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Electronic Supplementary Information

Hierarchically controlled protonation/aggregation of a porphyrin–spermine derivative.

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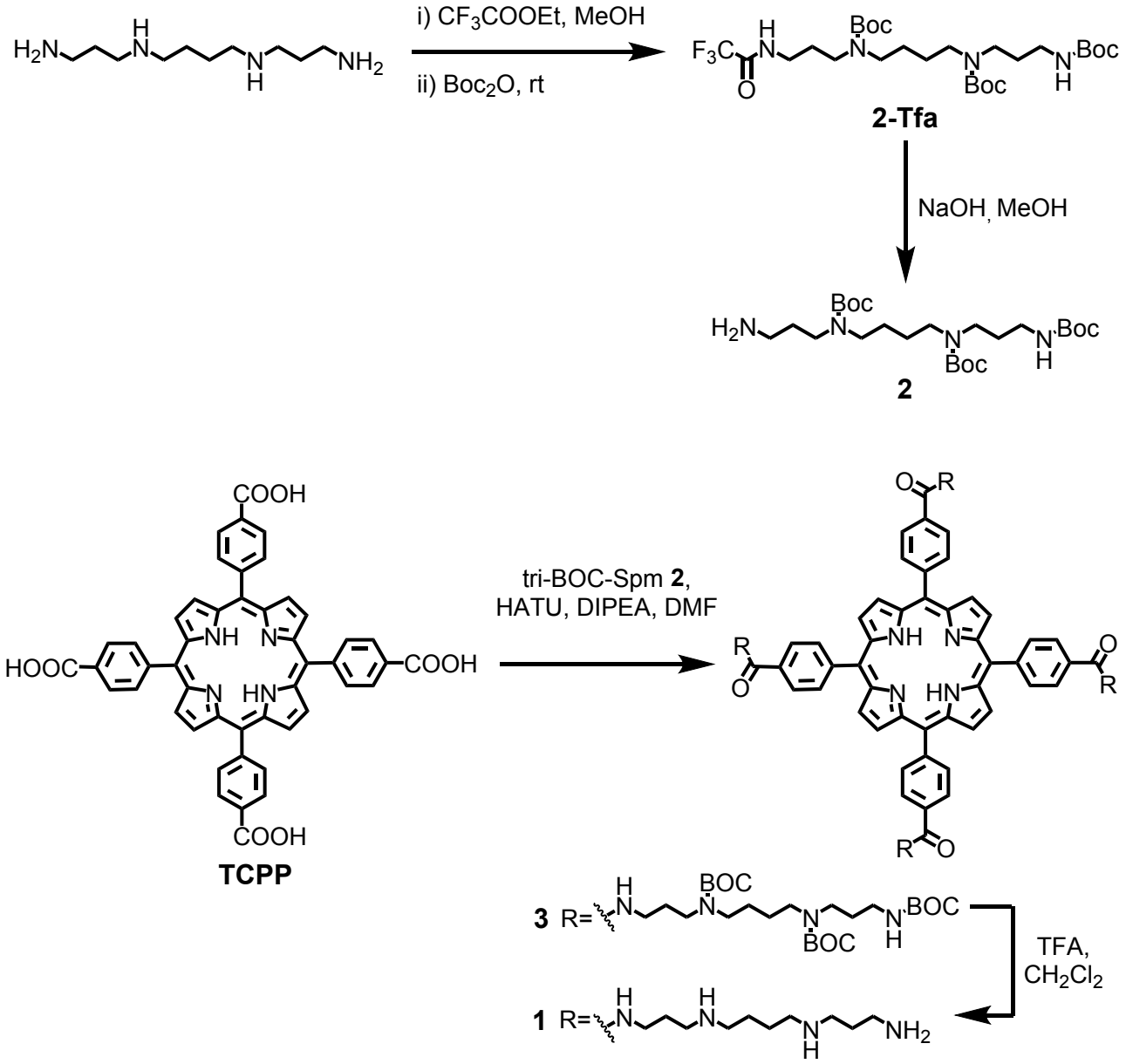
General experimental methods.

All stock solutions of H_2TCPPSpm4 were prepared dissolving the solid in ultrapure water obtained from Elga Purelab Flex system by Veolia, at pH 1 for HCl, in order to achieve concentrations ranging from $2x10^{-4}$M to $3x10^{-4}$M. The concentration of these stock solutions was checked by spectrophotometric experiments using $\varepsilon_{\lambda 435nm}$ = 338000 $M^{-1}cm^{-1}$ at pH=1. To prepare independent solutions we dilute in several cuvettes at different pH, the calculated amount of H_2TCPPSpm4 stock solution to obtain in each cuvettes 2 μM work solutions. As soon as we prepared the solution, we recorded the spectrum. For the continuous titration we dilute the calculated amount of stock solution to obtain 2 μM work solution, at pH 1. Then, every 15 minutes, we added small amounts of NaOH to increase the pH of the solution and performed the spectrum. A JASCO V-560 UV-vis spectrophotometer equipped with a 1 cm path-length cell was used for the UV-vis measurements. For RLS and fluorescence data, a fluorolog FL-11 Jobin-Yvon Horiba was used.
SEM analysis of H_2TCPPSpm4 drop casted on silicon substrates at different pHs, has been performed by using a Field Emission Scanning Electron Microscope (Zeiss Supra 55VP). The EDS analysis has been performed using an Oxford x-Act 10 mm2 SSD detector.

