Supplementary Data

Synthesis and Biological Evaluation of the [D-MeAla^{11}]-Epimer of Coibamide A

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Table of contents

Experimental procedures

Synthetic general methods. Optical rotations were measured with a JASCO P-1020 polarimeter. ^{1}H and ^{13}C NMR spectra were recorded using a JEOL ECA-500 spectrometer, and chemical shifts are reported in δ (ppm) relative to a TMS internal standard (at δ_H 0 in $CDCl_3$), and the residual $CHCl_3$ signal (δ_C 77.23 ppm), respectively. ^{1}H NMR spectra are tabulated as follows: chemical shift, multiplicity (br = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), number of protons, and coupling constant(s). Electrospray ionization mass spectrometry (ESI-MS) data were recorded using a Quattro micro API (Waters) spectrometer. High resolution mass spectra were recorded on a Shimadzu LC-ESI-IT-TOF-MS. Infrared (IR) spectra were obtained on a JASCO FT/IR-4100 FT-IR spectrometer with JASCO ATR PRO410-S. For flash chromatography, Wakogel C-300E was employed. For analytical reversed phase HPLC, a Cosmosil 5C_{18}-ARII column (4.6 × 250 mm, Nacalai Tesque Inc., Kyoto, Japan) was employed with a linear gradient of CH_3CN containing 0.1% (v/v) TFA (solvent B) in 0.1% TFA/H_2O (solvent A) at a flow rate of 1 mL/min on a Hitachi L-2130 system (Hitachi corporation, Ltd, Tokyo, Japan). Preparative HPLC was performed using a Cosmosil 5C_{18}-ARII column (20 × 250 mm, Nacalai Tesque Inc.) with a linear gradient of solvent B in solvent A at a flow rate of 8 mL/min on a Hitachi L-7150 system (Hitachi corporation, Ltd).

Fmoc-MeSer(Me)-OH. Fmoc-MeSer(Me)-OH was synthesized by the identical procedure reported previously.[S1] To a suspension of Fmoc-Ser(Me)-OH (5.0 g, 14.7 mmol) in toluene (293 mL), paraformaldehyde (2.93 g, 97.6 mmol) and *p*-toluenesulfonic acid monohydrate (293 mg, 1.54 mmol) were added, and the mixture was refluxed for 1 h. The solution was cooled, washed with 1 N $NaHCO_3$ aq. and dried over Na_2SO_4. Concentration in vacuo gave a crystalline product. The crystalline product was dissolved in $CHCl_3$/TFA (1:1, 147 mL), and triethylsilane (7.02 mL, 44.0 mmol) were added. The solution was stirred at room temperature for 23 h followed by concentration to give an oily residue. Purification by flash chromatography on silica gel ($CHCl_3$:MeOH = 95:5 to 90:10) provided Fmoc-MeSer(Me)-OH as a colorless oil (4.98 g, 2 steps 96%): $[\alpha]^{26}_{D}$ −2.11 (*c* 1.17, $CHCl_3$); IR (neat): 2918, 1739, 1699, 1451, 1404, 1323, 1160, 911, 740 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) *δ* 2.95 and 3.03 (2 s, 3H), 3.26 and 3.39 (2 s, 3H), 3.50-3.66 (m, 1H), 3.73-3.94 (m, 1H), 4.19-4.33 (m, 1H), 4.35-4.65 (m, 2H), 4.96 (dd, *J* = 7.8, 4.4 Hz, 1H), 7.24-7.47 (m, 4H), 7.52-7.68 (m, 2H), 7.70-7.83 (m, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) *δ* 32.3, 47.1, 58.9, 59.0, 67.5, 68.0, 69.9, 70.1, 120.0, 124.7, 125.0, 127.1, 127.7, 141.3, 143.8, 156.0, 157.0, 174.0; HRMS (ESI) *m/z* calcd for $C_{20}H_{22}NO_5$ ($[M+H]^+$) 356.1498, found: 356.1493.

H-Hva-OBn (6). To a suspension of (*S*)-2-hydroxyisovaleric acid (500 mg, 4.23 mmol), $NaHCO_3$ (711 mg, 21.2 mmol) in dry DMF (12.8 mL) was added BnBr (2.51 mL, 4.23 mmol) dropwise, and the mixture was stirred under Ar at room temperature for 23 h. The resulting mixture was diluted with EtOAc and the extract was washed with water, saturated NH_4Cl and brine, and dried over Na_2SO_4. After concentration, the residue was purified by flash chromatography on silica gel (*n*-hexane:EtOAc = 9:1) to provide the ester **6** as a colorless oil (708 mg, 80%): $[\alpha]^{27}_D$ −10.1 (*c* 2.00, $CHCl_3$); IR (neat): 3500, 2964, 2933, 2875, 1732, 1456, 1370, 1262, 1213, 1137, 1071, 1029, 908, 733, 697 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.83 (d, J = 6.9 Hz, 3H), 1.01 (d, J = 6.9 Hz, 3H), 2.09 (m, 1H), 2.76 (s, 1H), 4.08 (d, J = 3.2 Hz, 1H), 5.19 (d, J = 11.9 Hz, 1H), 5.24 (d, J = 11.9 Hz, 1H), 7.31-7.41 (m, 5H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.8, 18.8, 32.1, 67.2, 75.0, 128.4, 128.5, 128.6, 135.1, 174.8; HRMS (ESI) *m/z* calcd for $C_{12}H_{16}NaO_3$ ($[M+Na]^+$) 231.0997, found: 231.1000.

Fmoc-Val-Hva-OBn (7). H-Hva-OBn **6** (300 mg, 1.44 mmol) and DMAP (17.6 mg, 0.144 mmol) were added to a stirred mixture of Fmoc-Val-OH (586 mg, 1.73 mmol) and DCC (594 mg, 2.88 mmol) in dry THF (3 mL). The mixture was stirred at room temperature for 24 h. The resulting mixture was poured into Et_2O, and the solution was filtered through a Celite pad. The filtrate was washed with saturated $NaHCO_3$, water and brine, and dried over Na_2SO_4. After concentration, the residue was purified by flash column chromatography on silica gel (*n*-hexane:DCM:EtOAc = 60:35:5) to provide the ester **7** as a colorless oil (463 mg, 61%): $[\alpha]^{25}_D$ −29.1 (*c* 1.00, $CHCl_3$); IR (neat): 2963, 1744, 1513, 1451, 1371, 1280, 1174, 1094, 1033, 911, 683 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 0.84-1.24 (m, 12H), 2.27 (m, 2H), 4.23 (t, J = 6.9 Hz, 1H), 4.35-4.56 (m, 3H), 4.94 (d, J = 4.0 Hz, 1H), 5.16 (d, J = 12.0 Hz, 1H), 5.20 (d, J = 12.0 Hz, 1H), 5.32 (d, J = 8.6 Hz, 1H), 7.28-7.50 (m, 9H), 7.60 (d, J = 6.9 Hz, 2H), 7.76 (d, J = 7.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.0, 17.1, 18.7, 18.9, 30.1, 31.3, 47.2, 58.6, 67.0, 67.1, 77.4, 120.0, 125.1, 127.0, 127.7, 128.4, 128.5, 128.6, 135.1, 141.3, 143.7, 143.9, 156.2, 169.1, 171.9; HRMS (ESI) *m/z* calcd for $C_{32}H_{35}NNaO_6$ ($[M+Na]^+$) 552.2362, found: 552.2369.

Fmoc-Val-Hva-OH (8). To a solution of Fmoc-Val-Hva-OBn **7** (430 mg, 0.82 mmol) in DCM (2.73 mL) was added 10% Pd/C (59.3 mg), and the mixture was stirred under H_2 at room temperature for 7 h. The resulting mixture was filtered through a Celite pad, and the filtrate was concentrated. Purification of the residue by flash column chromatography on silica gel (EtOAc:EtOH = 1:0 to 0:1) provided the acid **8** as a colorless oil (246 mg, 68%): $[\alpha]^{26}_D$ −28.3 (*c* 1.00, $CHCl_3$); IR (neat): 3459, 2964, 1723, 1617, 1513, 1450, 1407, 1277, 1109, 1014, 907, 683, 649 cm^{-1}; ^1H NMR (400 MHz,

$CDCl_3$) δ 0.86-1.08 (m, 12H), 2.28 (m, 2H), 4.23 (m, 1H), 4.35-4.49 (m, 3H), 4.93 (m, 1H), 5.35 (m, 1H), 6.54 (s, 1H), 7.24-7.32 (m, 2H), 7.35-7.40 (m, 2H), 7.60 (m, 2H), 7.74 (d, J = 7.5 Hz, 2H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.0, 17.1, 18.8, 18.9, 30.0, 31.2, 47.1, 58.7, 67.2, 77.1, 120.0, 125.1, 127.0, 127.7, 141.3, 143.7, 143.8, 156.4, 172.0, 173.9; HRMS (ESI) *m/z* calcd for $C_{25}H_{30}NO_6$ ($[M+H]^+$) 440.2073, found: 440.2079.

