Supporting Information for

Synergism between Genome Sequencing, Tandem Mass Spectrometry and Bio-Inspired Synthesis Reveals Insights into Nocardioazine B Biogenesis

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S1. Abbreviations

$(BOC)_2O$	-	di-*tert*-butyl dicarbonate
BINOL	-	1, 1'-bi-2, 2'-naphthol
BOP-Cl	-	*bis*(2-oxo-3-oxazolidinyl)phosphonic chloride
Cbz-Cl	-	benzyloxycarbonyl chloride
NCS	-	*N*-chlorosuccinimide
KOH	-	potassium hydroxide
DMAc	-	*N*,*N*-dimethylacetamide
AIBN	-	2,2'-azo*bis*isobutyronitrile
DMF	-	*N*,*N*-dimethylformamide
NMR	-	nuclear magnetic resonance
CD_3OD	-	deuterated methanol
DKP	-	diketopiperazine
TLC	–	thin layer chromatography
THF	-	tetrahydrofuran
MHz	-	mega Hertz
$CDCl_3$	-	deuterated chloroform
HR-MS	-	high resolution mass spectrometry
LC-MS	-	liquid chromatography-mass spectrometry
L-Trp	-	L-tryptophan
D-Trp	-	D-tryptophan
rt	-	room temperature
h	-	hour
d	-	day(s)
satd.	-	saturated
anhyd.	-	anhydrous
BLASTP	-	Protein Basic Local Alignment Search Tool

S2. Culturing of *Nocardiopsis sp*. CMB M0322 and Extraction of Alkaloidal DKP Metabolites

Nocardiopsis sp. strain CMB-M0232, originally-isolated by the Capon group from a sediment sample obtained from South Molle Island from a depth of 55 m[1] was obtained from the Capon laboratory as pure strains of individual colonies. This organism was maintained and propagated for further culturing using standard microbiological techniques. *Nocardiopsis* sp. CMB-M0232 were grown to dense colonies on a single agar plate (comprising 25 mL of 1% starch, 0.4% yeast extract, 0.2% peptone, 1.8% agar, and 0.0005% rifampicin) under incubation at 27 °C for four weeks. For larger laboratory culture and for generating extracts, a frozen glycerol stock culture (1.2 mL) of *Nocardiopsis* sp. (CMB-M0232) was used to inoculate a 250 mL Schott flask containing 80 mL of M1 broth (1% starch, 0.4% yeast extract and 0.2% peptone dissolved in deionized water, supplemented with 3% (by weight) ocean salt (Instant Ocean®, USA). The flask was shaken at 225 rpm in a rotary shaking incubator for ~6-14 d at 27 °C depending on the maturity of each inoculation, as measured by OD_{600}. An aliquot of this seed culture (5.0 mL, average OD_{600} = 0.6) was used to inoculate each of six 2 L Schott flasks containing 500 mL of M1 broth, and fermentation was continued for a further 8-21 d (at 27 °C and with rotary shaking at 225 rpm). Following fermentation, the culture was extracted with an equal volume of EtOAc (i.e. 500 mL per flask) and the combined organic phase concentrated *in vacuo* to yield a crude extract (250 mg). The crude extract was triturated sequentially with hexane (25 mL), CH_2Cl_2 (25 mL) and MeOH (25 mL), to afford individual fractions of 71.2 mg, 56.3 mg and 13.6 mg

[1] See supporting information of: Raju, R., Piggott, A. M., Huang, X.-C., and Capon, R. J. Nocardioazines: A Novel Bridged Diketopiperazine Scaffold from a Marine-Derived Bacterium Inhibits P-Glycoprotein, *Org. Lett.* **2011**, *13*, 2770-2773.

respectively. Fractions were concentrated to dryness in vacuo, and the CH_2Cl_2 fraction was subsequently subjected to HPLC fractionation (Zorbax CN 5 μm, 250 × 9.4 mm column, 4 mL/min gradient elution from 60% H_2O/MeOH to 100% MeOH over 55 min, with a hold at 100% MeCN for 5 min) to yield multiple metabolites as described in section **S10**. Genomic DNA was isolated from *Nocardiopsis sp*. CMB M0322 and was subjected to sequencing.

S3. Draft Genome Sequencing of *Nocardiopsis sp*. CMB M0322 and Pathway Annotation, Cloning and Heterologous expression

The draft genome sequence and assembly of *Nocardiopsis* sp. CMB-M0232 was completed by Cofactor Genomics (St. Louis, MO) through a combination of Illumina and 454 sequencing technologies. Open reading frames (ORFs) were predicted using GeneMark Version 2.5. BLASTP searches were employed to determine ORFs with homology to those in the NCBI database.

Construction of Nocardiopsis sp. *CMB-M0232 cosmid clone library*

A ~2,000-member cosmid clone library of *Nocardiopsis* sp. CMB-M0232 gDNA was prepared using SuperCos 1 vector and following manufacturer protocols (Agilent Technologies). Briefly, gDNA was digested with Sau3A1 to afford ~30-50kB fragments, which were ligated into SuperCos 1. MaxPlax lambda packaging extracts (EpiCenter) were used to package constructs, which were introduced into *E. coli* XL1-MRF for propagation. Individual members of the clone library were stored at -80 °C as glycerol-preserved stocks in 96-well microtiter plates.

Screening of cosmid clone library for contig #1 gene cluster

To identify cosmid library members carrying contig #1, clones from individual microtiter plates

were pooled and screened by PCR using the primer pair CDPS1F (5'-GTCGGTGACGAGCCATGCCC-3') and CDPS1R (5'-CTTCGCGCAACGCGCCAAAT-3'), which flank *nozA*. Each PCR contained 20.2 μL of molecular biology grade water, 2.5 μL of 10X ThermoPol buffer, 0.5 μL of dNTPs (200 μM of each dNTP), 1 μL DMSO, 0.1 μL of each primer (0.4 □M), 0.5 μL of template, and 0.1 μL of ThermoPol Taq polymerase (New England Biolabs). PCR was conducted with an initial denaturation cycle of 94 °C for 3:00, followed by 30 cycles of 94 °C for 45s, 60 °C for 60 s, and 72 °C for 60 s, a final extension cycle of 72 °C for 5 min. Once plates containing contig #1 were identified, clones from individual rows and columns within these plates were pooled and re-screened to determine the specific well(s) containing cosmids with contig #1. For each resulting clone (e.g. pAL557), sequencing of ~2 kB of each end of the gDNA insert was conducted to verify the entirety of contig #1 was contained within the insert.

Adaptation of cosmid construct for expression of contig #1 and introduction into S. coelicolor M1146

The SuperCos 1 cosmid clone pAL557, carrying contig #1, was adapted for introduction into and expression in *Streptomyces* hosts by following the method described by Smanski et al.[2] Specifically, pAL557 was modified by using λ-RED *E. coli* recombination approaches to introduce an origin of conjugal transfer (*oriT*), *Streptomyces θ*C31 integrase for integration into the *attB* site of the *Streptomyces* chromosome, and an apramycin resistance gene (*aac(3)IV*). Using previously described methods and primers 3'AmpF and 3'AmpR,[2] a 270 bp fragment from the 3'-end of the *bla* gene from SuperCos 1 was PCR amplified and cloned into the XbaI/BamHI site of *Streptomyces*-integrating pSET152 vector,[3] which encodes *aac(3)IV*, *oriT*, and *θ*C31 integrase. The resulting pSET152/3'bla construct was then linearized by digestion with BamHI and EcoRI. This linear construct was introduced into λ-RED recombination proficient *E. coli* BW25113/pIJ790 carrying pAL557, to afford homologous recombination between the

[2] M. J. Smanski, J. Casper, R. M. Peterson, Z. Yu, S. R. Rajski and B. Shen, *J. Nat. Prod.* **2012,** *75*, 2158-2167.

[3] M. Bierman, R. Logan, K. O'Brien, E. Seno, R. Rao and B. Schoner, *Gene*, 1992, **116**, 43-49.

homologous pUC site and 3'-*bla* end. This yielded pAL5571, for which the presence of *aac(3)IV*, *oriT*, and θC31 integrase was confirmed by PCR and DNA sequencing. pAL5571 was introduced into *S. coelicolor* M1146 by interconjugal transfer from *E. coli* ET12567/pUZ8002 using standard methods.[4] Integration of pAL5571 contig #1 genes into the *S. coelicolor* M1146 chromosome was confirmed by PCR amplification and sequencing of selected genes spanning the entire contig. The gDNA insert size was approximated by restriction digesting pAL5571 with BamHI and evaluating resulting DNA fragment sizes by agarose gel electrophoresis.

Cultivation and chemical extraction of S. coelicolor M1146

Fifty microliters of *S. coelicolor* M1146/pAL5571 6-day starter culture in M1 media was used to inoculate 10 mL of M1 media (without Instant Ocean) supplemented with 50 μg/mL apramycin in 50 mL Falcon tubes. Control cultures were equivalently prepared using *S. coelicolor* M1146 and omitting apramycin. Cultures were incubated at 30 °C with shaking at 225 rpm for six days. Treatment and control fermentations were conducted in triplicate. Cultures were extracted with 10 mL EtOAc and the resulting chemical extracts concentrated to dryness *in vacuo.* Chemical extracts were resolubilized with 100 μL of MeOH for analysis by HPLC and LC/MS.

Evaluation of chemical profiles of S. coelicolor M1146/pAL5571 treatments and M1146 controls

[4] (a) J. P. Gomez-Escribano and M. J. Bibb, *Microbiol. Biotechnol.* **2011,** *4*, 207-215.
(b) T. Kieser, M. J. Bibb, M. J. Buttner, K. F. Chater and D. A. Hopwood, *Practical Streptomyces Genetics*, The John Innes Foundation, Norwich, 2000.

Metabolite profiles were compared between treatment and control cultures by HPLC and LC/MS. Twenty microliters of each treatment and control extract (n=3) were analyzed by HPLC with diode array detection, and two microliters were evaluated by LC/MS. HPLC was conducted using an Agilent 1100 HPLC system with diode array detector, Agilent Zorbax SB-C18 column (4.1 × 150 mm, 5 um), and a flow rate of 0.75 mL/min. Elution began with a hold at 80/20 H_2O/ACN for 3 min, then a linear gradient from 80/20 to 10/90 H_2O/ACN over the next 22 min, followed by a linear gradient from 10/90 H_2O/ACN to 100% ACN over the next 2 min, and a final hold at 100% ACN for 5 min. Chemical profiles were compared using ChemStation (Agilent). LC/MS analyses were conducted as described elsewhere in Methods.

S4. General Experimental and Instrumentation

I. Reagents, Solvents and Glassware. All small-scale dry reactions were carried out under a blanket of nitrogen, using standard syringe-septum, and cannulation techniques.[5] AIBN was recrystallized from ether and stored at 0-5 °C in an amber bottle. Dry THF was obtained by distillation over sodium-benzophenone ketyl. Dry triethyl amine and diisobutyl ethyl amine was obtained after distillation over KOH. Dry dichloromethane and dry DMF were prepared by distilling over calcium hydride. Anhydrous ether and hexanes were obtained from an m-Braun solvent purification system (charged with A2 alumina as a desiccant).[6] All other solvents were purified according to specific literature procedures.[7]

[5] Pirrung, M. C.; Chapter 8: Conducting the Reaction Itself, *The Synthetic Organic Chemist's Companion*, John Wiley & Sons Inc., Hoboken, NJ, 2007, 69-91.

[6] Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K. Timmers, F. J. Safe and Convenient Procedure for Solvent Purification. *Organometallics*, **1996**, *15*, 1518 -1520.

II. Chromatography. Analytical thin-layer chromatography (TLC) was performed with silica Gel 60 Å (230-400 mesh) specifically to monitor the progress of each chemical reaction and used as a guide for purification of the ensuing mixtures. These were conducted on glass plates (7.5 x 2.5 and 7.5 x 5.0 cm) coated with silica gel G containing 13% calcium sulphate as binder or on pre-coated 0.2 mm thick 60 F_{254} silica plates and various combinations of ethyl acetate and hexane were used as eluent. Visualization of spots after TLC was accomplished by exposure to iodine vapour and/or UV light (254 nm). All compounds were purified using flash column chromatography[8] (Silica gel grade: 200-400 mesh, 40-63 μm) at medium pressure (20 psi). Preparatory thin-layer chromatography (TLC) (to obtain purified compounds) for select products were performed on glass plates (7.5 x 2.5 and 7.5 x 5.0 cm) coated with 60 Å silica gel. Yields refer to compounds isolated to analytical purity after chromatography.

III. Structural Characterization of Synthetic and Biosynthetic Intermediates. NMR spectroscopic analyses (^{1}H NMR, ^{13}C NMR) were conducted for all new compounds. ^{1}H (400 MHz) and ^{13}C (100 MHz) spectra were recorded on a 400 MHz spectrometer, with the exception of a few compounds recorded on a 600 MHz spectrophotometer (^{1}H: 600 MHz and ^{13}C: 150 MHz). Pertinent frequency is specifically reported for each compound. Chemical shift values (δ) for NMR spectra are reported in parts per million (ppm) relative to the residual (indicated) solvent peak ($CDCl_3$ or CD_3OD). Additional peaks other than the compound in question, if any, are calibrated based on reported values for trace impurities.[9] Coupling constants are reported in

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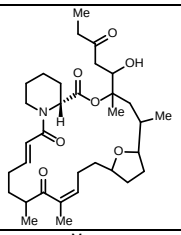
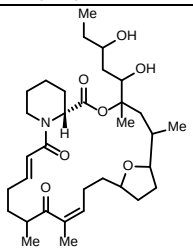
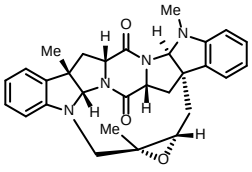
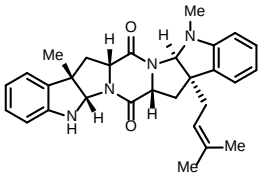
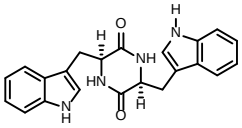
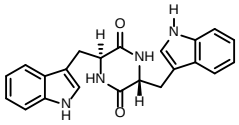
[7] Armarego, W. L. F.; Chai, C. L. L.; *Purification of Laboratory Chemicals*, 5th Ed. Elsevier Butterworth-Heinemann, 2003.

[8] Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.* **1978**, *43*, 2923-2925.

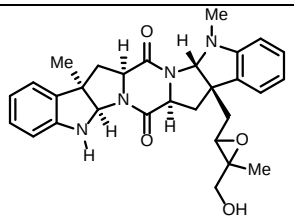
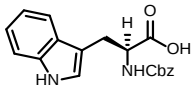
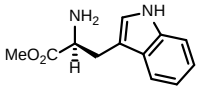
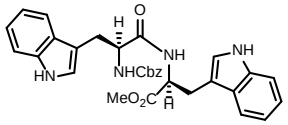
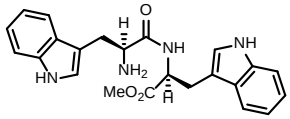
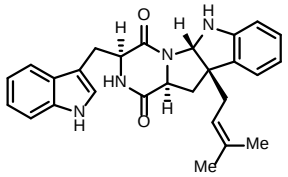
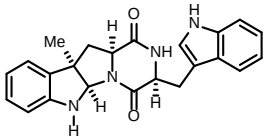
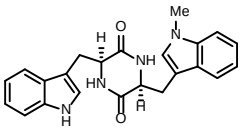
[9] (a). Gottileb, H. E.; Kotlyar, V.; Nudelman, A. NMR Chemical Shifts of Common Laboratory Solvents as Tace Impurities *J. Org. Chem.* **1997**, *62*, 7512-7515 and (b). Fulmer, G. R.; Miller, A. J. M.; Sherden, N. H.; Gottileb, H. E.; Nudelman, A.; Stoltz, B. M.; Bercaw, J. E.; Goldberg, K. I. NMR Chemical Shifts of Trace Impurities: Common

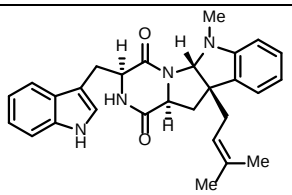
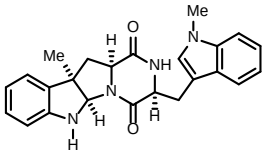
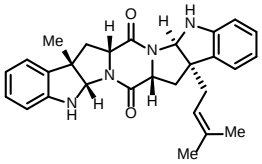
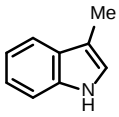
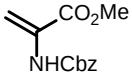
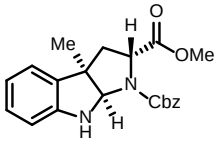
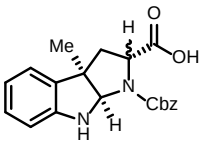
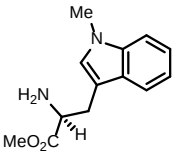
Hz. Data for ^{1}H NMR are reported as follows: chemical shift (δ, ppm), multiplicity (s = singlet, brs = broad singlet, d = doublet, t = triplet, q = quartet, ddd = double double doublet, m = multiplet, cm = complex multiplet), integration corresponding to the number of protons followed by coupling constants in Hz. For ^{13}C NMR spectra, the nature of the carbons (C, CH, CH2 or CH3) was determined by recording the Distortionless Enhancement by Polarization Transfer (DEPT) experiment, and notations are provided in parentheses.^{13}C NMR data is reported in parts per million (δ) relative to the residual (indicated) solvent peak. All melting points for solids were determined on a Buchi B-540 instrument and are reported uncorrected. pH determination was performed with a standard pH meter. IR spectra were recorded on a FT-IR spectrophotometer. Chiroptical measurements ($[\alpha]_D$) were obtained on a polarimeter in a 100 × 2 mm cell. Chiral HPLC analyses for enantio-enriched synthetic intermediates were performed using a Shimadzu LC-20-AT Series separations module equipped with Shimadzu SPD-M20A PDA (photo diode array) multiple wavelength detector (180nm-800nm). The overall system, CBM-20 was controlled using LC Solutions software. Raw data was plotted using Origin® software program after exporting absorbance data as an ASCII-formatted file. Analytical separations of enantioenriched mixtures were carried out on Daicel® (normal phase) AS chiral column. High-resolution mass spectrometry (HRMS) data for synthetic compounds reported herein were obtained by direct infusion of methanolic solutions on a HDMS QTOF mass spectrometer. Accurate LC-MS-MS data of biological extracts were recorded with a Waters Acquity I-Class UPLC system and a Waters Synapt G2 HDMS mass spectrometer as described in **Section S10**.

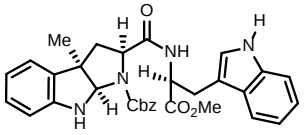
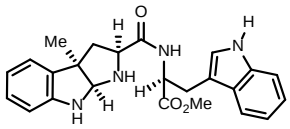
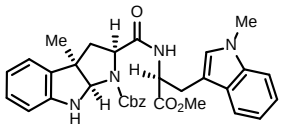
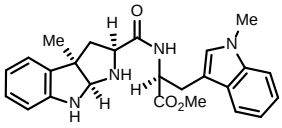
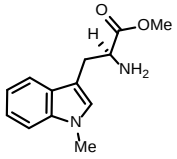
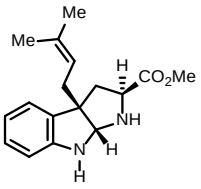
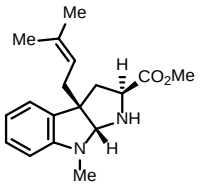
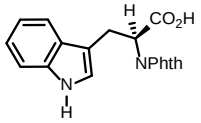
240 **Table S1**[10]. List of chemical structures and corresponding numbers assigned.
241
242

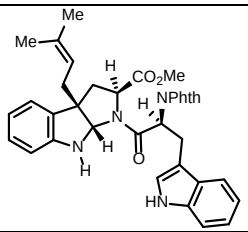
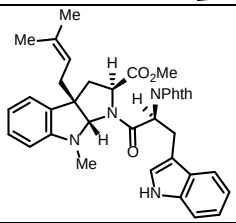
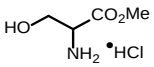
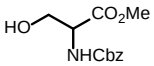
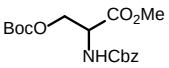
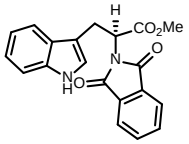
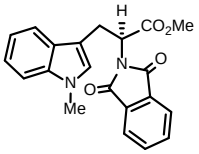
Compound #	Structure	Published / reference name given in this study
1		nocardiopsin A
2		nocardiopsin B
3		nocardioazine A
4		nocardioazine B
5* and (*ent*-5)*		*cyclo*-L-Trp-L-Trp DKP and *cyclo*-D-Trp-D-Trp DKP
6		*cyclo*-L-Trp-D-Trp DKP

[10] Note: Synthetic targets assembled in this study as putative intermediates in nocardioazine biosynthetic pathway are denoted by *.

7		Late stage epoxide intermediate
8 and *ent*-8		N-Cbz-L-Trp-acid or N-Cbz-D-Trp-acid
9 and *ent*-9		L-Trp methyl ester or D-Trp methyl ester
10 and *ent*-10		L-Trp-*N*-Cbz-L-Trp-COOMe-dimer or D-Trp-*N*-Cbz-D-Trp-COOMe-dimer
11 and *ent*-11		L-Trp-L-Trp-COOMe-dimer or D-Trp-D-Trp-COOMe-dimer
12*		*cyclo*-L-Trp- C3′-^{n}prenyl-L-Trp DKP
13*		*cyclo*-C3-Me-L-Trp -L-Trp DKP
14*		*cyclo-N1¢-Me-L-Trp-L-Trp DKP*

15*		*cyclo*-L-Trp-*N*1′-Me-C3′-^{n}prenyl-L-Trp DKP
16*		*cyclo*-C3-Me-L-Trp-*N*1′-Me-L-Trp DKP
17*		Des-*N1′*-Me-nocardioazine B
18		C3-Methyl-indole
19		Methyl 2-(benzyloxy)carbonyl-amino acrylate
20a, b		(2S,3aR,8aR)-1-((benzyloxy)carbonyl)-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carboxylic acid methyl ester
20c		(2S,3aR,8aR)-1-((benzyloxy)carbonyl)-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carboxylic acid
21		*N*-Me-L-Trp-Methyl Ester

22		Benzyl-(2S,3aR,8aR)-2-((3-(1H-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)carbamoyl)-3a-methyl-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate
23		Methyl((2S,3aR,8aS)-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carbonyl)tryptophanate
24		Benzyl-(2S,3aR,8aR)-2-((1-methoxy-3-(1-methyl-1H-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-3a-methyl-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate
25		*N*-methyl-(2S,3aR,8aS)-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carbonyl) methyl-1-tryptophanate
26		*N*-Me-L-Trp-COOMe ester
27a/b		C3′-^{n}prenyl-L-Trp-pyrroloindoline methyl ester
28a/b		*N*1′-Me-C3′-^{n}prenyl-L-Trp-pyrroloindoline methyl ester
29		N1′-phthalyl-L-Trp-acid

30		C3'-^{n}prenyl-pyrroloindoline-methyl-ester-*N*1-phthalyl-L-Trp-amide
31		*N*1'-Me-C3'-^{n}prenyl-pyrroloindoline-methyl-ester-*N*1-phthalyl-L-Trp-amide
32		L-Serine methyl ester hydrochloride
33		*N*-Cbz-L-Serine methyl ester
34		*O*-Boc-*N*-Cbz-L-Serine methyl ester
35		Methyl (S)-2-(1,3-dioxoisoindolin-2-yl)-3-(1H-indol-3-yl)propanoate
36		*N1-Me-N2*-phth-L-Trp-methyl ester

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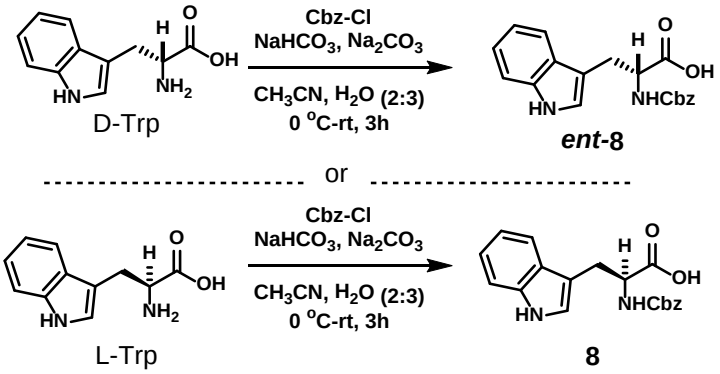
S6. Synthesis of *cyclo*-L-Trp-L-Trp DKP and *cyclo*-D-Trp-D-Trp DKP

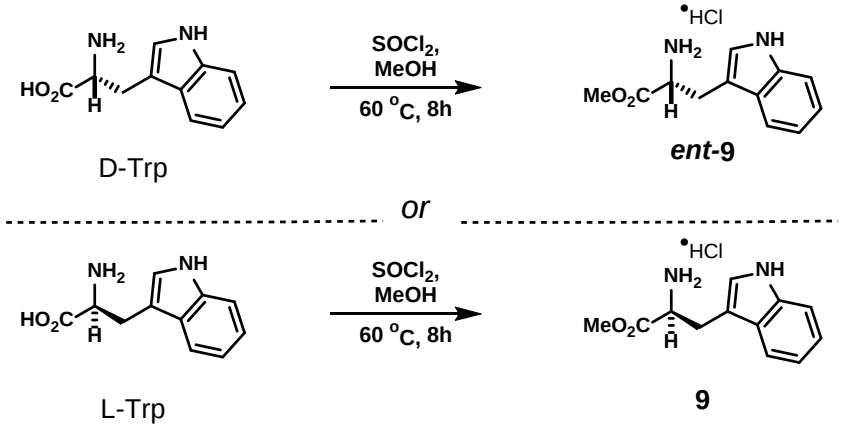


L/D-Trp-*N*-Cbz-carbamate (8 or *ent*-8)[11]

To a clear solution of L/D-Trp (500 mg, 2.45 mmol) in 20 mL of CH_3CN-H_2O (2:3) were added $NaHCO_3$ (308 mg, 3.68 mmol) and Na_2CO_3 (390 mg, 3.68 mmol). The resulting turbid solution (pH = 10~11) was cooled to 0 °C (H_2O/ice bath) and stirred for 15 min. To this mixture was added Cbz-Cl (350 μL, 1.20 mmol) drop wise. The resulting solution was stirred for 15-20 min at 0 °C, the ice bath was removed and reaction was stirred at rt for 3h, at which time no starting material remained (TLC analysis). After acidification by drop wise addition of a 1 N HCl solution and removal of CH_3CN by rotary evaporation, the reaction mixture was transferred to a separatory funnel and washed three times with EtOAc. The combined organic phase was washed with brine, dried over anhyd. Na_2SO_4, and filtered. Concentration under reduced pressure gave 813 mg (98%) of **8** or ***ent*-8** as a colorless powder which was directly used for the next step without further purification. mp: 125-126 °C; IR (KBr): 3413, 3020, 2934, 1702, 1596, 1519, 1415, 1345, 1218, 1137, 1067, 760, 672 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ 7.58 (d, J = 8.0

[11] Shao, Y.-M.; Yang, W.-B.; Peng, H.-P.; Hsu, M.-F.; Tsai, K.-C.; Kuo, T.-H.; Wang, A. H.-J.; Liang, P.-H.; Lin, C.-H.; Yang, A.-S. and Wong, C.-H. *ChemBioChem* **2007**, *8*, 1654–1657.

Hz, 1H), 7.33-7.23 (m, 7H), 7.06 (s, 1H), 7.09-7.02 (m, 1H), 6.95 (ddd, *J* = 7.8, 7.0, 0.8 Hz, 1H), 5.04 (AB, *J* = 12.5 Hz, 1H), 4.98 (AB, *J* = 12.5, Hz, 1H), 4.42 (dd, *J* = 7.4, 4.8 Hz, 1H), 3.36 (ABX, *J* = 14.6, 4.8 Hz, 1H), 3.14 (ABX, *J* = 14.6, 7.5 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD, DEPT): δ 158.1 (C), 138.3 (C), 137.9 (C), 129.4 (2 * CH), 129.3 (C), 129.2 (C), 128.8 (CH), 128.7 (CH), 124.4 (CH), 122.1 (CH), 119.6 (CH), 119.5 (2 * CH), 112.1 (CH), 111.7 (C), 67.3 (CH_2), 57.8 (CH), 29.2 (CH_2). HRMS (EI, M^+): m/z calcd. for $C_{19}H_{18}O_4N_2$ 338.1267, found 338.1262.

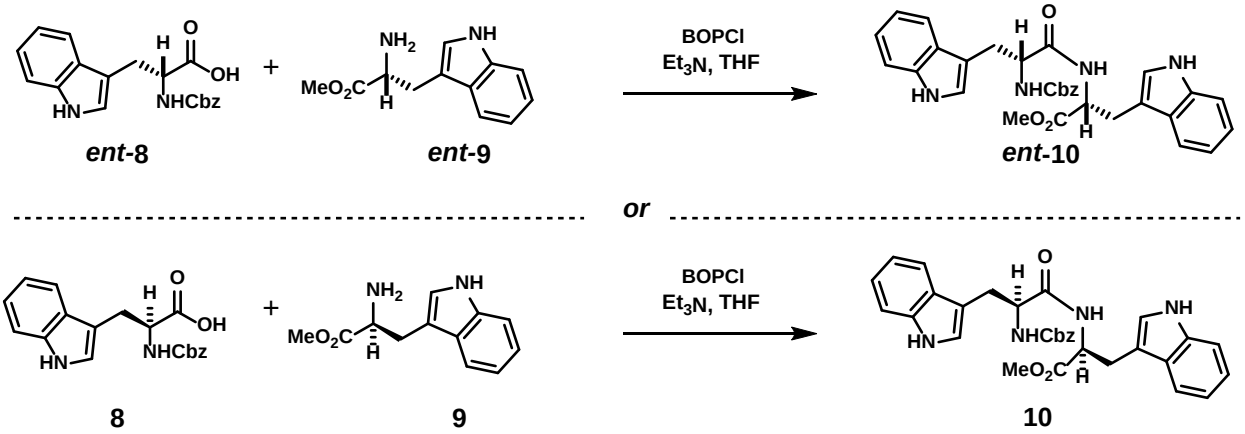


L/D-Trp methyl ester hydrochloride (9 or *ent*- 9)[12]

Thionyl chloride (7.15 mL, 98 mmol) was added drop wise to a cold (0 °C) solution of anhydrous methanol (220 mL) under magnetic stirring. The solution was stirred at 0 °C for 30

[12] This compound displayed satisfactory characterization data as published in the literature and was used without further purification or recrystallization: (a) Isaacs, N. S. and Coulson, M. Effect of pressure on processes modelling the Maillard reaction *J. Phys. Org. Chem.* **1996**, *9*, 639-644. (b) Robaa, D.; Enzensperger, C.; AbulAzm, S. E.; Hefnawy, M. M.; El-Subbagh, H. I.; Wani, T. A. and Lehmann, J. Chiral Indolo[3,2-f][3]benzazecine-Type Dopamine Receptor Antagonists: Synthesis and Activity of Racemic and Enantiopure Derivatives *J. Med. Chem.* **2011**, *54*, 7422–7426.

min. and then L/D-Trp (8.00 g, 39.2 mmol) was added and the resulting solution was heated at 60 °C for 18 h. After evaporation of the solvent, a white residue of hydrochloride salt was obtained, which was neutralized by a satd. Na_2CO_3 solution (25 mL) and the ester was extracted with equal volume of ethyl acetate three times. The organic layer was dried over anhyd. Na_2SO_4 and evaporated under reduced pressure yielding pale yellow oil, which solidified upon standing to a pale yellow crystalline solid. Yield: 8.48 g (99%, 38.8 mmol) of L/D-Trp methyl ester (**9** or *ent*-**9**). mp: 91-93 °C IR (KBr): 3386, 3017, 1730, 1582, 1441, 1351, 1215, 1101, 1014, 758, 665 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.27 (brs, 1H), 7.62 (d, *J* = 7.9 Hz, 1H), 7.35 (td, *J* = 8.1, 0.9 Hz, 1H), 7.20 (ddd, *J* = 8.2, 7.1, 1.2 Hz, 1H), 7.13 (ddd, *J* = 8.0, 7.1, 1.1 Hz, 1H), 7.04 (d, *J* = 2.3 Hz, 1H), 3.85 (dd, *J* = 7.7, 4.8 Hz, 1H), 3.72 (s, 3H), 3.29 (ABXY, *J* = 14.4, 4.8, 0.8 Hz, 1H), 3.06 (ABXY, *J* = 14.4, 7.7, 0.4 Hz, 1H), 1.60 (brs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 175.9 (C), 136.4 (C), 127.6 (C), 123.1 (CH), 122.3 (CH), 119.6 (CH), 118.7 (CH), 111.4 (CH), 111.2 (C), 55.1 (CH), 52.2 (CH_3), 30.8 (CH_2). HRMS (EI, M^+): m/z calcd. for $C_{12}H_{14}O_2N_2$ 218.1055, found 218.1055.

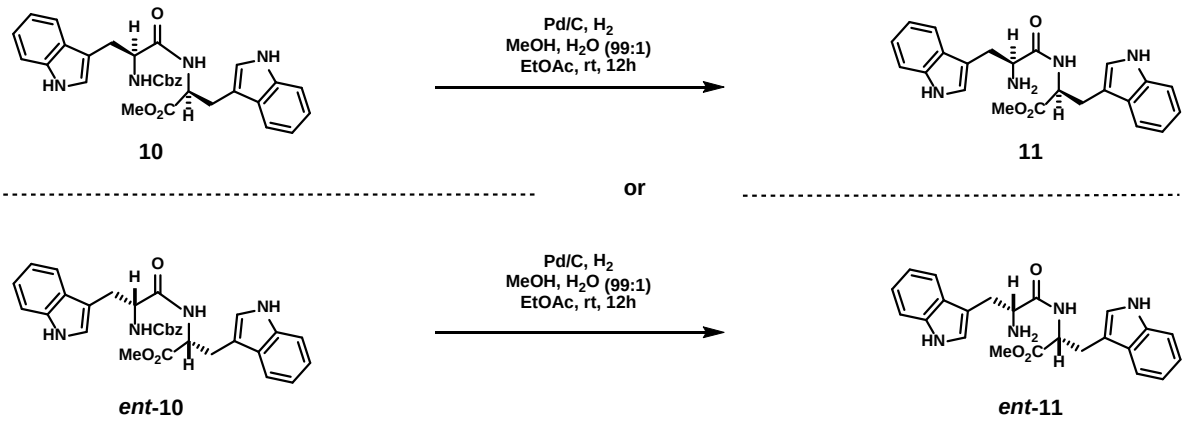


***ent*-8** ***ent*-9** ***ent*-10**

8 **9** **10**

Methyl ((benzyloxy)carbonyl)-L/D-tryptophyl-L/D-tryptophanate (10 or ***ent*-10)**

To a cold (−10 °C) magnetically stirred solution of *N*-Cbz-acid **8** or ***ent*-8** (400 mg, 1.18 mmol) with D/L-Trp-methyl ester (***ent*-9** or **9**) (284.0 mg, 1.30 mmol) in dry THF (5.0 mL) was added Et_3N (0.66 mL, 4.73 mmol) followed by BOP-Cl (903 mg, 3.55 mmol) and the resulting mixture was stirred at same temperature overnight and was then quenched by addition of water (20 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic layer was washed with brine and dried (anhyd. Na_2SO_4). Evaporation of the solvent under reduced pressure and purification of the residue on a silica gel column using ethyl acetate–hexanes (1:1) as eluent furnished the coupled-product **10** or ***ent*-10** as a colorless dense liquid in 93% yield (595 mg, 1.11 mmol). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.80 (brs, 2H), 7.67 (d, *J* = 8.2 Hz, 1H), 7.37-7.27 (m, 7H), 7.25 (d, *J* = 8.4 Hz, 1H), 7.20 (t, *J* = 7.3 Hz, 1H), 7.17-7.08 (m, 2H), 6.93 (t, *J* = 7.3 Hz, 1H), 6.89 (s, 1H), 6.55 (s, 1H), 6.15 (d, *J* = 7.3 Hz, 1H), 5.42 (d, *J* = 7.6 Hz, 1H), 5.08 (s, 2H), 4.79 (td, *J* = 7.8, 5.4 Hz, 1H), 4.51 (q, *J* = 4.4 Hz, 1H), 3.61 (s, 3H), 3.35 (ABX, *J* = 13.7, 3.0 Hz, 1H), 3.21-3.05 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 171.9 (C), 171.1 (2 × C), 136.2 (C), 136.1 (C), 128.6 (4 × CH), 128.3 (CH), 128.1 (2 × CH), 127.4 (CH), 123.7 (CH), 123.1 (C), 122.3 (C), 122.2 (2 × CH), 119.9 (CH), 119.7 (CH), 119.0 (C), 118.5 (CH), 111.4 (CH), 111.3 (CH), 109.4 (CH), 67.0 (CH_2), 55.5 (CH_3), 52.8 (CH) , 52.5 (CH), 28.6 (CH_2), 27.5 (CH_2). HRMS (EI, M^+): m/z calcd. for $C_{31}H_{30}O_5N_4$ 538.2216, found 538.2182.



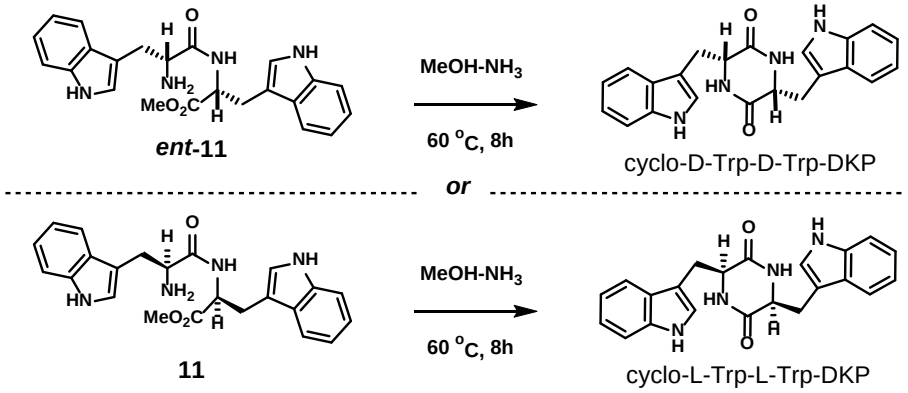
307

308

309 **Methyl L/D-tryptophyl-L/D-tryptophanate (11** or ***ent*-11)**

310 To a homogenous solution (under stirring with a magnetic bar) of coupling compound **10** or ***ent*-**
311 **10** (235 mg, 0.44 mmol) in MeOH and ethyl acetate (1:1) was added 10% palladium on activated
312 charcoal (5 mg, 0.04 mmol) and the reaction mixture stirred under hydrogen at 1 atm for 7 h. The
313 solution was then filtered over a pad of celite and washed with ethyl acetate. Evaporation of the
314 solvent under reduced pressure yielded **11** or ***ent*-11** (98%, 173 mg, 0.43 mmol) in sufficiently
315 pure form as an off-white solid and was subjected to the next step. mp: 207 °C. IR (KBr): 3407,
316 3018, 2938, 1723, 1672, 1515, 1350, 1221, 1060, 757, 678 cm^{-1}. ^{1}H NMR (400 MHz, CD_3OD):
317 δ 7.89 (s, 2H), 7.59 (td, *J* = 8.0, 1.0 Hz, 1H), 7.34 (td, *J* = 8.1, 1.0 Hz, 1H), 7.29 (td, *J* = 8.1, 1.0
318 Hz, 1H), 7.24 (td, *J* = 8.0, 1.0 Hz, 1H), 7.10 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 7.07 (s, 1H), 7.06-
319 6.99 (m, 2H), 6.89 (ddd, *J* = 8.0, 7.0, 1.0 Hz, 1H), 6.85 (s, 1H), 4.72 (t, *J* = 6.0 Hz, 1H), 3.61 (s,
320 3H), 3.59 (dd, *J* = 6.8, 5.6 Hz, 1H), 3.13 (ABXY, *J* = 14.6, 6.6, 0.6 Hz, 1H), 3.09 (ABXY, *J* =
321 14.6, 5.6, 0.6 Hz, 1H), 3.03 (ABXY, *J* = 14.6, 5.6, 0.6 Hz, 1H), 2.98 (ABXY, *J* = 14.2, 6.6, 0.6
322 Hz, 1H). ^{13}C NMR (100 MHz, CD_3OD, DEPT): δ 177.1 (C), 173.7 (C), 138.1 (C), 137.9 (C),
323 128.9 (C), 128.6 (C), 124.9 (CH), 124.5 (CH), 122.5 (CH), 122.4 (CH), 119.8 (CH), 119.8 (CH),

119.5 (CH), 119.2 (CH), 112.3 (CH), 112.3 (CH), 110.9 (C), 110.1 (C), 56.4 (CH), 54.4 (CH), 52.7 (CH_3), 31.6 (CH_2), 28.4 (CH_2). HRMS (EI, $[M\text{-}NH_3]^+$): m/z calcd. for $C_{23}H_{21}O_3N_3$ 387.1583, found 387.1585.

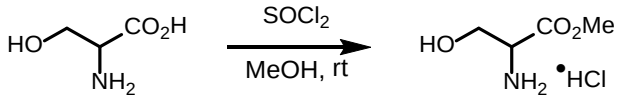


***cyclo*-L/D-Trp-L/D-Trp DKP (5** or *ent*-**5)**

A homogenous solution (under stirring with a magnetic bar) of amine **11** or ***ent*-11** (396 mg, 0.98 mmol) was refluxed overnight in 14 M methanolic ammonia (15.0 mL). Evaporation of the solvent under reduced pressure and washing of the resulting residue with chloroform furnished the pure diketopiperazine **5** or ***ent*-5** (345 mg, 95% yield, 0.93 mmol) as a pale yellow solid. mp: 242 ºC. IR (KBr): 3409, 3326, 3018, 2926, 2481, 1659, 1536, 1453, 1336, 1225, 1088, 1018, 932, 758, 669 cm^{-1}. ^1H NMR (400 MHz, CD_3OD): δ 7.45 (td, *J* = 8.0, 1.0 Hz, 2H), 7.30 (td, *J* = 8.0, 1.0 Hz, 2H), 7.09 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 2H), 7.01 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 2H), 6.46 (s, 2H), 4.04 (dd, *J* = 6.7, 3.9 Hz, 2H), 2.92 (dd, *J* = 14.4, 3.8 Hz, 2H), 2.17 (dd, *J* = 14.4, 7.2 Hz, 2H). ^{13}C NMR (100 MHz, CD_3OD, DEPT): δ 169.7 (2 × C), 138.0 (2 × C), 128.6 (2 × C), 125.9 (2 × CH), 122.5 (2 × CH), 120.1 (2 × CH), 119.7 (2 × CH), 112.4 (2 × CH), 109.4 (2 × C), 56.8 (2 × CH), 31.4 (2 × CH_2). HRMS (EI, M^+): m/z calcd. for $C_{22}H_{20}O_2N_4$ 372.1586, found 372.1595. $[\alpha]^{21}{}_D$ − 52 (*c* 0.05, MeOH) for *cyclo*-L-Trp-L-Trp DKP (**5**) and $[\alpha]^{21}{}_D$ + 52 (*c*

0.05, MeOH) for *cyclo*-D-Trp-D-Trp DKP (***ent*-5**)[13]. HPLC of individual enantiomer provided below in Fig. S14 and Fig S15.

S7. Asymmetric Synthesis of *cyclo*-C3-Me-L-Trp-L-Trp DKP (13)

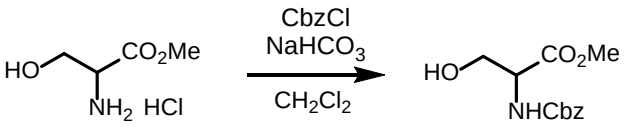


L-Serine **(32)**

L-serine methyl ester hydrochloride[14] (32)

Thionyl chloride (3.79 mL, 52.0 mmol) was added drop wise to a cold (0 ^{o}C) solution of anhydrous methanol (50 mL) under magnetic stirring. The solution was stirred at 0 ^{o}C for 30 min and then *L*-Serine (5.0 g, 47.6 mmol) was added. The reaction mixture was stirred at room temperature for 24 h and TLC analysis ($CHCl_3$/CH_3OH, 9:1) indicated complete disappearance of L-serine. The reaction mixture was evaporated under reduced pressure and the residue was triturated with petroleum ether (~5 times) to provide 7.2 g (98%, 46.6 mmol) of *L*-serine methyl ester hydrochloride salt (**32**) as a colorless powder which was directly used in the subsequent step without further purification. mp: 161–162 ^{o}C; IR (KBr): 3402, 3024, 2951, 2691, 1739, 1625, 1524, 1448, 1244, 1072, 893 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.13 (t, J = 3.8 Hz, 1H), 4.01 (ABX, J = 11.9, 4.4 Hz, 1H), 3.91 (ABX, J = 11.9, 3.4 Hz, 1H), 3.85 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 169.3 (C), 60.6 (CH_2), 56.1 (CH), 53.8 (CH_3). HRMS (ESI, M+H^{+}): m/z calcd. for $C_4H_{10}O_3N$ 120.0655, found 120.0660.

[13] Data matched published report: Raju, R., Piggott, A. M., Huang, X.-C., and Capon, R. J. Nocardioazines: A Novel Bridged Diketopiperazine Scaffold from a Marine-Derived Bacterium Inhibits P-Glycoprotein, *Org. Lett.* **2011**, *13*, 2770-2773.
[14] Gu, K.; Bi, L.; Zhao, M.; Wang, C.; Ju, J. and Peng, S. *Bioorg. Med. Chem.* **2007**, *15*, 6273–6290.



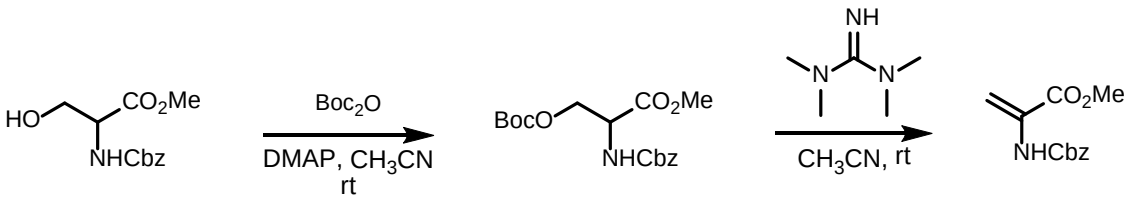
(32) **(33)**

359

360 ***N*-Cbz-*L*-serine methyl ester** (**33**)

361 *L*-serine methyl ester hydrochloride (4.0 g, 25.7 mmol) was dissolved in a mixture of saturated
362 $NaHCO_3$ (11.0 g in 50 mL H_2O) and CH_2Cl_2 (70.0 mL). To this solution, benzyl chloroformate
363 (3.85 mL, 27.0 mmol) was added at 0 ^oC and the reaction mixture was stirred for 6 h at rt. After
364 quenching the reaction with 1.0 M aqueous HCl at 0 ^oC, the organic layer was washed with water
365 and brine and dried over anhyd. Na_2SO_4. The solvent was evaporated under reduced pressure,
366 and the resultant residue was purified by column chromatography (EtOAc/hexane 1:1) to yield
367 *N*-Cbz-*L*-serine methyl ester (**33**) (6.3 g, 24.9 mmol, 97% yield) as colorless oil. IR (KBr): 3393,
368 3024, 2951, 1715, 1532, 1443, 1342, 1224, 1066, 893 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.40-
369 7.27 (m, 5H), 5.75 (d, *J* = 6.56 Hz, 1H), 5.12 (s, 2H), 4.45 (dd, *J* = 7.80, 3.68 Hz, 1H), 5.05-3.85
370 (m, 2H), 3.78 (s, 3H), 2.35 (t, *J* = 5.76 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 171.1
371 (C), 156.4 (C), 136.1 (C), 128.6 (2 × CH), 128.4 (2 × CH), 128.2 (CH), 67.3 (CH_2), 63.3 (CH_2),
372 56.1 (CH), 52.9 (CH_3). HRMS (EI, M^+): m/z calcd. for $C_{12}H_{15}O_5N$ 253.0950, found 253.0954.

373



(33) **(34)** **(19)**

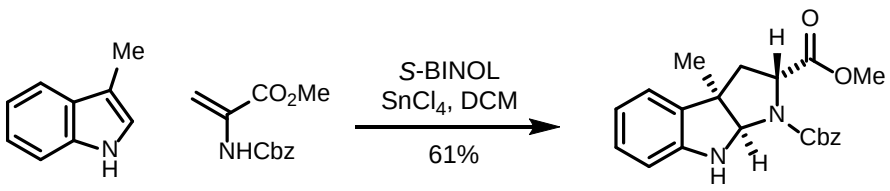
374

375

376 **Methyl 2-(benzyloxy)carbonyl-amino acrylate (19)**

To a homogenous solution (under stirring) of (6.3 g, 24.8 mmol) *N*-Cbz-L-serine methyl ester (**33**) in dry CH_3CN (50 mL) was added DMAP (0.5 g, 4.1 mmol) followed by di-*tert*-butyl dicarbonate (5.16 g, 23.6 mmol) under rapid stirring at room temperature. The reaction was monitored by TLC (diethyl ether/*n*-hexane, 1:1) until all the reactants had been consumed. Tetra-methyl guanidine (1.57 mL, 12.4 mmol) was added at room temperature and the reaction mixture was further stirred overnight. Evaporation of the solvent at reduced pressure gave a residue that was partitioned between diethyl ether (100 mL) and water. The organic phase was washed with brine and dried (anhyd. Na_2SO_4). The solvent was evaporated under reduced pressure and the residue was purified by column chromatography to yield the pure olefin product (**19**) (3.03 g, 12.9 mmol, 52% yield) as a colorless liquid. IR (KBr): 3402, 3024, 2951, 2691, 1719, 1640, 1522, 1448, 1320, 1210, 1072, 893 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.40-7.29 (m, 5H), 6.27 (s, 1H), 5.80 (d, *J* = 1.5 Hz, 1H), 5.17 (s, 2H), 3.81 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 164.2 (C), 153.1 (C), 135.8 (C), 131.0 (C), 128.6 (2 × CH), 128.4 (CH), 128.3 (2 × CH), 106.1 (CH_2), 67.1 (CH_2), 53.0 (CH_3). HRMS (EI, M^+): m/z calcd. for $C_{12}H_{13}O_4N$ 235.0845, found 235.0845.

The intermediate *O*-Boc-*N*-Cbz-L-serine methyl ester (**34**) was characterized as a colorless liquid. IR (KBr): 3366, 2972, 1735, 1524, 1453, 1366, 1274, 1165, 1075, 853 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.38-7.28 (m, 5H), 5.61 (d, *J* = 8.3 Hz, 1H), 5.12 (s, 2H), 4.61 (dd, *J* = 8.4, 3.6 Hz, 1H), 4.48 (ABX, *J* = 11.2, 3.6 Hz, 1H), 4.34 (ABX, *J* = 11.2, 3.6 Hz, 1H), 3.78 (s, 3H), 1.46 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 169.9 (C), 155.8(C), 153.1 (C), 136.1 (C), 128.6 (2 × CH), 128.3 (CH), 128.2 (2 × CH), 83.0 (C), 67.3 (CH_2), 66.2 (CH_2), 53.5 (CH), 53.0 (CH_3), 27.7 (3 × CH_3). HRMS (ESI, $M+H^+$): m/z calcd. for $C_{17}H_{24}O_7N$ 354.1547, found 354.1552.



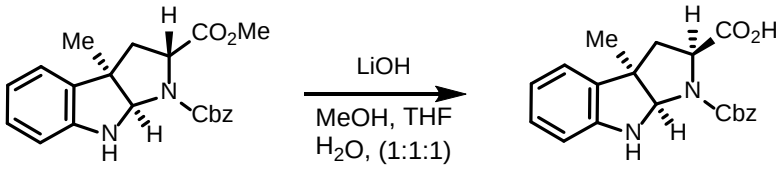
(**18**) (**19**) (**20a**)

1-benzyl-2-methyl-(2R,3aR,8aR)-3a-methyl-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1,2(2H)-dicarboxylate (20a)

Enantioselective formation of **20a** followed literature procedure reported by Repka et al.[15] Accordingly, to a homogenous solution (under stirring) of 3-methyl indole (0.9 g, 6.86 mmol) in dry CH_2Cl_2 (40.0 mL) was added (*S*)–BINOL (0.393 g, 1.37 mmol) and methyl 2-(benzyloxy)carbonyl-amino acrylate (**19**) (1.61 g, 6.86 mmol) followed by slow addition of $SnCl_4$ (1.2 equiv. in 1.0 M CH_2Cl_2) at room temperature over a period of 30 minutes and stirring was continued for 4 h. To the reaction mixture 1M HCl was added and the organic layers extracted with CH_2Cl_2. The organic phase was washed with brine and dried (anhyd. Na_2SO_4). The solvent was evaporated under reduced pressure and the residue was purified by column chromatography to yield the cyclic product **20** in 61% yield (1.54 g, 4.20 mmol). The product was detected to be present as a mixture of rotational isomers in 3:2 ratio as indicated by ^{1}H NMR signals. IR (KBr): 3396, 3034, 2950, 1743, 1703, 1607, 1448, 1454, 1416, 1344, 1271, 1207, 1172, 1127, 1062, 1001, 959, 917, 818, 750, 696 cm^{-1}. **Data for major isomer:** ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.43-7.28 (m, 5H), 7.12-7.03 (m, 2H), 6.81-6.74 (m, 1H), 6.64 (d, *J* = 7.7 Hz, 1H), 5.41 (brs, 1H), 5.27 (s, 1H), 5.19 (AB, *J* = 12.2 Hz, 1H), 4.93 (AB, *J* = 12.2 Hz, 1H), 4.11

[15] Repka, L. M.; Ni, J.; Reisman, S. E. *J. Am. Chem. Soc.* **2010**, *132*, 14418-20.

