Supporting Information

Fluorinated Porphyrin-Based Theranostics for Dual Imaging and Chemo-Photodynamic Therapy

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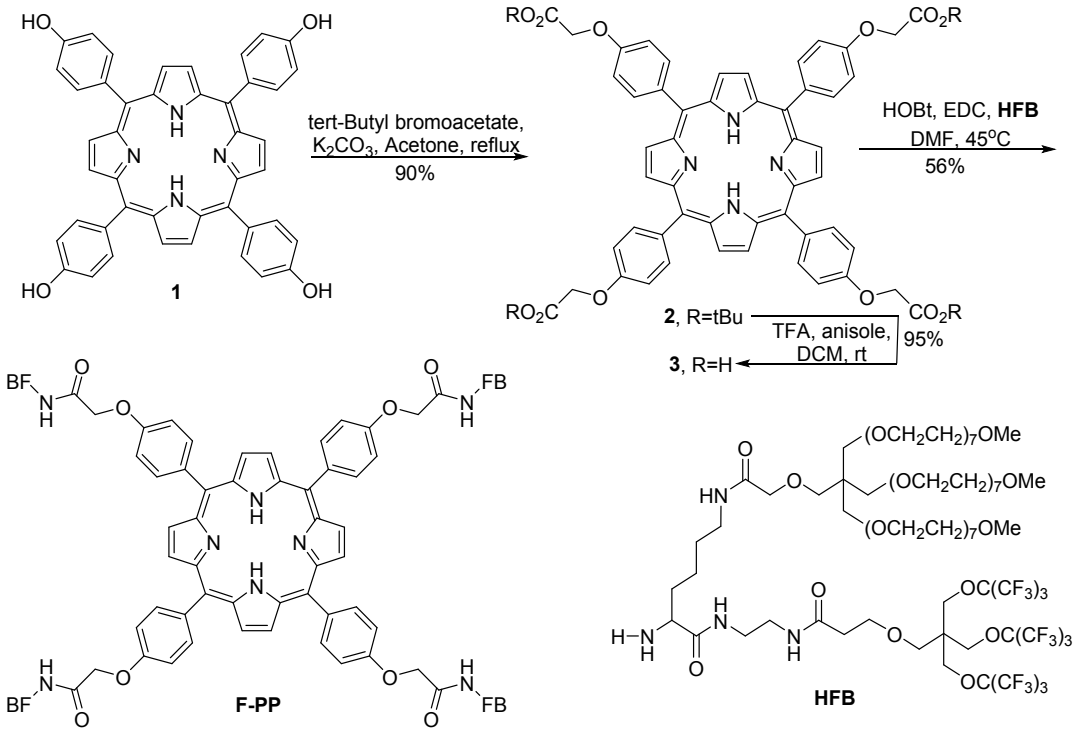
1. General information

^{1}H, ^{19}F and ^{13}C NMR spectra were recorded on a 400 MHz Bruker NMR spectrometer. 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 $CDCl_3$ and 73 mM trifluoroacetic acid (s, -79.61) 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). Mass spectra were recorded on an ESI mass spectrometer for compounds below 3,000 Da and a MALDI-TOF spectrometer with α-cyano-4-hydroxylcinnamic acid as matrix using the reflection mode for positive ions for compounds above 3,000 Da.

Unless otherwise indicated, all reagents were obtained from commercial supplier and used without prior purification. All solvents were analytical or HPLC grade. Deionized water was used unless otherwise indicated. DMF, Et_3N, MeOH and THF were dried and freshly distilled prior to use. Flash chromatography was performed on silica gel (200-300 mesh) with petroleum ether (PE)/EtOAc (EA) or CH_2Cl_2/MeOH as eluents.

2. Synthesis scheme and HPLC chromatogram of F-PP



Scheme S1. Synthesis of fluorinated amphiphile **F-PP.**

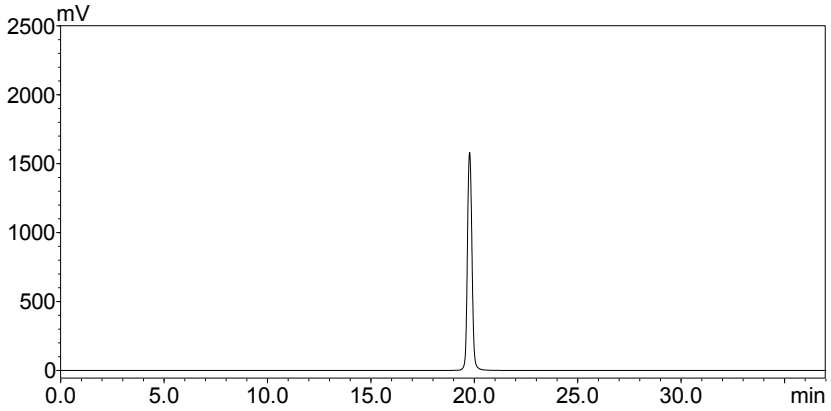


Figure S1. HPLC chromatogram of **F-PP** (Conditions: 0-15 min: 30%-100% MeOH 15-40 min: 100% MeOH).

