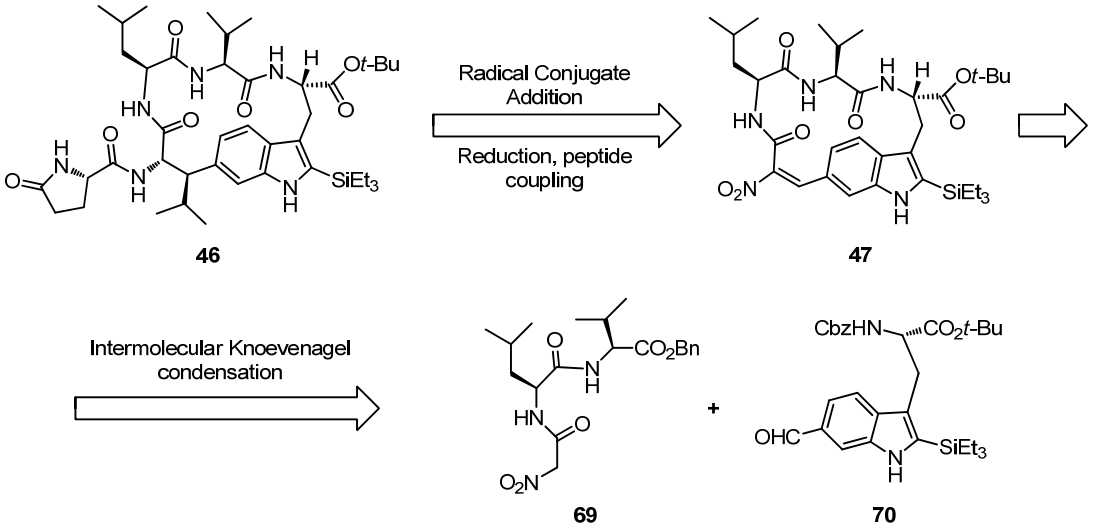


Figure 15. The 3rd generation approach to the synthesis of the left-hand ring

In this methodology the α, β-unsaturated nitro compound **47** is obtained as the product of the intermolecular Knoevenagel condensation of the nitroacetamide dipeptide **69** and the central tryptophan residue-derived aldehyde **70**.

Preparation of the nitroacetamide dipeptide **69** turned out to be a straightforward task (Figure **16**). Commercial L-valine was protected as a benzyl ester under standard Fischer esterification conditions with azeotropic water removal. Peptide coupling with the N-Boc-protected L-leucine gave ester **72** in excellent yield. Boc group removal with TFA quantitatively gave us free amine **73**, which was subsequently protected using Rajappa's protocol.[35] Removal of the vinyl thioether with mercuric (II) chloride in acetonitrile–water gave us desired compound **69** in a good overall yield (Figure **16**).

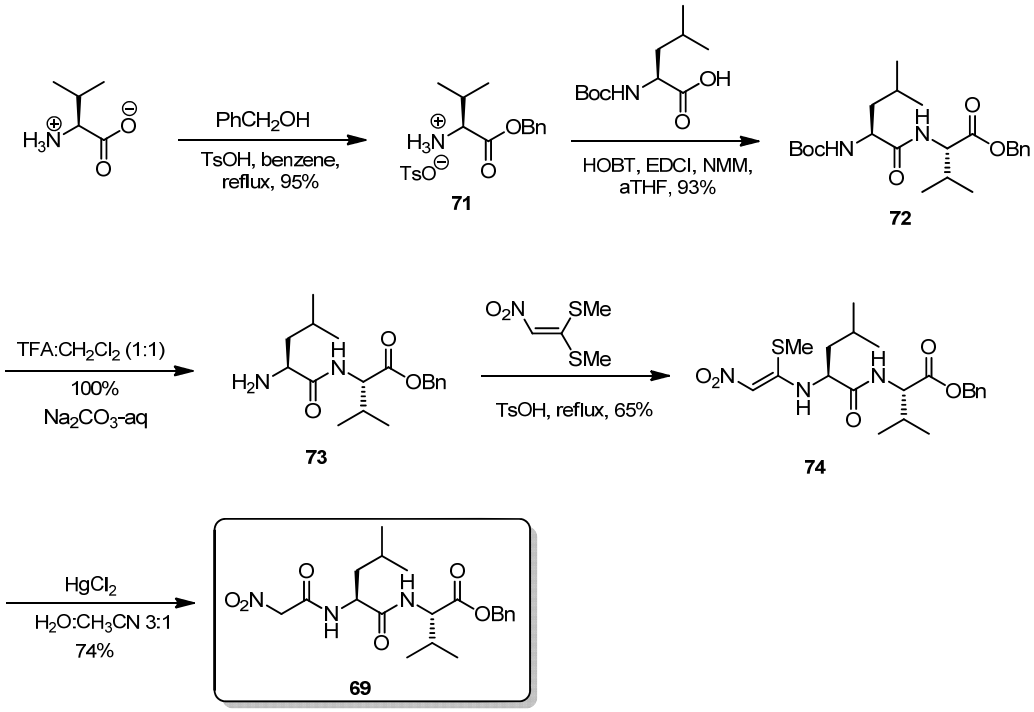
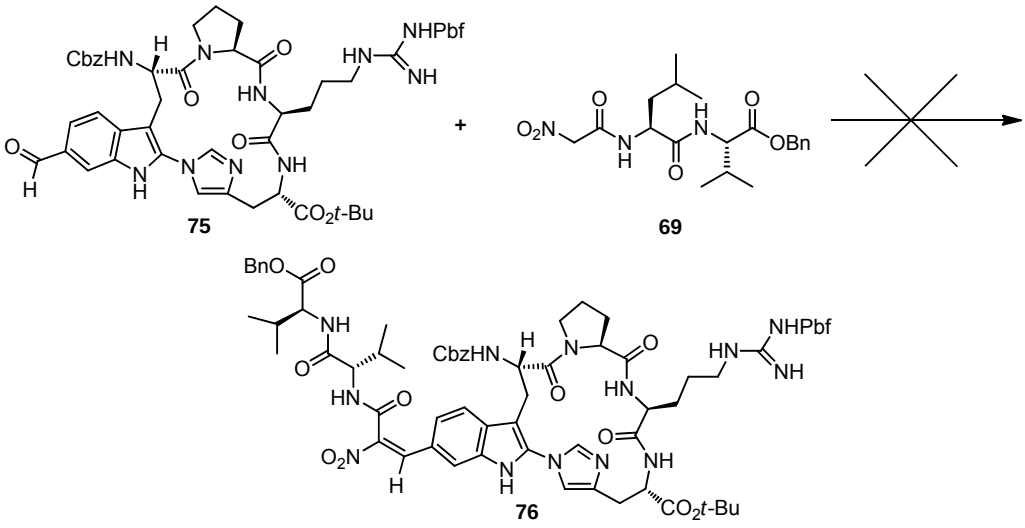


Figure 16. Synthesis of the nitroacetyl peptide **69**

Initially we attempted intermolecular Knoevenagel condensation of the nitro compound **69** with the aldehyde **75** derived from the assembled right-hand ring of celogentin C. We hoped to assemble the left-hand ring using the right-hand ring as the scaffold, thus dramatically reducing number of steps in the total synthesis. To our dismay, all of the attempts to attach the linear chain

by means of Knoevenagel condensation were unsuccessful (Figure **17**). The aldehyde **75** is an extremely polar compound and we experienced solubility issues with the solvent systems of our choice. Also, we speculated on the possibility of Lewis acids binding to the macrocycle due to the presence of multiple Lewis basic sites capable of acting as n-type ligands.



Entry	Conditions	Results
1.	$MgBr_2 \cdot OEt_2$, Et_3N, THF or DCM	No reaction
2.	$TiCl_4$, *N*-methyl morpholine (NMM), THF	No reaction
3.	Toluene, 4Å MS, 75 ^oC	No reaction

Figure 17. Intermolecular Knoevenagel condensation with the right-hand ring aldehyde **75**

Preparation of the chiral tryptophan residue-derived aldehyde **70** was uneventful and afforded the product in excellent yield (Figure **18**).

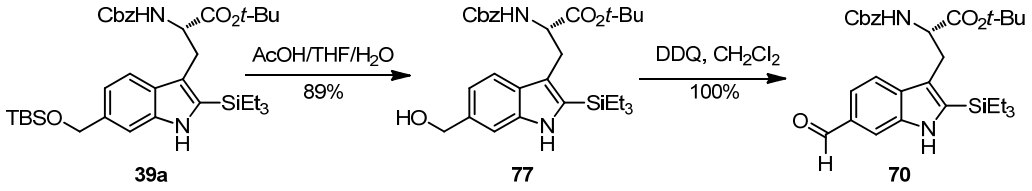


Figure 18. Preparation of the tryptophan-derived aldehyde **70**

Intermolecular Knoevenagel condensation of this aldehyde to the previously made nitro peptide **69** under TiCl4/NMM conditions[20(a)] gave us the desired product **78** in good yields. (Figure **19**).

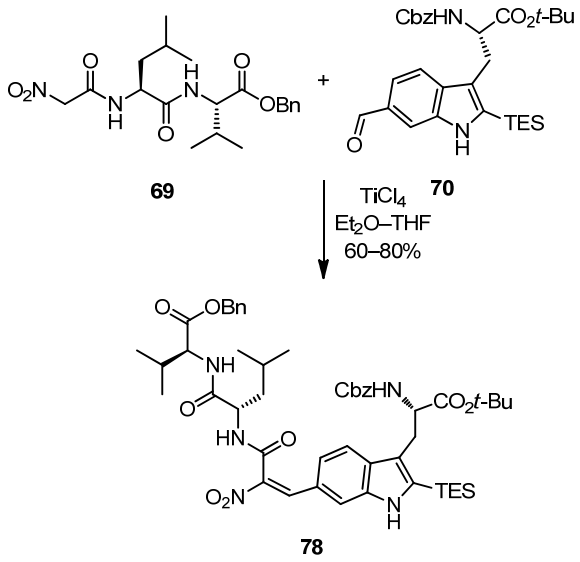


Figure 19. Intermolecular Knoevenagel condensation

This reaction has been further optimized by Bing Ma. Deeply colored product **78** was reduced with sodium borohydride as the model step for the radical conjugate addition. Bing Ma was able to successfully couple the resulting amine with L-pyroglutamic acid, to deprotect the product and successfully perform the macrolactamization, finally succeeding in the synthesis of the model left-hand ring of celogentin C. This success ultimately led to the completion of the total synthesis of

celogentin C a year later[36] with the left-hand ring acting as the scaffold for the assembly of the right-hand ring utilizing already developed methodology.[21]

1.6 Conclusions

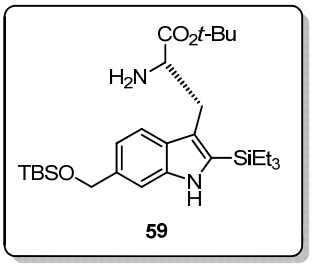
Two attempts to synthesize the left-hand ring of celogentin C have been made. The key substrate for the first method (**59**) was prepared via Cornblum methodology.[31] We could not achieve macrocyclization by attempting intramolecular alkylation of this substrate. Attempts to oxidize it to produce the aldehyde **60** also failed.

The second generation approach involved attempted introduction of the aldehyde group prior to introduction of the nitroacetyl fragment utilizing Rajappa's technique.[35] Our efforts to deprotect the thioether-protected substrate **68** were unsuccessful.

We have succeeded in preparation of the intermolecular Knoevenagel condensation adduct (**78**) from the nitroacetyl-Leu-Val-OBn peptide **69** and the tryptophane-derived aldehyde **70**. This product was further used in the total synthesis of celogentin C.

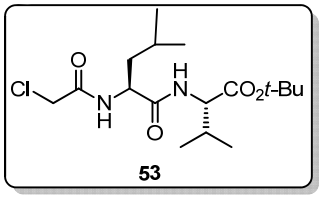
1.7 Experimental details

Dimethyl formamide, methanol, methylene chloride, and tetrahydrofuran were dried by passage through a Glass Contour solvent drying system containing cylinders of activated alumina.[37] Flash chromatography was carried out using 60-230 mesh silica gel. ^{1}H NMR spectra were acquired on 300 or 500 MHz spectrometers with chloroform (7.27ppm) or dimethyl sulfoxide (2.50 ppm) as internal reference. Signals are reported as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), dt (doublet of triplets), br s (broad singlet), m (multiplet). Coupling constants are reported in hertz (Hz). ^{13}C NMR spectra were acquired on spectrometers operating at 75 or 125 MHz with chloroform (77.23 ppm) or dimethyl sulfoxide (39.51 ppm) as internal reference. Infrared spectra were obtained on an FT-IR spectrometer. Mass spectral data were obtained using FAB or ESI techniques.



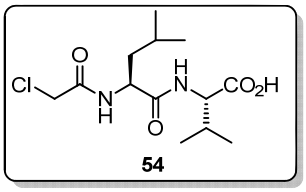
59

6-(*tert*-Butyldimethylsilyloxymethyl)-2-triethylsilyl-L-tryptophan *tert*-butyl ester (59). A solution of Cbz-protected tryptophan **39a** (300 mg, 0.460 mmol) in MeOH (7.0 mL) was treated with ammonium acetate (17 mg, 0.22 mmol) and 10% Pd/C (30 mg). The resultant mixture was stirred under H_2 (1 atm) for 18 h, then filtered through Celite and concentrated *in vacuo*. Flash chromatography (SiO_2, 3 × 20 cm, 20–25% EtOAc in hexanes gradient elution) afforded **6** (180 mg, 0.347 mmol, 76%) as a light yellow film: $[\alpha]_D^{25}$ +3.0 (*c* 4.2, CH_3OH); IR (film): 3485, 1736, 1382, 1152, 1122 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.98 (s, 1H), 7.60 (d, *J* = 8.5 Hz, 1H), 7.38 (s, 1H), 7.02 (d, *J* = 8.5 Hz, 1H), 4.85 (s, 2H), 3.70 (dd, *J* = 4.0, 10.5 Hz, 1H), 3.33 (dd, *J* = 4.0, 14.0 Hz, 1H), 2.86 (dd, *J* = 10.5, 14.0 Hz, 1H), 1.97 (br s, 2H), 1.46 (s, 9H), 1.16–0.76 (m, 24H), 0.13 (s, 6H);^{13}C NMR (125 MHz, $CDCl_3$): δ 174.8, 139.0, 136.3, 132.6, 128.6, 121.8, 119.1, 118.3, 108.6, 81.1, 65.7, 57.3, 32.9, 28.3 (3C), 26.3 (3C), 18.7, 7.7 (3C), 4.0 (3C), –4.9 (2C); HRMS–FAB: *m/z* $[M + H]^+$ calcd for $C_{28}H_{50}N_2O_3Si_2$: 519.3440; found: 519.3437.



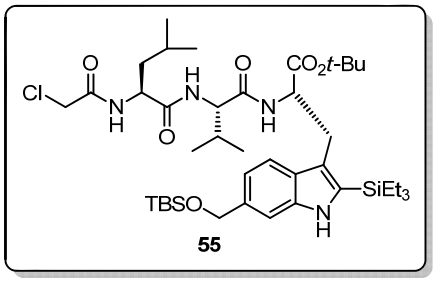
53

(*N*-Chloroacetyl-L-leucyl)-L-valine *tert*-butyl ester (53). A solution of valine *tert*-butyl ester (1.00 g, 5.77 mmol) in anhydrous THF (20 mL) at 0 °C under N_2 was treated with *N*-chloroacetyl leucine (**52**, 1.25 g, 6.02 mmol), HOBt (1.00 g, 7.40 mmol), EDCI (1.40 g, 7.30 mmol), and *N*-methyl morpholine (1.20 mL, 1.10 g, 10.9 mmol). The resultant mixture was stirred at 0 °C for 1 h then at rt for 18 h. H_2O (10 mL) was added to the mixture, and the layers were separated. The aqueous layer was extracted with EtOAc (2 × 15 mL), and the combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (SiO_2, 5 × 22 cm, 20–30% EtOAc in hexanes gradient elution) afforded **53** (1.40 g, 3.86 mmol, 67%) as a white foam: $[\alpha]_D^{25}$ –50 (*c* 0.50, CH3OH); IR (film): 3283, 2963, 1734, 1652, 1545, 1155 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.55 (d, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 9.0 Hz, 1H), 4.67–4.57 (m, 1H), 4.27 (dd, *J* = 8.5, 5.0 Hz, 1H), 3.95 (s, 2H), 2.08–1.88 (m, 1H), 1.62–1.48 (m, 3H), 1.38 (s, 9H), 0.84 (d, *J* = 5.5 Hz, 3H), 0.81 (d, *J* = 6.5 Hz, 6H), 0.80 (d, *J* = 7.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 172.0, 170.6, 166.4, 81.7, 57.9, 52.1, 42.4, 41.4, 31.1, 28.0 (3C), 24.7, 22.9, 22.2, 18.9, 17.8; HRMS–FAB: *m/z* $[M + Na]^+$ calcd for $C_{17}H_{31}N_2O_4ClNa$: 385.1870; found: 385.1857.



54

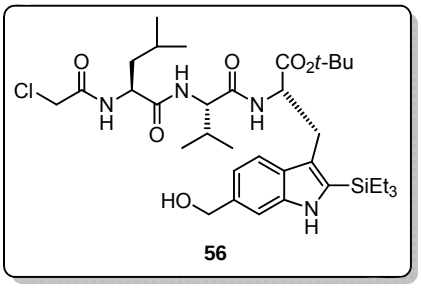
(*N*-Chloroacetyl-L-leucyl)-L-valine (54). A solution of **53** (0.40 g, 1.10 mmol) in anhydrous CH_2Cl_2 (3.0 mL) at 0 °C under N_2 was treated with BCl_3 (1.0 M in CH_2Cl_2, 2.20 mL, 2.20 mmol). The resultant mixture was stirred at 0 °C for 50 min and at rt for 10 min. MeOH (0.50 mL) was added to quench the reaction, and the mixture was diluted with CH_2Cl_2 (5 mL) and washed with H_2O (2 × 5 mL). The organic layer was dried (Na_2SO_4), and concentrated *in vacuo* to afford a quantitative yield of crude **54** (0.34 g theoretical yield) that was used directly in the following reaction.



55

(*N*-Chloroacetyl-L-leucyl)-(L-valinyl)-6-(*tert*-butyldimethylsilyloxymethyl)-2 triethylsilyl-L-tryptophan *tert*-butyl ester (55). A solution of **59** (0.240 g, 0.463 mmol) in anhydrous THF (5.0 mL) at 0 °C under N_2 was treated with crude **54** (0.300 g, 0.980 mmol), HOBt (81 mg, 0.60 mmol), EDCI (115 mg, 0.600 mmol), and *N*-methyl morpholine (88 μL, 81 mg, 0.80 mmol). The resultant mixture was stirred at 0 °C for 1 h then at rt for 18 h. H_2O (10 mL) was added to the mixture, and the layers were separated. The aqueous layer was extracted with EtOAc (2 × 15 mL), and the combined organic layers were washed with brine (10 mL), dried (Na_2SO_4), and

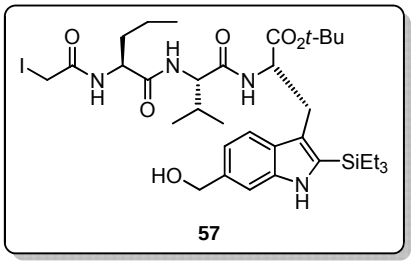
concentrated *in vacuo*. Flash chromatography (SiO_2, 5 × 22 cm, 20–30% EtOAc in hexanes gradient elution) afforded **55** (0.241 g, 0.298 mmol, 64%) as a light yellow solid: $[\alpha]_D^{25}$ –26 (*c* 2.4, CH_3OH); IR (film): 3281, 2959, 2928, 1728, 1644, 1537, 1250, 1135 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.99 (s, 1H), 7.53 (d, *J* = 8.5 Hz, 1H), 7.37 (s, 1H), 7.02 (d, *J* = 5.5 Hz, 1H), 7.01 (d, *J* = 8.5 Hz, 1H), 6.55 (d, *J* = 9.0 Hz, 1H), 6.20 (d, *J* = 6.5 Hz, 1H), 4.84 (s, 2H), 4.68–4.60 (m, 1H), 4.50–4.42 (m, 1H), 4.16 (dd, *J* = 5.5, 8.5 Hz, 1H) 4.05 (s, 2H), 3.27 (dd, *J* = 7.0, 14.5 Hz, 1H), 3.12 (dd, *J* = 9.0, 14.5 Hz, 1H), 2.08–2.02 (m, 1H), 1.62–1.45 (m, 3H), 1.27 (s, 9H), 1.04–0.99 (m, 8H), 0.96 (s, 9H), 0.97–0.92 (m, 9H), 0.91–0.86 (m, 10H), 0.11 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.3, 171.2, 170.4, 166.2, 138.9, 136.5, 132.8, 128.1, 119.4, 118.6, 118.4, 108.6, 81.9, 65.5, 58.2, 55.0, 52.3, 42.6, 41.4, 31.9, 29.3, 28.0 (3C), 26.2 (3C), 24.9, 23.1, 22.2, 19.1, 18.7, 18.0, 7.7 (3C), 3.9 (3C), –4.9 (2C); HRMS–ESI: *m/z* $[M + Na]^+$ calcd for $C_{41}H_{71}N_4O_6ClSi_2$: 829.44929; found: 829.44929.



56

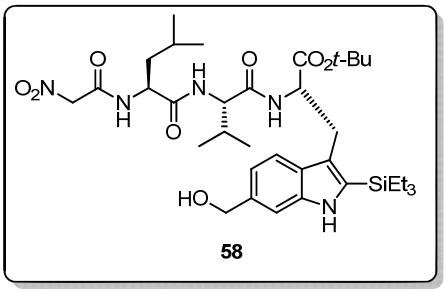
(*N*-Chloroacetyl-L-leucyl)-(L-valinyl)-6-(hydroxymethyl)-2-triethylsilyl-L-tryptophan *tert*-butyl ester (56). A solution of **55** (20.0 mg, 0.0248 mmol) in AcOH–THF–H_2O (3:2:2, 3.0 mL) was stirred at rt for 2 h. The resultant mixture was neutralized with sat aq $NaHCO_3$ (3 mL), extracted with EtOAc (3 × 5 mL), dried (Na_2SO_4), and concentrated *in vacuo*. Flash chromatography (SiO_2, 1.5 × 10 cm, 50% EtOAc in hexanes elution) afforded **56** (12.0 mg,

0.0173 mmol, 70%) as a white solid: $[\alpha]_D^{25}$ –3.3 (*c* 1.0, $CHCl_3$); IR (film): 3283, 2958, 1728, 1644, 1546, 1156 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 8.10 (s, 1H), 7.55 (d, *J* = 8.1 Hz, 1H), 7.37 (s, 1H), 7.15 (dd, *J* = 1.2, 8.1 Hz, 1H), 6.92 (d, *J* = 8.7 Hz, 1H), 6.32 (d, *J* = 9.0 Hz, 1H), 6.10 (d, *J* = 7.8 Hz, 1H), 4.79 (d, *J* = 12.0 Hz, 1H), 4.74–4.64 (m, 2H), 4.45–4.37 (m, 1H), 4.06–4.00 (m, 1H), 4.02 (s, 2H), 3.34 (dd, *J* = 6.0, 14.4 Hz, 1H), 3.10 (dd, *J* = 10.5, 14.4 Hz, 1H), 2.63 (br s, 1H), 2.06–1.95 (m, 1H), 1.67–1.48 (m, 3H), 1.38 (s, 9H), 1.07–0.91 (m, 21H), 0.83 (d, *J* = 6.6 Hz, 3H), 0.71 (d, *J* = 6.9 Hz, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ 171.2, 171.1, 170.1, 166.4, 138.7, 135.9, 133.6, 129.0, 119.9, 119.8, 118.6, 110.4, 82.2, 66.3, 58.1, 55.2, 52.4, 42.6, 41.1, 31.7, 29.3, 28.1 (3C), 25.0, 23.2, 22.2, 19.1, 17.6, 7.7 (3C), 3.9 (3C); HRMS–ESI: *m/z* $[M + Na]^+$ calcd for $C_{35}H_{57}N_4O_6ClSi$: 715.36281; found: 715.36232.



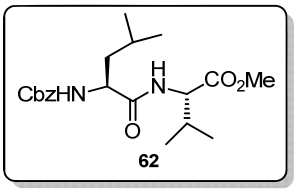
57

(*N*-Iodoacetyl-L-leucyl)-(L-valinyl)-6-(hydroxymethyl)-2-triethylsilyl-L-tryptophan *tert*-butyl ester (57). A solution of **56** (30.0 mg, 0.0433 mmol) in acetone (1.0 mL) was treated with KI (20.0 mg, 0.120 mmol) and stirred at rt for 3 h. The mixture was concentrated *in vacuo*, the residue was washed with $CHCl_3$, and the organic solution was concentrated *in vacuo* to afford **57** (33.0 mg, 0.0421 mmol, 97%) as a light yellow solid that was used directly in the following reaction.



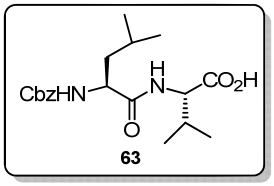
58

(*N*-Nitroacetyl-L-leucyl)-(L-valinyl)-6-(hydroxymethyl)-2-triethylsilyl-L-tryptophan *tert*-butyl ester (58). A solution of **57** (10.0 mg, 0.0128 mmol) in anhydrous Et_2O–CH_3CN (10:1, 440 μL) was treated with $AgNO_2$ (12.0 mg, 0.0780 mmol). The resultant mixture was stirred at rt for 12 h, then concentrated *in vacuo*. Flash chromatography (SiO_2, 2 × 10cm, 33–100% EtOAc in hexanes gradient elution) afforded **58** (3.2 mg, 0.00455 mmol, 36%, 71% based on recovered **57**) as a greenish yellow solid, along with recovered **57** (5.0 mg, 50%) and the corresponding alcohol (0.9 mg, 10%): $[\alpha]_D^{25}$ –1.4 (*c* 0.70, $CHCl_3$); IR (film): 3293, 2957, 1732, 1645, 1564, 1155 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$): δ 7.98 (s, 1H), 7.54 (d, *J* = 8.5 Hz, 1H), 7.33 (s, 1H), 7.14 (d, *J* = 8.0 Hz, 1H), 6.97 (d, *J* = 7.5 Hz, 1H), 6.12 (d, *J* = 8.5 Hz, 1H), 5.95 (d, *J* =8.0 Hz, 1H), 5.10 (d, *J* = 14.0 Hz, 1H), 5.05 (d, *J* = 14.0 Hz, 1H), 4.80 (d, *J* = 12.0 Hz, 1H), 4.71 (d, *J* = 11.5 Hz, 1H), 4.70–4.65 (m, 1H), 4.40–4.35 (m, 1H), 3.93 (dd, *J* = 5.5, 8.5 Hz, 1H), 3.36 (dd, *J* = 5.5, 14.5 Hz, 1H), 3.08 (dd, *J* = 10.0, 14.5 Hz, 1H), 2.85 (br s, 1H), 2.05–1.98 (m, 1H), 1.75–1.44 (m, 3H), 1.42 (s, 9H), 1.06–0.81 (m, 27H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.23, 171.17, 170.3, 160.4, 138.7, 135.5, 133.7, 129.0, 120.0, 119.9, 118.7, 110.3, 82.3, 77.9, 66.3, 58.4, 55.3, 52.5, 41.6, 32.1, 29.3, 28.1 (3C), 24.9, 23.1, 22.0, 19.1, 18.0, 7.6 (3C), 3.9 (3C); HRMS–ESI: *m/z* $[M + Na]^+$ calcd for $C_{35}H_{57}N_5O_8Si$: 726.38686; found: 726.38629.



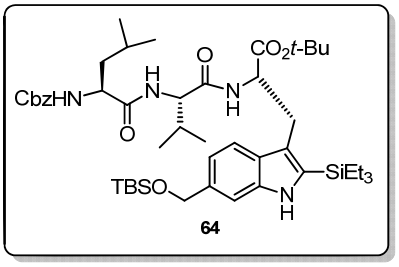
62

Compound 62. Into a 6 dram straight wall vial containing 1.0557g (3.98 mmol) of Cbz-protected L-Leucine (**61**), L-Valine Methyl Ester (0.5g, 3.78 mmol) was added, followed by 13.3 mL of anhydrous tetrahydrofuran. The mixture was cooled to 0 ºC on an ice-water bath while stirring, and 0.6637g (5.04 mmol) of HOBT was added followed by 0.929g (5.04 mmol) of EDCI and 0.8 mL (0.7499g, 7.433 mmol) of N-methyl morpholine was added to the reaction mixture. The mixture was allowed to warm up to room temperature and stirred for additional 18h, at which point it was quenched with 20 mL of water and extracted with EtOAc (10 × 5 mL). Column chromatography on silica gel (29.5 × 4.5 cm, Hex:EtOAc 2:1) afforded 0.6097g (43%) of pure **62**.



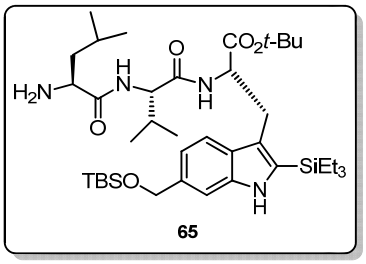
63

Compound 63. Into a 6 dram straight wall vial containing 0.225g (0.5945 mmol) of the methyl ester **62**, 19.7 mL of *t*-BuOH:H_2O solvent mixture (2:1) and the mixture was cooled to 0 ºC. 1M lithium hydroxide monohydrate solution in *t*-BuOH:H_2O mixture (2:1) (0.82 mL, 0.8114 mmol), (pre-cooled to 0 ºC) was added, and the mixture was stirred for 7h and worked up with 1N aqueous HCl. Gradient column chromatography on silica gel (Hex:EtOAc 2:1 -> 10% MeOH in EtOAc) afforded 0.1834g (84.6 %) of pure **63**.



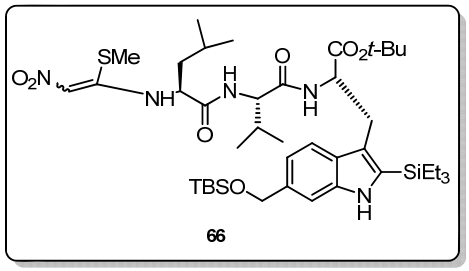
64

Compound 64. Into a 6 dram straight wall vial containing 0.0955g (0.2621 mmol) of Cbz-protected acid **63**, chiral tryptophan amine **59** (0.1295g, 0.2496 mmol) was added, followed by 0.9 mL of anhydrous tetrahydrofuran. The mixture was cooled to 0 ºC on an ice-water bath while stirring, and 0.0438g (0.3268 mmol) of HOBT was added followed by 0.0613g (0.3268 mmol) of EDCI and 53.7 µL (0.0504g, 0.4992 mmol) of N-Methyl morpholine was added to the reaction mixture. The mixture was allowed to warm up to room temperature and stirred for additional 18h, at which point it was quenched with 5 mL of water and extracted with EtOAc (10 x 2 mL). Column chromatography on silica gel (Hex:EtOAc 2:1) afforded 0.2031g (94%) of pure **64**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.90 (s, 1H), 7.42 (d, *J* = 10.0 Hz, 1H), 7.23–7.12 (m, 5H), 6.87 (d, *J* = 10.0 Hz, 1H), 6.65 (d, *J* = 6.0 Hz, 1H), 6.54 (d, *J* = 8.5 Hz, 1H), 5.57 (d, *J* =8.0 Hz, 1H), 4.99 (s, 2H), 4.72 (s, 2H), 4.57 (dd, *J* = 7.5, 8.5 Hz, 1H), 4.25 (br t, *J* = 7.0 Hz, 1H), 4.13 (dd, *J* = 7.0, 8.0 Hz, 1H), 3.15 (dd, *J* = 6.5, 7.5 Hz, 1H), 3.03 (dd, *J* = 5.5, 8.0 Hz, 1H), 1.99–1.88 (m, 1H), 1.60–150 (m, 1H), 1.45 (br t, 2H), 1.14 (d, *J* = 14.5 Hz, 1H), 1.07 (br s, 9H), 0.92–0.87 (m, 9H), 0.86–0.72 (m, 33H); ^{13}C NMR (125 MHz, CDCl3): δ 172.3, 171.5, 170.6, 156.4, 138.8, 136.4, 136.2, 132.6, 128.6, 128.2, 119.6, 118.7, 118.3, 108.4, 81.8, 67.0, 65.5, 58.1, 54.9, 53.8, 41.6, 31.8, 29.5, 27.8 (3C), 26.2, 24.8, 23.1, 22.1, 19.1, 18.6, 18.1, 7.6 (3C), 3.8 (3C).



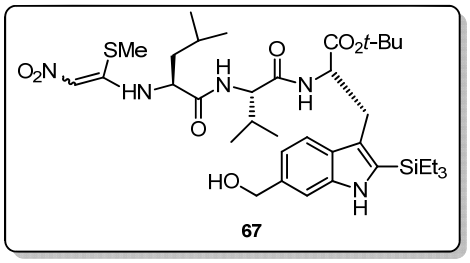
65

Compound 65. Into a 100 mL round bottom flask equipped with magnetic stir bar were placed 0.2031 g (0.2349 mmol) of **64** followed by 3.5 mL of anhydrous methyl alcohol, at which point 0.0306g of Pd on activated charcoal was added under stirring, followed by 0.0362g (0.4698 mmol) of ammonium acetate. The flask was capped with a septum, evacuated, and an H_2 gas balloon – syringe assembly was injected through the septum. The mixture was allowed to stir at room temperature for 24h, at which point the syringe was removed, the flask was once again evacuated, septum removed and Pd/C was filtered off on a celite pad. The solvent and celite pad washings were combined and concentrated. Column chromatography on silica gel (Hex:EtOAc 2:1) afforded 0.17g (72%) of pure **65**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.95 (s, 1H), 7.48 (d, J = 9.5 Hz, 1H), 7.43 (d, J = 8.5 Hz, 1H), 6.88 (d, J = 8.5 Hz, 1H), 6.22 (d, J = 7.5 Hz, 1H), 4.71 (br s, 2H), 4.57–4.50 (m, 1H), 4.05 (dd, J = 3.0, 6.0 Hz, 1H), 4.01 (dd, J = 7.0, 7.5 Hz, 1H), 3.24–3.16 (m, 6H), 3.00 (dd, J = 4.5, 10 Hz, 1H), 2.01–1.92 (m, 1H), 1.61–1.53 (m, 1H), 1.52–1.44 (m, 1H), 1.36–1.06 (m, 13H), 0.94–0.66 (m, 37H).



