Mitochondrial Affinity of Guanidine-rich Molecular Transporters Built on Monosaccharide Scaffolds: Stereochemistry and Lipophilicity

Woo Sirl Lee, Wanil Kim,[†] Kyong-Tai Kim,[†] and Sung-Kee Chung*

Department of Chemistry, Pohang University of Science and Technology, Pohang 790-784, Korea

*E-mail: skchung@postech.ac.kr

[†]Department of Life Science, Pohang University of Science and Technology, Pohang 790-784, Korea

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We synthesized eight G8 molecular transporters (MTs) based on 4 different monosaccharide scaffolds, and studied their biological properties with a special focus on possible mitochondrial targeting and tissue selectivity. The mitochondrial affinity of these MTs was found to be clearly related to the scaffold stereochemistry and also tenuously with the lipophilicity. It may be suggested that in the practical delivery strategy of drugs for the brain and mitochondrial diseases the BBB permeability and mitochondrial affinity should be considered as key parameters, and that an enhanced mitochondrial affinity appears possible by further research on the structure-property relationship of guanidine-rich molecular transporters.

Key Words : Drug delivery, Mitochondrial affinity, Scaffold stereochemistry, Monosaccharide, Blood-brain barrier

Introduction

Good bioavailability at the target tissue is an essential requirement of any new drug candidate. Because of poor pharmacokinetic profiles including absorption, distribution, metabolism or excretion (ADME), many drug candidates with promising *in vitro* activity either fail completely or end up with only limited usages. Human body has many layers of membrane barrier, such as plasma membrane, nuclear membrane, and blood-brain barrier (BBB). A successful drug must be able to overcome one or more of the bio-membranes. Against this backdrop there has been a high demand for molecular transporters (MTs) that can help a promising drug candidate to be taken up by cells and delivered to the desired tissues. Much research efforts have been focused on cell-penetrating peptides (CPPs) in terms of their uptake mechanisms and their utility as delivery vectors for the purpose of enhancing pharmacological properties of various drug candidates, including small molecules, proteins, genes, etc.¹⁻⁶

We have recently explored novel classes of synthetic molecular transporters, in which multiple units of guanidine are attached through linear or branched chain carboxylate linkers to a variety of scaffolds such as inositol dimers, sorbitol, lactose, and sucrose.⁷⁻¹² These guanidine-rich MTs generally exhibited good uptake properties and displayed rather diverse intracellular organellar and tissue selectivity. A series of G8 molecular transporters based on the sorbitol scaffold showed colocalization with MitoTracker Red in HeLa as well as CD34⁺ stem cell-like KG1a leukaemia cells, together with interesting tissue distribution patterns.⁸ The mitochondrial localization of some molecular transporters seemed of significance because of their potential as mitochondria targeting delivery vector in connection with develop-

ing either diagnostic or therapeutic agents for several important mitochondrial diseases: familial amyotrophic lateral sclerosis (ALS), Alzheimer's disease, and Huntington's disease, and also for studying apoptosis and aging, in which mitochondria are known to play essential roles.¹³⁻¹⁸ In the design of the guanidine-rich MTs, it has usually been assumed that the intracellular localization patterns, in particular the mitochondrial affinity must be related to a number of factors including 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 relationship of these MTs in terms of the mitochondrial affinity, we previously studied G8 MTs based on *myo*- and *scyllo*-inositol scaffolds. Their intracellular localization patterns suggested that the mitochondrial affinity appeared better related to the stereochemistry of the inositol scaffold than the reverse phase HPLC-based lipophilicity.¹⁹ Now we have more systematically studied the mitochondrial affinity of a series of guanidine-rich MTs built on stereoisomeric monosaccharide scaffolds: D-glucose, mannose, allose and galactose, and wish to report the results herein.

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), analytical reverse-phase TLC on a Merck RP-8 F254s, and visualization was done with

UV light (254 nm and 365 nm), 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 C₈-reversed phase silica gel. NMR spectra were recorded on a Bruker DPX 300 (¹H-NMR at 300 MHz; ¹³C-NMR at 75 MHz) and Bruker DRX 500 (¹H-NMR at 500 MHz; ¹³C-NMR at 125 MHz) spectrometers. Tetramethylsilane was used as reference for ¹H 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 (5 μ m, 4.6 mm ID $\times$ 25 cm). Low resolution mass spectra were determined on a Micromass PLATFORM II (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 Na₂SO₄ or MgSO₄, and evaporating the solvent.

2,3,4,6-Tetra-*O*-benzoyl- α -D-glucosyl Benzoate (5): To a solution of D-glucose (2 g, 11 mmol) in dry pyridine (24 mL) at rt, was added dropwise benzoyl chloride (8 mL, 67 mmol). The mixture was stirred at rt for 8 h, and cold water (10 mL) was added to quench the reaction. The reaction mixture was diluted with CH₂Cl₂ (100 mL) and the organic phase was washed first with 1N HCl solution (2 $\times$ 20 mL) and sat. NaHCO₃ solution to neutral pH. The extract was dried (Na₂SO₄) and evaporated under reduced pressure to afford a crude product as yellow oil, which was crystallized in methanol and acetone to give **5** as a white solid (7.51 g, 97.4%). *R*_f 0.7 (EtOAc:*n*-Hexane = 1:2); mp 175-178 °C; [α]_D²⁵ 26.4 (*c* 1.39 in CHCl₃); ¹H-NMR(CDCl₃): δ 4.38-4.44 (m, 1H, H-5), 4.48 (dd, *J* = 12.3, 4.8 Hz, 1H, H-6), 4.64 (dd, *J* = 12.3, 2.9 Hz, 1H, H-6), 5.80-5.89 (m, 2H, H-2 & H-4), 6.04 (t, *J* = 9.4 Hz, 1H, H-3), 6.29 (d, *J* = 7.9 Hz, 1H, H-1), 7.20-8.05 (m, 25H, aromatic); ¹³C-NMR (CDCl₃): δ 63.10, 69.50, 71.27, 73.24, 73.60, 93.12, 128.75, 128.82, 128.85, 128.92, 129.13, 129.96, 130.23, 130.60, 133.48, 133.74, 133.84, 133.92, 134.22, 165.00, 165.52, 166.08, 166.50 ppm; MS(FAB) *m/z* calcd. for C₄₁H₃₂O₁₁Na 723.08 [M+Na]⁺, found 723.69 [M+Na]⁺; HRMS (FAB) *m/z* found 723.1765 [M+Na]⁺.

2,3,4,6-Tetra-*O*-benzoyl- α -D-mannosyl Benzoate (6): To a solution of D-mannose (1 g, 5.55 mmol) in dry pyridine (28 mL) at 0 °C, was added dropwise benzoyl chloride (4 mL, 33.5 mmol). The mixture was stirred at rt for 6 h, and cold water (10 mL) was added to quench the reaction. The reaction mixture was diluted with CH₂Cl₂ (100 mL) and the organic phase was washed first with 1N HCl solution (2 $\times$ 20 mL) and sat. NaHCO₃ solution to neutral pH. The extract was dried (Na₂SO₄) and evaporated under reduced pressure to afford a crude product as yellow oil, which was purified on silica gel (EtOAc:*n*-Hexane = 1:3) to give **6** as a white

foamy solid (3.22 g, 82.8 %). *R*_f 0.6 (EtOAc:*n*-Hexane = 1:4); mp 118-119 °C; [α]_D²⁵ -22.2 (*c* 1.05 in CHCl₃); ¹H-NMR (CDCl₃): δ 4.36-4.60 (m, 2H, H-5 & H-6), 4.72 (dd, *J* = 16.7, 2.6 Hz, 1H, H-6), 5.93(t, *J* = 2.8 Hz, 1H, H-2), 6.06 (dd, *J* = 10.2, 3.2 Hz, 1H, H-3), 6.30 (t, *J* = 10.0 Hz, 1H, H-4), 6.64 (d, *J* = 1.7 Hz, 1H, H-1), 7.25-8.22 (m, 25H, aromatic); ¹³C-NMR (CDCl₃): δ 62.75, 66.55, 69.84, 70.42, 71.60, 73.73, 91.79, 128.86, 129.10, 129.20, 129.25, 129.40, 130.20, 130.38, 130.57, 133.50, 133.84, 133.99, 134.10, 134.51, 164.25, 165.56, 165.71, 166.11, 166.47 ppm; MS (FAB) *m/z* calcd. for C₄₁H₃₂O₁₁Na 723.08 [M+Na]⁺, found 723.39 [M+Na]⁺; HRMS (FAB) *m/z* found 723.1842 [M+Na]⁺.

2,3,4,6-Tetra-*O*-benzoyl- α -D-allosyl Benzoate (7): To a solution of D-allose (0.47 g, 2.608 mmol) in dry pyridine (14 mL) at 0 °C, was added dropwise benzoyl chloride (4 mL, 33.5 mmol). The mixture was stirred at rt for 6 h, and cold water (10 mL) was added to quench the reaction. The reaction mixture was diluted with CH₂Cl₂ (100 mL) and the organic phase was washed first with 1N HCl solution (2 $\times$ 20 mL) and sat. NaHCO₃ solution to neutral pH. The extract was dried (Na₂SO₄) and evaporated under reduced pressure to afford a crude product as yellow oil, which was purified on silica gel (Ethyl acetate:*n*-Hexane = 1:3) to give **7** as a white foamy solid (2.4 g, quant.). *R*_f 0.5 (EtOAc:*n*-Hexane = 1:2); mp 162-168 °C; [α]_D²⁵ -2.8 (*c* 0.99 in CHCl₃); ¹H-NMR (CDCl₃): δ 4.47 (dd, *J* = 12.3, 4.8 Hz, 1H, H-6), 4.69 (dd, *J* = 12.3, 2.5 Hz, 1H, H-6), 4.84 (m, 1H, H-5), 5.67 (dd, *J* = 10.1, 3.0 Hz, 1H, H-4), 5.75 (dd, *J* = 8.4, 3.0 Hz, 1H, H-2), 6.36 (t, *J* = 2.9 Hz, 1H, H-3), 6.65 (d, *J* = 8.5 Hz, 1H, H-1), 7.25-8.14 (m, 25H, aromatic); ¹³C-NMR (CDCl₃): δ 63.39, 67.49, 69.20, 69.64, 72.13, 91.73, 128.82, 128.91, 129.20, 129.66, 129.73, 129.95, 130.24, 130.63, 133.60, 133.88, 133.94, 134.12, 134.21, 165.18, 165.24, 165.36, 165.71, 166.61, 172.46 ppm; MS (FAB) *m/z* calcd. for C₄₁H₃₂O₁₁Na 723.08 [M+Na]⁺, found 723.39 [M+Na]⁺; HRMS (FAB) *m/z* found 723.1842 [M+Na]⁺.

2,3,4,6-Tetra-*O*-benzoyl- α -D-galactosyl Benzoate (8): To a solution of D-galactose (1 g, 5.55 mmol) in dry pyridine (28 mL) at 0 °C, was added dropwise benzoyl chloride (4 mL, 33.5 mmol). The mixture was stirred at rt for 6 h, and cold water (10 mL) was added to quench the reaction. The reaction mixture was diluted with CH₂Cl₂ (100 mL) and the organic phase was washed first with 1 N HCl solution (2 $\times$ 20 mL) and sat. NaHCO₃ solution to neutral pH. The extract was dried (Na₂SO₄) and evaporated under reduced pressure to afford a crude product as yellow oil, which was purified on silica gel (Ethyl acetate:*n*-Hexane = 1:3) to give **8** as a white foamy solid (3.27 g, 84.1%). *R*_f 0.5 (EtOAc:*n*-Hexane = 1:3); [α]_D²⁵ 122.2 (*c* 0.90 in CHCl₃); ¹H-NMR(CDCl₃): δ 4.40 (dd, *J* = 11.3, 7.0 Hz, 1H, H-6), 4.61 (dd, *J* = 11.3, 6.4 Hz, 1H, H-6), 4.84 (t, *J* = 6.5 Hz, 1H, H-5), 6.01 (dd, *J* = 10.6, 3.5 Hz, 1H, H-2), 6.12 (dd, *J* = 10.7, 3.1 Hz, 1H, H-3), 6.20 (d, *J* = 2.1 Hz, 1H, H-4), 6.96 (d, *J* = 3.5 Hz, 1H, H-1), 7.25-8.14 (m, 25H, aromatic); ¹³C-NMR (CDCl₃): δ 62.23, 68.09, 68.86, 68.94, 69.84, 91.08, 128.78, 128.83, 129.10, 129.16, 129.36, 129.68, 130.18, 130.37, 130.61, 133.66, 133.83, 133.90, 134.16, 134.35, 164.95, 165.90, 165.98,

166.12, 166.33 ppm; MS (FAB) m/z calcd. for $C_{41}H_{33}O_{11}$ 701.70 $[M+H]^+$, found 701.38 $[M+H]^+$; HRMS (FAB) m/z found 723.2023 $[M+H]^+$.

