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Syntheses of model compounds related to an antigenic epitope in pectic polysaccharides from *Bupleurum falcatum* L.

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Abstract

Stereocontrolled syntheses of model compounds related to a category of the major antigenic epitope against anti-bupleurum 2IIc/PG-1-IgG from an anti-ulcer pectic polysaccharide are described. Glycosylation of the glucuronic acid donors methyl(2,3-di-*O*-benzoyl-4-*O*-methyl-α-D-glucopyranosyl trichloroacetimidate)uronate and methyl (2,3-di-*O*-benzoyl-4-*O*-methyl-β-D-glucopyranosyl)uronate-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-galactopyranosyl trichloroacetimidate with the common acceptor 2-(trimethylsilyl)ethyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside in the presence of trimethylsilyl triflate (TMSOTf) gave the desired di- and trisaccharide derivatives. Furthermore the products were transformed into the oligo-valent clustering saccharides, *N*,*N*′,*N*″-tri-{5-[4-*O*-methyl-β-D-glucopyranosyluronic acid-(1→6)-β-D-galactopyranosyloxy]pentylcarbonylaminoethyl}-1,3,5-benzenetriamide and *N*,*N*′,*N*″-tri-{5-[4-*O*-methyl-β-D-glucopyranosyluronic acid (1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyloxy]-pentylcarbonylaminoethyl}-1,3,5-benzenetriamide.

Keywords: Antigenic epitope; Anti-ulcer pectic polysaccharide; *Bupleurum falcatum*; Oligovalent di- and trisaccharides; Chemical synthesis

1. Introduction

In the last few years, it has become apparent that pectic polysaccharides including pectin from medicinal herbs display various in vitro and in vivo pharmacological activities in addition to the application for drug delivery. Therefore, it is possible to consider pectic polysaccharides, not only as one of the active ingredients of medicinal herbs, but also as medicinals.[1] Recently, Yamada et al.[2] have reported that the potent anti-ulcer pectic polysaccharide (Bupleuran 2IIc) was isolated from the hot-water extract of the roots of *Bupleurum falcatum*. Bupleuran 2IIc consists of a galacturonan region, a 'ramified' region (PG-1) composed of a rhamnogalacturonan core having neutral sugar side chains, and a rhamnogalacturonan II-like region;[3] the 'ramified' region has been considered to be an important part of the expression of the activity. The backbone of PG-1 is composed of an α-L-Rha-(1→4)-α-D-GalA-(1→2)-repeating unit, which was reported in our previous paper[4] as the synthetic tetrasaccharide.

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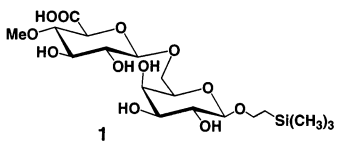
On the other hand, a polyclonal antibody (anti-bupleuran 2IIc/PG-1-IgG) against the PG-1 of the bupleuran 2IIc was prepared, and the major antigenic epitope against this antibody was characterized as a 6-linked galactosyl chain containing terminal glucuronic acid (GlcA) or 4-*O*-methyl-glucuronic acid (GlcA4Me), which were substituted to (1→3)-β-D-galactosyl chains in the 'ramified' region of bupleuran 2IIc.[5] Furthermore, among the structural region of bupleuran 2IIc, the 'ramified' region (PG-1), showed potent mitogenic activity, suggesting it was the active site.[6] The proposed structure of the antigenic epitope in PG-1 was the target for synthetic studies as part of our investigations on the synthesis of oligosaccharides of biological interest. In this time, di-, and trisaccharide derivatives including GlcA4Me were chosen as target compounds. Concerning the synthesis of oligosaccharides, suitable glycomimetics that are able to compete or perform even better than the naturally occurring carbohydrate ligands are needed for the development of suitably bioactive compounds. For this purpose clustering glycosides[7] have proved to be advantageous in many instances, as the multipresentation of specific sugar epitope in one molecule can result in remarkably increased adhesion. It is expected that multivalent saccharides would bind to the cell-surface adhesion molecule more tightly than monovalent ones.

We therefore initiated the synthesis of simple readily accessible oligo-valent saccharides, and we report herein the synthesis of mono- and trivalent analogs (**1**–**4**) of β-D-GlcA4Me-(1→6)-β-D-Gal- and β-D-GlcA4Me-(1→6)-β-D-Gal-(1→6)-β-D-Gal.

2. Results and discussion

Syntheses of monosaccharide derivatives.— Syntheses of the additional glucuronic acid methyl ester building blocks **9** were carried out as depicted in Scheme 1. Compound **6** was prepared from known 2-(trimethylsilyl)ethyl 2,3-di-*O*-benzyl-β-D-glucopyranoside (**5**)[8] by the following three-step procedure. Regioselective tritylation of the starting material with trityl chloride, followed by methylation and subsequent acid-hydrolysis of the trityl group, gave compound **6**. Oxidation and esterifica-



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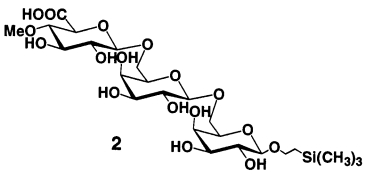

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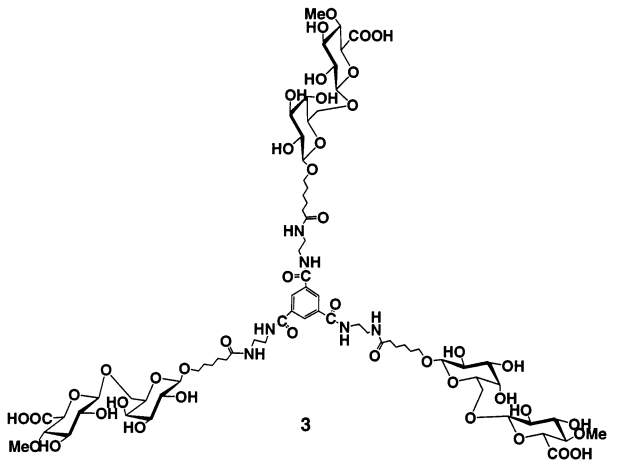

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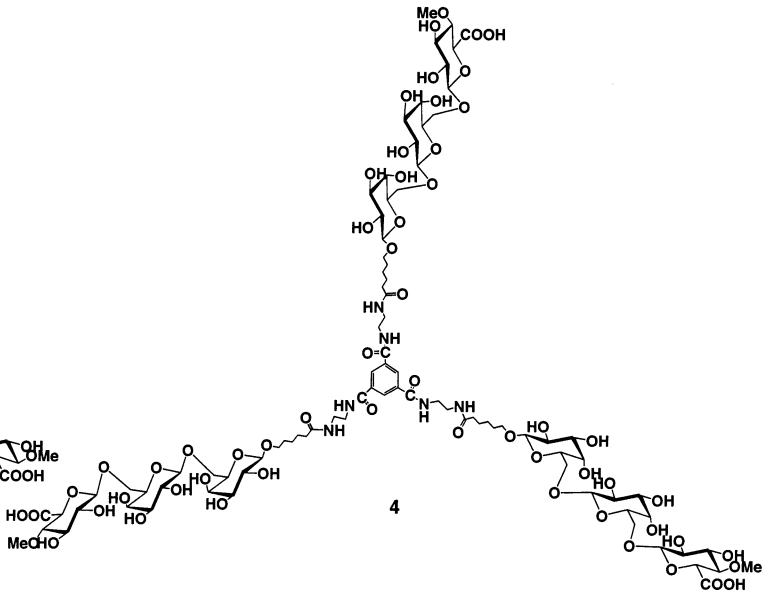

