PNAS

Supporting Information for

Fluorinated Lipid Nanoparticles Enable Real-Time Tracking of mRNA Delivery and Uncover Spatiotemporal Mechanisms of Immune Activation

Kairu Xie[a,1], Lijun Zhu[a,b,1], Mojie Duan[a], Yu Fu[a,b], Haiqiang Wang[a,b], Yuhan Shen[a,c], Yu Li[a,b], Ruifang Wang[a,b], Zhong-Xing Jiang[a.b], Shizhen Chen[a,b,c], Jung Soo Suk[d], Daiqin Chen[a,b,*], Xin Zhou[a,b,c,*]

Corresponding authors: Xin Zhou, Daiqin Chen
Email: xinzhou@wipm.ac.cn; chendychin@apm.ac.cn

This PDF file includes:

Synthesis of compounds

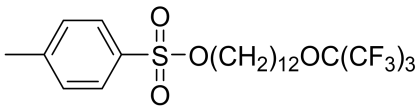
Synthesis and characterization of bis(6-(perfluoro-*tert*-butoxyl)hexyl)amine (FC6) and bis(12-(perfluoro-*tert*-butoxyl)dodecyl)amine (FC12).

$HO(CH_2)_{12}OC(CF_3)_3$

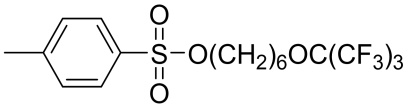
Compound 1. 1-Bromo-12-dodecanol (16.6 g, 62.7 mmol) was dissolved in DMF, and potassium perfluoro-*tert*-butoxide (14.3 g, 52.2 mmol) was added to the solution. The reaction mixture was stirred at 110°C for 12 h. After thin-layer chromatography (TLC) indicated the reaction was completed, DMF was removed under reduced pressure. The residue was diluted with CH_2Cl_2 and washed with water three times. The organic phase was collected, concentrated, and purified by flash column chromatography on silica gel to yield compound 1 as a clear oil (17.2 g, 78% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.98 (t, J = 6.5 Hz, 2H), 3.63 (t, J = 6.7 Hz, 2H), 1.70 – 1.61 (m, 2H), 1.59 – 1.51 (m, 2H), 1.40 – 1.22 (m, 16H).

$HO(CH_2)_6OC(CF_3)_3$

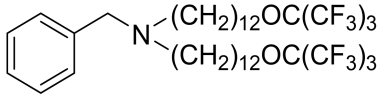
Compound 2. Compound **2** was obtained from 6-bromo-1-hexanol (15.0 g, 82.8 mmol) as a clear oil (22.1 g，79% yield) by employing the same synthetic procedures as compound **1**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.99 (t, J = 6.4 Hz, 2H), 3.63 (t, J = 6.6 Hz, 2H), 1.68 (p, J = 6.6 Hz, 2H), 1.57 (p, J = 6.7 Hz, 2H), 1.44 – 1.37 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.8, 120.6 (t, J = 293.6.0 Hz), 114.6, 80.5 – 79.4 (m), 69.84, 69.83, 62.7, 32.6, 29.8, 25.4, 25.3. ^{19}F NMR (471 MHz, $CDCl_3$) δ -73.51. HRMS (ESI): calculated for $C_{10}H_{13}F_9KO_2^+$ $[M+K]^+$ 375.0403, found 375.0321.



Compound 3. To a solution of compound **1** (1.0 g, 2.4 mmol) in CH_2Cl_2 (10 mL) was added Et_3N (0.48 g, 0.66 mL, 4.8 mmol). After the solution was cooled to 0°C, a solution of *p*-toluenesulfonyl chloride (0.92 g，4.8 mmoll) in CH_2Cl_2 (10 mL) was slowly added, and the resulting mixture was stirred at rt for 5 h. After TLC showed that the reaction was completed, the reaction mixture was washed with saturated aqueous ammonium chloride, and extracted with CH_2Cl_2. The organic phase was separated, concentrated, and purified by flash column chromatography on silica gel to provide compound **3** as a clear oil (1.1 g, 81% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.78 (d, J = 8.4 Hz, 2H), 7.33 (d, J = 8.0 Hz, 2H), 4.00 (dt, J = 12.1, 6.5 Hz, 4H), 2.43 (s, 3H), 1.69 – 1.59 (m, 4H), 1.35 (q, J = 7.2, 6.8 Hz, 2H), 1.32 – 1.17 (m, 14H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.8, 139.3, 133.4, 129.9, 128.0, 124.1, 120.6 (t, J = 294.2 Hz), 114.2, 80.4 – 79.2 (m), 70.7, 69.8, 33.8, 29.7, 29.42, 29.40, 29.38, 29.3, 29.1, 28.9, 28.8, 25.30, 25.26. ^{19}F NMR (471 MHz, $CDCl_3$) δ -73.48. HRMS (ESI): calculated for $C_{23}H_{31}F_9NaO_4S^+$ $[M+Na]^+$, 597.1692, Found 597.1691.

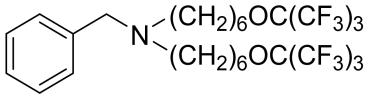


Compound 4. Compound **4** was obtained from compound **2** (7.4 g, 22.0 mmol) as a clear oil (8.7 g, 80% yield) by employing the same synthetic procedures as compound **3**. ^{1}H NMR (500 MHz, Acetone-d_6) δ 7.80 (d, J = 8.3 Hz, 2H), 7.48 (d, J = 8.1 Hz, 2H), 4.07 (dt, J = 18.3, 6.3 Hz, 4H), 2.46 (s, 3H), 1.67 (dt, J = 13.4, 6.5 Hz, 4H), 1.48 – 1.19 (m, 4H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 144.9, 138.0, 133.3, 129.9, 128.0, 120.5 (t, J = 294.8 Hz), 115.2, 80.4 – 79.5 (m), 70.5, 69.68, 69.67, 29.6, 28.8, 25.1, 24.9, 21.68, 21.65. ^{19}F NMR (471 MHz, Acetone-d_6) δ -71.42. HRMS (ESI): calculated for $C_{17}H_{19}F_9KO_4S^+$ $[M+K]^+$ 529.0492, found 529.0494.

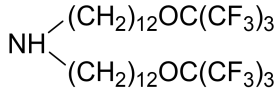


Compound 5. Under an atmosphere of Ar, to a suspension of K_2CO_3 (400.9 mg, 2.9 mmol) and KI (120.4 mg, 0.73 mmol) in dry CH_3CN (5 mL) was added a solution of benzylamine (77.7 mg, 0.73 mmol) and compound **3** (500.0 mg, 0.87 mmol) in dry CH_3CN (10 mL), respectively. The resulting mixture was stirred at 80°C for 6 h. Then, a solution of compound **3** (500.0 mg, 0.87 mmol) in dry CH_3CN (10 mL) was added to the mixture, and the reaction was stirred at 80°C for

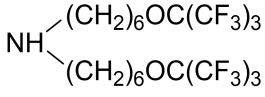
another 6 h. After TLC showed that the reaction was completed, the reaction mixture was washed with saturated aqueous ammonium chloride, and extracted with EtOAc. The organic phase was separated, concentrated, and purified by flash column chromatography on silica gel to provide compound **5** as a clear oil (599.8 mg, 91% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.28 (m, 4H), 7.26 – 7.21 (m, 1H), 4.02 (t, J = 6.5 Hz, 4H), 3.57 (s, 2H), 2.42 (t, J = 7.4 Hz, 4H), 1.73 – 1.64 (m, 4H), 1.52 – 1.45 (m, 4H), 1.42 – 1.36 (m, 4H), 1.34 – 1.26 (m, 28H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 140.3, 139.2, 128.8, 128.0, 126.6, 120.4 (t, J = 294.2 Hz), 114.0, 80.4 – 79.2 (m), 69.8, 58.7, 53.8, 33.8, 32.0, 29.7, 29.65, 29.59, 29.54, 29.46, 29.2, 29.0, 27.5, 27.0, 25.3, 22.7. ^{19}F NMR (471 MHz, $CDCl_3$) δ -73.54.



Compound 6. Compound **6** was obtained from compound **4** (4.2 g, 8.6 mmol) as a clear oil (2.3 g, 86% yield) by employing the same synthetic procedures as compound **5**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 – 7.28 (m, 4H), 7.26 – 7.21 (m, 1H), 3.99 (t, J = 6.5 Hz, 4H), 3.55 (s, 2H), 2.41 (t, J = 7.2 Hz, 4H), 1.66 (p, J = 6.6 Hz, 4H), 1.48 (p, J = 7.2 Hz, 4H), 1.40 – 1.27 (m, 8H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 140.3, 129.0, 128.2, 126.8, 120.6 (t, J = 292.3 Hz), 115.2, 80.5 – 79.4 (m), 70.0, 58.8, 53.7, 29.8, 27.0, 25.3. ^{19}F NMR (471 MHz, $CDCl_3$) δ -73.48. HRMS (ESI): calculated for $C_{27}H_{31}F_{18}KNO_2^+$ $[M+K]^+$ 782.1699, found 782.1656.

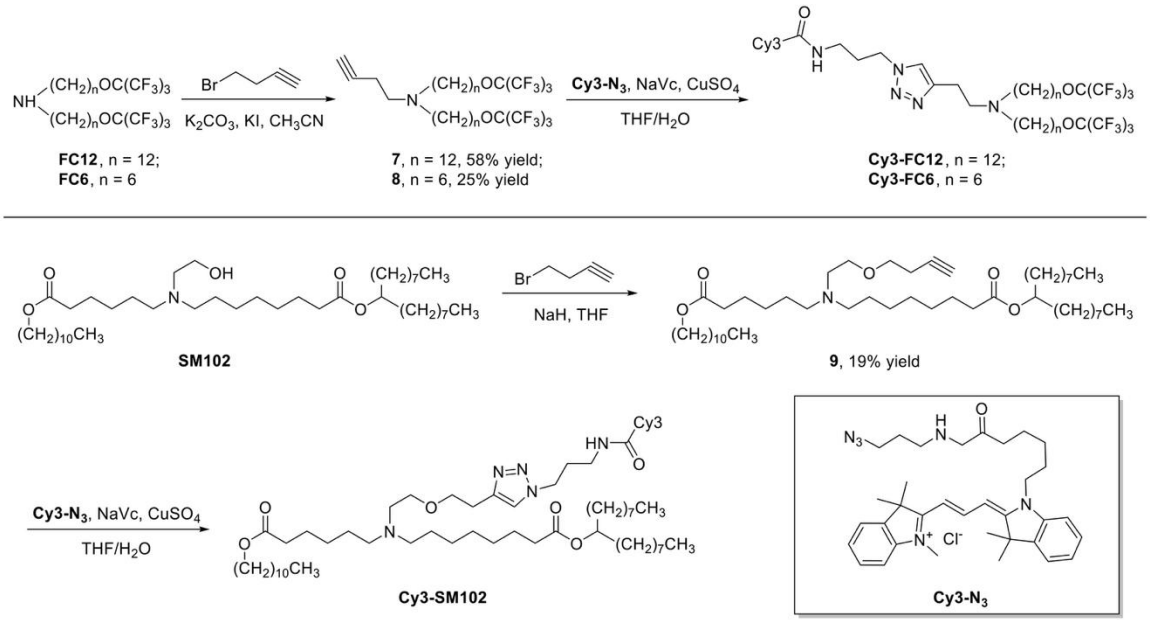


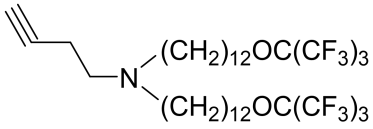
Compound FC12. Under an atmosphere of H_2, a mixture of compound **5** (1.0 g, 1.1 mmol) and Pd/C (10% on carbon, 23.3 mg) in dry MeOH (10 mL) was stirred at rt for 12 h. After TLC showed that the reaction was completed, the mixture was filtrated through a pad of Celite, and the filtrate was concentrated. The residue was purified by column chromatography on silica gel to give lipid **FC12** as a clear oil (720.9 mg, 80% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.97 (t, J = 6.5 Hz, 4H), 2.62 – 2.57 (m, 4H), 1.65 (d, J = 6.6 Hz, 4H), 1.50 (d, J = 7.4 Hz, 4H), 1.35 (t, J = 7.6 Hz, 4H), 1.26 (q, J = 4.8, 3.7 Hz, 28H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.4 (t, J = 294.8 Hz), 80.4 – 79.4 (m), 69.8, 49.9, 31.9, 29.8, 29.7, 29.54, 29.52, 29.47, 29.4, 29.3, 29.1, 27.4, 25.2, 22.7. ^{19}F NMR (471 MHz, $CDCl_3$) δ -73.53. HRMS (ESI) calculated for $C_{32}H_{50}F_{18}NO_2^+$ 822.3549, found 822.3547.



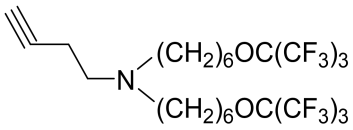
Compound FC6. Lipid **FC6** was obtained from compound **6** (1.0 g, 1.3 mmol) as a clear oil (0.72 g, 82% yield) by employing the same synthetic procedures as lipid **FC12**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.97 (t, J = 6.4 Hz, 4H), 2.60 (t, J = 7.4 Hz, 4H), 1.65 (p, J = 6.7 Hz, 4H), 1.52 (p, J = 7.4 Hz, 4H), 1.43 – 1.27 (m, 8H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 120.6 (t, J = 293.6 Hz), 80.3 – 79.4 (m), 69.80, 69.79, 49.7, 29.8, 29.7, 29.5, 27.0, 25.3. ^{19}F NMR (471 MHz, $CDCl_3$) δ -73.40. HRMS (ESI) calculated for $C_{20}H_{25}F_{18}KNO_2^+$ $[M+K]^+$ 692.1229, found 692.1229.

Synthesis of Cy3-labeled fluorinated lipids.

