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Supporting Information

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Interface Engineering of Synthetic Pores: Toward Hypersensitive Biosensors

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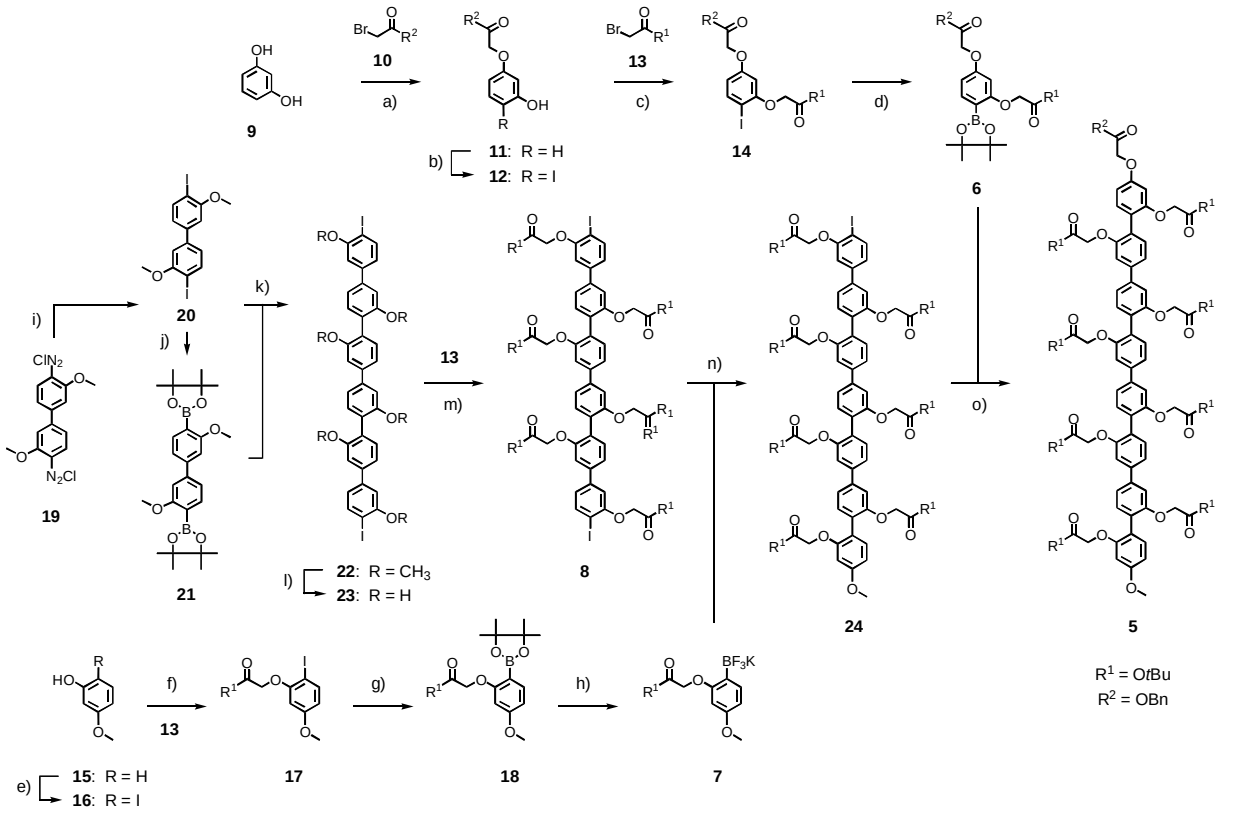
1. Materials and methods. As in refs. [S1-S3], Supporting Information. In brief, reagents for synthesis were purchased from Fluka, Aldrich, amino acid derivatives from Novabiochem and Bachem, egg yolk phosphatidylcholine (EYPC) from Avanti polar lipids, HATU from Applied Biosystems, HPTS from Molecular Probes, buffers and salts from Sigma or Fluka-Aldrich. All reactions were performed under argon atmosphere. Unless stated otherwise, column chromatography was carried out on silica gel 60 (Fluka, 40-63 μm). Analytical (TLC) and preparative thin layer chromatography (PTLC) was performed in silica gel 60 (Fluka, 0.2 mm) and silica gel GF (Analtech, 1000 μm), respectively. HPLC was performed using either Jasco HPLC system (PU-980, UV-970, FP-920) or an Agilent 1100 series apparatus with a photo diode array detector. $[\alpha]^{20}_{D}$ values were recorded on a Jasco P-1030 Polarimeter, melting points (Mp) on a heating table from Reichert (Austria), IR spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer (ATR, Golden Gate, unless stated) and are reported as wavenumbers ν in cm^{-1} with band intensities indicated as s (strong), m (medium), w (weak). ESI-MS were performed on a Finnigan MAT SSQ 7000 instrument or a ESI API 150EX and are given in *m*/*z* (peak intensity in %). MALDI-TOF on a Axima CFR^{+} (Shimadzu). ^{1}H and ^{13}C spectra were recorded (as indicated) either on a Bruker 300 MHz, 400 MHz or 500 MHz spectrometer and are reported as chemical shifts (δ) in ppm relative to TMS (δ = 0). Spin multiplicities are reported as a singlet (s), doublet (d), triplet (t), quartet (q) and quintet (quint) with coupling constants (*J*) given in Hz, or multiplet (m). ^{1}H and ^{13}C resonances were assigned with the aid of additional information from 2D NMR spectra (H,H-COSY, DEPT 135, HSQC and HMBC). UV-Vis spectra were measured on a Varian Cary 1 Bio spectrophotometer equipped with a stirrer and a temperature controller (25 ºC) and are reported as maximal absorption wavelength λ in nm (extinction coefficient ε in $mM^{-1}cm^{-1}$). Fluorescence measurements were preformed either on a FluoroMax-2 or a FluoroMax-3, Horiba Jobin Yvon, both equipped with an injector port, a stirrer and a temperature controller (25 ºC). Fluorescence spectra are not corrected. The Mini-Extruder with a polycarbonate membrane, pore size 100 nm, used for LUV preparation was from Avanti polar lipids.

2. Abbreviations. ADP: Adenosine 5'- diphosphate; ATP: Adenosine 5' triphosphate;

AMPSO: N-(1,1-Dimethyl-2-hydroxyethyl)-3-amino-2-hydroxypropanesulfonic acid; ANTS: 8-aminonaphthalene-1,3,6-trisulfonic acid; Boc: *t*-Butoxycarbonyl; calcd: Calculated; CB: Cascade blue; CF: 5(6)-carboxyfluorescein; DCM: Dichloromethane; DMF: N,N-Dimethylformamide; DPX: *p*-Xylene-bis(N-pyridinium bromide); EtOAc: Ethyl acetate; EYPC LUVs: Egg yolk phosphatidylcholine large unilamellar vesicles; EYPC/PG LUVs: Egg yolk phosphatidylcholine/phoshpatidylcholesterol large unilamellar vesicles; Fmoc: Fluorenylmethoxycarbonyl; Lys: Lysine; *Gla*: Glycolic acid ($HOCH_2CO_2H$, $-OCH_2CO-$); HATU: 2-(1H-7-Azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate; HBTU: 2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate; HEPES: 4-(2-Hydroxyethyl)-1-piperazine-ethanesulfonic acid; HMBC: Heteronuclear multiple bond correlation; HPTS: 8-Hydroxy-1,3,6-pyrenetrisulfonate; MALDI: Matrix assisted laser desorption ionization; MES: 2-(N-morpholine)-ethane sulphonic acid; PTLC: Preparative thin layer chromatography; RPHPLC: Reverse phase high performance liquid chromatography; RT: Room temperature; R_f: Retention factor; TEA: Triethylamine; TES: 2-[(2-Hydroxy-1,1-bis(hydroxymethyl)ethyl)amino]ethanesulfonic acid; *t*But: *tert*-butyl; Z: Carboxybenzyl.

