Supporting Information

Structure-activity relationship studies of 3-*epi*-deoxynegamycin derivatives as potent readthrough drug candidates

Keisuke Hamada, Akihiro Taguchi, Masaya Kotake, Suguru Aita, Saori Murakami, Kentaro Takayama, Fumika Yakushiji, and Yoshio Hayashi*

Department of Medicinal Chemistry, School of Pharmacy, Tokyo University of Pharmacy and Life Science, 1432-1, Horinouchi, Hachioji, Tokyo, 192-0392, Japan.

*Corresponding author; Yoshio Hayashi, E-mail; yhayashi@toyaku.ac.jp. Phone: +81 -42-676-3275. Fax: +81-676-4475.

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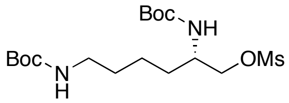
1. General information

All reaction mixtures were stirred magnetically. ^{1}H NMR spectra were measured in $CDCl_3$ and D_2O solutions, and referenced to TMS (0.00 ppm) and D_2O (4.79 ppm) using Bruker AVANCE-III (400 MHz), Bruker DPX-400 NMR Spectrometer (400 MHz) and Varian Mercury-300 NMR (300 MHz) spectrophotometers. ^{13}C NMR spectra were measured in $CDCl_3$ and D_2O solutions, and referenced to $CDCl_3$ (77.05 ppm) using Bruker AVANCE-III (400 MHz) and Bruker DPX-400 NMR (400 MHz) spectrophotometers. When peak multiplicities are reported, the following abbreviations are used: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br s, broad singlet; br d, broad doublet. Melting points were measured with Yanaco MP–500D melting point apparatuses. Mass spectra were obtained on Waters MICRO MASS LCT-premier. Optical rotations were measured with a JASCO Polarimeter P-1030 at the sodium-D line (589 nm) at the concentrations (*c*, g 100 mL^{-1}). The measurements were carried out between 24-25 ºC in a cell with path length (*l*) of 1 dm. Specific rotations $[\alpha]_D$ are given in 10^{-1} deg cm^2 g^{-1}. Column chromatography was performed on silicagel 60N (spherical, neutral) (4-50 μm or 63-210 μm), thin layer chromatography (TLC) was performed on precoated plates (0.25 mm, silica gel Merk Kieselgel $60F_{254}$), and compounds were visualized with UV light, phosphomolybdic acid stain, and ninhydrin stain. Preparative HPLC was performed using a C18 reversed-phase column (250 x 20 mm; YMC-Pack ODS-AM) with a binary solvent system. Analytical HPLC was performed using a C18 reversed-phase column (COSMOSIL Packed Column, Protein–R, 4.6ID x 150 mm) with a binary solvent system. Solvents and reagents were purchased from Kanto Chemical Co., Inc., Kokusan Chemical Co., Ltd., Wako Pure Chemical Industries, Ltd., and Watanabe Chemical Industries, Ltd.

2. Synthesis of derivatives 9a-c

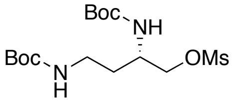
The derivatives **9a-c** were synthesized by previously reported synthetic procedure of 3-*epi*-deoxynegamycin (**2**) [1].

(*S*)-2,6-bis(*tert*-Butoxycarbonylamino)hexylmethanesulfonate (5a)



N-methylmorphorine (140 μL, 1.25 mmol) and isobutylchloroformate (165 μL, 1.25 mmol) were added to a solution of Boc-Lys(Boc)-OH·DCHA **4a** (600 mg 1.14 mmol) in THF (1.5 mL) at –15 ºC. The reaction mixture was then stirred at –15 ºC. After stirring for 10 min at this temperature, the mixture was filtration and the cake was washed with THF. $NaBH_4$ (65 mg, 1.71 mmol) in H_2O (1 mL) was added to a solution of the intermediate in THF at –15 ºC. After stirring for 10 min at this temperature, the mixture was added to 1 M HCl at –15 ºC and extracted with AcOEt. The extracts were washed with brine, dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was used in the next step without further purification. Et_3N (237 μL, 1.71 mmol) and MsCl (264 μL, 3.42 mmol) were added to a solution of above residue in CH_2Cl_2 (7.5 mL) at 0 ºC. After stirring for overnight at room temperature, the mixture was added H_2O at 0 ºC and extracted with $CHCl_3$. The extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography with $CHCl_3$/MeOH (100 : 1) to give **5a** (270 mg, 0.658 mmol, 2 steps 58%) as a white solid; $[\alpha]_D^{25} = -11.2$ (*c* 2.07, MeOH); m.p. 83.6-85.4 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 4.89-4.73 (br d, 1H), 4.64 (br s, 1H), 4.34-4.22 (m, 1H), 4.18 (dd, J = 10 and 4.2 Hz, 1H), 3.81 (br s, 1H), 3.12 (br s, 2H), 3.04 (s, 3H), 1.64-1.34 (m, 24H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.1, 155.4, 79.8, 79.1, 71.1, 49.6, 39.9, 37.3, 30.6, 29.7, 28.4 (3 carbons), 28.3 (3 carbons), 22.8; HRMS (ES+) calcd for $C_{17}H_{34}N_2O_7SNa$ $[M+Na]^+$ 433.1984 found 433.1991.

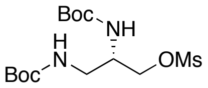
(*S*)-2,4-bis(*tert*-Butoxycarbonylamino)butylmethanesulfonate (5b)



5b was prepared in the same manner as described for compound **5a** using Boc-Dab(Boc)-OH **4b** (10.0 g, 31.4 mmol).

5b (7.97 g, 20.9 mmol, 2 steps 66%) was obtained as a colorless oil; $[\alpha]_D^{25}$ = –38.1 (*c* 1.97, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.07 (br s, 1H), 5.03-4.78 (br d, 1H), 4.30 (dd, *J* = 10 and 3.5 Hz, 1H), 4.23 (dd, *J* = 10 and 4.4 Hz, 1H), 4.01-3.88 (m, 1H), 3.38 (br s, 1H), 3.12-2.94 (m, 4H), 1.87-1.55 (m, 2H), 1.44 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 155.7, 80.1, 79.4, 71.4, 47.3, 37.3, 36.9, 31.9, 28.4 (3 carbons), 28.3 (3 carbons); HRMS (ES+) calcd for $C_{15}H_{30}N_2O_7SNa$ $[M+Na]^+$ 405.1671, found 405.1671.

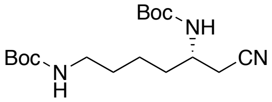
(*S*)-2,3-bis(*tert*-Butoxycarbonylamino)propylmethanesulfonate (5c)



5c was prepared in the same manner as described for compound **5a** using Boc-Dap(Boc)-OH **4c** (383 mg, 1.26 mmol).

5c (270 mg, 0.733 mmol, 2 steps 58%) was obtained as a white powder; $[\alpha]_D^{25}$ = –4.74 (*c* 0.27, MeOH); m.p. 90.8-92.1 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 5.46-5.26 (br d, 1H) 4.99 (br s, 1H), 4.34-4.17 (m, 2H), 4.02-3.83 (m, 1H), 3.33 (t, *J* = 6.1 Hz, 2H), 3.06 (s, 3H), 1.44 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.9, 155.6, 80.1 (2 carbons), 68.5, 50.6, 40.8, 37.4, 28.3 (6 carbons); HRMS (ES+) calcd for $C_{14}H_{28}N_2O_7SNa$ $[M+Na]^+$ 391.1515, found 391.1512.

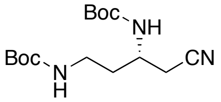
(*S*)-*tert*-Butyl 6-cyanohexane-1,5-diyldicarbamate (6a)



18–Crown–6–ether (860 mg, 3.25 mmol) and KCN (353 mg, 5.42 mmol) were added to a solution of **5a** (198 mg, 0.499 mmol) in DMF (14 mL) at room temperature. After stirring for 2 h at 100 ºC, the mixture was cooled to room temperature, a saturated aqueous $NaHCO_3$ solution was added at 0 ºC and extracted with AcOEt. The extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography with hexane/AcOEt (2 : 1) to give **6a** (475 mg, 1.39 mmol, 51%) as a white solid; $[\alpha]_D^{25}$ = –43.5 (*c* 0.53, $CHCl_3$); m.p. 79.0-79.8 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.93-4.77 (br d, 1H), 4.60 (br s, 1H), 3.89-3.68 (m, 1H), 3.23-3.03 (m, 2H), 2.72 (dd, *J* = 17 and 5.5 Hz, 1H), 2.56 (dd, *J* = 17 and 3.6 Hz, 1H), 1.83-1.32 (m, 24H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.2, 155.2, 117.4, 80.1, 79.2, 47.2, 39.8, 32.9, 29.8, 28.4 (3 carbons), 28.3

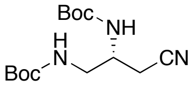
(3 carbons), 23.8, 22.8; HRMS (ES+) calcd for $C_{17}H_{31}N_3O_4Na$ $[M+Na]^+$ 364.2212, found 364.2216.

(*S*)-*tert*-Butyl 4-cyanobutane-1,3-diyldicarbamate (6b)



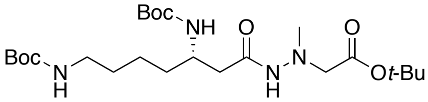
6b was prepared by stirring a solution of **5b** (7.97 g, 20.9 mmol) in acetonitrile (105 mL) for overnight at 40 ºC using the same reagents as described for compound **6a**.
6b (4.08 g, 13.0 mmol, 62%) was obtained as a white powder; $[\alpha]_D^{25} = -64.1$ (*c* 1.14, $CHCl_3$); m.p. 84.7-85.6 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 5.03-4.72 (m, 2H), 4.00-3.83 (m, 1H), 3.48-3.26 (m, 1H), 3.16-2.97 (m, 1H), 2.74 (dd, *J* = 17 and 5.4 Hz, 1H), 2.62 (dd, *J* = 17 and 4.6 Hz, 1H), 1.90-1.67 (m, 2H), 1.45 (s, 9H), 1.44 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 156.0, 155.3, 117.2, 80.4, 79.6, 45.0, 36.9, 34.3, 28.4 (3 carbons), 28.3 (3 carbons), 23.9; HRMS (ES+) calcd for $C_{15}H_{27}N_3O_4Na$ $[M+Na]^+$ 336.1899, found 336.1896.

(*S*)-*tert*-Butyl 3-cyanopropane-1,2-diyldicarbamate (6c)



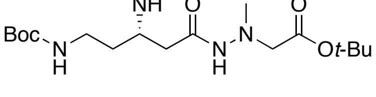
6c was prepared by stirring a solution of **5c** (205 mg, 0.557 mmol) in acetonitrile (3 mL) for 3 h under reflux using the same reagents as described for compound **6a**.
6c (100 mg, 0.334 mmol, 60%) was obtained as a white powder; $[\alpha]_D^{25} = -31.1$ (*c* 0.63, $CHCl_3$); m.p. 120.4-120.9 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 5.63-5.36 (br d, 1H), 4.94 (br s, 1H), 4.00-3.83 (m, 1H), 3.50-3.22 (m, 2H), 2.77-2.51 (m, 2H), 1.44 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.0, 155.4, 117.1, 80.4, 80.2, 48.8, 43.0, 28.31 (3 carbons), 28.29 (3 carbons), 21.2; HRMS (ES+) calcd for $C_{14}H_{25}N_3O_4Na$ $[M+Na]^+$ 322.1743, found 322.1738.

(*S*)-*tert*-Butyl2-{2-[3,7-bis(*tert*-butoxycarbonylamino)heptanoyl]-1-methylhydrazinyl}acetate (8a)



KOH (124 mg, 2.20 mmol) was added to a solution of **6a** (75.4 mg, 0.221 mmol) in EtOH/H_2O (2 : 1, 2 mL) at room temperature. After stirring for overnight at 80 ºC, the mixture was removed under reduced pressure. The resulting aqueous phase was acidified to pH 1 upon the addition of 1 M HCl at 0 ºC and extracted with AcOEt. The extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was used in the next step without further purification. PTSA·$H_2NN(Me)CH_2CO_2$*t*-Bu **7** (147 mg, 0.442 mmol) and HOBt·H_2O (67.7 mg, 0.442 mmol) were added to a solution containing above residue in DMF (2 mL) at room temperature. Et_3N (61.3 μL, 0.442 mmol) and EDC·HCl (84.7 mg, 0.442 mmol) were added to the mixture at 0 ºC. After stirring for overnight at room temperature, the mixture was poured into 10% citric acid aqueous solution and extracted with AcOEt. The extracts were washed with saturated aqueous $NaHCO_3$ solution, H_2O, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography with $CHCl_3$/MeOH (100 : 1) to give **8a** (42.0 mg, 83.6 μmol, 2 steps 38%) as a white solid; $[\alpha]_D^{25} = -12.4$ (*c* 0.56, $CHCl_3$); m.p. 86.5-87.3 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90 (s) and 7.44-7.31 (br d, total 1H), 5.49-5.17 (m, 1H), 4.75-4.52 (br s, 1H), 3.98-3.77 (m, 1H), 3.67-3.33 (m, 2H), 3.20-3.03 (m, 2H), 2.88-2.48 (m, 4H), 2.42-2.21 (m, 1H), 1.88-1.20 (m, 33H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 169.1, 156.1, 155.7, 82.4, 79.2, 79.0, 58.3, 47.7, 43.9, 40.3, 39.4, 34.4, 29.6, 28.4 (6 carbons), 28.2 (3 carbons), 23.3; HRMS (ES+) calcd for $C_{24}H_{46}N_4O_7Na$ $[M+Na]^+$ 525.3264, found 525.3265.

(*S*)-*tert*-Butyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methylhydrazinyl} acetate (8b)

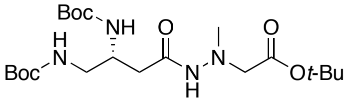


8b was prepared in the same manner as described for compound **8a** using **6b** (90.2 mg, 0.288 mmol).

8b (51.3 mg, 0.108 mmol, 2 steps 38%) was obtained as a white powder; $[\alpha]_D^{25} = -40.1$ (*c* 0.52, $CHCl_3$); m.p. 108.9-110.8 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.94 (s) and 7.47-7.35 (br d, total 1H), 5.83-5.49 (m, 1H), 5.49-5.11 (m, 1H), 4.15-3.80 (m, 1H), 3.69-3.46 (m, 2H), 3.46-3.20 (m, 1H), 3.05-2.81 (m, 1H), 2.81-2.48 (m, 4H), 2.48-2.18

(m, 1H), 1.75-1.18 (m, 29H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 170.2, 169.1, 156.1, 82.4, 79.3, 79.0, 58.2, 45.1, 43.9, 38.9, 37.1, 35.2, 28.44 (3 carbons), 28.36 (3 carbons), 28.2 (3 carbons); HRMS (ES+) calcd for $C_{22}H_{42}N_4O_7Na$ $[M+Na]^+$ 497.2951, found 497.2959.

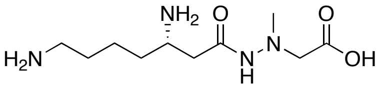
(*S*)-*tert*-Butyl 2-{2-[3,4-bis(*tert*-butoxycarbonylamino)butanoyl]-1-methylhydrazinyl} acetate (8c)



8c was prepared in the same manner as described for compound **8a** using **6c** (76.9 mg, 0.257 mmol).

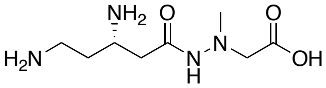
8c (46.0 mg, 0.100 mmol, 2 steps 39%) was obtained as a white powder; $[\alpha]_D^{25}$ = –5.90 (*c* 0.62, $CHCl_3$); m.p. 84.7-85.9 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.11 (s) and 7.33 (s, total 1H); 5.78-5.36 (m, 1H), 5.08 (br s, 1H), 4.08-3.83 (m, 1H), 3.63-3.49 (m, 2H), 3.45-3.19 (m, 2H), 3.09- 2.50 (m, 4H), 2.34 (d, *J* = 5.1 Hz, 1H), 1.57-1.35 (m, 27H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 168.9, 156.6, 155.8, 82.3, 79.5, 79.3, 58.8, 48.6, 45.1, 43.9, 36.7, 28.4 (6 carbons), 28.2 (3 carbons); HRMS (ES+) calcd for $C_{21}H_{40}N_4O_7Na$ $[M+Na]^+$ 483.2795, found 483.2792.

(*S*)-2-[2-(3,7-Diaminoheptanoyl)-1-methylhydrazinyl]acetic acid·2TFA (9a)



4 M HCl/dioxane (2 mL) was added to **8a** (22.0 mg, 43.8 μmol) at 0 ºC. After stirring for 1 h at room temperature, the mixture was removed under reduced pressure. The residue was purified by preparative HPLC (gradient: milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 100 : 0 to milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 95 : 5 over 40 min, flow late 5 mL/min, UV: 222 nm) to give **9a** (14.4 mg, 30.4 μmol, 69%) as TFA salts; m.p. 66.0-67.0 ºC; ^{1}H NMR (400 MHz, D_2O) δ 3.66-3.54 (m, 3H), 3.01 (t, *J* = 7.6 Hz, 2H), 2.68 (s, 3H), 2.60 (dd, *J* = 16 and 5.6 Hz, 1H), 2.51 (dd, *J* = 16 and 7.0 Hz, 1H), 1.81-1.63 (m, 4H), 1.57-1.40 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 173.3, 169.4, 58.6, 48.4, 44.1, 39.0, 35.0, 31.3, 26.3, 21.6; HRMS (ES+) calcd for $C_{10}H_{23}N_4O_3$ $[M+H]^+$ 247.1770, found 247.1770.

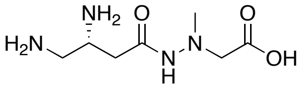
(*S*)-2-[2-(3,5-Diaminopentanoyl)-1-methylhydrazinyl]acetic acid·2TFA (9b, TCP-112)



9b was prepared in the same manner as described for compound **9a** using **8b** (29.3 mg, 61.8 μmol).

9b (14.6 mg, 32.6 μmol, 53%) was obtained as a colorless solid; $[\alpha]_D^{25} = +8.21$ (*c* 1.22, H_2O); m.p. 127.9-129.0 ºC; ^1H NMR (400 MHz, D_2O) δ 3.81-3.72 (m, 1H), 3.71 (s, 2H), 3.18-3.08 (m, 2H), 2.75-2.57 (m, 5H), 2.16-2.00 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 172.3, 168.9, 58.3, 46.1, 44.4, 35.7, 34.6, 29.7; HRMS (ES+) calcd for $C_8H_{19}N_4O_3$ $[M+H]^+$ 219.1457, found 219.1455.

(*S*)-2-[2-(3,4-Diaminobutanoyl)-1-methylhydrazinyl]acetic acid·2TFA (9c)

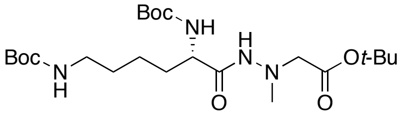


9c was prepared in the same manner as described for compound **9a** using **8c** (25.4 mg, 55.2 μmol).

9c (12.4 mg, 28.6 mg, 52%) was obtained as a colorless solid; m.p. 108.1-108.7 ºC; ^1H NMR (400 MHz, D_2O) δ 4.00 (q, *J* = 6.2 Hz, 1H), 3.64 (s, 2H), 3.48-3.33 (m, 2H), 2.85-2.62 (m, 5H); ^{13}C NMR (100 MHz, D_2O) δ 173.3, 168.1, 58.5, 46.1, 44.1, 40.6, 33.4; HRMS (ES+) calcd for $C_7H_{17}N_4O_3$ $[M+H]^+$ 205.1301, found 205.1303.

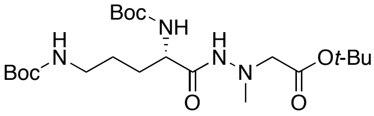
3. Synthesis of derivatives 12a-d

(*S*)-*tert*-Butyl 2-[1-methyl-2-(2,2,14,14-tetramethyl-4,12-dioxo-3,13-dioxa-5,11-diazapentadecancarbonyl)hydrazinyl]acetate (11a)



PTSA·$H_2NN(Me)CH_2CO_2$*t*-Bu **7** (62.8 mg, 0.189 mmol) and HOBt·H_2O (29.0 mg, 0.189 mmol) were added to a solution of Boc-Lys(Boc)-OH·DCHA **4a** (50.0 mg, 94.7 μmol) in DMF (2 mL). Et_3N (26.2 μL, 0.189 mmol) and EDC· HCl (36.2 mg, 0.189 mmol) were added to the mixture at 0 ºC. After stirring for overnight at room temperature, the mixture was poured into 10% citric acid aqueous solution and extracted with AcOEt. The extracts were washed with saturated aqueous $NaHCO_3$ solution, H_2O and brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography with $CHCl_3$/MeOH (100 : 1) to give **11a** (45.1 mg, 92.3 μmol, 98%) as a colorless oil; $[\alpha]_D^{25} = -3.08$ (*c* 0.50, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.16 (s) and 7.54 (br d, *J* = 24 Hz, total 1H), 5.32-5.06 (m, 1H), 4.90-4.52 (m, 1H), 4.08-3.86 (m, 1H), 3.78-3.33 (m, 2H), 3.26-3.03 (m, 2H), 2.75 (s, 3H), 1.90-1.20 (m, 33H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.1, 169.3, 156.1, 155.6, 82.4, 80.0, 79.5, 58.4, 53.3, 43.9, 40.1, 32.3, 29.7, 28.46 (3 carbons), 28.41 (3 carbons), 28.1 (3 carbons), 22.5; HRMS (ES+) calcd for $C_{23}H_{45}N_4O_7$ $[M+H]^+$ 489.3288, found 489.3278.

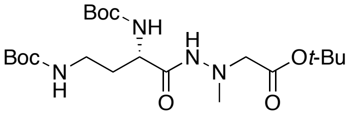
(*S*)-*tert*-Butyl 2-[1-methyl-2-(2,2,13,13-tetramethyl-4,11-dioxo-3,12-dioxa-5,10-diazatetradecanecarbonyl)hydrazinyl]acetate (11b)



11b was prepared in the same manner as described for compound **11a** using Boc-Orn(Boc)-OH **10** (100 mg, 0.303 mmol).

11b (131 mg, 0.277 mmol, 91%) was obtained as a colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.28 (s) and 7.63 (d, *J* = 25 Hz, total 1H), 5.38-5.18 (m, 1H), 4.79 (br s, 1H), 4.13-3.95 (m, 1H), 3.77-3.38 (m, 2H), 3.29-3.10 (m, 2H), 2.75 (s, 3H), 1.89-1.34 (m, 31H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 169.1, 156.0, 155.4, 82.2, 79.5, 79.0, 58.4, 52.8, 43.8, 39.8, 29.9, 28.3 (3 carbons), 28.2 (3 carbons), 28.1 (3 carbons), 26.1; HRMS (ES+) calcd for $C_{22}H_{43}N_4O_7$ $[M+H]^+$ 475.3132, found 475.3127.

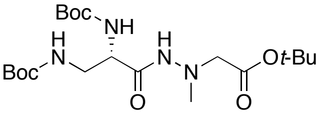
(*S*)-*tert*-Butyl 2-[1-methyl-2-(2,2,12,12-tetramethyl-4,10-dioxo-3,11-dioxa-5,9-diazatridecanecarbonyl)hydrazinyl]acetate (11c)



11c was prepared in the same manner as described for compound **11a** using **4c** (60.6 mg, 0.190 mmol).

11c (59.4 mg, 0.129 mmol, 68%) was obtained as a colorless oil; $[\alpha]_D^{25}$ = –20.4 (*c* 0.63, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.30 (s) and 7.69-7.48 (m, total 1H), 5.46-5.26 (m, 1H), 5.13 (br s) and 4.90-4.72 (m, total 1H), 4.15-4.00 (m, 1H) and 3.78-3.50 (m, 2H), 3.48-3.29 (m, 1H), 3.11-2.92 (m, 1H), 2.76 (s, 3H), 2.06-1.68 (m, 2H), 1.68-1.19 (m, 27H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 169.8, 156.1, 155.7, 82.4, 80.1, 79.7, 58.4, 50.8, 43.4, 36.8, 34.0, 28.4 (3 carbons), 28.3 (3 carbons), 28.2 (3 carbons); HRMS (ES+) calcd for $C_{21}H_{41}N_4O_7$ $[M+H]^+$ 461.2975, found 461.2978.

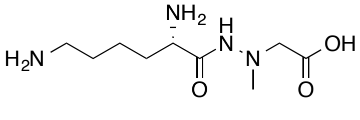
(*S*)-*tert*-Butyl 2-[1-methyl-2-(2,2,11,11-tetramethyl-4,9,dioxo-3,10-dioxa-5,8-diazadodecanecarbonyl)hydrazinyl]acetate (11d)



11d was prepared in the same manner as described for compound **6a** using **4c** (172 mg, 0.565 mmol).