The NMR experiments were carried out at 27° C on a Varian UNITY Inova 500 MHz spectrometer (^{1}H at 499.88 MHz, ^{13}C-NMR at 125.7 MHz) equipped with pulse field gradient module (Z axis) and a tuneable 5 mm Varian inverse detection probe (ID-PFG). ESI mass spectra were acquired on an ES-MS Thermo-Finnigan LCQ-DECA using MeOH (positive ion mode). Positive MALDI-TOF mass spectra were acquired by a Voyager DE (PerSeptive Biosystem) equipped with a nitrogen laser (emission at 337 nm for 3 ns) and a flash AD converter (time base 2 ns).
All of the chemicals were purchased from Sigma-Aldrich and used without further purification, H_2TCPP was purchased from Frontier Scientific and used as received. Ultra-pure water (18.2 M) was used in all experiments.



Synthesis of tri-BOC-Spm (2)

Compound **2** was obtained by a modified literature method [1] with a two-step procedure as shown in Scheme 1.

Synthesis of 2-Tfa

A solution of Spermine (2.123 g, 10.5 mmol) in MeOH (120 mL) was stirred under nitrogen at -78 °C. After 10 min, ethyl trifluoroacetate (1.24 mL, 10.5 mmol) was added dropwise over 1 hour and the temperature was slowly increased to 0°C. Then a solution of Boc_2O (11.46 g, 52.5 mmol) in MeOH (10 mL) was added to reaction mixture during which temperature was brought back to 25° C. After 24 h, the solvent was removed under reduced pressure and the residue was extracted with dichloromethane. The organic phase was washed with H_2O and then dried over anhydrous Na_2SO_4. The product was purified by column chromatography (EtOAc) to give the desired compound (**2-Tfa**) as a yellow oil (3.1 g, 49% yield). ^{1}H NMR (500 MHz, acetone-d_6): δ 8.54 (br s, 1H); 5.95 (br s, 1H); 3.34-3.24 (m, 10H); 3.07 (m, 2H); 1.82 (br s, 2H); 1.69 (br s, 2H); 1.54-1.41 (m, 31H) ppm. ^{13}C NMR (126 MHz, acetone-d_6): δ 155.7, 155.2, 146.7, 116.2, 84.8, 78.4, 77.5, 46.7, 44.0, 37.5, 36.6, 27.7, 27.6, 26.5, 25.5 ppm.

Synthesis of tri-BOC-Spm (2)

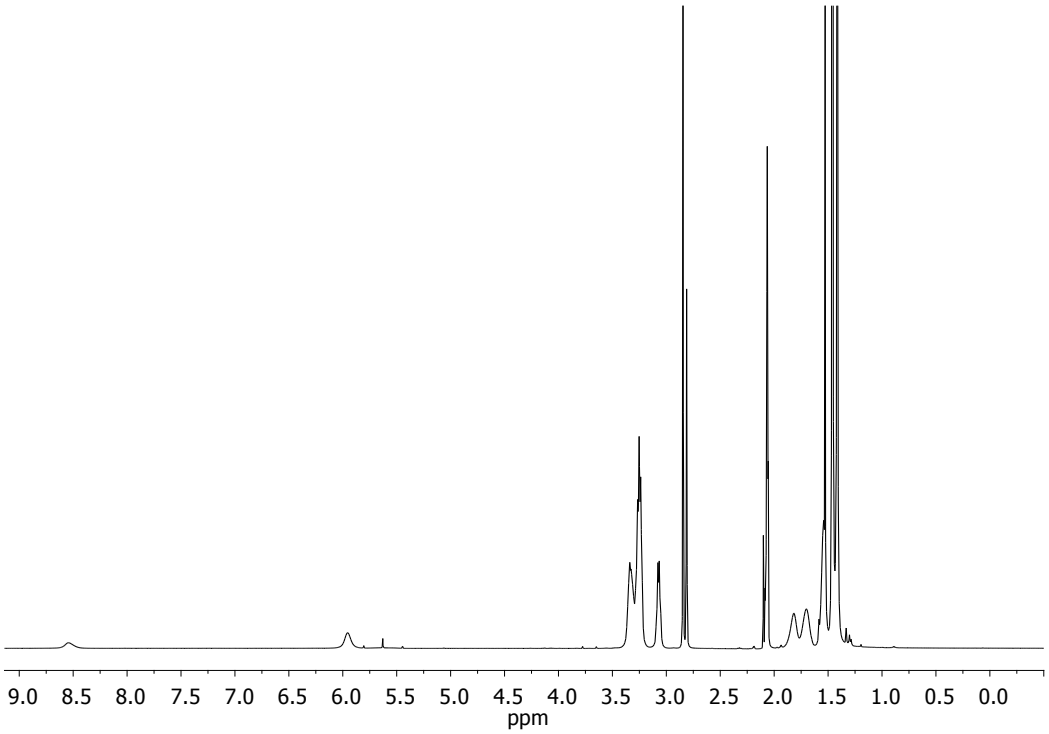
The trifluoroacetate protecting group of **2-Tfa** (1.41 g, 2.35 mmol in 5 mL MeOH) was removed with NaOH after stirring for 15 h at room temperature. The solution was concentrated in vacuum and the residue was extracted with dichloromethane. The organic phase was washed with H_2O and then dried over anhydrous Na_2SO_4. The solvent was removed under reduced pressure to give the desired product **2** as a yellow oil (1.089 g, 92% yield). ^{1}H NMR (500 MHz, acetone-d_6): δ 5.98 (br s, 1H), 3.30-3.23 (m, 8H), 3.17 (t, *J* =7.0 Hz, 2H), 3.08 (m, 2H), 1.80 (m, 2H), 1.70 (br s, 2H), 1.53 (br s, 4H), 1.46 -1.41 (m, 27H) ppm.; ESI-MS: Mass Calcd. for $C_{25}H_{51}N_4O_6$ 503.38. Found *m/z* = 503.4 $[M+H]^+$.

