Synthesis of tetrafluorinated aromatic amino acids with distinct signatures in ^{19}F-NMR

Luoheng Qin, Christopher Sheridan and Jianmin Gao

Department of Chemistry, Merkert Chemistry Center, Boston College,
Chestnut Hill, Massachusetts, 02467-3801

Supporting Information

I. General information

Chemicals were obtained from Fisher and Aldrich, unless indicated otherwise. Boc-pentafluorophenylalanine (Boc-Z) was purchased from Peptech (Burlington, MA). Fmoc-OSu, Rink-Amide-MBHA resin, and O-Benzotriazole-N,N,N',N'-tetramethyl-uronium-hexafluoro-phosphate(HBTU) were purchased from Novabiochem (San Diego, CA). All Fmoc-protected natural amino acids were purchased from Advanced Chemtech (Louisville, KL). All amino acids described in this submission are in the L-configuration. Peptide synthesis was carried out on a Tribute peptide synthesizer (Protein Technologies, Tucson, AZ). ^{1}H-NMR, ^{13}C-NMR and ^{19}F-NMR data were collected on Varian Gemini 400 MHz or Varian 500 MHz NMR spectrometers. HR-MS data were generated by the Boston College Mass-Spec facility.

II. Synthesis procedures

General Procedure I: Boc- deprotection

A Boc-protected amino acid (0.1 mmol) was dissolved in a mixture of 95/2.5/2.5 TFA/$^{i}Pr_3SiH/H_2O$ (1 mL), and stirred at r.t. for 1 h. The solvents were then removed under vacuum, and the residue was directly used in General Procedure II.

General Procedure II: Fmoc- protection

The crude free amino acid (0.1 mmol) prepared via General Procedure I or III was dissolved in 10% Na_2CO_3 aq. (1 mL), followed by an addition of CH_3CN (0.5 mL) and Fmoc-OSu (32 mg, 0.095 mmol). The resulting mixture was stirred vigorously at r.t. for 2.5 h, then poured into 1 N HCl (20 mL) and extracted with DCM (3 × 10 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. After the solvent was evaporated under vacuum, the product was isolated with silica gel column (9:1 toluene/HOAc).

*(S)-2-(tert-butoxycarbonylamino)-3-(2,3,5,6-tetrafluoro-4-(4-methoxybenzylthio)phenyl)propanoic acid (**2**, Boc-ZpSPMB-OH)*

(S)-2-(tert-butoxycarbonylamino)-3-(perfluorophenyl) propanoic acid (Boc-Z, **1**, 200 mg, 0.56 mmol) and (4-methoxyphenyl)methanethiol (125 μL, 0.90 mmol) were dissolved in isopropanol (2 mL), followed by an addition of 1 N NaOH aq. (6 mL). The resulting solution was sealed in a pressure bottle under N_2 protection and stirred at 70 ^{o}C for 24 h. Upon cooling to room temperature, the reaction mixture was poured into a mixture of 1 N HCl (50 mL) and 1 M $CuSO_4$ aq. (10 mL), swirled thoroughly, and extracted with DCM (3 × 30 mL). The combined organic layers were washed with a 10:1 mixture of brine and 1N HCl and dried over Na_2SO_4. After the solvent was evaporated under vacuum, the product was isolated with silica gel column (3:1 hexanes/EtOAc with 3% HOAc). Yield: 243 mg (88%). ^{1}H NMR (500 MHz, Acetone –*d*$_6$) δ: 7.18 (d, *J* = 8.5 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 6.25 (d, *J* = 9.0 Hz, 1H), 4.48 (m, 1H), 4.13 (s, 2H), 3.76 (s, 3H), 3.34 (m, 1H), 3.21 (m, 1H), 1.34 (s, 9H). ^{13}C NMR (125 MHz, Acetone-*d*$_6$) δ: 172.8, 160.6, 156.5, 149.0, 147.8, 147.1, 145.8, 131.3, 130.1, 118.7, 115.2, 80.0, 55.9, 53.8, 39.5, 28.8, 27.2. ^{19}F NMR (470 MHz, Acetone-*d*$_6$) δ: -131.2 (2F), -138.7 (2F). HRMS (ESI+): *m/z* calculated for $C_{22}H_{24}F_4NO_5S$ $[M]^+$, 490.13113; found 490.13237.

*(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(2,3,5,6-tetrafluoro-4-(4-methoxybenzylthio)phenyl)propanoic acid (**3**, Fmoc-ZpSPMB-OH)*

Following General Procedure I and II. The product was purified with silica gel column (10:1 toluene/HOAc). Yield: 80%, over 2 steps. ^{1}H NMR (500 MHz, Acetone –*d*$_6$) δ: 7.83 (d, *J* = 7.5 Hz, 2H), 7.66 (d, *J* = 7.5 Hz, 2H), 7.40 (m, 2H), 7.31 (m, 2H), 7.09 (d, *J* = 8.5 Hz, 2H), 6.92 (d, *J* = 9.0 Hz, 1H), 6.78 (d, *J* = 8.5 Hz, 2H), 4.61 (m, 1H), 4.17-4.33 (m, 3H), 4.05 (s, 2H), 3.71 (s, 3H), 3.40 (m, 1H), 3.30 (m, 1H). ^{13}C NMR (100 MHz, Acetone-*d*$_6$) δ: 172.0, 160.2, 156.8, 149.0, 147.4, 146.6, 145.0, 144.9, 142.1, 130.9, 129.6, 128.6, 128.0, 126.1, 120.8, 118.1, 114.7, 113.2, 67.5, 55.5, 53.8, 47.9, 39.1, 26.5. ^{19}F NMR (470 MHz, Chloroform-*d*) δ: -130.8 (2F), -138.5 (2F). HRMS (ESI+): *m/z* calculated for $C_{32}H_{26}F_4NO_5S$ $[M]^+$, 612.14678; found 612.14419.

*(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(2,3,5,6-tetrafluoro-4-mercaptophenyl)propanoic acid (**4**, Fmoc-ZpSH-OH)*

Compound **3** Fmoc-ZpSPMB-OH (312 mg, 0.51 mmol) was dissolved in a mixture of methanol (8 mL) and acetonitrile (8 mL). $Hg(OAc)_2$ (195 mg, 0.61 mmol) was added. The resulting solution was stirred at

60 ^{o}C for 6 h in a sealed vial. Upon cooling to r.t., the reaction was quenched by bubbling H_2S into the reaction solution over 20 min. EtOAc (20 mL) was then added, and HgS was removed by vacuum filtration. 1N HCl (20 mL) was added to the filtrate, swirled thoroughly, and the organic layer was separated. The aqueous layer was extracted with EtOAc (2 × 30 mL), and the combined organic layers were dried over Na_2SO_4. After the solvent was evaporated under vacuum, the product was isolated with silica gel column (1:1 Et_2O/hexane first, to remove less-polar byproducts; followed by 9:1 toluene/HOAc). Yield: 215 mg (86%). ^{1}H NMR (500 MHz, Acetone –d_6) δ: 7.81 (d, J = 7.5 Hz, 2H), 7.64 (d, J = 8.0 Hz, 2H), 7.39 (m, 2H), 7.29 (m, 2H), 6.95 (d, J = 8.5 Hz, 1H), 4.63 (m, 1H), 4.14-4.33 (m, 3H), 3.39 (m, 1H), 3.28 (m, 1H). ^{13}C NMR (125 MHz, Acetone –d_6) δ: 172.3, 156.9, 147.5, 145.3, 145.1, 144.9, 143.3, 142.0, 128.5, 127.9, 126.1, 114.1, 111.5, 67.5, 54.0, 47.9, 26.3. ^{19}F NMR (470 MHz, Acetone-d_6) δ: -139.7 (2F), -143.6 (2F). HRMS (ESI+): *m/z* calculated for $C_{24}H_{18}F_4NO_4S$ $[M]^+$, 492.08927; found 492.08680.

*(S)-tert-butyl 2-(tert-butoxycarbonylamino)-3-(perfluorophenyl)propanoate (**5**, Boc-Z-O^{t}Bu)*

t-Butyl trichloroacetimidate (4.04 mL, 22.5 mmol) was slowly added to a solution of Boc-Z (Compound **1**, 4.0 g, 11.3 mmol) in 120 mL DCM/cyclohexane (1:2). The resulting solution was stirred at r.t. for 16 h. White crystal precipitated out. $BF_3{\cdot}H_2O$ (0.22 mL) was then added, and the reaction was stirred at r.t. for another 1 h. The reaction was quenched with saturated $NaHCO_3$ aq. (100 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (100 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. After the solvent was evaporated under vacuum, the product was isolated with silica gel column (9:1 hexanes/EtOAc) as white crystal. Yield: 4.4 g (95%). ^{1}H NMR (400 MHz, Chloroform–*d*) δ: 5.12 (d, J = 8.8 Hz, 1H), 4.46 (m, 1H), 3.21 (m, 1H), 3.05 (m, 1H), 1.45 (s, 9H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, Chloroform–*d*) δ: 169.9, 155.0, 146.9, 144.5, 140.9, 138.7, 136.2, 110.9, 82.9, 79.9, 53.3, 28.1, 27.8, 26.3. ^{19}F NMR (400 MHz, Chloroform–*d*) δ: -142.3 (2F), -156.5 (1F), -163.1 (2F). HRMS (ESI+): *m/z* calculated for $C_{18}H_{23}F_5NO_4$ $[M]^+$, 412.15472; found 412.15470.

*(S)-tert-butyl 3-(4-azido-2,3,5,6-tetrafluorophenyl)-2-(tert-butoxycarbonylamino)propanoate (**6**, Boc-ZpN$_3$-O^{t}Bu)*

A mixture of **5** (400 mg, 0.97 mmol), tetrabutylammonium azide (28 mg, 0.097 mmol) and NaN_3 (126 mg, 1.94 mmol) in anhydrous DMF (4 mL) was sealed in a pressure vessel and stirred at 80 ^{o}C for 16 h. Upon cooling to room temperature, the reaction mixture was poured into saturated $NaHCO_3$ aq. (40 mL), swirled thoroughly, and extracted with DCM (40 mL) and chloroform (2 × 40 mL). The combined organic layers were washed with brine and dried over Na_2SO_4. After the solvent was evaporated under vacuum, the product was isolated with silica gel column (6:1 hexanes/EtOAc) as white solid. Yield: 342 mg (81%). ^{1}H NMR (400 MHz, Chloroform–*d*) δ: 5.11 (d, *J* = 7.6 Hz, 1H), 4.44 (m, 1H), 3.22 (m, 1H), 3.03 (m, 1H), 1.45 (s, 9H), 1.40 (s, 9H). ^{13}C NMR (100 MHz, Chloroform–*d*) δ: 170.0, 155.1, 146.9, 144.5, 141.8, 139.3, 119.5, 111.5, 83.0, 80.2, 53.5, 28.4, 28.1, 26.6. ^{19}F NMR (400 MHz, Chloroform–*d*) δ: -142.6 (2F), -153.0 (2F). HRMS (ESI+): *m/z* calculated for $C_{18}H_{23}F_4N_4O_4$ $[M]^+$, 435.16554; found 435.16337.

*(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(4-azido-2,3,5,6-tetrafluorophenyl)propanoic acid (**7**, Fmoc-ZpN$_3$-OH)*

Boc-ZpN$_3$-O^{t}Bu **6** was treated following General Procedure I and II. Yield: 85%. Known compound.[S1] ^{1}H NMR (400 MHz, Methanol–*d$_4$*) δ: 7.77 (d, *J* = 7.2 Hz, 2H), 7.60 (m, 2H), 7.40 (m, 2H), 7.30 (m, 2H), 4.53 (m, 1H), 4.12-4.34, (m, 3H), 3.33 (m, 1H), 3.19 (m, 1H).

*(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(4-amino-2,3,5,6-tetrafluorophenyl)propanoic acid (**8**, Fmoc-ZpNH$_2$-OH)*

1,3-propanedithiol (138 μL , 1.38 mmol) was added into a solution of Fmoc-ZpN$_3$-OH **7** (345 mg, 0.69 mmol) and DIPEA (240 μL, 1.38 mmol) in 5 mL methanol/acetonitrile (1:1). The resulting solution was stirred at r.t. for 5 min, then poured into 1N HCl (20 mL) and extracted with DCM (5 × 20 mL). The combined organic layers were dried over Na_2SO_4. DCM was evaporated under vacuum, and the product was isolated with silica gel column (3:2 hexanes/EtOAc, 2% HOAc) as white crystal. Yield: 314 mg (96%). ^{1}H NMR (400 MHz, Methanol–*d$_4$*) δ: 7.77 (d, *J* = 7.6 Hz, 2H), 7.60 (d, *J* = 7.6 Hz, 2H), 7.36 (m, 2H), 7.28 (m, 2H), 4.41 (m, 1H), 4.12-4.32 (m, 3H), 3.20 (m, 1H), 3.05 (m, 1H). ^{13}C NMR (100 MHz, Methanol–*d$_4$*) δ: 172.9, 156.9, 146.7, 144.4, 143.7, 141.0, 137.5, 134.9, 127.3, 126.7, 124.8, 119.4, 100.6, 66.8, 53.6, 24.4. ^{19}F NMR (400 MHz, Acetone-*d$_6$*) δ: -148.1 (2F), -164.4 (2F). HRMS (ESI+): *m/z* calculated for $C_{24}H_{19}F_4N_2O_4$ $[M]^+$, 475.12809; found 475.12717.

