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Mitochondrial Affinity of Guanidine-rich Molecular Transporters Built on *myo*- and *scyllo*-Inositol Scaffolds: Stereochemistry Dependency

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We prepared several novel molecular transporters built on *myo*- and *scyllo*-inositol scaffolds with variations in the number of guanidine residues, linker chain lengths and patterns. Some of these transporters were found to localize in mitochondria, and the mitochondrial affinity seems to be substantially related to the scaffold stereochemistry.

Key Words: Blood-brain barrier, Cellular membrane, Drug delivery, Inositol scaffold, Mitochondrial affinity

Introduction

Efficient drug delivery and good bioavailability at target tissues are important factors in the new drug development, because many drug candidates with promising *in vitro* activity fail or find only limited applications due to poor absorption, distribution, metabolism or excretion (ADME) profiles. Highly desired are novel molecular transporters that can help a promising drug candidate to be taken up by cells and delivered to the desired organelles or tissues. In this connection, substantial research efforts have been extended to several classes of cell-penetrating peptides (CPPs) such as HIV-1 Tat protein. CPPs and related peptides have been extensively studied for their potential utilities as delivery vehicles in order to enhance pharmacological properties of drugs having poor bioavailability, including small molecules, proteins, genes, *etc*.[1-5] However, the CPPs have a number of limitations, including instability toward endogenous peptidases, immunogenic liability, inter-tissue permeation despite their interesting *in vitro* characteristics. In efforts to improve upon these limitations, several different types of synthetic molecular transporters (MTs), e.g. peptoids,[6] oligocarbamates,[7] β-peptides,[8] peptide nucleic acids,[9] and others[10-13] have been investigated.

Recently we have explored novel classes of synthetic molecular transporters, in which multiple units of the guanidine functionality are attached through linear or branched chain carboxylate linkers to various scaffolds such as inositol dimers, sorbitol, lactose, and sucrose.[14-17] Many of these MTs exhibited good cellular uptake properties, and moreover diverse intracellular organellar and tissue selectivity was observed somewhat unexpectedly. For example, several G8 (containing eight guanidine residues) molecular transporters based on the sorbitol scaffold and branched chain linkers showed intracellular selectivity, namely a good affinity toward mitochondria in HeLa as well as $CD34^+$ stem cell-like KG1a leukaemia cells.[15] This observation was deemed highly interesting because of their potential as mitochondria selective delivery vectors, especially in view of a dearth of viable mitochondrial transporters.[18-20] It is now recognized that mitochondria play essential roles in many intractable neural and neuromuscular diseases such as familial amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and Huntington's disease, as well as in apoptosis and aging.[21-25] In addition, the sorbitol-based G8 transporters also displayed more affinity for heart muscle and brain sections in mice than any other tissues examined. Studies with lactose-based G7 molecular transporters[16] and sucrose-based G7 molecular transporters[17] showed that the mitochondrial affinity and the organellar distribution pattern in general appear to be related to the charge (number of guanidine residues), the nature of scaffold, flexibility and lipophilicity of the linker chains, and even the cargo attached. In order to better understand the structure-property relationships with a particular focus on the mitochondrial affinity, we have now studied the effect of the scaffold stereochemistry in a series of G8 molecular transporters built on two inositol stereoisomers, namely *myo*- and *scyllo*-inositols.

Experimental

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 (0.25 mm thickness) and analytical reverse-phase TLC on a Merck RP-8 F254s, and visualization was done with UV light (254 nm and 365 nm), and/or by spraying with a 5% solution of phosphomolybdic acid or ninhydrin, 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 C8-reversed phase silica gel. Melting points were determined on a Thomas-Hoover MP apparatus and uncorrected. 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 500 MHz; ^{13}C-NMR at 125MHz) spectrometers. Tetramethysilane was used as reference, and the chemical shift were reported in δ ppm and the coupling constant in Hz. Analytical HPLC was performed on Agilent 1100-HPLC Chemstation with an analytical column ZORBAX SB-C8 (5 μm, 4.6 mm ID × 25cm). Low resolution mass spectra were determined on a Micromass PLATFORM ll (EI and FAB). High resolution mass spectra were done on a JMS-700 at Korea Basic Science Institute (Daegu), and MALDI-TOF mass spectra on a Voyager-DE STR system at POSTECH. The standard extractive work-up procedure consisted of pouring into a large amount of water, extracting thoroughly with the organic solvent indicated, washing the combined extract successively with water and brine, drying the extract over anhydrous Na_2SO_4 or $MgSO_4$, and evaporating the solvent.

DL-4-*O*-Benzoyl-1-*O*-(6-*N*-Cbz-aminohexanoyl)-2,3:5,6-di-*O*-isopropylidene-*myo*-inositol (7): A solution of compound **6**[26] (350 mg, 0.960 mmol), 6-*N*-Cbz-amino hexanoic acid (290 mg, 1.15 mmol), EDC (221 mg, 1.15 mmol) and DMAP (35 mg, 0.288 mmol) in DMF (5 mL) was stirred overnight at rt under N_2. The reaction mixture was diluted with EtOAc, washed with H_2O, $NaHCO_3$ and brine. Organic phase was dried and concentrated to give the crude product, which was purified by column chromatography (EtOAc:*n*-Hexane = 1:2) to afford compound **7** (505 mg, 86%) as a white solid. mp 160 - 162 °C; R_f 0.29 (EtOAc:*n*-Hexane = 1:2); ^{1}H NMR ($CDCl_3$) δ 1.32, 1.43, 1.48, 1.64 (4s, each 3H, 2 -CMe_2), 1.37-1.58 (m, 6H), 2.47 (t, *J* = 7.2 Hz, 2H), 3.20-3.22 (m, 2H), 3.65 (dd, *J* = 11.0, 9.3 Hz, 1H, H-6), 4.23 (t, *J* = 10.0 Hz, 1H, H-5), 4.33 (dd, *J* = 6.7 z, 4.9 Hz, 1H, H-3), 4.67 (t, *J* = 4.5 Hz, 1H, H-2), 4.79 (br. s, 1H, -NH-), 5.10 (s, 2H, -CH_2-Ph), 5.18 (dd, *J* = 10.5, 4.2 Hz, 1H, H-1), 5.55 (dd, *J* = 11.0, 6.8 Hz, 1H, H-4), 7.32-8.10 (m, 10H, 2 Ph); ^{13}C NMR ($CDCl_3$): 24.4, 25.9, 26.8, 26.9, 27.7, 29.5, 33.9, 40.8, 66.6, 70.4, 74.6, 74.9, 75.2, 76.5, 79.5, 110.7, 113.0, 128.1, 128.2, 128.3, 128.5, 129.7, 130.0, 133.2, 136.7, 156.4, 165.4, 173.1; HRMS (FAB): *m*/*z* calcd for $C_{33}H_{42}NO_{10}$: 612.2806; found 612.2809 $[M+H]^+$.

DL-4-*O*-Benzoyl-1-*O*-(6-*N*-Cbz-aminohexanoyl)-*myo*-inositol (8): To a solution of compound **7** (256 mg, 0.418 mmol) in CH_2Cl_2: MeOH (1:5, 3 mL) was added gaseous HCl saturated ethyl acetate, and stirring was continued at rt for 30 min. The reaction mixture was concentrated and washed with ether to give the crude product, which was further purified by flash column chromatography to afford the compound **8** (202 mg, 91%) as a white sticky solid. R_f 0.47 (CH_2Cl_2:MeOH = 9:1); ^{1}H NMR (CD_3OD) δ 1.35-1.43 (m, 2H), 1.50-1.56 (m, 2H), 1.66-1.72 (m, 2H), 2.45 (t, *J* = 7.2 Hz, 2H), 3.13 (t, *J* = 6.9 Hz, 2H), 3.57 (t, *J* = 9.6 Hz, 2H), 3.78 (dd, *J* = 2.7, 10.2 Hz, 1H), 4.00 (t, *J* = 9.6 Hz, 1H), 4.14 (t, *J* = 2.6 Hz, 1H), 4.75 (dd, *J* = 2.7, 10.2 Hz, 1H), 5.08 (s, 2H), 5.48 (t, *J* = 9.8 Hz, 1H), 7.31-7.37 (m, 5H, aromatic), 7.45-7.51(m, 2H, aromatic), 7.56-7.61 (m,1H, aromatic), 8.09-8.12 (m, 2H); ^{13}C NMR (CD_3OD) δ 25.7, 27.4, 30.7, 35.1, 41.8, 67.5, 71.4, 72.0, 72.1, 74.6, 75.6, 77.0, 128.9, 129.1, 129.5,129.6, 130.9, 132.1, 134.2, 138.6, 159.1, 168.3, 175.2; HRMS (FAB): *m*/*z* calcd for $C_{27}H_{34}NO_{10}$: 531.2178; found 531.2183 $[M+H]^+$.

