Stabilizing self-assembled Fmoc-F_5-Phe hydrogels by co-assembly with PEG-functionalized monomers

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Electronic supporting information (ESI)

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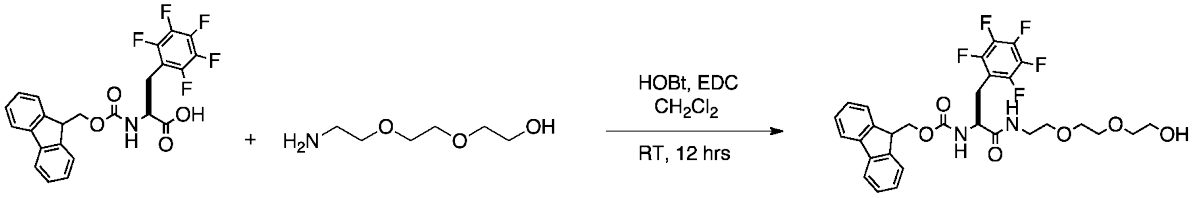
Materials and Methods

Fmoc-amino acids and organic solvents were purchased commercially and used without further purification. CH_2Cl_2 was purified as described in: Pangborn, A. B.; Giardello, M. A.; Grubbs, R. H.; Rosen, R. K.; Timmers, F. J. *Organometallics* **1996**, *15*, 1518–1520. Water for the gelation experiments was purified using a nanopure filtration system prior to use.

Synthesis and Purification of Fmoc-F_5-Phe-PEG



1 **2** **3**

1 (120 mg, 250umol), hydroxybenzotriazole (45 mg, 300 μmol) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (58 mg, 300 μmol) were dissolved in CH_2Cl_2 (10 mL) under dry argon at room temperature. After the mixture had stirred for 10 min **2** (0.88 mL, 500 μmol) was added drop wise. The reaction mixture was then stirred for 12 hr at room temperature. Due to the water solubility of the product, no water washes were performed, instead the volume of CH_2Cl_2 was reduced by rotary evaporation, and the mother liquor was purified by chromatography (ethyl acetate:hexanes, 98:2 *v*/*v*) to give **3** as a colorless solid. ^{1}H NMR:(400 MHz, $CDCl_3$): δ 7.75 (d, *J* = 12 Hz, 2H), 7.53 (t, *J* = 8 Hz, 2H), 7.38 (t, *J* = 8 Hz, 2H), 7.29 (t, *J* = 8 Hz, 2H), 7.16 (t, *J* = 8 Hz, 1H), 5.97 (d, *J* = 8 Hz, 1H), 4.51 (q, *J* = 8 Hz, 1H), 4.37 (q, *J* = 8 Hz, 1H), 4.26 (t, *J* = 8 Hz, 1H), 4.14 (t, *J* = 8 Hz, 1H), 3.70 (t, *J* = 4 Hz, 2H), 3.59-3.51 (m, 9H), 3.39 (q, *J* = 4 Hz, 1H), 3.26 (q, *J* = 4 Hz, 1H), 3.06 (q, *J* = 4 Hz, 1H) ppm; ^{13}C NMR (100 MHz, $CDCl_3$): δ 185.1, 169.8, 155.9, 143.6, 143.2, 141.2, 127.7, 127.0, 124.9, 119.9, 110.4, 72.3, 70.1, 70.0, 69.3, 67.1, 53.8, 46.9, 39.3, 26.0 ppm; ^{19}F NMR (376 MHz, $CDCl_3$): δ -79.8 (m, 2F), -93.1 (t, 1F), -99.7 (m, 2F) ppm; IR (neat) cm^{-1} 3290, 2924, 2888, 2870, 1697, 1693, 1656, 1650, 1531, 1537, 1519, 1501 1451, 1445. HRMS (ESI) *m*/*z* 631.1836 (631.1843 calcd for $C_{30}H_{29}N_2O_6F_5Na^+$ $[MNa]^+$).

