Electronic Supplementary Information for

Synthesis of hydrophilic conjugated porphyrin dimers for one-photon and two-photon photodynamic therapy at NIR wavelengths

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Materials and Methods

HPLC purification

The HPLC purification of conjugated porphyrin dimers was done on a VWR Hitachi ELITE LaChrom HPLC system equipped with L-2130 quaternary pump, L-2455 diode array detector, L-2200 autosampler and FoxyJr fraction collector. The chromatographic separations were monitored in the range 260 nm – 800 nm.

Analytical HPLC were carried out using C_8 5 µm, 3.9 × 150 mm Eclipse XDB-C8 column (Agilent) using 1 mL / min flow and stepwise gradient. The column was heated to 40 ^oC. Semipreparative HPLC separations were carried out using C_8 5 µm, 9.4 × 250 mm Eclipse XDB-C8 column from Agilent using 4 ml / min flow rate and a stepwise gradient. The column was heated to 40 ^oC.

NMR spectra

NMR spectra were recorded at ambient probe temperature using either a Brucker DPX400 (400 MHz), or Brucker AVANCE AV400 (400 MHz). Chemical shifts are quoted as parts per million (ppm) relative to tetramethylsilane and coupling constants (*J*) are quoted in Hertz (Hz). UV/Vis spectra were recorded on a Perkin Elmer Lambda 20 UV-Vis.

MS spectra

Mass spectra were carried out using Matrix Assisted Laser Desorption Ionization-Time of Flight (MALDI-TOF) only molecular ions and major peaks are reported. Melting points are reported uncorrected and boiling points were taken from the vapor temperature of the distilling product. Matrix: 2-[(2E)-3-(4-tert-Butylphenyl)-2-methylprop-2-enylidene] malononitrile (DCTB).

Purity of porphyrin dimers

Proton NMR spectra and MALDI-TOF spectra of RP-HPLC purified dimers did not show presence of any impurities. The purity of all conjugated porphyrin dimers was further confirmed by the RP-HPLC. A small amount of the each purified dimer was dissolved in THF or DMF and injected into the HPLC system. The HPLC profile was monitored between 280 nm and 800 nm (DAD detector). The HPLC chromatographs monitored at two different wavelengths (440 nm and 695 nm) can be found in the ESI section of this manuscript. HPLC profiles did not show presence of any impurities and confirmed the high purity of all hydrophilic porphyrin dimers required for *in vitro* or *in vivo* biological testing.

Conjugated porphyrin dimer 2 (P_2-NMeI)

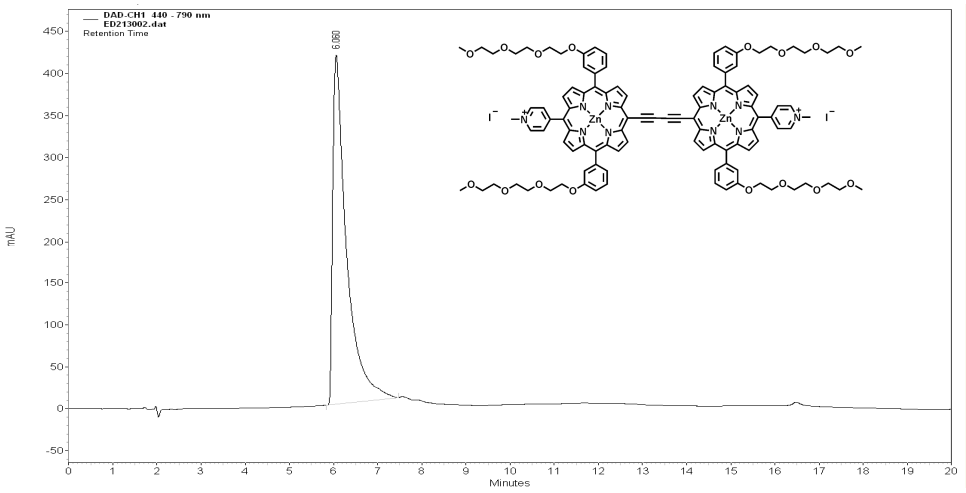


Figure S1. HPLC chromatograph of the porphyrin dimer **2** monitored 440 nm (Soret band).

Chromatographic condition: T = 40°C, flow = 4 mL / min, gradient solvent system: water / MeOH / THF / 1% acetic acid in water, Table S1. The porphyrin dimer was dissolved in THF and 20 μL was injected each time.

Table S1. Solvent gradient used for HPLC purification of the porphyrin dimer **2** (Method **A**)

Time (min)	Water	Methanol	THF	2% aqueous acetic acid
0.0	10 %	15 %	15 %	60 %
9.0	17 %	50 %	25 %	8 %
11.0	12 %	24 %	52 %	12 %
12.0	5 %	10 %	80 %	5 %
14.0	2.5 %	15 %	80 %	2.5 %

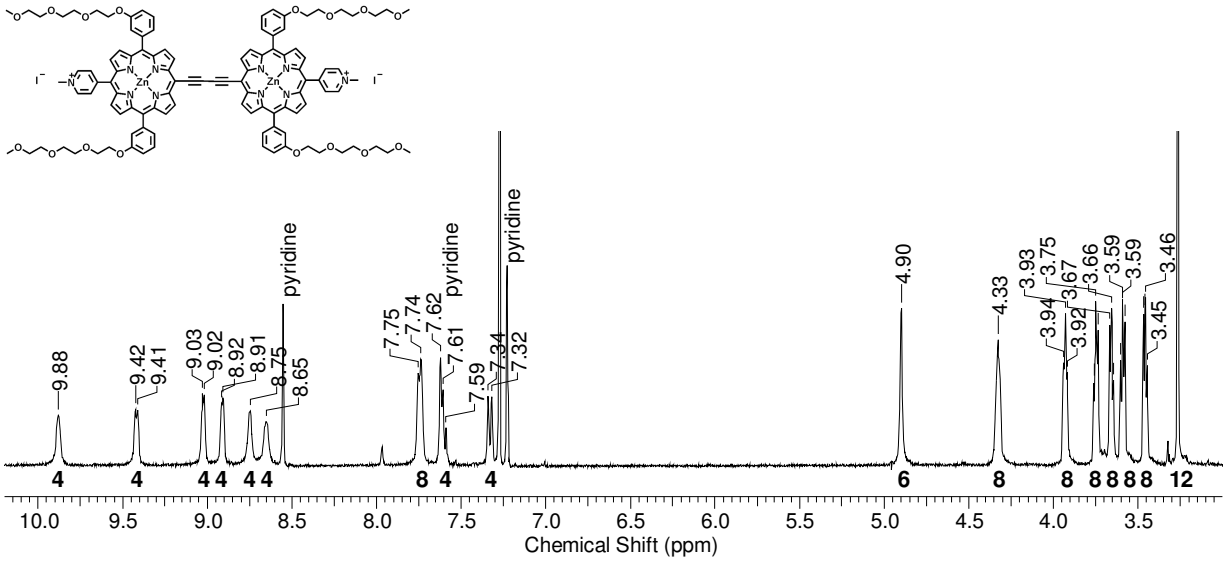


Figure S2. ^{1}H NMR spectrum of porphyrin dimer **2** ($CDCl_3$ / 5% pyridine-d_5, 400MHz).

