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Supporting Information

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All-in-One Heptamethine Cyanine Amphiphiles for Dual Imaging-Guided Chemo-Photodynamic-Photothermal Therapy of Breast Cancer

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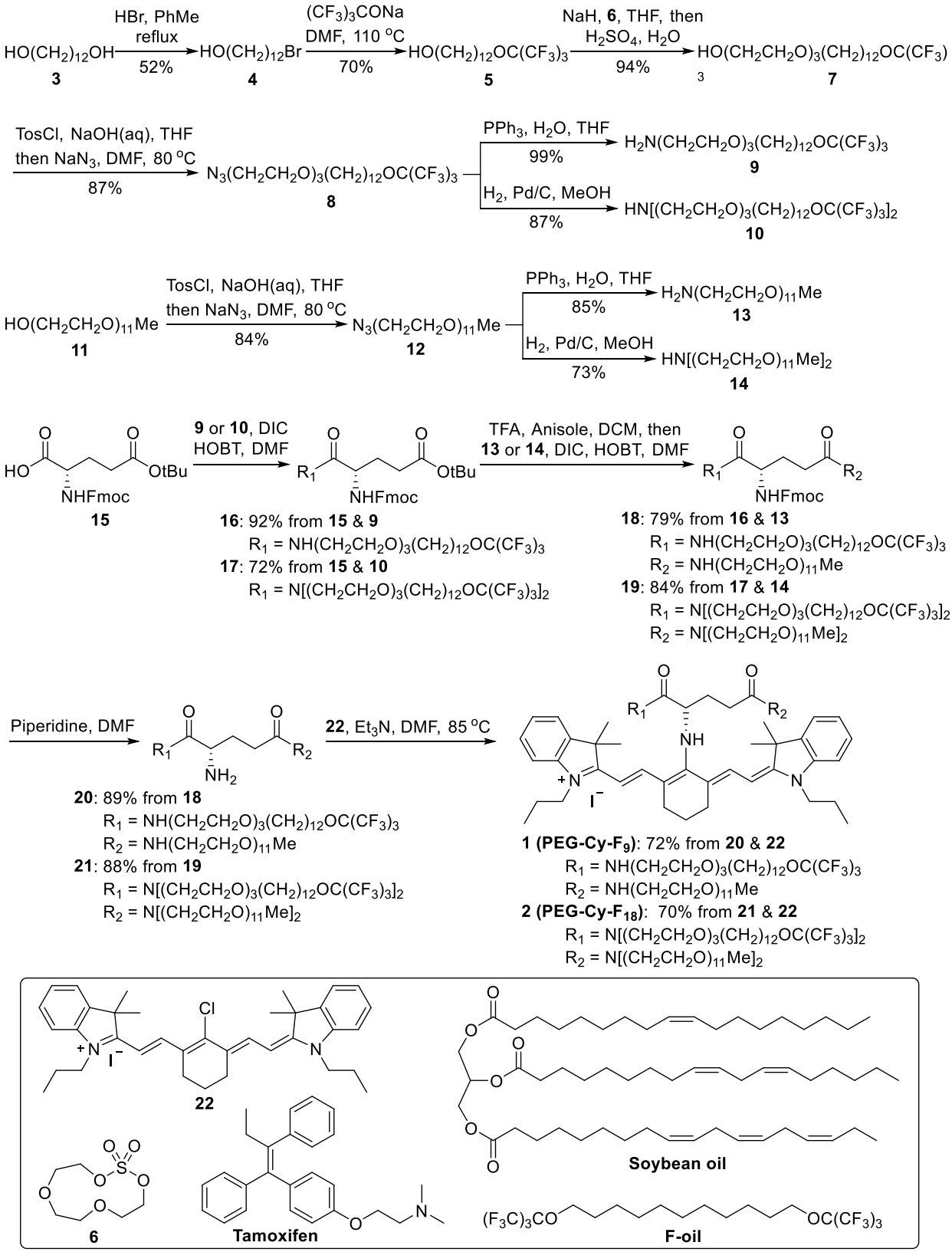
1. General Information

Unless otherwise indicated, all reagents were obtained from commercial supplier and used without prior purification. Column flash chromatography was performed on silica gel (200-300 mesh) with the eluent as indicated in procedures. ^{1}H, ^{19}F and ^{13}C NMR spectra were recorded on a Bruker 400 MHz. Chemical shifts are in ppm and coupling constants (*J*) are in Hertz (Hz). ^{1}H NMR spectra were referenced to tetramethylsilane (d, 0.00 ppm) using $CDCl_3$ as solvent, ^{13}C NMR spectra were referenced to solvent carbons (77.16 ppm for $CDCl_3$). ^{19}F NMR spectra were referenced to 2% perfluorobenzene (s, -164.90 ppm) in $CDCl_3$ or 2% sodium triflate (s, -79.61 ppm) in D_2O. The splitting patterns for ^{1}H NMR spectra are denoted as follows: s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), b (broad) and combinations thereof. MS spectra were recorded on an Asilent LCMS-1100 spectrometer for compounds below 2,000 Da. MALDI-TOF mass spectra were recorded on a Bruker Ultraflex III TOF/TOF spectrometer.

2. Synthesis of Compounds



Scheme S1. Synthetic route of amphiphiles PEG-Cy-Fs

$HO[CH_2]_{12}Br$

4

12-bromododecan-1-ol (4).[1] To a stirring solution of the 1,12-dodecanediol (9.00 g, 44.48 mmol) in 100 mL of toluene was added HBr 47% (4.83 mL, 88.96 mmol). The reaction was stirred at 110 °C for 24 h. The solvent was removed under reduced pressure, and the residue was purified by column chromatography on silica gel with ethyl acetate/petroleum ether (1/15) as eluents to provide compound **4** as a light yellow oil (6.19 g, 52% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.62 (t, *J* = 6.7 Hz, 2H), 3.41 (t, *J* = 6.9 Hz, 2H), 1.85 (dt, *J* = 14.5, 7.0 Hz, 3H), 1.56 (p, *J* = 6.7 Hz, 2H), 1.42 (p, *J* = 7.1 Hz, 2H), 1.34 - 1.28 (m, 14H).

$HO[CH_2]_{12}OC[CF_3]_3$

5

12-((1,1,1,3,3,3-hexafluoro-2-(trifluoromethyl)propan-2-yl)oxy)dodecan-1-ol (5). To a solution of NaOH (9.52 g, 0.17 mol) in H_2O (40 mL) was added perfluoro-tert-butanol (36.40 g, 0.15 mol) at 0 °C. After stirring for 3 h, the reaction mixture was lyophilized and the solid was dissolved in DMF. Then compound **4** (46.66 g, 0.18 mol) was added to the solution, and the reaction was heated to 110 °C. After reacting overnight, H_2O was added to the mixture. The organic phase was separated and purified by flash column chromatography on silica gel with ethyl acetate/petroleum ether (1/20) as eluents to provide compound **5** as a light yellow oil (45.42 g, 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99 (t, *J* = 6.5 Hz, 2H), 3.64 (t, *J* = 6.7 Hz, 2H), 1.67 (dt, *J* = 14.6, 6.6 Hz, 2H), 1.57 (p, *J* = 6.7 Hz, 3H), 1.40 – 1.25 (m, 16H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 120.6 (q, *J* = 293.0 Hz), 79.9 (dd, *J* = 59.1, 29.3 Hz), 70.0, 63.2, 32.9, 29.8, 29.72, 29.67, 29.63, 29.56, 29.3, 25.9, 25.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.57. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{16}H_{25}F_9NaO_2^+$, 443.1603; found, 443.1613.

$HO[CH_2CH_2O]_3[CH_2]_{12}OC[CF_3]_3$

7

24,24,24-trifluoro-23,23-bis(trifluoromethyl)-3,6,9,22-tetraoxatetracosan-1-ol (7). Under an atmosphere of N_2, to a suspension of NaH (8.65 g, 60% in mineral oil, 216.10 mmol) in dry THF (150 mL) was added a solution of compound **5** (45.42 g, 108.05 mmol) in THF (150 mL) at 0 °C, and the mixture was stirred for 30 min. Then a solution of cyclic sulfate (34.40 g, 162.08 mmol) in THF (150 mL) was added and the resulting mixture was stirred overnight at rt. Then, water (3.9 mL) was added and H_2SO_4 was added to adjust the pH to 3.0. The mixture was stirred until hydrolysis was completed. The reaction mixture was neutralized with saturated $NaHCO_3$ solution, concentrated under vacuum and purified by flash chromatography on silica gel with ethyl acetate/petroleum ether (1/8) as eluents to provide

compound **7** as a clear oil (56.29 g, 94% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99 (t, *J* = 6.5 Hz, 2H), 3.77 – 3.71 (m, 2H), 3.71 – 3.64 (m, 6H), 3.60 (dt, *J* = 9.1, 4.4 Hz, 4H), 3.45 (t, *J* = 6.8 Hz, 2H), 1.71 – 1.63 (m, 2H), 1.59 (p, *J* = 7.0 Hz, 2H), 1.40 – 1.25 (m, 16H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 120.5 (q, *J* = 293.0 Hz), 79.4 – 80.3 (m), 72.7, 71.7, 70.69, 70.68, 70.4, 70.1, 70.0, 61.8, 29.8, 29.7, 29.63, 29.59, 29.56, 29.5, 29.2, 26.2, 25.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.54. HRMS (ESI) m/z: $[M + K]^+$ calcd for $C_{22}H_{37}F_9KO_5^+$, 591.2129; found, 591.2127.

$N_3[CH_2CH_2O]_3[CH_2]_{12}OC[CF_3]_3$

8

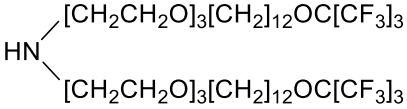
1-azido-24,24,24-trifluoro-23,23-bis(trifluoromethyl)-3,6,9,22-tetraoxatetracosane (8). To a solution of compound **7** (20.30 g, 36.74 mmol) in THF (100 mL) was added a solution of NaOH (5.14 g, 128.59 mmol) in water (16 mL). After the solution cooled to 0 °C, a solution of p-toluenesulfonyl chloride (8.41 g, 44.09 mmol) in THF (50 mL) was slowly added, and the resulting mixture was stirred overnight at rt. The reaction mixture was concentrated under vacuum. The residue was dissolved in water (100 mL) and extracted with DCM. The organic layers were concentrated under vacuum to provide crude sulfonate as light yellow oil. To a solution of crude sulfonate in DMF (120 mL) was added NaN_3 (3.58 g, 55.07 mmol), and the resulting mixture was stirred at 80 °C for 12 h. Then, the reaction mixture containing excess NaN_3 was filtered through a pad of Celite, the filtrate was concentrated under vacuum. The residue was dissolved in water (100 mL), and extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated under vacuum, and purified by flash chromatography on silica gel with ethyl acetate/petroleum ether (1/20) as eluents to obtain compound **8** as a light yellow oil (18.47 g, 87% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99 (t, *J* = 6.5 Hz, 2H), 3.71 – 3.64 (m, 8H), 3.59 (dd, *J* = 5.7, 3.4 Hz, 2H), 3.45 (t, *J* = 6.8 Hz, 2H), 3.40 (t, *J* = 5.1 Hz, 2H), 1.71 – 1.63 (m, 2H), 1.58 (p, *J* = 6.9 Hz, 2H), 1.39 – 1.24 (m, 16H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 120.6 (q, *J* = 292.8 Hz), 79.5 – 80.4 (m), 71.7, 70.9, 70.8, 70.19, 70.17, 70.0, 50.8, 29.82, 29.76, 29.70, 29.67, 29.63, 29.61, 29.55, 29.3, 26.2, 25.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.60. HRMS (ESI) m/z: $[M + K]^+$ calcd for $C_{22}H_{36}F_9N_3KO_4^+$, 616.2194; found, 616.2191.

$NH_2[CH_2CH_2O]_3[CH_2]_{12}OC[CF_3]_3$

9

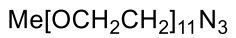
24,24,24-trifluoro-23,23-bis(trifluoromethyl)-3,6,9,22-tetraoxatetracosan-1-amine (9). To a solution of compound **8** (2 g, 3.46 mmol) in THF (20 mL) was added triphenylphosphine (1.36 g, 5.18 mmol), and the resulting mixture was stirred for 1 h at 40 ºC. Then H_2O (0.31

mL, 17.2 mmol) was added and the reaction mixture was stirred overnight at this temperature. The solution was concentrated and purified by flash chromatography on silica gel with MeOH/DCM (1/10) as eluents to provide compound **9** as a light yellow oil (1.89 g, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99 (t, *J* = 6.4 Hz, 2H), 3.69 – 3.61 (m, 6H), 3.61 – 3.55 (m, 2H), 3.53 (t, *J* = 5.1 Hz, 2H), 3.45 (t, *J* = 6.8 Hz, 2H), 2.88 (t, *J* = 5.1 Hz, 2H), 1.72 – 1.62 (m, 2H), 1.58 (p, *J* = 6.9 Hz, 2H), 1.39 – 1.25 (m, 16H). ^{13}C NMR (101 MHz, $CDCl_3$) 120.5 (q, *J* = 293.9 Hz), 79.1 – 80.0 (m), 73.1, 71.6, 70.7, 70.6, 70.3, 70.1, 69.9, 41.7, 29.8, 29.68, 29.66, 29.63, 29.58, 29.56, 29.5, 29.2, 26.2, 25.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.60. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{22}H_{38}F_9NNaO_4^+$, 574.2549; found, 574.2487.



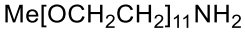
10

bis(24,24,24-trifluoro-23,23-bis(trifluoromethyl)-3,6,9,22-tetraoxatetracosyl)amine (10). Under an atmosphere of H_2, a mixture of compound **8** (6.00 g, 10.39 mmol) and Pd/C (10% on carbon, 2.21 g, 2.08 mmol) in dry MeOH (207 mL) was stirred at rt overnight. The mixture was filtrated through a pad of Celite, and the filtrate was concentrated. The residue was purified by column chromatography on silica gel to give product **10** as a light yellow oil (4.92 g, 87% yield) with MeOH/DCM (1/40) as eluents. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.99 (t, *J* = 6.5 Hz, 4H), 3.71 – 3.54 (m, 20H), 3.45 (t, *J* = 6.8 Hz, 4H), 2.86 (t, *J* = 5.3 Hz, 4H), 1.67 (p, *J* = 6.6 Hz, 4H), 1.58 (p, *J* = 6.9 Hz, 4H), 1.39 – 1.24 (m, 32H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 120.5 (q, *J* = 293.0 Hz), 79.4 – 80.3 (m), 71.6, 70.7, 70.6, 70.4, 70.2, 70.1, 70.0, 49.2, 29.8, 29.71, 29.66, 29.63, 29.59, 29.57, 29.5, 29.2, 26.2, 25.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.55, -164.90. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{44}H_{73}F_{18}N_3NaO_8^+$, 1108.4941; found, 1108.4943.



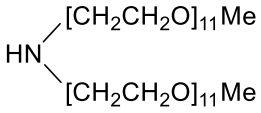
12

34-azido-2,5,8,11,14,17,20,23,26,29,32-undecaoxatetratriacontane (12). Compound **12** was obtained from compound **11** as a light yellow oil in 84% yield (6.09 g) by employing the same synthetic procedures as compound **8**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.64 – 3.54 (m, 40H), 3.50 – 3.46 (m, 2H), 3.33 (q, *J* = 5.1 Hz, 2H), 3.31 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 71.8, 70.58, 70.56, 70.52, 70.46, 70.4, 70.0, 59.0, 50.6. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{22}H_{47}F_9N_3NaO_{11}^+$, 564.3103; found, 564.3109.



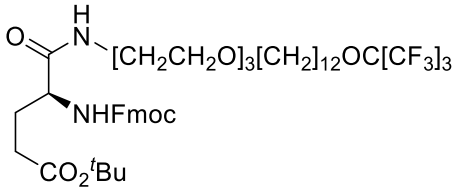
13

2,5,8,11,14,17,20,23,26,29,32-undecaoxatetratriacontan-34-amine (13). Compound **13** was obtained from compound **12** as a light yellow oil in 85% yield (2.43 g) by employing the same synthetic procedures as compound **9**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.68 – 3.63 (m, 38H), 3.57 – 3.54 (m, 2H), 3.52 (t, *J* = 5.2 Hz, 2H), 3.38 (s, 3H), 2.87 (t, *J* = 5.2 Hz, 2H).



14

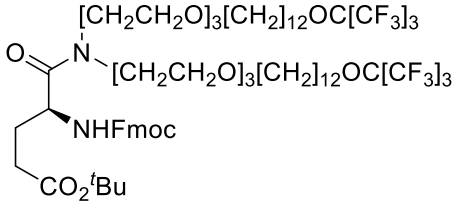
di(2,5,8,11,14,17,20,23,26,29,32-undecaoxatetratriacontan-34-yl)amine (14). Compound **14** was obtained from compound **12** as a light yellow oil in 73% yield (1.36 g) by employing the same synthetic procedures as compound **10**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.70 – 3.59 (m, 80H), 3.57 – 3.54 (m, 4H), 3.38 (s, 6H), 2.86 (t, *J* = 5.3 Hz, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 71.8, 70.4, 70.3, 70.1, 70.0, 58.9, 48.9. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{46}H_{95}F_9NNaO_{22}^+$,1036.6238; found, 1036.6248.



16

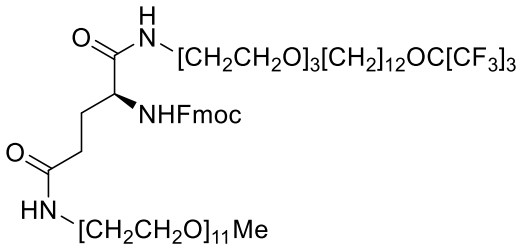
tert-butyl(S)-27-((((9H-fluoren-9-yl)methoxy)carbonyl)amino)-1,1,1-trifluoro-26-oxo-2,2-bis(trifluoromethyl)-3,16,19,22-tetraoxa-25-azatriacontan-30-oate (16). Under an atmosphere of N_2, a solution of Fmoc-L-glutamic acid 5-tert-butyl ester (3.05 g, 7.17 mmol), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI·HCl, 1.38 g, 7.17 mmol), and 1-Hydroxybenzotriazole (HOBt, 968.6 mg, 7.17 mmol) in DMF (30 mL) was stirred at rt for 15 min. Then, a solution of compound **9** (2.64 g, 4.78 mmol) in DMF (10 mL) was added and the resulting mixture was stirred for 12 h. The reaction was quenched with water and extracted with DCM three times. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by flash chromatography on silica gel with EA /PE (1/2) as eluents to provide product **16** as a light yellow oil (4.22 g, 92% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.60 (d, *J* = 7.5 Hz, 2H), 7.39 (t, *J* = 7.4 Hz, 2H), 7.31 (td, *J* = 7.5, 1.2 Hz, 2H), 5.91 (d, *J* = 8.0 Hz, 1H), 4.38 (d, *J* = 7.9 Hz, 2H), 4.21 (t, *J* = 7.2 Hz, 1H), 3.99 (t, *J* = 6.5 Hz, 2H), 3.64 – 3.54 (m, 10H), 3.50 – 3.39 (m, 4H), 2.45 – 2.25 (m, 2H), 2.11 (q, *J* = 6.2 Hz, 1H), 1.93 (dd, *J* = 14.5, 7.2 Hz, 1H), 1.65 (dt, *J* = 8.2, 6.4 Hz, 2H), 1.56 (p, *J* = 7.0 Hz, 2H), 1.45 (s, 9H), 1.37 – 1.23 (m, 16H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.7, 171.4, 156.2, 143.9, 143.8, 141.3, 127.8, 127.1, 125.2, 120.5 (q, *J*

= 292.6 Hz), 120.0, 80.8, 79.4 – 80.2 (m), 71.6, 70.5, 70.3, 70.0, 69.9, 69.7, 67.0, 54.3, 47.2, 39.4, 31.6, 29.74, 29.67, 29.63, 29.60, 29.58, 29.55, 29.53, 29.48, 29.2, 28.5, 28.1, 26.1, 25.3. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{46}H_{63}F_9N_2NaO_9^+$, 981.4282; found, 981.4296.



17

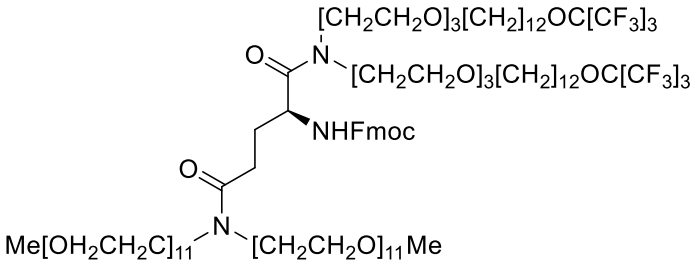
tetraoxatetracosyl)-2,2-bis(trifluoromethyl)-3,16,19,22-tetraoxa-25-azatriacontan-30-oate (17). Compound **17** was obtained from compound **10** as a light yellow oil in 72% yield (3.70 g) by employing the same synthetic procedures as compound **16**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.61 (dd, *J* = 7.5, 2.8 Hz, 2H), 7.40 (tt, *J* = 7.5, 1.5 Hz, 2H), 7.32 (tdd, *J* = 7.4, 2.7, 1.2 Hz, 2H), 5.73 (d, *J* = 8.6 Hz, 1H), 4.82 – 4.72 (m, 1H), 4.43 – 4.28 (m, 2H), 4.21 (t, *J* = 7.2 Hz, 1H), 3.99 (t, *J* = 6.5 Hz, 4H), 3.80 (td, *J* = 10.8, 9.7, 5.6 Hz, 2H), 3.68 – 3.51 (m, 21H), 3.42 (dt, *J* = 11.3, 6.9 Hz, 5H), 2.43 – 2.23 (m, 2H), 2.03 (dt, *J* = 11.5, 3.9 Hz, 1H), 1.78 (dtd, *J* = 14.0, 8.3, 7.8, 5.7 Hz, 1H), 1.71 – 1.62 (m, 4H), 1.61 – 1.52 (m, 4H), 1.45 (s, 9H), 1.39 – 1.33 (m, 4H), 1.31 – 1.24 (m, 28H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.4, 172.3, 156.1, 144.0, 143.9, 141.3, 127.7, 127.1, 125.3, 125.2, 120.5 (q, *J* = 291.9 Hz), 120.0, 80.6, 79.1 – 80.0 (m), 71.63, 71.60, 70.8, 70.7, 70.6, 70.54, 70.48, 70.10, 70.07, 69.9, 69.3, 69.1, 67.0, 50.2, 48.4, 47.2, 46.6, 31.0, 29.8, 29.7, 29.65, 29.62, 29.57, 29.5, 29.2, 28.1, 26.1, 25.3. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.61. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{68}H_{98}F_{18}N_2NaO_{13}^+$,1515.6679; found, 1515.6713.



18

(9H-fluoren-9-yl)methyl(S)-(65,65,65-trifluoro-36,40-dioxo-64,64-bis(trifluoromet-hyl)-2,5,8,11,14,17,20,23,26,29,32,44,47,50,63-pentadecaoxa-35,41-diazapentahexacontan-39-yl)carbamate (18). Compound **16** (521 mg, 0.54 mmol) was dissolved in DCM (6 mL), anisole (0.089 mL, 0.81mmol) and TFA (2 mL) were added and the resulting solution was stirred for 12 h. The reaction mixture was neutralized with saturated $NaHCO_3$ solution and washed with DCM. The combined aqueous phases were concentrated to give a residue. Then,

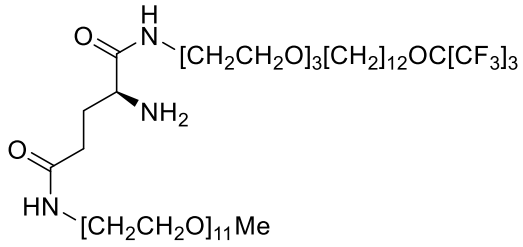
the residue and HOBt (88.1 mg, 0.65 mmol) were dissolved in DMF (4 mL), and *N*,*N*'-Diisopropylcarbodiimide (DIC, 0.10 mL, 0.65 mmol) was added to the mixture under an atmosphere of N_2. After 15 min, a solution of compound **13** (336.1 mg, 0.65 mmol) in DCM (2 mL) was added and the resulting mixture was stirred for 12 h at rt. The reaction was quenched with water and extracted with DCM three times. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated under vacuum, and purified by flash chromatography on silica gel with MeOH/DCM (1/25) as eluents to provide compound **18** as a light yellow oil (601 mg, 79% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.34 – 7.29 (m, 2H), 6.27 (d, *J* = 7.4 Hz, 1H), 4.36 (dd, *J* = 7.3, 4.1 Hz, 2H), 4.21 (t, *J* = 7.1 Hz, 1H), 3.99 (t, *J* = 6.5 Hz, 2H), 3.66 – 3.53 (m, 52H), 3.44 (dt, *J* = 13.7, 5.8 Hz, 6H), 3.38 (s, 3H), 2.36 – 2.22 (m, 2H), 2.06 (td, *J* = 13.9, 13.0, 6.8 Hz, 2H), 1.71 – 1.62 (m, 2H), 1.59 – 1.52 (m, 2H), 1.38 – 1.33 (m, 2H), 1.30 – 1.24 (m, 16H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.0, 171.6, 156.4, 144.0, 143.9, 141.3, 127.8, 127.2, 125.3, 120.5 (q, *J* = 293.6 Hz), 120.0, 79.4 – 80.3 (m), 72.0, 71.6, 70.64, 70.59, 70.52, 70.48, 70.3, 70.2, 70.0, 69.8, 69.6, 67.0, 59.1, 54.5, 47.2, 39.4, 32.4, 29.8, 29.7, 29.63, 29.59, 29.56, 29.5, 29.2, 26.1, 25.4. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.62. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{65}H_{102}F_9N_3NaO_{19}^+$, 1422.6856; found, 1422.6885.



