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

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Received 18 January 2001; accepted 19 May 2001

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-<sup>O</sup>-benzoyl-4-<sup>O</sup>-methyl-α-D-glucopyranosyl trichloroacetimidate)uronate and methyl (2,3-di-<sup>O</sup>-benzoyl-4-<sup>O</sup>-methyl-β-D-glucopyranosyl)uronate-(1→6)-2,3,4-tri-<sup>O</sup>-benzoyl-α-D-galactopyranosyl trichloroacetimidate with the common acceptor 2-(trimethylsilyl)ethyl 2,3,4-tri-<sup>O</sup>-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-<sup>O</sup>-methyl-α-D-glucopyranosyluronic acid-(1→6)-β-D-galactopyranosyloxy]pentylcarbonylaminooethyl}-1,3,5-benzenetrimetiamide and N,N′,N″-tri-{5-[4-<sup>O</sup>-methyl-β-D-glucopyranosyluronic acid (1→6)-β-D-galactopyranosyl-(1→6)-β-D-galactopyranosyloxy]-pentylcarbonylaminooethyl}-1,3,5-benzenetramide. © 2001 Elsevier Science Ltd. All rights reserved.

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. Recently, Yamada et al. 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; 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-1-Rha-(1→4)-α-D-GalA-(1→2)-repeating unit, which was reported in our previous paper 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. Furthermore, among the structural region of bupleuran 2IIc, the ‘ramified’ region (PG-1), showed potent mitogenic activity, suggesting it was the active site. 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 have proved to be advantageous in many instances, as the multi-presentation 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 monovalent 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) 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-
tion of 6 was carried out with K$_2$Cr$_2$O$_7$ 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-glucopyranosiduronate] (8). For selective removal of the 2-(trimethylsilyl)ethyl (SE) group, 8 was treated with trifluoroacetic acid in dichloromethane for 1 h at 0 °C to give the 1-hydroxy compound, which on further treatment 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 1H NMR spectroscopy (H-1′, 4.81 ppm, $J_{7.9}$...
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 1H 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 debenzylation 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-trichloroethoxy carbonyl)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 benzylolation gave benzyl 5-methoxycarbonylpentyl ether (17). Compound 17 was converted to the primary amine 18 by reaction with neat anhydrous ethylenediamine 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-trichloroethoxy carbonyl)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 1H 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
by Zn–AcOH gave the primary amines 23 and 24 (Scheme 4). We chose 1,3,5-benzenetricarbonyl trichloride 12 as 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 afforded the desired trivalent 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 was evaluated, and the results will be reported in detail elsewhere.

