

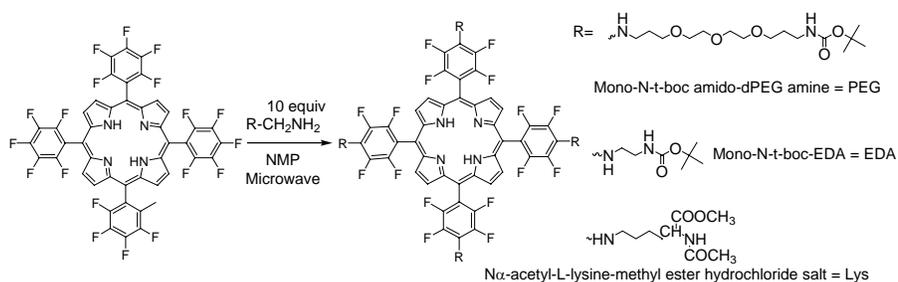
Efficient microwave-assisted synthesis of amine substituted pentafluorophenyl porphyrin

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Material & Methods

UV-Vis spectra were recorded on a Varian Bio3 spectrophotometer. Steady-state fluorescence (emission) spectra were measured with a Fluorolog $\tau 3$, Jobin-SPEX Instruments S.A., Inc. Flash column chromatography was performed using 230-400 mesh ASTM Merck silica gel-60. Electrospray ionization (ESI) MS spectrometric analyses were performed at the Mass Spectrometry Facility of the Department of Chemistry, Hunter College using an Agilent Technologies HP-1100 LC/MSD instrument. ^1H NMR was run in CD_3OD on a Bruker Avance 500MHz spectrometer. Chemical shifts are reported in parts per million (ppm). X-ray structure for Por-EDA₄ was obtained from a Bruker-Nonius KappaCCD X-ray diffractometer at 100K. High Resolution Mass Spectrometry (HRMS) was performed at CUNY Mass Spectrometry Facility at Hunter College. All reagents were obtained from commercial sources and used without further purification.

