Electronic Supplementary Information

for

Synthesis and Cellular Uptake Properties of Guanidine-containing Molecular Transporters built on the Sucrose Scaffold

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Experimental Section

I. Synthesis

General Methods. All non-hydrolytic reactions were carried out in oven-dried glassware under an inert atmosphere of dry argon or nitrogen. All commercial chemicals were used as received except for solvents, which were purified and dried by standard methods prior to use. Analytical TLC was performed on a Merck 60 F254 silica gel plate (0.25mm thickness), analytical reverse-phase TLC on a Merck RP-8 F254s, and visualization was done with UV light (254nm and 365nm), or by spraying with a 5% solution of phosphomolybdic acid or ninhydrin solution followed by charring with a heat gun. Column chromatography was performed on Merck 60 silica gel (70-230 or 230-400 mesh), and MPLC was performed on Fluka 100 C_8-reversed phase silica gel. NMR spectra were recorded on a Bruker DPX 300 (^{1}H-NMR at 300MHz; ^{13}C-NMR at 75 MHz) and Bruker DRX 500 (^{1}H-NMR at 500MHz; ^{13}C-NMR at 125MHz) spectrometers. Tetramethysilane was used as reference for ^{1}H NMR, and the chemical shifts were reported in δ ppm and the coupling constants in Hz. Analytical HPLC was performed on Agilent 1100-HPLC Chemstation with an analytical column ZORBAX SB-C8 (5μm, 4.6mm ID x 25cm). Low resolution mass spectra were determined on a Micromass PLATFORM ll (EI and FAB). High resolution mass spectra were determined on a JMS-700, and MALDI-TOF mass spectra on a Voyager-DE STR system at the Korea Basic Science Support Center. The standard extractive work-up procedure consisted of pouring into a large amount of water, extracting with organic solvent indicated, washing the combined extracts successively with water and brine, drying the extract over anhydrous Na_2SO_4 or $MgSO_4$, and evaporating the solvent.

2,2,2-Trifluoroethyl 3-bromopropionate (1): To a solution of 2,2,2-trifluoroethanol (15 mL, 208 mmol) in CH_2Cl_2 (30mL) at 0°C, were added triethylamine (4.5 mL, 32 mmol) and 3-bromopropionyl chloride (2.94 mL, 29 mmol). The mixture was stirred at rt for 5 h. The reaction was quenched with water. The standard extractive workup with EtOAc gave the crude product of which an impurity was precipitated by addition of hexane followed by filtration to give **1** (3.53g, 52 %) as yellow liquid. R_f 0.6 (n-Hex : CH_2Cl_2 = 1:2, twice); ^{1}H-NMR ($CDCl_3$): δ 3.05 (t, *J* = 6.7Hz, 2H, $\underline{CH_2}$Br), 3.60 (t, *J* =6.7Hz, 2H, CO$\underline{CH_2}$), 4.54 (q, *J* =8.4Hz, 2H, $\underline{CH_2}CF_3$) ppm; ^{13}C-NMR($CDCl_3$) δ 20.89, 25.23, 37.20, 60.41, 169.20 ppm; MS (FAB): m/z calcd for $C_5H_6BrF_3O_2$ 233.95, found 233.78 $[M]^+$.

2,2,2-Trifluoroethyl 3-azidopropionate (2): To a solution of **1** (3.71 g, 15.8 mmol) in dimethylform amide (DMF, 15 mL), were added sodium azide (2.72 g, 63.2 mmol) and tetrabutylammonium iodide (TBAI, 0.69 g, 3.2 mmol). The mixture was stirred at rt for 12 h. The reaction was quenched with water. The standard extractive workup with EtOAc gave the crude product of which an impurity was precipitated by addition of hexane followed by filtration to give **2** (2.76g, 89 %) as yellow liquid. R_f 0.3 (EtOAc: n-Hex = 1:4); ^{1}H-NMR ($CDCl_3$): δ 2.70 (t, *J* = 6.4 Hz, 2H, $\underline{CH_2}N_3$), 3.63 (t, *J* =6.4Hz, 2H, CO$\underline{CH_2}$), 4.54 (q, *J* =8.4Hz, 2H, $\underline{CH_2}CF_3$) ppm; ^{13}C-NMR ($CDCl_3$) δ 21.21, 33.65, 46.60, 60.58, 169.21 ppm; HRMS (FAB) m/z calcd for $C_5H_{67}F_3N_3O_2$ 198.0578, found 198.0486 $[M+H]^+$.

