Supplementary Information

Total Synthesis of COPD Biomarker Desmosine that Crosslinks Elastin

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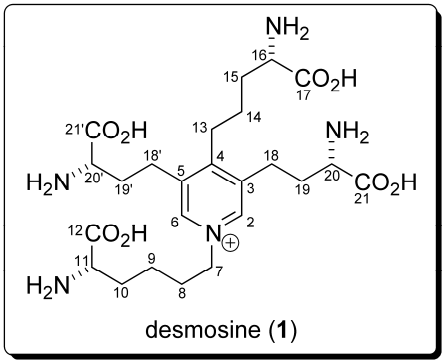
Supplementary Information

General:

All non-aqueous reactions were conducted under an atmosphere of nitrogen with magnetic stirring using freshly distilled solvents unless otherwise indicated. Tetrahydrofuran (THF) was dried by distillation from sodium/benzophenone ketyl and stored over activated molecular sieves. Dichloromethane (CH_2Cl_2), diethyl ether (Et_2O), diisopropylethylamine (iPr$_2$NEt), and acetonitrile (MeCN) were dried by distillation and stored over activated molecular sieves. Dimethylformamide (DMF) was distilled by $MgSO_4$ and stored over activated molecular sieves. Dehydrated methanol (MeOH) and ethanol (EtOH) were purchased from Kanto Chemicals (Tokyo, Japan). All reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. Analytical thin layer chromatography (TLC) was performed on Silica gel 60 F_{254} plates produced by Merck. Column chromatography was performed with acidic Silica gel 60 (spherical, 40-50 μm) or neutral Silica gel 60N (spherical, 40-50 μm) produced by Kanto Chemicals. Natural desmosine **1** was purchased from Elastin Products (St. Louis, MO).

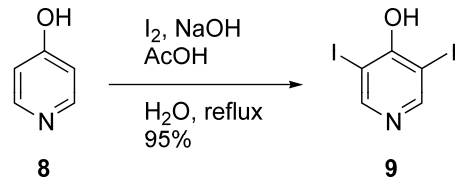
Melting points were measured by an AS one ATM-01 apparatus. Optical rotations were measured on a JASCO P-2200 digital polarimeter at the sodium lamp (λ = 589 nm) D line and are reported as follows: $[\alpha]_D^T$ (c g/100 mL, solvent). UV spectra were recorded on a JASCO V-560 UV/VIS spectrophotometer. Infrared (IR) spectra were recorded on a JASCO FT-IR 4100 spectrometer and are reported in wavenumbers (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EXC 300 spectrometer (300 MHz) or on a JEOL JNM-ECA 500 spectrometer (500 MHz). ^{1}H NMR data are reported as follows: chemical shift (δ, ppm), integration, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants (J) in Hz, assignments. ^{13}C NMR data are reported in terms of chemical shift (δ, ppm). EI-MS spectra were recorded on a Shimadzu GCMS QP-5050 instrument or on a JEOL JMS-700 instrument. FAB-MS spectra were also observed on the JEOL JMS-700. ESI-MS spectra were recorded on a JEOL JMS-T100LC instrument or on a Thermo Exactive spectrometer. JASCO HPLC systems PU-2085, MD-2010, and CO-2060 were used for the purification of **1**.

The carbon numbering on ^{1}H NMR of all compounds is corresponding with desmosine **1**.



desmosine (**1**)

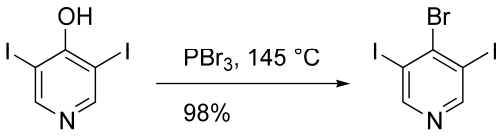
4-Hydroxy-3,5-diiodopyridine (9):



8 **9**

To a solution of starting material 4-hydroxypyridine **8** (5.01 g, 52.6 mmol, 1.0 eq) in H_2O (67 mL) was added a solution of NaOH (13.2 g, 330 mmol, 6.3 eq) and NaOAc (40.1 g, 489 mmol, 9.3 eq) in H_2O (167 mL). The solution was stirred and refluxed, and then powdered I_2 (46.9 g, 184 mmol, 3.5 eq) was added to the solution. The solution was acidified with 50% AcOH and then neutralized with 40% NaOH. This acidification-neutralization procedure was made under the reflux condition and repeated with three times in 20 min. The forth acidification was made until free iodine was precipitated. After sublimation of the I_2 by boiling, the residue was filtered, washed with boiling water, and dried to give the product **9** as a colorless powder (17.18 g, 49.5 mmol, 94%); mp 280 °C with decomposition; IR (KBr, cm^{-1}) 3166, 2952, 2880, 2805, 2360, 1605, 1525, 1334, 1270, 1038, 846, 746, 712, 597; ^{1}H NMR (300 MHz, DMSO-d_6) δ 11.95 (1H, s, OH), 8.27 (2H, s, H2/6); ^{13}C NMR (75 MHz, DMSO-d_6) δ 170.52, 143.04, 86.59; ESI-HRMS (*m*/*z*) calcd for $C_5H_2I_2NO$ $[M-H]^-$ 345.8231, found 345.8231.

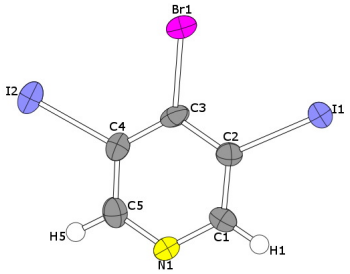
4-Bromo-3,5-diiodopyridine (5a):



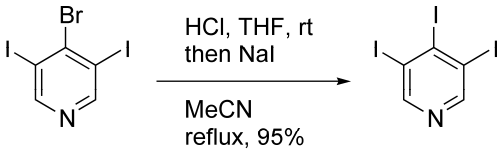
9 **5a**

To a solution of **9** (34.7 mg, 0.10 mmol, 1.0 eq) was added PBr_3 (1.0 mL, 10.6 mmol, 106 eq). The solution was heated to 145 °C, and stirring for 4.5 h at this temperature. The mixture was then cooled to room temperature, diluted with EtOAc, and quenched with a NaOH aqueous solution to pH 9 at 0 °C. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (hexane/EtOAc = 5:1) afforded **5a** as a yellow powder (40.0 mg, 0.098 mmol, 98%); R_f 0.57 (hexane/EtOAc = 5:1); mp = 158-161 °C; IR (KBr, cm^{-1}) 3066, 1815, 1524, 1496, 1406, 1684, 1205, 1161, 1086, 1003, 885, 725, 692, 517; ^{1}H NMR (300 MHz, DMSO-d_6) δ 8.85 (2H, s, H2/6); (300 MHz, $CDCl_3$)

δ8.81 (2H, s, H2/6); ^{13}C NMR (75 MHz, DMSO-d_6) δ156.1, 145.3, 102.3; (75 MHz, $CDCl_3$) δ156.6, 145.8, 100.7; EI-MS (*m/z*) calcd for $C_5H_2BrI_2N$ $[M]^+$ 408.75, found 408.85; ESI-HRMS (*m/z*) calcd for $C_5H_3BrI_2N$ $[M+H]^+$ 409.7538, found 409.7532. The structure of **5a** was also confirmed by single-crystal X-ray analysis.

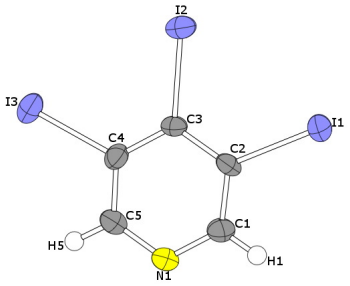


3,4,5-Triiodopyridine (5b):

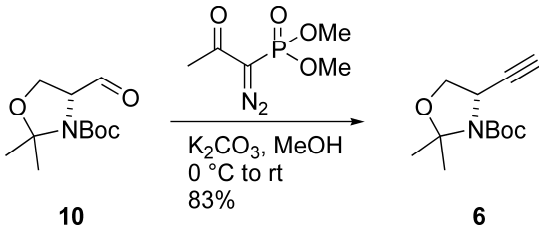


5a **5b**

To a solution of **5a** (39.9 mg, 0.097 mmol, 1.0 eq) in THF (0.5 mL) was added a 3 M HCl solution, which was diluted with Et_2O. After stirring for 5 min at room temperature, the mixture was evaporated *in vacuo*. To the mixture was added NaI (307.9 mg, 2.05 mmol, 21.2 eq) in MeCN (1.5 mL). After stirring for 24 h under reflux condition, the mixture was diluted with CH_2Cl_2, and quenched with 10% K_2CO_3 solution and 5% $NaHSO_3$ solution. The aqueous layer was then extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (hexane/EtOAc = 5:1) afforded **5b** as a colorless powder (42.0 mg, 0.092 mmol, 94%). Recrystallization of the product from benzene or hexane gave **5b** as colorless needles; R_f 0.60 (hexane/EtOAc = 5:1); mp = 150-152 °C; IR (KBr, cm^{-1}) 3434, 3060, 1817, 1520, 1485, 1376, 1198, 1157, 1072, 885, 722, 695, 676, 504; ^{1}H NMR (300 MHz, $CDCl_3$) δ8.79 (2H, s, H2/6); ^{13}C NMR (75 MHz, $CDCl_3$) δ154.3, 107.7; EI-MS (*m/z*) calcd for $C_5H_2I_3N$ $[M]^+$ 456.73, found 456.73; ESI-HRMS (*m/z*) calcd for $C_5H_3I_3N$ $[M+H]^+$ 457.7400, found 457.7391. The structure of **5b** was also confirmed by single-crystal X-ray analysis.

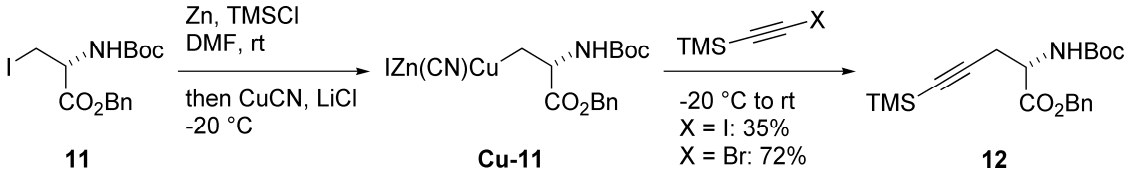


2,2-(*S*)-Dimethyl-3-(*tert*-butoxycarbonyl)-4-ethynyloxazolidine (6):



To a solution of Garner's aldehyde **10**[1] (1.28 g, 5.6 mmol, 1.0 eq) and dimethyl-1-diazo-2-oxopropyl phosphonate (1.61 g, 8.4 mmol, 1.5 eq) in MeOH (20 mL) was added K_2CO_3 (1.55 mg, 11.2 mmol, 2.0 eq) in one portion at 0 °C. After stirring for 1 h at 0 °C, then for 2 h at room temperature, the mixture was diluted with EtOAc, quenched with a saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (hexane/EtOAc = 20:1) afforded **6** as a colorless oil (1.05 g, 4.65 mmol, 83%); R_f 0.50 (hexane/EtOAc = 5:1); $[\alpha]_D^{20}$ 90.9 (*c* 0.1, $CHCl_3$) (lit value: $[\alpha]_D^{20}$ -96.5 (*c* 1.23, $CHCl_3$)); IR (neat, cm^{-1}) 3293, 3261, 2981, 2935, 2879, 2116, 1702, 1477, 1458, 1377, 1265, 1245, 1210, 1180, 1150, 1095, 1067, 1055, 855, 806, 770, 676; ^1H NMR (300 MHz, $CDCl_3$) δ 4.48-4.58 (1H, m, H20), 3.97-4.05 (2H, m, CH_2), 2.26 (1H, s, H19), 1.47-1.61 (15H, m, Me/*t*Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.5, 151.3, 94.3, 93.9, 82.7, 82.3, 80.7, 80.2, 70.5, 70.1, 68.6, 48.3, 28.3, 26.8, 25.8, 25.1, 24.3; EI-MS (*m/z*) calcd for $C_{11}H_{16}NO_3$ $[M\text{-}CH_3]^+$ 210.11, found 210.10.

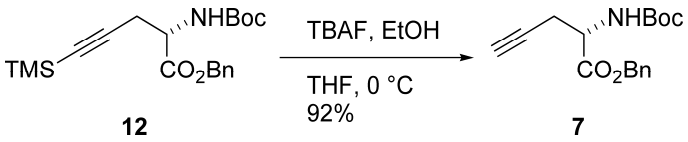
Benzyl 2-(*S*)-[(*tert*-butoxycarbonyl)amino]-5-(trimethylsilyl)pent-4-ynoate (12):



CuCN (465.6 mg, 5.20 mmol, 1.0 eq) and LiCl (437.4 mg, 10.3 mmol, 2.0 eq) were dried together by heating under vacuum at 150 °C for 2 h in one flask. While the CuCN and LiCl were drying, zinc powder (2.09 g, 32.0 mmol, 6.2 eq) dried under vacuum with heating using a heat gun over 5 min. To the flask containing the zinc powder were added DMF (2.6 mL) and TMSCl (198 μL, 1.56 mmol, 5 mol% to Zn) at room temperature. After stirring for 30 min at room temperature, to this suspension was slowly added a solution of benzyl 2-(*S*)-[(*tert*-butoxycarbonyl)amino]-3-iodopropanoate **11**[2] (2.10 g, 5.17 mmol, 1.0 eq) in DMF (2.6 mL, washed with 0.3 mL × 2). After confirming generation of Zn-inserted **11** judged on TLC, to another flask containing the CuCN and LiCl in DMF (10.0 mL) cooling to -20 °C was added a solution of Zn-inserted **11** over 30 min to produce **Cu-11**.

After stirring for 15 min at -20 °C, freshly prepared (2-bromoethynyl)trimethylsilane (1.14 g, 6.44 mmol, 1.25 eq) (or, (2-iodoethynyl)trimethylsilane) was added dropwise to the reaction mixture of **Cu-11** over 5 min. The mixture was then allowed to slowly warm to room temperature and continue stirring for 16 h. The mixture was diluted with EtOAc, and quenched with a saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (hexane/EtOAc = 10:1) afforded **12** as a yellow oil (1.40 g, 3.74 mmol, 72%); R_f 0.47 (hexane/EtOAc = 5:1); $[\alpha]_D^{25}$ +6.8 (*c* 0.1, $CHCl_3$); IR (neat, cm^{-1}) 3442, 3373, 3091, 3066, 3034, 2960, 2927, 2856, 2179, 1949, 1748, 1717, 1500, 1456, 1367, 1344, 1251, 1215, 1165, 1051, 1029, 1008, 842, 760, 698, 641; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30-7.40 (5H, m, Bn), 5.31 (1H, d, J = 8.1 Hz, NH), 5.24 (1H, d, J = 12.3 Hz, Bn), 5.15 (1H, d, J = 12.3 Hz, Bn), 4.48-4.54 (1H, m, H16), 2.82 (1H, dd, J = 16.8, 4.8 Hz, H15), 2.72 (1H, dd, J = 16.8, 4.8 Hz, H15), 1.45 (9H, s, *t*Bu), 0.13 (9H, s, TMS); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 155.2, 135.4, 128.7, 128.5, 128.3, 100.9, 88.6, 80.2, 67.4, 52.3, 28.4, 24.3, 0.1; EI-MS (*m/z*) calcd for $C_{16}H_{21}NO_4Si$ $[M-(CH_3)_3]^+$ 319.12, found 319.20; FAB-MS (*m/z*) calcd for $C_{20}H_{30}NO_4Si$ $[M+H]^+$ 376.19, found 376.22.

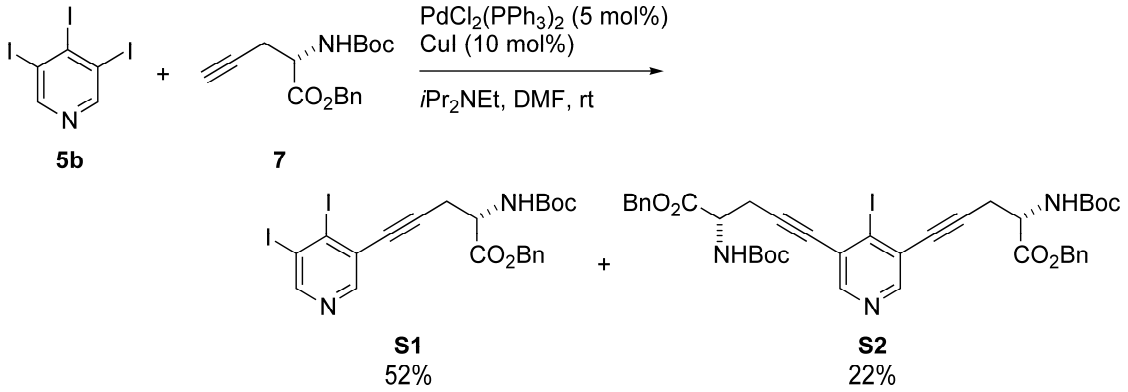
Benzyl 2-(*S*)-[(*tert*-butoxycarbonyl)amino]pent-4-ynoate (7):



12 **7**

To a solution of **12** (462.3 mg, 1.23 mmol, 1.0 eq) in THF (16.4 mL) and EtOH (358 μL, 6.16 mmol, 5.0 eq) cooled to 0 °C was added TBAF (607 μL, 616 μmol, 1.0 M solution in THF, 0.5 eq). After stirring at 0 °C for 1.5 h, the reaction mixture was diluted with EtOAc, and quenched with saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (hexane/EtOAc = 5:1) afforded **7** as a yellow oil (358.1 mg, 1.18 mmol, 92%); R_f 0.43 (hexane/EtOAc = 5:1); $[\alpha]_D^{20}$ +8.1 (*c* 0.1, $CHCl_3$); IR (neat, cm^{-1}) 3390, 3298, 3090, 3066, 3034, 2978, 2933, 2123, 1955, 1455, 1428, 1155, 1696, 1506, 1062, 1022, 988, 753, 698, 653; ^1H NMR (300 MHz, $CDCl_3$) δ 7.30-7.40 (5H, m, Bn), 5.37 (1H, d, J = 7.8 Hz, NH), 5.24 (1H, d, J = 12.3 Hz, Bn), 5.18 (1H, d, J = 12.0 Hz, Bn), 4.54-4.49 (1H, m, H16), 2.83-2.67 (2H, m, H15), 2.01 (1H, t, J = 2.7 Hz, H13), 1.44 (9H, s, *t*Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.7, 155.2, 135.3 128.7, 128.6 128.4, 80.3, 78.6, 71.8, 67.6, 52.2, 28.4, 23.0; FAB-MS (*m/z*) calcd for $C_{17}H_{22}NO_4Si$ $[M+H]^+$ 304.15, found 304.24.

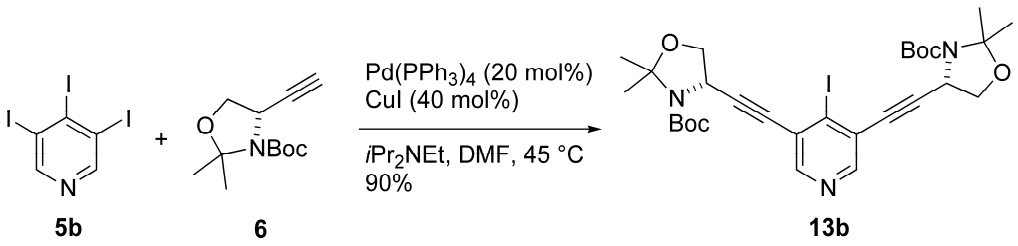
Benzyl 2-(*S*)-[(*tert*-butoxycarbonyl)amino]-5-(4',5'-diiodopyridin-3'-yl)pent-4-ynoate (S1), and benzyl 5-{5'-[4"-(*S*)-benzyloxycarbonyl-4"-(*tert*-butoxycarbonyl)amino-but-1"-ynyl]-4'-iodopyridin-3'-yl}-2-(*S*)-[(*tert*-butoxycarbonyl)amino]pent-4-ynoate (S2):



5b **7** **S1** 52% **S2** 22%

A solution of **5b** (16.2 mg, 0.035 mmol, 1.0 eq), **7** (22.5 mg, 0.074 mmol, 2.1 eq) in DMF (0.4 mL) and *i*Pr_2NEt (61.7 μL) was added CuI (0.6 mg, 3.15 μmol, 10 mol%) and $PdCl_2(PPh_3)_2$ (1.2 mg, 1.71 μmol, 5 mol%). After stirring at room temperature for 15.5 h, the reaction mixture was diluted with EtOAc, and quenched with saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (hexane/Et_2O = 4:1) afforded **S1** (11.7 mg, 0.019 mmol, 52%), **S2** (6.4 mg, 0.008 mg, 22%), and recovered **5b** as a slightly yellow solid. **S1**: a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.73 (1H, s, H6), 8.27 (1H, s, H2), 7.27-7.37 (5H, m, Bn), 5.51 (1H, d, J = 8.1 Hz, NH), 5.27 (1H, d, J = 12.3 Hz, Bn), 5.19 (1H, d, J = 12.0 Hz, Bn), 4.64 (1H, dt, J = 8.1, 4.8 Hz, CH), 3.08 (2H, d, J = 4.8 Hz, CH_2), 1.45 (9H, s, *t*Bu); EI-MS (*m*/*z*) calcd for $C_{22}H_{22}I_2N_2O_4$ $[M]^+$ 631.97, found 632.10. **S2**: a yellow oil; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.21 (2H, s, H2/6), 7.28-7.38 (10H, m, Bn), 5.53 (2H, d, J = 8.4 Hz, NH), 5.27 (2H, d, J = 12.0 Hz, Bn), 5.19 (2H, d, J = 12.0 Hz, Bn), 4.64 (2H, dt, J = 8.1, 4.2 Hz, CH), 3.07 (4H, d, J = 4.2 Hz, CH_2), 1.45 (18H, s, *t*Bu).