19

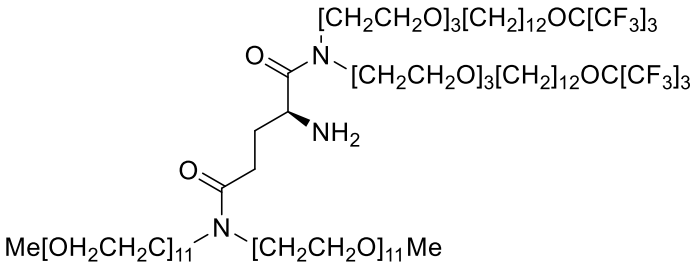
Amide 19. Amide **19** was obtained from compound **17** as a light yellow oil in 84% yield (2.94 g) by employing the same synthetic procedures as compound **18**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.76 (d, *J* = 7.5 Hz, 2H), 7.61 (dd, *J* = 9.6, 7.5 Hz, 2H), 7.40 (t, *J* = 7.4 Hz, 2H), 7.35 – 7.29 (m, 2H), 5.88 (d, *J* = 8.4 Hz, 1H), 4.81 – 4.64 (m, 1H), 4.30 (qd, *J* = 10.4, 7.4 Hz, 2H), 4.21 (t, *J* = 7.3 Hz, 1H), 3.99 (t, *J* = 6.5 Hz, 4H), 3.68 – 3.49 (m, 110H), 3.42 (dt, *J* = 14.0, 7.0 Hz, 6H), 3.38 (s, 6H), 2.58 – 2.39 (m, 2H), 2.18 – 2.07 (m, 1H), 1.80 – 1.71 (m, 1H), 1.71 – 1.63 (m, 4H), 1.61 – 1.52 (m, 4H), 1.40 – 1.33 (m, 4H), 1.31 – 1.24 (m, 28H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 172.5, 172.3, 156.2, 144.0, 143.8, 141.2, 127.7, 127.1, 125.3, 125.2, 120.4 (q, *J* = 293.5 Hz), 120.0, 79.3 – 80.2 (m), 71.9, 71.6, 71.5, 70.6, 70.5, 70.45, 70.0, 69.8, 69.6, 69.58, 69.2, 69.0, 59.0, 48.6, 48.2, 47.1, 46.6, 46.4, 29.7, 29.6, 29.57, 29.54, 29.49, 29.4, 29.1, 28.9,

26.1, 25.3. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.62. MS (MALDI) m/z: $[M + Na]^+$ calcd for $C_{110}H_{183}F_{18}N_3NaO_{34}^+$, 2455.229; found, 2455.126.



20

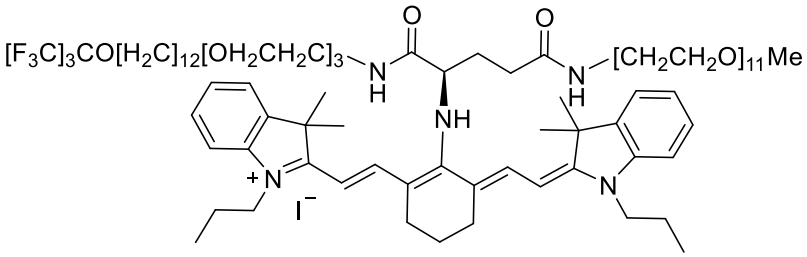
(S)-2-amino-N5-(2,5,8,11,14,17,20,23,26,29,32-undecaoxatetratriacontan-34-yl)-N1-(24,24,24-trifluoro-23,23-bis(trifluoromethyl)-3,6,9,22-tetraoxatetracosyl)pentanediamide (20). In dark, piperidine (0.32 mL, 3.29 mmol) was added to a stirring solution of compound **18** (576 mg, 0.41 mmol) in DCM (7 mL), the resulting mixture was stirred for 12 h. Then, the reaction was quenched with water and extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to provide product **20** as a light yellow oil (431 mg, 89% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.85 (t, *J* = 5.7 Hz, 1H), 7.44 (t, *J* = 5.6 Hz, 1H), 3.99 (t, *J* = 6.4 Hz, 2H), 3.69 – 3.62 (m, 44H), 3.57 (ddd, *J* = 9.6, 6.0, 4.0 Hz, 8H), 3.49 – 3.41 (m, 7H), 3.38 (s, 3H), 2.35 (dtd, *J* = 28.3, 14.4, 7.0 Hz, 2H), 2.07 (dq, *J* = 13.4, 6.7 Hz, 1H), 1.90 (dq, *J* = 14.2, 7.1 Hz, 1H), 1.72 – 1.63 (m, 2H), 1.58 (p, *J* = 7.0 Hz, 2H), 1.37 (t, *J* = 7.7 Hz, 2H), 1.31 – 1.25 (m, 14H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 174.6, 173.1, 120.4 (q, *J* = 294.7 Hz), 79.0 – 79.9 (m), 71.9, 71.6, 70.5, 70.47, 70.43, 70.40, 70.2, 70.05, 69.96, 69.8, 69.7, 59.0, 54.3, 39.2, 39.0, 32.6, 30.9, 29.7, 29.6, 29.53, 29.48, 29.46, 29.4, 29.1, 26.0, 25.2. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.62. HRMS (ESI) m/z: $[M + Na]^+$ calcd for $C_{50}H_{92}F_9N_3NaO_{17}^+$, 1200.6175; found, 1200.6208.



21

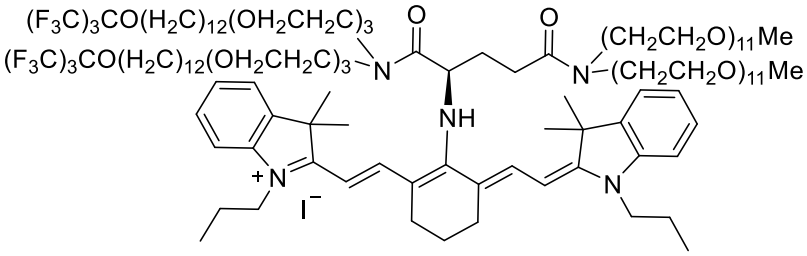
Amine 21. Amine **21** was obtained from compound **19** as a light yellow oil in 88% yield (913 mg) by employing the same synthetic procedures as compound **20**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.92 (t, *J* = 6.6 Hz, 4H), 3.61 – 3.47 (m, 111H), 3.37 (td, *J* = 6.9, 1.7 Hz, 5H), 3.31

(s, 6H), 3.29 – 3.23 (m, 1H), 2.72 – 2.57 (m, 1H), 2.45 (dt, *J* = 16.3, 5.9 Hz, 1H), 1.93 (dt, *J* = 14.6, 5.7 Hz, 1H), 1.64 – 1.55 (m, 5H), 1.51 (q, *J* = 6.6 Hz, 4H), 1.31 – 1.18 (m, 32H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 175.4, 172.8, 120.2 (q, *J* = 293.3 Hz), 79.0 – 80.0 (m), 71.6, 71.3, 70.4, 70.3, 70.1, 69.8, 69.7, 69.6, 69.2, 69.1, 68.9, 58.8, 58.7, 49.9, 48.4, 47.9, 46.0, 29.43, 29.36, 29.3, 29.25, 29.19, 29.17, 28.9, 28.5, 25.8, 25.0, 25.0. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.59. MS (MALDI) m/z: $[M + Na]^+$ calcd for $C_{95}H_{173}F_{18}N_3NaO_{32}^+$, 2233.161; found, 2233.085.



PEG-Cy-F_9

Fluorinated amphiphile PEG-Cy-F_9. Under an atmosphere of N_2, triethylamine (0.18 mL, 1.27 mmol) was added to a stirring solution of IR-780 iodide (283.1 mg, 0.42 mmol) and the compound **20** (500 mg, 0.42 mmol) in DMF (6 mL). The resulting mixture was stirred for 12 h at 85 °C. Then, the reaction was quenched with water and extracted with DCM. The combined organic layers were dried over anhydrous Na_2SO_4, concentrated under vacuum and purified by flash chromatography on silica gel with MeOH/DCM (1/30) as eluents to provide amphiphile PEG-Cy-F_9 as a blue oil (469 mg, 72% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.02 (s, 1H), 7.87 (d, *J* = 13.0 Hz, 2H), 7.77 (s, 1H), 7.57 (d, *J* = 17.0 Hz, 1H), 7.31 – 7.26 (m, 4H), 7.09 (t, *J* = 7.4 Hz, 2H), 6.89 (d, *J* = 7.9 Hz, 2H), 5.72 (d, *J* = 13.0 Hz, 2H), 4.55 (d, *J* = 8.3 Hz, 1H), 3.99 (t, *J* = 6.5 Hz, 2H), 3.82 (t, *J* = 7.3 Hz, 3H), 3.71 – 3.51 (m, 56H), 3.42 (t, *J* = 7.1 Hz, 4H), 3.38 (s, 3H), 2.53 – 2.38 (m, 6H), 2.32 (t, *J* = 7.5 Hz, 1H), 1.83 (q, *J* = 7.2 Hz, 5H), 1.77 – 1.58 (m, 14H), 1.58 – 1.52 (m, 2H), 1.43 – 1.36 (m, 2H), 1.30 – 1.24 (m, 16H), 1.04 (t, *J* = 7.4 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.8, 171.5, 169.0, 168.2, 143.0, 140.6, 139.7, 128.1, 123.2, 122.2, 121.8, 120.5 (q, *J* = 293.6 Hz), 108.8, 95.9, 79.4 – 80.2 (m), 71.9, 71.5, 70.61, 70.57, 70.52, 70.49, 70.21, 70.15, 70.0, 69.9, 69.7, 69.2, 62.9, 59.0, 48.1, 45.0, 39.2, 32.4, 31.9, 31.7, 31.0, 29.7, 29.6, 29.6, 29.6, 29.4, 29.1, 28.5, 28.4, 26.1, 25.3, 25.2, 22.7, 21.6, 20.2, 14.1, 11.8. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.65. HRMS (ESI) m/z: $[M]^+$ calcd for $C_{86}H_{135}F_9N_5O_{17}^+$, 1680.9704; found, 1680.9548.



PEG-Cy-F_{18}

Fluorinated amphiphile PEG-Cy-F_{18} 2. Amphiphile PEG-Cy-F_{18} **2** was obtained from compound **21** as a light yellow oil in 70% yield (854 mg) by employing the same synthetic procedures as PEG-Cy-F_9 **1**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 13.0 Hz, 2H), 7.35 – 7.31 (m, 3H), 7.14 (t, *J* = 7.4 Hz, 2H), 6.99 (d, *J* = 8.0 Hz, 2H), 6.83 (d, *J* = 8.9 Hz, 1H), 5.84 (d, *J* = 13.1 Hz, 2H), 4.97 (s, 1H), 3.99 (t, *J* = 6.4 Hz, 4H), 3.91 (d, *J* = 7.4 Hz, 4H), 3.73 – 3.40 (m, 116H), 3.38 (s, 6H), 3.37 (s, 1H), 2.60 (t, *J* = 7.6 Hz, 2H), 2.41 – 2.32 (m, 5H), 1.85 (p, *J* = 7.1 Hz, 5H), 1.73 (d, *J* = 16.0 Hz, 12H), 1.68 – 1.61 (m, 4H), 1.54 (dt, *J* = 12.2, 6.7 Hz, 4H), 1.41 (d, *J* = 12.3 Hz, 2H), 1.38 – 1.32 (m, 4H), 1.26 (d, *J* = 5.5 Hz, 28H), 1.06 (t, *J* = 7.3 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 172.6, 172.2, 169.6, 166.9, 142.9, 140.4, 140.1, 128.4, 123.7, 122.1, 122.0, 120.5 (q, *J* = 293.2 Hz), 109.4, 96.7, 79.4 – 80.1 (m), 71.9, 71.57, 71.55, 70.58, 70.55, 70.5, 70.3, 69.93, 69.90, 59.0, 57.4, 48.8, 48.3, 46.4, 45.2, 29.7, 29.6, 29.6, 29.5, 29.4, 29.4, 29.1, 28.6, 28.4, 28.3, 26.1, 25.3, 24.9, 22.7, 21.7, 20.4, 14.1, 11.7. ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.59. MS (MALDI) m/z: $[M]^+$ calcd for $C_{131}H_{216}F_{18}N_5O_{32}^+$, 2713.514; found, 2713.426.

3. ^{19}F NMR Spectra of PEG-Cy-F_9, PEG-Cy-F_{18} and F-oil

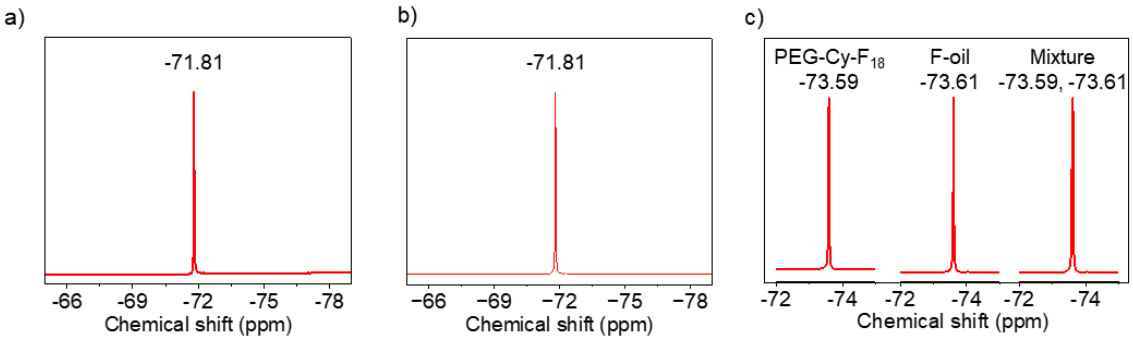


Figure S1. ^{19}F NMR spectra of PEG-Cy-F_9 (a) and PEG-Cy-F_{18} (b) in MeOH (Using CF_3SO_3Na in D_2O as the internal standard). ^{19}F NMR spectra of PEG-Cy-F_{18}, F-oil and their mixture in $CDCl_3$ (c).

4. *In vitro* ^{19}F MRI Experiments

The ^{19}F magnetic resonance imaging (^{19}F MRI) experiments were performed on a 400 MHz Bruker BioSpec MRI system. The ^{19}F *in vitro* images were acquired using a spin-echo pulse sequence, method = RARE, matrix size = 32 × 32, SI = 20 mm, FOV = 3.0 cm, TR = 4000 ms, TE = 3.0 ms, scan time = 256 s.

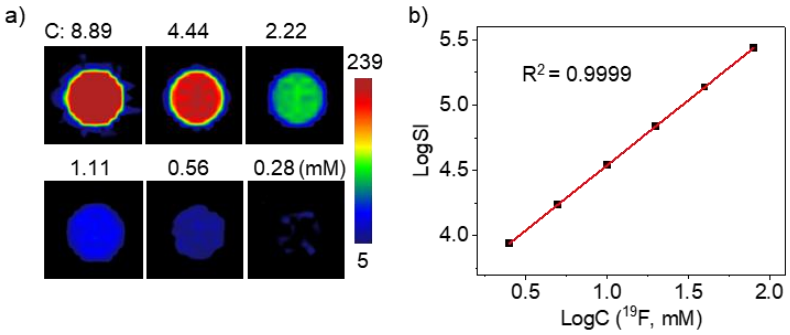


Figure S2. ^{19}F MRI phantom images (a, concentration of PEG-Cy-F$_9$ as indicated), the logarithm plot of ^{19}F signal intensity (SI) versus ^{19}F concentrations (b) of PEG-Cy-F$_9$.

5. Optical Properties of PEG-Cy-Fs

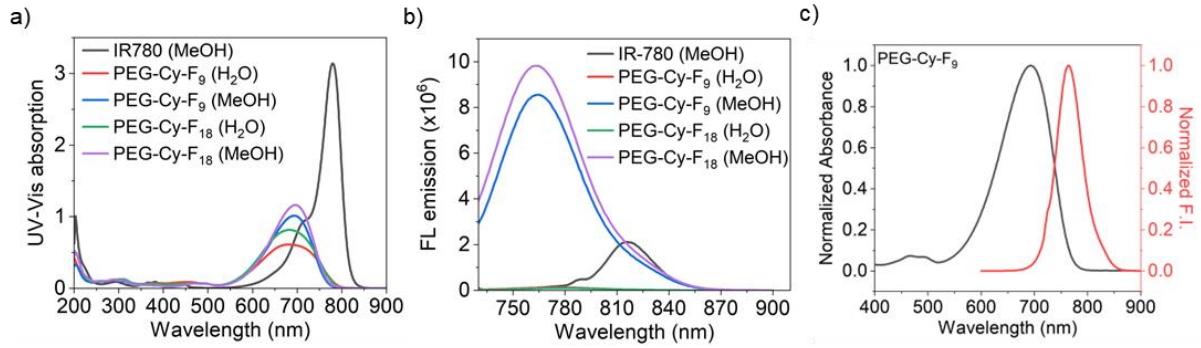


Figure S3. UV-Vis absorption spectra (a) and NIR FL emission spectra (b) of PEG-Cy-Fs. Normalized absorption (black) and fluorescence emitting (red) spectra of PEG-Cy-F$_9$ (c).

6. CMC and LogP Measurement

The *n*-octanol/water partition coefficients (LogP) values of PEG-Cy-F$_9$ and PEG-Cy-F$_{18}$ were measured following shake-flask method.[2] The maximum UV absorption (Shimadzu UV-2600 spectrophotometer) was measured and compared with calibration curve to obtain the concentration of the compound. LogP = Lg[(C_s-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.

The CMCs of PEG-Cy-F_9 and PEG-Cy-F_{18} were detected using pyrene as a fluorescent indicator (excitation wavelength, 334 nm). The ratio of fluorescence intensity at 373 nm and 384 from the pyrene was plotted against the amphiphile concentration to calculate the CMC.

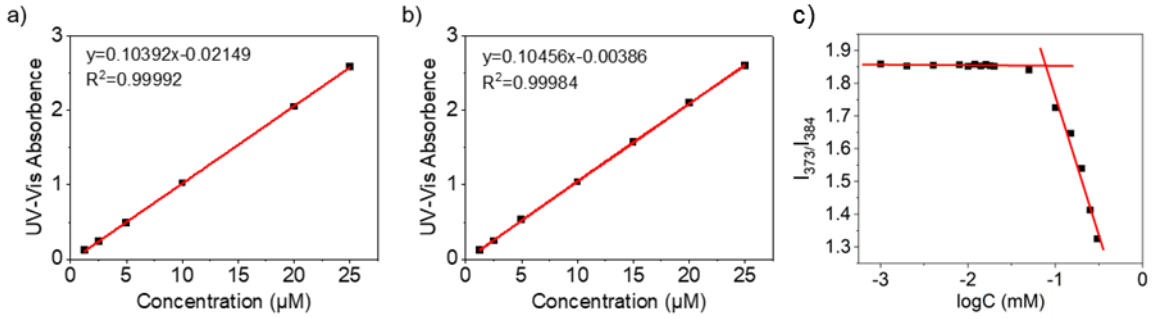


Figure S4. UV-Vis absorption standard curves of PEG-Cy-F_9 (a) and PEG-Cy-F_{18} (b), CMC measument of PEG-Cy-F_9 (c).

7. Solvent-Dependent ^{19}F NMR and TEM Image of PEG-Cy-F_9

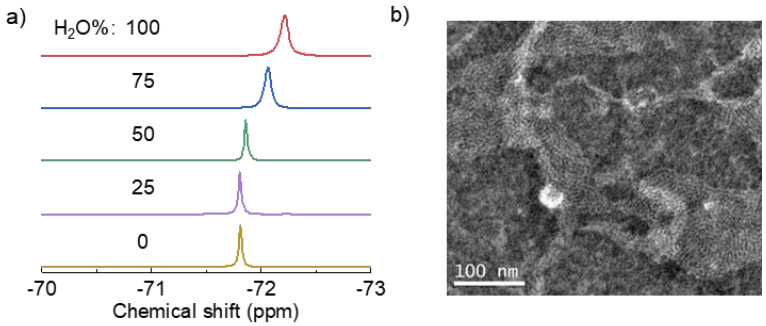


Figure S5. Solvent-dependent ^{19}F NMR spectra (a), and TEM image (b) of PEG-Cy-F_9.

8. Characterization of Nanoemulsions

8.1. DLS Measurement and TEM of Nanoemulsions

Dynamic light scattering (DLS) measurement was performed to determine the average particle size and zeta potential of nanoparticles using Malvern Zetasizer (Malvern, Nano ZS 90, UK). Data were given as mean ± standard deviation (SD) based on three independent measurements.

Transmission electron microscopy (TEM, JEM-2100, JEOL, negative staining with phosphotungstic acid at 1%, w/v) was used to observe the morphology of nanoemulsions.

8.2. Encapsulation Efficiency and Drug Loading Content

Encapsulation efficiency (EE%) and drug loading content (DLC%) of nanoemulsions were determined using HPLC (Sunfire C18 column (5 μm, 4.6 × 250 mm). Briefly, 100 μL of SoFoTm/PEG-Cy-F_{18} was diluted with 900 μL of MeOH, the mixture was sonicated for 20 minutes, centrifuged at 12,000 rpm for 20 minutes, and the tamoxifen content in the supernatant was determined by HPLC as the total tamoxifen content in nanoemulsions (W_t). Meanwhile, 100 μL of SoFoTm/PEG-Cy-F_{18} was diluted with H_2O, the sample was transferred to an ultrafiltration centrifuge tube, and centrifuged at 12,000 rpm for 20 min. MeOH was added to dissolve the trapped nanoemulsions, the content of tamoxifen in the MeOH solution was determined by HPLC as the encapsulated tamoxifen content in nanoemulsions (W_o). EE% and DLC% were calculated by equations (1) and (2), respectively. Where W_d is the weight of the nanoemulsions.

$EE\% = W_o/W_t \times 100\%$ (1)

$DLC\% = W_o/W_d \times 100\%$ (2)

9. Celluar Uptake of SoFoTm/PEG-Cy-F_{18} NPs

MCF-7 cells were co-incubated with SoFoTm/PEG-Cy-F_{18} (PEG-Cy-F_{18} concentration: 4 μM) at 37 ^oC for 2, 6, 12, 24 h, respectively. The cellular uptake of SoFoTm/PEG-Cy-F_{18} was studied by laser scanning confocal microscopy (A1R/A1, Nikon). Fluorescent dye DAPI was used as the nucleus dye.

10. Detection of Singlet Oxygen

TEMP was selected as a singlet oxygen capture agent. 190 μL of SoFoTm/PEG-Cy-F_{18} (0.5 mM) was irradiated with a laser (750 nm, 1 W cm^{-2}) for 120 s. Then, 10 μL of TEMP (0.1 M) was immediately added to the solution and the mixture was measured by electron spin resonance (ESR).

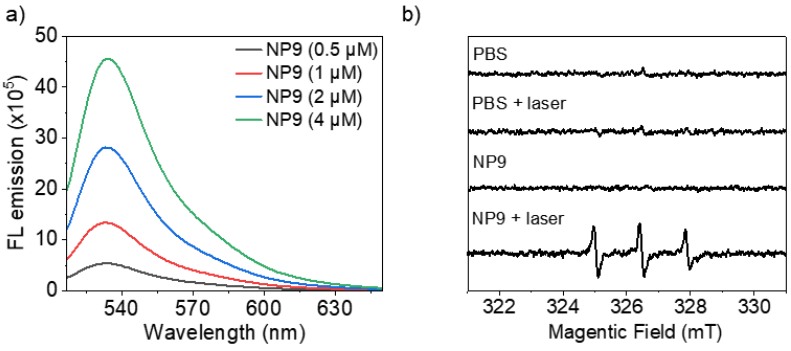


Figure S6. Fluorescence spectra of the SOSG with **NP9** after photoirradiation at wavelengths of 750 nm at 1 W cm^{-2} for 120 s (a, concentration as indicated). ESR spectra of PBS, PBS + laser, **NP9**, **NP9** + laser (b). **NP9**: SoFoTm/PEG-Cy-F_{18}.