Fmoc-MeAla-(2-Cl)Trt resin (1). A solution of Fmoc-MeAla-OH (650.7 mg, 2.0 mmol) and (i-Pr)$_2$NEt (2.32 mL, 13.3 mmol) in DCM (22.2 mL) was added to (2-Cl)Trt chloride resin (2.22 g, 3.33 mmol), and the reaction was continued for 2 h at room temperature. The reagent solution was filtered off, and the resin was washed with DCM/DMF/(i-Pr)$_2$NEt (17:2:1) to yield the desired resin **1** (0.616 mmol/g, 2.81 g, 86% loading).

Fmoc deprotection during solid-phase peptide synthesis. The peptidyl resin was treated with piperidine/DMF (2:8) for 20 min. For the Fmoc deprotection for Tyr(Me)^{10} and MeLeu^9, Fmoc-protected resin was treated with piperidine/DMF (2:8) for 2 min followed by additional 8 min.

Coupling reaction using HATU/(i-Pr)$_2$NEt [for Ala^8 and Tyr(Me)^{10}]. To a solution of Fmoc amino acids (4.33 mmol) in DMF (8 mL) were added HATU (1.61 g, 4.24 mmol) and (i-Pr)$_2$NEt (1.51 mL, 8.65 mmol). After 2 min, the whole was poured to the peptidyl resin (0.865 mmol), and the reaction was continued for 2 h.

Coupling reaction using DIC/HOBt [for MeIle^7 and MeLeu^9]. To a solution of Fmoc amino acid (4.33 mmol) in DMF (8 mL) were added DIC (670 μL, 4.33 mmol) and HOBt·H_2O (663 mg, 4.33 mmol). After 2 min, the whole was poured to the peptidyl resin (0.865 mmol), and the reaction was continued for 2 h.

Coupling reaction using DIC/HOAt [for Val^1-Hva^2, Thr(Trt)^5 and Ser(Me)^6]. To a solution of Fmoc amino acid (4.33 mmol) in DMF (8 mL) were added DIC (670 μL, 4.33 mmol) and HOAt (589 mg, 4.33 mmol). After 2 min, the whole was poured to the peptidyl resin (0.865 mmol), and the reaction was continued for 16 h.

Coupling reaction using BTC [for MeSer(Me)^3 and MeLeu^4]. To a solution of BTC (295 mg, 0.995 mmol) in dry THF (14.6 mL) were added Fmoc-amino acids (3.03 mmol), 2,4,6-collidine

(1.14 mL, 8.65 mmol) and (*i*-Pr)$_2$NEt (1.21 mL, 6.92 mmol). This solution was added to the peptidyl resin in dry THF (6.27 mL), and the reaction was continued for 2.5 h (MeSer(Me)^3) or for 4 h (MeLeu^4).

***N*-Methylation on solid support by Ns-strategy.** 2,4,6-collidine (1.14 mL, 8.65 mmol) and NsCl (767 mg, 3.46 mmol) were added to the peptidyl resin (0.865 mmol) in NMP (5 mL), and the reaction was continued for 15 min at room temperature.

After removal of the reagent solution, PPh$_3$ (1.13 g, 4.33 mmol) and MeOH (350 μL, 8.65 mmol) in dry THF (3 mL) was added to a suspension of the resin, followed by dropwise addition of DIAD (2.27 mL, 4.33 mmol) in dry THF (3 mL), and the reaction was continued for 30 min at room temperature. This Mitsunobu reaction was repeated twice.

To the methylated peptidyl resin in NMP (6 mL), DBU (648 μL, 4.33 mmol) and 2-mercaptoethanol (607 μL, 8.65 mmol) were added, and the reaction was continued for 5 min. This process was repeated twice.

Reductive amination for N-terminal methylation and cleavage from the resin: linear peptide (4). NaBH(OAc)$_3$ (1.83 g, 8.65 mmol) in DCE (3 mL) and formalin (644 μL, 8.65 mmol) in DCE (3 mL) were added to the peptidyl resin (0.865 mmol), and the reaction was continued for 2 h at room temperature. The resulting peptidyl resin was washed with DCE and MeOH, and the filtrate was concentrated. The residue was dissolved in DCM, and the solution was washed with water, and dried over Na_2SO_4. The resulting oil was treated with TFA/DCM (5:95, 5 mL) for 30 min, and the mixture was concentrated. Separately, the peptidyl resin was also treated with TFA/DCM (5:95, 5 mL) for 30 min, and the mixture was concentrated. The combined crude linear peptide was treated with Et$_3$N/DCM (8:1, 9 mL) for 1 day. After concentration, the residue was purified by reverse-phase preparative HPLC (32-52% linear gradient of solvent B in solvent A over 60 min) to yield the linear peptide **4** (327 mg, 29% from the resin) as colorless powder: t_R: 21.4 min (35–55% linear gradient of solvent B in solvent A over 40 min); purity: >99%; MS (ESI+) 1327.9 $[M+Na]^+$, 1306.0 $[M+H]^+$.

Synthesis of [D-MeAla^{11}]-coibamide A. To a solution of the linear peptide **4** (49.9 mg, 0.038 mmol) in DCM/DMF/NMI (90:8:2, 38.0 mL) were added MSNT (226 mg, 0.764 mmol) and (*i*-Pr)$_2$NEt (397 μL, 2.28 mmol), and the reaction was stirred at 30 °C for 3 days. After concentration of the reaction mixture, the residue was purified by preparative HPLC [MeOH-H_2O (9:1)] to provide [D-MeAla^{11}]-coibamide A (1.87 mg, 3.8%): t_R: 35.1 min (40–70% linear gradient of solvent B in

solvent A over 60 min); $[\alpha]^{28}_{D}$ –48.1 (*c* 0.02, $CHCl_3$); IR (neat): 3401, 2963, 2251, 1732, 1644, 1466, 1407, 1248, 1095, 729; ^{1}H NMR (500 MHz, $CDCl_3$) δ 0.80–1.10 (m, 37H), 1.26–1.29 (m, 4H), 1.37 (m, 2H), 1.51 (m, 4H), 2.05 (m, 2H), 2.19–2.20 (m, 2H), 2.40 (s, 9H), 2.75 (s, 3H), 2.86 (m, 6H), 3.00–3.03 (m, 4H), 3.13–3.16 (m, 6H), 3.30–3.31 (m, 6H), 3.49–3.53 (m, 2H), 3.64 (m, 2H), 3.72–3.78 (m, 6H), 4.75 (br m, 1H), 5.11 (d, *J* = 6.3 Hz, 1H), 5.13 (m, 1H), 5.30 (m, 1H), 5.36 (m, 1H), 5.50 (m, 1H), 5.71 (m, 1H), 5.83 (m, 1H), 6.31 (br s, 1H), 6.60 (br s, 1H), 6.70 (br s, 1H), 6.80 (d, *J* = 8.6 Hz, 2H), 7.11 (d, *J* = 8.6 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 11.6, 12.9, 15.7, 17.9, 18.6, 18.8, 19.3, 19.6, 21.1, 21.9, 22.8, 23.2, 24.2, 25.0, 25.3, 27.7, 28.9, 29.6, 29.9, 30.0, 31.0, 31.9, 36.4, 38.0, 38.8, 39.5, 41.2, 47.0, 49.9, 51.0, 51.1, 52.1, 52.9, 55.2, 58.76, 58.80, 63.5, 64.7, 68.2, 68.6, 69.1, 74.0, 74.6, 113.8, 128.2, 130.4, 158.6, 167.2, 168.6, 169.7, 169.8, 170.3, 170.4, 171.4, 171.6; MS (ESI+) 1309.9 $[M+Na]^+$, 1287.9 $[M+H]^+$, 644.5 $[M+2H]^{2+}$.

