Electronic Supplementary Material (ESI) for Soft Matter.

Effects of fluoro substitutions and electrostatic interactions on self-assembled structures and hydrogelation of tripeptides: Tuning the mechanical properties of co-assembled hydrogels

Abdelreheem Abdelfatah Saddik, Rajan Deepan Chakravarthy, Mohiuddin Mohammed and Hsin-Chieh Lin*

Department of Materials Science and Engineering, National Chiao Tung University, Hsinchu 300, Taiwan, ROC.

E-mail: hclin45@nctu.edu.tw

Supporting Information

Contents **Page Number**

1. Experimental Methods

Hydrogel preparation: Hydrogels were prepared by weighing 4.0 mg of compound in a screw-capped 2 mL vial (diameter 10 mm). Aqueous NaOH solution was added and the resulting solution was vortexed and sonicated for few minutes to get a clear solution. Then pH and concentration of the solution were carefully adjusted by HCl solution to trigger the hydrogelation.

Transmission Electron Microscopy (TEM): TEM Images were obtained with a Hitachi HT7700 transmission electron microscope at an accelerating voltage of 100 kV. Hydrogels were applied directly onto 200 mesh carbon-coated copper grids. Excess amount of the hydrogel was carefully removed by capillary action (filter paper), and the grids were then immediately stained with uranyl acetate for 30 s. Excess stain was removed by capillary action, and the grids were allowed to air dry.

Scanning electron microscopy (SEM): Hydrogels were applied directly onto silicon wafers and the samples were allowed to air dry. Samples were visualized with a JEOL JSM-6700F scanning electron microscope at an accelerating voltage of 5 kV and a working distance of 6.3 mm.

Rheological tests: Rheological tests were conducted on TA discovery. 20 mm parallel plate was used during the experiment. 100 μL of hydrogel sample (2 wt %) was placed on the parallel plate. Angular frequency sweep test: Test range (0.1 to 100 rads-1 frequency, strain = 0.8 %), 13 points per decade. Sweep mode is "log" and temperature was carried out at 25 ^{o}C and 37 ^{o}C.

Circular dichroism (CD) spectra: CD spectra were recorded in the UV-Vis region (200-350 nm) using a 0.1 mm quartz cuvette for all the hydrogelators at various concentrations.

NMR spectra: NMR spectra were recorded on 300 MHz Bruker NMR instrument at 298 K using partially deuterated solvents as internal standards for ^{1}H-NMR. Coupling constants (*J*) are denoted in Hz and chemical shifts (δ) in ppm. Multiplicities are denoted as follows: s = singlet, d = doublet, t = triplet, m = multiplet.

FTIR: Spectra were measured using a Perkin Elmer spectrum 100 series spectrometer and were collected at a resolution of 4 cm^{-1} using a detector by averaging scans (20 scans).

Powder XRD: PXRD study was carried out to understand the periodic arrangement of molecules during self-assembly process. **PFB-FFK**, **TFB-FFK, MFB-FFK** and **B-FFK** hydrogels and/or (hydrogel to precipitate) were prepared based on the procedure discussed above and the sample was frozen and dried using Lyophilizer (FDM-2, UNISS) to get the xerogel. The xerogel sample was analysed by PXRD. The measurements were performed on Bragg–Brentano-type powder diffractometer (Bruker D8 Advance diffractometer, operated at 40 kV and 40 mA, with Cu Kα radiation, λ = 1.5418 Å). The data were collected in the 2θ range of 5–30° using the step size of 0.016°.

Cell Line:The L929 cells (NCTC clone 929, ATCC CCL-1) were purchased from Food Industry Research and Devlopment Institute Bioresource Collection and Research Center, Taiwan, R.O.C.

Cell Viability: The cell viability assay was performed using a colorimetric assay (MTT) The L929 cells were seeded at density 4×10^{4} cells per well in a 24-well tissue culture plate and cells were then incubated for 24 hrs at 37 ℃ under 5% CO_2 atmosphere. After 24 hrs incubation, the medium was replaced with freshly prepared medium containing samples at different concentrations (10 to 500 μM) for the period of 24, and 48 hrs. The medium was then replaced at each time point with fresh medium containing MTT reagent 0.5 mg/mL final concentration and incubated for additional 4 hrs, then MTT reagent was removed and DMSO was added to dissolve

the formazan crystals, the 100 µL of each 24 well plate was transferred to 96-well plate and the optical density of resulting solution was measured at 595 nm using TECAN Infinite F50 microplate reader. Cells without the treatment of peptide solutions were used as control.

Cell viability (Gel extraction medium):

The hydrogels were prepared under sterile conditions in a 15mL sterile tube and pH was then adjusted to 7.4. the hydrogel was then leached by Eagle's Minimum Essential Medium (EMEM, ATCC, Cat # 30-2003) containing 10% Horse serum supplemented with Penicillin/Streptomycin 100 units/mL (HyClone Cat #SV30010) was placed on top of hydrogel and incubated for 3 days under a humidified atmosphere of 5% CO_2 at 37°C. The L929 cells were seeded on to a 24 well tissue culture plate at a density of 4×10^4 cells in each well and then incubated at 37 ℃ under 5% CO_2 atmosphere for 24 hrs. After 24 hrs of incubation, the medium was removed, and cells were then incubated with gel extraction for 24 hrs, and 48 hrs. The medium was replaced at each time point with fresh medium containing MTT reagent 0.5 mg/mL final concentration and incubated for another 4 hrs, then MTT reagent was removed and DMSO was added to dissolve the formazan crystals, the 100 µL of each 24 well plate was transferred to 96-well plate and the optical density of resulting solution was measured at 595 nm using TECAN Infinite F50 microplate reader. Cells without the treatment of gel extraction were used as control.

2. Supplementary Figures.

Fig. S1. Stability of **TFB-FFK** hydrogel.

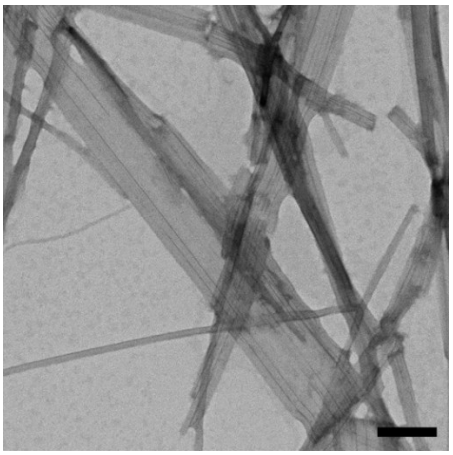

Fig. S2. TEM of **TFB-FFK** hydrogel after 1 h (Scale bar = 250 nm).

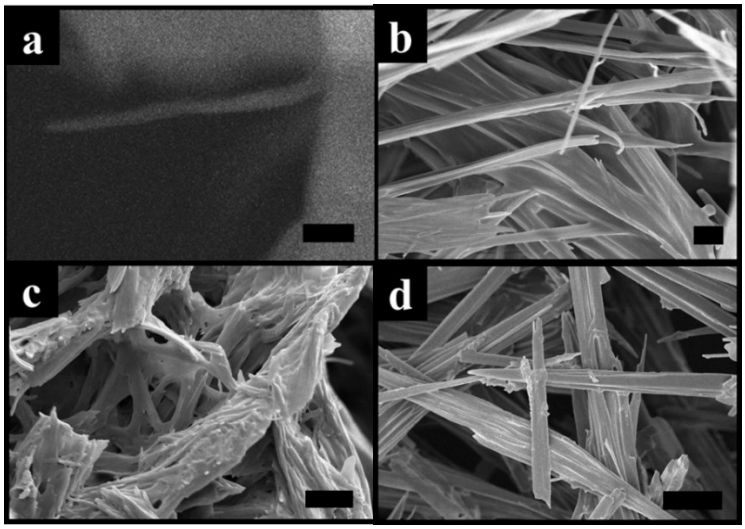

Fig. S3. SEM images for a) **PFB-FFK**. Scale bar: 100 nm; b) **TFB-FFK**. Scale bar: 2 µm; c) **MFB-FFK**. Scale bar: 2 µm, and d) **B-FFK**. Scale bar: 5 µm at pH=7.

