Supplementary Material (ESI) for Chemical Communications

Synthesis of cationic porphyrin modified amino acids

*Eric Biron and Normand Voyer**

Département de chimie and CREFSIP, Faculté des sciences et de génie, Université Laval, Québec, Québec, Canada G1K 7P4

Normand.Voyer@chm.ulaval.ca

ELECTRONIC SUPPLEMENTARY INFORMATION :

Experimental details

EXPERIMENTAL

General

NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 with a Bruker AC-F-300 spectrometer. The chemical shifts (δ) are given ppm with respect to the residual solvent signal and coupling constants (J) are given in Hz. The following abbreviations were used to explain the multiplicities: s, single; d, doublet; t, triplet; q, quartet; dd, doublet of doublet; m, multiplet; b, broad. Mass spectra (ES) were performed on a Perkin Elmer API-III instrument (University of Toronto) and (CI, EI) on a ZAB-IF VG instrument (University of Sherbrooke). UV-visible analysis were performed with a UV-visible Hewlett Parkard HP 8452-A spectrophotometer. RP-HPLC analyses and separations were conducted on a Hewlett Packard HP 1050 instrument using a Vydac colunm (analytical: 4.6 mm x 250 mm, 5 μm C_{18}, 1 mL/min) and a Phenomenex column (semi-preparative: 25 mm × 250 mm, 10 μm C_{18}, 8 mL/min) with different 30 min linear gradients from water (0.1% TFA) and CH_3CN (0.1% TFA) and detection at 220 nm.

5-*p*-Nitrophenyl-10-15-20-(tri-4-pyridyl)porphyrine

p-Nitrobenzaldehyde (85 mmol) and 4-pyridine carboxaldehyde (157 mmol) were added to a 1 L round bottom flask containing propionic acid (750 mL) and acetic anhydride (75 mL) at 110°C. Pyrrole (208 mmol) was then added portionwise and the reaction mixture was refluxed for 1.5 h. The propionic acid and the acetic anhydride were removed by distillation until 75 mL to 100 mL was left in the flask The remaining mixture was neutralized with a solution 1N NaOH on ice, filtered and washed with a solution 1N NaOH (3x) and with water (3x). The solid was dried overnight, dissolved in DCM and filtered. The DCM was removed under reduce pressure and the resulting purple solid was purified by silica gel chromatography (gradient of 2.5% to 10% ethanol/DCM) affording 2.8 g (4.22 mmol, 8%) of purple solid. ^{1}H NMR (300 MHz; $CDCl_3$), δ = -2,7 (s, 2H, NH pyrrole), 8.16-8.18 (m, 6H, 2,6 pyridine), 8.38-8.41 (d, 2H, H_{ortho} NO_2), 8.66-8.68 (d, 2H, H_{meta} NO_2), 8.77-8.92 (m, 8H, H pyrrole), 9.05-9.15 (m, 6H, 3,5 pyridine); EI-MS (70eV): 662 (M^+); UV/visible ($CHCl_3$), λ/nm (ε/10^{-3} $M^{-1}cm^{-1}$): 418 (353), 514 (18.2), 548 (6.3), 588 (5.8), 644 (1.9).

5-*p*-Aminophenyl-10-15-20-(tri-4-pyridyl)porphyrine (1)

The nitro-porphyrine (4 mmol) was dissolved in a solution 6N HCl (400 mL) followed by addition $SnCl_2$ (20 mmol) and the mixture was stirred at room temperature for 24 h. The reaction mixture was neutralized with a solution 1N NaOH and NaOH on ice. The aqueous basic solution was extracted with DCM and the resulting organic phase washed with water (3x). The organic phase was dried over $MgSO_4$ and the solvent evaporated under reduced pressure affording 2.5 g (3.96 mmol, 98%) of purple solid. ^{1}H NMR (300 MHz; $CDCl_3$), δ = -2.7 (s, 2H, NH pyrrole), 7.05-7.10 (d, 2H, J=5 Hz, H_{ortho} NH_2), 7.95-8.00 (d, 2H, J=5 Hz, H_{meta} NH_2) 8.16-8.20 (m, 6H, 2,6 pyridine), 8.80-8.88 (m, 6H, 3,5 pyridine), 9.05-9.12 (m, 8H, H pyrrole); EI-MS (70eV): 632 (M^+); UV/visible ($CHCl_3$), λ/nm (ε/10^{-3} $M^{-1}cm^{-1}$): 420 (255), 514 (15), 548 (7.7), 588 (5), 644 (6.4).

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-γ-benzyloxycarbonyl-L-glutamic acid methyl ester**

Boc-Glu(Bzl)-OH **2** (6 mmol) was dissolved in a minimum of DCM at 0°C followed by addition of DCC (7.1 mmol) and HOBt (7.1 mmol). The mixture was stirred for 30 min and filtered. Methanol (35 mmol) was added to the filtrate and the solution stirred for 16 h. The reaction mixture was washed with a solution 0.05N HCl (3x), water (3x), a saturated solution of $NaHCO_3$ (3x), and water (3x). The organic phase was dried over $MgSO_4$ and the solvent evaporated under reduced pressure to afford 2.1 g (5.88 mmol, 98%) of white solid. $[\alpha]_D$: -17,0 (c=1, MeOH); mp: 38-41°C; ^{1}H NMR (300MHz, $CDCl_3$), δ = 1.42 (s, 9H, t-butyl), 1.92-2.01 (m, 1H, $H^{\beta}2$), 2.16-2.22 (m, 1H, $H^{\beta}1$), 2.41-2.48 (m, 2H, H^{γ}), 3.72 (s, 3H, OCH_3), 4.32-4.34 (m, 1H, H^{α}), 5.11 (m, 3H, CH_2 bzl + NH), 7.31-7.34 (m, 5H, H_{arom} benzyl); CI-MS (NH_3): 352 $(M+H)^+$, 365 $(M+NH_4)^+$.