*(S)-3-(4-azido-2,3,5,6-tetrafluorophenyl)-2-(2,2,2-trifluoroacetamido)propanoic acid (**9**, CF$_3$CO-ZpN$_3$-OH)*

Boc-ZpN$_3$-O^{t}Bu **6** (300 mg, 0.69 mmol) was dissolved in TFA (2.5 mL) at 0 ^{o}C. After stirred 0.5 h at 0 ^{o}C, trifluoroacetic anhydride (125 μL, 0.90 mmol) was added dropwise. The resulting solution was stirred at 0 ^{o}C for 2 h and at r.t. for another 16 h. TFA was removed under vacuum, and the residue was directly purified with silica gel column (9:1 toluene/HOAc). Yield: 258 mg (100%). ^{1}H NMR (400 MHz, Methanol–d_4) δ: 4.68 (m, 1H), 3.42 (m, 1H), 3.20 (m, 1H). ^{13}C NMR (100 MHz, Methanol–d_4) δ: 170.5, 157.3, 146.7, 144.3, 141.6, 139.1, 118.9, 114.4, 111.1, 51.7, 24.1. ^{19}F NMR (400 MHz, Acetone-d_6) δ: -77.0 (3F), -144.9 (2F), -154.8 (2F). HRMS (ES-): *m/z* calculated for $C_{11}H_4F_7N_4O_3$ $[M]^-$, 373.0172; found 373.0163.

*(S)-3-(4-amino-2,3,5,6-tetrafluorophenyl)-2-(2,2,2-trifluoroacetamido)propanoic acid (**10**, CF_3CO-ZpNH$_2$-OH)*

1,3-propanedithiol (138 μL , 1.38 mmol) was added into a solution of **9** (258 mg, 0.69 mmol) and DIPEA (240 μL, 1.38 mmol) in 5 mL methanol/acetonitrile (1:1). The resulting solution was stirred at r.t. for 5 min, then poured into 1N HCl (20 mL) and extracted with DCM (5 × 20 mL). The combined organic layers were dried over Na_2SO_4. DCM was evaporated under vacuum, and the product was isolated with silica gel column (3:2 hexanes/EtOAc, 2% HOAc) as white crystal. Yield: 228 mg (95%). ^{1}H NMR (400 MHz, Acetone –d_6) δ: 8.70 (d, J = 8.4 Hz, 1H), 4.77 (m, 1H), 3.38 (m, 1H), 3.22 (m, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 169.6, 156.5, 146.8, 144.5, 137.3, 134.9, 127.1, 114.5, 100.0, 52.0, 23.7. ^{19}F NMR (400 MHz, Acetone-d_6) δ: -77.0 (3F), -148.4 (2F), -164.4 (2F). HRMS (ESI+): *m/z* calculated for $C_{11}H_8F_7N_2O_3$ $[M]^+$, 349.04231; found 349.04386.

General Procedure III: CF_3CO- deprotection

CF_3CO- protected amino acid (0.1 mmol) was suspended in 1.2 N HCl (3 mL) and refluxed for 16 h. HCl was removed on rotovap. And the residue was directly used in general procedure II.

*(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(2,3,5,6-tetrafluoro-4-iodophenyl)propanoic acid (**12c**, Fmoc-ZpI-OH)*

t-Butyl nitrite (57 μL, 0.43 mmol) was added dropwise into a mixture of **10** (100 mg, 0.287 mmol) and iodine (146 mg, 0.576 mmol) in acetonitrile (5.8 mL) at 0 °C. The resulting solution was stirred at 0 °C for 1.5 h. The solvent was removed on rotovap, and the residue was treated with 2 M $NaHSO_3$ (30 mL) then extracted with DCM (3 × 30 mL). The combined organic layers were dried over Na_2SO_4. DCM was evaporated under vacuum, and the crude product was isolated with silica gel column (9:1 toluene/HOAc) as a mixture of CF_3CO-ZpI/ZpH (20:1). The ZpI/ZpH mixture was treated sequentially as General Procedure III and II, and finally purified with RP-HPLC (Waters Prep LC, Jupiter 10u C18 300A Column). Eluent condition: 70%-100% B over 20 min (Buffer A: 95% water, 5% acetonitrile and 0.1%

TFA; Buffer B: 5% water, 95% acetonitrile and 0.1% TFA). Yield: 114 mg (68%, over 3 steps). ^{1}H NMR (400 MHz, Acetonitrile–*d₃*) δ: 7.79 (d, *J* = 7.8 Hz, 2H), 7.58 (d, *J* = 7.8 Hz, 2H), 7.40 (m, 2H), 7.30 (m, 2H), 6.18 (d, *J* = 7.8 Hz, 1H), 4.49 (m, 1H), 4.10-4.28, (m, 3H), 3.29 (m, 1H), 3.19 (m, 1H). ^{13}C NMR (100 MHz, Acetonitrile-d_3) δ: 171.5, 156.2, 148.3, 146.4, 145.9, 144.2, 141.3, 128.0, 127.5, 127.4, 125.4, 120.2, 71.3, 66.9, 52.9, 47.1, 25.9. ^{19}F NMR (400 MHz, Chloroform–*d*) δ: -120.7 (2F), -140.7 (2F). HRMS (ESI+): *m/z* calculated for $C_{24}H_{17}F_4INO_4$ [M]$^+$, 586.01384; found 586.01431.

General Procedure IV: Sandmeyer reaction

t-Butyl nitrite (57 μL, 0.43 mmol) was added dropwise into a mixture of **10** (100 mg, 0.287 mmol), CuX (0.576 mmol) and CuX_2 (0.576 mmol, X = Cl, Br) in acetonitrile (5.8 mL) at 0 °C. The resulting solution was stirred at 0 °C for 1.5 h. The solvent was removed on rotovap, and the residue was treated with 1 M HCl (30 mL) then extracted with DCM (3 × 30 mL). The combined organic layers were dried over Na_2SO_4. DCM was evaporated under vacuum, and the crude product was isolated with silica gel column (9:1 toluene/HOAc) as a mixture of CF_3CO-ZpX/ZpH, which was directly subjected to General Procedure III.

*(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(4-bromo-2,3,5,6-tetrafluorophenyl)propanoic acid (**12b**, Fmoc-ZpBr-OH)*

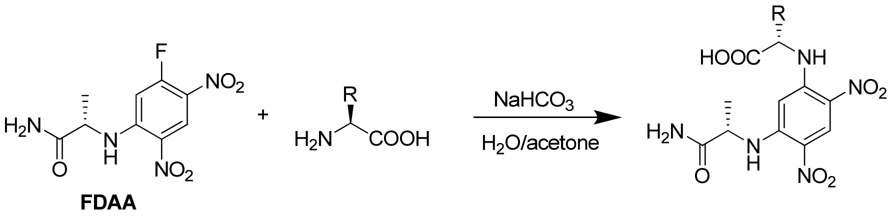
Following General Procedure IV, III and II. The final product was purified with RP-HPLC (Waters Prep LC, Jupiter 10u C18 300A Column). Eluent condition: 70%-100% B over 20 min (Buffer A: 95% water, 5% acetonitrile and 0.1% TFA; Buffer B: 5% water, 95% acetonitrile and 0.1% TFA). Yield: 71%, over 3 steps. ^{1}H NMR (500 MHz, Acetonitrile–d_3) δ: 7.81 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 8.0 Hz, 2H), 7.40 (m, 2H), 7.32 (m, 2H), 6.13 (d, *J* = 8.0 Hz, 1H), 4.64 (m, 1H), 4.16-4.30, (m, 2H), 4.16 (m, 1H), 3.30 (m, 1H), 3.20 (m, 1H). ^{13}C NMR (100 MHz, Methanol-d_4) δ: 172.0, 156.9, 146.7, 145.8, 144.2, 143.7, 143.3, 141.0, 127.3, 126.5, 124.7, 119.3, 116.1, 97.8, 66.8, 52.8, 46.8, 25.3. ^{19}F NMR (400 MHz, Chloroform–*d*) δ: -133.5 (2F), -139.9 (2F). HRMS (ESI+): *m/z* calculated for $C_{24}H_{17}BrF_4NO_4$ [M]$^+$, 538.02771; found 538.02805.

*(S)-2-(((9H-fluoren-9-yl)methoxy)carbonylamino)-3-(4-chloro-2,3,5,6-tetrafluorophenyl)propanoic acid (**12a**, Fmoc-ZpCl-OH)*

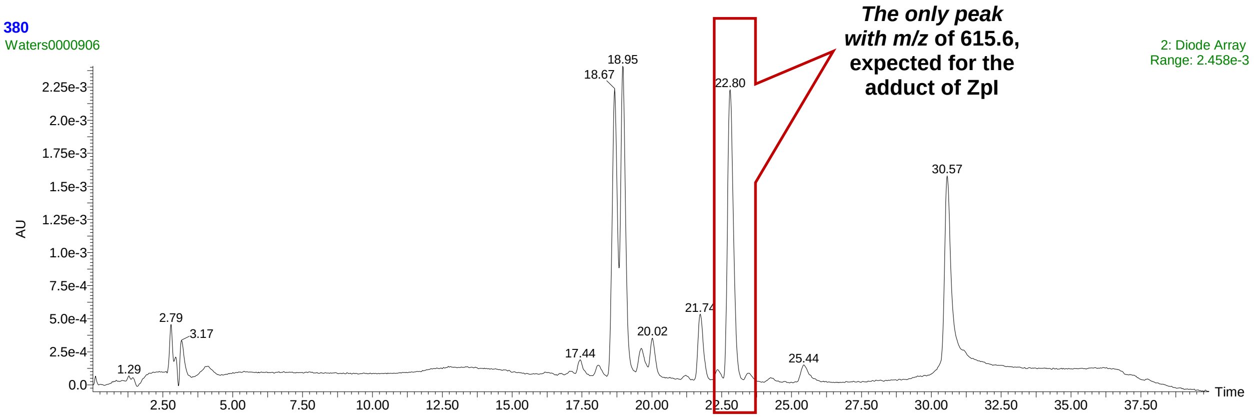
Following General Procedure IV, III and II. The final product was purified with RP-HPLC (Waters Prep LC, Jupiter 10u C18 300A Column). Alluent condition: 70%-100% B over 20 min (Buffer A: 95% water, 5% acetonitrile and 0.1% TFA; Buffer B: 5% water, 95% acetonitrile and 0.1% TFA). Yield: 62%, over 3 steps. ^{1}H NMR (500 MHz, Acetonitrile–d_3) δ: 7.82 (d, J = 7.5 Hz, 2H), 7.60 (d, J = 8.0 Hz, 2H), 7.40 (m, 2H), 7.32 (m, 2H), 6.13 (d, J = 8.0 Hz, 1H), 4.64 (m, 1H), 4.14-4.30, (m, 3H), 3.30 (m, 1H), 3.20 (m, 1H). ^{13}C NMR (100 MHz, Acetone-d_6) δ: 171.2, 155.9, 147.0, 145.0, 144.5, 144.0, 142.6, 141.1, 127.6, 127.1, 125.1, 119.9, 115.9, 110.0, 66.5, 53.0, 47.0, 25.5. ^{19}F NMR (400 MHz, Chloroform–d) δ: -141.2 (2F), -141.8 (2F). HRMS (ESI+): m/z calculated for $C_{24}H_{17}ClF_4NO_4$ $[M]^+$, 494.07822; found 494.07935.

III. Marfey's test[S2] to confirm on the stereochemistry of ZpXs

In order to examine the chirality of the new amino acids reported here, Marfey's test was carried out.



Fmoc- ZpXs were deprotected with piperidine in DMF, and the free amino acids were isolated by Prep HPLC. The amino acids were dissolved in water to give stock solutions of 50 mM. Then 50 μL of an amino acid stock solution was mixed with 50 μL water, 20 μL of 1 M $NaHCO_3$ aq., and 100 μL of the Marfey's reagent (1-Fluoro-2,4-dinitrophenyl-5-L-alanine amide, FDAA) stock solution (1% FDAA in acetone). The resulting mixture was incubated with gentle shaking at 35 °C for 1 h. The reaction was quenched by addition of 10 μL 2 N HCl. The acidified solution was diluted to 1 mL with methanol, and examined by LC-MS by monitoring absorption at 340 nm. For all amino acids examined, a single peak was observed confirming lack of epimerization. Shown below is the result of ZpI as an example. ***Only one peak was identified with the molecular weight of the FDAA-coupled product, indicating lack of epimerization.***



***The only peak with m/z* of 615.6, expected for the adduct of ZpI**

IV. Peptide synthesis

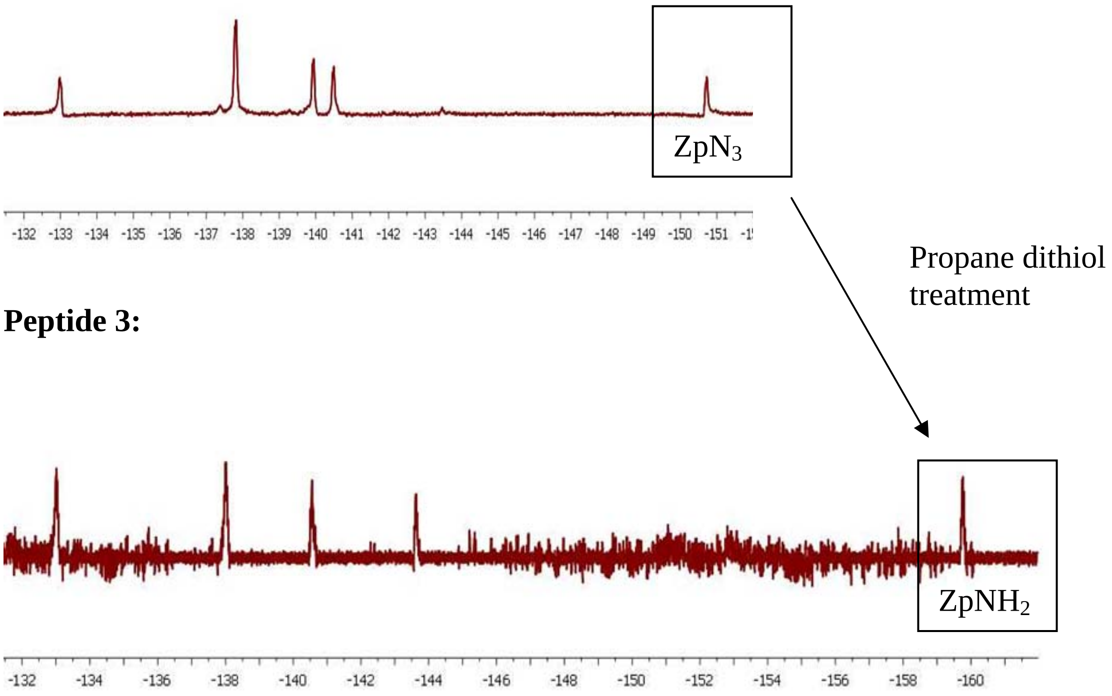
In order to confirm that Fmoc-ZpXs are compatible with solid phase peptide synthesis, we have synthesized three peptides based on the well known membrane-lytic peptide magainin 2. The sequences of the peptides are shown below:

