

Synthesis and Evaluation of Linear and Macrocyclic Dolastatin 10 Analogues Containing Pyrrolidine Ring Modifications

Michinori Akaiwa[¶], Tioga Martin[†], and Brian A. Mendelsohn^{#}*

Agensys Inc. an affiliate of Astellas Pharma Inc.,

1800 Stewart St, Santa Monica, CA, USA, 90404.

Supporting Information

Reactions were carried out at ambient temperature with exposure to air, unless otherwise noted. All reagents and solvents were purchased from commercial sources and used as received. NMR spectra were obtained on a Bruker AV 500 MHz, 400 MHz or 300 MHz spectrometer at 25 °C. The NMR spectra were obtained from DMSO-*d*₆ and were referenced to the residual solvent peak (¹H: 2.50 ppm; ¹³C: 39.5 ppm). High-resolution mass spectrometry samples were injected without column onto a Dionex 3000-Orbitrap Velos LC-MS. Flash chromatography was carried out on a Yamazen purification system using prepacked Yamazen Universal columns. Preparative HPLC was carried out using a Phenomenex Gemini-NX 10 μm, C18 110 Å column (150 x 30 mm) using a 5% to 95% gradient of MeCN / 0.05% aqueous TFA mixture over 13 min unless another column or solvent system is noted. Drug compounds purified by preparative HPLC were assumed to be salts containing one molecule of trifluoroacetic acid (TFA).

Liquid chromatography mass spectrometry (LCMS) retention times were acquired on an Acquity UPLC BEH C8 1.7 μm 2.1 x 50 mm column, 40 °C. 0-0.5 min: isocratic 85: 5: 10 H₂O / MeCN / 0.5% TFA in H₂O; 0.5-1.6 min: linear gradient 85: 5: 10 H₂O / MeCN / 0.5% TFA in H₂O to 98: 2 MeCN / 0.5% TFA in H₂O; 1.60-1.9 min linear gradient 98: 2 MeCN / 0.5% TFA in H₂O to 85: 5: 10 H₂O / MeCN / 0.5% TFA in H₂O; 1.9-2.0 isocratic 85: 5: 10 H₂O / MeCN / 0.5% TFA in H₂O.

in vitro cell proliferation assay were conducted using the method below.

Day 1: Plated appropriate cell density 1500 cells/well (50μL per well); 2000 cells/well for MOLM13

Day 2: Compound treatment

All compounds at 10 μ M start concentration, 1:10 dilution (50 μ L per well at 2x concentration dispensed from master mix); tested in triplicate. Treatment for 5 days.

Day 6: Assay End point

Add 20 μ L of Presto Blue, Incubate @ 37 °C for 2 h

Read signal on Biotek synergy H4 plate reader

Subtract Media Background and Calculate % survival: (drug / no drug)*100

Plot Survival Values in GraphPad Prism by transforming values so that $x=\log(x)$ and Analyzing transformed values using Non-linear regression curve fit Sigmoidal dose response function

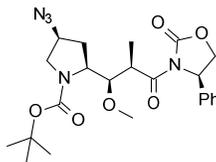


Figure S1. Boc-Dap (4-N₃)-Phenyloxazolidinone (Compound **8**)

To a stirred solution of (4R)-4-phenyl-3-propanoyl-1,3-oxazolidin-2-one (1.33 g, 6.06 mmol) in CH₂Cl₂ (36 mL) that was cooled to 0 °C, *n*-Bu₂BOTf (1.78 mL, 8.27 mmol) and *N,N*-Diisopropylethylamine (DIEA) (1.5 mL, 8.43 mmol) were added. Then stirred for 45 min. The resulting solution was cooled to -78 °C, added dropwise of a solution of aldehyde **5** (1.82 g, 5.51 mmol) in CH₂Cl₂ (36 mL), and stirred for 1 h. After stirring was further conducted at 0 °C, for an additional 1 h, analysis by LCMS showed the reaction was complete (High diastereoselectivity, see Page S60). The reaction was terminated with methanol and saturated sodium bicarbonate. The reaction mixture was extracted with CH₂Cl₂. The combined organic fractions were washed with brine, dried over a pad of magnesium sulfate, filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (40 μm, 60 Å, 3.0 x 16.5 cm) using 0% to 5% MeOH in CH₂Cl₂ as the eluent. A total of 1.40 g of compound **7** was obtained (2.55 mmol, 46%) as a white amorphous solid. LCMS *t*_R = 1.83 min; ESIMS *m/z* 549.33 [M + H]⁺

To a stirred at 5 °C solution of compound **7** (0.114 g, 0.21 mmol), proton sponge (0.325 g, 1.51 mmol) and Molecular sieves, 4 Å in CH₂Cl₂ (3.5 mL) were added Me₃OBf₄ (0.219 g, 1.48 mmol). The reaction mixture was stirred at room temperature (rt). After 68 h, analysis by LCMS showed the reaction was complete. The reaction solution was filtered, and concentrated *in vacuo*. The residue was purified by flash chromatography on silica gel (30 μm, 60 Å, 2.3 x 12.3 cm) using 2% to 25% EtOAc in Hexane as the eluent. A total of 0.073 g of Boc-Dap (4-OTBS)-Phenyloxazolidinone was obtained (0.13 mmol, 63%) as a white amorphous solid.

To a stirred solution of Boc-Dap (4-OTBS)-Phenyloxazolidinone (0.073 g, 0.13 mmol) in THF (3 mL) was slowly added HF-Py (140 μ L, 1.56 mmol) and then stirred at rt. After 5 h, analysis by LCMS showed the reaction was complete. The reaction mixture was added with 30 mL of saturated sodium bicarbonate and then extracted with EtOAc. The organic layers were washed with 30 mL of 1 N HCl, dried over anhydrous magnesium sulfate, concentrated *in vacuo* and dried further under high vacuum. A total of 58 mg of Boc-Dap (4-OH)-Phenyloxazolidinone was obtained (0.13 mmol, quant.) as a white amorphous solid that was used without further purification.

To a stirred at 5 °C solution of Boc-Dap(4-OH)-Phenyloxazolidinone (58 mg, 0.13 mmol) in THF (2 mL) were added DPPA (42 μ L, 0.20 mmol), DIAD (77 μ L, 0.39 mmol) and PPh₃ (103 mg, 0.20 mmol). After 15 h, analysis by LCMS showed the reaction was complete. The reaction solution was concentrated *in vacuo* and the residue was purified by flash chromatography on silica gel (30 μ m, 60 Å, 2.3 x 12.3 cm) using 2% to 50% EtOAc in hexanes as the eluent. A total of 37 mg of the title compound was obtained (78.0 μ mol, 59%) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 7.42-7.24 (m, 5H), 5.49 (dd, *J* = 8.6, 3.6 Hz, 1H), 4.73 (t, *J* = 8.7 Hz, 1H), 4.20-4.08 (m, 2H), 4.00-3.76 (m, 4H), 2.88 (s, 3H), 2.87-2.74 (m, 1H), 2.39-2.23 (m, 1H), 2.05-1.83 (m, 1H), 1.39 (s, 9H), 1.03 (d, *J* = 6.0 Hz, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 174.77, 153.97, 139.97, 129.02, 128.24, 126.21, 83.35, 79.61, 70.22, 60.54, 57.71, 57.60, 57.31, 51.07, 50.56, 46.39, 29.86, 28.46, 14.23; LCMS *t*_R = 1.66 min; ESIMS *m/z* 474.30 [M + H]⁺; HRESIMS *m/z* 474.2355 [M + H]⁺ (calcd for C₂₃H₃₂N₅O₆, 474.2347).

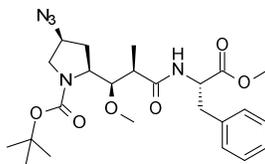


Figure S2. Boc-Dap (4-N₃)-Phe-OMe (Compound 10)

To a stirred at 5 °C solution of Boc-Dap(4-N₃)-Phenyloxazolidinone (241 mg, 0.51 mmol) in THF (10 mL) were added with 30% H₂O₂ (0.81 mL, 8.14 mmol) and 0.5 M LiOH (5 mL, 2.50 mmol). After 15 h, analysis by LCMS showed the reaction was complete. The reaction was quenched with 1 M sodium thiosulfate, and the reaction mixture was stirred at rt for 10 min and then extracted with saturated sodium bicarbonate and CH₂Cl₂, which were cooled to 0-5 °C previously. The aqueous layer was adjusted to a pH of 2, followed by extracted with EtOAc (3 times). The combined organic layer was dried over anhydrous magnesium sulfate and concentrated *in vacuo* to afford Boc-Dap (4-N₃)-OH **9** as a white solid that was used without further purification.

To a stirred rt suspension of H-Phe-OMe HCl salt (54 mg, 0.250 mmol), Boc-Dap (4-N₃)-OH **9** (90 mg, 0.274 mmol), EDCI (75 mg, 0.391 mmol) and HOBT (40 mg, 0.261 mmol) in CH₂Cl₂ (2 mL) was added Et₃N (60 μL, 0.430 mmol). After 2 h, analysis by LCMS showed that the reaction was complete. The mixture was purified by preparatory RP-HPLC with a Phenomenex Gemini-NX 10 μm, C18 110 Å column (150 x 30 mm) using a 5% to 95% MeCN in 0.05% aqueous TFA solution as the eluent. A total of 50 mg of the title compound was obtained as the TFA salt (0.102 mmol, 41%) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.32 (d, *J* = 8.0 Hz, 1H), 7.31-7.16 (m, 5H), 4.49 (ddd, *J* = 9.7, 7.9, 5.3 Hz, 1H), 4.16-4.02 (m, 1H), 3.95-3.71 (m, 3H), 3.61 (s, 3H), 3.18 (s, 3H), 3.05 (dd, *J* = 13.7, 5.4 Hz, 1H), 2.95 – 2.71 (m, 2H), 2.33-2.12 (m, 2H), 1.95-1.75 (m, 1H), 1.41 (s, 9H), 0.73 (d, *J* = 6.8 Hz, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 174.33, 172.42, 153.28, 137.74, 129.55, 128.56, 126.90, 82.39, 79.50, 60.58,

57.57, 57.21, 53.72, 52.20, 50.94, 50.35, 43.15, 37.19, 29.79, 28.49, 14.63; LCMS t_R = 1.61 min, ESIMS m/z 490.45 [M + H]⁺; HRESIMS m/z 490.2671 [M + H]⁺ (calcd for C₂₄H₃₆N₅O₆, 490.2660)

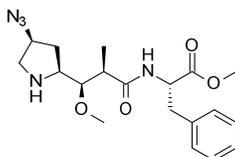


Figure S3. H-Dap (4-N₃)-Phe-OMe (Compound **11**)

A solution of Boc-Dap (4-N₃)-Phe-OMe **10** (24 mg, 0.05 mmol) in 4.0 M HCl in dioxane (1 mL, 4.00 mmol) was stirred rt. After 2 h, analysis by LCMS showed that the reaction was complete. The solution was concentrated *in vacuo* and dried further under high vacuum. A total of 20.5 mg the title compound (0.05 mmol, 98%) was obtained as the HCl salt as a pale brown solid that was used without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.62 (d, J = 8.1 Hz, 1H), 8.55 (s, 1H), 7.33 – 7.17 (m, 5H), 4.60 – 4.43 (m, 2H), 3.78-3.57 (m, 4H), 3.46-3.35 (m, 1H), 3.33 – 3.25 (m, 4H), 3.16 – 3.05 (m, 2H), 2.88 (dd, J = 13.8, 9.9 Hz, 1H), 2.49 – 2.34 (m, 2H), 1.76 (ddd, J = 13.3, 9.9, 6.1 Hz, 1H), 0.73 (d, J = 7.0 Hz, 3H); LCMS t_R = 1.03 min, ESIMS m/z 390.32 [M + H]⁺; HRESIMS m/z 390.2146 [M + H]⁺ (calcd for C₁₉H₂₈N₅O₄, 390.2136)

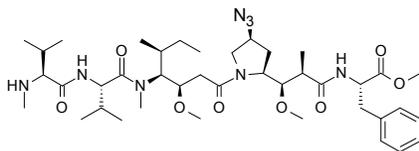


Figure S4. MeVal-Val-Dil-Dap (4-N₃)-Phe-OMe (Compound **13a**)

To a stirred rt solution of H-Dap (4-N₃)-Phe-OMe **11** (7 mg, 0.02 mmol), Fmoc-MeVal-Val-Dil-OH **12a** (11 mg, 16.31 μ mol) and Et₃N (8 μ L, 0.06 mmol) in N, N-dimethylacetamide

(DMAc) (1 mL) were added EDCI (7 mg, 0.04 mmol) and HOBt (2 mg, 0.01 mmol) and the mixture was stirred for 15 hours. To the mixture was added Et₂NH (40 μL, 386.67 μmol). After 1 h, analysis by LCMS showed the reaction was complete. The reaction mixture was diluted with H₂O and DMAc and then the mixture was purified by preparatory RP-HPLC with a Phenomenex Gemini-NX 10 μm, C18 110 Å column (150 x 30 mm) using a 5% to 95% MeCN in 0.05% aqueous TFA solution as the eluent. A total of 6 mg of the title compound was obtained as the TFA salt (6.66 μmol, 41%) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆, a complex spectrum was observed, presumably due to *cis/trans* conformational isomers) δ 8.90-8.75 (m, 2H), [8.42 (d, *J* = 7.9 Hz), 8.32 (d, *J* = 8.0 Hz) 1H], 7.31 – 7.17 (m, 5H), 4.72-4.63 (m, 1H), 4.59 (t, *J* = 8.5 Hz, 1H), 4.48 (ddd, *J* = 9.8, 8.0, 5.4 Hz, 1H), 4.14 – 4.02 (m, 2H), 4.00-3.63 (m, 5H), [3.61(s), 3.60 (s) 3H], [3.19(s), 3.17 (s) 3H], 3.12 (s, 3H), 3.07-2.96 (m, 4H), 2.88 (dd, *J* = 13.7, 9.7 Hz, 1H), 2.47 (t, *J* = 5.0 Hz, 3H), 2.32 – 2.16 (m, 3H), 2.12 – 1.94 (m, 2H), 1.92-1.66 (m, 2H), 1.34-1.19 (m, 1H), 0.98 – 0.83 (m, 17H), 0.80-0.70 (m, 6H); ¹³C NMR (75 MHz, DMSO) δ 174.38, 173.90, 172.44, 169.46, 167.30, 166.43, 164.91, 137.73, 129.55, 128.98, 128.57, 128.30, 126.91, 85.77, 82.20, 80.61, 78.65, 65.99, 63.66, 60.12, 59.02, 57.89, 57.59, 57.35, 56.07, 55.30, 55.13, 53.72, 52.20, 50.97, 49.36, 43.32, 38.89, 37.48, 37.26, 32.46, 32.09, 32.01, 30.51, 29.90, 25.85, 25.13, 21.50, 20.63, 18.96, 18.90, 18.73, 18.05, 15.95, 15.95, 14.67, 10.73; LCMS *t*_R = 1.31 min, ESIMS *m/z* 787.72 [M + H]⁺; HRESIMS *m/z* 787.5096 [M + H]⁺ (calcd for C₄₀H₆₇N₈O₈, 787.5076)

