Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Crown Ether Size and Stereochemistry Affect the Self-Assembly, Hydrogelation, and Cellular Interactions of Crown Ether/Peptide Conjugates

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Supporting Information

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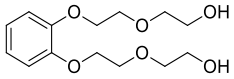
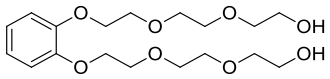
2. Synthesis and characterization of crown ether acids:

Synthesis of 1,2-Bis [2-(2-hydroxyethoxy)ethoxy]benzene (3a). [1]



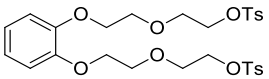
A solution of catechol (6.18 g, 0.0561 mol) and anhydrous K_2CO_3 (24.5 g, 0.177 mol) in anhydrous MeCN (220 mL) was heated under reflux for 45 min under N_2 gas, whereupon KI (3.15 g, 0.0189 mol) was added. The reaction mixture was heated under reflux for another 10 min, before 2-(2-chloroethoxy)ethanol (18 ml, 15.25 g, 0.168 mol) was added over a period of 1 h. Thereafter, the reaction mixture was stirred under reflux for 19 h. After being cooled down to room temperature, the reaction mixture was filtered and concentrated under vacuum. The resulting residue was dissolved in DCM (200 mL), washed with aqueous K_2CO_3 (10% w/v, 100 mL), aqueous NaCl (100 mL), H_2O (3×100 mL), and dried ($MgSO_4$). Removal of the solvent gave an orange-brown oil, which was purified using column chromatography using silica gel with ethyl acetate: hexane (4:1) eluent affording the compound **3a** the pale yellow oil (6.8 g, 42%).^{1}H NMR (300 MHz, $CDCl_3$, 25^{o}C): δ=3.65-3,70 (m, 5H; OH, $2CH_2$), 3.70-3.80 (m, 5H; OH, $2CH_2$), 3.90-3.95 (m, 4H; $2CH_2$), 4.15-4.20 (m, 4H; $2CH_2$), 6.91 (d, *J*=0.9 Hz, 4H; 4CH); MS [ESI$^+$]: m/z(%): calcd. 286.33, obsvd. 309.2 $[M+Na]^+$.

Synthesis of 1,2-Bis{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}benzene (3b).



A solution of catechol (5.15 g, 0.0465 mol) and anhydrous K_2CO_3 (20.2 g, 0.145 mol) in anhydrous MeCN (125 mL) was heated under reflux for 45 min under N_2 gas, whereupon KI (2.6 g, 0.0155 mol) was added. The reaction mixture was heated under reflux for another 10 min, before 2-(2-(2-chloroethoxy)ethoxy)ethanol (20.5 ml, 23.8 g, 0.14 mol) was added over a period of 1 h. Thereafter, the reaction mixture was stirred under reflux for 19 hours. After being cooled down to room temperature, the reaction mixture was filtered and concentrated under vacuo. The resulting residue was dissolved in DCM (200 mL), washed with aqueous K_2CO_3 (10% w/v, 100 mL), aqueous NaCl (100 mL), H_2O (3×100 mL), and dried ($MgSO_4$). Removal of the solvent gave an orange-brown oil, which was purified using column chromatography using silica gel with ethyl acetate: hexane (4:1) eluent affording the compound **3b** as pale yellow oil (12.5 g, 59%).^{1}H NMR (300 MHz, $CDCl_3$, 25^{o}C): δ=3.44 (s, 2H; 2OH), 3.50-3.55 (m, 4H; $2CH_2$), 3.55-3.70 (m, 12H; $6CH_2$), 3.81 (t, *J*=4.95 Hz, 4H; $2CH_2$), 4.10 (t, *J*=4.8 Hz, 4H; $2CH_2$), 6.84 (s, 4H; CH); MS [ESI$^+$]: m/z(%): calcd. 374.44, obsvd. 397.2 $[M+Na]^+$.

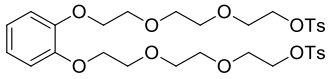
Synthesis of 1,2-Bis [2-(2-tosyloxyethoxy)ethoxy]benzene (4a). [1]



The mixture of **3a** (5 g, 17.48 mmol), DMAP (0.4 g, 3.3 mmol), Et_3N (27 mL) and anhydrous DCM (60 mL) was cooled to 0 °C. TsCl (17 g, 89.14 mmol) dissolved in anhydrous DCM (120 mL) was added dropwise to the cooled reaction mixture over a period of 1 h. The reaction mixture was allowed to reach room temperature and stirred for

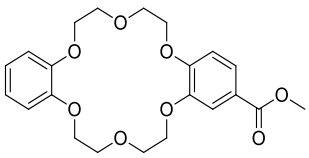
19 h, where after the reaction mixture was poured into 4 M HCl (300 mL) and H_2O (300 mL). The organic phase was washed with 1 M HCl (2 × 200 mL), aqueous NaCl (300 mL), H_2O (300 mL), and dried ($MgSO_4$). After evaporation of the solvent, the deep red oil was purified using column chromatography by silica gel with ethyl acetate: hexane (4:1) eluent affording the title compound **2a** as a yellow oil (8.14 g, 78.4%).^{1}H NMR (300 MHz, $CDCl_3$, 25^{o}C): δ=2.41 (s, 6H; $2CH_3$), 3.75-3.80 (m, 8H; $4CH_2$), 4.05-4.10 (m, 4H; $2CH_2$), 4.15-4.20 (m, 4H; $2CH_2$), 6.85-6.95 (m, 4H; 4CH), 7.29 (t, *J*=5.1 Hz, 4H; 4CH), 7.79 (dd, *J*=6.6, 1.8 Hz, 4H; 4CH); MS [ESI$^+$]: m/z(%): calcd. 594.71, obsvd. 618.2 $[M+Na]^+$.

Synthesis of 1,2-Bis[2-[2-(2-tosyloxyethoxy)ethoxy]ethoxy]benzene (4b).



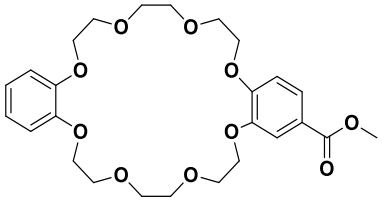
The mixture of **3b** (12.5 g, 33 mmol), DMAP (0.745 g, 6.15 mmol), Et_3N (44 mL) and anhydrous DCM (100 mL) was cooled to 0 °C. TsCl (31.5 g, 165.2 mmol) dissolved in anhydrous DCM (220 mL) was added dropwise to the cooled reaction mixture over a period of 3 h. The reaction mixture was allowed to reach room temperature and stirred for 19 h, where after the reaction mixture was poured into 4 M HCl (300 mL) and H_2O (300 mL). The organic phase was washed with 1 M HCl (2 × 200 mL), aqueous NaCl (300 mL), H_2O (300 mL), and dried ($MgSO_4$). After evaporation of the solvent, the deep red oil was purified using column chromatography by silica gel with ethyl acetate: hexane (4:1) eluent affording the title compound **2c** as a yellow oil (18 g, 71.1%).^{1}H NMR (300 MHz, $CDCl_3$, 25^{o}C): δ=2.42 (s, 6H; $2CH_3$), 3.55-3.60 (m, 4H; $2CH_2$), 3.65-3.70 (m, 8H; $4CH_2$), 3.83 (t, *J*=4.95 Hz, 4H; $2CH_2$), 4.10-4.20 (m, 8H; $4CH_2$), 6.91 (s, 4H; 4CH), 7.33 (d, *J*=8.1 Hz, 4H; 4CH), 7.79 (d, *J*=8.4 Hz, 4H; 4CH); MS [ESI$^+$]: m/z(%): calcd. 682.81, obsvd. 705.3 $[M+Na]^+$.

Synthesis of 4-Carbomethoxydibenzo-18-crown-6 (5a). [2]



Ditosylate **4a** (4.3 g, 6.9 mmol), methyl 3,4-dihydroxybenzoate (1.25 g, 7.4 mmol) and KPF_6 (1.65 g, 8.8 mmol) were dissolved in MeCN (220 mL). The solution was degassed by bubbling N_2 (g) through it. The flask was wrapped with aluminum foil to protect from light, and anhydrous K_2CO_3 (4.1 g, 29.3 mmol) was added. The mixture was heated at reflux under N_2 (g) for 5 days, allowed to cool to room temperature and the solids were filtered. The solvent was removed by rotary evaporation and the solid product was filtered and washed by DCM. The pure compound dried under vacuo, product as white solid. Yield (2.72 gm, 90%).^{1}H NMR (300 MHz, [D_6]DMSO, 25^{o}C): δ=3.82 (s, 3H; CH_3), 3.90 (s, 8H; $4CH_2$), 4.12 (s, 4H; $2CH_2$), 4.22 (s, 4H; $2CH_2$), 6.85-6.95 (m, 2H; 2CH), 6.99 (t, *J*=4.65 Hz, 2H; 2CH), 7.13 (d, *J*=8.7 Hz, 1H; CH), 7.48 (d, *J*=1.5 Hz, 1H; CH), 7.61 (t, *J*=4.2 Hz, 1H; CH); MS [ESI$^+$]: m/z(%): calcd. 418.45, obsvd. 457.1 $[M+K]^+$.

Synthesis of 4-Carbomethoxydibenzo-24-crown-8 (5b).