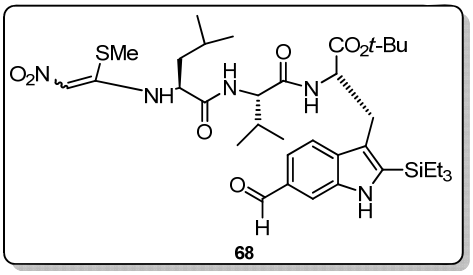
66

Compound 66. Into a 6 dram straight wall vial containing 0.0594 g (0.08124 mmol) of **65**, 0.0134 g (0.08124 mmol) of 1,1 – bis(methylthio)-2-nitroethylene (Aldrich) was added, followed by 0.4 mL of acetonitrile. At this point 0.00035g (0.0016248 mmol, 2 mol%) of pyridinium p-toluene sulfonate (PPTS) was added, and the mixture was allowed to stir at 80 ºC for 18h. Evaporation of the solvent and the following column chromatography on silica gel (Hex:EtOAc 1:1) afforded 0.0327g (47%) of pure **66**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.34 (d, *J* = 7.0 Hz, 1H), 8.05 (br s, 1H), 7.50 (d, *J* = 8.0 Hz, 1H), 7.37 (br s, 1H), 6.99 (d, *J* = 4.5 Hz, 1H), 6.57 (s, 1H), 6.45 (d, *J* =8.5 Hz, 1H), 6.04 (d, *J* = 7.0 Hz, 1H), 4.83 (s, 2H), 4.18–4.08 (m, 5H), 3.30 (dd, *J* = 6.5, 8.0 Hz, 1H), 3.07 (dd, *J* = 4.5, 9.5 Hz, 1H), 2.33 (s, 3H), 2.10–2.02 (m, 5H), 1.73–1.65 (m, 3H), 1.33 (s, 9H), 1.24 (t, *J* = 7.5 Hz, 6H), 1.06–0.86 (m, 24H), 0.82 (d, *J* = 6.5 Hz, 3H), 0.12 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.2, 170.2, 169.9, 164.1, 140.0, 136.6, 132.9, 128.0, 119.4, 118.5, 118.4, 108.8, 108.1, 82.0, 65.5, 60.6, 58.1, 57.8, 55.0, 42.1, 31.8, 29.3, 28.0 (3C), 26.2, 25.0, 23.1, 21.6, 21.2, 19.2, 18.7, 17.9, 7.7 (3C), 4.0 (3C).



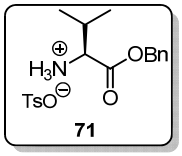
67

Compound 67. Into a 6 dram straight wall vial containing 0.0061g (0.00719 mmol) of **66** was added 0.95 mL of AcOH:THF:H_2O solvent mixture (3:2:2). The mixture was stirred for 12 h at room temperature, quenched with 5.76 mL of saturated aqueous sodium hydrocarbonate solution and extracted with ethyl acetate (10 × 1 mL), solvent evaporated. Column chromatography (Hex:EtOAc 1:1 + 1% MeOH) afforded 0.004 g (76%) of pure **67**: ^{1}H NMR (500 MHz, $CDCl_3$): δ 10.31 (d, *J* = 7.5 Hz, 1H), 8.01 (br s, 1H), 7.53 (d, *J* = 7.5 Hz, 1H), 7.37 (br s, 1H), 7.10 (d, *J* = 7.8 Hz, 1H), 6.54 (s, 1H), 6.31 (d, *J* =4.3 Hz, 1H), 5.94 (d, *J* = 7.0 Hz, 1H), 4.77 (dd, *J* = 4.0, 12.5 Hz, 2H), 4.66–4.60–4.09 (m, 2H), 4.05 (dd, *J* = 2.5, 5.5 Hz, 1H), 3.32 (dd, *J* = 5.5, 9.0 Hz, 1H), 3.07 (dd, *J* = 4.5, 10.0 Hz, 1H), 2.35 (s, 3H), 2.08–1.95 (m, 3H), 1.72–1.60 (m, 3H), 1.55 (br s, 4H), 1.37 (s, 9H), 1.33 (s, 1H), 1.28–1.24 (m, 2H), 1.06–0.87 (m, 15H), 0.81 (d, *J* = 6.5 Hz, 3H).



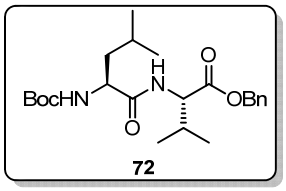
68

Compound 68. Into a 6 dram straight wall vial containing 0.023g (0.0314 mmol) of **67** 1.64 mL of aCH_2Cl_2 was added followed by 4 Å powdered molecular sieves (0.021g), N-methyl morpholine (NMO) (0.0105g, 0.0965 mmol) and tetrabutylammonium perruthenate (TPAP) (0.0011g, 3µmol). The mixture was stirred at room temperature for 30 min and the solvent was concentrated on vacuum. Column chromatography (Hex:EtOAc 1:1) afforded 0.0111 g (48%) of pure **68**.



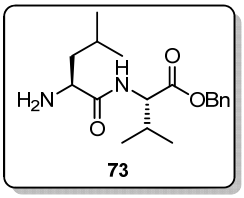
71

Compound 71. To the suspension of the L-Valine (4.000 g, 34.180 mmol) in 200 mL benzene was treated with benzyl alcohol (11.135 g, 10.66 mL, 103.1 mmol), followed by *p*-toluenesulfonic acid (7.860 g, 41.38 mmol). The resulting mixture was refluxed for 6 hours (Generated water was removed by using a Barret trap). Cooled to r.t. and concentrated in vacuo. Treated with 150 mL ether and cooled to 0 ºC. Filtered and washed with ether (5×50 mL). The residue was kept under high vacuum over night to afford the product (**71**, 12.306 g, 32.471 mmol, 95%) as a white solid.



72

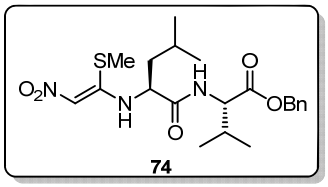
Compound 72. To the solution of Val-OBn (**71**, 11.000 g, 29.000 mmol) and Boc-Leu-OH (6.720 g, 29.000 mmol) in anhydrous THF (150 mL) was treated with *N*-methyl morpholine (14.685 g, 15.50 mL, 145.400 mmol) and HOBt (4.540 g, 34.900 mmol) at 0 ^{o}C, followed by EDCI (6.540 g, 34.900 mmol). The resulting mixture was stirred at 0 ^{o}C → r.t. for 12 hours. Treated with 100 mL sat. aq. $NaHCO_3$ and extracted with CH_2Cl_2 (5 × 30mL). Dried and concentrated in vacuo. Flash chromatography (SiO_2, 3.5×25 cm, 30% EtOAc/Hexanes) afforded **72** (11.327 g, 26.970 mmol, 93% yield) as a white solid.



73

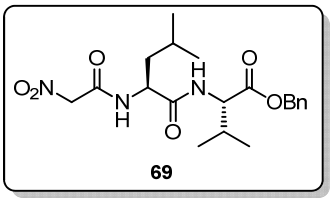
Compound 73. To the solution of Boc-Leu-Val-OBn (**72**, 5.680 g, 13.520 mmol) in 19.0 mL anhydrous CH_2Cl_2 was treated with trifluoroacetic acid (17.760g, 12.0 mL, 155.789 mmol) dropwise at 0 ^{o}C. The resulting mixture was stirred at r.t. for 1 hour. Concentrated in vacuo. The residue was dissolved in 100 mL CH_2Cl_2 and basicified with 2M NaOH (ca. 80 mL). The organic layer was separated. The aqueous layer was extracted with CH_2Cl_2 (3 × 30 mL). Combined

organic layers was dried and concentrated to afford the product **73** (4.326 g, 13.520 mmol, 100%) as a yellow oil.



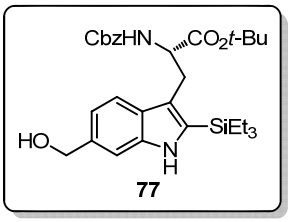
74

(*S*)-benzyl 3-methyl-2-((*S*)-4-methyl-2-((Z)-1-(methylthio)-2-nitrovinylamino) pentanamido)butanoate (**74**). To the solution of (*S*)-benzyl 2-((S)-2-amino-4-methylpentanamido)-3-methylbutanoate (**73**) (4.300 g, 13.440 mmol) in 77 mL MeCN was treated with (2-nitroethene-1,1-diyl)bis(methylsulfane) (Aldrich, 2.200 g, 13.440 mmol), followed by *p*-toluenesulfonic acid (254.0 mg, 1.340 mmol). The resulting mixture was refluxed for 18 h. Cooled to r.t. and concentrated in vacuo. Flash chromatography (SiO_2, 3.5×35 cm, EtOAc/Hexanes 1:10 → 1:1, then pure EtOAc) afforded **74** (3.810 g, 8.720 mmol, 65% yield) as a yellow solid: $[\alpha]_D^{25}$ +165.7 (*c* 0.77, CHCl3); ^{1}HNMR ($CDCl_3$, 500 MHz) δ 10.39 (d, *J* = 7.0 Hz, 1H), 7.40─7.32 (m, 5H), 6.55 (s, 1H), 6.36 (d, *J* = 8.5 Hz, 1H), 5.20 (d, *J* = 12.5 Hz, 1H), 5.11 (d, *J* = 12.5 Hz, 1H), 4.60 (dd, *J* = 9.0, 5.0 Hz, 1H), 4.27─4.23 (m, 1H), 2.36 (s, 3H), 2.25─2.18 (m, 1H), 1.86─1.74 (m, 3H), 0.98 (d, *J* = 6.5 Hz, 3H), 0.93 (t, *J* = 6.5 Hz, 6H), 0.86 (d, *J* = 7.0 Hz, 3H); ^{13}CNMR ($CDCl_3$, 125 MHz) δ 171.5, 170.3, 164.4, 135.3, 128.9 (2C), 128.8, 128.6 (2C), 108.2, 67.4, 58.0, 57.3, 42.3, 31.6, 25.1, 23.1, 21.6, 19.2, 17.8, 14.8; IR (film) νmax 3303, 2961, 1741, 1663, 1560, 1467, 1332, 1223, 1153, 1077, 1003, 763 cm^{-1}; HRMS (ESI) *m/z* 438.20560 (MH^+, $C_{21}H_{31}N_3O_5SH^+$ requires 438.20572).



69

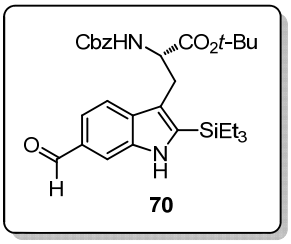
***S*)-benzyl 3-methyl-2-((*S*)-4-methyl-2-(2-nitroacetamido)pentanamido)butanoate** (**69**). To the suspension of **74** (3.810 g, 8.720 mmol) in 60 mL MeCN/H_2O (3:1) was treated with mercuric chloride (2.710 g, 10.000 mmol). Stirred at 45 ^oC for 40 hours. The mixture was concentrated in vacuo. H_2O was removed under high vacuum. The residue was treated with 100 mL CH_2Cl_2 and 20 g silica gel (60─200 mesh), which was then concentrated in vacuo. Flash chromatography (SiO_2, 3.5×35 cm, EtOAc/Hexanes 1:10 → 1:3, then pure EtOAc) afforded **69** (2.230 g, 5.480 mmol, 74% yield) as a white solid: $[\alpha]^{25}_D$ –40.9 (*c* 0.44, $CHCl_3$); ^{1}HNMR ($CDCl_3$, 500 MHz) δ 7.78 (d, *J* = 8.5 Hz, 1H), 7.38─7.33 (m, 5H), 6.68 (d, *J* = 9.0 Hz, 1H), 5.21 (d, *J* = 12.0 Hz, 1H), 5.12 (d, *J* = 12.0 Hz, 1H), 5.10 (s, 2H), 4.62─4.57 (m, 1H), 4.48 (dd, *J* = 8.5, 5.0 Hz, 1H), 2.18 (m, 1H), 1.68─1.55 (m, 3H), 0.92─0.85 (m, 12H); ^{13}CNMR ($CDCl_3$, 125 MHz) δ 172.1, 171.3, 160.7, 135.3, 128.8 (2C), 128.8, 128.7 (2C), 77.8, 67.4, 57.8, 52.5, 41.5, 31.2, 24.9, 22.9, 22.3, 19.0, 17.8; IR (film) νmax 3277, 2963, 1741, 1651, 1564, 1375, 1263, 1003, 751, 697 cm^{-1}; HRMS (ESI) *m/z* 408.21250 (MH^+, $C_{20}H_{29}N_3O_6H^+$ requires 408.21291).



77

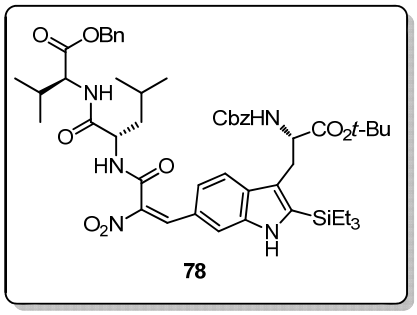
(*S*)-tert-butyl 2-(benzyloxycarbonylamino)-3-(6-(hydroxymethyl)-2-(triethylsilyl)

-1H-indol-3-yl)propanoate (**77**)**.** A solution of the (*S*)-tert-butyl 2-(benzyloxycarbonylamino)-3-(6-((tert-butyldimethylsilyloxy)methyl)-2-(triethylsilyl)-1H-indol-3-yl)propanoate (**39a**) (3.400 g, 5.210 mmol) in 160 mL AcOH-THF-H_2O (3:2:2) was stirred at r.t. for 4 hours. Diluted with 200 mL H_2O and extracted with $CHCl_3$ (3×50 mL). The combined organic layers were dried (Na_2SO_4) and concentrated in vacuo. Acetic acid was removed under high vacuum. Flash chromatography (SiO_2, 3.5×30 cm, EtOAc/Hexanes 1:2 → 1:1) afforded **77** (2.500 g, 4.640 mmol, 89% yield) as a white solid: $[\alpha]^{25}_{D}$ -2.2 (*c* 2.27, $CHCl_3$); ^{1}HNMR ($CDCl_3$, 500 MHz) δ 8.06 (br s, 1H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.35 (s, 1H), 7.30─7.21 (m, 5H), 7.05 (d, *J* = 7.5 Hz, 1H), 5.02 (d, J = 8.5 Hz, 1H), 4.96 (d, *J* = 12.0 Hz, 1H), 4.91 (d, *J* = 12.0 Hz, 1H), 4.75 (d, *J* = 5.5 Hz, 2H), 4.53─4.48 (m, 1H), 3.29 (dd, *J* = 5.5, 14.5 Hz, 1H), 3.12 (dd, *J* = 10, 15 Hz, 1H), 1.72 (t, *J* = 5.5 Hz, 1H), 1.34 (s, 9H), 0.99 (m, 6H), 0.93 (m, 9H); ^{13}CNMR ($CDCl_3$, 125 MHz) δ 171.8, 155.8, 138.8, 136.5, 135.5, 133.4, 128.7, 128.6 (2C), 119.2 (3C), 120.2, 119.4, 119.2, 109.7, 82.0, 66.8, 66.2, 56.2, 29.9, 28.0 (3C), 7.6 (3C), 3.8 (3C); IR (film) νmax 3353, 2954, 2874, 1705, 1499, 1393, 1240, 1153, 1005, 738 cm^{-1}; HRMS (ESI) *m/z* 539.29369 (MH^+, $C_{30}H_{42}N_2O_5SiH^+$ requires 539.29358).



70

Compound 70. To a solution of the alcohol (**77**, 3.990 g, 7.410 mmol) in 100 mL absolute CH_2Cl_2 was treated with the suspension of DDQ (1.690 g, 7.410 mmol) in 40 mL CH_2Cl_2 at 0 °C dropwise over 10 min. The resulting mixture was treated with 20 g silica gel (60─200 mesh) and concentrated *in vacuo*. Flash chromatography (SiO_2, 3.5×30 cm, 10% → 30% EtOAc/Hexanes) afforded **70** (3.970 g, 7.410 mmol, 100% yield) as a yellow solid: $[\alpha]^{25}_D$ +4.2 (*c* 1.38, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 10.02 (s, 1H), 8.48 (br s, 1H), 7.88 (s, 1H), 7.71 (d, *J* = 8 Hz, 1H), 7.59 (d, *J* = 8.5 Hz, 1H), 7.32─7.28 (m, 3H), 7.22─7.18 (m, 2H), 5.30 (d, *J* = 8.0 Hz, 1H), 4.93 (d, *J* = 12.0 Hz, 1H), 4.89 (d, *J* = 12.0 Hz, 1H), 4.55─4.49 (m, 1H), 3.30 (dd, *J* = 14.0, 6.0 Hz, 1H), 3.16 (dd, *J* = 14.5, 9.0 Hz, 1H), 1.30 (s, 9H), 1.20─0.94 (m, 15H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 192.8, 171.5, 155.8, 139.2, 138.0, 136.4, 133.9, 131.4, 128.6 (2C), 128.3, 128.2 (2C), 121.0, 120.6, 119.6, 114.1, 82.3, 66.9, 56.3, 30.2, 28.0 (3C), 7.6 (3C), 3.7 (3C); IR (film) νmax 3346, 2955, 2875, 1701, 1606, 1508, 1368, 1224, 1153, 740 cm^{-1}; HRMS (ESI) *m/z* 537.27470 (MH^+, $C_{30}H_{40}N_2O_5SiH^+$ requires 537.27793).



78

(*S*)-benzyl 2-((*S*)-2-((*E*)-3-(3-((*S*)-2-(benzyloxycarbonylamino)-3-tert-butoxy-3-oxopropyl)-2-(triethylsilyl)-1H-indol-6-yl)-2-nitroacrylamido)-4-methylpentanamido)-3-methylbutanoate (**78**). The solution of the aldehyde (**70**, 774.0 mg, 1.440 mmol) and the nitro compound (**69**, 877.0 mg, 2.150 mmol) in THF-Et_2O (10.0 mL, 2:1) was cooled to 0 ^oC. Treated with 1 M $TiCl_4$ in CH_2Cl_2 (3.03 mL, 3.030 mmol) dropwise under Ar, followed by *N*-methyl morpholine (583.8 mg, 0.63 mL, 5.780 mmol). The resulting mixture was then stirred at 40 ^oC for 6 hours. The reaction was cooled to r.t. and quenched with 1 mL H_2O and diluted with 50 mL CH_2Cl_2. Dried over Na_2SO_4 and concentrated in vacuo. Flash chromatography (SiO2, 3.5×30 cm, EtOAc/Hexanes 1:6 → 1:3) afforded **78** (857.2 mg, 0.922 mmol, 64% yield) as a yellow solid: $[\alpha]^{25}_D$ −44.8 (*c* 1.2, CHCl3); ^{1}HNMR ($CDCl_3$, 500 MHz) δ 8.72 (s, 1H), 8.20 (s, 1H), 7.86 (s, 1H), 7.58 (d, *J* = 8.5 Hz, 1H), 7.36—7.33 (m, 6H), 7.31—7.27 (m, 2H), 7.23—7.21 (m, 2H), 7.13 (d, *J* = 8.5 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 6.65 (d, *J* = 6.0Hz, 1H), 5.23 (d, *J* = 12.0 Hz, 1H), 5.22 (d, *J* = 4.5 Hz, 1H), 5.15 (d, *J* = 11.5 Hz, 1H), 4.93 (dd, *J* = 22.0, 12.0, Hz, 2H), 4.63—4.57 (m, 2H), 4.52—4.48 (m, 1H), 3.26 (dd, *J* = 15.0, 6.0 Hz, 1H), 3.10 (dd, *J* = 14.0, 9.0 Hz, 1H), 2.18 (m, 1H), 1.71—1.65 (m, 1H), 1.64—1.55 (m, 2H), 1.30 (s, 9H), 1.01—0.92 (m, 14H), 0.92—0.83 (m, 13H); ^{13}CNMR ($CDCl_3$, 125 MHz) δ 171.6, 171.5, 171.2, 162.0, 155.8, 141.1, 139.6, 139.5, 138.8, 136.4, 135.4, 132.6, 128.8 (2C), 128.7, 128.63 (2C), 128.60 (3C),

128.3, 128.2, 123.9, 122.2, 120.8, 119.7, 114.3, 82.3, 67.3, 66.9, 57.8, 56.1, 53.4, 40.6, 31.4, 30.1, 28.0 (3C), 24.7, 22.9, 22.0, 19.1, 17.8, 7.5 (3C), 3.7 (3C); IR (film) νmax 3323, 2957, 2874, 1729, 1651, 1519, 1301, 1153, 1093, 1003, 751, 697 cm-1; HRMS (ESI) *m/z* 943.49920 (MH^+, $C_{50}H_{67}N_5O_{10}H^+$ requires 943.49955).

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Chapter II. Computational and Synthetic Studies of Thioviridamide

2.1 Significance of nuclear magnetic resonance in organic chemistry

The history of nuclear magnetic resonance is very typical for the 20th century science, when war effort culminated in creation of the most advance modern analytical technique. Initial discovery of the resonance by Isidor Rabi[1] in 1938 was followed by almost simultaneous discovery of NMR by Edward M. Purcell from Harvard[2] and Felix Bloch from Stanford[3] upon irradiation of bulk matter with radio frequency electromagnetic field. Although the initial goal was the accurate determination of magnetic moments for various elements, it soon became clear that these "constants" depended upon the chemical surroundings of a nucleus. The concept of chemical shift was born. The first NMR spectrum of ethanol, showing three distinct resonance signals was acquired. In 1951 Hahn and Maxwell reported[4] a *spin echo* effect in dichloroacetaldehyde, indicating existence of the interaction between the two protons. The mechanism was proposed by Ramsey and Purcell the same year, and the term "J coupling" was coined. For these breakthrough achievements Felix Bloch and Edward Mills Purcell were awarded the Nobel Prize in physics in 1952.

Soon after Arnold constructed the first homogenous magnet and spinning sample was proposed by Bloch to remove remaining inhomogeneity, the chemists realized the enormous potential of this new technique. Recent decades have seen enormous progress in development of both increasingly strong and homogenous magnets and progressively sophisticated NMR experimental techniques. Introduction of Fourier transform NMR brought at least two orders of magnitude improvement in sensitivity, while introduction of two-dimensional FT NMR allowed observation of complex correlations including those normally forbidden in NMR transitions.

2.2 Molecular mechanics in organic chemistry

As *Ab Initio* methods of simulation remain computationally expensive for larger systems, researchers have naturally turned to molecular mechanics as the fast and inexpensive tool of structure optimization. The concept of molecular mechanics involves a *force field* – a system of force and energy equations and parameter sets used to produce the potential energy of a system of particles (most often, but not necessarily atoms). In this approximation atoms are represented by oscillating masses, coupled by elastic strings, representing chemical bonds. As such, a molecule appears to be an oscillator with some degree of anharmonicity.

Description and parameterization of force fields progressed steadily over time as molecular mechanics encompassed new applications. As Hooke's law (Figure **20**) describes ideally harmonic case, simple potentials like Morse potential[5] accounted for anharmonicity of real world systems, and Lennard-Jones potential[6] included terms for Pauli repulsion (a quantum effect) and long range attraction (van der Waals force) in diatomic molecules.

$$F = -kx$$

Figure 20. Hooke's law

Realistic applications required more sophisticated force fields. The new generations included multiple parameters derived from spectroscopic data and, later on, from the accurate *Ab Initio* calculations. MM2 force field was developed by Norman Allinger for the purpose of conformational analysis of hydrocarbons.[7] This was rapidly followed by AMBER,[8] CHARMM[9] and GROMOS,[10] developed for the purpose of molecular dynamics of macromolecules. OPLS (Optimized Potential of Liquid Simulation) force field,[11] and its derivative, OPLS-AA (all atom)

were developed by William L. Jorgensen[12] of Yale University, and they are re-parameterizations of AMBER intended to produce accurate geometries and conformational energies of peptides in polar media.

2.3 *Ab Initio* Methods of Chemical Simulation

Ab Initio, meaning "from the beginning", applies to the methods of quantum chemistry not using any empirical parameters and relying solely on fundamental physical constants. In quantum physics, the Schrödinger equation describes how a quantum state of a system changes in time. In the standard interpretations, the quantum state, or a wave function, is the most complete description available for a physical system. For stationary (not evolving) systems, time-independent Schrödinger equation is a sufficient description used to produce energy levels and other properties of molecules and atoms (Figure **21**).

$$E\psi(r) = -\frac{\hbar^2}{2m}\nabla^2\psi(r) + V(r)\psi(r)$$

Figure 21. Time-independent Schrödinger equation for a single particle

As the Schrödinger equation cannot be solved precisely for systems of many particles, a number of approximations must be introduced. Born and Oppenheimer assumed independency of nuclear and electronic motion.[13] Thus, the energies of nuclear repulsion and the electronic energy are produced independently. The molecular orbital approach, introduced by Friedrich Hund and Robert S. Mulliken, allowed for separation of a many-electron wave function into a system of individual one-electron "orbitals". Electronic motions in atoms however, are *correlated*, due to the Pauli exclusion principle and Coulomb interaction, meaning that a many-electron wave

function is not a simple linear combination of individual one-electron terms, and electrons do "feel" the presence of each other and their motion is not independent. Further approximations and refinements were required to account for this complexity. Douglas Hartree and Vladimir Fock introduced[14] the "equations with exchange terms", or the self-consistent field procedure (SCF), allowing iterative numerical solution of time-independent Schrödinger equation for multi-particle systems. This method, however, completely neglects relativistic effects and effects arising from electronic correlation, thus leading to significant deviations from the experiment. Further refinements have been introduced (post-SCF methods), such as Møller-Plesset perturbation theory[15] (Mp*n*), configuration interaction (CI),[16] complete active space SCF (CASSCF),[17] all adding superpositions of states or harmonic terms with varying speeds of convergence to the original SCF equations.

An alternative to Hartree-Fock that rapidly evolved in recent decades is density functional theory (DFT), giving approximate solutions to the exchange and correlation energies. In the Kohn-Sham formulation[18] of DFT, Hartree-Fock theory can be regarded as a special case of density functional theory. While being not precisely *Ab initio* techniques in nature, DFT methods however are much less computationally expensive, compared to the MP*n* or CI. Such hybrid functionals like the ubiquitous B3LYP[19] or MPW1PW91,[20] which is used in this work, became the workhorses of organic chemists.

2.4 Simulation of NMR chemical shifts with *Ab Initio* methods

Such properties as chemical shift depend on the interaction of static magnetic fields (external and internal) with the magnetic field created by the electronic motion inside of a molecule. These

static fields affect the *kinetic* energy term of the Hamiltonian, and in case of finite basis the results of the calculation with such an operator depend on the *origin* of the coordinate system (the "gauge"). Two major methods have been proposed in order to deal with this problem: the GIAO (gauge invariant atomic orbitals)[21, 22] and the CSGT (continuous set of gauge transformation).[23] It has been shown that the GIAO method generally produces more accurate values of magnetic shielding tensors while being less computationally expensive as well.

NMR spectroscopy remains one of the most powerful tools of structure determination. However, even with an arsenal of sophisticated 1D and 2D experiments, incorrect assignments of stereochemistry remain rather common. It is a particularly challenging task in case of flexible molecules existing as sets of shallow minima on potential energy surface (PES) at ambient temperatures. Unlike very rapid relaxation of electronic and vibrational energy levels in molecules, systems of nuclear spins possess significant relaxation times in the order of seconds or even minutes. This slow relaxation causes averaging of chemical shifts at ambient temperatures over time as flexible molecules remain in vibrational and rotational motion. This necessitates use of abbreviated Boltzmann distributions (Figure **22**) in order to accurately predict chemical shifts in such flexible systems.

$$\delta = \sum_i \left(\frac{\delta_i e^{-G_i^0 / RT}}{\sum_j e^{-G_j^0 / RT}} \right)$$

Figure 22. Abbreviated Boltzmann distribution for chemical shift simulation

Despite significant progress in the area of incremental prediction of chemical shifts and spin-spin coupling constants, it only remained applicable to rigid, conformationally stable systems,

for which sets of tabulated empirical parameters can produce acceptable agreement to the experimental data. Assignment of stereochemistry, determination of relative configuration by NMR remains the most challenging, rapidly evolving task at the cutting edge of synthetic organic chemistry.

Recent years have seen increased interest in the area of *Ab Initio* prediction of chemical shifts. A number of natural products, such as maitotoxin,[24] hexacyclinol,[25] applidinones A-C,[26] gloriosaols A and B,[27] kadlongilactones D and F,[28] artarborol[29] and others had their configuration assigned or re-assigned by quantum-chemical means.

Giuseppe Bifulco proposed the use of ^{13}C chemical shifts as a tool of differentiation among constitutional isomers of natural products.[30, 31] He also pioneered the use of conformational searching and Boltzmann averaging for determination of the relative stereochemistry. In both cases GIAO-produced ^{13}C chemical shifts were used. Hoye and Cramer pointed out, however, that ^{1}H chemical shifts are more reliable at discriminating between stereoisomers despite the smaller chemical shift window for protons.[32]

NMR shift assignments have been used in cases where a mixture of diastereomers is forming in a chemical reaction, for example a pair of bicyclic peroxides[33] and carene epoxides.[34] Effects of using various levels of theory in the NNR chemical shift calculations have been studied[32] and this area has been extensively reviewed.[35]

2.5 Thioviridamide

Thioviridamide (**79**), isolated[36, 37] by Hayakawa and co-workers from the broth of the gram-positive bacteria *Streptomyces olivoviridis*, is a cyclic oligopeptide possessing a number of challenging structural motifs. The cyclic portion of **79** is comprised of five amino acids, two of which are novel: β-hydroxy-*N*1,*N*3-dimethylhistidinium (hdmHis), and *S*-(2-aminovinyl)cysteine (avCys). Although an *N′*-acylated avCys is not present in any known natural products other than **79**, similar structural fragments are incorporated into some of the lantibiotics, a family of cyclic peptide antibiotics.[38] The acyclic side chain of **79** is attached to the avCys *N*-terminus and consists of six amino acids with the 2-hydroxy-2-methyl-4-oxopentanoyl (HMOP) moiety at the terminus. The side chain contains five consecutive thioamide linkages. No stereochemical details of **79** are known, other than the *cis* configuration of the olefin (Figure **23**).

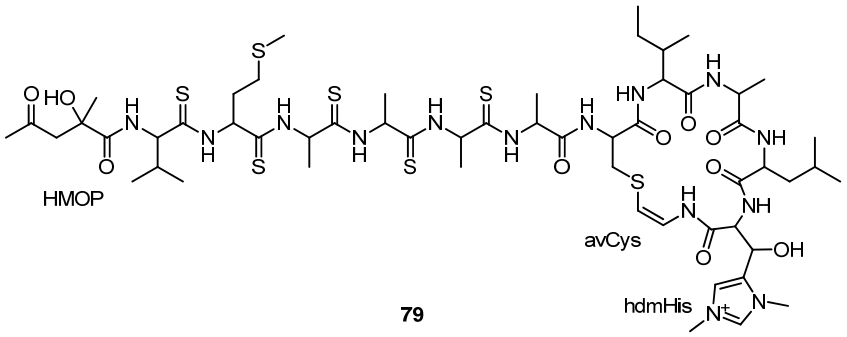


Figure 23. Thioviridamide

Thioviridamide possesses extraordinarily potent and selective anticancer activity. It induces apoptosis in Ad12-3Y1 cells (IC_{50} = 3.9 ng/mL) and E1A-3Y1 cells (IC_{50} = 32 ng/mL).[36] These cell lines have both been transfected with the E1A oncogene, which inactivates the retinoblastoma tumor suppressor protein (pRB). pRB inactivation is implicated in a variety of human cancers.[39, 40] The potency of thioviridamide is comparable to that of apoptolidin (IC_{50} =

17 ng/mL in Ad12-3Y1 cells), a well-known antitumor agent also isolated by the Hayakawa group.[41, 42] Thioviridamide is also selective for cells containing the E1A oncogene, as it inhibits the growth of other tumor cell lines and normal rat fibroblasts at concentrations ranging from 200 to 890 ng/mL, without causing the death of normal cells. Consequently, thioviridamide may be an effective chemotherapeutic agent for cancers that employ an E1A-like oncogene. To date, no synthetic studies of **79** have appeared in the literature.

A major stumbling block on the way to the total synthesis of thioviridamide is the lack of stereochemical information. There are 16384 (214) possible isomers of **79**, of which only one is known to occur in nature. Our approach to solving this problem was to reduce the number of potential structures by making reasonable assumptions and then to further narrow the field through the use of molecular modeling. In this way, we have arrived at a manageable number of candidate structures for total synthesis. We began by focusing on the structure of the cyclic pentapeptide moiety of thioviridamide. This subunit contains seven stereocenters, resulting in 128 possible isomers. Our strategy for reducing this number is summarized in Figure **24**.

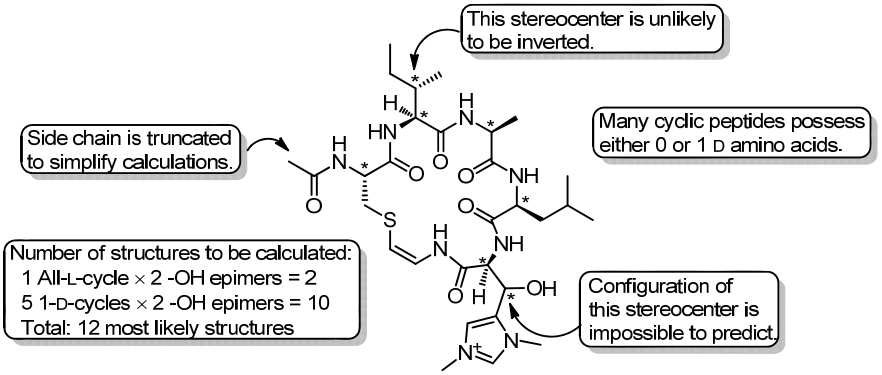


Figure 24. Number and type of thioviridamide cyclic isomers for molecular modeling

It is likely that most of the amino acids present in the ring are of the natural L configuration. However, the orientation of the β-carbon of the β-hydroxy-*N*1,*N*3-dimethylhistidinium (hdmHis) residue is impossible to predict, as both *syn* and *anti* β-hydroxy amino acids are produced in nature.[43] In regards to the isoleucine residue, it is unlikely that an enzyme would invert the configuration of the β-carbon of the abundant L enantiomer due to its much lower acidity compared to the α-stereocenter. In other words, this residue is likely either L-isoleucine or D-*allo*-isoleucine; it is probably not D-isoleucine or L-*allo*-isoleucine. By assuming that the ring contains no more than one D amino acid, we are left with twelve isomers. We have built truncated models of these 12 thioviridamide structures, cutting the side chain off as an acetyl group in order to reduce computational expense. It is possible that our assumption regarding the number of D amino acids present in the macrocycle may turn out to be invalid. Nevertheless, we believed that this plan will allow us to generate a set of computational "lead structures" from which further virtual optimization could be conducted.