11. H&E Staining Assay of Major Organs

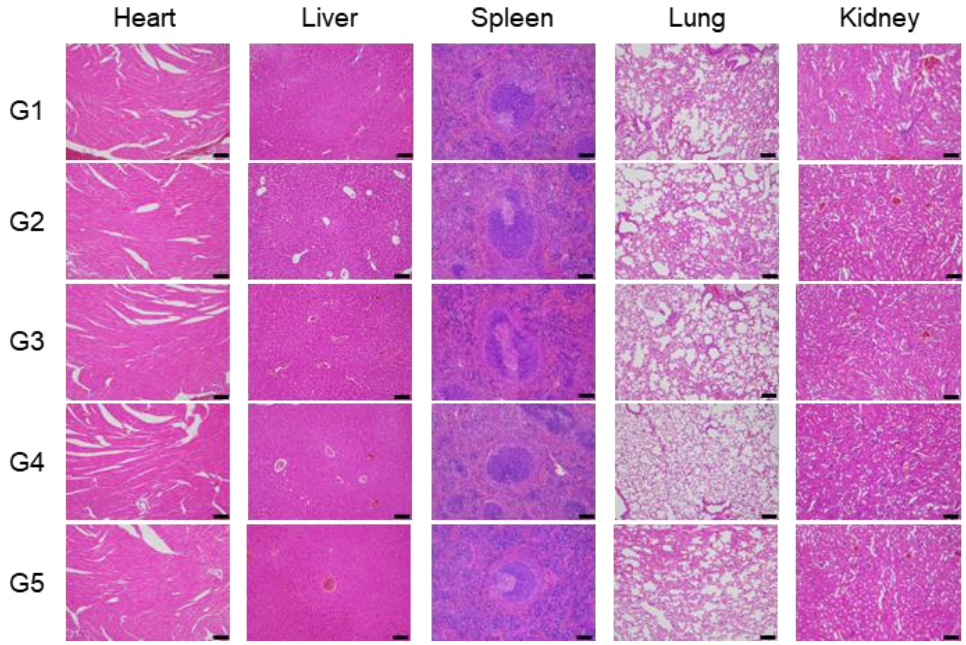
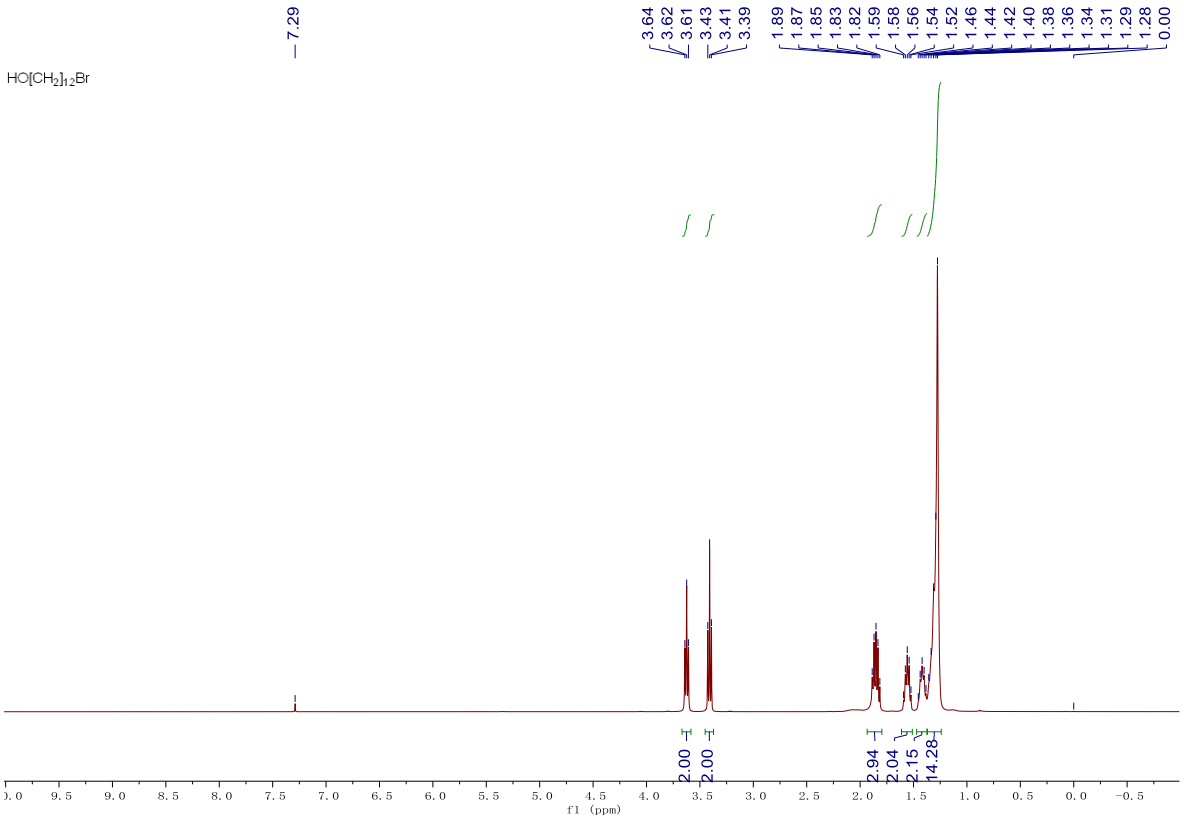


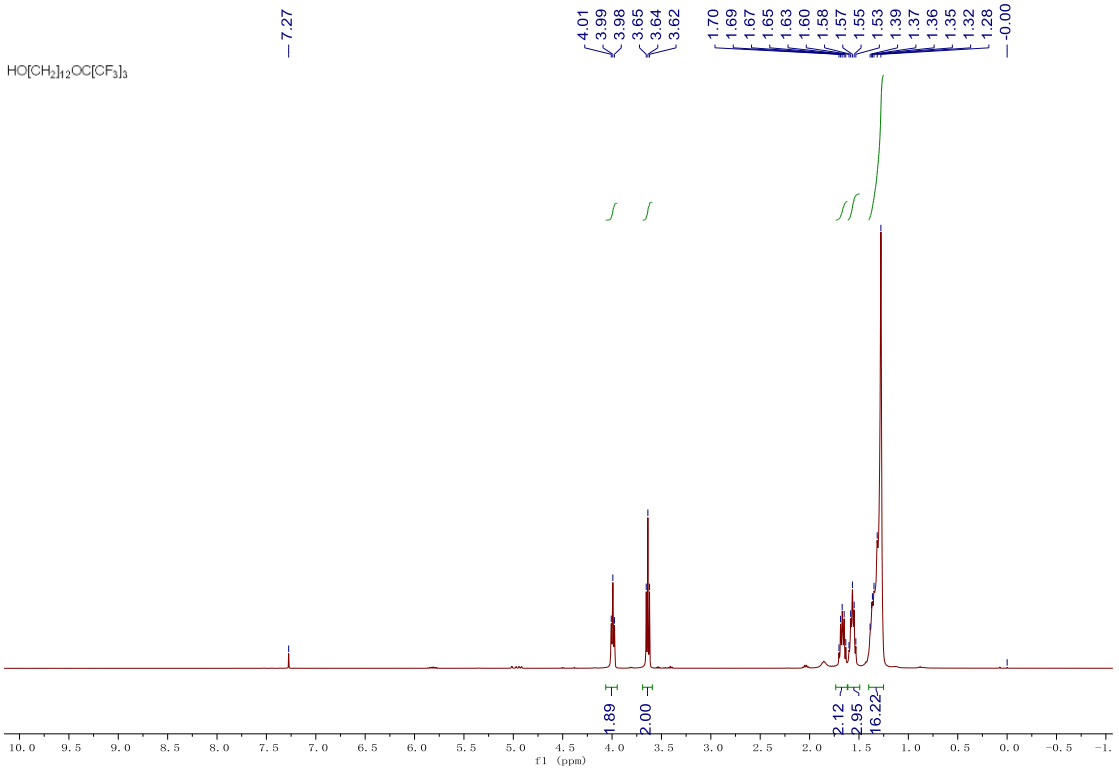
Figure S7. H&E staining assay results of major organs, scale bar: 100 μm.

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

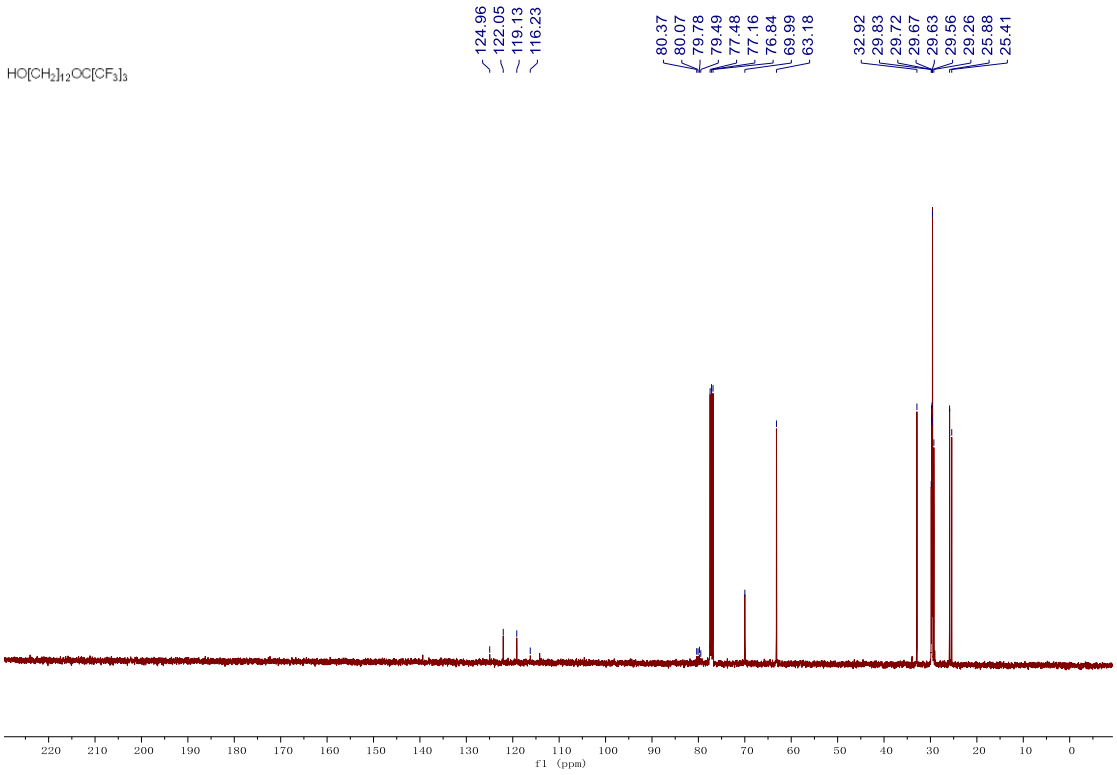
^{1}H NMR of compound **4**



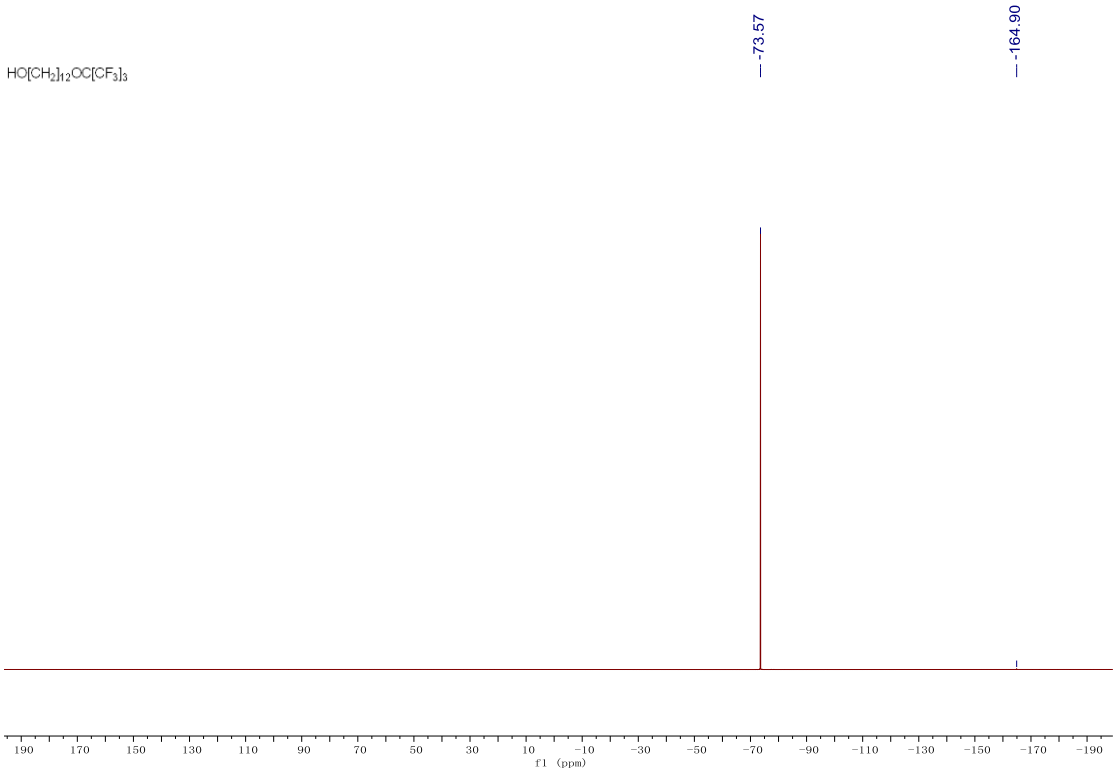
^{1}H NMR of compound **5**



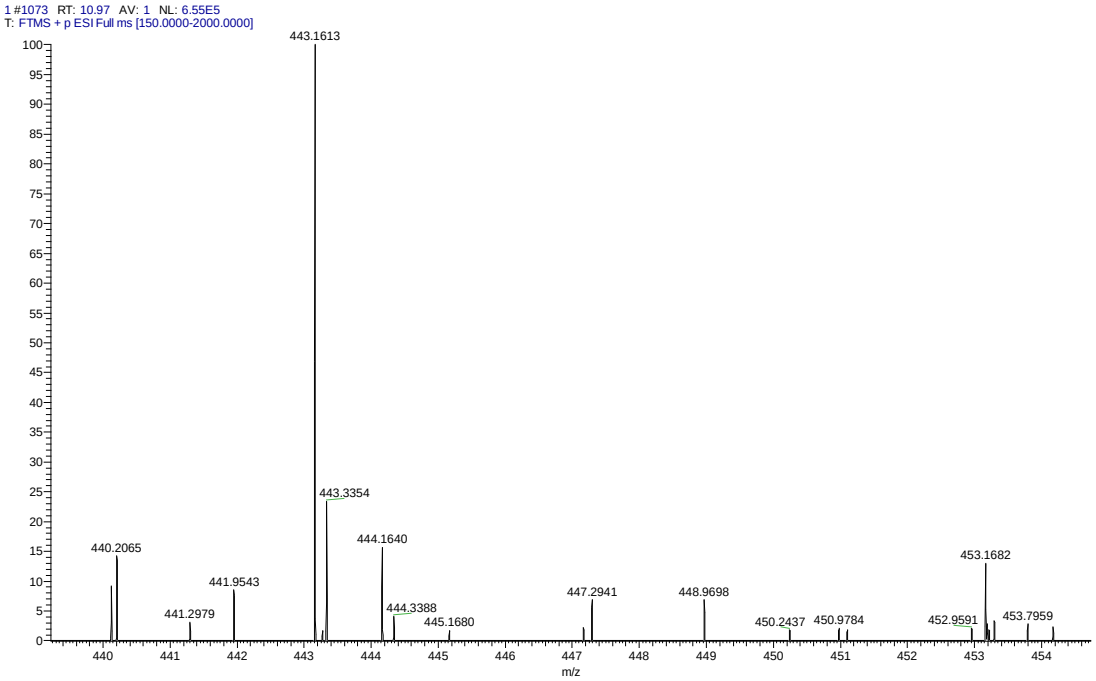
^{13}C NMR of compound **5**



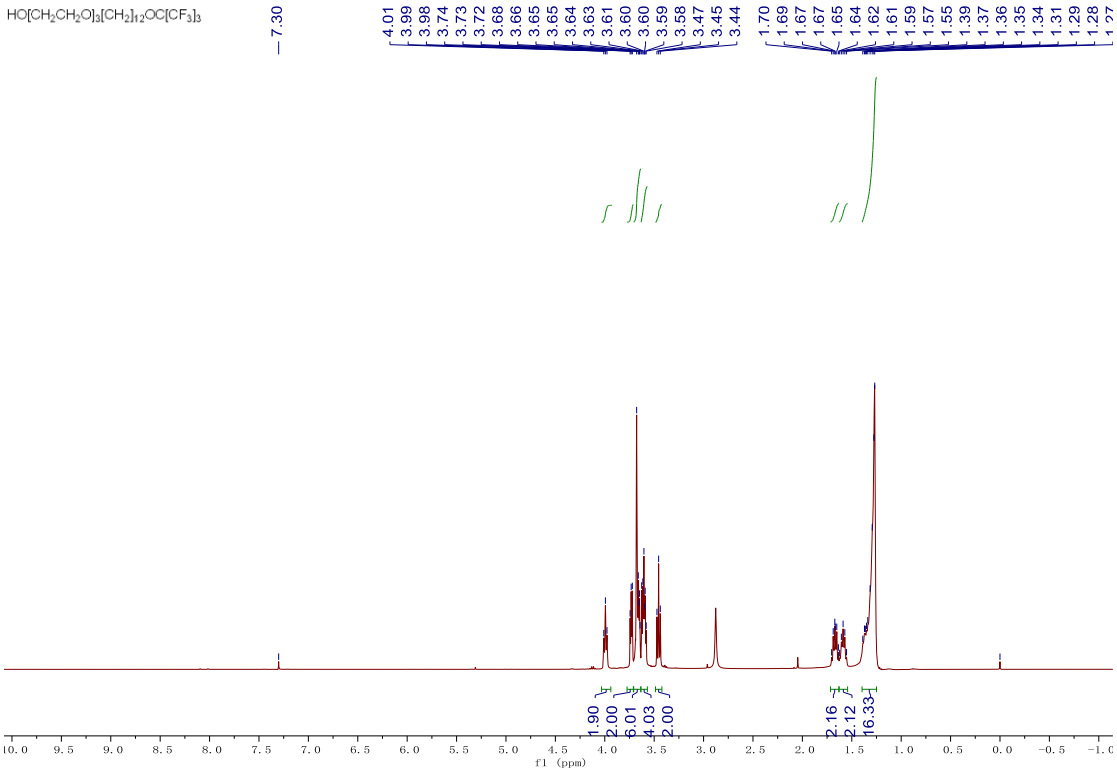
^{19}F NMR of compound **5**



HRMS of compound **5**



^{1}H NMR of compound **7**



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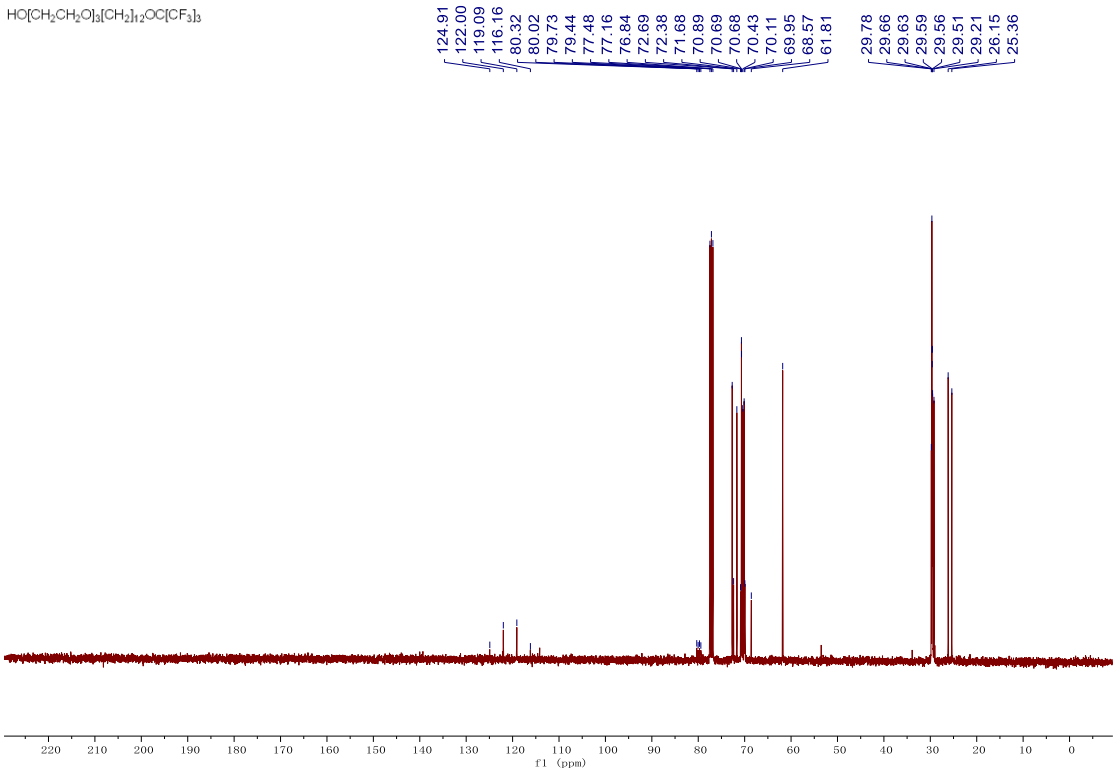
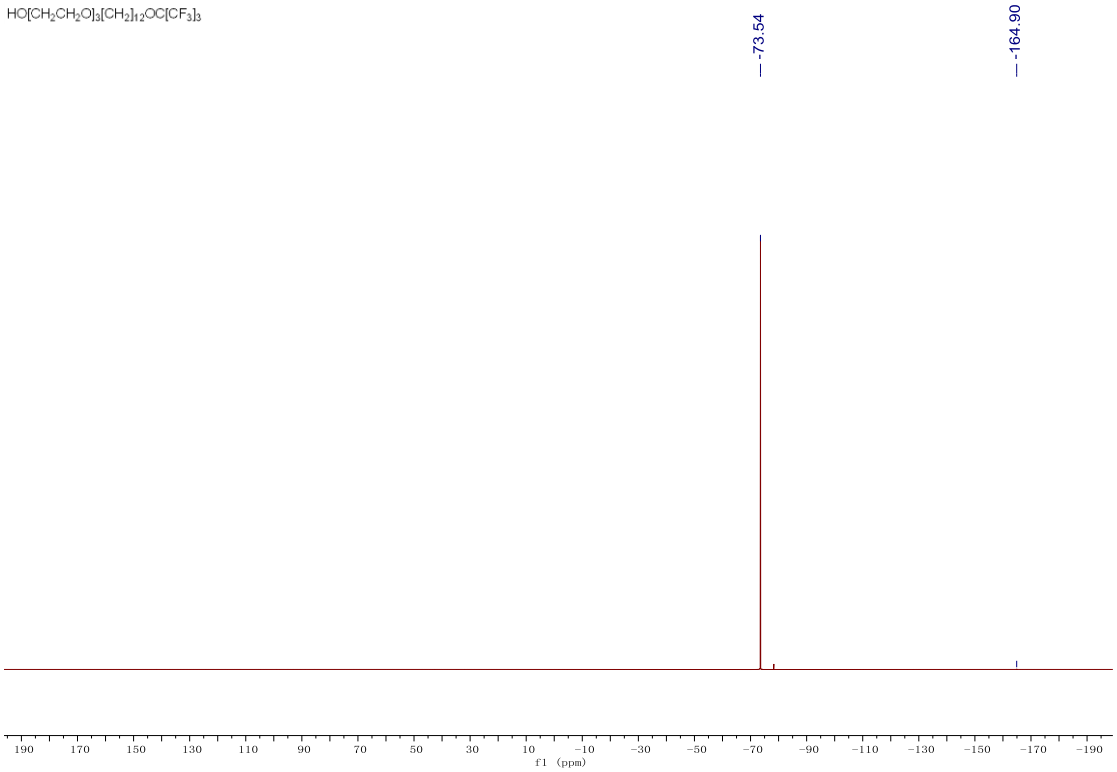
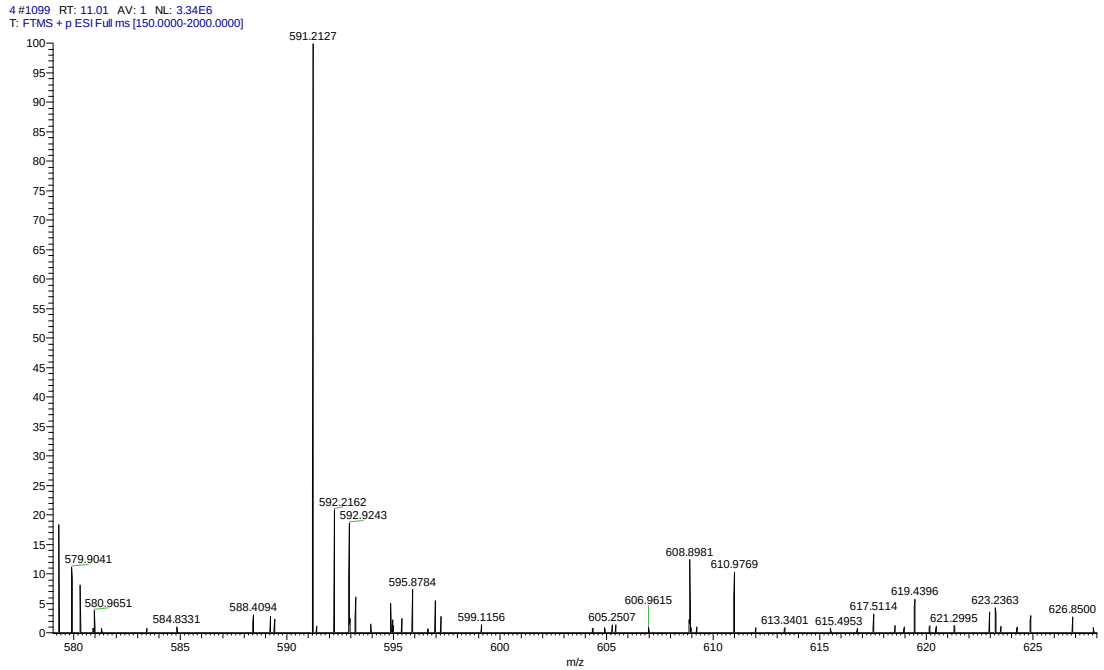


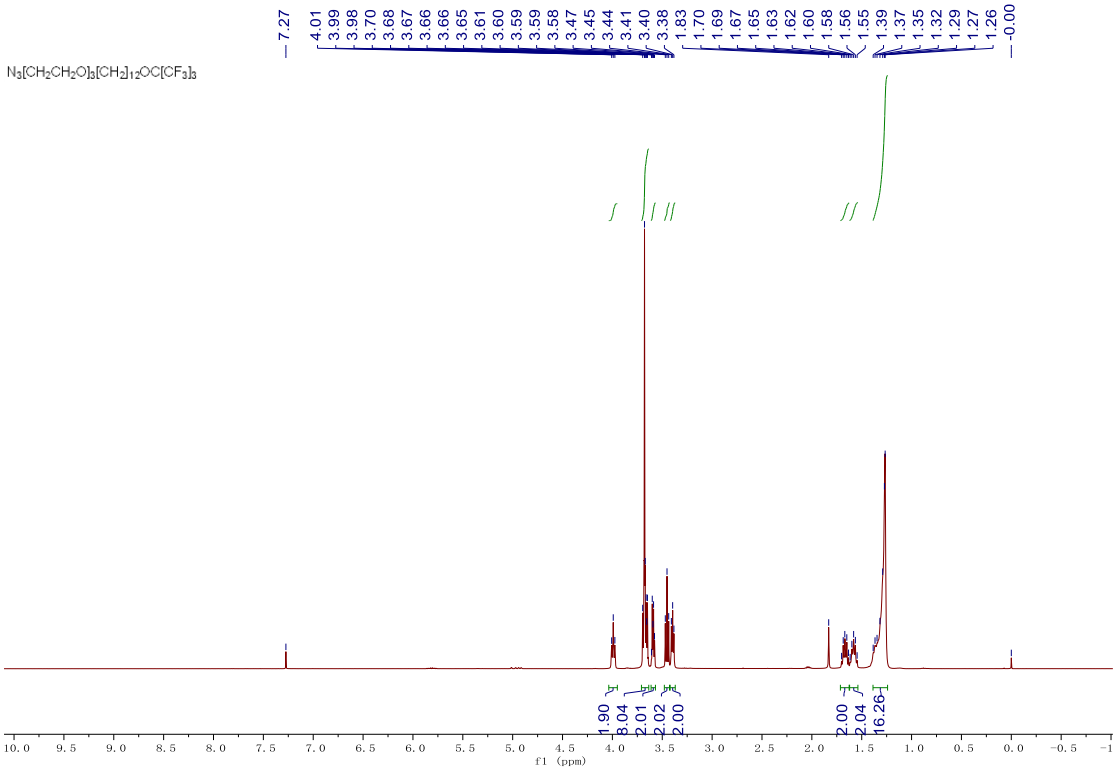
^{19}F NMR of compound **7**



HRMS of compound **7**



^{1}H NMR of compound **8**



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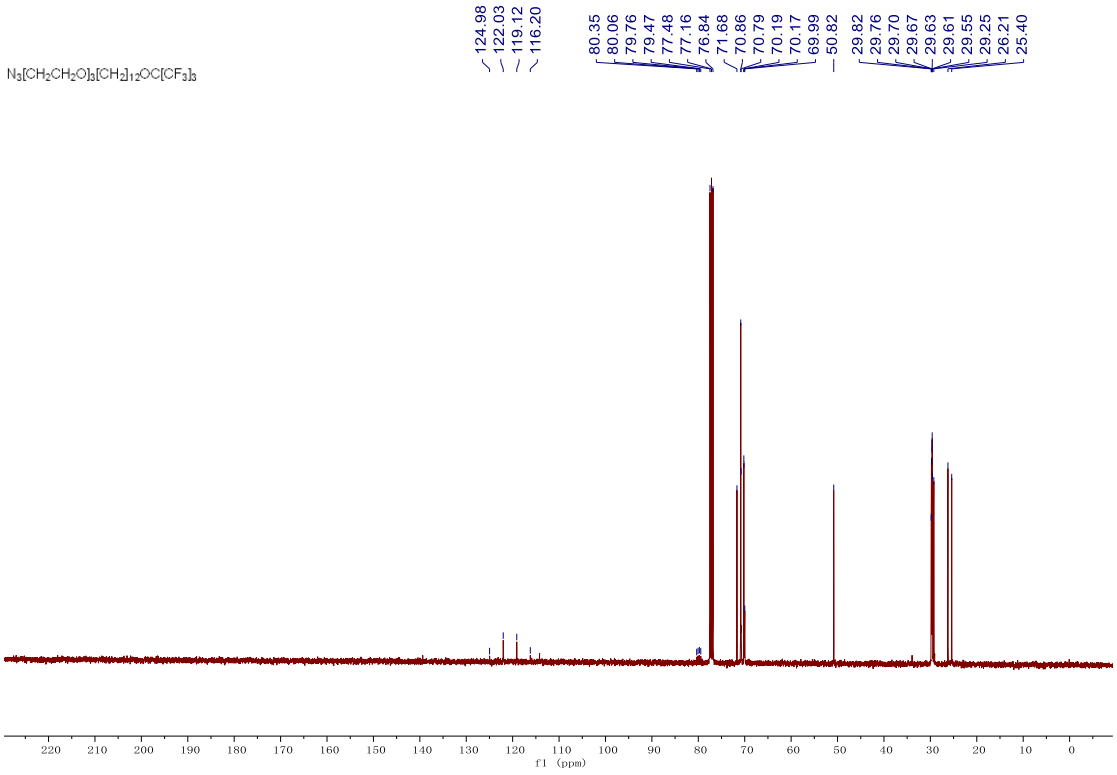