11d (158 mg, 0.353 mmol, 62%) was obtained as a white solid; $[\alpha]_D^{25}$ = –12.4 (*c* 0.38, $CHCl_3$); m.p. 64.6-65.9 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.55 (s) and 7.73-7.30 (m, total 1H), 5.82 (br s, 1H), 5.19 (br s, 1H), 4.16 (br s, 1H), 3.56 (d, *J* = 5.2 Hz, 2H), 3.55-3.39 (m, 2H), 2.75 (s, 3H), 1.49 (s, 9H), 1.46-1.33 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.8, 168.4, 157.0, 155.8, 82.3, 80.1, 79.8, 58.4, 54.7, 43.9, 42.3, 28.28 (3 carbons), 28.25 (3 carbons), 28.1 (3 carbons); HRMS (ES+) calcd for $C_{20}H_{39}N_4O_7$ $[M+H]^+$ 447.2819, found 447.2827.

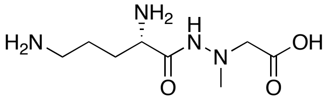
(*S*)-2-[2-(2,6-Diaminohexanoyl)-1-methylhydrazinyl]acetic acid·2TFA (12a)



4 M HCl/dioxane (2 mL) was added to **11a** (26.7 mg, 54.6 μmol) at 0 ºC. After stirring for 1 h at room temperature, the mixture was removed under reduced pressure. The residue was purified by preparative HPLC (gradient: milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 100 : 0 to milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 95 : 5 over 40 min, flow late 5 mL/min, UV: 222 nm) to give **9a** (17.4 mg, 37.8 μmol, 69%) as TFA salts; m.p. 71.1-72.5 ºC; ^{1}H NMR (400 MHz, D_2O) δ 3.87 (t, *J* = 6.7 Hz,

1H), 3.65 (q, *J* = 17 Hz, 2H), 3.00 (t, *J* = 7.7 Hz, 2H), 2.70 (s, 3H), 2.00-1.82 (m, 2H), 1.76-1.65 (m, 2H), 1.41 (q, *J* = 7.7 Hz, 2H); ^{13}C NMR (100 MHz, D_2O) δ 173.2, 167.4, 58.6, 51.8, 44.2, 38.9, 30.2, 26.3, 21.2; HRMS (ES+) calcd for $C_9H_{21}N_4O_3$ $[M+H]^+$ 233.1614, found 233.1613.

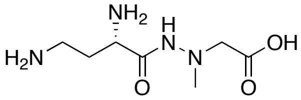
(*S*)-2-[2-(2,4-Diaminopentanoyl)-1-methylhydrazinyl]acetic acid·2TFA (12b)



12b was prepared in the same manner as described for compound **12a** using **11b** (86.8 mg, 0.183 mmol).

12b (36.4 mg, 81.7 μmol, 45%) was obtained as a light yellow solid; m.p. 51.6-52.5 ºC; ^{1}H NMR (400 MHz, D_2O) δ 3.92 (t, *J* = 6.7 Hz, 1H), 3.78-3.62 (m, 2H), 3.02 (t, *J* = 7.6 Hz, 2H), 2.70 (s, 3H), 2.03-1.86 (m, 2H), 1.78-1.63 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 172.5, 167.0, 58.2, 51.5, 44.4, 38.7, 27.7, 22.3; HRMS (ES+) calcd for $C_8H_{19}N_4O_3$ $[M+H]^+$ 219.1457, found 219.1456.

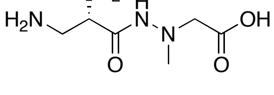
(*S*)-2-[2-(2,4-Diaminobutanoyl)-1-methylhydrazinyl]acetic acid·2TFA (12c)



12c was prepared in the same manner as described for compound **12a** using **11c** (29.9 mg, 64.9 μmol).

12c (12.7 mg, 29.3 μmol, 45%) was obtained as a yellow solid; m.p. 71.7-72.4 ºC; ^{1}H NMR (400 MHz, D_2O) δ 4.00 (t, *J* = 6.7 Hz, 1H), 3.78-3.61 (m, 2H), 3.17-3.02 (m, 2H), 2.71 (s, 3H), 2.34-2.20 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 172.8, 166.3, 58.2, 49.7, 44.3, 35.1, 28.3; HRMS (ES+) calcd for $C_7H_{17}N_4O_3$ $[M+H]^+$ 205.1301, found 205.1300.

(*S*)-2-[2-(2,3-Diaminopropanoyl)-1-methylhydrazinyl]acetic acid·2TFA (12d)

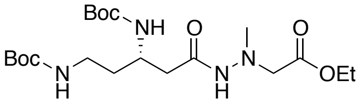


12d was prepared in the same manner as described for compound **11a** using **11d** (24.4 mg, 53.0 μmol).

12d (4.83 mg, 11.6 μmol, 22%) was obtained as a yellow solid; m.p. 113.2-115.1 ºC; ^{1}H NMR (400 MHz, D_2O) δ 4.28 (t, *J* = 6.0 Hz, 1H), 3.83-3.63 (m, 2H), 3.63-3.43 (m, 2H), 2.73 (s, 3H); ^{13}C NMR (100 MHz, D_2O) δ 175.6, 167.2, 60.8, 52.6, 47.0, 42.3; HRMS (ES+) calcd for $C_6H_{15}N_4O_3$ $[M+H]^+$ 191.1144, found 191.1143.

4. Synthesis of derivatives 15a-d

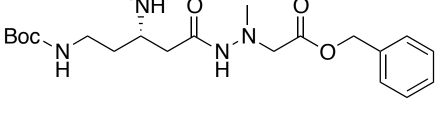
(*S*)-Ethyl 2-{2-[3,5-bis(*tert*-Butoxycarbonylamino)pentanoyl]-1-methylhydrazinyl}acetate (14a)



14a was prepared in the same manner as described for compound **8b** using **6b** (105 mg, 0.335 mmol) and $H_2NN(Me)CH_2CO_2Et$ **13a** (0.670 mmol).

14a (46.5 mg, 0.104 mmol, 2 steps 31%) was obtained as a colorless solid; $[\alpha]_D^{25}$ = –44.6 (*c* 1.01, $CHCl_3$); m.p. 101.4-102.0 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s) and 7.28 (br d, total 1H), 5.77 (m, 1H), 5.44-5.20 (m, 1H), 4.21 (q, *J* = 7.2 Hz, 2H), 4.08-3.88 (m, 1H), 3.75-3.46 (m, 2H), 3.38 (br s, 1H), 2.92 (br s 1H), 2.88-2.49 (m, 2H), 2.48-2.39 (m) and 2.24 (dd, *J* = 15 and 5.8 Hz, total 1H), 1.75-1.55 (m, 2H), 1.44 (s, 18H), 1.32-1.23 (m, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 169.7, 156.14, 156.04, 79.4, 79.1, 60.1, 57.7, 45.4, 44.1, 38.9, 37.2, 35.1, 28.5 (3 carbons), 28.4 (3 carbons), 14.2; HRMS (ES+) calcd for $C_{20}H_{39}N_4O_7$ $[M+H]^+$ 447.2819, found 447.2812.

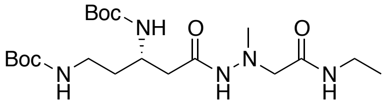
(*S*)-Benzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methylhydrazinyl} acetate (14b)



14b was prepared in the same manner as described for compound **8b** using **6b** (101 mg, 0.322 mmol) and $H_2NN(Me)CH_2CO_2Bn$ **13b** (87.3 mg, 0.450 mmol).

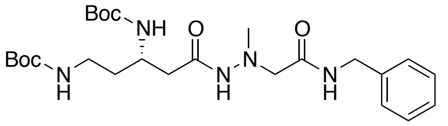
14b (64.9 mg, 0.127 mmol, 2 steps 57%) was obtained as a white solid; $[\alpha]_D^{25}$ = –34.5 (*c* 2.17, $CHCl_3$); m.p. 93.1-95.0 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.77 (s) and 7.42-7.27 (m, total 6H), 5.72-5.54 (m, 1H), 5.42-5.09 (m, 3H), 4.04-3.86 (m, 1H), 3.81-3.49 (m, 2H), 3.37 (br s, 1H), 3.04-2.50 (m, 5H), 2.49-2.35 (m) and 2.29-2.17 (m, total 1H), 1.78-1.57 (m, 2H), 1.43 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.5, 169.5, 156.2, 156.0, 135.1, 128.7 (2 carbons), 128.6, 128.5 (2 carbons), 79.3, 79.0, 66.7, 57.7, 45,4, 44.1, 38.9, 37.2, 34.8, 28.5 (3 carbons), 28.4 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{41}N_4O_7$ $[M+H]^+$ 509.2975, found 509.2981.

(*S*)-di-*tert*-Butyl {5-[2-(2-ethylamino-2-oxoethyl)-2-methylhydrazinyl]-5-oxopentane-1,3-diyl}dicarbamate (14c)



10 % Pd/C (7.5 mg) was added to a solution of **14b** (75 mg, 0.148 mmol) in MeOH (2 mL) at room temperature. The resulting mixture was subjected to three cycles of vacuum followed by flush with H_2 before stirring for 15 min under an atmosphere of H_2. The mixture was filtered through a pad of Celite® with MeOH, and the resulting filtrate was concentrated under reduced pressure. The residue was used in the next step without further purification. Ethylamine·HCl (24.1 mg, 0.296 mmol) and HOBt·H_2O (45.3 mg, 0.296 mmol) were added to a solution containing above residue in DMF (2 mL) at room temperature. Et_3N (41.0 μL, 0.296 mmol) and EDC·HCl (56.7 mg, 0.296 mmol) were added to the mixture at 0 ºC. After stirring for overnight at room temperature, the mixture was poured into 10% citric acid aqueous solution and extracted with AcOEt. The extracts were washed with saturated aqueous $NaHCO_3$ solution, H_2O and brine and dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography with $CHCl_3$/MeOH (100 : 1) to give **14c** (46.0 mg, 0.103 mmol, 2 steps 70%) as a white solid. $[\alpha]_D^{25}$ = –16.4 (*c* 1.12, $CHCl_3$); m.p. 123.0-124.0 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.90 (br s, 1H), 5.53 (br s, 1H), 5.22 (br, s, 1H), 4.06-3.82 (m, 1H), 3.55-3.14 (m, 5H), 2.96 (br s, 1H), 2.68 (s, 3H), 2.52-2.27 (m, 2H), 2.04 (s, 1H), 1.70-1.56 (m, 2H), 1.43 (s, 18H), 1.14 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 168.9, 156.3, 156.0, 79.6, 79.3, 62.6, 46.7, 45.6, 38.8, 37.1, 35.2, 34.1, 28.5 (3 carbons), 28.4 (3 carbons), 14.5; HRMS (ES+) calcd for $C_{20}H_{39}N_5O_6Na$ $[M+Na]^+$ 468.2798 found 468.2799.

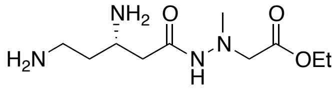
(*S*)-di-*tert*-Butyl {5-[2-(2-benzylamino-2-oxoethyl)-2-methylhydrazinyl]-5-oxopentane-1,3-diyl}dicarbamate (14d)



14d was prepared in the same manner as described for compound **8b** using **6b** (98.0 mg, 0.313 mmol) and $H_2NN(Me)CH_2CONHBn$ **13c** (97.8 mg, 0.506 mmol).

14d (41.3 mg, 81.3 μmol, 2 steps 26%) was obtained as a white solid; $[\alpha]_D^{25} = -22.4$ (*c* 0.62, $CHCl_3$); m.p. 168.5-169.7 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.35-8.21 (m, 1H), 7.69 (s, 1H), 7.39-7.20 (m, 5H), 5.38 (br d, 1H), 5.15 (br s, 1H), 4.53-4.38 (m, 2H), 3.89-3.76 (m, 1H), 3.38 (s, 2H), 3.36-3.14 (m, 1H), 3.01-2.94 (m, 1H), 2.64 (s, 3H), 2.31 (dd, *J* = 16 and 5.1 Hz) and 2.21 (dd, *J* = 14 and 6.3 Hz, total 2H), 1.78-1.50 (m, 2H), 1.44 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.9, 168.9, 156.3, 155.9, 138.3, 128.6 (2 carbons), 128.0 (2 carbons), 127.3, 79.6, 79.4, 62.6, 46.6, 45.6, 43.1, 38.6, 37.2, 34.9, 28.5 (3 carbons), 28.4 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{42}N_5O_6$ $[M+H]^+$ 508.3135, found 508.3116.

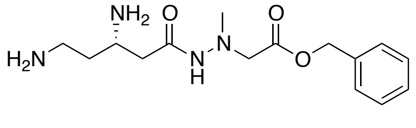
(*S*)-Ethyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (15a)



15a was prepared in the same manner as described for compound **8b** using **14a** (29.1 mg, 65.2 mmol). The residue was purified by preparative HPLC (gradient: milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 95 : 5 to milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 90 : 10 over 40 min, flow late 5 mL/min, UV: 222 nm).

15a (11.0 mg, 23.3 μmol, 36%) was obtained as a colorless solid; $[\alpha]_D^{25} = +8.44$ (*c* 0.43, H_2O); m.p. 72.3-73.8 ºC; ^1H NMR (300 MHz, D_2O) δ 4.17 (q, *J* = 7.2 Hz, 2H), 3.74-3.70 (m, 1H), 3.61 (s, 2H), 3.10 (t, *J* = 8.2 Hz, 2H), 2.67-2.56 (m, 5H), 2.11-2.01 (m, 2H), 1.23 (t, *J* = 7.1 Hz, 3H); HRMS (ES+) calcd for $C_{10}H_{23}N_4O_3$ $[M+H]^+$ 247.1770, found 247.1764.

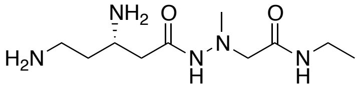
(*S*)-Benzyl 2-[2-(3,5-diaminohexanoyl)-1-methylhydrazinyl]acetate·2TFA (15b)



15b was prepared in the same manner as described for compound **8b** using **14b** (64.9 mg, 0.128 mmol). The residue was purified by preparative HPLC (gradient: milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 85 : 15 to milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 55 : 45 over 40 min, flow late 5 mL/min, UV: 222 nm).

15b (34.6 mg, 64.5 mmol, 50%) was obtained as a colorless solid; $[\alpha]_D^{25}$ = +5.80 (*c* 1.15, H_2O); m.p. 65.5-66.3 ºC; ^{1}H NMR (400 MHz, D_2O) δ 7.44-7.39 (m, 5H), 5.20 (s, 2H), 3.68-3.60 (m, 3H), 3.07 (t, *J* = 7.2 Hz, 2H), 2.63 (s, 3H), 2.59-2.44 (m, 2H), 2.14-1.98 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 173.6, 171.8, 138.2, 131.7 (2 carbons), 131.6, 131.3 (2 carbons), 70.1, 61.3, 48.9, 47.2, 38.5, 37.6, 32.7; HRMS (ES+) calcd for $C_{15}H_{25}N_4O_3$ $[M+H]^+$ 309.1927, found 309.1915.

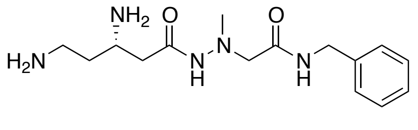
(*S*)-2-(2-(3,5-Diaminopentanoyl)-1-methylhydrazinyl)-*N*-ethylacetamide·2TFA (15c)



15c was prepared in the same manner as described for compound **15b** using **14c** (21.3 mg, 47.8 μmol). The residue was purified by preparative HPLC (gradient: milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 95 : 5 to milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 85 : 15 over 40 min, flow late 5 mL/min, UV: 222 nm).

15c (19.6 mg, 41.4 μmol, 87%) was obtained as a white solid; $[\alpha]_D^{25}$ = +20.4 (*c* 0.38, H_2O); m.p. 133.7-134.4 ºC; ^{1}H NMR (400 MHz, D_2O) δ 3.80-3.69 (m, 1H), 3.45 (s, 2H), 3.22 (q, *J* = 7.3 Hz, 2H), 3.17-3.03 (m, 2H), 2.70-2.54 (m, 4H), 2.18-1.98 (m, 2H), 1.10 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (100 MHz, D_2O) δ 174.0, 172.4, 64.3, 49.1, 48.0, 38.6, 37.6, 37.3, 32.8, 16.4; HRMS (ES+) calcd for $C_{10}H_{24}N_5O_2$ $[M+H]^+$ 246.1930, found 246.1928.

(*S*)-*N*-Benzyl-2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetamide·2TFA (15d)

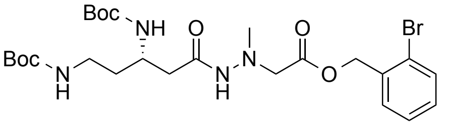


15d was prepared in the same manner as described for compound **15b** using **14c** (34.7 mg, 68.3 μmol).

15d (14.8 mg, 27.6 μmol, 40%) was obtained as a white solid; $[\alpha]_D^{25}$ = +0.90 (*c* 0.49, H_2O); m.p. 77.3-78.2 ºC; ^1H NMR (400 MHz, D_2O) δ 7.43-7.22 (m, 5H), 4.41 (q, *J* = 15 Hz, 2H), 3.67-3.25 (m, 3H), 3.11-2.98 (m, 2H), 2.62 (s, 3H), 2.67-2.40 (m, 2H), 2.08-1.89 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 174.1, 172.4, 140.7, 131.7 (2 carbons), 131.7 (2 carbons), 130.5, 64.0, 48.9, 48.0, 45.7, 38.6, 37.8, 32.9; HRMS (ES+) calcd for $C_{15}H_{26}N_5O_2$ $[M+H]^+$ 308.2085, found 308.2087.

5. Synthesis of derivatives 17a-l

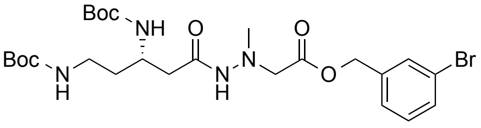
(*S*)-2-Bromobenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16a)



10 % Pd/C (10.3 mg) was added to a solution of **14b** (103 mg, 0.203 mmol) in MeOH (2 mL) at room temperature. The resulting mixture was subjected to three cycles of vacuum followed by flush with H_2 before stirring for 15 min under an atmosphere of H_2. The mixture was filtered through a pad of Celite® with MeOH, and the resulting filtrate was concentrated under reduced pressure. The residue was used in the next step without further purification. *o*-Bromobenzyl alcohol (45.6 mg, 0.244 mmol) and DMAP (2.48 mg, 20.3 μmol) were added to a solution containing above residue in DMF (2 mL) at room temperature. DCC (46.0 mg, 0.223 mmol) was added to the mixture at 0 ºC. After stirring for overnight at room temperature, the mixture was concentrated under reduced pressure. The residue was filtered with $CHCl_3$. The organic layer was washed with H_2O, brine, dried over Na_2SO_4, filtered and concentrated under reduced pressure. The residue was purified by silica gel flash column chromatography with $CHCl_3$/MeOH (100 : 1) to give **16a** (56.1 mg, 95.5 μmol, 2 steps 47%) as a white solid; $[\alpha]_D^{25}$ = –30.7 (*c* 1.11, $CHCl_3$); m.p. 122.0-123.4 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 (s) and 7.44-7.28 (m, total 3H), 7.60 (d, *J* = 8.0 Hz, 1H), 7.25-7.20 (m, 1H), 5.74-5.57 (m, 1H), 5.40-5.20 (m, 3H), 4.06-3.89 (m, 1H), 3.83-3.56 (m, 2H), 3.37 (br s, 1H), 3.00-2.89 (m, 1H), 2.83-2.72 (m) and 2.63-2.50 (m, total 4H), 2.46-2.38 (m) and 2.23 (dd, *J* = 15 and 5.8 Hz, total 1H), 1.74-1.57 (m, 2H), 1.44 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 169.3, 156.1, 134.4, 133.0, 130.5, 130.3, 130.2, 127.7, 123.7, 79.3, 79.0, 66.3, 57.7,

45.4, 44.1, 38.9, 37.1, 35.0, 28.5 (3 carbons), 28.4 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{40}N_4O_7Br$ $[M+H]^+$ 587.2080, found 587.2092.

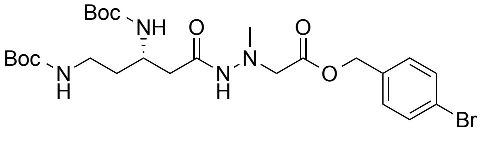
(*S*)-3-Bromobenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16b)



16b was prepared in the same manner as described for compound **16a** using **14b** (108 mg, 0.212 mmol) and *m*-bromobenzyl alcohol (47.5 mg, 0.254 mmol).

16b (84.6 mg, 0.144 mmol, 2 steps 68%) was obtained as a white solid; $[\alpha]_D^{25}$ = –27.7 (*c* 1.24, $CHCl_3$); m.p. 82.5-83.6 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.82 (s) and 7.34 (br d, total 1H), 7.57-7.44 (m, 2H), 7.32-7.20 (m, 3H), 5.73-5.57 (m, 1H), 5.41-5.20 (m, 1H), 5.14 (s, 2H), 4.06-3.88 (m, 1H), 3.82-3.47 (m, 2H), 3.36 (br s, 1H), 3.04-2.88 (m, 1H), 2.87-2.49 (m, 3H), 2.48-2.38 (m) and 2.24 (dd, *J* = 15 and 5.7 Hz, total 1H), 1.86-1.57 (m, 2H), 1.43 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.8, 169.3, 156.0, 137.2, 131.7, 131.4, 130.3, 126.9, 122.6, 79.3, 79.0, 65.7, 58.9, 57.8, 45.5, 44.1, 38.9, 37.1, 35.0, 28.4 (3 carbons), 28.3 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{40}N_4O_7Br$ $[M+H]^+$ 587.2080, found 587.2080.

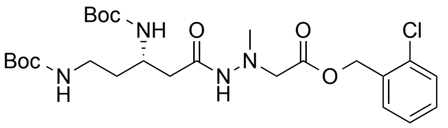
(*S*)-4-Bromobenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16c)



16c was prepared in the same manner as described for compound **16a** using **14b** (120 mg, 0.236 mmol) and *p*-bromobenzyl alcohol (53.0 mg, 0.283 mmol).

16c (71.6 mg, 0.122 mmol, 2 steps 52%)was obtained as a white solid; $[\alpha]_D^{25}$ = –31.7 (*c* 1.10, $CHCl_3$); m.p. 121.7-122.4 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.77 (s) and 7.28 (br s, total 1H), 7.51 (d, *J* = 8.3 Hz, 2H), 7.25-7.21 (m, 2H), 5.71-5.52 (m, 1H), 5.40-5.18 (m, 1H), 5.12 (s, 2H), 4.07-3.85 (m, 1H), 3.69-3.50 (m, 2H), 3.36 (br s, 1H), 3.00-2.86 (m, 1H), 2.68-2.48 (m, 4H), 2.45-2.38 (m) and 2.23 (dd, *J* = 15 and 5.8 Hz, total 1H), 1.74-1.57 (m, 2H), 1.43 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 169.4, 156.1, 134.0, 131.9 (2 carbons), 130.2 (2 carbons), 122.7, 79.3, 79.0, 65.9, 58.9, 57.7, 45.4, 44.1, 38.9, 37.1, 35.0, 28.5 (3 carbons), 28.4 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{40}N_4O_7Br$ $[M+H]^+$ 587.2080, found 587.2078.

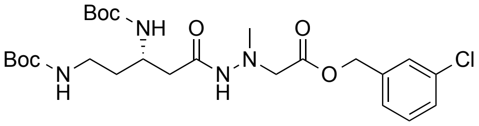
(*S*)-2-Chlorobenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16d)



16d was prepared in the same manner as described for compound **16a** using **14b** (98.0 mg, 0.193 mmol) and *o*-chlorobenzyl alcohol (32.9 mg, 0.232 mmol).

16d (37.2 mg, 0.069 mmol, 2 steps 36%) was obtained as a white solid; $[\alpha]_D^{25}$ = –38.0 (*c* 1.00, $CHCl_3$); m.p. 90.3-91.3 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.88 (s, 1H), 7.46-7.25 (m, 4H), 5.74-5.56 (m, 1H), 5.46-5.20 (m, 3H), 4.06-3.88 (m, 1H), 3.82-3.45 (m, 2H), 3.37 (br s, 1H), 3.02-2.48 (m, 5H), 2.42-2.36 (m) and 2.24 (dd, *J* = 15 and 6.0 Hz, total 1H), 1.79-1.54 (m, 2H), 1.43 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 169.5, 156.1, 156.0, 133.9, 132.7, 130.4, 130.0, 129.7, 127.1, 79.2, 78.9, 64.0, 57.7, 45.4, 44.0, 38.9, 37.2, 34.8, 28.45 (3 carbons), 28.36 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{39}N_4O_7NaCl$ $[M+Na]^+$ 565.2405, found 265.2393.

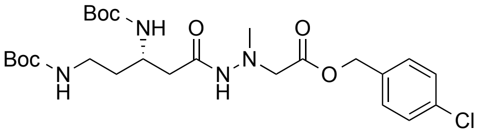
(*S*)-3-Chlorobenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16e)



16e was prepared in the same manner as described for compound **16a** using **14b** (100 mg, 0.197 mmol) and *m*-chlorobenzyl alcohol (34.4 mg, 0.242 mmol).

