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Supporting Information

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for

Surface Binding Inhibitors of the SCF-KIT Protein–Protein Interaction

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ELISA assay

The entire extracellular (EC) domain of KIT (Figure 1) was expressed in insect cells and purified to homogeneity as described before.[1] The receptor-binding portion of SCF was expressed in *E. coli* into inclusion bodies, solubilized and refolded as previously reported.[2] The purified SCF was then conjugated to HRP by mixing HRP with SCF dimer in a 1:1 ratio, according to the manufacturer's instructions (ZYMED Laboratories, 01-2002 Kit). EC KIT was diluted to a concentration of 10 µg/mL in PBS and 50 µL were set in each well for overnight incubation (this way, each well would hold 0.5 µg KIT). 96-well Nunc-Immuno plates with MaxiSorp surface were used in all assays (Figure 2). The plate was washed three times with PBS and incubated at room temperature for 2 h in 3% BSA (300 µL per well), followed by additional washing. In order to determine the apparent Kd of the SCF-KIT interaction, different concentrations of HRP-labeled SCF were incubated with the KIT-coated plate in a volume of 100 µL for 90 min, at room temperature. The plates were washed three times with PBS and an HRP activity assay was performed using the ABTS color reaction, according to the manufacturer's instructions (PIERCE, cat. No 34026). After 30 min, the reaction was quenched with 1% SDS and the plates were read with a spectrophotometer set at 410 nm. In order to estimate the inhibitory effect of the various compounds on the SCF-KIT interaction, the plates were incubated with the compound of interest (70 µL) for 90 min at room temperature, followed by HRP conjugated SCF addition. Competition and saturation binding data were fitted by a sigmodial dose response (variable slope) and one site saturation curves, respectively. As control experiments, SCF was incubated with KIT without the addition of inhibitors, without KIT after BSA incubation, as well as without BSA or KIT and with inhibitors.

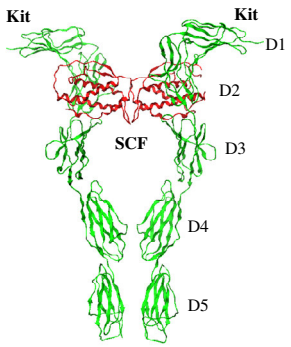


Figure 1. Crystal structure of the SCF-KIT complex.[1] To identify potential inhibitors of the SCF-KIT interaction, an ELISA assay that utilizes a whole extra cellular KIT (domains D1-D5) was developed.