3,5-Bis{2'-(*S*)-[2",2"-dimethyl-3"-(*tert*-butoxycarbonyl)-4"-ethynyloxazolidin]ethyn-1'-yl}-4-iodopyridine (13b):

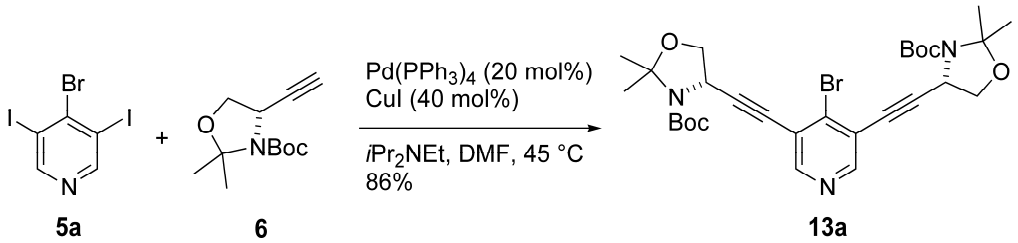


5b **6** **13b**

A solution of **5b** (14.8 mg, 32.4 μmol, 1.0 eq), **6** (27.6 mg, 122.5 μmol, 3.8 eq), $Pd(PPh_3)_4$ (7.4 mg, 6.40 μmol, 20 mol%), and CuI (2.6 mg, 13.7 μmol, 42 mol%) in DMF (1.6 mL, 20 μM to **5b**) was degassed by freeze/pump/thaw techniques. *i*Pr_2NEt (320 μL) was added to the resulting solution. After stirring at 45 °C for 30 h, the reaction mixture was diluted with EtOAc, and quenched with saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (hexane/Et_2O = 1:1) afforded **13b** (19.1 mg, 29.3 μmol, 90%) as a colorless solid; R_f 0.40 (hexane/EtOAc = 1:2); $[\alpha]_D^{25}$ +148.6 (*c* 0.1, $CHCl_3$); IR (neat, cm^{-1}) 3530, 3385, 2980, 2925, 2873, 2234, 1698, 1541, 1475, 1457, 1373, 1262, 1208, 1169, 1095, 1057, 945, 845, 806, 768, 672, 538, 513; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.34 (1H, s, H2/6), 4.78-4.89 (2H, m, H20/20'),

4.11-4.19 (4H, m, H21/21'), 1.70 (6H, s, Me), 1.54 (6H, s, Me), 1.43 (18H, s, *t*Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.1, 150.0, 127.4, 95.5, 94.6, 94.1, 80.6, 68.7, 65.9, 49.2, 28.6, 27.2, 26.1, 25.3, 24.4; ESI-HRMS (*m*/*z*) calcd for $C_{29}H_{38}IN_3NaO_6$ $[M+Na]^+$ 674.1703, found 674.1693.

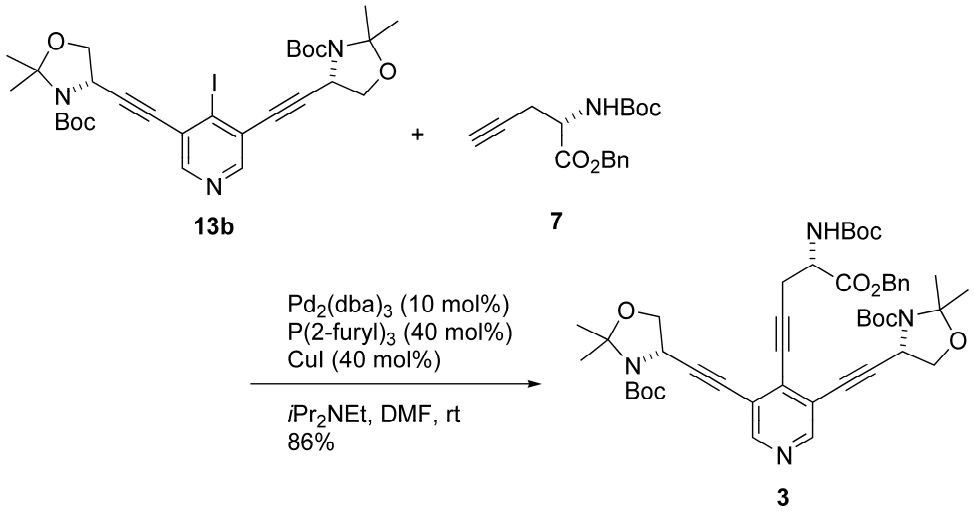
3,5-Bis{2'-(*S*)-[2",2"-dimethyl-3"-(*tert*-butoxycarbonyl)-4"-ethynyloxazolidin]ethyn-1'-yl}-4-bromopyridine (13a):



5a **6** **13a**

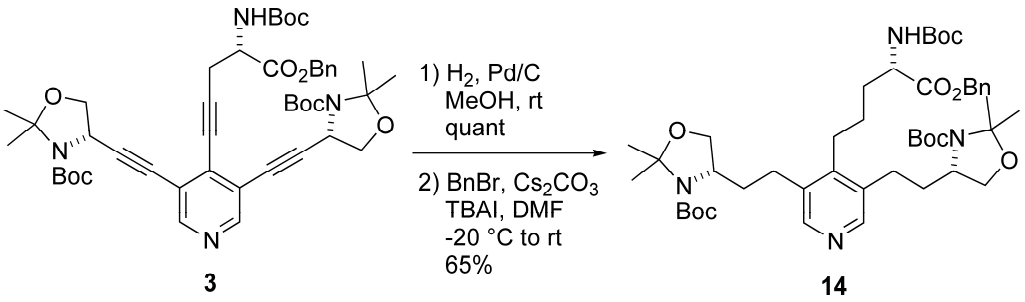
A solution of **5a** (13.6 mg, 33.1 μmol, 1.0 eq), **6** (28.6 mg, 126.9 μmol, 3.8 eq), $Pd(PPh_3)_4$ (7.5 mg, 6.49 μmol, 20 mol%), and CuI (2.4 mg, 12.6 μmol, 38 mol%) in DMF (1.6 mL, 20 μM to **5a**) was degassed by freeze/pump/thaw techniques. *i*Pr_2NEt (320 μL) was added to the resulting solution. After stirring at 45 °C for 8 h, the reaction mixture was diluted with EtOAc, and quenched with saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (hexane/Et_2O = 1:1) afforded **13a** (17.3 mg, 28.6 μmol, 86%) as a colorless solid; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.44 (1H, s, H2/6), 4.78-4.88 (2H, m, H20/20'), 4.14 (4H, m, H21/21'), 1.68 (6H, s, Me), 1.54 (6H, s, Me), 1.42 (18H, s, *t*Bu); ESI-MS (*m*/*z*) calcd for $C_{29}H_{38}BrN_3NaO_6$ $[M+Na]^+$ 626.16, found 626.24.

Benzyl 2-(*S*)-[(*tert*-butoxycarbonyl)amino]-5-{bis[2”-(*S*)-(2”’,2”’-dimethyl-3”’-(*tert*-butoxycarbonyl)-4”’-oxazolidin)-ethyn-1”-yl]pyridin-3’,5’-yl}pent-4-ynoate (3):



A solution of **13b** (16.8 mg, 25.8 μmol, 1.0 eq), **7** (23.7 mg, 78.1 μmol, 3.0 eq), $Pd_2(dba)_3$ (3.2 mg, 2.62 μmol, 10 mol%), P(2-furyl)$_3$ (2.5 mg, 10.8 μmol, 42 mol%) and CuI (2.0 mg, 10.5 μmol, 41 mol%) in DMF (1.29 mL, 20 μM to **13b**) was degassed by freeze/pump/thaw techniques. *i*Pr_2NEt (22.6 μL) was added to the resulting solution. After stirring at room temperature for 8.5 h, the reaction mixture was diluted with EtOAc, and quenched with saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (hexane/Et_2O = 1:1) afforded **3** (18.3 mg, 22.1 μmol, 86%) as a colorless oil; R_f 0.30 (hexane/EtOAc = 1:2); $[\alpha]_D^{25}$ +148.6 (*c* 0.1, $CHCl_3$); IR (neat, cm^{-1}) 3431, 3345, 2979, 2934, 2876, 2359, 2251, 2230, 1746, 1705, 1499, 1454, 1379, 1261, 1171, 1103, 1054, 914, 844, 735, 698, 580, 542;[1] H NMR (300 MHz, $CDCl_3$) δ 8.49 (1H, s, H2/6), 7.30-7.44 (5H, m, Bn), 5.22 (1H, d, *J* = 12.3 Hz, Bn), 5.15 (2H, d, *J* = 12.3 Hz, Bn/NH), 4.81-4.95 (2H, m, H20/20’), 4.57 (1H, m, H16), 4.11 (4H, m, H21/21’), 3.00-3.15 (2H, m, H15), 1.66 (6H, s, Me), 1.53 (6H, s, Me), 1.48 (18H, s, *t*Bu), 1.44 (9H, s, *t*Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ170.4, 155.2, 151.4, 150.9, 135.3, 128.3, 121.4, 97.9, 95.5, 94.3, 80.9, 80.4, 78.8, 68.7, 67.3, 65.9, 52.2, 49.3, 28.4, 27.1, 25.9, 25.2, 24.5; ESI-HRMS (*m*/*z*) calcd for $C_{46}H_{58}N_4NaO_{10}$ $[M+Na]^+$ 849.4051, found 849.4053.

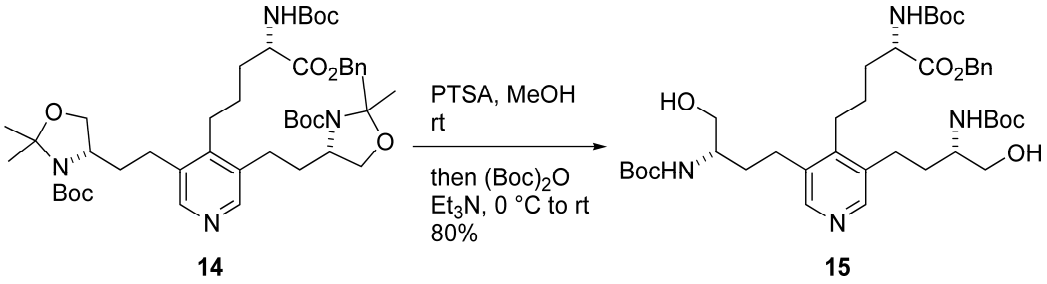
Benzyl 2-(*S*)-[(*tert*-butoxycarbonyl)amino]-5-{3',5'-bis[2"-(*S*)-(2"',2"'-dimethyl-3"'-(*tert*-butoxycarbonyl)-4"'-oxazolidin]-ethyn-1"-yl}-pentanoate (14):



A solution of **3** (66.7 mg, 80.7 μmol, 1.0 eq) in MeOH (2.9 mL) was treated with 10% Pd/C (53.8 mg, 50.5 μmol, 63 mol%) and hydrogenated at balloon pressure at room temperature. After stirring for 4 days at room temperature, the insoluble was separated by filtration through neutral silica gel and Celite eluting with MeOH. The filtrate was then concentrated in *vacuo*. Concentration of the filtrate yielded carboxylic acid (62.9 mg, 84.0 μmol, quant) as a colorless solid; ^{1}H NMR (500 MHz, CD_3OD) δ 8.16 (2H, s, H2/6), 3.90-4.05 (8H, m, NH, H16/20/20'), 2.63-2.84 (6H, m, H13/18/18'), 1.95-1.97 (2H, m, H14), 1.79-1.86 (4H, m, H19/19'), 1.43-1.57 (41H, m, H15/Me/*t*Bu); ESI-MS (*m*/*z*) calcd for $C_{39}H_{65}N_4O_{10}$ $[M+H]^+$ 749.47, found 749.48.

To a solution of the obtained carboxylic acid (20.2 mg, 27.0 μmol, 1.0 eq) and Cs_2CO_3 (29.2 mg, 8.94 mmol, 3.3 eq) in DMF (0.27 mL) was added TBAI (11.6 mg, 31.4 μmol, 1.2 eq) and BnBr (3.4 μL, 28.6 μmol, 1.06 eq) at -20 °C. After stirring for 4 h at -20 °C followed by for 12 h at room temperature, the mixture was diluted with EtOAc, and quenched with saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (hexane/EtOAc = 1:3) afforded **14** (14.6 mg, 17.4 μmol, 65%) as a colorless oil; R_f 0.48 (hexane/EtOAc = 1:5); $[\alpha]_D^{25}$ -28.5 (*c* 0.07, $CHCl_3$); IR (neat, cm^{-1}) 2979, 2933, 2873, 2357, 1739, 1696, 1389, 1365, 1253, 1166, 1094, 858, 681; ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.20 (2H, s, H2/6), 7.29-7.33 (5H, m, Bn), 5.25 (1H, s, NH), 5.18 (1H, d, J = 12.0 Hz, Bn), 5.19 (1H, d, J = 12.5 Hz, Bn), 4.33 (1H, m, H16), 3.78-3.98 (6H, H20/20'/21/21'), 2.54 (6H, H13, H18/18'), 1.94-2.00 (2H, m, H14), 1.79 (4H, m, H19/19'), 1.44-1.59 (41H, H15/Me/*t*Bu); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.8, 171.7, 156.0, 152.8, 152.2, 148.9, 135.9, 135.3, 219.1, 128.9, 94.4, 93.9, 80.7, 80.2, 67.3, 60.9, 58.1, 53.9, 35.7, 35.1, 31.1, 29.0, 28.1, 27.5, 27.3, 26.8, 25.0, 23.6, 21.6, 14.7; ESI-HRMS (*m*/*z*) calcd for $C_{46}H_{71}N_4O_{10}$ $[M+H]^+$ 839.5170, found 839.5151.

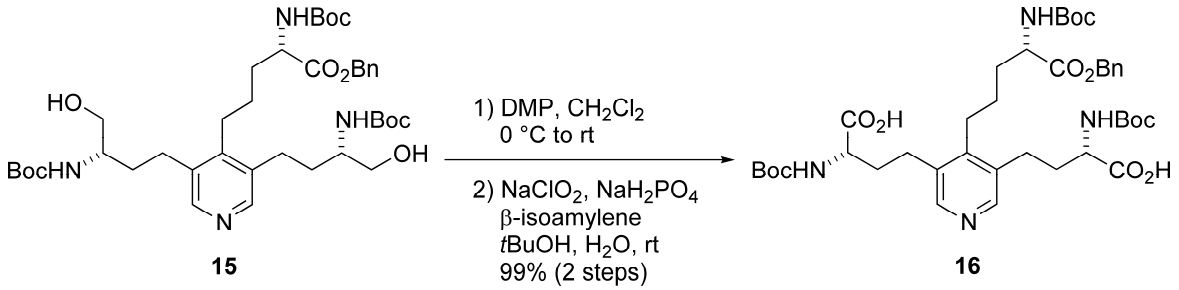
Benzyl 5-{3',5'-bis[3"-(*S*)-(*tert*-butoxycarbonyl)amino-4"-hydroxy-butyl]pyridin-4'-yl}-2-(*S*)-[(*tert*-butoxycarbonyl)amino]pentanoate (15):



14 **15**

To a solution of **14** (15.3 mg, 18.2 μmol, 1.0 eq) in MeOH (0.7 mL) was added PTSA monohydrate (8.0 mg, 42.1 μmol, 2.3 eq) at room temperature. After stirring for 48 h at room temperature, Et_3N (23.0 μL, 165 μmol, 9.0 eq) was added to the resulting solution. The solution was cooled to 0 °C, and then a solution of $(Boc)_2O$ (24.8 mg, 113 μmol, 6.2 eq) in MeOH (0.30 mL, washed with 0.15 mL × 2) was slowly added. After stirring for additional 17 h at room temperature, the mixture was diluted with CH_2Cl_2, and quenched with saturated $NaHCO_3$ solution. The aqueous layer was then extracted with CH_2Cl_2. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (CH_2Cl_2/MeOH = 20:1→10:1) afforded **15** (11.1 mg, 14.6 μmol, 80%) as a colorless oil; R_f 0.74 (CH_2Cl_2/MeOH = 5:1); $[\alpha]_D^{25}$ +37.8 (*c* 0.10, MeOH); IR (oil, cm^{-1}) 3344, 2929, 2356, 1692, 1517, 1455, 1365, 1248, 1165, 1054, 751, 716, 699, 462, 418, 406; ^1H NMR (300 MHz, CD_3OD) δ 8.16 (2H, s, H2/6), 7.30-7.34 (5H, m, Bn), 7.08 (1H, d, J = 8.7 Hz, NH), 6.55 (1H, d, J = 7.5 Hz, NH), 5.21 (1H, d, J = 12.3 Hz, Bn), 5.11 (1H, d, J = 12.3 Hz, Bn), 4.18 (1H, m, H16), 3.43-3.56 (6H, m, H20/20'/21/21'), 2.67-2.72 (6H, m, H13/18/18'), 1.93-1.99 (2H, m, H14), 1.82 (2H, m, H15), 1.51-1.68 (4H, m, H19/19'), 1.47, 1.45, 1.52 (27H, s, *t*Bu); ^{13}C NMR (75 MHz, CD_3OD) δ 174.0, 158.3, 158.1, 148.4, 137.3, 129.6, 129.4, 129.3, 80.7, 80.1, 67.8, 65.2, 55.1, 53.5, 52.7, 34.2, 34.2, 32.9, 30.8, 28.8, 28.7, 28.0, 27.6; ESI-HRMS (*m*/*z*) calcd for $C_{40}H_{63}N_4O_{10}$ $[M+H]^+$ 759.4544, found 759.4537.

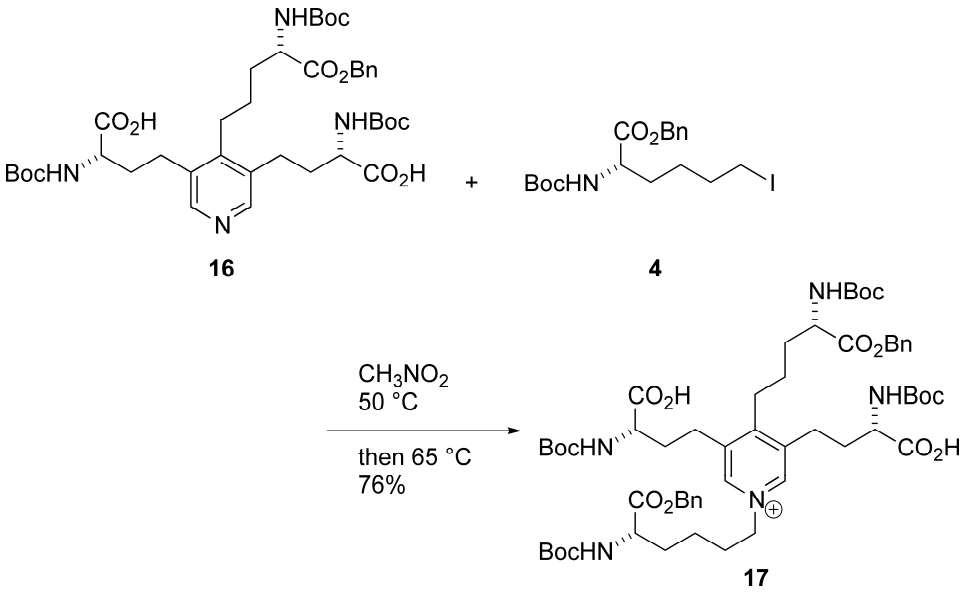
Benzyl 5-[3',5'-bis(3"-(*S*)-*tert*-butoxycarbonylamino-3"-carboxy-propyl)-pyridin-4'-yl]-2-(*S*)-[(*tert*-butoxycarbonyl)amino]pentanoate (16):



To a solution of Dess-Martin periodinane (79.7 mg, 188 μmol, 8.3 eq) in CH_2Cl_2 (0.5 mL) was slowly added a solution of **15** (17.2 mg, 22.7 μmol, 1.0 eq) in CH_2Cl_2 (0.3 mL, washed with 0.1 mL × 2) at 0 °C. After stirring for 2 h at room temperature, the mixture was diluted with Et_2O, quenched with an ice-cold saturated $Na_2S_2O_3$ solution and an ice-cold saturated $NaHCO_3$ solution. The aqueous layer was then extracted with Et_2O. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. The dialdehyde was obtained as a colorless oil, and was used to the next reaction without further purification.

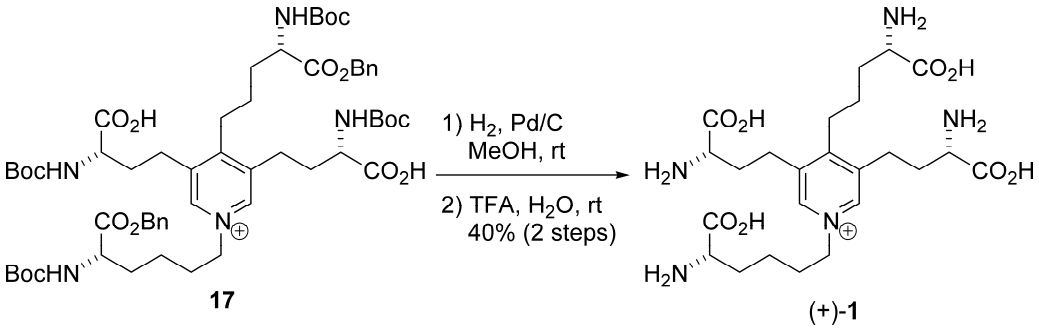
To a solution of the dialdehyde in *t*BuOH (1.0 mL) and cold β-isoamylene (98 μL, 876 μmol, 39 eq) was added a solution of NaH_2PO_4 dihydrate (50.3 mg, 322 μmol, 14 eq) and $NaClO_2$ (47.5 mg, 415 mmol, 18 eq) in deionized water (0.4 mL, washed with 0.2 mL × 2) with using a plastic disposable syringe (not using a steel needle) at room temperature. After stirring for 1 h at room temperature, the mixture was diluted with EtOAc, quenched with saturated NH_4Cl solution. The aqueous layer was then extracted with EtOAc. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated *in vacuo*. Purification on silica gel column chromatography (CH_2Cl_2/MeOH = 20:1→1:1) afforded **16** (17.7 mg, 22.5 mmol, 99% over two steps) as a colorless solid; R_f 0.39 (CH_2Cl_2/MeOH = 1:1); $[\alpha]_D^{20}$ +3.1 (*c* 0.1, MeOH); IR (neat, cm^{-1}) 3408, 2979, 2931, 2803, 1698, 1525, 1456, 1368, 1252, 1165, 1028, 860, 754, 699; ^1H NMR (300 MHz, CD_3OD) δ 8.16 (2H, s, H2/6), 7.32-7.35 (5H, m, Bn), 5.22 (1H, d, J = 12.3 Hz, Bn), 5.12 (1H, d, J = 12.3 Hz, Bn), 4.20 (1H, m, H16), 4.10 (2H, m, H20/20'), 2.89 (6H, H13, H18/18'), 1.88-2.25 (4H, m, H19/19'), 1.52-1.67 (4H, m, H14/15), 1.46 (18H, s, *t*Bu), 1.45 (9H, s, *t*Bu); ^{13}C NMR (75 MHz, CD_3OD) δ 175.6, 173.7, 157.8, 157.8, 147.4, 137.3, 137.0, 129.3, 129.1, 129.0, 80.4, 80.3, 67.6, 54.8, 54.4, 34.2, 32.5, 30.4, 28.5, 28.0, 27.6, 27.0; ESI-HRMS (*m*/*z*) calcd for $C_{40}H_{59}N_4O_{12}$ $[M+H]^+$ 787.4129, found 787.4102.