Conjugated porphyrin dimer 2

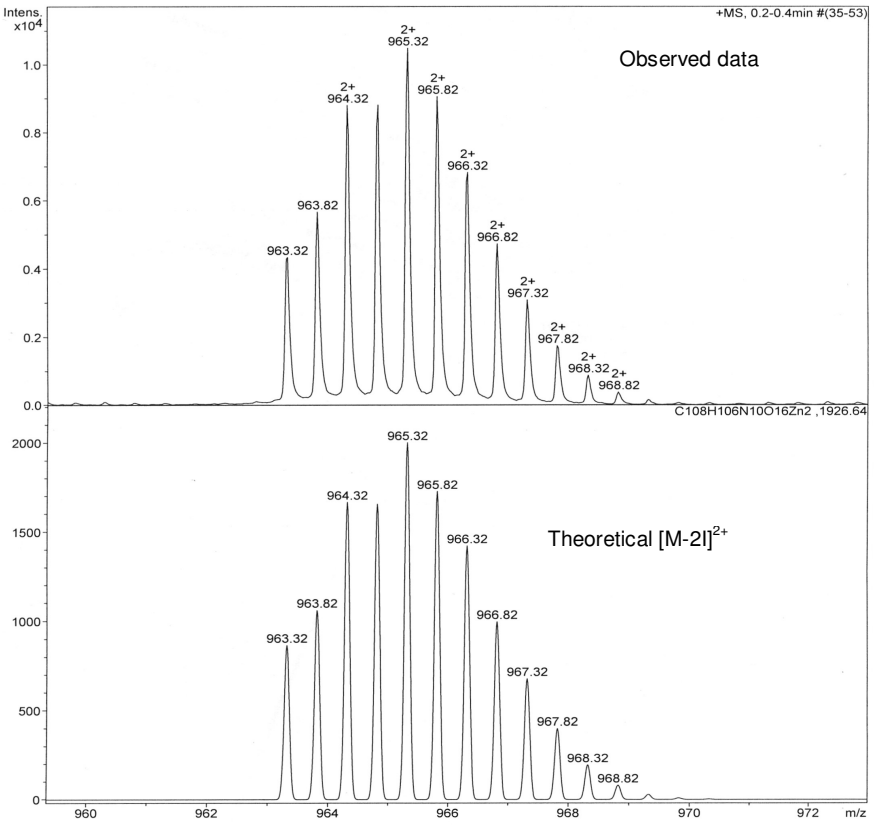


Figure S3. ESI-TOF of the porphyrin dimer **2** *m/z* ESI-TOF 963.32 ($C_{108}H_{106}N_{10}O_{16}Zn_2^{2+}$, $[M]^{2+}$, requires 963.32)

Conjugated porphyrin dimer 5 (P_2-SO_3NH_4)

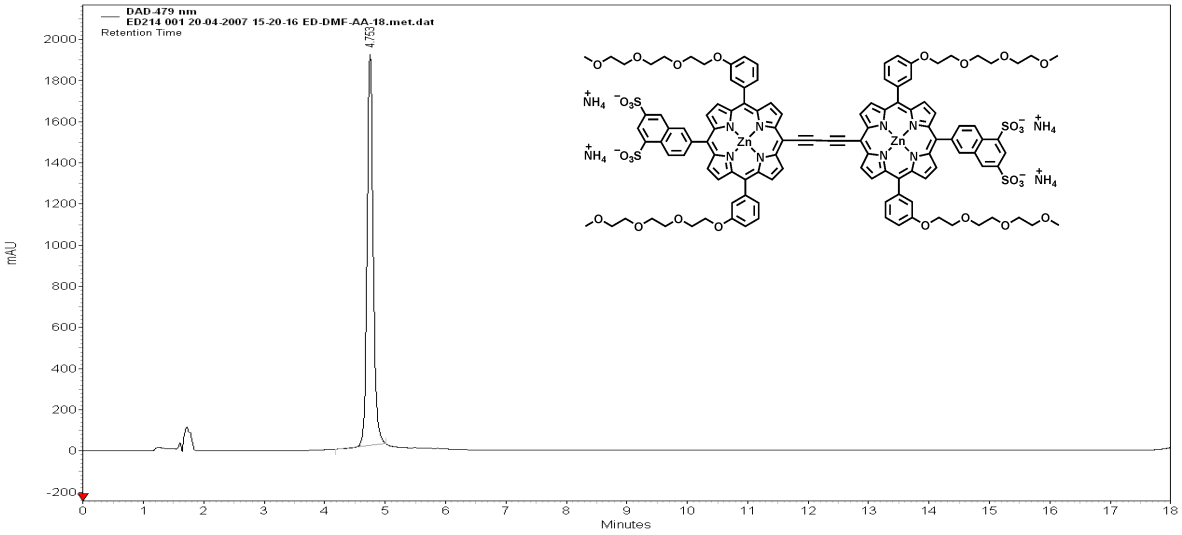


Figure S4. HPLC chromatograph of the porphyrin dimer **5** monitored 440 nm (Soret band).

Chromatographic condition: T = 40°C, flow = 4 mL / min, gradient solvent system: MeOH / DMF / ammonium acetate in water (1g/L), Table S2. The porphyrin dimer **5** was dissolved in DMF and 20 µL was injected each time.

Table S2. Solvent gradient used for HPLC purification of the porphyrin dimer **5** (Method **B**)

Time (min)	Methanol	DMF	aqueous ammonium acetate (1g/L)
0.0	20 %	40 %	40 %
3.0	6.7 %	80 %	13.3 %
8.0	6.7 %	80 %	13.3 %
10.0	0 %	100 %	0 %
14.0	0 %	100 %	0 %