16e (24.4 mg, 0.045 mmol, 2 steps 23%) was obtained as a white sold; $[\alpha]_D^{25}$ = –19.3 (*c* 1.00, $CHCl_3$); m.p. 95.9-96.3 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.78 (s) and 7.38-7.18 (m, total 5H), 5.71-5.51 (m, 1H), 5.40-5.06 (m, 3H), 4.06-3.86 (m, 1H), 3.81-3.51 (m, 2H), 3.42-3.28 (m, 1H), 3.02-2.86 (m, 1H), 2.86-2.46 (m, 4H), 2.46-2.36 (m) and 2.24 (dd, *J* = 15 and 5.9 Hz, total 1H), 1.75-1.52 (m, 2H), 1.52-1.30 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.3, 169.4, 156.2 (2 carbons), 137.1, 134.6, 130.0, 128.8, 128.5, 126.4, 79.3, 79.0, 65.7, 57.7, 45.5, 44.1, 38.9, 37.1, 34.9, 28.5 (3 carbons), 28.4 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{39}N_4O_7NaCl$ $[M+Na]^+$ 565.2405, found 565.2405.

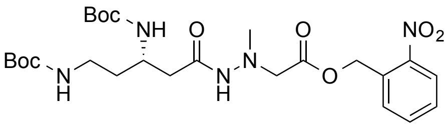
(*S*)-4-Chlorobenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16f)



16f was prepared in the same manner as described for compound **16a** using **14b** (100 mg, 0.197 mmol) and *p*-chlorobenzyl alcohol (35.5 mg, 0.250 mmol).

16f (42.0 mg, 0.077 mmol, 2 steps 39%) was obtained as a white solid; $[\alpha]_D^{25}$ = –30.6 (*c* 0.85, $CHCl_3$); m.p. 102.6-103.9 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 7.76 (s), 7.42-7.22 (m, total 5H), 5.72-5.51 (m, 1H), 5.42-5.06 (m, 3H), 4.06-3.86 (m, 1H), 3.79-3.50 (m, 2H), 3.46-3.28 (m, 1H), 3.03-2.85 (m, 1H), 2.85-2.46 (m, 4H), 2.46-2.34 (m) and 2.23 (dd, *J* = 15 and 5.8 Hz, total 1H), 1.75-1.54 (m, 2H), 1.54-1.28 (m, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.4, 169.4, 156.2 (2 carbons), 134,6, 133.6, 129.9 (2 carbons), 128.9 (2 carbons), 79.3, 79.0, 65.9, 57.7, 45.5, 44.1, 38.9, 37.2, 34.8, 28.5 (3 carbons), 28.4 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{40}N_4O_7Cl$ $[M+H]^+$ 543.2586, found 543.2590.

(*S*)-2-Nitrobenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16g)

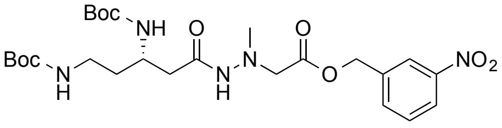


16g was prepared in the same manner as described for compound **16a** using **14b** (80.6 mg, 0.158 mmol) and *o*-nitrobenzyl alcohol (35.2 mg, 0.230 mmol).

16g (28.3 mg, 51.1 μmol, 2 steps 27%) was obtained as a white solid; $[\alpha]_D^{25}$ = –33.0 (*c* 1.40, $CHCl_3$); m.p. 124.2-125.8 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.13 (d, *J* = 8.1 Hz, 1H), 7.79-7.63 (m) and 7.26 (br s, total 2H), 7.62-7.50 (m, 2H), 5.77-5.50 (m, 3H), 5.40-5.19 (m, 1H), 4.09-3.88 (m, 1H), 3.87-3.51 (m, 2H), 3.37 (br s, 1H), 3.03-2.48 (m, 5H), 2.42 (dd, *J* = 15 and 4.5 Hz) and 2.35 (dd, *J* = 15 and 6.0 Hz, total 1H), 1.77-1.54

(m, 2H), 1.43 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.0, 169.1, 156.2 (2 carbons), 147.7, 133.9, 131.1, 129.6, 129.3, 125.2, 79.3, 79.0, 63.4, 57.6, 45.4, 44.1, 39.0, 37.1, 34.9, 28.44 (3 carbons), 28.36 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{40}N_5O_9$ $[M+H]^+$ 554.2826, found 554.2826.

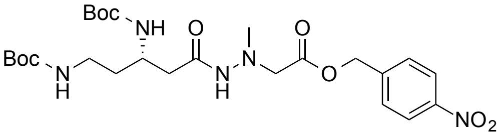
(*S*)-3-Nitrobenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16h)



16h was prepared in the same manner as described for compound **16a** using **14b** (60.3 mg, 0.118 mmol) and *m*-nitrobenzyl alcohol (21.7 mg, 0.142 mmol).

16h (41.4 mg, 74.8 μmol, 2 steps 63%) was obtained as a white solid; $[\alpha]_D^{25}$ = –32.0 (*c* 1.49, $CHCl_3$); m.p. 86.9-87.8 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.23 (s, 2H), 7.76 (s) and 7.25 (br s, total 1H), 7.70 (t, *J* = 7.5 Hz, 1H), 7.59 (t, *J* = 7.9 Hz, 1H), 5.71-5.52 (m, 1H), 5.40-5.18 (m, 3H), 4.06-3.85 (m, 1H), 3.84-3.57 (m, 2H), 3.36 (br s, 1H), 3.03-2.50 (m, 5H), 2.42 (dd, *J* = 15 and 4.8 Hz) and 2.25 (dd, *J* = 15 and 5.9 Hz, total 1H), 1.78-1.58 (m, 2H), 1.43 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 169.3, 156.1 (2 carbons), 148.4, 137.1, 134.1, 129.8, 123.5, 123.1, 79.3, 79.0, 65.2, 57.7, 45.5, 44.1, 39.0, 37.1, 34.9, 28.44 (3 carbons), 28.35 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{40}N_5O_9$ $[M+H]^+$ 554.2826, found 554.2817.

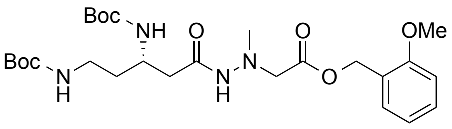
(*S*)-4-Nitrobenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16i)



16h was prepared in the same manner as described for compound **16a** using **14b** (82.5 mg, 0.197 mmol) and *p*-nitrobenzyl alcohol (36.3 mg, 0.237 mmol).

16h (45.3 mg, 81.9 μmol, 2 steps 42%) was obtained as a white solid; $[\alpha]_D^{25}$ = –30.5 (*c* 0.77, $CHCl_3$); m.p. 98.7-100.5 ºC; ^1H NMR (400 MHz, $CDCl_3$) δ 8.25 (d, *J* = 8.7 Hz, 2H), 7.71 (s) and 7.23 (br d, total 1H), 7.53 (t, *J* = 8.5 Hz, 2H), 5.74-5.48 (m, 1H), 5.40-5.17 (m, 3H), 4.08-3.84 (m, 1H), 3.83-3.75 (m, 2H), 3.36 (br s, 1H), 3.03-2.83 (m, 1H), 2.82-2.49 (m, 4H), 2.42 (dd, J = 16 and 5.8 Hz) and 2.24 (dd, J = 15 and 5.8 Hz, total 1H), 1.76-1.55 (m, 2H), 1.43 (s, 9H), 1.42 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.2, 169.3, 156.2 (2 carbons), 147.9, 142.3, 128.7 (2 carbons), 123.9 (2 carbons), 79.4, 79.1, 65.1, 57.7, 45.5, 44.1, 39.0, 37.1, 34.9, 28.45 (3 carbons), 28.37 (3 carbons); HRMS (ES+) calcd for $C_{25}H_{28}N_5O_9Na$ $[M+Na]^+$ 576.2645, found 576.2633.

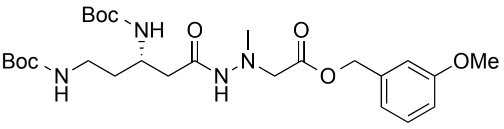
(*S*)-3-Methoxybenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16j)



16j was prepared in the same manner as described for compound **16a** using **14b** (114 mg, 0.224 mmol) and *o*-methoxybenzyl alcohol (37.1 mg, 0.269 mmol).

16j (59.6 mg, 0.111 mmol, 2 steps 49%) was obtained as a white solid; $[\alpha]_D^{25}$ = –38.0 (c 0.75, $CHCl_3$); m.p. 77.1-79.0 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.80 (s) and 7.39-7.25 (m, total 3H), 7.04-6.86 (m, 2H), 5.77-5.56 (m, 1H), 5.39-5.17 (m, 3H), 4.05-3.89 (m, 1H), 3.86 (s, 3H), 3.78-3.50 (m, 2H), 3.37 (br s, 1H), 3.00-2.83 (m, 1H), 2.81-2.49 (m, 4H), 2.48-2.37 (m) and 2.20 (dd, J = 15 and 5.6 Hz, total 1H), 1.78-1.57 (m, 2H), 1.43 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 169.6, 157.7, 156.2, 130.4, 130.2, 123.3, 120.5, 110.6, 79.3, 79.0, 62.3, 59.0, 57.8, 55.5, 45.3, 44.0, 38.9, 37.1, 35.2, 28.4 (3 carbons), 28.3 (3 carbons); HRMS (ES+) calcd for $C_{26}H_{43}N_4O_8$ $[M+H]^+$ 539.3081, found 539.3079.

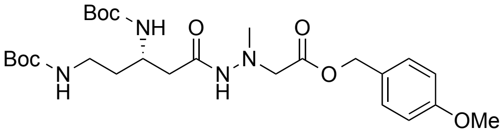
(*S*)-3-Methoxybenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16k)



16k was prepared in the same manner as described for compound **16a** using **14b** (79.7 mg, 0.157 mmol) and *m*-methoxybenzyl alcohol (26.0 mg, 0.188 mmol).

16k (41.0 mg, 76.2 µmol, 2 steps 49%) was obtained as a white solid; $[\alpha]_D^{25}$ = –33.8 (c 0.94, $CHCl_3$); m.p. 79.3-80.5 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (s) and 7.36-7.25 (m, total 2H), 6.98-6.86 (m, 3H), 5.76-5.56 (m, 1H), 5.40-5.19 (m, 1H), 5.15 (s, 2H), 4.04-3.94 (m, 1H), 3.82 (s, 3H), 3.79-3.49 (m, 2H), 3.37 (br s, 1H), 3.02-2.88 (m, 1H), 2.87-2.48 (m, 4H), 2.47-2.38 (m) and 2.25 (dd, *J* = 15 and 5.7 Hz, total 1H), 1.76-1.56 (m, 2H), 1.43 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 169.5, 159.8, 156.2 (2 carbons), 136.5, 129.8, 120.6, 114.1, 114.0, 79.3, 79.0, 66.6, 57.7, 55.3, 45.4, 44.1, 38.9, 37.2, 34.9, 28.5 (3 carbons), 28.4 (3 carbons); HRMS (ES+) calcd for $C_{26}H_{43}N_4O_8$ $[M+H]^+$ 539.3081, found 539.3072.

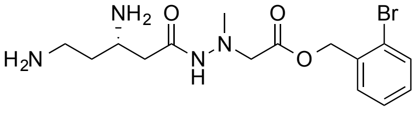
(*S*)-4-Methoxybenzyl 2-{2-[3,5-bis(*tert*-butoxycarbonylamino)pentanoyl]-1-methyl hydrazinyl}acetate (16l)



16l was prepared in the same manner as described for compound **16a** using **14b** (106 mg, 0.209 mmol) and *p*-methoxybenzyl alcohol (34.7 mg, 0.251 mmol).

16l (49.9 mg, 92.7 μmol, 2 steps 44%) was obtained as a white solid; $[\alpha]_D^{25}$ = –29.6 (*c* 0.15, $CHCl_3$); m.p. 89.6-90.2 ºC; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.81 (s) and 7.38-7.23 (m, total 3H), 6.90 (d, *J* = 7.2 Hz, 2H), 5.74-5.57 (m, 1H), 5.41-5.23 (m, 1H), 5.11 (s, 2H), 4.06-3.85 (m, 1H), 3.81 (s, 3H), 3.78-3.46 (m, 2H), 3.37 (br s, 1H), 3.00-2.86 (m, 1H), 2.85-2.45 (m, 4H), 2.44-2.37 (m) and 2.22 (dd, *J* = 15 and 5.7 Hz, total 1H), 1.76-1.56 (m, 2H), 1.43 (s, 18H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.7, 169.6, 159.9, 156.1, 130.4 (2 carbons), 127.1, 114.0 (2 carbons), 79.2, 78.9, 66.6, 57.7, 55.3, 45.4, 44.0, 38.9, 37.1, 34.7, 28.44 (3 carbons), 28.35 (3 carbons); HRMS (ES+) calcd for $C_{26}H_{42}N_4O_8Na$ $[M+Na]^+$ 561.2900, found 561.2889.

(*S*)-2-Bromobenzyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17a)

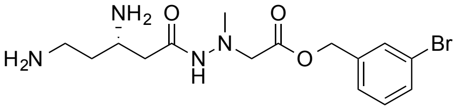


17a was prepared in the same manner as described for compound **9b** using **16a** (52.0 mg, 88.5 μmol). The residue was purified by preparative HPLC (gradient: milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 75 : 25 to milli–Q water (TFA 0.1%) : CH_3CN (TFA 0.1%) = 55 : 45 over 40 min, flow late 5 mL/min, UV: 222 nm).

17a (26.9 mg, 43.9 μmol, 50%) was obtained as a white solid; $[\alpha]_D^{25}$ = +4.87 (*c* 0.10,

H_2O); m.p. 84.7-85.8 ºC; ^1H NMR (400 MHz, D_2O) δ 7.73 (d, *J* = 8.0 Hz, 1H), 7.57-7.43 (m, 2H), 7.40-7.37 (m, 1H), 5.33 (s, 2H), 3.82-3.69 (m, 3H), 3.16 (t, *J* = 7.3 Hz, 2H), 2.71 (s, 3H), 2.70-2.55 (m, 2H), 2.22-2.01 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 170.6, 168.8, 134.2, 133.0, 130.9, 130.7, 128.0, 123.3, 66.9, 58.3, 46.1, 44.3, 35.7, 34.6, 29.8; HRMS (ES+) calcd for $C_{15}H_{24}N_4O_3Br$ $[M+H]^+$ 387.1032, found 387.1033.

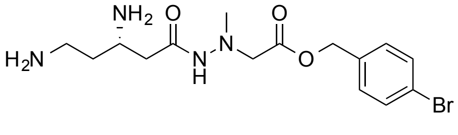
(*S*)-3-Bromobenzyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17b)



17b was prepared in the same manner as described for compound **17a** using **16b** (44.9 mg, 76.4 μmol).

17b (10.7 mg, 17.3 μmol, 23%) was obtained as a white solid; $[\alpha]_D^{25}$ = +8.08 (*c* 0.77, D_2O); m.p. 102.4-103.0 ºC; ^1H NMR (400 MHz, D_2O) δ 7.70-7.57 (m, 2H), 7.44-7.32 (m, 3H), 5.20 (s, 2H), 3.79-3.64 (m, 3H), 3.10 (t, *J* = 7.5 Hz, 2H), 2.67 (s, 3H), 2.62-2.45 (m, 2H), 2.18-1.98 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 170.6, 168.8, 137.7, 131.5, 130.9, 130.5, 127.0, 121.9, 66.2, 58.4, 46.0, 44.3, 35.6, 34.5, 29.8; HRMS (ES+) calcd for $C_{15}H_{24}N_4O_3Br$ $[M+H]^+$ 387.1032, found 387.1035.

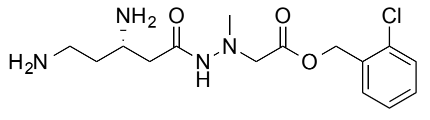
(*S*)-4-Bromobenzyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17c)



17c was prepared in the same manner as described for compound **17a** using **16c** (37.2 mg, 63.3 μmol).

17c (18.4 mg, 29.9 μmol, 47%) was obtained as a white solid; $[\alpha]_D^{25}$ = +6.67 (*c* 0.50, H_2O); m.p. 88.5-89.4 ºC; ^1H NMR (400 MHz, D_2O) δ 7.59 (d, *J* = 8.4 Hz, 2H), 7.33 (d, *J* = 8.2 Hz, 2H), 5.16 (s, 2H), 3.74-3.62 (m, 3H), 3.09 (t, *J* = 8.3 Hz, 2H), 2.65 (s, 3H), 2.60-2.45 (m, 2H), 2.20-1.98 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 173.5, 171.7, 137.4, 134.6 (2 carbons), 133.1 (2 carbons), 124.9, 69.3, 61.4, 48.9, 47.2, 38.6, 37.5, 32.7; HRMS (ES+) calcd for $C_{15}H_{24}N_4O_3Br$ $[M+H]^+$ 387.1032, found 387.1024.

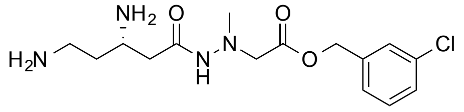
(*S*)-2-Chlorobenzyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17d)



17d was prepared in the same manner as described for compound **17a** using **16d** (37.2 mg, 68.5 μmol).

17d (35.1 mg, 61.6 μmol, 90%) was obtained as a white solid; $[\alpha]_D^{25}$ = +4.99 (*c* 1.00, H_2O); m.p. 78.5-79.5 ºC; ^{1}H NMR (400 MHz, D_2O) δ 7.53-7.44 (m, 2H), 7.44-7.32 (m, 2H), 5.30 (s, 2H), 3.80-3.60 (m, 3H), 3.09 (t, *J* = 8.9 Hz, 2H), 2.64 (s, 3H), 2.61-2.39 (m, 2H), 2.15-1.96 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 170.5, 168.8, 133.5, 132.5, 130.8, 130.4, 129.7, 127.3, 64.7, 58.3, 46.0, 44.2, 35.6, 34.5, 29.7; HRMS (ES+) calcd for $C_{15}H_{24}N_4O_3Cl$ [M+H]$^+$ 343.1537, found 343.1535.

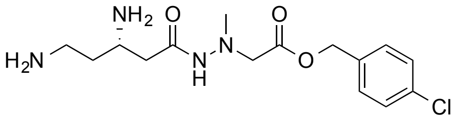
(*S*)-3-Chlorobenzyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17e)



17e was prepared in the same manner as described for compound **17a** using **16e** (24.4 mg, 45.0 μmol).

17e (14.0 mg, 24.5 μmol, 54%) was obtained as a white solid; $[\alpha]_D^{25}$ = +5.27 (*c* 1.00, H_2O); m.p. 69.9-70.8 ºC; ^{1}H NMR (400 MHz, D_2O) δ 7.45-7.31 (m, 4H), 5.18 (s, 2H), 3.76-3.60 (m, 3H), 3.08 (t, *J* = 8.6 Hz, 2H), 2.65 (s, 3H), 2.60-2.45 (m, 2H), 2.13-1.95 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 170.6, 168.8, 137.4, 133.8, 130.2, 128.5, 128.0, 126.5, 66.2, 58.4, 46.0, 44.3, 35.6, 34.5, 29.7; HRMS (ES+) calcd for $C_{15}H_{24}N_4O_3Cl$ [M+H]$^+$ 343.1537, found 343.1535.

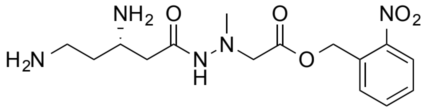
(*S*)-4-Chlorobenzyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17f)



17f was prepared in the same manner as described for compound **17a** using **16f** (22.6 mg, 41.7 μmol).

17f (13.1 mg, 23.0 μmol, 55%) was obtained as a white solid; $[\alpha]_D^{25}$ = +2.52 (*c* 0.50, H_2O); m.p. 72.1-73.2 ºC; ^{1}H NMR (400 MHz, D_2O) δ 7.47-7.30 (m, 4H), 5.17 (s, 2H), 3.80-3.55 (m, 3H), 3.08 (t, *J* = 8.7 Hz, 2H), 2.63 (s, 3H), 2.59-2.40 (m, 2H), 2.15-1.92 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 170.6, 168.8, 134.0, 133.8, 129.9 (2 carbons), 128.7 (2 carbons), 66.4, 58.4, 46.0, 44.2, 35.6, 34.5, 29.7; HRMS (ES+) calcd for $C_{15}H_{24}N_4O_3Cl$ $[M+H]^+$ 343.1537, found 343.1534.

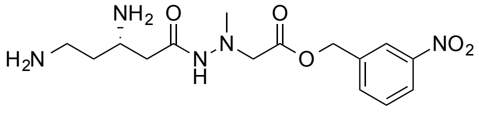
(*S*)-2-Nitrobenzyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17g)



17g was prepared in the same manner as described for compound **17a** using **16g** (28.3 mg, 51.1 μmol).

17g (21.8 mg, 37.5 μmol, 74%) was obtained as a white solid; $[\alpha]_D^{25}$ = +6.10 (*c* 0.50, H_2O); m.p. 79.9-80.4 ºC; ^{1}H NMR (400 MHz, D_2O) δ 8.15 (d, *J* = 7.3 Hz, 1H), 7.75 (t, *J* = 7.5 Hz, 1H), 7.69-7.58 (m, 2H), 5.52 (s, 2H), 3.79-3.68 (m, 3H), 3.11 (t, *J* = 7.7 Hz, 2H), 2.65 (s, 3H), 2.62 (m, 2H), 2.13-1.99 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 173.3, 171.9, 150.3, 137.4, 133.34, 133.27, 132.6, 128.2, 67.0, 61.2, 49.0, 47.2, 38.6, 37.6, 32.7; HRMS (ES+) calcd for $C_{15}H_{24}N_5O_5$ $[M+H]^+$ 354.1777, found 354.1782.

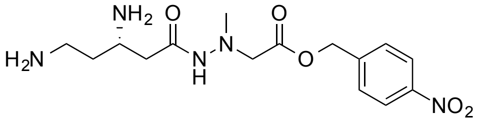
(*S*)-3-Nitrobenzyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17h)



17h was prepared in the same manner as described for compound **17a** using **16h** (36.9 mg, 66.6 μmol).

17h (21.6 mg, 37.2 μmol, 56%) was obtained as a white solid; $[\alpha]_D^{25}$ = +6.49 (*c* 0.50, H_2O); m.p. 76.0-76.7 ºC; ^{1}H NMR (400 MHz, D_2O) δ 8.25 (s, 1H), 8.21 (d, *J* = 8.3 Hz, 1H), 7.80 (d, *J* = 7.7 Hz, 1H), 7.62 (t, *J* = 8.0 Hz, 1H), 5.30 (s, 2H), 3.79-3.63 (m, 3H), 3.11 (t, *J* = 7.3 Hz, 2H), 2.66 (s, 3H), 2.60 (dd, *J* = 17 and 5.3 Hz) and 2.53 (dd, *J* = 17 and 7.5 Hz, total 2H), 2.18-1.97 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 173.5, 171.8, 150.8, 140.1, 137.6, 132.8, 126.3, 125.7, 68.7, 61.3, 49.0, 47.2, 38.6, 37.6, 32.8; HRMS (ES+) calcd for $C_{15}H_{24}N_5O_5$ $[M+H]^+$ 354.1777, found 354.1773.

(*S*)-4-Nitrobenzyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17i)

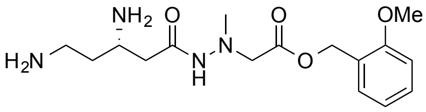


17i was prepared in the same manner as described for compound **17a** using **16i** (22.1 mg, 39.9 μmol).

17i (15.7 mg, 27.0 μmol, 68%) was obtained as a white solid; $[\alpha]_D^{25}$ = +3.72 (*c* 0.50, H_2O); m.p. 52.6-53.0 ºC; ^{1}H NMR (400 MHz, D_2O) δ 8.24 (d, *J* = 8.7 Hz, 2H), 7.60 (d, *J* = 8.7 Hz, 2H), 5.30 (s, 2H), 3.80-3.61 (m, 3H), 3.09 (t, *J* = 7.3 Hz, 2H), 2.65 (s, 3H), 2.59 (dd, *J* = 16 and 5.3 Hz) and 2.52 (dd, *J* = 17 and 7.4 Hz, total 2H), 2.15-1.98 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 173.4, 171.9, 150.3, 145.9, 131.5 (2 carbons), 126.7 (2 carbons), 68.7, 61.2, 48.9, 47.2, 38.6, 37.8, 32.8; HRMS (ES+) calcd for $C_{15}H_{24}N_5O_5$ $[M+H]^+$ 354.1777, found 354.1771.

(*S*)-2-Methoxybenzyl

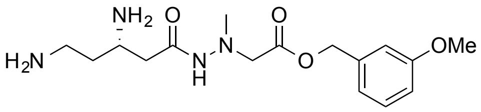
2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17j)



17j was prepared in the same manner as described for compound **17a** using **16j** (30.4 mg, 56.5 μmol).