The NMR data for **79** were collected in CD_3OD solution.[42] Thus, we planned to minimize the macrocyclic structures in a highly dielectric environments according to the protocol of Jacobson and Lokey.[44] It is likely that the cyclic pentapeptides will exhibit multiple low-energy conformations in a high dielectric environment, as the stability derived from internal hydrogen bonding would be minimized. Thus, we have to perform a conformational search and produce populations of conformers for each of the 12 structures of our choice. Chemical shifts calculated by *Ab Initio* methods and averaged over the population of conformers within 1 kcal/mol of the global minimum for each compound will hopefully allow us to assign the relative configurations of all the 7 stereocenters of interest in the thioviridamide macrocycle.

2.6 Computational Procedure

The 12 isomers (**80a-l**) (from Figure **5**) of required stereochemistry were initially produced with the ChemBioDraw Ultra software (v. 12.0), then transferred into ChemBio3D Ultra (v. 12.0), and preliminarily optimized with molecular mechanics using the MMFFs force field. The resulting files were converted into the SYBYL .mol2 format, as we have determined that this format was the one most effectively retaining atom and bond type information upon transfer from the PC-based Chem3D to the UNIX-based Macromodel (v. 7.1).[45] The structures were assigned positive partial charges of +0.5 on both imidazole nitrogens (by setting both "Charge 1" and "Charge 2" options in Macromodel to "+0.5"), as we have assumed such charge distribution shall as least partially approximate the real-world charge delocalization. We have decided to perform the comformational search with the OPLS-AA force field, as it gave us the least number of "weak" (low quality) parameters, especially torsions, which are of utmost importance in conformational searches, as they account for dihedral angle variation (Table **1**).

Force field	Weak stretches	Weak bends	Weak torsions
MMFFs	5	22	62
AMBER	3	4	2
OPLS-AA	0	0	0

Table 1. Number of unoptimized parameters vs. force field

Since the experimental NMR data for thioviridamide (**79**) was collected in CD_3OD (with the relative permittivity of 33.0), and Macromodel 7.1 offers only water and chloroform as the choices of solvents, we set the option of dielectric constant in Macromodel to 33.0, effectively optimizing our conformers in a highly permeable vacuum. Setting the "cutoff" option to "extended" allowed for the long-range van der Waals interactions to be accurately treated. The width of the

comformational energy window was set to 50 kcal/mol and the optimization method to PRCG (Polak-Ribiere conjugate gradient).[46, 47]

Another issue we faced was the number of steps of the MCMM (Monte Carlo Multiple Minimum)[47] conformational search that we should attempt. Macromodel manuals advocate performing comformational searches until the global minimum is found multiple times and no new conformations are further produced. However, given the size of our system of interest, performing an exhaustive comformational search was impractical due to time constraints and inability to occupy computational resources for prolonged periods of time. Most literature sources recommend 10,000-50,000 of MCMM steps, however for systems of significantly smaller size. With these considerations in mind, we performed a benchmark comformational search for the compound **2j** with OPLS-AA force field and 300,000 steps of MCMM search, which was completed within 1 week of wall time (Figure **25**).

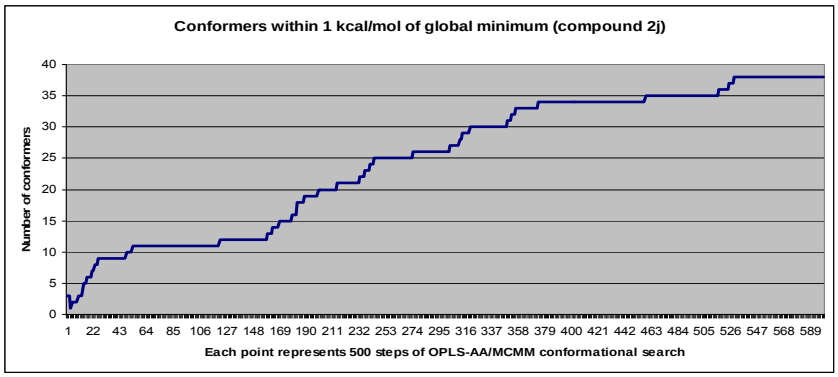


Figure 25. Number of conformers within 1kcal/mol of global minimum for the compound **80j**

The likely global minimum was rapidly found after barely 25,000 steps of MCMM search (Figure **26**). In order to conserve computational time but still produce a reasonable number of conformers within 1 kcal/mol we decided to perform MCMM comformational searches on 100,000 steps.

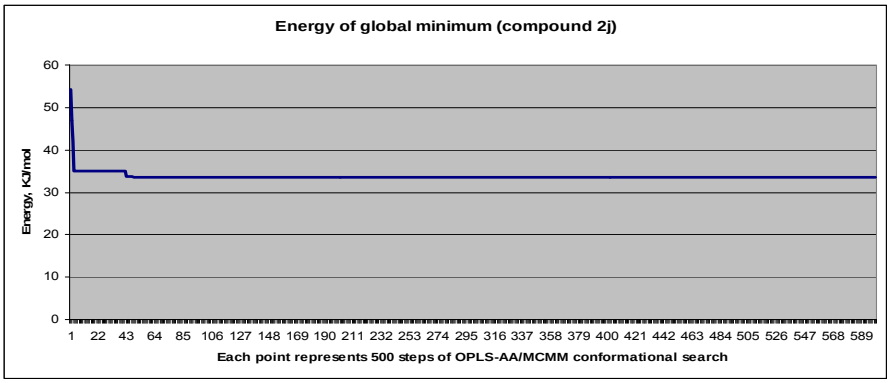


Figure 26. Energy of the global minimum for the compound **80j**

Despite existing literature precedents advocating *Ab Initio* optimization of the conformer families,[32] the data favoring omission of such optimization also exists.[49] We have attempted optimization of the OPLS-AA global minimum for the compound **80j** at the PM3MM level in vacuum. It took approximately 8 hours of wall time on 8 CPU cores (1 node) of the Marylou5 supercomputer at Brigham Young University (two Quad-core Intel Nehalem (2.8 GHz) CPUs with 24 GB of memory per node, running CentOS (based on Red Hat Enterprise Linux v5.1)) to complete the optimization. As it can be seen from the Figure **27**, the forces in the system diminish rather rapidly, while the maximum displacement remains significant. This is usually an indication of a shallow potential energy surface (PES), and, particularly, of an existing free rotation in the system. Based on these facts and a possibility for the generated conformers to be perturbed and degrade into the true global minimum, we decided to omit the *Ab Initio* optimization of the conformer families altogether.

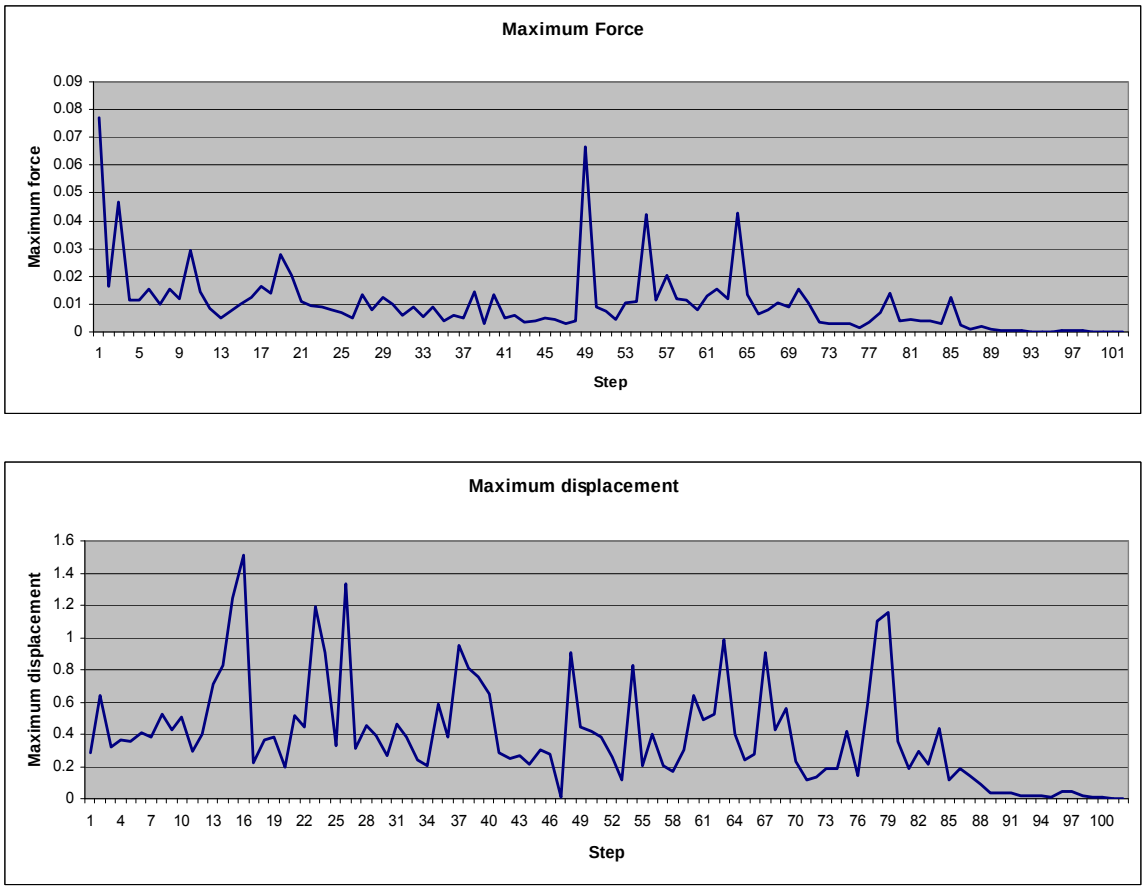


Figure 27. Berny optimization of the **80j** macrocycle at PM3MM level

Populations of conformers produced at the OPLS-AA level were converted to the Gaussian 03 (Rev. D.01)[48] input file format, and transferred to the Marylou5 supercomputer. Single point *Ab Initio* energy calculations were performed at the 6-311+G(2d, p)[49] level of theory with the MPW1PW91[20] density functional. The solvent cavity was modeled with the Integral Equation Formalism Polarizable Continuum Model (IEFPCM),[50] with parameters set for methyl alcohol. Tight convergence criteria were applied (SCF=Tight). Isotropic magnetic shielding tensors were calculated with GIAO method (NMR=GIAO).

2.7 Data Analysis

Our approach to the assignment of stereochemistry was to calculate the isotropic gauge-independent magnetic shielding tensors (GIAOs) and then obtain calculated chemical shifts by scaling GIAOs relative to the corresponding values for ^{1}H chemical shifts of tetramethylsilane TMS, which was also calculated at the discussed level of theory. The data was Boltzmann-averaged (Figure **22**), with *Ab Initio* SCF energies used in lieu of the free energies of individual conformers. The issue of how to quantify the agreement between the experimental and theoretical data depends on how the data is processed. We cannot expect a perfect match, as we have abbreviated the conformational search and the side chain of thioviridamide.

Various approaches for data analysis are described in the literature:[51]

1. Correlation coefficient, r, between calculated and experimental shifts.
2. Mean absolute error (MAE) $MAE = \frac{1}{N}\sum_{i}^{N}\left|\delta_{calc} - \delta_{\exp}\right|$
3. Corrected mean absolute error (CMAE) $CMAE = \frac{1}{N}\sum_{i}^{N}\left|\delta_{scaled} - \delta_{\exp}\right|$,

where $\delta_{scaled} = \frac{\delta_{calc} - intercept}{slope}$

The latter procedure involves plotting experimental (x axis) vs theoretical (y axis) data, performing linear regression analysis, and extracting the value of the slope and y-intercept from the linear equation.

Having analyzed our data using the above-mentioned criteria, we have obtained possible best fit candidates for the stereochemical structure of the thioviridamide macrocycle (Figure **28** and Table **2**):

Structure	Slope	Y intercept	R	MAE	CMAE	Note
80a	1.1371	-0.2608	0.96623	0.507399	0.324973	Natural (all L), R-alcohol
80b	1.2267	-0.4696	0.97242	0.454076	0.289233	Natural (all L), S-alcohol
80c	1.2129	-0.4696	0.95984	0.514962	0.304128	D-avCys, R-alcohol
80d	**1.2161**	**-0.4182**	**0.98025**	**0.470800**	**0.227371**	**D-avCys, S-alcohol**
80e	1.1791	-0.3470	0.94799	0.626550	0.461198	D-allo-Ile, R-alcohol
80f	1.2043	-0.3728	0.95063	0.635425	0.465851	D-allo-Ile, S-alcohol
80g	1.1919	-0.4231	0.97113	0.463800	0.316063	D-Ala, R-alcohol
80h	1.2124	-0.4486	0.96643	0.458952	0.318783	D-Ala, S-alcohol
80i	1.2170	-0.6446	0.95656	0.522734	0.354181	D-Ala, R-alcohol
80j	1.2280	-0.5232	0.96302	0.509023	0.359652	D-Ala, S-alcohol
80k	1.1947	-0.4155	0.97196	0.454910	0.314153	D-hdmHis, R-alcohol
80l	**1.2044**	**-0.4943**	**0.98214**	**0.390459**	**0.213663**	**D-hdmHis, S-alcohol**

Table 2. Statistical data for the compounds **80a-l**

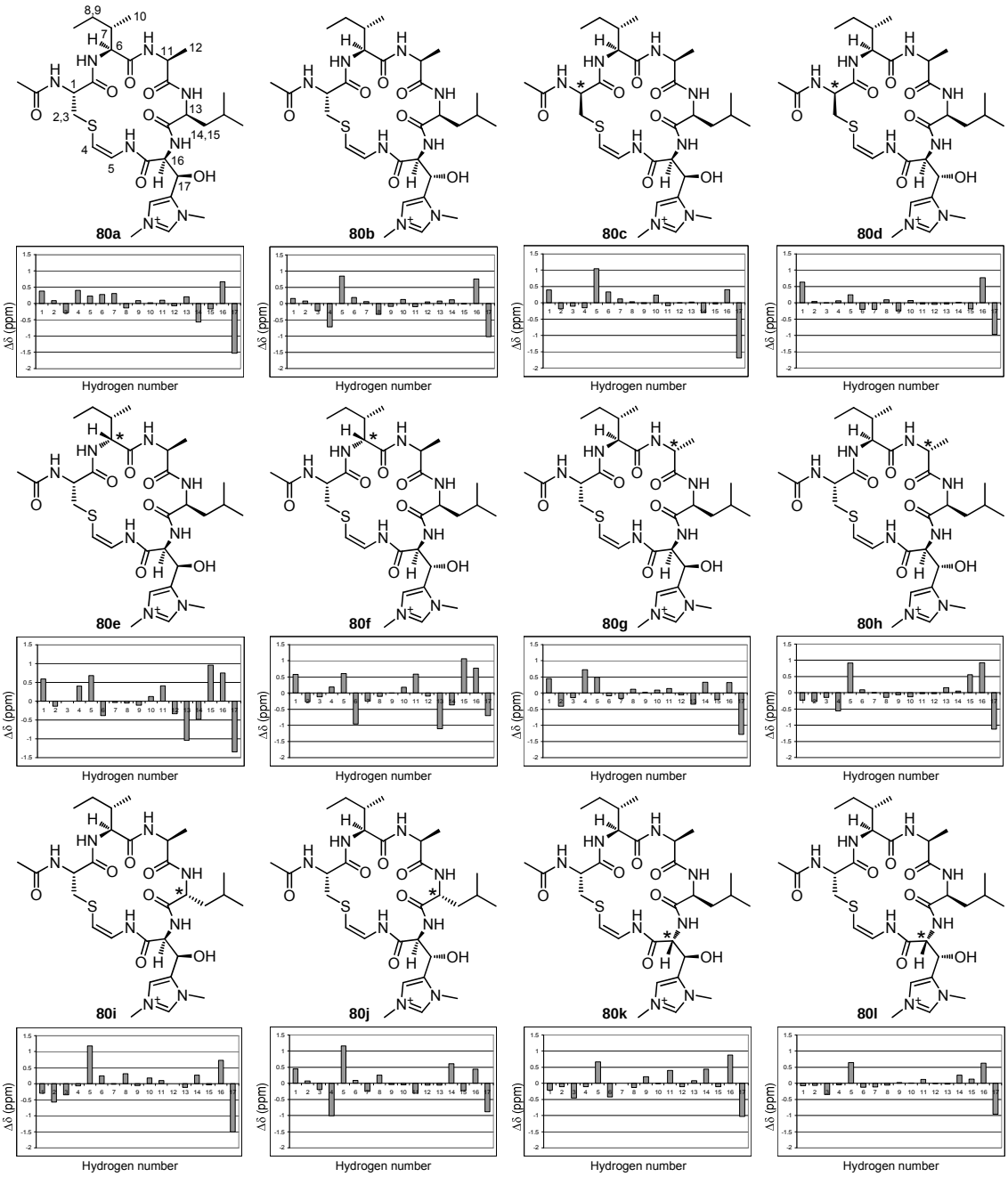


Figure 28. Difference between calculated and experimental ^{1}H NMR chemical shifts for compounds **80a-l**. D-Amino acids are denoted by asterisks.

2.8 Results and Discussion

Upon a closer look at the Table **2,** we can derive two likely candidates for the thioviridamide macrocycle. Compounds **80d** with inverted (2-aminovinyl)cysteine (D-avCys) and the S-configuration at the alcohol center, and **80l** with D-β-hydroxy-*N*1,*N*3-dimethylhistidinium (D-hdmHys) and the S-configuration at the alcohol center. These compounds have the highest values of the correlation coefficients and the lowest values of corrected mean absolute errors (CMAE) (Figure **29**). This is indeed the likely case, as most of the thioviridamide macrocycle framework is comprised of natural (L) amino acids.

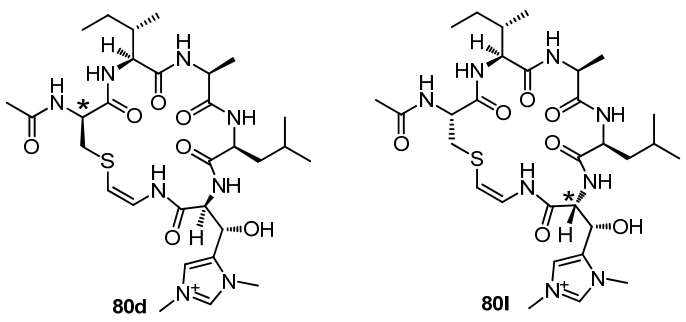


Figure 29. Likely candidates for the stereochemical configuration of thioviridamide

2.9 Conclusions

We have performed extensive computational analysis of the 12 most likely candidate structures to fit the stereochemical profile of thioviridamide. Populations of conformers were produced with the OPLS-AA force field, and *Ab Initio* single point energy calculations were performed at the MPW1PW91/6-311+G(2d, p) level of theory with IEFPCM approximation of solvent cavity. Theoretical chemical shifts were produced through scaling of the GIAO isotropic magnetic tensors for the protons of interest with the averaged value of the 1H chemical shift of

TMS produced at the same level of theory. Statistical analysis of the data by means of method of linear regression allows preliminary assignment of thioviridamide stereochemistry: **D-avCys**-L-Ile-L-Ala-L-Leu-L-hdmHys(S-alcohol), or the L-avCys-L-Ile-L-Ala-L-Leu-**D-hdmHys**(S-alcohol). In both cases, only an unusual amino acid is inverted.

2.10 Synthetic Studies of the *β*-Thioenamide Subunit of Thioviridamide

The *cis*- β-thioenamide subunit of thioviridamide (Figure **30**), connecting β-hydroxy-*N*1,*N*3-dimethylhistidinium (hdmHis), and *S*-(2-aminovinyl)cysteine (avCys) amino acids is the only unusual cross-linkage within the thioviridamide macrocycle. Existing methods of β-thioenamide synthesis, such as Pummerer rearrangement[52] or imine acylation[53] produce predominantly *trans* β-thioenamides. Assembly of this *cis*- β-thioenamide subunit of thioviridamide shall be the key step towards the total synthesis of the natural product, and we have initiated research aimed at development of methods that would allow us to assemble such structural motifs.

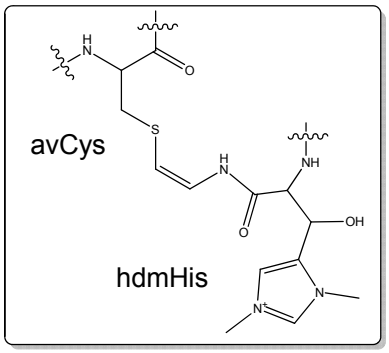


Figure 30. *β*-Thioenamide subunit of thioviridamide

2.10.1 Ynamides

Ynamides are a relatively new class of compounds whose chemistry has evolved rapidly in recent years.[54] They are electron-rich alkynes, with the alkyne moiety directly connected to an amide nitrogen. As the presence of an N-acyl group is implied, the reactivity of ynamides can be modified by varying the nature of the N-acyl substituent. It has been recently demonstrated that ynamides can act as radical acceptors.[55, 56] We have speculated that β-thioenamides can form via the addition of thiyl radicals to ynamides. A recent development by Yorimitsu and Oshima confirmed this hypothesis – addition of arenethiols to disubstituted tosylynamides does produce β-thioenamides. (Figure **31**).[57]

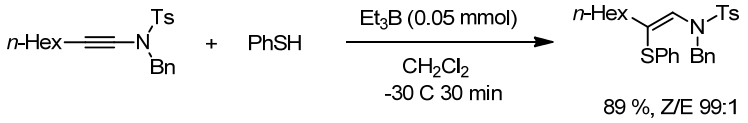


Figure 31. Yorimitsu synthesis of *β*-Thioenamides subunit of thioviridamide

2.10.2 Addition of Thiyl Radicals to Terminal Ynamides

Synthesis of the avCys-hdmHis subunit of thioviridamide (**79**) requires radical addition to a terminal ynamide, something that Yorimitsu and Oshima did not report. We have proposed a thiyl radical addition reaction to terminal ynamides (Figure **32**).

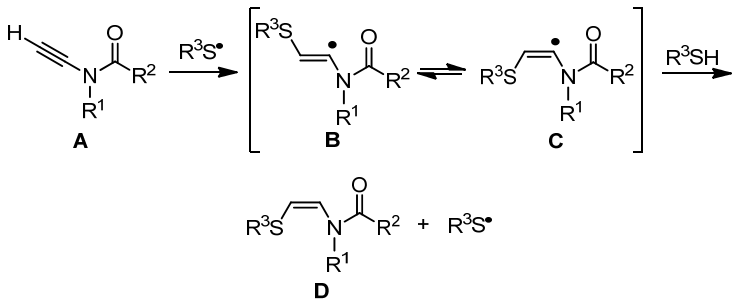


Figure 32. Proposed addition reaction of thiyl radicals to terminal ynamides

Vinyl radicals B and C, the products of addition of a thiyl radical to the terminal carbon of ynamide A, should rapidly equilibrate and abstract a hydrogen atom from the thiol, forming *Z* or *E* β-thioenamides. Hydrogen abstraction by the less hindered vinyl radical C shall produce kinetic *Z*- β-thioenamides, the goal of our synthesis, while the thermodynamically more stable *E*-β-thioenamides can form via addition/elimination[58] of the 2nd equivalent of thiyl radical, and this pathway shall be favored in the excess of thiol (Figure **33**).

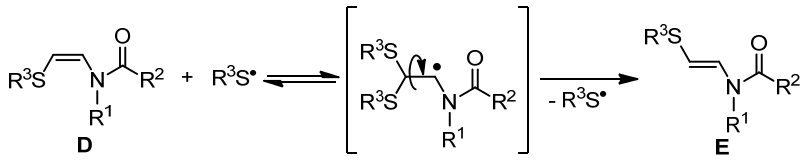


Figure 33. Proposed mechanism of isomerization of the *cis*- β-thioenamides

We began studying this reaction by utilizing commercially available thiols and simple ynamides. As my labmate Junghoon Kang performed studies with the acyclic *N*-benzyl-*N*-ethynylbenzamide (**81**), I was working on the synthesis and radical additions to the cyclic oxazolidin-2-one-derived ynamide (**82**) (Figure **34**), both of which have been synthesized before.[59, 60]

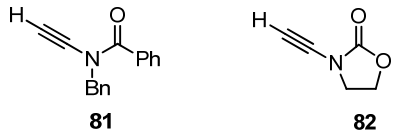


Figure 34. Simple ynamides used in the study

Depending on how one may look at ynamides, they do possess one challenging property – methods of their preparation, or, rather the outcomes of such methods are strongly substrate-dependent. Despite well known literature procedures for the preparation of **82**, none of the listed procedures for its preparation produced it in a high yield. The preparation of the TMS- or TIPS-protected ynamides **83**-**84** has been attempted with the variety of methods (Figure **35**).

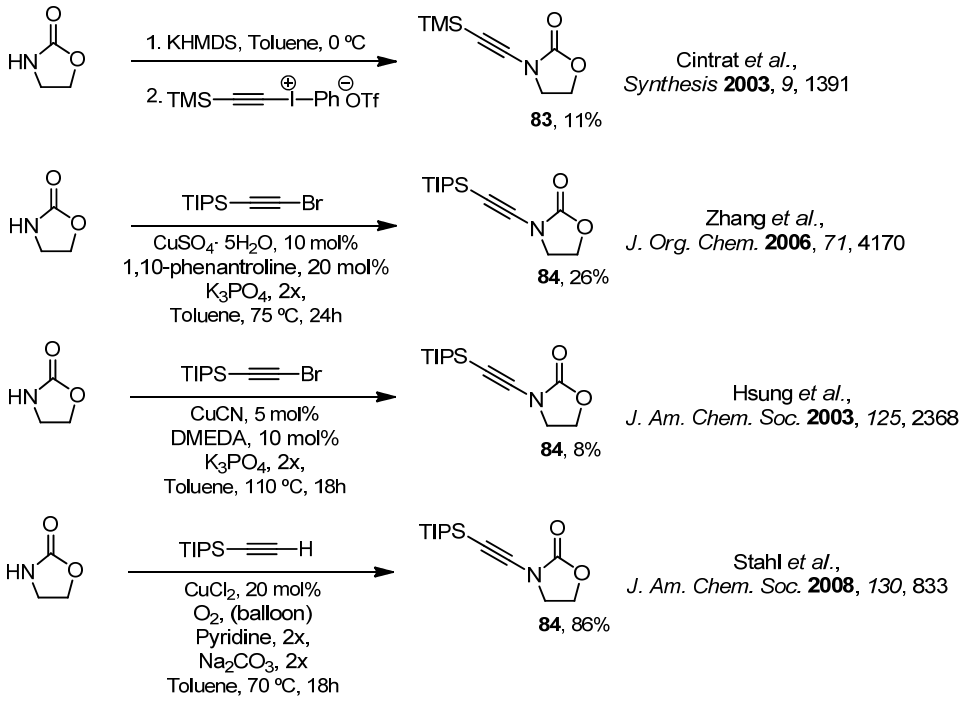


Figure 35. Synthesis of the protected ynamides **83**-**84**

In our hands, only the copper(II)-catalyzed oxidative coupling protocol of Stahl[44] provided high yields of **84**. This came as a surprise to us, and we attempted to use same method to prepare the

acyclic ynamide **81**. However, we could only obtain traces of the desired product, which is another indication of how strongly the methods of ynamide synthesis depend on the nature of substrate.

The TIPS-protecting group was subsequently removed by the action of TBAF.[61] (Figure **36**).

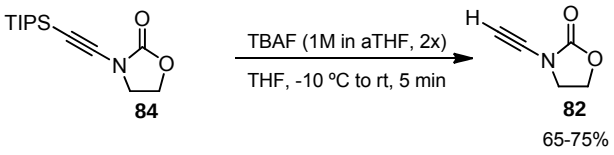


Figure 36. TBAF deprotection of the protected ynamide **84**

Addition of an excess of *n*-butyl thiol to the ynamide **82** in *tert*-butanol at 85 ºC with AIBN as initiator[62] produced predominantly *E*-β-thioenamide **85a**. On the contrary, employing only 1 equivalent of thiol, 50 mol % of the initiator, and running the reaction for 10 min instead of 3 hours produced mostly the *Z*-β-thioenamide **85a** with the excellent stereoselectivity of 1:6 (*E*/*Z*). Use of phenyl thiol showed similar results. These results are summarized in Table **3**. Interestingly, both the *E*- and *Z*- β-thioenamides survived column chromatography on silica gel and were separable in all cases.

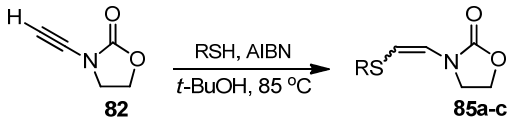


Figure 37. Radical addition of simple thiols to the ynamide **82**

RSH (equiv)	equiv AIBN	time	product	% yield (*E*:*Z*)
n-BuSH (4)	2	3 h	**85a**	82 (8.4:1)
n-BuSH (1)	0.5	10 min	**85a**	84 (1:6.0)
PhSH (4)	2	5 h	**85b**	81 (8.5:1)
PhSH (1)	0.5	10 min	**85b**	88 (1:5.1)
t-BuSH (4)	2	6 h	**85c**	86 (1:5.2)

Table 3. Radical addition of simple thiols to the ynamide **82**.

E:*Z* ratios were determined from 1H NMR spectra of the crude reaction mixtures.

Of course we attempted to utilize protocol of Yorimitsu and Oshima[57] of radical addition of thiyl radicals to internal ynamides with low temperature initiation (Et3B, –78 °C). We could have expected improved selectivity towards the *Z*-β-thioenamides **85a**, however we have observed significantly diminished *Z*-selectivity under these conditions (Figure **38**).

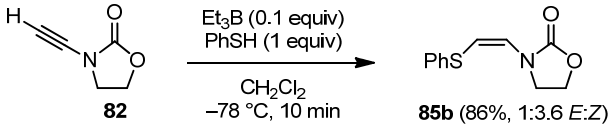


Figure 38. Addition of phenyl thiol to the ynamide **82** under the Yorimitsu and Oshima conditions

We also wished to examine the use of a thiol which would have approximated better the "real world" *S*-(2-aminovinyl)cysteine (avCys), and we decided to use the methyl ester of N-Cbz-protected cysteine (**87,** Figure **21**) for this purpose (Figure **39**, Table **4**).

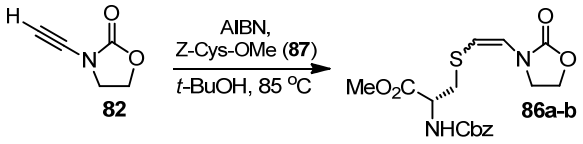


Figure 39. Addition of N-Cbz-protected cysteine methyl ester (**87**) to the ynamide **82**

equiv of **87**	equiv of AIBN	time	product	% yield[a] (*E*:*Z*)
2	1	2.5 h	**86a**	78 (10:1)
1	0.5	10 min	**86b**	81 (1:2.9)

Table 4. Radical addition of N-Cbz-protected cysteine methyl ester (87) to the ynamide **82**.

E:*Z* ratios were determined from 1H NMR spectra of the crude reaction mixtures.

We have observed similar trends with this cysteine-derived thiol, albeit the diastereomeric ratio in the 2nd case (compound **86b**) was not as great.

2.10.3 Model Synthesis of the *β*-Thioenamide Subunit of Thioviridamide

At this point we moved on to synthesize a model β-thioenamide subunit. We speculated that the β-thioenamide fragment of thioviridamide can be modeled with the β-thioenamide assembled by radical addition of the thiyl radical, derived from the protected L-cysteine **87** and the L-phenylalanine-derived ynamide **88** (Figure **40**). We moved on to synthesize these compounds and assemble the model β-thioenamide fragment.

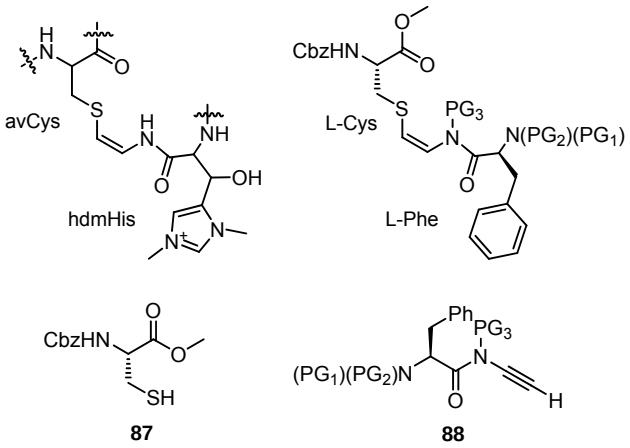


Figure 40. Model *β*-thioenamide subunit of thioviridamide and its precursors (**87**, **88**). PG indicates protecting group.

We embarked on the preparation of protected phenylalanine ynamides **88**. At first we attempted preparation of bis(Boc), PMB-protected compound **89** (Figure **41**). Commercially available N-Boc-L-phenylalanine **90** was protected as a benzyl ester, and the 2nd Boc group was installed (**92**). Upon benzyl ester cleavage and peptide coupling with *p*-methoxybenzylamine, compound **94** was obtained. Various synthetic procedures for the preparation of ynamides that we had tried before (Figure **35**), had failed to produce the desired ynamide. Copper(II)-catalyzed coupling under mild conditions, proposed by professor Danheiser produced an unidentified compound, containing an ynamide fragment, but the Boc-protecting groups did not integrate

properly in the 1H NMR. Apparently there was loss of protection due to the basic nature of KHMDS used in the Danheiser protocol.