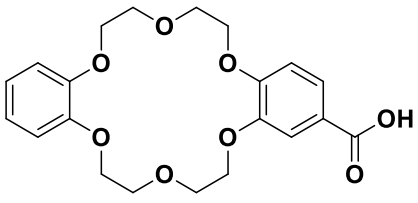


Ditosylate **4b** (4.8 g, 7 mmol), methyl 3,4-dihydroxybenzoate (1.18 g, 7 mmol) and KPF_6 (1.56 g, 8.4 mmol) were dissolved in MeCN (220 mL). The solution was degassed by bubbling N_2 (g) through it. The flask was wrapped with aluminum foil to protect from light, and anhydrous K_2CO_3 (3.92 g, 28.0 mmol) was added. The mixture was heated at reflux under N_2 (g) for 5 days, allowed to cool to room temperature and the solids were filtered. The solvent was removed by rotary evaporation and the residue was dissolved in DCM. The solution was washed with pyridinium hydrochloride (12x15 mL), water (15 mL), $NaHCO_3$ (satd.) (4x15 mL), and water (1x15 mL), dried over $MgSO_4$, filtered, and the solvent was removed by rotary evaporation to yield a gray white solid, this solid was purified by column chromatography using silica with ethyl acetate: hexane (4:1) eluent affording the title compound as white solid. Yield (3.2 gm, 89.9%).^{1}H NMR (300 MHz, $CDCl_3$, 25^{o}C): δ=3.85 (d, *J*=1.8 Hz, 8H; $4CH_2$), 3.88 (s, 3H; CH_3), 3.90-3.70 (m, 8H; $4CH_2$), 4.10-4.25 (m, 8H; $4CH_2$), 6.80-6.90 (m, 5H; 5CH), 7.53 (d, *J*=2.1 Hz, 1H; CH), 7.65 (dd, *J*=8.4, 1.8 Hz, 1H; CH); MS [ESI$^+$]: m/z(%): calcd. 506.55, obsvd. 529.2 $[M+Na]^+$.

Synthesis of Crown acid (6a & 6b).[3]

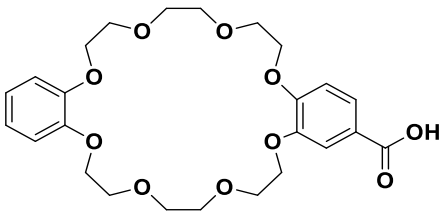
General procedure: Crown ester (5.53 mmol) and EtOH (88 mL). A solution of aqueous KOH (4M, 8.5 mL) was added dropwise and the reaction mixture was heated at reflux for 12 h. Upon completion of the reaction the solvent was removed to give an off white solid, which was redissolved in H_2O (100 mL) and neutralized with H_2SO_4. The solution was extracted with DCM (2x100 mL), and the organic layers were combined, dried over $MgSO_4$, and concentrated to give a white solid, which was recrystallized from EtOH to give a white solid compounds, compound (**6a**) purify by dissolved it is $NaHCO_3$ then filter the solution to remove any insoluble compounds then the filtrate was acidified by conc. HCl until acidic, the solid product obtained was filtrated and dried in oven at 70^{o}C.

4-Carboxy dibenzo[18]crown-6 (6a).



Yield (1.3 gm, 45%).^{1}H NMR (300 MHz, [D_6]DMSO, 25^{o}C): δ=3.85 (s, 8H; $4CH_2$), 4.06 (d, *J*=3.9 Hz, 4H; $2CH_2$), 4.12 (d, *J*=7.2 Hz, 4H; $2CH_2$), 6.85-6.95 (m, 4H; 4CH), 7.04 (d, *J*=8.4 Hz, 1H; CH), 7.43 (d, *J*=2.1 Hz, 1H; CH), 7.55 (dd, *J*=8.4, 1.8 Hz, 1H; CH); MS [ESI$^+$]: m/z(%): calcd. 404.45, obsvd. 427.1 $[M+Na]^+$.

4-Carboxy dibenzo[24]crown-8 (6b).



Yield (1.5 gm, 55%).^{1}H NMR (300 MHz, $CDCl_3$, 25^{o}C): δ=3.85 (d, *J*=2.7 Hz, 8H; $4CH_2$), 3.93 (s 8H; $4CH_2$), 4.18 (q, *J*=4.2 Hz, 8H; $4CH_2$), 6.85 (d, *J*=1.2 Hz, 5H; 5CH), 7.57 (s, 1H; CH), 7.72 (d, *J*=8.1 Hz, H; CH); MS [ESI^{+}]: m/z(%): calcd. 492.53, obsvd. 515.55 $[M+Na]^{+}$.

3. Supplementary Figures and Tables

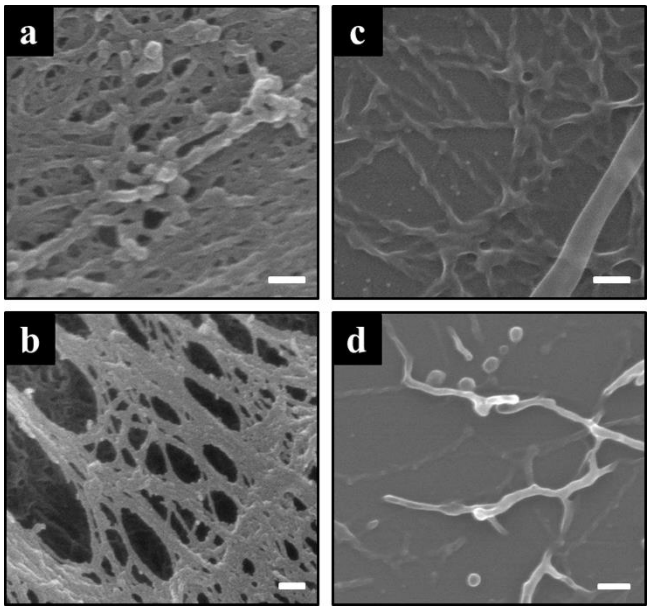


Figure S1. SEM images for a) DB18C6^{L}F^{L}F; b) DB18C6^{D}F^{D}F; c) DB24C8^{L}F^{L}F and d) DB24C8^{D}F^{D}F at pH=7. Scale bar: 100 nm.

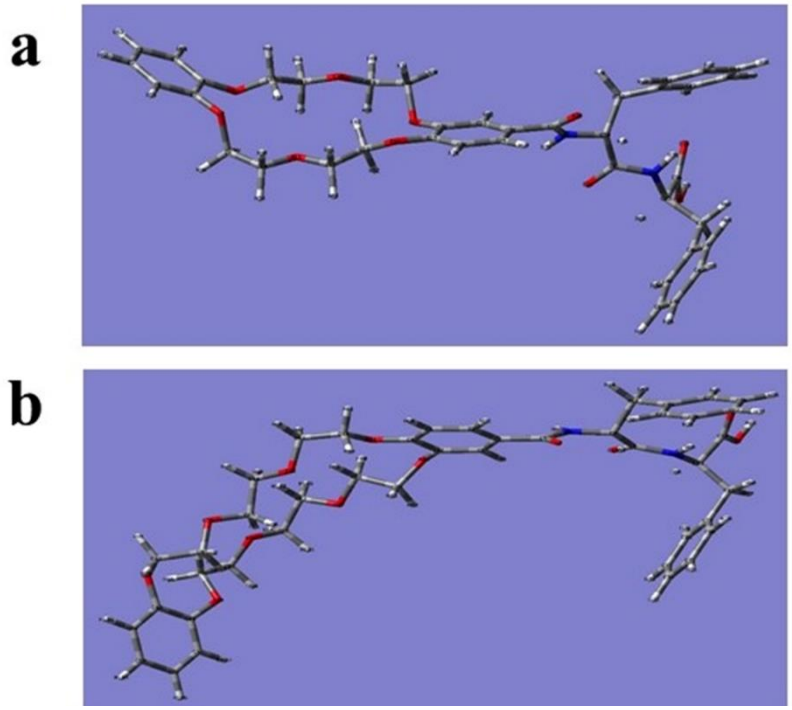


Figure S2. Optimized structure of a) DB18C8^{L}F^{L}F, and b) DB24C8^{L}F^{L}F compounds calculated using semiempirical method (AM1).

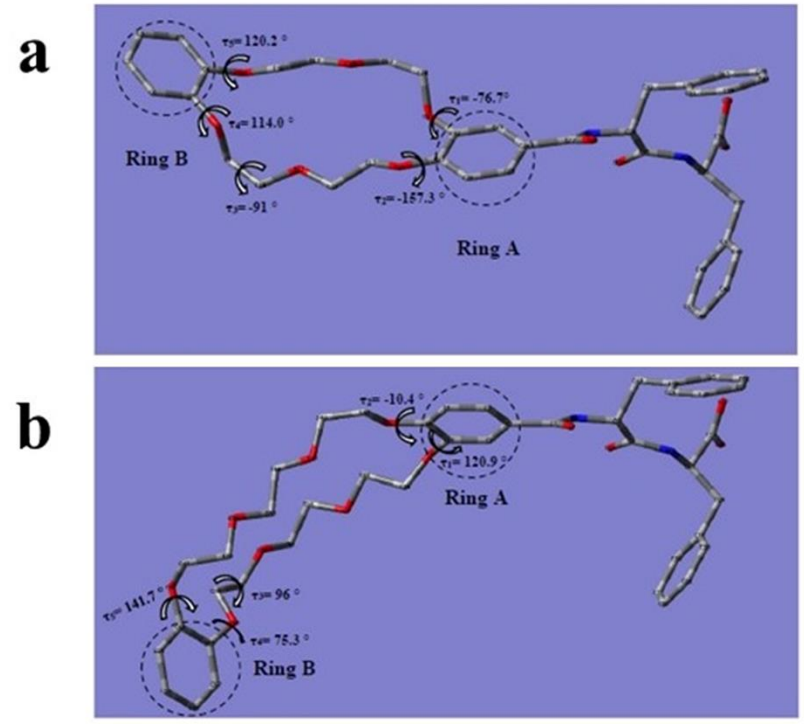


Figure S3. Optimized structure after removing of hydrogen atoms of a) DB18C6^{L}F^{L}F, and b) DB24C8^{L}F^{L}F compounds without hydrogen atoms calculated using semiempirical method (AM1).

