Supplementary Material (ESI) for Chemical Communications

Supporting Information for

Efficient Gene Transfection with Functionalised Multicalixarenes

Ruth Lalor,[a] Jessica L. DiGesso,[a] Anja Mueller[a] and Susan E. Matthews*

School of Chemical Sciences and Pharmacy, University of East Anglia Norwich, UK. Fax: 44 1603 592003; Tel: 44 1603 593986; E-mail: susan.matthews@uea.ac.uk

Experimental Details

All chemicals were purchased from Sigma-Aldrich Ltd. (Aldrich, Sigma and Fluka/Riedel de Haën brands), Lancaster or Acrôs Organics, and were used without further purification. Deuterated solvents for NMR use were purchased from Cambridge Isotope Laboratories, Inc., or Apollo Scientific. DMF was stored over molecular sieves (4Å). Analytical thin layer chromatography (TLC) was performed using Merck Kieselgel 60 F_{254} silica gel plates. Column chromatography was run using silica gel 60 (70-230 mesh ASTM). Visualisation was by UV light (254 nm), or by immersion in 5% ninhydrin (in ethanol) solution. NMR spectra were recorded at 293 K, unless otherwise stated, using a 400 MHz Varian Unity Plus Spectrometer, operating at 399.92 MHz or 399.96 MHz (proton), 100.56 MHz or 100.57 MHz (carbon) and 376.24 MHz (fluorine), or a 300 MHz Gemini 2000 spectrometer operating at 300.05 MHz (proton) and 75.45 MHz (carbon). Shifts are referenced relative to the internal reference standard, tetramethylsilane (TMS), with chemical shifts expressed in parts per million (ppm or δ) downfield from the standard and coupling constants (*J*) expressed in Hz. NMR data was processed using MestReC software. MALDI-TOF mass spectra were recorded by the EPSRC National Mass Spectrometry Service, Swansea, or using a Kratos Axima CFR MALDI Mass Spectrometer. ESI mass spectra were recorded on a Shimadzu LC-MS 2010EV Spectrometer, with HPLC grade CH_3OH, water or CH_3CN as carrier solvents. Melting points were measured using an electrothermal Mel-temp® melting point apparatus and are reported uncorrected. Infrared spectra were recorded using an Avatar 360 FT-IR Spectrometer.

Compounds **6**[1],**14**[2],**15**[3],**19**[4],**20**[5] were prepared according to literature procedures

5,11,17,23-*p*-*Tert*-butyl-25,26,27-tripropoxy-28-phthalimidopropoxycalix[4]arene (7) To a solution of **6**[1] (25.00 g, 32.13 mmol) stirring in DMF (300 mL) under an atmosphere of Ar for 1 h were added NaH (95%, 3.08 g, 128.50 mmol) and DMF (300 mL). After 1 h, *n*-3(bromopropyl)phthalimide (34.45 g, 128.50 mmol) was added and the solution stirred for 5 days. H_2O (300 mL) was added and the resulting precipitate filtered and reprecipitated from DCM/MeOH

to yield **7** as a white powder (21.87 g, 71%). **Mp** 193-196 °C; **IR** *v*Phth=1716 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$); 7.85 (2H, dd, *J* = 5.3 Hz, *J* = 3.0 Hz, ArH_{pht}), 7.70 (2H, dd, *J* = 5.3 Hz, *J* = 3.0 Hz, ArH_{pht}), 6.88 (2H, s, Ar*H*), 6.86 (2H, s, Ar*H*), 6.65 (2H, d, *J* = 2.4 Hz, Ar*H*), 6.63 (2H, d, *J* = 2.4 Hz, Ar*H*), 4.40 (2H, d, *J* = 12.4 Hz, ArCH_2Ar), 4.35 (2H, d, *J* = 12.4 Hz, ArCH_2Ar), 3.97 (2H, t, *J* = 7.8 Hz, OC$H_2$$CH_2CH_2N$), 3.83 (4H, m, OC$H_2$$CH_2CH_3$), 3.75-3.70 (4H, m, OC$H_2$$CH_2CH_3$, $OCH_2CH_2CH_2N$, coincident), 3.09 (2H, d, *J* = 12.4 Hz, ArCH_2Ar), 3.08 (2H, d, *J* = 12.4 Hz, ArCH_2Ar), 2.48 (2H, m, $OCH_2CH_2CH_2N$), 1.92 (6H, m, $OCH_2CH_2CH_3$), 1.16 (9H, s, $(CH_3)_3Ar$), 1.15 (9H, s, $(CH_3)_3Ar$), 0.96 (6H, t, *J* = 7.8 Hz, $OCH_2CH_2CH_3$), 0.95 (18H, s, $(CH_3)_3Ar$), 0.87 (3H, t, *J* = 7.8 Hz, $OCH_2CH_2CH_3$); **^{13}C NMR** (100 MHz, $CDCl_3$); 168.4, 154.3, 153.9, 153.4, 144.5, 144.2, 134.8, 134.1, 133.3, 133.2, 132.4, 125.3, 125.2, 124.9, 124.8, 116.5, 105.6, 101.5, 77.1, 123.4, 72.7, 35.7, 34.1, 33.9, 31.8, 31.7, 31.5, 31.3, 31.2, 29.8, 23.6, 23.4, 10.7, 10.2; **ESI MS** 1001.5 m/z $[M+K]^+$.