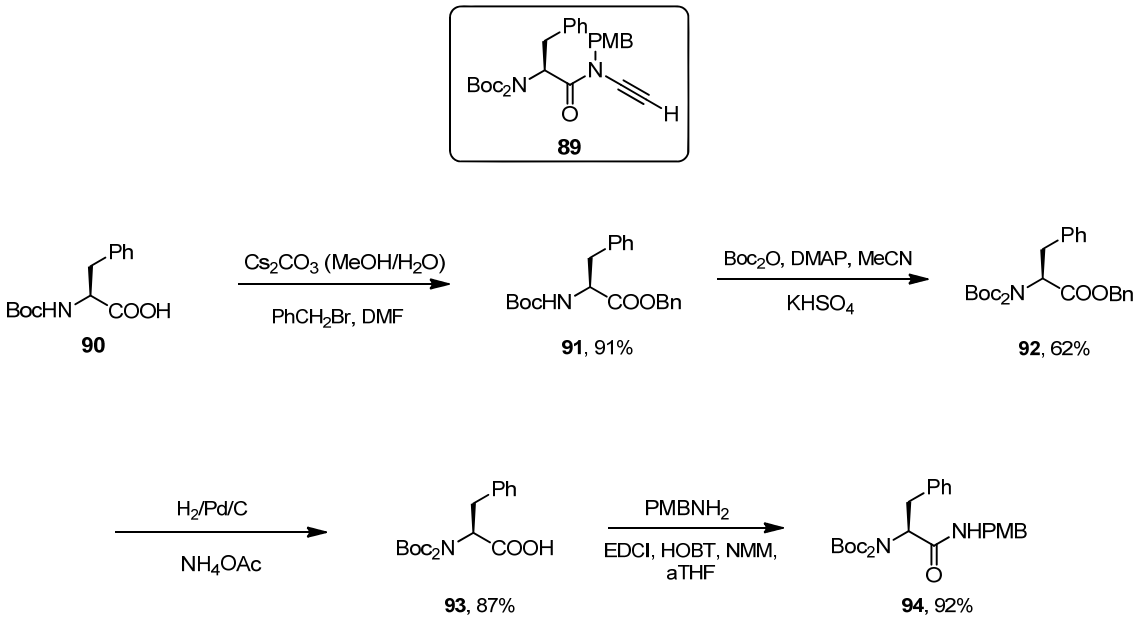


Figure 41. Preparation of the $(Boc)_2$, PMB-protected L-phenylalanine.

We decided to avoid use of electrophilic protecting groups at the amine nitrogen, and attempted preparation of azide, PMB-protected L-phenylalanine **95**. (Figure **42**) L-phenylalanine was converted into azido acid **96**, which was subsequently PMB-protected. However, all attempts at alkynylation did not produce the desired ynamide. Employment of the Danheiser protocol, seemingly the best for such acyclic compounds, led to the evolution of N_2 upon addition of CuI to the deprotonated compound **97**. No ynamide was detected. The possibility of click chemistry cannot be ruled out under such conditions.

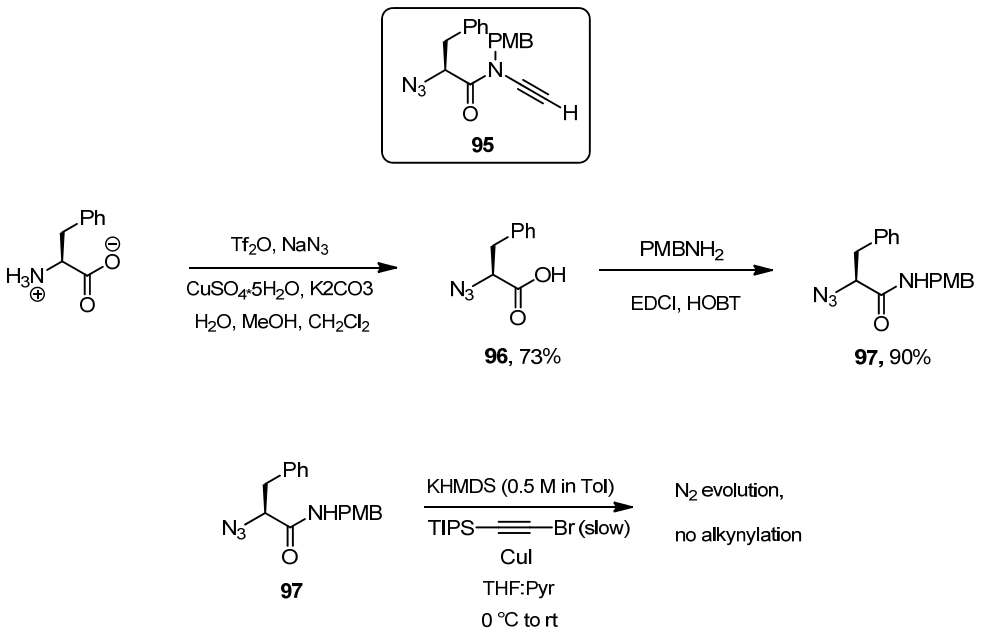


Figure 42. Preparation of the N_3, PMB-protected L-phenylalanine.

At this point, we decided to work with the alcohol protecting groups (Figure **23**). L-phenylalanine was converted into L-3-phenyllactic acid **99** by diazotization. Subsequent PMB and silicon ether protections afforded protected L-3-phenyllactic PMB amide (**101**). Attempted alkynylation by the Danheiser protocol afforded the corresponding ynamide **103** in a good yield (Figure **43**). However, we have discovered loss of the alkyne during the TBAF deprotection of the TiPS-protecting group at the terminal alkyne carbon. We are not aware of the mechanism of this transformation, but we have speculated that the alcohol protecting group (which was also lost) may have participated in this process.

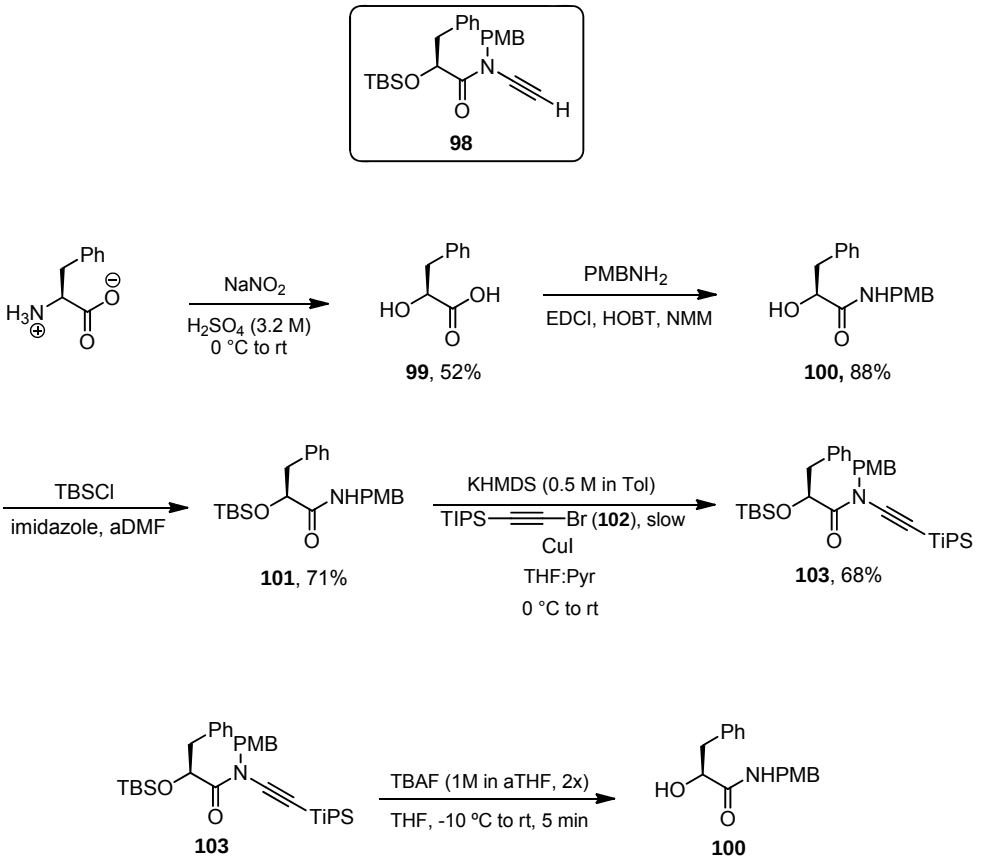


Figure 43. Preparation of the silicon ether, PMB-protected L-phenylalanines.

Finally we moved on to work with the triazone-protected compounds. PMB protection of the commercially available N-Boc L-phenylalanine and subsequent Boc group removal afforded free amine **105** in a good overall yield. Triazone protection of this compound proved to be a challenging reaction. We have optimized the reaction conditions and it was discovered that complete water removal from the reaction mixture before addition of N,N´-dimethylurea is crucial for obtaining high yields of triazone **106**. Alkynylation of this compound afforded ynamide **108** in a good yield (Figure **44**).

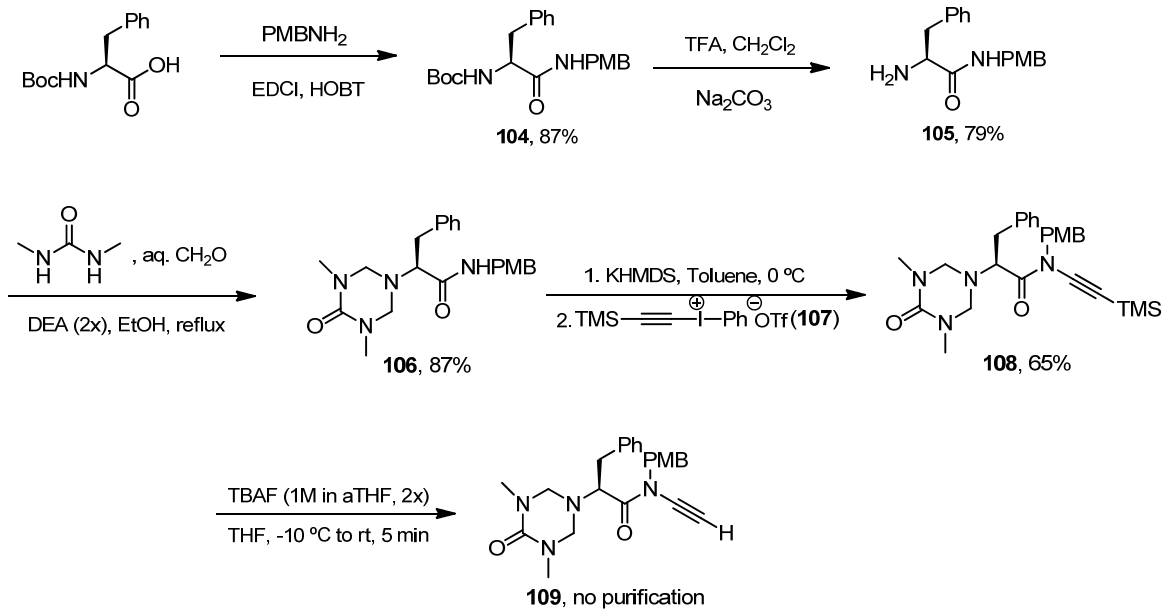


Figure 44. Preparation of the triazone, PMB-protected L-penylalanine-derived ynamide **109**

This ynamide was deprotected with TBAF. The resulting deprotected ynamide **109** did not survive the column purification on SiO_2 and was unstable at room temperature, so no purification was attempted on this compound, and it was engaged into the radical reaction immediately upon preparation. Unfortunately, despite detection of the addition products **110** by mass spectrometry we were not able to isolate any product of radical addition from this reaction (Figure **45**). We have tried a wide variety of purification techniques, however all our attempts were not successful.

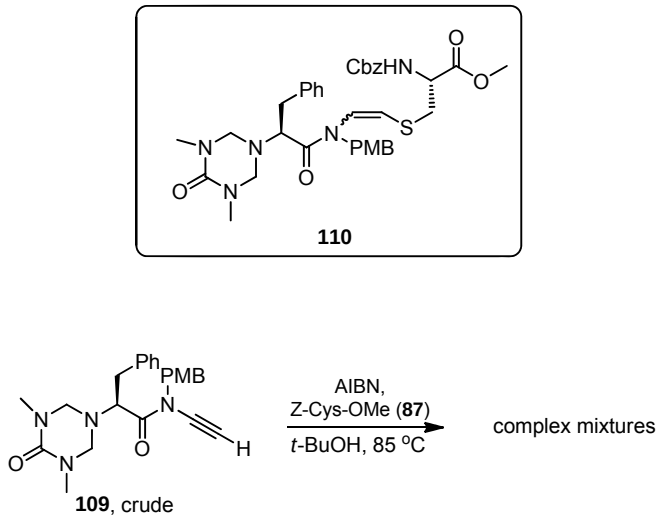


Figure 45. Attempted assembly of the model β-thioenamide subunit **110**

2.10.4 Conclusions

We have performed extensive computational studies of the thioviridamide (**79**) macrocycle. Even though we did not obtain a single, unambiguous answer as to which of the 12 isomers corresponds to the configuration of the natural product, the two most likely candidate structures were produced (**80d** and **80l**). We can also confidently state that besides the avCys-hdmHys junction, the rest of the macrocycle framework is comprised of natural (L) amino acids.

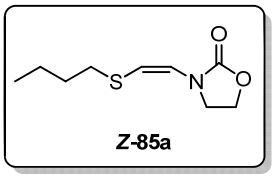
We have discovered the novel addition reaction of thiyl radicals to terminal ynamides and obtained two sets of conditions (kinetic and thermodynamic), allowing us to obtain *Z*- or *E*- β-thioenamides at will.

Our effort to synthesize the model β-thioenamide subunit was met with partial success. A number of ynamides were synthesized (**103**, **108**, **109**) and characterized.

2.11 Experimental details

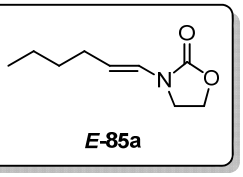
Flash chromatography was carried out using 60–230 mesh silica gel. ^{1}H NMR spectra were acquired on a 500 MHz spectrometer with chloroform (7.27 ppm) as internal reference. Signals are reported as follows: s (singlet), d (doublet), t (triplet), dd (doublet of doublets), br s (broad singlet), m (multiplet). Coupling constants are reported in hertz (Hz). 13C NMR spectra were acquired on a spectrometer operating at 125 MHz with chloroform (77.23 ppm) as internal reference. Infrared spectra were obtained on an FT-IR spectrometer. Mass spectral data were obtained using ESI techniques.

General Procedure for Thiyl Radical Additions to Ynamides. A solution of the ynamide (1 equiv) and thiol (1–4 equiv) in dry *t*-BuOH (stored over 4 Å MS) at rt under Ar was treated with AIBN (0.5–2 equiv). The resulting mixture was stirred at 85 °C for the appropriate length of time (10–360 min), then concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (1 mL) and stirred with sat aq $NaHCO_3$ for 2 h. The layers were separated, and the aqueous layer was extracted with CH_2Cl_2 (3 × 1 mL). The combined organic layers were washed with H_2O (2 × 1 mL) and brine (1 mL), dried (Na_2SO_4), and concentrated in vacuo. Flash chromatography (SiO_2) afforded the β-thioenamide adducts in diastereomerically pure form.



***Z*-85a**

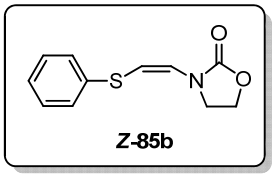
(*Z*)-3-(2-(butylthio)vinyl)oxazolidin-2-one (*Z*-85a). Prepared from **82** (24.0 mg, 0.216 mmol), *n*-butyl thiol (23 μL, 19.2 mg, 0.213 mmol, 1 equiv), *t*-BuOH (300 μL), and AIBN (17.7 mg, 0.108 mmol, 0.5 equiv) according to the General Procedure with a 10 min reaction time. The crude product (*E*:*Z* = 1:6.0) was purified by flash chromatography (SiO_2, 30% EtOAc in hexanes elution) to afford *Z*-**85a** (31.3 mg, 0.155 mmol, 72%) as an oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.59 (d, *J* = 8.5 Hz, 1H), 5.27 (d, *J* = 9.0 Hz, 1H), 4.41 (dd, *J* = 8.7, 6.7 Hz, 2H), 4.23 (dd, *J* = 8.7, 7.2 Hz, 2H), 2.65 (t, *J* = 7.2 Hz, 2H), 1.63–1.59 (m, 2H), 1.44–1.40 (m, 2H), 0.93 (t, *J* = 7.5 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.5, 123.2, 106.5, 62.6, 44.9, 35.1, 31.9, 21.6, 13.6; IR (film) μ_{max} 3027, 2956, 2925, 2871, 1760, 1653, 1617, 1558, 1540, 1506, 1479, 1407, 1326, 1267, 1205, 1078, 1031, 976 cm^{-1}; HRMS (ESI) *m/z* 202.0901 (MH^+, $C_9H_{15}NO_2SH^+$ requires 202.0896).



***E*-85a**

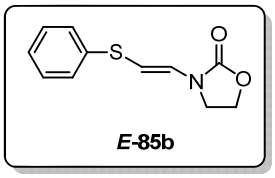
(*E*)-3-(2-(butylthio)vinyl)oxazolidin-2-one (*E*-85a). Prepared from **82** (30 mg, 0.27 mmol), *n*-butyl thiol (115 μL, 96.2 mg, 1.07 mmol, 4 equiv), *t*-BuOH (300 μL), and AIBN (88.7 mg, 0.54 mmol, 2 equiv) according to the General Procedure with a 3 h reaction time. The crude product (*E*:*Z* = 8.4:1) was purified by flash chromatography (SiO_2, 30% EtOAc in hexanes elution) to

afford *E*-**85a** (39.6 mg, 0.197 mmol, 73%) as an oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.97 (d, *J* = 13.5 Hz, 1H), 5.35 (d, *J* = 14.0 Hz, 1H), 4.46 (dd, *J* = 8.2, 8.0 Hz, 2H), 3.75 (dd, *J* = 9.0, 7.0 Hz, 2H), 2.62 (t, *J* = 7.5 Hz, 2H), 1.61–1.56 (m, 2H), 1.44–1.40 (m, 2H), 0.93 (t, *J* = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.7, 127.6, 103.6, 62.1, 42.5, 34.2, 31.3, 21.8, 13.6; IR (film) ν_{max} 3058, 2956, 2923, 2871, 1996, 1755, 1628, 1558, 1506, 1479, 1399, 1371, 1332, 1274, 1231, 1070, 1035, 977 cm^{-1}; HRMS (ESI) *m/z* 202.0899 (MH^+, $C_9H_{15}NO_2SH^+$ requires 202.0896).



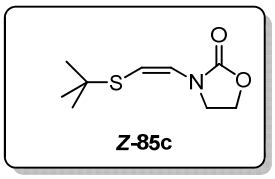
***Z*-85b**

(*Z*)-3-(2-(phenylthio)vinyl)oxazolidin-2-one (*Z*-85b). Prepared from **82** (30 mg, 0.27 mmol), thiophenol (28 μL, 30.1 mg, 0.273 mmol, 1 equiv), *t*-BuOH (300 μL), and AIBN (22.2 mg, 0.135 mmol, 0.5 equiv) according to the General Procedure with a 10 min reaction time. The crude product (*E*:*Z* = 1:5.1) was purified by flash chromatography (SiO_2, 30% EtOAc in hexanes elution) to afford *Z*-**85b** (44.2 mg, 0.200 mmol, 74%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.34–7.29 (m, 4H), 7.23–7.19 (m, 1H), 6.95 (d, *J* = 8.5 Hz, 1H), 5.52 (d, *J* = 8.0 Hz, 1H), 4.41 (t, *J* = 8.5 Hz, 2H), 4.29 (t, *J* = 8.5 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.4, 136.6, 129.2 (2C), 128.3, 127.5 (2C), 126.2, 100.8, 62.7, 44.8; IR (film) ν_{max} 3068, 3044, 2998, 1746, 1625, 1578, 1475, 1437, 1402, 1235, 1180, 1083, 1034, 980 cm^{-1}; HRMS (ESI) *m/z* 222.05867 (MH^+, $C_{11}H_{11}NO_2SH^+$ requires 222.05833).



***E*-85b**

(*E*)-3-(2-(phenylthio)vinyl)oxazolidin-2-one (*E*-85b). Prepared from **82** (23.0 mg, 0.207 mmol), thiophenol (85 μL, 91.5 mg, 0.830 mmol, 4 equiv), *t*-BuOH (300 μL), and AIBN (68.0 mg, 0.414 mmol, 2 equiv) according to the General Procedure with a 5 h reaction time. The crude product (*E*:*Z* = 8.5:1) was purified by flash chromatography (SiO_2, 30% EtOAc in hexanes elution) to afford *E*-**85b** (33.4 mg, 0.151 mmol, 73%) as a colorless oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.32–7.24 (m, 5H), 7.20–7.17 (m, 1H), 5.52 (d, *J* = 13.5 Hz, 1H), 4.50 (t, *J* = 8.0 Hz, 2H), 3.81 (t, *J* = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.6, 137.0, 132.0, 129.0 (2C), 127.7 (2C), 126.1, 100.6, 62.2, 42.5; IR (film) μ_{max} 3068, 2922, 1748, 1623, 1582, 1510, 1479, 1439, 1418, 1329, 1279, 1210, 1085, 1031, 976 cm^{-1}; HRMS (ESI) *m/z* 222.0592 (MH^+, $C_{11}H_{11}NO_2SH^+$ requires 222.0583).

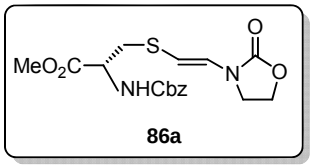


***Z*-85c**

(*Z*)-3-(2-(*tert*-butylthio)vinyl)oxazolidin-2-one (*Z*-85c). Prepared from **82** (30 mg, 0.27 mmol), *tert*-butyl thiol (121 μL, 96.8 mg, 1.07 mmol, 4 equiv), *t*-BuOH (300 μL), and AIBN (88.7 mg, 0.54 mmol, 2 equiv) according to the General Procedure with a 6 h reaction time. The crude product (*E*:*Z* = 1:5.2) was purified by flash chromatography (SiO_2, 20% acetone in hexanes elution) to afford *Z*-**85c** (39.1 mg, 0.194 mmol, 72%) and *E*-**85c** (7.6 mg, 0.038 mmol, 14%),

both as colorless oils. For *Z*-**85c**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 6.72 (d, *J* = 9.0 Hz, 1H), 5.42 (d, *J* = 9.0 Hz, 1H), 4.39 (t, *J* = 8.5 Hz, 2H), 4.23 (t, *J* = 8.5 Hz, 2H), 1.35 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.6, 125.6, 101.8, 62.6, 45.4, 44.8, 30.3 (3C); IR (film) μ_{max} 2963, 2239, 1755, 1623, 1582, 1480, 1402, 1234, 1079, 1037 cm^{-1}; HRMS (ESI) *m/z* 202.0891 (MH^+, $C_9H_{15}NO_2SH^+$ requires 202.0896).

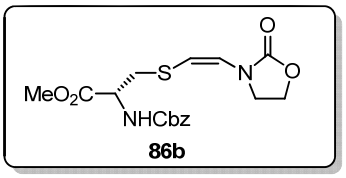
For *E*-**85c**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.12 (d, *J* = 14.0 Hz, 1H), 5.41 (d, *J* = 13.5 Hz, 1H), 4.46 (t, *J* = 8.0 Hz, 2H), 3.77 (t, *J* = 8.0 Hz, 2H), 1.30 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 154.6, 132.6, 99.8, 62.1, 44.2, 42.5, 30.4 (3C); IR (film) μ_{max} 2963, 1745, 1614, 1481, 1416, 1363, 1286, 1213, 1090, 1032 cm^{-1}; HRMS (ESI) *m/z* 202.0884 (MH^+, $C_9H_{15}NO_2SH^+$ requires 202.0896).



86a

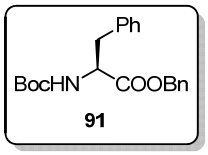
(*E*)-Methyl-2-(benzyloxycarbonylamino)-3-(2-(2-oxooxazolidin-3-yl)vinylthio) propanoate (86a). Prepared from **82** (10.0 mg, 0.090 mmol), **87**63 (48.4 mg, 0.18 mmol, 2 equiv), *t*-BuOH (300 μL), and AIBN (14.8 mg, 0.090 mmol, 1 equiv) according to the General Procedure with a 2.5 h reaction time. The crude product (*E*:*Z* = 10:1) was purified by flash chromatography (SiO2, 20–30% acetone in hexanes gradient elution) to afford **86a** (24.3 mg, 0.064 mmol, 71%) as a colorless oil: $[\alpha]^{25}_{D}$ +11 (*c* 0.18, CH_2Cl_2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.37–7.31 (m, 5H), 7.07 (d, *J* = 13.5 Hz, 1H), 5.56 (d, *J* = 8.0 Hz, 1H), 5.27 (d, *J* = 14.0 Hz, 1H), 5.16 (d, *J* = 12.0 Hz, 1H), 5.09 (d, *J* = 12.5 Hz, 1H), 4.65–4.61 (m, 1H), 4.40–4.33 (m, 2H), 3.76 (s, 3H), 3.63–3.55 (m, 2H), 3.08 (dd, *J* = 14.0, 5.0 Hz, 1H), 3.01 (dd, *J* = 14.0, 5.5 Hz, 1H); ^{13}C NMR ($CDCl_3$,

125 MHz) δ 170.9, 155.5, 154.4, 136.1, 130.5, 128.5 (2C), 128.2, 128.1 (2C), 101.3, 67.1, 62.1, 53.7, 52.7, 42.2, 37.3; IR (film) ν_{max} 3333, 3032, 2953, 1746, 1620, 1524, 1409, 1329, 1210, 1085, 1036 cm^{-1}; HRMS (ESI) *m/z* 398.1362 ($MNH4^+$, $C_{17}H_{20}N_2O_6SNH_4^+$ requires 398.1380).

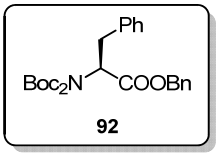


86b

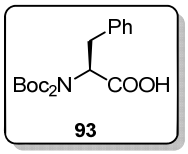
(*Z*)-Methyl-2-(benzyloxycarbonylamino)-3-(2-(2-oxooxazolidin-3- yl)vinylthio)propanoate (86b). Prepared from **82** (10.0 mg, 0.090 mmol), **87**63 (24.2 mg, 0.090 mmol, 1 equiv), *t*-BuOH (300 μL), and AIBN (7.4 mg, 0.045 mmol, 0.5 equiv) according to the General Procedure with a 10 min reaction time. The crude product (*E*:*Z* = 1:2.9) was purified by flash chromatography (SiO2, 20–30% acetone in hexanes gradient elution) to afford **86b** (20.5 mg, 0.054 mmol, 60%) as a colorless oil: $[\alpha]^{25}_D$ +22 (*c* 0.15, CH2Cl2); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.39–7.33 (m, 5H), 6.59 (d, *J* = 8.0 Hz, 1H), 5.57 (d, *J* = 7.0 Hz, 1H), 5.15 (d, *J* = 8.5 Hz, 1H), 5.14 (d, *J* = 12.5 Hz, 1H), 5.10 (d, *J* = 12.5 Hz, 1H), 4.68–4.63 (m, 1H), 4.38–4.31 (m, 2H), 4.15 (t, *J* = 7.8 Hz, 2H), 3.75 (s, 3H), 3.18 (dd, *J* = 14.5, 4.0 Hz, 1H), 3.06 (dd, *J* = 14.2, 4.8 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.6, 156.4, 155.5, 136.0, 128.6 (2C), 128.4, 128.2 (2C), 125.2, 103.7, 67.2, 62.6, 54.0, 52.7, 44.7, 38.0; IR (film) ν_{max} 3337, 2954, 1748, 1630, 1519, 1403, 1341, 1237, 1039 cm^{-1}; HRMS (ESI) *m/z* 398.1377 (MNH_4^+, $C_{17}H_{20}N_2O_6SNH_4^+$ requires 398.1380).



(S)-benzyl 2-((tert-butoxycarbonyl)amino)-3-phenylpropanoate (91). N-Boc-protected L-phenylalanine (**90**, 5.0g, 18.84 mmol) was dissolved in 80 mL of methyl alcohol and 8 ml of H_2O were added. The solution was titrated to pH = 7 with the 20% aqueous solution of Cs_2CO_3 (ca. 22 mL), the mixture evaporated to dryness. 50 mL of dry DMF were added, and removed on rotary evaporator at 45 ºC, this step was repeated twice. Another 50 mL of dry DMF were added, followed by benzyl bromide (3.56g, 20.8 mmol). The mixture was stirred at room temperature for 6 hours, concentrated on rotary evaporator, the oil residue was diluted with 200 mL of water and extracted with ethyl acetate, dried over Na_2SO_4. Column chromatography (SiO_2, 30% ethyl acetate in hexanes) afforded 7.83g (91%) of **91**.

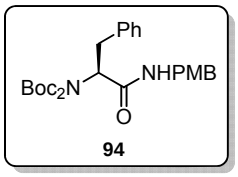


Compound 92. To 0.207g (1mmol) of **91** in 3 mL of dry MeCN was added DMAP (0.012g, 0.1 mmol, 10 mol %), Boc_2O (0.240g, 1.1 mmol). The mixture was stirred at room temperature for 5 h, concentrated on rotary evaporator, diluted with 25 mL of aqueous $KHSO_4$, extracted with ethyl acetate (3×20 mL) and dried over Na_2SO_4. Column chromatography (SiO_2, 30% ethyl acetate in hexanes) afforded 0.179g (62%) of pure benzyl ester **92**.



93

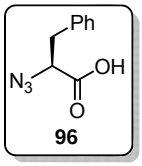
Compound 93. 4.2221g (9.2683 mmol) of bis(N-Boc) protected benzyl ester **92** was placed into 100 mL round bottom flask, 1.5717g (20.3905 mmol) of ammonium acetate was added, followed by 0.2111g (5% w/w) of 10% palladium on activated charcoal and 25 mL of methyl alcohol. The flask was evacuated under stirring and hydrogen gas-filled balloon was inserted through the septum. The reaction was allowed to stir for 18 h at room temperature, re-evacuated, the catalyst was filtered through a celite pad, the filtrate was concentrated on a rotary evaporator. Column chromatography (SiO_2, 30% ethyl acetate in hexanes, 100% ethyl acetate, gradient elution) afforded 2.943g (87%) of pure bis(N-Boc) protected acid **93**.



94

Compound 94. 1.1041g (3.0214 mmol) of compound **93** was placed into a 50 mL round bottom flask and 10mL of anhydrous THF were added. The flask was cooled down to 0 ºC on an ice-water bath, when 0.3947g (2.8775 mmol) of *p*-methoxybenzylamine was added with stirring. To this mixture were sequentially added: N-hydroxybenzotriazole (HOBT, 0.5049g, 3.8357 mmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 0.7068g, 3.8357 mmol) and N-methylmorpholine (619 µL, 0.5695g, 5.755 mmol). The mixture was stirred for 2 h at 0 ºC and for additional 18 h at room temperature, quenched with water, extracted with ethyl acetate (5×25 mL) and concentrated on a rotary evaporator. Column chromatography (SiO_2, 20% ethyl acetate

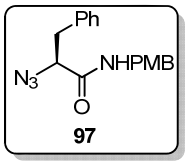
in hexanes, 50% ethyl acetate in hexanes, gradient elution) afforded (1.29g, 92%) of pure **94**: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.25 (br t, *J* = 7.5 Hz, 2H), 7.22–7.15 (m, 5H), 6.84 (d, *J* = 8.5 Hz, 2H), 6.22 (t, *J* = 5.5 Hz, 1H), 5.01 (dd, *J* = 10.5, 4.5 Hz, 1H), 4.43 (dd, *J* = 14.5, 6.0 Hz, 1H), 4.39 (dd, *J* = 14.7, 6.0 Hz, 1H), 3.77 (s, 3H), 3.49 (dd, *J* = 14.3, 4.5 Hz, 1H), 3.29 (dd, *J* = 14.0, 11.0 Hz, 1H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 169.8, 159.0, 152.1, 138.0, 130.2, 129.5, 129.2, 128.4, 126.5, 114.0, 83.3, 61.13, 61.09, 55.3, 55.2, 43.2, 35.3, 27.8.



96

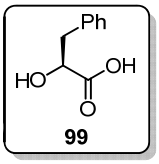
(S)-2-azido-3-phenylpropanoic acid (**96**). Triflyl azide preparation: A solution of sodium azide (5.34g, 82.35 mmol) was dissolved in de-ionized H_2O (13.5 mL) with CH_2Cl_2 (22.5 mL) and cooled to 0 ºC on ice-water bath. Triflyl anhydride (2.79 mL, 4.6788g, 16.65 mmol) was added slowly over 5 min through a glass pipette with stirring continued for 2 h. The mixture was placed in a separatory funnel and the CH_2Cl_2 phase removed. The aqueous portion was extracted with CH_2Cl_2 (2×11.25 mL). The organic fractions, containing the triflyl azide, were combined together and washed once with saturated Na_2CO_3 and used without further purification. L-Phenylalanine (1.3827g, 8.37 mmol) was combined with K_2CO_3 (1.7373g, 12.57 mmol) and $CuSO_4{\cdot}5H_2O$ (0.0209g, 87.3 μmol), de-ionized water (27 mL) and methyl alcohol (54 mL). The triflyl azide in CH_2Cl_2 (45 mL) was added and the mixture was stirred at room temperature overnight. The organic solvents were removed on a rotary evaporator, and the aqueous slurry was diluted with water (150 mL). This was acidified to pH=6 with conc. HCl and diluted with 0.25 M, pH=6.2 phosphate buffer (50 mL), extracted with ethyl acetate (5×25 mL) to remove

sulfonamide byproduct. The aqueous phase was then acidified to pH=2 with conc. HCl. The product was obtained from another round of ethyl acetate extractions (5×25 mL), the extracts were combined, dried over $MgSO_4$ and evaporated to dryness, affording 1.167g (73%) of **96** as an off-white foam.