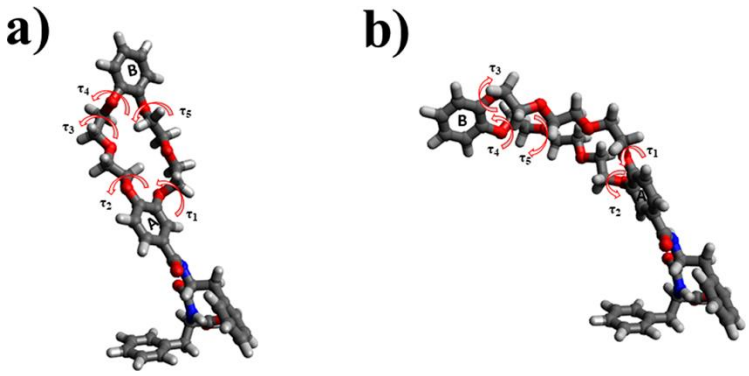


Figure S4. Optimized structure of a) DB18C6^{L}F^{L}F, and b) DB24C8^{L}F^{L}F compounds calculated using AM1 calculations.

Entry	AM1 method					DFT method				
	torsional angles					torsional angles				
	τ_1	τ_2	τ_3	τ_4	τ_5	τ_1	τ_2	τ_3	τ_4	τ_5
DB18C6^{L}F^{L}F	-76.7°	-157.3°	91°	114.0°	120.2°	-28.8°	144.5°	-72.2°	107.6°	116.7°
DB24C8^{L}F^{L}F	120.9°	-10.4°	96°	75.3°	141.7°	129.1°	-17.3°	70.1°	77.6°	132.1°

Table S1. Torsional angles for DB18C6^{L}F^{L}F, and DB24C8^{L}F^{L}F compounds calculated by AM1 and DFT calculations.

From the AM1 calculations, we obtained the optimized structures of DB18C6^{L}F^{L}F, and DB24C8^{L}F^{L}F (Figure S2). Further, using DFT calculations showed the optimized structures for DB18C6^{L}F^{L}F, and DB24C8^{L}F^{L}F, respectively (Figure 1e, and 1f). From the optimized structures, we found that all of these compounds with the same side chain (FF) conjugated with phenyl ring A of different crown ring size existing as different molecular structure, thus indicating the size of crown ether also play a crucial role in molecular packing in these self-assembled systems (Figure S3 and S4), and the proposed molecular packing for DB18C6^{L}F^{L}F, and DB24C8^{L}F^{L}F showed in Figure S5. The torsional angles τ_1/τ_2 between rings A and B in DB24C8^{L}F^{L}F obtained from DFT calculations were 129.1°/-17.3°, these angles differed considerably from those in DB18C6^{L}F^{L}F (144.5°/-28.8°, Table S1). The torsional angles τ_4/τ_5 between rings B and A in DB24C8^{L}F^{L}F were 77.6°/132.1°, while those of DB18C6^{L}F^{L}F were 107.6°/116.7° (similar results were obtained by AM1 calculations, see Table S1).

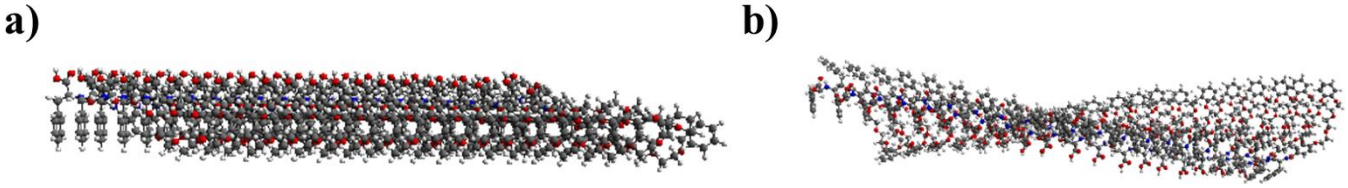


Figure S5. Proposed molecular packing for a) DB18C6^{L}F^{L}F, and b) DB24C8^{L}F^{L}F.

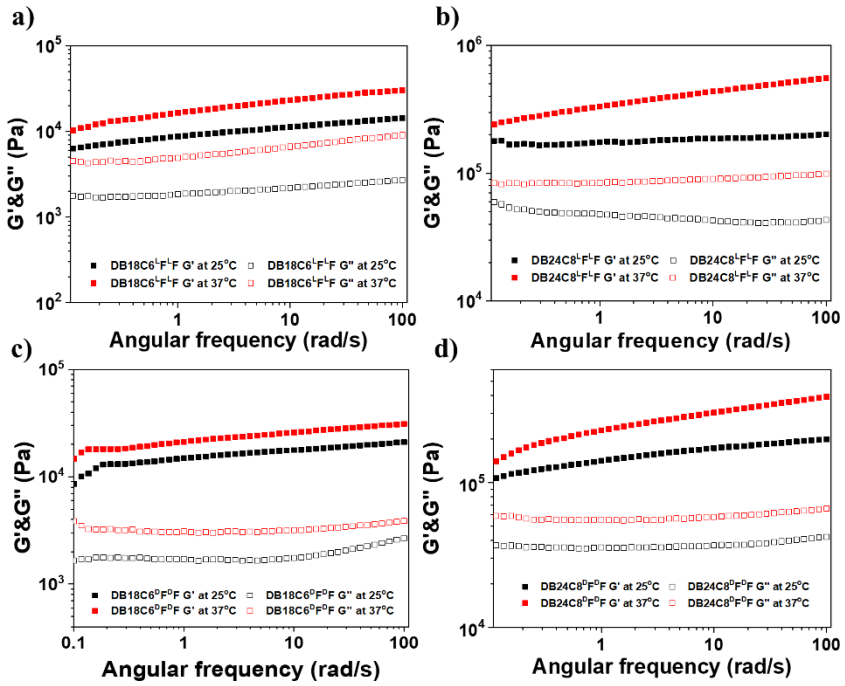


Figure S6. Rheological measurement of DB18C6^{L}F^{L}F, DB18C6^{D}F^{D}F, DB24C8^{L}F^{l}F and DB24C8^{D}F^{D}F hydrogels of frequency sweep at a strain of 0.8% over a range of frequency of 0.1-100 rad/s, a) DB18C6^{L}F^{L}F at temperature 25^{o}C and 37^{o}C respectively, b) DB18C6^{D}F^{D}F at temperature 25^{o}C and 37^{o}C respectively, c) DB24C8^{L}F^{L}F at temperature 25^{o}C and 37^{o}C respectively, and d) DB24C8^{D}F^{D}F at temperature 25^{o}C and 37^{o}C respectively.

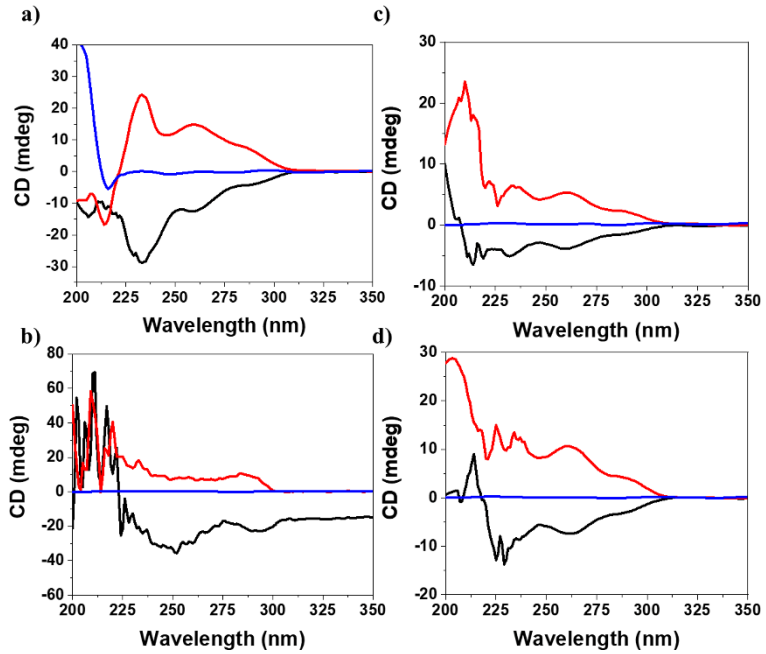


Figure S7. CD spectra for DB18C6-COOH, and DB24C8-COOH respectively (blue), a) DB18C6^{L}F^{L}F (black), DB18C6^{D}F^{D}F (red) at 1000 µM, b) DB18C6^{L}F^{L}F (black), DB18C6^{D}F^{D}F (red) at 2500 µM, c) DB24C8^{L}F^{L}F (black), and DB24C8^{D}F^{D}F (red) at 500 µM concentration d) DB24C8^{L}F^{L}F (black), and DB24C8^{D}F^{D}F (red) at 1000 µM concentration respectively.

