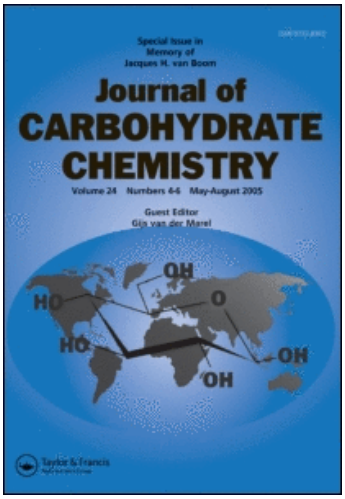

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SYNTHESIS OF SIMPLE MULTIVALENT β-D-GalNAc-(1→4)-β-D-Gal OLIGOMERS AS PROBES FOR INVESTIGATING THE INTERACTIONS OF *P. AERUGINOSA* PILI WITH MULTIVALENT RECEPTORS[1]

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ABSTRACT

Five multivalent β-D-GalNAc-(1→4)-β-D-Gal oligomers were selected and synthesized as probes for investigating the adhesin-receptor interactions of *P. aeruginosa* pili with multivalent receptors. They were synthesized by the amide coupling reactions of 8-(*N*-2-aminoethyl)carboxamidooctyl 4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranoside (7) with EDTA dianhydride, EDTA, Kemp's triacid and adipic acid with EDC, DIC and DCC combined with HOBt as coupling reagents and by the reaction of per-*O*-acetylated 7 with 1,3,5-benzenetricarbonyl trichloride followed by de-*O*-acetylation. These resulting multivalent compounds contain flexible C_9 spacer arms as linkers attached to either flexible hydrophilic moieties or rigid hydrophobic cores.

INTRODUCTION

Microbial adherence to the host epithelial cell surface is a key step in the initial stage of the infection process. Adhesins are structures on microbial surfaces which are used for adherence to host cells. *Pseudomonas aeruginosa* is an opportunistic pathogen

which employs adhesins, called fimbriae or pili, to mediate attachment to host epithelial cells[2-5] and initiate many infections and diseases.[6,7] Previous studies have shown that pili adhesins on *P. aeruginosa* interact with the glycosphingolipid asialo-GM_1 [β-D-Gal-(1→3)- β-D-GalNAc-(1→4)-β-D-Gal-(1→4)-β-D-Glc-OCer] receptor via its internal disaccharide sequence β-D-GalNAc-(1→4)-β-D-Gal, which is therefore suggested to play a crucial role in pili mediated adhesion of *P. aeruginosa.*[8-16] In order to gain a more detailed understanding of the pilus-carbohydrate interaction, a chemical mapping approach employing single hydroxy-modified octyl β-D-GalNAc-(1→4)-β-D-Gal disaccharide analogs was performed.[17,18] In this study, individual hydroxy groups were replaced by a hydrogen atom, a methoxy group or a propyloxy group. Such a study is particularly useful to produce an understanding of the pilus-carbohydrate interaction and for developing inhibitors of adhesins that are simpler and have higher affinity than the natural oligosaccharide ligands.[19] The previous research involved the interaction of monovalent carbohydrates with the adhesins. Because of the multivalent nature of *P. aeruginosa*, it is expected that multivalent saccharides would bind to the cell surface adhesins more tightly than monovalent ones.

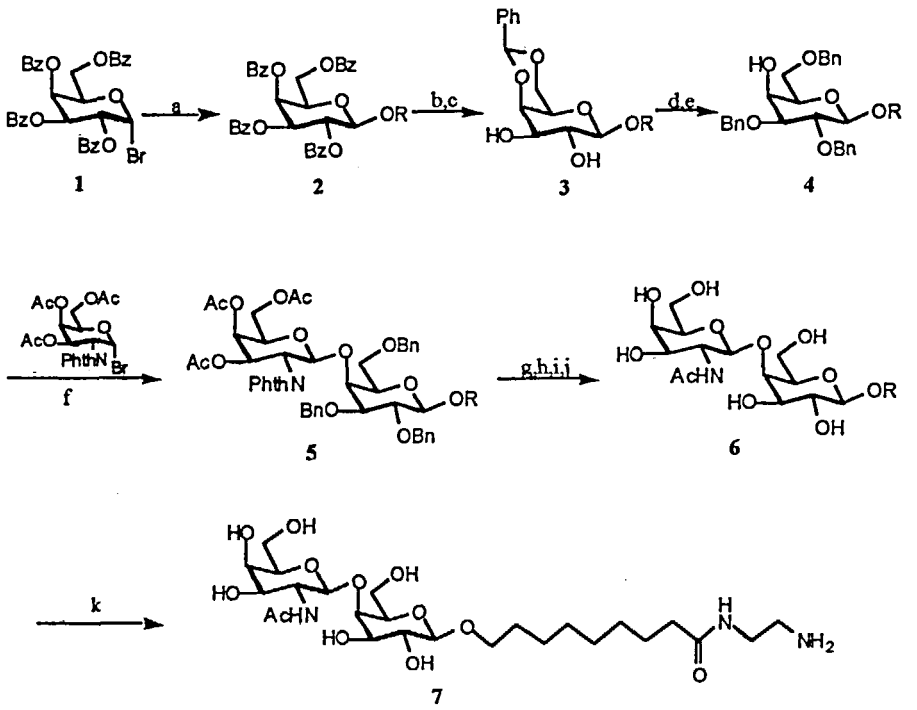
The advantage of using multivalent inhibitors arises from the fact that cell surfaces usually contain multiple receptor structures and adhesins possess multiple binding sites.[20-22] Many adhesion processes mediated by protein-carbohydrate interactions employ multiple protein-carbohydrate complexes to provide the necessary avidity for tight binding to the cell surface.[23] There has correspondingly been a great interest in the development of glycopolymers and dendrimers to achieve high-avidity binding.[24-27] To obtain a better insight into the nature of the receptor-adhesin interactions and more information for the design of anti-adhesive therapeutics, we therefore initiated the synthesis of simple readily accessible oligo-valent saccharides. Simple templates were chosen, based on the need for eventual commercially viable inhibitors. In this communication, we report the synthesis of divalent (**8, 9**), trivalent (**10, 15**) and tetravalent (**11**) analogs of β-D-GalNAc-(1→4)-β-D-Gal. The assay results will be reported separately.[28]