A representative procedure for exhaustive acylation - A solution of compounds **8** (53 mg, 0.1 mmol), **12a** (438 mg, 0.6 mmol), EDC (115 mg, 0.6 mmol) and DMAP (18 mg, 0.15 mmol) in DMF (2 mL) was stirred at rt under N_2 for 48h. The reaction mixture was diluted with EtOAc, washed several times with H_2O, $NaHCO_3$ and brine. The organic phase was dried and concentrated. The crude product was purified by column chromatography on silica gel (CH_2Cl_2:MeOH = 9:1) to afford compound **9a** as a sticky solid (314 mg, 92%).

DL-4-O-Benzoyl-1-*O*-(6-*N*-Cbz-aminohexanoyl)-2,3,5,6-tetra-*O*-[6-[bis-(3-*N*,*N*'-di-Boc-guanidino-propyl)-amino]-hexanoyl]-*myo*-inositol (9a): Colorless sticky solid; R_f 0.36 (CH_2Cl_2:MeOH = 9:1); ^{1}H NMR ($CDCl_3$) δ 1.01-1.67 (m, 190H), 2.06-2.46 (m, 32H), 3.14-3.20 (m, 4H), 3.34-3.48 (m, 16H), 4.20-5.81 (m, 6H), 5.02 (s, 2H, CH_2Ph) 7.30-7.45 (m, 7H), 7.56-7.59 (m, 1H), 7.92-7.95 (m, 2H), 8.49 (brs, 8H), 11.49 (brs, 8H); ^{13}C NMR ($CDCl_3$): 22.8, 24.1, 24.6, 24.8, 25.1, 25.5, 26.2, 26.4, 26.8, 27.0, 27.3, 28.2, 28.5, 28.7, 29.8, 31.7, 33.8, 34.0, 34.2, 38.1, 39.0, 39.7, 40.2, 41.0, 51.6, 51.7, 52.5, 53.2, 53.7, 53.9, 66.6, 66.7, 68.4, 69.5, 70.2, 70.9, 71.1, 77.4, 79.2, 79.6, 83.0, 128.1, 128.2, 128.7, 129.2, 130.0, 133.6, 133.7, 136.8, 136.9, 153.2, 153.6, 156.2, 156.6, 163.8, 165.3, 172.4, 172.5, 172.6; MALDI-TOF MS: *m*/*z* calcd for $C_{163}H_{280}N_{29}O_{46}$: 3380.0; found: 3380.3 $[M+ 3H]^+$.

DL-4-*O*-Benzoyl-1-*O*-(6-*N*-Cbz-aminohexanoyl)-2,3,5,6-tetra-*O*-[8-[bis-(3-*N*,*N*'-di-Boc-guanidino-propyl)-amino]-octanoyl]-*myo*-inositol (9b): was similarly prepared from compound **8** (53 mg, 0.1 mmol), **12b** (454 mg, 0.6 mmol), EDC (115 mg, 0.6 mmol) and DMAP (18 mg, 0.15 mmol) as a colorless sticky solid (331 mg, 95%): R_f 0.39 (CH_2Cl_2:MeOH = 9:1); ^{1}H NMR ($CDCl_3$) δ 0.97-1.63 (m, 206H), 1.84-2.45 (m, 32H), 3.09-3.19 (m, 4H), 3.35-3.48 (m, 16H), 4.20-5.71 (m, 6H), 5.01 (s, 2H, CH_2Ph) 7.26-7.28 (m, 5H), 7.29-7.38 (m, 2H), 7.42-7.48 (m, 1H), 7.80-7.89 (m, 2H), 8.45 (brs, 8H), 11.43 (brs, 8H). ^{13}C NMR ($CDCl_3$) δ 24.9, 25.0, 25.1, 25.3, 26.4, 26.6, 27.6, 28.4, 28.7, 28.9, 29.4, 29.5, 29.7, 30.0, 30.1, 31.8, 34.2, 34.3, 34.4, 39.9, 41.2, 41.5, 51.9, 52.8, 54.2, 66.8, 67.0, 68.7, 69.6, 69.7, 70.4, 71.0, 71.5, 79.6,78.0, 83.3, 128.4, 128.5, 128.9, 129.1, 129.5, 130.2, 133.8, 134.0, 137.0, 137.1, 153.4, 153.6, 156.5, 156.8, 162.9, 163.9, 165.6, 172.5, 172.7, 172.8, 173.0, 174.4; MALDI-TOF MS: *m*/*z* calcd for $C_{171}H_{294}N_{29}O_{46}$: 3490.2; found: 3490.4 $[M+ H]^+$.

A representative procedure for the removal of the *N*-benzyloxycarbonyl (Cbz) group - A mixture of compound **9a** (150 mg, 0.044 mmol) in a mixed solvent of CH_2Cl_2:MeOH (1:9, 5 mL) was hydrogenated (40 psi) at rt over 10% Pd/C (30 mg). After 24 h, the catalyst was filtered through a celite bed and the filtrate was evaporated to afford free amine **10a** (141 mg, 98%) as an off-white foamy solid.

DL-4-*O*-Benzoyl-1-*O*-(6-aminohexanoyl)-2,3,5,6-tetra-*O*-[6-[bis-(3-*N*,*N*'-di-Boc-guanidino-propyl)-amino]-hexanoyl]-*myo*-inositol (10a): Off-white foamy solid: ^{1}H NMR (CD_3OD) δ 1.22-1.74 (m, 190 H), 1.98-2.40 (m, 32H), 2.67-3.82 (m, 20H), 4.28-5.88 (m, 6H), 7.49-7.53 (m, 2H), 7.64-7.66 (m, 1H), 7.95-7.99 (m, 2H); MALDI-TOF MS: m/z calcd for $C_{155}H_{274}N_{29}O_{44}$: 3246.0094; found: 3246.0450 $[M+ 3H]^+$.

DL-4-*O*-Benzoyl-1-*O*-(6-aminohexanoyl)-2,3,5,6-tetra-*O*-[8-[bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-octanoyl]-*myo*-inositol (10b): 10b was similarly prepared from compound **9b** (150 mg, 0.042 mmol) as an off-white foamy solid (145 mg, 98%): ^{1}H NMR (CD_3OD) δ 1.15-1.74 (m, 206 H), 2.05-2.40 (m, 32H), 2.87-3.50 (m, 20H), 4.28-5.88 (m, 6H), 7.50-7.54 (m, 2H), 7.65-7.67 (m, 1H), 7.96-7.99 (m, 2H); MALDI-TOF MS: *m/z* calcd for $C_{163}H_{291}N_{29}O_{44}$: 3359.1; found: 3359.5 $[M+4H]^+$.

A representative procedure for the FITC-I attachment - To a solution of **10a** (140 mg, 0.0432 mmol) in a mixed solvent THF: abs. ethanol (5 mL, 2:3), were added fluorescein-5-isothiocyanate (20.2 mg, 0.052 mmol) and triethylamine (18 μL, 0.13 mmol). The reaction mixture was stirred for 24 h at rt in dark, concentrated, diluted with ethyl acetate and washed with water. Organic phase was dried and concentrated. The crude product (160 mg) was used without further purification in the next reaction.

DL-4-*O*-Benzoyl-1-*O*-[6-(*N*-fluoresceinyl-5-thioureido)-hexanoyl]-2,3,5,6-tetra-*O*-[6-[bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-hexanoyl]-*myo*-inositol (11a): R_f 0.39 (CH_2Cl_2:MeOH = 9:1); ^{1}H NMR ($CDCl_3$) δ 0.9-1.68 (m, 190H), 2.02-2.48 (m, 36H), 3.40-3.48 (m, 16H), 4.20-5.81 (m, 6H), 6.48-7.12 (m, 6H), 7.41-7.94(m, 8H), 8.50 (brs, 8H), 11.48 (brs, 8H).

DL-4-*O*-Benzoyl-1-*O*-[6-(*N*-fluoresceinyl-5-thioureido)-hexanoyl]-2,3,5,6-tetra-*O*-[8-[bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-octanoyl]-*myo*-inositol (11b): was similarly prepared from **10b** (144 mg, 0.43 mmol), FITC-1 (20.2 mg, 0.052 mmol) and triethylamine (18 μL, 0.13 mmol) as a greenish yellow sticky solid (162 mg): R_f 0.39 (CH_2Cl_2:MeOH = 9:1); ^{1}H NMR ($CDCl_3$) δ 0.82-1.69 (m, 206H), 2.02-2.63 (m, 36H), 3.40-3.79 (m, 16H), 4.05-5.81 (m, 6H), 6.62-7.94 (m, 14H), 8.50 (brs, 8H), 11.47 (brs, 8H).

A representative procedure for the removal of the *N*-Boc group - A solution of **11a** (160 mg crude) in gaseous HCl saturated ethyl acetate was stirred at rt for 24 h. The solution was concentrated and repeatedly washed with ethyl acetate and ether, and evaporated to give the crude product, which was purified by MPLC on Fluka 100 C8-reversed phase silica gel (CH_3CN:H_2O = 1:1, containing 0.1% TFA in both CH_3CN and H_2O). After freeze drying, compound **1a** (as HCl-salt) was obtained (40 mg, 40% over two steps).