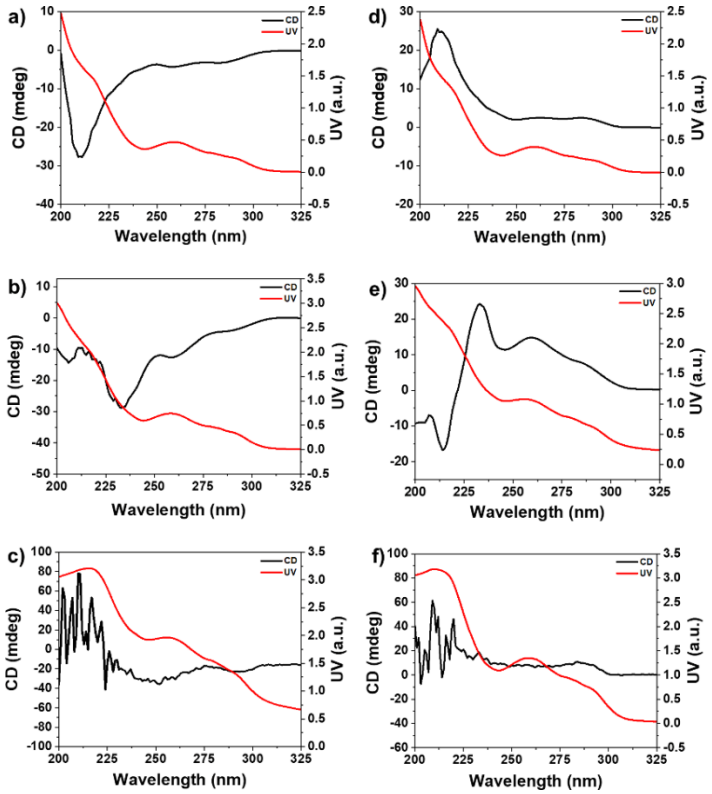


Figure S8. CD and UV spectra for a, b and c) DB18C6^{L}F^{L}F at 500 uM, 1000 uM and 2500 uM concentration respectively, and d, e and f) DB18C6^{D}F^{D}F at 500 uM, 1000 uM and 2500 uM concentration respectively**.**

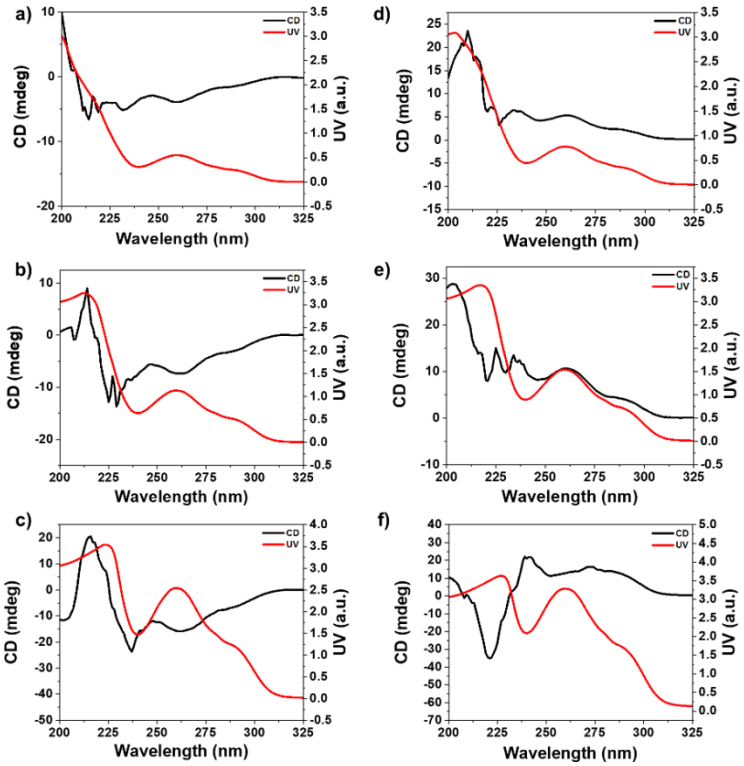


Figure S9. CD and UV spectra for a, b and c) DB24C8^{L}F^{L}F at 500 uM, 1000 uM and 2500 uM concentration respectively, and d, e and f) DB24C8^{D}F^{D}F at 500 uM, 1000 uM and 2500 uM concentration respectively**.**

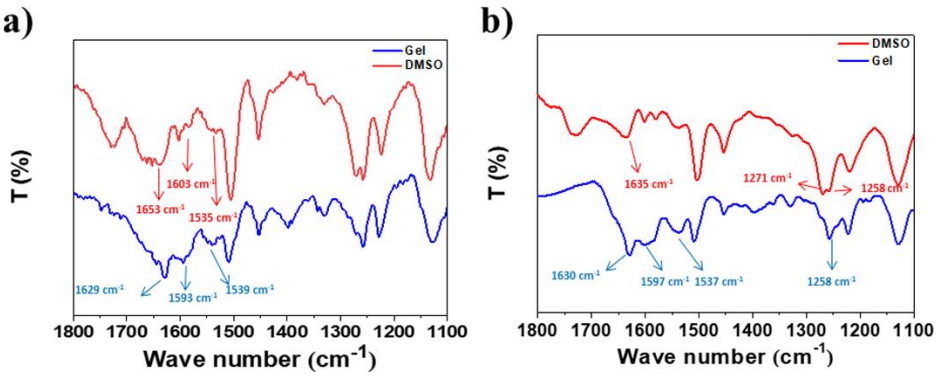


Figure S10. FT-IR spectra for a) DB18C6^{L}F^{L}F, and b) DB24C8^{L}F^{L}F**.**

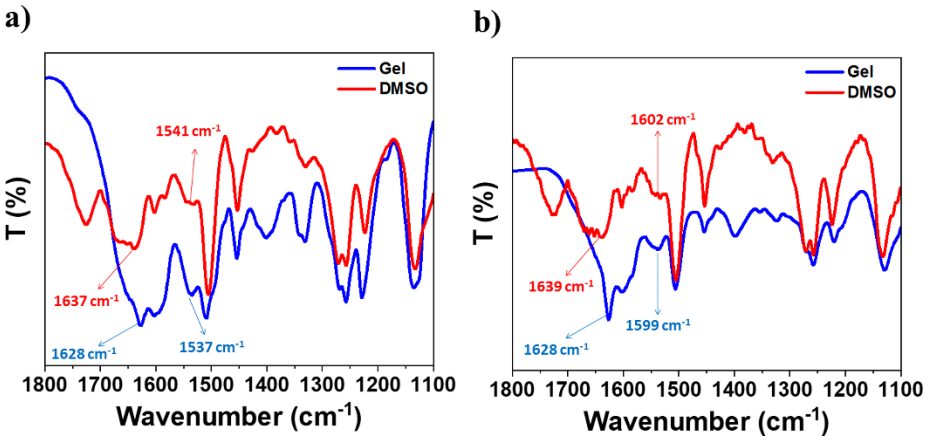


Figure S11. FT-IR spectra for a) DB18C6^{D}F^{D}F, and b) DB24C8^{D}F^{D}F**.**

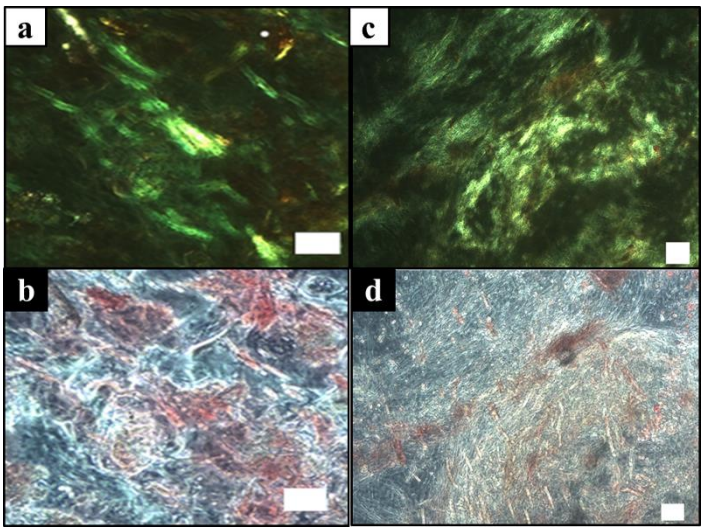


Figure S12. a) Polarized optical microscopic image of DB18C6^{L}F^{L}F and b) Bright field image of DB18C6^{L}F^{L}F hydrogel at 2 wt % stained with Congo red dye, c) Polarized optical microscopic image of DB24C8^{L}F^{L}F and d) Bright field image of DB24C8^{L}F^{L}F hydrogel at 2 wt % stained with Congo red dye. Scale bar = 100 µm**.**

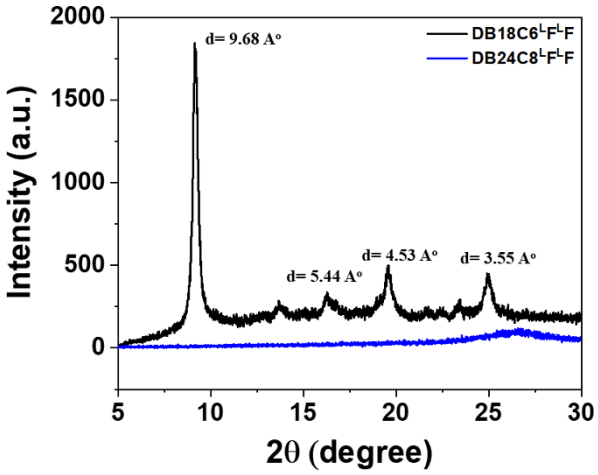


Figure S13. XRD analysis of the xerogel for DB18C6^{L}F^{L}F at 2 wt %, and DB24C8^{L}F^{L}F at 2 wt %.