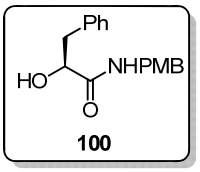
97

(S)-2-azido-N-(4-methoxybenzyl)-3-phenylpropanamide (**97**). 0.6581g (3.4419 mmol) of azide-protected acid **96** was placed into a 50 mL round bottom flask and 12 mL of anhydrous THF were added. The flask was cooled down to 0 ºC on an ice-water bath, when 0.4497g (3.278 mmol) of *p*-methoxybenzylamine was added with stirring. To this mixture were sequentially added: N-hydroxybenzotriazole (HOBT, 0.5752g, 4.2919 mmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 0.8051g, 4.2919 mmol) and N-methylmorpholine (705 µL, 6.5560 mmol). The mixture was stirred for 2 h at 0 ºC and for additional 18 h at room temperature, quenched with water, extracted with ethyl acetate (5×25 mL) and concentrated on a rotary evaporator. Column chromatography (SiO_2, 20% ethyl acetate in hexanes, 50% ethyl acetate in hexanes, gradient elution) afforded (0.9603g, 90%) of pure **97** as a white foam: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.35–7.22 (m, 5H), 7.07 (d, *J* = 8.7 Hz, 2H), 6.83 (d, *J* = 8.7 Hz, 2H), 6.46–6.36 (br, 1H), 4.36 (dd, *J* = 13.2, 6.0 Hz, 1H), 4.30 (dd, *J* = 12.9, 2.7 Hz, 1H), 4.23 (dd, *J* = 7.7, 4.2 Hz, 1H), 3.79 (s, 3H), 3.36 (dd, *J* = 14.1, 4.2 Hz, 1H), 3.07 (dd, *J* = 13.8, 4.2 Hz, 1H).



99

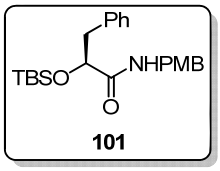
(S)-2-hydroxy-3-phenylpropanoic acid (**99**). 10.5g (150 mmol) of $NaNO_2$ was dissolved in 25 mL of water and cooled down to 0 ºC on an ice-water bath, when 25 mL of 3.2 M H_2SO_4 was slowly added to the solution. In a 250 mL round bottom flask 5.0g (30.5 mmol) of L-Phenylalanine was dissolved in 100 mL of 3.2 M H_2SO_4, and also cooled down to 0 ºC. The solution of nitrous acid was slowly added with stirring to the acidified solution of L-Phenylalanine. The mixture was stirred at 0 ºC for 2 h, warmed up to 50 ºC, then cooled down to room temperature. Extraction with ethyl acetate (5×25 mL), drying over Na_2SO_4 and concentration under reduced pressure yielded yellow oil. Re-crystallization from Hexanes-Ethyl alcohol afforded 2.633g (52%) of pure **99**.



100

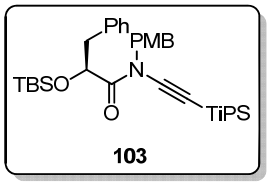
(S)-2-hydroxy-N-(4-methoxybenzyl)-3-phenylpropanamide (**100**). 1.0942g (6.5847 mmol) of the acid **99** was placed into a 100 mL round bottom flask. The flask was cooled down to 0 ºC on an ice-water bath, when 0.9857g (6.2711 mmol) of *p*-methoxybenzylamine was added with stirring. To this mixture were sequentially added: N-hydroxybenzotriazole (HOBT, 1.1004g, 8.2108 mmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 1.5402g, 8.2108 mmol) and N-methylmorpholine (1349 μL, 12.5423 mmol). The mixture was stirred for 2 h at 0 ºC and for additional 18 h at room temperature, quenched with water, extracted with ethyl acetate (5×25

mL) and concentrated on a rotary evaporator. Column chromatography (SiO_2, 50% ethyl acetate in hexanes) afforded 1.5728g (88%) of **100** as a white foam.



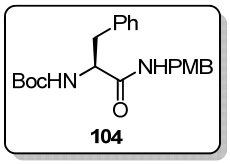
101

(S)-2-((tert-butyldimethylsilyl)oxy)-N-(4-methoxybenzyl)-3-phenylpropanamide (**101**). Into a 50 mL round bottom flask was added the alcohol **100** (0.2529g, 0.8839 mmol), followed by 11 mL of anhydrous dimethyl formamide. To this solution was added *tert*-butyl dimethylsilyl chloride (0.1332g, 0.8839 mmol) and imidazole (0.0602g, 0.8839 mmol). The mixture was stirred at room temperature overnight, when DMF was removed in high vacuum. Column chromatography (10% Et_2O in Hexanes) afforded 0.2510g (71%) of **101** as transparent oil: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.56–7.43 (m, 5H), 7.34 (d, J = 8.4 Hz, 2H), 7.08 (d, J = 8.7 Hz, 2H), 6.98–6.90 (br, 1H), 4.70–4.48 (m, 3H), 4.02 (s, 3H), 3.40 (dd, J = 13.6, 3.0 Hz, 1H), 3.16 (dd, J = 13.5, 7.5 Hz, 1H), 1.05 (s, 9H), 0.11 (s, 3H), 0.00 (s, 3H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 172.5, 158.9, 137.2, 130.1, 129.9, 128.9, 128.0, 126.5, 113.9, 74.8, 55.1, 42.4, 41.6, 25.6, 17.9, -5.5, -5.7.

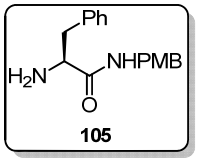


103

(S)-2-((tert-butyldimethylsilyl)oxy)-N-(4-methoxybenzyl)-3-phenyl-N ((triisopropylsilyl)ethynyl)propanamide (**103**). Into a 25 mL round bottom flask 0.4014g (1.0045 mmol) of **101** was placed and the flask was flushed with argon for 5 min. Anhydrous tetrahydrofuran (3.8 mL) and anhydrous pyridine (2.0 mL, 1.9742g, 25.171 mmol) were added into the flask through the septum and the flask was cooled down to 0 ºC when KHMDS (0.5 M in toluene, 2.0 mL, 1.0045 mmol) was added dropwise over 10 min. The flask was allowed to warm up to room temperature over 15 min and CuI (0.1913g, 1.0045 mmol) was added at once by removal and rapid replacement of the septum. The flask was stirred at room temperature for 2 h when (bromoethynyl)triisopropylsilane (**102**, 0.3504g, 1.1552 mmol) in 1.3 mL of anhydrous tetrahydrofuran was slowly added into the flask via syringe pump over 2 h. The reaction mixture was stirred at room temperature for 20 h, diluted with CH_2Cl_2 (30 mL), washed with brine/ammonium hydroxide solution (3:1) twice, then with 1N citric acid solution. The organic layers were dried over $MgSO_4$ and concentrated under reduced pressure. Column chromatography (Pure hexanes, 30% EtOAc in hexanes, graduate elution) afforded 0.3960g (68%) of **103** as dark yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.48 (d, J = 9.5 Hz, 1H), 7.46–7.40 (m, 6H), 7.05 (d, J = 9.0 Hz, 2H), 5.36 (dd, J = 9.5, 3.5 Hz, 1H), 4.91 (d, J = 14 Hz, 1H), 4.86 (d, J = 14 Hz, 1H), 4.02 (s, 3H), 3.25 (dd, J = 13.3, 3.0 Hz, 1H), 3.09 (dd, J = 13.7, 9.5 Hz, 1H), 1.29–1.25 (m, 21H), 0.97 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H).

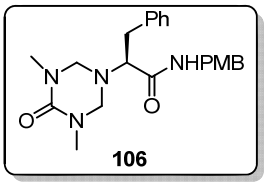


(S)-tert-butyl (1-((4-methoxybenzyl)amino)-1-oxo-3-phenylpropan-2-yl)carbamate (**104**). N-Boc-protected L-phenylalanine (**90**, 2.004g, 7.5535 mmol) was placed into a 100 mL round bottom flask. The flask was cooled down to 0 ºC on an ice-water bath, when 0.9867g (7.1937 mmol) of *p*-methoxybenzylamine was added with stirring. To this mixture were sequentially added: N-hydroxybenzotriazole (HOBT, 1.2623g, 9.5892 mmol), 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, 1.7667g, 9.5892 mmol) and N-methylmorpholine (1682 µL, 1.5475g, 14.3875 mmol). The mixture was stirred for 2 h at 0 ºC and for additional 18 h at room temperature, quenched with water, extracted with ethyl acetate (5×25 mL) and concentrated on a rotary evaporator. Column chromatography (SiO_2, 30% ethyl acetate in hexanes) afforded 2.403g (87%) of **104** as fluffy white crystals.



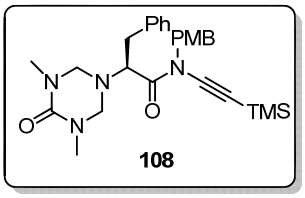
(S)-2-amino-N-(4-methoxybenzyl)-3-phenylpropanamide (105). 0.8889g (2.3120 mmol) of compound **104** was placed into a 100 mL round bottom flask and 3.5 mL of CH_2Cl_2 were added. Then trifluoroacetic acid (TFA, 3.46 mL, 5.308g, 46.55 mmol) was added dropwise into the flask and it was loosely capped with a septum. The flask was stirred at room temperature for 1 h, the solution was concentrated in reduced pressure, diluted with CH_2Cl_2 and shaken in a separatory

funnel with the saturated Na_2CO_3 solution. Repeated dichloromethane extraction (5×15 mL) and drying over $MgSO_4$ afforded 0.5187g (79%) of **105**.



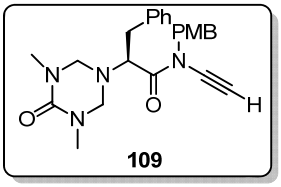
106

(S)-2-(3,5-dimethyl-4-oxo-1,3,5-triazinan-1-yl)-N-(4-methoxybenzyl)-3-phenylpropanamide (28). 0.9955g (3.5003 mmol) of the amine **105** was placed into a 50 mL round bottom flask, 4 mL of 36% aqueous formaldehyde solution were added, followed by 1.43 mL of diisopropylethylamine and 0.4 mL of ethyl alcohol. The mixture was stirred under argon for 1 h when 20 mL of dry toluene were added and the mixture was concentrated under reduced pressure at 50 ºC. This last step was repeated two times. Eventually, 0.3591g of N,N´-dimethylurea were added to the solid residue, followed by 20 mL of dry toluene. The mixture was refluxed overnight in an oil bath at 110 ºC, concentrated under reduced pressure. Column chromatography (pure CH_2Cl_2, 2% MeOH in CH_2Cl_2, gradient elution) afforded 1.16g (87%) of **106** as yellow crystals: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.26 (d, *J* = 7.5 Hz, 2H), 7.23 (d, *J* = 7.0 Hz, 1H), 7.19 (d, *J* = 7.0 Hz, 2H), 6.97 (d, *J* = 8.0 Hz, 2H), 6.79 (d, *J* = 7.0 Hz, 2H), 6.52-6.49 (bm, 1H), 4.27 (d, *J* = 14.0 Hz, 2H), 4.24 (dd, *J* = 4.0 Hz, *J* = 2.0 Hz, 2H), 4.14 (d, *J* = 14.0 Hz, 2H), 3.81 (dd, *J* = 4.0 Hz, *J* = 2.0 Hz, 1H), 3.78 (s, 3H), 3.18 (dd, *J* = 13.5 Hz, *J* = 8.0 Hz, 1H), 3.02 (dd, *J* = 13.5 Hz, *J* = 6.0 Hz, 1H), 2.68 (s, 6H);



108

(S)-2-(3,5-dimethyl-4-oxo-1,3,5-triazinan-1-yl)-N-(4-methoxybenzyl)-3-phenyl-N-((trimethylsilyl)ethynyl)propanamide (108). Triazone-protected amine **106** (0.6159g, 1.6668 mmol) was placed in a 100 mL round bottom flask and dried overnight in high vacuum. The flask was flushed with argon gas and 50 mL of dry toluene were added into the flask, followed by KHMDS (0.5 M in toluene, 3.7 mL, 1.8335 mmol). The flask was sonicated for 2 h and phenyl((trimethylsilyl)ethynyl)iodonium triflate (**107**) was added. The flask was now heated up to 75 ºC for 75 min, allowed to cool to room temperature and kept at room temperature for 5 h. Concentration under reduced pressure followed by column chromatography (pure CH_2Cl_2, 1% MeOH in CH_2Cl_2, gradient elution) afforded 0.533g (65%) of **108** as intensely yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.23–7.20 (m, 3H), 7.15 (dd, *J* = 7.5, 2.5 Hz, 2H), 6.99 (d, *J* = 9.0 Hz, 2H), 6.77 (d, *J* = 8.5 Hz, 2H), 4.87 (dd, *J* = 9.0, 6.5 Hz, 1H), 4.57 (d, *J* = 14.0 Hz, 1H), 4.35 (d, *J* = 13.5 Hz, 1H), 4.31–4.25 (m, 4H), 3.79 (s, 3H), 3.08 (dd, *J* = 13.3, 9.0 Hz, 1H), 3.02 (dd, *J* = 13.0, 6.5 Hz, 1H), 2.75 (s, 6H), 0.12 (s, 9H);



109

(S)-2-(3,5-dimethyl-4-oxo-1,3,5-triazinan-1-yl)-N-ethynyl-N-(4-methoxybenzyl)-3-phenylpropanamide (109). 0.039g (0.0792 mmol) of protected ynamide **108** was added into a 1

dram vial. 1.3 mL of anhydrous tetrahydrofuran was added into the vial, and the vial was cooled down to 0 ºC on a ice-water bath. 0.1029 mL (102.9 μL, 0.1029 mmol) of TBAF was added into the vial and the mixture was stirred at 0 ºC for 15 min. 1 mL of saturated NH_4Cl solution was added into the vial and the organic layer was extracted with CH_2Cl_2 (5×1 mL) and concentrated under reduced pressure. Compound **109** was used in further reaction without purification.

2.12 References

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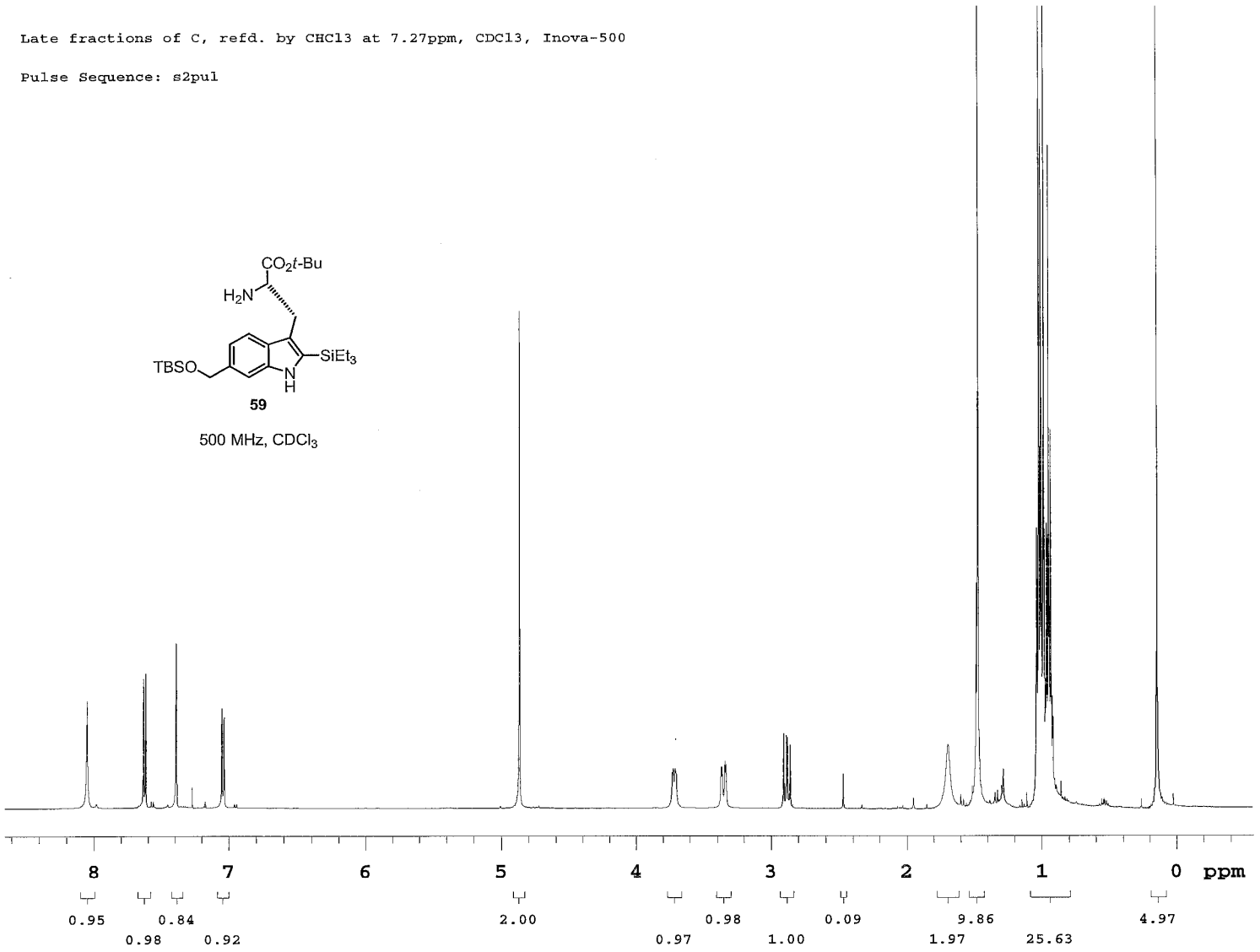
Selected NMR Spectra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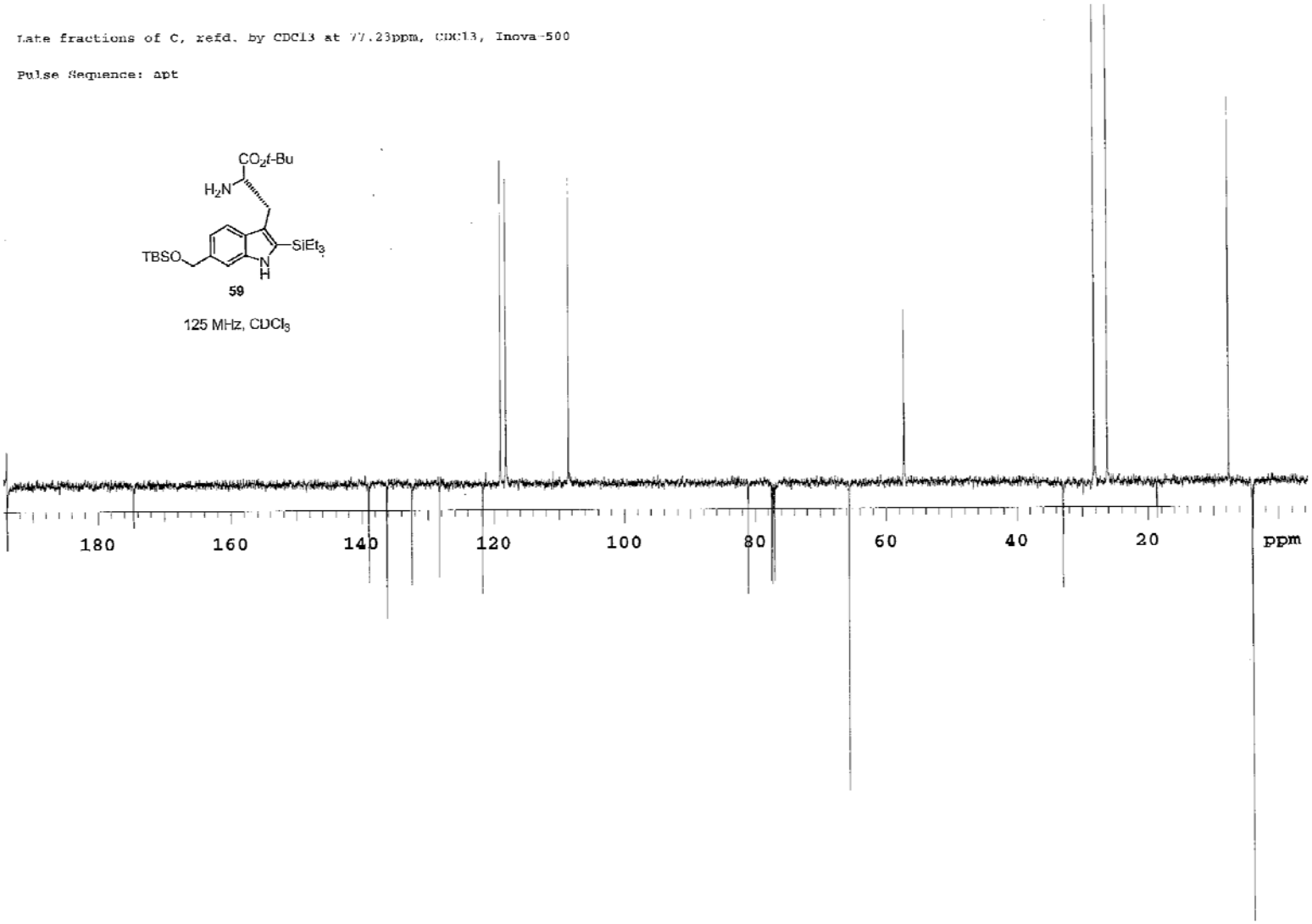
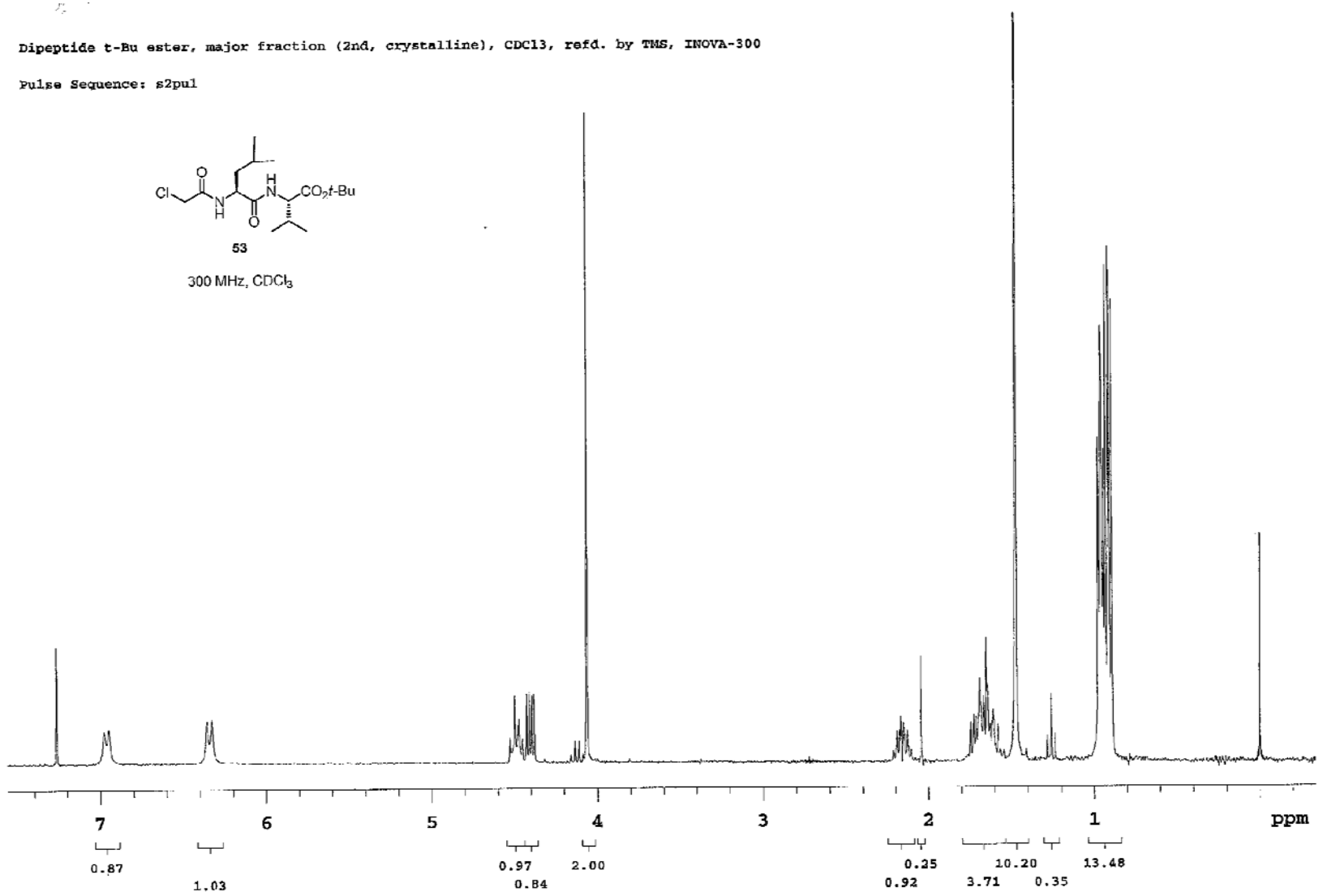
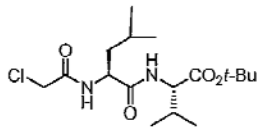
CO2t-Bu
H2N
TBSO
SiEt3
N
H
59
500 MHz, CDCl3
8
7
6
5
4
3
2
1
0
ppm
0.95
0.98
0.84
0.92
2.00
0.97
0.98
1.00
0.09
1.97
9.86
25.63
4.97


Late fractions of C, refd. by CDCl3 at 77.23ppm, CDCl3, Inova-500
Pulse Sequence: apt
CO2t-Bu
H2N
TBSO
SiEt3
N
H
59
125 MHz, CDCl3
180
160
140
120
100
80
60
40
20
ppm


Dipeptide t-Bu ester, major fraction (2nd, crystalline), CDCl3, refd. by TMS, INOVA-300
Pulse Sequence: s2pul
Cl
O
N
H
H
N
O
CO2t-Bu
53
300 MHz, CDCl3
7
6
5
4
3
2
1
ppm
0.87
1.03
0.97
0.84
2.00
0.92
0.25
3.71
10.20
0.35
13.48

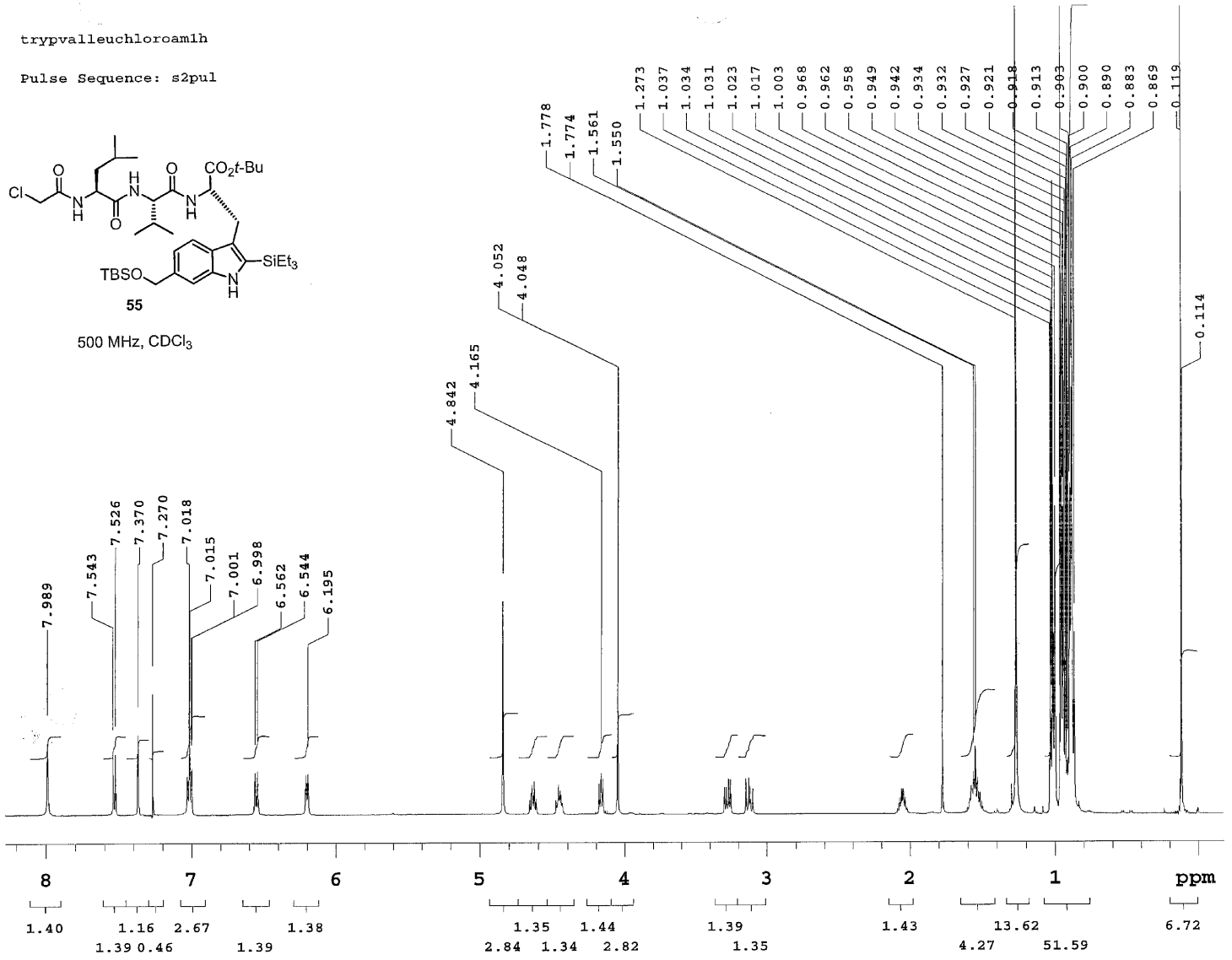
Dipeptide t-Bu ester, major fraction (2nd, crystalline), CDCl3, refd. by TMS, INOVA-300

Pulse Sequence: apt



53

75 MHz, $CDCl_3$


trypvalleuchloroam1h
Pulse Sequence: s2pul
55
500 MHz, CDCl3
7.989
7.543
7.526
7.370
7.270
7.018
7.015
7.001
6.998
6.562
6.544
6.195
4.842
4.165
4.052
4.048
1.778
1.774
1.561
1.550
1.273
1.037
1.034
1.031
1.023
1.017
1.003
0.968
0.962
0.958
0.949
0.942
0.934
0.932
0.927
0.921
0.918
0.913
0.903
0.900
0.890
0.883
0.869
0.119
0.114
8
7
6
5
4
3
2
1
ppm
1.40
1.16
1.39
0.46
2.67
1.39
1.38
1.35
2.84
1.44
1.34
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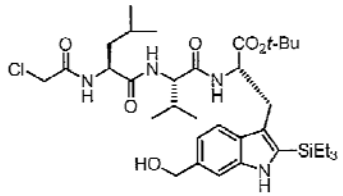
1.43
13.62
4.27
51.59
6.72

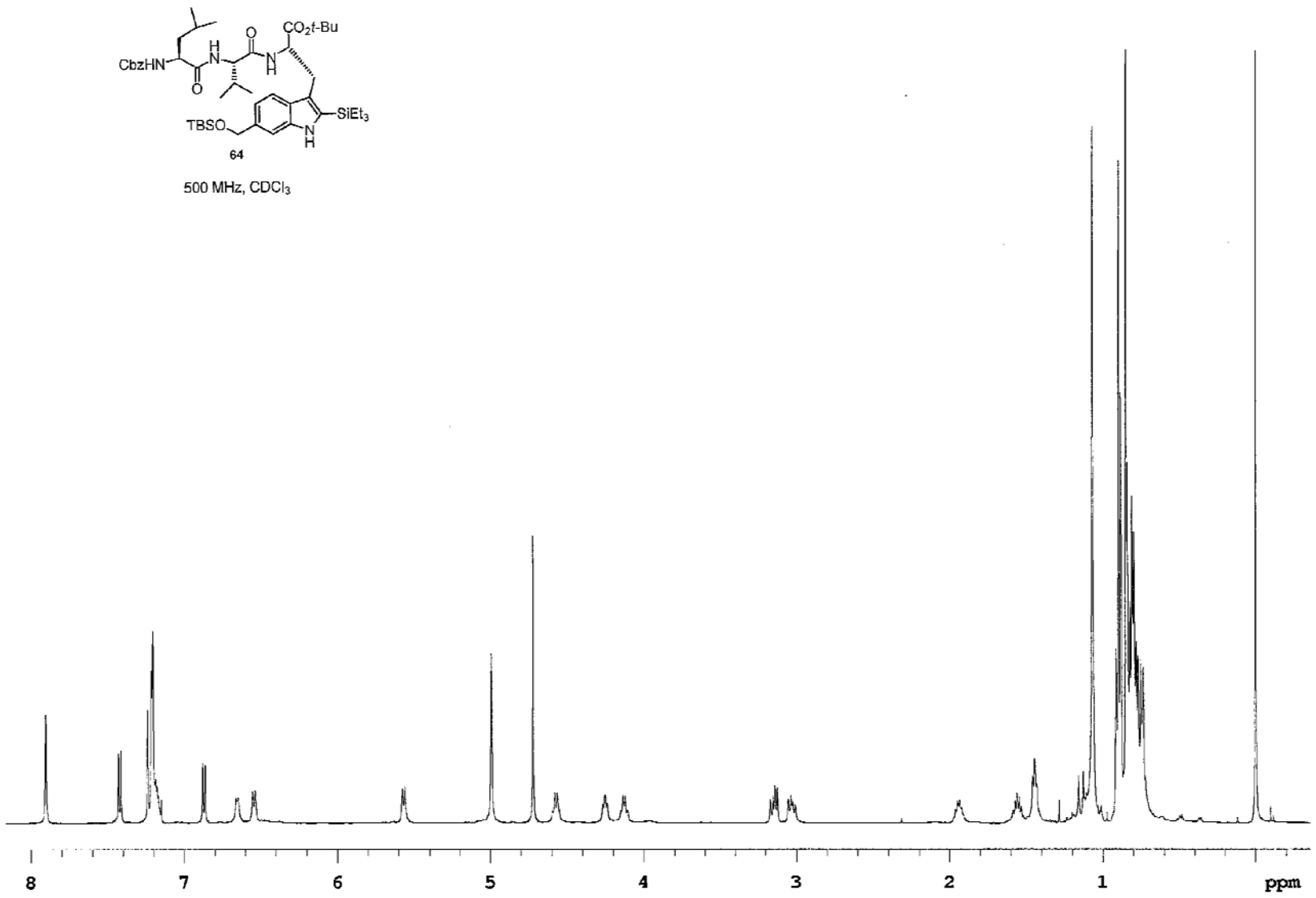
STANDARD 1H OBSERV

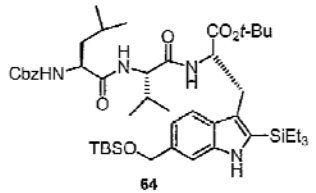
Pulse Sequence: s2



56

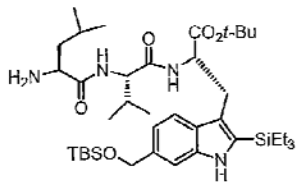
300 MHz, $CDCl_3$


CbzHN
O
H
N
O
N
H
CO2t-Bu
TBSO
SiEt3
N
H
64
500 MHz, CDCl3
8
7
6
5
4
3
2
1
ppm



64

125 MHz, $CDCl_3$



65

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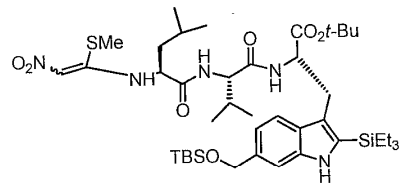