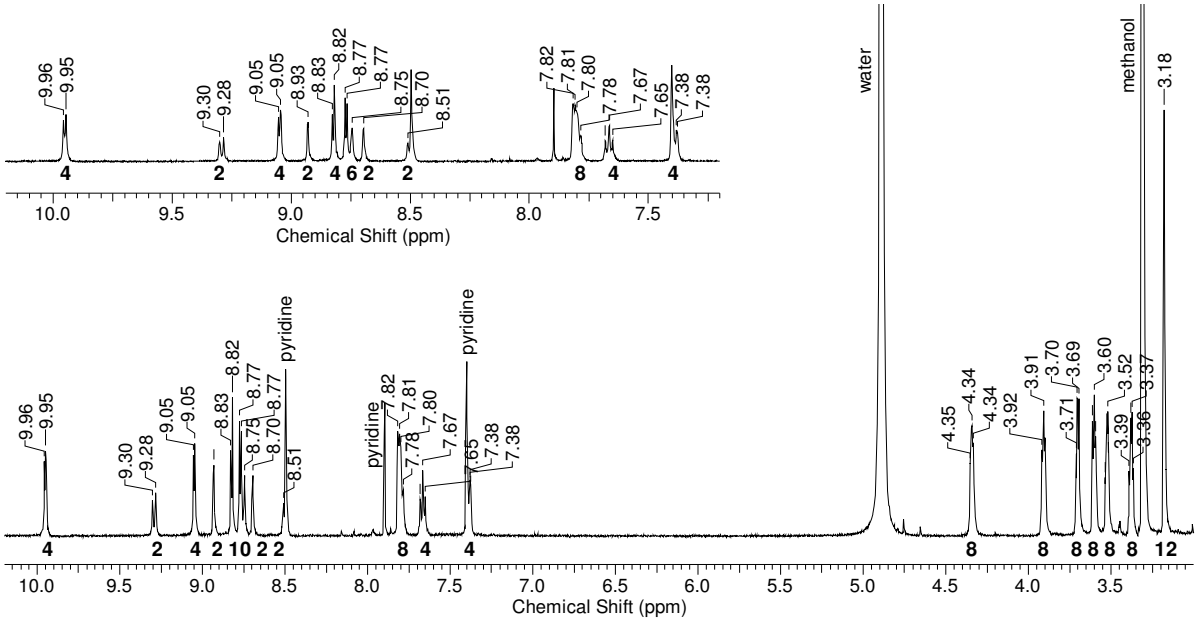


Figure S5. ^{1}H NMR spectrum of porphyrin dimer **5** (MeOD / 5% pyridine-d_5, 500 MHz).

Conjugated porphyrin dimer 5

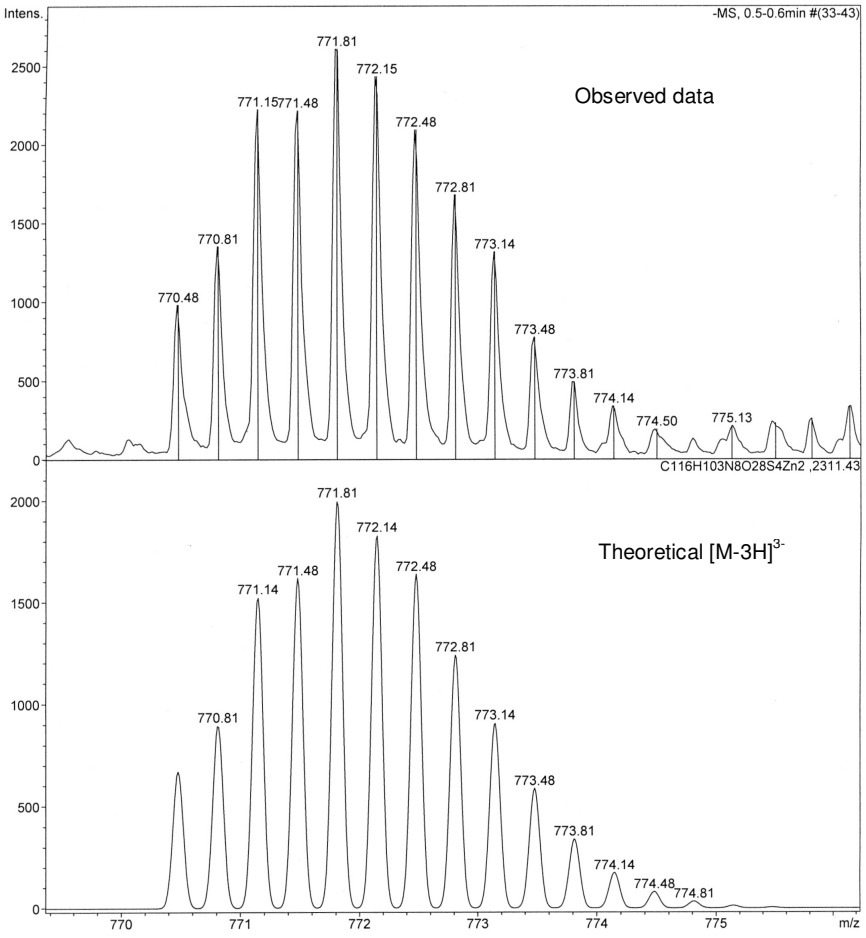


Figure S6. ESI-TOF of the porphyrin dimer **5** *m/z* ESI-TOF 770.48 ($C_{116}H_{103}N_8O_{28}S_4Zn_2^{3-}$, $[M]^{3-}$, requires 770.47).

Conjugated porphyrin dimer 3 (P_2C_2-NMeI)

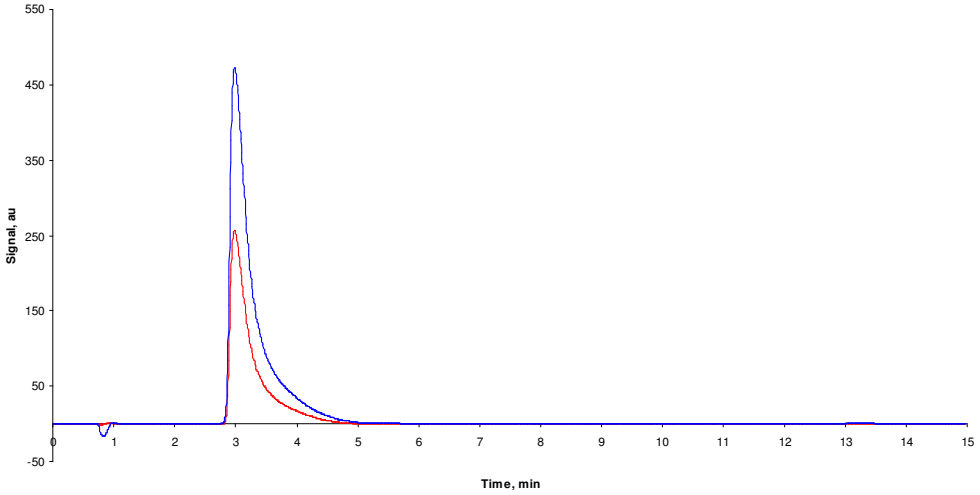


Figure S7. HPLC chromatograph of the porphyrin dimer **3** monitored 440 nm (Soret band, blue line) and 770 nm (Q band, red line).

Table S3. Solvent gradient used for HPLC purification of the porphyrin dimer **3** (Method **C**)

Time/min	MeOH	DMF	1g/L NH_4OAc
0	20 %	40 %	40 %
6	6.7 %	80 %	13.3 %
13	6.7 %	80 %	13.3 %
13.01	20 %	40 %	40 %
15	20 %	40 %	40 %