***N*$^{\alpha}$-*tert*-Butyloxycarbonyl-L-glutamic acid methyl ester (3)**

Boc-Glu(Bzl)-OMe (5.5 mmol) was dissolved in MeOH followed by addition of the catalyst (10% Pd/C, 0.5 g) and the mixture stirred under hydrogen atmosphere at 40 psi for 12 h. The reaction mixture was filtered on cellite and the solvent evaporated under reduced pressure to afford 1.43 g (5.45 mmol, 98%) of white solid. $[\alpha]_D$: -24,9 (c=1, MeOH); mp: 42-45°C ; ^{1}H NMR (300MHz, $CDCl_3$) δ = 1.43 (s, 9H, *t*-butyl), 1.90-1.99 (m, 1H, $H^{\beta}2$), 2.14-2.21 (m, 1H, $H^{\beta}1$), 2.38-2.51 (m, 2H, H^{γ}), 3.74 (s, 3H, OCH_3), 4.34-

4.36 (m, 1H, H^{α}), 5.16-5.18 (d, 1H, J = 7 Hz, NH); CI-MS (NH_3): 262 = $(M+H)^+$, 279 = $(M+NH_4)^+$.

N^{α}-*tert*-Butyloxycarbonyl-γ-(5-*p*-amidophenyl-10,15,20-(tri-4-pyridyl)porphyrine)-L-glutamic acid methyl ester (4)

Boc-Glu-OMe **3** (3.8 mmol) was dissloved in dry DCM at 0°C followed by addition of triethylamine (4.6 mmol) and ethyl chloroformate (3.8 mmol). The reaction mixture was stirred for 30 min under N_2 atmosphere and evaporated to dryness under reduced pressure. The resulting solid was redissolved in dry DCM, at 0°C followed by the addition of a solution of porphyrine **1** (1.3 mmol) in dry DCM and triethylamine (4.6 mmol). The reaction mixture was stirred for 1 h at 0°C and 1h at room temperature under N_2 atmosphere. The reaction mixture was washed with a solution of 5% $NaHCO_3$ (m/v) (3x) and water (3x). The organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The resulting solid was purified by silica gel chromatography (2.5% to 10% ethanol/DCM) to afford 0.91g (1.04 mmol, 80.3%) of purple solid. ^{1}H NMR (300MHz, DMSO-d_6), δ = -2.98 (s, 2H, NH pyrrole), 1.45 (s, 9H, *t*-butyl), 1.95-2.05 (m, 1H, $H^{\beta}2$), 2.15-2.25 (m, 1H, $H^{\beta}1$), 2.59-2.68 (m, 2H, H^{γ}), 3.75 (s, 3H, OCH_3), 4.14-4.21 (m, 1H, H^{α}), 7.41-7.45 (d, 1H, J = 6 Hz, NH), 8.05-8.12 (d, 2H, J = 8 Hz, H_{ortho} NHCO) 8.14-8.18 (d, 2H, J = 8 Hz, H_{meta} NHCO), 8.22-8.30 (m, 6H, 2,6 pyridine), 8.87-8.98 (m, 8H, H pyrrole), 9.05-9.10 (m, 6H, 3,5 pyridine), 10.44 (s, 1H, NH_{δ}); ES-MS: m/*z* 876 = $(M+H)^+$; UV/visible ($CHCl_3$), λ/nm (ε/10^{-3} $M^{-1}cm^{-1}$): 420 (344), 516 (16.9), 552 (8.2), 590 (6.0), 642 (3.7).

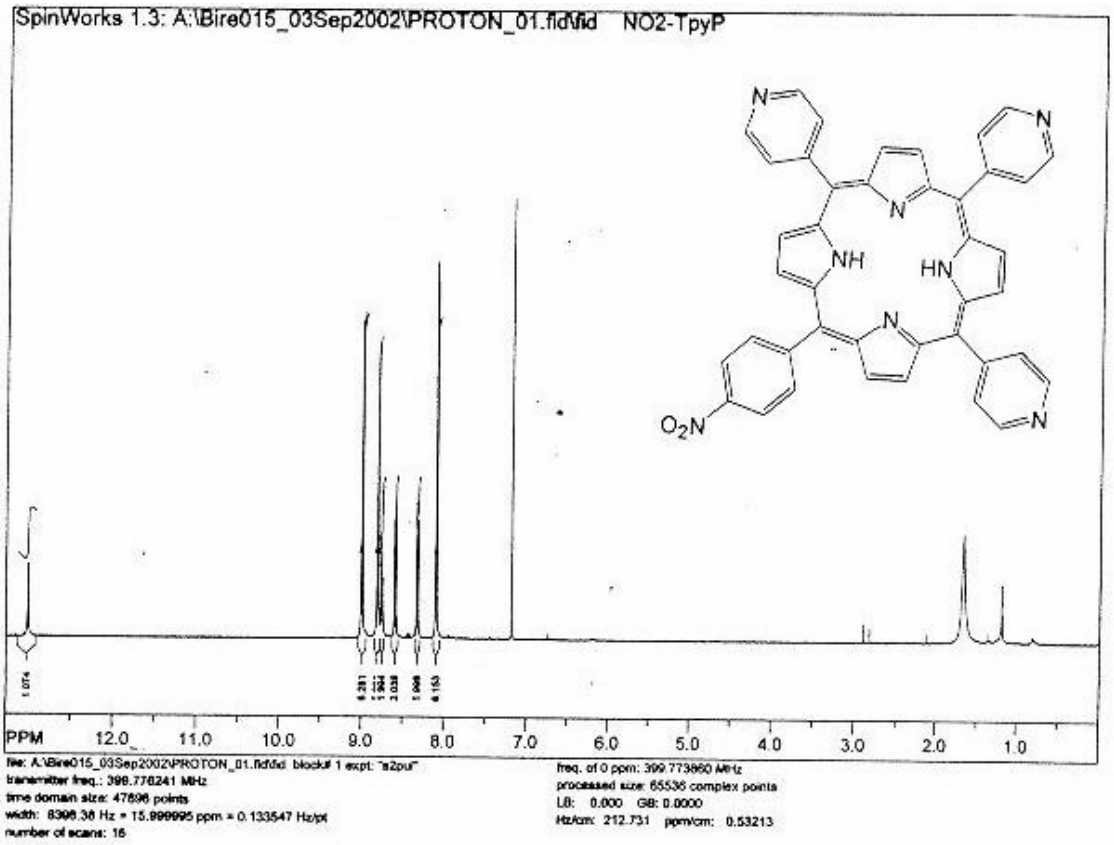
N^{α}-*tert*-Butyloxycarbonyl-γ-(5-*p*-amidophenyl-10,15,20-(tri-4-pyridyl)porphyrine)-L-glutamic acid (5)