(t, *J* = 7.8 Hz, 1H), 3.47 (s, 3H), 2.65 (ABX, *J* = 12.9, 7.7 Hz, 1H), 2.16 (ABX, *J* = 12.9, 8.4 Hz, 1H), 1.40 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 173.1 (C), 154.3 (C), 147.9 (C), 135.8 (C), 133.3 (C), 128.8 (CH), 128.4 (2 * CH), 128.2 (CH), 128.0 (2 * CH), 122.4 (CH), 119.3 (CH), 109.9 (CH), 83.5 (CH), 67.3 (CH_2), 59.4 (CH), 52.2 (CH_3), 52.1 (C), 41.7 (CH_2), 24.1 (CH_3). HRMS (EI, M^+): m/z calcd. for $C_{21}H_{22}O_4N_2$ 366.1580, found 366.1580.



(20a) **(20c)**

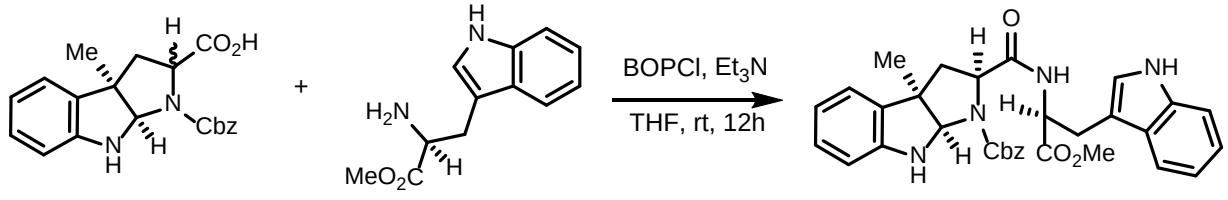
(2S,3aR,8aR)-1-((benzyloxy)carbonyl)-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carboxylic acid (20c)

To a homogenous solution (under stirring with a magnetic bar) of *N*-Cbz-methyl ester **20a** (373 mg, 1.02 mmol) in MeOH (7.0 mL) and THF (7.0 mL) was added aqueous solution of LiOH (98.0 mg, 4.0 mmol in 7.0 mL H_2O) and the reaction mixture stirred at room temperature overnight. It was then quenched with 1N HCl at 0 °C till the pH is between 4-5 followed by extraction with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine and dried (anhyd. Na_2SO_4). Evaporation of the solvent under reduced pressure and purification of the residue on a silica gel column using ethyl acetate–hexanes (1:1) as eluent furnished the acid **20c** in 69% yield (241 mg, 0.69 mmol).

A similar prep was executed for synthesis of 20c on a gram-scale: To a magnetically stirred solution of N-Cbz-methyl ester **20a** (1.0 g, 2.73 mmol) in MeOH (10.0 mL) and THF (10.0 mL)

was added excess aqueous solution of LiOH (656.0 mg, 10.0 mmol in 10.0 mL H_2O) and the reaction mixture stirred at room temperature overnight. It was then quenched with 1N HCl at 0 °C till the pH of solution become 4-5 and extracted with ethyl acetate (3 × 50 mL). The combined organic layer was washed with brine and dried (anhyd. Na_2SO_4). Evaporation of the solvent under reduced pressure and purification of the residue on a silica gel column using ethyl acetate–hexanes (1:1) as eluent furnished the acid **20c** in 50% yield (480 mg, 1.36 mmol). Pale yellow liquid.

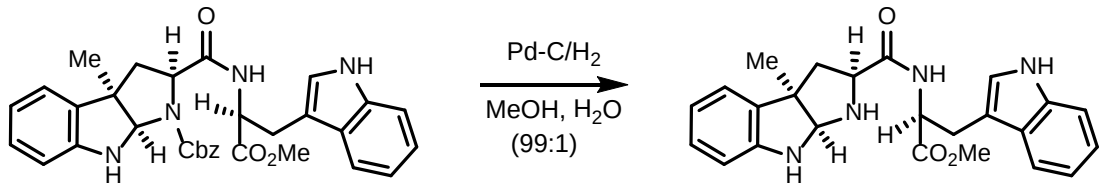
IR (KBr): 3391, 2961, 1701, 1608, 1462, 1414, 1354, 1317, 1205, 1157, 1127, 1050, 1016, 978, 917, 746 cm^{-}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.45-7.36 (m, 2H), 7.33-7.20 (m, 3H), 7.13-7.03 (m, 2H), 6.82-6.75 (m, 1H), 6.65 (d, *J* = 7.7 Hz 1H), 5.28 (s, 1H), 5.14 (AB, *J* = 12.4 Hz, 1H), 5.01 (AB, *J* = 12.4 Hz, 1H), 4.18 (t, *J* = 8.0 Hz, 1H), 2.72 (ABX, *J* = 13.0, 8.1 Hz, 1H), 2.22 (ABX, *J* = 13.0, 8.0 Hz, 1H), 1.41 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 178.3 (C), 154.5 (C), 147.9 (C), 135.9 (C), 133.3 (C), 129.0 (CH), 128.5 (2 × CH), 128.2 (CH), 127.7 (2 × CH), 122.5 (CH), 119.4 (CH), 110.1 (CH), 83.8 (CH), 67.5 (CH_2), 59.2 (CH), 52.3 (C), 41.8 (CH_2), 24.2 (CH_3). HRMS (ESI, M^{+}): m/z calcd. for $C_{20}H_{20}N_2O_4$ 352.1423, found 352.1428.



(20c) **(9)** **(22)**

Benzyl-(2S,3aR,8aR)-2-((3-(1H-indol-3-yl)-1-methoxy-1-oxopropan-2-yl)carbamoyl)-3a-methyl-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (22)

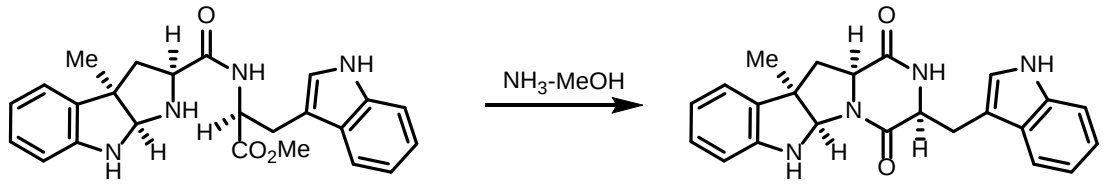
To a homogenous solution of *N*-Cbz-acid (**20c**) (218.0 mg, 0.62 mmol) and L-Trp-methyl ester hydrochloride (**9**) (151.0 mg, 0.69 mmol) in dry THF (4.0 mL) (under stirring with a magnetic bar) at −10 °C was added Et_3N (0.38 mL, 2.75 mmol) followed by BOP-Cl (395.0 mg, 1.55 mmol) and the resulting mixture was stirred at −10 °C overnight. It was then quenched with water (5.0 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layers were washed with brine and dried (anhyd. Na_2SO_4). Evaporation of the solvent under reduced pressure and purification of the residue on a silica gel column using ethyl acetate–hexanes (1:1) as eluent furnished the coupled product (**22**) as a colorless dense liquid in 90% yield (306 mg, 0.55 mmol). IR (KBr): 3359, 3012, 2960, 2927, 1684, 1612, 1519, 1418, 1349, 1216, 1155, 1127, 748 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 8.54 (brs, 1H), 7.44 (d, *J* = 7.8 Hz, 1H), 7.38 (brs, 2H), 7.32 (d, *J* = 8.0 Hz, 1H), 7.30-6.95 (m, 8H), 6.84 (d, *J* = 2.2 Hz, 1H), 6.76 (t, *J* = 7.4 Hz, 1H), 6.60 (d, *J* = 7.8 Hz, 1H), 5.40 (brs, 1H), 5.11 (s, 1H), 4.97 (AB, *J* = 12.3 Hz, 1H), 4.88 (AB, *J* = 12.3 Hz, 1H), 4.73 (q, *J* = 5.6 Hz, 1H), 3.93 (t, *J* = 7.8 Hz, 1H), 3.62 (s, 3H), 3.31 (dd, *J* = 5.4, 2.3 Hz, 1H), 3.17 (t, *J* = 6.0 Hz, 1H), 2.41 (dd, *J* = 13.0, 7.8 Hz, 1H), 2.09 (dd, *J* = 13.0, 8.0 Hz, 1H), 1.26 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 172.0 (C), 171.9 (C), 154.6 (C), 147.8 (C), 136.2 (C), 135.9 (C), 133.7 (C), 128.9 (CH), 128.4 (2 * CH), 128.1 (CH), 127.9 (2 * CH), 127.6 (C), 123.0 (CH), 122.4 (CH), 122.3 (CH), 119.7 (CH), 119.2 (CH), 118.4 (CH), 111.5 (CH), 109.7 (CH), 109.5 (C), 84.1 (CH), 67.2 (CH_2), 61.4 (CH), 52.9 (C), 52.3 (CH), 51.9 (CH_3), 42.2 (CH_2), 27.4 (CH_2), 23.8 (CH_3). HRMS (ESI, M^+): m/z calcd. for $C_{32}H_{32}O_5N_4$ 552.2373, found 552.2367.



(22) **(23)**

Methyl((2S,3aR,8aS)-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carbonyl)tryptophanate (23)

To a homogenous solution (under stirring) of coupling compound **22** (95.0 mg, 0.188 mmol) in MeOH and ethyl acetate (1:1) was added 10% palladium on activated charcoal (18 mg, 0.017 mmol) and the reaction mixture stirred under hydrogen at 1 atm. for 7h. It was then filtered with celite pad and washed with ethyl acetate. Evaporation of the solvent under reduced pressure yielded 97% (70.0 mg, 0.167 mmol) of product **23** which was subjected for the next step without any purification. IR (KBr): 3359, 3012, 2960, 2927, 1684, 1519, 1418 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (d, *J* = 7.9 Hz, 1H), 7.38 (d, *J* = 8.1 Hz, 1H), 7.19-7.00 (m, 5H), 6.82 (t, *J* = 7.4 Hz, 1H), 6.68 (d, *J* = 7.9 Hz, 1H), 5.20 (s, 1H), 4.84 (dd, *J* = 9.6, 5.0 Hz, 1H), 3.84 (dd, *J* = 11.9, 5.9 Hz, 1H), 3.70 (s, 3H), 3.35 (ABX, *J* = 14.6, 4.8 Hz, 1H), 3.08 (ABX, *J* = 14.6, 9.6 Hz, 1H), 2.23 (dd, *J* = 13.2, 6.0 Hz, 1H), 1.62 (t, *J* = 12.8 Hz, 1H), 1.30 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 173.2 (C), 168.3 (C), 149.2 (C), 137.9 (C), 133.8 (C), 130.0 (CH), 128.7 (C), 124.6 (CH), 124.0 (CH), 122.4 (CH), 121.3 (CH), 119.9 (CH), 119.2 (CH), 112.4 (CH), 110.8 (C), 110.4 (CH), 85.2 (CH), 59.5 (CH), 55.5 (C), 54.8 (CH), 52.9 (CH_3), 44.1 (CH_2), 28.5 (CH_2), 25.2 (CH_3). HRMS (ESI, M^+): m/z calcd. for $C_{24}H_{26}O_3N_4$ 418.2005, found: 418.2008.

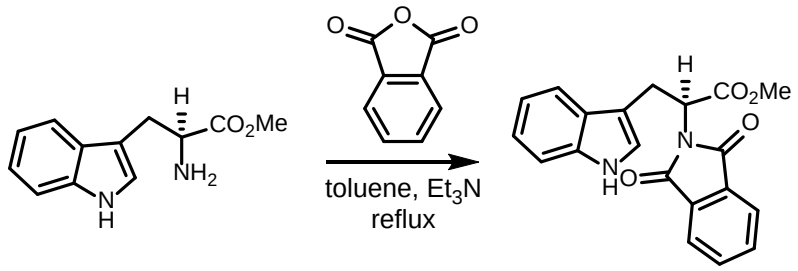


(23) **(13)**

***Cyclo*-C3-Me-L-Trp-L-Trp DKP (13)**

To a homogenous solution (under stirring with a magnetic bar) of amine **23** (77.0 mg, 0.184 mmol) in 14 M methanolic ammonia (4.0 mL) was refluxed for overnight. Evaporation of the solvent under reduced pressure and the residue was washed with chloroform furnished the pure diketopiperazine **13** (44.0 mg, 68% yield) as a pale yellow color solid. IR (KBr): 3431, 3297, 3025, 1666, 1631, 1528, 1447, 1340, 1286, 1072, 911, 754, 625 cm^{-1}. ^{1}H NMR (600 MHz, CD_3OD): δ 7.90 (s, 1H), 7.51 (dd, *J* = 6.8, 1.5 Hz, 1H), 7.11 (dd, *J* = 7.1, 1.5 Hz,1H), 7.07-7.00 (m, 2H), 6.97 (dt, *J* = 7.7, 0.8 Hz, 1H), 6.93 (s, 1H), 6.85 (d, *J* = 7.9 Hz, 1H), 6.62 (t, *J* = 7.4 Hz, 1H), 6.39 (d, *J* = 7.8 Hz, 1H), 5.09 (s, 1H), 4.59 (brs, 1H), 4.23 (t, *J* = 3.7 Hz, 1H), 3.42 (ABX, *J* = 14.7, 3.7 Hz, 1H), 3.08 (ABX, *J* = 14.7, 4.3 Hz, 1H), 2.38 (dd, *J* = 12.0, 5.8 Hz, 1H), 2.24 (dd, *J* = 12.4, 5.8 Hz, 1H), 1.84 (t, *J* = 12.0 Hz, 1H), 1.28 (s, 3H). ^{13}C NMR (150 MHz, CD_3OD, DEPT): δ 171.0 (C), 168.1 (C), 150.0 (C), 137.6 (C), 133.0 (C), 129.2 (CH), 128.7 (C), 125.6 (CH), 123.1 (CH), 122.8 (CH), 120.1 (2 × CH), 119.2 (CH), 112.5 (CH), 110.7 (CH), 108.7 (C), 82.1 (CH), 59.4 (CH), 59.1 (CH), 51.9 (C), 43.1 (CH_2), 31.0 (CH_2), 24.7 (CH_3). HRMS (ESI, $M+H^+$): m/z calcd. for $C_{23}H_{23}O_2N_4$ 387.1816, found 387.1824.

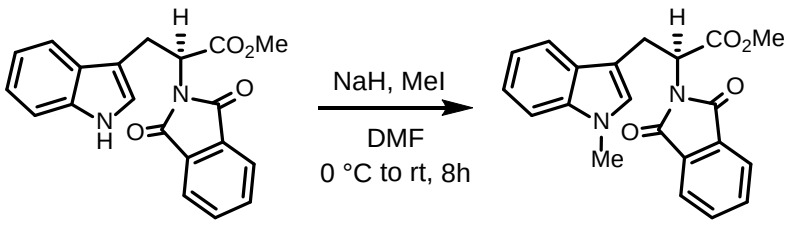
S8. Asymmetric Synthesis of *cyclo*-C3-Me-L-Trp-*N*1′-Me-L-Trp DKP (16)



(9) **(35)**

Methyl (S)-2-(1,3-dioxoisoindolin-2-yl)-3-(1H-indol-3-yl)propanoate *or* *N*-phth-L-Trp-methyl ester (35)

To a refluxing solution of L-Trp-methyl ester (**9**) (1.0 g, 4.58 mmol) and phthalic anhydride (0.747 g, 5.04 mmol) in toluene (35 mL) was added triethylamine (0.702 mL, 5.04 mmol) and the reflux was continued overnight. Evaporation of the solvent under reduced pressure yielded 98% (1.57 g, 4.51 mmol) of product **35** which was subjected for the next step without any purification as an orange fluffy solid. mp: 80 °C. IR (KBr): 3611, 3417, 2941, 1853, 1717, 1635, 1585, 1524, 1455, 1385, 1254, 1187, 1093, 1018, 880.6, 733.1 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.84 (brs, 1H), 7.65 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.30 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.49 (ddd, *J* = 7.9, 6.8, 0.72 Hz, 1H), 7.16 (ddd, *J* = 8.0, 6.6, 0.96 Hz, 1H), 7.02 (ddd, *J* = 8.1, 6.8, 1.1 Hz, 1H), 6.95 (ddd, *J* = 8.1, 6.9, 1.2 Hz, 1H), 6.89 (d, *J* = 2.4 Hz, 1H), 5.17 (dd, *J* = 9.5, 6.4 Hz, 1H), 3.69 (s, 3H), 3.65 (dd, *J* = 4.3, 0.92 Hz, 1H), 3.63 (dd, *J* = 2.0, 0.88 Hz, 1H). **^{13}C** NMR (100 MHz, $CDCl_3$, DEPT): δ 169.7 (C), 167.6 (2 × C), 136.7 (C), 134.0 (2 × CH), 131.7 (2 × C), 127.6 (C), 127.3 (CH), 123.4 (2 × CH), 121.6 (CH), 119.0 (CH), 118.6 (CH), 109.5 (C), 109.2 (CH), 52.8 (CH), 32.6 (CH_3), 24.8 (CH_2). HRMS (EI, M^+): m/z calcd. for $C_{20}H_{16}N_2O_4$ 348.1110, found 348.1109.

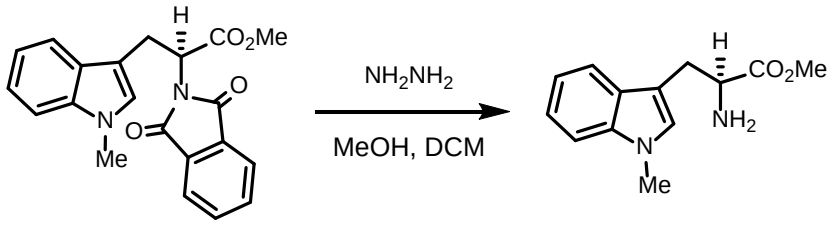


35 **36**

Methyl (S)-2-(1,3-dioxoisoindolin-2-yl)-3-(1-methyl-1H-indol-3-yl)propanoate *or* N^1-Me-N^2-phth-L-Trp-methyl ester (36)

To a cold (0 °C) solution of *N*-phth-L-Trp-methyl ester (**35**) (800 mg, 2.30 mmol) in dry DMF (8.0 mL) was added NaH (101 mg, 2.53 mmol) followed by slow addition of methyl iodide (215 μL, 3.45 mmol) and the resulting mixture was stirred at same temperature for 8h (reaction monitored by TLC). It was then quenched with water (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic layer was washed with brine and dried (anhyd. Na_2SO_4). Evaporation of the solvent under reduced pressure and purification of the residue on a silica gel column using ethyl acetate–hexanes (1:20) as eluent furnished the product **36** in 58% yield (480 mg, 1.32 mmol). Crystalline solid. mp: 124-125 °C; IR (KBr): 3026, 2952, 1745, 1714, 1614, 1553, 1470, 1435, 1390, 1328, 1256, 1210, 1127, 1103, 1017, 967, 917, 880, 750, 720, 662 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.77 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.67 (dd, *J* = 5.5, 3.0 Hz, 2H), 7.59 (d, *J* = 8.0 Hz, 1H), 7.21 (d, *J* = 8.2 Hz, 1H), 7.15 (ddd, *J* = 8.0, 6.8, 1.0 Hz, 1H), 7.04 (ddd, 8.0, 6.9, 1.2 Hz, 1H), 6.87 (s, 1H), 5.26 (dd, *J* = 8.8, 7.1 Hz, 1H), 3.79 (s, 3H), 3.74 (d, *J* = 1.2 Hz, 1H) 3.72 (s, 1H), 3.65 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 169.8 (C), 167.7 (2 × C), 136.9 (C), 134.1 (2 × CH), 131.8 (2 × C), 127.7 (C), 127.4 (CH), 123.5 (2 × CH), 121.7

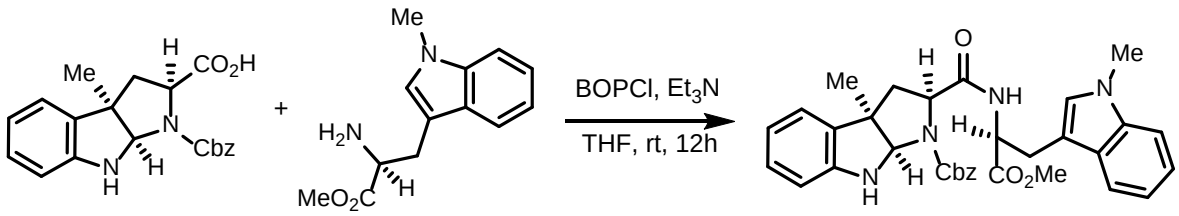
(CH), 119.0 (CH), 118.7 (CH), 109.6 (C), 109.2 (CH), 52.9 (CH), 52.8 (CH_3), 32.7 (CH_3), 24.9 (CH_2). HRMS (EI, M^+): m/z calcd. for $C_{21}H_{18}N_2O_4$ 362.1267, found 362.1253.



36 **21**

_N_-Me-L-Trp-Methyl Ester (21)

To a solution of phthalyl amine (**36**) (525 mg, 1.45 mmol) in MeOH (7.0 mL) and CH_2Cl_2 (7.0 mL) was added hydrazine hydrate (78 μL, 1.59 mmol) and the reaction mixture stirred for 24h. It was then filtered on a celite pad and washed with ethyl acetate. Evaporation of the solvent under reduced pressure and purification of the residue on silica gel (pre-neutralized with Et_3N) column using MeOH–$CHCl_3$ (1:20) as eluent furnished the amine (**21**) in (180 mg, 0.77 mmol) 54% yield. IR (KBr): 3369, 2950, 2922, 1736, 1608, 1546, 1473, 1439, 1373, 1325, 1249, 1207, 1173, 1128, 1062, 1014, 837, 742 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.61 (td, *J* = 8.0, 1.0 Hz, 1H), 7.30 (td, *J* = 8.2, 1.0 Hz, 1H), 7.23 (ddd, *J* = 8.1, 6.9, 1.1 Hz, 1H), 7.12 (ddd, *J* = 8.0, 6.9, 1.1 Hz, 1H), 6.93 (s, 1H), 3.82 (dd, 7.7, 4.8 Hz, 1H), 3.75 (s, 3H), 3.72 (s, 3H), 3.28 (ABXY, *J* = 14.4, 4.8, 0.8 Hz, 1H), 3.04 (ABXY, *J* = 14.4, 7.7, 0.6 Hz, 1H), 1.58 (brs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 175.9 (C), 137.1 (C), 128.0 (C), 127.8 (CH), 121.8 (CH), 119.1 (CH), 119.0 (CH), 109.7 (C), 109.4 (CH), 55.2 (CH), 52.1 (CH_3), 32.8 (CH_3), 30.7 (CH_2). HRMS (EI, M^+): m/z calcd. for $C_{13}H_{16}O_2N_2$ 232.1212, found 232.1208.

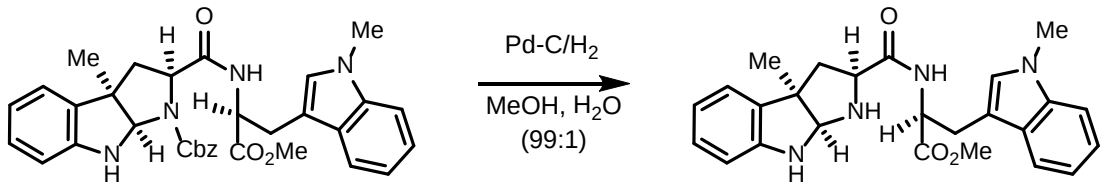


20c **21** **24**

Benzyl-(2S,3aR,8aR)-2-((1-methoxy-3-(1-methyl-1H-indol-3-yl)-1-oxopropan-2-yl)carbamoyl)-3a-methyl-3,3a,8,8a-tetrahydropyrrolo[2,3-b]indole-1(2H)-carboxylate (24)

To a cold (0 °C) magnetically stirred solution of *N*-Cbz-acid **20c** (135.0 mg, 0.38 mmol) with *N*-Me-L-Trp-methyl ester **21** (98.0 mg, 0.42 mmol) in dry THF (3 mL) was added Et_3N (213.0 µL, 1.53 mmol) followed by BOP-Cl (254.0 mg, 1.00 mmol) and the resulting mixture was stirred at room temperature for 12 h (reaction monitored by TLC). It was then quenched with water (3 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layer was washed with brine and dried over anhyd. Na_2SO_4. Evaporation of the solvent under reduced pressure and purification of the residue on silica gel column using ethyl acetate–hexanes (1:3) as eluent furnished the coupled amide **24** in 81% yield (175 mg, 0.31 mmol) as a mixture of two rotational isomers. IR (KBr): 3381, 3019, 2954, 2927, 1740, 1692, 1610, 1515, 1481, 1465, 1443, 1416, 1355, 1328, 1252, 1214, 1157, 1126, 1054, 1012, 986, 749, 696 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.51 (d, *J* = 7.9 Hz, 0.5H), 7.40 (d, *J* = 8.1 Hz, 1H), 7.37 (s, 2H), 7.30-7.20 (m, 5H), 7.20-7.05 (m, 5H), 6.99 (d, *J* = 7.5 Hz, 1H), 6.89 (s, 1H), 6.78-6.71 (m, 1.5H), 6.70 (s, 1H), 6.59 (d, *J* = 7.8 Hz, 1H), 6.55 (d, *J* = 7.8 Hz, 0.5H), 6.43 (d, *J* = 8.0 Hz, 0.5H), 6.12 (d, *J* = 7.6

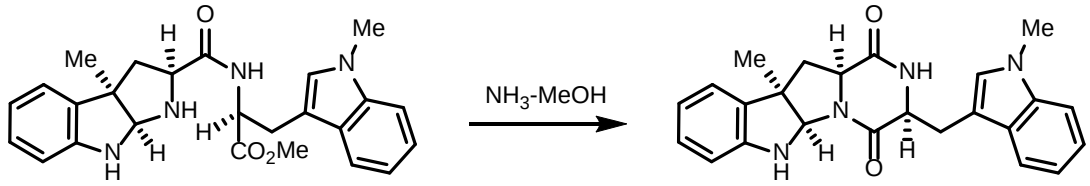
Hz, 1H), 5.41 (brs, 1H), 5.31 (AB, *J* = 12.4 Hz, 0.5H), 5.20 (s, 0.5H), 5.12 (s, 1H), 5.09 (AB, *J* = 12.4 Hz, 0.5H), 4.96 (AB, *J* = 12.4 Hz, 1H), 4.89 (AB, *J* = 12.4 Hz, 1H), 4.67 (dd, *J* = 12.8, 5.4 Hz, 1H), 4.07 (t, *J* = 7.9 Hz, 0.5H), 3.90 (t, *J* = 7.9 Hz, 1H), 3.73 (s, 1.5H), 3.72 (s, 3H), 3.68 (s, 1.5H), 3.64 (s, 3H), 3.68-3.62 (m, 0.5H), 3.32 (t, *J* = 4.8 Hz, 0.5H), 3.17 (ABX, *J* = 14.8, 5.5 Hz, 1H), 3.11 (ABX, *J* = 14.8, 5.5, Hz, 1H), 2.42 (ABX, *J* = 13.0, 7.8, Hz, 1H), 2.32 (ABX, *J* = 13.0, 7.8, Hz, 0.5H), 2.21 (ABX, *J* = 13.0, 7.0, Hz, 0.5H), 2.10 (ABX, *J* = 13.0, 8.2, Hz, 1H), 1.34 (s, 1.5H), 1.30 (s, 3.0H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 172.2 (C), 172.0 (C), 171.7 (C), 171.2 (C), 154.8 (C), 154.6 (C), 147.8 (C), 147.2 (C), 136.9 (2 * C), 136.1 (C), 136 (C), 133.8 (C), 133.6 (C), 128.9 (2 * CH), 128.6 (C), 128.5 (4 * CH), 128.4 (C), 128.1 (4 * CH), 128.0 (CH), 127.9 (2 * CH), 127.4 (CH), 122.4 (CH), 122.3 (CH), 122.0 (CH), 121.8 (CH), 119.4 (CH), 119.3 (CH), 119.2 (CH), 119.1 (CH), 118.7 (CH), 118.6 (CH), 109.8 (CH), 109.5 (CH), 109.4 (CH), 109.3 (CH), 108.3 (C), 108.2 (C), 84.1 (CH), 83.8 (CH), 67.6 (CH_2), 67.1 (CH_2), 61.8 (CH), 61.5 (CH), 53.3 (C), 52.8 (C), 52.7 (CH), 52.4 (CH_3), 52.3 (CH), 51.9 (CH_3), 42.1 (CH_2), 41.4 (CH_2), 32.8 (2 * CH_3), 27.5 (CH_2), 27.3 (CH_2), 23.8 (CH_3), 23.6 (CH_3). HRMS (EI, M^+): m/z calcd for $C_{33}H_{34}O_5N_4$: 566.2529 found: 566.2529.



24 **25**

N-methyl-(2S,3aR,8aS)-3a-methyl-1,2,3,3a,8,8a-hexahydropyrrolo[2,3-b]indole-2-carbonyl) methyl-1-tryptophanate (25)

To a magnetically stirred solution of **24** (75 mg, 0.13 mmol,) in MeOH (2 mL) and ethyl acetate (1 mL) was added 10% palladium on activated charcoal (14 mg, 0.1 equiv.) and the reaction mixture was stirred under hydrogen at 1 atm. Pressure for 6h. It was then filtered with Celite pad and washed with ethyl acetate. Evaporation of the solvent under reduced pressure furnished the amine **25** (57 mg, 0.13 mmol) in quantitative yield. The identity of the compound was confirmed by HRMS and subjected for the next step without any purification. HRMS (EI, M^+): m/z calcd. for $C_{25}H_{28}N_4O_3$ 432.2161; found, 432.2164.



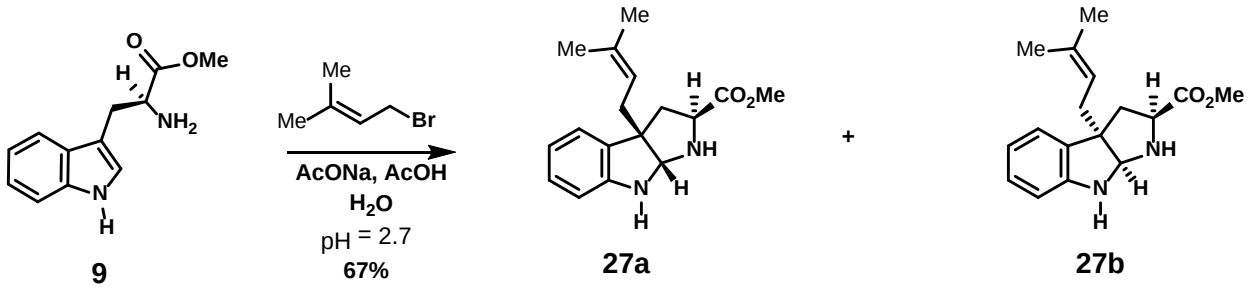
25 **16**

C3-Me-L-Trp-*N*1′-Me-L-Trp DKP (16)

To a magnetically stirred solution of amine (57 mg, 0.13 mmol) in 10M methanolic ammonia (4.0 mL) was refluxed for overnight. Evaporation of the solvent under reduced pressure and the residue was washed with chloroform furnished the pure diketopiperazine **16** (48 mg, 0.12 mmol) in 91% yield. IR (KBr): 3431, 3297, 3025, 1666, 1631, 1528, 1447, 1340, 1286, 1072, 911, 754, 625 cm^{-1}. ^1H NMR (600 MHz, CD_3OD): δ 7.51 (d, *J* = 5.2 Hz, 1H), 7.10-7.07 (m, 2H), 7.06-6.98 (m, 2H), 6.91 (d, *J* = 4.9 Hz, 1H), 6.86 (s, 1H), 6.65 (t, *J* = 4.9 Hz, 1H), 6.51 (d, *J* = 5.2 Hz, 1H), 5.15 (s, 1H), 4.59 (brs, 2H), 4.22 (s, 1H), 3.40 (s, 3H), 3.35 (dd, *J* = 9.8, 2.0 Hz, 1H), 3.12 (dd, *J* = 9.8, 3.0 Hz, 1H), 2.36 (dd, *J* = 7.9, 3.9 Hz, 1H), 2.25 (dd, *J* = 8.3, 3.9 Hz, 1H), 1.84 (t, *J* = 8.1 Hz, 1H), 1.29 (s, 3H). ^{13}C NMR (150 MHz, CD_3OD, DEPT): δ 170.8 (C), 168.2 (C), 150.2 (C), 138.0 (C), 133.2 (C), 129.8 (CH), 129.5 (C), 129.4 (CH), 123.4 (CH), 122.7 (CH), 120.3

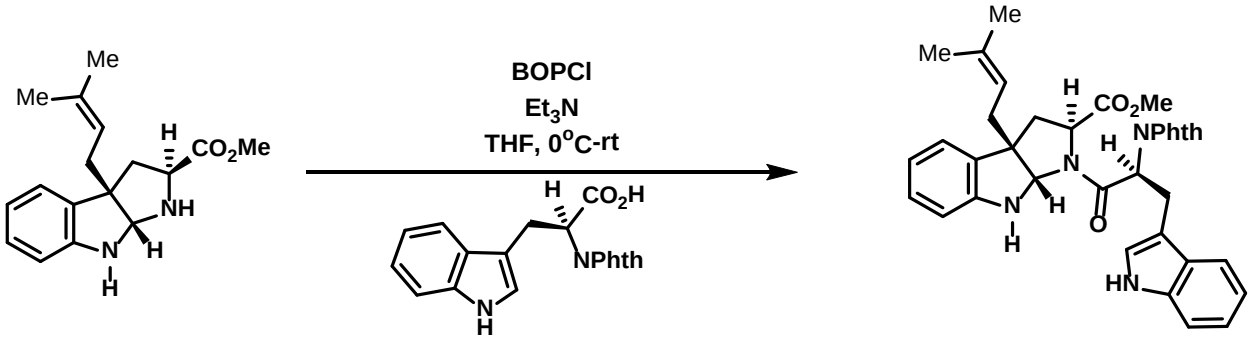
(CH), 120.1 (CH), 119.7 (CH), 110.5 (CH), 110.3 (CH), 108.3 (C), 82.3 (CH), 59.6 (CH), 59.1 (CH), 52.0 (C), 42.7 (CH_3), 32.9 (CH_2), 30.7 (CH_2), 24.9 (CH_3). HRMS (EI, M^+): m/z calcd. for $C_{24}H_{24}O_2N_4$ 400.1899, found 400.1897.

S9. Synthesis of *cyclo*-L-Trp-C3′-^{n}prenyl-L-Trp DKP (12) and *cyclo*-L-Trp-*N*1′-Me-C3′-^{n}prenyl-L-Trp DKP (15)



To a magnetically stirred solution of L-Trp-methyl ester (**9**) (400 mg, 1.83 mmol) in sodium acetate-acetic acid solution (pH = 2.7) (30 mL) was added prenyl bromide (635 μL, 5.50 mmol) over a period for 45-50 minutes at room temperature. The resulting mixture was stirred at same temperature for overnight. Evaporation of acetic acid under reduced pressure resulted in a solid residue which was dissolved in ethyl acetate. Solution was neutralized by addition of sodium carbonate solution and the ester was extracted with ethyl acetate three times. The combined organic layers were dried over anhyd. Na_2SO_4 and evaporated under reduced pressure yielding a diastereomeric mixture of the cyclic product **27a** and **27b** (in a 4:1 ratio) (358 mg, 1.25 mmol) in 67% overall yield (based on recovered starting material). Data for major diastereomer **27a**: IR (KBr): 3366, 3044, 2933, 1823, 1737, 1672, 1605, 1477, 1365, 1225, 1110, 1024, 942, 833, 747 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ 7.06-7.01 (m, 2H), 6.72 (dt, *J* = 7.4, 0.9 Hz, 1H), 6.56 (d, *J*

= 7.9 Hz, 1H), 5.09 (t, *J* = 7.3 Hz, 1H), 4.91 (s, 1H), 3.71 (dd, *J* = 10.5, 5.8 Hz, 1H), 3.70 (s, 3H), 3.42 (br's, 2H), 2.47-2.41 (m, 2H), 2.38 (dd, *J* = 12.0, 5.8 Hz, 1H), 2.00 (dd, *J* = 12.0, 10.7 Hz, 1H), 1.68 (s, 3H), 1.55 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 174.4 (C), 150.0 (C), 134.7 (C), 133.1 (C), 128.2 (CH), 123.6 (CH), 119.6 (CH), 118.8 (CH), 109.1 (CH), 82.2 (CH), 59.4 (CH), 58.8 (CH_3), 52.2 (CH_2), 44.2 (C), 36.9 (CH_2), 26.0 (CH_3), 18.2 (CH_3). HRMS (ESI, $M+H^+$): m/z calcd. for $C_{17}H_{23}O_2N_2$ 287.1681, found 287.1758.

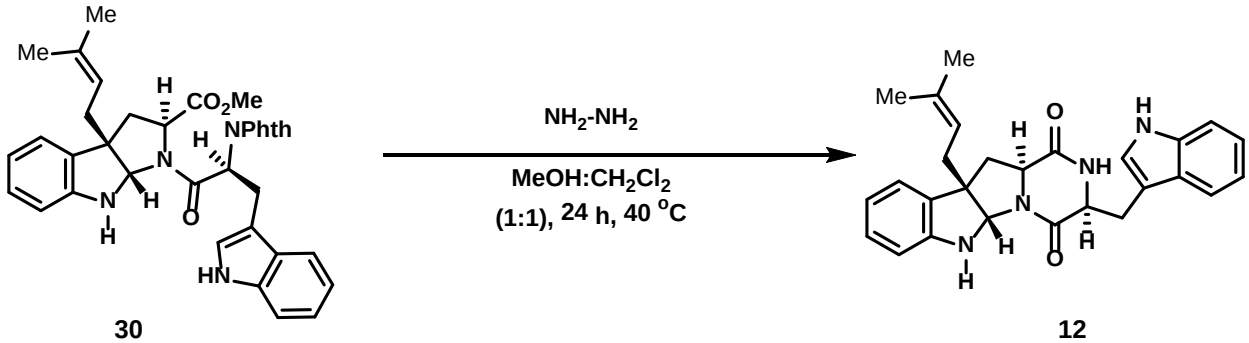


27a N-Phth-L-Trp (**29**) **30**

C3'-^{n}prenyl-pyrroloindoline-methyl-ester-*N*1-phthalyl-L-Trp-amide (30)

To a cold (0 °C) magnetically stirred solution of C3-^{n}Prenyl-pyrrolo indole methyl ester **27a** (42.0 mg, 0.15 mmol) with L-Trp-*N*-phth-acid **29** (44.0 mg, 0.13 mmol) in dry THF (1.5 mL) was added Et_3N (101 µL, 0.73 mmol) followed by BOP-Cl (93 mg, 0.37 mmol) and the resulting mixture was stirred at room temperature for overnight. It was then quenched with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layer was washed with brine and dried (anhyd. Na_2SO_4). Evaporation of the solvent under reduced pressure and purification of the residue on a silica gel column using ethyl acetate–hexanes (4:6) as eluent furnished the coupling compound as a mixture of rotamers in 59 mg, 67% yield. The identity of the compound was confirmed by HRMS and subjected for the next step without any purification. IR (KBr):

3372, 3057, 2937, 1717, 1646, 1449, 1371, 1193, 1101, 913, 730 cm^{-1}. HRMS (ESI, $M+Na^+$): m/z calcd. for $C_{36}H_{34}O_5N_4Na$ 625.2427, found 625.2441.



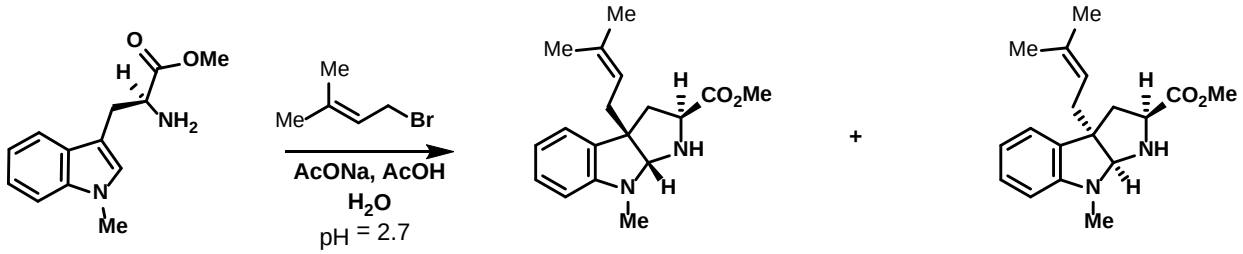
30 **12**

***cyclo*-L-Trp-C3′-^{n}prenyl-L-Trp DKP (12)**

To a magnetically stirred solution of **30** (56 mg, 0.09 mmol) in MeOH (1.0 mL) and CH_2Cl_2 (1.0 mL) was added hydrazine hydrate (45 μL, 0.93 mmol) and the reaction mixture stirred under nitrogen atmosphere for 24h. It was then quenched with water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The combined organic layer was washed with brine and dried (anhyd. Na_2SO_4). Evaporation of the solvent under reduced pressure and purification of the residue on a silica gel coloumn chromatography using EtOAc as eluent furnished the *cyclo*-L-Trp-C3′-^{n}prenyl-L-Trp DKP (**12**) in 70 % yield (29.0 mg, 0.06 mmol) as a single diasteromer. IR (KBr): 3297, 3058, 1663, 1452, 1324, 1192, 1088, 922, 814, 740, 608 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.74 (brs, 1H), 7.59 (d, *J* = 7.3 Hz, 1H), 7.20 (m, 3H), 7.05 (dt, *J* = 7.7, 1.2 Hz, 1H), 6.89 (d, *J* = 6.9 Hz, 1H), 6.86 (d, *J* = 2.4 Hz, 1H), 6.70 (t, *J* = 7.4 Hz, 1H), 6.40 (d, *J* = 7.8 Hz, 1H), 6.04 (brs, 1H), 5.22 (s, 1H), 5.04 (t, *J* = 7.3 Hz, 1H), 4.44 (brs, 1H), 4.26 (dd, *J* = 9.2, 4.0 Hz, 1H), 3.37 (ABX, *J* = 14.6, 5.4 Hz, 1H), 3.12 (ABX, *J* = 14.6, 3.8 Hz, 1H), 2.72 (dd, *J* = 12.0, 5.5 Hz, 1H), 2.36-2.26 (m, 3H), 2.00 (t, *J* = 12.0 Hz, 1H), 1.65 (s, 3H), 1.49 (s, 3H).

^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 169.3 (C), 165.9 (C), 149.2 (C), 136.0 (C), 135.8 (C), 131.0 (C), 128.3 (CH), 127.3 (C), 123.7 (CH), 123.3 (CH), 122.8 (CH), 120.0 (CH), 119.0 (CH), 118.7 (CH), 118.5 (CH), 111.5 (CH), 109.3 (CH), 108.9 (C), 79.1 (CH), 58.5 (CH), 57.7 (CH), 55.1 (C), 39.2 (CH_2), 35.5 (CH_2), 30.6 (CH_2), 26.1 (CH_3), 18.1 (CH_3). HRMS (ESI, M+Na^+): m/z calcd. for $C_{27}H_{28}O_2N_4Na$ 463.2110, found 463.2104.

Asymmetric Synthesis of *cyclo*-L-Trp-*N*1′-Me-C3′-^{n}prenyl-L-Trp DKP (15)

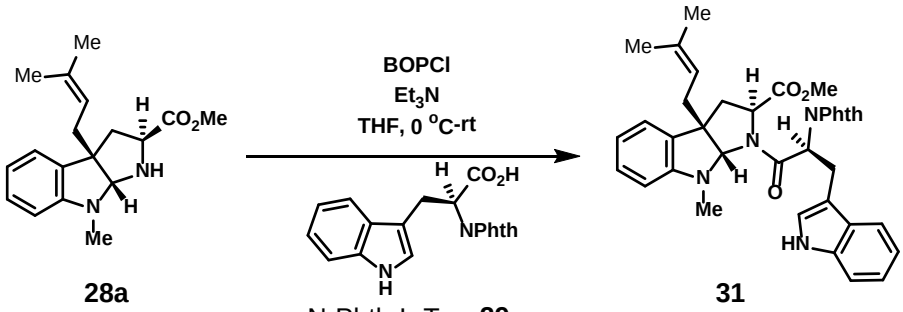


***N*1′-Me-C3′-^{n}prenyl-L-Trp-pyrroloindoline methyl ester (28)**

To a magnetically stirred solution of *N*-Me-*L*-Trp-methyl ester **26** (680 mg, 2.93 mmol) in acetate-acetic acid solution (pH = 2.7) (30 mL) was added prenyl bromide (1.29 ml, 8.79 mmol) over a period for 45-50 minutes at room temperature. The resulting mixture was stirred at same temperature overnight. After evaporation of acetic acid under reduced pressure the resulting solid residue was dissolved in ethyl acetate. Then the mixture was neutralized through addition of sodium carbonate solution and the aqueous layer was extracted with ethyl acetate three times. The collected organic layer was dried over anhyd. Na_2SO_4 and evaporated under reduced pressure yielding diastereomeric mixture of the cyclic product in 11% yield for **28b** (99 mg, 0.33 mmol) and 21% yield for **26a** (185 mg, 0.62 mmol) along with a 40% recovery of **26** amounting

to a 53% overall yield based on recovered starting material. **Data for diastereoisomer 28a:** IR (KBr): 3366, 3044, 2933, 1823, 1737, 1672, 1605, 1477, 1365, 1225, 1110, 1024, 942, 833, 747, 662 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.08 (dt, *J* = 7.7, 1.2 Hz, 1H), 7.00 (dd, *J* = 7.3, 1.2 Hz, 1H), 6.63 (dt, *J* = 7.4, 0.9 Hz, 1H), 6.34 (d, *J* = 7.8 Hz, 1H), 5.09 (qt, *J* = 6.7, 1.3 Hz, 1H), 4.67 (s, 1H), 3.71 (s, 3H), 3.66 (dd, *J* = 10.3, 6.2 Hz, 1H), 3.08 (brs, 1H), 2.83 (s, 3H), 2.45-2.38 (m, 2H), 2.35 (dd, *J* = 12.2, 6.2 Hz, 1H), 2.00 (t, *J* = 11.2 Hz, 1H), 1.68 (s, 3H), 1.56 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 174.6 (C), 151.20 (C), 134.6 (C), 133.4 (C), 128.3 (CH), 123.0 (CH), 119.7 (CH), 117.0 (CH), 105.6 (CH), 88.8 (CH), 59.5 (CH), 57.1 (C), 52.2 (CH_3), 44.0 (CH_2), 36.7 (CH_2), 31.7 (CH_3), 26.0 (CH_3),18.2 (CH_3). HRMS (EI, M^+): m/z calcd. for $C_{18}H_{24}O_2N_2$ 300.1838, found 300.1838.

Data for diastereoisomer 28b: IR (KBr): 3366, 3044, 2933, 1823, 1737, 1672, 1605, 1477, 1365, 1225, 1110, 1024, 942, 833, 747, 662 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.05 (dt, *J* = 7.6, 1.2 Hz, 1H), 6.98 (dd, *J* = 7.3, 0.9 Hz, 1H), 6.60 (dt, *J* = 7.4, 0.8 Hz, 1H), 6.29 (d, *J* = 7.8 Hz, 1H), 5.13 (qt, *J* = 6.7, 1.2 Hz, 1H), 4.63 (s, 1H), 3.91 (dd, *J* = 7.8, 3.1 Hz, 1H), 3.50 (brs, 1H), 3.33 (s, 3H), 2.86 (s, 3H), 2.49-2.36 (m, 3H), 2.33 (ABX, *J* = 12.8, 7.9 Hz, 1H), 1.69 (s, 3H), 1.58 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$, DEPT): δ 174.6 (C), 150.8 (C), 134.5 (C), 133.6 (C), 128.3 (CH), 123.2 (CH), 119.9 (CH), 117.0 (CH), 105.6 (CH), 88.7 (CH), 60.0 (CH), 56.0 (CH), 52.0 (CH_3), 41.0 (CH_2), 36.2 (CH_2), 31.4 (CH_3), 26.1 (CH_3), 18.2 (CH_3). HRMS (ESI, M^+): m/z calcd. for $C_{18}H_{24}O_2N_2$ 300.1838, found 300.18375.

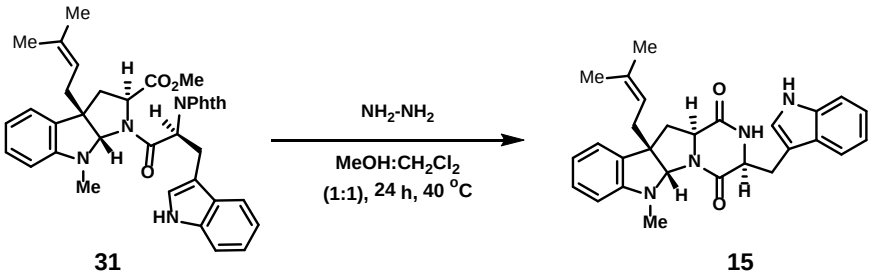


727

728

729 A cold (−10 °C) solution of *N*1'-Me-C3'-^{n}prenyl-*L*-Trp-pyrroloindoline methyl ester **28a** (100
730 mg, 0.33 mmol) was mixed with *N*-Phth-*L*-Trp acid **29** (101, 0.30 mmol) under stirring in dry
731 THF (8 mL) and Et_3N (168 µL, 1.21 mmol) was added followed by BOP-Cl (192 mg, 0.75
732 mmol) and the resulting mixture was stirred at same temperature for 8 h. TLC was used for
733 monitoring the progress. The reaction was then quenched with water (10 mL) and extracted with
734 ethyl acetate (3 × 15 mL). The combined organic layers were washed with brine and dried over
735 anhyd. Na_2SO_4. Evaporation of the solvent under reduced pressure and purification of the residue
736 on a silica gel column using ethyl acetate–hexanes (1:20) as eluent furnished the coupled product
737 **31** as a mixture of rotational isomers in 97% yield (180 mg, 0.29 mmol). This material was
738 subjected to the DKP-forming step directly. HRMS (EI, M^+): m/z calcd. for $C_{29}H_{32}N_3O_3$
739 470.2444, found 470.2446.

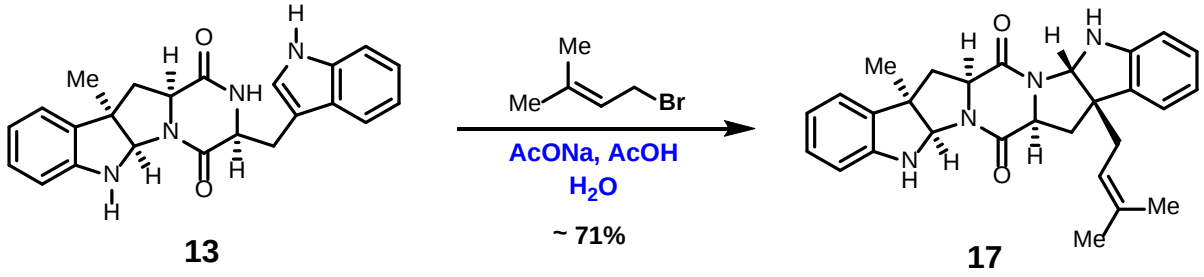
740



741

742 **Cyclo-*L*-Trp-*N*1'-Me-C3'-^{n}prenyl-*L*-Trp DKP (15)**

To a magnetically stirred solution of **31** (180 mg, 0.29 mmol) in MeOH (5.0 mL) and CH_2Cl_2 (5.0 mL) was added hydrazine hydrate (152 μL, 0.31 mmol) and the reaction mixture stirred under nitrogen atmosphere for 24 h. It was then quenched with water (10 mL) and extracted with ethyl acetate (3 × 15 mL). The combined organic layers were washed with brine and dried (anhyd. Na_2SO_4). Evaporation of the solvent under reduced pressure and purification of the residue on preparative TLC using MeOH–$CHCl_3$ (1:40) as eluent furnished the Cyclo-*L*-Trp-*N*1'-Me-C3'-^{n}prenyl-*L*-Trp DKP (**15**) in 74% yield (99.0 mg, 0.22 mmol). IR (KBr): 3308, 3243, 3059, 2927, 2864, 1788, 1670, 1501, 1442, 1359, 1305, 1233, 1172, 1104, 984, 919,733 $cm^{-}1$. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.21 (brs, 1H), 7.61 (d, *J* = 8.1 Hz, 1H), 7.39 (d, *J* = 8.1 Hz, 1H), 7.22 (t, *J* = 7.2 Hz, 1H), 7.21 (t, *J* = 7.4 Hz, 2H), 7.18 (brs, 1H), 7.00 (d, *J* = 7.3 Hz, 1H), 6.74 (t, *J* = 7.2 Hz, 1H), 6.48 (d, *J* = 7.7 Hz, 1H), 5.98 (brs, 1H), 5.41 (s, 1H), 4.97 (t, *J* = 7.2 Hz, 1H), 4.43 (d, *J* = 7.6 Hz, 1H), 4.02 (dd, *J* = 11.0, 6.2 Hz, 1H), 3.68 (dd, *J* = 14.6, 3.1 Hz, 1H), 3.09 (s, 3H), 3.07 (dd, *J* = 14.6, 10.0 Hz, 1H), 2.52 (dd, *J* = 12.0, 6.0 Hz, 1H), 2.36-2.24 (m, 2H), 1.90 (t, *J* = 12.0 Hz, 1H), 1.67 (s, 3H), 1.52(s, 3H). ^{13}C NMR (150 MHz, $CDCl_3$, DEPT): δ 168.8 (C), 165.4 (C), 136.6 (2 × C), 135.6 (C), 131.8 (C), 129.0 (CH), 126.9 (CH), 124.0 (CH), 123.3 (CH), 122.9 (CH), 120.2 (CH), 120.1 (C), 118.9 (CH), 118.7 (CH), 111.6 (CH), 109.5 (CH), 109.0 (C), 85.6 (CH), 58.7 (CH), 55.0 (CH) 54.5 (C), 41.0 (CH_3), 36.7 (CH_2), 29.8 (CH_2), 28.5 (CH_2), 26.1 (CH_3), 18.2 (CH_3). HRMS (EI, M^{+}): m/z calcd. for $C_{28}H_{30}N_4O_2$ 454.2369, found 454.2368.