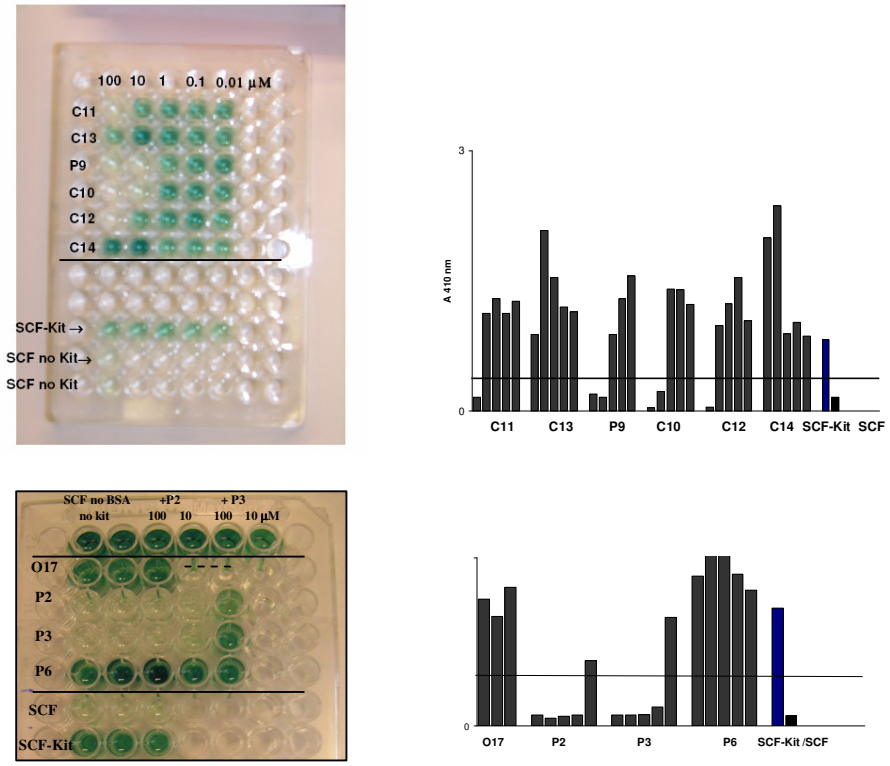


Figure 2. Two representative ELISA assays for naked eye visualization of the different inhibitors potencies. The synthetic molecules were incubated along with HRP conjugated SCF at five different concentrations: 100 µM (left), 10 µM, 1 µM, 0.1 µM and 0.01 µM (right). Top: Carboxy-modified calixarenes (C10-12) as well as porphyrin (P9) inhibited the SCF-Kit interaction at concentration range of 10-100 µM, while positively charged C13 or hydrophobic C14 calixarenes showed no inhibitory effect. Bottom: comparison between the most potent carboxy-modified porphyrins P2 and P3 (IC_{50} < 0.1 µM) and the positively charged porphyrin P6 or oligopyridylamide O17 that lacks a hydrophobic core (only three concentrations are shown). Control experiments include incubation of SCF without the inhibitors (SCF-KIT), without KIT after BSA incubation (SCF), as well as without BSA or KIT and with inhibitors.

Kit phosphorylation assay

Starved 3T3 cell that stably express KIT were incubated with various compounds in a final concentration of 10 μM for 30 minutes at 37 ºC. SCF was then added for five minutes.[1] The cells were lysed, KIT was immunoprecipitated and analyzed by SDS-PAGE and immunobloting. KIT expression level was visualized using anti-KIT antibodies, while KIT phosphorylation was visualized using anti-PhosphoTyrosine antibodies. Control experiments include lack of SCF stimulation, SCF stimulation with no compounds added, as well as addition of DMSO in parallel to the addition of the tested compounds.

Synthesis

C12 To a solution of tetrakis(butyloxy)calix[4]arene[3] (50 mg, 0.061mmol) in 8 mL dry dichloromethane oxalyl chloride (158 mg, 1.8 mmol, 30 eq) and a catalytic amount of DMF (30 μL) were added. The mixture was stirred at RT overnight. The reaction mixture was evaporated *in vacuo* to give the tetra-acid chloride. A solution of L-aspartic acid ditertbutyl ester (HCl salt) (6 eq) and DIPEA (8 eq) in dry methylene chloride (10 mL) was added and the mixture was stirred at RT overnight. The solvent and excess reagents were evaporated *in vacuo* and the residue was redissolved in 50 mL dichloromethane and washed with 0.5 N HCl (2x 50mL), 1N NaOH (2x 50 mL) and brine (2x50 mL). The combined organic extracts were evaporated in vacuo, and purified by SiO_2 (MeOH/ dichloromethane, 1:15, v:v). The product containing fractions were combined, evaporated *in vacuo* and treated with TFA/ water (95:5, v:v) to afford the final product. Yield: (45 mg, 60% (over 2 steps)) ^{1}H NMR (500 MHz, Acetone-d_6): δ = 7.63 (d, J= 8 HZ, 2H, NH), 7.27 (s, 4H, Ar, calix), 7.16 (s, 4H, Ar calix), 4.93 (m, 4H,), 4.52 (d, J= 13.5 Hz, 4 H, H_{ax} of Ar CH_2Ar), 4.02 (m, 8H, $OCH_2CH_2CH_2CH_3$), 3.67 (d, J= 9 Hz, 2H, NH), 3.35 (d, *J* = 13.5 Hz, 4H, H_{eq} of $ArCH_2Ar$), 3.13 (dd, J= 11Hz, 6 Hz, 4H), 2.95 (dd, J= 11Hz, 6 Hz, 4H), 1.95 (m, 8H, $OCH_2CH_2CH_2CH_3$), 1.52 (m, 8H, $OCH_2CH_2CH_2CH_3$), 1.01 (t, *J* = 7.0 Hz, 12H, $OCH_2CH_2CH_2CH_3$). ^{13}C NMR (500 MHz, Acetone-d_6): δ = 173.84 (CO), 173.65 (CO), 168.88 (CO), 161.30 (4°, Ar), 136.82 (4°, Ar), 136.64 (4°, Ar), 130.00 (4°, Ar), 129.88 (3°, Ar), 129.32 (3°, Ar), 76.93, 50.92, 37.25, 34.11, 32.51 ($ArCH_2Ar$), 21.09 ($OCH_2CH_2CH_2CH_3$), 15.36 ($OCH_2CH_2CH_2CH_3$). MALDI-TOF: calcd. $[M+Na]^+$ 1307.47 found 1307.91.