1'-*O*-(3-Azidopropionyl)sucrose (3): To a solution of sucrose (4.70 g, 13.73 mmol) in DMF-H_2O (VH_2O/Vtot=7 %, 11.6 mL) were added **2** (0.90 g, 4.56 mmol) and PN (Proteinase N which is commercially available in Fluka, 7.2 U/mg, 691 mg); lyophilized enzyme was prepared by dissolving the crude enzyme in water (5 mg/mL), adjusting pH 7.8 with 0.1 M KOH and then freeze-drying the solution. The mixture was stirred at 45°C for 3 days. The mixture was filtered and filtrate was evaporated to give the crude product, which was purified by column chromatography on silica gel to obtain **3** (1.25 g, 62 %) as a white foamy solid. R_f= 0.33 (CH_2Cl_2:Me_2CO:MeOH:H_2O=14:5:5:1); $[\alpha]_D^{25}$ 46.84 (*c* 1.00 in MeOH); ^{1}H-NMR (CD_3OD): δ 2.65 (t, *J* = 6.3Hz, 2H, Hβ), 3.30-3.43 (m, 2H, H2, H4), 3.59 (t, *J* =6.3Hz, 2H, Hα), 3.65-3.83 (m, 7H, H5, H6a, H6´a, H´5, H6b, H3, H6´b), 4.02-4.09 (m, 2H, H3´, H4´), 4.21 (d, *J* = 12.0 Hz, 1H, H1´b), 4.41 (d, *J* = 12.0 Hz, , H1´a), 5.40 (d, *J* =3.8 Hz, 1H, H1) ppm; ^{13}C-NMR (CD_3OD): δ 33.64, 46.89, 61.18, 62.18, 63.28, 70.35, 72.00, 73.42, 73.56, 73.92, 77.83, 82.76, 93.11, 102.89, 171.18 ppm; HRMS (FAB) m/z calcd for $C_{15}H_{25}N_3O_{12}Na$ 462.1336, found 462.1336 $[M+Na]^+$.

A representative per-acylation with *N*-Boc-protected-aminocarboxylic acid: A solution of **3** (200 mg, 0.46 mmol), 6-aminohexanoic acid (2.11g, 9.10 mmol), EDC (1.75 mg, 9.10 mmol) and DMAP(84 mg, 0.68 mmol) in DMF (8 mL) was stirred at rt for 36 h under N_2, treated with EtOAc, and washed several times with saturated $NaHCO_3$, water and brine. The organic phase was dried and concentrated to give the crude product, which was purified by column chromatograghy on silica gel to afford **4** (772 mg, 88 %) as colorless sticky oil.

1'-*O*-(3-Azidopropionyl)-2,3,3',4,4',6,6'-hepta-*O*-[6-*N*-(*t*-butyloxycarbonyl)-aminohexanoyl]sucrose (4): Colorless sticky oil; R_f 0.67 (EtOAc: n-Hex = 1:1); ^{1}H-NMR ($CDCl_3$): δ1.23-1.65 (m, 105H), 2.20-2.46 (m, 14H), 2.66 (t, *J* =6.4 Hz, 2H), 3.09-3.11 (m, 14H), 3.62 (t, *J* =6.5 Hz, 2H), 4.09-4.37 (m, 8H), 4.59-4.95 (brs, 6H), 4.88 (dd, *J* = 10.4, 3.5 Hz, 1H), 5.08 (t, *J* =9.7 Hz, 1H,), 5.35-5.49 (m, 3H), 5.64 (d, *J* = 3.7Hz, 1H) ppm; ^{13}C-NMR ($CDCl_3$): δ 24.88, 26.55, 28.82, 30.11, 31.80, 34.01, 36.87, 40.83, 46.91, 60.75, 61.79, 63.66, 64.00, 68.10, 69.03, 69.91, 70.38, 74.63, 75.48, 79.39, 170.45, 172.31, 172.66, 172.86, 172.90, 173.43, 173.59 ppm; MALDI-TOF MS: *m/z* calcd for $C_{92}H_{158}N_{10}O_{33}Na$ 1954.089; found 1954.062 $[M+Na]^+$.