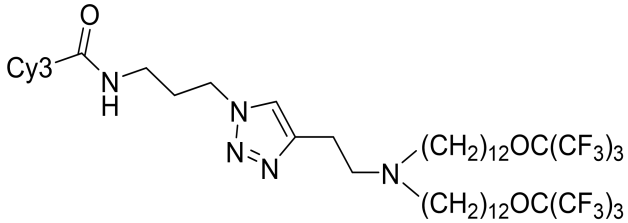




Compound 7. Under an atmosphere of Ar, to a suspension of K_2CO_3 (30.0 mg, 0.21 mmol) and KI (5.0 mg, 0.03 mmol) in dry CH_3CN (2 mL) was added a solution of 4-bromobut-1-yne (20.0 mg, 0.15 mmol) and compound **FC12** (60.0 mg, 0.07 mmol) in dry CH_3CN (5 mL), respectively. The resulting mixture was stirred at 80°C for 12 h. After TLC showed that the reaction was completed, the reaction mixture was washed with saturated aqueous ammonium chloride, and extracted with EtOAc. The organic phase was separated, concentrated, and purified by flash column chromatography on silica gel to provide compound **7** as a clear oil (31.0 mg, 58% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.00 (t, J = 6.6 Hz, 4H), 3.00 – 2.85 (m, 4H), 1.85 (t, J = 8.1 Hz, 4H), 1.68 (t, J = 7.3 Hz, 4H), 1.30 – 1.27 (m, 35H), 0.89 (t, J = 6.7 Hz, 2H). ^{19}F NMR (471 MHz, $CDCl_3$) δ -73.58.

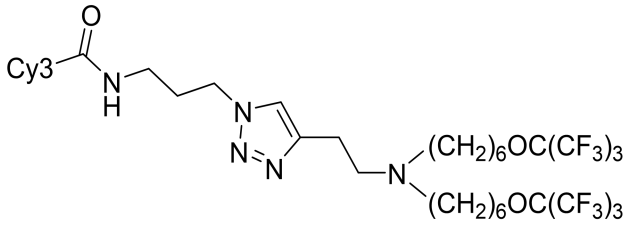


Compound 8. Compound **8** was obtained from compound **FC6** (60.0 mg, 0.09 mmol) as a clear oil (16.1 mg，25% yield) by employing the same synthetic procedures as compound **7**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.00 (t, J = 6.4 Hz, 4H), 3.08 – 2.84 (m, 4H), 1.88 (p, J = 8.1 Hz, 4H), 1.69 (t, J = 6.8 Hz, 4H), 1.59 – 1.00 (m, 12H), 1.07 – 0.74 (m, 1H). ^{19}F NMR (471 MHz, $CDCl_3$) δ -73.58.

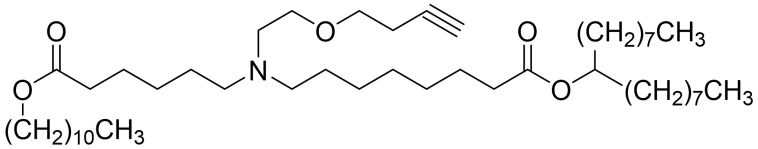


Compound Cy3-FC12. Under an atmosphere of Ar, to a solution of **Cy3-N$_3$** (3.0 mg, 0.005 mmol) in dry THF (2 mL) was added a solution of compound **7** (7.0 mg, 0.08 mmol) in dry THF (2 mL). Then, 1 mL $CuSO_4$ (0.2 mg, 0.001 mmol) aqueous solution and 1 mL NaVc (0.5 mg, 0.002 mmol) aqueous solution were successively added to the reaction mixture. The reaction mixture was stirred at room temperature for 24 h. After TLC showed that the reaction was completed, the reaction mixture was washed with water three times. The organic phase was concentrated to

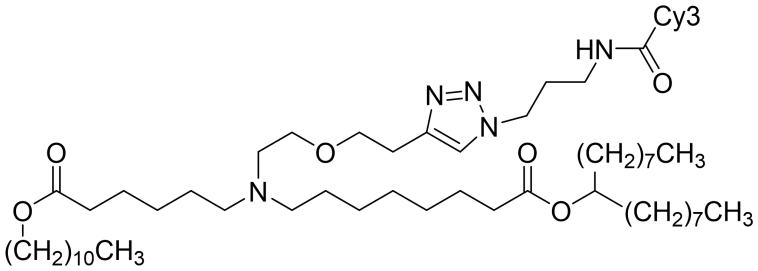
provide compound **Cy3-FC12**. HRMS (ESI) calculated for $C_{69}H_{97}F_{18}N_7O_3^+$ [M/2+H]$^+$ 707.9008, found 707.1145.



Compound Cy3-FC6. Compound **Cy3-FC6** was obtained from compound **8** (6.0 mg, 0.01 mmol) by employing the same synthetic procedures as compound **Cy3-FC12**. HRMS (ESI) calculated for $C_{57}H_{73}F_{18}N_7O_3^+$ [M/2+H]$^+$ 623.7350, found 624.1447.



Compound 9. Under an atmosphere of Ar, to a suspension of NaH (7.0 mg, 0.29 mmol) in dry THF (2 mL) was added a solution of **SM102** (100.0 mg, 0.14 mmol) in dry THF (5 mL). The reaction mixture was stirred at 0 °C for 0.5 h. Then, dry THF (5 mL) solution of 4-bromobut-1-yne (37.0 mg, 0.28 mmol) was added to the reaction solution, and the mixture was stirred at room temperature for 12 h. After TLC showed that the reaction was completed, the mixture was quenched with ice water. Then THF was evaporated under vacuum and extracted with EtOAc. The organic phase was separated, concentrated, and purified by flash column chromatography on silica gel to provide compound **9** as a clear oil (20.4 mg, 19% yield). ^{1}H NMS (600 MHz, $CDCl_3$) δ 5.18 – 4.61 (m, 1H), 4.33 – 4.05 (m, 2H), 2.72 – 2.70 (m, 3H), 2.56 – 2.42 (m, 4H), 2.32 – 2.28 (m 6H), 1.79 – 1.59 (m, 6H), 1.52 – 1.36 (m, 10H), 1.37 – 1.28 (m, 50H), 0.90 (t, J = 7.0 Hz, 9H).



Compound Cy3-SM102. Compound **Cy3-SM102** was obtained from compound **9** (6.0 mg, 0.008 mmol) by employing the same synthetic procedures as compound **Cy3-FC12**. HRMS (ESI) calculated for $C_{81}H_{134}N_7O_6$ [M/2] 651.1349, found 650.0997.

Molecular docking. Molecular docking was performed using the MOE2019 software package. The Triangle Matcher algorithm was used for conformational sampling, and the London dG algorithm was used for scoring. A total of 50 conformations were collected from the docking. The optimal binding conformation was selected by ranking the conformations based on the docking score. The 2D interaction diagram was generated using the MOE2019 software, and the 3D binding schematic was created using PyMOL(1).

MD simulation. Lipid bilayer modeling was conducted using the packmol software package(2). The SM102 system contained the lipid molecules SM102, DSPC, Cholesterol, and PEG2000 in a molar ratio of 200: 40: 154: 6. The FC6 system was composed of FC6: SM102: DSPC: Cholesterol: PEG2000 at a ratio of 100: 100: 40: 154: 6. Similarly, the FC12 system consisted of FC12: SM102: DSPC: Cholesterol: PEG2000 at a ratio of 100: 100: 40: 154: 6. Each system also included 19800 water molecules and 200 Cl- ions to neutralize the system charge. The box sizes of SM102, FC6, FC12 systems are 10.5×10.5×8.0 nm^3, 12.0×12.0×8.0 nm^3 and 13.0×13.0×8.0 nm^3 respectively.

The molecular dynamics simulations followed the standard protocol. Initially, energy minimization was performed for a maximum of 5000 steps using the steepest descent algorithm. This was followed by a 5-step NPT ensemble pre-equilibration for a total of 1875 ps, during which restraints were applied to the protein and the bilayer. The production phase of the simulation was run for 500 ns using Gromacs2023 software(3). A cutoff distance of 1.2 nm was used for both Coulombic and Van der Waals interactions. The simulation temperature was maintained at 300 K using the Nose-Hoover temperature coupling method, and the pressure was controlled at 1 bar using the Parrinello-Rahman method with a semiisotropic pressure coupling scheme. The Particle-mesh Ewald (PME) method was employed to handle long-range electrostatic interactions. All analyses were performed on the final 200 ns of the trajectory.

The Area per Lipid (APL) is defined as the average area occupied by a lipid in the plane of the lipid bilayer. It is calculated using the following formula:

$$APL = \frac{2S}{N} \tag{1}$$

Where S represents the area of the bilayer plane and N is the total number of lipids in the bilayer system.

The Lateral Diffusion (LD) coefficient measures the mobility of lipids within the bilayer system. A higher LD value indicates greater fluidity. It is calculated from the Mean Squared Displacement (MSD) as follows:

$$MSD(t) = \langle |r(t) - r(0)|^2 \rangle \tag{2}$$

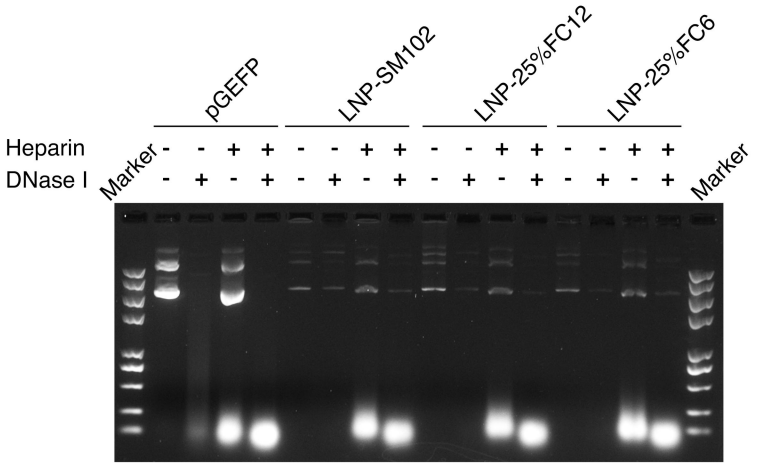
$$LD = \langle \frac{MSD(t)}{2dt} \rangle \tag{3}$$

Here, MSD(t) is the mean squared displacement of the lipid at time t, r(t) is the coordinate of the center of mass of the lipid type in the bilayer plane at time t, r(0) is its initial coordinate, and d represents the dimensionality (d=2 for this system).

The Deuterium Order Parameter (S_{CD}) is used to describe the flexibility of the lipid tails. A larger S_{CD} value indicates that the fatty acid long chains of the lipid tails are straighter, more rigid, and more ordered. The formula for its calculation is:

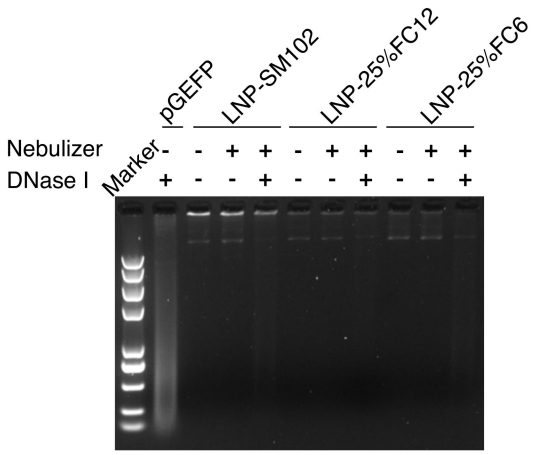
$$S_{CD} = \left| \frac{1}{2} \langle 3cos^2\theta_i - 1 \rangle \right| \tag{4}$$

i denotes the carbon atom number in the lipid tail, and θ represents the angle between the C-H bond vector of that carbon atom and the vector of the positive z-axis.



198

199 **Fig. S1.** LNP encapsulation protects pDNA from degradation by DNase I. Agarose gel retardation
200 assays of LNPs treated with or without DNase I and heparin, free pEGFP was used as a control.
201



202

203 **Fig. S2.** LNP encapsulation protects pDNA from degradation by DNase I or treatment with a
204 nebulizer. Agarose gel retardation assays of LNPs treated with or without DNase I and nebulizer,
205 free pEGFP was used as a control.

206

207

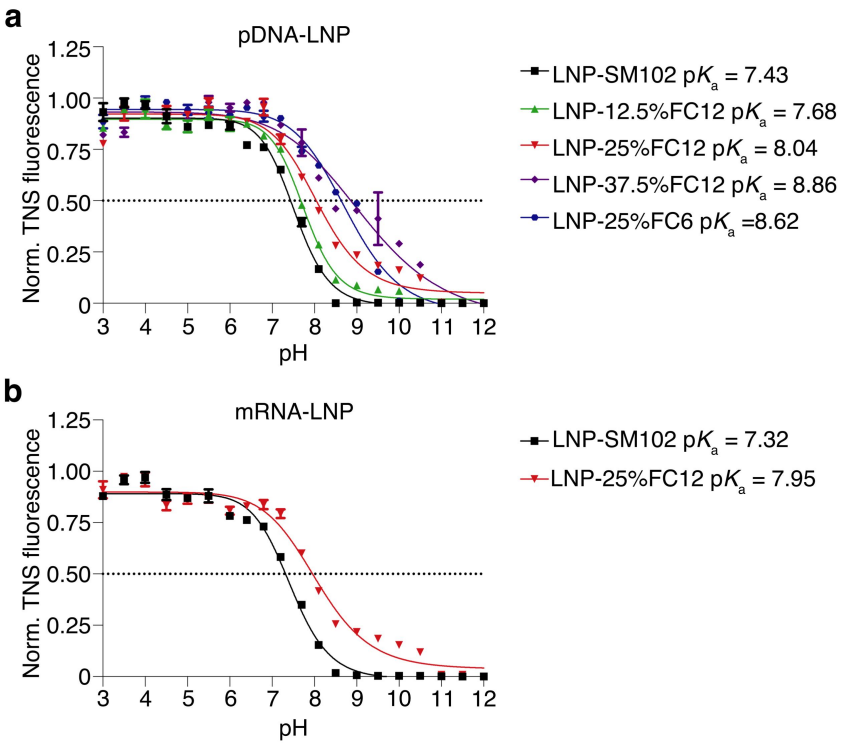
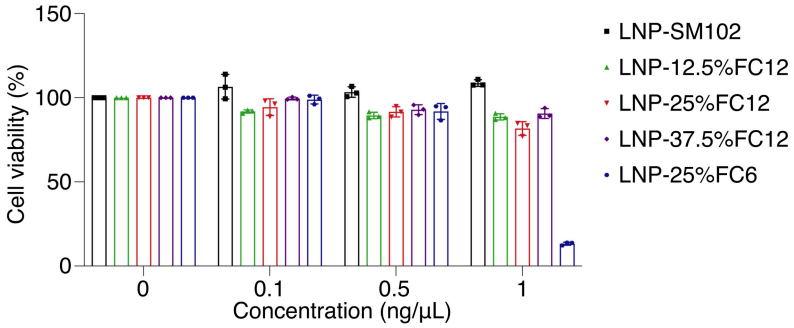


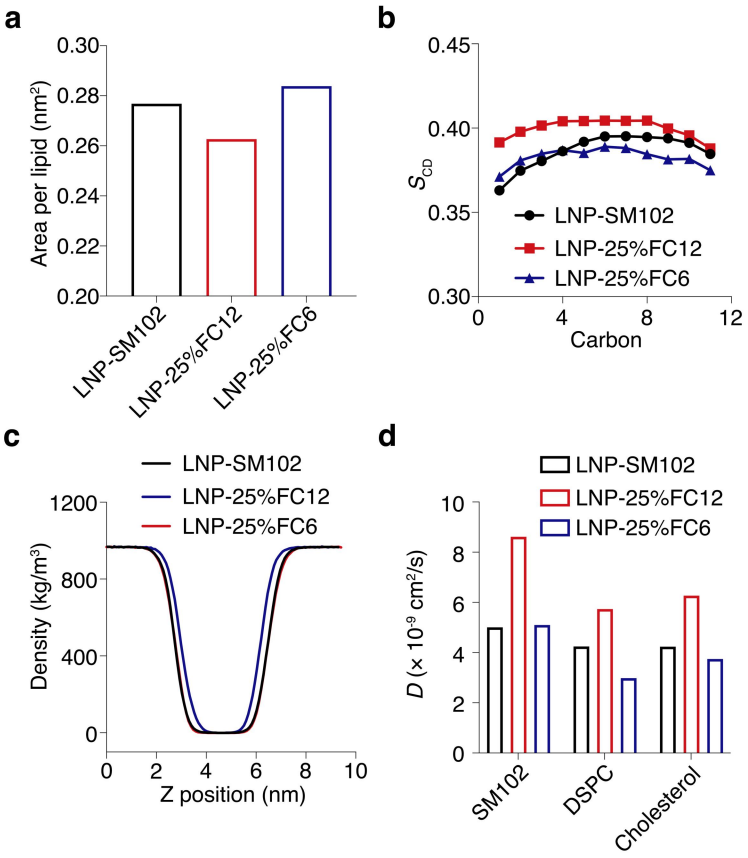
Fig. S3. Normalized TNS fluorescence intensity as a function of pH for LNPs loaded with DNA (a) or mRNA (b). The apparent pK_a was determined by nonlinear regression and defined as the pH at which fluorescence reached half of its maximum intensity.