DL-4-*O*-Benzoyl-1-*O*-[6-(*N*-fluoresceinyl-5-thioureido)-hexanoyl]-2,3,5,6-tetra-*O*-[6-[bis-(3-guanidino-propyl)-amino]- hexanoyl]-*myo*-inositol·8HCl (1a): Greenish yellow foamy solid; UV (H_2O): λ_{max} (ε) = 493 nm (32843 $cm^{-1}M^{-1}$); ^{1}H NMR (CD_3OD) δ 1.14-1.81 (m, 46H), 2.0-2.60 (m, 34H), 2.98-3.85 (m, 18H), 4.10-5.82 (m, 6H), 6.65-6.80 (m, 4H), 7.20-8.37 (m, 10H); MALDI-TOF MS: *m/z* calcd for $C_{96}H_{155}N_{30}O_{17}S$: 2032.2; found: 2032.2 $[M+H]^+$; analytical HPLC: t_R = 2.359 min (flow rate 1 mL/min; UV: λ = 220 nm; H_2O:CH_3CN = 40:60 with 0.1% TFA), purity > 95%.

DL-4-*O*-Benzoyl-1-*O*-[6-(*N*-fluoresceinyl-5-thioureido)-hexanoyl]-2,3,5,6-tetra-*O*-[8-[bis-(3-guanidino-propyl)-amino]-octanoyl]-*myo*-inositol·8HCl (1b): was similarly prepared from **11b** (162 mg crude) as a light yellow foamy solid (43 mg, 42% over two steps): UV (H_2O): λ_{max} (ε) = 496 nm (36838 cm^{-1} M^{-1}); ^{1}H NMR (CD_3OD) δ 1.11-1.75 (m, 62H), 2.02-2.55 (m, 34H), 2.99-3.65 (m, 18H), 5.01-5.80 (m, 6H), 6.54-6.70 (m, 4H), 7.17-8.37 (m, 10H); MALDI-TOF MS: *m/z* calcd for $C_{104}H_{170}N_{30}O_{17}S$: 2143.3; found: 2143.3 $[M]^+$; analytical HPLC: t_R = 3.162 min (flow rate 1 mL/min; UV: λ = 220 nm; H_2O:CH_3CN = 60:40 with 0.1% TFA), purity > 95%.

1-*O*-Benzoyl-2,3:5,6-di-*O*-isopropylidine-*scyllo*-inositol (14a) and DL-1-*O*-Benzoyl-2,3:4,5-di-*O*-isopropylidine-*scyllo*-inositol (14b): To a solution of 1-benzoyl-2,3,4,5-tetrabenzyl-*scyllo*-inositol (compound **20**)[27] (9.96 g, 35.05 mmol) and p-TSA (3.33 mg, 17.5 mmol) in DMF (110 mL) at rt, was added dropwise 2-methoxypropene (33.5 mL, 350 mmol). After 1day, the reaction mixture was poured into aq. $NaHCO_3$ with vigorous stirring, and extracted with EtOAc. The organic layer was dried, concentrated, diluted with EtOAc (100 mL), and filtered to give compound **14a** (2.9 g, 22.8%), and the filtrate was evaporated and chromatographed on silica gel to give compounds **14b** (1.4 g, 11.5%). **14a :** R_f 0.25 (EtOAc:*n*-Hexane = 1:2); mp 284 - 285 °C; ^{1}H NMR ($CDCl_3$) δ 1.44, 1.47 (each s, each 6H, 2 CMe_2), 2.46 (d, *J* = 2.7Hz, 1H, OH), 3.76 (app. t, *J* = 9.3 Hz, 2H, H-3 & H-5), 3.85 (app. t, *J* = 9.3 Hz, 2H, H-2 & H-6), 4.13 (dt, *J* = 2.7, 8.9 Hz, 1H, H-4), 5.60 (t, *J* = 9.2 Hz, 1H, H-1), 7.26-8.10 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ 27.1, 69.4, 70.1, 78.7, 80.7, 114.0, 128.7, 130.0, 130.5, 133.6, 165.7; MS (FAB) *m/z* clacd for $C_{19}H_{25}O_7$ 365.1600, found 365 $[M+H]^+$. **14b :** R_f 0.40 (EtOAc: *n*-Hexane = 1:2); mp 206 - 207 °C; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 6H), 1.49 (s, 3H), 1.51 (s, 3H), 2.87 (d, *J* = 4.1 Hz, 1H, OH), 3.67-3.95 (m, 4H, H-2, H-3, H-4 & H-5), 4.13 (ddd, *J* = 4.1, 7.9, 10.2 Hz, 1H, H-6), 5.43 (dd, *J*=7.9, 10.4 Hz, 1H, H-1), 7.44-8.11 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ 26.9, 27.0, 27.1, 73.0, 75.6, 75.8, 76.3, 79.3, 81.7, 114.3, 114.4, 128.8, 129.7, 130.5, 134.0, 167.1; MS (FAB) *m/z* clacd for $C_{19}H_{25}O_7$ 365.1600, found 365 $[M+H]^+$.

1-*O*-Benzoyl-4-*O*-(6-*N*-Cbz-aminohexanoyl)-2,3:5,6-di-*O*-isopropylidine-*scyllo*-inositol (15): A solution of compound **14a** (172 mg, 0.472 mmol), 6-*N*-Cbz-aminohexanoic acid (238 mg, 0.944 mmol), EDC (181 mg, 0.944 mmol) and DMAP (17 mg, 0.144 mmol) in DMF (5 mL) was stirred overnight at rt under N_2. The reaction mixture was diluted with EtOAc, washed with H_2O, $NaHCO_3$ and brine. Organic phase was dried and concentrated to give the crude product, which was purified by column chromatography (CH_2Cl_2:MeOH = 35:1) to afford compound **15** (257 mg, 89.1%) as a white solid. R_f 0.29 (CH_2Cl_2:MeOH = 25:1); mp 217 - 218 °C; ^{1}H NMR ($CDCl_3$) δ 1.39-1.45 (m, 14H), 1.52-1.58 (m, 2H), 1.66-1.75 (m, 2H), 2.42 (t, 2H, *J* = 7.31), 3.21 (q, 2H, *J* = 6.38 Hz), 3.86 (m, 4H), 5.10 (s, 2H), 5.40 (t, 1H, *J* = 9.3Hz), 5.59 (t, 1H, *J* = 9.26 Hz), 7.32-7.38 (m, 5H), 7.44-7.49 (m, 2H), 7.56-7.61 (m, 1H), 8.09-8.12(m, 2H); ^{13}C NMR ($CDCl_3$) δ 25, 26.6, 27.3, 30.2, 34.7, 41.6, 67.3, 69.4, 70.1, 79.1, 79.1, 114.3, 128.9, 128.8, 129.0, 129.2, 130.3, 130.8, 134.0, 137.3, 157.0, 166.0, 173.0; HRMS (FAB) *m/z* calcd for $C_{33}H_{42}NO_{10}$ 612.2809, found 612.2808 $[M+H]^+$.

1-*O*-Benzoyl-4-*O*-(6-*N*-Cbz-aminohexanoyl)-*scyllo*-inositol (16): A solution of compound **15** (60 mg, 0.0981 mmol) in gaseous HCl saturated EtOAc (3 mL) was stirred at rt for 30 min and evaporated under reduced pressure. The residue was washed several times with ether to afford compound **16** (49.6 g, 95.2%) as a white solid. mp 193 - 194 °C; ^{1}H NMR (CD_3OD) δ 1.38-1.42 (m, 2H), 1.48-1.55 (m, 2H), 1.66-1.71 (m, 2H), 2.43 (t, 2H, *J* = 7.18 Hz), 3.12 (t, 2H, *J* = 6.76 Hz), 3.50 (t, 2H, *J* = 9.55 Hz), 3.60 (t, 2H, *J* = 9.50 Hz), 4.90-5.12 (m, 4H), 7.27-7.36 (m, 5H), 7.45-

7.51 (m, 2H), 7.58-7.63 (m, 1H), 8.08-8.10 (m, 2H); ^{13}C NMR (CD_3OD) δ 23.8, 25.3, 28.7, 33.2, 39.8, 65.4, 71.9, 71.9, 74.5, 75.4, 126.9, 127.0, 127.5, 127.6, 128.9, 129.7, 132.2, 157.2, 165.9, 173.2; HRMS (FAB) *m/z* calcd for $C_{27}H_{34}NO_{10}$ 532.2183, found 532.2177 $[M+H]^+$, calcd for $C_{27}H_{33}NO_{10}Na$ 554.2002, found 554.2040 $[M+Na]^+$.