3. Pore Synthesis



Scheme 1. a) Cs_2CO_3, acetone, 60 ºC, 30 min, 75%; b) I_2, AgOTf, $CHCl_3$, 2.5 h, RT, 40%; c) Cs_2CO_3, acetone, 65 ºC, 30 min, 92%; d) pinacolborane, $PdCl_2$(dppf), Et_3N, CH_3CN, 2 h, 85 ºC, 60%; e)-h), see ref [39]; (e) AgOTf, I_2, 72%; f) Cs_2CO_3, 96%; g) pinacolborane, $PdCl_2$(dppf), 88%; h) KHF_2, quant; i)-m), see ref [30]; (i), KI, 70%; (j), pinacolborane, $PdCl_2$(dppf), 69%; (k) $Pd(PPh_3)_4$, 14%; l) BBr_3; m) Cs_2CO_3, 92% (from **22**); n) $PdCl_2$(dppf), c.y. 42% (from ref [39]); o) $Pd(PPh_3)_4$, toluene/EtOH 10:1, Na_2CO_3, 80%.

Benzyl 2-(3'-Hydroxyphenoxy)acetate (11). A mixture of resorcinol **9** (1.00 g, 9.08 mmol) and Cs_2CO_3 (3.00 g, 9.08 mmol) in acetone (35 ml) was stirred for 30 min at 60 °C. Then, benzylbromoacetate (350 µl, 2.24 mmol) was added. The mixture was stirred for 30 min at 60 ºC. Cooled to RT, the reaction mixture was filtered through cotton, and the solid was washed with acetone (5 x 5 ml). The organic phase was then concentrated *in vacuo*. Purification of the crude product by column chromatography (CH_2Cl_2/MeOH 100:1, R_f = 0.15) yielded **11** (1.63 g, 75%) as a colorless solid. Mp: 65 - 66 ºC; IR (neat): 3398 (s), 3031 (w), 2909 (w), 1746 (s), 1603 (s), 1514 (m), 1437 (m), 1215 (s), 1161 (s), 1088 (m), 1022 (w), 868 (m), 748 (m); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): 7.39 - 7.34 (m, 5H), 7.14 (t, ^3J(H,H) = 8.1 Hz, 1H), 6.50 – 6.47 (m, 2H), 6.43 (t, ^4J(H,H) = 2.3 Hz, 1H), 5.71 (s, 1H), 5.24 (s, 2H), 4.64 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): 169.4 (s), 159.1 (s), 157.2 (s), 135.3 (s), 130.5 (d), 128.9 (d), 128.8 (d), 128.7 (d), 109.4 (d), 106.7 (d), 102.8 (d), 67.4 (t), 65.5 (t); MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1): 259 (100, $[M+H]^+$), 276 (88, $[M+NH_4]^+$), 181 (76, $[M-C_6H_5+NH_4]^+$).

Benzyl 2-(3'-Hydroxy-4'-Iodophenoxy)acetate (12). To a solution of **11** (700 mg, 2.89 mmol) and CF_3COOAg (640 mg, 2.896 mmol) in chloroform (30 ml) was added a solution of I_2 (733 mg, 2.89 mmol) in chloroform (40 ml) dropwise over a period of 1.5 h and stirred at RT. Once the addition was finished, the solution was stirred for 1 hour after which the remaining solid was filtered through cotton and washed with chloroform (5 x 5 ml). The organic phase was successively washed with a freshly prepared solution of 5% $Na_2S_2O_3$ (2 x 10 ml), sat $NaHCO_3$ (2 x 20 ml), and brine (2 x 10 ml). It was then dried over Na_2SO_4 and concentrated *in vacuo*. Purification of the crude product by column chromatography (CH_2Cl_2, R_f = 0.35) yielded **12** (912 mg, 40%) as a colorless solid. Mp: 133-135 ºC; IR (neat): 3330 (s), 1736 (m), 1583 (w), 1499 (w), 1190 (w), 1081 (s), 822 (w); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): 7.53 (d, ^3J(H,H) = 8.8 Hz, 1H), 7.39 - 7.34 (m, 5H), 6.61 (d, ^4J(H,H) = 2.9 Hz, 1H), 6.36 (dd, ^3J(H,H) = 8.8 Hz, ^4J(H,H) = 2.9 Hz, 1H), 5.27 (s, 2H), 4.66 (s, 2H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): 168.6 (s), 160.0 (s), 155.9 (s), 138.4 (d), 135.1 (s), 128.9 (d), 128.8 (d), 128.7 (d), 109.9 (d), 102.0 (d), 76.1 (s), 67.4 (t), 65.5 (t); MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1): 407 (100, $[M+Na]^+$), 402 (78, $[M+NH_4]^+$), 385 (81, $[M+H]^+$), 258 (54, $[M-I]^+$).

Benzyl 2-(3'-*tert*-Butoxycarbonylmethoxy-4'-Iodophenoxy)acetate (14). To a solution of **12** (797 mg, 2.07 mmol) in dry acetone (40 ml) was added Cs_2CO_3 (675 mg, 2.07 mmol). After stirring of the resulting suspension was stirred at 65 °C for 30 min, *tert*-butylbromoacetate (307 µl, 2.07 mmol) was added. The reaction was stirred for an additional 30 min and filtered through cotton. The solid washed with acetone (2 x 5 ml), and the organic phase was concentrated *in vacuo*. Purification of the crude product by column chromatography (CH_2Cl_2, R_f = 0.55) yielded **14** (940 mg, 92%) as a colorless solid. Mp: 70 - 71 ºC; IR (neat): 2979 (w), 2922 (w), 1744 (s), 1735 (s), 1583 (m), 1492 (m), 1149 (m), 1062 (m), 840 (m); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): 7.65 (d, ^3J(H,H) = 8.6 Hz, 1H), 7.39 - 7.34 (m, 5H), 6.42 (d, ^4J(H,H) = 2.6 Hz, 1H), 6.30 (dd, ^3J(H,H) = 8.6 Hz, ^4J(H,H) = 2.6 Hz, 1H), 5.26 (s, 1H), 4.65 (s, 2H), 4.53 (s, 2H), 1.52 (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): 168.6 (s), 167.2 (s), 159.4 (s), 157.8 (s), 139.8 (d), 135.2 (s), 128.9 (d), 128.8 (d), 128.7 (d), 108.5 (d), 101.5 (d), 82.8 (s), 77.4 (s), 67.4 (t), 66.8 (t), 65.7 (t), 28.3 (q); MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1): 519 (100, $[M+NH_4]^+$), 460 (78, $[M+NH_4-{}^t\text{butyl}]^+$), 316 (56, $[M+H-{}^t\text{butyl-I}]^+$).