RESULTS AND DISCUSSION

To synthesize the multivalent β-D-GalNAc-(1→4)-β-D-Gal analogs, we chose the C_9 spacer arm as a linker, developed by Lemieux *et al.*,[29] which has been frequently used to prepare artificial carbohydrate antigens via covalent attachment to proteins. It is planned to examine other linkers in response to the results of the biological evaluation of the analogs prepared using Lemieux's linker. We also varied the properties of the core spanning a range of hydrophobicities; 1,3,5-benzenetriamide, 1,3,5-trimethyl-1,3,5-cyclohexane-triamide and adipamide. Ethylenediaminetetraacetamide and adipamide provide flexible cores, while 1,3,5-benzenetriamide and 1,3,5-trimethyl-1,3,5-cyclohexanetriamide provide relatively rigid cores. All of the cores can place the β-D-GalNAc-(1→4)-β-D-Gal ligands on one "side", offering the possibility of multiple ligand binding to surface-clustered receptors.

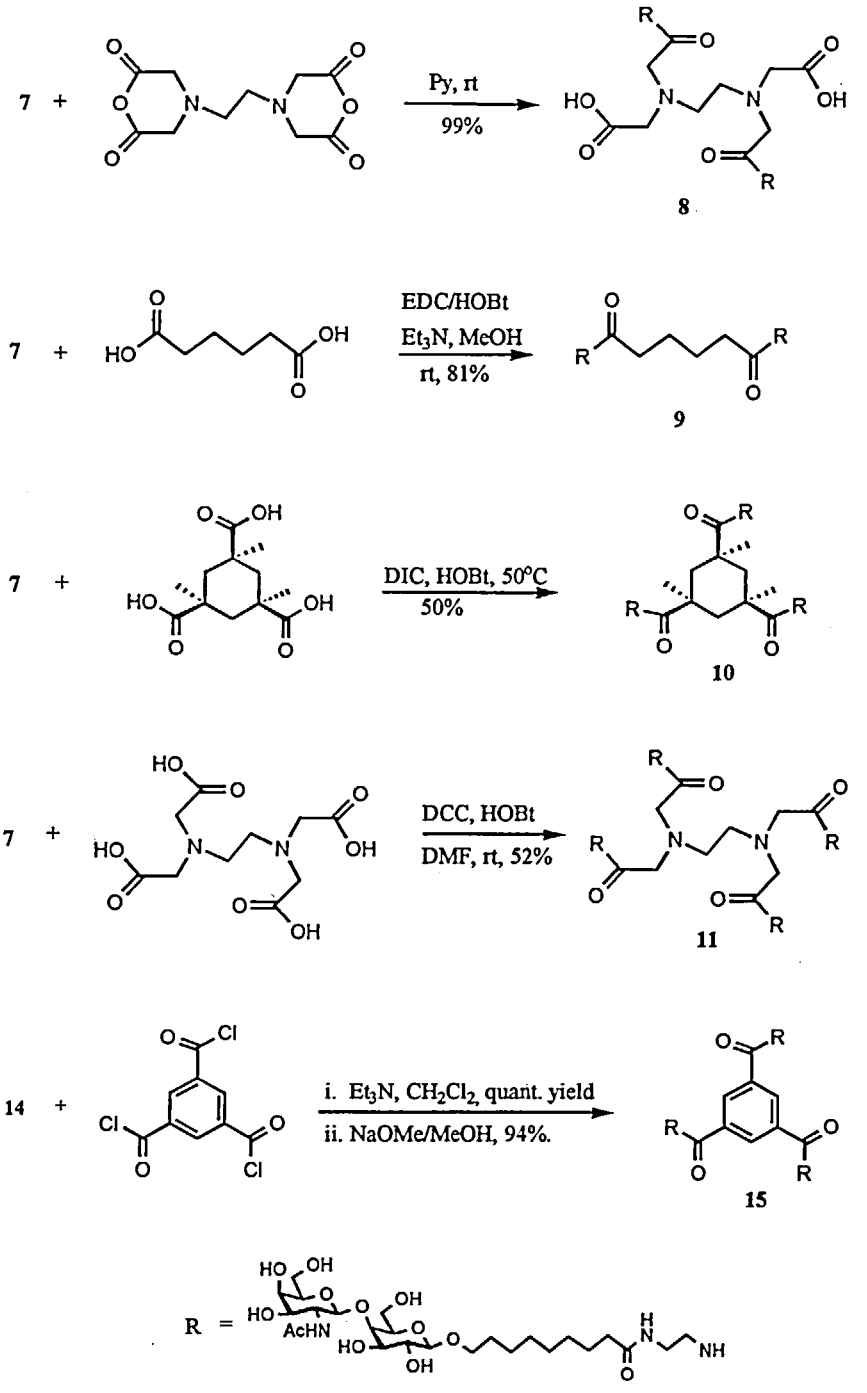
The synthesis of **7** with the 8-(*N*-2-aminoethyl)carboxamidooctyl aglycon was performed as shown in Scheme 1. 8-Methoxycarbonyloctyl 4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranoside (**6**) was previously synthesized by Lemieux.[30] In the present procedure, tetra-*O*-benzoyl-α-D-galactopyranosyl bromide (**1**) was coupled to with 8-methoxycarbonyloctan-1-ol using $Hg(CN)_2/HgBr_2$ as the promoter system to give **2** (90%). Debenzoylation of **2**, followed by benzylidenation, benzylation and benzylidene ring opening, gave **4** (63% yield from **2**). Glycosylation of **4** with tri-*O*-acetyl-2-deoxy-2-phthalimido-α-D-galactopyranosyl bromide using silver trifluoromethanesulfonate as a promoter gave **5** in 91% yield. Deprotection then furnished disaccharide **6**. Treatment of **6** with neat anhydrous ethylenediamine at 70 °C for two days[31] yielded **7** (81%) after purification by column chromatography on Iatrobeads. The chromatography was essential to rid **7** of contaminating ethylenediamine which otherwise interfered in the subsequent amide coupling reactions. The ^{1}H NMR spectrum confirmed the structure showing three triplet signals for CONHCH_2 (3.26 ppm, *J* 6.5 Hz), for CH_2NH_2 (2.75 ppm, *J* 6.5 Hz) and for CH_2CONH (2.20 ppm, *J* 7.5 Hz).