1'-*O*-(3-Azidopropionyl)-2,3,3',4,4',6,6'-hepta-*O*-[6-*N*-(*t*-butyloxycarbonyl)-aminooctanoyl]sucrose (5): Colorless sticky oil (712 mg, 74 %); R_f 0.85 (EtOAc: n-Hex = 1:1); ^{1}H-NMR ($CDCl_3$): δ1.31-1.60 (m, 133H), 2.18-2.41 (m, 14H), 2.65 (t, *J* =6.4Hz, 2H), 3.07-3.11 (m, 14H), 3.60 (t, *J* =6.4Hz, 2H), 4.09-4.30 (m, 8H), 4.41-4.82 (brs, 6H), 4.87 (dd, *J* = 10.3, 3.6Hz, 1H), 5.09 (t, *J* = 9.6Hz, 1H,), 5.37-5.48 (m, 3H), 5.64 (d, *J* = 3.6Hz, 1H) ppm; ^{13}C-NMR ($CDCl_3$): δ 24.97, 25.16, 28.82, 29.34, 30.40, 34.06, 41.08, 46.94, 61.78, 63.84, 68.12, 69.04, 69.84, 70.41, 74.60, 75.54, 79.38, 90.12, 103.82, 156.42, 170.33, 172.40, 172.80, 172.89, 173.05, 173.53, 173.70 ppm; MALDI-TOF MS: *m/z* calcd for $C_{106}H_{186}N_{10}O_{33}Na$ 2150.308; found 2150.265 $[M+Na]^+$.

A representative procedure for the *N*-Boc-deprotection: To the HCl (gas) saturated solution of EtOAc (5 mL) at rt, was added **4** (134 mg, 0.069 mmol), and the solution was stirred for 3 h. The precipitate was separated and dried under vacuum to give the white hydrochloride salt **6** (101 mg, quant.).

1'-*O*-(3-Azidopropionyl)-2,3,3',4,4',6,6'-hepta-*O*-[6-aminohexanoyl hydrochloride]sucrose (6): White solid salt; ^{1}H-NMR (CD_3OD): δ1.28 -1.58 (m, 42H), 2.19-2.39 (m, 14H), 2.58 (t, *J* =6.2Hz, 2H), 2.83-2.90 (m, 14H), 3.52 (t, *J* =6.2Hz, 2H), 4.05-4.22 (m, 8H), 4.77-4.94 (m, 1H), 5.01 (t, *J* = 9.8Hz, 1H), 5.29-5.47 (m, 3H), 5.62 (d, *J* = 3.6Hz, 1H) ppm; ^{13}C-NMR (CD_3OD): δ 24.44, 26.03, 27.26, 33.51, 33.63, 33.72, 33.85, 33.91, 39.78, 47.09,51.26, 62.01, 63.78, 63.90, 68.36, 69.09, 70.05, 70.57, 74.75, 75.95, 79.22, 90.09, 103.80, 171.08, 172.56, 172.64, 172.89, 172.69, 172.89, 173.02, 173.21, 173.68, 173.76, 174.73 ppm; MALDI-TOF MS: *m/z* calcd for $C_{57}H_{102}N_{10}O_{19}Na$ 1253.722; found 1253.920 $[M+Na]^+$.

1'-*O*-(3-Azidopropionyl)-2,3,3',4,4',6,6'-hepta-*O*-[6-aminooctanoyl hydrochloride]sucrose (7): White solid salt (127 mg, quant.); ^{1}H-NMR (CD_3OD): δ1.38 -1.66 (m, 70H), 2.25-2.44 (m, 14H), 2.66 (brs, 2H), 2.89-2.91 (m, 14H), 3.60 (t, *J* =5.8Hz, 2H), 4.09-4.40 (m, 8H), 4.73-4.93 (m, 1H), 5.08 (t, *J* = 9.6Hz, 1H,), 5.39-5.54 (m, 3H), 5.70 (d, *J* = 3.3 Hz, 1H) ppm; ^{13}C-NMR (CD_3OD): δ 24.79, 26.35, 27.53, 28.96, 33.69, 39.78, 46.94, 60.12, 60.54, 63.73, 68.34, 69.04, 69.96, 70.52, 75.84, 90.02, 103.78, 170.89, 172.74, 173.00, 173.25, 173.78, 173.88 ppm; MALDI-TOF MS: *m/z* calcd for $C_{71}H_{130}N_{10}O_{19}Na$ 1449.941; found 1449.710 $[M+Na]^+$.

A representative procedure for guanidinylation: To a solution of **6** (101 mg, 0.068 mmol) in a mixed solvent (1,4-dioxane:water=5:1, 5 mL), were added Et_3N (0.30 mL, 2.037 mmol) and *N,N′*-di-Boc-*N″*-trifluoromethanesulfonylguanidine (532 mg, 1.358 mmol), and the reaction mixture was stirred at rt for 3 days. The mixture was diluted with EtOAc, and washed with 1N-$NaHSO_4$, saturated $NaHCO_3$ and brine. The organic layer was separated, dried and concentrated to give the crude product, which was purified by column chromatography on silica gel to afford **8** (133 mg, 67 %) as a white foamy solid.