Benzyl 2-[3'-*tert*-Butoxycarbonylmethoxy-4'-(4'',4'',5'',5''-Tetramethyl-[1'',3'',2'']dioxaborolan-2''-yl)phenoxy]acetate (6). To a solution of **14** (182 mg, 0.36 mmol) and $Pd(dppf)Cl_2$ (7 mg, 10 mol%) in degassed acetonitrile (10 ml) was added TEA (240 µl, 1.65 mmol). After stirring at 85 °C for 5 min, 4,4,5,5-tetramethyl-1,3,2-dioxaborolane (130 µl, 0.83 mmol) was added. The reaction mixture was stirred for an additional 2 h at 85 ºC. Cooled to RT, the reaction mixture was passed through a silica column (Petroleum ether/EtOAc 2:1, R_f = 0.4) to yield a yellow-brown oil. The crude product was dissolved in diethyl ether (20 ml), and activated charcoal (30 mg) was added. After stirring for 15 min at RT, the resulting suspension was filtered through filter paper, and the filtrate was concentrated *in vacuo* to yield a pale yellow oil (109 mg, 60%). IR (neat): 2979 (m), 2931 (w), 1756 (m), 1604 (s), 1575 (m), 1145 (s), 1077 (m), 914 (w); ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): 7.68 (d, ^3J(H,H) = 8.2 Hz, 1H), 7.39 - 7.34 (m, 5H), 6.51 (dd, ^3J(H,H) = 8.2 Hz, ^4J(H,H) = 2.1 Hz, 1H), 6.45 (d, ^4J(H,H) = 2.1 Hz, 1H), 5.27 (s, 2H), 4.69 (s, 2H), 4.53 (s, 2H), 1.53 (s, 9H), 1.39 (s, 12H); ^{13}C NMR (100 MHz,

$CDCl_3$, 25 °C): 168.6 (s), 168.1 (s), 164.6 (s), 161.5 (s), 138.5 (d), 135.3 (s), 128.8 (d), 128.7 (d), 107.8 (s), 106.3 (s), 83.5 (s), 82.1 (s), 77.4 (s), 67.4 (t), 67.2 (t), 65.4 (t), 28.3 (q), 25.1 (q); MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1): 1015 (100, $[2M+NH_4]^+$), 556 (80, $[M+K+NH_4]^+$), 539 (76, $[M+Na+NH_4]^+$), 443 (65, $[M-^t butyl]^+$).

7^4-Iodo-1^4-Methoxy-1^2,2^2,3^3,4^2,5^3,6^2,7^3-Octakis(*Gla*-OtBu)-*p*-Septiphenyl (24). This compound was prepared following previously reported procedures.[S1]

1^4-Methoxy-8^4-(*Gla*-OBn)-1^2,2^2,3^3,4^2,5^3,6^2,7^3,8^2-octakis(*Gla*-OtBu)-*p*-Octiphenyl (5). To a solution of **24** (44 mg, 0.027 mmol), **6** (33 mg, 0.068 mmol) and $Pd(PPh_3)_4$ (10 mg, 0.0014 mmol) in 7 ml of a mixture of degassed and dry toluene/ethanol (10:1) was added 3 ml 2 M Na_2CO_3. The resulting solution was stirred at 83 ºC for 2 h. The reaction mixture was then diluted with toluene (10 ml), washed with brine (2 x 20 ml), dried over Na_2SO_4, and concentrated under reduce pressure. The resulting crude product was purified using silica gel column chromatography (CH_2Cl_2/acetone 100:0.5, R_f = 0.3) followed by PTLC (CH_2Cl_2/acetone 100:1, R_f = 0.35) to yield **5** (40 mg, 80%) as a colorless solid. ^{1}H NMR (500 MHz, $CDCl_3$, 25 °C): 7.54 (d, ^3J(H, H) = 8.0 Hz, 2H), 7.53 (d, ^3J(H, H) = 7.0 Hz, 2H), 7.47 (d, ^3J(H,H) = 7.8 Hz, 1H), 7.45 (d, ^3J(H,H) = 7.8 Hz, 1H), 7.39 - 7.35 (m, 7H), 7.32 - 7.32 (m, 2H), 7.30 - 7.29 (m, 2H), 7.29 - 7.27 (m, 2H), 7.13 (d, ^4J(H,H) = 1.5 Hz, 2H), 7.12 - 7.11 (m, 2H), 7.10 - 7.10 (m, 2H), 6.64 (dd, ^3J(H,H) = 7.8 Hz, ^4J(H,H) = 2.0 Hz, 1H), 6.56 (dd, ^3J(H,H) = 7.8 Hz, ^4J(H,H) = 2.0 Hz, 1H), 6.54 (d, ^4J(H,H) = 3.0 Hz, 1H), 6.47 (d, ^4J(H,H) = 3.0 Hz, 1H), 5.28 (s, 2H), 4.70 (s, 2H), 4.60 (s, 4H), 4.59 (s, 4H), 4.55 (s, 2H), 4.55 (s, 2H), 4.51 (s, 2H), 4.48 (s, 2H), 3.85 (s, 3H), 1.51 (s, 72H), Fig. S1; ^{13}C NMR (125 MHz, $CDCl_3$, 25 °C): 168.72 (s), 168.12 (s), 168.10 (s), 168.08 (s), 168.07 (s), 167.87 (s), 160.12 (s), 158.25 (s), 156.50 (s), 156.43 (s), 155.84 (s), 155.79 (s), 155.76 (s), 141.65 (s), 141.60 (s), 141.57 (s), 141.55 (s), 141.30 (s), 141.15 (s), 135.11 (s), 132.52 (d), 132.50 (d), 132.49 (d), 132.38 (d), 132.35 (d), 128.64 (d), 128.53 (d), 128.46 (d), 126.78 (d), 126.58 (d), 126.56 (d), 126.50 (d), 126.45 (d), 121.12 (s), 120.16 (s), 119.88 (s), 111.57 (s), 111.49 (s), 111.47 (s), 111.44 (s), 105.91 (d), 105.69 (d), 101.06 (d), 99.98 (d), 81.97 (s), 67.01 (t), 66.64 (t), 66.59 (t), 66.57 (t), 66.47 (t), 65.58 (t), 55.34 (q), 28.10 (q), Fig. S1; MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1):

1864 (100, $[M+NH_4]^+$).

Fmoc-Lys(Z)-Lys(Z)-NH_2. *General procedure A:* To a solution of HBTU (2.05 g, 5.4 mmol), Fmoc-Lys(Z)-OH (1.75 g, 3.5 mmol) and H-Lys(Z)-NH_2 (1.00 g, 3.16 mmol) in dry DMF (40 ml) at 0 °C was added dropwise TEA (2.7 ml, 19 mmol). The reaction mixture was allowed to warm up to rt and stirred at the same time for 1 h after which the solvent was removed. The crude product was purified by column chromatography (CH_2Cl_2/MeOH 15:1, R_f = 0.32) to yield pure Fmoc-Lys(Z)-Lys(Z)-NH_2 (1.70 g, 74%) as a colorless solid. $[\alpha]^{20}_D$ = -5.2 (*c* 1.00, MeOH); Mp: 205 - 207 °C; IR (neat): 3427 (w), 3018 (w), 2716 (w), 1736 (m), 1633 (s), 1230 (m), 1087 (w), 866 (w); ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): 7.88 (d, ^3J(H,H) = 7.6 Hz, 2H), 7.81 (d, ^3J(H,H) = 7.9 Hz, 1H), 7.72 (t, ^3J(H,H) = 7.3 Hz, 2H), 7.52 (d, ^3J(H,H) = 7.9 Hz, 1H), 7.38 (t, ^3J(H,H) = 7.3 Hz, 2H), 7.33 – 7.31 (m, 14H), 7.21 (s, 1H), 7.00 (s, 1H), 4.98 (s, 4H), 4.25 – 4.20 (m, 4H), 3.96 (dd, ^3J(H,H) = 7.5 Hz, ^4J(H,H) = 2.4 Hz, 1H), 2.95 – 2.93 (m, 4H), 1.62 – 1.60 (m, 2H), 1.50 – 1 48 (m, 2H), 1.33 – 1.29 (m, 4H), 1.27 – 1.24 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C): 173.5 (s), 171.8 (s), 156.1 (s), 156.0 (s), 143.9 (s), 143.7 (d), 140.7 (d), 137.3 (d), 128.3 (d), 127.6 (d), 127.3 (d), 127.1 (d), 125.3 (d), 120.1 (d), 65.6 (t), 65.1 (t), 54.9 (d), 54.7 (d), 46.7 (d), 40.1 (t), 31.8 (t), 31.5 (t), 29.1 (t), 22.9 (t), 22.5 (t); MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1): 786 (100, $[M+Na]^+$)

