Electronic supplementary information

Synthesis and cell phototoxicity of a triply bridged fused diporphyrin appended with six thioglucose units

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Pages

The pioneering work by the Osuka group in making bridged, fused, and other porphyrin tapes has explored a variety of synthetic conditions[1-9] and the Kim group has been instrumental in exploring the excited state dynamics of the fused porphyrin systems.[10] Annulated porphyrins by the Bruckner group have enhanced our understanding of HOMO-LUMO gap tuning.[11]

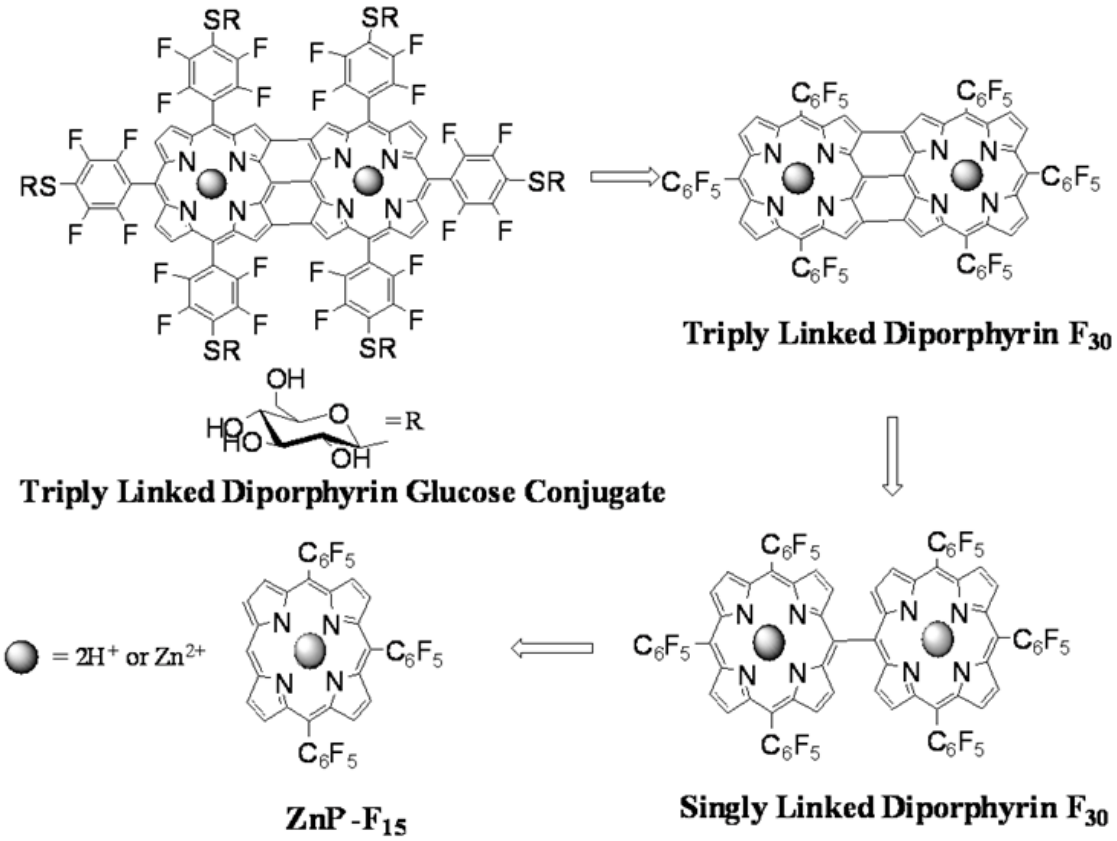
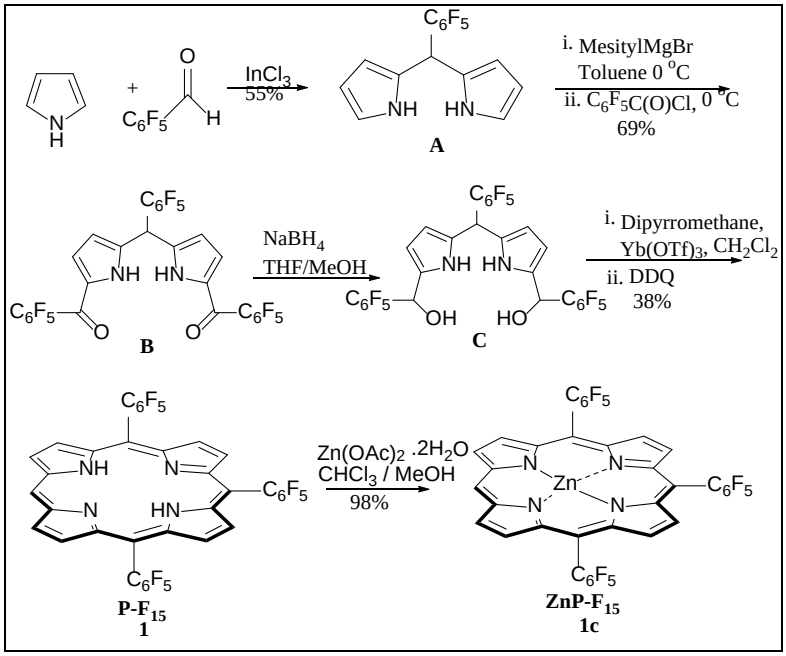


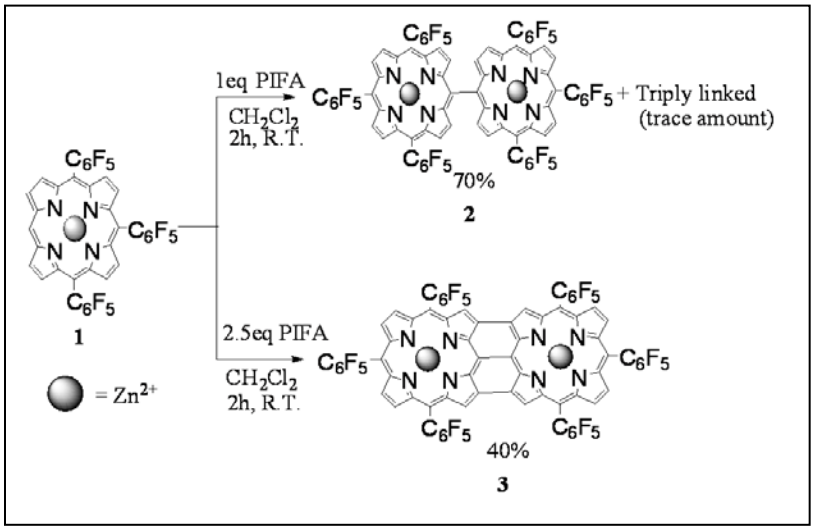
Figure ESI 1: Retrosynthesis of triply linked diporphyrin appended with thioglucose.

5,10,15-tris(pentafluorophenyl)porphyrinatozinc(II) (ZnP-F_{15}) is the key parent molecule for the synthesis of singly and triply bridged diporphyrins. This porphyrin was synthesized using reported procedure by the Nocera group[12] as shown in Scheme **ESI 1**. 5-Pentafluorophenyl dipyrromethane **A** and 1,9-diacyldipyrromethane **B** were synthesized according to reported procedures.[13-15] Reduction of **B** gave the dipyrromethane dicarbinol (2-OH), which upon condensation with dipyrromethane, gave the corresponding free base porphyrin P-F_{15} in 38% yield. This porphyrin was then metalated using $Zn(OAc)_2$ affording ZnP-F_{15} porphyrin in 98% yield.[16]

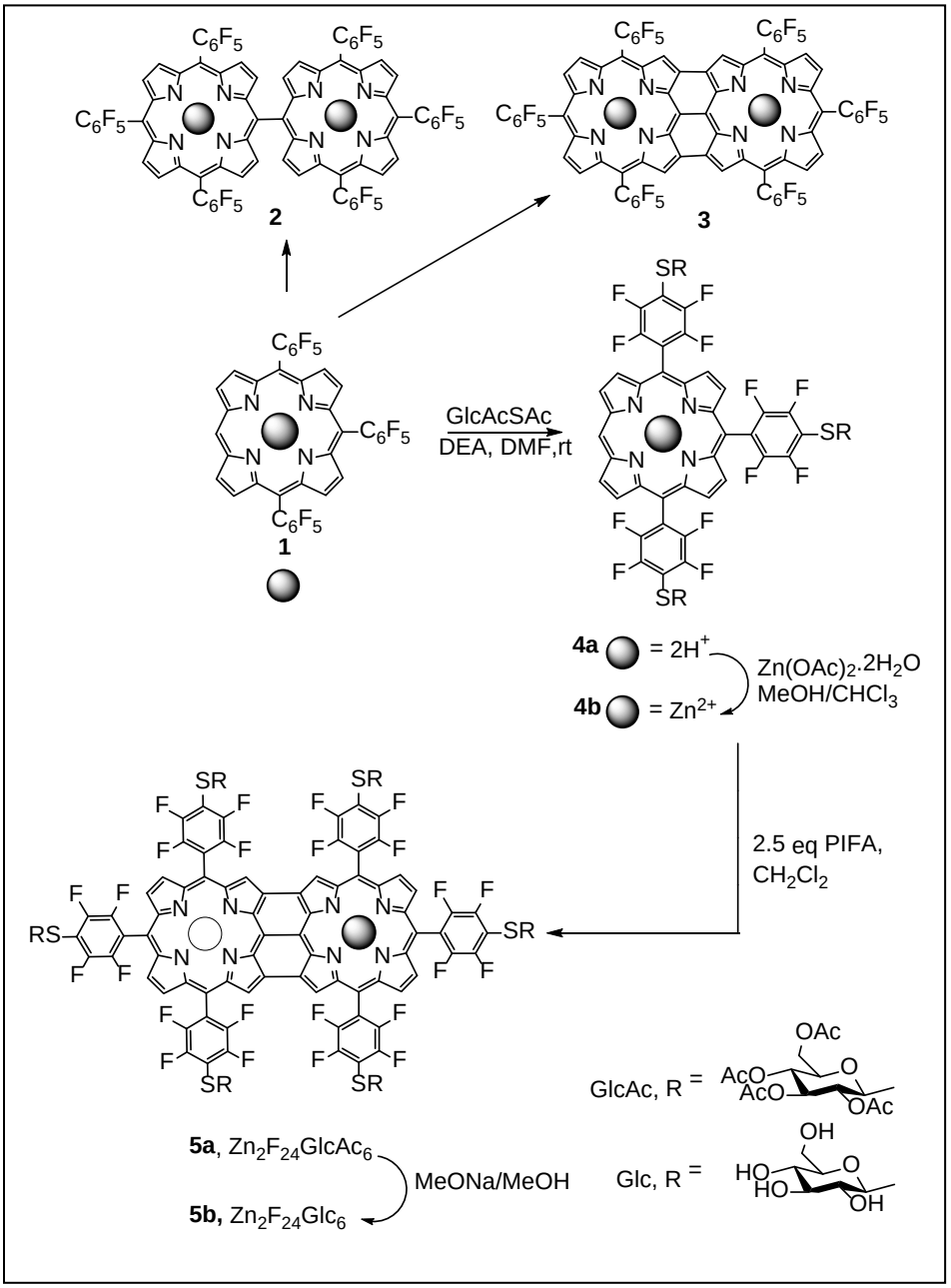


Scheme ESI 1. Synthesis of ZnP-F_{15} (1c) porphyrin according the procedures by Nocera and coworkers [12].

To target the dye to cancer, compound **3** was demetalated and treated with 6.5 equivalents of thioglucose in the presence of diethylamine as a base. Under a variety of conditions, this substitution reaction gave a mixture of **4 - 8** substituted compounds and isomers that were difficult to separate as shown by the MALDI-TOF spectra (see below, Figure ESI 25).



Scheme ESI 2. Synthesis of singly and triply linked diporphyrins, **2** and **3.**



Scheme ESI 3. Synthesis of *meso-meso*, *β-β, β-β* triply bridged Zn^{II} diporphyrin appended with six thioglucose units, **5b** from the glycosylated monomer **4b**.

Experimental Procedure

Materials and Methods

General. ^{1}H and ^{13}C NMR spectra were recorded in a Brüker Avance 500 MHz spectrometer and the ^{19}F spectra in a JEOL 400 MHz spectrometer. Electrospray ionization mass spectrometric analyses were performed at the CUNY Mass Spectrometry Facility at Hunter College using an Agilent Technologies HP-1100 LC/MSD instrument. The electrospray ionization was run in methanol, with 0.1% formic acid. UV-visible spectra were recorded on a Varian Bio3 spectrophotometer. All reagents were obtained from commercial sources and used without further purification. Flash column chromatography was performed using silica gel-60, and the analytical TLC was carried out on precoated sheets with silica gel (0.2 mm thick), both from Sorbent Technologies. The porphyrin ZnP-F_{15} (**1)** was prepared according to reported procedures and results were consistent with previous work.[12,14] The iodine(III) reagent PIFA was purchased from Aldrich.

Meso-Meso Singly Bridged Zn^{II}-diporphyrin (2)

A sample of 5,10,15-tris(pentafluorophenyl)porphyrinatozinc(II) (ZnP-F_{15}) (43 mg, 0.05 mmol) in 15 mL of CH_2Cl_2 was cooled to -78^{o}C and then PIFA (22 mg, 0.05 mmol) was added. Then, the cooling bath was removed and the mixture was stirred at room temperature for about 2 h. The resulting yellow-brown mixture was then washed with water several times; the organic layer was dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/hexane = 1:1) to give **2** (18 mg, 70% yield). ^{19}F NMR (CD_2Cl_2-d_2): δ -138.40 to -138.23 (m, 8F, Ar-o-F), -153.95 to -153.80 (m, 4F, Ar-p-F), -163.42 to -163.19 (m, 8F, Ar-m-F). ^{1}H NMR (CD_2Cl_2-d_2): δ 8.23-8.24 ppm (d, 2H, Por-β) 8.74-8.75 ppm (d, 2H, Por-β), 9.15-9.17 ppm (2d, 4H, Por-β) ^{13}C NMR (CD_2Cl_2-d_2): δ 103.59, 104.74, 116.33, 116.35, 121.02, 130.74, 131.92, 135.85, 136-146 (C_6F_4), 149.81, 150.38, 155.31. HRMS Calcd for $C_{76}H_{16}F_{30}N_8Zn_2$ $(M)^{+\cdot}$: 1737.9602 found 1737.9618.

Meso-meso, *β-β, β-β* triply bridged Zn^{II} diporphyrin (3)

A sample of 5,10,15-tris(pentafluorophenyl)porphyrinatozinc(II) (ZnP-F_{15}) (43 mg, 0.05 mmol) in 15 mL of CH_2Cl_2 was cooled to -78^{o}C and then PIFA (50 mg, 0.125 mmol) was added. Then, the cooling bath was removed, and the mixture was stirred at room temperature for about 2 h. The resulting yellow-brown mixture was then washed with water several times; the organic layer was dried with anhydrous Na_2SO_4, filtered, and concentrated in vacuum. The residue was purified by flash column chromatography (silica gel, CH_2Cl_2/hexane = 1:1) to give **3** (20 mg, 40% yield). ^{19}F NMR (CD_2Cl_2-d_2): δ -138.36 to -138.00 (m, 8F, Ar-*o*-F), -153.31 to -153.23 (m, 4F, Ar-p-F), -162.28 to -162.05 (m, 8F, Ar-m-F). ^{1}H NMR ($CDCl_3$-d): δ 7.71-7.72 ppm (d, 4H, Por-β) 7.67-7.70 ppm (d, 4H, Por-β), 7.08 ppm (s, 4H, Por-β) ^{13}C NMR (CD_2Cl_2-d_2): δ 107.37, 114.52, 114.68, 118..67, 120.36, 126.43, 130.97, 136.33, 137.48, 142.76-146.76 (C_6F_4), 152.88, 153.06, 153..93, 155.17. HRMS Calcd for $C_{76}H_{12}F_{30}N_8Zn_2$ $(M)^{+\cdot}$: 1733.9289 found 1733.9301.

For **2** and **3**, in addition, a cluster of peaks with the same pattern as one calculated based upon the isotopic distribution of the formula was observed. Additional evidence of the structure of the compounds **2** and **3** were obtained from UV-visible spectrum, which shows for compound

2, split Soret absorption bands at 412 and 448 nm of nearly equal intensity, this pattern is typical for *meso-meso* singly linked fused diporphyrins.[1,17-19] For diporphyrin **3**, broad peak is observed at 415 nm with a shoulder at 455 nm and the Q-bands are observed at 557 and 1068 nm. UV-visible spectra of compounds **1**, **2** and **3** are shown below, Figure ESI 40.

Synthesis of $FBGlc_3$ (4a)

To a solution of the free baseF_{15} **1** (10 mg, 12.3μmol) and 2,3,4,6-tetra-*O*-acetyl-glucosylthioacetate (18 mg, 36.9 μmol, 3.0 equiv) in DMF (1.0 mL), diethyl amine (0.2 mL) was added. The reaction mixture was stirred at room temperature for 4h. The reaction mixture was precipitated with methanol/H_2O and the solid filtered through a short column of Celite and washed with water. The crude mixture was recovered in CH_2Cl_2 and purified by flash chromatography (silica gel) using a mixture of ethyl acetate/hexanes (3:1) as eluent. $FBGlc_3$ **4a** (19 mg, 86%) was obtained after crystallization in CH_2Cl_2/hexanes, as a pink powder. ^{19}F NMR ($CDCl_3$): δ -154.0 to -153.6 (m, 6F, Ar-m-F), -158.0 to -157.6 (m, 6F, Ar-*o*-F). ^{1}H NMR ($CDCl_3$): δ 10.42 (s, 1H, meso-H), 9.50-9.51 (d, 2H, pyrrolic β-H), 9.03-9.05 (m, 6H, pyrrolic β-H), 5.19-5.28 (m, 12H, Glc-H), 4.35-4.36 (m, 6H, Glc-H), 3.93-3.95 (m, 3H, Glc-H), 2.11-2.26 (m, 36H, acetyl-H), -3.05 (s, 2H, NH). ^{13}C NMR ($CDCl_3$): δ 20.68 (CH_3CO_2), 61.93, 68.13, 70.68, 73.95,76.46, 84.70 (Glc), 103.16, 107.69, 111.69, 122.23, 130.35, 131.02-131.16, 133.43-133.50, 145.36-147.35 (C_6F_4), 169.44, 169.52, 170.23 and 170.70 (CH_3CO_2). HRMS calcd for $C_{80}H_{68}F_{12}N_4O_{27}S_3$ $(M)^{+\cdot}$ 1840.3041 found 1840.3003.

Synthesis of $ZnGlc_3$ (4b)

A solution of porphyrin **4a** (15 mg, 8 μmol) in a mixture of $CHCl_3$/methanol (3:1, 9 mL: 3 mL) was treated with $Zn(OAc)_2.2H_2O$ (0.130 g, 0.6 mmol). The reaction mixture was stirred overnight at room temperature after which it was concentrated to dryness. The crude product was dissolved in CH_2Cl_2 (50 mL), washed with water and brine, dried over Na_2SO_4 and concentrated to dryness. $ZnGlc_3$ **4b** (14 mg, 95%) was obtained after crystallization in CH_2Cl_2/hexanes, as a pink powder. ^{19}F NMR ($CDCl_3$): δ -154.0 to -153.5 (m, 6F, Ar-m-F), -158.0 to -157.4 (m, 6F, Ar-*o*-F). ^{1}H NMR ($CDCl_3$): 10.42 (s, 1H, meso-H), 9.54-9.55 (d, 2H, pyrrolic β-H), 9.07-9.13 (m, 6H, pyrrolic β-H), 5.18-5.35 (m, 21H, Glc-H), 4.30 (m, 6H, Glc-H), 3.90-3.92 (m, 3H, Glc-H), 2.06-2.23 (m, 36H, acetyl-H). ^{13}C NMR ($CDCl_3$): δ 20.58 (CH_3CO_2), 61.95, 68.11, 70.67, 73.92, 76.39, 84.87 (Glc), 103.16, 107.69, 111.69, 122.23, 130.35, 131.25-131.82, 133.43-133.50, 145.36-147.35 (C_6F_4), 150.55, 169.42, 169.49, 170.22 and 170.71 (CH_3CO_2). HRMS calcd for $C_{80}H_{66}F_{12}N_4O_{27}S_3Zn$ $(M)^{+\cdot}$ 1902.2176 found 1902.2176.