213

214 **Fig. S4.** The cell viability of 293T cells after treatment with pDNA-loaded LNPs for 48h.

215



216

217 **Fig.S5.** The structural properties of LNP lipid bilayers. a. The area per lipid. b. The S_{CD} order
218 parameter value. c. The water density across the bilayers. d. The diffusion constants of lipids on
219 the bilayers.

220

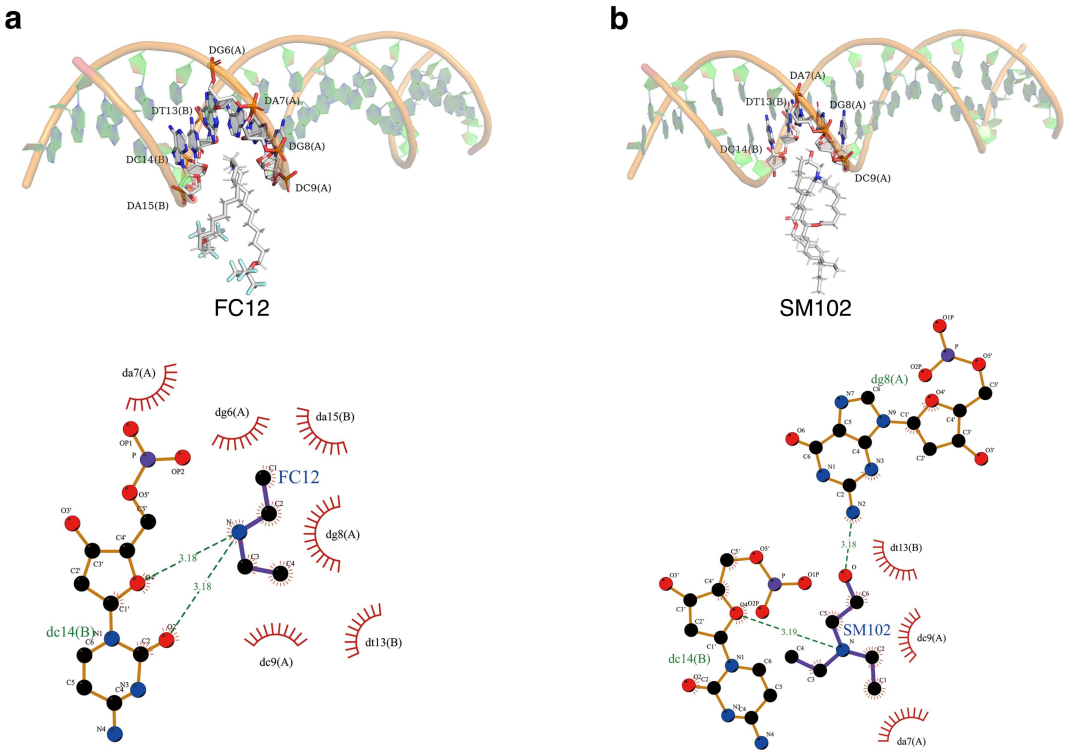


Figure S6. The binding modes of ionizable lipids with pDNA. a. FC12. b. SM102.

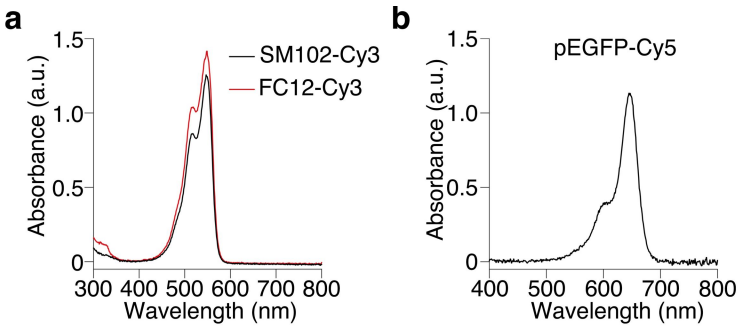
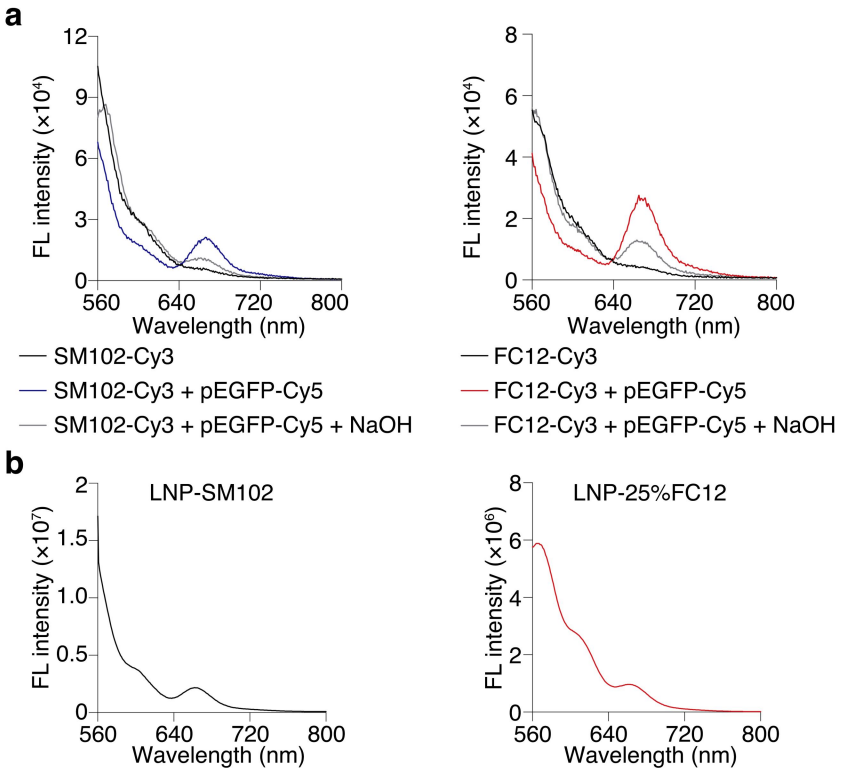


Fig. S7. a. UV-vis absorption of SM102 and FC12 labeled with Cy3. b. UV-vis absorption of pEGFP labeled with Cy5.



228

229 **Fig. S8.** (a) Fluorescence spectra of Cy3-labeled ionizable lipid alone, with pEGFP-Cy5, and with
230 pEGFP-Cy5 in the presence of NaOH. The concentrations of Cy3 and Cy5 were 5 μM and 20 μM,
231 respectively. (b) Fluorescence spectra of Cy3- and Cy5-labeled LNP-SM102 and LNP-25%FC12.
232 Excitation wavelength (λ_{ex}) = 550 nm.

233

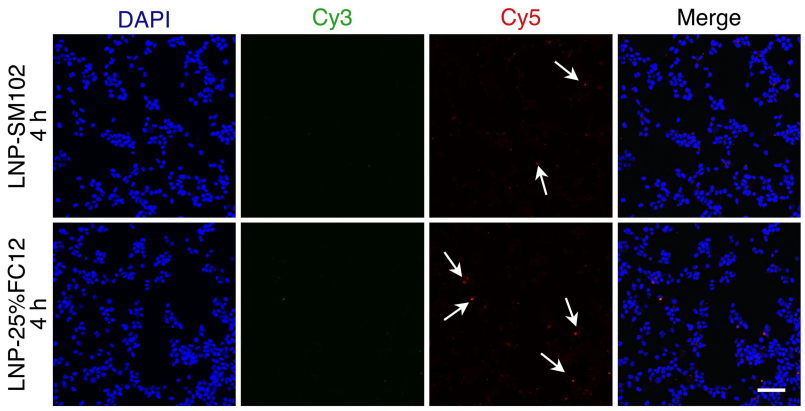


Fig. S9. Representative confocal images showing cellular uptake of Cy5-labeled LNPs after 4 hours. Nuclei stained with DAPI (Blue). Scale bar = 100 μm.

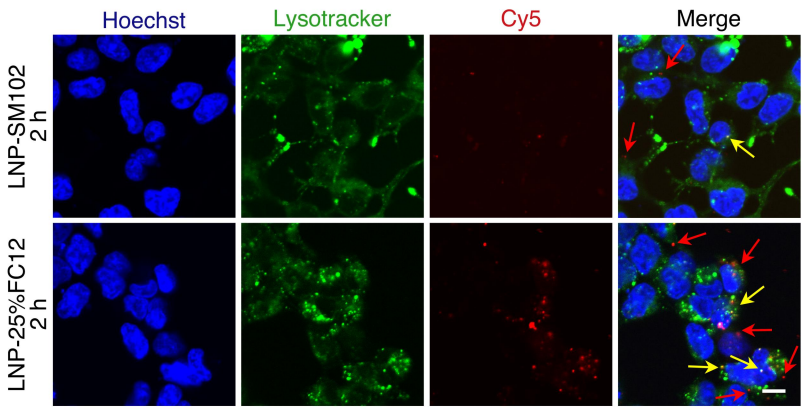


Fig. S10. Representative confocal images of LNP endosomal escape in 293T cells treated with Cy5-labeled LNPs for 2 h, stained with LysoTracker Green and Hoechst 33342. Scale bar = 10 μm.

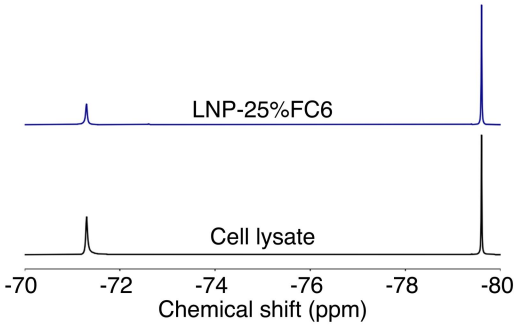


Fig. S11. ^{19}F MRS of LNP-25%FC6 and 293T cells treated with LNP-25%FC6 for 48 h. 2 mM of CF_3NaO_3S (-79.6 ppm) were used as the ^{19}F MRS reference.

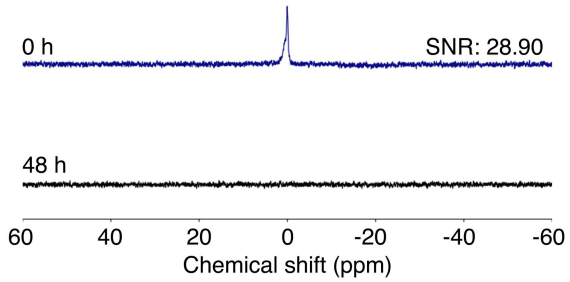


Fig. S12. The ^{19}F MRS of LNP-25%FC6 (-71.3 ppm) and mice intramuscularly injected with LNP-25%FC6 for 48 h.

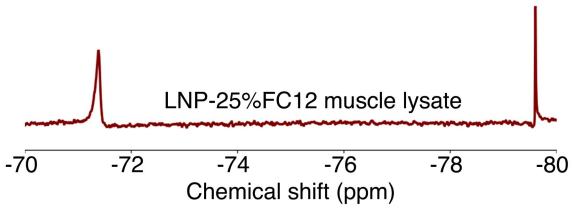


Fig. S13. The ^{19}F MRS of gastrocnemius muscle lysate after treatment with LNP-25%FC12 for 48h. 1 mM of CF_3NaO_3S (-79.6 ppm) was used as the ^{19}F MRS reference.

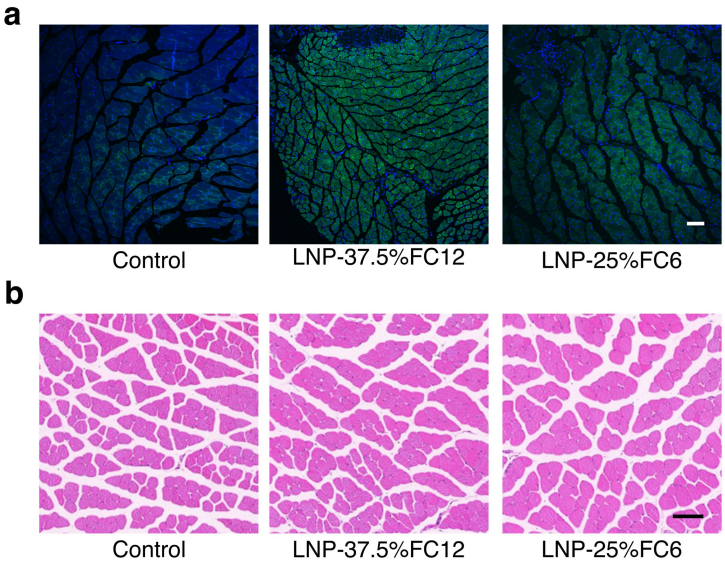
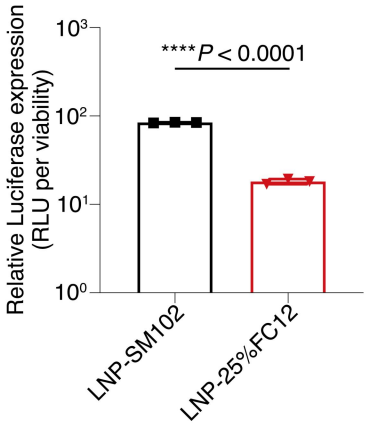


Fig. S14. a. EGFP protein expression detected by immunohistochemistry staining of gastrocnemius muscle after treatment with pEGFP-loaded LNPs or PBS for 48 h. Scale bar = 100 μm. b. Representative H&E staining of gastrocnemius muscle tissues harvested from the mice receiving different LNP treatments. Scale bars: 100 μm.