3. Experimental

Optical rotations were determined with a JASCO digital polarimeter. ¹H and ¹³C NMR spectra were recorded on a JNM A 500 FT NMR spectrometer in CDCl₃ with Me₄Si as the internal standard. MALDI-TOFMS was recorded on a Perceptive Voyager RP mass spectrometer. TLC was performed on Silica Gel 60 F₂₅₄ (E. Merck) with detection by quenching of UV fluorescence and by spraying with 10% H₂SO₄. 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 CHCl₃ and washed with 5% HCl, aq NaHCO₃ and water, dried (Na₂SO₄), 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 CHCl₃. The extract was successively washed with aq NaHCO₃ and water, dried (Na₂SO₄), 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, 70%): ¹H NMR (CDCl₃): δ 7.34–7.25 (m, 10 H, 2 Ph), 4.95–4.69 (m, 4 H, 2 PhCH₂), 4.42 (d, 1 H, J₁₂ 7.9 Hz, H-1), 4.00–3.95 (m, 1 H, –OCH₂CH₂–), 3.93–3.76 (brd, 2 H, H-6a,b), 3.63–3.26 (m, 5 H, H-2,3,4,5, –OCH₂CH₂–), 3.55 (s, 3 H, OMe), 0.97–0.86 (m, 2 H, –OCH₂CH₂–), −0.06 (s, 9 H, Si(CH₃)₃); MALDI-TOFMS: Calcd for C₁₅H₂₇O₅Si: 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-glucopyranosiduronate (7).—To a solution of 6 (1.22 g, 5.06 mmol) in acetonitrile (30 mL) was added K₂Cr₂O₇ (2.8 g), 3.5 M H₂SO₄ (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 CHCl₃, and the washings were successively washed with water, dried (Na₂SO₄), 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 (CDCl₃): δ 7.36–7.26 (m, 10 H, 2 Ph), 4.94–4.68 (m, 4 H, 2 PhCH₂), 4.42 (d, 1 H, J₁₂ 7.5 Hz, H-1), 4.00–3.94 (m, 1 H, –OCH₂CH₂–), 3.81 (s, 3 H, COOMe), 3.78 (d, 1 H, J₄,5 10.5 Hz, H-5), 3.62–3.39 (m, 4 H, H-2,3,4, –OCH₂CH₂–), 3.49 (s, 3 H, OMe), 0.96–0.86 (m, 2 H, –OCH₂CH₂–), −0.03 (s, 9 H, Si(CH₃)₃); ¹³C NMR (CDCl₃): 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 PhCH₂), 74.3 (C-5), 67.8
Methyl [2-(trimethylsilyl)ethyl 2,3-di-O-benzoyl-4-O-methyl-β-D-glucopyranosiduronate (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 CHCl₃. The extract was washed sequentially with 5% HCl, qq NaHCO₃ and water, dried (Na₂SO₄), 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%). 1H NMR: δ 8.07–7.34 (10 H, m, 2 Ph), 5.68 (t, 1 H, J₂,₃ = J₃,₄ 9.2 Hz, H-3), 5.43 (t, 1 H, J₁,₂ 7.6 Hz, H-2), 4.82 (dd, 1 H, H-1), 4.15 (d, 1 H, J₄,₅ 9.6 Hz, H-5), 4.12–3.97 (m, 2 H, H-4, −OCH₂CH₂), 3.92 (s, 3 H, COOMe), 3.69–3.50 (m, 1 H, −OCH₂CH₂−), 3.48 (s, 3 H, OMe), 0.92–0.87 (m, 2 H, −CH₂CH₂−), 0.03 (s, 9 H, Si(CH₃)₃); 13C NMR (CDCl₃): δ 168.6 (C-6), 100.8 (C-1), 79.1 (C-4), 74.4 (C-3,5), 72.0 (C-2), 67.8 (−OCH₂CH₂−), 60.4 (COOMe), 52.7 (OMe), 17.8 (−OCH₂CH₂−), −1.5 (SiMe₃). MALDI-TOFMS: Calcd for C₂₇H₃₄O₉Si: m/z 525.1. Found: m/z 525.1 [M + Na]⁺.