Synthesis of protected thioglycosylated triply bridged porphyrin, $Zn_2F_{24}GlcAc_6$ (5a)

A sample of porphyrin **4b** (20 mg, 0.0105 mmol) in 15 mL of CH_2Cl_2 was cooled to -78^{o}C and then PIFA (12 mg, 0.026 mmol) was added. Then, the cooling bath was removed, and the mixture was stirred at room temperature for about 2 h. The resulting ink-blue mixture was then washed with water several times; the organic layer was dried with anhydrous Na_2SO_4, filtered, and concentrated in vaccum. The residue was purified by flash column chromatography (silica gel, ethylacetate/hexane = 3:2) to give **5a** (8 mg, 40% yield). ^{19}F NMR (THF-d_8): δ -134.48 to -133.18 (m, 12F, Ar-m-F), -140.39 to -139.51 (m, 12F, Ar-*o*-F). ^{1}H NMR (DMSO-d_6): 7.93-7.94 (d, 4H, pyrrolic β-H), 7.85-7.86 (d, 4H, pyrrolic β-H), 7.35 (s, 4H, pyrrolic β-H), 5.36-5.51 (m, 10H, Glc-H), 5.01-5.05 (m, 13H, Glc-H), 4.05-4.16 (m, 19H, Glc-H), 2.03-2.13 (m, 72H, acetyl-H). ^{13}C NMR (DMSO-d_6): δ 20.78 (CH_3CO_2), 62.25, 68.41, 70.80, 73.55, 76.39,

84.92 (Glc), 107.83, 131.72, 131.81, 146.42-148.53 (C_6F_4), 152.77, 155.01, 169.71, 169.80, 170.08 (CH_3CO_2). MALDI $C_{160}H_{126}F_{24}N_8O_{54}S_6Zn_2$ $(M+H)^+$ 3804.960, found 3804.587.

Synthesis of thiogylcosylated triply bridged porphyrin, $Zn_2F_{24}Glc_6$ (5b)

Compound **5a** (6 mg, 15.7 μmol) was dissolved in methanol/CH_2Cl_2 (3:1, 4 mL) and treated with sodium methoxide (0.5 M solution in methanol, 1 mL). The reaction mixture was stirred at room temperature for 1 h and then neutralized by an aqueous citric acid solution. The mixture was filtered through Waters Sep-Pak C18 35 cm^3 reverse-phase prep column and washed with water. The deprotected porphyrin **5b** was eluted with methanol and purified by flash chromatography (silica gel) using a mixture of ethyl acetate/methanol (3:2) as an eluent. Porphyrin **5b** (4 mg, 91%) was obtained after crystallization in methanol/CH_2Cl_2 as a voilet powder. mp > 250 °C The ortho-flourine atom resonances remain near -135 ppm are nearly the same for **3** and **5a**. ^{19}F NMR (DMSO-d_6): δ -132.78 to -132.60 (m, 12F, Ar-m-F), -139.83 to -138.87 (m, 12F, Ar-*o*-F) ^{1}H NMR (DMSO-d_6): 8.00 ppm (d, 4H, Por-β) 7.92 ppm (d, 4H, Por-β), 7.42 ppm (s, 4H, Por-β) 5.56-5.58 (m, 12H, Glc-H), 5.08-5.12, (m, 12H, Glc-H), 4.11-4.18 (m, 18H, Glc-H), 3.23-3.26 (m, 24H), Glc-H).^{13}C NMR (DMSO-d_6): δ 61.78, 67.94, 70.33, 73.08, 74.76 (Glc),88.49, 107.38, 114.68, 118.67, 120.36, 126.44, 130.97, 136.37, 137.49, 138.49, 142.77-146.77(C_6F_4), 152.89, 153.93, 155.18 MALDI calcd. for $C_{112}H_{78}F_{24}N_8O_{30}S_6Zn_2$ $(M+H)^+$ 2795.9, found 2795.6.

NMR Spectra

The ^{1}H NMR spectra of 5a and 5b show diagnostic porphyrin pyrrole β protons as a singlet at 7.35 ppm (inner) and doublet of doublets at 7.90 ppm (outer). The ^{1}H NMR spectra of 5a show the acetyl resonance at 2.00 and 2.13 ppm, the other carbohydrate protons unit appear between 4 and 6 ppm, and the anomeric protons appear as doublets at 5.36 ppm to 5.50 ppm. The ^{19}F NMR spectra confirm the substitution of the para-fluorine atom by the sugar unit, showing the disappearance of the para-fluorine resonances in 3 and 5b at -153 ppm. An important diagnostic is that the meta-fluorine signal shifts from -162 ppm in 3 to -140 ppm in 5b.

UV-visible spectroscopy

Ultrospec 3300 pro from GE healthcare was used to record the UV-visible spectra of dilute solutions, typically ~ 3μM, of triply linked $Zn_2F_{24}Glc_6$ compounds in DMSO, toluene, ethyl acetate, ethanol, phosphate buffer saline (PBS) solvents. The spectra were recorded from 350 to 1120 nm using 1 cm quartz cuvettes. For triply linked Zn_2F_{30} compound, UV-visible spectra were recorded on a Varian Bio3 spectrophotometer using dilute solutions, typically ~ 2μM, of compound in DMSO, toluene, ethyl acetate, ethanol, and DCM.

Emission spectroscopy, fluorescence quantum yield, and fluorescence lifetime

Steady-state fluorescence (emission) spectra and fluorescence lifetime were measured with a Fluorolog τ3, Jobin-SPEX Instrument S.A., Inc. For singly linked compound emission spectra were recorded in DMSO, toluene, ethyl acetate, ethanol, and phosphate buffer saline (PBS) solvent in air and under nitrogen atmosphere by purging N_2 gas through these solutions for ca. 10 minutes. The concentrations of each compound in these solutions were typically ~3μM. In each case the compounds were excited at 483 nm where the O.D for each compound was 0.047. Both, the excitation and emission monochromators had a band pass of 2 nm. The corrected emission (for instrument response) and absorption (UV-visible) spectra were used to calculate the quantum yield. The quantum yields were calculated relative to ZnTPP in toluene,

which has a fluorescence quantum yield of 0.033.[20,21] The quantum yields were measured indirectly using ZnTPP; thus these values may have some systematic error. All experiments were carried out on the same day, using identical concentrations and similar experimental conditions to minimize any experimental errors. To measure the fluorescence decay lifetime, 401nm laser was used to excite the molecule, average power = 13.6 pJ/pulse, and emission decay was recorded at 620 nm. The band pass for both excitation and emission monochromators were 5 nm. The decays were obtained with 2000 counts or more in the peak channel. The data was fitted with first exponential fit curve and the goodness of the fit was judged by reduced χ^2 values, the random distribution of residuals and the autocorrelation of the residuals.

The UV-visible spectra of **2** and **3**, and the fluorescence spectra of **2** (ESI 35-37) are consistent with those reported by Kim and coworkers.[9] The UV-visible and fluorescence spectra of thioglycosylated monomer, 4a and 4b (ESI 24-25) are similar to those reported by Nocera and coworkers.[12] The UV-visible spectra of 5b and 6b are similar to previous report for **3** and **2**, respectively,[9] but with a stronger solvent dependence.

Dynamic Light Scattering (DLS) for particle size measurement

A solution of triply linked $Zn_2F_{24}Glc_6$ was found to form aggregates in various studied solvents. A Precision Detector PD2000DLS Cool-Batch dynamic light scattering (DLS) instrument was used in batch mode at 25 ^oC to determine particle size. These solutions were also sonicated in Fisher SF15 sonicator to break the large aggregates into the small ones. To measure the size of triply linked $Zn_2F_{24}Glc_6$ in 2% solution of DMSO in PBS, the solution was sonicated for ca. 30 minutes and left to rest for another 30 min and then the size was measured. Without sonication large sized particle were observed mainly but after sonication the large sized particles, 284±15 nm broke down to smaller sized particles, 82±7 nm.

Quantum yield of singlet oxygen production

The quantum yield of singlet oxygen ($^1O_2$) production (Φ_Δ) for $Zn_2F_{24}Glc_6$ in DMSO was determined by exciting the chromophore at 532 nm by time-resolved Nd:YAG laser as previously reported, in which the $^1O_2$ phosphorescence at 1270 nm was monitored [22,23] *meso*-tetra(4-sulfonatophenyl)porphine dihydrochloride (TSPP) was employed as a reference sensitizer ($\Phi_{\Delta,TSPP}$ = 0.63 in D_2O).[24] $Zn_2F_{24}Glc_6$ was tested in DMSO. The absorbance from $Zn_2F_{24}Glc_6$ and TSPP at 532 nm was matched within the range of 0.05-0.2. $^1O_2$ luminescence was monitored as a function of sensitizer absorbance. Slopes were analyzed from a plot of $^1O_2$ intensity via absorbance (Figure ESI 2). Φ_Δ can be calculated according to the following equation.

$$\frac{\Phi_{\Delta,\,Zn_2F_{24}Glc_6}}{\Phi_{\Delta,\,TSPP}} = \frac{Slope_{\,Zn_2F_{24}Glc_6}}{Slope_{\,TSPP}}$$

Here, $\Phi_{\Delta,\,Zn2F24Glc6}$ and $\Phi_{\Delta,\,TSPP}$ are the Φ_Δ from Zn_2F_{24} Glc_6 and reference TSPP, respectively, and $S_{Zn2F24\ Glc6}$ and S_{TSPP} represent the slopes obtained from the plot of initial intensities of $^1O_2$ via the absorbance at an excitation wavelength of 532 nm for $Zn_2F_{24}Glc_6$ and reference TSPP, respectively. System errors may exist in different sample media, which deviated intercepts from origin. Those deviations do not affect the slope calculations and can be corrected by the parallel shift of $^1O_2$ intensities along y-axis.

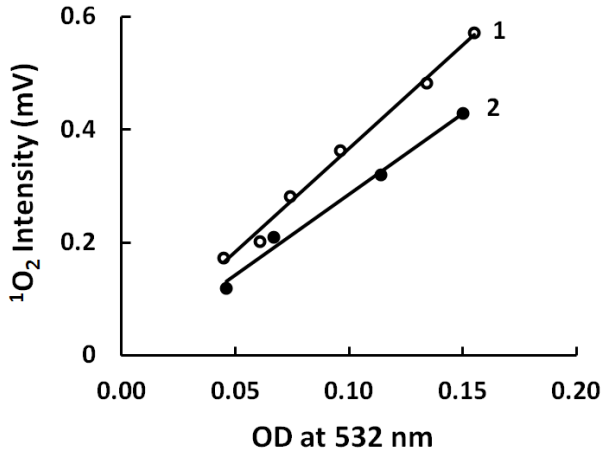


Figure ESI 2. Changes in $^1O_2$ Intensity as a function of OD at 532 nm for Zn_2F_{24} Glc_6 in DMSO (1) and TSPP in D_2O (2)

Cell Culture. MDA-MB-231, a human breast cancer cells were maintained in DMEM, 10% FBS, 1% antimycotic at 37 °C and in 5% CO_2 atmosphere were plated onto coverslips in cell culture dishes for 24 hours. Cells were then incubated with triply linked $Zn_2F_{24}Glc_6$ compound dissolved in DMSO to a final concentration of 50 nM such that there was never more than 0.5% DMSO in the solution. After 24 h incubation, cells were washed with PBS 5-6 times to remove the unbound compound and then fixed with 4% paraformaldehyde solution for 15 min at room temperature. The cells were then washed with PBS 3 times again and then mounted on glass slides with Dako fluorescent mounting medium and left to air-dry for 1 hour. Corners were sealed with clear nail polish. The cells on slides were then visualized using a Nikon Optiphot 2 fluorescence microscope. Images were captured as JPEG files at 20X magnification, with a 505-565 nm excitation band-pass filter and a 565-685 nm emission band-pass filter. Four days later, the same fixed slides were again visualized under same conditions of original images. For each set of experiments, cells were cultured and the fluorescence images were taken under identical culture and microscopic conditions.

Two photon fluorescence for **5b** was investigated using MDA-MB-231 cell lines. The specimens were excited using a tuneable Ti-Sapphire at 820 nm. The emitted light was collected between 500 and 670 nm because the compound has very low or almost no absorption at this excitation wavelength. No significant two photon fluorescence was observed in this band pass filter This implies a significant fraction of the excited state enters the triplet manifold. Two photon microscopy also reveals no significant two photon fluorescence using excitations at 900 nm or 960 nm.[9]

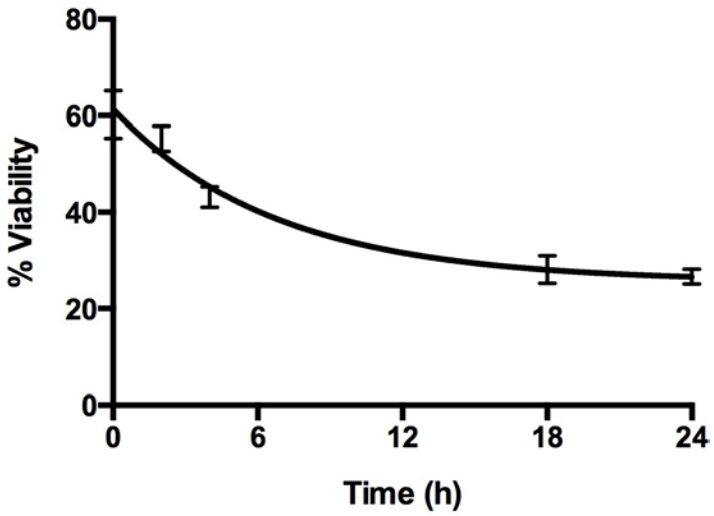


Figure ESI 3: After treating MDA-MB-231 with **5b** (10 μM) for 24 hours and removing the unbound dye, cell viability studies were assayed at different time intervals after photodynamic treatment with white light with (0.92 mW cm^{-2}; 11.04 kJ m^{-2} for 20 min).

PDT Studies

Cell Culture. All tissue culture medium and reagents were purchased. Dulbecco's Modified Eagle Medium (DMEM) from Sigma Aldrich and FBS and antibiotic (Penicillin Streptomycin) from Invitrogen (Carlsbad, CA). MDA-MB-231 cells were purchased from ATCC and cultured in DMEM in 10% FBS and 1% antibiotic. The cells were split once every two days to maintain a sub-confluent stock.

(a) Dark toxicity

Then cells (10000) were seeded in a 24 well plate and incubated at 37^0C and in CO_2 atmosphere for 24 h until 70% confluence was observed. Porphyrin stock solutions (32 mM) was prepared in DMSO and then diluted with DMEM into final working concentrations (1.56, 3.15, 6.25, 12.5, 25 μM). Compound with different concentrations were added to the 70% confluent cells and incubated for 24 h. Then the medium containing porphyrin was removed and washed once with PBS (pH ~ 7.4). 0.4% Trypan blue (Life Technologies™) was added to cells. The mixture was incubated at room temperature for 10 min, and trypan blue uptake was determined by counting on a hemacytometer. The IC_{50} values were calculated from dose-response curves, which were obtained using GraphPad Prism software.

(b) Photo toxicity:

MDA-MB-231 cells were plated as described above. Then working concentrations (1.56, 3.15, 6.25, 12.5, 25 μM) of compound 5b was added and incubated for 24 h. Then the medium was replaced with the fresh medium. The cells were then exposed to a white 13 W fluorescent light (0.92 mW cm^{-2} or 11.04 kJ m^{-2}) for 20 min. Then the medium containing porphyrin was removed. 0.4% Trypan blue (Life Technologies™) was added to cells. The mixture was incubated at room temperature for 10 min, and trypan blue uptake (dead cells) was determined by counting on a hemacytometer. The IC_{50} values were calculated from dose-response curves, which were

obtained using GraphPad Prism software.

(c) Photo toxicity (indications of apoptosis):

To the MDA-MB-231 cells compound 5b at 25 μM concentration was added and incubated for 24 h. Then the medium was replaced with the fresh growth medium. The cells were then exposed to a white 13 W fluorescent light (0.92 mW cm^{-2} or 11.04 kJ m^{-2}) for 20 min. Then the cells were incubated for several time intervals (0, 2, 4, 18 and 24 h) before replacing the medium with 0.4% Trypan blue and incubated for 10 min, and trypan blue uptake (dead cells) was determined by counting on hemacytometer.

Data File	HCMDSS49A.d	**Sample Name**	FBF15
Sample Type	Sample	**Position**	P1-A4
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	8/4/2010 2:36:17 PM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=808.0744 M=HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 2: C38H11F15N4	0.275	808.0747	46509	C38H11F15N4	808.0744	0.4
Cpd 1: C18H35NO	0.275	281.2721	27995	C18H35NO	281.2719	0.75

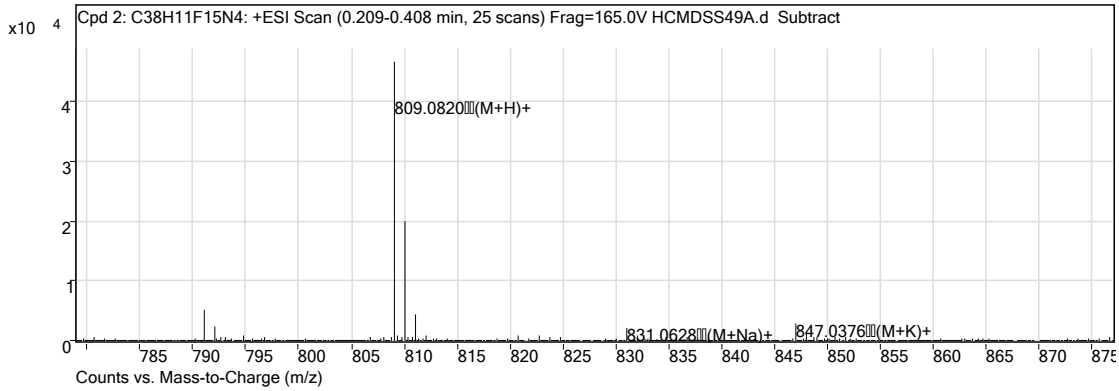


Figure ESI 4: Porphyrin P-F_{15} (**1a**) HRMS.