66

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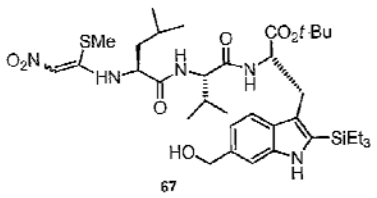
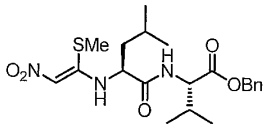


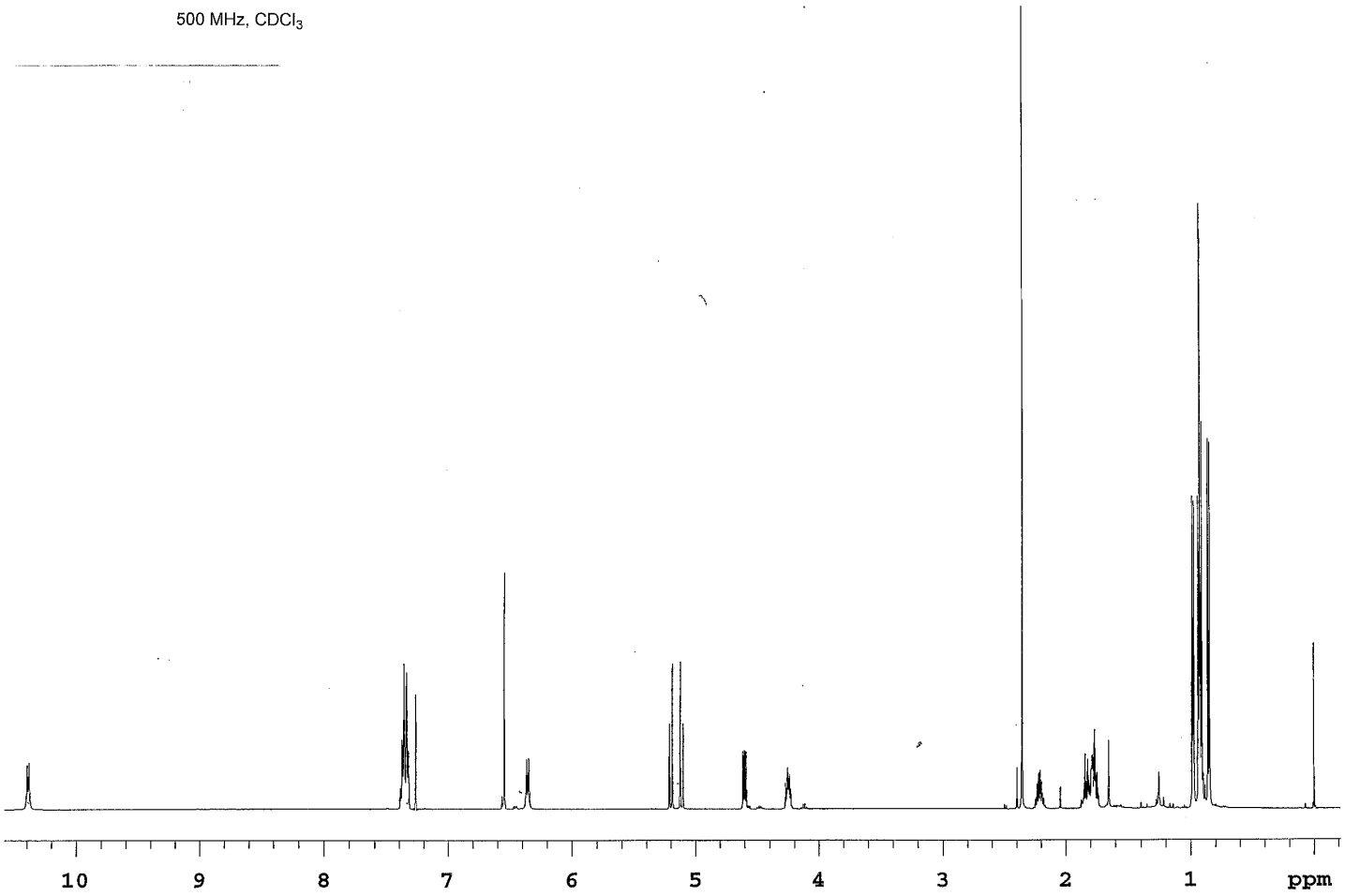
67

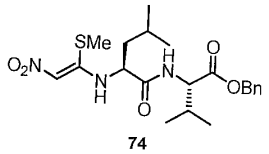
500 MHz, $CDCl_3$



74

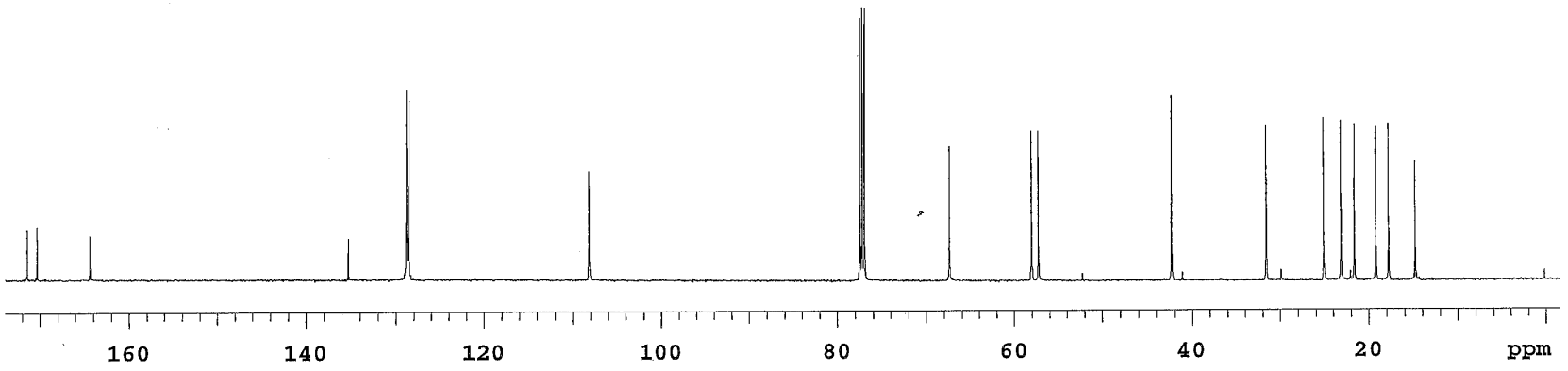
500 MHz, $CDCl_3$

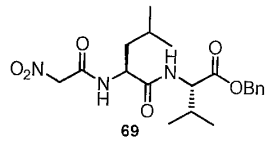




74

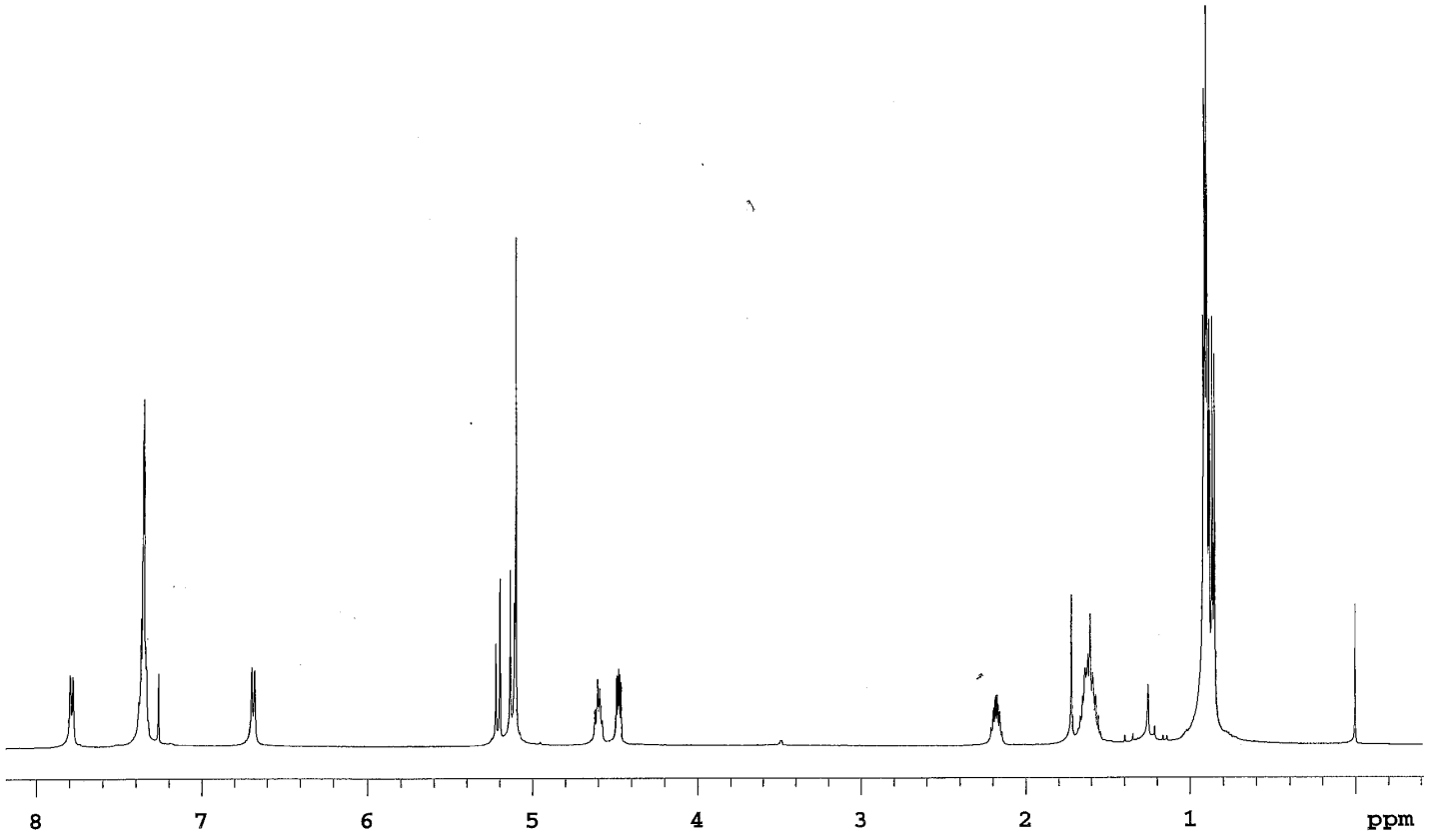
125 MHz, CDCl3

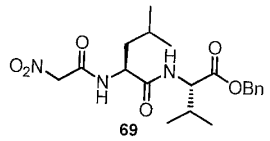




69

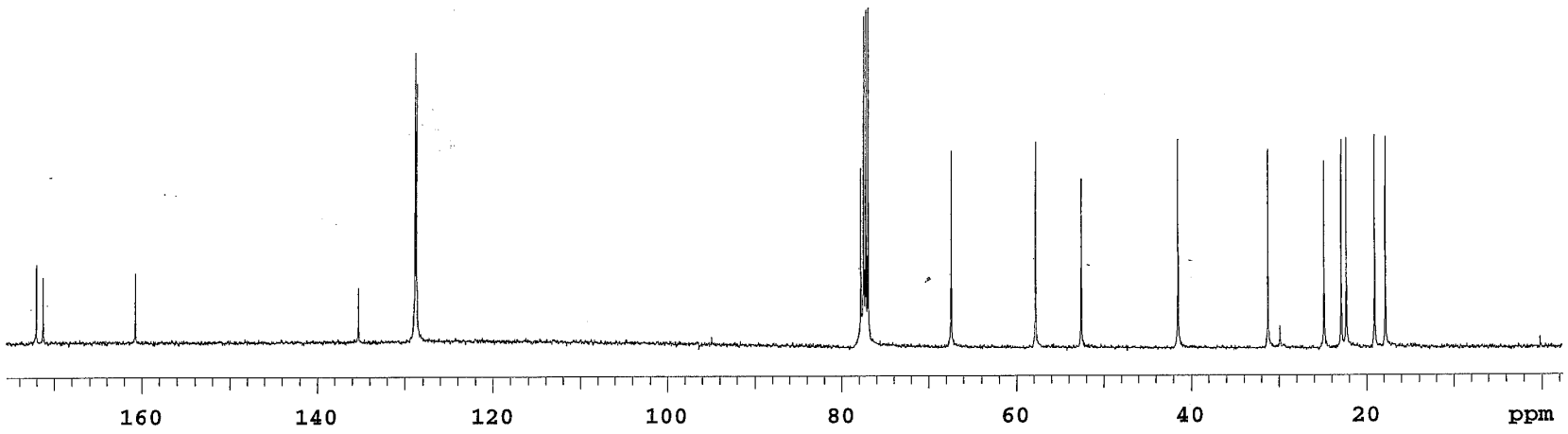
500 MHz, CDCl3

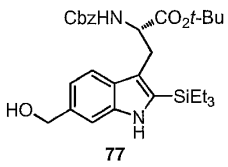




69

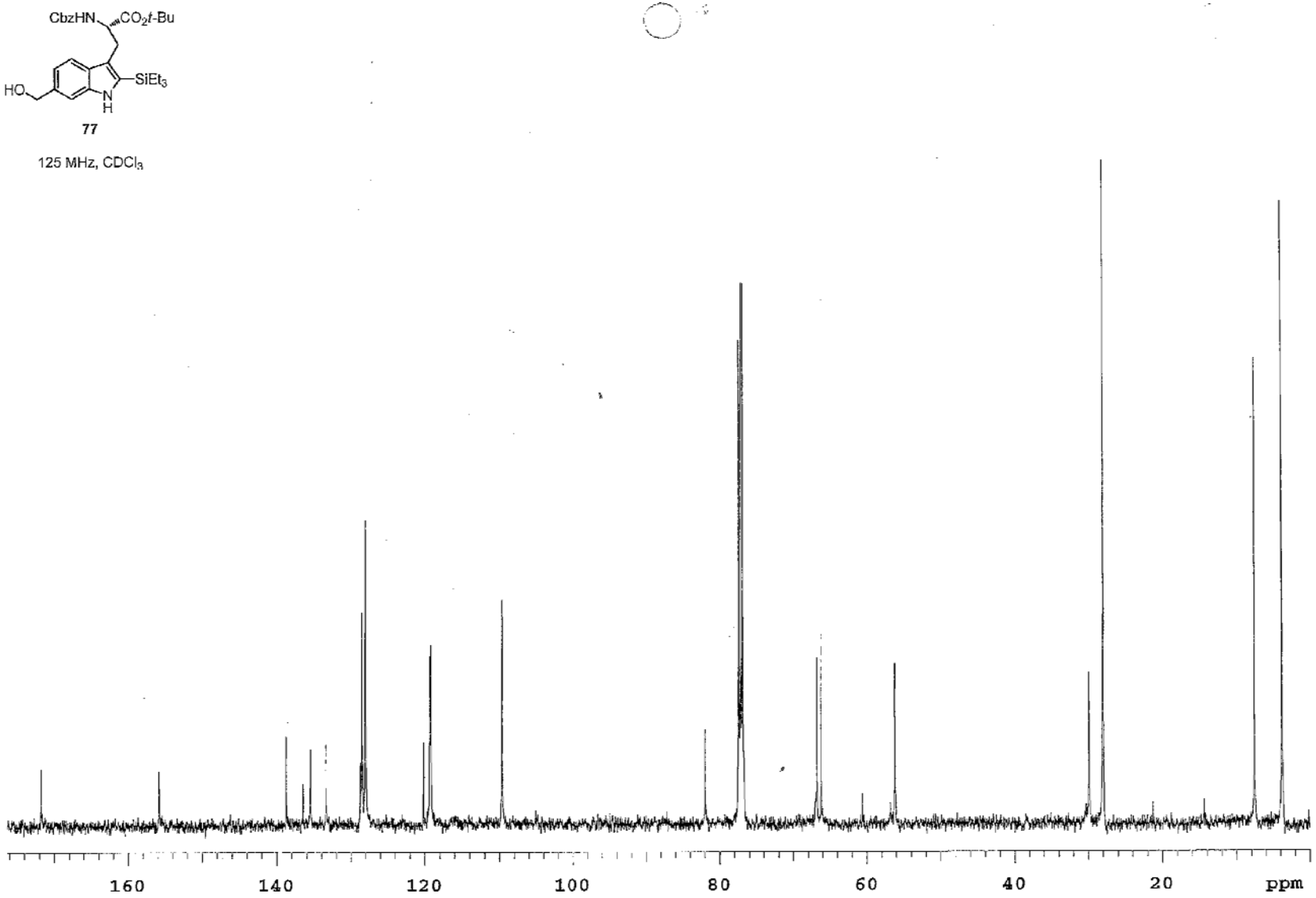
125 MHz, CDCl3

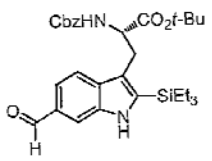




77

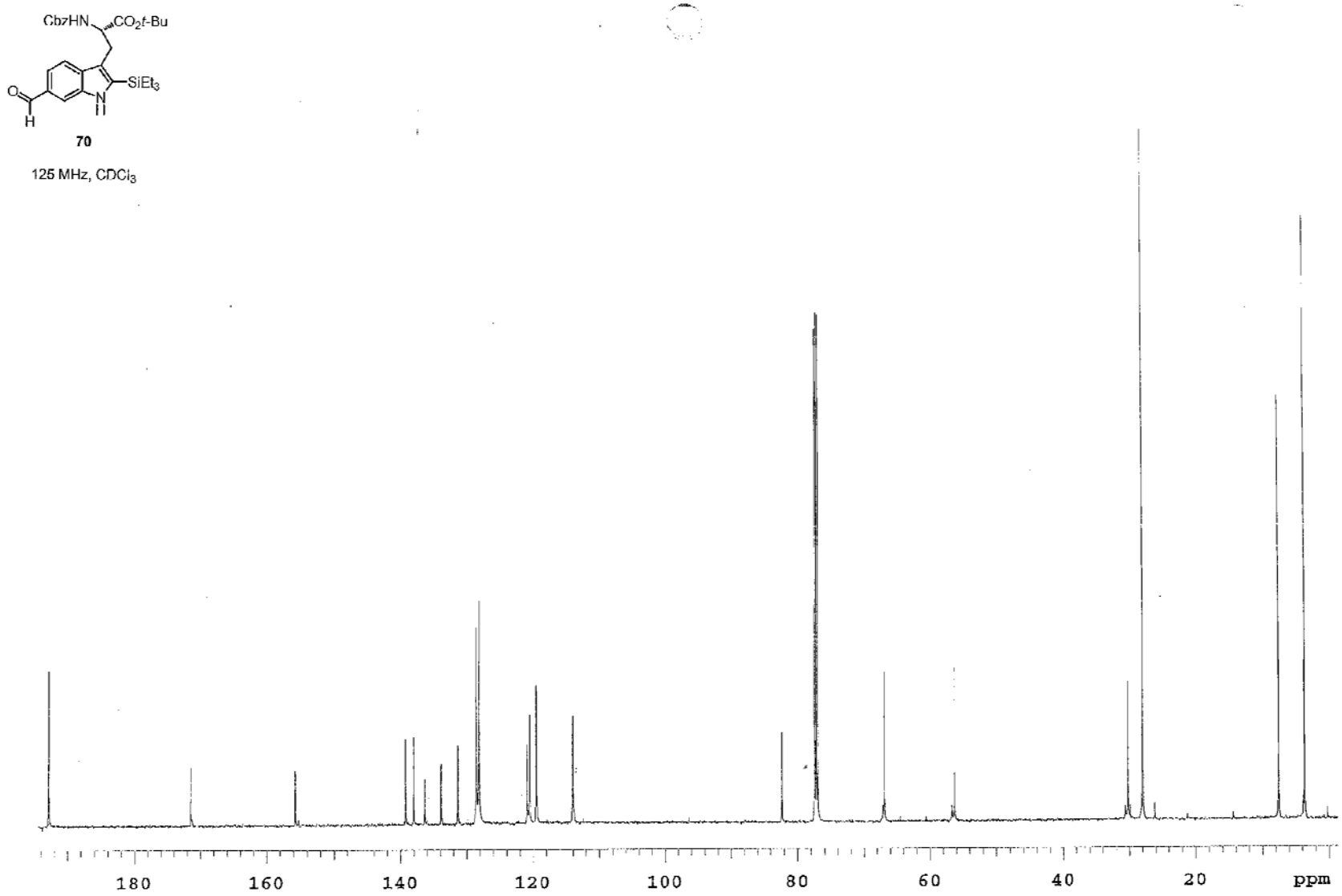
500 MHz, $CDCl_3$

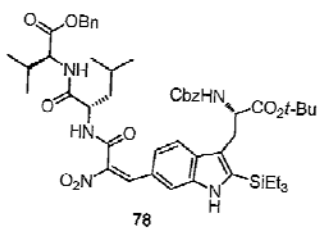

CbzHN
CO2t-Bu
SiEt3
HO
N
H
77
125 MHz, CDCl3
160
140
120
100
80
60
40
20
ppm



70

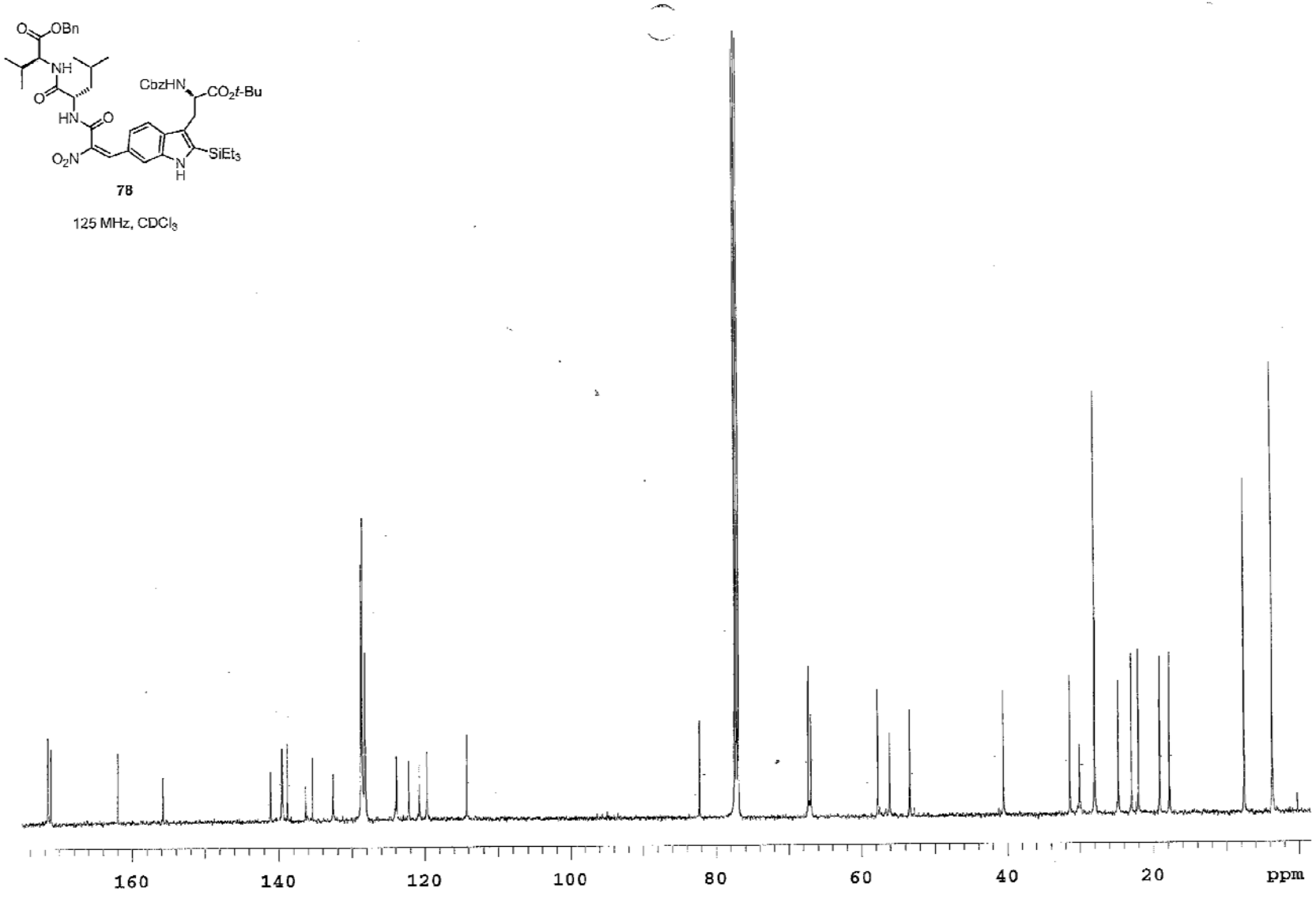
500 MHz, CDCl3

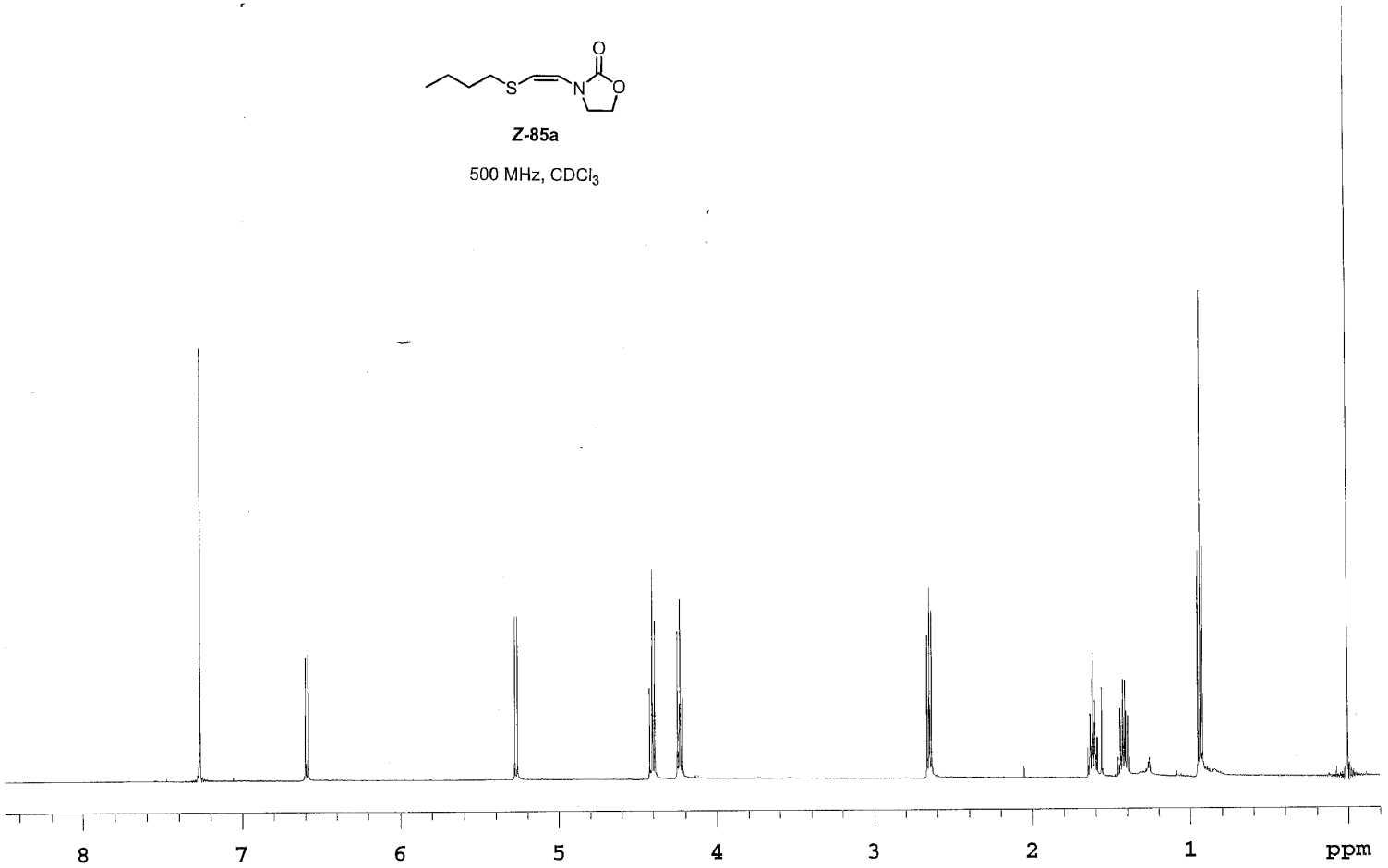

CbzHN
CO2t-Bu
SiEt3
O
H
N
H
70
125 MHz, CDCl3
180
160
140
120
100
80
60
40
20
ppm



78

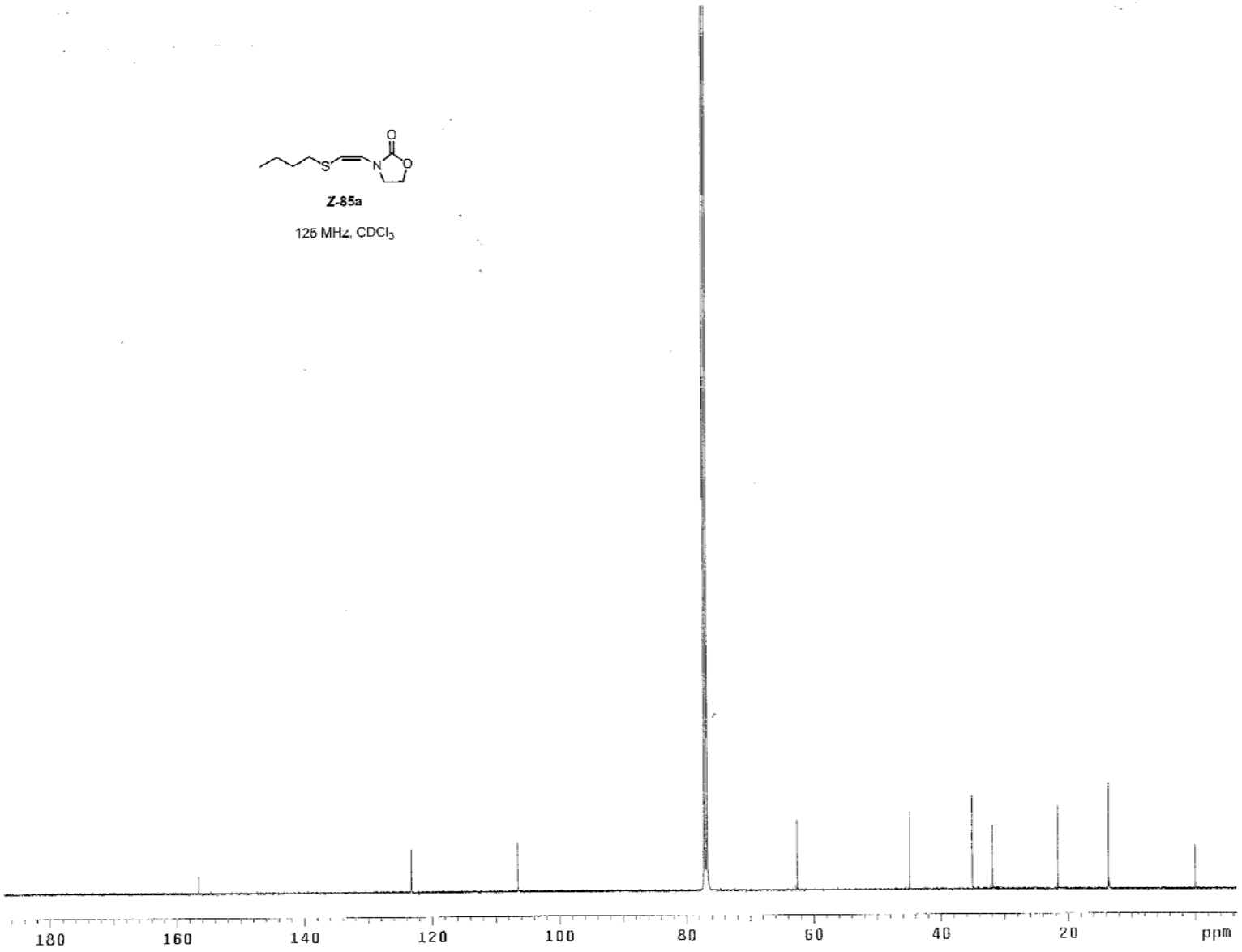
500 MHz, $CDCl_3$

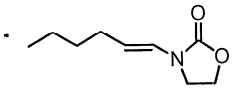

OBn
NH
CbzHN
CO2t-Bu
HN
O2N
SiEt3
N
H
78
125 MHz, CDCl3
160
140
120
100
80
60
40
20
ppm