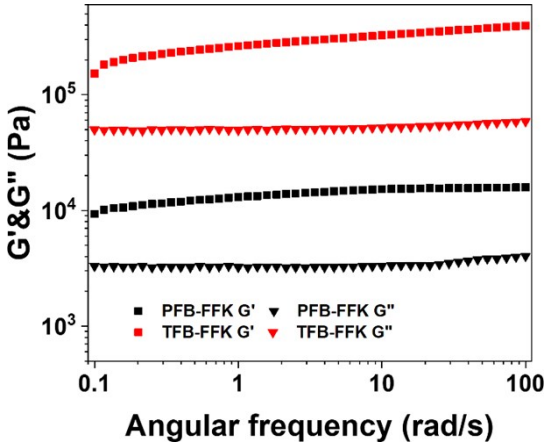

Fig. S4 Rheological measurement of **PFB-FFK** and **TFB-FFK** hydrogels at 37°C (Frequency sweep over a range of frequency of 0.1-100 rad/s at a critical strain of 0.8 %).

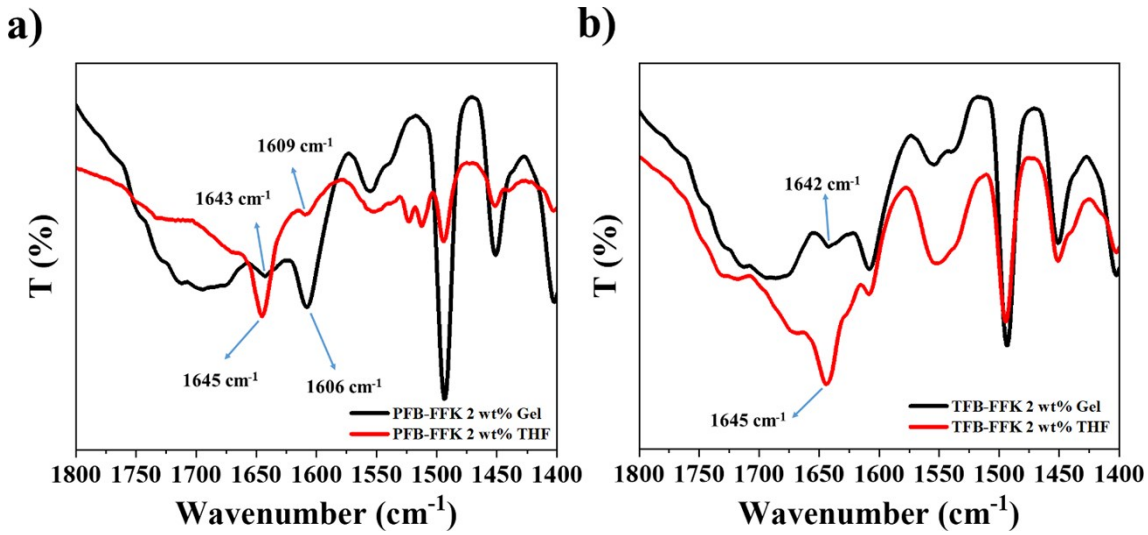

Fig. S5. FT-IR spectra for a) **PFB-FFK** and b) **TFB-FFK.**

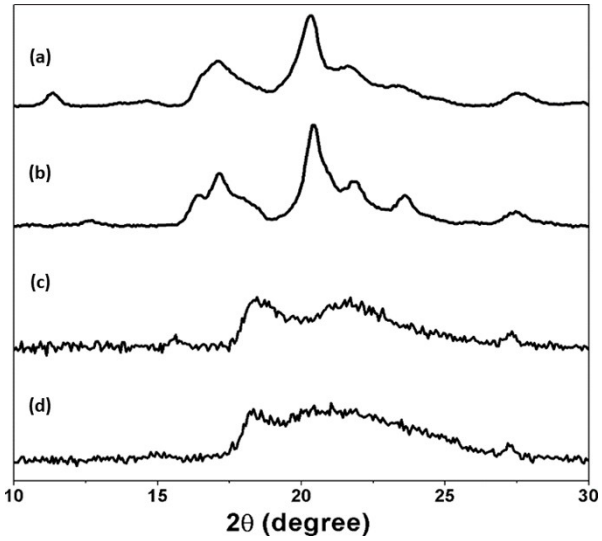

Fig. S6. Powder XRD pattern of a) B-FFK (pH neutralized solid sample) b) MFB-FFK (pH neutralized solid sample), c) TFB-FFK (freeze-dried sample) and d) PFB-FFK (freeze-dried sample).

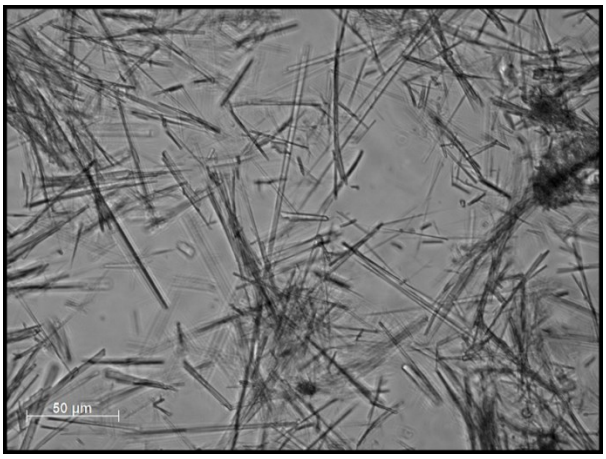

Fig. S7. Optical microscopic image of self-assembled **TFB-FFK** tripeptide (after 3 hr suspension in aqueous condition). The peptide sample was air-dried for 12 hours before being imaged.

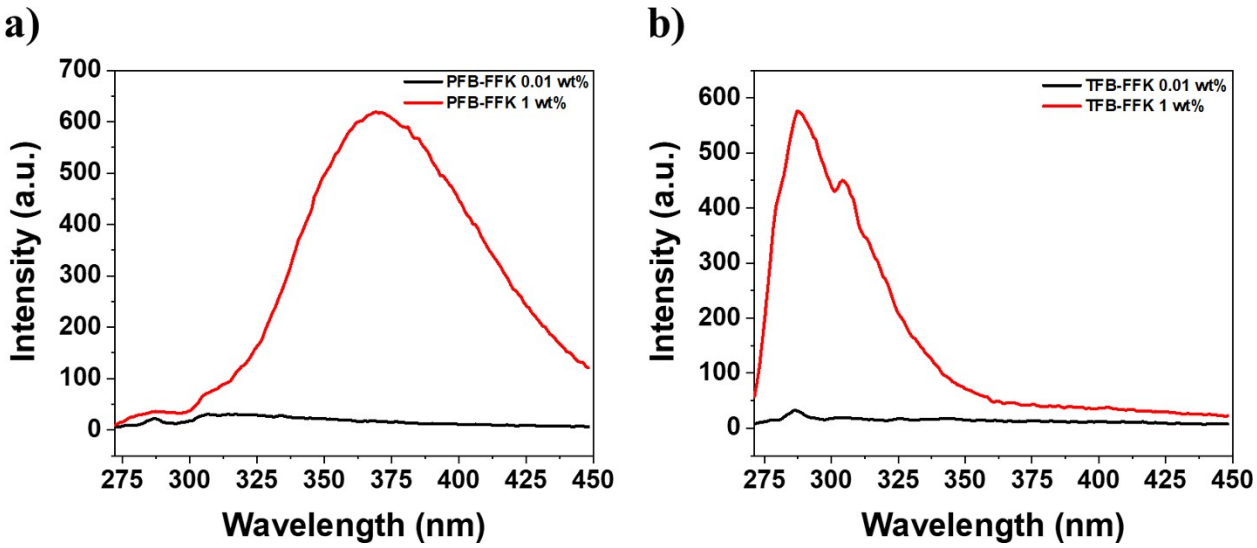

Fig. S8. PL spectra of a) **PFB-FFK** (black line: 1 wt %; red line: 0.01 wt %) and b) **TFB-FFK** (black line: 1 wt %; red line: 0.01 wt %).