Synthesis of 3

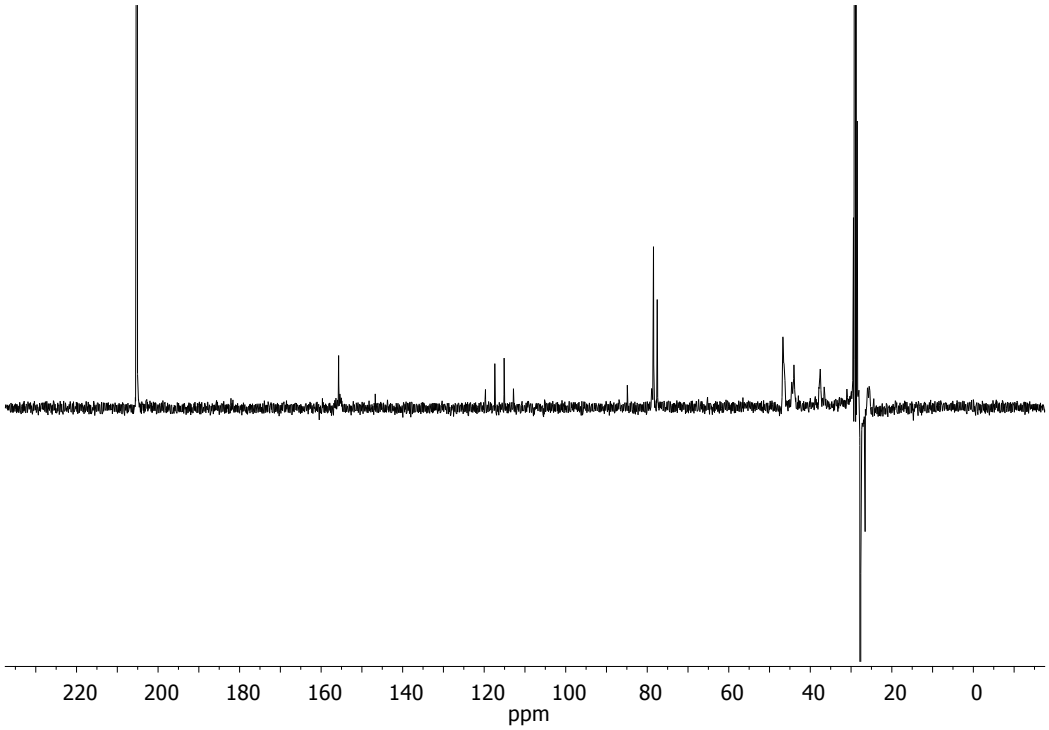
To a solution of H_2TCPP (200 mg, 0.252 mmol) in dry DMF(2 mL), a solution of HATU (445 mg, 1.17 mmol in 1 mL of DMF) was added dropwise and stirred for 20 min at room temperature under N_2 atmosphere. Then a solution of **2** (510 mg, 1.02 mmol) in dry DMF (2 mL) was added to the reaction mixture. After 40 min *N*,*N*-diisopropylethylamine (200 μL, 1.17 mmol) was added to the reaction, which was stirred for 24 h at rt. The solvent was removed under reduced pressure and CH_2Cl_2 was added obtaining a precipitate that was removed by filtration. The resulting solution was concentrated under vacuum and purified by column chromatography (CH_2Cl_2/EtOH 90:10) to afford the desired compound **3** as a purple powder (107.6 mg, 16% yield). ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.84 (s, 8H), 8.31(br s, 16H), 3.63-3.00 (br s, 48H), 1.91 (br s, 8H), 1.69 (br s, 8H), 1.55-1.28 (m, 124 H), -2.72 (s, 2H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) 134.5, 125.5, 80.1, 79.6, 55.5, 46.8, 43.5, 37.5, 28.5, 28.47, 28.43, 27.7 ppm. MALDI: Mass Calcd. for $C_{148}H_{223}N_{20}O_{28}$ 2728.66. Found *m/z* 2728.92 $[M+H]^+$