3. Preparation of L-PP

The liposome **L-PP** was prepared with the film dispersion method according to our previous method.[1] To a flask was added a mixture of HSPC/CHOL/**F-PP**/DOX (15 mg/5 mg/15 mg/4 mg), 3.0 mL organic solvent (chloroform/methanol = 2/1) and triethylamine (3 equivalent to DOX). The mixture was stirred to form a homogeneous solution. Then, the

organic solvent was removed by rotary evaporation under vacuum and the residue formed a dry film on the wall of the flask. Water (2.0 mL) was added to the flask. The flask was rotated on a rotary evaporation at normal pressure for 2 min and sonicated at 60 ^{o}C for 2 h. Liposome was collected after filtration through a 0.45 μm and a 0.22 μm polycarbonate membrane. The amount of DOX encapsulated in the liposomes was measured by HPLC. The drug-loading content and drug encapsulation efficiency were calculated as below:

$$\text{Drug loading content (\%)} = Wt/Ws \times 100\%$$

$$\text{Drug encapsulation efficiency (\%)} = Wt/Wo \times 100\%$$

Wt: the amount of DOX loaded into nanoparticles; Ws: the amount of nanoparticles after lyophilization; Wo: the initial amount of DOX added.

4. Characterization of L-PP

The size distribution and zeta potential of liposome were determined by DLS (Nano ZS 90, Malvern, UK) and TEM (Tecnai G20, FEI, USA). The stability of L-PP was monitored by DLS at 4 °C.

Table S1. Composition and characterization of liposome **L-PP**.

	liposome compositions	**Liposome characterization**				
	HSPC/CHOL/**F-PP**/DOX (w/w/w/w)	Size (nm)	PDI	Zeta (mV)	Drug loading content	Drug encapsulating efficiency
L-PP	15:5:15:4	121.0	0.146	14.2	6%	86%

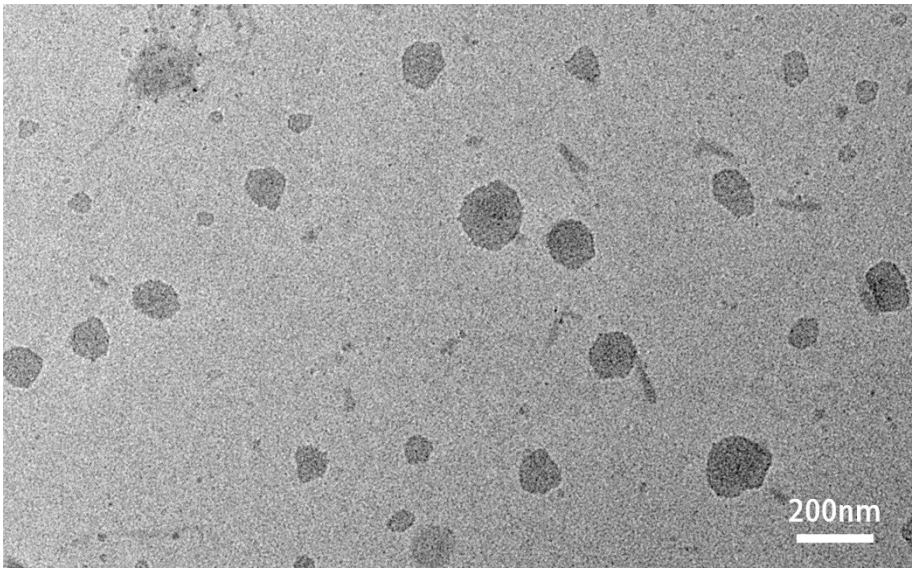


Figure S2. TEM image of **L-PP**.

