Quantitatively Fine-Tuning the Physicochemical and Biological Properties of Peptidic Polymers through Monodisperse PEGylation

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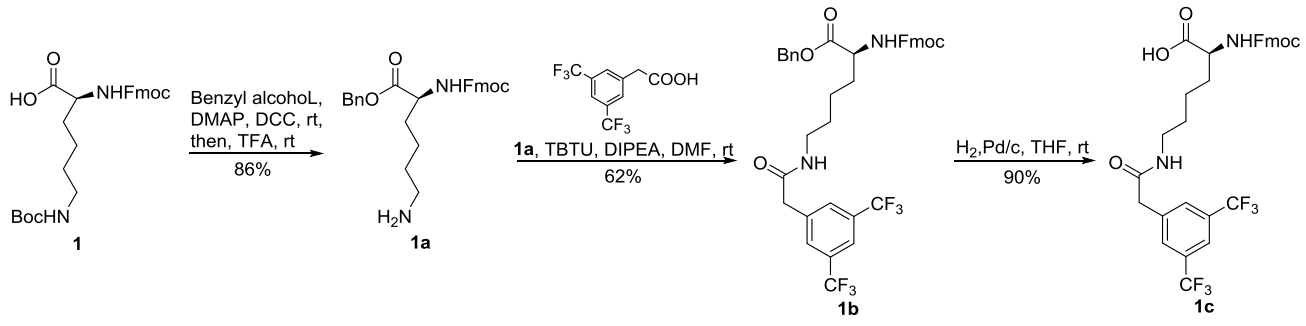
1. General information

Unless otherwise indicated, all reagents were obtained from commercial supplier and used without prior purification. All solvents were analytical or HPLC grade. DCM, DMF, Et_3N, DIPEA and THF were dried and freshly distilled prior to use. Flash chromatography was performed on silica gel (200−300 mesh) with eluents as indicated in procedures. Chemical shifts are expressed in ppm and coupling constants (*J*) are in Hertz (Hz). The splitting patterns for ^1H NMR spectra are denoted as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), and m (multiplet). LC-MS mass spectra were recorded on Bruker UltiMate 3000 & Compact mass spectrometer. MALDI-TOF mass spectra were recorded on a Bruker Ultraflex III TOF/TOF spectrometer using the reflection mode for positive ions with α-cyano-4-hydroxylcinnamic acid or 2,5-dihydroxybenzoic acid as matrix.

All HPLC analysis were performed on SHIMADZU SIL-20A. For M-PEGylated peptidic polymers HPLC analysis: SPD-20A UV detector (254 nm), RP C18 column (5 μm, 4.6 × 100 mm), a gradient elution of 70% methanol in water to 100% methanol over 15 min (flow rate 0.7 mL/min) then 100% methanol for 5 min. For DOX HPLC analysis: SPD-20A UV detector (480 nm), RP C18 column (5 μm, 4.6 ×100 mm), a gradient elution of solvent A (ammonium dihydrogen phosphate buffer, water containing 0.5% v/v acetic acid and 0.01 M of ammonium dihydrogen phosphate, 0.35 mL/min) and solvent B (acetonitrile, 0.35 mL/min) over 20 min.

2. Synthesis of compounds

1）Synthesis of amino acid 1c

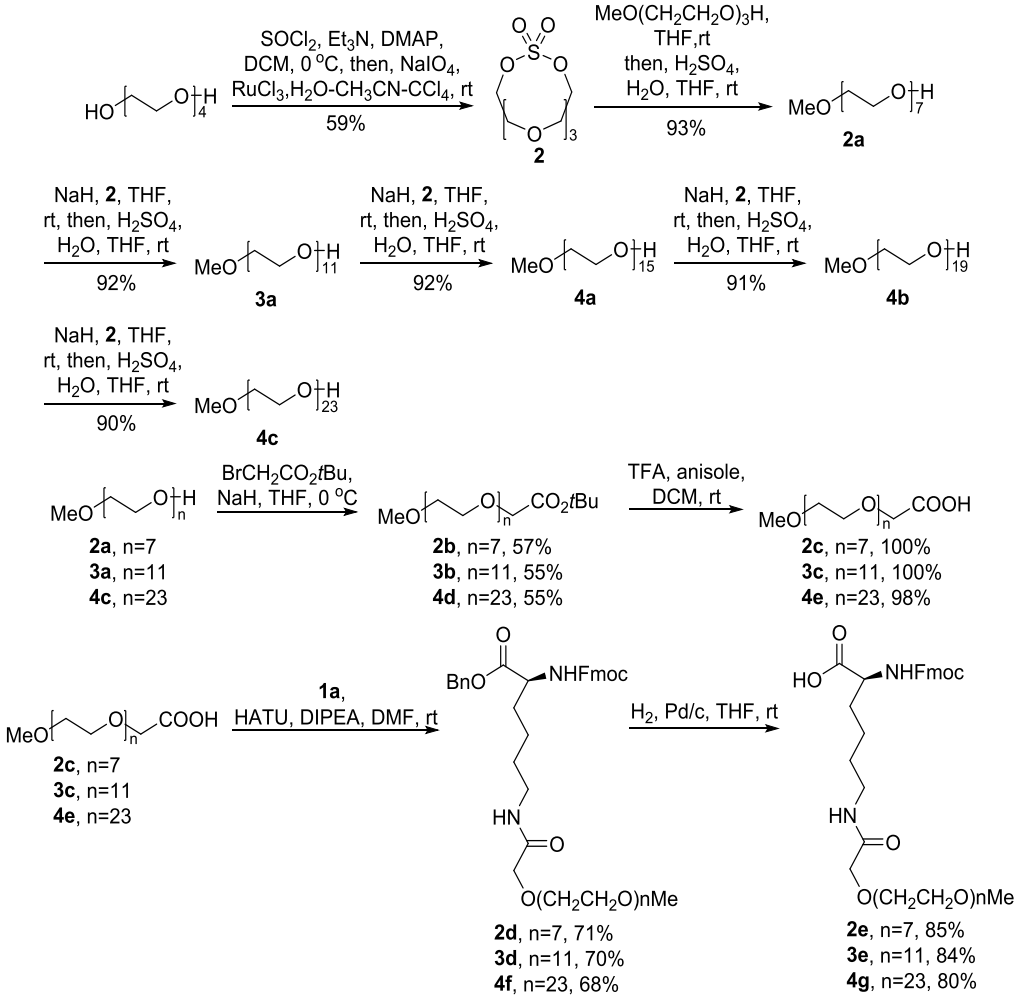


Compound 1a was prepared according to a literature method.[1] ^1H NMR (400 MHz, $CDCl_3$) δ 1.25-1.43 (m, 2H), 1.64 (s, 3H), 1.81(s, 1H), 2.87 (s, 2H), 4.02-4.26 (m, 2H), 4.36 (d, *J* = 7.4 Hz, 2H), 5.01-5.25 (m, 2H), 7.29 -7.66 (m, 9H), 7.75 (d, *J* = 7.4 Hz, 2H), 7.93 (s, 2H).

Compound 1b. TBTU and DIPEA were added to a solution of 2-(3,5-bis(trifluoromethyl)phenyl) acetic acid (1.0 g, 3.7 mmol) in dry DMF (35 mL) at rt. Then, a solution of **1a** (1.9 g, 4.0 mmol) in dry DMF (15 mL) was added to the reaction, and the resulting mixture was stirred overnight at rt. Then DMF was evaporated under vacuum and the residue was washed with H_2O and extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to give a residue. The residue was purified with flash chromatography on silica gel (PE:EA = 6:1 to 2:1) to give the desired product **1b** (1.6 g, 62% yield) as white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31-1.46 (m, 2H), 1.53 (d, *J* = 6.8 Hz, 2H), 1.65-1.78 (m, 1H), 1.88 (d, *J* = 7.1 Hz, 1H), 3.16-3.32 (m, 2H), 3.49-3.64 (m, 2H), 4.24 (t, *J* = 6.9 Hz, 1H), 4.33-4.53 (m, 3H), 5.21 (q, *J* = 12.2 Hz, 2H), 7.28-7.48 (m, 9H), 7.60 (d, *J* = 7.3 Hz, 2H), 7.71-7.86 (m, 5H).

Compound 1c was prepared by employing the a literature method.[1] ^{1}H NMR (400 MHz, DMSO-d_6) δ 1.29-1.43 (m, 4H), 1.54-1.71 (m, 2H), 3.02-3.07 (m, 2H), 3.66 (s, 2H), 3.85-3.92 (m, 1H), 4.18-4.32 (m, 3H), 7.32 (t, *J* = 7.4 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.72 (d, *J* = 7.4 Hz, 2H), 7.89 (d, *J* = 7.5 Hz, 2H), 7.96 (d, *J* = 9.0 Hz, 3H).

2）Synthesis of M-PEGylated *L*-lysine 2e, 3e, 4g



Compound 2. Cyclic sulfate **2** was prepared by employing the a literature method.[1]

Compound 2a. Under an atmosphere of Ar, to a suspension of NaH (1.8 g, 60% in mineral oil, 45.7 mmol) in dry THF (40 mL) was added a solution of triethylene glycol monomethyl ether (5.0 g, 30.5 mmol) in dry THF (15 mL) at 0 ℃. After stirring for 2 h, a solution of macrocyclic sulfate **2** (10.1 g, 39.6 mmol) in dry THF (15 mL) was added to the reaction, and the resulting mixture was stirred overnight at rt. Then, H_2O (1.4 mL, 76.1 mmol) was added and the pH was adjusted to 3 with H_2SO_4. After stirring at rt for 3 h, the reaction was quenched with saturated $NaHCO_3$ solution and concentrated under vacuum, the residue was purified with flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to give **2a** (9.6 g, yield: 93%) as clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40 (s, 3H), 3.55-3.80 (m, 28H).

Compound 3a was prepared from **2a** by following the same procedure for **2a** as light yellow oil (12.9 g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40 (s, 3H), 3.55-3.76 (m, 44H).

Compound 4a was prepared from **3a** by following the same procedure for **2a** as light yellow oil (14.5 g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40 (s, 3H), 3.54-3.77 (m, 60H).

Compound 4b was prepared from **4a** by following the same procedure for **2a** as light yellow oil (23 g, 91% yield). ^{1}H NMR (400 MHz, $CDCl_3$) 3.40 (s, 3H), 3.49-3.80 (m, 76H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 77.5, 77.2, 76.9, 72.6, 71.9, 70.5, 70.2, 61.6, 59.0. HRMS (ESI) calcd for $C_{39}H_{80}NaO_{20}^+$ $[M+Na]^+$ 891.5141, found 891.5126.

Compound 4c was prepared from **4b** by following the same procedure for **2a** as light yellow oil (6.6 g, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40 (s, 3H), 3.47-3.90 (m, 92H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 77.60, 77.28, 76.96, 72.47, 71.81, 70.40, 70.29, 61.38, 58.91. HRMS (ESI) calcd for $C_{47}H_{96}NaO_{24}^+$ $[M+Na]^+$ 1067.6189, found 1067.6182.

Compound 2b. Under an atmosphere of Ar, to a suspension of NaH (0.2 g, 60% in mineral oil, 5.1 mmol) in dry THF (3 mL) was added a solution of **2a** (0.5 g, 1.5 mmol) in dry THF (10 mL) at 0 ℃ and the mixture was stirred at this temperature for 2 h. Then *tert*-butyl bromoacetate (1.0 g, 0.8 mL, 5.1 mmol) was added to the reaction, and the resulting mixture was stirred overnight at rt. The reaction was neutralized with saturated NH_4Cl, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified with flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to give **2b** (0.4 g, 57% yield) as light yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (s, 9H), 3.40 (s, 3H), 3.48-3.80 (m, 28H), 4.04 (s, 2H).

Compound 2c. A solution of compound **2b** (7.8 g, 19.3 mmol), anisole (3.1 mL, 28.9 mmol) and TFA (35.8 mL, 481.8 mmol) in DCM (100 mL) was stirred overnight at 30 ℃. After concentrated under vacuum, the residue was dissolved in water and washed with Et_2O, then the water phase was extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated under vacuum to give **2c** (7.7 g, 100% yield) as yellow oil.

Compound 2d. HATU and DIPEA were added to a solution of **2c** (7.0 g, 17.6 mmol) in dry DMF (130 mL) at rt. Then, a solution of **1a** (6.2 g, 13.5 mmol) in dry DMF (30 mL) was added to the reaction, and the resulting mixture was stirred overnight at rt. Then DMF was evaporated and the residue was washed with H_2O, extracted with DCM and dried over anhydrous Na_2SO_4. The combined organic layers were purified with flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to give **2d** (8.1 g, 71% yield) as clear oil. ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.44-1.60 (m, 4H), 1.78-1.92 (m, 2H), 3.22-3.27 (m, 2H), 3.29 (s, 3H), 3.47 (dd, J = 5.8, 3.8 Hz, 2H),

3.53-3.73 (m, 28H), 4.07-4.30 (m, 3H), 4.30-4.51 (m, 3H), 5.14-5.25(m, 2H), 7.32-7.45 (m, 9H), 7.74 (d, *J* = 7.4 Hz, 2H), 7.89 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.4, 170.1, 156.1, 143.9, 141.3, 135.4, 128.6, 128.4, 127.7, 127.1, 125.1, 120.0, 71.9, 70.9, 70.3, 70.0, 67.0, 59.0, 53.9, 47.2, 31.9, 29.7, 29.4, 22.5. HRMS (ESI) calcd for $C_{45}H_{62}N_2NaO_{13}^+$ $[M+Na]^+$ 861.4150, found 861.4131.

Compound 2e. A mixture of **2d** (5.2 g, 6.2 mmol) and Pd/C (0.6 g, 10% on carbon) in MeOH (50 mL) was stirred at rt under H_2 (1 atm) for 5 h. The mixture was filtered through Cite and purified with flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to give **2e** (3.9 g, 85% yield) as clear oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33-1.66 (m, 4H), 1.69-2.00 (m, 2H), 3.29-3.42 (m, 5H), 3.54-3.70 (m, 28H), 4.02 (d, *J* = 16.5 Hz, 2H), 4.09-4.58 (m, 4H), 7.33 (t, *J* = 7.4 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.63 (dd, *J* = 7.3, 3.4 Hz, 2H), 7.78 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 173.9, 170.5, 156.1, 143.9, 141.3, 127.7, 127.1, 125.1, 120.0, 71.9, 70.9, 70.4, 66.8, 59.0, 53.6, 47.2, 38.3, 31.6, 28.7, 22.0. HRMS (ESI) calcd for $C_{38}H_{55}N_2O_{13}^-$ $[M-H]^-$ 747.3704, found 747.3725.

Compound 3b was prepared from **3a** by following the same procedure for **2b** as light yellow oil (15.4 g, 55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.50 (s, 9H), 3.40 (s, 3H), 3.57 (dd, *J* = 5.7, 3.5 Hz, 2H), 3.64-3.76 (m, 42H), 4.05 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.6, 81.5, 71.9, 70.6, 69.0, 59.0, 28.1. HRMS (ESI) calcd for $C_{29}H_{58}NaO_{14}^+$ $[M+Na]^+$ 653.3724, found 653.3702.

Compound 3c was prepared from **3b** by following the same procedure for **2c** as light yellow oil (13.3 g, 100% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.40 (s, 3H), 3.48-3.87 (m, 44H), 4.18 (s, 2H).

Compound 3d was prepared from **3c** by following the same procedure for **2d** as light yellow oil (6.6 g, 70% yield). ^{1}H NMR (400 MHz, $(CD_3)_2CO$) δ 1.48 (d, *J* = 7.3 Hz, 2H), 1.54 (d, *J* = 7.7 Hz, 2H), 1.80-1.91 (m, 2H), 3.23-3.28 (m, 2H), 3.31 (s, 3H), 3.48-3.51 (m, 2H), 3.55-3.69 (m, 42H), 3.94 (s, 2H), 4.26 (d, *J* = 7.1 Hz, 2H), 4.34-4.41 (m, 2H), 5.20 (d, *J* = 3.6 Hz, 2H), 7.35-7.47 (m, 9H), 7.74 (d, *J* = 7.4 Hz, 2H), 7.89 (d, *J* = 7.2 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.3, 170.2, 156.1, 143.9 , 141.3 , 135.36, 128.8 , 128.5, 128.3, 127.7, 127.1, 125.1, 120.0, 71.8, 70.8, 70.2, 67.1, 66.8, 59.0, 54.0, 47.2, 38.3, 31.7, 29.7, 22.5. HRMS (ESI) calcd for $C_{53}H_{78}N_2NaO_{17}^+$ $[M+Na]^+$ 1037.5198, found 1037.5203.

Compound 3e was prepared from **3d** by following the same procedure for **2e** as light yellow oil (5.0 g, 84% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33-1.66 (m, 4H), 1.72-2.01 (m, 2H), 3.32 (d, *J* = 5.1 Hz, 2H), 3.38 (s, 3H), 3.50-3.79 (m, 44H), 4.00 (s, 2H), 4.22 (t, *J* = 6.7 Hz, 1H), 4.30-4.55 (m, 3H), 7.23-7.36 (m, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.62 (s, 2H), 7.77 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 174.0, 170.5, 156.1, 143.9, 141.3, 127.7, 127.1, 125.1, 120.0, 71.9, 71.0, 70.4, 69.9, 66.8, 59.0, 53.6, 47.2, 38.3, 31.6, 28.7, 22.1. HRMS (ESI) calcd for $C_{46}H_{71}N_2O_{17}^-$ $[M-H]^-$ 923.4753, found 923.4750.

Compound 4d was prepared from **4c** by following the same procedure for **2b** as light yellow oil (14.0 g, 55% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (s, 9H), 3.40 (s, 3H), 3.56-3.59 (m, 2H), 3.64-3.75 (m, 90H), 4.04 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.5, 81.3, 71.79, 70.5, 68.9, 58.9, 28.09. HRMS (ESI) calcd for $C_{53}H_{106}NaO_{26}^+$ $[M+Na]^+$ 1181.6870, found 1181.6873.

Compound 4e was prepared from **4d** by following the same procedure for **2c** as light yellow oil (10.8 g, 98% yield). ^{1}H NMR (400 MHz, $(CD_3)_2CD$) δ 3.31 (s, 3H), 3.47-3.51 (m, 2H), 3.53-4.00 (m, 90H), 4.14 (s, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.8, 71.8, 70.6, 70.4, 68.4, 58.8. HRMS (ESI) calcd for $C_{49}H_{97}O_{26}^-$ $[M-H]^-$ 1101.6268, found 1101.6237.

Compound 4f was prepared from **4e** by following the same procedure for **2d** as light yellow oil (5.8 g, 68% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.27 (d, *J* = 7.1 Hz, 2H), 1.56 (s, 2H), 2.07 (s, 2H), 3.27 (s, 2H), 3.40 (s, 3H), 3.41-3.97 (m, 92H), 4.12-4.18 (m, 2H), 4.24 (s, 2H), 4.38-4.46 (m, 3H), 5.20 (d, *J* = 4.3 Hz, 2H), 7.31-7.45 (m, 9H), 7.62 (s, 2H), 7.79 (d, *J* = 7.8 Hz, 2H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.3, 170.1 , 156.1, 143.8, 141.2 , 135.4, 129.1, 128.5, 128.2, 127.7, 127.0, 125.1, 119.9, 71.8, 70.8, 70.1, 69.3, 68.3, 67.1, 66.8, 59.0, 54.0, 47.2, 38.3, 31.6, 29.3, 22.5. HRMS (ESI) calcd for $(C_{73}H_{128}N_2Na_4O_{29}^{2+})/2$ $[(M+Na)/2]^+$ 794.4097, found 794.4108.

Compound 4g was prepared from **4f** by following the same procedure for **2e** as light yellow oil (4.4 g, 80% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45-1.69 (m, 4H), 1.97-1.79 (m, 2H), 3.32 (d, *J* = 5.6 Hz, 2H), 3.40 (s, 3H), 3.59-3.54 (m, 2H), 3.76-3.60 (m, 90H), 3.99 (s, 2H), 4.24 (t, *J* = 6.6 Hz, 1H), 4.35-4.48 (m, 3H), 7.34 (t, *J* = 7.3 Hz, 2H), 7.42 (t, *J* = 7.4 Hz, 2H), 7.63 (d, *J* = 6.9 Hz, 2H), 7.78 (d, *J* = 7.5 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 169.3, 81.0, 71.7, 70.7, 70.3, 70.2, 70.1, 69.6, 69.0, 58.7, 51.6, 45.2, 27.9. HRMS (ESI) calcd for $C_{70}H_{119}N_2O_{29}^-$ $[M-H]^-$ 1451.7899, found 1451.7940.

Peptide P_7-8. ^{1}H NMR (400 MHz, CD_3CO) δ 1.76-1.22 (m, 48H), 3.05-3.28 (m, 16H), 3.36 (d, *J* = 3.2 Hz, 15H), 3.75-3.52 (m, 146H), 4.06-4.53 (m, 17H), 7.81-7.89 (m, 5H), 7.90-8.00 (m, 9H). ^{19}F NMR (376 MHz, D_3O) δ -63.40, -63.41, -63.43, -63.48, -63.51. MS (MALDI) m/z calcd for $C_{173}H_{275}F_{18}N_{17}KO_{57}^+$ $[(M+K)]^+$ 3883.8, found 3882.4.

Peptide P_7-12. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.16-1.37 (m, 24H), 1.38-1.69 (m, 48H), 3.21 (s, 24H), 3.38 (s, 21H), 3.43-3.75 (m, 206H), 3.79-4.12 (m, 25H), 7.66-7.74 (m, 7H), 7.75-7.89 (m, 15H). ^{19}F NMR (376 MHz, D_3O) δ -63.41,-63.43. MS (MALDI) m/z calcd for $C_{251}H_{394}F_{30}N_{25}Na_2O_{81}^+$ $[(M+2Na-H)]^+$ 5670.6, found 5669.9.

Peptide P_{11}-8. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.82-1.01 (m, 48H), 3.12-3.32 (m, 16H), 3.39 (s, 15H), 3.94-3.43 (m, 226H), 5.35-3.99 (m, 17H), 7.67-7.76 (m, 5H), 7.77-7.93 (m, 9H). ^{19}F NMR (376 MHz, D_3O) δ -63.29, -63.36, -63.37, -63.44, -63.48. MS (MALDI) m/z calcd for $C_{215}H_{359}F_{18}N_{18}O_{77}^+$ $[(M+ACN+H)]^+$ 4767.4, found 4766.5.

Peptide P_{11}-12. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31-1.68 (m, 48H), 1.72-2.05 (m, 24H), 3.00-3.35 (m, 24H), 3.39 (d, *J* = 3.2 Hz, 21H), 3.77-3.53 (m, 318H), 4.21-4.42 (m, 25H), 7.67-7.74 (m, 7H), 7.77-7.89 (s, 15H). ^{19}F NMR (376 MHz, D_3O) δ -63.38, -63.40. MS (MALDI) m/z calcd for $C_{308}H_{512}F_{30}N_{25}O_{110}^+$ $[(M+CH_3OH+H)]^+$ 6895.5, found 6896.9.

Peptide P_{23}-8. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38-1.07 (m, 16H), 1.40-1.69 (m, 32H), 3.01-3.34 (m, 16H), 3.40 (s, 15H), 3.98-3.48 (m, 466H), 4.02-4.47 (m, 17H), 7.68-7.77 (m, 5H), 7.79-7.88 (m, 9H). ^{19}F NMR (376 MHz, D_3O) δ -63.24, -63.32, -63.40, -63.43. MS (MALDI) m/z calcd for $C_{335}H_{598}F_{18}N_{18}O_{137}^+$ $[(M+ACN+H)]^+$ 7411.9, found 7407.2.

3. Solvent-dependent ^{19}F NMR

^{19}F NMR spectra of peptidic polymers in mixed solvents of methanol and water in different proportions were measured by a Bruker NMR 376 MHz spectrometer and referenced to 2% perfluorobenzene (s, -164.90 ppm) in $CDCl_3$ and 73 mM sodium trifluomethanesulfonate (s, -79.61 ppm) in D_2O.

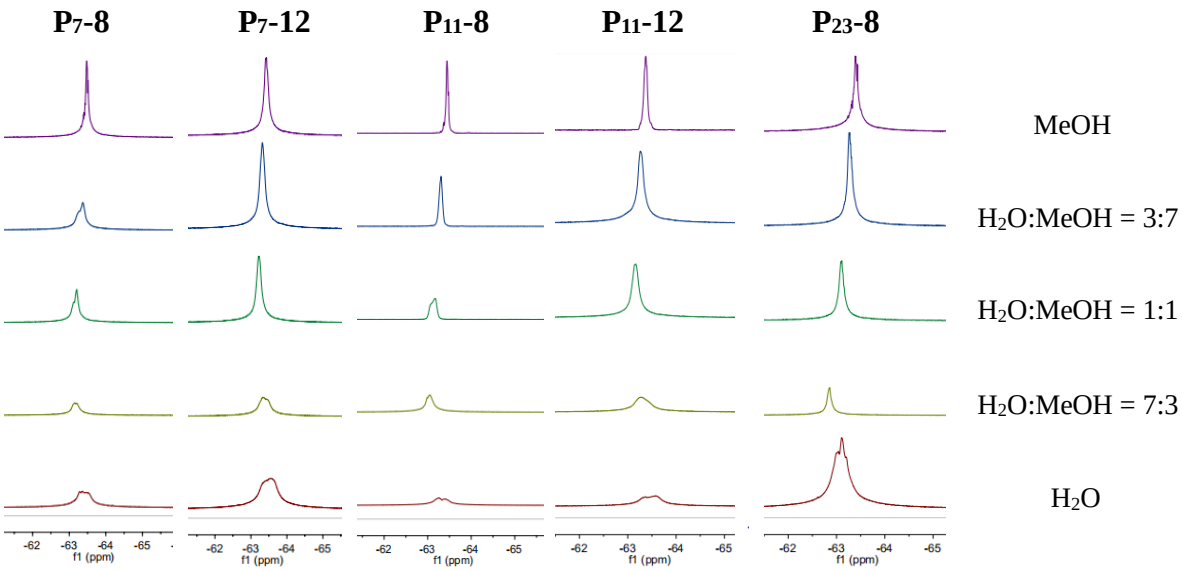


Figure S1. Solvent-dependent ^{19}F NMR spectra of the M-PEGs peptidic polymers

4. Determination of n-octanol/water partition coefficients

The log*P* values of peptidic polymers were detected by a shake-flask method. Specifically, a peptide was dissolved in distilled water saturated with *n*-octanol. Then 1.5 mL of this solution was mixed with an equal volume of *n*-octanol saturated with distilled water and the mixture was shock overnight, then the *n*-octanol phase was separated by centrifugation. Equal-volume samples of the shaken water phase and the starting solution were subsequently taken and analyzed by HPLC. The peak area was measured at λ = 254 nm, and compared with calibration curve to obtain the concentration of the peptide. log*P* values were determined from: $\log[(C_s\text{-}C_w)/C_w]$, where C_s and C_w are the concentrations of the starting water solution and the water phase of the compound, respectively.