1'-*O*-(3-Azidopropionyl)-2,3,3',4,4',6,6'-hepta-*O*-[*N,N′*-bis-Boc-*N″*-aminohexanoylguanidine] sucrose (8): White foamy solid; R_f 0.38 (EtOAc: n-Hex = 1:2); ^{1}H-NMR ($CDCl_3$): δ1.27 -1.69 (m, 168H), 2.19-2.44 (m, 14H), 2.64 (t, *J* =6.4Hz, 2H), 3.32-3.49 (m, 14H), 3.60 (t, *J* =6.4Hz, 2H), 4.08-4.36 (m, 8H), 4.86 (dd, *J* = 10.3, 3.6Hz, 1H), 5.07 (t, *J* = 9.6Hz, 1H,), 5.30-5.46 (m, 3H), 5.61 (d, *J* = 3.5Hz, 1H), 8.30 (brs, 7H), 11.49 (brs, 7H) ppm; ^{13}C-NMR ($CDCl_3$): δ 14.54, 21.38, 24.67, 26.65, 26.72, 28.41, 28.64, 29.10, 30.01, 33.92, 34.02, 34.07, 34.28, 41.04, 46.88, 60.72, 61.61, 63.57, 64.16, 67.98, 69.02, 69.82, 70.41, 74.79, 75.43, 79.51, 83.31, 90.13, 103.90, 153.60, 156.44, 170.33, 171.46, 172.18, 172.56, 172.66, 172.80,

173.38, 176.98 ppm; MALDI-TOF MS: *m/z* calcd for $C_{134}H_{228}N_{24}O_{47}Na$ 2948.61; found 2948.75 $[M+Na]^+$.

1'-*O*-(3-Azidopropionyl)-2,3,3',4,4',6,6'-hepta-*O*-[*N*,*N'*-bis-Boc-*N''*-aminohexanoylguanidine] sucrose (9): White foamy solid (383 mg, 77 %); R_f 0.59 (EtOAc: n-Hex = 1:2); ^{1}H-NMR ($CDCl_3$): δ1.27 -1.56 (m, 196H), 2.21 -2.37 (m, 14H), 2.65 (t, *J* =6.4Hz, 2H), 3.37-3.48 (m, 14H), 3.61 (t, *J* =6.5Hz, 2H), 4.07-4.38 (m, 8H), 4.87 (dd, *J* = 10.3, 3.6Hz, 1H), 5.10 (t, *J* = 9.6Hz, 1H,), 5.35-5.51 (m, 3H), 5.64 (d, *J* = 3.6Hz, 1H) 8.30 (brs, 7H), 11.49 (brs, 7H) ppm; ^{13}C-NMR ($CDCl_3$): δ 24.96, 27.09, 28.16, 28.25, 28.34, 28.43, 29.33, 34.04, 41.27, 46.91, 61.70, 63.70, 63.97, 68.04, 69.03, 69.79, 70.40, 74.73, 75.49, 76.62, 77.08, 79.50, 83.30, 90.15, 103.89, 153.65, 156.45, 163.98, 170.28, 172.34, 172.75, 172.82, 172.95, 173.00, 173.46, 173.51, 173.60 ppm; MALDI-TOF MS: *m/z* calcd for $C_{148}H_{256}N_{24}O_{47}Na$ 3144.83; found 3144.83 $[M+Na]^+$.

A representative procedure for reduction of the terminal azide moiety: A mixture of **8** (128 mg, 0.044 mmol) and 10 wt.% Pd/C (250 mg) in abs. EtOH was hydrogenated by using a hydrogenator apparatus (50 psi). After stirring for 3 h at rt, the catalyst was filtered and the filtrate was evaporated to give the crude product **10** (102 mg, 80 %) as a white foamy solid.

1'-*O*-(3-Aminopropionyl)-2,3,3',4,4',6,6'-hepta-*O*-[*N*,*N'*-bis-Boc-*N''*-aminohexanoylguanidine] sucrose (10): White foamy solid; R_f 0.40 (CH_2Cl_2:MeOH=15:1); ^{1}H-NMR ($CDCl_3$): δ1.18 -1.58 (m, 168H), 2.22-2.48 (m, 14H), 2.66-3.02 (m, 4H), 3.29 -3.48 (m, 14H), 4.02-4.38 (m, 8H), 4.87 (d, *J* = 10.0 Hz, 1H), 5.06 (t, *J* = 9.5Hz, 1H), 5.33-5.48 (m, 3H), 5.61 (brs, 1H), 8.30 (brs, 7H), 11.48 (brs, 7H) ppm; MALDI-TOF MS: *m/z* calcd for $C_{134}H_{230}N_{22}O_{47}Na$ 2922.618, found 2923.072 $[M+Na]^+$.