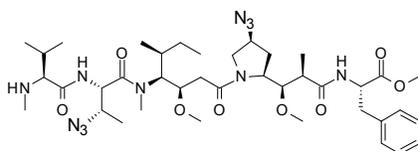


Figure S5. MeVal-Abu (3-N₃)-Dil-Dap (4-N₃)-Phe-OMe (Compound **13b**)

To a stirred rt solution of H-Dap (N₃)-Phe-OMe **11** (18 mg, 0.04 mmol), Fmoc-MeVal-Abu(3-N₃)-Dil-OH **12b** (26 mg, 37.08 μmol) and Et₃N (20 μL, 0.14 mmol) in DMAc (2 mL, 16.23 mmol) were added EDCI (18 mg, 0.09 mmol) and HOBt (4.00 mg, 0.03 mmol) and the mixture was stirred for 15 hours. To the mixture was added Et₂NH (40 μL, 0.386 mmol). After 3 h, analysis by LCMS showed the reaction was complete. The reaction mixture was diluted with H₂O and DMAc and then the mixture was purified by preparatory RP-HPLC with a Phenomenex Gemini-NX 10 μm, C18 110 Å column (150 x 30 mm) using a 5% to 95% MeCN in 0.05% aqueous TFA solution as the eluent. A total of 22 mg of the title compound was obtained as the TFA salt (23.71 μmol, 64%) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆, a complex spectrum was observed, presumably due to *cis/trans* conformational isomers) δ [9.10 (d, *J* = 8.6 Hz), 8.71 (d, *J* = 9.1 Hz) 1H], 8.90 (br-s, 1H), [8.41 (d, *J* = 7.9 Hz), 8.33 (d, *J* = 8.0 Hz) 1H], [7.49-7.33 (m), 7.32 – 7.07 (m) 5H], 4.86 (t, *J* = 8.3 Hz, 1H), 4.59 (br-s, 1H), 4.48 (ddd, *J* = 9.7, 7.9, 5.4 Hz, 1H), 4.33 – 3.86 (m, 6H), 3.78 – 3.66 (m, 1H), [3.61 (s), 3.60 (s) 3H], [3.21 (s), 3.18 (s) 3H], [3.13 (s), 3.09 (s) 3H], 3.09 – 3.01 (m, 2H), 2.98 (s, 3H), 2.93-2.81 (m, 1H), 2.49-2.43 (m, 3H), 2.36 – 2.17 (m, 3H), 2.09 (dq, *J* = 13.1, 6.5 Hz, 1H), 1.94 – 1.64 (m, 2H), 1.36-1.21 (m, 4H), 0.99 – 0.83 (m, 11H), 0.83-0.66 (m, 6H); ¹³C NMR (101 MHz, DMSO) δ 174.38, 172.43, 170.27, 169.45, 167.86, 166.84, 137.79, 137.73, 129.54, 128.80, 128.56, 126.90, 124.86, 80.61, 77.61, 65.98, 60.16, 58.23, 57.90, 57.81, 57.34, 53.71, 52.80, 52.18, 51.44, 43.31, 37.19, 32.67, 32.46, 29.82, 28.82, 25.85, 18.65, 18.22, 18.04, 16.08, 15.97, 14.65, 13.42, 10.94; LCMS *t*_R = 1.32 min, ESIMS *m/z* 814.66 [M + H]⁺; HRESIMS *m/z* 814.4972 [M + H]⁺ (calcd for C₃₉H₆₄N₁₁O₈, 814.4934)

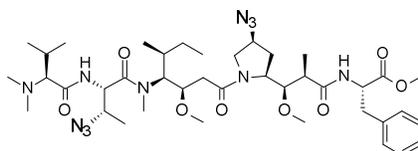


Figure S7. Fmoc-MeVal-Abu (3-N₃)-Dil-OtBu (Compound 15)

To a stirred rt solution of Fmoc-MeVal-Abu (3-N₃)-Dil-OtBu (130 mg, 0.18 mmol) in EtOH (3 mL) was added Pd on carbon (150 mg, 70.48 μ mol) under nitrogen atmosphere. The reaction was stirred rt under hydrogen atmosphere. After 4 h, analysis by LCMS showed the reaction was complete. After insoluble materials were removed by filtration, the filtrate was concentrated under reduced pressure. The residue was purified by preparatory RP-HPLC with a Phenomenex Gemini-NX 10 μ m, C18 110 Å column (150 x 30 mm) using a 5% to 95% MeCN in 0.05% aqueous TFA solution as the eluent. A total of 90 mg of the title compound was obtained as the TFA salt (0.13 mmol, 72%) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.66 (d, *J* = 8.3 Hz, 1H), 7.94-7.81 (m, 4H), 7.64 (d, *J* = 7.6 Hz, 2H), 7.47-7.38 (m, 2H), 7.37-7.26 (m, 2H), 4.86 (t, *J* = 7.5 Hz, 1H), 4.56 – 4.19 (m, 4H), 3.88-3.77 (m, 1H), 3.57-3.44 (m, 1H), 3.28 (s, 3H), 2.99 – 2.74 (m, 6H), 2.60 – 2.53 (m, 1H), 2.28 – 2.00 (m, 2H), 1.79 (br-s, 1H), 1.48-1.27 (m, 10H), 1.22-1.08 (m, 3H), 0.97 – 0.66 (m, 14H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 171.27, 170.78, 170.01, 156.49, 144.28, 144.18, 141.24, 128.10, 127.51, 125.41, 120.58, 80.51, 78.47, 67.18, 63.77, 57.96, 51.48, 48.05, 47.19, 38.67, 33.0, 30.08, 28.15, 28.07, 27.25, 25.69, 19.41, 19.19, 16.25, 14.90, 11.04; LCMS *t*_R = 1.65 min, ESIMS *m* / *z* 695.65 [M + H]⁺; HRESIMS *m* / *z* 695.4350 [M + H]⁺ (calcd for C₃₉H₅₉N₄O₇, 695.4378)

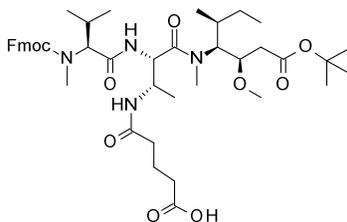


Figure S8. Fmoc-MeVal-Abu (3-NHCOCH₂CH₂CH₂CO₂H)-Dil-OtBu (Compound 16)

To a stirred rt solution of Fmoc-MeVal-Abu (3-NH₂)-Dil-OtBu (43 mg, 0.06 mmol) in DMAc (1 mL) was added glutaric anhydride (7 mg, 0.06 mmol). The reaction was stirred at at 60 °C. After 4 h, analysis by LCMS showed the reaction was complete. The mixture was purified by preparatory RP-HPLC with a Phenomenex Gemini-NX 10 μm, C18 110 Å column (150 x 30 mm) using a 5% to 95% MeCN in aqueous TFA solution as the eluent. A total of 40 mg of the title compound was obtained as the TFA salt (43.34 μmol, 70%) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 11.99 (s, 1H), 8.17 (d, *J* = 8.5 Hz, 1H), 7.90 (d, *J* = 7.6 Hz, 2H), 7.79 – 7.60 (m, 3H), 7.42 (td, *J* = 7.6, 1.2 Hz, 2H), 7.32 (t, *J* = 7.3 Hz, 2H), 4.98 – 4.86 (m, 1H), 4.56 – 4.18 (m, 5H), 4.14 – 4.01 (m, 2H), 3.90-3.63 (m, 1H), 3.22 (d, *J* = 8.7 Hz, 3H), 2.99 (s, 3H), 2.78 (s, 3H), 2.21 – 2.13 (m, 2H), 2.19-1.89 (m, 4H), 1.87 – 1.60 (m, 4H), 1.40 (s, 9H), 1.37-1.29 (s, 2H), 1.08 – 0.57 (m, 14H); LCMS *t*_R = 1.81 min, ESIMS *m/z* 809.70 [M + H]⁺

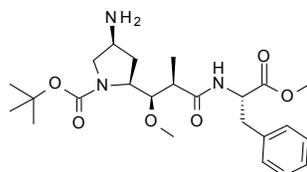


Figure S9. Boc-Dap (4-NH₂)-Phe-OMe (Compound 17)

To a stirred rt solution of Boc-Dap (4-N₃)-Phe-OMe (35 mg, 0.06 mmol) in EtOH (2 mL) was added Pd on carbon (30 mg, 28.19 μmol) under nitrogen atmosphere. The reaction was stirred rt under hydrogen atmosphere. After 4 h, analysis by LCMS showed the reaction was complete. After insoluble materials were removed by filtration, the filtrate was concentrated under reduced pressure.

A total of 26 mg of the title compound was obtained (0.06 mmol, 97%) as a white amorphous solid that was used without further purification. ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.30 (d, *J* = 7.9 Hz, 1H), 8.00 (s, 2H), 7.35 – 7.12 (m, 5H), 4.52 (ddd, *J* = 9.6, 7.9, 5.4 Hz, 1H), 4.01-3.39 (m,

7H), 3.21 (s, 3H), 3.05 (dd, $J = 13.8, 5.4$ Hz, 2H), 2.89 (dd, $J = 13.7, 9.6$ Hz, 1H), 2.34 (m, 2H), 1.88 (br-s, 1H), 1.42 (s, 9H), 0.75 (d, $J = 6.6$ Hz, 3H); LCMS $t_R = 1.13$ min, ESIMS m/z 464.45 $[M + H]^+$; HRESIMS m/z 464.2790 $[M + H]^+$ (calcd for $C_{24}H_{37}N_3O_6$, 464.2755)

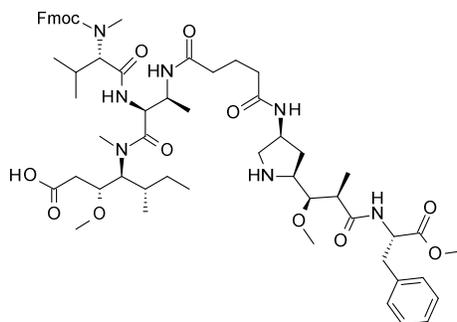


Figure S10. Fmoc-MeVal-Abu (3-NHCOCH₂CH₂CH₂CO-[Boc-Dap (4-NH-)]-Phe-OMe)-Dil-OH (Compound **18**)

To a stirred rt solution of Fmoc-MeVal-Abu (3-NHCOCH₂CH₂CH₂COOH)-Dil-OtBu (61 mg, 0.08 mmol), Boc-Dap (NH₂)-Phe-OMe (33 mg, 0.07 mmol) and DIEA (37 μ L, 0.21 mmol) in DMAc (1.5 mL) was added 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU) (70 mg, 184.10 μ mol). After 4 h, analysis by LCMS showed the reaction was complete. To the mixture was added 1N HCl aq. and then the mixture was stirred for 1 hour. After separation, the organic layer was washed with saturated sodium bicarbonate, brine and dried over anhydrous magnesium sulfate. The organic layer was concentrated under reduced pressure, the residue was dried further under high vacuum. A total of 95 mg of Fmoc-MeVal-Abu (3-NHCOCH₂CH₂CH₂CO-[Boc-Dap (4-NH-)]-Phe-OMe)-Dil-OtBu (75.72 μ mol, 102%) was obtained as a white amorphous solid. that was used without further purification.

A solution of Fmoc-MeVal-Abu (3-NHCOCH₂CH₂CH₂CO-[Boc-Dap (4-NH-)]-Phe-OMe)-Dil-*O*tBu (90 mg, 0.07 mmol) in 4 M HCl in dioxane (3 mL, 12.00 mmol) was stirred rt. After 2 h, analysis by LCMS showed the reaction was complete. The crude reaction mixture was concentrated under reduced pressure and dried further under high vacuum. The residue was purified by preparatory RP-HPLC with a Phenomenex Gemini-NX 10 μm, C18 110 Å column (150 x 30 mm) using a 5% to 95% MeCN in 0.05% aqueous TFA solution as the eluent. A total of 81 mg of the title compound was obtained as the TFA salt (0.07 mmol, 99%) as a white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆) δ 12.24 (s, 1H), 8.95 (s, 1H), 8.56 (d, *J* = 8.1 Hz, 1H), 8.26 – 8.11 (m, 1H), 8.08 (d, *J* = 6.6 Hz, 1H), 7.90 (d, *J* = 7.7 Hz, 2H), 7.64 (d, *J* = 7.3 Hz, 2H), 7.47-7.38 (m, 3H), 7.38 – 7.16 (m, 8H), 6.56-6.48 (m, 2H), 4.62 – 4.16 (m, 4H), 4.13-3.64 (m, 4H), 3.62 (s, 3H), 3.46-3.16 (m, 9H), 3.14 – 2.84 (m, 4H), 2.79 (s, 3H), 2.23 – 1.95 (m, 8H), 1.89 – 1.58 (m, 2H), 1.18-0.83 (m, 1H), 1.12-0.83 (m, 6H), 0.83 – 0.56 (m, 17H); LCMS *t*_R = 1.54 min, ESIMS *m/z* 1099.03 [M + H]⁺; HRESIMS *m/z* 1098.6164 [M + H]⁺ (calcd for C₅₉H₈₄N₇O₁₃, 1098.6122)

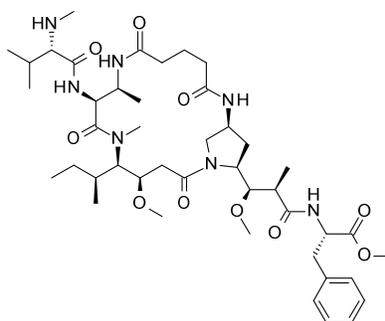


Figure S11. Macrocyclic analogue (Compound 19)

To a stirred rt solution of **18** (81 mg, 0.0714 mmol) in EtOAc (500 mL) were added CMPI (100 mg, 0.391 mmol) and DIEA (62 μL, 0.36 mmol). After 16 h, analysis by LCMS showed the macrocyclic condensation reaction was complete. The mixture was evaporated to give a yellow

To a stirred rt solution of Dov-Abu (3-N₃)-Dil-OtBu (180 mg, 0.29 mmol) and N-(but-3-yn-1-yl)-2-chloroacetamide (75 mg, 0.52 mmol) in DMF (5 mL) was added CuBr (76 mg, 0.53 mmol). After 15 h, analysis by LCMS showed the reaction was complete. The crude suspension was diluted with 15 mL of DMF, 2 mL of 0.5 M ethylenediaminetetraacetic acid (EDTA) and 10 mL of water. The mixture was purified by preparatory RP-HPLC using MeCN in 0.05% aqueous TFA as the eluent. A total of 120 mg Dov-Abu (3-triazole-CH₂CH₂NH-acetyl-Cl)-Dil-OtBu (160 μ mol, 54%) was obtained as a white amorphous solid.