4-[4'-(*S*)-Benzyloxycarbonyl-4'-(*tert*-butoxycarbonyl)amino-butyl]-1-[5"-(*S*)-benzyloxycarbonyl-5"-(*tert*-butoxycarbonyl)amino-pentyl]-3,5-bis[3"'-(*S*)-(*tert*-butoxycarbonyl)amino-3"'-carboxy-propyl]-pyridinium (17):



A mixture of **16** (7.3 mg, 9.2 μmol, 1.0 eq) and benzyl 2-(*S*)-[(*tert*-butoxycarbonyl)amino]-6-iodohexanoate **4**[2] (12.5 mg, 27.9 μmol, 3.0 eq) in CH_3NO_2 (1.2 mL) was heated at 50 °C for 81 h, then warmed up to 60 °C for 18.5 h. The reaction mixture was concentrated *in vacuo*. Purification on silica gel column chromatography (CH_2Cl_2/MeOH = 20:1→3:1) afforded **17** (7.8 mg, 7.0 μmol, 76%) as a yellow oil; R_f 0.48 (CH_2Cl_2/MeOH = 1:1); $[\alpha]_D^{20}$ -1.8 (*c* 0.1, MeOH); IR (neat, cm^{-1}) 3369, 2978, 2931, 1709, 1518, 1455, 1367, 1252, 1165, 1052, 1027, 861, 752, 699, 584; ^{1}H NMR (300 MHz, CD_3OD) δ 8.62 (2H, s, H2/6), 7.32-7.35 (5H, m, Bn), 5.09-5.24 (4H, m, Bn), 4.47 (2H, t, J = 6.9 Hz, H7), 4.21 (1H, m, H16), 4.12 (3H, m, H11/20/20'), 2.89 (6H, m, H13/18/18'), 2.15 (2H, m, H19/19'), 2.02 (4H, m, H8/19/19'), 1.66-1.76 (4H, m, H10/15), 1.57-1.60 (2H, m, H14), 1.42-1.45 (20H, m, H9/*t*Bu); ^{13}C NMR (75 MHz, CD_3OD) δ 175.6, 174.2, 160.7, 158.4, 143.5, 142.3, 137.5, 130.1, 130.0, 130.0, 129.9, 129.7, 129.7, 129.6, 129.5, 81.1, 81.0, 68.2, 62.2, 55.1, 54.4, 33.8, 32.9, 32.2, 31.8, 29.8, 29.1, 29.0, 28.6, 27.9, 27.6, 23.9; ESI-HRMS (*m*/*z*) calcd for $C_{58}H_{84}N_5O_{16}$ $[M]^+$ 1106.5908, found 1106.5976.

4-(4'-(*S*)-Amino-4'-carboxy-butyl)-1-(5"-(*S*)-amino-5"-carboxy-pentyl)-3,5-bis-(3"'-(*S*)-amino-3"'-carboxy-propyl)-pyridinium, Desmosine (1):



A solution of **17** (20.5 mg, 18.5 μmol, 1.0 eq) in MeOH (0.3 mL) was treated with 10% Pd/C (130.9 mg, 124 μmol, 6.7 eq) and hydrogenated at balloon pressure at room temperature. After stirring for 24 h at room temperature, the insoluble was separated by filtration through a celite pad on neutral silica gel eluting with MeOH. The filtrate was then concentrated *in vacuo*. Concentration of the filtrate yielded crude tetracarboxylic acid as a colorless solid. The product was used to the next reaction without further purification.

A mixture of TFA and distilled water (2.0 mL, 95:5 ratio) was added to the crude tetracarboxylic acid at room temperature and stirred for 2 h. The solvent was removed on a rotary evaporator. Purification on C18 silica gel column chromatography (0.1% TFA in distilled water) afforded the crude product as a colorless solid (20.2 mg). The crude product was then purified by reversed phase HPLC system. The conditions were as follows: column, Cosmosil 5C_{18}-AR-II (10 × 250 mm, Nacalai tesque, Kyoto); solvent, linear gradient of 10% MeOH and 90% H_2O; flow rate, 1.5 mL/min; detection, 254 nm; temperature, 40 °C; R_t = 6.8 min (desmosine **1**). As a result, 4.7 mg of pure desmosine **1** was obtained as a colorless amorphous in 40% over two steps.

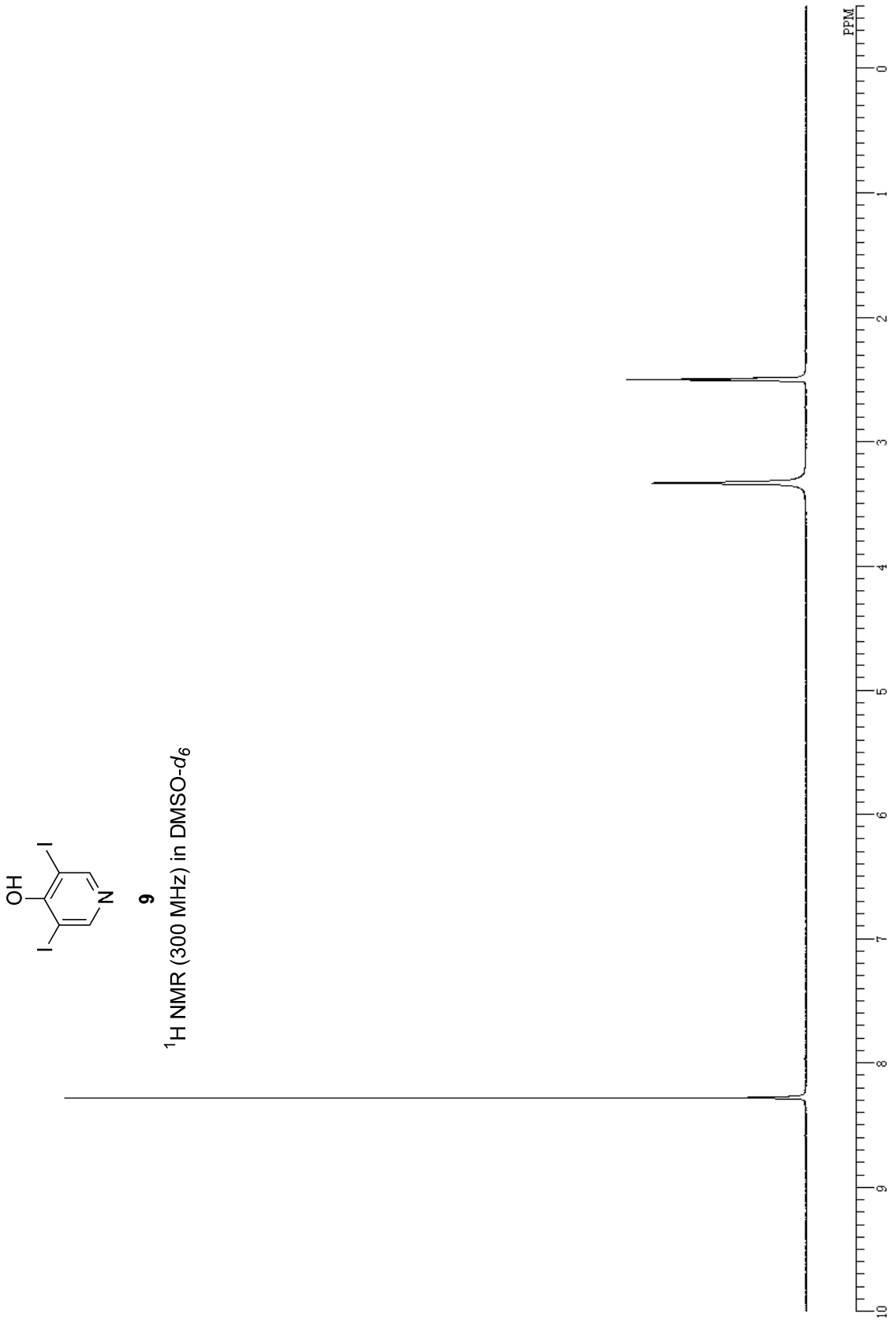
Desmosine (Synthetic): R_f 0.28 [MeOH (0.1% TFA)/H_2O (0.1% TFA) = 1:9]; $[\alpha]_D^{20}$ +7.6 (*c* 0.10, H_2O); UV λ_{max}: 268, 237 nm in H_2O (lit. value: λ_{max}: 269, 235 nm in 0.05 M HCl)[3]; ^{1}H NMR (500 MHz, D_2O) δ 8.58 (2H, s, H2/6), 4.53 (2H, t, J = 7.2 Hz, 7H), 4.08-4.10 (1H, m, H20), 4.01-4.03 (1H, m, H16), 3.96-3.98, (1H, m, H11), 3.06-3.12 (2H, m, H13), 2.92-2.98 (4H, m, H18/18'), 2.22-2.26 (4H, m, H19/19'), 2.11-2.15 (2H, m, H15), 2.02-2.08 (2H, m, H8), 1.95-2.00 (2H, m, H10), 1.42-1.72 (4H, m, H9/14); ^{13}C NMR (125 MHz, D_2O) δ 175.1, 175.1, 174.8 (C12/17/21),161.0 (C4), 144.2 (C2/6), 142.4 (C3/5), 63.0 (C7), 55.6 (C11/16/20/20'), 32.9, 32.5, 32.3, 31.7 (C8/10/15/19/19'), 30.6 (C8), 28.1 (C13), 23.5 (C9/14); ESI-HRMS (*m*/*z*) calcd for $C_{24}H_{40}N_5O_8$ $[M]^+$ 526.2877, found 526.2877.

Desmosine (Natural): R_f 0.28 [MeOH (0.1% TFA)/H_2O (0.1% TFA) = 1:9]; $[\alpha]_D^{20}$ +10.0 (*c* 0.10, H_2O); UV λ_{max}: 268, 234 nm in H_2O; ^{1}H NMR (500 MHz, D_2O) δ 8.57 (2H, s, H2/6), 4.51 (2H, t, J = 7.5 Hz, 7H), 4.11 (1H, m, H20), 4.04 (1H, m, H16), 3.99, (1H, m, H11), 3.05-3.11 (2H, m, H13), 2.91-2.96 (4H, m, H18/18'), 2.19-2.28 (4H, m, H19/H19'), 2.10-2.16 (2H, m, H15), 2.02-2.07 (2H, m, H8), 1.92-1.99 (2H, m, H10), 1.45-1.76 (4H, m, H9/14); ^{13}C NMR (125 MHz, D_2O) δ 174.7, 174.7, 174.6 (C12/17/21),161.0 (C4), 144.2 (C2/6), 142.3

(C3/5), 63.0 (C7), 55.3, 55.2, 55.2 (C11/16/20/20'), 32.7, 32.4, 32.4, 31.6 (C8/10/15/19/19'), 30.6 (C8), 28.1 (C13), 24.0, 23.6 (C9/14).

References:

1) A. Dondoni and D. Perrone, *Org. Synth.* 2000, **77**, 64-70.
2) Y. Koseki, H. Yamada and T. Usuki, *Tetrahedron: Asymmetry* 2011, **22**, 580-586.
3) S. M. Partridge, D. F. Elsden and J. Thomas, *Nature* 1963, **197**, 1297-1298.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N
9
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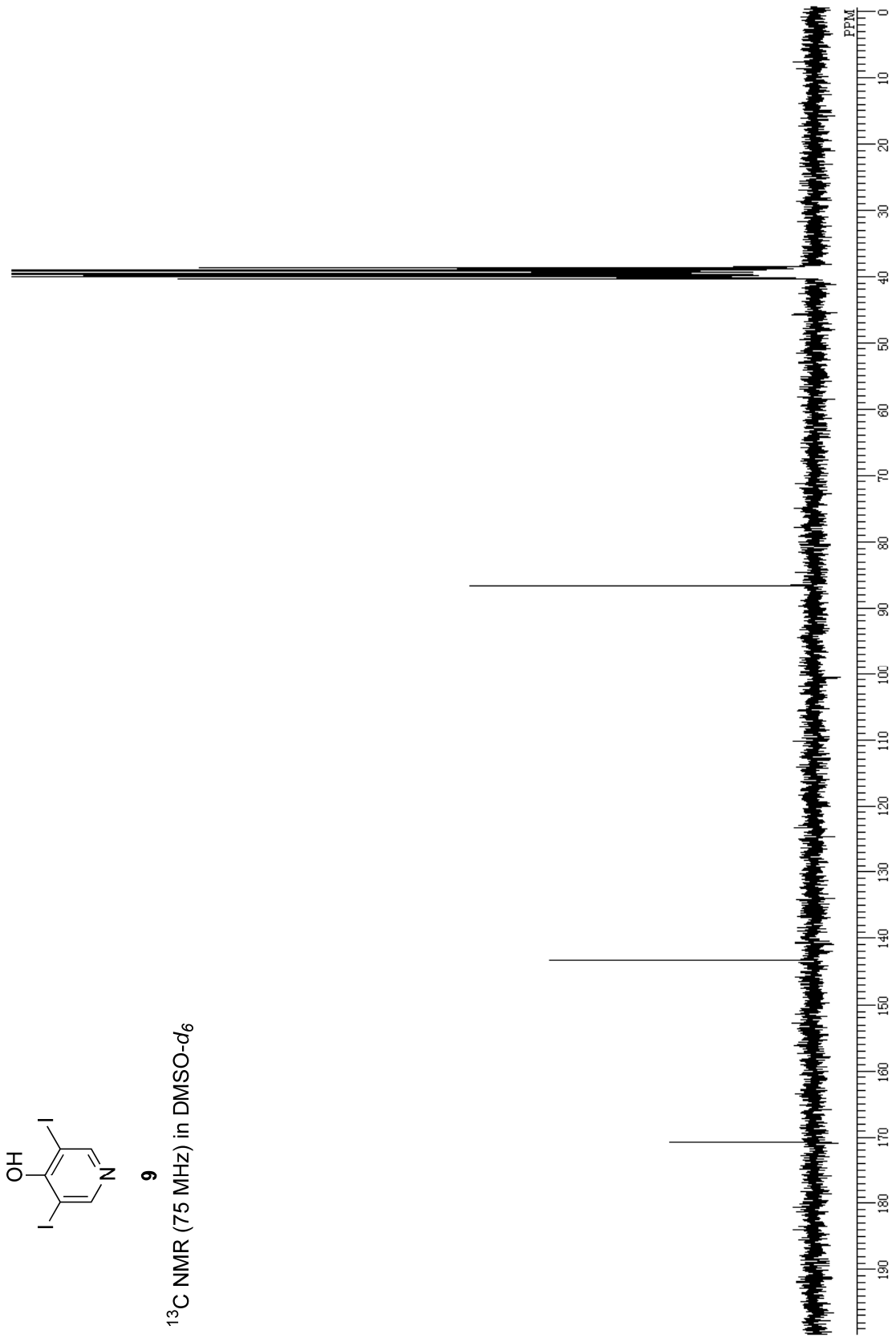
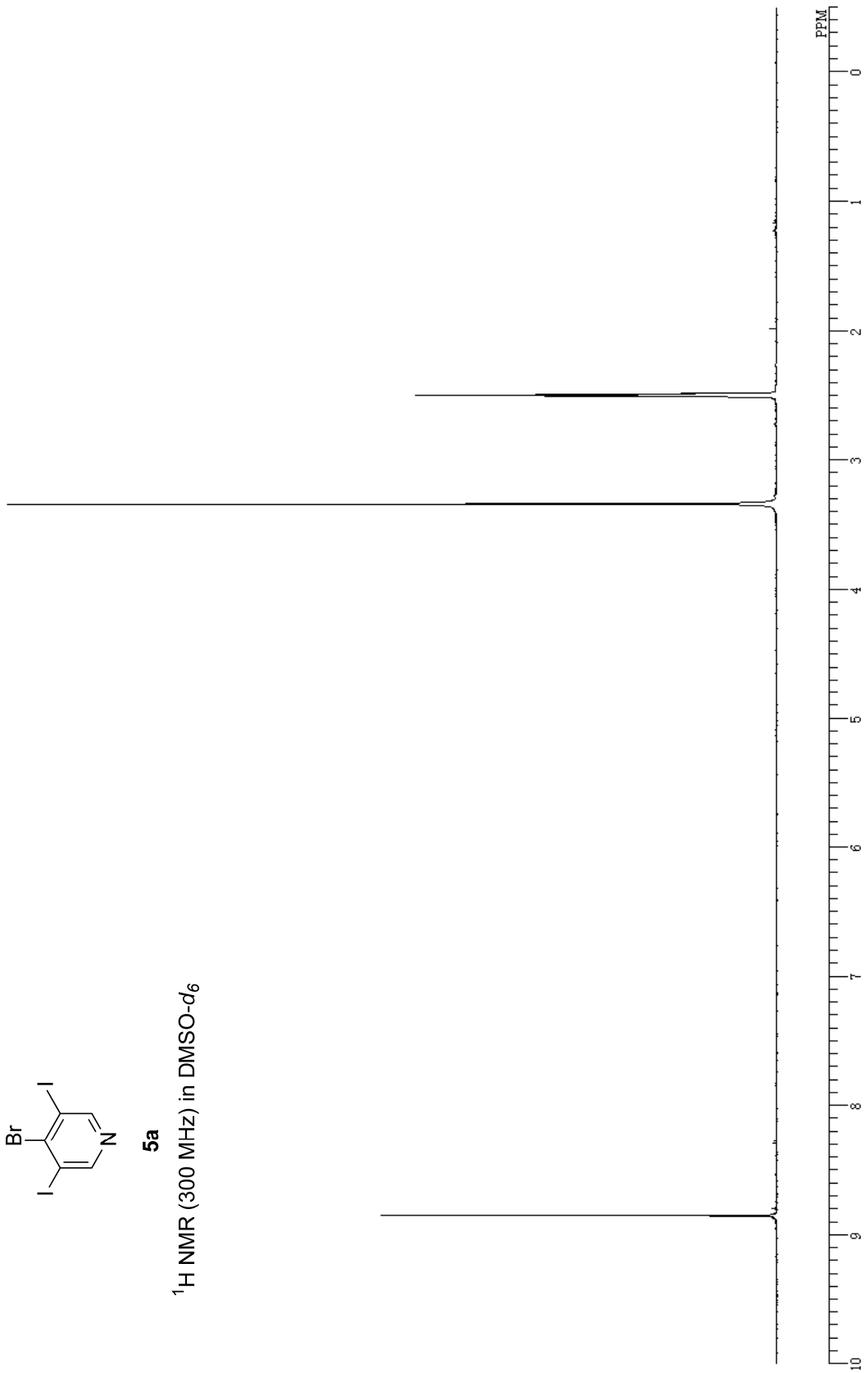
2
3
4
5
6
7
8
9
10



