Electronic supplementary information (ESI)

Synthesis and *in vitro* assessment of a bifunctional closomer probe for fluorine (^{19}F) magnetic resonance and optical bimodal cellular imaging

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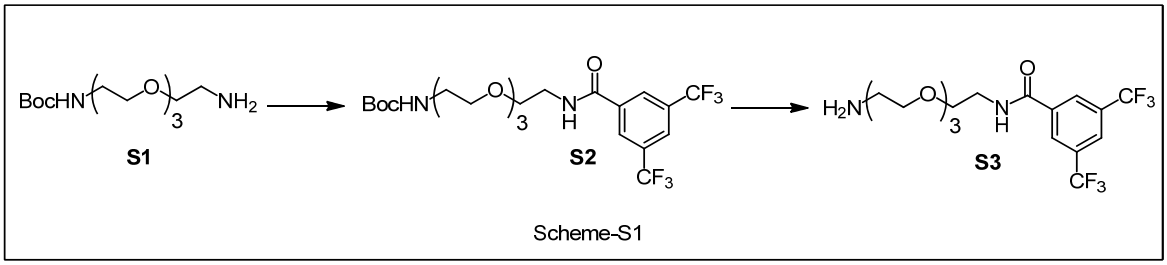
Contents

Experimental procedures

General. Common reagents and chromatographic solvents were obtained from commercial suppliers (Sigma-Aldrich, Fisher Scientific) and used without any further purification. Lipophilic Sephadex LH-20 was obtained from GE Healthcare. NMR spectra were recorded on Bruker Avance 400 or 500 MHz spectrometers. The high-resolution mass spectrometry analysis was performed using Applied Biosystems Mariner ESI-TOF. Fluorescence spectra were recorder on a Varian Cary Eclipse fluorescence spectrophotometer.



Scheme-S1

Synthesis of tert-butyl (1-(3,5-bis(trifluoromethyl)phenyl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)carbamate (**S1**):

Compound **S1** was prepared according to the literature reported procedure.[1]

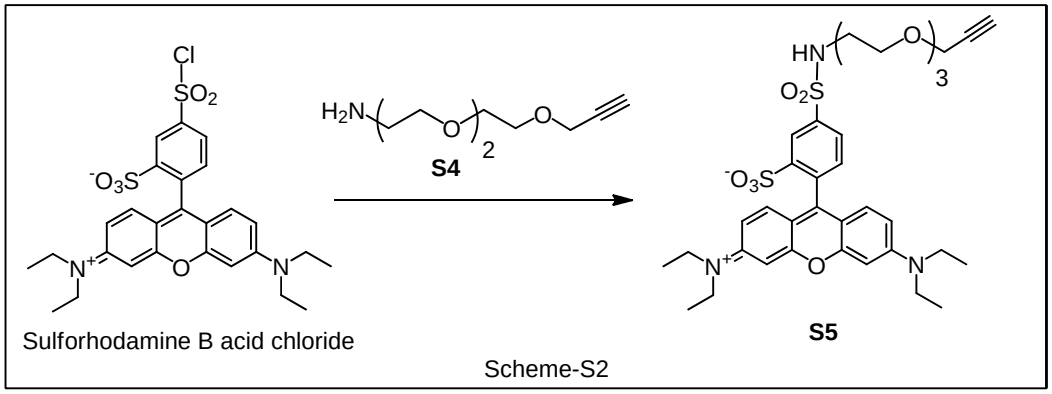
Synthesis of tert-butyl (1-(3,5-bis(trifluoromethyl)phenyl)-1-oxo-5,8,11-trioxa-2-azatridecan-13-yl)carbamate (**S2**):

In a 100 mL round bottom flask (RBF), amine **S1** (1.50 g, 5.13 mmol), 3,5-Bis(trifluoromethyl) benzoic acid (1.32 g, 5.13 mmol), *N*-Ethyl-*N*′-(3-dimethylaminopropyl) carbodiimide hydrochloride (1.28 g, 6.67 mmol) and 4-dimethylamino pyridine (0.81 g, 6.67 mmol) were dissolved in 30 mL dry dichloromethane (DCM). The resultant mixture was stirred for 12 h at room temperature (RT) under argon (Ar) atmosphere. Reaction mixture was then diluted with dichloromethane (100 ml) and washed with 5% HCl/water (100 mL) and then with aq. $NaHCO_3$ (100 mL). Organic layer was separated, dried over sodium sulfate (Na_2SO_4) and concentrated. Product **S2** was purified by silica gel column chromatography using 1-3% methanol (MeOH)-DCM gradient as mobile phase. The pure product was obtained as viscous oil. Yield: 2.30 g (84%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.31 (s, 2H), 7.95 (s, 1H), 7.51 (brs, 1H), 5.04 (brs, 1H), 3.70 - 3.56 (m, 10H), 3.51 – 3.47 (m, 2H), 3.22 (q, 2H, *J* = 5.3 & 10.6 Hz), 1.39 (m, 9H). ^{13}C NMR

(100 MHz, $CDCl_3$): δ 165.43, 164.63, 156.76, 137.54, 133.10, 132.83, 132.49, 132.16, 130.56, 128.46, 125.44, 122.44, 119.73, 79.99, 71.43, 71.38, 71.34, 71.25, 71.13, 71.04, 70.99, 70.93, 70.85, 70.29, 69.69, 54.15, 41.06 and 29.06. ^{19}F NMR (376 MHz, $CDCl_3$): δ -62.92. HRMS (m/z): calcd. for $C_{22}H_{30}F_6N_2O_6$ $[M+Na]^+$ 555.1900. Found: 555.0855.

N-(2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethyl)-3,5-bis(trifluoromethyl)benzamide (**S3**):

Compound **S2** (2.30 g, 4.32 mmol) was stirred with 20% trifluroacetic acid (TFA)-DCM (15 mL) at 0 °C for 3 h under Ar atmosphere. Reaction mixture was then concentrated under vacuum. The residue was then dissolved in 100 mL DCM and washed with aq. $NaHCO_3$ solution (100 mL). Organic layer was then separated, dried over Na_2SO_4 and concentrated. The crude product was purified via column chromatography over alumina (IV) using 1-5% MeOH-DCM gradient as mobile phase. The pure product was obtained as colorless oil. Yield: 1.63 g (87%). ^1H NMR (400 MHz, $CDCl_3$): δ 8.84 (brs, 1H), 8.30 (s, 2H), 7.86 (s, 1H), 3.56 - 3.51 (m, 12H), 3.36 (m, 2H), 2.66 (m, 2H), 2.17-1.27 (brs, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.53, 137.72, 132.86, 132.52, 132.19, 131.85, 128.67, 127.87, 125.16, 125.10, 122.45, 119.74, 73.77, 71.21, 71.04, 70.95, 70.62, 42.09 and 40.99. ^{19}F NMR (376 MHz, $CDCl_3$): δ -63.00. HRMS (m/z): calcd. for $C_{17}H_{22}F_6N_2O_4$ $[M+H]^+$ 433.1557. Found: 433.1916.



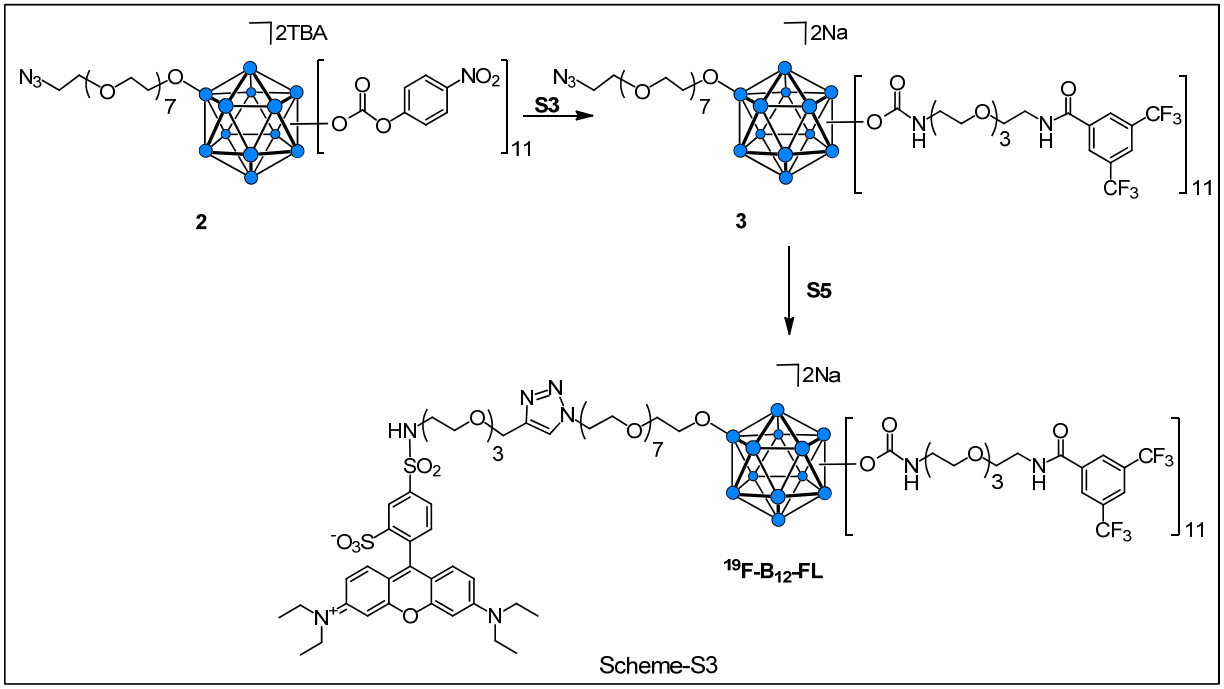
Scheme-S2

2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethanamine (**S4**):

Compound **S4** was prepared according to the literature reported procedure.[2]

2-(6-(diethylamino)-3-(diethyliminio)-3H-xanthen-9-yl)-5-(N-(2-(2-(2-(prop-2-yn-1-yloxy)ethoxy)ethoxy)ethyl)sulfamoyl)benzenesulfonate (**S5**):