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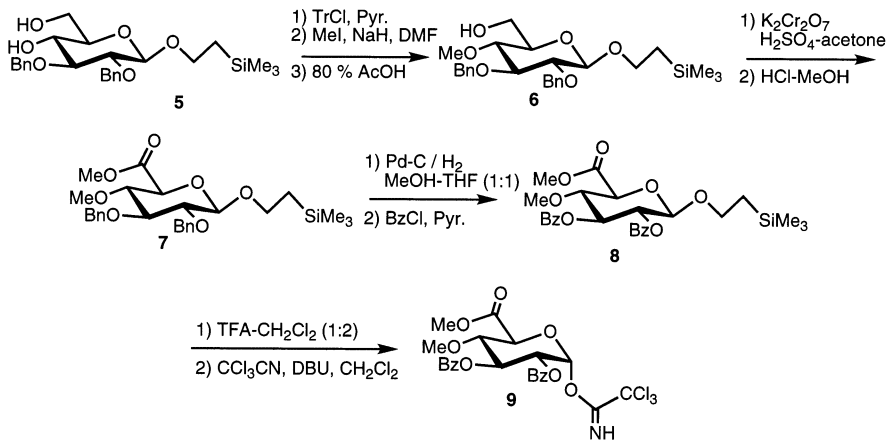
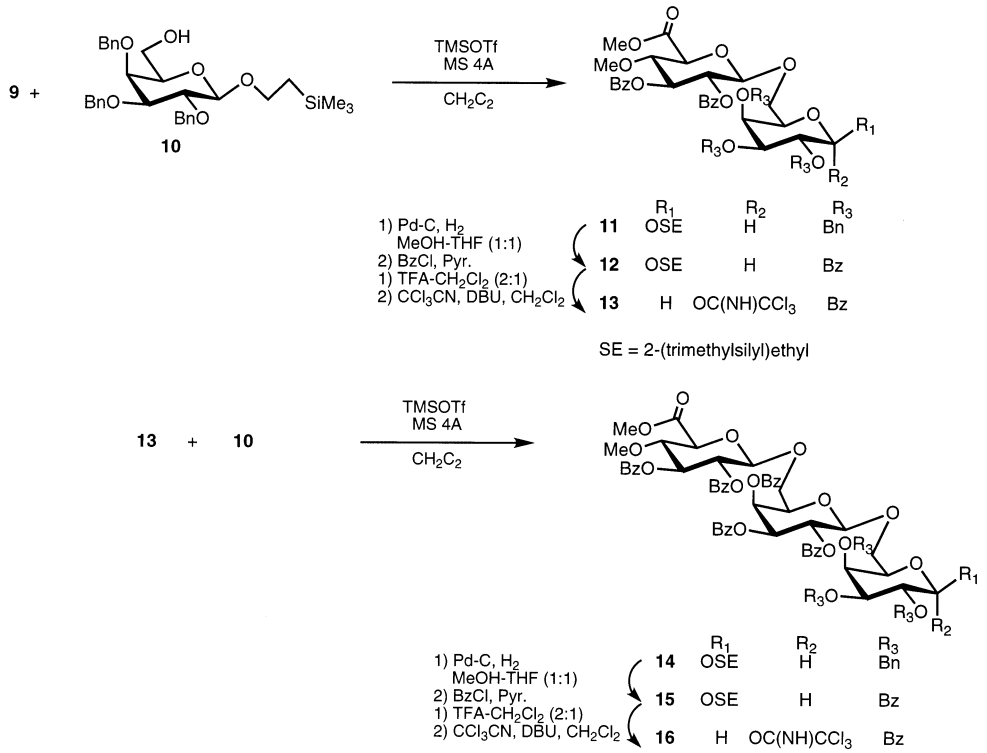
tion of **6** was carried out with $K_2Cr_2O_7$ and HCl–MeOH. Removal of the benzyl groups from **7** by catalytic hydrogenolysis over 10% Pd–C and subsequent benzoylation gave methyl[2-(trimethylsilyl)ethyl(2,3-di-*O*-benzoyl-4-*O*-methyl-β-D-glucopyranosid)uronate)] (**8**). For selective removal of the 2-(trimethylsilyl)ethyl (SE) group, **8** was treated[9] with trifluoroacetic acid in dichloromethane for 1 h at 0 °C to give the 1-hydroxy compound, which on further treatment[10] with trichloroacetonitrile in the presence of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) in dichloromethane for 2 h at 0 °C, gave the corresponding α-trichloroacetimidate **9** (70%) as the sole product (Scheme 1).

Syntheses of target oligosaccharide analogues.—The glycosylation of **9** with 2-(trimethylsilyl)ethyl 2,3,4-tri-*O*-benzyl-β-D-galactopyranoside (**10**) in the presence of trimethylsilyl triflate (TMSOTf) and 4 Å MS in dichloromethane for 1 h at 0 °C gave the desired disaccharide **11** (81%), as evidenced by ^{1}H NMR spectroscopy (H-1′, 4.81 ppm, *J* 7.9



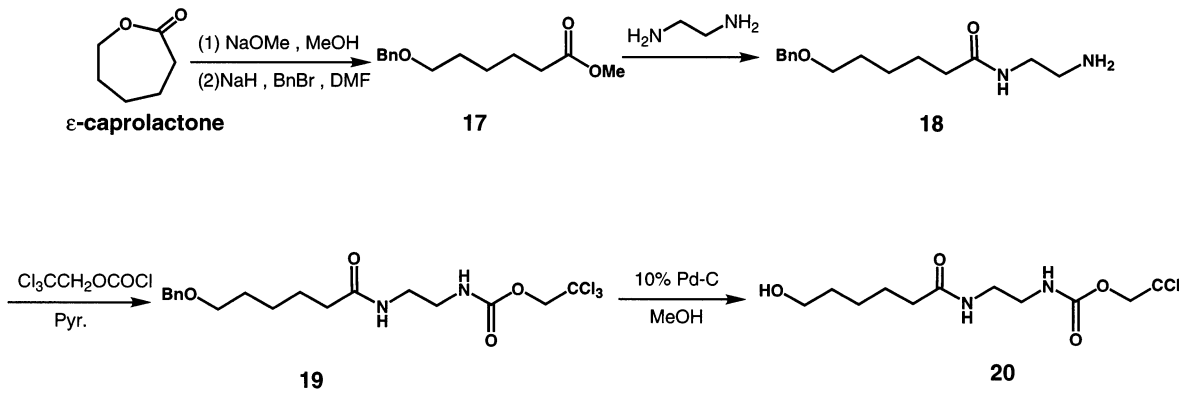
Scheme 1.



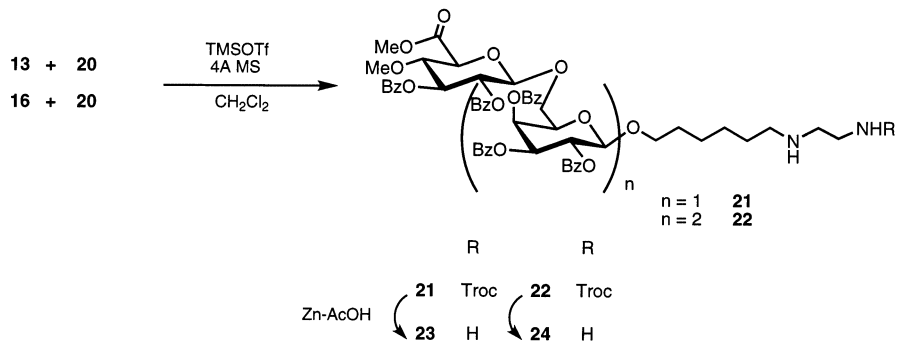
Scheme 2.