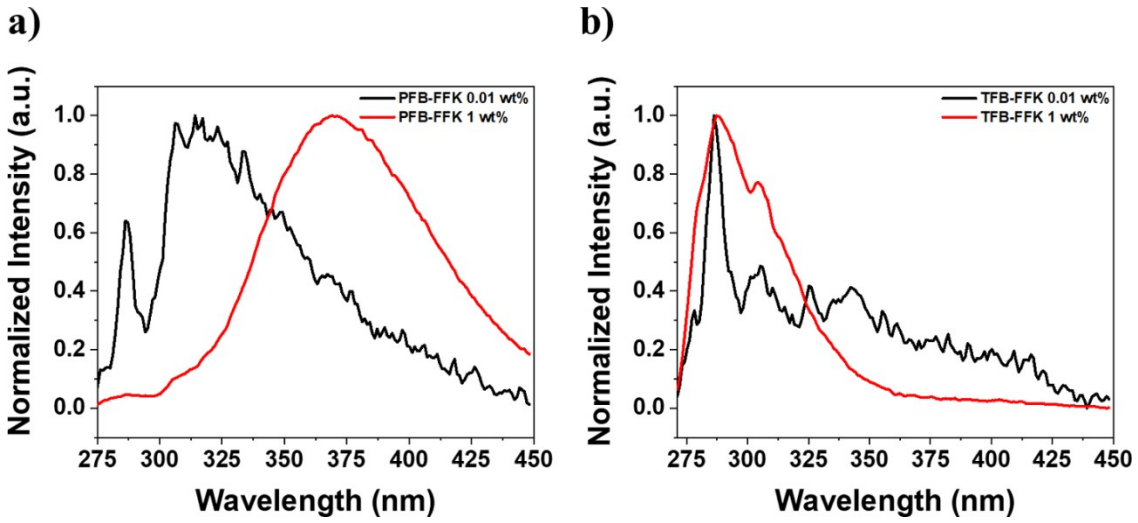

Fig. S9. PL for a) **PFB-FFK** at 1 wt % and 0.01 wt % after normalization, b) **TFB-FFK** at 1 wt % and 0.01 wt % after normalization.

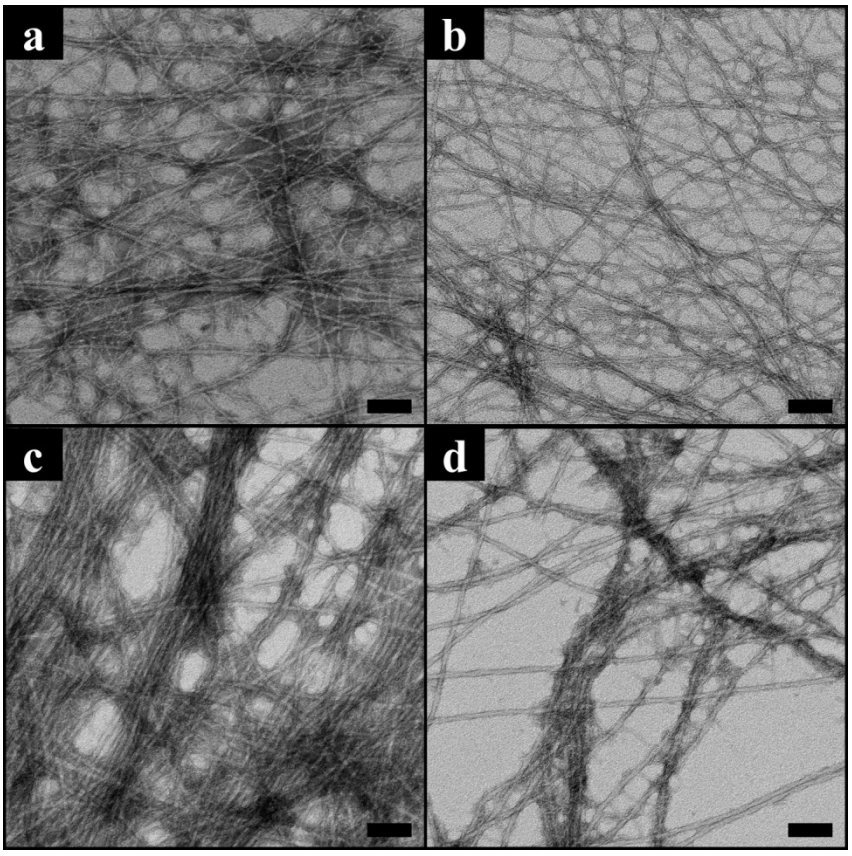

Figure. S10. Negatively stained TEM images for a, b) mixture of **PFB-FFD** + **PFB-FFK** in the ratio of (1.5:0.5) hydrogel, and c, d) mixture of **PFB-FFD** + **PFB-FFK** in the ratio of (0.5:1.5) hydrogel (scale bar: 100 nm) at pH=7; inset: magnified images of the hydrogels.

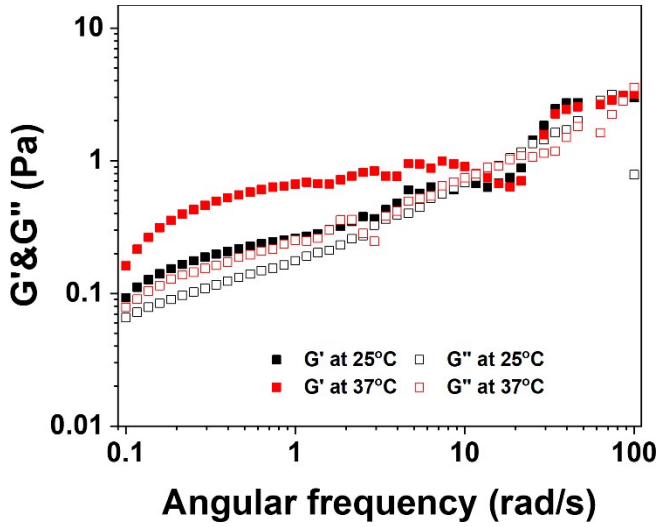

Fig. S11 Rheological measurement of **PFB-FFD** hydrogel at 25°C and 37°C (Frequency sweep over a range of frequency of 0.1-100 rad/s at a critical strain of 0.8 %).

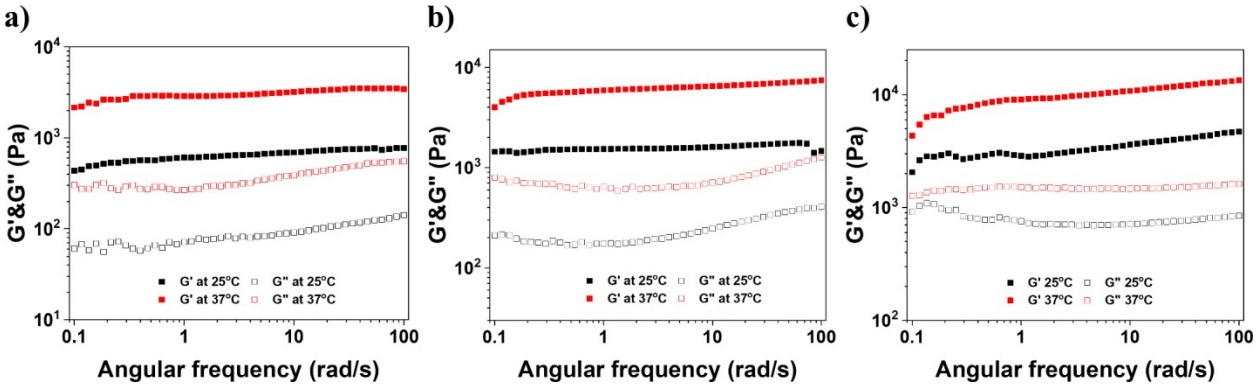

Fig. S12 Rheological measurement of a) **PFB-FFK/PFB-FFD** mix(0.5:1.5), b) **PFB-FFK/PFB-FFD** mix (1:1), and c) **PFB-FFK/PFB-FFD** mix (1.5:0.5) with total 2 wt% hydrogels at 25°C and 37°C (Frequency sweep over a range of frequency of 0.1-100 rad/s at a critical strain of 0.8 %).

