SUPPLEMENTARY INFORMATION

NOVEL OCTAVALENT CROSS-LINKER DISPLAYS EFFICIENT TRAPPING OF PROTEIN-PROTEIN INTERACTIONS

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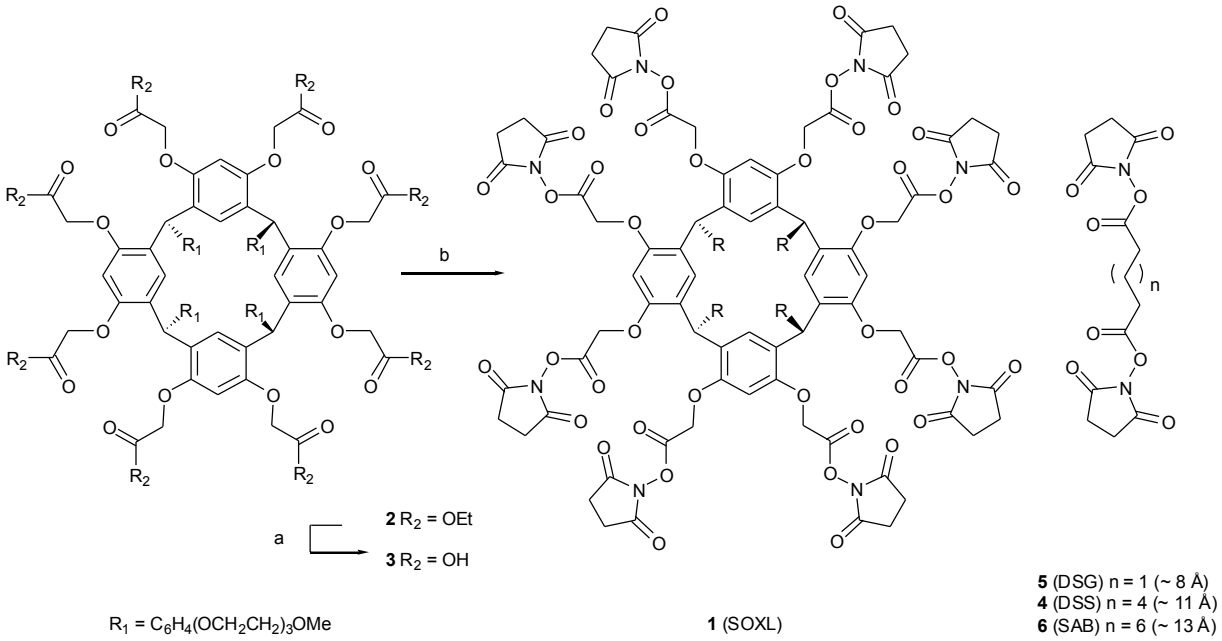
Synthesis of SOXL **1**



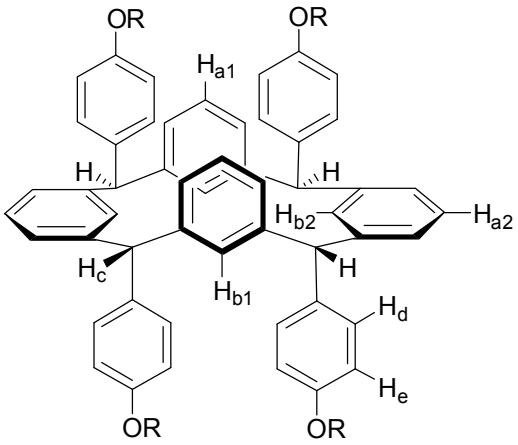


Figure 1. Numbering scheme for NMR assignment of C_{2h} resorcin[4]arene derivatives.

2,2',2'',2''',2'''',2''''',2'''''',2'''''''-[(2,8,14,20- Tetra{4-[2-(2-methoxyethoxy)ethoxy]phenyl}pentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-4,6,10,12,16,18,22,24-octayl)octakis(oxy)]octakis-acetic acid octaethyl ester (2).