2,3,4,6-Tetra-*O*-benzoyl- α -D-glucopyranose (9): To a solution of **5** (3.66 g, 5.223 mmol) in CH_2Cl_2 (20 mL) at 0 °C, was added dropwise 30% HBr/HOAc (1.33 mL). The mixture was stirred at rt for 6 h, and ice water was added to quench the reaction. The mixture was washed with sat. $NaHCO_3$ and brine, then the organic extract was dried over Na_2SO_4 and evaporated under reduced pressure to afford yellow oil. The crude product was dissolved in acetone (18 mL) and water (0.9 mL). The solution was treated with Ag_2CO_3 (2.16 g, 7.834 mmol), and stirred at rt for 2 h. The mixture was filtered over a bed of celite and concentrated to give **9** (α,β mixture) as a white foamy solid (3.24 g, quant.). R_f 0.3 (EtOAc:*n*-Hex = 1:2); 1H -NMR ($CDCl_3$): δ 4.16-4.22 (m, 1H, H-5 β), 4.41-4.53 (m, 1H, H-6 α & H-6 β), 4.62-4.70 (m, 1H, H-6 β & H-5 α), 5.08 (brs, 1H, H-1), 5.28-5.41 (m, 1H, H-2 α & H-2 β), 5.68-5.77 (m, 1H, H-1 α , H-5 α & H-5 β), 5.97 (t, J = 9.7 Hz, 1H, H-3 β), 5.97 (t, J = 9.9 Hz, 1H, H-3 α), 7.28-8.04 (m, 20H, aromatic); ^{13}C -NMR ($CDCl_3$): δ 63.27, 63.46, 68.19, 69.89, 70.54, 72.66, 72.73, 72.88, 74.70, 90.89, 96.52, 128.78, 128.87, 129.10, 129.13, 129.30, 129.55, 129.91, 130.15, 130.24, 130.40, 133.58, 133.78, 133.94, 134.09, 165.62, 166.24, 166.67, 167.24 ppm; MS (FAB) m/z calcd. for $C_{34}H_{29}O_{10}$ 597.18 $[M+H]^+$, found 597.18 $[M+H]^+$; HRMS (FAB) m/z found 597.1766 $[M+H]^+$.

2,3,4,6-Tetra-*O*-benzoyl- α -D-mannopyranose (10): To a solution of **6** (1.95 g, 2.8 mmol) in CH_2Cl_2 (10 mL) at 0 °C, was added dropwise 30% HBr/HOAc (1.30 mL). The mixture was stirred at rt for 6 h, and ice water was added to quench the reaction. The mixture was washed with sat. $NaHCO_3$ and brine, then the organic extract was dried over Na_2SO_4 and evaporated under reduced pressure to afford yellow oil. The crude product was dissolved in acetone (6 mL) and water (0.3 mL). The solution was treated with Ag_2CO_3 (0.383 g, 1.39 mmol), and stirred at rt for 2 h. The mixture was filtered over a bed of celite and concentrated to give **10** (α,β mixture) as a white foamy solid (1.67 g, quant.). R_f 0.2 (EtOAc:*n*-Hexane = 1:2); 1H -NMR ($CDCl_3$): δ 4.41 (dd, J = 12.1, 3.5 Hz, 1H, H-6 $\alpha\beta$), 4.41-4.53 (m, 2H, H-5 $\alpha\beta$, H-6 $\alpha\beta$), 5.51 (brs, 1H, H-1 β), 5.54 (d, J = 1.5 Hz, 1H, H-1 α), 5.68-5.76 (m, 1H, H-2 $\alpha\beta$), 5.99 (dd, J = 10.2, 3.2 Hz, 1H, H-3 $\alpha\beta$), 6.12-6.22 (m, 1H, H-2 $\alpha\beta$), 7.26-8.11 (m, 20H, aromatic); ^{13}C -NMR ($CDCl_3$): δ 63.12, 67.26, 69.28, 70.19, 71.28, 92.76, 128.72, 128.87, 128.98, 129.49, 129.68, 129.93, 130.23, 133.51, 133.60, 133.85, 165.91, 166.00, 166.80 ppm; MS (FAB) m/z calcd. for $C_{34}H_{29}O_{10}$ 597.18 $[M+H]^+$, found 597.30 $[M+H]^+$; HRMS (FAB) m/z found 597.1761 $[M+H]^+$.

2,3,4,6-Tetra-*O*-benzoyl- α -D-allopyranose (11): To a solution of **7** (1.8 g, 2.6 mmol) in CH_2Cl_2 (10 mL) at 0 °C, was added dropwise 30% HBr/HOAc (1.5 mL). The mixture was stirred at rt for 6 h, and ice water was added to quench the reaction. The mixture was washed with sat. $NaHCO_3$ and brine, then the organic extract was dried over Na_2SO_4 and evaporated under reduced pressure to afford yellow oil. The

crude product was dissolved in acetone (6 mL) and water (0.3 mL). The solution was treated with Ag_2CO_3 (1.1 g, 3.9 mmol), and stirred at rt for 2 h. The mixture was filtered over a bed of celite and concentrated to give **11** (α,β mixture) as a white foamy solid (1.67 g, quant.). R_f 0.2 (EtOAc:*n*-Hexane = 1:2); 1H -NMR ($CDCl_3$): δ 4.44-4.51 (m, 1H, H-6 $\alpha\beta$), 4.61-4.92 (m, 2H, H-5 $\alpha\beta$, H-6 $\alpha\beta$), 5.32 (dd, J = 8.1, 2.9 Hz, 1H, H-2), 5.40-5.77 (m, 2H, H-1 $\alpha\beta$ & H-4 $\alpha\beta$), 6.17-6.35 (m, 1H, H-3 $\alpha\beta$), 7.26-8.08 (m, 20H, aromatic); ^{13}C -NMR ($CDCl_3$): δ 63.69, 67.18, 67.92, 69.89, 71.58, 72.21, 94.47, 128.82, 129.16, 129.23, 129.81, 129.96, 130.21, 133.63, 133.90, 134.06, 165.25, 165.69, 166.69; MS (FAB) m/z calcd. for $C_{34}H_{29}O_{10}$ 597.18 $[M+H]^+$, found 597.28 $[M+H]^+$; HRMS (FAB) m/z found 597.1761 $[M+H]^+$.

2,3,4,6-Tetra-*O*-benzoyl- α -D-galactopyranose (12): To a solution of **8** (2.1 g, 3.0 mmol) in CH_2Cl_2 (10 mL) at 0 °C, was added dropwise 30% HBr/HOAc (1.4 mL). The mixture was stirred at rt for 6 h, and ice water was added to quench the reaction. The mixture was washed with sat. $NaHCO_3$ and brine, then the organic extract was dried over Na_2SO_4 and evaporated under reduced pressure to afford yellow oil. The crude product was dissolved in acetone (6 mL) and water (0.3 mL). The solution was treated with Ag_2CO_3 (0.414 g, 1.5 mmol), and stirred at rt for 2 h. The mixture was filtered over bed of celite and concentrated to give **12** (α,β mixture) as a white foamy solid (1.73 g, 97%). R_f 0.3 (EtOAc:*n*-Hexane = 1:2); 1H -NMR ($CDCl_3$): δ 4.35-4.47 (m, 2H, H-5 & H-6), 4.59-4.71 (m, 1H, H-6), 5.06 (t, J = 7.7 Hz, 1H, H-2), 5.61-5.76 (m, 2H, H-1 & H-3), 6.01-6.11 (m, 1H, H-4), 7.24-8.11 (m, 20H, aromatic); ^{13}C -NMR ($CDCl_3$): δ 62.49, 62.81, 67.28, 68.36, 68.58, 69.65, 69.89, 71.35, 91.52, 128.68, 128.74, 128.83, 128.88, 129.06, 129.49, 129.60, 129.83, 130.13, 130.21, 130.36, 133.59, 133.65, 133.86, 133.97, 134.09, 166.00, 166.48 ppm; MS (FAB) m/z calcd. for $C_{34}H_{29}O_{10}$ 597.18 $[M+H]^+$, found 597.28 $[M+H]^+$; HRMS (FAB) m/z found 597.1761 $[M+H]^+$.

2,3,4,6-Tetra-*O*-benzoyl- α -D-glucopyranosyl Trichloroacetimidate (13): To a solution of **9** (3.24 g, 5.223 mmol) in dry CH_2Cl_2 (30 mL) were added trichloroacetonitrile (10.5 mL, 104.46 mmol) and CS_2CO_3 (0.85 g, 2.612 mmol). The reaction mixture was stirred at rt for 12 h, then the mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:*n*-Hexane = 1:4) to afford **13** (3.56 g, 92%) as colorless foam. R_f 0.8 (EtOAc:*n*-Hexane = 1:2 x2); 1H -NMR ($CDCl_3$): δ 4.46 (dd, J = 12.8, 5.5 Hz, 1H, H-5), 4.62-4.68 (m, 2H, H-5 & H-6), 5.60 (dd, J = 10.2, 3.7 Hz, 2H, H-2), 5.82 (t, J = 9.8 Hz, 1H, H-4), 6.28 (t, J = 10.0 Hz, 1H, H-3), 6.84 (d, J = 3.7 Hz, 1H, H-1), 7.26-8.05 (m, 20H, aromatic), 8.64 (s, 1H, NH); ^{13}C -NMR ($CDCl_3$): δ 62.89, 69.07, 70.60, 71.11, 91.14, 93.53, 128.61, 128.76, 128.80, 128.86, 128.96, 129.02, 129.26, 129.98, 130.14, 130.18, 130.34, 133.57, 133.71, 133.98, 160.93, 165.61, 165.82, 165.06, 166.46 ppm.

2,3,4,6-Tetra-*O*-benzoyl- α -D-mannopyranosyl Trichloroacetimidate (14): To a solution of **10** (1.60 g, 2.682 mmol) in dry CH_2Cl_2 (12 mL) were added trichloroacetonitrile (2.7

mL, 26.82 mmol) and Cs_2CO_3 (175 mg, 0.536 mmol). The reaction mixture was stirred at rt for 12 h, then the mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:*n*-Hexane = 1:4) to afford **14** (1.78 g, 81%) as colorless foam. R_f 0.8 (EtOAc:*n*-Hexane = 1:2 x2); $^1\text{H-NMR}$ (CDCl_3): δ 4.47 (dd, J = 12.2, 4.0 Hz, 1H, H-6), 4.61-4.66 (m, 1H, H-5), 4.71 (dd, J = 12.2, 2.3 Hz, 1H, H-6), 5.93-6.00 (m, 2H, H-2 & H-3), 6.24 (t, J = 10.0 Hz, 1H, H-4), 6.57 (d, J = 1.2 Hz, 1H, H-1), 7.26-8.10 (m, 20H, aromatic), 8.86 (s, 1H, NH); $^{13}\text{C-NMR}$ (CDCl_3): δ 62.76, 66.43, 69.23, 70.21, 71.93, 95.05, 128.79, 128.86, 128.90, 129.08, 129.21, 129.32, 130.17, 130.27, 130.36, 133.52, 133.76, 134.00, 134.11, 160.26, 165.52, 165.77, 165.89, 166.44 ppm.

2,3,4,6-Tetra-*O*-benzoyl- α -D-allopyranosyl Trichloroacetimidate (15): To a solution of **11** (1.37 g, 2.30 mmol) in dry CH_2Cl_2 (15 mL) were added trichloroacetonitrile (5.2 mL, 52.00 mmol) and Cs_2CO_3 (424 mg, 1.30 mmol). The reaction mixture was stirred at rt for 12 h, then the mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:*n*-Hexane = 1:4) to afford **15** (1.79 g, 86%) as colorless foam. R_f 0.7 (EtOAc:*n*-Hexane = 1:2 x2); $^1\text{H-NMR}$ (CDCl_3): δ 4.45 (dd, J = 12.2, 5.4 Hz, 1H, H-6), 4.67 (dd, J = 12.2, 3.0 Hz, 1H, H-6), 4.96-5.01 (m, 1H, H-5), 5.63-5.72 (m, 2H, H-2 & H-4), 6.34 (t, J = 3.3 Hz, 1H, H-3), 6.81 (d, J = 4.5 Hz, 1H, H-1), 7.26-8.24 (m, 20H, aromatic), 8.75 (s, 1H, NH); $^{13}\text{C-NMR}$ (CDCl_3): δ 63.14, 66.89, 66.97, 67.09, 67.78, 91.45, 92.74, 128.82, 129.10, 129.18, 129.74, 130.00, 130.16, 130.24, 130.33, 130.56, 130.75, 133.60, 133.94, 161.58, 165.10, 165.26, 166.35, 166.51 ppm.

