Supporting Information

Water-Soluble, Membrane-Permeable Organic Fluorescent Nanoparticles with Large Tunability in Emission Wavelengths and Stokes Shifts

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Contents

General method and Materials. Unless otherwise mentioned，all chemicals were commercially available and used as received. ^{1}H NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz) using TMS as internal standard. Mass spectra (EI) were obtained in the positive ion mode on a Waters GCT premier. Dynamic light scattering (DLS) was performed on a Dynapro nanostar dynamic light scattering detector. Transmission electron microscopic (TEM) images were obtained using a JEOL-2100 microscope with an accelerating voltage of 200 kV. Fluorescence spectra were recorded at ambient temperature on a Hitachi 4600 Spectrophotometer. The absorption spectra were recorded on a Hitachi U-3900 spectrophotometer. The general preparation and characterization of the SCMs were previously reported.[1] The SCMs were characterized additionally by DLS and TEM. The crosslinking was confirmed by the cleavage of the 1, 2-diol groups on the SCMs by periodic acid, followed by ESI-MS of the digested products.[1]

Caution: *Small organic azides are potentially explosive* and must be handled with care and avoid high temperatures, particularly in concentrated forms and/or in large quantities.

Synthesis of F2. F2 was synthesized as an orange solid via the reported procedure starting from o-methyl-benzaldehyde and pyrrole.[2] Overall yield was 34%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.93 (s, 2H, pyrrole-H); 7.47 (d, 2H, J = 8.0 Hz, ArH); 7.33 (d, 2H, J = 7.6 Hz, ArH); 6.96 (d, 2H, J = 4.4 Hz, pyrrole-H); 6.54(d, 2H, J = 4.0 Hz, pyrrole-H); 2.48 (s, 3H, CH3); MS (EI): m/z calcd for $C_{16}H_{13}BF_2N_2$ 282.11; found 282.11.

Synthesis of F3. F3 was synthesized as a blue solid via the reported procedure starting from 2, 4-dimethylpyrrole and 4-hydroxybenzaldehyde.[3] Overall yield was 11%. ^{1}H NMR ($CDCl_3$, 400 MHz): δ 7.61 (d, J = 16.0 Hz, 2 H, CH=CH), 7.58 (d, J = 8.4 Hz, 4 H, ArH), 7.21 (d, J = 16.0 Hz, 2 H, CH=CH), 7.18 (d, J = 8.8 Hz, 2 H, ArH), 6.96 (d, J = 8.4 Hz, 2 H, ArH), 6.93 (d, J = 8.8 Hz, 4 H, ArH), 6.61 (s, 2 H, pyrrole-H), 4.97 (s, 1 H, OH), 3.86 (s, 6 H, OCH3), 1.51 (s, 6 H, CH3); MS (TOF): m/z calcd for $C_{35}H_{31}BF_2N_2O_3$ 576.24, found 576.06.

Preparation of the SCM F1-F4. A 10 mM aqueous solution of surfactant **1** was prepared using Millipore water. An aliquot of the dye (5–10 μL) in DMSO (0.12 mol/L) was injected into a stirred aqueous solution of the surfactant (3.0 mL). Cross-linker **2** (5.3 mg), $CuCl_2$ (15 μL, 6.7 mg/mL), and sodium ascorbate (15 μL, 99 mg/mL) were added. The reaction mixture was allowed to stir slowly at room temperature for 24 h. The mixture was dialyzed against deionized water (1 L) for three times using a 2000 Da molecular weight cut-off tubing.

Fluorescence quantum yields of SCM F1-F3. Quantum yields of SCM **F1-F3** were determined using quinine sulfate, rhodamine 6G, and rhodamine 101 as standards according to a published method,[4] and were measured at least 3 times. The quantum yields were calculated according to the following equation:

$$\Phi=\Phi_S\times I/I_S\times OD_S/OD\times\eta^2/\eta_S^2 \qquad (1)$$

in which Φ is the quantum yield, I is the integrated intensity, η is the refractive index (η_{H2O} = 1.333 was used here), OD is the optical density. The subscript S refers to the standard.