Determination of the configuration of component amino acids. A portion of the synthetic product (1.2 mg) was hydrolyzed in 6N HCl (2 mL) at 105 °C for 24 h and evaporated to dryness. A solution of 1% 1-fluoro-2-4-dinitrophenyl-5-L-leucinamide (FDLA) in acetone (300 µL), and saturated $NaHCO_3$ (1 mL), were added to a portion of the hydrolysate (1 mg) and the mixture was heated (40 °C, 1 h). The solution was evaporated to dryness, and the residue resuspended in MeOH for filtration through a C_{18} solid phase extraction cartridge (100 mg) before analysis by LC-MS. The amino acid standards were derivatized following the same procedure. LC-MS analyses were performed on a Thermo Aquasil C_{18} column (2 × 150 mm, 5 µm) using two different MeCN-H_2O linear solvent gradients and negative mode ESIMS. For Tyr(Me) and MeLeu analyses (40 min total run time, flow rate 0.6 mL/min): hold 5 min at 10% MeCN-H_2O, ramp to 45% MeCN-H_2O over 25 min and hold 10 min. For MeSer(Me), Ala and MeAla analyses (60 min total run time, flow rate 0.8 mL/min): hold 10 min at 10% MeCN-H_2O, ramp to 35% MeCN-H_2O over 40 min and hold 10 min. The retention times (Table S1) of the derivatized residues in the synthetic product hydrolysate matched L-Tyr(Me), L-MeLeu, L-MeSer(Me), L-MeThr, L-Ala, and D-MeAla. The co-eluting L-MeSer(Me) and L-Ala HPLC peaks were distinguished by their MS data. Further analyses were also performed in which the enantiomeric standards (D-MeSer(Me) and L-MeAla) were separately coinjected with the hydrolysate, resulting in the detection of two distinct peaks with identical *m/z*, matching the retention times of L- and D- standards in each case, as expected. MS fragmentation patterns were also useful for differentiating between MeSer(Me) and MeThr residues. To assign the Hva residue by GC-MS analysis, L- and D-Hva standards and the remaining portion of the synthetic product acid hydrolysate (0.2 mg) were each treated with *i*-PrOH and acetyl chloride (105 °C, 1 h),

dried under a stream of N_2 gas, and derivatized with pentafluoropropanoic anhydride (PFPA) in CH_2Cl_2 in a sealed vial (105 °C, 10 min) before drying under N_2 again. The resulting residues were analyzed using a chiral Cyclosil B column (30.0 m × 250 μm, 0.25 μm), leading to the assignment of L-Hva (t_R = 11.1 min; D-Hva, 11.4 min).

Growth inhibition/viability assay (Table 1).[S2] A549, HCT-116, MCF-7 (generous gifts from Dr. Akira Hirasawa, Graduate School of Pharmaceutical Sciences, Kyoto University) and B16 melanoma cells (generous gift from Dr. Hiroshi Nose, Kola-Gen Pharma) were cultured in DMEM medium (Sigma), McCoy's 5A medium (GIBCO), EMEM medium (Wako), and DMEM medium (Sigma), respectively, supplemented with 10% (v/v) FBS at 37 °C in a 5% CO_2 incubator. A549, HCT-116, MCF-7 and B16 melanoma cells were seeded at 500, 5000, 5000 and 2000 cells/well in 50 μL of culture media in 96-well plates (BD Falcon), respectively, and incubated for 6 h. Chemicals in DMSO were diluted 250-fold with the culture medium in advance. After addition of fresh culture medium (40 μL), the chemical diluents (30 μL) were added to the cell cultures. The cells under chemical treatment were incubated for a further 72 h. The wells in the plates were washed twice with phenol-red minus medium. After 1 h incubation with 100 μL of the medium, the cells were supplemented with 20 μL of the MTS reagent (Promega), followed by incubation for an additional 40 min. Absorbance at 490 nm of each well was measured using a Wallac 1420 ARVO SX multilabel counter (Perkin Elmer).

Growth inhibition/viability assay (Table 2). Human PC-3 prostate cancer, MDA-MB-231 breast cancer and H292 lung cancer cell lines were purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA). Human SF-295 glioblastoma cells were obtained from the NCI cell line repository (Frederick, MD, USA). PC-3 cells were maintained in DMEM (MediaTech Inc., Manassas, VA, USA) supplemented with 10% FBS (HyClone, Logan, UT), L-glutamine (2 mM) and 1% penicillin/streptomycin (Mediatech Inc.). SF-295, MDA-MB-23, and H292 cells were cultured in RPMI-1640 medium supplemented with L-glutamine (2 mM), sodium pyruvate (1 mM), sodium bicarbonate (1.5 g/L), 1% penicillin/streptomycin and 10% FBS. All cells were maintained in a humidified chamber containing 5% CO_2 and were seeded (3,000 cells per well) into 96-well flat-bottom plates (BD Biosciences, Franklin Lakes, NJ) 16 h before the addition of the test compound or vehicle (DMSO). Treated cells were then maintained under standard cell culture conditions for a further 72 h. The viability of all cells was assessed by MTT assay as described previously[S3] with the viability of vehicle-treated cells defined as 100%.

References

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S3 Hau, A. M.; Greenwood, J. A.; Löhr, C. V.; Serrill, J. D.; Proteau, P. J.; Ganley, I. G.; McPhail, K. L.; Ishmael J. E. *PLoS One* **2013**, 8, e65250.

Table S1. Retention times for Marfey's derivatives of amino acid standards and the corresponding amino acid in the hydrolysate of synthetic [D-MeAla^{11}]-coibamide A

Amino Acid (AA)	Observed *m/z* (ESI neg.) for FDLA-AA	t_R (min): authentic standard	t_R (min): hydrolysate of synthetic coibamide
L-Ala	382	48.35	48.25
D-Ala	382	54.35	
L-MeAla	396	51.27	
D-MeAla	396	51.82	51.75
L-MeLeu	438	35.56	35.68
D-MeLeu	438	39.08	
L-MeSer(Me)	426	48.30	48.25
D-MeSer(Me)	426	49.17	
L-Tyr(Me)	488	34.08	34.11
D-Tyr(Me)	488	38.81	
L-MeThr	426	43.19	43.44
D-MeThr	426	47.12	