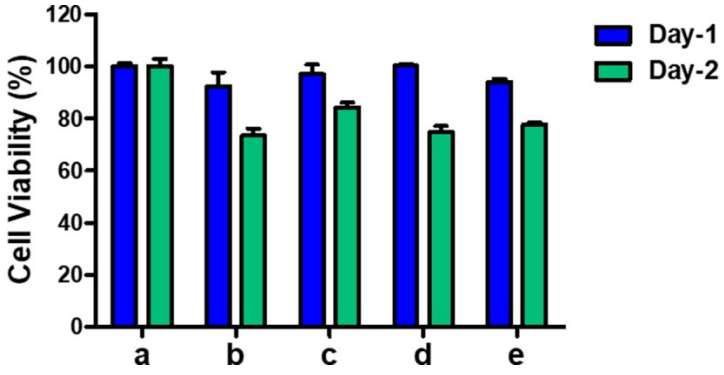

Fig. S13 Cell viability using MTT assay up to 48 hours of gel extraction using L929 cells. a) Control, b) **PFB-FFK** 2wt%, c) **PFB-FFD/PFB-FFK** mix (1:1), d) **PFB-FFD/PFB-FFK** mix (1.5:0.5) and e) **PFB-FFD/PFB-FFK** mix (0.5:1.5).

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

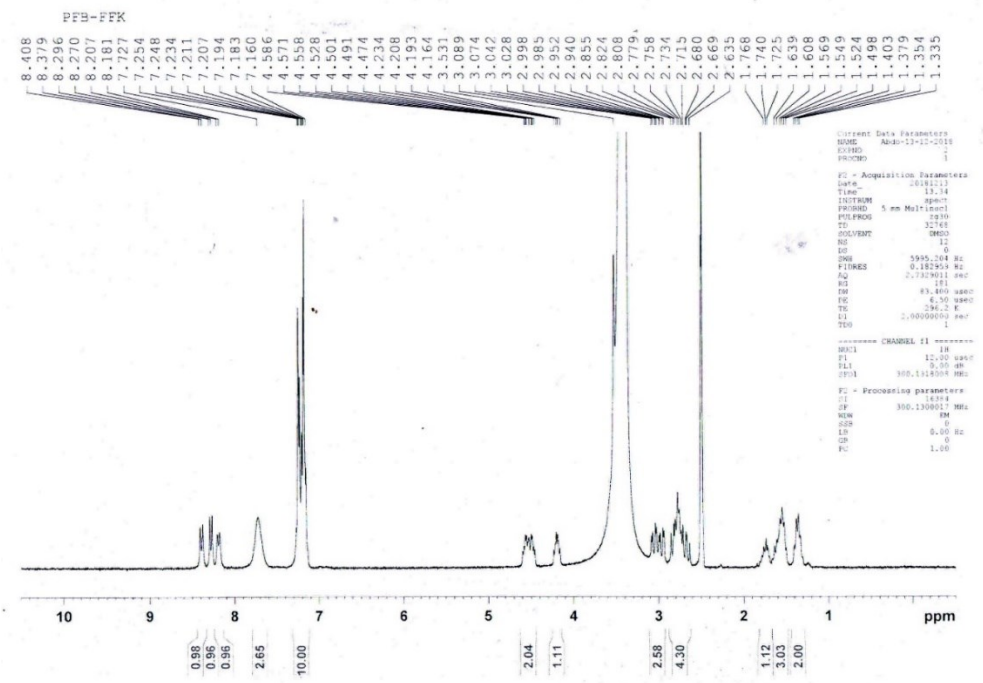

Fig. S14. The ^{1}H NMR spectrum of **PFB-FFK** in DMSO-d_6.

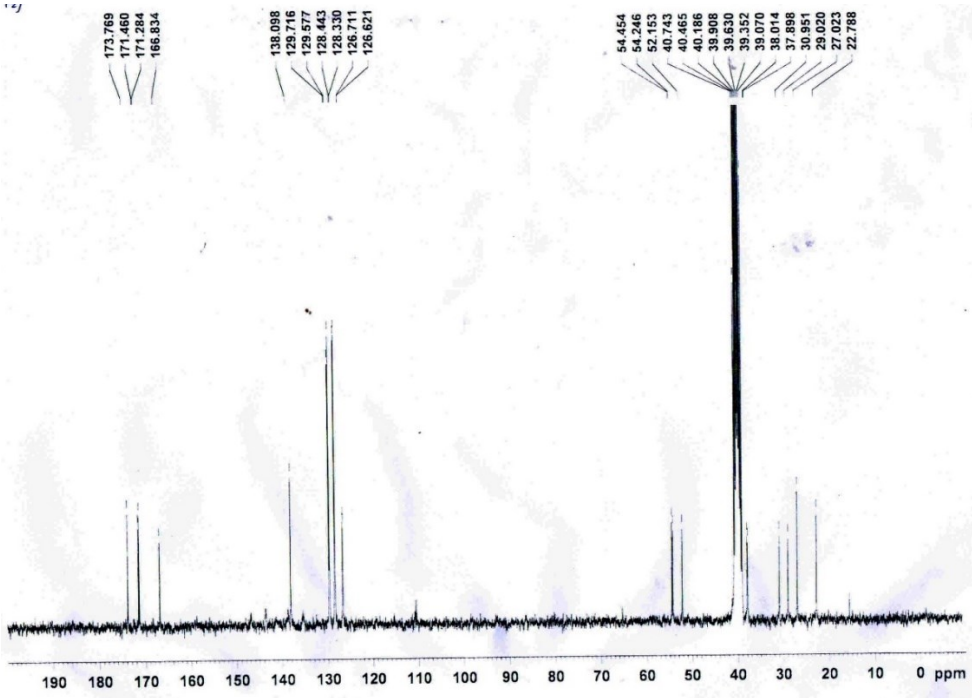

Fig. S15. The ^{13}C NMR spectrum of **PFB-FFK** in DMSO-d_6.

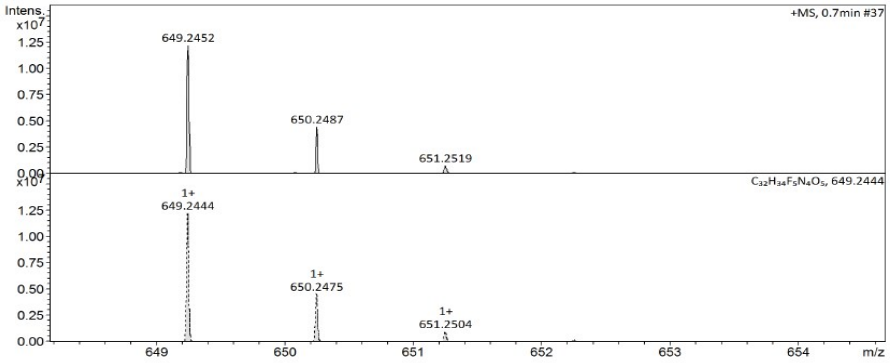

Fig. S16. The HRMS spectrum of **PFB-FFK**.