Energy transfer calculations. In order to estimate the Förster radius of donor-accepter, the spectral overlap integral was then calculated using the following equation: [5,6]

$$J=\int_0^\infty f_D(\lambda)\varepsilon_A(\lambda)\lambda^4 d\lambda \qquad (2)$$

Where λ is the wavelength of light (nm), $\varepsilon_A(\lambda)$ is the molar absorptivity of the acceptor at that wavelength (M^{-1} cm^{-1}), and f_D (λ) is the donor fluorescence spectrum normalized on the wavelength scale according to

$$1=\int_0^\infty f_D(\lambda)d\lambda \qquad (3)$$

The overlap integral was estimated to be 4.97×10^{14} $M^{-1}cm^{-1}nm^4$ for transfer between **F1** and **F2**. The Förster radius can be calculated using the following equation[5]

$$R_0(\text{Å})=0.211\times(J\phi\kappa^2\eta^{-4})^{1/6} \qquad (4)$$

in which k^2 as the orientation value was assumed to be 2/3, the refractive index of water (η**)** was 1.33, and fluorescence quantum yield of donor (Φ) was 0.30. The

Förster radius was thus found to be 3.8 nm for the energy transfer between the donor and the acceptor.

The energy transfer efficiency (E.T.E) was calculated from[7]:

$$E = 1-\Phi_2/\Phi_1 \qquad (5)$$

Where Φ_1 and Φ_2 are the quantum yield of **F1** in SCM, and the fluorescence quantum yield of **F1** after FRET to **F2** in SCM, respectively. Φ_1= 0.30 and Φ_2= 0.062 with quinine sulfate as standard. Thus, the FRET efficiency was estimated to be 79%. Each SCM is estimated to encapsulate ca. one **F1** and one **F2** in this case. Thus, the distance between donor and acceptor was estimated to be 3.0 nm according to the following equation[7]:

$$E = 1/[1+ (R/ R_0)^6] \qquad (6)$$

Cell culture and cell imaging. HeLa cells were cultured in culture medium (DMEM / F12 supplemented with 10% FBS, 50 unit/mL penicillin, and 50 μg/mL of streptomycin) at 37 ºC under a humidified atmosphere containing 5% CO_2. After 24 h, the cells were incubated with the SCM-**F3** (1 μM) for 30 min at 37 ºC. After the medium was removed and the cells were carefully washed with HBSS buffer for two times, fluorescence imaging of living HeLa cells was observed under Nikon AlR-Si laser scanning microscope with a 60 × oil-immersion objective lens. The differential interference contrast (DIC) and the fluorescence images were captured, digitized, and processed to generate the pseudo-color images using NIS element C software.

Cytotoxicity of the assembly. MTT assays were performed to assess the metabolic activity of Hela cells. Hela cells were seeded in 96-well plates at an intensity of 4 × 10^4 cells mL^{-1}. After 24 h incubation, the medium was replaced by the SCM-**F3** in culture media at concentrations of 1, 2, 4, 10 μM, and the cells were then incubated for 1 h. After that, the wells were washed once with culture medium and incubated for another 24 h. Then 20 μL of freshly prepared MTT (5 mg mL^{-1}) solution in Millipore water was added to each well. The MTT wells were removed after 4h incubation in the incubator. DMSO (100 μL) was then added into each well, and the plate was gently shaken for 5 min at room temperature to dissolve all precipitates formed. The absorbance of MTT at 492 nm was monitored by the microplate reader (Thermo Scientific Multiskan MK3). Cell viability was expressed by the ratio of absorbance of the cells incubated with SCM-**F3** to that of the cells incubated with culture medium only.