5. Turbidity Test

The turbidity tests were recorded by a UV-visible Lambda 35 spectrometer (Perkin Elmer, USA) at 700 nm. All peptidic polymers with 3 concentrations were dissolved in water (3.0 mM , 1.2 mM or 0.342 mM). The transmittance was measured between 30 ℃ and 99 ℃ through temperature-controlled heating and cooling cycles, and the sample was equilibrated for 10 min at

each temperature before measurement.

Table S1. LCST of M-PEGs peptidic polymers at 3.0 mM, 1.2 mM and 0.342 mM

Concentration	**P_7-8**	**P_7-12**	**P_{11}-8**	**P_{11}-12**	**P_8-12**	**P_{4+4}-10**
0.342（mM）	75 ℃	63 ℃	90 ℃	84 ℃	26 ℃	66 ℃
1.2（mM）	71 ℃	61 ℃	87 ℃	82 ℃	24 ℃	60 ℃
3（mM）	68.5 ℃	60 ℃	84 ℃	80 ℃	23 ℃	56 ℃

6. Dynamic light scattering

The dynamic light scattering (DLS) analysis, including particle size and polydispersity index (PDI), was performed on DLS Analyzer (Malvern Zetasizer Nano 3690). The peptidic polymers (**P_7-8, P_7-12, P_8-12** and **P_{4+4}-10**) were dissolved in water with desired concentration and measured at an angle of 90° in a 10 mm diameter cell at the set temperatures (25 ℃, LCST and LCST + 5 ℃) and recorded by Malvern software. Each measurement was repeated 3 times.

Table S2. DLS of M-PEGs peptidic polymers (0.9 mg/mL) at room temperature

	P_7-8	**P_7-12**	**P_{11}-8**	**P_{11}-12**	**P_{23}-8**
Size(nm)	76.85	84.26	165.0	153.3	199.0
PDI	0.592	0.626	0.439	0.663	0.380

Table S3. DLS of M-PEGs peptidic polymers (0.3 mg/mL) at different temperatures

	P_7-8		**P_7-12**		**P_8-12**		**P_{4+4}-10**	
	Size(nm)	PDI	Size(nm)	PDI	Size(nm)	PDI	Size(nm)	PDI
rt	177.3	0.362	176.3	0.348	208.0	0.315	257.4	0.498
LCST	584.2	0.102	551.2	0.155	658.0	0.179	1113	0.464
LCST+5℃	427.7	0.079	300.6	0.042	843.4	0.049	826.6	0.170

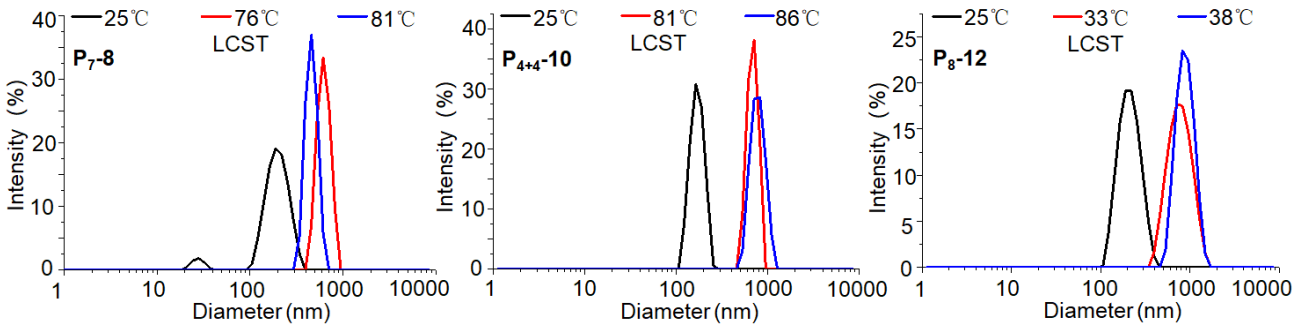


Figure S2. DLS M-PEGs peptidic polymers (0.3 mg/mL) at different temperature

7. Transmission electron microscopy

The M-PEGs peptidic polymers **P7-8**, **P7-12** and drug-loaded nanoemulsion **P7-12**/DOX dissolved in water at a concentration of 0.3 mg/mL were allowed to dry in the oven at 25 ℃ for 30 minutes and then air-dried for 2 hours. After the TEM images were taken on JEM-1230 at an acceleration voltage of 200 kV.

8. UV and Fluorescent property of P7-8

The UV property of **P7-8** was detected by UV 2400 and the fluorescent property was detected by F-4600 FL Spectrophotometer with concentration of 0.3 mg/mL at room temperature.

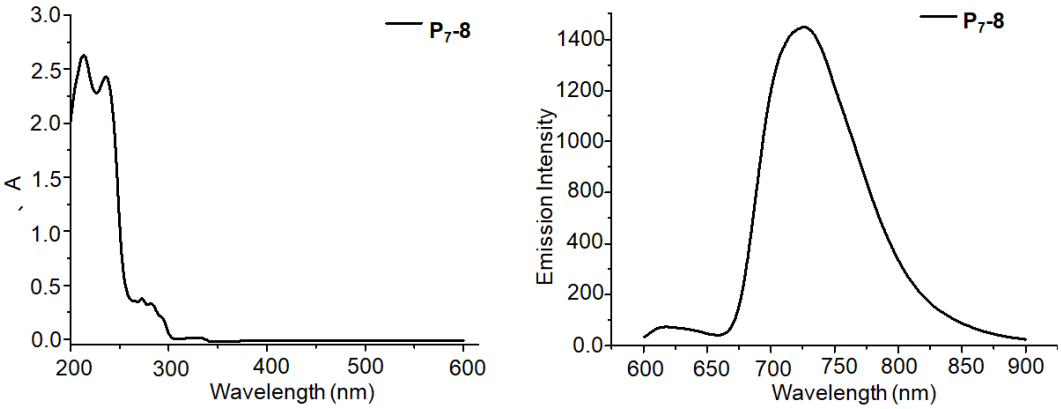


Figure S3. UV and Fluorescent spectrum of **P7-8**

9. Biocompatibility assay

L929 cells were cultured in alpha-MEM medium containing 10% FBS. $HepG_2$ cells were cultured in DMEM medium containing 10% FBS. All cells were cultured at 37 ℃ in humidified atmosphere containing 5% CO_2 and the growth medium was replaced with fresh media every 24 h.

The biocompatibility studies of all peptidic polymers were researched in L929 cell lines *in vitro*

by MTT assay. Briefly, L929 cells were seeded into a 96-well plate and allowed for adherent culture at 37 ℃ for 24 hours. Afterwards, the cells were incubated with a gradient concentration of the M-PEGs peptide ranging from 8 μg/mL to 1000 μg/mL. Every concentration was set with five wells at least. Cells treated with only media were used as control. After incubation 24 hours, MTT stock solution (0.1 mg/mL in PBS, 200 μL) was added to each well and incubated for another 4 h. The media were replaced by 100 μL DMSO to dissolve the formazan blue crystal. The relative cell viability (%) was determined spectrophotometrically by comparing the absorbance of each well at 490 nm with control well using a microplate reader (Bio Tek Instruments, USA). All of the experiments were repeated in three times at least.

10. Stability study in rat plasma

In the *in vitro* stability studies, peptidic polymers **P_7-8**, **P_7-12**, **P_8-12** and **P_{11}-12** were selected as experimental object to study the effects of various factors on the stability of the peptide in SD rat plasma, such as polyethylene glycol size, peptidic polymers chain length and amino acids arrangement in the peptidic polymers. In brief, 2.0 mg of peptide was incubated with 600 μL of rat plasma at 37 ℃ for 72 h with gentle stirring. After 2, 4, 8, 18, 30, 42, 54, 66 and 72 h, a sample (20 μL) was collected and mixed with 1.0 mL methanol. The mixture was filtered and analyzed by HPLC. The integrated values of these peaks were compared to those of t = 0 min in plasma for each peptide and expressed as a fraction of the initial compound that was remaining at the given time point.

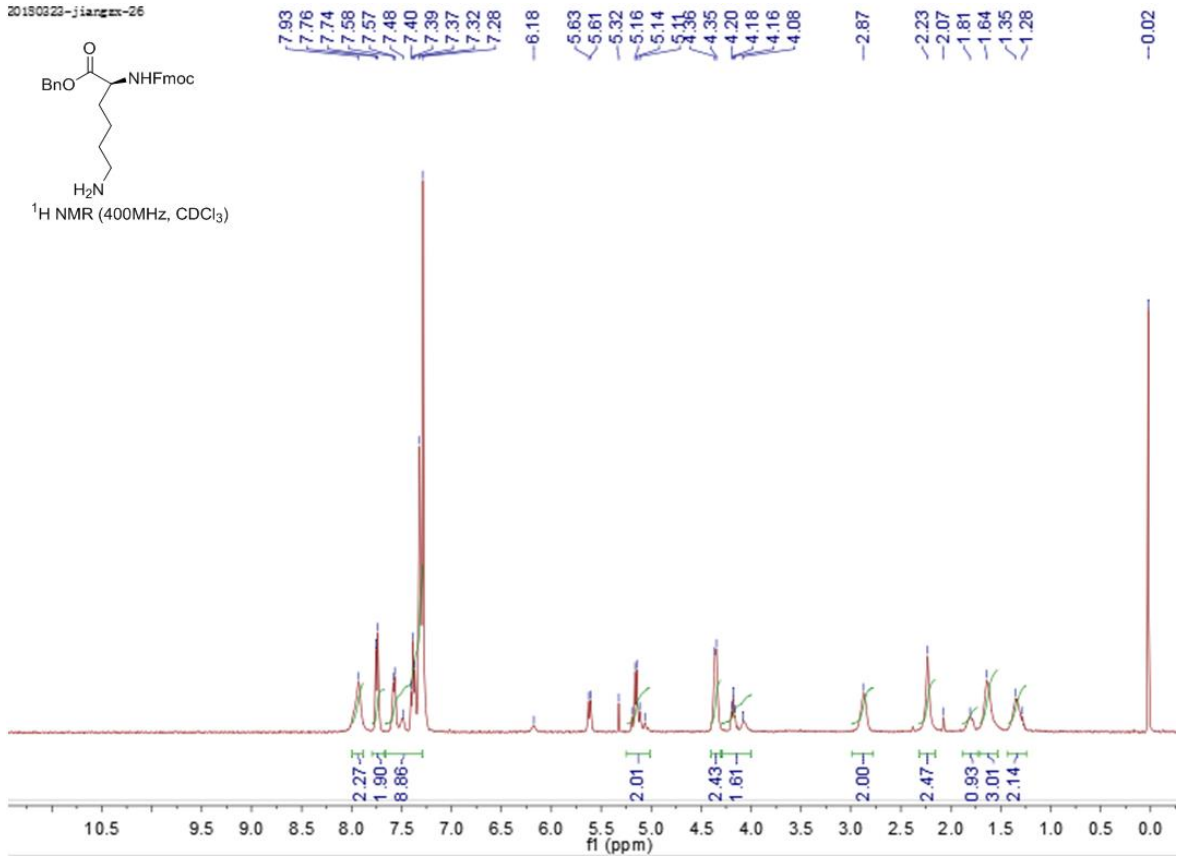
11. IC_{50} of P_7-12/DOX and DOX at 37 ℃ on $HepG_2$ cells

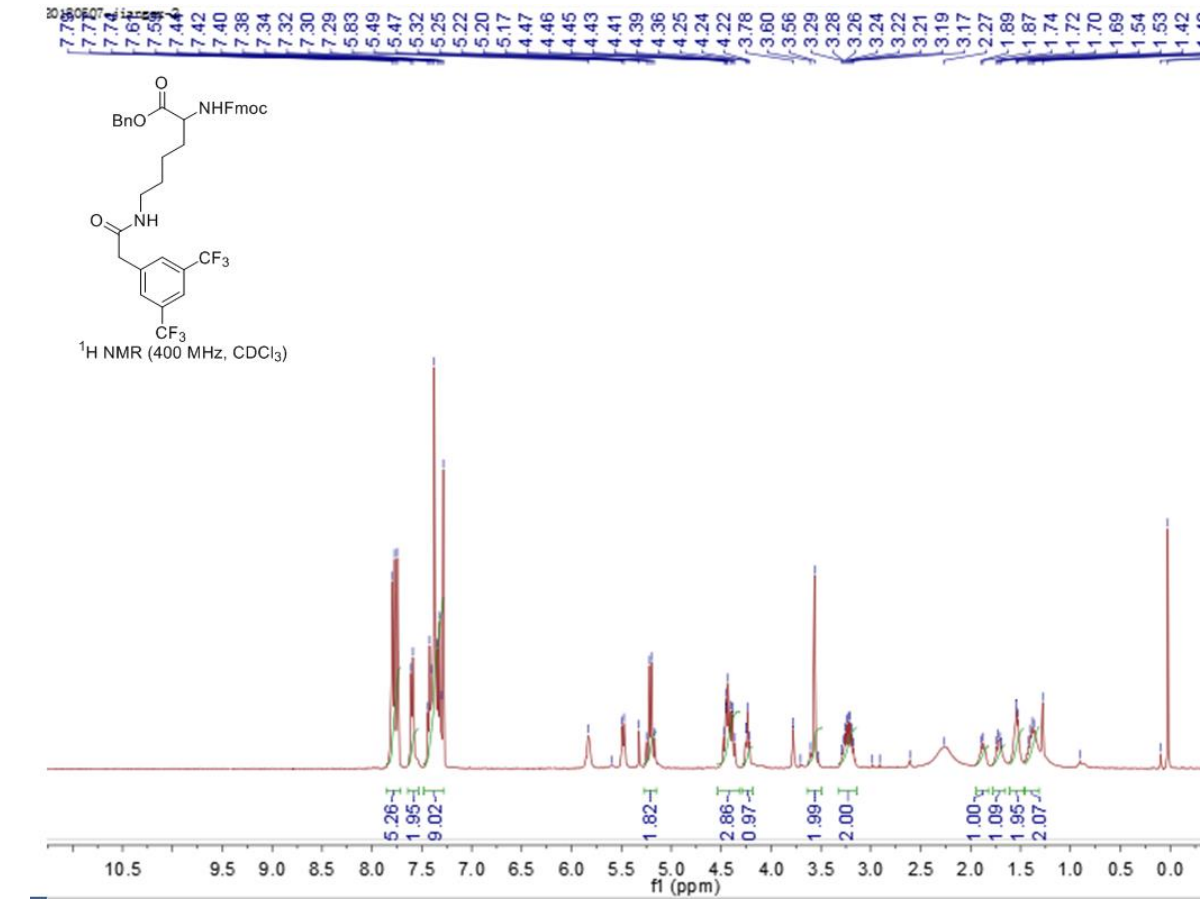
$HepG_2$ cells were seeded into a 96-well plate and allowed for adherent culture at 37 ℃ for 24 hours. Subsequently, the cells were incubated with a gradient concentration of free DOX and **P_7-12**/DOX nanoemulsion and eventual concentration is 0.1, 1, 5, 10, 20, 50 μg/mL, respectively. Every concentration was set with five wells at least. Cells treated with only media were used as control. After incubation 24 hours at 37 ℃, followed by replacing the medium with 200 μL MTT (0.1 mg/mL in PBS) solution and incubated for another 4 h at 37 ℃. Then the medium was replaced with 100 μL DMSO and the absorbance values was measured at 490 nm using a microplate reader (Bio Tek Instruments, USA). All of the experiments were repeated three times.

Reference

1. Zhu, J.; Xiao, Y.; Zhang, H.; Li, Y.; Yuan, Y.; Yang, Z.; Chen, S.; Zheng, X.; Zhou, X.; Jiang, Z.-X., Peptidic monodisperse PEG “combs” with fine-tunable LCST and multiple imaging modalities. *Biomacromolecules.* **2019**, *20*, 1281-1287.

12. Copies of $^{1}H/^{13}C/^{19}F$ NMR, HRMS spectra of compounds

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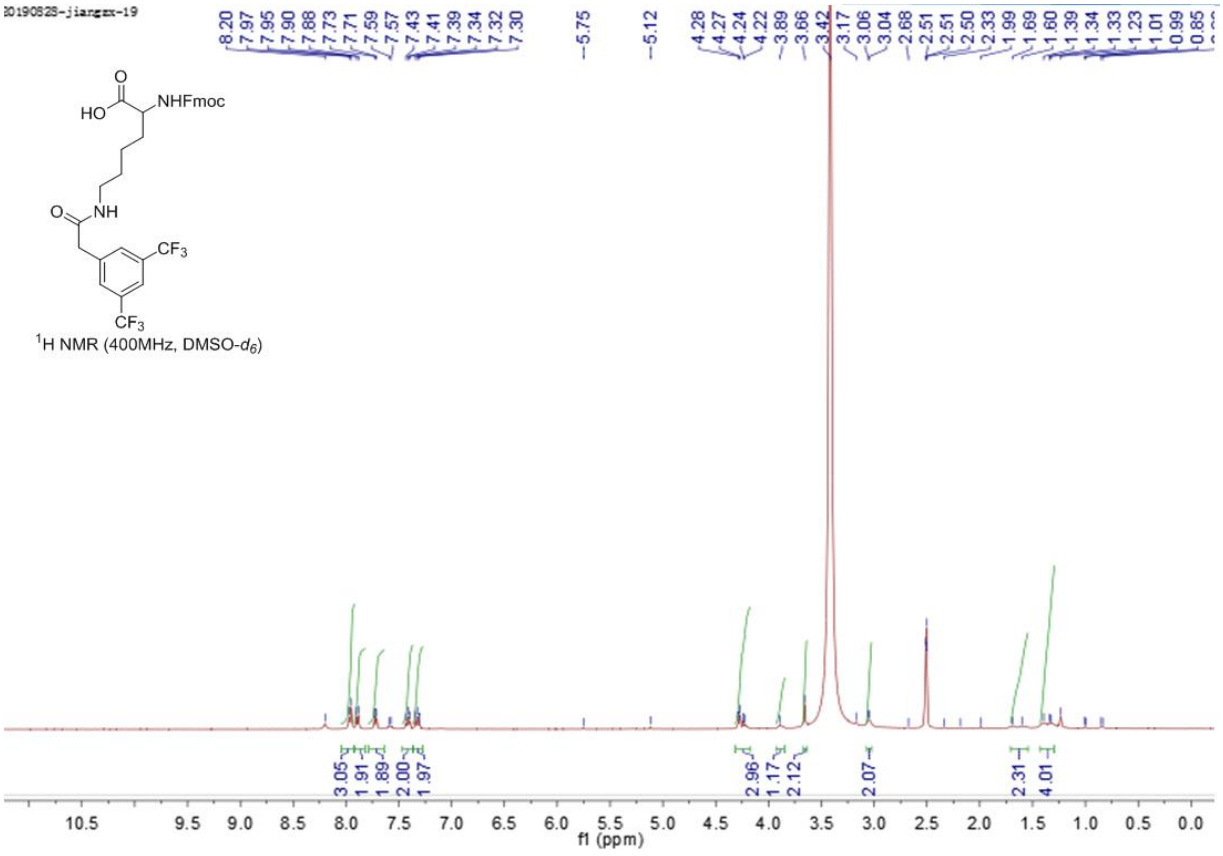
NH
CF3
CF3
1H NMR (400 MHz, CDCl3)
f1 (ppm)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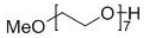
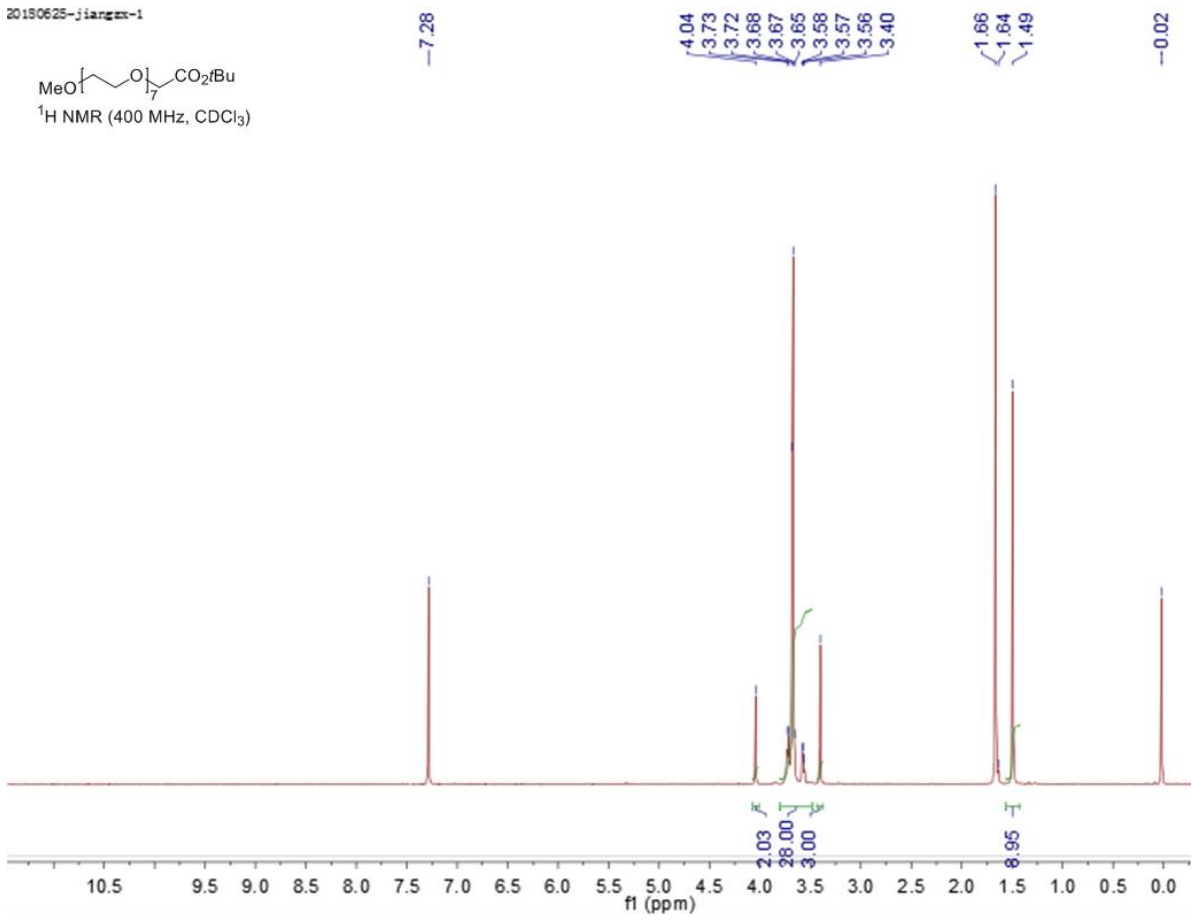
NHFmoc
NH
CF3
CF3
1H NMR (400MHz, DMSO-d6)
f1 (ppm)

20180305-jiangsx-2



^{1}H NMR (400 MHz, $CDCl_3$)