Circular Dichroism Spectroscopy

CD spectra were recorded on an AVIV 202 circular dichroism spectrometer. Spectra of monomeric (unassembled) compounds were obtained by preparing 4.9 mM methanol (MeOH) solutions of the Fmoc amino acid and co-assemblies and gel samples were prepared by diluting concentrated MeOH stock solutions of Fmoc amino acids (247 mM) in water to final concentrations of 4.9 mM Fmoc amino acid in 2% MeOH/H_2O (*v*/*v*). The solutions were then immediately transferred into 0.1 mm path length quartz cuvette and gelation was completed in the cuvette. Spectra of the transparent gels were collected at 25 °C from 350 to 190 nm with a 1.0 nm step, 1.0 nm bandwidth, and 6 sec. averaging time per step.

Transmission Electron Microscopy

Images were taken with a Hitachi 7650 transmission electron microscope with an accelerating voltage of 80 kV. Samples of gel were applied directly onto 200 mesh carbon coated copper grids and allowed to stand for 30–45 s. Excess gel was carefully removed by capillary action (filter paper) and the grids were then immediately stained with uranyl acetate (20 μL for 45 s). Excess stain was removed by capillary action and the grids were allowed to air dry for 10–15 minutes.

Rheology

Measurements were conducted on a TA Instruments AR-G2 rheometer operating in oscillatory mode, with a 20 mm parallel plate geometry equipped with a solvent trap filled with silicon oil. DMSO stock solutions of Fmoc amino acid were diluted into water and immediately applied to the stage (1.4 mm gap) and covered with the solvent trap in order to prevent sample evaporation. Rheological analyses of Fmoc-F_5-Phe-PEG were conducted with and without heating of the system and were found to be identical. The rheological properties of the gels stabilize before optical clarification is complete. As such, all experiments were conducted at room temperature without heat. Following application of the sample to the stage a dynamic time sweep was immediately performed at 25 °C for 35–45 minutes with an angular frequency of 6.283 rad s^{-1} and 0.2% strain. A dynamic frequency sweep was performed immediately following the time sweep experiment from 0.1–50 rad s^{-1} with 0.2% strain at 25 °C. Shear recovery experiments were performed by applying a fresh sample to the stage, and immediately performing an oscillatory time sweep with 0.2 strain at 6.283 rad s^{-1} for 45 minutes. After the initial time sweep experiment, a second time sweep was performed with 100% strain at 6.283 rad s^{-1} for 30 seconds. Immediately following this step, a third time sweep was performed with 0.2% strain at 6.283 rad s^{-1} for 30 minutes in order to monitor shear recovery. All reported values for G´ and G´´ are an average of at least three runs.

UV-Vis Spectroscopy

UV absorption spectra of each sample were obtained by preparing fresh gels (4.9 mM Fmoc amino acid in 2% MeOH/H_2O) in a 0.1 mm path length quartz cuvette. Spectra of monomeric (unassembled) compounds were obtained with 4.9 mM Fmoc amino acid in MeOH. Spectra were collected from 200–500 nm using a Shimadzu UV-2401 PC UV-vis spectrophotometer.

Fluorescence Spectroscopy

Fluorescence spectra were obtained using a Fluorolog-3 spectrofluorimeter. Spectra of monomeric (unassembled) Fmoc amino acid were obtained using 4.9 mM monomer in MeOH. Hydrogel spectra were obtained by transferring 4.9 mM Fmoc amino acid in 2% MeOH/H_2O into a 0.1 mm path-length quartz cuvette and allowing gelation to occur. Spectra were recorded from 295–500 nm with an excitation wavelength of 265 nm; cutoff points were dictated by the limits of the photomultiplier tube in the spectrofluorimeter.

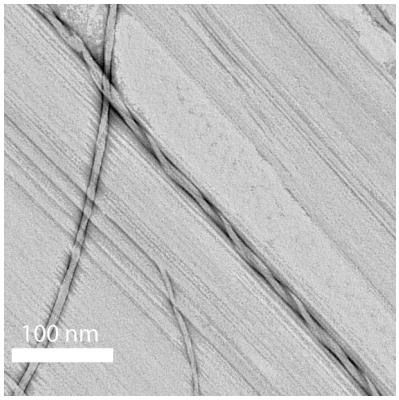


Figure S1. TEM images of helical structures formed by Fmoc-F_5-Phe-PEG

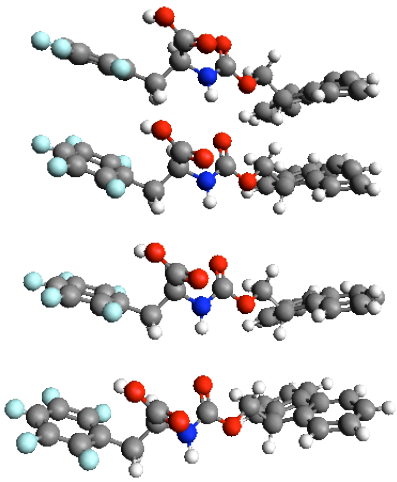


Figure S2. Model of monomer packing in Fmoc-F_5-Phe-OH fibrils.