Linear ramping between solvent mixtures

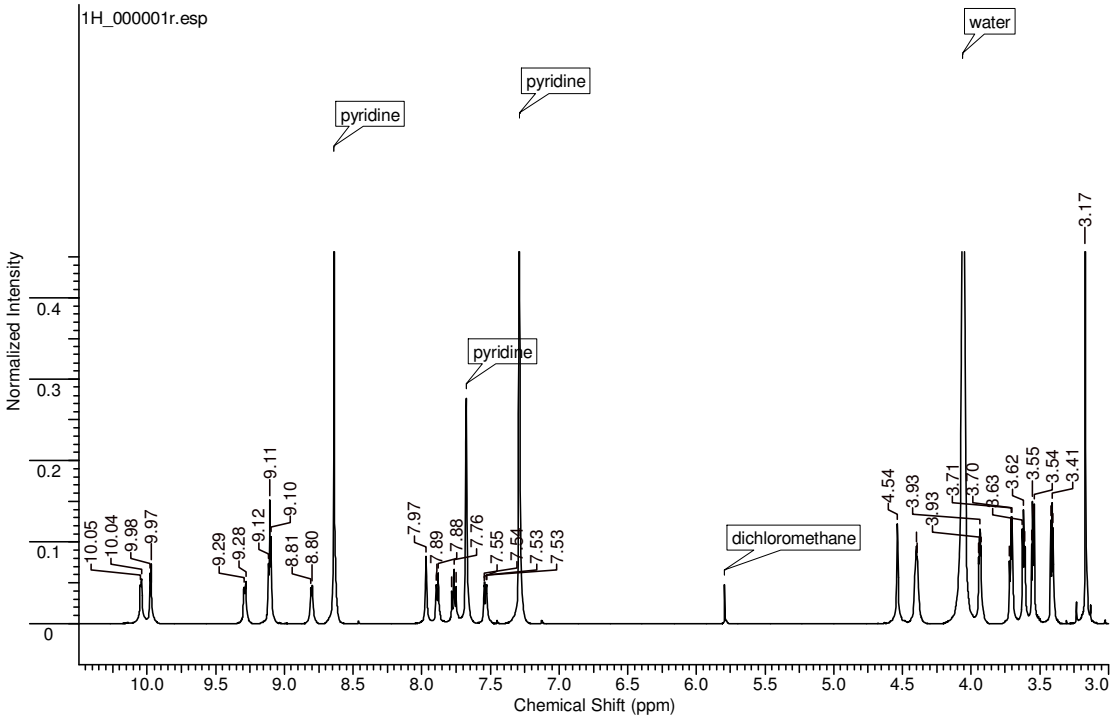


Figure S8. ^{1}H NMR spectrum of porphyrin dimer **3** (MeOD / 5% pyridine-d_5, 400 MHz).

Conjugated porphyrin dimer 3

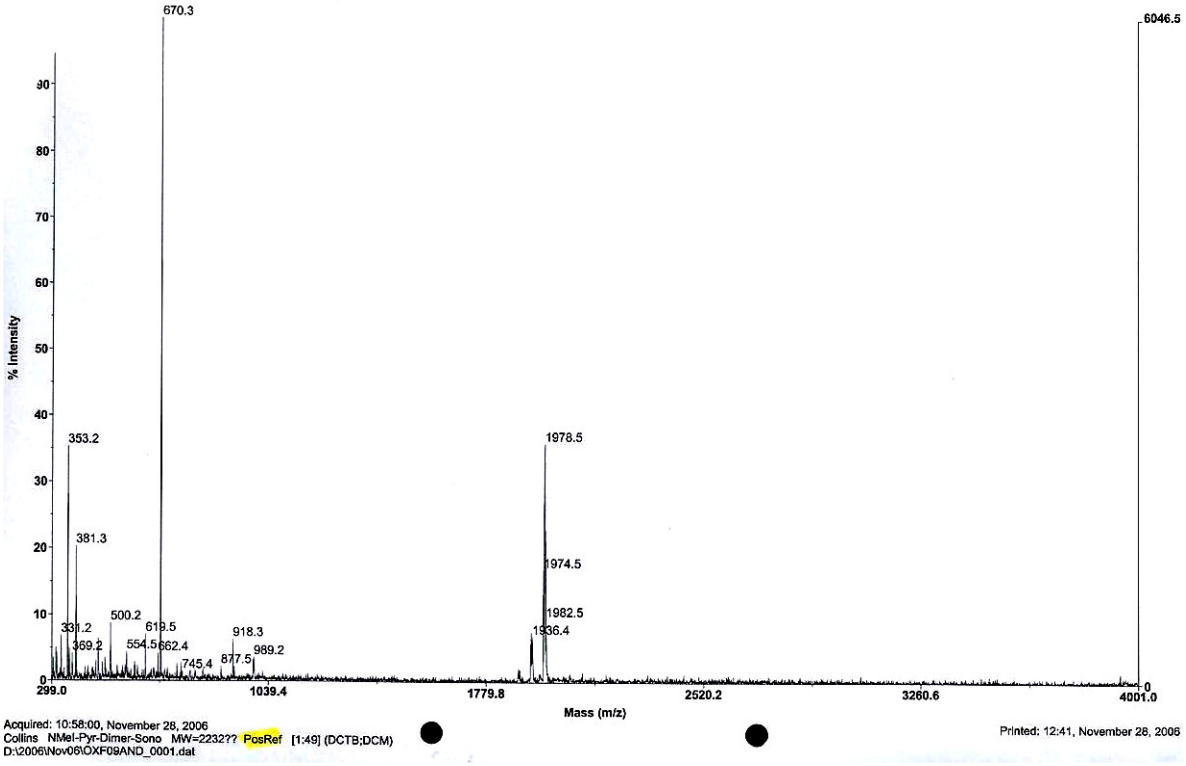


Figure S9. ESI-TOF of the porphyrin dimer **3** *m/z* MALDI-TOF 1978.5 ($C_{112}H_{106}N_{10}O_{16}Zn_2$, $[M-2I]^+$, requires 1978.6, 100%)

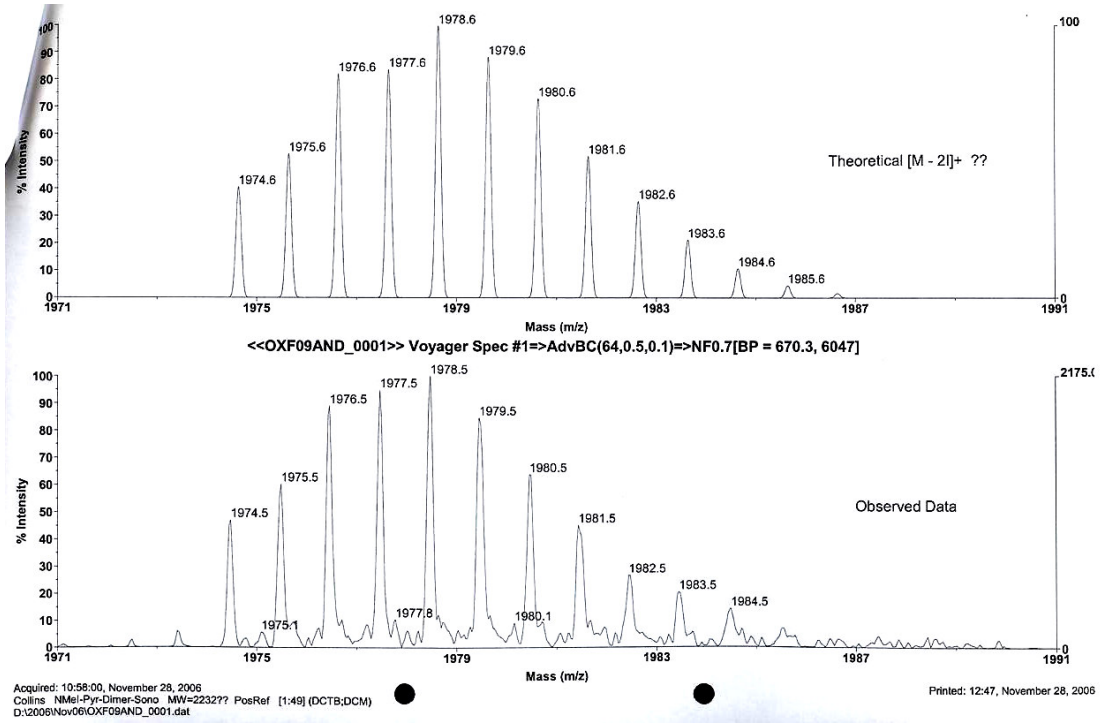


Figure S10. ESI-TOF of the porphyrin dimer **3** *m/z* MALDI-TOF 1978.5 ($C_{112}H_{106}N_{10}O_{16}Zn_2$, $[M-2I]^+$, requires 1978.6, 100%).