17j (20.8 mg, 36.8 μmol, 65%) was obtained as a white solid; $[\alpha]_D^{25}$ = +2.35 (*c* 1.00, H_2O); m.p. 66.5-66.8 ºC; ^{1}H NMR (400 MHz, D_2O) δ 7.46-7.35 (m, 2H), 7.10 (d, *J* = 8.3 Hz, 1H), 7.03 (t, *J* = 6.9 Hz, 1H), 5.22 (s, 2H), 3.85 (s, 3H), 3.70-3.60 (m, 3H), 3.08 (t, *J* = 7.2 Hz, 2H), 2.63 (s, 3H), 2.59-2.46 (m, 2H), 2.18-1.97 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 173.6, 171.8, 160.4, 133.7, 133.5, 126.0, 123.8, 114.6, 65.8, 61.2, 58.6, 48.9, 47.0, 38.6, 37.6, 32.7; HRMS (ES+) calcd for $C_{16}H_{27}N_4O_4$ $[M+H]^+$ 339.2032, found 339.2025.

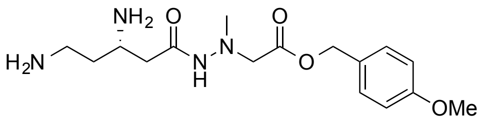
(*S*)-3-Methoxybenzyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17k)



17k was prepared in the same manner as described for compound **17a** using **16k** (22.1 mg, 41.1 μmol).

17k (15.0 mg, 26.5 μmol, 64%) was obtained as a white solid; $[\alpha]_D^{25}$ = +8.44 (*c* 0.43, H_2O); m.p. 58.1-58.6 ºC; ^1H NMR (400 MHz, D_2O) 7.38 (t, *J* = 19 Hz, 1H), 7.05-6.99 (m, 3H), 5.18 (s, 2H), 3.83 (s, 3H), 3.68 (s) and 3.67-3.65 (m, total 3H), 3.08 (t, *J* = 18 Hz, 2H), 2.64 (s, 3H), 2.58-2.46 (m, 2H), 2.08-1.99 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 170.6, 168.8, 159.0, 137.0, 130.1, 120.9, 114.2, 113.8, 66.9, 58.4, 55.4, 46.0, 44.2, 35.6, 34.6, 29.8; HRMS (ES+) calcd for $C_{16}H_{27}N_4O_4$ $[M+H]^+$ 339.2032, found 339.2021.

(*S*)-4-Methoxybenzyl 2-[2-(3,5-diaminopentanoyl)-1-methylhydrazinyl]acetate·2TFA (17l)



17l was prepared in the same manner as described for compound **17a** using **16k** (30.8 mg, 57.2 μmol).

17l (18.2 mg, 32.1 μmol, 56%) was obtained as a white solid; $[\alpha]_D^{25}$ = +0.25 (*c* 0.50, H_2O); m.p. 71.0-72.1 ºC; ^1H NMR (400 MHz, D_2O) δ 7.50 (d, *J* = 8.7 Hz, 2H), 7.02 (d, *J* = 8.7 Hz, 2H), 5.15 (s, 2H), 2.84 (s, 3H), 3.73-3.61 (m, 3H), 3.09 (t, *J* = 7.5 Hz, 2H), 2.64 (s, 3H), 2.55-2.49 (m, 2H), 2.15-1.94 (m, 2H); ^{13}C NMR (100 MHz, D_2O) δ 170.7, 168.8, 159.1, 130.4 (2 carbons), 127.8, 114.2 (2 carbons), 66.9, 58.4, 55.4, 46.0, 44.2, 35.6, 34.5, 29.7; HRMS (ES+) calcd for $C_{16}H_{27}N_4O_4$ $[M+H]^+$ 339.2032, found 339.2036.

6. Biological evaluation

6-1. Chemicals

Geneticin (G418) solution was purchased from Roche Diagnostics K.K., Swizerland.

6-2. Plasmid

The previously reported plasmids[1] were used in this study. The dual-reporter plasmid for mammalian cells expression encodes the β-galactosidase and luciferase genes connected with the premature termination codon (PTC), a 27-mer stretch of DNA that contains the sequence surrounding the PTC in exon 23 of the *mdx* gene for mouse dystrophin: TTGAAAGAGCAA<u>TAA</u>AATGGCTTCAAC. The PTC was originally TAA. We used TGA in this study.

The *in vitro* eukaryotic expression study involved the insertion of the β-galactosidase-TGA-luciferase fragment downstream of the IRES sequence of the pT7CFE1-CGST-HA-His vector (Thermo Fisher Scientific).

6-3. The cell-based readthrough activity evaluation

The cell-based readthrough activity was evaluated by previously reported procedure[1].

COS-7 cells were maintained in D-MEM (high glucose, Wako Pure Chemical Industries, Ltd., Japan) containing 10% fetal bovine serum (FBS, Nichirei BIiosciences Inc., Japan) at 37 ºC in a humidified 5% CO_2 atmosphere. Cells were plated in 96-well plates at 8000 cells/well. After incubation at 37 °C for 12 h, cells were transfected with a plasmid with PTC using the FuGene® HD Transfect reagent (Roche Diagnostics K.K., Swizerland). The medium was removed from the well, and the medium containing the compounds at a concentration of 50-200 μM were added to the well. As a control, the medium without compounds was also added. The cells were incubated at 37 °C for 48 h, cells were collected, and β-galactosidase activity in the cell lysates was measured according to the manufacturer's protocol for the β-Galactosidase Enzyme Assay with Reporter Lysis Buffer (Promega KK, USA). The β-galactosidase activity was measured by TECAN SAFIRE (TECAN Japan Co., Ltd, Japan) at 420 nm (reference 0 nm). The luciferase activity in the cell lysates was measured according to the manufacturer's protocol for using the PicaGene® BrillianStar-LT (TOYO INK CO., LTD., Japan). The luciferase activity was measured using a Berthold Luminometer MicroLumat Plus LB96V (Berthold Japan K.K., Japan). The readthrough activity was determined as the ratio of luciferase activity to β-galactosidase activity. The activities of compounds were expressed as a ratio relative to control (=1).

6-4. The readthrough activity evaluation of cell-free protein synthesis system

The cell-free readthrough activity was evaluated by previously reported procedure[1].

The readthrough activity of the cell-free protein synthesis system was evaluated according to the manufacturer's protocol for the Human Cell-Free Protein Expression System (TAKARA Bio Inc, Japan). Compounds (final concentration, 20 μM) were added to the lysate before incubation at 37 °C for 3 h. The β-galactosidase and luciferase activities were measured in the same way as in the cell-based assay, as described above.

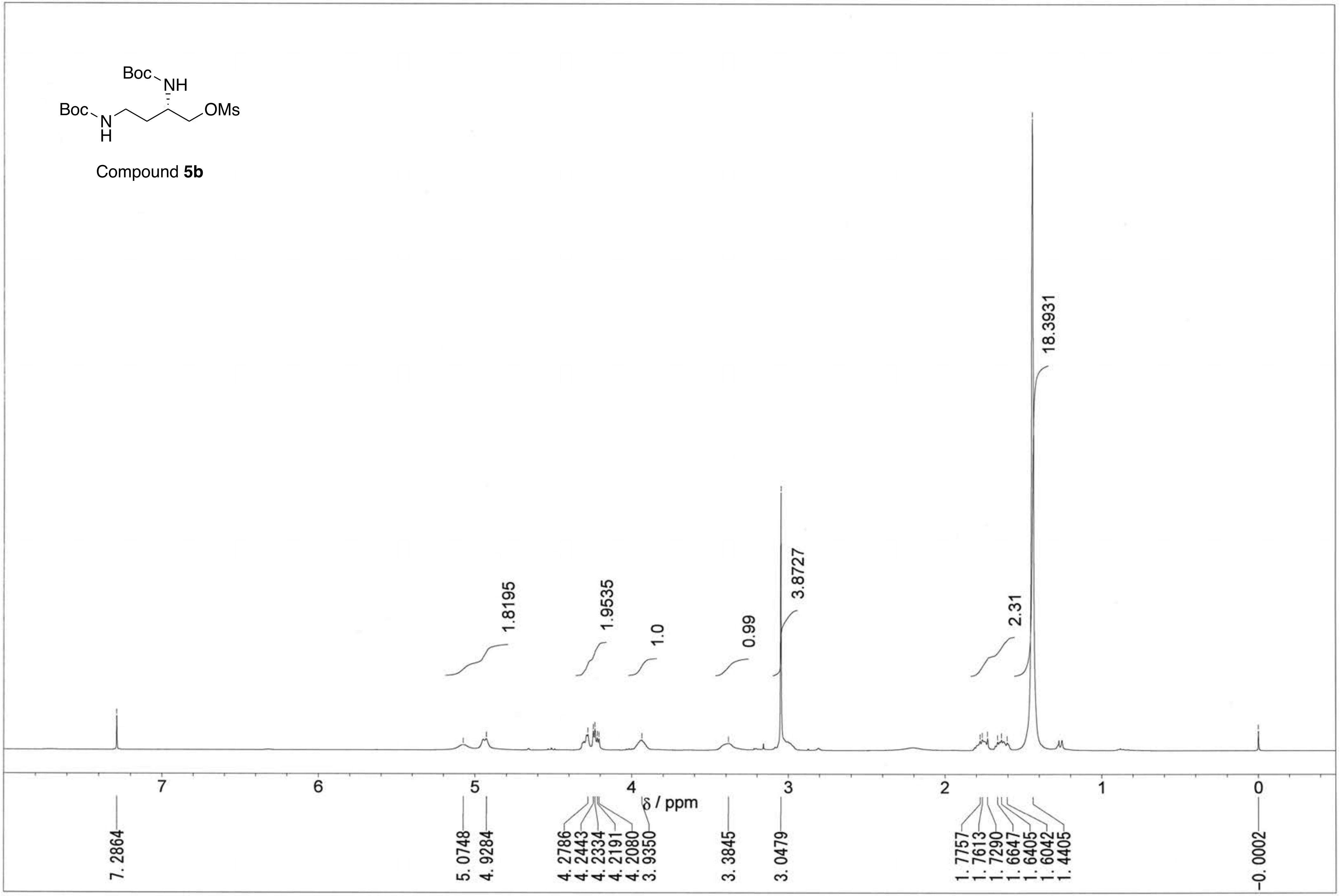
6-5. The antimicrobial activity assay (MIC assay)

The antimicrobial activity assay of **9b** was evaluated at Hygiene & Microbiology Research Center. As a bacterial strain, S*taphylococcus aureus* NBRC13276, *Escherichia coli* NBRC3972 were used.

6-6. Hydrolysis of benzyl ester of **17e** by porcine liver esterase

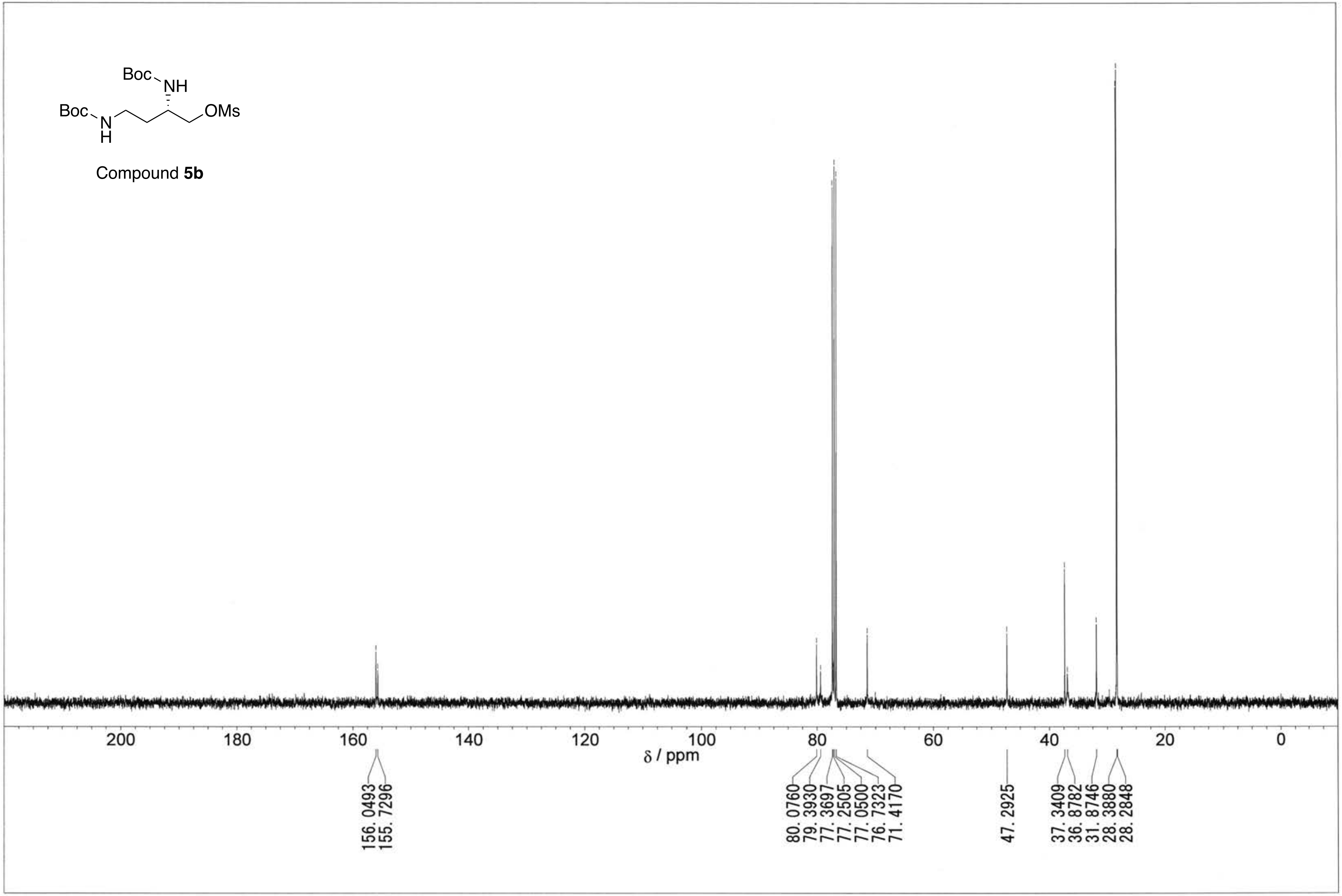
The stock solution of **17e** (20 mM in 100 mM phosphate buffer, pH 7.4) was diluted to 2 mM with 100 mM phosphate buffer (pH 7.4). The suspension of the porcine liver esterase (9 μL; containing 110 μunit/mL; PLE: carboxylic-ester hydrolase, Aldrich, USA) was added to the solution of **17e** (2 mM, 450 μL). The residual solution of **17e** (2 mM, 50 μL) was used as a sample of time zero without addition of esterase. Each solution (2 mM, 50 μL) containing PLE was incubated at 37 ºC for appropriate times. After the incubation, the mixture was filtered through the centrifugal filter (0.2 μm filter unit, NANOSEP® MF centrifugal devices, PALL) and immediately frozen at –78 ºC to neutralize a PLE. After melting, the filtrate containing **17e** and its metabolites were analyzed by RP-HPLC and high-resolution mass spectrometry for identification of some metabolites appeared as a new HPLC peak.