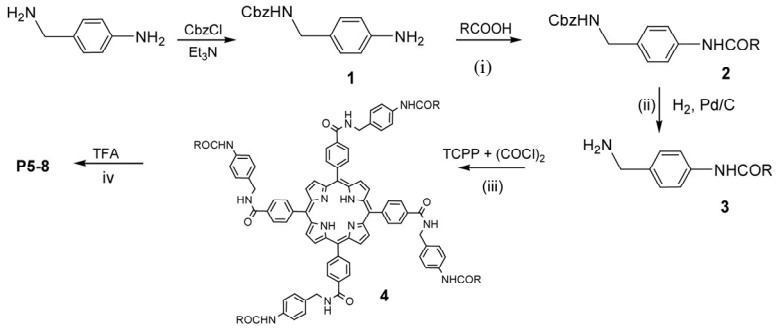
C11 was prepared in an analogous manner to **C12** (L-glutamic acid ditertbutyl ester hydrochloride was used as the amino acid). Yield: (58 mg, 72% (over 2 steps)) ^{1}H NMR (500 MHz, Acetone-d_6): δ 7.96 (d, J= 8 HZ, 3H, NH), 7.39 (s, 4H, Ar, calix), 7.29 (s, 4H, Ar calix), 4.54 (d, J= 13.5 Hz, 4 H, H_{ax} of Ar CH_2Ar), 4.49 (m, 4H), 4.02 (t, J= 8Hz, 8H, $OCH_2CH_2CH_2CH_3$), 3.64 (d, J=10 Hz, 1H, NH), 3.36 (d, *J* = 13.5 Hz, 4H, H_{eq} of $ArCH_2Ar$), 2.60 (m, 8H), 2.13 (m, 8H), 1.99 (m, 8H, $OCH_2CH_2CH_2CH_3$), 1.52 (m, 8H, $OCH_2CH_2CH_2CH_3$), 1.03 (t, *J* = 7.5 Hz, 12H, $OCH_2CH_2CH_2CH_3$). ^{13}C NMR (500 MHz, Acetone-d_6): δ = 177.03 (CO), 174.08 (CO), 169.84 (CO), 161.32 (4°, Ar), 136.63 (4°, Ar), 136.44 (4°,

Ar), 130.86 (4°, Ar), 129.57 (3°, Ar), 129.22 (3°, Ar), 76.98, 55.15, 34.14, 32.56, 32.23 ($ArCH_2Ar$), 27.49, 21.06 ($OCH_2CH_2CH_2CH_3$), 15.39 ($OCH_2CH_2CH_2CH_3$). MALDI-TOF calcd. $[M+Na]^+$ 1363.54 found 1364.11.