Methyl (2,3-di-O-benzoyl-4-O-methyl-α-D-glucopyranosyl trichloroacetimidate)uronate (9).—To a solution of 8 (115 mg, 0.22 mmol) in CH₂Cl₂ (2 mL) cooled to 0 °C was added CF₃COOH (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 CH₂Cl₂ (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: 1H NMR (CDCl₃): δ 8.63 (s, 1 H, NH), 8.04–7.26 (m, 10 H, 2 Ph), 6.76 (d, 1 H, J₁,₂ 3.3 Hz, H-1), 6.07 (t, 1 H, J₃,₄ = J₄,₅ 9.5 Hz, H-3), 5.46 (dd, 1 H, H-2), 4.54 (d, 1 H, J₄,₅ 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 C₂₇H₂₂Cl₃NO₉: 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 (10).—A solution of 9 (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 benzyolated 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 CHCl₃. The extract was washed sequentially with 5% HCl, aq NaHCO₃, and water, dried (Na₂SO₄), 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%): [α]D²⁰ + 103.4° (c 1.8 CHCl₃); ¹H NMR (CDCl₃): 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), 5.38 (t, 1 H, H-2), 4.85 (d, 1 H, J₁,₂ 6.7 Hz, H-1'), 4.67 (d, 1 H, J₁,₂ 8.6 Hz, H-1), 4.10 (d, 1 H, J₁,₂ 7.9 Hz, H-1), 3.70 (s, 3 H, COOMe), 3.39 (s, 3 H, OMe). ¹³C NMR (CDCl₃): 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-2'), 71.7 (C-2'), 69.3 (C-3), 69.0 (C-2), 69.0 (C-4), 68.2 (C-3), 3.70 (s, 3 H, COOMe), 3.39 (s, 3 H, OMe). MALDI-TOFMS: Caled for C₄₅H₄₄Cl₅NO₇; 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-galactopyranosyl)-uronate]-(1 → 6)-2,3,4-tri-O-benzoyl-β-D-galactopyranosyl-(1 → 6)-2,3,4-tri-O-benzoyl-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: [α]D²⁰ + 58.2° (c 0.9 CHCl₃); ¹H NMR (CDCl₃): δ 4.80 (d, 1 H, J₁,₂ 7.9 Hz, H-1'), 4.76 (d, 1 H, J₁,₂ 9.1 Hz, H-1”), 4.29 (d, 1 H, J₁,₂ 79 Hz, H-1), 3.55 (s, 3 H, COOMe), 3.39 (s, 3 H, OMe); ¹³C NMR (CDCl₃): δ 104.8 (C-1), 102.6 (C-1’), 102.3 (C-1”). MALDI-TOFMS: Caled for C₄₅H₄₅O₁₇Si; 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-galactopyranosyl)-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: ²⁰[α]D + 98.4° (c 0.7 CHCl₃); ¹H NMR (CDCl₃): δ 4.81 (d, 1 H, J₁,₂ 7.9 Hz, H-1), 4.74 (d, 1 H, J₁,₂ 7.9 Hz, H-1”), 4.61 (d, 1 H, J₁,₂ 7.3 Hz, H-1”), 3.70 (s, 3 H, COOMe), 3.40 (s, 3 H, OMe). ¹³C NMR (CDCl₃): δ 103.0 (C-1”), 102.9 (C-1’), 102.3 (C-1) MALDI-TOFMS: Caled for C₄₅H₄₅O₁₇Si; m/z 1479.6. Found: m/z 1502.6 [M + Na]⁺.

Methyl (2,3-di-O-benzoyl-4-O-methyl-β-D-galactopyranosyl)uronate-(1 → 6)-2,3,4-tri-O-benzoyl-β-D-galactopyranosyl-(1 → 6)-2,3,4-tri-O-benzoyl-β-D-galactopyranosyl trichloroacetimide (16).—Compound 16 was prepared from 15 (100 mg, 67.6 μmol) as described for the preparation of 13, yielding 103 mg (quant) of a syrup: ¹H NMR (CDCl₃): 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 (CDCl3): $\delta$ 100.9 (C-1), 100.7 (C-1'), 93.7 (C-1). MALDI-TOFMS: Caled for $C_{78}H_{66}Cl_3NO_2$: $m/z$ 1522.7. Found: $m/z$ 1545.6 [M + Na]$^+$.  

2-(Trimethylsilyl)ethyl 4-O-methyl-$\beta$-D-glucopyranosyluronic acid-(1→6)-$\beta$-D-galactopyranoside (1).—To a solution of 12 (26 mg, 25.6 $\mu$mol) 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): $[z]_D^{25} = -43.5^\circ$ (c 0.6 MeOH); MALDI-TOFMS: Caled for $C_{18}H_{34}O_12Si$: $m/z$ 470.5. Found: $m/z$ 493.2 [M + Na]$^+$, 515.2 [M + 2Na – H]$^+$.

2-(Trimethylsilyl)ethyl 4-O-methyl-$\beta$-D-glucopyranosyluronic acid-(1→6)-$\beta$-D-galactopyranosyl - (1→6) - $\beta$ - D – galactopyranoside (2).—To a solution of 15 (27 mg, 17.9 $\mu$mol) 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): $[z]_D^{25} = 32.6^\circ$ (c 0.6 MeOH); MALDI-TOFMS: Caled for $C_{36}H_{44}O_{17}$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 $\varepsilon$-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 CHCl3–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 CHCl3. The extract was successively washed withaq NaHCO3 and water, dried (Na2SO4), 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 (CDCl3): $\delta$ 7.23–7.12 (m, 5 H, Ph), 4.44 (s, 2 H, CH2Ph), 3.59 (s, 3 H, COOMe); MALDI-TOFMS: Caled for $C_{11}H_{19}NO_4$: $m/z$ 236.1. Found: $m/z$ 259.1 [M + Na]$^+$.