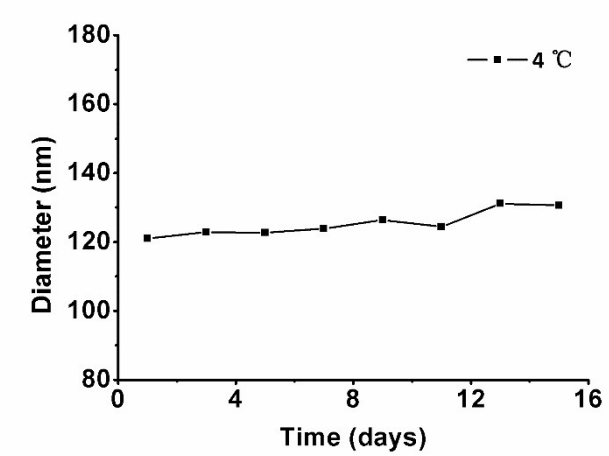


Figure S3. Stability of **L-PP** at 4 °C.

5. *In vitro* DOX release graph of L-PP

The release of DOX from **L-PP** was performed using dialysis method. Briefly, the solution of **L-PP** was transferred to dialysis membrane tubes (molecular weight cutoff: 1000 Da), and the tubes were immersed into 300 mL of PBS solution with different pH value and stirred at 37 °C for 24 h. At the time points of 0.5 h, 1 h, 2 h, 4 h, 8 h, 12 h, and 24 h, 1.0 mL of the external buffer was collected and replaced by 1.0 mL of fresh buffer. The DOX concentration in the collected buffer was measured using fluorescence Spectrometer.

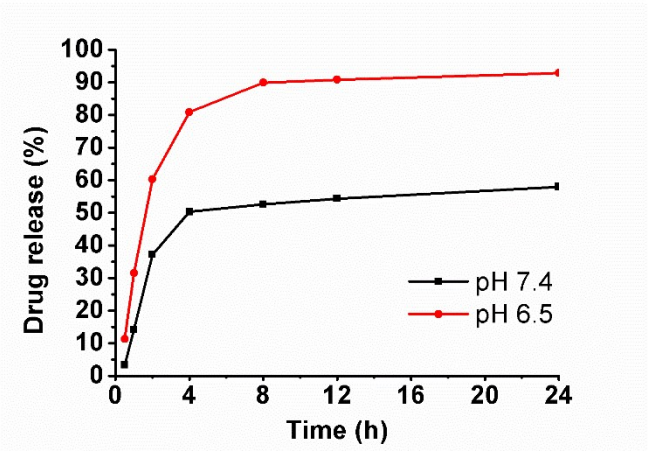


Figure S4. DOX release graph of **L-PP**.

6. Cell culture and cytotoxicity assay

HepG2 cells and MCF-7 cells were cultured in Gibico DMEM medium containing 10% FBS. L02 cells were cultured in Gibico 1640 medium containing 15% FBS. All cells were cultured at 37 °C in a humidified atmosphere containing 5% CO_2.

The cell viability of **F-PP** against cells (L02, MCF-7 and HepG2) were evaluated using a microculture tetrazolium (MTT) method. L02 cells, MCF-7 cells and HepG2 cells were seeded in 96-well plates at a density of 2×10^5 /mL, respectively, and incubated 24 h to adhere. Then the cells were incubated with free **F-PP** at different concentrations ranging from 0.15 mM to 0.75 mM for 24 h, followed by replacing the medium with 100 μL MTT (1.0 mg/mL) solution and incubated for another 4 h. Cells treated with normal medium were used as control. Then the medium was replaced with 200 μL DMSO solution and the absorbance values was measured at 490 nm wavelength using a microplate reader. All of the experiments were carried out in three times.

Cell viability (%) was calculated as the formula:

Cell viability (%) = $[(A_{Test}-A_{Blank}) / (A_{Control}-A_{Blank})] \times 100\%$

A_{Test}, $A_{Control}$ and A_{Blank} represented the absorbance of cells with different treatments, untreated cells and blank culture media, respectively.

7. *In vitro* ^{19}F MRI experiments

All MRI experiments were performed on a 376 MHz MRI system. The temperature of the magnet room was maintained 25 ^{o}C during the entire MRI experiment.

In vitro ^{19}F MRI of **F-PP** and **L-PP**: solution of 170 mM ^{19}F was serially diluted 1×, 2×, 4×, 8× times by PBS, forming **F-PP** solutions with ^{19}F concentrations of 170 mM, 85 mM, 42.5 mM, 21.3 mM, 10.7 mM, respectively. The ^{19}F *in vitro* images were acquired using a gradient-echo (GRE) pulse sequence, method = RARE, matrix size = 32×32, FOV = 30 mm×30 mm, TR = 2500 ms, TE = 3.77 ms, RARE factor = 1, slice thickness = 20 mm, number of average = 4, scan time = 160 s. The longitudinal relaxation time T_1 was measured through the inversion recovery method and the transverse relaxation time T_2 was measured through the spin-echo method (Table S2).