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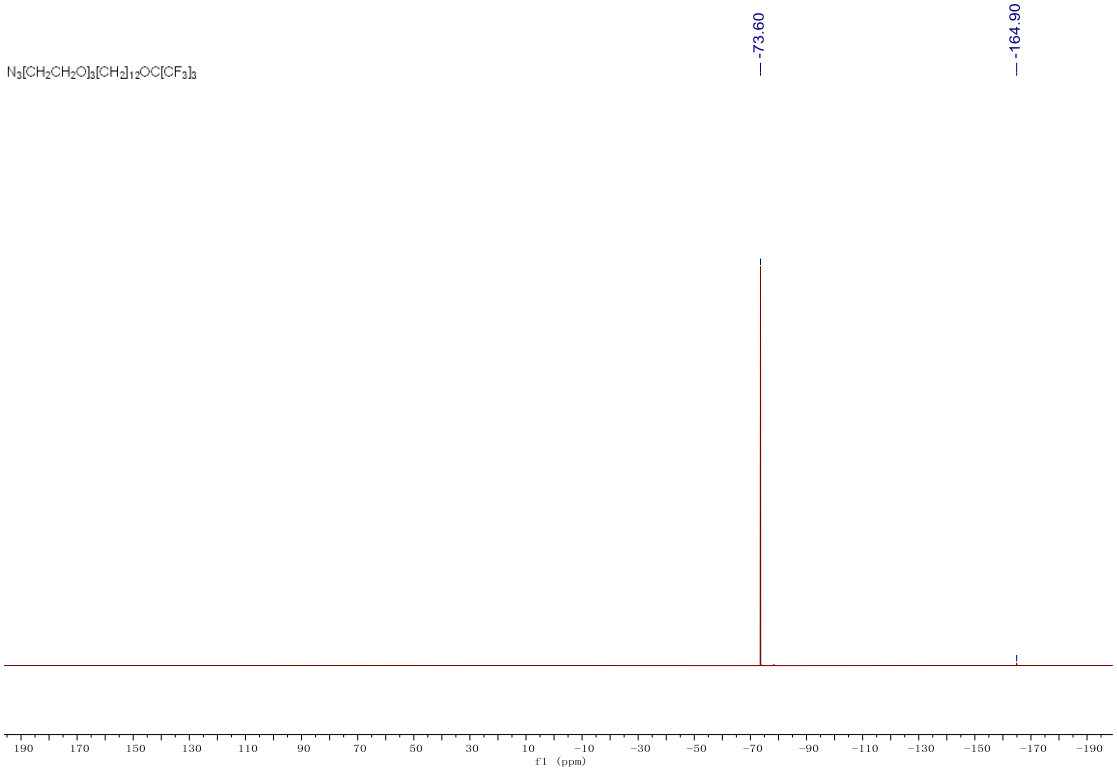

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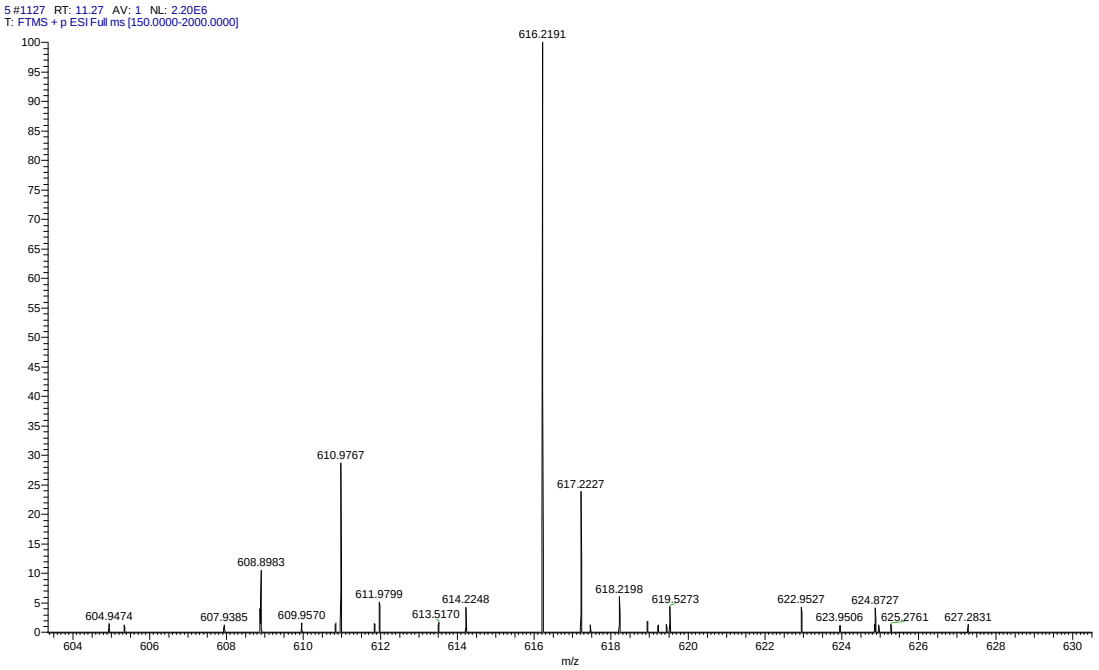

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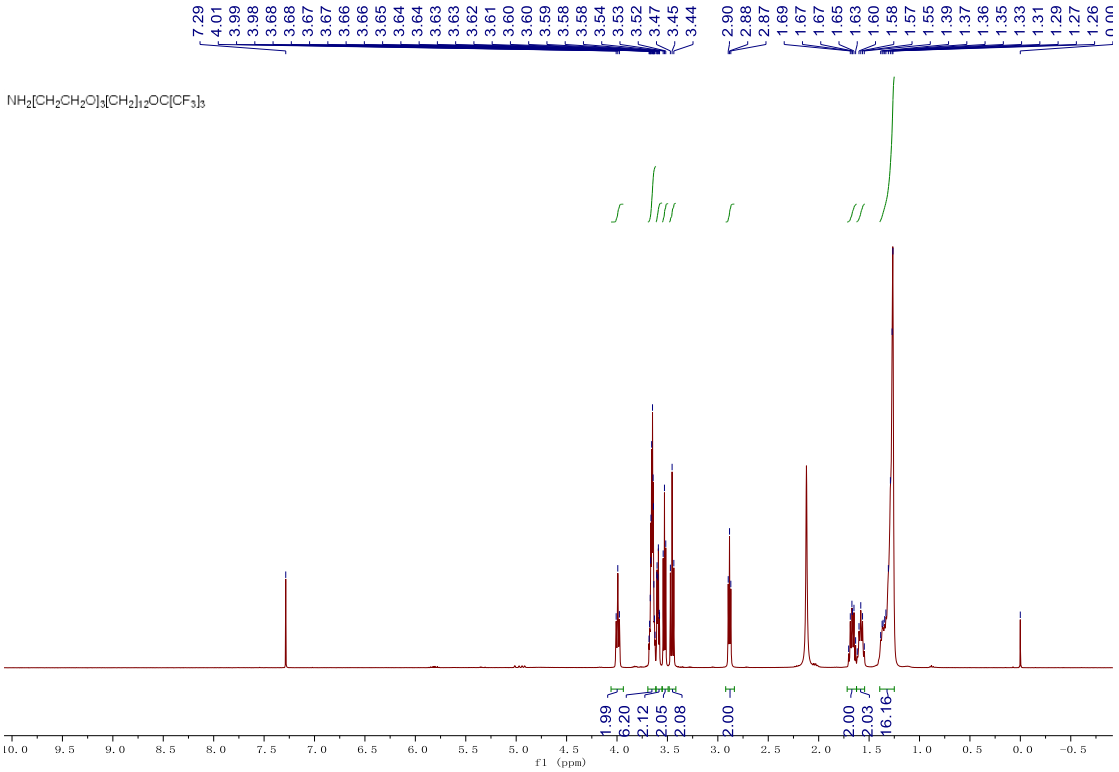

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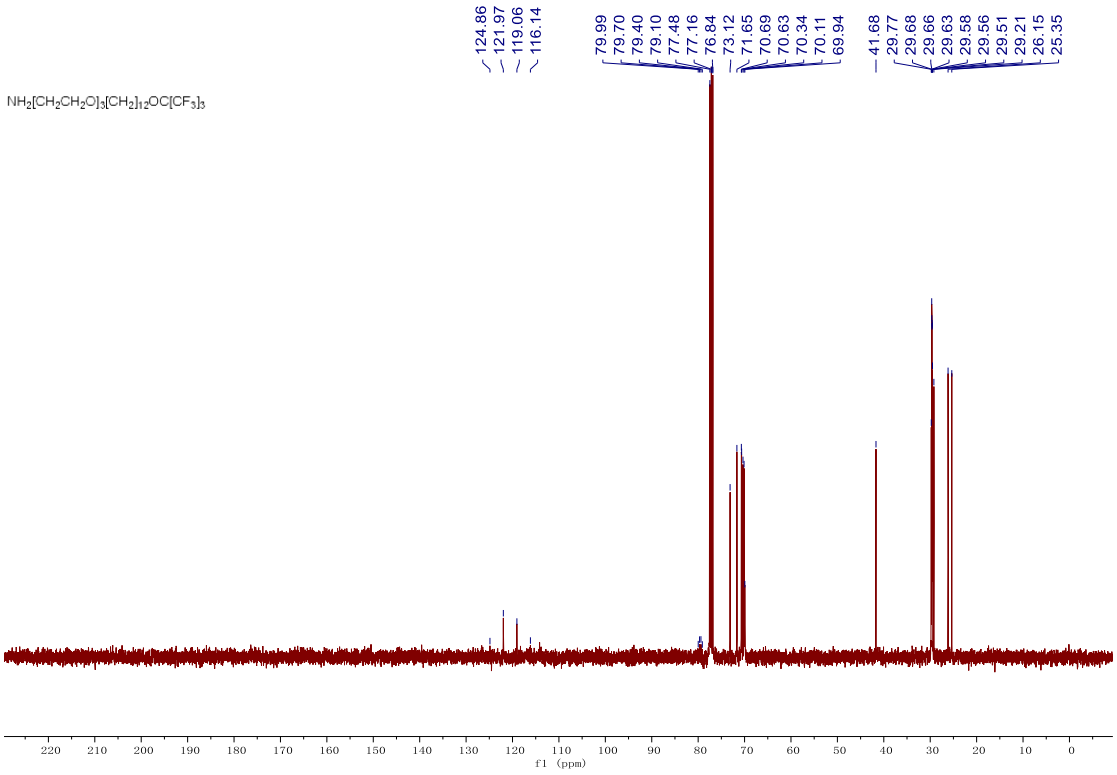

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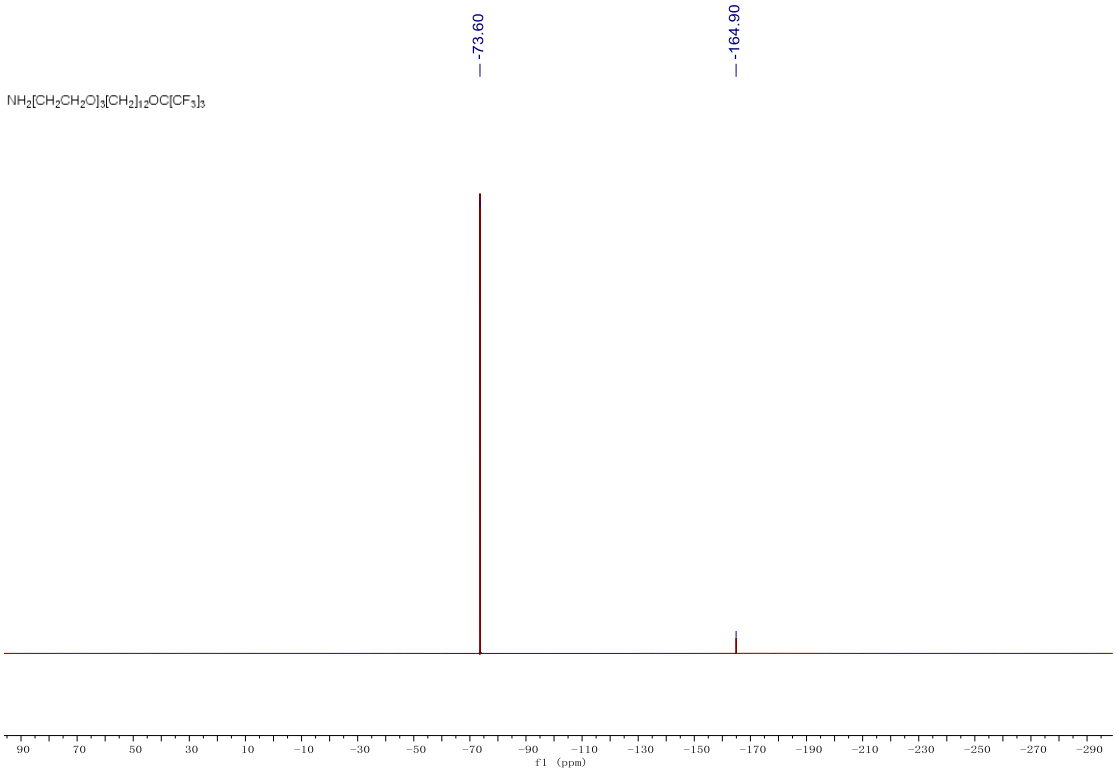

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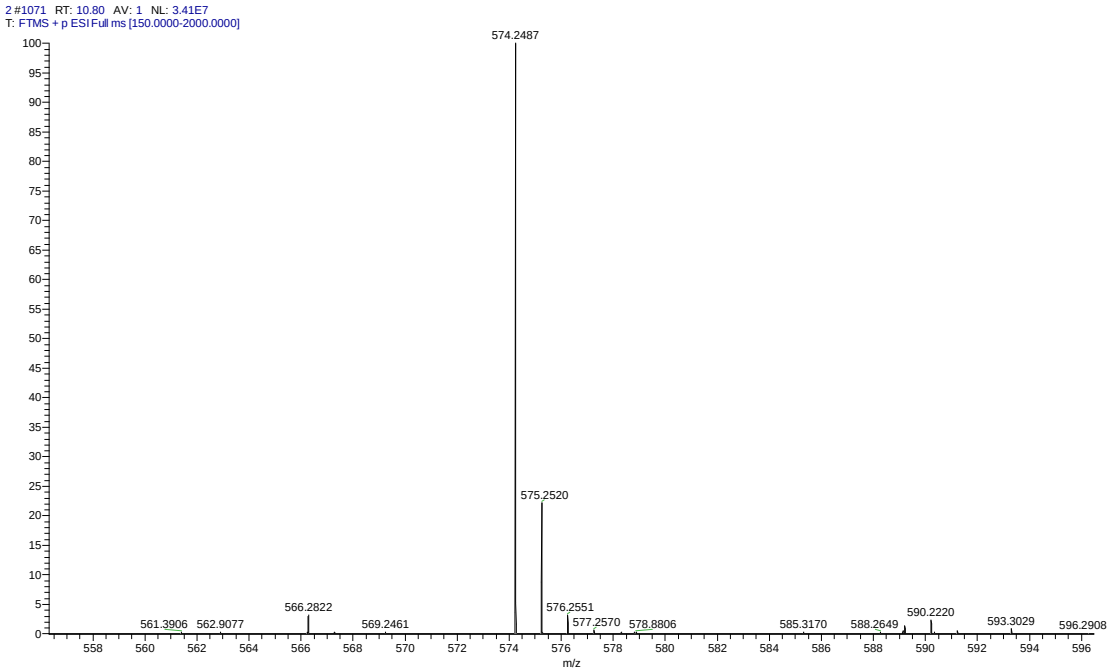

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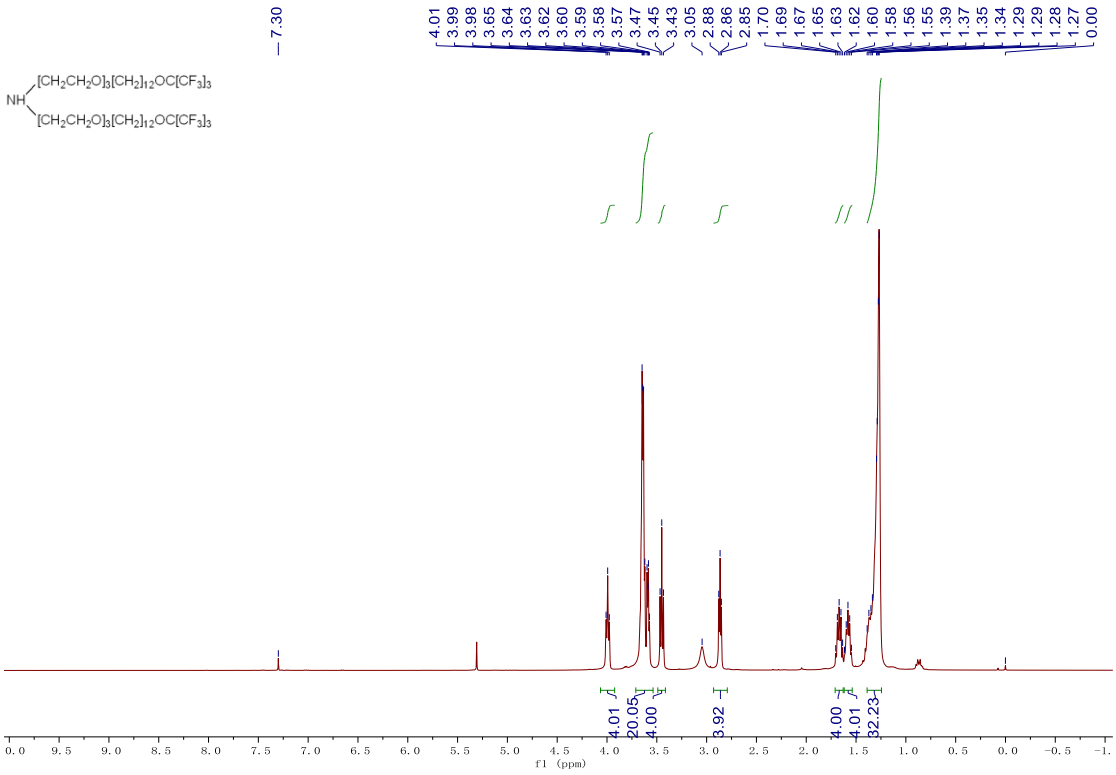

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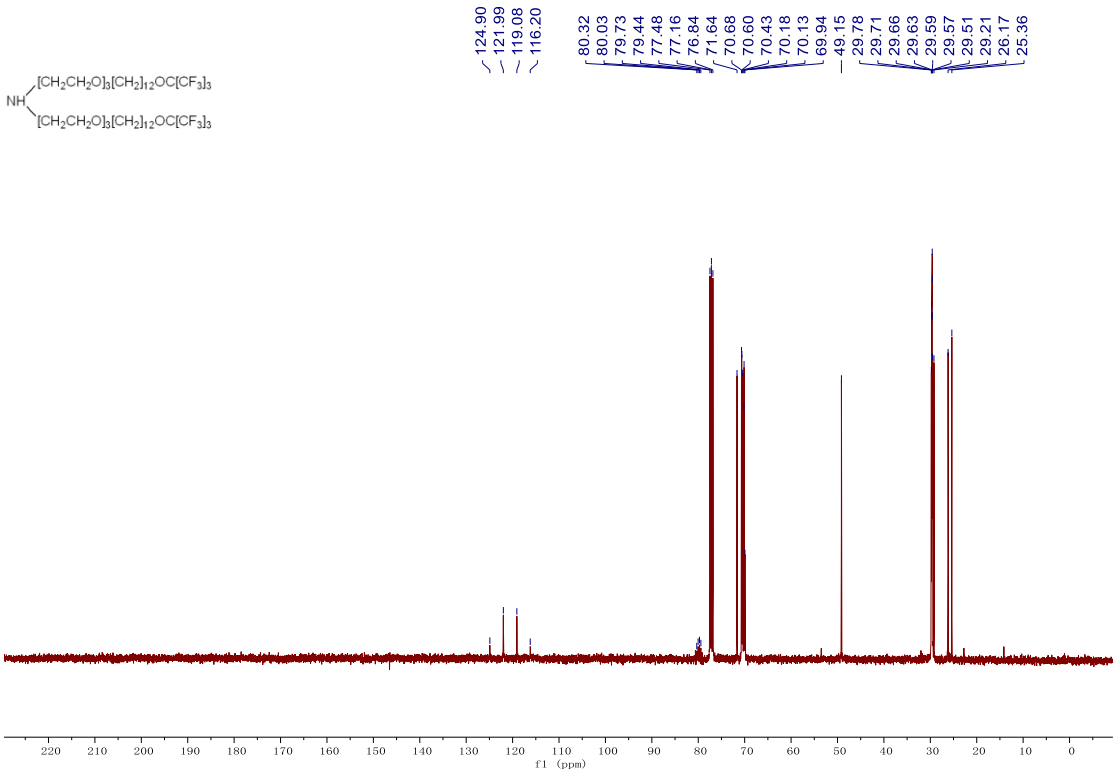

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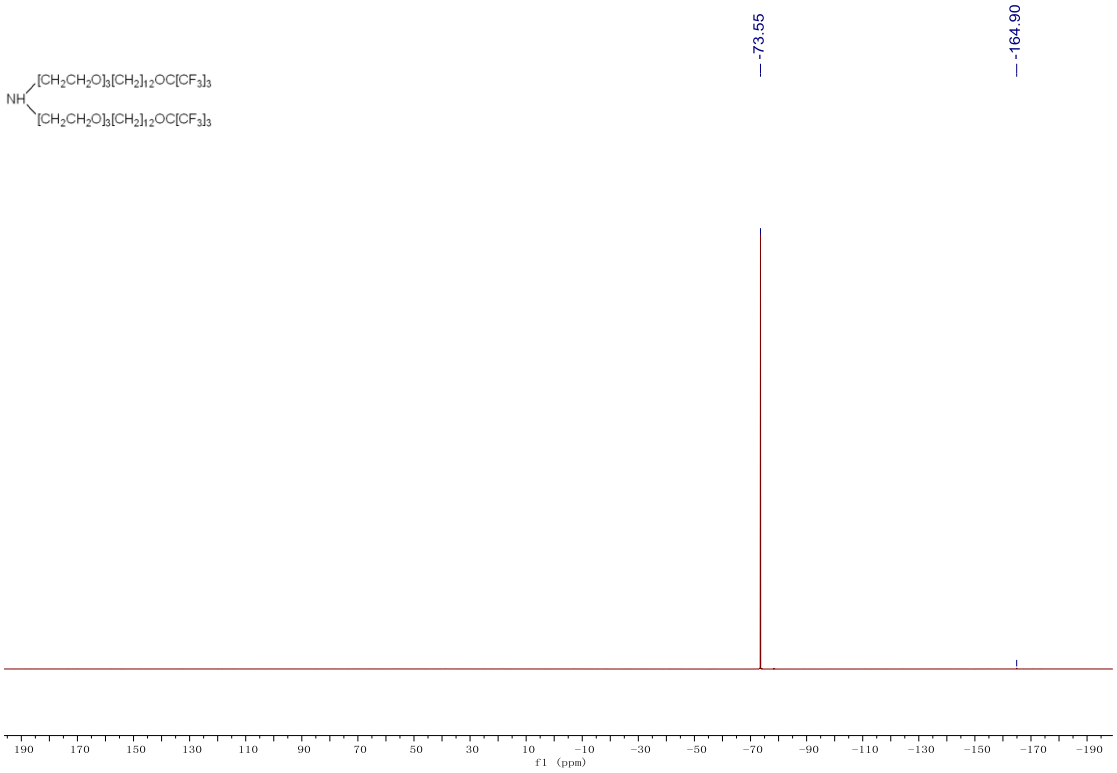

