CHEMBIOCHEM

Supporting Information

CHEMBIOCHEM

Supporting Information

for

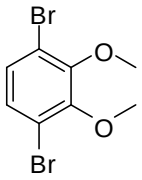
Modular sequential Palladium coupling gives easy access to the SMoC class of cellular transporters

*Anne-Sophie Rebstock, Cristina Visintin, Elisabetta Leo, Cristina Garcia Posada, Sarah R. Kingsbury, Gareth H. Williams, Kai Stoeber and David L. Selwood**

Chemistry: experimental methods

All starting materials were either commercially available or synthesised by methods reported in the literature. ^{1}H and ^{13}C spectra were recorded on a Bruker AMX-300 spectrometer. Chemical shifts are reported as ppm relative to TMS as internal standard. Mass spectra were recorded on either a VG ZAB SE spectrometer (EI, FAB) or a Gilson-Finningan AQA LC-mass spectrometer using C-18 column (Hypersil BDS 100 x 4.6 mm, 5 µm). Microanalysis was carried out by the Analytical Services Section, Department of Chemistry, University College London. Purification was by reverse-phase HPLC (Gilson) using preparative C-18 columns (Hypersil PEP 100 x 21 mm, 5µm). Melting points were determined on a Gallenkamp melting point apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer Spectrum One series FT-IR spectrophotometer. The microwave experiments were run on a Biotage Initiator 60 microwave.

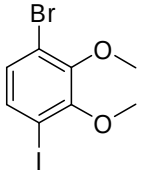
1,4-Dibromo-2,3-dimethoxy-benzene 2[1]



n-BuLi 1.6M in hexane (75 ml, 120 mmol) was added dropwise at 0 °C to a solution of veratrole 1 (3.3 g, 24 mmol) and TMEDA (22.5 ml, 150 mmol) in anhydrous ether (250 ml) under nitrogen and stirred at room temperature for 3 days. The reaction mixture was cooled to –78 °C and Br_2 (7.5mL, 147 mmol) was added, after stirring for further day at room temperature. The reaction mixture was diluted with ether (150 mL) and washed with water (150 mL), 1N HCl (150 mL×2) and brine (150 mL), dried over Na_2SO_4. The solvent was removed under vacuum and purified by silica gel flash chromatography using cyclohexane/AcOEt as eluent (5/1) to afford 1,4-dibromo-2,3-dimethoxy-benzene **2** as e colourless oil (1.15 g, 16% yield).

^{1}H-NMR ($CDCl_3$): 3.83 (s, 6H), 7.18 (s, 2H).

1, 2-Dimethoxy-3-bromo-6-iodobenzene 3



To a solution of veratrole **1** (12.7 mL, 0.1 mol) in dry ether (200 mL) was added, under nitrogen at 0 °C, TMEDA (16.4 mL, 0.12 mol) followed by BuLi (1.6M in hexane, 75 mL, 0.12 mol). The mixture was allowed to warm to room temperature and stirred for 1 h. After cooling to 0 °C, TMSCl (15.3 mL, 0.12 mol) was added. The reaction mixture was stirred at 0 °C for 30 minutes. After stirring overnight at room temperature, the reaction was cooled again to 0 °C and TMEDA (16.4 mL, 0.12 mol) and BuLi (1.6M in hexane, 75 mL, 0.12 mol) were added. After stirring for 2 h at room temperature, the reaction mixture was cooled to - 40 °C and a solution of $(CBrCl_2)_2$ (32 g, 0.1 mol) in dry ether (50 mL) was added. After stirring for 1 h at minus??30 °C, the reaction mixture was allowed to warm to 0 °C and water (100 mL) was added slowly. The organic layer was

separated and washed with water (2 x 100 mL), dried over Na_2SO_4 and filtered. The solvent was removed under vacuum and the crude product purified by flash chromatography using DCM/Et_2O (85/15) as eluent to afford the title compound (25.7 g, 89% yield) as a yellow oil.

^{1}H-NMR ($CDCl_3$): δ 0.29 (s, 9H), 3.85 (s, 3H), 3.91 (s, 3H), 6.98 (d, *J* = 7.9 Hz, 1H), 7.27 (d, *J* = 8 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ-0.63, 60.2, 60.4, 119.7, 127.9, 130.4, 149.4, 158.6.

Theoretical Mass: (M + H) 288.01811. Measured Mass: (M + H) 288.01863

To a solution of 1,2-dimethoxy-3-bromo-6-trimethylsilylbenzene (5.8 g, 20 mmol) in DCM (60 mL) was added dropwise at - 50 °C a solution of iodine chloride (3.6 g, 22 mmol) in DCM (20 mL) keeping the temperature below - 40 °C. After stirring for 2 h at - 50 °C, the reaction mixture was quenched with $Na_2S_2O_3$ 1 M (100 mL). The layers were separated and the aqueous layer was extracted with DCM (100 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated under vacuum. The residue was then crystallized from MeOH (30 mL) to afford **3** (1.82 g, 26% yield) as a white solid.

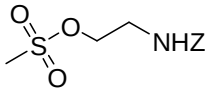
^{1}H-NMR ($CDCl_3$): δ3.86 (s, 6H), 7.02 (d, *J* = 8.7 Hz, 1H), 7.35 (d, *J* = 8.6 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ60.8, 60.8, 91.3, 118.4, 129.7, 134.4, 150.7, 154.1.

Theoretical Mass: (M + H) 342.88306. Measured Mass: (M + H) 342.88334

M.p. 30-32°C

2-(Benzyloxycarbonylamino)ethyl methanesulfonate 4



To a solution of 2-(benzyloxycarbonylamino)ethanol (25 g, 0.128 mol) and Et_3N (70 mL, 0.51 mol) in DCM (250 mL) was added methanesulfonyl chloride (25 mL, 0.32 mol) dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature and stirred overnight. The organic layer was washed with HCl 1M (2 x 50 mL), sat. aq. $NaHCO_3$ (2 x 50 mL), brine (2 x 50 mL), and dried over $MgSO_4$ and filtered. The solvent

was removed under vacuum to afford **4** (35 g, 100% yield) as an orange solid which was used in the next step without further purification.

^{1}H-NMR ($CDCl_3$): δ 2.92 (s, 3H), 3.54 (q, J = 5 Hz, 2H), 4.39 (t, J = 5 Hz, 2H), 5.08 (s, 2H), 5.84(bs, 1 H), 7.31(m, 5H).

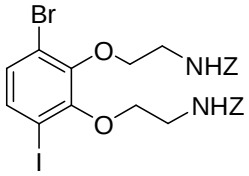
^{13}C-NMR ($CDCl_3$): δ 37.2, 40.3, 66.9, 68.6, 128.1, 128.6, 136.3, 156.5.

Theoretical Mass: (M + H) 274.07491. Measured Mass: (M + H) 274.07510

Microanalysis: %C 47.86 (48.34), %H 5.66 (5.66), %N 5.08 (5.12)

M.p. 44-46°C

Dibenzyl 2,2 ' -(3-bromo-6-iodo-1,2-phenylene)bis(oxy)bis(ethane-2,1-diyl)dicarbamate



To a solution of 1, 2-dimethoxy-3-bromo-6-iodophenyl **3** (5.7 g, 16.5 mmol) in DCM (50 mL) was added a 1.0 M DCM solution of BBr_3 (16.5 mL, 16.5 mmol) dropwise at 0 °C. The reaction mixture was allowed to warm to room temperature. After stirring overnight, the reaction mixture was cooled to 0 °C and MeOH (2 mL) was added dropwise. After stirring for 10 minutes at room temperature, the organic layer was washed with 1N HCl (2 × 20 mL), water (20 mL) and brine (20 mL), dried over $MgSO_4$, filtered and concentrated under vacuum. The resulting 3-bromo-6-iodocatechol was then dissolved in DMF (70 mL) and Na_2CO_3 (5.25 g, 49.5 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **4** (13.5 g, 49.5 mmol) were added. After stirring at 80°C under nitrogen for 24 h, the reaction mixture was poured onto water (200 mL) and extracted with EtOAc (2 x 100 mL). The organic layer was washed with water (100 mL), dried over Na_2SO_4 and filtered. The solvent was then removed under vacuum and the crude compound was purified by flash chromatography using cyclohexane/EtOAc (8/2) as eluent to afford the product (6.5 g, 59% yield) as a white solid.

ESMS; *m/z* 669, 671 (M+1), 686,688 (M+18)

^{1}H-NMR ($CDCl_3$): δ 3.53 (q, J = 5 Hz, 4H), 4.05 (m, 4H), 5.12 (s, 4H), 5.63 (bs, 1H), 5.65 (bs, 1H), 7.03 (d, J = 8.5 Hz, 1H), 7.34 (m, 11H).

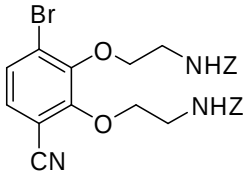
^{13}C-NMR ($CDCl_3$): δ 41.1, 41.2, 66.8, 72.8, 72.9, 91.4, 118.5, 128.1, 128.5, 130.2, 134.9, 136.6, 149.4, 152.8, 156.5.

Theoretical Mass: (M + Na) 690.99166. Measured Mass: (M + Na) 690.98946

Microanalysis: %C 47.01 (46.66), %H 4.01 (3.92), %N 4.13 (4.19)

M.p. 79°C

Dibenzyl 2,2'-(3-bromo-6-cyano-1,2-phenylene)bis(oxy)bis(ethane-2,1-diyl)dicarbamate 5



A mixture of dibenzyl 2,2'-(3-bromo-6-iodo-1,2-phenylene)bis(oxy)bis(ethane-2,1-diyl)dicarbamate (1.34 g, 2 mmol), zinc cyanide (0.12 g, 1 mmol), Pd_2dba_3 (37 mg, 0.04 mmol), dppf (44 mg, 0.08 mmol), zinc acetate (75 mg, 0.4 mmol) and zinc (26 mg, 0.4 mmol) in dimethylacetamide (10 mL) was heated at 140°C for 10 minutes under microwave irradiation. The reaction mixture was diluted in EtOAc (25 mL) and water (100 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (7/3) as eluent to afford **5** (0.69 g, 61% yield) as a white solid.

ESMS; *m/z* 568-570 (M+1), 585-587 (M+18).

^{1}H-NMR ($CDCl_3$): δ3.54 (bs, 4H), 4.08 (bs, 2H), 4.25 (bs, 2H), 5.10 (s, 2H), 5.11 (s, 2H), 5.52 (bs, 2H), 7.21-7.38 (m, 12H).

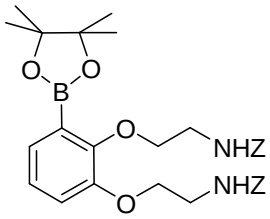
^{13}C-NMR ($CDCl_3$): δ 41.1, 66.9, 73.0, 74.3, 107.4, 115.4, 124.5, 128.1, 128.2, 128.5, 128.6, 128.8, 129.2, 136.4, 150.0, 155.0, 156.4.

Theoretical Mass: (M + Na) 590.09026. Measured Mass: (M + Na) 590.09091

IR ν_{Max} KBR/cm^{-} 3322 (br NH, NH_2), 2231 (CN), 1694 (C=ON)

M.p. 91-92°C

Dibenzyl 2,2'-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-phenylene)bis(oxy)bis(ethane-2,1-diyl)dicarbamate <u>6</u>



$Pd(dppf)Cl_2$ (1.5 g, 1.9 mmol), potassium acetate (5.4 g, 56 mmol) and bispinacolatodiboran (10 g, 41 mmol) were weighted in a three necked round bottom flask and DMSO (100 ml) was added. After stirring for 2 minutes, the bromo derivative **<u>13</u>** (20 g, 37 mmol) was added. The reaction mixture was heated to 80°C for 72 h. After cooling the mixture at room temperature, 1 L of water and 0.5 L of toluene were added. Toluene was separated and washed with brine, dried over $MgSO_4$, filtered and evaporated to dryness. The resulting oil was purified by silica chromatography using DCM to DCM/EtOAc (9/1) as eluent yielding **<u>6</u>** (6.4g, 30%) as a white solid.

ESMS; *m/z* 591(M+1)

^{1}H-NMR ($CDCl_3$): δ1.30 (s 12H), 3.54 (t, J = 4.6 Hz, 4H), 4.04 (m, 2H), 4.14 (t, J = 4.4 Hz, 2H), 5.11 (s, 4H), 5.12 (bs, 1H), 5.25 (bs, 1H), 6.49 (bs, 1H), 6.98 (m, 2H), 7.33 (bs, 10H).

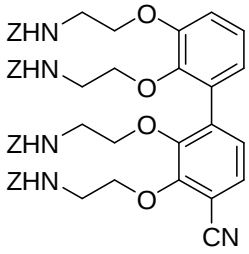
^{13}C-NMR ($CDCl_3$): δ24.6, 40.7, 41.5, 66.7, 66.8, 72.7, 83.9, 117.8, 124.2, 128.0, 128.1, 128.4, 128.5, 129.2, 136.5, 136.7, 151.1, 153.1, 156.5, 156.6

Theoretical Mass: (M + Na) 613.26970. Measured Mass: (M + Na) 613.26890

Microanalysis: %C 65.12 (65.09), %H 6.76 (6.66), %N 4.74 (4.74)

M.p. 59°C

Tetrabenzyl 2,2',2'',2'''-(4-cyanobiphenyl-2,2',3,3'-tetrayltetrakis(oxy))tetrakis(ethane-2,1-diyl)tetracarbamate 7



A degassed mixture of dibenzyl 2,2'-(3-bromo-6-cyano-1,2-phenylene) bis(oxy)bis(ethane-2,1-diyl)dicarbamate **5** (0.3 g, 0.5 mmol), dibenzyl 2,2'-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-phenylene)bis(oxy)bis(ethane-2,1-diyl)dicarbamate **6** (0.35 g, 0.6 mmol), $PdCl_2dppf.CH_2Cl_2$ (20 mg, 0.025 mmol), potassium phosphate (0.21 g, 1 mmol), toluene (1 mL) and water (0.1 mL) was heated at 100°C for 18 h. The reaction mixture was diluted in EtOAc (25 mL) and water (25 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (1/1) as eluent to afford **7** (0.48 g, 100% yield) as a yellow oil.

ESMS; *m/z* 952 (M+1), 969 (M+18)

^{1}H-NMR ($CDCl_3$): δ3.20 (m, 4H), 3.50 (m, 4H), 3.80 (m, 4H), 3.93 (m, 2H), 4.26 (m, 2H), 4.85 (bs, 1H), 4.98 (s, 2H), 5.01 (s, 2H), 5.08 (s, 4H), 5.31(bs, 1H), 5.56 (bs, 1H), 5.68 (bs, 1H), 6.86 (m, 2H), 7.05 (m, 3H), 7.30 (m, 20H).

^{13}C-NMR ($CDCl_3$): δ 40.6, 41.1, 66.8, 68.0, 70.9, 72.7, 73.9, 75.0, 112.0, 114.1, 116.0, 122.6, 124.5, 127.0, 128.0, 128.2, 128.5, 131.1, 136.4, 136.5, 139.3, 145.8, 149.5, 151.6, 154.2, 156.2, 156.5.

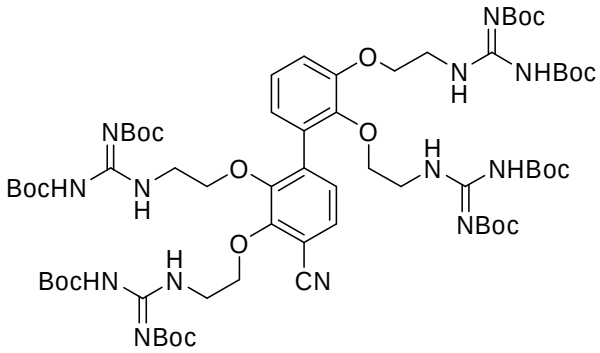
Theoretical Mass: (M + Na) 974.35882. Measured Mass: (M + Na) 974.360673

Microanalysis ($C_{53}H_{53}N_5O_{12}.2H_2O$): %C 64.92 (64.63), %H 5.51 (5.81), %N 6.81(7.09)

IR ν_{Max} KBR/cm$^-$ 3418 (br NH), 3335 (br NH), 2230 (CN), 1713 (C=O)

Mp 72-74°C

2, 3, 2', 3'-Tetra{2-[*N, N*'-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-4-cyanobiphenyl 9



Benzyl 2,2',2",2'"-(4-cyanobiphenyl-2,2',3,3'-tetrayl)tetrakis(oxy)tetrakis(ethane-2,1-diyl)tetracarbamate **7** (2 g, 2.1 mmol) was dissolved in DCM (100 mL) and HBr (30% in acetic acid, 20 mL) was added dropwise. After stirring at room temperature for 1.5 h, water (25 mL) was added to the mixture, the layers were separated and the aqueous layer was washed with DCM (2 x 25 mL). The water was then removed under vacuum and the crude tetra-amine was carefully dried under vacuum for several hours.

The resulting solid was suspended in DCM (10 mL) and Et_3N (2.9 mL, 21 mmol) and N, *N*-di-boc-*N*'-trifluoromethanesulfonyl-guanidine **8** (3.3 g, 8.4 mmol) were added. The mixture was stirred overnight at room temperature, diluted with DCM (40 mL) then washed with 2M $NaHSO_4$ 2M (25 mL) followed by sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography using cyclohexane/EtOAc (8/2) as eluent to afford the title compound **9** (1.87 g, 65% yield) as a white solid.

ESMS; *m/z* 1385 (M+1), 693 (M+2), 462 (M+3)

^{1}H-NMR ($CDCl_3$): δ1.47 (m, 72H), 3.47 (m, 2H), 3.54 (m, 2H), 3.90 (m, 8H), 4.16 (t, *J* = 5 Hz, 2H), 4.38 (t, *J* = 5 Hz, 2H), 7.00 (m, 3H), 7.18 (d, *J* = 8 Hz, 1H), 7.29 (d, *J* = 8 Hz, 1H), 8.45 (m, 2H), 8.79 (m, 2H), 11.40 (s, 1H), 11.42 (s, 1H), 11.48 (bs, 2H).