Data File	HCMDSS53A.d	**Sample Name**	ZnF15
Sample Type	Sample	**Position**	P1-A1
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	9/17/2010 4:54:46 PM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=869.9879 M=HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 2: C38H9F15N4Zn	0.263	869.9875	21608	C38H9F15N4Zn	869.9879	-0.53

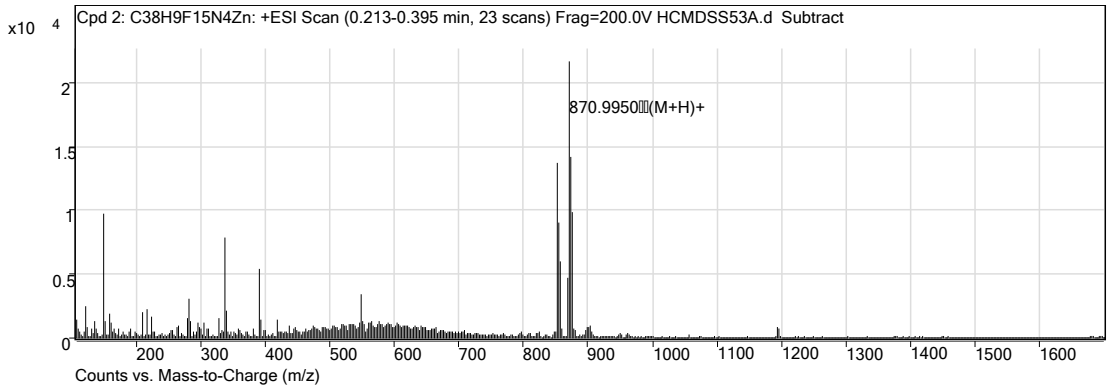


Figure ESI 5: Porphyrin ZnP-F_{15} (**1b**) HRMS.

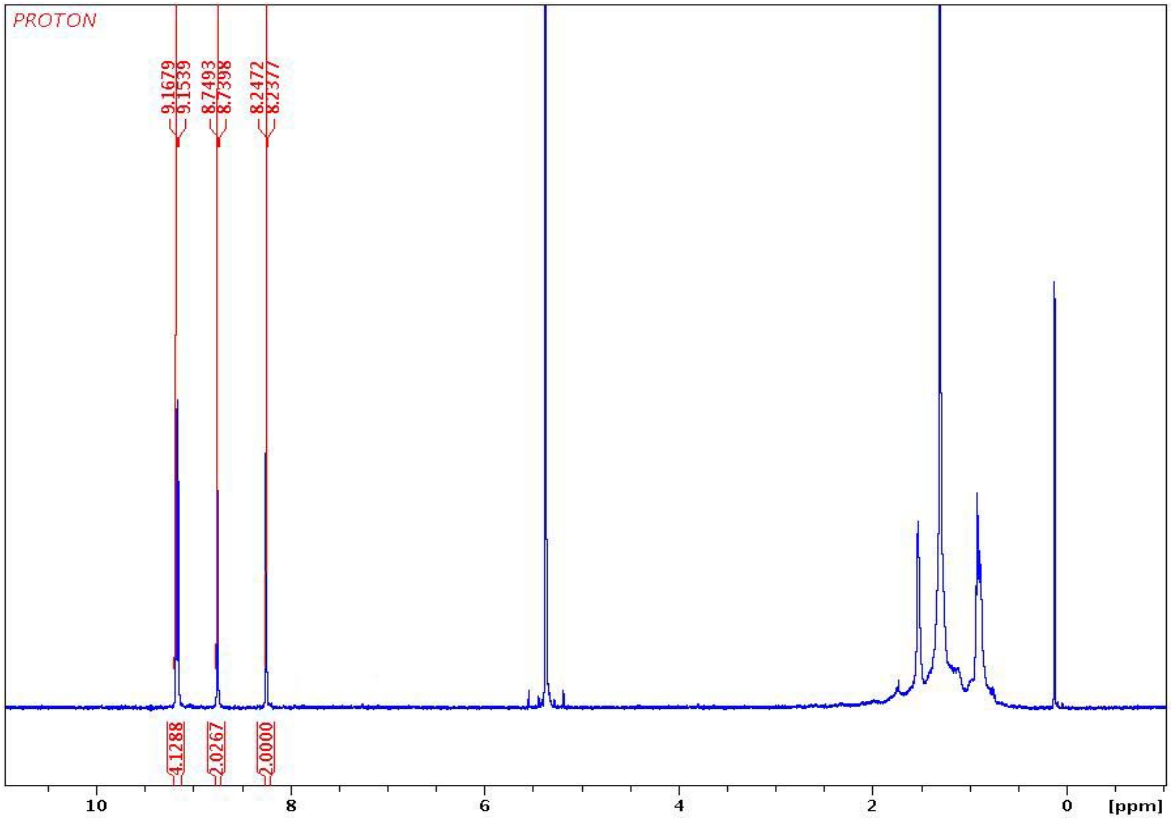


Figure ESI 6: *Meso-Meso* singly linked Zn^{II}-diporphyrin **(2)** ^{1}H NMR CD_2Cl_2(5.32 ppm, water 1.5 ppm)

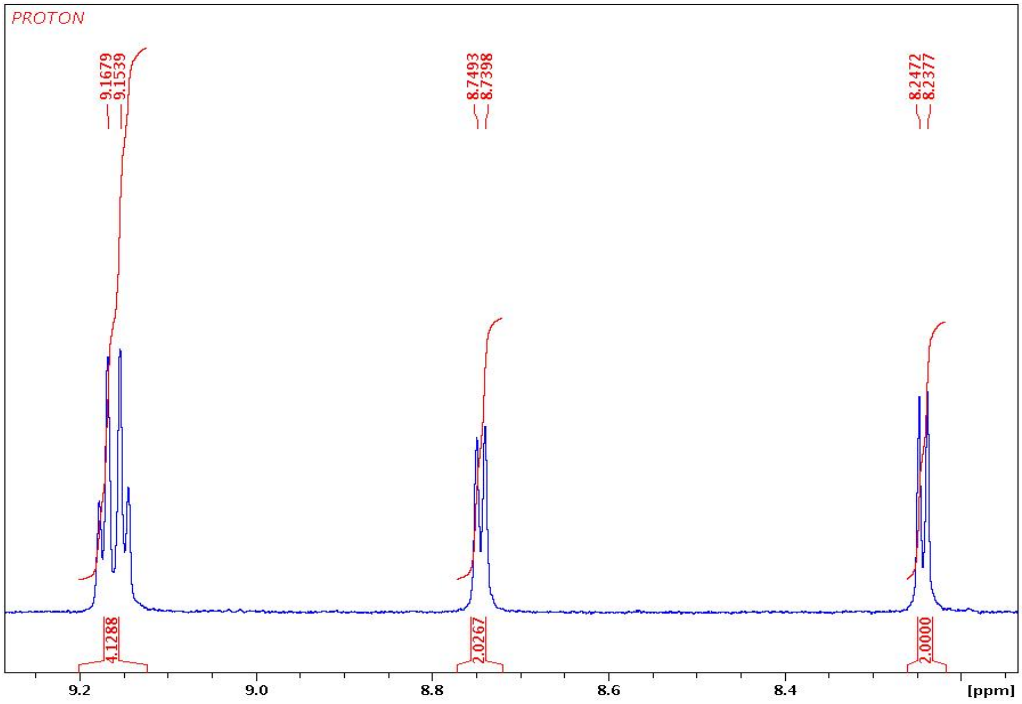


Figure ESI 7: Expanded ^{1}H NMR spectrum of compound **2.**

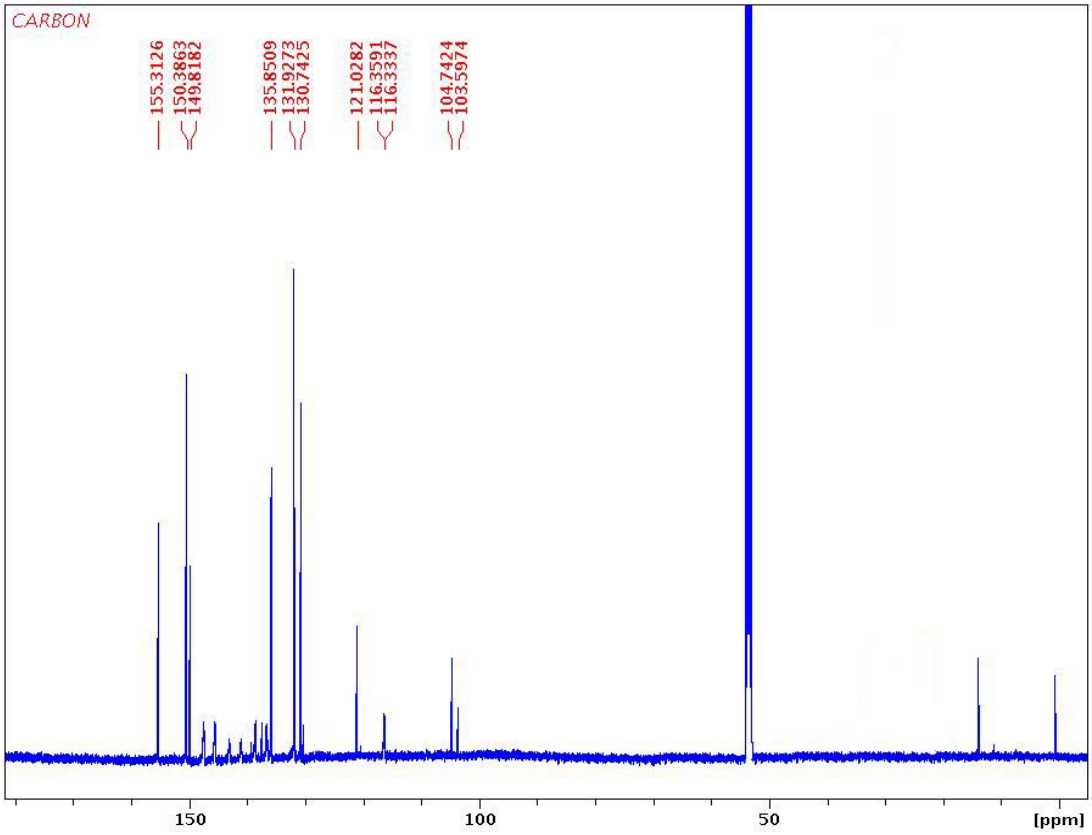


Figure ESI 8: *meso-meso* singly linked Zn^{II}-diporphyrin **(2)** ^{13}C NMR (CD_2Cl_2).

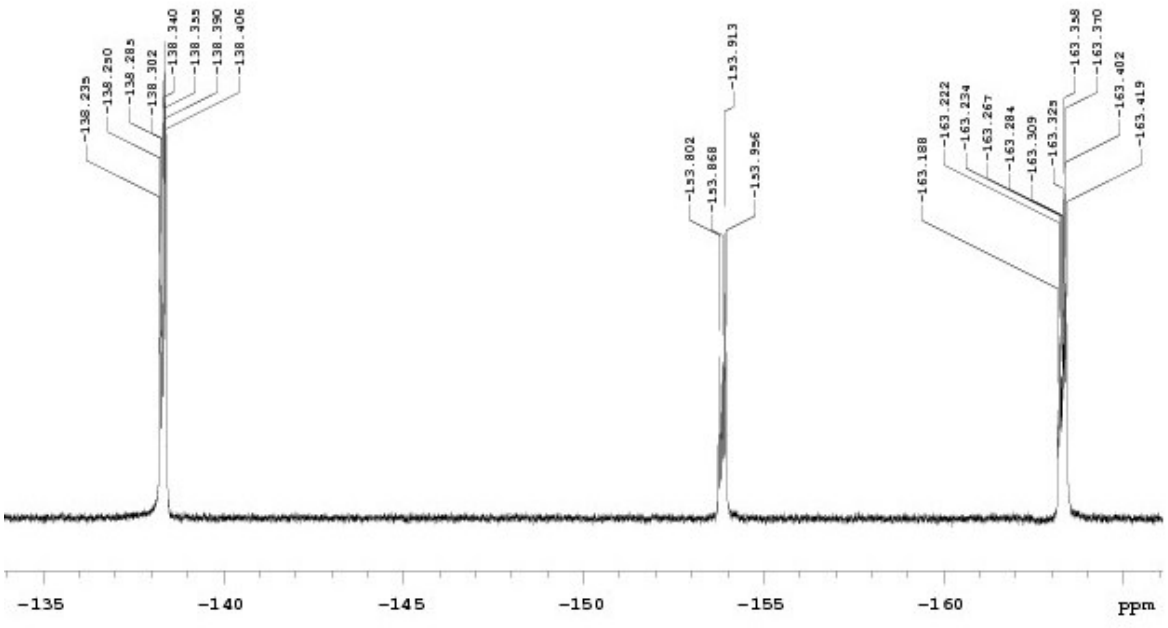


Figure ESI 9: *meso-meso* singly linked Zn^{II}-diporphyrin **(2)** ^{19}F NMR (CD_2Cl_2).

Data File	HCMDSS60E.d	**Sample Name**	Zndiporphyrin-2
Sample Type	Sample	**Position**	P1-A1
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	12/29/2010 3:12:18 PM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=1737.9602 EM=HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C76H16F30N8Zn2	0.212	1737.9618	12355	C76H16F30N8Zn2	1737.9602	0.96

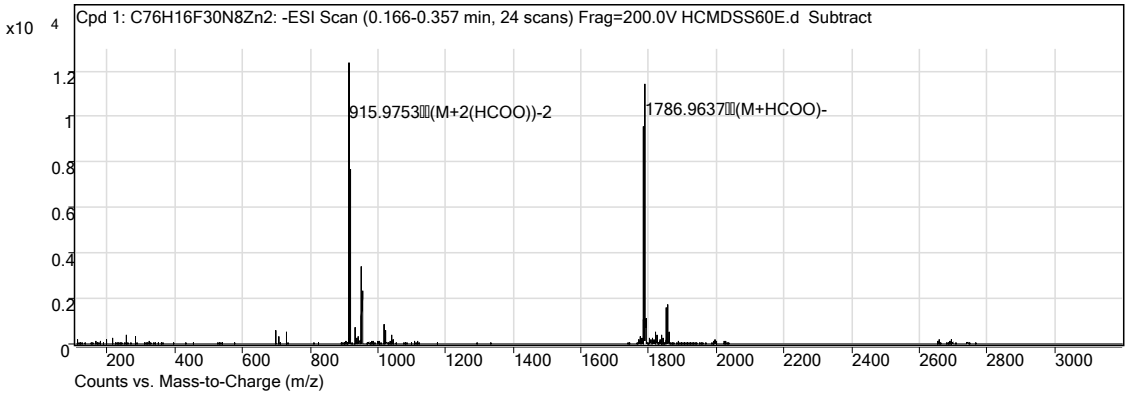


Figure ESI 10: *meso-meso* singly linked Zn^{II}-diporphyrin **(2)** HRMS.

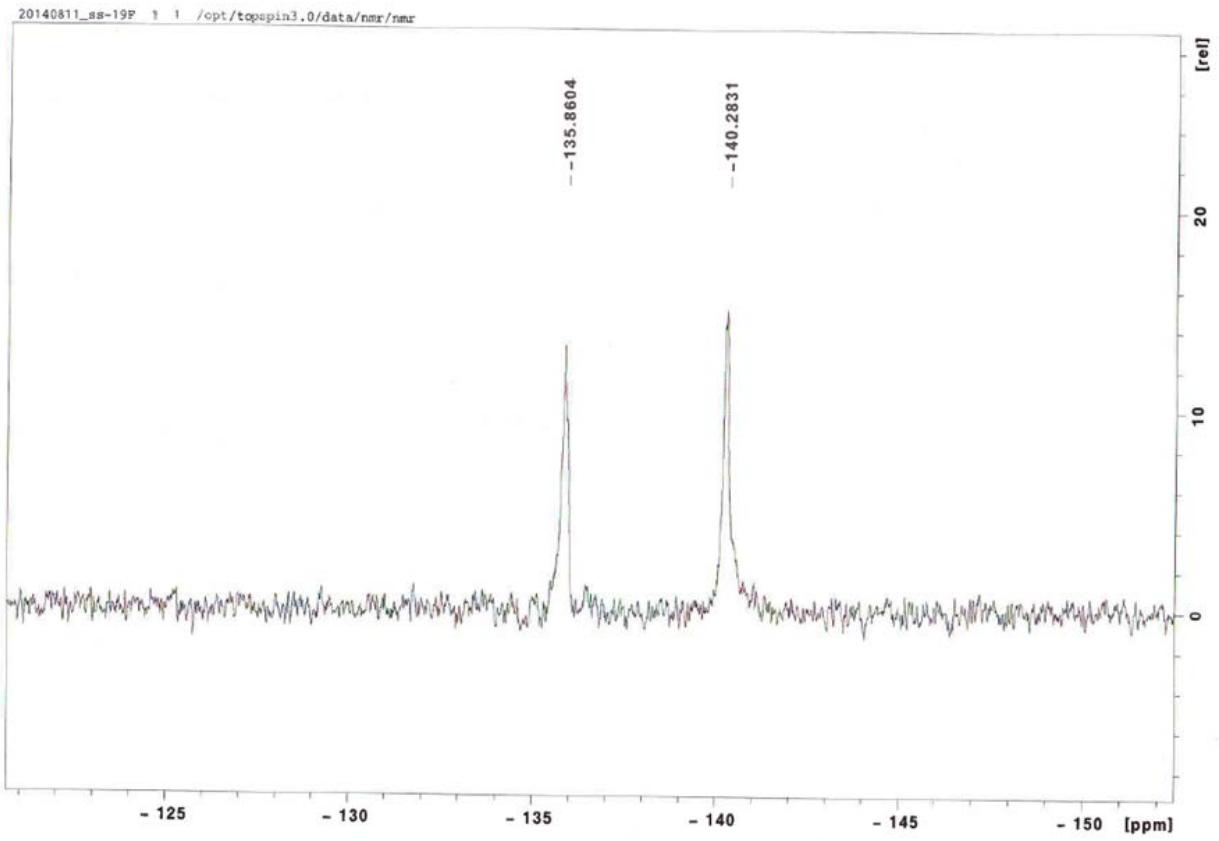


Figure ESI 11: *meso-meso* singly linked glycosylated Zn^{II}-diporphyrin **(6b)** ^{19}F NMR (MeOD-d_4).