261

262 **Fig. S15.** LNP-mediated mLuc mRNA delivery. 293T cells were treated with mLuc-loaded LNP
263 (10 ng) or free mRNA (10 ng per well) for 24 h. (n = 3)

264

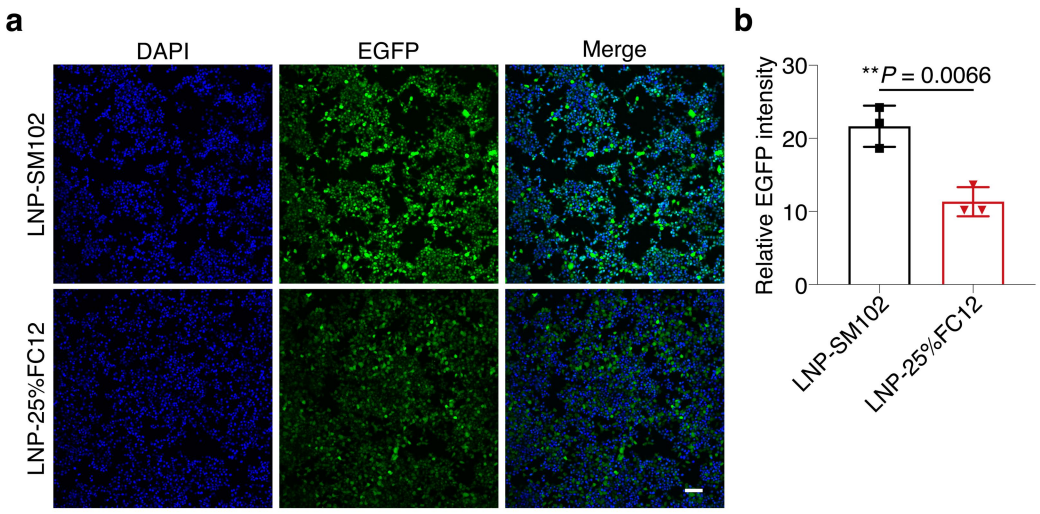


Fig. S16. Representative fluorescent images of EGFP protein expression in 293T cells following treatment with mEGFP-loaded LNPs for 24 h. Scale bar = 100 μm. B. LNP-mediated mEGFP delivery (n = 3). 293T cells were treated with mEGFP-loaded LNP (400 ng) or free mEGFP (400 ng per well) for 24 h.

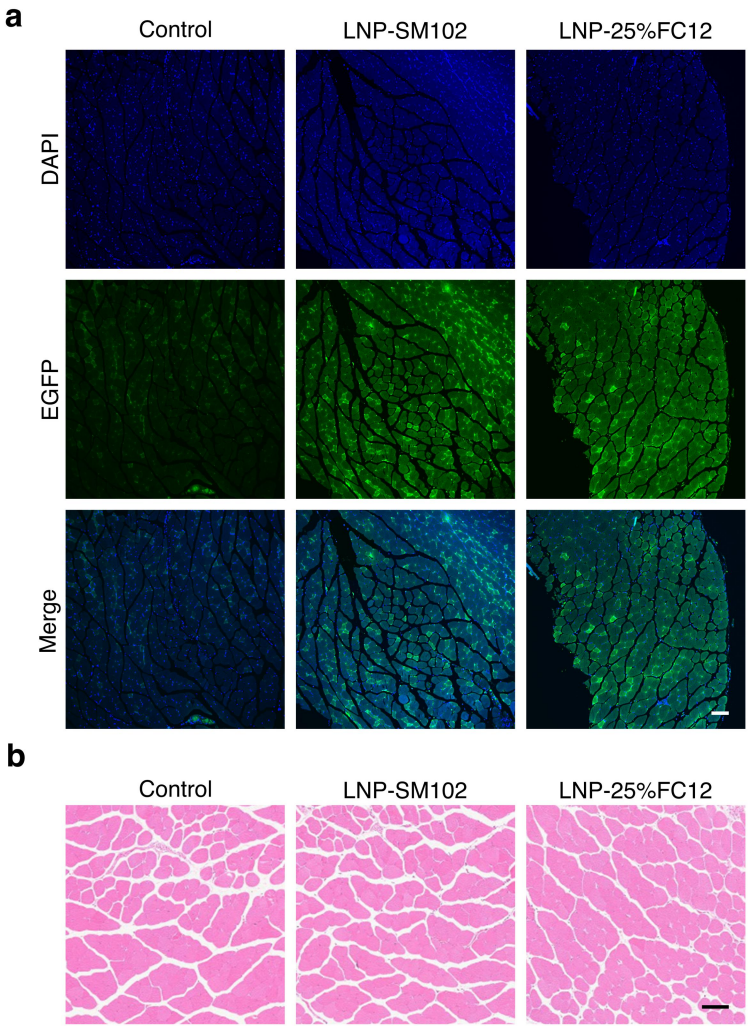


Fig. S17. a. EGFP protein expression detected by immunohistochemistry staining of gastrocnemius muscle after treatment with mEGFP-loaded LNPs or PBS for 24 h. Scale bar = 100 μm. b. Representative H&E staining of gastrocnemius muscle tissues harvested from the mice receiving different mEGFP-loaded LNPs treatments. Scale bars = 100 μm.

Table S1. The formulation and characterization of pDNA-loaded LNPs. The hydrodynamic diameter and PDI of LNPs were obtained by DLS measurement in PBS (pH = 7.4). ζ-potential was obtained by DLS measurement in H_2O. Data are presented as mean ± SEM (n = 3).

LNP	Recipe	Diameter (nm)	PDI	Zeta potential (mV)	Encapsulation efficiency (%)
LNP-SM102	SM102:DSPC:Chol:DMG-PEG2000 = 50:10:38.5:1.5	107 ± 7	0.141 ± 0.014	4 ± 3	80.0 ± 3.4
LNP-25%FC12	FC12:SM102:DSPC:Chol:DMG-PEG2000 = 25:25:10:38.5:1.5	117 ± 5	0.194 ± 0.003	7 ± 4	82.5 ± 2.4
LNP-25%FC6	FC6:SM102:DSPC:Chol:DMG-PEG2000 = 25:25:10:38.5:1.5	101 ± 4	0.144 ± 0.003	10 ± 2	79.8 ± 3.9

Table S2. The T_1 and T_2 of fluorinated lipids and FLNPs.

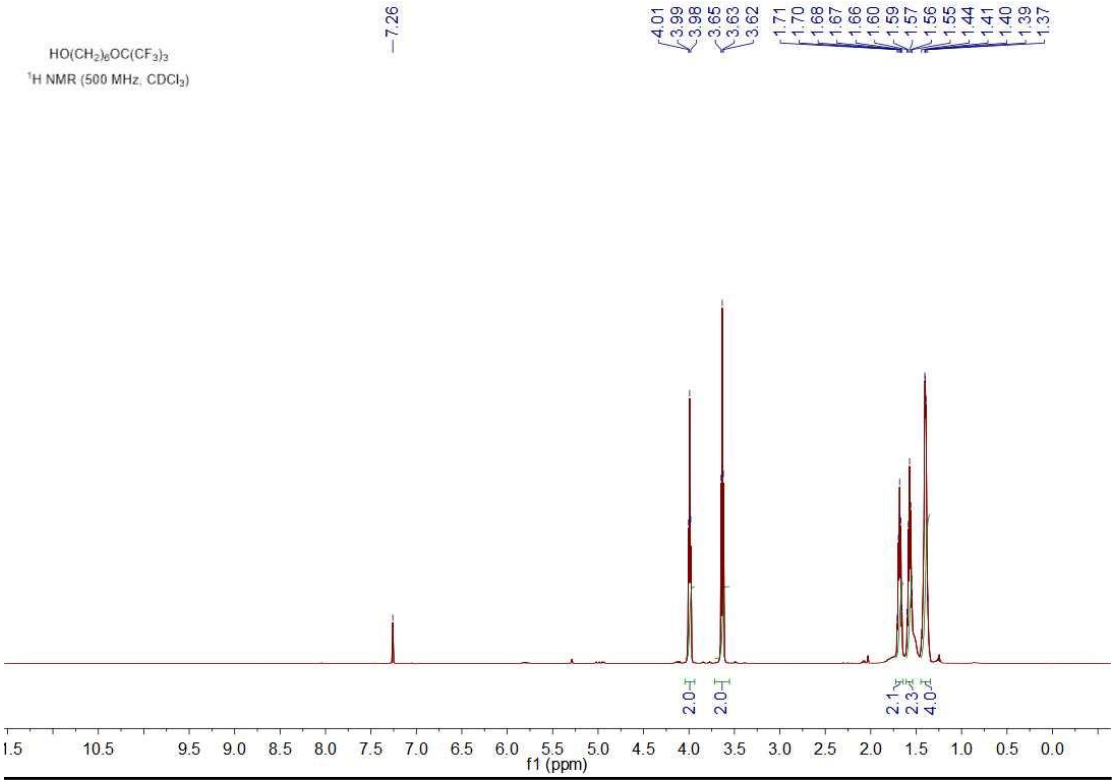
Category	Title	T_1 (ms)	T_2 (ms)
Fluorinated lipids	FC12	603.1	444.1
	FC6	561.9	164.4
FLNPs	LNP-25%FC12	156.5	5.5
	LNP-25%FC6	442.2	10.3

Table S3. The formulation and characterization of mRNA-loaded LNPs. The hydrodynamic diameter and PDI of LNPs were obtained by DLS measurement in PBS (pH = 7.4). ζ-potential was obtained by DLS measurement in H_2O. Data are presented as mean ± SEM (n = 3).

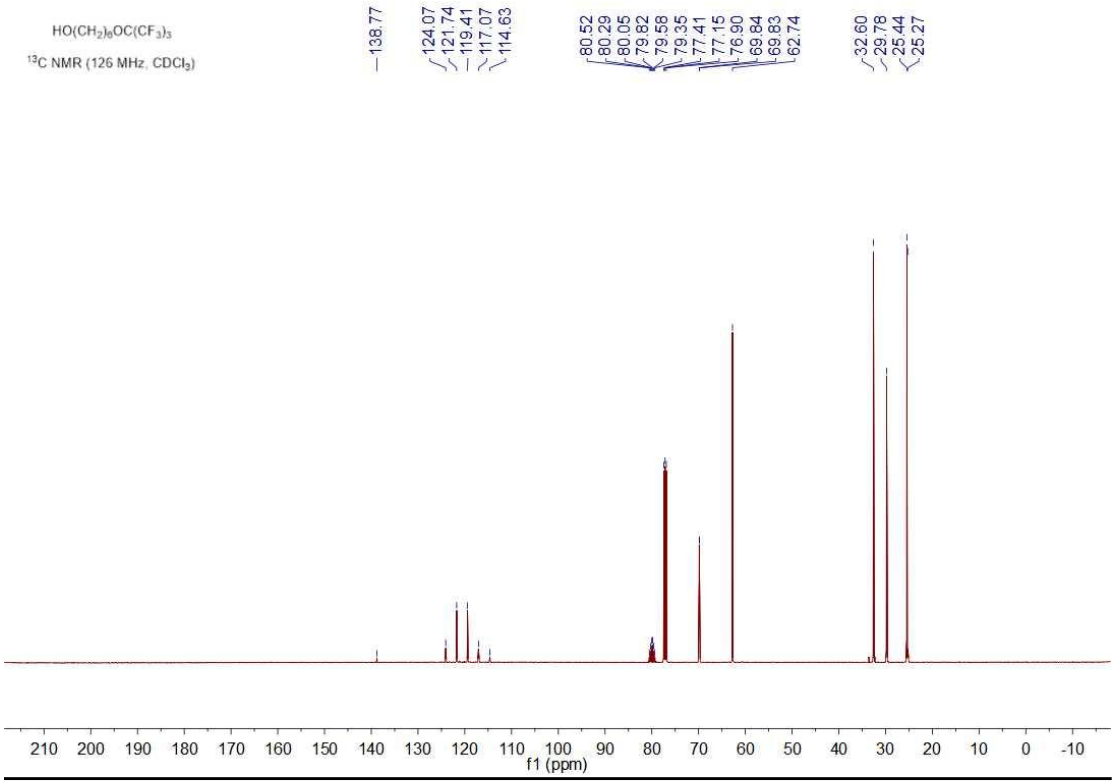
LNP	Recipe	Diameter (nm)	PDI	Zeta potential (mV)	Encapsulation efficiency (%)
LNP-SM102	SM102:DSPC:Chol:DMG-PEG2000 = 50:10:38.5:1.5	75 ± 5	0.140 ± 0.014	2 ± 1	92.3 ± 3.2
LNP-25%FC12	FC12:SM102:DSPC:Chol:DMG-PEG2000 = 25:25:10:38.5:1.5	79 ± 10	0.154 ± 0.022	15 ± 1	98.6 ± 1.5
LNP-25%FC6	FC6:SM102:DSPC:Chol:DMG-PEG2000 = 25:25:10:38.5:1.5	87 ± 6	0.177 ± 0.014	9 ± 2	96.1 ± 0.4