13 **17**

Des-N1′-Me-Nocardioazine B (17)

To a magnetically stirred solution of *Cyclo*-C3-Me-L-Trp-L-Trp DKP **(13)** (88 mg, 0.23 mmol) in sodium acetate-acetic acid solution (pH = 2.7) (5 mL) was added prenyl bromide (26.0 μL, 0.23 mmol) drop wise over a period for 30-40 minutes at room temperature. The resulting mixture was stirred at same temperature for overnight. Evaporation of acetic acid under reduced pressure resulted in a solid residue which was dissolved in ethyl acetate. Solution was neutralized by addition of sodium carbonate solution and the prenylated DKP was extracted with ethyl acetate three times. The combined organic layers were dried over Na_2SO_4 and evaporated under reduced pressure yielding a diastereomeric mixture of Des-N1′-Me-Nocardioazine B (**17**) (in a 4:1 ratio) (73 mg, 0.16 mmol) in 71% overall yield (based on recovered starting material). Copies of NMR spectra reflect a mixture of inseparable diastereomeric mixture. IR (KBr): 3430, 3298, 3026, 1667, 1631, 1529, 1447, 1340, 1286, 1072, 911, 754, 625 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) (*major diastereomer*): δ 7.80 (s, 1H), 7.59 (dd, *J* = 6.8, 1.5 Hz, 1H), 7.20-7.00 (m, 3H), 6.97 (dd, *J* = 7.7, 0.8 Hz, 1H), 6.93 (s, 1H), 6.70 (t, *J* = 7.9 Hz, 1H), 6.39 (d, *J* = 7.4 Hz, 1H), 6.02 (brs, 1H), 5.19 (s, 1H), 4.40 (brs, 1H), 4.25 (t, *J* = 3.7 Hz, 1H), 3.79 (m, 1H), 3.62 (m, 1H), 3.39 (ABX, *J* = 14.7, 3.7 Hz, 1H), 3.08 (ABX, *J* = 14.7, 4.3 Hz, 1H), 2.75 (dd, 14.7, 3.7 Hz, 1H), 2.38 (dd, *J* = 12.0, 5.8 Hz, 1H), 1.84 (t, *J* = 12.0 Hz, 1H), 1.64 (s, 1H), 1.28 (s, 3H) 1.40 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) (*major diastereomer*): 174.6; 170.5; 145.4; 134.0;

133.8; 132.8; 123.9; 123.3; 122.4; 120.5; 113.2; 80.7; 73.3; 72.8; 65.9; 63.3; 60.74; 50.46; 45.0; 38.9; 35.2; 31.0; 27.9; 27.5; 26.9; 23.9; 23.4; 18.1. HRMS (ESI, $M+H^+$): m/z calcd. for $C_{28}H_{31}N_4O_2$ 455.2469, found 455.2554.

S10. Conditions for HRMS, HPLC, LC-MS and MS^2 Characterization and Identification of signature peaks.

A 2.1x50 mm column packed with BEH C18 1.7 μm particles (Waters) was held at 45 °C throughout the separation; mobile phase A was 5% v/v Omnisolve grade CH_3CN (EMD Millipore, Billerica, MA), 0.1% v/v formic acid (Sigma Aldrich) in Omnisolve grade water (EMD). Mobile phase B was 5% v/v Omnisolve water, 0.1% v/v formic acid in acetonitrile and the flow rate was maintained at 0.3 mL/min. The gradient profile was: Start at 10% B, linear gradient to 100% B over 30 minutes, hold 3 minutes at 100% B, and a linear gradient to 0% B over two minutes followed by a 3 minute re-equilibration period between injections. All effluent was directed into the ESI source of the G2 (3.0 kV on capillary, 120 °C source temperature, 850 L/h of nitrogen desolvation gas @ 600 °C, 20 L/h of cone gas, 40 V on sample cone, 4 V on extraction cone) which was used in resolution mode (20,000 resolving power). Separate chromatograms were recorded simultaneously with a 0.2 sec MS1 scan from m/z 275-600 for the LC eluent with no collision energy, along with nine 0.2 second MS-MS scans directing the quadrupole to sequentially pass 205.10 (L/D-tryptophan, $[M+H]^+$); 373.17 (*cyclo*-L-Trp-L/D-Trp DKP (**5**), R_t for $[M-H]^-$, 5.05 min and R_t for $[M+H]^+$7.05 min.); m/z 387.18 (*cyclo*-C3-Me-L-Trp-L-Trp DKP (**13**), $M+H^+$, R_t 10.32 min.); m/z 401.19 (*cyclo*-C3-Me-L-Trp-*N*1′-Me-L-Trp DKP (**16**), $[M+H]^+$, R_t 12.15 min.); m/z 441.23 (*cyclo*-L-Trp-C3′-^{n}prenyl-L-Trp DKP (**12**),

804 $[M+H]^+$, R_t 12.12 min.); m/z 455.24 (*cyclo*-L-Trp-*N*1′-Me-C3′-^{n}prenyl-L-Trp DKP (**15**), $[M+H]^+$,
805 R_t 18.56 min. (17.25-19.25 min)); m/z 483.24 (nocardioazine A (**3**), $[M+H]^+$, R_t 8.8 min.), 469.26
806 (nocardioazine B (**4**), $[M+H]^+$, R_t 18.40 min.), and m/z 574.37 (nocardiopsin A (**1**), $M+H^+$).

807 During the nine MS^2 experiments, the trap voltage (collision energy) was scanned from
808 20-50 V over 0.2 seconds; the trap was filled with $7.9*10^{-3}$ mbar of UHP Ar. The 9th
809 chromatogram was another MS1 scan of the lock spray nozzle which had 5 μL/min of a 2 mg/L
810 solution of Leucine-enkephalin (Sigma) in 50% v/v methanol, 0.1% v/v formic acid in water
811 using the m/z 556.2765 $(M+H)^+$ ion to dynamically correct the mass axis calibration throughout
812 the experiment. After acquisition, extracted ion chromatograms for fragment ions were
813 generated using MassLynx 4.1 software (Waters). Each metabolite, synthesized or extracted was
814 observed and identified through MSM data. For example, the location of the methyl group of the
815 des-Me-nocardioazine B intermediates was determined by the presence of either 198.129 as the
816 base peak (indicating an H on N1') or 212.144 (indicating a CH3 on N1'). These identification
817 details are further elaborated in elsewhere.[16]
818

[16] Srinivasan, J.; Porwal, S. K.; Alqahtani, N.; James, E. Bis, D. Lane, A. L.; Viswanathan, R.; Karty, J. K.; "Collision-Induced Dissociation of Nocardioazines A and B and Related Tryptophan Cyclic Diketopiperzaines" Article *In preparation* for the Journal of the American Society for Mass Spectrometry.

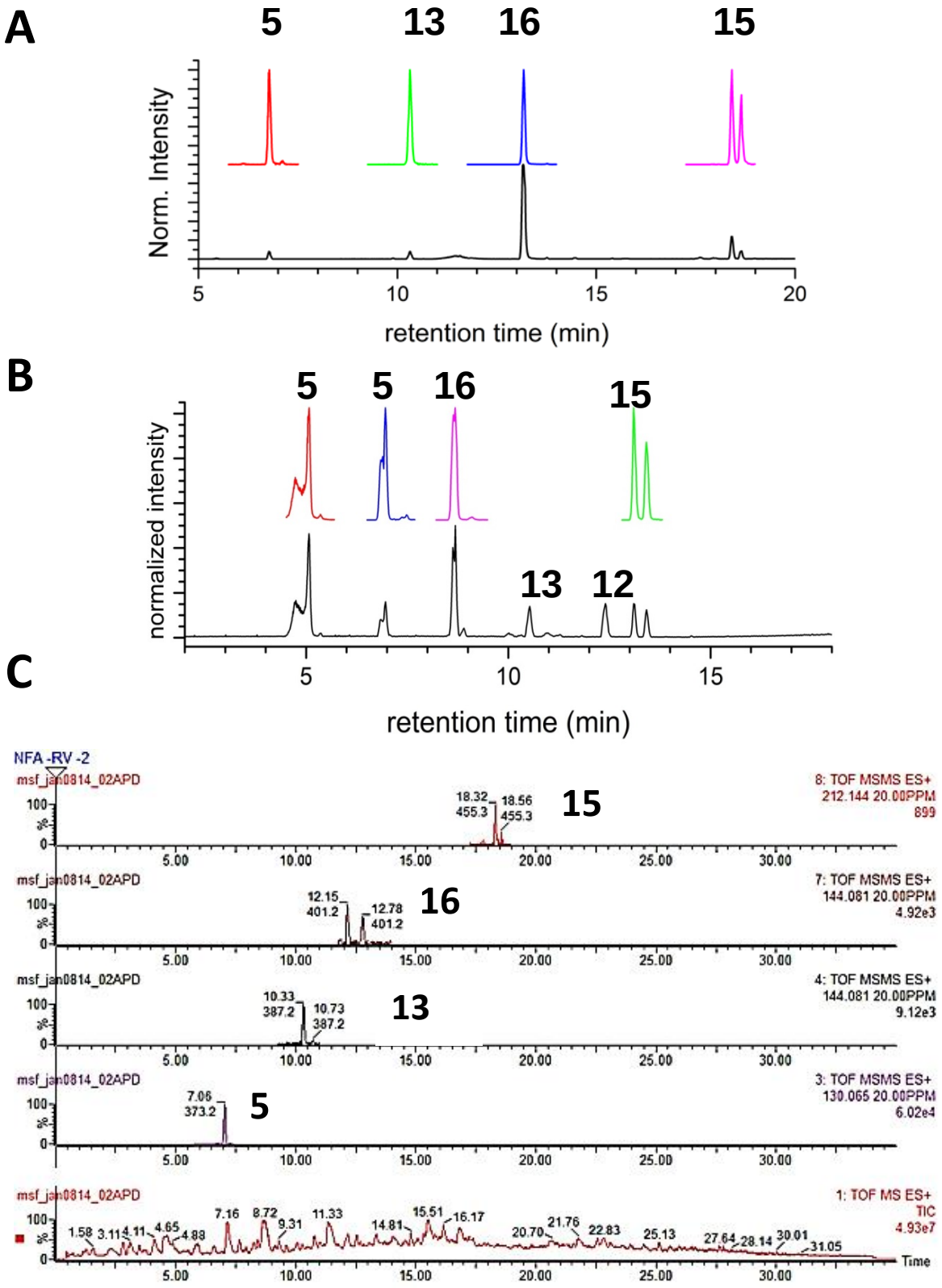


Figure S1. LC-ESI-TOF MS spectra for biosynthetic intermediates in their purely synthesized state. **A**: LC-ESI (+) ions from mixture of synthesized standards **5**, **13 15** and **16**; **B**: LC-ESI (-) ions from mixture of synthesized standards **5**, **13 15** and **16**. **C**. Extracted ion chromatograms for positive ion tandem mass spectra of **5**, **8**, **10**, and **11** from biological extract.

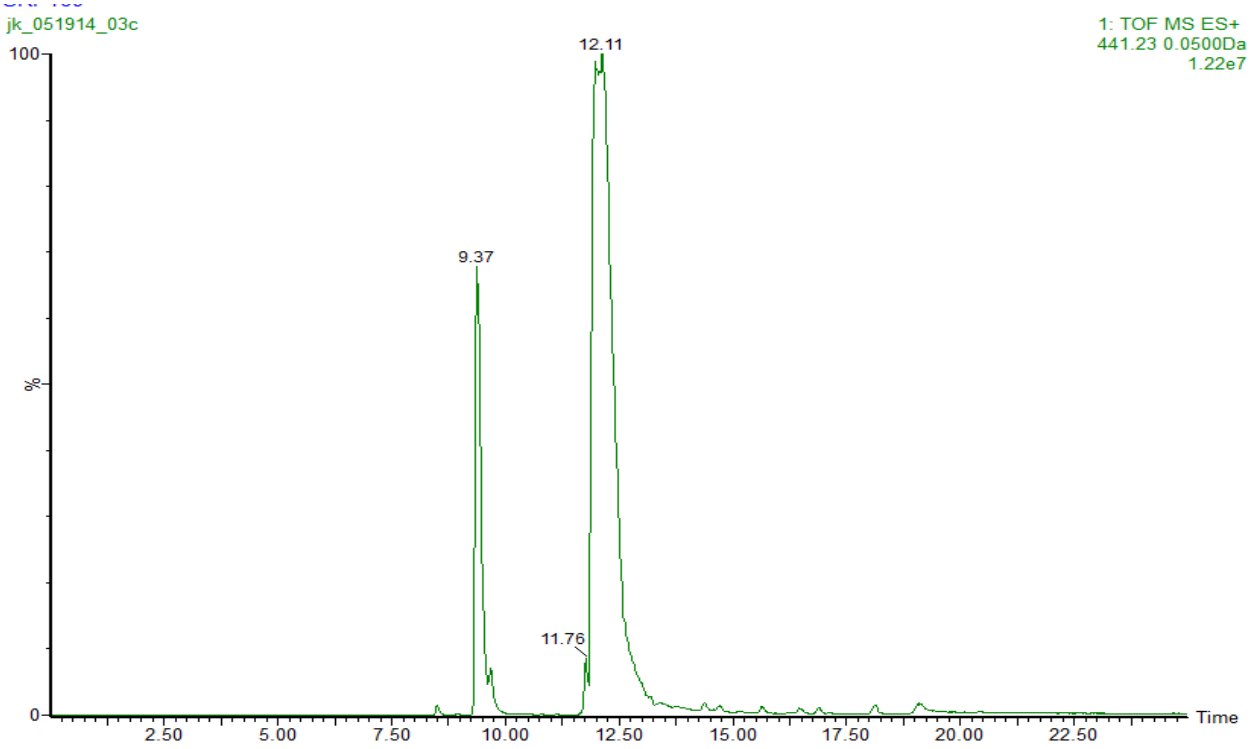
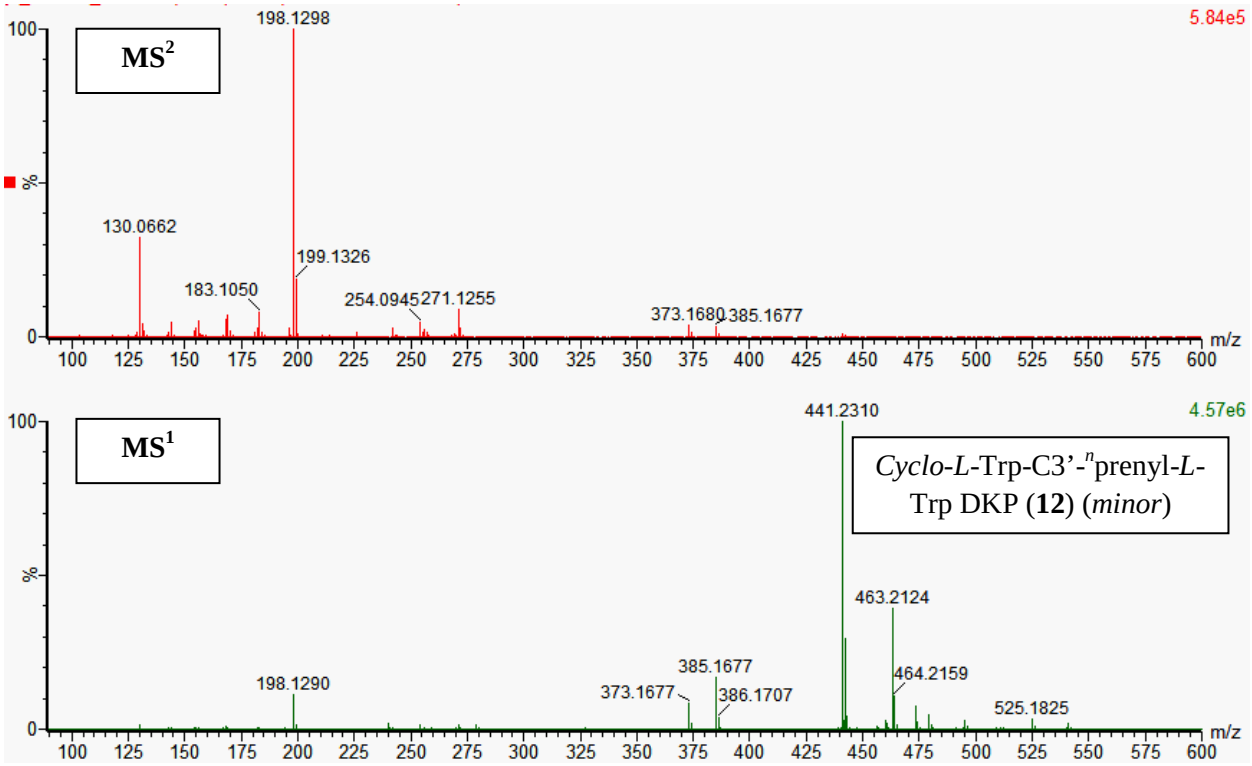


Figure S2. Two diastereomers of *cyclo*-L-Trp-C3′-^{n}prenyl-L-Trp DKP (**12**) were synthesized and their LC traces were seen as distinct peaks at R_t 9.37 (*minor*) and 12.11 (*major*) min respectively.



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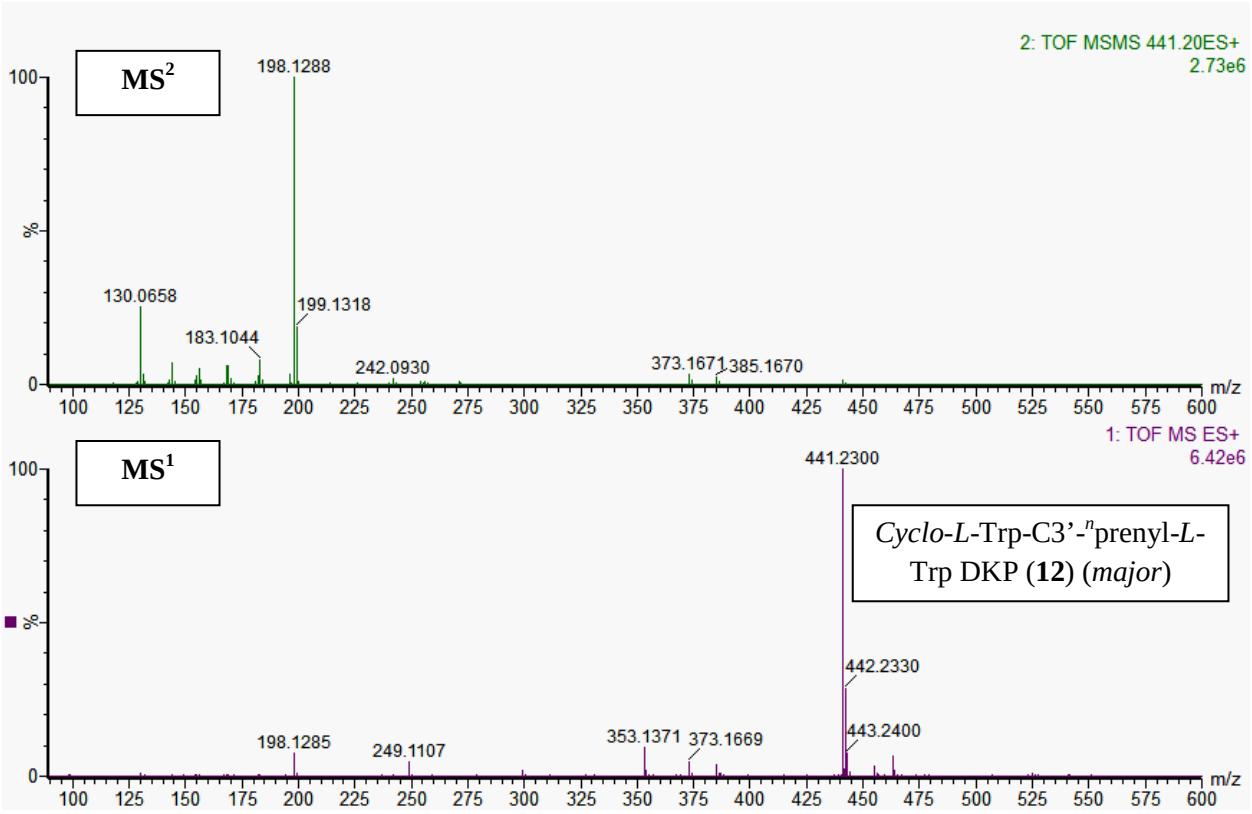

825

826 **Figure S3.** MS^1 and MS^2 for two diastereomers of *cyclo*-L-Trp-C3′-^{n}prenyl-L-Trp DKP (**12**).

827 **Table S2.** MS^2 fragments for $[M+H]^+$ peak at m/z = 373.1659 corresponding to *cyclo*-L-Trp-L-Trp DKP (**5**).

828

Observed Mass	% Rel. Int.	*Predicted Mass*	Mass Diff. (ppm)	Ion Composition
373.1662	2.8	*373.1659*	0.80	$C_{22}H_{21}N_4O_2^+$
242.0930	2.7	*242.0924*	2.48	$C_{13}H_{12}N_3O_2^+$
214.0980	1.6	*214.0975*	2.34	$C_{12}H_{12}N_3O^+$
169.0759	5.7	*169.0760*	-0.59	$C_{11}H_9N_2^+$
159.0924	2.5	*159.0917*	4.40	$C_{10}H_{11}N_2^+$
144.0809	1.3	*144.0808*	0.69	$C_{10}H_{10}N^+$
130.0654	100	*130.0651*	2.31	$C_9H_8N^+$
103.0544	2.8	*103.0542*	1.94	$C_8H_7^+$

829

830

Table S3. MS^2 fragments for $[M+H]^+$ peak at m/z = 387.1816 corresponding to *cyclo*-C3-Me-L-Trp-L-Trp DKP (**13**).

Observed Mass	% Rel. Int.	*Predicted Mass*	Mass Diff. (ppm)	Ion Composition
387.1825	14.7	*387.1816*	2.32	$C_{22}H_{21}N_4O_2^+$
242.0936	3.0	*242.0924*	4.96	$C_{13}H_{12}N_3O_2^+$
184.0761	10.1	*184.0757*	2.17	$C_{12}H_{10}NO^+$
159.0923	3.2	*159.0917*	3.77	$C_{10}H_{11}N_2^+$
156.0815	3.9	*156.0808*	4.48	$C_{11}H_{10}N^+$
144.0813	100	*144.0808*	3.47	$C_{10}H_{10}N^+$
130.0656	24.5	*130.0651*	3.84	$C_9H_8N^+$
103.0547	1.0	*103.0542*	4.85	$C_8H_7^+$

836

837 **Table S4.** MS^2 fragments for $[M+H]^+$ peak at m/z = 401.1972 corresponding to *cyclo*-C3-Me-L-Trp-*N*1′-Me-L-Trp DKP (**16**).

Observed Mass	% Rel. Int.	*Predicted Mass*	Mass Diff. (ppm)	Ion Composition
401.1981	8.8	*401.1972*	2.24	$C_{24}H_{25}N_4O_2^+$
384.1707	0.5	*384.1707*	0.00	$C_{24}H_{22}N_3O_2^+$
256.1089	1.0	*256.1081*	3.12	$C_{14}H_{14}N_3O_2^+$
201.1023	0.5	*201.1022*	0.50	$C_{12}H_{13}N_2O^+$
184.0761	2.6	*184.0757*	2.17	$C_{12}H_{10}NO^+$
173.1078	0.5	*173.1079*	-0.58	$C_{11}H_{13}N_2^+$
156.0813	0.8	*156.0808*	3.20	$C_{11}H_{10}N^+$
144.0813	100	*144.0808*	3.47	$C_{10}H_{11}N^+$

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839

840 **Table S5.** MS^2 fragments for $[M+H]^+$ peak at m/z = 441.2285 corresponding to *cyclo*-L-Trp-C3′-^{n}prenyl-L-Trp DKP (**12**) .

Observed Mass	% Rel. Int.	*Predicted Mass*	Mass Diff. (ppm)	Ion Composition
441.2292	1.5	*441.2285*	1.59	$C_{27}H_{29}N_4O_2^+$
385.1664	2.6	*385.1659*	1.30	$C_{23}H_{21}N_4O_2^+$
373.1665	3.6	*373.1659*	1.61	$C_{22}H_{21}N_4O_2^+$
271.1235	1.2	*271.1230*	1.84	see below
242.0930	2.3	*242.0924*	2.48	$C_{13}H_{12}N_3O_2^+$
198.1287	100	*198.1277*	5.05	$C_{14}H_{16}N^+$
183.1044	8.0	*183.1043*	0.55	$C_{13}H_{13}N^{+\bullet}$
168.0815	6.3	*168.0808*	4.16	$C_{12}H_{10}N^+$
156.0809	5.6	*156.0808*	0.64	$C_{11}H_{10}N^+$
144.0810	7.0	*144.0808*	1.39	$C_{10}H_{10}N^+$
130.0656	25.0	*130.0651*	3.84	$C_9H_8N^+$

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843

844 **Table S6.** MS^2 fragments for $[M+H]^+$ peak at m/z = 455.2442 corresponding to *Cyclo-L*-Trp-*N*1'-Me-C3'-^{n}prenyl-*L*-Trp DKP (**15**).

845

Observed Mass	% Rel. Int.	*Predicted Mass*	Mass Diff. (ppm)	Ion Composition
455.2435	0.8	*455.2442*	-1.54	$C_{28}H_{31}N_4O_2^+$
399.1806	2.1	*399.1816*	-2.51	$C_{24}H_{23}N_4O_2^+$
387.1823	2.0	*387.1816*	1.81	$C_{23}H_{23}N_4O_2^+$
285.1396	3.8	*285.1387*	3.16	$C_{20}H_{17}N^{2+}$
268.1089	2.1	*268.1081*	2.98	$C_{15}H_{14}N_3O^{2+}$
212.1441	100	*212.1434*	3.30	$C_{15}H_{18}N^+$
197.1207	9.7	*197.1199*	4.06	$C_{14}H_{15}N^{+\bullet}$
182.0972	8.6	*182.0964*	4.39	$C_{13}H_{12}N^+$
158.0969	4.1	*158.0964*	3.16	$C_{11}H_{12}N^+$
144.0814	52.1	*144.0808*	4.16	$C_9H_8N^+$
130.0657	6.5	*130.0651*	4.61	$C_8H_7^+$

846

847

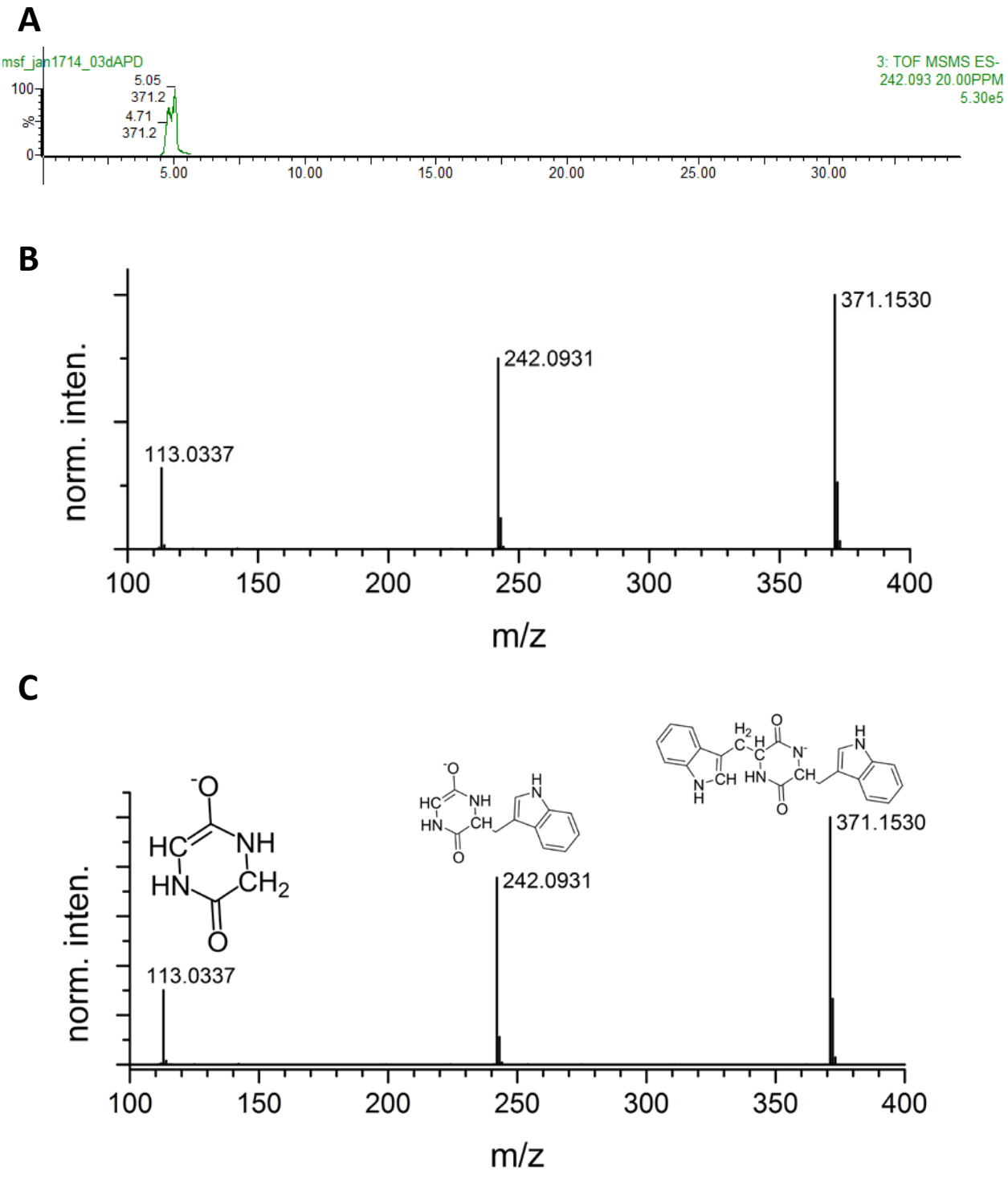

Figure S4. *cyclo*-L-Trp-L-Trp DKP (**5**). **A**. LC-ESI(-)-TOF-MSMS spectrum from *Nocardiopsis sp.* CMB-M0232. **B**. ESI(-) MS^2 for **5** extracted from *Nocardiopsis sp.* CMB-M0232. **C**. ESI(-) MS^2 for synthetic **5**.

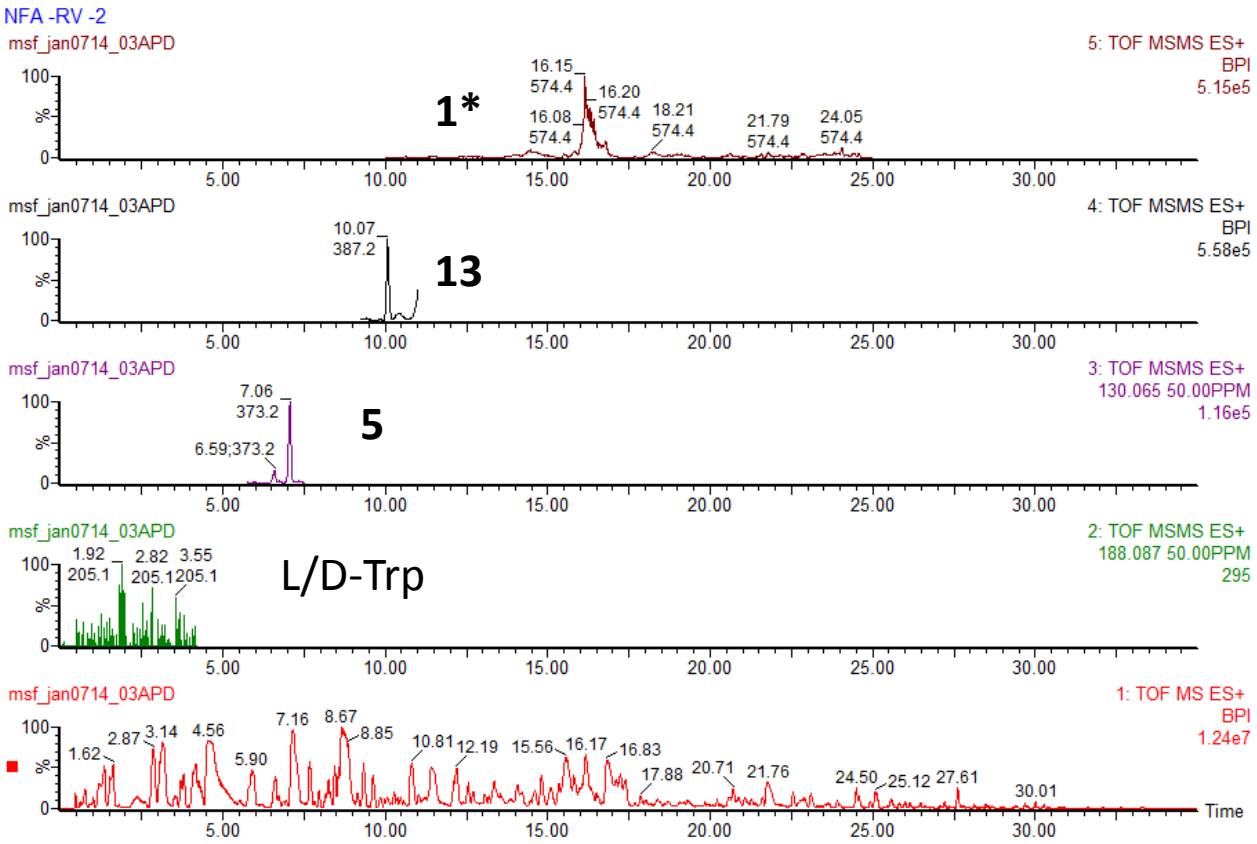

Figure S5. LC- ESI(+)-TOF-MSMS traces from *Nocardiopsis sp.* CMB-M0232 confirming presence of tabulated metabolites as given in **Table 1** for m/z = 373.2 (**5**); 387.2 (**13**) and 574.4 (nocardiopsin A, * - pending full confirmation).

856

857

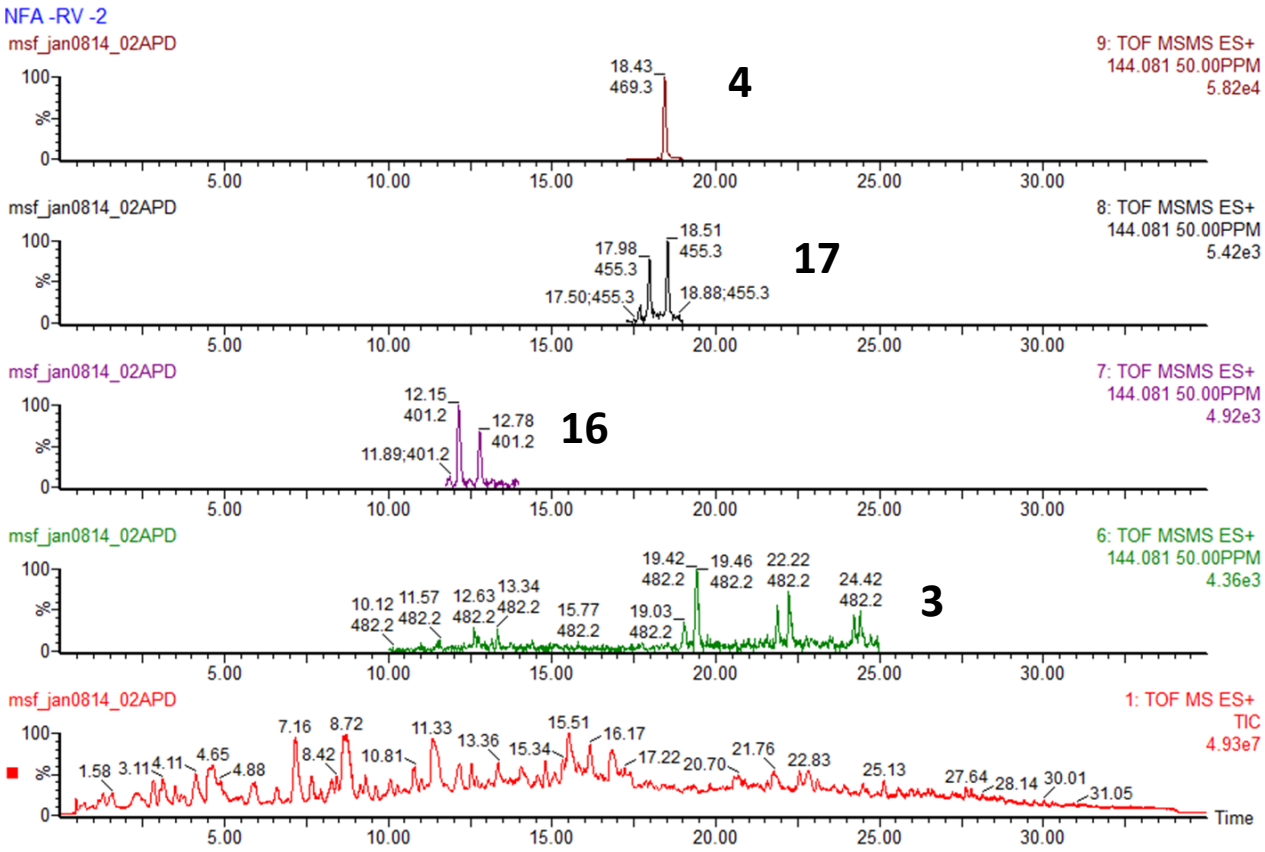

Figure S6. LC-ESI(+)-TOF-MSMS traces from *Nocardiopsis sp.* CMB-M0232 confirming presence of tabulated metabolites as given in Table 1 for m/z = 401.2 (**16**); 455.3 (des-N1′-Me-nocardioazine B (**17**)) and 469.3 (nocardioazine B (**4**)).

858

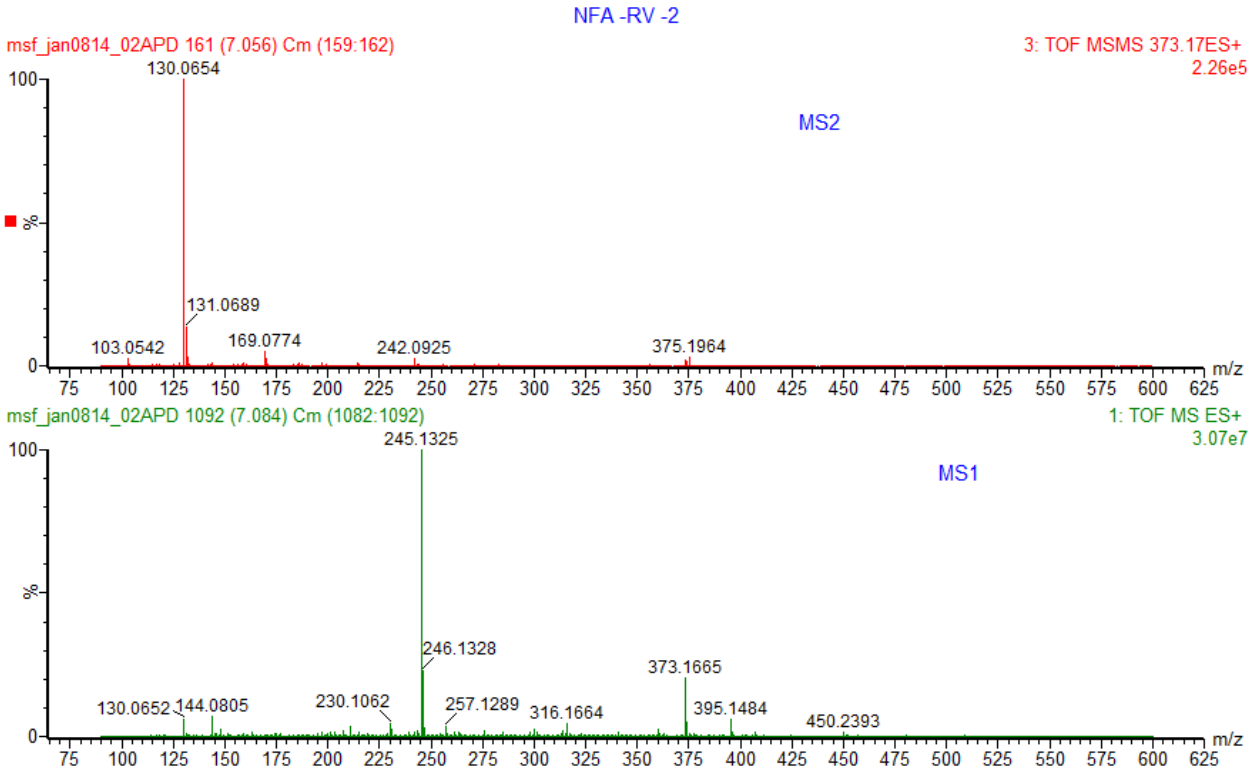

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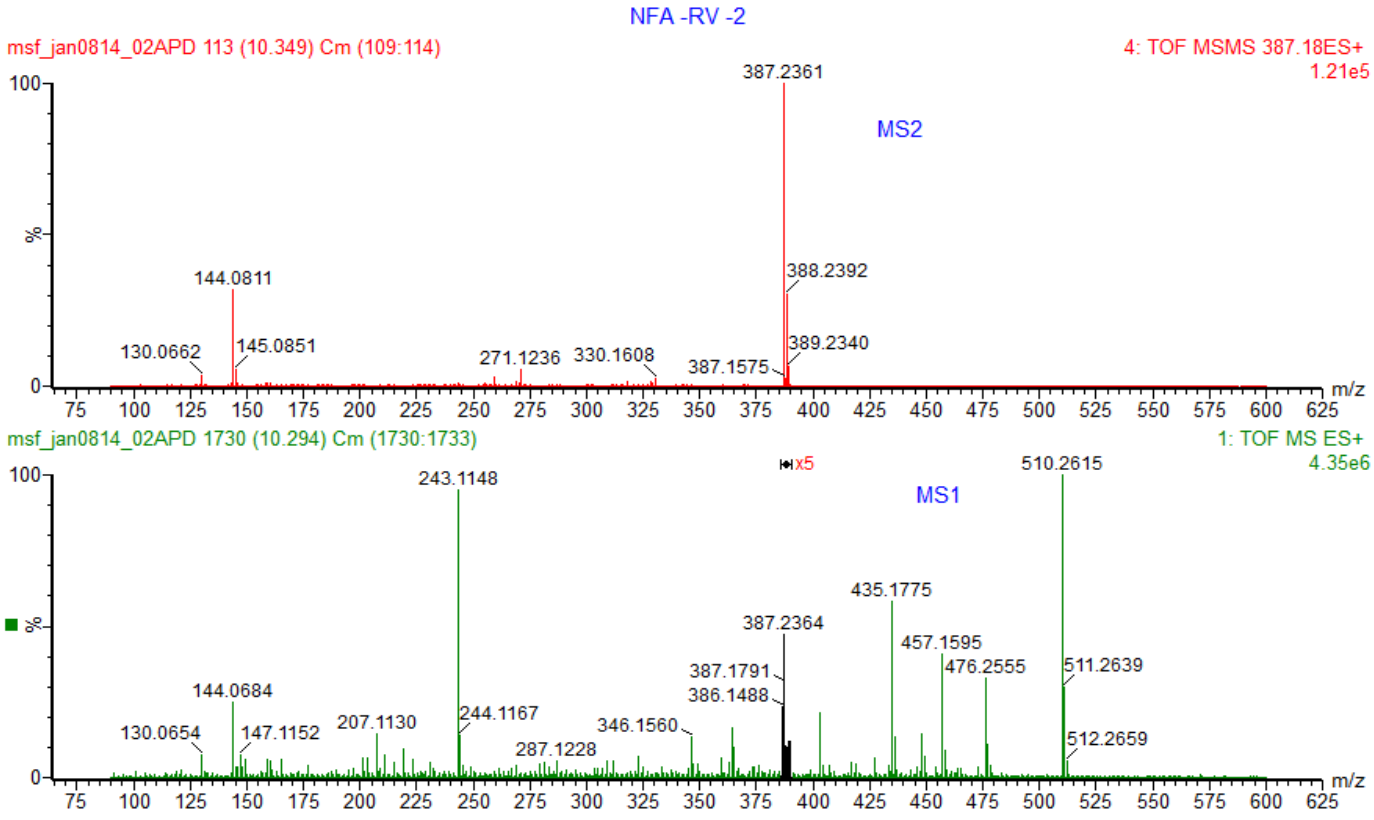
Figure S7. ESI(+)-TOF MS^1 and MS^2 spectra spectra for m/z = 373.1665 (**5**) extracted from from *Nocardiopsis sp.* CMB-M0232.

861



862

863

Figure S8. ESI(+)-TOF MS^1 and MS^2 spectra for m/z = 387.1791 (**13**) extracted from *Nocardiopsis sp.* CMB-M0232. *cyclo*-C3-Me-L-Trp-L-Trp DKP (**13**) (7.7 ppm error) is shown with blue arrow, 387.2384 (148 ppm error) is not **13,** and is a different unidentified metabolite. Also, 387.2361 not fragmented during MSMS where *Cyclo*-C3-Me-*L*-Trp-*L*-Trp DKP (**13**) was totally fragmented in standard sample. Also, a coeluting, metabolite at m.z 387.2361 was not fragmented. The presence of 144.0811 fragment (2.1 ppm error) indicates *cyclo*-C3-Me-L-Trp-L-Trp DKP (**13**).

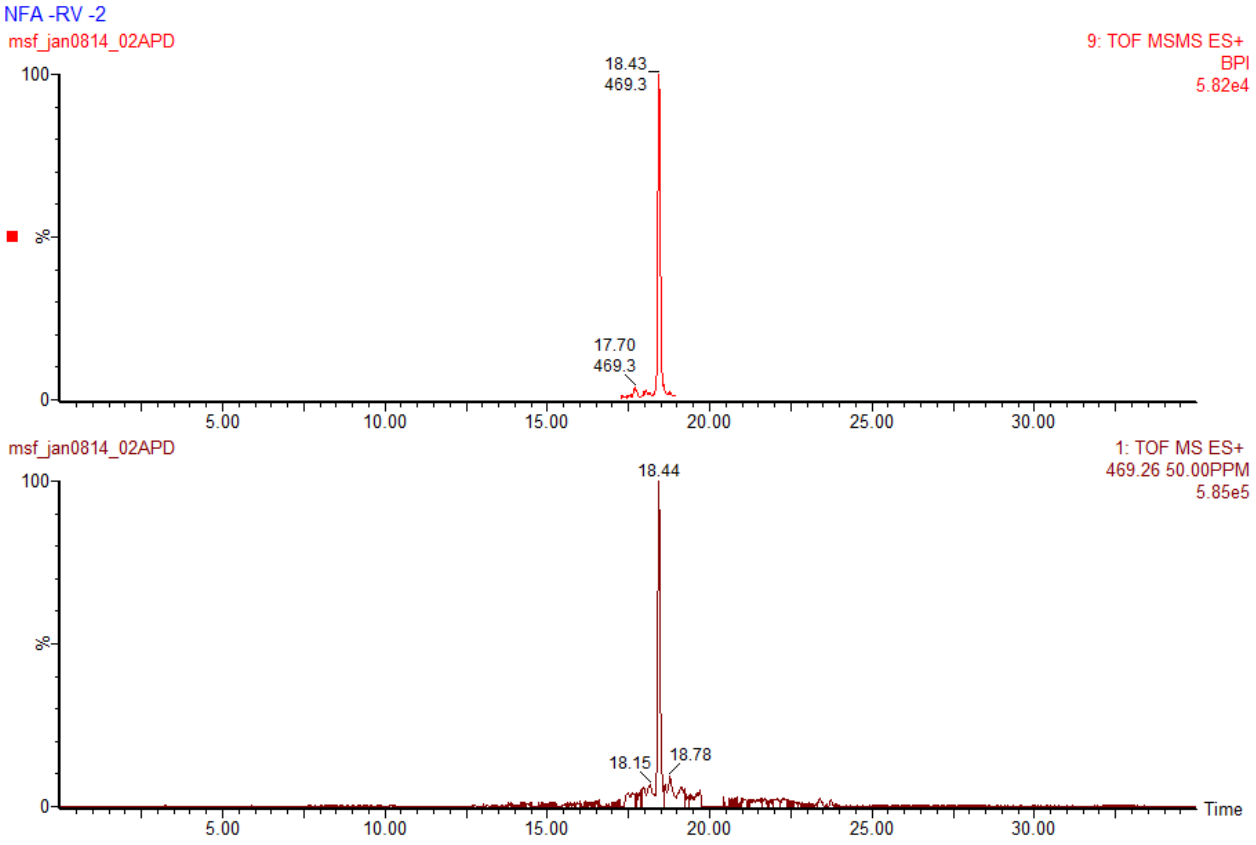
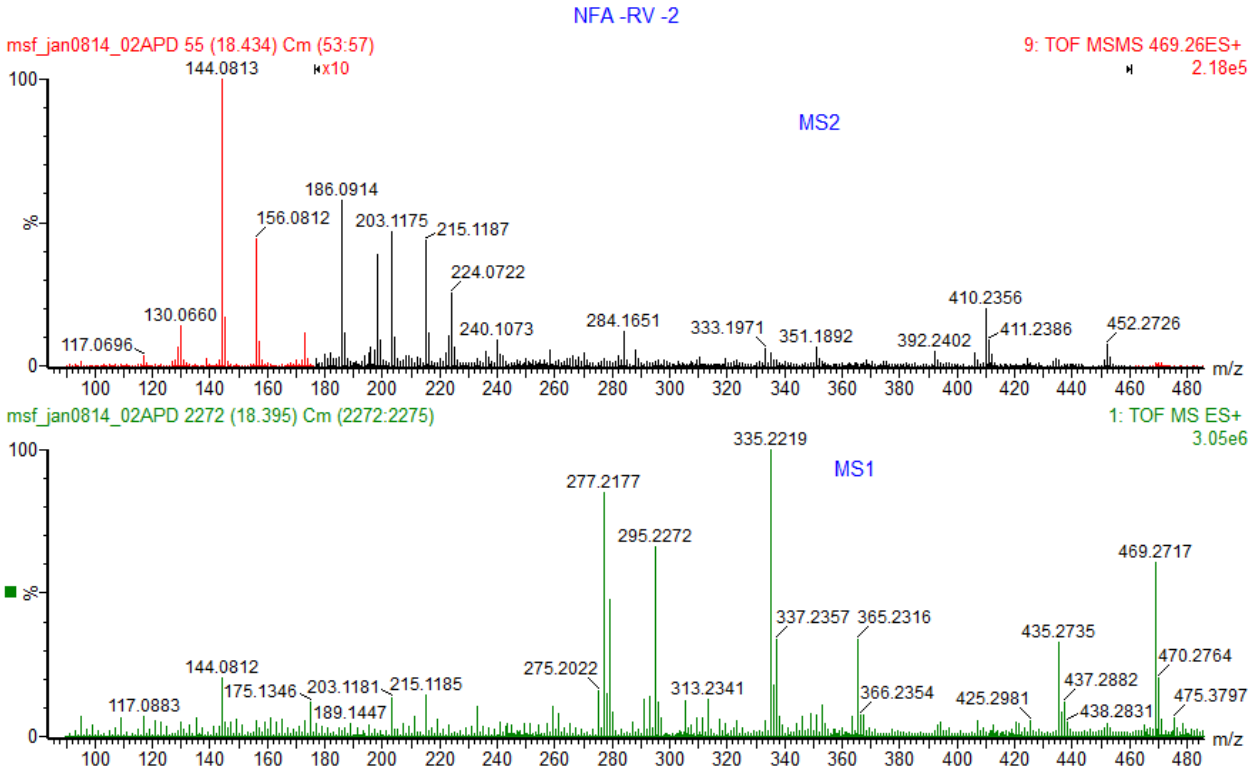


Figure S9. LC-ESI+ TOF MSMS spectra for m/z = 469.27 (nocardioazine B, **4**) extracted from from *Nocardiopsis sp.* CMB-M0232.

868



869

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Figure S10. Preliminary data for ESI(+)-TOF MS^1 and MS^2 spectra for m/z = 469.27; probably indicating nocardioazine B (**4**) from *Nocardiopsis sp.* CMB-M0232.