5,11,17,23-Tetra-nitro-25,26,27-tripropoxy-28-phthalimidopropoxycalix[4]arene (8)

To a solution of **7** (12.40 g, 12.78 mmol) in DCM (250 mL) stirring under an atmosphere of Ar at 0 ^{o}C, was added TFA (13.33 mL) and nitric acid (65%, 20 mL). The solution was stirred for 1 h before pouring into H_2O (500 mL) and DCM (250 mL). The organic phase was separated, the aqueous phase extracted with DCM (2 x 250 mL), the organic layers combined, dried over sodium sulfate and the solvent removed under reduced pressure. The residue was precipitated from MeOH to yield **8** as a pale yellow powder (10.43 g, 89%). **Mp** > 152 °C (decomp.); **IR** *v*Phth=1712 cm^{-1}, *v*NO_2=1528 cm^{-1}, 1345 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$); 7.79 (2H, dd, *J* = 5.4 Hz, *J* = 3.0 Hz, ArH_{pht}), 7.69 (2H, dd, *J* = 5.4 Hz, *J* = 3.0 Hz, ArH_{pht}), 7.51 (2H, s, Ar*H*), 7.50 (4H, s, Ar*H*), 7.48 (2H, s, Ar*H*), 4.46 (2H, d, *J* = 14.0 Hz, ArCH_2Ar), 4.44 (2H, d, *J* = 14.0 Hz, ArCH_2Ar), 4.02 (2H, t, *J* = 7.0 Hz, OC$H_2$$CH_2CH_2N$), 3.90 (6H, m, OC$H_2$$CH_2CH_3$), 3.77 (2H, t, *J* = 7.0 Hz, $OCH_2CH_2CH_2N$), 3.35 (2H, d, *J* = 14.0 Hz, ArCH_2Ar), 3.33 (2H, d, *J* = 14.0 Hz, ArCH_2Ar), 2.21 (2H, m, $OCH_2CH_2CH_2N$), 1.83 (6H, m, $OCH_2CH_2CH_3$), 0.93 (9H, t, *J* = 7.5 Hz, $OCH_2CH_2CH_3$). **^{13}C NMR** (100 MHz, $CDCl_3$); 168.4, 161.9, 161.6, 143.2, 143.0, 135.6, 135.5, 134.5, 132.1, 124.2, 124.1, 123.6, 109.9, 78.1, 77.9, 73.7, 35.1, 31.4, 31.3, 29.7, 23.5, 23.4, 10.3; ***M/z*** **(MALDI TOF)** 940.3 m/z $[M+Na]^+$

5,11,17,23-Tetra-amino-25,26,27-tripropoxy-28-phthalimidopropoxycalix[4]arene (9)

To a solution of **7** (12.00 g, 13.08 mmol) in EtOH (300 mL) was added $Sn(II)Cl_2.2H_2O$ (59.05 g, 261.71 mmol), and the resultant solution heated at reflux for 16 h. The solution was poured into iced H_2O (200 mL), DCM (200 mL) was added and the biphasic solution stirred for 1 h, after which NaOH (aq., 1N, 200 mL) was added, followed by stirring for 1 h. The organic layer was separated,

washed with H_2O and brine, and the solvent was removed under reduced pressure to yield **9** as a pale brown glass (10.24 g, 98%). **Mp** > 163 °C (decomp.); **IR** νNH_2=3409 cm^{-1}, νPhth=1710 cm^{-1}, νNH_2=1611 cm^{-1}, νCN=1215 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$); 7.83 (2H, dd, *J* = 5.4 Hz, *J* = 3.0 Hz, ArH_{pht}), 7.69 (2H, dd, *J* = 5.4 Hz, *J* = 3.0 Hz, ArH_{pht}), 6.12 (2H, s, Ar*H*), 6.10 (2H, s, Ar*H*), 5.96 (4H, s, Ar*H*), 4.29 (2H, d, *J* = 13.2 Hz, ArCH_2Ar), 4.26 (2H, d, *J* = 13.2 Hz, ArCH_2Ar), 3.87 (2H, t, *J* = 7.4 Hz, OC$H_2CH_2CH_2$N), 3.73 (4H, m, OC$H_2CH_2CH_3$), 3.68 (4H, m, OC$H_2CH_2CH_3$, $OCH_2CH_2CH_2$N, coincident), 3.00 (8H, b s, ArNH_2), 2.91 (2H, d, *J* = 13.2 Hz, ArCH_2Ar), 2.89 (2H, d, *J* = 13.2 Hz, ArCH_2Ar), 2.24 (2H, m, $OCH_2CH_2CH_2$N), 1.81 (6H, m, $OCH_2CH_2CH_3$), 0.92 (9H, m, $OCH_2CH_2CH_3$); **^{13}C NMR** (100 MHz, $CDCl_3$); 168.4, 150.4, 150.1, 149.9, 140.7, 140.4, 140.3, 136.2, 135.4, 135.3, 134.1, 132.4, 123.4, 116.1, 116.0, 115.9, 76.9, 72.6, 50.9, 35.8, 31.4, 31.3, 29.6, 23.4, 23.3, 10.7, 10.5; ***M/z* (MALDI TOF)** 820.3 m/z $[M+Na]^+$.