To a stirred rt solution of Dov-Abu(3-triazole-CH₂CH₂NH-acetyl-Cl)-Dil-OtBu (120 mg, 160 μ mol) in 4.0 M HCl in dioxane (2.4 mL, 4.00 mmol). After 3 h, analysis by LCMS showed the reaction was complete. The crude reaction mixture was concentrated under reduced pressure. A total of 89 mg of the title compound was obtained as the HCl salt (0.19mmol, 99%) as an off-white amorphous solid. ¹H NMR (400 MHz, DMSO-*d*₆- conformational isomers) δ 9.83 (s, 1H), [9.58 (d, *J* = 8.7 Hz) and 9.35 (d, *J* = 8.7 Hz), 1H], 8.35 (t, *J* = 5.7 Hz, 1H), [7.80 (s) and 7.94 (s), 1H], 5.46 (t, *J* = 8.2 Hz, 1H), 5.09-4.98 (m, 1H), 4.38 (br-s, 1H), [4.07 (s) and 4.05 (s), 2H], 3.82 (d, *J* = 5.9 Hz, 1H), 3.64 (br-s, 1H), 3.42-3.31 (m, 2H), 3.18 (s, 3H), [2.93 (s) and 2.90 (s), 3H], 2.84-2.67 (m, 8H), 2.37 – 2.13 (m, 2H), 1.99 (dd, *J* = 15.7, 9.3 Hz, 1H), 1.83 – 1.68 (m, 1H), 1.63 – 1.45 (m, 4H), 1.36-1.19 (m, 1H), 0.96 (d, *J* = 6.8 Hz, 3H), 0.91-0.80 (m, 6H), 0.75 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (101 MHz, DMSO-*d*₆) δ 173.28, 169.78, 166.33, 165.96, 159.43, 159.06, 158.71, 158.35, 144.30, 122.75, 78.31, 72.33, 71.78, 57.54, 56.57, 53.24, 50.27, 43.07, 41.93, 41.60, 39.04, 38.23, 37.28, 32.72, 31.64, 28.92, 27.03, 25.82, 25.66, 24.01, 19.55, 16.91, 16.20, 10.90; LCMS *t*_R = 0.94 min, *m/z* 602.49, 604.50 [M + H]⁺; HRESIMS *m/z* 602.3425 [M + H]⁺ (calcd for C₂₇H₄₉ClN₇O₆, 602.3427)

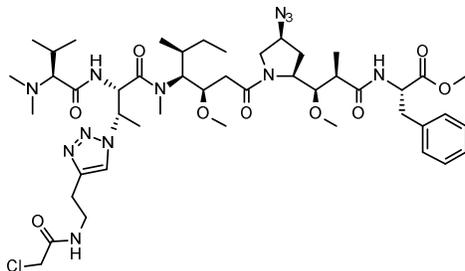


Figure S13. Dov-Abu (3-triazole-CH₂CH₂NH-acetyl-Cl)-Dil-Dap (4-N₃)-Phe-OMe
(Compound **22**)

To a stirred rt solution of compound **21** (24 mg, 0.04 mmol), H-Dap (N₃)-Phe-OMe HCl salt **11** (15 mg, 0.04 mmol) in CH₂Cl₂ (3 mL) were added DIEA (25 μL, 0.140 mmol), EDCI (18 mg, 0.09 mmol), HOBt (5 mg, 0.03 mmol). After 18 h, analysis by LCMS showed the reaction was complete. The reaction mixture was diluted with H₂O and DMAc and then the mixture was purified by preparatory RP-HPLC with a Phenomenex Gemini-NX 10 μm, C18 110 Å column (150 x 30 mm) using a 5% to 95% MeCN in 0.05% aqueous TFA solution as the eluent. A total of 24 mg of the title compound was obtained as the TFA salt (22.07 μmol, 59%) as a white amorphous solid. LCMS *t*_R = 1.29 min, *m/z* 973.83, 975.85 [M + H]⁺; HRESIMS *m/z* 973.5387 [M + H]⁺ (calcd for C₄₆H₇₄ClN₁₂O₉, 973.5385)

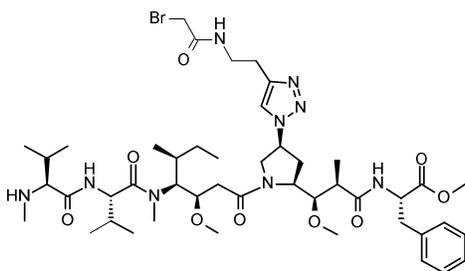
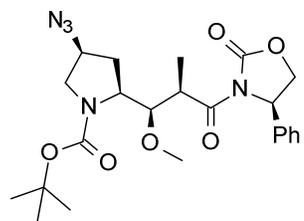


Figure S14. MeVal-Val-Dil-Dap (4-triazole-CH₂CH₂NH-acetyl-Br)-Phe-OMe (Compound **23**)

To a stirred rt solution of compound **13a** (15 mg, 16.65 μmol), N-(but-3-yn-1-yl)-2-bromoacetamide (5 mg, 26.31 μmol) in DMF (1 mL) was added CuBr (9.55 mg, 66.59

μmol). After 15 h, analysis by LCMS showed the reaction was complete. The crude suspension was diluted with 7 mL of DMAc, 2 mL of 0.5 M EDTA and 5 mL of water. The mixture was purified by preparatory RP-HPLC using MeCN in 0.05% aqueous TFA as the eluent. A total of 5 mg of the title compound (4.58 μmol , 28%) was obtained as a white amorphous solid. ^1H NMR (400 MHz, $\text{DMSO-}d_6$, a complex spectrum was observed, presumably due to *cis/trans* conformational isomers) δ 8.80 (d, $J = 8.6$ Hz, 2H), 8.65 (s, 1H), 8.38-8.29 (m, 1H), 8.03 – 7.92 (m, 1H), 7.32 – 7.14 (m, 5H), 5.12 – 4.90 (m, 1H), 4.67 (br-s, 1H), 4.57 (t, $J = 8.7$ Hz, 1H), 4.52-4.41 (m, 1H), 4.24 (t, $J = 8.5$ Hz, 1H), 4.09 – 3.94 (m, 2H), 3.89 – 3.39 (m, 9H), 3.35 – 3.24 (m, 2H), 3.23 – 3.11 (m, 6H), 3.08 – 2.94 (m, 4H), 2.92 – 2.56 (m, 3H), 2.45 (t, $J =$ Hz, 3H), 2.35 – 2.14 (m, 2H), 2.12 – 1.92 (m, 2H), 1.87-1.67 (m, 2H), 1.35-1.18 (m, 1H), 1.02-0.83 (m, 17H), 0.81-0.66 (m, 6H); LCMS $t_{\text{R}} = 1.19$ min, m / z 976.79, 978.77 $[\text{M} + \text{H}]^+$; HRESIMS m / z 976.4874, 978.4873 $[\text{M} + \text{H}]^+$ (calcd for $\text{C}_{46}\text{H}_{75}\text{BrN}_9\text{O}_9$, 976.4866)



Compound **8**

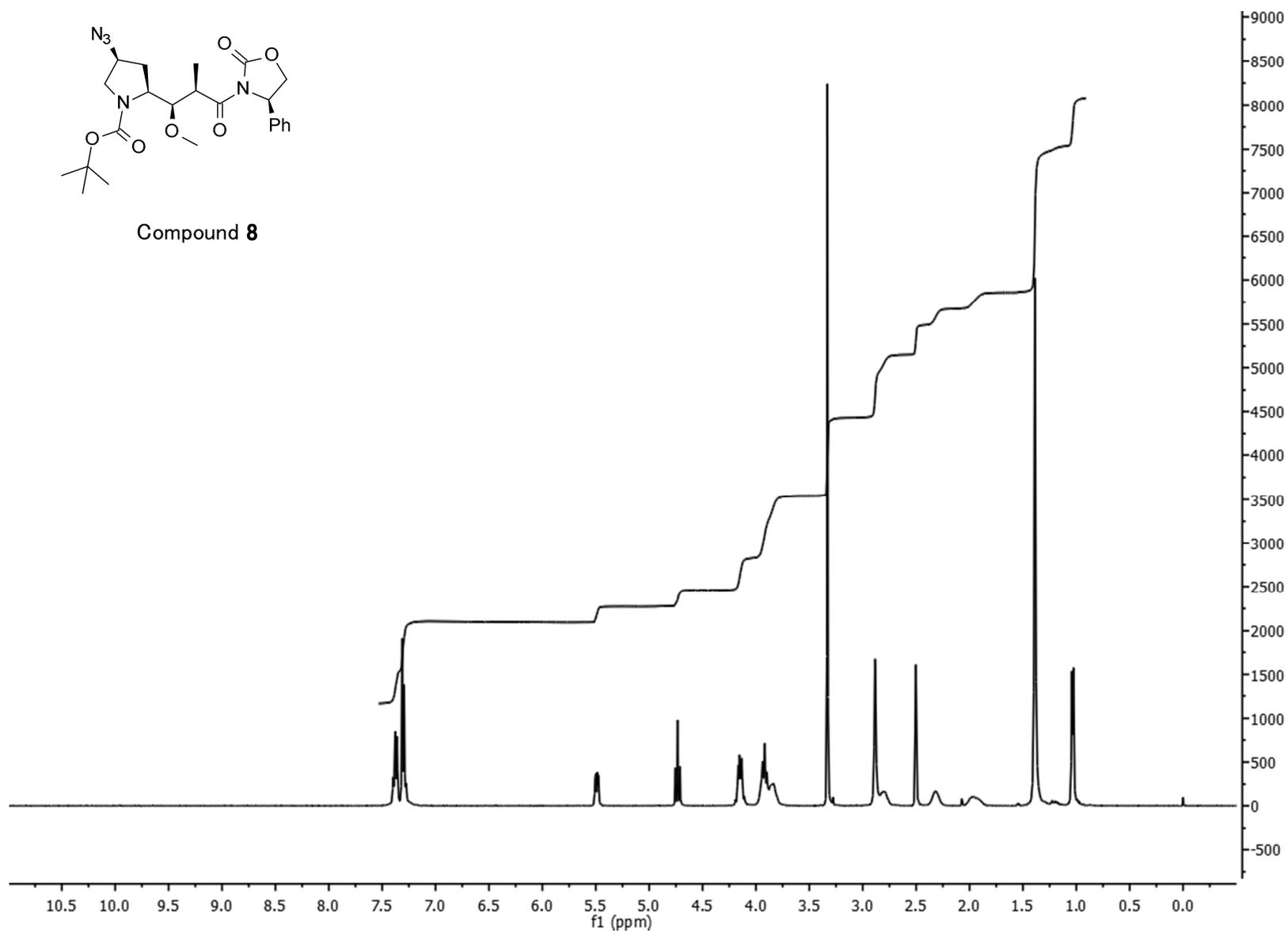
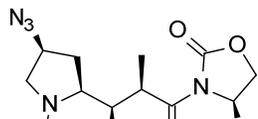


Figure S15. ¹H NMR (400 MHz, DMSO-*d*₆) of Compound **8**



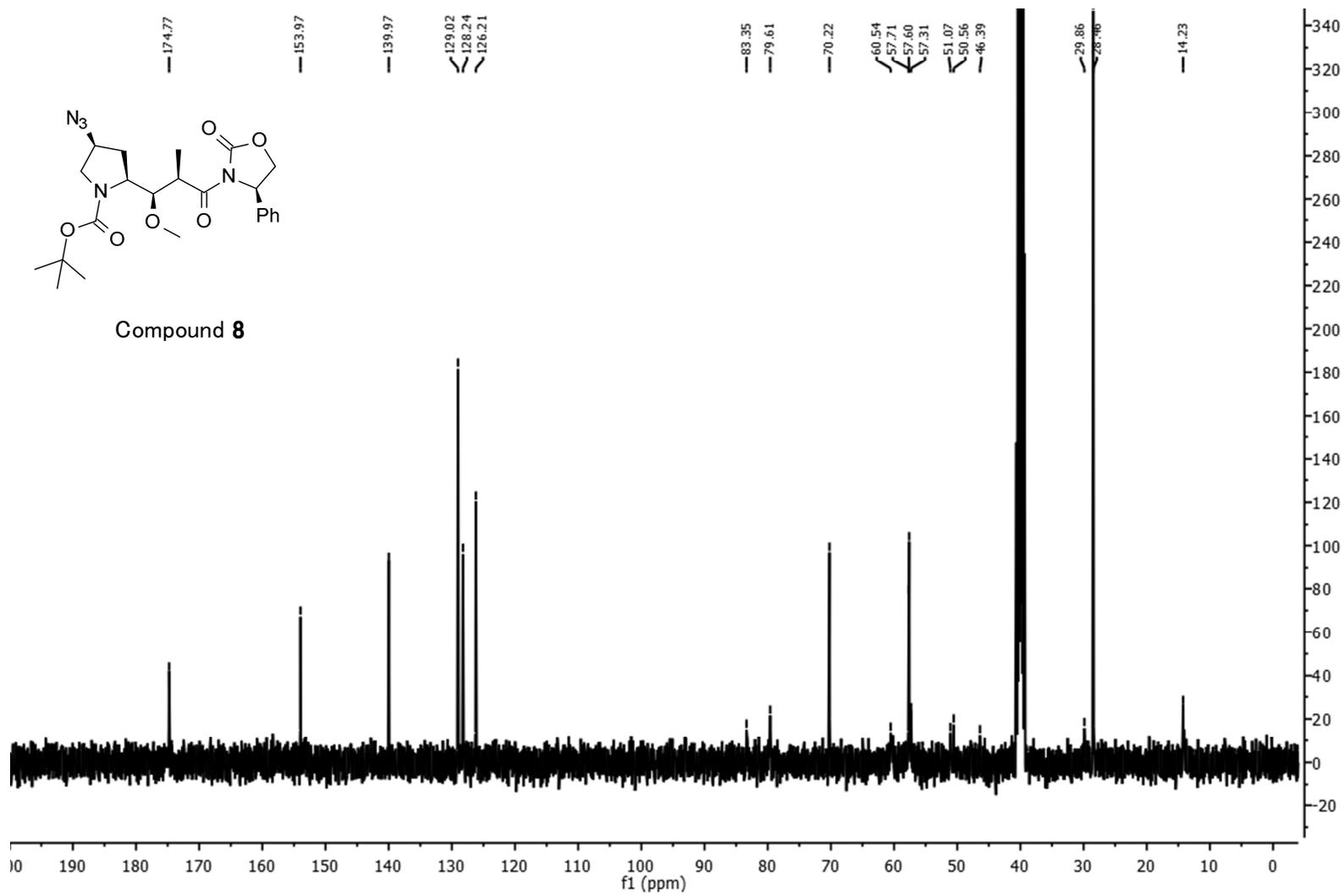
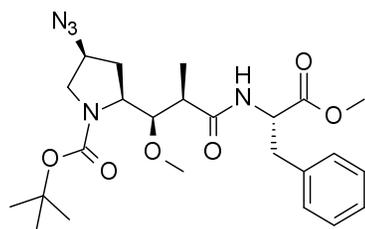