$^{1}H/^{13}C/^{19}F$ NMR and MS Spectra of Compounds

^{1}H NMR of compound **2**



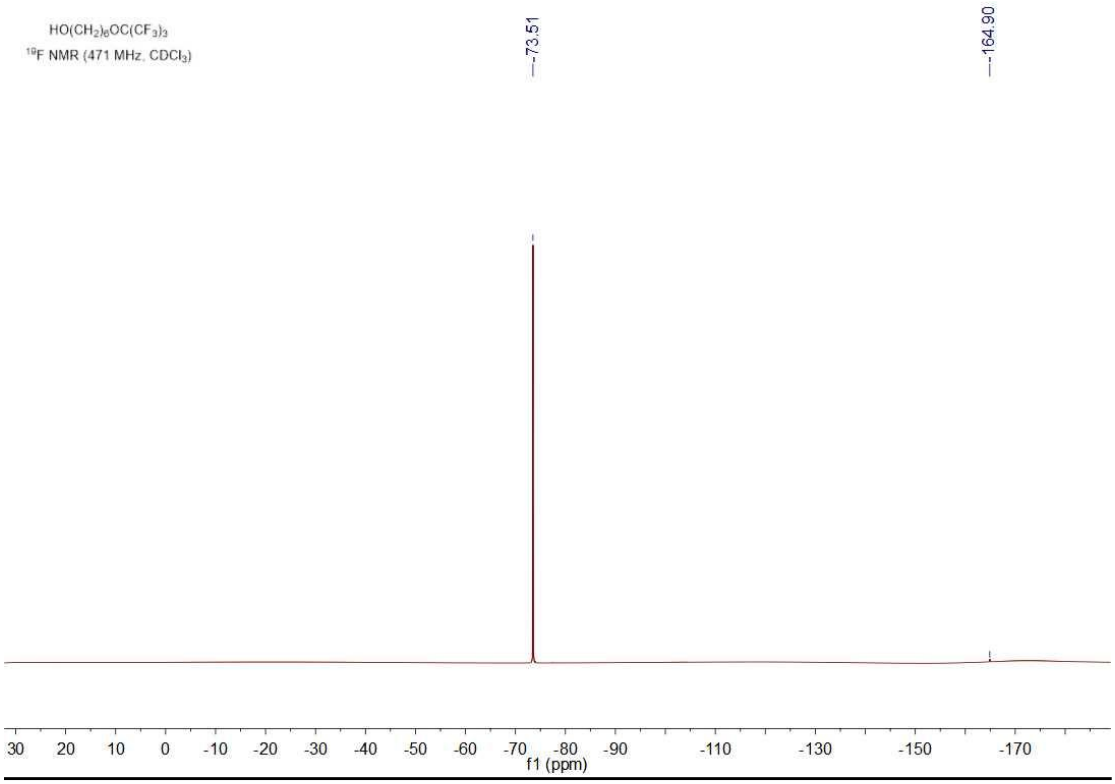
294 ^{13}C NMR of compound **2**



295

296

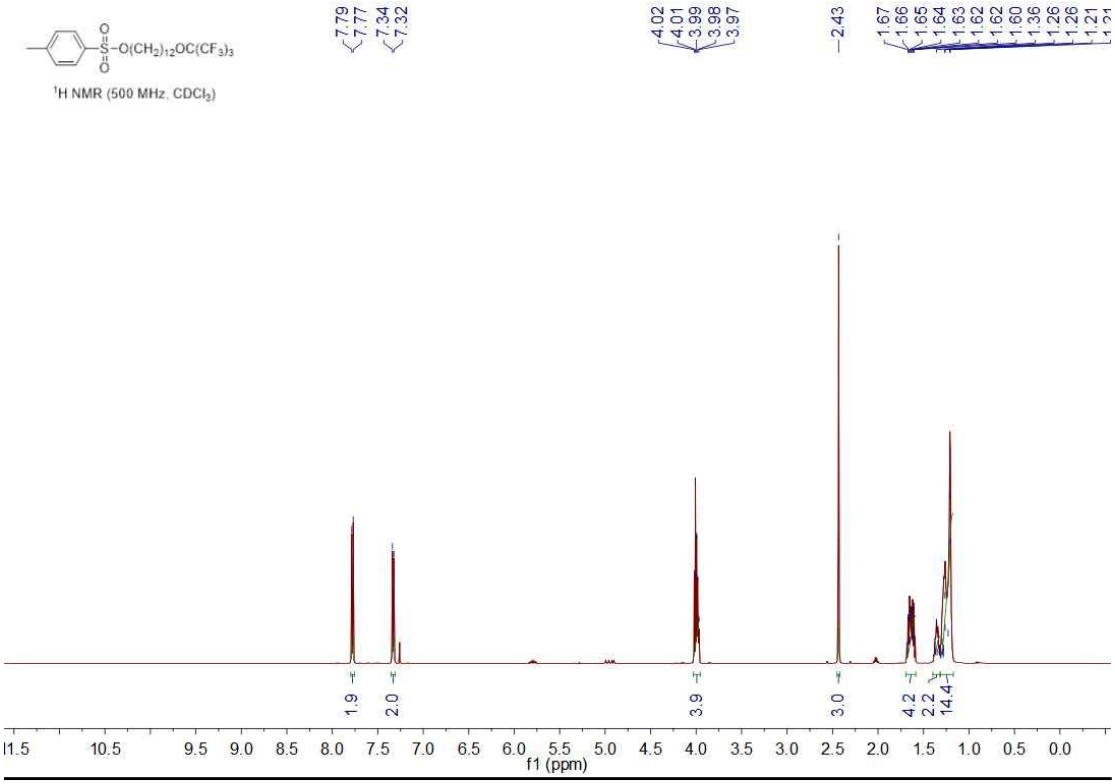
297 ^{19}F NMR of compound **2**



298

299

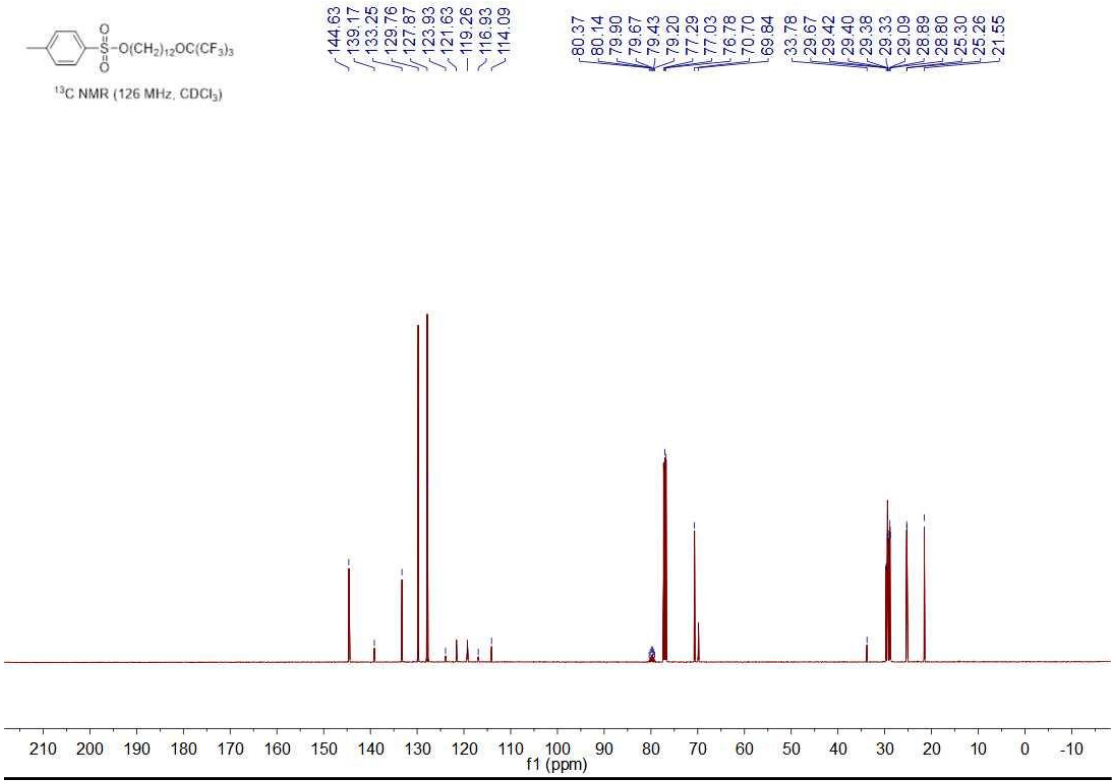
300 ^{1}H NMR of compound **3**



301

302

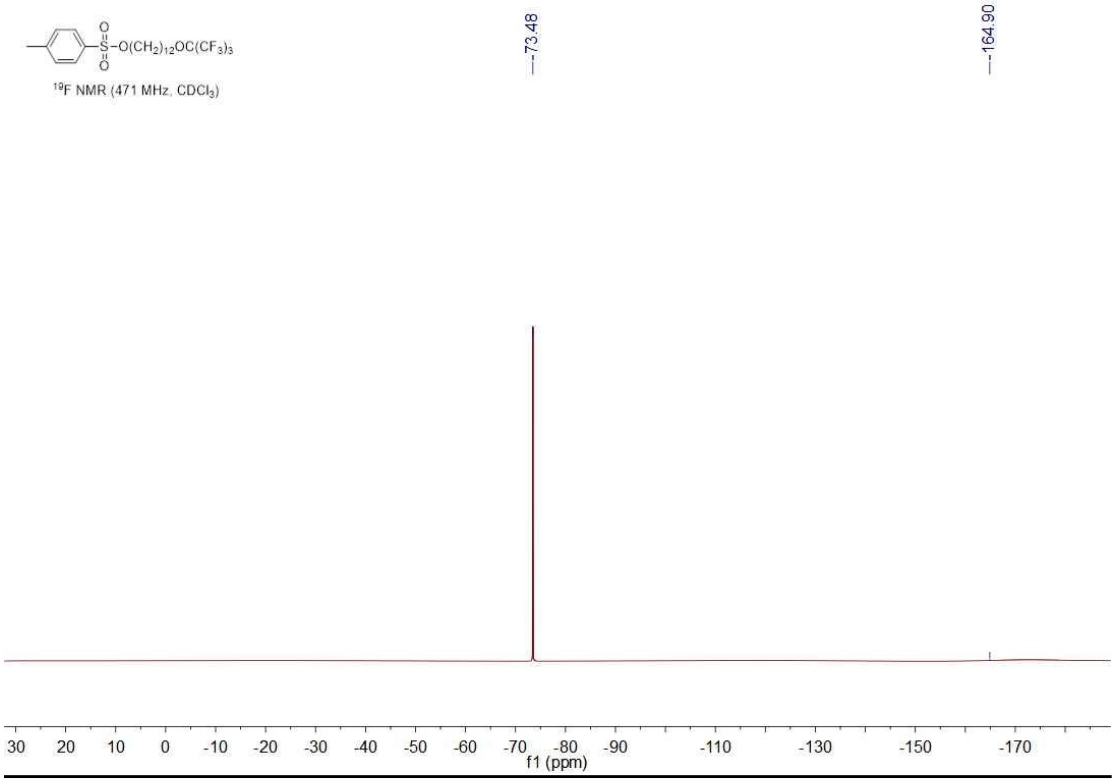
303 ^{13}C NMR of compound **3**



304

305

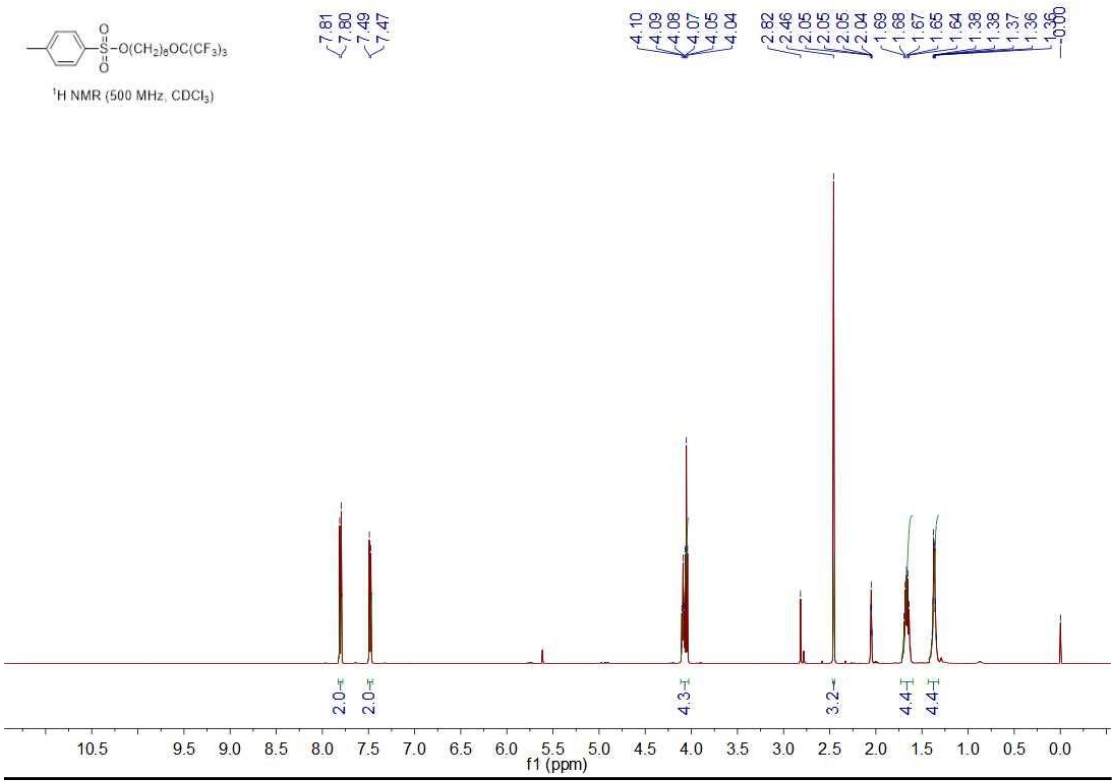
306 ^{19}F NMR of compound **3**