1-*O*-Benzoyl-4-*O*-(6-*N*-Cbz-aminohexanoyl)-2,3,5,6-tetra-*O*-[6-[bis-(3-*N,N*'-di-Boc-guanidino-propyl)-amino]-hexanoyl]- *scyllo*-inositol (17a): A solution of compound **16** (20 mg, 0.038 mmol), **12a** (165 mg, 0.23 mmol), EDC (48.6 mg, 0.23 mmol), and DMAP (7 mg, 0.057 mmol) in CH_2Cl_2 (1.5 mL) was stirred at rt under N_2 for 48 h. The reaction mixture was diluted with CH_2Cl_2, washed several times with H_2O, $NaHCO_3$ and brine. The organic phase was dried and concentrated to give the crude product, which was purified on silica gel (CH_2Cl_2: MeOH = 10:1) to afford compound **17a** (109 mg, 85%); R_f 0.41 (CH_2Cl_2:MeOH = 10:1); ^{1}H NMR ($CDCl_3$) δ 0.95-1.96 (m, 178H), 1.97-2.04 (m, 8H), 2.05-2.16 (m, 8H), 2.17-2.27 (m, 8H), 2.28-2.56 (m, 24H), 3.35-3.43 (m, 16H), 5.09 (s, 2H), 5.31-5.53 (m, 6H), 7.34-7.35 (m, 5H), 7.39-7.47 (m, 2H), 7.56-7.62 (m, 1H), 7.92-7.95 (m, 2H), 8.47 (brs, 8H), 11.48 (brs, 8H) ; ^{13}C NMR ($CDCl_3$) δ 24.87, 26.07, 26.88, 27.49, 29.80, 33.93, 34.17, 39.56, 40.88, 51.54, 54.12, 63.37, 69.71, 70.00, 70.32, 70.47, 79.28, 83.06, 128.12, 128.58, 128.83, 130.05, 153.20, 156.22, 156.64, 163.70, 165.50, 172.06, 173.64; MS (MALDI-TOF) *m/z* calcd for $C_{163}H_{277}N_{29}O_{46}$ 3377.0, found 3377.1 $[M]^+$.

1-*O*-Benzoyl-4-*O*-(6-*N*-Cbz-aminohexanoyl)-2,3,5,6-tetra-*O*-[8-[bis-(3-*N,N*'-di-Boc-guanidino-propyl)-amino]-octanoyl]-*scyllo*-inositol (17b): was similarly prepared from compound **16** (75 mg, 0.14 mmol), **12b** (637.5 mg, 0.84 mmol), EDC (180 mg, 0.84 mmol), and DMAP (25.8 mg, 0.21 mmol). **17b** (432.4 mg, 88.5%): R_f 0.46 (CH_2Cl_2:MeOH = 9:1); ^{1}H NMR ($CDCl_3$) δ 0.96-1.95 (m, 194H), 1.96-2.16 (m, 8H), 2.17-2.26 (m, 8H), 2.18-2.57 (m, 32H), 3.32-3.57 (m, 16H), 5.09 (s, 2H), 5.26-5.56 (m, 6H), 7.18-7.35 (m, 5H), 7.37-7.50 (m, 2H), 7.52-7.65 (m, 1H), 7.88-7.93 (m, 2H), 8.50 (brs, 8H), 11.49 (brs, 8H); ^{13}C NMR ($CDCl_3$) δ 24.90, 26.43, 26.73, 27.70, 29.42, 29.51, 29.88, 33.97, 34.14, 39.82, 40.96, 51.81, 54.19, 69.76, 70.05, 70.51, 70.54, 79.31, 80.03, 128.23, 128.68, 130.10, 153.29, 156.26, 156.61, 163.83, 165.20, 172.33; MS (MALDI-TOF) *m/z* calcd for $C_{171}H_{293}N_{29}O_{46}$ 3489.1, found 3489.3 $[M]^+$.

1-*O*-Benzoyl-4-*O*-(6-aminohexanoyl)-2,3,5,6-tetra-*O*-[6-[bis-(3-*N,N*'-di-Boc-guanidino-propyl)-amino]-hexanoyl]-*scyllo*-inositol (18a): A mixture of compound **17a** (150 mg, 0.0444 mmol) in a mixed solvent of CH_2Cl_2: MeOH (1:9, 5 mL) was hydrogenated (40 psi) at rt over 10% Pd/C (30 mg). After 24 h, the catalyst was filtered through celite and the filtrate was evaporated to afford free amine **18a** (141mg, 98%) as an off-white foamy solid. ^{1}H NMR (CD_3OD) δ 1.18-1.85 (m, 166H), 1.88-2.10 (m, 16H), 2.11-2.21 (m, 4H), 2.22-2.42 (m, 8H), 2.75-2.95 (m, 8H), 3.01-3.23 (m, 24H), 3.37-3.51 (m, 16H), 5.48-5.65 (m, 6H), 7.50-7.55 (m, 2H), 7.65-7.69 (m, 1H), 7.95-7.97 (m, 2H).

1-*O*-Benzoyl-4-*O*-(6-aminohexanoyl)-2,3,5,6-tetra-*O*-[8-[bis-(3-*N,N*'-di-Boc-guanidino-propyl)-amino]-octanoyl]-*scyllo*-inositol (18b) was similarly prepared. **18b** (142 mg, 98%) as an off-white foamy solid: ^{1}H NMR (CD_3OD) δ 1.04-1.82 (m, 184H), 1.96-2.16 (m, 24H), 2.18-2.42 (m, 8H), 2.98-3.23 (m, 28H), 3.43-3.58 (m, 24H), 5.41-5.73 (m, 6H), 7.46-7.54 (m, 2H), 7.60-7.72 (m, 1H), 7.90-8.00 (m, 2H).

1-*O*-Benzoyl-4-*O*-[6-(*N*-fluoresceinyl-5-thioureido)-hexanoyl]-2,3,5,6-tetra-*O*-[6-[bis-(3-*N,N*'-di-Boc-guanidino-propyl)-amino]-hexanoyl]-*scyllo*-inositol (19a): To a solution of **18a** (114 mg, 0.035 mmol) in a mixed solvent THF : abs. ethanol (5 mL, 2:3), were added fluorescein-5-isothiocyanate (16.4 mg, 0.042 mmol) and triethylamine (15 μL, 0.105 mmol), and the reaction mixture was stirred for 24 h at rt in dark. The reaction mixture was concentrated, diluted with ethyl acetate, and washed with water. Organic phase was dried and concentrated to give the crude product **19a** (130 mg) as a yellow sticky glass, which was directly used in the next step. ^{1}H NMR ($CDCl_3$) δ 1.05-2.95 (m, 218H), 3.24-3.51 (m, 24H), 5.30-5.65 (m, 6H), 6.50-7.95 (m, 14H), 8.50 (brs, 8H), 11.42 (brs, 8H).

1-*O*-Benzoyl-4-*O*-[6-(*N*-fluoresceinyl-5-thioureido)-hexanoyl]-2,3,5,6-tetra-*O*-[8-[bis-(3-*N,N*'-di-Boc-guanidino-propyl)-amino]-octanoyl]-*scyllo*-inositol (19b): was similarly prepared from **18b** (112 mg, 0.033 mmol). **19b** (127 mg) as a yellow sticky glass: ^{1}H NMR ($CDCl_3$) δ 0.84-2.34 (m, 216H), 2.58-2.93 (m, 24H), 3.27-3.56 (m, 18H), 2.58-2.93 (m, 24H), 3.27-3.56 (m, 18H), 5.22-5.57 (m, 6H), 6.47-7.92 (m, 14H), 8.48 (brs, 8H), 11.42 (brs, 8H).

1-*O*-Benzoyl-4-*O*-[6-(*N*-fluoresceinyl-5-thioureido)-hexanoyl]-2,3,5,6-tetra-*O*-[6-{bis-(3-guanidino-propyl)-amino}-hexanoyl]-*scyllo*-inositol (2a): A solution of **19a** (130 mg crude) in gaseous HCl saturated ethyl acetate was stirred at rt for 24h. The solution was concentrated and repeatedly washed with ethyl acetate and ether, and the crude product was purified by MPLC on Fluka 100 C8-reversed phase silica gel (CH_3CN: H_2O = 1:1, containing 0.1% TFA in both CH_3CN and H_2O). Upon freeze drying compound **2a** (HCl-salt) was obtained as a greenish yellow foamy solid (30 mg, 37% over two steps). UV (H_2O): $\lambda_{max}(\varepsilon)$ = 497 nm (30416 cm^{-1} M^{-1}); ^{1}H NMR (CD_3OD) δ 1.09 (m, 4H), 1.37-1.47 (m, 12H), 1.52-1.58 (m, 4H), 1.59-1.72 (m, 8H), 1.73-1.83 (m, 6H), 1.97-2.11 (m, 18H), 2.12-2.24 (m, 8H), 2.28-2.37 (m, 6H), 2.89-2.97 (m, 4H), 3.18-3.23 (m, 12H), 3.23-3.37 (m, 16H), 5.53-5.72 (m, 6H), 6.60-6.62 (m, 2H), 6.73-6.77 (m, 4H), 7.21 (m, 2H), 7.53 (m, 2H), 7.68 (m, 1H), 7.73 (m, 1H), 7.98 (m,2H); ^{13}C NMR (CD_3OD) δ 22.81, 23.16, 23.75, 23.96, 25.39, 25.60, 25.72, 29.26, 33.02, 33.19, 38.18, 38.22, 50.26, 50.34, 52.82, 53.07, 70.08, 70.20, 102.21, 112.55, 115.45, 117.77, 128.85, 128.58, 129.02, 129.60, 153.11, 157.46, 161.33, 161.61, 165.05, 171.93, 171.98, 172.31, 181.30; MS (MALDI-TOF) *m/z* calcd for $C_{96}H_{154}N_{30}O_{17}S$ 2031.2, found 2031.3 $[M]^+$; analytical HPLC: t_R = 3.588 min (flow rate 1 mL/min; UV: λ = 220 nm; H_2O:CH_3CN = 70:30 with 0.1% TFA), purity > 95%.