2,3,4,6-Tetra-*O*-benzoyl- α -D-galactopyranosyl Trichloroacetimidate (16): To a solution of **12** (1.70 g, 2.85 mmol) in dry CH_2Cl_2 (15 mL) were added trichloroacetonitrile (8.7 mL, 57.00 mmol) and Cs_2CO_3 (464 mg, 1.425 mmol). The reaction mixture was stirred at rt for 12 h, then the mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:*n*-Hexane = 1:5) to afford **16** (1.68 g, 84%) as colorless foam. R_f 0.7 (EtOAc:*n*-Hexane = 1:2 x2); $^1\text{H-NMR}$ (CDCl_3): δ 4.41 (dd, J = 11.4, 6.1 Hz, 1H, H-6), 4.59 (dd, J = 11.4, 6.9 Hz, 1H, H-6), 4.87 (t, J = 6.4 Hz, 1H, H-5), 5.94 (dd, J = 10.6, 3.6 Hz, 1H, H-2), 6.06 (dd, J = 10.6, 3.2 Hz, 1H, H-3), 6.16 (d, J = 2.5 Hz, 1H, H-4), 6.91 (d, J = 3.6 Hz, 1H, H-1), 7.26-8.11 (m, 20H, aromatic), 8.64 (s, 1H, NH); $^{13}\text{C-NMR}$ (CDCl_3): δ 62.60, 68.26, 68.78, 68.92, 70.92, 91.18, 94.16, 126.30, 128.75, 128.82, 128.86, 129.14, 129.27, 129.33, 129.77, 130.17, 130.26, 130.38, 133.67, 133.77, 133.99, 134.15, 161.03, 165.86, 165.92, 166.04, 166.33, 175.42, 179.94 ppm.

5-(*N*-Benzyloxycarbonyl)-aminopentyl 2,3,4,6-tetra-*O*-benzoyl- β -D-glucopyranoside (17): A mixture of **13** (2.8 g, 3.779 mmol) and *N*-Cbz-5-aminopentanol (1.08 g, 4.535 mmol) in dry CH_2Cl_2 (25 mL) with 4 Å molecular sieve was stirred for 0.5 h under N_2 , cooled to 10 °C, and $\text{Sn}(\text{OTf})_2$

(158 mg, 0.378 mmol) was added. After stirring for 12 h at rt, the mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:*n*-Hexane = 1:3) to afford **17** (2.46 g, 80%) as foamy solid. R_f 0.5 (EtOAc:*n*-Hexane = 1:2 x2); $[\alpha]_D^{25}$ 8.8 (*c* 1.15 in CHCl_3); $^1\text{H-NMR}$ (CDCl_3): δ 1.20-1.57 (m, 6H), 2.91 (q, J = 6.5 Hz, 2H), 3.49-3.54 (m, 1H), 3.90-3.93 (m, 1H), 4.13-4.17 (m, 1H, NHCBz), 4.47 (dd, J = 12.1, 5.2 Hz, 1H, H-6), 4.62 (dd, J = 12.1, 3.2, 2H, H-5 & H-6), 4.80 (d, J = 7.8 Hz, 1H, H-1), 5.08 (s, 2H), 5.49 (dd, J = 9.7, 7.8 Hz, 1H, H-2), 5.64 (t, 1H, H-4), 5.90 (t, J = 9.6 Hz, 1H, H-3), 7.25-8.03 (m, 25H, aromatic); $^{13}\text{C-NMR}$ (CDCl_3): δ 22.04, 23.43, 29.32, 29.78, 41.21, 63.56, 66.91, 70.21, 70.38, 72.34, 72.61, 73.27, 101.70, 128.47, 128.71, 128.82, 128.91, 129.22, 129.72, 130.00, 130.15, 130.24, 133.56, 133.65, 133.71, 133.85, 137.12, 156.70, 165.46, 165.61, 166.23, 166.56, 166.99 ppm; MS (FAB) m/z calcd for $\text{C}_{47}\text{H}_{46}\text{NO}_{12}$ 816.30 $[\text{M}+\text{H}]^+$, found 816.30 $[\text{M}+\text{H}]^+$; HRMS (FAB) m/z found 816.3027 $[\text{M}+\text{H}]^+$.

5-(*N*-Benzyloxycarbonyl)-aminopentyl 2,3,4,6-tetra-*O*-benzoyl- α -D-mannopyranoside (18): A mixture of **14** (1.65 g, 2.227 mmol) and *N*-Cbz-5-aminopentanol (634 mg, 2.672 mmol) in dry CH_2Cl_2 (15 mL) with 4 Å molecular sieve was stirred for 0.5 h under N_2 , cooled to 10 °C, and $\text{Sn}(\text{OTf})_2$ (93 mg, 0.223 mmol) was added. After stirring for 12 h at rt, the mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:*n*-Hexane = 3:7) to afford **18** (1.34 g, 74%) as foamy solid. R_f 0.4 (EtOAc:*n*-Hexane = 1:2 x2); $[\alpha]_D^{25}$ -33.0 (*c* 1.02 in CHCl_3); $^1\text{H-NMR}$ (CDCl_3): δ 1.46-1.75 (m, 6H), 3.21 (q, J = 6.6 Hz, 2H), 3.53-3.60 (m, 1H), 3.80-3.85 (m, 1H), 4.41-4.52 (m, 2H, H-5 & H-6), 4.68 (dd, J = 12.0, 2.4 Hz, 1H, H-6), 4.95 (brs, 1H, NHCBz), 5.08 (d, J = 1.3 Hz, 1H, 1-H), 5.10 (s, 2H), 5.68 (dd, J = 3.2, 1.8 Hz, 1H, H-2), 5.89 (dd, J = 10.1, 3.3 Hz, 1H, H-3), 6.11 (t, J = 10.0 Hz, 1H, H-4), 7.25-8.10 (m, 25H, aromatic); $^{13}\text{C-NMR}$ (CDCl_3): δ 23.89, 24.22, 29.38, 30.21, 41.39, 60.81, 63.38, 67.00, 67.42, 69.00, 69.29, 70.55, 71.02, 98.05, 128.45, 128.72, 128.86, 128.91, 129.00, 129.38, 129.47, 129.74, 130.14, 130.25, 133.51, 133.61, 133.86, 137.09, 156.88, 165.89, 165.94, 166.23, 165.97, 166.59, 168.36 ppm; MS (FAB) m/z calcd for $\text{C}_{47}\text{H}_{46}\text{NO}_{12}$ 816.30 $[\text{M}+\text{H}]^+$, found 816.40 $[\text{M}+\text{H}]^+$; HRMS (FAB) m/z found 816.3020 $[\text{M}+\text{H}]^+$.

5-(*N*-Benzyloxycarbonyl)-aminopentyl 2,3,4,6-tetra-*O*-benzoyl- β -D-allopyranoside (19): A mixture of **15** (1.08 g, 1.46 mmol) and *N*-Cbz-5-aminopentanol (415 mg, 1.75 mmol) in dry CH_2Cl_2 (10 mL) with 4 Å molecular sieve was stirred for 0.5 h under N_2 , cooled to 10 °C, and $\text{Sn}(\text{OTf})_2$ (61 mg, 0.14 mmol) was added. After stirring for 12 h at rt, the mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography (EtOAc:*n*-Hexane=3:7) to afford **19** (1.05 g, 88%) as foamy solid. R_f 0.4 (EtOAc:*n*-Hexane = 1:2 x2); $[\alpha]_D^{25}$ 0.3 (*c* 1.08 in CHCl_3); $^1\text{H-NMR}$ (CDCl_3): δ 1.23-1.62 (m, 6H), 2.95 (q, J = 6.3 Hz, 2H), 3.53-3.58 (m, 1H), 3.92-3.97 (m, 1H), 4.44 (dd, J = 11.8, 5.2 Hz, 1H, H-6), 4.53-4.58

(m, 2H, H-5 & NHCH_2Cbz), 4.68 (dd, $J = 11.8, 2.7$ Hz, 1H, H-6), 5.06 (s, 2H), 5.14 (d, $J = 8.0$ Hz, 1H, H-1), 5.38 (dd, $J = 8.0, 3.1$ Hz, 1H, H-2), 5.54 (dd, $J = 9.8, 2.9$ Hz, 1H, H-4), 6.21 (t, $J = 3.0$ Hz, 1H, H-3), 7.26–8.05 (m, 25H, aromatic); ^{13}C -NMR (CDCl_3): δ 23.51, 29.46, 29.84, 63.84, 66.93, 68.05, 69.83, 70.12, 70.24, 71.20, 99.75, 128.50, 128.78, 128.83, 128.91, 129.11, 129.34, 129.79, 129.98, 130.15, 133.61, 133.82, 133.93, 137.08, 156.71, 165.18, 165.64, 166.63 ppm; MS (FAB) m/z calcd for $\text{C}_{47}\text{H}_{46}\text{NO}_{12}$ 816.30 $[\text{M}+\text{H}]^+$, found 816.30 $[\text{M}+\text{H}]^+$; HRMS (FAB) m/z found 816.3020 $[\text{M}+\text{H}]^+$.

5-(*N*-Benzyloxycarbonyl)-aminopentyl 2,3,4,6-tetra-*O*-benzoyl- β -D-galactopyranoside (20): A mixture of **16** (1.67 g, 2.25 mmol) and *N*-Cbz-5-aminopentanol (642 mg, 2.70 mmol) in dry CH_2Cl_2 (17 mL) with 4 Å molecular sieve was stirred for 0.5 h under N_2 , cooled to 10 °C, and $\text{Sn}(\text{OTf})_2$ (95 mg, 0.22 mmol) was added. After stirring for 12 h at rt, the mixture was filtered and the filtrate was concentrated under reduced pressure. The crude product was purified by column chromatography ($\text{EtOAc}:\text{n-Hexane} = 3:7$) to afford **20** (1.35 g, 74%) as foamy solid. R_f 0.3 ($\text{EtOAc}:\text{n-Hexane} = 1:2 \times 2$); $[\alpha]_{\text{D}}^{25}$ 48.0 (c 1.13 in CHCl_3); ^1H -NMR (CDCl_3): δ 1.21–1.66 (m, 6H), 3.21 (q, $J = 6.4$ Hz, 2H), 3.52–3.59 (m, 1H), 3.95–4.02 (m, 1H), 4.32 (t, $J = 6.7$ Hz, 1H, H-6), 4.30 (dd, $J = 11.1, 6.7$ Hz, 1H, H-6), 4.58 (brs, 1H, NHCH_2Cbz), 4.64 (dd, $J = 11.3, 4.9$ Hz, 1H, H-5), 4.78 (d, $J = 7.9$ Hz, 1H, H-1), 5.07 (s, 2H), 5.59 (dd, $J = 10.4, 3.4$ Hz, 1H, H-2), 5.76 (dd, $J = 10.4, 7.9$ Hz, 1H, H-3), 5.99 (d, $J = 2.8$ Hz, 1H, H-4), 7.24–8.11 (m, 25H, aromatic); ^{13}C -NMR (CDCl_3): δ 23.46, 29.36, 29.84, 41.21, 62.40, 66.94, 68.52, 70.27, 70.66, 71.69, 72.05, 102.17, 127.83, 128.49, 128.70, 128.89, 129.03, 129.18, 129.43, 129.79, 130.09, 130.19, 133.46, 133.73, 134.00, 137.08, 165.62, 165.99, 166.47 ppm; MS (FAB) m/z calcd for $\text{C}_{47}\text{H}_{46}\text{NO}_{12}$ 816.30 $[\text{M}+\text{H}]^+$, found 816.40 $[\text{M}+\text{H}]^+$; HRMS (FAB) m/z found 816.3020 $[\text{M}+\text{H}]^+$.

5-(*N*-Benzyloxycarbonyl)-aminopentyl β -D-glucopyranoside (21): To a solution of **17** (1.89 g, 2.28 mmol) in MeOH (70 mL) was added NaOMe (0.35 mL, 1.14 mmol, 25% w/v), and the solution was stirred at rt for 12 h. The mixture was neutralized by filtration through acidic resin. The filtrate was concentrated and the crude product was washed with 5% EtOAc in *n*-hexane to remove the by-product, methyl benzoate. The washed product was dried under vacuum to afford **21** as colorless sticky mass (1.28 g, quant.). R_f 0.1 ($\text{MC}:\text{MeOH} = 10:1$); $[\alpha]_{\text{D}}^{25}$ –18.5 (c 1.35 in CH_3OH); ^1H -NMR (CD_3OD): δ 1.51–1.68 (m, 6H), 3.14 (t, $J = 7.0$ Hz, 2H, OCH_2), 3.20 (t, $J = 8.5$ Hz, 1H, H-2), 3.26–3.31 (m, 2H, H-4 & H-6), 3.37 (t, $J = 8.5$ Hz, 1H, H-3), 3.54–3.59 (m, 1H, CH_2NHCbz), 3.67 (dd, $J = 12.0, 5.0$ Hz, 1H, H-5), 3.87–3.94 (m, 2H, H-6 & CH_2NHCbz), 4.26 (d, $J = 8.0$ Hz, 1H, H-1), 5.08 (s, 2H, CH_2Ph), 7.30–7.36 (m, 5H, aromatic); ^{13}C -NMR (CD_3OD): 22.87, 28.97, 29.22, 40.35, 61.42, 65.92, 69.26, 70.32, 73.75, 76.52, 76.76, 102.98, 127.37, 127.54, 128.06, 137.10, 157.54 ppm; MS (FAB) m/z calcd for $\text{C}_{19}\text{H}_{30}\text{NO}_8$ 400.20 $[\text{M}+\text{H}]^+$, found 400.32 $[\text{M}+\text{H}]^+$; HRMS (FAB) m/z found 400.1971 $[\text{M}+\text{H}]^+$.