Table S2. T_1 and T_2 of **F-PP** and **L-PP**.

	T_1 (ms)	T_2 (ms)
F-PP	375	10
L-PP	439	9

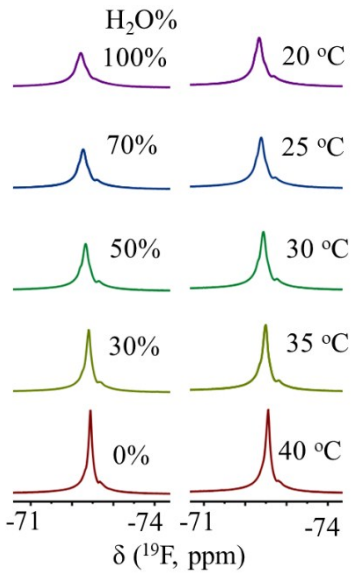


Figure S5. ^{19}F NMR of **F-PP** in different ratios of water and methanol, and at different temperatures in water.

8. *Ex vivo* histological staining

HepG2 tumour-bearing mice in the therapeutic study were sacrificed on day 21 from which the tumors and major organs from saline group, **L-PP** group and **L-PP** + laser group were collected and fixed with paraformaldehyde or cryosectioned for hematoxylin-eosin (H&E) staining. The tumors and major organs of DOX group were collected at day 18.

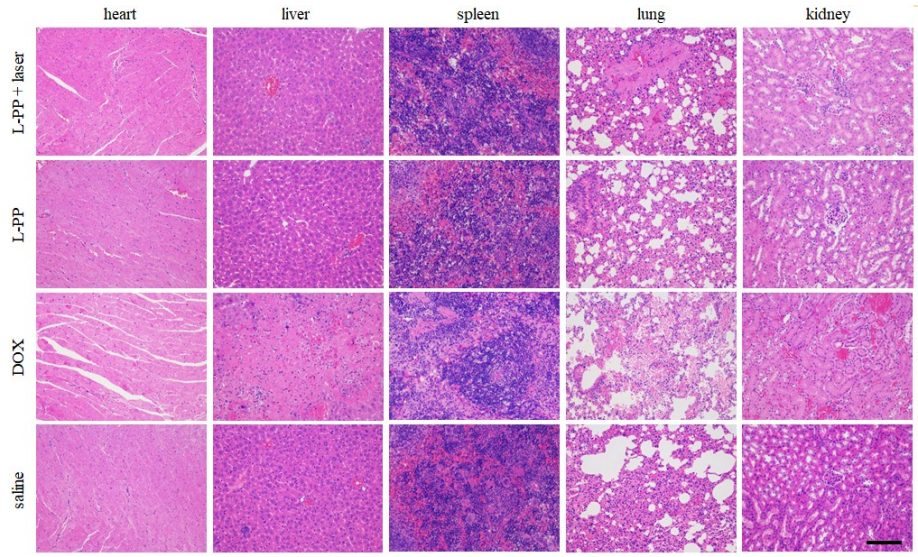


Figure S6. Representative H&E staining of major organs after group treatments. Scale bar, 100 μm.

9. Synthetic procedures of F-PP

compound 2. To a suspension of compound **1** (800 mg, 1.2 mmol) and K_2CO_3 (1.7 g, 12.0 mmol) in acetone (80 mL) was added *tert*-butyl bromoacetate (1.9 g, 9.6 mmol), and the reaction mixture was heated at reflux for 12 h. After filtration and removal of the solvent, the residue was purified by flash chromatography on silica gel (PE/EA 10:1) to afford compound **2** as a reddish brown solid (1.2 g, 90% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.89 (s, 8H), 8.16 (d, *J* = 8.0 Hz, 8H), 7.31 (d, *J* = 8.0 Hz, 8H), 4.585 (s, 8H), 1.64 (s, 36H), -2.77 (s, 2H).