H-Lys(Z)-Lys(Z)-NH_2. *General procedure B*: To a solution of Fmoc-Lys(Z)-Lys(Z)-NH_2 (2.14 g, 2.80 mmol) in DMF (60 ml) was added piperidine (3 ml, 30 mmol). After stirring for 30 min at RT, the solution was concentrated *in vacuo.* Purification of the crude product by column chromatography (CH_2Cl_2/MeOH 15:1, 1% TEA, R_f = 0.31) yielded pure H-Lys(Z)-Lys(Z)-NH_2 (1.15 g, 76%) as a colorless solid. $[\alpha]^{20}_D$ = -2.7 (*c* 1.00, MeOH); Mp: 100 – 101 °C; IR (neat): 3306 (m), 2937 (m), 2497 (m), 1684 (w), 1538 (s), 1265 (m), 839 (w); ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): 8.28 (s, 1H,), 7.45 (s, 1H), 7.33 – 7.30 (m, 12H), 7.01 (s, 1H), 4.98 (s, 4H), 4.19 (dd, ^3J(H,H) = 8.0 Hz, ^3J(H,H) = 4.4 Hz, 1H), 3.45 (t, ^3J(H,H) = 8.4 Hz, 1H), 2.95 – 2.89 (m, 4H), 1.62 – 1.59 (m, 2H), 1.50 – 1.48 (m, 2H), 1.33 – 1.29 (m, 4H), 1.27 – 1.25 (m, 4H); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C): 173.5 (s), 171.8 (s), 156.1 (s), 137.3 (d), 128.4 (d), 127.3 (d), 65.1 (t),

53.2 (d), 52.2 (d), 40.1 (t), 32.6 (t), 32.0 (t), 29.1 (t), 22.9 (t), 22.5 (t); MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1): 559 (100, $[M+NH_4]^+$).

Fmoc-Lys(Z)-Lys(Z)-Lys(Z)-NH_2. Following *general procedure A*, H-Lys(Z)-Lys(Z)-NH_2 (1.15 g, 2.12 mmol) was coupled with Fmoc-Lys(Z)-OH (1.17 g, 2.34 mmol) in the presence of HBTU (1.36 g, 3.6 mmol) and TEA (2 ml, 12.72mmol). Purification of the crude product by silica gel column chromatography (CH_2Cl_2/MeOH 20:1, R_f = 0.3) yielded Fmoc-Lys(Z)-Lys(Z)-Lys(Z)-NH_2 (891 mg, 40%) as a colorless solid. $[\alpha]^{20}_D$ = -10.8 (*c* 1.00, DMSO); Mp: 145-147 °C IR (neat): 3300 (m), 2943 (w), 1685 (s), 1633 (s), 1529 (s), 1230 (s), 833 (s); ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): 7.89 (d, ^3J(H,H) = 7.6 Hz, 2H), 7.81 (d, ^3J(H,H) = 8.1 Hz, 1H), 7.77 – 7.67 (m, 2H), 7.58 (d, ^3J(H,H) = 8.1 Hz, 1H), 7.38 (t, ^3J(H,H) = 7.6 Hz, 2H), 7.33 – 7.31 (m, 18H), 7.21 (s, 1H), 4.98 (s, 6H), 4.25 – 4.20 (m, 6H), 3.97 – 3.95 (m, 2H), 2.95 – 2.89 (m, 6H), 1.62 – 1.59 (m, 3H), 1.50 – 1.45 (m, 3H), 1.33 – 1.29 (m, 6H), 1.27 – 1.24 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C): 173.5 (s), 172.1 (s), 156.1 (s), 156.0 (s), 145.6 (d), 143.1 (d), 141.1 (d), 137.25 (s), 128.3 (d), 127.6 (d), 127.3 (d), 127.1 (d), 125.3 (d), 120.1 (d), 65.6 (t), 65.1 (t), 54.9 (d), 54.7 (d), 46.7 (d), 40.1 (t), 31.8 (t), 31.5 (t), 29.1 (t), 22.9 (t), 22.5 (t); MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1): 1049 (100, $[M+Na]^+$), 1044 (81, $[M+NH_4]^+$).

H-Lys(Z)-Lys(Z)-Lys(Z)-NH_2. Following the g*eneral procedure B*, Fmoc-Lys(Z)-Lys(Z)-Lys(Z)-NH_2 (860 mg, 0.84 mmol) in DMF (60 ml) was treated with 5% piperidine (3 ml). The purification of the crude product by silica gel column chromatography (CH_2Cl_2/MeOH, 10:1 1% TEA, R_f = 0.30) yielded H-Lys(Z)-Lys(Z)-Lys(Z)-NH_2 (538 mg, 80%) as a colorless solid. $[\alpha]^{20}_D$ = -8.4 (*c* 1.00, MeOH); Mp: 151 – 152 °C; IR (neat): 3255 (m), 2973 (m), 1687 (w), 1612 (m), 1247 (w), 823 (m); ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): 7.33 – 7.29 (m, 18H), 4.98 (s, 6H), 4.17 – 4.09 (m, 2H), 3.66 (t, ^3J(H,H) = 6.4 Hz, 1H), 2.95 – 2.92 (m, 6H), 1.62 – 1.58 (m, 3H), 1.50 – 1.47 (m, 3H), 1.33 – 1.29 (m, 6H), 1.27 – 1.24 (m, 6H); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C): 173.5 (s), 172.1 (s), 178.8 (s), 156.1 (s), 137.3 (d), 128.4 (d), 127.3 (d), 65.1 (t), 53.1 (d), 52.2 (d), 40.1 (t), 32.6 (t), 32.0 (t), 29.1 (t), 22.9 (t), 22.5 (t); MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1): 826 (100, $[M+Na]^+$).