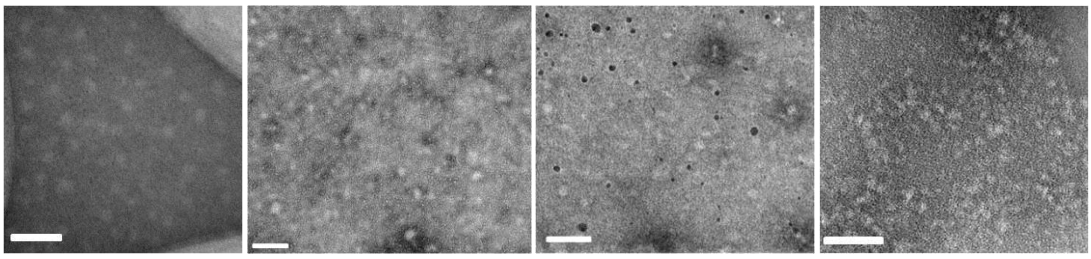
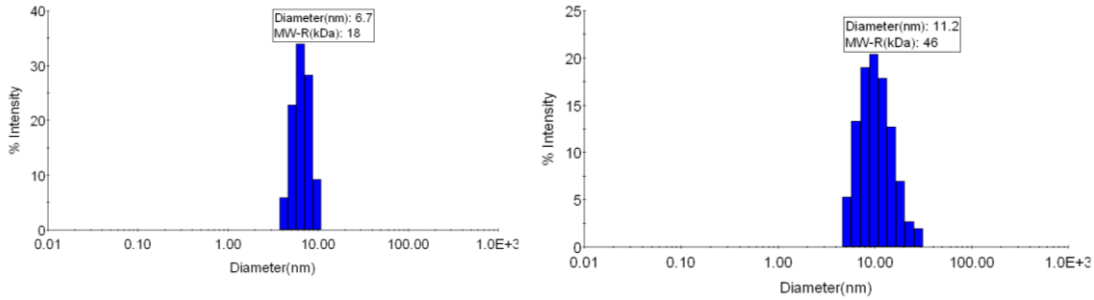
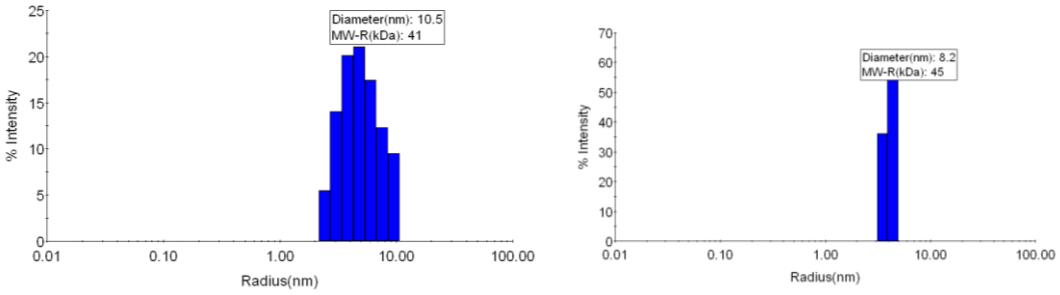


Figure 1s. TEM micrographs of SCMs, SCM-**F1**, SCM-**F2**, and SCM-**F3** from left to right. Scale bar = 50 nm.

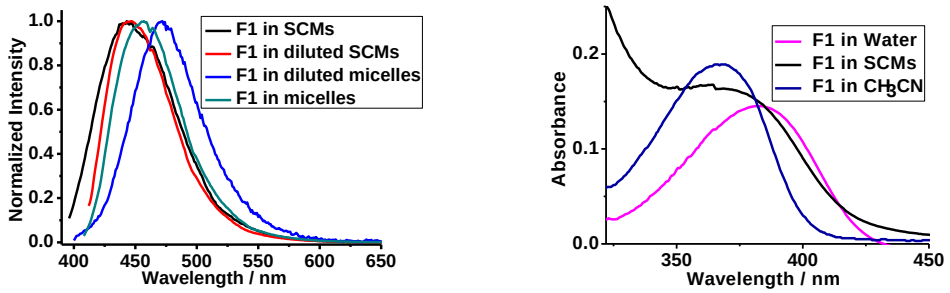


(a) (b)



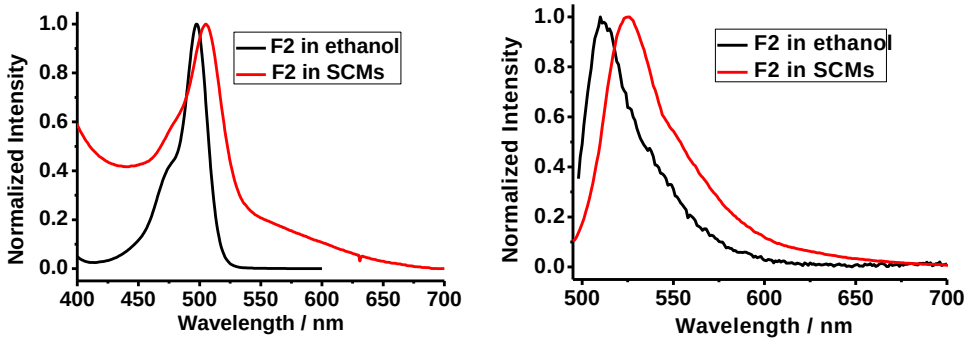
(c) (d)

Figure 2s. Distribution of the hydrodynamic diameters of SCMs (a) , SCM-**F1**(b), SCM-**F2** (c), and SCM-**F3** (d) determined by DLS. A small amount (<2% by weight) of larger particles could also be detected by DLS but was not shown.