1'-*O*-(3-Aminopropionyl)-2,3,3',4,4',6,6'-hepta-*O*-[*N*,*N'*-bis-Boc-*N''*-aminohexanoylguanidine] sucrose (11): White foamy solid (179mg, 90 %); R_f 0.45 (CH_2Cl_2:MeOH=15:1); ^{1}H-NMR ($CDCl_3$): δ1.15 -1.86 (m, 196H), 2.13-2.51 (m, 14H), 2.65-3.07 (m, 4H), 3.38-3.53 (m, 14H), 4.09-4.42 (m, 8H), 4.87-4.96 (m, 1H), 5.08 (t, *J* = 9.6Hz, 1H,), 5.32-5.56 (m, 3H), 5.63 (s, 1H), 8.30 (s, 7H), 11.51 (s, 7H) ppm; MALDI-TOF MS: *m/z* calcd for $C_{148}H_{258}N_{22}O_{47}Na$ 3118.837; found 3118.480 $[M+Na]^+$.

A representative procedure for the FITC-I attachment: To a solution of **10** (76 mg, 0.026 mmol) in a mixed solvent THF and Abs. ethanol (5 mL, THF:EtOH=3:2), were added fluorescein-5-isothiocyanate (12 mg, 0.031 mmol) and triethylamine (12 μL, 0.078 mmol). The reaction mixture was stirred for 24 h at rt in dark, and concentrated. The crude product was purified by column chromatography on silica gel to afford **12** (53 mg, 62 %) as yellow sticky oil.

1'-*O*-(3-(*N*-fluoresceinyl-5-thioureido)-propionyl)-2,3,3',4,4',6,6'-hepta-*O*-[*N*,*N'*-bis-Boc-*N''*-aminohexanoylguanidine] sucrose (12): light-yellow sticky oil; R_f 0.50 (CH_2Cl_2:MeOH=20:1); ^{1}H-NMR ($CDCl_3$): δ1.18 -1.78 (m, 168H), 1.98-2.32 (m, 14H), 2.82-3.12 (m, 2H), 3.18-3.46 (brs, 14H), 3.70-4.44 (m, 10H), 4.75-5.07 (m, 2H), 5.18-5.46 (m, 3H), 5.56 (brs, 1H), 6.52-6.73 (m, 6H), 6.94 (s, 1H), 7.44 (br s, 1H), 8.28 (brs, 8H), 11.43 (brs, 7H) ppm; MALDI-TOF MS: *m/z* calcd for $C_{155}H_{241}N_{23}O_{52}SNa$ 3311.654; found 3311.292 $[M+Na]^+$.

1'-*O*-(3-(*N*-fluoresceinyl-5-thioureido)-propionyl)-2,3,3',4,4',6,6'-hepta-*O*-[*N*,*N'*-bis-Boc-*N''*-aminohexanoylguanidine] sucrose (13): light-yellow sticky oil (72 mg, 70 %); R_f 0.55 (CH_2Cl_2:MeOH=20:1); ^{1}H-NMR ($CDCl_3$): δ1.18 -1.63 (m, 196H), 2.10-2.43 (m, 14H), 2.82-3.12 (m, 2H), 3.32 (brs, 14H), 3.79-4.31 (m, 10H), 4.72-5.08 (m, 2H), 5.28-5.45 (m, 3H), 5.56 (s, 1H), 6.48-6.77 (m, 6H), 6.86-6.96 (m, 1H), 7.41-7.56 (m, 1H), 8.30 (brs, 8H), 11.43 (brs, 7H) ppm; MALDI-TOF MS: *m/z* calcd for $C_{169}H_{269}N_{23}O_{52}SNa$ 3507.873; found 3507.664 $[M+Na]^+$.

A representative procedure for the *N*-Boc-deprotection from the guanidine moiety: To a solution of **12** (53 mg, 0.016 mmol) in EtOAc (1 mL) at rt, was added the HCl (g) saturated solution of EtOAc (4 mL). After stirring for 20 h, the solution was concentrated and the residue was washed with a mixture of diethyl ether and MeOH (20:1) to remove less polar impurities. The residue was dried and purified by MPLC on reverse phase C-8 silica gel (H_2O/CH_3CN = 3:2 with 0.1 % TFA). The purified product was dissolved in de-ionized water, filtered through PTFE syringe filter, and lyophilized to give **14** (28 mg, 83 %) as a light greenish-yellow foamy solid (HCl salt).