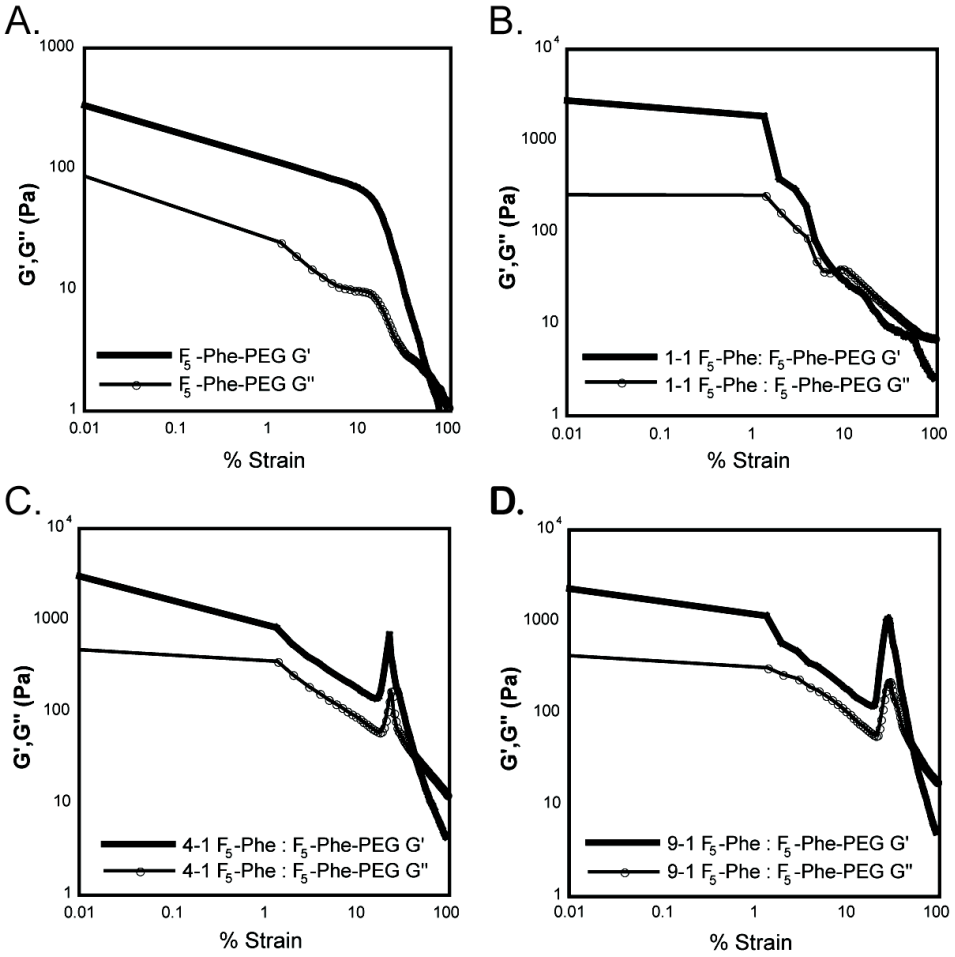


Figure S3. Rheological strain sweep data for Fmoc-F_5-Phe : Fmoc-F_5-Phe-PEG mixtures. A. Fmoc-F_5-Phe-PEG; B. Fmoc-F_5-Phe : Fmoc-F_5-Phe-PEG (1:1); C. Fmoc-F_5-Phe : Fmoc-F_5-Phe-PEG (4:1); D. Fmoc-F_5-Phe : Fmoc-F_5-Phe-PEG (9:1).