(a) (b)

Figure 3s. The emission spectra (λ_{ex} = 367 nm) (a) and absorption (b) of **F1** in different medium. ($C_{\mathbf{F1}}$ = 15 μM except for the diluted SCMs and micelles, in which the solution was diluted to 0.025 mM for the surfactant.)



(a) (b)

Figure 4s. Normalized absorption spectra (a) and emission spectra (λ_{ex} = 490 nm) (b) of **F2**. ($C_{\mathbf{F2}}$ = 10 μM)

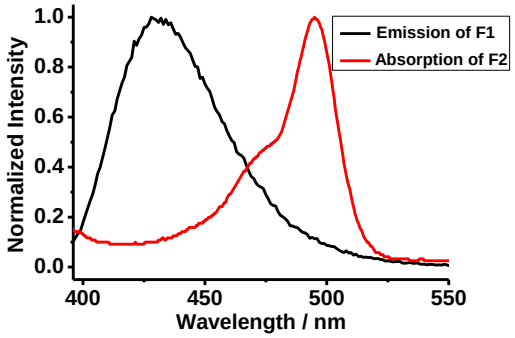


Figure 5s. The overlap between the emission of **F1** and absorption of **F2** in CH_3CN. ($C_{\mathbf{F1}}$ = $C_{\mathbf{F2}}$ = 5 μM)

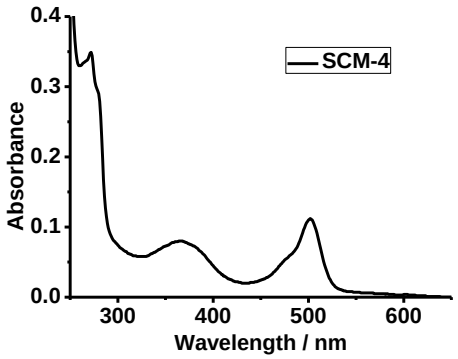


Figure 6s. The absorption of SCM-**F4**. (C_{SCM}= 5 μM, $C_{\mathbf{F2}}$= 3.9 μM, $C_{\mathbf{F3}}$= 3.6 μM)

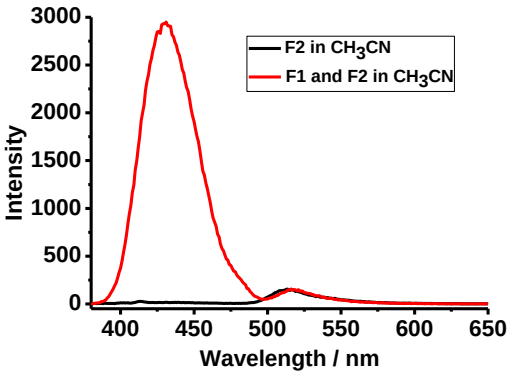


Figure 7s. Fluorescence of **F2** in acetonitrile (black line) and fluorescence of **F1** and **F2** in acetonitrile (red line). (λ_{ex} = 367 nm, $C_{\mathbf{F1}}$ = 5 μM, $C_{\mathbf{F2}}$ = 5 μM, with otherwise identical condition of the FRET system in SCM-**F4**).

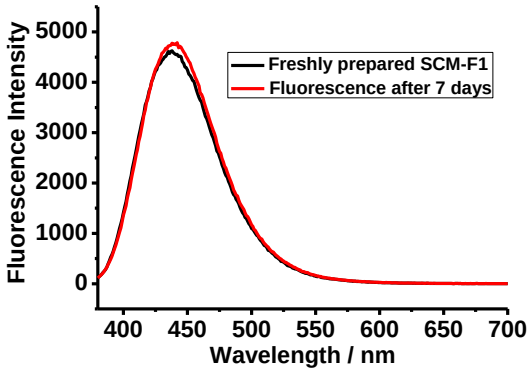


Figure 8s. Fluorescence spectra of freshly prepared SCM-**F1** (black line) aqueous solution and its fluorescence after 7 days of storage (red line). ($C_{\mathbf{F1}}$ = 5 μM).