Figure S16. ^{13}C NMR (101 MHz, DMSO- d_6) of Compound 8



Compound 10

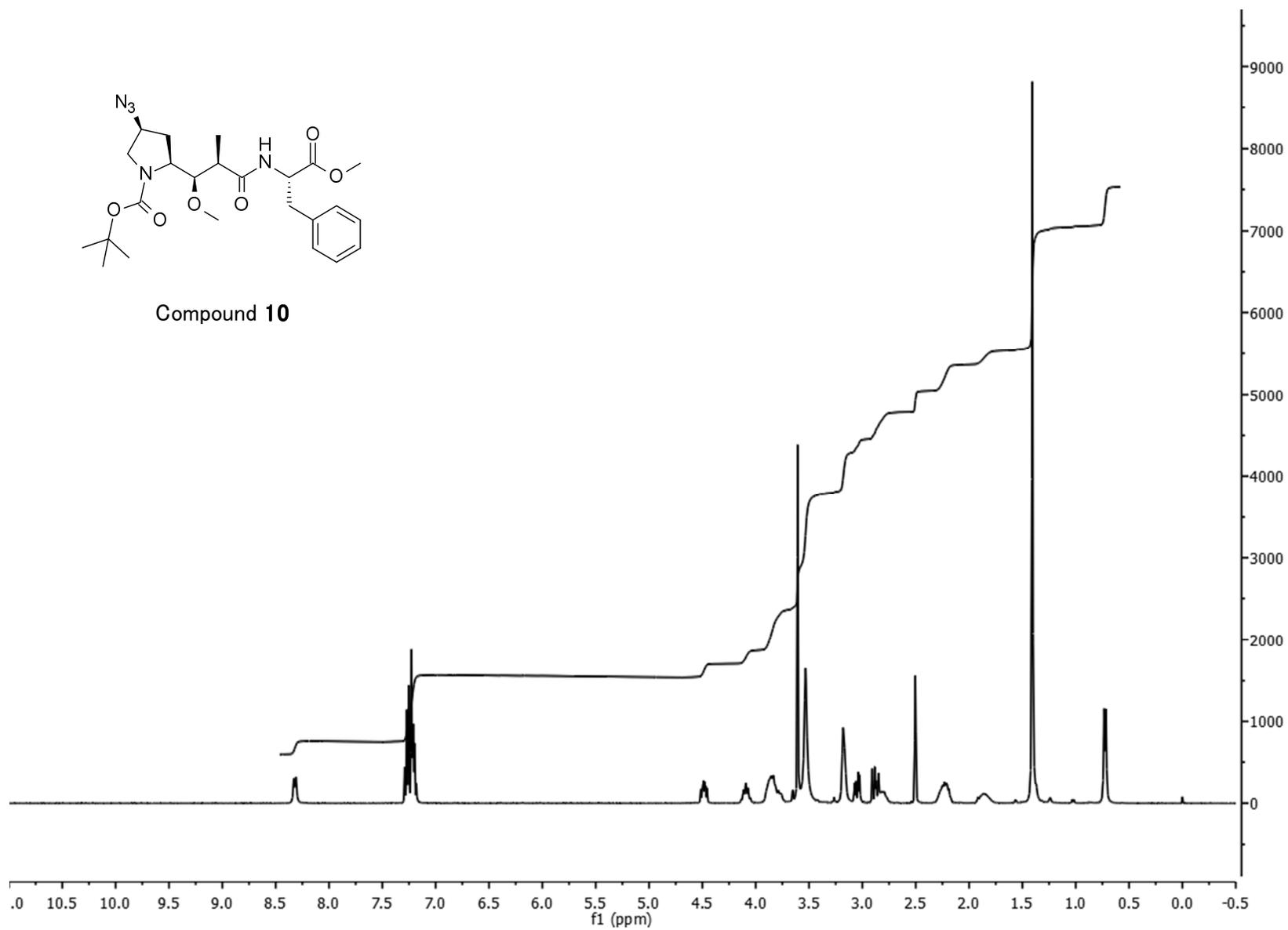


Figure S17. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) of Compound 10

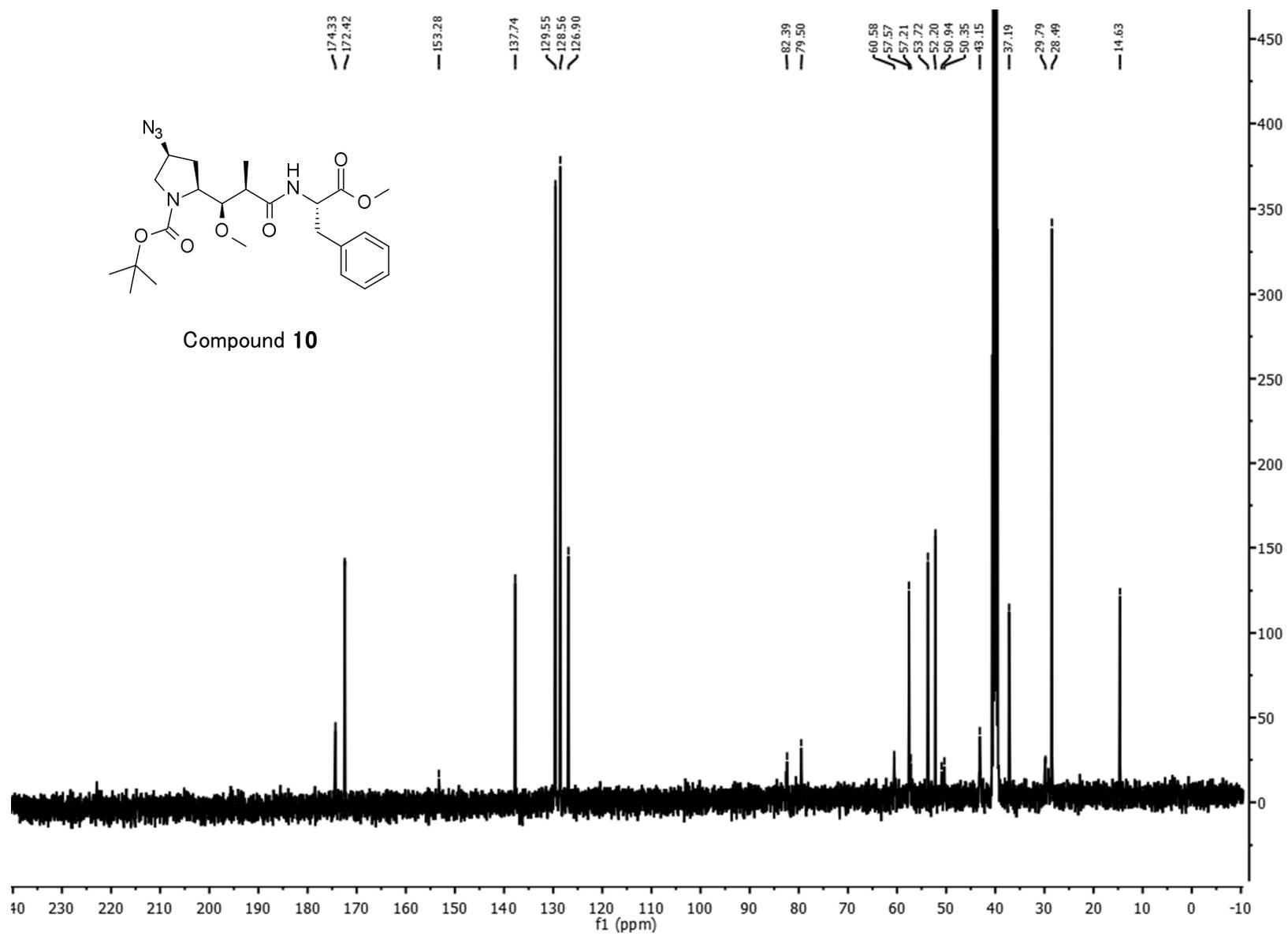
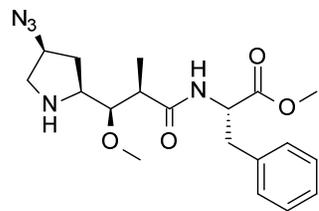


Figure S18. ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) of Compound 10