5,11,17,23-Tetra-BOC-amino-25,26,27-tripropoxy-28-phthalimidopropoxycalix[4]arene (10)

9 (1.50 g, 1.87 mmol) was dissolved in DCM (120 mL), to which was added BOC anhydride (1.64 mL, 7.52 mmol), dropwise. Stirring was continued overnight, and the solvent removed under reduced pressure. The product was purified by a short, rapid column over silica gel, eluting with DCM followed by EtOAc, to yield **10** as a pale brown glass (2.14 g, 95%). **Mp** 174-176 °C; **IR** νNH=3324 cm^{-1}, νCO=1767 cm^{-1}, νPhth=1715 cm^{-1}, νNH=1598 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$); 7.83 (2H, dd, *J* = 5.4 Hz, *J* = 3.0 Hz, ArH_{pht}), 7.69 (2H, dd, *J* = 5.4 Hz, *J* = 3.0 Hz, ArH_{pht}), 6.70 (2H, s, Ar*H*), 6.69 (2H, s, Ar*H*), 6.49 (2H, s, Ar*H*), 6.48 (2H, s, Ar*H*), 6.18 (1H, s, ArN*H*), 6.17 (1H, s, ArN*H*), 6.05 (2H, s, ArN*H*), 4.34 (2H, d, *J* = 13.3 Hz, ArCH_2Ar), 4.31 (2H, d, *J* = 13.3 Hz, ArCH_2Ar), 3.91 (2H, t, *J* = 7.4 Hz, OC$H_2CH_2CH_2$N), 3.77 (4H, m, OC$H_2CH_2CH_3$, $OCH_2CH_2CH_2$N, coincident), 3.71 (4H, t, *J* = 7.4 Hz, OC$H_2CH_2CH_3$), 3.07 (2H, d, *J* = 13.3 Hz, ArCH_2Ar), 3.06 (2H, d, *J* = 13.3 Hz, ArCH_2Ar), 2.25 (2H, m, $OCH_2CH_2CH_2$N), 1.83 (6H, m, $OCH_2CH_2CH_3$), 1.47 (18H, s, $C(CH_3)_3$), 1.45 (18H, s, $C(CH_3)_3$), 0.92 (9H, m, $OCH_2CH_2CH_3$); **^{13}C NMR** (100 MHz, $CDCl_3$); 168.4, 153.6, 153.5, 153.4, 152.8, 135.9, 135.8, 135.0, 134.8, 134.1, 132.6, 132.3, 132.2, 132.1, 129.7, 123.4, 120.2, 120.1, 119.9, 80.4, 80.3, 80.1, 77.1, 72.7, 35.6, 31.4, 31.2, 29.6, 28.6, 23.4, 23.2, 10.6, 10.4; ***M/z* (MALDI TOF)** 1220.6 m/z $[M+Na]^+$.

5,11,17,23-Tetra-Boc-glycine-25,26,27-tripropoxy-28-phthalimidopropoxycalix[4]arene (11)

A solution of **9** (0.50 g, 0.63 mmol) and BOC GlycineONSu (1.50 g, 5.50 mmol) with Hünig's base (0.95 mL, 6.26 mmol) and a catalytic amount of DMAP, in $CHCl_3$ (10 mL), was stirred over 48 h. The reaction was quenched with H_2O (10 mL), and the organic layer separated and washed with citric acid (10 %, 10 mL), NaOH (10 %, 10 mL), brine (10 mL) and H_2O (10 mL). The solvent was removed under reduced pressure and the residue purified by column chromatography over silica gel,

eluting with DCM:MeOH 19:1, to yield **11** (0.59 g, 0.42 mmol) as a pale orange solid in a 67% yield. **Mp** >178 °C (decomp.); **IR** *v*NH=3401 cm^{-1}, 3309 cm^{-1}, *v*Phth=1716 cm^{-1}, *v*CO=1680 cm^{-1}, *v*NH=1593 cm^{-1}; **^{1}H NMR** (400 MHz, MeOD); 7.83 (2H, dd, *J* = 5.6 Hz, *J* = 2.9 Hz, ArH_{pht}), 7.77 (2H, dd, *J* = 5.6 Hz, *J* = 2.9 Hz, ArH_{pht}), 6.99 (4H, s, Ar*H*), 6.65 (4H, s, Ar*H*), 4.39 (2H, d, *J* = 13.0 Hz, ArCH_2Ar), 4.37 (2H, d, *J* = 13.0 Hz, ArCH_2Ar), 3.98 (2H, t, *J* = 7.5 Hz, C$H_2$$CH_2$), 3.85 (2H, t, *J* = 7.5 Hz, C$H_2$$CH_2$), 3.75 (10 H, m, C$H_2$$CH_2$, CH_2CH_2CH_2, NHC(O)CH_2, coincident), 3.64 (4H, s, NHC(O)CH_2), 3.06 (2H, d, *J* = 13.0 Hz, ArCH_2Ar), 3.04 (2H, d, *J* = 13.0 Hz, ArCH_2Ar), 2.33 (2H, m, CH_2C$H_2$$CH_2$), 1.84 (6H, m, CH_2C$H_2$$CH_3$), 1.43 (9H, s, C(C$H_3$)$_3$), 1.42 (9H, s, C(C$H_3$)$_3$), 1.41 (18H, s, C(C$H_3$)$_3$), 0.96 (6H, t, *J* = 7.4 Hz, CH_2CH_2CH_3), 0.82 (3H, t, *J* = 7.4 Hz, CH_2CH_2CH_3); **^{13}C NMR** (100 MHz, MeOD); 168.8, 168.6, 168.4, 157.3, 135.7, 135.6, 134.3, 134.2, 132.1, 132.0, 123.0, 120.8, 120.5, 79.5, 77.2, 76.7, 72.7, 43.8, 43.6, 35.1, 31.0, 30.9, 29.4, 27.6, 23.3, 23.0, 9.9, 9.5; **ESI MS** 1426.35 m/z $[M]^+$.