4. Cell Cultures.

Cell Line: The 3A6-RFP cell line (hMSC) were a generous gift from Dr. Shih-Chieh Huang (China Medical University, ROC Taiwan) and L929 mouse fibroblast cell line was purchased from ATCC

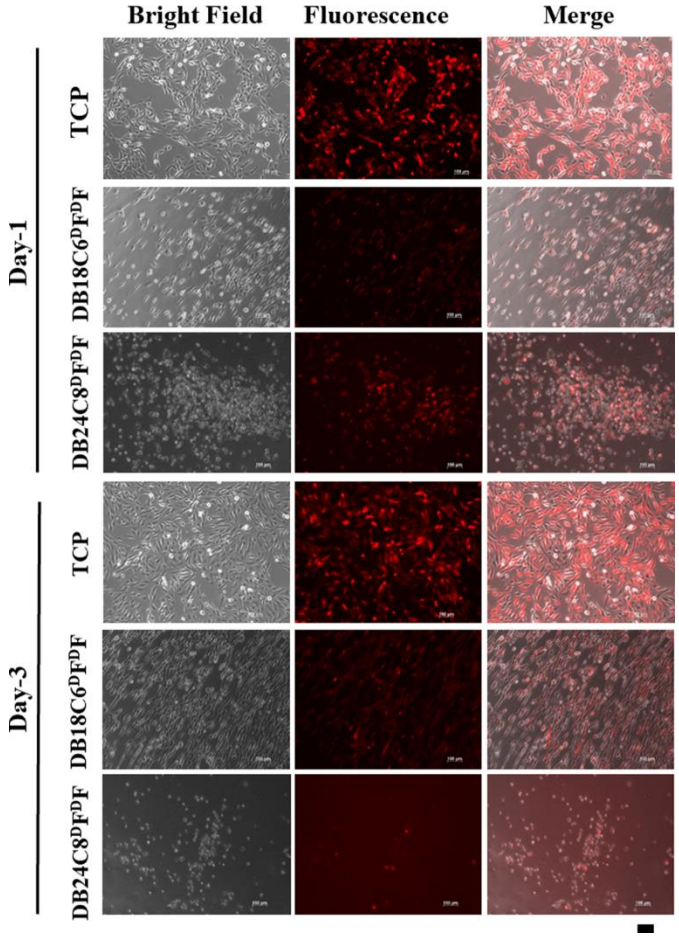


Figure S14. 3A6-RFP cells were seeded onto a tissue culture plate (TCP), DB18C6^{D}F^{D}F, and DB24C8^{D}F^{D}F hydrogels coated surfaces for day 1 and day 3. The cell morphology was imaged by fluorescence microscopy under 10X magnification. (scale bar: 100 μm).

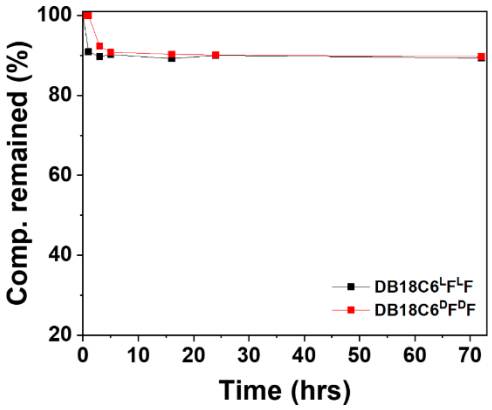


Figure S15. The time-dependent course of the digestions DB18C6^{L}F^{L}F and DB18C6^{D}F^{D}F by proteinase K.