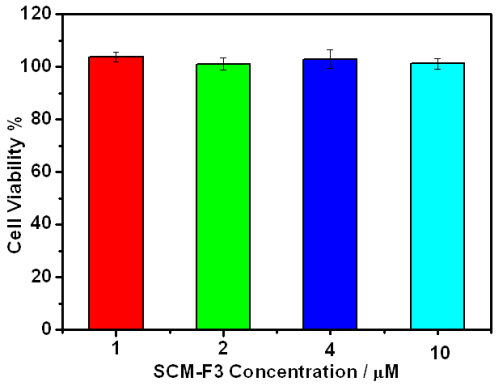
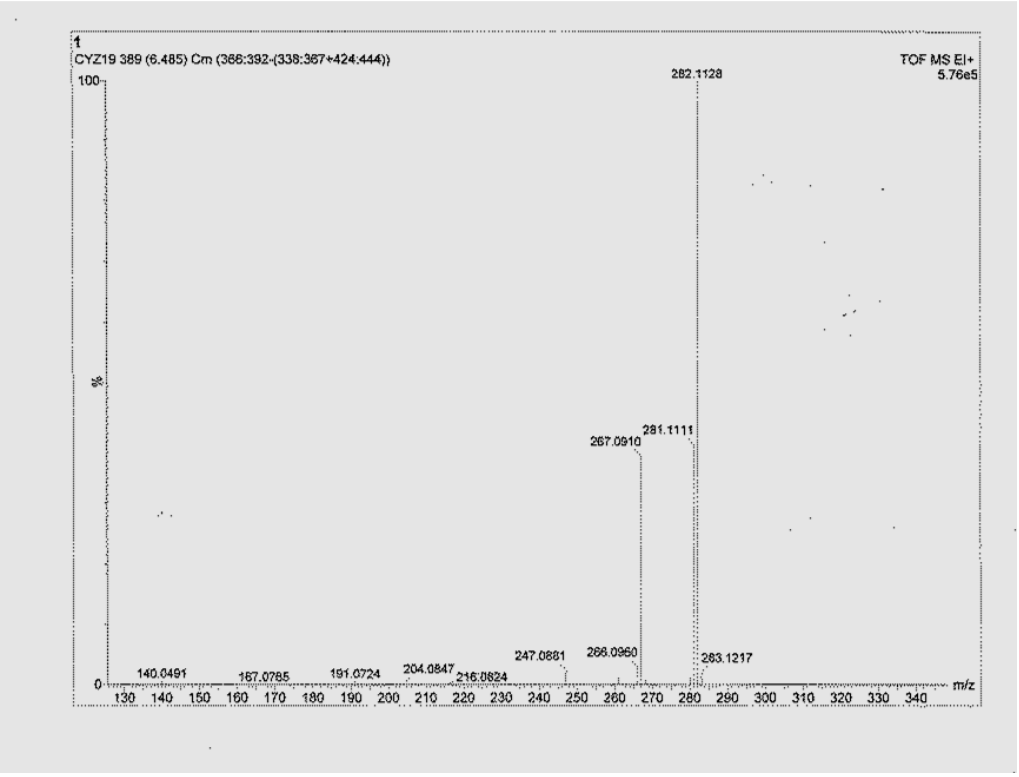