In a 100 mL round-bottom flask, sulforhodamine B acid chloride (0.50 g, 0.87 mmol) was dissolved in 10 mL tetrahydrofuran (THF) and 2 mL *N*, *N*-dimethylformamide (DMF). This mixture was cooled to 0 °C, and added DIPEA (0.17 g, 1.30 mmol) followed by the slow addition of amine **S4** (0.21 g, 1.13 mmol) in 10 ml THF. The reaction mixture was gradually allowed warmed to room temperature and stirred for 12 h under an Ar atmosphere. The reaction mixture was then concentrated under vacuum, and the pure product was obtained as pink colored power after purification by column chromatography over alumina (IV) using 1-5 % MeOH/DCM gradient as the mobile phase. Yield: 0.40 g (63%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.56 (s, 1H), 7.89 (d, 1H, *J* = 7.6 Hz), 7.15 (d, 1H, *J* = 7.8 Hz), 7.02 (d, 2H, *J* = 9.4 Hz), 6.69 (d, 2H, *J* = 9.4 Hz), 6.58 (s, 2H), 4.04 (d, 2H, *J* = 1.5 Hz), 3.55 - 3.43 (m, 18H), 3.07 (t, 2H, *J* = 5.0 & 10 Hz), 2.37 (s, 1H), 1.16 (t, 12H, *J* = 6.8 &13.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.92, 157.58, 155.60, 146.87, 142.35, 133.74, 132.84, 130.32, 127.53, 126.89, 114.14, 113.63, 95.76, 79.40, 74.82, 70.42, 70.24, 70.16, 69.73, 68.93, 58.27, 45.83, 42.90 and 12.39. HRMS (m/z): calcd. for $C_{36}H_{44}N_3O_9S_2$ $[M+H]^+$ 728.2675. Found: 727.9888. calcd. for $C_{36}H_{44}N_3O_9S_2$ $[M+Na]^+$ 750.2495. Found: 749.9365. calcd. for $C_{36}H_{44}N_3O_9S_2$ $[2M+Na]^+$ 1477.5092. Found: 1477.6736.



Scheme-S3

*Synthesis of Closomer **2**.*

Closomer **2** was prepared according to the literature reported procedure.[3]

*Synthesis of Closomer **3**.*

A mixture of Closomer **2** (0.10 g, 0.03 mmol) and amine **S3** (0.86 g, 2.00 mmol) in 15 mL acetonitrile (ACN) was stirred at RT for 3 days. Progress of the reaction was monitored via mass spectrometry and ^{11}B NMR analysis. After completion, the reaction mixture was concentrated in rotavap and dried under vacuum. The crude product was then purified by gel filtration chromatography over Lipophilic Sephadex LH-20 using MeOH as the mobile phase to obtain pure product as colorless viscous oil. Purified product was passed through an ion exchange resin column to exchange TBA^+ with Na^+ ion. Yield: 0.16 g (84%). ^{1}H NMR (400 MHz, CD_3CN): δ 8.35 (s, 22H), 8.11 (s, 11H), 7.84 (brs, 11H), 6.56 (brs, 11H), 3.99 (m, 2H), 3.62-3.46 (m, 184H), 3.10 (m, 22H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.09, 156.98, 137.77, 132.58, 132.17, 131.83, 131.49, 128.71, 125.51, 125.45, 122.74, 70.95 – 70.68 (multiple peaks-PEG-CH_2-), 70.29, 69.73, 51.32, 41.81 and 40.61. ^{11}B NMR (128 MHz, CD_3CN): δ -16.07 and -18.34. ^{19}F NMR (376 MHz, CD_3CN): δ -63.35. HRMS (m/z): calcd. for $C_{214}H_{263}B_{12}F_{66}N_{25}O_{74}$ $[M]^{2-}$ 2876.5638. Found: 2876.6501.

*Synthesis of ^{19}**F-B$_{12}$-FL***

In a 50 mL round bottom flask, the azide containing Closomer **3** (0.37 g, 0.064 mmol), alkyne terminated sulforhodamine B, **S5**, (60.8 mg, 0.084 mmol), and copper (I) iodide (12.2 mg, 0.064 mmol) were dissolved in a 1:1 mixture of THF and ACN (10 mL). To this mixture DIPEA (13.0 mg, 0.10 mmol) was added and the reaction mixture was vigorously stirred at RT for 12 h under an Ar atmosphere. The progress of the reaction was monitored by mass spectrometry analysis. After completion, the reaction mixture was concentrated to dryness, dissolved in ethyl acetate and filtered through a celite pad. The filtrate was concentrated and purified via gel filtration column chromatography over Lipophilic Sephadex LH-20 using DCM as an eluent to afford the pure product as pink solid after drying. Yield: 380 mg (91%). ^{1}H NMR (400 MHz, CD_3OD): δ 8.68 (s, 1H), 8.44 (m, 23H), 8.13 (m, 13H), 7.55 (m, 1H), 7.14 (m, 2H), 7.00 (m, 2H), 6.98 (m, 2H), 4.64 (m, 4H), 3.90 (m, 8H), 3.65 (m, 206H), 3.33 (m, 34H), 1.30 (m, 12H). ^{13}C NMR (100 MHz, $CDCl_3$ + CD_3OD drops): δ 165.71, 158.63, 157.15, 156.30, 148.05, 143.08, 137.34, 134.40, 132.95, 132.62, 132.28, 131.95, 137.34, 128.59, 128.18, 127.85, 125.28, 125.14, 122.43, 119.72, 114.94, 114.31, 96.45, 71.08 – 70.20 (multiple peaks-PEG-CH_2-), 46.55, 43.70, 41.63, 40.74 and 13.15. ^{11}B NMR (128 MHz, $CDCl_3$ + CD_3OD drops): δ -16.39 and -18.74. ^{19}F NMR (376 MHz,

$CDCl_3$ + CD_3OD drops): δ -63.05. HRMS (m/z): calcd. for $C_{250}H_{308}B_{12}F_{66}N_{28}O_{83}S_2$ $[M]^{2-}$ 3240.0425. Found: 3241.0366 $[M]^{2-}$+H.

Cell Culture and maintenance: A549, DLD1, T47D and EMT-6 (ATCC, Manassas, VA) cells were maintained in DMEM + 10% FBS, 5% CO_2 and humidified atmosphere. When the flasks reached 90% confluence, cells were harvested using TrypLE (GIBCO, Grand Island, NY) according to the manufacturer's protocol. Briefly, cells were rinsed using 1X PBS to remove FBS and incubated with TrypLE for 10 min at 37 °C. Cells were then centrifuged at 323*g* for 8 min in a Fisher Scientific Accuspin 3R centrifuge (Pittsburgh, PA). Cell were resuspended in DMEM + 10% FBS and counted in an automatic cell counter (Invitrogen, Grand Island, NY).

Time dependent cellular uptake of ^{19}F-B^{12}-FL in A549 cells: A549 cells at the density of 300000 cells/time point were incubated with 100 μM of **^{19}F-B$_{12}$-FL** (dissolved in DMSO) for different time points (5 min, 30 min, 1 h and 3 h). Final DMSO concentration on the cells was less than 2%. Cells were harvested using TrypLE cell detachment solution and washed 3 times with 1X PBS at 323g for 8 mins. Pellets were frozen at -20 ^{0}C and lyophilized. The residue was extracted with DMSO (500 μL each) and the filtrates were analyzed by ^{19}F NMR and fluorescence spectroscopy (Figure-S1).

Dose dependent cellular uptake of ^{19}F-B^{12}-FL in A549 cells: A549 cells at the density of 300000 cells/dose were incubated with different doses of **^{19}F-B$_{12}$-FL** (0.1-80 μM, dissolved in DMSO) for 1hr. Final DMSO concentration on the cells was less than 2%. Cells were harvested using TrypLE cell detachment solution and washed 3 times with 1X PBS at 323g for 8 min. Pellets were frozen at -20 ^{0}C and lyophilized. The residue was extracted with DMSO (500 μL each) and the filtrates were analyzed by ^{19}F NMR and fluorescence spectroscopy.

Evaluation of toxicity of ^{19}F-B$_{12}$-FL on A549, DLD1, T47D and EMT-6 cells: A549, DLD1, T47D and EMT-6 cells at the density of 10000/well were plated in 96 well plates. Cells were treated with different doses of **^{19}F-B$_{12}$-FL** ranging from 0.1-100 μM for 72 h followed by addition of MTT reagent for 4 h. The reaction was stopped after 4 h with stop solution provided with MTT kit. Plates were read at 570 nm using microplate reader.

Intracellular localization of ^{19}F-B$_{12}$-FL in T47D by confocal microscopy: T47D cells at the density of 100000 cells/chamber were plated in chamber slide overnight for adherence and then

incubated with green Lysotracker at 37 ^{o}C for 30 min followed by washing with 1X PBS for 3-4 times. Cells were then incubated with **^{19}F-B$_{12}$-FL** (10 μM) for 3 h following which cells were washed 3-4 times with 1X PBS. Slides were mounted and observed under confocal and fluorescent microscope and pictures were taken.