5-(*N*-Benzyloxycarbonyl)-aminopentyl α -D-mannopyra-

noside (22): To a solution of **18** (1.34 g, 1.642 mmol) in MeOH (50 mL) was added NaOMe (0.25 mL, 0.821 mmol, 25% w/v), and the solution was stirred at rt for 12 h. The mixture was neutralized by filtration through acidic resin. The filtrate was concentrated and the crude product was washed with 5% EtOAc in *n*-hexane to remove the by-product, methyl benzoate. The washed product was dried under vacuum to afford **22** as colorless sticky mass (0.72 g, quant.). R_f 0.1 ($\text{MC}:\text{MeOH} = 10:1$); $[\alpha]_{\text{D}}^{25}$ 26.3 (c 1.04 in CH_3OH); ^1H -NMR (CD_3OD): δ 1.41–1.64 (m, 6H), 3.14 (t, $J = 7.5$ Hz, 2H, OCH_2), 3.43–3.45 (m, 1H, CH_2NHCbz), 3.54–3.56 (m, 1H, H-5), 3.63 (t, $J = 9.5$ Hz, 1H, H-4), 3.70–3.76 (m, 3H, H-3, H-6 & CH_2NHCbz), 3.80–3.81 (m, 1H, H-2), 3.83 (dd, $J = 11.5, 2.5$ Hz, 1H, H-6), 4.76 (brs, 1H, H-1), 5.08 (s, 2H, CH_2Ph), 7.30–7.36 (m, 5H, aromatic); ^{13}C -NMR (CD_3OD): δ 23.16, 28.83, 29.28, 40.35, 61.53, 65.94, 67.05, 67.30, 70.87, 71.29, 73.19, 100.16, 127.36, 127.53, 128.05, 137.09, 157.54, 164.80 ppm; MS (FAB) m/z calcd for $\text{C}_{19}\text{H}_{30}\text{NO}_8$ 400.20 $[\text{M}+\text{H}]^+$, found 400.32 $[\text{M}+\text{H}]^+$; HRMS (FAB) m/z found 400.1971 $[\text{M}+\text{H}]^+$.

5-(*N*-Benzyloxycarbonyl)-aminopentyl β -D-allopyranoside (23): To a solution of **19** (315 mg, 0.39 mmol) in MeOH (15 mL) was added NaOMe (0.06 mL, 0.193 mmol, 25% w/v), and the solution was stirred at rt for 12 h. The mixture was neutralized by filtration through acidic resin. The filtrate was concentrated and the crude product was washed with 5% EtOAc in *n*-hexane to remove the by-product, methyl benzoate. The washed product was dried under vacuum to afford **23** as colorless sticky mass (176 mg, quant.). R_f 0.1 ($\text{MC}:\text{MeOH} = 10:1$); $[\alpha]_{\text{D}}^{25}$ –15.4 (c 1.30 in CH_3OH); ^1H -NMR (CD_3OD): δ 1.42–1.66 (m, 6H), 3.14 (t, $J = 6.5$ Hz, 2H, OCH_2), 3.30 (dd, $J = 8.0, 3.0$ Hz, 1H, H-2), 3.48 (dd, $J = 10.0, 2.5$ Hz, 1H, H-4), 3.54–3.56 (m, 1H, CH_2NHCbz), 3.66–3.70 (m, 2H, H-5 & H-6), 3.85–3.90 (m, 2H, H-6 & CH_2NHCbz), 4.06 (t, $J = 3.0$ Hz, 1H, H-3), 4.61 (d, $J = 8.0$ Hz, 1H, H-1), 5.08 (s, 2H, CH_2Ph), 7.31–7.36 (m, 5H, aromatic); ^{13}C -NMR (CD_3OD): δ 22.88, 29.00, 29.20, 40.34, 61.79, 65.91, 67.61, 69.16, 70.99, 71.54, 74.02, 100.46, 127.36, 127.52, 128.05, 137.11, 157.53 ppm; MS (FAB) m/z calcd for $\text{C}_{19}\text{H}_{30}\text{NO}_8$ 400.20 $[\text{M}+\text{H}]^+$, found 400.32 $[\text{M}+\text{H}]^+$; HRMS (FAB) m/z found 400.1971 $[\text{M}+\text{H}]^+$.

5-(*N*-Benzyloxycarbonyl)-aminopentyl β -D-galactopyranoside (24): To a solution of **20** (1.35 g, 1.65 mmol) in MeOH (50 mL) was added NaOMe (0.25 mL, 0.821 mmol, 25% w/v), and the solution was stirred at rt for 12 h. The mixture was neutralized by filtration through acidic resin. The filtrate was concentrated and the crude product was washed with 5% EtOAc in *n*-hexane to remove the by-product, methyl benzoate. The washed product was dried under vacuum to afford **24** as colorless sticky mass (0.65 g, 98%). R_f 0.1 ($\text{MC}:\text{MeOH} = 10:1$); $[\alpha]_{\text{D}}^{25}$ –9.9 (c 1.11 in CH_3OH); ^1H -NMR (CD_3OD): δ 1.42–1.67 (m, 6H), 3.14 (t, $J = 7.0$ Hz, 2H, OCH_2), 3.46 (dd, $J = 6.5, 3.0$ Hz, 1H, H-6), 3.50–3.58 (m, 3H, H-2, H-3 & CH_2NHCbz), 3.75–3.77 (m, 2H, H-4 & H-5), 3.85 (d, $J = 3.0$ Hz, 1H, H-6), 3.90–3.92 (m, 1H, CH_2NHCbz), 4.21 (d, $J = 7.5$ Hz, 1H, H-1), 5.08 (s, 2H, CH_2Ph), 7.30–7.36 (m, 5H, aromatic); ^{13}C -NMR (CD_3OD):

δ 24.41, 30.53, 30.74, 41.88, 62.63, 67.43, 70.45, 70.73, 72.72, 75.18, 76.72, 105.11, 128.89, 129.05, 129.57, 138.61, 159.04 ppm; MS (FAB) m/z calcd for $C_{19}H_{30}NO_8$ 400.20 $[M+H]^+$, found 400.32 $[M+H]^+$; HRMS (FAB) m/z found 400.1971 $[M+H]^+$.

5-Amino-*N*-benzyloxycarbonylpentyl 2,3,4,6-tetra-*O*-[6-[bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-hexanoyl]- β -D-glucopyranoside (25a): A solution of compound **21** (53 mg, 0.135 mmol), linker acid (**R-OH** ($n = 5$), 590 mg, 0.808 mmol), EDC (207 mg, 1.078 mmol) and DMAP (25 mg, 0.202 mmol) in dry DMF (2 mL) was stirred for 72 h at rt under N_2 . The reaction mixture was diluted with CH_2Cl_2 , washed several times with water and brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (CH_2Cl_2 :MeOH = 15:1) to afford compound **25a** (257 mg, 83%) as a white foamy solid. R_f 0.5 (MC:MeOH = 15:1x2); 1H NMR ($CDCl_3$): δ 1.16-1.70 (m, 190H), 2.04-2.47 (m, 32H), 3.13 (q, $J = 6.6$ Hz, 2H), 3.41-3.47 (m, 16H), 3.66-3.84 (m, 3H), 4.16 (brs, 1H), 4.22 (t, $J = 5.0$ Hz, 1H), 4.47 (d, $J = 7.8$ Hz, 1H), 4.93-5.24 (m, 5H), 7.29-7.34 (m, 5H), 8.51 (s, 8H), 11.50 (s, 8H); ^{13}C -NMR ($CDCl_3$): δ 23.43, 25.12, 25.19, 25.33, 26.62, 27.52, 28.44, 28.70, 29.44, 30.05, 31.61, 34.49, 36.82, 39.99, 41.28, 51.99, 54.22, 62.29, 63.58, 66.78, 69.49, 70.01, 71.59, 72.96, 79.45, 83.20, 128.36, 128.83, 137.16, 153.47, 156.43, 156.82, 164.02, 172.25, 172.96, 173.49 ppm.

5-Amino-*N*-benzyloxycarbonylpentyl 2,3,4,6-tetra-*O*-[8-[bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-octanoyl]- β -D-glucopyranoside (25b): A solution of compound **21** (57 mg, 0.144 mmol), linker acid (**R-OH** ($n = 7$), 540 mg, 0.714 mmol), EDC (182 mg, 0.951 mmol) and DMAP (22 mg, 0.178 mmol) in dry DMF (2 mL) was stirred for 72 h at rt under N_2 . The reaction mixture was diluted with CH_2Cl_2 , washed several times with water and brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (CH_2Cl_2 :MeOH = 15:1) to afford compound **25b** (322 mg, 93%) as a white foamy solid. R_f 0.5 (MC:MeOH = 10:1); 1H NMR ($CDCl_3$): δ 1.27-1.73 (m, 206H), 2.17-2.69 (m, 32H), 3.17 (t, $J = 6.5$ Hz, 2H), 3.31-3.58 (m, 16H), 3.60-3.91 (m, 3H), 4.09-4.53 (m, 3H), 4.78-5.24 (m, 5H), 7.31-7.34 (m, 5H), 8.53 (s, 8H), 11.49 (s, 8H); ^{13}C -NMR ($CDCl_3$): δ 23.40, 25.09, 25.24, 25.40, 26.44, 27.80, 28.41, 28.67, 28.92, 29.47, 29.55, 29.96, 31.58, 31.77, 34.39, 35.92, 36.81, 39.88, 41.25, 51.89, 53.81, 54.24, 66.80, 68.73, 69.43, 70.00, 72.34, 74.60, 79.47, 80.20, 83.23, 101.13, 103.35, 128.36, 128.82, 137.11, 153.43, 156.46, 156.79, 162.90, 163.95, 172.36, 173.63, 174.35 ppm.

5-Amino-*N*-benzyloxycarbonylpentyl 2,3,4,6-tetra-*O*-[6-[bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-hexanoyl]- α -D-mannopyranoside (26a): A solution of compound **22** (119 mg, 0.298 mmol), linker acid (**R-OH** ($n = 5$), 1.30 g, 1.788 mmol), EDC (457 mg, 2.384 mmol) and DMAP (55 mg, 0.447 mmol) in dry DMF (2 mL) was stirred for 72 h at rt under N_2 . The reaction mixture was diluted with CH_2Cl_2 , washed several times with water and brine. The organic phase was dried and concentrated. The residue was purified

by column chromatography (CH_2Cl_2 :MeOH = 15:1) to afford compound **26a** (849 mg, 89%) as a white foamy solid. R_f 0.5 (MC:MeOH = 15:1x2); 1H NMR ($CDCl_3$): δ 1.28-1.69 (m, 190H), 2.17-2.46 (m, 32H), 3.17 (q, $J = 6.7$ Hz, 2H), 3.46-3.52 (m, 16H), 3.66-3.71 (m, 2H), 3.92-3.99 (m, 1H), 4.09-4.24 (m, 2H), 4.76 (brs, 1H), 5.00 (brs, 1H), 5.09 (s, 2H), 5.16-5.22 (m, 2H), 5.31 (dd, $J = 10.0, 3.3$ Hz, 1H), 7.31-7.36 (m, 5H), 8.51 (s, 8H), 11.50 (s, 8H); ^{13}C -NMR ($CDCl_3$): δ 24.80, 24.85, 26.31, 27.15, 27.22, 27.90, 28.07, 28.22, 28.33, 28.48, 28.89, 29.67, 29.80, 31.23, 33.86, 33.99, 34.06, 34.11, 39.63, 40.93, 51.39, 51.65, 53.87, 62.78, 63.20, 66.29, 66.50, 68.07, 68.73, 68.98, 69.12, 69.53, 76.77, 79.05, 79.08, 82.80, 82.82, 97.49, 128.01, 128.33, 128.47, 129.56, 136.74, 153.10, 156.05, 156.41, 163.65, 172.28, 172.34, 172.56, 173.02, 173.51 ppm. δ 23.43, 25.12, 25.19, 25.33, 26.62, 27.52, 28.44, 28.70, 29.44, 30.05, 31.61, 34.49, 36.82, 39.99, 41.28, 51.99, 54.22, 62.29, 63.58, 66.78, 69.49, 70.01, 71.59, 72.96, 79.45, 83.20, 128.36, 128.83, 137.16, 153.47, 156.43, 156.82, 164.02, 172.25, 172.96, 173.49 ppm.