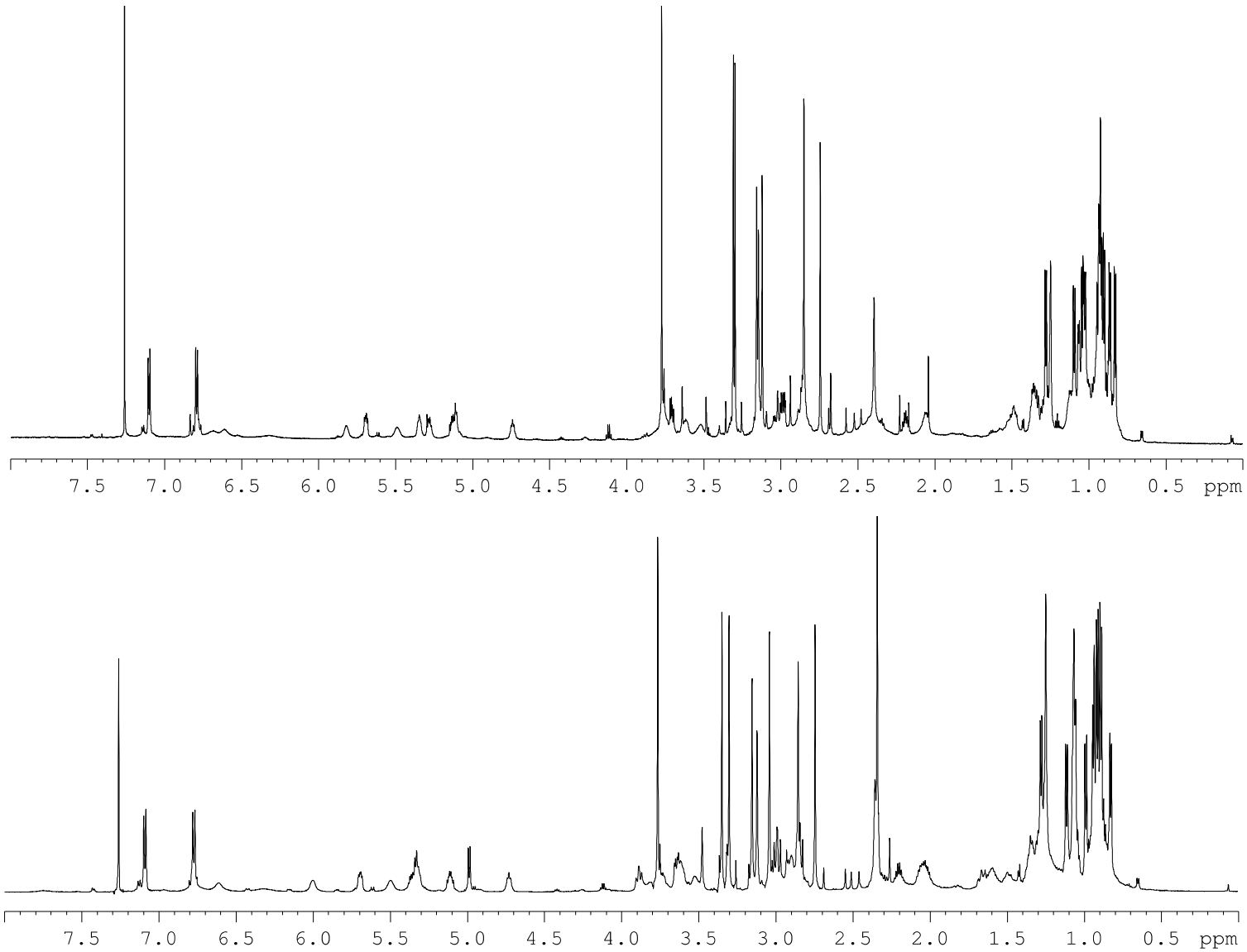
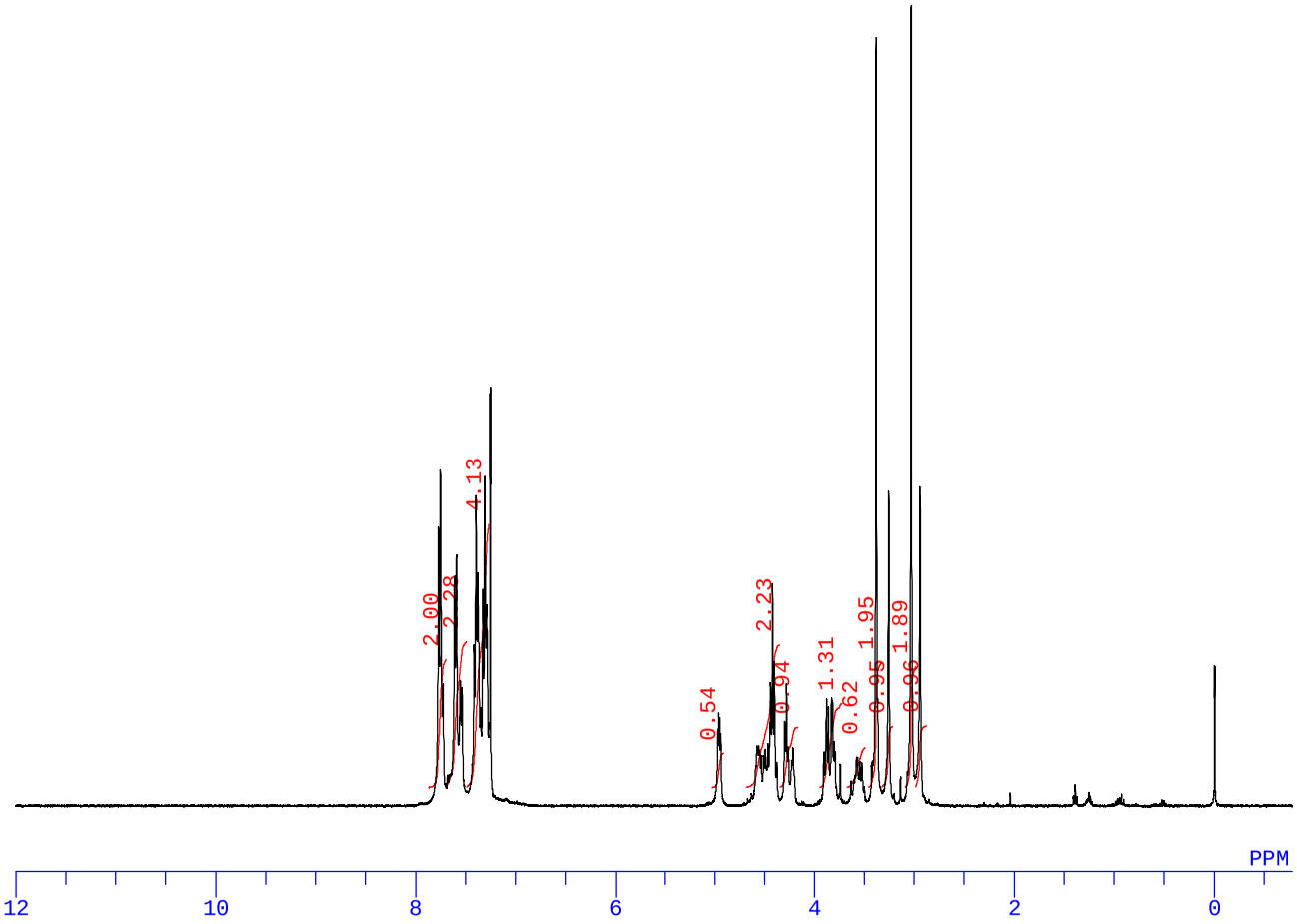


Figure S1. Comparative ^{1}H NMR spectra for [D-MeAla^{11}]-coibamide A (upper) and natural coibamide A (lower)

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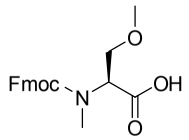

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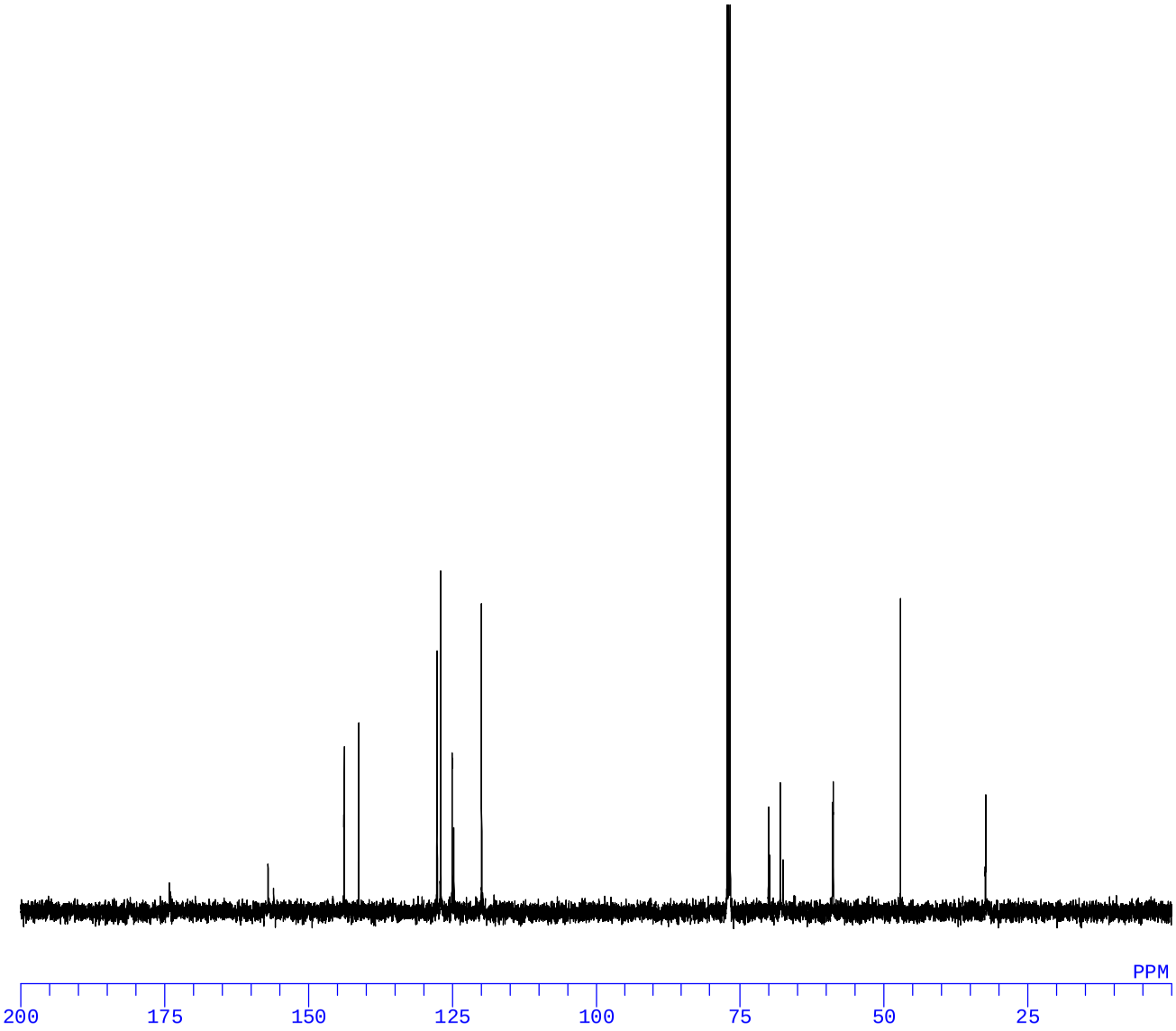