Hz). Compound **11** was subjected to O-debenzylation, followed by benzoylation, to give compound **12**. Selective removal of the SE group and treatment with trichloroacetonitrile in the presence of DBU gave only the corresponding α-trichloroacetimidate **13**. Glycosylation of **13** with **10** in the presence of TMSOTf and 4 Å MS in dichloromethane for 1 h at 0 °C gave the desired trisaccharide **14** (85%), as evidenced by ^{1}H NMR spectroscopy (H-1′, 4.80 ppm, *J* 7.9 Hz), showing the newly formed glycosidic linkages to be β. Compound **14** was converted by O-debenzylation and subsequent benzoylation to compound **15**. Selective removal of the SE group, and treatment with trichloroacetonitrile in the presence of DBU gave only the corresponding α-trichloroacetimidate **16**. In an alternate procedure, a part of **12** and **15** was converted by debenzoylation and hydrolysis of the ester to give the target compounds **1** and **2** (Scheme 2).

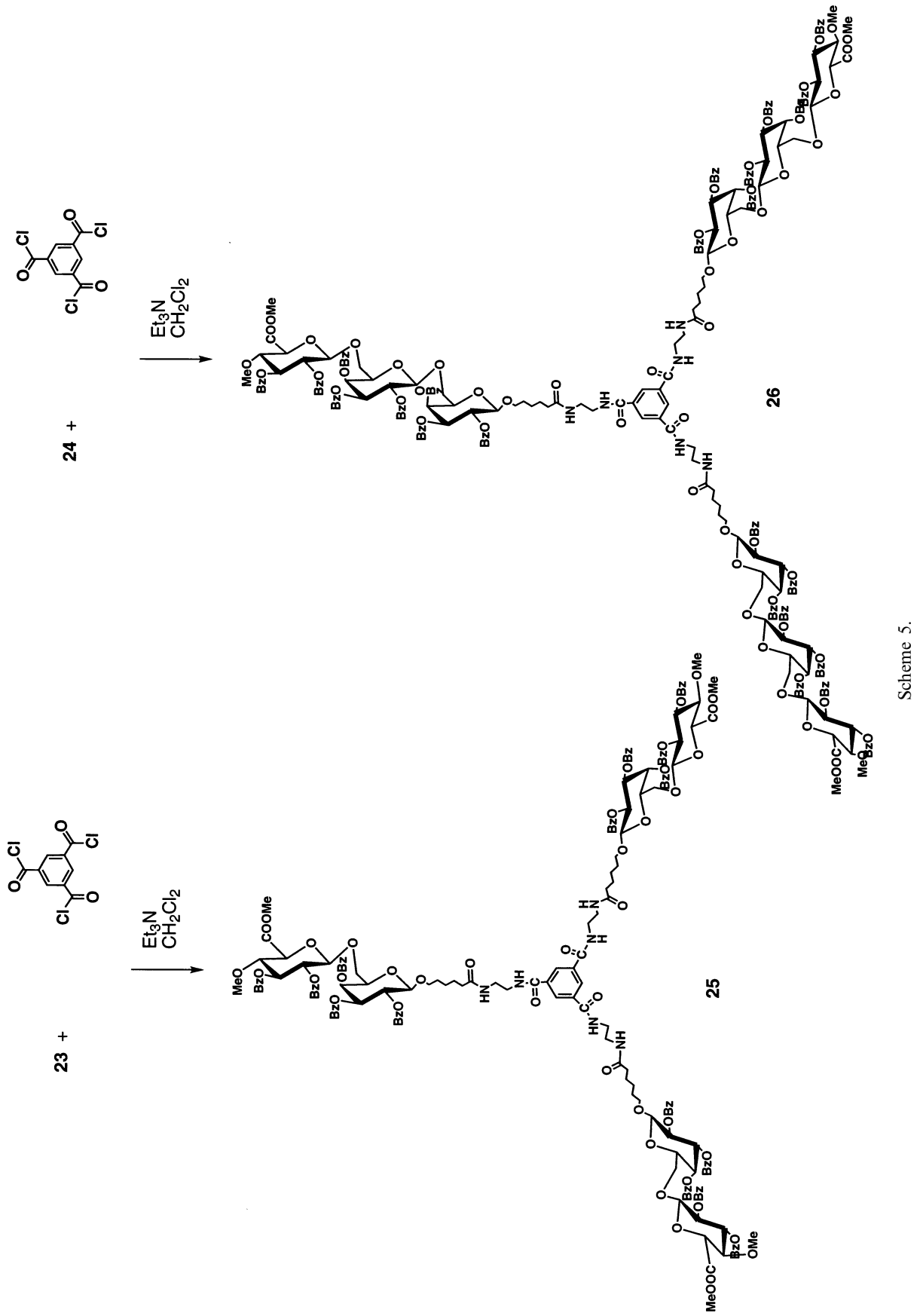
Next, concerning the synthesis of sugar clusters, spacer groups are needed as linkers between the core and the carbohydrate chain. We chose 5-[2-(2,2,2-trichloroethoxycarbonyl)aminoethyleneaminocarbonyl]pentanol (**20**) as a spacer, which was prepared as depicted in Scheme 3. Compound **20** was prepared from ε-caprolactone by the following procedure. ε-Caprolactone was converted to 5-methoxycarbonylpentanol by NaOMe, and subsequent benzylation gave benzyl 5-methoxycarbonylpentyl ether (**17**). Compound **17** was converted to the primary amine **18** by reaction with neat anhydrous ethylenediamine[11] and was protected by 2,2,2-trichloroethyl carbamate (**19**). Finally removal of the benzyl groups by catalytic hydrogenolysis over 10% Pd–C gave the target spacer, 5 - [2 - (2,2,2 - trichloroethoxycarbonyl)aminoethyleneaminocarbonyl]pentanol (TOF-MS *m*/*z*: 371.3 $[M + Na]^+$) (**20**) (Scheme 3). Coupling of **13** and/or **16** with the spacer **20** in the presence of TMSOTf and 4 Å MS for 1 h at 0 °C afforded the desired di- and trisaccharide derivatives **21** (65%) and **22** (60%), as evidenced by ^{1}H NMR spectroscopy (**21**: H-1, 4.58 ppm, *J* 7.9 Hz, **22**: H-1, 4.68 ppm, *J* 7.9 Hz). Removal of the Troc group from **21** and



Scheme 3.



Scheme 4.



Scheme 5.

22 by Zn–AcOH gave the primary amines **23** and **24** (Scheme 4). We chose 1,3,5-benzenetricarbonyl trichioride[12] a core for the synthesis of the sugar cluster. Coupling of **23** and/or **24** with the core, 1,3,5-benzenetricarbonyl trichloride in the presence of triethylamine afforded the desired trivalent oligosaccharide cluster **25** (64%) and **26** (78%), as evidenced by TOF-MS (**25**: $[M + Na]^+ = 3360$, **26**: $[M + Na]^+ = 4782$) (Scheme 5). Finally, removal of all acyl groups and esters with sodium methoxide in 5:1 methanol–water for 4 h at room temperature afforded the desired oligosaccharide clusters **3** and **4**. Compound **3** revealed an $[M + Na]^+$ ion peak at m/z 1757 and compound **4** revealed an $[M + Na]^+$ ion peak at m/z 2243 in the TOF-MS spectrum.