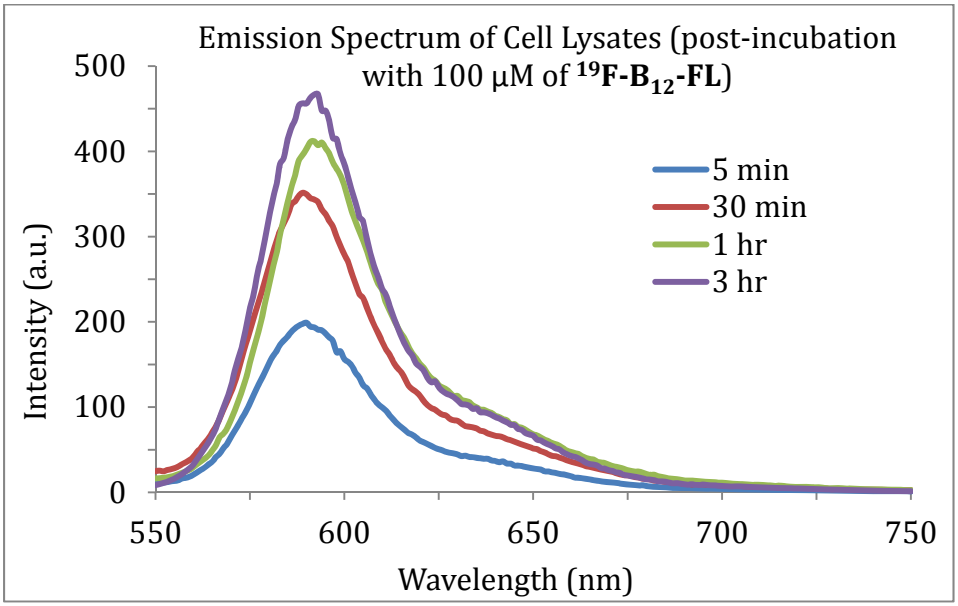


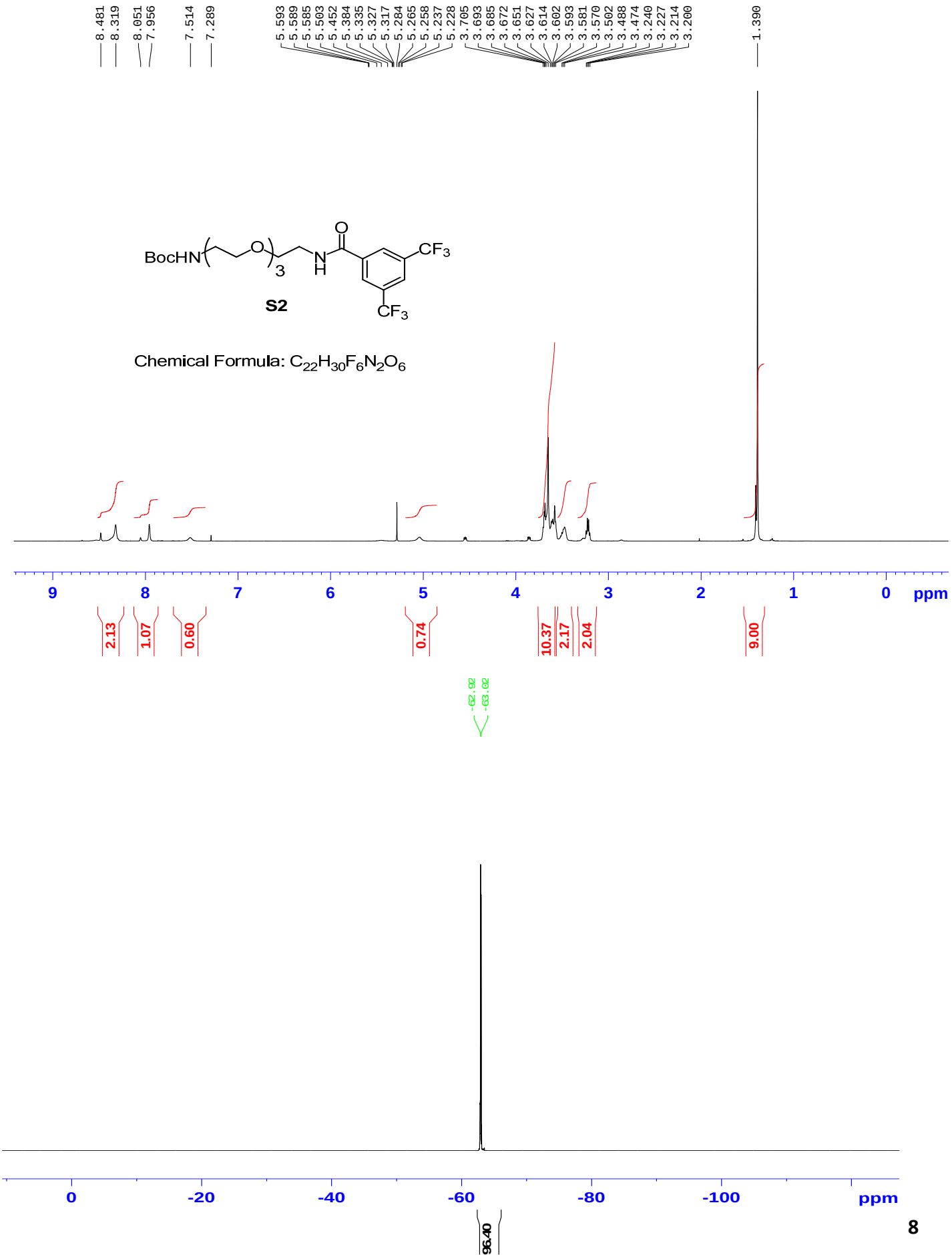
Fig. S1 Time dependent uptake of **^{19}F-B$_{12}$-FL** by human A549 cells. Figure shows fluorescence intensity of cell lysates post incubation of 100 μM of **^{19}F-B$_{12}$-FL** at various time-points

References:

(1) K. D. McReynolds, A. Bhat, J. C. Conboy, S. S. Saavedra and J. Gervay-Hague, *Bioorg. Med. Chem.*, 2002, **10**, 625.

(2) L. N. Goswami, L. Ma, S. Chakravarty, Q. Cai, S. S. Jalisatgi and M. F. Hawthorne, *Inorg. Chem.*, 2013, **52**, 1694.

(3) L. N. Goswami, Z. H. Houston, S. S. Sarma, H. Li, S.S. Jalisatgi, M. F. Hawthorne, *J. Org. Chem.*, 2012, **77**, 11333.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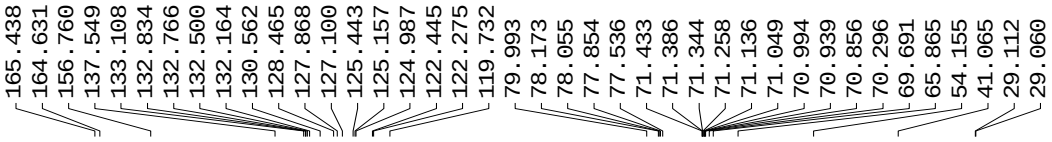
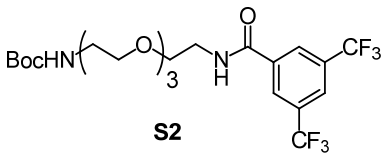
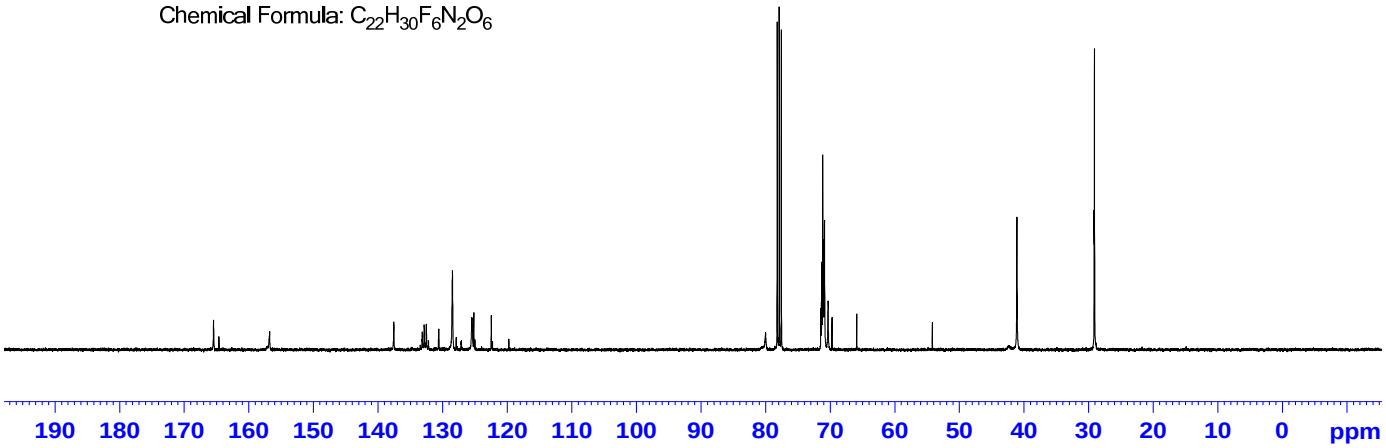




S2

Chemical Formula: $C_{22}H_{30}F_6N_2O_6$



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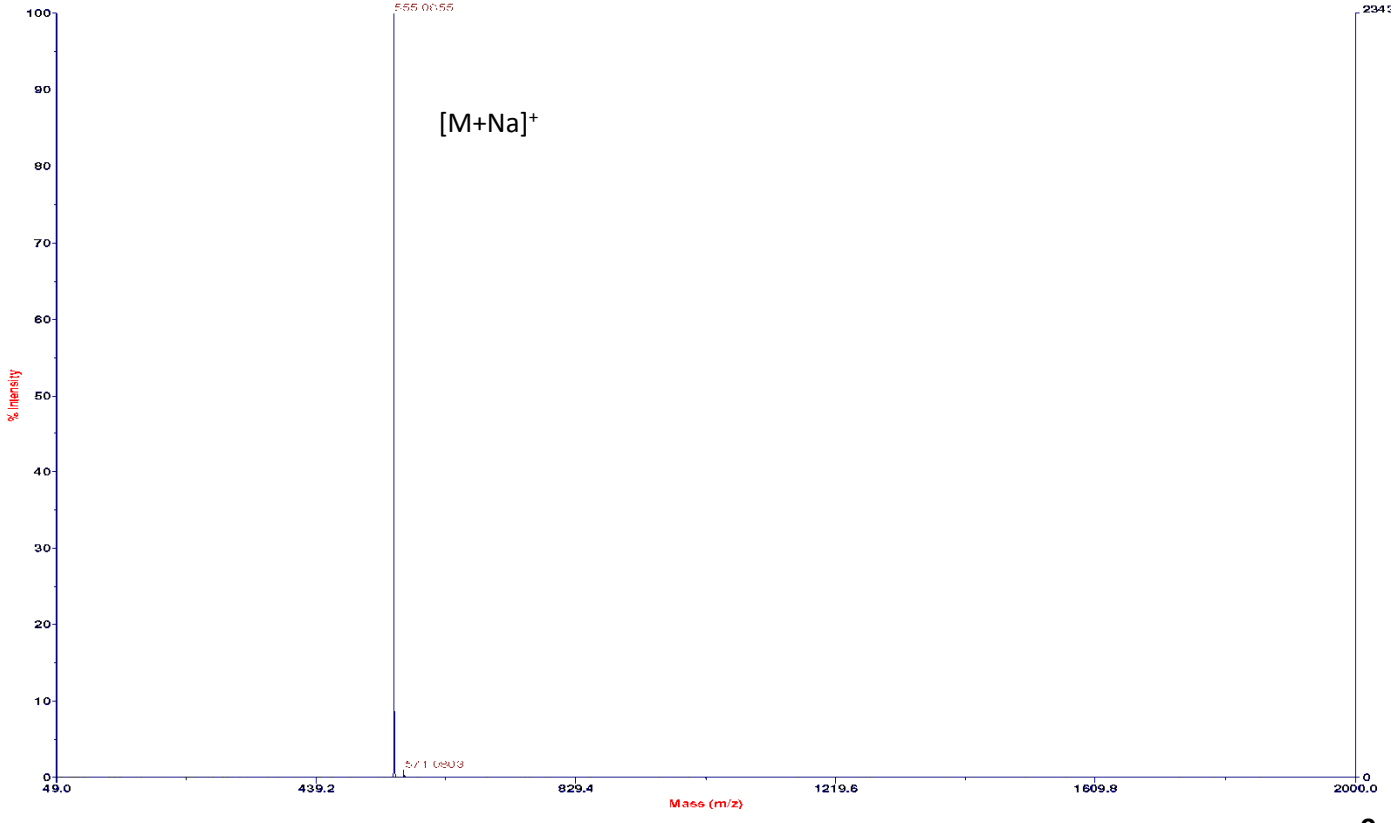