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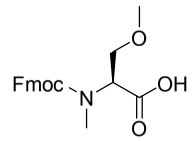

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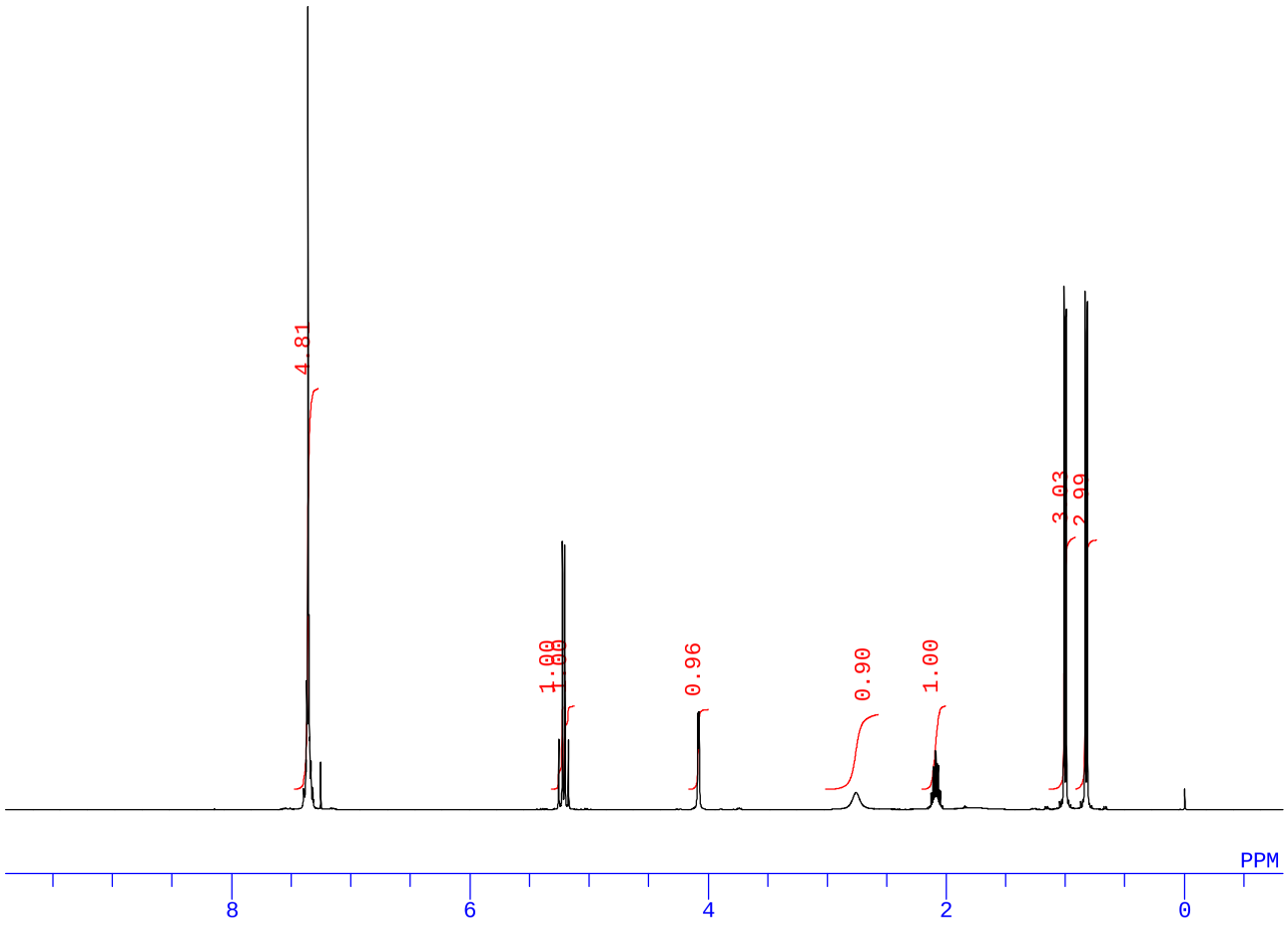