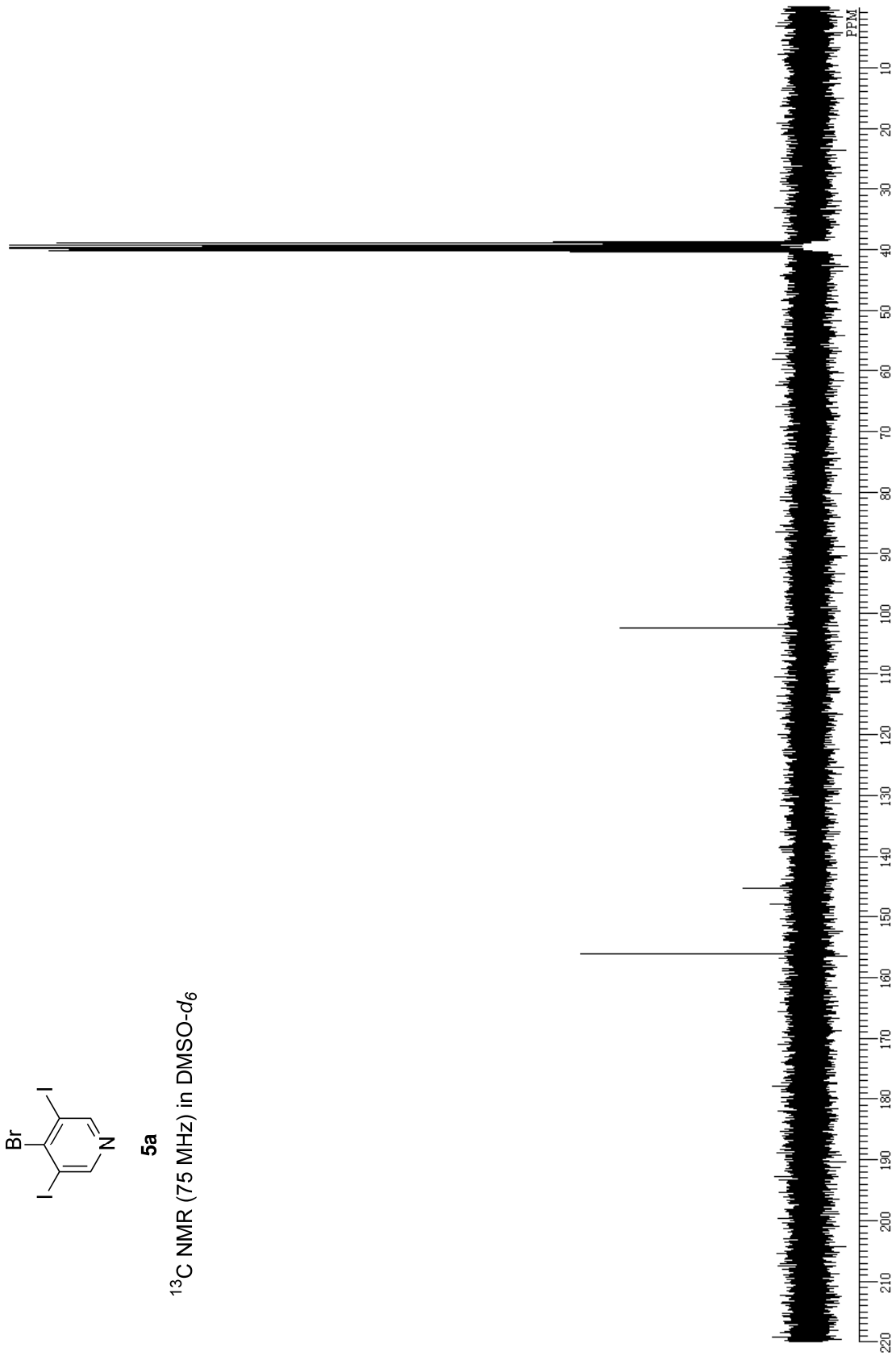
9

^{13}C NMR (75 MHz) in DMSO-d_6



5a

^{1}H NMR (300 MHz) in DMSO-d_6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N
5a
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150
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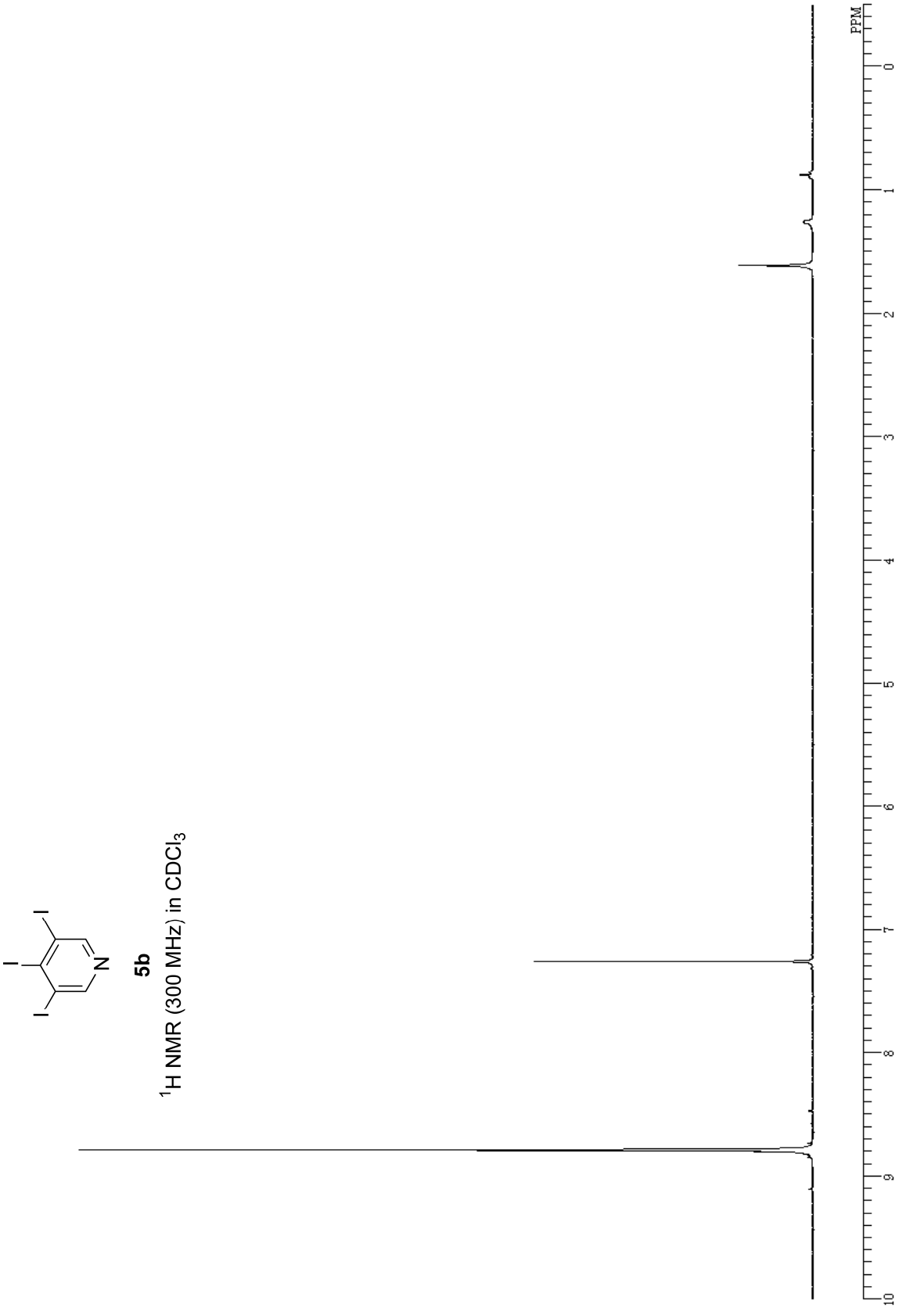
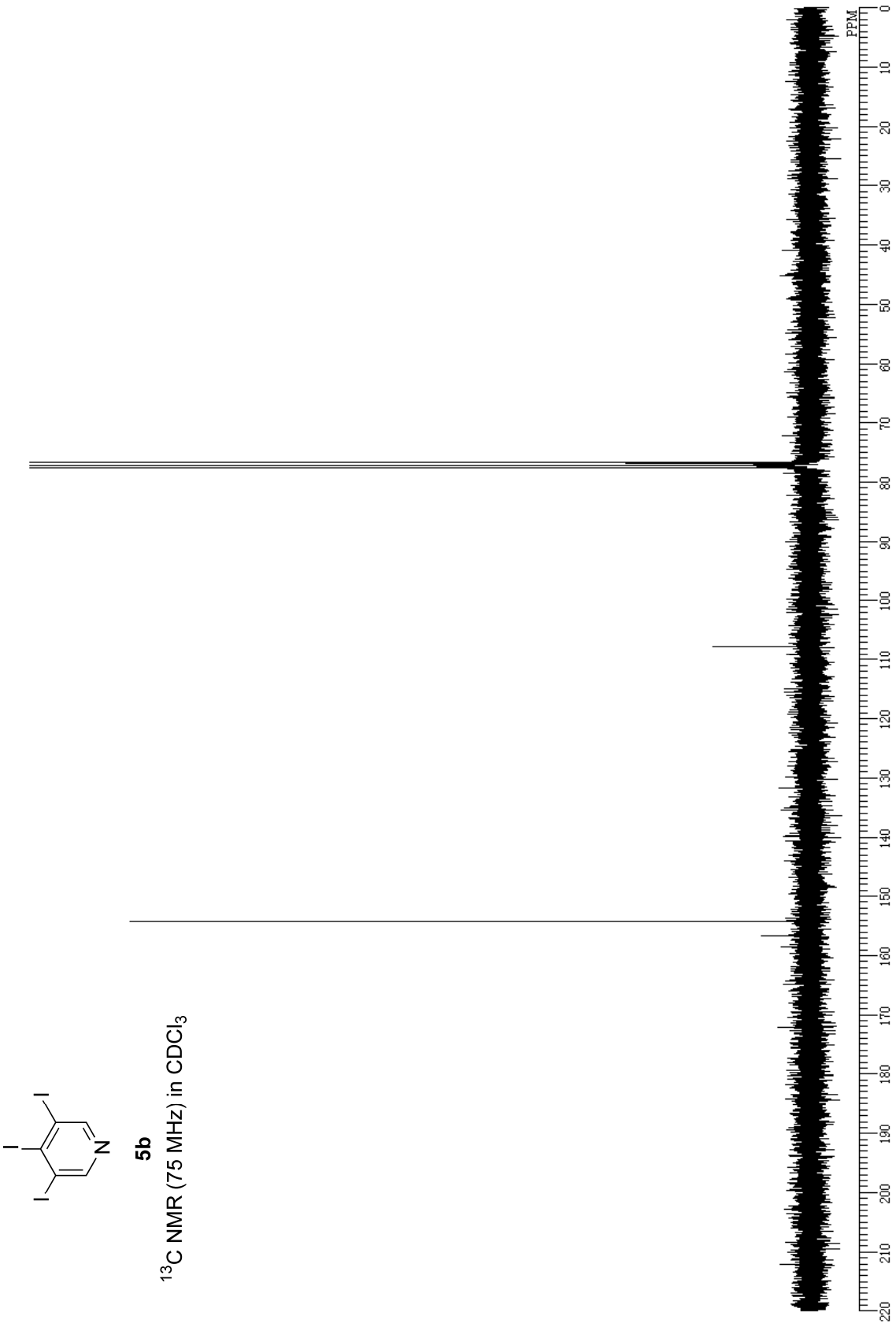
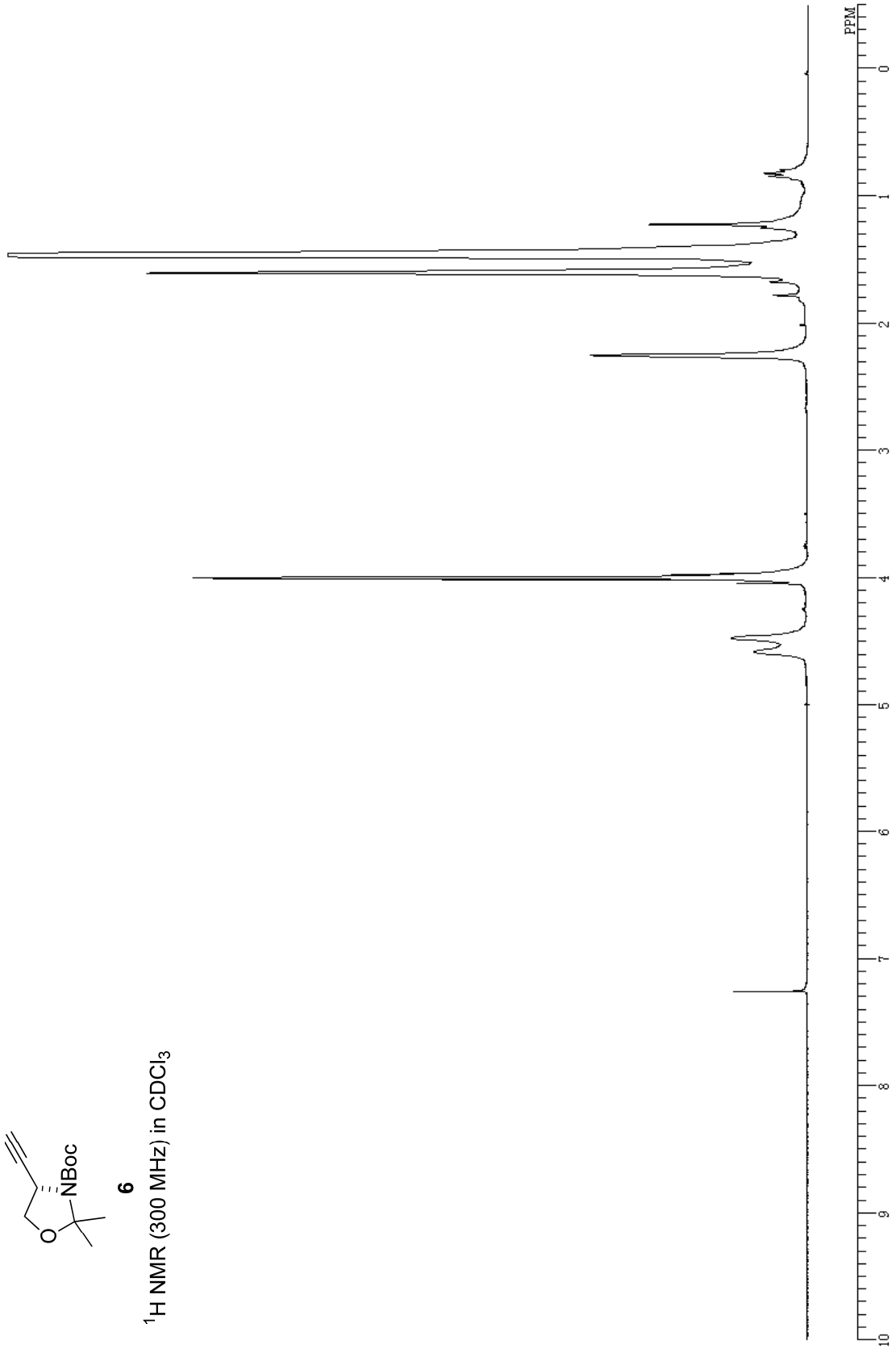