Compound 11

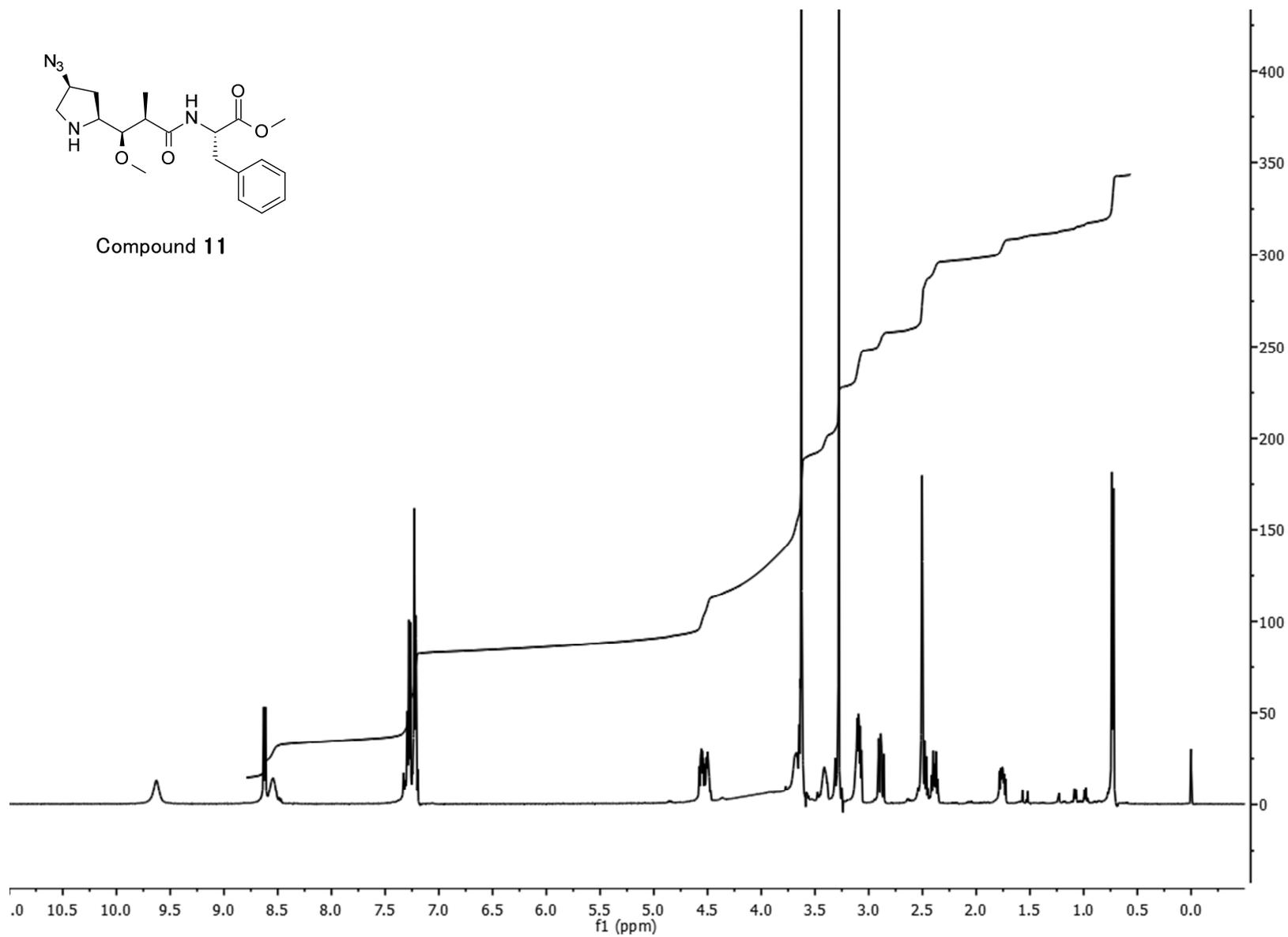


Figure S19. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) of Compound 11

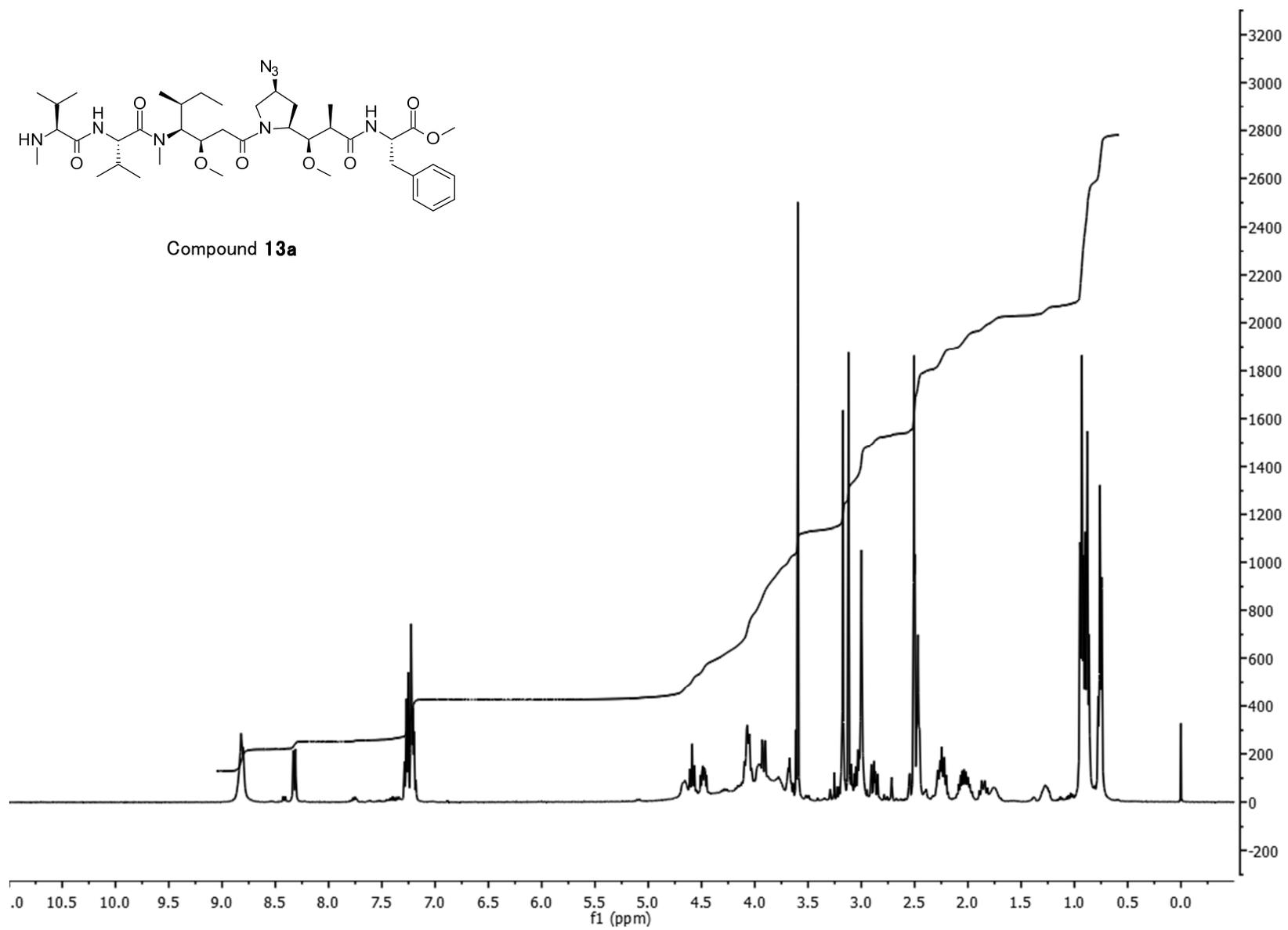


Figure S20. 1H NMR (400 MHz, $DMSO-d_6$) of Compound 13a

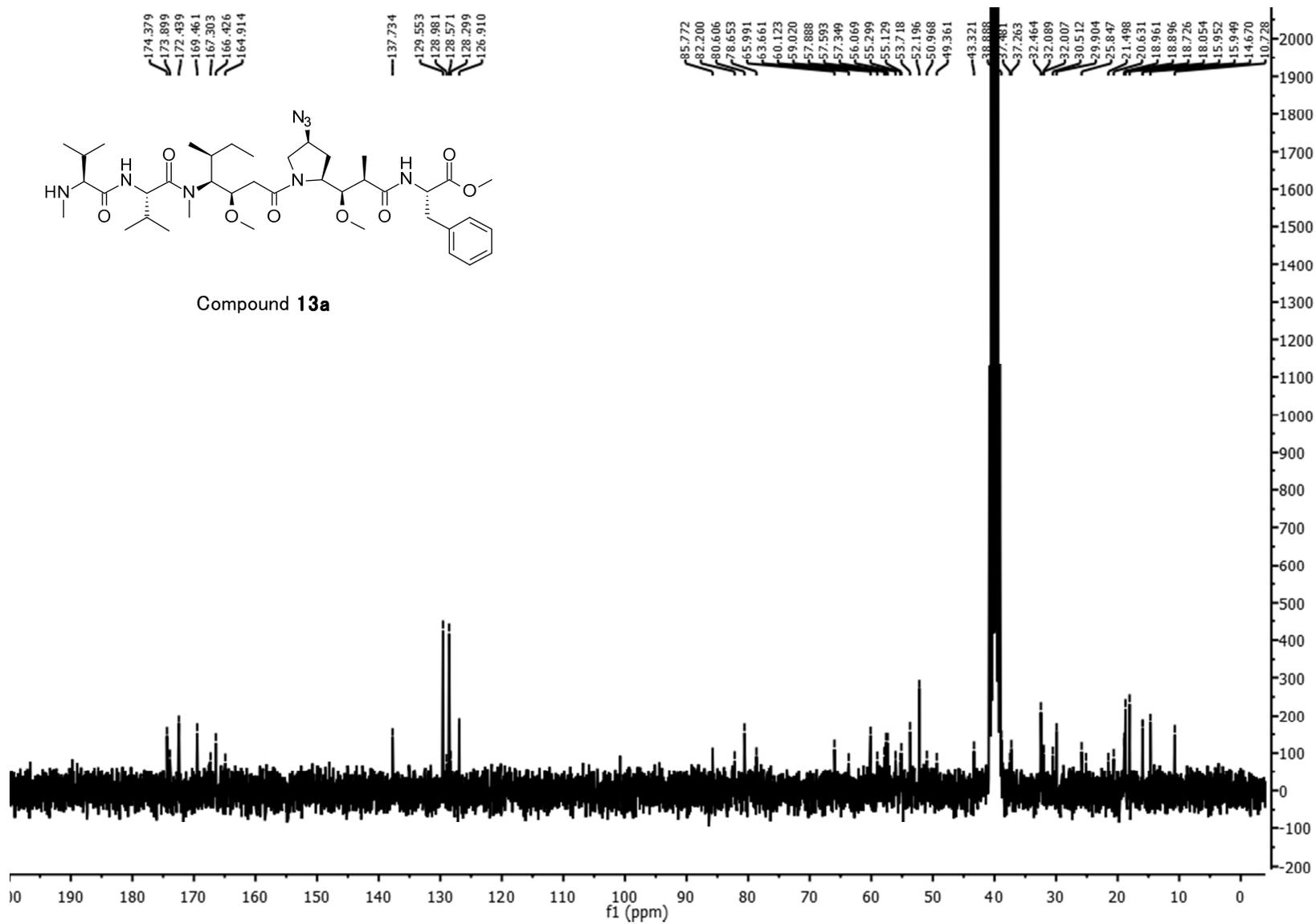


Figure S21. ^{13}C NMR (75 MHz, $\text{DMSO-}d_6$) of Compound 13a

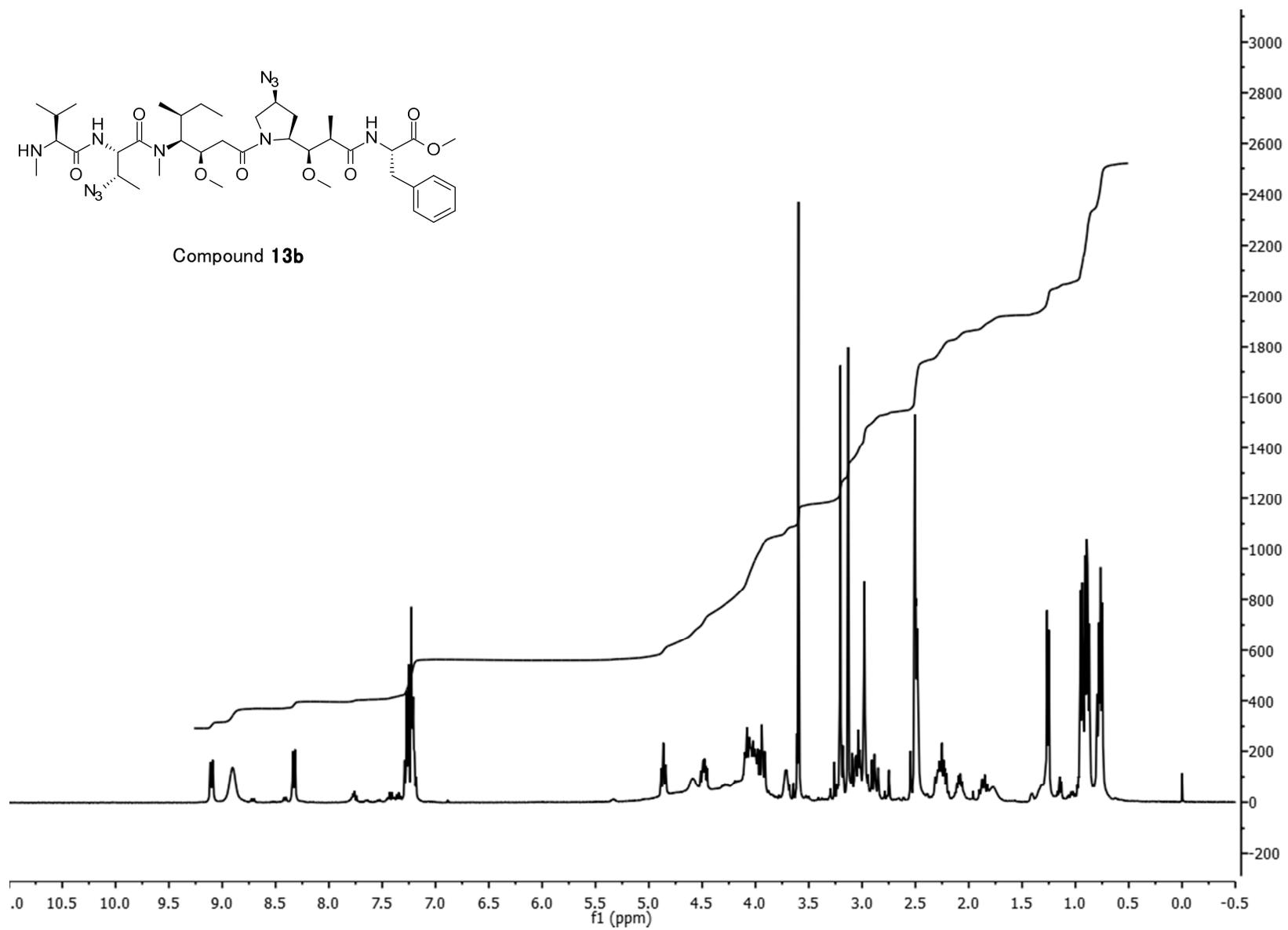


Figure S22. 1H NMR (400 MHz, DMSO- d_6) of Compound 13b

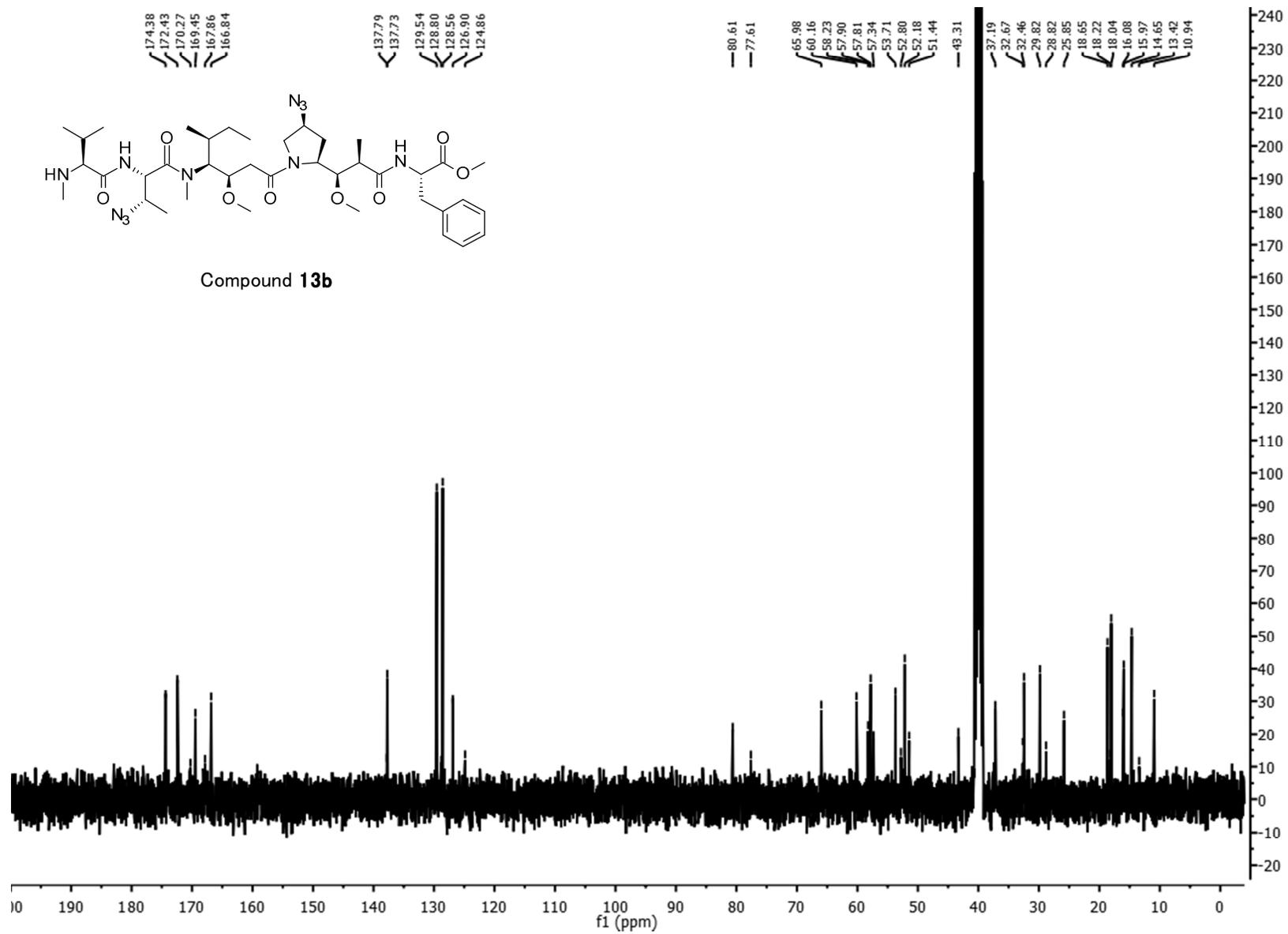


Figure S23. ^{13}C NMR (101 MHz, DMSO- d_6) of Compound 13b

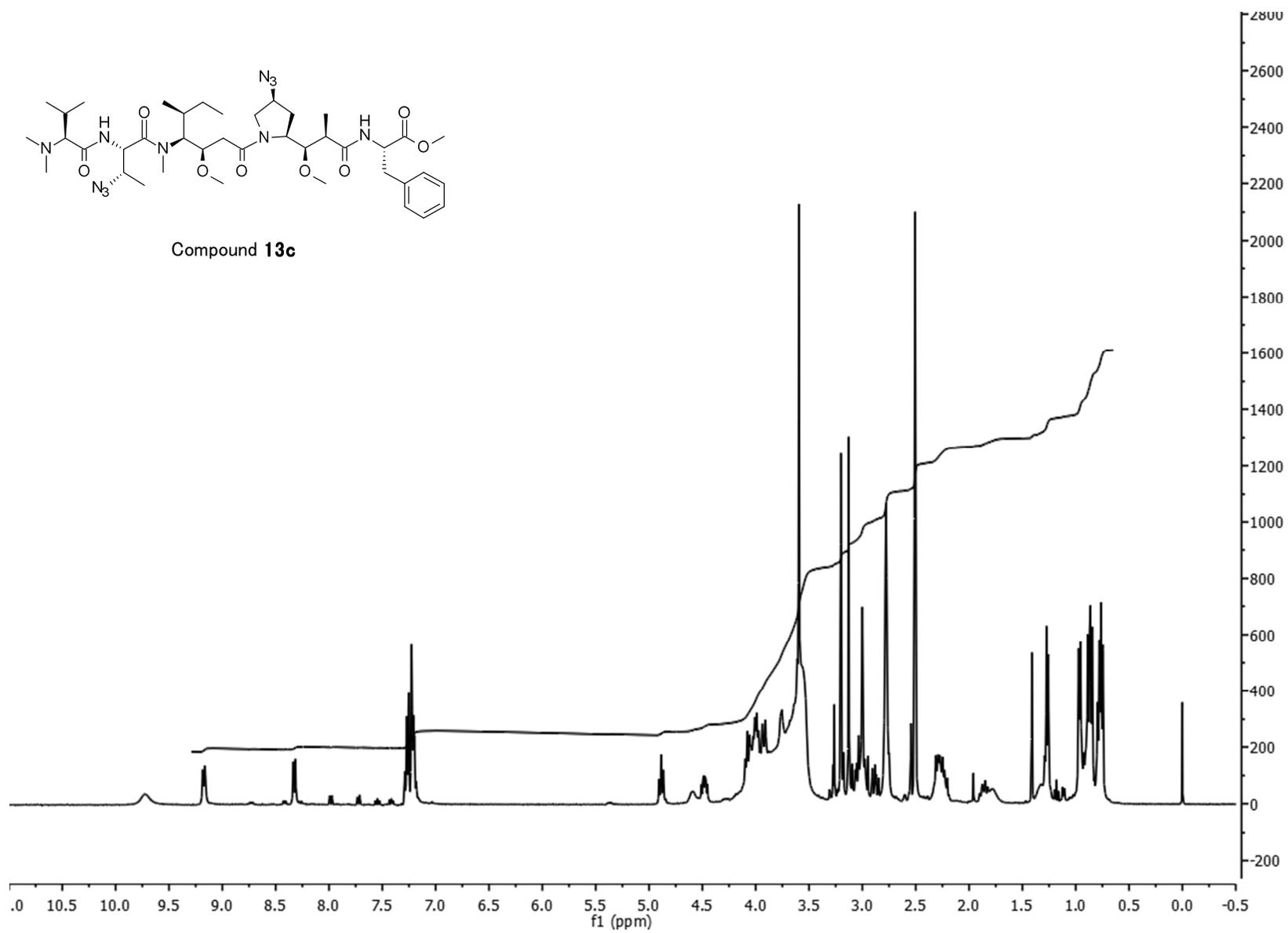


Figure S24. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) of Compound 13c