Conjugated porphyrin dimer 6 (P_2C_2-CO_2NH_4)

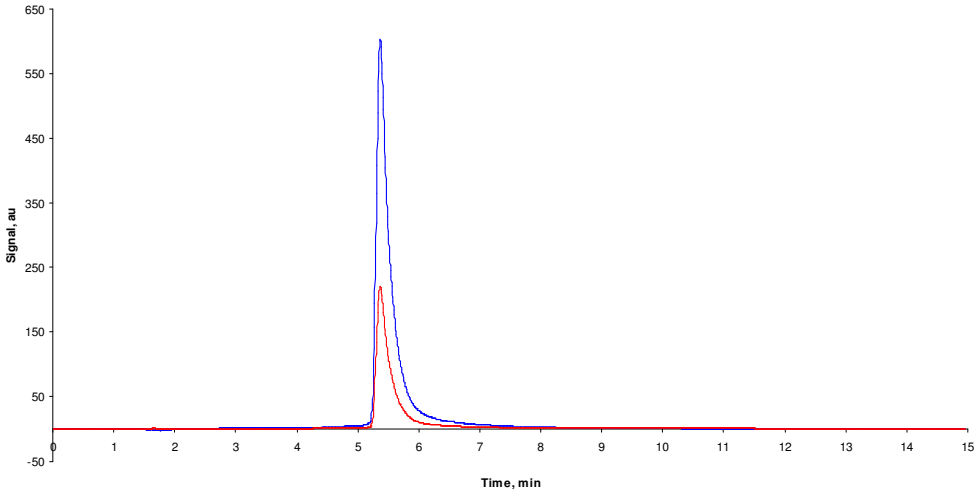


Figure S11. HPLC chromatograph of the porphyrin dimer **6** monitored 440 nm (Soret band, blue line) and 680 nm (Q band, red line).

Table S4. Solvent gradient used for HPLC purification of the porphyrin dimer **6** (Method **C**)

Time/min	MeOH	DMF	1g/L NH_4OAc
0	20 %	40 %	40 %
6	6.7 %	80 %	13.3 %
13	6.7 %	80 %	13.3 %
13.01	20 %	40 %	40 %
15	20 %	40 %	40 %

Linear ramping between solvent mixtures

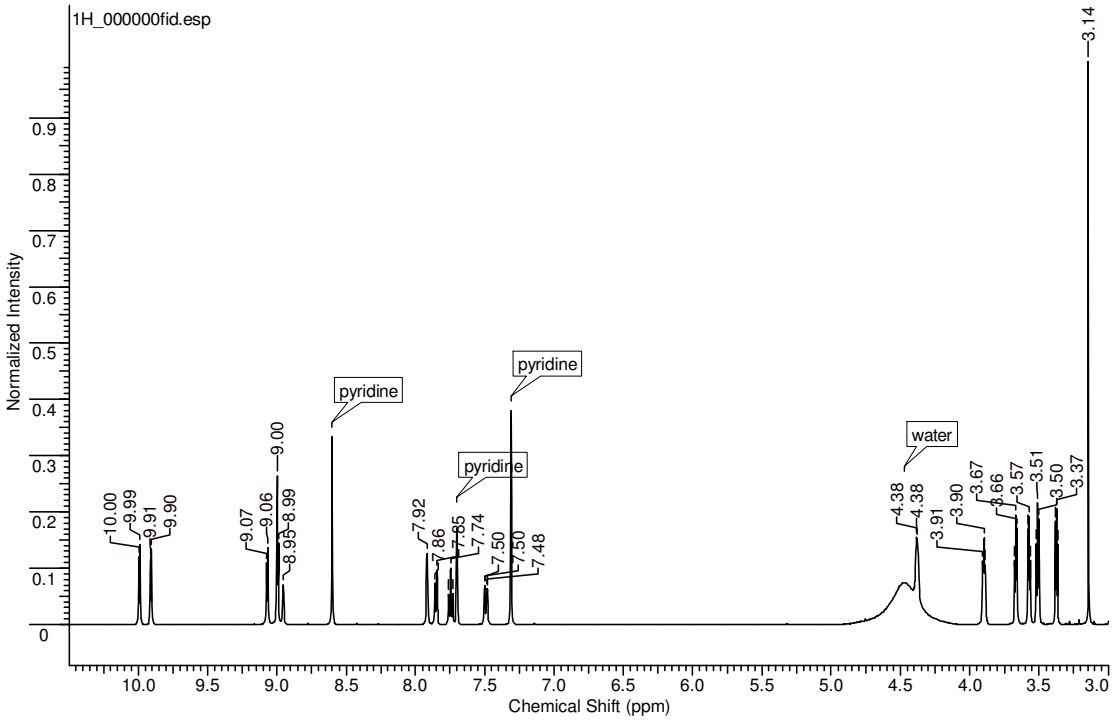


Figure S12. ^{1}H NMR spectrum of porphyrin dimer **6** (DMSO / 5% pyridine-d_5, 400 MHz).