C13 was prepared in an analogous manner to **C12** (N-Boc-1,3 diaminopropane was used). Yield: (48 mg, 80% (over 2 steps)) ^1H NMR (500 MHz, Acetone-d_6): δ = 8.30 (t, *J* = 5.7 Hz, 4H), 7.85 (s, 8H), 7.35 (s, 8H), 4.25 (d, *J* = 12.5 Hz, 4H), 3.92 (t, *J* = 7 Hz 8H), 3.40 (m, 2 H), 3.32 (d, J = 12.5 Hz, 4H), 2.94 (s, 2 H), 1.89 (m, 2H), 1.44 (m, 2H), 0,98 (t, J= 7 Hz, 12H). ^{13}C NMR (500 MHz, Acetone-d_6): δ 167.01, 159.09, 158.69, 158.45, 134.42, 128.43, 128.15, 75.17, 39.14, 37.52, 32.08, 19.19, 14.24. MALDI-TOF calcd. $[M+H]^+$ 992.61 found 992.89.

C14,[3] **P1,**[4] **P2-4,**[5] **O15-16**[6] **O17-18**[7] were prepared according to previously described procedures.

General procedure for the preparation of P5-8:



Scheme 1. General route for the preparation of P5-8, R= Boc and/or t-Butyl protected amino acid.

Cbz-protected 4-(aminobenzyl) amine (**1**): To a stirring solution of 4-aminobenzylamine (5 g, 40.9 mmol), Et_3N (10.4 mL, 74.6 mmol) in anhydrous THF (200 mL) at 0ºC, a solution of CbzCl (6.98 g, 40.9 mmol) in anhydrous THF (100 mL) was added over 30 minutes. The reaction mixture was allowed to stir at RT for 16 hours. The solvent was removed *in vacuo* and the residue was redissolved in dichloromethane, washed with 10% $NaHCO_3$, dried over $MgSO_4$, concentrated and purified by SiO_2 (EtOAc: Hex, 1:1). Yield: (5.19 g, 49.3 %). ^1H NMR (400 MHz, CD_2Cl_2): δ = 7.33 (m, 5H), 7.06 (d, J=7.0 Hz, 2H), 6.64 (d, J=7.0 Hz, 2H), 5.09 (s, 2H), 4.22 (d, 2H), 3.70 (br s, 2H).

P8 (i) To a stirring solution of *N*-(*tert*-Butoxycarbonyl)glycine (175 mg, 1 mmol) in freshly distilled methylene chloride (20 mL) at 0 ºC, N-methylmorpholine (NMM) (110 µL, 1 mmol) and isobutyl chloroformate (130 µL, 1 mmol) were added. After 5 minutes Cbz-protected 4-(aminobenzyl) amine (**1**) was added (256 mg, 1 mmol), the solution was allowed to warm to RT and stir over night. The crude product **2** was purified by silica gel flash column chromatography (Hexane: EtOAc, 2:1→1:1) in 95% yield (393 mg). ^1H NMR (400 MHz): δ = 8.09 (b, 1H), 7.47 (d, J = 7.6 Hz, 2H), 7.20-7.37 (m, 7H), 5.13 (s, 2H), 5.04 (b, 1H), 4.35 (d, 2H), 3.92 (d, J=4.8 Hz, 2H), 1.59 (br, 1H), 1.48 (s, 9H).

(ii) Compound **2** (343 mg, 0.83 mmol) and 10% Pd/C (58 mg) in MeOH (15 mL) were stirred under H_2 for 4 hours. After this time, the reaction was filtered through Celite, washing with MeOH. The filtrate was concentrated and the product **3** was purified by silica gel flash column chromatography (CH_2Cl_2: MeOH: Et_3N, 92: 7: 1). Yield: 91% (210 mg). ^{1}H NMR (400 MHz, DMSO-d6): δ = 9.85 (s, 1H), 7.51 (d, J=7.5 Hz, 2H), 7.26 (d, J=7.45 Hz, 2H), 7.05 (br t, 1H), 3.72 (d, 2H), 3.64 (s, 2H), 3.35 (br s, 1H) 1.92 (br s, 1H), 1.37 (s, 9H).