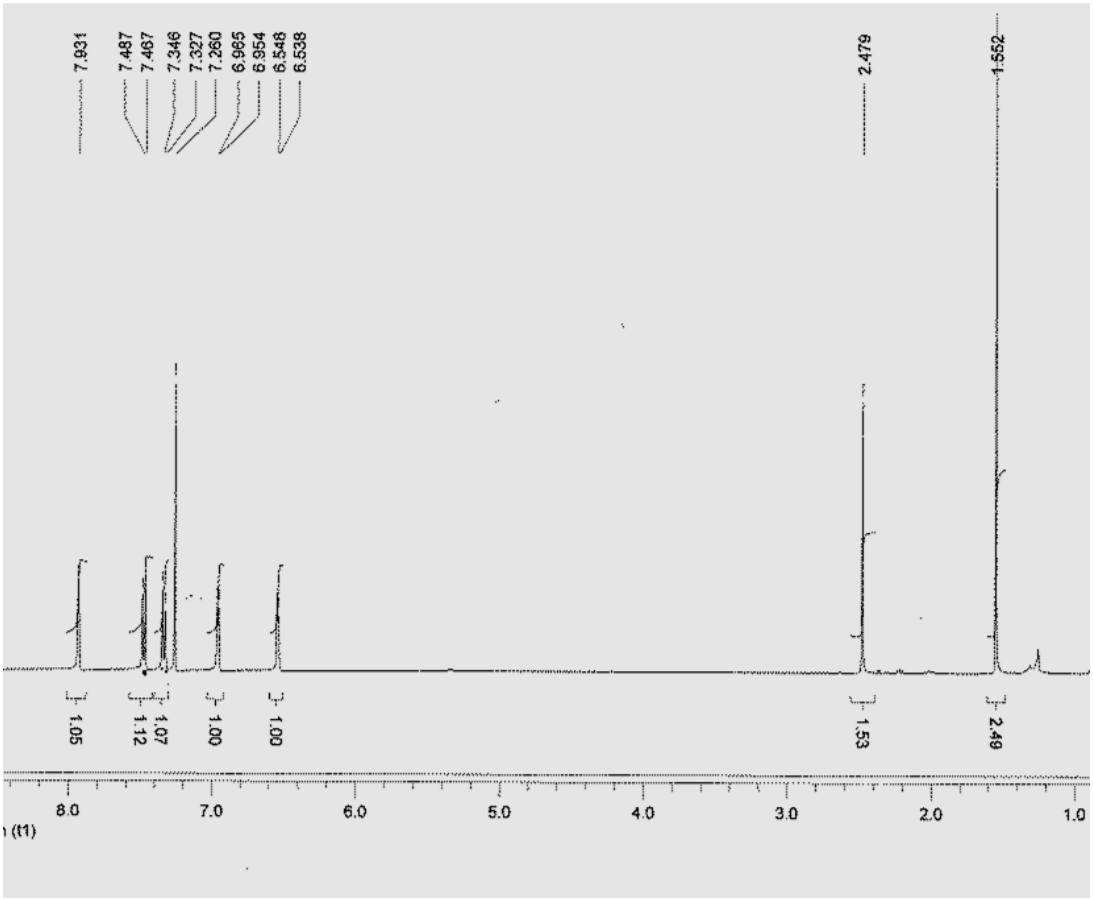