***Z*-85a**

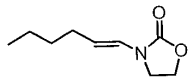
500 MHz, $CDCl_3$


Z-85a
125 MHz, CDCl3
180
160
140
120
100
80
60
40
20
ppm



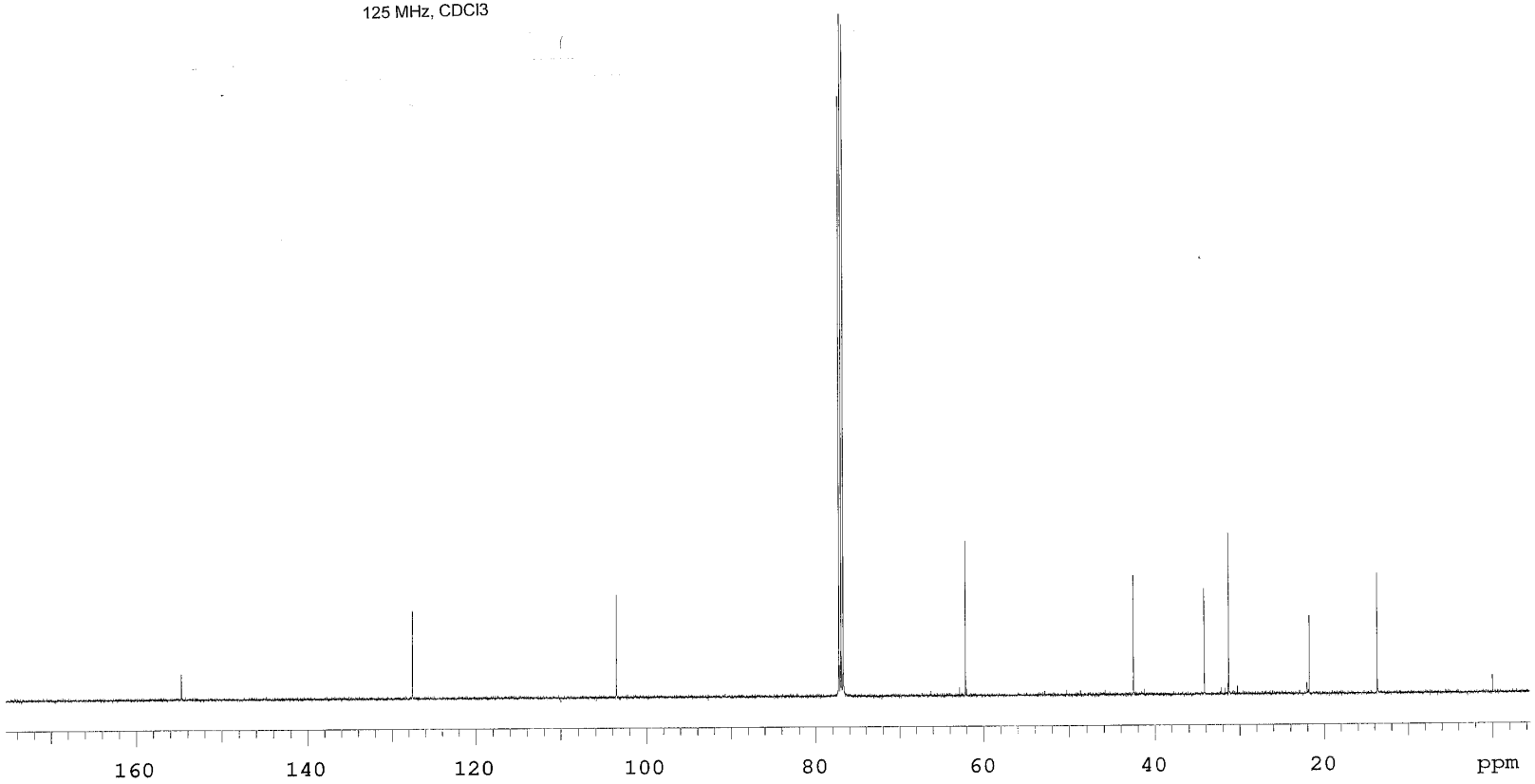
***E*-85a**

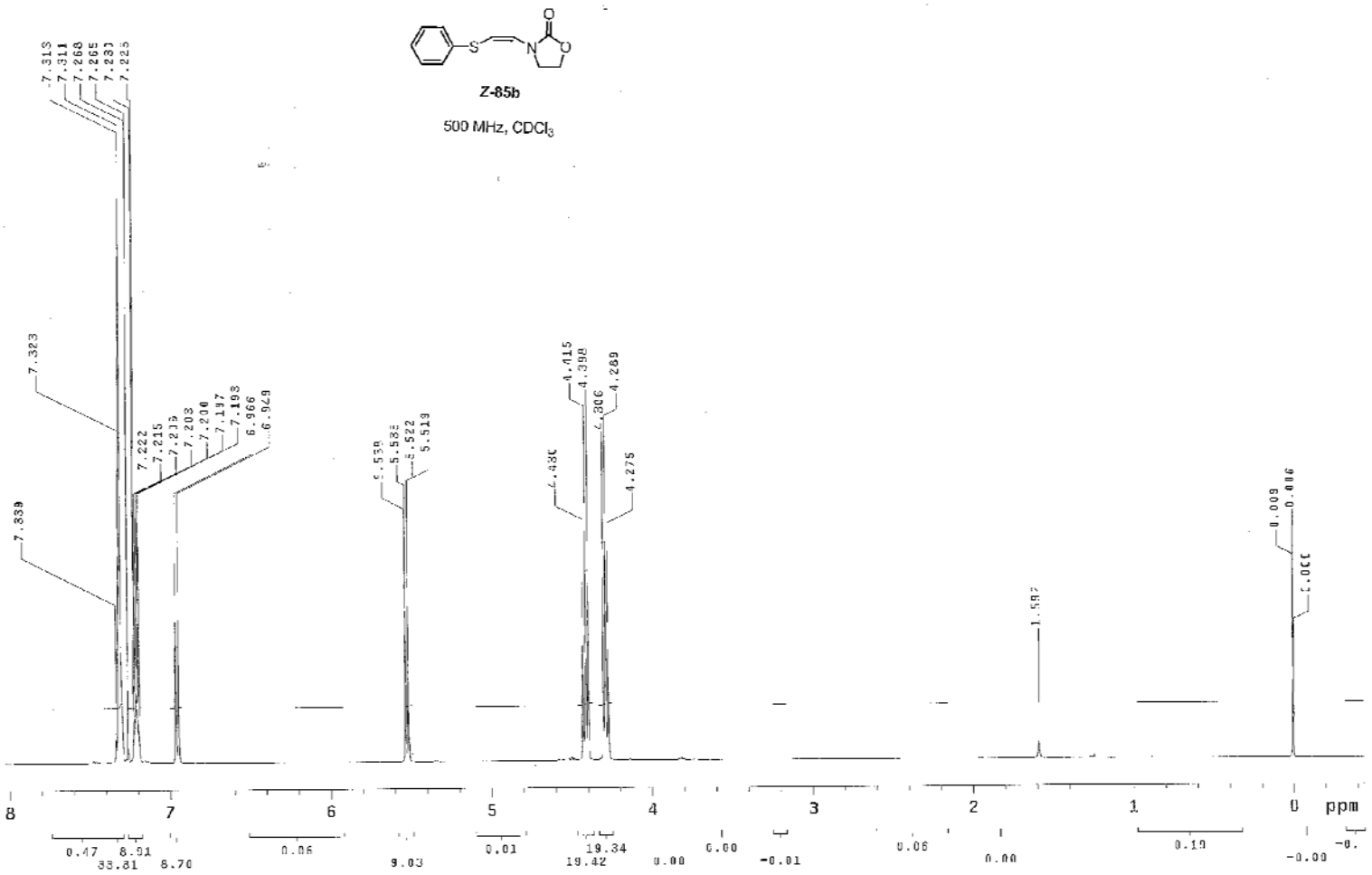
500 MHz, CDCl3

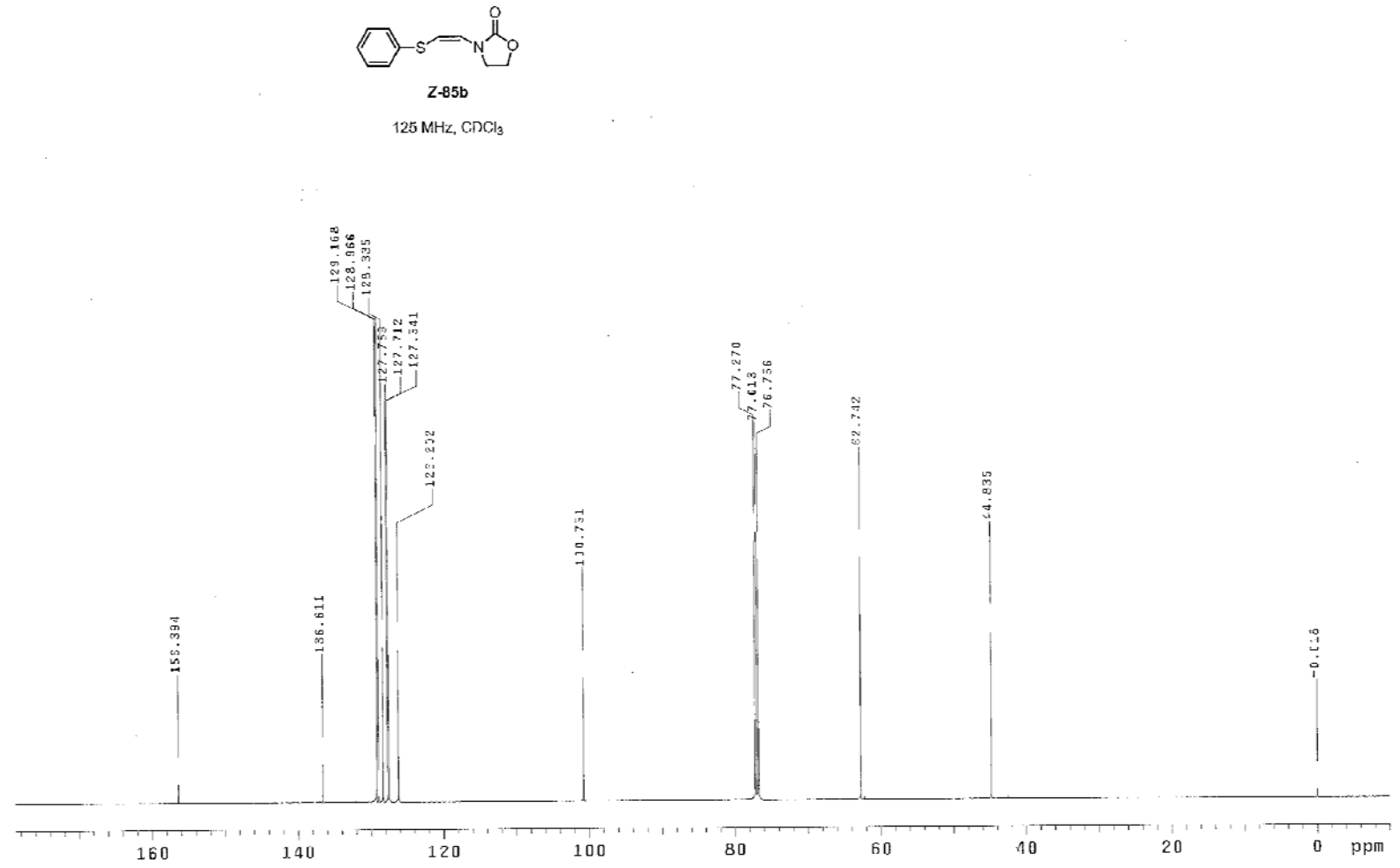


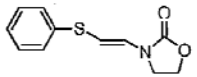
***E*-85a**

125 MHz, CDCl3



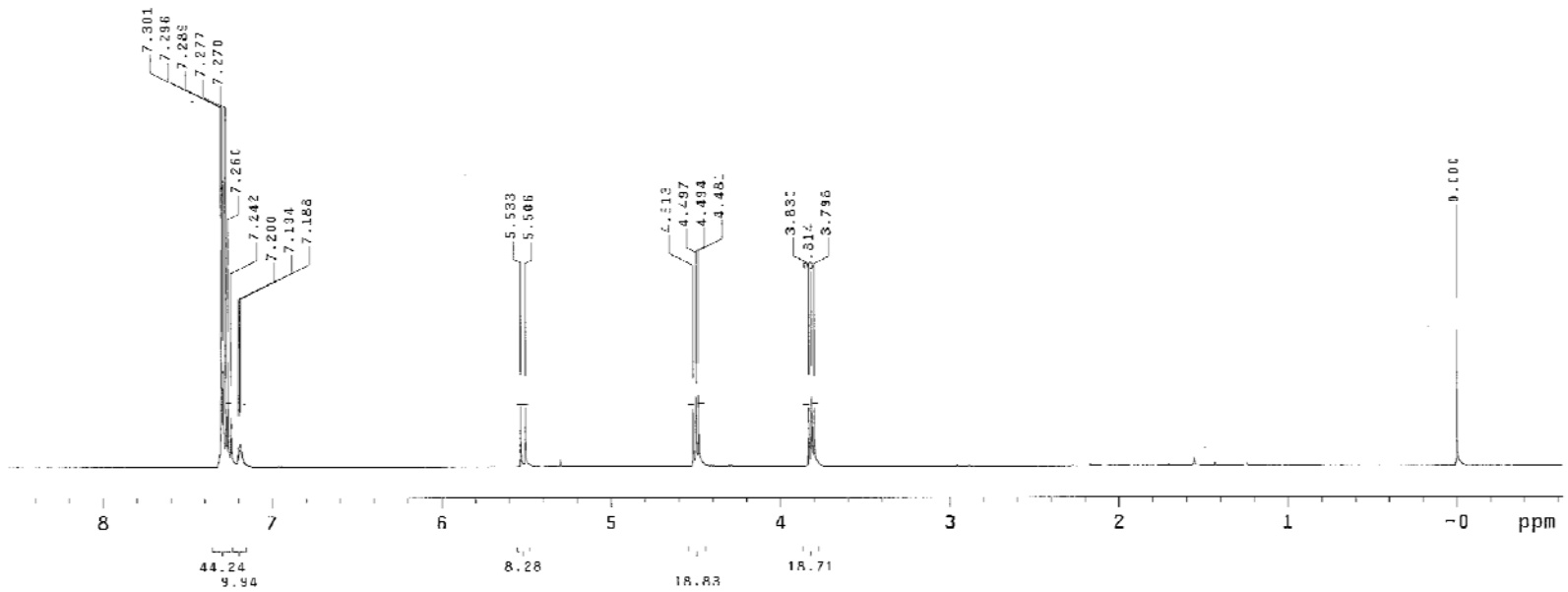

Z-85b
500 MHz, CDCl3
7.313
7.311
7.268
7.265
7.233
7.225
7.323
7.339
7.222
7.215
7.209
7.203
7.200
7.197
7.193
6.966
6.949
5.539
5.538
5.522
5.519
4.415
4.398
4.389
4.306
4.269
4.275
1.582
0.009
0.006
0.000
8
7
6
5
4
3
2
1
0
ppm

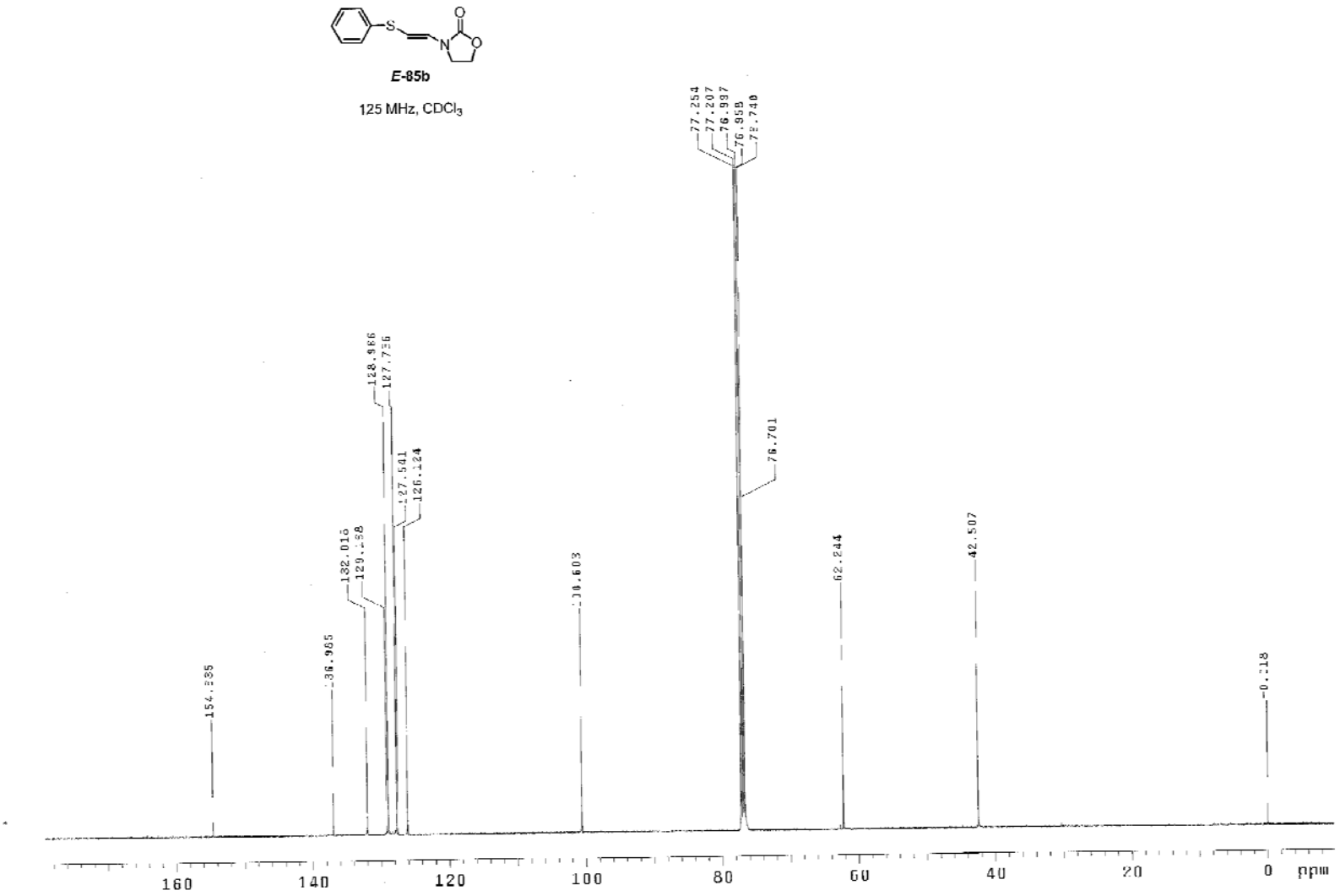

Z-85b
125 MHz, CDCl3
129.168
128.966
128.325
127.753
127.712
127.541
122.232
110.731
77.270
77.018
76.756
62.742
54.835
155.394
136.611
160
140
120
100
80
60
40
20
0
ppm

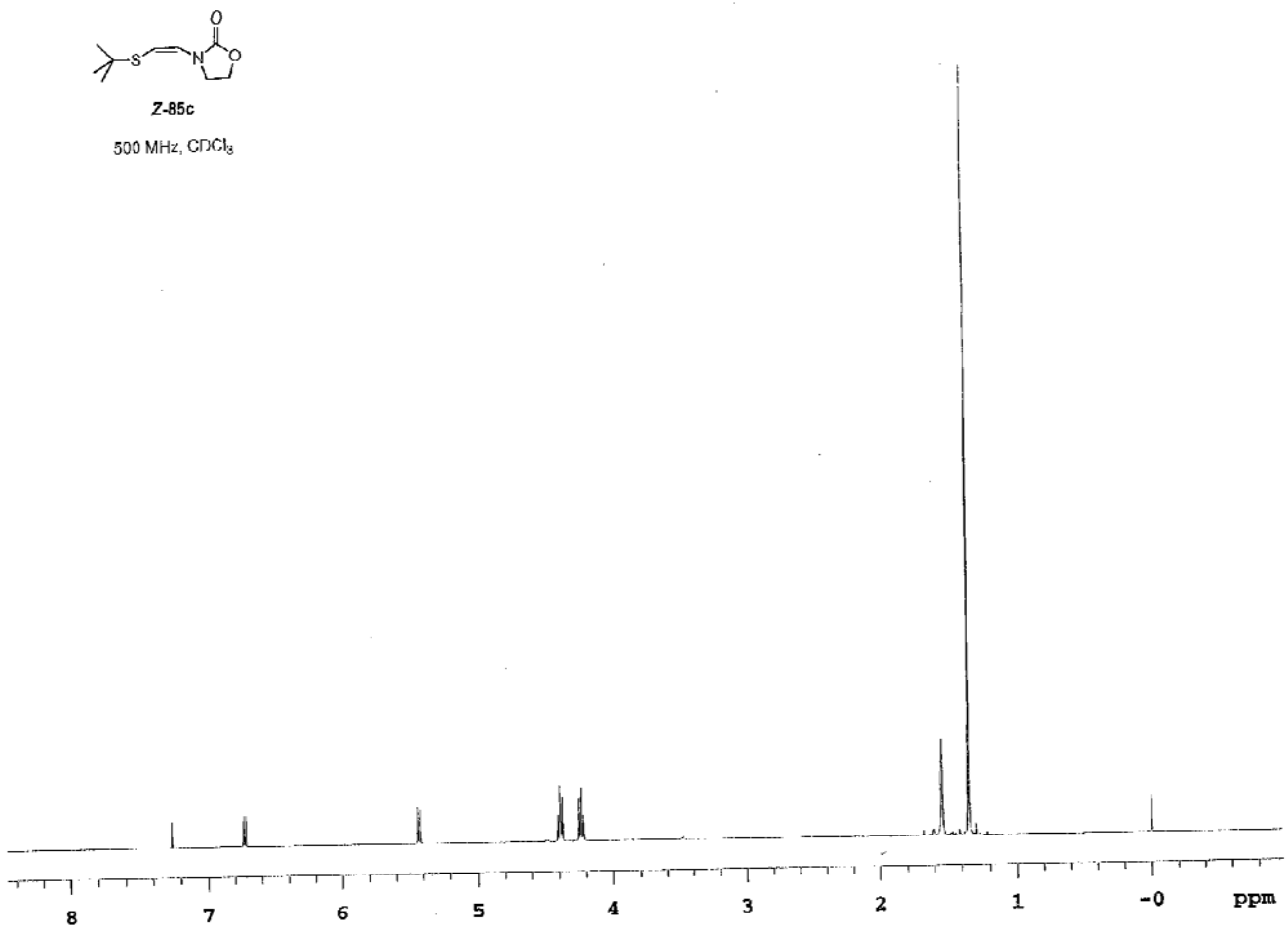


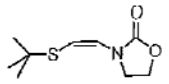
***E*-85b**

500 MHz, $CDCl_3$



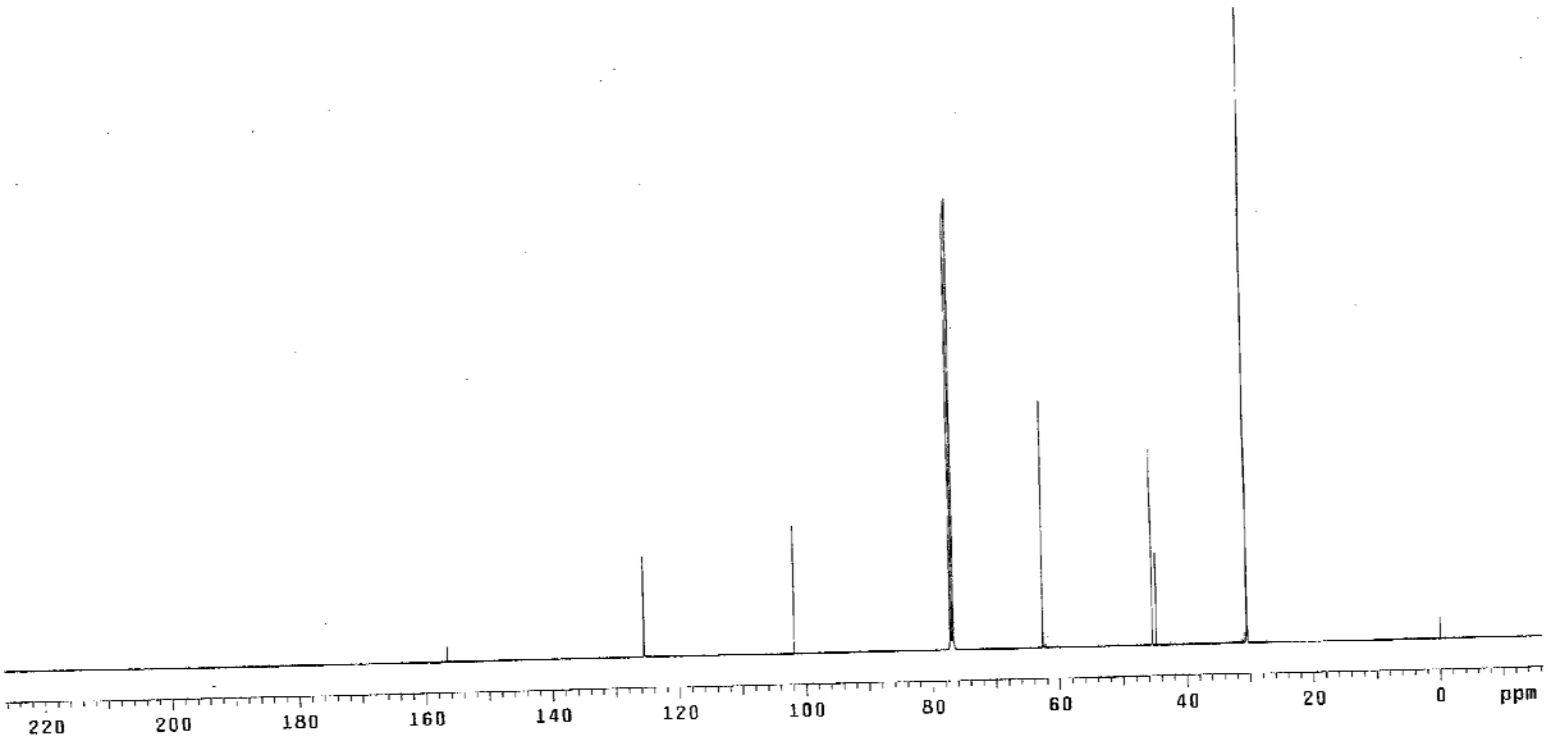

E-85b
125 MHz, CDCl3
154.385
136.965
132.016
129.258
128.966
127.736
127.541
126.124
100.603
77.254
77.207
76.997
76.955
76.740
76.701
62.244
42.507
0.118
160
140
120
100
80
60
40
20
0
ppm

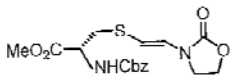

Z-85c
500 MHz, CDCl3
8
7
6
5
4
3
2
1
-0
ppm



Z-85c

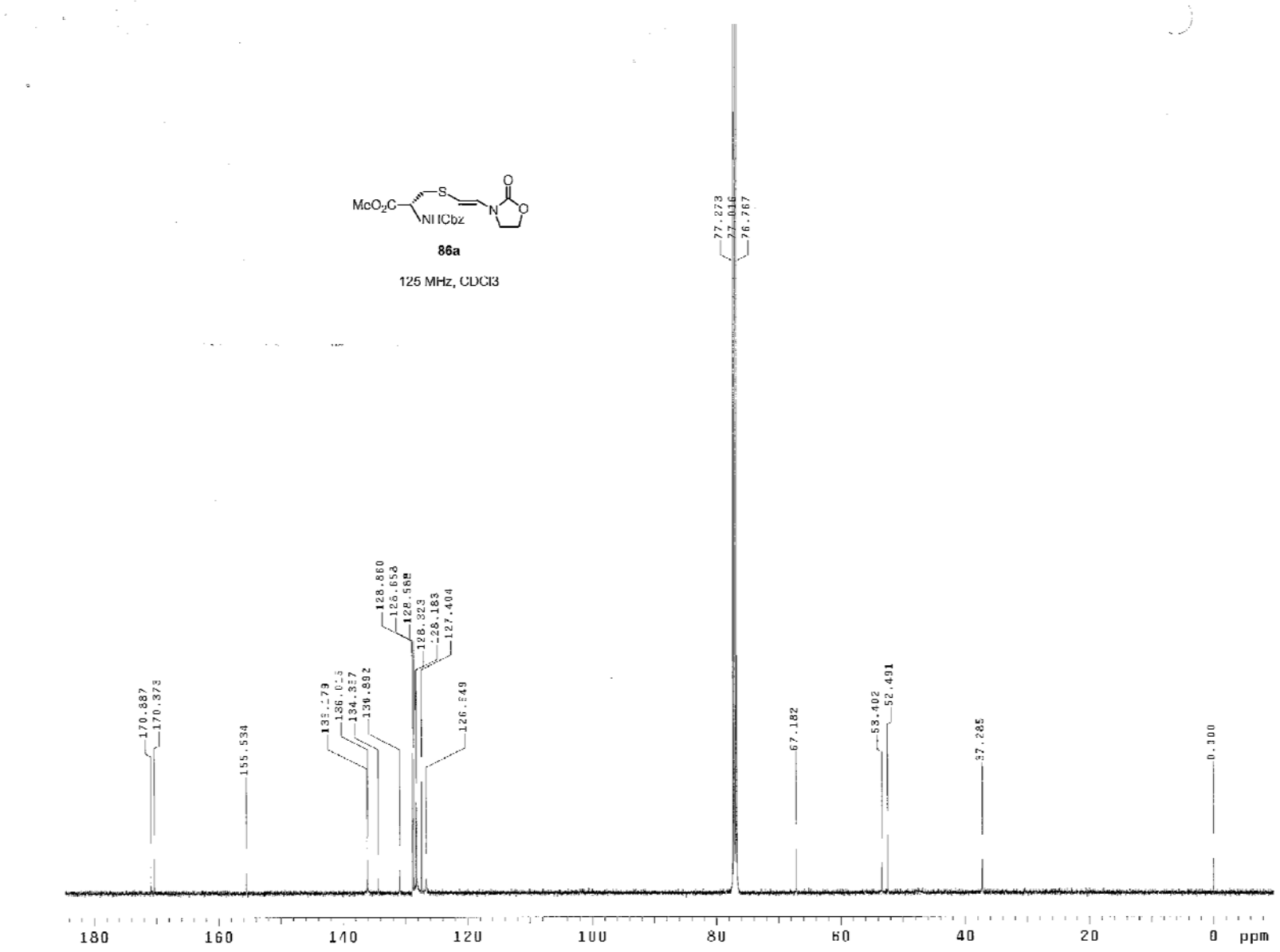
125 MHz, $CDCl_3$

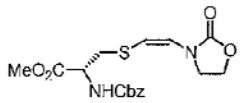




86a

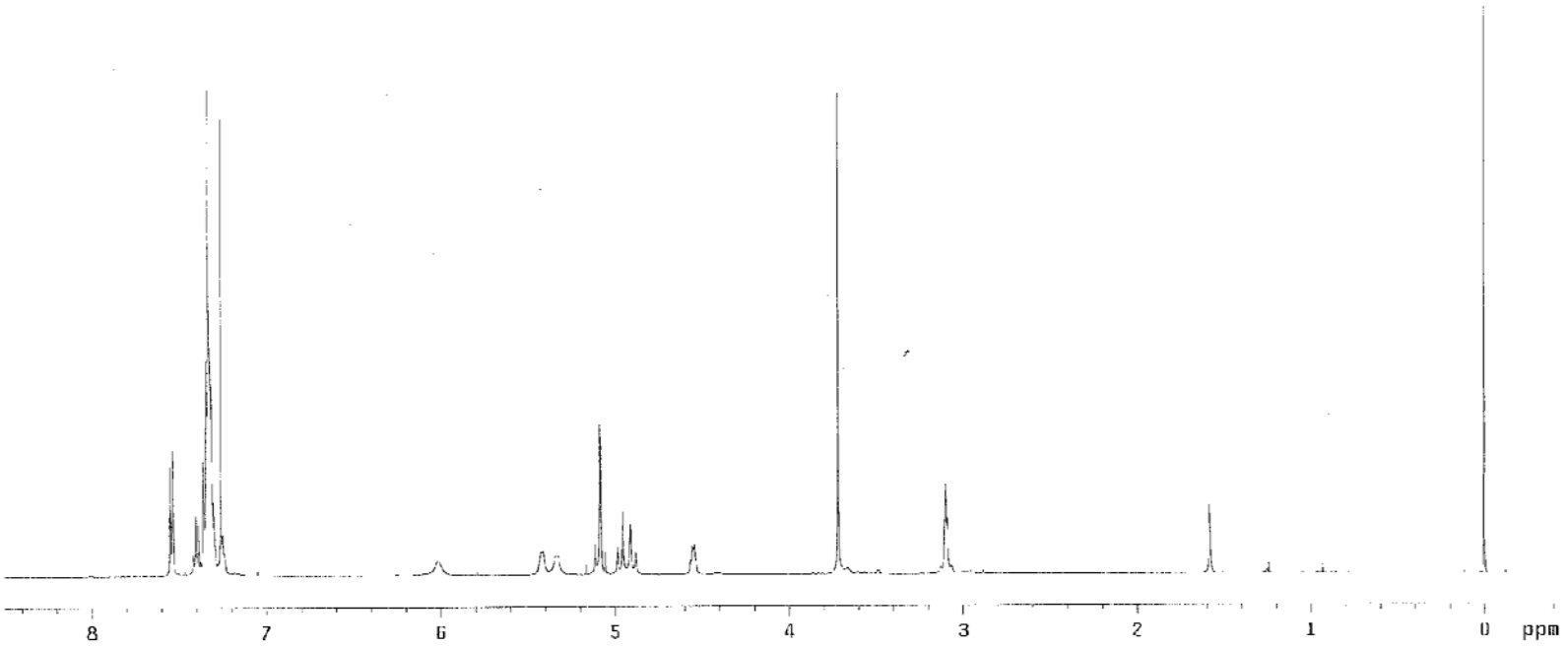
500 MHz, CDCl3

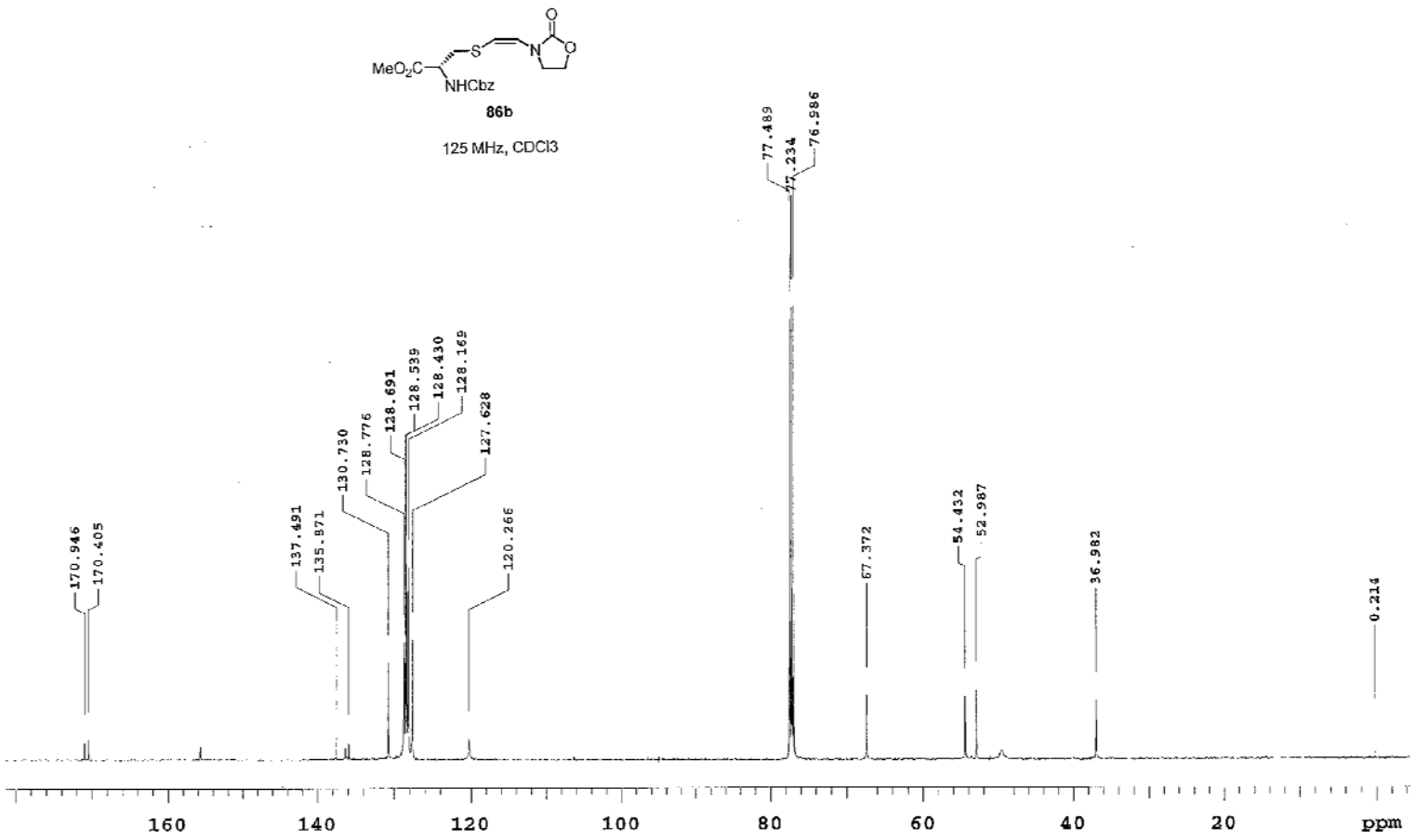

MeO2C
NHCbz
S
N
O
O
86a
125 MHz, CDCl3
77.273
77.016
76.767
170.887
170.373
155.534
135.479
134.327
130.892
128.860
128.658
128.568
128.323
128.183
127.404
126.849
67.182
53.402
52.491
37.285
0.300
180
160
140
120
100
80
60
40
20
0
ppm