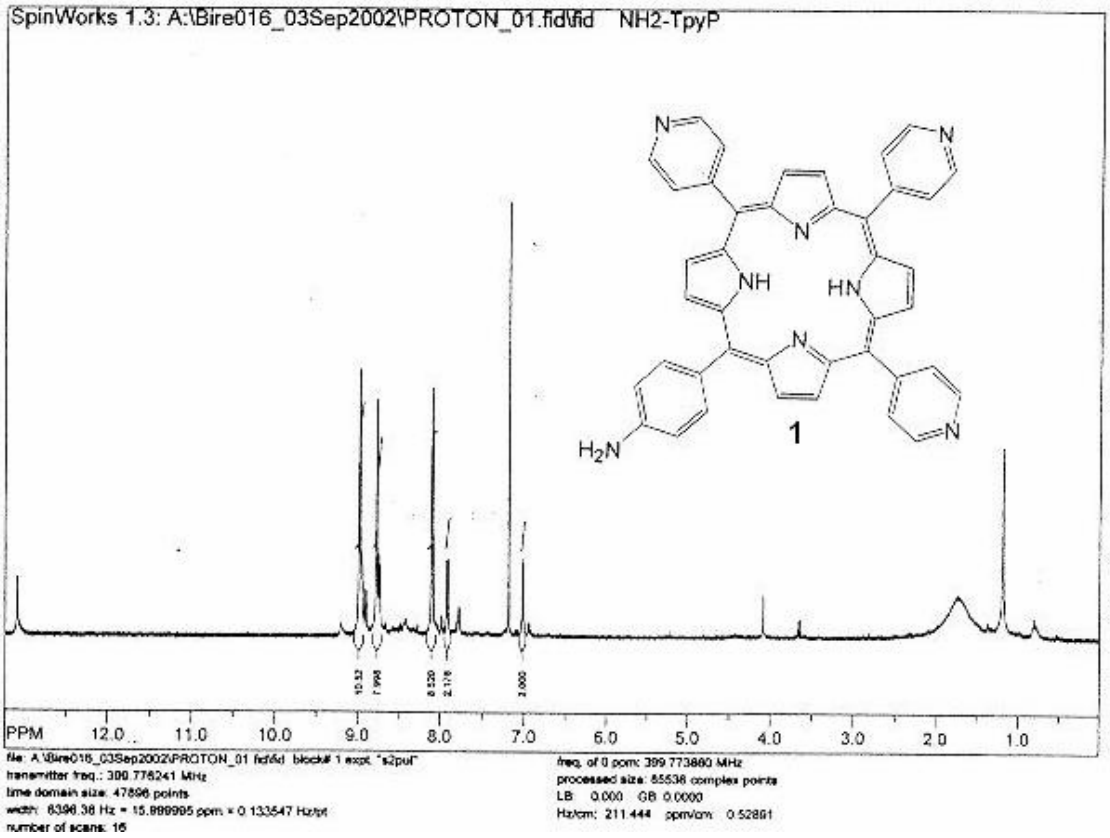
Boc-Glu(TPyP)-OMe **4** (0.8 mmol) was dissolved in THF at 0°C followed by the addition of a solution 1N NaOH (3.2 mmol). The reaction mixture was stirred at room temperature until complete conversion was observed and then THF was evaporated under reduced pressure. The remaining aqueous phase was extracted with DCM (2x) combined with the addition of acetic acid. The acetic acid breaks the emulsion and pushes the amino acid in the organic phase. The colored organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The remaining acetic acid