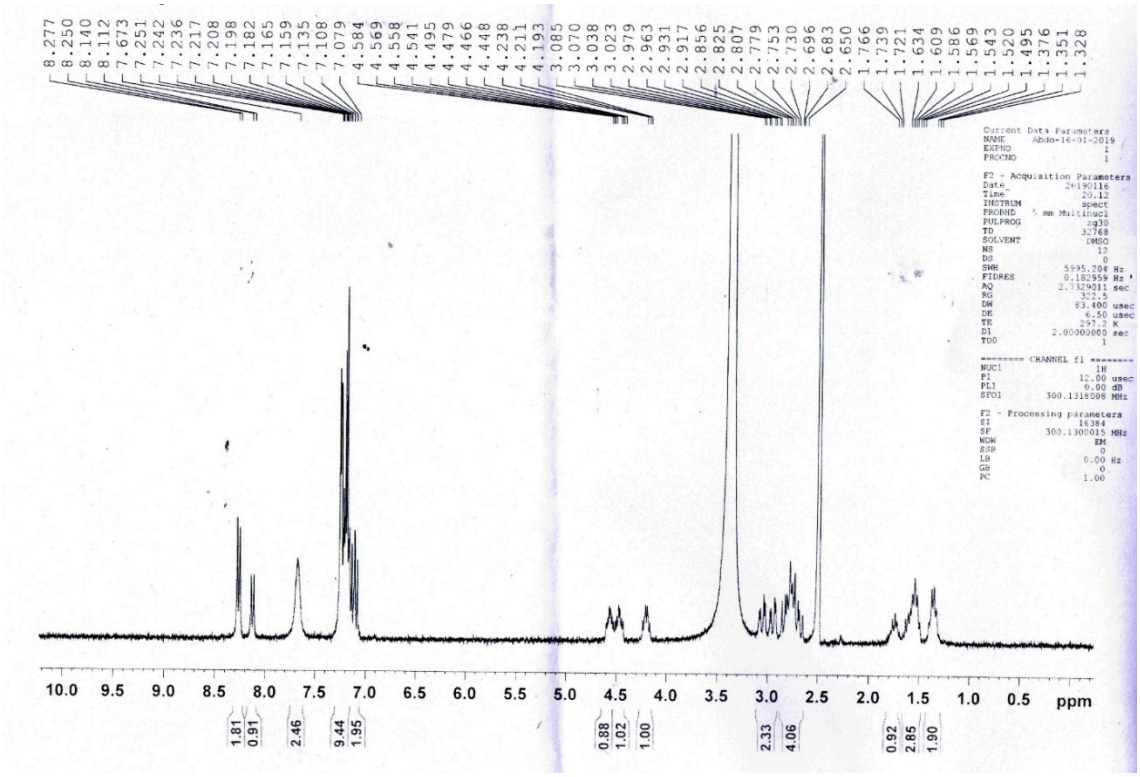

Fig. S17. The ^{1}H NMR spectrum of **TFB-FFK** in DMSO-d_6.

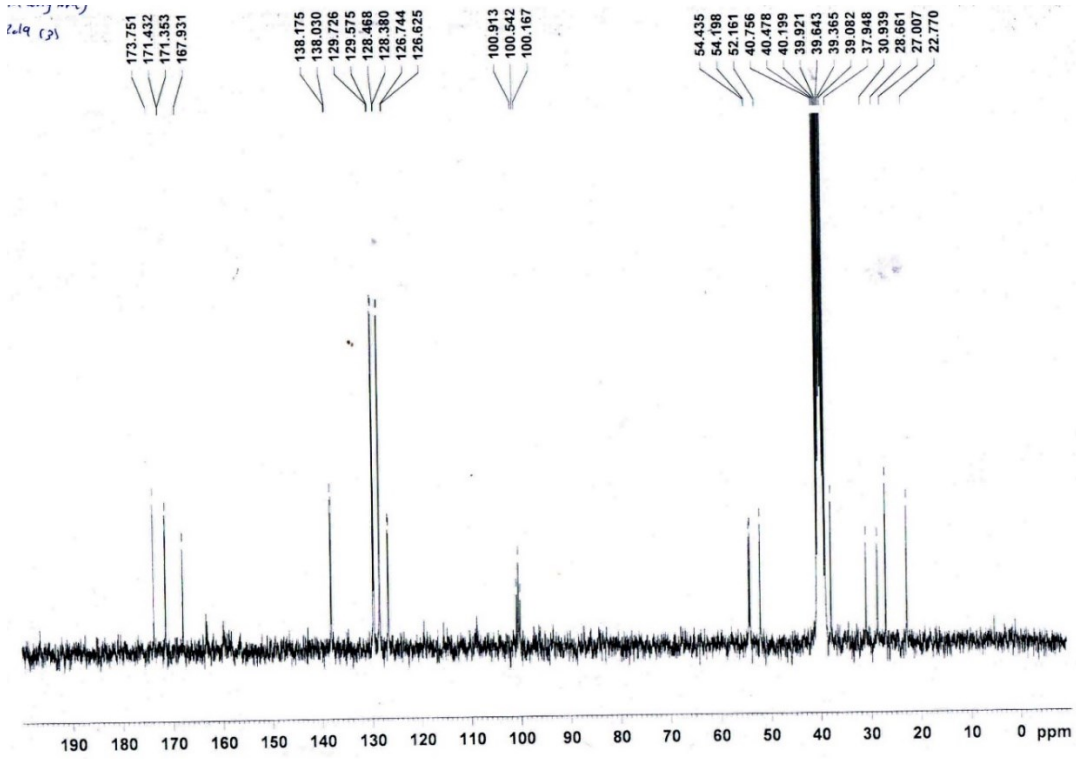

Fig. S18. The ^{13}C NMR spectrum of **TFB-FFK** in DMSO-d_6.

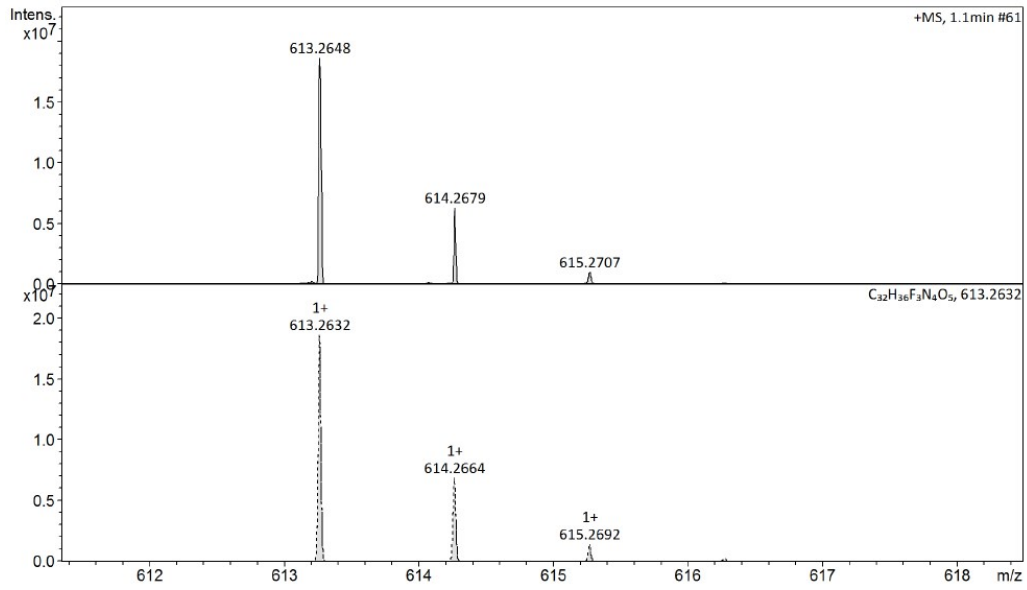

Fig. S19. The HRMS spectrum of **TFB-FFK**.