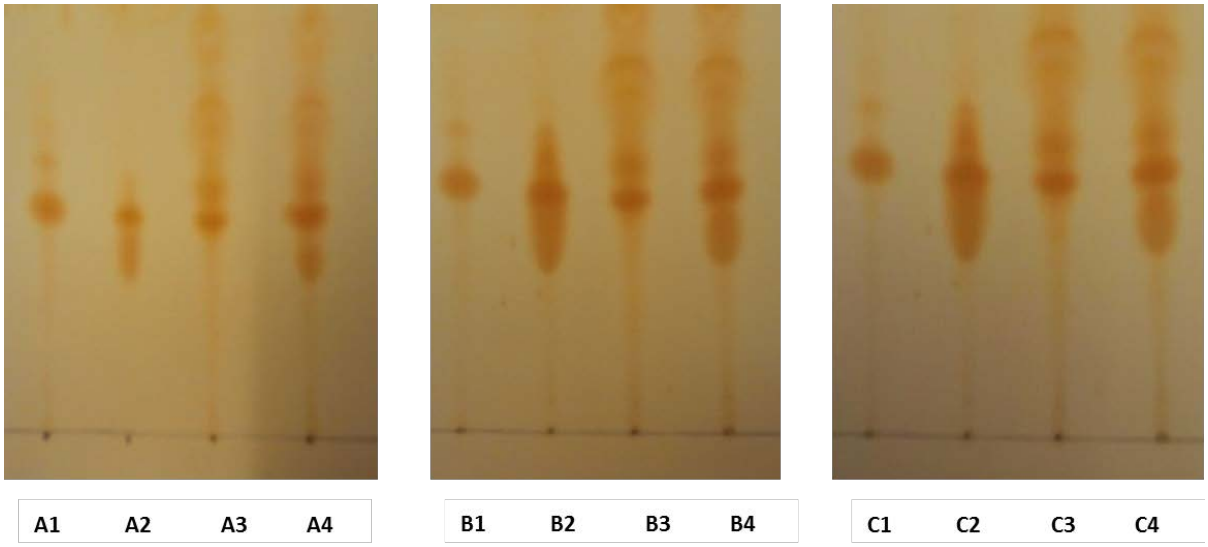
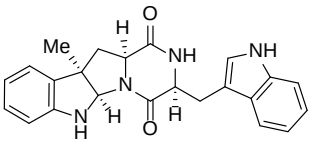


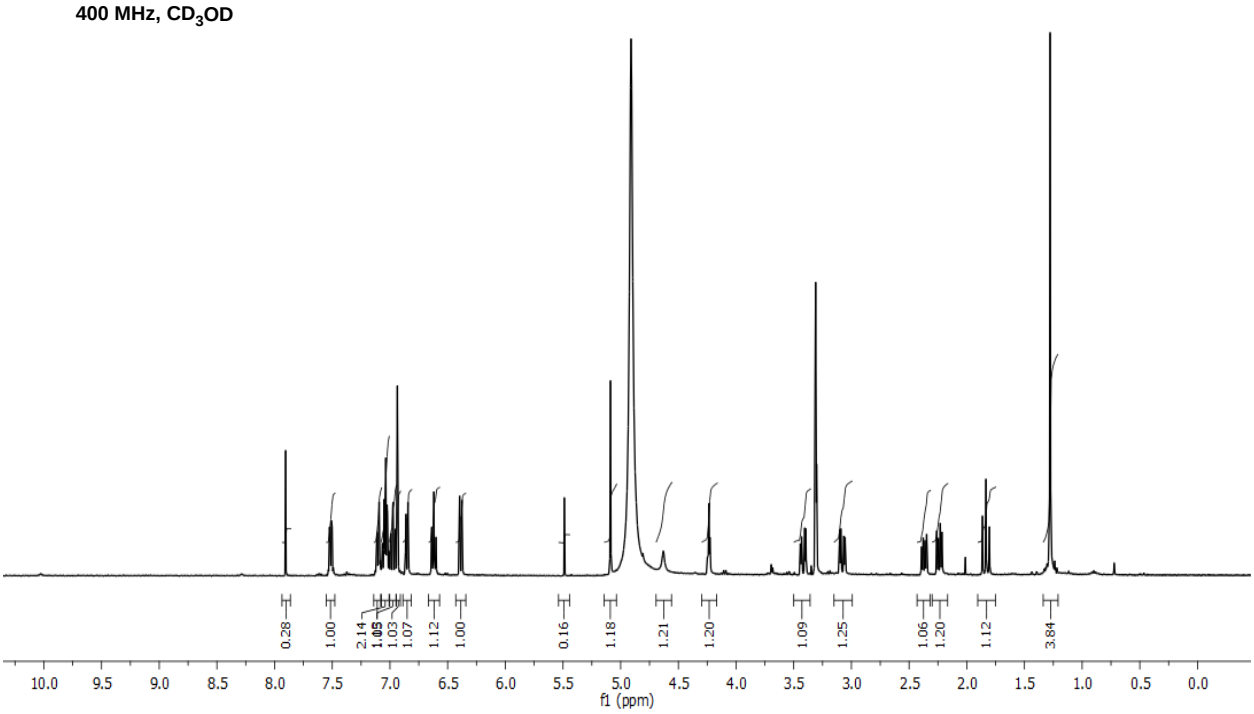
Figure S11. TLC images of intermediates extracted from *Nocardiopsis sp*. CMB M0322 corresponding to alkaloidal fractions. A1, B1 and C1 are synthetic standards of *Cyclo*-C3-Me-*L*-Trp-*L*-Trp DKP (**13**). A2 and A3are extracts of *Nocardiopsis sp*. CMB M0322 at 7 days from the time of inoculation. B2 and B3 are extracts of *Nocardiopsis sp*. CMB M0322 at 14 days from the time of inoculation. C2 and C3 are extracts of *Nocardiopsis sp*. CMB M0322 at 21 days from the time of inoculation. A4, B4 and C4 are co-spots between respective extracts and synthetic standard of **13**.

886

887 **NMR for compound from extract**:



13



888

889 **Figure S12.** ^{1}H NMR spectrum for **13** extracted from *Nocardiopsis sp.* CMB-M0232 after 21 days of culture followed by fractionation and purification. Purification methods followed procedures adopted for the synthesis of **13** except culture extracts were used instead of crude reaction mixtures.

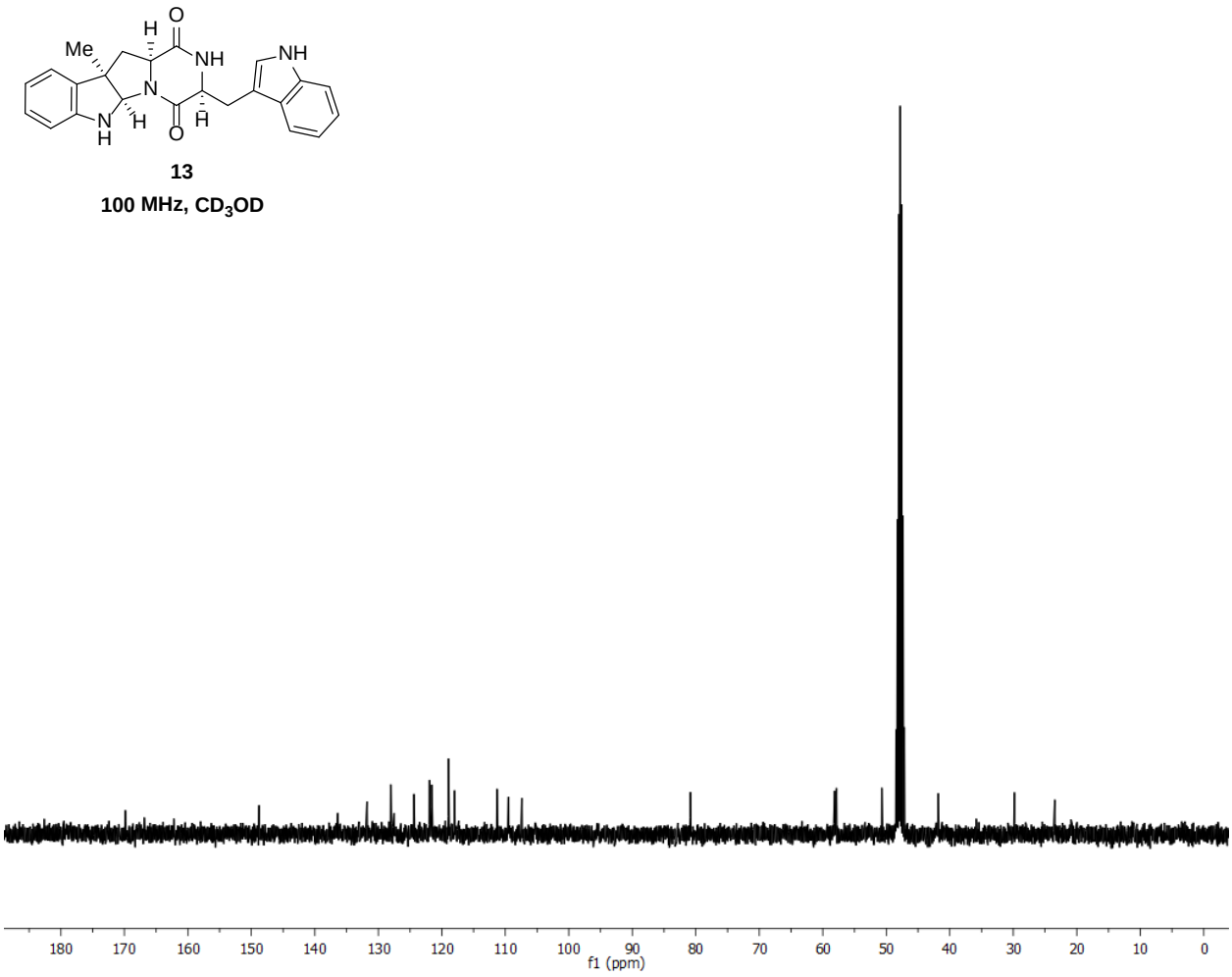


Figure S13. ^{13}C NMR spectrum for **13** extracted from *Nocardiopsis sp.* CMB-M0232 – 6.9-7.15 ppm.

S29. Cyclo-L-Trp-L-Trp-DKP (**5**) HPLC analysis[17]

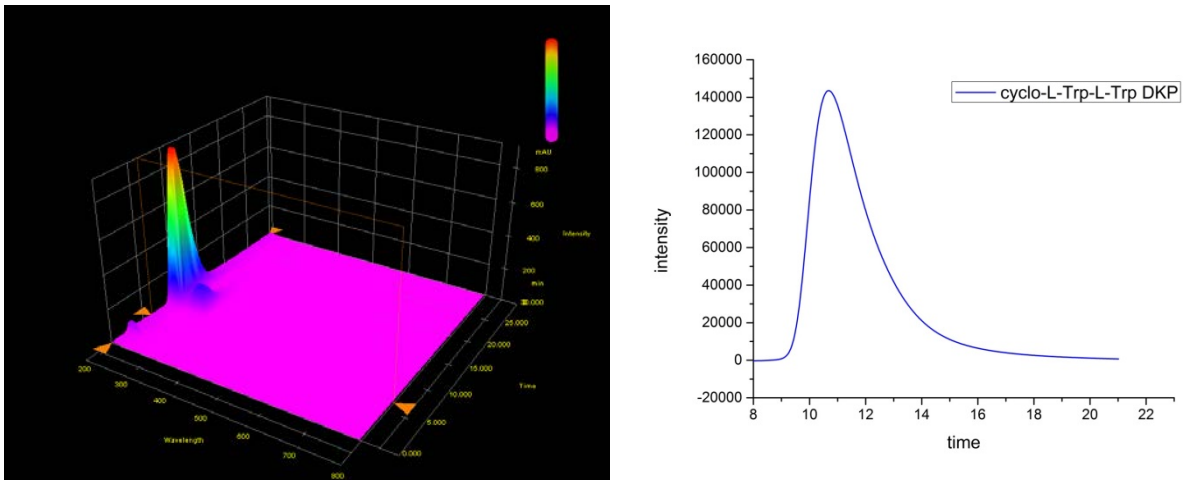


Figure S14. HPLC analyses of *cyclo*-L-Trp-L-Trp-DKP (**5**).

S30. Cyclo-D-Trp-D-Trp-DKP (***ent*-5**) HPLC analysis.

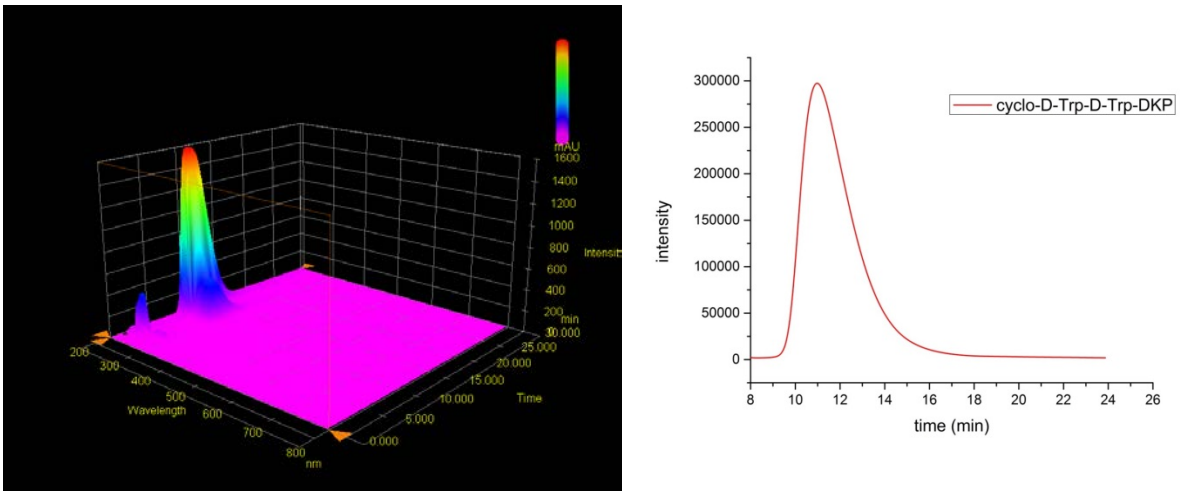
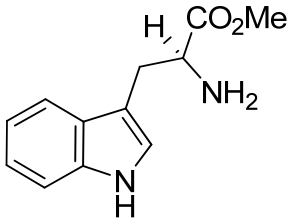


Figure S15. HPLC analyses of *cyclo*-D-Trp-D-Trp-DKP (*ent*-**5**).

[17] A considerable asymmetry is noticeable in the HPLC traces and we attribute this to the fact that **5** and ***ent*-5** are polar entities and show significant "tailing" effect on a normal phase chiral AS column under the conditions observed.

S31. COPIES OF NMR SPECTRA



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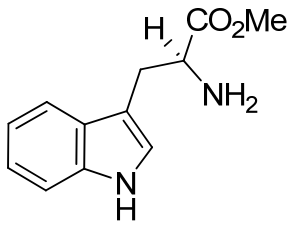

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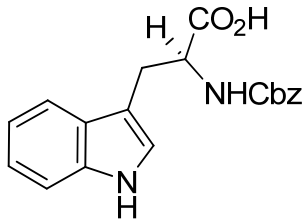

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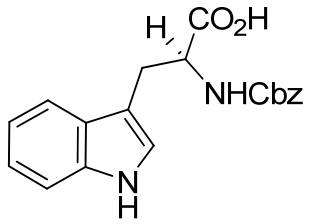

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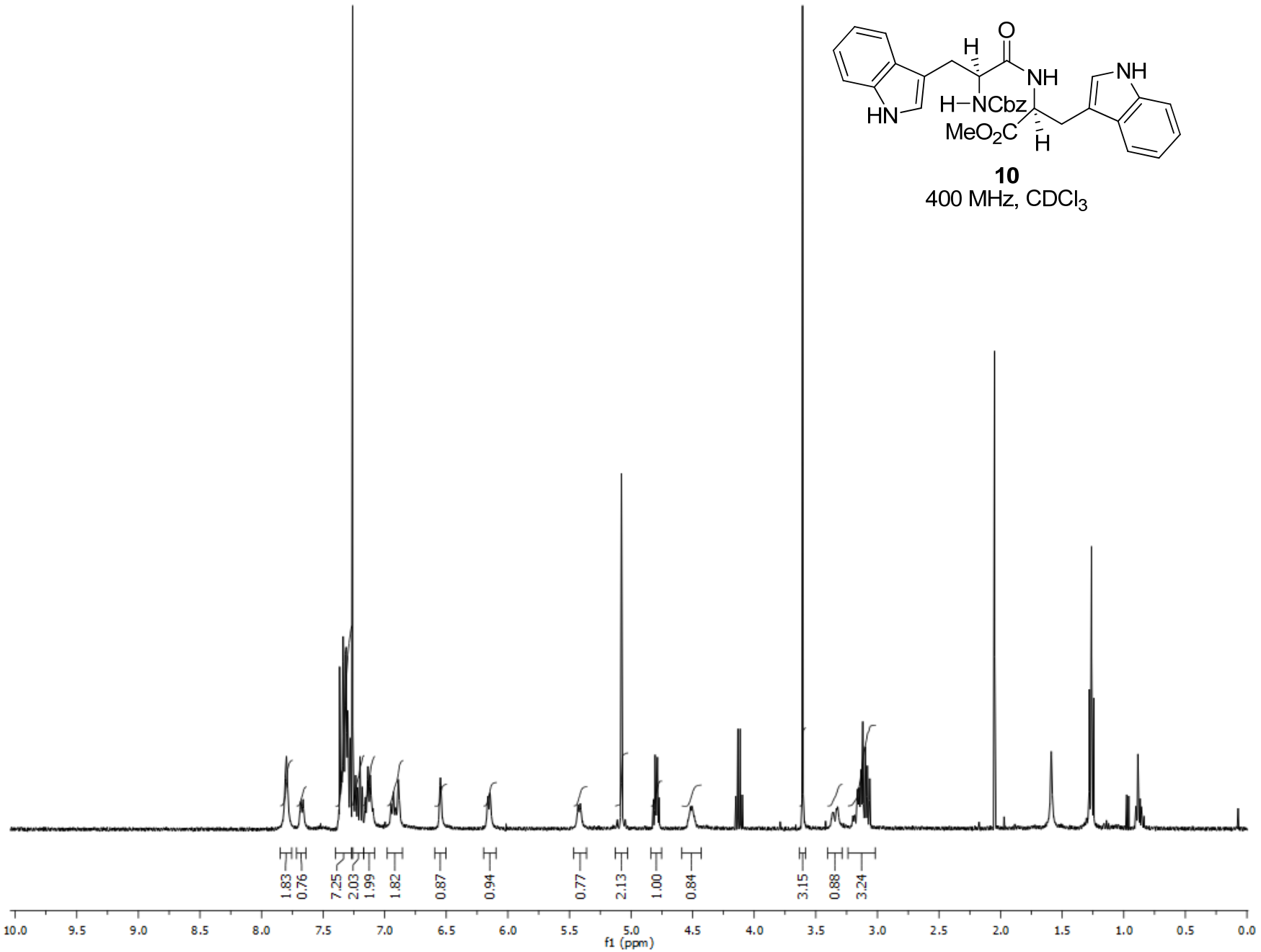

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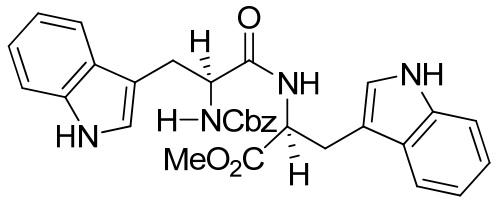

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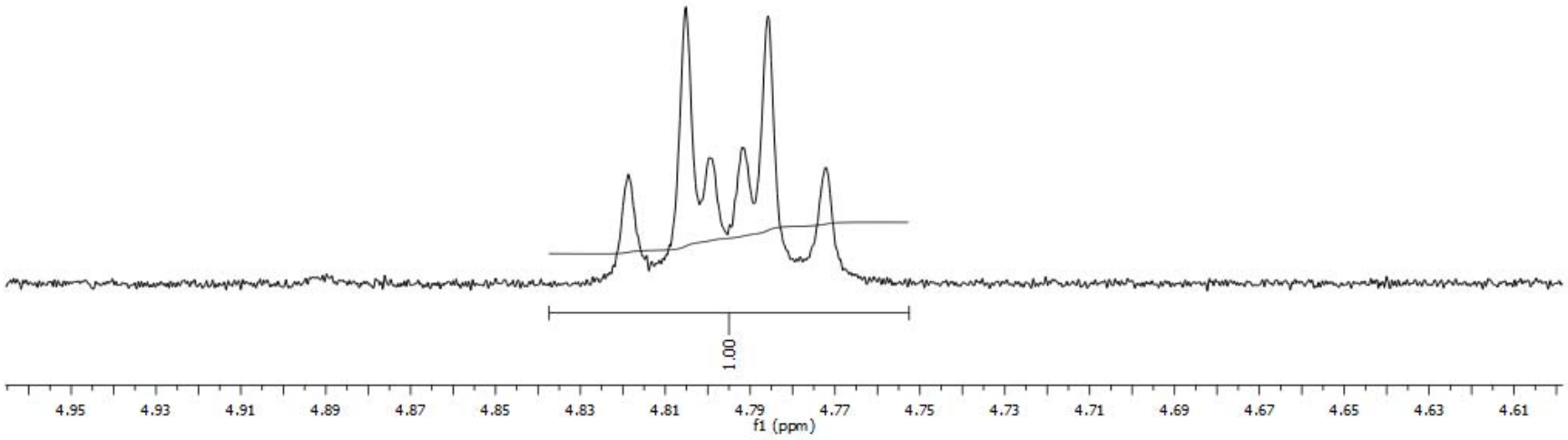

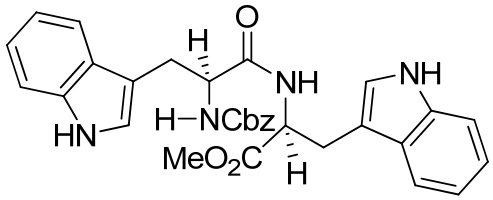

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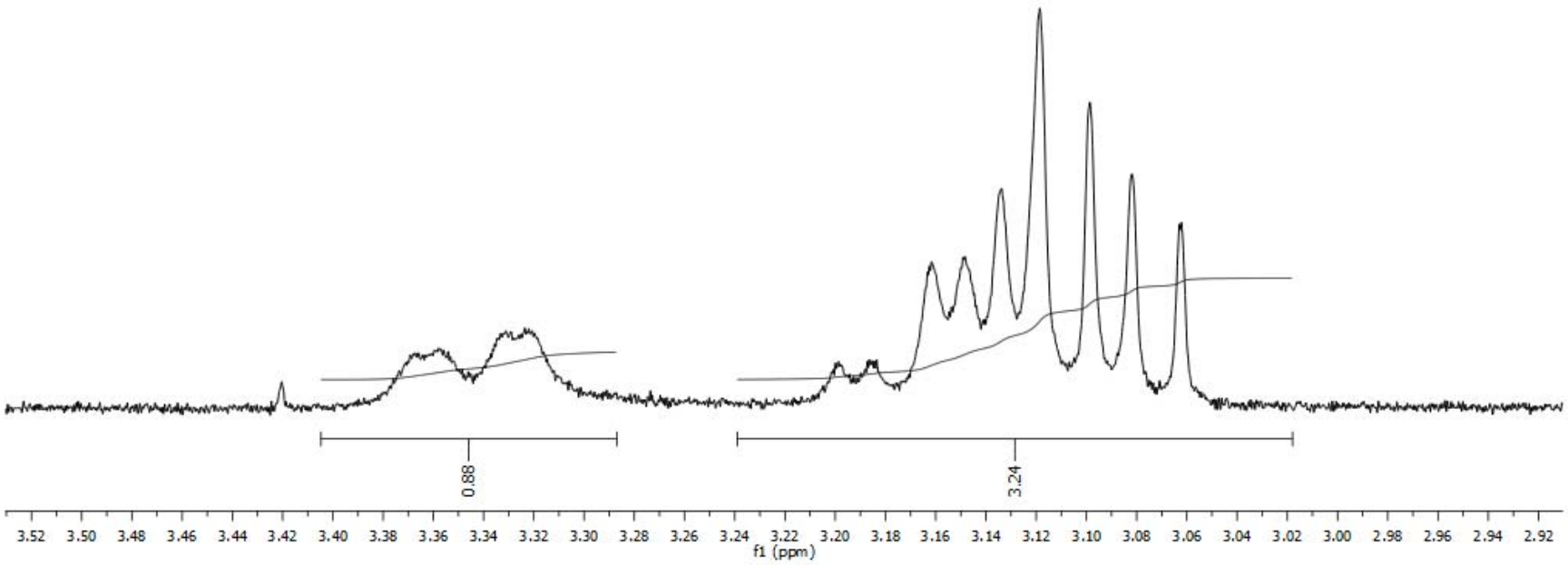

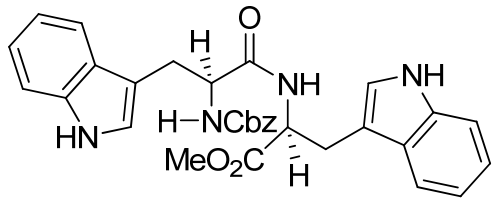

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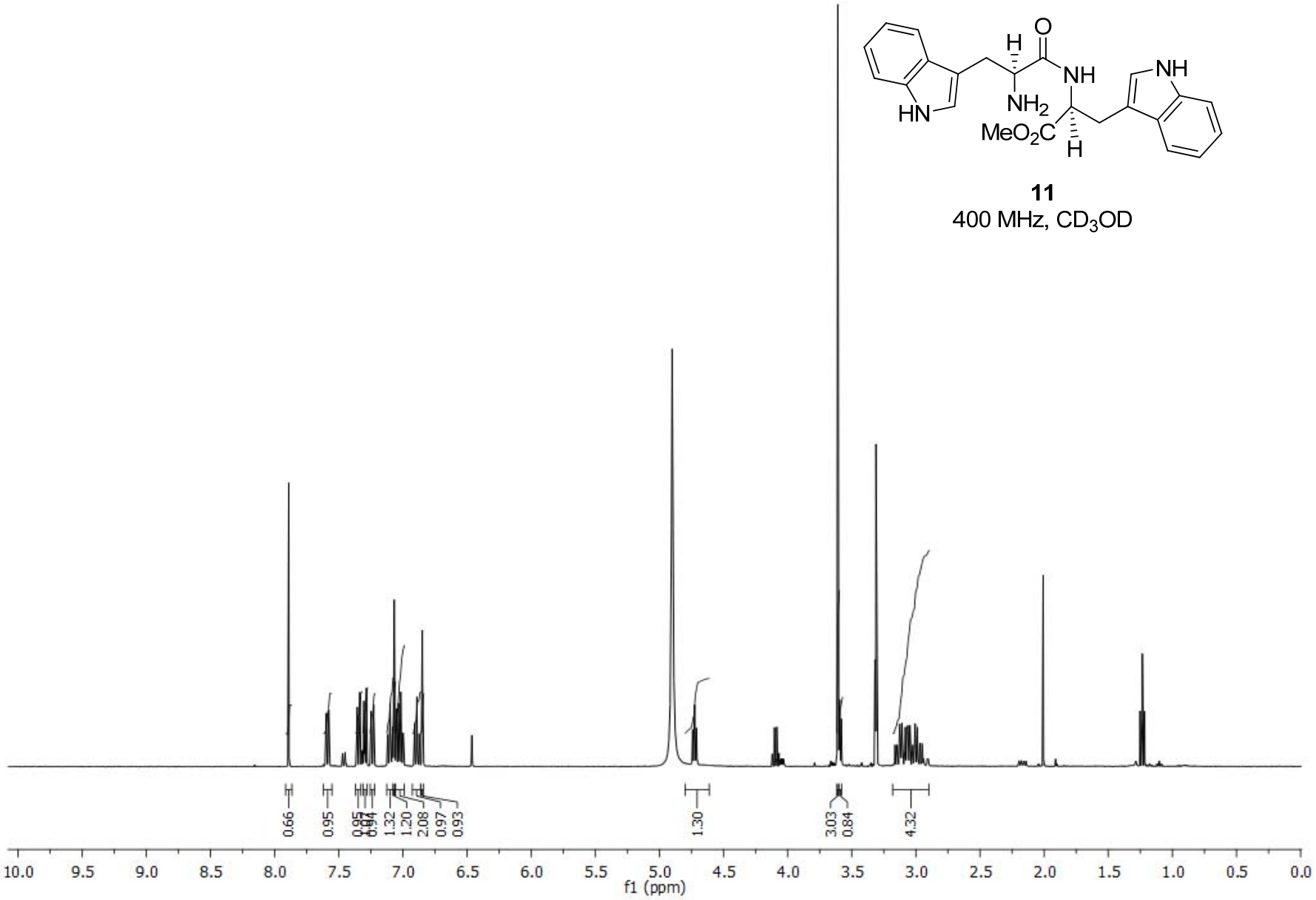
O
H
NH
NH
NH2
HN
MeO2C
H
11
400 MHz, CD3OD
0.66
0.95
0.85
0.94
1.32
1.20
2.08
0.97
0.93
1.30
3.03
0.84
4.32
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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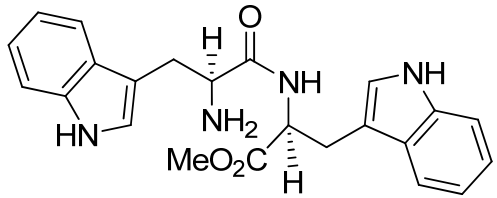
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



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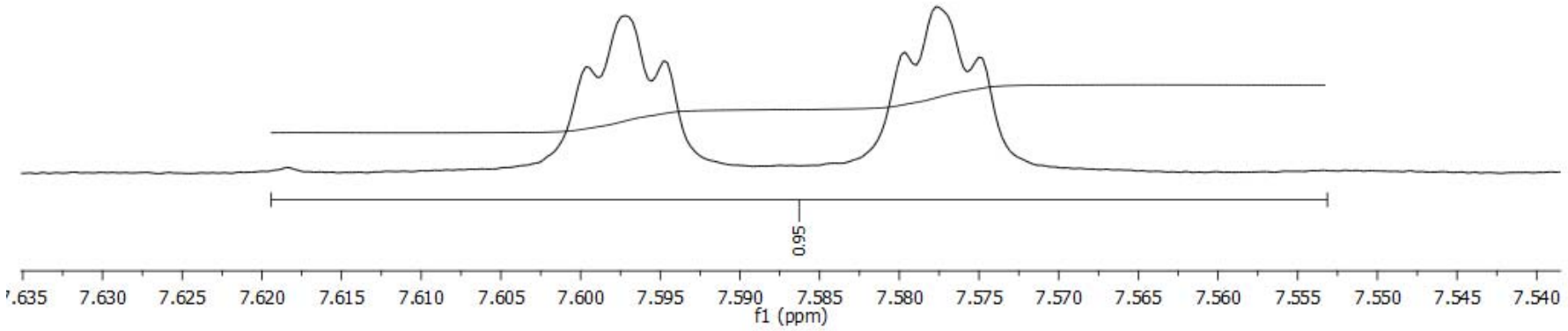

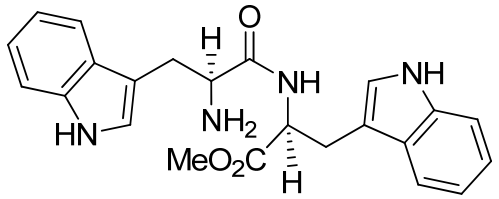

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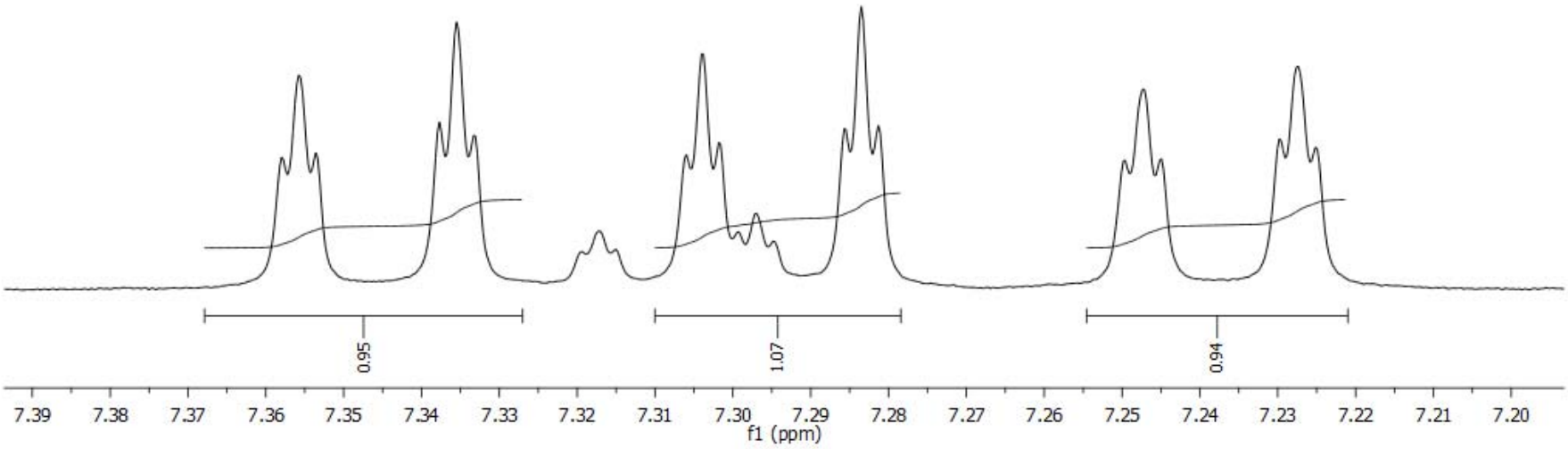

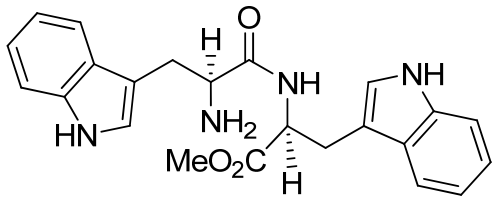

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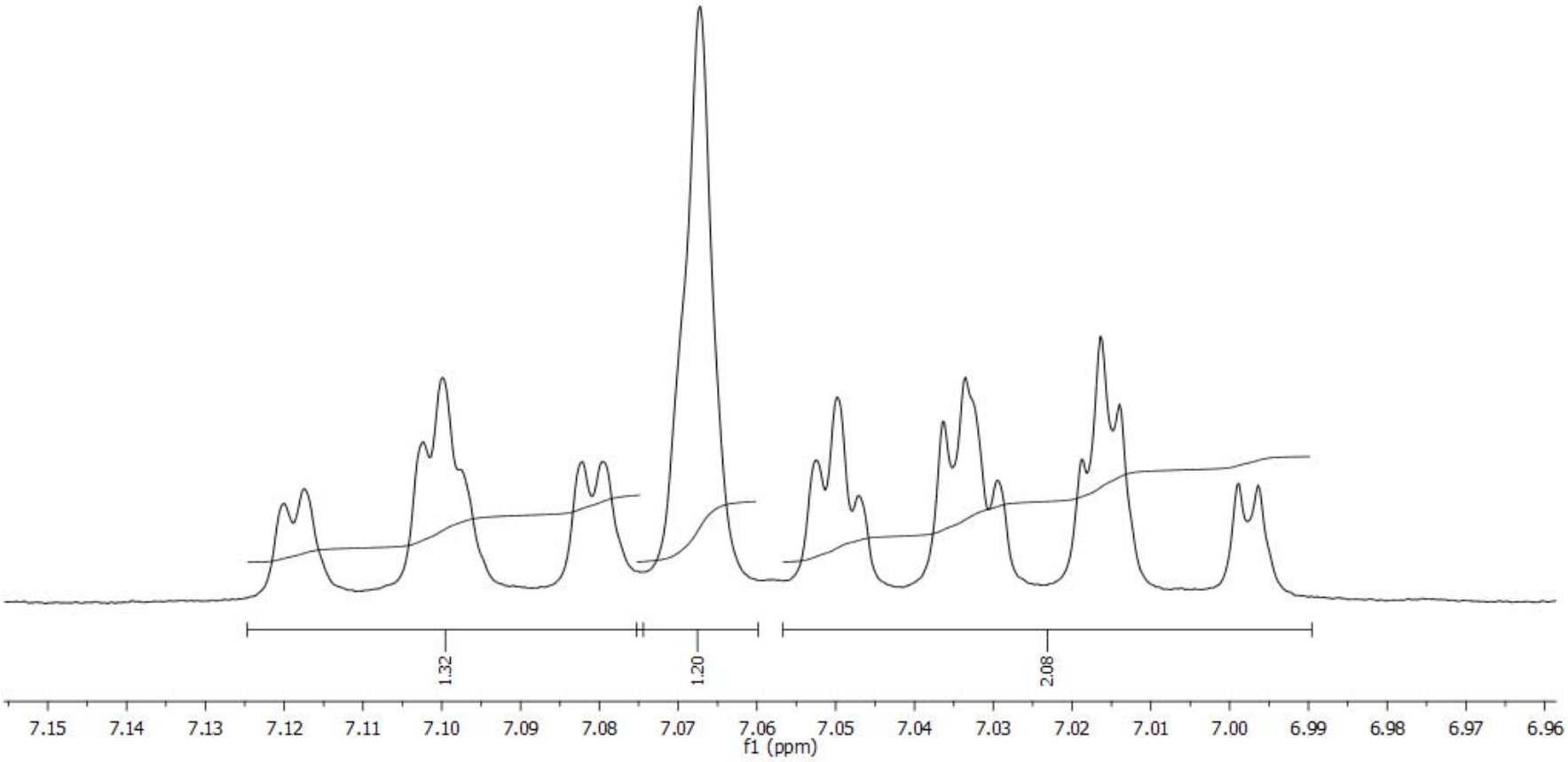

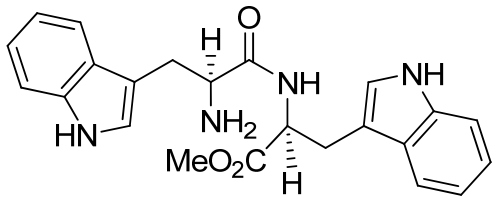

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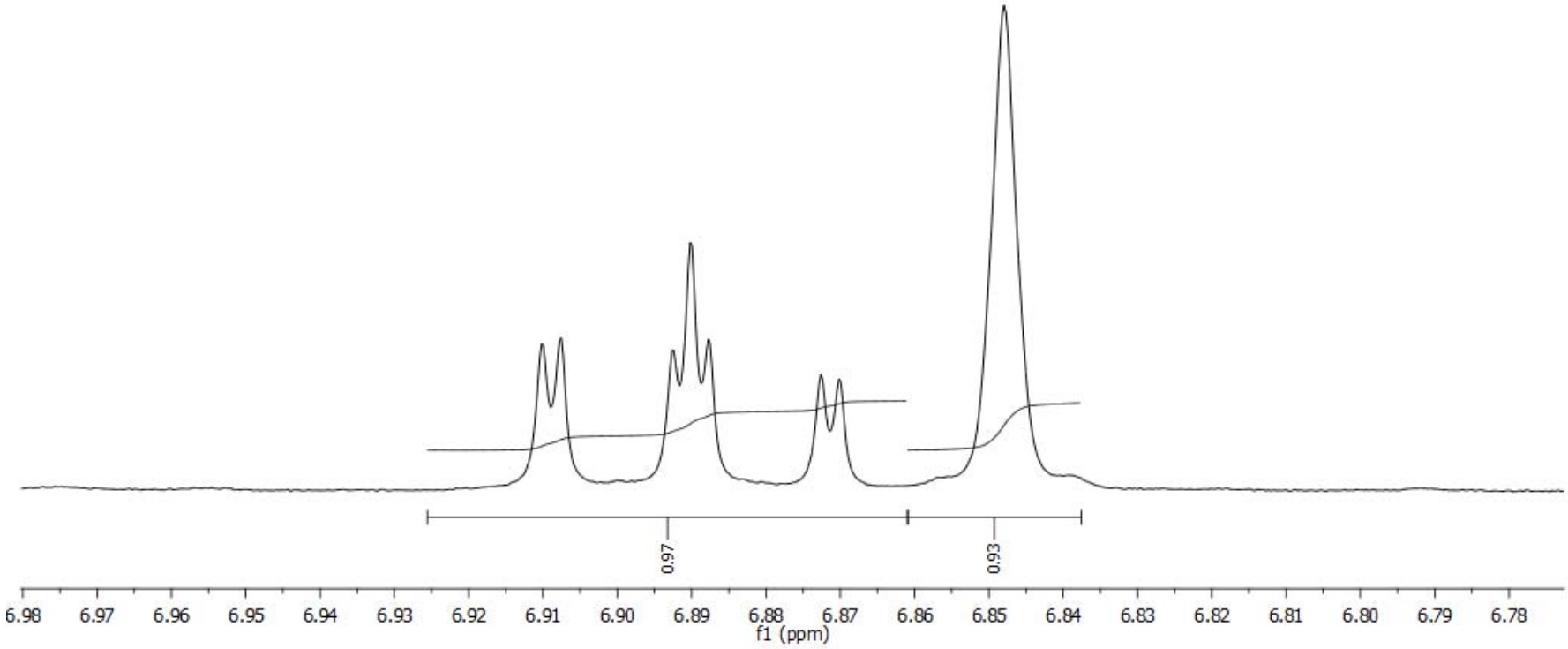

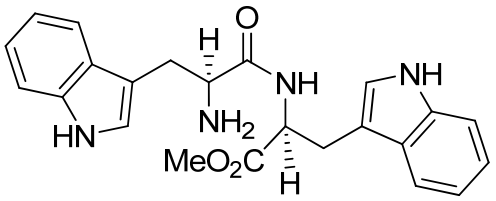

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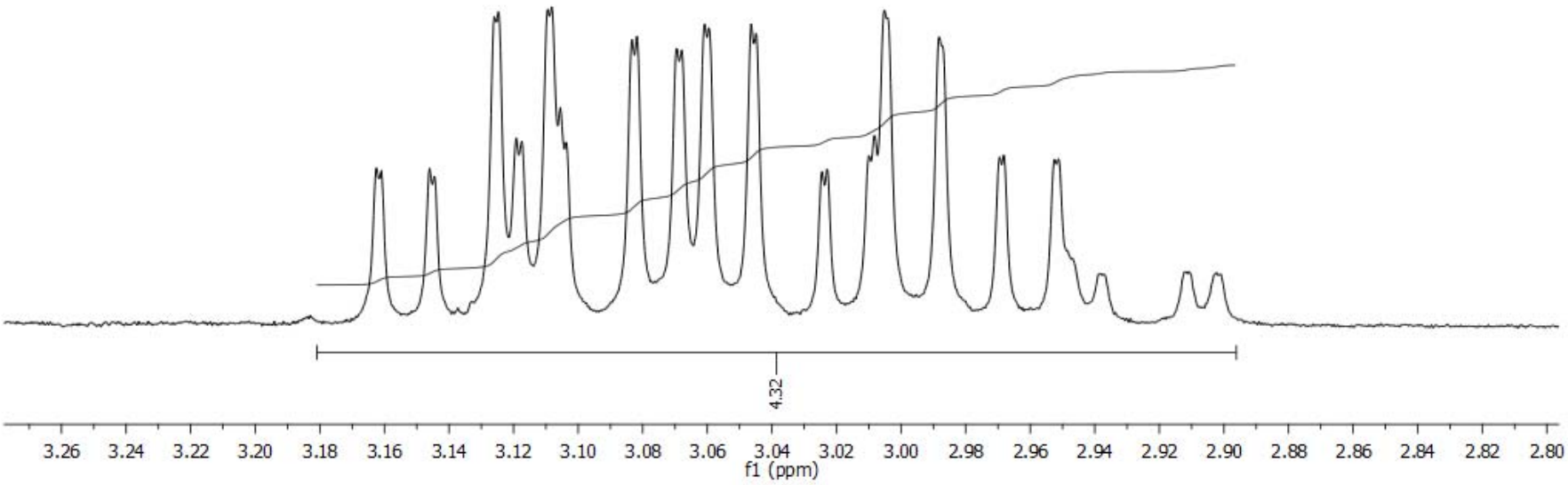

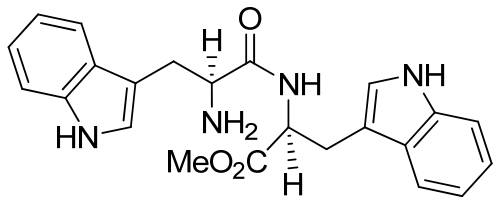

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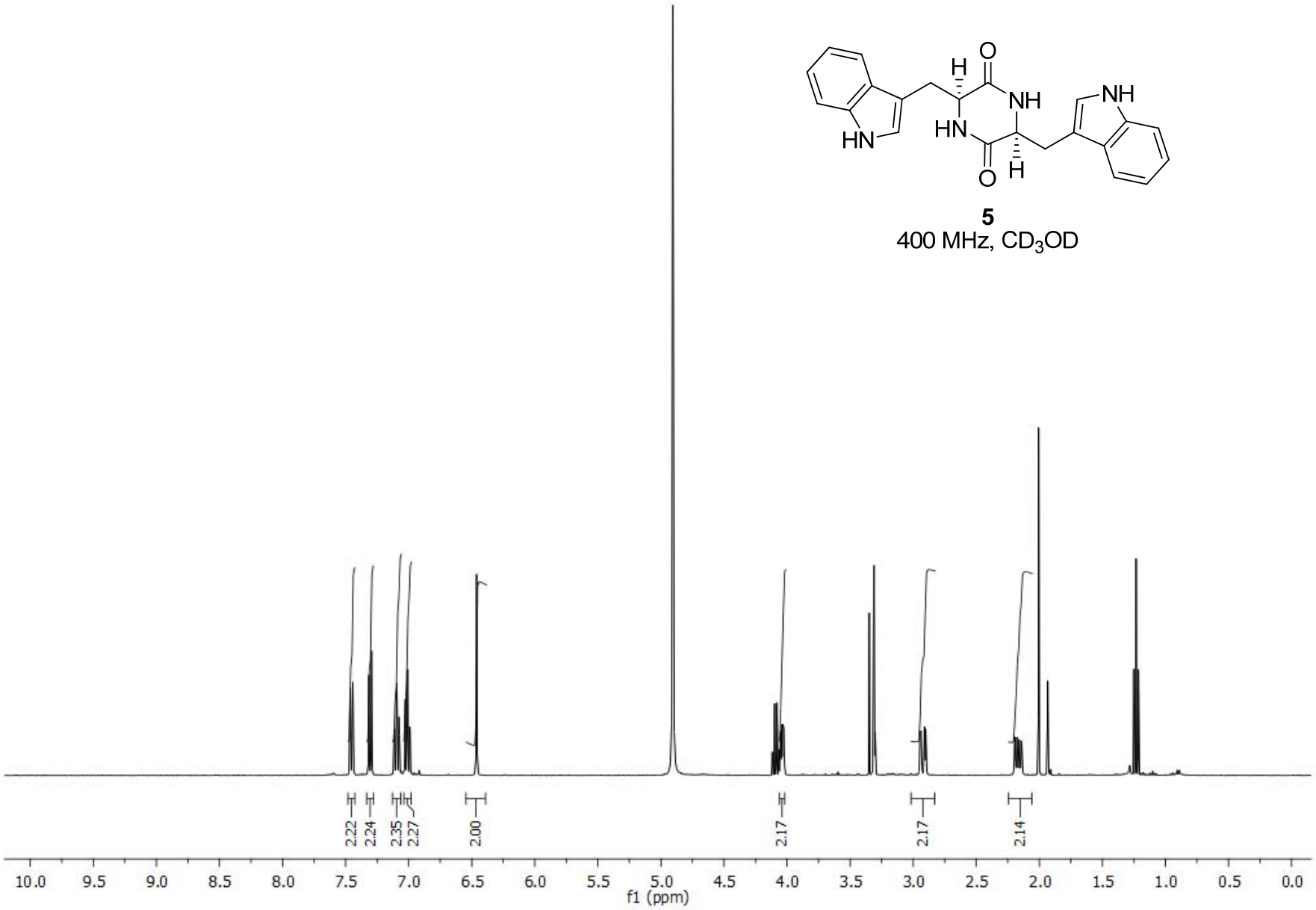

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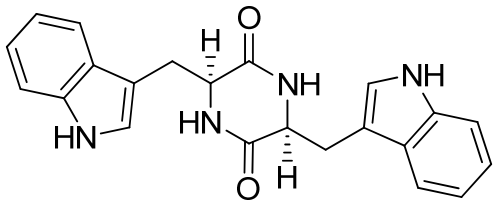

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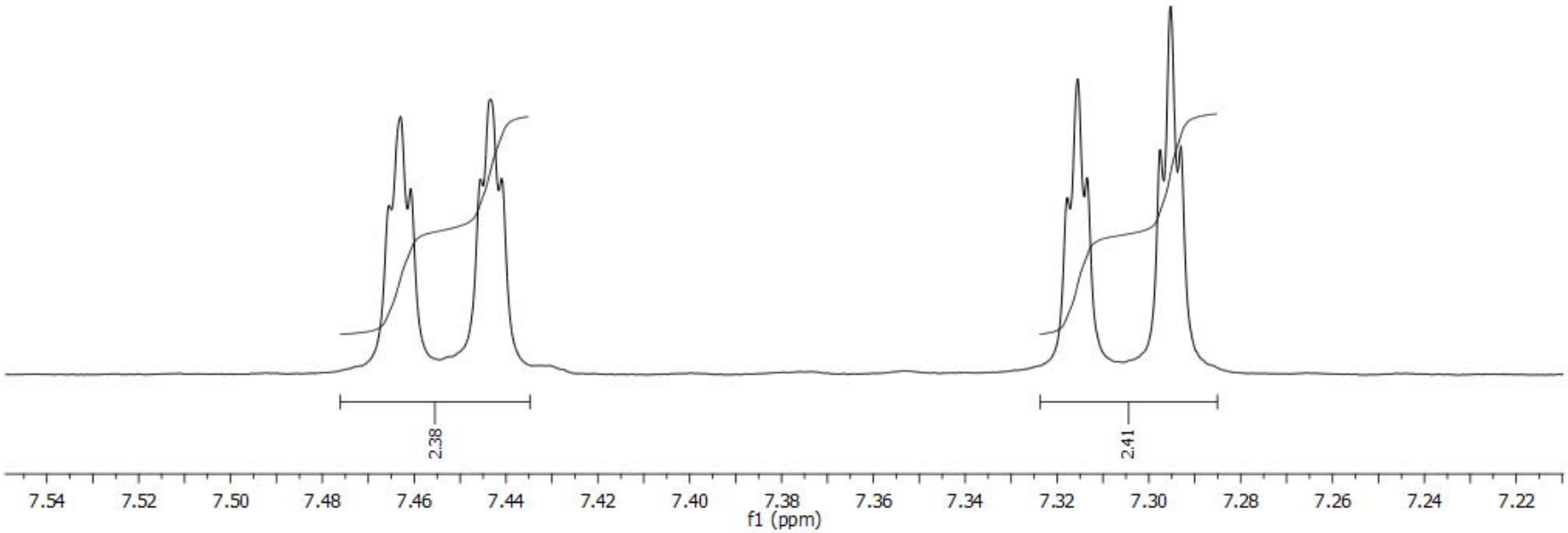

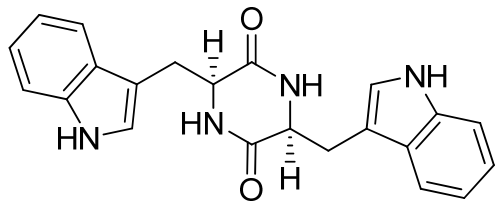

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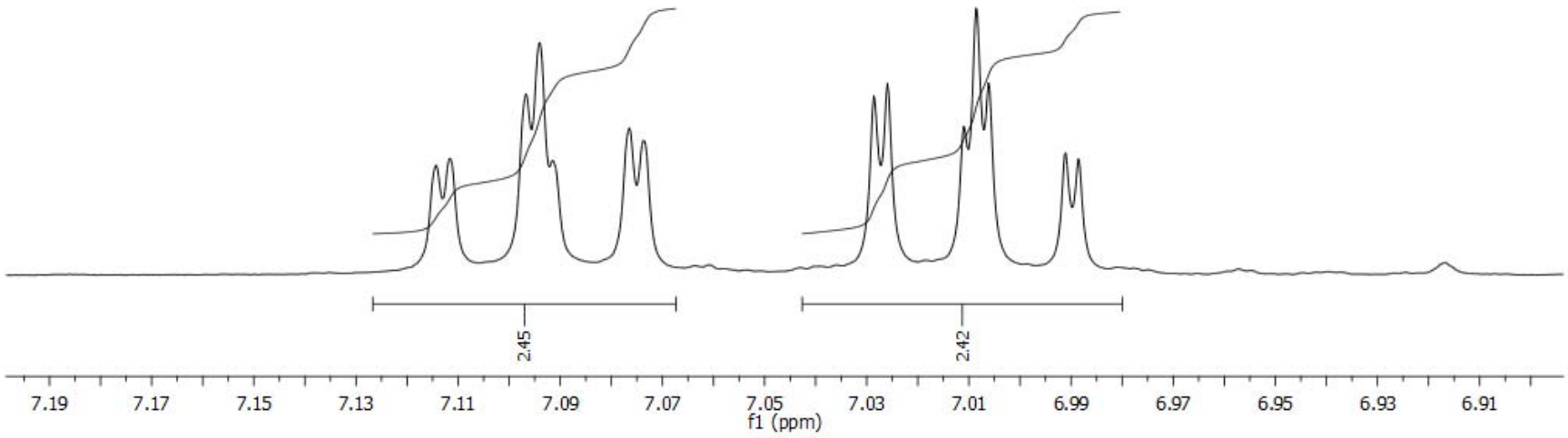

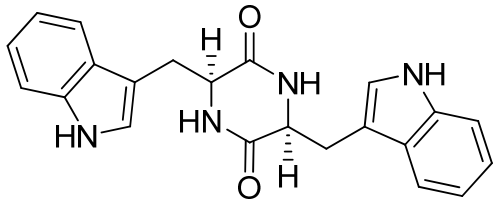

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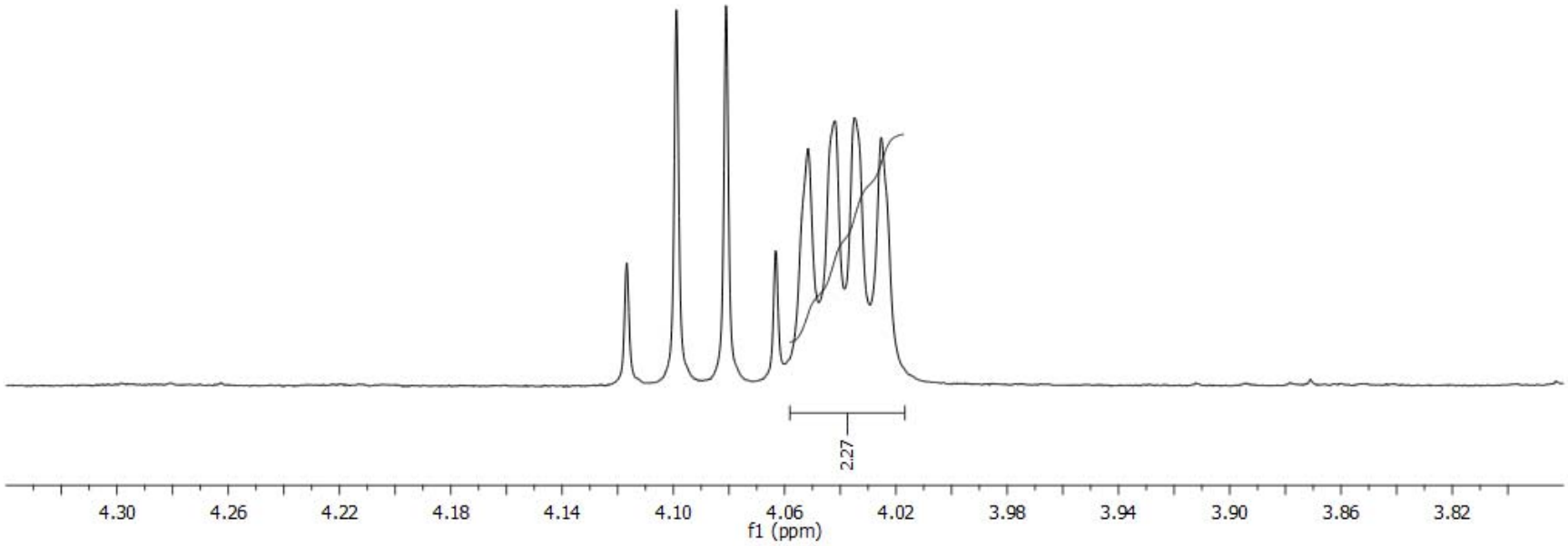