5-Amino-*N*-benzyloxycarbonylpentyl 2,3,4,6-tetra-*O*-[8-[bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-octanoyl]- α -D-mannopyranoside (26b): A solution of compound **22** (120 mg, 0.300 mmol), linker acid (**R-OH** ($n = 7$), 1.37 g, 1.802 mmol), EDC (460 mg, 2.400 mmol) and DMAP (55 mg, 0.450 mmol) in dry DMF (2 mL) was stirred for 72 h at rt under N_2 . The reaction mixture was diluted with CH_2Cl_2 , washed several times with water and brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (CH_2Cl_2 :MeOH = 15:1) to afford compound **26b** (892 mg, 91%) as a white foamy solid. R_f 0.5 (MC:MeOH = 10:1); 1H NMR ($CDCl_3$): δ 1.27-1.71 (m, 206H), 2.30-2.47 (m, 32H), 3.17 (t, $J = 6.7$ Hz, 2H), 3.44-3.48 (m, 16H), 3.66-3.73 (m, 2H), 3.90-4.01 (m, 1H), 4.10-4.22 (m, 2H), 4.76 (brs, 1H), 4.96 (brs, 1H), 5.09 (s, 2H), 5.18 (t, $J = 10.1$ Hz, 2H), 5.32 (dd, $J = 10.0, 3.3$ Hz, 1H), 7.29-7.36 (m, 5H), 8.52 (s, 8H), 11.49 (s, 8H); ^{13}C -NMR ($CDCl_3$): δ 25.20, 26.62, 27.92, 28.46, 28.72, 29.66, 40.03, 51.77, 54.36, 54.42, 59.56, 66.94, 69.10, 69.23, 75.93, 76.98, 78.55, 79.04, 79.23, 83.22, 85.66, 105.09, 116.20, 128.43, 128.88, 129.96, 142.40, 145.80, 153.49, 156.47, 156.81, 160.84, 164.04, 169.63, 171.79, 172.81 ppm.

5-Amino-*N*-benzyloxycarbonylpentyl 2,3,4,6-tetra-*O*-[6-[bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-hexanoyl]- β -D-allopyranoside (27a): A solution of compound **23** (58 mg, 0.146 mmol), linker acid (**R-OH** ($n = 5$), 639 mg, 0.876 mmol), EDC (224 mg, 1.168 mmol) and DMAP (27 mg, 0.219 mmol) in dry DMF (2 mL) was stirred for 72 h at rt under N_2 . The reaction mixture was diluted with CH_2Cl_2 , washed several times with water and brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (CH_2Cl_2 :MeOH = 15:1) to afford compound **27a** (331 mg, 83%) as a white foamy solid. R_f 0.5 (MC:MeOH = 15:1x2); 1H NMR ($CDCl_3$): δ 1.26-1.75 (m, 190H), 2.22-2.52 (m, 32H), 3.13 (q, $J = 6.7$ Hz, 2H), 3.44-3.46 (m, 17H), 3.83-4.06 (m, 2H), 4.19-4.24 (m, 2H), 4.63-

4.96 (m, 3H), 5.08 (s, 2H), 5.59-5.64 (m, 1H), 7.31-7.36 (m, 5H), 8.51 (s, 8H), 11.48 (s, 8H); ^{13}C -NMR (CDCl_3): δ 23.47, 24.06, 24.28, 25.08, 25.45, 26.23, 27.36, 28.44, 28.69, 28.94, 29.52, 30.05, 31.62, 33.94, 34.17, 34.34, 39.72, 41.33, 50.82, 51.78, 54.02, 62.76, 63.62, 66.80, 69.33, 69.48, 69.64, 69.96, 70.58, 79.54, 83.33, 128.39, 128.85, 137.15, 153.46, 156.50, 156.84, 163.94, 171.96, 172.62, 173.55, 173.86 ppm.

5-Amino-N-benzoyloxycarbonylpentyl 2,3,4,6-tetra-O-[8-[bis-(3-N,N'-di-Boc-guanidino-propyl)-amino]-octanoyl]- β -D-allopyranoside (27b): A solution of compound **23** (48 mg, 0.120 mmol), linker acid (**R-OH** ($n = 7$), 546 mg, 0.721 mmol), EDC (184 mg, 0.960 mmol) and DMAP (22 mg, 0.180 mmol) in dry DMF (2 mL) was stirred for 72 h at rt under N_2 . The reaction mixture was diluted with CH_2Cl_2 , washed several times with water and brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (CH_2Cl_2 :MeOH = 15:1) to afford compound **27b** (339 mg, 93%) as a white foamy solid. R_f 0.5 (MC:MeOH = 10:1); ^1H NMR (CDCl_3): δ 1.27-1.71 (m, 206H), 2.30-2.52 (m, 32H), 3.14 (q, $J = 6.5$ Hz, 2H), 3.44-3.46 (m, 17H), 3.65-4.22 (m, 4H), 4.61-4.89 (m, 3H), 5.08 (s, 2H), 5.59-5.64 (m, 1H), 7.29-7.35 (m, 5H), 8.53 (s, 8H), 11.49 (s, 8H); ^{13}C -NMR (CDCl_3): δ 23.46, 24.92, 25.02, 25.08, 25.17, 26.50, 27.84, 28.44, 28.70, 29.50, 29.60, 29.70, 30.00, 31.61, 34.40, 34.57, 39.59, 39.94, 51.60, 51.96, 54.34, 66.86, 69.87, 79.48, 79.71, 83.23, 83.78, 98.98, 128.40, 128.85, 153.45, 156.46, 156.79, 163.99, 172.46, 173.76 ppm.

5-Amino-N-benzoyloxycarbonylpentyl 2,3,4,6-tetra-O-[6-[bis-(3-N,N'-di-Boc-guanidino-propyl)-amino]-hexanoyl]- β -D-galactopyranoside (28a): A solution of compound **24** (111 mg, 0.280 mmol), linker acid (**R-OH** ($n = 5$), 1.22 g, 1.678 mmol), EDC (430 mg, 2.237 mmol) and DMAP (51 mg, 0.419 mmol) in dry DMF (2 mL) was stirred for 72 h at rt under N_2 . The reaction mixture was diluted with CH_2Cl_2 , washed several times with water and brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (CH_2Cl_2 :MeOH = 15:1) to afford compound **28a** (856 mg, 95%) as a white foamy solid. R_f 0.5 (MC:MeOH = 15:1x2); ^1H NMR (CDCl_3): δ 1.25-1.69 (m, 190H), 2.15-2.47 (m, 32H), 3.13 (q, $J = 6.5$ Hz, 2H), 3.43-3.45 (m, 17H), 3.84-3.91 (m, 2H), 4.04-4.24 (m, 2H), 4.43 (d, $J = 7.8$ Hz, 1H), 4.99 (dd, $J = 10.6$, 3.3 Hz, 1H), 5.08 (s, 2H), 5.16-5.22 (m, 1H), 5.37 (dd, $J = 3.3$ Hz, 1H), 7.31-7.34 (m, 5H), 8.52 (s, 8H), 11.50 (s, 8H); ^{13}C -NMR (CDCl_3): δ 23.48, 24.89, 25.25, 25.40, 25.62, 26.47, 26.65, 27.52, 28.09, 28.46, 28.72, 29.51, 30.07, 31.63, 34.30, 34.46, 40.01, 41.29, 41.84, 52.02, 53.80, 54.23, 55.46, 66.83, 69.51, 71.23, 76.12, 76.98, 79.50, 83.23, 101.72, 128.39, 128.85, 137.22, 138.37, 149.87, 153.49, 156.45, 161.66, 164.04, 172.27, 173.12, 173.35 ppm.

5-Amino-N-benzoyloxycarbonylpentyl 2,3,4,6-tetra-O-[8-[bis-(3-N,N'-di-Boc-guanidino-propyl)-amino]-octanoyl]- β -D-galactopyranoside (28b): A solution of compound **24** (103 mg, 0.258 mmol), linker acid (**R-OH** ($n = 7$), 1.17 g, 1.548 mmol), EDC (396 mg, 2.064 mmol) and DMAP (47

mg, 0.387 mmol) in dry DMF (2 mL) was stirred for 72 h at rt under N_2 . The reaction mixture was diluted with CH_2Cl_2 , washed several times with water and brine. The organic phase was dried and concentrated. The residue was purified by column chromatography (CH_2Cl_2 :MeOH = 15:1) to afford compound **28b** (796 mg, 94%) as a white foamy solid. R_f 0.5 (MC:MeOH = 10:1); ^1H NMR (CDCl_3): δ 1.27-1.71 (m, 206H), 2.26-2.47 (m, 32H), 3.14 (t, $J = 6.5$ Hz, 2H), 3.44-3.46 (m, 17H), 3.84-3.91 (m, 2H), 4.08-4.17 (m, 2H), 4.43 (d, $J = 7.8$ Hz, 1H), 4.99 (dd, $J = 10.4$, 3.3 Hz, 1H), 5.09 (s, 2H), 5.15-5.21 (m, 1H), 5.38 (d, $J = 3.3$ Hz, 1H), 7.31-7.36 (m, 5H), 8.52 (s, 8H), 11.49 (s, 8H); ^{13}C -NMR (CDCl_3): δ 25.13, 25.38, 26.56, 27.88, 28.46, 28.71, 29.45, 29.56, 29.63, 29.69, 30.09, 34.30, 39.95, 41.31, 51.96, 54.36, 66.90, 69.14, 70.16, 71.30, 79.51, 83.26, 101.71, 128.42, 128.87, 132.39, 137.11, 153.48, 156.47, 156.80, 157.00, 164.01, 172.41, 173.06, 173.25, 173.50 ppm.

5-Aminopentyl 2,3,4,6-tetra-O-[6-[bis-(3-N,N'-di-Boc-guanidino-propyl)-amino]-hexanoyl]- β -D-glucopyranoside (29a): A mixture of compound **25a** (104 mg, 0.032 mmol) in a mixed solvent of CH_2Cl_2 :MeOH (1:9, 10 mL) was hydrogenated (50 psi) over 10% Pd/C (200 mg) at rt. After 24 h, the catalyst was filtered through a celite bed and the filtrate was evaporated to afford free amine **29a** (81 mg, 81%) as a sticky solid. R_f 0.1 (MC:MeOH = 9:1); ^1H NMR (CDCl_3): δ 1.10-2.04 (m, 198H), 2.24-2.98 (m, 26H), 3.45-3.47 (m, 16H), 4.09-4.24 (m, 3H), 4.08-4.24 (m, 2H), 4.51-4.54 (d, $J = 7.9$ Hz, 1H), 4.91-5.34 (m, 3H), 8.50 (s, 8H), 11.48 (s, 8H) ppm.

5-Aminopentyl 2,3,4,6-tetra-O-[8-[bis-(3-N,N'-di-Boc-guanidino-propyl)-amino]-octanoyl]- β -D-glucopyranoside (29b): A mixture of compound **25b** (112 mg, 0.033 mmol) in a mixed solvent of CH_2Cl_2 :MeOH (1:9, 10 mL) was hydrogenated (50 psi) over 10% Pd/C (250 mg) at rt. After 24 h, the catalyst was filtered through a celite bed and the filtrate was evaporated to afford free amine **29b** (99 mg, 92%) as a sticky solid. R_f 0.1 (MC:MeOH = 9:1); ^1H NMR (CDCl_3): δ 1.10-1.70 (m, 214H), 2.20-3.01 (m, 26H), 3.47 (brs, 16H), 4.06-4.16 (m, 3H), 4.32-4.51 (m, 3H), 4.78-5.34 (m, 3H), 8.50 (s, 8H), 11.43 (s, 8H) ppm.

5-Aminopentyl 2,3,4,6-tetra-O-[6-[bis-(3-N,N'-di-Boc-guanidino-propyl)-amino]-hexanoyl]- α -D-mannopyranoside (30a): A mixture of compound **26a** (165 mg, 0.051 mmol) in a mixed solvent of CH_2Cl_2 :MeOH (1:9, 10 mL) was hydrogenated (50 psi) over 10% Pd/C (170 mg) at rt. After 24 h, the catalyst was filtered through a celite bed and the filtrate was evaporated to afford free amine **30a** (134 mg, 85%) as a sticky solid. R_f 0.1 (MC:MeOH = 9:1); ^1H NMR (CDCl_3): δ 1.26-2.04 (m, 198H), 2.35-3.01 (m, 26H), 3.49 (brs, 16H), 3.67-3.70 (m, 2H), 3.92-4.24 (m, 3H), 4.78 (brs, 1H), 5.09-5.34 (m, 3H), 8.50 (s, 8H), 11.47 (s, 8H) ppm.