(iii) To a stirring solution of TCPP (110 mg, 0.139 mmol) in anhydrous dichloromethane, oxalyl chloride (429 μL, 5 mmol) and 5 drops of DMF were added. After 16 hours the reaction mixture was concentrated and dried under high vacuum for 8 hours. The residue was redissolved in THF (7 mL) and added dropwise to a solution of **3** and DIPEA in CH_2Cl_2/THF (1:3, 15 mL) at O ºC. The reaction mixture was loaded on silica column and the product **4** was purified by flash chromatography (MeOH:CH_2Cl_2, 1:9). Yield: 63%. ^{1}H NMR (400 MHz, DMSO-d6): δ = 9.98 (s, 4H), 9.43 (br, 4H), 8.88 (s, 8H), 8.36 (s, 16H), 7.63 (d, J=7.5 Hz, 8H), 7.43 (d, J=7.5 Hz, 8H), 7.08 (br t, 4H), 4.62 (s, 8H), 3.76 (d, 8H), 1.40 (s, 36H).

(iv) Compound **4** (40 mg, 21.8 mmol) was dissolved in 5 mL TFA-CH_2Cl_2 (1:1) with 5% H_2O. The solution was stirred for 5 hours and then evaporated to dryness. The crude product was dissolved in HPLC grade water (+0.1% TFA) and washed thoroughly with dichloromethane. The aqueous layer was concentrated and lyophilized. ^{1}H NMR (400 MHz, DMSO-d6): δ = 10.48 (s, 4H), 9.47 (br, 4H), 8.89 (s, 8H), 8.37 (s, 16H), 8.13 (s, 12H), 7.64 (d, J=8.1 Hz, 8H), 7.48 (d, J=8.2 Hz, 8H), 4.63 (br, 8H), 3.82 (d, 8H). MALDI-TOF calculated. $[MH]^+$ 1435.60, found 1435.52.

P5 (i) To a stirring solution of Boc-L-glutamic acid 5-*tert*-butyl ester (304 mg, 1 mmol) in freshly distilled dichloromethane (20 mL) at 0 ºC, N-methylmorpholine (NMM) (110 μL, 1 mmol) and isobutyl chloroformate (130 μL, 1 mmol) were added. After 5 minutes compound **1** was added (256 mg, 1 mmol) and the solution was stirred over night at RT. The crude product **2** was purified by silica gel flash column chromatography (Hexane: EtOAc, 2:1→1:1) in 90% yield (485 mg). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.56 (br, 1H), 7.50 (d, J=8.0 Hz, 2H), 7.20-7.40 (m, 7H), 5.39 (br s, 1H), 5.15 (s, 2H), 5.04 (br, 1H), 4.35 (d, 2H), 4.32 (br, 1H), 2.29-2.57 (m, 2H), 1.86-2.19 (m, 2H), 1.42 (s, 18H). ESMS (m/z): $[M+Na]^+$ 564.3.

(ii) H_2 was bubbled through a solution of **2** (398 mg, 0.74 mmol) and 10% Pd/C (52 mg) in 13 mL MeOH. The reaction mixture was stirred under H_2 for 30 minutes. The reaction was then filtered through Celite, which was washed further with MeOH. The filtrate was concentrated and the product **3** was purified by silica gel flash column chromatography (96: 3.5: 0.5, CH_2Cl_2: MeOH: Et_3N). Yield: 278 mg, 92%. ^{1}H NMR (400 MHz, DMSO-d6): δ = 9.89 (s, 1H), 7.52 (d, J=8.0 Hz, 2H), 7.26 (d, J=8.0 Hz, 2H), 7.06 (d, 1H), 4.08 (m, 1H), 3.68 (s, 2H), 2.25 (br, 2H), 1.64-1.94 (br m, 2H), 1.40 (s, 18H).