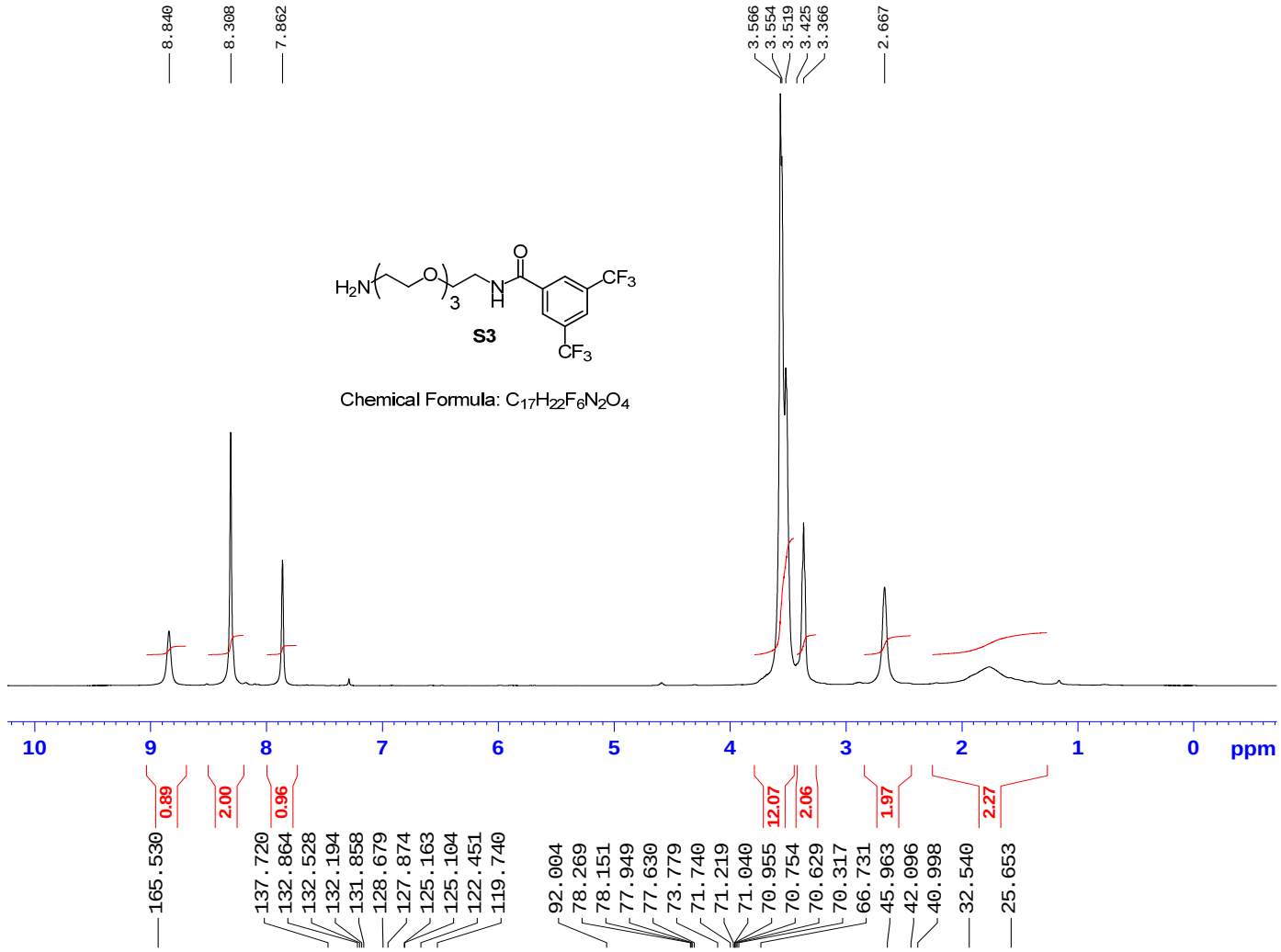
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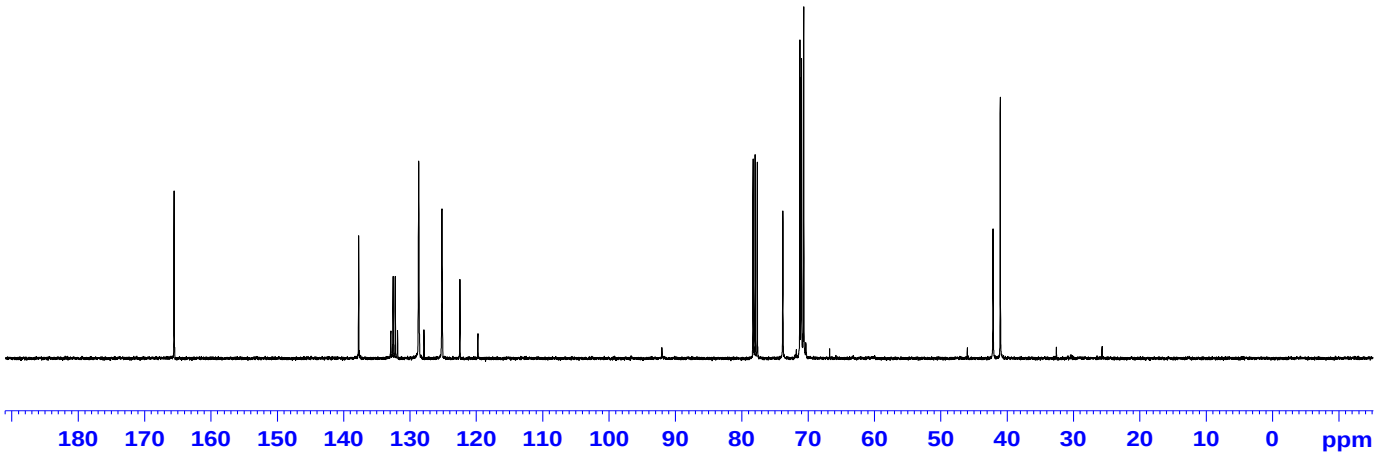
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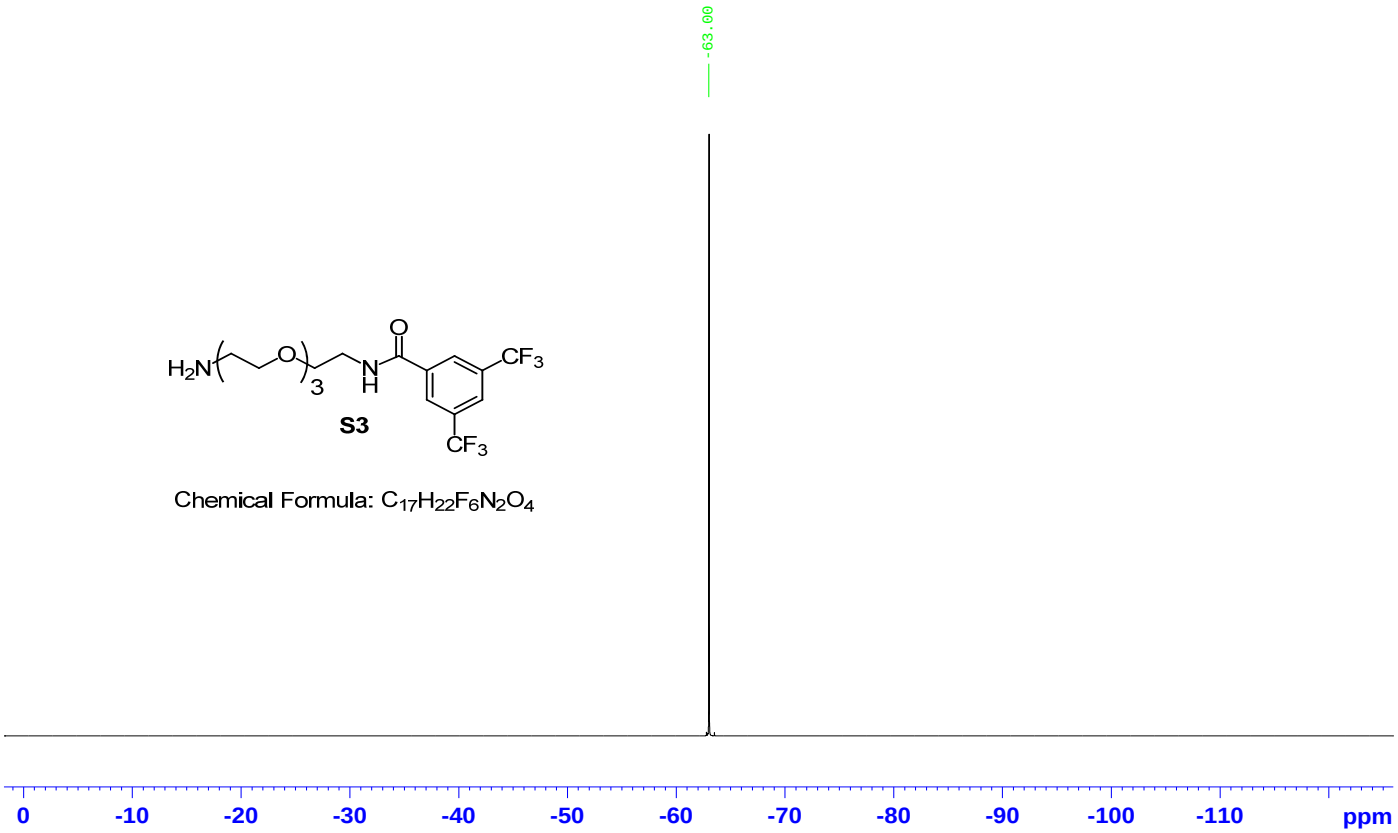

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Chemical Formula: $C_{17}H_{22}F_6N_2O_4$