Mitogenic activity of these compounds on spleen cells and Peyer's patch cells were evaluated, and the results will be reported in detail elsewhere.

3. Experimental

Optical rotations were determined with a JASCO digital polarimeter. ^1H and ^{13}C NMR spectra were recorded on a JNM A 500 FT NMR spectrometer in $CDCl_3$ with Me_4Si as the internal standard. MALDI-TOFMS was recorded on a Perceptive Voyager RP mass spectrometer. TLC was performed on Silica Gel 60 F_{254} (E. Merck) with detection by quenching of UV fluorescence and by spraying with 10% H_2SO_4. Column chromatography was carried out on Silica Gel 60 (E. Merck). 2-(Trimethylsilyl)ethyl 2,3-di-*O*-benzyl-β-D-glucopyranoside (**5**) was prepared by a literature method.[8]

2-(Trimethylsilyl)ethyl 2,3-di-O-*benzyl-4*-O-*methyl-β*-D-*glucopyranoside* (**6**).—To a solution of 2-(trimethylsilyl)ethyl 2,3-di-*O*-benzyl-β-D-glucopyranoside (**5**) (1.7 g, 3.93 mmol) in pyridine (15 mL) was added triphenylmethyl chloride (2.2 g, 7.89 mmol). The reaction mixture was stirred at 70 °C for 12 h, then diluted with $CHCl_3$ and washed with 5% HCl, aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica-gel column chromatography using 100:1 benzene–acetone as eluent to give 2-(trimethylsilyl)ethyl 2,3-di-*O*-benzyl-6-*O*-trityl-β-D-glucopyranoside. To a solution of this compound in DMF (15 mL) was added NaH in oil (110 mg) and iodomethane (1 mL). The mixture was stirred for 4 h at rt, and then MeOH was added to destroy excess NaH. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extract was successively washed with aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. This compound was treated with 80% AcOH (10 mL) at 55 °C for 10 h, and following removal of AcOH and water by coevaporation with toluene, the crude product was purified by column chromatography (50:1 benzene–acetone) on silica gel to give compound **6** (1.31 g, three steps 70%): ^1H NMR ($CDCl_3$): δ 7.34–7.25 (m, 10 H, 2 × Ph), 4.95-4.69 (m, 4 H, 2 PhCH_2), 4.42 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.00–3.95 (m, 1 H, –OCH_2CH_2–), 3.93–3.76 (br,d, 2 H, H-6a,6b), 3.63–3.26 (m, 5 H, H-2,3,4,5, –OCH_2CH_2–), 3.55 (s, 3 H, OMe), 0.97–0.86 (m, 2 H, –OCH_2CH_2–), −0.06 (s, 9 H, $Si(CH_3)_3$): MALDI-TOFMS: Calcd for $C_{26}H_{38}O_6Si$: m/z 474.2. Found: m/z 497.2 $[M + Na]^+$.

Methyl [2-(trimethylsilyl)ethyl 2,3-di-O-*benzyl-4*-O-*methyl-β*-D-*glucopyranosid]uronate* (**7**).—To a solution of **6** (1.22 g, 5.06 mmol) in acetone (30 mL) was added $K_2Cr_2O_7$ (2.8 g), 3.5 M H_2SO_4(11 mL), and the mixture was stirred for 1 h at 55 °C, at the end of which time water was added. The residue was washed with $CHCl_3$, and the washings were successively washed with water, dried (Na_2SO_4), and concentrated. The resulting compound was treated with 10% HCl–MeOH (60 mL) at rt for 2 h and then concentrated. The product was purified by silica-gel column chromatography using 8:1 hexane–EtOAc as eluent to give **7** (1.88 g, 73.9%): ^1H NMR ($CDCl_3$): δ 7.36–7.26 (m, 10 H, 2 Ph), 4.94–4.68 (m, 4 H, 2 PhCH_2), 4.42 (d, 1 H, $J_{1,2}$ 7.5 Hz, H-1), 4.00–3.94 (m, 1 H, –OCH_2CH_2–), 3.81 (s, 3 H, COOMe), 3.78 (d, 1 H, $J_{4,5}$ 10.5 Hz, H-5), 3.62–3.39 (m, 4 H, H-2,3,4, –OCH_2CH_2–), 3.49 (s, 3 H, OMe), 0.96–0.86 (m, 2 H, –OCH_2CH_2–), −0.03 (s, 9 H, $Si(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 169.1 (C-6), 103.4 (C-1), 83.7 (C-4), 81.6 (C-2), 81.1 (C-3), 75.5, 74.8 (2 PhCH_2), 74.3 (C-5), 67.8

(–O*C*H$_2$CH$_2$–), 60.6 (COO*Me*), 52.4 (OMe), 18.4 (–OCH$_2$*C*H$_2$–), −1.5 (SiMe$_3$). MALDI-TOFMS: Calcd for $C_{27}H_{38}O_7Si$: *m*/*z* 502.2. Found: *m*/*z* 525.1 [M + Na]+.

Methyl [2-(trimethylsilyl)ethyl 2,3-di-O-*benzoyl-4*-O-*methyl-β*-D-*glucopyranosid]uronate* (**8**).—A solution of **7** (1.87 g, 3.73 mmol) in MeOH (10 mL) and THF (10 mL) was hydrogenated over 10% Pd–C (230 mg) for 3 h at rt, filtered through Celite, and the residue was washed with MeOH and concentrated. The residue was benzoylated with BzCl (4 mL) in pyridine (15 mL) for 10 h at rt. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed sequentially with 5% HCl, aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica-gel column chromatography using 5:1 hexane–EtOAc as eluent to give **8** (1.84 g, 93.1%): ^{1}H NMR: δ 8.07–7.34 (10 H, m, 2 Ph), 5.68 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.2 Hz, H-3), 5.43 (t, 1 H, $J_{1,2}$ 7.6 Hz, H-2), 4.82 (d, 1 H, H-1), 4.15 (d, 1 H, $J_{4,5}$ 9.6 Hz, H-5), 4.12-3.97 (m, 2 H, H-4, –OC*H*$_2$CH$_2$–), 3.92 (s, 3 H, COOMe), 3.69–3.50 (m, 1 H, –OCH$_2$CH$_2$–), 3.48 (s, 3 H, OMe), 0.92–0.87 (m, 2 H, –OCH$_2$CH$_2$), −0.03 (s, 9 H, Si(CH$_3$)$_3$); ^{13}C NMR ($CDCl_3$): δ 168.6 (C-6), 100.8 (C-1), 79.1 (C-4), 74.4 (C-3,5), 72.0 (C-2), 67.8 (–O*C*H$_2$CH$_2$–), 60.4 (COO*Me*), 52.7 (OMe), 17.8 (–OCH$_2$*C*H$_2$–), −1.5 (SiMe$_3$). MALDI-TOFMS: Calcd for $C_{27}H_{34}O_9Si$: *m*/*z* 530.2. Found: *m*/*z* 553.3 [M + Na]+.