I
I
I
N
5b
^{1}H NMR (300 MHz) in $CDCl_3$
PPM
0
1
2
3
4
5
6
7
8
9
10



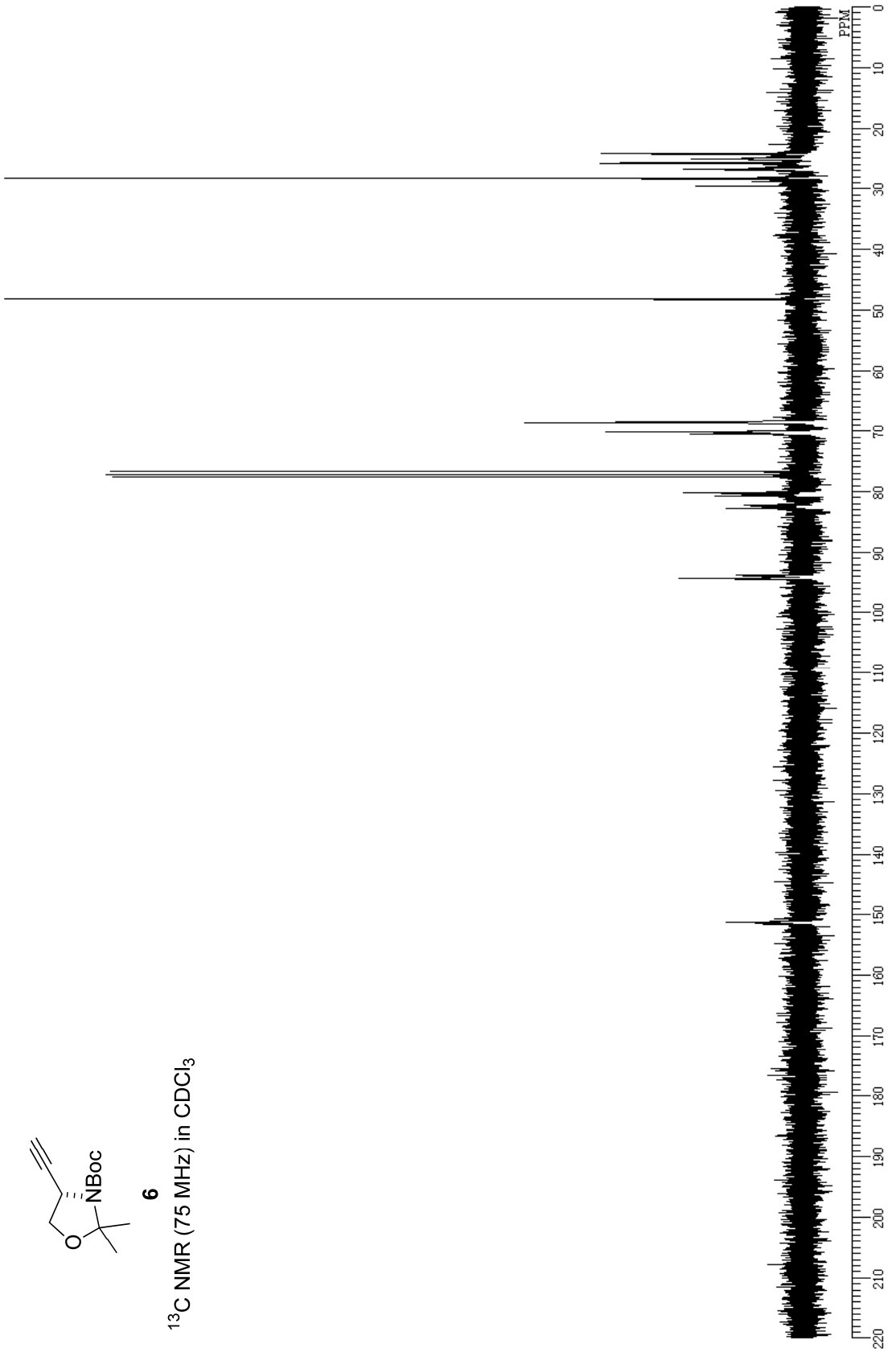
5b

^{13}C NMR (75 MHz) in $CDCl_3$



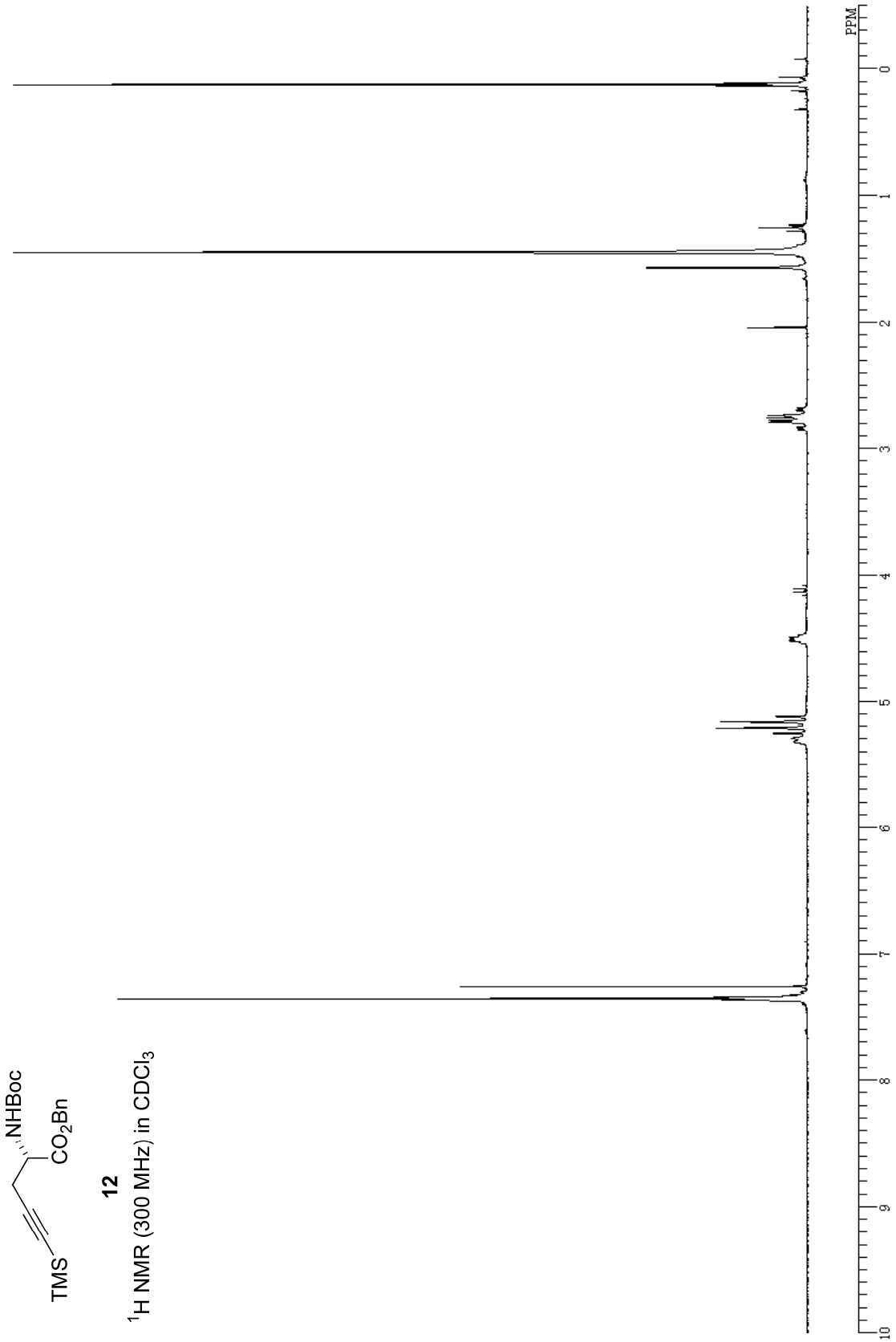
6

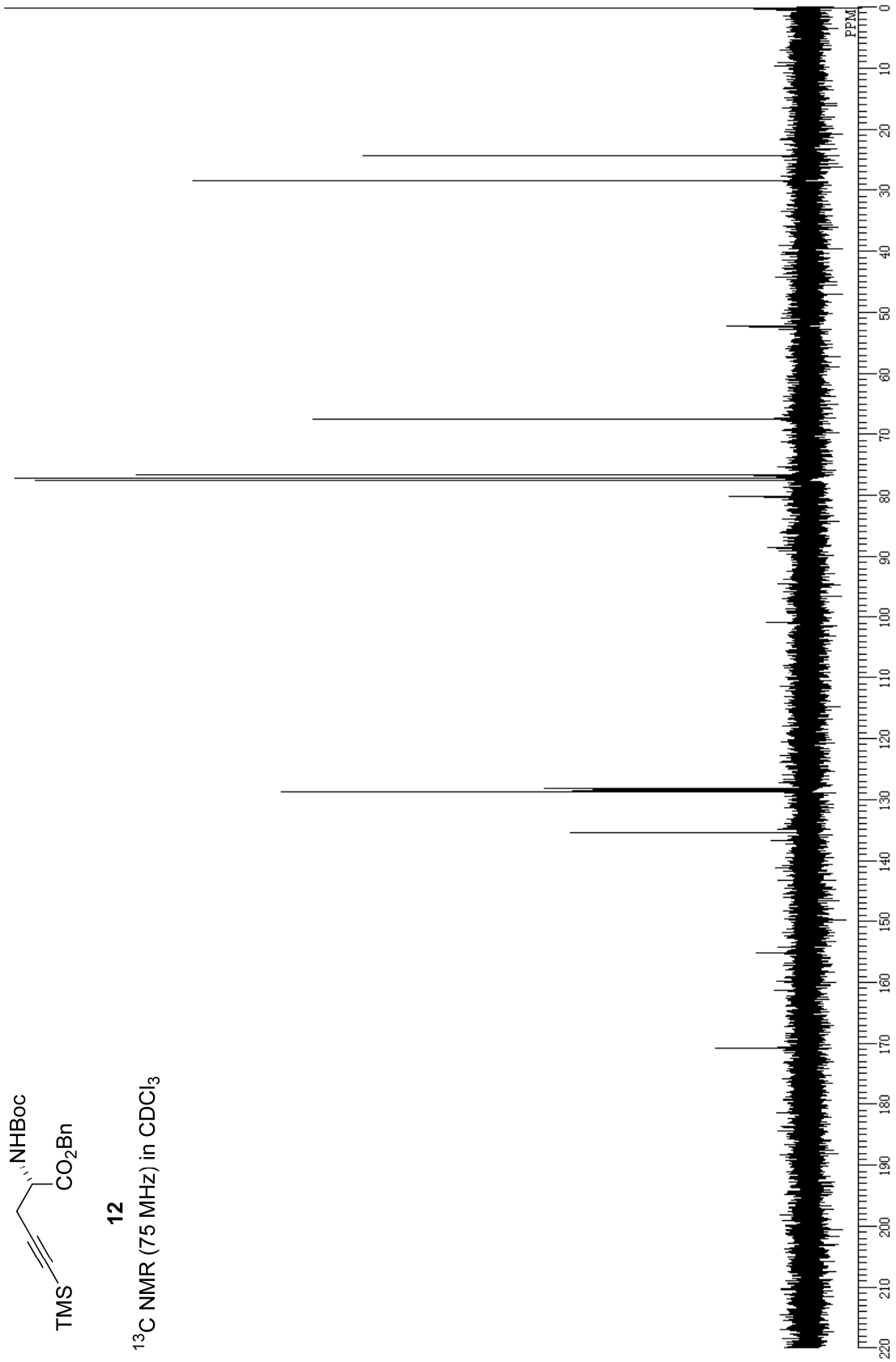
^{1}H NMR (300 MHz) in $CDCl_3$

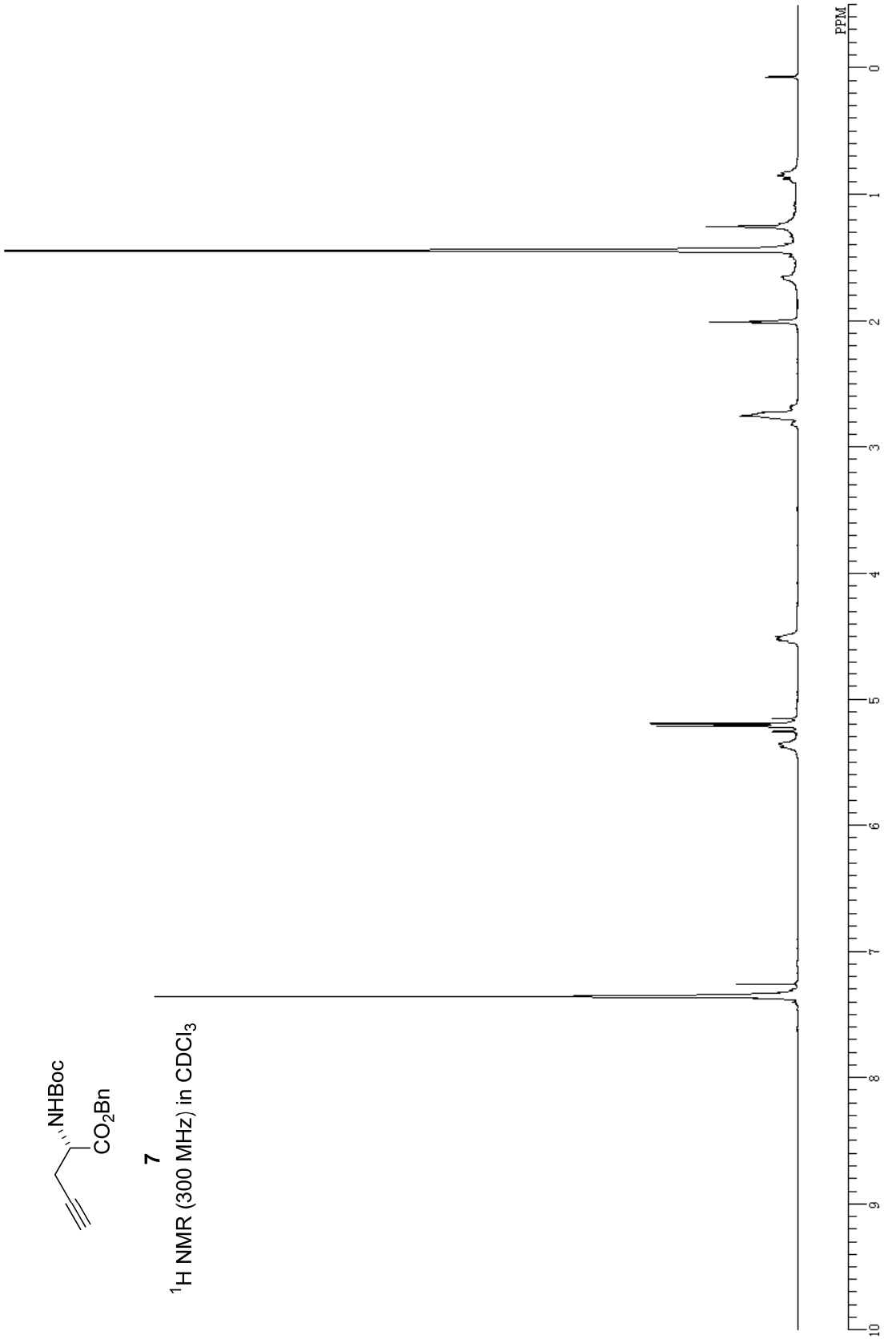


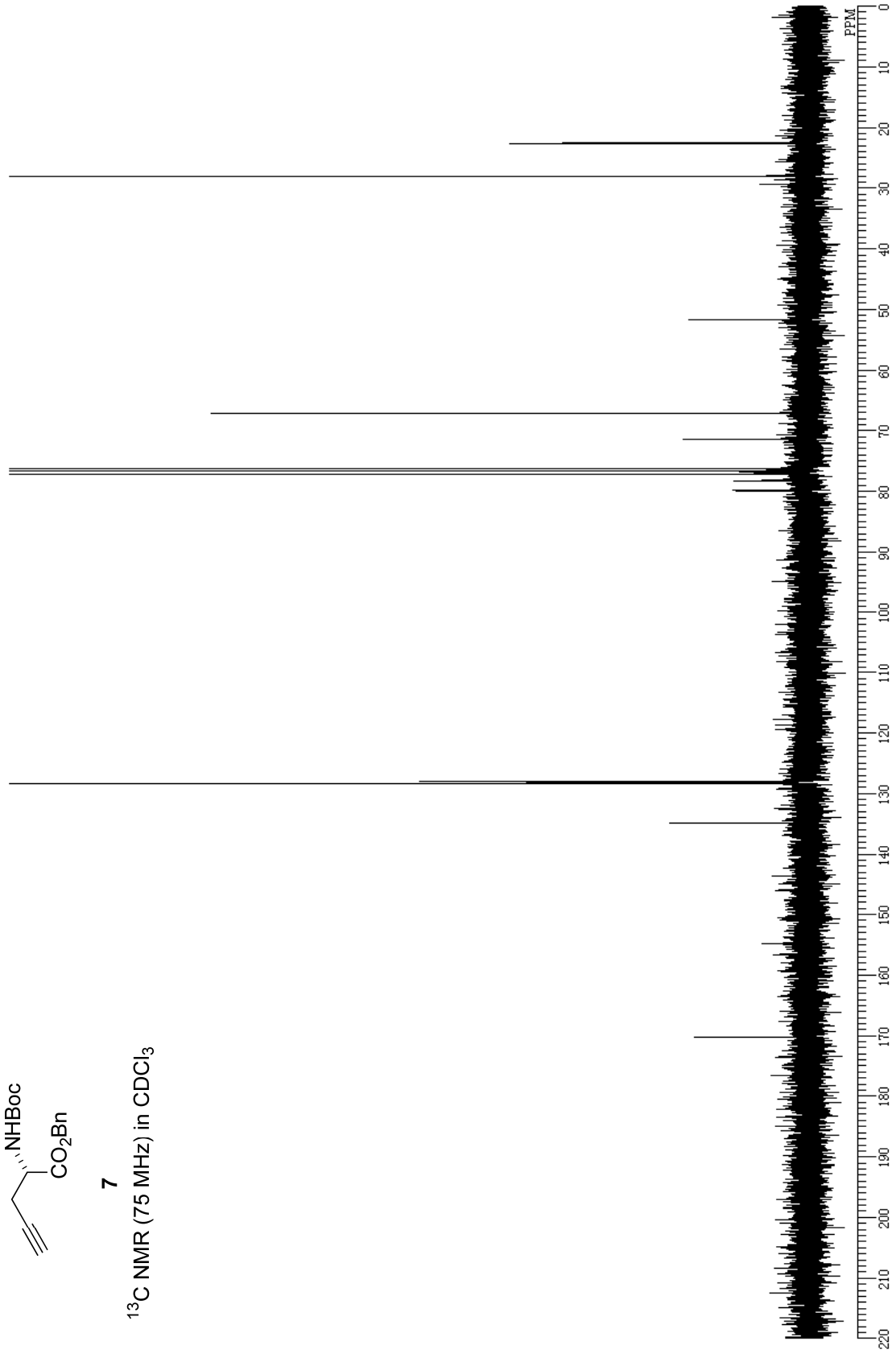
6

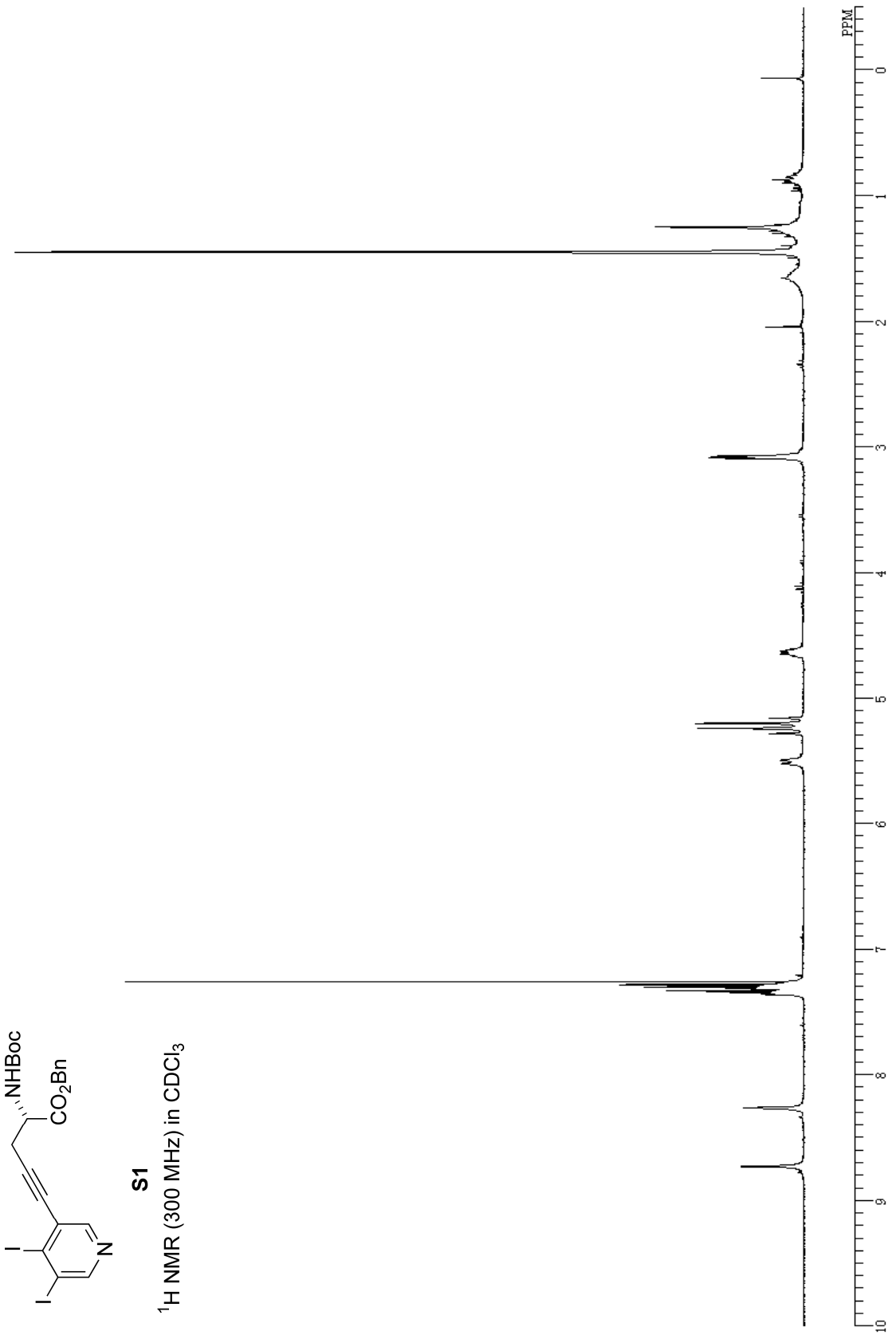
^{13}C NMR (75 MHz) in $CDCl_3$

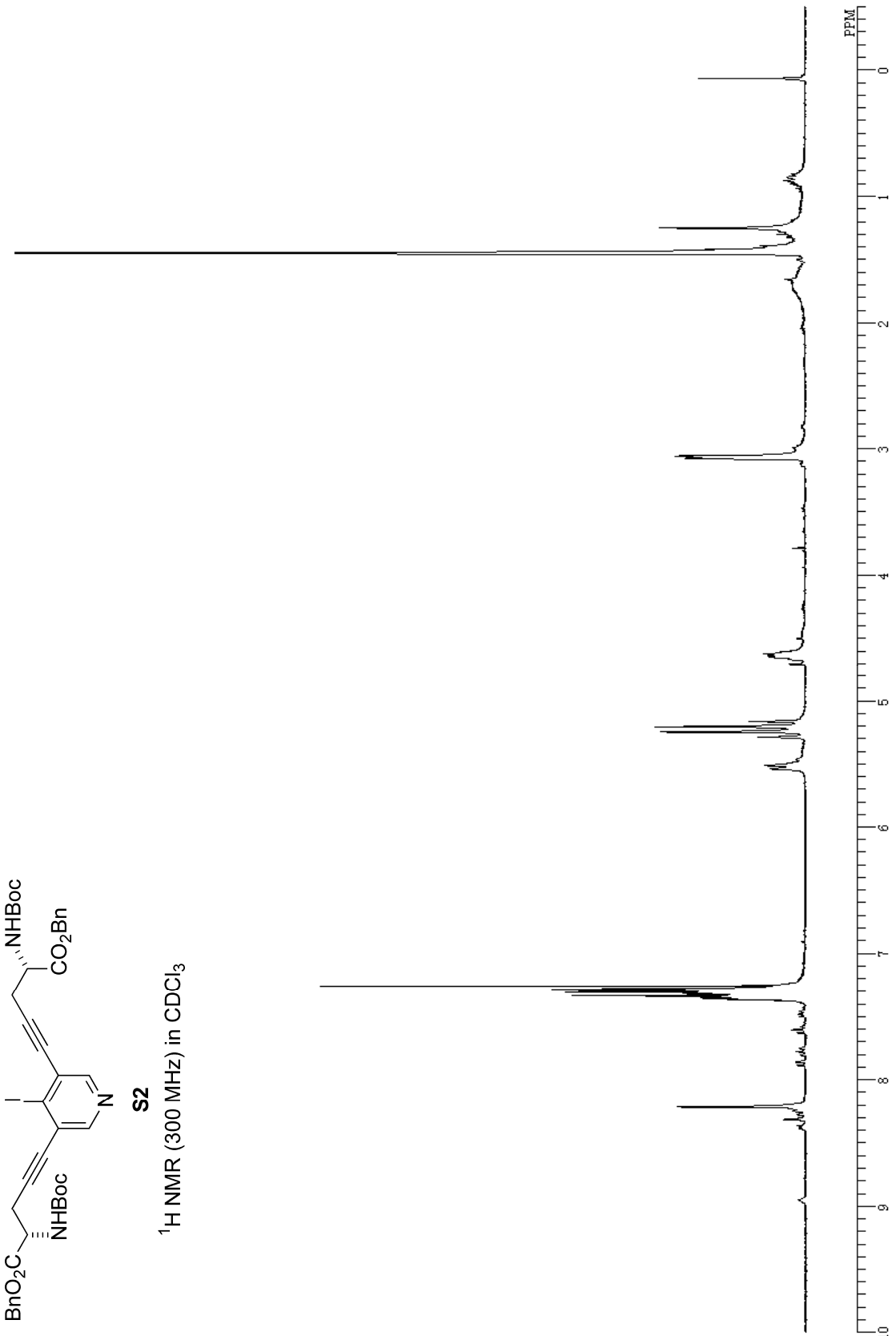

NHBoc
CO2Bn
TMS
12
1H NMR (300 MHz) in CDCl3
PPM
0
1
2
3
4
5
6
7
8
9
10


NHBoc
CO2Bn
TMS
12
13C NMR (75 MHz) in CDCl3
PPM
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220


NHBoc
CO2Bn
7
1H NMR (300 MHz) in CDCl3
PPM
0
1
2
3
4
5
6
7
8
9
10

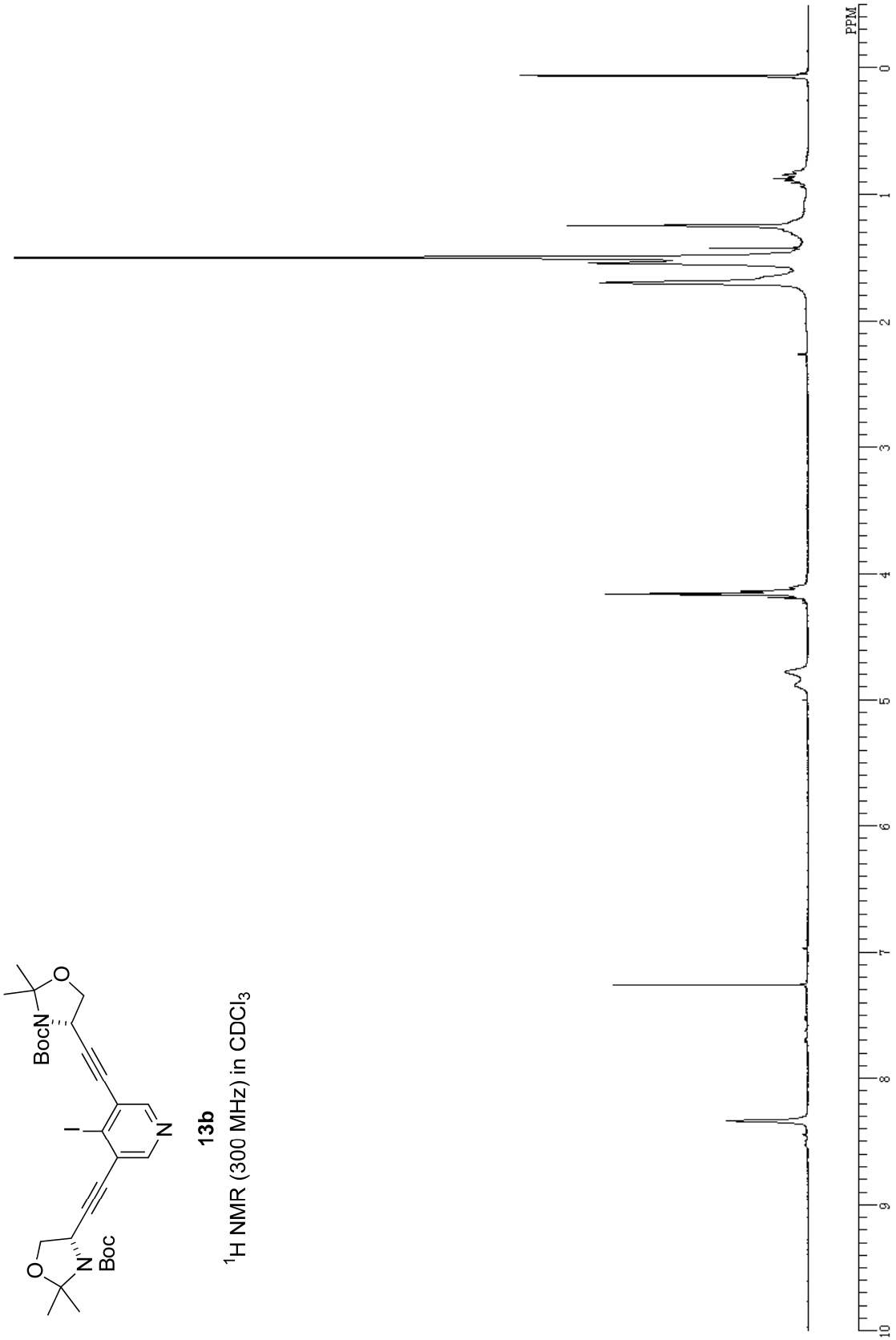

NHBoc
CO2Bn
7
13C NMR (75 MHz) in CDCl3
PPM
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220