5. ^{1}H, ^{13}C NMR and HRMS spectra of all new compounds

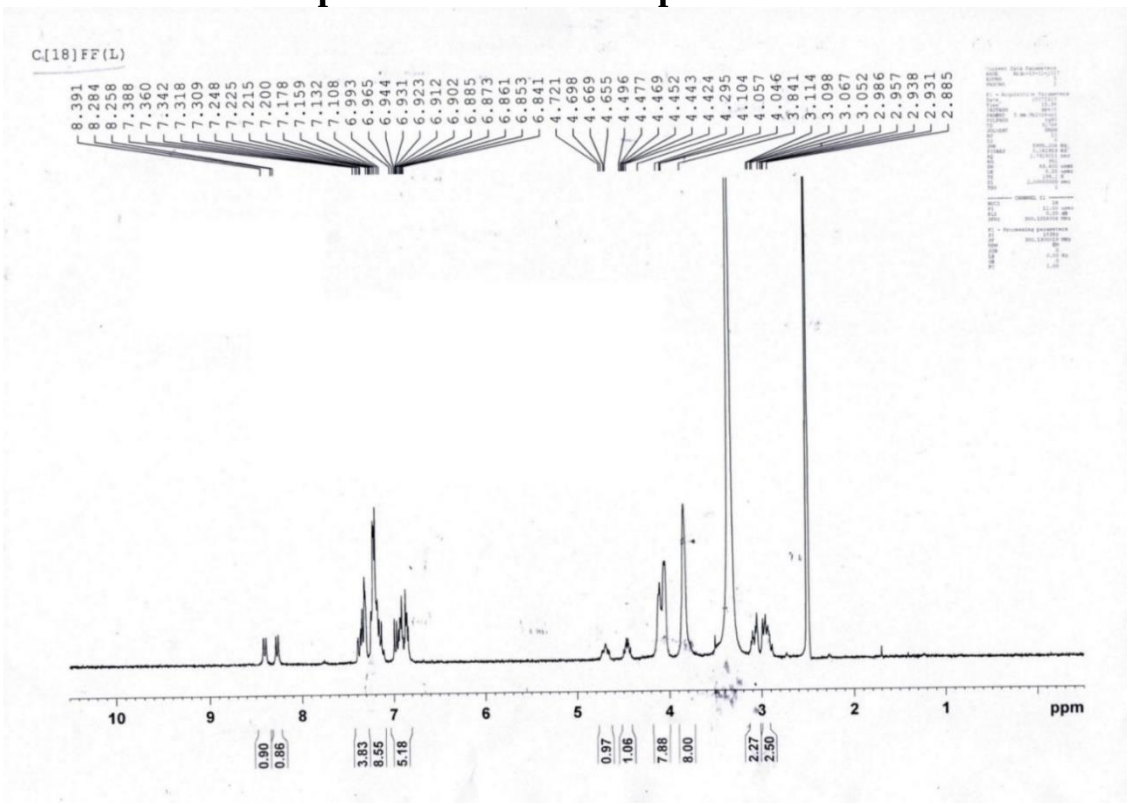


Figure S16. The ^{1}H-NMR spectrum of DB18C6^{L}F^{L}F in DMSO-d_6

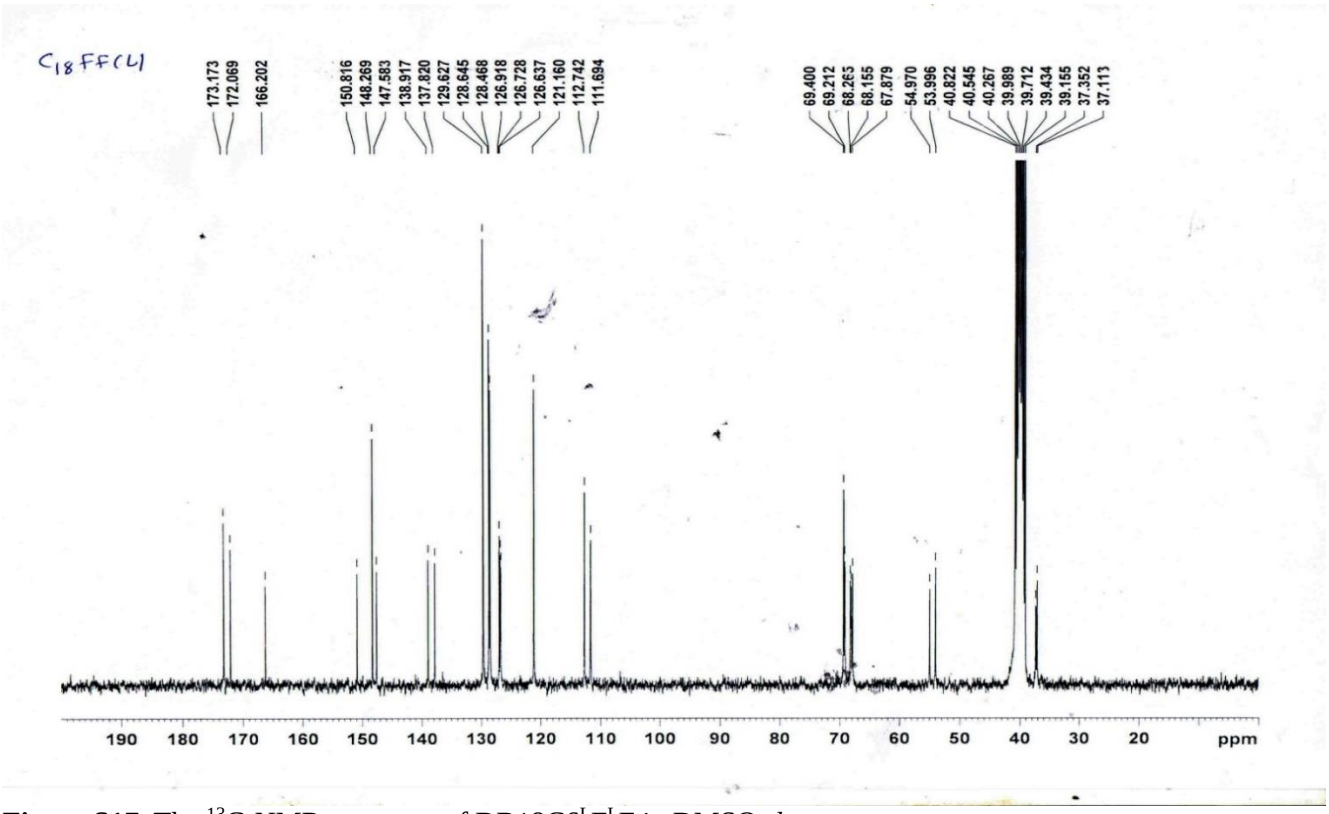


Figure S17. The ^{13}C-NMR spectrum of DB18C6^{L}F^{L}F in DMSO-d_6

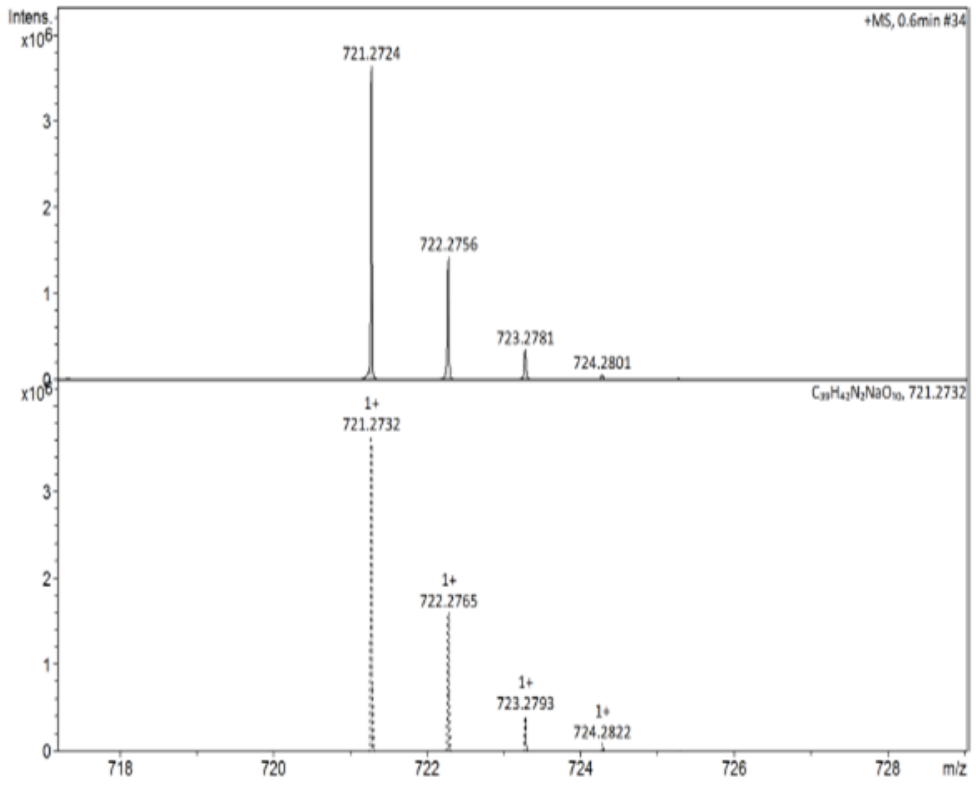


Figure S18. The HRMS of DB18C6^{L}F^{L}F

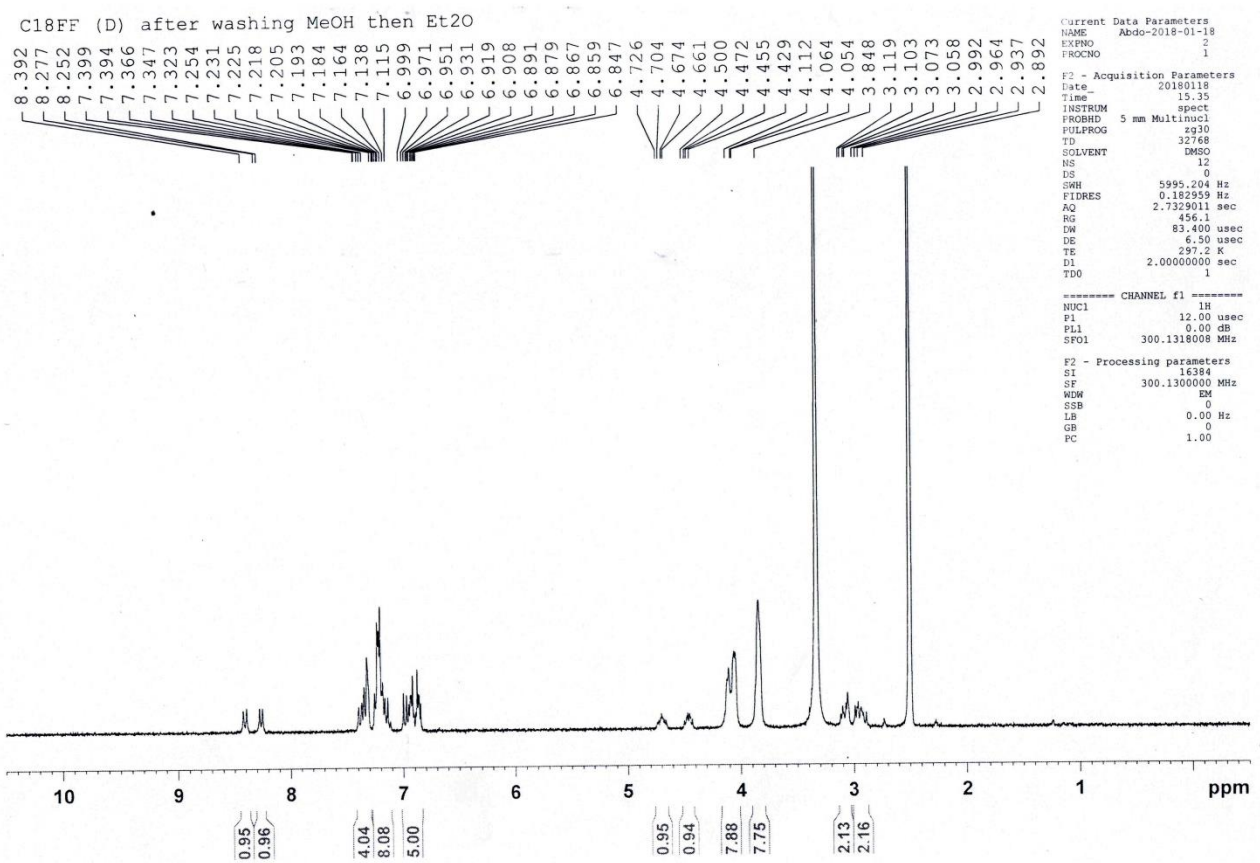


Figure S19. The ^{1}H-NMR spectrum of DB18C6^{D}F^{D}F in DMSO-d_6