Methyl (2,3-di-O-*benzoyl-4*-O-*methyl-α*-D-*glucopyranosyl trichlornacetimidate)uronate* (**9**).—To a solution of **8** (115 mg, 0.22 mmol) in CH_2Cl_2 (2 mL) cooled to 0 °C was added CF_3COOH (1 mL), and the mixture was stirred for 1 h at 0 °C and concentrated. Ethyl acetate and toluene (1:2) were added, and the solvent was evaporated to give the 1-hydroxy compound. To a solution of the residue in CH_2Cl_2 (1 mL), cooled at 0 °C were added trichloroacetonitrile (0.7 mL, 7 mmol) and DBU (60 μL, 0.4 mmol). The mixture was stirred for 2 h at 0 °C. After completion of the reaction, the mixture was concentrated. Column chromatography (3:1 hexane–EtOAc) of the residue on silica gel gave **9** (89 mg, 70%) as an amorphous mass: ^{1}H NMR ($CDCl_3$): δ 8.63 (s, 1 H, NH), 8.04–7.26 (m, 10 H, 2 Ph), 6.76 (d, 1 H, $J_{1,2}$ 3.3 Hz, H-1), 6.07 (t, 1 H, $J_{2,3} = J_{3,4}$ 9.5 Hz, H-3), 5.46 (dd, 1 H, H-2), 4.54 (d, 1 H, $J_{4,5}$ 99 Hz, H-S), 3.98 (t, 1 H, H-4), 3.84 (s, 3 H, COOMe), 3.45 (s, 3 H, OMe); MALDI-TOFMS: Calcd for $C_{24}H_{22}Cl_3NO_9$: *m*/*z* 573.0. Found: *m*/*z* 596.1 [M + Na]+.

2-(Trimethylsilyl)ethyl [methyl (2,3-di-O-*benzoyl-4*-O-*methyl-β*-D-*glucopyranosyl)-uronate]-(1→6)-2,3,4-tri*-O-*benzyl-β*-D-*galactopyranoside* (**11**).—To a solution of the trichloroacetimidate **9** (636 mg, 1.11 mmol) and **10** (300 mg, 0.55 mmol) in CH_2Cl_2 (2 mL) were added 4 Å MS (500 mg), and the mixture was stirred for 3 h at rt, then cooled to 0 °C. TMSOTf (32 μL, 160 μmol) was added, and the mixture was stirred for 1 h at 0 °C, then neutralized with Et_3N. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica-gel column chromatography using 2:1 hexane–EtOAc as eluent to give **11** (429 mg, 80.8%): $[\alpha]_D^{24}$ + 21.6° (*c* 1.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 7.98–7.18 (m, 25 H, 5 Ph), 5.63 (t, 1 H, H-3′), 5.35 (t, 1 H, H-2′), 4.88–4.51 (m, 6 H, 3 × C*H*$_2$Ph), 4.81 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.24 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.05 (d, 1 H, H-5′), 3.97, 3.75 (dt, 2 H, H-6), 3.94 (m, 1 H, H-4′), 3.87, 3.46 (dt, 2 H, C*H*$_2$CH$_2$SiMe$_3$), 3.78 (s, 3 H, COOMe), 3.77 (d, 1 H, H-4), 3.75 (m, 1 H, H-5), 3.72 (t, 1 H, H-2), 3.40 (s, 3 H, OMe), 3.35, 3.34 (dd, 1 H, H-3), 0.96 (t, 2 H, C*H*$_2$SiMe$_3$), −0.01 (s, 9 H, SiMe$_3$); ^{13}C NMR ($CDCl_3$): δ 168.4 (C-6′), 165.5, 165.1 (2 C=O), 103.3 (C-1), 101.2 (C-1′), 82.0 (C-3), 79.3 (C-2), 79.1 (C-4′), 75.1 (C-4, 2Ph*C*H$_2$), 74.6 (C-5, Ph*C*H$_2$), 74.3 (C-3′), 74.1 (C-5′), 72.0 (C-2′), 67.9 (C-6), 67.3 (–O*C*H$_2$CH$_2$–), 60.5 (COO*Me*), 52.7 (OMe), 18.4 (–OCH$_2$*C*H$_2$–), −1.1 (SiMe$_3$) MALDI-TOFMS: Calcd for $C_{54}H_{62}O_{14}Si$: *m*/*z* 962.2. Found: *m*/*z* 985.3 [M + Na]+.

2-(Trimethylsilyl)ethyl [methyl (2,3-di-O-*benzoyl-4*-O-*methyl-β*-D-*glucopyranosyl)-uronate]-(1→6)-2,3,4-tri*-O-*benzoyl-β*-D-*galactopyranoside* (**12**).—A solution of **11** (121 mg, 0.13 mmol) in MeOH (4 mL) and THF (2 mL) was hydrogenated over 10%

Pd–C (100 mg) for 30 min at rt, then filtered through Celite. The filtrate was concentrated, and the residue was benzoylated with BzCl (0.5 mL) in pyridine (1.5 mL) for 1 h at rt. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extract was washed sequentially with 5% HCl, aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The product was purified by silica-gel column chromatography using 30:1 benzene–EtOAc as eluent to give **12** (102 mg, 80.7%): $[\alpha]_D^{25}$ +103.4° (*c* 1.8 $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.06–7.19 (m, 25 H, 5 Ph), 5.84 (d, 1 H, H-4), 5.70 (t, 1 H, H-2), 5.62 (t, 1 H, H-3′), 5.48, 5.46 (dd, 1 H, H-3), 5.38 (t, 1 H, H-2′), 4.85 (d, 1 H, $J_{1',2'}$ 6.7 Hz, H-1′), 4.67 (d, 1 H, $J_{1,2}$ 8.6 Hz, H-1), 4.10 (m, 1 H, H-5), 4.06 (d, 1 H, H-5′), 4.05, 3.85 (dt, 2 H, H-6), 3.93 (t, 1 H, H-4′), 3.87, 3.44 (dt, 2 H, $CH_2CH_2SiMe_3$), 3.72 (s, 3 H, COOMe), 3.38 (s, 3 H, OMe), 0.73 (t, 2 H, CH_2SIMe_3), −0.12 (s, 9 H, $SiMe_3$); ^{13}C NMR ($CDCl_3$): δ 168.4 (C-6′), 165.5, 165.2, 165.1 (5 C=O), 101.1 (C-1′), 100.8 (C-1), 78.8 (C-4′), 73.2 (C-5), 74.2 (C-3′), 74.2 (C-5′), 71.9 (C-2′), 71.9 (C-3), 69.9 (C-2), 69.9 (C-6), 68.8 (C-4), 67.5 (–OCH_2CH_2–), 60.4 (COO*Me*), 52.7 (OMe), 17.7 (–OCH_2CH_2–), −1.4 ($SiMe_3$). MALDI-TOFMS: Calcd for $C_{54}H_{56}O_{17}Si$: *m*/*z* 1004.3. Found: *m*/*z* 1027.4 $[M + Na]^+$.