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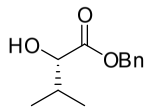
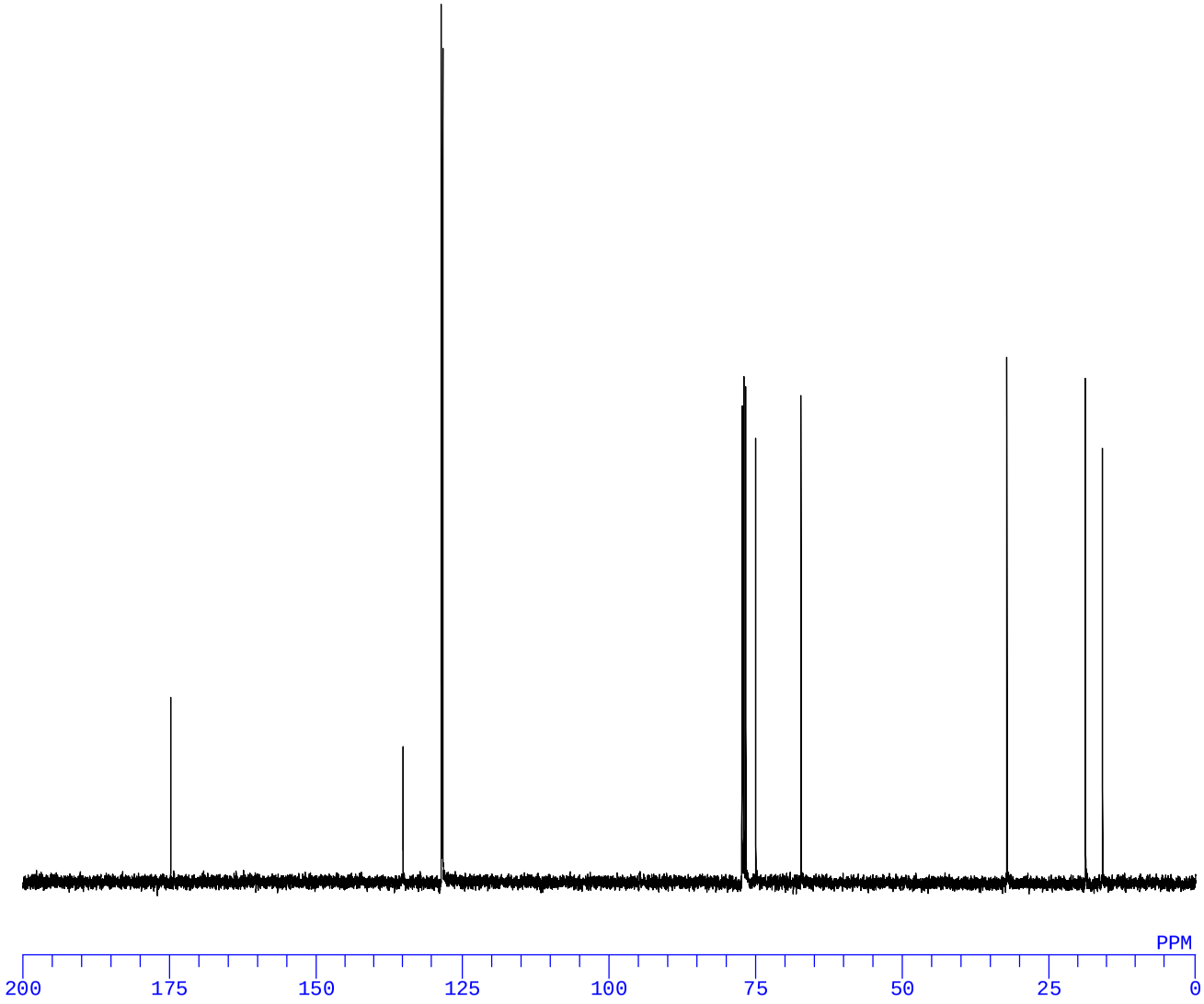
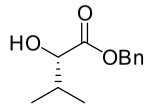
DATIM  2014-07-08 18:03:15
OBNUC  1H
EXMOD  proton.jxp
OBFRQ        399.78 MHz
OBSET          4.19 KHz
OBFIN          7.29 Hz
POINT         13107
FREQU       6002.40 Hz
SCANS            16
ACQTM         2.1837 sec
PD            5.0000 sec
PW1             3.06 usec
IRNUC  1H
CTEMP          22.0 c
SLVNT  CDCL3
EXREF          0.00 ppm
BF             0.12 Hz
RGAIN            28
```



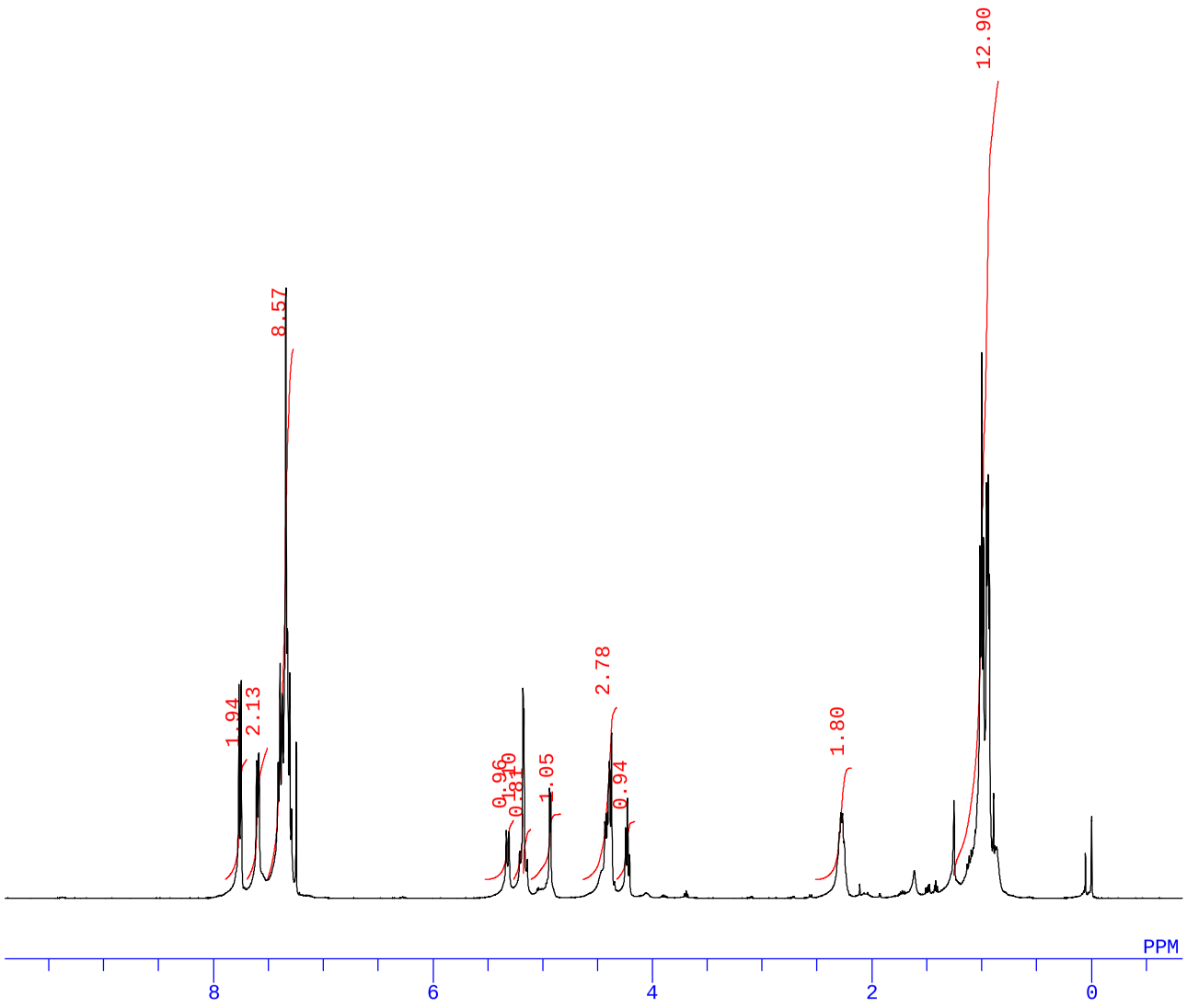
6



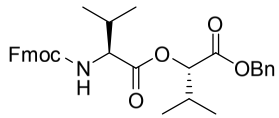

```
DFILE  140708-Hva-OBn-13C_Carbon
COMNT  single pulse decoupled ga
DATIM  2014-07-08 18:17:11
OBNUC  13C
EXMOD  carbon.jxp
OBFRQ        100.53 MHz
OBSET          5.35 KHz
OBFIN          5.86 Hz
POINT         26214
FREQU      25125.63 Hz
SCANS           200
ACQTM         1.0433 sec
PD            2.0000 sec
PW1             3.77 usec
IRNUC  1H
CTEMP          22.0 c
SLVNT  CDCL3
EXREF         77.00 ppm
BF             1.20 Hz
RGAIN            60
```



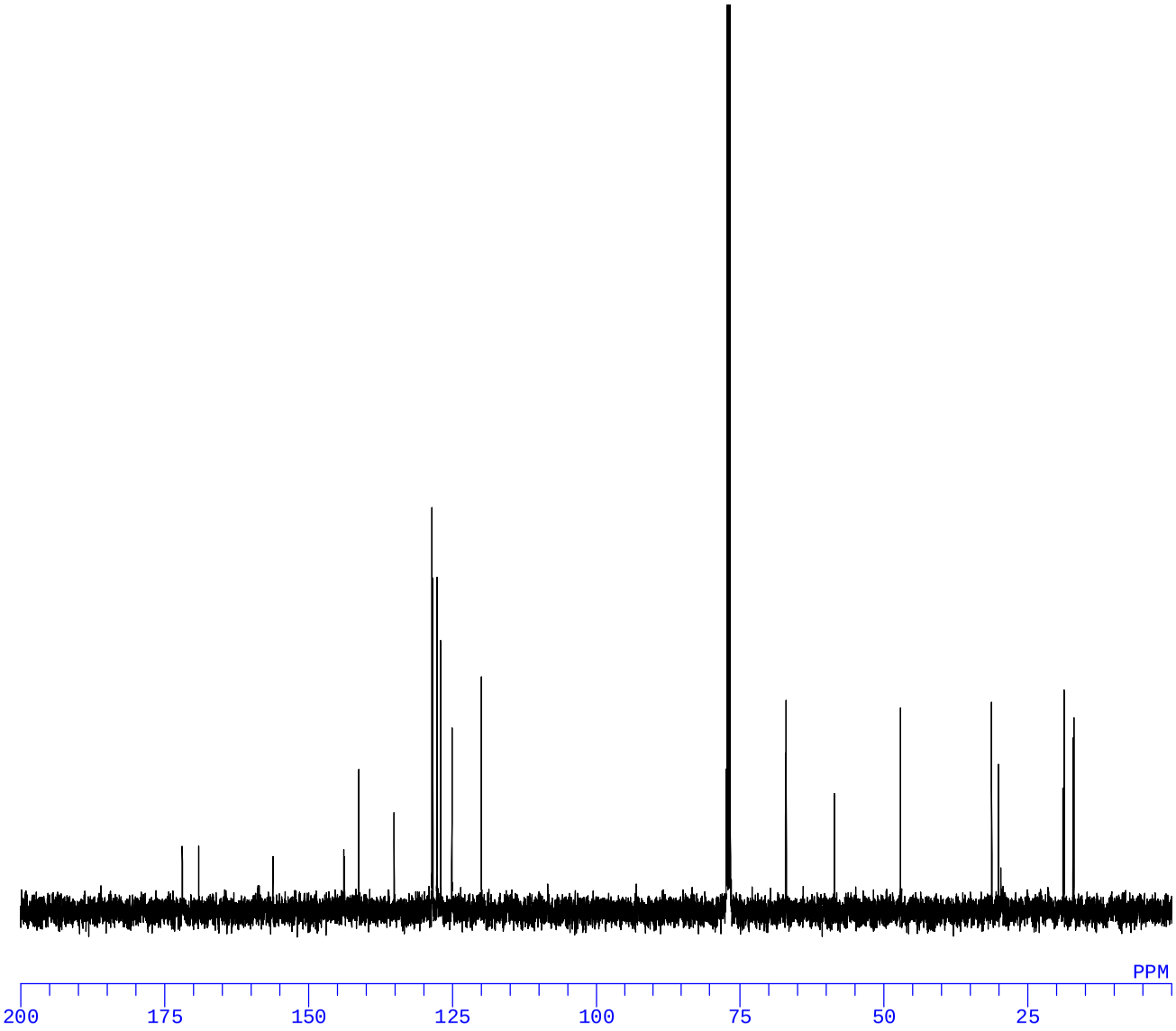
6



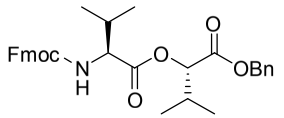