Magainin 2: **GIGKFLHSAKKFGKAFVGEIMNS**
Peptide 1: **GIGKFL(ZpN$_3$)SAKK(ZpBr)GKA(ZpCl)VGEIMNS**
Peptide 2: **Ac-SAKK(ZpI)GKA(ZpN$_3$)VGEIMNS**
Peptide 3*: **GIGKFL(ZpNH$_2$)SAKK(ZpBr)GKA(ZpCl)VGEIMNS**

Peptides 1 and **2** were synthesized on Rink-Amide-MBHA resin (90 mg, 0.56 mmol/g, 0.05 mmol) using standard Fmoc/HBTU chemistry. Five equivalents of the commercially available amino acids were used for the coupling reaction. For the fluorinated amino acids, the incorporation was accomplished by using three equivalents of the amino acids and extended coupling time (1 hour). After cleavage of the N-terminal Fmoc group from the fully protected peptide on resin, the peptide was cleaved off the resin and deprotected fully with reagent K (80% TFA, 5% H_2O, 2.5% EDT, 5% Thioanisole and 7.5% Phenol) at room temperature for two hours. The beads were filtered off and the filtrate was treated with Et_2O three times. The precipitate was collected and dried under vacuum. The crude peptides were purified by RP-HPLC (Waters Prep LC, Jupiter 10u C4 300A Column). The identity of the final product was confirmed by ESI-MS. **Peptide 1**: for $[M+3H]^{3+}$, *m/z* calculated 950.3, found 951.3; for $[M+4H]^{4+}$, *m/z* calculated 713.0, found 713.2. **Peptide 2**: $[M+2H]^{2+}$, *m/z* calculated 1034.5, found 1034.5.

Peptide 3 was obtained by specifically reducing ZpN_3 with 1,3-propanedithiol in DMF in presence of DIPEA, the condition similar to the synthesis of Compounds **8** and **10** (see section II). The clean conversation was evident in 19F-NMR of the peptide 1 and 3 (shown below); it was also confirmed by ESI-MS (**Peptide 3**: for $[M+3H]^{3+}$, *m/z* calculated 941.7, found 941.2). These results indicate that ZpN_3 serves as a protected from of $ZpNH_2$ for peptide synthesis.

Peptide 1:



V. ^{19}F-NMR chemical shifts in $CDCl_3$ and CD_3OD

Experimental conditions: Fmoc protected ZpX amino acids (Fmoc-ZpX-OH) were dissolved in $CDCl_3$ and CD_3OD respectively at ~ 1 mg/mL. Fluorobenzene was sealed into capillary tubes and put into each NMR test tube to serve as pseudo-internal ^{19}F-NMR standards (-113.15 ppm).[S3] The NMR data were collected on Varian Gemini 400 MHz NMR spectrometer.

Table S1. ^{19}F chemical shifts of the tetrafluorinated phenylalanine analogues.

<table>
<tr><td></td><td colspan="6">$CDCl_3$</td><td colspan="6">CD_3OD</td></tr>
<tr><td>Z</td><td colspan="2">-161.5</td><td colspan="2">-154.4</td><td colspan="2">-142.0</td><td colspan="2">-165.0</td><td colspan="2">-159.0</td><td colspan="2">143.6</td></tr>
<tr><td>ZpH</td><td colspan="3">-138.7</td><td colspan="3">-142.6</td><td colspan="3">-141.2</td><td colspan="3">-144.4</td></tr>
<tr><td>ZpCl</td><td colspan="3">-140.7</td><td colspan="3">-141.3</td><td colspan="3">-142.7</td><td colspan="3">-143.7</td></tr>
<tr><td>ZpBr</td><td colspan="3">-133.0</td><td colspan="3">-140.8</td><td colspan="3">-135.9</td><td colspan="3">-142.3</td></tr>
<tr><td>ZpI</td><td colspan="3">-120.1</td><td colspan="3">-140.2</td><td colspan="3">-123.1</td><td colspan="3">-142.2</td></tr>
<tr><td>ZpN3</td><td colspan="3">-151.3</td><td colspan="3">-142.5</td><td colspan="3">-154.3</td><td colspan="3">-144.6</td></tr>
<tr><td>ZpNH2</td><td colspan="3">-161.5</td><td colspan="3">-145.3</td><td colspan="3">-164.0</td><td colspan="3">-148.0</td></tr>
<tr><td>^{a}ZpOH</td><td colspan="3">-163.4</td><td colspan="3">-144.8</td><td colspan="3">-164.9</td><td colspan="3">-147.4</td></tr>
<tr><td>^{a}ZpO$^-$</td><td colspan="3">-</td><td colspan="3">-</td><td colspan="3">-168.2</td><td colspan="3">-152.1</td></tr>
<tr><td>ZpSH</td><td colspan="3">-137.3</td><td colspan="3">-142.2</td><td colspan="3">-139.8</td><td colspan="3">-144.4</td></tr>
<tr><td>ZpS$^-$</td><td colspan="3">-</td><td colspan="3">-</td><td colspan="3">138.4</td><td colspan="3">148.7</td></tr>
</table>

^{a}Fmoc-ZpOH-OH is insoluble in $CDCl_3$. Boc-ZpOH-OMe was used instead in this group.

VI. 2D ^{19}F-^{19}F COSY experiment for a fluorinated magainin.

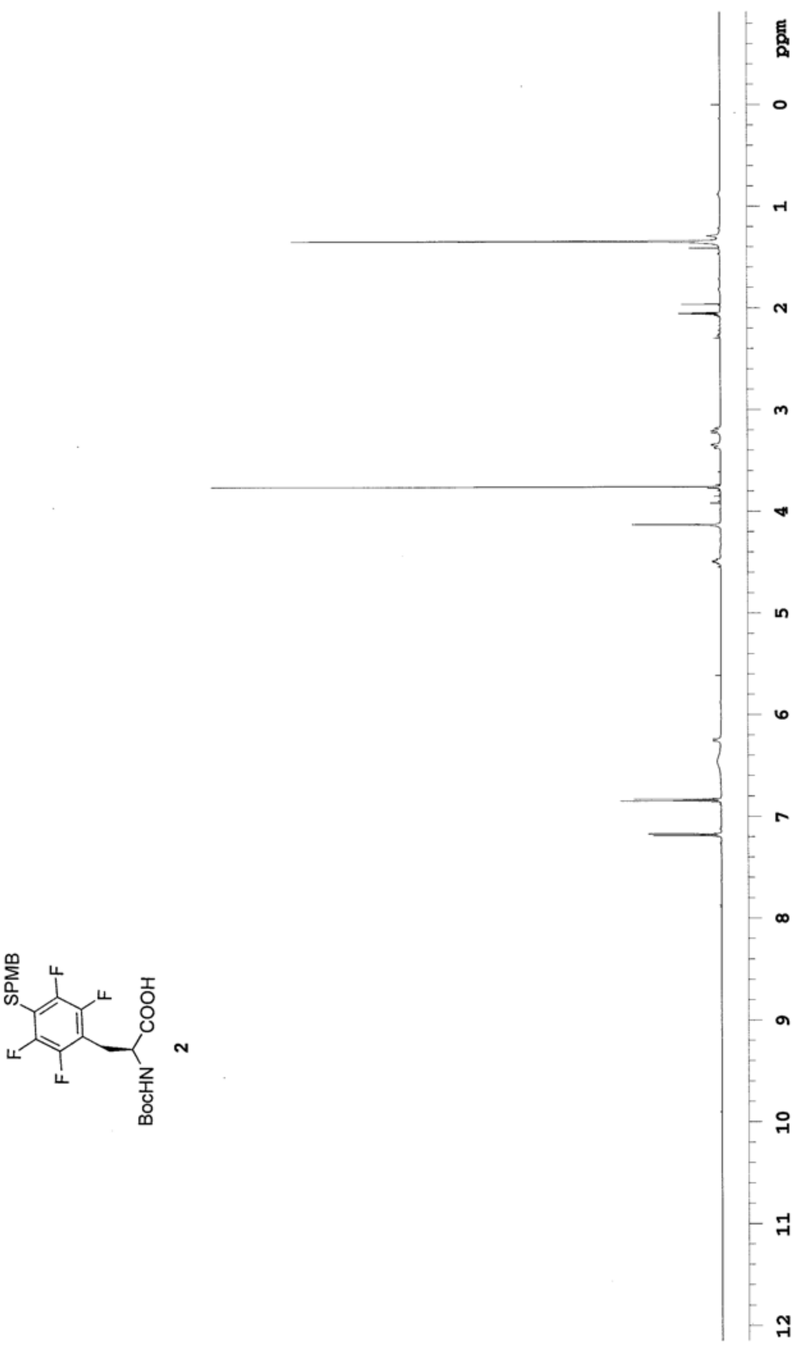
The magainin mutant (**peptide 1** incorporating ZpBr, ZpCl and ZpN_3) was dissolved in 90% DMSO-*d6* and 10% TRIS buffer (pH = 7.4) at the final concentration of 1 mM. The NMR data were collected on Varian 600 MHz NMR spectrometer and shown in **Figure 1** in the main text.

VII. References

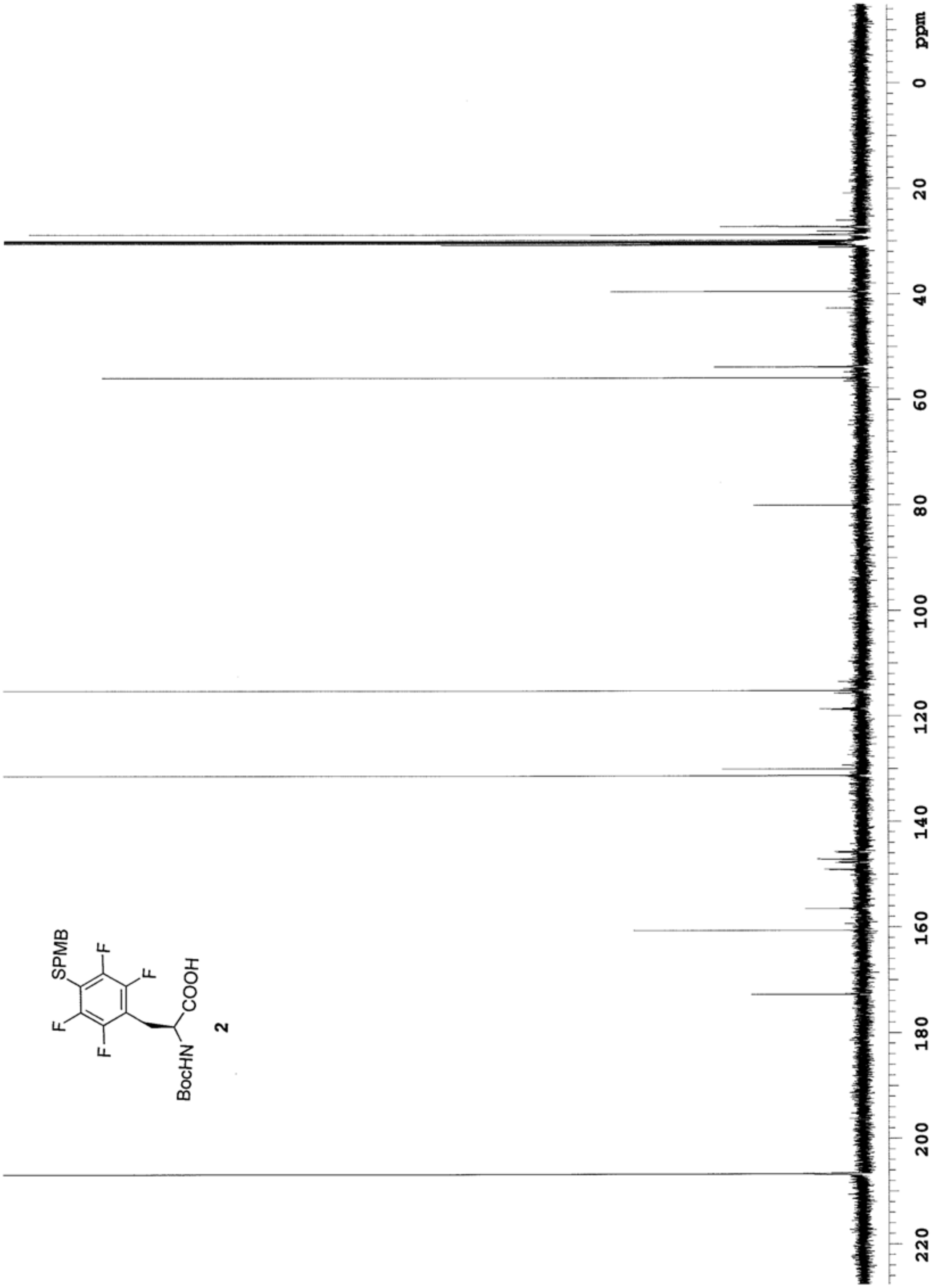
S1. Redman, J; Ghadiri, M. *Org. Lett.* **2002**, *4*, 4467.
S2. Bhushan, R.; Bruckner, H. *Amino Acids* **2004**, *27*, 231.
S3. http://chemnmr.colorado.edu/manuals/19F_NMR_Reference_Standards.pdf