Conjugated porphyrin dimer 6

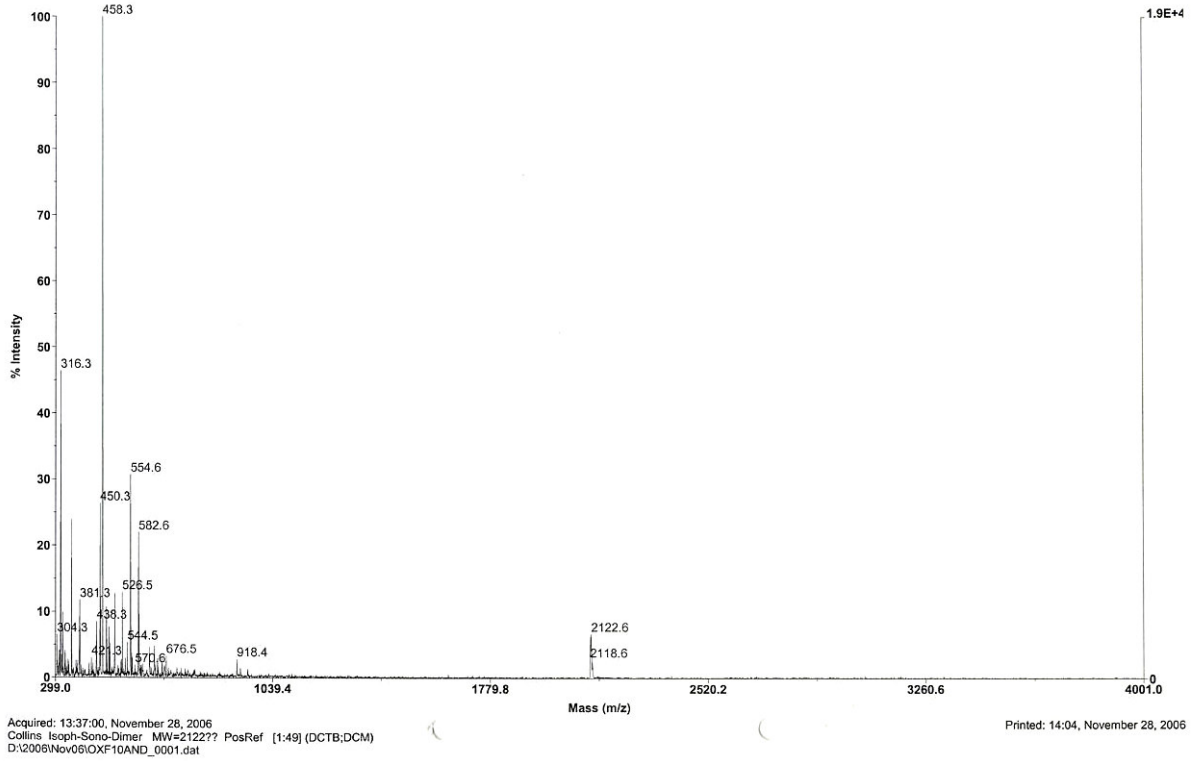


Figure S13. ESI-TOF of the porphyrin dimer **6** *m/z* MALDI-TOF 2122.6 ($C_{116}H_{102}N_8O_{24}Zn_2$, M^+, requires 2122.6, 100%)

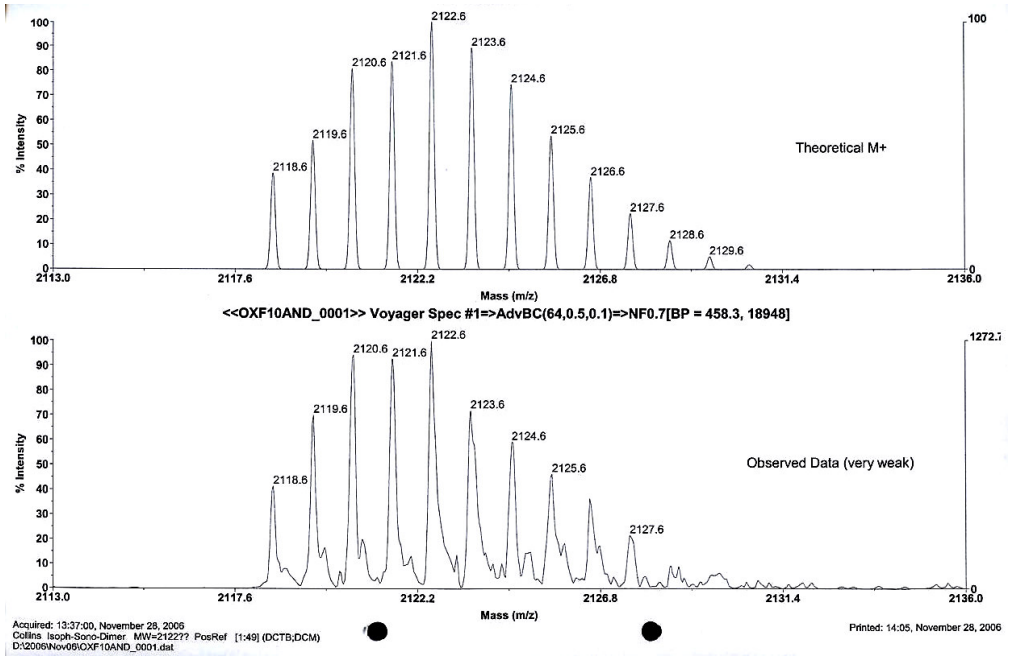


Figure S14. ESI-TOF of the porphyrin dimer **6** *m/z* MALDI-TOF 2122.6 ($C_{116}H_{102}N_8O_{24}Zn_2$, M^+, requires 2122.6, 100%)

Conjugated porphyrin dimer 4 (P_2-NMe_3OAc)

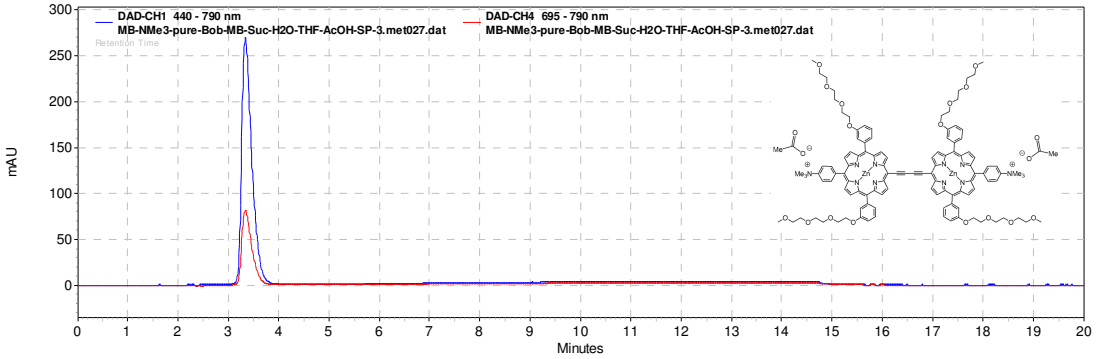


Figure S15. HPLC chromatogram of the porphyrin dimer **4** monitored by at two different wavelengths, 440 nm (Soret band, blue line) and 695 nm (Q band, red line).

Chromatographic condition: T = 40°C, flow = 4 mL / min, gradient solvent system: 1% acetic acid in water / THF, Table S5. Porphyrin dimer **4** was dissolved in THF and 30 µL was injected.