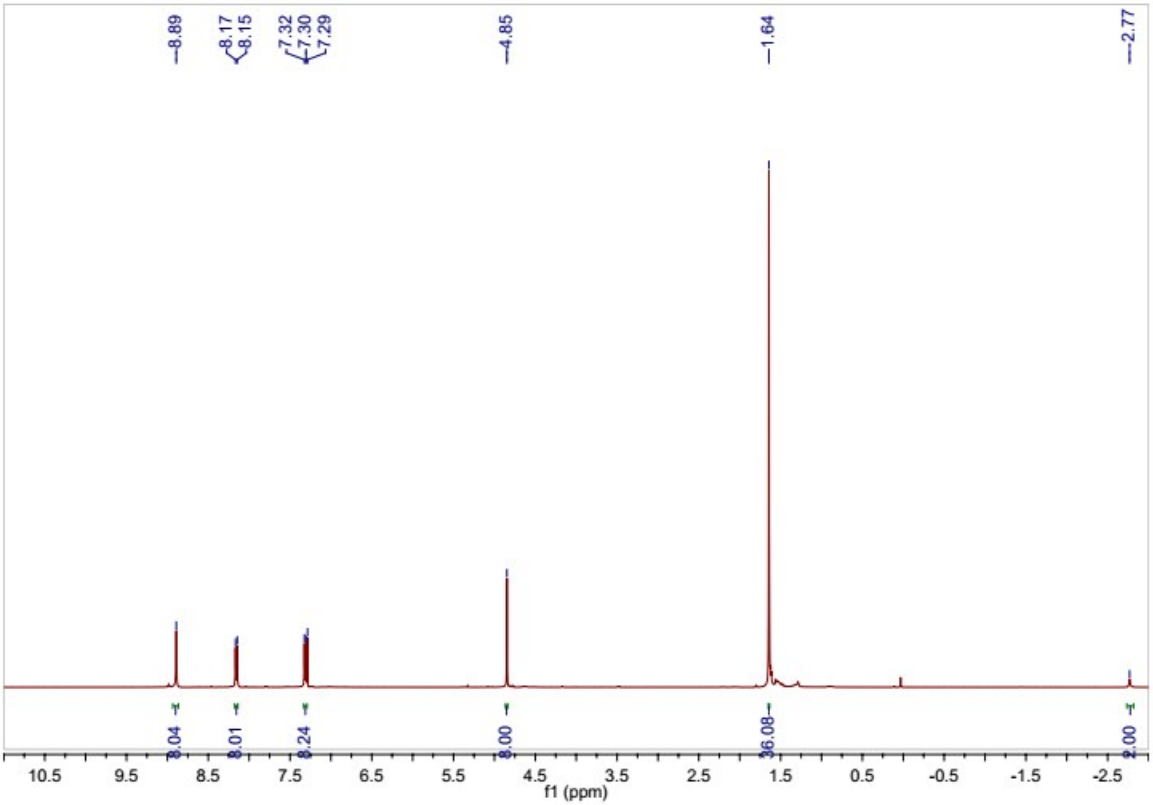
compound 3. To a solution of trifluoroacetic acid (9.1 g, 80.0 mmol) in dry CH_2Cl_2 (60 mL) was added compound **2** (1.1 g, 1.0 mmol). The reaction mixture was stirred at room temperature overnight and then concentrated in vacuo to get compound **3**. Compound **3** was used for next step without further purification.

compound F-PP. Under an atmosphere of argon, to a stirring solution of HOBt (243 mg, 1.8 mmol) and compound **3** (182 mg, 0.3 mmol) in DMF (60 mL) was added EDC (345 mg, 1.8 mmol) at 0 °C. After 20 min, HFB[1] (3.9 g, 1.8 mmol) was added in one portion and the reaction mixture was stirred at 45 °C for 24 h. Then the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (CH_2Cl_2/MeOH = 10/1) to give compound **F-PP** as purple oil (2.1 g, 62% yield). ^{1}H NMR (400 MHz, CD_3OD) δ