VIII. Appendix, ^{1}H and ^{13}C NMR spectra

^{1}H NMR



^{13}C NMR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F
F
F
COOH
BocHN
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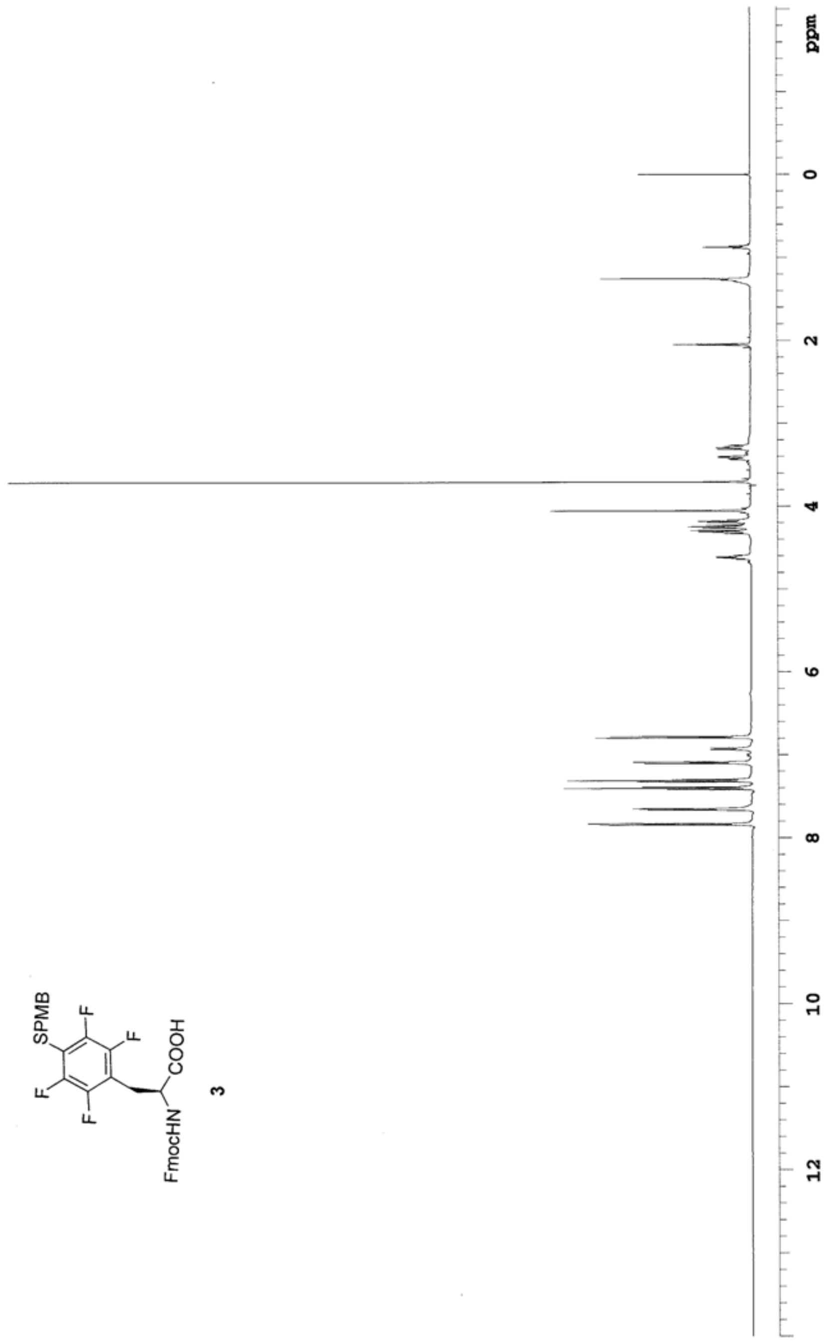
200
220

^{1}H NMR



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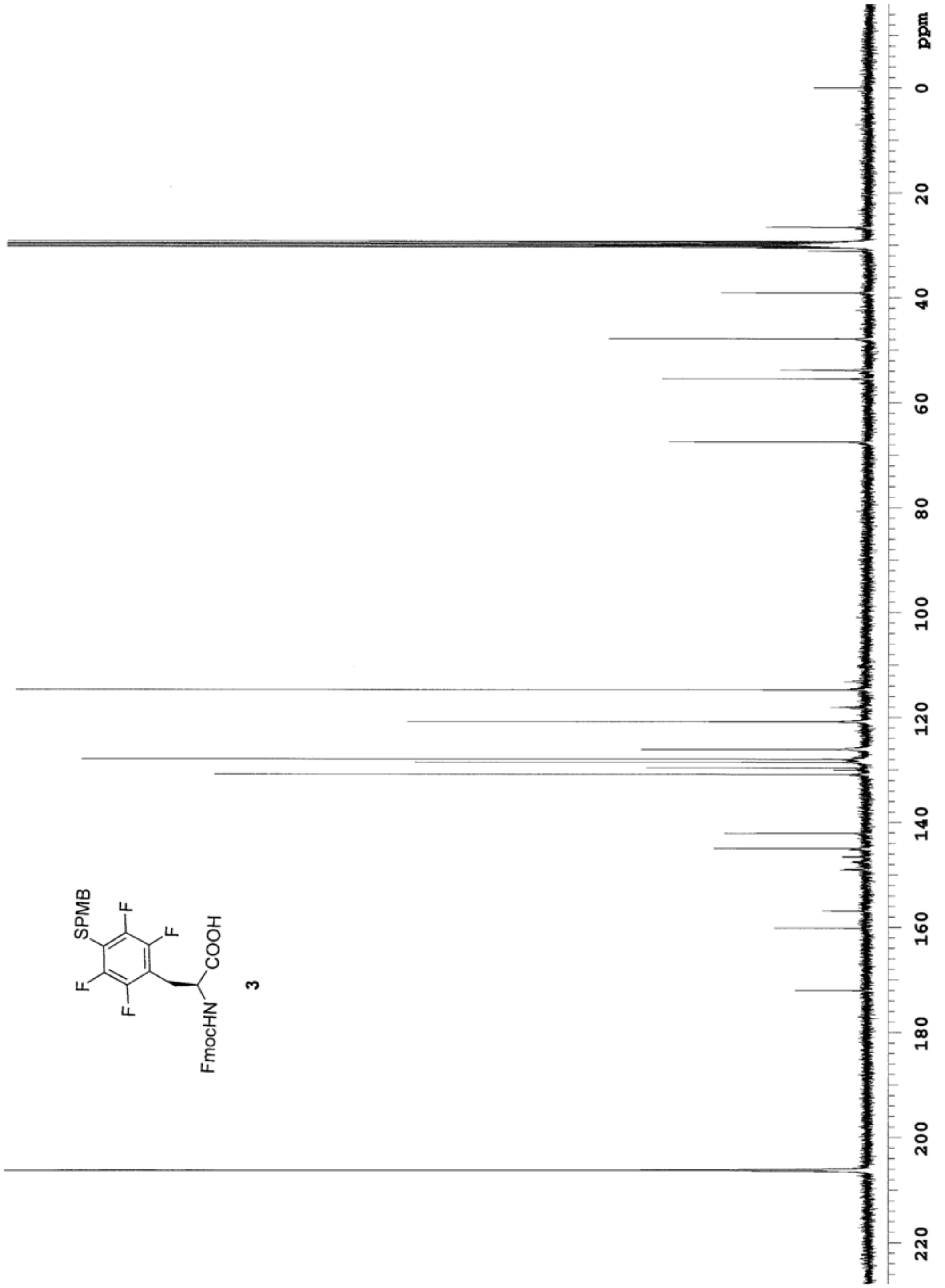
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F
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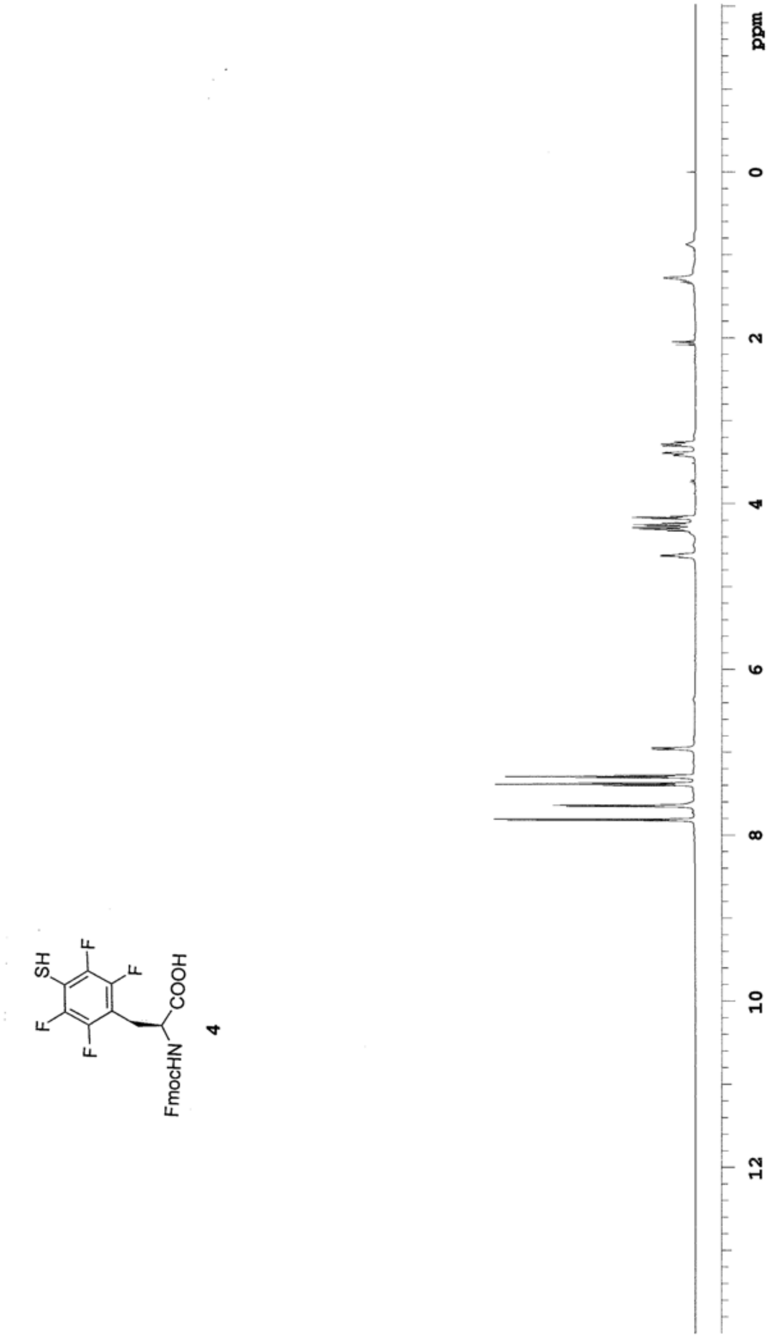
COOH
3