General Procedures

Preparation of EDA Derivative of TPPF₂₀ by Microwave Irradiation

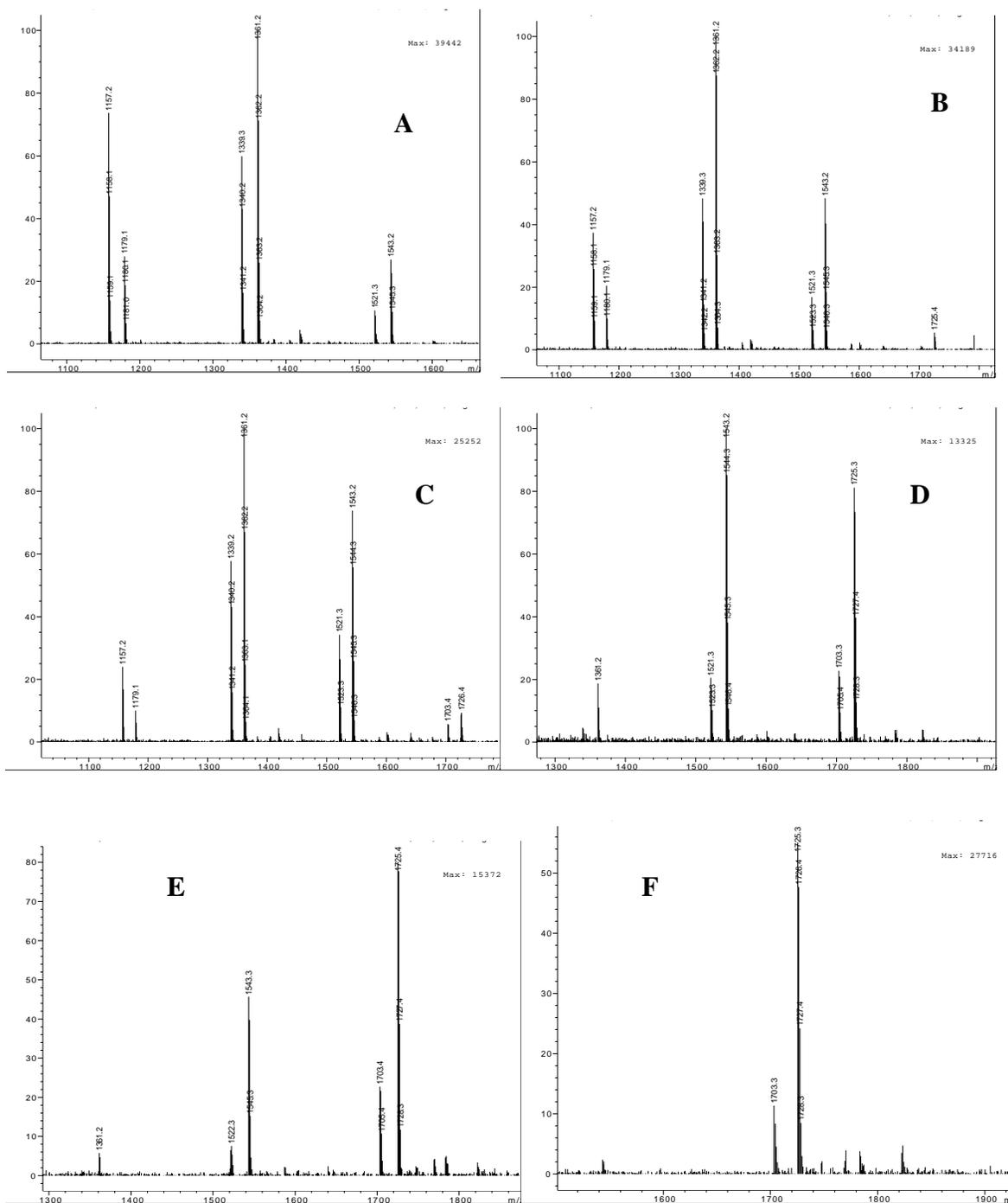
Into a 3.4 mL vial, 5 mg of 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin (5.1 μmol) and 10 equivalents of mono-N-t-boc EDA (Quanta BioDesign) in NMP (0.1 mL) were added. The closed vial was irradiated using a domestic microwave oven (1100 W, Samsung MW4250W) at 2 min intervals until no starting material was visualized by TLC (10 min). After the vial was cooled, the solvent (NMP) was removed in vacuo. The reaction mixture was purified on a silica gel prep TLC plate (hexane: ethyl acetate: methanol, 2:1.5:0.5). The porphyrin was re-dissolved in methanol and evaporated under reduced pressure to afford >90 % yield. **EDA porphyrin** (Por-EDA₄): ^1H NMR (CD_3OD , 500 MHz) δ 9.12 (br, pyrrole βH , 8H), 3.77 (t, amine or amide CH_2 , 8H), 3.52 (t, amine or amide CH_2 , 8H), 1.54 (s, t-boc, 36H). HRMS (ESI): calculated for $\text{C}_{72}\text{H}_{70}\text{F}_{16}\text{N}_{12}\text{O}_8$ (M+H)⁺ 1535.52568, found (M+H)⁺ 1535.52566, Δ 0.02 ppm.