1-*O*-Benzoyl-4-*O*-[6-(*N*-fluoresceinyl-5-thioureido)-hexanoyl]-2,3,5,6-tetra-*O*-[8-{bis-(3-guanidino-propyl)-amino}-octanoyl]-*scyllo*-inositol (2b) was similarly prepared from **19b** (127 mg crude). **2b** (HCl-salt) as a greenish yellow foamy solid (33.3 mg, 41% over two steps): UV (H_2O): $\lambda_{max}(\varepsilon)$ = 499 nm (29705 cm^{-1} M^{-1}); ^{1}H NMR (CD_3OD) δ 1.02-1.09 (m, 4H), 1.10-1.17 (m, 8H), 1.28-1.45 (m, 24H), 1.55-1.58 (m, 4H), 1.62-1.67 (m, 6H), 1.68-1.74 (m, 2H), 1.74-1.80 (m, 4H), 2.03-2.09 (m, 16H), 2.13-2.15 (m, 4H), 2.25-2.29((m, 4H), 2.31-2.35 (m, 2H), 3.08-3.13 (m, 4H), 3.16-3.37 (m, 32H), 5.52-5.53 (m, 3H), 5.59-5.63 (m, 2H), 5.67-5.71 (m, 1H), 6.57-6.60 (m, 2H), 6.70-6.74

(m, 4H), 7.19-7.21 (m, 2H), 7.49-7.52 (m, 2H), 7.65-7.68 (m, 1H), 7.72-7.74 (m, 1H), 7.96-7.97 (m, 2H); ^{13}C NMR (CD_3OD) δ 22.80, 23.16, 23.18, 23.26, 23.40, 24.07, 24.26, 24.43, 25.79, 25.97, 26.07, 28.08, 28.20, 28.30, 28.48, 28.63, 29.26, 33.37, 34.47, 38.20, 50.27, 50.33, 53.20, 53.27, 56.08, 69.96, 70.10, 70.30, 70.75, 102.22, 110.17, 110.95, 112.39, 113.35, 115.53, 117.86, 120.26, 124.37, 127.76, 128.45, 128.88, 129.57, 133.62, 152.96, 157.44, 157.46, 161.49, 161.77, 165.16, 169.91, 172.24, 172.25, 172.32; MS (MALDI-TOF) *m/z* calcd for $C_{104}H_{170}N_{30}O_{17}S$ 2143.3, found 2143.3 $[M]^+$; analytical HPLC: t_R = 4.958 min (flow rate 1 mL/min; UV: λ = 220 nm; H_2O:CH_3CN = 70:30 with 0.1% TFA), purity > 95%.

1-*O*-Benzoyl-2-*O*-(6-*N*-Cbz-aminohexanoyl)-3,4,5,6-tetra-*O*-benzyl-*scyllo*-inositol (21): A solution of compound **20**[27] (250 mg, 0.388 mmol), 6-*N*-Cbz-aminohexanoic acid (150 mg, 0.565 mmol), EDC (110 mg, 0.565 mmol) and DMAP (14 mg, 0.14 mmol) in CH_2Cl_2 (4 mL) was stirred for 24 h at rt under N_2. The reaction mixture was diluted with CH_2Cl_2, washed several times with saturated $NaHCO_3$. The organic phase was dried and concentrated. The residue was purified by column chromatography (EtOAc:*n*-Hexane = 1: 3-1:2) to afford compound **14** (338 mg, 98%) as a white sticky solid. R_f 0.45 (EtOAc:*n*-Hexane = 1:2); ^{1}H NMR ($CDCl_3$) δ 0.91-1.29 (m, 6H), 2.01 (t, *J* = 7.1 Hz, 2H), 2.88 (br.s, 2H), 3.66-3.70 (m, 4H), 4.56-4.88 (m, 8H), 5.08 (s, 2H), 5.27-5.37 (m, 2H), 7.03-7.53 (m, 28H), 7.94 (d, *J* = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 24.2, 25.9, 29.3, 33.8, 40.5, 66.4, 71.7, 72.6, 75.4, 75.5, 75.9, 80.0, 80.3, 82.6, 82.7, 127.5, 127.62, 127.65, 127.7, 127.8, 127.9, 128.0, 128.2, 128.3, 128.4, 128.5, 129.4, 129.8, 133.3, 136.7, 137.5, 138.1, 138.16, 138.2, 156.2, 165.5, 172.4. HRMS (FAB) *m/z* calcd for $C_{55}H_{58}NO_{10}$ 892.4061, found 892.4069 $[M+H]^+$.

1-*O*-Benzoyl-2-*O*-(6-aminohexanoyl)-*scyllo*-inositol (22). A mixture of compound **21** (338 mg, 0.379 mmol) and 20 wt % $Pd(OH)_2$/C (moisture 60%) (170 mg) in CH_2Cl_2: EtOH (2: 3, 5 mL) was hydrogenated under H_2 (pressure: 50 psi) for 48 h. The reaction mixture was filtered through a celite-bed and the filtrate was concentrated to afford compound **22** (140 mg, quantitative) as a white sticky solid. R_f 0.12 (CH_2Cl_2:MeOH = 5:1 × 2); ^{1}H NMR (CD_3OD) δ 1.09-1.20 (m, 2H), 1.37-1.50 (m, 4H), 2.14-2.35 (m, 2H), 2.67 (t, J = 7.7 Hz, 2H), 3.38-3.45 (m, 2H), 3.54-3.67 (m, 2H), 5.12-5.25 (m, 2H), 7.47-7.52 (m, 2H), 7.61-7.63 (m, 1H), 8.00-8.03 (m, 2H); ^{13}C NMR (CD_3OD) δ 25.3, 26.7, 28.2, 34.8, 40.5, 73.5, 73.7, 74.6, 75.4, 75.5, 129.8, 130.9, 131.3, 134.7, 167.5, 174.4; HRMS (FAB) *m/z* calcd for $C_{19}H_{28}NO_8$ 398.1815, found 398.1816 $[M+H]^+$.

1-*O*-Benzoyl-2-*O*-(6-*N*-Cbz-aminohexanoyl)-*scyllo*-inositol (23). To a solution of compound **22** (130 mg, 0.352 mmol) in 1,4-dioxane: H_2O (1:1, 3 mL) at 0 °C was added triethyl amine (59 μL, 0.422 mmol), and the solution was stirred for 5 min. Benzyl chloroformate (98 μL, 0.7 mmol) was added to the reaction mixture and the solution stirred for overnight at rt. The reaction mixture was evaporated to dryness, diluted with EtOAc and washed with water and brine solution. The organic phase was dried and concentrated. The crude residue was purified by flash chromatography (CH_2Cl_2:MeOH = 12:1) to afford compound **23** (120 mg, 68%) as a white solid. R_f 0.28 (CH_2Cl_2: MeOH = 10:1); mp = 105 - 106 °C; ^{1}H NMR (CD_3OD) δ 1.04-1.36 (m, 6H), 2,14-2.23 (m, 2H), 2.86-2.89 (m, 2H), 3.38-3.41 (m, 2H), 3.52-3.62 (m, 2H), 5.05 (s, 2H), 5.10-5.20 (m, 2H), 7.35-7.57 (m, 8H), 7.99-8.01 (m, 2H); ^{13}C NMR (CD_3OD) δ 25.6, 27.2, 30.5, 35.1, 41.6, 67.4, 73.6, 73.7, 74.5, 75.4, 75.6, 128.9, 129.1, 129.6, 129.7, 131.0, 131.3, 134.6, 138.6, 158.9, 167.5, 174.7; HRMS (FAB) *m/z* calcd for $C_{27}H_{34}NO_{10}$ 532.2183, found 532.2180 $[M+H]^+$.