NHBoc
CO2Bn
I
I
N
S1
1H NMR (300 MHz) in CDCl3
PPM
0
1
2
3
4
5
6
7
8
9
10



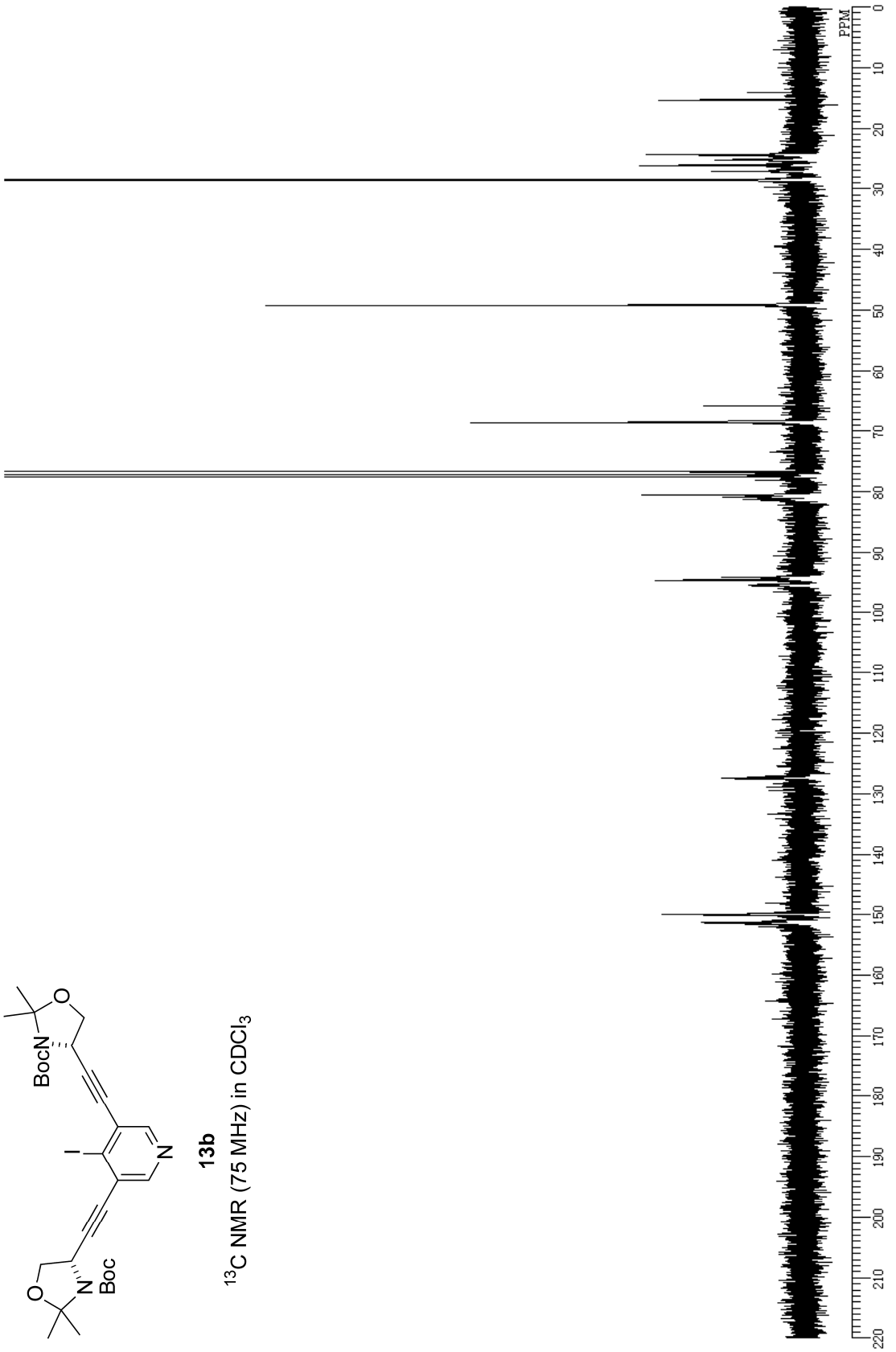
S2

^{1}H NMR (300 MHz) in $CDCl_3$



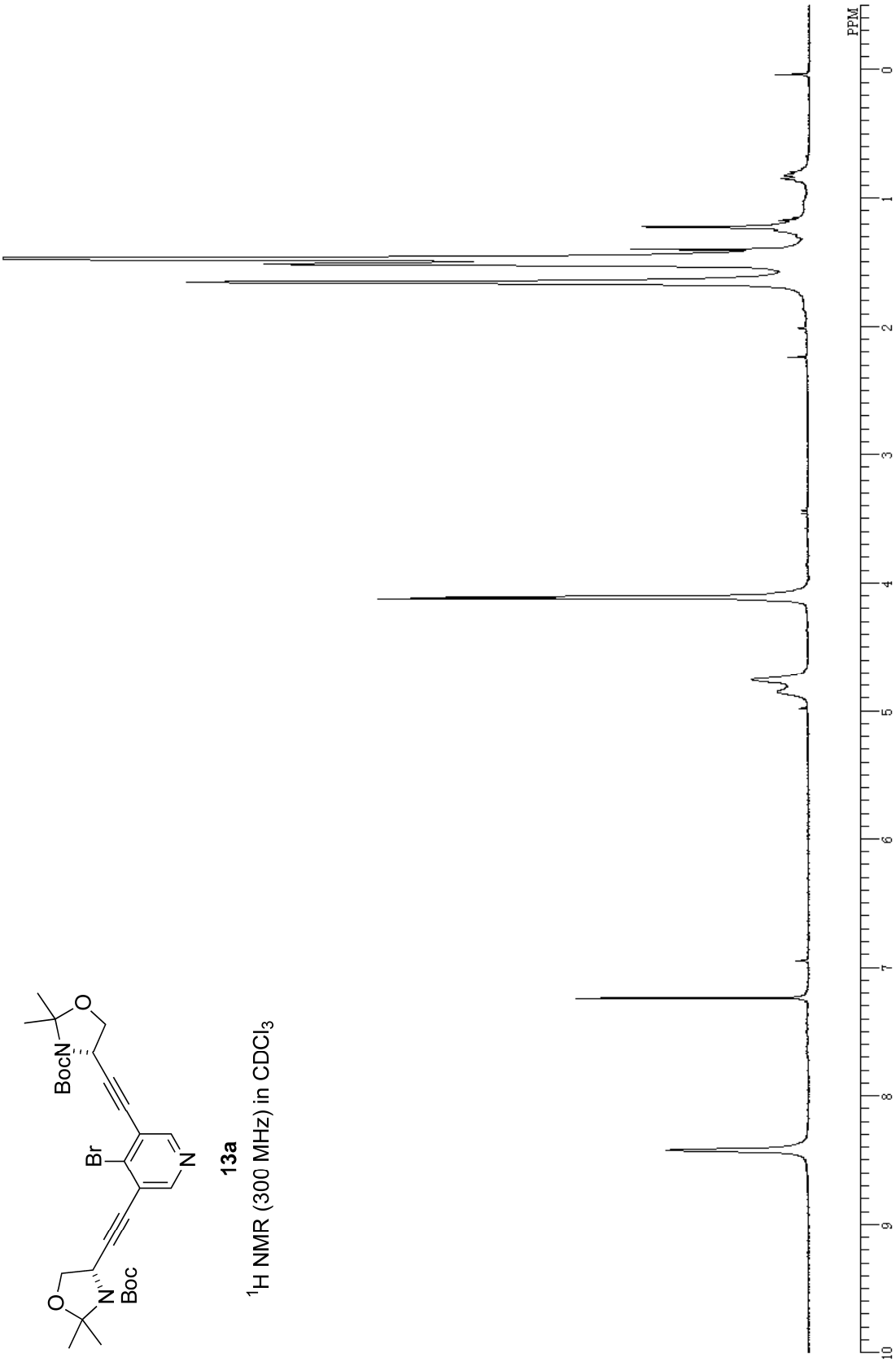
13b

^{1}H NMR (300 MHz) in $CDCl_3$



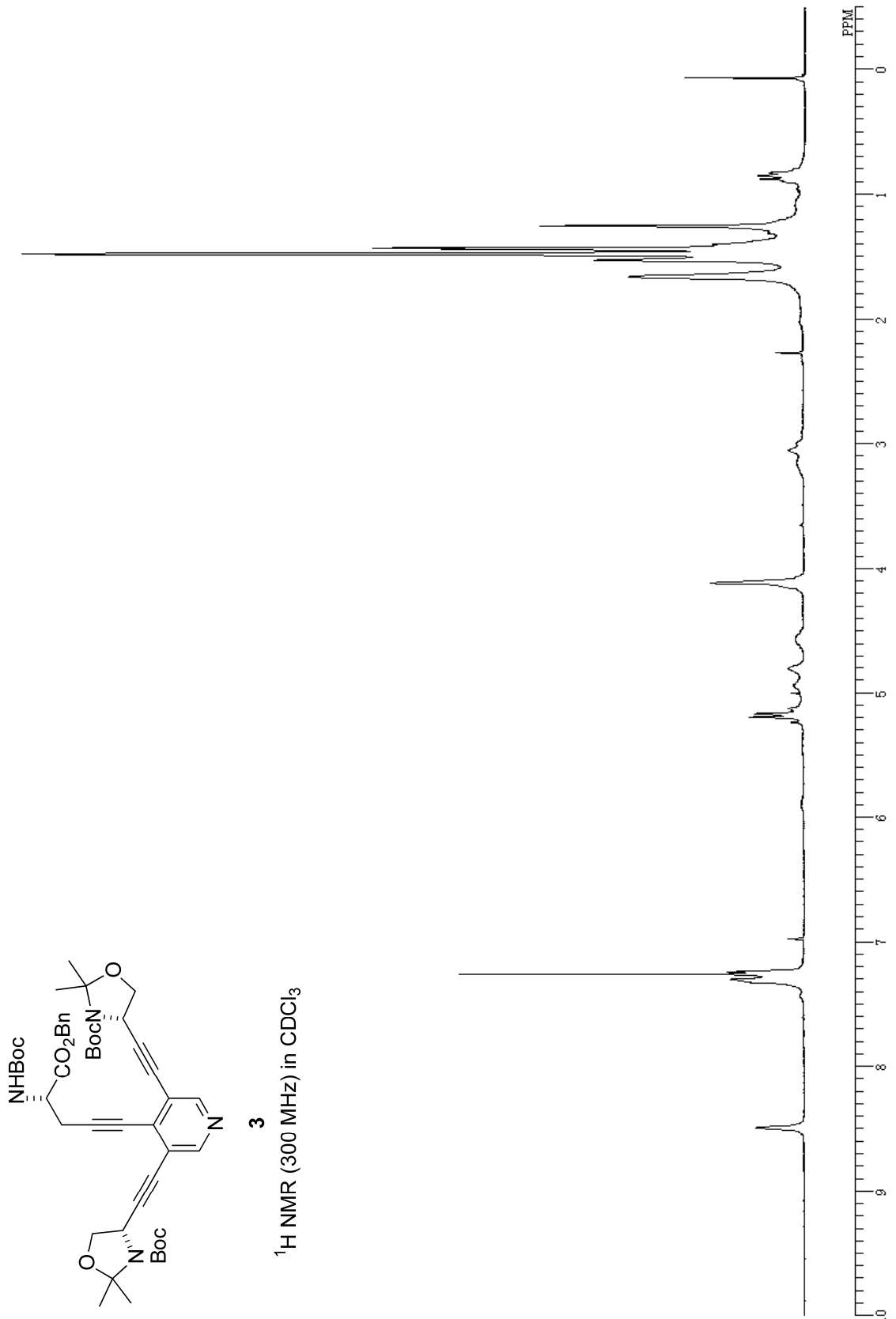
13b

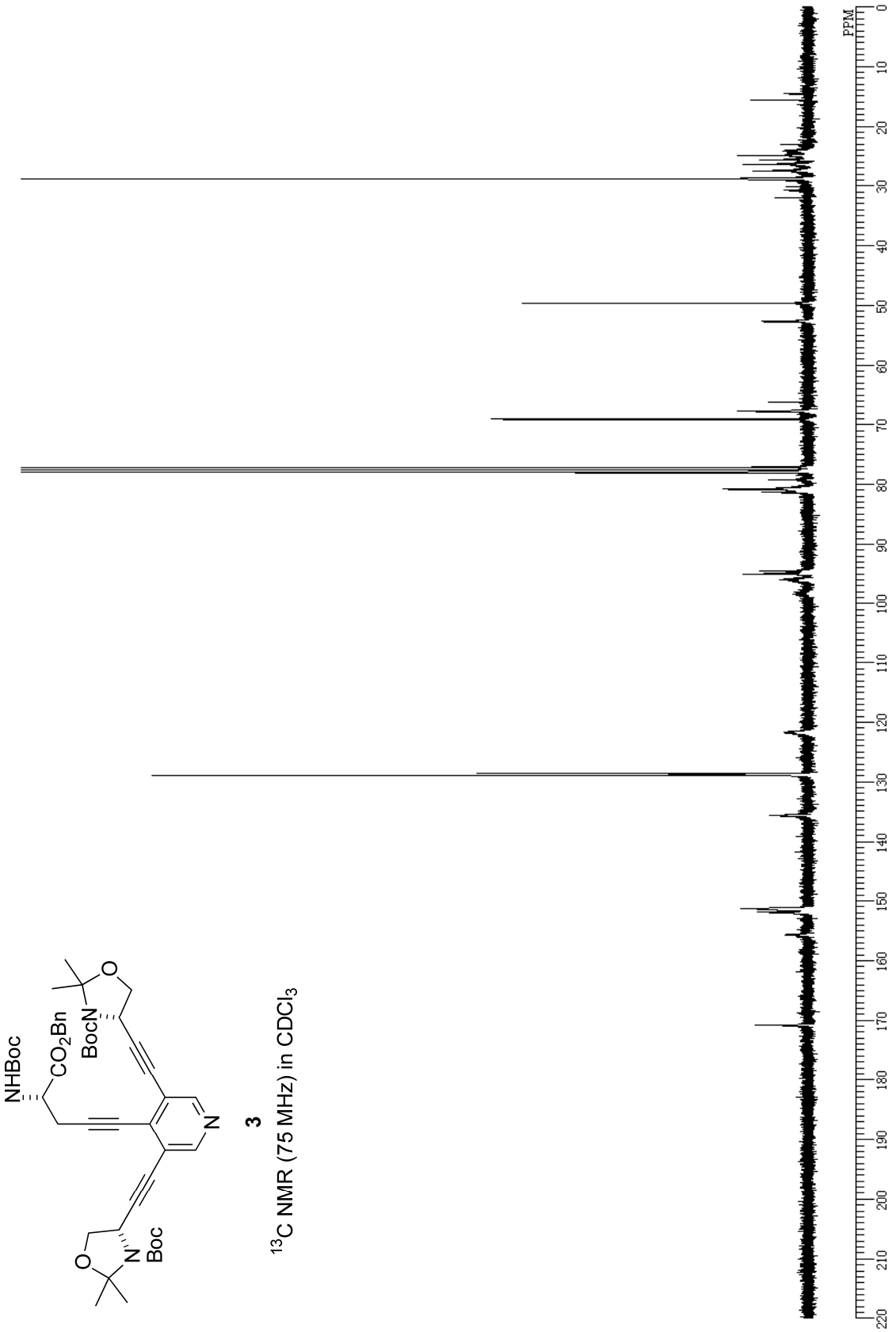
^{13}C NMR (75 MHz) in $CDCl_3$

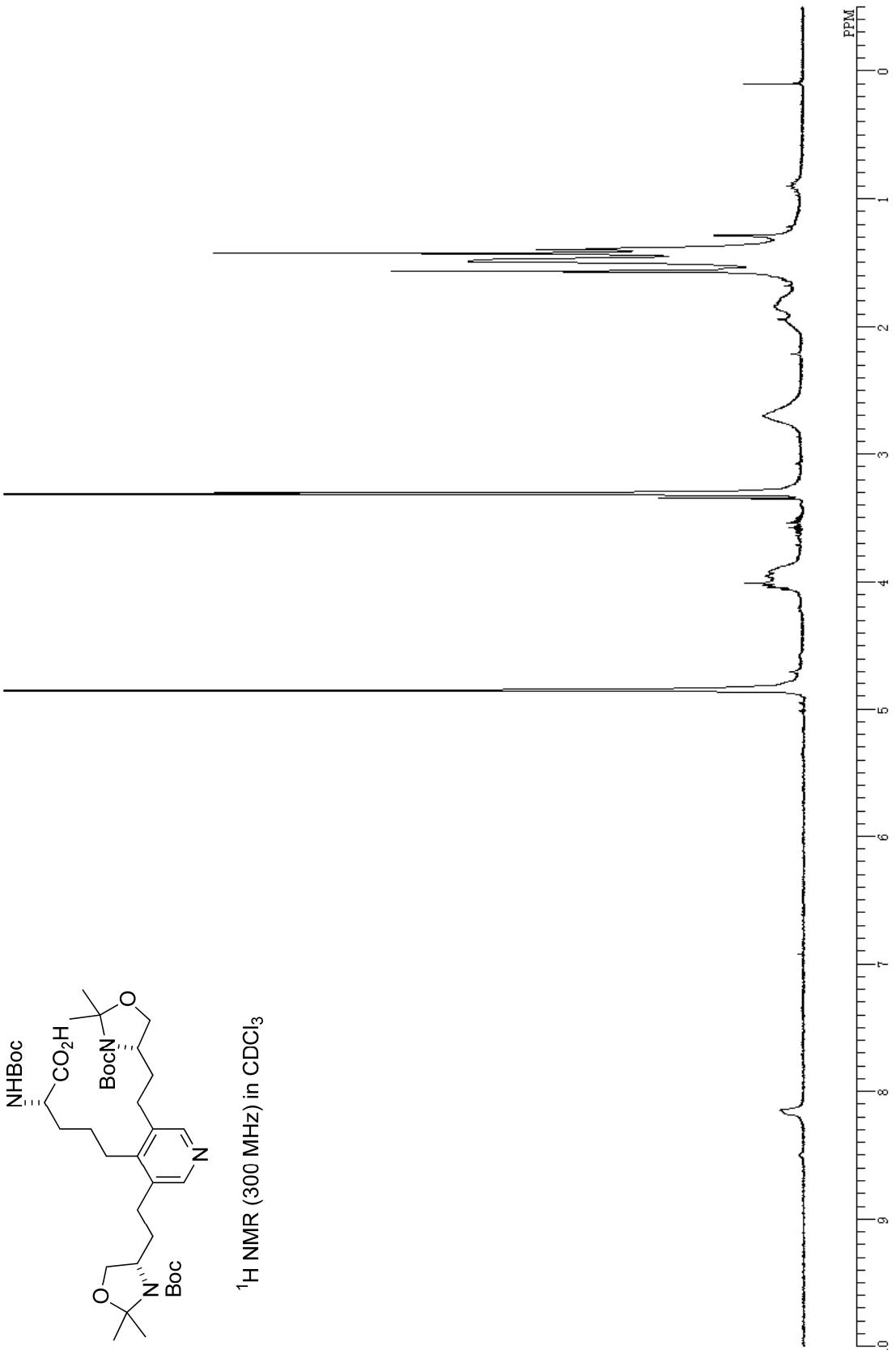


13a

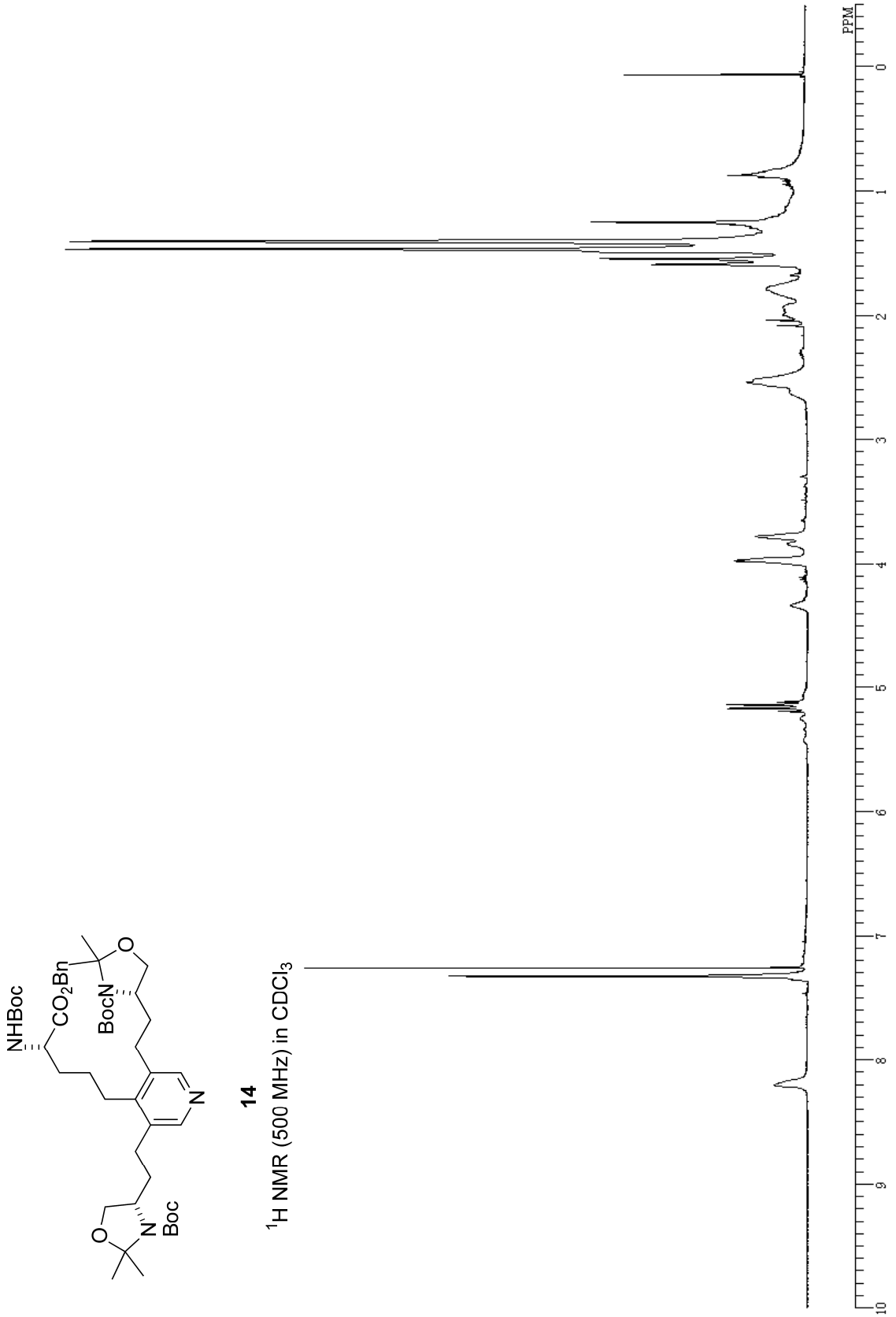
^{1}H NMR (300 MHz) in $CDCl_3$




NHBoc
CO2Bn
BocN
O
N
Boc
O
N
3
13C NMR (75 MHz) in CDCl3
PPM
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220