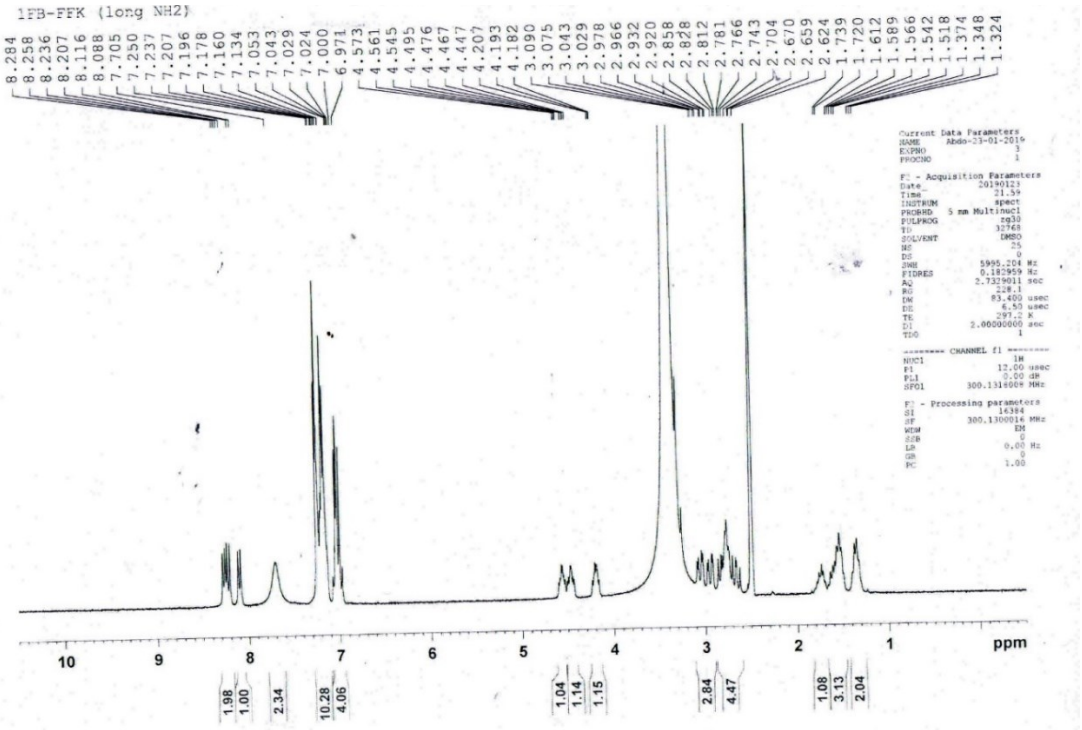

Fig. S20. The ^{1}H NMR spectrum of **MFB-FFK** in DMSO-d_6.

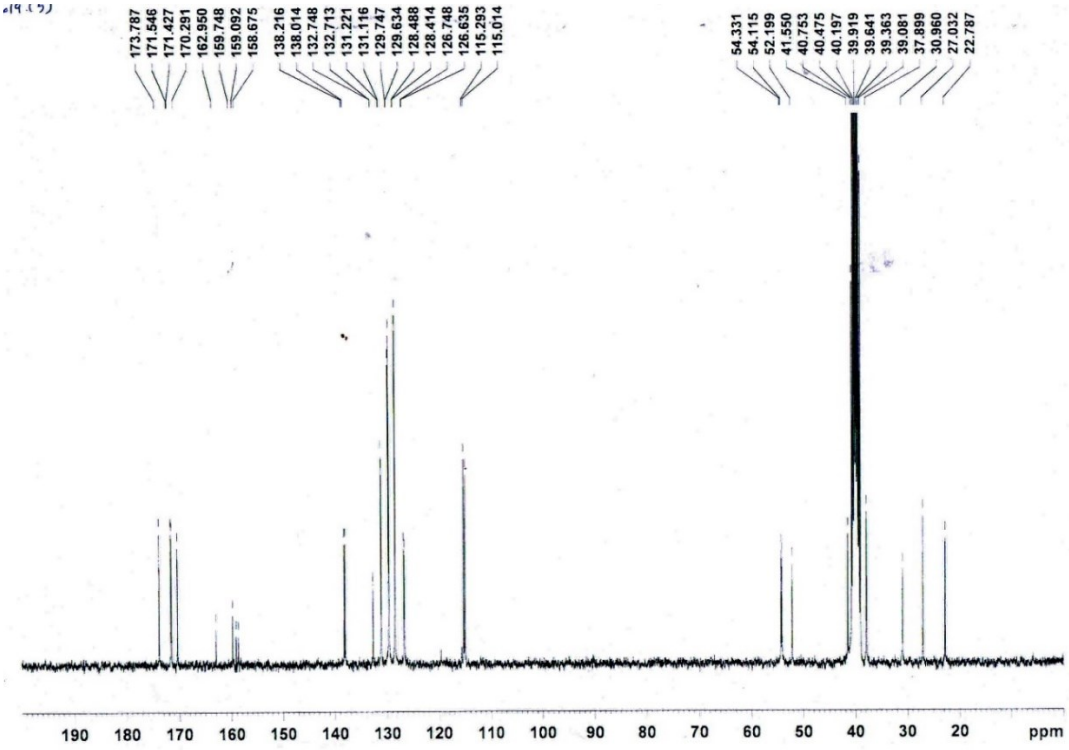

Fig. S21. The ^{13}C NMR spectrum of **MFB-FFK** in DMSO-d_6.

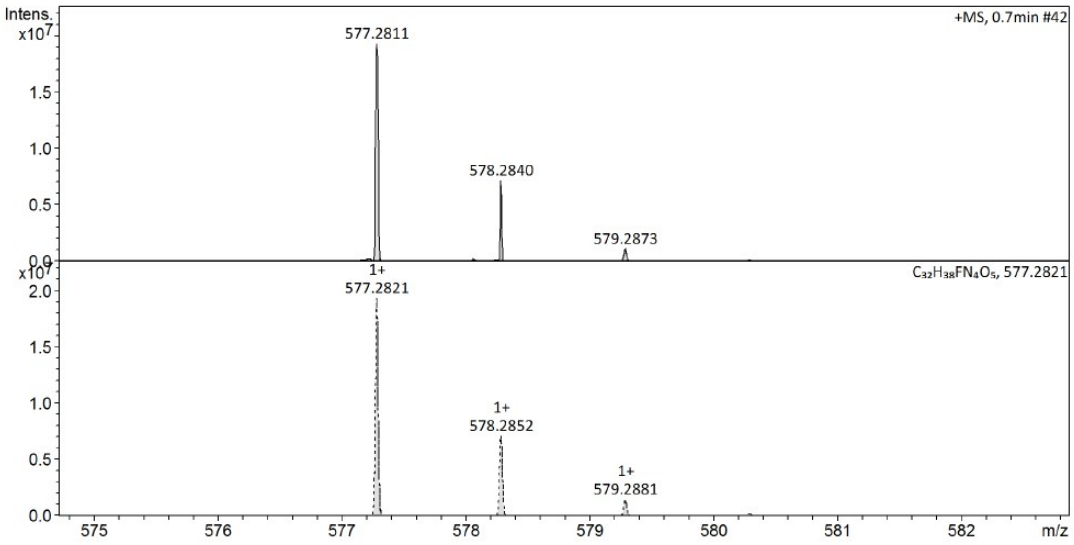

Fig. S22. The HRMS spectrum of **MFB-FFK**.