Preparation of Lys Derivative of TPPF₂₀ by Microwave Irradiation

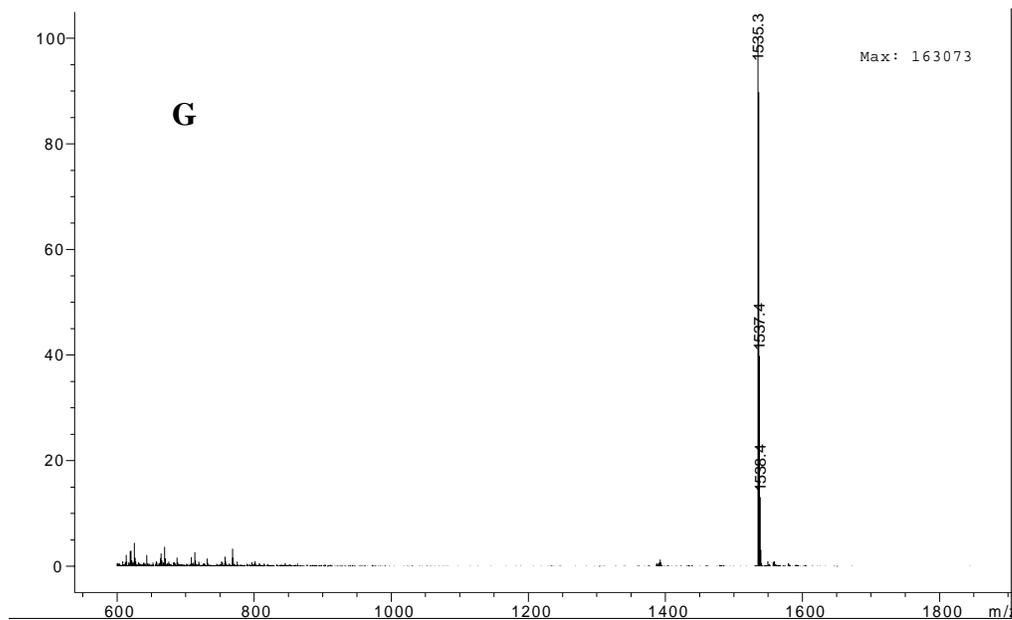
Into a 3.4 mL vial, 5 mg of 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin (5.1 μmol) and 10 equivalents of N α -acetyl-L-lysine-methyl ester hydrochloride salt (Aldrich) and sodium bicarbonate (10equiv) in NMP (0.1 mL) were added. The closed vial was irradiated using a domestic microwave oven (1100 W, Samsung MW4250W) at 2 min intervals until no starting material was visualized by TLC (30 min). After the vial was cooled, the solvent (NMP) was removed in vacuo. The reaction mixture was purified on a silica gel prep TLC plate (hexane: ethyl acetate: methanol, 2: 1.5: 0.5). The porphyrin was re-dissolved in methanol and evaporated under reduced pressure to afford >70 % yield. **Lysine porphyrin** (Por-Lys₄): ^1H NMR (CD_3OD , 500 MHz) δ 9.10 (br, pyrrole βH , 8H), 4.54 (m, amine or amide CH, 4H), 3.79 (s, COOCH_3 , 12H), 3.70 (m, amine or amide CH_2 , 8H), 2.06 (s, OCH_3 , 12H), 1.99 (m, CH_2 , 8H), 1.90 (m, CH_2 , 8H), 1.68 (m, CH_2 , 8H). HRMS (ESI): calculated for $\text{C}_{80}\text{H}_{78}\text{F}_{16}\text{N}_{12}\text{O}_{12}$ (M+H)⁺ 1703.56794, found (M+H)⁺ 1703.56946, Δ 0.89 ppm.