Synthesis of 1

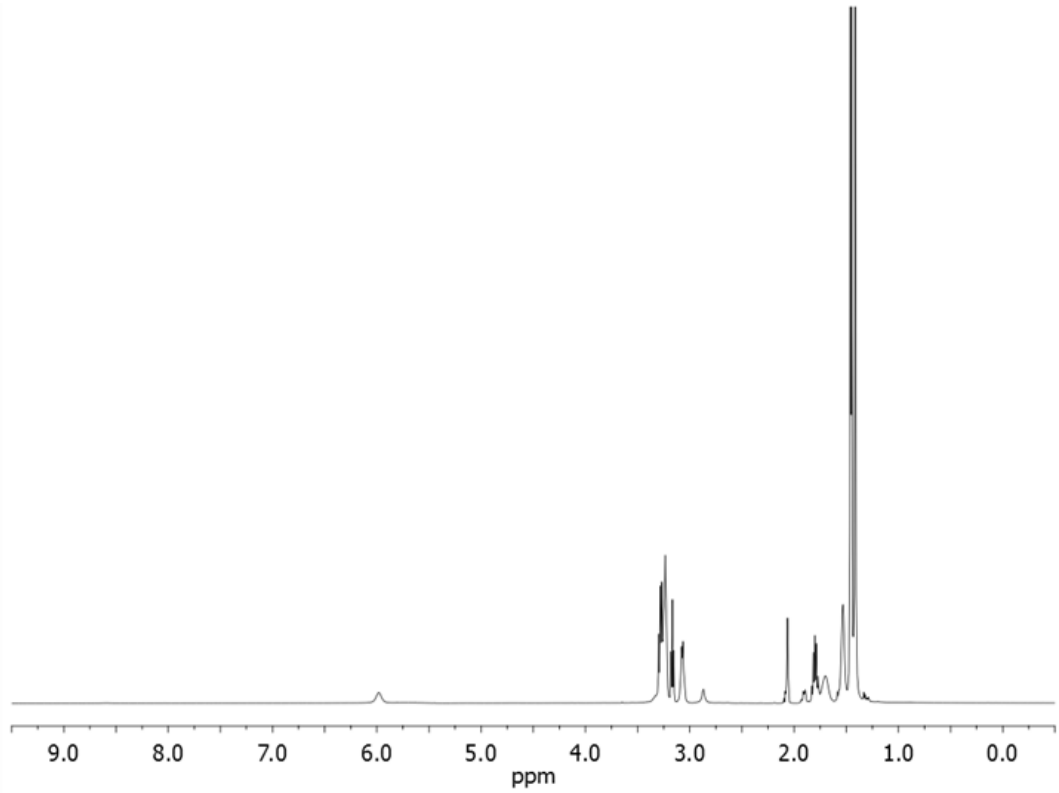
To a solution of **3** in CH_2Cl_2 (0.04 mmol in 1.5 mL), TFA (20 eq.) was added dropwise under nitrogen and the reaction was stirred at rt for 2h. The reaction was monitored by TLC (CH_2Cl_2/EtOH 90:10) and the solvent was removed under reduced pressure affording the corresponding trifluoroacetate salt compound **1** as a green-purple solid in nearly quantitative yield. ^{1}H NMR (500 MHz, CD_3OD): δ 8.88 (br, s, 8H), 8.37 (d, *J* = 8.0 Hz, 8H), 8.33 (d, *J* = 8.0 Hz, 8H), 3.71 (br, s, 8H), 3.26-3.06 (m, 40H), 2.19-2.09 (m, 16H), 1.91 (br, s, 16H) ppm. ^{13}C NMR (126 MHz, CD_3OD): δ 170.8, 136.4, 135.2, 127.3, 146.5, 121.4, 46.6, 45.8, 37.8, 27.8, 25.3, 24.4, 24.3 ppm. ESI-MS: Mass Calcd. for $C_{88}H_{127}N_{20}O_4$ 1528.03. Found *m/z* 1527.7 $[M+H]^+$ (100%), 1453.7 $[M-(C_3H_9N_2)]^+$, 1399.6 $[M-(C_7H_{17}N_2]^+$, 1325.6 $[M-(C_{10}H_{26}N_4]^+$.



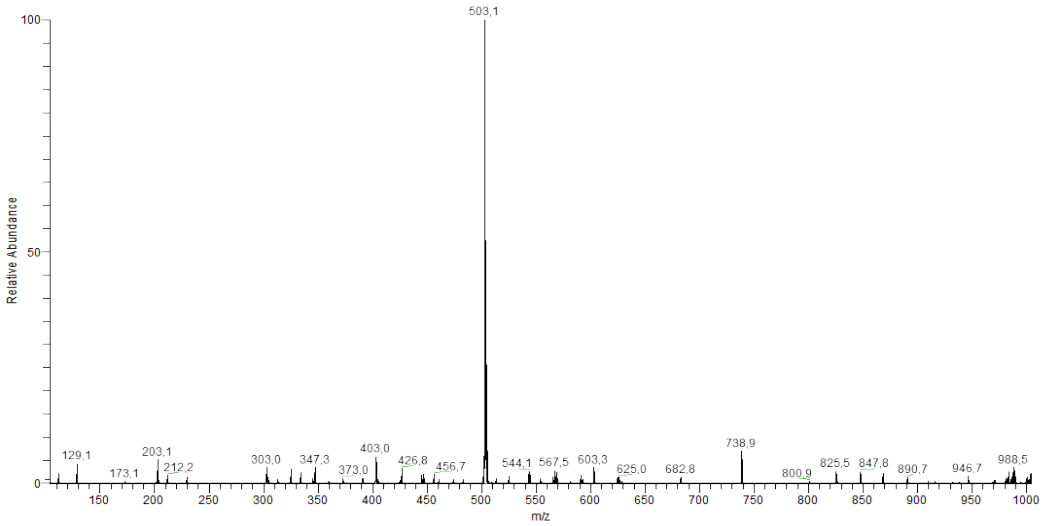
^{1}H-NMR of compound **2-Tfa** in acetone-d_6