5-(2-Aminoethyleaminocarbonyl)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 CHCl3–MeOH) of the residue on silica gel to give 18 (917 mg, 77.1%) as an amorphous mass: $^1$H NMR (CD2OD): $\delta$ 7.38–7.32 (m, 5 H, aromatic), 4.48 (s, 2 H, CH2Ph), 3.28 (br,s, 2 H, CH2NHCOO), 2.79 (br,s, 2 H, CH2NH2); MALDI-TOFMS: Caled for $C_{18}H_{25}Cl_3N_2O_4$: $m/z$ 264.1. Found: $m/z$ 287.1 [M + Na]$^+$.  

Benzyl 5-[2-(2,2,2-trichloroethoxy carbonyl)aminoethyleaminocarbonylpentyl]pentyl benzyl ether (19).—To a solution of 18 (37 mg, 0.14 mmol) in pyridine (1 mL) cooled to 0°C was added TrocCl (58 $\mu$L, 0.42 mmol), and the mixture was stirred for 30 min at rt and concentrated. Column chromatography (90:1 CHCl3–MeOH) of the residue on silica gel to give 19 (50 mg, 80.3 %); $^1$H NMR (CDCl3): $\delta$ 7.34–7.27 (m, 5 H, Ph), 6.20 (br,s, 1 H, NHCOO), 5.91 (br,s, 1 H, NHCOO), 4.71 (s, 2 H, CH2ClCl), 4.49 (s, 2 H, CH2Ph), 3.36 (t, 2 H, CH2NHCOO), 3.32 (t, 2 H, CH2NH2); MALDI-TOFMS: Caled 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-Trichloroethoxy carbonyl)aminoethyleaminocarbonylpentyl]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 CHCl3–MeOH as eluent to give 20 (178 mg, 65.8%); $^1$H NMR (CDCl3): $\delta$ 6.73 (br,s, 1 H, NHCOO), 6.32 (br,s, 1 H, NHCOO), 4.72 (s, 2 H, CH2ClCl), 3.39 (t, 2 H, CH2NHCOO), 3.37 (t, 2 H, CH2NH2); MALDI-TOFMS: Caled for $C_{11}H_{19}Cl_3N_2O_4$: $m/z$ 348.0. Found: $m/z$ 371.3 [M + Na]$^+$.  

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5-[2-(2,2,2-Trichloroethoxy-carbonyl)amino-ethyleneariminocarbonyl]-pentyl [methyl (2,3-di-O-benzoyl-4-O-methyl-β-D-galactopyranosyl)-uronate]- (1 → 6)-2,3,4-tri-O-benzoyl-β-D-galactopyranoside (21).—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 CHCl₃. The combined filtrate and washings were successively washed with water, dried (Na₂SO₄), and concentrated. The product was purified by silica-gel column chromatography using 1:3 benzene–EtOAc as eluent to give 23 (92 mg, 58.0%): [α]D²⁰ + 74.8° (c 0.6 CHCl₃); †H NMR (CDCl₃): δ 8.06–7.21 (m, 25 H, 5 Ph), 5.99 (br.s, 1 H, NHCO), 5.80 (d, 1 H, H-4), 5.64 (d, 1 H, H-2), 5.61 (d, H, H-3), 5.48 (dd, 1 H, H-5), 5.37 (t, 1 H, H-2), 4.85 (d, 1 H, J₁₂ = 7.3 Hz, H-1'), 4.69 (q, 2 H, OCH₂C₃Cl), 4.58 (d, 1 H, J₁₂ = 7.9 Hz, H-1), 4.10 (m, 1 H, H-5), 4.07 (d, 1 H, H-5'), 4.03 (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, OCH₂CH₂), 3.26 (m, 2 H, CH₂NHCOO), 1.89 (t, 2 H, CH₂CO), 1.44 (m, 2 H, CH₂CH₂CO), 1.14 (m, 2 H, OCH₂CH₂), 0.88 (t, 2 H, OCH₂CH₂); MALDI-TOFMS: Calcd for C₆₃H₇₀Cl₃N₂O₂₈: m/z 1235.7 Found: m/z 1258.4 [M + Na]⁺.