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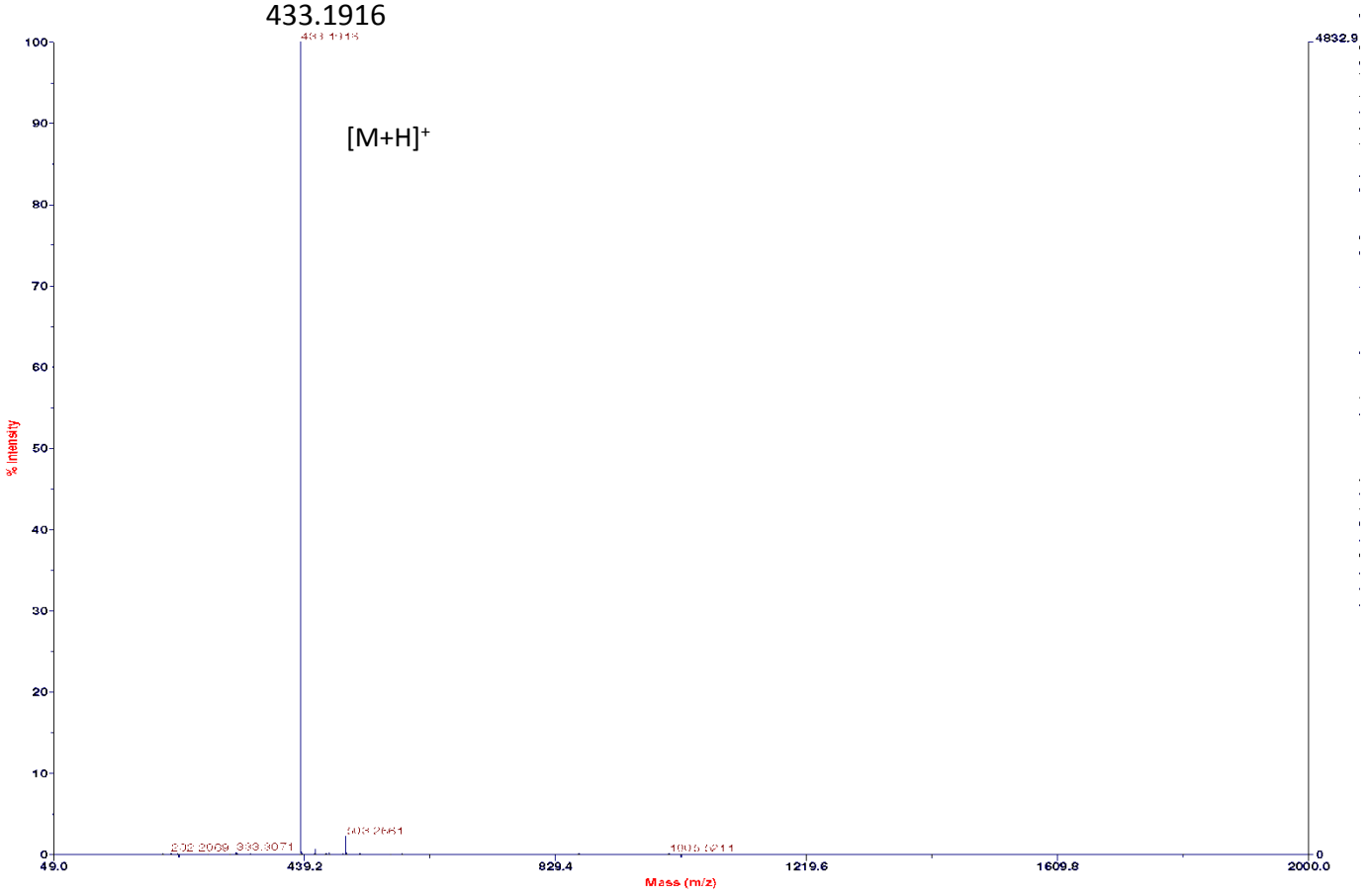

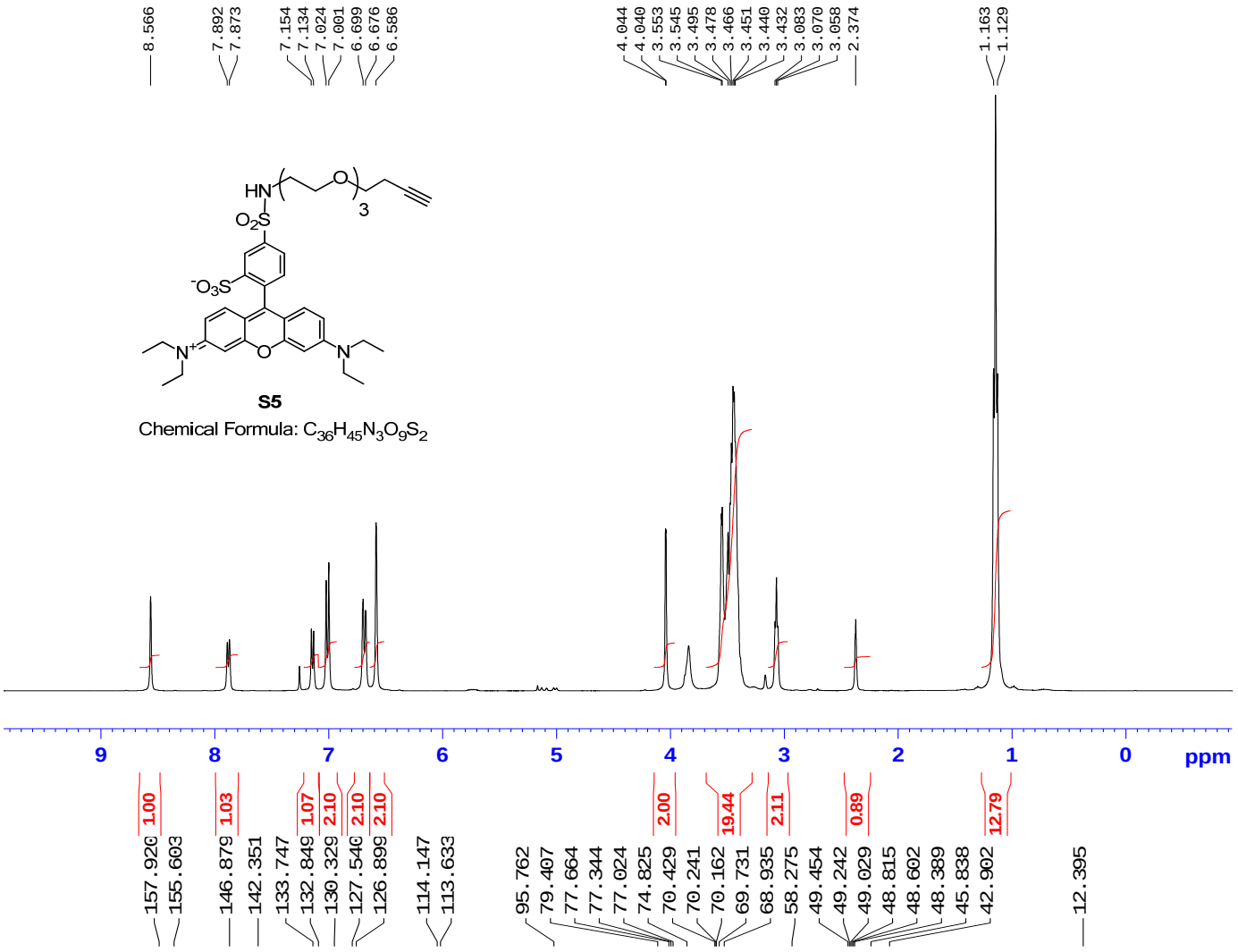
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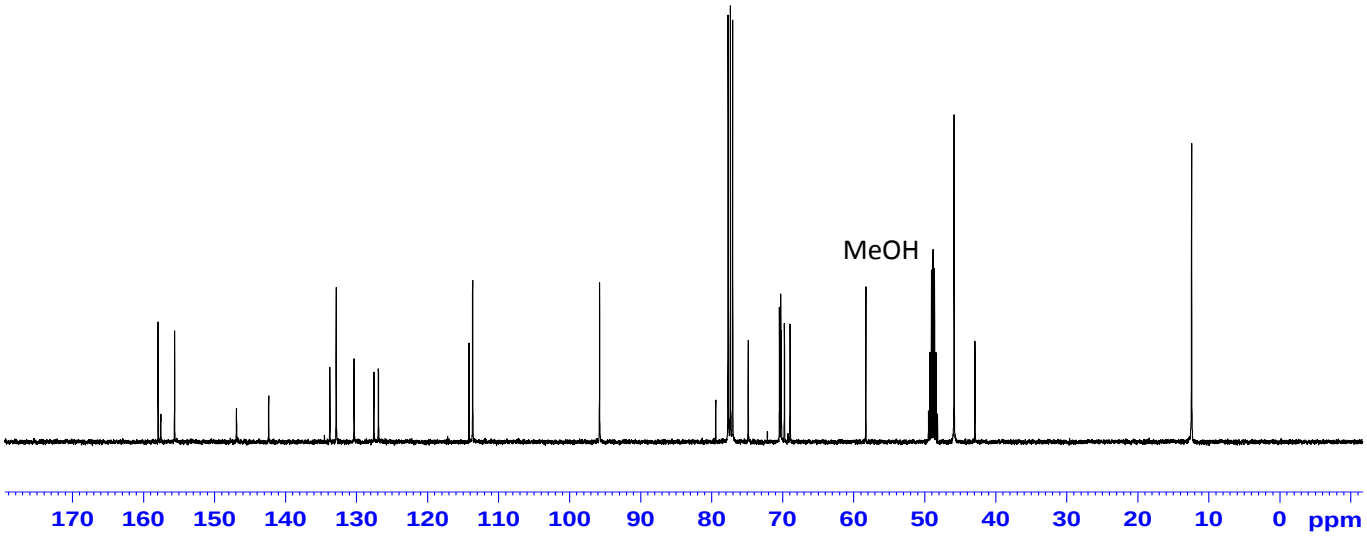
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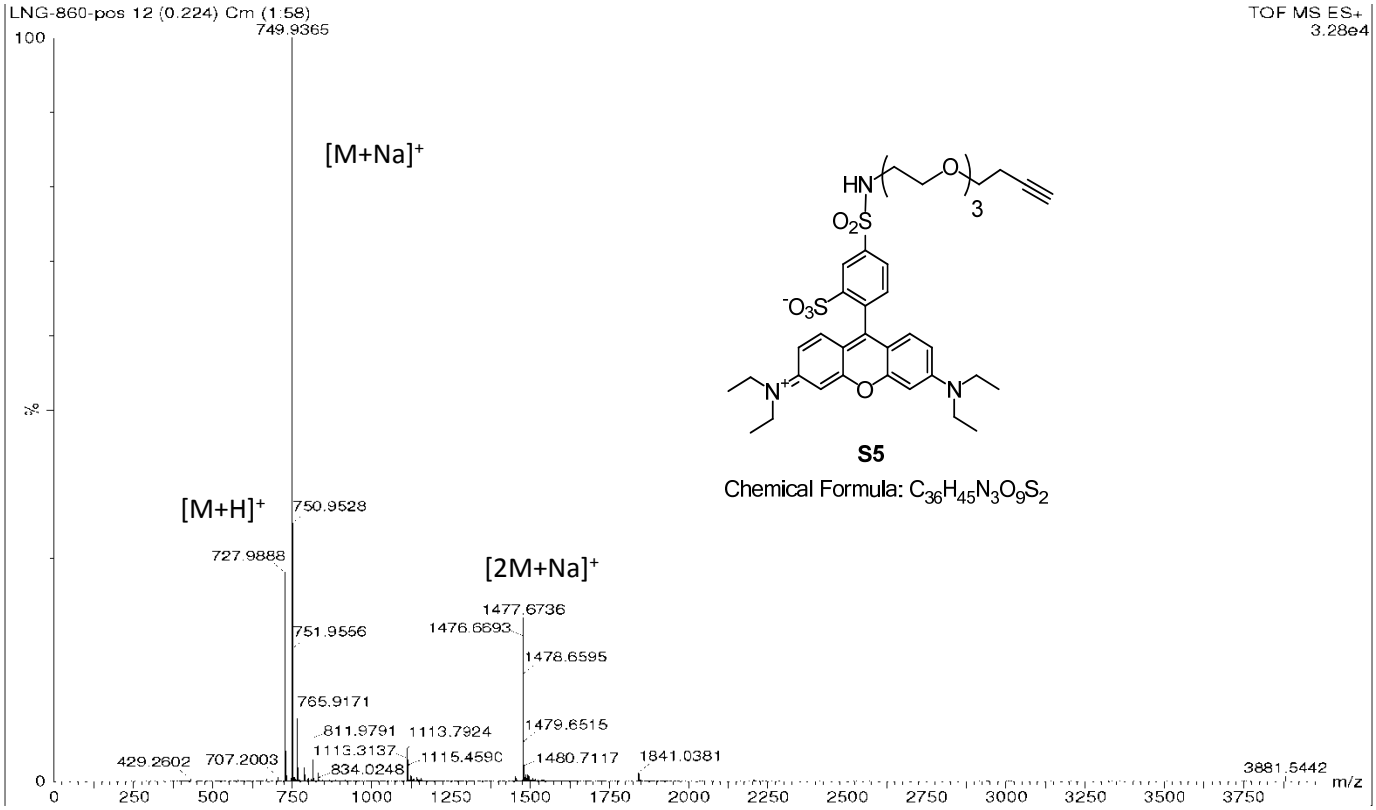
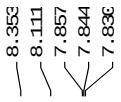
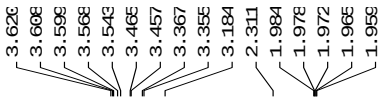
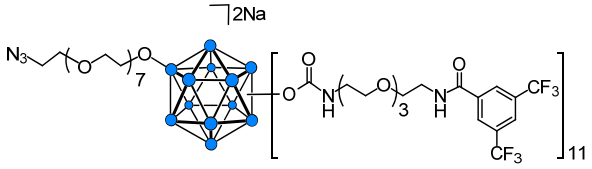