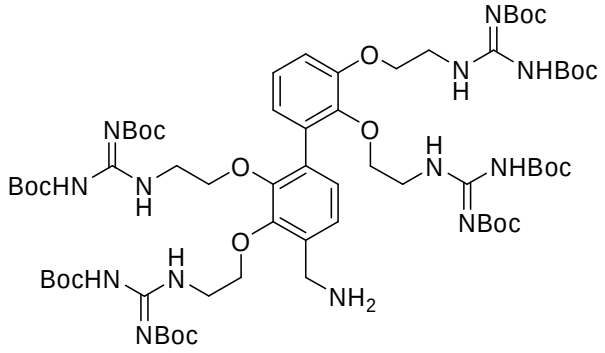
^{13}C-NMR ($CDCl_3$): δ 26.8, 28.0, 28.0, 28.2, 39.9, 40.6, 40.8, 41.3, 73.2, 78.8, 78.9, 79.0, 79.2, 82.9, 82.9, 83.1, 107.1, 113.7, 115.7, 123.2, 124.0, 127.6, 127.7, 130.8, 138.4, 144.8, 149.6, 151.6, 152.8, 152.9, 153.0, 153.0, 154.2, 156.0, 156.3, 163.3, 163.4.

Theoretical Mass: (M + Na) 1406.71832. Measured Mass: (M + Na) 1406.71565

IR ν_{Max} KBR/cm$^-$ 3335 (br, NH), 2231 (CN), 1722 (C=O), 1641 (C=N)

Mp 101-103°C

2, 3, 2', 3'-Tetra{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-4-(aminomethyl)biphenyl



Tert-butyl (2,2',2",2"'-(4-cyanobiphenyl-2,2',3,3'-tetrayl)tetrakis(oxy)tetrakis (ethane-2,1-diyl))tetrakis(azanediyl)tetrakis((tert-butoxycarbonylamino)methan-1-yl-1-ylidene)tetracarbamateylidene)tetracarbamate **9** (0.47 g, 0.34 mmol) was dissolved in THF (7 mL) and NH_4OH (1.4 mL) was added followed by Raney Nickel (0.5 g). The mixture was stirred vigorously under H_2 atmosphere for 16 h. The catalyst was filtered off and washed several times with MeOH. Diethylenetriamine (1eq) was added and the mixture stirred for 30 minutes then concentrated under vacuum. The residue was dissolved in EtOAc (10 mL) and washed with sat. aq. $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography using DCM/MeOH (97/3) as eluent to afford (0.210g, 44% yield) as a white solid.

ESMS; *m/z* 1388 (M+1), 695 (M+2), 464 (M+3)

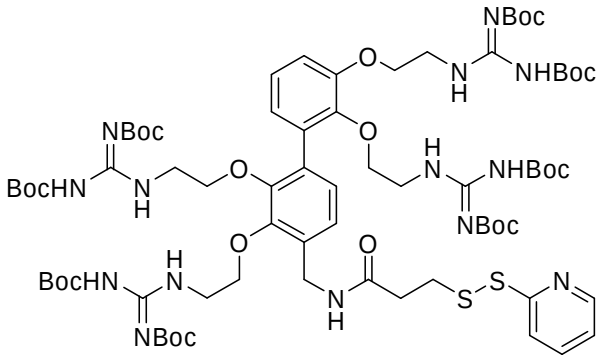
^{1}H-NMR ($CDCl_3$): δ1.41 (m, 72H), 3.44 (m, 4H), 3.78 (m, 10H), 4.13 (m, 4H), 6.93 (m, 5H), 8.43 (bt, 1H), 8.49 (bt, 1H), 8.78 (m, 2H), 11.38 (m, 2H), 11.44 (bs, 1H), 11.48 (bs, 1H).

^{13}C-NMR ($CDCl_3$): δ 28.1, 28.3, 40.0, 40.9, 41.2, 41.4, 41.7, 67.2, 70.6, 71.0, 71.4, 78.8, 78.9, 79.0, 79.1, 82.6, 82.8, 83.0, 112.8, 123.3, 123.8, 126.8, 131.4, 132.5, 136.9, 145.2, 149.2, 149.4, 151.7, 152.8, 152.9, 153.0, 156.0, 156.2, 156.3, 163.4, 163.5.

IR ν_{Max} KBR/cm$^-$ 3335 (br NH, NH_2), 1722 (C=O), 1641 (C=N)

Mp 105-107°C

***N*-{2, 3, 2', 3'-Tetra{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-4-ylmethyl}-3-[2-pyridyl)dithio]propionamide**



To a solution of 2, 3, 2', 3'-tetra{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-4-(aminomethyl)biphenyl (0.10 g, 0.072 mmol) and *i*-Pr_2EtN (20 µL, 0.10 mmol) in DCM (1.5 mL) was added N-succinimidyl-3-(2-pyridyldithio)propionate **<u>10</u>** (22 mg, 1 eq) and the mixture was stirred in the dark for 24 h. The solvent was removed under vacuum and the crude oil was purified by preparative TLC (ethyl acetate/hexane 1/1) to afford the title product (13 mg, 11% yield).

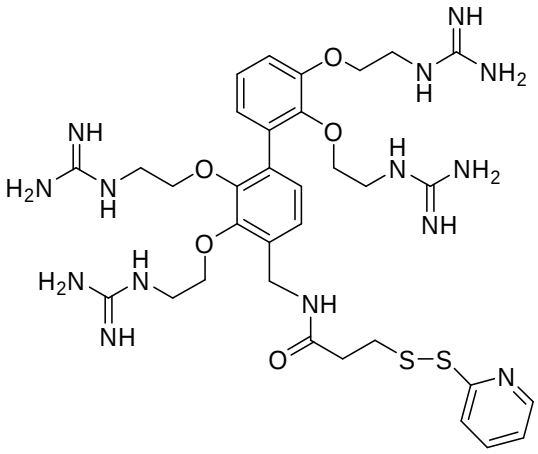
ESMS; *m/z* 1587 (M+1), 794 (M+2), 530 (M+3)

^{1}H-NMR ($CDCl_3$): δ1.43-1.51 (m, 72H), 2.70 (t, *J* = 7.2 Hz, 2H), 3.11 (t, *J* = 7.2 Hz, 2H), 3.45-3.50 (m, 4H), 3.84-3.93 (m, 8H), 4.15-4.21 (m, 4H), 4.46 (d, *J* = 5.6 Hz, 2H), 6.89-6.92 (m, 2H), 6.99-7.08 (m, 4H), 7.57-7.68 (m, 2H), 8.36 (m, 1H), 8.46 (br t, 1H), 8.55 (br t, 1H), 8.80 (br t, 2H), 11.35 (bs, 1H), 11.41 (bs, 1H), 11.47 (bs, 1H), 11.53 (bs, 1H).

IR ν_{Max} KBR/cm^- 3336 (NH), 1722 (C=O), 1641 (C=N)

Theoretical Mass: (M + H) 1585.76459. Measured Mass: (M + H) 1585.05043

***N*-[2, 3, 2', 3'-Tetra(2-guanidino-ethyloxy)-biphenyl-4-ylmethyl]-3-[2-pyridyl)dithio]propionamide, trifluoroacetate salt <u>11</u>**



N'-Bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-4-ylmethyl}-3-[2-pyridyl)dithio]propionamide (15 mg, 0.0094 mmol) was dissolved in a mixture of TFA/ H_2O/triisopropylsilane 95/2.5/2.5 (1 mL) and stirred at room temperature for 3 h. The solvent was removed under vacuum to give **<u>11</u>** as colourless oil (15 mg, 100%yield).

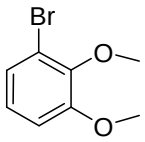
ESMS; *m/z* 785(M+1), 395 (M+2)

^{1}H-NMR (MeOH-d4): δ 2.73 (t, *J* = 6.6 Hz, 2H), 3.10 (t, *J* = 6.6 Hz, 2H), 3.23-3.30 (m, 4H), 3.62-3.67 (m, 4H), 3.88 (m, 2H), 3.96 (m, 2H), 4.22 (m, 4H), 4.47 (s, 2H), 6.87 (dd, *J* = 7.4, 1.7 Hz, 1H), 7.02 (d, *J* = 7.9 Hz, 1H), 7.08-7.24 (m, 4H), 7.76-7.80 (m, 2H), 8.38 (d, *J* = 4.7 Hz, 1H), 8.70 (br t, 1H).

IR ν_{Max} KBR/cm$^-$ 3376 (br NH, NH_2), 1681 (C=O)

Theoretical Mass: 748.33734. Measured Mass: 748.33682

1-Bromo-2,3-dimethoxy-benzene <u>12</u>[2]



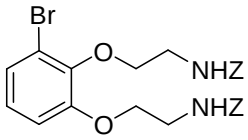
n-BuLi 1.6M in hexane (70 mL, 112 mmol) was added dropwise at 0 °C to a solution of veratrole (10 g, 72.3 mmol) and TMEDA (10.9 mL, 72.3 mmol) in anhydrous ether (50 mL) under nitrogen and stirred at room temperature for 2 h. The reaction mixture was cooled to –78 °C and $(CBrCl_2)_2$ (31.2 g, 112 mmol) was added, after stirring for a further

10 min, the cooling bath was removed and the reaction vessel allowed to warm to room temperature. The reaction mixture was diluted with ether (50 mL), washed with water (50 mL), 1N HCl (2 x 50 mL), brine (50 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and the crude product was purified by silica gel flash chromatography using hexane/DCM as eluent (5/1) to afford 1-bromo-2,3-dimethoxy-benzene **12** as a colourless oil (12.1 g, 77% yield).

^{1}H-NMR ($CDCl_3$): 3.81 (s, 3H), 3.83 (s, 3H), 6.85 (d, *J* = 8.3 Hz, 1H), 6.61-6.93 (m, 1H), 6.96 (d, *J* = 8.3 Hz, 1H).

^{13}C-NMR ($CDCl_3$): 60.3, 60.6, 109.6, 111.7, 117.4, 132.1, 149.4, 151.5.

Dibenzyl 2,2'-(3-bromo-1,2-phenylene)bis(oxy)bis(ethane-2,1-diyl)dicarbamate 13



The 1-bromo-2,3-dimethoxybenzene **12** (2.17 g, 10 mmol) was dissolved in DCM (60 ml) and treated with 1.0 M DCM solution of BBr_3 (15 mL, 15 mmol) at 0 °C and then allowed to warm to room temperature. After stirring overnight, the reaction mixture was cooled to 0 °C, 2 mL of MeOH was added, and the solvent was removed under vacuum. The residue was dissolved in 20 mL of EtOAc and washed with 1N HCl (2 x 50 mL), water, brine and dried over Na_2SO_4. The solvent was removed under vacuum. The crude residue was then dissolved in DMF (40 mL) and Cs_2CO_3 (9.75 g, 30 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **3** (6.8 g, 25 mmol) were added. After stirring at 100 °C under nitrogen for 2 h, a further amount of Cs_2CO_3 (9.75 g, 30 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **4** (6.8 g, 25 mmol) were added. After stirring at 100 °C for 2 h, the reaction mixture was poured onto water (900 mL) and extracted with EtOAc (3 x 100 mL). The organic layers were combined, washed with water (50 mL) and dried over Na_2SO_4. The solvent was removed under vacuum and purified by flash chromatography using cyclohexane/EtOAc (8/2) as eluent to afford **13** (2.45 g, 45% yield) as a white solid.

ESMS; *m/z* 543, 545 (M+1), 560, 562 (M+18)

^{1}H-NMR ($CDCl_3$): δ3.54 (m, 4H), 4.06 (m, 4H), 5.09 (s, 4H), 5.62(bs, 1H), 5.75(bs, 1H), 6.82 (d, *J* = 8 Hz, 1H), 6.91 (t, *J* = 8 Hz, 1H), 7.13 (dd, *J* = 8 Hz, *J* = 1.3 Hz, 1H), 7.32 (m, 10H).

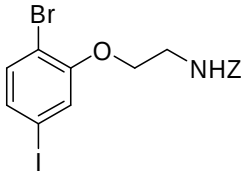
^{13}C-NMR ($CDCl_3$): δ40.5, 41.3, 66.8, 68.2, 71.8, 112.9, 118.0, 125.4, 128.1, 128.2, 128.5, 136.4, 152.6, 156.5.

Theoretical Mass: (M + Na) 565.09501. Measured Mass: (M + Na) 565.09508

Microanalysis: %C 57.58 (57.47), %H 5.10 (5.01), %N 5.16 (5.04)

M.p. 54°C

Benzyl 2-(2-bromo-5-iodophenoxy)ethylcarbamate <u>15</u>



To a solution of 2-bromo-5-iodoanisole **<u>14</u>** (12.5 g, 40 mmol) in DCM (200 mL) was added a 1.0 M DCM solution of BBr_3 (40 mL) dropwise at 0 °C. The reaction mixture was then allowed to warm to room temperature. After stirring overnight, the reaction mixture was cooled to 0 °C and MeOH (5 mL) was added dropwise. After stirring for 10 minutes at room temperature, the reaction mixture was washed with 1N HCl (2 x 50 mL), water (50 mL) and brine (50 mL). The organic layer was dried over $MgSO_4$. filtered and concentrated under vacuum. The residue was then dissolved in DMF (100 mL) and Cs_2CO_3 (26 g, 80 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **<u>3</u>** (11 g, 40 mmol) were added. After stirring at 80°C under nitrogen for 4 h, the reaction mixture was dissolved in EtOAc (100 mL). The organic layer was washed with brine (3 x 50 mL), dried over $MgSO_4$ and filtered. The solvent was removed under vacuum and the crude compound was purified by flash chromatography using cyclohexane/EtOAc (85/15) as eluent to afford **<u>15</u>** (11.5 g, 60% yield) as a white solid.

ESMS; *m/z* 476, 478 (M+1), 493, 495 (M+18)

^{1}H-NMR ($CDCl_3$): δ 3.64 (q, *J* = 5.2 Hz, 2H), 4.05 (t, *J* = 4.7 Hz, 2H), 5.12 (s, 2H), 5.33 (bs, 1H), 7.15-7.24 (m, 3H), 7.35 (bs, 5H).

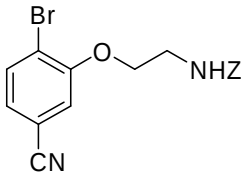
^{13}C-NMR ($CDCl_3$): δ 40.4, 67.0, 68.7, 92.4, 112.4, 122.7, 128.2, 128.2, 128.6, 131.5, 134.6, 136.4, 155.4, 156.4.

Theoretical Mass: (M + Na) 497.91777. Measured Mass: (M + Na) 497.91638

Microanalysis: %C 40.41 (40.36), %H 3.24 (3.18), %N 2.86 (2.94)

M.p. 70°C

Benzyl 2-(2-bromo-5-cyanophenoxy)ethylcarbamate <u>16</u>



A mixture of benzyl 2-(2-bromo-5-iodophenoxy)ethylcarbamate **<u>15</u>** (1.9 g, 4 mmol), zinc cyanide (0.23 g, 2 mmol), Pd_2dba_3 (73 mg, 0.08 mmol), dppf (88 mg, 0.16 mmol), zinc acetate (0.14 g, 0.8 mmol) and zinc (52 mg, 0.8 mmol) in dimethylacetamide (20 mL) was heated at 140 °C for 10 minutes under microwave irradiation. The reaction mixture was diluted in EtOAc (25 mL) and water (100 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (7/3) as eluent to afford **<u>16</u>** (1.13 g, 75% yield) as a white solid.

ESMS; *m/z* 375, 377 (M+1), 392, 394 (M+18)

^{1}H-NMR ($CDCl_3$): δ 3.69 (q, *J* = 5.2 Hz, 2H), 4.11 (t, *J* = 4.7 Hz, 2H), 5.12 (s, 2H), 5.26 (bs, 1H), 7.08 (s, 1H), 7.15 (dd, *J* = 8.1 Hz, *J* = 1.7 Hz, 1H), 7.35 (bs, 5H), 7.64 (d, *J* = 8.1 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ 40.3, 67.0, 68.7, 112.3, 115.8, 118.0, 118.4, 125.9, 128.2, 128.3, 128.6, 134.4, 136.3, 155.3, 156.4.

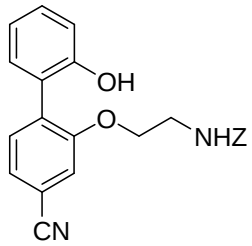
Microanalysis: %C 54.48 (54.42), %H 4.09 (4.03), %N 7.35 (7.47)

Theoretical Mass: (M + Na) 397.01637. Measured Mass: (M + Na) 397.01504

IR ν_{Max} KBR/cm^{-}3322 (br NH, NH_2), 2231 (CN), 1694 (C=ON)

M.p. 86-87°C

Benzyl 2-(4-cyano-2'-hydroxybiphenyl-2-yloxy)ethylcarbamate



A degassed mixture of benzyl 2-(2-bromo-5-cyanophenoxy)ethylcarbamate **16** (0.7 g, 1.8 mmol), 2-hydroxyphenylboronic acid (0.3 g, 2.2 mmol), $PdCl_2$dppf.CH_2Cl_2 (73 mg, 0.09 mmol), potassium phosphate (0.83 g, 3.6 mmol), toluene (4 mL) and water (0.4 mL) was heated at 100°C for 18 h. The reaction mixture was diluted in EtOAc (25 mL) and water (25 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography using cyclohexane/EtOAc (7/3) as eluent to afford the title compound (0.34 g, 49% yield) as transparent oil.