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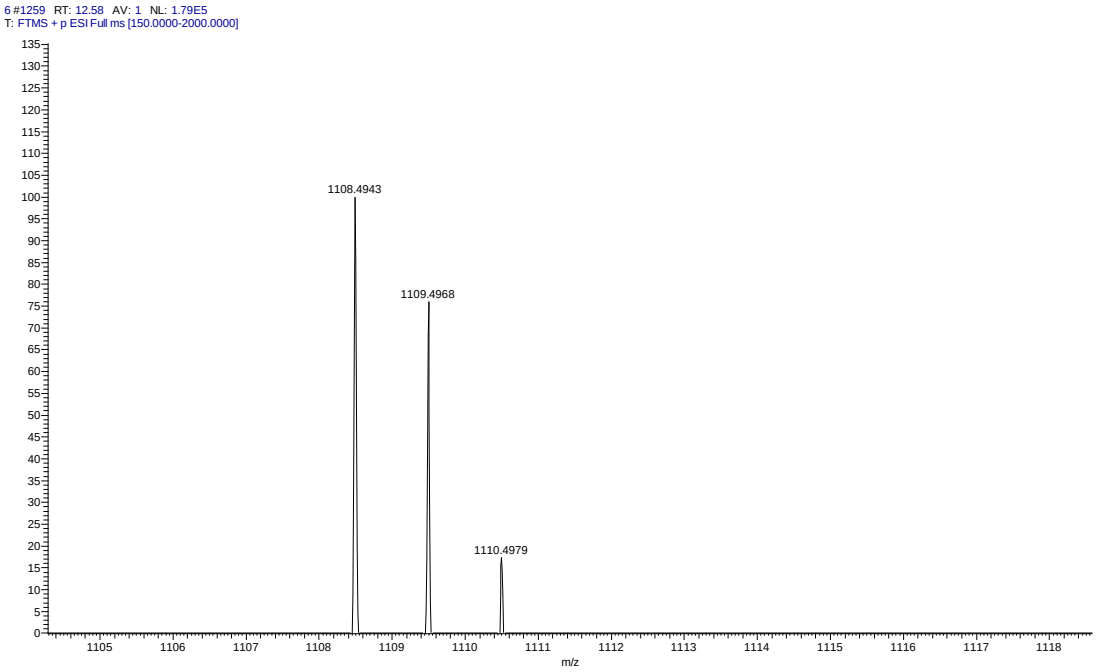
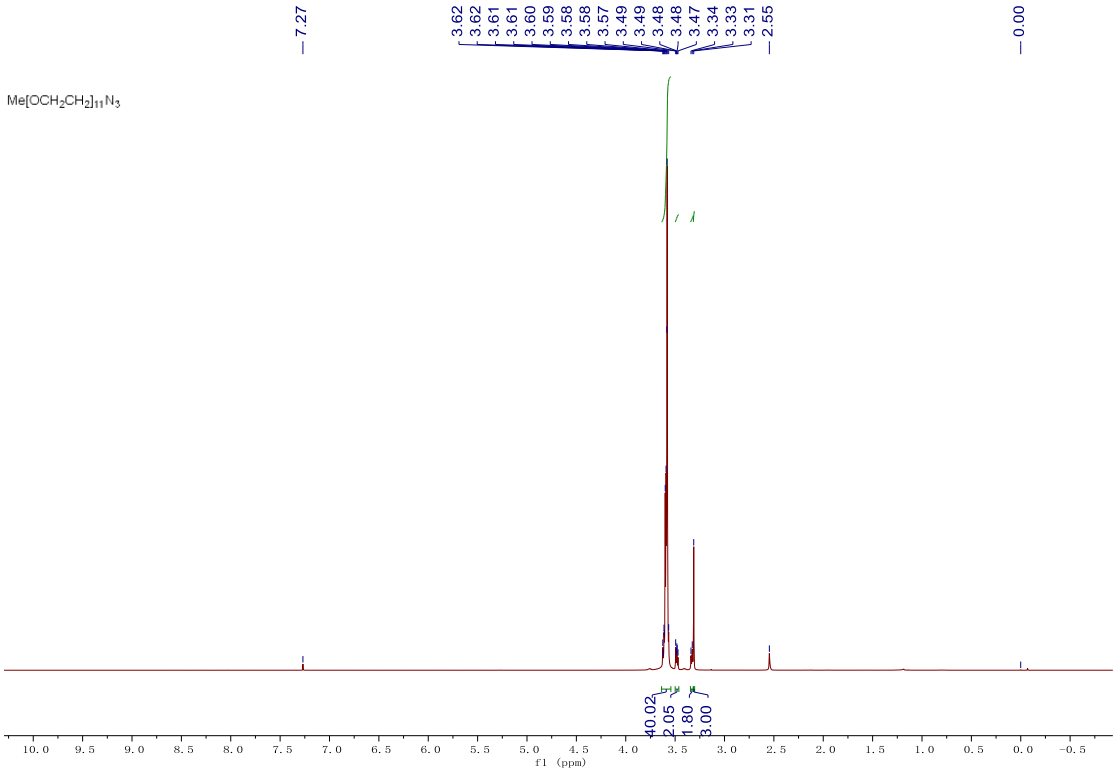


^{1}H NMR of compound **12**



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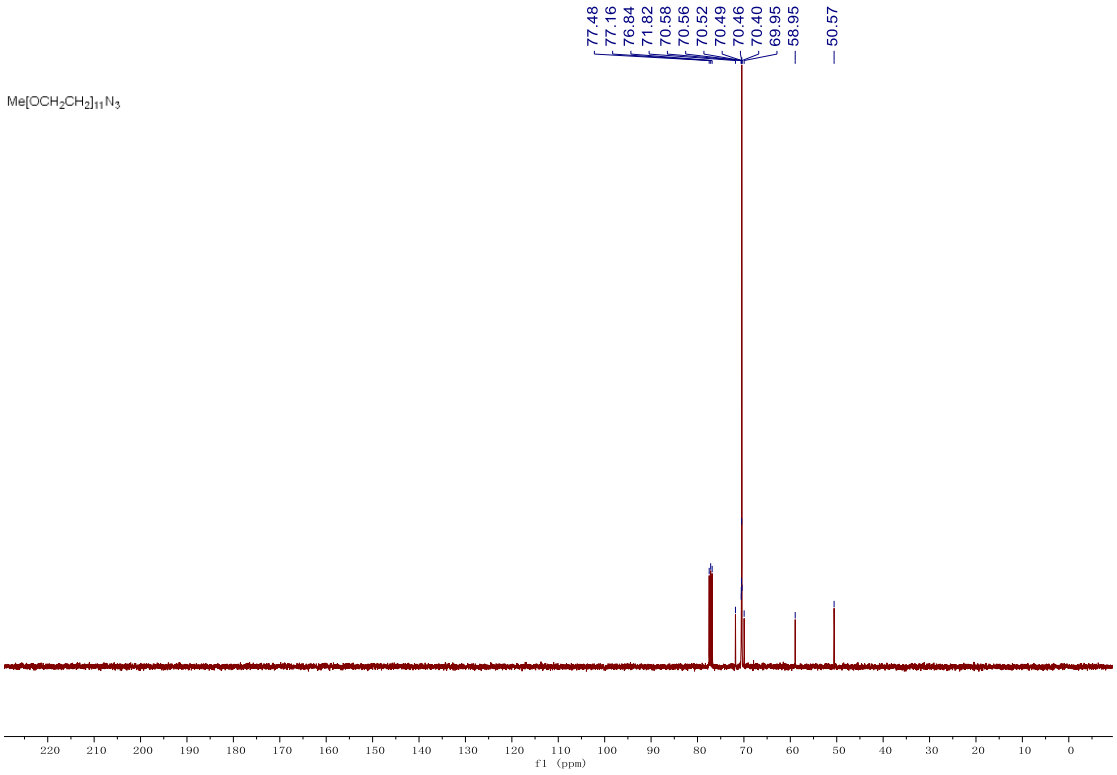
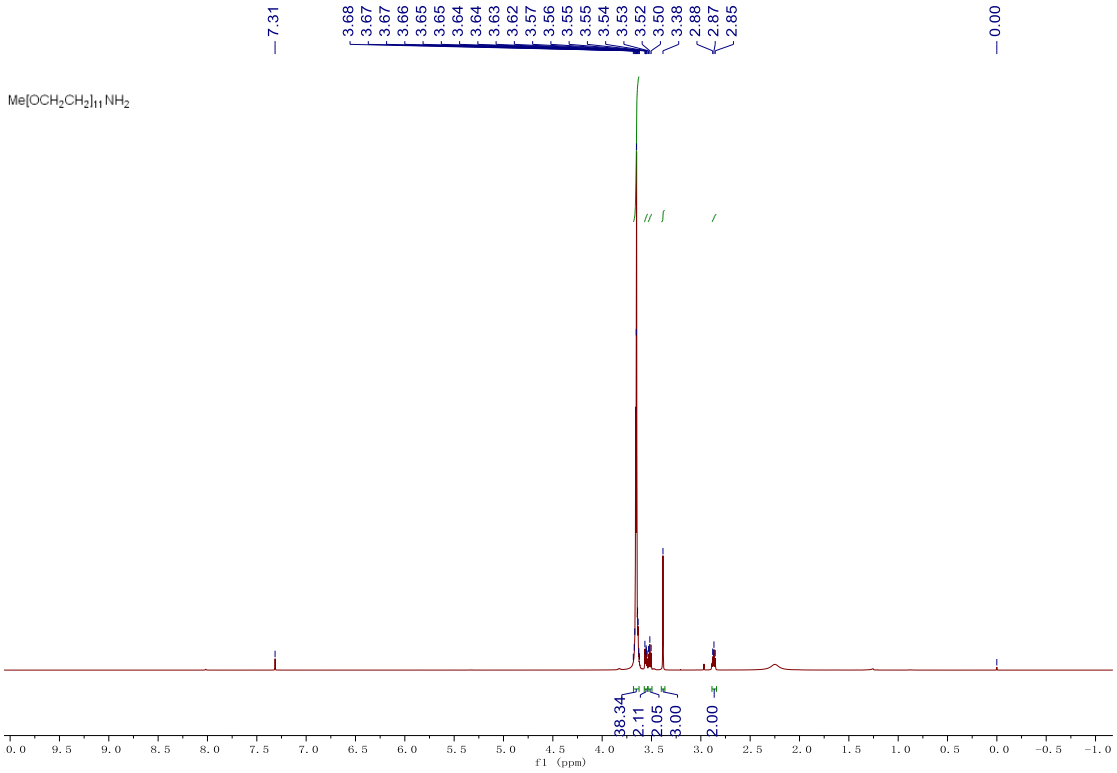


^{1}H NMR of compound **13**



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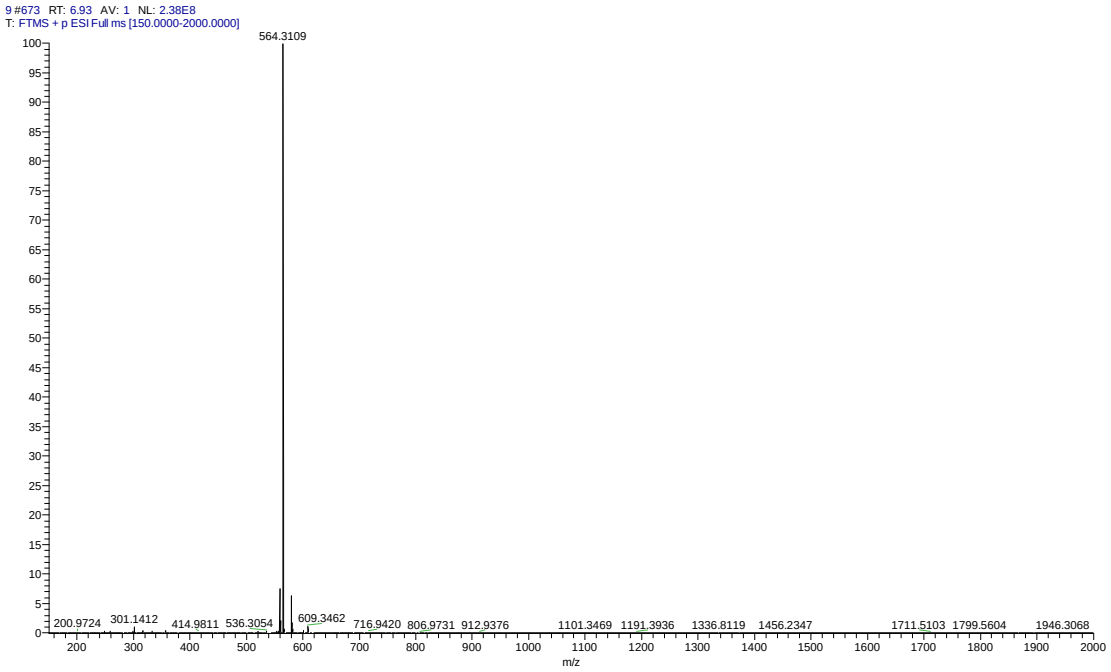

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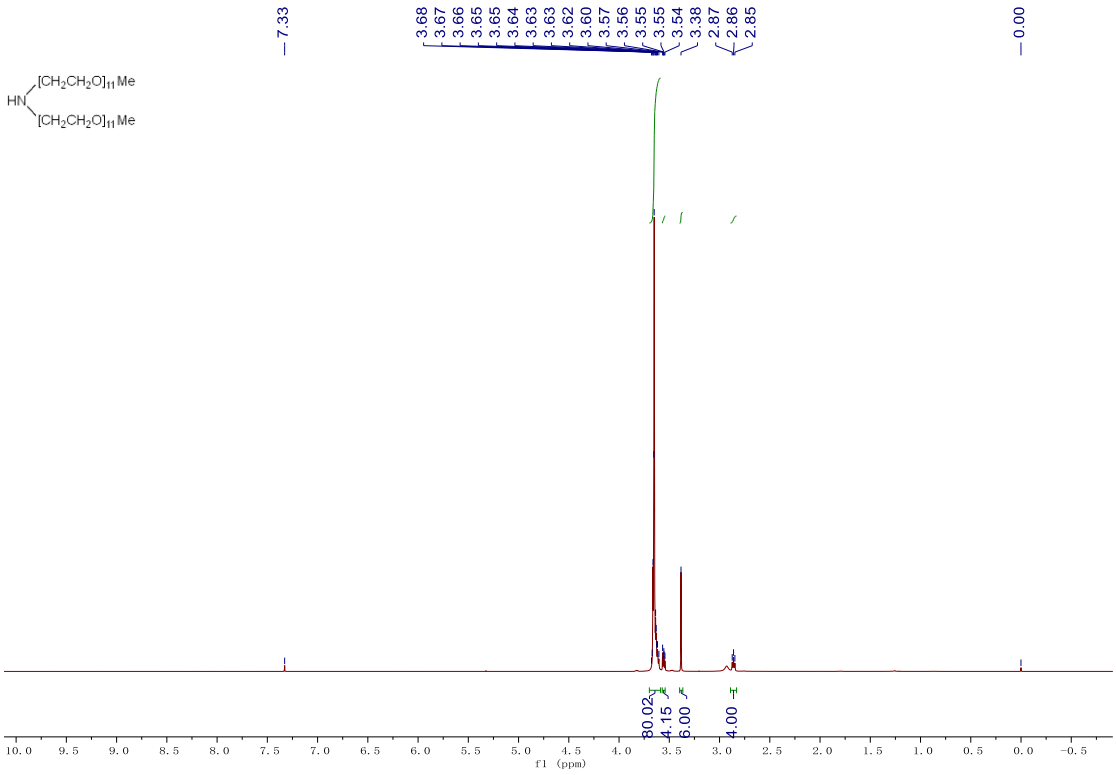

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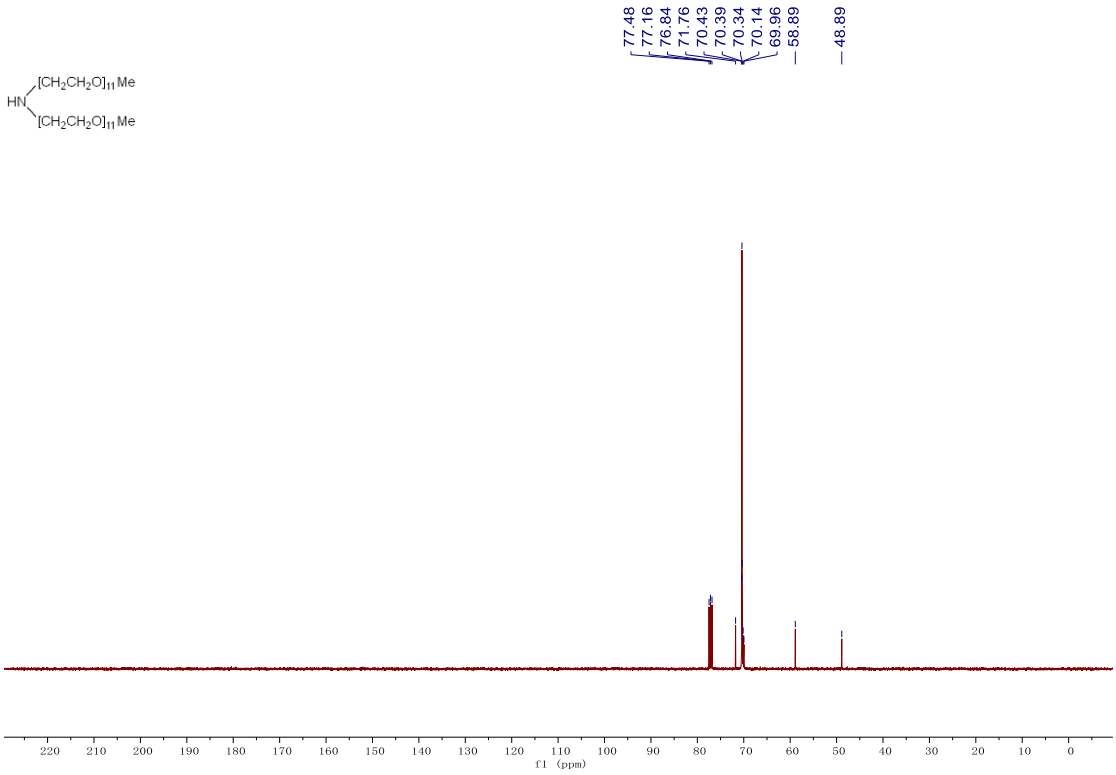
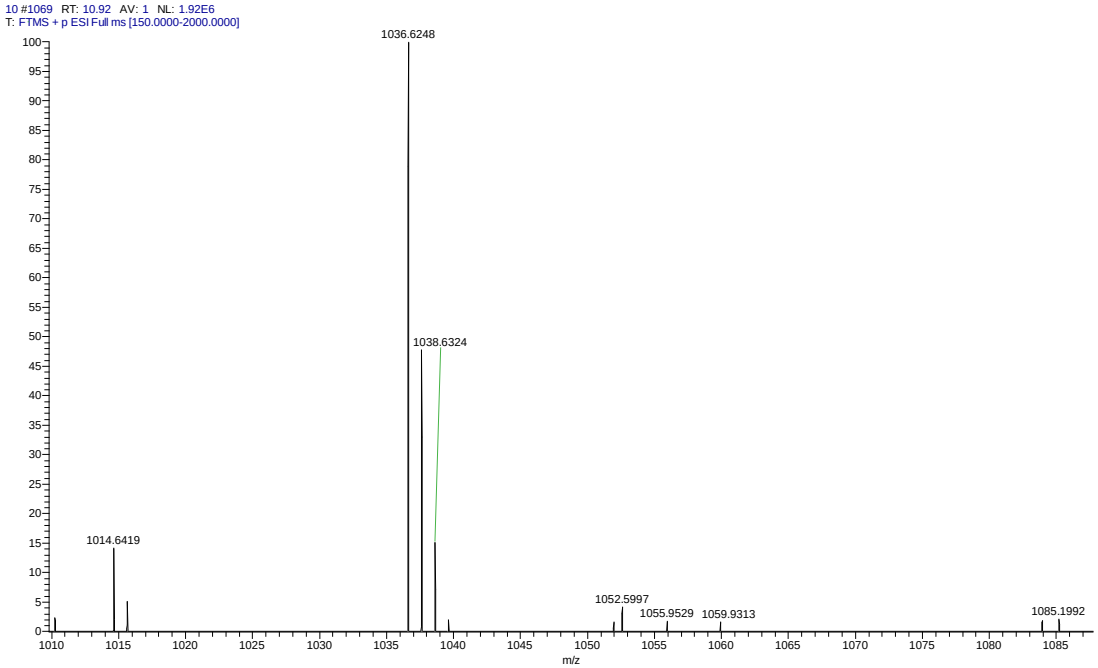
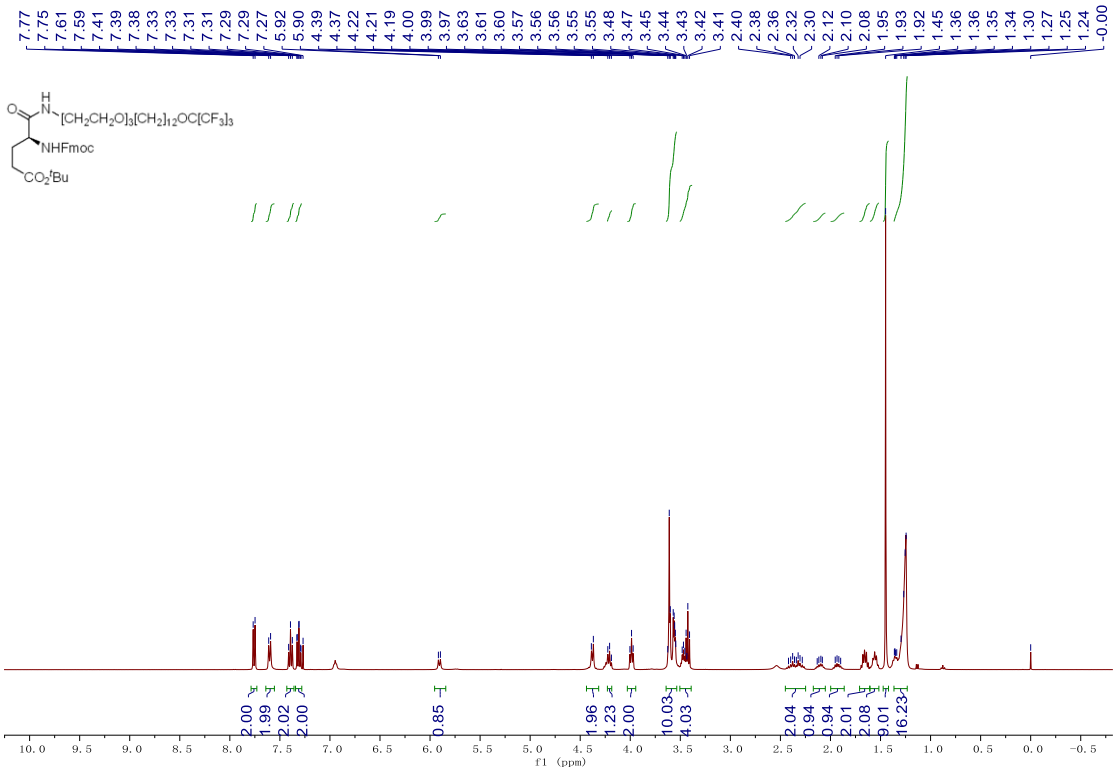