Preparation of PEG Derivative of TPPF₂₀ by Microwave Irradiation

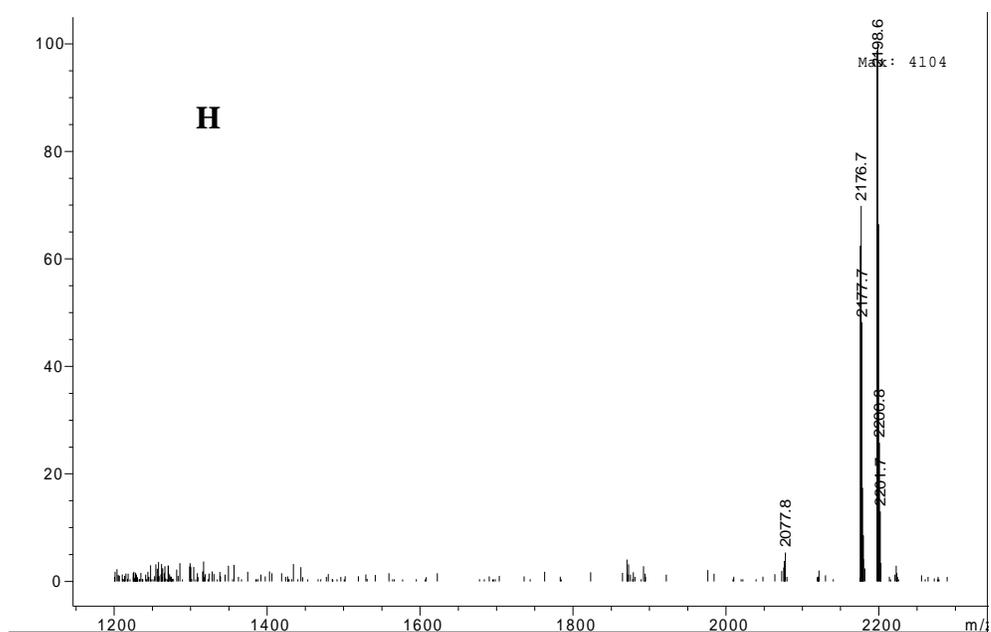
Into a 3.4 mL vial, 5 mg of 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin (5.1 μmol) and 10 equivalents of mono-N-t-boc amido-dPEG amine (Quanta BioDesign) in NMP (0.1 mL) were added. The closed vial was irradiated using a domestic microwave oven (1100 W, Samsung MW4250W) at 2 min intervals until no starting material was visualized by TLC (12 min). After the vial was cooled, the solvent (NMP) was removed in vacuo. The crude reaction product was purified via silica gel chromatography using hexane/ethyl acetate/methanol solvent systems to afford >90 % yield. **PEG porphyrin** (Por-PEG₄): ^1H NMR (CD_3OD , 500 MHz) δ 9.15 (br s, pyrrole βH , 8H), 3.85-3.6 (m, CH_2O , 6 methylene groups next to O, 48H), 3.53 (t, amine or amide CH_2 , 8H), 3.2 (t, amine or amide CH_2 , 8H), 2.1 (t, CH_2 , 8H), 1.7 (t, CH_2 , 8H), 1.37 (s, t-boc, 36H). HRMS (ESI): calculated for $\text{C}_{104}\text{H}_{134}\text{F}_{16}\text{N}_{12}\text{O}_{20}$ (M+H)⁺ 2175.96546, found (M+H)⁺ 2175.96280, Δ 1.22ppm.