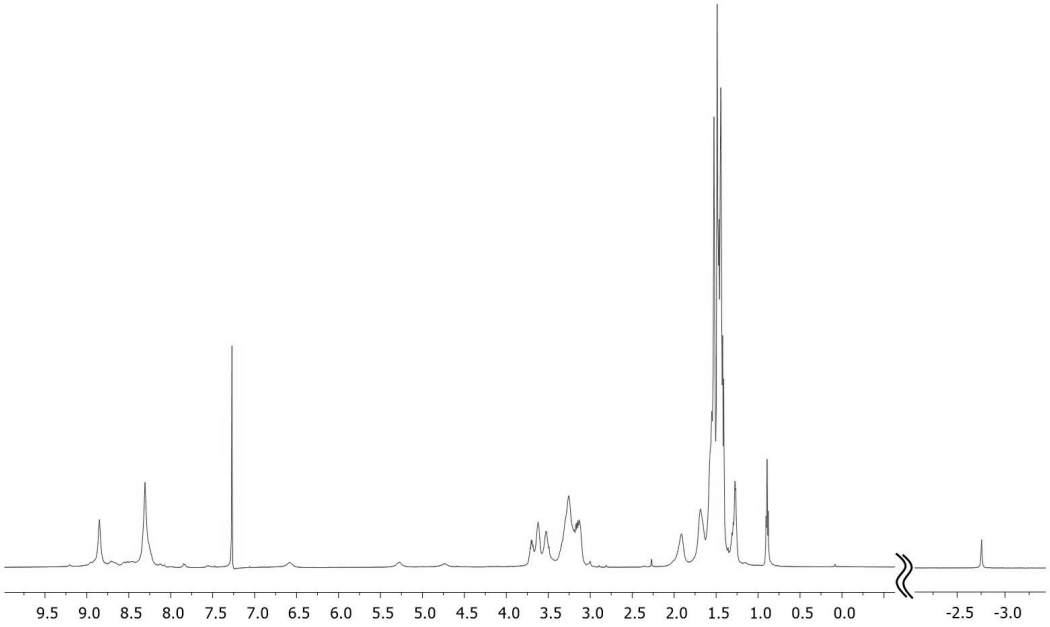
APT of compound **2-Tfa** in acetone-d_6



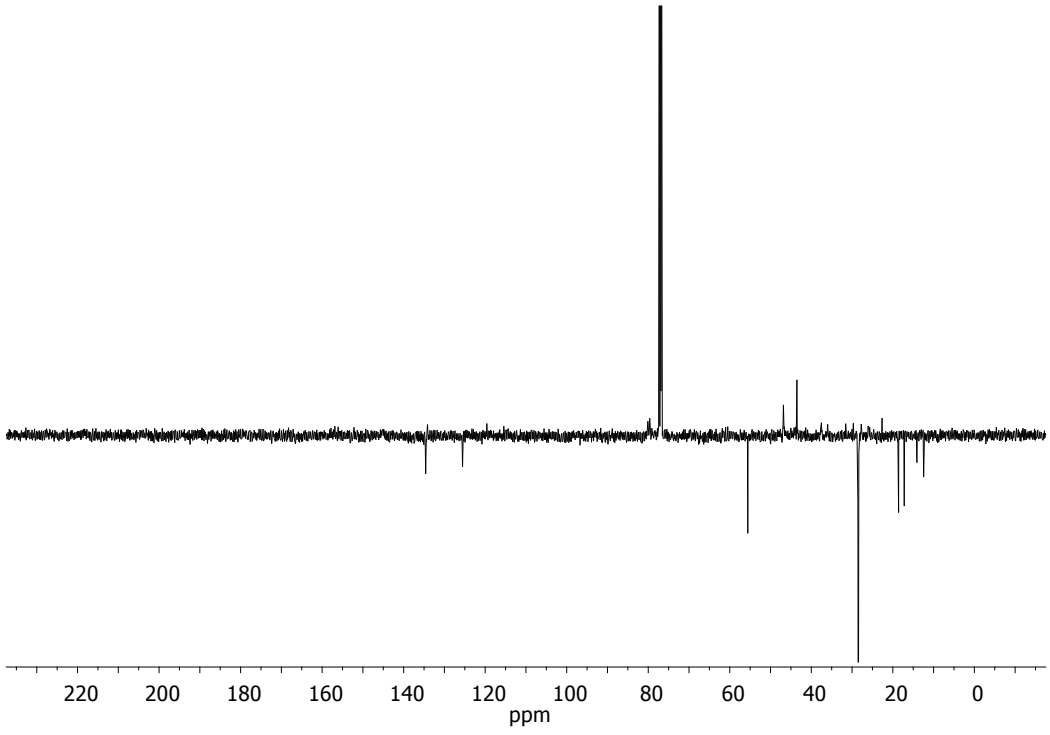
^{1}H-NMR of compound **2** in acetone-d_6