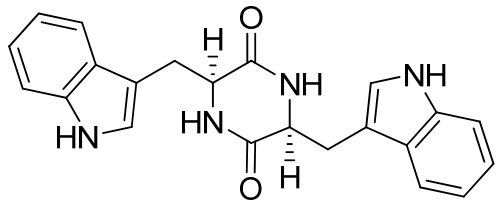

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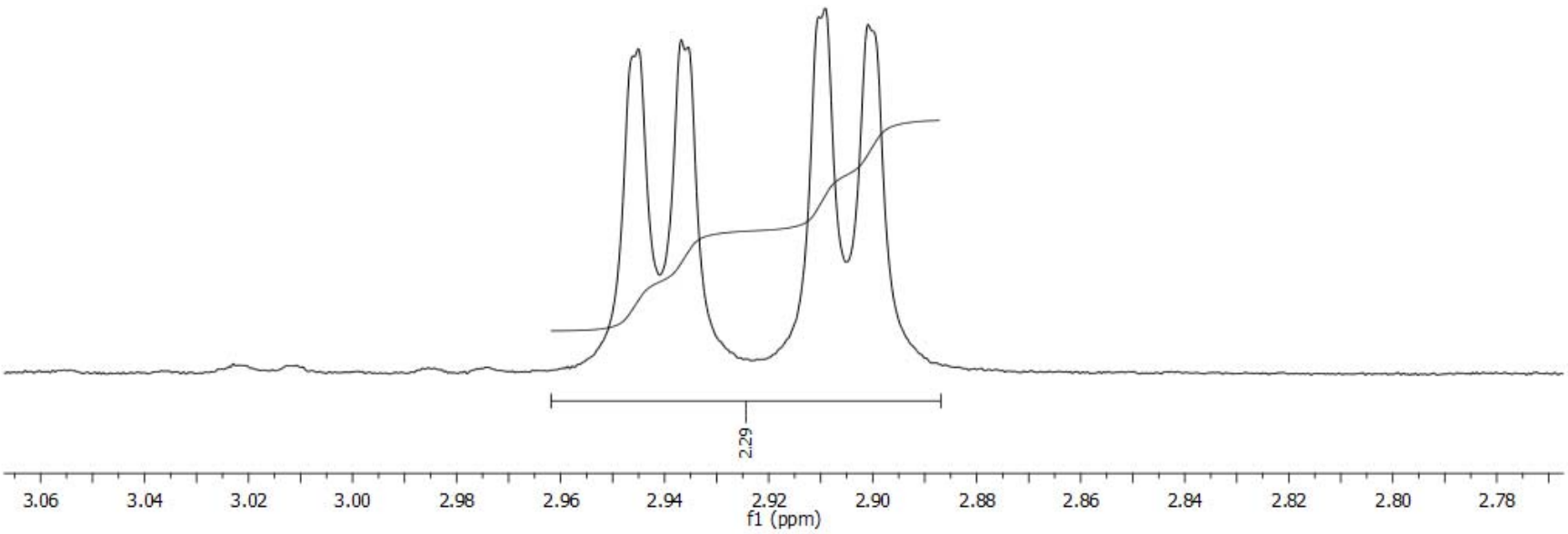

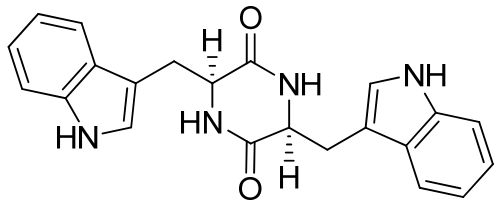

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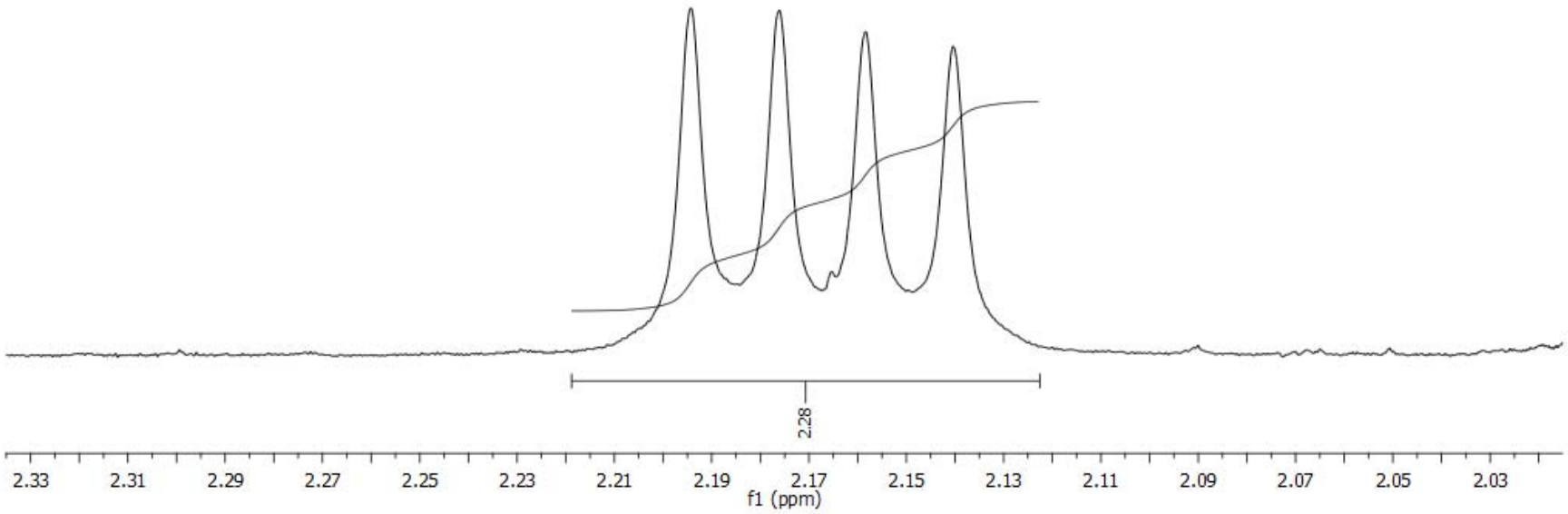
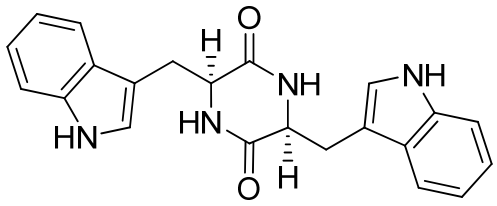
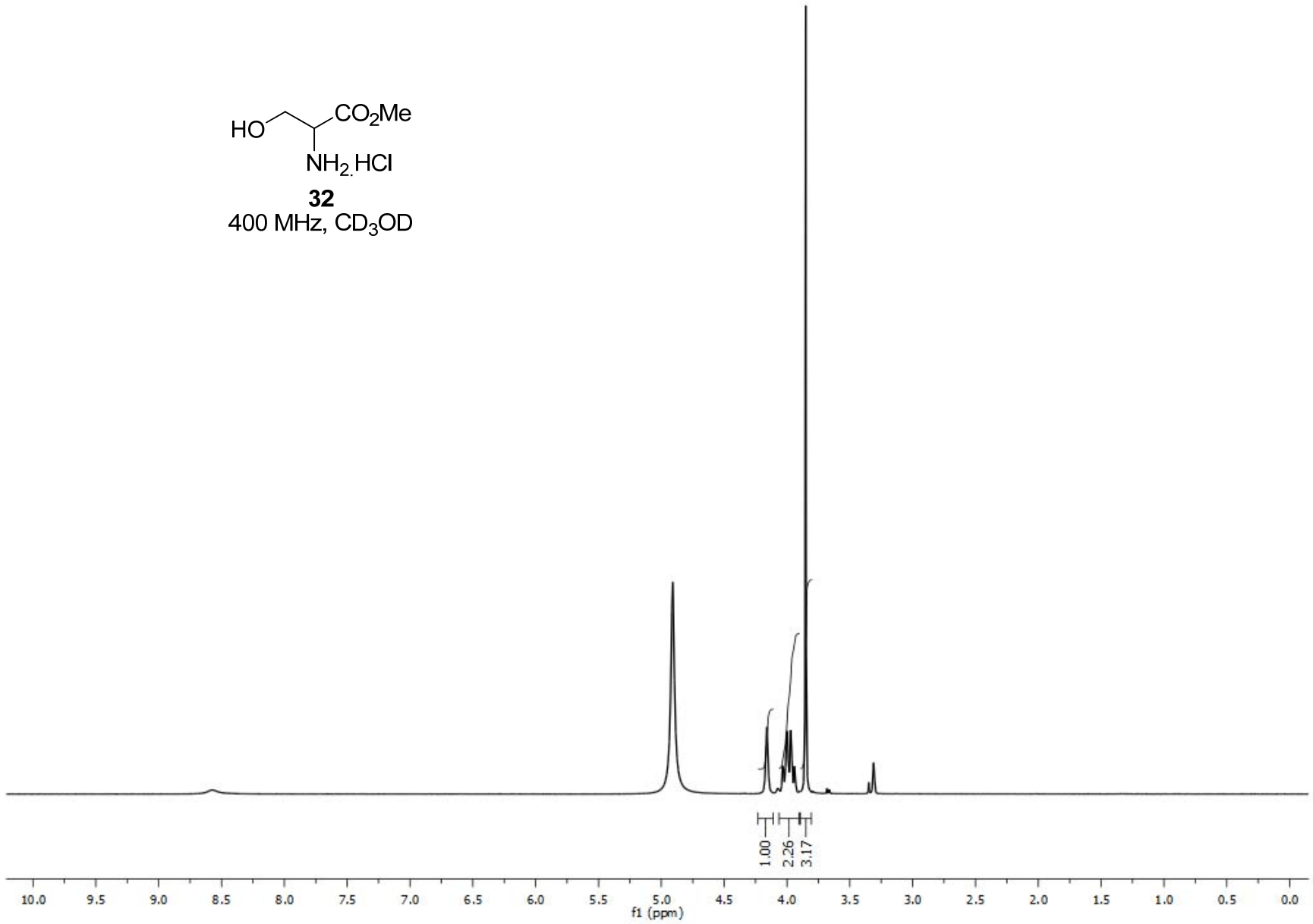
400 MHz, CD_3OD

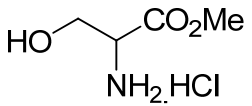




5

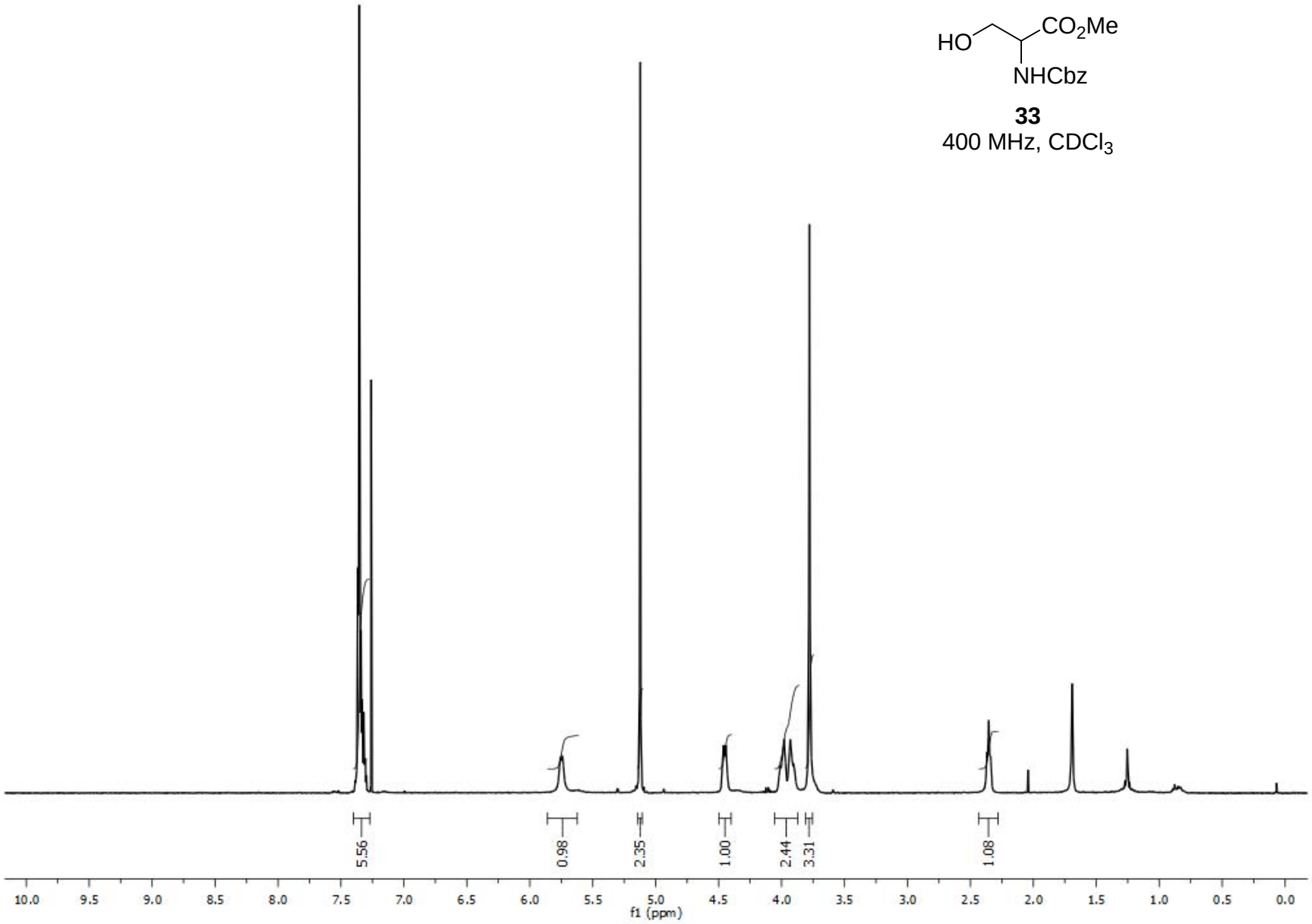
100 MHz, CD_3OD

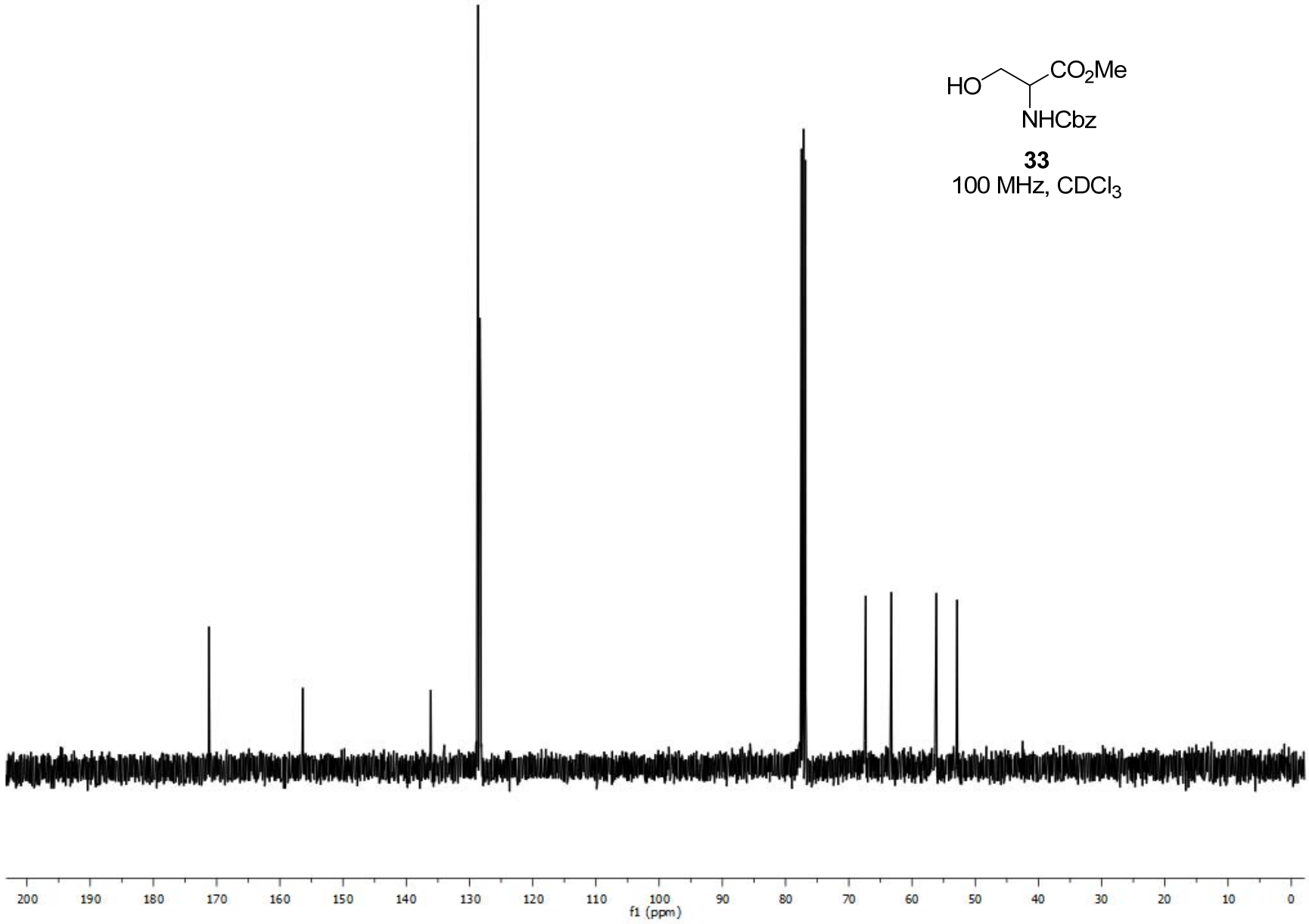

HO
CO2Me
NH2.HCl
32
400 MHz, CD3OD
1.00
2.26
3.17
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

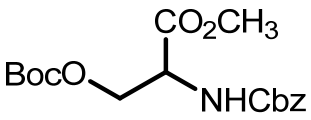


32

100 MHz, CD_3OD

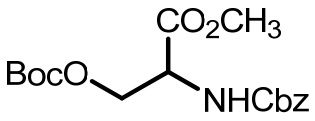

HO
CO2Me
NHCbz
33
400 MHz, CDCl3
5.56
0.98
2.35
1.00
2.44
3.31
1.08
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


HO
CO2Me
NHCbz
33
100 MHz, CDCl3
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



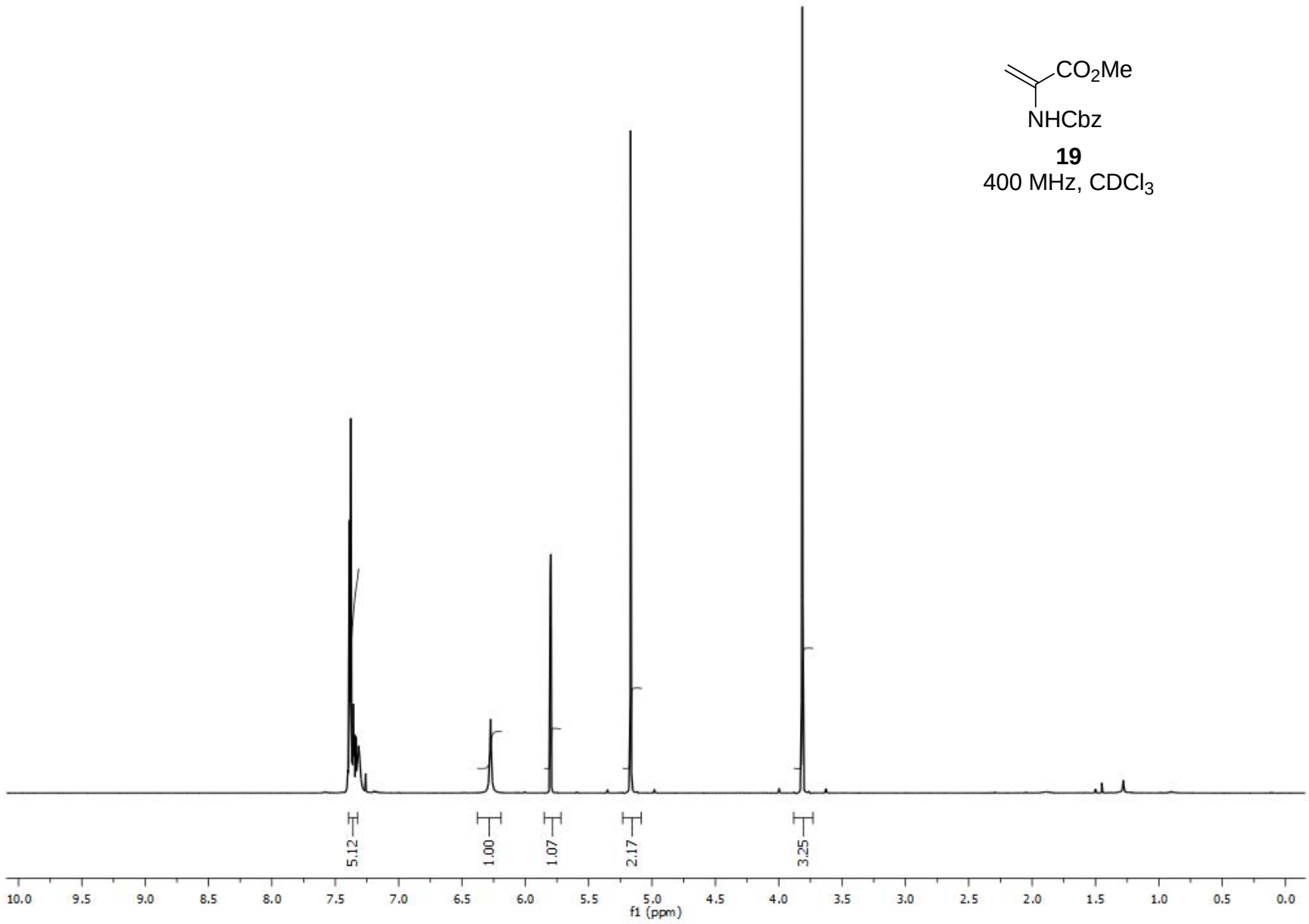
34

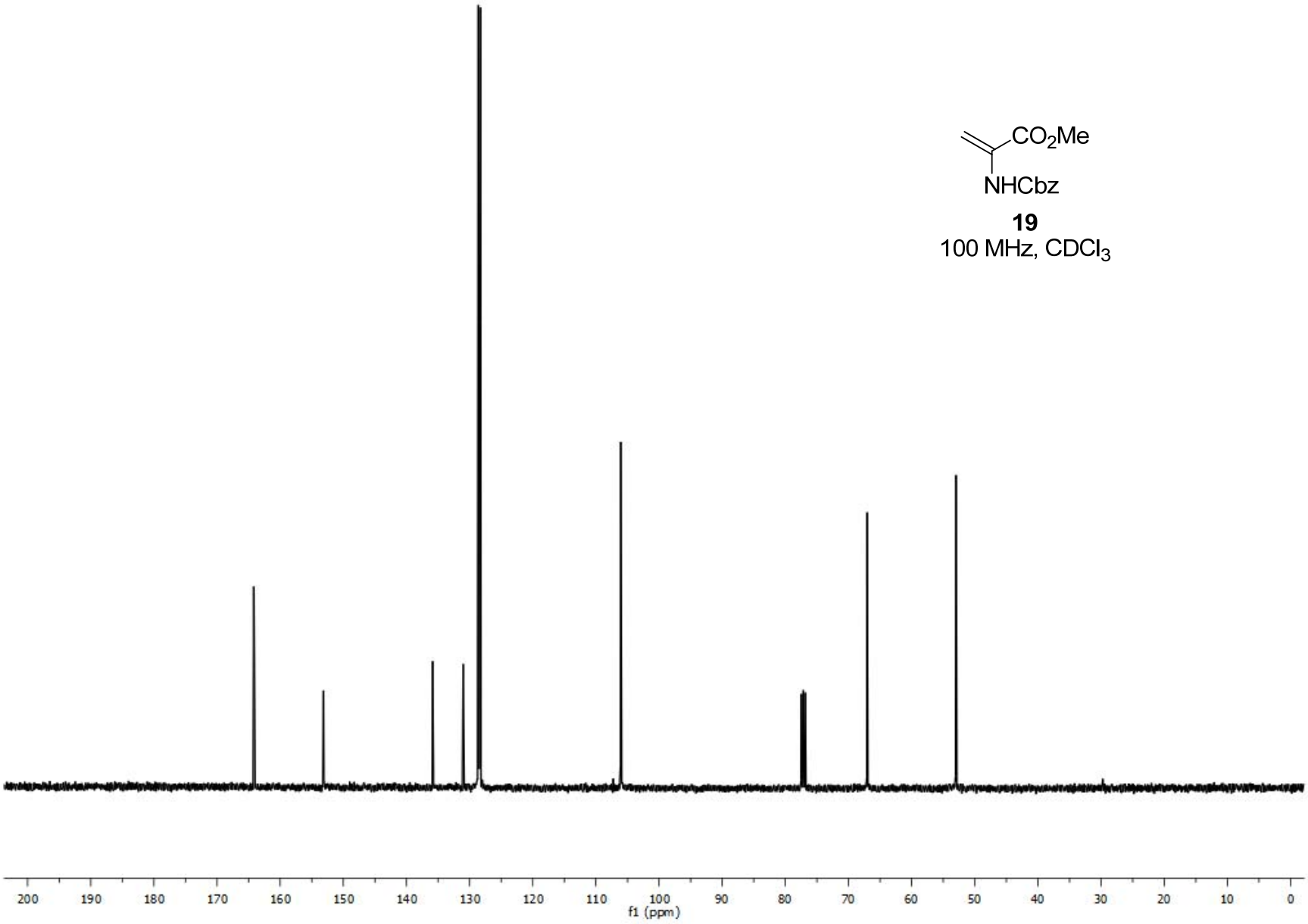
400 MHz, $CDCl_3$

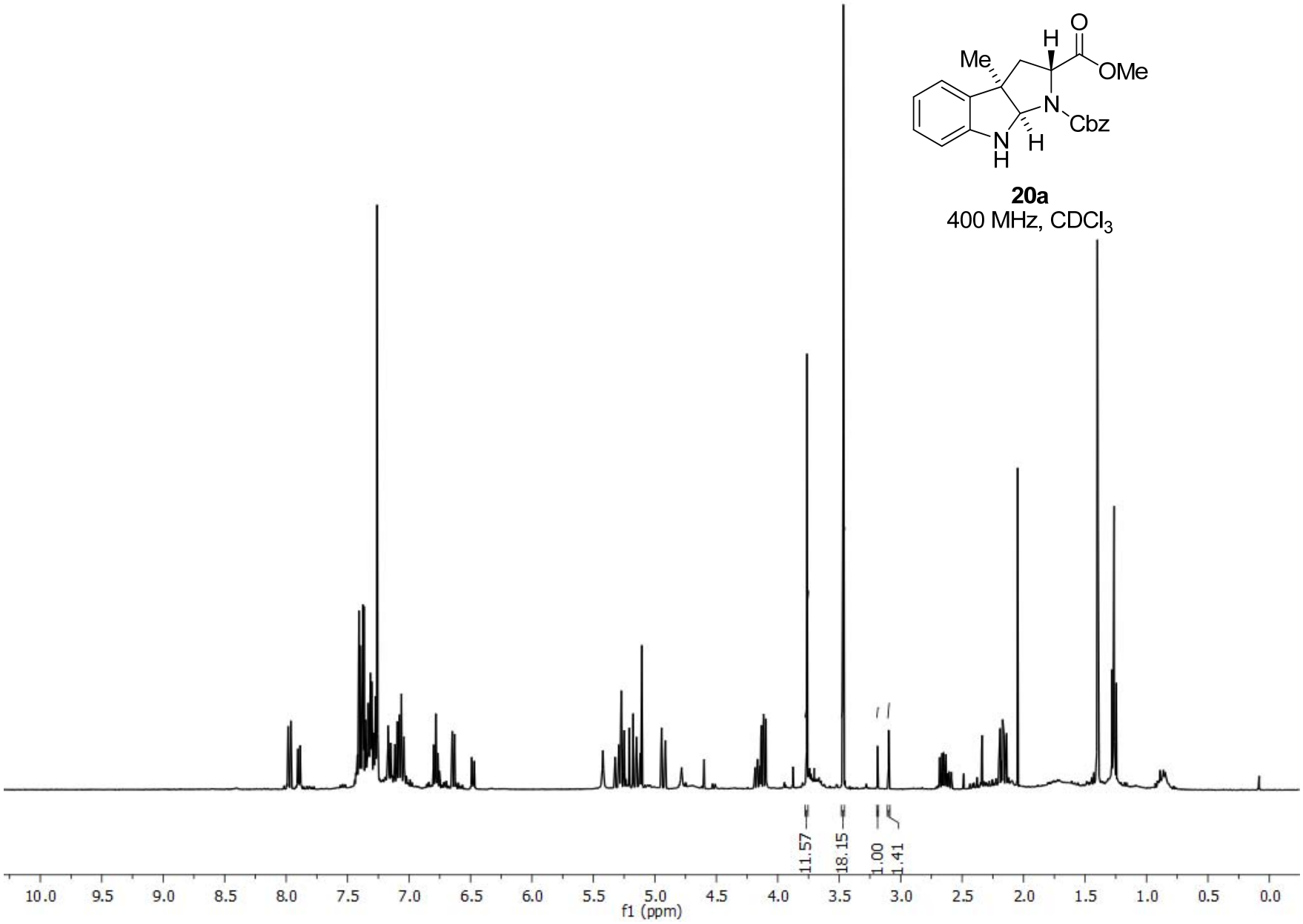


34

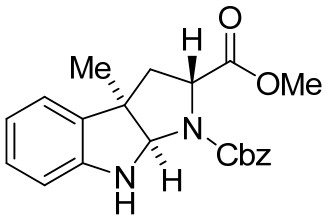
100 MHz, $CDCl_3$


CO2Me
NHCbz
19
400 MHz, CDCl3
5.12
1.00
1.07
2.17
3.25
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

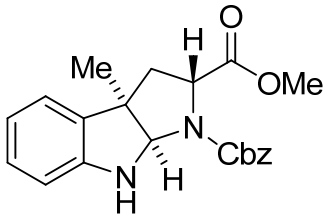

CO2Me
NHCbz
19
100 MHz, CDCl3
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)