20180625-jiangzx-1
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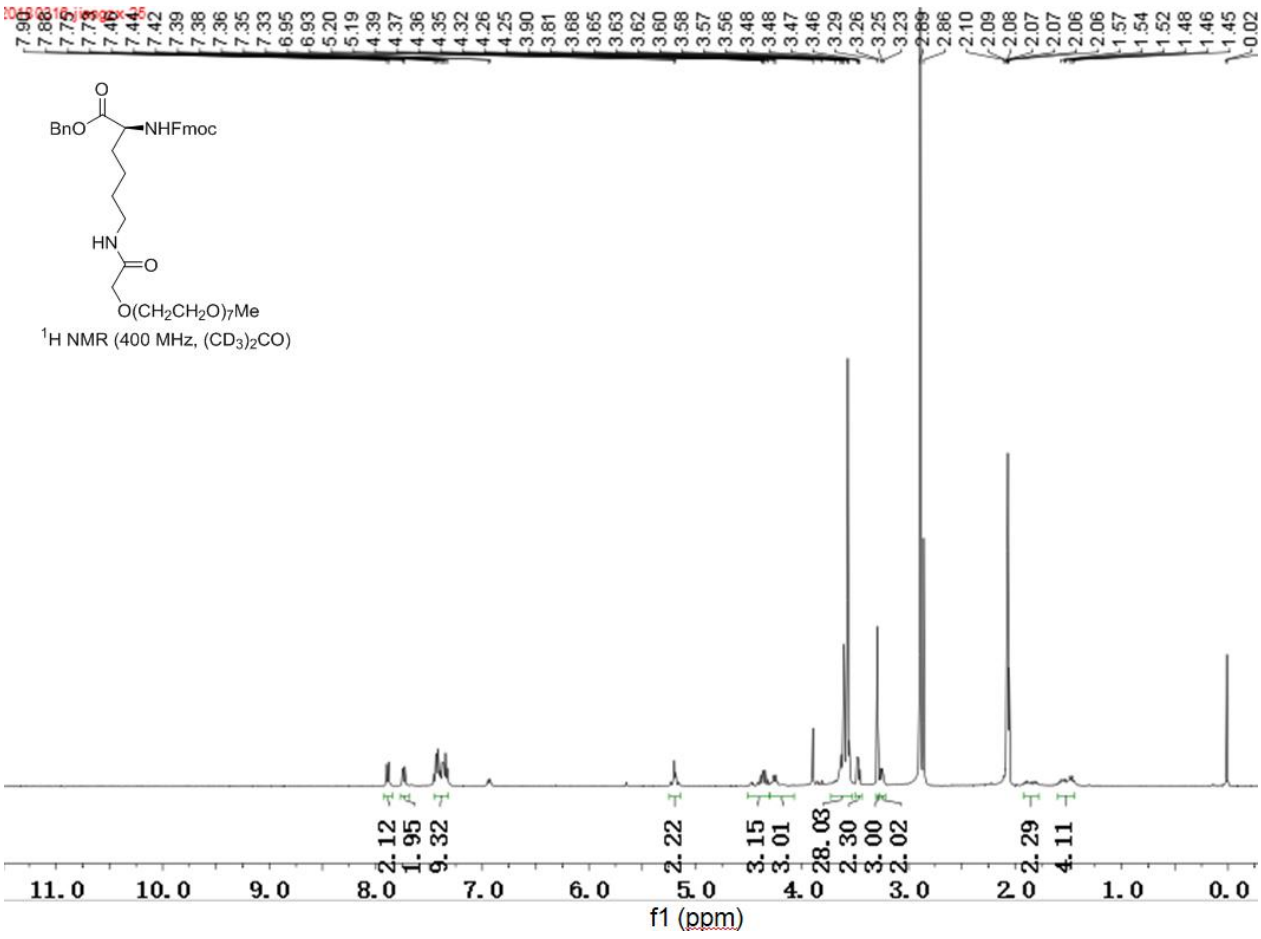
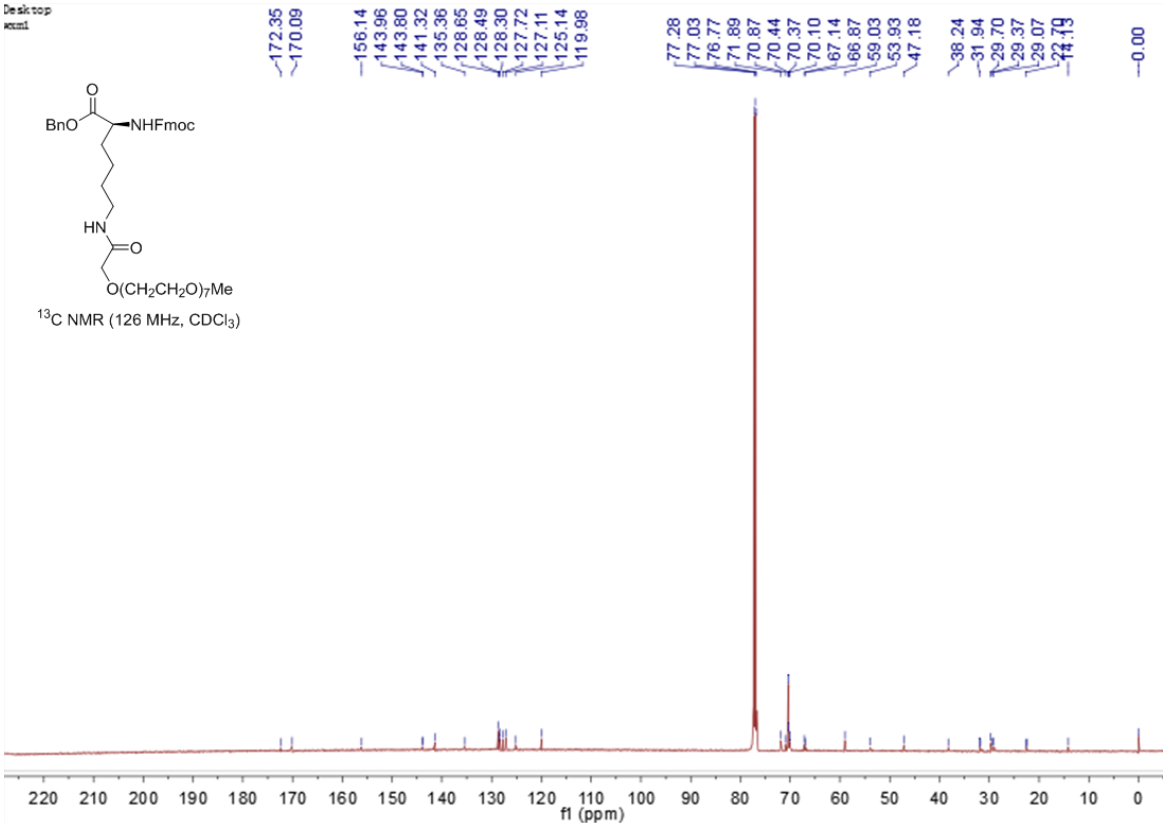
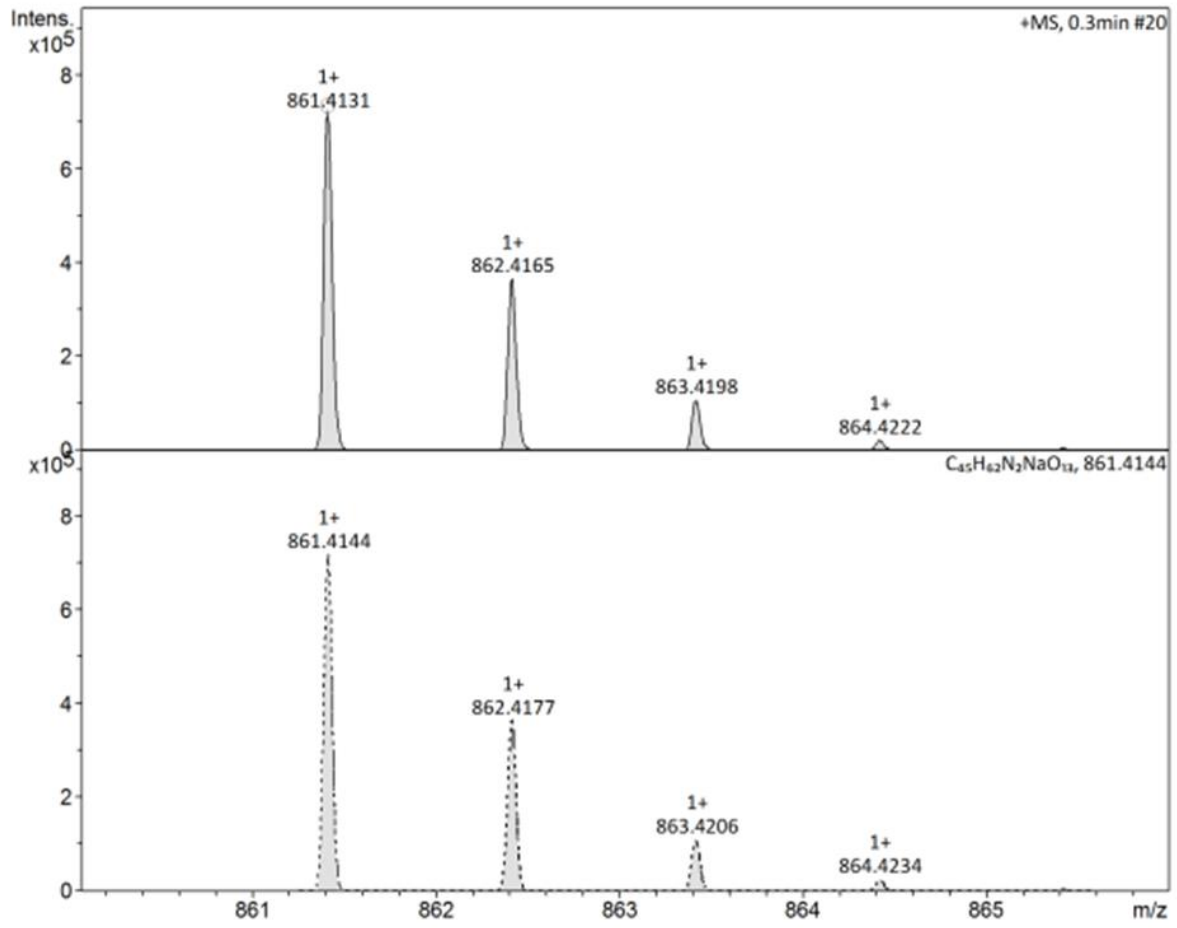
O
7
CO2tBu
1H NMR (400 MHz, CDCl3)
7.28
4.04
3.73
3.72
3.68
3.67
3.65
3.58
3.57
3.56
3.40
1.66
1.64
1.49
0.02
2.03
28.00
3.00
8.95
f1 (ppm)


BnO
NHFmoc
HN
O
O(CH2CH2O)7Me
1H NMR (400 MHz, (CD3)2CO)
2.12
1.95
9.32
2.22
3.15
3.01
28.03
2.30
3.00
2.02
2.29
4.11
f1 (ppm)


Desktop
exm1
172.35
170.09
156.14
143.96
143.80
141.32
135.36
128.65
128.49
128.30
127.72
127.11
125.14
119.98
77.28
77.03
76.77
71.89
70.87
70.44
70.37
70.10
67.14
66.87
59.03
53.93
47.18
38.24
31.94
29.70
29.37
29.07
22.79
14.13
0.00
BnO
NHFmoc
HN
O
O(CH2CH2O)7Me
13C NMR (126 MHz, CDCl3)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

+MS, 0.3min #20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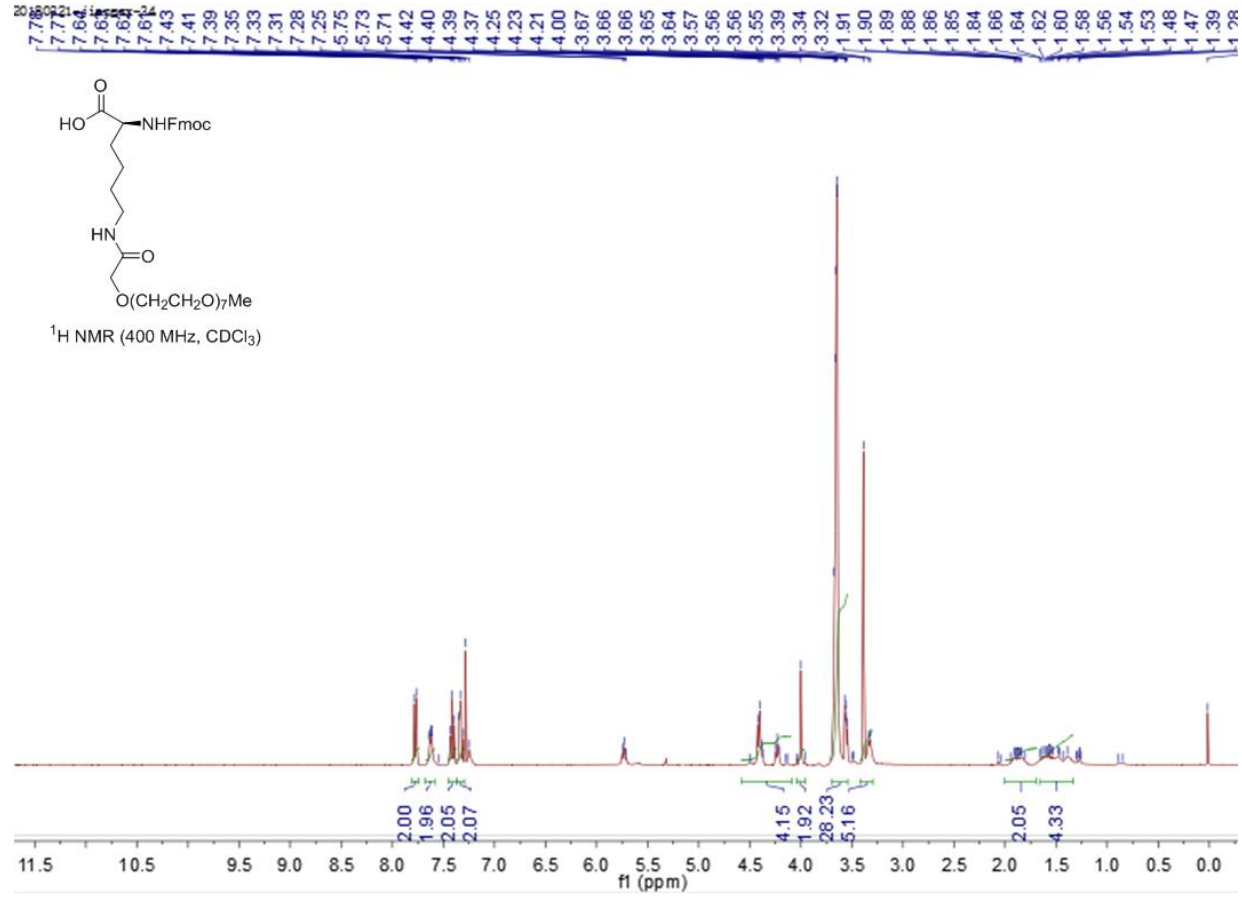
1+
862.4165
1+
863.4198
1+
864.4222
C45H62N2NaO13, 861.4144
1+
861.4144
1+
862.4177
1+
863.4206
1+
864.4234
861
862
863
864
865
m/z


HO
NHFmoc
HN
O
O(CH2CH2O)7Me
1H NMR (400 MHz, CDCl3)
2.00
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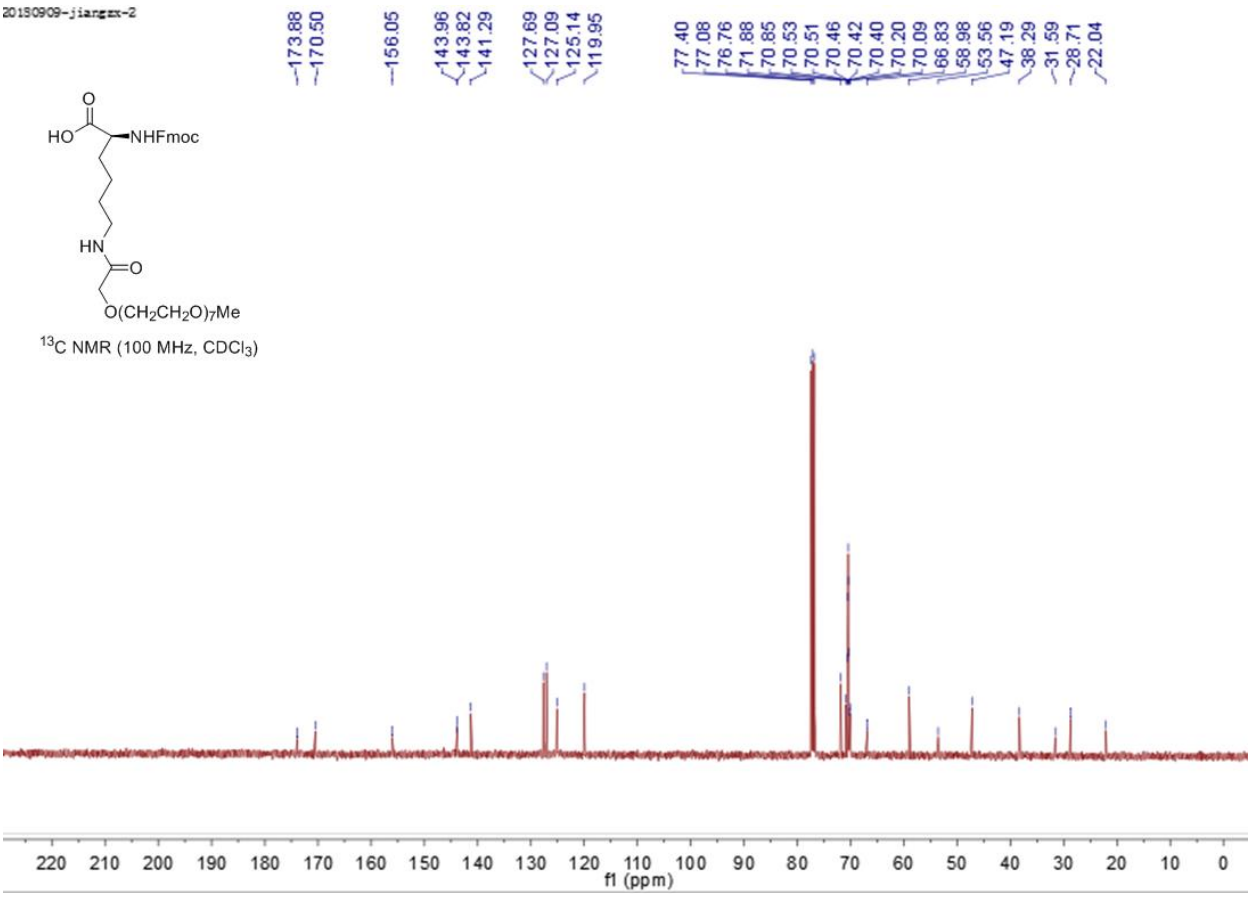
2.05
2.07
4.15
1.92
28.23
5.16
2.05
4.33
f1 (ppm)


20150909-jiangzx-2
HO
NHFmoc
HN
O
O(CH2CH2O)7Me
13C NMR (100 MHz, CDCl3)
f1 (ppm)

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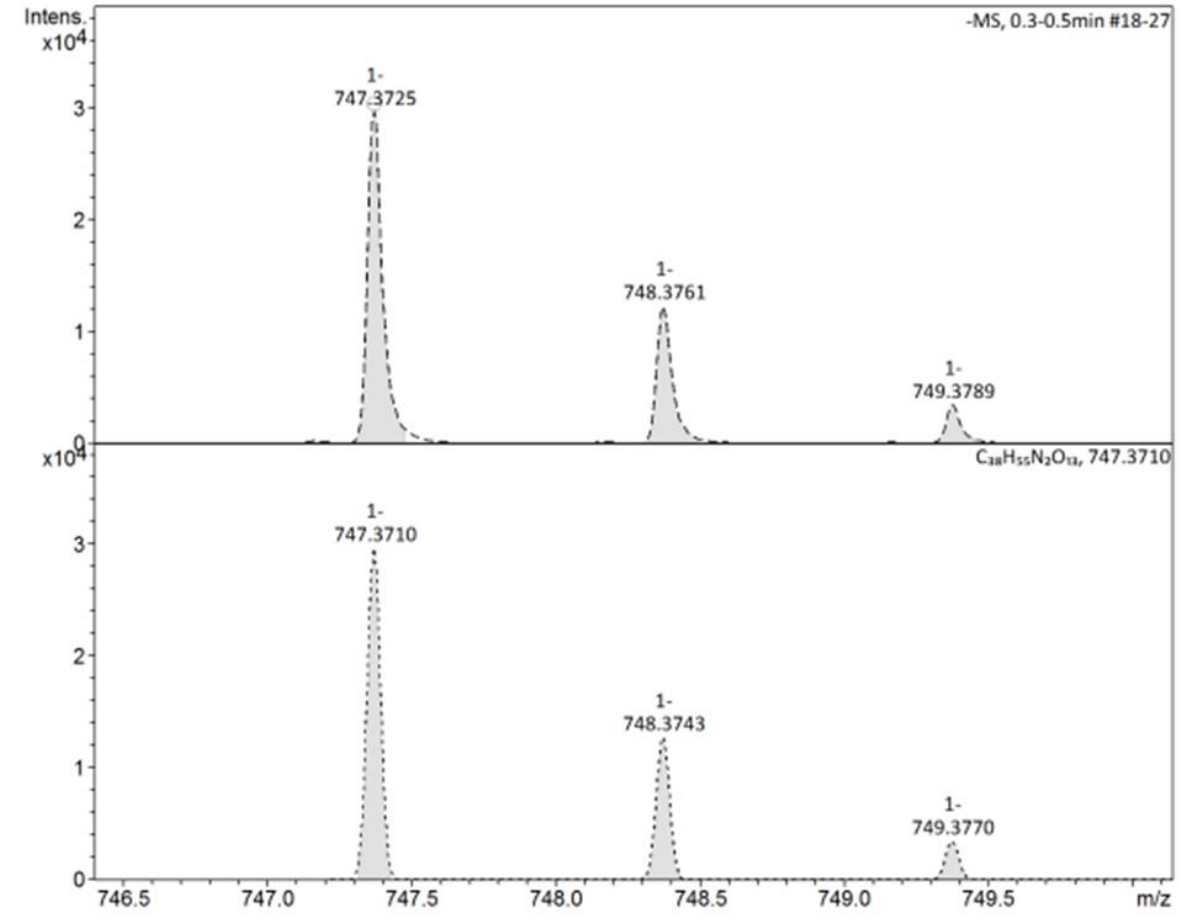
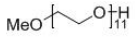
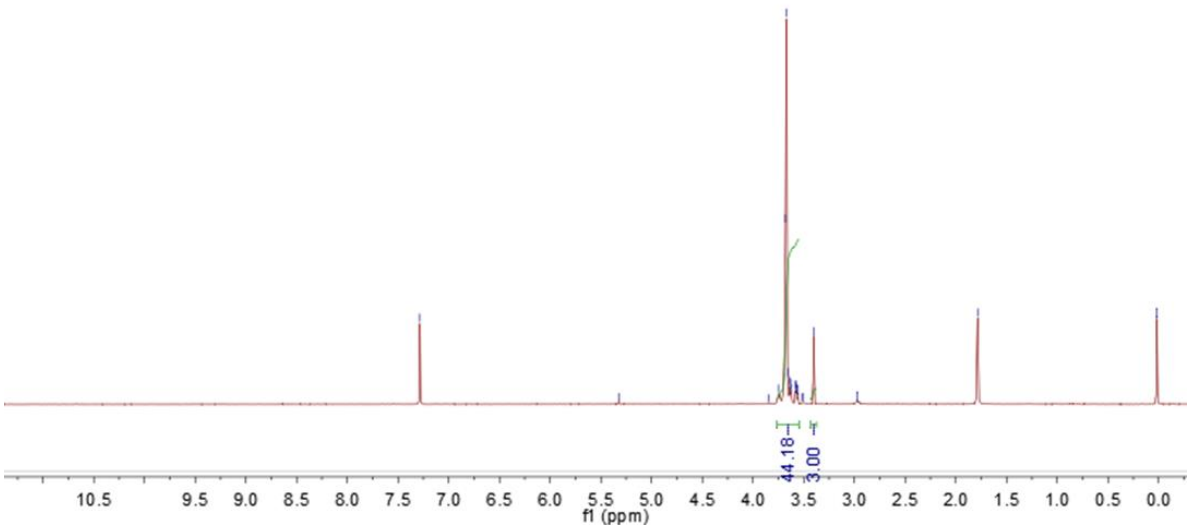
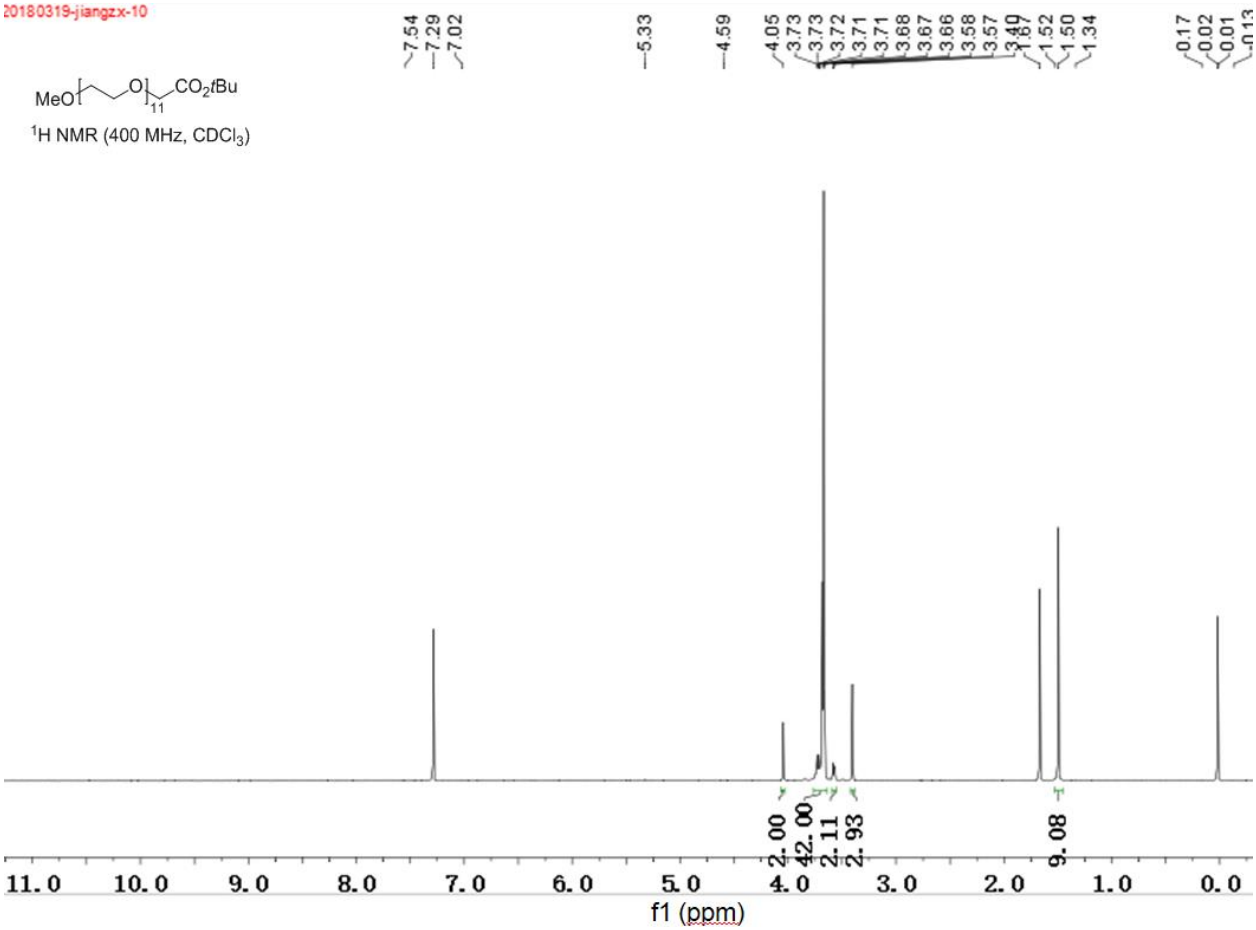




^{1}H NMR (400 MHz, $CDCl_3$)