Resorcinarene (**3**) (2.09 g, 1.45 mmol) and Cs_2CO_3 (4.72 g, 14.5 mmol) were combined in dry DMF (15 mL) and stirred at 50˚C for 1 h. ethyl bromoacetate (7.26 g, 43.5 mmol) was added and the mixture stirred at 50˚C for 18 h. After evaporation of the DMF under reduced pressure, the residue was partitioned between CH_2Cl_2 and water. The organic phase was washed with water and dried over $MgSO_4$. After evaporation of the solvent, the crude material was purified by silica flash chromatography [CH_2Cl_2 – CH_3OH (10:1), R.f = 0.56]. The fractions containing product were collected and the solvent removed *in vacuo* to give the octaester(**4**) as a pale yellow solid (1.8 g, 58 %); m.p. 62-64 °C; ν_{max}/cm^{-1} (KBr) 3432vs, 2928s, 2361s, 1752s, 1610w, 1585w, 1512m, 1498m, 1456s, 1406s, 1381s, 1381w, 1297m, 1247s, 1208m, 1176m, 1158m, 1112m, 1081w, 1023w, 932w, 857w, 825w; δ_H(400.13 MHz; DMSO; Me_4Si) 1.16 (12 H, t, *J* 8 Hz, CH_2CH_3), 1.17 (12 H, t, *J* 8 Hz, CH_2CH_3), 3.24 (12 H, s, OCH_3), 3.44 (8 H, m, CH_3OCH_2), 3.53 (8 H, m, $CH_3OCH_2CH_2$), 3.55 (8 H, m, $CH_3OCH_2CH_2OCH_2$), 3.60 (8 H, m, $ArOCH_2CH_2OCH_2$), 3.73 (8 H, t, *J* 5 Hz, $ArOCH_2CH_2$), 3.97 (8 H, t, *J* 5 Hz, $ArOCH_2$), 4.10 (8 H, q, *J* 8 Hz, CH_3CH_2), 4.11 (8 H, q, *J* 8 Hz, CH_3CH_2), 4.28 (4 H, d, *J* 16 Hz, CH_AH_BCOO), 4.46 (4 H, d, *J* 16 Hz, CH_AH_BCOO), 4.57 (4 H, d, *J* 16 Hz, CH_AH_BCOO), 4.65 (4 H, d, *J* 16 Hz, CH_AH_BCOO), 5.69 (4 H, s, H_c), 5.94 (2 H, s, H_{b1}), 6.19 (2 H, s, H_{b2}), 6.41 (2 H, s, H_{a2}), 6.55 (8 H, d, *J* 9 Hz, H_e), 6.59 (8 H, d, *J* 9 Hz, H_d), 6.65 (2 H, s, H_{a1}); δ_C(100.5 MHz; $CDCl_3$; Me_4Si) 14.2 (CH_3), 42.1 (CH), 59.0 (CH_3), 61.0 (CH_2), 61.1 (CH_2), 66.9 (CH_2), 67.2 (CH_2), 67.3 (CH_2), 69.8 (CH_2), 70.6 (CH_2), 70.7 (CH_2), 70.8 (CH_2), 71.9 (CH_2), 99.5 (CH), 100.9 (CH), 113.8 (CH), 126.8 (C), 127.8 (C), 128.6 (CH), 130.0 (CH), 132.2 (CH), 134.5 (C), 154.5 (C), 154.7 (C), 156.6 (C), 168.8 (C), 169.2 (C); LRMS (MALDITOF) m/z = calcd for $C_{112}H_{144}O_{40}$ M^+ requires 2128.9; found 2128.0.

Crystallographic information: Colourless tablet, 1.12 x 1.12 x 0.58 mm, triclinic, $P\bar{1}$, a = 11.7830(8), b = 15.3349(11), c = 31.054(2) Å, α = 91.283(2), β = 91.879(2), γ = 102.979(2)°, V = 5462.3(6) Å^3, Z = 2, D_{calcd} = 1.295 g/cm^3, 2θmax = 55°, Mo $K\alpha$, λ = 0.71073 Å, ω scans, T = 150(2) K, 50321 reflections measured, all 24449 unique used in the refinement, no absorption or extinction corrections applied, structure solution by direct and difference Fourier methods using SHELXS97, structure refinement used SHELXL97, 1374 parameters, H atoms geometrically placed and refined using a riding model, R = 0.0758, wR = 0.235, full-matrix least-squares on F^2, final residual electron density 1.19 and –0.75 e Å^{-3}. CCDC 297190 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from the Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.".