The synthesis of multivalent analogs **8**, **9**, **10** and **15** was performed as shown in Scheme 2. The amine **7** was coupled with ethylenediaminetetraacetic dianhydride in dry pyridine to give the divalent compound **8** in almost quantitative yield based on the



Scheme 1. R = $(CH_2)_8CO_2Me$; (a) $HO(CH_2)_8CO_2Me$, $Hg(CN)_2$-$HgBr_2$, 3Å MS, MeCN, 90%; (b) NaOMe/MeOH; (c) $PhCH(OMe)_2$, *p*-TsOH, MeCN, 85%; (d) BnBr/NaH, DMF, 90%; (e) $NaCNBH_3$, HCl, THF, 82%; (f) AgOTf, collidine, 4Å MS, 91%; (g) NH_2NH_2-HOAc, MeOH; (h) Ac_2O/pyridine; (i) NaOMe/MeOH; (j) $Pd(OH)_2/C$, H_2, MeOH, 80% from **5**; (k) ethylenediamine, 70 °C, 2 d, 95%.

dianhydride. Treatment of **7** with adipic acid in the presence of 1-[3-(dimethylamino)propyl]ethylcarbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBt)[32] in Et_3N and MeOH gave the divalent conjugate **9** (81%) as a white powder. Using *N,N'*-diisopropylcarbodiimide (DIC) and HOBt coupling between **7** and Kemp's triacid in DMF at room temperature for two days did not give any trivalent **10**. However, heating the reaction mixture at 50 °C overnight gave **10** in 50% yield. The synthesis of the tetravalent **11** was not so straightforward. Attempted reaction of the divalent compound **8** with amine **7** using EDC and HOBt as coupling reagents failed. Similarly, reactions of EDTA with **7** in the presence of DIC and HOBt or EDC and HOBt as coupling reagents in different solvents and temperatures did not produce compound **11**.



Scheme 2

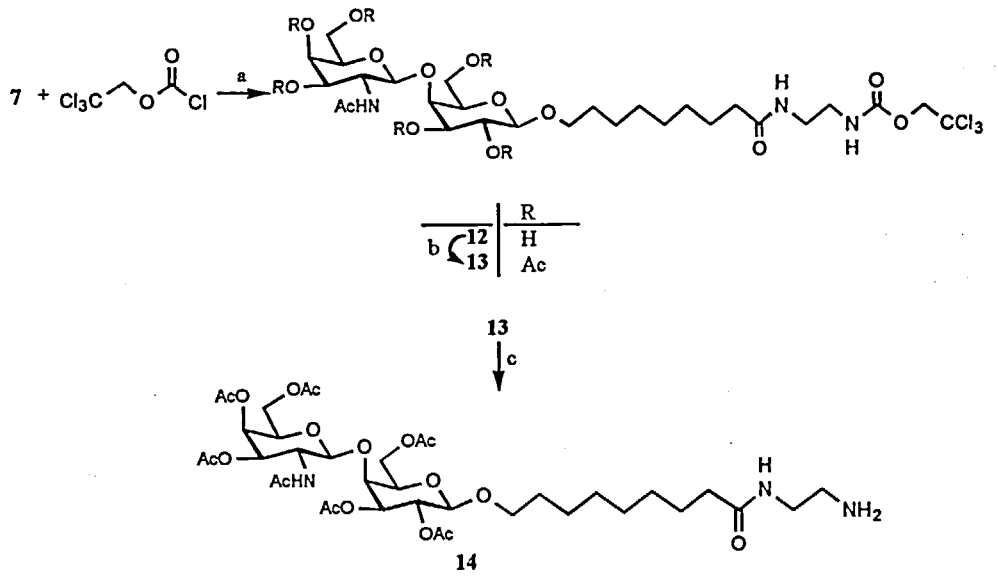
Treatment of 7 with EDTA in the presence of 1,3-dicyclohexylcarbodiimide (DCC)[33] and HOBt in dry DMF at room temperature for four days finally furnished **11** as a white powder (52%, 30% starting material 7 recovered).

Attempted reaction of 7 with 1,3,5-benzenetricarbonyl trichloride in pyridine, or DMF, in the presence of DMAP, or in MeOH in the presence of K_2CO_3 failed to give trivalent analog **15**, which was ascribed to solubility problems. Disaccharide 7 was therefore *O*-acetylated as shown in Scheme 3.