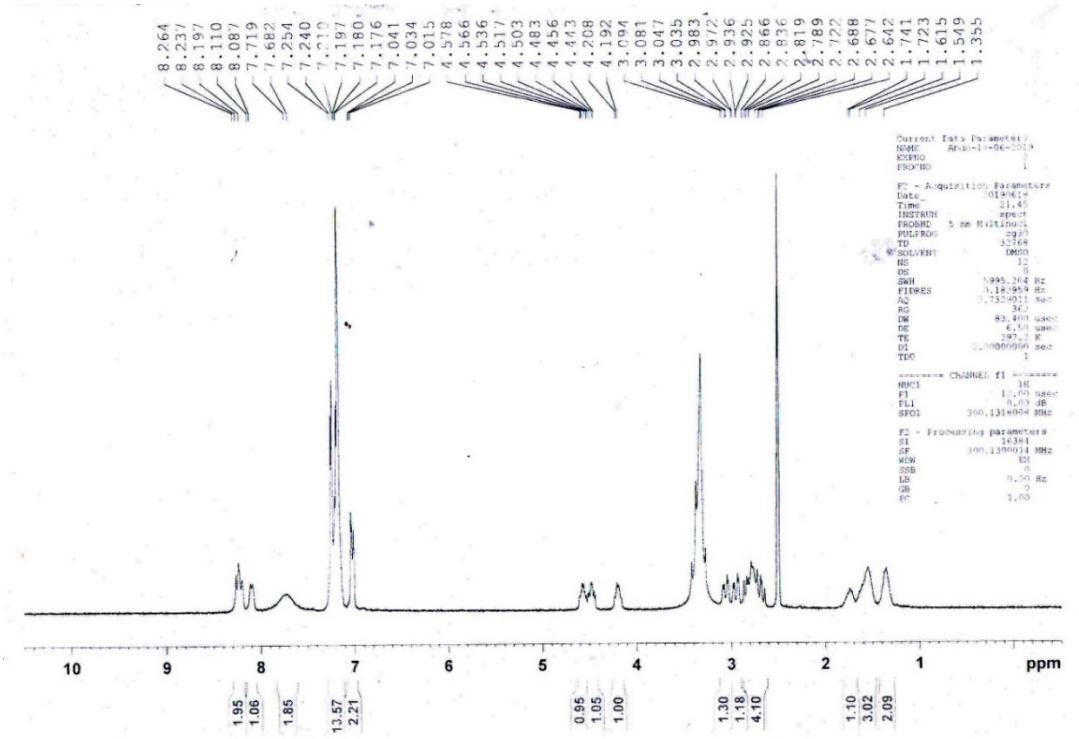

Fig. S23. The ^{1}H NMR spectrum of **B-FFK** in DMSO-d_6.

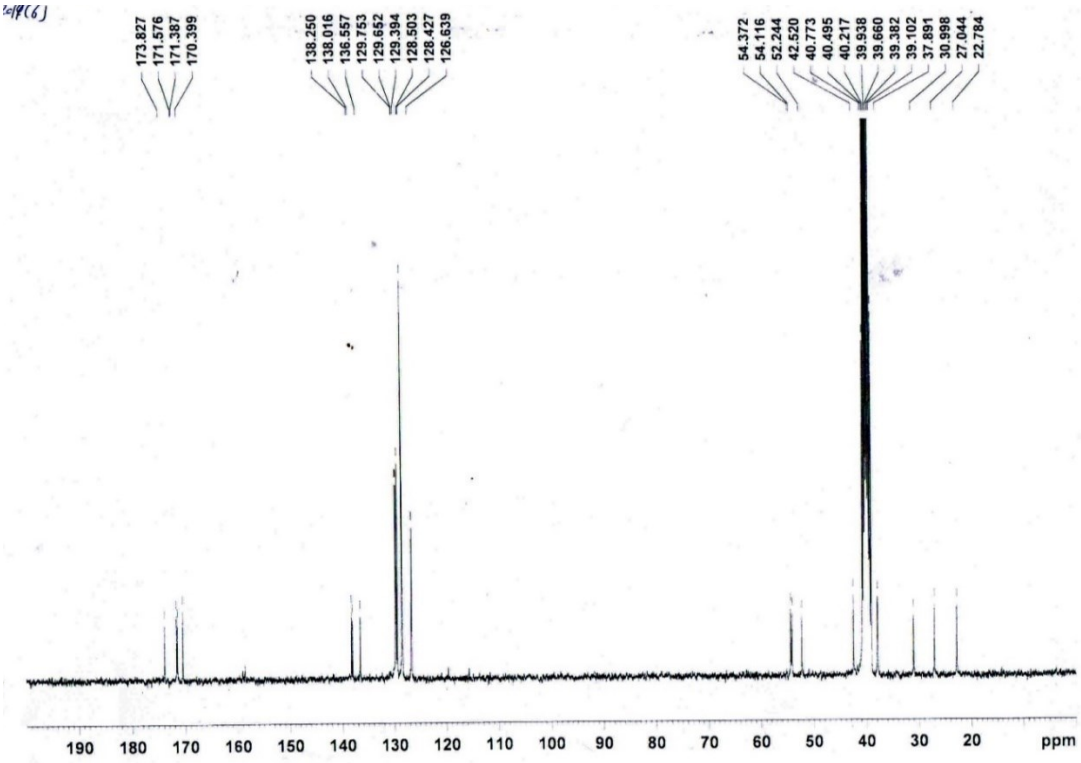

Fig. S24. The ^{13}C NMR spectrum of **B-FFK** in DMSO-d_6.

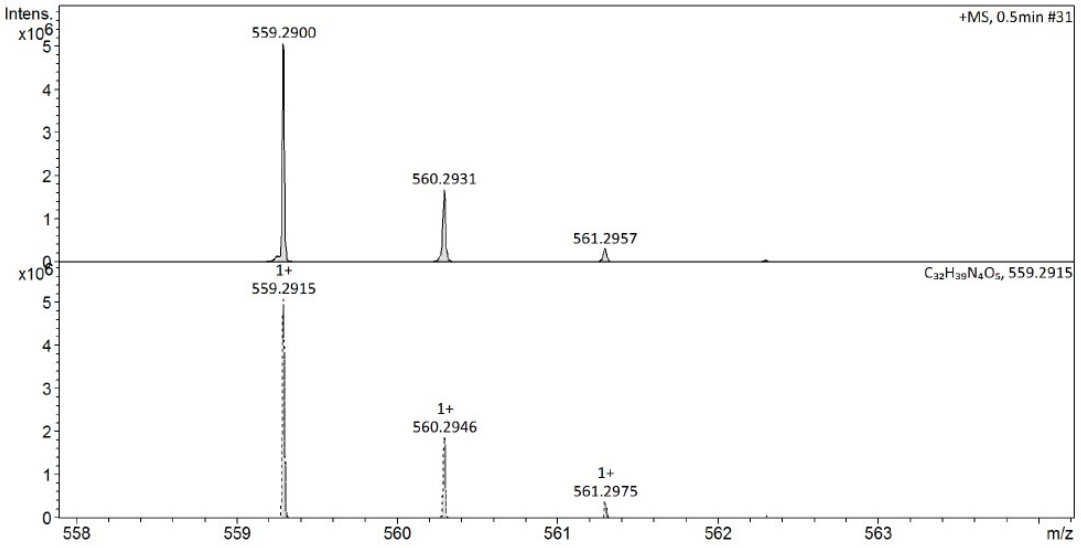

Fig. S25. The HRMS spectrum of **B-FFK**.