*Methyl (2,3-di-*O*-benzoyl-4-*O*-methyl-β-*D*-glucopyranosyl)uronate - (1→6) - 2,3,4 - tri - *O*-benzoyl-α-*D*-galactopyranosyl trichloroacetimidate* (**13**).—To a solution of **12** (102 mg, 0.10 mmol) in CH_2Cl_2 (2 mL) cooled to 0 °C was added CF_3COOH (0.6 mL), and the mixture was stirred for 1 h at rt and concentrated. Ethyl acetate and toluene (1:2) were added, and the solvents were evaporated to give the 1-hydroxy compound. To a solution of the residue in CH_2Cl_2 (1 mL), cooled at 0 °C were added trichloroacetonitrile (0.4 mL, 4 mmol) and DBU (100 μL, 0.67 mmol). The mixture was stirred for 1 h at 0 °C. After completion of the reaction, the mixture was concentrated. Column chromatography (10:1 benzene–EtOAc) of the residue on silica gel gave **13** (87 mg, 83.0%) as an amorphous mass: ^1H NMR ($CDCl_3$): δ 8.32 (s, 1 H, NH), 8.08–7.22 (m, 25 H, 5 Ph), 6.73 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 6.00 (d, 1 H, H-4), 5.96 (m, 1 H, H-3), 5.84 (t, 1 H, H-2), 5.57 (t, 1 H, H-3′), 5.33 (t, 1 H, H-2′), 4.82 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1′), 4.05 (m, 1 H, H-5), 4.05 (d, 1 H, H-5′), 4.05, 3.80 (dt, 2 H, H-6), 3.89 (t, 1 H, H-4′), 3.72 (s, 3 H, COOMe), 3.36 (s, 3 H, OMe)); ^{13}C NMR ($CDCl_3$): δ 168.4 (C-6′), 165.6, 165.4, 165.1, 160.4 (5 C=O), 100.9 (C-1′), 93.5 (C-1), 78.8 (C-4′), 74.2 (C-5), 74.2 (C-3′), 74.2 (C-5′), 71.7 (C-2′), 69.3 (C-3), 69.0 (C-2), 69.0 (C-4), 68.2 (C-6), 60.4 (COO*Me*), 52.7 (OMe). MALDI-TOFMS: Calcd for $C_{51}H_{44}Cl_3NO_{17}$: *m*/*z* 1048.6. Found: *m*/*z* 1071.6 $[M + Na]^+$.

*2-(Trimethylsilyl)ethyl [methyl (2,3-di-*O*-benzoyl - 4 - O - methyl - β - D - glucopyranosyl)-uronate] - (1→6) - 2,3,4 - tri - O - benzoyl - β - D-galactopyranosyl - (1→6) - 2,3,4 - tri - O - benzyl-β-D-galactopyranoside* (**14**).—Compound **14** was prepared from **13** (700 mg, 0.67 mmol) and **10** (180 mg, 0.33 mmol) as described in the previous glycosylation procedure of disaccharide **11**, yielding 474 mg (84.5%) of a syrup: $[\alpha]_D^{25}$ +58.2° (*c* 0.9 $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.80 (d, 1 H, $J_{1,2}$. 7.9 Hz, H-1′), 4.76 (d, 1 H, $J_{1',2'}$ 9.1 Hz, H-1″), 4.29 (d, 1 H, $J_{1,2}$ 79 Hz, H-1), 3.55 (s, 3 H, COOMe), 3.39 (s, 3 H, OMe); ^{13}C NMR ($CDCl_3$): δ 104.8 (C-1), 102.6 (C-1′), 102.3 (C-1″). MALDI-TOFMS: Calcd for $C_{81}H_{84}O_{22}Si$: *m*/*z* 1436.6. Found: *m*/*z* 1459.6 $[M + Na)]^+$.

*2-(Trimethylsilyl)ethyl [methyl (2,3-di-*O*-benzoyl - 4 - O - methyl - β - D - glucopyranosyl)-uronate] - (1→6) - 2,3,4 - tri - O - benzoyl - β - D-galactopyranosyl - (1→6) - 2,3,4 - tri - O - benzoyl-β-D-galactopyranoside* (**15**).—Compound **15** was prepared from **14** (360 mg, 0.25 mmol) as described for the preparation of **13**, yielding 307 mg (83.0%) of a syrup: $[\alpha]_D^{25}$ +98.4° (*c* 0.7 $CHCl_3$);^1H NMR ($CDCl_3$): δ 4.81 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.74 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.61 (d, 1 H, $J_{1'',2''}$ 7.3 Hz, H-1″), 3.70 (s, 3 H, COOMe), 3.40 (s, 3 H, OMe). ^{13}C NMR ($CDCl_3$): δ 103.0 (C-1″), 102.9 (C-1′), 102.3 (C-1). MALDI-TOFMS: Calcd for $C_{81}H_{78}O_{25}$ Si: *m*/*z* 1479.6. Found: *m*/*z* 1502.6 $[M + Na]^+$.

*Methyl (2,3-di-*O*-benzoyl-4-*O*-methyl-β-*D*-glucopyranosyl)uronate - (1→6) - 2,3,4 - tri - O-benzoyl - β - D - galactopyranosyl - (1→6) - 2,3,4-tri-O-benzoyl-α-D-galactopyranosyl trichloroacetimidate* (**16**).—Compound **16** was prepared from **15** (100 mg, 67.6 μmo1) as described for the preparation of **13**, yielding 103 mg (quant) of a syrup: ^1H NMR ($CDCl_3$): δ

8.38 (s, 1 H, NH), 6.75 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.71 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.44 (d, 1 H, $J_{1'',2''}$ 7.3 Hz, H-1′), 3.62 (s, 3 H, COOMe), 3.36 (s, 3 H, OMe). ^{13}C NMR ($CDCl_3$): δ 100.9 (C-1″), 100.7 (C-1′), 93.7 (C-1). MALDI-TOFMS: Calcd for $C_{78}H_{66}Cl_3NO_{25}$: m/z 1522.7. Found: m/z 1545.6 $[M + Na]^+$.

2-(Trimethylsilyl)ethyl 4-O-methyl-β-D-glucopyranosyluronic acid-(1→6)-β-D-galactopyranoside (**1**).—To a solution of **12** (26 mg, 25.6 μmol) in 2:1 MeOH–water (3 mL) was added NaOMe (40 mg), and the mixture was stirred for 2 h at rt, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with MeOH. The filtrate and washings were combined and concentrated. Column chromatography (3:1 MeOH–water) of the residue on Sephadex LH-20 gave **1** (12.0 mg, quant): $[\alpha]_D^{25}$ −43.5° (*c* 0.6 MeOH); MALDI-TOFMS: Calcd for $C_{18}H_{34}O_{12}Si$: m/z 470.5. Found: m/z 493.2 $[M + Na]^+$, 515.2 $[M + 2Na - H]^+$.

2-(Trimethylsilyl)ethyl 4-O-methyl-β-D-glucopyranosyluronic acid-(1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranoside (**2**).—To a solution of **15** (27 mg, 17.9 μmol) in 5:1 MeOH–water (2.4 mL) was added NaOMe (40 mg), and the mixture was stirred for 2 h at rt, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with MeOH. The filtrate and washings were combined and concentrated. Column chromatography (3:1 MeOH–water) of the residue on Sephadex LH-20 gave **2** (11.3 mg, quant): $[\alpha]_D^{25}$ −32.6° (*c* 0.6 MeOH); MALDI-TOFMS: Calcd for $C_{24}H_{44}O_{17}Si$: m/z 632.3. Found: m/z 655.3 $[M + Na]^+$, 677.3 $[M + 2Na - H]^+$.

Benzyl 5-methoxycarbonylpentyl ether (**17**).—To a solution of ε-caprolactone (10 mL, 90.2 mmol) in MeOH (100 mL) was added NaOMe (80 mg), and the mixture was stirred for 3 h at rt, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with 1:1 $CHCl_3$–MeOH. The filtrate and washings were combined and concentrated. To a solution of this compound in DMF (262 mL) was added NaH in oil (19 g) and BnBr (56 mL). The mixture was stirred for 4 h at rt, and then MeOH was added to destroy excess NaH. The reaction mixture was poured into ice-water and extracted with $CHCl_3$. The extract was successively washed with aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. This compound was purified by column chromatography (5:1 hexane–EtOAc) on silica gel to give compound **17** (10.2 g, 47.7%): ^{1}H NMR ($CDCl_3$): δ 7.23–7.12 (m, 5 H, Ph), 4.44 (s, 2 H, CH_2Ph), 3.59 (s, 3 H, COOMe); MALDI-TOFMS: Calcd for $C_{14}H_{20}O_3$: m/z 236.1. Found: m/z 259.1 $[M + Na]^+$.