Amine 7 was treated with 2,2,2-trichloroethoxycarbonyl chloride (TrocCl) in 1N $NaHCO_3$ at room temperature[34] to yield the *N*-Troc product **12** (97%). Pyridine could not be used as solvent since the hydroxy groups would then also react with TrocCl.[35] Acetylation of **12** with Ac_2O and pyridine then gave acetylated disaccharide **13** (88%). The Troc group shows a sharp and characteristic two proton singlet at 4.75 ppm in the ^1H NMR spectrum. Compound **13** was stirred with zinc dust in glacial acetic acid at room temperature overnight to give the required *O*-acetylated β-D-GalNAc-(1→4)-β-D-Gal amine **14** in 67% yield. This compound had to be utilized immediately to avoid *O*→*N* acetyl group migration. Compound **14** was coupled with 1,3,5-benzenetricarbonyl trichloride in dichloromethane and triethylamine for ten minutes yielding the trivalent acetylated analog in a quantitative yield. After deacetylation, the target compound **15** was obtained (94%, Scheme 2).

EXPERIMENTAL

General methods. TLC was performed on Silica Gel 60-F_{254} (E. Merck) with detection by charring with H_2SO_4. Column chromatography was performed on Silica Gel 60 (E. Merck, 40-63 μm), on Iatrobeads (Iatron Laboratories Inc.) or on 10% C_{18}-SiO_2 (Toronto Research Chemicals). C-18 Sep-Pak sample-preparation cartridges were from Waters Associates. Millex-GV (0.22 mm) filter units were from Millipore. Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 22 ± 2 °C. ^1H NMR spectra were recorded at 300 MHz (Bruker AM 300), at 360 MHz (Bruker WM 360), or at 500 (Varian UNITY 500) on solutions in $CDCl_3$ (internal Me_4Si, δ 0), CD_3OD (δ



Scheme 3. (a) aq $NaHCO_3$ 1N, 98%; (b) Ac_2O/Pyridine, 88%; (c) Zn-HOAc, 67%.

3.30), or D_2O (δ 4.82). ^{13}C NMR spectra were recorded at 75 MHz, at 90 MHz, or at 125 MHz, on the same instruments in $CDCl_3$ (δ 77.07) or in D_2O (internal acetone, δ 31.07) and in CD_3OD (δ 49.0). FAB-mass spectra (FABMS) were obtained on a Kratos AEIMS9 or a Micromass ZabSpec Hybrid Sector-TOF.

8-Methoxycarbonyloctyl 4-*O*-(2-acetamido-2-deoxy-β-D-galacto-pyranosyl)-β-D-galactopyranoside (6). For preparation, see Scheme 1 and ref. 30. ^1H NMR (D_2O) δ 4.64 (d, 1H, $J_{1'2'}$ =8.5 Hz, H-1'), 4.18 (d, 1H, $J_{1,2}$ =7.9 Hz, H-1), 4.02 (d, 1H, $J_{3,4}$ =3.0 Hz, H-4), 3.91-3.47 (m, 12H, H-2', H-3', H-4', H-5', 2 x H-6', H-3, H-5, 2H-6, $OCH_2(CH_2)_7COOMe$), 3.43 (dd, 1H, $J_{1,2}$ =7.9 Hz and $J_{2,3}$ 10.0 Hz, H-2), 3.65 (s, 3H, OMe), 2.31 (t, 2H, J =7.5 Hz, CH_2COMe), 2.03 (s, 3H, Ac), 1.60 and 1.35 (b, 12H, $OCH_2(CH_2)_6CH_2COOMe$); ^{13}C NMR (D_2O) δ 178.7, 175.8, 103.6, 103.4, 76.7, 75.7, 74.9, 73.7, 71.8, 71.7, 71.3, 68.7, 61.9, 61.2, 53.6, 52.9, 34.6, 29.7, 29.07, 29.01, 28.96, 25.8, 25.1, 23.2.

8-(*N*-2-Aminoethyl)carboxamidooctyl 4-*O*-(2-acetamido-2-deoxy-β-D-galacto-pyranosyl)-β-D-galactopyranoside (7). Disaccharide **6** (350 mg) was dissolved in neat anhydrous ethylenediamine (refluxed and distilled from sodium) (100 mL). The stirred solution was heated at 70 °C for 2 d and then concentrated and co-concentrated with toluene (3 x 50 mL) to remove the excess ethylenediamine. The residue was chromatographed on an Iatrobeads column (MeOH-CH_2Cl_2, 3:1 containing 1% aq ammonia) and then further purified using C-18 Sep-Pak adsorption.[36] An aqueous solution of the product was passed through a Millipore filter and the filtrate was lyophilized to provide 7 as a white powder (359 mg, 95%): $[\alpha]_D$ -7.1° (*c* 0.4, MeOH); R_f 0.23 (MeOH-CH_2Cl_2, 3:1 containing 1% aq ammonia); ^{1}H NMR (CD_3OD) δ 4.63 (d, 1H, $J_{1',2'}$=8.5 Hz, H-1'), 4.18 (d, 1H, $J_{1,2}$=7.9 Hz, H-1), 4.02 (d, 1H, $J_{3,4}$ =3.0 Hz, H-4), 3.75 (d, 1H, $J_{3',4'}$ =3.0 Hz, H-4'), 3.91-3.77 and 3.74-3.46 (m, 11H, H-2', H-3', H-5', 2 x H-6', H-3, H-5, 2 x H-6, OCH_2(CH_2)$_7$CONH), 3.44 (dd, 1H, $J_{1,2}$ =7.9 Hz and $J_{2,3}$ =10.0 Hz, H-2), 3.26 (t, 2H, *J* 6.5 =Hz, CONHCH_2), 2.75 (t, 2H, *J* =6.5 Hz, CH_2 NH_2), 2.20 (t, 2H, *J* =7.5 Hz, CH_2CONH), 2.03 (s, 3H, Ac), 1.60 and 1.35 (b, 12H, OCH$_2$(CH_2)$_6$$CH_2$CONH); ^{13}C NMR ($D_2O$) δ 178.8, 175.9, 103.6, 103.4, 76.7, 75.7, 74.9, 73.6, 71.8, 71.7, 71.3, 68.7, 61.9, 61.2, 53.5, 40.3, 40.2, 36.6, 29.6, 29.1, 29.0, 28.9, 26.0, 25.7, 23.2. HR-MS(ES) ($C_{25}H_{48}N_3O_{12}$: 582.3238 $[M+H]^+$): *m/z* 582.3235 $[M+H]^+$.