1-*O*-Benzoyl-2-*O*-(6-*N*-Cbz-aminohexanoyl)-3,4,5,6-tetra-*O*-[6-[bis-(3-*N*,*N*'-di-Boc-guanidino-propyl)-amino]-hexanoyl]-*scyllo*-inositol (24). A solution of compound **23** (30 mg, 0.059 mmol), linker **12a** (261 mg, 0.357 mmol), EDC (68 mg, 0.357 mmol) and DMAP (8.7 mg, 0.071 mmol) in dry DMF (2 mL) was stirred for 48 h at rt under N_2. The reaction mixture was diluted with EtOAc, washed several times with saturated $NaHCO_3$. The organic phase was dried and concentrated. The residue was purified by column chromatography (CH_2Cl_2: MeOH = 10: 1) to afford compound **24** (185 mg, 92%) as a white foamy solid. R_f 0.36 (CH_2Cl_2:MeOH = 10:1); ^{1}H NMR ($CDCl_3$) δ 0.91-1.69 (m, 190H), 2.10-2.46 (m, 32H), 2.90-2.95 (m, 4H), 3.38-4.22 (m, 16H), 4.88-5.51 (m, 6H), 5.07 (s, 2H, CH_2Ph) 7.30-7.36 (m, 5H), 7.39-7.45 (m, 2H), 7.54-7.60 (m, 1H), 7.91-7.95 (m, 2H), 8.49 (brs, 8H), 11.49 (brs, 8H); ^{13}C NMR (CD_3OD) δ 24.3, 24.8, 24.9, 26.0, 26.3, 26.9, 27.2, 27.5, 27.8, 28.1, 28.4, 28.6, 29.4, 29.7, 33.7, 34.0, 34.1, 34.2, 39.6, 40.6, 51.5, 51.7, 53.1, 53.6, 53.8, 63.3, 66.5, 69.2, 69.6, 69.8, 69.9, 70.1, 70.4, 77.4, 79.1, 82.9, 128.1, 128.5, 128.7, 129.9, 133.9, 136.8, 153.1, 156.1, 156.4, 162.5, 163.7, 165.0, 171.8, 171.9, 172.0, 173.6, 174.1; MS (MALDI-TOF) *m/z* calcd for $C_{163}H_{281}N_{29}O_{46}$ 3381.0541, found 3381.0720 $[M+4H]^+$.

1-*O*-Benzoyl-2-*O*-(6-aminohexanoyl)-3,4,5,6-tetra-*O*-[6-[bis-(3-*N*,*N*'-di-Boc-guanidino-propyl)-amino]-hexanoyl]-*scyllo*-inositol (25). A mixture of compound **24** (100 mg, 0.03 mmol) in a mixed solvent of CH_2Cl_2:MeOH (1:9, 5 mL) was hydrogenated (40 psi) over 10% Pd/C (25 mg) at rt. After 24 h, the catalyst was filtered through a celite bed and the filtrate was evaporated to afford free amine **25** (96 mg, 99%) as a sticky solid. ^{1}H NMR ($CDCl_3$) δ 1.06-2.26 (m, 202H), 2.88-3.70 (m, 40H), 5.31-5.56 (m, 6H), 7.45-7.50 (m, 2H), 7.61-7.64 (m, 1H), 7.93-7.95 (m, 2H), 8.47 (s, 8H), 11.43 (m, 8H); MS (MALDI-TOF) *m/z* calcd for $C_{155}H_{273}N_{29}O_{44}$: 3245.0016, found 3245.1240 $[M+2H]^+$.

1-*O*-Benzoyl-2-*O*-[6-(*N*-fluoresceinyl-5-thioureido)-hexanoyl]-3,4,5,6-tetra-*O*-[6-[bis-(3-*N*,*N*'-di-Boc-guanidino-propyl)-amino]-hexanoyl]-*scyllo*-inositol (26). To a solution of **25** (88 mg, 0.027 mmol) in a mixed solvent THF:abs. ethanol (5 mL, 2:3), were added fluorescein-5-isothiocyanate (12.7 mg, 0.032 mmol) and triethylamine (11.3 μL, 0.081 mmol). The reaction mixture was stirred for 24 h at rt in dark, concentrated, diluted with ethyl acetate, and washed with water. Organic phase was dried and concentrated. The crude product **26** (99 mg) was used in the next reaction without further purification. ^{1}H NMR ($CDCl_3$) δ 1.02-2.88 (m, 226H), 3.32-3.51 (m, 16H), 3.62-4.12 (m, 2H), 5.05-5.61 (m, 6H), 6.56-7.90(m, 14H), 8.50 (brs, 8H), 11.44 (brs, 8H).

1-*O*-Benzoyl-2-*O*-[6-(*N*-fluoresceinyl-5-thioureido)-hexanoyl]-3,4,5,6-tetra-*O*-[6-{bis-(3-guanidino-propyl)-amino}-hexanoyl]-*scyllo*-inositol (3a). A solution of **26** (99 mg) in gaseous HCl saturated ethyl acetate was stirred at rt for 24 h, concentrated and repeatedly washed with ethyl acetate and ether

to give the crude product, which was purified by MPLC on Fluka 100 C8-reversed phase siica gel ($CH_3CN:H_2O$ = 1:1, containing 0.1% TFA in both CH_3CN and H_2O). Upon freeze drying compound **3a** (as HCl salt) was obtained as a greenish yellow foamy solid (41.2 mg, 73% over two steps): UV (H_2O): λ_{max} (ε) = 497 nm (48139 cm^{-1} M^{-1}); ^1H NMR (CD_3OD) δ 1.15-2.38 (m, 80H), 2.88-3.66 (m, 16H), 5.56-5.72 (m, 6H), 7.20-7.70 (m, 10H), 7.91-8.59 (m, 4H); MS (MALDI-TOF) *m/z* calcd for $C_{96}H_{155}N_{30}O_{17}S$ 2032.2, found 2032.2 $[M+H]^+$; analytical HPLC: t_R = 4.699 min (flow rate 1 mL/min; UV: λ = 254 nm; H_20: CH_3CN 70: 30 with 0.1% TFA), purity > 95%.

Bioassays.

Cell culture: 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 (Gifco) in an incubator at 37 °C, under an atmosphere containing air and 5% CO_2.

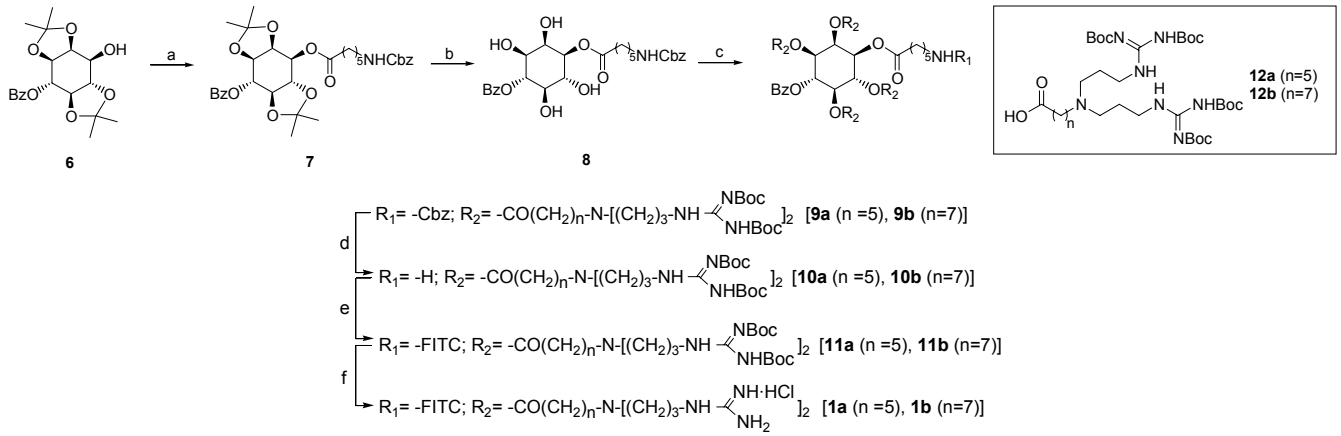
Imaging analysis: The cells (1.0×10^4/well) were seeded into 96-well plate, cultured overnight and treated with compounds at the indicated concentration in the presence of 250 nM MitoTracker Deep Red (Molecular Probes) for 10 min. After washing with serum-free DMEM, images were captured by Image Xpress 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 the 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 with DMSO only as a negative control and 500 nM MitoTracker Deep Red or MitoTracker Green FM (Molecular Probes) was used as a positive control, respectively. Data represent uptake percent for 10,000 cells per sample.