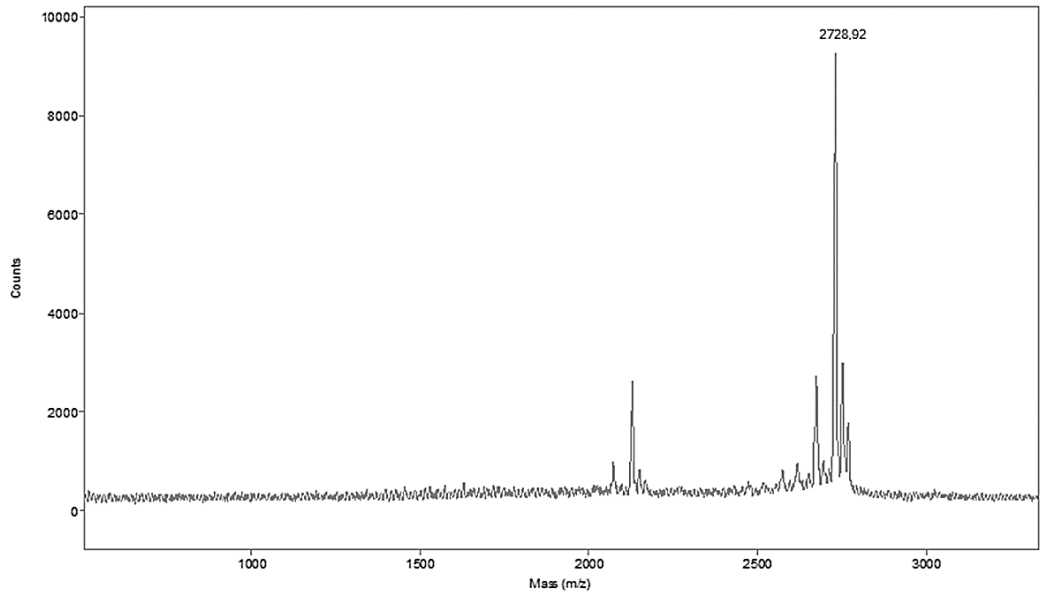
ESI-MS of compound **2**



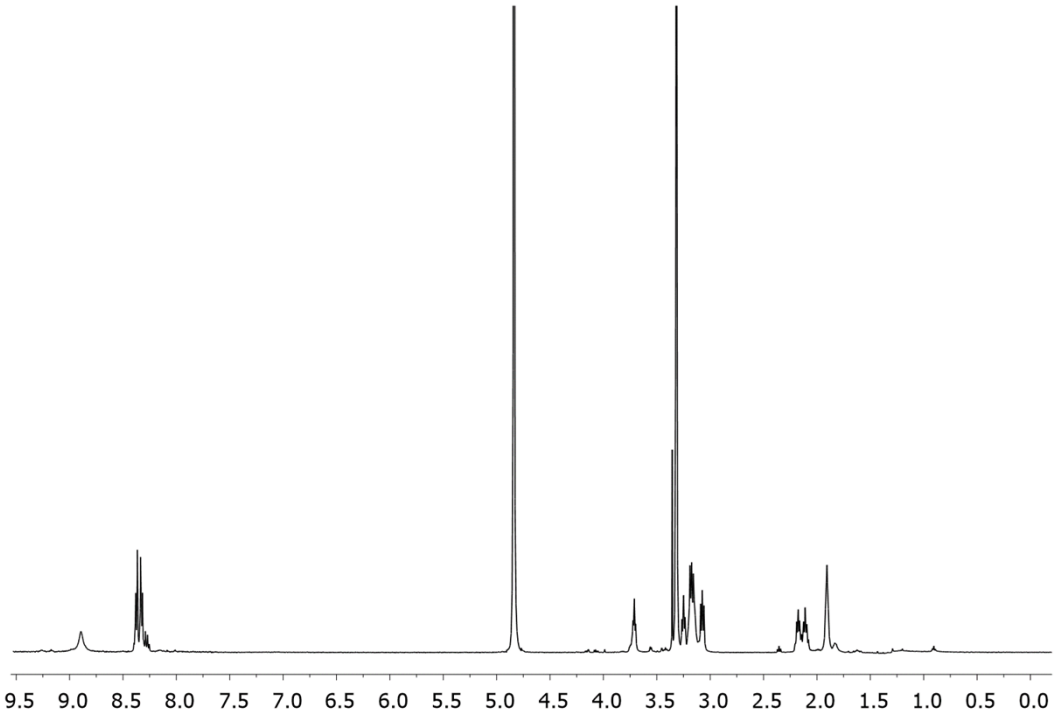
^{1}H-NMR of compound **3** in $CDCl_3$



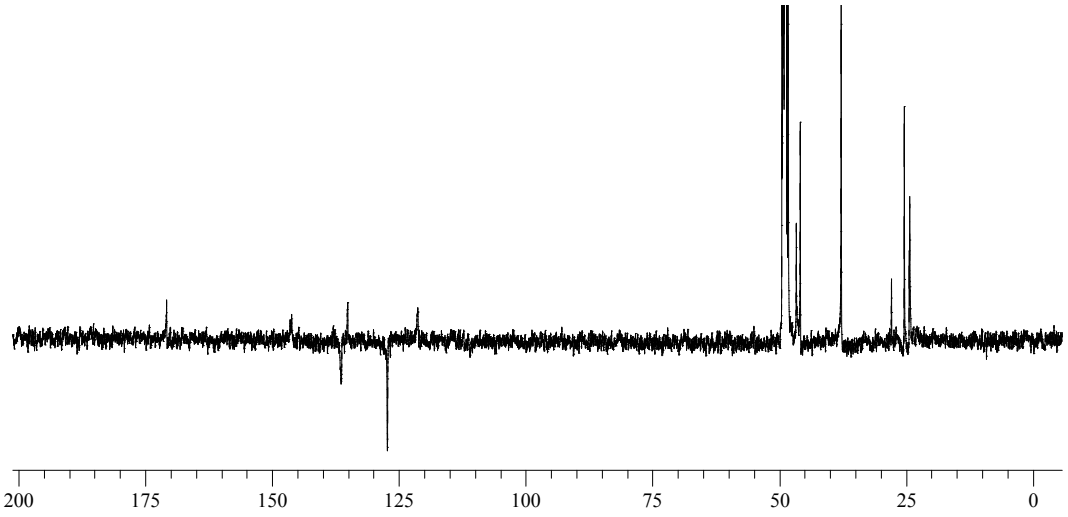
APT of compound **3** in $CDCl_3$



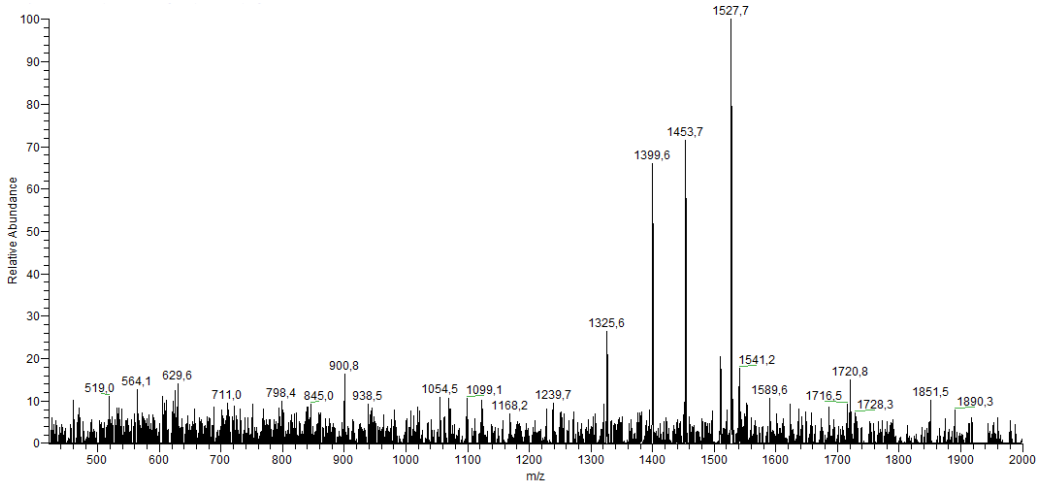
MALDI of compound **3**



^{1}H-NMR of compound **1** in CD_3OD

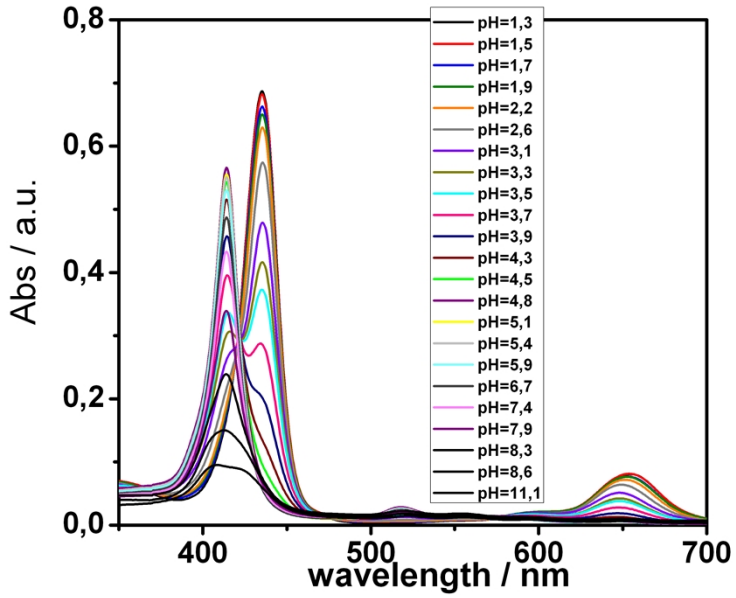


APT of compound **1** in CD_3OD

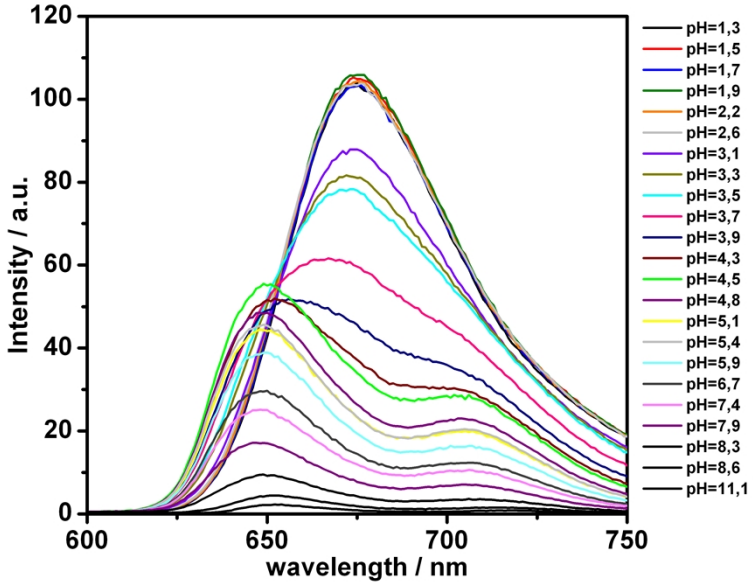


ESI-MS of compound **1**

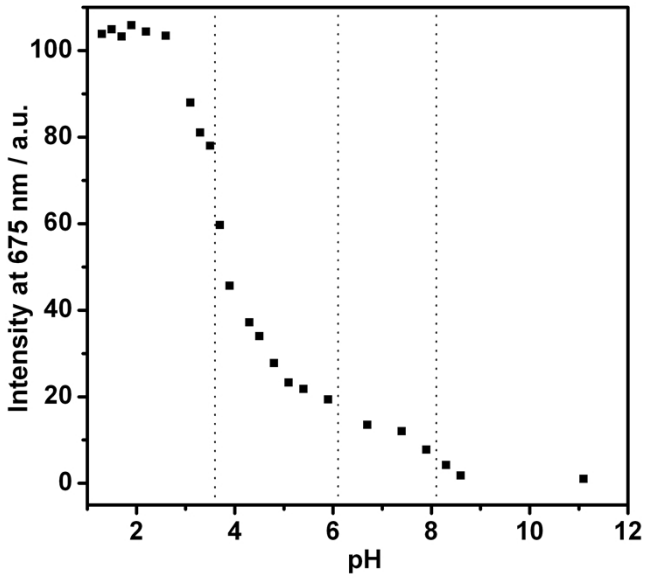
[1] a) Geall, A. J.; Blagbrough, I. S.; Tetrahedron, 2000, 56, 2449–2460. b) US2007197658A1.



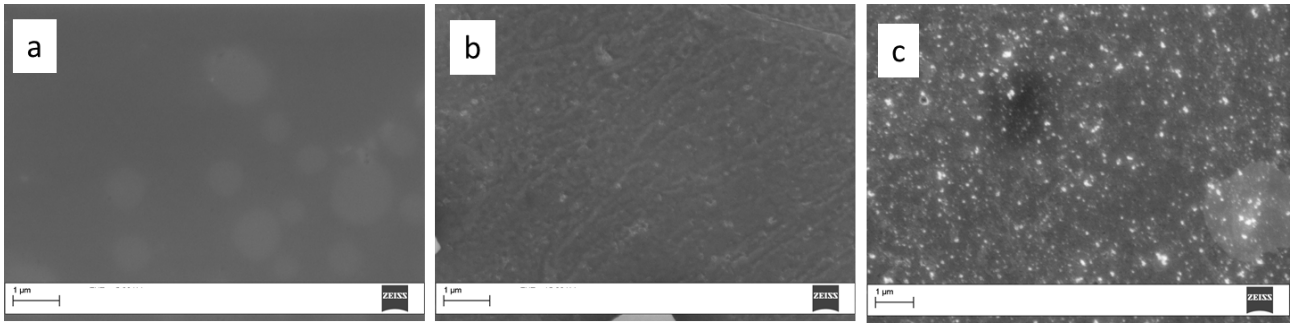