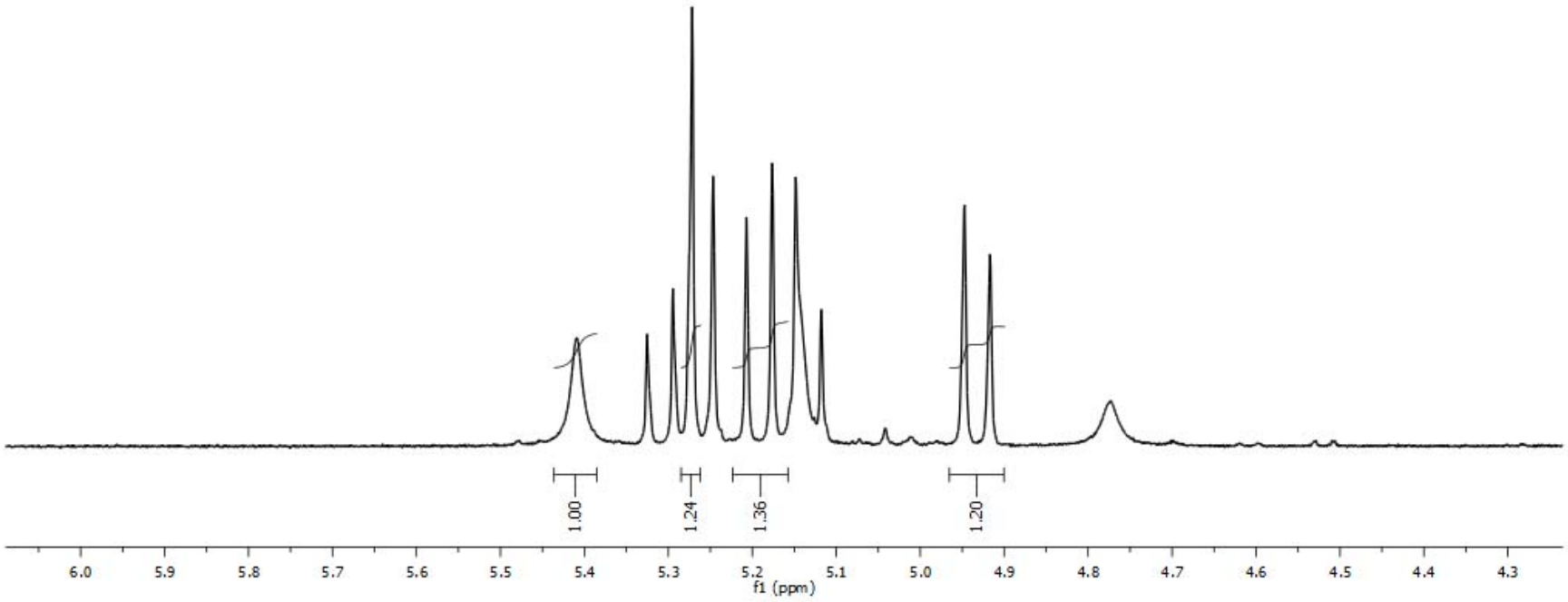
20a
400 MHz, $CDCl_3$

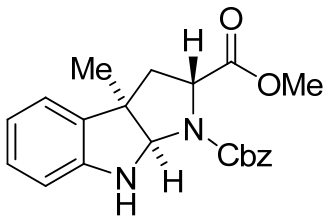


20a
400 MHz, $CDCl_3$

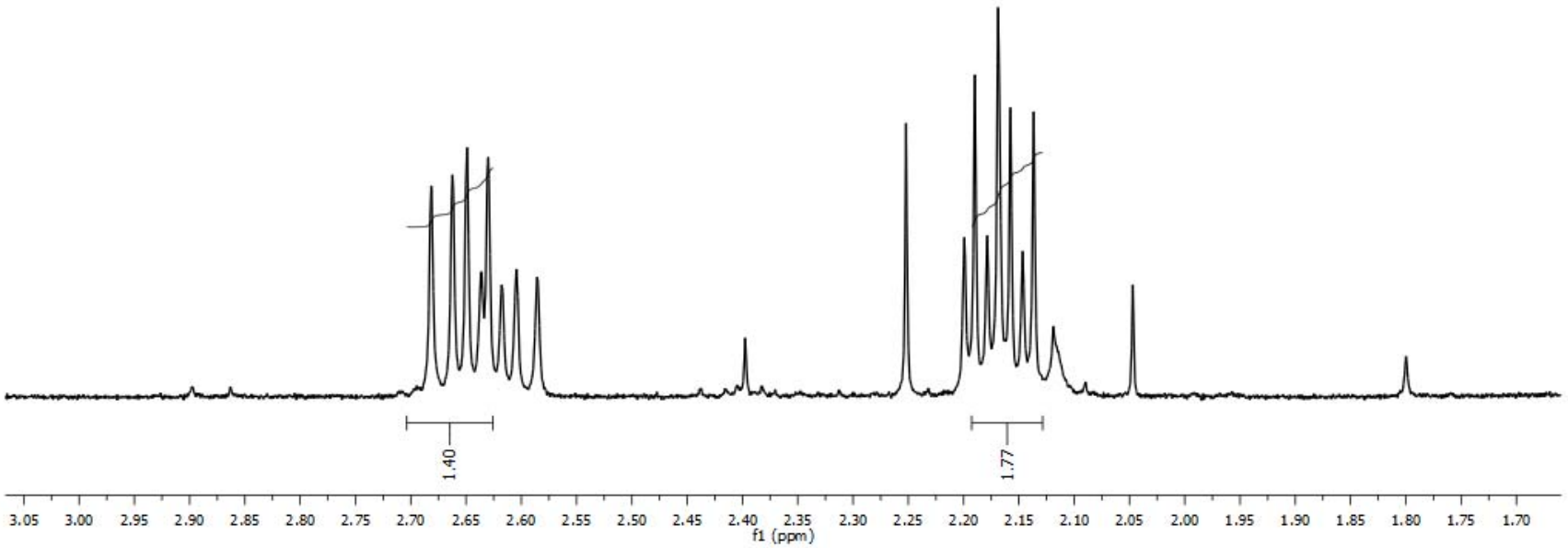


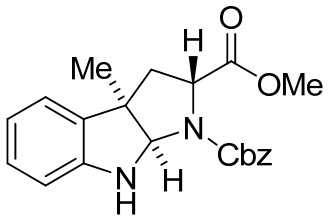
20a
400 MHz, $CDCl_3$



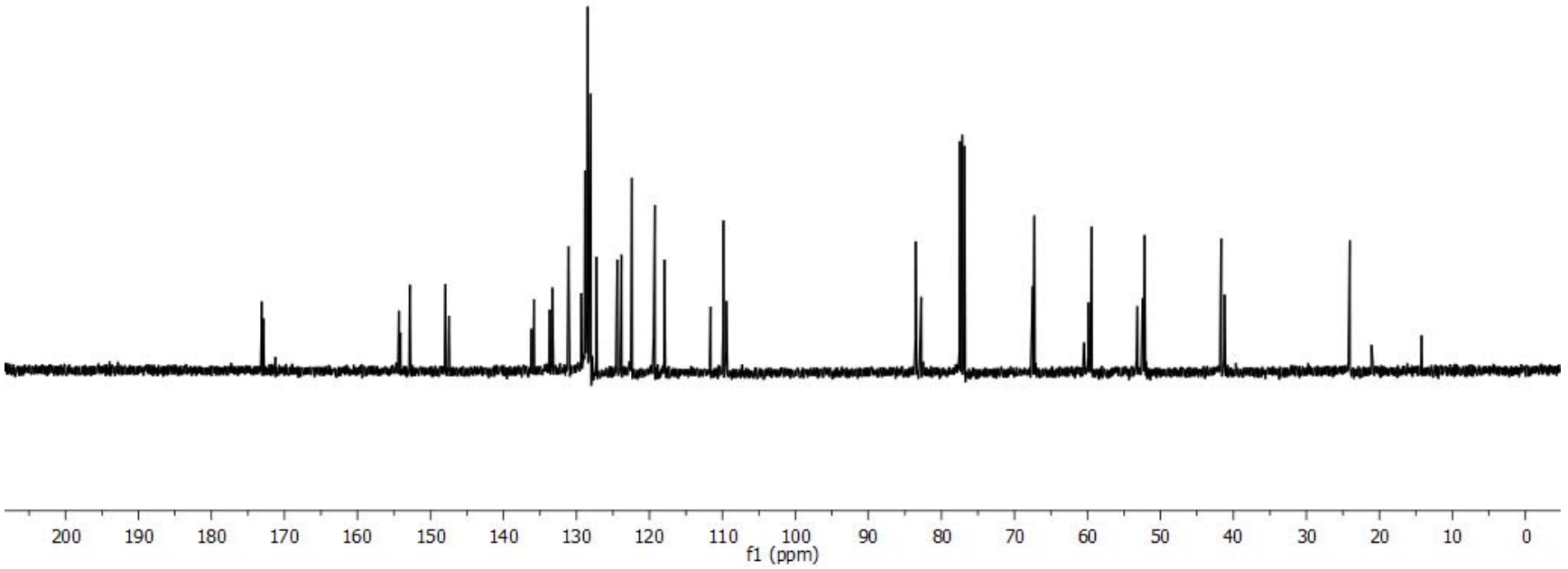


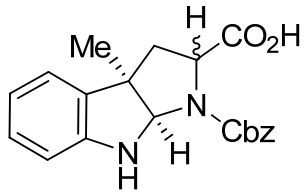
20a
400 MHz, $CDCl_3$



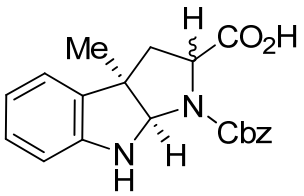


20a
100 MHz, $CDCl_3$

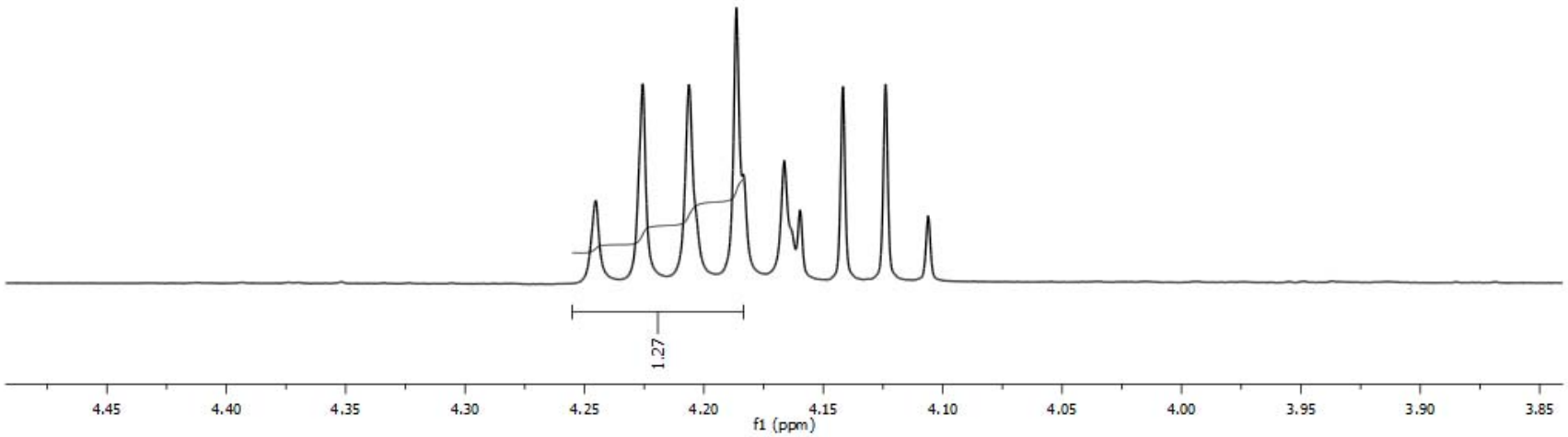


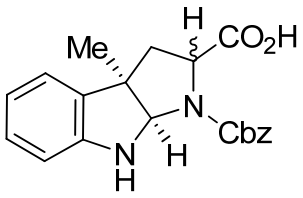


20c
400 MHz, $CDCl_3$



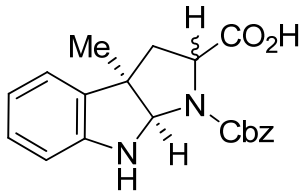
20c
400 MHz, $CDCl_3$



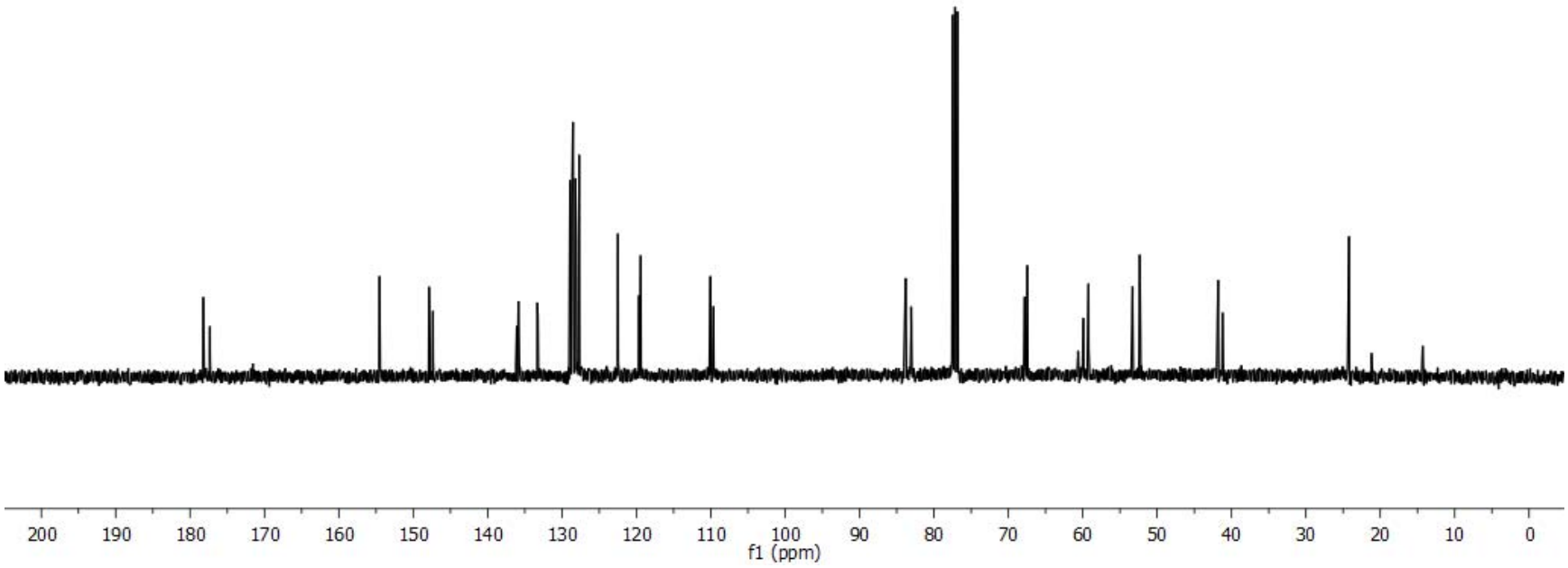


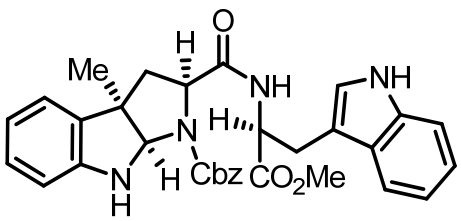
20c

400 MHz, $CDCl_3$

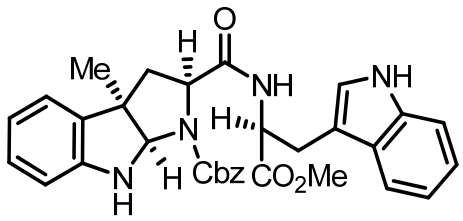


20c
100 MHz, $CDCl_3$

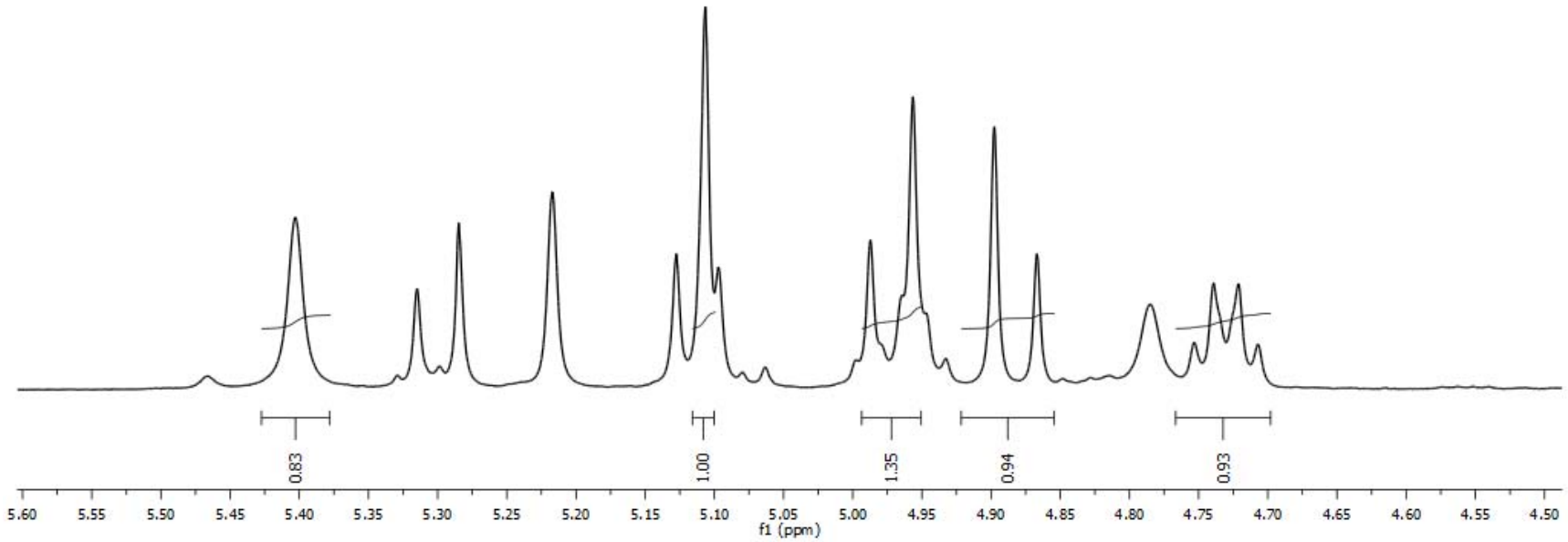


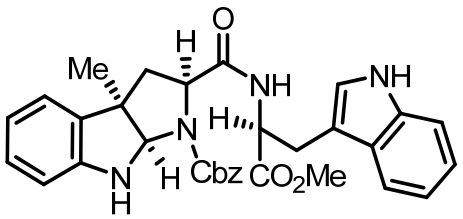


22
400 MHz, $CDCl_3$

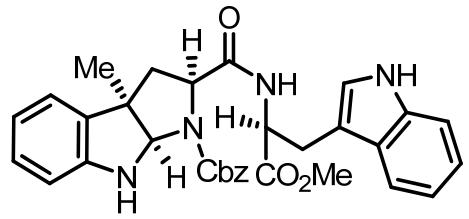


22
400 MHz, $CDCl_3$

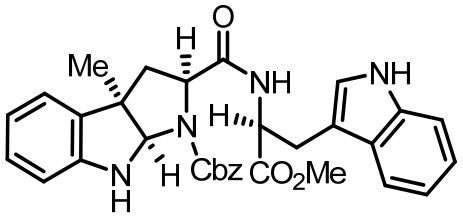




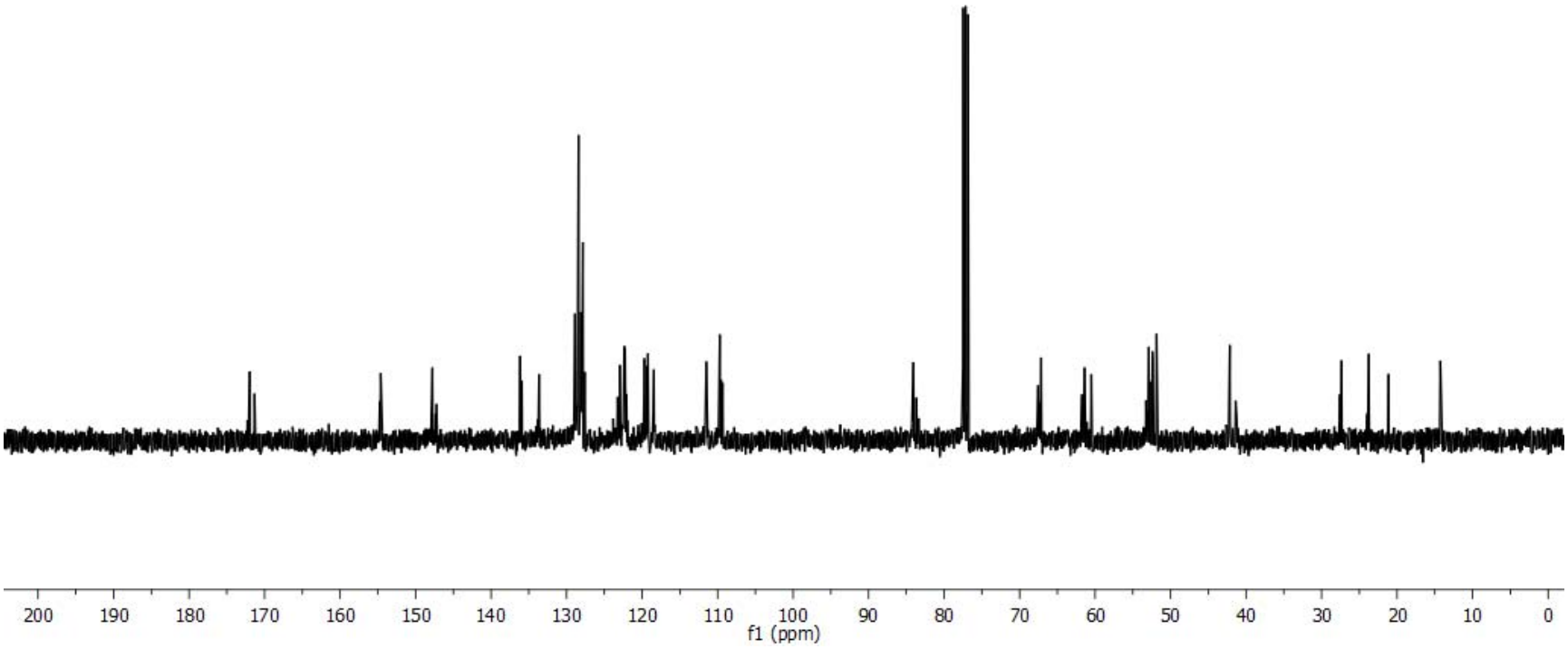
22
400 MHz, $CDCl_3$

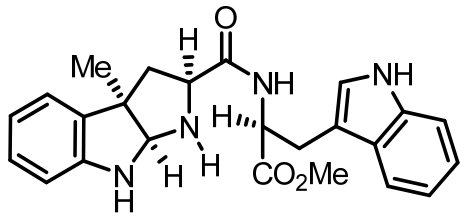


22
400 MHz, $CDCl_3$



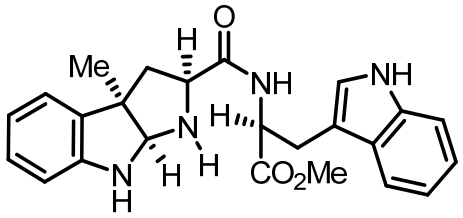
22
100 MHz, $CDCl_3$





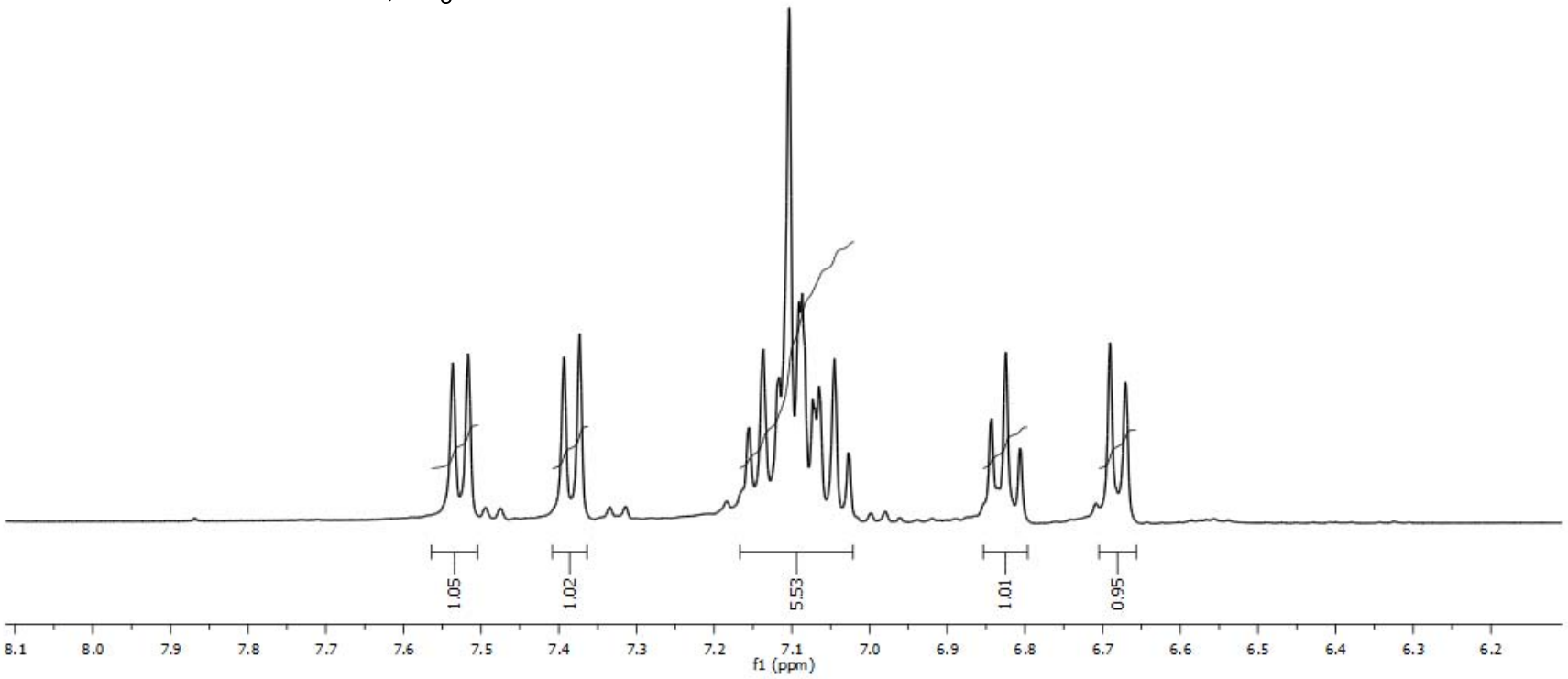
23

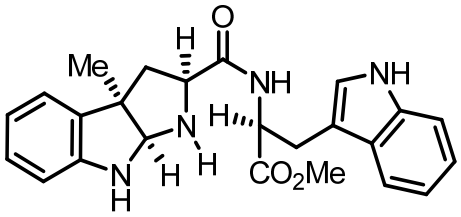
400 MHz, CD_3OD



23

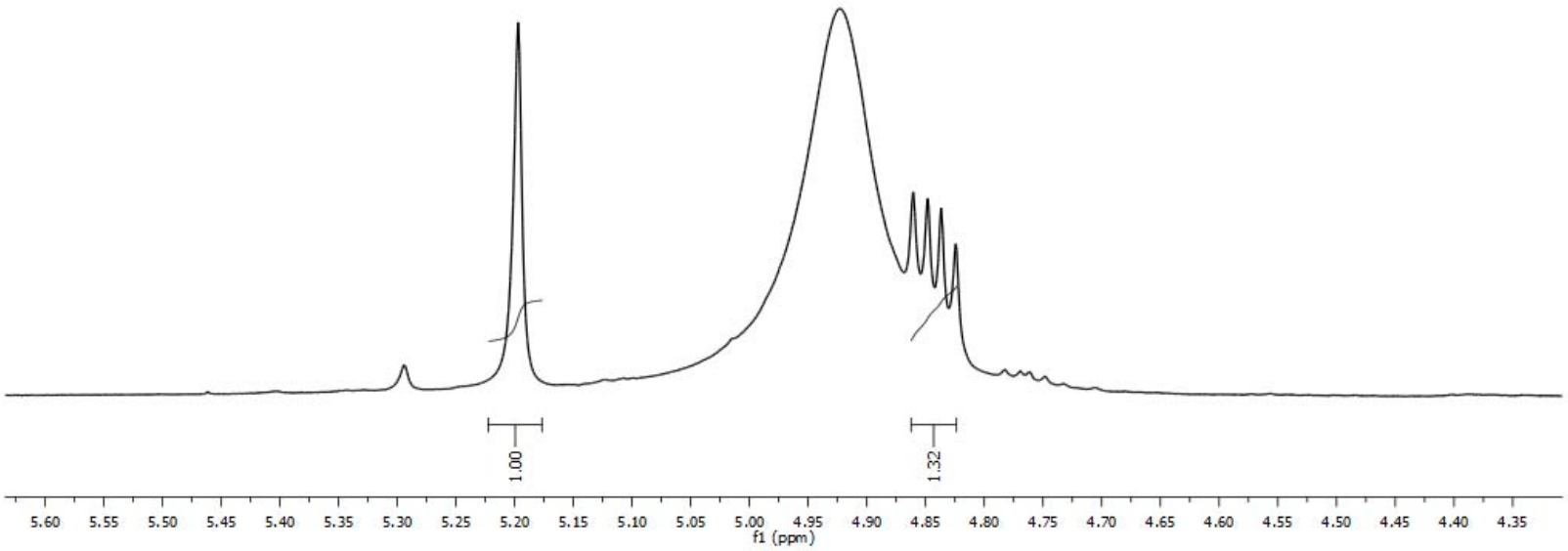
400 MHz, CD_3OD

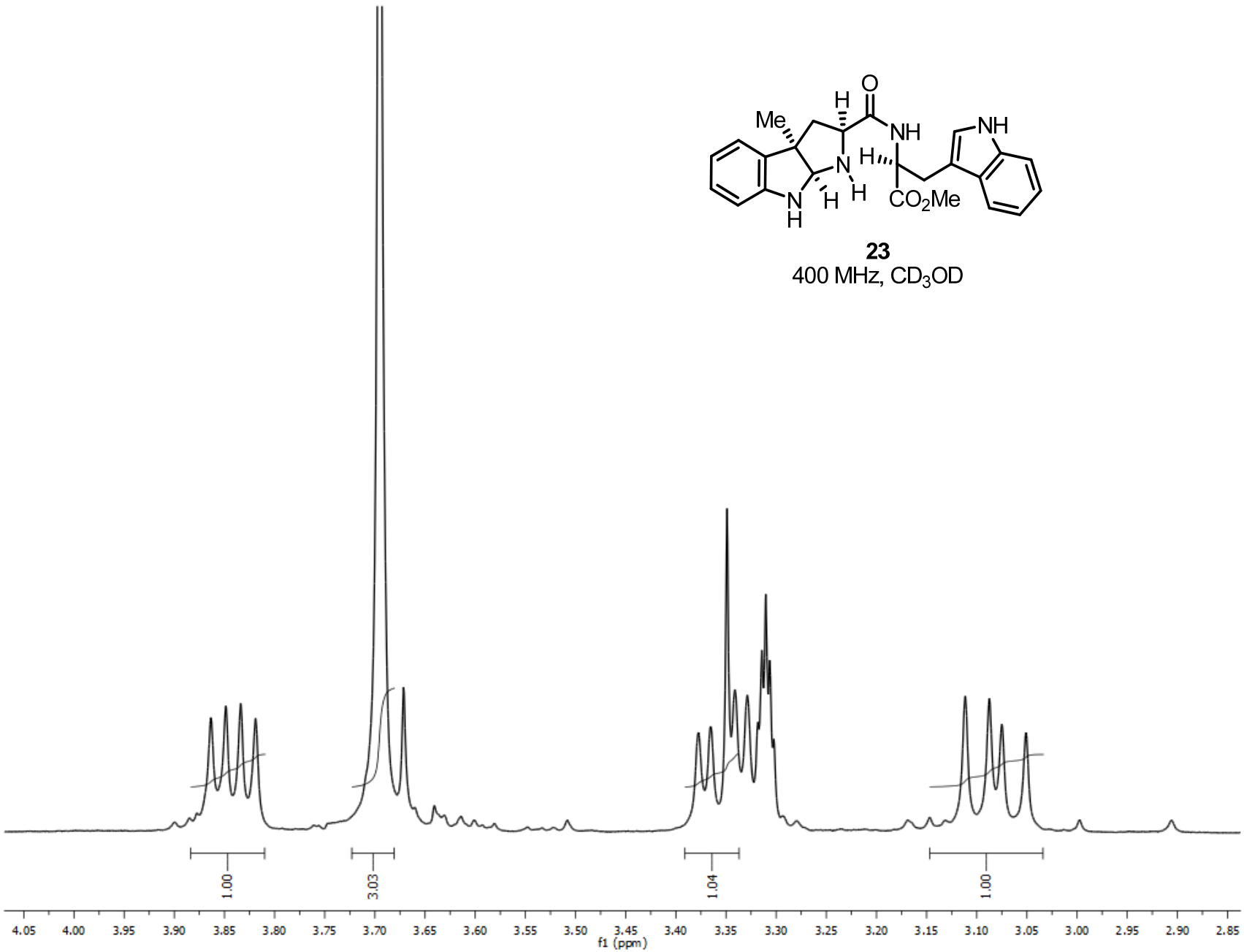




23

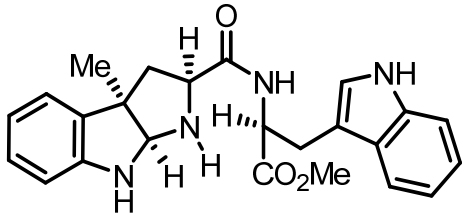
400 MHz, CD_3OD



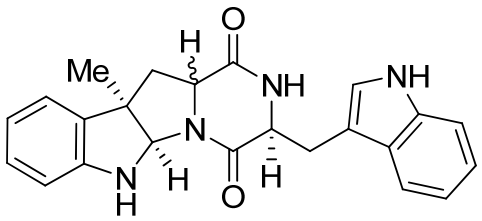


23

400 MHz, CD_3OD

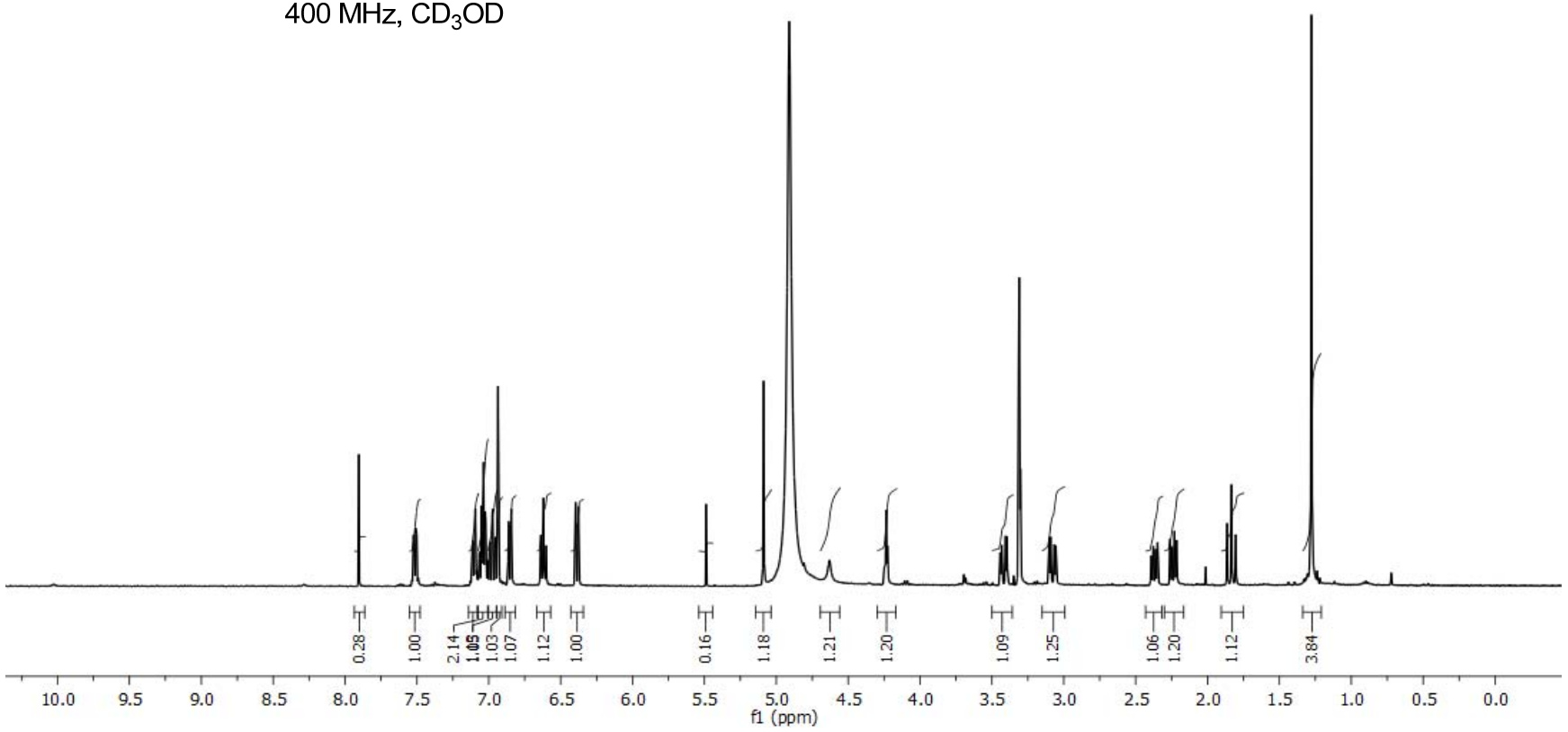


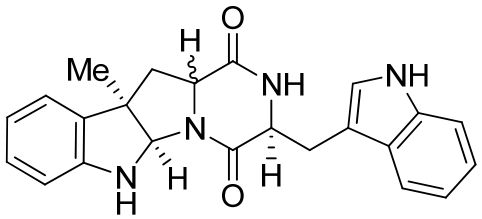
23
100 MHz, CD_3OD



13*

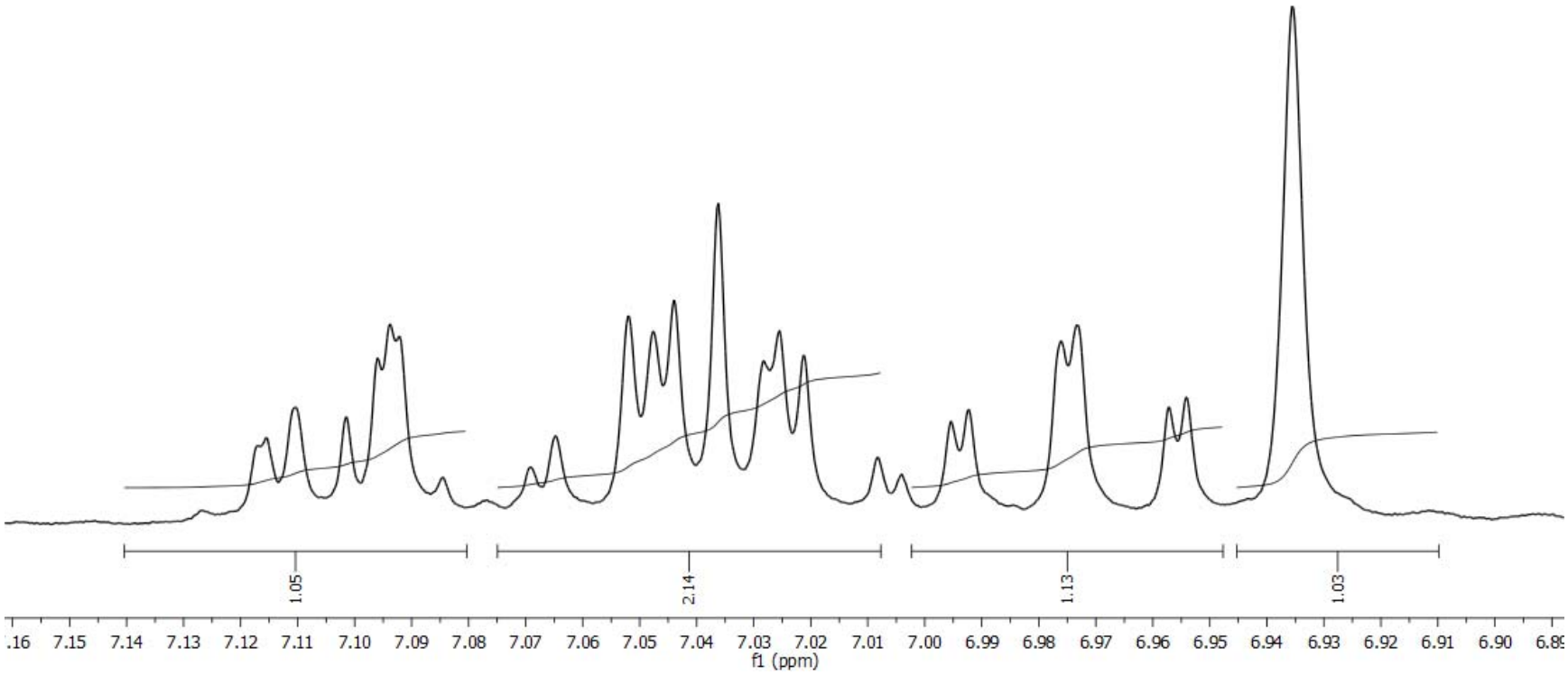
400 MHz, CD_3OD

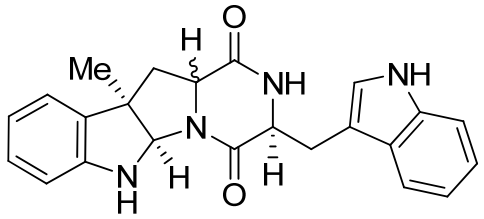




13*

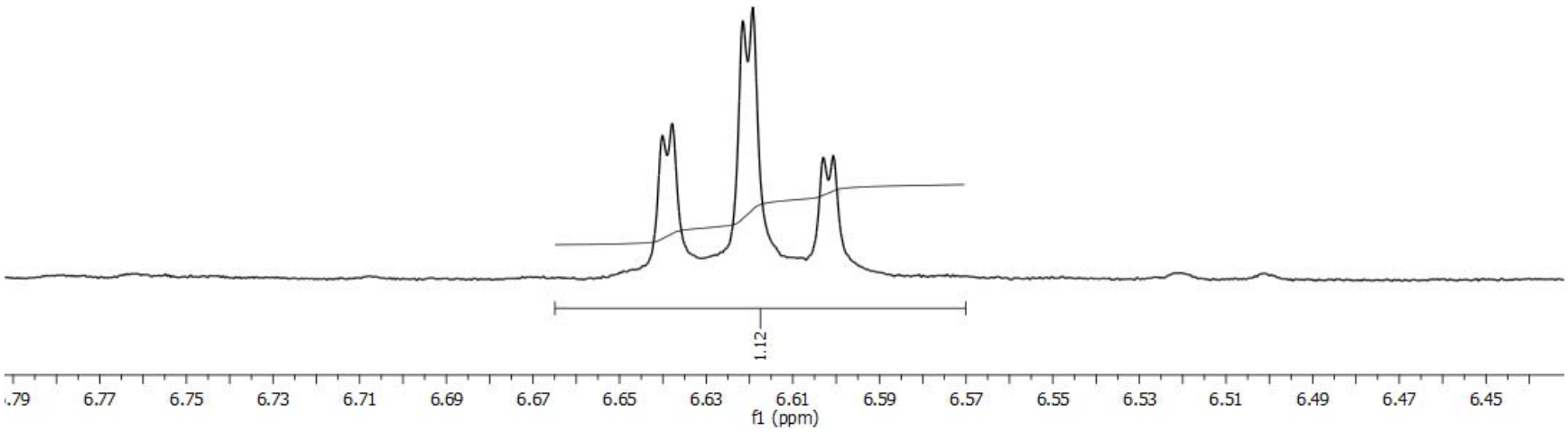
400 MHz, CD_3OD

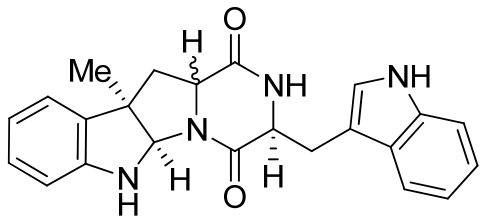




13*

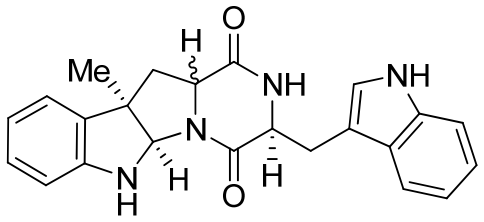
400 MHz, CD_3OD





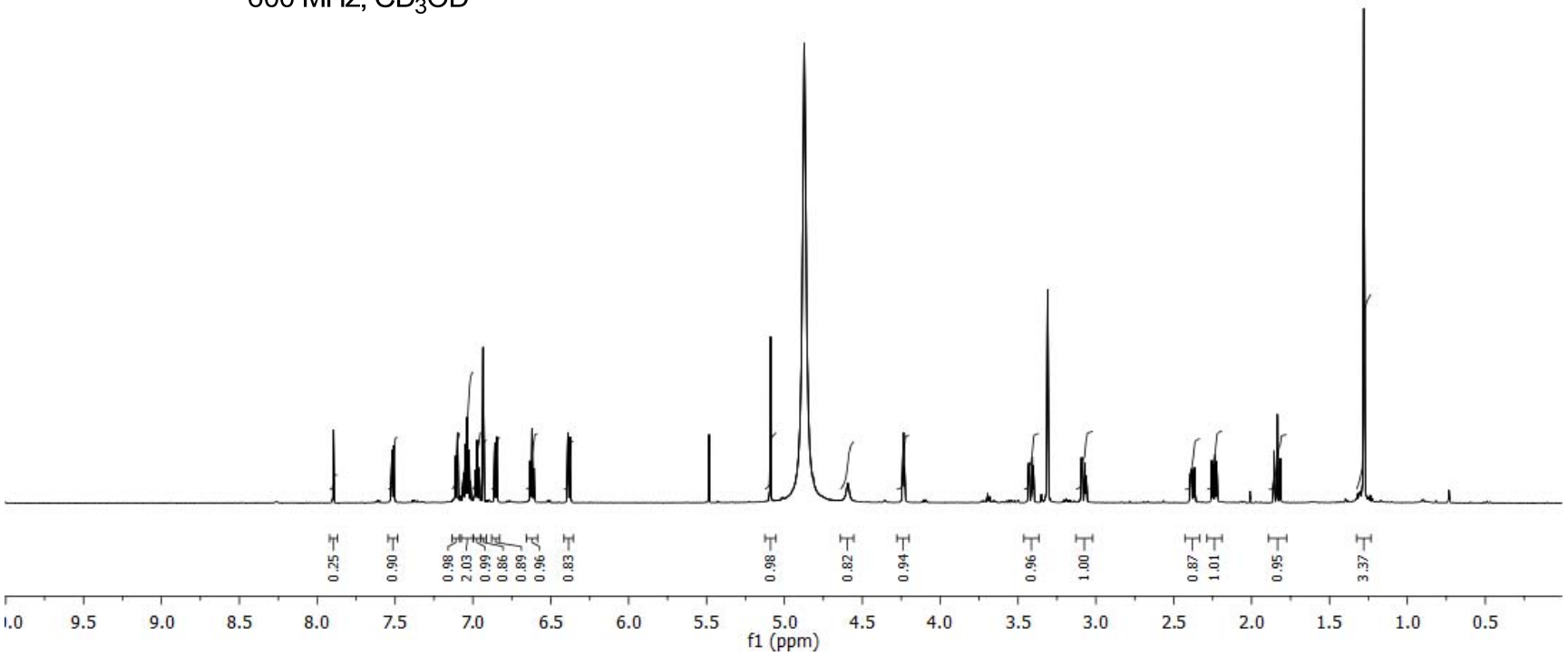
13*

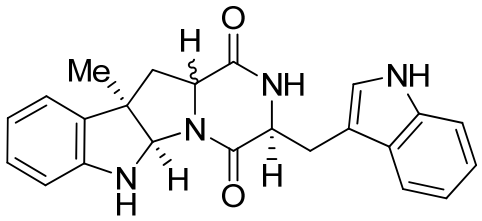
400 MHz, CD_3OD



13*

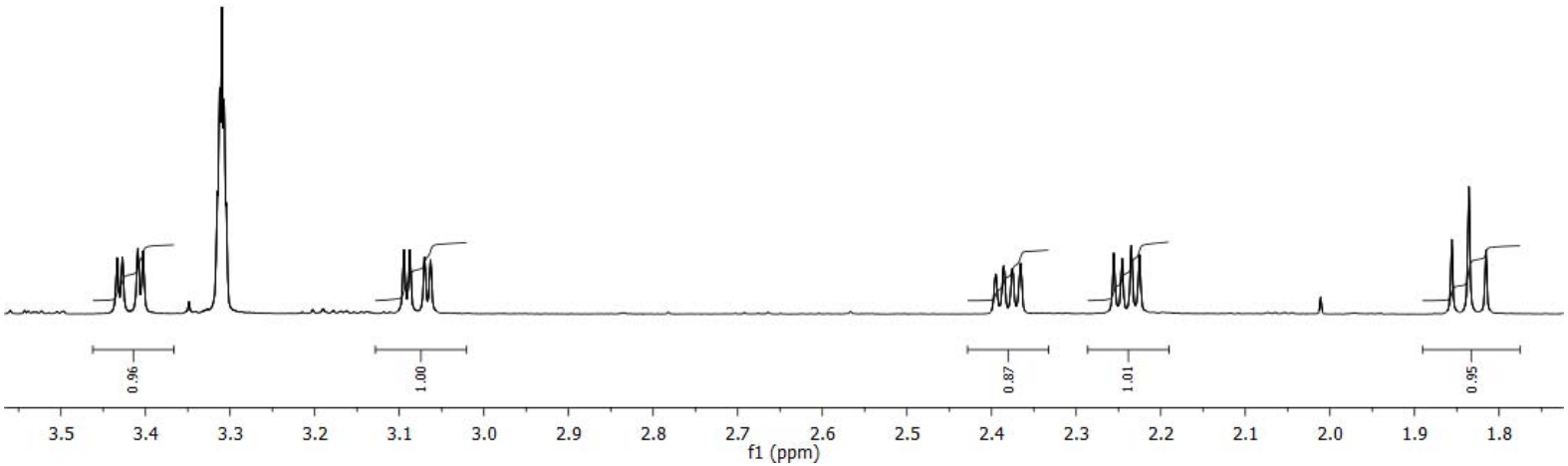
600 MHz, CD_3OD

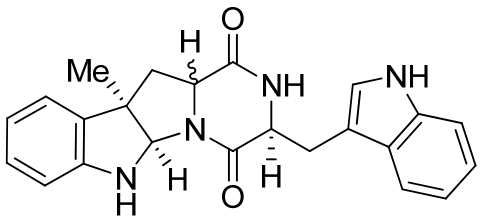




13*

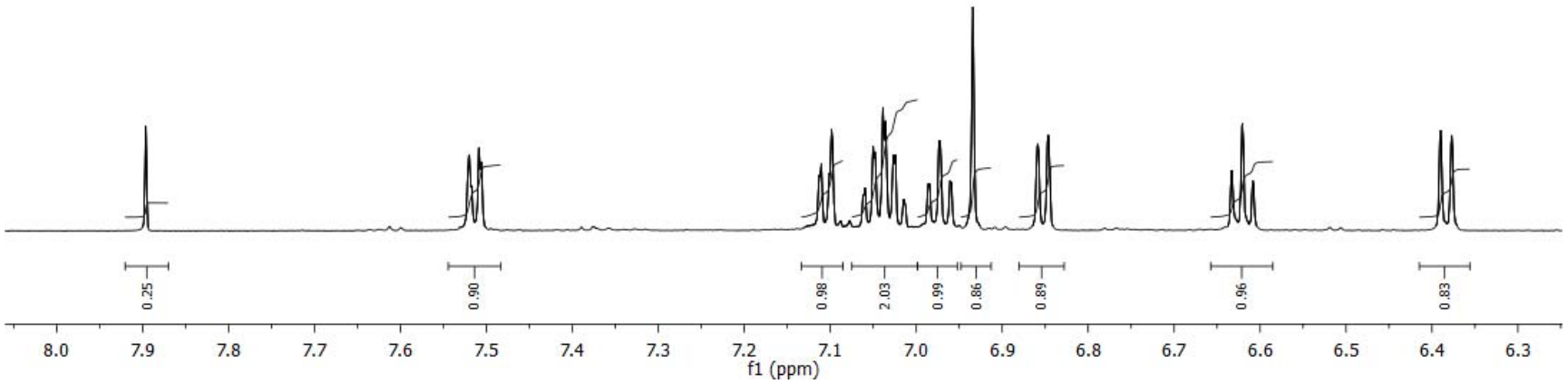
600 MHz, CD_3OD

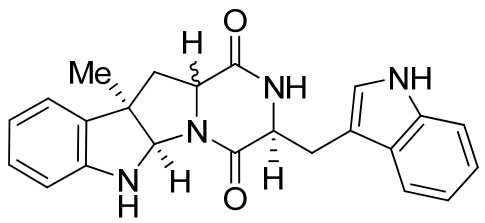




13*

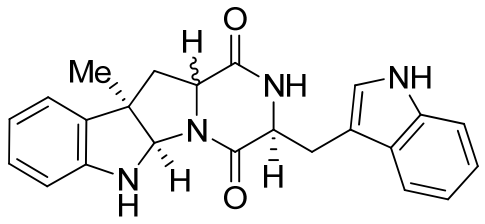
600 MHz, CD_3OD





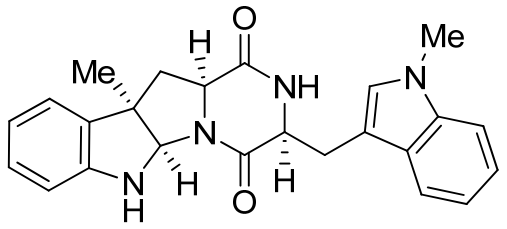
13*

150 MHz, CD_3OD



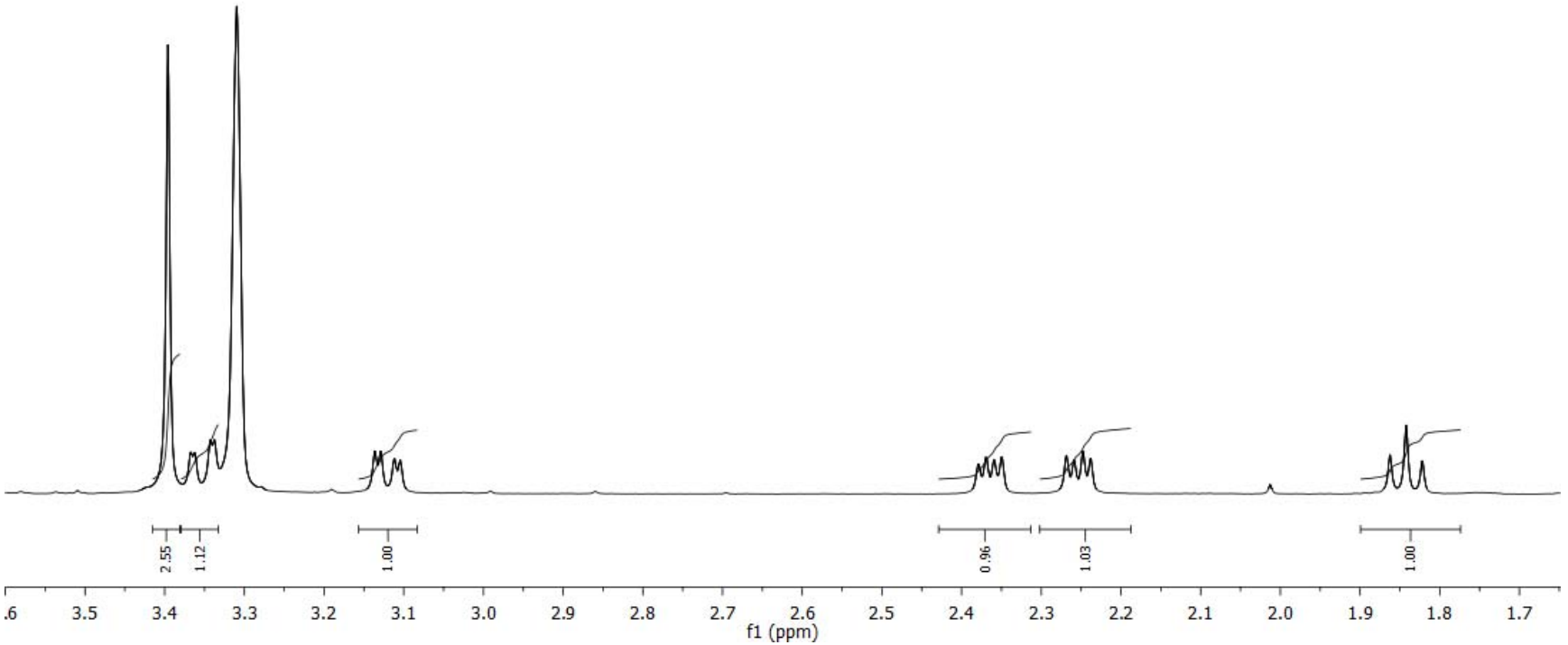
13*

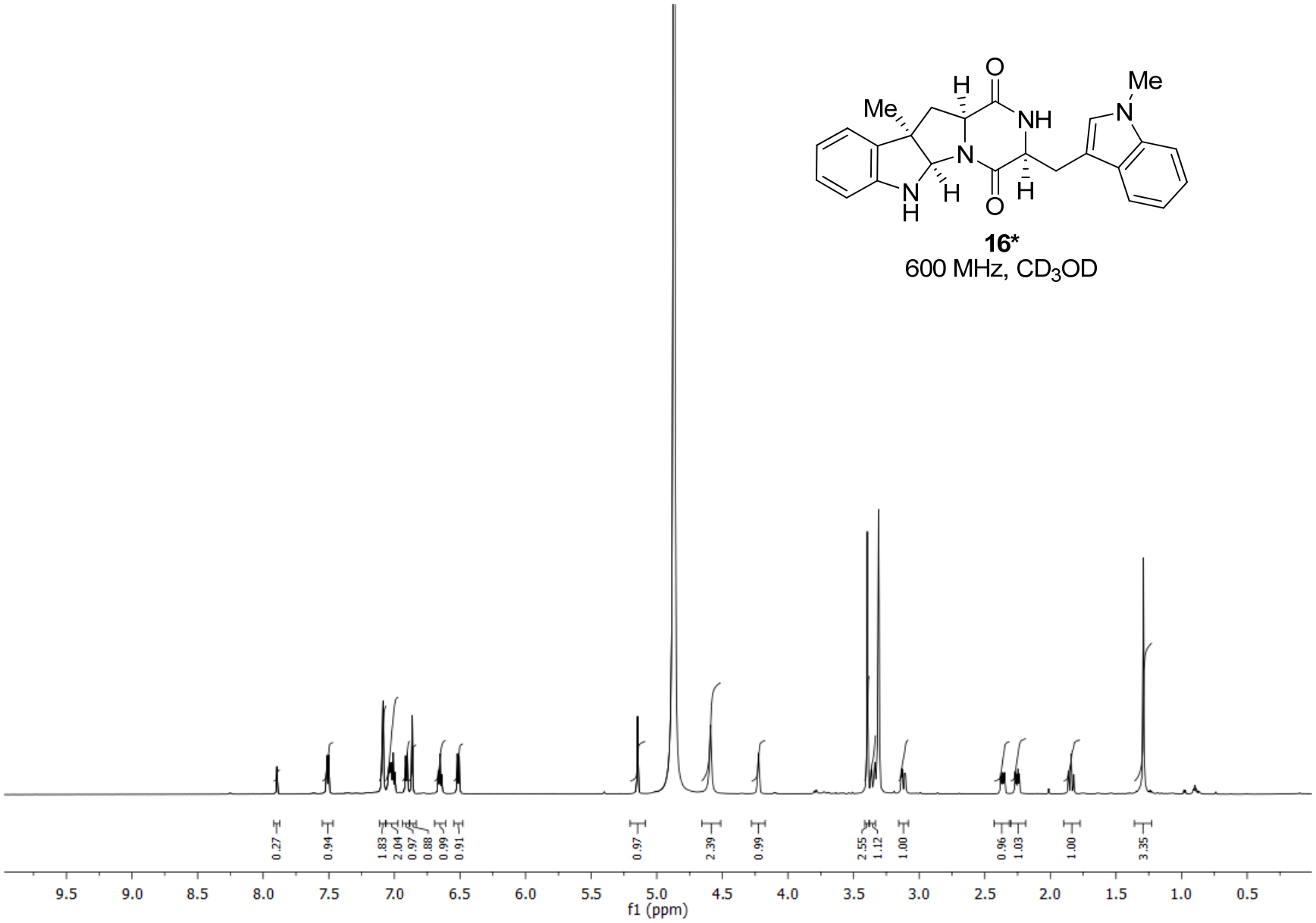
100 MHz, CD_3OD



16*

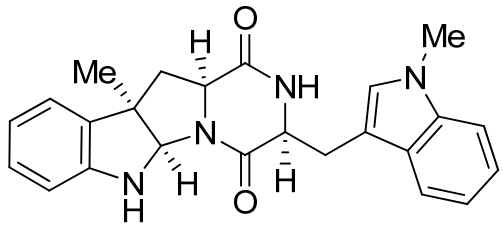
600 MHz, CD_3OD





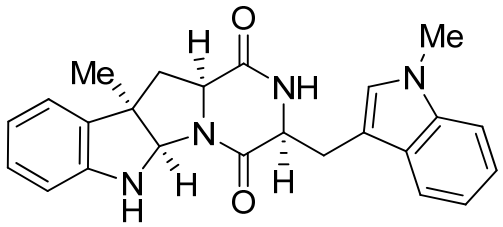
16*

600 MHz, CD_3OD



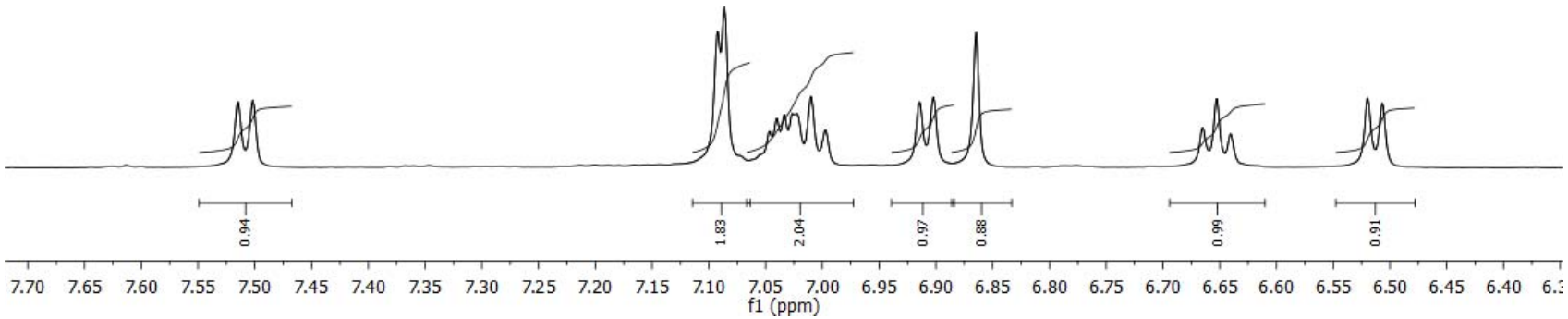
16*

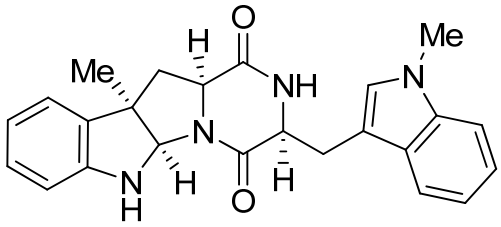
600 MHz, CD_3OD



16*

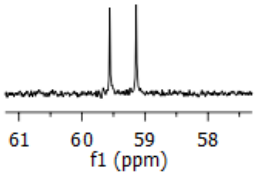
600 MHz, CD_3OD

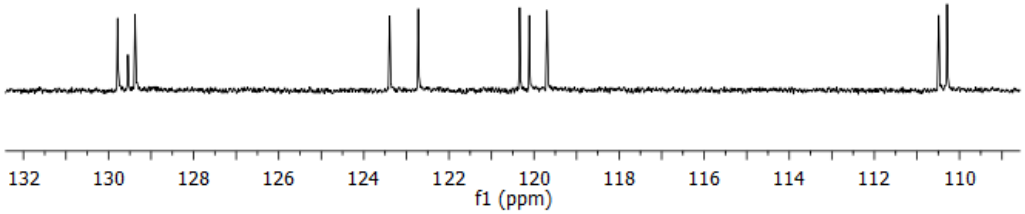


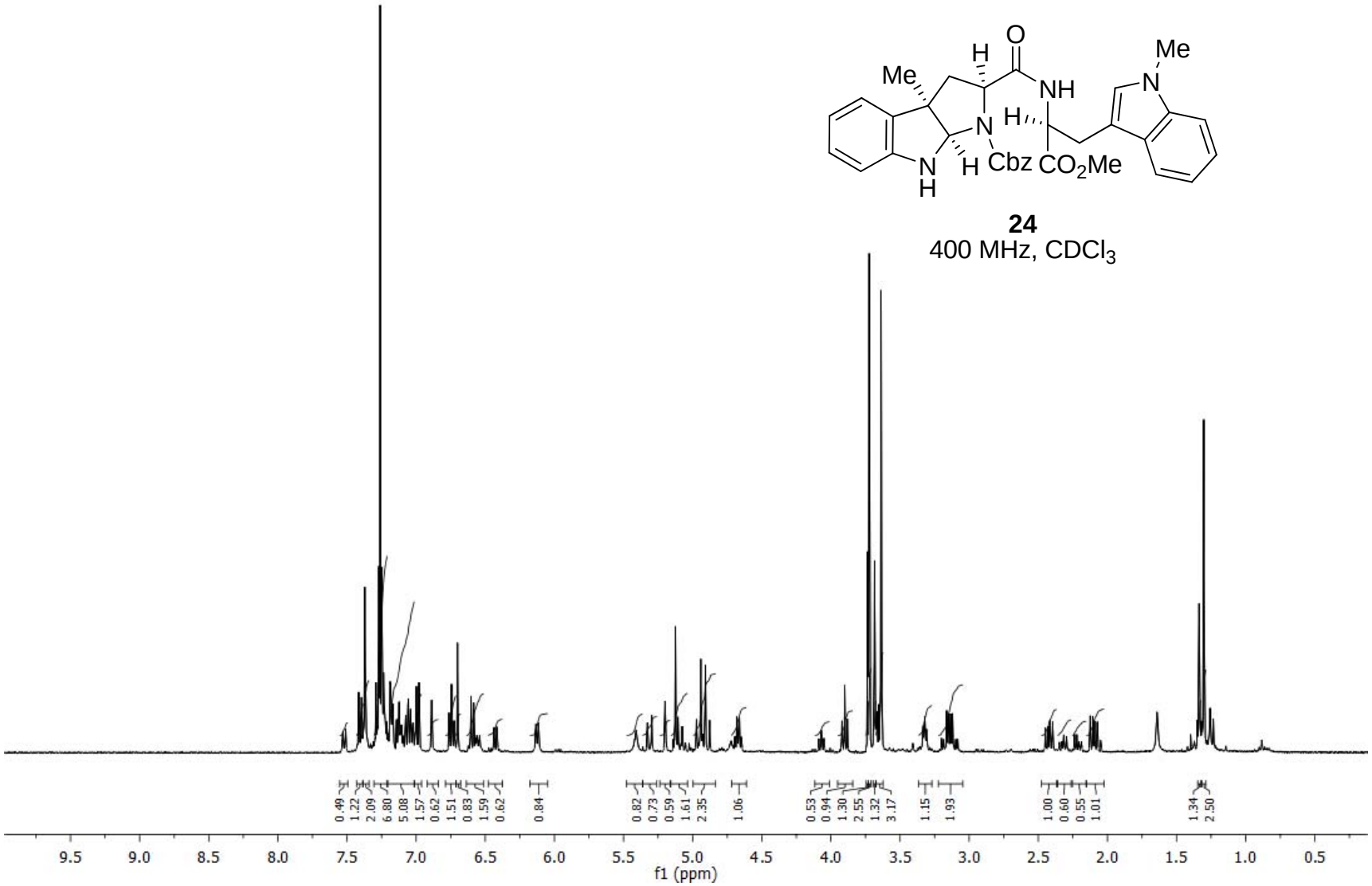


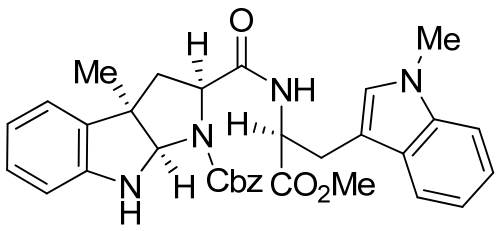
16*

150 MHz, CD_3OD



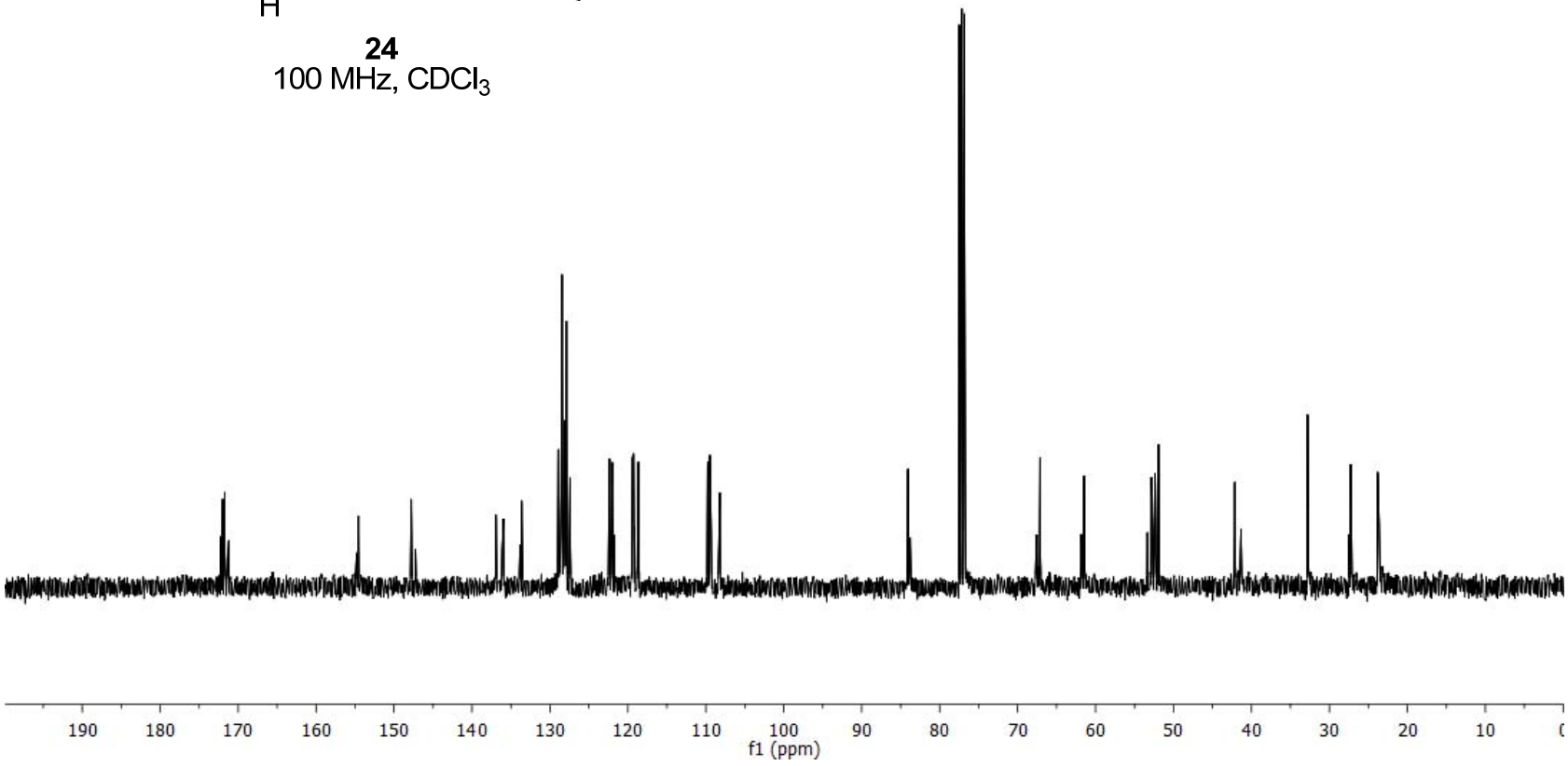


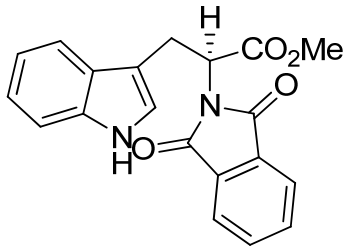

O
H
Me
NH
Me
N
H
N
N
H
H
Cbz
CO2Me
24
400 MHz, CDCl3
0.49
1.22
2.09
6.80
5.08
1.57
0.62
1.51
0.83
1.59
0.62
0.84
0.82
0.73
0.59
1.61
2.35
1.06
0.53
0.94
1.30
2.55
1.32
3.17
1.15
1.93
1.00
0.60
0.55
1.01
1.34
2.50
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)