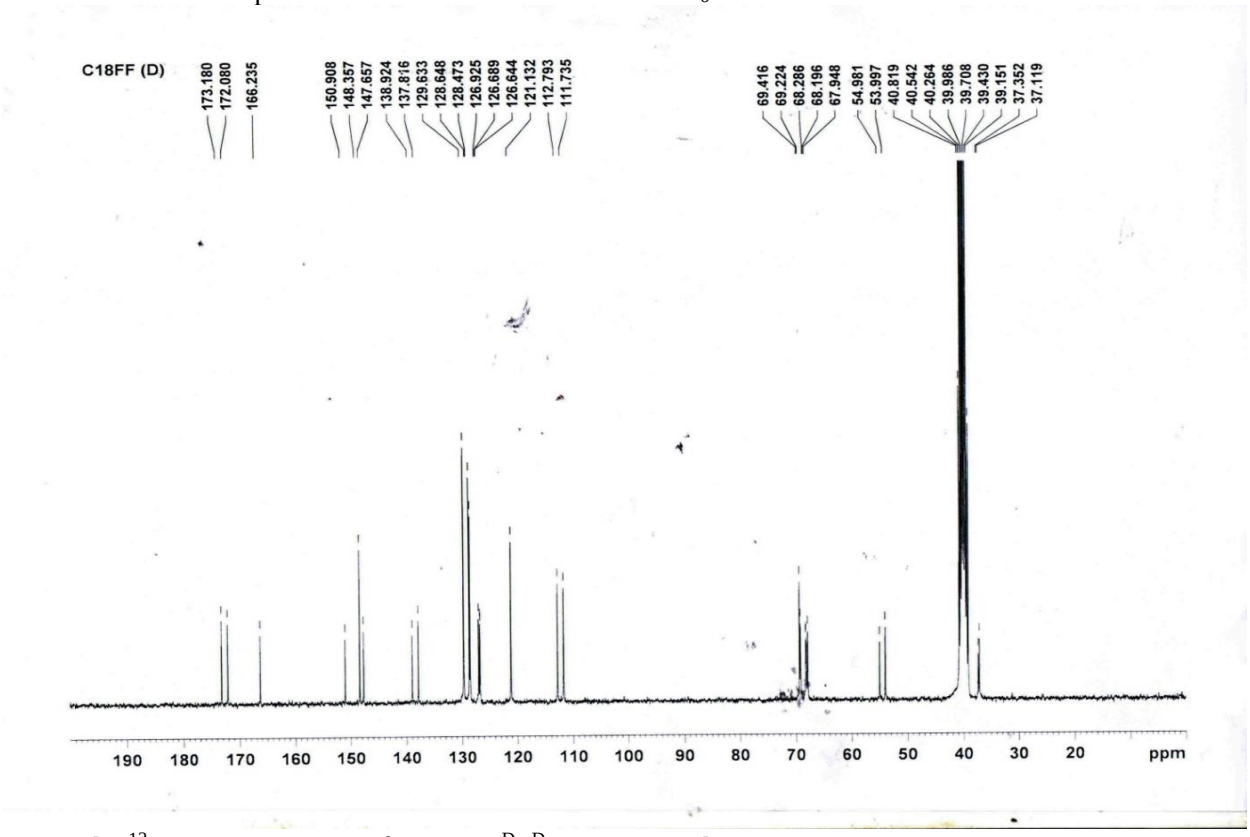


Figure S20. The ^{13}C-NMR spectrum of DB18C6^{D}F^{D}F in DMSO-d_6

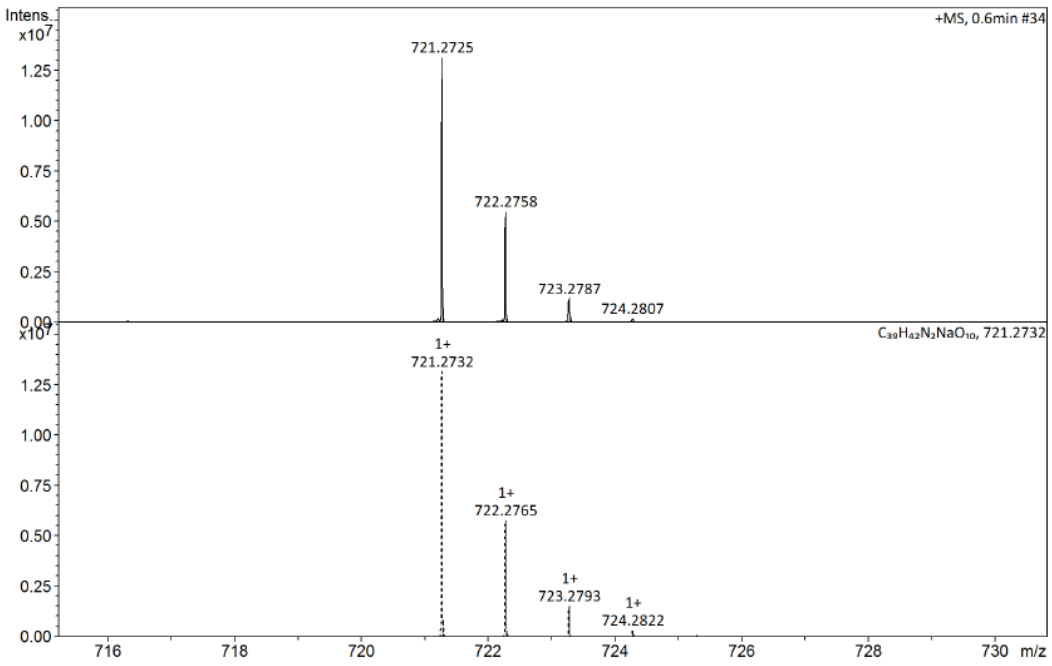


Figure S21. The HRMS of DB18C6^{D}F^{D}F

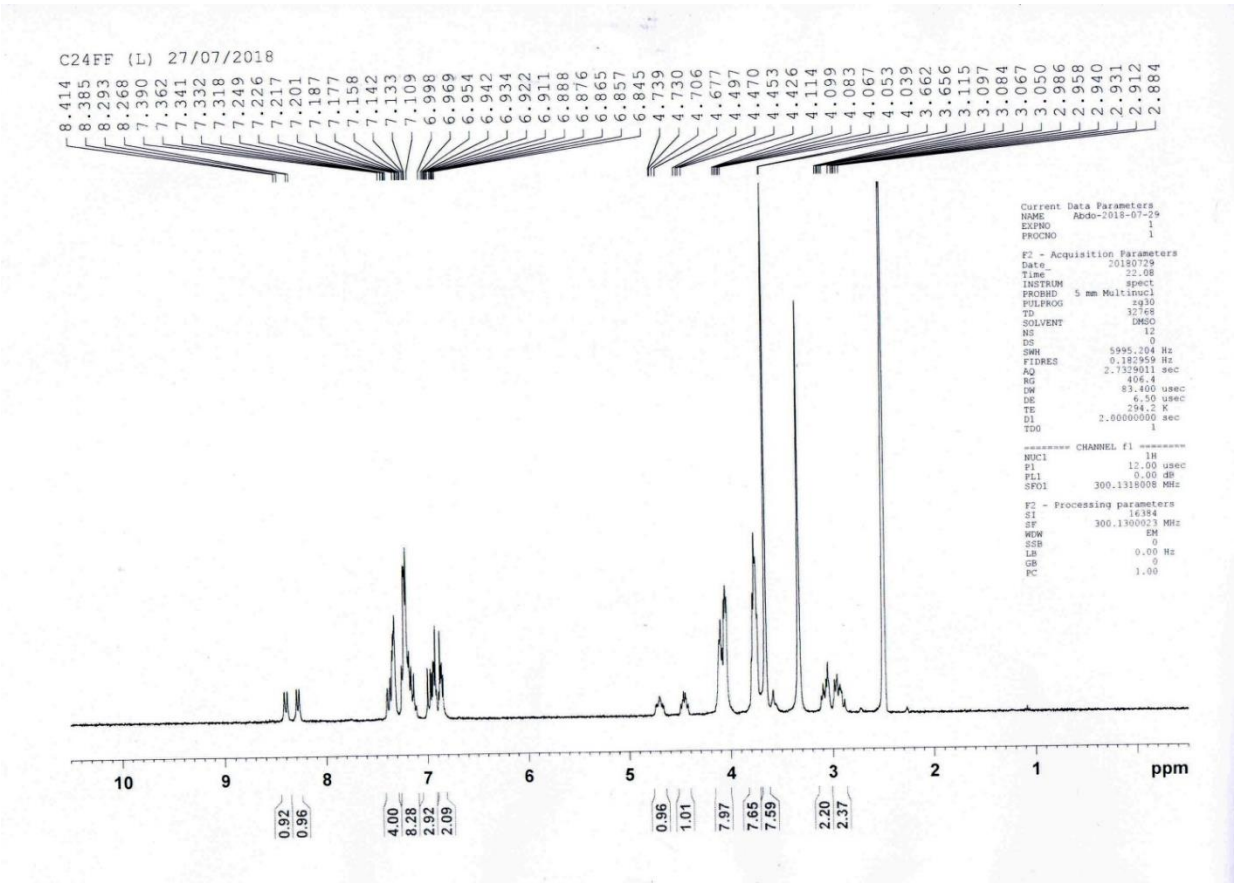


Figure S22. The ^{1}H-NMR spectrum of DB24C8^{L}F^{L}F in DMSO-d_6

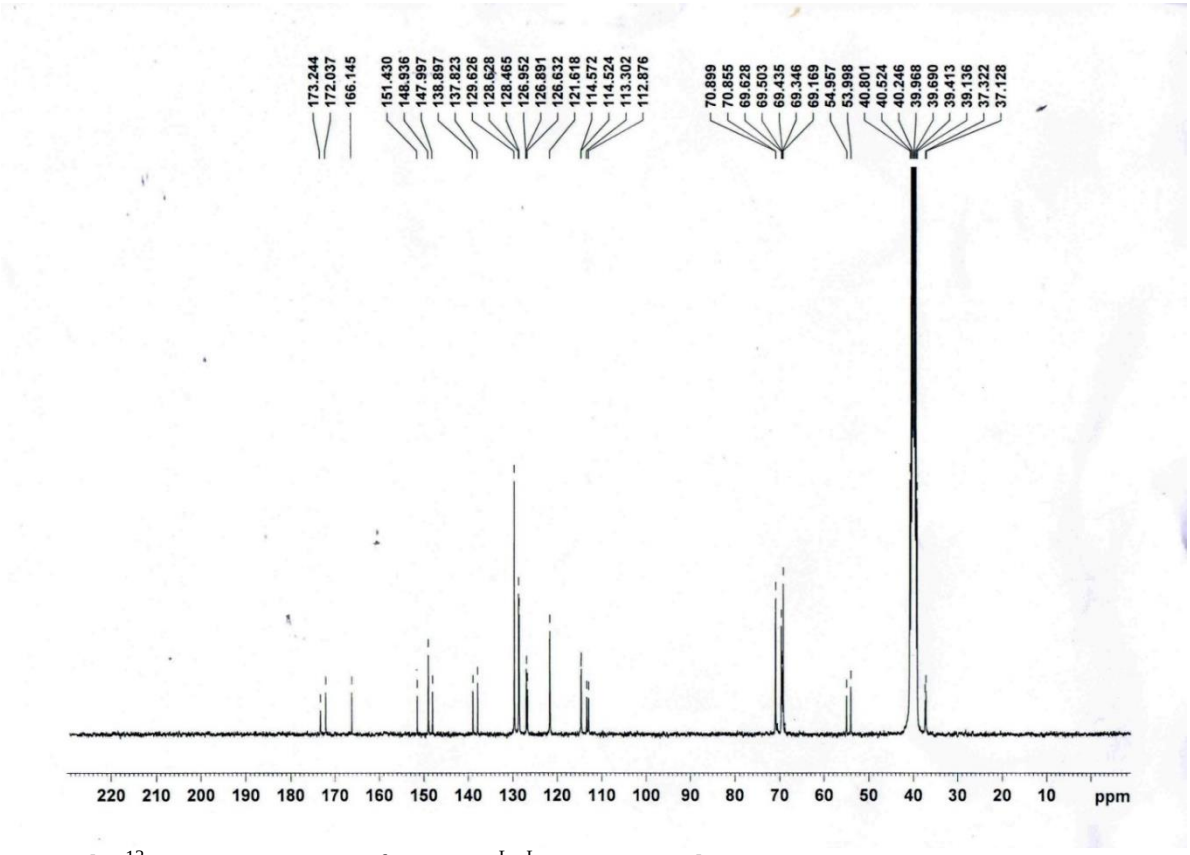


Figure S23. The ^{13}C-NMR spectrum of DB24C8^{L}F^{L}F in DMSO-d_6