5,11,17,23-Tetra-Boc-amino-25,26,27-tripropoxy-28-aminopropoxycalix[4]arene (12)

A solution of **10** (2.00 g, 1.66 mmol) and hydrazine hydrate (0.52 mL, 16.68 mmol) was stirred in EtOH (40 mL) at reflux for 5 h, after which time H_2O (40 mL) was added. The organic components were extracted into DCM, washed with brine and the solvent removed under reduced pressure to yield **12** as a brown glass (1.53 g, 86%). **Mp** 182-184 °C; **IR** *v*NH=3322 cm^{-1}, *v*CO=1678 cm^{-1}, *v*NH=1599 cm^{-1}, 1547 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$); 6.63 (2H, s, Ar*H*), 6.54 (2H, s, Ar*H*), 6.22 (2H, s, Ar*H*), 6.17 (2H, s, Ar*H*), 4.32 (2H, d, *J* = 13.3 Hz, ArCH_2Ar), 4.31 (2H, d, *J* = 13.3 Hz, ArCH_2Ar), 3.84 (2H, t, *J* = 7.4 Hz, OC$H_2$$CH_2$$CH_2$N), 3.74 (6H, m, OC$H_2$$CH_2$$CH_3$), 3.06 (2H, d, *J* = 13.3 Hz, ArCH_2Ar), 3.05 (2H, d, *J* = 13.3 Hz, ArCH_2Ar), 2.82 (2H, t, *J* = 7.0 Hz, OCH_2CH_2C$H_2$$NH_2$), 2.25 (2H, m, OCH_2C$H_2$$CH_2$N), 1.83 (6H, m, OCH_2C$H_2$$CH_3$), 1.47 (18H, s, C($CH_3$)$_3$), 1.45 (18H, s, C($CH_3$)$_3$), 0.92 (9H, m, OCH_2CH_2CH_3); **^{13}C NMR** (100 MHz; $CDCl_3$); 153.6, 153.2, 142.3, 135.4, 135.2, 135.1, 132.2, 120.1, 120.0, 110.0, 96.2, 80.2, 39.6, 31.3, 28.6, 23.3, 19.8, 10.6, 10.5; ***M/z* (MALDI TOF)** 1068.6 m/z $[M]^+$.

5,11,17,23-Tetra-Boc-glycine-25,26,27-tripropoxy-28-aminopropoxycalix[4]arene (13)

A solution of **11** (0.50 g, 0.35 mmol) and hydrazine hydrate (0.11 mL, 3.50 mmol) was stirred in EtOH (20 mL) at reflux for 12 h, after which time H_2O (20 mL) was added. The organic components were extracted into DCM, washed with brine and the solvent removed under reduced pressure to yield **13** as a brown glass (0.29 g, 64%). **Mp** > 140 °C (decomp.); **IR** *v*NH=3457 cm^{-1}, 3042 cm^{-1}; **^{1}H NMR** (400 MHz, MeOD); 7.21-6.61 (6H, m, Ar*H*), 5.99 (1H, s, Ar*H*), 5.81 (1H, s, Ar*H*), 4.44 (2H, d, *J* = 13.1 Hz, ArCH_2Ar), 4.43 (2H, d, *J* = 13.1 Hz, ArCH_2Ar), 3.76 (16 H, m, C$H_2$$CH_2$$CH_3$, C$H_2$$CH_2$$CH_2$$NH_2$, C(O)C$H_2$NH, coincident), 3.13 (2H, d, *J* = 13.1 Hz, ArCH_2Ar),

3.10 (2H, d, *J* = 13.1 Hz, ArC*H*$_2$Ar), 3.02 (2H, m, $CH_2CH_2CH_2NH_2$), 2.08 (2H, m, $CH_2CH_2CH_2NH_2$), 1.91 (6H, m, $CH_2CH_2CH_3$), 1.46 (36H, m, $C(CH_3)_3$), 0.99 (9H, m, $CH_2CH_2CH_3$); **ESI m/z** 1297 m/z $[M]^+$.

25,26,27,28-Tetra(pentafluorophenoloate)methoxycalix[4]arene (1,3 alternate) (16)

15 (2.33 g, 3.55 mmol) was suspended in EtOAc (35 mL) and cooled to 0 ^oC. DCC (2.93 g, 14.20 mmol) and pentafluorophenol (2.62 g, 14.20 mmol) were added and the solution stirred overnight. The solution was filtered, the filtrate concentrated under reduced pressure, the residue dissolved in EtOAc and filtered to yield **16** (3.82 g, 81%) as a white powder. **Mp** 195-197 °C; **IR** *v*C(O)O=1737 cm^{-1}, 1249 cm^{-1}, 1205 cm^{-1}, 1120 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$); 7.21 (8H, d, *J* = 7.6 Hz, Ar*H*). 6.89 (4H, t, *J* = 7.6 Hz, Ar*H*), 4.05 (8H, s, OC*H*$_2$OPFP), 3.96 (8H, s, ArC*H*$_2$Ar); **^{13}C NMR** (100 MHz, $CDCl_3$); 165.6, 155.5, 134.5, 134.4, 131.4, 131.3, 131.2, 124.7, 67.6, 37.7, 31.1; **^{19}F NMR** (376 MHz, $CDCl_3$); -153.0 (8F, m, F_{ortho}), -157.9 (4F, m, F_{para}), -162.5 (8F, m, F_{meta}); ***M/z* (MALDI-TOF)** 1343.2 m/z $[M+Na]^+$ 1360.2 m/z $[M+K]^+$.