Compound 5b proton



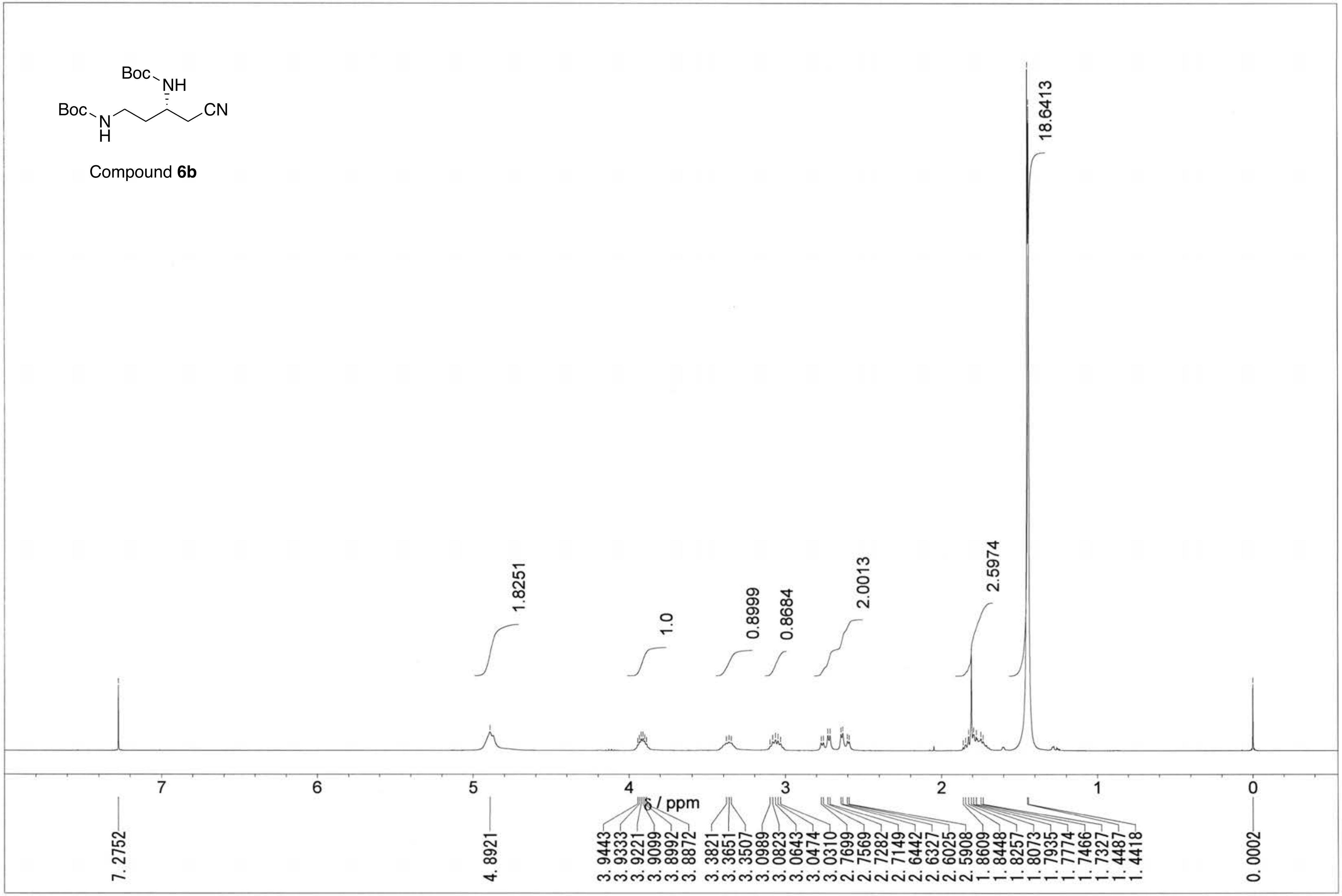
Compound **5b**

Compound 5b carbon

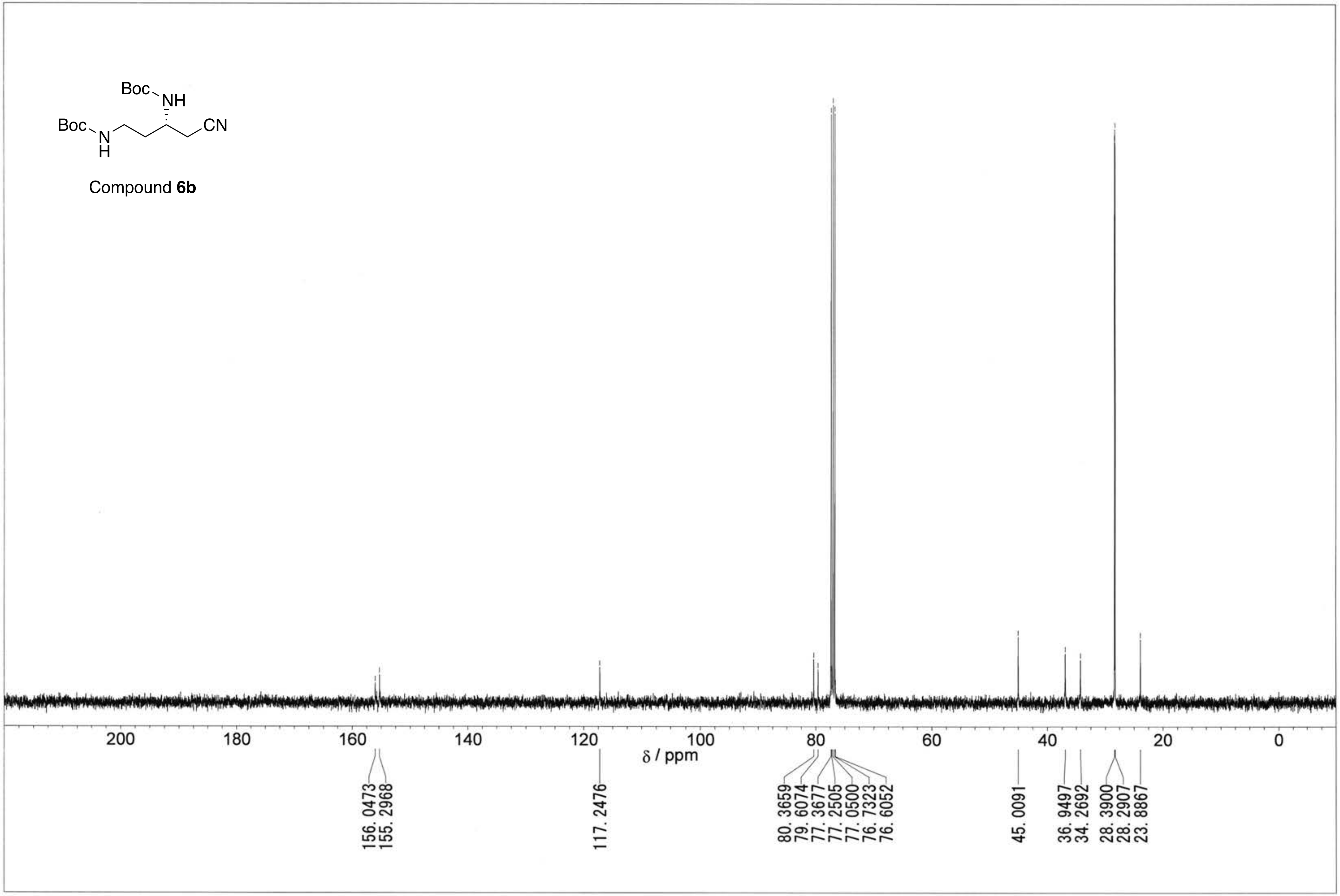


Compound **5b**

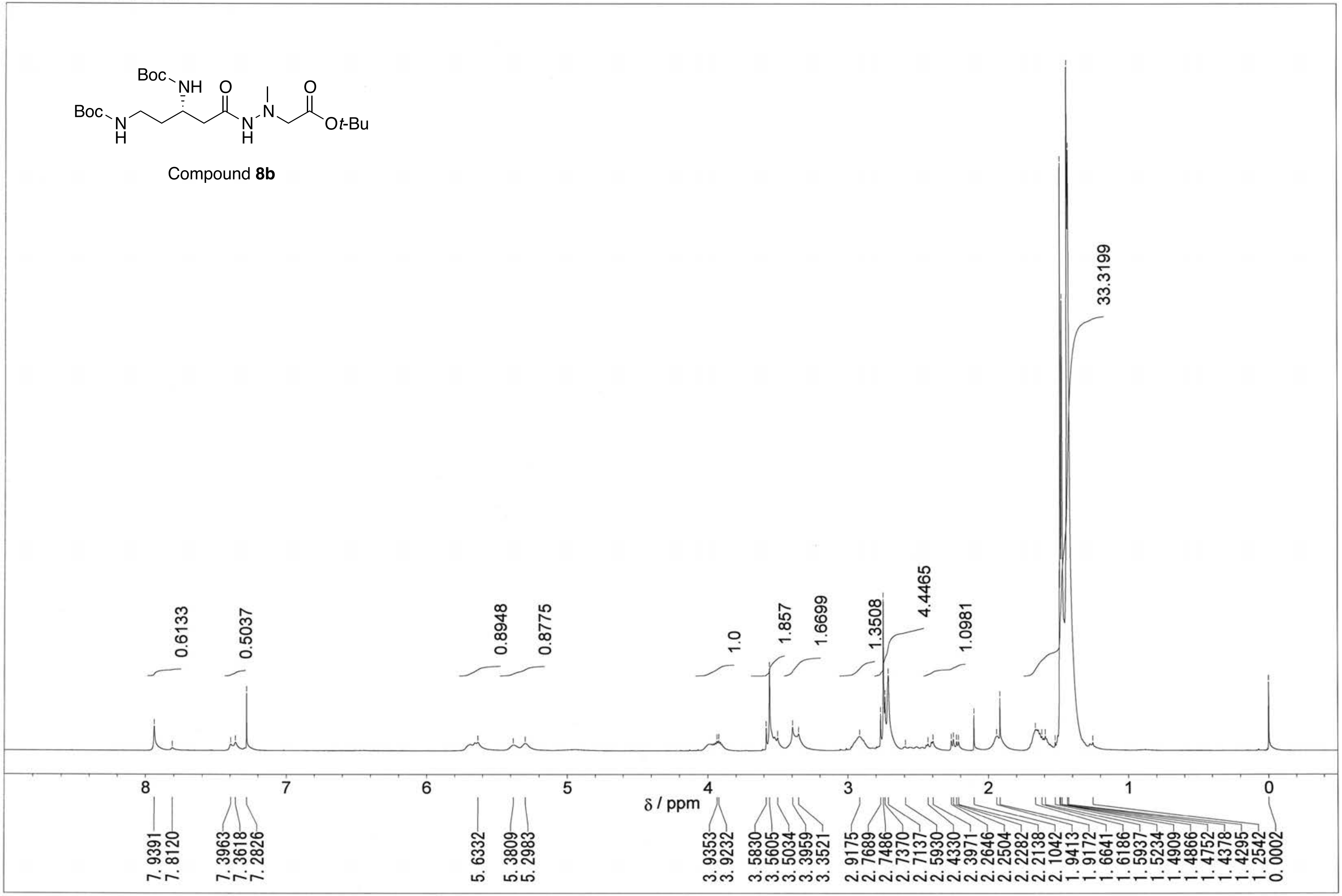
Compound 6b proton



Compound 6b carbon

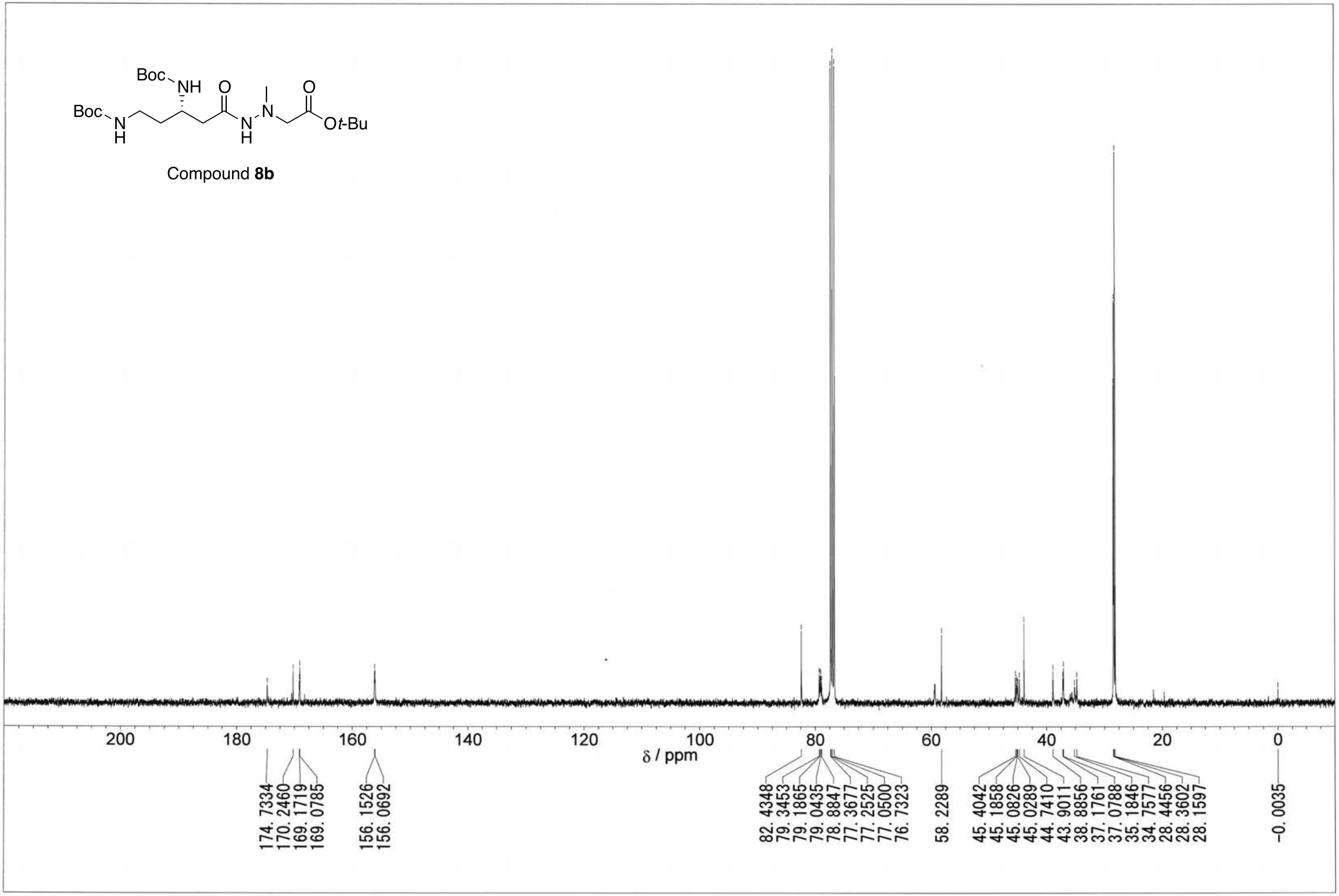


Compound 8b proton

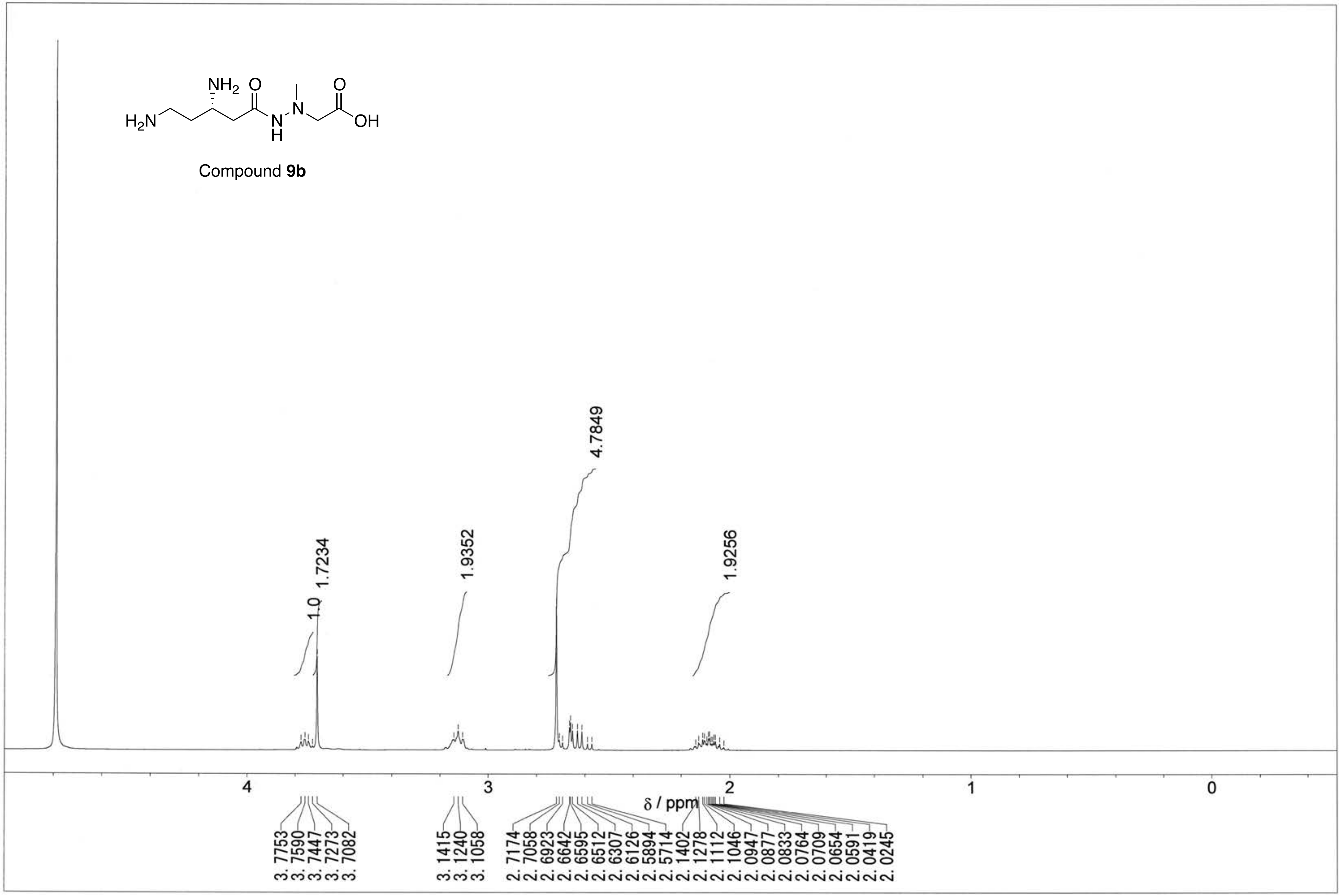


Compound **8b**

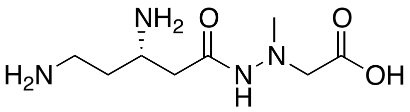
Compound 8b carbon



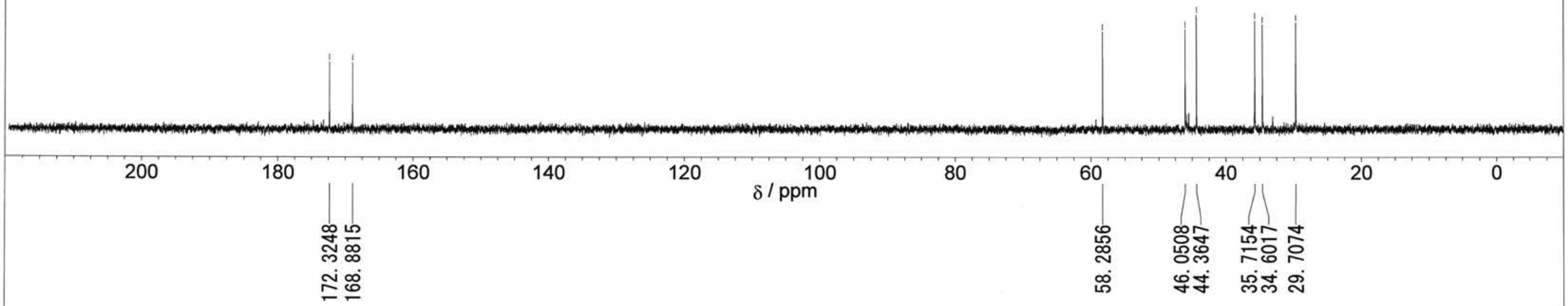
Compound 9b proton


Compound **9b**

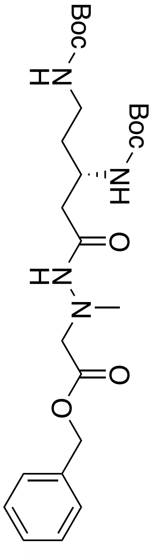
Compound 9b carbon



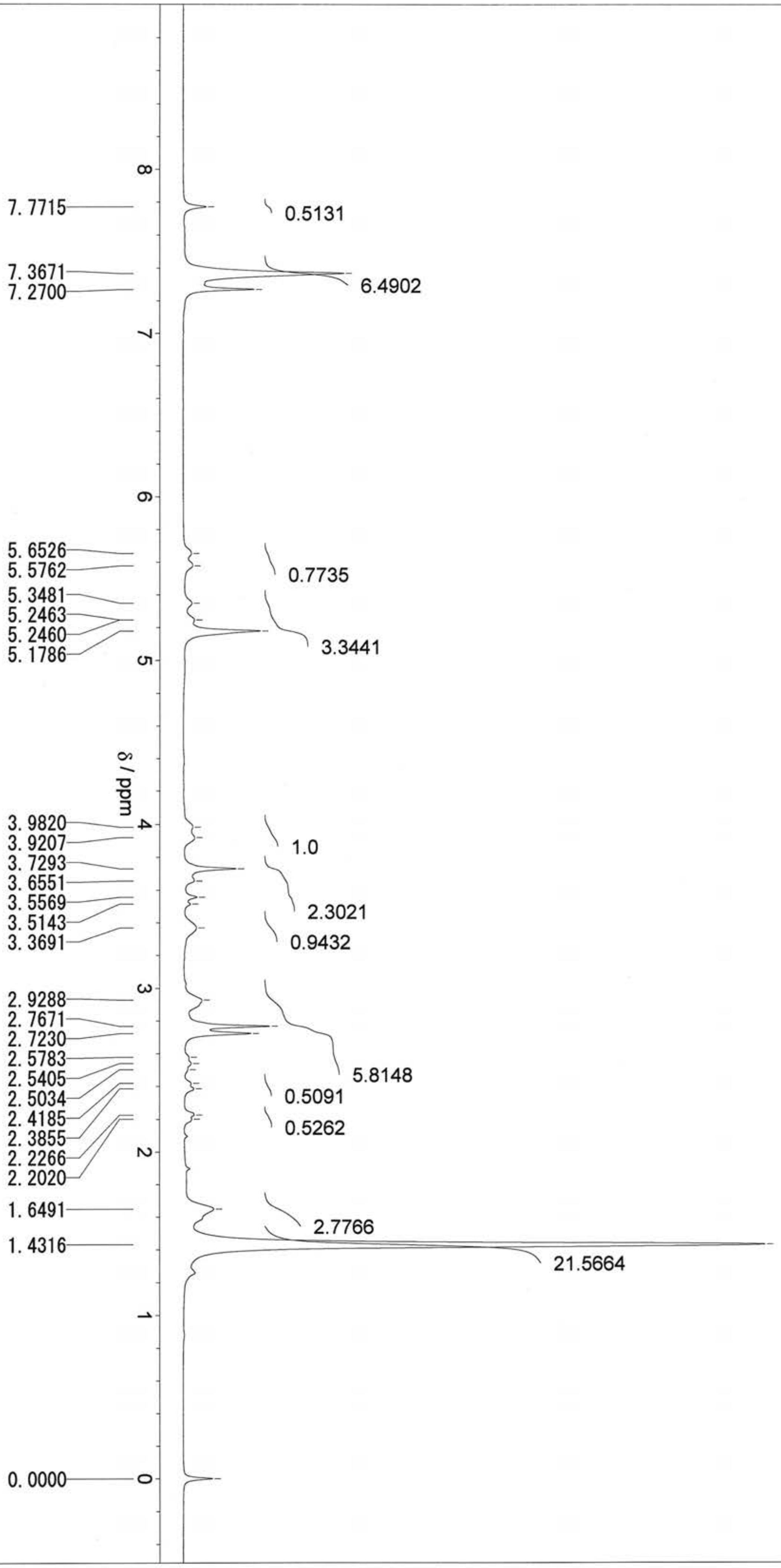
Compound **9b**



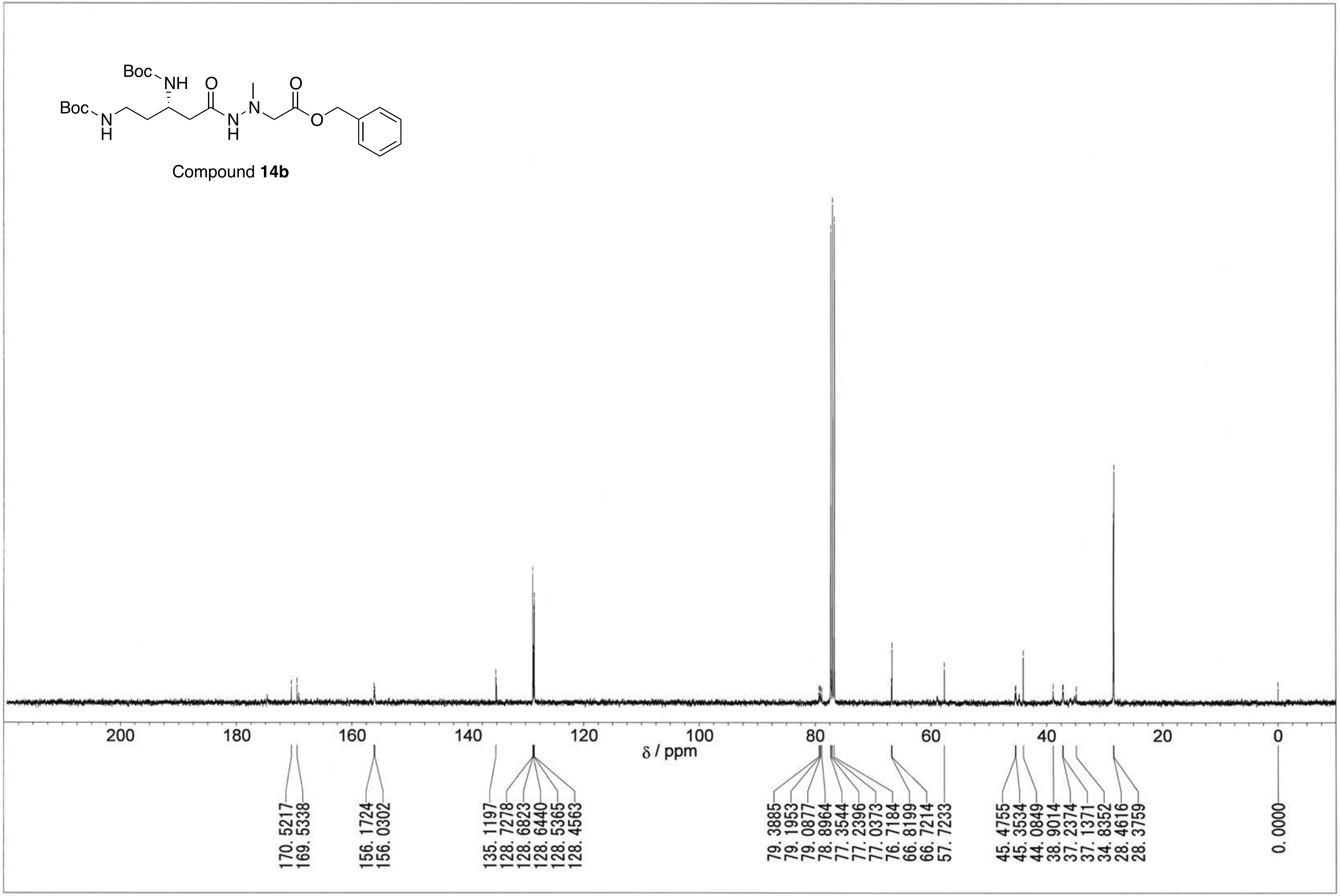
Compound 14b proton



Compound **14b**

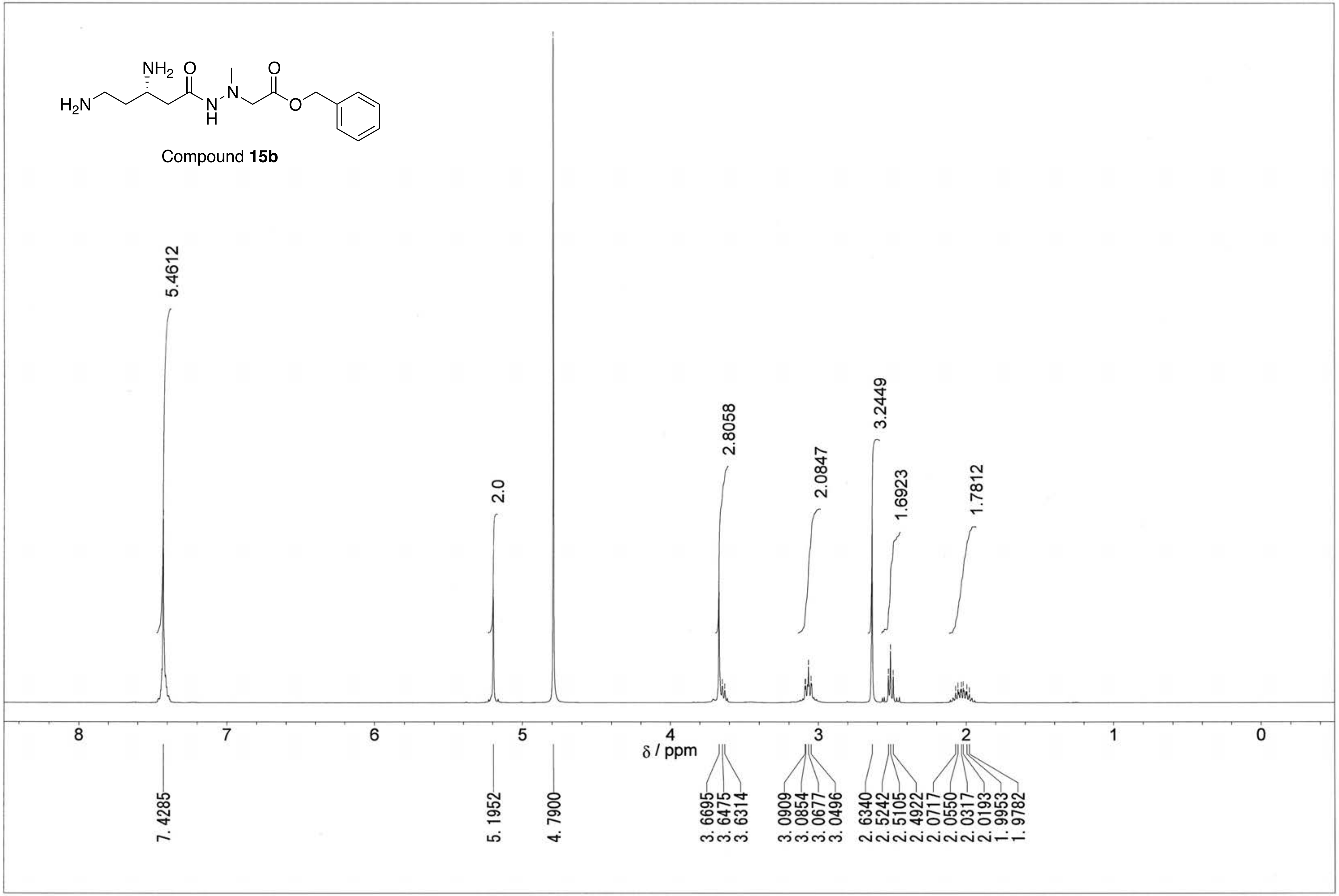


Compound 14b carbon