1'-*O*-(3-(*N*-fluoresceinyl-5-thioureido)-propionyl)-2,3,3',4,4',6,6'-hepta-*O*-[aminohexanoylguanidinium] sucrose·7HCl (14): yellow foamy solid; UV λ_{max}(H_2O) 496 nm

[ε =11,100 ($cm^{-1}M^{-1}$)]; ^{1}H-NMR (CD_3OD): δ1.30 -1.74 (m, 42H), 2.30 -2.54 (m, 14H), 2.85-2.89 (m, 2H), 3.17-3.26 (m, 14H), 3.99 (t, *J* =6.0Hz, 2H), 4.16-4.38 (m, 8H), 4.91 (dd, *J* = 10.0, 3.0Hz, 1H), 5.13 (t, *J* = 9.5Hz, 1H,), 5.40-5.48 (m, 2H), 5.609 (d, *J* = 6.5 Hz, 1H), 5.74 (d, *J* = 3.5 Hz, 1H), 6.62 (d, *J* = 3.5 Hz, 3H), 6.71 (s, 3H), 7.22(d, *J* = 8.0 Hz, 1H), 7.71 (d, *J* = 7.0 Hz, 1H), 8.24 (s, 1H) ppm; MALDI-TOF MS: *m/z* calcd for $C_{85}H_{130}N_{23}O_{24}S$ 1888.940; found 1888.956 $[M+H]^+$ Analytical HPLC (ZORBAX-SB-C8) R_t = 3.36 min (Flow rate = 1mL/min, 220 nm, CH_3CN: H_2O = 40:60 containing 0.1 % TFA), purity 90+ %.

1'-*O*-(3-(*N*-fluoresceinyl-5-thioureido)-propionyl)-2,3,3',4,4',6,6'-hepta-*O*-[aminohexanoylguanidinium] sucrose·7HCl (15): yellow foamy solid (42 mg, 87 %); UV λ_{max}(H_2O) 497 nm [ε =13,200 ($c5m^{-1}M^{-1}$)]; ^{1}H-NMR (CD_3OD): δ1.32 -1.60 (m, 70H), 2.31-2.51 (m, 14H), 2.84-2.88 (m, 2H), 3.17-3.24 (m, 14H), 3.97 (brs, 2H), 4.16 -4.39 (m, 8H), 5.12-5.14 (m, 1H), 5.44-5.56 (m, 3H), 5.60 (d, *J* = 6.5, 1H), 5.75 (d, *J* = 3.5Hz, 1H), 6.56-6.72 (m, 6H), 7.18 (d, *J* = 8.0Hz , 1H), 7.72-7.79 (m, 1H), 8.29 (s, 1H) ppm; MALDI-TOF MS: *m/z* calcd for $C_{99}H_{158}N_{23}O_{24}S_{33}$ 2085.157; found 2085.407 $[M+H]^+$ Analytical HPLC (ZORBAX-SB-C8) R_t = 3.43 min (Flow rate = 1mL/min, 220 nm, CH_3CN: H_2O = 40:60 containing 0.1 % TFA), purity 90+ %.

1'-*O*-[3-(2-(6-Amino-1-methyl-3-oxo-7-sulfo-3,4-dihydro-naphthalen-2-yl)-acetylamino)-propionyl]-2,3,3',4,4',6,6'-hepta-*O*-[aminohexanoylguanidinium] sucrose·7HCl (16): white foamy solid (7 mg, 67 % over two steps); UV λ_{max}(H_2O) 347 nm [ε =2,800 ($cm^{-1}M^{-1}$)]; ^{1}H-NMR (CD_3OD): δ1.34 -1.59 (m, 70H), 1.91 (s, 3H), 2.27-2.44 (m, 14H), 2.61-2.64 (m, 2H), 2.84-2.91 (m, 2H), 3.14-3.16 (m, 14H), 3.40-3.42 (m, 2H), 4.18 -4.35 (m, 8H), 5.07-5.13 (m, 1H), 5.41-5.56 (m, 4H), 5.71 (brs, 1H), 6.63 (s, 1H), 8.07 (s, 1H) ppm; MALDI-TOF MS: *m/z* calcd for $C_{90}H_{156}N_{23}O_{25}S$ 1991.132; found 1991.088 $[M+H]^+$ Analytical HPLC (ZORBAX-SB-C8) R_t = 3.16 min (Flow rate = 1mL/min, 220 nm, CH_3CN: H_2O = 40:60 containing 0.1 % TFA), purity 85+ % .