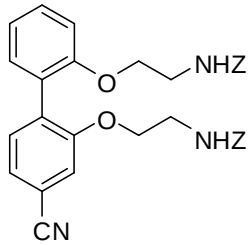
ESMS; *m/z* 389 (M+1), 406 (M+18)

^{1}H-NMR ($CDCl_3$): δ 3.47 (m, 2H), 4.05 (m, 2H), 5.06 (s, 2H), 5.30 (bs, 1H), 6.92-7.04 (m, 2H), 7.15 (m, 2H), 7.28-7.39 (m, 8H).

^{13}C-NMR ($CDCl_3$): δ 40.3, 66.9, 68.4, 112.5, 115.8, 116.9, 118.5, 121.1, 124.3, 125.8, 128.1, 128.3, 128.6, 130.1, 131.0, 132.8, 133.2, 136.3, 153.2, 155.5, 156.5.

Theoretical Mass: (M + Na) 411.13207. Measured Mass: (M + Na) 411.13099

Dibenzyl 2,2'-(4-cyanobiphenyl-2,2'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate 17



Benzyl 2-(4-cyano-2'-hydroxybiphenyl-2-yloxy)ethylcarbamate (0.21 g, 0.54 mmol) was dissolved in DMF (7 mL) and Cs_2CO_3 (0.46 g, 1.4 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **4** (0.21 g, 0.77 mmol) were added.

After stirring at 80°C under nitrogen for 2 h, the reaction mixture was dissolved in EtOAc (25 mL). The organic layer was washed with brine (3 x 20 mL) and dried over $MgSO_4$. The solvent was removed under vacuum and the crude compound was purified by flash chromatography using cyclohexane/EtOAc (65/35) as eluent to afford (0.16g, 54% yield) as colourless oil.

ESMS; *m/z* 566 (M+1), 583 (M+18)

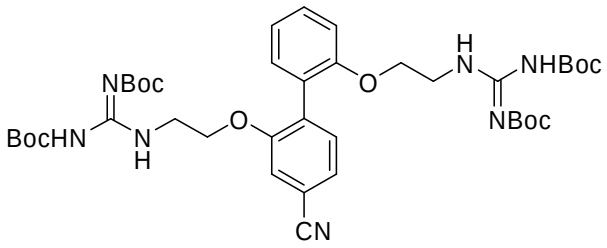
^{1}H-NMR ($CDCl_3$): δ 3.33 (m, 4H), 3.90 (m, 4H), 4.80 (bs, 1H), 4.97 (bs, 5H), 6.88 (d, *J* = 8.1 Hz, 1H), 7.04 (m, 2H), 7.14 (dd, *J* = 7.5 Hz, *J* = 1.6 Hz, 1H), 7.30 (m, 13H).

^{13}C-NMR ($CDCl_3$): δ 40.3, 66.9, 68.2, 68.3, 112.4, 113.3, 116.2, 119.9, 121.8, 125.6, 128.3, 128.6, 128.7, 129.9, 130.8, 132.1, 134.5, 136.4, 155.5, 156.0, 156.1.

Theoretical Mass: (M + H) 566.22910. Measured Mass: (M + H) 566.22799

IR ν_{Max} KBR/cm$^-$ 3347 (br, NH), 2228 (CN), 1715 (C=O)

2, 2'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-4-cyanobiphenyl



Dibenzyl 2,2'-(4-cyanobiphenyl-2,2'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate **17** (0.12 g, 0.21 mmol) was dissolved in DCM (10 mL) and HBr (30% in acetic acid, 1.2 mL) was added dropwise. After stirring at room temperature for 1.5 h, the solvent was removed under vacuum and the crude diamine was carefully dried under vacuum for several hours.

The resulting solid was suspended in DCM (5 mL) and Et_3N (0.29 mL, 2.1 mmol) and N, N-di-boc-N'-trifluoromethanesulfonyl-guanidine **8** (0.17 g, 0.42 mmol) were added. The mixture was stirred over night at room temperature, diluted with DCM (20 mL) then washed with 2M $NaHSO_4$ 2M (25 mL) followed by sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using

cyclohexane/EtOAc (8/2) as eluent to afford the title compound (0.09 g, 54% yield) as a white solid.

^{1}H-NMR ($CDCl_3$): δ 1.49 (m, 36H), 3.71 (q, *J* = 5 Hz, 4H), 4.05 (t, *J* = 4.6 Hz, 4H), 6.96 (d, *J* = 8.3 Hz, 1H), 7.03 (t, *J* = 7.1 Hz, 1H), 7.17 (d, *J* = 1.2 Hz, 1H), 7.30-7.34 (m, 3H), 7.46 (d, *J* = 7.8 Hz, 1H), 8.46 (m, 2H), 11.46 (bs, 2H).

^{13}C-NMR ($CDCl_3$): δ 28.1, 28.3, 39.9, 40.0, 66.7, 67.3, 79.4, 83.1, 83.3, 111.8, 112.0, 115.5, 118.7, 120.8, 124.9, 125.3, 129.5, 131.6, 132.9, 132.9, 153.0, 155.2, 155.8, 156.2, 163.4.

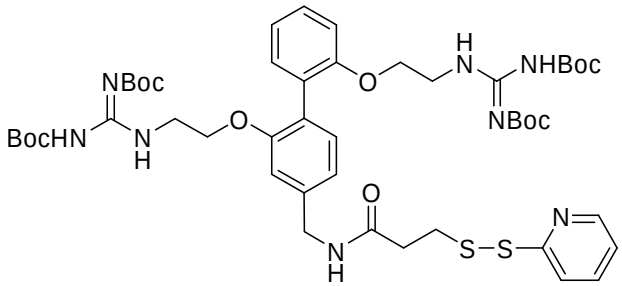
Theoretical Mass: (M + H) 782.40885. Measured Mass: (M + H) 782.40963

Microanalysis ($C_{39}H_{55}N_7O_{10}.2H_2O$): %C 56.90 (57.27), %H 7.12 (7.09), %N 11.58 (11.99)

IR ν_{Max} KBR/cm^{-} 3333 (br NH), 2228 (CN), 1721 (C=O), 1640 (C=N), 1618 (C=N)

M.p. 82°C

_N_-{2, 2'-Bis{2-[_N_, _N_'-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-4-ylmethyl}-3-[2-pyridyl)dithio]propionamide



2, 2'-Bis{2-[*N*, *N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-4-cyanobiphenyl (0.06 g, 0.077 mmol) was dissolved in THF (2 mL) and NH_4OH (0.2 mL) was added followed by Raney Nickel (0.05 g). The mixture was stirred vigorously under H_2 atmosphere for 16 h. The catalyst was filtered off and washed several times with MeOH. Diethylenetriamine (1eq) was added and the mixture stirred for 30 minutes then concentrated under vacuum. The residue was dissolved in EtOAc (10 mL) and washed with sat. aq. $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. To a solution of the crude amine and *i*-Pr_2EtN (26 µl, 0.15 mmol) in DCM (1.5 mL) was added N-succinimidyl-3-(2-pyridyldithio)propionate **10** (34

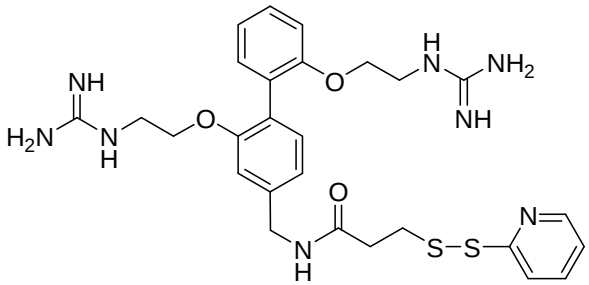
mg, 0.11 mmol) and the mixture was stirred in the dark for 24 h. The solvent was removed under vacuum and the crude oil was purified by preparative TLC (ethyl acetate/hexane 1/1) to afford the title compound (20 mg, 26% yield).

^{1}H-NMR ($CDCl_3$): δ 1.48 (m, 36H), 2.68 (t, *J* = 5.0 Hz, 2H), 3.03 (t, *J* = 5 Hz, 2H), 3.67 (m, 4H), 3.99 (m, 4H), 4.49 (d, *J* = 5.2 Hz, 2H), 6.90-7.05 (m, 4H), 7.21-7.33 (m, 3H), 7.57 (m, 2H), 8.20 (d, *J* = 4.7 Hz, 1H), 8.47 (m, 2), 11.46 (bs, 2H).

^{13}C-NMR ($CDCl_3$): δ 28.1, 28.3, 29.7, 35.2, 35.8, 40.2, 53.4, 66.9, 79.3, 82.9, 83.1, 112.3, 112.7, 120.4, 121.0, 126.9, 128.5, 132.0, 132.3, 136.9, 138.4, 149.6, 152.9, 155.6, 155.8, 156.2, 159.4, 163.5, 170.6.

Theoretical Mass: (M + Na) 1005.41899. Measured Mass: (M + Na) 1005.41622

_N_-[2, 2'-Bis(2-guanidino-ethyloxy)-biphenyl-4-ylmethyl]-3-[2-pyridyl)dithio]propionamide, formiate salt <u>18</u>

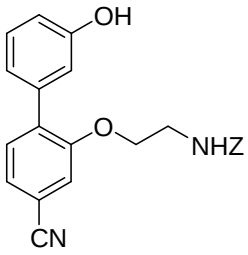


_N_-{2, 2'-Bis{2-[*N*, **N**'-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-4-ylmethyl}-3-[2-pyridyl)dithio]propionamide (15 mg, 0.015 mmol) was dissolved in formic acid (1 mL) and stirred at room temperature overnight. The mixture was then heated at 50°C for 1h. Water was added and the product was lyophilised to give **<u>18</u>** as colourless oil (13.6 mg, 100%).

^{1}H-NMR (MeOH-d4): δ 2.76 (t, *J* = 6.6 Hz, 2H), 3.11 (t, 2H, *J* = 6.3 Hz, 2H), 3.42 (m, 4H), 4.04 (m, 4H), 4.421 (s, 2H), 6.99-7.24 (m, 6H), 7.35 (t, *J* = 6.9 Hz, 1H), 7.78-7.83 (m, 2H), 8.15 (m, 1H), 8.39 (d, *J* = 4.6 Hz, 1H), 8.57 (bs, 2H).

Theoretical Mass: (M + H) 583.22734. Measured Mass: (M + H) 583.22651

Benzyl 2-(4-cyano-3'-hydroxybiphenyl-2-yloxy)ethylcarbamate



A degassed mixture of benzyl 2-(2-bromo-5-cyanophenoxy)ethylcarbamate **16** (0.7 g, 1.8 mmol), 3-hydroxyphenylboronic acid (0.3 g, 2.2 mmol), $PdCl_2dppf.CH_2Cl_2$ (73 mg, 0.09 mmol), potassium phosphate (0.83 g, 3.6 mmol), toluene (4 mL) and water (0.4 mL) was heated at 100°C for 18 h. The reaction mixture was diluted in EtOAc (25 mL) and water (25 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (7/3) as eluent to afford the title compound (0.57 g, 81% yield) as transparent oil.

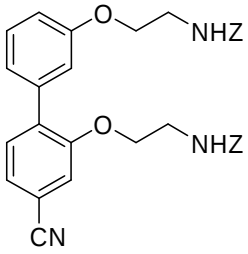
ESMS; *m/z* 389 (M+1), 406 (M+18)

^{1}H-NMR ($CDCl_3$): δ 3.63 (m, 2H), 4.01 (m, 2H), 5.10 (bs, 3H), 5.19 (bs, 1H), 6.89 (m, 2H), 6.99 (d, *J* = 7.6 Hz, 1H), 7.09-7.40 (m, 8H), 7.48 (d, *J* = 7.8 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ 40.4, 67.3, 67.9, 111.8, 115.6, 116.4, 117.0, 118.7, 121.0, 125.5, 128.2, 128.4, 128.6, 129.6, 131.3, 135.2, 136.0, 137.4, 155.5, 155.9, 156.8.

Theoretical Mass: (M + H) 389.15012. Measured Mass: (M + H) 389.15110

Dibenzyl 2,2'-(4-cyanobiphenyl-2,3'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate 19



To benzyl 2-(4-cyano-3'-hydroxybiphenyl-2-yloxy)ethylcarbamate (0.49 g, 1.26 mmol) dissolved in DMF (12 mL) was added Cs_2CO_3 (0.82 g, 2.5 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **4** (0.38 g, 1.4 mmol). After stirring at

80 °C under nitrogen for 3 h, further Cs_2CO_3 (0.41 g, 1.25 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **3** (0.2 g, 0.7 mmol) were added. After stirring for an additional 2 h, the reaction mixture was dissolved in EtOAc (25 mL). The organic layer was washed with brine (3 x 20 mL) and dried over $MgSO_4$. The solvent was removed under vacuum and the crude compound was purified by flash chromatography using cyclohexane/EtOAc (65/35) as eluent to afford the title compound **19 (**0.44g, 62% yield) as a colourless oil.

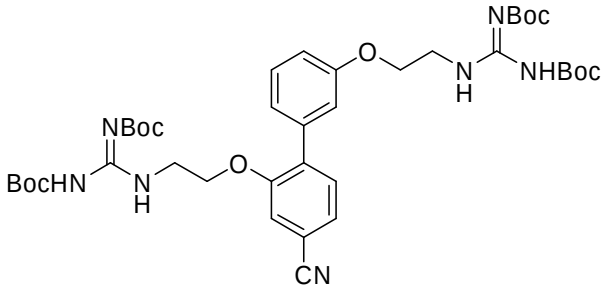
ESMS; *m/z* 566 (M+1), 583 (M+18)

^{1}H-NMR ($CDCl_3$): δ 3.51 (bs, 4H), 4.03 (bs, 4H), 5.07 (m, 5H), 5.32 (bs, 1H), 6.87 (d, *J* = 7.6 Hz, 1H), 7.04 (m, 2H), 7.21 (s, 1H), 7.32-7.38 (m, 13H).

^{13}C-NMR ($CDCl_3$): δ 40.4, 40.6, 67.0, 68.0, 112.1, 114.0, 115.9, 118.6, 122.0, 125.5, 128.2, 128.3, 128.6, 129.5, 131.5, 135.8, 136.3, 136.4, 138.0, 155.5, 156.3, 156.5, 158.4.

Theoretical Mass: (M + H) 566.22910. Measured Mass: (M + H) 566.22854

2, 3'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-4-cyanobiphenyl



Dibenzyl 2,2'-(4-cyanobiphenyl-2,3'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate **19** (0.362 g, 0.64 mmol) was dissolved in DCM (10 mL) and HBr (30% in acetic acid, 3.5 mL) was added dropwise. After stirring at room temperature for 1.5 h, the solvent was removed under vacuum and the crude diamine was carefully dried under vacuum for several hours.

The resulting solid was suspended in DCM (5 mL) and Et_3N (0.88 mL, 6.4 mmol) and N, N-di-boc-N'-trifluoromethanesulfonyl-guanidine **8** (0.47 g, 1.2 mmol) were added. The mixture was stirred overnight at room temperature, diluted with DCM (20 mL) then washed with 2M $NaHSO_4$ 2M (25 mL), sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The

residue was purified by flash chromatography using cyclohexane/EtOAc (8/2) as eluent to afford the title compound (0.33 g, 66% yield) as a white solid.

^{1}H-NMR ($CDCl_3$): δ 1.48 (m, 36H), 3.79 (q, J = 5.2 Hz, 2H), 3.84 (q, J = 5 Hz, 2H), 4.09 (m, 4H), 6.94 (dd, J = 8.1 Hz, J = 1.7 Hz, 1H), 6.99 (d, J = 1.4 Hz, 1H), 7.18-7.22 (m, 2H), 7.34 (m, 2H), 7.40 (d, J = 7.8 Hz, 1H), 8.63 (t, J = 5.1Hz, 1H), 8.75 (bt, 1H), 11.45 (bs, 2H).

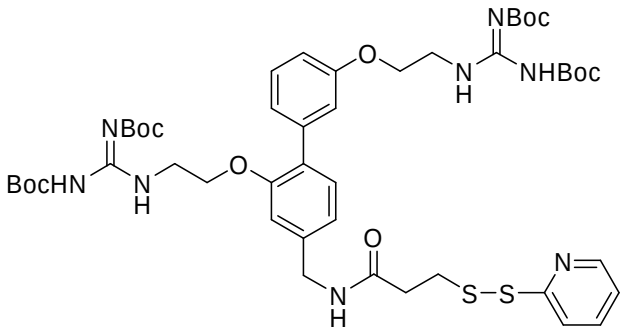
^{13}C-NMR ($CDCl_3$): δ 28.0, 28.3, 39.8, 40.2, 66.5, 67.4, 79.4, 79.5, 83.2, 83.2, 112.0, 114.2, 115.5, 115.8, 118.7, 122.7, 125.3, 129.2, 131.6, 135.8, 137.8, 153.0, 155.5, 156.3, 156.4, 158.6, 163.4, 163.5.

Theoretical Mass: (M + H) 782.40885. Measured Mass: (M + H) 782.40733

M.p. 89-91°C

IR ν_{Max} KBR/cm$^-$ 3333 (br NH), 2229 (CN), 1722 (C=O), 1641 (C=N), 1618 (C=N)

***N*-{2, 3'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-4-ylmethyl}-3-[2-pyridyl)dithio]propionamide**



2, 3'-Bis{2-[*N, N*'-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-4-cyanobiphenyl (0.50g, 0.7mmol) was dissolved in THF (14 mL) and NH_4OH (2.4 mL) was added followed by Raney Nickel (0.50 g). The mixture was stirred vigorously under H_2 atmosphere for 16 h. The catalyst was filtered off and washed several times with MeOH. Diethylenetriamine (1eq) was added and the mixture stirred for 30 minutes then concentrated under vacuum. The residue was dissolved in EtOAc (10 mL) and washed with sat. aq. $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. To a solution of the crude (0.040 g, 0.050 mmol) and *i*-Pr_2EtN (10 µL, 0.10 mmol) in DCM (0.7 mL) was added N-succinimidyl-3-(2-pyridyldithio)propionate **10** (15 mg, 0.053

mmol) and the mixture was stirred in the dark for 24 h. The solvent was removed under vacuum and the crude oil was purified by preparative TLC (ethyl acetate/hexane 1/1) to afford the title compound (8.5 mg, 16% yield).