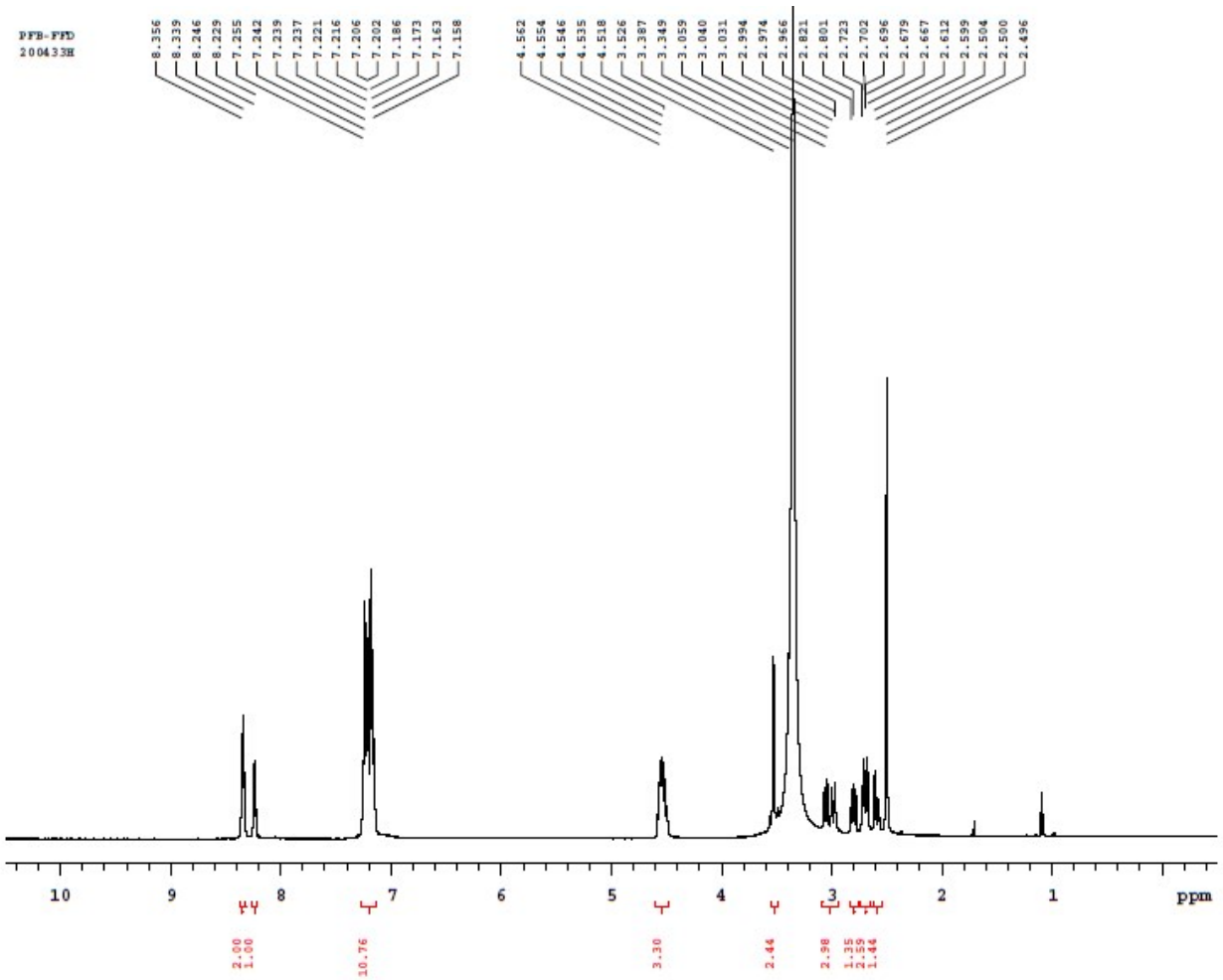

Fig. S26. The ^{1}H NMR spectrum of **PFB-FFD** in DMSO-d_6.

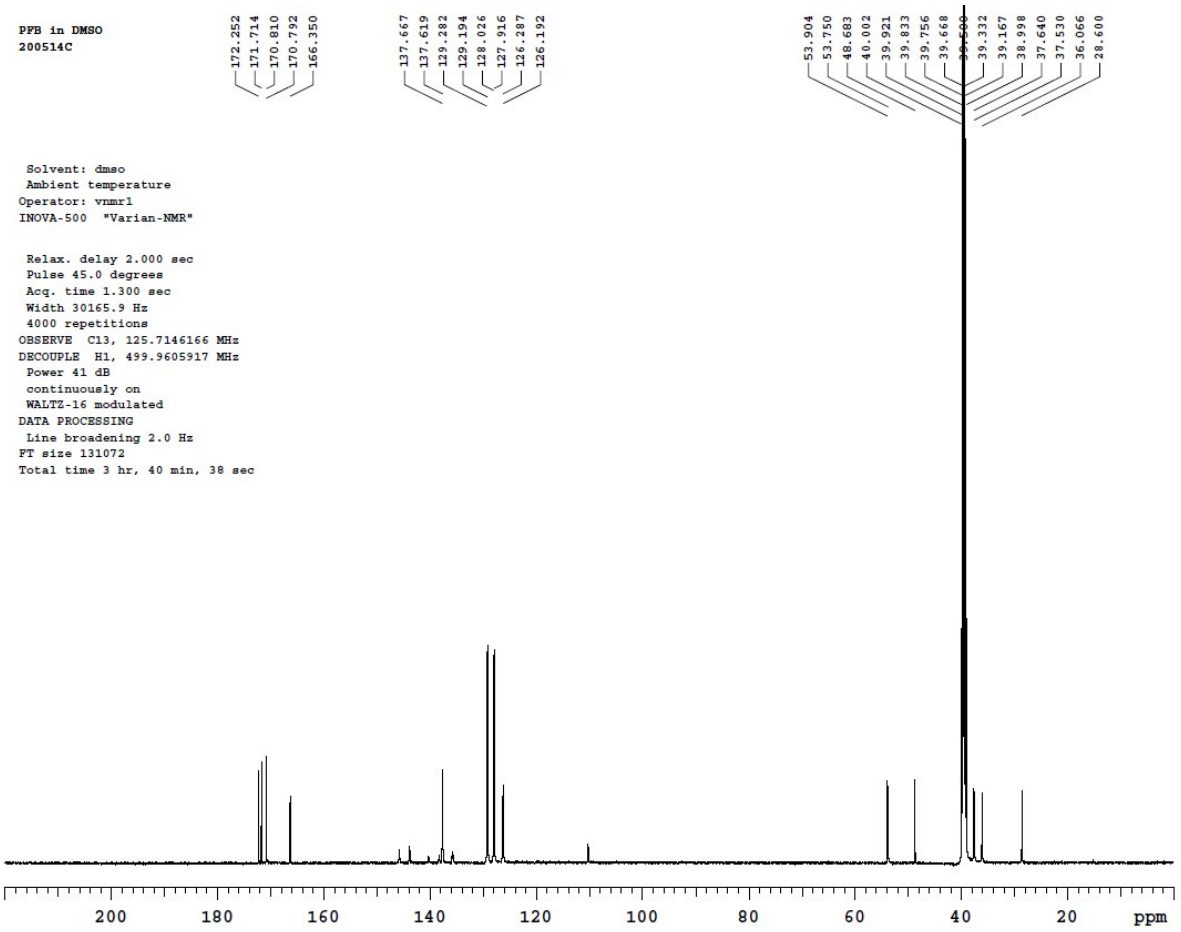

Fig. S27. The ^{13}C NMR spectrum of **PFB-FFD** in DMSO-d_6.

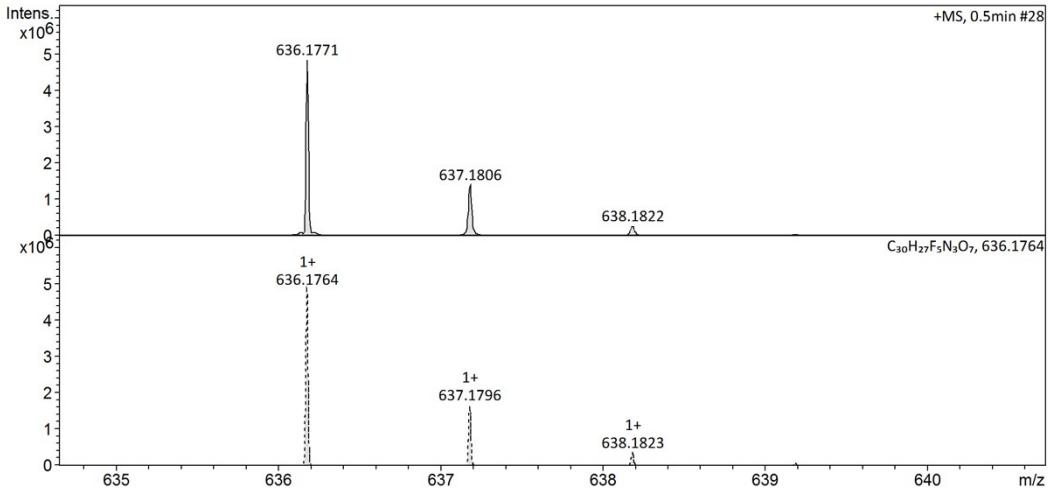

Fig. S28. The HRMS spectrum of **PFB-FFD**.