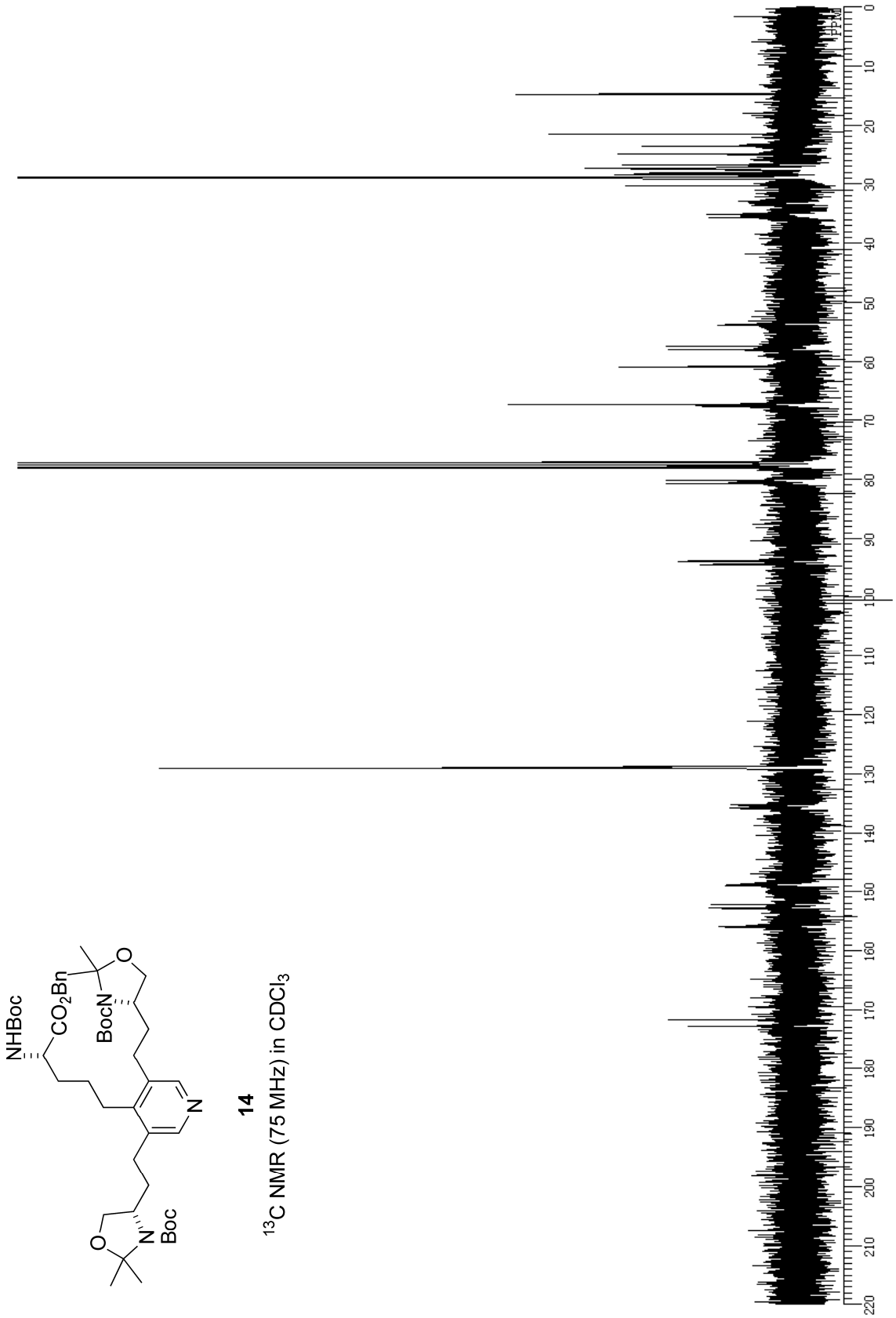


^{1}H NMR (300 MHz) in $CDCl_3$



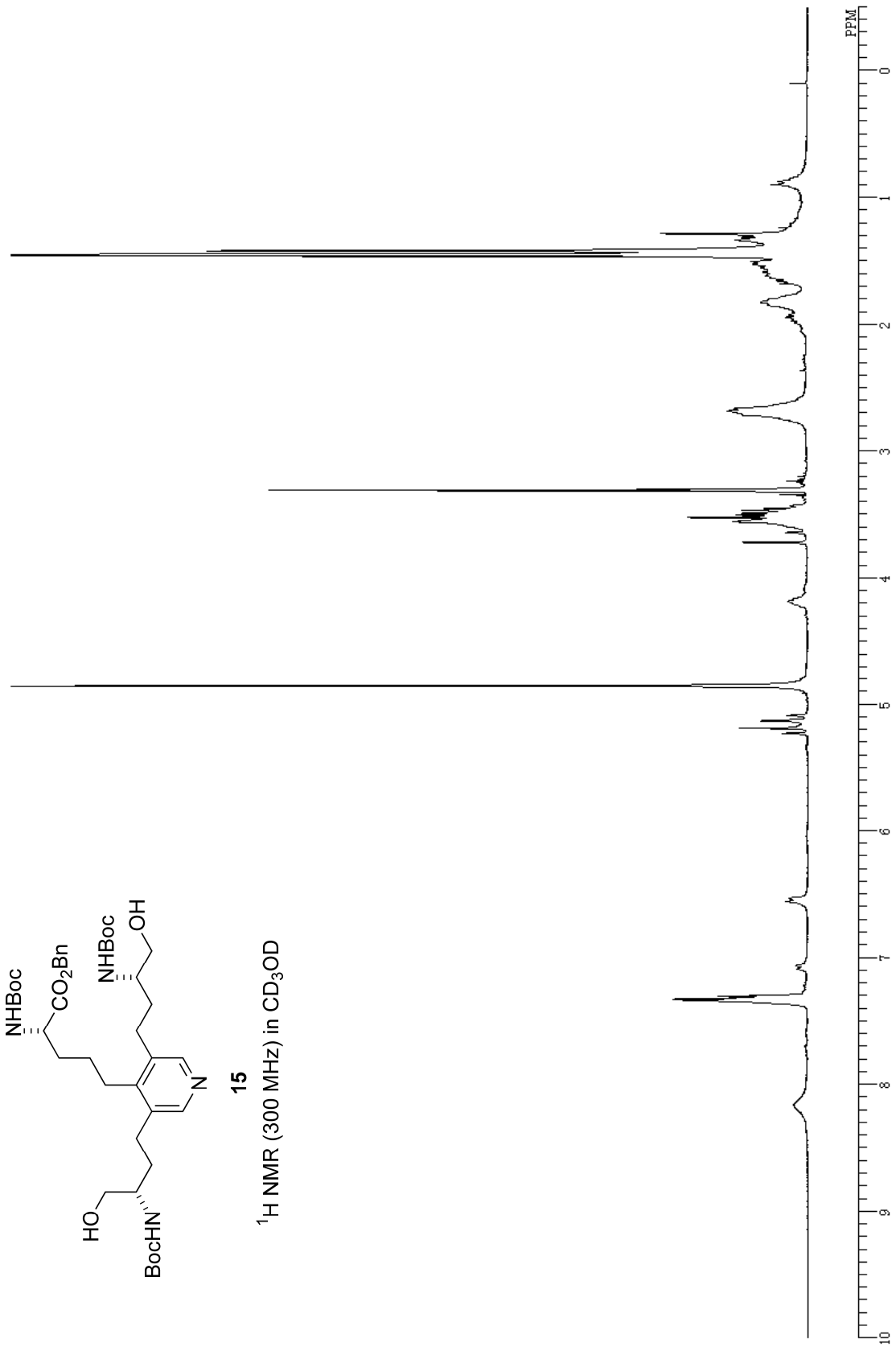
14

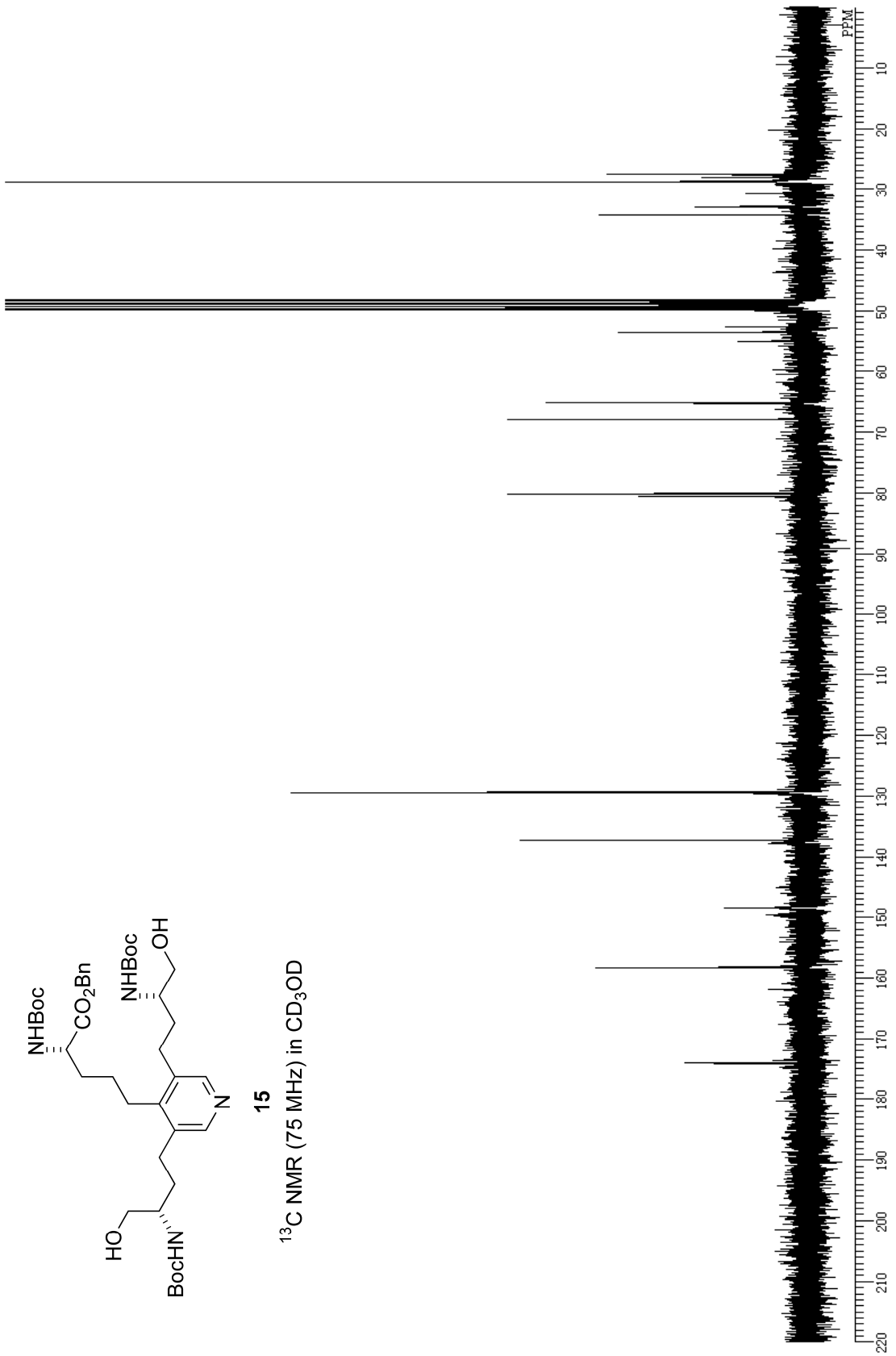
^{1}H NMR (500 MHz) in $CDCl_3$



14

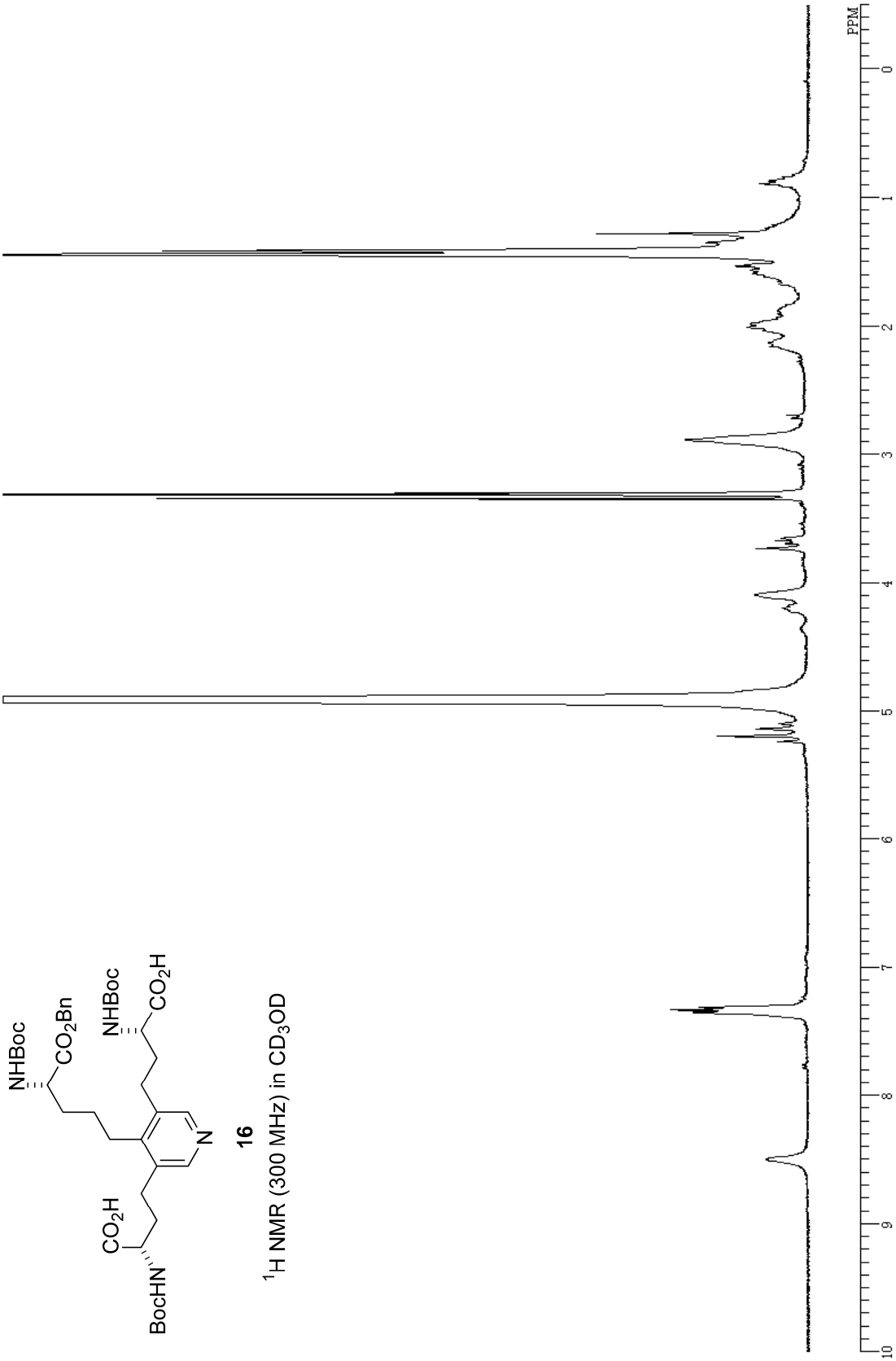
^{13}C NMR (75 MHz) in $CDCl_3$

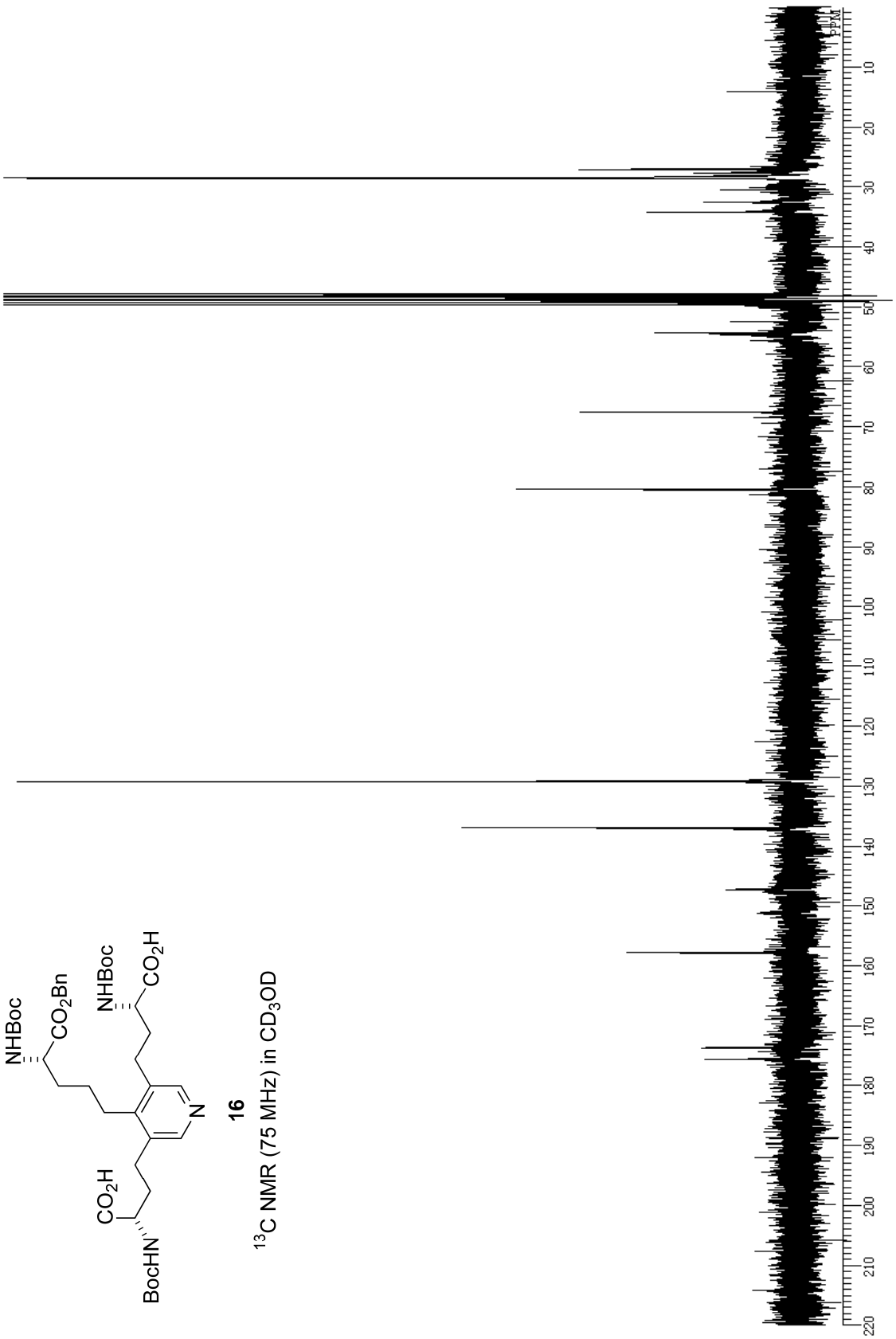




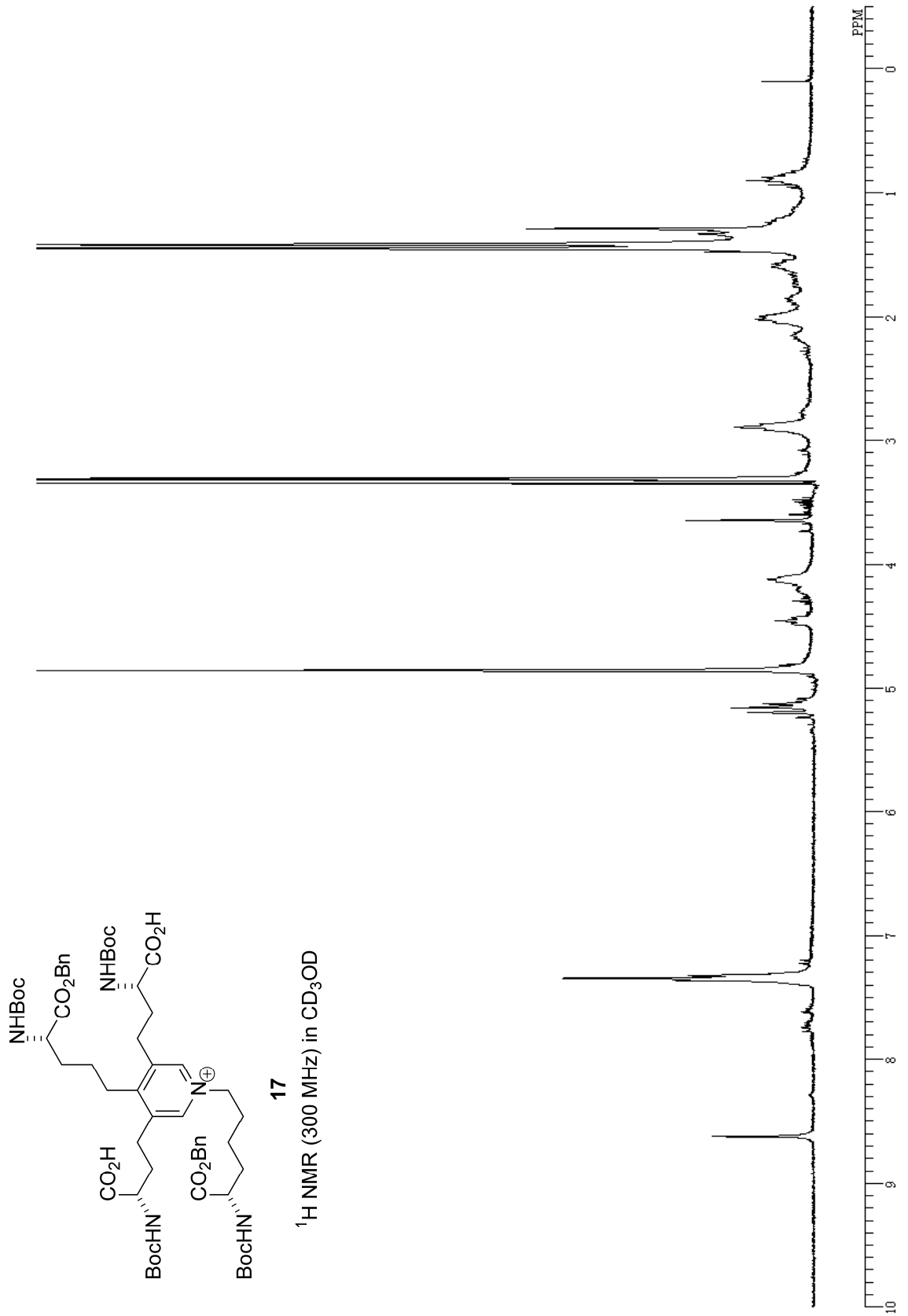
15

^{13}C NMR (75 MHz) in CD_3OD


NHBoc
CO2Bn
NHBoc
CO2H
CO2H
BocHN
N
16
1H NMR (300 MHz) in CD3OD
PPM
0
1
2
3
4
5
6
7
8
9
10