Fmoc-Lys(Z)-Lys(Z)-Lys(Z)-Lys(Z)-NH_2. Following *general procedure A*, H-Lys(Z)-Lys(Z)-Lys(Z)-NH_2 (270 mg, 0.335 mmol) was coupled with Fmoc-Lys(Z)-OH (186 mg, 0.37 mmol) in the presence of HBTU (220 mg, 0.57 mmol) and TEA (1 ml, 2.05 mmol). Purification of the crude product by silica gel column chromatography (CH_2Cl_2/MeOH 20:1, R_f = 0.35) yielded HPLC-pure (YMC-Pack SIL, DCM/MeOH 90:10, 2 ml/min, R_t 6.98 min) Fmoc-Lys(Z)-Lys(Z)-Lys(Z)-Lys(Z)-NH_2 (420 mg, 92%) as a colorless solid. $[\alpha]^{20}_D$ = -5.4 (*c* 1.00, MeOH); Mp: 201 – 202 °C; IR (neat): 3257 (m), 1685 (s), 1631 (s), 1532 (m), 1257 (s), 1026 (m), 840 (m); ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): 7.89 (d, ^3J(H,H) = 7.6 Hz, 2H), 7.71 (t, ^3J(H,H) = 7.3 Hz, 2H), 7.46 (d, ^3J(H,H) = 8.0 Hz, 12H), 7.38 (t, ^3J(H,H) = 7.6 Hz, 2H), 7.33 – 7.31 (m, 25H), 4.97 (s, 8H), 4.25 – 4.23 (m, 7H), 3.97 – 3.96 (m, 2H), 2.95 – 2.93 (m, 8H), 1.62 – 1.60 (m, 4H), 1.50 – 1.48 (m, 4H), 1.33 – 1.30 (m, 8H), 1.27 – 1.25 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C): 173.5 (s), 172.1 (s), 156.1 (s), 156.0 (s), 145.6 (d), 143.1 (d), 141.1 (d), 137.2 (d), 128.3 (d), 127.6 (d), 127.3 (d), 127.1 (d), 125.3 (d), 120.1 (d), 65.6 (t), 65.1 (t), 54.9 (d), 54.7 (d), 46.7 (d), 40.1 (t), 31.8 (t), 31.5 (t), 29.1 (t), 22.9 (t), 22.5 (t); MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1): 1301 (100, $[M+NH_4]^+$).

H-Lys(Z)-Lys(Z)-Lys(Z)-Lys(Z)-NH_2 (25). Following the g*eneral procedure B*, Fmoc-

Lys(Z)-Lys(Z)-Lys(Z)-Lys(Z)-NH_2 (207 mg, 0.15 mmol) in DMF (20 ml) was treated with 5% piperidine. (1 ml) Purification of the crude product by silica gel column chromatography (CH_2Cl_2/MeOH 15/1 1% TEA, R_f = 0.35) yielded HPLC-pure (YMC-Pack SIL, DCM/MeOH/TEA 90:9:1, 2 ml/min, R_t 7.5 min) H-Lys(Z)-Lys(Z)-Lys(Z)-Lys(Z)-NH_2 (206 mg, quant) as a colorless solid. $[\alpha]^{20}_D$ = -7.9 (*c* 1.00, MeOH); Mp: 178 -179 °C; IR (neat): 3286 (m), 2604 (m), 2497 (m), 1685 (s), 1537 (s), 1258 (s), 1035 (m), 840 (m); ^{1}H NMR (400 MHz, DMSO-d_6, 25 °C): 7.33 – 7.31 (m, 20H), 7.23 – 7.00 (m, 3H), 4.97 (s, 8H), 4.30 – 4.12 (m, 4H), 3.60 – 3.42 (m, 1H), 2.95 – 2.96 (m, 6H), 1.62 – 1.60 (m, 4H), 1.50 – 1.48 (m, 4H), 1.33 – 1.30 (m, 8H), 1.27 – 1.25 (m, 8H); ^{13}C NMR (100 MHz, DMSO-d_6, 25 °C): 173.5 (s), 172.1 (s), 156.1 (s), 156.0 (s), 145.6 (d), 143.1 (d), 128.3 (d), 127.6 (d), 65.1 (t), 54.9 (d), 54.7 (d), 40.1 (t), 31.8 (t), 31.5 (t), 29.1 (s), 22.9 (t), 22.5 (t); MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1): 1066 (100, $[M+H]^+$). HRMS (ESI): Found 1066.5608; calcd for $C_{56}H_{76}O_{12}N_9$, 1066.5607.

H-Leu-Arg(Pmc)-Leu-His(Trt)-Leu-NH_2 (26). This compound was prepared in 7 steps following reported procedure with minor modifications.[S2]

1^4-Methoxy-8^4-(*Gla*-OH)-1^2,2^2,3^3,4^2,5^3,6^2,7^3,8^2-Octakis(*Gla*-OtBu)-*p*-Octiphenyl (27). To a solution of **5** (40 mg, 0.022 mmol) in degassed THF (4 ml), $Pd(OH)_2$/C (5 mg) was added. The suspension was degassed several times, and set to under hydrogen atmosphere. After stirring for 1.5 h at RT, the reaction mixture was filtered (celite), and the filtrate was concentrated under reduced pressure. Purification of the crude product by PTLC (CH_2Cl_2/Acetone, 2:1, R_f = 0.4) yielded **27** (36 mg, 96%) as a light yellow solid. ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): 7.42 – 7.49 (m 4H), 7.34 – 7.33 (m, 2H), 7.32 – 7.30 (m, 8H), 7.11 – 7.09 (m, 6H), 6.65 – 6.62 (m, 2H), 6.58 (d, ^4J(H,H) = 3.0 Hz, 1H), 6.46 (d, ^4J(H,H) = 3.0 Hz, 1H), 4.60 – 4.51 (m, 18H), 3.84 (s, 3H), 1.48 (s, 72H); MS (ESI, 1 mM NH_4HCO_3 AcCN/MeOH 1:1): 1778 (100, $[M+ Na]^+$), 898 (52, $[M+H+NH_4]^{2+}$).

1^4-Methoxy-8^4-(*Gla*-Lys(Z)-Lys(Z)-Lys(Z)-Lys(Z)-NH_2)-1^2,2^2,3^3,4^2,5^3,6^2,7^3,8^2-Octakis(*Gla*-OtBu)-*p*-Octiphenyl (28). To a solution of **27** (35 mg, 0.02 mmol), **25** (43 mg, 0.04 mmol) and HATU (15 mg, 0.04 mmol) in freshly distilled DMF (1 ml), TEA (0.5