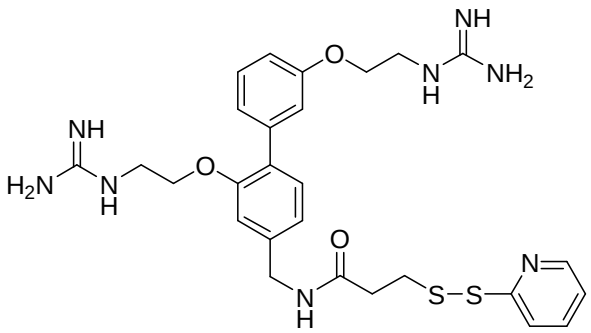
^{1}H-NMR ($CDCl_3$): δ 1.49 (m, 36H), 2.67 (t, *J* = 5 Hz, 2H), 3.13 (t, *J* = 5 Hz, 2H), 3.73 (m, 2H), 3.84 (m, 2H), 4.05 (m, 4H), 4.50 (d, *J* = 4.1 Hz, 2H), 6.98-6.94 (m, 4H), 7.21-7.730 (m, 3H), 7.55 (m, 2H), 8.19 (d, *J* = 3.5 Hz, 1H), 8.62 (t, *J* = 4 Hz, 1H), 8.76 (m, 1H), 11.46 (bs, 2H).

^{13}C-NMR ($CDCl_3$): δ 27.0, 28.0, 28.2, 28.5, 35.3, 35.8, 39.9, 40.2, 43.6, 66.3, 66.7, 79.3, 79.4, 83.1, 112.0, 113.1, 115.6, 120.6, 120.7, 121.1, 122.9, 128.8, 129.9, 131.1, 137.0, 139.0, 139.3, 149.51, 152.9, 155.47, 156.2, 156.3, 158.4, 159.1, 163.4, 163.5, 170.7, 171.9.

Theoretical Mass: (M + H) 983.43705. Measured Mass: (M + H) 983.43839

IR ν_{Max} KBR/cm^{-}3332 (NH), 1722 (C=O), 1641 (C=O).

_N_-[2, 3'-Bis(2-guanidino-ethyloxy)-biphenyl-4-ylmethyl]-3-[2-pyridyl)dithio]propionamide, formate salt <u>20</u>

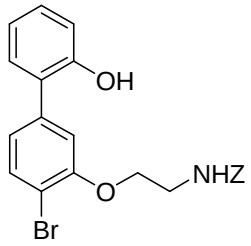


N-{2, 3'-Bis{2-[*N*, *N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-4-ylmethyl}-3-[2-pyridyl)dithio]propionamide (11 mg, 0.011 mmol) was dissolved in formic acid (1 mL) and stirred at room temperature overnight. The mixture was then heated at 50°C during 1h. Water was then added and the product was lyophilised to give **<u>20</u>** as colourless oil (10 mg, 100%).

^{1}H-NMR (MeOH-d4): δ 2.71 (t, *J* = 6.6 Hz, 2H), 3.11 (t, 2H, *J* = 6.9 Hz, 2H), 3.50 (bt, 2H), 3.60 (bt, 2H), 4.12 (m, 4H), 4.41 (s, 2H), 6.92 (d, *J* = 7.5 Hz, 1H), 6.99-7.12 (m, 4H), 7.18-7.34 (m, 3H), 7.73-7.83 (m, 2H), 8.37 (d, *J* = 3.9 Hz, 1H), 8.44 (bs, 2H).

Theoretical Mass: (M + H) 583.22734. Measured Mass: (M + H) 583.22816

Benzyl 2-(4-bromo-2'-hydroxybiphenyl-3-yloxy)ethylcarbamate



A degassed mixture of benzyl 2-(2-bromo-5-iodophenoxy)ethylcarbamate **15** (1.43 g, 3 mmol), 2-hydroxyphenylboronic acid (0.37 g, 2.7 mmol), $PdCl_2$dppf.CH_2Cl_2 (0.12 g, 0.15 mmol), potassium phosphate (1.27 g, 6 mmol), toluene (6 mL) and water (0.6 mL) was heated at 100°C for 18 h. The reaction mixture was diluted in EtOAc (25 mL) and water (25 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (75/25) as eluent to afford the title compound (0.90 g, 75% yield) as transparent oil.

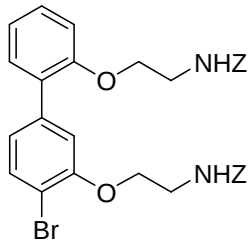
ESMS; *m/z* 442, 444 (M+1), 459, 461 (M+18)

^{1}H-NMR ($CDCl_3$): δ 3.64 (m, 2H), 4.10 (m, 2H), 5.11 (s, 2H), 5.43 (bs, 1H), 6.02 (s, 1H), 6.97-7.03 (m, 4H), 7.25 (m, 2H), 7.35 (bs, 5H), 7.59 (d, J = 8 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ 40.5, 67.0, 68.4, 111.6, 114.7, 116.2, 120.9, 123.1, 127.2, 128.2, 128.2, 128.6, 129.4, 130.2, 133.6, 136.3, 138.4, 152.6, 155.0, 156.6

Theoretical Mass: (M + Na) 464.04733. Measured Mass: (M + Na) 464.04803

Dibenzyl 2,2'-(4-bromobiphenyl-3,2'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate 21



Benzyl 2-(4-bromo-2'-hydroxybiphenyl-3-yloxy)ethylcarbamate (0.8 g, 1.8 mmol) was dissolved in DMF (10 mL) and Cs_2CO_3 (1.17 g, 3.6 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **4** (0.55 g, 2 mmol) were added. After stirring at 80 °C under nitrogen for 3.5 h, the reaction mixture was dissolved in EtOAc (25 mL). The organic layer was washed with brine (3 x 20 mL) and dried over $MgSO_4$. The solvent was removed under vacuum and the crude compound was purified by flash chromatography using cyclohexane/EtOAc (7/3) as eluent to afford the title compound **21** (0.6 g, 54% yield) as a colourless oil.

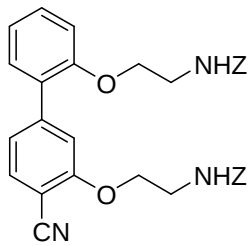
ESMS; *m/z* 619, 621 (M+1), 636, 638 (M+18)

^{1}H-NMR ($CDCl_3$): δ 3.50 (q, J = 5.1 Hz, 2H), 3.58 (m, 2H), 4.04 (t, J = 4.8 Hz, 2H), 4.08 (m, 2H), 5.08 (m, 5H), 5.39 (bs, 1H), 6.97 (m, 2H), 7.06 (m, 2H), 7.25-7.33 (m, 12H), 7.53 (d, J = 8.1 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ 40.6, 66.9, 67.7, 68.5, 111.1, 113.2, 115.2, 121.7, 123.6, 128.1, 128.5, 128.6, 129.3, 130.0, 130.7, 132.8, 136.4, 136.5, 139.2, 154.5, 155.2, 156.4, 156.5.

Theoretical Mass: (M + Na) 641.12631. Measured Mass: (M + Na) 641.12487

Dibenzyl 2,2'-(4-cyanobiphenyl-3,2'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate 22



A mixture of dibenzyl 2,2'-(4-bromobiphenyl-3,2'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate **21** (0.35 g, 0.56 mmol), zinc cyanide (0.1 g, 0.85 mmol), Pd_2dba_3 (26

mg, 0.03 mmol), dppf (39 mg, 0.07 mmol), zinc acetate (51 mg, 0.28 mmol) and zinc (18 mg, 0.28 mmol) in dimethylacetamide (4 mL) was heated at 160 °C for 10 minutes under microwave irradiation. The reaction mixture was diluted in EtOAc (25 mL) and water (100 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography using cyclohexane/EtOAc (65/35) as eluent to afford **22** (0.24 g, 76% yield) as a colourless oil.

ESMS; *m/z* 566 (M+1), 583 (M+18)

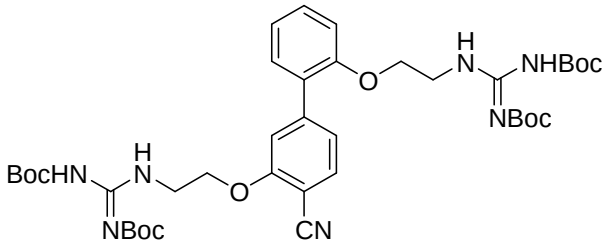
^{1}H-NMR ($CDCl_3$): δ 3.50 (q, *J* = 5.2 Hz, 2H), 3.58 (m, 2H), 4.06 (t, *J* = 4.9 Hz, 2H), 4.14 (m, 2H), 5.07 (m, 5H), 5.30 (bs, 1H), 6.97 (d, *J* = 8.2 Hz, 1H), 7.06-7.14 (m, 3H), 7.28-7.38 (m, 12H), 7.53 (d, *J* = 7.9 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ 40.3, 40.4, 66.9, 67.5, 68.1, 100.4, 113.0, 113.8, 116.4, 121.7, 122.5, 128.1, 128.2, 128.3, 128.5, 128.6, 129.2, 130.1, 130.7, 133.1, 136.3, 145.2, 155.2, 156.3, 156.4, 159.9.

Theoretical Mass: (M + H) 566.22910. Measured Mass: (M + H) 566.22854

IR ν_{Max} KBR/cm$^-$ 3336 (br NH), 2224 (CN), 1712 (C=O)

3, 2'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-4-cyanobiphenyl



Dibenzyl 2,2'-(4-cyanobiphenyl-3,2'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate **22** (0.21 g, 0.371 mmol) was dissolved in DCM (10 mL) and HBr (30% in acetic acid, 2 mL) was added dropwise. After stirring at room temperature for 1.5 h, the solvent was removed under vacuum and the crude diamine was carefully dried under vacuum for several hours.

The resulting solid was suspended in DCM (5 mL) and Et_3N (0.51 mL, 3.7 mmol) and N, N-di-boc-N'-trifluoromethanesulfonyl-guanidine **8** (0.29 g, 0.74 mmol) were added. The

mixture was stirred overnight at room temperature, diluted with DCM (20 mL) then washed with 2M $NaHSO_4$ 2M (25 mL) follow by sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (8/2) as eluent to afford the title compound (0.17 g, 59% yield) as a white solid.

^{1}H-NMR ($CDCl_3$): δ 1.48 (m, 36H), 3.78 (q, *J* = 4.9Hz, 2H), 3.90 (q, *J* = 5.1Hz, 2H), 4.08(t, *J* = 4.8Hz, 2H), 4.23 (t, *J* = 5Hz, 2H), 6.98 (d, *J* = 8.2Hz, 1H), 7.05 (m, 2H), 7.26-7.37 (m, 4H), 8.60 (t, *J* = 5.1Hz, 1H), 8.82 (t, *J* = 5Hz, 1H), 11.45 (bs, 2H).

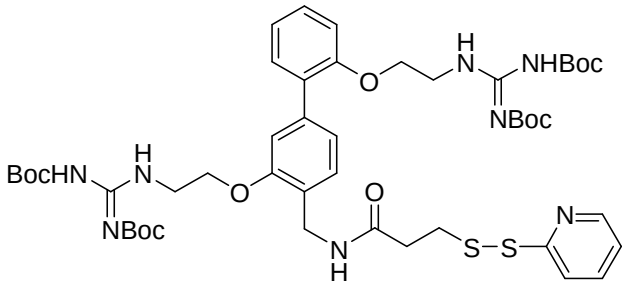
^{13}C-NMR ($CDCl_3$): δ 28.1, 28.3, 39.9, 66.8, 67.5, 79.3, 79.4, 83.3, 83.3, 100.6, 112.3, 113.5, 116.3, 121.5, 123.2, 129.1, 130.0, 130.7, 133.2, 144.9, 153.0, 153.1, 155.1, 156.3, 156.5, 159.9, 163.4.

Theoretical Mass: (M + H) 782.40885. Measured Mass: (M + H) 782.40887

IR ν_{Max} KBR/cm$^-$ 3333 (br NH), 2226 (CN), 1723 (C=O), 1643 (C=N), 1615 (C=N)

M.p. 88-90°C

_N_-{3, 2'-Bis{2-[_N, N'_-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-4-ylmethyl}-3-[2-pyridyl)dithio]propionamide



To a solution of palladium on carbon 10% (25 mg), Raney nickel (25 mg) and NH_4OH (20 µL) in dioxane (40 mL) and water (10 mL) was added 3, 2'-bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-cyanobiphenyl (0.070 g, 0.09 mmol). The mixture was then hydrogenated (6 bar) overnight. The catalyst was filtered off and washed several times with MeOH. Diethylenetriamine (1eq) was added and the mixture was stirred for 30 minutes then concentrated under vacuum. The residue was dissolved in EtOAc (10 mL) and washed with sat. aq. $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. To a solution of the crude and *i*-

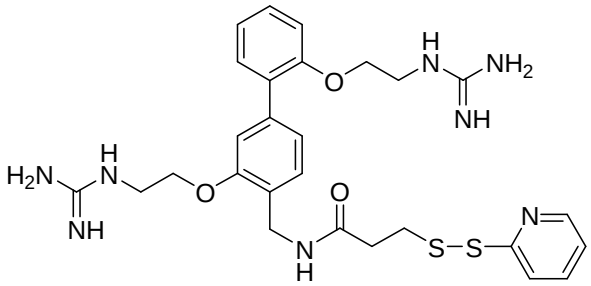
Pr_2EtN (31 µL, 0.18 mmol) in DCM (2 mL) was added N-succinimidyl-3-(2-pyridyldithio)propionate **10** (39 mg, 0.126 mmol) and the mixture was stirred in the dark for 24 h. The solvent was removed under vacuum and the crude oil was purified by preparative TLC (ethyl acetate/hexane 1/1) to afford the title compound (14 mg, 16% yield).

^{1}H-NMR ($CDCl_3$): δ 1.48 (m, 36H), 2.64 (t, 2H), 3.09 (t, *J* = 6.9 Hz, 2H), 3.76 (q, *J* = 5.1 Hz , 2H), 3.84 (q, *J* = 4.5 Hz, 2H), 4.05 (t, *J* = 5.1 Hz, 2H), 4.13 (t, *J* = 4.8 Hz, 2H), 4.51 (d, *J* = 6.0 Hz, 2H), 6.70 (t, *J* = 5.1 Hz, 1H), 6.72-7.06 (m, 4H), 7.19 (d, *J* = 6.3 Hz, 1H), 7.27-7.38 (m, 4H), 7.53 (t, *J* = 6.3 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 1H), 8.33 (d, *J* = 3.9 Hz, 1H), 8.63 (bt, 1H), 8.78 (bt, 1H), 11.45 (bs, 1H), 11.52 (bs, 1H).

^{13}C-NMR ($CDCl_3$): δ 28.0, 28.3, 34.6, 35.8, 39.0, 40.1, 40.2, 66.5, 67.0, 79.3, 79.4, 83.2, 83.4, 112.5, 112.8, 119.9, 120.7, 121.5, 122.8, 125.2, 128.8, 129.9, 130.6, 130.9, 136.2, 149.6, 153.0, 153.3, 155.3, 156.0, 156.3, 163.5, 170.3.

Theoretical Mass: (M + H) 983.43705. Measured Mass: (M + H) 983.43757

_N_-[3, 2'-Bis(2-guanidino-ethyloxy)-biphenyl-4-ylmethyl]-3-[2-pyridyl)dithio]propionamide, formate salt 23



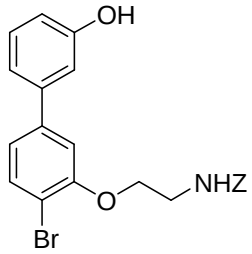
N-{3, 2'-Bis{2-[*N*, *N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-4-ylmethyl}-3-[2-pyridyl)dithio]propionamide (14 mg, 0.014 mmol) was dissolved in formic acid (1 mL) and stirred at room temperature overnight. The mixture was then heated at 50°C during 1h. Water was then added and the product was lyophilised to give **23** as a colourless oil (11.4 mg, 93%).

^{1}H-NMR (MeOH-d_4): δ 2.72 (t, *J* = 6.1 Hz, 2H), 2.97 (t, 2H, *J* = 6.0 Hz, 2H), 3.49 (bs, 2H), 3.64 (bs, 2H), 4.08 (bs, 2H), 4.17 (bs, 2H), 4.45 (s, 2H), 7.05-7.09 (m, 3H), 7.20 (t, *J*

= 5.7 Hz, 1H), 7.28-7.35 (m, 3H), 7.70-7.92 (m, 2H), 8.37 (d, *J* = 4.5 Hz, 1H), 8.40 (bs, 2H).

Theoretical Mass: (M + H) 583.22734. Measured Mass: (M + H) 583.22816

Benzyl 2-(4-bromo-3'-hydroxybiphenyl-3-yloxy)ethylcarbamate



A degassed mixture of benzyl 2-(2-bromo-5-iodophenoxy)ethylcarbamate **15** (1.43 g, 3 mmol), 2-hydroxyphenylboronic acid (0.37 g, 2.7 mmol), $PdCl_2dppf.CH_2Cl_2$ (0.12 g, 0.15 mmol), potassium phosphate (1.27 g, 6 mmol), toluene (6 mL) and water (0.6 mL) was heated at 100°C for 18 h. The reaction mixture was diluted in EtOAc (25 mL) and water (25 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (75/25) as eluent to afford (0.54 g, 45% yield) as a transparent oil.