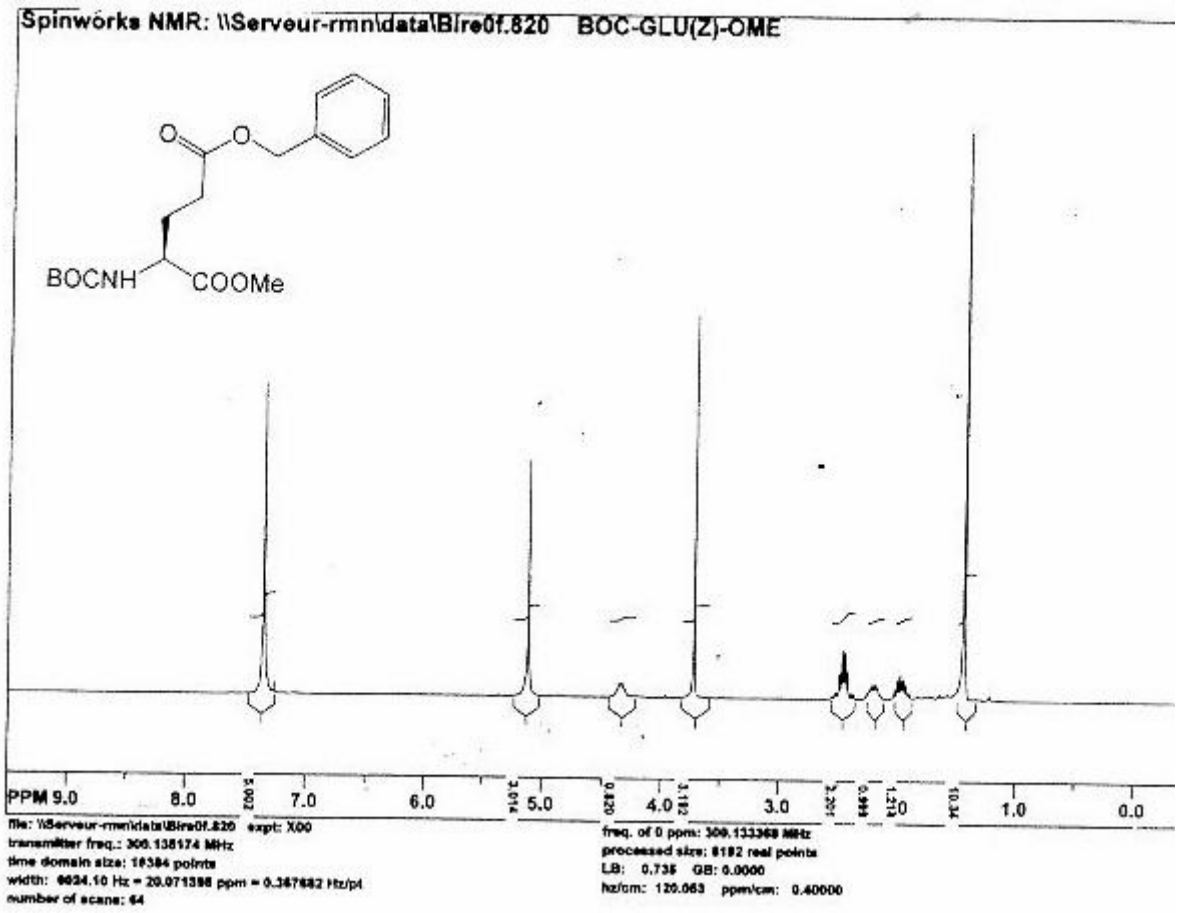
was co-evaporated with toluene (4x) under reduced pressure or lyophilized. The resulting purple solid was purified by short column silica gel chromatography (DCM, 10% ethanol/DCM and finally MeOH/DCM (1:1)) to afford 620 mg (0.72 mmol, 90%) of purple solid. ^{1}H NMR (300MHz, DMSO-d_6), δ = -2.98 (s, 2H, NH pyrrole), 1.45 (s, 9H, *t*-butyl), 2.01-2.19 (m, 2H, H^{β}), 2.52-2.58 (m, 2H, H^{γ}), 3.85-3.91 (m, 1H, H^{α}), 6.38-6.42 (d, 1H, J = 6 Hz, NH Glu), 8.05-8.18 (m, 4H, H_{ortho} NH + H_{meta} NH), 8.22-8.30 (m, 6H, 2,6 pyridine), 8.87-8.98 (m, 8H, H pyrrole), 9.05-9.10 (m, 6H, 3,5 pyridine), 10.44 (s, 1H, NH^{δ} Glu); ES-MS: m/*z* 862 = $(M+H)^+$; UV/visible (MeOH), λ/nm (ε/10^{-3} $M^{-1}cm^{-1}$): 416 (265), 512 (12.9), 546 (5.9), 588 (4.0), 650 (4.9)

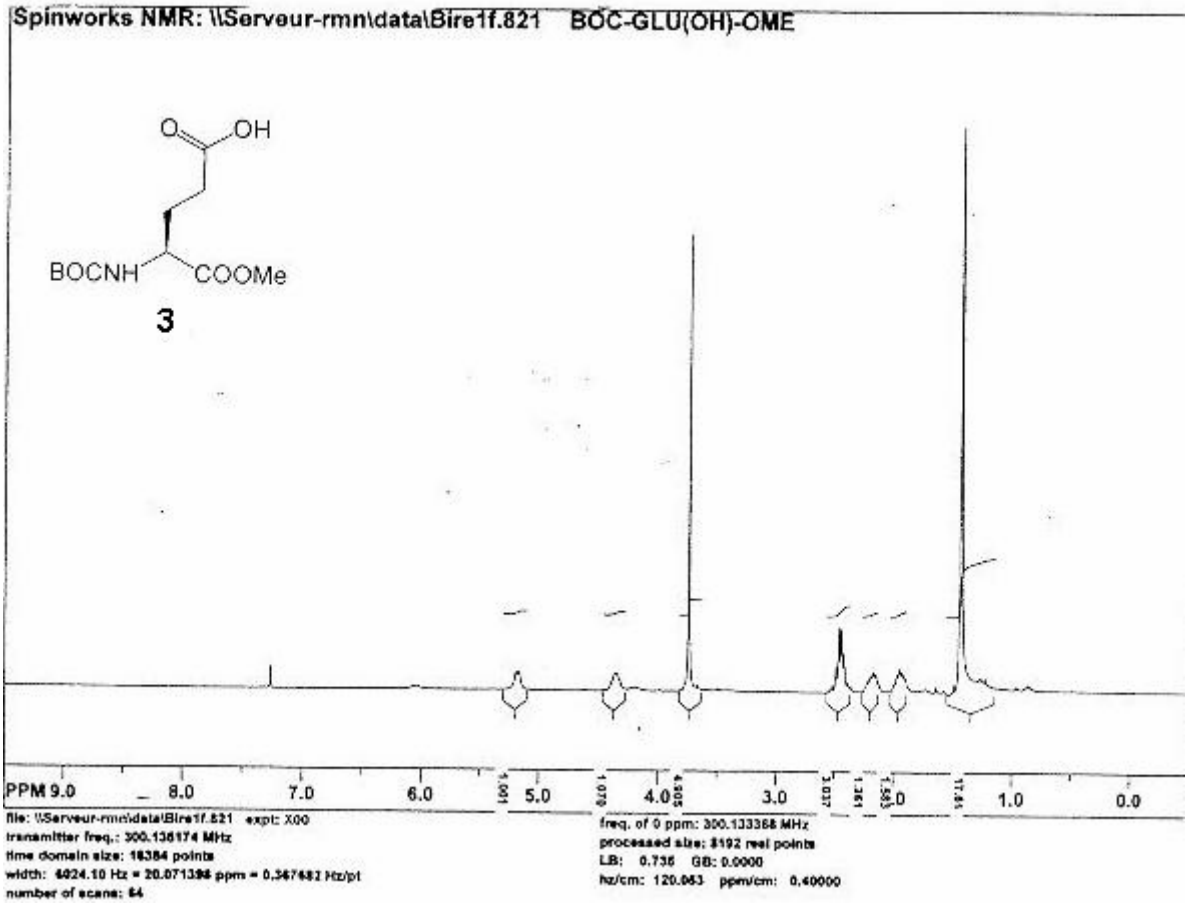
N^{α}-9-Fluorenylmethoxycarbonyl-γ-(5-*p*-amidophenyl-10,15,20-(tri-4-pyridyl)porphyrine)-L-glutamic acid (6)