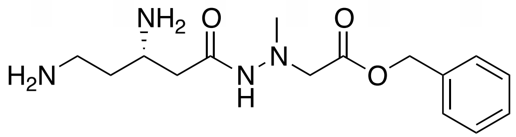
Compound **14b**

Compound 15b proton

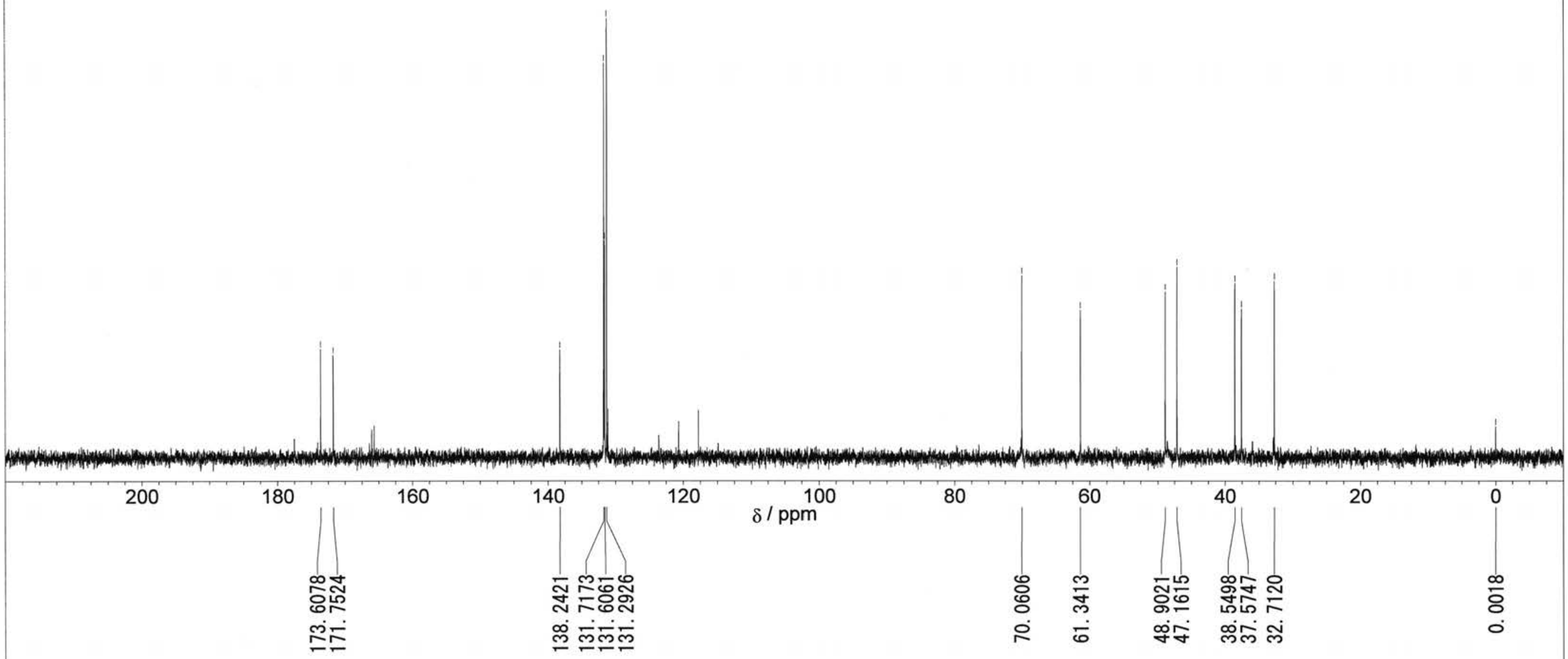


Compound **15b**

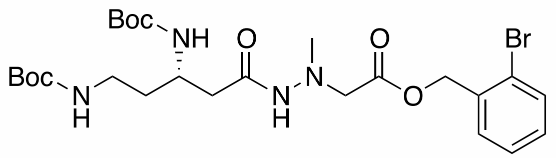
Compound 15b carbon



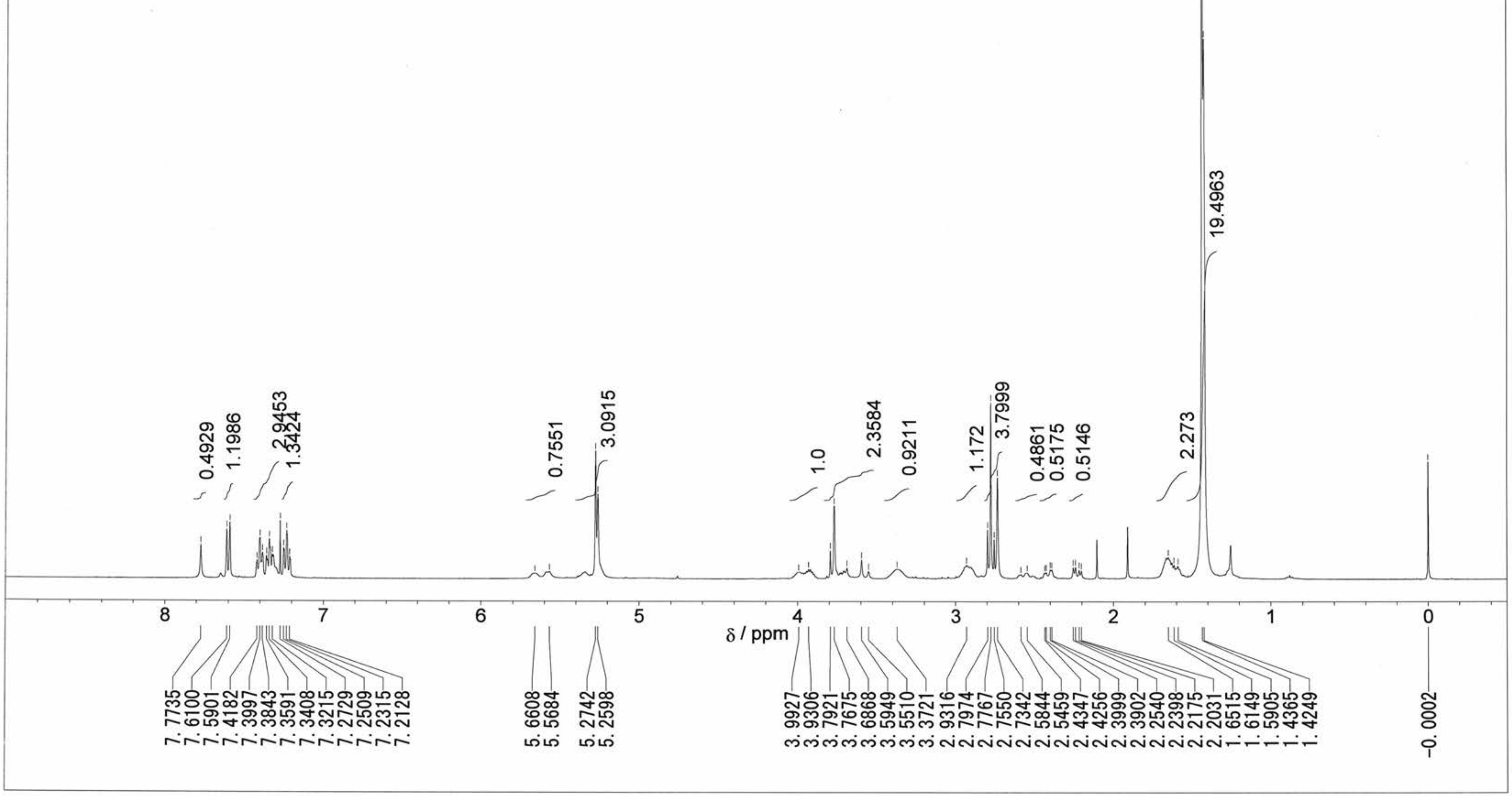
Compound **15b**



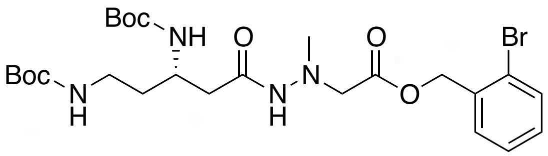
Compound 16a proton



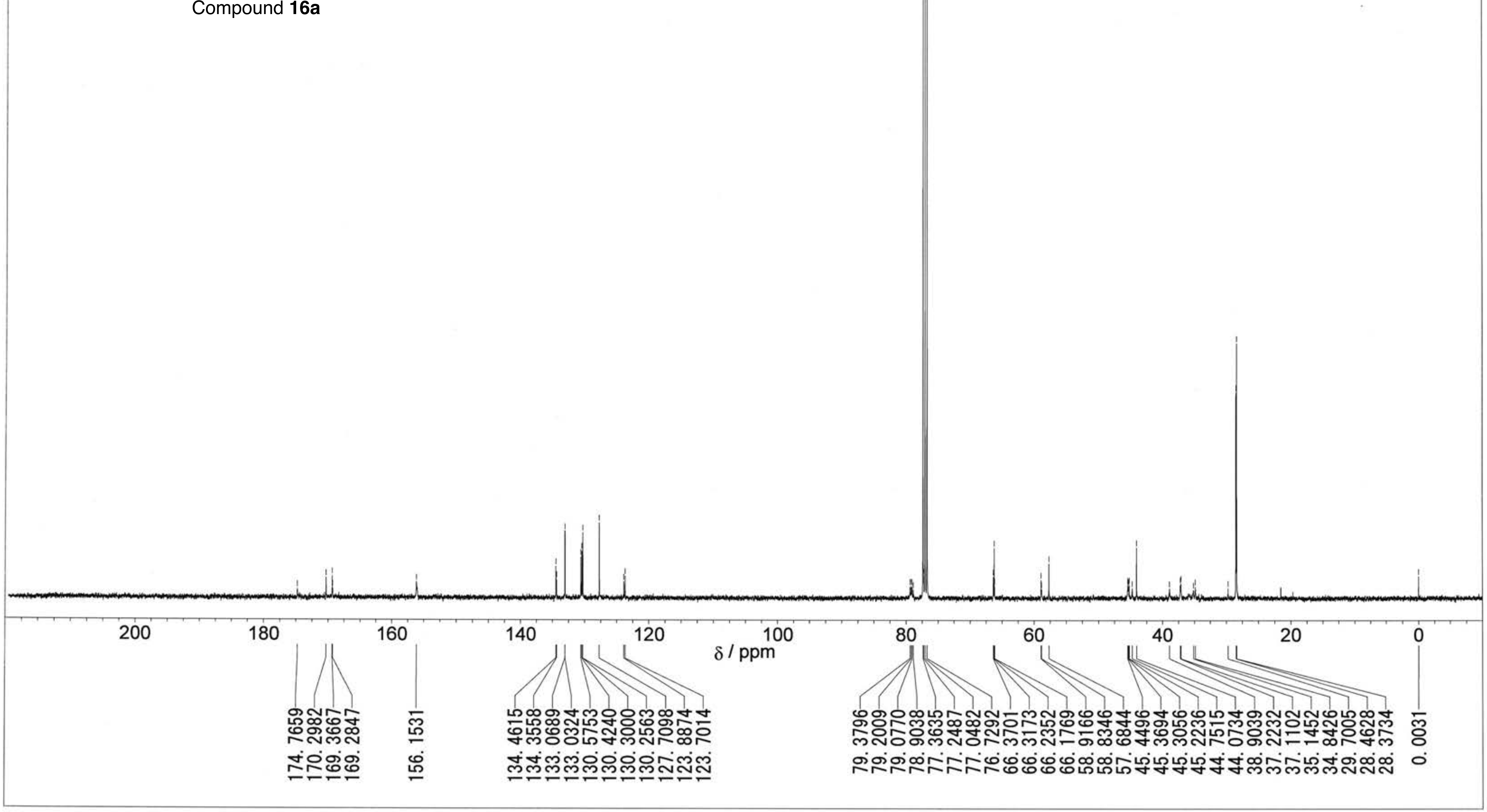
Compound **16a**



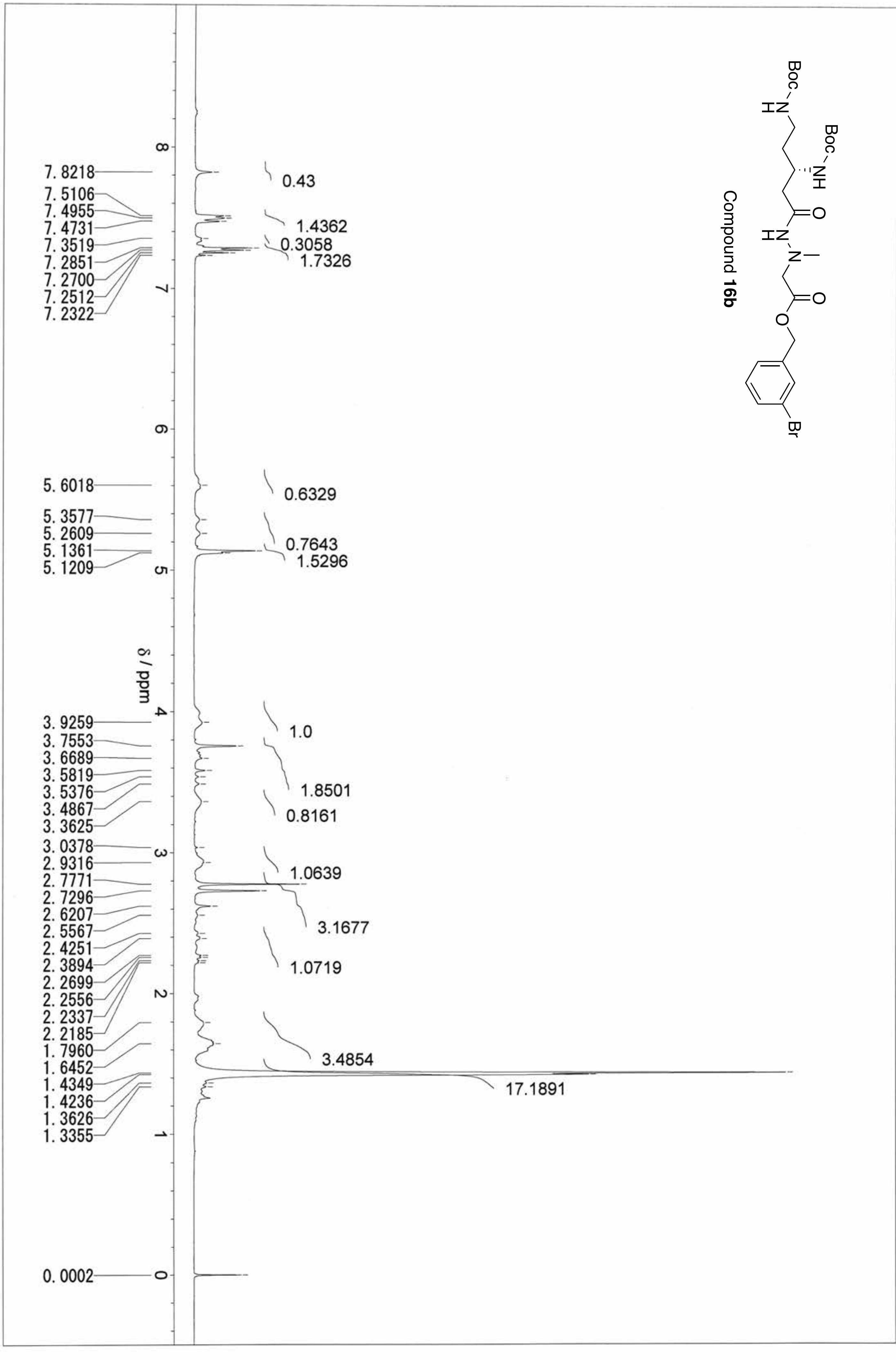
Compound 16a carbon



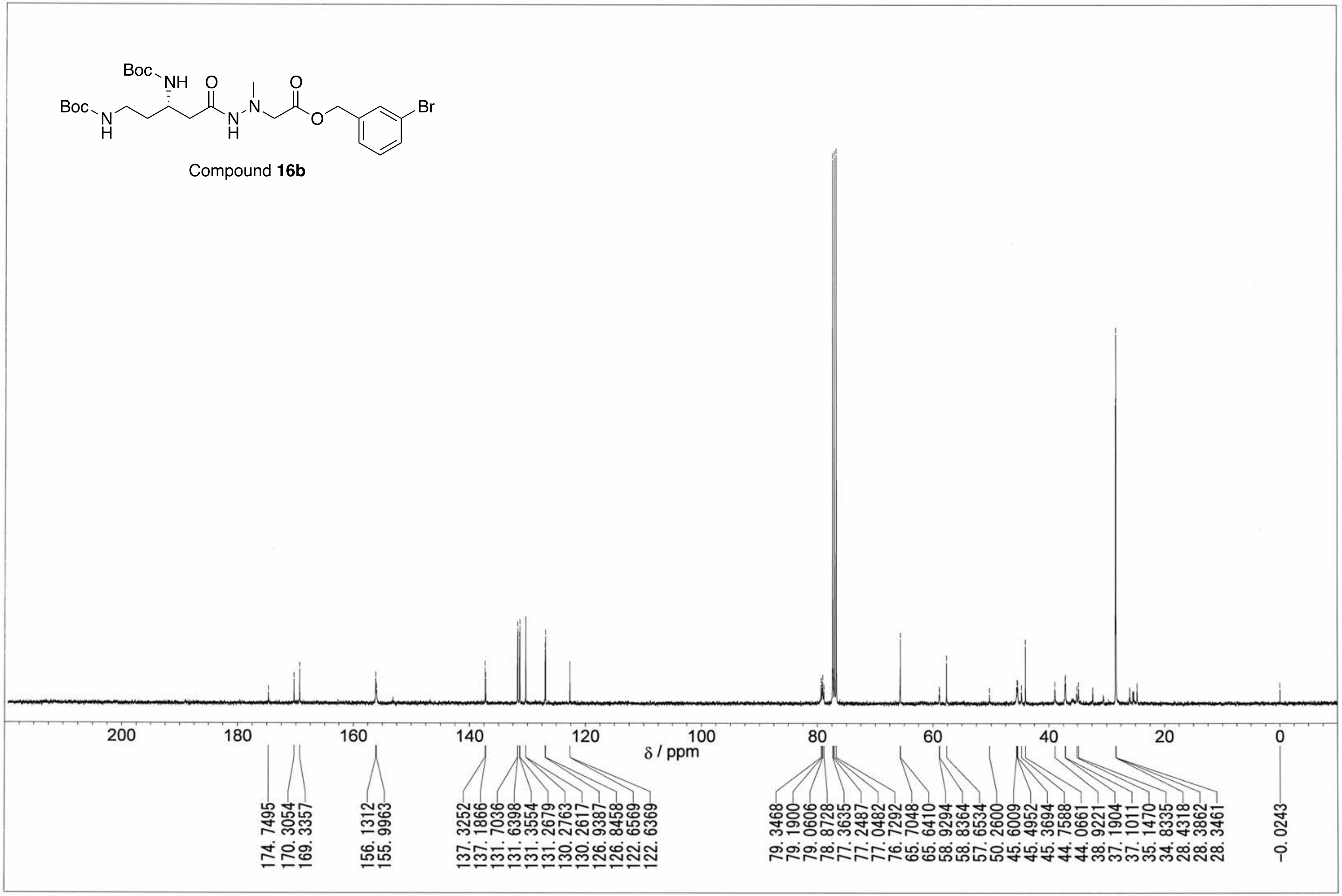
Compound **16a**



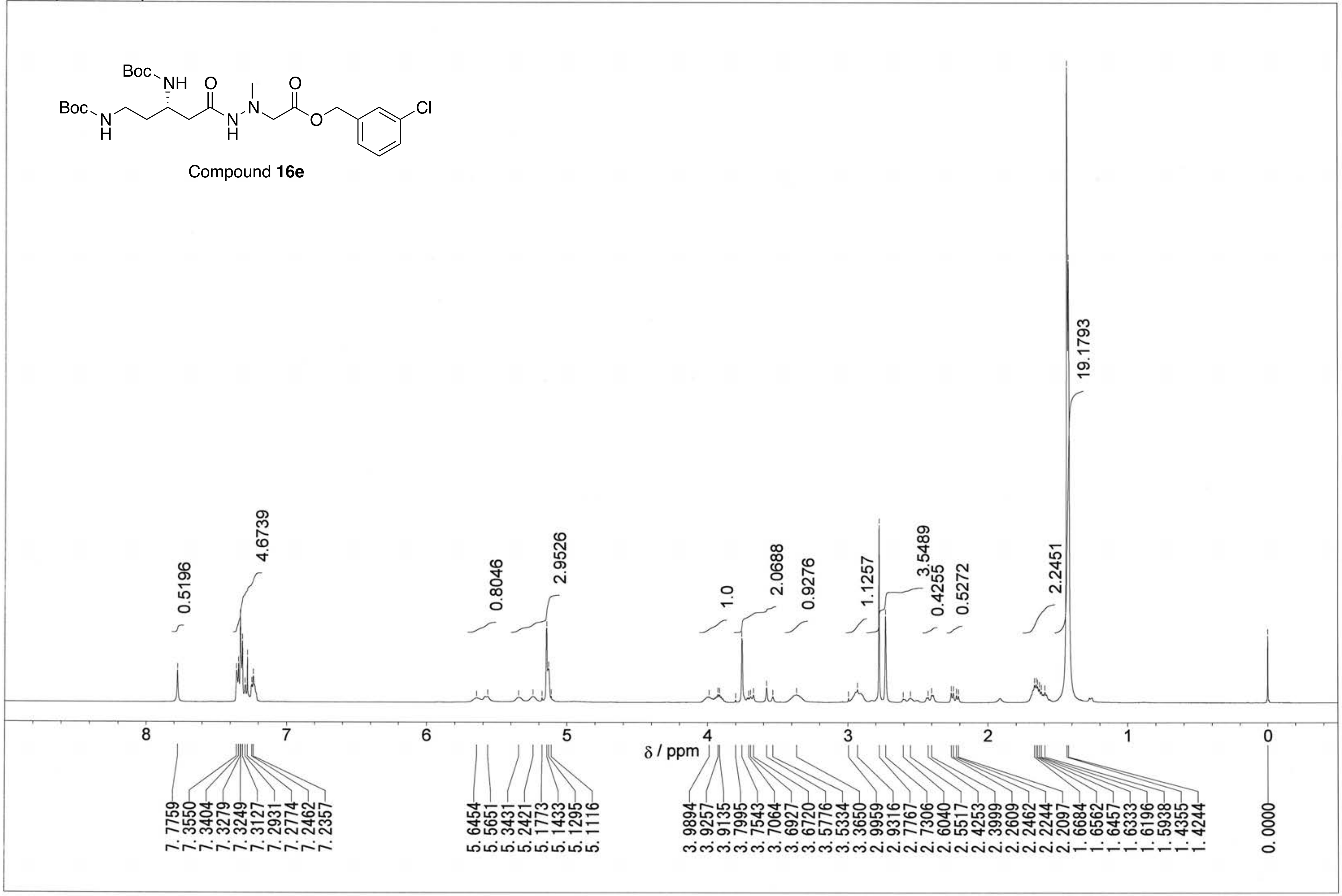
Compound 16b proton



Compound 16b carbon

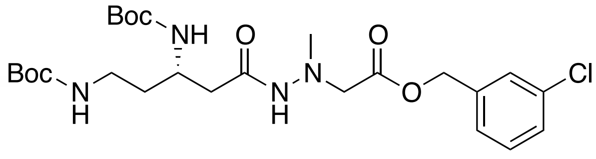


Compound 16e proton

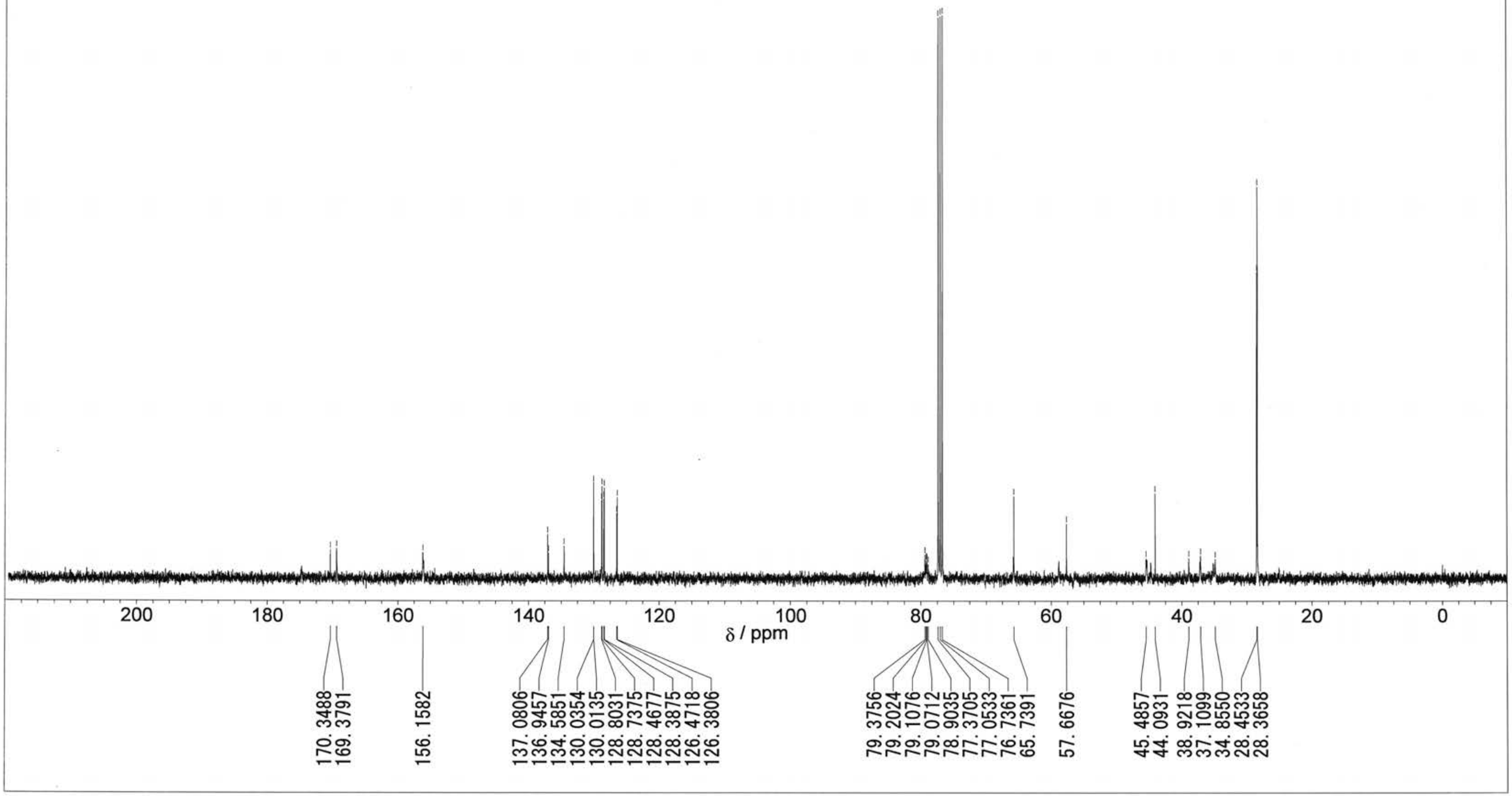


Compound **16e**

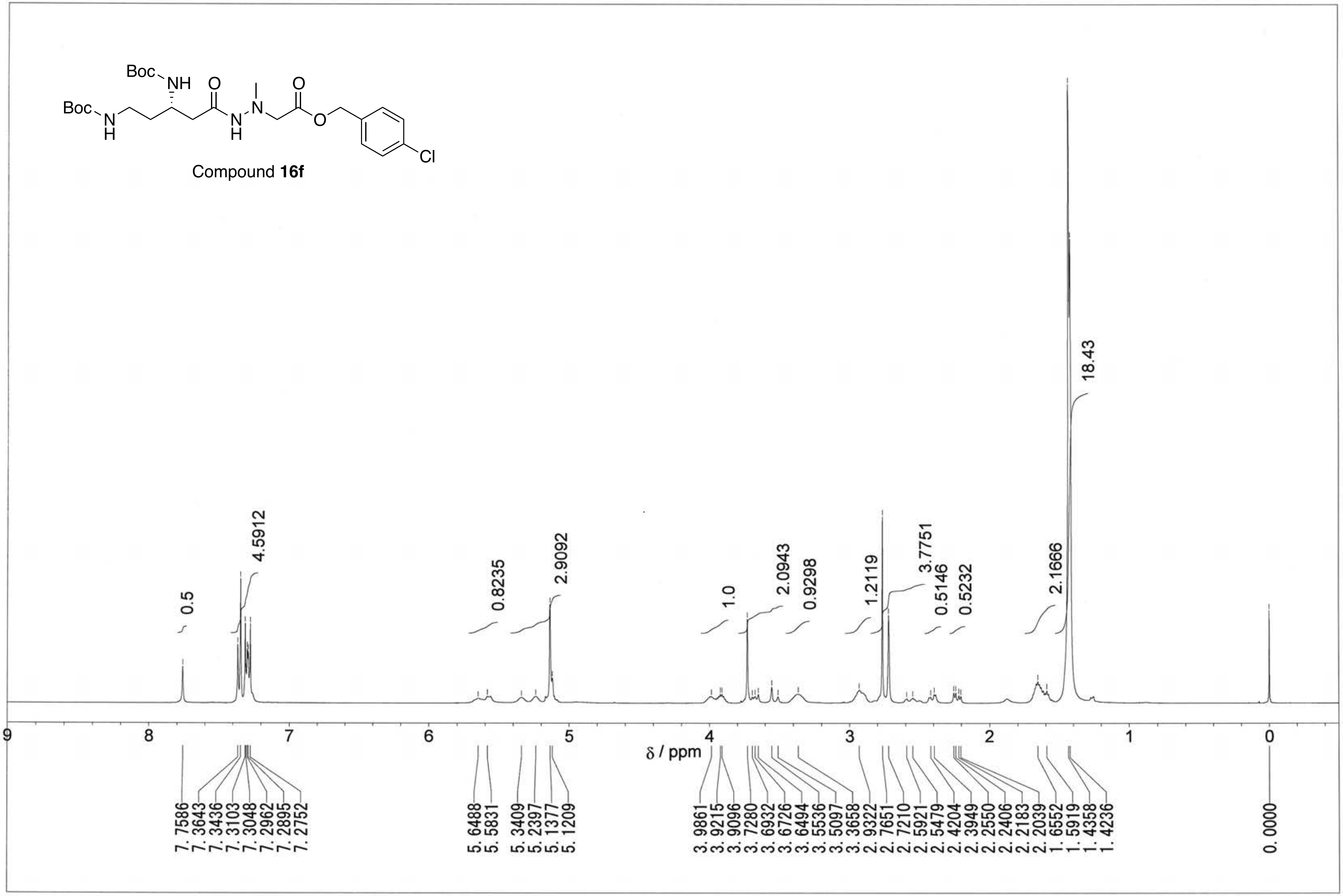
Compound 16e carbon