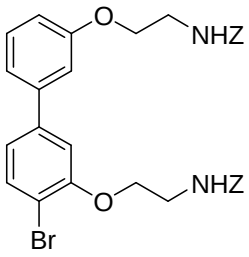
ESMS; *m/z* 442, 444 (M+1), 459, 461 (M+18)

^{1}H-NMR ($CDCl_3$): δ 3.65 (q, *J* = 5 Hz, 2H), 4.08 (t, *J* = 4.8 Hz, 2H), 5.14 (s, 2H), 5.43 (bs, 1H), 6.77 (s, 1H), 6.88 (dd, *J* = 8.1 Hz, *J* = 1.7Hz, 1H), 6.98-7.06 (m, 4H), 7.24-7.36 (m, 6H), 7.52 (d, *J* = 8 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ 40.6, 67.2, 68.3, 111.5, 112.4, 114.1, 114.9, 119.1, 121.2, 128.2, 128.3, 128.6, 130.1, 133.4, 136.1, 141.6, 141.8, 154.9, 156.6, 156.9.

Theoretical Mass: (M + Na) 464.04733. Measured Mass: (M + Na) 464.04628

Dibenzyl 2,2'-(4-bromobiphenyl-3,3'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate 24



Benzyl 2-(4-bromo-3'-hydroxybiphenyl-3-yloxy)ethylcarbamate (0.46 g, 1.8 mmol) was dissolved in DMF (10 mL) and Cs_2CO_3 (1.17 g, 3.6 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **4** (0.55 g, 2 mmol) were added. After stirring at 80 °C under nitrogen for 3.5 h, the reaction mixture was dissolved in EtOAc (25 mL). The organic layer was washed with brine (3 x 20 mL) and dried over $MgSO_4$. The solvent was removed under vacuum and the crude compound was purified by flash chromatography using cyclohexane/EtOAc (7/3) as eluent to afford the title compound **24** (0.33 g, 52 % yield) as a colourless oil.

ESMS; *m/z* 619, 621 (M+1), 636, 638 (M+18)

^{1}H-NMR ($CDCl_3$): δ 3.65 (m, 4H), 4.10 (t, *J* = 4.5 Hz, 2H), 4.16 (t, *J* = 4.4 Hz, 2H), 5.12 (s, 4H), 5.32 (bs, 1H), 5.40 (bs, 1H), 6.88 (d, *J* = 6.8 Hz, 1H), 7.05 (m, 3H), 7.14 (d, *J* = 7.7 Hz, 1H), 7.35 (bs, 11H), 7.57 (d, *J* = 8.6 Hz, 1H).

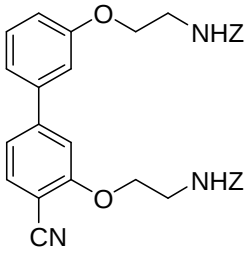
^{13}C-NMR ($CDCl_3$): δ 40.6, 66.9, 67.0, 68.5, 111.7, 112.5, 113.5, 119.9, 121.2, 128.2, 128.6, 130.1, 133.5, 136.4, 141.7, 141.8, 155.1, 156.5, 158.9.

Theoretical Mass: (M + Na) 641.12631. Measured Mass: (M + Na) 641.12715

Microanalysis: %C 62.08 (62.04), %H 5.13 (5.04), %N 4.39 (4.52)

IR ν_{Max} KBR/cm$^-$ 3339 (br, NH), 2226 (CN), 1723 (C=O), 1641 (C=N), 1620 (C=N)

Dibenzyl 2,2'-(4-cyanobiphenyl-3,3'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate 25



A mixture of dibenzyl 2,2'-(4-bromobiphenyl-3,3'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate **24** (0.26 g, 0.42 mmol), zinc cyanide (74 mg, 0.63 mmol), Pd_2dba_3 (19 mg, 0.02 mmol), dppf (29 mg, 0.05 mmol), zinc acetate (39 mg, 0.21 mmol) and zinc (14 mg, 0.21 mmol) in dimethylacetamide (3 mL) was heated at 160°C for 10 minutes under microwave irradiation. The reaction mixture was diluted in EtOAc (25 mL) and water (100 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (65/35) as eluent to afford **25** (0.2 g, 84% yield) as a colourless oil.

ESMS; *m/z* 566 (M+1), 583 (M+18)

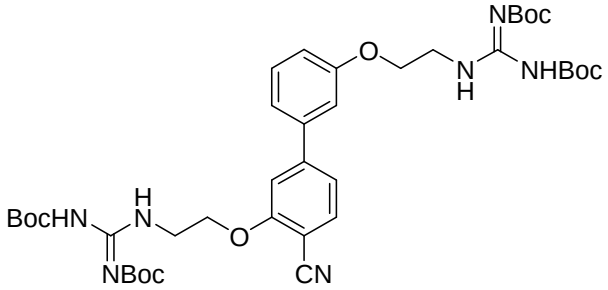
^{1}H-NMR ($CDCl_3$): δ 3.61-3.71 (m, 4H), 4.10 (t, *J* = 4.6 Hz, 2H), 4.22 (t, *J* = 4.7 Hz, 2H), 5.11 (s, 4H), 5.27 (bs, 1H), 5.37 (bs, 1H), 6.93 (d, *J* = 8.1 Hz, 1H), 7.07-7.22 (m, 4H), 7.29-7.40 (m, 11H), 7.59 (d, *J* = 8 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ 40.3, 40.4, 66.9, 67.1, 68.2, 100.9, 111.1, 113.7, 114.5, 120.2, 128.1, 128.2, 128.2, 128.5, 128.6, 130.3, 134.0, 136.3, 140.9, 147.4, 156.5, 159.0, 160.4.

Theoretical Mass: (M + H) 566.22910. Measured Mass: (M + H) 566.22964

IR ν_{Max} KBR/cm$^-$ 3339 (br, NH), 2225 (CN), 1713 (C=O)

3, 3'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-4-cyanobiphenyl



Dibenzyl 2,2'-(4-cyanobiphenyl-3,3'-diyl)bis(oxy)bis(ethane-2,1-diyl)dicarbamate **25** (0.15 g, 0.26 mmol) was dissolved in DCM (10 mL) and HBr (30% in acetic acid, 1.5 mL) was added dropwise. After stirring at room temperature for 1.5 h, the solvent was removed under vacuum and the crude diamine was carefully dried under vacuum for several hours.

The resulting solid was suspended in DCM (5 mL) and Et_3N (0.36 mL, 2.6 mmol) and N, N-di-boc-N'-trifluoromethanesulfonyl-guanidine **8** (0.21 g, 0.53 mmol) were added. The mixture was stirred overnight at room temperature, diluted with DCM (20 mL) then washed with 2M $NaHSO_4$ 2M (25 mL) followed by sat.aq. $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (8/2) as eluent to afford the title compound (0.18 g, 86% yield) as a white solid.

^{1}H-NMR ($CDCl_3$): δ 1.50 (m, 36H), 3.86 (q, *J* = 5.1 Hz, 2H), 3.93 (q, *J* = 5.2 Hz, 2H), 4.15 (t, *J* = 5 Hz, 2H), 4.29 (t, *J* = 5.1 Hz, 2H), 6.99 (dd, *J* = 8.1 Hz, *J* =2 Hz, 1H), 7.10-7.16 (m, 3H), 7.21 (d, *J* = 8 Hz, 1H), 7.38 (t, *J* = 8 Hz, 1H), 7.60 (d, *J* = 8 Hz, 1H), 8.62 (bt, 1H), 8.82 (bt, 1H), 11.45 (bs, 2H).

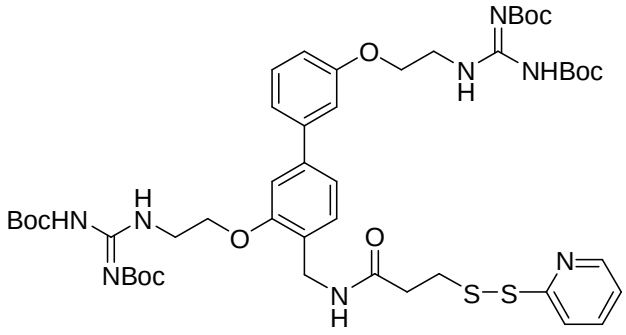
^{13}C-NMR ($CDCl_3$): δ 27.8, 28.1, 28.3, 39.9, 40.2, 66.6, 67.6, 79.4, 83.2, 83.3, 101.2, 111.3, 114.1, 114.7, 116.0, 120.2, 120.3, 130.2, 134.1, 141.0, 147.4, 153.0, 153.1, 156.4, 156.5, 159.1, 160.4, 163.5.

Theoretical Mass: (M + H) 782.40885. Measured Mass: (M + H) 782.40656

IR ν_{Max} KBR/cm$^-$ 3332 (br, NH), 2226 (CN), 1723 (C=O), 1641 (C=N), 1620 (C=N)

M.p. 82-84°C

***N*-{3, 3'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-4-ylmethyl}-3-[2-pyridyl)dithio]propionamide**



To a solution of palladium on carbon 10% (25 mg), Raney nickel (25 mg) and NH_4OH (20 μL) in dioxane (40 mL) and water (10 mL) was added 3, 3'-Bis{2-[N, N'-bis(tert-

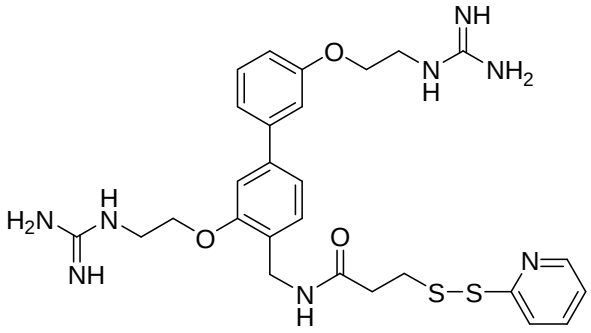
butoxycarbonyl)guanidino]-ethyloxy}-5-cyanobiphenyl (0.070 g, 0.09 mmol). The mixture was then hydrogenated (6 bar) overnight. The catalyst was then filtered off and washed several times with MeOH. Diethylenetriamine (1eq) was added and the mixture was stirred for 30 minutes then concentrated under vacuum. The residue was dissolved in EtOAc (10 mL) and washed with sat. aq. $NaHCO_3$ 10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. To a solution of the crude and *i*-Pr_2EtN (31 µL, 0.18 mmol) in DCM (2 mL) was added N-succinimidyl-3-(2-pyridyldithio)propionate **10** (39 mg, 0.126 mmol) and the mixture was stirred in the dark for 24 h. The solvent was removed under vacuum and the crude oil was purified by preparative TLC (ethyl acetate/hexane 1/1) to afford the title compound (8 mg, 11% yield).

^{1}H-NMR ($CDCl_3$): δ 1.50 (m, 36H), 2.64 (t, 2H), 3.08 (t, *J* = 6.9 Hz, 2H), 3.89 (m , 4H), 4.17 (m, 4H), 4.50 (d, *J* = 5.7 Hz, 2H), 6.81 (bt, 1H), 6.92 (dd, *J* = 7.8 Hz, *J* = 2.4 Hz, 1H), 7.02-7.15 (m, 5H), 7.32 (t, *J* = 4.5 Hz, 1H), 7.42 (d, *J* = 8.1 Hz, 1H), 7.53-7.63 (m, 2H), 8.34 (d, *J* = 3.6 Hz, 1H), 8.78 (m, 2H), 8.78 (bt, 1H), 11.49 (bs, 1H), 11.54 (bs, 1H).

^{13}C-NMR ($CDCl_3$): δ 28.0, 28.3, 34.4, 35.3, 38.5, 39.9, 40.1, 66.3, 66.7, 79.3, 79.5, 83.1, 83.4, 111.5, 112.9, 120.1, 120.4, 125.7, 129.2, 130.1, 136.0, 140.9, 141.3, 148.0, 153.7, 156.9, 159.6, 163.5, 170.5.

Theoretical Mass: (M + H) 983.43705. Measured Mass: (M + H) 983.43928

_N_-[3, 3'-Bis(2-guanidino-ethyloxy)-biphenyl-4-ylmethyl]-3-[2-pyridyl)dithio]propionamide, formate salt 26



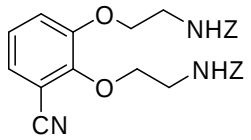
N-{3, 3'-Bis{2-[N, N'-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-4-ylmethyl}-3-[2-pyridyl)dithio]propionamide (8 mg, 0.0081 mmol) was dissolved in

formic acid (1 mL) and stirred at room temperature overnight. The mixture was then heated at 50°C for 1h. Water was added and the product was freeze-dried to give **26** as colourless oil (7.1 mg, 100%).

^{1}H-NMR (MeOH-d_4): δ 2.69 (t, *J* = 6.8 Hz, 2H), 3.09 (t, 2H, *J* = 6.5 Hz, 2H), 3.60-3.68 (m, 4H), 4.18(t, *J* = 4.9 Hz, 2H), 4.24 (t, *J* = 4.8 Hz, 2H), 4.46 (s, 2H), 6.95 (d, *J* = 7.5 Hz, 1H), 7.18-7.24 (m, 4H), 7.31-7.39 (m, 2H), 7.71-7.78 (m, 2H), 8.36 (d, *J* = 4.6 Hz, 1H), 8.43 (bs, 2H).

Theoretical Mass: (M + H) 583.22734. Measured Mass: (M + H) 583.22796

Dibenzyl 2,2'-(3-cyano-1,2-phenylene)bis(oxy)bis(ethane-2,1-diyl)dicarbamate



A mixture of dibenzyl 2,2'-(3-bromo-1,2-phenylene)bis(oxy)bis(ethane-2,1-diyl)dicarbamate **13** (1.10 g, 2 mmol), zinc cyanide (0.35 g, 3 mmol), Pd_2dba_3 (92 mg, 0.1 mmol), dppf (0.14 g, 0.25 mmol), zinc acetate (0.18 g, 1 mmol) and zinc (65 mg, 1 mmol) in dimethylacetamide (10 mL) was heated at 160 °C for 10 minutes under microwave irradiation. The reaction mixture was diluted in EtOAc (25 mL) and water (100 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were washed with water (50 mL) and brine (50 mL), dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (1/1) as eluent to afford the title compound (0.70 g, 72% yield) as a white solid.

ESMS; *m/z* 490 (M+1), 507 (M+18)

^{1}H-NMR ($CDCl_3$): δ 3.54 (m, 4H), 4.05 (m, 2H), 4.21 (m, 2H), 5.09 (s, 2H), 5.65 (bs, 1H), 5.74 (s, 1H), 7.09-7.13 (m, 3H), 7.30 (bs, 5H).

^{13}C-NMR ($CDCl_3$): δ 40.5, 41.2, 66.8, 68.2, 107.8, 116.2, 118.0, 124.7, 125.0, 128.1, 128.5, 136.4, 150.3, 151.7, 156.6.

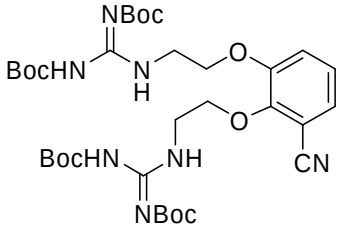
Theoretical Mass: (M + Na) 512.17975. Measured Mass: (M + Na) 512.18046

Microanalysis: %C 65.86 (66.25), %H 5.56 (5.56), %N 8.47 (8.58)

IR ν_{Max} KBR/cm$^-$ 3334 (br NH), 2232 (CN), 1702 (C=O)

M.p. 78°C

1,2-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-3-cyanophenyl 27



Dibenzyl 2,2'-(3-cyano-1,2-phenylene)bis(oxy)bis(ethane-2,1-diyl)dicarbamate (0.44 g, 0.9 mmol) was dissolved in DCM (20 mL) and HBr (30% in acetic acid, 4 mL) was added dropwise. After stirring at room temperature for 1.5 h, water (10 mL) was added to the mixture, the layers were separated and the aqueous layer was washed with DCM (2 x 10 mL). The water was then removed under vacuum and the crude diamine was carefully dried under vacuum for several hours.

The resulting solid was suspended in DCM (2.5 mL) and *i*-Pr_2EtN (0.65 mL, 4.5 mmol) and N, N-di-boc-N'-trifluoromethanesulfonyl-guanidine **8** (0.7 g, 1.8 mmol) were added. The mixture was stirred overnight at room temperature, diluted with DCM (20 mL) then washed with 2M $NaHSO_4$ 2M (20 mL) followed by sat. aq. $NaHCO_3$ (20 mL) and brine (20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (92/18) as eluent to afford **27** (0.29 g, 45% yield) as a white solid.

ESMS; *m/z* 706 (M+1), 354 (M+2)

^{1}H-NMR ($CDCl_3$): δ 1.49 (m, 36H), 3.86 (m, 4H), 4.16 (t, *J* = 5 Hz, 2H), 4.32 (t, *J* = 5 Hz, 2H), 7.07 (t, *J* = 8 Hz, 1H), 7.14 (dd, *J* = 8 Hz, *J* = 2 Hz, 1H), 7.18 (dd, *J* = 8 Hz, *J* = 2Hz, 1H), 8.75 (m, 2H), 11.42 (bs, 2H).

^{13}C-NMR ($CDCl_3$): δ 28.0, 28.1, 28.3, 39.7, 41.2, 67.4, 72.6, 79.2, 79.4, 82.9, 83.4, 108.0, 115.9, 118.2, 124.8, 125.0, 150.2, 151.7, 153.1, 156.4, 163.3, 163.4.