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11
CO2tBu
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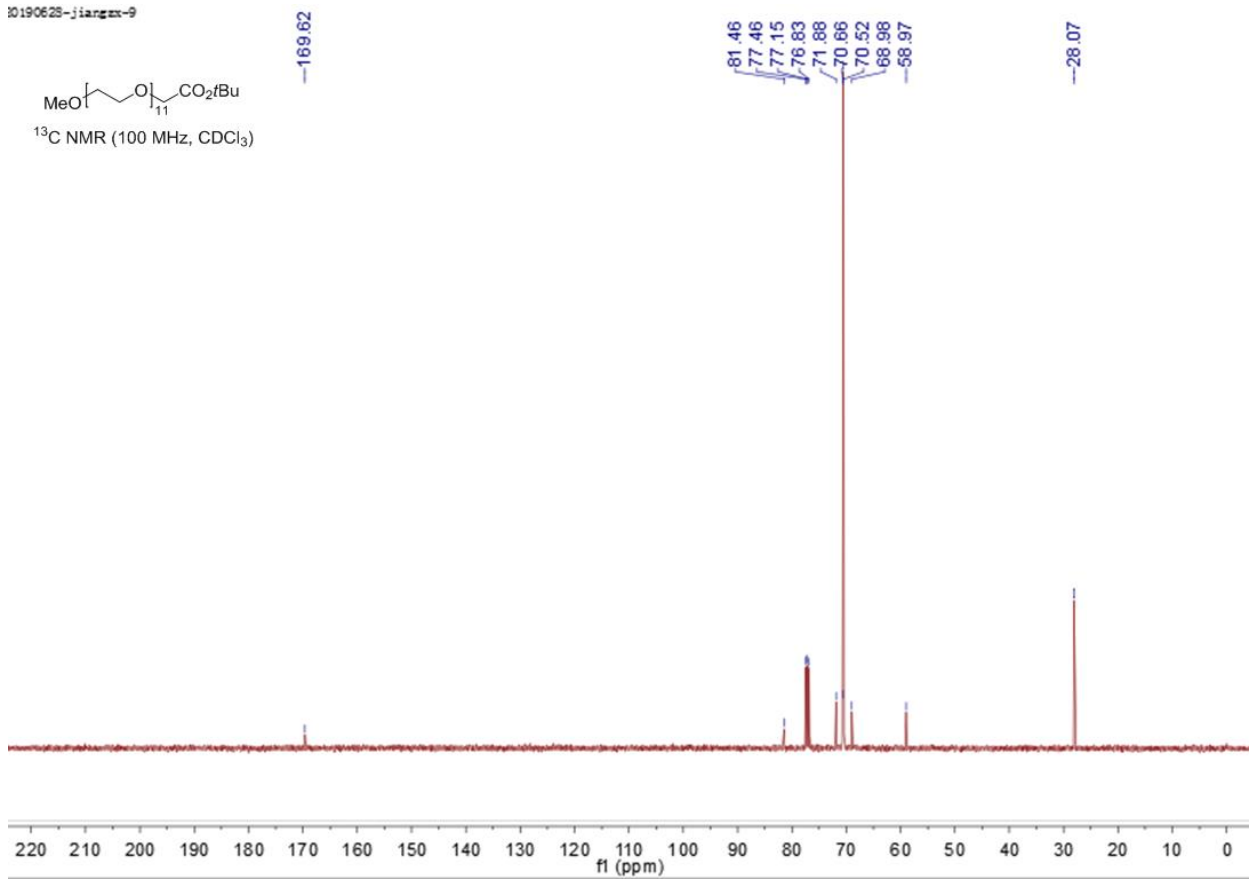
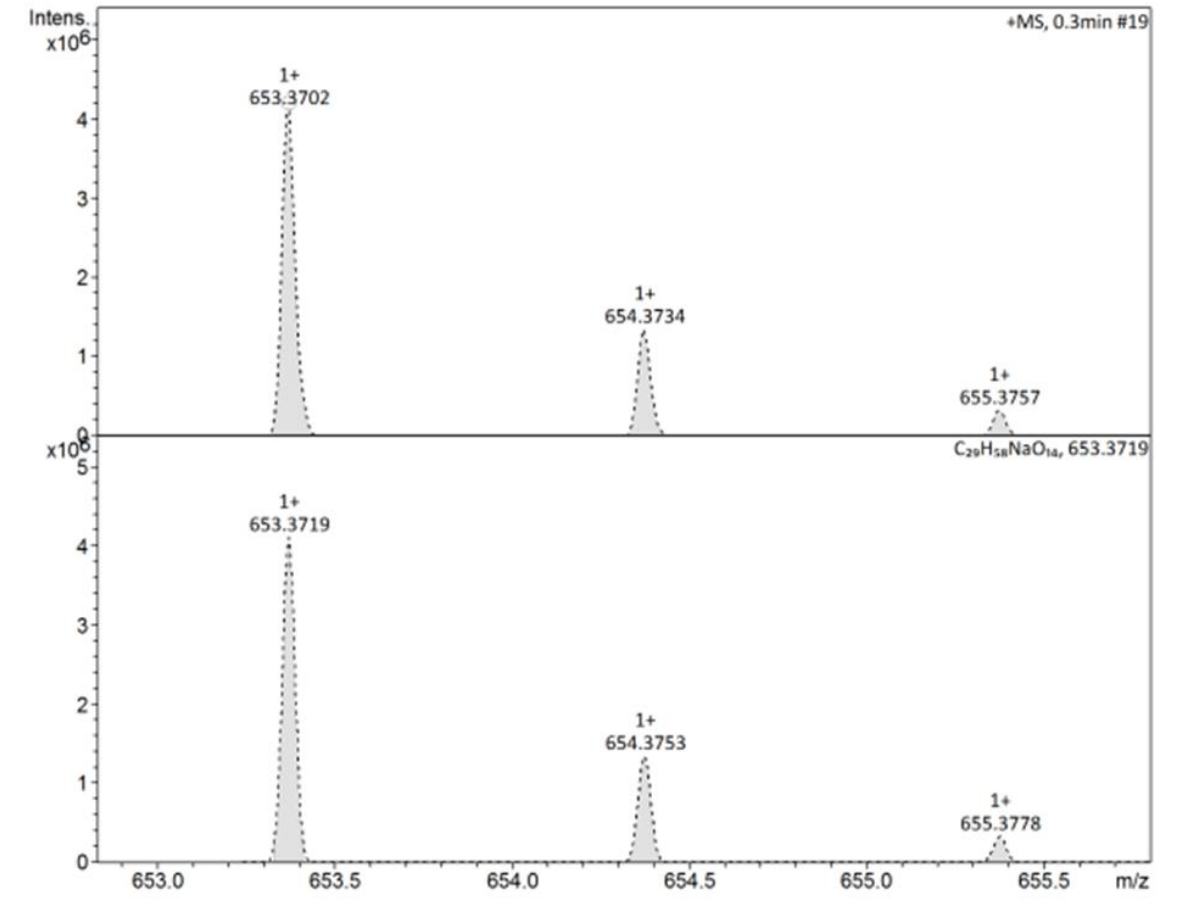
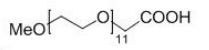
7.54
7.29
7.02
5.33
4.59
4.05
3.73
3.72
3.71
3.68
3.67
3.66
3.58
3.57
1.52
1.50
1.34
0.17
0.02
0.01
2.00
42.00
2.11
2.93
9.08
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)

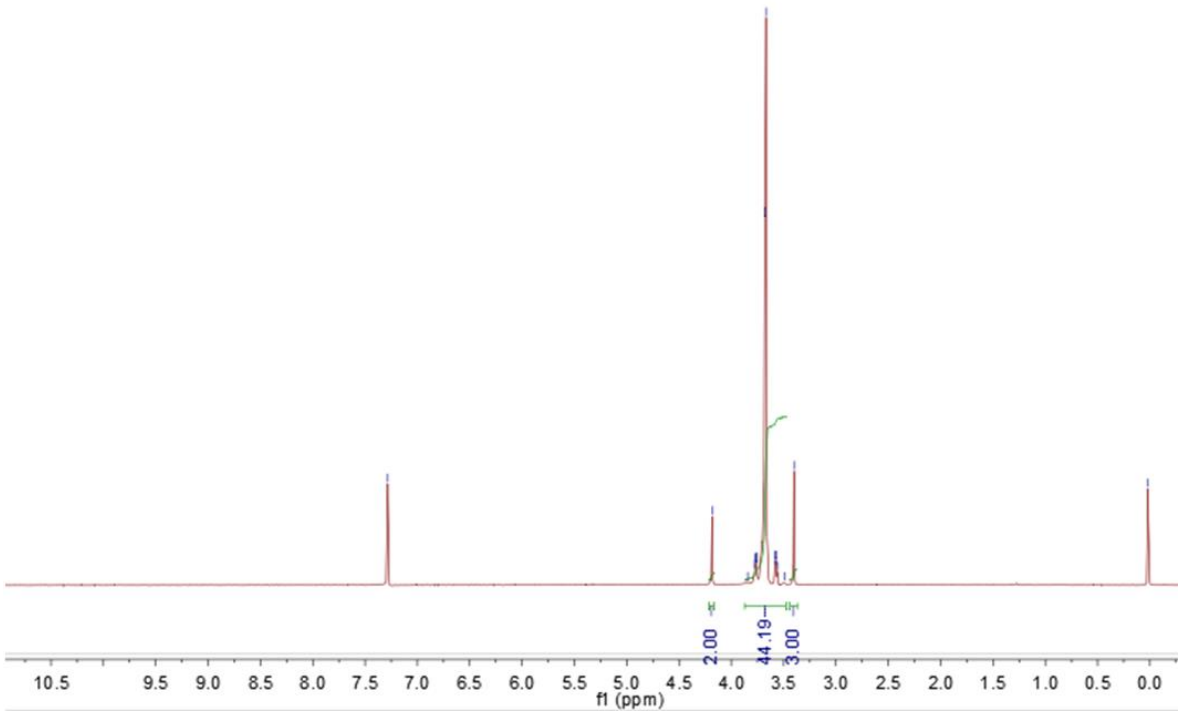

20190628-jiangzx-9
MeO
11
CO2tBu
13C NMR (100 MHz, CDCl3)
169.62
81.46
77.46
77.15
76.83
71.88
70.66
70.52
68.98
58.97
28.07
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

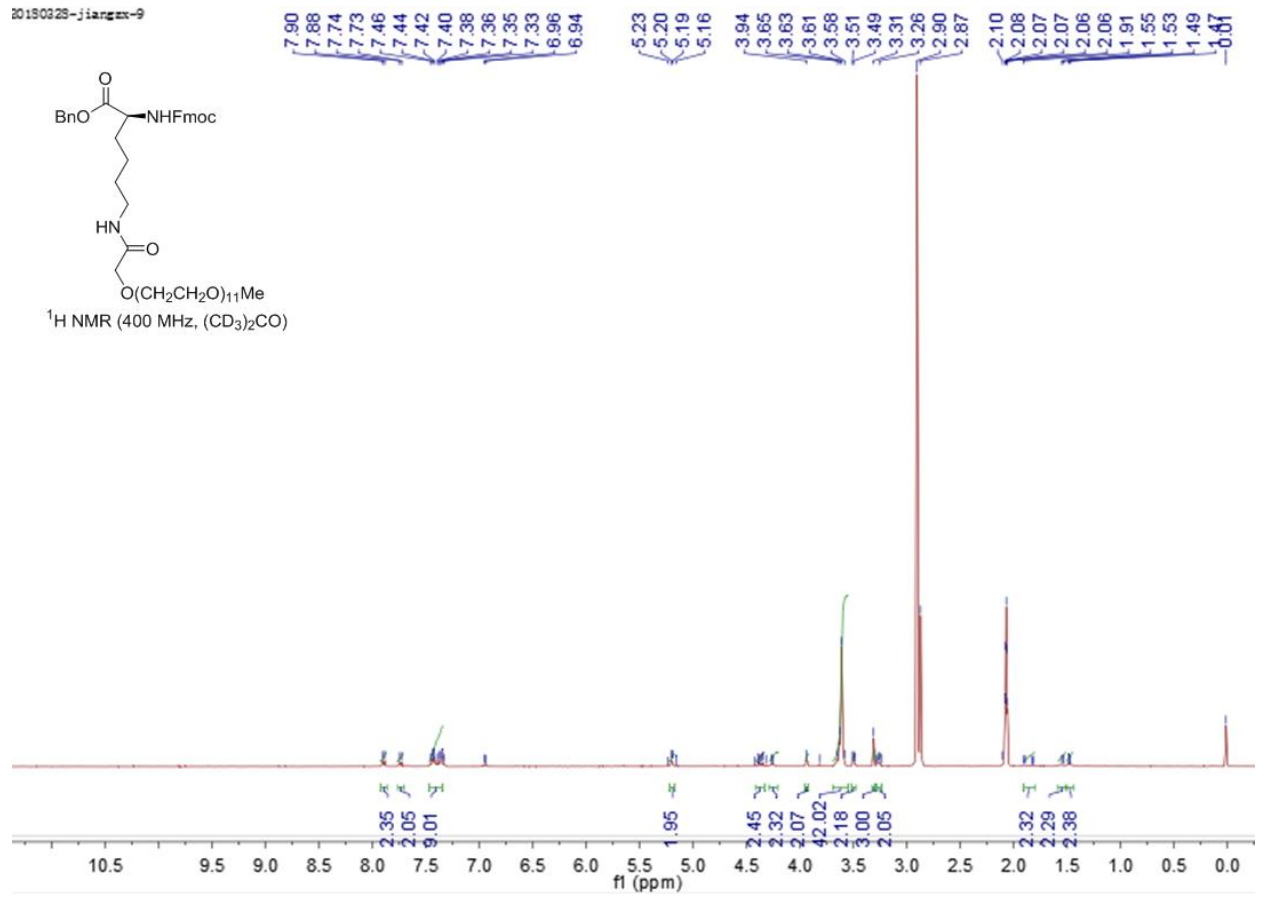
+MS, 0.3min #19





^{1}H NMR (400 MHz, $CDCl_3$)




20190328-jiangzx-9
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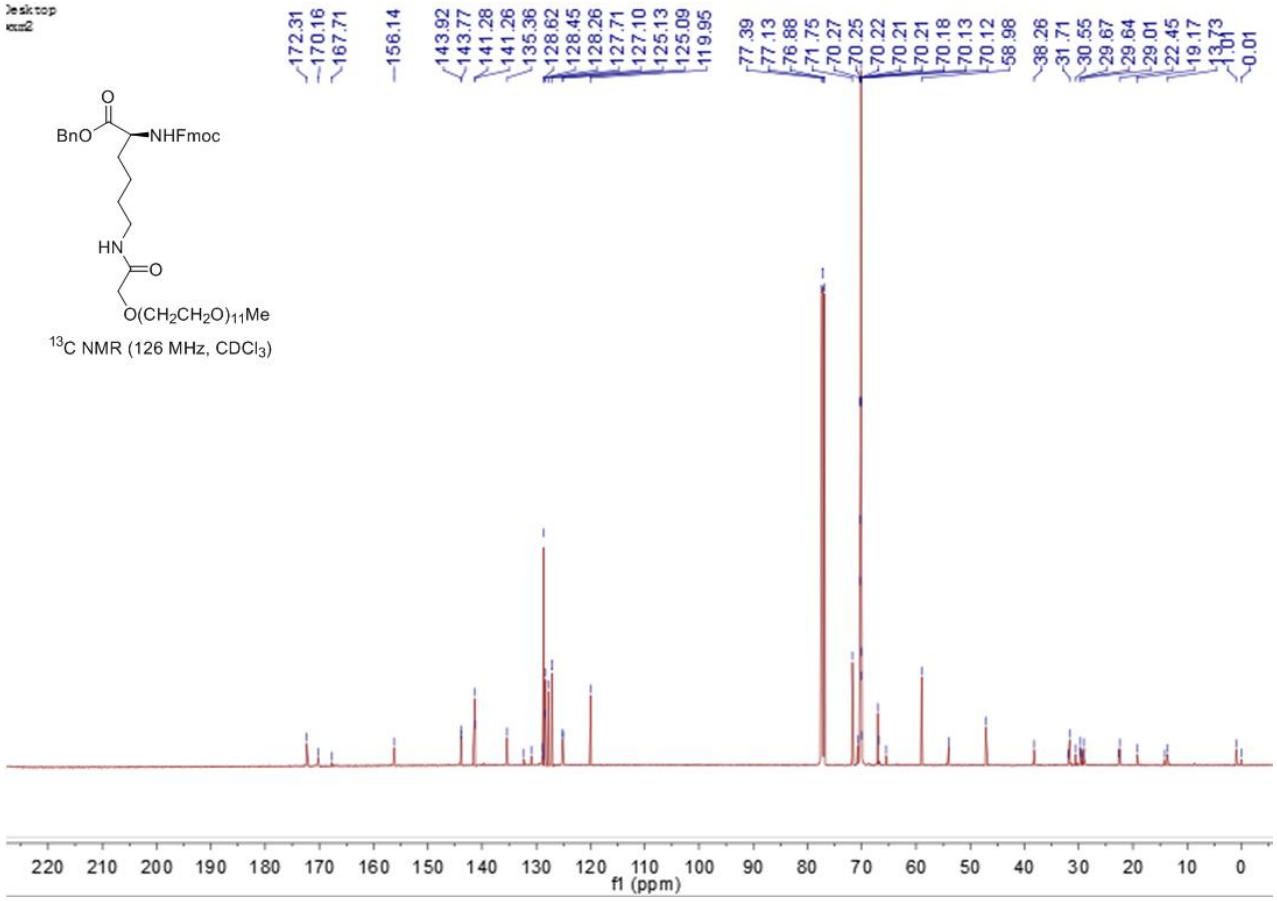
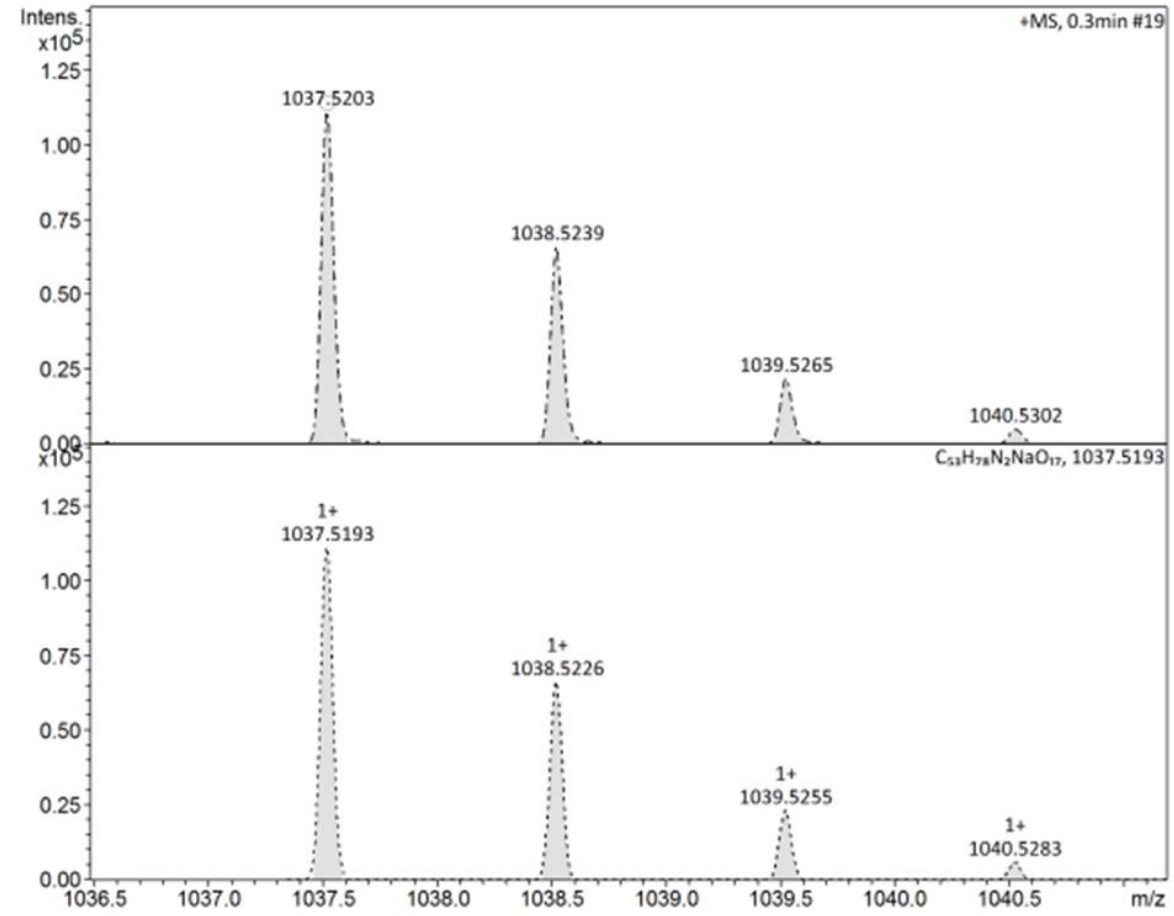
HN
O
O(CH2CH2O)11Me
1H NMR (400 MHz, (CD3)2CO)
f1 (ppm)


BnO
NHFmoc
HN
O
O(CH2CH2O)11Me
13C NMR (126 MHz, CDCl3)
f1 (ppm)

+MS, 0.3min #19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x10^5
+MS, 0.3min #19
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1038.5239
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1040.5302
C53H78N2NaO17, 1037.5193
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1037.5193
1+
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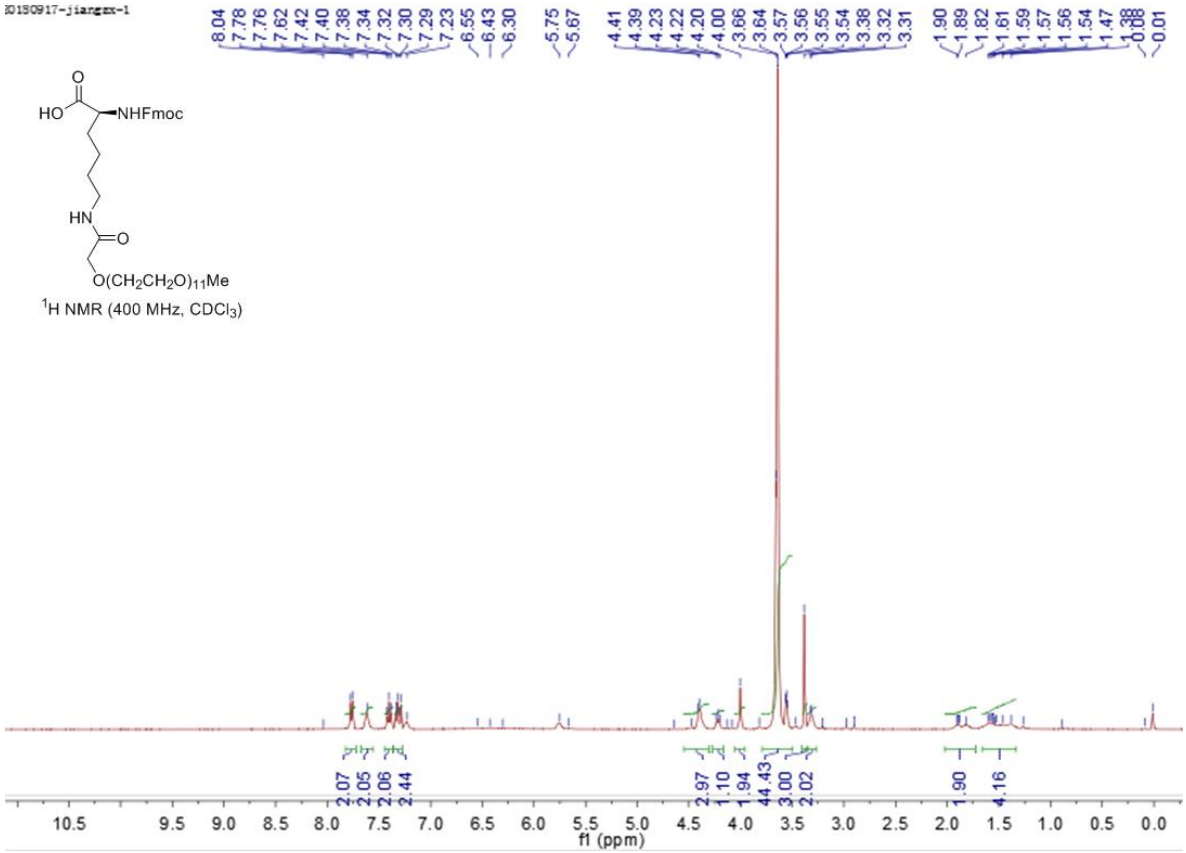
1+
1039.5255
1+
1040.5283
m/z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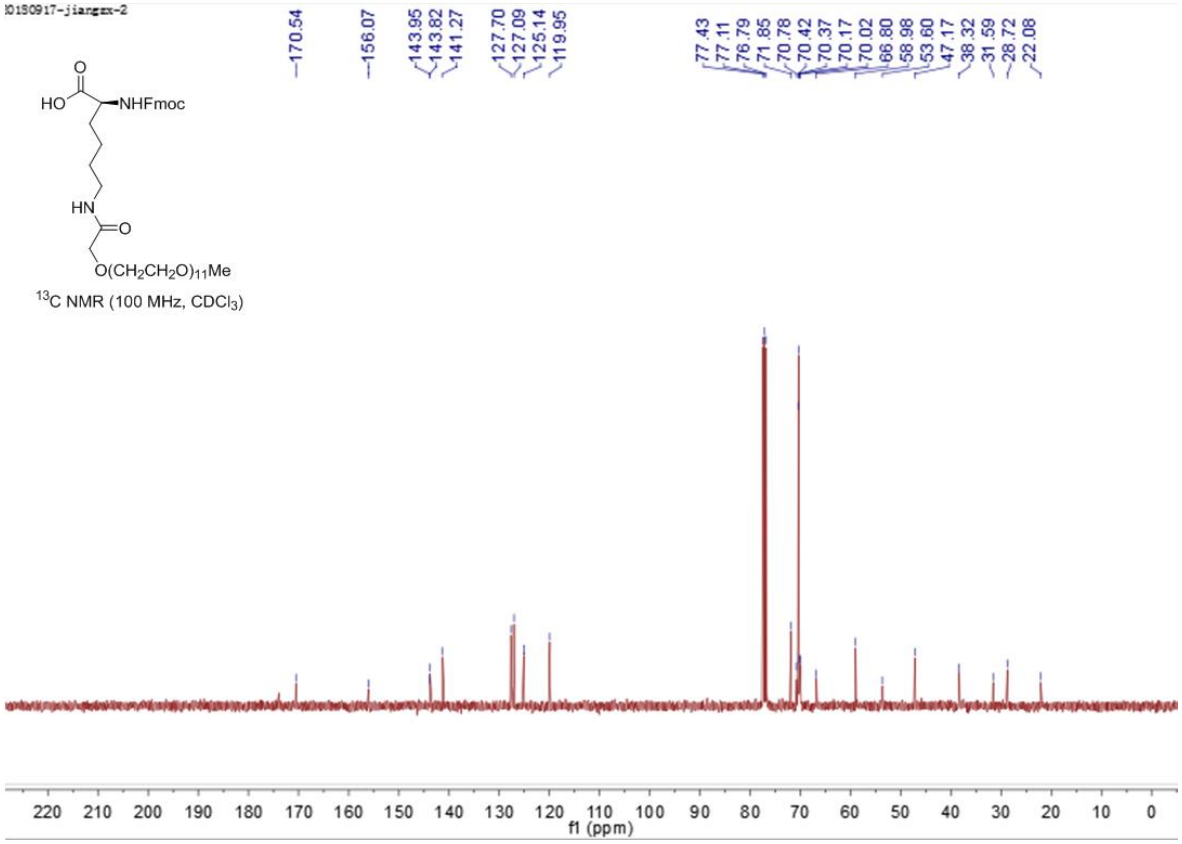
HN
O
O(CH2CH2O)11Me
1H NMR (400 MHz, CDCl3)
f1 (ppm)