Multi-calixarene (17)

A solution of **16** (1.24 g, 0.94 mmol), **12** (4.00 g, 3.74 mmol), Hünig's base (1.54 mL, 9.36 mmol) and a catalytic amount of DMAP was stirred overnight in DCM (70 mL). The reaction was quenched with H_2O (70 mL), washed with dilute HCl (10%, 70 mL), NaOH (10%, 70 mL), brine (70 mL), dried over anhydrous $MgSO_4$ and the solvent evaporated under reduced pressure. The product was precipitated from DCM/MeOH yield **17** as a pale pink powder (3.55 g, 78%). **Mp** > 205 °C (decomp.); **IR** *v*NH=3405 cm^{-1}, 3327 cm^{-1}, *v*CO=1703 cm^{-1}, *v*NH=1599 cm^{-1}, 1530 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$); 6.98 (8H, d, *J* = 7.4 Hz, Ar*H*), 6.76 (4H, s, Ar*H*), 6.69 (4H, t, *J* = 7.4 Hz, Ar*H*), 6.64 (4H, s, Ar*H*), 6.46 (12H, s, Ar*H*), 6.44 (4H, s, Ar*H*), 6.36 (8H, s, Ar*H*), 6.07 (4H, s, N*H*), 4.35 (8H, d, *J* = 13.2 Hz, ArC*H*$_2$Ar), 4.33 (8H, d, *J* = 13.2 Hz, ArC*H*$_2$Ar), 3.81, 3.47 (24H, 8H, m, s, OC*H*$_2$CH$_2$CH$_2$N, OC*H*$_2$CH$_2$CH$_3$, coincident, OC*H*$_2$C(O)N, ArC*H*$_2$Ar), 3.71 (16H, t, *J* = 6.9 Hz, OC*H*$_2$CH$_2$CH$_3$), 3.30 (8H, m, OCH$_2$CH$_2$C*H*$_2$N), 3.08 (8H, d, *J* = 13.2 Hz, ArC*H*$_2$Ar), 3.01 (8H, d, *J* = 13.2 Hz, ArC*H*$_2$Ar), 2.03 (8H, m, OCH$_2$C*H*$_2$CH$_2$N), 1.86 (24H, m, OCH$_2$C*H*$_2$CH$_3$), 1.65 (16H, b s, ArN*H*C(O)OC(CH$_3$)$_3$), 1.48 (72H, s, C(C*H*$_3$)$_3$), 1.46 (36H, s, C(C*H*$_3$)$_3$), 1.44 (36H, s, C(C*H*$_3$)$_3$), 0.98 (12H, t, *J* = 7.4 Hz, OCH$_2$CH$_2$C*H*$_3$), 0.92 (24H, t, *J* = 7.4 Hz, OCH$_2$CH$_2$C*H*$_3$); ***M/z* (MALDI TOF)** 4885.65 m/z $[M+Na+4H]^+$.

Multi-calixarene (1)

Multi-calixarene **17** (1.00 g, 0.21 mmol) was dissolved in DCM (20 mL), through which was bubbled $HCl_{(g)}$ until the solution turned pink and a precipitate formed, after which time it was left to react for a further 10 mins to ensure the reaction had gone to completion. The solvent was removed

under reduced pressure to yield **1** as a pale pink powder (0.75 g, 95%). **Mp** > 263 °C (decomp.); **IR** *v*NH=3358 cm^{-1}, *v*C(O)=1626 cm^{-1}, *v*CN=1456 cm^{-1}, *v*NH_2=1214 cm^{-1}; ^{1}H NMR (400 MHz, D_2O); 7.10 (8H, b s, Ar*H*), 6.95 (12H, b s, Ar*H*), 6.78 (8H, b s, Ar*H*), 6.62 (16H, b s, Ar*H*), 4.42 (8H, d, *J* = 13.5 Hz, ArCH_2Ar), 4.38 (8H, d, *J* = 13.5 Hz, ArCH_2Ar), 4.12 (4H, b s, N*H*), 3.90 (24H, b s, ArCH_2Ar, OCH_2C(O)N, OC$H_2$$CH_2CH_2$N, coincident), 3.76 (24H, b s, OC$H_2$$CH_2CH_3$), 3.38 (8H, d, *J* = 13.5 Hz, ArCH_2Ar), 3.35 (8H, d, *J* = 13.5 Hz, ArCH_2Ar), 3.26 (8H, b s, OCH_2CH_2CH_2N), 2.07 (8H, m, OCH_2C$H_2$$CH_2$N), 1.90 (24H, m, OCH_2C$H_2$$CH_3$), 0.93 (36H, m, OCH_2CH_2CH_3); ***M/z* (MALDI TOF)** 3279.2 m/z $[M+Na]^+$.