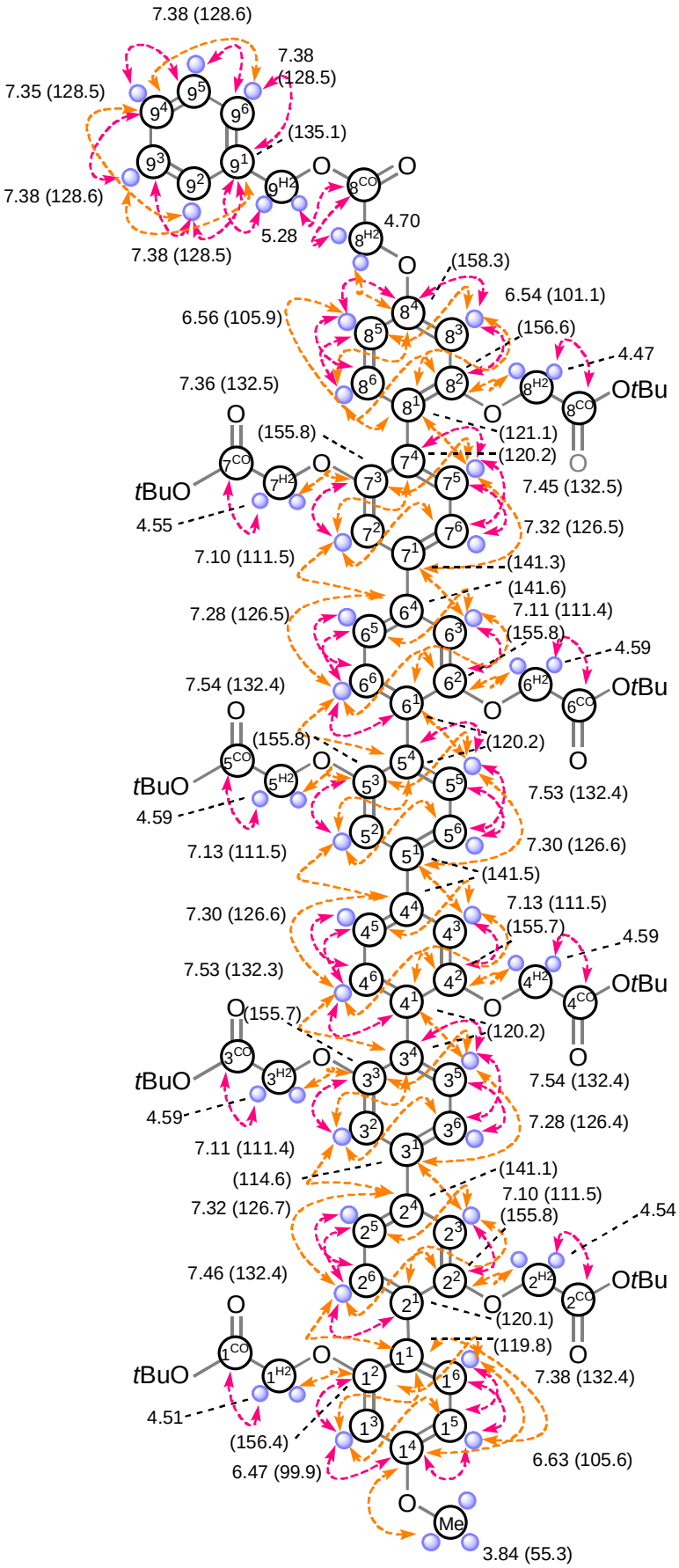
ml, 3.7 mmol) was added. After stirring for 3.5 h in the dark, the solvent was removed under reduced pressure. Purification of the crude product by silica gel column chromatography (CH_2Cl_2/acetone, 2:1, R_f = 0.3) and then by PTLC (CH_2Cl_2/MeOH 20:1, R_f = 0.4) gave HPLC-pure (YMC-Pack SIL, CH_2Cl_2/MeOH/TEA, 90:10, 2 ml/min, R_t 6.39 min) **28** (35 mg, 63%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$/methanol-d_4 1:1, 25 °C): 7.47 – 7.44 (m, 4H), 7.31 – 7.27 (m, 30H), 7.09 – 7.07 (m, 6H), 6.61 – 6.42 (m, 4H), 6.25 – 6.12 (m, 4H), 5.01 – 4.92 (m, 9H), 4.56 – 4.45 (m, 18H), 3.80 (s, 3H), 3.08 – 2.98 (m, 8H), 1.98 – 1.55 (m, 96H); MS (MALDI, dithranol): 2825.4 (100, $[M+Na]^+$).

1^4-Methoxy-8^4-(*Gla*-Lys(Z)-Lys(Z)-Lys(Z)-Lys(Z)-NH_2)-1^2,2^2,3^3,4^2,5^3,6^2,7^3,8^2-Octakis(*Gla*-OH)-*p*-Octiphenyl (29). To a solution of **28** (35 mg, 0.012 mmol) in CH_2Cl_2 (1 ml), TFA was added (1 ml). After stirring for 1 h at RT, the solvent was removed with a stream of nitrogen. The resulting solid was dissolved in CH_2Cl_2/TFA 1:1. After stirring for another 1 h at RT, the solvent was removed with a stream of nitrogen. The crude product was purified by solid liquid extraction with hexane (3 x 5 ml). The resulting solid was dissolved in toluene (10 ml) and subjected to azeotropic evaporation (2 x). The resulting crude **29** (27 mg, 94%) was used for the next step without further purification. ^{1}H NMR (400 MHz, $CDCl_3$/methanol-d_4 1:1, 25 °C): 7.44 – 7.42 (m, 4H), 7.39 – 7.08 (m, 39H), 6.63 – 6.49 (m, 4H), 5.01 – 4.81 (m, 8H), 4.64 – 4.54 (m, 18H), 4.26 – 4.16 (m, 4H) 3.82 (s, 3H), 3.13 – 2.90 (m, 8H), 1.98 – 1.55 (m, 37H); MS (ESI, – ve, CH_2Cl_2/MeOH 1:1): 1177 (100, $[M-2H]^{2-}$), 784 (80, $[M-3H]^{3-}$).

1^4-Methoxy-8^4-(*Gla*-Lys(Z)-Lys(Z)-Lys(Z)-Lys(Z)-NH_2)-1^2,2^2,3^3,4^2,5^3,6^2,7^3,8^2-Octakis(*Gla*-Leu-Arg(Pmc)-Leu-His(Trt)-Leu-NH_2)-*p*-Octiphenyl (30). To a solution of **29** (25 mg, 0.01 mmol), **26** (147 mg, 0.127 mmol) and HATU (48 mg, 0.127 mmol) in freshly distilled DMF (1 ml), TEA (0.5 ml) was added. After stirring for 5.5 h in the dark at RT, the reaction mixture was loaded on a Sephadex LH-20 column and eluted with DMF. After evaporation of the solvent, the product was further purified by PTLC ($CHCl_3$/MeOH 8:1, R_f = 0.4) to yield **30** (87 mg, 75%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$/Methanol-d_4 1:1, 25 °C): 7.52 – 6.92 (m, 167H), 6.81 – 6.43 (m, 19H), 5.03 (s, 8H), 4.60 – 4.11 (m, 40H), 3.82 (s, 3H), 3.20 – 2.80 (m, 26H), 2.60 – 2.30 (m, 60H),

2.10 – 1.90 (m, 24H), 1.85 – 1.20 (m, 120H), 1.00 – 0.50 (m, 144H); MS (MALDI, dithranol): 11483 (100, $[M+H]^+$), 5725 (20, $[M+2H]^{2+}$).

1^4-Methoxy-8^4-(*Gla*-Lys-Lys-Lys-Lys-NH_2)-1^2,2^2,3^3,4^2,5^3,6^2,7^3,8^2-Octakis(*Gla*-Leu-Arg-Leu-His-Leu-NH_2)-*p*-Octiphenyl (4). To a solution of **30** (15 mg, 1.3 µmol) in TFA (1.5 ml) containing pentamethylbenzene (50 mg, 0.33 mmol), thioanisole (60 µl, 0.51 mmol), HBr/AcOH (50 µl of 5.7 M in acetic acid) was added. After stirring the solution for 90 min at RT, the solution was concentrated under reduced pressure. The crude product was purified by solid liquid extraction with diethyl ether (3 x 25 ml) and toluene (10 ml) and subjected to azeotropic evaporation (2 x). Purification of the crude product by RP HPLC (YMC-Pack ODS-A 250 x 10 mm, H_2O/MeOH/TFA 15 : 84 : 1, 2 ml/min, R_t 5.77 min, Fig. S2) yielded **4** (6.0 mg, 68%) as a colorless solid. ^{1}H NMR (400 MHz, $CDCl_3$/MeOD 1:1, 25 °C): 8.65 – 8.52 (m, 7H), 7.62 – 7.11 (m, 34H), 6.80 – 6.60 (m, 6H), 4.80 – 4.77 (m, 18H), 4.45 – 4.38 (m, 36H), 3.86 (s, 3H), 3.29 – 3.11 (m, 26H), 2.98 – 2.95 (m, 10H), 2.01 – 1.45 (m, 137H), 1.01 – 0.61 (m, 144H); MS (MALDI, dithranol): 6869.4 (100, $[M+H]^+$), Fig. S3.