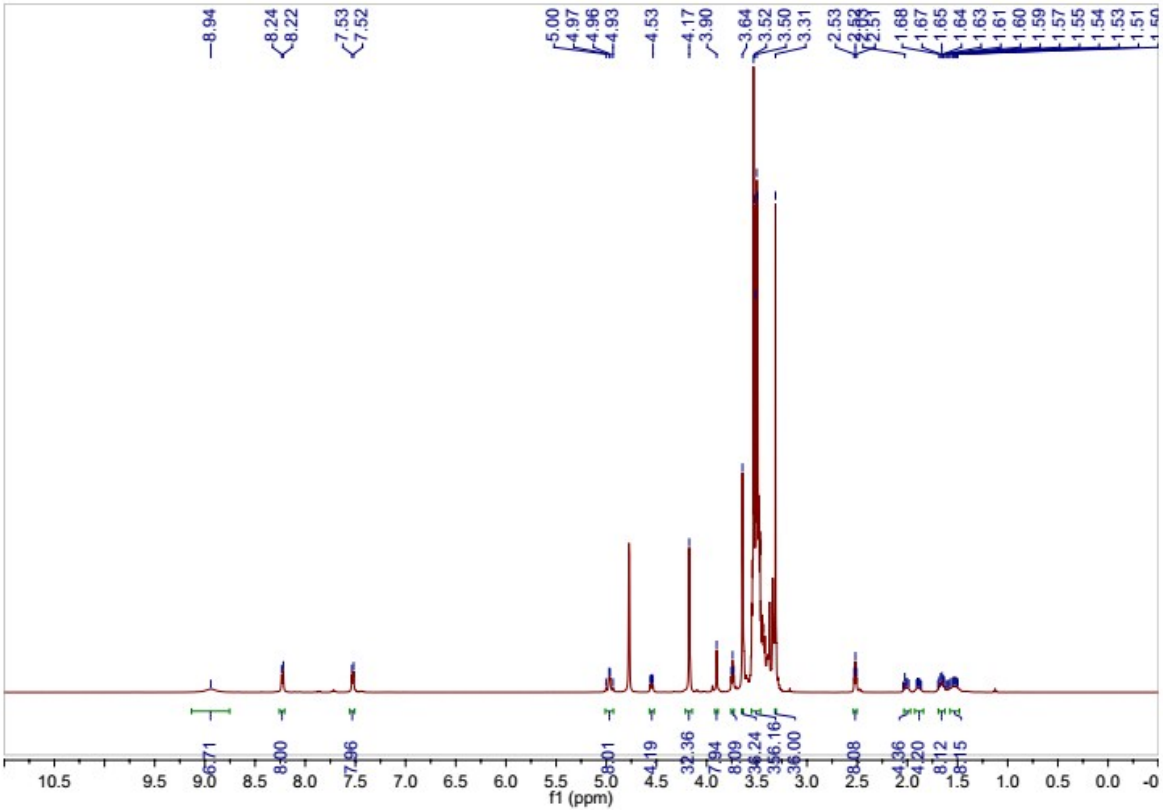
8.94 (s, 8H), 8.21 (d, *J* = 8.0 Hz, 8H), 7.52 (d, *J* = 4.0 Hz, 8H), 4.93-5.0 (m, 8H), 4.53-3.56 (m, 4H), 4.17 (s, 32H), 3.90 (s, 8H), 3.74 (t, *J* = 4.0 Hz, 8H), 3.64 (s, 36H), 3.49-3.53(m, 356 H), 3.31 (s, 36H), 2.52 (t, *J* = 4.0 Hz, 8H), 1.99-2.04 (m, 4H), 1.87-1.91 (m, 4H), 1.63-1.70 (m, 8H), 1.50-1.57 (m, 8H); ^{19}F NMR (376 MHz, $CDCl_3$) δ -73.53; ^{13}C NMR (100 MHz, CD_3OD) ^{13}C NMR (126 MHz, CD_3OD) δ 174.2, 173.4, 172.6, 170.9, 159.4, 137.0, 136.6, 126.7,121.5 (q, *J* = 232.0 Hz) 121.1, 114.6, 80.3-81.5 (m), 72.9, 72.1, 72.0, 71.8, 71.2-71.60 (m), 70.9, 70.7, 68.7, 68.6, 67.3, 67.2, 64.8, 59.1, 54.50, 47.4, 46.5, 40.3, 39.9, 39.7, 39.6, 37.3, 32.8, 30.4, 24.8, 22.6, 15.7; MS (MALDI-TOF) calcd for $C_{372}H_{566}F_{108}N_{21}O_{136}^+$ $[M+NH_4]^+$ 9555.6, found 9556.9.