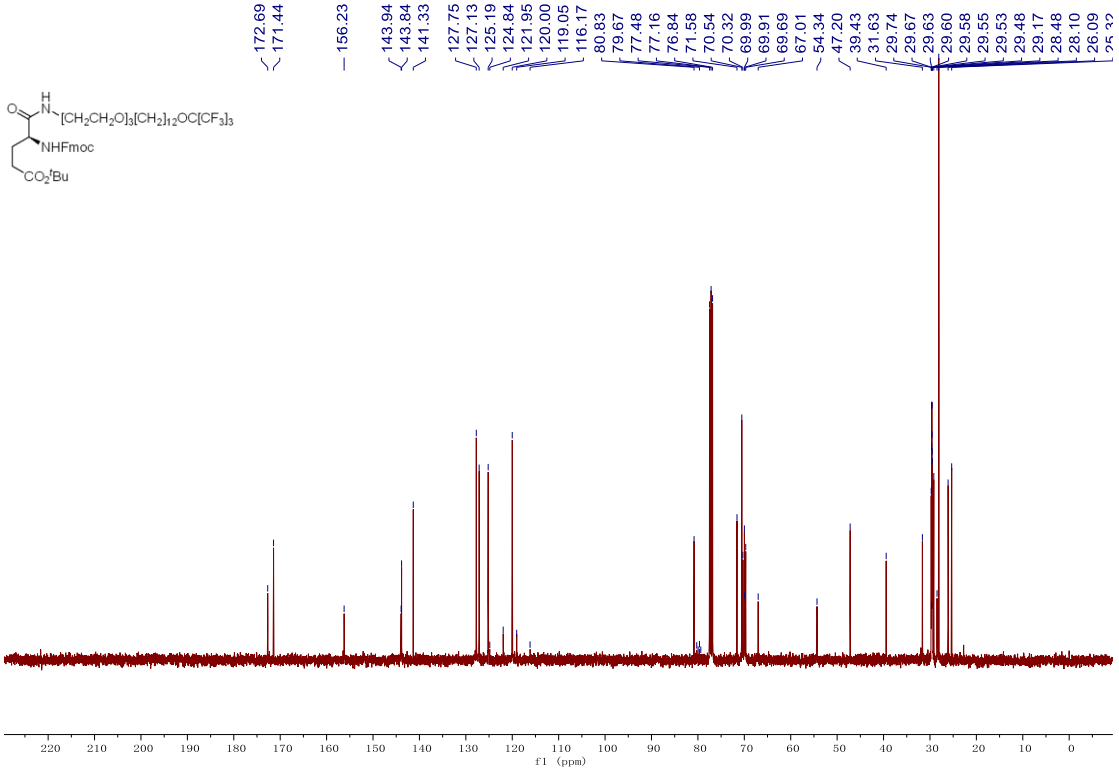
HRMS of compound **14**



^{1}H NMR of compound **16**



^{13}C NMR of compound **16**



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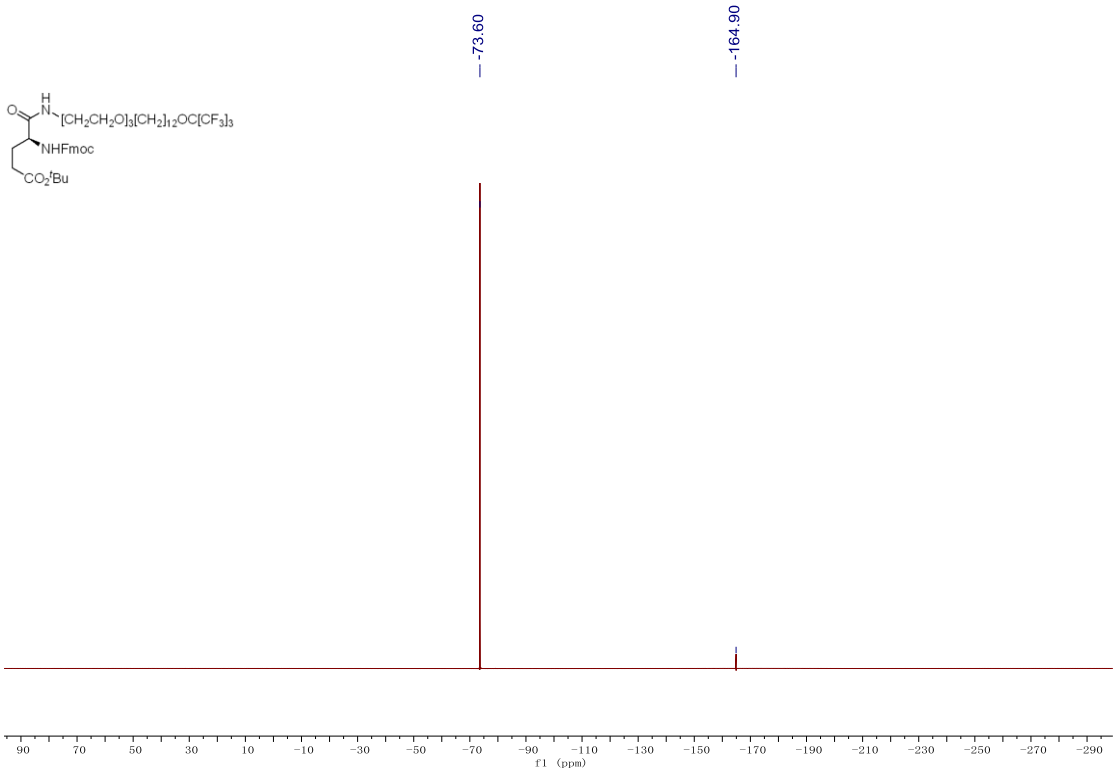
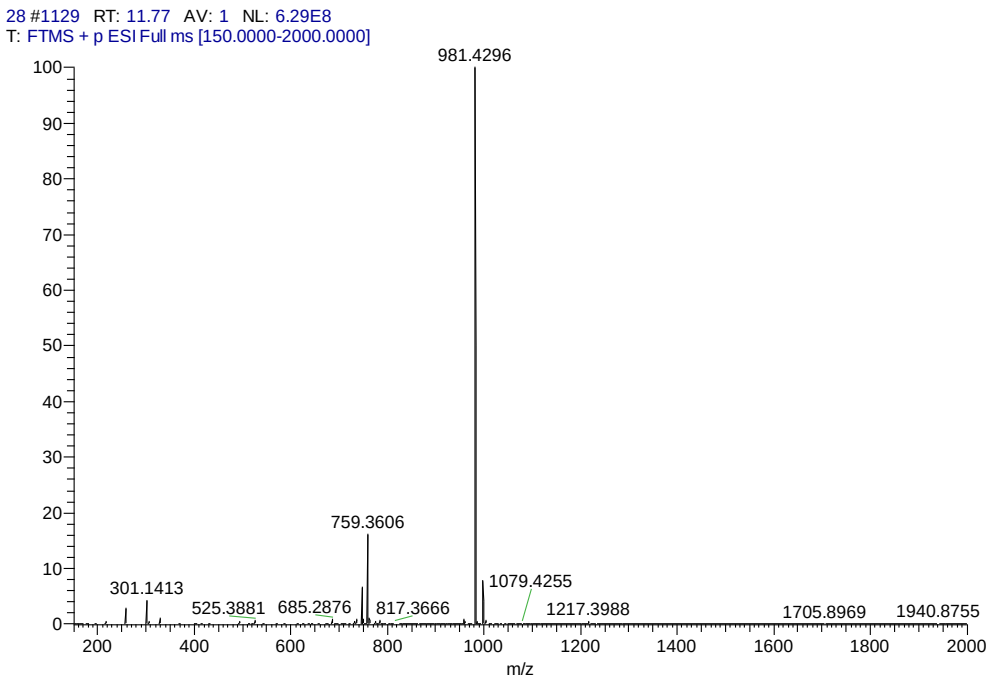


HRMS of compound **16**



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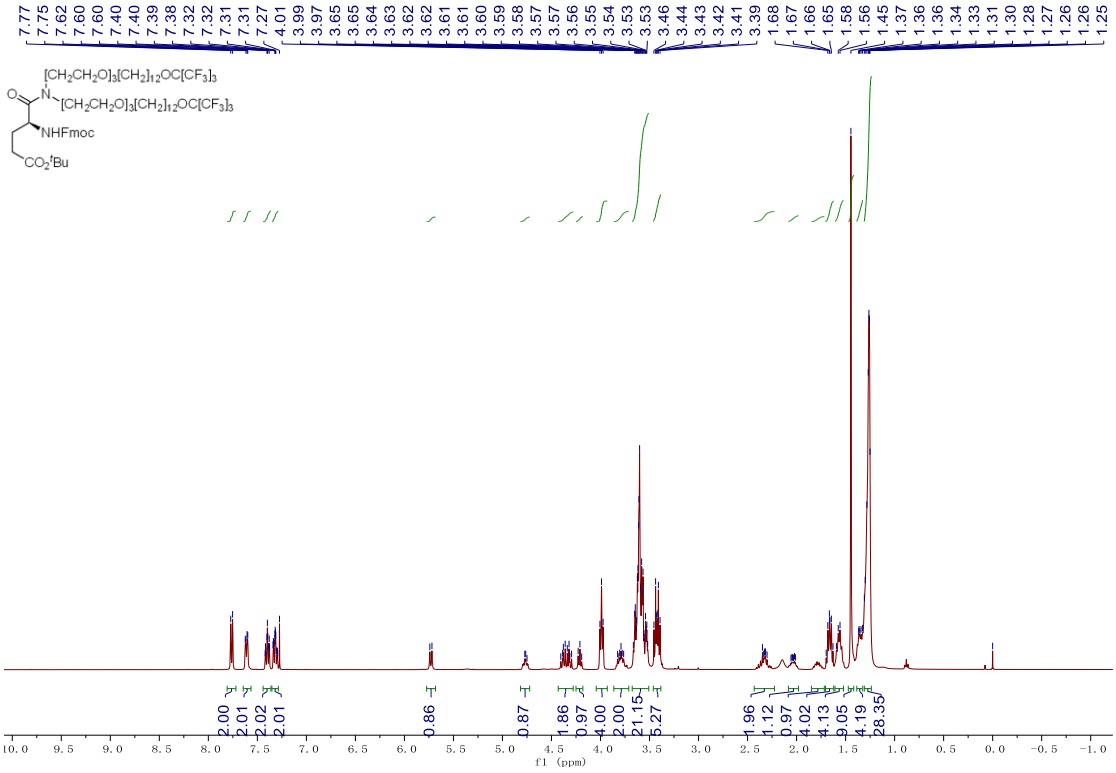

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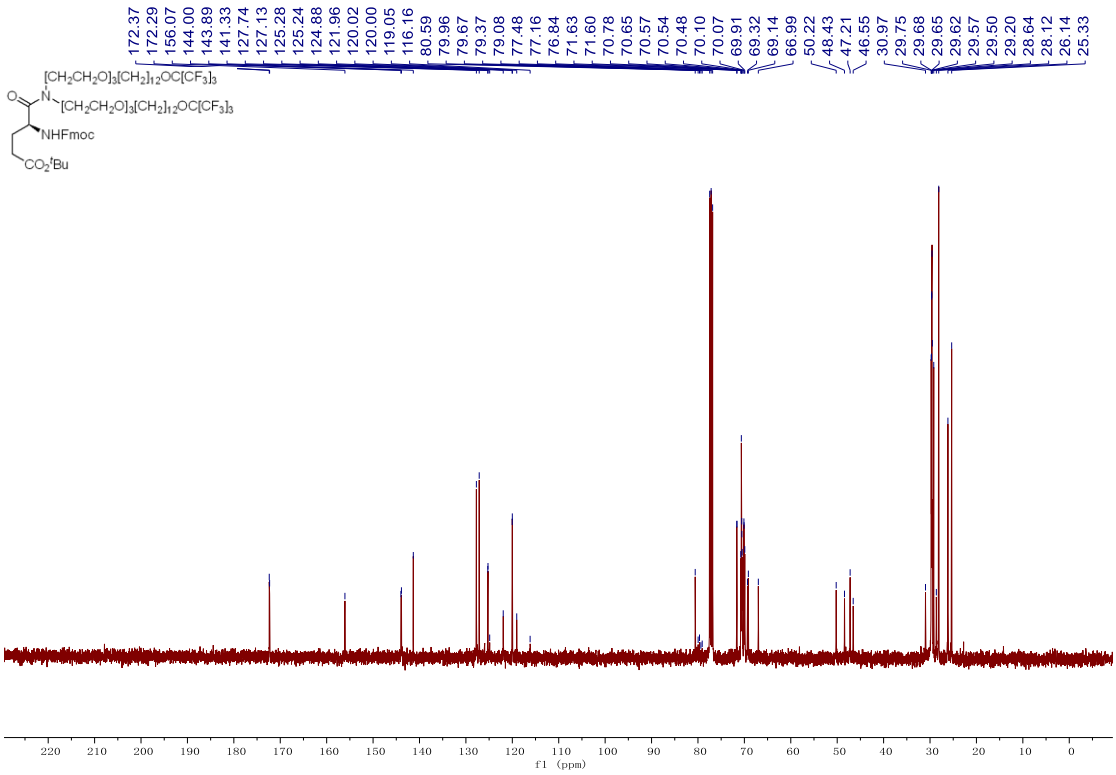

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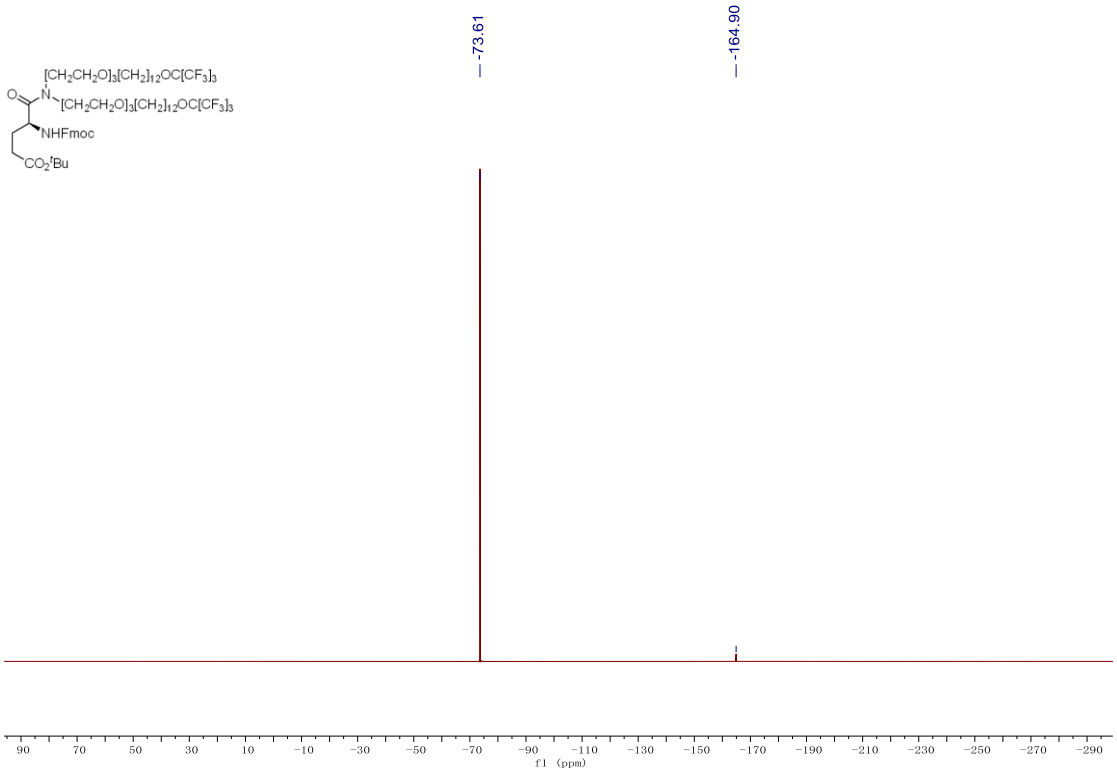
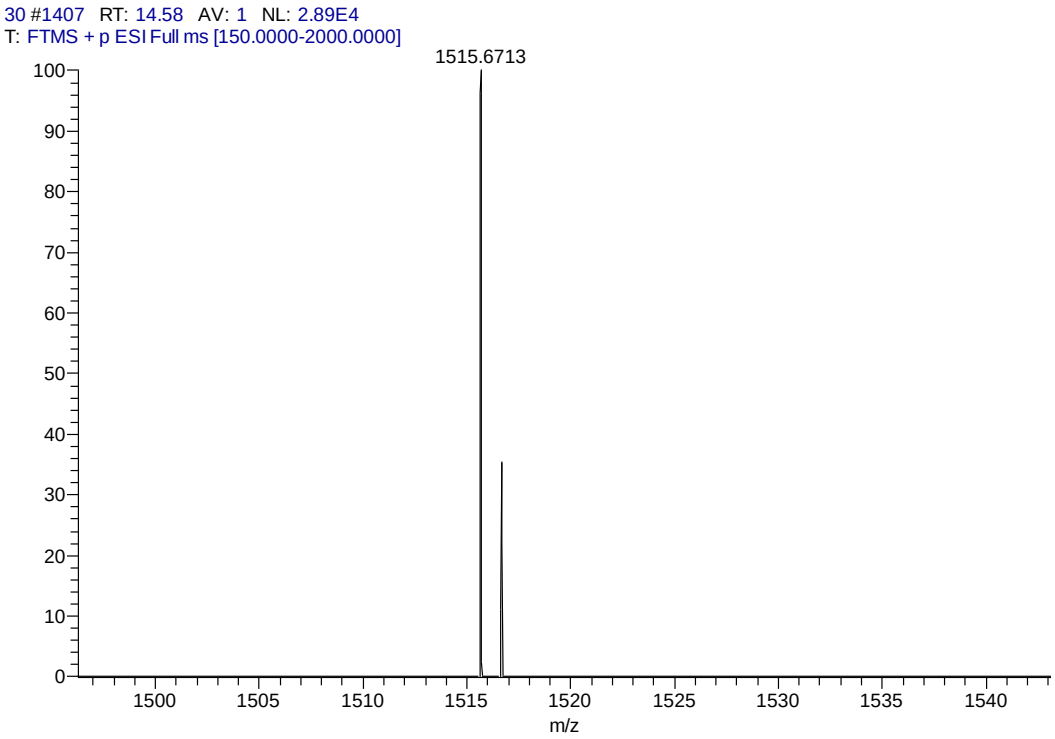
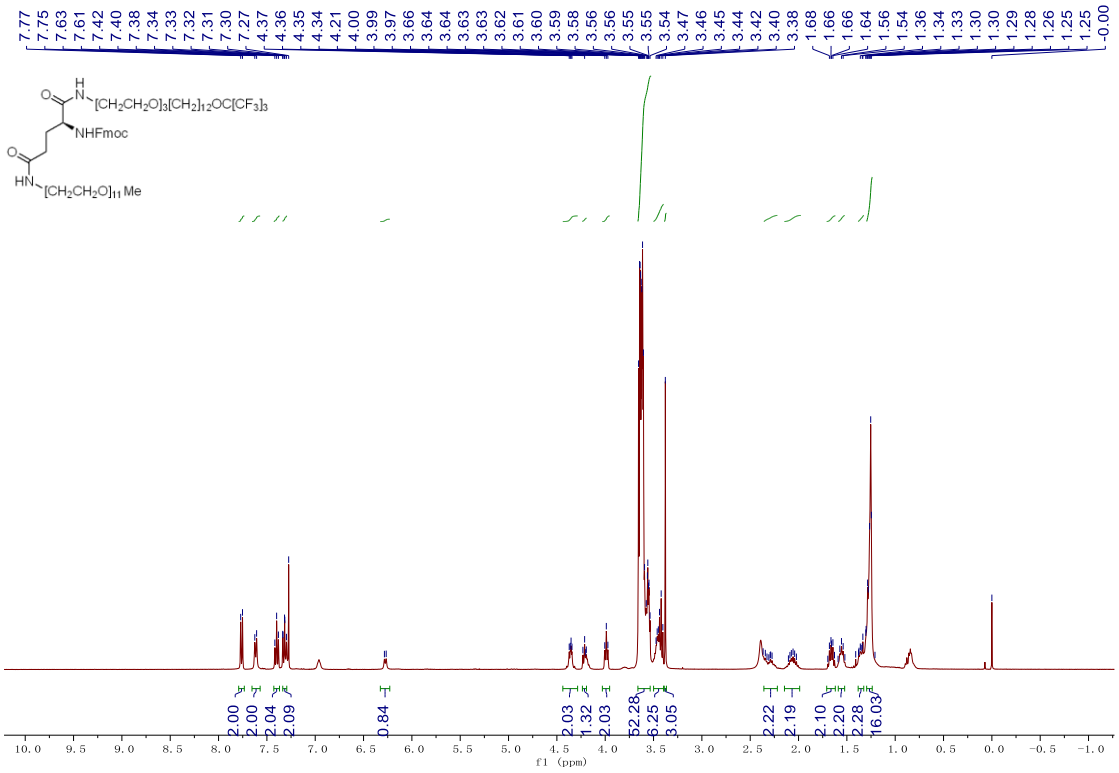


HRMS of compound **17**



^{1}H NMR of compound **18**



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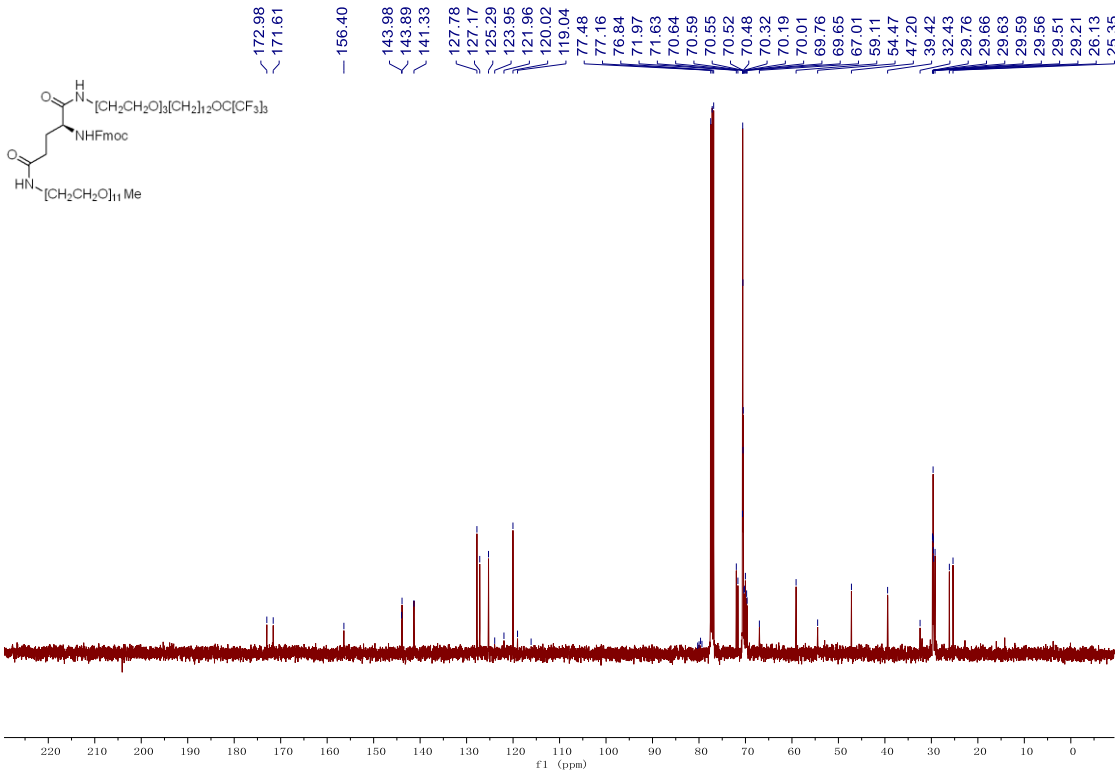

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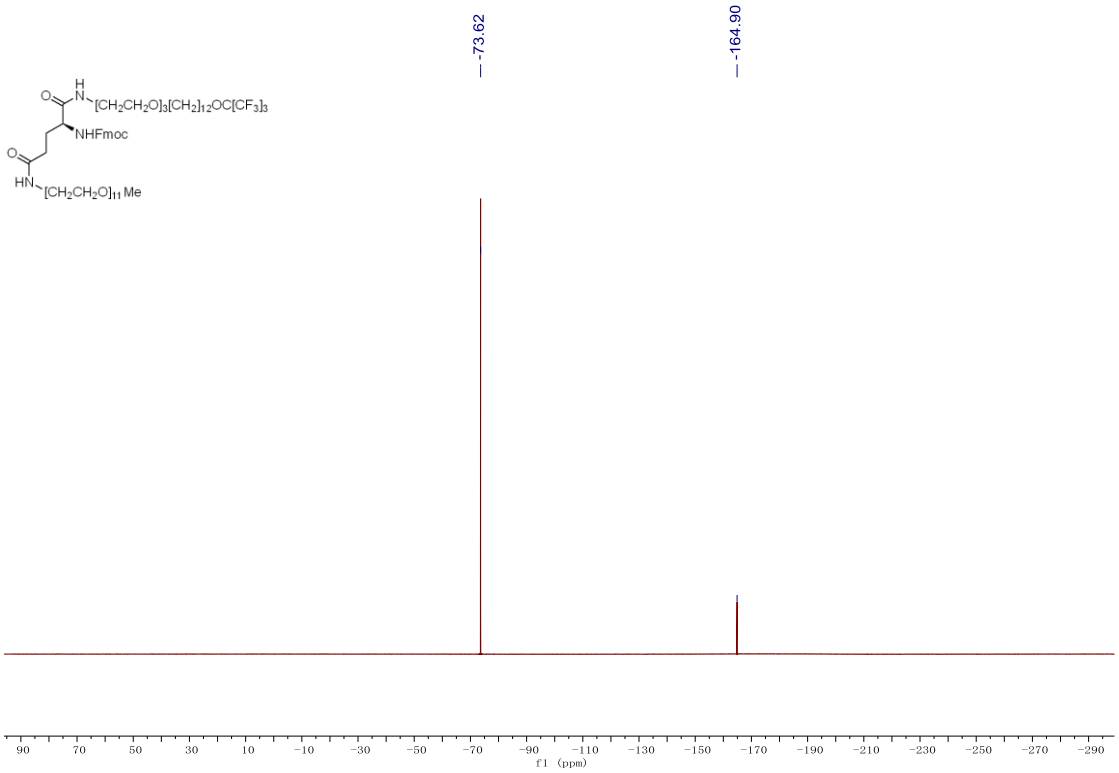

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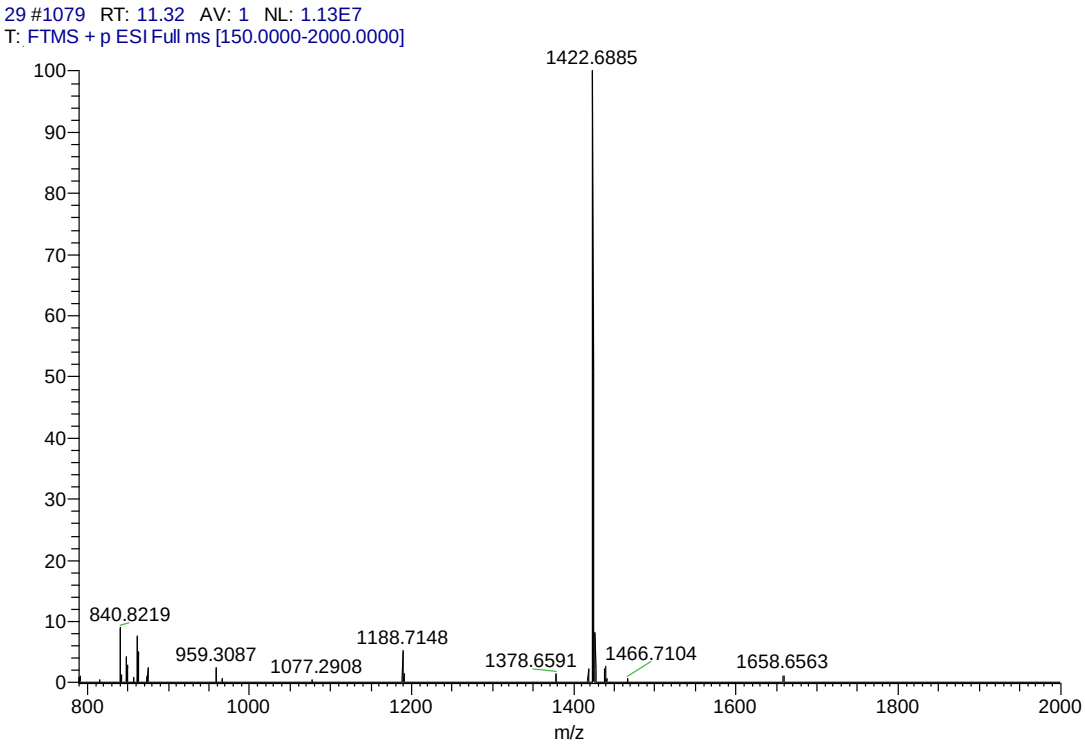