```
DFILE  141009-F183-Fmoc-Val-Hva-
COMNT  single_pulse
DATIM  2014-10-11 10:43:01
OBNUC  1H
EXMOD  proton.jxp
OBFRQ        399.78 MHz
OBSET          4.19 KHz
OBFIN          7.29 Hz
POINT         13107
FREQU       6002.40 Hz
SCANS             8
ACQTM         2.1837 sec
PD            5.0000 sec
PW1             3.06 usec
IRNUC  1H
CTEMP          20.9 c
SLVNT  CDCL3
EXREF          0.00 ppm
BF             0.12 Hz
RGAIN            30
```



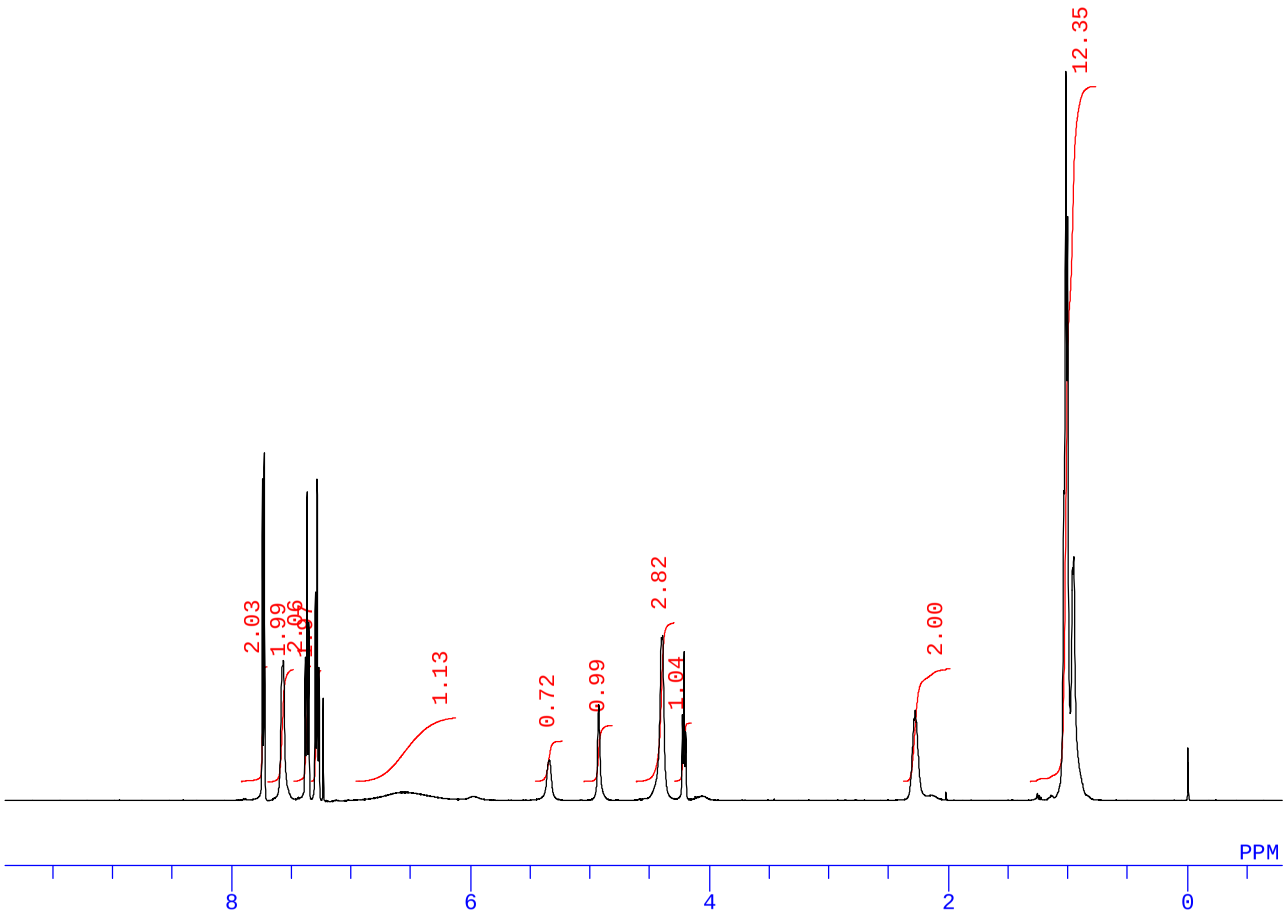
7



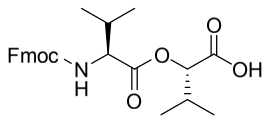

```
DFILE  140826-Fmoc-Val-Hva-OBn-1
COMNT  single pulse decoupled ga
DATIM  2014-08-26 21:37:24
OBNUC  13C
EXMOD  single_pulse_dec
OBFRQ        125.77 MHz
OBSET          7.87 KHz
OBFIN          4.21 Hz
POINT         26214
FREQU      31446.06 Hz
SCANS           256
ACQTM         0.8336 sec
PD            2.0000 sec
PW1             3.50 usec
IRNUC  1H
CTEMP          23.6 c
SLVNT  CDCL3
EXREF          77.00 ppm
BF              1.20 Hz
RGAIN             52
```



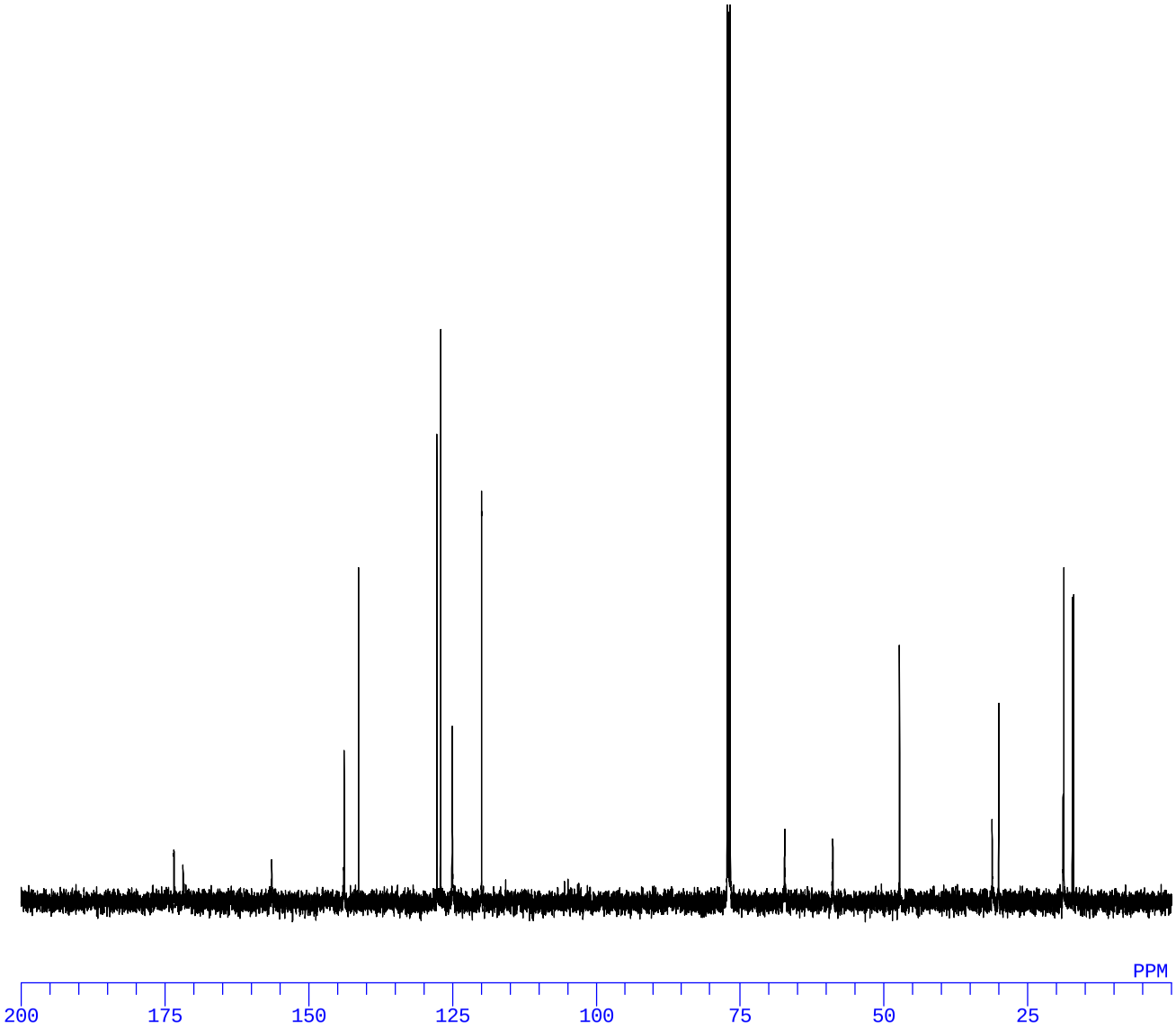
7



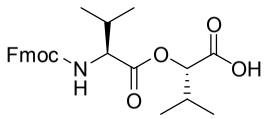