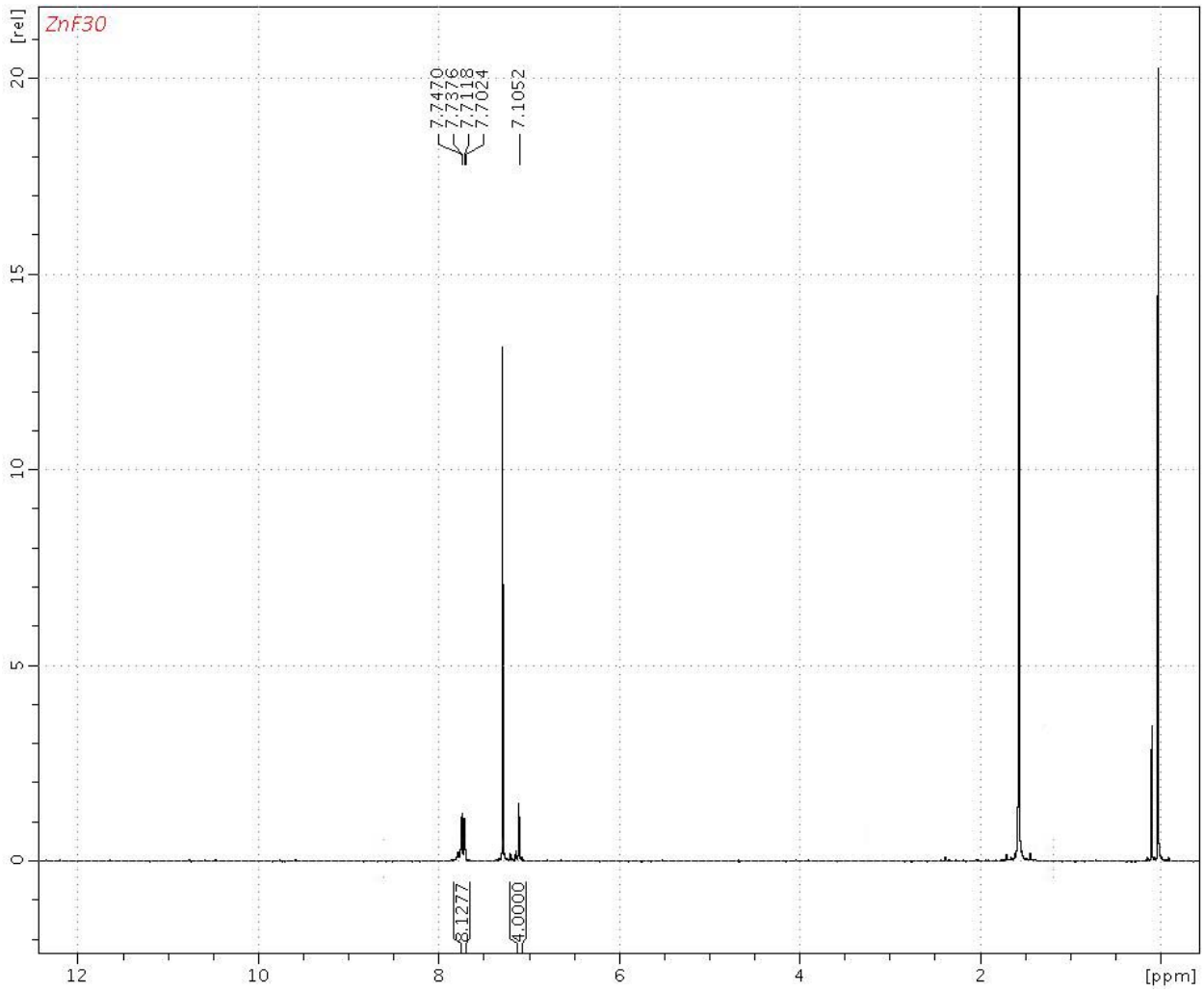


Figure ESI 12: *meso-meso*, β-β, β-β triply linked Zn^{II}-diporphyrin **(3)** ^{1}H NMR $CDCl_3$ (7.28 ppm, water 1.5 ppm)

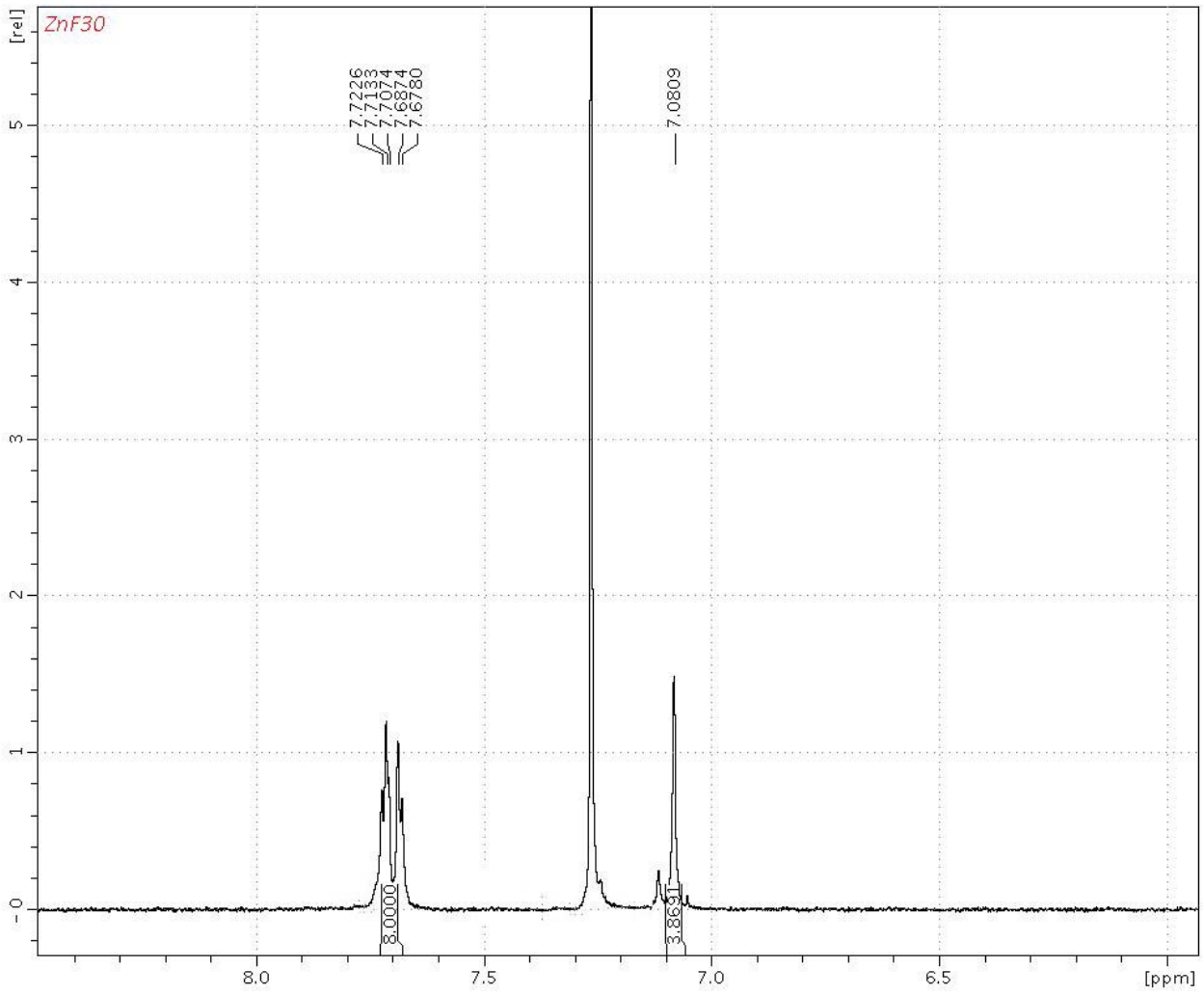


Figure ESI 13: Expanded spectrum ^{1}H NMR *meso-meso*, *β-β*, *β-β* triply linked Zn^{II}-diporphyrin **(3)**

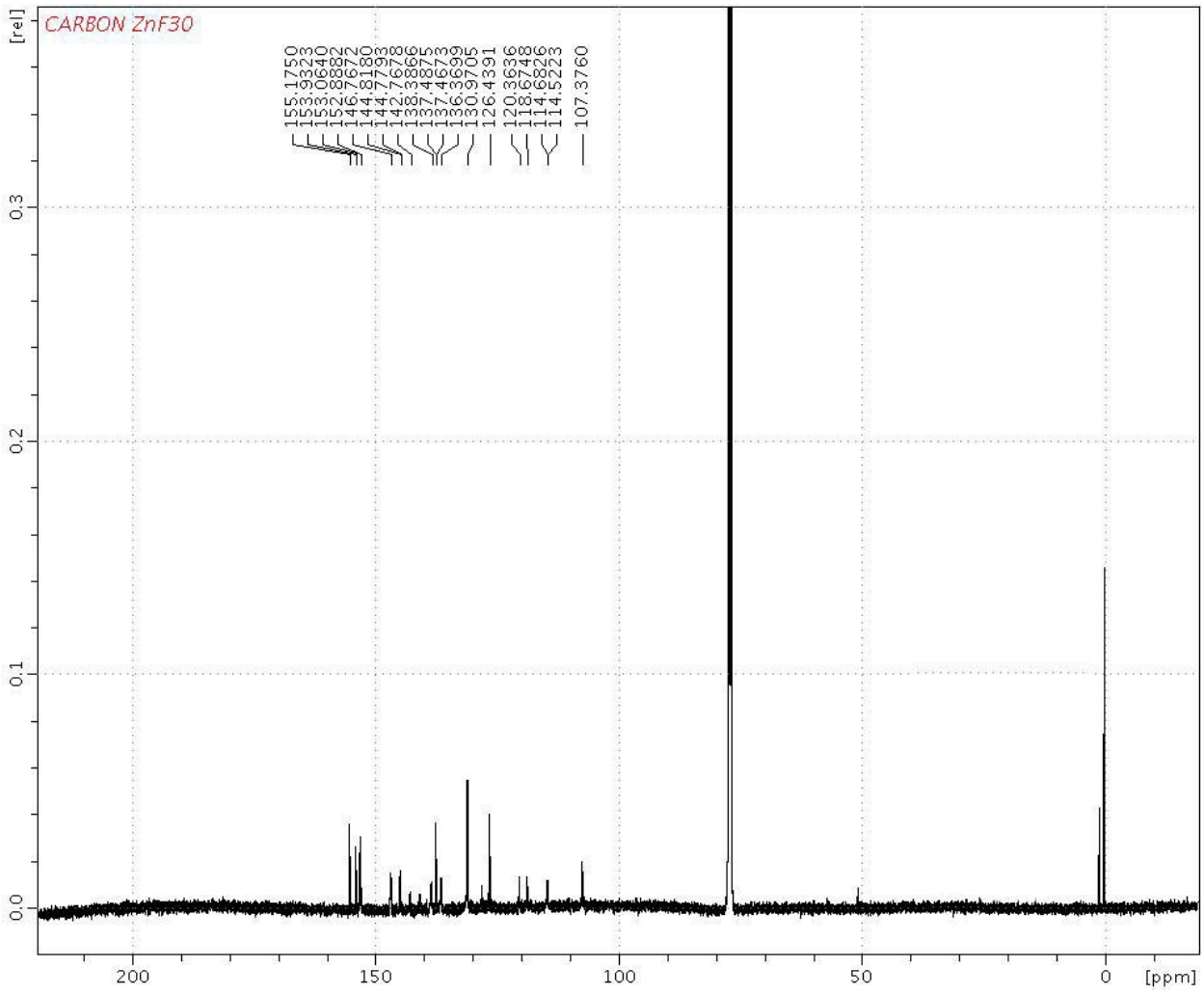


Figure ESI 14: *meso-meso, β-β, β-β* triply linked Zn^{II}-diporphyrin **(3)** ^{13}C NMR $CDCl_3$.

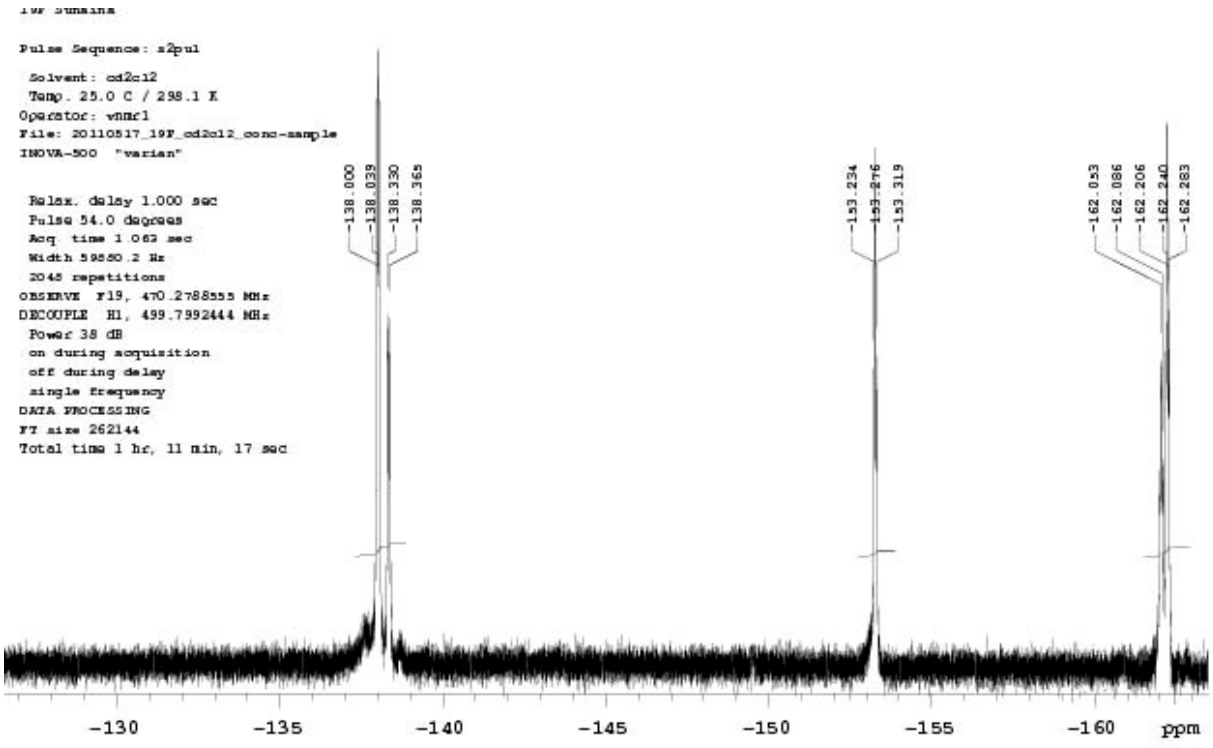


Figure ESI 15: *meso-meso*, *β-β*, *β-β* triply linked Zn^{II}-diporphyrin **(3)** ^{19}F NMR (CD_2Cl_2)

Data File	HCMDSS61A.d	**Sample Name**	Zndiporphyrin-1
Sample Type	Sample	**Position**	P1-A2
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	12/29/2010 4:51:03 PM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=1733.9289 EM=HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C76H12F30N8Zn2	0.219	1733.9301	6155	C76H12F30N8Zn2	1733.9289	0.68

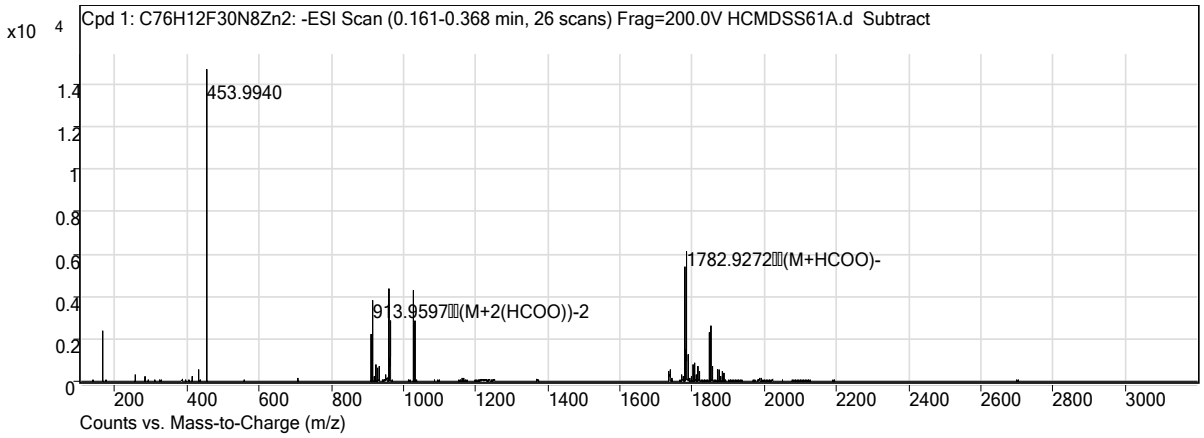


Figure ESI 16 : *meso-meso, β-β, β-β* triply linked Zn^{II} diporphyrin **(3)** HRMS.

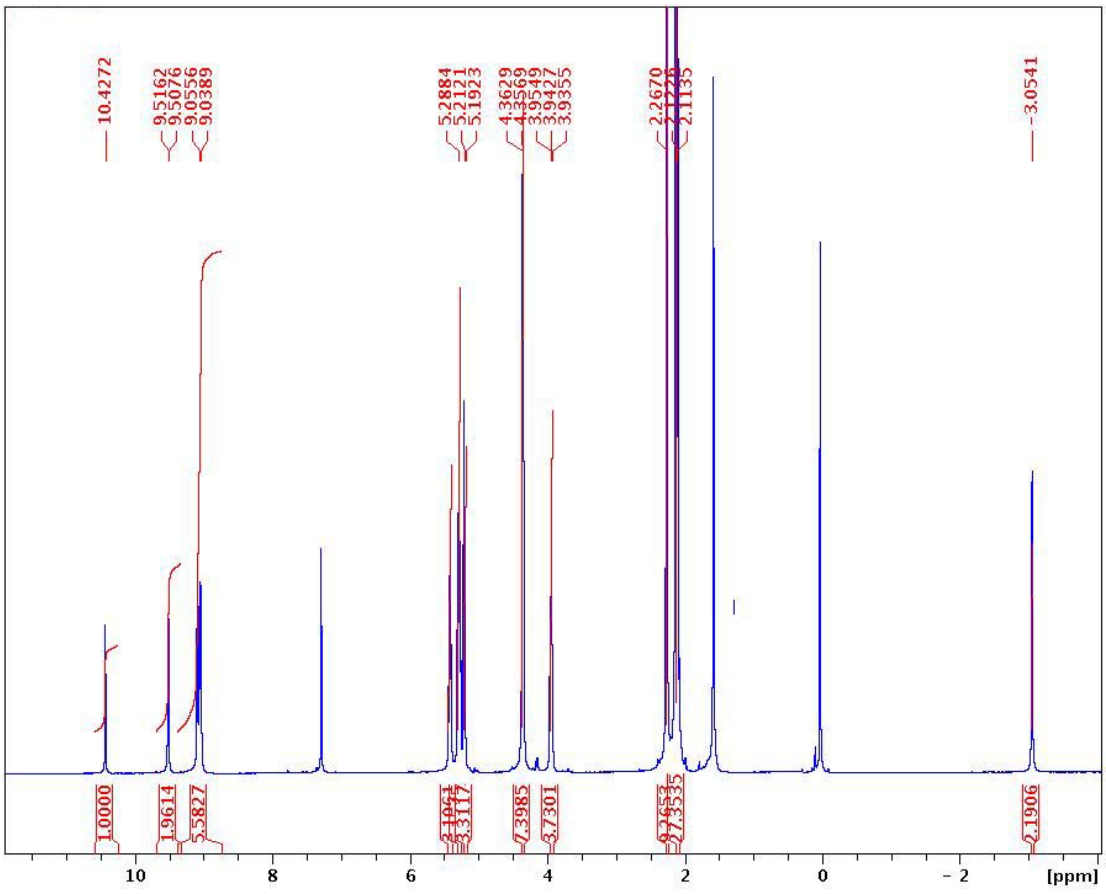


Figure ESI 17: $FBGlc_3$ **(4a)** ^1H NMR in $CDCl_3$ ($CHCl_3$ 7.28 ppm, water 1.6 ppm)

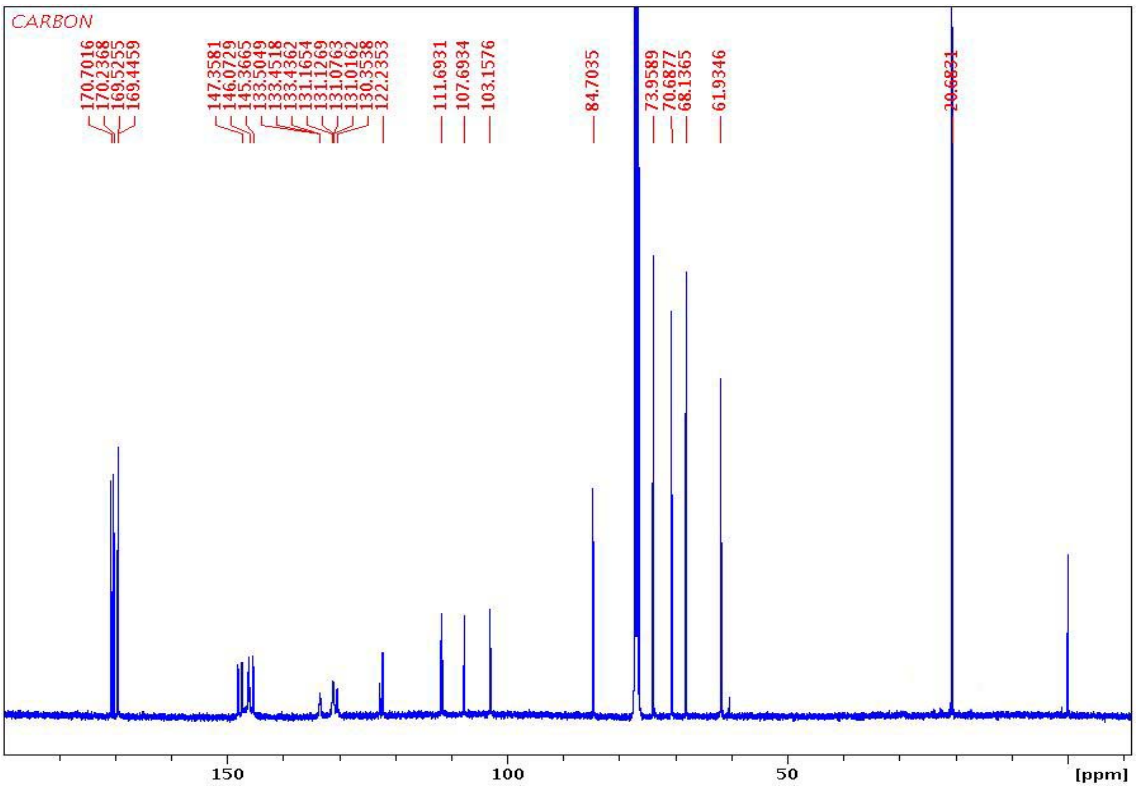


Figure ESI 18: $FBGlc_3$ **(4a)** ^{13}C NMR ($CDCl_3$).