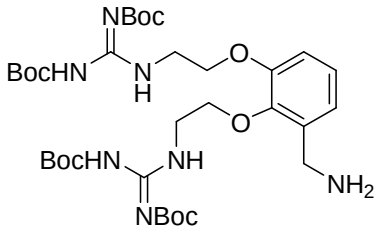
$C_{33}H_{53}N_7O_{11}\cdot H_2O$: %C 54.93 (54.76), %H 7.48 (7.38), %N 12.88 (13.55).

Theoretical Mass: (M + Na) 728.35949. Measured Mass: (M + Na) 728.35986

IR ν_{Max} KBR/cm^- 3332 (br, NH), 2234 (CN), 1723 (C=O), 1642 (C=N), 1620 (C=N)

M.p. 99-101°C

1,2-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-3-(aminomethyl)phenyl



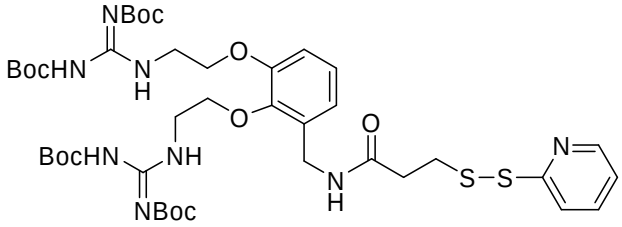
1,2-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-3-cyanophenyl **27** (0.07 g. 0.1 mmol) was dissolved in THF (7 mL) and NH_4OH (1.4 mL) was added followed by Raney Nickel (0.07 g) . The mixture was stirred vigorously under H_2 atmosphere for 16 h. The catalyst was filtered off and washed several times with MeOH. Diethylenetriamine (1eq) was added and the mixture stirred for 30 minutes then concentrated under vacuum. The residue was dissolved in EtOAc (10 mL) and washed with sat. aq. $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using DCM/MeOH (97/3) as eluent to afford the product (0.020 g, 28% yield).

ESMS; *m/z* 710 (M+1), 355 (M+2)

^{1}H-NMR ($CDCl_3$): δ 1.48 (m, 36H), 3.82 (m, 6H), 4.10 (m, 4H), 6.95 (m, 3H), 8.45 (m, 1H), 8.52 (m, 1H).

IR ν_{Max} KBR/cm^{-}3337 (br NH, NH_2), 1722 (C=O), 1641 (C=ON)

***N*-(2,3-bis([N, N'-bis(tert-butoxycarbonyl)guanidino]-ethyloxy)benzyl)-3-(pyridin-2-yldisulfanyl)propanamide**



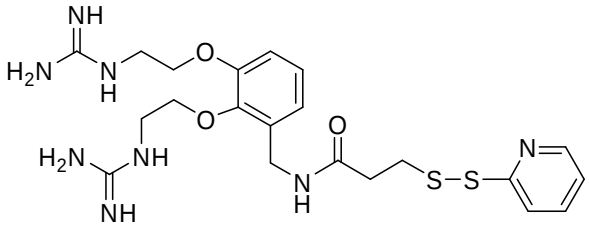
To a solution of 1,2-bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-3-(aminomethyl)phenyl (0.020 g, 0.028 mmol) and *i*-Pr_2EtN (10 µL, 0.05 mmol) in DCM (0.6 mL) was added N-succinimidyl-3-(2-pyridyldithio)propionate **10** (12.8 mg, 1.4 eq)

and the mixture was stirred in the dark for 24 h. The solvent was removed under vacuum and the crude oil was purified by preparative TLC (EtOAc/cyclohexane 1/1) to afford the title compound (5.2mg, 20% yield).

IR ν_{Max} KBR/cm^{-}3338 (br NH, NH_2), 1723 (C=O), 1641 (C=ON)

^{1}H-NMR ($CDCl_3$): δ 1.48 (m, 36H), 2.61 (t, *J* = 7 Hz, 2H), 3.06 (t, *J* = 7 Hz, 2H), 3.86 (m, 4H), 4.12 (t, *J* = 5 Hz, 2H), 4.21 (t, *J* = 5 Hz, 2H), 7.02 (m, 5H), 7.59 (m, 2H), 8.34 (m, 1H), 8.76 (m, 2H), 11.49 (bs, 1H), 11.52 (bs, 1H).

***N*-(2,3-bis(2-guanidinoethoxy)benzyl)-3-(pyridin-2-yldisulfanyl)propanamide <u>28</u>**



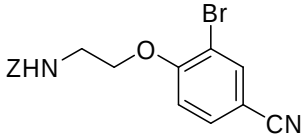
N-(2,3-bis([N, N′-bis(tert-butoxycarbonyl)guanidino]-ethyloxy)benzyl)-3-(pyridin-2-yldisulfanyl)propanamide (5.2 mg, 0.006 mmol) was dissolved in a mixture of TFA/ H_2O/triisopropylsilane 95/2.5/2.5 (1 mL) and stirred at room temperature for 3 h. The solvent was removed under vacuum to give **<u>28</u>** as colourless oil (5 mg, 100% yield).

^{1}H-NMR (MeOH-d4): δ 2.66 (t, *J* = 6.7 Hz, 2H), 3.07 (t, *J* = 6.7 Hz, 2H), 3.56 (m, 2H), 3.63 (m, 2H), 4.17 (m, 4H), 4.42 (s, 2H), 6.99 (m, 4H), 7.19 (m, 1H), 7.74 (m, 2H), 8.35 (bd, *J* = 4.1 Hz, 1H).

Theoretical Mass: (M + H) 507.19604. Measured Mass: (M + H) 507.19498

IR ν_{Max} KBR/cm^{-}3369 (br NH, NH_2), 1671 (C=ON)

***N*-(2-(4-cyanophenoxy)ethyl)-2-phenylacetamide**



3-Bromo-4-hydroxybenzonitrile **<u>29</u>** (7.25 g, 36.6 mmol) was dissolved in DMF (20 mL) and Na_2CO_3 (3.7 g, 73.2 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **<u>4</u>** (12 g, 42 mmol) were added. After stirring at 80 °C under nitrogen for 15 h, the reaction mixture was dissolved in EtOAc (25 mL). The organic layer was washed with

brine (3 x 20 mL), dried over $MgSO_4$ and filtered. The solvent was removed under vacuum and the crude compound was purified by flash chromatography using cyclohexane/EtOAc (8/2) as eluent to afford the title compound (9.4 g, 68% yield) as a white solid.

^{1}H-NMR ($CDCl_3$): δ 3.70 (q, *J* = 5.3 Hz, 2H), 4.13 (m, 2H), 5.11 (s, 2H), 5.23 (bs, 1H), 6.90 (d, *J* = 8.5 Hz, 1H), 7.35 (m, 5H), 7.56 (d, *J* = 8.5 Hz, 1H), 7.81 (d, *J* = 1.9 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ 40.2, 67.0, 68.5, 105.7, 112.7, 113.0, 117.6, 128.2, 128.3, 128.6, 133.2, 136.3, 136.8, 156.4, 158.4.

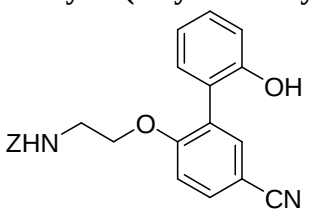
Theoretical Mass: (M + Na) 397.01637. Measured Mass: (M + Na) 397.01539.

Microanalysis: %C 54.10 (54.42), %H 4.23 (4.03), %N 7.19 (7.47)

IR ν_{Max} KBR/cm^{-}3351(br NH, NH_2), 2227 (CN), 11704 (C=ON)

M.p. 65-68°C

Benzyl 2-(5-cyano-2'-hydroxybiphenyl-2-yloxy)ethylcarbamate



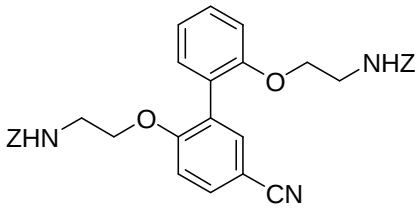
A degassed mixture of *N*-(2-(4-cyanophenoxy)ethyl)-2-phenylacetamide (1.9 g, 5 mmol), 2-hydroxyphenyl boronic acid (0.83 g, 6 mmol), $Pd(OAc)_2$ (10 mg, 0.05 mmol) , 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (40 mg, 0.1 mmol), potassium phosphate (2.1 g, 10 mmol), toluene (10 mL) and water (1 mL) was heated at 100°C for 1 h. The reaction mixture was diluted in EtOAc (50 mL) and water (50 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (7/3) as eluent to afford the title compound (1.07 g, 55% yield) as a colourless oil.

^{1}H-NMR ($CDCl_3$): δ 3.48 (m, 2H), 4.13 (m, 2H), 5.06 (bs, 2H), 5.32 (bs, 1H), 6.34 (bs, 1H), 6.91-7.02 (m, 3H), 7.13 (d, *J* = 7Hz, 1H), 7.27-7.33 (m, 6H), 7.58 (m, 2H).

^{13}C-NMR ($CDCl_3$): δ 40.2, 66.9, 68.0, 105.2, 112.8, 116.7, 118.8, 121.1, 123.7, 128.1, 128.3, 128.6, 128.9, 130.0, 131.2, 133.6, 135.8, 136.3, 153.1, 156.5, 158.7.

Theoretical Mass: (M + H) 389.15012. Measured Mass: (M + H) 389.15078

Dibenzyl 2,2'-(5-cyanobiphenyl-2,2'-diylbis(oxy))bis(ethane-2,1-diyl)tetracarbamate <u>30</u>



Benzyl 2-(5-cyano-2'-hydroxybiphenyl-2-yloxy)ethylcarbamate (1.06 g, 2.7 mmol) was dissolved in DMF (7 mL) and Cs_2CO_3 (1.8 g, 5.4 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **<u>4</u>** (0.75 g, 2.7 mmol) were added. After stirring at 80 °C under nitrogen overnight, further Cs_2CO_3 (0.9 g, 2.7 mmol) and 2-(benzyloxycarbonylamino)ethyl methanesulfonate **<u>4</u>** (0.4 g, 1.5 mmol) were added. After stirring for a further 4 h, the reaction mixture was dissolved in EtOAc (25 mL). The organic layer was washed with brine (3 x 20 mL) and dried over $MgSO_4$. The solvent was removed under vacuum and the crude compound was purified by flash chromatography using cyclohexane/EtOAc (1/1) as eluent to afford **<u>30</u>** (1.23 g, 80% yield) as a colourless oil.

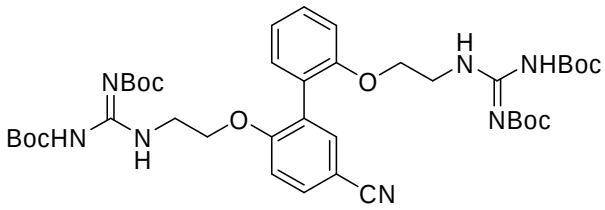
^{1}H-NMR ($CDCl_3$): δ 3.32 (m, 2H), 3.80 (t, *J* = 8.9 Hz, 2H), 4.00 (m, 2H), 4.39 (t, *J* = 8.7 Hz, 2H), 5.04 (s, 2H), 5.22 (s, 2H), 5.30 (bs, 1H), 5.54 (bs, 1H), 6.86 (m, 2H), 7.02 (t, *J* = 7.3 Hz, 1H), 7.13 (dd, *J* = 8.9 Hz, *J* = 1.7 Hz, 1H), 7.34 (m, 11H), 7.46 (d, *J* = 2 Hz, 1H), 7.53 (dd, *J* = 8.5 Hz, 1.8 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ 40.1, 40.3, 66.8, 68.0, 68.3, 104.7, 112.8, 113.5, 118.9, 121.9, 126.4, 128.1, 128.3, 128.5, 128.6, 129.7, 129.8, 131.0, 133.5, 134.9, 135.4, 136.4, 155.6, 156.2, 159.2, 163.6.

Theoretical Mass: (M + Na) 588.21104. Measured Mass: (M + Na) 588.21104

IR ν_{Max} KBR/cm^{-} 3342 (br, NH), 2224 (CN), 1721 (C=O)

2, 2'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-cyanobiphenyl



Dibenzyl 2,2'-(5-cyanobiphenyl-2,2'-diylbis(oxy))bis(ethane-2,1-diyl)tetracarbamate **30** (0.9 g, 1.6 mmol) was dissolved in DCM (50 mL) and HBr (30% in acetic acid, 8 mL) was added dropwise. After stirring at room temperature for 1.5 h, water (25 mL) was added to the mixture, the layers were separated and the aqueous layer was washed with DCM (2 x 25 mL). The water was then removed under vacuum and the crude diamine was carefully dried under vacuum for several hours.

The residue was suspended in DCM (7 mL) and Et_3N (1.2 mL, 8 mmol) and N, N-di-boc-N'-trifluoromethanesulfonyl-guanidine **8** (1.3 g, 3.2 mmol) were added. The mixture was stirred overnight at room temperature, diluted with DCM (20 mL) then washed with 2M $NaHSO_4$ 2M (25 mL) follow by sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was purified by flash chromatography using cyclohexane/EtOAc (8/2) as eluent to afford the title compound (0.28 g, 22% yield) as a white solid.

ESMS; *m/z* 782(M+1), 391(M+2)

^{1}H-NMR ($CDCl_3$): δ1.48 (m, 36H), 3.69 (m, 4H), 4.05 (t, *J* = 5.2 Hz, 2H), 4.13 (t, *J* = 5.2 Hz, 2H), 6.95-7.06 (m, 3H), 7.22 (dd, *J* =7.5, 1.7 Hz, 1H), 7.32 (td, *J* = 7.2, 1.7 Hz, 1H), 7.55 (d, *J* = 2 Hz, 1H), 7.59 (dd, *J* = 8.5, 2.1 Hz, 1H), 8.40 (m, 2H), 11.42 (bs, 2H).

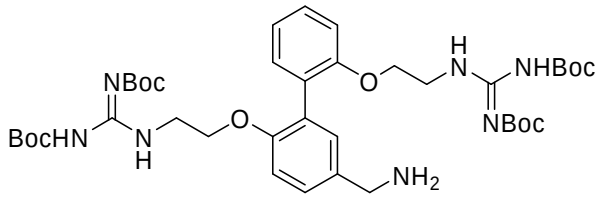
^{13}C-NMR ($CDCl_3$): δ28.1, 28.3, 39.8, 40.1, 66.7, 66.9, 79.3, 79.4, 83.1, 104.1, 112.2, 112.4, 119.2, 121.0, 125.6, 129.1, 129.4, 131.3, 133.2, 135.2, 152.9, 155.6, 156.3, 159.2, 163.4.

Theoretical Mass: (M + Na) 804.39079. Measured Mass: (M + Na) 804.39290

IR ν_{Max} KBR/cm$^-$ 3333 (br, NH), 2226 (CN), 1722 (C=O), 1642 (C=N), 1619 (C=N)

M.p. 82°C

2, 2'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-(aminomethyl)biphenyl



2, 2'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-cyanobiphenyl (0.10 g, 0.12 mmol) was dissolved in THF (7 mL) and NH_4OH (1.4 mL) was added followed by Raney Nickel (0.1 g). The mixture was stirred vigorously under H_2 atmosphere for 16 h. The catalyst was filtered off and washed several times with MeOH. Diethylenetriamine (1eq) was added and the mixture stirred for 30 minutes then concentrated under vacuum. The residue was dissolved in EtOAc (10 mL) and washed with sat. aq. $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using DCM/MeOH (97/3) as eluent to afford the product (45 mg, 48% yield).

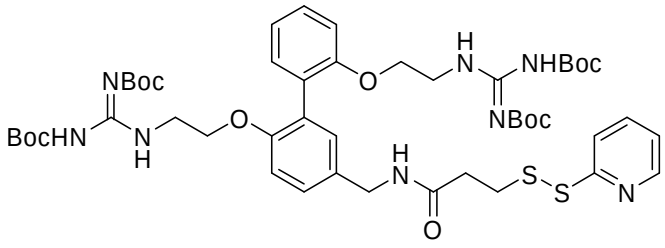
ESMS; *m/z* 786(M+1), 394(M+2)

^{1}H-NMR ($CDCl_3$): δ1.47 (m, 36H), 3.63 (m, 4H), 3.81 (s, 2H), 4.02 (m, 4H), 6.90-6.99 (m, 3H), 7.22 (m, 4H), , 8.40 (m, 2H), 11.42 (bs, 2H).

^{13}C-NMR ($CDCl_3$): δ28.1, 28.3, 40.2, 40.3, 67.1, 79.1, 79.2, 82.8, 82.9, 112.9, 113.0, 121.0, 127.0, 127.8, 128.0, 128.5, 130.6, 131.8, 135.7, 152.8, 154.7, 155.7, 156.2, 163.4.

IR ν_{Max} KBR/cm$^-$ 3338 (NH, NH_2), 1723 (C=O), 1641(C=N), 1614 (C=N)

***N*-{2, 2'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-5-ylmethyl}-3-[2-pyridyl)dithio]propionamide**



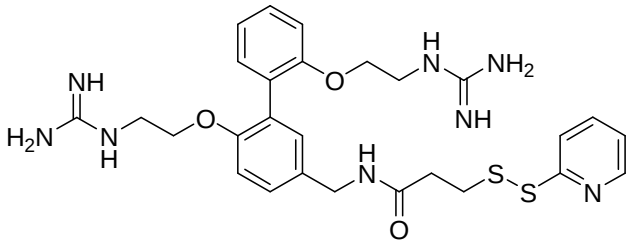
To a solution of 2, 2'-Bis{2-[N, N'-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-(aminomethyl)biphenyl (0.042 g, 0.053 mmol) and *i*-Pr_2EtN (10 µL, 0.10 mmol) in DCM (0.7 mL) was added N-succinimidyl-3-(2-pyridyldithio)propionate **10** (15 mg, 0.053 mmol) and the mixture was stirred in the dark for 24 h. The solvent was removed under vacuum and the crude oil was purified by preparative TLC (ethyl acetate/hexane 1/1) to afford the title compound (8.5 mg, 16% yield).