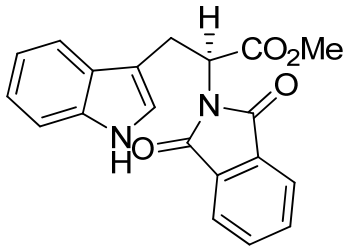
24

100 MHz, $CDCl_3$

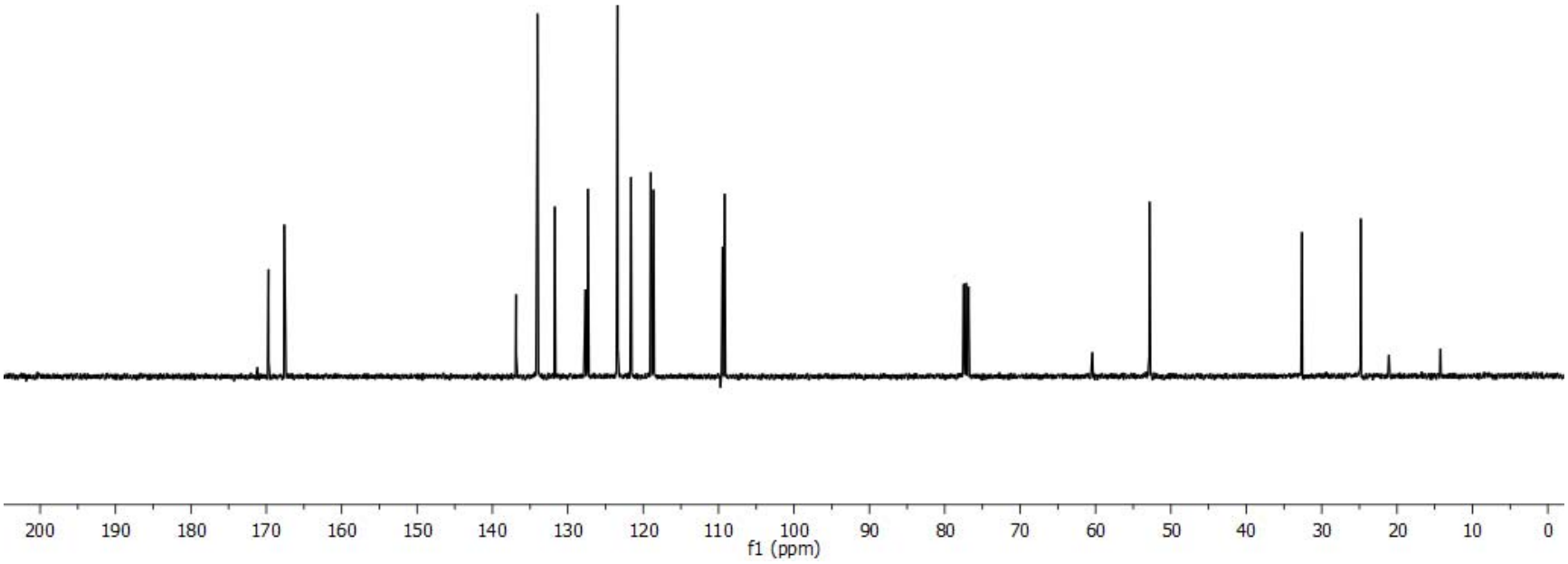


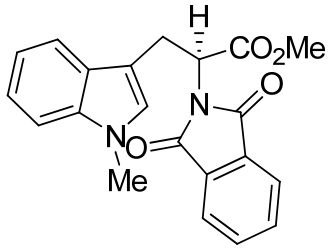


35
400 MHz, $CDCl_3$



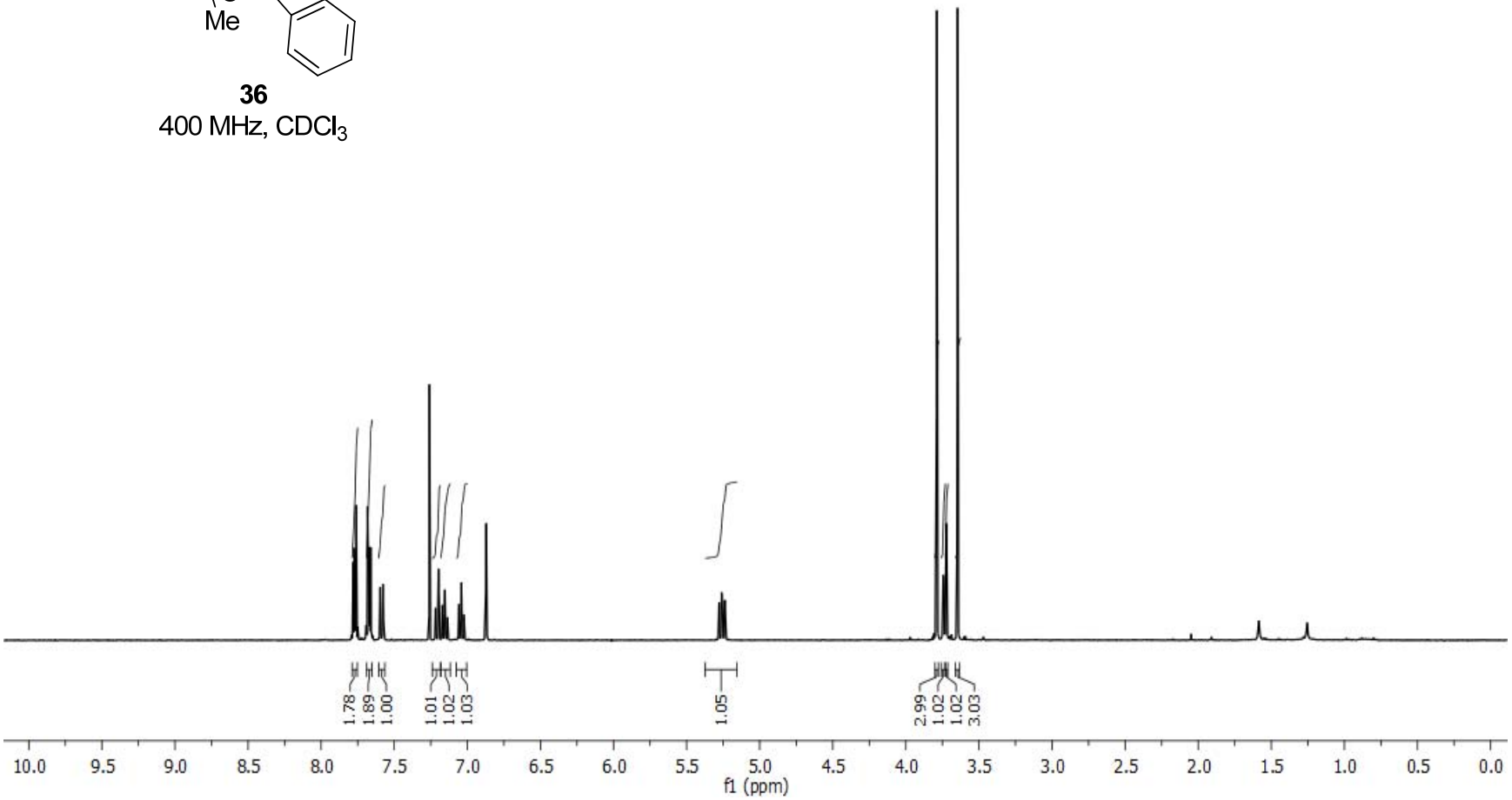
35
100 MHz, $CDCl_3$

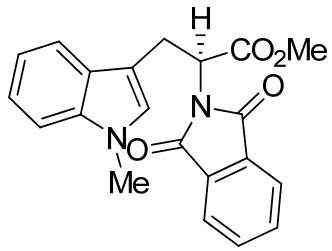




36

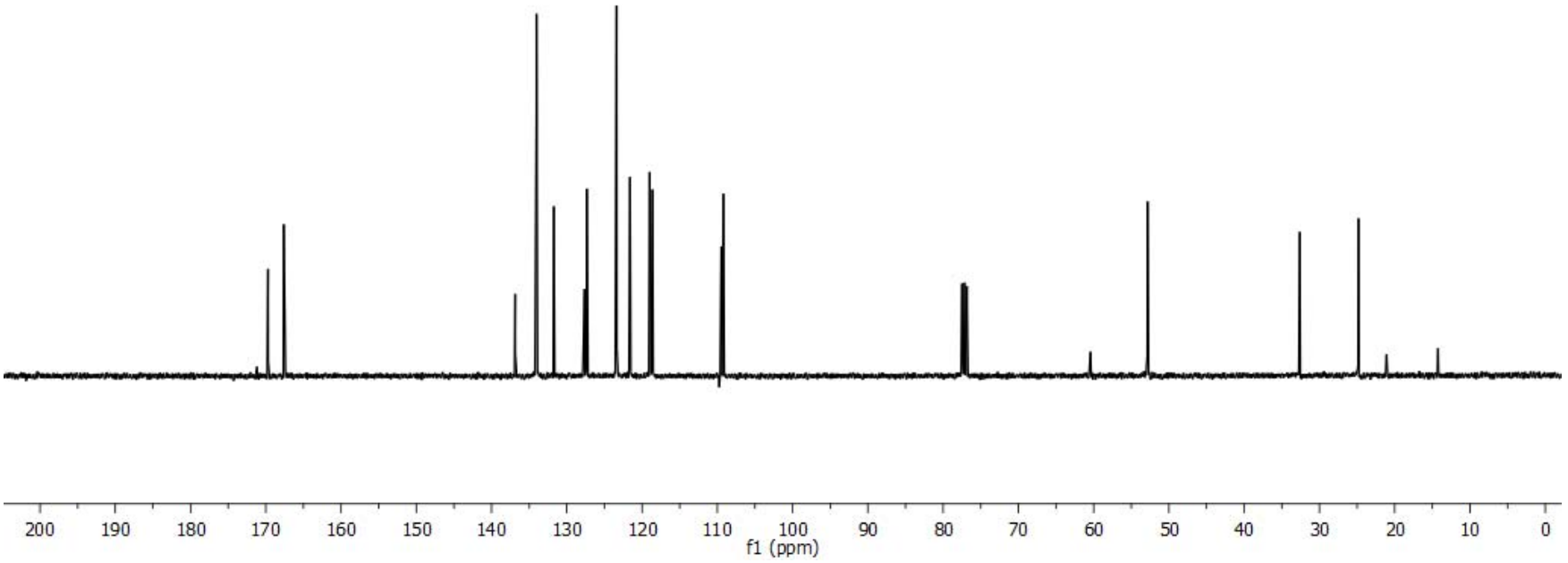
400 MHz, $CDCl_3$

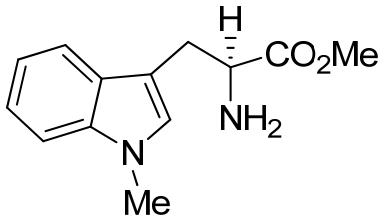




36

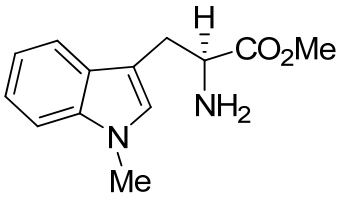
100 MHz, $CDCl_3$





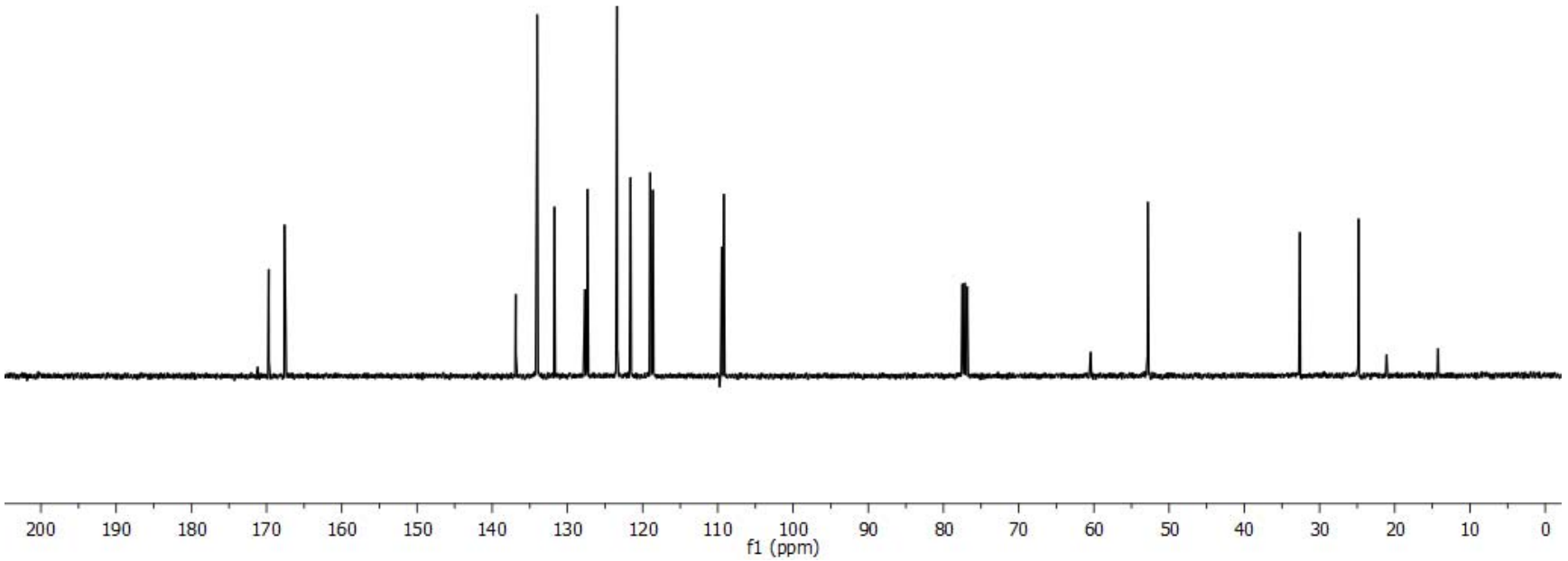
26

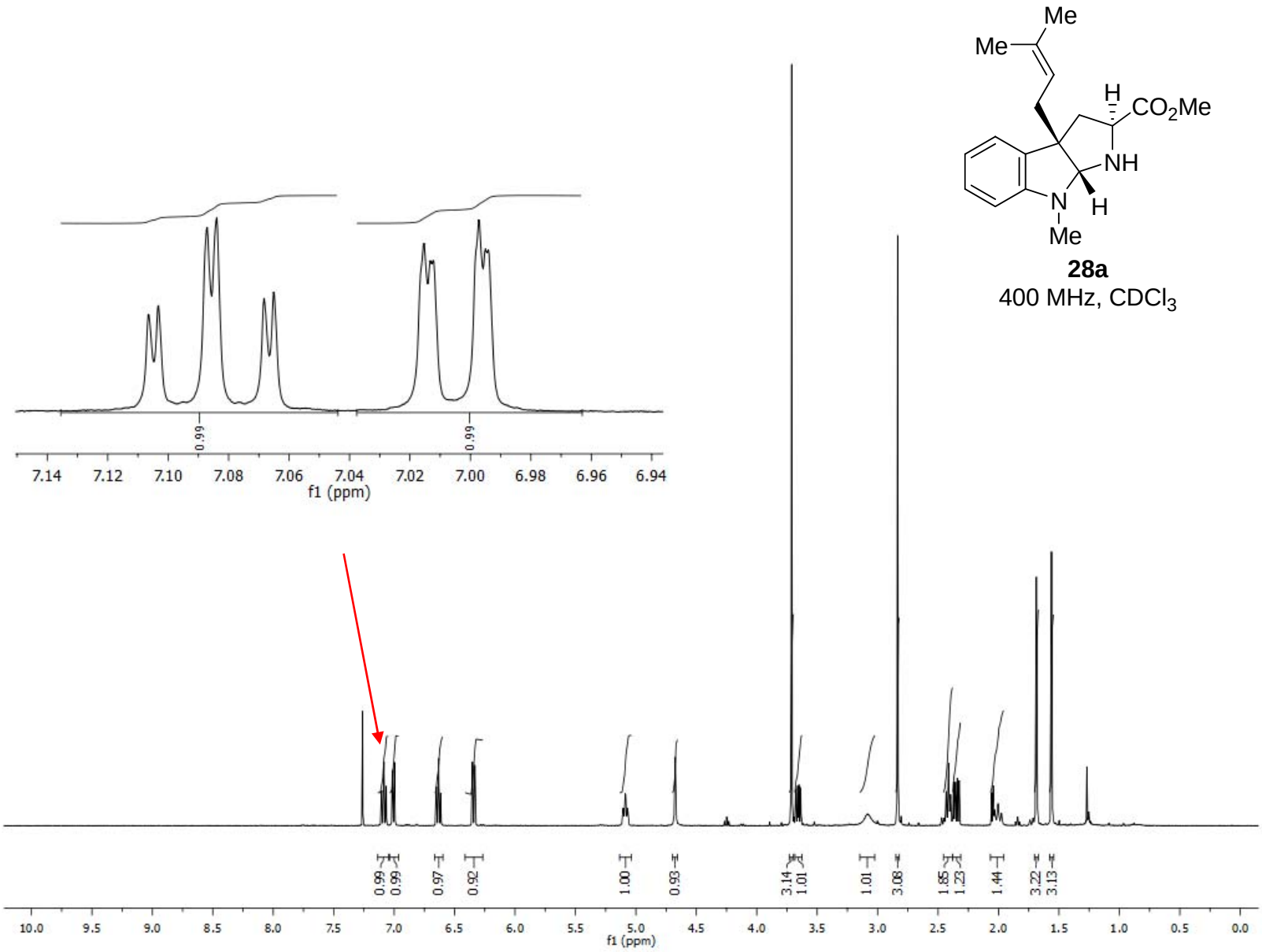
400 MHz, $CDCl_3$

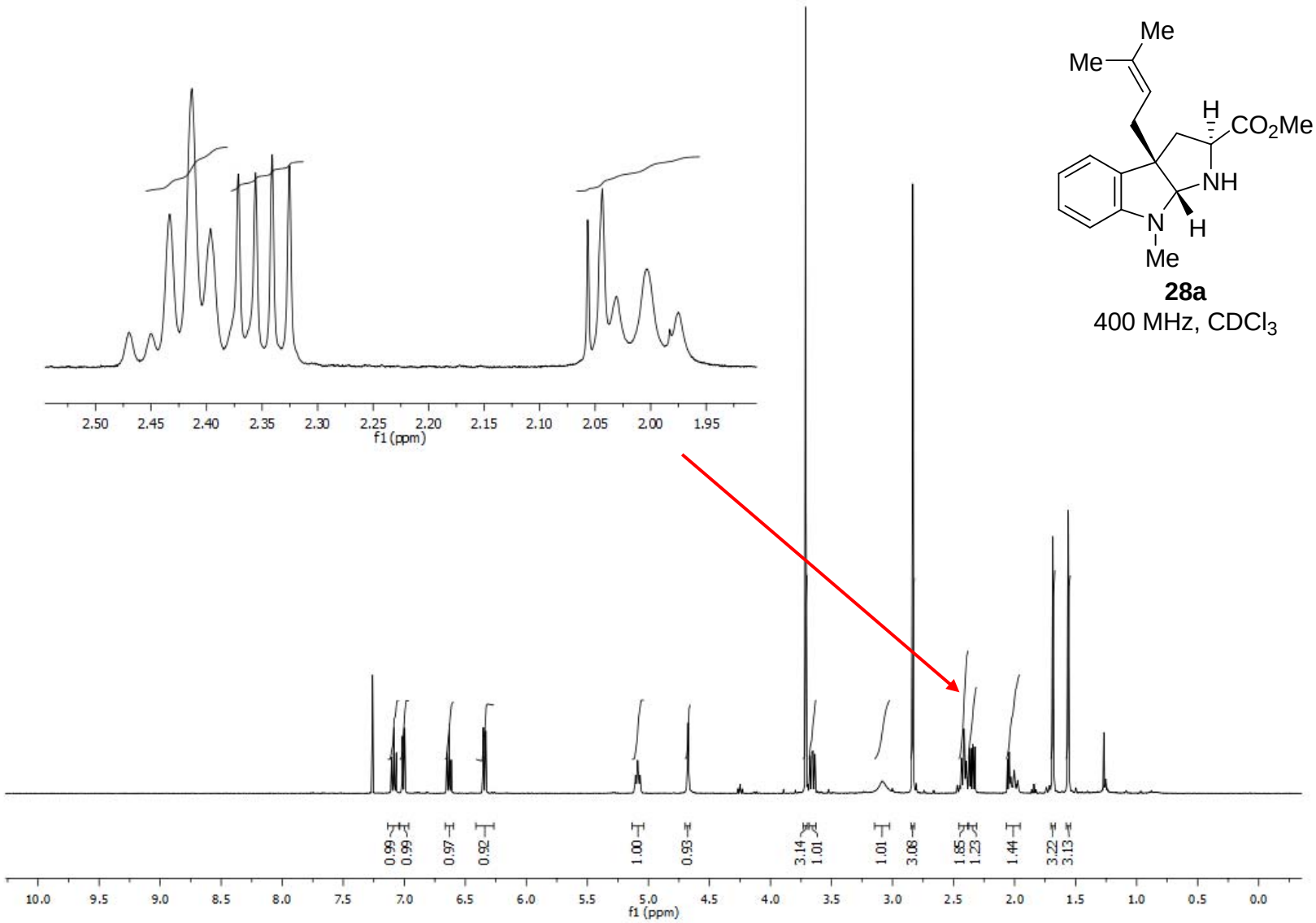


26

100 MHz, $CDCl_3$

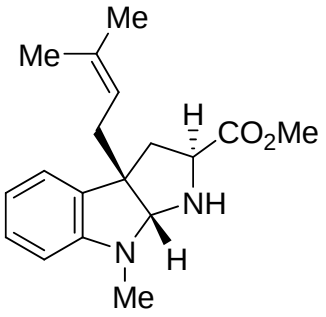



Me
Me
H
CO2Me
NH
N
H
Me
28a
400 MHz, CDCl3
0.99
0.99
7.14
7.12
7.10
7.08
7.06
7.04
7.02
7.00
6.98
6.96
6.94
f1 (ppm)
0.99
0.99
0.97
0.92
1.00
0.93
3.14
1.01
1.01
3.08
1.85
1.23
1.44
3.22
3.13
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)



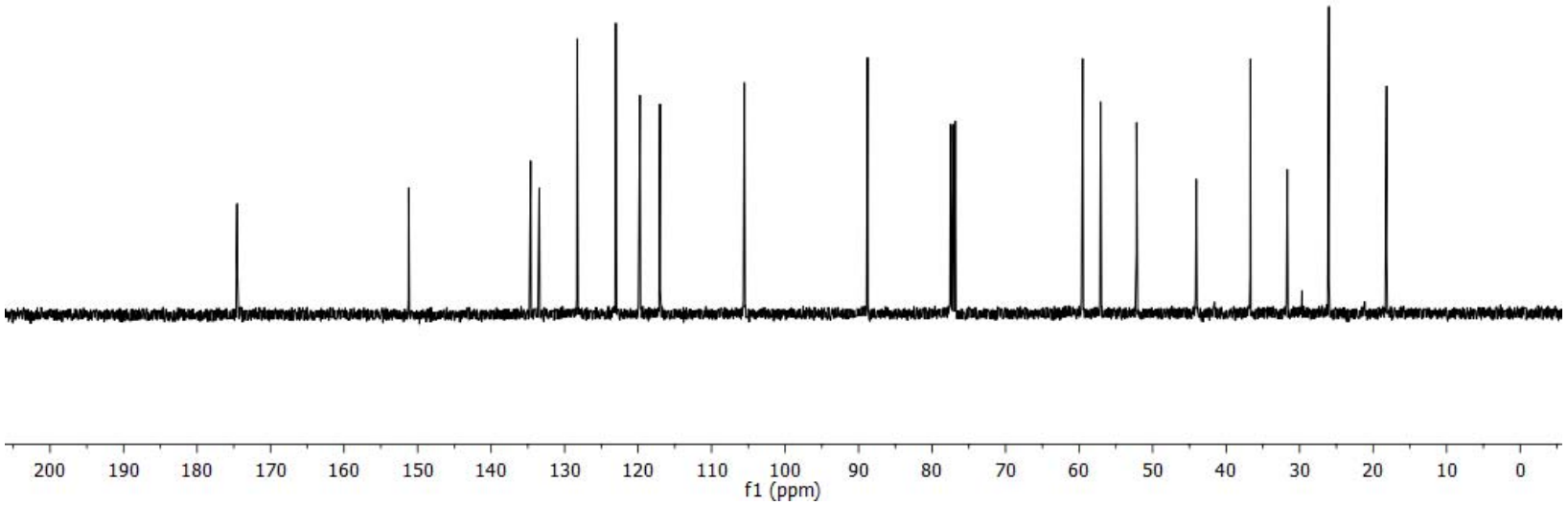
28a

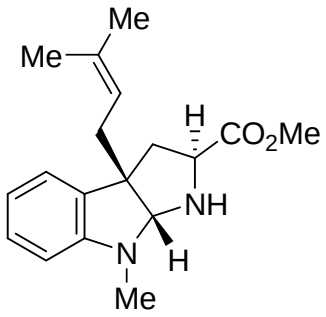
400 MHz, $CDCl_3$



28a

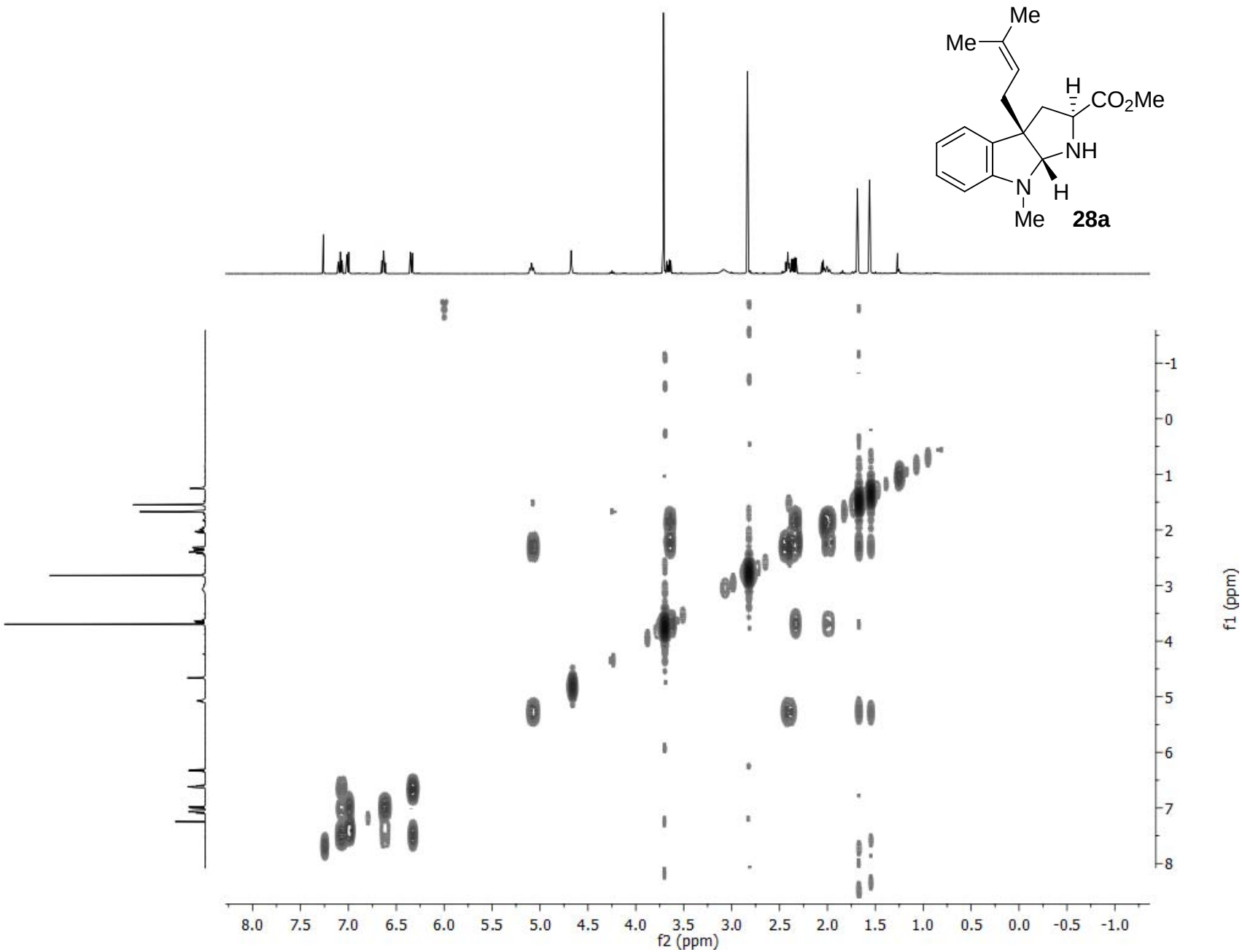
100 MHz, $CDCl_3$

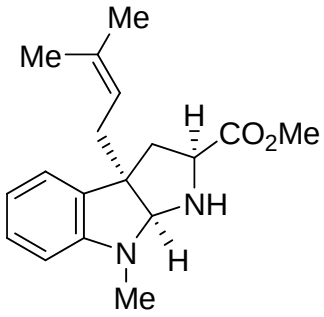




28a

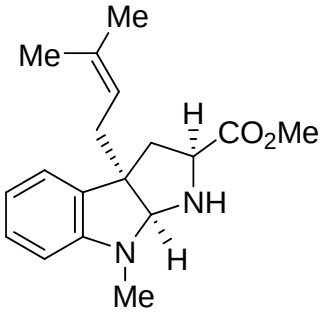
100 MHz, $CDCl_3$


Me
Me
H
CO2Me
NH
N
H
Me
28a
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
f2 (ppm)
-1
0
1
2
3
4
5
6
7
8
f1 (ppm)



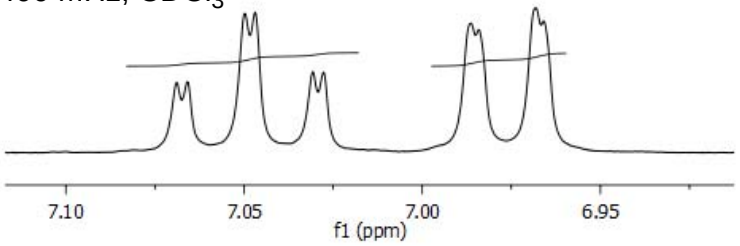
28b

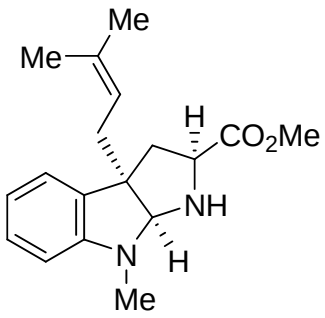
400 MHz, $CDCl_3$



28b

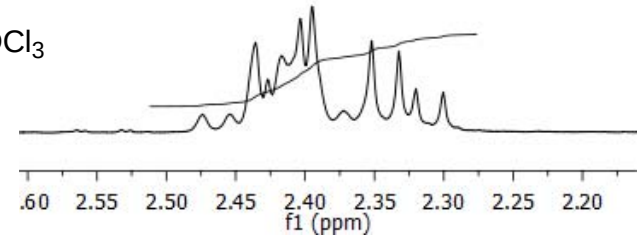
400 MHz, $CDCl_3$

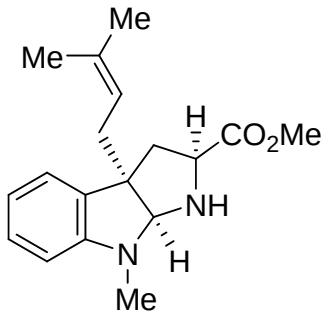




28b

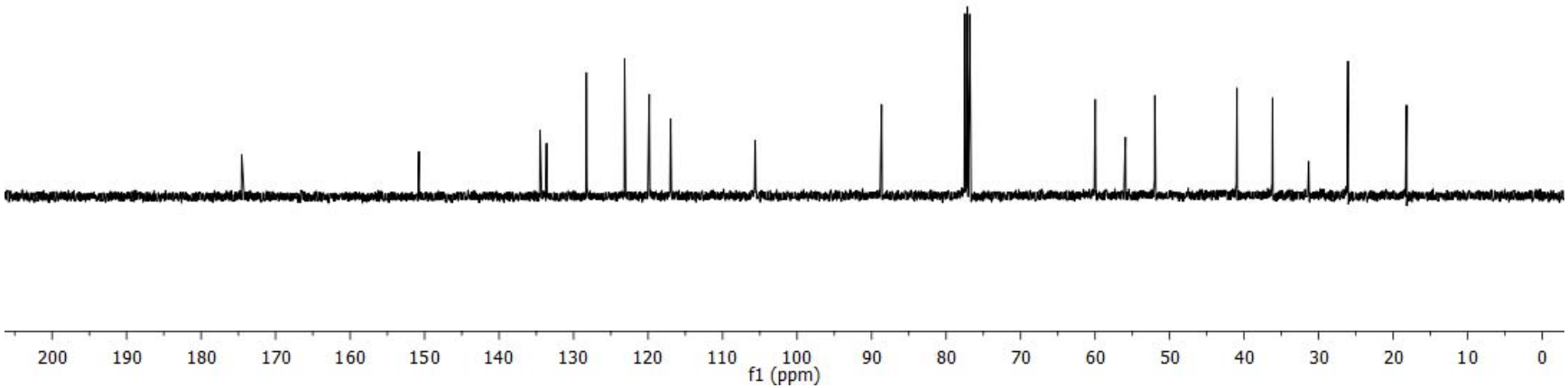
400 MHz, $CDCl_3$

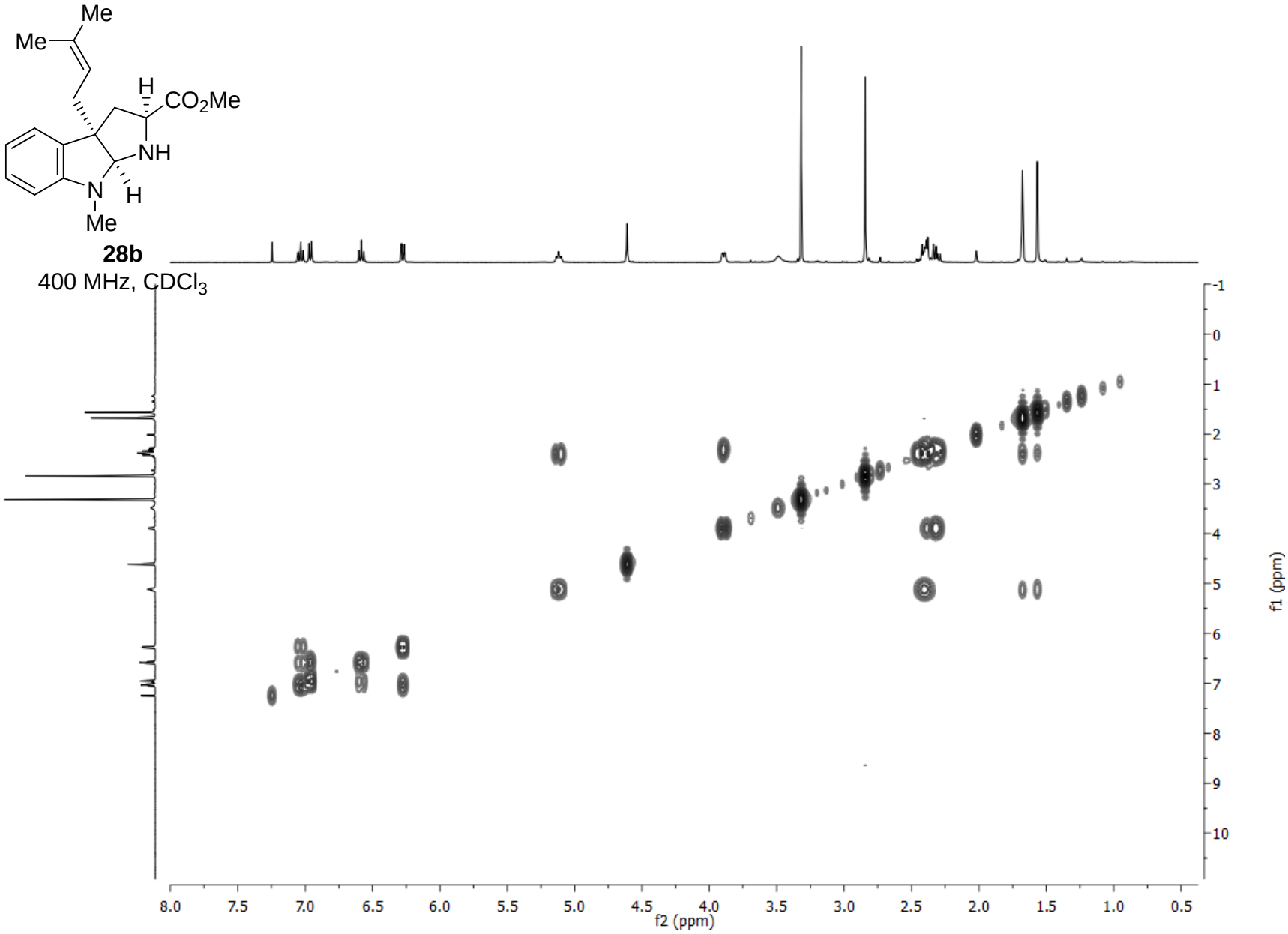


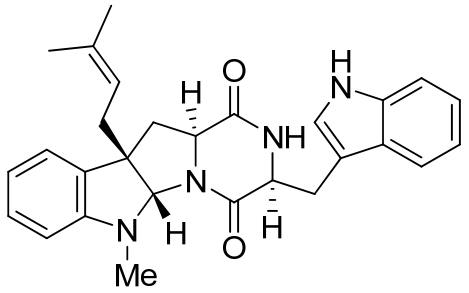


28b

100 MHz, $CDCl_3$

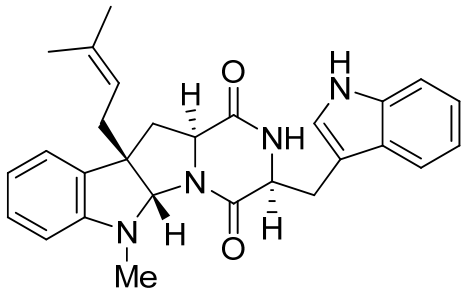



Me
Me
H
CO2Me
NH
N
H
Me
28b
400 MHz, CDCl3
f2 (ppm)
f1 (ppm)



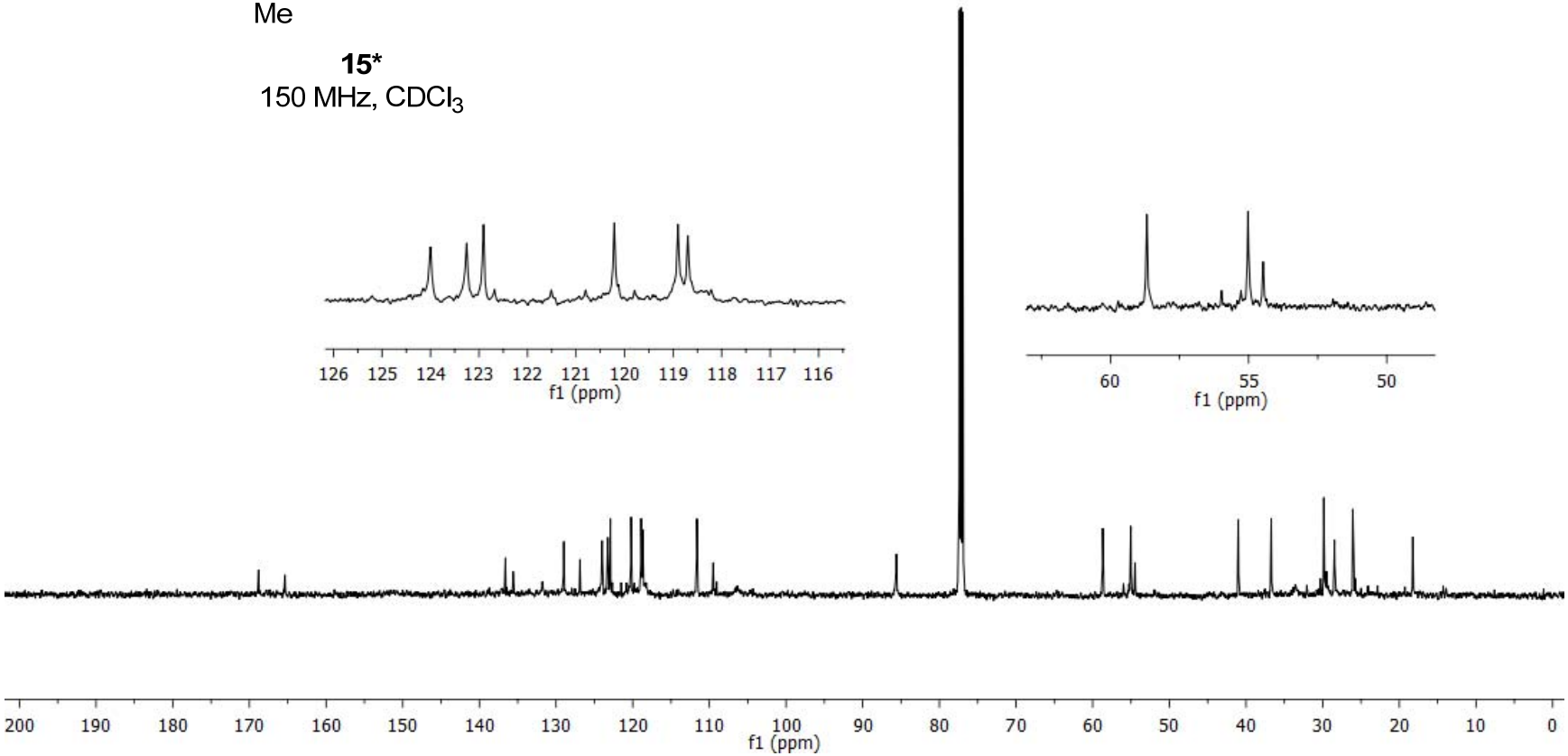
15*

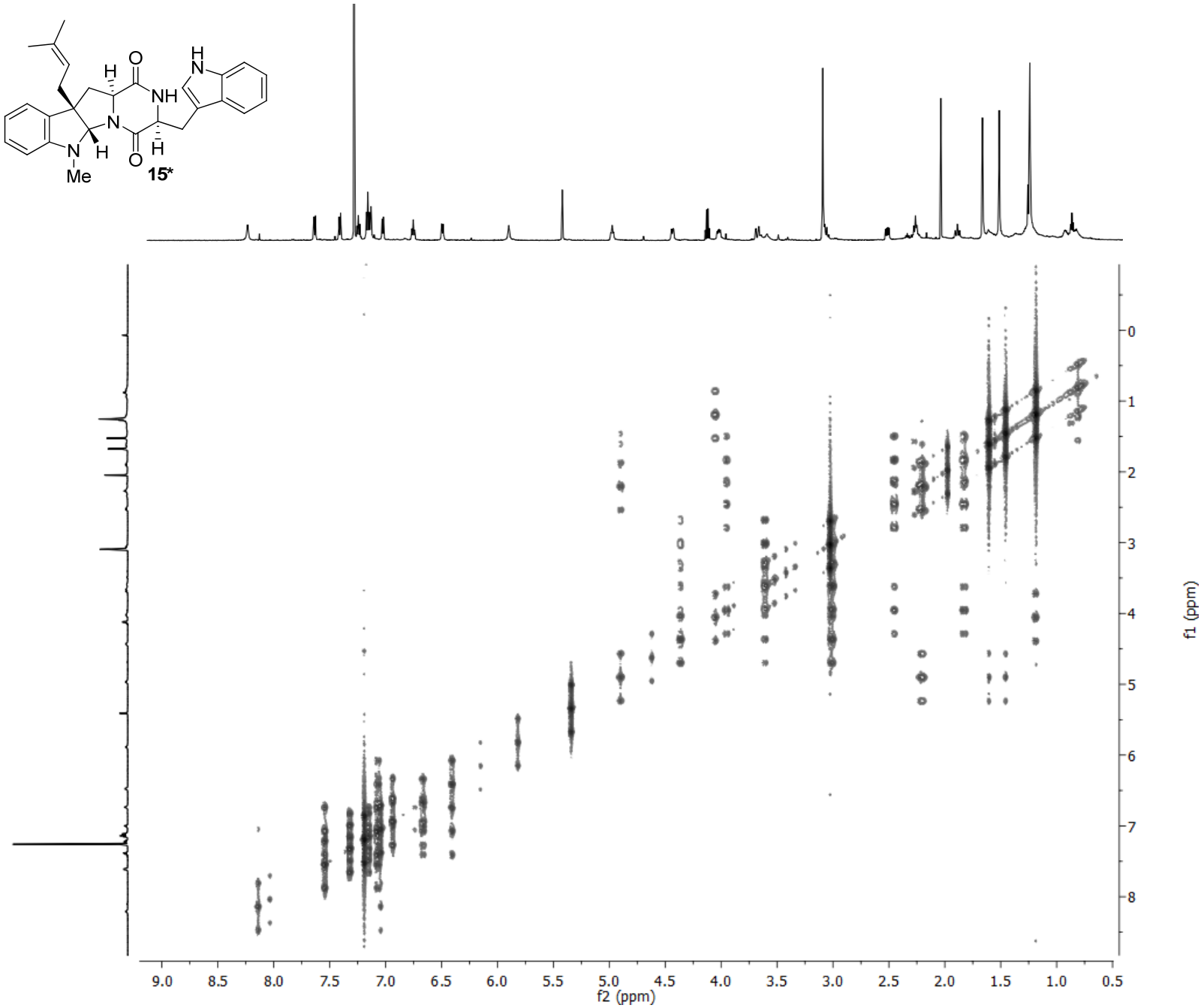
600 MHz, $CDCl_3$

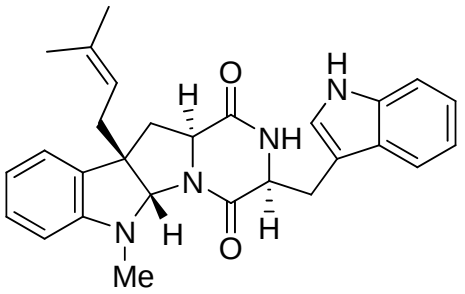


15*

150 MHz, $CDCl_3$

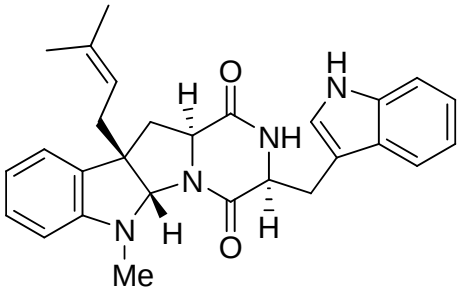



O
H
NH
N
H
N
H
O
H
N
Me
15*
f2 (ppm)
f1 (ppm)



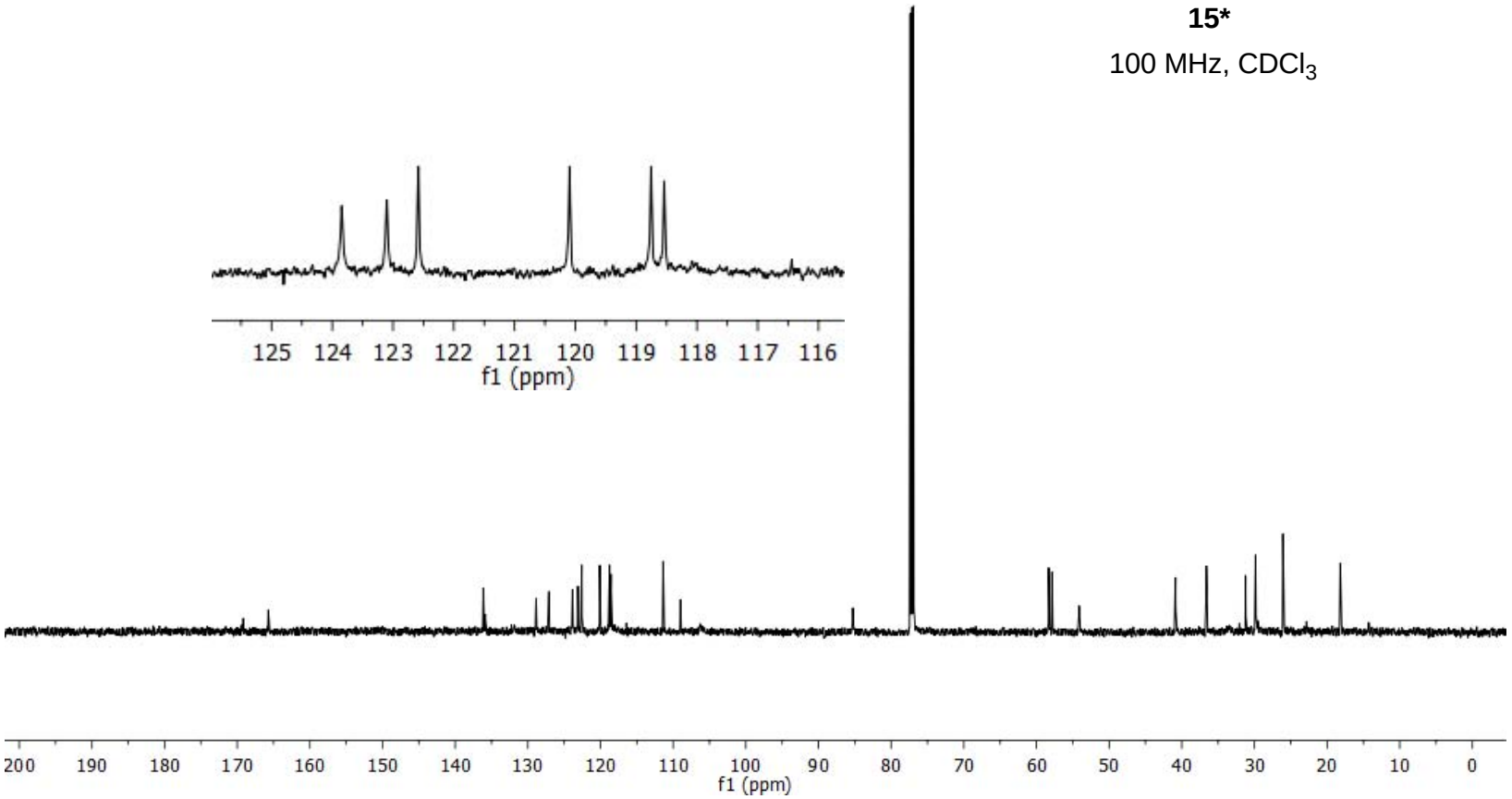
15*

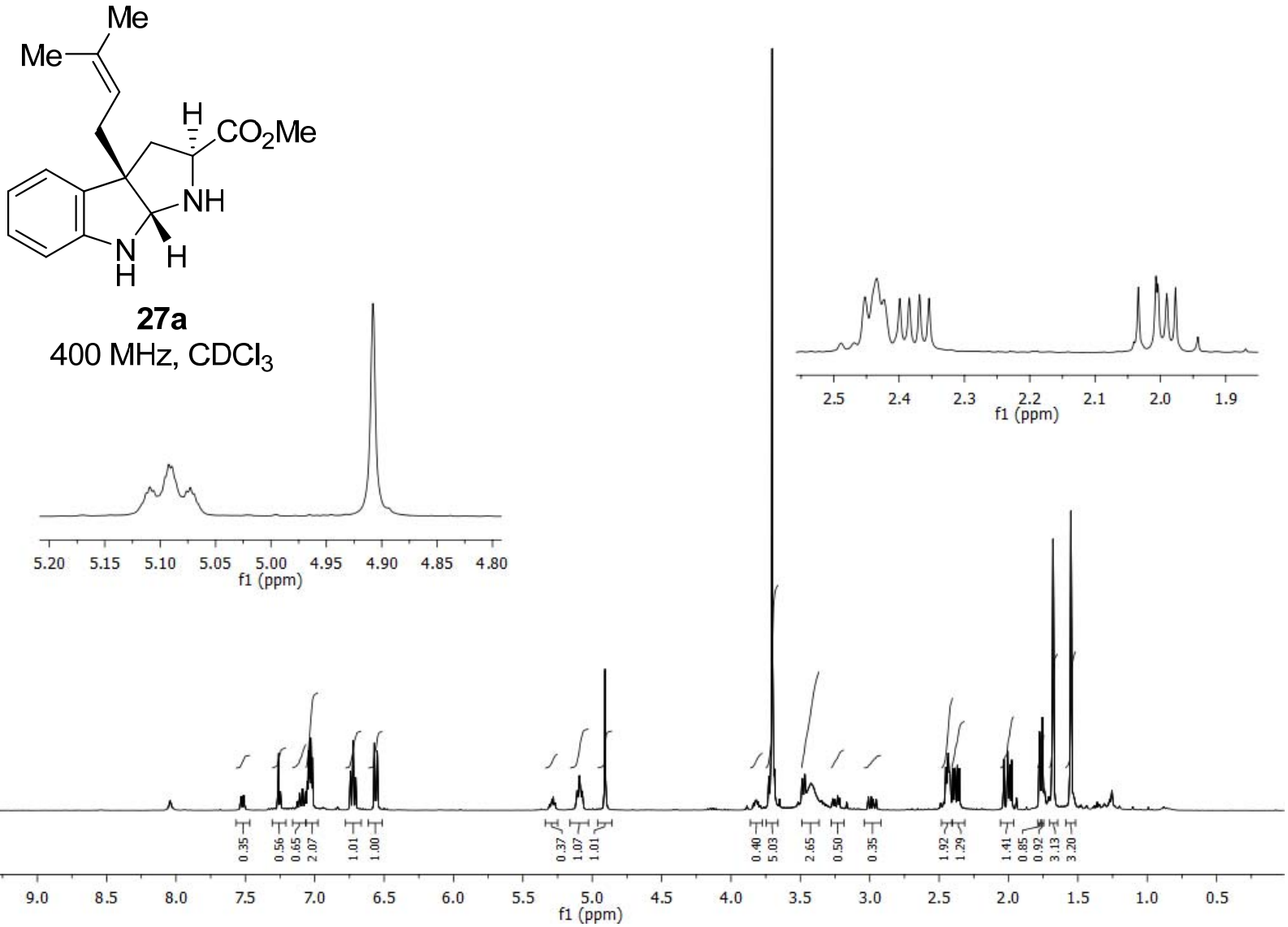
600 MHz, $CDCl_3$

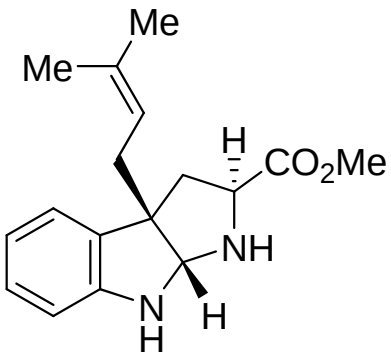


15*

100 MHz, $CDCl_3$

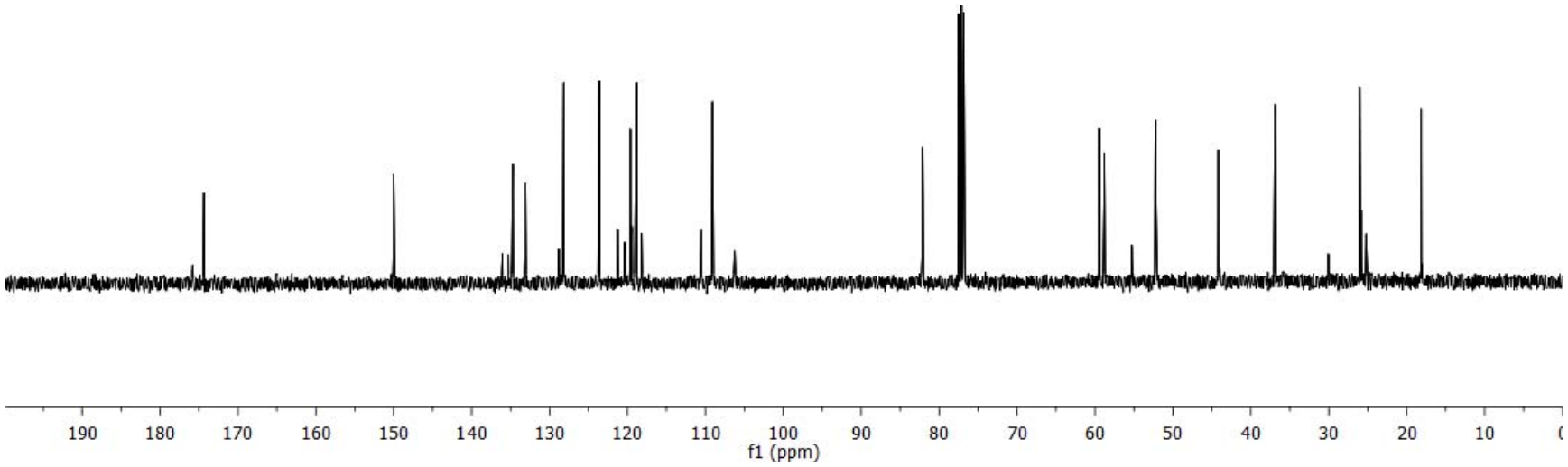


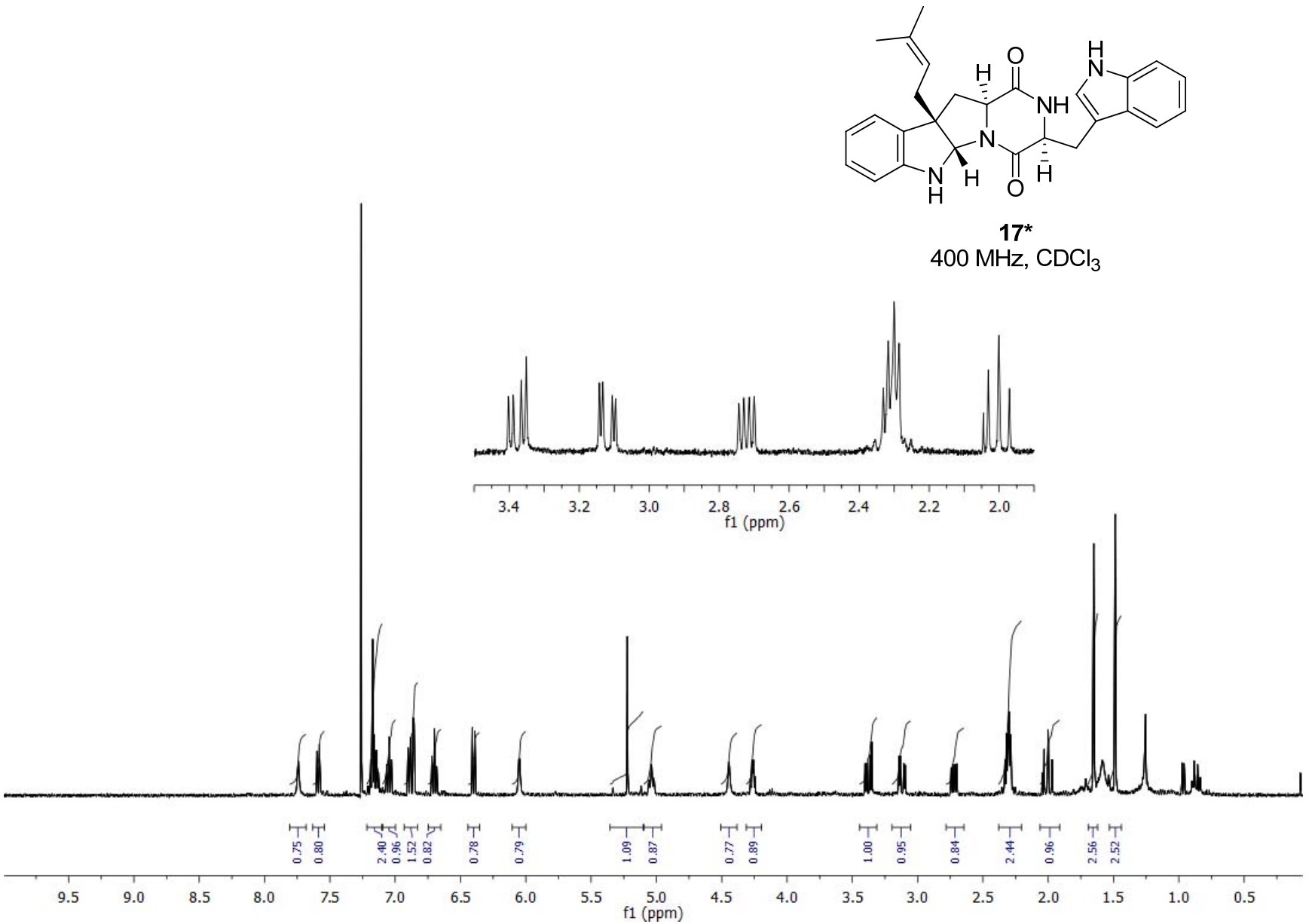

Me
Me
H
CO2Me
NH
N
H
H
27a
400 MHz, CDCl3
5.20 5.15 5.10 5.05 5.00 4.95 4.90 4.85 4.80
f1 (ppm)
2.5 2.4 2.3 2.2 2.1 2.0 1.9
f1 (ppm)
0.35 0.56 0.65 2.07 1.01 1.00 0.37 1.07 1.01 0.40 5.03 2.65 0.50 0.35 1.92 1.29 1.41 0.85 0.92 3.13 3.20
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
f1 (ppm)