^{1}H NMR



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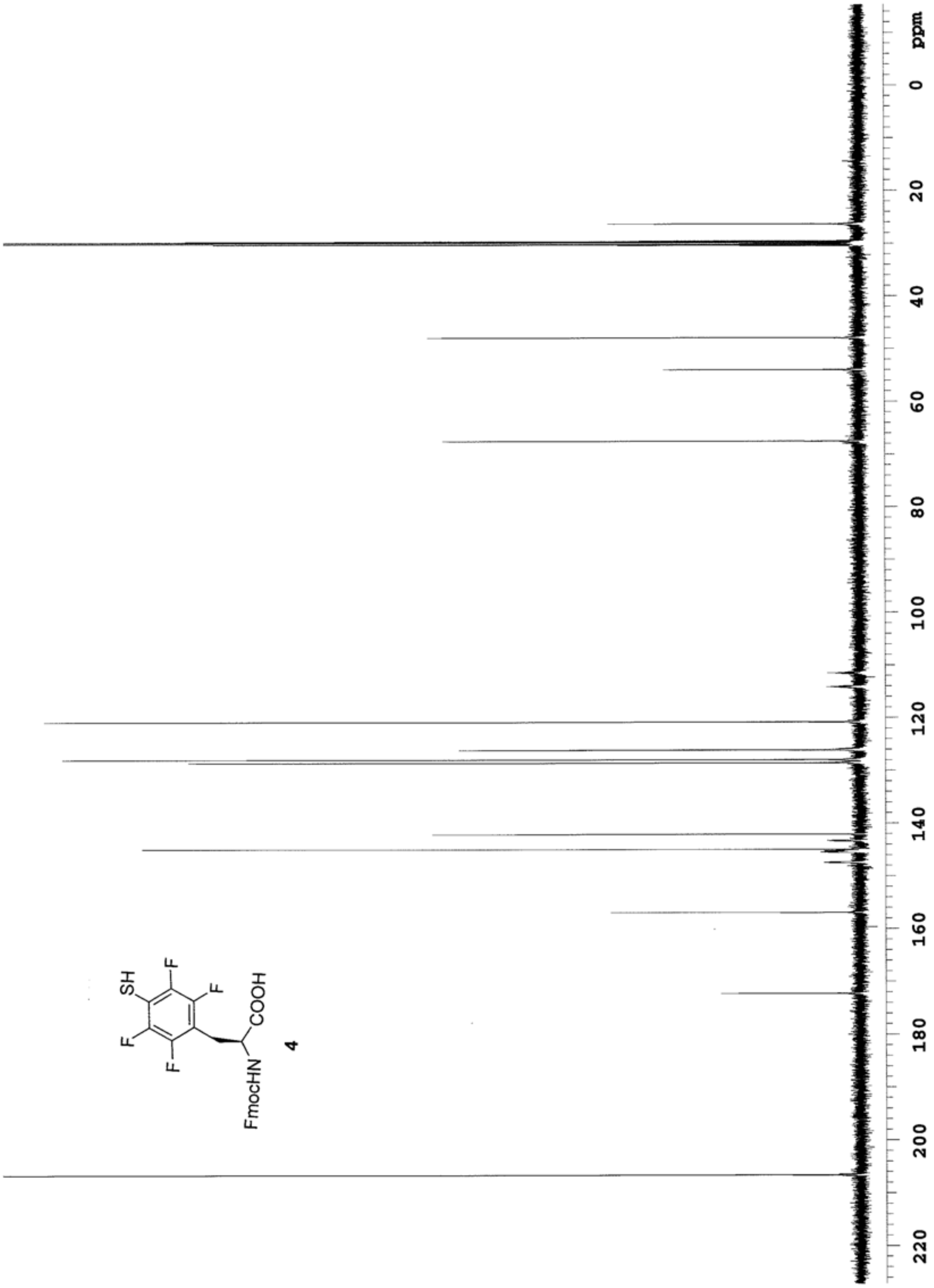
^{13}C NMR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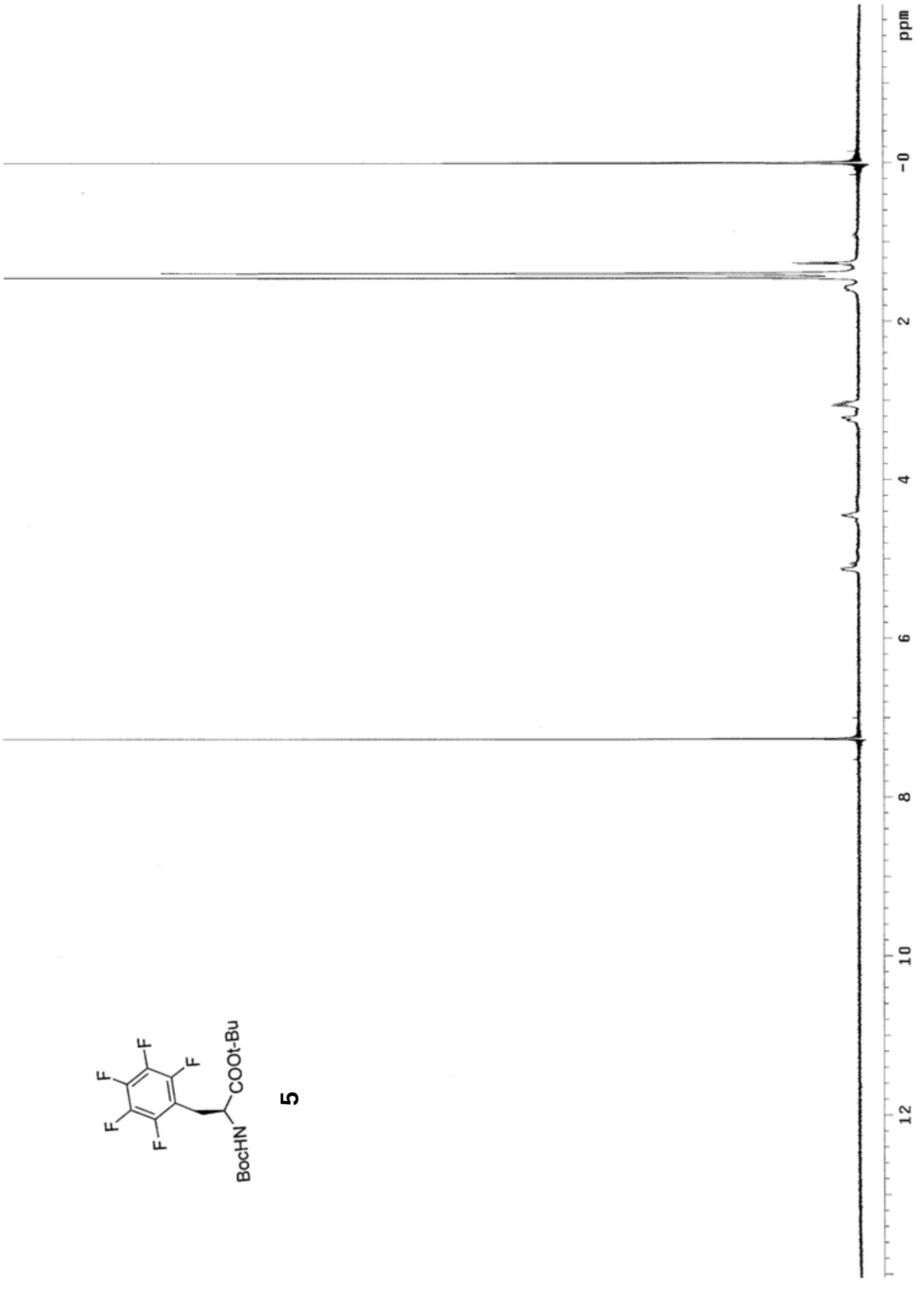
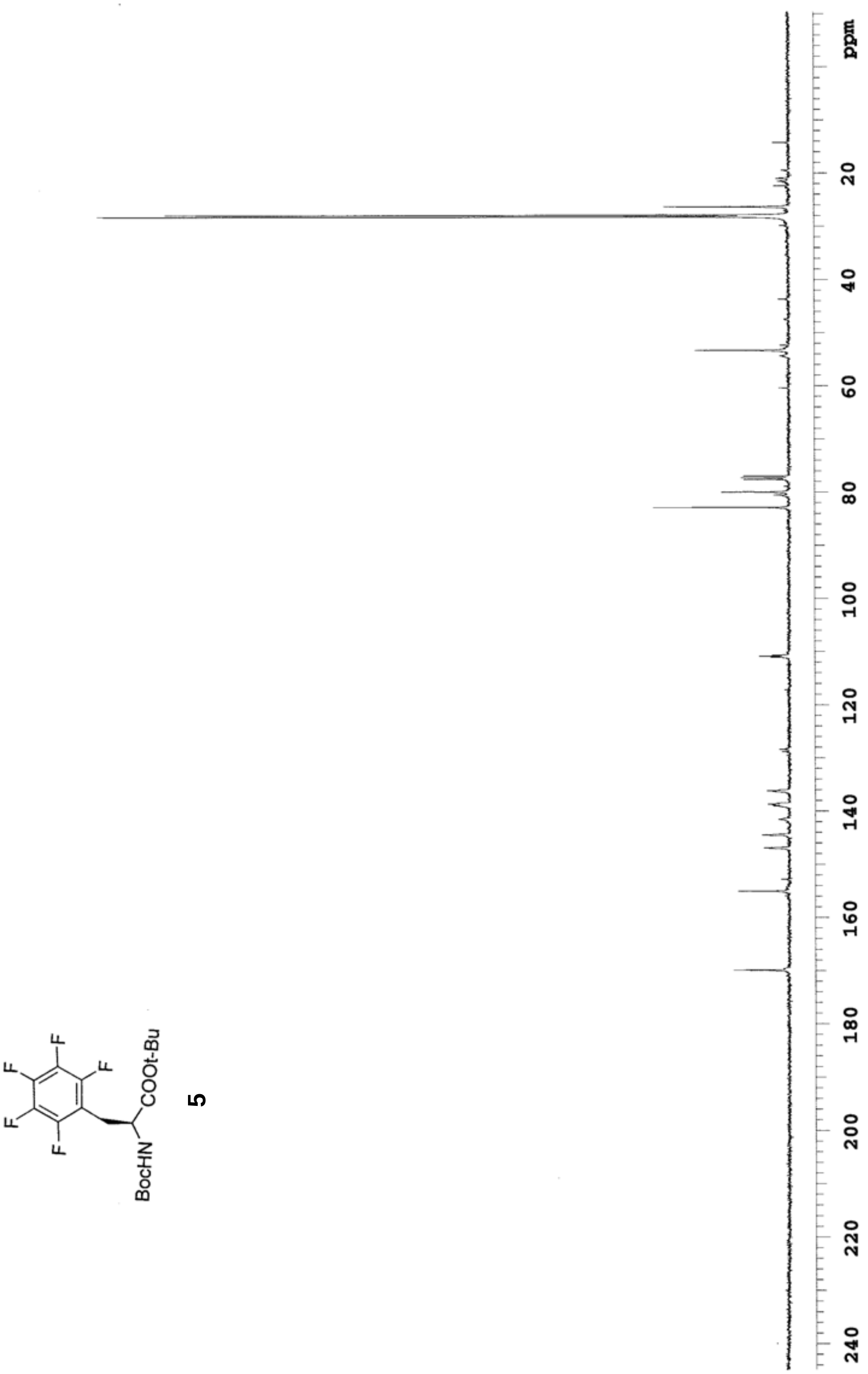
FmocHN
4

^{1}H NMR



^{13}C NMR


ppm
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40
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80
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120
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240
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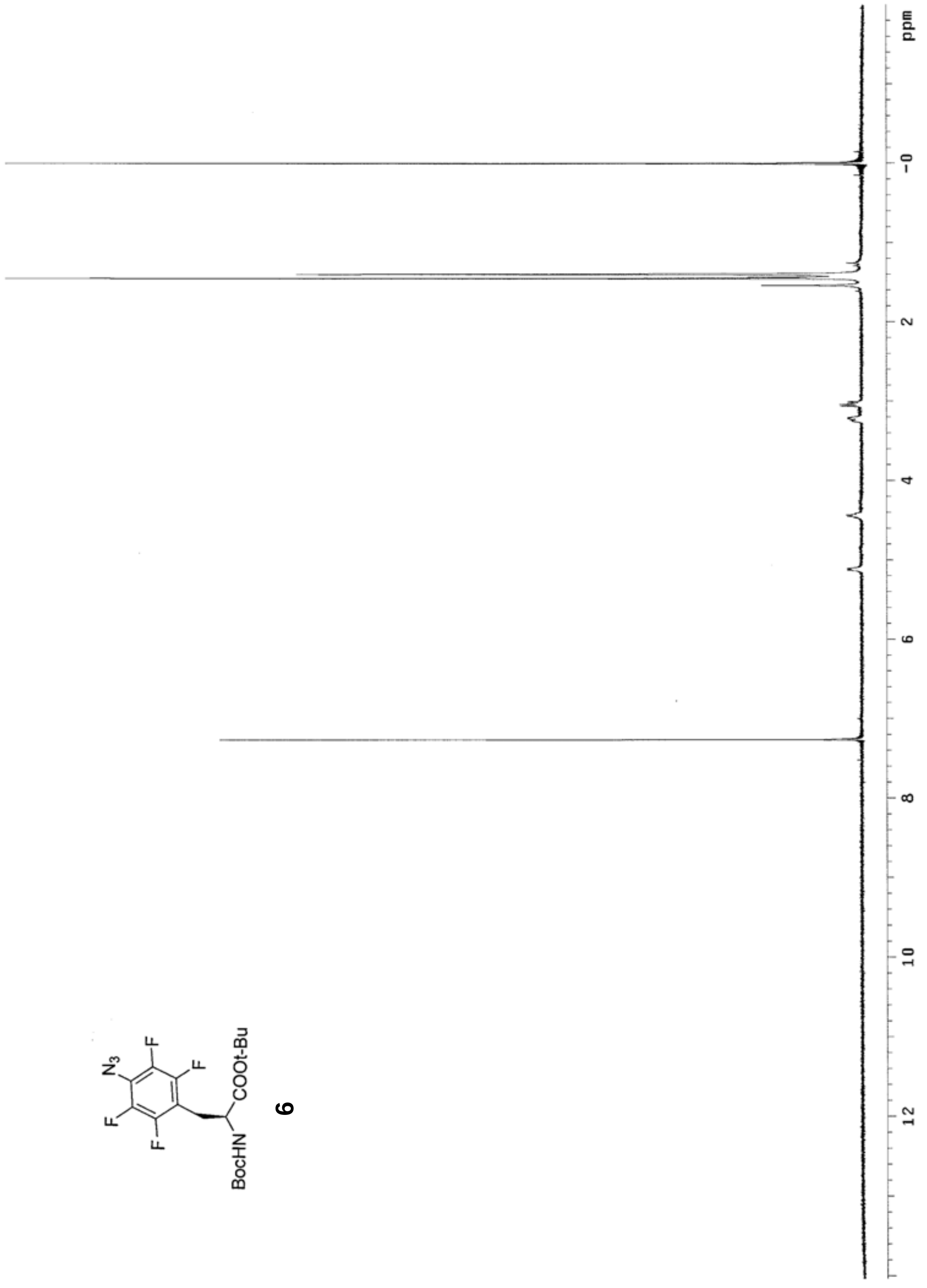
F
F
F
F
BocHN
COOt-Bu