^{1}H-NMR ($CDCl_3$): δ1.48 (m, 36H), 2.61 (t, *J* = 6.8 Hz, 2H), 3.09 (t, *J* = 6.7 Hz, 2H), 3.65 (m, 4H), 4.02 (m, 4H), 4.42 (d, *J* = 5.4 Hz, 2H), 6.89-7.03 (m, 6H), 7.23-7.30 (m, 3H), 7.58 (m, 2H), 8.23 (m, 2H), 8.23 (dd, *J* = 3.8, 1.5Hz, 1H), 8.36 (bt, 1H), 8.47 (bt, 1H), 11.66 (bs, 2H).

Theoretical Mass: (M + H) 983.43705. Measured Mass: (M + H) 983.43763

IR ν_{Max} KBR/cm$^-$ 3332 (NH), 1722 (C=O), 1641 (C=O).

_N_-[2, 3'-Bis(2-guanidino-ethyloxy)-biphenyl-5-ylmethyl]-3-[2-pyridyl)dithio]propionamide, trifluoroacetate salt 31



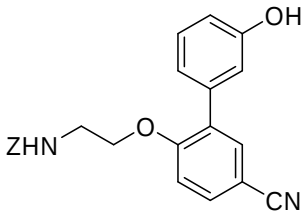
N-{2, 3'-Bis{2-[N, N'-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-5-ylmethyl}-3-[2-pyridyl)dithio]propionamide (8.5 mg, 0.0086 mmol) was dissolved in a mixture of TFA/ H_2O/triisopropylsilane 95/2.5/2.5 (1 mL) and stirred at room temperature for 3 h. The solvent was removed under vacuum to give **31** as a colourless oil (8 mg, 100%).

^{1}H-NMR (MeOH-d4): δ 2.71 (t, *J* = 6.7Hz, 2H), 3.07 (t, 2H, *J* = 6.7Hz, 2H), 3.41 (m, 4H), 4.05 (m, 4H), 4.35 (s, 2H), 7.07 (m, 4H), 7.18 (m, 4H), 7.27 (m, 2H), 7.35 (m, 2H), 7.75 (m, 2H), 8.37 (m, 1H).

Theoretical Mass: (M + H) 583.22734. Measured Mass: (M + H) 583.22566

IR ν_{Max} KBR/cm$^-$3336 (NH), 1642 (C=O)

Benzyl 2-(5-cyano-3'-hydroxybiphenyl-2-yloxy)ethylcarbamate



A degassed mixture of *N*-(2-(4-cyanophenoxy)ethyl)-2-phenylacetamide (3.7 g, 9.8 mmol), 3-hydroxyphenyl boronic acid (1.6 g, 11.6 mmol), $Pd(OAc)_2$ (22 mg, 0.1 mmol) 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (82 mg, 0.2 mmol) potassium phosphate (4.2 g, 20 mmol), toluene (20 mL) and water (2 mL) was heated at 100°C for 1 h. The reaction mixture was diluted in EtOAc (50 mL) and water (50 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (7/3) as eluent to afford the title compound (2.2 g, 59% yield) as a colourless solid.

ESMS; *m/z* 389 (M+1)

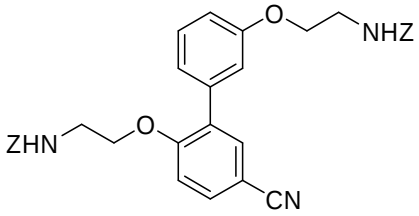
^{1}H-NMR ($CDCl_3$): δ 3.40 (m, 2H), 3.60 (m, 2H), 5.08 (s, 2H), 5.23 (m, 2H), 6.89 (m, 4H), 6.95 (2, 1H), 7.18 (m, 56H), 7.53 (m, 1H).

Microanalysis: $C_{23}H_{20}N_2O_4 \cdot H_2O$: %C 67.81 (67.97), %H 5.41 (5.46), %N 6.85 (6.89).

IR ν_{Max} KBR/cm$^-$3347(br NH, NH_2), 2225(CN), 1714 (C=ON)

Mp >200^{o}C

***N*-{2-[5'-Cyano-2'-(2-phenylacetylamino-ethoxy)-biphenyl-3-yloxy]-ethyl}-2-phenyl-acetamide <u>32</u>**



Benzyl 2-(5-cyano-3'-hydroxybiphenyl-2-yloxy)ethylcarbamate (0.62 g, 1.6 mmol) was dissolved in DMF (8 mL) and Cs_2CO_3 (1.04 g, 3.2 mmol) and 2-

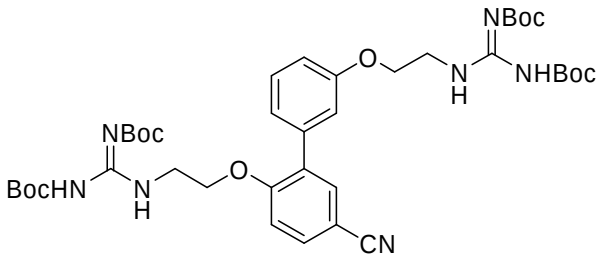
(benzyloxycarbonylamino)ethyl methanesulfonate **4** (0.44 g, 1.6 mmol) were added. After stirring at 80 °C under nitrogen for 3.5 h, the reaction mixture was dissolved in EtOAc (25 mL). The organic layer was washed with brine (3 x 20 mL) and dried over $MgSO_4$. The solvent was removed under vacuum and the crude compound was purified by flash chromatography using cyclohexane/EtOAc (1/1) as eluent to afford **32** (0.86 g, 95% yield).

^{1}H-NMR ($CDCl_3$): δ 3.70 (q, J = 5.3 Hz, 2H), 4.13 (m, 2H), 5.11 (s, 2H), 5.23 (bs, 1H), 6.90 (d, J = 8.5 Hz, 1H), 7.35 (m, 5H), 7.56 (d, J = 8.5 Hz, 1H), 7.81 (d, J = 1.9 Hz, 1H).

^{13}C-NMR ($CDCl_3$): δ 40.2, 67.0, 68.5, 105.7, 112.7, 113.0, 117.6, 128.2, 128.3, 128.6, 133.2, 136.3, 136.8, 156.4, 158.4.

IR ν_{Max} KBR/cm^{-}3335(br NH, NH_2), 2225(CN), 1714 (C=ON)

2, 3'-Bis{2-[*N, N*'-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-cyanobiphenyl



N-{2-[5'-Cyano-2'-(2-phenylacetylamino-ethoxy)-biphenyl-3-yloxy]-ethyl}-2-phenyl-acetamide **32** (1.7 g, 3 mmol) was dissolved in DCM (25 mL) and HBr (30% in acetic acid, 4.0 mL) was added dropwise. After stirring at room temperature for 1.5 h, water (25 mL) was added to the mixture, the layers were separated and the aqueous layer was washed with DCM (2 x 25 mL). The water was then removed under vacuum and the crude diamine was carefully dried under vacuum for several hours.

The resulting solid was suspended in DCM (20 mL) and *i*-Pr_2EtN (3mL, 39 mmol) and N, N-di-boc-N'-trifluoromethanesulfonyl-guanidine **8** (3.7 g, 9 mmol) were added. The mixture was stirred overnight at room temperature, diluted with DCM (40 mL) then washed with 2M $NaHSO_4$ 2M (25 mL) followed by sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using

cyclohexane/EtOAc (8/2) as eluent to afford the title compound (1.102 g, 47% yield) as a white solid.

ESMS; *m/z* 782(M+1), 391(M+2)

^{1}H-NMR ($CDCl_3$): δ 1.48 (m, 36H), 3.80 (m, 2H), 3.83 (m, 2H), 4.12 (m, 4H), 6.81-6.98 (m, 2H), 7.09 (d, *J* = 9.2 Hz, 1H), 7.16 (d, *J* = 7.8 Hz, 1H), 7.34 (t, *J* = 7.9 Hz, 1H), 7.60 (m, 2H), 8.60 (t, *J* = 5.3 Hz, 1H), 8.76 (t, *J* = 4.9 Hz, 1H), 11.54 (bs, 2H).

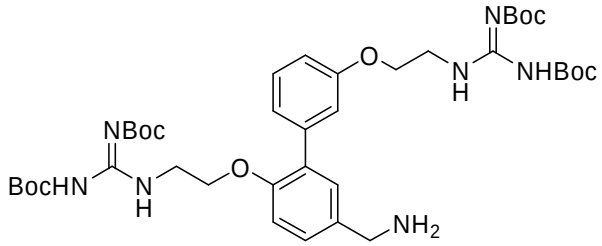
^{13}C-NMR ($CDCl_3$): δ 28.0, 28.3, 39.6, 40.2, 66.5, 67.0, 79.3, 79.4, 83.2, 83.3, 104.6, 112.7, 113.9, 115.9, 119.0, 122.7, 129.2, 131.9, 133.2, 134.5, 137.5, 153.0, 156.4, 158.6, 158.7, 163.4, 163.5.

Microanalysis: $C_{39}H_{55}N_7O_{10}$: %C 60.30 (59.91), %H 7.20 (7.09), %N 12.45 (13.54).

IR ν_{Max} KBR/cm^{-} 3333 (br, NH), 2226 (CN), 1722 (C=O), 1642 (C=N), 1619 (C=N)

Mp 120-121^{o}C

2, 3'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-(aminomethyl)biphenyl



2, 3'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-cyanobiphenyl (0.54 g, 0.7 mmol) was dissolved in THF (14 mL) and NH_4OH (2.4 mL) was added followed by Raney Nikel (0.5 g). The mixture was stirred vigorously under H_2 atmosphere for 16 h. The catalyst was filtered off and washed several times with MeOH. Diethylenetriamine (1eq) was added and the mixture stirred for 30 minutes then concentrated under vacuum. The residue was dissolved in EtOAc (10 mL) and washed with sat. aq. $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using DCM/MeOH (97/3) as eluent to afford the product (0.213 g, 39% yield).

ESMS; *m/z* 787 (M+1), 394 (M+2)

^{1}H-NMR ($CDCl_3$): δ 1.47 (m, 36H), 3.59 (m, 2H), 3.78 (m, 4H), 4.03 (t, *J* = 4.9 Hz, 2H), 4.10 (t, *J* = 4.9 Hz, 2H), 6.86 (dd, *J* = 8, 2Hz, 1H), 6.93 (d, *J* = 8.3 Hz, 1H), 7.20-7.30 (m, 4H), 8.63 (t, *J* = 5.2 Hz, 1H), 8.74 (t, *J* = 5.1 Hz, 1H), 11.54 (bs, 2H).

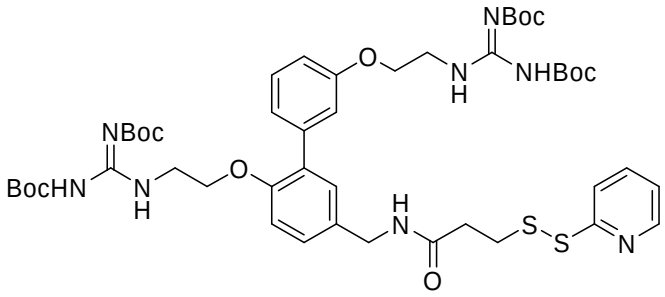
^{13}C-NMR ($CDCl_3$): δ 28.1, 28.3, 40.1, 40.3, 45.9, 66.3, 67.1, 79.3, 83.0, 83.1, 112.9, 113.2, 115.6, 123.0, 127.3, 128.9, 129.9, 130.9, 136.2, 139.7, 152.9, 153.0, 154.2, 156.3, 156.3, 158.4, 163.5, 163.5.

Microanalysis: $C_{39}H_{59}N_7O_{10}\cdot H_2O$: %C 58.60 (58.27), %H 7.4 (7.65), %N 12.20 (11.91)

IR ν_{Max} KBR/cm^{-} 3338 (br NH, NH_2), 1722 (C=ON), 1641 (C=O)

Mp 124-125^{o}C

***N*-{2, 3'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-5-ylmethyl}-3-[2-pyridyl)dithio]propionamide**



To a solution of 2, 3'-Bis{2-[N, N'-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-(aminomethyl)biphenyl (0.10 g, 0.12 mmol) and *i*-Pr_2EtN (40 µL, 1eq) in DCM (1.5 mL) was added N-succinimidyl-3-(2-pyridyldithio)propionate **9** (44 mg, 0.12 mmol) and the mixture was stirred in the dark for 24 h. The solvent was removed under vacuum and the crude oil was purified by preparative TLC (ethyl acetate/hexane 1/1) to afford the title compound (25 mg, 21% yield).

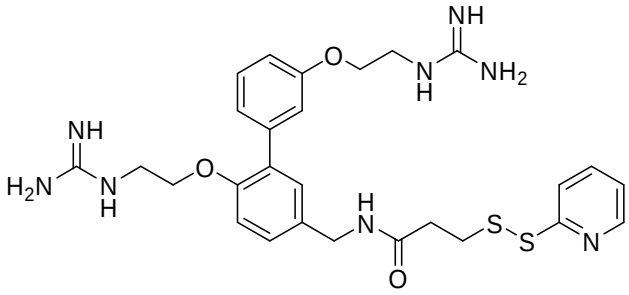
ESMS; *m/z* 983(M+1), 492(M+2)

^{1}H-NMR ($CDCl_3$): δ 1.49 (m, 36H), 2.62 (t, *J* = 6.5 Hz, 2H), 2.72 (t, *J* = 6.5 Hz, 2H), 3.76 (m, 2H), 3.83 (m, 2H), 4.08 (m, 4H), 4.45 (d, *J* = 5.4 Hz, 2H), 6.95 (m, 4H), 7.21 (m, 4H), 7.55 (m, 2H), 8.10 (d, *J* = 4.8 Hz, 1H), 8.46 (t, *J* = 4.7 Hz, 1H), 8.74 (bs, 1H), 11.47 (bs, 2H).

^{13}C-NMR (MeOH-d4): 28.0, 28.3, 34.5, 35.3, 39.5, 39.8, 43.3, 66.3, 67.0, 79.3, 81.3, 83.1, 110.5, 111.2, 113.3, 120.5, 120.9, 122.8, 128.1, 128.9, 130.9, 131.0, 1369, 149.5, 151.2, 153.0, 156.3, 158.8, 159.1, 163.5, 170.5.

IR ν_{Max} KBR/cm^{-}3332 (NH), 1722 (C=O), 1641 (C=O)

***N*-[2, 3'-Bis(2-guanidino-ethyloxy)-biphenyl-5-ylmethyl]-3-[2-pyridyl)dithio]propionamide, trifluoroacetate salt <u>33</u>**



N-{2, 3'-Bis{2-[*N*, *N*'-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-5-ylmethyl}-3-[2-pyridyl)dithio]propionamide (25 mg, 0.025 mmol) was dissolved in a mixture of TFA/H_2O/triisopropylsilane 95/2.5/2.5 (1 mL) and stirred at room temperature for 3 h. The solvent was removed under vacuum to give **<u>33</u>** as colourless oil (20 mg, 80%).

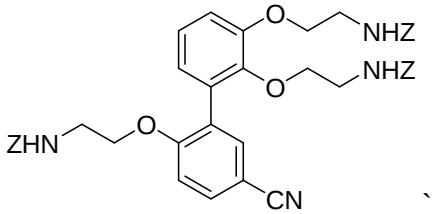
ESMS; *m/z* 582(M+1), 292(M+2)

^{1}H-NMR (MeOH-d4): δ 2.65 (t, *J* = 6.7 Hz, 2H), 3.07 (t, 2H, *J* = 6.7 Hz, 2H), 3.50 (m, 2H), 3.60 (m, 2H), 4.10 (m, 4H), 4.35 (s, 2H), 6.91 (d, *J* = 7.4 Hz, 1H), 7.10 (m, 7H), 7.74 (m, 2H), 8.35 (d, *J* = 4.7 Hz, 1H).

^{13}C-NMR (MeOH-d4): δ26.9, 29.7, 40.4, 67.2, 67.7, 104.6, 112.8, 115.4, 116.8, 119.0, 121.0, 128.1, 128.3, 128.6, 129.6, 131.4, 133.1, 134.9, 135.9, 137.1, 156.0, 156.8, 158.7.

IR ν_{Max} KBR/cm^{-}3376 (NH), 1681(C=ON)

Tribenzyl 2,2',2''-(5-cyanobiphenyl-2,2',3'-triyltris(oxy))tris(ethane-2,1-diyl)tetracarbamate 34

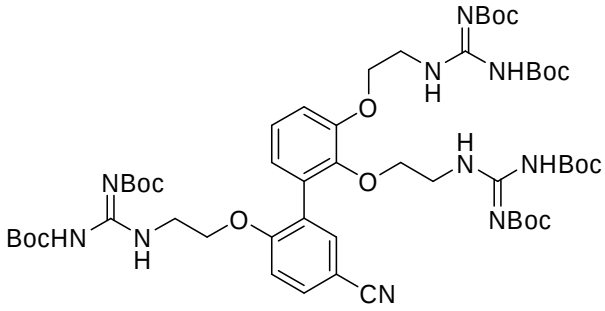


A degassed mixture of *N*-(2-(4-cyanophenoxy)ethyl)-2-phenylacetamide **29** (1.0 g, 2.67 mmol) 2,2'-(3-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1,2-phenylene)bis(oxy)bis (ethane-2,1-diyl)dicarbamate **6** (2.07 g, 3.47 mmol), $PdCl_2dppf.CH_2Cl_2$ (100 mg, 4 mol%), potassium phosphate (1.13 g, 5.34 mmol), toluene (2 mL) and water (0.133 mL) was heated at 100°C for 18 h. The reaction mixture was diluted in EtOAc (25 mL) and water (25 mL) was added. The layers were separated and the aqueous layer was extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over MgSO4, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (75/35) as eluent to afford **34** (1.9 g, 93% yield).