10. ^{1}H NMR, ^{19}F NMR, ^{13}C NMR, MS and HRMS Spectra of Compounds

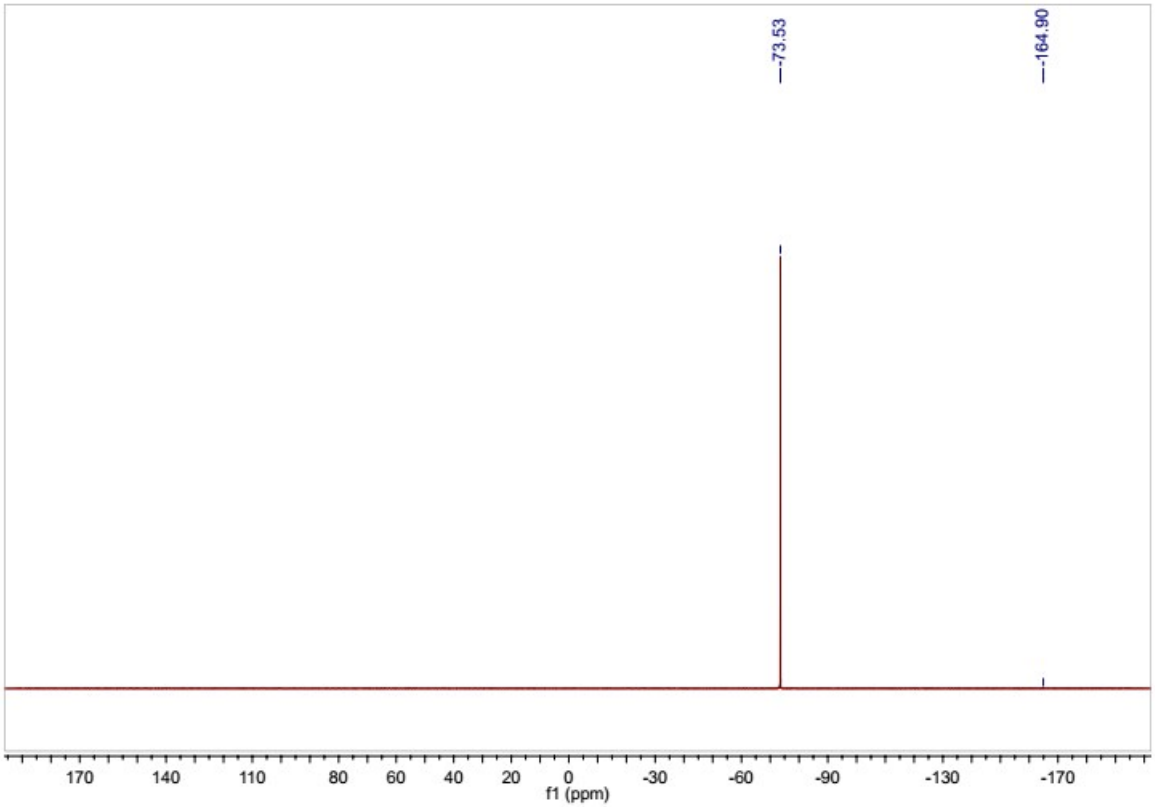
^{1}H NMR spectra of Compound **2** (400 MHz, $CDCl_3$)



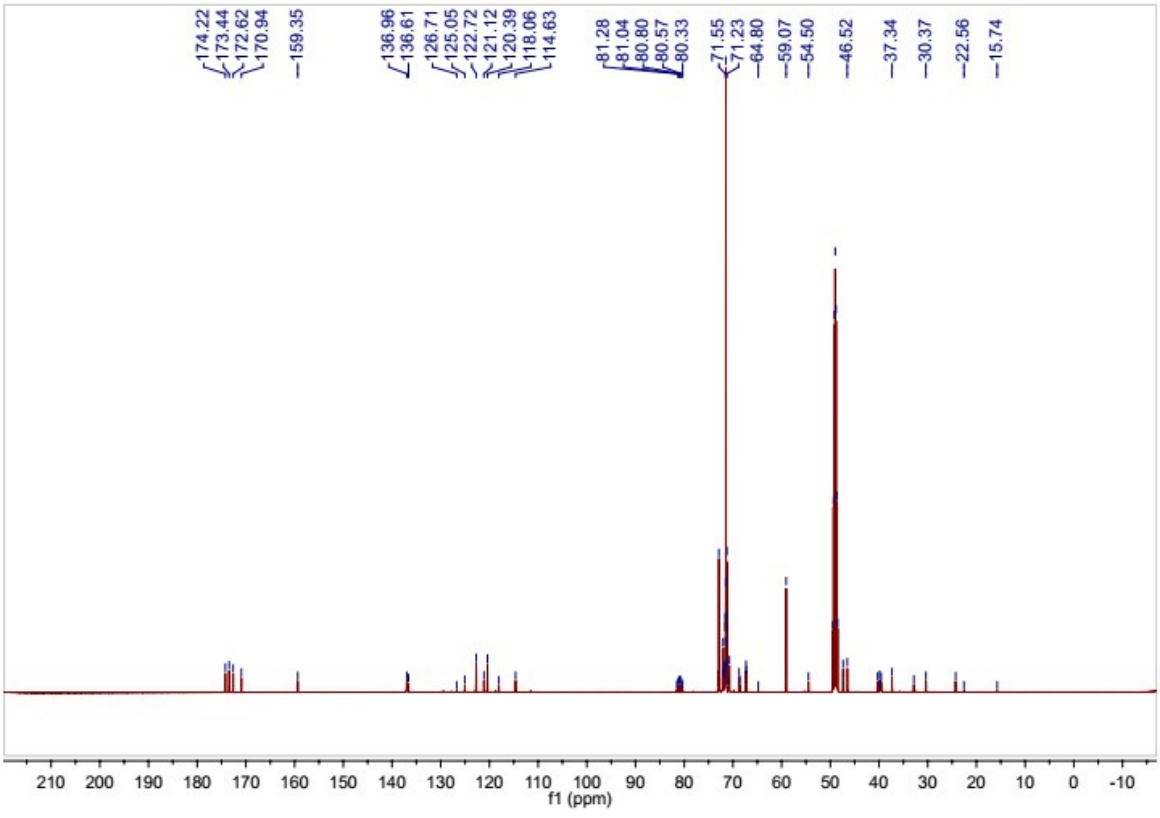
^{1}H NMR spectra of **F-PP** (400 MHz, CD_3OD)