UV–vis spectra of a solution of H_2TCPPSpm4 (2 μM) increasing the pH from 1.3 to 11.1



Fluorescence spectra of a solution of H_2TCPPSpm4 (2 μM) increasing the pH from 1.3 to 11.1. The λex 424 nm

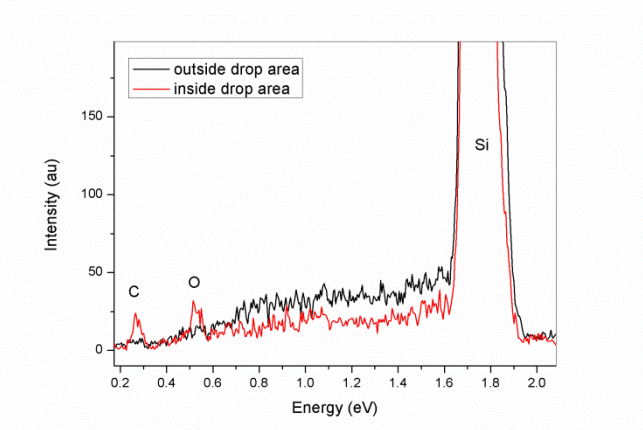


Fluorescence *vs* pH, for the continuous titration at 675 nm (solid black square), of 2 μM H_2TCPPSpm4 solution.



SEM images of H_2TCPPSpm4 deposited on silicon by drop casting of 100μl of 100 μM solution at pH=1,5 (a), pH=5 (b) and pH=8)c)

SEM images show silicon surface after evaporation of 100 μl of H_2TCPPSpm4 aqueous solution (10 μM) at different pH. Clearly, working at pH 1,5 (a) the substrate surface doesn't reveal the presence of any aggregates, while a more structured surface is well evident upon increasing pH value. In particular, small spherical nanostructures are visible at pH 5 (b), while at pH 8 a high density of particles is well detectable (c).
EDS analyses of regions shown in (b) and (c) confirm the carbonaceous nature of such small nanostructures.



EDS spectra of silicon inside (red line) and outside (black line) the H_2TCPP-Spm4 drop spot (1 μM, pH=8).

Nanoaggregate dimensions (below tenth of nanometers) confirm the RLS measurements that have shown a signal associable to small aggregates, as expected by the use of extremely diluted solutions.