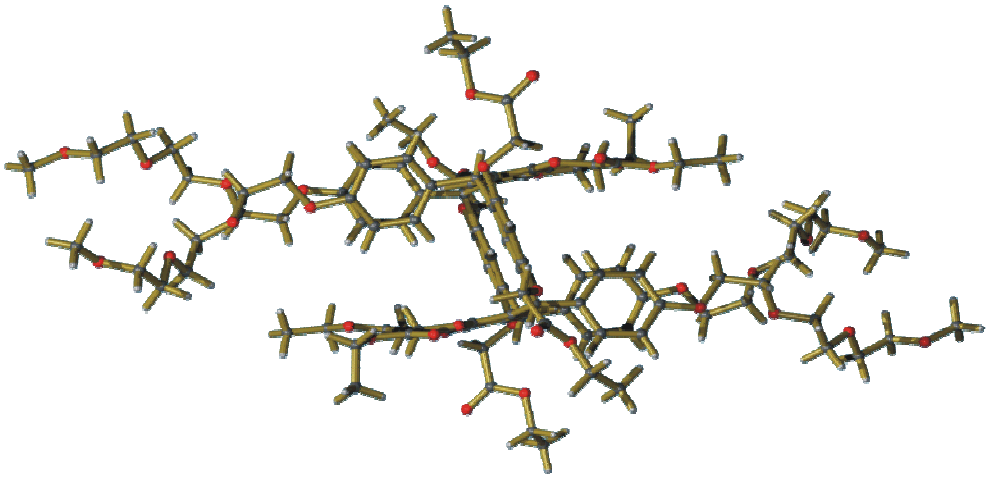


Figure 2: One centrosymmetric dimer from octaester **2** crystal structure. [18]
(the asymmetric unit contains two independent half molecules; each lying about an inversion centre.)

2,2',2'',2''',2'''',2''''',2'''''',2'''''''-[(2,8,14,20- Tetra{4-[2-(2-methoxyethoxy)ethoxy]phenyl}pentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-4,6,10,12,16,18,22,24-octayl)octakis(oxy)]octakis-acetic acid (3).

Octaester **(4)** (1.8 g, 0.85 mmol) was dissolved in THF (50 mL). To this was added a KOH solution (5.7 g KOH in 30 mL water) and the reaction mixture was stirred vigorously at room temperature for 1 h. The mixture was washed with ether and the aqueous phase adjusted to pH 2. The mixture was left at -20 ºC for 18 h and the resultant precipitate was collected by suction filtration. The solid was dried (0.1 mmHg) to give the octaacid **(5)** as a white solid (1.30 g, 80 %); m.p. 168-172 °C; ν_{max}/cm^{-1} (KBr) 3398s, 2931vs, 1754vs, 1610m, 1584m, 1510s, 1440m, 1403m, 1299m, 1248m, 1186m, 1160m, 1111m, 1075m, 931w, 844w, 823w, 733w; δ_H(399.8 MHz; $CDCl_3$; Me_4Si) 3.37 (12 H, s, OCH_3), 3.56 (8 H, m, CH_3OCH_2), 3.66 (8 H, m, $CH_3OCH_2CH_2$), 3.69 (8 H, m, $CH_3OCH_2CH_2OCH_2$), 3.74 (8 H, m, $ArOCH_2CH_2OCH_2$), 3.85 (8 H, t, *J* 5 Hz, $ArOCH_2CH_2$), 4.08 (8 H, t, *J* 5 Hz, $ArOCH_2$), 4.36 (4 H, d, *J* 16 Hz, CH_AH_BCOO), 4.42 (4 H, d, *J* 16 Hz, CH_AH_BCOO), 4.50 (4 H, d, *J* 16 Hz, CH_AH_BCOO), 4.52 (4 H, d, *J* 16 Hz, CH_AH_BCOO), 5.89 (4 H, s, H_c), 5.95 (2 H, s, H_{b1}), 6.27 (2 H, s, H_{b2}), 6.40 (2 H, s, H_{a2}), 6.47 (2 H, s, H_{a1}), 6.58 (16 H, m, $H_{e,d}$); δ_C(100.5 MHz; $CDCl_3$; Me_4Si) 42.1 (CH), 58.2 (CH_3), 66.5 (CH_2), 66.7 (CH_2), 67.2 (CH_2), 69.7 (CH_2), 70.2 (CH_2), 70.3 (CH_2), 70.5 (CH_2), 71.7 (CH_2), 99.5 (CH), 100.5 (CH), 113.7 (CH), 126.7 (C), 127.6 (C), 128.7 (CH), 130.0 (CH), 132.2 (CH), 134.5 (C), 154.4 (C), 154.6 (C), 156.7 (C), 169.8 (C), 170.1 (C); HRMS-ES$^-$(m/z): $[M]^{2-}$ calcd for $[C_{96}H_{110}O_{40}]^{2-}$: 951.8322; found 951.8224.