^{1}H-NMR ($CDCl_3$): δ 3.15 (m, 2H), 3.38 (m, 2H), 3.46 (m, 2H), 3.60 (m, 2H), 3.68 (m, 2H), 4.12 (m, 4H), 5.00 (bs, 2H), 5.03 (bs, 2H) 5.07 (bs, 2H), 5.25 (bs, 1H), 5.32 (bs, 1H), 5.97 (bs, 1H), 6.61-6.93 (m, 6H), 7.07-7.61 (m, 15H).

IR ν_{Max} KBR/cm 3335(br, NH), 2225(CN), 1714 (C=O)

2, 2', 3'-Tris{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-cyanobiphenyl



Tribenzyl 2,2',2"-(5-cyanobiphenyl-2,2',3'-triyltris(oxy))tris(ethane-2,1-diyl) tetracarbamate **34** (1.6 g, 2.11 mmol) was dissolved in DCM (10 mL) and HBr (30% in acetic acid, 10mL) was added dropwise. After stirring at room temperature for 2 h, water

(25 mL) was added to the mixture, the layers were separated and the aqueous layer was washed with DCM (2 x 25 mL). The water was then removed under vacuum and the crude diamine was carefully dried under vacuum for several hours.

ESMS; *m/z* 357 (M+1)

^{1}H-NMR (MeOH-d_4): δ 3.05 ((t, *J* = 5.1 Hz, 2H), 3.30 (m, 2H), 3.52 (t, *J* = 4.7 Hz, 2H), 3.98 (t, *J* = 5.3 Hz, 2H), 3.68 (m, 2H), 4.12 (m, 4H), 5.00 (bs, 2H), 5.03 (bs, 2H) 5.07 (bs, 2H), 5.25 (bs, 1H), 5.32 (bs, 1H), 5.97 c, 6.99 (dd, *J* = 6.9, 2.3Hz, 1H), 7.17-7.26 (m, 2H), (d, *J* = 8.6 Hz, 1H), 7.65 (s, 1H), 7.80 (dd, *J* = 8.6, 2.1 Hz, 1H).

The resulting solid was suspended in DCM (10 mL) and *i*-Pr_2EtN (1.6 mL) and N, N-di-boc-N'-trifluoromethanesulfonyl-guanidine **8** (2.47 g, 3eq) were added. The mixture was stirred overnight at room temperature, diluted with DCM (40 mL) then washed with 2M $NaHSO_4$ (25 mL) followed by sat. aq. $NaHCO_3$ (25 mL) and brine (25 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using cyclohexane/EtOAc (80/20) as eluent to afford the title compound (1.3 g, 57% yield) as a white solid.

ESMS; *m/z* 1084(M+1), 542(M+2)

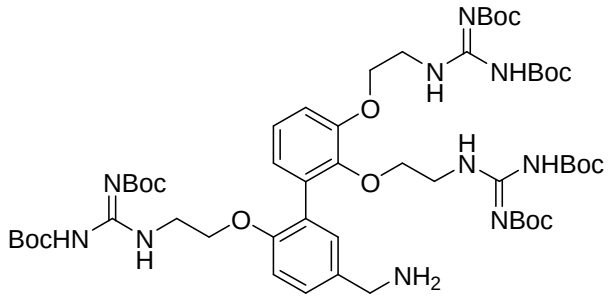
^{1}H-NMR ($CDCl_3$): δ1.43-1.53 (m, 54H), 3.43 (m, 2H), 3.73 (m, 2H), 3.84 (m, 2H), 3.91 (m, 2H), 4.07-4.18 (m, 5H), 6.88 (m, 1H), 6.97 (m, 1H), 7.06 (m, 2H), 7.56 (bs, 1H), 8.41 (bt, 1H), 8.44 (bt, 1H), 8.80 (bt,1H), 11.42 (bs, 1H), 11.47 (bs, 1H), 11.10 (bs, 1H).

^{13}C-NMR ($CDCl_3$): δ 27.8, 28.0, 28.1, 28.3, 38.9, 39.7, 40.6, 66.5, 71.0, 73.5, 79.3, 82.8, 83.1, 85.9, 104.9, 112.7, 114.7, 124.5, 124.1, 127.7, 130.8, 135.9 , 145.8, 149.0, 151.4, 153.0, 156.2, 156.3, 159.5, 163.4.

IR ν_{Max} KBR/cm^{-} 3326 (br NH, NH_2), 2226 (CN), 1724 (C=O), 1619 (C=O)

Mp 74-75^{o}C

2, 2', 3'-Tris{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-(aminomethyl)biphenyl



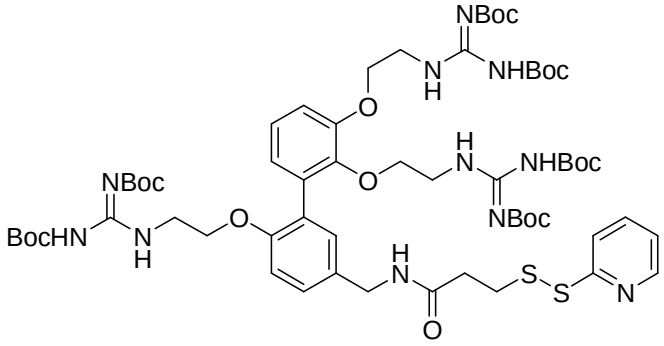
2,2',3'-Tris{2-[*N,N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-cyanobiphenyl (0.100 g, 0.092 mmol) was dissolved in THF (1.2 mL) and NH_4OH (0.30 mL) was added followed by Raney Nickel (0.010 g). The mixture was stirred vigorously under H_2 atmosphere for 16 h. The catalyst was filtered off and washed several times with MeOH. Diethylenetriamine (1eq) was added and the mixture stirred for 30 minutes then concentrated under vacuum. The residue was dissolved in EtOAc (10 mL) and washed with sat. aq. $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated under vacuum. The residue was then purified by flash chromatography using MeOH/DCM (5/95) as eluent to afford the title compound (70 mg, 69% yield).

^{1}H-NMR ($CDCl_3$): δ1.47 (m, 54H), 3.38 (m, 2H), 3.70 (m, 2H), 3.79 (m, 4H), 3.89 (m, 2H), 3.70 (m, 2H), 4.01 (m, 2H), 6.70-6.95 (m, 4H), 7.05 (m, 2H), 8.34 (bt, 1H), 8.42 (bt, 1H), 8.80 (m, 1H), 11.43 (m, 2H), 11.46 (bs, 1H).

^{13}C-NMR ($CDCl_3$): δ 28.1, 28.9, 30.34, 38.72, 40.1, 41.5, 41.4, 45.8, 50.6, 66.7, 67.2, 68.1, 71.0, 72.4, 78.8, 79.2, 82.6, 82.8, 83.0, 112.8, 123.8, 124.4, 127.1, 127.2, 128.5, 131.0, 131.4, 132.5, 136.0, 145.2, 151.6, 152.8, 152.9, 154.3, 156.0, 156.3, 156.3, 163.4.

IR ν_{Max} KBR/cm$^-$ 3335 (br NH, NH_2), 1722 (C=O), 1641 (C=ON)

***N*-{2, 2',3'-Tris{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-5-ylmethyl}-3-[2-pyridyl)dithio]propionamide**



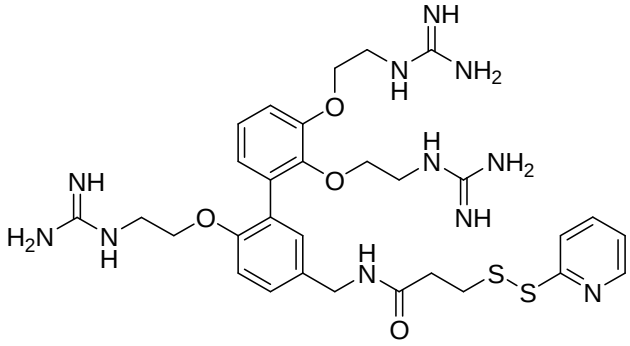
To a solution of 2,2',3'-Tris{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-5-(aminomethyl)biphenyl (50 mg, 0.045 mmol) and *i*-Pr_2EtN (10 µL, 0.10 mmol) in DCM (0.7 mL) was added N-succinimidyl-3-(2-pyridyldithio)propionate **10** (13 mg, 0.045 mmol) and the mixture was stirred in the dark for 24 h. The solvent was removed under vacuum and the crude oil was purified by preparative TLC (ethyl acetate/hexane 35/75) to afford the title compound (35 mg, 60 % yield).

ESMS; *m/z* 1285 (M+1), 643 (M+2), 429 (M+3).

^{1}H-NMR ($CDCl_3$): δ1.43-1.53 (m, 54H), 2.64 (t, *J* = 7.1 Hz, 2H), 3.10 (t, *J* = 6.8 Hz, 2H), 3.40 (m, 2H), 3.71 (m, 2H), 3.83 (m, 2H), 3.91 (m, 2H), 4.03 (m, 2H), 4.15 (m, 2H), 4.38 (d, *J* = 5.6 Hz, 2H), 6.84-7.06 (m, 7H), 7.23 (m, 1H), 7.61 (m, 2H), 8.31 (dd, *J* = 4.1, 1Hz, 1H), 8.39 (bt, 1H), 8.52 (bt, 1H), 8.80 (bt,1H), 11.40 (bs, 1H), 11.45 (bs, 1H), 11.05 (bs, 1H).

^{13}C-NMR ($CDCl_3$): δ21.0, 25.5, 28.0, 28.1, 28.3, 34.7, 35.6, 40.0, 41.3, 43.0, 60.3, 66.7, 67.1, 70.6, 79.1, 79.2, 82.9, 83.0, 112.1, 112.7, 119.9, 120.7, 123.8, 124.3, 127.0, 128.8, 130.5, 131.4, 132.7, 136.9, 145.4, 1495, 151.6, 152.8, 153.0, 154.8, 156.1, 156.2, 156.3, 159.7, 163.3, 163.5, 170.5.

***N*-[2, 2', 3'-Tris(2-guanidino-ethyloxy)-biphenyl-5-ylmethyl]-3-[2-pyridyl)dithio]propionamide, trifluroacetate salt <u>35</u>**



N-{2, 3'-Bis{2-[*N, N'*-bis(tert-butoxycarbonyl)guanidino]-ethyloxy}-biphenyl-5-ylmethyl}-3-[2-pyridyl)dithio]propionamide (6 mg) was dissolved in a mixture of TFA/ H_2O/triisopropylsilane 95/2.5/2.5 (1 mL) and stirred at room temperature for 3 h. The solvent was removed under vacuum to give **<u>35</u>** as colourless oil (4 mg, 60%).

ESMS; *m/z* 343 (M+2)

^{1}H-NMR (MeOH-d_4): δ 2.68 (t, *J* = 6.8Hz, 2H), 3.07 (t, *J* = 6.7 Hz, 2H), 3.20 (m, 2H), 3.42 (m, 2H), 3.65 (m, 2H), 3.90 (m, 2H), 4.08 (m, 2H), 4.17 (m, 2H), 4.37 (bs, 2H), 6.86 (m, 1H), 7.05-7.29 (m, 6H), 7.73 (m, 2H), 8.37 (m, 1H).

^{13}C-NMR ($CDCl_3$): δ 35.4, 35.8, 42.3, 43.0, 43.6, 68.3, 68.8 67.1, 72.7, 79.1, 114.1, 114.5, 121.2, 122.4, 125.0, 125.6, 128.9, 129.1, 130.9, 132.7, 134.6, 139.1, 146.5, 150.4, 152.7, 156.1, 159.3, 161.0, 173.6.

Theoretical Mass: (M + H) 684.28572. Measured Mass: (M + H) 684.28417

IR ν_{Max} KBR/cm^{-}3412 (br NH, NH_2), 1677 (C=ON)

Biology: experimental methods

Protein expression and coupling to xG-SMoC and Alexa Fluor 488

Geminin and 15k were expressed and purified as described.[3] We conjugated geminin with Alexa Fluor 488 using the Molecular Probes Protein Labelling kit, according to the manufacturer's protocol (Invitrogen). Coupling reactions of proteins with the different SMoCs have been described in Okuyama et al. 2007 (ref 3). The efficiency of coupling was assessed by spectrophotometric quantification of pyridyl at 343 nm and monitoring release of sulfhydryl groups using Ellman's Reagent (Pierce, Rockford, IL, USA) according to Pierce protocol 22582.

Cell Culture

Human U2OS and WI-38 human diploid fibroblasts (HDF), were cultured in DMEM supplemented with 10% FCS, 100 U/mL penicillin and 0.1 mg/mL streptomycin.

Subcellular localization studies

For detection of different xG-SMoC-geminin-AlexaFluor488 uptake into live cells, exponentially growing cells (WI-38 HDF and U2OS) were cultured on glass coverslips. Cells were washed in PBS, and incubated with fresh medium containing 10 μM xG-SMoC-Geminin-AlexaFluor488. Coverslips were washed extensively in PBS, placed in a plate containing medium without Red Phenol (Gibco) and observed by live confocal fluorescence microscopy (MP-UV, Leica Microsystems GmbH, Wetzlar, Germany) using 40× and 63× water immersion objectives. In order to obtain similar fluorescence intensities, WI-38, and U2OS cells required incubation with the protein conjugate for 1 and 5 hours respectively.

Fluorescence detection by FACS analysis

To evaluate and compare the ability of different SMoCs to carry biomolecules into different human cells and provide a measure of the efficiency of cell penetration we treated xG-SMoC-geminin-AlexaFluor488 molecules with WI-38 and U2OS.

Cells were seeded in 6-well plates and incubated with 10 μM xG-SMoC-geminin-AlexaFluor488 for 3 h (WI-38) or 5 h (U2OS).

Cells were then washed extensively with PBS, trypsinised, resuspended in medium, washed again in cold PBS and immediately analysed by Flow Cytometry (FACSCalibur). A total of 10,000 cells per sample were counted. The mean fluorescence intensity of AF488-15k-SMoCs treated cell populations was compared with untreated control cells and cells incubated with AF488 or AF488-15k only.

Cell proliferation assay

To assess whether 15k geminin retains its ability to inhibit DNA replication in cycling cells when coupled with xG-SMoC, 10 μM of xG-SMoC-15k was added to WI-38 and U2OS and replenished every 24 h, for a total 72 h treatment. Seventy-one hours after the initial treatment, cells were pulse-labelled for 1 h with 10 μM BrdU, then fixed with 4% paraformaldehyde, and treated as described.[3] Confocal fluorescence microscopy was performed on a Leica TCS DMRE confocal microscope.

Reference List

[1] M. Albrecht, *Synthesis-Stuttgart* **1996,** 230-&.

[2] R. V. Stevens, G. S. Bisacchi, *Journal of Organic Chemistry* **1982,** *47* 2396-2399.

[3] M. Okuyama, H. Laman, S. R. Kingsbury, C. Visintin, E. Leo, K. L. Eward, K. Stoeber, C. Boshoff, G. H. Williams, D. L. Selwood, *Nat.Methods* **2007,** *4* 153-159.

Figure S1

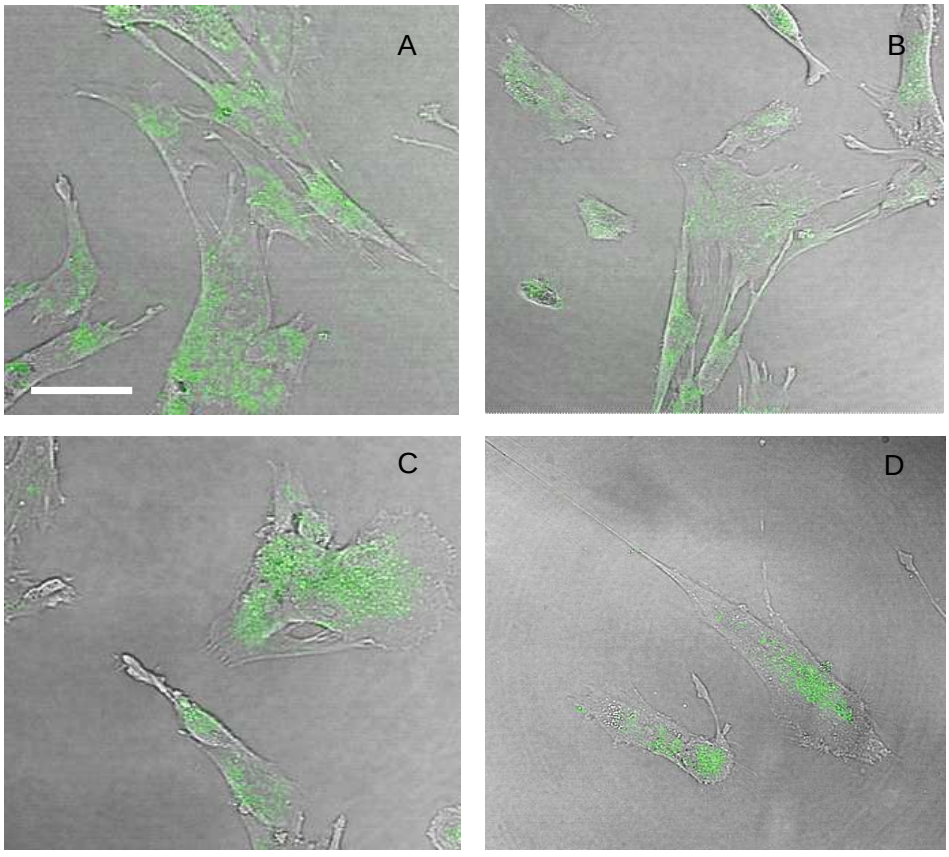


Fig. S1. Live microscopy Visualisation of delivery of SMoC analogues coupled to geminin labelled with Alexa Fluor 488 in WI-38 HDF. Final concentrations of geminin 10 μM. Analogues are as in Fig. 2 in the manuscript.