5-Aminopentyl 2,3,4,6-tetra-O-[8-[bis-(3-N,N'-di-Boc-guanidino-propyl)-amino]-octanoyl]- α -D-mannopyranoside (30b): A mixture of compound **26b** (164 mg, 0.045 mmol) in a mixed solvent of CH_2Cl_2 :MeOH (1:9, 10 mL) was hydrogenated (50 psi) over 10% Pd/C (320 mg) at rt. After 24 h, the catalyst was filtered through a celite bed and

the filtrate was evaporated to afford free amine **30b** (132 mg, 78%) as a sticky solid. R_f 0.1 (MC:MeOH = 9:1); ^1H NMR (CDCl_3): δ 1.21–2.39 (m, 214H), 3.13 (brs, 26H), 3.53 (brs, 16H), 3.67–3.72 (m, 2H), 3.96–4.21 (m, 3H), 4.77 (s, 1H), 5.09–5.31 (m, 3H), 4.06–4.16 (m, 3H), 4.32–4.51 (m, 3H), 4.78–5.34 (m, 3H), 8.46 (s, 8H), 11.42 (s, 8H) ppm.

5-Aminopentyl 2,3,4,6-tetra-O-[6-bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-hexanoyl]- β -D-allopyranoside (31a): A mixture of compound **27a** (130 mg, 0.040 mmol) in a mixed solvent of CH_2Cl_2 :MeOH (1:9, 10 mL) was hydrogenated (50 psi) over 10% Pd/C (220 mg) at rt. After 24 h, the catalyst was filtered through a celite bed and the filtrate was evaporated to afford free amine **31a** (112 mg, 90%) as a sticky solid. R_f 0.1 (MC:MeOH = 9:1); ^1H NMR (CDCl_3): δ 1.27–2.46 (m, 198H), 2.65–3.08 (m, 26H), 3.51 (brs, 17H), 3.86–4.24 (m, 4H), 4.75–4.93 (m, 3H), 5.60 (brs, 1H), 8.48 (s, 8H), 11.45 (s, 8H) ppm.

5-Aminopentyl 2,3,4,6-tetra-O-[8-bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-octanoyl]- β -D-allopyranoside (31b): A mixture of compound **27b** (100 mg, 0.029 mmol) in a mixed solvent of CH_2Cl_2 :MeOH (1:9, 10 mL) was hydrogenated (50 psi) over 10% Pd/C (200 mg) at rt. After 24 h, the catalyst was filtered through a celite bed and the filtrate was evaporated to afford free amine **31b** (82 mg, 85%) as a sticky solid. R_f 0.1 (MC:MeOH = 9:1); ^1H NMR (CDCl_3): δ 1.26–2.41 (m, 214H), 3.07 (brs, 26H), 3.52 (brs, 17H), 3.78–4.20 (m, 4H), 4.75–4.94 (m, 3H), 5.63 (s, 1H), 8.49 (s, 8H), 11.42 (s, 8H) ppm.

5-Aminopentyl 2,3,4,6-tetra-O-[6-bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-hexanoyl]- β -D-galactopyranoside (32a): A mixture of compound **28a** (146 mg, 0.050 mmol) in a mixed solvent of CH_2Cl_2 :MeOH (1:9, 10 mL) was hydrogenated (50 psi) over 10% Pd/C (216 mg) at rt. After 24 h, the catalyst was filtered through a celite bed and the filtrate was evaporated to afford free amine **32a** (124 mg, 88%) as a sticky solid. R_f 0.1 (MC:MeOH = 9:1); ^1H NMR (CDCl_3): δ 1.26–2.36 (m, 198H), 2.13 (brs, 26H), 3.52 (brs, 17H), 3.88–3.95 (m, 2H), 4.06–4.24 (m, 2H), 4.54 (d, J = 7.6 Hz, 1H), 5.02–5.13 (m, 2H), 5.36 (d, J = 2.6 Hz, 1H), 8.48 (s, 8H), 11.45 (s, 8H) ppm.

5-Aminopentyl 2,3,4,6-tetra-O-[8-bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-octanoyl]- β -D-galactopyranoside (32b): A mixture of compound **28b** (152 mg, 0.045 mmol) in a mixed solvent of CH_2Cl_2 :MeOH (1:9, 10 mL) was hydrogenated (50 psi) over 10% Pd/C (300 mg) at rt. After 24 h, the catalyst was filtered through a celite bed and the filtrate was evaporated to afford free amine **32b** (132 mg, 90%) as a sticky solid. R_f 0.1 (MC:MeOH = 9:1); ^1H NMR (CDCl_3): δ 1.26–2.42 (m, 214H), 2.58–3.35 (m, 26H), 3.51 (brs, 17H), 3.85–3.94 (m, 2H), 4.05–4.20 (m, 2H), 4.47 (d, J = 7.7 Hz, 1H), 5.00–5.19 (m, 2H), 5.38 (d, J = 2.6 Hz, 1H), 8.47 (s, 8H), 11.44 (s, 8H) ppm.

5-(*N*-Fluoresceinyl-5-thioureido)-aminopentyl 2,3,4,6-tetra-O-[6-bis-(3-guanidino-propyl)-amino]-hexanoyl]- β -D-glucopyranoside (1a): To a solution of **29a** (81 mg, 0.026 mmol) in a mixed solvent THF and abs. ethanol (3 mL, EtOH:THF = 3:2), were added fluorescein-5-isothio-

cyanate (12 mg, 0.029 mmol) and triethylamine (30 μL , 0.208 mmol). The reaction mixture was stirred for 24 h at rt in dark, concentrated. The crude product was washed with water and brine, and the organic layer was dried over Na_2SO_4 . The crude product was dissolved in dry CH_2Cl_2 , then trifluoroacetic acid (TFA, 0.5 mL) in CH_2Cl_2 (5 mL) was added and the mixture was stirred at rt in dark for 6 h. The mixture was concentrated and repeatedly washed with CH_2Cl_2 to give a crude product, which was purified by MPLC on Fluka 100 C8-reversed phase silica gel ($\text{CH}_3\text{CN}:\text{H}_2\text{O}$ = 30:70, with 0.1% TFA). Upon freeze drying compound **1a** (as TFA salt) was obtained as a yellow foamy solid (18 mg, 33% over two steps). UV $\lambda_{\text{max}}(\text{H}_2\text{O})$ 496 nm [ϵ = 21,200 ($\text{cm}^{-1}\text{M}^{-1}$)]; ^1H NMR (CD_3OD): δ 1.32–1.76 (m, 46H), 2.03 (brs, 24H), 2.25–2.41 (m, 8H), 3.18–3.53 (m, 18H, overlapped with CD_3OD), 3.65–3.70 (m, 3H), 3.78–3.92 (m, 1H), 4.18–4.22 (m, 2H), 4.71–5.07 (m, 2H, overlapped with CD_3OD), 5.27 (t, J = 9.5 Hz, 1H), 6.54–6.71 (m, 6H), 7.17–7.22 (m, 1H), 7.67–7.71 (m, 1H), 8.36 (s, 1H) ppm; MALDI-TOF MS: m/z calcd for $\text{C}_{88}\text{H}_{150}\text{N}_{30}\text{O}_{15}\text{S}$ 1899.16; found 1899.140 [M] $^+$; analytical HPLC (ZORBAX SB-C8): t_R = 2.451 min (flow rate 1 mL/min; UV: λ = 220 nm; $\text{H}_2\text{O}:\text{CH}_3\text{CN}$ = 70:30 with 0.1% TFA), purity > 94%.

5-(*N*-Fluoresceinyl-5-thioureido)-aminopentyl 2,3,4,6-tetra-O-[8-bis-(3-*N,N'*-di-Boc-guanidino-propyl)-amino]-octanoyl]- β -D-glucopyranoside (1b): To a solution of **29b** (82 mg, 0.025 mmol) in a mixed solvent THF and abs. ethanol (3 mL, EtOH:THF = 3:2), were added fluorescein-5-isothiocyanate (11 mg, 0.028 mmol) and triethylamine (30 μL , 0.203 mmol). The reaction mixture was stirred for 24 h at rt in dark, concentrated. The crude product was washed with water and brine, and the organic layer was dried over Na_2SO_4 . The crude product was dissolved in dry CH_2Cl_2 , then trifluoroacetic acid (TFA, 0.5 mL) in CH_2Cl_2 (5 mL) was added and the mixture was stirred at rt in dark for 6 h. The mixture was concentrated and repeatedly washed with CH_2Cl_2 to give a crude product, which was purified by MPLC on Fluka 100 C8-reversed phase silica gel ($\text{CH}_3\text{CN}:\text{H}_2\text{O}$ = 30:70, with 0.1% TFA). Upon freeze drying compound **1b** (as TFA salt) was obtained as a yellow foamy solid (41 mg, 71% over two steps). UV $\lambda_{\text{max}}(\text{H}_2\text{O})$ 498 nm [ϵ = 25,800 ($\text{cm}^{-1}\text{M}^{-1}$)]; ^1H NMR (CD_3OD): δ 1.33–1.74 (m, 62H), 2.05 (brs, 24H), 2.26–2.53 (m, 8H), 3.15–3.48 (m, 18H, overlapped with CD_3OD), 3.54–3.84 (m, 3H), 3.79–3.92 (m, 2H), 4.12–4.38 (m, 2H), 4.71–5.07 (m, 2H, overlapped with CD_3OD), 5.28 (t, J = 9.5 Hz, 1H), 6.53–6.74 (m, 6H), 7.17–7.20 (m, 1H), 7.69–7.73 (m, 1H), 8.39 (s, 1H) ppm; MALDI-TOF MS: m/z calcd for $\text{C}_{96}\text{H}_{167}\text{N}_{30}\text{O}_{15}\text{S}$ 2012.30, found 2012.281 [$\text{M}+\text{H}$] $^+$; analytical HPLC (ZORBAX SB-C8): t_R = 2.488 min (flow rate 1 mL/min; UV: λ = 220 nm; $\text{H}_2\text{O}:\text{CH}_3\text{CN}$ = 70:30 with 0.1% TFA), purity > 96%.

5-(*N*-Fluoresceinyl-5-thioureido)-aminopentyl 2,3,4,6-tetra-O-[6-bis-(3-guanidino-propyl)-amino]-hexanoyl]- α -D-mannopyranoside (2a): To a solution of **30a** (134 mg, 0.043 mmol) in a mixed solvent THF and abs. ethanol (3 mL, EtOH:THF = 3:2), were added fluorescein-5-isothiocyanate (19 mg, 0.047 mmol) and triethylamine (50 μL ,

0.344 mmol). The reaction mixture was stirred for 24 h at rt in dark, concentrated. The crude product was washed with water and brine, and the organic layer was dried over Na_2SO_4 . The crude product was dissolved in dry CH_2Cl_2 , then trifluoroacetic acid (TFA, 0.5 mL) in CH_2Cl_2 (5 mL) was added and the mixture was stirred at rt in dark for 6 h. The mixture was concentrated and repeatedly washed with CH_2Cl_2 to give a crude product, which was purified by MPLC on Fluka 100 C8-reversed phase silica gel ($\text{CH}_3\text{CN}:\text{H}_2\text{O} = 30:70$, with 0.1% TFA). Upon freeze drying compound **2a** (as TFA salt) was obtained as a yellow foamy solid (53 mg, 57% over two steps). UV $\lambda_{\text{max}}(\text{H}_2\text{O})$ 498 nm [$\epsilon = 21,000 \text{ (cm}^{-1}\text{M}^{-1})$]; $^1\text{H NMR (CD}_3\text{OD)}$: δ 1.33-1.77 (m, 46H), 2.03 (brs, 24H), 2.35-2.46 (m, 8H), 3.15-3.48 (m, 18H, overlapped with CD_3OD), 3.54-3.87 (m, 3H), 4.17-4.28 (m, 2H), 4.71-5.07 (m, 1H, overlapped with CD_3OD), 5.17-5.29 (m, 3H), 6.55-6.73 (m, 6H), 7.15-7.18 (m, 1H), 7.69-7.72 (m, 1H), 8.32 (s, 1H) ppm; MALDI-TOF MS: m/z calcd for $\text{C}_{88}\text{H}_{150}\text{N}_{30}\text{O}_{15}\text{S}$ 1899.16, found 1899.194 $[\text{M}]^+$; analytical HPLC (ZORBAX SB-C8): $t_R = 2.482$ min (flow rate 1 mL/min; UV: $\lambda = 220$ nm; $\text{H}_2\text{O}:\text{CH}_3\text{CN} = 70:30$ with 0.1% TFA), purity > 97%.