2,2',2'',2''',2'''',2''''',2'''''',2'''''''-[(2,8,14,20- Tetra{4-[2-(2-methoxyethoxy)ethoxy]phenyl}pentacyclo[19.3.1.1^{3,7}.1^{9,13}.1^{15,19}]octacosa-1(25),3,5,7(28),9,11,13(27),15,17,19(26),21,23-dodecaene-4,6,10,12,16,18,22,24-octayl)octakis(oxy)] octakis-acetic acid octa-2,5-dioxo-pyrrolidin-1-yl ester (1).

To a solution of octaacid **(5)** (100 mg, 0.052 mmol) in dry CH_2Cl_2 (1 mL) were added oxalyl chloride (109.8 µL, 1.26 mmol) and a catalytic amount of DMF (1 drop of a solution of 1 drop of DMF in 1 mL CH_2Cl_2) at 0 ºC. After 5 min, the solution was allowed to warm to room temperature and stirred for 1 h. The solvent was removed by evaporation, and CH_2Cl_2 was evaporated twice from the remaining octaacid chloride which was then dissolved in CH_2Cl_2 (1 mL). In a separate flask, *N*-hydroxysuccinimide was co-evaporated with dry toluene (3 x 1 mL), dissolved in dry THF (1 mL), and cooled to -10 ºC. Then the solution of the octaacid chloride and piperidinomethyl polystyrene HL (200 - 400 mesh) (413.5 mg, 1.65 mmol) was added, and the mixture was stirred for 2 h at room temperature. After 2 h the mixture was filtered through celite to remove

the piperidinomethyl polystyrene HL (200 - 400 mesh) and the resulting octasuccinimidyl ester, SOXL **1** was used without further purification to prepare cross-linked GST; (139 mg, quant.); ^{1}H NMR (399.8 MHz, $CDCl_3$, 25°C): δ = 2.74 (s, 16H), 2.82 (br s, 16H), 3.29 (s, 12H; OCH_3), 3.46 (m, 8H; CH_3OCH_2), 3.55 (m, 8H; $CH_3OCH_2CH_2$), 3.57 (m, 8H; $CH_3OCH_2CH_2OCH_2$), 3.60 (m, 8H; $ArOCH_2CH_2OCH_2$) 3.70 (t, ^{3}J(H,H) = 5 Hz, 8H; $ArOCH_2CH_2$), 3.92 (t, ^{3}J(H,H) = 5 Hz, 8H; $ArOCH_2$), 4.70 (d, ^{2}J(H,H) = 16 Hz, 4H; CH_AH_BCOO), 4.78 (d, ^{2}J(H,H) = 16 Hz, 4H; CH_AH_BCOO), 4.83 (d, ^{2}J(H,H) = 16 Hz, 4H; CH_AH_BCOO), 4.92 (d, ^{2}J(H,H) = 16 Hz, 4H; CH_AH_BCOO), 5.77 (s, 4H; H_c), 5.91 (s, 2H; H_{b1}), 6.24 (s, 2H; H_{b2}), 6.35 (s, 2H; H_{a2}), 6.37 (s, 2H; H_{a1}), 6.39 (d, ^{3}J(H,H) = 9 Hz, 8H; H_e), 6.53 (d, ^{3}J(H,H) = 9 Hz, 8H; H_d); ^{13}C NMR (100.5 MHz, $CDCl_3$, 25°C, TMS): δ = 25.5 (CH_2), 25.6 (CH_2), 42.0 (CH), 58.9 (CH_3), 63.6 (CH_2), 64.5 (CH_2), 66.8 (CH_2), 69.7 (CH_2), 70.5 (CH_2), 70.6 (CH_2), 71.8 (CH_2), 98.5 (CH), 100.5 (CH), 113.9 (CH), 125.8 (C), 127.5 (C), 128.5 (CH), 130.2 (CH), 132.4 (CH), 134.0 (C), 153.6 (C), 156.4 (C), 164.9 (C), 165.0 (C), 168.9 (C), 169.3 (C); IR(KBr): ν = 3503 cm^{-1}, 2936, 1830, 1791, 1735, 1507, 1436, 1361, 1302, 1250, 1202, 1076 ; Anal. Calc. for $C_{128}H_{136}N_8O_{56}.H_2O$: C, 56.93; H, 5.15, N, 4.15; found: C, 56.90; H, 5.30, N, 4.06.