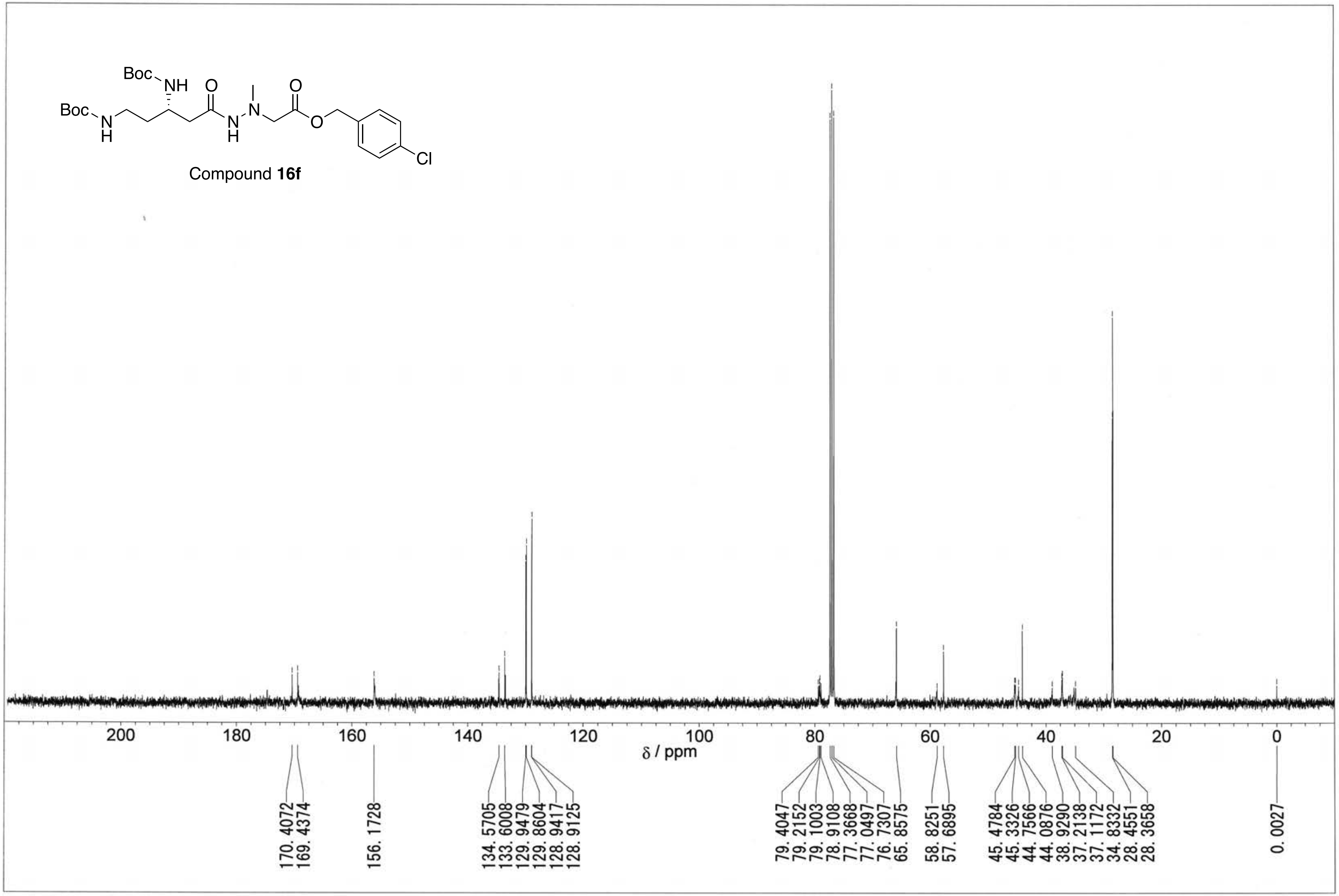
Compound **16e**



Compound 16f proton

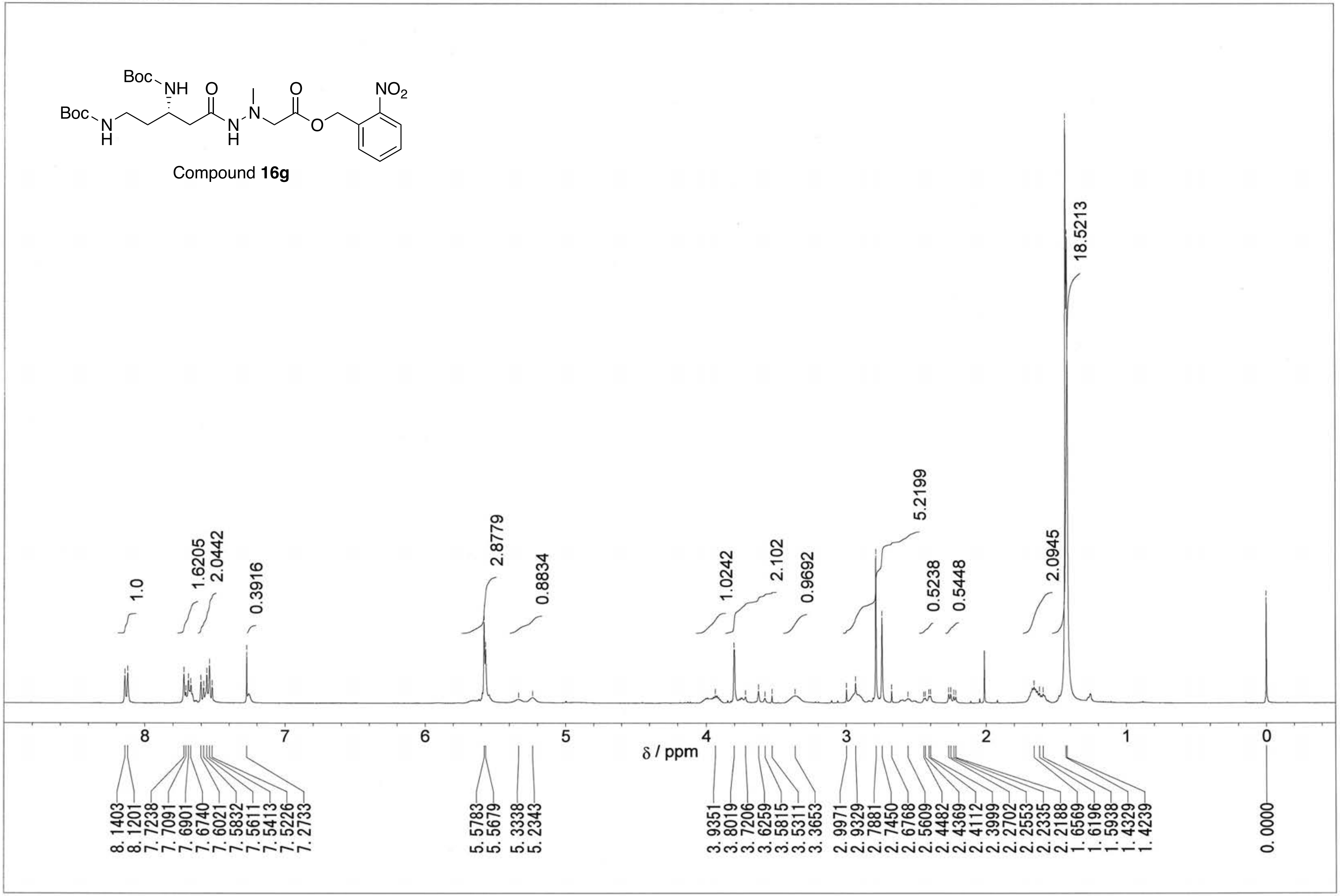


Compound 16f carbon

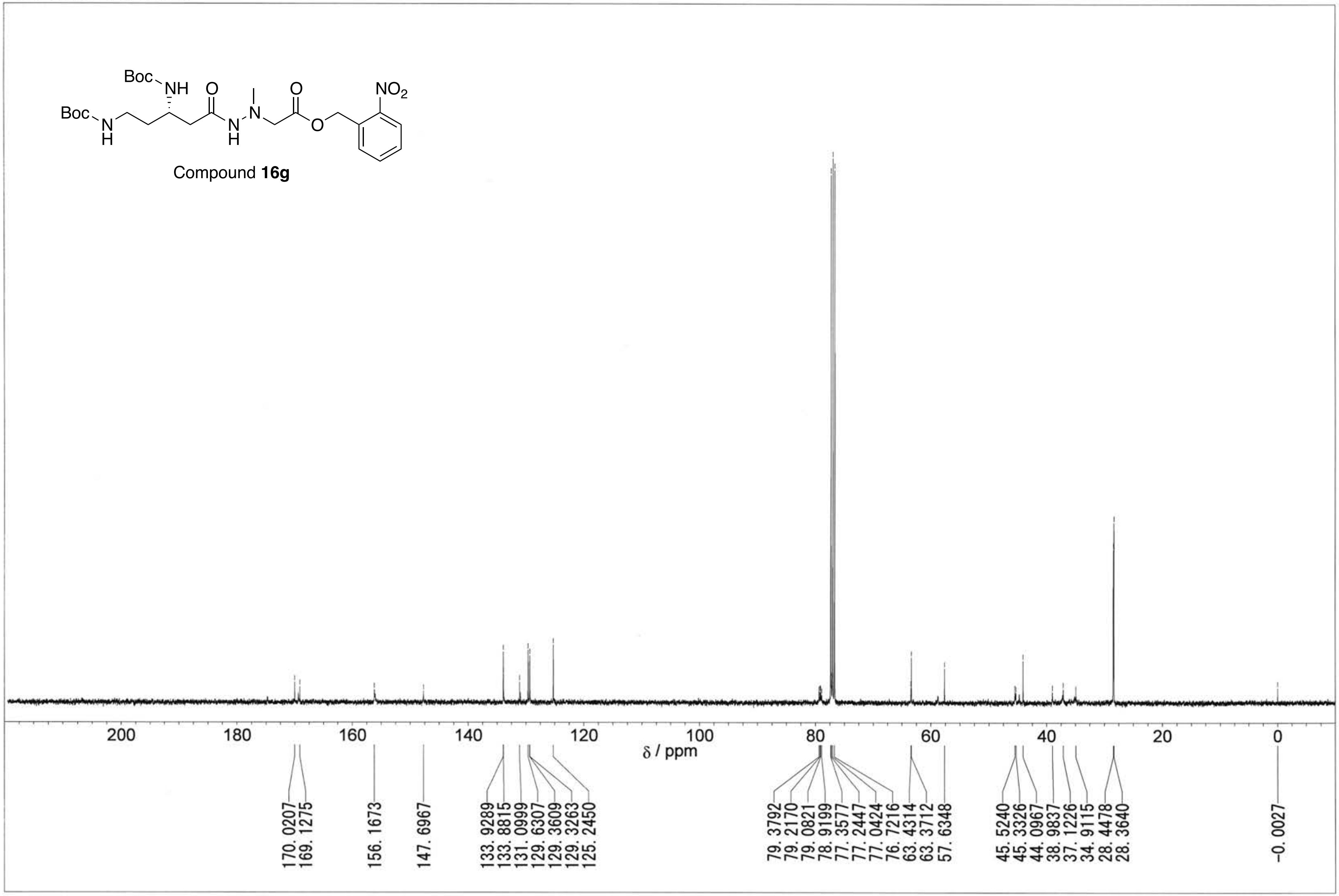


Compound **16f**

Compound 16g proton

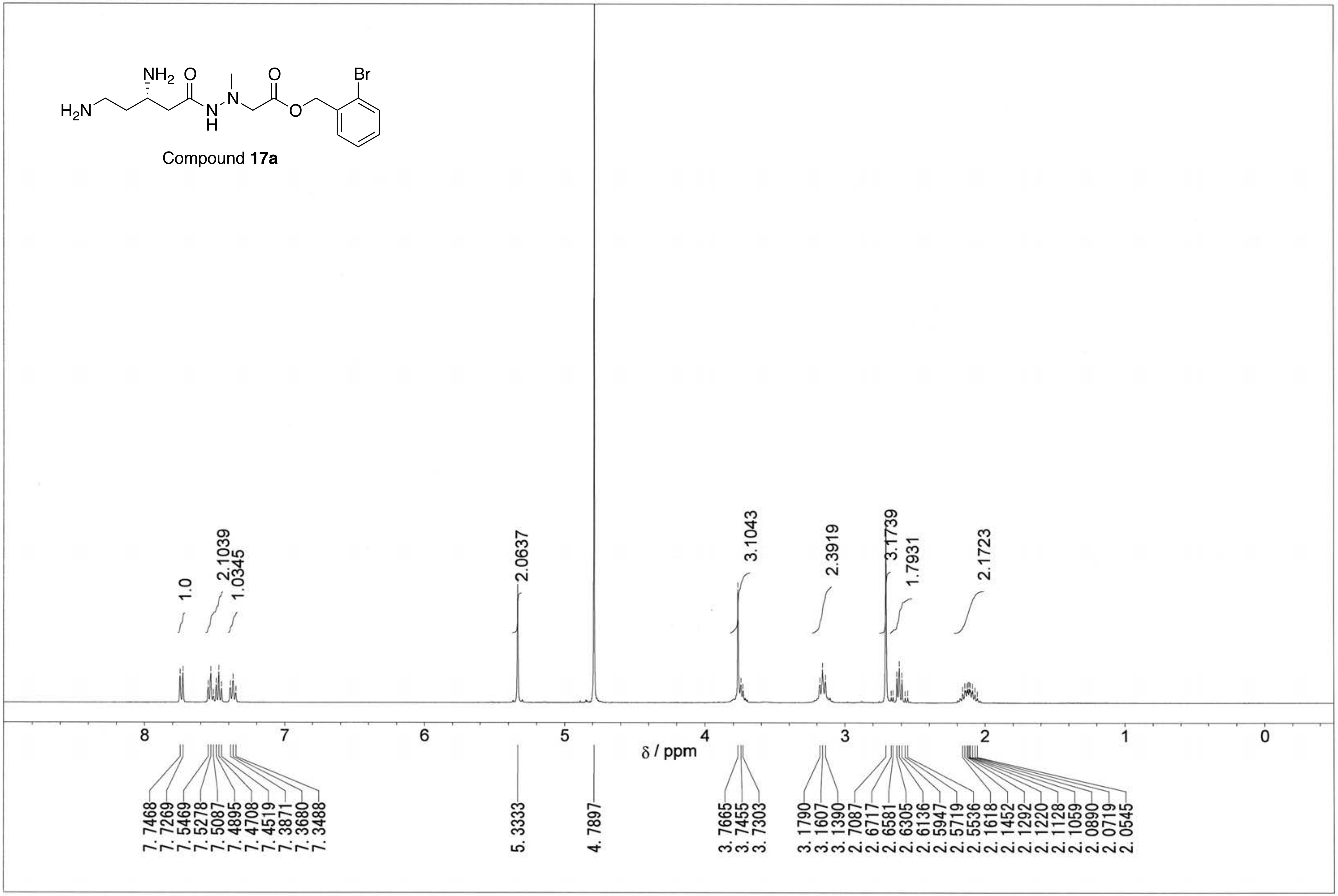


Compound 16g carbon

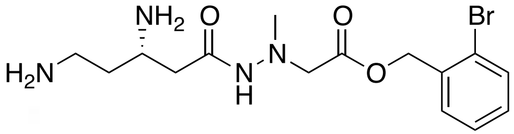


Compound **16g**

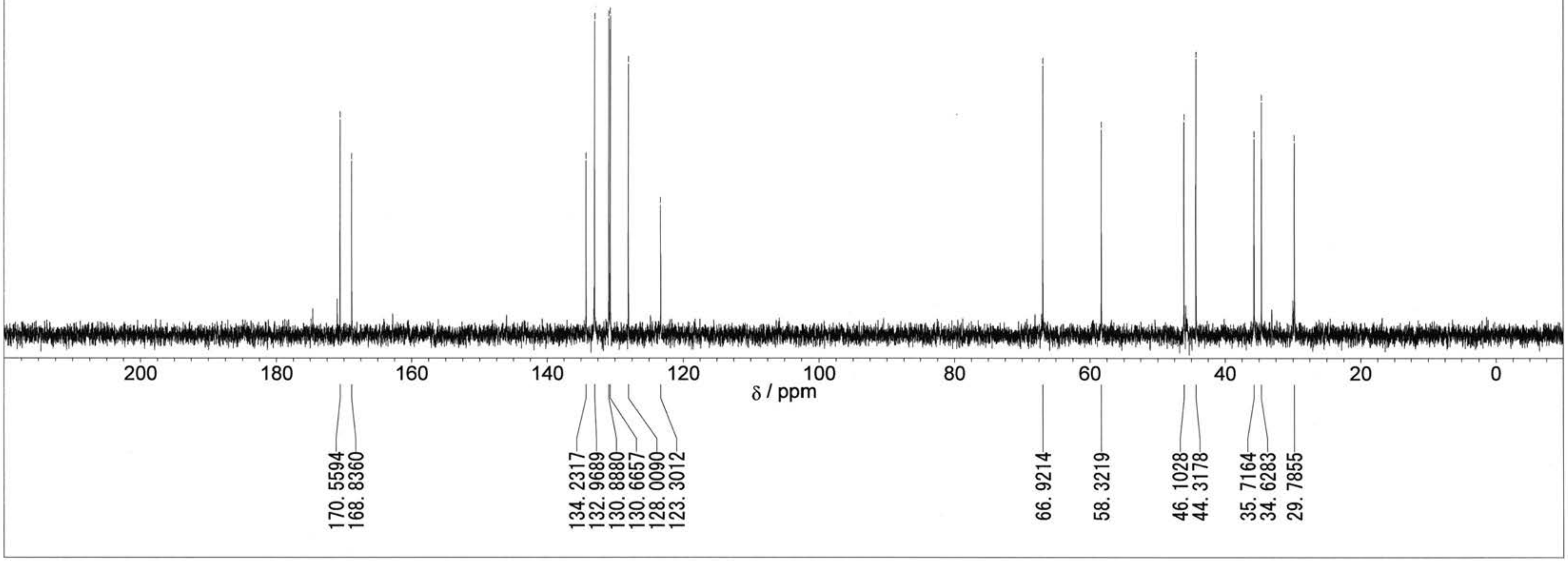
Compound 17a proton



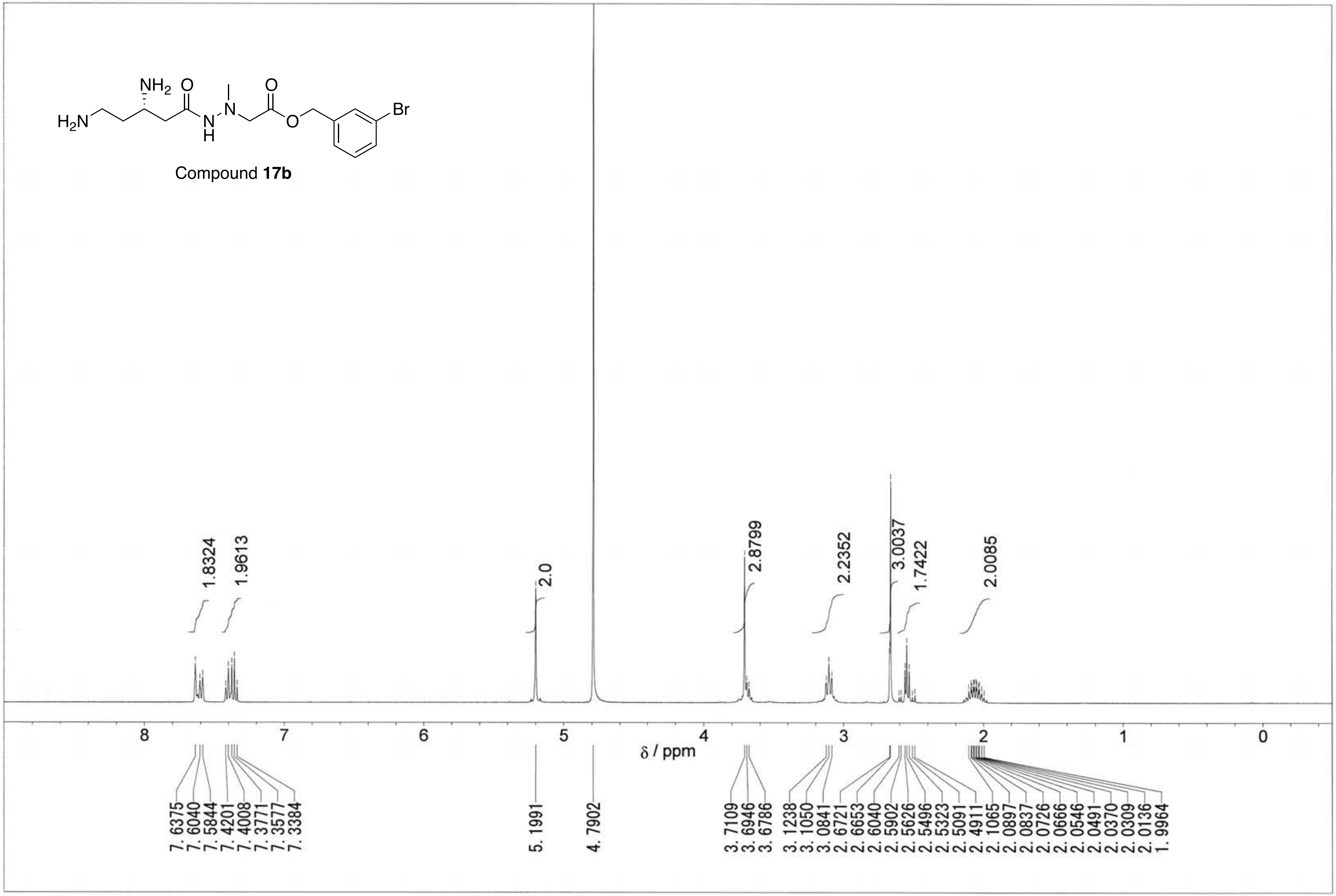
Compound 17a carbon



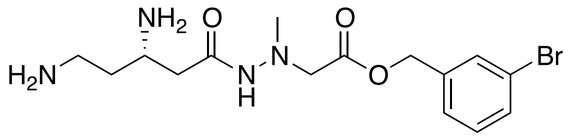
Compound **17a**



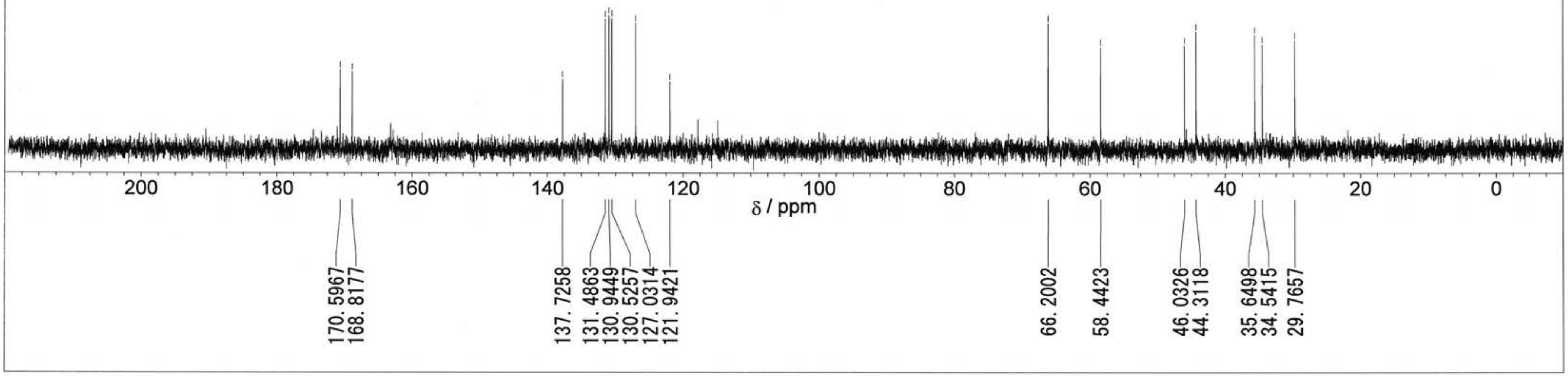
Compound 17b proton



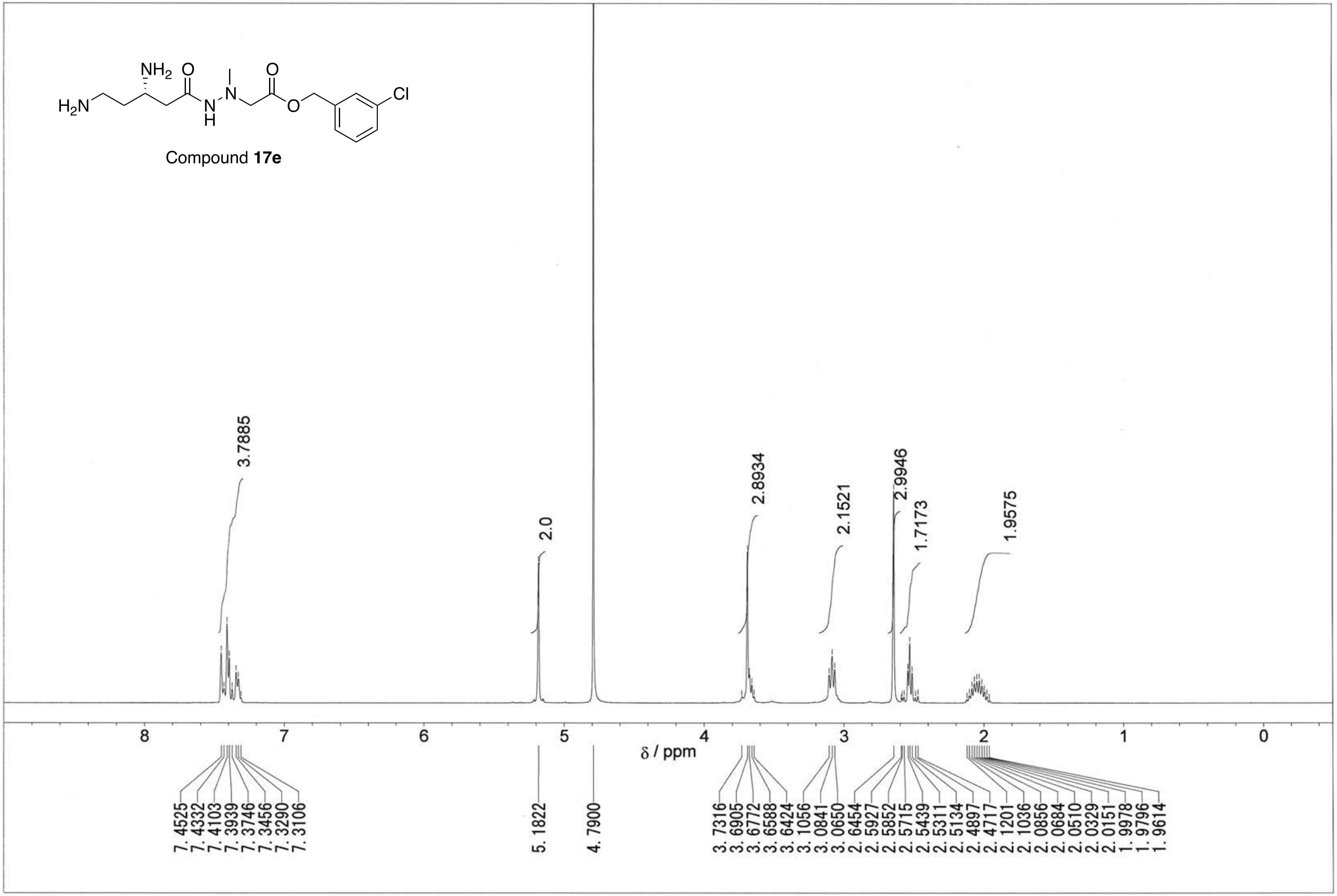
Compound 17b carbon



Compound **17b**

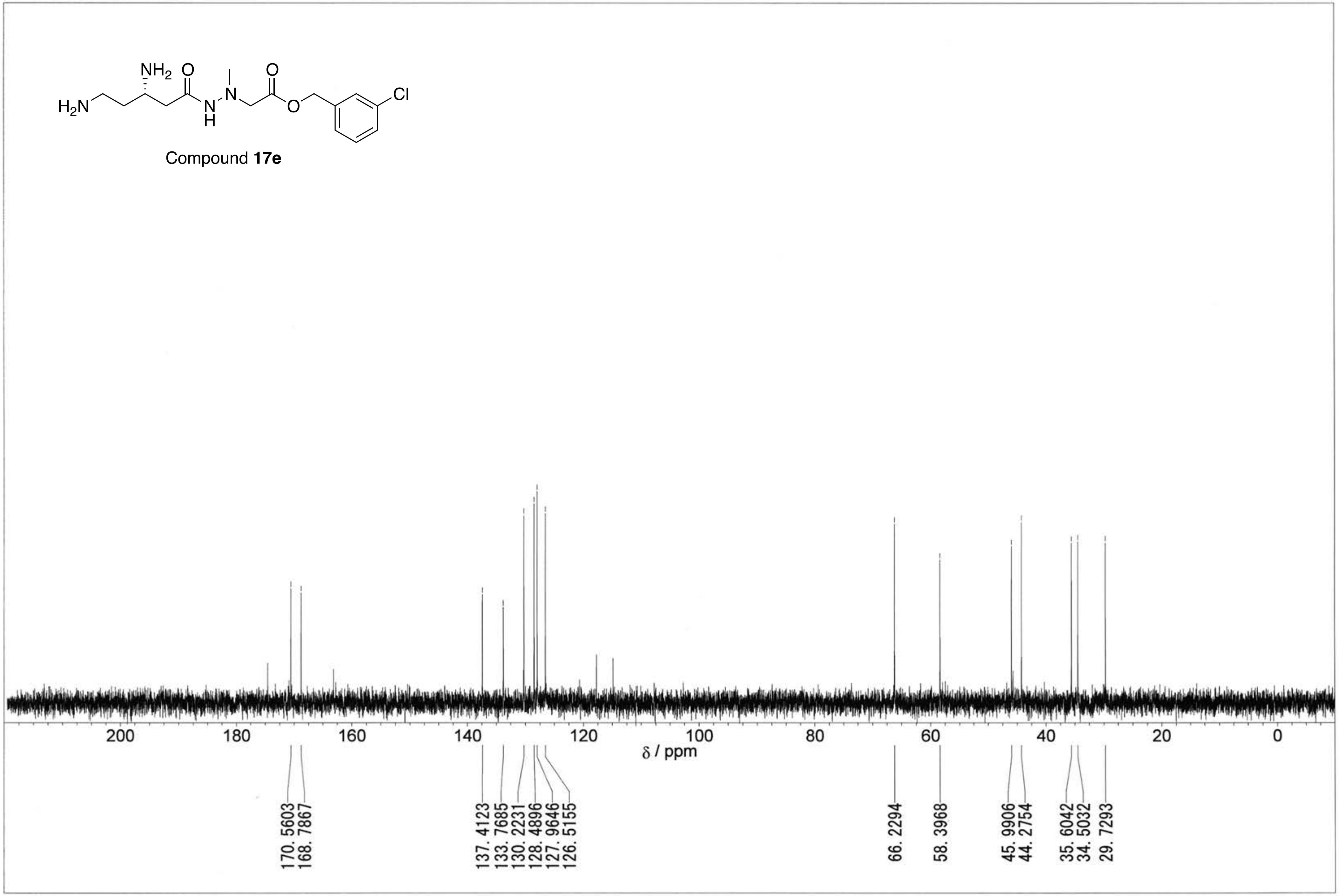


Compound 17e proton



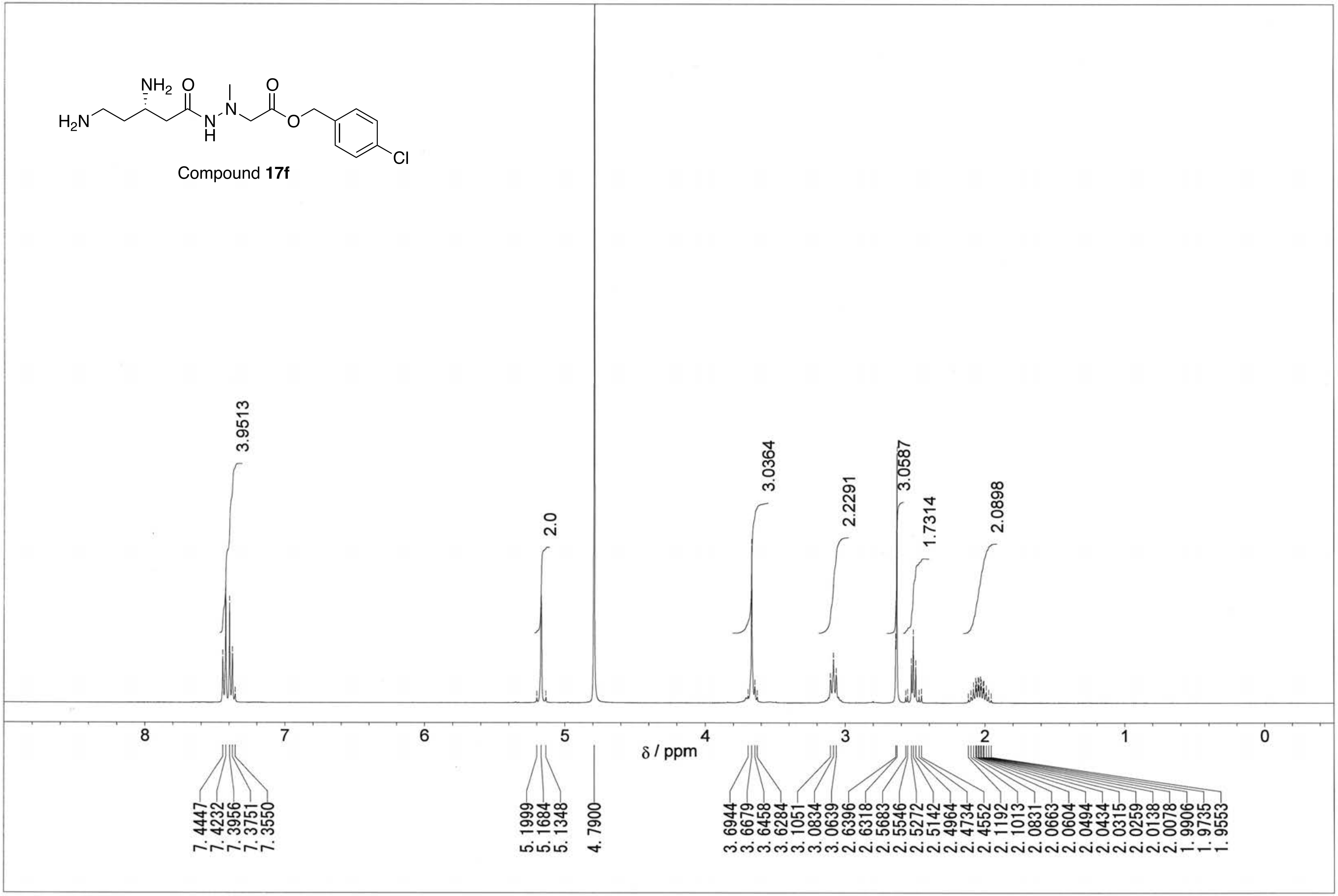