10180917-jiangzx-2
170.54
156.07
143.95
143.82
141.27
127.70
127.09
125.14
119.95
77.43
77.11
76.79
71.85
70.78
70.42
70.37
70.17
70.02
66.80
58.98
53.60
47.17
38.32
31.59
28.72
22.08
HO
NHFmoc
HN
O
O(CH2CH2O)11Me
13C NMR (100 MHz, CDCl3)
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

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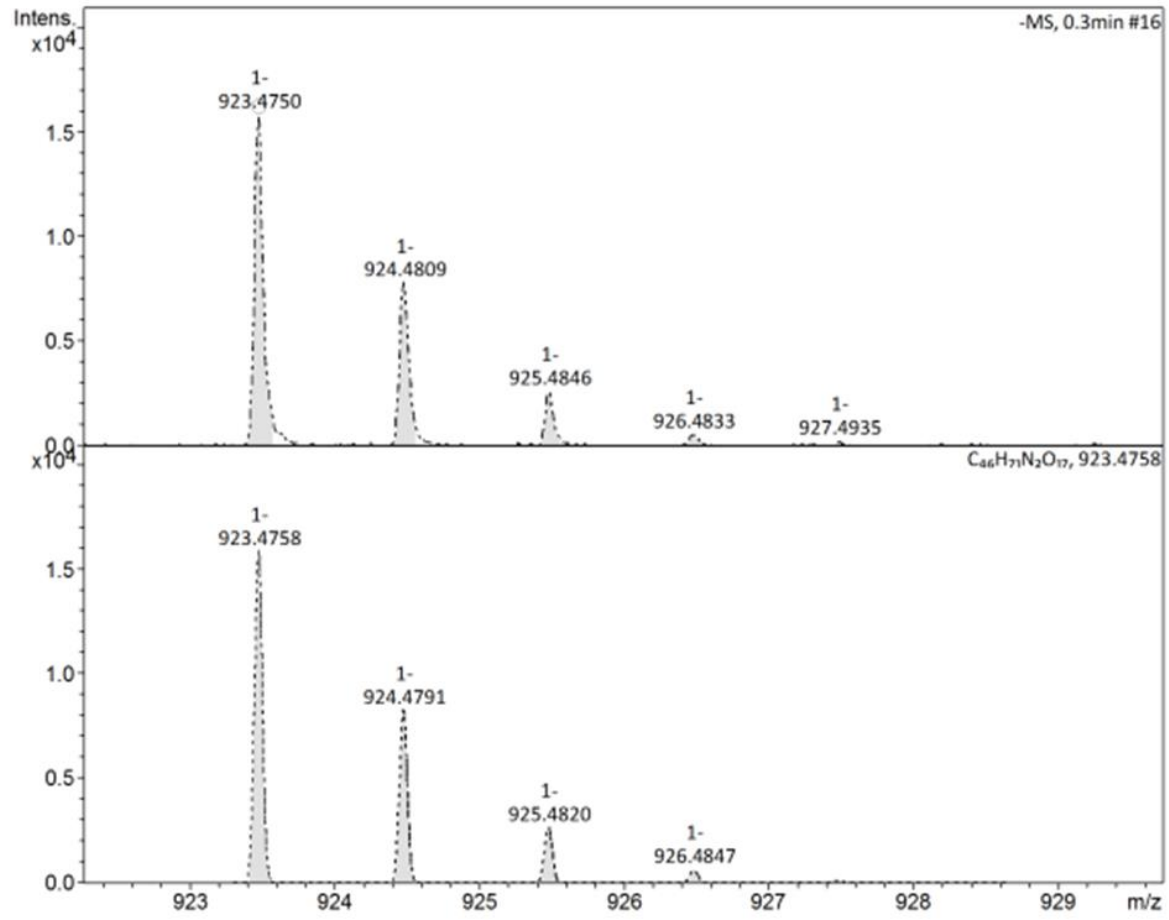
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1-
924.4809
1-
925.4846
1-
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1-
927.4935
C46H71N2O17, 923.4758
1-
923.4758
1-
924.4791
1-
925.4820
1-
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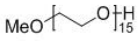
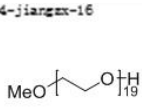
923 924 925 926 927 928 929 m/z

20150323-jiangzx-24

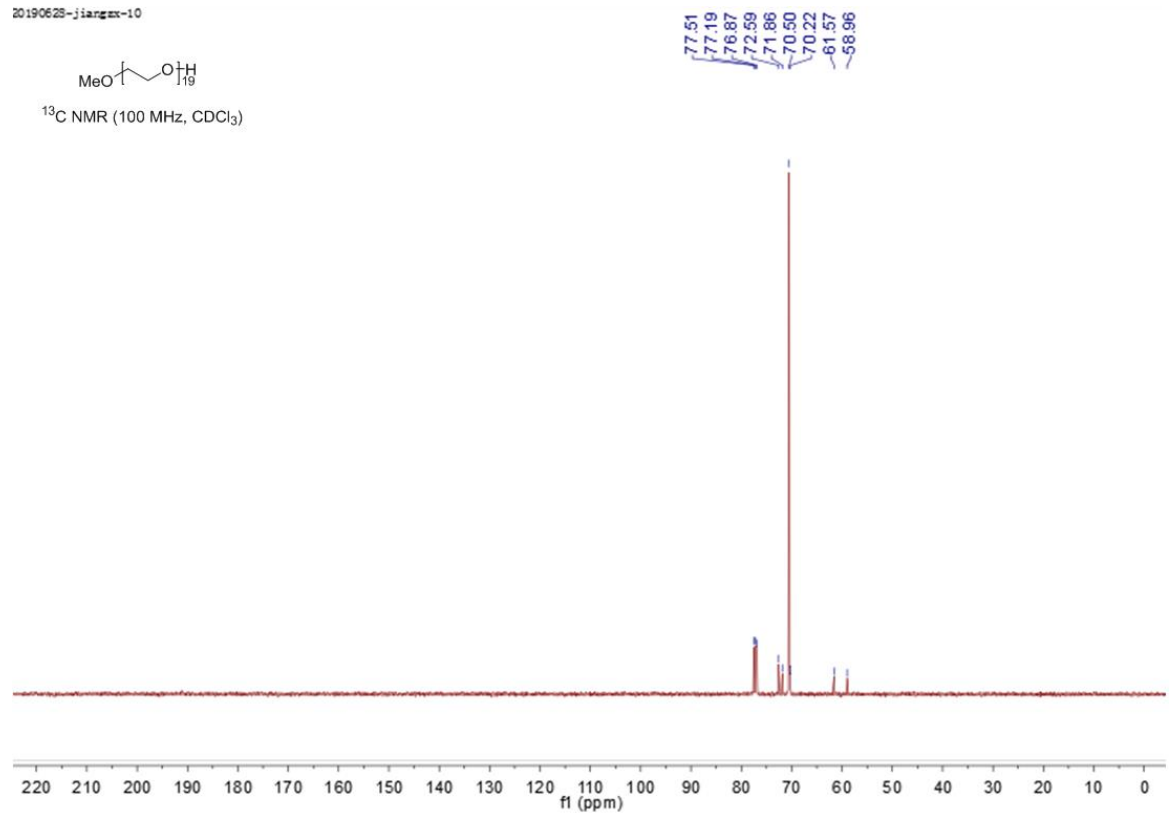


^{1}H NMR (400 MHz, $CDCl_3$)

20150404-jiangzx-16



^{1}H NMR (400 MHz, $CDCl_3$)


20190628-jiangsx-10
MeO
OH
19
13C NMR (100 MHz, CDCl3)
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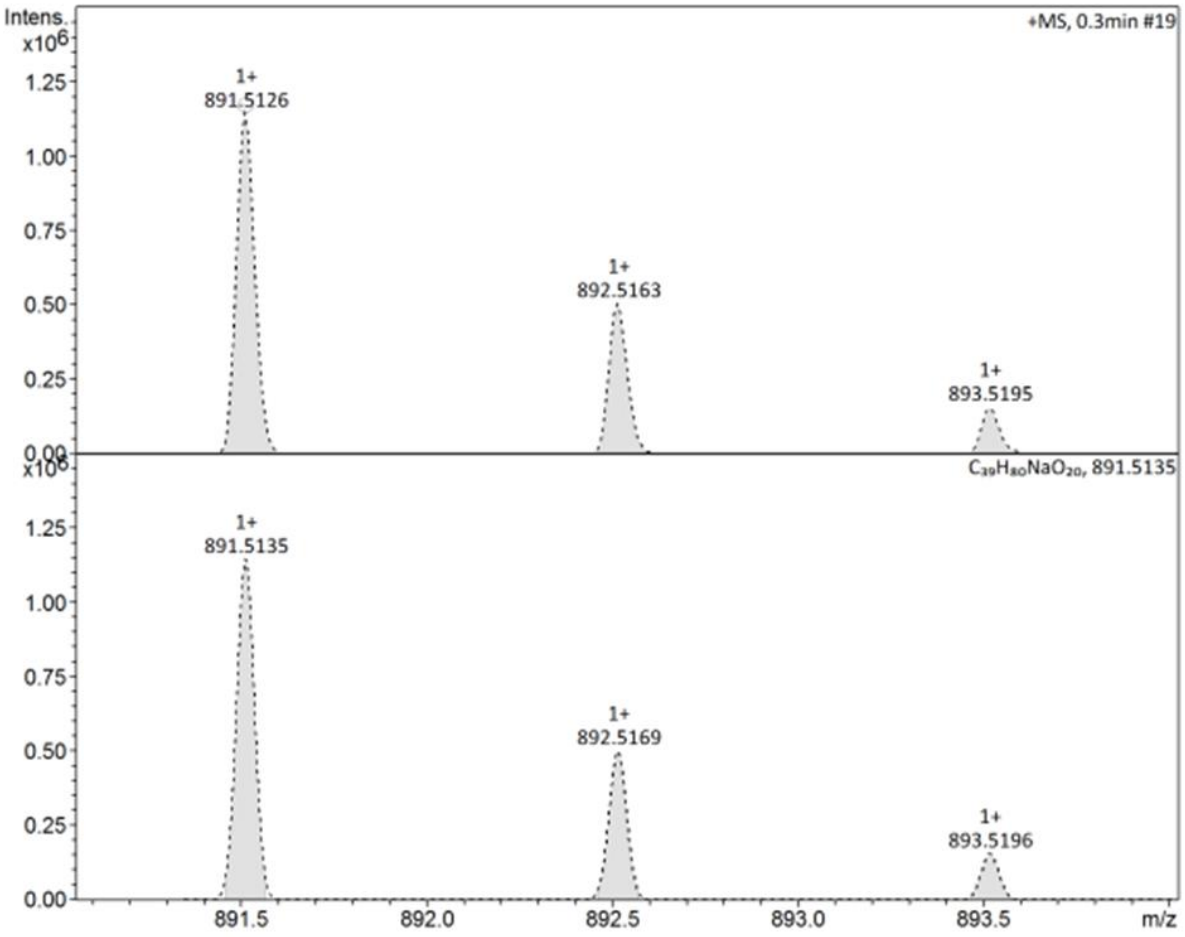
77.19
76.87
72.59
71.86
70.50
70.22
61.57
58.96
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

+MS, 0.3min #19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0.75
0.50
0.25
0.00
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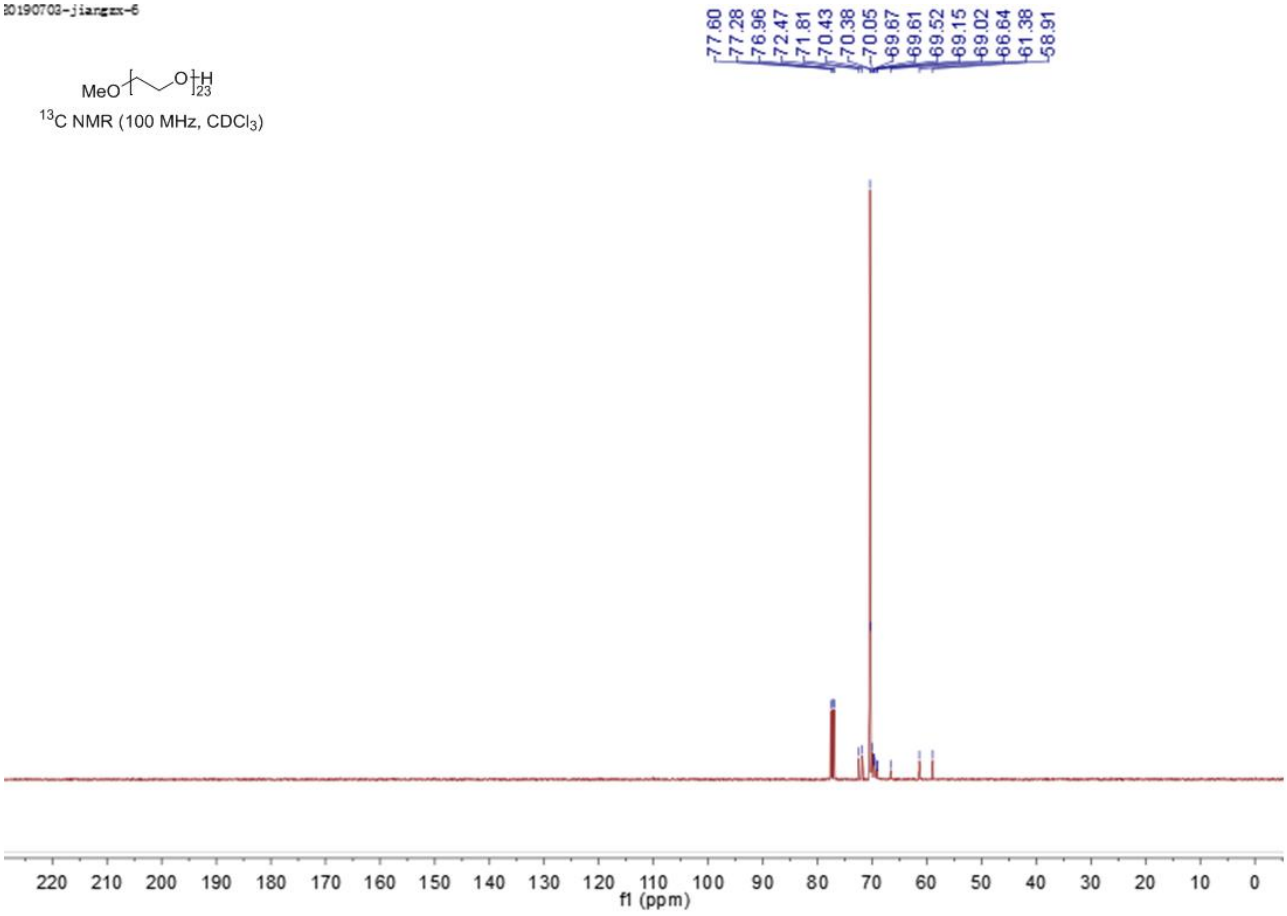
1+
892.5163
1+
893.5195
C39H80NaO20, 891.5135
1+
891.5135
1+
892.5169
1+
893.5196
891.5
892.0
892.5
893.0
893.5
m/z

20180404-jiangzx-40

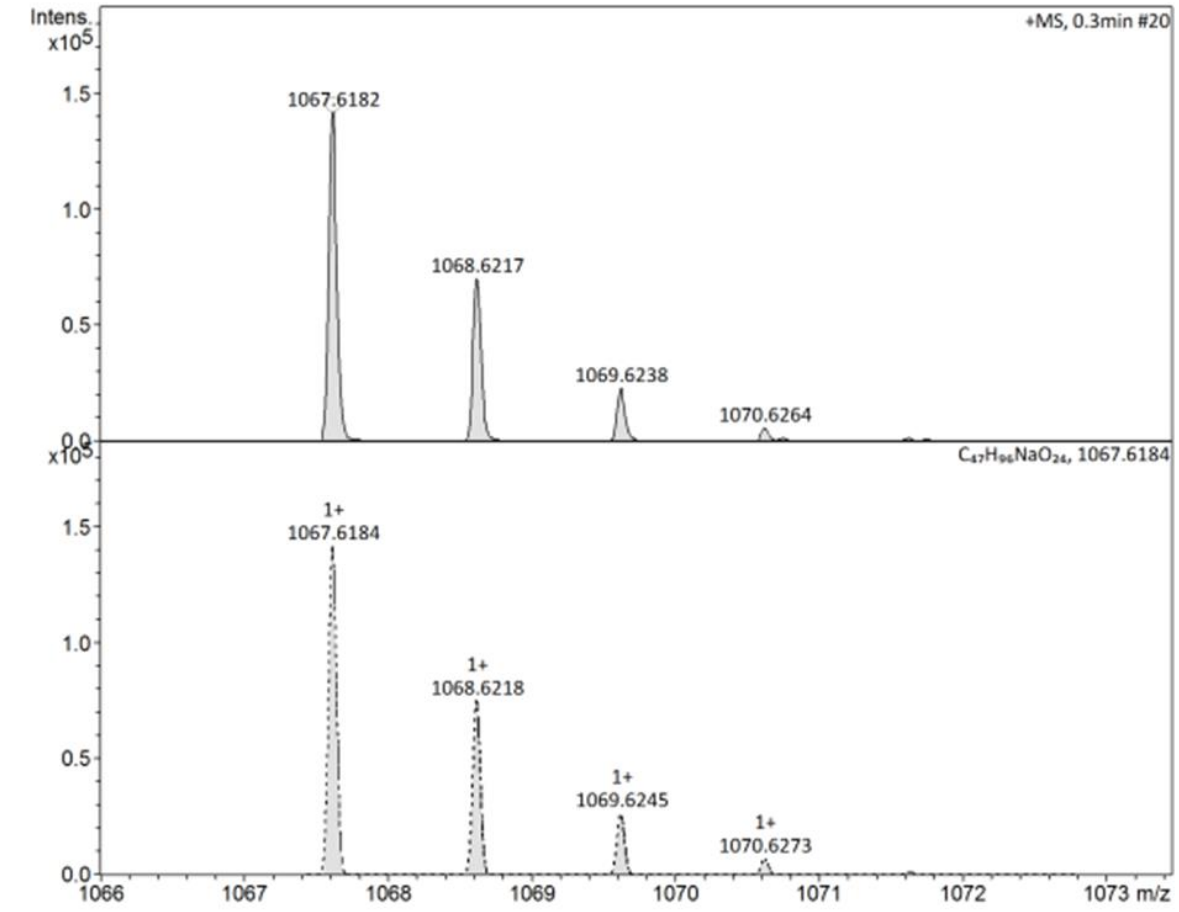
^{1}H NMR (400 MHz, $CDCl_3$)

20190703-jiangzx-6

^{13}C NMR (100 MHz, $CDCl_3$)



+MS, 0.3min #20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1070.6264
C47H96NaO24, 1067.6184
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1067.6184
1+
1068.6218
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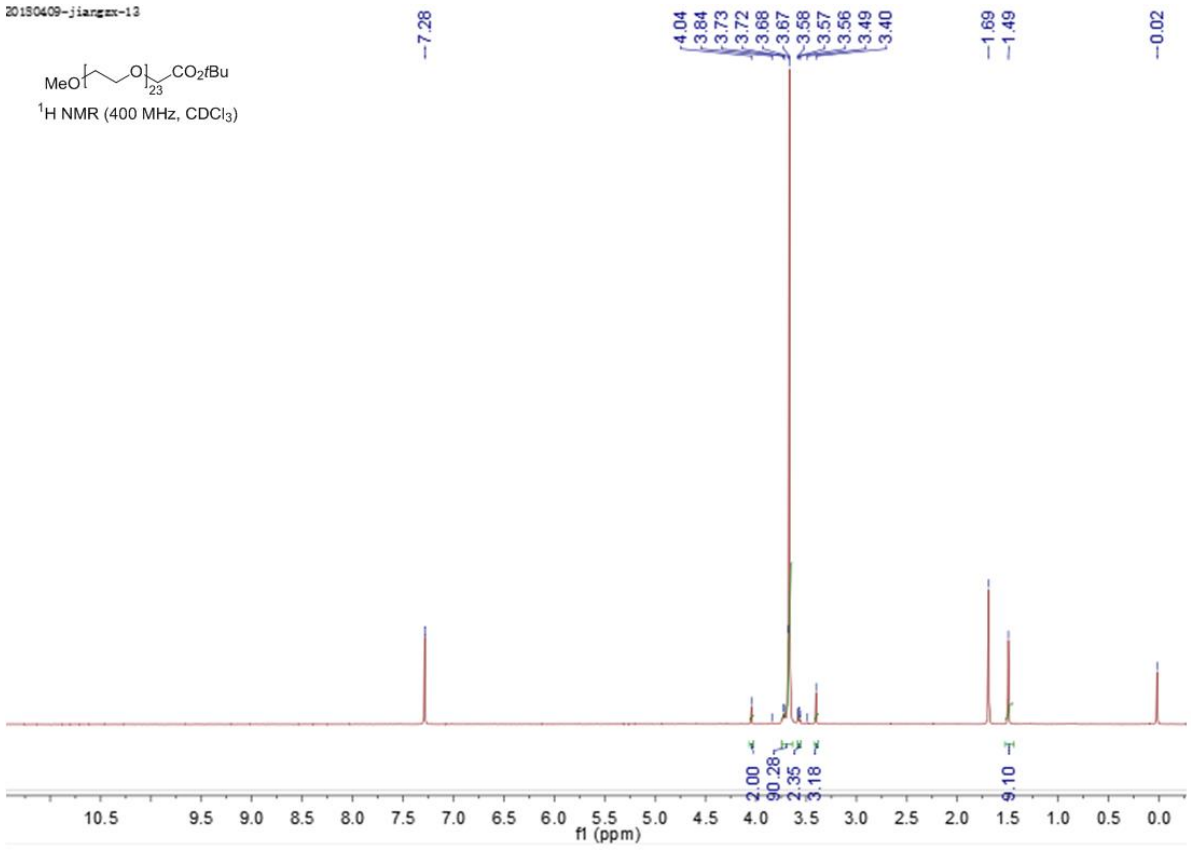
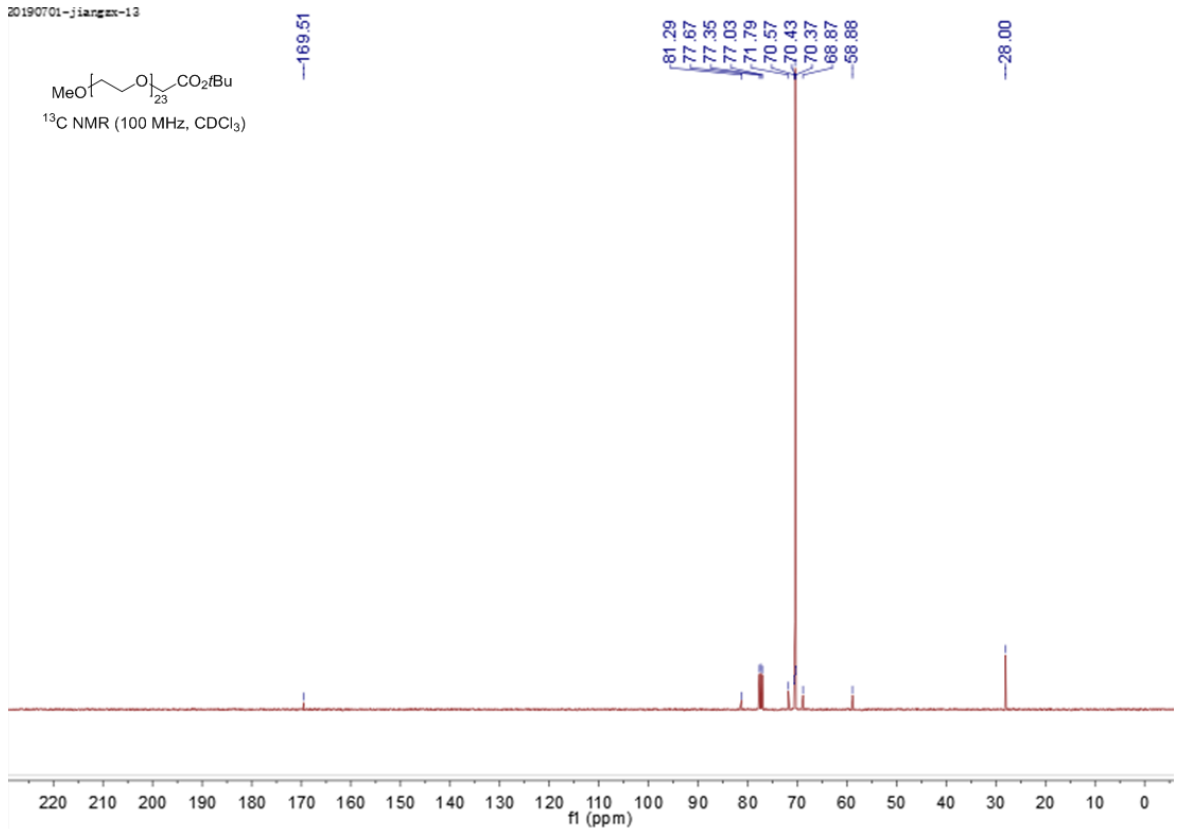
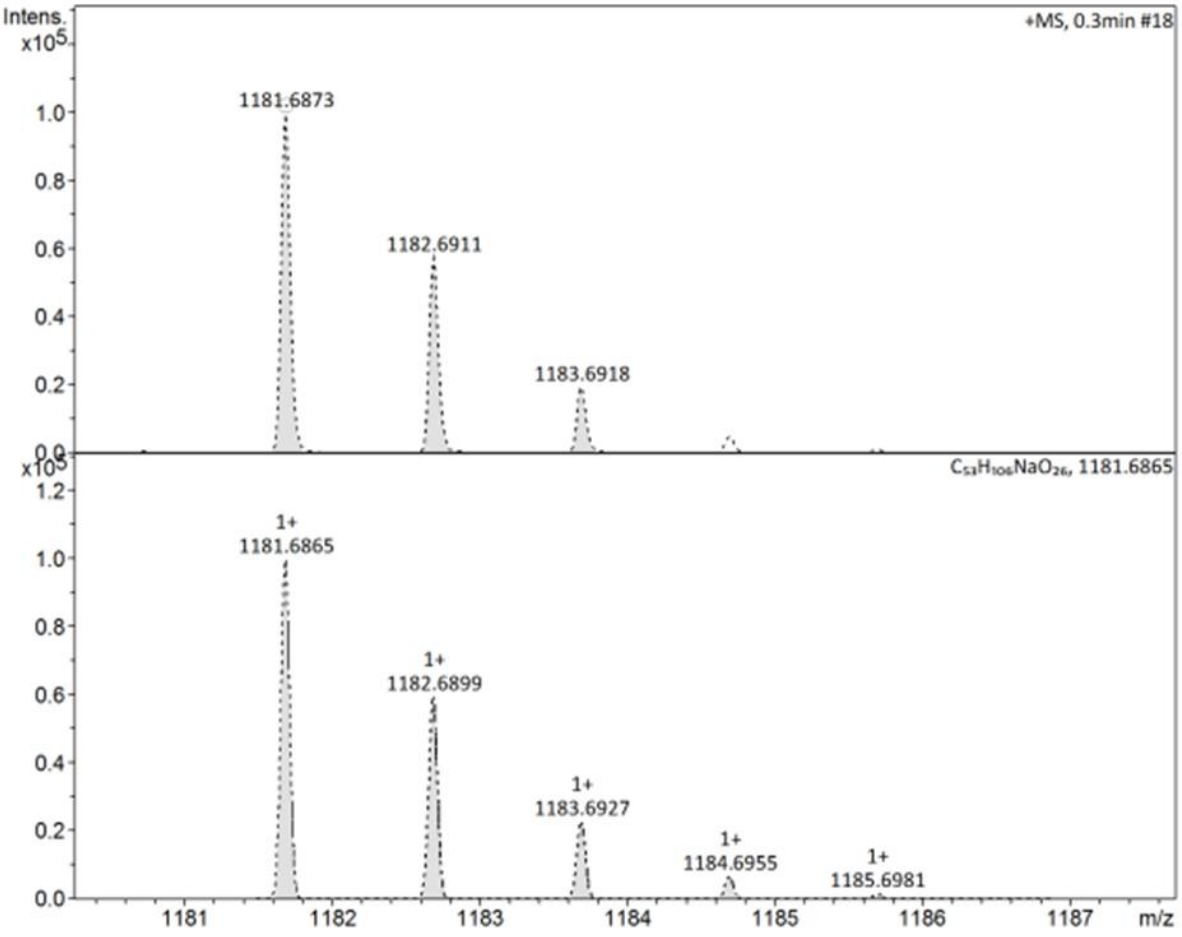
1068
1069
1070
1071
1072
1073 m/z