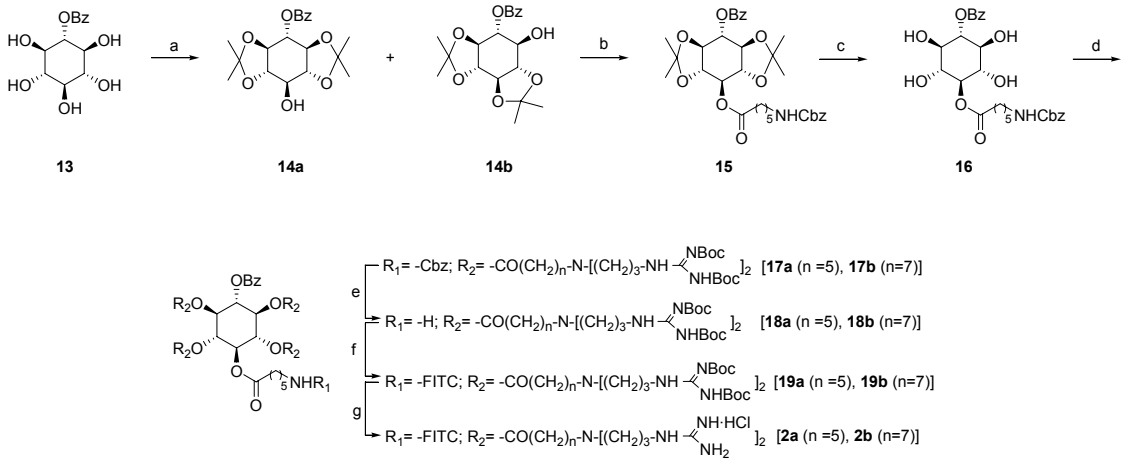
Tissue distribution study: Each transporter [**1a** (84.5 mg kg^{-1}), **1b** (88.6 mg kg^{-1}), **2a** (84.5 mg kg^{-1}), **2b** (85.0 mg kg^{-1}), **3a** (84.5 mg kg^{-1}),] was dissolved in sterile distilled water (1 mL) and injected into 8-week-old mice (C57BL/6) intraperitoneally. The treated mice were perfused after 20 minutes with 4% paraformaldehyde in PBS (pH 7.4). The organs were incubated overnight in 0.5 M sucrose in PBS. Placed in cryoprotectant, they were cut into 15-μm sections with cryostat, and transferred to coated glass slides. After drying at 37 °C, the sections were washed with PBS and treated with 0.3% Triton X-100 for 15 minutes at room temperature and analyzed with an Axioplan2 fluorescence imaging microscope.

Results and Discussion

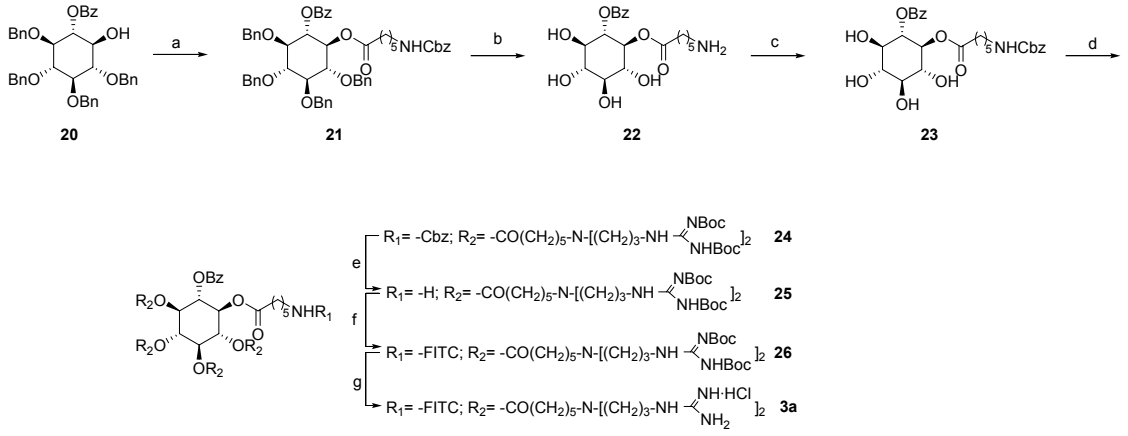
All transporters used in the present studies (**1-3**) were prepared from the *myo*-inositol derivative **6**,[26] essentially according to the reported procedures.[14-17] For the synthesis of transporter **1**, the C1-OH of compound **6** was condensed with *N*-Cbz protected 6-aminohexanoic acid using 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) and 4-dimethylaminopyridine (DMAP), and then the acetonide protecting groups were removed by treatment with EtOAc saturated with HCl (g) to provide compound **8**. Exhaustive coupling of **8** with bisguanidinylated carboxylic acid **12a** and **12b**[15] in the presence of EDC and DMAP gave the acylated products in good yields. Removal of the *N*-Cbz protecting group in the side chain of the acylated products by hydrogenolysis, attachment of the fluorescent probe on the primary amino group with fluorescein-5-isothiocyanate (FITC-I), and finally removal of the Boc groups on the guanidine groups in EtOAc saturated with HCl (g) gave desired MTs **1a** and **1b** (Scheme 1). The *scyllo*-inositol based transporters **2** and **3a** were similarly prepared from **13, 20** and requisite components respectively (Scheme 2 and 3). The transporters (**1-3**) obtained as their HCl salt were rigorously purified by medium-pressure liquid chromatography (MPLC) on a reverse phase (C8) silica gel column, and preparative high performance liquid chromatography (HPLC) on a reverse phase (C18) column when necessary. They were all satisfactorily characterized by ^1H NMR spectroscopy and MALDI-TOF mass spectral analyses. The sample purity was assessed by analytical HPLC on a ZORBAX SB-C8 reverse phase column (5 μm, 4.6 mm ID × 25 cm; flow rate 1 mL min^{-1} of $CH_3CN:H_2O$ = 30:70 with 0.1% TFA; detection UV at λ = 220 nm). The molecular transporters showed the following



Scheme 1. a. $HO_2C(CH_2)_5$-NHCbz, EDC, DMAP, DMF, 12 h, RT, 86%; b. HCl (g), EtOAc, DCM, MeOH, 0.5 h, RT, 91%; c. **12a** or **12b**, EDC, DMAP, DMF, 48 h, 92% & 95%; d. 10% Pd/C, H_2 (50 psi), DCM, MeOH, 24 h, RT, 98%; e. FITC-I, TEA, THF, EtOH, 24 h, RT; f. HCl (g), EtOAc, 24 h, RT, 40% & 42% over two steps



Scheme 2. a. 2-methoxypropene, *p*-TSA, DMF, 24 h, rt, 22.8% (**14a**) & 11.5% (**14b**); b. $HO_2C(CH_2)_5NHCbz$, EDC, DMAP, DCM, 24 h, rt, 89%; c. HCl (g), EtOAc, 0.5 h, rt, 95%; d. **12a** or **12b**, EDC, DMAP, DCM, 48h, rt, 85% (**17a**) & 88% (**17b**); e. 10% Pd/C, H_2 (50 psi), DCM, MeOH, 24 h, rt, 98%; f. FITC-I, TEA, EtOH, THF, 24 h, rt; g. HCl (g), EtOAc, rt, 24 h, 37 - 41% over two steps



Scheme 3. a. $HO_2C(CH_2)_5NHCbz$, EDC, DMAP, DCM, 24 h, rt, 98%; b. 20% $Pd(OH)_2/C$, H_2 (50 psi), DCM, EtOH, 24 h, rt, quant.; c. CbzCl, TEA, 1,4-dioxane, H_2O, rt, 68%; d. **12a**, EDC, DMAP, DCM, 48 h, rt, 92%; e. 10% Pd/C, H_2 (50 psi), DCM, MeOH, 24 h, rt, 99%; f. FITC-I, TEA, EtOH, THF, rt, 24 h; g. HCl (g), EtOAc, 24 h, rt, 73% over two steps

retention times on the C8 column: **1a**, 3.254; **1b**, 3.910; **2a**, 3.617; **2b**, 4.690; **3a**, 3.754 min.

First, the cellular uptake property of compounds **1-3** was examined in live HeLa cells by confocal laser scanning microscopy (CLSM) with FITC-labeled arginine octamer (R8-F1) as the reference. After incubation with 5.0 μM of each transporter for 10 min at 37 °C, the cells were found substantially marked with the green fluorescence. The quantitative uptake efficiencies were measured by fluorescence-activated cell-sorter (FACS) for all five transporters, and the results are summarized in Fig. 1. The FACS analyses show that all MTs are internalized substantially better than R8-F1. The MTs with longer linker chains (**1b** and **2b**) show more efficient uptake than the shorter chain versions (**1a** and **2a**) as expected, and the MT with the *ortho*-like substitution (**3a**) shows better uptake than the one with *para*-like substitution (**2a**). The intracellular localization patterns of all MTs (2.5 - 5.0 μM) were examined without fixing in HeLa cells by co-incubating with MitoTracker Red (500 nM, Invitrogen) at 37 °C for 10 min, and the results are shown in Fig. 2. The *myo*-inositol based transporters (**1a** and **1b**) showed good localization in mitochondria (Fig. 2a), whereas the *scyllo*-inositol based transporters (**2a**, **2b**, and **3a**) displayed little affinity toward mitochondria showing only punctate patterns with poorly defined localization. (Fig. 2b). Previously, with the lactose- and sucrose-based transporters the lipophilicity reflected in the HPLC retention time on a reverse phase column,[28-30] appeared to be somewhat correlated to the mitochondrial affinity.[16,17] However, the present cases show poor correlation between the HPLC-based lipophilicity and the mitochondrial localization. Instead the mitochondrial affinity appears better correlated with the stereochemistry of the scaffold; transporters **1a** and **1b** (*myo*-inositol, *para*-like substitution) show good affinity toward mitochondria, whereas transporters **2a** and **2b** with identical structures except the scaffold stereochemistry (*scyllo*-inositol, *para*-like), and **3a** (*scyllo*-inositol, *ortho*-like) do not exhibit much affinity for mitochondria.