5-(2-Aminoethyleneariminocarbonyl)pentyl [methyl (2,3-di-O-methyl-β-D-galactopyranosyl)-uronate]- (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: [α]D²⁰ + 73.3° (c 0.6 CHCl₃). †H NMR (CDCl₃): δ 4.78 (d, 1 H, J₁₂ = 7.9 Hz, H-1'), 4.76 (d, 1 H, J₁₂ = 7.9 Hz, H-1'), 4.68 (d, 1 H, J₁₂ = 7.9 Hz, H-1'), 3.72 (s, 3 H, COOMe), 3.41 (s, 3 H, OMe). †C NMR (CDCl₃): δ 101.7 (C-1'), 101.5 (C-1), 101.4 (C-1); MALDI-TOFMS: Calcd for C₈₈H₈₃Cl₃N₂O₂₆: m/z 1534.6. Found: m/z 1557.9 [M + Na]⁺.
ture was stirred for 5 min at rt. After completion of the reaction, the mixture was concentrated. Column chromatography (20:1 CHCl₃–MeOH) of the residue on silica gel gave 25 (11 mg, 64.1%); ¹H NMR (CDCl₃): δ 5.80 (d, 3 H, J₃,₄ 3.0 Hz, H-4), 5.61 (t, 6 H, J 9.1 Hz, H-2, H-3), 5.50 (dd, 3 H, J₃,₄ 3.7, J₂,₃ 10.4 Hz, H-3), 5.36 (t, 3 H, H-2'), 4.85 (d, 3 H, J₁,₂ 7.3 Hz, H-1'), 4.57 (d, 3 H, J₁,₂ 7.9 Hz, H-1), 4.10 (m, 3 H, J₁,₂ 4.0 Hz, J₂,₃ 4.0 Hz, H-5), 4.07 (d, 3 H, H-5), 4.03, 3.91 (dt, 6 H, J 9.1 Hz, H-2, H-3), 3.73 (s, 9 H, COOMe), 3.37 (s, 9 H, OMe); ¹³C NMR (CDCl₃): 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 C₁₉₀H₂₄₀N₆O₅₇: m/z 3371.1. Found: m/z 3360.2 [M + Na]⁺.

N,N',N''-Tri-{5-[methyl (2,3-di-O-benzoyl-4-O- methyl -β - d - glucopyranosyl)uronic acid]-(1 → 6)-2,3,4-tri-O-benzoyl-β- d - galactopyranosyl -(1 → 6)-2,3,4-tri-O-β- d - galactopyranosylpentylcarbonylamineolthyl} - 1,3,5 - benzzenetramide (26).—To a solution of 24 (26 mg, 16.8 µmol) in CH₂Cl₂ (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 CHCl₃–MeOH) of the residue on silica gel gave 26 (8.6 mg, 77.8%); MALDI-TOFMS: Calcd for C₂₆₁H₃₄₆N₆O₄₁: m/z 4759.5. Found: m/z 4782.0 [M + Na]⁺.

N,N',N''-Tri-{5-[4-O-methyl-β - d - glucopyranosyluronic acid-(1 → 6)-β- d - galactopyranosylpentylcarbonylamineolthyl} - 1,3,5 - benzzenetramide (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.): [α]₂⁰D⁺ 17.8° (c 0.2 water); MALDI-TOFMS: Calcd for C₇₂H₁₁₄NO₄₂: 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 - glucopyranosy luronic acid (1 → 6)-β - d - galactopyranosy l-(1 → 6)-β - d - galactopyranosylpentylcarbonylamineolthyl}-1,3,5-benzzenetramide (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 combined and concentrated. Column chromatography (3:1 MeOH–water) of the residue on Sephadex LH-20 gave 4 (2.0 mg, quant) [α]₂⁰D⁺ 23.2° (c 0.1 water); MALDI-TOFMS: Calcd for C₉₀H₁₄₄N₆O₅₇: m/z 2220.8. Found: m/z 2243.2 [M + Na]⁺, 2309.5 [M – 3H + 4Na]⁺.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research (No. 12672062) from the Ministry of Education, Science, Sports and Culture of Japan. We gratefully acknowledge the financial support of Uehara Memorial Foundation. The authors are grateful to Mrs J. Hada for providing NMR and MS data.

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