Monitoring the Por-Lys₄ reaction every 5 minutes via ESI-MS (atmospheric photo ionization, positive ion mode)---shows how the reaction proceeds to the Por-Lys₄ product only. **[A]**, shows the mono-substituent (1157 and Na⁺ adduct 1179), di-substituent (1339 and Na⁺ adduct 1361), tri-substituent (1521 and Na⁺ adduct 1543). **[B, C]**, shows all four substituent including the tetra-substituent at 1703 (Na⁺ adduct 1725). **[D, E]**, shows the di-, tri-, and tetra-substituent. Going from **D** to **E** it is noticeable that the tri-substituent is decreased and the tetra-substituent is increased. **F** shows the tetra-substituent only (1703 and Na⁺ adduct 1725).

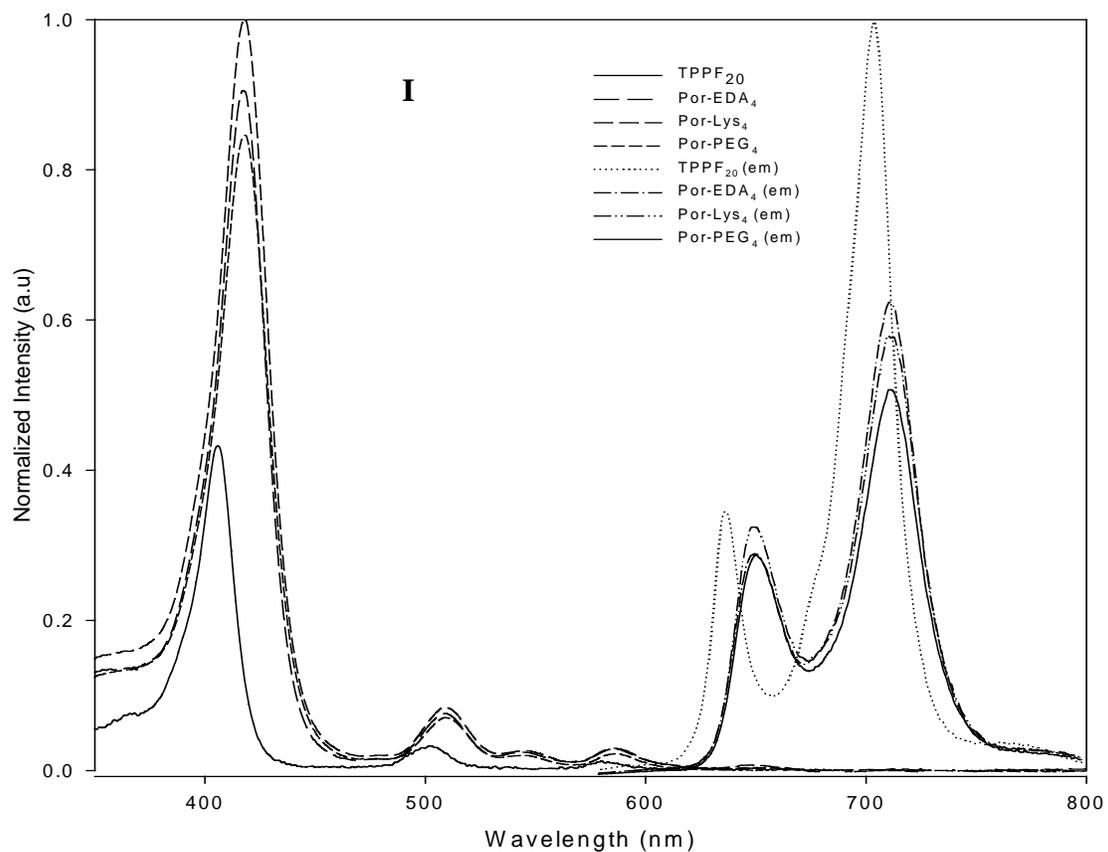


G: ESI-MS (API, positive ion mode) of Por-EDA₄ (peak at 1535)

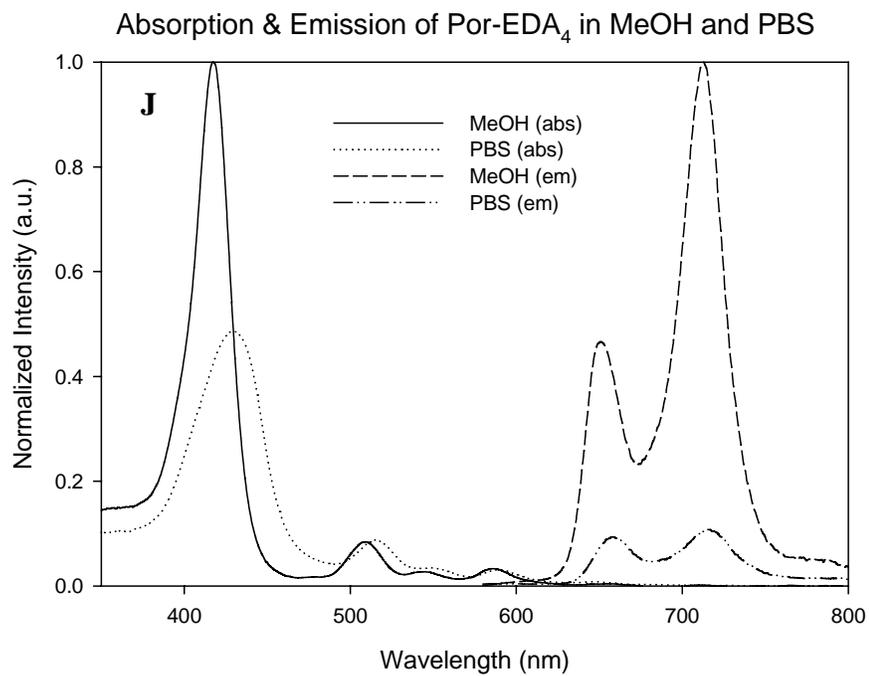


H: ESI-MS (API, positive ion mode) of Por-PEG₄ (parent peak at 2176 and Na⁺ adduct 2198)