307

308

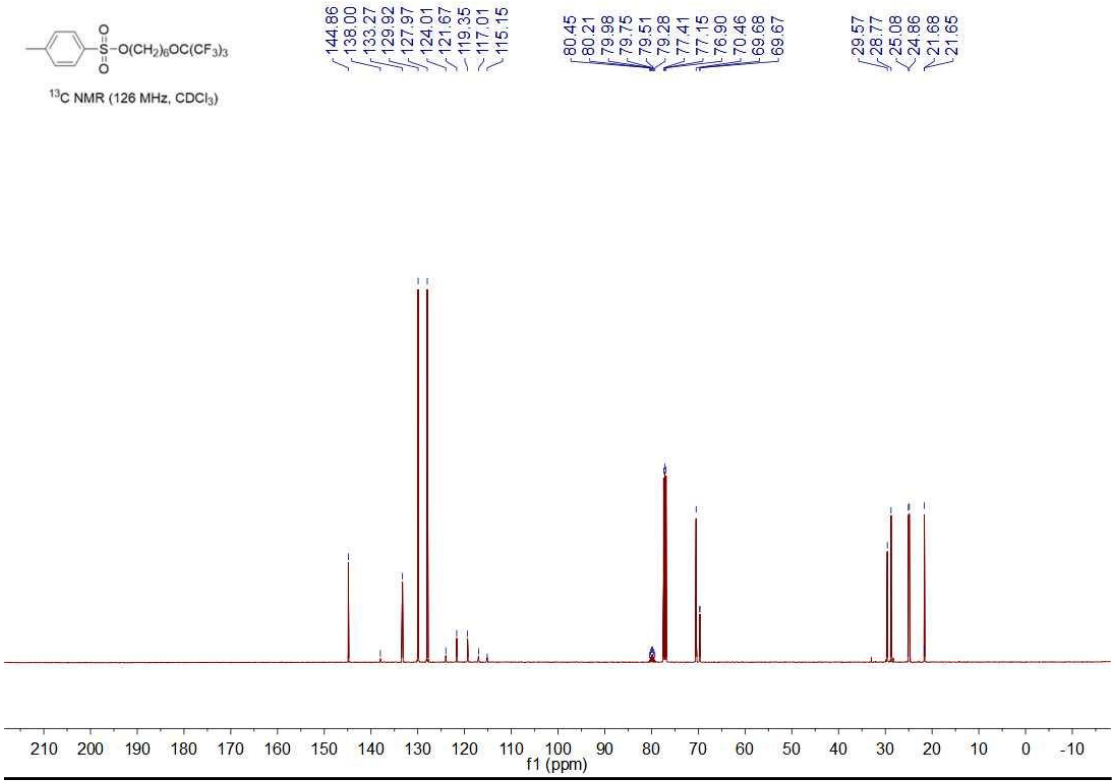
309 ^{1}H NMR of compound **4**



310

311

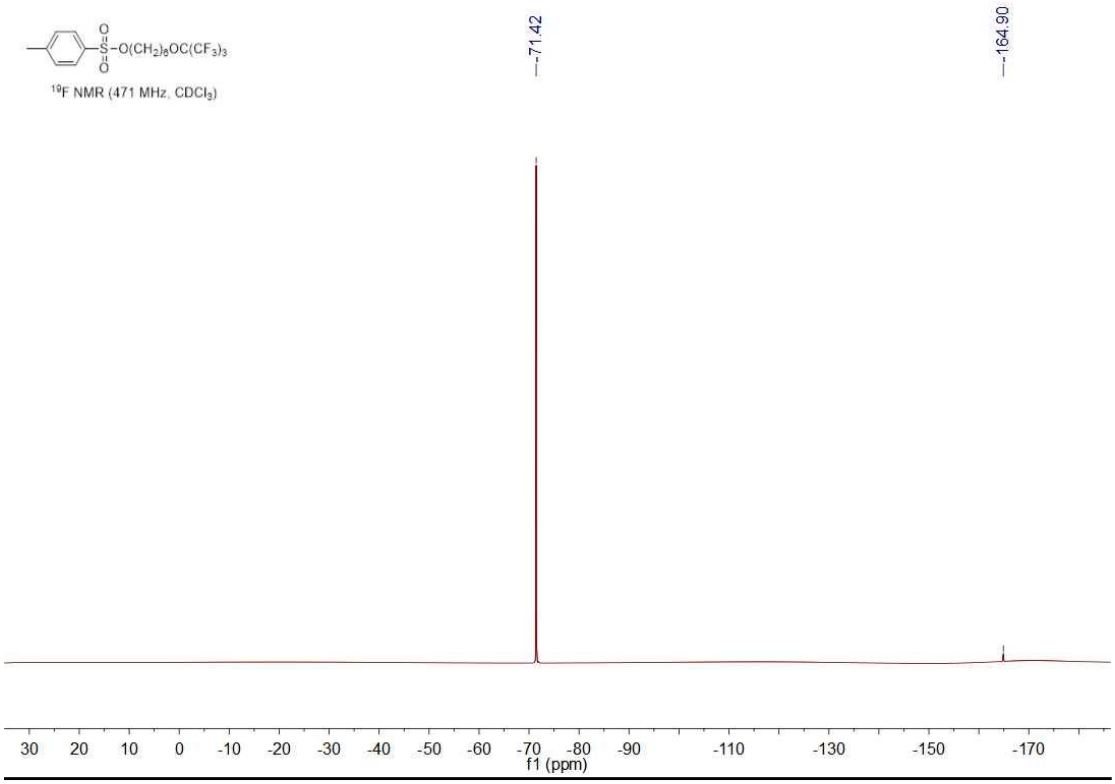
312 ^{13}C NMR of compound **4**



313

314

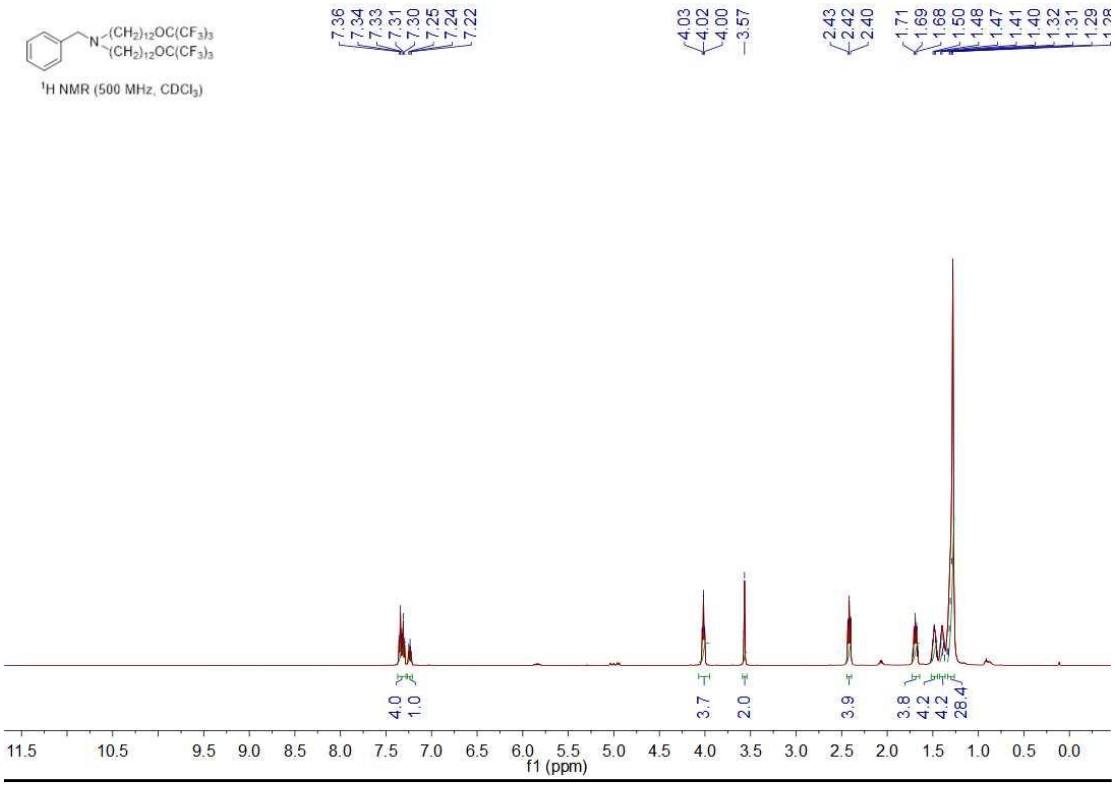
315 ^{19}F NMR of compound **4**



316

317

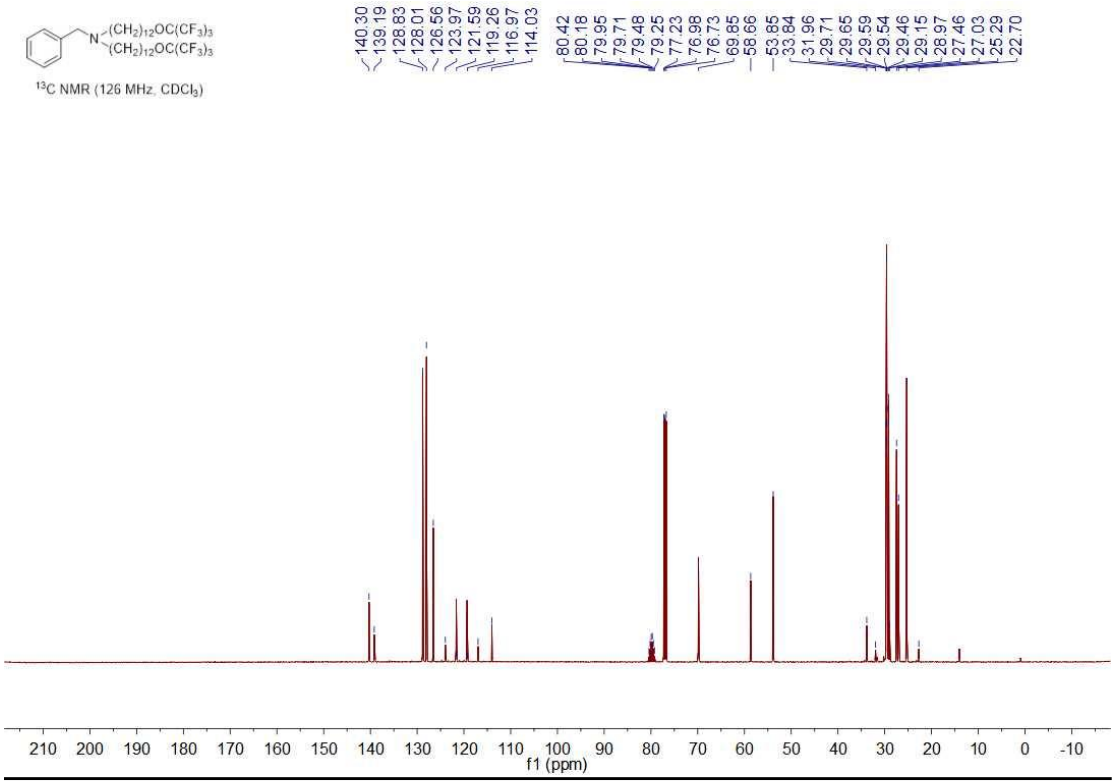
318 ^{1}H NMR of compound **5**



319

320

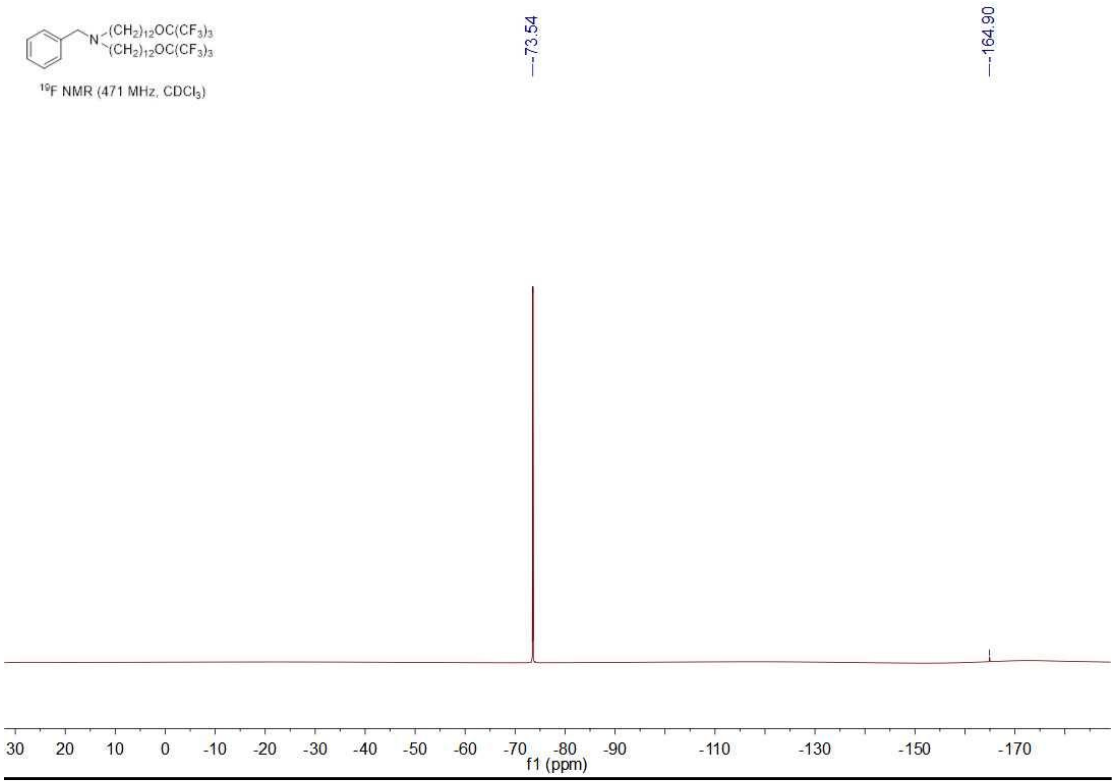
321 ^{12}C NMR of compound **5**



322

323

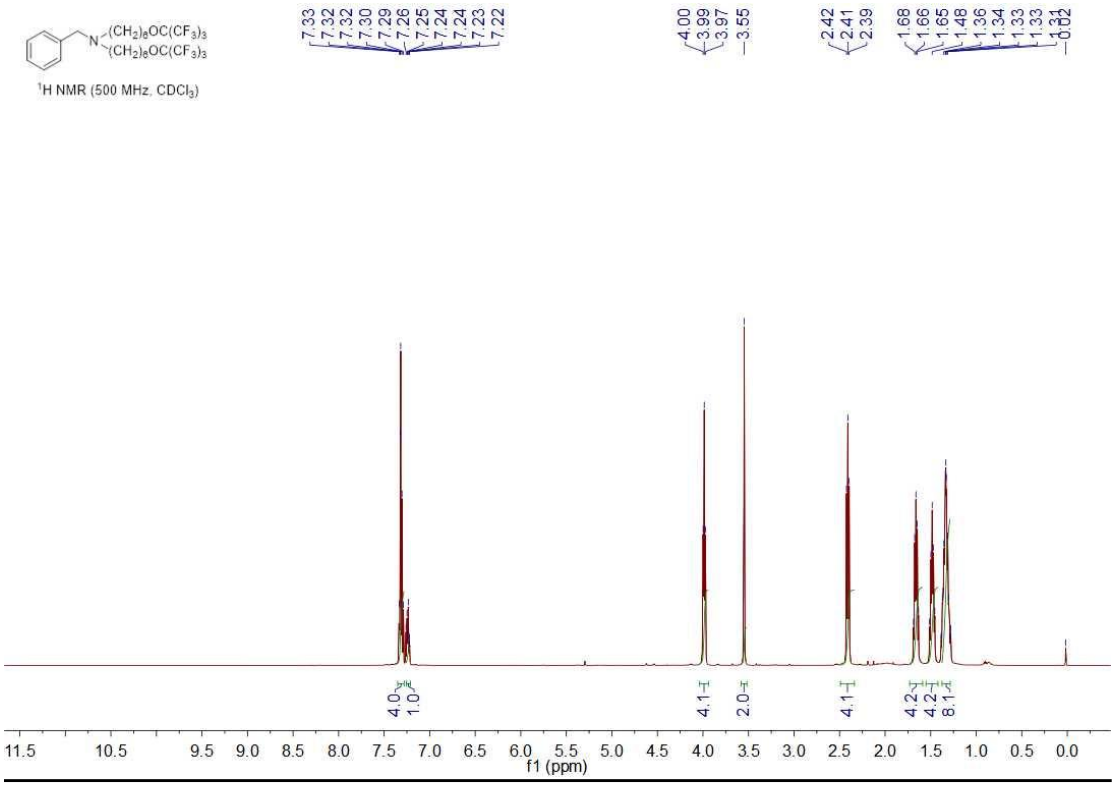