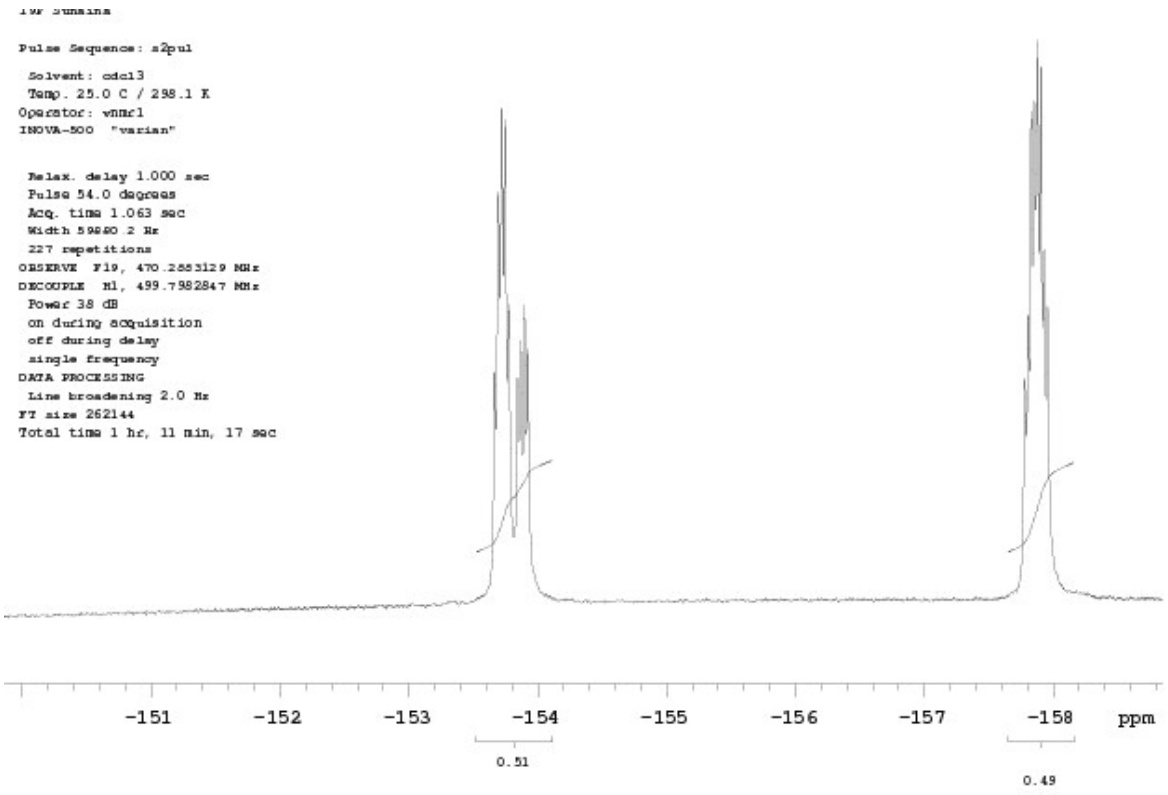


Figure ESI 19: $FBGlc_3$ **(4a)** ^{19}F NMR ($CDCl_3$)

Data File	HCMDSS56B.d	**Sample Name**	FBGlu3
Sample Type	Sample	**Position**	P1-A1
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	9/27/2010 6:11:40 PM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=1840.3041 EM=HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C80H68F12N4O27S3	0.239	1840.3056	1605	C80H68F12N4O27S3	1840.3041	0.81
Cpd 2: C80H71F12N5O27S3	0.288	1857.331	19655	C80H71F12N5O27S3	1857.3307	0.14

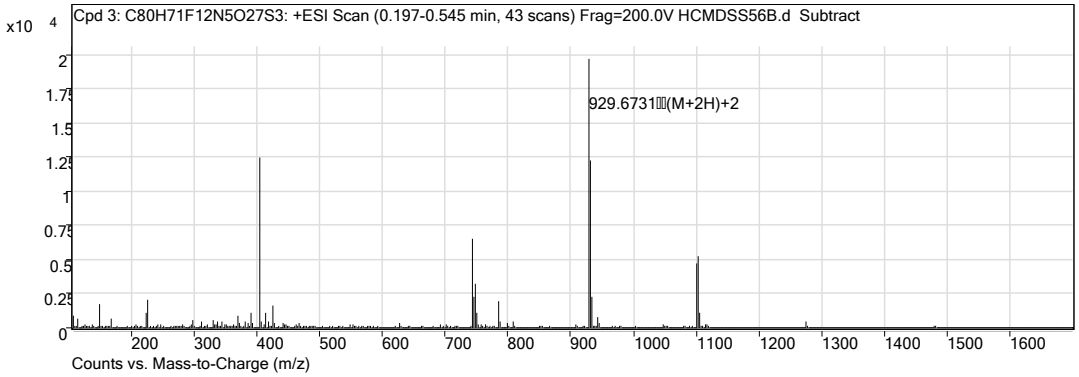


Figure ESI 20: $FBGlc_3$ **(4a)** HRMS

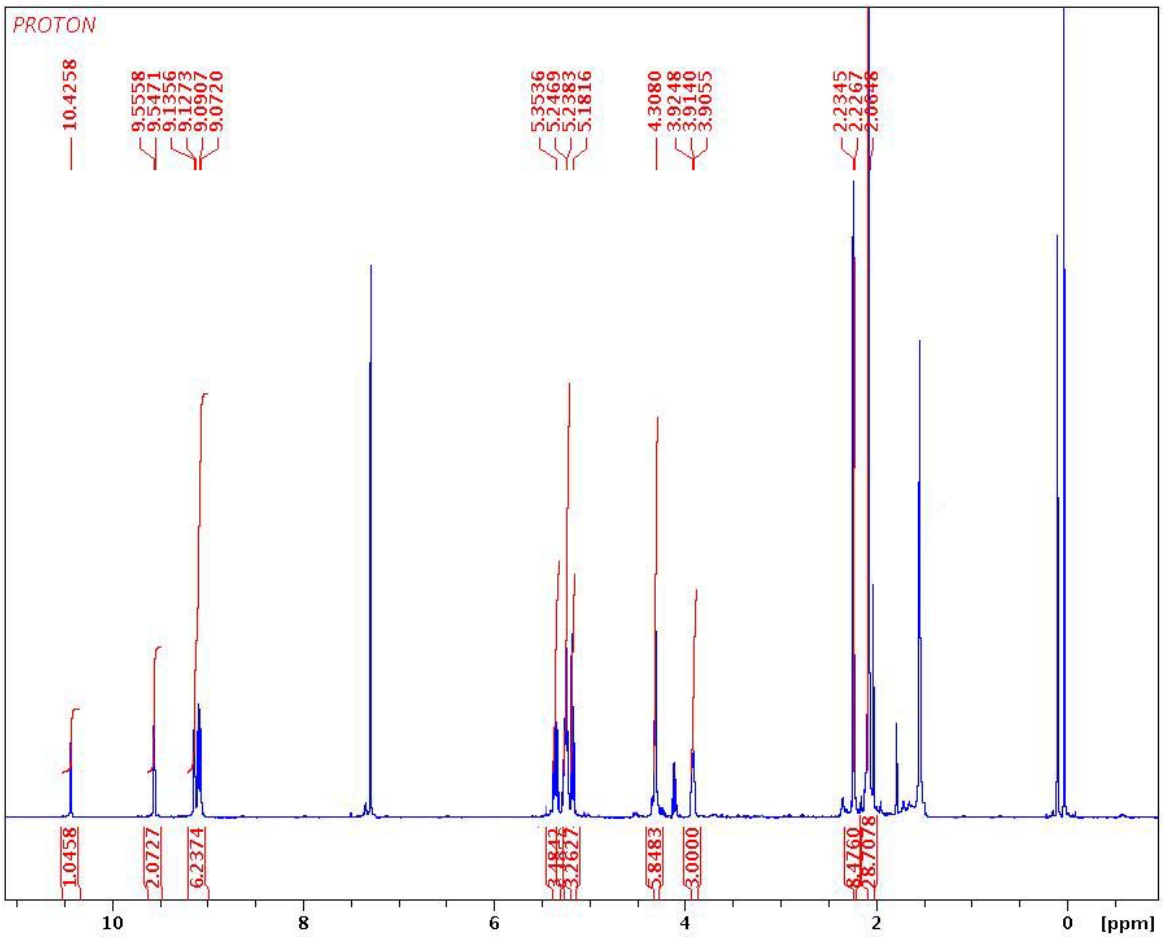


Figure ESI 21: $ZnGlc_3$ **(4b)** ^1H NMR in $CDCl_3$ ($CDCl_3$ 7.28 ppm, water 1.6 ppm)

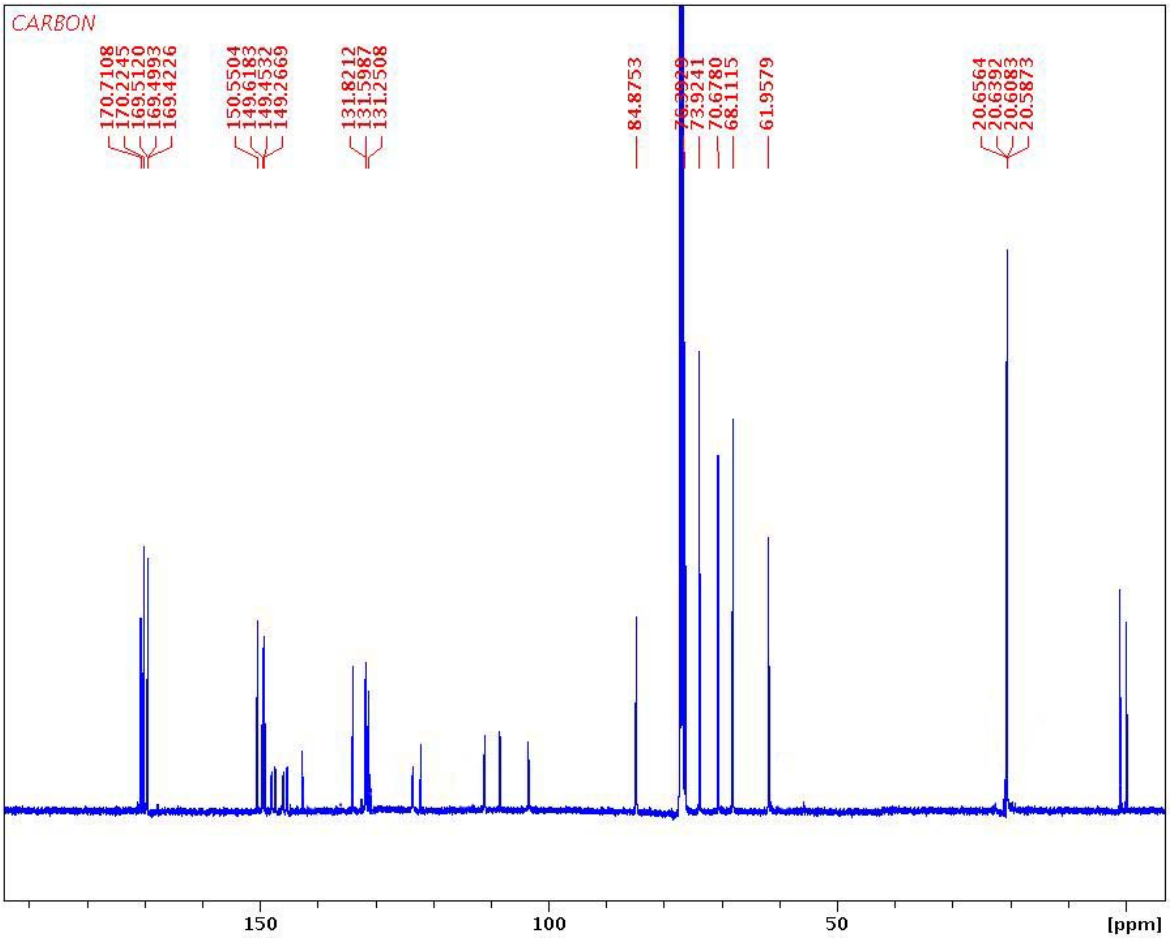


Figure ESI 22: $ZnGlc_3$ **(4b)** ^{13}C NMR $CDCl_3$

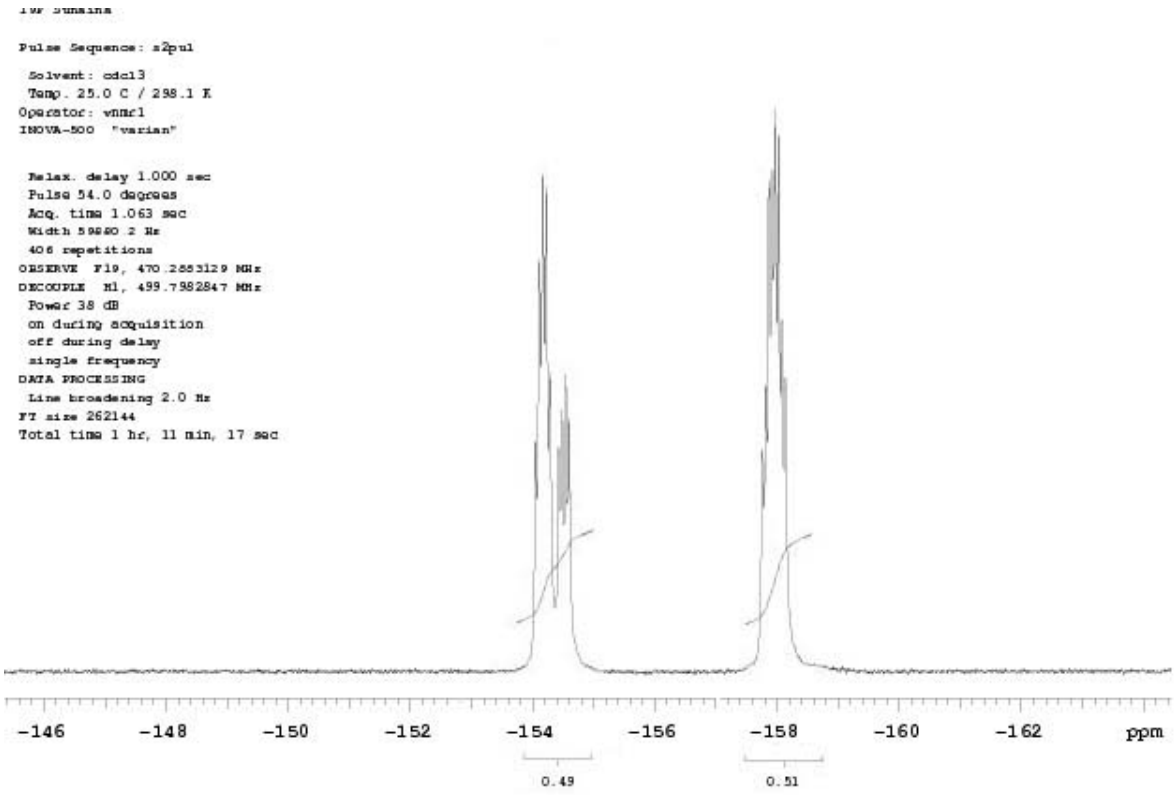


Figure ESI 23: $ZnGlc_3$ **(4b)** ^{19}F NMR $CDCl_3$

Dat File	HCMDSS57A.d	**Sample Name**	ZnGlu3
Sample Type	Sample	**Position**	P1-A1
Instrument Name	Instrument 1	**User Name**	
Acq Method		**Acquired Time**	9/29/2010 6:21:31 PM
IRM Calibration Status	Success	**DA Method**	HCEmpirical1.m
Comment	EM=1902.2176 EM=HC ESI Pos Small Molecule No HPLC.m		

Compound Table

Compound Label	RT	Mass	Abund	Formula	Tgt Mass	Diff (ppm)
Cpd 1: C80H66F12N4O27S3Zn	0.261	1902.2176	23190	C80H66F12N4O27S3Zn	1902.2176	-0.03