5

^{1}H NMR



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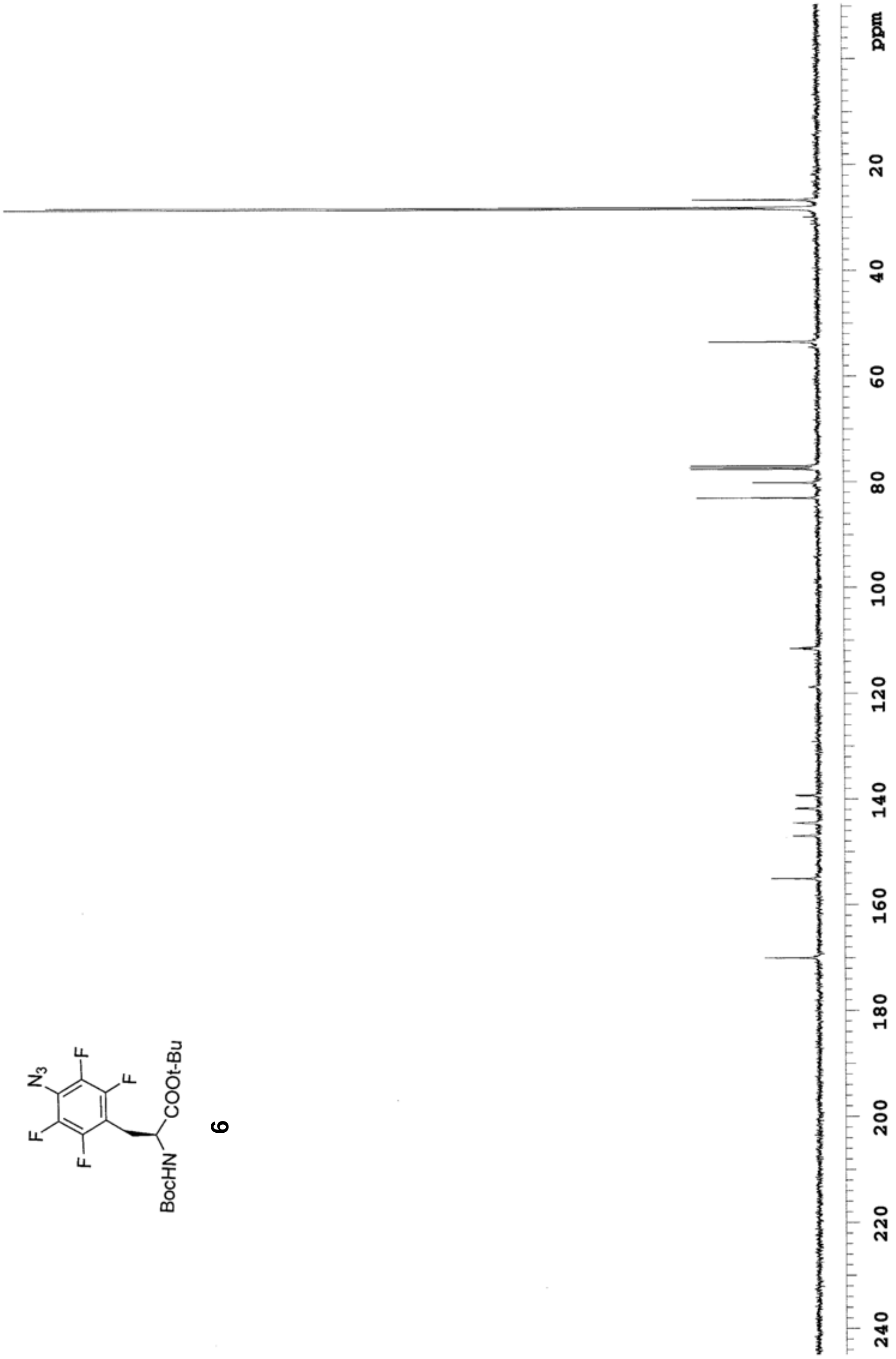
^{13}C NMR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F
F
F
F
BocHN
COOt-Bu
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ppm
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60
80
100
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140
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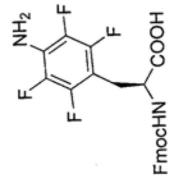
200
220
240

^{1}H NMR



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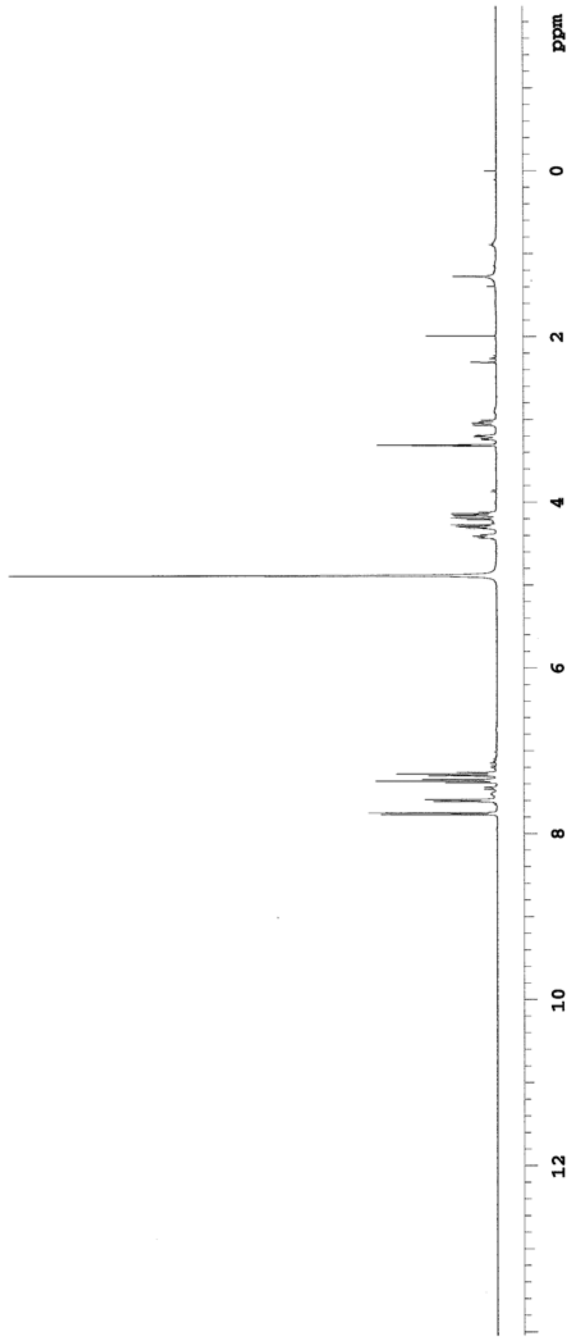
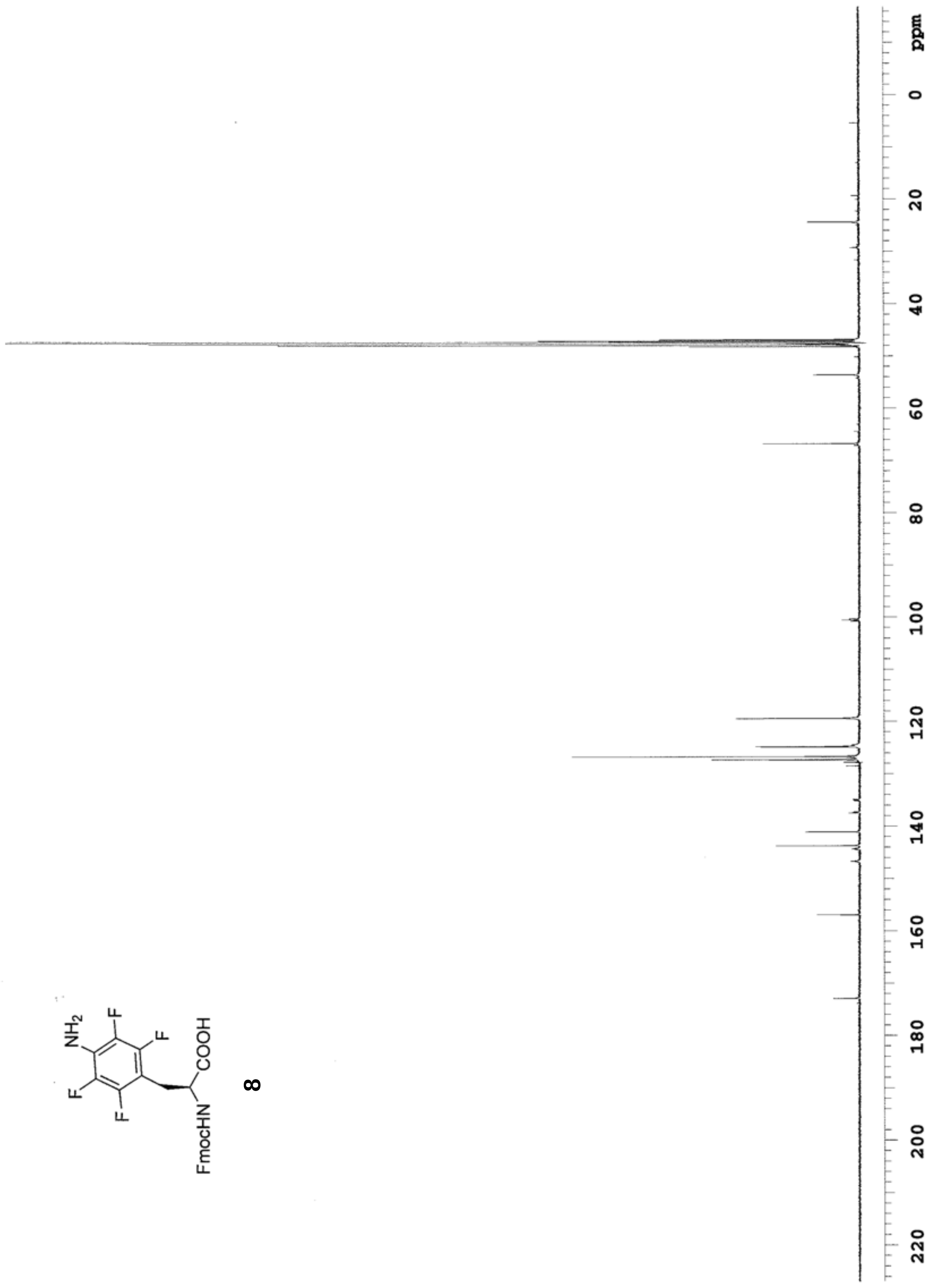


^{13}C NMR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20
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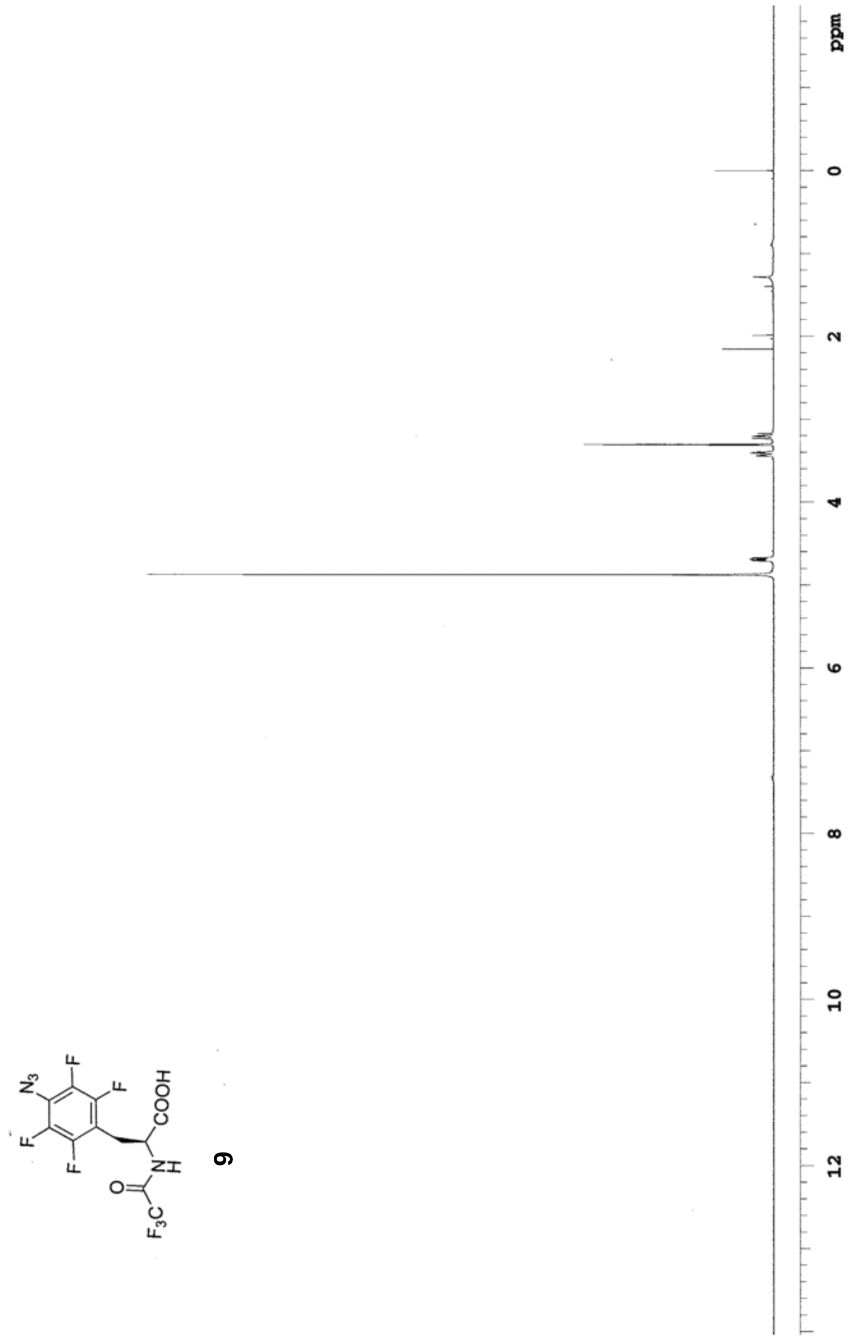
COOH