324 ^{19}F NMR of compound **5**



325

326

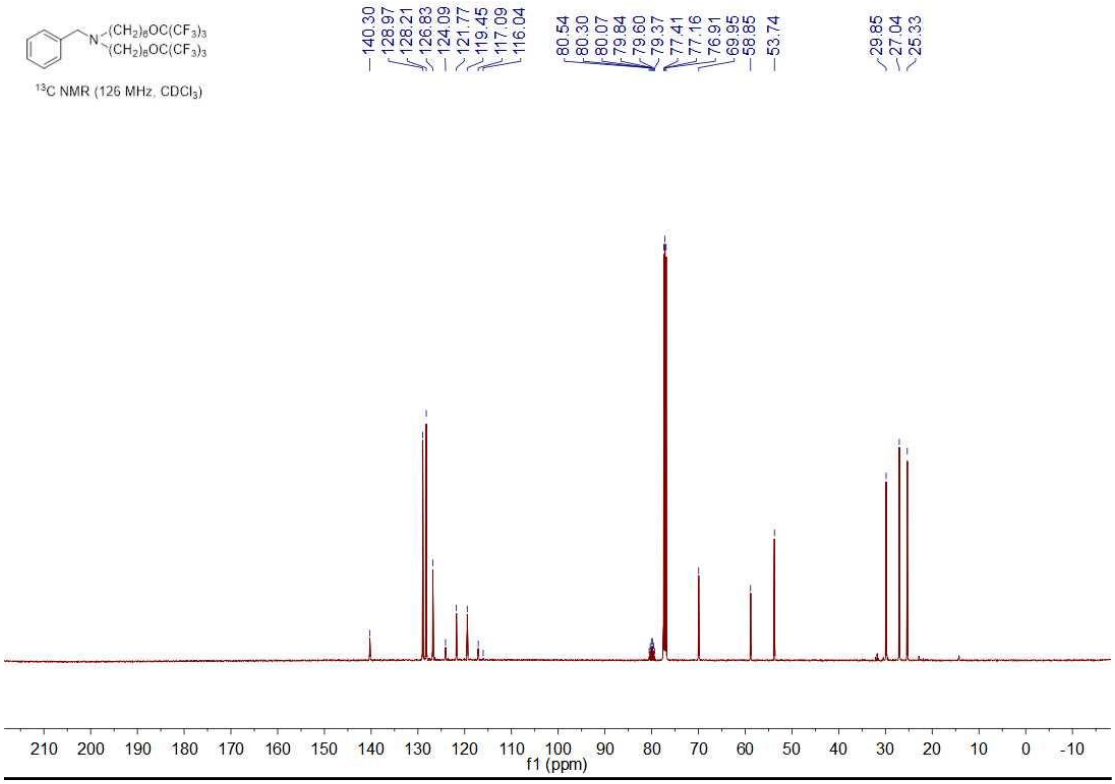
327 ^{1}H NMR of compound **6**



328

329

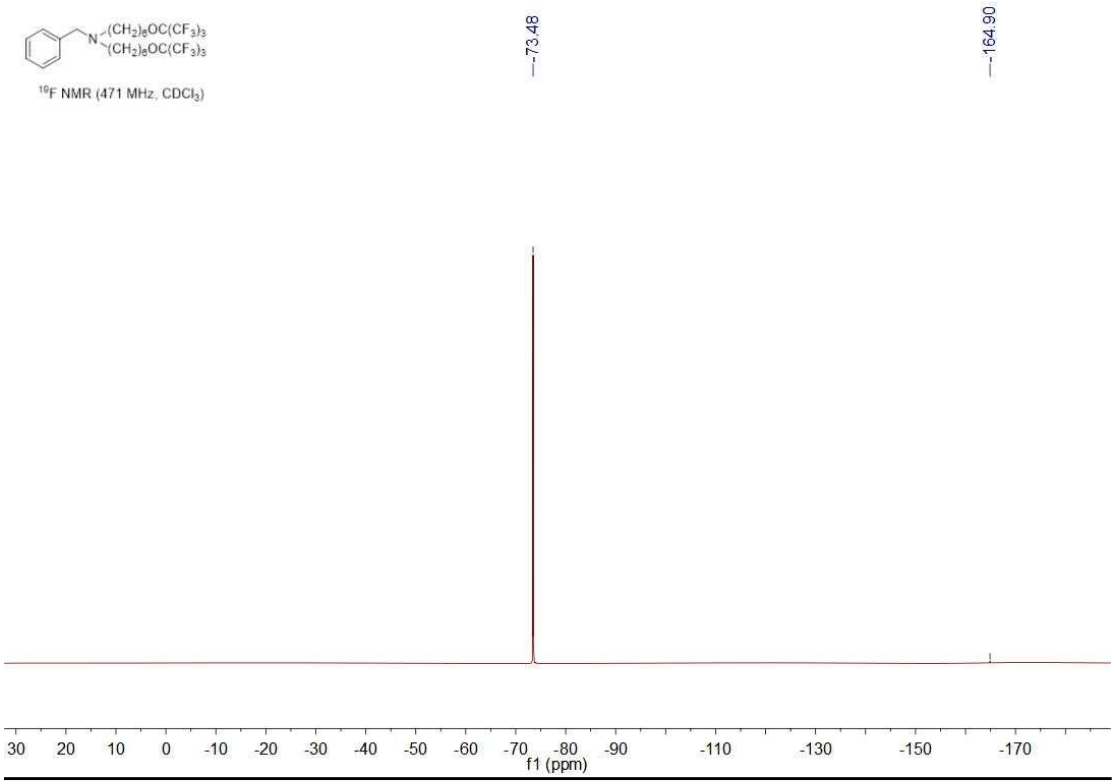
330 ^{13}C NMR of compound **6**



331

332

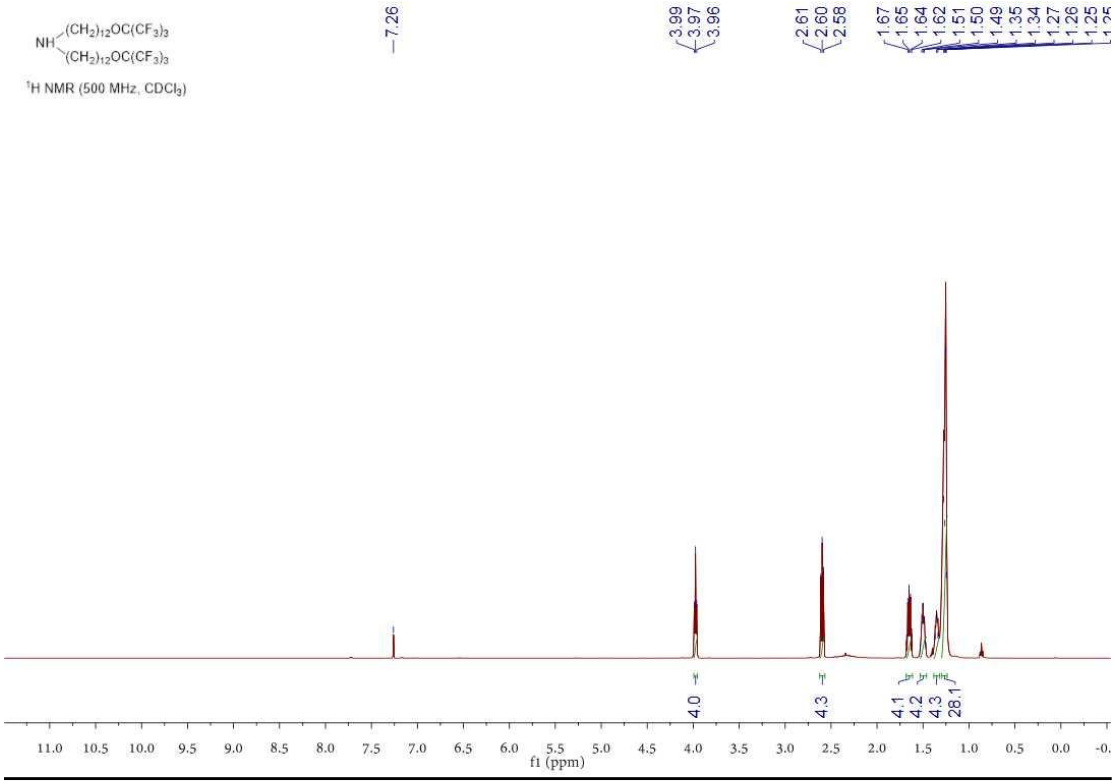
333 ^{19}F NMR of compound **6**



334

335

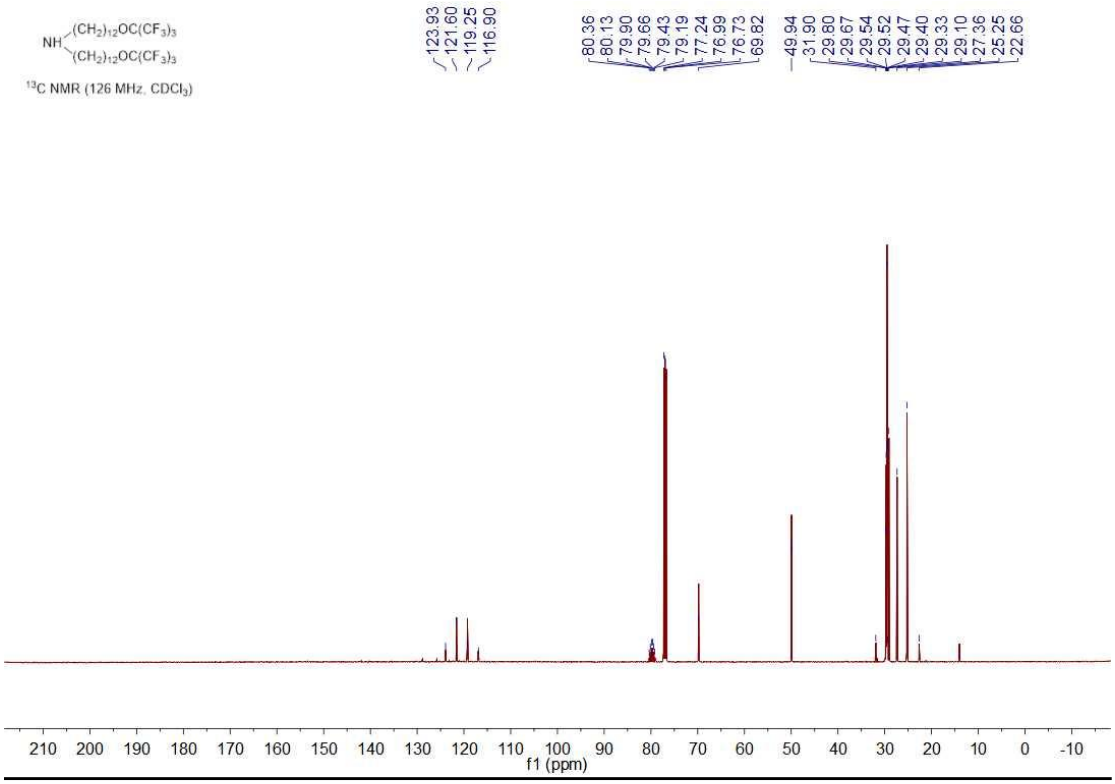
336 ^{1}H NMR of compound Lipid **FC12**



337

338

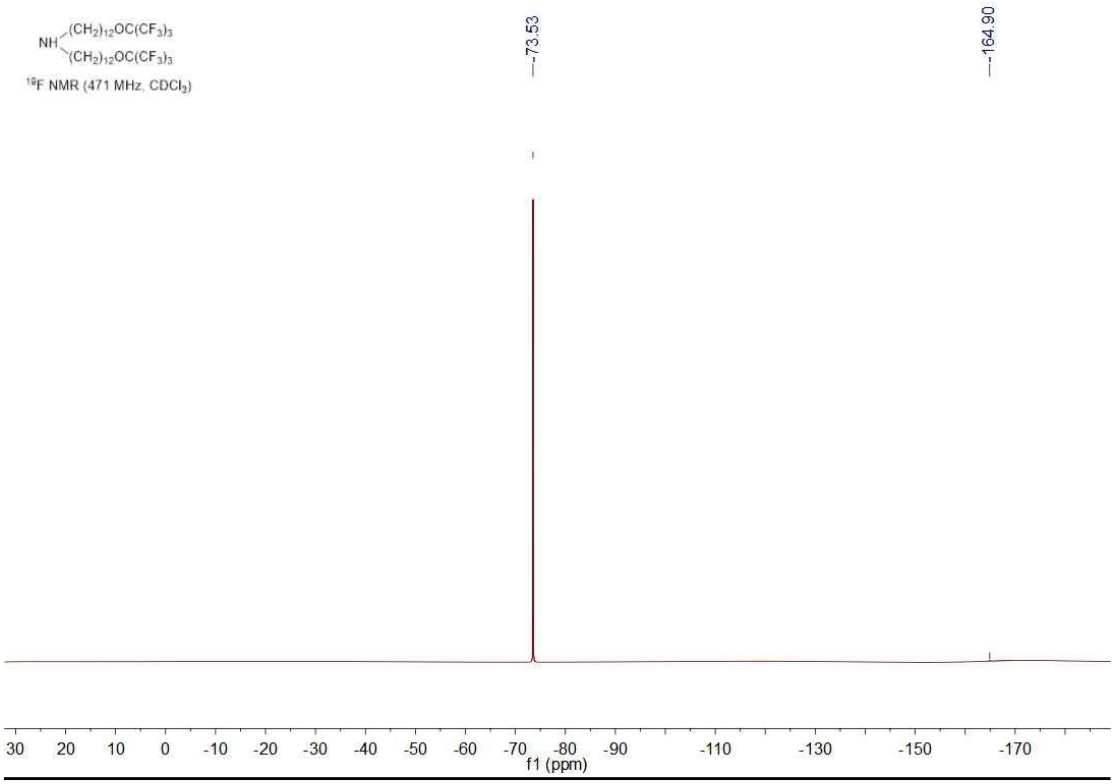
339 ^{13}C NMR of compound Lipid **FC12**