S5

Chemical Formula: $C_{36}H_{45}N_3O_9S_2$







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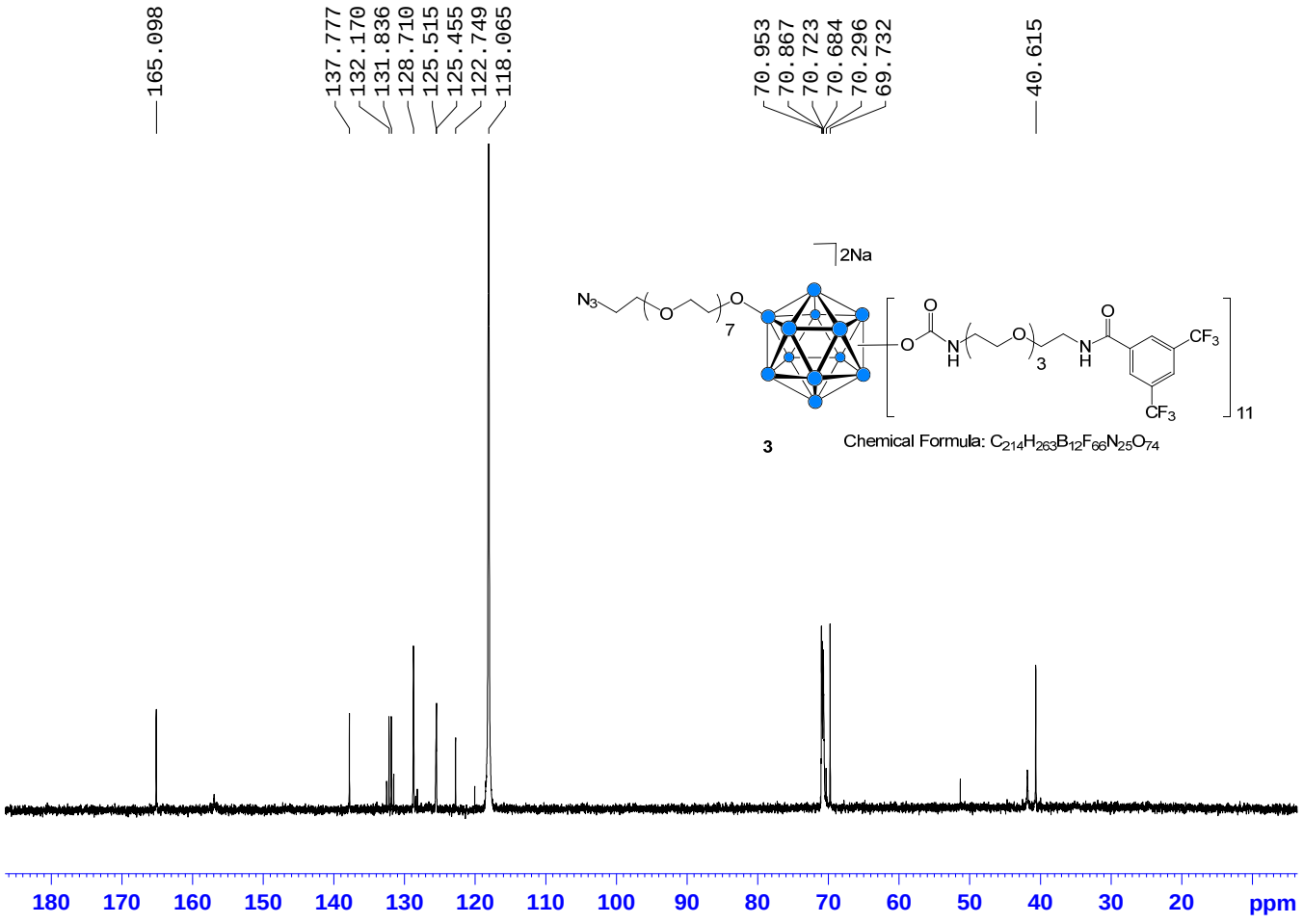
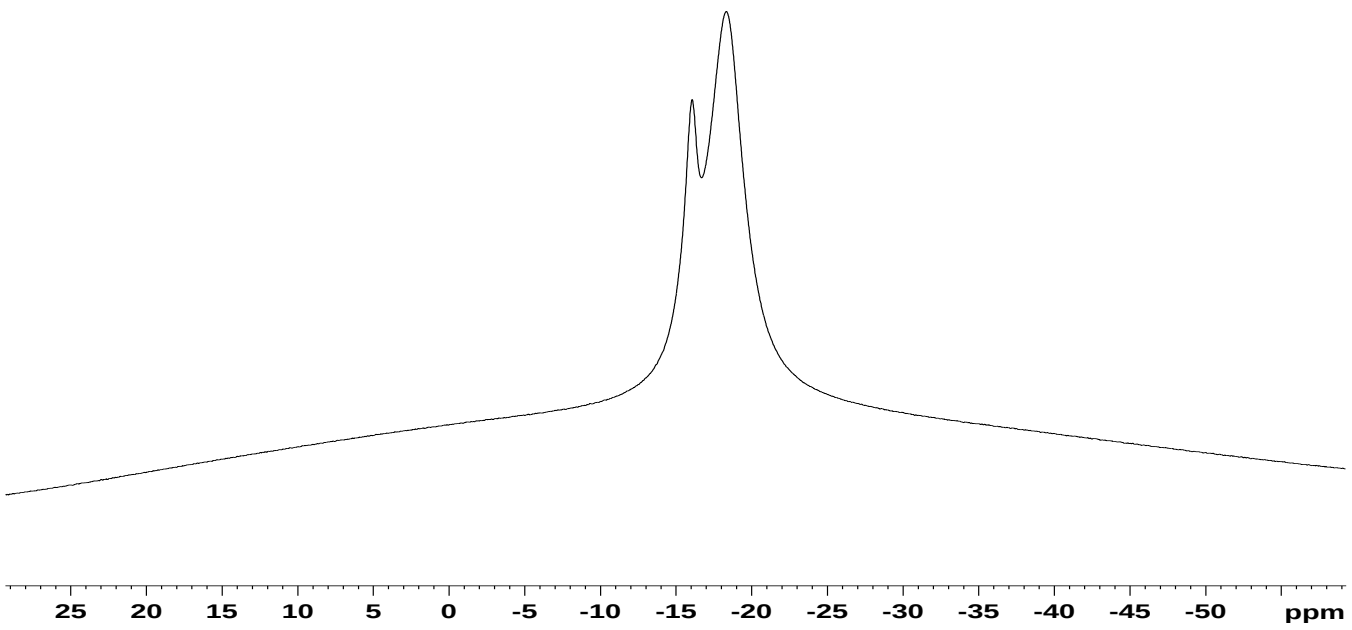
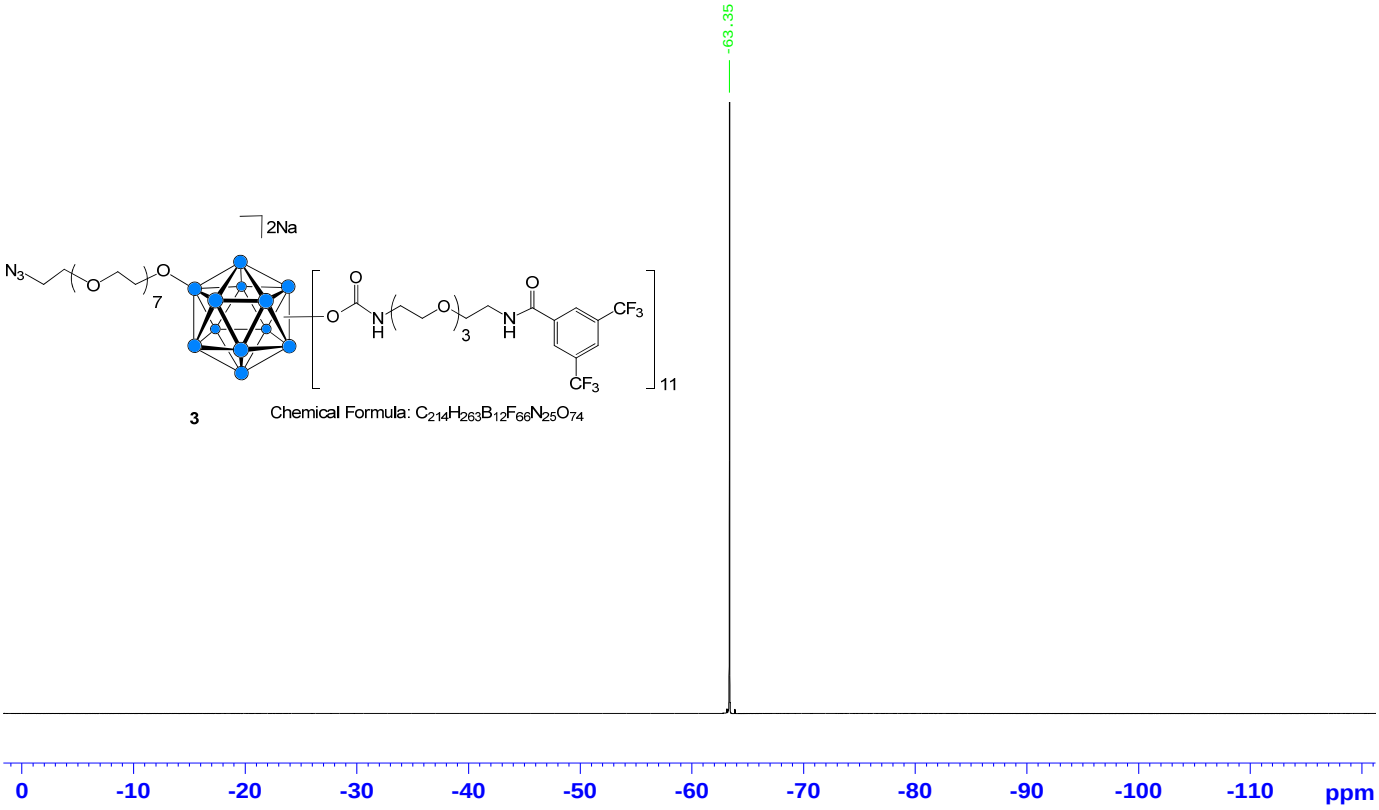