Compound **17e**

Compound 17e carbon



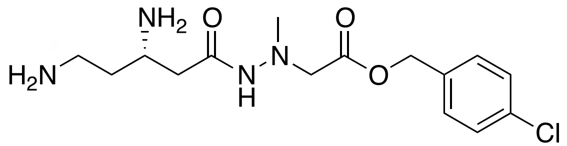
Compound **17e**

Compound 17f proton

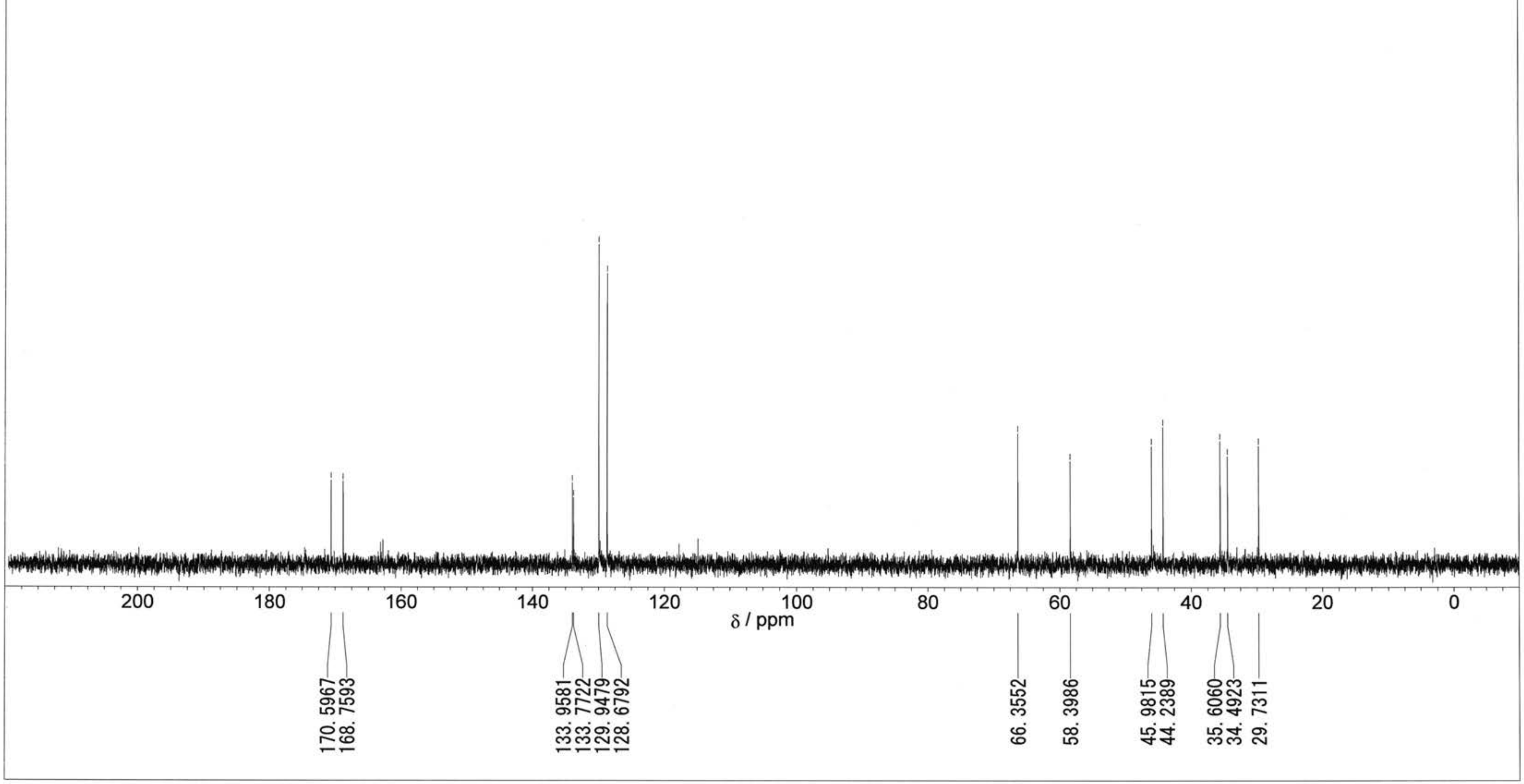


Compound **17f**

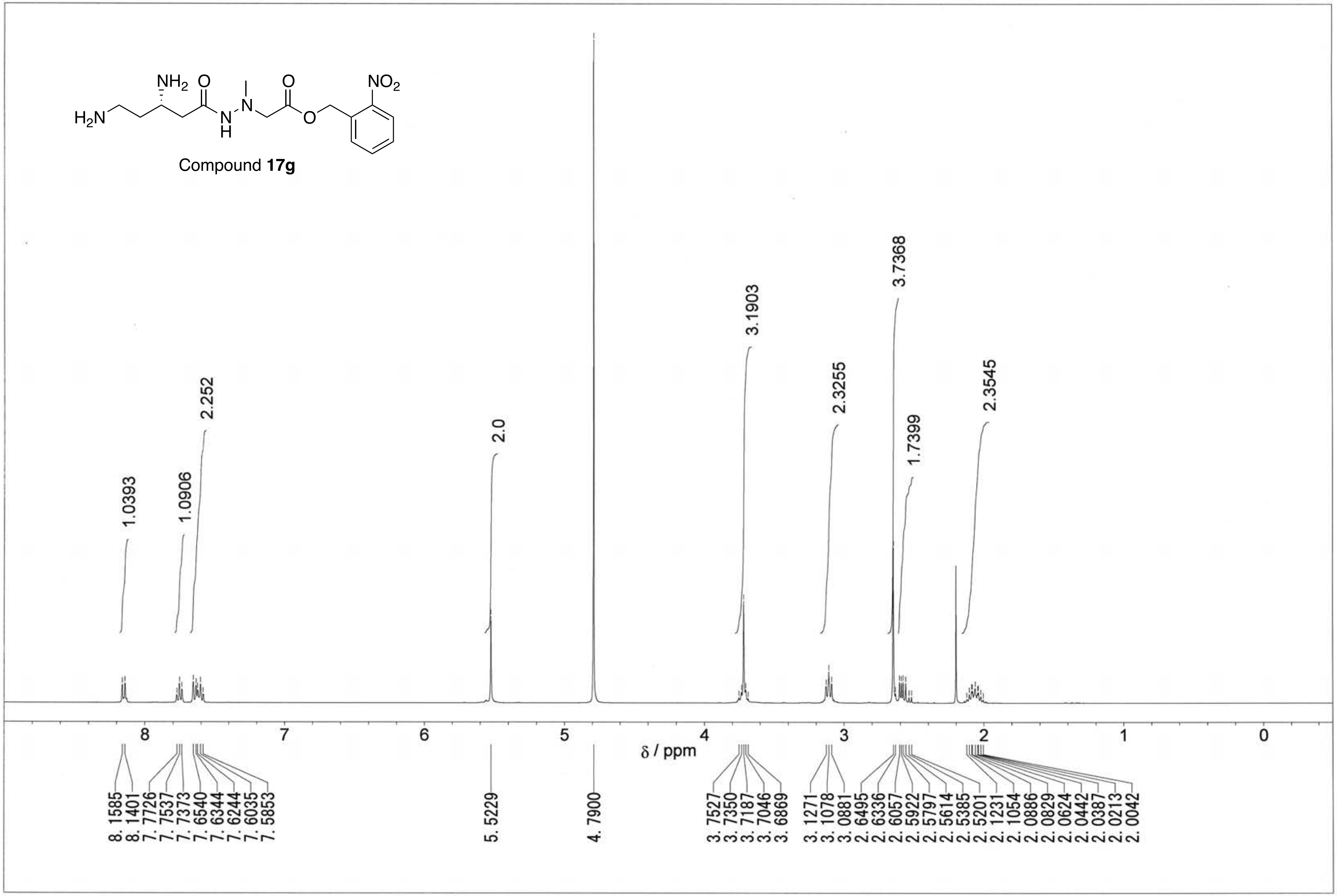
Compound 17f carbon



Compound **17f**

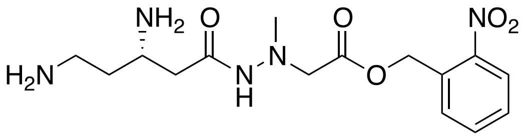


Compound 17g proton

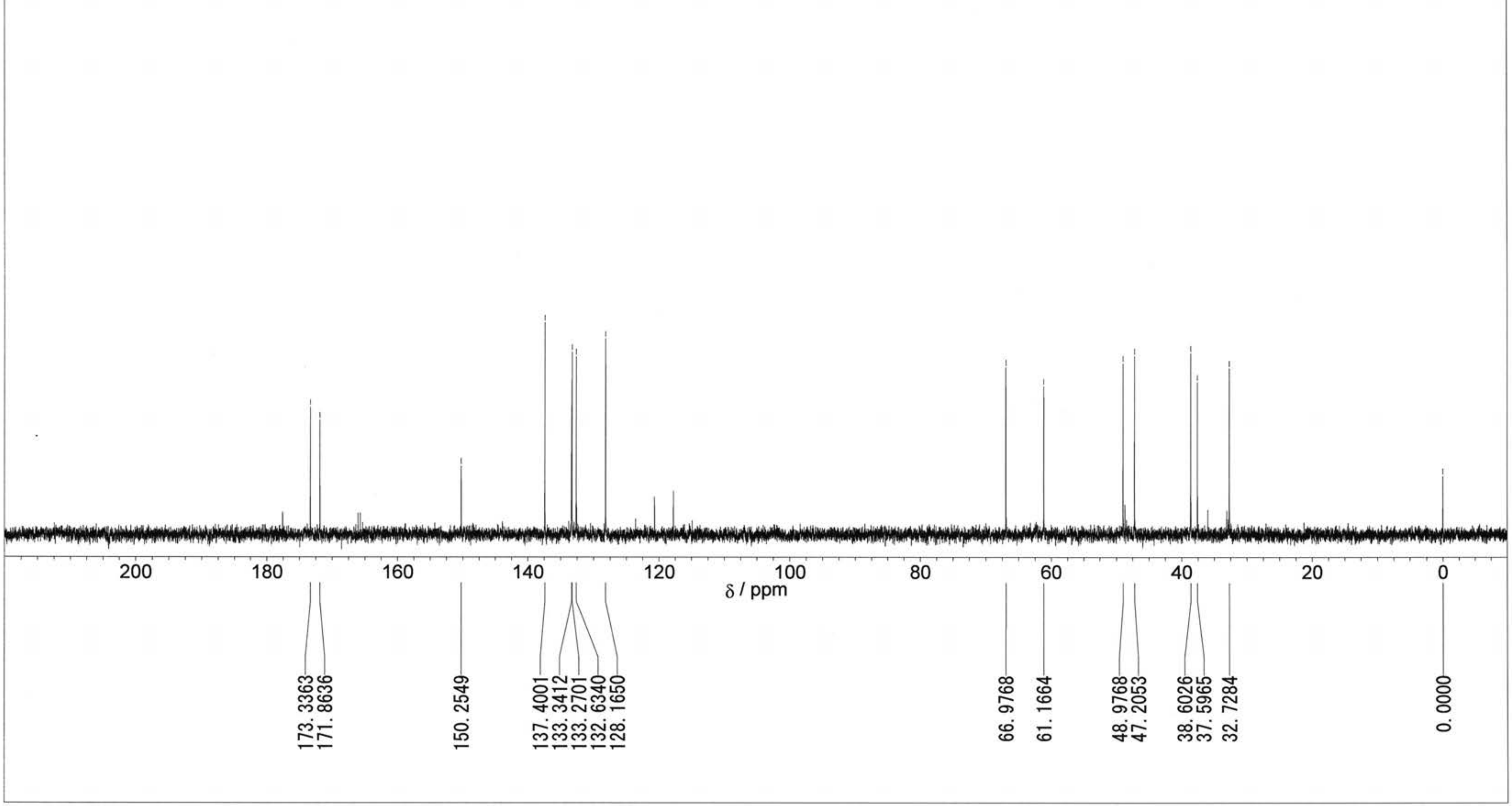


Compound **17g**

Compound 17g carbon



Compound **17g**



8. References

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(2) Hayashi, Y.; Regnier, T.; Nishiguchi, S.; Sydnes, M. O.; Hashimoto, D.; Hasegawa, J.; Katoh, T.; Kajimoto, T.; Shiozuka, M.; Matsuda, R.; Node, M.; Kiso, Y. Efficient total synthesis of (+)-negamycin, a potential chemotherapeutic agent for genetic diseases. *Chem. Commun*. **2008**, 2379-2381.