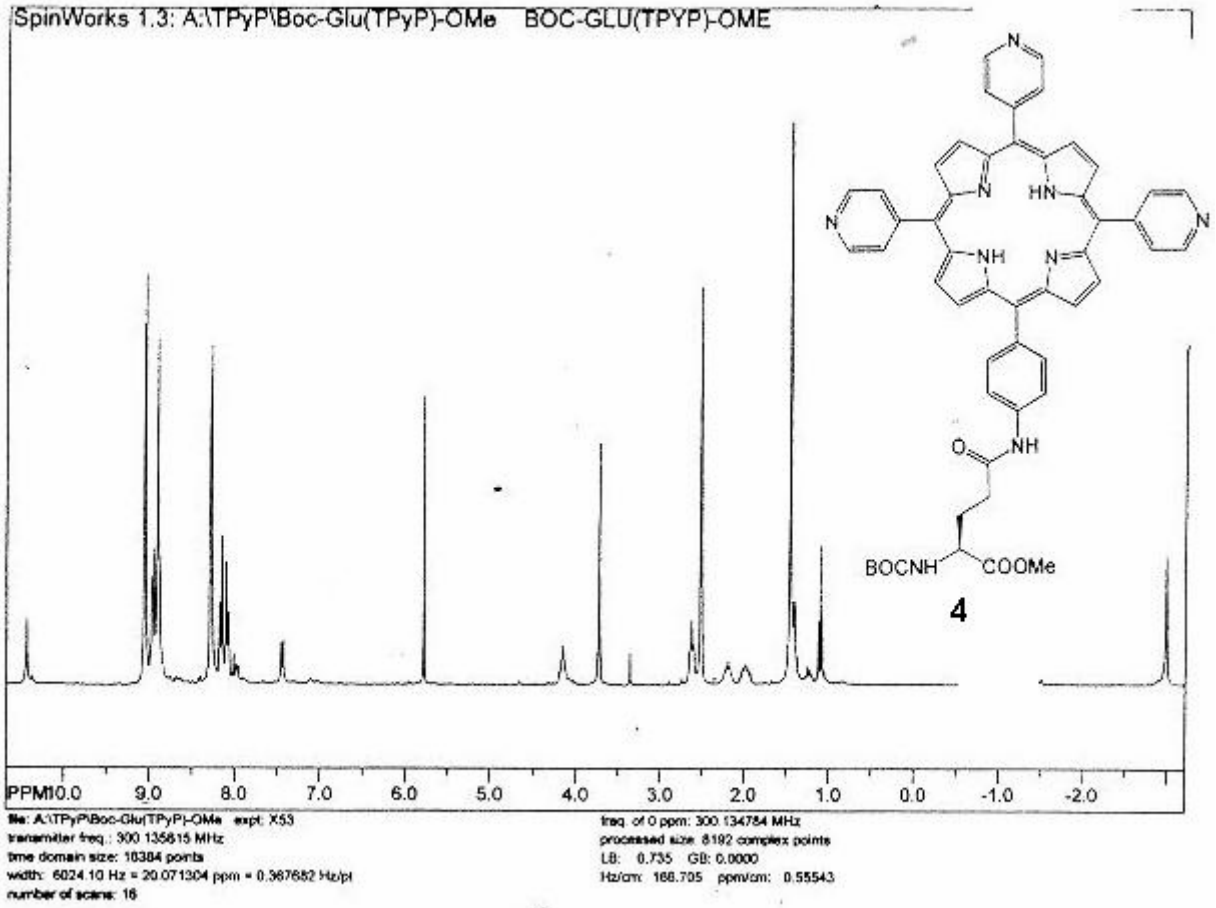
Boc-Glu(TPyP)-OH **5** (0.7 mmol) was dissolved in a solution 4M HCl/dioxane (1.4 mmol) at 0°C and stirred for 30 min at room temperature. The reaction mixture was evaporated to dryness and the resulting solid dried under vacuum. The deprotected amino acid was dissolved in H_2O/Acetonitrile (1:9) at 0°C followed by addition of DIEA (4.2 mmol) and Fmoc-OSu (0.77 mmol). The reaction mixture was stirred for 3 h at room temperature and the acetonitrile evaporated under reduced pressure. The resulting solution was extracted with DCM (2x) combined with the addition of acetic acid like previously described. The colored organic phase was dried over Na_2SO_4 and the solvent evaporated under reduced pressure. The remaining acetic acid was co-evaporated with toluene (4x) under reduced pressure or lyophilized. The resulting purple solid was purified by short column silica gel chromatography (10% ethanol/DCM and MeOH/DCM (1:1)) to afford 550 mg (0.56 mmol, 80%) of purple solid. ^{1}H NMR (300MHz, DMSO-d_6) δ = -2.98 (s, 2H, NH pyrrole), 2.05-2.35 (m, 2H, H^{β}), 2.54-2.60 (m, 2H, H^{γ}), 3.97-4.01 (m, 1H, H^{α}), 4.20-4.41 (m, 3H, H9 fluorenyl + OCH_2-fluorenyl), 6.90-6.95 (d, 1H, J = 6 Hz, NH Glu), 7.32-7.41 (m, 4H, H_{arom} fluorenyl), 7.70-7.78 (d, 2H, H_{arom} fluorenyl), 7.80-7.88 (d, 2H, H_{arom} fluorenyl), 8.05-8.26 (m, 10H, H_{ortho} NH + H_{meta} NH + 6H, 2,6 pyridine), 8.71-8.95 (m, 8H, H pyrrole), 9.00-9.10 (m, 6H, 3,5 pyridine), 10.44 (s, 1H, NH^{δ}) ; ES-MS : m/*z* 985 = $(M+H)^+$; UV/visible (MeOH), λ/nm (ε/10^{-3} $M^{-1}cm^{-1}$): 416 (181), 514 (10.7), 548 (6.7), 590 (5.3), 650 (5.0).