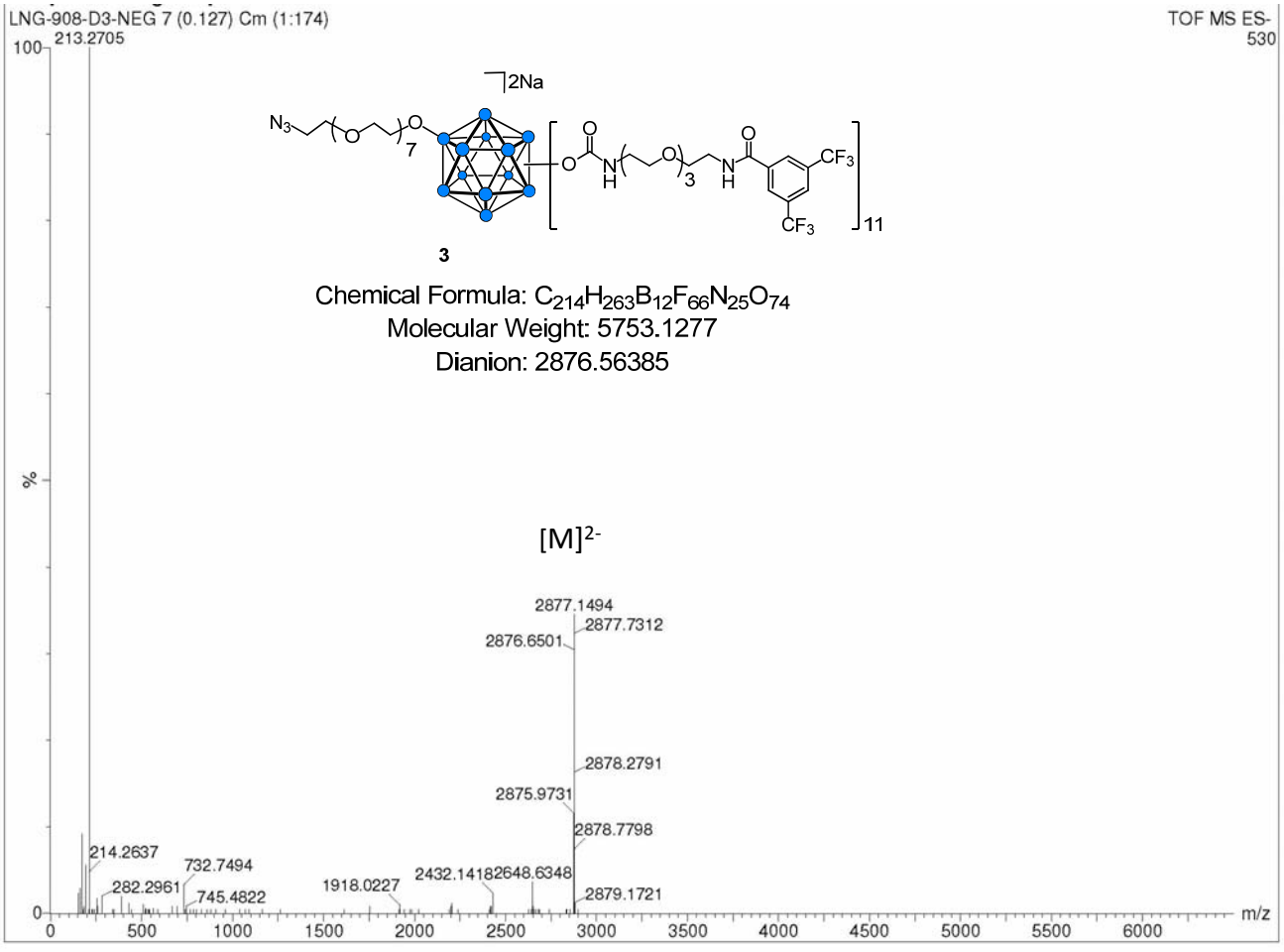
3 Chemical Formula: $C_{214}H_{263}B_{12}F_{66}N_{25}O_{74}$