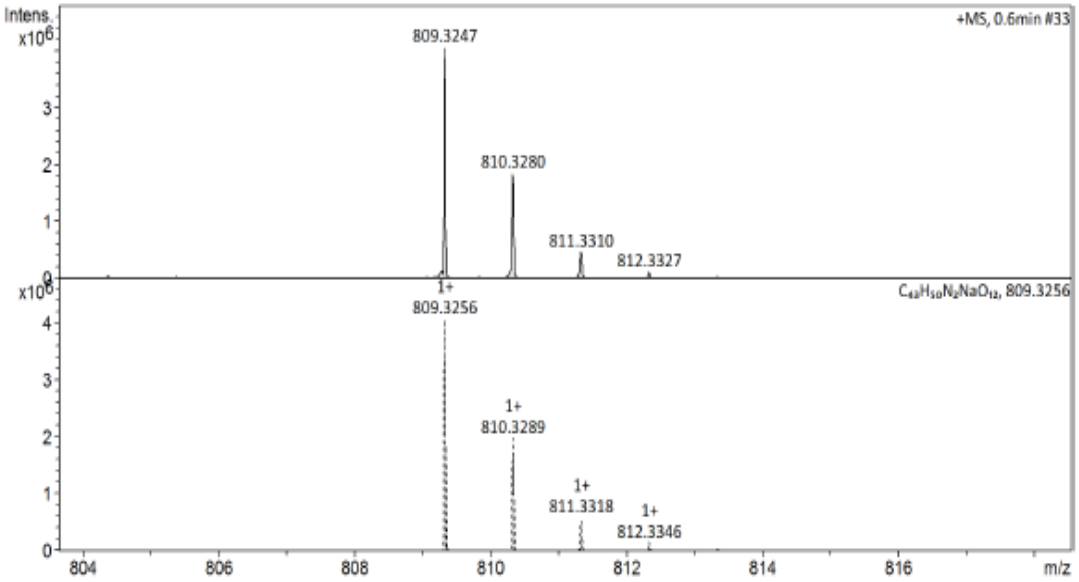


Figure S24. The HRMS of DB24C8^{L}F^{L}F

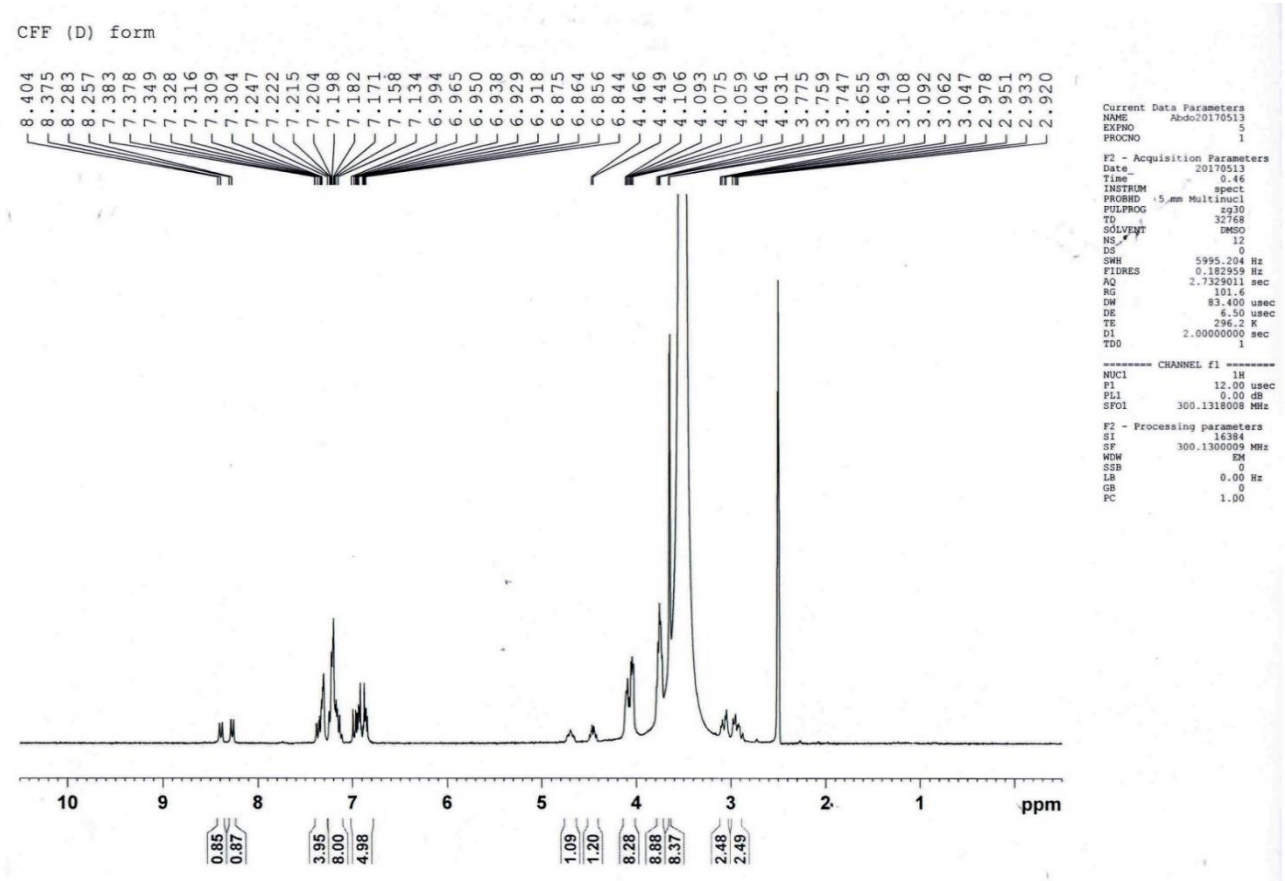


Figure S25. The ^{1}H-NMR spectrum of DB24C8^{D}F^{D}F in DMSO-d_6

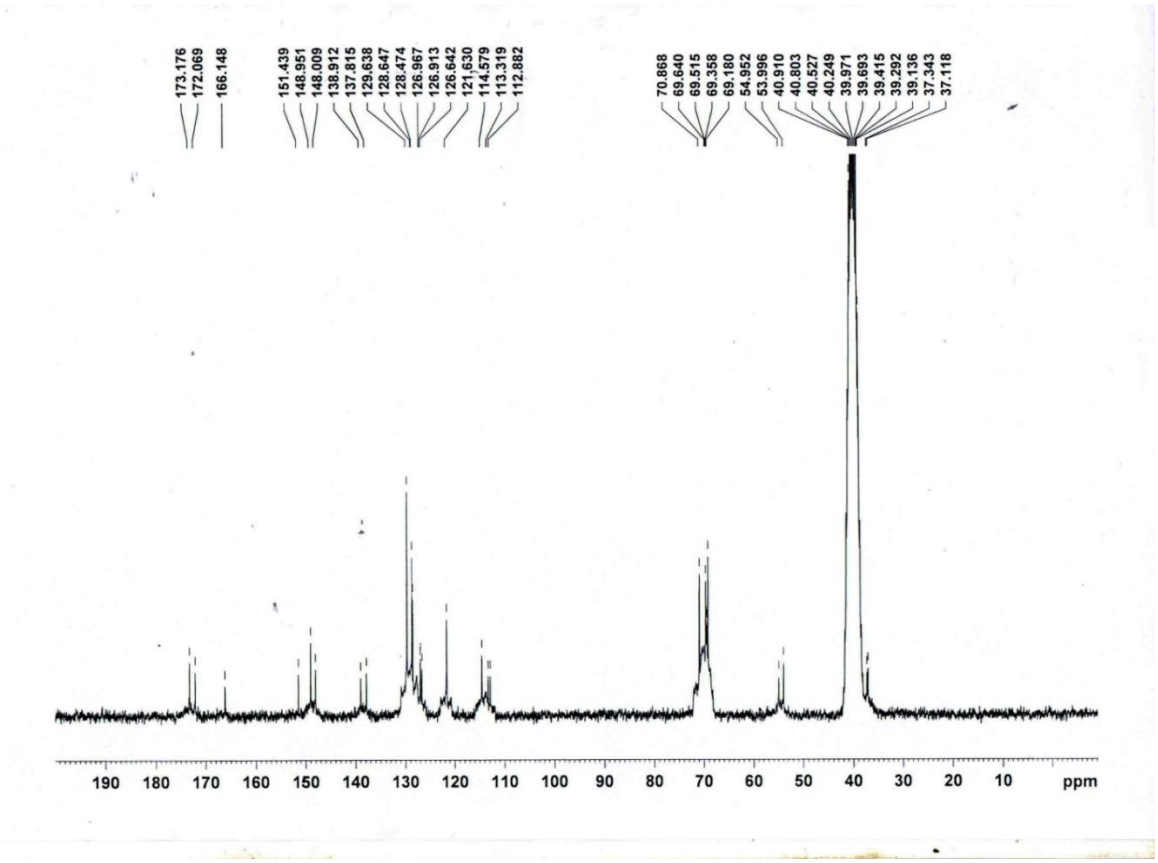


Figure S26. The ^{13}C-NMR spectrum of DB24C8^{D}F^{D}F in DMSO-d_6

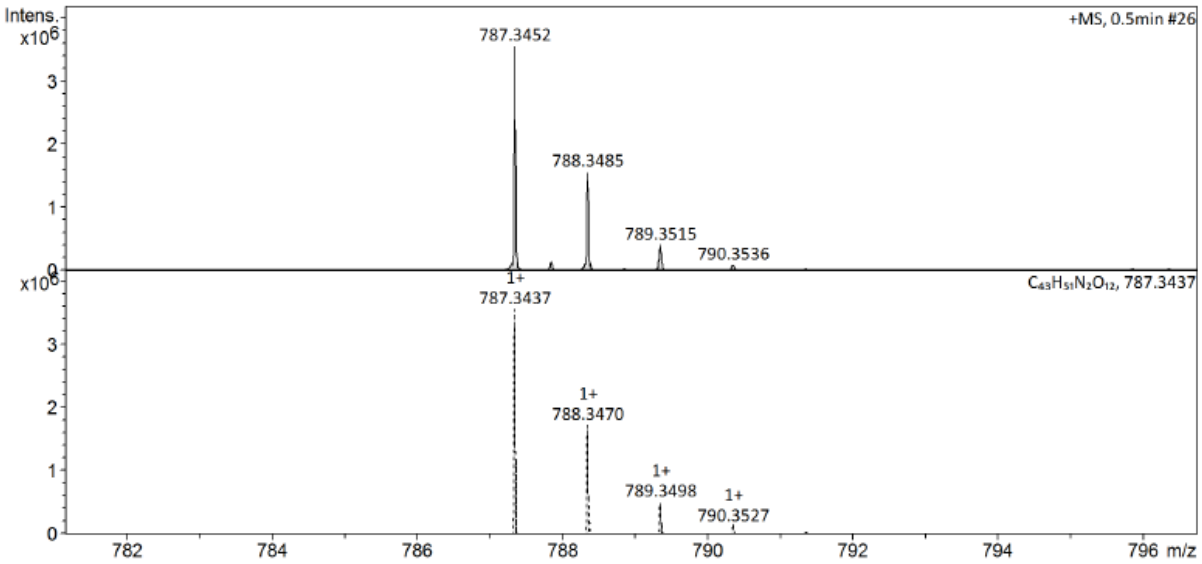


Figure S27. The HRMS of DB24C8^{D}F^{D}F

6. References

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