7.38 (128.6)
7.38 (128.5)
7.35 (128.5)
(135.1)
7.38 (128.6)
5.28
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7.38 (128.5)
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6.56 (105.9)
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4.47
7.36 (132.5)
OtBu
(155.8)
(121.1)
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tBuO
7.45 (132.5)
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7.10 (111.5)
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6.63 (105.6)
Me
3.84 (55.3)

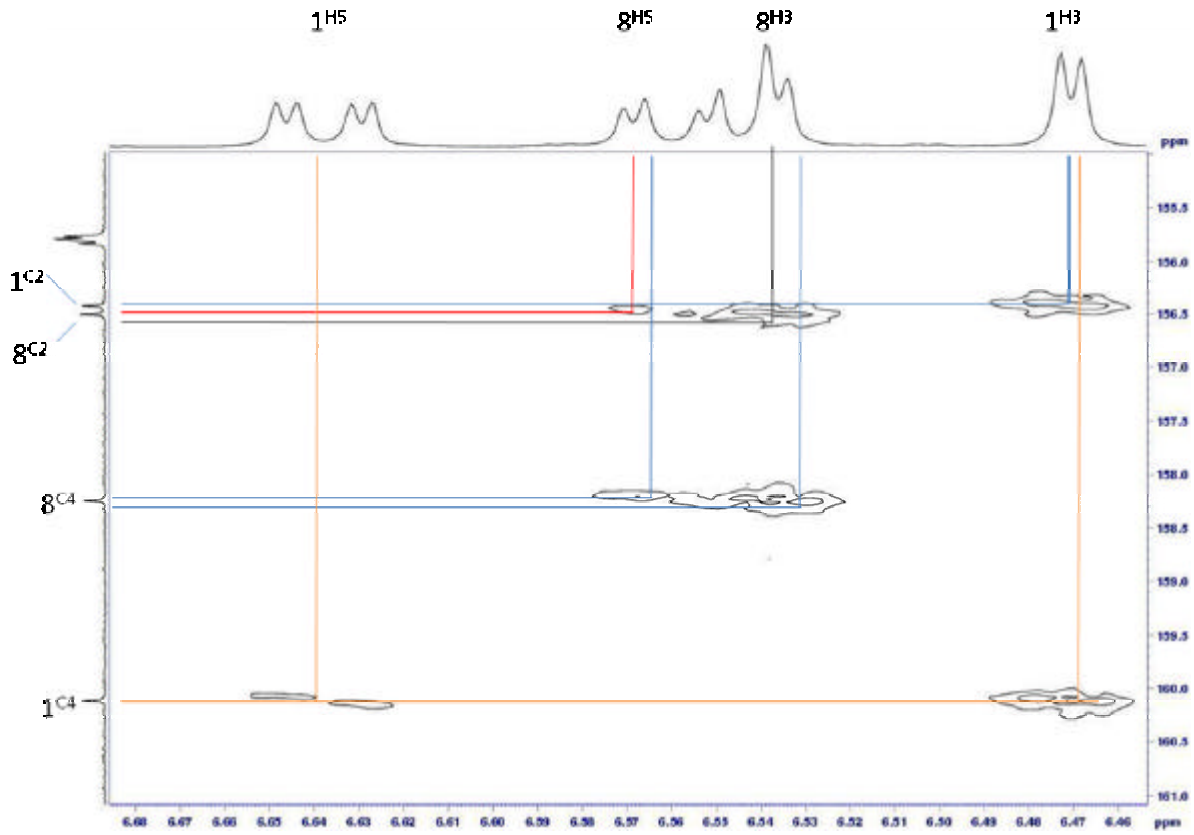

Figure S1. Representative assignment of chemical shifts from ^{13}C NMR and ^{1}H NMR spectra of **5** from two bond (magenta arrows) and three bond coupling (orange arrows) in the HMBC spectrum (top) with example of coupling network (bottom).

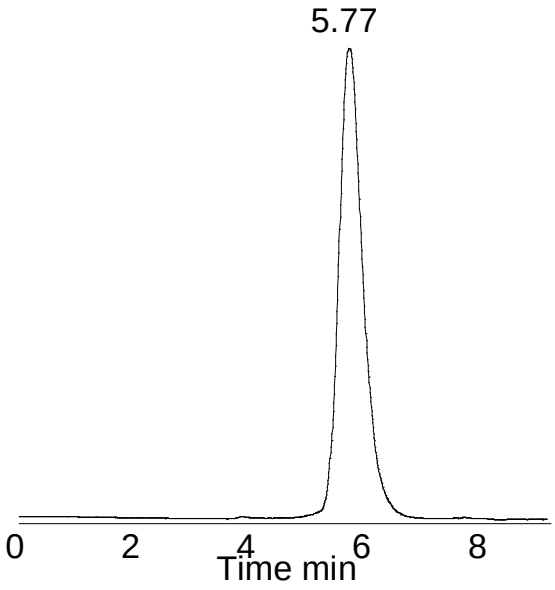

Figure S2. RP HPLC of **4** (detection at 254 nm, YMC-Pack ODS-A 250 x 10 mm, H_2O/MeOH/TFA 15 : 84 : 1, 2 ml/min, R_t 5.77 min).

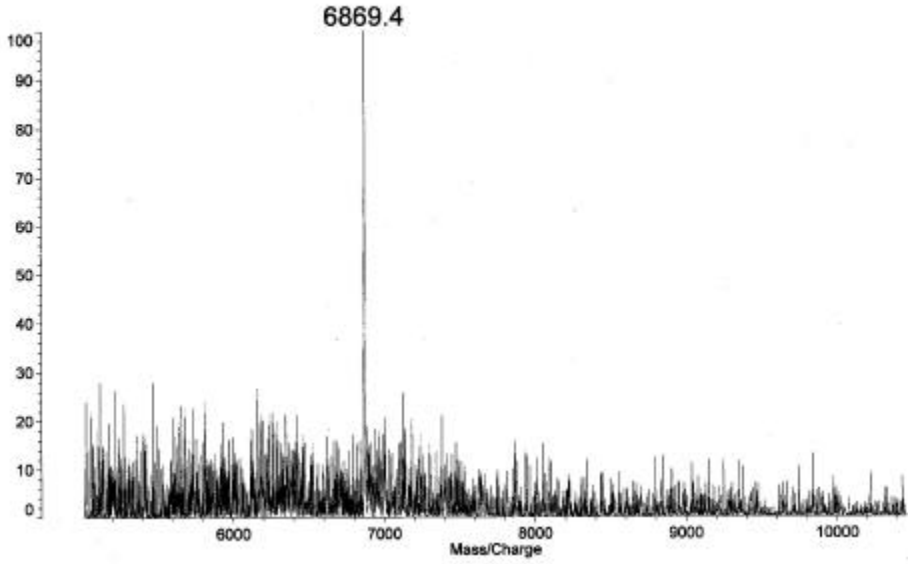

Figure S3. MALDI MS of **4**.

4. Vesicle and Blocker Preparation

EYPC-LUVsÉANTS/DPX. *General procedure C:* As in ref [S3]. In brief, to a solution of EYPC in ethanol (25 µl of 1 g/ml) were added MeOH (1 ml) and $CHCl_3$ (1 ml). A thin film was obtained by slow solvent evaporation on a rotary evaporator, dried *in vacuo* (= 2 h), hydrated (1 ml; 12.5 mM ANTS, 45.0 mM DPX, 5 mM TES, 20 mM NaCl, pH 7.0), subjected to freeze-thaw cycles (=5x) and extruded using a Mini-Extruder with a polycarbonate membrane (pore size 100 nm, =10x). Extravesicular ANTS and DPX were removed by gel filtration (Sephadex G-50) with 10 mM TES, 100 mM NaCl, pH 7.0. The LUV fractions were combined and diluted to 6 ml with the same buffer. Lipid concentrations were estimated from the amount of entrapped dye, the estimated values were consistent with previous results from phosphate analysis. The final stock solutions had the following characteristics: ~2.5 mM EYPC, inside: 12.5 mM ANTS, 45.0 mM DPX, 5 mM TES, 20 mM NaCl, pH 7.0, outside: 10 mM TES, 100 mM NaCl, pH 7.0 and were used within 4 days.