5-(2-Aminoethyleneaminocarbonyl)pentyl benzyl ether (**18**).—Compound **17** (1 g, 4.5 mmol) was treated with ethylenediamine (10 mL) at 70 °C for 18 h. After completion of the reaction, the mixture was concentrated. Column chromatography (2:1 $CHCl_3$–MeOH) of the residue on silica gel to give **18** (917 mg, 77.1%) as an amorphous mass: ^{1}H NMR (CD_3OD): δ 7.38–7.32 (m, 5 H, aromatic), 4.48 (s, 2 H, CH_2Ph), 3.28 (br,s, 2 H, CH_2NHCOO), 2.79 (br,s, 2 H, CH_2NH$_2$); MALDI-TOFMS: Calcd for $C_{15}H_{24}N_2O_2$: m/z 264.1. Found: m/z 287.1 $[M + Na]^+$.

Benzyl 5-[2-(2,2,2-trichloroethoxycarbonyl)aminoethyleneaminocarbonyl]pentyl ether (**19**).—To a solution of **18** (37 mg, 0.14 mmol) in pyridine (1 mL) cooled to 0 °C was added TrocCl (58 μL, 0.42 mmol), and the mixture was stirred for 30 min at rt and concentrated. Column chromatography (90:1 $CHCl_3$–MeOH) of the residue on silica gel gave **19** (50 mg, 80.3 %); ^{1}H NMR ($CDCl_3$): δ 7.34–7.27 (m, 5 H, Ph), 6.20 (br,s, 1 H, NHCO), 5.91 (br,s, 1 H, NHCOO), 4.71 (s, 2 H, CH_2CCl_3), 4.49 (s, 2 H, CH_2Ph), 3.36 (t, 2 H, CH_2NHCO), 3.32 (t, 2 H, CH_2NHCOO); MALDI-TOFMS: Calcd for $C_{18}H_{25}Cl_3N_2O_4$: m/z 438.1. Found: m/z 461.0 $[M + Na]^+$.

5-[2-(2,2,2-Trichloroethoxycarbonyl)aminoethyleneaminocarbonyl]pentanol (**20**).—A solution of **19** (340 mg, 0.77 mmol) in MeOH (4 mL) was hydrogenated over 10% Pd–C (60 mg) for 20 min at rt, then filtered through Celite. The filtrate was concentrated, and the residue was purified by silica-gel column chromatography using 10:1 $CHCl_3$–MeOH as eluent to give **20** (178 mg, 65.8%); ^{1}H NMR ($CDCl_3$): δ 6.73 (br,s, 1 H, NHCO), 6.32 (br,s, 1 H, NHCOO), 4.72 (s, 2 H, CH_2CCl_3), 3.39 (t, 2 H, CH_2NHCO), 3.37 (t, 2 H, CH_2-NHCOO); MALDI-TOFMS: Calcd for $C_{11}H_{19}Cl_3N_2O_4$: m/z 348.0. Found: m/z 371.3 $[M + Na]^+$.

5-[2-(2,2,2,-Trichloroethoxycarbonyl)aminoethyleneaminocarbonyl]pentyl [methyl (2,3-di-O-benzoyl-4-O-methyl-β-D-glucopyranosyl)uronate]-(1→6)-2,3,4-tri-O-benzoyl-β-D-galactopyranoside (**21**).—To a solution of the trichloroacetimidate **13** (244 mg, 0.23 mmol) and **20** (40 mg, 0.12 mmol) in CH_2Cl_2 (1 mL) were added molecular sieves 4 Å (300 mg), and the mixture was stirred for 1 h at rt, then cooled to 0 °C. TMSOTf (22 μL, 120 μmol) was added, and the mixture was stirred for 1 h at 0 °C, then neutralized with Et_3N. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica-gel column chromatography using 1:3 benzene–EtOAc as eluent to give **21** (100 mg, 64.7%): $[\alpha]_D^{24}$ +74.2° (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 8.06–7.21 (m, 25 H, 5 × Ph), 5.80 (d, 1 H, H-4), 5.64 (t, 1 H, H-2), 5.61 (t, 1 H, H-3′), 5.48 (dd, 1 H, H-3), 5.37 (t, 1 H, H-2′), 4.85 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1′), 4.69 (q, 2 H, OCH_2CCl_3), 4.58 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.10 (m, 1 H, H-5), 4.07 (d, 1 H, H-5′), 4.03, 3.91 (dt, 2 H, H-6), 3.91 (t, 1 H, H-4′), 3.78 (s, 3 H, COOMe), 3.38 (s, 3 H, OMe), 3.36 (m, 2 H, OCH_2CH_2), 3.36 (m, 2 H, CH_2NHCO–), 3.26 (m, 2 H, CH_2NHCOO), 1.89 (t, 2 H, CH_2CO), 1.44 (m, 2 H, CH_2CH_2CO), 1.14 (m, 2 H, OCH_2CH_2), 0.88 (t, 2 H, $OCH_2CH_2CH_2$); MALDI-TOFMS: Calcd for $C_{60}H_{61}Cl_3N_2O_{20}$: *m*/*z* 1235.7 Found: *m*/*z* 1258.4 $[M+Na]^+$.

5-[2-(2,2,2,-Trichloroethoxycarbonyl)aminoethyleneaminocarbonyl]pentyl [methyl (2,3-di-O-benzoyl-4-O-methyl-β-D-glucopyranosyl)uronate]-(1→6)-2,3,4-tri-O-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-β-D-galactopyranoside (**22**).—Compound **22** was prepared from **16** (86 mg, 56.5 μmol) and **20** (10 mg, 28.7 μmol) as described in the previous glycosylation procedure of **21**, yielding 29 mg (59.7%) of a syrup: $[\alpha]_D^{25}$ +72.1° (*c* 1.3 $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 4.77 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.76 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.68 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 3.72 (s, 3 H, COOMe), 3.41 (s, 3 H, OMe). ^{13}C NMR ($CDCl_3$): δ 101.6 (C-1), 101.5 (C-1″), 101.4 (C-1′); MALDI-TOFMS: Calcd for $C_{87}H_{83}Cl_3N_2O_{28}$: *m*/*z* 1709.6. Found: *m*/*z* 1732.8 $[M+Na]^+$.