```
DFILE  140708-Fmoc-Val-Hva-OH-1H
COMNT  single_pulse
DATIM  2014-07-08 11:31:27
OBNUC  1H
EXMOD  single_pulse.ex2
OBFRQ        500.16 MHz
OBSET          2.41 KHz
OBFIN          6.01 Hz
POINT         13107
FREQU       7507.39 Hz
SCANS             8
ACQTM         1.7459 sec
PD            5.0000 sec
PW1             6.82 usec
IRNUC  1H
CTEMP           50.0 c
SLVNT  CDCL3
EXREF           0.00 ppm
BF              0.12 Hz
RGAIN             36
```



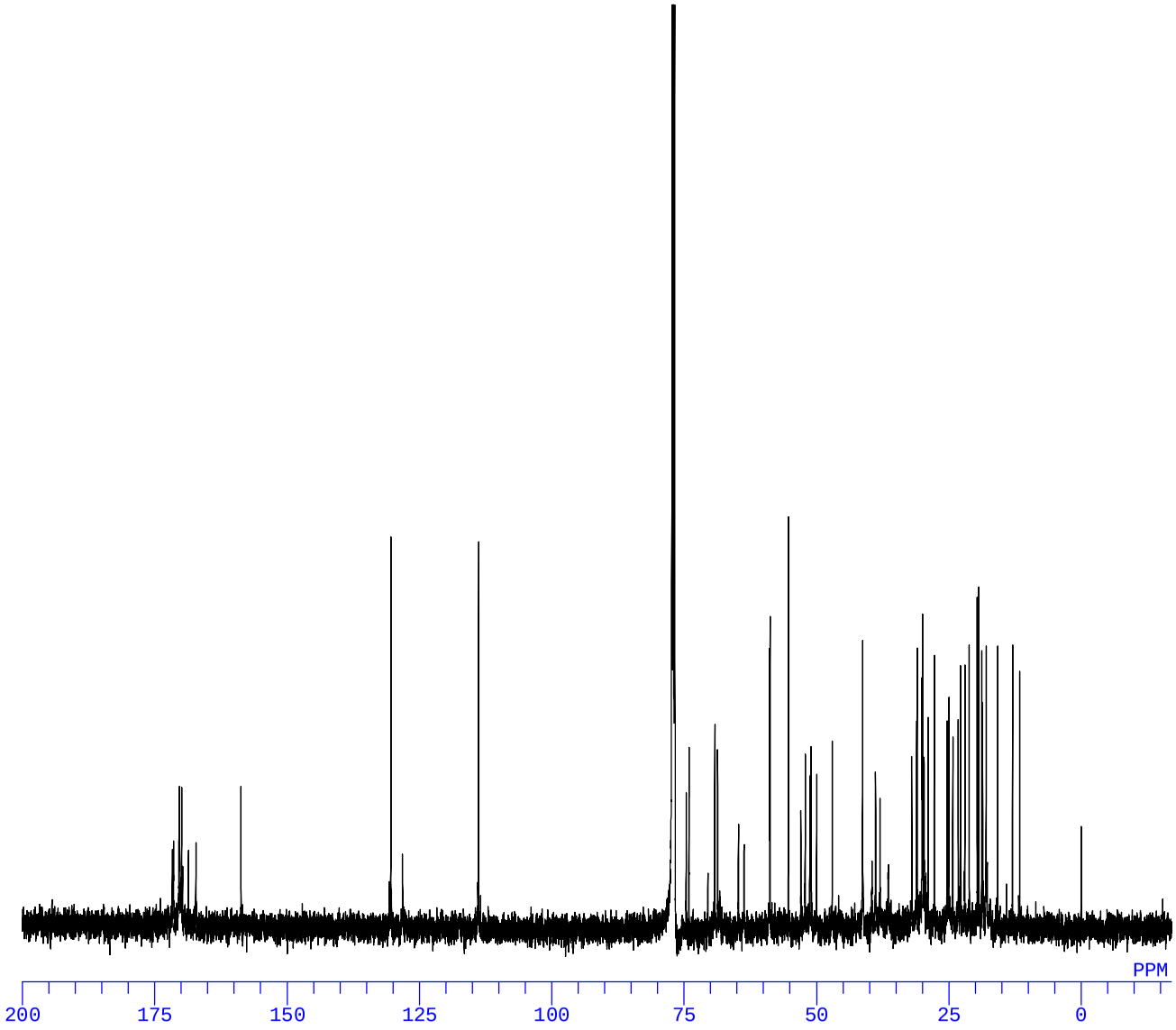
8



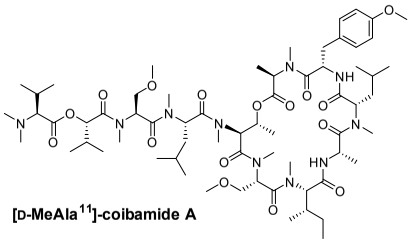

```
DFILE  140708-Fmoc-Val-Hva-OH-13
COMNT  single pulse decoupled ga
DATIM  2014-07-08 13:58:57
OBNUC  13C
EXMOD  single_pulse_dec
OBFRQ        125.77 MHz
OBSET          7.87 KHz
OBFIN          4.21 Hz
POINT         26214
FREQU      31446.06 Hz
SCANS           256
ACQTM        0.8336 sec
PD           2.0000 sec
PW1            3.50 usec
IRNUC  1H
CTEMP          50.0 c
SLVNT  CDCL3
EXREF         77.00 ppm
BF             1.20 Hz
RGAIN            52
```



8




```
DFILE  110702-13C-Thrbody-overwe
COMNT  single pulse decoupled ga
DATIM  04-07-2011 02:32:32
OBNUC  13C
EXMOD  single_pulse_dec
OBFRQ        125.77 MHz
OBSET          7.87 KHz
OBFIN          4.21 Hz
POINT         26214
FREQU      31446.06 Hz
SCANS         32768
ACQTM        0.8336 sec
PD           2.0000 sec
PW1            3.73 usec
IRNUC  1H
CTEMP          28.6 c
SLVNT  CDCL3
EXREF          0.00 ppm
BF             1.20 Hz
RGAIN            56
```



[D-MeAla[11]]-coibamide A