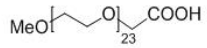

20150409-jiangzx-13
MeO
O
23
CO2tBu
1H NMR (400 MHz, CDCl3)
7.28
4.04
3.84
3.73
3.72
3.68
3.67
3.58
3.57
3.56
3.49
3.40
1.69
1.49
0.02
2.00
90.28
2.35
3.18
9.10
f1 (ppm)


20190701-jiangzx-13
MeO
O
23
CO2tBu
13C NMR (100 MHz, CDCl3)
169.51
81.29
77.67
77.35
77.03
71.79
70.57
70.43
70.37
68.87
58.88
28.00
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
f1 (ppm)

+MS, 0.3min #18

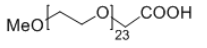

Intens.
x10^5
+MS, 0.3min #18
1181.6873
1182.6911
1183.6918
C53H106NaO26, 1181.6865
1+
1181.6865
1+
1182.6899
1+
1183.6927
1+
1184.6955
1+
1185.6981
1181 1182 1183 1184 1185 1186 1187 m/z

20190708-jiangzx-5



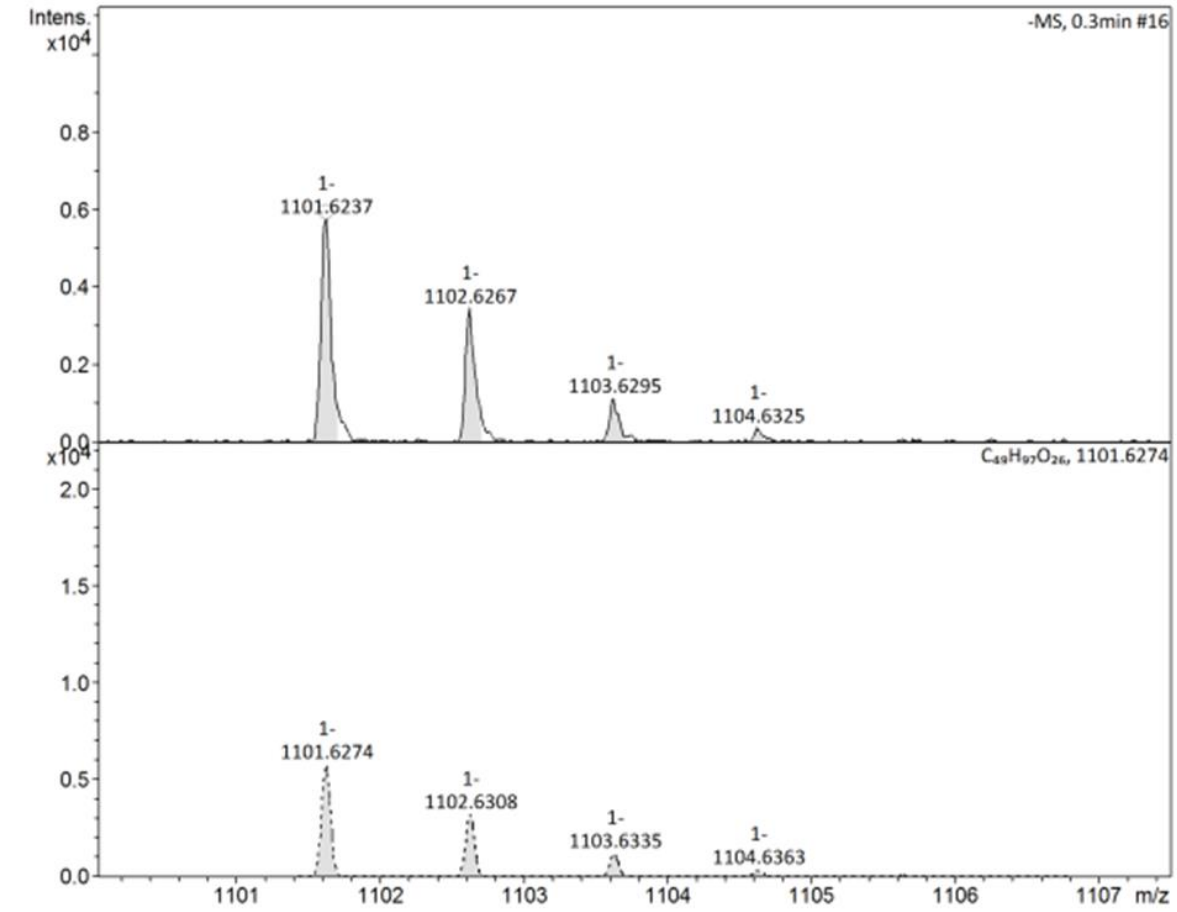
^{1}H NMR (400 MHz, $(CD_3)_2CO$)

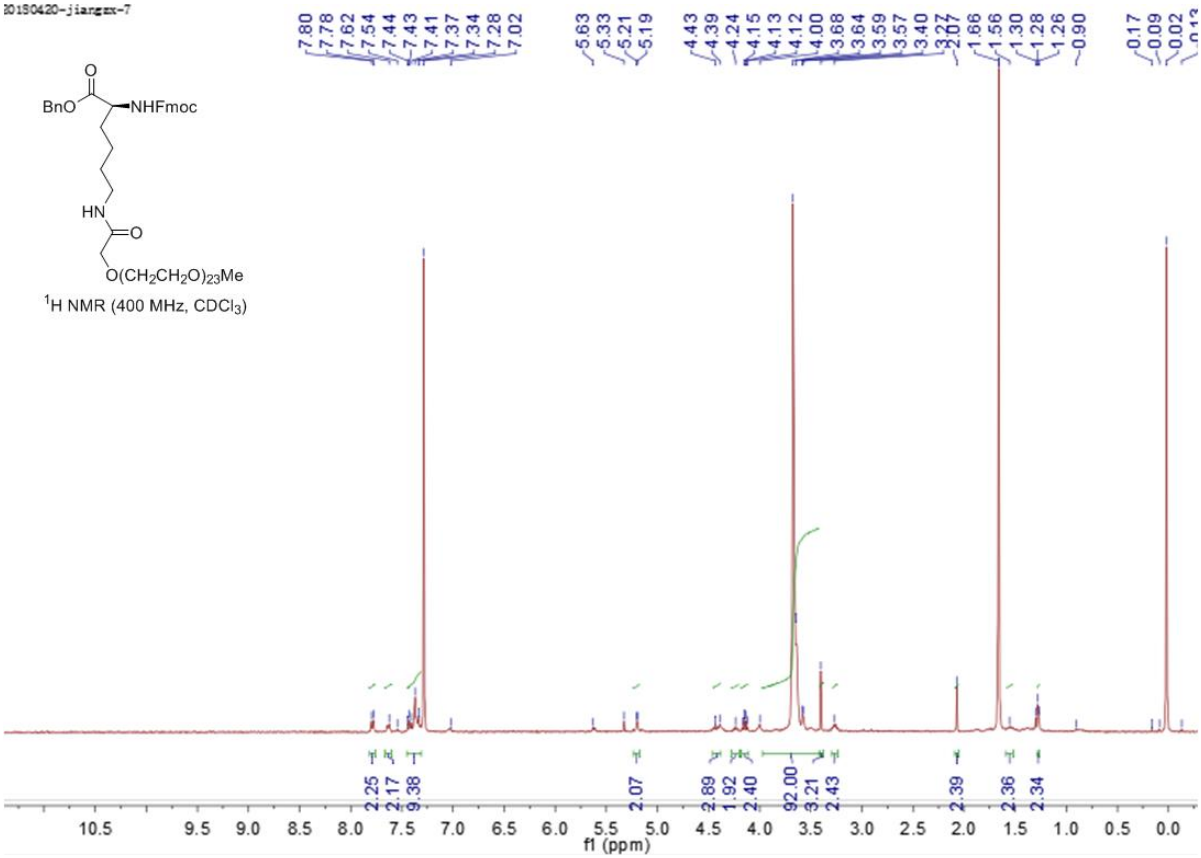
20190701-jiangzx-14

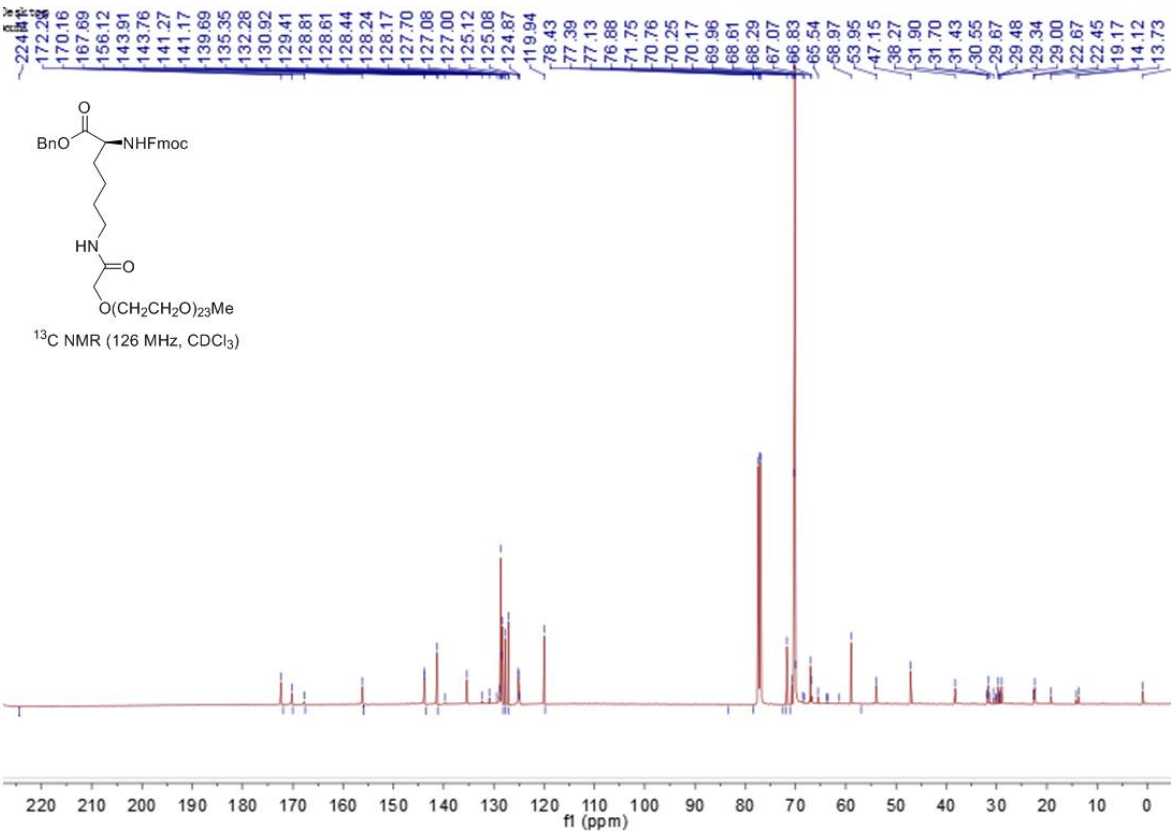


^{13}C NMR (100 MHz, $CDCl_3$)

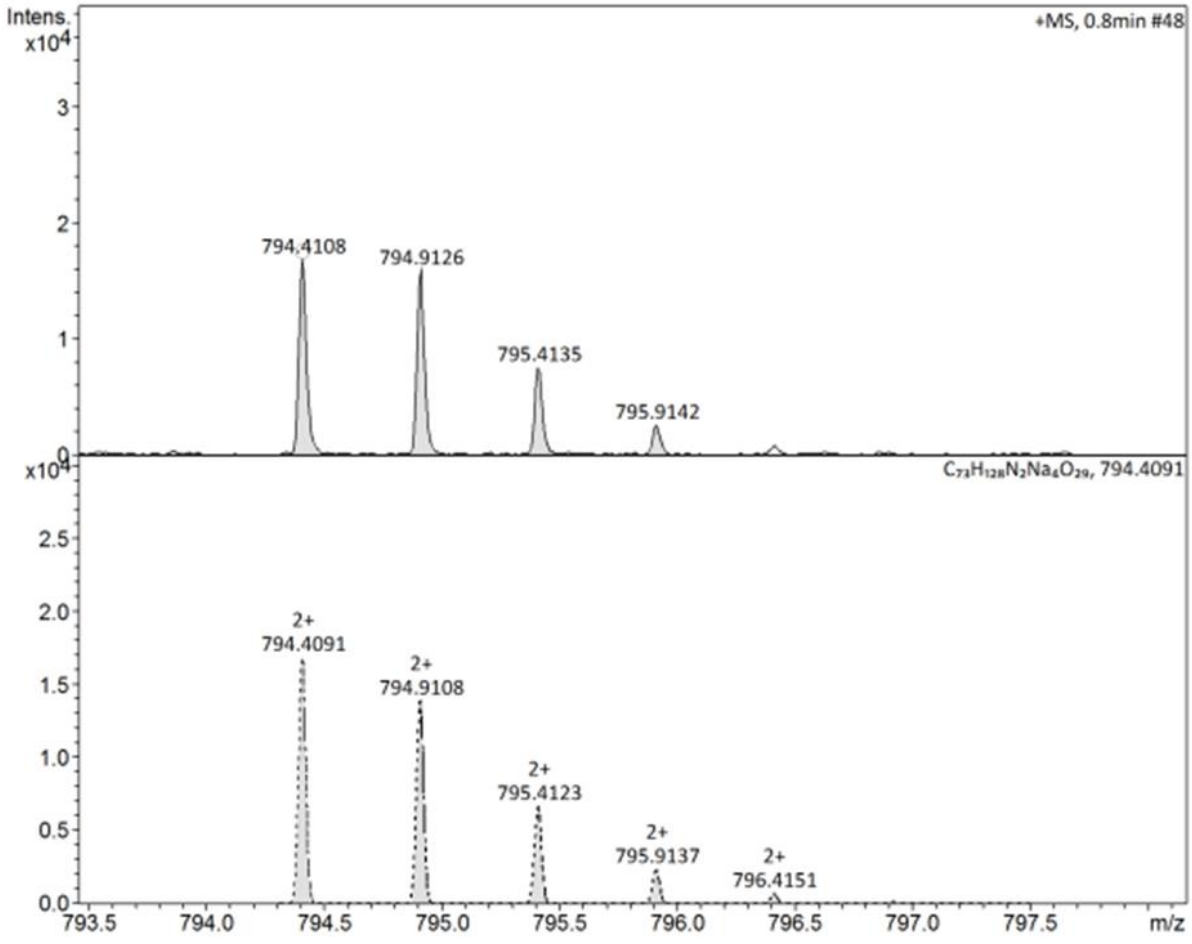
-MS, 0.3min #16

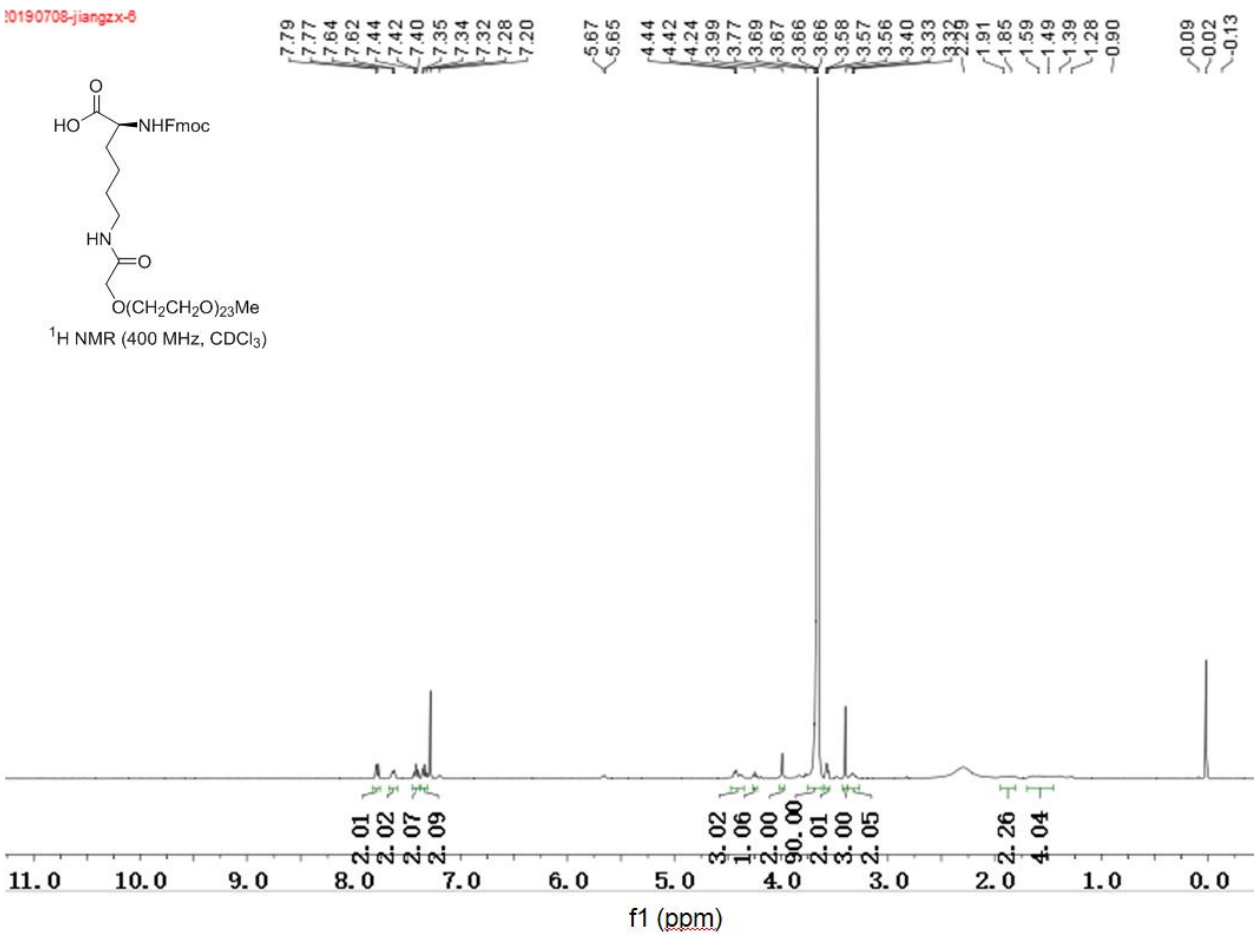

Intens.
x10^4
-MS, 0.3min #16
1-
1101.6237
1-
1102.6267
1-
1103.6295
1-
1104.6325
C49H97O26, 1101.6274
1-
1101.6274
1-
1102.6308
1-
1103.6335
1-
1104.6363
m/z

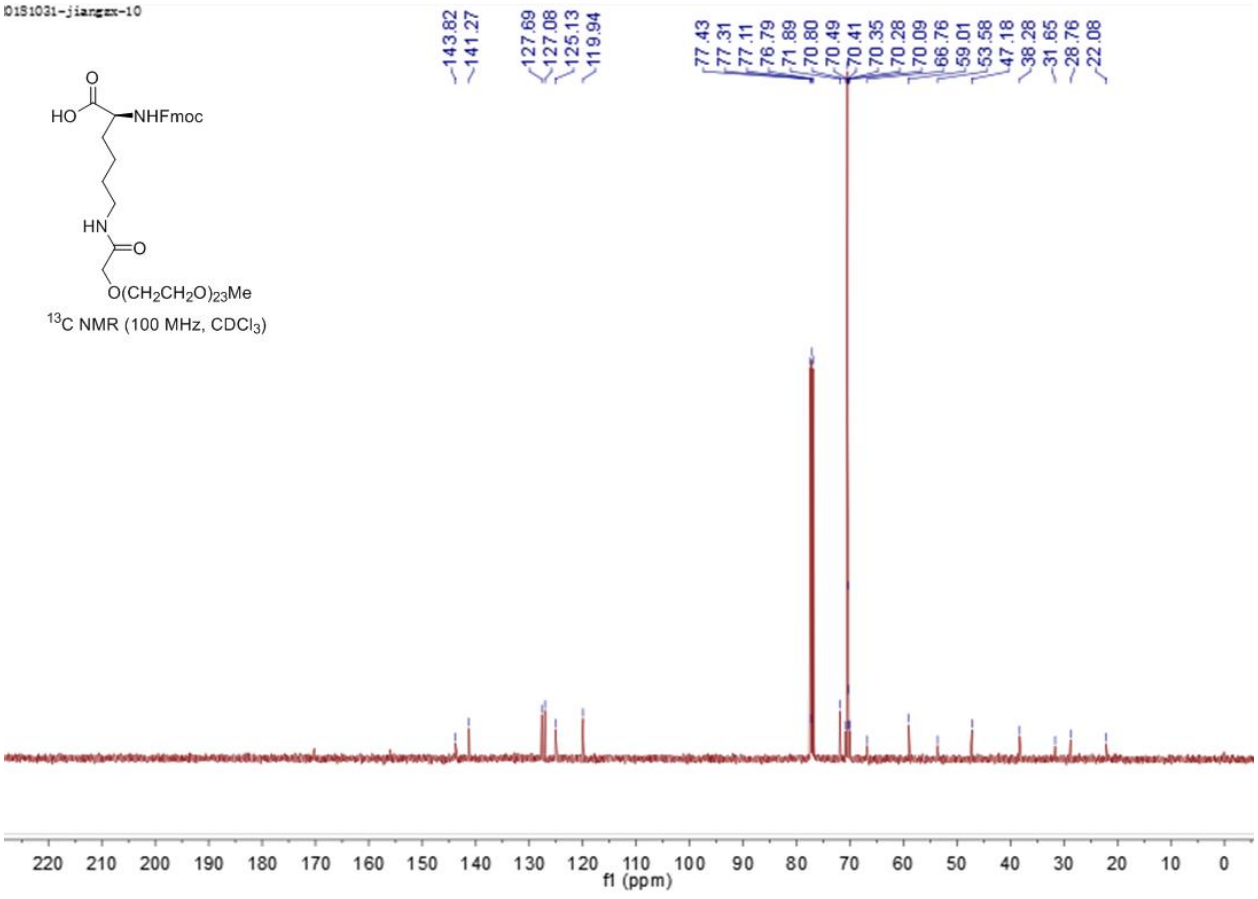

20150420-jiangzx-7
BnO
NHFmoc
HN
O
O(CH2CH2O)23Me
1H NMR (400 MHz, CDCl3)
f1 (ppm)


BnO
NHFmoc
HN
O
O(CH2CH2O)23Me
13C NMR (126 MHz, CDCl3)
f1 (ppm)