Multi-calixarene (18)

A solution of **13** (0.2 g, 0.15 mmol), **16** (0.05 g, 0.04 mmol), Hünig's base (0.06 mL, 0.36 mmol) and a catalytic amount of DMAP were stirred together in DCM (4 mL) over 48 h. H_2O (5 mL) and DCM (5 mL) were added, and the organic phase separated, and washed with H_2O (2 x 7 mL). The solvent was removed under reduced pressure, and the residue purified by column chromatography over silica gel, eluting with DCM:MeOH 10:1. This yielded **18** (0.08 g, 39%) as a pale orange solid. **Mp** > 250 °C (decomp.); **IR** *v*NH=3400 cm^{-1}, 3302 cm^{-1}, *v*C(O)NH=1670 cm^{-1}, 1590 cm^{-1}, *v*C(O)O=1298 cm^{-1}, 1157 cm^{-1}; **^{1}H NMR** (400 MHz, MeOD); 7.02 (16 H, m, Ar*H*), 6.86 (20 H, m, Ar*H*), 6.74 (6H, m, Ar*H*), 6.51 (2H, b s, Ar*H*), 5.89 (m, N*H*), 4.43 (8H, d, *J* = 13.6 Hz, ArCH_2Ar), 4.37 (8H, d, *J* = 13.0 Hz, ArCH_2Ar), 4.00-3.58 (88H, m, ArCH_2Ar, C$H_2CH_2CH_3$, C$H_2CH_2CH_2$NH, CH_{2gly}, OCH_2C(O)NH, CH_2CH_2CH_2NH, coincident), 3.10 (8H, d, *J* = 13.6 Hz, ArCH_2Ar), 3.00 (8H, d, *J* = 13.0 Hz, ArCH_2Ar), 2.05 (8H, m, CH_2C$H_2$$CH_2$NH), 1.94 (24H, m, CH_2C$H_2$$CH_3$), 1.45 (144H, s, C(C$H_3$)$_3$), 1.02 (36H, m, CH_2CH_2CH_3); ***M/z* (MALDI TOF)** 5801.4 m/z $[M+Cl]^+$, 5823.8 m/z $[M+Cl+Na]^+$.

Multi-calixarene 2

16 (0.25 g, 0.04 mmol) was dissolved in DCM (10 mL), through which was bubbled $HCl_{(g)}$ until a precipitate formed and the solution turned pink, after which time it was left to react for a further 10 mins to ensure the reaction had gone to completion. The solvent was removed under reduced pressure to yield the title compound **2** as a pale pink powder (quantitative, quaternary chloride salt). **Mp** > 230 °C (decomp.); **IR** *v*NH=3411 cm^{-1}, 3216 cm^{-1}, *v*CO=1680 cm^{-1}, *v*NH=1598 cm^{-1}, 1547 cm^{-1}, **^{1}H NMR** (400 MHz, $(CD_3)_2SO$); 10.34 (8H, b s, ArN*H*C(O)CH_2), 10.06 (4H, b s, ArN*H*C(O)CH_2), 10.02 (4H, b s, ArN*H*C(O)CH_2), 8.27 (32H, m, ArNHC(O)CH_2NH_2), 7.04 (24H, m, Ar*H*), 6.69 (12H, m, Ar*H*), 6.59 (8H, m, Ar*H*), 4.37 (8H, d, *J* = 12.3 Hz, ArCH_2Ar), 4.34 (8H, d, *J* = 12.3 Hz, ArCH_2Ar), 3.85-3.63 (96H, m, NHC(O)C$H_2$$NH_2$, ArC$H_2Ar, OCH_2$C(O)N, OC$H_2$$CH_2$-$CH_2$N, N*H*C(O)$CH_2NH_2$, OC$H_2$$CH_2CH_3$, coincident), 3.17 (8H, b s, OCH_2CH_2CH_2N), 3.08 (16H,

d, *J* = 12.6 Hz, ArC*H*$_2$Ar, coincident), 1.99 (8H, m, OCH$_2$C*H*$_2$CH$_2$N), 1.85 (24H, m, OCH$_2$C*H*$_2$CH$_3$), 0.98 (12H, t, *J* = 7.1 Hz, OCH$_2$CH$_2$C*H*$_3$), 0.91 (24H, t, *J* = 7.1 Hz, OCH$_2$CH$_2$C*H*$_3$); ***M/z* (MALDI TOF)** 4189.3 m/z $[M+H+Na]^+$.

25,26,27,28-Tetra(pentafluorophenoloate)methoxycalix[4]arene (21)

A solution of **20** (3.00 g, 4.50 mmol) in EtOAc (50 mL) was cooled to 0 °C. DCC (3.71 g, 18.00 mmol) and pentafluorophenol (3.31 g, 18.00 mmol) were added and the solution stirred overnight. The solution was filtered, the filtrate concentrated under reduced pressure, the residue dissolved in EtOAc and filtered to yield **21** as a white powder (1.90 g, 32%). **Mp** 184-185 °C; **IR** *ν*C(O)=1808 cm^{-1}, *ν*CO=1125 cm^{-1}, 1091 cm^{-1}, 1065 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$); 6.68 (12H, m, Ar*H*), 5.05 (8H, s, OC*H*$_2$CO$_2$PFP), 4.81(4H, d, *J* = 13.7 Hz, ArC*H*$_2$Ar), 3.26(4H, d, *J* = 13.7 Hz, ArC*H*$_2$Ar). **^{13}C NMR** (100 MHz, $CDCl_3$); 166.3, 155.6, 142.5, 139.9, 134.4, 129.1, 128.9, 124, 70.2, 32.1, 31.7; **^{19}F NMR** (376 MHz, $CDCl_3$); -152 (8F, m, F_{ortho}), -157 (4F, m, F_{para}), -162 (8F, m, F_{meta}); **ESI-MS** 1358 m/z $[M+K-H]^+$.