(iii) To a stirring solution of TCPP (108 mg, 0.137 mmol) in anhydrous dichloromethane (25 mL) and oxalyl chloride (579 μL, 6.85 mmol), a drop of DMF was added. After 16 hours the reaction mixture was concentrated and dried under high vacuum for 8 hours. The residue was redissolved in THF (8 mL) and added dropwise to a solution of **3** and DIPEA in CH_2Cl_2/THF (1:3, 15 mL) at OC. The reaction mixture was loaded on silica column and the product **4** purified by flash chromatography (MeOH:CH_2Cl_2, 2:8). Yield: 53%. ^{1}H NMR (400 MHz, DMSO-d6): δ = 10.00 (s, 4H), 9.42 (br, 4H), 8.87 (s, 8H), 8.34 (s, 16H), 7.63 (d, J=8.1 Hz), 7.41 (d, J=8.1 Hz, 8H), 7.09 (d, 4H), 4.61 (br s, 8H), 4.09 (m, 4H), 2.27 (m. 8H), 1.91-1.79 (br m, 8H) 1.40 (s, 72H).

(iv) Porphyrin **4** (50 mg, 21.3 mmol) was dissolved in 6 mL TFA-CH_2Cl_2 (1:1) with 5% H_2O. The solution was stirred for 4 hours and then evaporated to dryness. The crude product was dissolved in HPLC grade water (+0.1% TFA) and washed thoroughly with dichloromethane. The aqueous layer was concentrated and lyophilized. ^{1}H NMR (400 MHz, DMSO-d6): δ = 10.55 (s, 4H), 9.48 (br, 4H), 8.90 (s, 8H), 8.37 (s, 16H), 8.31 (s, 12H), 7.66 (d, J=8.2 Hz, 8H), 7.49 (d, J=8.2 Hz, 8H), 4.65 (br, 8H), 4.01 (m, 4H), 2.42 (t, 8H), 2.10 (m, 8H).

P6 Cbz-protected 4-(aminobenzyl) amine **1** (282 mg, 1.1 mmol), N,N-Bis-(tert-butoxycarbonyl)-L-lysine (346 mg, 1 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI) (201 mg, 1.05 mmol) and dimethylaminopyridine (DMAP) (12.2 mg, 0.1 mmol) were dissolved in 20 mL dichloromethane at 0 ºC and stirred at RT under N_2 for 16 hours. The product was purified by silica gel flash column chromatography (Hexane: EtOAc, 2:1→1:1), followed by hydrogenolysis and coupling to *meso*-tetrakis-(4-carboxyphenyl) porphyrin (TCPP) according to the steps described above. The functionalized pophyrin **4** (50 mg, 19.8 mmol) was dissolved in 6 mL TFA-CH_2Cl_2 (1:1) with 5% H_2O. The solution was stirred for 4 hours and then evaporated to dryness. The crude product was dissolved in HPLC grade water (+0.1% TFA) and washed thoroughly with dichloromethane. The aqueous layer was concentrated and lyophilized. ^{1}H NMR (400 MHz, DMSO-d6): δ = 10.59 (s, 4H), 9.46 (br t, 4H), 8.87 (s, 8H), 8.34 (br, 28H), 7.77 (s, 12H), 7.63 (d, J= 8.2 Hz, 8H), 7.45 (d, J= 7.9 Hz, 8H), 4.61 (d, 8H), 3.95 (m, 4H), 2.76 (m, 8H), 1.82 (m, 8H), 1.56 (m, 8H), 1.39 (m, 8H). MALDI-TOF MS *m/z* calculated 1720.88, found 1722.20 $[M + H]^+$.