Production and purification of glutathione S-transferase (GST).

XL-1Blue *E.coli* (Stratagene, CA, USA) were transformed with the pGEX2T plasmid (AmerhsamBioscience). This plasmid drives bacterial expression of the *Schistosoma japonicum* form of GST. XL-1 Blue harboring pGEX2T were cultured in 2xYT containing 100 μg/mL ampicillin at 37 °C, to an optical density between 0.6-0.8 OD_{600} before being transferred to 28 °C, at which time isopropyl-β-D-thiogalactoside was added to a final concentration of 0.1 mM, as previously described.[2] After 16 h incubation, cells were harvested, lysed and GST purified over Glutathione-Sepharose beads according to the manufacturer's recommendations (AmershamBioscience). Eluted GST was dialysed extensively against 4 changes of PBS and the protein concentration determined by Bradford assay. The integrity of the GST was verified by SDS-PAGE and Coomassie blue staining. Purified GST was stored in 100 μL aliquots at -80 °C until required.

Cross-linking experiments

GST was used at a constant final concentration of 2.7 μM in 30% (v/v) phosphate buffered saline, pH 7.2 (Invitrogen) 70% (v/v) water. Where stated, BSA was added in equimolar concentrations. Stock solutions of cross-linkers (SOXL**1,** DSS **4,** DSG **5,** SAB **6)** were dissolved in DMSO to identical concentrations and between 1 and 8 molar equivalents added to GST solution to a final volume of 100 μl. A constant DMSO concentration (8% v/v) used for all experiments and provided complete dissolution of reagents. Reactions

were performed for 60 minutes at room temperature and quenched by addition of 1 µl of 1M Tris-HCl pH 7.5. After cross-linking, samples were heated with 2-mercaptoethanol, 20 µl of each sample were loaded per lane and separated through 10% poly-acrylamide SDS gels.

SDS-PAGE and Immunoblotting

SDS-PAGE and immunoblotting were carried out as described previously.[3,4] A monoclonal anti-GST antibody (BD Bioscience Pharmingen, Oxford) was used for detection of GST and its cross-linked forms at a concentration of 5 $\mu g ml^{-1}$. Rabbit anti-rat secondary antibody conjugated to horseradish peroxidase were used at a concentration of 0.05 µg/mL (Dako, Denmark). Anti BSA rabbit polyclonal (Upstate Biotechnology New York, USA) antibody was used for detection of BSA at a concentration of 5 $\mu g\ mL^{-1}$. Goat anti-rabbit secondary antibody conjugated to horseradish peroxidase were used at a concentration of 0.05 µg/mL (Dako, Denmark). Immunoblots were developed using the ECL system (AmershamBioscience) and Kodak X-AR 5 film.

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3. Welham, M. J., U. Dechert, K. B. Leslie, F. Jirik, and J. W. Schrader. *J. Biol. Chem.,* **1994**. *269***,**23764-23768.
4. Welham, M. J., and J. W. Schrader. *J. Immunol.,* **1992**. *149***,** 2772-2783.