1'-*O*-(3-(*N*-6-tetramethyl-rhodamine-5-thioureido)-propionyl)-2,3,3',4,4',6,6'-hepta-*O*-[aminohexanoylguanidinium] sucrose·7HCl (17): pinky red foamy solid (8 mg, 74 % over two steps); UV λ_{max}(H_2O) 551 nm [ε =17,200 ($cm^{-1}M^{-1}$)]; ^{1}H-NMR (CD_3OD): δ1.32 -1.60 (m, 70H), 2.23-2.38 (m, 20H), 2.84-2.88 (m, 8H), 3.14-3.18 (m, 14H), 3.44 (brs, 2H), 4.15 -4.33 (m, 8H), 5.07-5.13 (m, 1H), 5.40-5.51 (m, 4H), 5.71 (brs, 1H), 6.98 -8.25 (m, 9H, aromatic) ppm;

MALDI-TOF MS: *m/z* calcd for $C_{103}H_{168}N_{23}O_{22}S$ 2139.243; found 2139.276 $[M+H]^+$ Analytical HPLC (ZORBAX-SB-C8) R_t = 3.35 min (Flow rate = 1mL/min, 220 nm, CH_3CN: H_2O = 40:60 containing 0.1 % TFA), purity 80+ %.

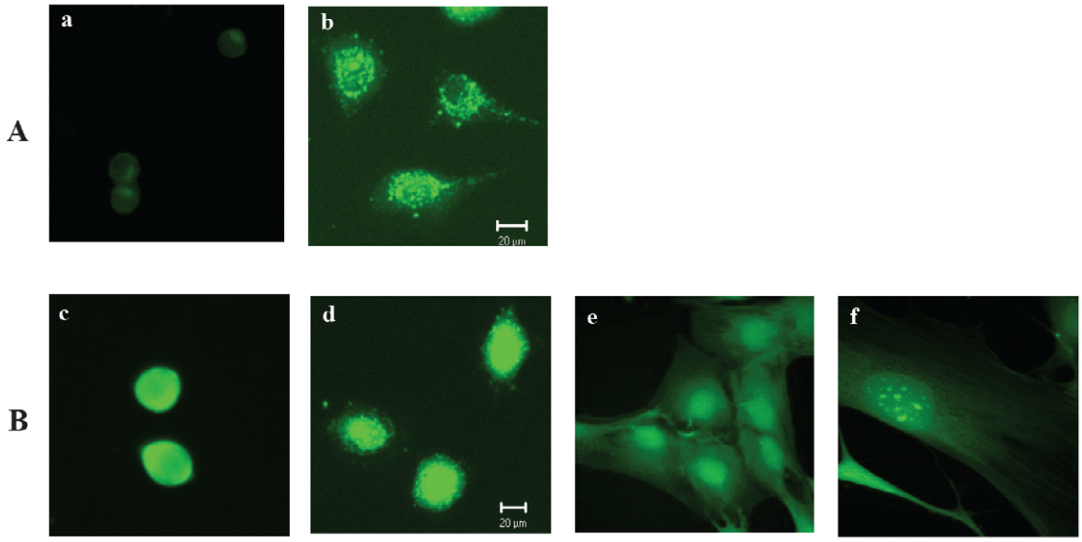
ll. Bioassays

Cell Culture. RAW264.7 cells were cultivated according to a literature procedure.[8] Human cervical cancer-derived HeLa cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM, Gibco) supplemented with 10 % (v/v) newborn calf serum (Gibco), 100 units/mL of penicillin, 100 μg/mL of streptomycin and 0.25 μg/mL of amphotercin B (Gibco) in an incubator at 37 °C, under an atmosphere containing air and 5 % CO_2.

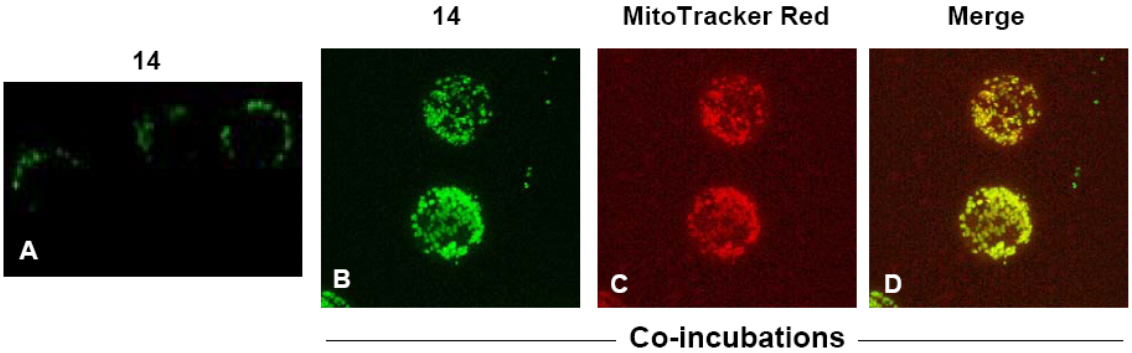
Imaging analysis. The cells (1.0×10^4 / well) were seeded into 96-well plate, cultured overnight and treated with compounds at a indicated concentration in the presence of 250 nM MitoTracker Deep Red (Molecular Probes) for 10 min. After washing with serum-free DMEM, images were captured by ImageXpress and analyzed by MetaXpress (Molecular Devices inc.).