3

Chemical Formula: $C_{214}H_{263}B_{12}F_{66}N_{25}O_{74}$



2Na

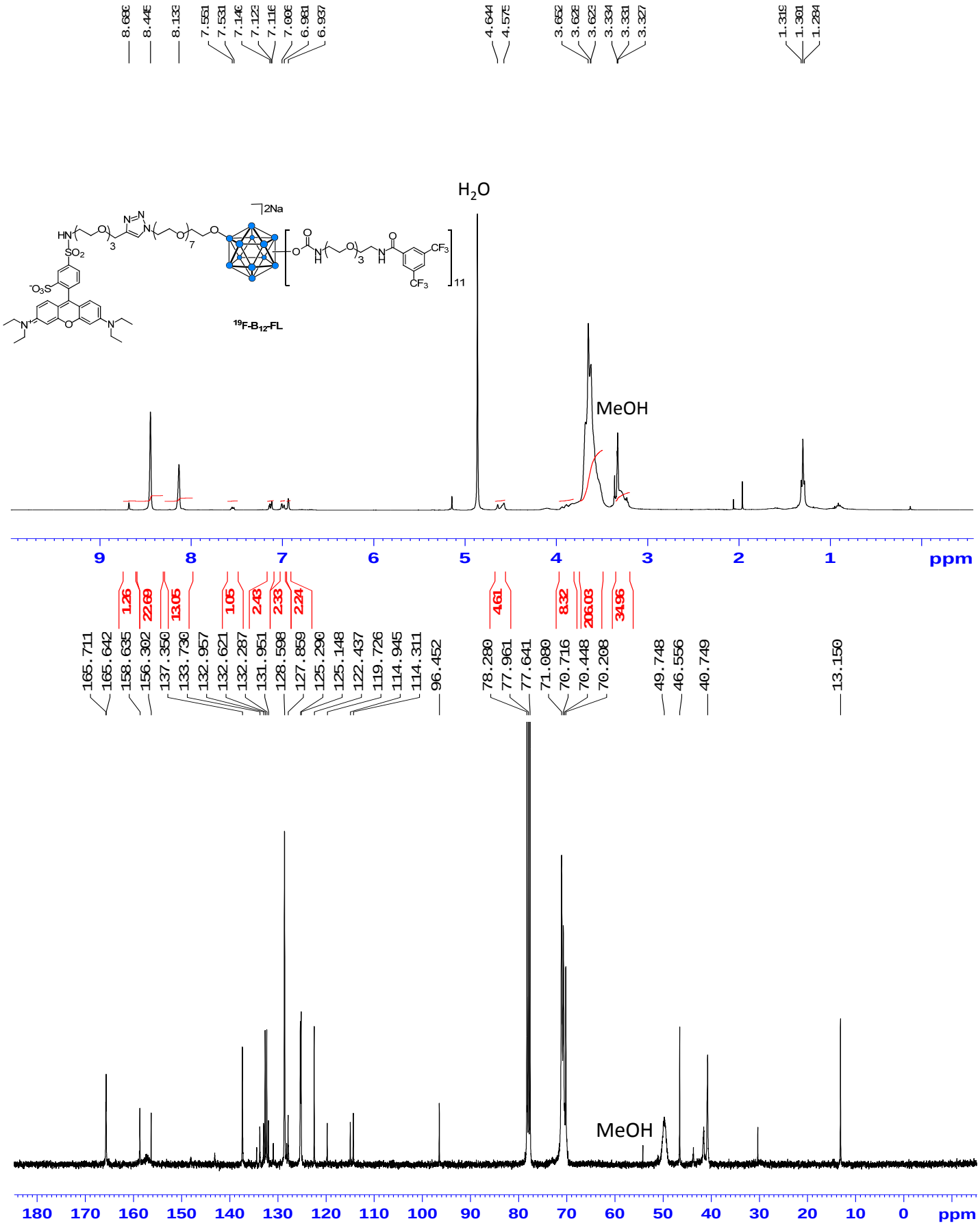
3

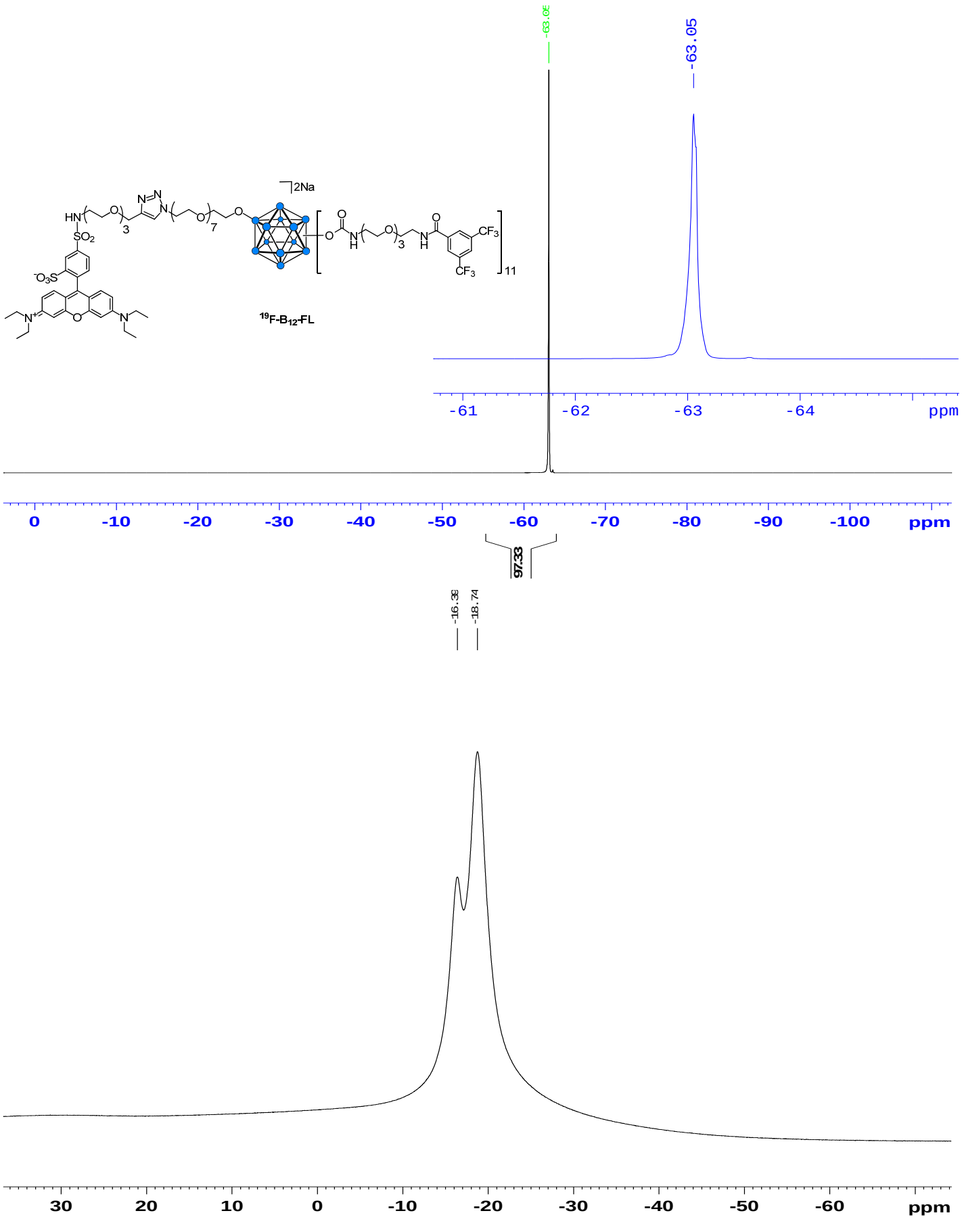
Chemical Formula: $C_{214}H_{263}B_{12}F_{66}N_{25}O_{74}$

Molecular Weight: 5753.1277

Dianion: 2876.56385

$[M]^{2-}$


8.688
8.445
8.133
7.551
7.531
7.146
7.123
7.116
7.086
6.981
6.937
4.644
4.575
3.652
3.628
3.622
3.334
3.331
3.327
1.319
1.301
1.284
H2O
MeOH
19F-B12-FL
9
8
7
6
5
4
3
2
1
ppm
1.26
22.69
13.05
1.05
2.43
2.33
2.24
4.61
8.32
206.03
34.96
165.711
165.642
158.635
156.302
137.350
133.730
132.957
132.621
132.287
131.951
128.598
127.859
125.290
125.148
122.437
119.726
114.945
114.311
96.452
78.280
77.961
77.641
71.080
70.716
70.448
70.208
49.748
46.556
40.749
13.150
MeOH
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
ppm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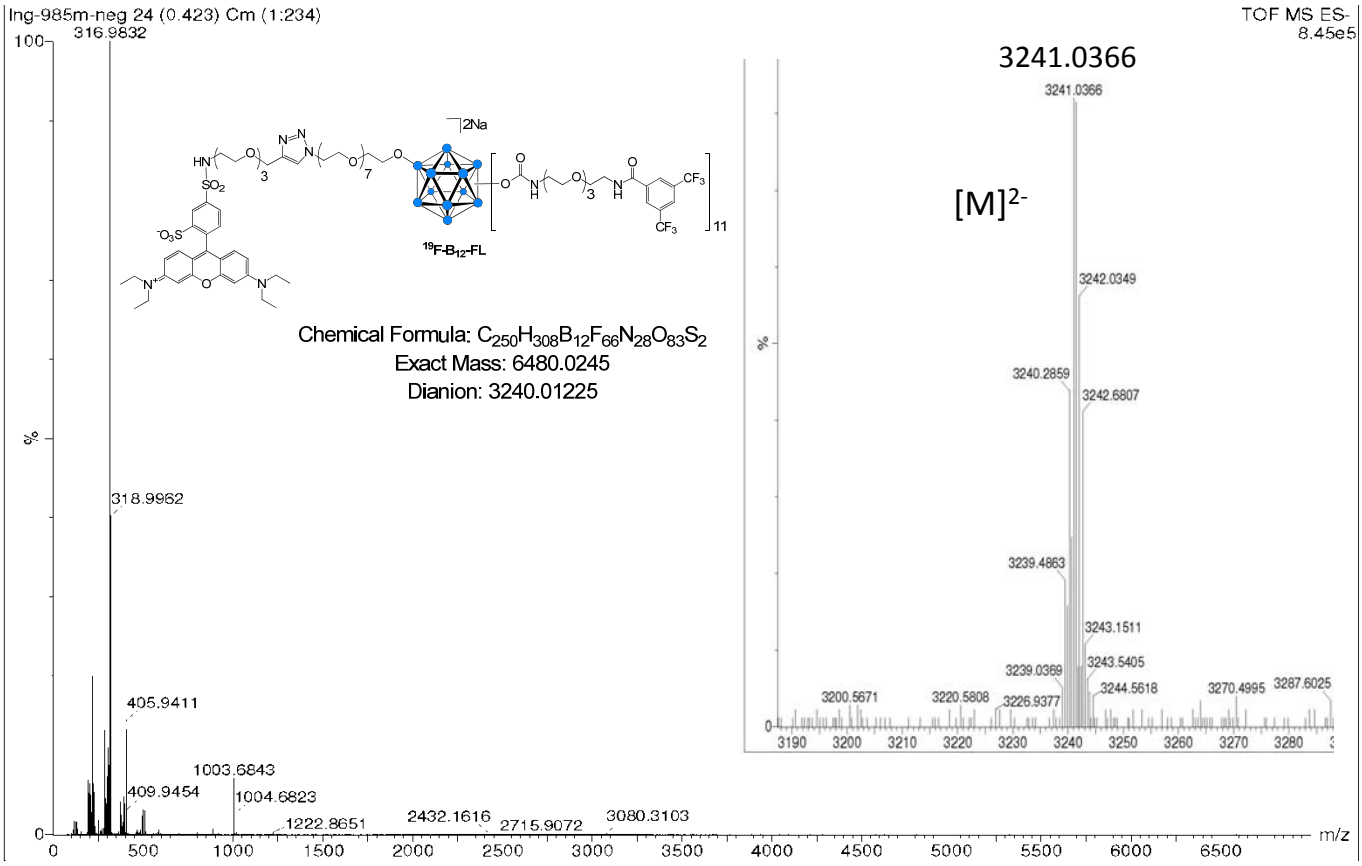
-61
-62
-63
-64
ppm
0
-10
-20
-30
-40
-50
-60
-70
-80
-90
-100
ppm
97.33
-16.38
-18.74
30
20
10
0
-10
-20
-30
-40
-50
-60
ppm

^{19}F-B$_{12}$-FL


lng-985m-neg 24 (0.423) Cm (1:234)
TOF MS ES-
8.45e5
316.9832
100
%
0
318.9962
405.9411
409.9454
1003.6843
1004.6823
1222.8651
2432.1616
2715.9072
3080.3103
m/z
0
500
1000
1500
2000
2500
3000
3500
4000
4500
5000
5500
6000
6500
2Na
11
^{19}F-B$_{12}$-FL
Chemical Formula: $C_{250}H_{308}B_{12}F_{66}N_{28}O_{83}S_2$
Exact Mass: 6480.0245
Dianion: 3240.01225
3241.0366
[M]$^{2-}$
3241.0366
3242.0349
3240.2859
3242.6807
3239.4863
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3239.0369
3243.5405
3200.5671
3220.5808
3226.9377
3244.5618
3270.4995
3287.6025
%
0
3190
3200
3210
3220
3230
3240
3250
3260
3270
3280