5-(2-Aminoethyleneaminocarbonyl)pentyl [methyl (2,3-di-O-benzoyl-4-O-methyl-β-D-glucopyranosyl)uronate]-(1→6)-2,3,4-tri-O-benzoyl-β-D-galactopyranoside (**23**).—To a solution of **21** (185 mg, 0.15 mmol) in AcOH (10 mL) was added zinc powder (200 mg), and the mixture was stirred for 12 h at rt. The solids were filtered off and washed with $CHCl_3$. The combined filtrate and washings were successively washed with water, dried (Na_2SO_4), and concentrated. The product was purified by silica-gel column chromatography using 10:1 $CHCl_3$–MeOH as eluent to give **23** (92 mg, 58.0%): $[\alpha]_D^{25}$ +74.8° (*c* 0.6 $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 8.06–7.21 (m, 25 H, 5 Ph), 5.99 (br,s, 1 H, NHCO), 5.80 (d, 1 H, H-4), 5.65 (t, 1 H, H-2), 5.61 (t, 1 H, H-3′), 5.49, 5.47 (dd, 1 H, H-3), 5.38 (t, 1 H, H-2′), 4.84 (d, 1 H, $J_{1',2'}$ 7.3 Hz, H-1′), 4.59 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1), 4.10 (m, 1 H, H-5), 4.06 (d, 1 H, H-5′), 4.07, 3.80 (m, 2 H, H-6), 3.92 (t, 1 H, H-4′), 3.75 (s, 3 H, COOMe), 3.38 (s, 3 H, OMe), 3.28 (m, 2 H, OCH_2CH_2), 3.28 (m, 2 H, CH_2NHCO–), 2.83 (br,d, 2 H, CH_2NH_2), 2.02 (br,s, 2 H, NH_2), 1.88 (t, 2 H, CH_2CO), 1.43 (m, 2 H, CH_2CH_2CO), 1.32 (m, 2 H, OCH_2CH_2), 1.12 (t, 2 H, $OCH_2CH_2CH_2$); MALDI-TOFMS: Calcd for $C_{57}H_{60}N_2O_{18}$: *m*/*z* 1060.4. Found: *m*/*z* 1083.4 $[M+Na]^+$.

5-(2-Aminoethyleneaminocarbonyl)pentyl [methyl (2,3-di-O-methyl-β-D-glucopyranosyl)uronate]-(1→6)-2,3,4-tri-O-benzoyl-β-D-galactopyranosyl-(1→6)-2,3,4-tri-O-benzoyl-β-D-galactopyranoside (***24***).—Compound **24** was prepared from **22** (56 mg) as described for the preparation of **23**, yielding 46 mg (91.5%) of a syrup: $[\alpha]_D^{25}$ +73.3° (*c* 0.6 $CHCl_3$). ^{1}H NMR ($CDCl_3$): δ 4.78 (d, 1 H, $J_{1'',2''}$ 7.9 Hz, H-1″), 4.76 (d, 1 H, $J_{1',2'}$ 7.9 Hz, H-1′), 4.64 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 3.70 (s, 3 H, COOMe), 3.40 (s, 3 H, OMe). ^{13}C NMR ($CDCl_3$): δ 101.7 (C-1), 101.5 (C-1′), 101.4 (C-1″); MALDI-TOFMS: Calcd for $C_{84}H_{82}N_2O_{26}$: *m*/*z* 1534.6. Found: *m*/*z* 1557.9 $[M+Na]^+$.

N,N′,N″-*Tri-{5-[methyl (2,3-di-O-benzoyl-4-O-methyl-β-D-glucopyranosyl)uronate]-(1→6)-2,3,4-tri-O-benzoyl-β-D-galactopyranosyloxypentylcarbonylaminoethyl}-1,3,5-benzenetriamide* (**25**).—To a solution of **23** (23 mg, 21.6 μmol) in CH_2Cl_2 (1 mL) were added triethylamine (3 μL) and 1,3,5-benzenetricarbonyl trichloride (1.3 mg, 5.0 μmol). The mix-

ture was stirred for 5 min at rt. After completion of the reaction, the mixture was concentrated. Column chromatography (20:1 $CHCl_3$–MeOH) of the residue on silica gel gave **25** (11 mg, 64.1%); ^1H NMR ($CDCl_3$): δ 5.80 (d, 3 H, $J_{3,4}$ 3.0 Hz, H-4), 5.61 (t, 6 H, J 9.1 Hz, H-2, H-3′), 5.50 (dd, 3 H, $J_{3,4}$ 3.7, $J_{2,3}$ 10.4 Hz, H-3), 5.36 (t, 3 H, H-2′), 4.85 (d, 3 H, $J_{1',2'}$ 7.3 Hz, H-1′), 4.57 (d, 3 H, $J_{1,2}$ 7.9 Hz, H-1), 4.10 (m, 3 H, H-5), 4.07 (d, 3 H, H-5′), 4.03, 3.91 (dt, 6 H, H-6), 3.91 (t, 3 H, H-4′), 3.73 (s, 9 H, COOMe), 3.37 (s, 9 H, OMe); ^{13}C NMR ($CDCl_3$): 168.5, 132.6–128.3, 101.3, 101.1, 78.9, 76.8, 74.1, 73.3, 71.9, 71.6, 70.0, 69.8, 68.8, 68.6, 60.4, 52.7, 29.7–11.5. MALDI-TOFMS: Calcd for $C_{180}H_{180}N_6O_{57}$: m/z 3337.1. Found: m/z 3360.2 $[M + Na]^+$.

N,N′,N″-*Tri-{5-[methyl (2,3-di*-O-*benzoyl-4*-O-*methyl*-β-D-*glucopyranosyl)uronate]-(1→6)-2,3,4-tri*-O-*benzoyl*-β-D-*galactopyranosyl*-(1→6)-2,3,4-tri-O-β-D-*galactopyranosyloxypentylcarbonylaminoethyl]*-1,3,5-*benzenetriamide* (**26**).—To a solution of **24** (26 mg, 16.8 μmol) in CH_2Cl_2 (1 mL) were added triethylamine (2.4 μL) and 1,3,5-benzenetricarbonyl trichloride (1 mg, 3.9 μmol). The mixture was stirred for 5 min at rt. After completion of the reaction, the mixture was concentrated. Column chromatography (20:1 $CHCl_3$–MeOH) of the residue on silica gel gave **26** (8.6 mg, 77.8%): MALDI-TOFMS: Calcd for $C_{261}H_{246}N_6O_{81}$: m/z 4759.5. Found: m/z 4782.0 $[M + Na]^+$.

N,N′,N″-*Tri-{5-[4*-O-*methyl*-β-D-*glucopyranosylurnnic acid-(1→6)*-β-D-*galactopyranosyloxy]pentylcarbonylaminoethyl}*-1,3,5-*benzenetriamide* (**3**).—To a solution of **25** (9.4 mg, 2.8 μmol) in 5:1 MeOH–water (1.2 mL) was added NaOMe (39 mg), and the mixture was stirred for 4 h at rt, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with MeOH. The filtrate and washings were combined and concentrated. Column chromatography (3:1 MeOH–water) of the residue on Sephadex LH-20 gave **3** (4.9 mg, quant.): $[\alpha]_D^{25}$ −17.8° (*c* 0.2 water); MALDI-TOFMS: Calcd for $C_{72}H_{114}NO_{42}$: m/z 1734.5. Found: m/z 1757.0 $[M + Na]^+$, 1823.0 $[M - 3H + 4Na]^+$.

N,N′,N″-*Tri-{5-[4*-O-*methyl*-β-D-*glucopyranosyluronic acid (1→6)*-β-D-*galactopyranosyl*-(1→6)-β-D-*galactopyranosyloxy]pentylcarbonylaminoethyl}*-1,3,5-*benzenetriamide* (**4**).—To a solution of **26** (4.3 mg, 0.9 μmol) in 5:1 MeOH–water (0.6 mL) was added NaOMe (16 mg), and the mixture was stirred for 4 h at rt, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off and washed with MeOH. The filtrate and washings were combinedand concentrated. Column chromatography (3:1 MeOH–water) of the residue on Sephadex LH-20 gave **4** (2.0 mg, quant) $[\alpha]_D^{25}$ −23.2° (*c* 0.1 water); MALDI-TOFMS: Calcd for $C_{90}H_{144}N_6O_{57}$: m/z 2220.8. Found: m/z 2243.2 $[M + Na]^+$, 2309.5 $[M - 3H + 4Na]^+$.

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