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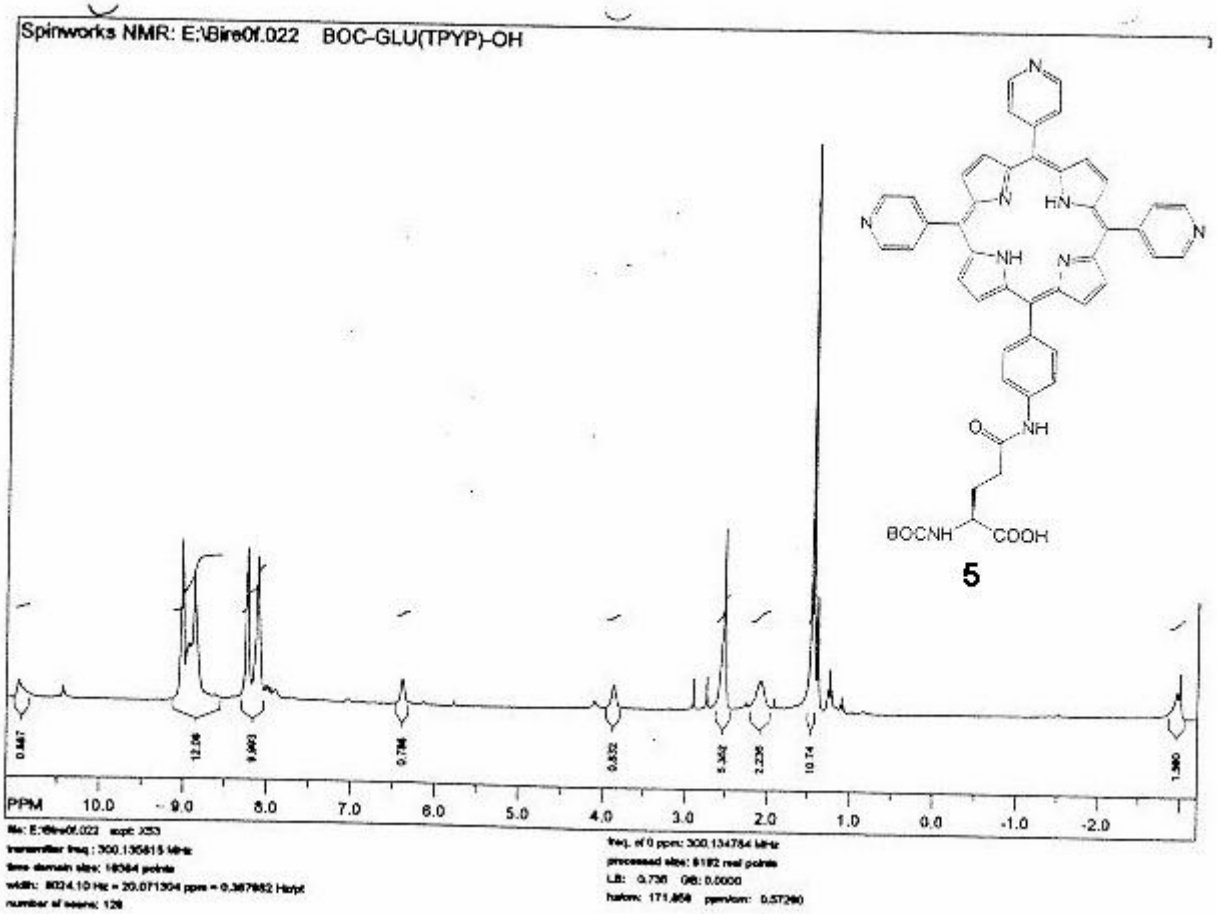
BOCNH COOMe
4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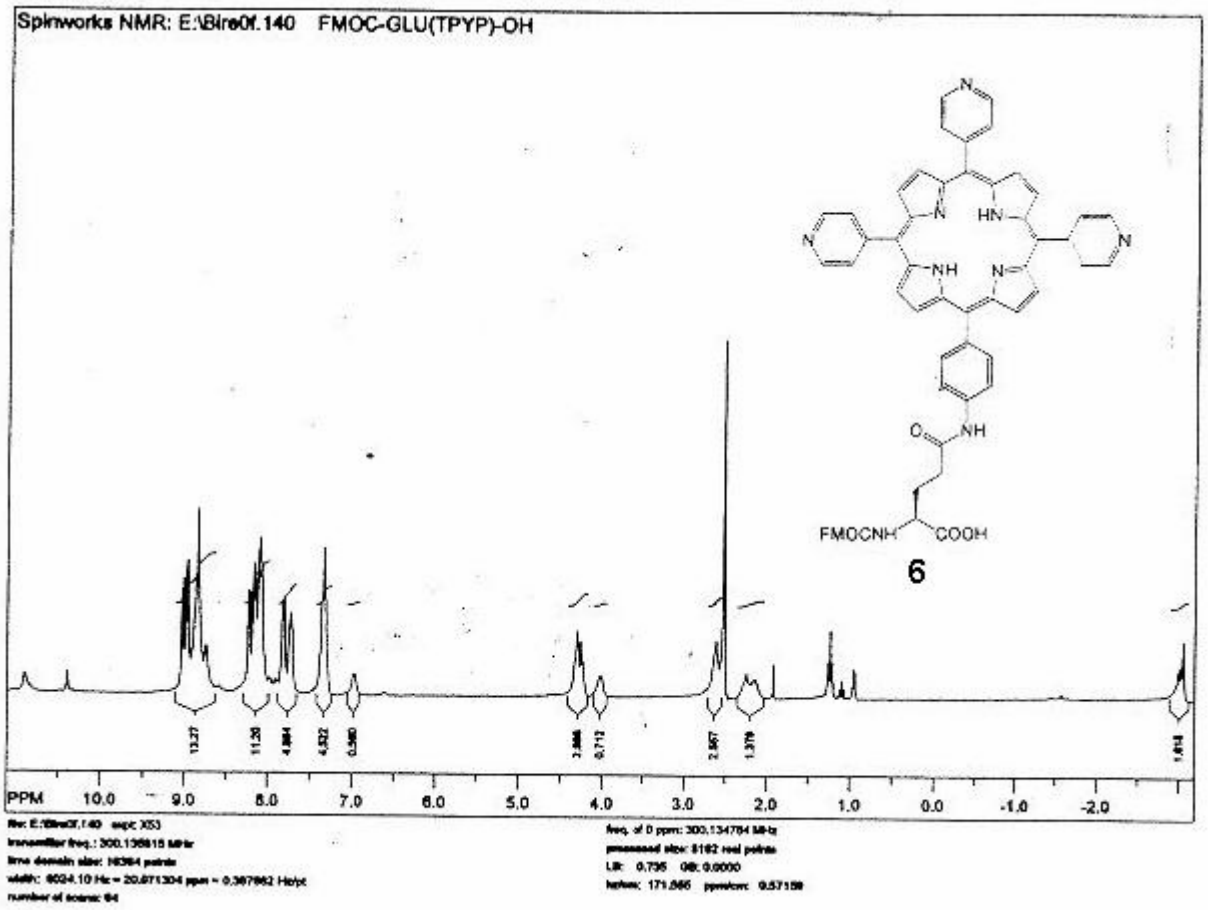