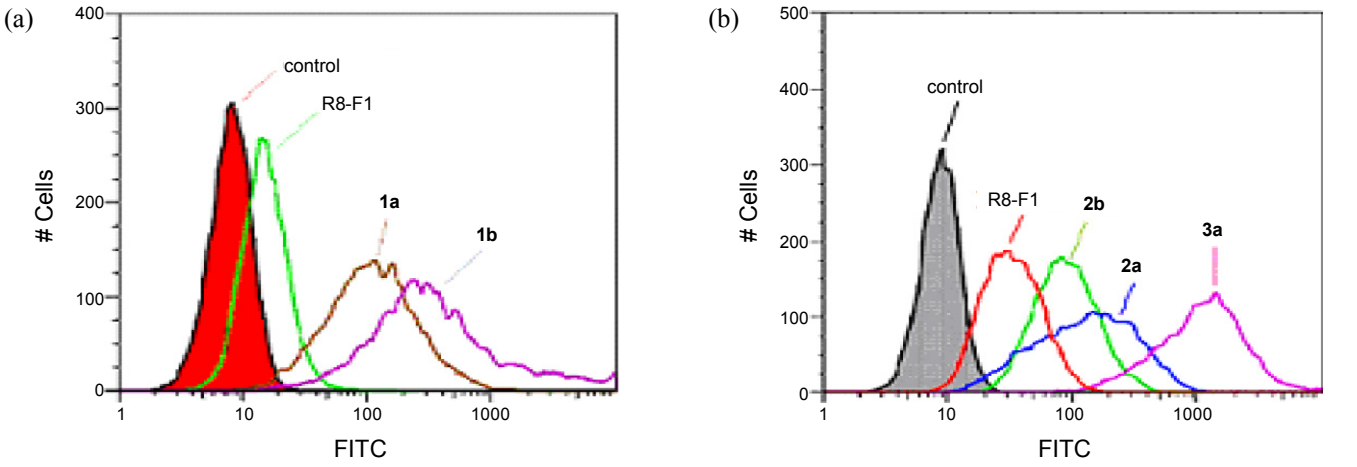


Figure 1. Cellular uptake studies of molecular transporters **1a**, **1b**, **2a**, **2b**, and **3a** (5.0 μM each) by flow cytometry. The HeLa cells were incubated with compounds or only DMSO (as control) in serum-containing DMEM at 37 °C for 10 min and analyzed for their average fluorescence intensity.

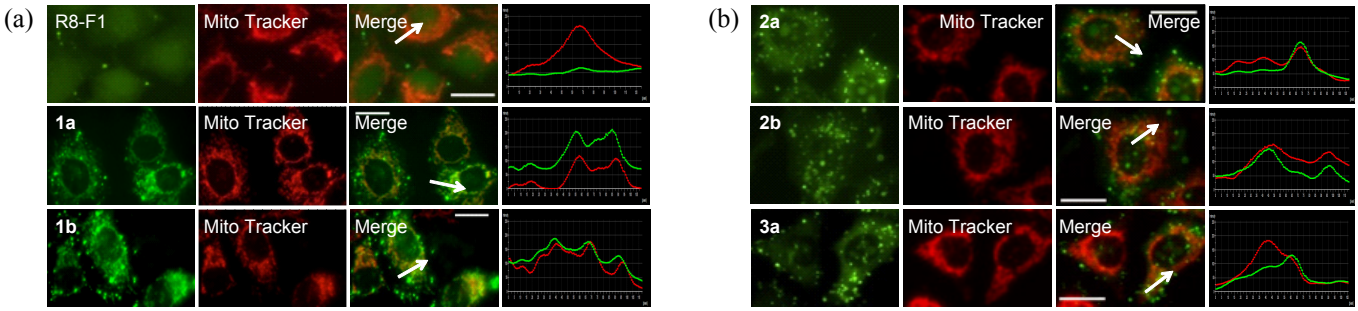


Figure 2. Mitochondrial localization studies of transporters. a) Fluorescence microscope images of compound **1a**, and **1b** and their localization in mitochondria. HeLa cells were treated with **1a** (2.5 μM), and **1b** (2.5 μM) and with MitoTracker Red (500 nM) at 37 °C for 10 min, with significant localization in mitochondria. b) Little colocalization from **2a** (5.0 μM), **2b** (5.0 μM), and **3a** (5.0 μM) with MitoTracker Red (500 nM) is seen. Intensity profiles for localization were analyzed for each arrow in the merged image. Scale bars: 20 μM.

The mouse tissue distribution patterns of the transporters were examined. Each transporter [**1a** (84.5 mg kg^{-1}), **1b** (88.6 mg kg^{-1}), **2a** (84.5 mg kg^{-1}), **2b** (85.0 mg kg^{-1}), **3a** (84.5 mg kg^{-1})] was dissolved in sterile distilled water and injected intraperitoneally (*ip*) into 8-week-old mice (C57BL/6). After 20 min, the treated mice were perfused with 4% paraformaldehyde in phosphate buffered saline solution (PBS; pH 7.4), and the major organs (heart, spleen, liver, kidneys, lungs, and brain) were incubated overnight in a solution of 0.5 M sucrose in PBS. Placed in cryoprotectant, they were cut into 15-μm sections with a cryostat and transferred to coated glass slides. After drying at 37 °C, the sections were washed with PBS and treated with 0.3% Triton X-100 for 15 min at rt, and analyzed with an Axioplan2 fluorescence imaging microscope (Fig. 3). Some selectivity trends in the tissue distribution are observed: 1) all transporters show a good affinity for brain; 2) *myo*-inositol based transporters (**1a**, **1b**) show an affinity toward liver and spleen to a degree; 3) *scyllo*-inositol based transporters (**2a**, **2b**, **3a**) are much widely distributed in all organs including kidney, lung, heart, spleen and liver. It is clear that all transporters can overcome the mouse blood-brain-barrier (BBB), when given *via* either the *ip* or per oral (*po*) route (Fig. 1 in Supplementary Materials).[31]

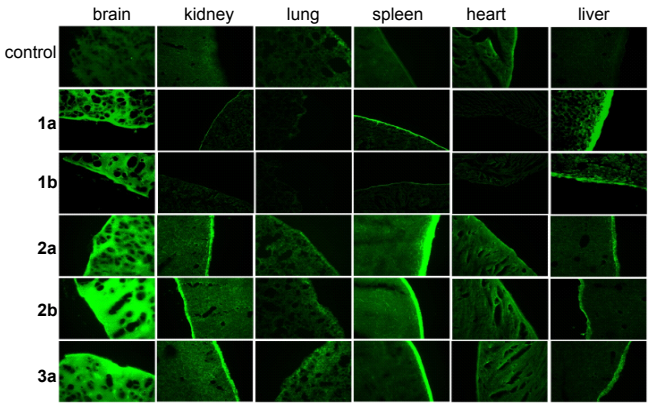


Figure 3. Distribution patterns of molecular transporters in mouse tissues by *ip* injection. Exposure times (ms): brain-10000, kidney-2500, lung-5000, spleen-1000, heart-1000, liver-2500. λ_{max} = 488 nm (green fluorescence from FITC).

Conclusion

In summary, we have prepared five G8 molecular transporters built on two stereoisomeric inositol (*myo*- and *scyllo*-) scaffolds,

and examined their cellular uptake properties and the intracellular localization patterns. Transporters **1a** and **1b** (*myo*-inositol scaffold with *para*-like substitution) were found to target mitochondria, whereas transporters, **2a** and **2b** (*scyllo*-inositol, *para*-like), and **3a** (*scyllo*-inositol, *ortho*-like) did not show much affinity for mitochondria. Although the mitochondrial affinity of guanidine-rich MTs generally appears to result from the subtle interplay of the number of charge (guanidine residues), geometric factors (scaffold and linker chain), and the overall lipophilicity, the present study demonstrates importance of the stereochemistry of the scaffold as a key parameter.[32]

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