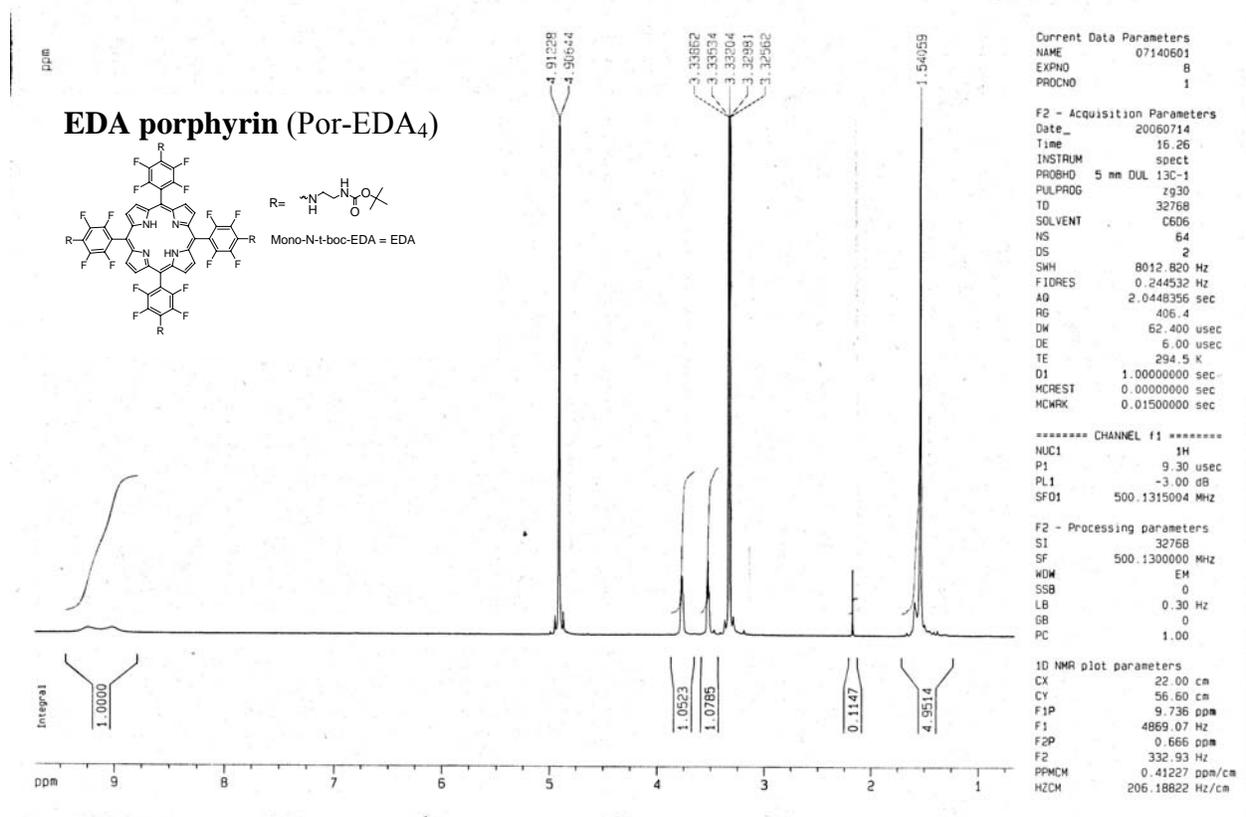
Absorption and Emission of Porphyrin Conjugates in MeOH



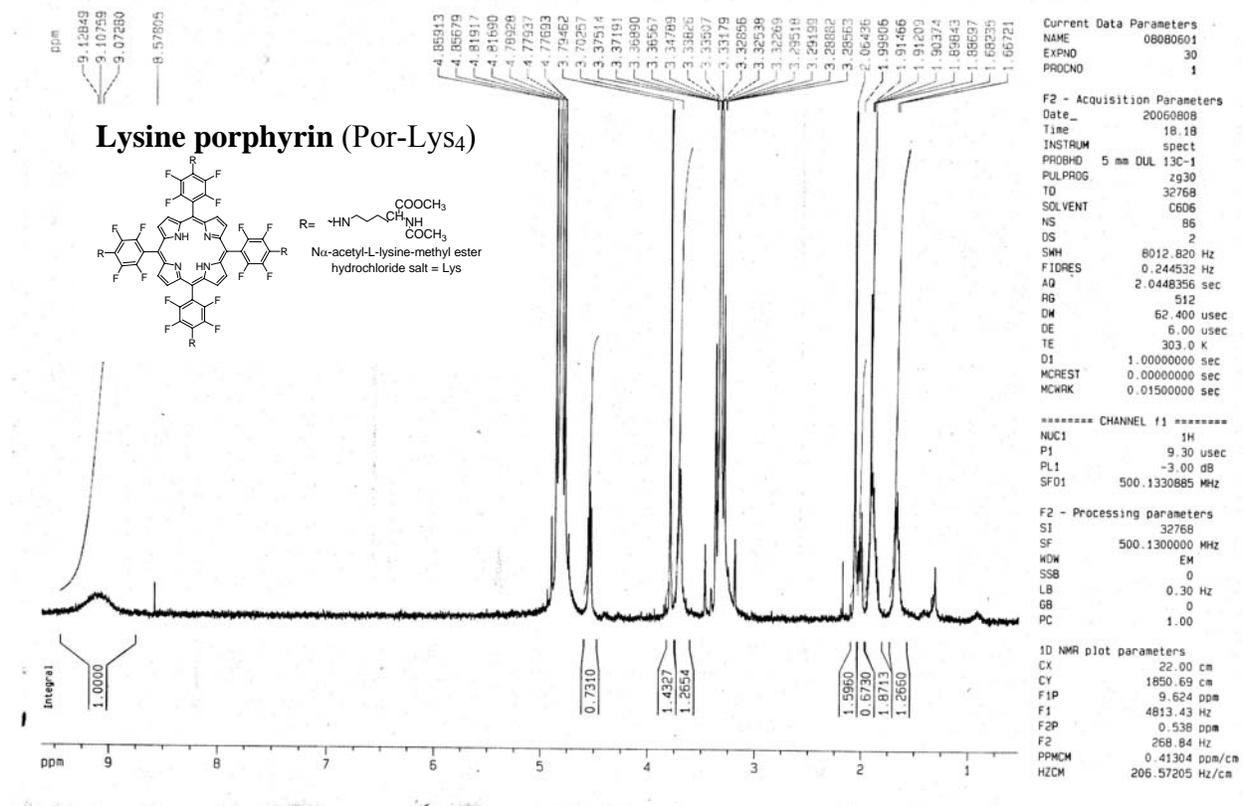
I: Absorption and emission of the parent porphyrin (TPPF₂₀) and the derivatives (Por-EDA₄, Por-Lys₄, Por-PEG₄) in MeOH.