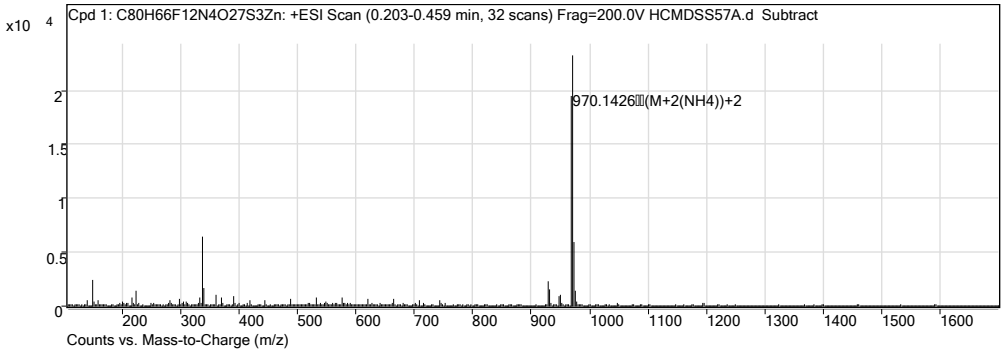


Figure ESI 24: $ZnGlc_3$ **(4b)** HRMS

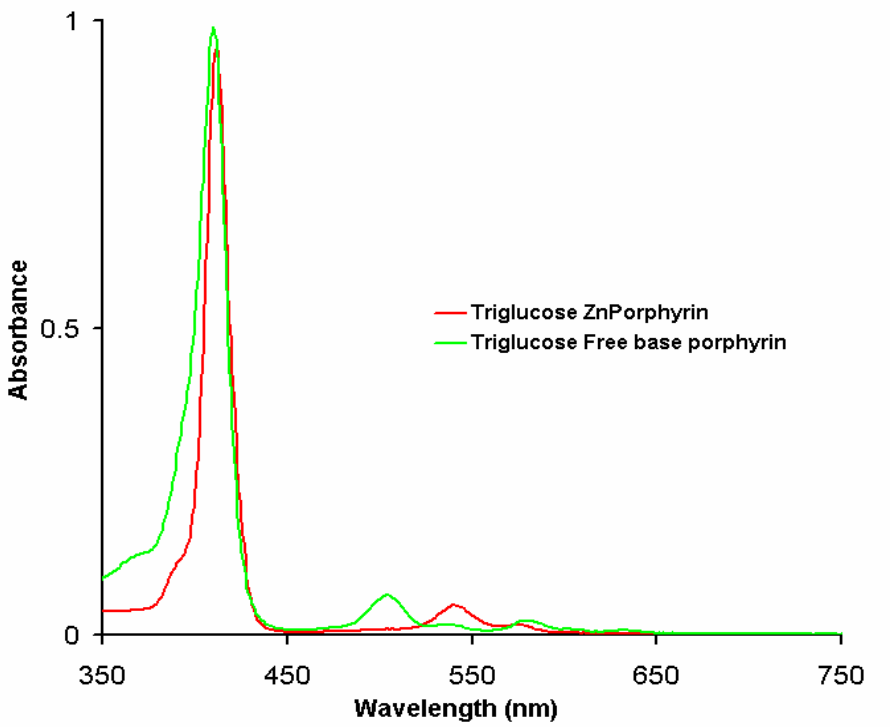


Figure ESI 25: UV-visible spectra of porphyrin **4a** and **4b.**

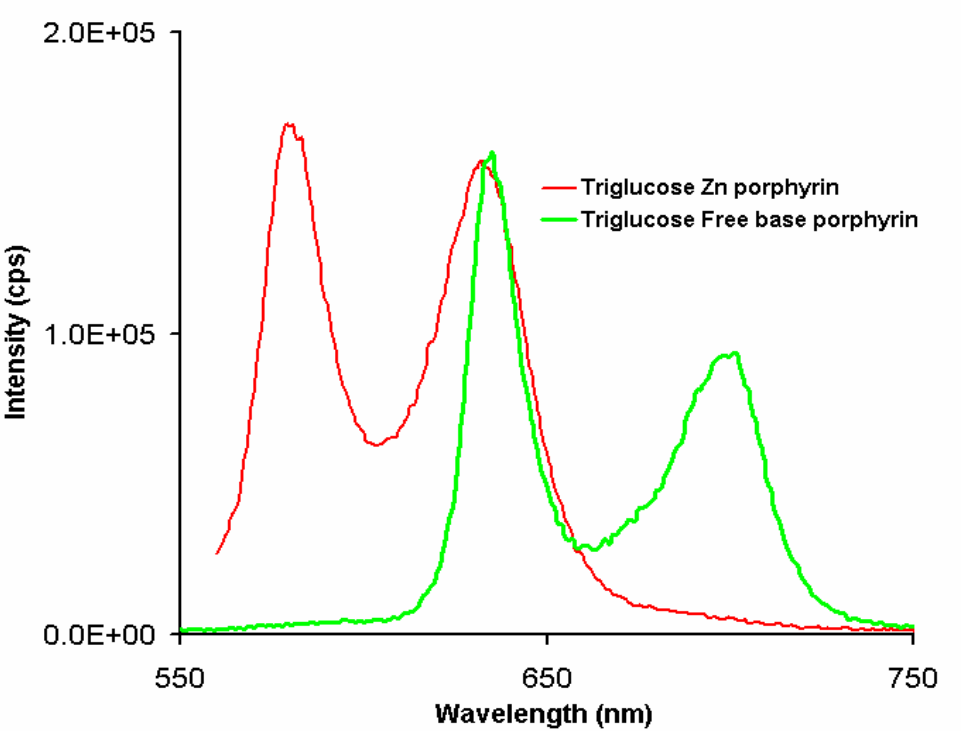


Figure ESI 26: Emission spectra of porphyrin **4a** and **4b**.

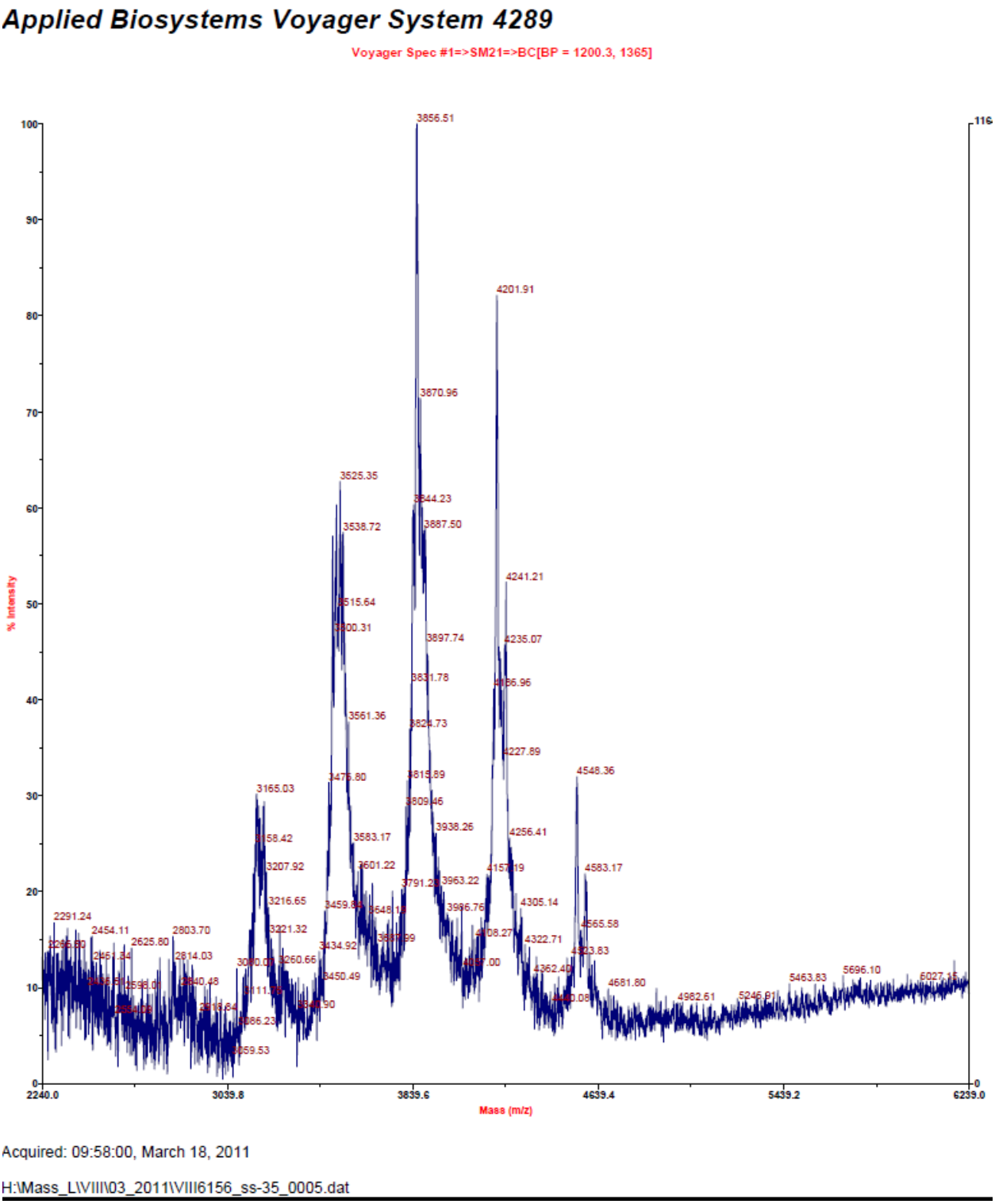


Figure ESI 27: MALDI-TOF spectrum of mixture of 4,5,6,7 and 8 glycosylated compounds/isomers resulting from reactions substituting the six *para* fluoro groups on compound **3** with the thioglucose.

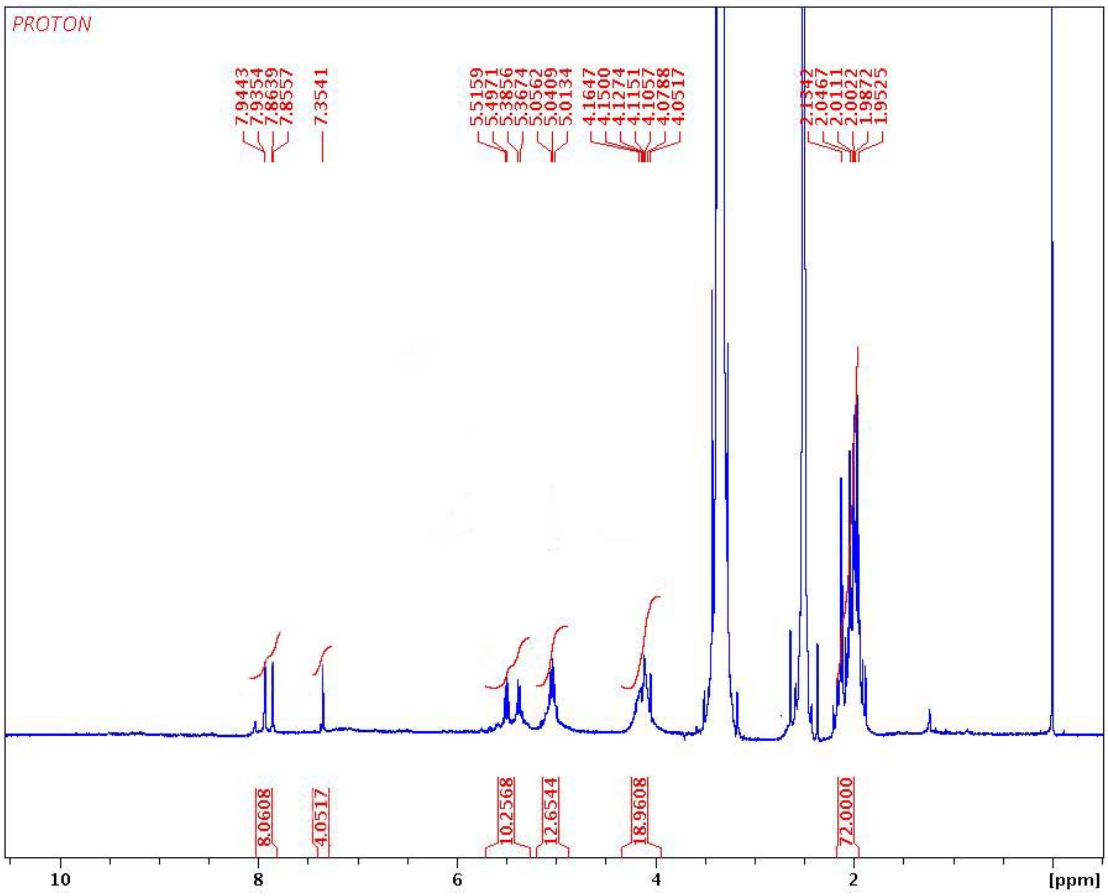


Figure ESI 28: $Zn_2F_{24}GlcAc_6$ **(5a)** ^{1}H NMR DMSO-d_6 (DMSO 2.5 ppm, water 3.34 ppm)

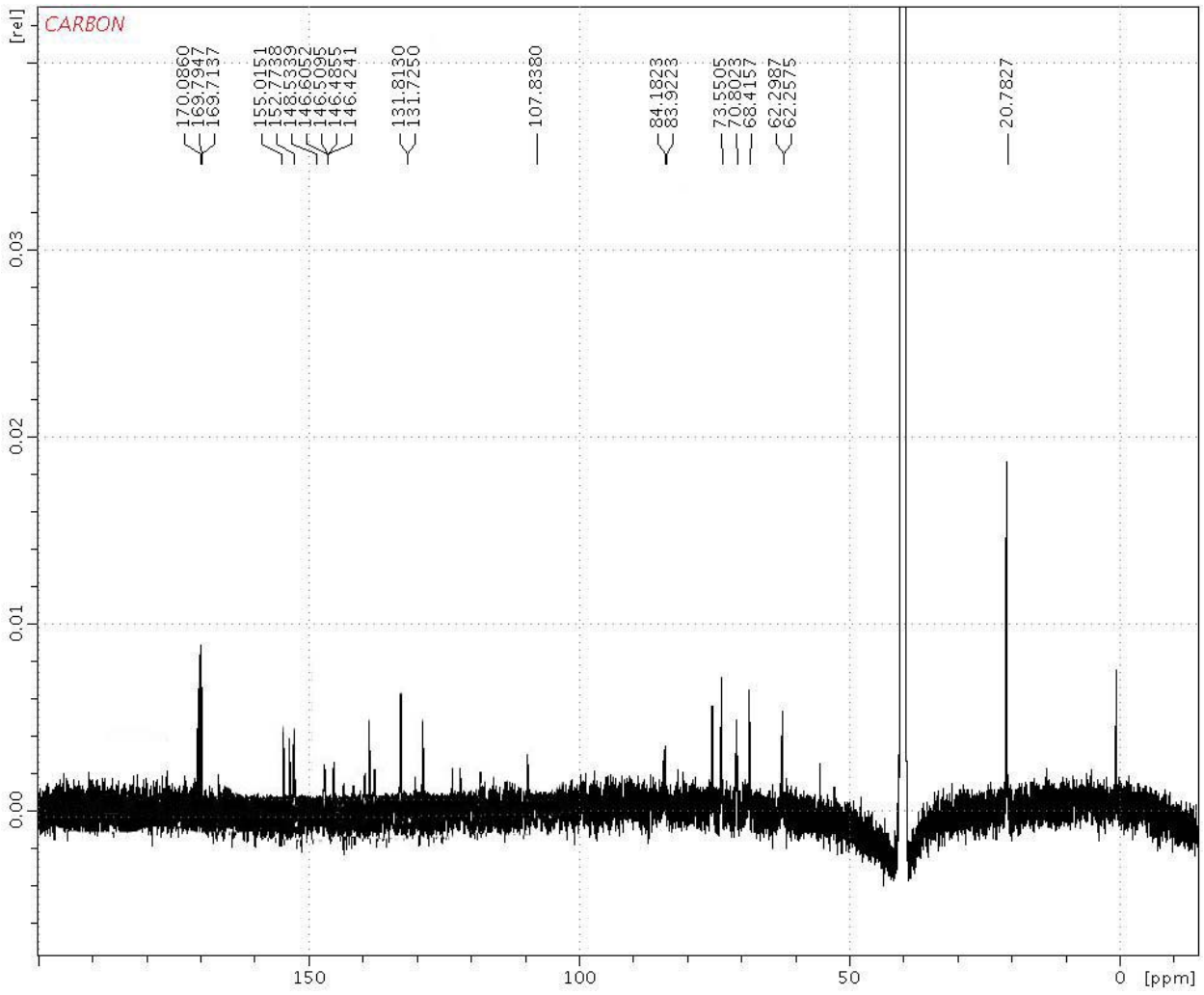


Figure ESI 29: $Zn_2F_{24}GlcAc_6$ **(5a)** ^{13}C NMR DMSO-d_6 (39.55 ppm)

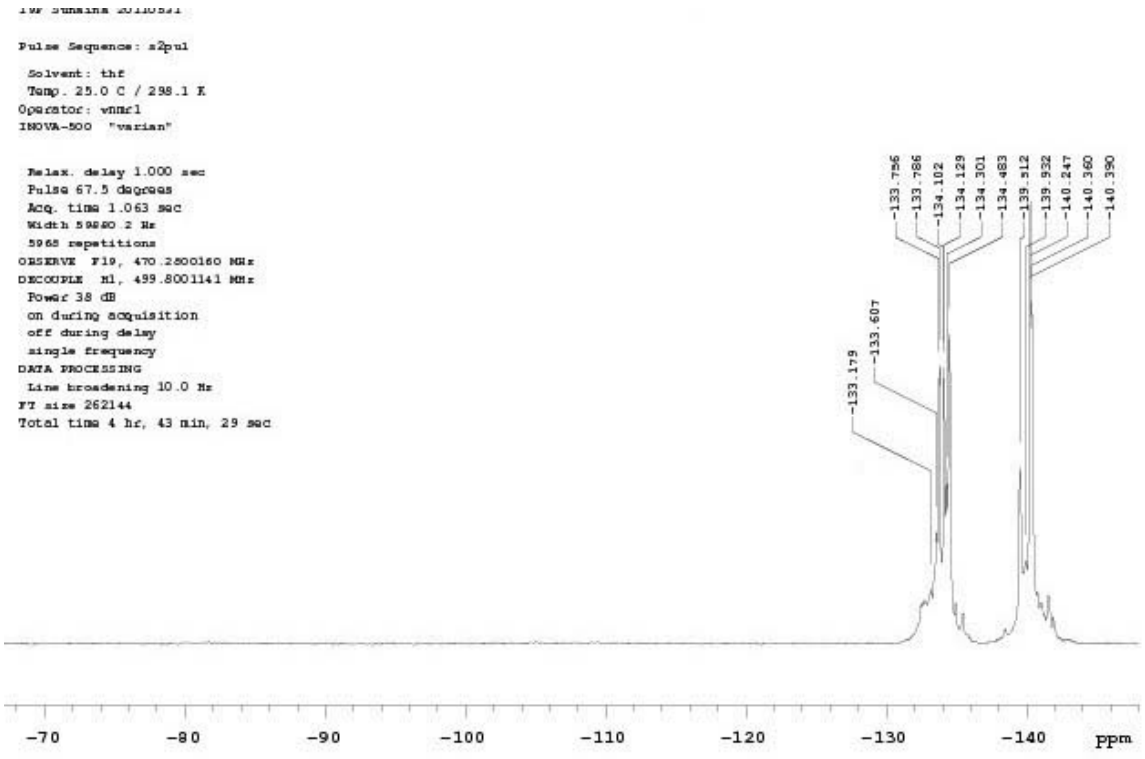
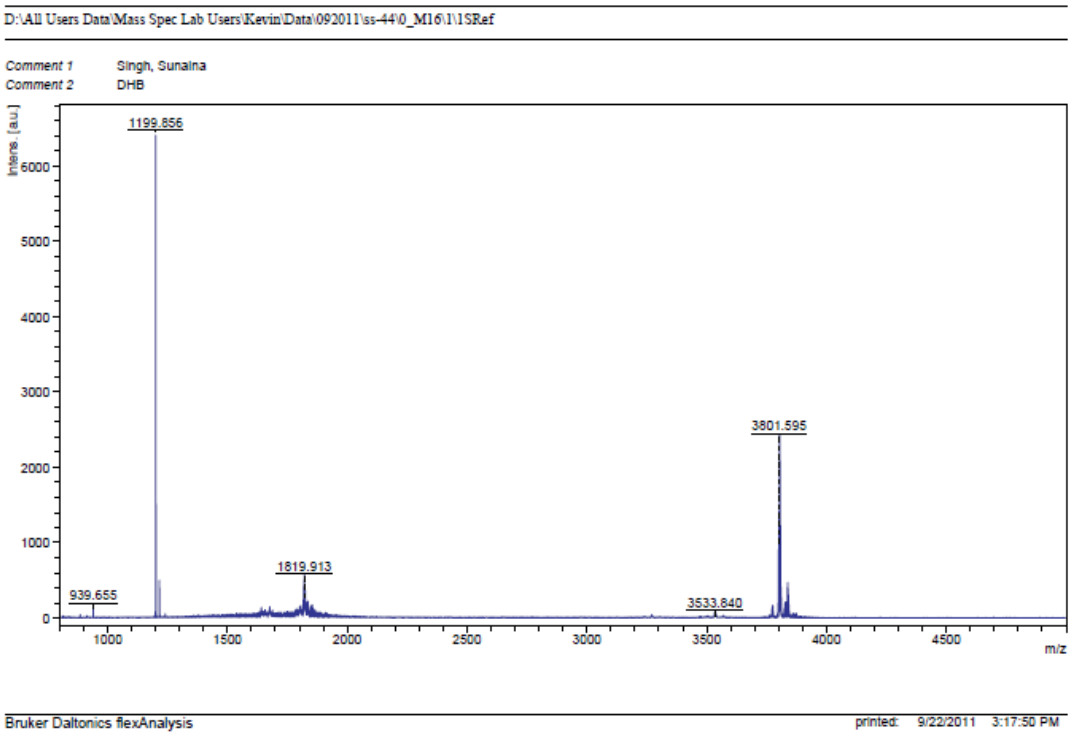


Figure ESI 30: $Zn_2F_{24}GlcAc_6$ **(5a)** ^{19}F NMR (THF-d_8)



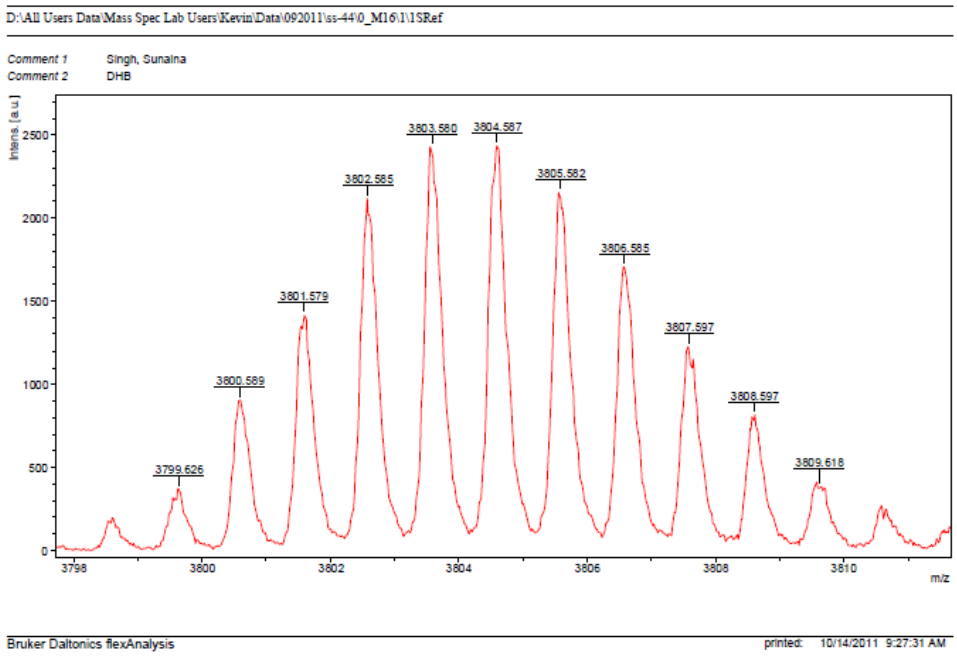


Figure ESI 31: $Zn_2F_{24}GlcAc_6$ **(5a)** MALDI-TOF spectrum.