***N,N'*-Di-{8-[4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galacto-pyranosyloxy]octylcarbonylaminoethyl}ethylenediaminediacetamido-*N,N'*-diacetic acid (8).** To a solution of 7 (5 mg, 8.6 μmol) in dry pyridine (0.6 mL), ethylenediaminetetraacetic dianhydride (0.85 mg, 3.3 μmol) was added at rt. TLC indicated the reaction had gone to completion (R_f 0.35, MeOH-CH_2Cl_2-H_2O, 4:2:1) after 4 h. The solution was concentrated and then co-evaporated with toluene (3x1 mL) to remove pyridine. The residue was applied to an Iatrobeads column (MeOH-CH_2Cl_2-H_2O, 4:2:1) and isolated as described for 7 to give product **8** (4.66 mg, 99%): $[\alpha]_D$ -5.3° (*c* 0.36, MeOH); ^{1}H NMR (D_2O) δ 4.64 (d, 2H, $J_{1',2'}$ =8.5 Hz, 2 x H-1'), 4.38 (d, 2H, $J_{1,2}$

=7.9 Hz, 2 x H-1), 4.08 (d, 2H, $J_{3,4}$ =3.0 Hz, 2 x H-4), 4.0-3.0 (m, 46H, 2 x H-2', 2 x H-3', 2 x H-4', 2 x H-5', 4 x H-6', 2 x H-3, 2 x H-2, 2 x H-5, 4 x H-6, 2 x $OCH_2(CH_2)_7CONH$, 12 x H for EDTA, and 2 x $CONHCH_2CH_2NH$), 2.23 (t, 4H, J =7.5 Hz, 2 x $O(CH_2)_7CH_2CONH$), 2.06 (s, 6H, 2 x Ac), 1.60 and 1.35 (b, 24H, 2 x $OCH_2(CH_2)_6CH_2CONH$); ^{13}C NMR (D_2O) δ 176.7, 175.2, 174.0, 104.9, 104.3, 78.4, 76.9, 75.5, 74.9, 74.7, 72.8, 70.8, 69.6, 62.7, 61.4, 60.9, 59.5, 55.6, 55.4, 54.2, 49.9, 49.6, 49.3, 49.0, 48.7, 48.4, 48.1, 40.0, 39.9, 37.1, 30.7, 30.3, 30.2, 26.9, 26.8, 23.1. FAB-MS ($C_{60}H_{106}N_8O_{30}$, MW: 1419): *m/z* 1442 $[M+Na]^+$, 1458 $[M+K]^+$, 1464 $[M-H+2Na]^+$ and 1480 $[M-H+2K]^+$.

***N,N'*-Di-{8-[4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galacto-pyranosyloxy]octylcarbonylaminoethyl}adipamide (9).** To a solution of 7 (5 mg, 8.6 μmol) and adipic acid (0.5 mg, 3.4 μmol) in dry MeOH (2 mL), 1-[3-(dimethylamino)propyl]ethylcarbodiimide hydrochloride (EDC) (6.5 mg, 33.9 μmol), 1-hydroxybenzotriazole (HOBt) (4.5 mg, μmol) and Et_3N (6.6 μL, 47.6 μmol) were added at rt. After stirring for 10 h, the solution was concentrated. Column chromatography (Iatrobeads, Pr^iOH-MeOH-aq ammonia, 3:3:2.5) and isolation as described for 7, followed by filtration through a Millipore filter and lyophilization gave 9 as a white powder (3.5 mg, 81%): R_f 0.08 (Pr^iOH-MeOH-aq ammonia, 3:3:2); $[\alpha]_D$ -8.6° (*c* 0.2, MeOH); ^{1}H NMR (D_2O) δ 4.63 (d, 2H, $J_{1'2'}$ =8.5 Hz, 2 x H-1'), 4.38 (d, 2H, $J_{1,2}$ =8.0 Hz, 2 x H-1), 4.08 (d, 2H, $J_{3,4}$ =2.6 Hz, 2 x H-4), 3.95-3.30 (m, 34H, 2 x H-2', 2 x H-3', 2 x H-4', 2 x H-5', 4 x H-6', 2 x H-2, 2 x H-3, 2 x H-5, 4 x H-6, 2 x $OCH_2(CH_2)_7CONH$, and 2 x $CONHCH_2CH_2NH$), 2.26-2.18 (m, 8H, 2 x $O(CH_2)_7CH_2CO$ and $CONHCH_2(CH_2)_2CH_2CONH$), 2.06 (s, 6H, 2 x Ac), 1.58 and 1.31 (b, 28H, $CONHCH_2(CH_2)_2CH_2CONH$ and 2 x $OCH_2(CH_2)_6CH_2CONH$); ^{13}C NMR (D_2O) δ 178.3, 177.5, 175.8, 103.6, 103.4, 76.7, 75.7, 74.9, 73.7, 71.8, 71.7, 71.3, 68.7, 61.9, 61.2, 53.6, 39.5, 39.3, 36.7, 36.3, 29.6, 29.2, 29.0, 26.2, 25.8, 25.6, 23.3. FAB-MS ($C_{56}H_{100}N_6O_{26}$, MW: 1273): *m/z* 1274 $[M+H]^+$, 1296 $[M+Na]^+$ and 1312 $[M+K]^+$.