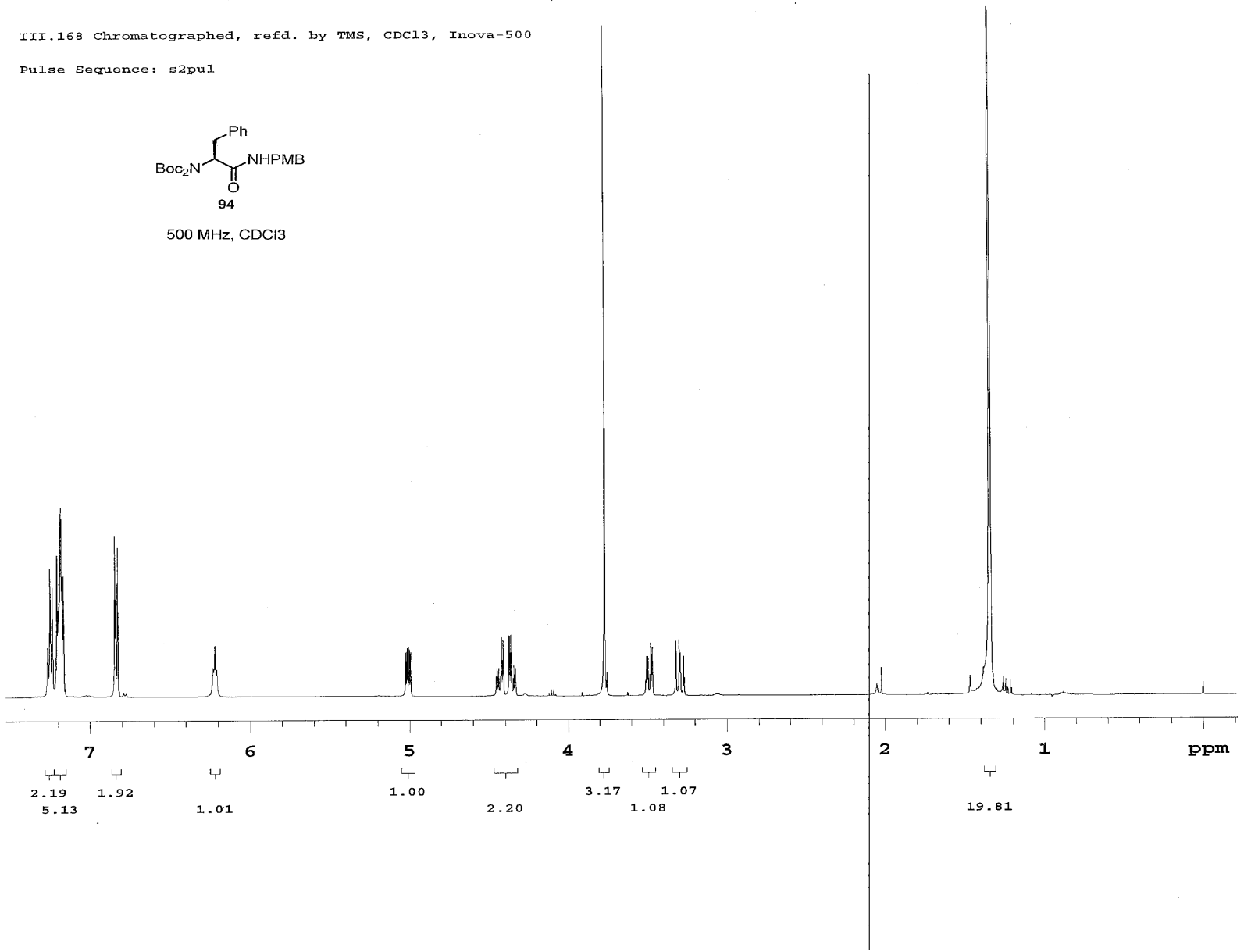


86b

500 MHz, CDCl3

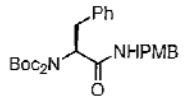



MeO2C
NHCbz
S
N
O
O
86b
125 MHz, CDCl3
170.946
170.405
137.491
135.871
130.730
128.776
128.691
128.539
128.430
128.169
127.628
120.266
77.489
77.234
76.986
67.372
54.432
52.987
36.982
0.214
160
140
120
100
80
60
40
20
ppm


III.168 Chromatographed, refd. by TMS, CDCl3, Inova-500
Pulse Sequence: s2pul
Ph
NHPMB
Boc2N
O
94
500 MHz, CDCl3
7
6
5
4
3
2
1
ppm
2.19
5.13
1.92
1.01
1.00
2.20
3.17
1.08
1.07
19.81

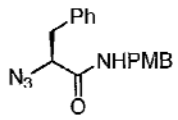
III.168 Chromatographed, refd. by CDCl3, CDCl3, Inova 500

Pulse Sequence: s2pul



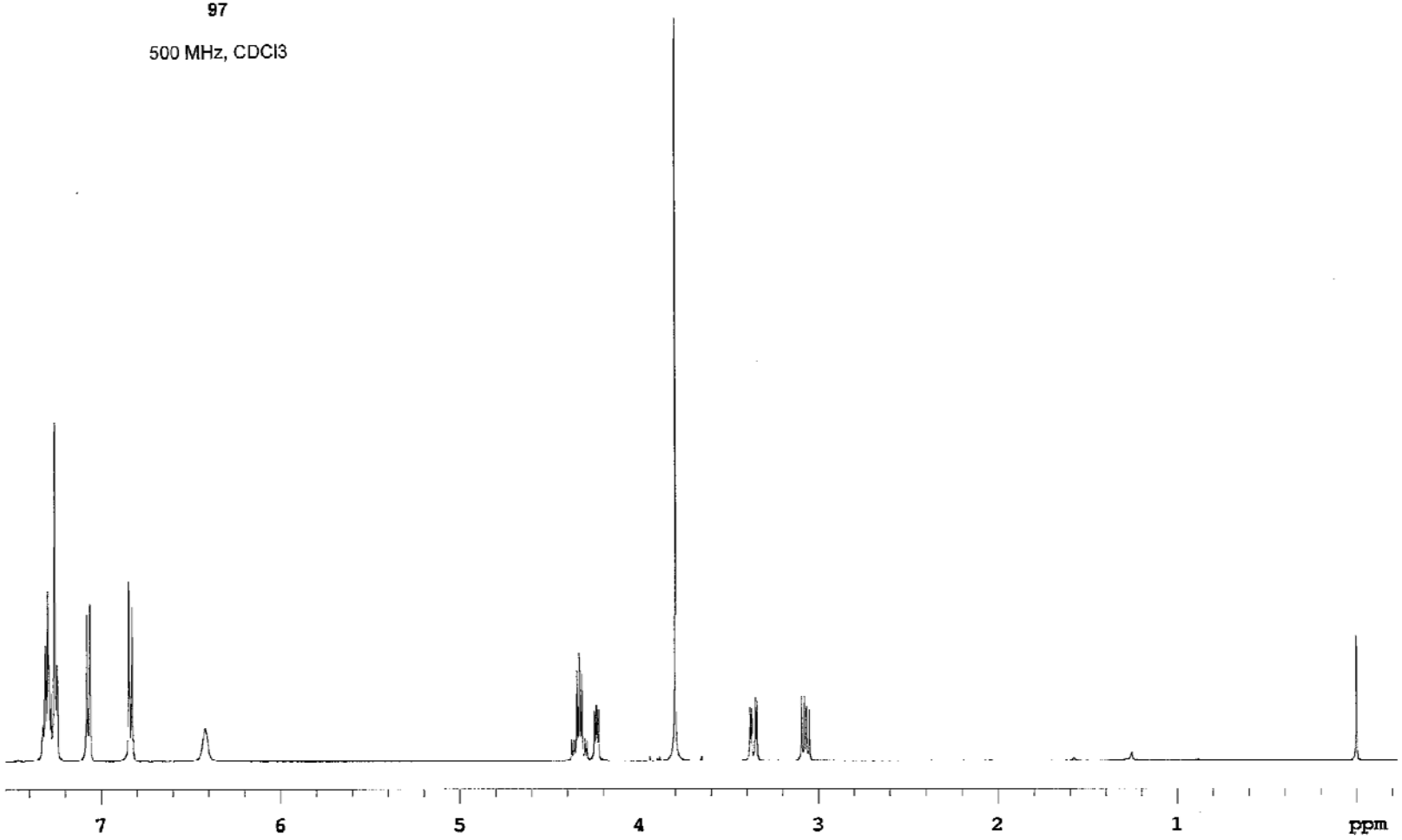
94

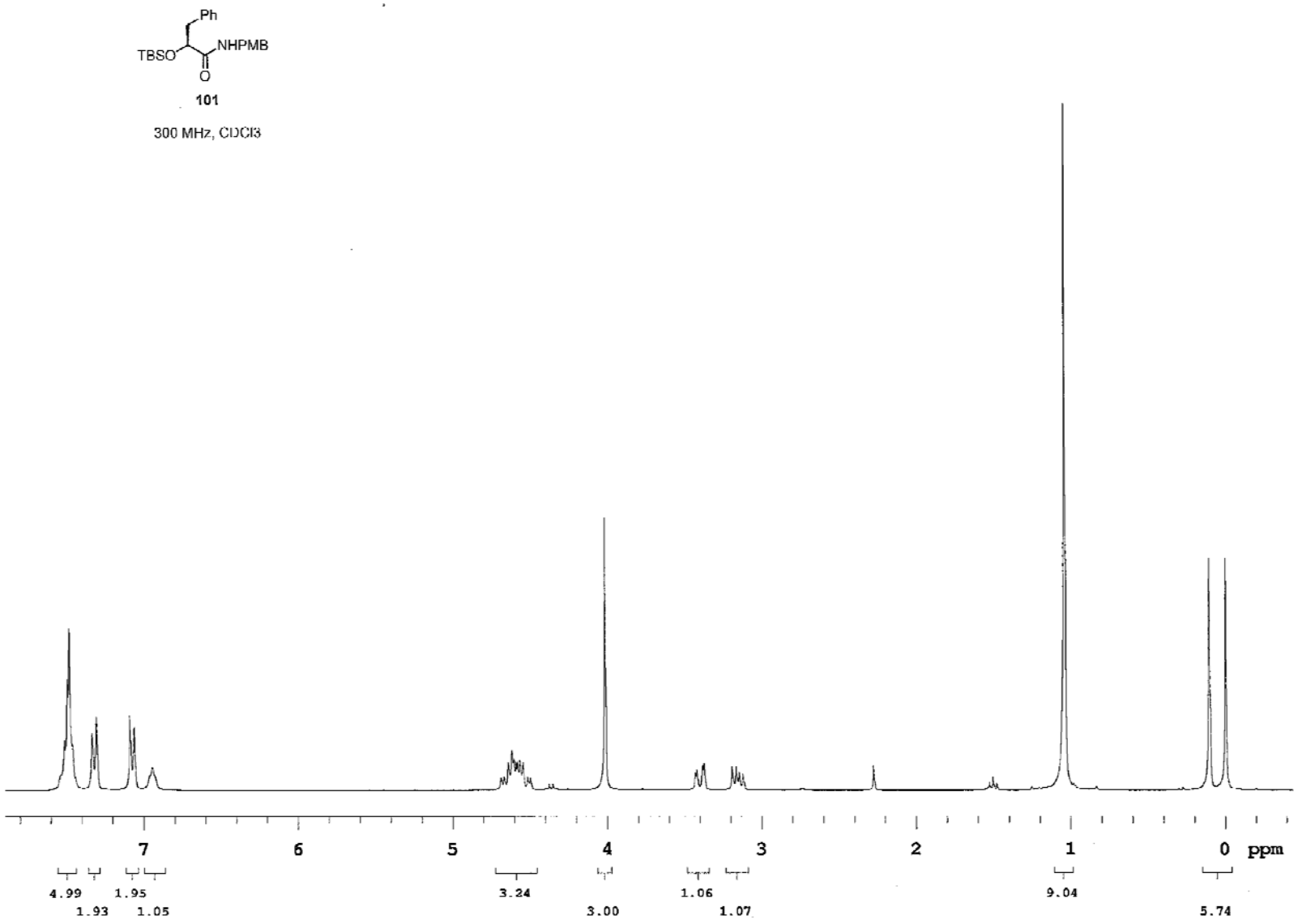
125 MHz, CDCl3

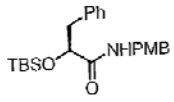


97

500 MHz, CDCl3

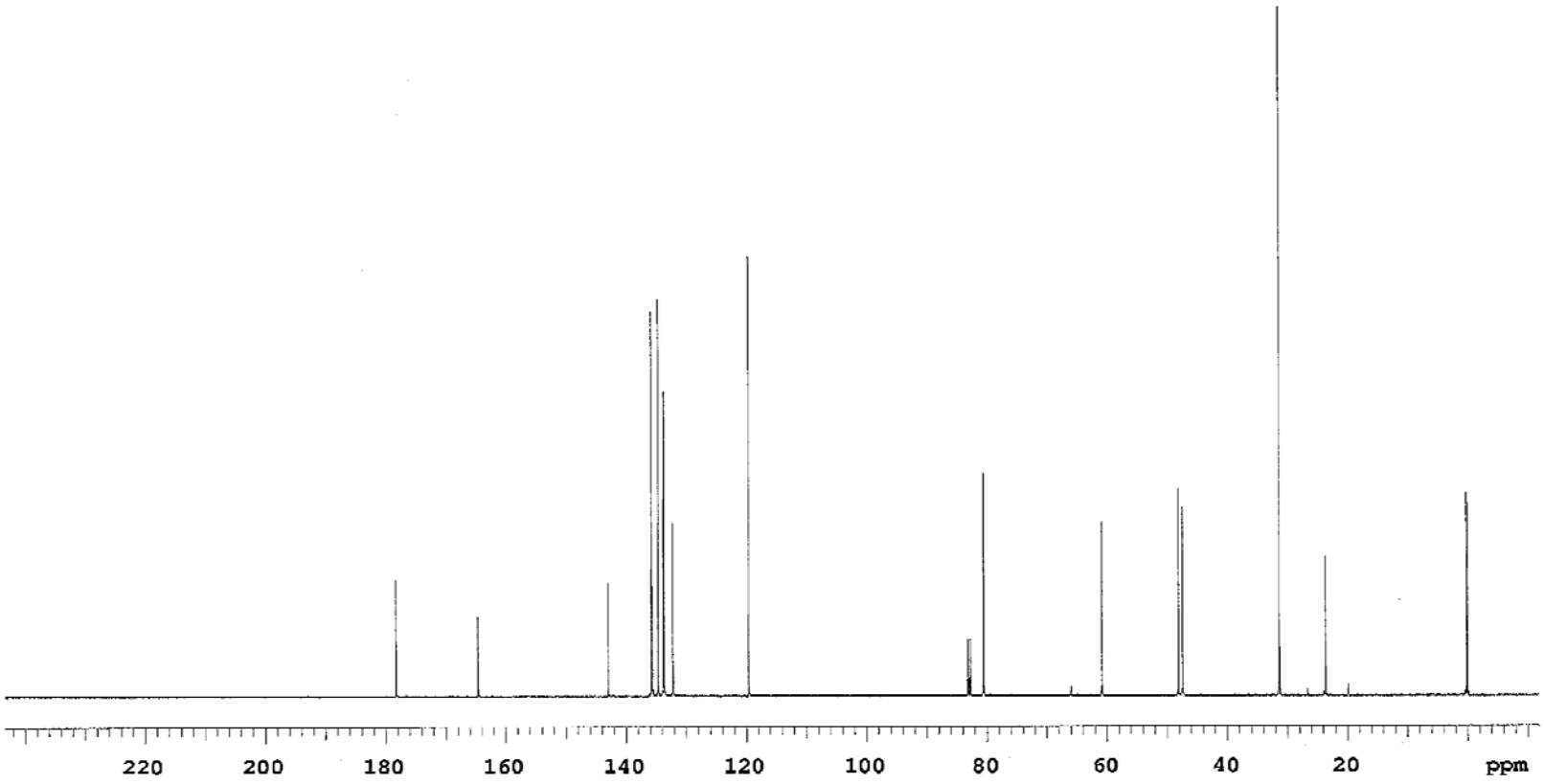


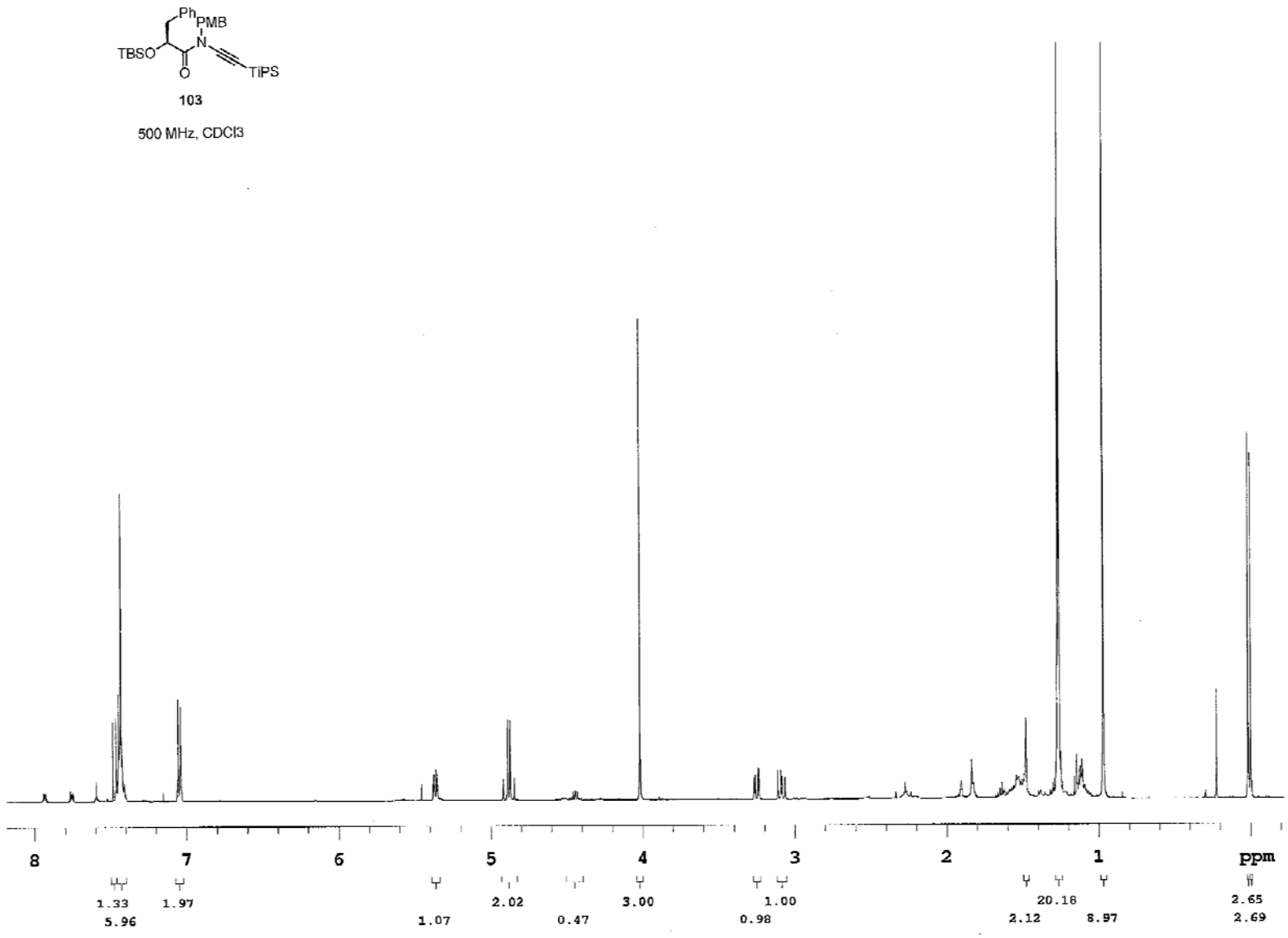

Ph
NHPMB
TBSO
O
101
300 MHz, CDCl3
7
6
5
4
3
2
1
0
ppm
4.99
1.93
1.95
1.05
3.24
3.00
1.06
1.07
9.04
5.74

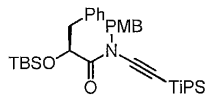


101

75 MHz, CDCl3

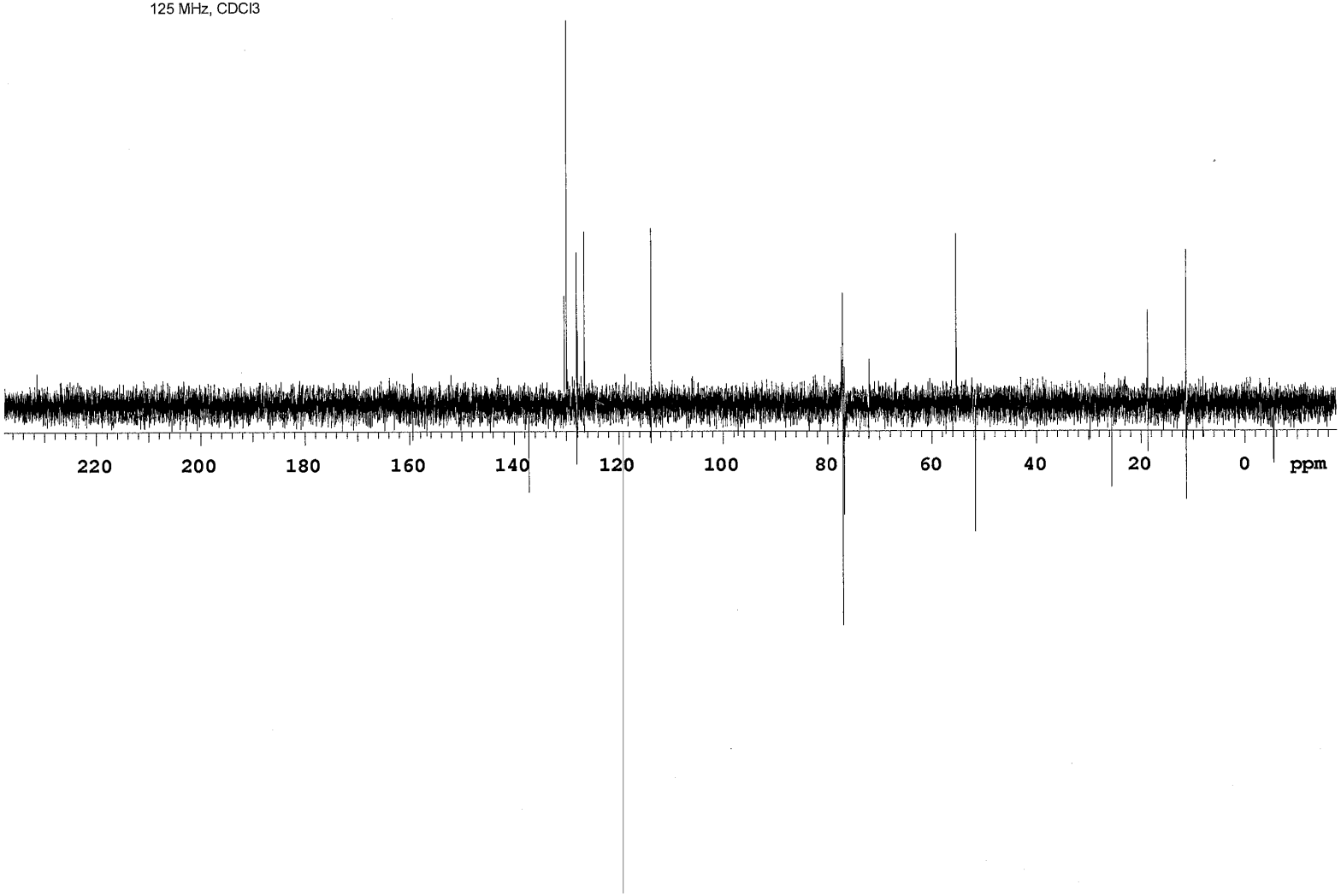


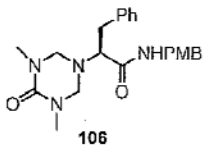

Ph
PMB
N
TBSO
O
TIPS
103
500 MHz, CDCl3
8
7
6
5
4
3
2
1
ppm
1.33
1.97
2.02
3.00
1.00
20.18
2.65
5.96
1.07
0.47
0.98
2.12
8.97
2.69



103

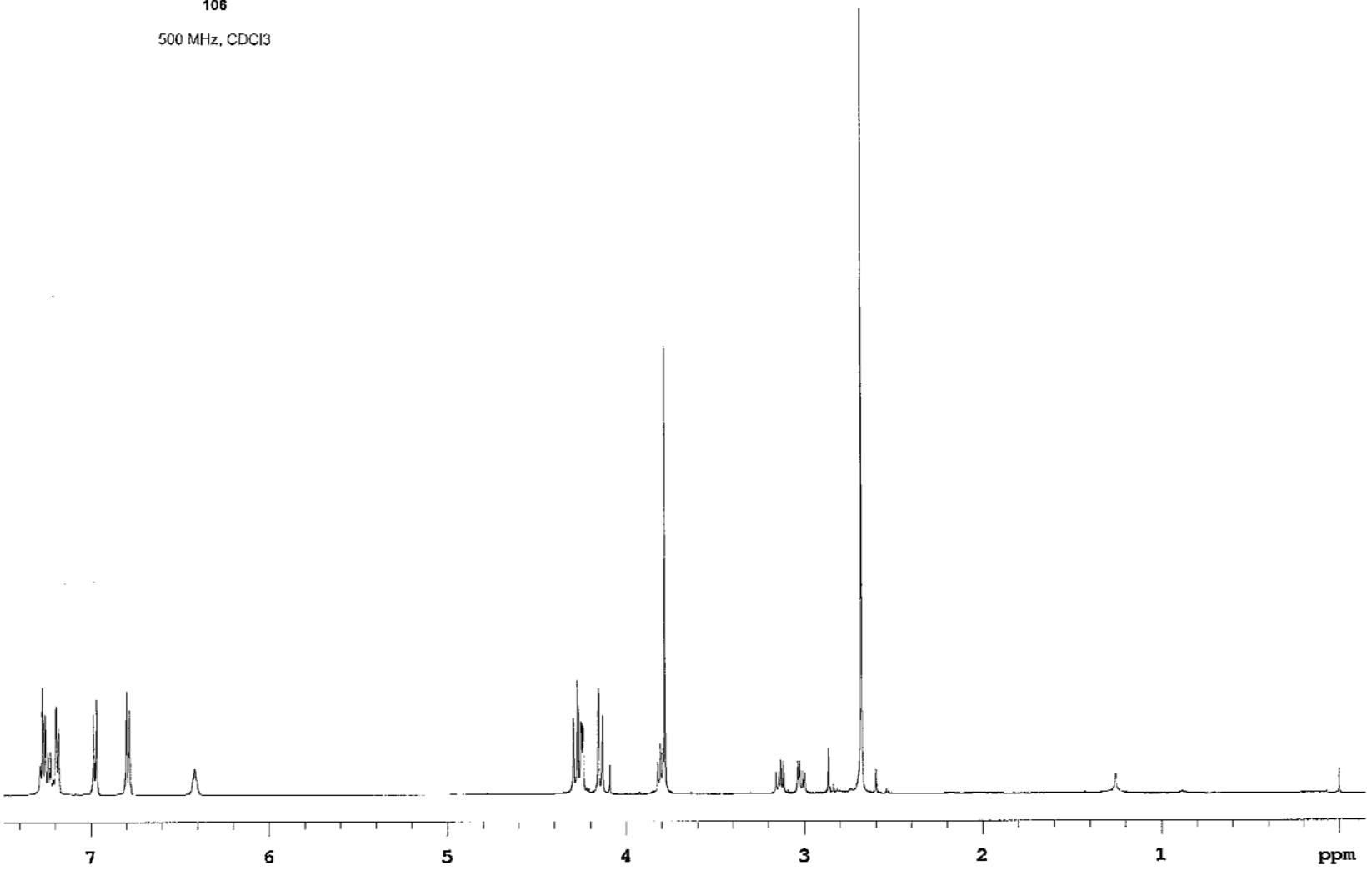
125 MHz, CDCl3

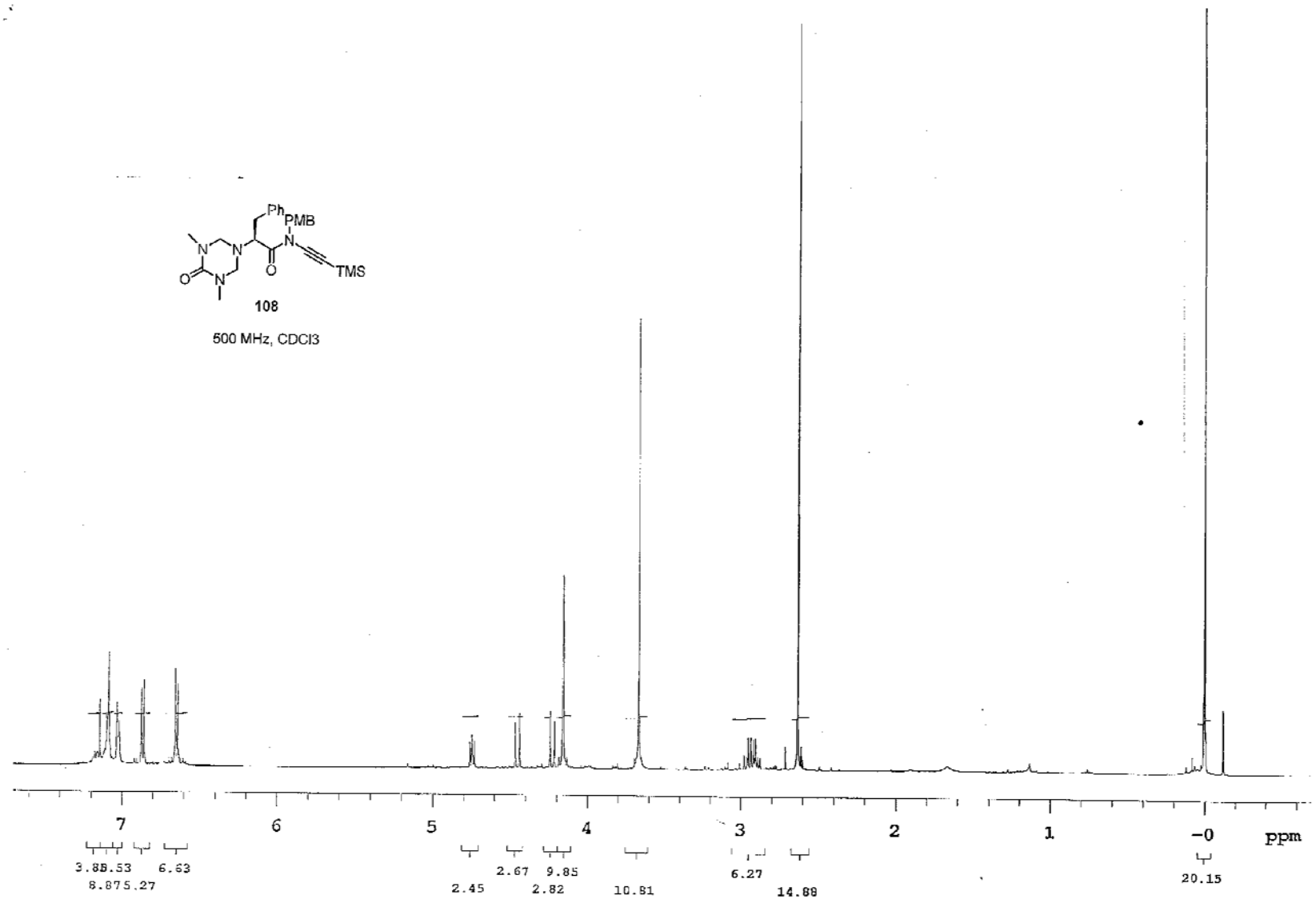


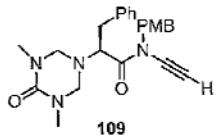


106

500 MHz, CDCl3




Ph
PMB
N
N
N
N
O
O
TMS
108
500 MHz, CDCl3
7
6
5
4
3
2
1
−0
ppm
3.88
5.53
6.63
8.87
5.27
2.45
2.67
9.85
2.82
10.81
6.27
14.88
20.15



109

500 MHz, CDCl3,
crude