Solid-Phase Peptide Synthesis

Loading of Wang-resin

Peptide synthesis was carried out using Wang resin (0.7 mmol/g) following standard Fmoc-strategy. Fmoc-Ala-OH (5 eq.) was activated with DIC (5 eq.) and HOBt (5 eq.) in DMF during 30 min at 0ºC and added to the resin swollen in DMF. Followed by addition of DIEA (1.5 eq.). The mixture was shaken mechanically for 24 h at room temperature. The resin was filtered and the remaining alcohol group were capped with a solution of Acetic anhydride/DIEA/DMF (7:2:91) for 15 min. The resin was filtered and washed thoroughly with DMF (3x), MeOH (3x), DMF (3 eq.), MeOH (3x) and dried *in vacuo*. The loading capacity was determined by the Kaiser test and ranged from 0.38-0.42 mmol/g.

Fmoc Deprotection (General Procedure)

The resin-bound Fmoc peptide was treated with 20% piperidine in DMF (v/v) for 15 minutes and a second time for 10 minutes. The resin was washed with DMF (3x), MeOH (3x) and DMF (3x).

Coupling of Fmoc-Glu(TPyP)-OH (6) to the resin

Fmoc-Glu(TPyP)-OH **6** (1.5 eq.) was using using HATU (1.5 eq.) and DIEA (2 eq.) in DMF for 3 h at room temperature. The resin was filtered and washed with DMF (3x), MeOH (3x) and DMF (3x).

DIC/HOBt Coupling (General Procedure)

Boc-Ala-OH (5 eq.) was activated with DIC (5 eq.) and HOBt (5 eq.) in DMF during 30 min at 0ºC and added to the resin swollen in DMF. Followed by addition of DIEA (1.5 eq.). The mixture was shaken mechanically for 1 h at room temperature. The resin was filtered and washed thoroughly with DMF (3x), MeOH (3x), DMF (3 eq.), MeOH (3x).

Methylation on solid support

N-Methylation of the porphyrin side chains on solid support was achieved with a mixture of iodimethane and DMF (6:94) for 24 h. The resin was filtered and washed with DMF (5x).

Cleavage from the resin

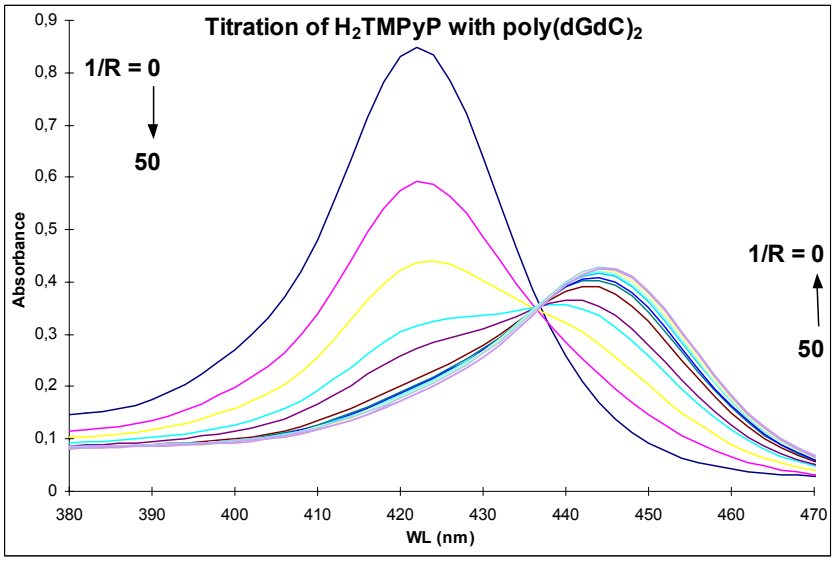
Peptides **7** and **8** were cleaved from the resin with a mixture of trifluoroacetic acid (TFA), triisopropylsilane (TIS) and water (95:2.5:2.5) for 4 h at room temperature. The resin was filtered and washed with DCM (2x) and MeOH (3x). The filtrate was evaporated under reduced pressure and the resulting mixture precipitated by adding diethylether. The solid was washed with diethylether (3x) and purified by RP-HPLC.