Table S5. Solvent gradient used for HPLC purification of the porphyrin dimer **4**: HPLC method **D**

time / min	1% aqueous acetic acid	THF
0	60 %	40 %
3	50 %	50 %
6	30 %	70 %
9	20 %	80 %
12	20 %	80 %
13	60 %	40 %
15	60 %	40 %

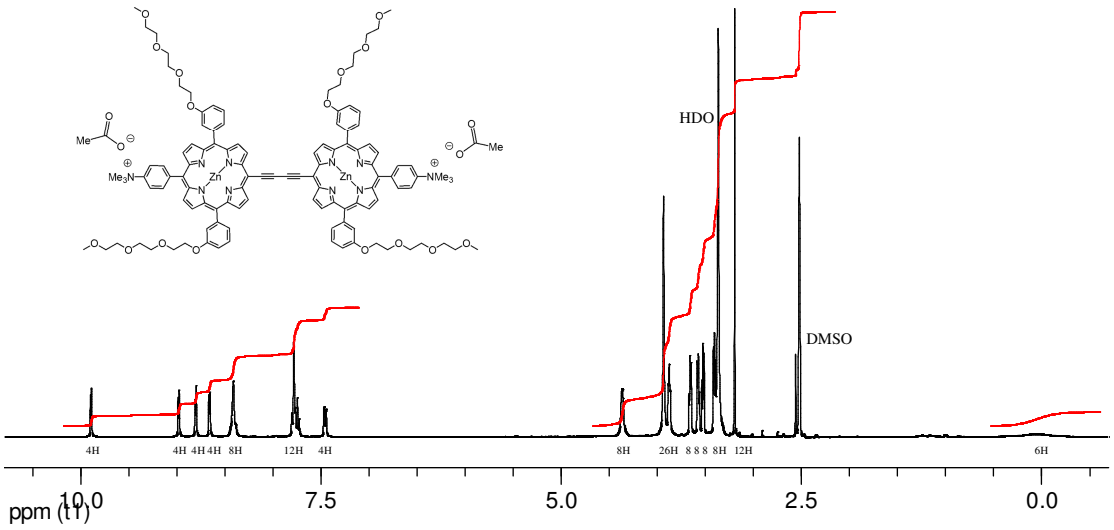


Figure S16. ^{1}H NMR spectrum of porphyrin dimer **4** (DMSO-d_6, 400MHz).

Conjugated porphyrin dimer 4

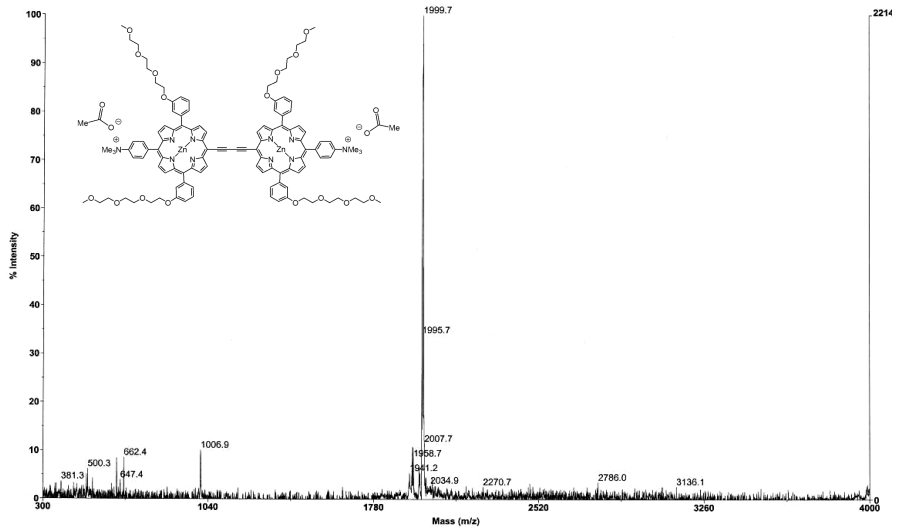
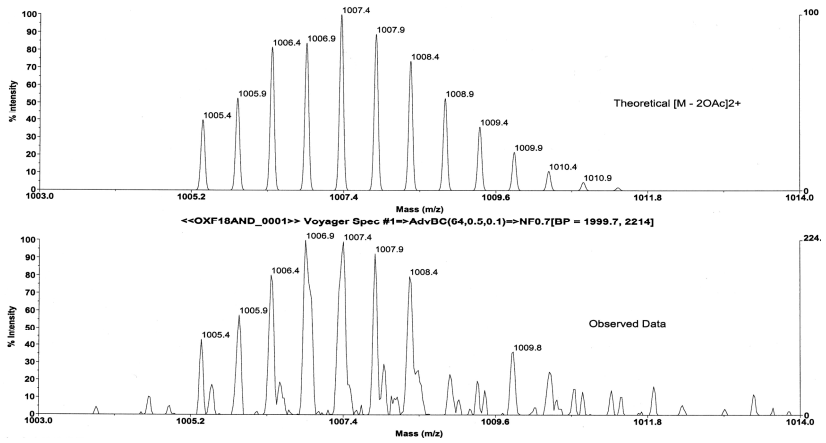
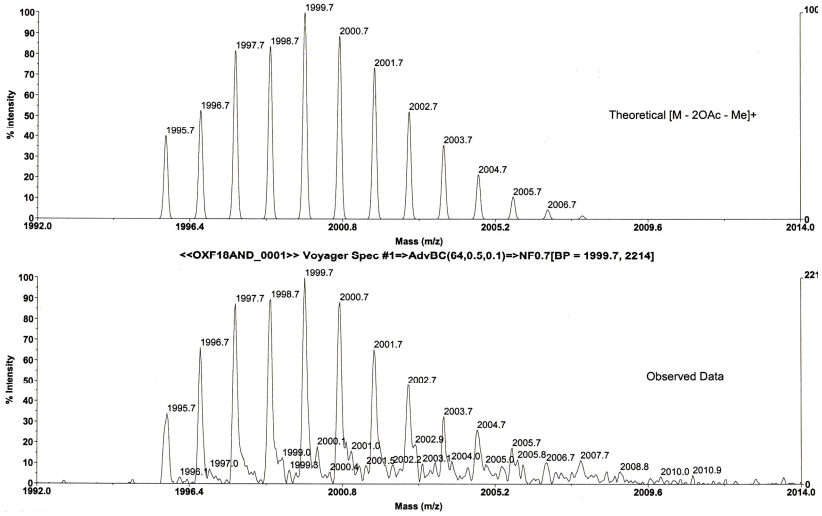


Figure S17. MALDI-TOF of the porphyrin dimer **4** . MALDI□TOF *m/z* 1006.9 ($C_{114}H_{118}N_{10}O_{16}Zn_2$, $[M\text{-}2OAc]^{2+}$, requires 1007.4).
Matrix: 2-[(2E)-3-(4-tert-Butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB)



MALDI-TOF *m/z* 1007.4 $[M\text{-}2OAc]^{2+}$, calculated for $C_{114}H_{118}N_{10}O_{16}Zn_2$ 1007.4



MALDI-TOF *m/z* 1998.7 $[M\text{-}2OAc\text{-}Me]^{+}$, calculated for $C_{113}H_{115}N_{10}O_{16}Zn_2$ 1998.7

Conjugated porphyrin dimer 7 (P_2-Suc)

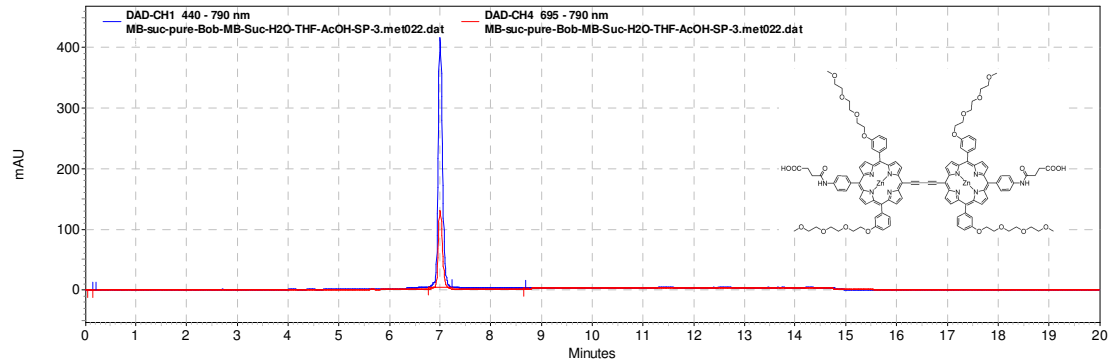


Figure S18. HPLC chromatograph of the porphyrin dimer **7** monitored at two different wavelengths, 440 nm (Soret band, blue line) and 695 nm (Q band, red line).