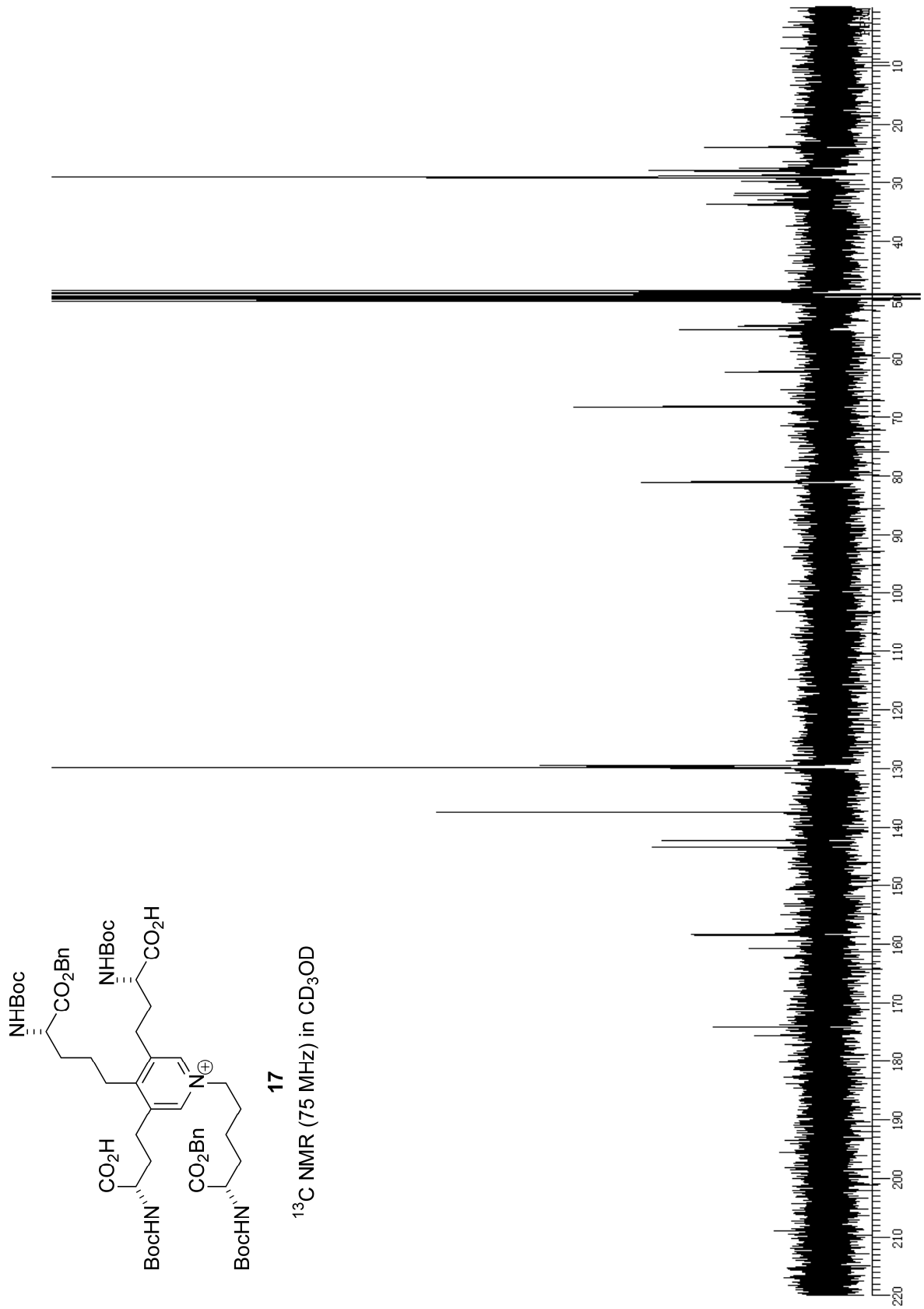

NHBoc
CO2Bn
NHBoc
CO2H
CO2H
BocHN
N
16
13C NMR (75 MHz) in CD3OD
PPM
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220

Supplementary Information



17

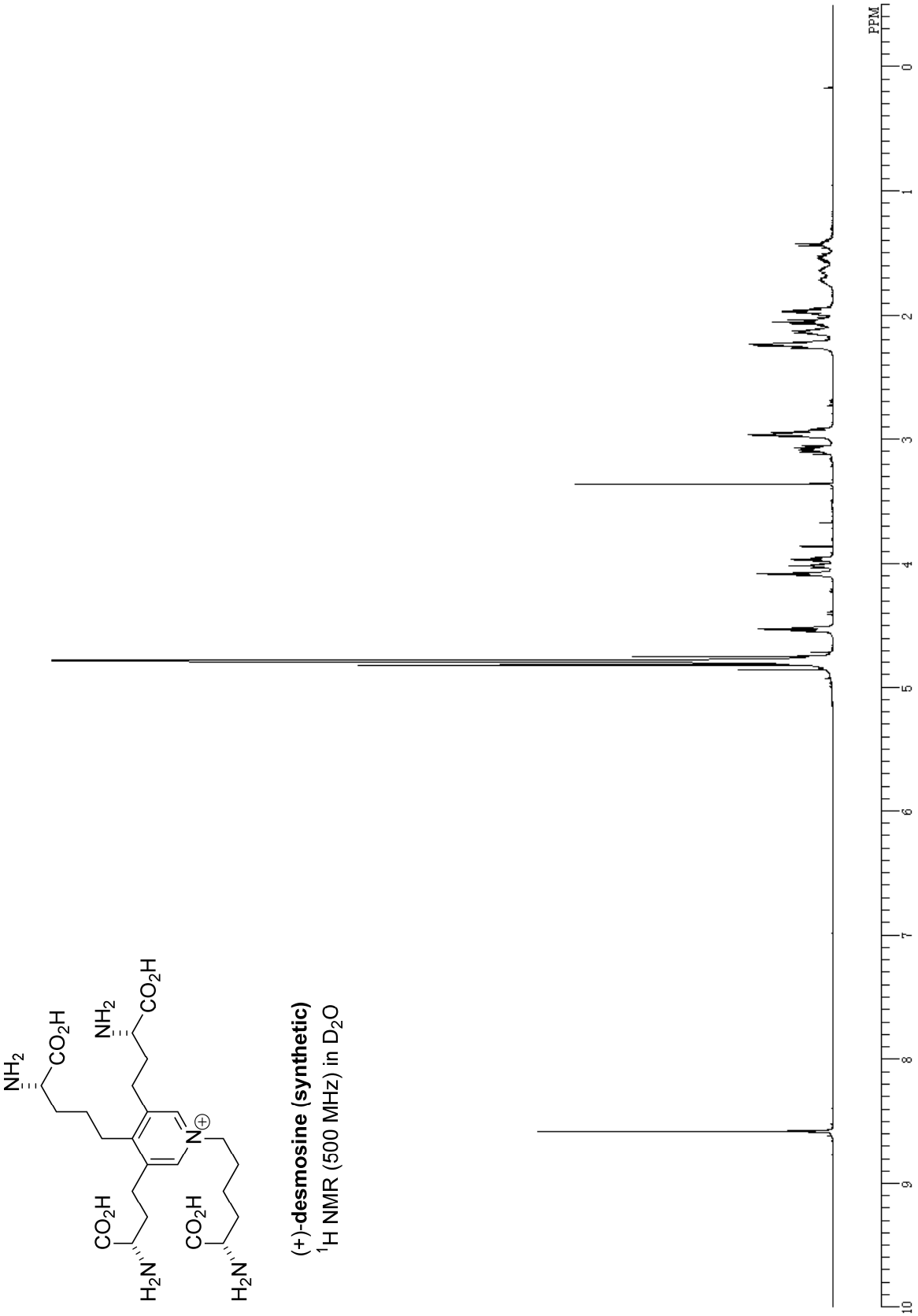
^{1}H NMR (300 MHz) in CD_3OD



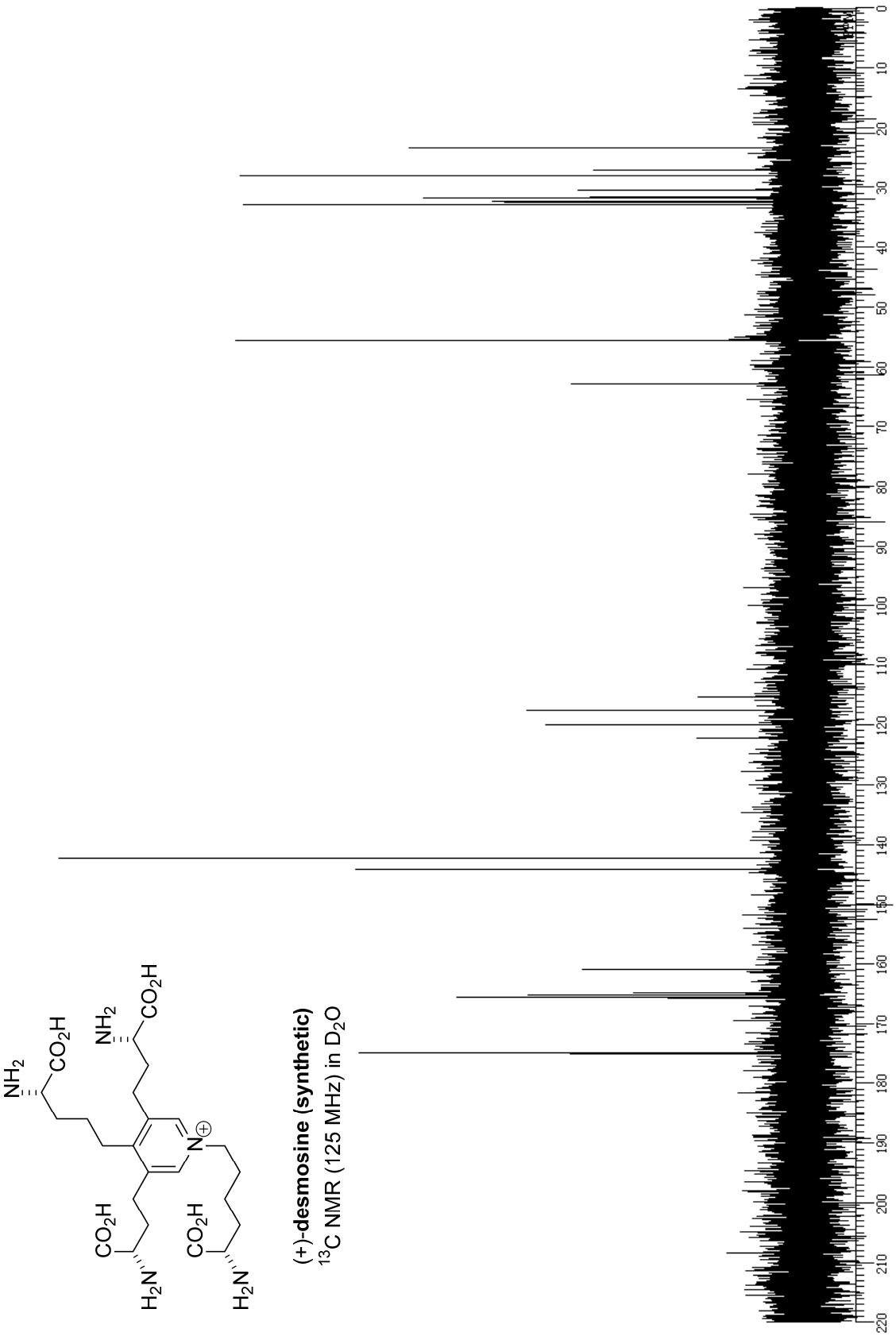
17

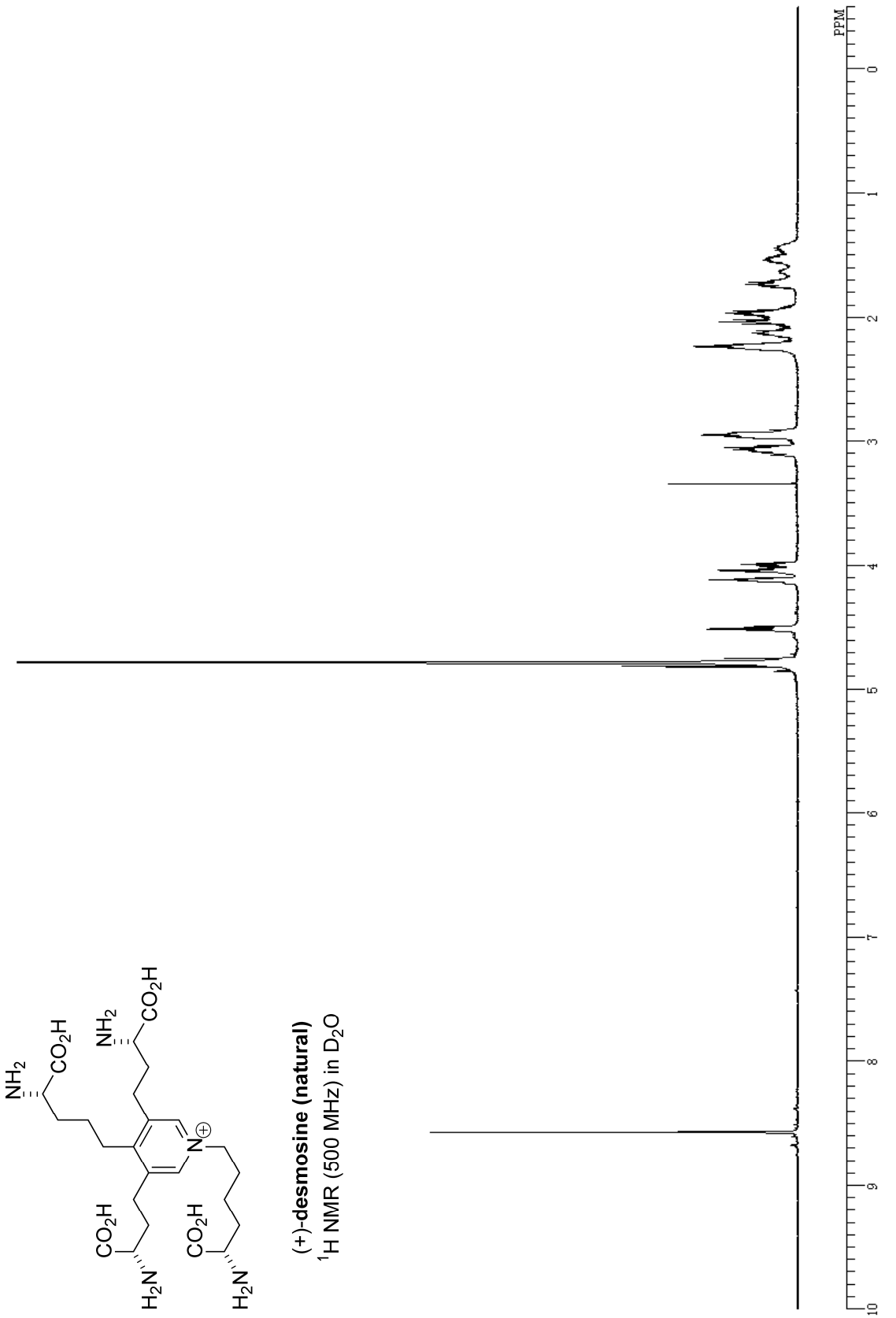
^{13}C NMR (75 MHz) in CD_3OD

Supplementary Information

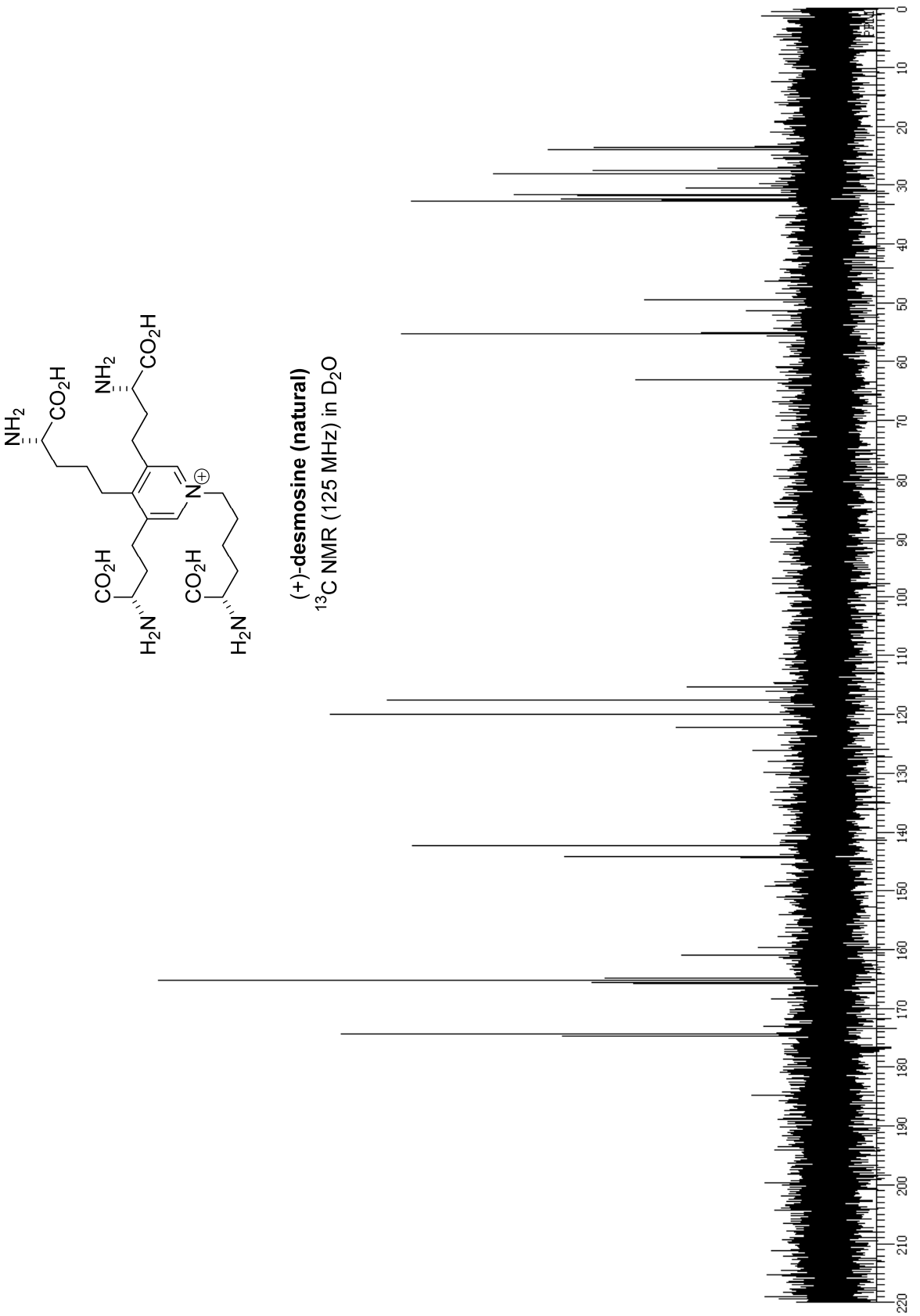


(+)-desmosine (synthetic)
^{1}H NMR (500 MHz) in D_2O


(+)-desmosine (synthetic)
^{13}C NMR (125 MHz) in D_2O
NH_2
CO_2H
NH_2
CO_2H
CO_2H
CO_2H
H_2N
H_2N
N⊕



(+)-desmosine (natural)
^{1}H NMR (500 MHz) in D_2O


NH2
CO2H
NH2
CO2H
CO2H
H2N
CO2H
H2N
N⊕
(+)-desmosine (natural)
13C NMR (125 MHz) in D2O
PPM
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210
220