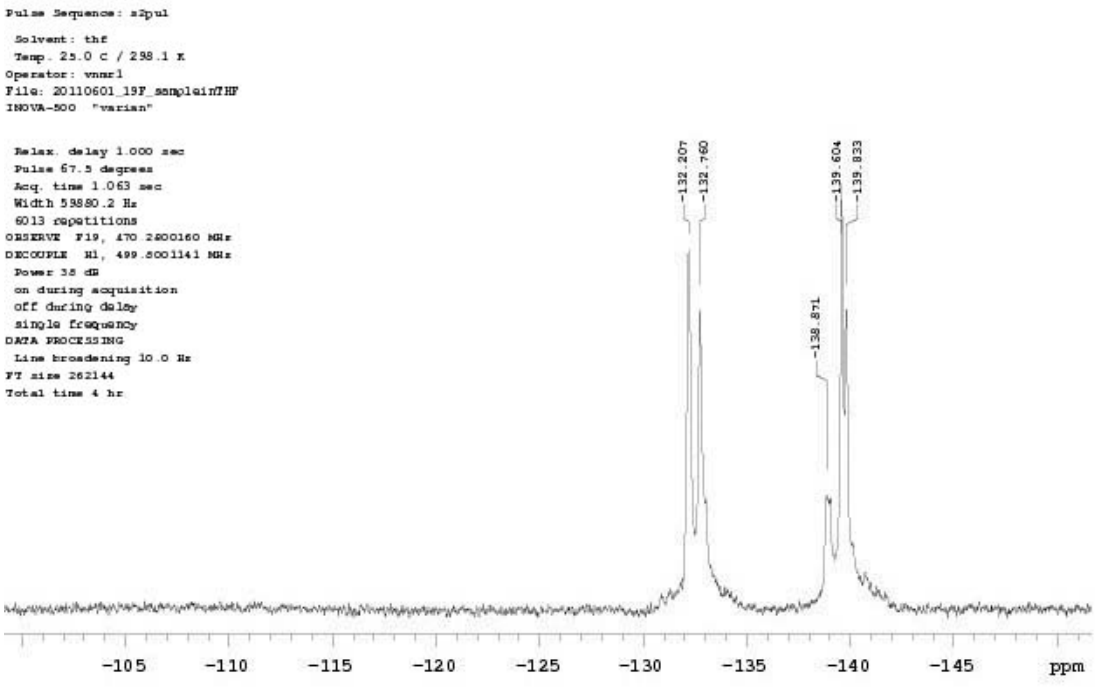


Figure ESI 32: $Zn_2F_{24}Glc_6$ **(5b)** ^{19}F NMR (DMSO-d_6)

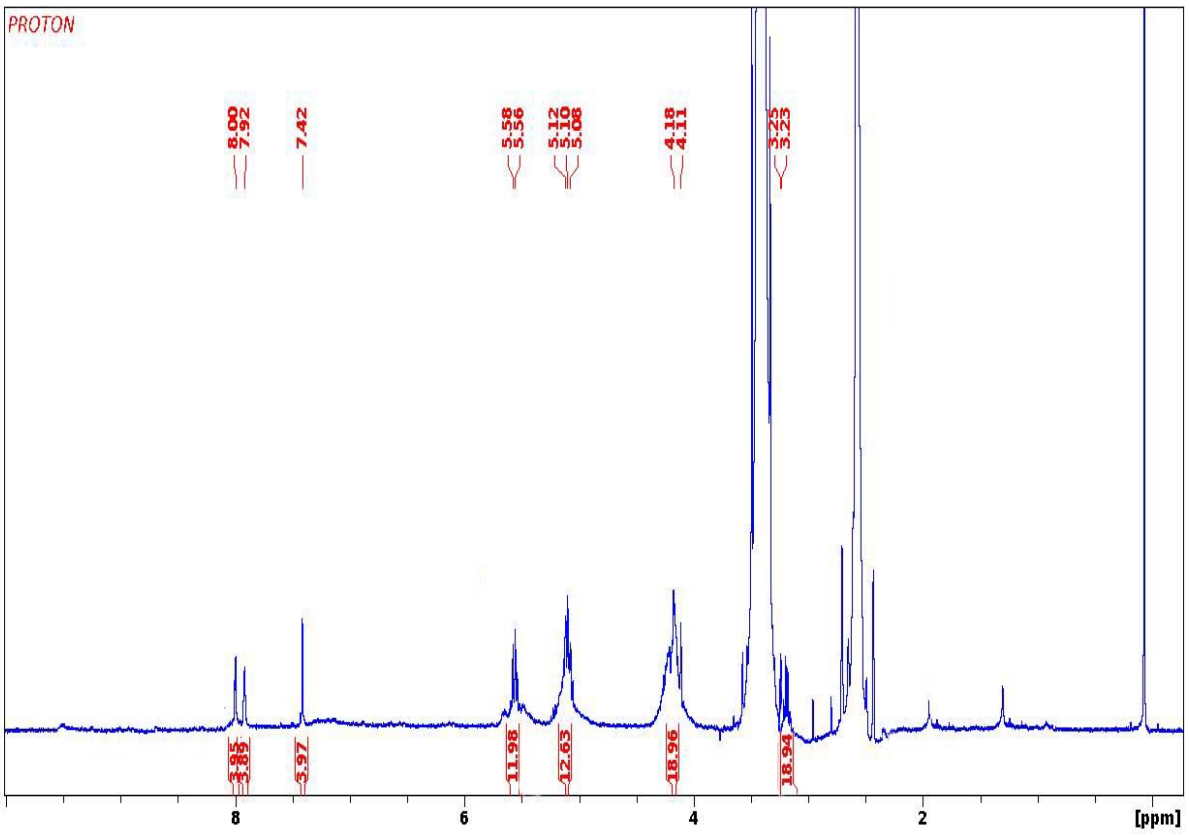


Figure ESI 33: $Zn_2F_{24}Glc_6$ **(5b)** ^{1}H NMR DMSO-d_6 (2.5 ppm, water 3.34 ppm)

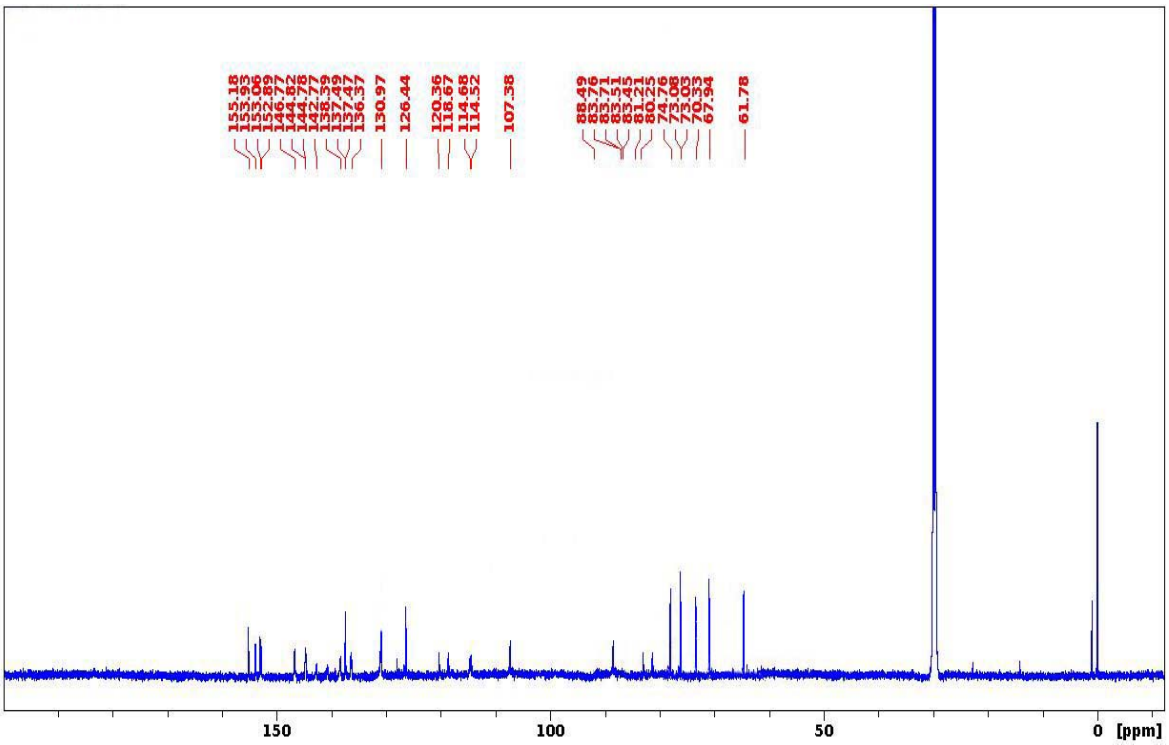


Figure ESI 34: $Zn_2F_{24}Glc_6$ **(5b)** ^{13}C NMR DMSO-d_6 (39.55 ppm)

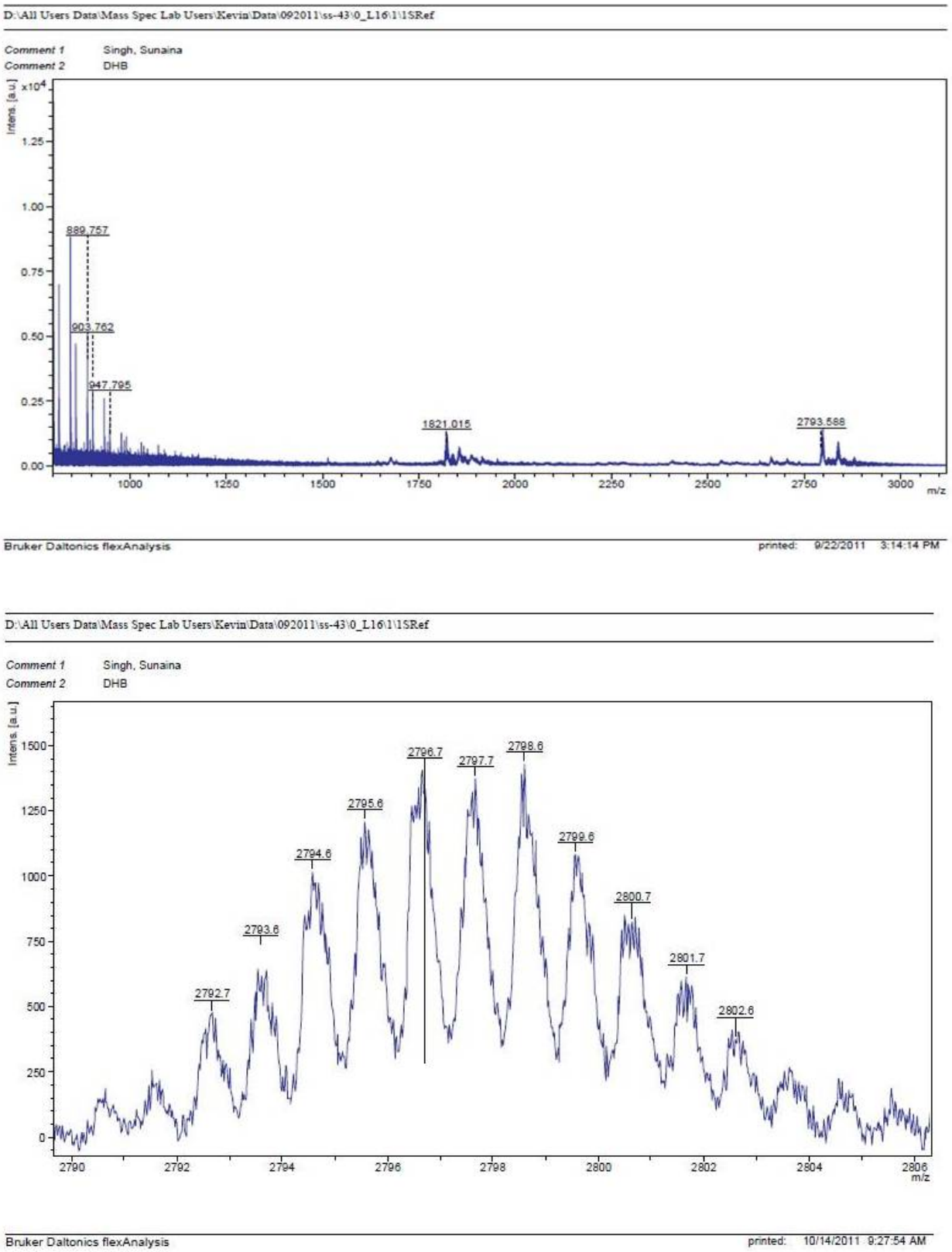


Figure ESI 35: $Zn_2F_{24}Glc_6$ **(5b)** MALDI-TOF spectrum.

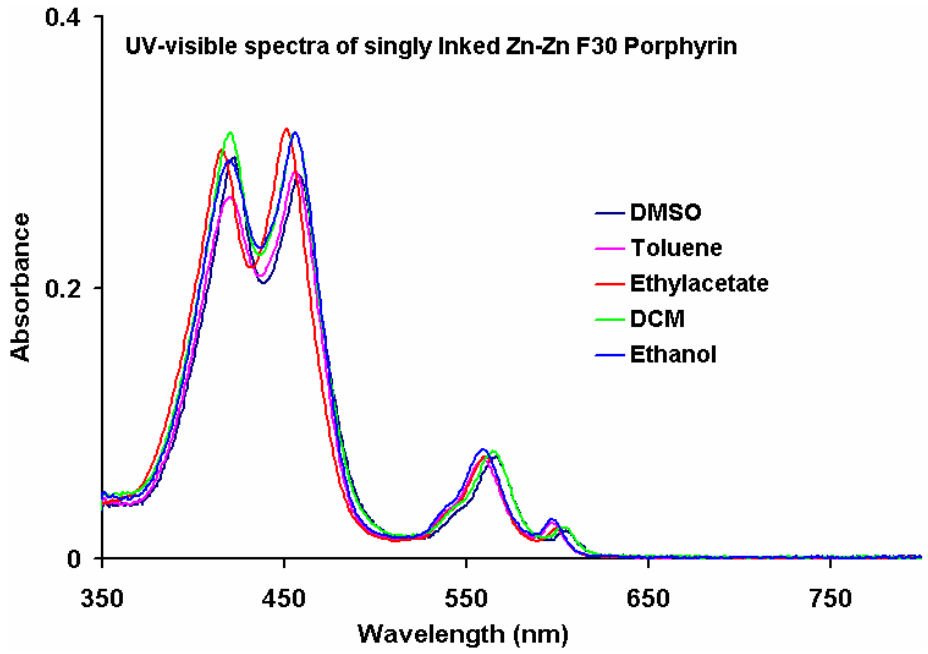


Figure ESI 36: UV-visible spectra of 5 µM solution of singly linked Zn-Zn F_{30} porphyrin (**2**) in different solvents, from a 1 mM solution of the compound in DMSO stock solution.

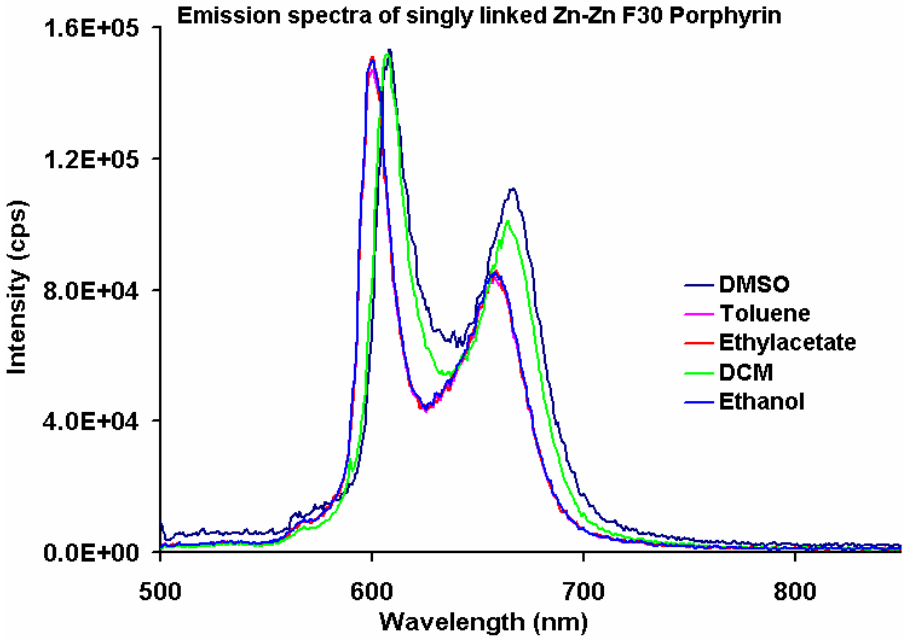


Figure ESI 37: Emission spectra of 5 µM solution of singly linked Zn-Zn F_{30} porphyrin **(2)** in different solvents, from a 1 mM solution of the compound in DMSO stock solution. Excitation = 483 nm, Band pass for each excitation and emission monochromator = 2 nm. Absorbance at 483 nm for each compound = 0.08.