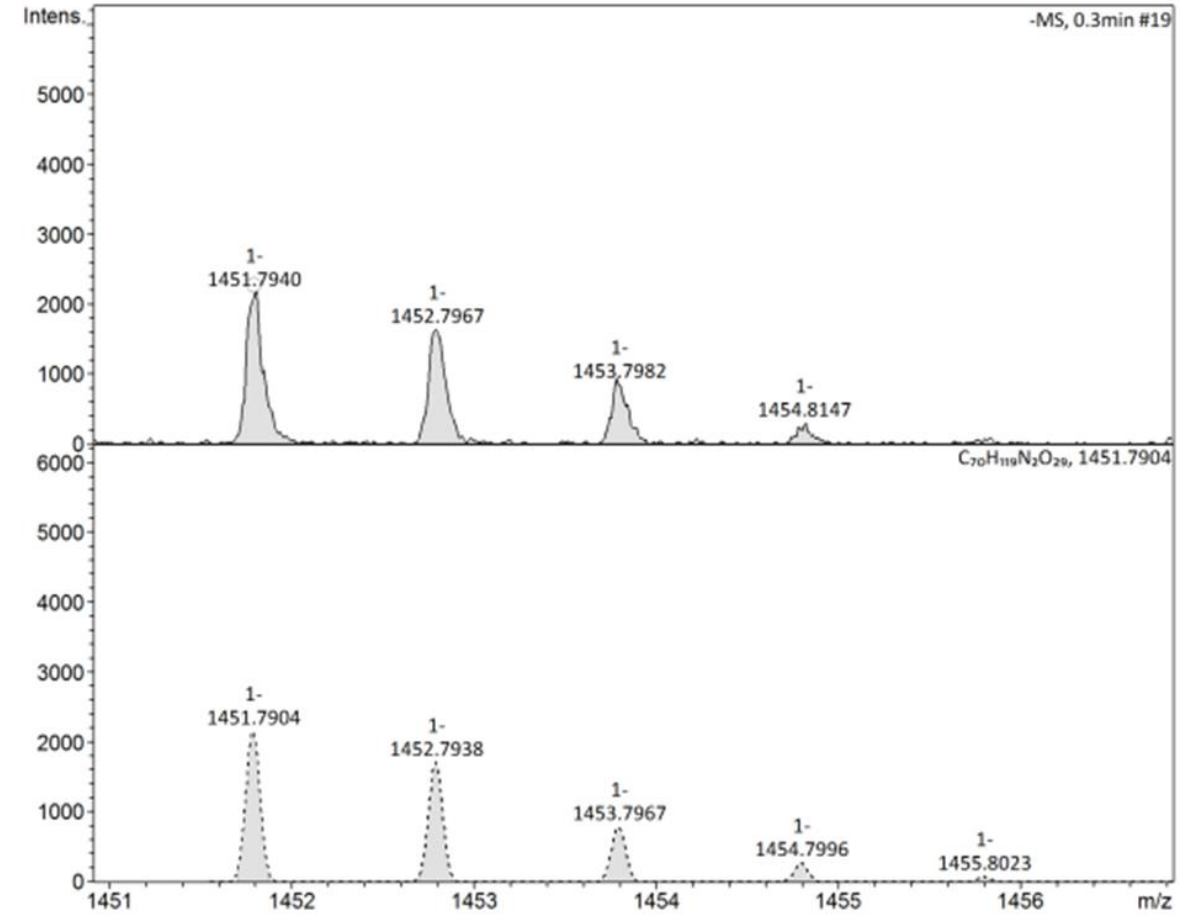
+MS, 0.8min #48

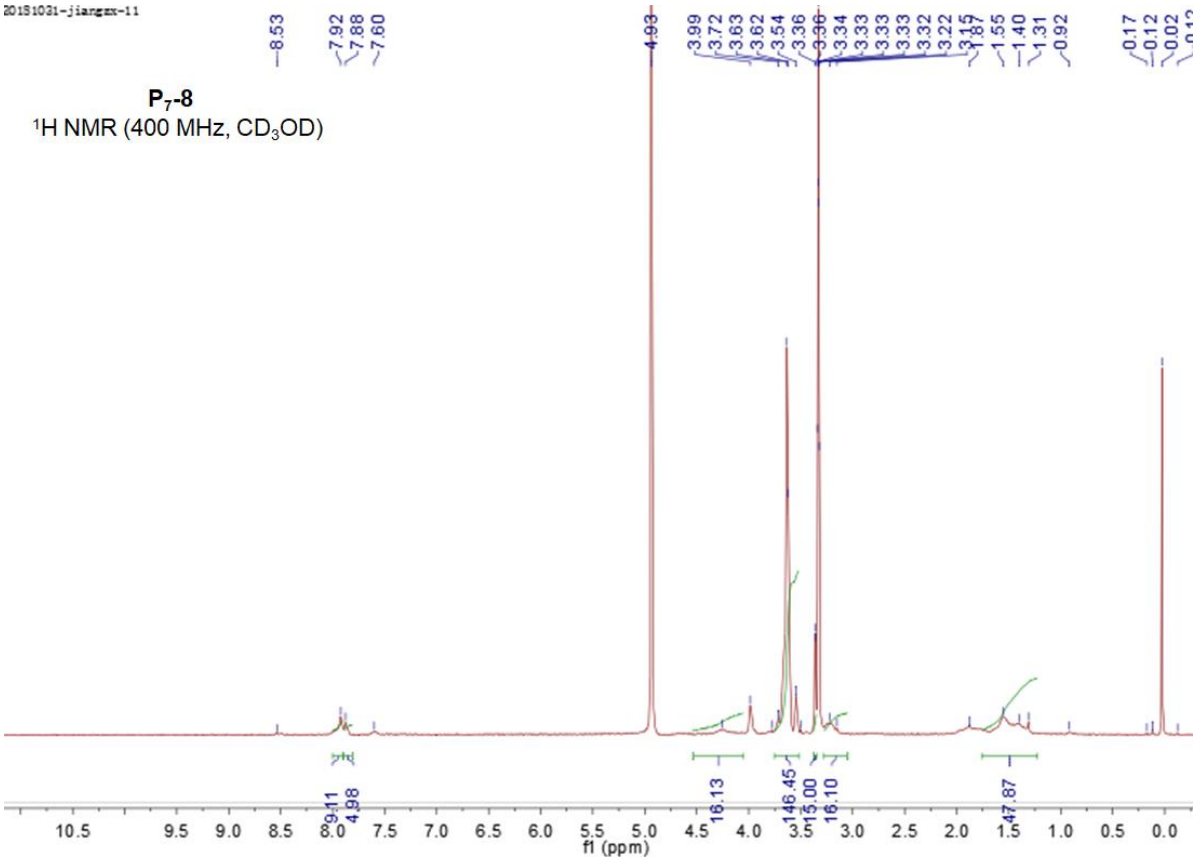

Intens.
x10^4
+MS, 0.8min #48
794.4108
794.9126
795.4135
795.9142
C73H128N2Na4O29, 794.4091
2+
794.4091
2+
794.9108
2+
795.4123
2+
795.9137
2+
796.4151
m/z


0190708-jiangzx-6
7.79
7.77
7.64
7.62
7.44
7.42
7.40
7.35
7.34
7.32
7.28
7.20
5.67
5.65
4.44
4.42
4.24
3.99
3.77
3.69
3.67
3.66
3.58
3.57
3.56
3.40
3.33
3.32
2.29
1.91
1.85
1.59
1.49
1.39
1.28
0.90
0.09
0.02
-0.13
HO
NHFmoc
HN
O
O(CH2CH2O)23Me
1H NMR (400 MHz, CDCl3)
2.01
2.02
2.07
2.09
3.02
1.06
2.00
90.00
2.01
3.00
2.05
2.26
4.04
11.0
10.0
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
f1 (ppm)


0151031-jiangzx-10
143.82
141.27
127.69
127.08
125.13
119.94
77.43
77.31
77.11
76.79
71.89
70.80
70.49
70.41
70.35
70.28
70.09
66.76
59.01
53.58
47.18
38.28
31.65
28.76
22.08
HO
NHFmoc
HN
O
O(CH2CH2O)23Me
13C NMR (100 MHz, CDCl3)
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

-MS, 0.3min #19





P_7-8

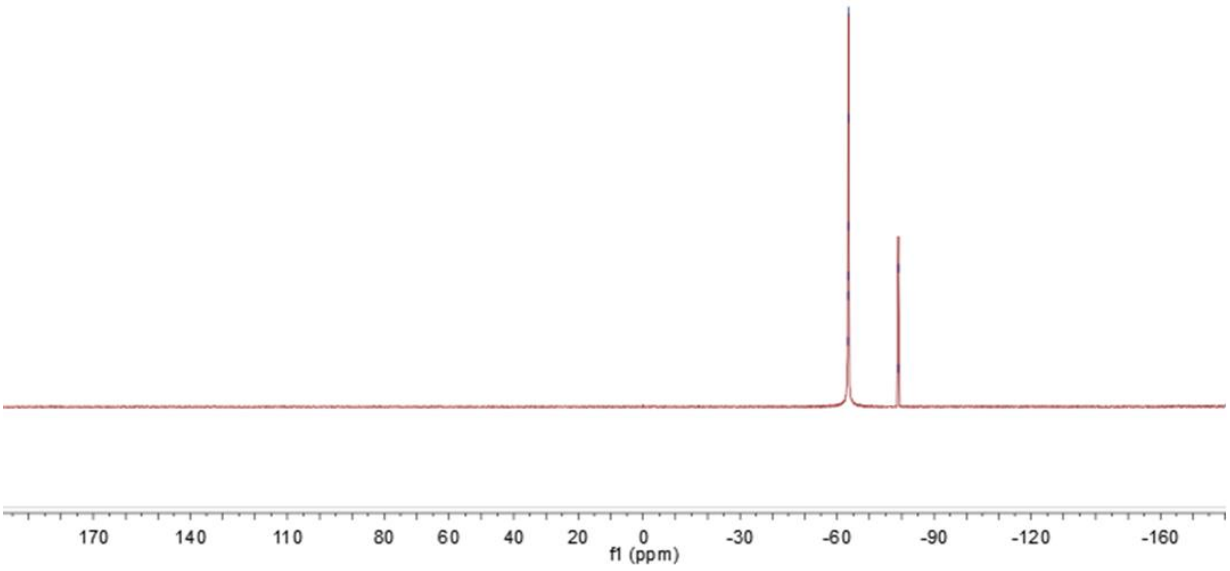
^{1}H NMR (400 MHz, CD_3OD)

20151210-jiangzx-5

-63.33
-63.40
-63.41
-63.43
-63.48
-63.51
-76.32
-78.96
-78.97

P_7-8

^{19}F NMR (376 MHz, D_2O)



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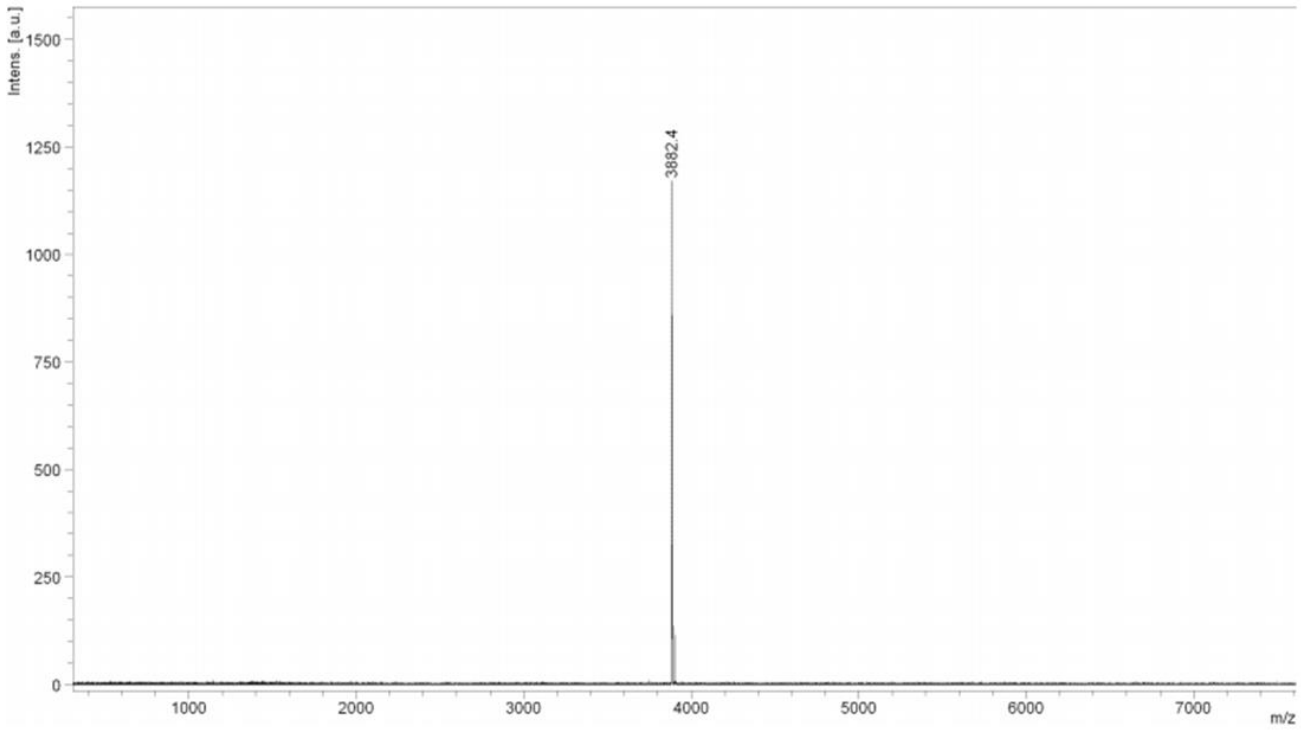
P_7-8

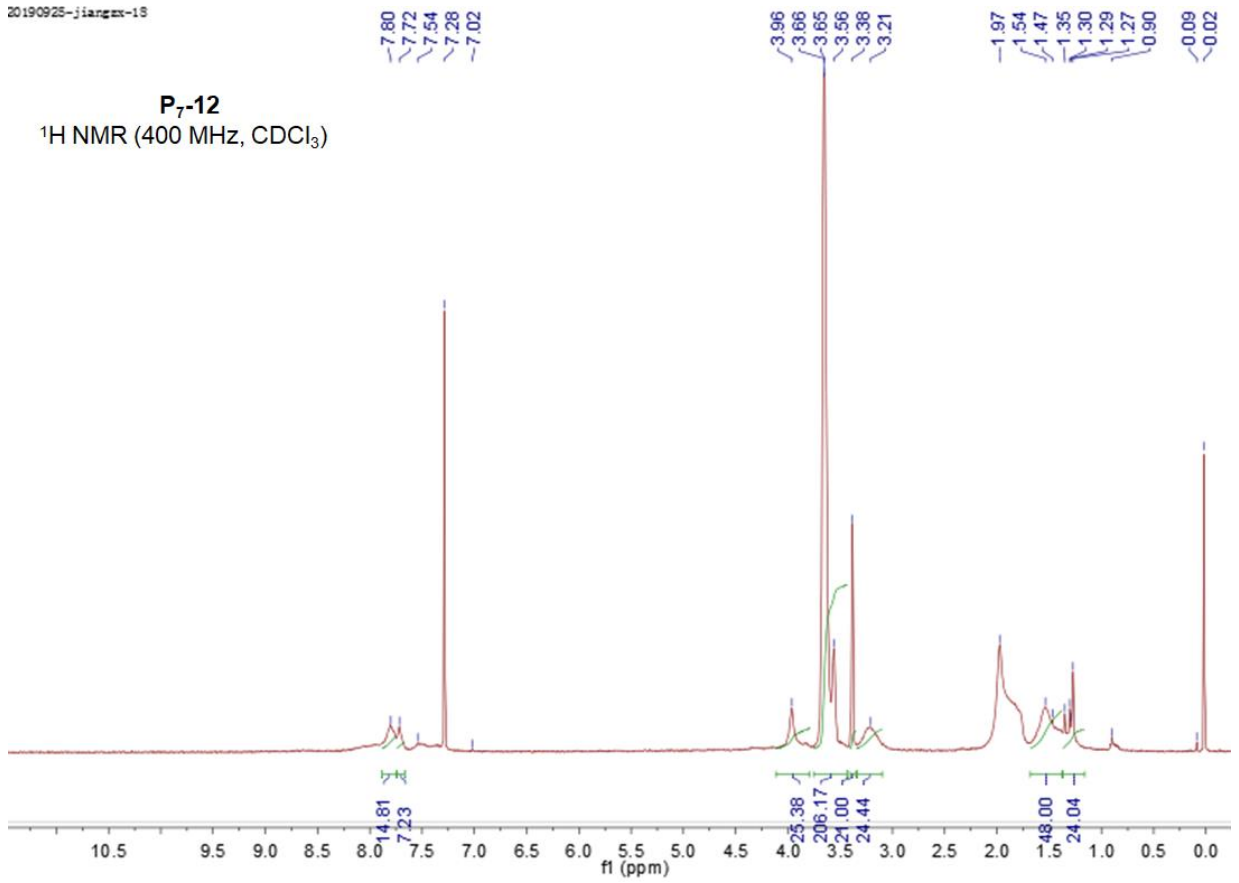
MS (MALDI)

MALDI-TOF,CCA,1,20180917



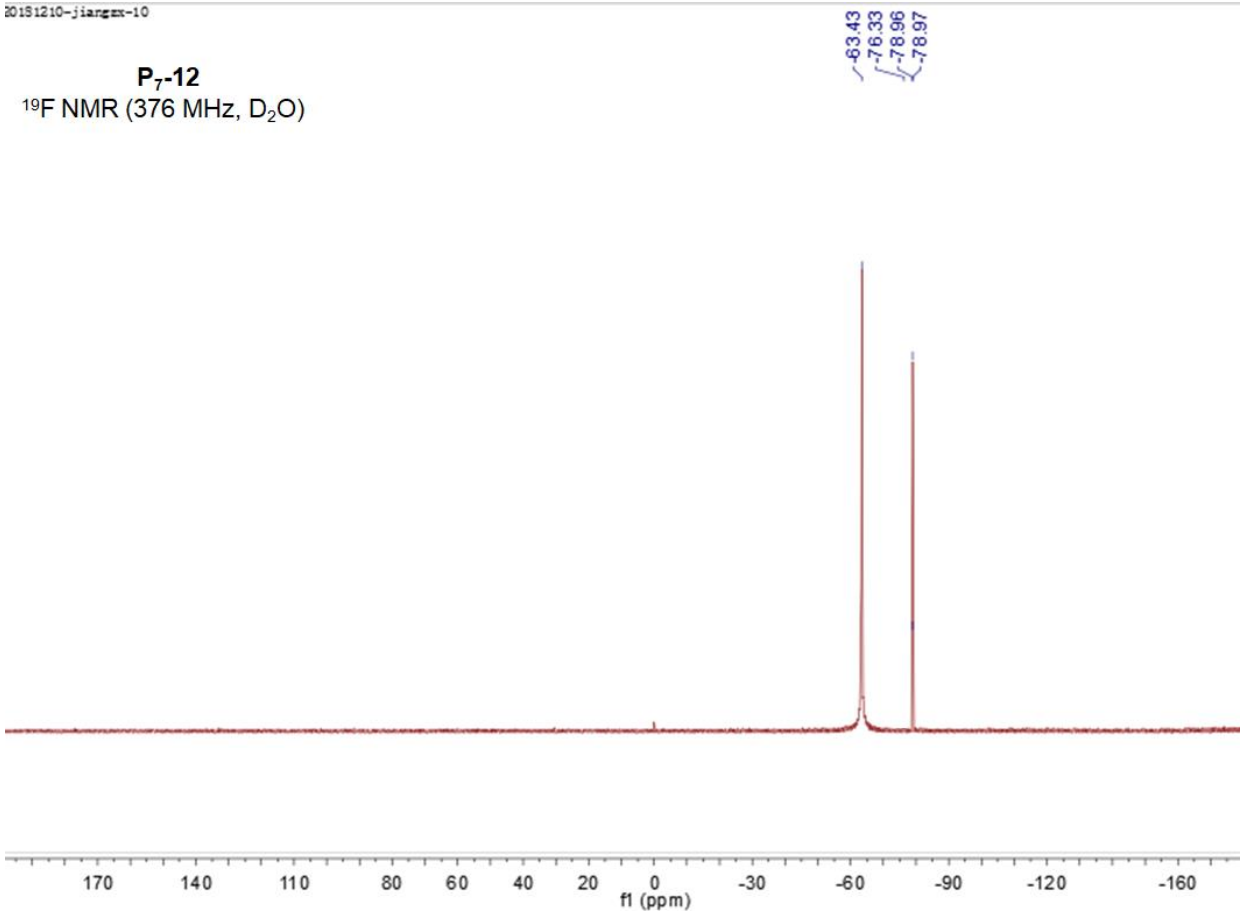
P_7-12

^{1}H NMR (400 MHz, $CDCl_3$)



P_7-12

^{19}F NMR (376 MHz, D_2O)



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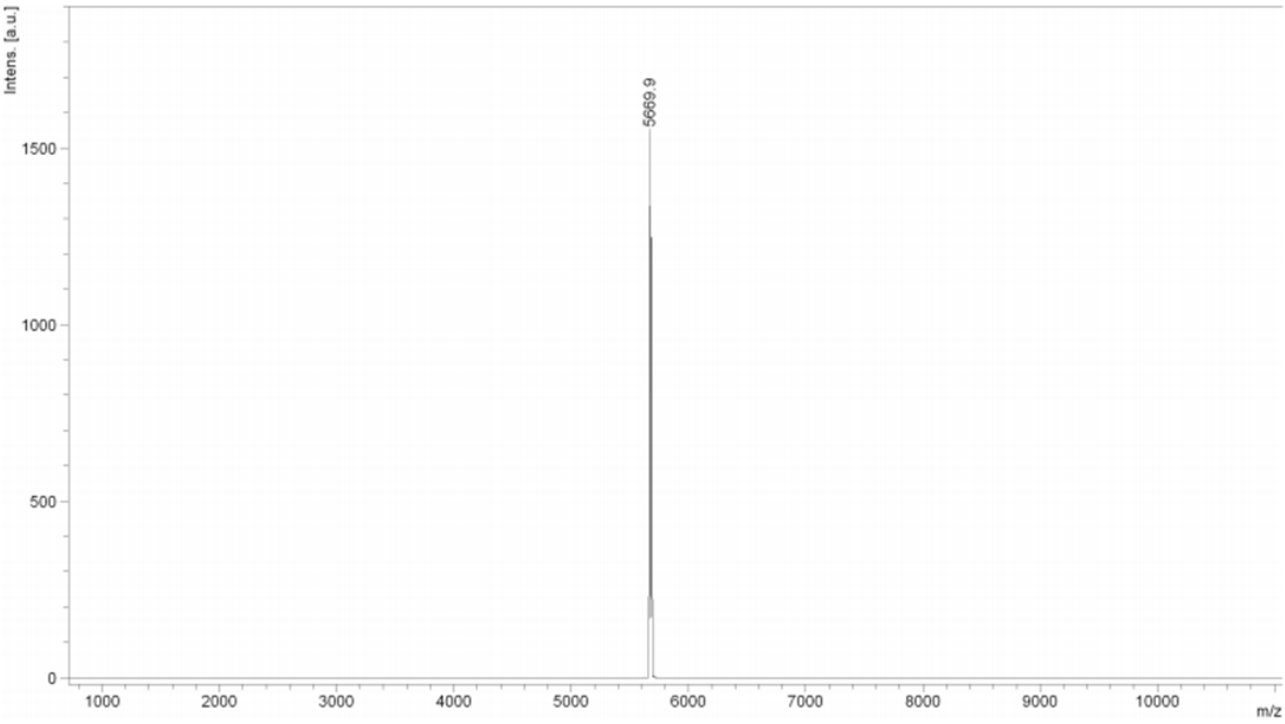
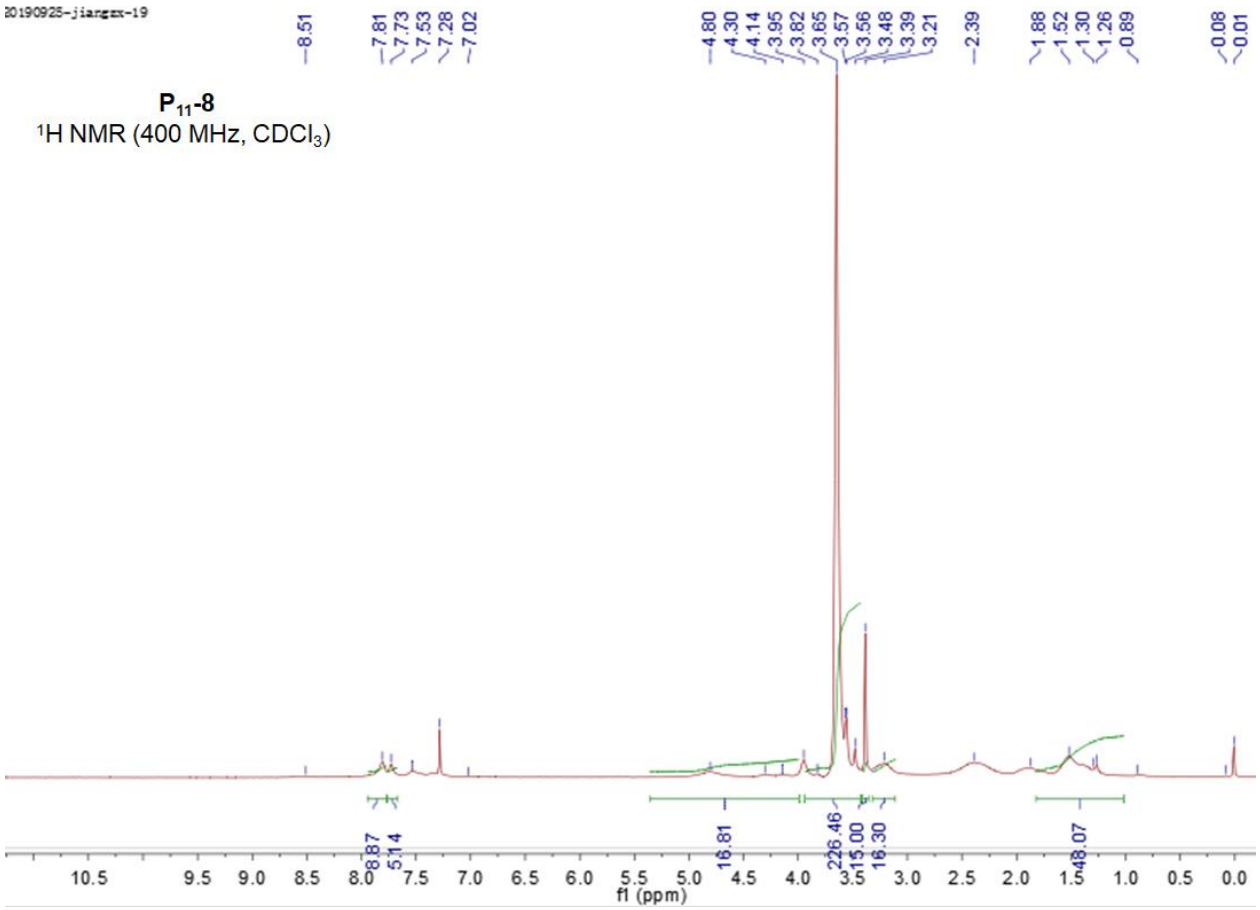
P_7-12
MS (MALDI)