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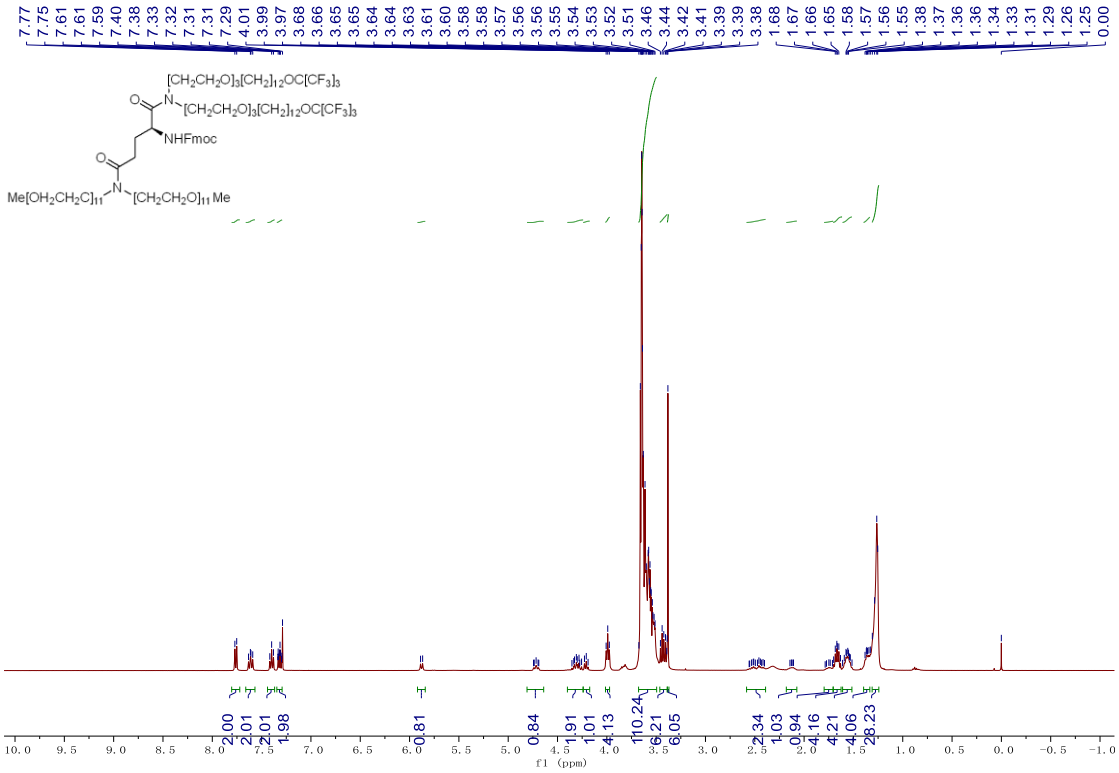
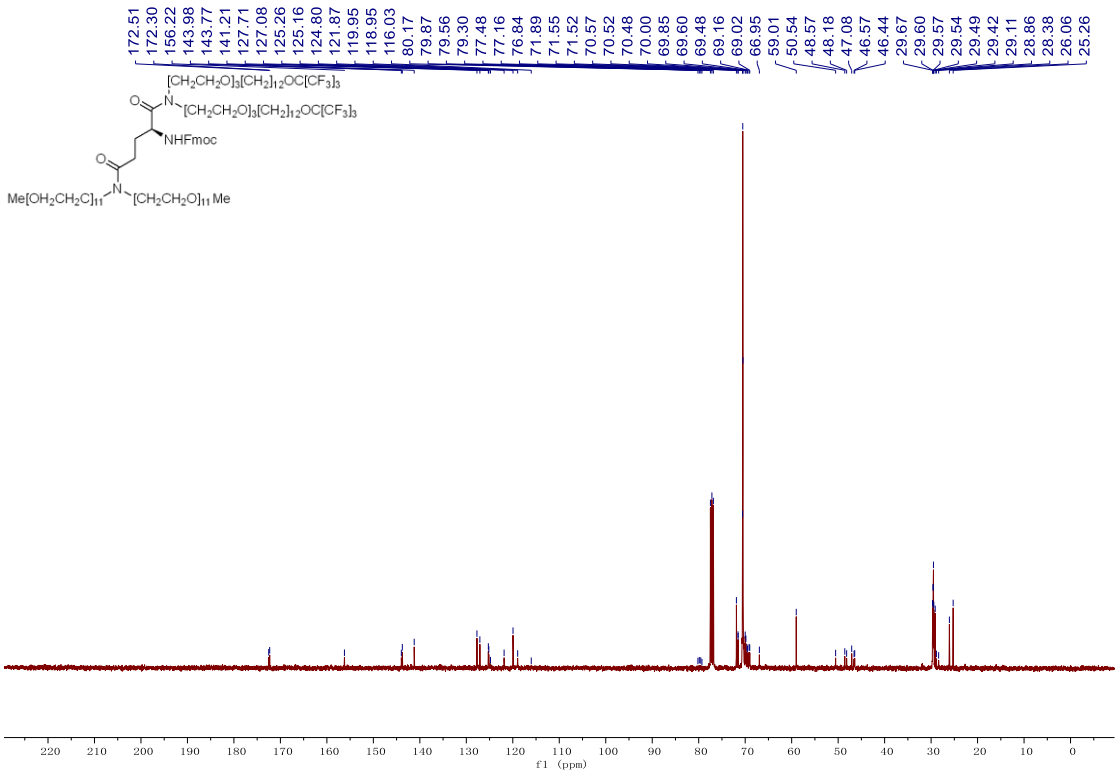
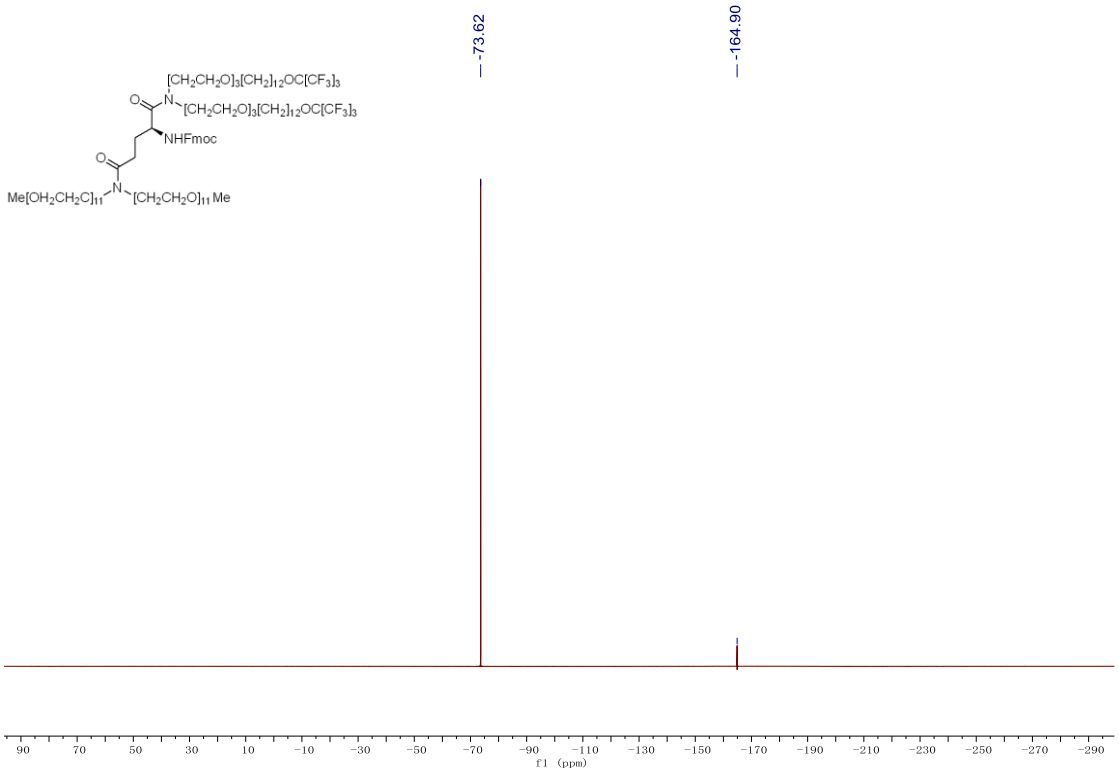


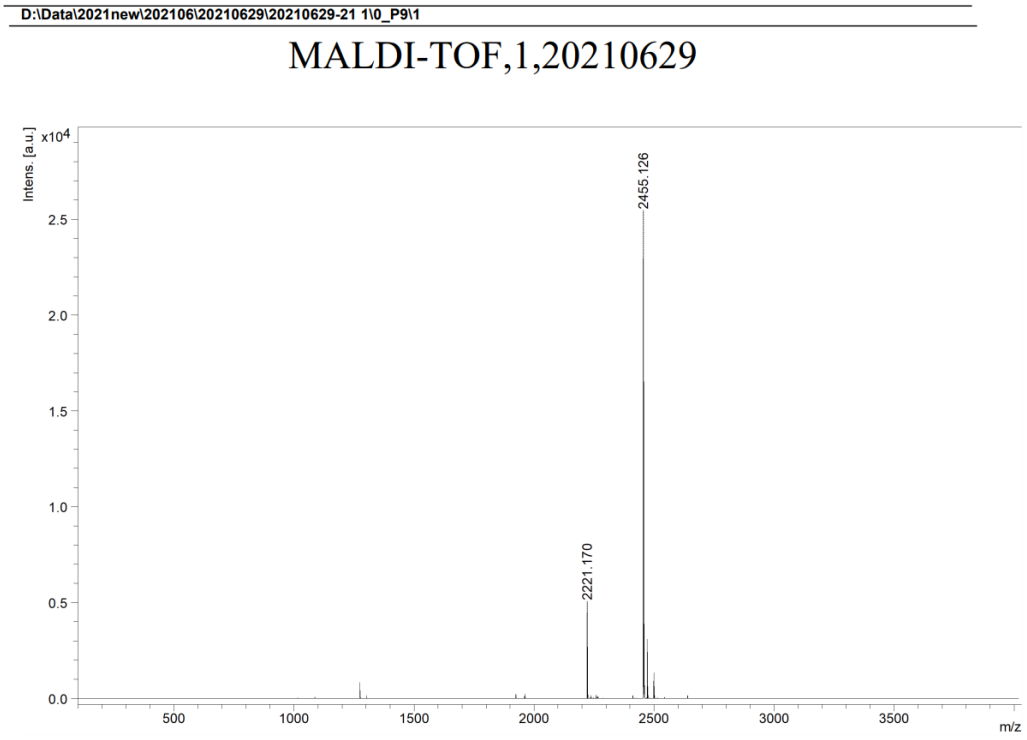
^{13}C NMR of compound **19**



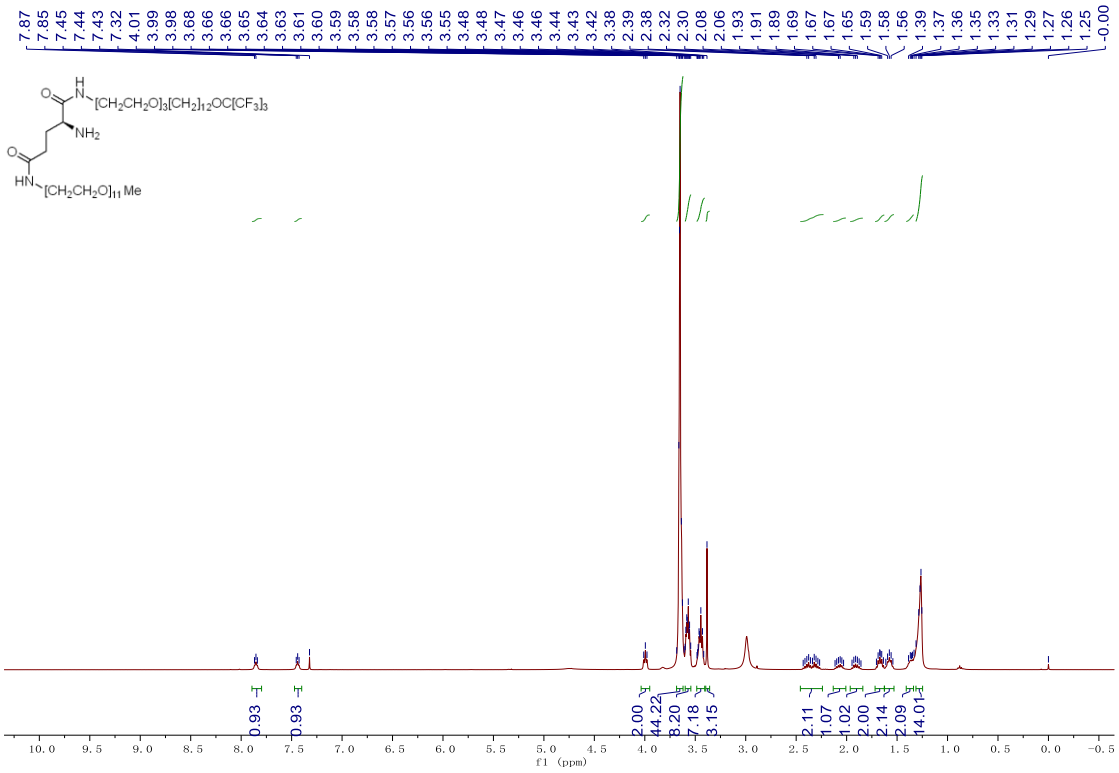
^{19}F NMR of compound **19**



HRMS(Maldi) of compound **19**



^{1}H NMR of compound **20**



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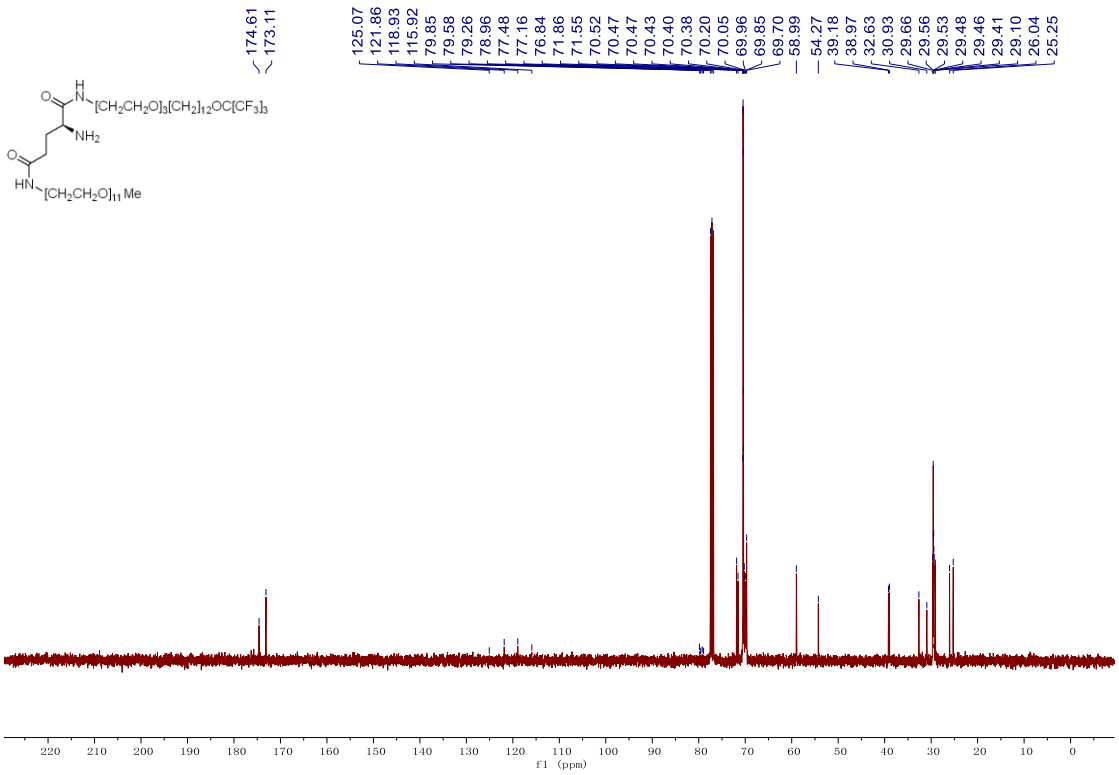

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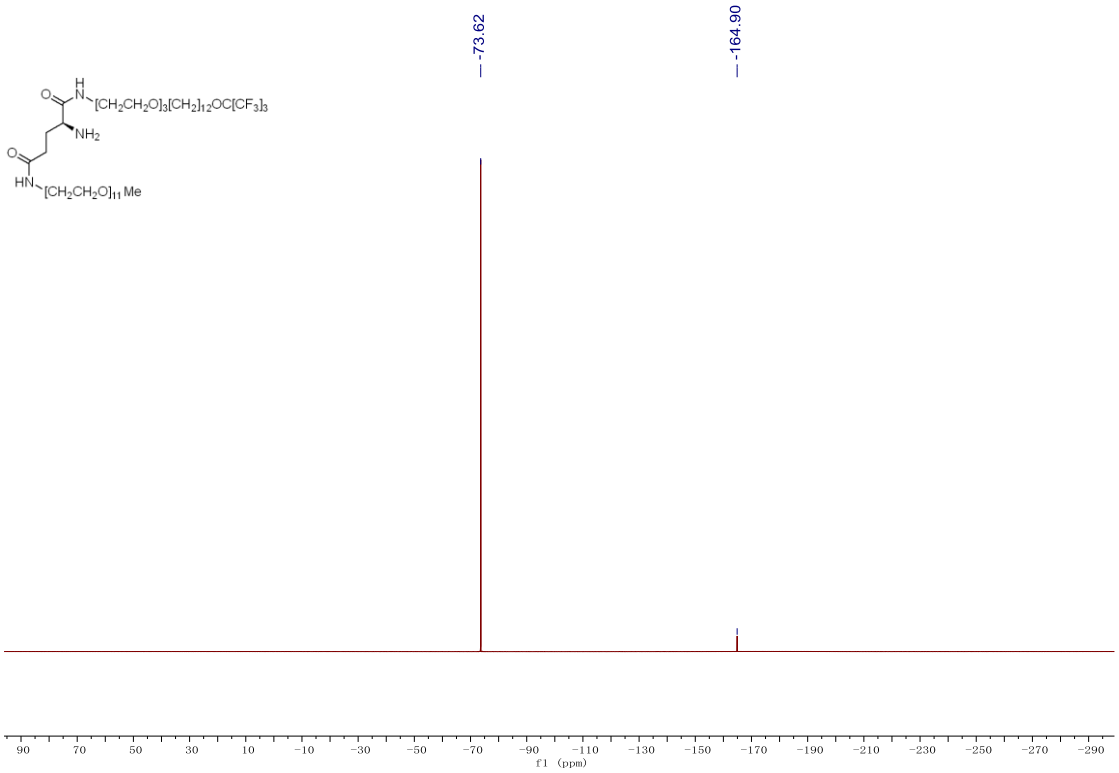
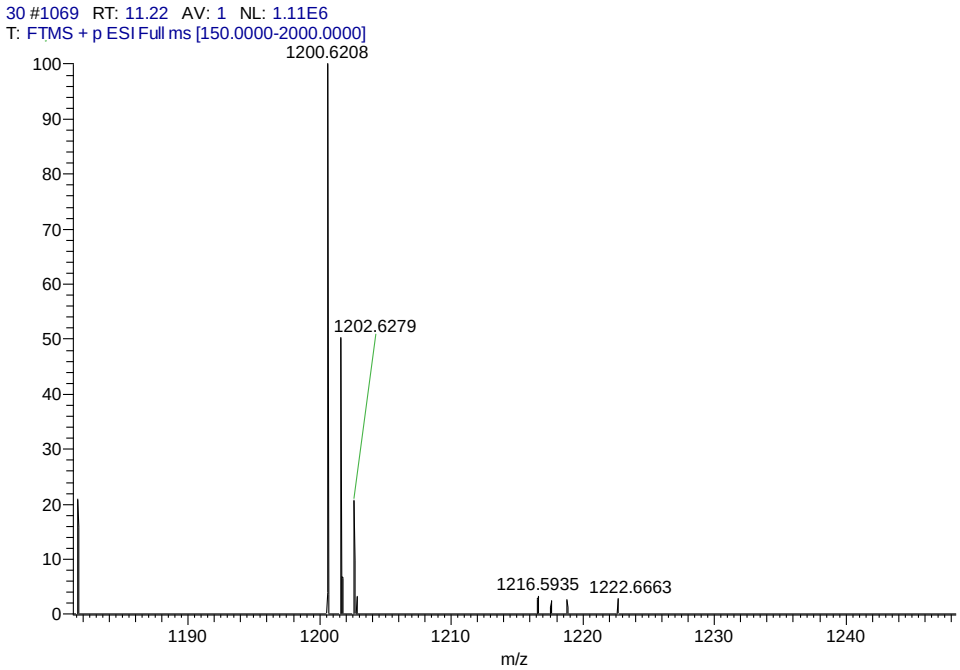
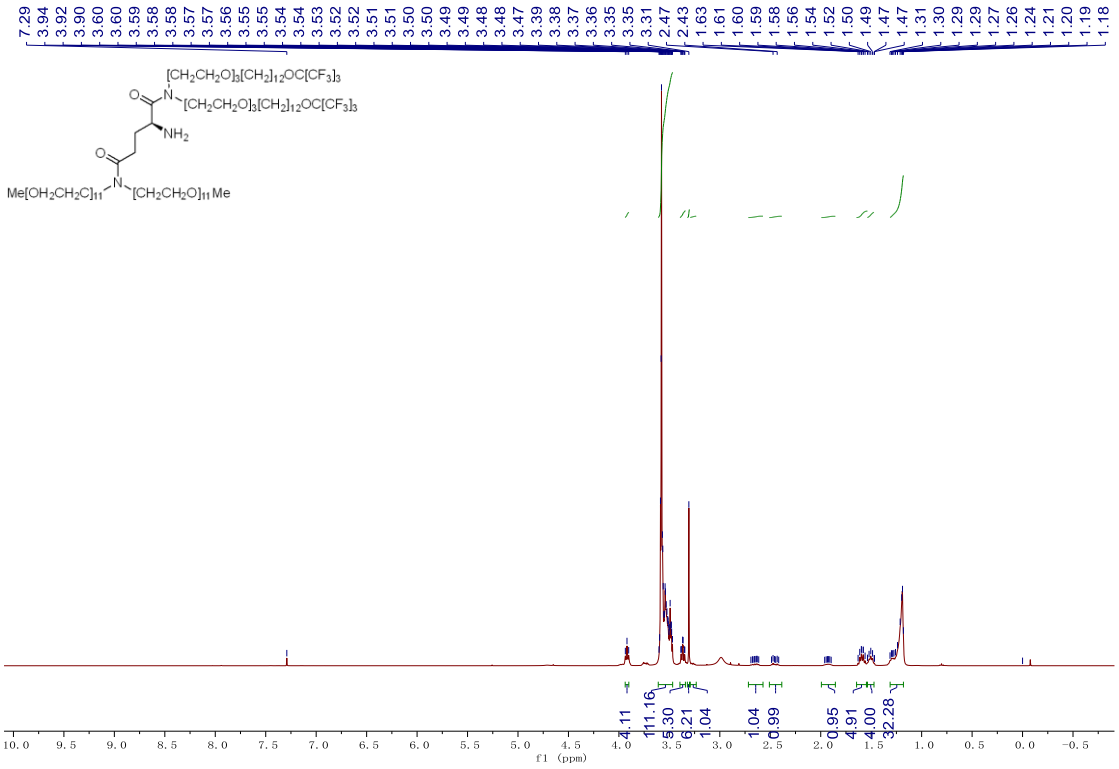


HRMS of compound **20**



^{1}H NMR of compound **21**



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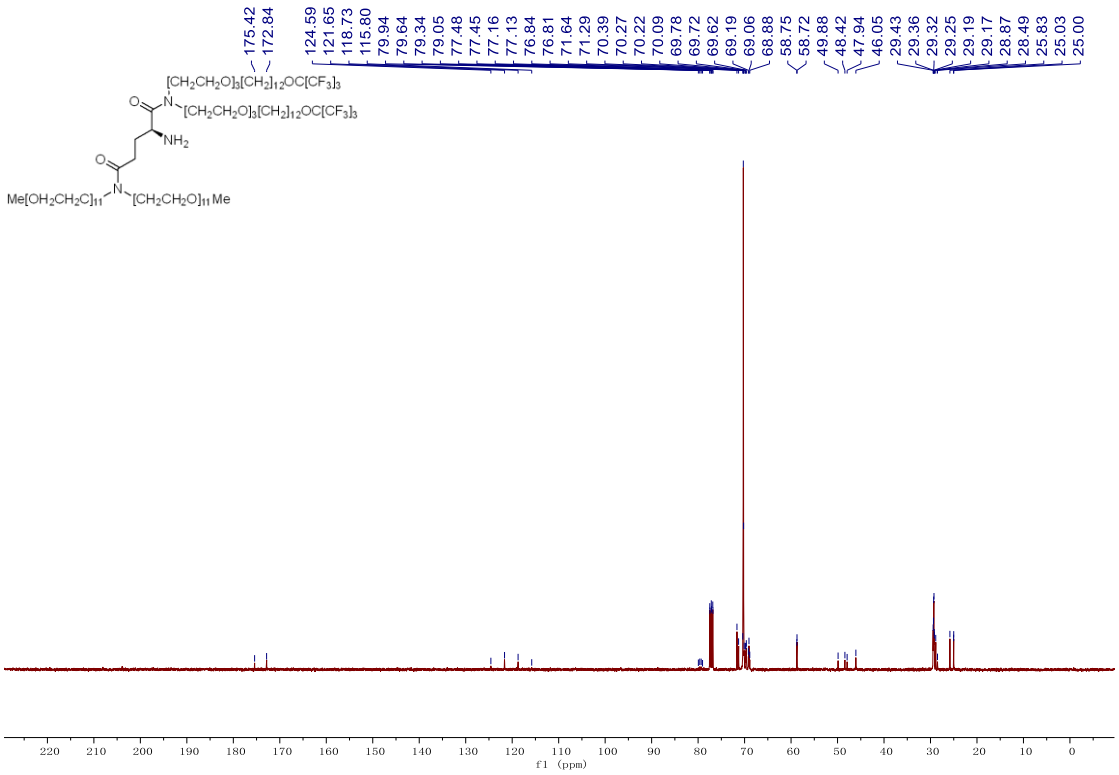