8

^{1}H NMR



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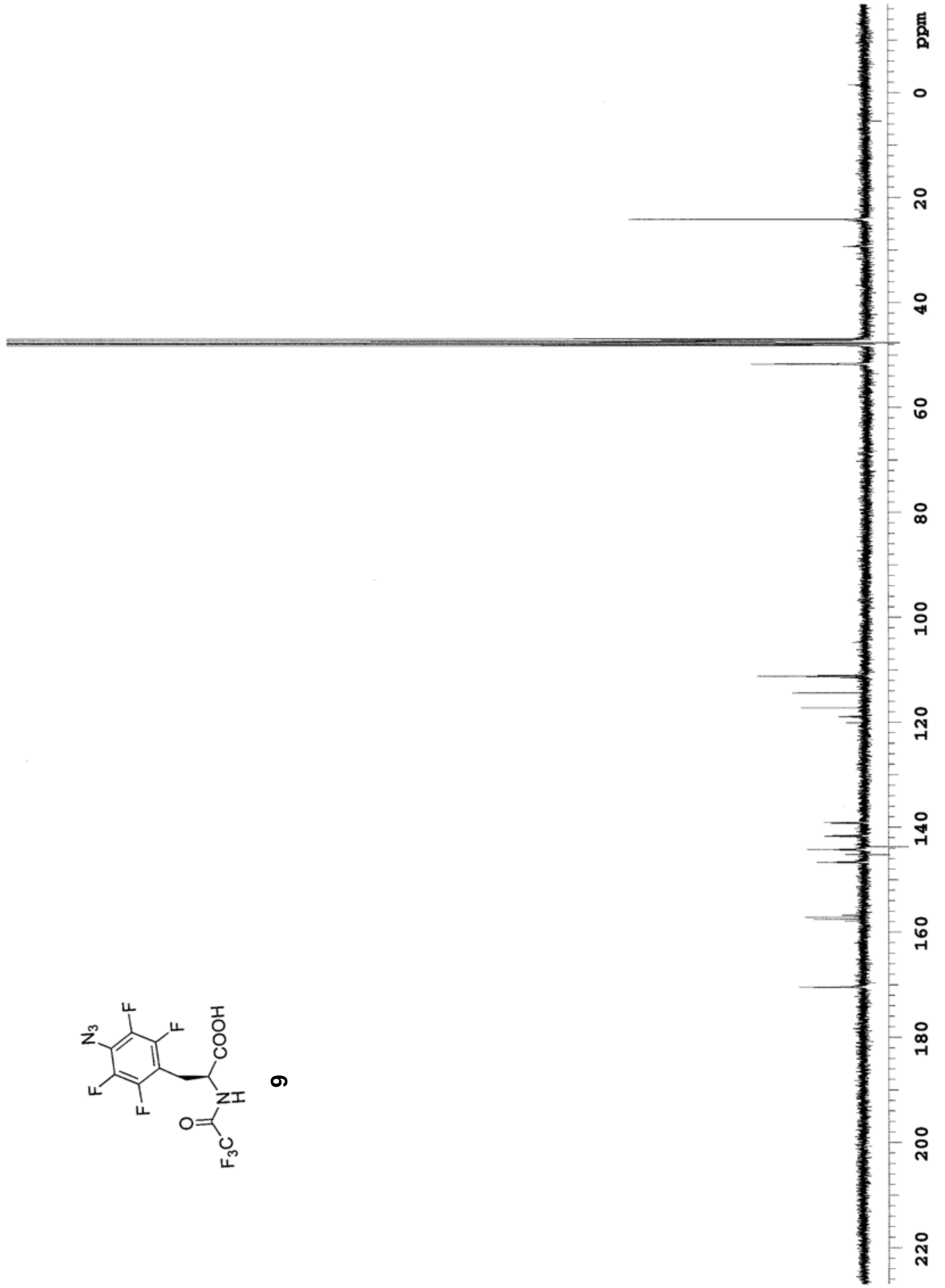
^{13}C NMR



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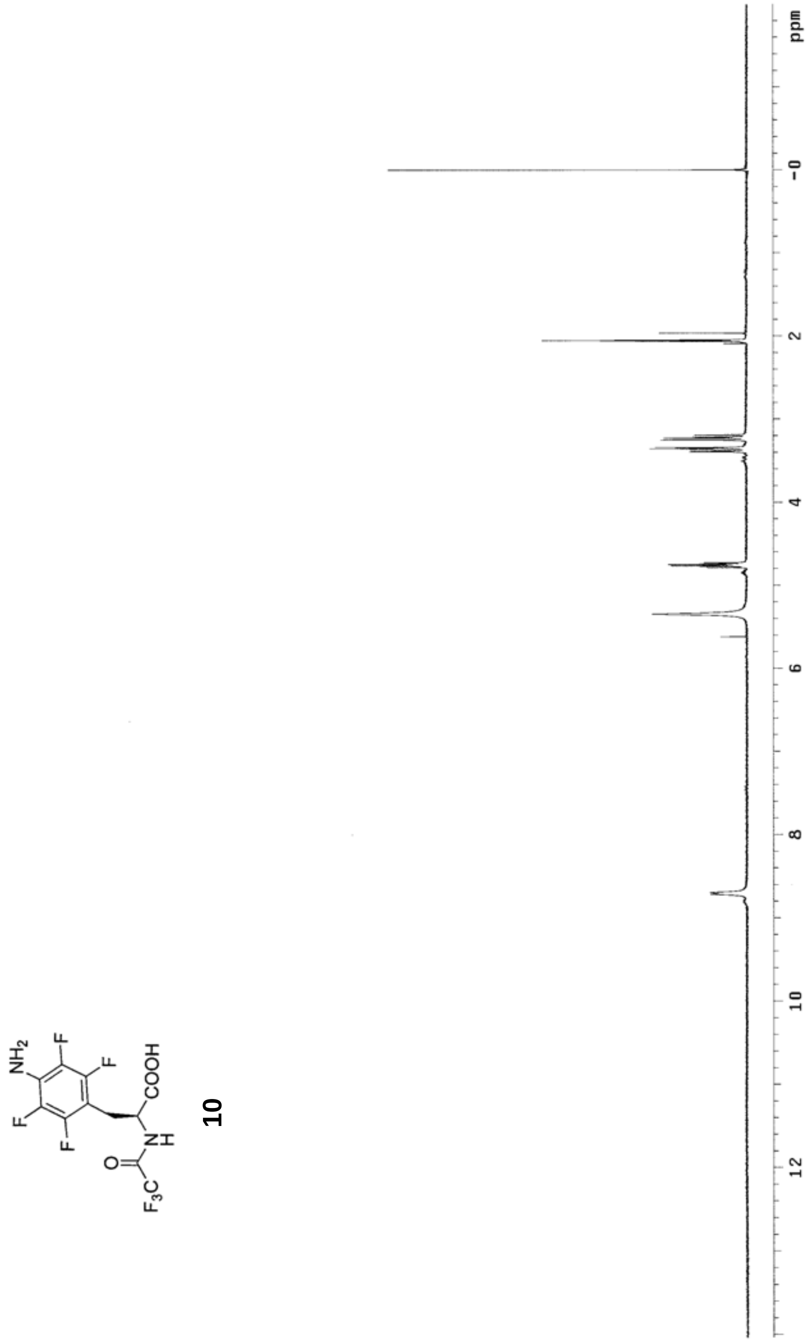
^{1}H NMR



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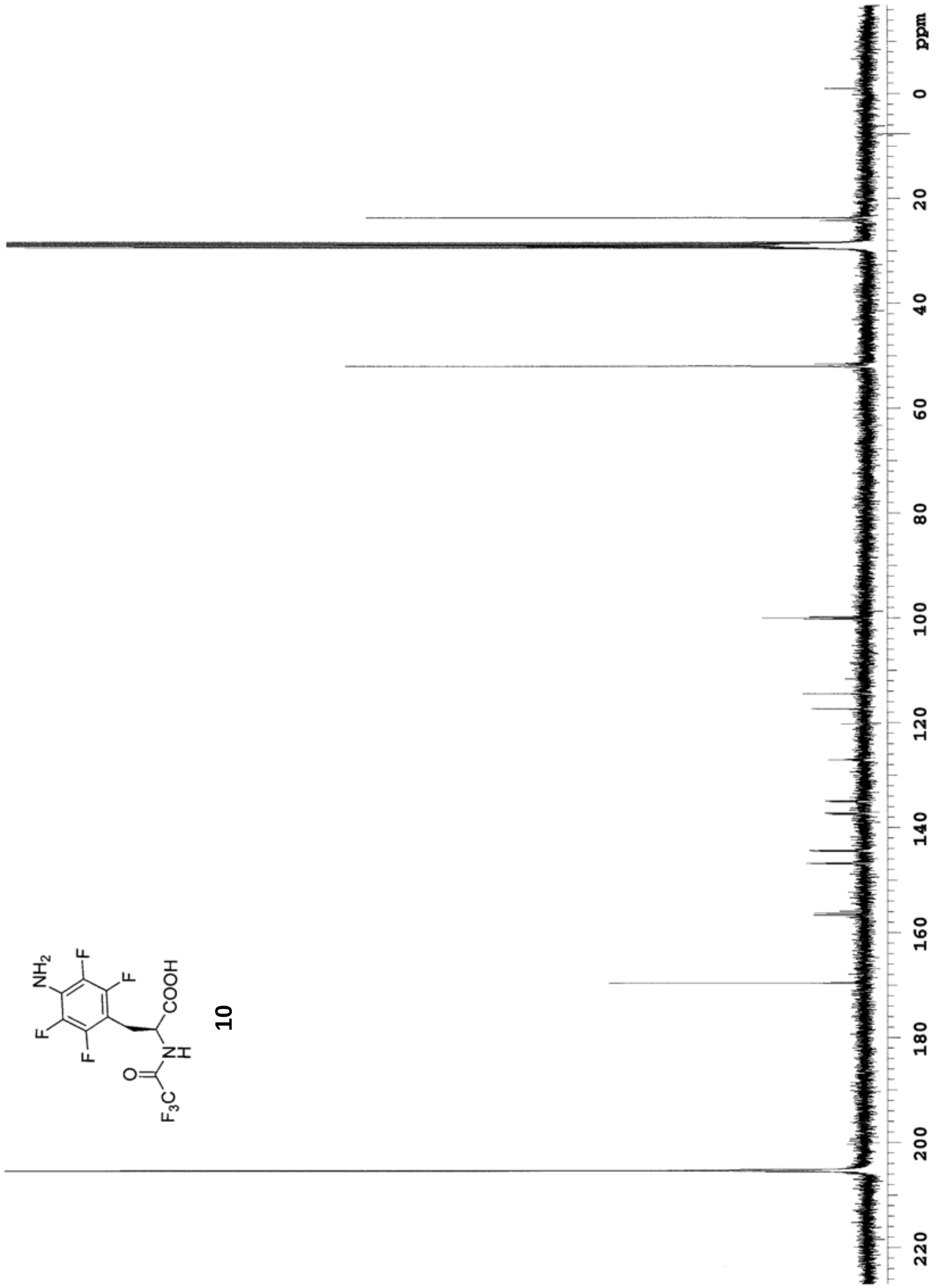
^{13}C NMR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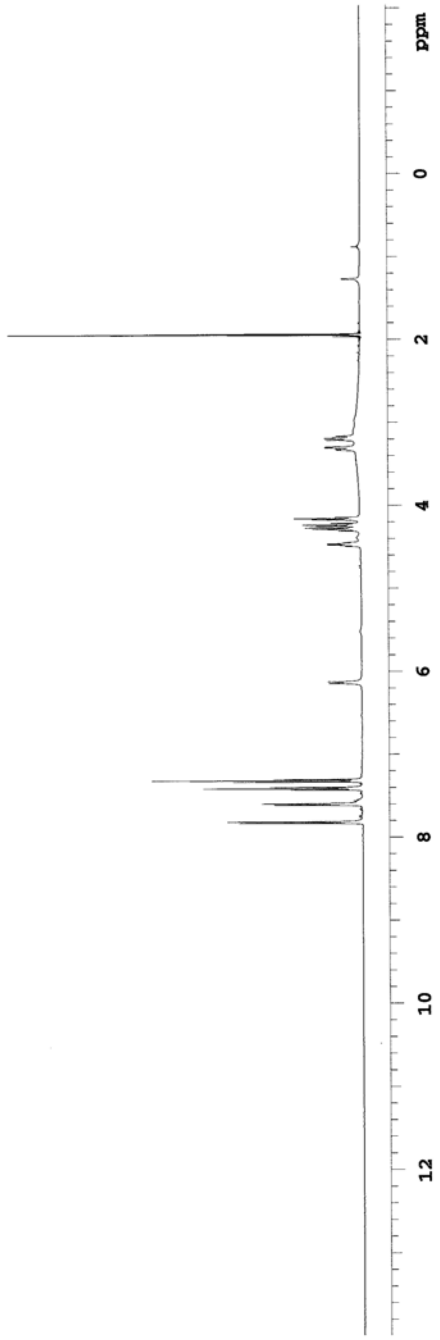
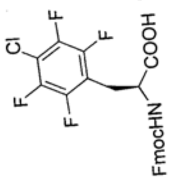
O
F$_3$C