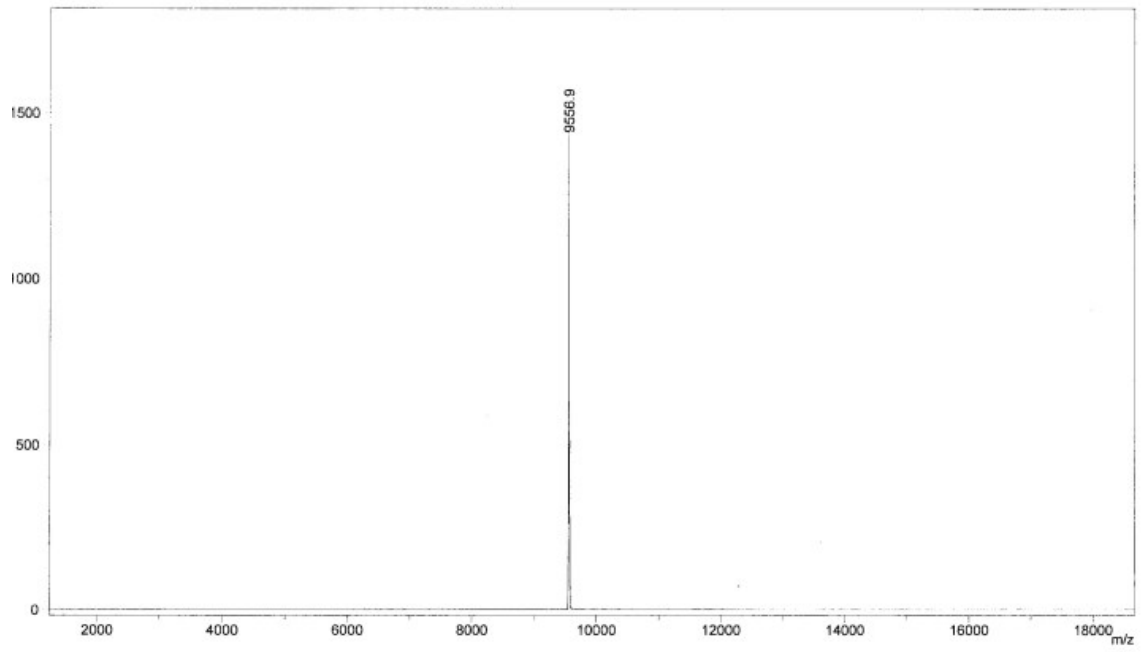
^{19}F NMR spectra of **F-PP** (376 MHz, $CDCl_3$)



^{13}C NMR spectra of **F-PP** (100 MHz, CD_3OD)



MALDI-TOF MS spectra of **F-PP**



[1] Bo, S.; Yuan, Y.; Chen, Y.; Yang, Z.; Chen, S.; Zhou, X.; Jiang, Z.-X. *Chem. Commun.* 2018, **54**, 3875.