***N,N',N''*-Tri-{8-[4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranosyloxy]octylcarbonylaminoethyl}-1,3,5-trimethyl-1,3,5-cyclohexanetriamide (10).** *N,N'*-Diisopropylcarbodiimide (DIC) (2.9 μL, 18.6 μmol) and HOBt (1.45 mg, 10.2 μmol) were added to a stirred solution of 7 (5.9 mg, 10.2 μmol) and Kemp's triacid (0.8 mg, 3.1 μmol) in dry DMF (0.2 mL) at 0 °C. The temperature was then increased to rt. The mixture was stirred for 2 d and then more DIC (3.0 μL) was added. The solution was heated at 50 °C overnight and concentrated under vacuum. The residue was applied to an Iatrobeads column ($CHCl_3$-MeOH-H_2O, 5:4:1) and isolated as described for 7. The main product was concentrated, filtered through a Millipore filter and lyophilized to give 10 (3 mg, 50%): R_f 0.35 ($CHCl_3$-MeOH-H_2O, 5:4:1); $[\alpha]_D$ -7.0° (*c* 0.27, MeOH); ^{1}H NMR (CD_3OD) δ 4.63 (d, 3H, $J_{1',2'}$ =8.5 Hz, 3 x H-1'), 4.18 (d, 3H, $J_{1,2}$ =8.0 Hz, 3 x H-1), 4.02 (d, 3H, $J_{3,4}$ =2.8 Hz, 3 x H-4), 3.95-3.20 (m, 51H, 3 x H-2', 3 x H-3', 3 x H-4', 3 x H-5', 6 x H-6', 3 x H-2, 3 x H-3, 3 x H-5, 6 x H-6, 3 x $OCH_2(CH_2)_7CONH$, and 3 x $CONHCH_2CH_2NH$), 2.74 and 1.16 (d, 3H for each, J_{gem} =15 Hz, 3 x CH_2 on Kemp's triamide ring), 2.20 (t, 6H, *J* =7.5 Hz, 3 x $O(CH_2)_7CH_2CO$), 2.02 (s, 9H, 3 x Ac), 1.60 and 1.33 (b, 36H, 3 x $OCH_2(CH_2)_6CH_2CO$), 1.22 (s, 9H, 3 x Me on Kemp's triamide ring); ^{13}C NMR (CD_3OD) δ 179.9, 176.5, 175.2, 104.9, 104.4, 78.5, 76.9, 75.5, 75.0, 74.8, 72.8, 70.9, 69.6, 62.7, 61.4, 55.6, 43.7, 43.4, 40.8, 39.6, 37.3, 33.5, 30.8, 30.4, 30.3, 27.04, 26.97, 23.1. FAB-MS ($C_{87}H_{153}N_9O_{39}$, MW: 1948): *m/z* 1971 $[M+Na]^+$.

***N,N,N',N'*-Tetra-{8-[4-*O*-(2-acetamido-2-deoxy-β-D-galactopyrano-syl)-β-D-galactopyranosyloxy]octylcarbonylaminoethyl}ethylenediaminetetraacetamide (11).** To a solution of 7 (10 mg, 17.2 μmol) and ethylenediaminetetraacetic acid (EDTA) (1.2 mg, 4.1 μmol) in dry DMF (1 mL) was added 1,3-dicyclohexylcarbodiimide (DCC) (8.5 mg, 41 μmol) and HOBt (2.8 mg, 17.2 μmol) at rt. The mixture was stirred for 4 d. After removal of DMF at 30 °C under vacuum, the residue was purified on an Iatrobeads column (2 g, MeOH-CH_2Cl_2-H_2O-aq ammonia, 9:6:3:1) and followed by C-18 Sep-Pak adsorption. The product was concentrated, filtered with a Millipore filter and lyophilized to give 11 as a white powder (4 mg, 52%, 30% of 7 recovered): R_f 0.1 (MeOH-CH_2Cl_2-

H_2O-aq ammonia, 9:6:3:1); $[\alpha]_D$ -4.4° (*c* 0.27, MeOH); ^{1}H NMR (D_2O) δ 4.65 (d, 4H, $J_{1',2'}$ =8.4 Hz, 4 x H-1'), 4.38 (d, 4H, $J_{1,2}$ =7.9 Hz, 4 x H-1), 4.09 (d, 4H, $J_{3,4}$ =2.8 Hz, 4 x H-4), 3.96-3.60 (m, 48H, 4 x H-2', 4 x H-3', 4 x H-4', 4 x H-5', 8 x H-6', 4 x H-3, 4 x H-5, 8 x H-6, 4 x O*CH*$_2$(CH$_2$)$_7$CO), 3.39 (dd, 4H, $J_{1,2}$ =7.9 Hz and $J_{2,3}$ =10.0 Hz, 4 x H-2), 3.36 (s, 16H, 4 x CONH*CH*$_2$*CH*$_2$NHCO), 3.27 (s, 8H, 4 x NH*CH*$_2$CONH), 2.70 (s, 4H, N*CH*$_2$*CH*$_2$N), 2.23 (t, 8H, *J* =7.5 Hz, 4 x O(CH$_2$)$_7$*CH*$_2$CO), 2.07 (s, 12H, 4 x Ac), 1.60 and 1.31 (m, 48H, 4 x OCH$_2$*(CH*$_2$*)*$_6$CH$_2$CO); ^{13}C NMR (D_2O) δ 178.1, 175.8, 174.2, 103.6, 103.4, 76.7, 75.7, 74.9, 73.7, 71.8, 71.7, 71.3, 68.7, 61.9, 61.2, 59.2, 53.6, 39.7, 36.8, 30.7, 29.6, 29.2, 29.1, 26.3, 25.9, 23.3. FAB-MS ($C_{110}H_{196}N_{14}O_{52}$, MW: 2545): 2568 $[M+Na]^+$.