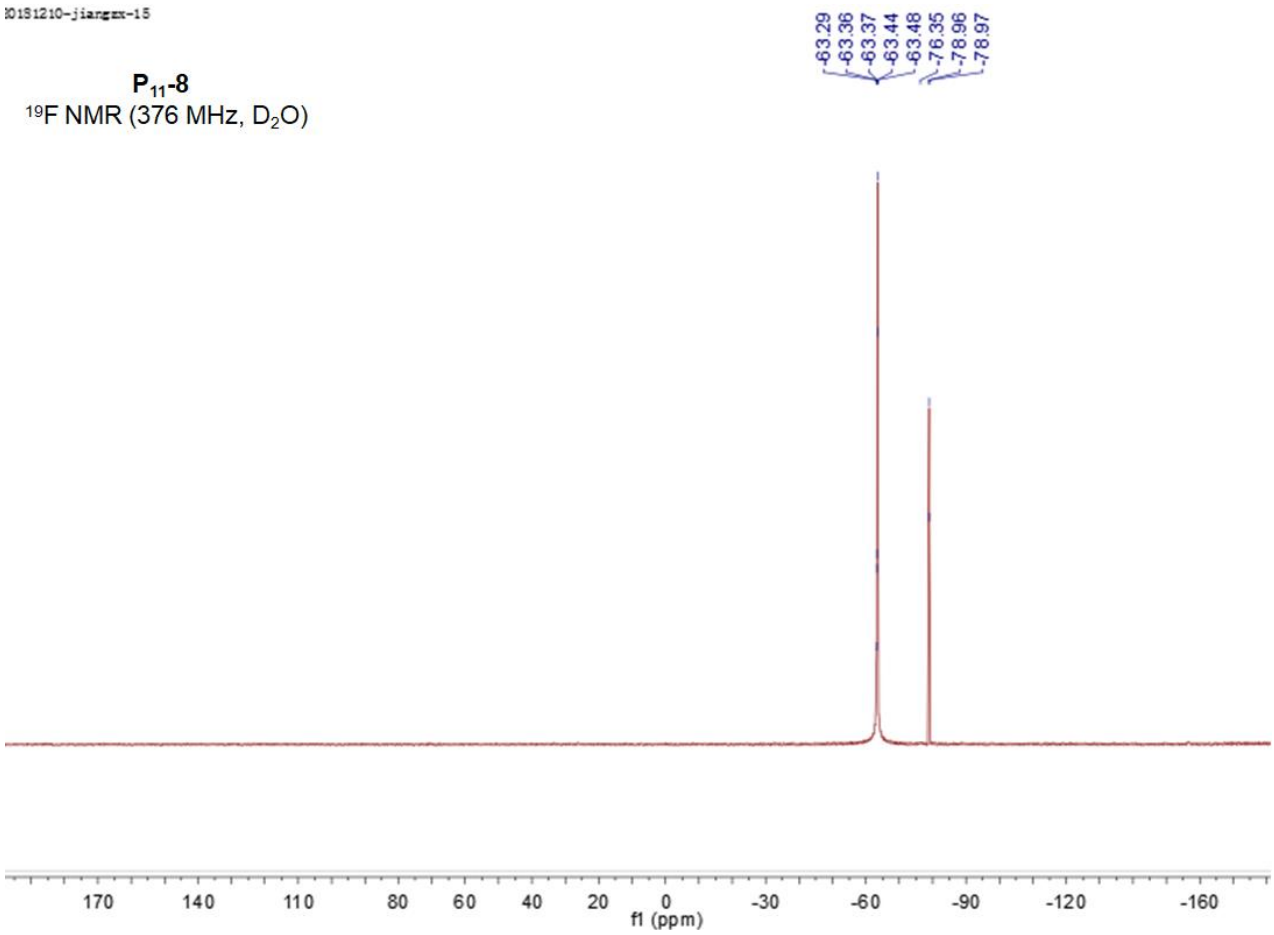




P_{11}-8
^{1}H NMR (400 MHz, $CDCl_3$)

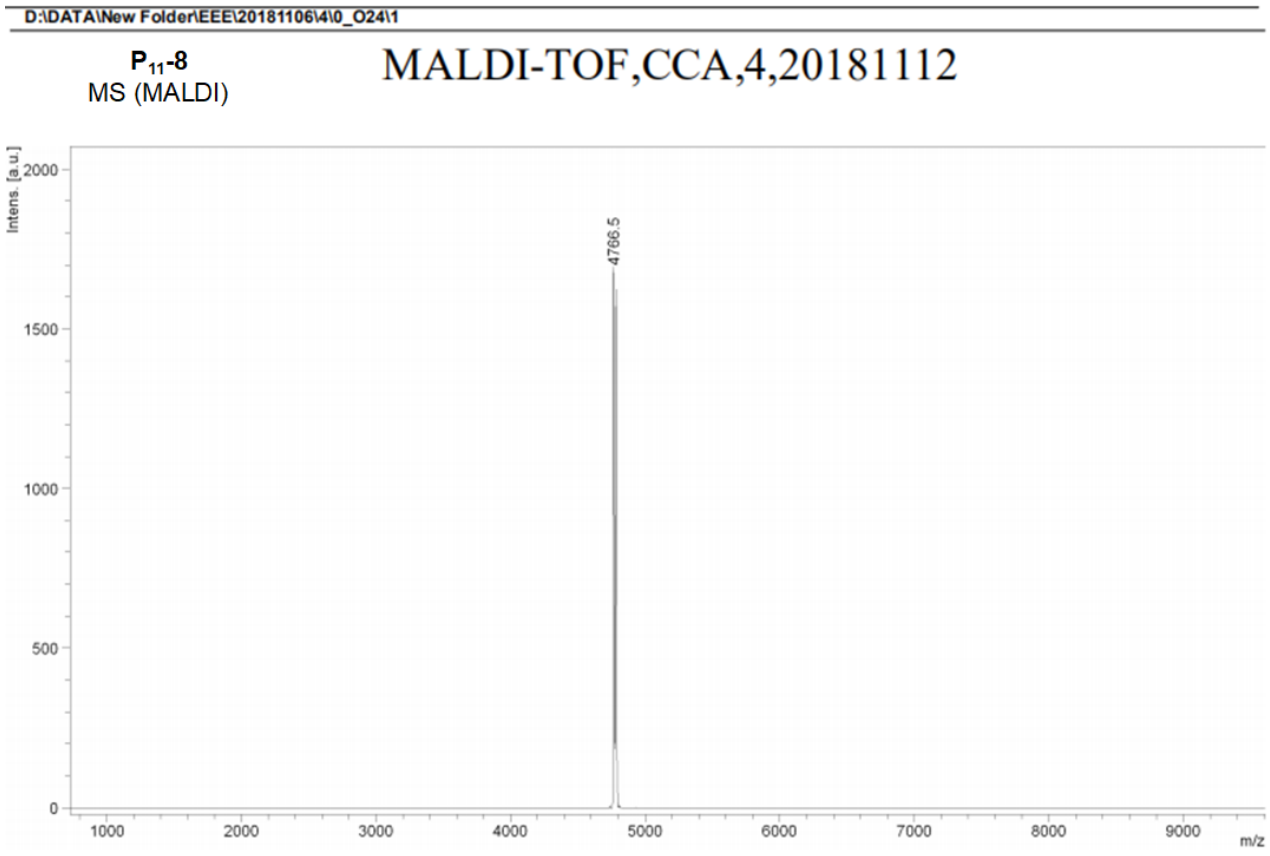
P_{11}-8

^{19}F NMR (376 MHz, D_2O)



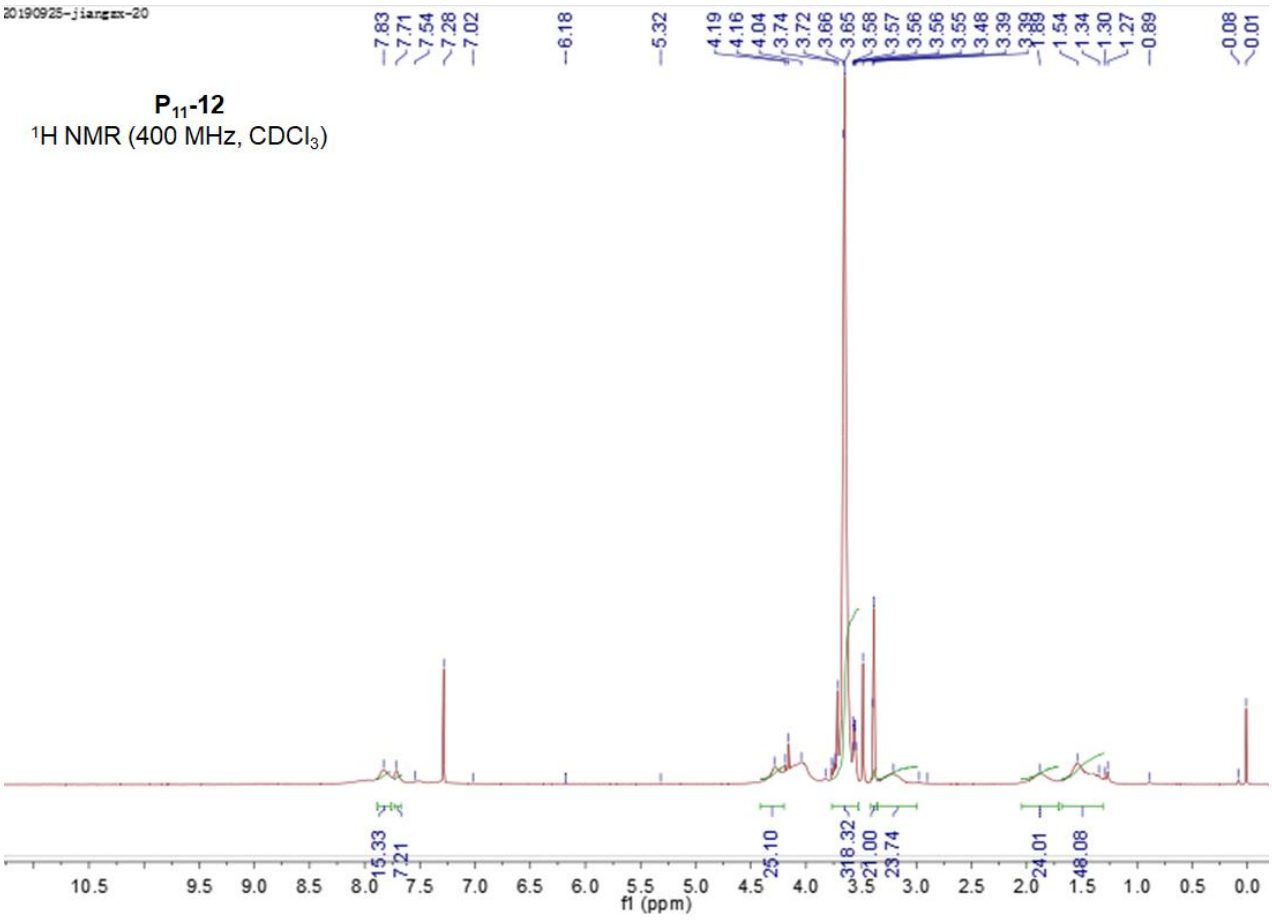
P_{11}-8

MS (MALDI)



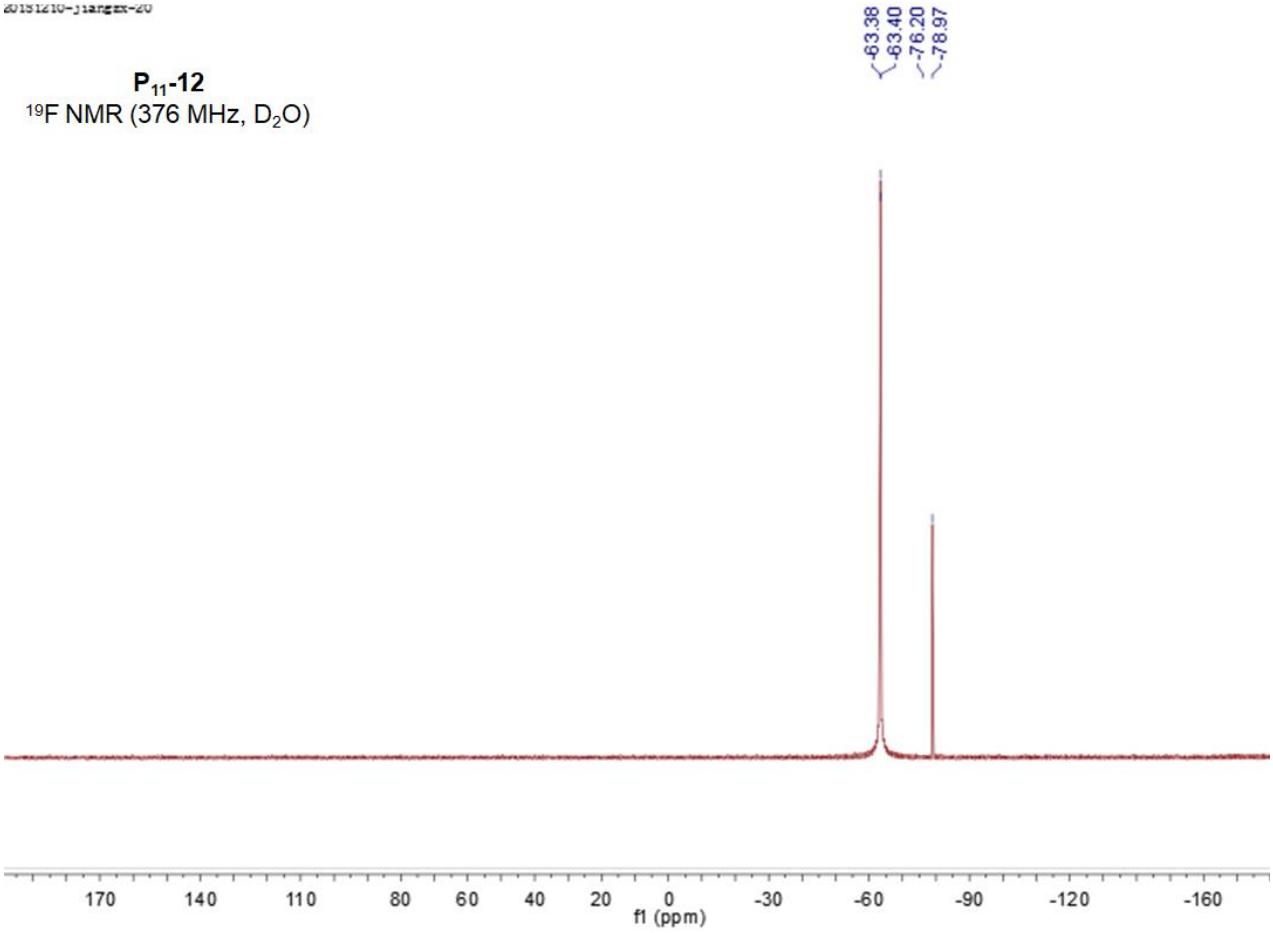
P_{11}-12

^{1}H NMR (400 MHz, $CDCl_3$)



P_{11}-12

^{19}F NMR (376 MHz, D_2O)



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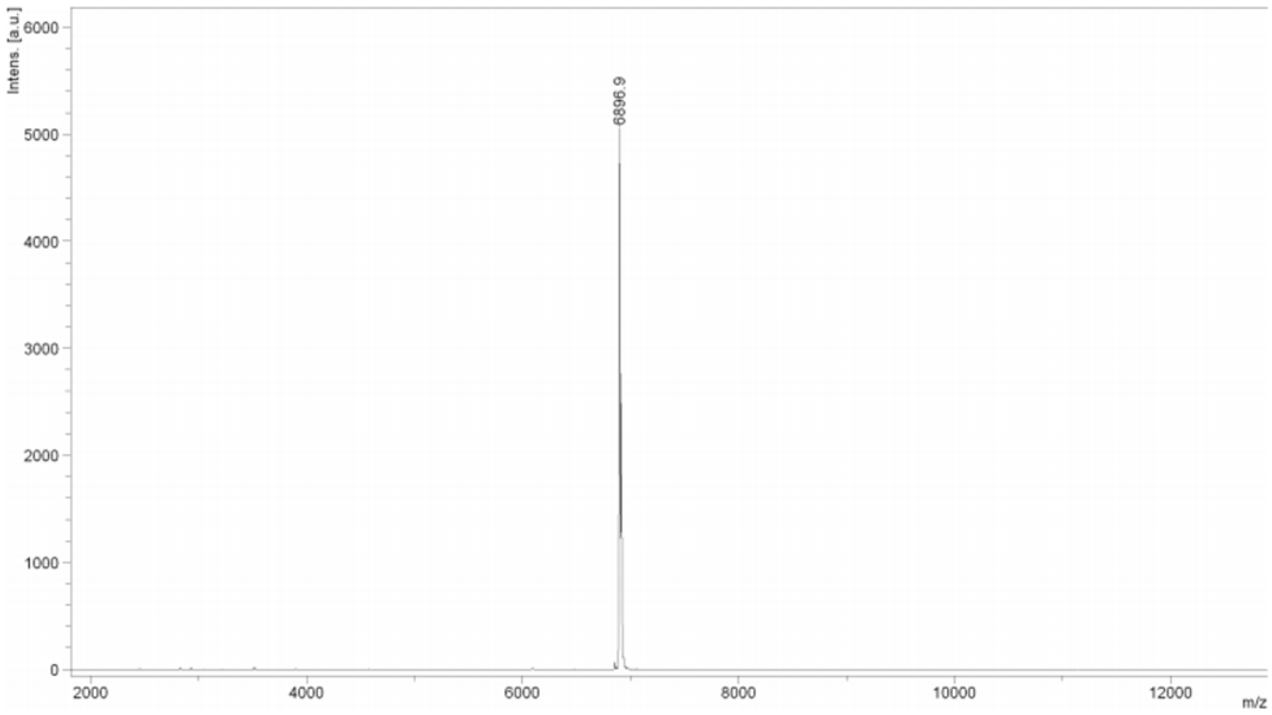
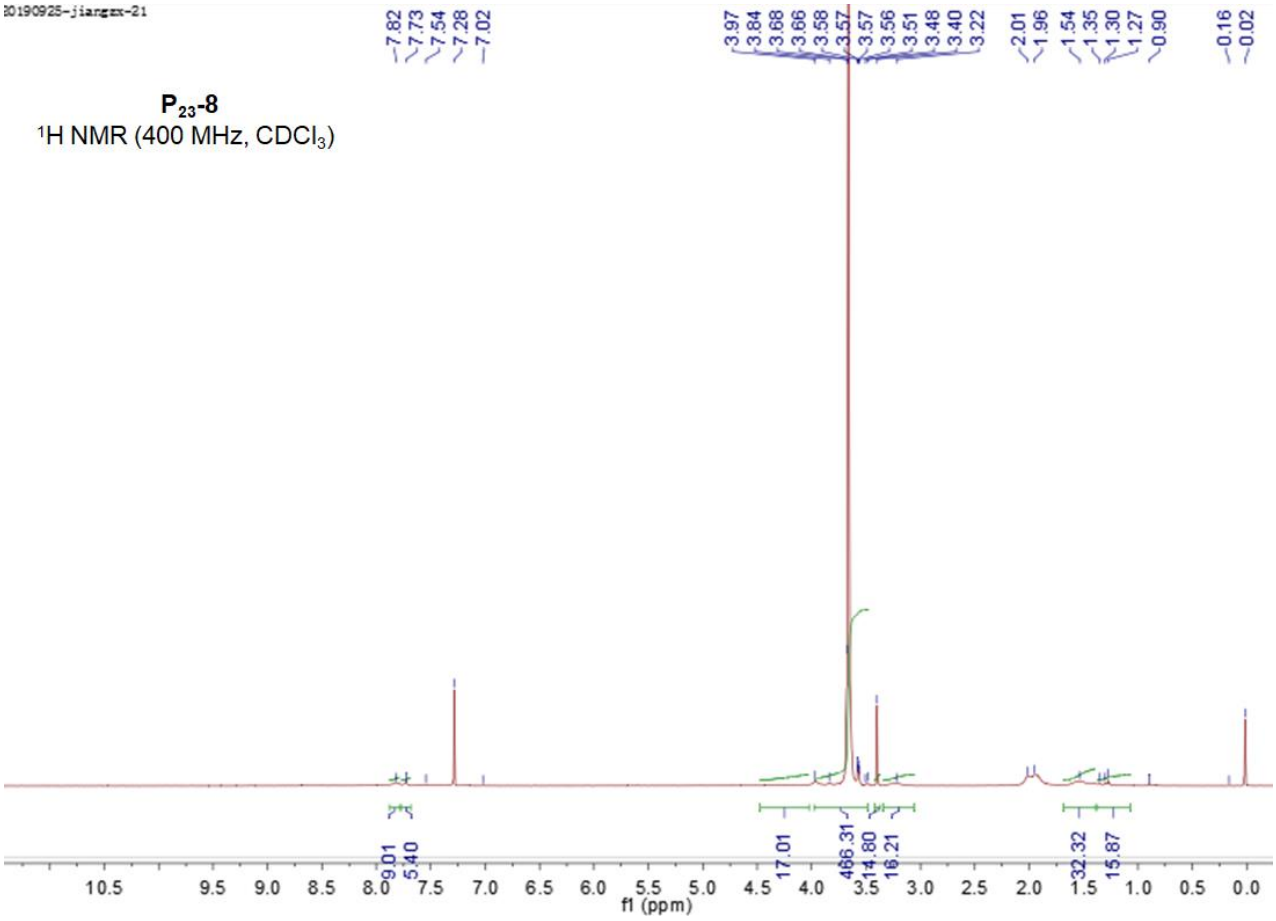
P_{11}-12
MS (MALDI)



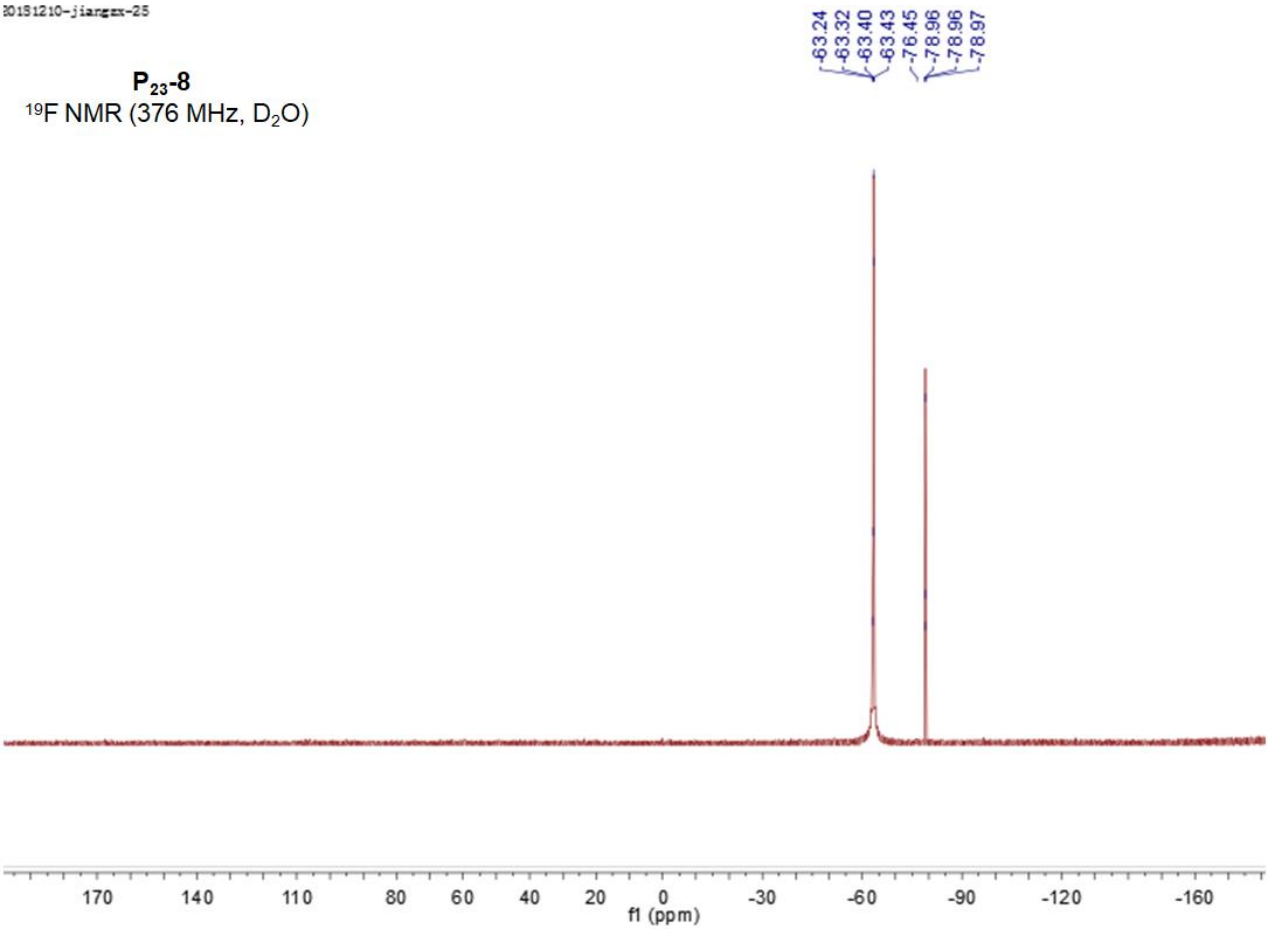
P_{23}-8
^{1}H NMR (400 MHz, $CDCl_3$)



20181210-jiangwx-25

P_{23}-8

^{19}F NMR (376 MHz, D_2O)



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P_{23}-8

MS (MALDI)