P7 (i) To a stirring solution of *N*-(*tert*-Butoxycarbonyl)-L-serine (205 mg, 1 mmol) in freshly distilled dichloromethane (20 mL) at 0 ºC, N-methylmorpholine (NMM) (110 μL, 1 mmol) and isobutyl chloroformare (130 μL, 1 mmol) were added. After 5 minutes Cbz-protected 4-(aminobenzyl) amine (**1**) was added (256 mg, 1 mmol) and the solution was stirred over night at RT. The product **2** was purified by silica gel flash column chromatography (Hexane: EtOAc, 2:1→1:1). Yield (384 mg, 87%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.80 (br, 1H), 7.46 (d, J=8.7 Hz, 2H), 7.20-7.37 (m, 7H), 5.67 (br,

1H), 5.14 (s, 2H), 5.05 (br, 1H), 4.34 (d, J=5.5 Hz, 2H), 4.26 (br, 2H), 3.68 (br, 1H), 1.48 (s, 9H). ESMS (m/z): $[M+Na]^+$ 466.6.

(ii) Compound **2** (321 mg, 0.724 mmol) and 10% Pd/C (51 mg) were stirred in MeOH (13 mL) under H_2 for 4 hours. The reaction mixture was filtered through Celite, washing with MeOH. The filtrate was concentrated and the product **3** was purified by silica gel flash column chromatography (92: 7: 1, CH_2Cl_2: MeOH: Et_3N). Yield: 217 mg, 97%. ^{1}H NMR (400 MHz, DMSO-d6): δ = 9.89 (s, 1H), 7.54 (d, J=7.0 Hz, 2H), 7.24 (d, J=7.0 Hz, 2H), 6.75 (d, 1H), 4.95 (br, 1H), 4.13 (m, 1H), 3.66 (s, 2H), 3.61 (br s, 2H), 3.35 (br, 2H), 1.38 (s, 9H).

(iii) To a stirring solution of TCPP (81 mg, 0.102 mmol) in anhydrous dichloromethane (20 mL), oxalyl chloride (316 µL, 3.73 mmol) and 5 drops of DMF were added. After 16 hours the reaction mixture was concentrated and dried under high vacuum for 8 hours. The residue was redissolved in THF (10 mL) and added dropwise to a solution of **3** (130 mg, 0.42 mmol) and DIPEA (146 µL, 0.84 mmol) in CH_2Cl_2/THF (1:3, 15 mL) at O ºC. The reaction mixture was dry loaded on silica column and the product **4** was purified by flash chromatography (MeOH 15%/ CH_2Cl_2). Yield: 96 mg, 48 %. ^{1}H NMR (400 MHz, DMSO-d6): δ = 9.98 (s, 4H), 9.42 (br t, 4H), 8.87 (s, 8H), 8.35 (s, 16H), 7.65 (d, J=7.6 Hz, 8H), 7.41 (d, J=7.6 Hz, 8H), 6.79 (br d, 4H), 4.96 (br t, 4H), 4.60 (s, 8H), 4.17 (m, 4H), 3.64 (br s, 8H), 1.40 (s, 36H).

(iv) The functionalized porphyrin **4** (40 mg, 20.4 mmol) was dissolved in 6 mL TFA-CH_2Cl_2 (1:1) with 5% H_2O. The solution was stirred for 4 hours and then evaporated to dryness. The crude product was dissolved in HPLC grade water (+0.1% TFA) and washed thoroughly with dichloromethane. The aqueous layer was concentrated and lyophilized. ^{1}H NMR (400 MHz, DMSO-d6): δ = 10.46 (s, 4H), 9.44 (m, 4H), 8.87 (br t, 8H), 8.36 (s, 16H), 8.25 (s, 12H), 7.63 (d, J=8.5 Hz, 8H), 7.47 (d, J=8.5 Hz, 8H), 4.64 (d, J=5.4 Hz, 8H), 4.0 (m, 4H), 3.86 (m, 8H). MALDI-TOF calculated. $[MH]^+$ 1556.69, found 1556.76.

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