NH_2-Ala-Glu(TPyP)-Ala-OH (7)

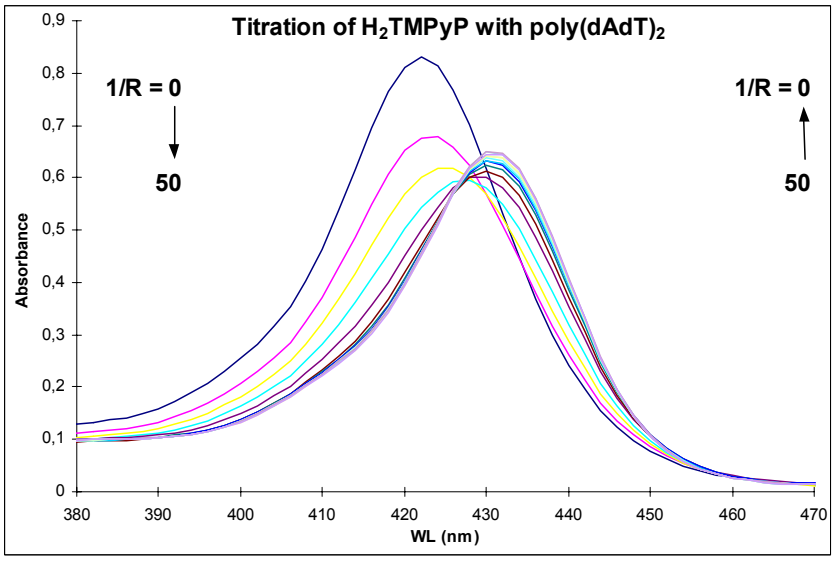
RP-HPLC: (90-50) t_R = 5.1 min; ES-MS: *m/z* 904 = $(M+H^+)$

NH_2-Ala-Glu(TMPyP)-Ala-OH (8)

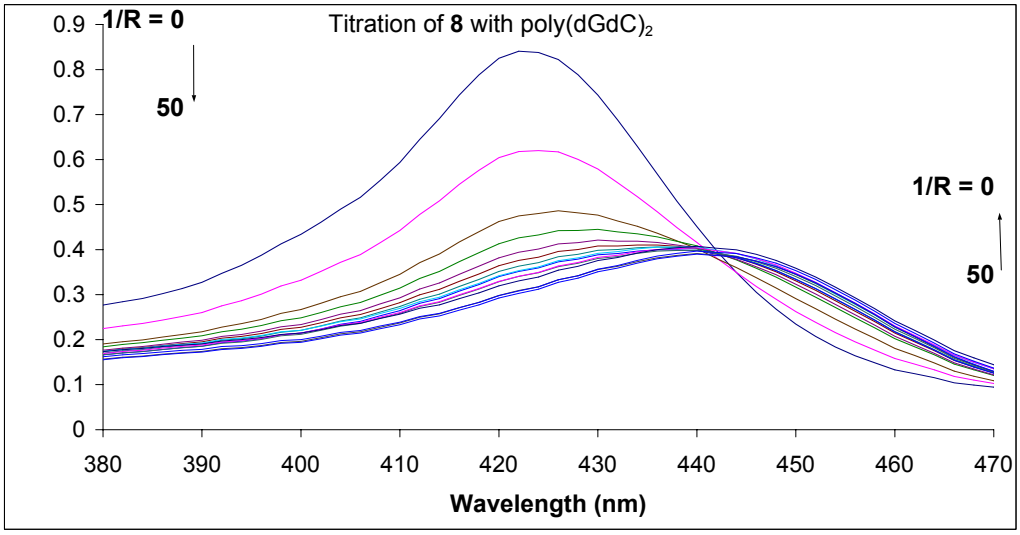
RP-HPLC: (90-50) t_R = 6.3 min; MS (ES): *m/z* 949 = M^+; UV/visible (buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0), λ_{max}/nm ($\varepsilon/10^{-3}$ $M^{-1}cm^{-1}$): 422 (56).



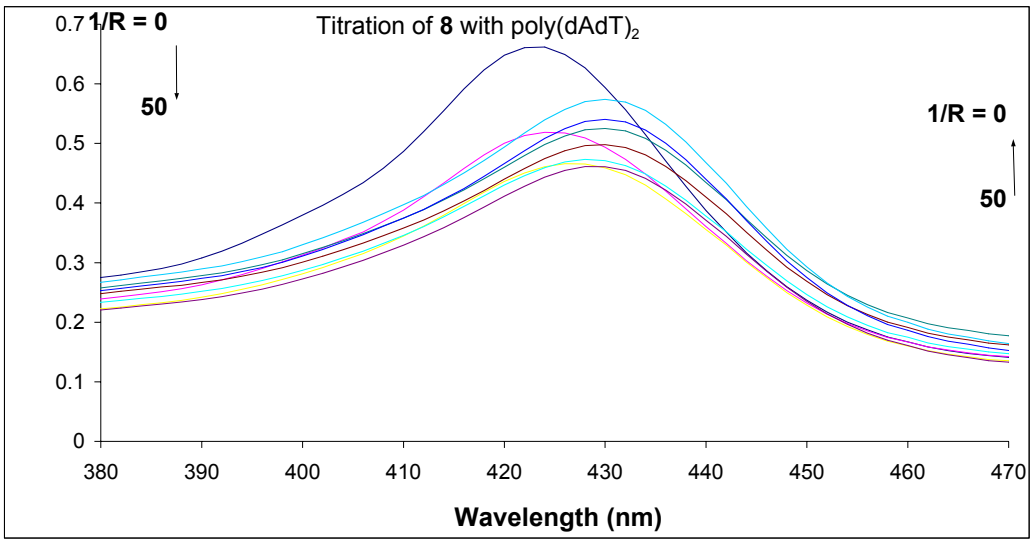
Titration of H_2TMPyP with poly(dGdC)$_2$ in buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0.



Titration of H_2TMPyP with poly(dAdT)$_2$ in buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0.



Titration of compound **8** with poly(dGdC)$_2$ in buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0.



Titration of compound **8** with poly(dAdT)$_2$ in buffer TE 10 mM, NaCl 50 mM, EDTA 1 mM, pH 7.0.