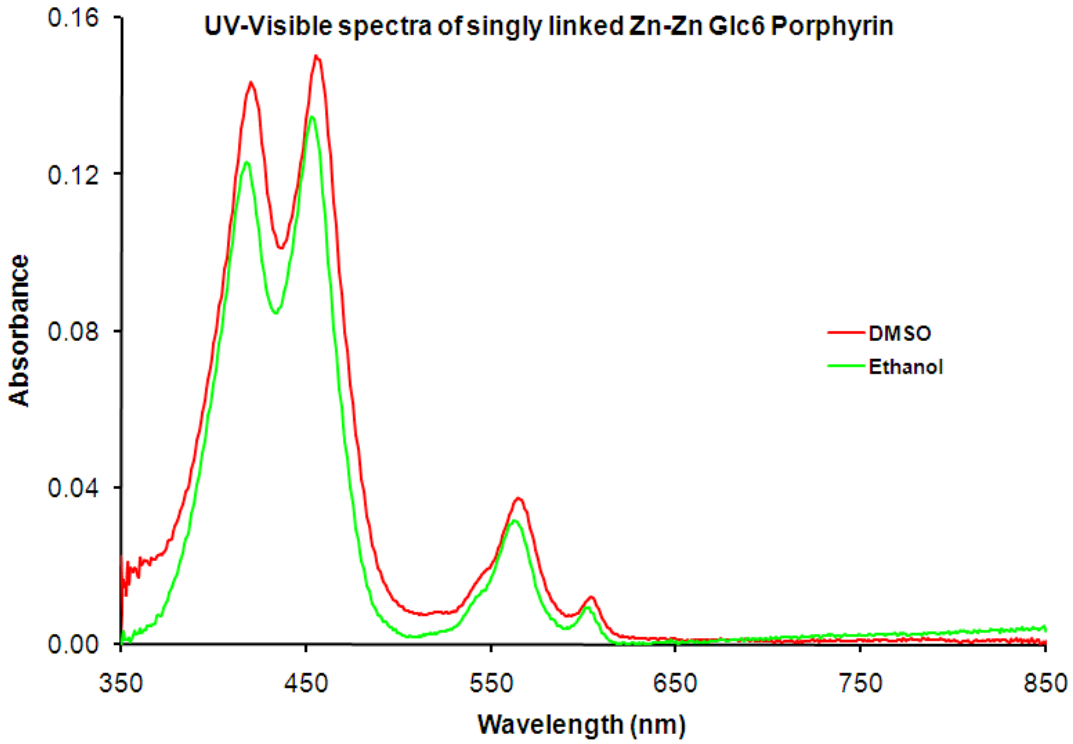


Figure ESI 38: UV-visible spectra of singly linked Zn-Zn Glc6 porphyrin (**6b**) in DMSO and ethanol solutions.

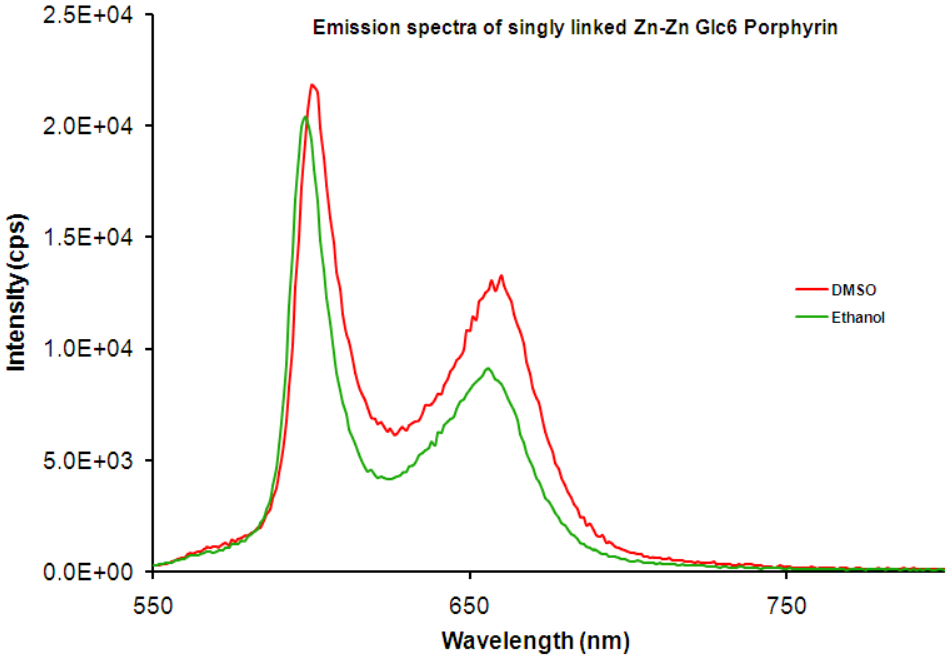


Figure ESI 39: Emission spectra of singly linked Zn-Zn Glc6 porphyrin **(6b)** in different DMSO and ethanol solutions. Excitation = 475 nm, Band pass for each excitation and emission monochromator = 2 nm. Absorbance at 475 nm for each compound = 0.03.

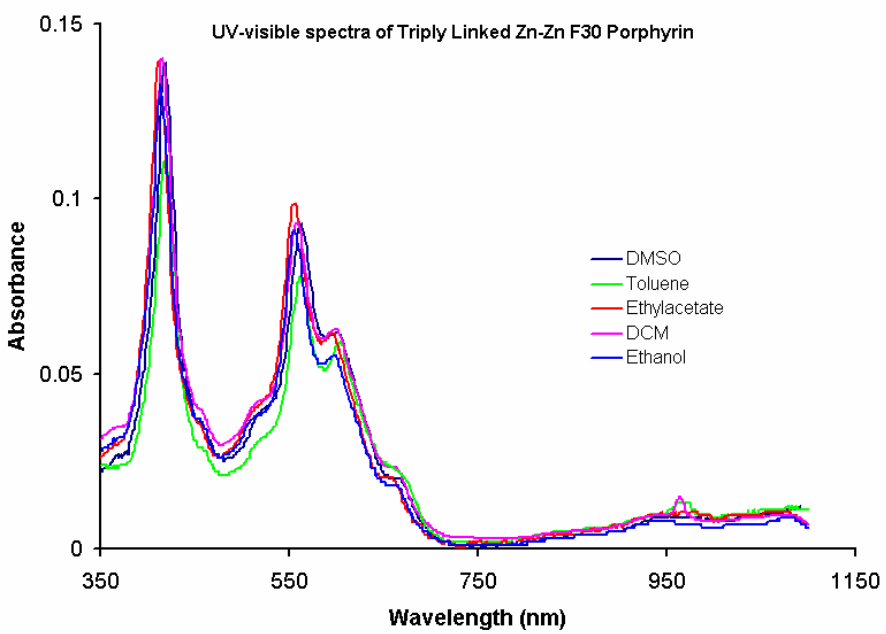


Figure ESI 40: UV-visible spectra of 1 µM solution of triply linked Zn-Zn F_{30} porphyrin (**3**) in different solvents, from a 1 mM solution of the compound in DMSO stock solution.

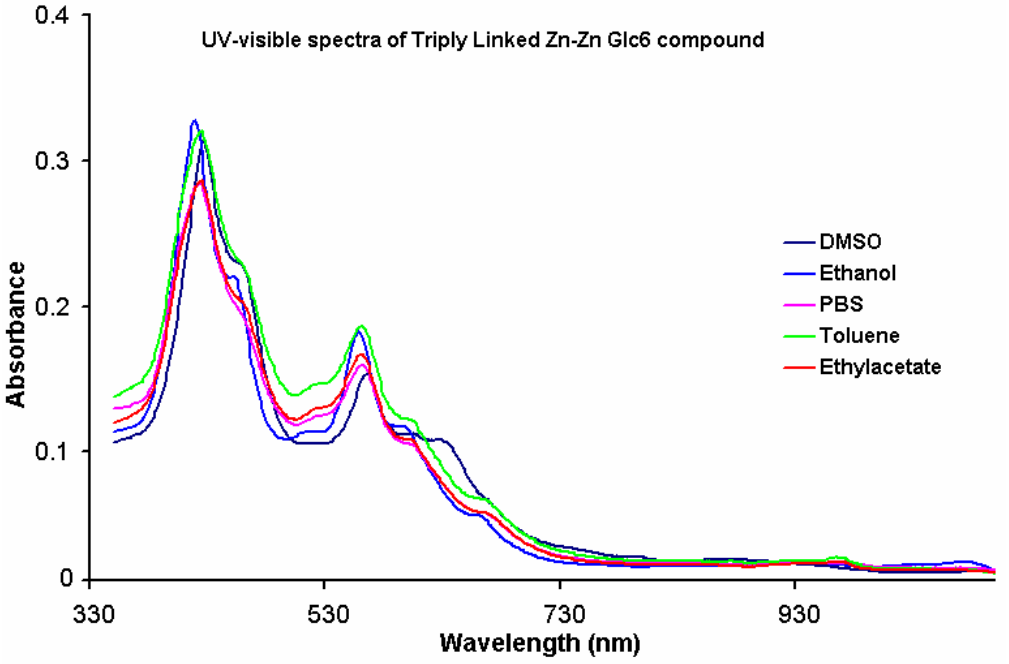


Figure ESI 41: UV-visible spectra of 2 µM solution of triply linked $Zn_2F_{24}Glc_6$ porphyrin (**5b**) in different solvents, from a 1 mM solution of the compound in DMSO stock solution.

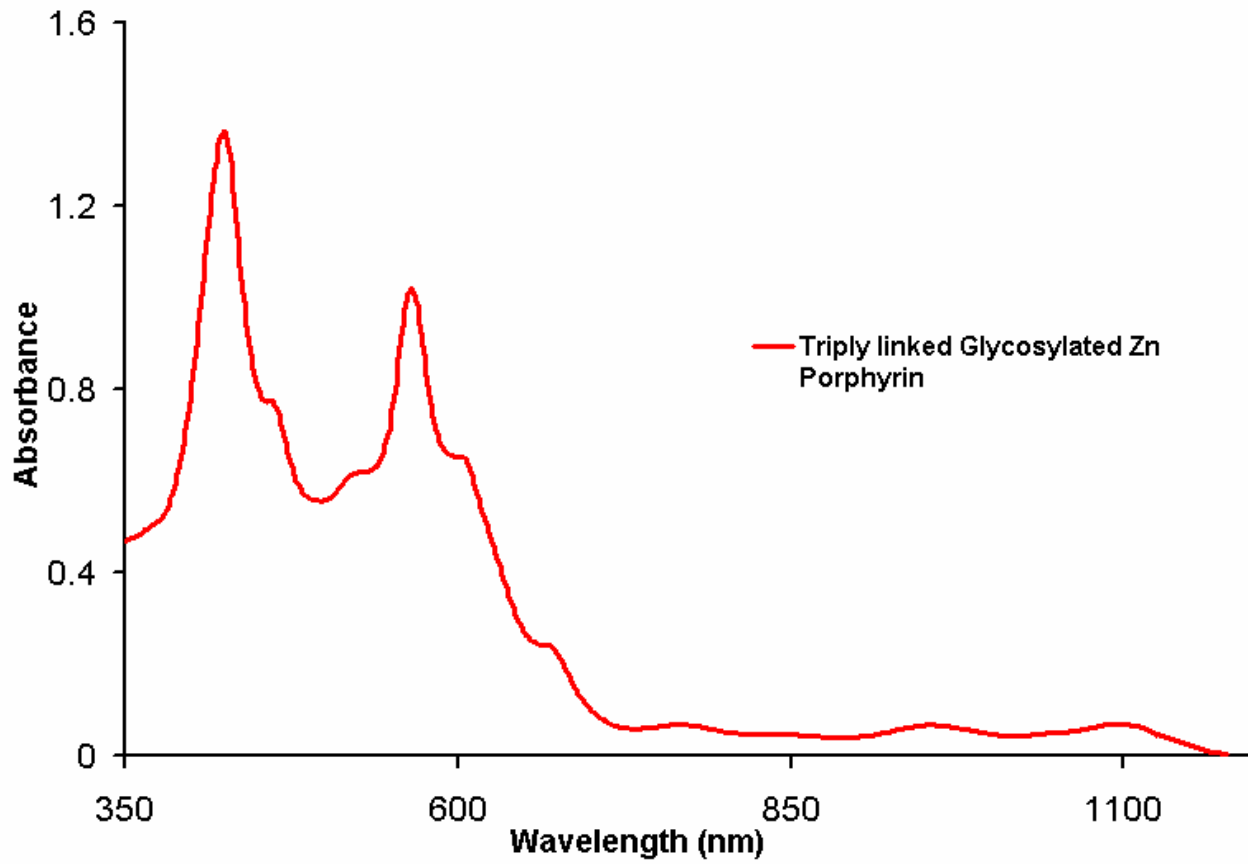


Figure ESI 42: UV-visible spectra of 2 µM solution of triply linked $Zn_2F_{24}GlcAc_6$ porphyrin (**5a**) in DMSO.

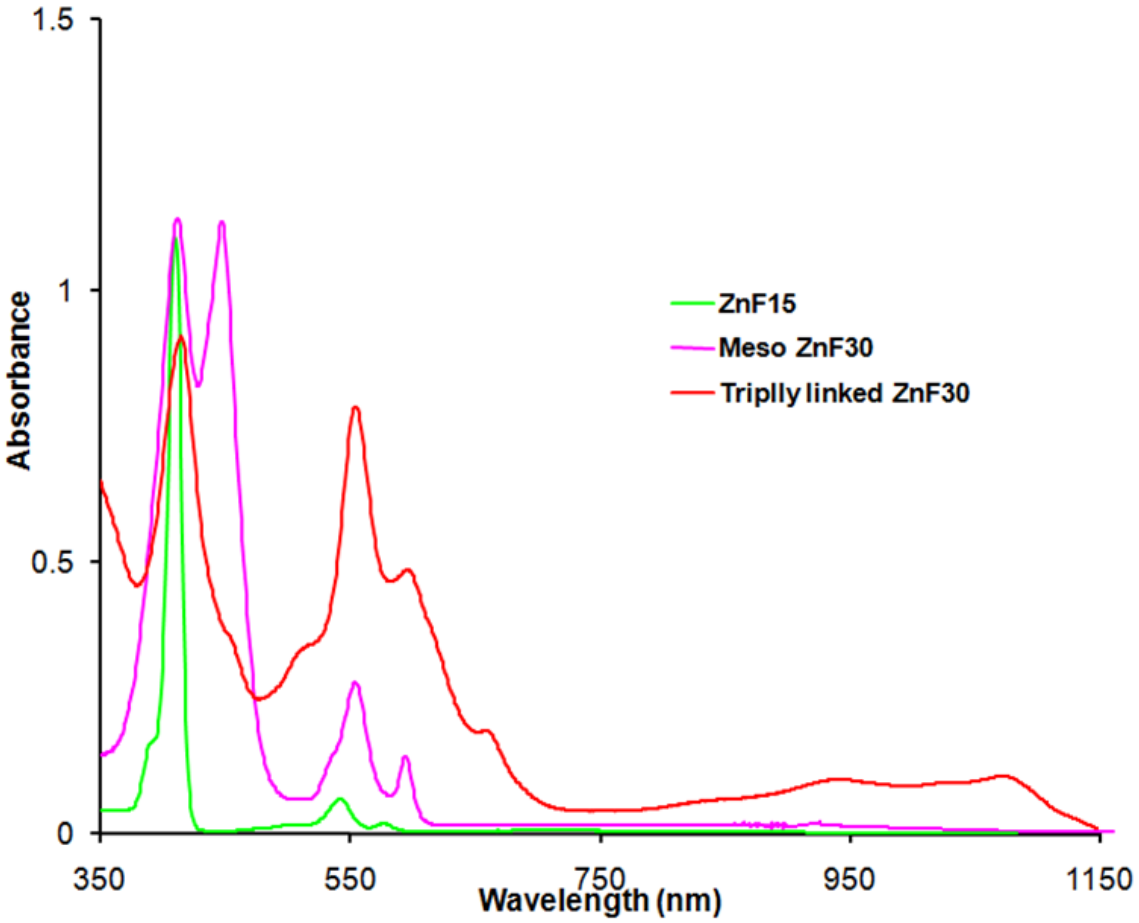


Figure ESI 43: UV-visible spectra of compounds **1(green)**, **2(pink)** and **3 (red)**.

ESI Table 1 : DLS Data of Triply Linked $Zn_2F_{24}Glc_6$ compound (**5b**)	
solvent	Size of aggregate
DMSO Toluene Ethylacetate Ethanol PBS	1160 ±100 35±7, 215±18 45±8, 225±22 30±6, 180±7 30±4, 284±15

ESI Table 2: Photophysical properties of singly Linked Zn_2F_{30} porphyrin (**2**)[a]						
Solvent	UV-Visible Peaks	Emission Peaks[b]	Quantum Yield[c]		Fluorescence life time, τ_f (nsec)[d]	
			Air	N_2	Air	N_2
DMSO	420, 457, 545, 565, 604	609, 667	0.096	0.097	T_1= 1.31 (20%) T_2= 2.79 (80%) (χ2 = 1.21)	T_1= 1.37 (26%) T_2= 2.83 (74%) (χ2 = 1.19)
Toluene	419, 455, 536, 557, 595	600, 656	0.099	0.095	T_1= 1.37 (21%) T_2= 2.94 (79%) (χ2 = 1.18)	T_1= 1.33 (19%) T_2= 3.01 (81%) (χ2 = 1.27)
Ethylacetate	414, 450, 537, 559, 596	601, 658	0.100	0.107	T_1= 1.39 (18%) T_2= 3.12 (82%) (χ2 = 1.21)	T_1= 1.33 (21%) T_2= 3.23 (79%) (χ2 = 1.17)
DCM	419, 455, 541, 562, 602	608, 664	0.125	0.123	T_1= 1.26 (22%) T_2= 2.69 (78%) (χ2 = 1.32)	T_1= 1.36 (23%) T_2= 2.63 (77%) (χ2 = 1.21)
EtOH ** Yellow highlighted peak values are shoulder at the ahead peak	420, 455, 535, 554, 596	598, 655	0.097	0.098	T_1= 1.31 (17%) T_2= 3.07 (83%) (χ2 = 1.25)	T_1= 1.37 (27%) T_2= 3.13 (73%) (χ2 = 1.15)

[a] This photophysical data is consistent with those reported by Osuka and coworkers in $CHCl_3$.[9] [b] λ_{ex} = 483 nm and O.D.= 0.047, [c] relative to Zn(II)tetraphenylporphyrin, [d] time correlated single photon counting

ESI Table 3: UV-visible properties of the triply linked Zn_2F_{30} porphyrin (**3**)		
Solvent	UV-Visible Peaks	
DMSO	419, 460, 520, 564, 605, 669, 963, 1089	In $CHCl_3$ the lifetime is 3.3 ps, but no quantum yield is reported.[9]
Toluene	420, 462, 517, 563, 607, 671, 969, 1087	
Ethylacetate	413, 456, 517, 557, 599, 660, 964, 1079	
DCM	417, 459, 517, 560, 603, 665, 967, 1080	
EtOH	414, 459, 513, 557, 600, 666, 948, 1083	

ESI Table 4: UV-visible properties of triply linked $Zn_2F_{24}Glc_6$ porphyrin **(5b)**	
Solvent	UV-Visible Peaks
DMSO	429, 462, 569, 610, 636, 749, 876, 943, 1090
Toluene	427, 462, 525, 565, 606, 667, 829, 966, 1079
Ethylacetate	428, 462, 524, 565, 606, 671, 848, 952, 1070
PBS	426, 461, 526, 565, 607, 671, 849, 950, 1071
EtOH	421, 455, 514, 561, 602, 664, 837, 935, 1029, 1078

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