Multi-calixarene (22)

A solution of **21** (0.10 g, 0.08 mmol), **12** (0.32 g, 0.30 mmol), Hünig's base (0.10 g, 0.76 mmol) and a catalytic amount of DMAP was stirred overnight in DCM (5 mL). The reaction was quenched with H_2O (5 mL), washed with dilute HCl (10%, 5 mL), NaOH (10%, 5 mL), brine (5 mL), dried over anhydrous $MgSO_4$ and the solvent evaporated under reduced pressure. The product was purified by column chromatography over silica gel, eluting with DCM:MeOH 40:1 to yield **22** as a cream powder (0.17 g, 46%). **Mp**; > 180 °C (decomp.); **IR** *ν*NH=3421 cm^{-1}, *ν*CO=1675 cm^{-1}, *ν*NH=1603 cm^{-1}, *ν*CO=1216 cm^{-1}, 1169 cm^{-1}; **^{1}H NMR** (400 MHz, $CDCl_3$); 6.85 (8H, m, Ar*H*), 6.51 (24H, m, Ar*H*), 6.27 (12H, m, Ar*H*), 5.97 (4H, b s, N*H*), 4.40 (4H, d, *J* = 13.2 Hz, ArC*H*$_2$Ar), 4.25 (8H, d, *J* = 13.3 Hz, ArC*H*$_2$Ar), 4.13 (8H, d, *J* = 13.3 Hz, ArC*H*$_2$Ar), 3.64 (40H, m, OC*H*$_2$C(O)N, OC*H*$_2$CH$_2$CH$_2$N, OC*H*$_2$CH$_2$CH$_3$, coincident), 3.37 (8H, m, OCH$_2$CH$_2$C*H*$_2$N), 3.14 (4H, d, *J* = 13.2 Hz, ArC*H*$_2$Ar), 3.00 (16H, d, *J* = 13.3 Hz, ArC*H*$_2$Ar, coincident), 2.74 (4H, b s, C(O)N*H*CH$_2$), 1.98 (8H, m, OCH$_2$C*H*$_2$CH$_2$N), 1.74 (24H, m, OCH$_2$C*H*$_2$CH$_3$), 1.44 (72H, s, C(C*H*$_3$)$_3$), 1.38 (72H, s, C(C*H*$_3$)$_3$), 0.91 (12H, t, *J* = 7.3 Hz, OCH$_2$CH$_2$C*H*$_3$), 0.79 (24H, t, *J* = 7.3 Hz, OCH$_2$CH$_2$C*H*$_3$); ***M/z* (MALDI TOF)** 4857 m/z $[M]^+$.

Multi-calixarene (3)

Multi-calixarene (**22**) (0.15 g, 0.03 mmol) was dissolved in DCM (5 mL), through which was bubbled $HCl_{(g)}$ until the solution turned pink and a precipitate formed, after which time it was left to react for a further 10 mins to ensure the reaction had gone to completion. The solvent was removed

under reduced pressure to yield **3** as a pale pink powder (quantitative). **Mp** > 220 °C (decomp.); **IR** *v*NH=3359 cm^{-1}, 3165 cm^{-1}, *v*C(O)NH=1650 cm^{-1}; **^{1}H NMR** (400 MHz, D_2O); 6.73 (24H, m, Ar*H*), 6.53 (20H, m, Ar*H*), 4.22 (20H, m, ArCH_2Ar), 3.63 (40H, m, OCH_2C(O)N, OC$H_2$$CH_2$$CH_2N, OCH_2$$CH_2$$CH_3$, coincident), 3.33 (8H, b s, $OCH_2CH_2CH_2N$), 3.20 (20H, m, ArCH_2Ar), 2.96 (4H, b s, C(O)N*H*CH_2), 2.02 (8H, m, $OCH_2CH_2CH_2N$), 1.68 (24H, m, $OCH_2CH_2CH_3$), 0.74 (36H, m, $OCH_2CH_2CH_3$); ***M/z* (MALDI TOF)** 3277.14 m/z $[M+Na]^+$.

Biological Methods

Graphs shown in the text are representative of the data. All experiments were reproduced at least three times.

A 1 mM solution, in distilled H_2O, of each compound evaluated was initially prepared. Subsequent dilutions to the desired concentrations were carried out using a HEPES (20 mmol), KCl (100 mmol), EDTA (0.2 mmol), DTT (0.5 mmol) and glycerol (20%) buffer, pH7.2. 1% agarose gels were prepared using agarose (Aldrich) in Tris Acetate EDTA Buffer (Fisher). Ethidium bromide (0.2 mL/100 mL) was added to the liquid agarose solution before setting. A 1Kb DNA marker and a control DNA were diluted with loading buffer and were additionally added to wells within each gel. Gel electrophoresis was carried out at 130 volts for 60 min, and visualisation was achieved using UV illumination. DNA was digested for 30 min prior to incubation with any compounds, using Eco-RI (Promega) digest at 37°C.