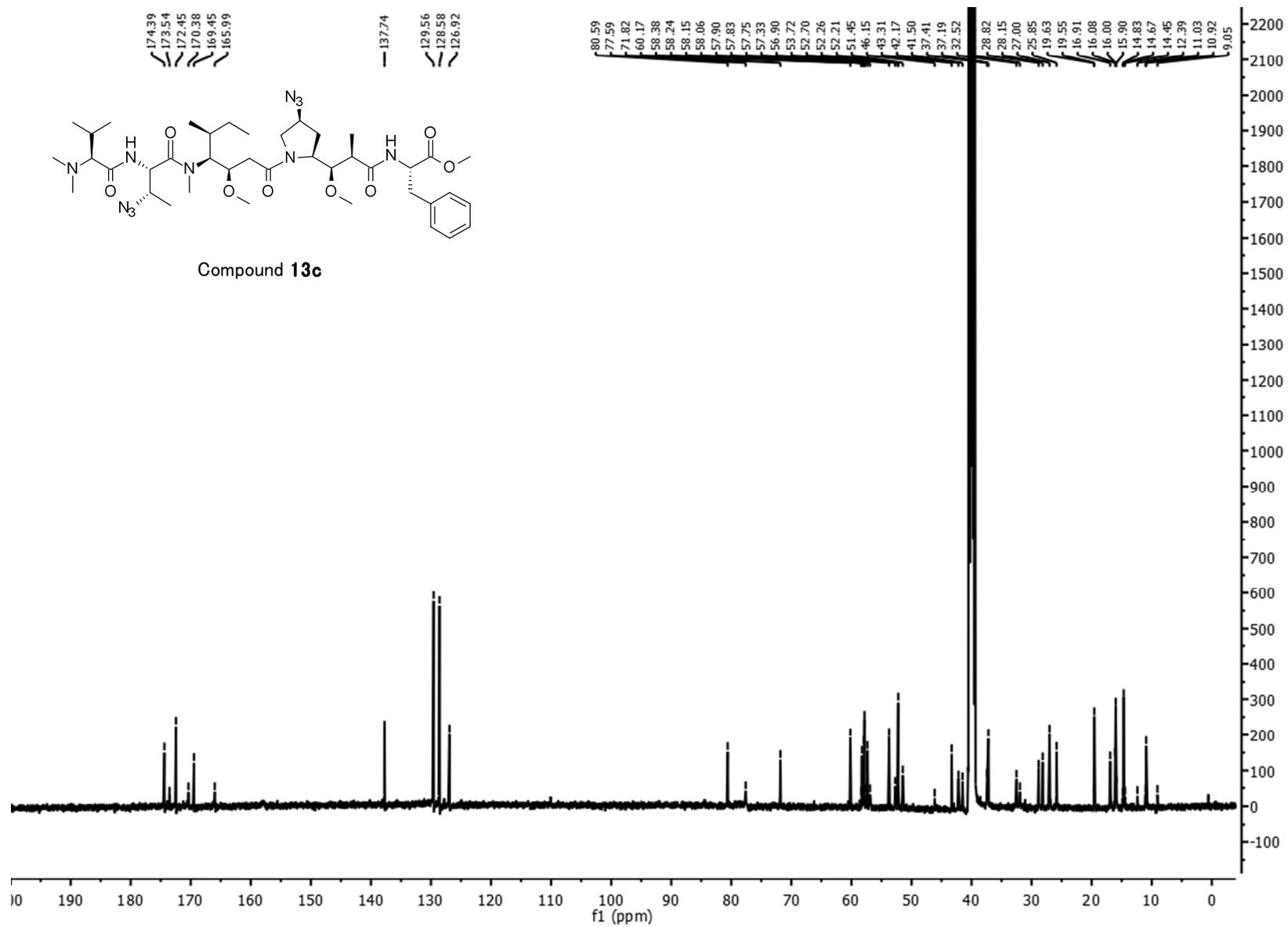
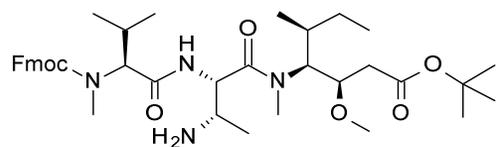


Figure S25. ^{13}C NMR (126 MHz, $\text{DMSO}-d_6$) of Compound 13c



Compound **15**

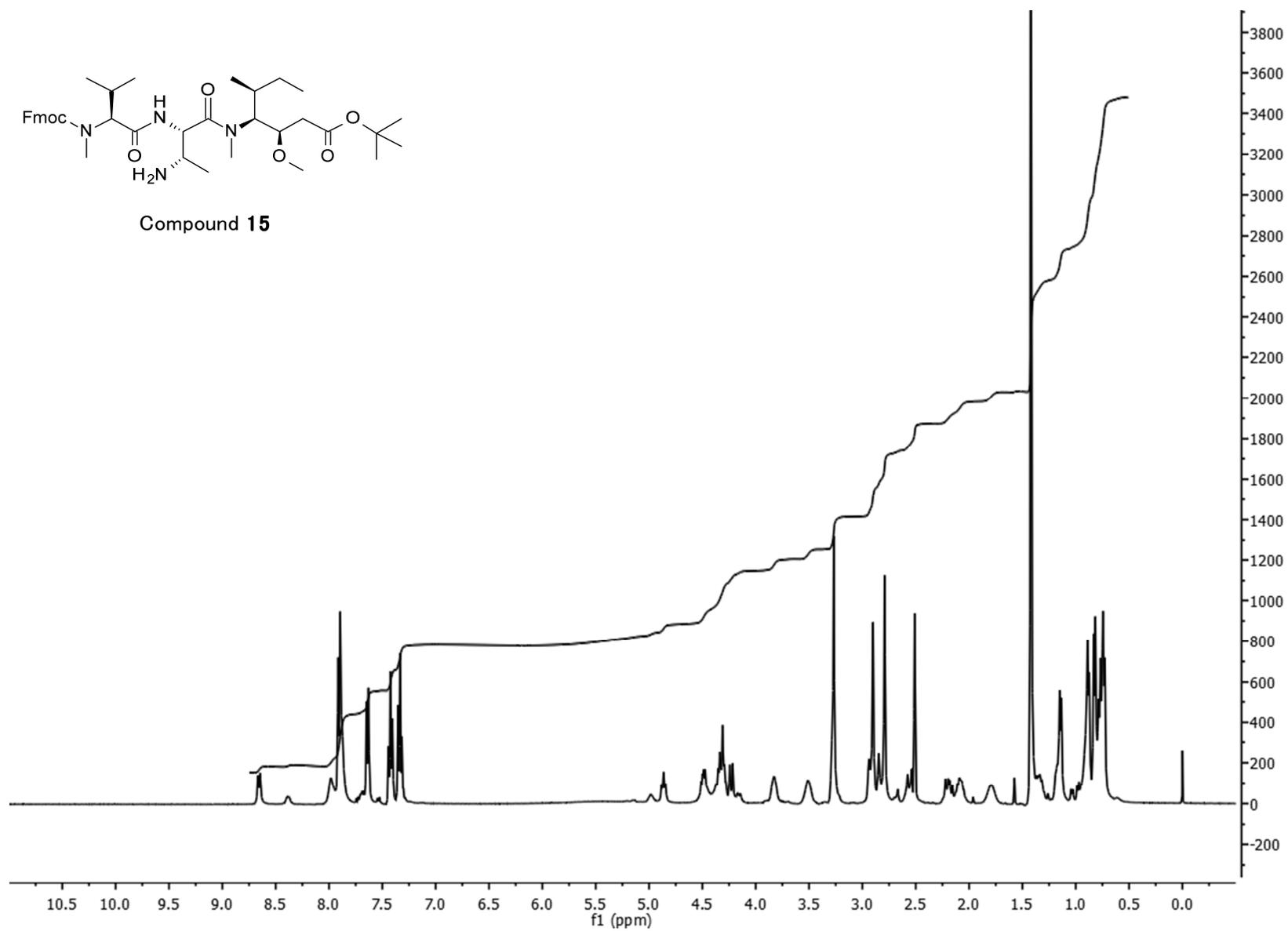


Figure S26. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) of Compound **15**