Cellular uptake experiment. The cells (5.0×10^5) were seeded into 60 mm dish, cultured overnight and treated with compounds at a indicated concentration for 10 min. The cells were washed in basal DMEM, and incubated for 30 min. After trypsinization, the cells were harvested, centrifuged and resuspended in PBS, and the fluorescence was measured via flow cytometry. Treatment of only DMSO as a negative control and 500 nM Mito Tracker Deep Red or MitoTracker Green FM (Molecular Probes) was used as a positive control, respectively. Data represent uptake per cent for 10,000 cells per sample.

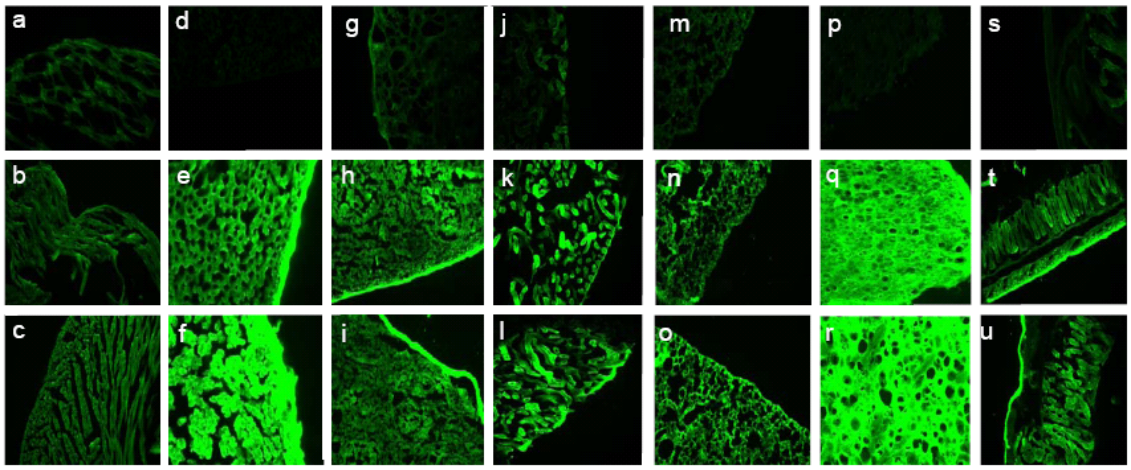
Protocols for the mouse tissue distribution study were previously described.[1]



Supplementary Figure S1. Fluorescence microscope images: A. a) RAW 264.7 cells (5 min), b) HeLa cells (15 min) were exposed to **14** (10 µM) at 37 °C. B. c) RAW 264.7 cells (10 µM for 5 min), d) HeLa cells (10 µM for 15 min), e) C2C12 cells (5 µM for 10 min), f) 3T3 cells (5 µM for 10 min) were exposed to **15** at 37 °C.



Supplementaty Figure S2. Colocalization of 14 with MitoTracker red. KG1a cells were (A) incubated with **14** (5µM) alone at 37˚C for 1 hour; (B-D) co-incubated for 1 hour at 37˚C with **14** (5µM) in the presence of MitoTracker red (5nM). The cells were then washed and imaged by CLSM. [We thank Professor Arwyn Jones of Cardiff University for the localization experiments in KG1a cells.]



Supplementary Figure S3. Tissue distribution of 14 (middle) and 15 (bottom) in mouse (top: control). Fluorescence Confocal microscope images of heart (a, b, c), spleen (d, e, f), liver (g, h, i), kidney (j, k. l), lung (m, n, o), brain (p, q, r) and stomach (s, t, u) tissue sections, isolated from mice 20 min after i.p. injection. Exposure time (ms): 1,000 (a, b, c), 1,000 (d, e, f), 2,500 (g, h, i), 2,500 (j, k, l), 5,000 (m, n, o), 10,000 (p, q, r), 2,000 (s, t, u); λ_{max}=488 nm (green fluorescence from FITC, magnification x 200)

1. K. K. Maiti, O. Y. Jeon, W. S. Lee, D. C. Kim, K. T. Kim, T. Takeuchi, S. Futaki, S. K. Chung, *Angew. Chem. Int. Ed.* 2006, *45,* 2907-2912.