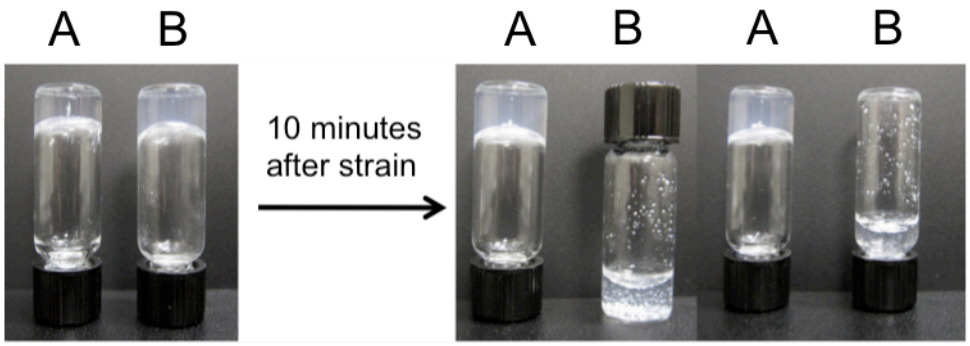


Figure S4. Gels of Fmoc-F_5-Phe-PEG (A) and Fmoc-F_5-Phe-OH (B) before and after the application of strain. Strain was applied by vigorous agitation of the gels by pipet or by manual shaking/agitation of the vials.

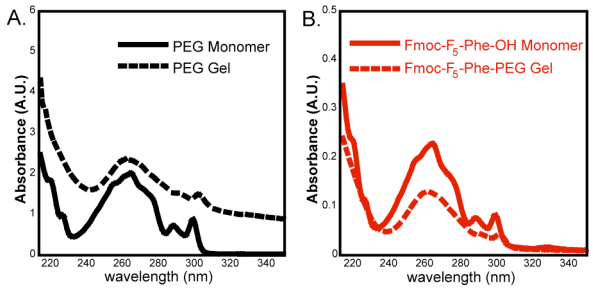


Figure S5. UV-vis spectra of A. Fmoc-F_5-Phe-PEG and B. Fmoc-F_5-Phe-OH.

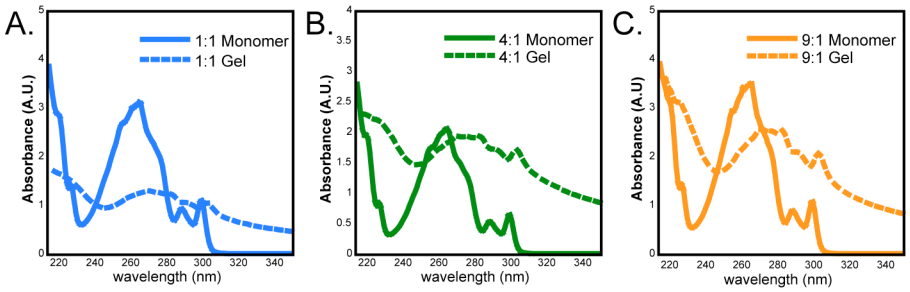


Figure S6. UV-vis spectra of A. 1:1, blue; B. 4:1, green; C. 9:1, orange mixtures of Fmoc-F_5-Phe OH : Fmoc-F_5-Phe-PEG.

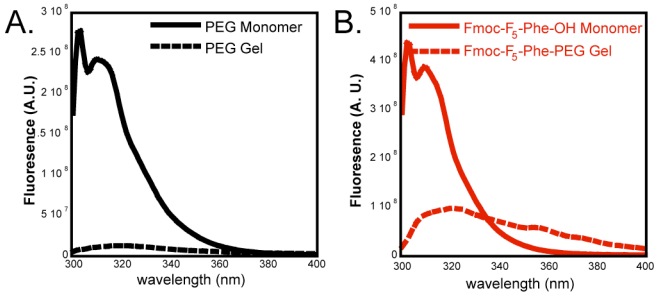


Figure S7. Fluorescence emission spectra of A. Fmoc-F_5-Phe-PEG and B. Fmoc-F_5-Phe-OH.