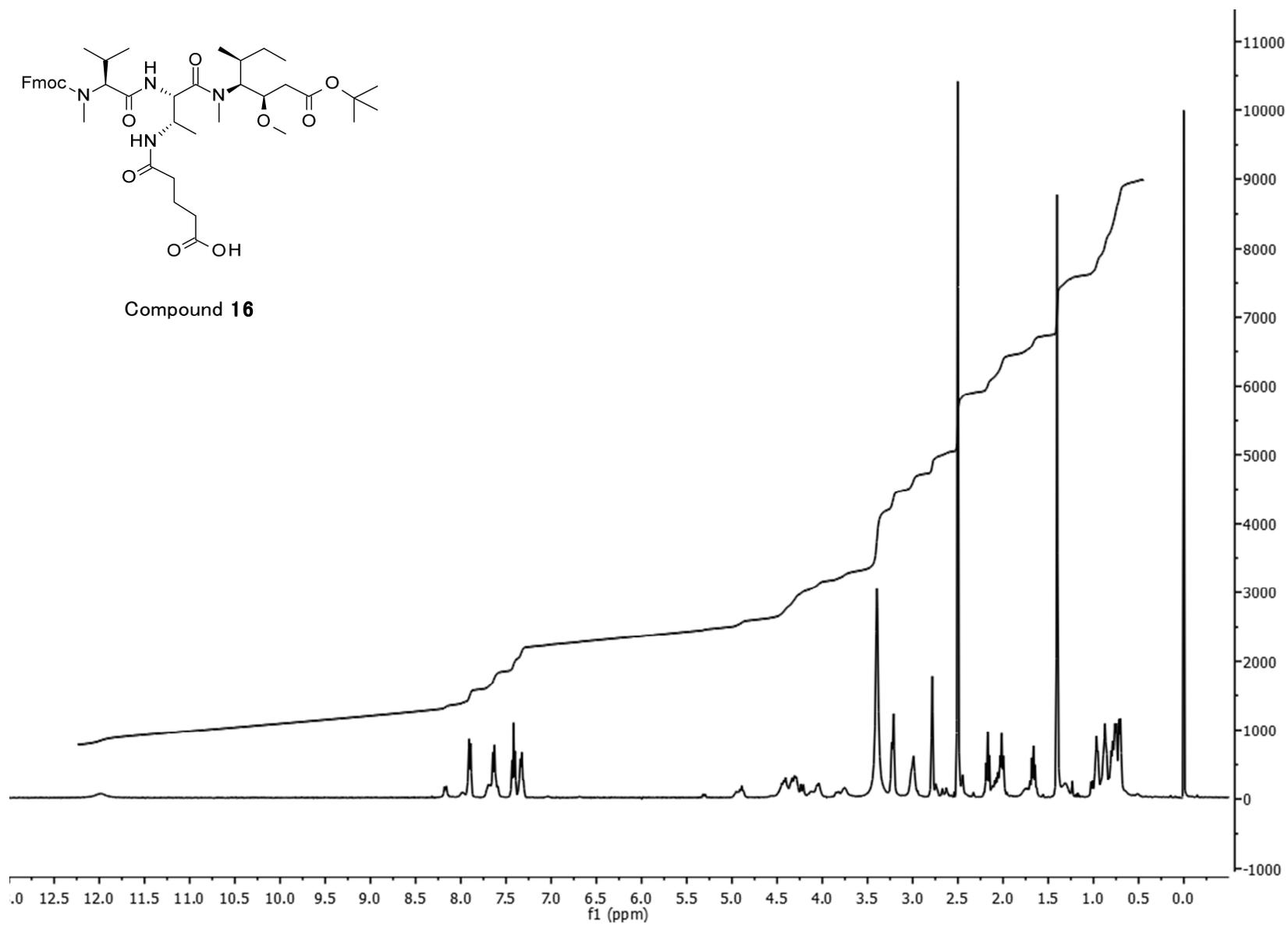


Figure S28. ^1H NMR (400 MHz, DMSO- d_6) of Compound 16

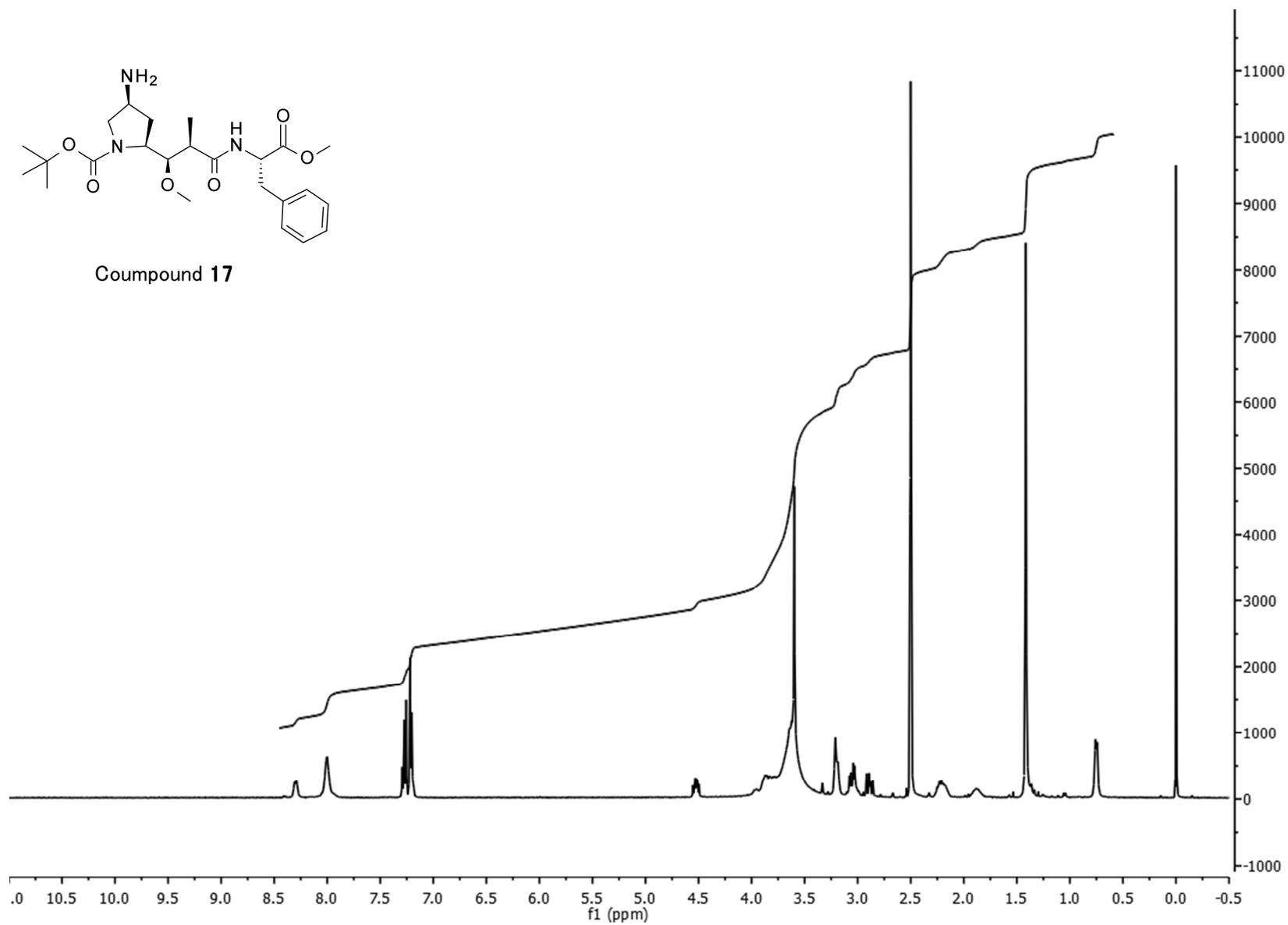


Figure S29. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) of Compound 17

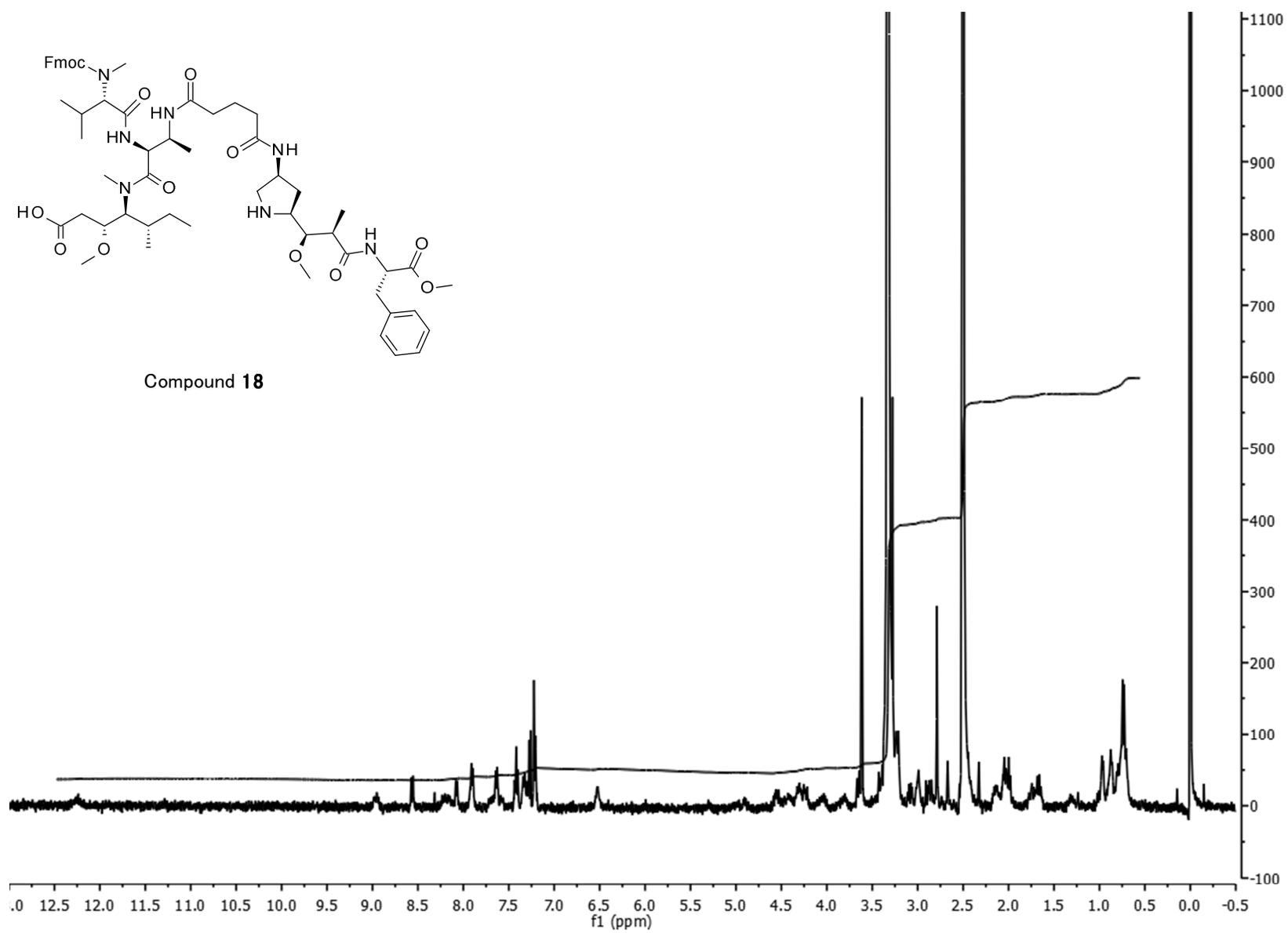


Figure S30. ^1H NMR (400 MHz, $\text{DMSO}-d_6$) of Compound 18

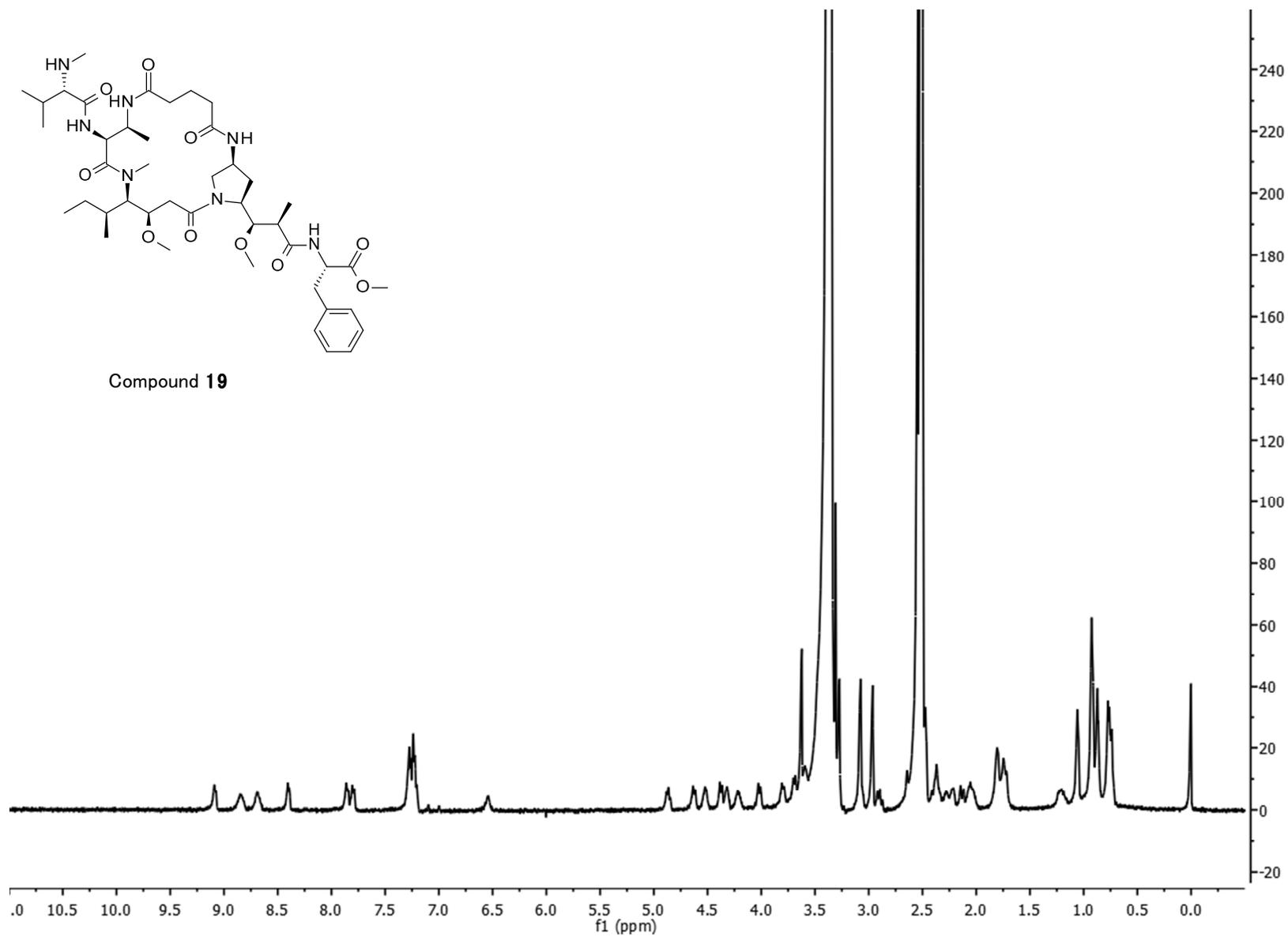


Figure S31. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) of Compound 19

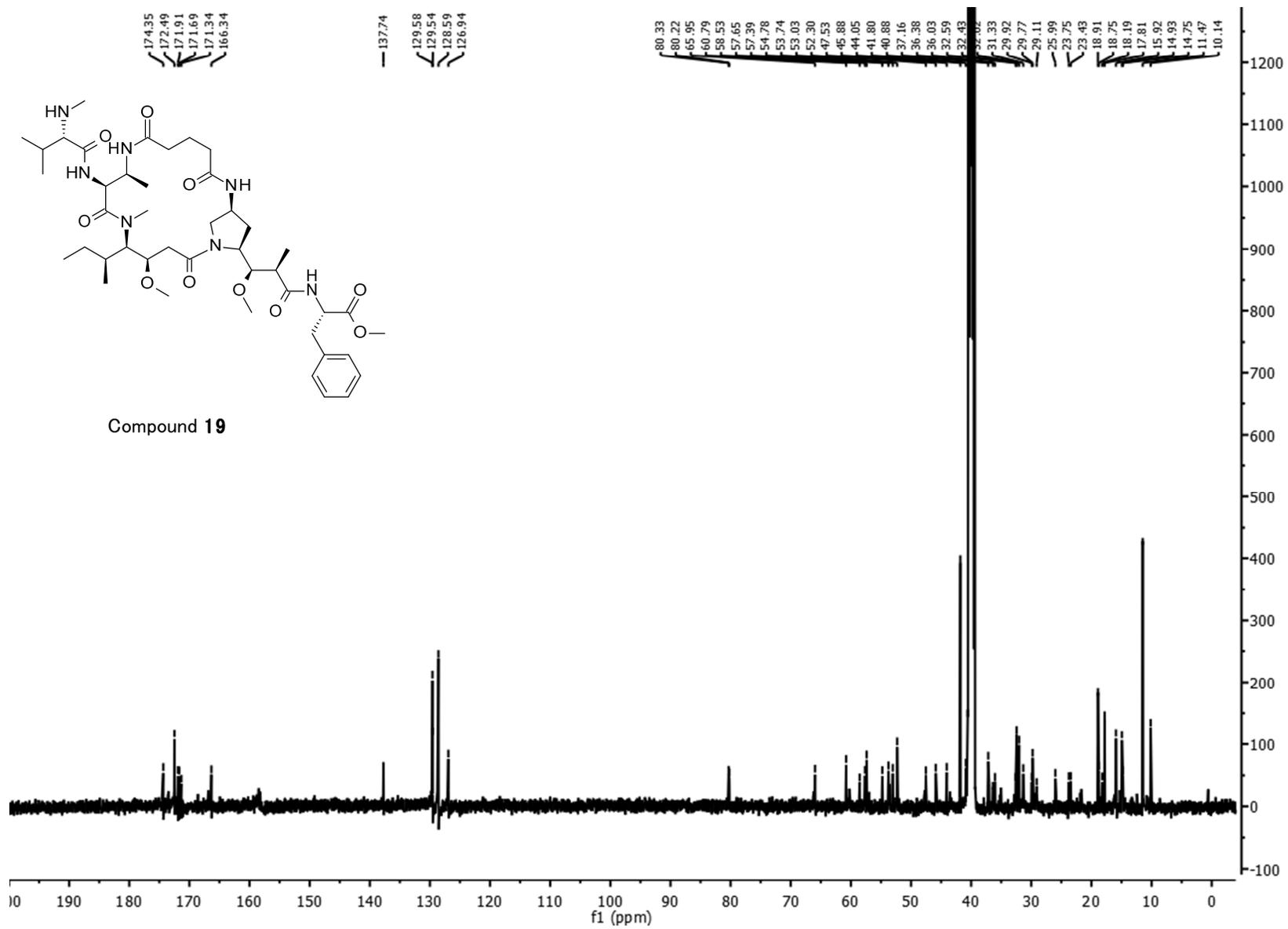


Figure S32. ^{13}C NMR (126 MHz, DMSO- d_6) of Compound 19

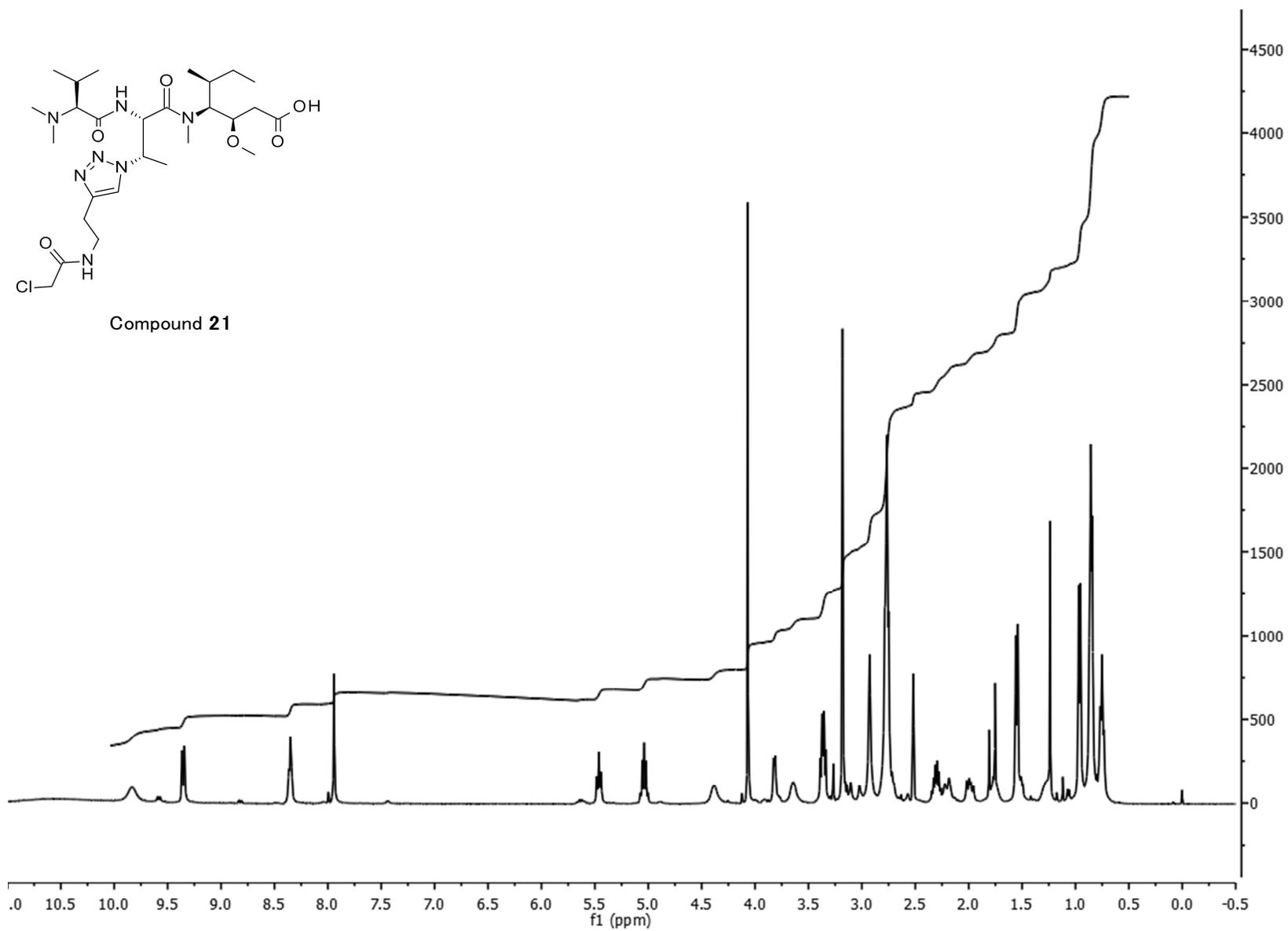


Figure S33. $^1\text{H NMR}$ (400 MHz, $\text{DMSO-}d_6$) of Compound 21