10

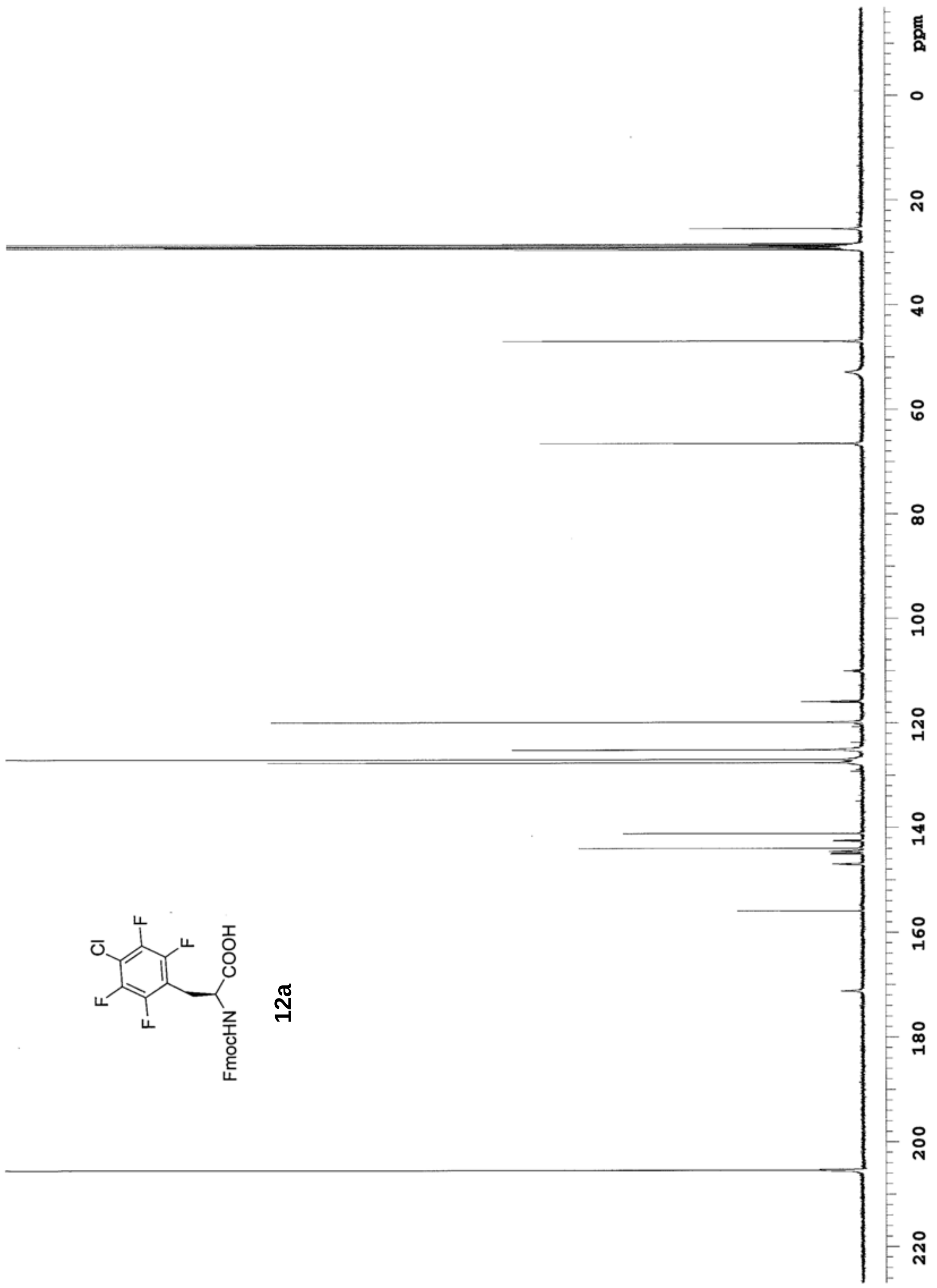
^{1}H NMR





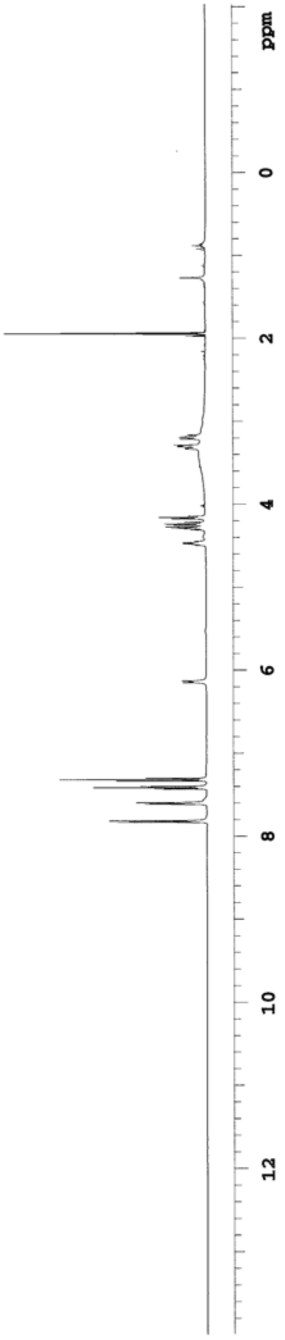
12a

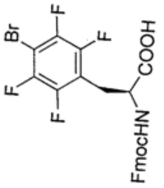
^{13}C NMR



12a

^{1}H NMR





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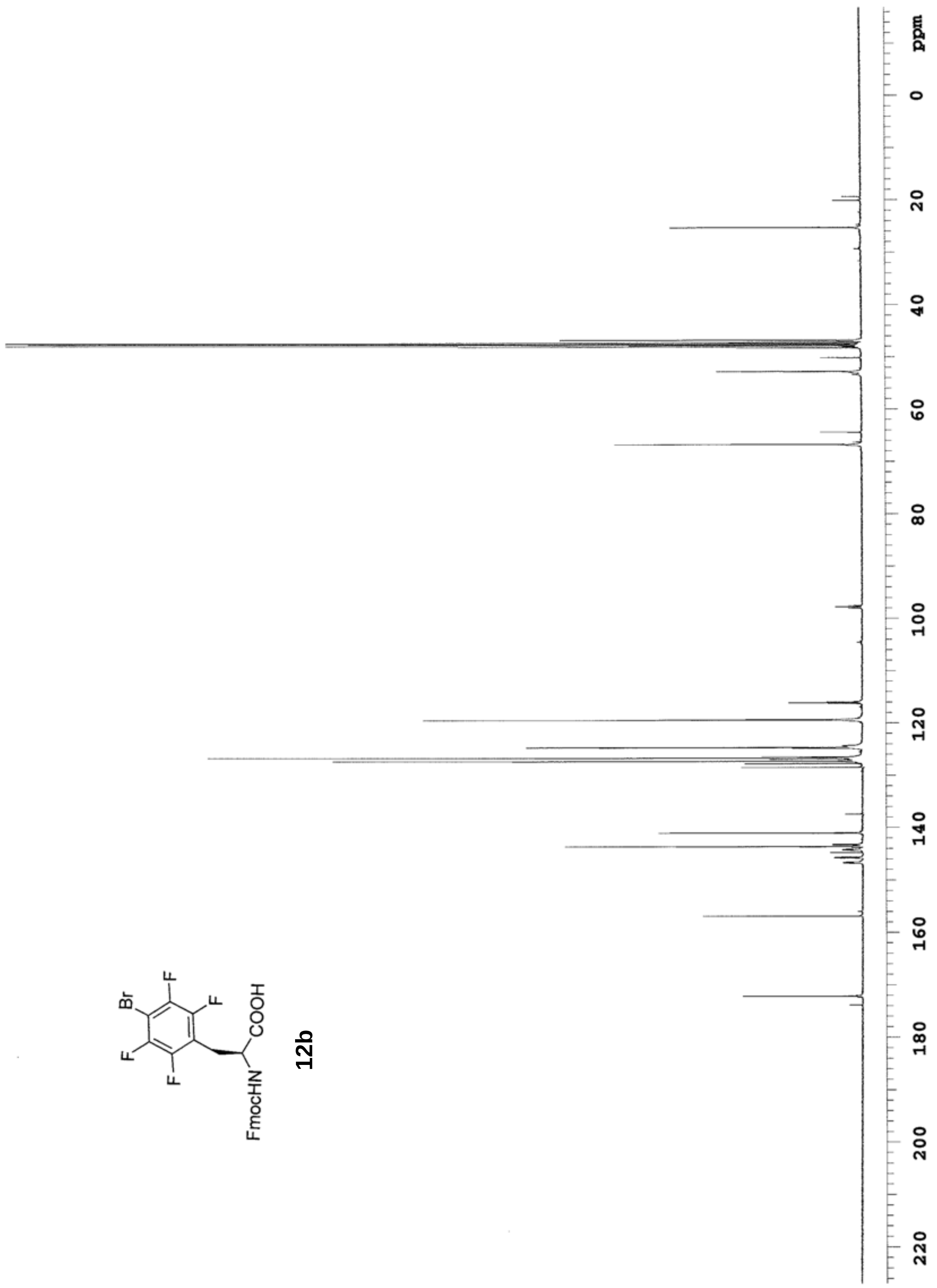
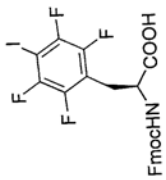
^{13}C NMR



12b

^{1}H NMR



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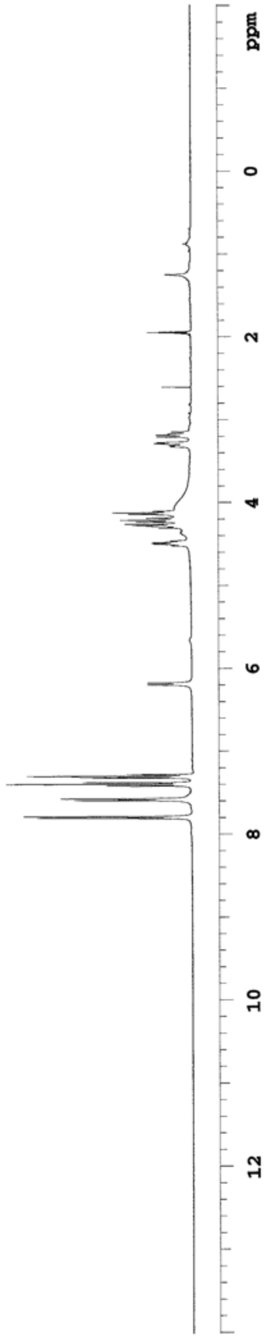

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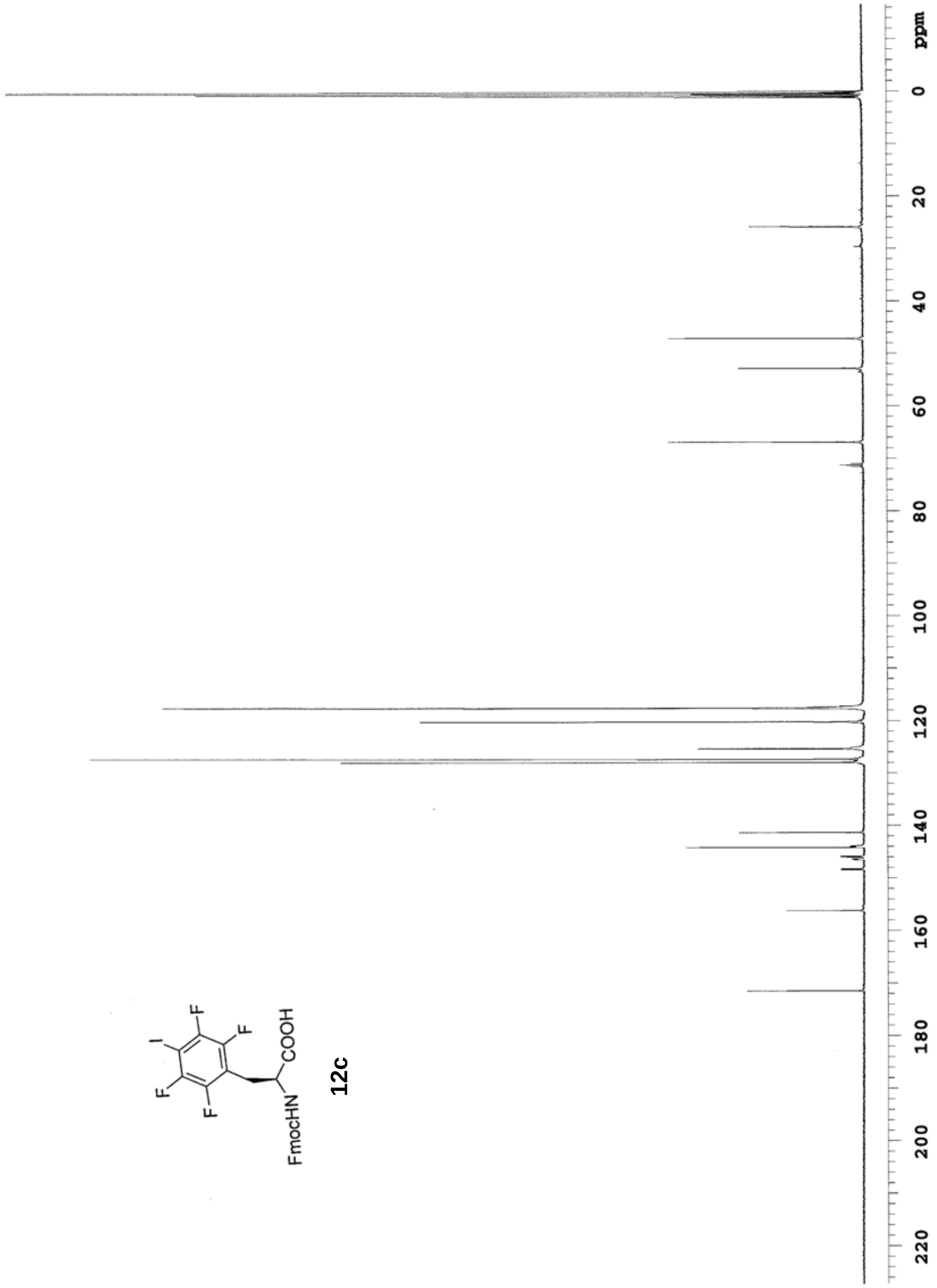

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