5-(N-Fluoresceinyl-5-thioureido)-aminopentyl 2,3,4,6-tetra-O-[8-[bis-(3-N,N'-di-Boc-guanidino-propyl)-amino]-octanoyl]- α -D-mannopyranoside (2b): To a solution of **30b** (123 mg, 0.037 mmol) in a mixed solvent THF and abs. ethanol (3 mL, $\text{EtOH}:\text{THF} = 3:2$), were added fluorescein-5-isothiocyanate (16 mg, 0.040 mmol) and triethylamine (50 μL , 0.329 mmol). The reaction mixture was stirred for 24 h at rt in dark, concentrated. The crude product was washed with water and brine, and the organic layer was dried over Na_2SO_4 . The crude product was dissolved in dry CH_2Cl_2 , then trifluoroacetic acid (TFA, 0.5 mL) in CH_2Cl_2 was added and the mixture was stirred at rt in dark for 6 h. The mixture was concentrated and repeatedly washed with CH_2Cl_2 (5 mL) to give a crude product, which was purified by MPLC on Fluka 100 C8-reversed phase silica gel ($\text{CH}_3\text{CN}:\text{H}_2\text{O} = 30:70$, with 0.1% TFA). Upon freeze drying compound **2b** (as TFA salt) was obtained as a yellow foamy solid (37 mg, 52% over two steps). UV $\lambda_{\text{max}}(\text{H}_2\text{O})$ 497 nm [$\epsilon = 28,300 \text{ (cm}^{-1}\text{M}^{-1})$]; $^1\text{H NMR (CDCl}_3)$: δ 1.32-1.73 (m, 62H), 2.03 (brs, 24H), 2.29-2.44 (m, 8H), 3.14-3.48 (m, 18H, overlapped with CD_3OD), 3.54-3.84 (m, 3H), 4.06-4.30 (m, 2H), 4.71-5.07 (m, 1H, overlapped with CD_3OD), 5.20-5.31 (m, 3H), 6.54-6.71 (m, 6H), 7.14-7.17 (m, 1H), 7.72-7.75 (m, 1H), 8.32 (s, 1H) ppm; MALDI-TOF MS: m/z calcd for $\text{C}_{96}\text{H}_{166}\text{N}_{30}\text{O}_{15}\text{S}$ 2011.29, found 2011.296 $[\text{M}]^+$; analytical HPLC (ZORBAX SB-C8): $t_R = 2.532$ min (flow rate 1 mL/min; UV: $\lambda = 220$ nm; $\text{H}_2\text{O}:\text{CH}_3\text{CN} = 70:30$ with 0.1% TFA), purity > 98%.

5-(N-Fluoresceinyl-5-thioureido)-aminopentyl 2,3,4,6-tetra-O-[6-[bis-(3-guanidino-propyl)-amino]-hexanoyl]- β -D-allopyranoside (3a): To a solution of **31a** (112 mg, 0.036 mmol) in a mixed solvent THF and abs. ethanol (3 mL, $\text{EtOH}:\text{THF} = 3:2$), were added fluorescein-5-isothiocyanate (16 mg, 0.039 mmol) and triethylamine (42 μL , 0.288 mmol). The reaction mixture was stirred for 24 h at rt

in dark, concentrated. The crude product was washed with water and brine, and the organic layer was dried over Na_2SO_4 . The crude product was dissolved in dry CH_2Cl_2 , then trifluoroacetic acid (TFA, 0.5 mL) in CH_2Cl_2 (5 mL) was added and the mixture was stirred at rt in dark for 6 h. The mixture was concentrated and repeatedly washed with CH_2Cl_2 to give a crude product, which was purified by MPLC on Fluka 100 C8-reversed phase silica gel ($\text{CH}_3\text{CN}:\text{H}_2\text{O} = 30:70$, with 0.1% TFA). Upon freeze drying compound **3a** (as TFA salt) was obtained as a yellow foamy solid (35 mg, 44% over two steps). UV $\lambda_{\text{max}}(\text{H}_2\text{O})$ 498 nm [$\epsilon = 19,900 \text{ (cm}^{-1}\text{M}^{-1})$]; $^1\text{H NMR (CD}_3\text{OD)}$: δ 1.37-1.76 (m, 46H), 2.03 (brs, 24H), 2.36-2.41 (m, 8H), 3.15-3.48 (m, 18H, overlapped with CD_3OD), 3.54-3.96 (m, 3H), 4.19-4.36 (m, 2H), 4.78-5.00 (m, 3H, overlapped with CD_3OD), 5.52-5.68 (m, 1H), 6.52-6.70 (m, 6H), 7.14-7.23 (m, 1H), 7.64-7.71 (m, 1H), 8.37 (s, 1H) ppm; MALDI-TOF MS: m/z calcd for $\text{C}_{88}\text{H}_{150}\text{N}_{30}\text{O}_{15}\text{S}$ 1899.16, found 1899.236 $[\text{M}]^+$; analytical HPLC (ZORBAX SB-C8): $t_R = 2.467$ min (flow rate 1 mL/min; UV: $\lambda = 220$ nm; $\text{H}_2\text{O}:\text{CH}_3\text{CN} = 70:30$ with 0.1% TFA), purity > 94%.

5-(N-Fluoresceinyl-5-thioureido)-aminopentyl 2,3,4,6-tetra-O-[8-[bis-(3-N,N'-di-Boc-guanidino-propyl)-amino]-octanoyl]- β -D-allopyranoside (3b): To a solution of **31b** (82 mg, 0.025 mmol) in a mixed solvent THF and abs. ethanol (3 mL, 3:2), were added fluorescein-5-isothiocyanate (11 mg, 0.028 mmol) and triethylamine (30 μL , 0.203 mmol). The reaction mixture was stirred for 24 h at rt in dark, concentrated. The crude product was washed with water and brine, and the organic layer was dried over Na_2SO_4 . The crude product was dissolved in dry CH_2Cl_2 , then trifluoroacetic acid (TFA, 0.5 mL) in CH_2Cl_2 (5 mL) was added and the mixture was stirred at rt in dark for 6 h. The mixture was concentrated and repeatedly washed with CH_2Cl_2 to give a crude product, which was purified by MPLC on Fluka 100 C8-reversed phase silica gel ($\text{CH}_3\text{CN}:\text{H}_2\text{O} = 30:70$, with 0.1% TFA). Upon freeze drying compound **3b** (as TFA salt) was obtained as a yellow foamy solid (35 mg, 40% over two steps). UV $\lambda_{\text{max}}(\text{H}_2\text{O})$ 499 nm [$\epsilon = 22,500 \text{ (cm}^{-1}\text{M}^{-1})$]; $^1\text{H NMR (CD}_3\text{OD)}$: δ 1.39-1.74 (m, 62H), 2.02 (brs, 24H), 2.28-2.47 (m, 8H), 3.15-3.48 (m, 18H, overlapped with CD_3OD), 3.54-3.98 (m, 3H), 4.20-4.24 (m, 2H), 4.70-5.10 (m, 3H, overlapped with CD_3OD), 5.58-5.65 (m, 1H), 6.52-6.73 (m, 6H), 7.15-7.24 (m, 1H), 7.65-7.72 (m, 1H), 8.31 (s, 1H) ppm; MALDI-TOF MS: m/z calcd for $\text{C}_{96}\text{H}_{166}\text{N}_{30}\text{O}_{15}\text{S}$ 2011.29, found 2011.321 $[\text{M}]^+$; analytical HPLC (ZORBAX SB-C8): $t_R = 2.491$ min (flow rate 1 mL/min; UV: $\lambda = 220$ nm; $\text{H}_2\text{O}:\text{CH}_3\text{CN} = 70:30$ with 0.1% TFA), purity > 95%.

5-(N-Fluoresceinyl-5-thioureido)-aminopentyl 2,3,4,6-tetra-O-[6-[bis-(3-guanidino-propyl)-amino]-hexanoyl]- β -D-galactopyranoside (4a): To a solution of **32a** (124 mg, 0.040 mmol) in a mixed solvent THF and abs. ethanol (3 mL, $\text{EtOH}:\text{THF} = 3:2$), were added fluorescein-5-isothiocyanate (17 mg, 0.044 mmol) and triethylamine (50 μL , 0.344 mmol). The reaction mixture was stirred for 24 h at rt in dark, concentrated. The crude product was washed with

water and brine, and the organic layer was dried over Na_2SO_4 . The crude product was dissolved in dry CH_2Cl_2 , then trifluoroacetic acid (TFA, 0.5 mL) in CH_2Cl_2 (5 mL) was added and the mixture was stirred at rt in dark for 6 h. The mixture was concentrated and repeatedly washed with CH_2Cl_2 to give a crude product, which was purified by MPLC on Fluka 100 C8-reversed phase silica gel ($\text{CH}_3\text{CN}:\text{H}_2\text{O} = 30:70$, with 0.1% TFA). Upon freeze drying compound **4a** (as TFA salt) was obtained as a yellow foamy solid (27 mg, 42% over two steps). UV $\lambda_{\text{max}}(\text{H}_2\text{O})$ 498 nm [$\epsilon = 25,100 \text{ (cm}^{-1}\text{M}^{-1})$]; $^1\text{H NMR}$ (CD_3OD): δ 1.32-1.75 (m, 46H), 2.04 (brs, 24H), 2.33-2.47 (m, 8H), 3.15-3.48 (m, 18H, overlapped with CD_3OD), 3.62-3.69 (m, 2H), 3.82-3.96 (m, 1H), 4.13-4.27 (m, 2H), 4.66 (d, $J = 7.6 \text{ Hz}$, 1H), 5.00-5.15 (m, 2H), 5.39 (d, $J = 3.1 \text{ Hz}$, 1H), 6.54-6.70 (m, 6H), 7.16-7.21 (m, 1H), 7.70-7.72 (m, 1H), 8.36 (s, 1H) ppm; MALDI-TOF MS: m/z calcd for $\text{C}_{88}\text{H}_{150}\text{N}_{30}\text{O}_{15}\text{S}$ 1899.16, found 1899.164 $[\text{M}]^+$; analytical HPLC (ZORBAX SB-C8): $t_{\text{R}} = 2.534 \text{ min}$ (flow rate 1 mL/min; UV: $\lambda = 220 \text{ nm}$; $\text{H}_2\text{O}:\text{CH}_3\text{CN} = 70:30$ with 0.1% TFA), purity > 97%.

5-(N-Fluoresceinyl-5-thioureido)-aminopentyl 2,3,4,6-tetra-O-[8-[bis-(3-N,N'-di-Boc-guanidino-propyl)-amino]-octanoyl]- β -D-galactopyranoside (4b**):** To a solution of **32b** (132 mg, 0.041 mmol) in a mixed solvent THF and abs. ethanol (3 mL, $\text{EtOH}:\text{THF} = 3:2$), were added fluorescein-5-isothiocyanate (18 mg, 0.045 mmol) and triethylamine (60 μL , 0.368 mmol). The reaction mixture was stirred for 24 h at rt in dark, concentrated. The crude product was washed with water and brine, and the organic layer was dried over Na_2SO_4 . The crude product was dissolved in dry CH_2Cl_2 , then trifluoroacetic acid (TFA, 0.5 mL) in CH_2Cl_2 (5 mL) was added and the mixture was stirred at rt in dark for 6 h. The mixture was concentrated and repeatedly washed with CH_2Cl_2 to give a crude product, which was purified by MPLC on Fluka 100 C8-reversed phase silica gel ($\text{CH}_3\text{CN}:\text{H}_2\text{O} = 30:70$, with 0.1% TFA). Upon freeze drying compound **4b** (as TFA salt) was obtained as a yellow foamy solid (51 mg, 57% over two steps). UV $\lambda_{\text{max}}(\text{H}_2\text{O})$ 498 nm [$\epsilon = 30,100 \text{ (cm}^{-1}\text{M}^{-1})$]; $^1\text{H NMR}$ (CD_3OD): δ 1.33-1.72 (m, 62H), 2.02 (brs, 24H), 2.29-2.42 (m, 8H), 3.15-3.48 (m, 18H, overlapped with CD_3OD), 3.61-3.65 (m, 2H), 3.82-3.98 (m, 1H), 4.13-4.38 (m, 2H), 4.65 (d, $J = 7.3 \text{ Hz}$, 1H), 4.95-5.14 (m, 2H), 5.39 (d, $J = 2.9 \text{ Hz}$, 1H), 6.54-6.73 (m, 6H), 7.12-7.19 (m, 1H), 7.69-7.73 (m, 1H), 8.37 (s, 1H) ppm; MALDI-TOF MS: m/z calcd for $\text{C}_{96}\text{H}_{167}\text{N}_{30}\text{O}_{15}\text{S}$ 2012.30, found 2012.296 $[\text{M}+\text{H}]^+$; analytical HPLC (ZORBAX SB-C8): $t_{\text{R}} = 2.585 \text{ min}$ (flow rate 1 mL/min; UV: $\lambda = 220 \text{ nm}$; $\text{H}_2\text{O}:\text{CH}_3\text{CN} = 70:30$ with 0.1% TFA), purity > 99%.

Bioassays.

Cell Culture: Human cervical cancer-derived HeLa cells were cultured as exponentially growing subconfluent monolayers on 100-mm dishes in Dulbecco's modified Eagle's medium (DMEM) supplanted with 10% (v/v) fetal bovine serum without antibiotics. A subculture was performed every 3-4 days.

Flow Cytometry. 1.0×10^5 HeLa cells were plated into 6-

well culture plate (SPL, Korea) and cultured for 24 h. After removing the medium, the cells were incubated with MT compounds in serum-free DMEM. After incubation, the cells were washed three times with PBS, and then incubated with 0.02% trypsin (500 μL) for 1 min at 37 °C. After addition of 1 mL of PBS, the cells were centrifuged at 5,000 rpm for 5 min. The cell pellet was resuspended and washed twice with 1 mL of PBS, and finally re-suspended in 1 mL of PBS to perform fluorescence analysis with a FACSCalibur (BD Biosciences) flow cytometer using a 488 nm laser excitation and a 515-545 nm emission filter. Each sample was analyzed 10,000 events.