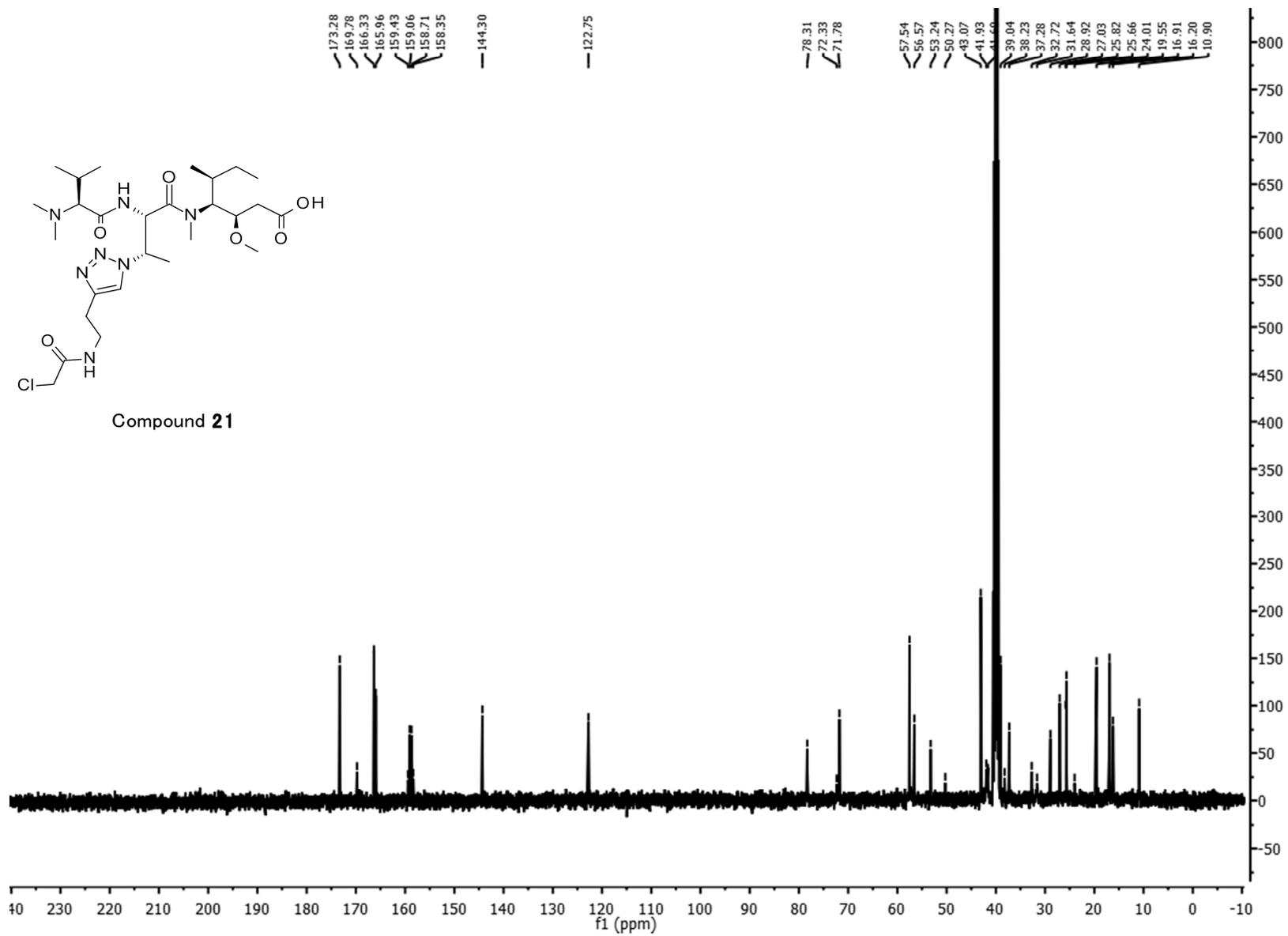


Figure S34. ^{13}C NMR (101 MHz, $\text{DMSO-}d_6$) of Compound 21

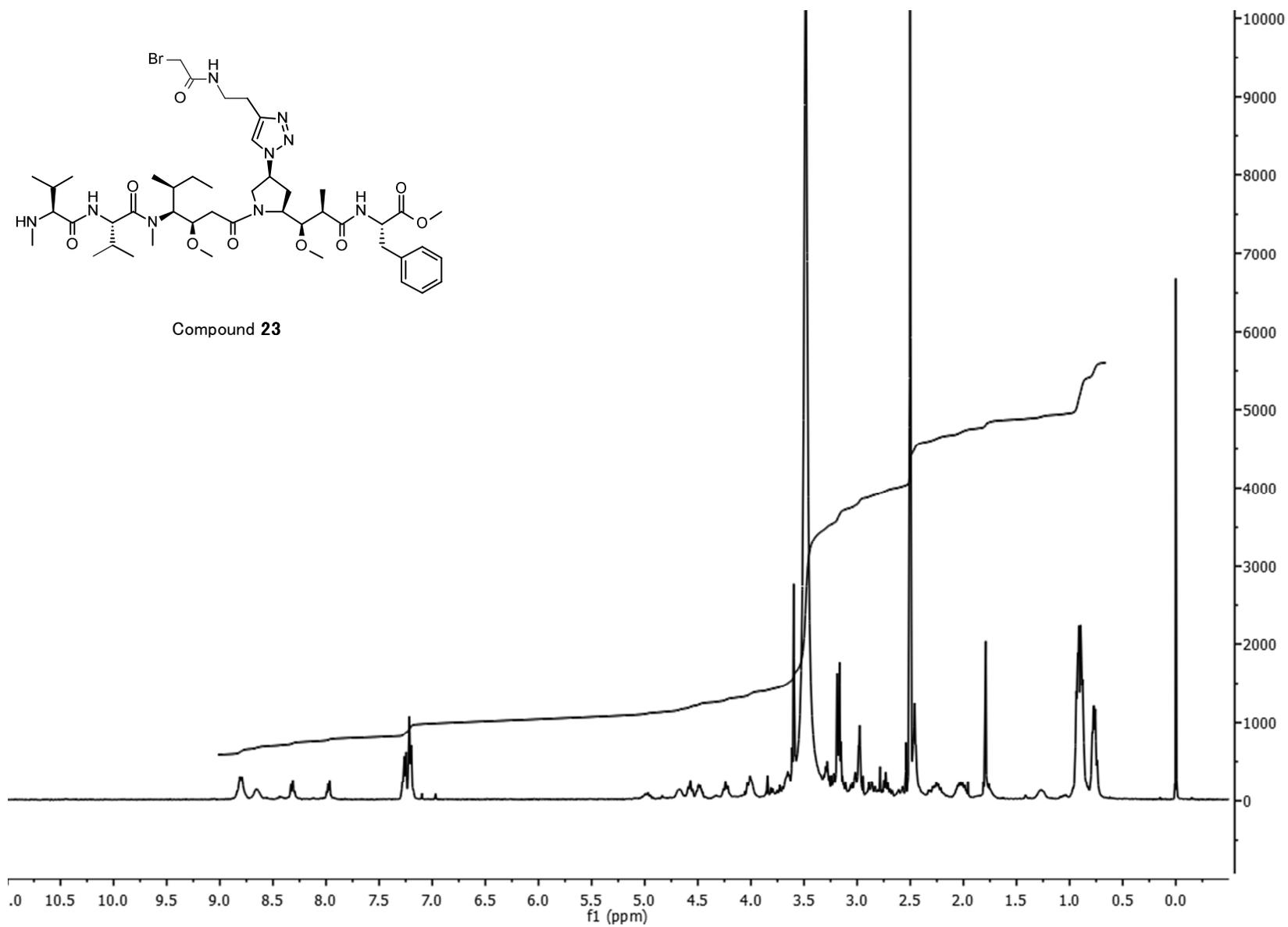
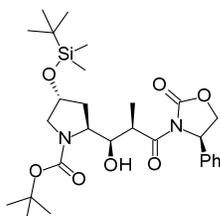


Figure S35. ^1H NMR (400 MHz, $\text{DMSO-}d_6$) of Compound 23

Chiral



Formula Weight : 548.74(2)
 Exact Mass : 548.29177831(2)
 Formula : C₂₈H₄₄N₂O₇Si
 Composition : C 61.29% H 8.08% N 5.11% O 20.41% Si 5.12%

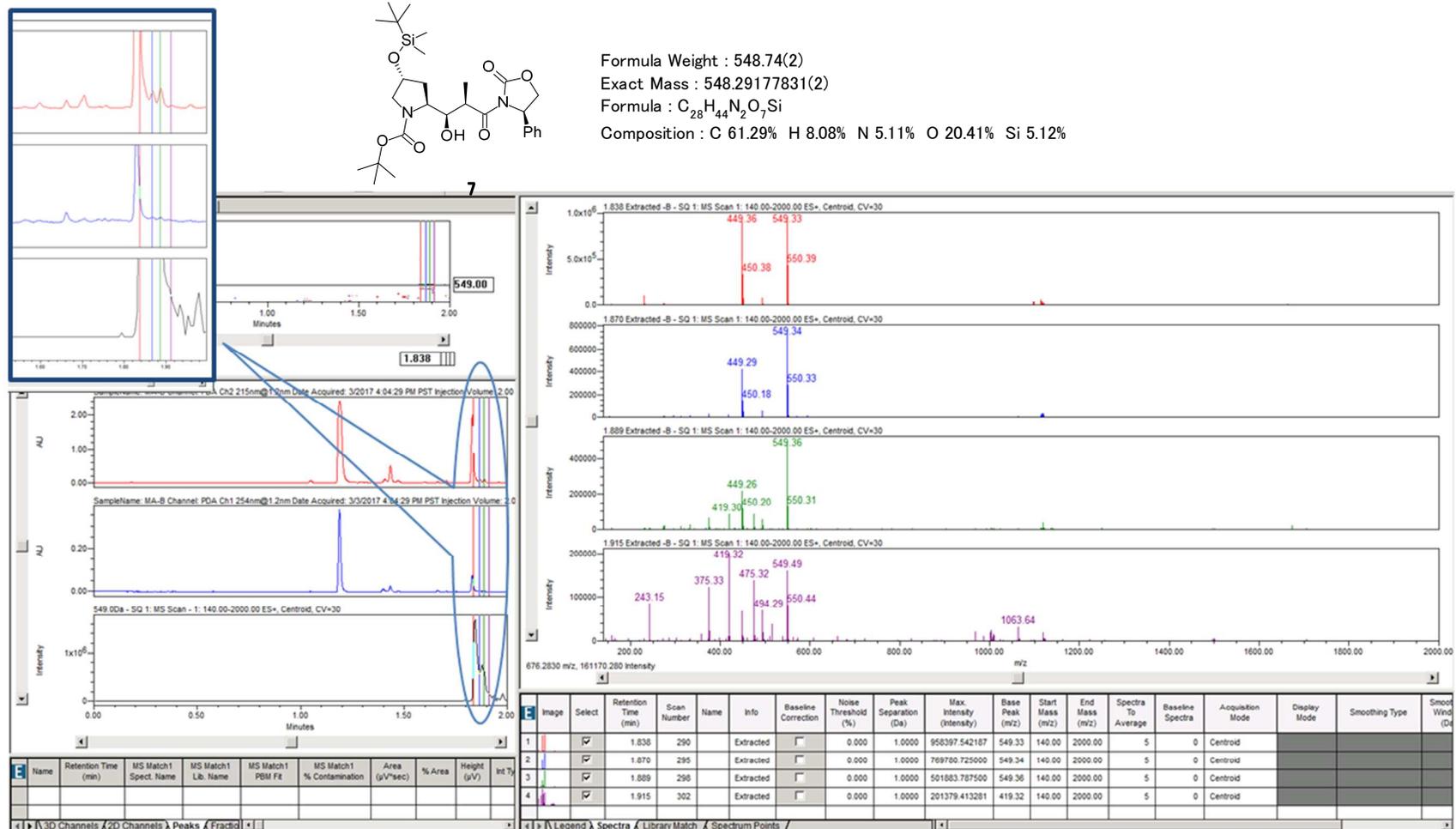


Figure S36. Aldol Reaction Diastereoselectivity

There were main peak (red line) and three slight peaks (blue, green and purple line) at 215 and 254 nm UV chart which have 215 nm ESIMS m / z 549 [M + H]⁺.

254 nm