Chromatographic condition: T = 40°C, flow = 4 mL / min, gradient solvent system: 1% acetic acid in water / THF, Table S6. The porphyrin dimer **7** was dissolved in THF and 30 µL was injected each time.

Table S6. Solvent gradient used for HPLC purification of porphyrin dimer **7**: HPLC method **D**

time / min	1% aqueous acetic acid	THF
0	60 %	40 %
3	50 %	50 %
6	30 %	70 %
9	20 %	80 %
12	20 %	80 %
13	60 %	40 %
15	60 %	40 %

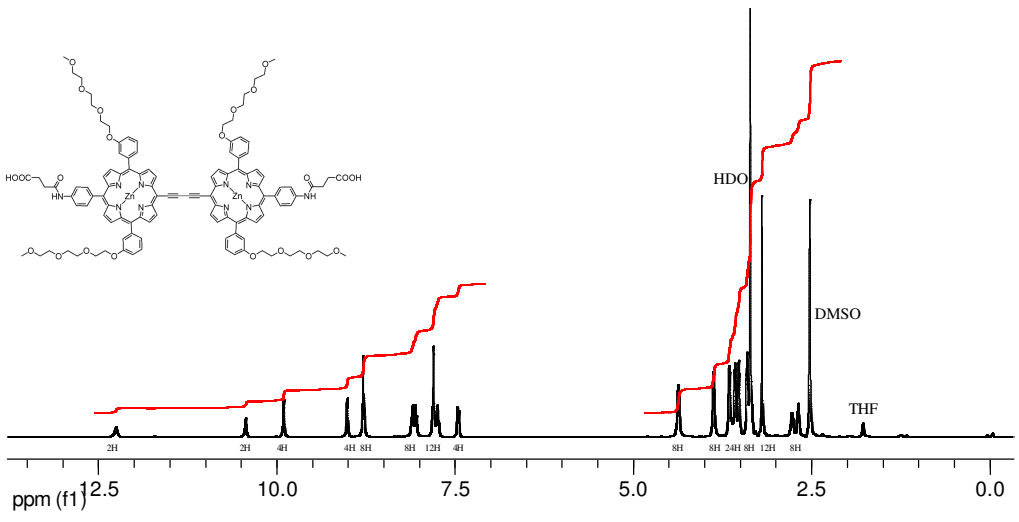


Figure S19. ^{1}H NMR spectrum of porphyrin dimer **7** (DMSO-d_6, 400MHz).

Conjugated porphyrin dimer 7

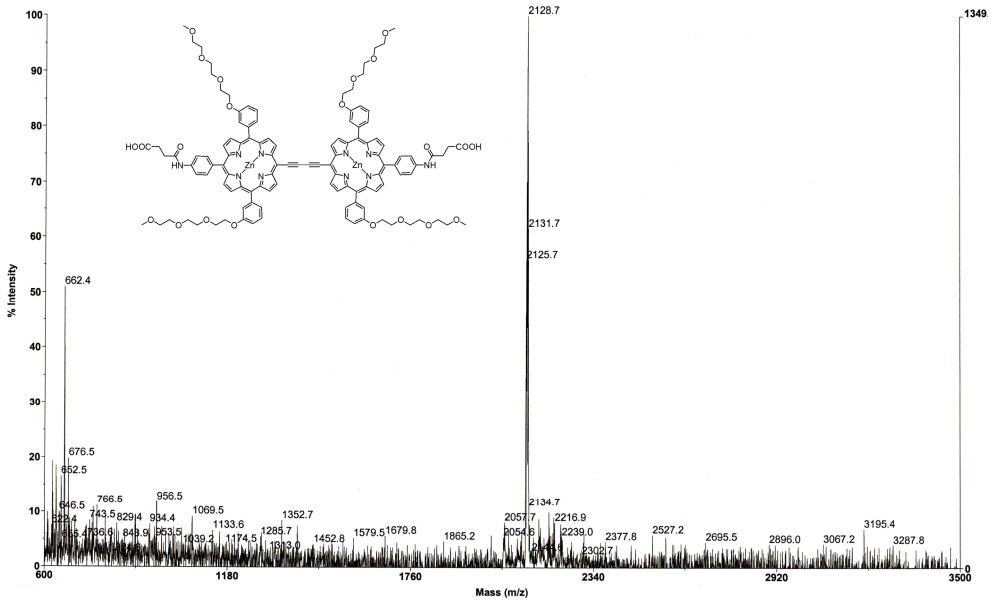
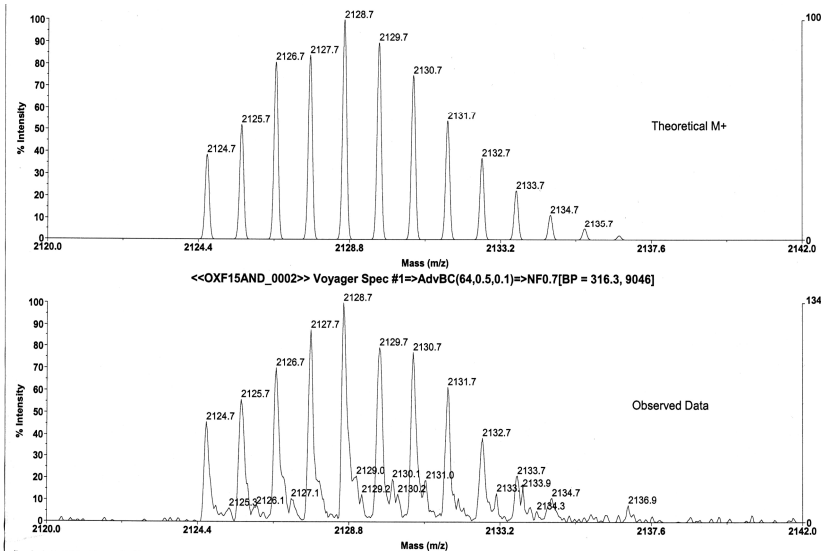


Figure S20. MALDI-TOF of the porphyrin dimer **7**. *m/z* MALDI-TOF 2128.7 ($C_{116}H_{112}N_{10}O_{22}Zn_2$, M^+, requires 2128.96).
Matrix: 2-[(2E)-3-(4-tert-Butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB)



MALDI-TOF *m/z* 2128.7 $[M]^+$, calculated for $C_{116}H_{112}N_{10}O_{22}Zn_2$ 2128.9643

HPLC method E (linear gradient)

time / min	1% aqueous acetic acid	THF
0	60 %	40 %
20	20 %	80 %
22	60 %	40 %
24	60 %	40 %