8-[2-(2,2,2-Trichloroethoxycarbonyl)aminoethyleneaminocarbonyl]-octyl 4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranoside (12). Compound 7 (20 mg, 34.4 μmol) was dissolved in aq $NaHCO_3$ (1N; 1 mL) and 2,2,2-trichloroethylchloroformate (14 μL, 103.3 μmol) was added at rt. The mixture was stirred for 30 min, concentrated and co-evaporated with toluene (3x1 mL). The residue was applied to an Iatrobeads column (Pr^iOH-MeOH-aq ammonia, 2:2:1) to give the Troc-derivative **12** (25 mg, 98%): R_f 0.53 (Pr^iOH-MeOH-aq ammonia, 3:3:2); $[\alpha]_D$ -6.9° (*c* 0.19, MeOH); ^{1}H NMR (CD_3OD) δ 4.74 (s, 2H, O*CH*$_2$Cl$_3$), 4.63 (d, 1H, $J_{1',2'}$ =8.5 Hz, H-1'), 4.18 (d, 1H, $J_{1,2}$ =7.9 Hz, H-1), 4.01 (d, 1H, $J_{3,4}$ =3.0 Hz, H-4), 3.90-3.45 (m, 12H, H-2', H-3', H-4', H-5', 2 x H-6', H-3, H-5, 2 x H-6, O*CH*$_2$(CH$_2$)$_7$CO), 3.42 (dd, 1H, $J_{1,2}$ =7.9 Hz and $J_{2,3}$ =10.0 Hz, H-2), 3.26 (m, 4H, CONH*CH*$_2$*CH*$_2$NHCO), 2.06 (t, 2H, *J* =7.5 Hz, O(CH$_2$)$_7$*CH*$_2$CO), 2.02 (s, 3H, Ac), 1.60 and 1.30 (bs, 12H, OCH$_2$*(CH*$_2$*)*$_6$CH$_2$CO). Compound **12** was used directly for preparation of **13**.

8-[2-(2,2,2-Trichloroethoxycarbonyl)aminoethyleneaminocarbonyl]-octyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-galactopyranoside (13). Compound **12** (25 mg, 33.7 μmol) was stirred in Ac_2O and pyridine (1:1, 1 mL) at rt overnight. The solution was concentrated and chromatographed (CH_2Cl_2-MeOH, 20:1) to give **13** (30 mg, 88%): R_f 0.68 (CH_2Cl_2-MeOH 10:1); $[\alpha]_D$ -

12.0° (*c* 0.19, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.40 (bs, 1H, CO*NH*CH_2), 6.02 (d, 1H, J =7.0 Hz, *NH*Ac), 5.91 (dd, 1H, $J_{2',3'}$ =11.5 Hz and $J_{2',3'}$ =3.0 Hz, H-3'), 5.75 (bs, 1H, CO*NH*CH_2), 5.38 (d, 1H, $J_{3',4'}$ =3.0 Hz, H-4'), 5.24 (dd, 1H, $J_{1,2}$ =7.9 Hz and $J_{2,3}$ =10.5 Hz, H-2), 5.12 (d, 1H, $J_{1',2'}$ =8.2 Hz, H-1'), 4.95 (dd, 1H, $J_{2,3}$ =10.5 Hz and $J_{2,3}$ =2.5 Hz, H-3), 4.55 (s, 2H, O*CH*$_2$$CCl_3$), 4.42 (d, 1H, $J_{1,2}$ =7.9 Hz, H-1), 4.28 (m, 2H, 2 x H-6), 4.14 (d, 1H, $J_{3,4}$ =2.5 Hz, H-4), 4.05 (d, 2H, $J_{5',6'}$ =6.5 Hz, 2 x H-6'), 3.92 (d, 1H, $J_{5',6'}$ =6.5 Hz, H-5'), 3.88 (m, 1H, O*CH*$(CH_2)_7$CO), 3.74 (t, 1H, $J_{5,6}$ =6.0 Hz, H-5), 3.50-3.33 (m, 5H, O*CH*$(CH_2)_7$CO and CONH*CH*$_2$*CH*$_2$NHCO), 2.18 (t, 2H, J =7.0 Hz, O$(CH_2)_7$*CH*$_2$CO), 2.14-1.97 (7s, 21H, 7Ac), 1.60 and 1.30 (bs, 12H, OCH$_2$*(CH$_2$)$_6$*CH$_2$CO). Compound **13** was used directly for preparation of **14**.