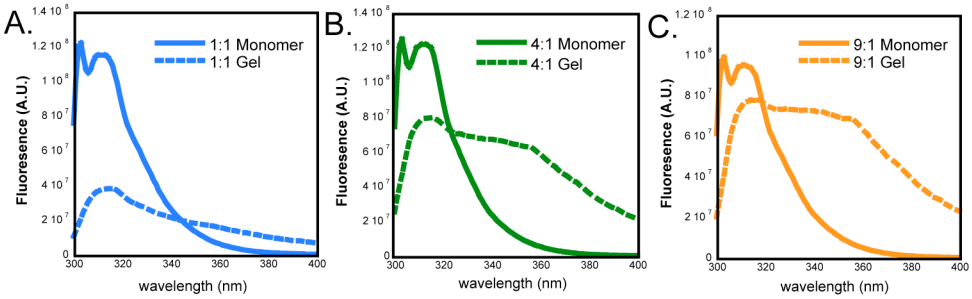
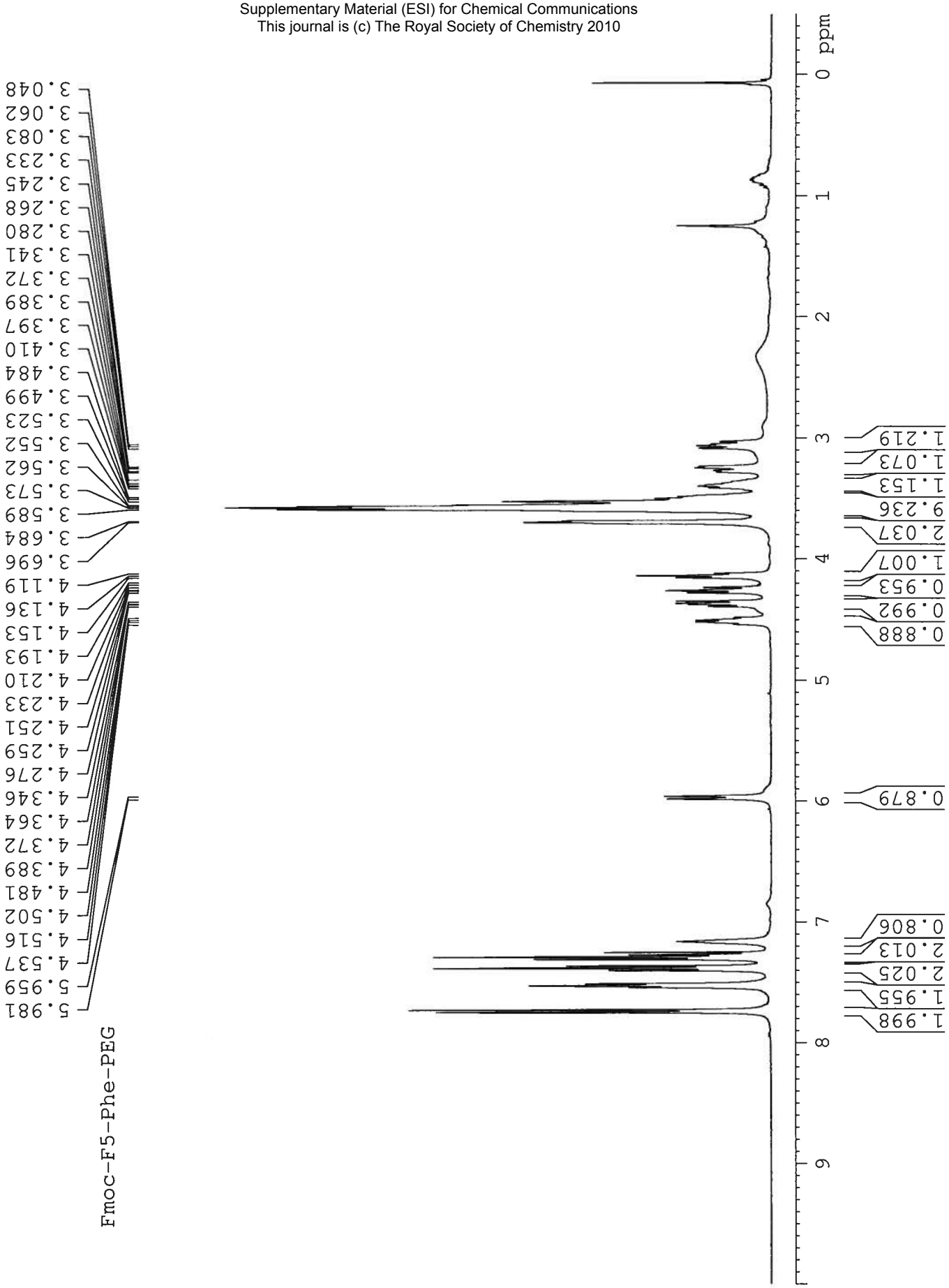