340

341

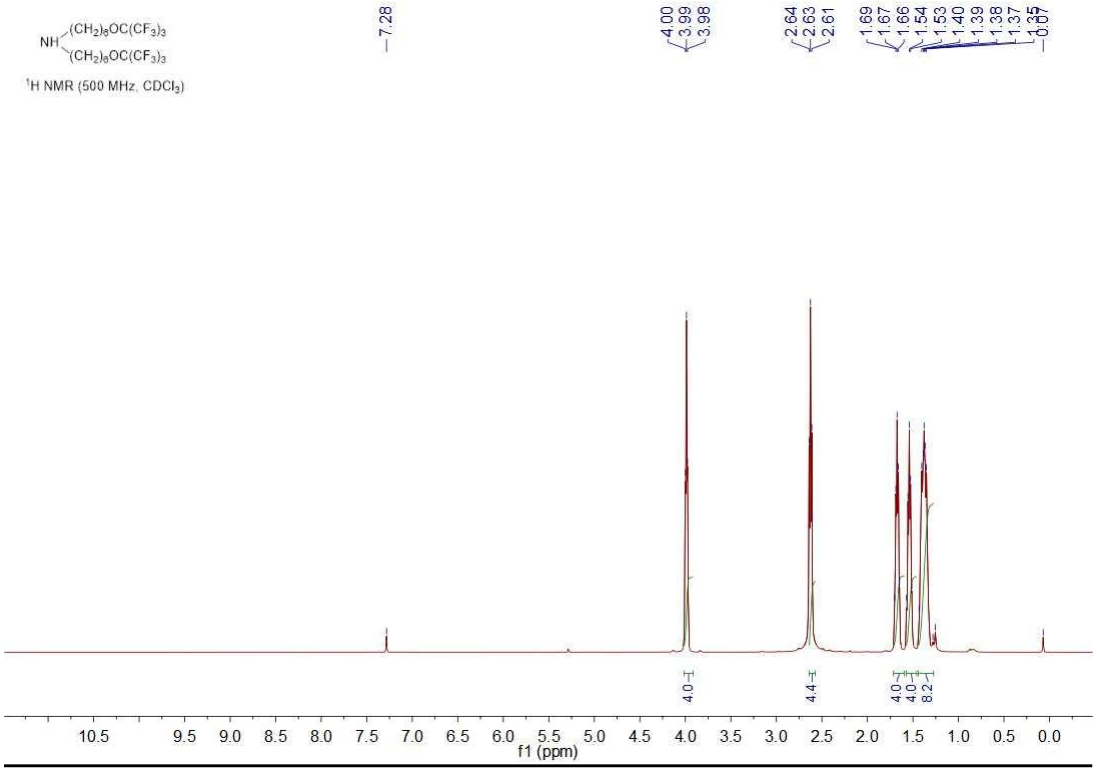
342 ^{19}F NMR of compound Lipid **FC12**



343

344

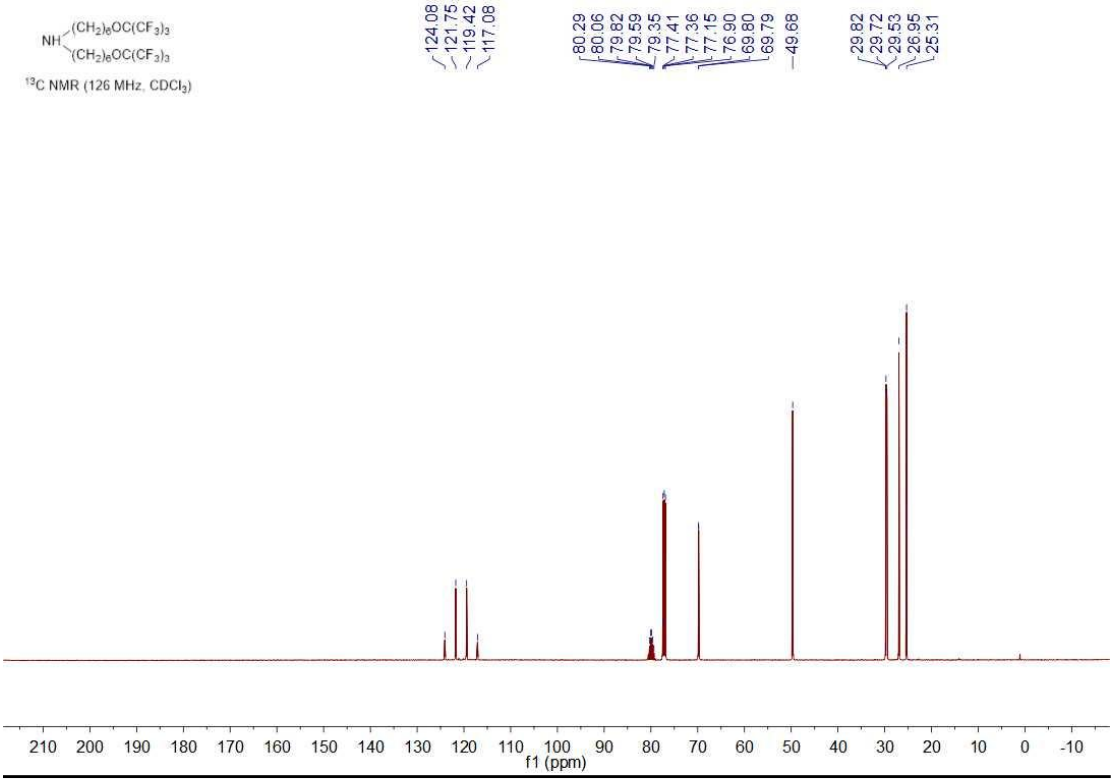
345 ^{1}H NMR of compound Lipid **FC6**



346

347

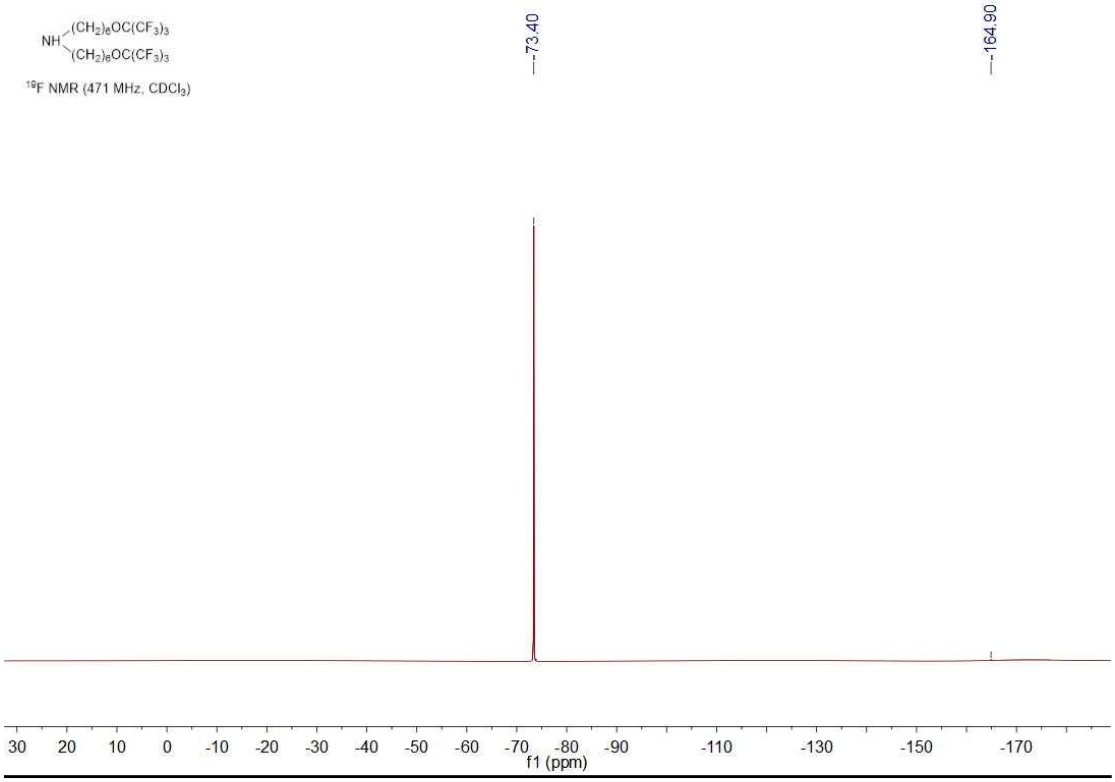
348 ^{13}C NMR of compound Lipid **FC6**



349

350

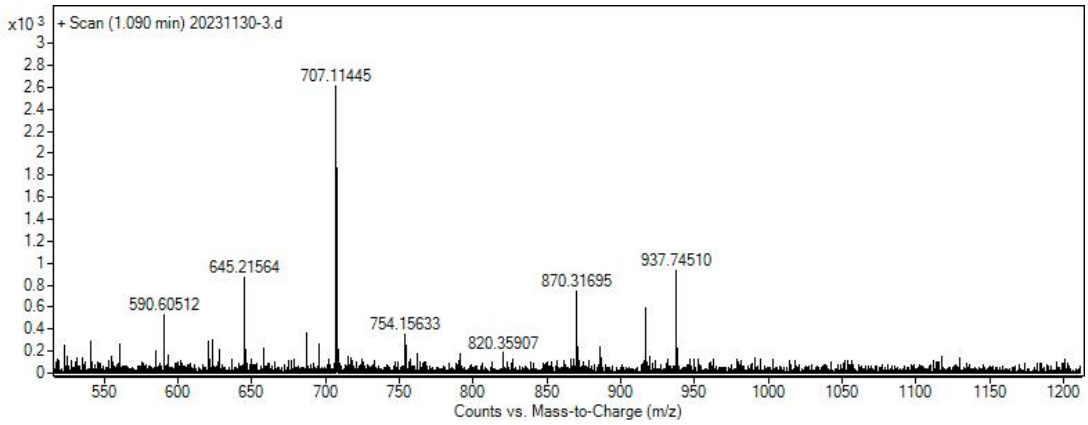
351 ^{19}F NMR of compound Lipid **FC6**



352

353

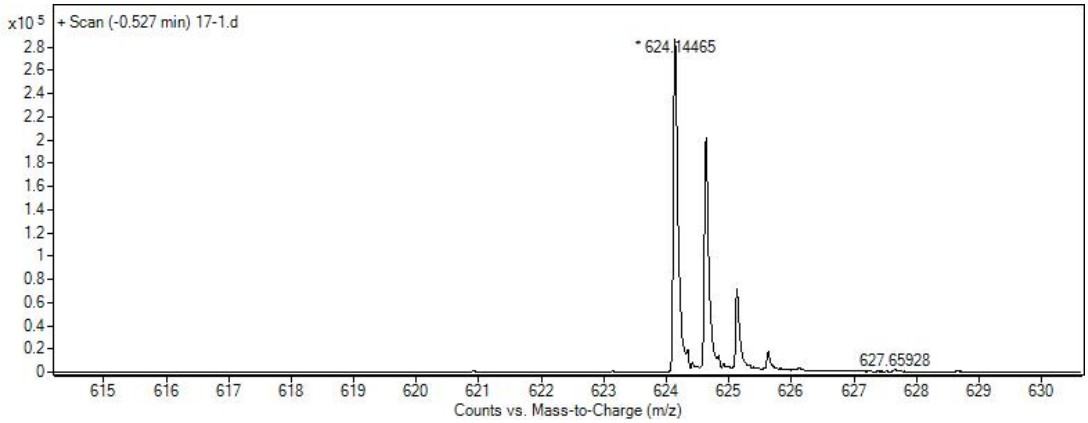
354 HRMS spectra of compound Cy3-FC12



355

356

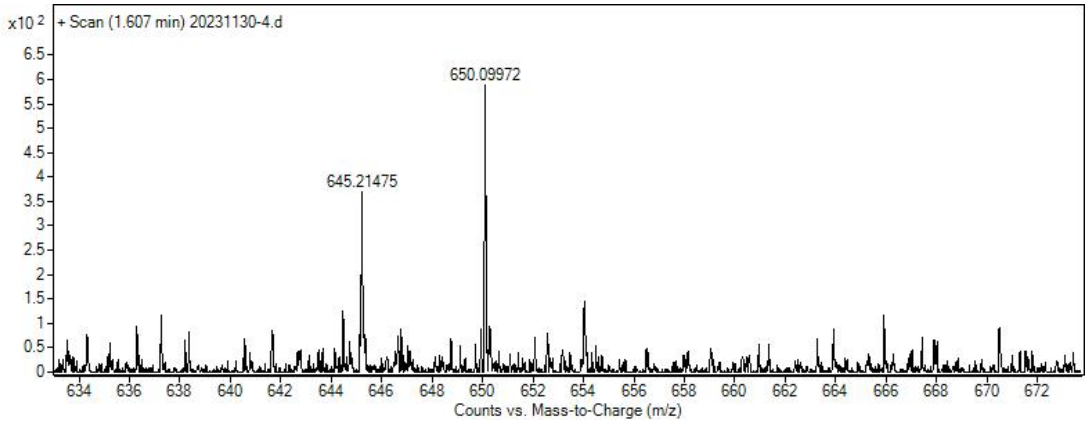
357 HRMS spectra of compound Cy3-FC6



358

359

360 HRMS spectra of compound Cy3-SM102



361

362

References

1. D. W. Schrödinger L, PyMOL. *http://www.pymol.org/pymol* (2020).
2. L. Martínez, R. Andrade, E. G. Birgin, J. M. Martínez, PACKMOL: A package for building initial configurations for molecular dynamics simulations. *J. Comput. Chem.* **30**, 2157-2164 (2009).
3. B. Hess, C. Kutzner, D. van der Spoel, E. Lindahl, GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. *J. Chem. Theory Comput.* **4**, 435-447 (2008).