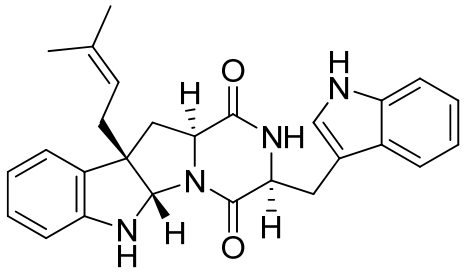


27a

100 MHz, $CDCl_3$

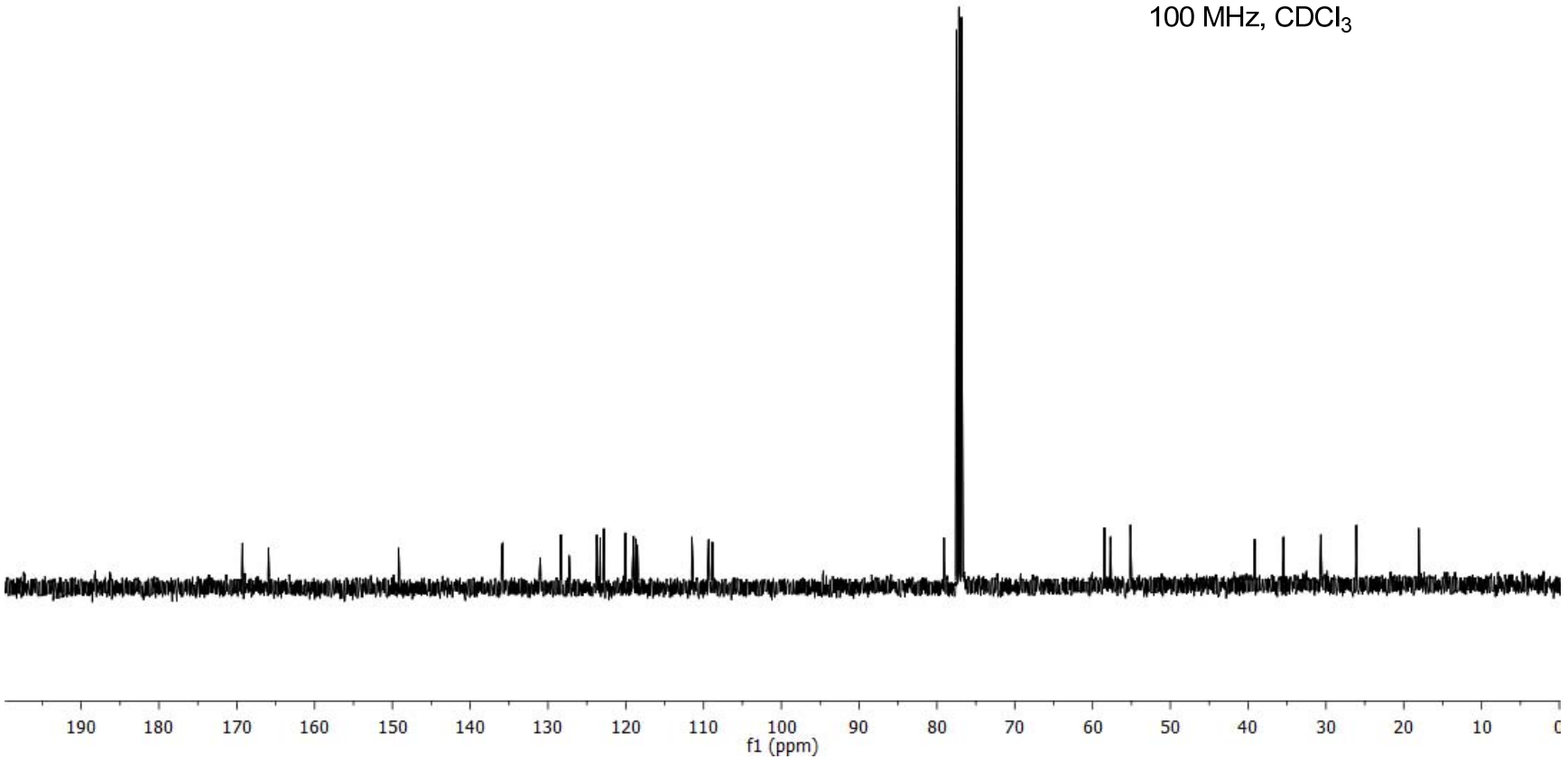


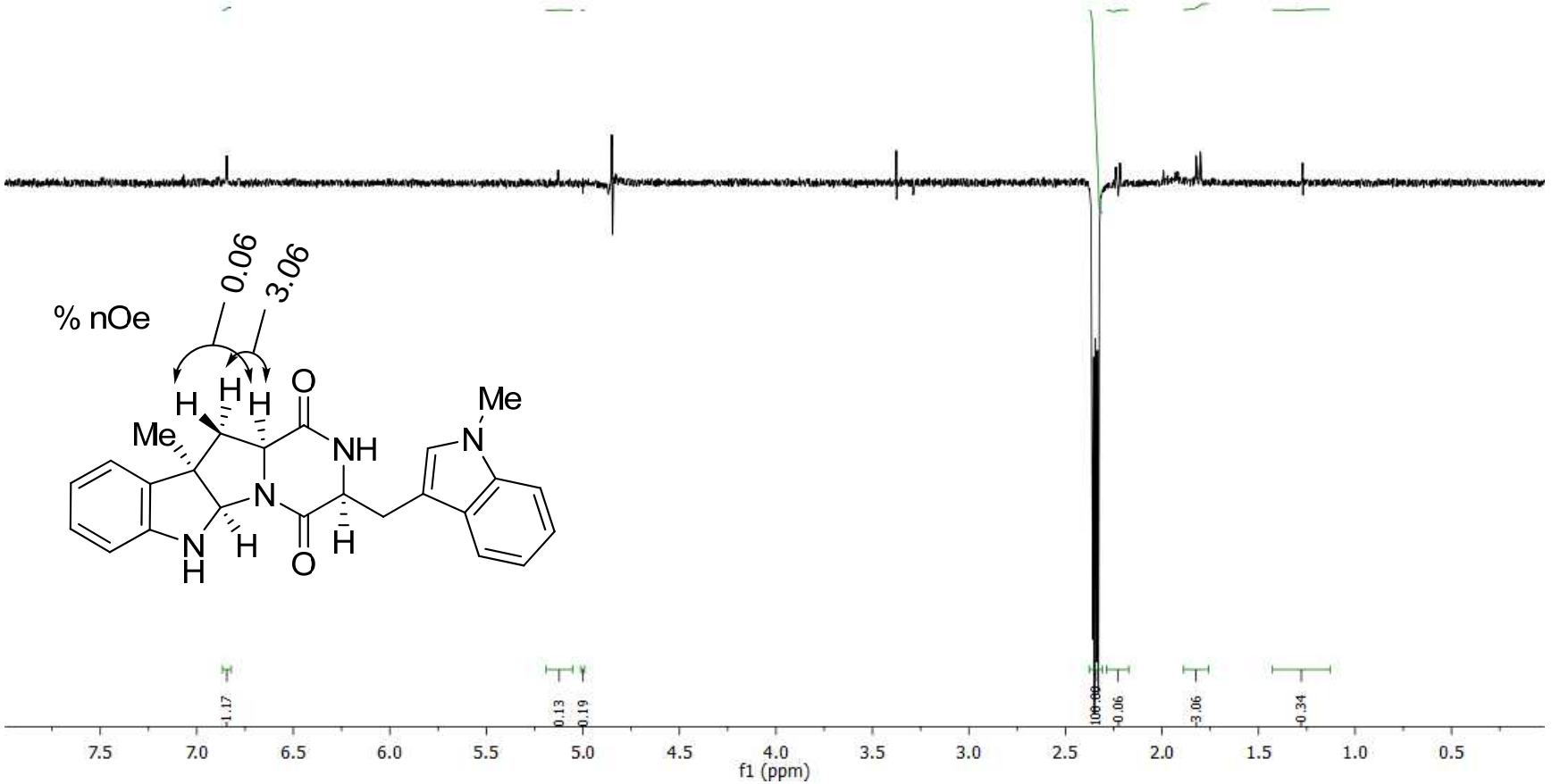

O
H
N
NH
N
H
N
H
H
O
17*
400 MHz, CDCl3
3.4
3.2
3.0
2.8
2.6
2.4
2.2
2.0
f1 (ppm)
0.75
0.80
2.40
0.96
1.52
0.82
0.78
0.79
1.09
0.87
0.77
0.89
1.00
0.95
0.84
2.44
0.96
2.56
2.52
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

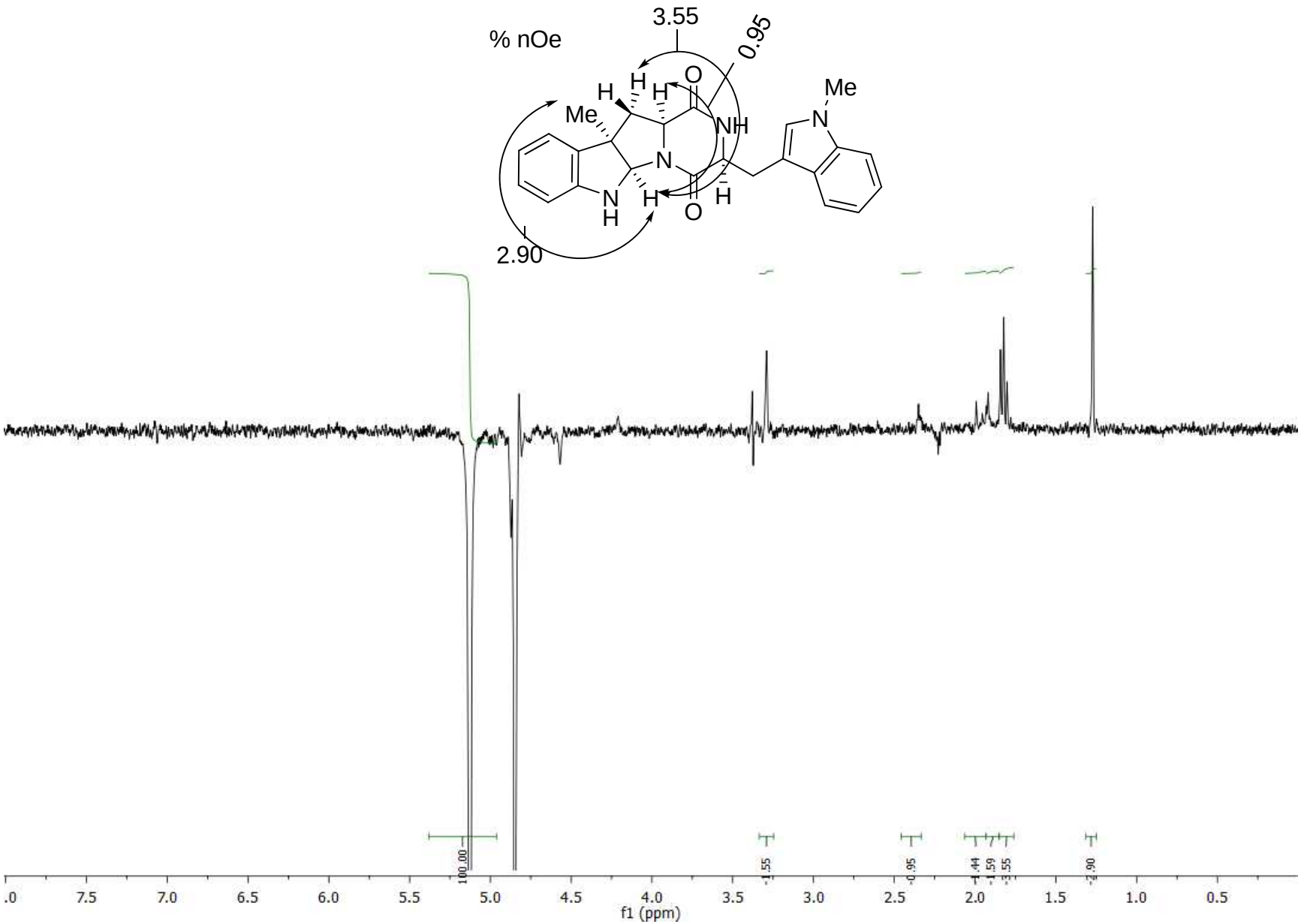


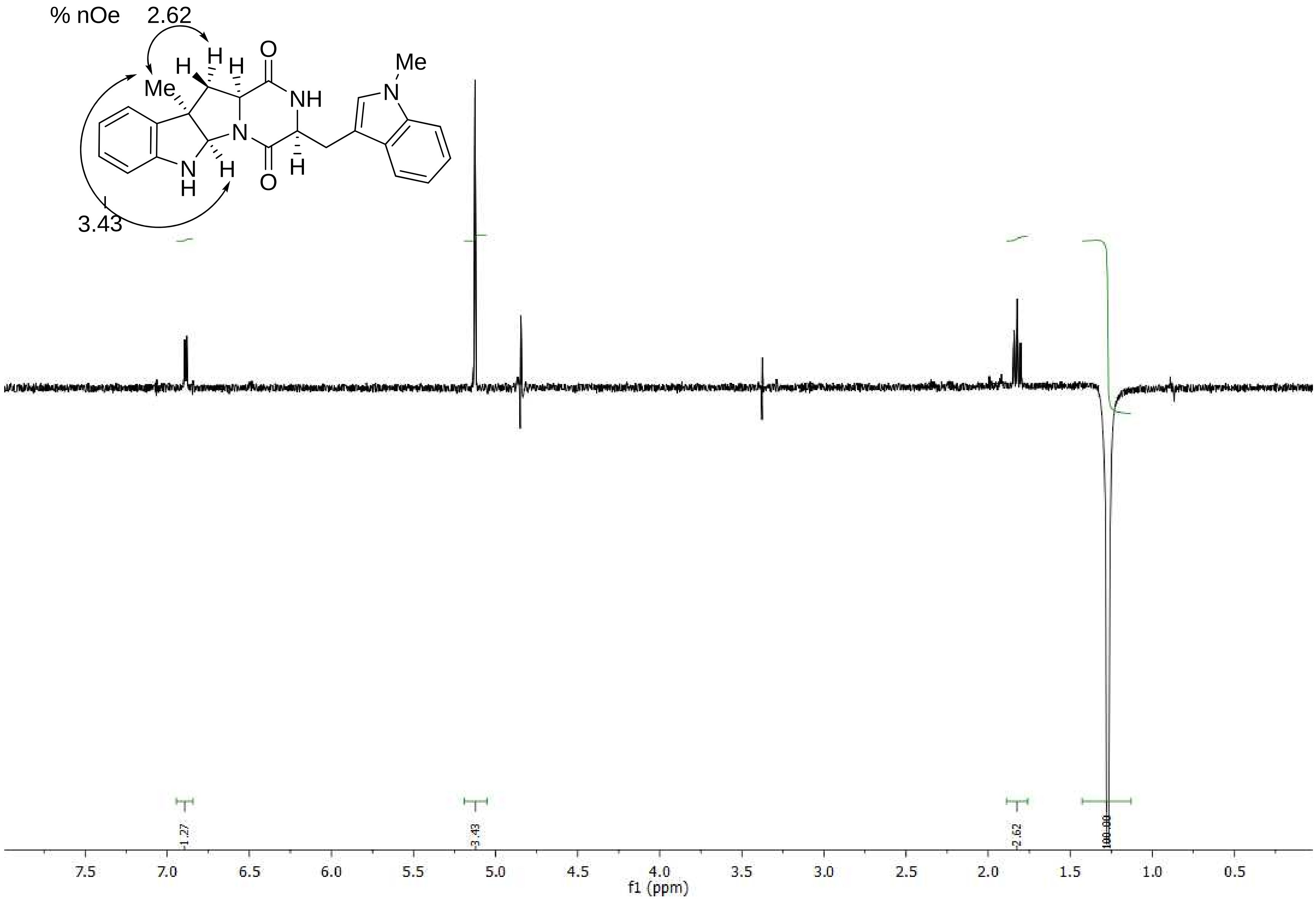
17*

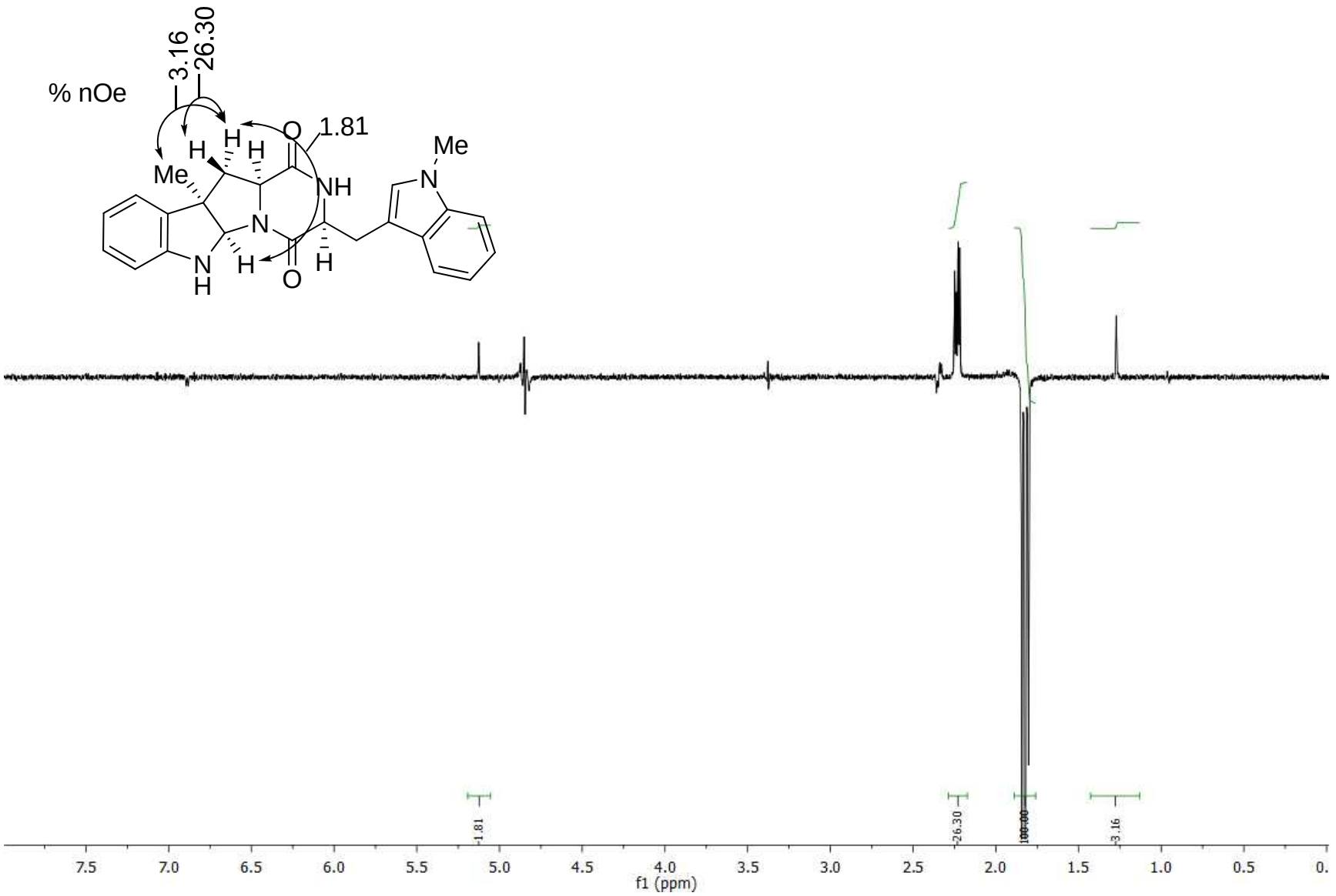
100 MHz, $CDCl_3$

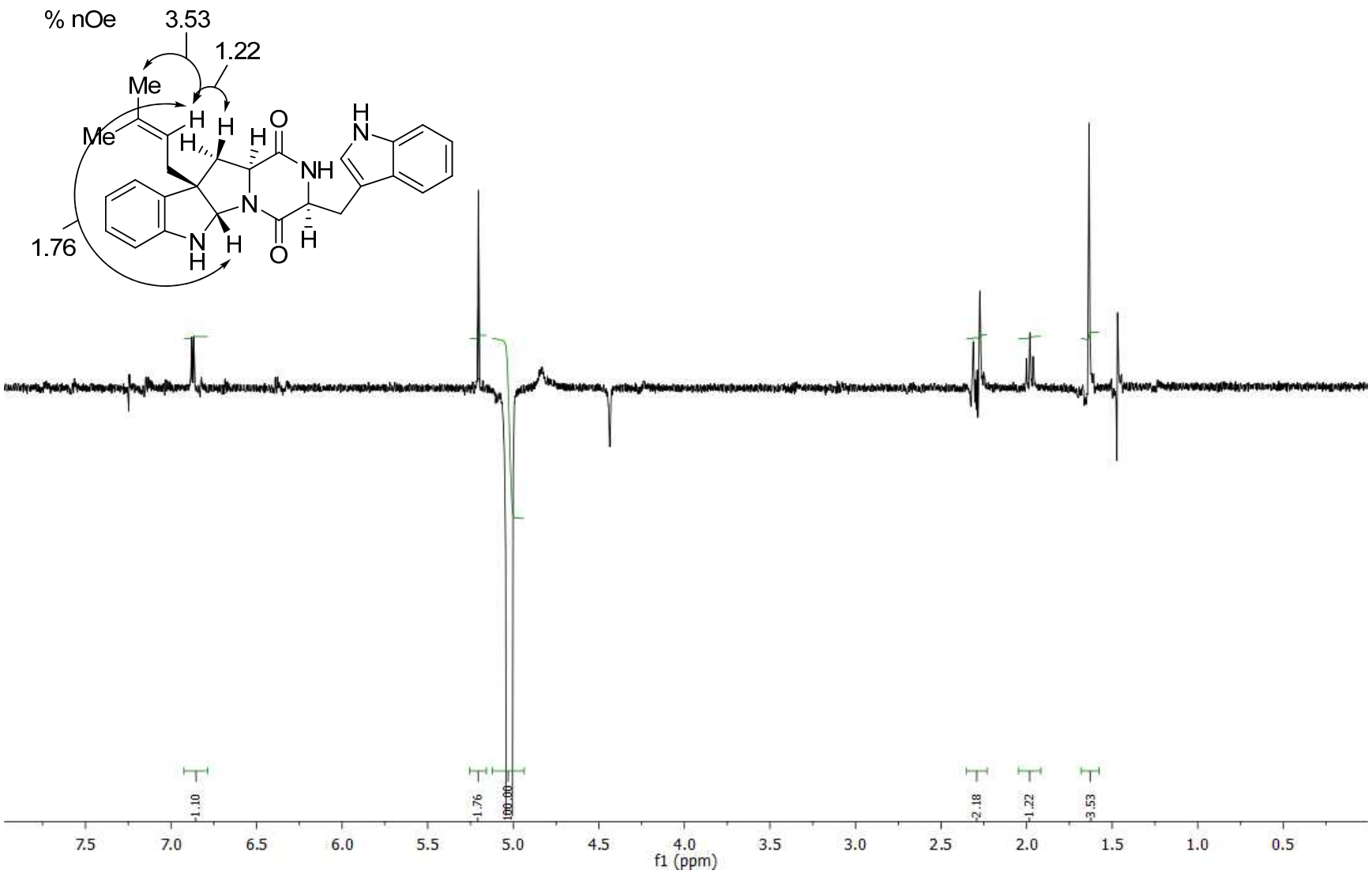


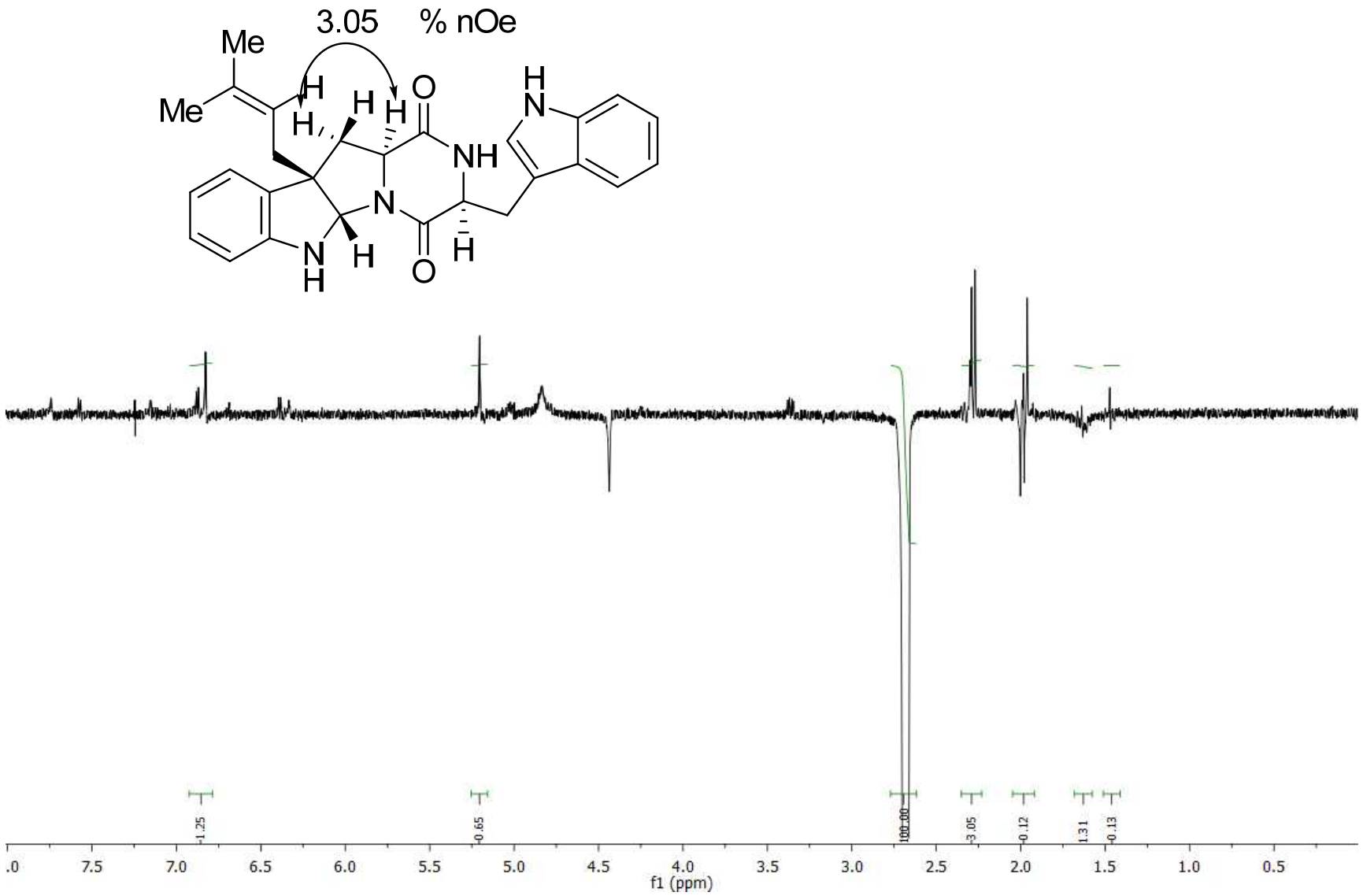

% nOe
0.06
3.06
H
H
H
O
NH
Me
N
Me
N
H
H
H
O
1.17
0.13
0.19
106.00
0.06
3.06
0.34
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

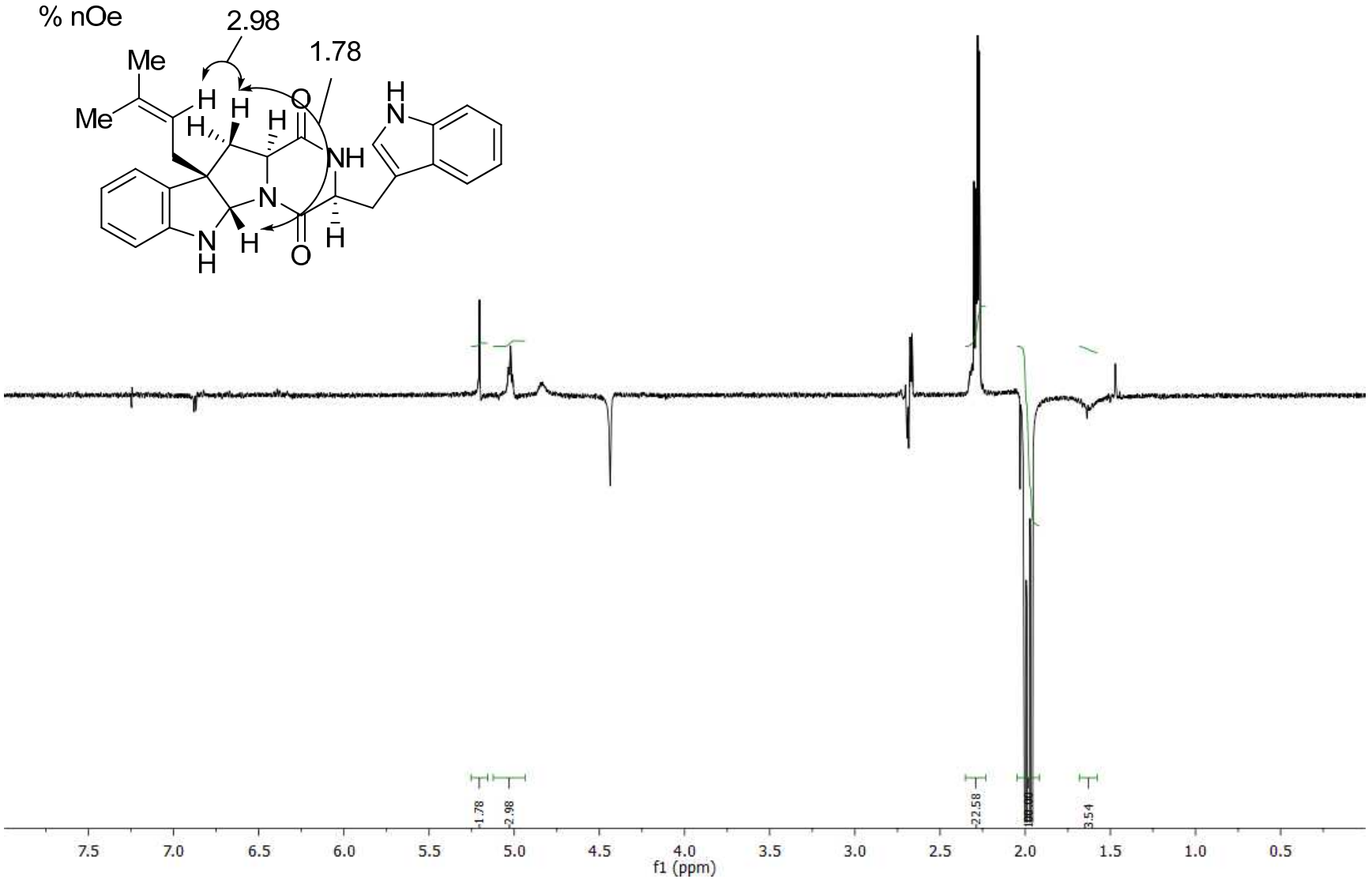

% nOe
3.55
0.95
2.90
Me
H
H
H
O
NH
N
Me
N
H
H
O
H
100.00
1.55
0.95
1.44
1.59
3.55
2.90
.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

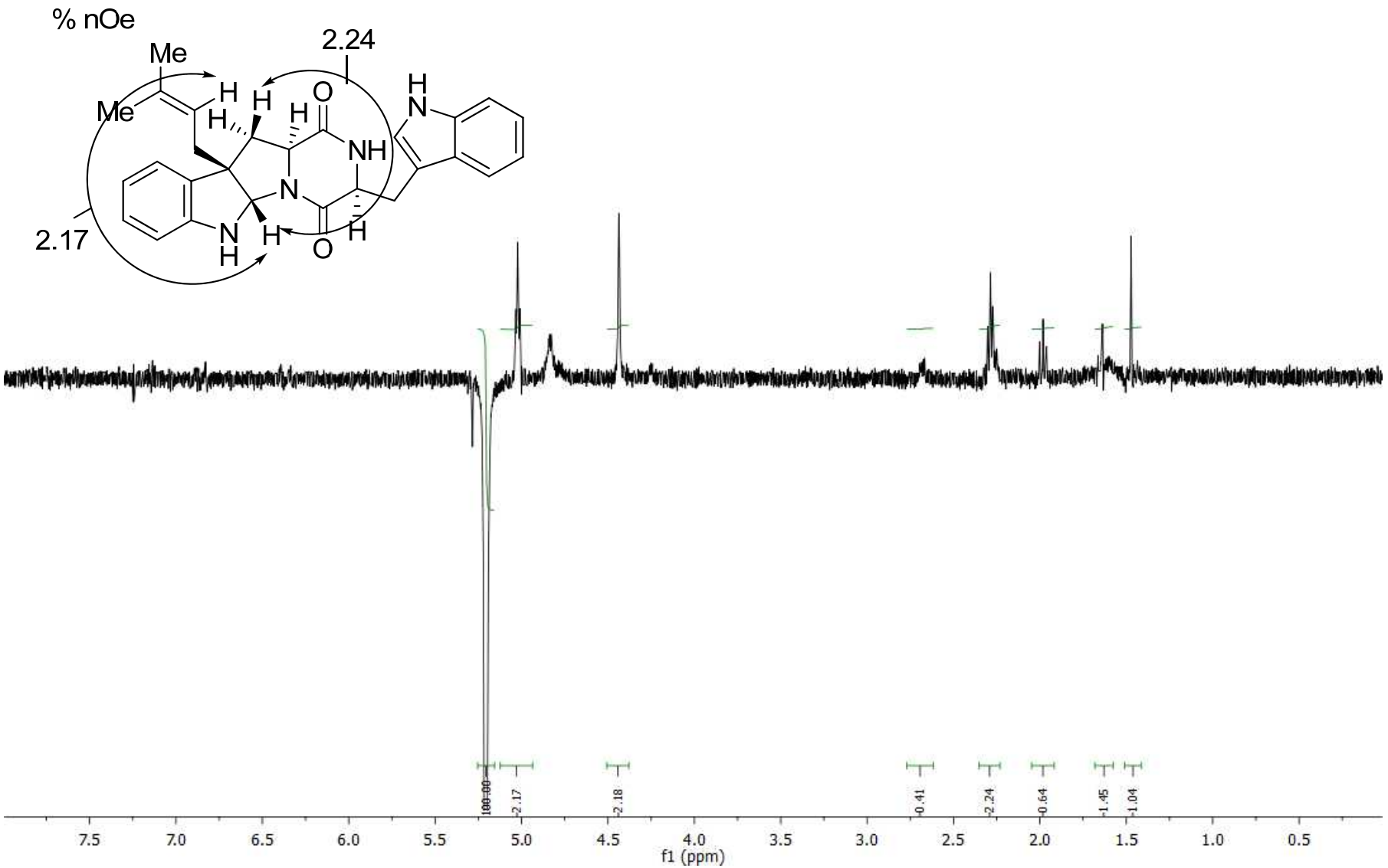

% nOe
2.62
3.43
Me
H
O
NH
N
H
1.27
3.43
2.62
100.00
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

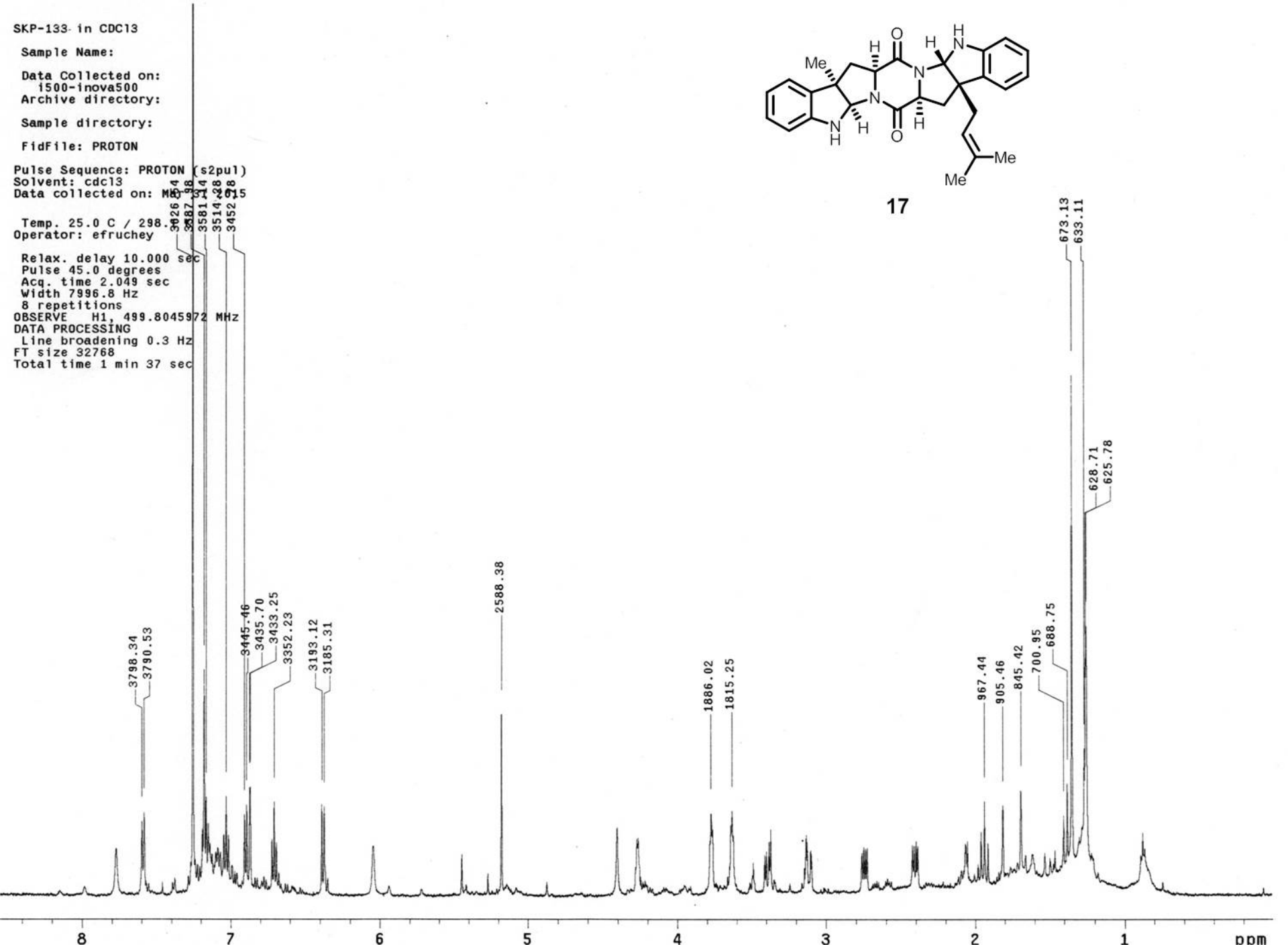

% nOe
3.16
26.30
1.81
Me
Me
H
H
H
O
NH
N
N
H
H
H
O
N
H
1.81
26.30
100.00
3.16
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.
f1 (ppm)

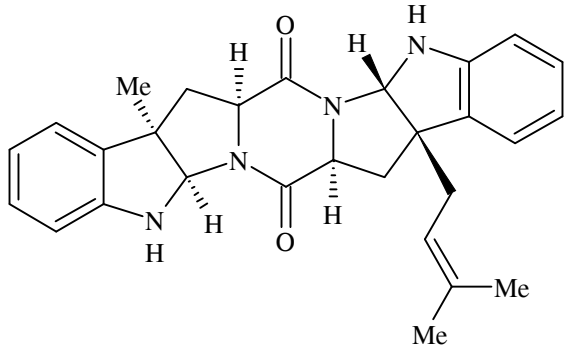

% nOe
3.53
1.22
Me
Me
1.76
H
H
H
H
O
NH
N
H
N
H
H
O
H
1.10
1.76
100.00
2.18
1.22
3.53
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

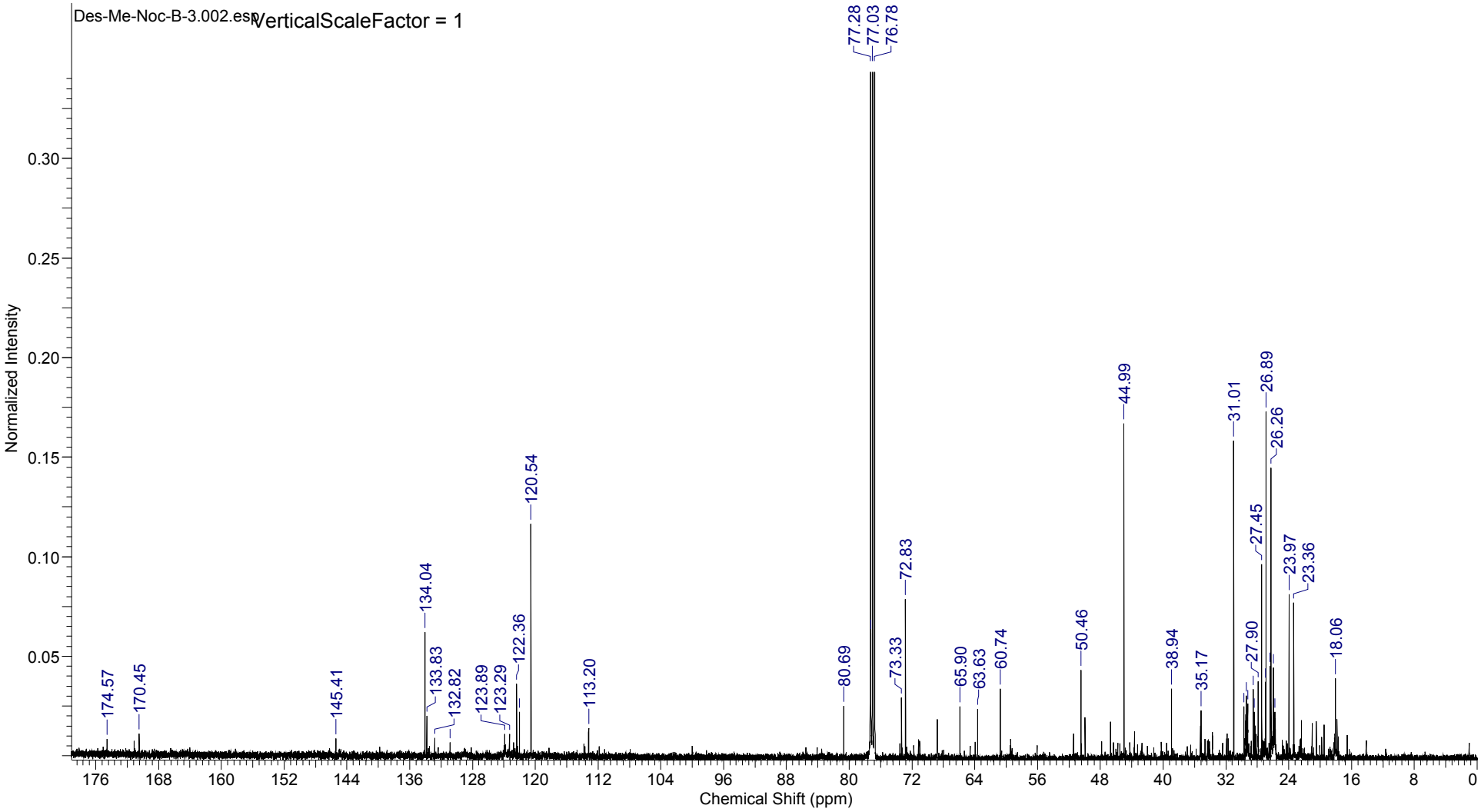

3.05 % nOe
Me
Me
H
H
H
O
H
N
NH
N
H
N
H
H
O
1.25
0.65
100.00
3.05
0.12
1.31
0.13
.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

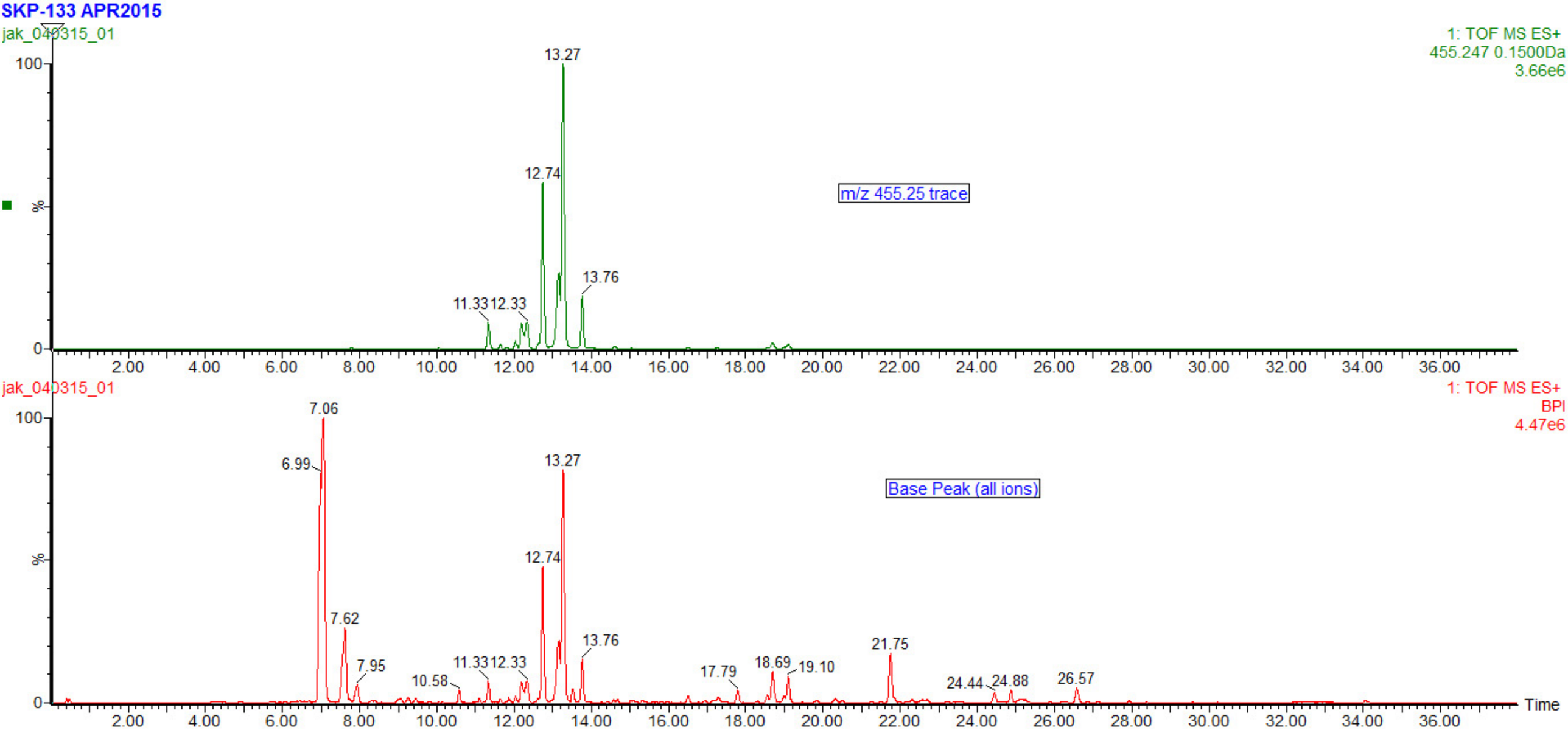

% nOe
2.98
1.78
Me
Me
H
H
H
H
O
NH
N
H
N
H
H
O
H
1.78
2.98
22.58
3.54
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


% nOe
Me
Me
2.24
2.17
H
H
H
H
O
NH
N
H
N
H
N
H
H
O
H
100.00
2.17
2.18
0.41
2.24
0.64
1.45
1.04
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)


SKP-133 in CDCl3
Sample Name:
Data Collected on:
i500-inova500
Archive directory:
Sample directory:
FidFile: PROTON
Pulse Sequence: PROTON (s2pul)
Solvent: cdcl3
Data collected on: Mar 31 2015
Temp. 25.0 C / 298.
Operator: efruchey
Relax. delay 10.000 sec
Pulse 45.0 degrees
Acq. time 2.049 sec
Width 7996.8 Hz
8 repetitions
OBSERVE H1, 499.8045972 MHz
DATA PROCESSING
Line broadening 0.3 Hz
FT size 32768
Total time 1 min 37 sec
3626.04
3587.38
3581.14
3514.28
3452.18
3798.34
3790.53
3445.46
3435.70
3433.25
3352.23
3193.12
3185.31
2588.38
1886.02
1815.25
967.44
905.46
845.42
700.95
688.75
673.13
633.11
628.71
625.78
Me
Me
Me
17
8
7
6
5
4
3
2
1
ppm


O
H
H
N
Me
N
N
H
H
H
O
H
N
Me
Me


Des-Me-Noc-B-3.002.esp
VerticalScaleFactor = 1
Normalized Intensity
0.30
0.25
0.20
0.15
0.10
0.05
77.28
77.03
76.78
174.57
170.45
145.41
134.04
133.83
132.82
123.89
123.29
122.36
120.54
113.20
80.69
73.33
72.83
65.90
63.63
60.74
50.46
44.99
38.94
35.17
31.01
27.90
27.45
26.89
26.26
23.97
23.36
18.06
176
168
160
152
144
136
128
120
112
104
96
88
80
72
64
56
48
40
32
24
16
8
0
Chemical Shift (ppm)


SKP-133 APR2015
jak_040315_01
1: TOF MS ES+
455.247 0.1500Da
3.66e6
100
%
0
13.27
12.74
13.76
11.33
12.33
m/z 455.25 trace
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00
jak_040315_01
1: TOF MS ES+
BPI
4.47e6
100
%
0
7.06
6.99
13.27
Base Peak (all ions)
12.74
7.62
13.76
7.95
11.33
12.33
10.58
17.79
18.69
19.10
21.75
24.44
24.88
26.57
Time
2.00
4.00
6.00
8.00
10.00
12.00
14.00
16.00
18.00
20.00
22.00
24.00
26.00
28.00
30.00
32.00
34.00
36.00