Figure S8. Fluorescence emission spectra of A. 1:1, blue; B. 4:1, green; C. 9:1, orange mixtures of Fmoc-F_5-Phe-OH:Fmoc-F_5-Phe-PEG.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4.276
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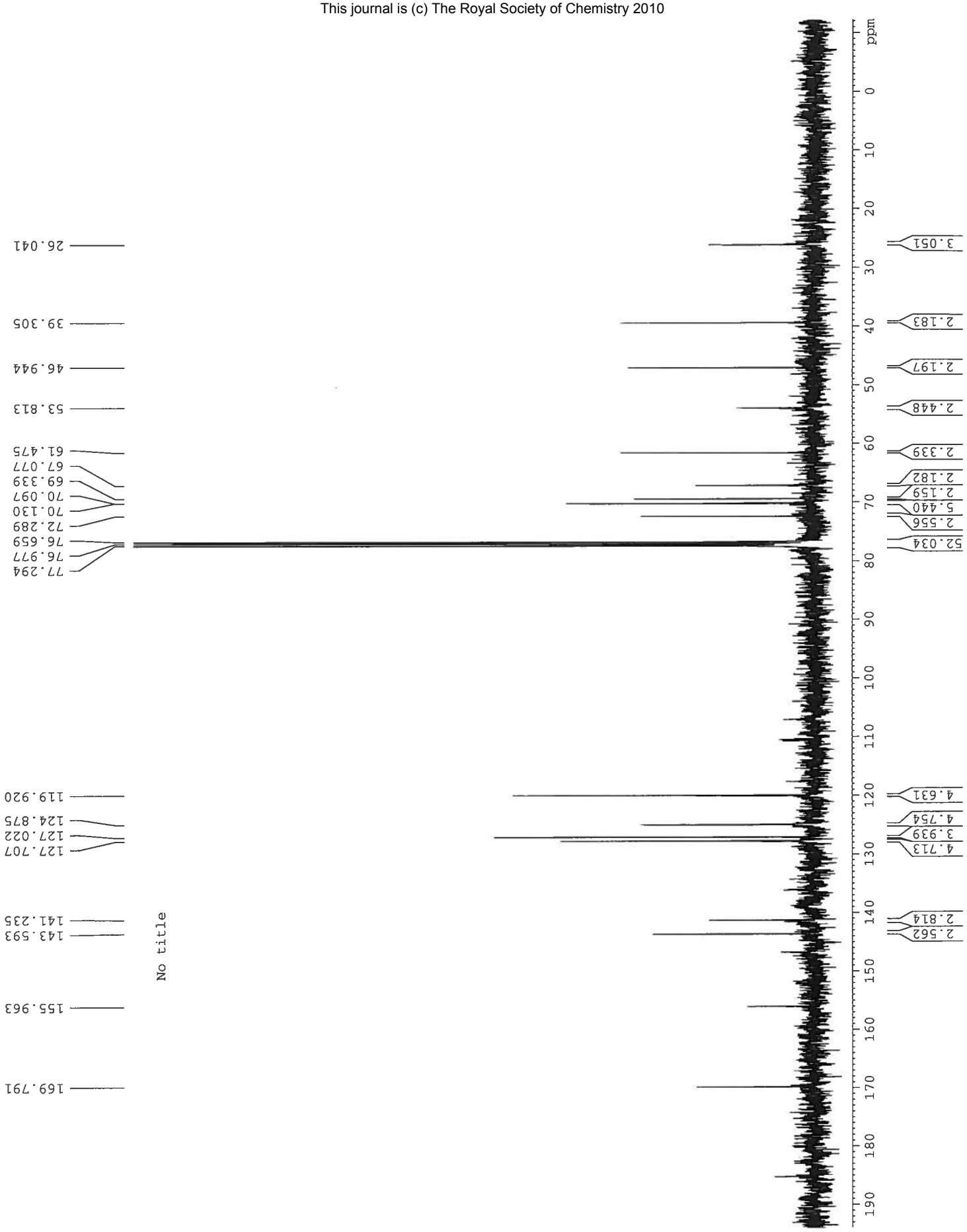
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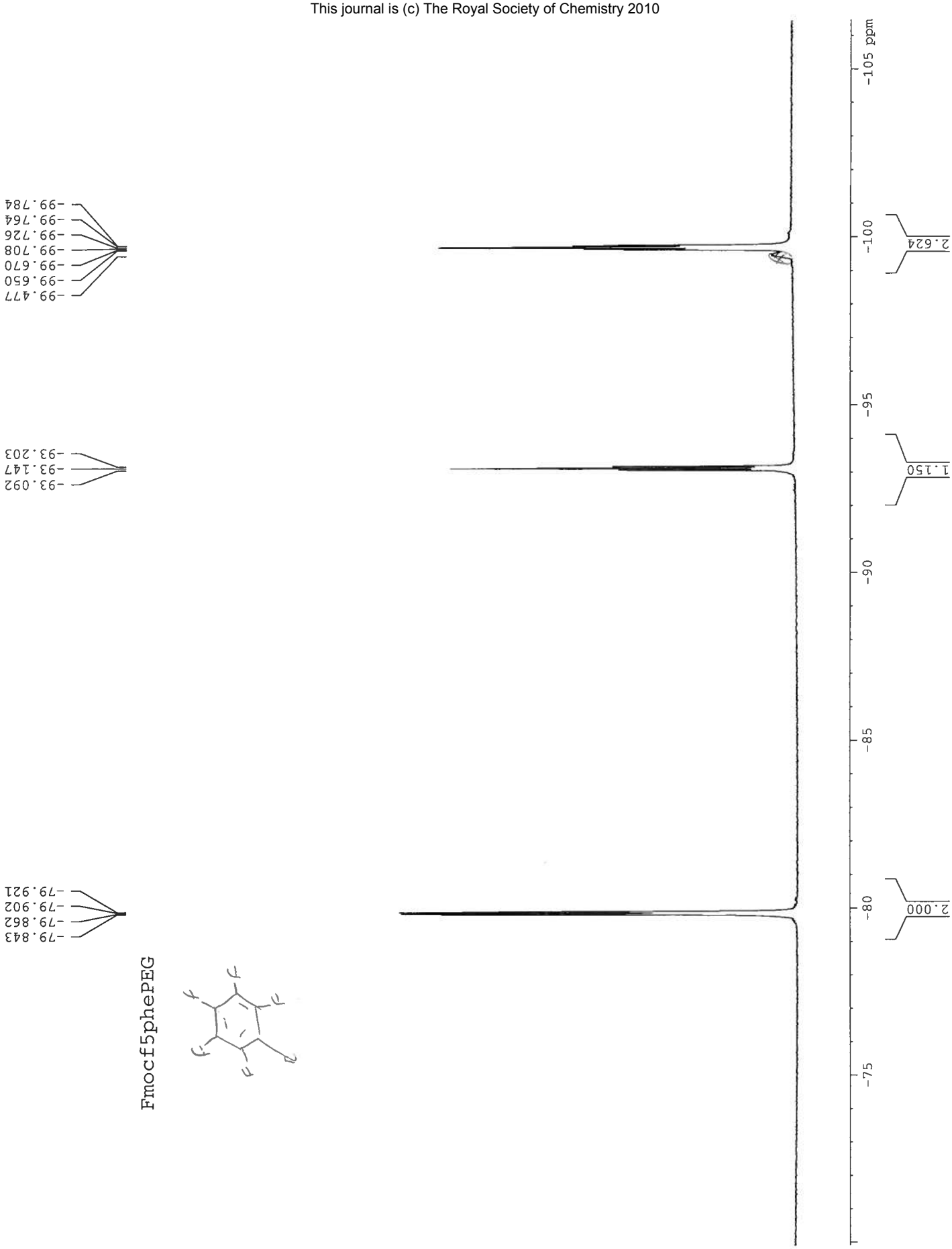
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