Cell Labeling and Imaging Analysis. The HeLa cells ($1.0 \times 10^5/\text{dish}$) were seeded into $35 \times 10 \text{ mm}$ cover glass bottom dish, cultured overnight, and the cells were washed twice with serum-free DMEM prior to addition and incubation with transporters. The cells were treated with MT compounds at the final concentration of 10 μM in the presence of 250 nM MitoTracker Red, 400 nM LysoTracker Red and 20 $\mu\text{g}/\text{mL}$ of Transferrin receptor-Texas Red (Molecular Probes), and then incubated at 37 °C for 15 min. After washing with serum-free DMEM three times, the live cells were immediately analyzed by laser scanning confocal microscopy and images were captured by Olympus FV1000 Laser Scanning Microscope (Olympus Optical, Tokyo, Japan) with a 60x, 1.35 N.A. oil lens.

Tissue Distribution Study. The MT compounds were dissolved in sterile distilled water (500 μL) and injected into 8-week-old mice (C57BL/6) intraperitoneally (*ip*) at the dosage level of sterile distilled water (500 μL ; control), **1a**, **2a**, **3a**, **4a** (80.5 mg/kg; 500 μL in water) and **1b**, **2b**, **3b**, **4b** (83.6 mg/kg; 500 μL in water), and saline as control respectively. The treated mice were perfused after 20 min 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 by fluorescence microscope.

Results and Discussion

In the present study four monosaccharides, where one stereochemical variation occurs at a time (D-glucose, mannose, allose and galactose), were employed as the core scaffolds for eight G8 molecular transporters (**1-4**). Glucose possesses all equatorial hydroxyl groups, except the anomeric position, whereas mannose, allose, and galactose have inverted hydroxyl group at C2, C3, and C4 position, respectively with reference to the glucose stereochemistry. The anomeric hydroxyl group was functionalized with 5-aminopentanol through glycosylation reaction for the attachment of a fluorescent probe, and the remaining 4 hydroxyl groups were used for the elaboration of guanidine functionalities via branched linker chains. First, the monosaccharides were perbenzoylated to compounds **5-8** with benzoyl chloride and

Scheme 1. (a) BzCl, pyridine, rt, 8 h, 83-97%; (b) (i) 30% HBr/HOAc, CH₂Cl₂, rt, 6 h, (ii) Ag₂CO₃, acetone:water = 20:1, rt, 2 h, quantitative; (c) CCl₃CN, Cs₂CO₃, CH₂Cl₂, rt, 12 h, 81-92%; (d) HO-(CH₂)₅-NHCbz, Sn(OTf)₂, 4 Å molecular sieve, rt, 12 h, 74-88%; (e) NaOMe, MeOH, rt, 12 h, quantitative.

pyridine at rt, and the anomeric benzoyl protecting group was selectively removed to give **9-12** by bromination followed by treatment with silver carbonate in acetone and water.²⁰ Compounds **9-12** were activated to the corresponding trichloroacetimidate derivatives **13-16** with trichloroacetonitrile and Cs₂CO₃, and then glycosylated with HO-(CH₂)₅-NHCbz in the presence of Sn(OTf)₂ as the activator and 4-Å molecular sieves to give **17-20** in good yields.²¹ The anomeric stereochemistry in **17-20** was expected to be β (equatorial) for glucose, allose and galactose, and α (axial) for mannose derivatives, and confirmed by the ¹H NMR analysis [*J* = 7.8 Hz (β-anomer, **17**), *J* = 1.3 Hz (α-anomer, **18**), *J* = 8.0 Hz (β-anomer, **19**), *J* = 7.9 Hz (β-anomer, **20**)]. The remaining benzoyl protecting groups in **17-20** were removed with NaOMe in MeOH at rt to provide **21-24** (Scheme 1).

Compounds **21-24** were exhaustively acylated with pre-guanidinylated branched linkers (R'-OH) in the presence of EDC and DMAP in DMF at rt for 48 h to provide **25-28** (each **a** and **b**) in good yields after column chromatography on silica gel. Removal of the *N*-Cbz protecting groups in **25-28** with H₂ (50 psi) over 10% Pd/C afforded the free amines **29-32**, which provided eight target MTs **1-4** (each **a** and **b**) with a fluorophore, upon treatment with fluorescein-5-isothiocyanate (FITC-I) in the presence of TEA, followed by removal of the Boc groups on guanidine with TFA. Compounds **1-4** were rigorously purified by medium-pressure liquid chromatography (MPLC) on reverse phase (C8) silica gel with acetonitrile and water as the mobile phase, and thoroughly characterized by HPLC on a ZORBAX SB-C8

reverse phase column (5 μm, 4.6 mm ID × 25 cm; flow rate 1 mL min⁻¹ of CH₃CN:H₂O = 30:70 with 0.1% TFA; detection UV at λ = 220 nm), NMR spectroscopy and MALDI-TOF mass spectrometry (Scheme 2). Molecular transporters showed the following retention times on the C8 column: glucose-based MTs (**1a**: *R_t* = 2.451, **1b**: *R_t* = 2.488); mannose-based MTs (**2a**: *R_t* = 2.482, **2b**: *R_t* = 2.532); allose-based MTs (**3a**: *R_t* = 2.467, **3b**: *R_t* = 2.491); galactose-based MTs (**4a**: *R_t* = 2.534, **4b**: *R_t* = 2.585).

The cellular uptake of the monosaccharide-based molecular transporters (**1-4**, 8 MTs) was carried out by confocal laser scanning microscopy (CLSM) and flow cytometry. The uptake was observed without fixing by CLSM in HeLa cells incubated at 37 °C for 15 min after treatment with transporters at 10 μM. The cells were well stained with green fluorescence from FITC of all transporter molecules. The uptake efficiency was measured by fluorescence-activated cell sorter (FACS) analysis, after incubation with compounds (1 μM each) in serum-free DMEM at 37 °C for 1 hr. The uptake efficiencies of these compounds were found to be higher than that of fluorescence-labeled octa-arginine (R8-FI). As expected, the molecular transporters with a longer linker (**b** series) are generally internalized better than those with a shorter linker (**a** series) (Figure 1). HeLa cells were treated with each MT (**1-4**, green fluorescence from FITC, at 10 μM) in the presence of MitoTracker Red (250 nM, Invitrogen), LysoTracker Red (400 nM, Invitrogen) and transferrin-TxRed (20 μg mL⁻¹, Invitrogen), respectively at 37 °C for 15 min. The glucose-based MTs **1a** (short chain)

Scheme 2. (a) EDC, DMAP, R'-OH (**a**. n=5; **b**. n=7), DMF, rt, 48 h, 83-95%; (b) 10 wt % Pd/C, H₂ (50 psi), CH₂Cl₂:MeOH = 1:9, rt, 24 h, 78-92%; (c) (i) FITC-I, TEA, THF:EtOH = 2:3, rt, dark, 24 h, (ii) TFA, CH₂Cl₂, rt, dark, 6 h, 42-57% over two steps.

Figure 1. Cellular uptake studies of monosaccharide-based molecular transporters using HeLa cells by FACS analysis. Glucose-based MTs (**1a** & **1b**), Mannose-based MTs (**2a** & **2b**), Allose-based MTs (**3a** & **3b**), Galactose-based MTs (**4a** & **4b**) taken up by HeLa cells. The cells were incubated with carrier compounds (1 μ M each) in serum-free DMEM at 37 $^{\circ}$ C for 1 hr and analyzed for their average fluorescent intensity.

and **1b** (long chain) were found significantly colocalized with MitoTracker (Figure 2), but not with LysoTracker or transferrin (a marker of the clathrin-mediated endocytosis) (see the supplementary Materials Figure S1). The mannose-based MT **2a** (short chain) was also significantly localized in mitochondria. Although it is not clear whether **2b** (long chain) was colocalized with MitoTracker, it clearly colocalized with LysoTracker, but not with transferrin (see the supplementary Materials Figure S2). The allose-based MT **3a** (short chain) was colocalized with LysoTracker (see the supplementary Materials Figure S3), while it is not evident that **3a** was colocalized with MitoTracker (Figure 2) or transferrin (see the supplementary Materials Figure S3).

Although colocalization of compound **3b** (long chain) with MitoTracker was not well defined (Figure 2), it was partially colocalized with LysoTracker and substantially with transferrin (see the supplementary Materials Figure S3). Lastly, the galactose-based MTs **4a** and **4b** were well colocalized with transferrin and partially with LysoTracker (see the supplementary Materials Figure S4), but not with MitoTracker (Figure 2). The intracellular localization patterns of these MTs are summarized in Table 1. The mannose-based MTs (**2**) possess two stereochemical variations at both C1 and C2, whereas the other three MTs (**1**, **3**, and **4**) have only one stereochemical difference with each other. Thus, the observed intracellular localization patterns suggest quite clearly that a subtle stereochemical variation of the sugar scaffold can have significant impacts on the mitochondrial affinity; the

Table 1. Intracellular co-localization of Molecular Transporters **1-4**: ++ (well co-localized); + (partially co-localized); - (not colocalized); nc (not clear)

	MitoTracker- Red	LysoTracker- Red	Transferrin- Tx Red
1a	++	—	—
1b	++	—	—
2a	++	—	—
2b	nc	++	—
3a	nc	++	nc
3b	nc	+	++
4a	—	+	++
4b	—	+	++

Figure 2. Fluorescence microscope images of monosaccharide-based MTs **1a-4b** and their localization. The HeLa cells were treated with MT compounds (green fluorescence from FITC, 10 μM each) with mitochondria (MitoTracker, 250 nM). Compound **1a**, **1b**, and **2a** were significantly localized in mitochondria, whereas it is not clear if **2b**, **3a**, and **3b** were colocalized with MitoTracker. However, colocalization of **4a** and **4b** with MitoTracker was not observed. [Scale bars: 5 μm, the intensity profiles were analyzed for each arrow in the merged image.]

Figure 3. Tissue distribution of monosaccharide-based MTs in mouse. [Glucose-based MTs (**1a** & **1b**), Mannose-based MTs (**2a** & **2b**), Allose-based MTs (**3a** & **3b**), and Galactose-based MTs (**4a** & **4b**)] Fluorescence microscope images of heart, spleen, liver, kidney, lung, and brain tissue sections, isolated from mice 20 min after i.p. injection. Exposure time (ms): heart (1,500), spleen (1,500), liver (2,000), kidney (2,000), lung 5,000, brain (7,000); $\lambda_{\text{max}} = 488$ nm (green fluorescence from FITC).

stereochemistry of the 3- and 4-OH of the monosaccharide scaffold appears important to the mitochondrial localization. It is also possible to see some tenuous correlations between the HPLC-based lipophilicity²²⁻²⁴ and the mitochondrial

affinity. For examples, all MTs with the R_f range of 2.451-2.491 (with exception of **2b**) showed some affinity toward mitochondria, whereas the MTs with $R_f = 2.534$ or longer show not much mitochondrial affinity. Furthermore, it is

interesting to note that the allose-based MT (**3b**) and the galactose-based MTs (**4a** and **4b**) colocalized with transferrin. The present data suggest that the scaffold stereochemistry has a significant influence on the mitochondrial affinity, and the lipophilicity may also be pseudo-correlated with the organellar localization pattern with a possible implication on the uptake mechanisms.

The tissue distribution patterns of the monosaccharide-based MTs in mice were also examined in the usual manner [control (sterile distilled water), **1a-4a** (80.5 mg kg⁻¹; 500 µL in water), **1b-4b** (83.6 mg kg⁻¹; 500 µL in water)] (Figure 3).²⁵ All monosaccharide-based MTs show good permeability to brain tissues. The glucose-based MTs appear to show some selectivity for liver (**1a**, **1b**) and kidney (**1a**), and the mannose-based MTs (**2a**, **2b**) and allose-based MTs (**3a**, **3b**) are widely distributed in liver and kidney, especially **3b** in liver.

Conclusion

In summary, we synthesized eight monosaccharide-based G8 MTs (**1-4**, each **a** and **b**), and studied their biological properties with special focus on the possible mitochondrial targeting and tissue selectivity. The mitochondrial affinity of these MTs was found to be clearly related to the scaffold stereochemistry, and also somewhat tenuously with the lipophilicity. It may be suggested that the practical delivery strategy of drugs for the therapy of brain and mitochondrial diseases should consider the BBB permeability and mitochondrial affinity as key parameters, and that enhanced mitochondrial affinity may be achieved through further research on the structure-property relationship of the guanidine-rich molecular transporters.

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25. All mouse experiments were performed in the POSTECH animal facility in compliance with the relevant laws and institutional guidelines.