EYPC-LUVsÉCF. As in [S3]. In brief, LUVs were prepared by freeze-thaw-extrusion following the general procedure C, using 10 mM HEPES, 50 mM CF, 10 mM NaCl, pH 7.4 for hydration and 10 mM HEPES, 107 mM NaCl, pH 7.4 for gel filtration. Stock solutions: ~2.5 mM EYPC, inside: 50 mM CF, 10.0 mM HEPES, 107 mM NaCl, pH 7.4, outside: 10 mM HEPES, 107 mM NaCl, pH 7.4.

EYPC/EYPG-LUVsÉCF. To a solution of EYPC in ethanol (12.5 µl of 1 g/ml) were added EYPG (12.5 mg), MeOH (1 ml) and $CHCl_3$ (1 ml). From this mixture, LUVs were prepared by freeze-thaw-extrusion following the general procedure C using 10 mM HEPES, 50 mM CF, 10 mM NaCl, pH 7.4 for hydration and 10 mM HEPES, 107 mM NaCl, pH 7.4 for gel filtration. Stock solutions: ~2.5 mM lipid (EYPC+EYPG), inside: 50 mM CF, 10.0 mM HEPES, 107 mM NaCl, pH 7.4, outside: 10 mM HEPES, 107 mM NaCl, pH 7.4.

Hydrazones 31 and 32. As in ref [S3]. Cascade blue hydrazide **35** (3 mg, 5 µmol) was

added to α-ketoglutarate **33** or pyruvate **34** (450 µL of 1 mM in 10 mM HEPES, 107 mM NaCl, pH 6.5). After stirring for 2 h at 50 °C, the solution was diluted with 2 ml of the same buffer. The pH adjusted to 6.5 and the final volume to 2.5 ml to give 2 mM stock solutions of **31** and **32**.

5. Pore Activity

(a) EYPC-LUVs É ANTS/DPX (100 µl), (b) EYPC-LUVs É CF (100 ~ 5 µl) or (c) EYPC/PG-LUVs É CF (100 ~ 5 µl) were added to gently stirred, thermostated buffer (1.90 ml; (a) 10 mM MES, 100 mM KCl, pH 4.5, (b, c) 10 mM HEPES, 107 mM NaCl, pH 6.5) in a fluorescence cuvette. Fluorescence emission intensity F_t (a) l_{em} = 520 nm, l_{ex} = 353 nm, (b, c) (l_{em} = 517 nm, l_{ex} = 492 nm) was monitored as a function of time (t) during the addition of **4** (20 µl in DMSO, final monomer concentration 8 nM to 500 nM) at t = 50 s and triton X-100 (40 µl, 1.2% aq). Fluorescence time courses F_t were normalized to fractional emission intensity I^n using equation (S1)

$$I^n = (F_t - F_0) / (F_8 - F_0) \quad \text{(S1)},$$

where $F_0 = F_t$ at pore addition, $F_8 = F_t$ at saturation after lysis. The obtained I^n was further converted into fractional pore activity Y using equation (S2)

$$Y = I^n / I^n_{MAX} \quad \text{(S2)},$$

where I^n_{MAX} is I^n at saturation obtained under the conditions giving the highest activity. The obtained fractional pore activities Y at 250 sec were plotted as a function of monomer concentration c_M and fitted to the Hill equation (S3) applied to self-assembly.

$$Y = Y_8 + (Y_0 - Y_8) / \{1 + (c_M / EC_{50})^n\} \quad \text{(S3)},$$

where Y_0 is Y without pore, Y_8 is Y with excess pore, EC_{50} the concentration for 50% pore activity and n the Hill coefficient (Fig. 2). The obtained EC_{50}'s were plotted as a function

of lipid concentration (Fig. 3A).

6. Pore Blockage

EYPC-LUVs ÉCF (5 ~ 100 µl) and blockers (**31**, **32**, ATP, ADP, 0.01 µM – 1000 µM final concentrations) were added to gently stirred, thermostated buffer (final volume 2.00 ml; 10 mM HEPES, 107 mM NaCl, pH 6.5) in a fluorescence cuvette. Fluorescence emission intensity F_t (l_{em} = 517 nm, l_{ex} = 492 nm) was monitored as a function of time (t) during the addition of **4** (20 µl in DMSO, final monomer concentration 60 nM for low lipid concentration or 200 nM for high lipid concentration) at t = 50 s and triton X-100 (40 µl, 1.2% aq). Fractional activities Y were obtained from fluorescence time courses F_t using (S1) and (S2) and fitted to the Hill equation for blockage

$$Y = Y_8 + (Y_0 - Y_8) / \{1 + (c_{BLOCKER} / IC_{50})^n\} \quad \text{(S4)}$$

where Y_0 is Y without ligand, Y_8 is Y with excess ligand, IC_{50} the concentration of blocker for 50% inhibition and n the Hill coefficient. The obtained IC_{50}'s were plotted as a function of lipid concentration (Tab. 1, Fig. 3B).

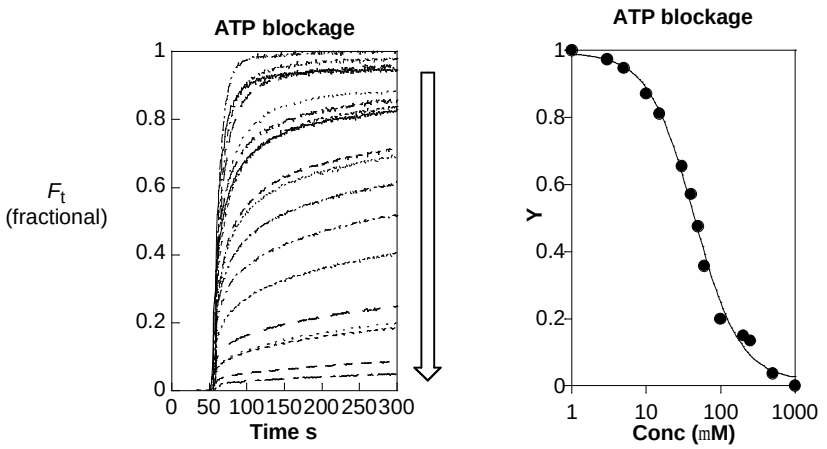

Figure S4. Representative examples for pore **1** blockage with increasing ATP concentration (left) and Hill analysis of the obtained dose response curve (right). The fluorescence kinetics show change in CF emission intensity F_t (l_{em} = 517 nm, l_{ex} = 492 nm) as a function of time during the addition of **4** after 50 s in the presence of ATP (increasing concentration with decreasing activity). Fractional activities Y obtained from (S1) and (S2) were plotted as a function of the ATP concentration and fitted to (S4).

9. References

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[S2] N. Sordé, S. Matile, *J. Supramol. Chem.* **2002**, *2*, 191-199.

[S3] H. Tanaka, G. Bollot, J. Mareda, S. Litvinchuk, D.-H. Tran, N. Sakai, S. Matile, *Org. Biomol. Chem.* **2007**, *5*, 1369-1380.