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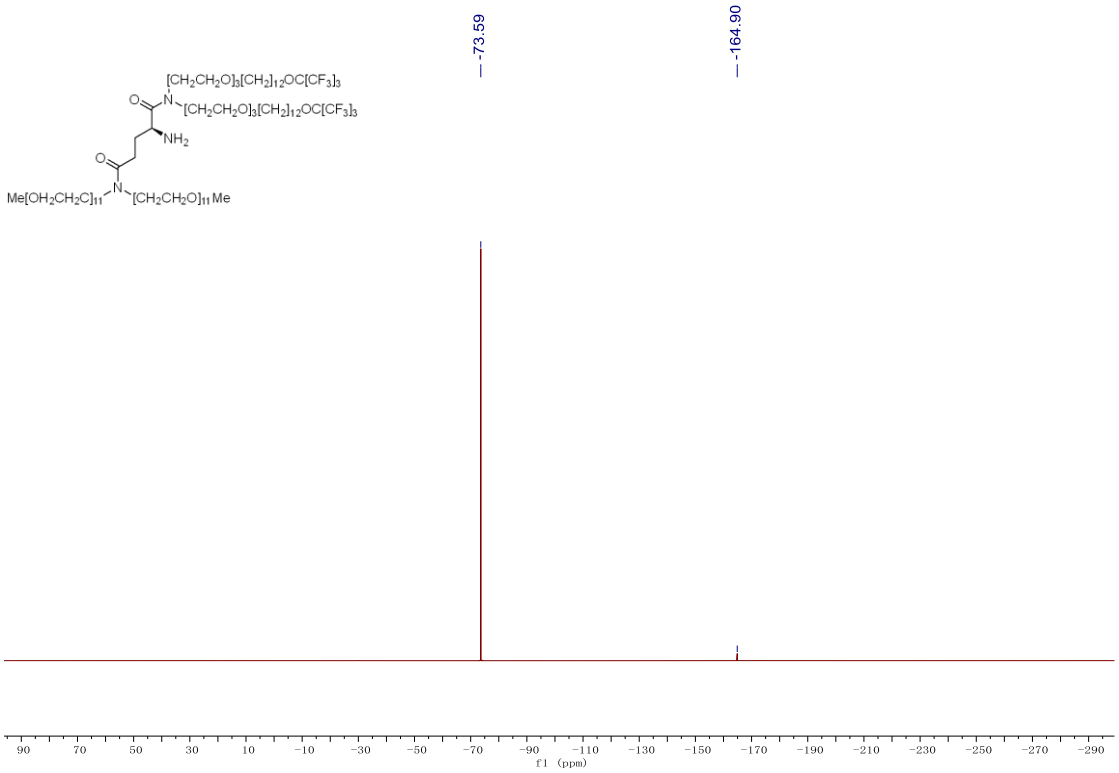
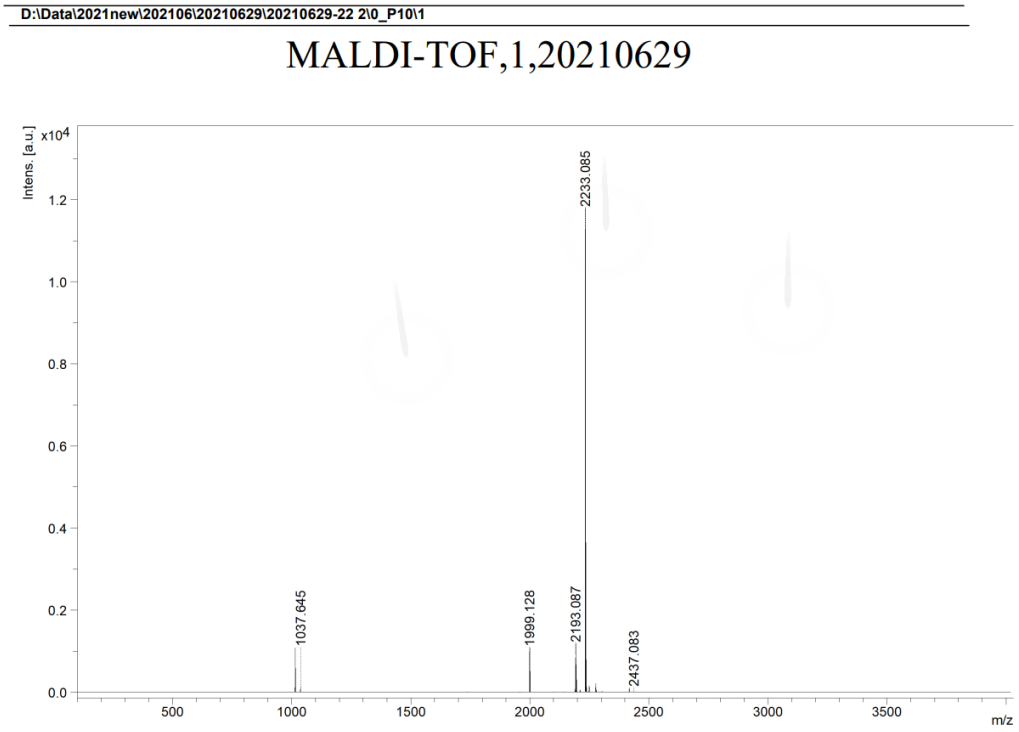
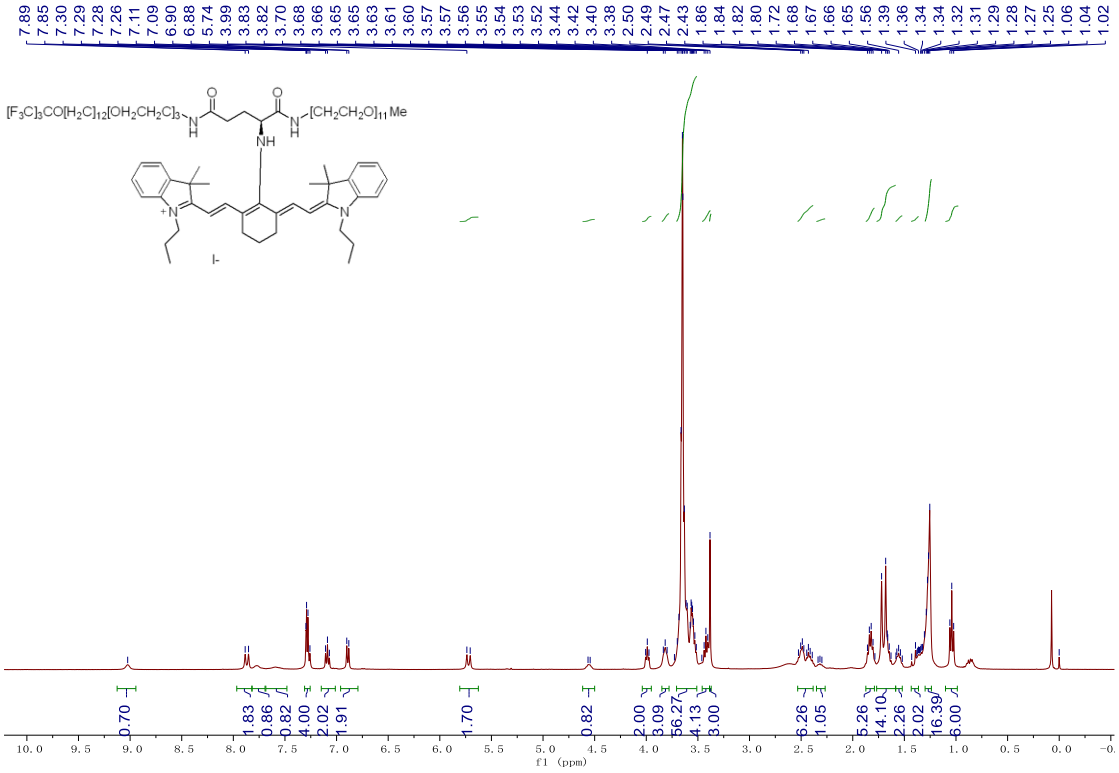


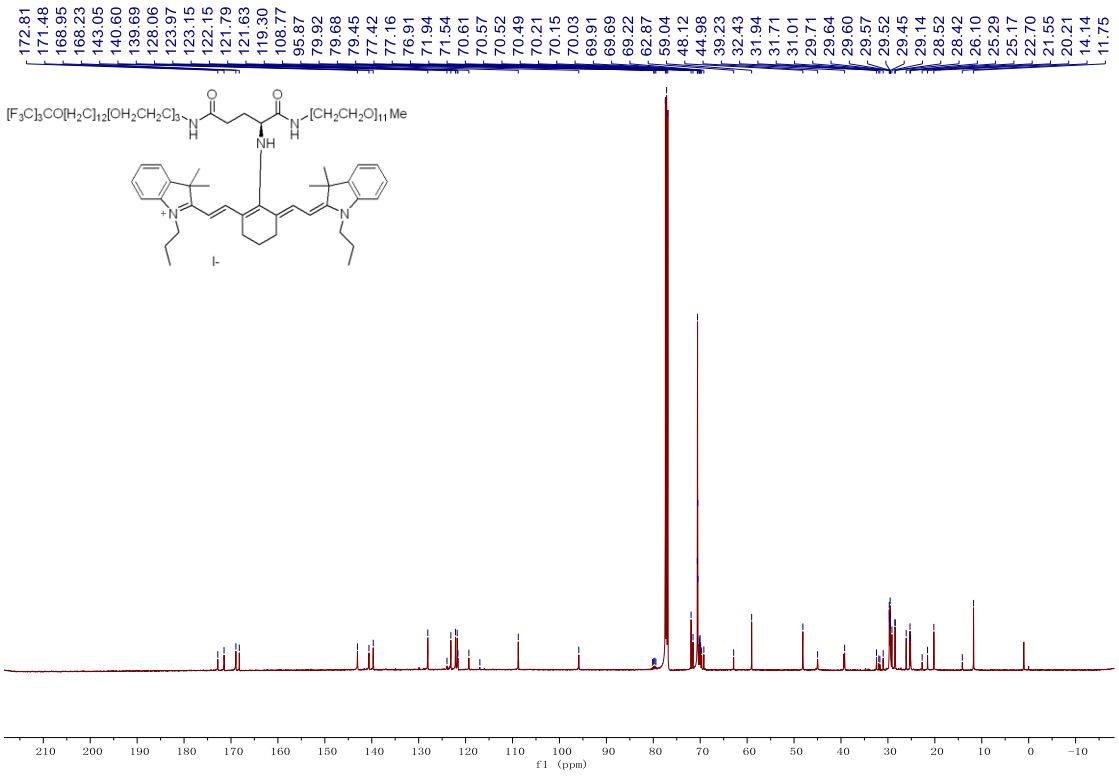
HRMS(Maldi) of compound **21**



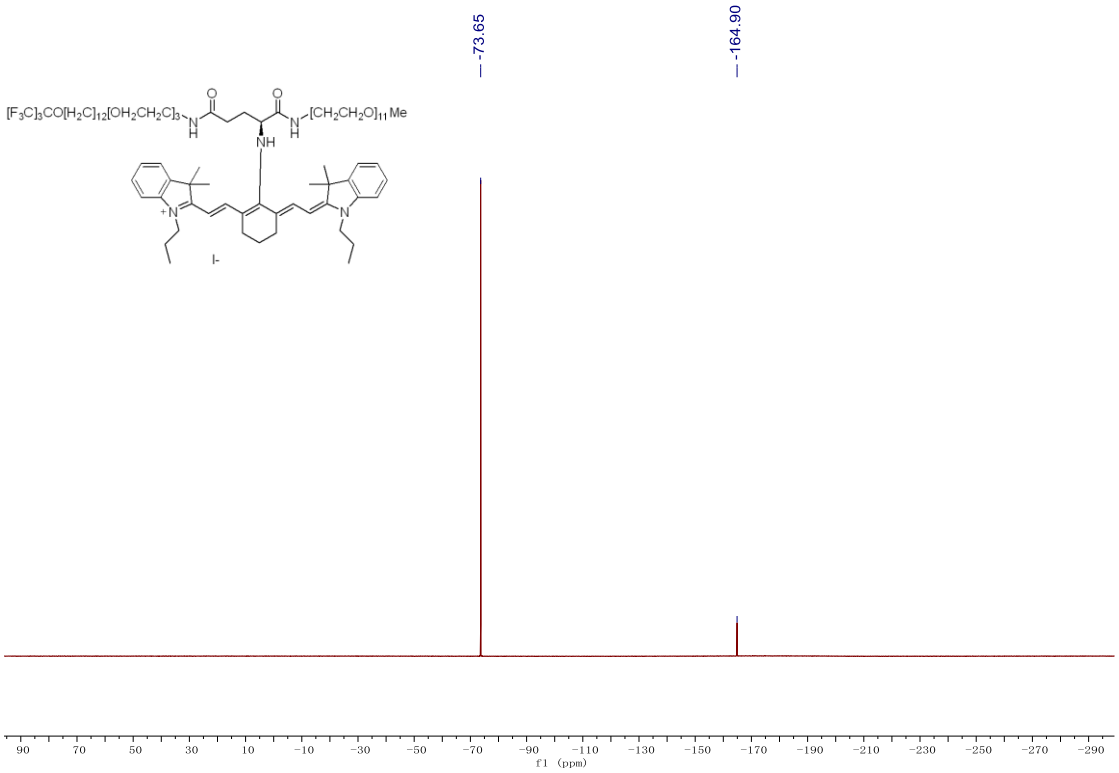
^{1}H NMR of compound **PEG-Cy-F$_9$**



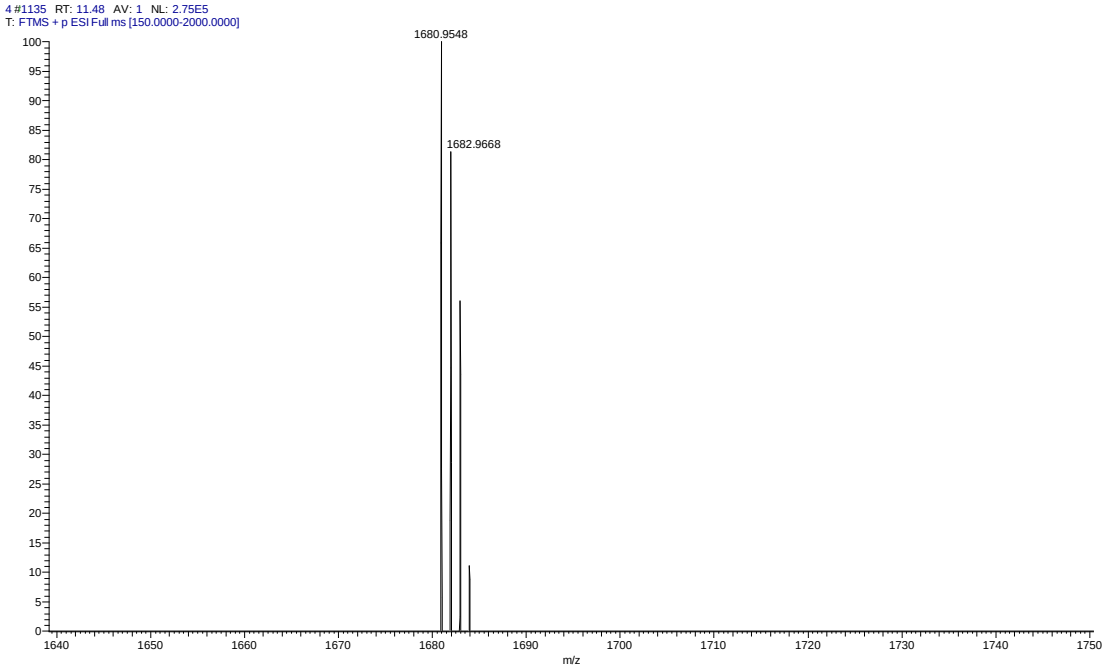
^{13}C NMR of compound **PEG-Cy-F$_9$**



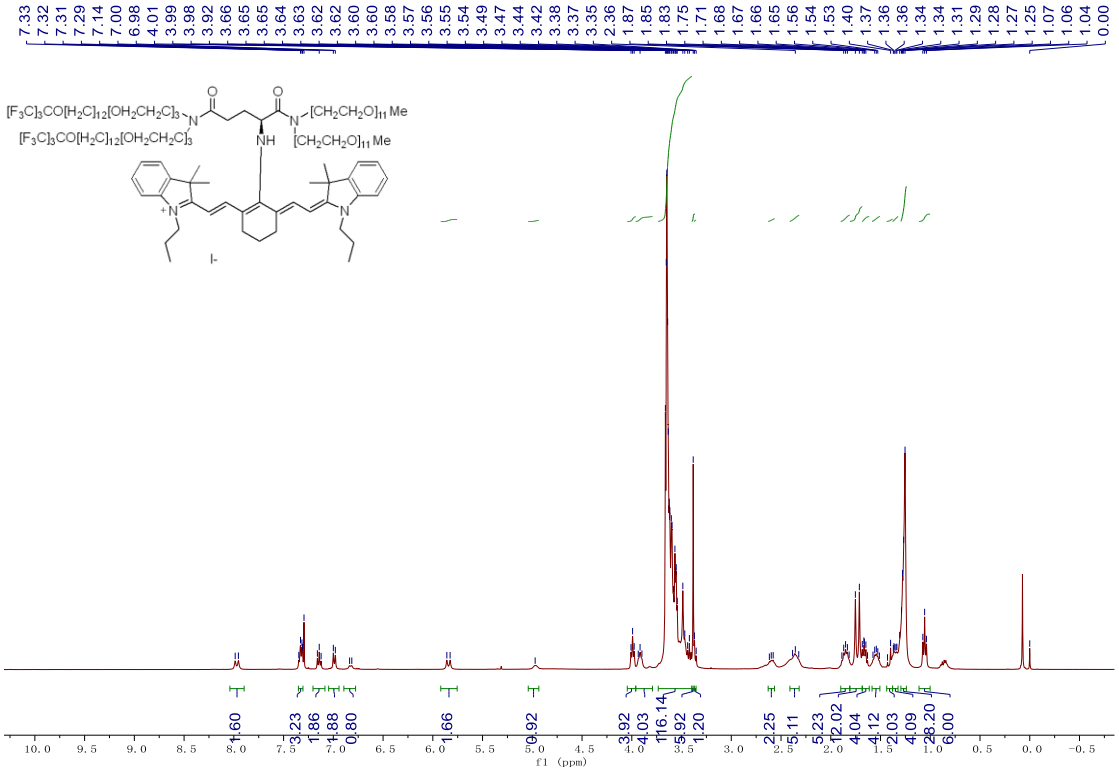
^{19}F NMR of compound **PEG-Cy-F$_9$**



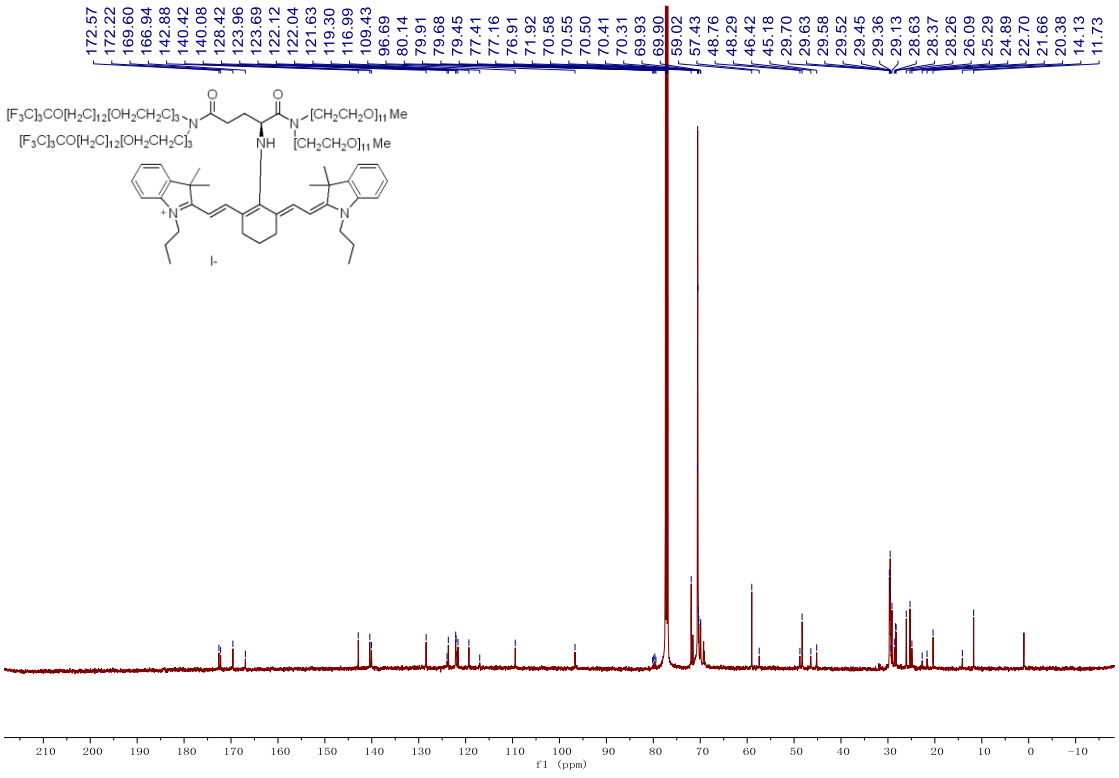
HRMS of compound **PEG-Cy-F$_9$**



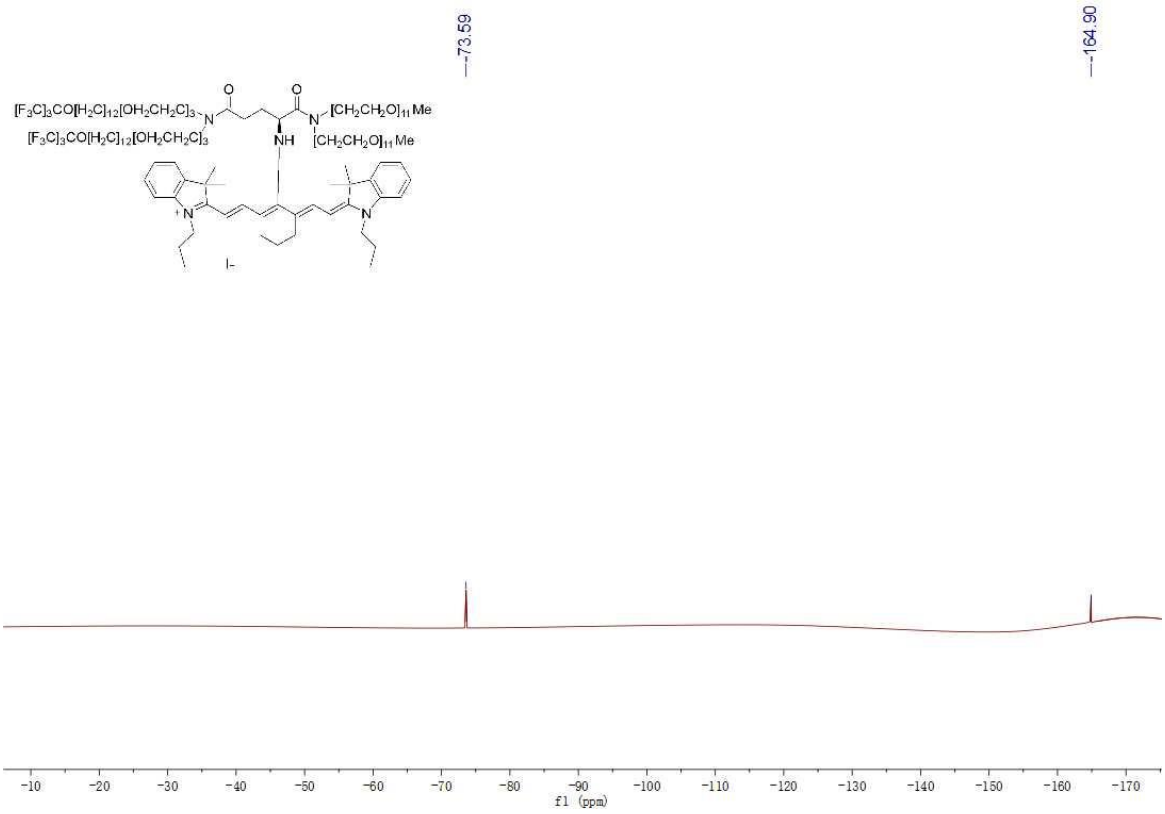
^{1}H NMR of compound **PEG-Cy-F$_{18}$**



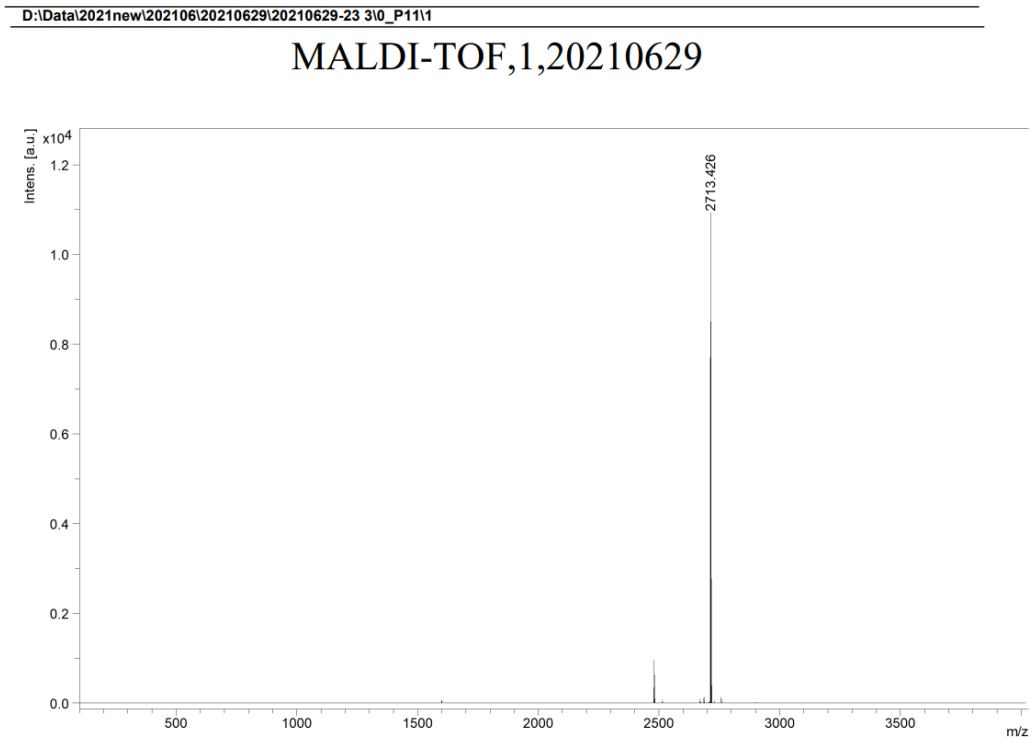
^{13}C NMR of compound **PEG-Cy-F$_{18}$**



^{19}F NMR of compound **PEG-Cy-F_{18}**



HRMS(Maldi) of compound **PEG-Cy-F_{18}**



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