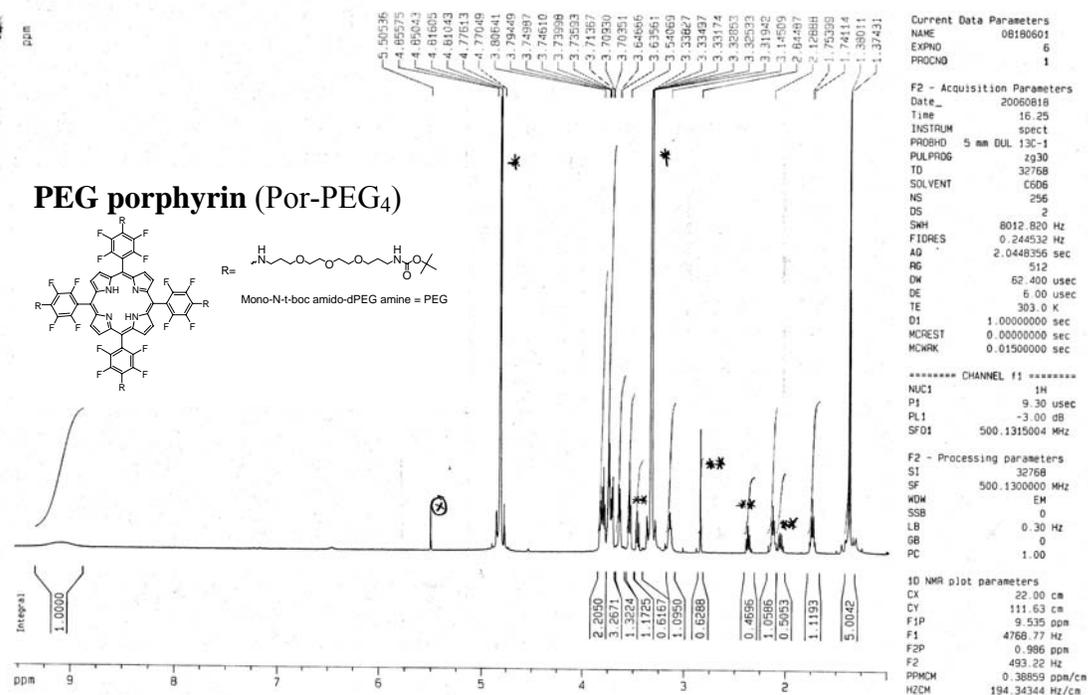
J: Absorption and emission of Por-EDA₄ in methanol and PBS. Note the shifts in absorption in the aqueous media.



K: 500 MHz ¹H NMR (CD₃OD) of Por-EDA₄



L: 500 MHz ¹H NMR (CD₃OD) of Por-Lys₄



M: 500 MHz ¹H NMR (CD₃OD) of Por-PEG₄: * = CH₃OH, ** = NMP, 5.50 ppm CH₂Cl₂