Figure 9s. Metabolic viability of Hela cells after incubation with SCM-**F3** at different concentrations for 24 h. The results indicated that the SCMs were of low toxicity to the cells at 1–10 μM.

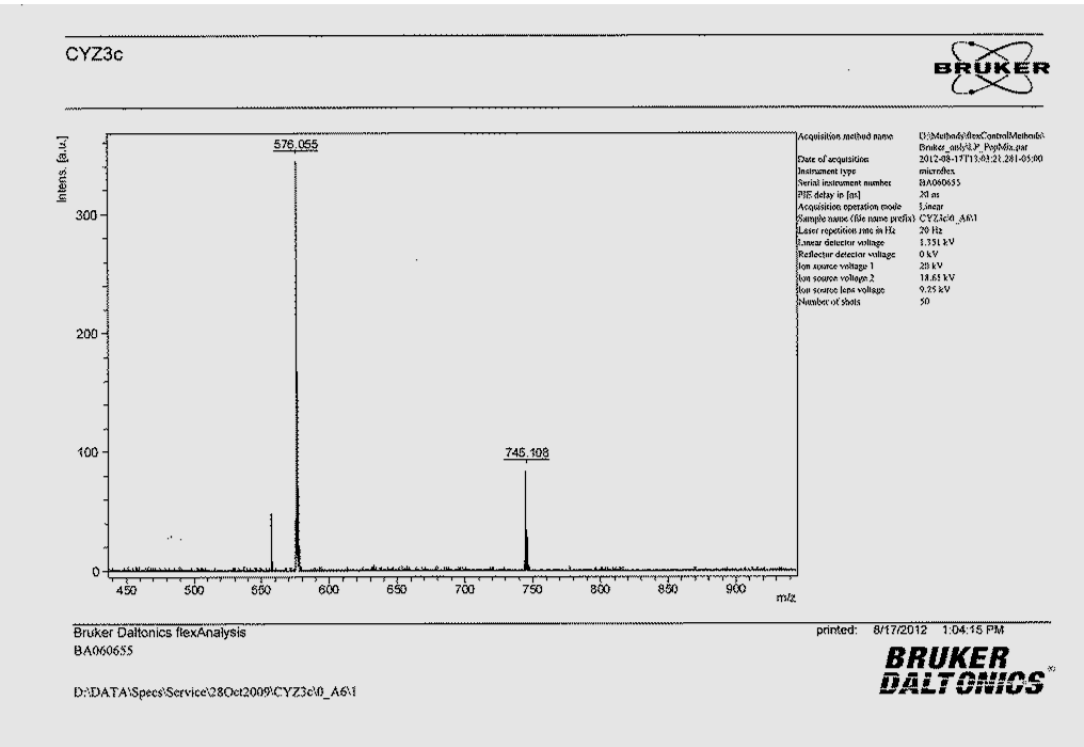
MS of **F2**



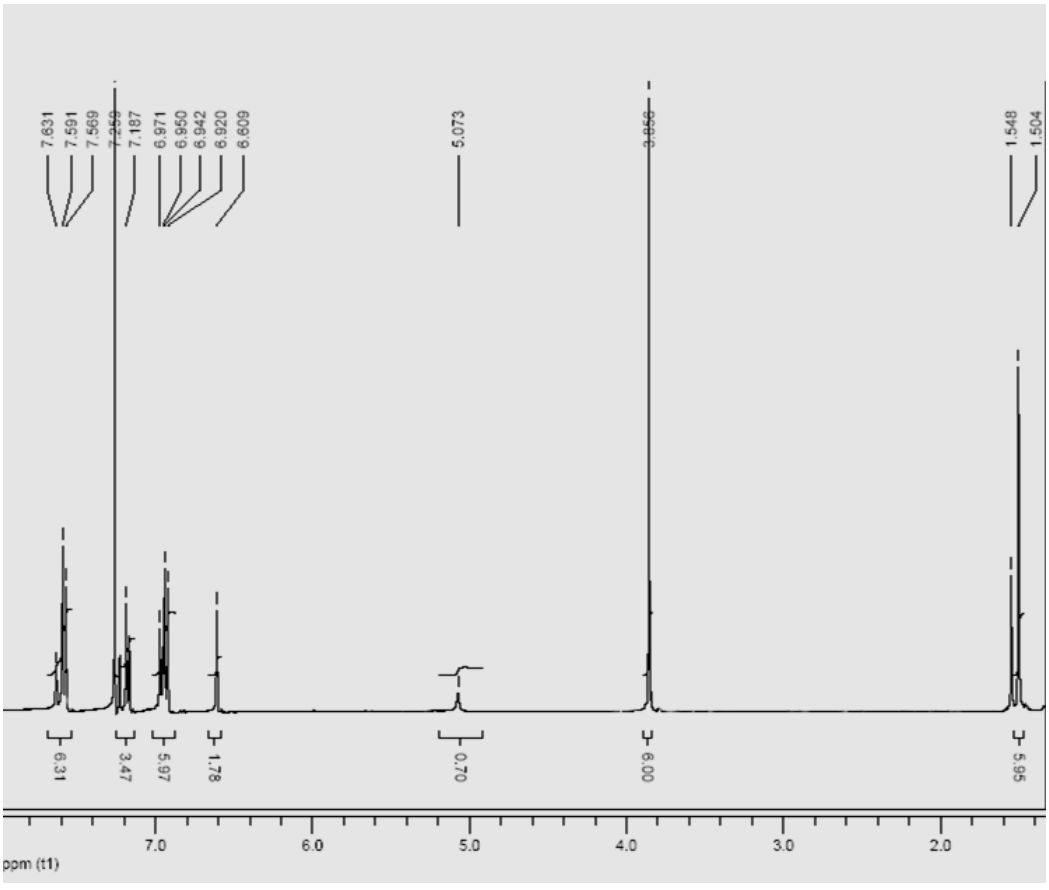
^{1}H NMR of **F2**



MS of **F3**



^{1}H NMR of **F3**



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