8-(2-Aminoethyleneaminocarbonyl)octyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-galactopyranosyl)-2,3,6-tri-*O*-acetyl-β-D-galacto-pyranoside (14). Zinc dust (30 mg) was added to a solution of **13** (29 mg, 28.7 μmol) in HOAc (0.5 mL) at rt. The suspension was stirred overnight and filtered through Celite and the Celite was washed with CH_2Cl_2. The combined filtrate and washings were partitioned with water, and the aqueous layer was washed with CH_2Cl_2. The dried solution was concentrated to a syrup which was dissolved in CH_2Cl_2 for chromatography (CH_2Cl_2-MeOH, 8:1). Evaporation of the main fraction gave **14** (20 mg, 84%): R_f 0.13 (CH_2Cl_2-MeOH, 8:1); $[\alpha]_D$ -15.6° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 6.28 (d, 1H, J =6.8 Hz, *NH*Ac), 6.12 (bs, 1H, CO*NH*CH_2CH_2), 5.95 (dd, 1H, $J_{2',3'}$ =11.5 Hz and $J_{2',3'}$ =3.3 Hz, H-3'), 5.40 (d, 1H, $J_{3',4'}$ =3.0 Hz, H-4'), 5.24 (dd, 1H, $J_{1,2}$ =8.0 Hz and $J_{2,3}$ =10.5 Hz, H-2), 5.14 (d, 1H, $J_{1',2'}$ =8.2 Hz, H-1'), 4.94 (dd, 1H, $J_{2,3}$ =10.5 Hz and $J_{2,3}$ =2.5 Hz, H-3), 4.42 (d, 1H, $J_{1,2}$ =8.0 Hz, H-1), 4.29 (d, 2H, $J_{5,6}$ =6.0 Hz, 2 x H-6), 4.13 (d, 1H, $J_{3,4}$ =2.5 Hz, H-4), 4.05 (d, 2H, $J_{5',6'}$ =6.5 Hz, 2 x H-6'), 3.92 (d, 1H, $J_{5',6'}$ =6.5 Hz, H-5'), 3.88 (m, 1H, O*CH*$(CH_2)_7$CO), 3.74 (t, 1H, $J_{5,6}$ =6.0 Hz, H-5), 3.47 (m, 1H, O*CH*$(CH_2)_7$CO), 3.34 (m, 3H, H-2', CONH*CH*$_2$ CH_2NH_2), 2.85 (bt, 2H, CONHCH$_2$*CH*$_2$NH$_2$), 2.19 (t, 2H, J =7.5 Hz, O$(CH_2)_7$*CH*$_2$CO), 2.15-1.97 (7s, 21H, 7Ac), 1.60 and 1.30 (bs, 12H, OCH$_2$*(CH$_2$)$_6$*CH$_2$CO). Compound **14** was used directly for preparation of **15.**

***N,N',N"*-Tri-{8-[4-*O*-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-β-D-galactopyranosyloxy]octylcarbonylaminoethyl}-1,3,5-benzenetriamide (15).** To a solution of compound **14** (8.3 mg, 9.9 μmol) and Et_3N (1.4 μL, 9.9 μmol) in dry CH_2Cl_2 (0.5 mL), was added 1,3,5-benzentricarbonyl trichloride (0.88 mg, 3 μmol) at rt. After 10 min, TLC showed the reaction had gone to completion. The solution was concentrated and the residue was purified by chromatography (CH_2Cl_2-MeOH, 15:1) to give the acetylated product (8.7 mg, 99%): R_f 0.56 (CH_2Cl_2-MeOH, 8:1); $[\alpha]_D$ -12.8° (*c* 0.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 8.65 (s, 3H, aromatic); ^{13}C NMR ($CDCl_3$) δ 193.9, 174.5, 171.6, 171.0, 170.7, 170.5, 170.2, 170.0, 169.7 (9 x Ac), and 134.7 and 129.0 (aromatic). This product (8.0 mg) was dissolved in dry MeOH (5 mL) and sodium methoxide (13.5 mg) was added at room temperature. After three hours, the solution was neutralized with Dowex-50W (H^+) exchange resin, filtered and concentrated, the residue was chromatographed on an Iatrobeads column (Pr^iOH-MeOH-H_2O, 3:3:1) and then isolated by C-18 Sep-Pak adsorption to give **15** (5.4 mg, 94%, as a white powder): R_f 0.28 (Pr^iOH-MeOH-H_2O, 3:3:1); $[\alpha]_D$ -5.3° (*c* 0.3, MeOH); ^1H NMR (D_2O) δ 8.35 (s, 3H, aromatic), 4.64 (d, 3H, $J_{1',2'}$ =8.5 Hz, 3 x H-1'), 4.34 (d, 3H, $J_{1,2}$ =7.9 Hz, 3 x H-1), 4.08 (d, 3H, $J_{3,4}$ =2.8 Hz, 3 x H-4), 3.94-3.48 (m, 48H, 3 x H-2', 3 x H-3', 3 x H-4', 3 x H-5', 6 x H-6', 3 x H-3, 3 x H-5, 6 x H-6, 3 x $OCH_2(CH_2)_7CONH$, and 3 x $CONHCH_2CH_2NH$), 3.38 (dd, 3H, $J_{1,2}$ =7.9 Hz and $J_{2,3}$ =10.0 Hz, 3 x H-2), 2.22 (t, 6H, *J* =7.0 Hz, 3 x $O(CH_2)_7CH_2CONH$), 2.07 (s, 9H, 3 x Ac), 1.48 and 1.11 (b, 18H, 3 x $OCH_2(CH_2)_6CH_2CONH$); ^{13}C NMR (D_2O) δ 178.4, 175.8, 169.0, 135.6, 129.9, 103.6, 103.4, 76.8, 75.7, 74.9, 73.7, 71.9, 71.7, 71.2, 68.7, 61.9, 61.1, 53.6, 40.4, 39.3, 36.7, 29.6, 29.2, 29.1, 19.0, 26.2, 25.8, 23.3. FAB-MS ($C_{84}H_{141}N_9O_{39}$, MW: 1899): *m/z* 1922 $[M+Na]^+$ and 1939 $[M+K]^+$.

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