Initial Binding Studies

10 μL each of a 1 mmol solution of multicalixarenes **1**-**3**, and **4** in H_2O was incubated with pGEM-EYFP plasmid DNA (0.5 μg) and loading buffer (10% glycerol, 5% EDTA, 0.1% Bromophenol Blue) (5 μL) for 3 min. Each sample was placed in an individual well in a 1% agarose gel.

Concentration Studies

10 μL each of multicalixarenes **1** and **3** at concentrations of 0.8 mmol, 0.4 mmol and 0.2 mmol, in buffer, was incubated with pEGFP-N1 (0.5 μg) DNA and loading buffer (5 μL) for 3 min. Each sample was placed in an individual well in a 1% agarose gel.

10 μL each of multi-calixarene **1**, multi-calixarene **2** and calix[4]arene **5** at concentrations of 0.8 mmol, 0.4 mmol and 0.2 mmol, in buffer, were incubated with pEGFP-N1 (0.5 μg) DNA and loading buffer (5 μL) for 3 min. Each sample was placed in an individual well in a 1% agarose gel.

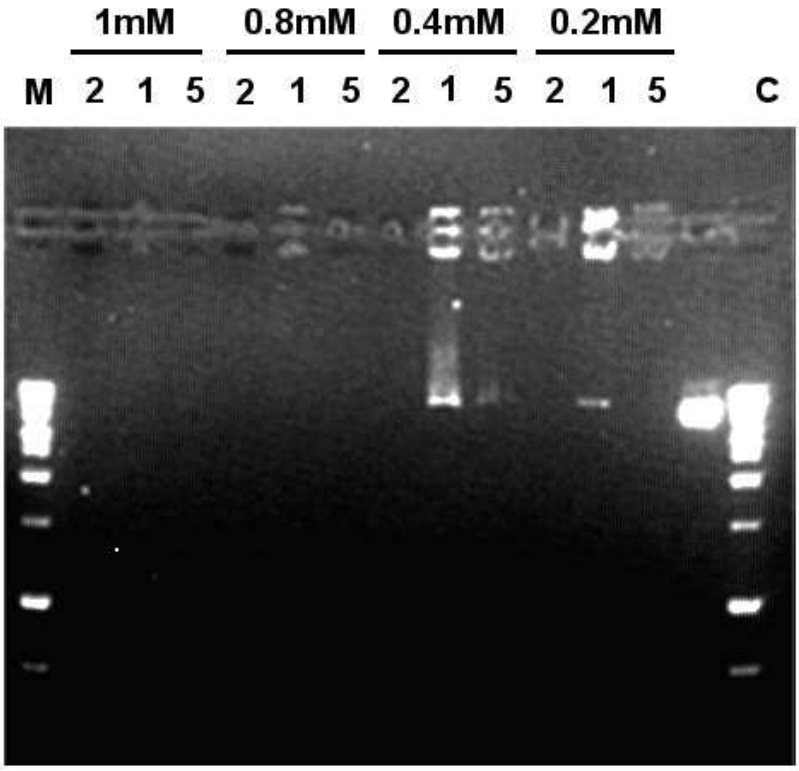

Fig. 5 Gel Electrophoresis studies were performed on a 1% agarose gel at 130V. Dilution study (concentrations shown in mM) pEGFP-N1. M is DNA ladder, C is DNA control with no added vector

Cell Viability Studies

MTS assays were performed using a CellTiter 96® AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Promega). All cell lines used have been described earlier.[6,7] All cells were incubated at 37 ˚C. Freshly harvested CHO Cells and HEK cells were suspended in DMEM (Invitrogen) with10% Foetal Calf serum and 2mM glutamine, THP-1 cells in RPMI (Invitrogen) with 10% Foetal Calf serum and 2mM glutamine, at a concentration of $1x10^5$ cells /mL. 50 μL of the cell solution was incubated with 30 μL of each of **1-5** and ß-cyclodextrin in buffer, at concentrations of 1 mmol, 100 μmol, 10 μmol, 100 nmol and 10 nmol. CHO cell viability was measured by MTS assay at 24 h, 48 h and 72 h. HEK and THP-1 cell viability was measured by MTS assay at 24 h and 48 h. Measurements were converted to percent viability by comparison to control experiments in which only PBS buffer had been added.

Transfection Studies

Transfection studies were carried out using multicalixarenes **1**-**3** and control calixarene **4**. FuGene® (Roche) was used as a commercially available comparative transfection agent. CHO.CCR5 cells were seeded and incubated in a 6-well plate 24 h prior to transfection. A 5% solution of each compound in DMEM was prepared, from a 10 mmol stock solution in buffer. An additional 3% solution of FuGENE® in DMEM was prepared. Following a 5 min solution incubation period, pDs2-mito (Clontech Laboratories, Inc.) (1 μg) was added to each solution, which was thoroughly mixed, incubated at RT for a further 15 min, added to the cell plate wells, and incubated at 37 ˚C overnight, cells were kept at physiological pH of 7.4 at all times. Visualisation of a positive transfection result was achieved using a Zeiss Axiovision 2 fluorescence microscope.

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