Supplementary data

Materials and methods

Preparation of compounds 3 and 4

Compounds 3 and 4 were prepared as follows (Scheme 1):

Scheme 1

*Bis-([1-acetamido-1-deoxy-2,3,6,3',6',2'',3'',4'',6''-nona-O-acetyl-β-D-Pk-2'-'yloxy]ethyl)oxycarbamoyl-pentane* (16).

Triethylamine (36.4 mg, 0.36 mmol) was added to a stirred solution of 1,5-diaminopentane (9 mg, 0.088 mmol) and *1-acetamido-1-deoxy-2,3,6-tri-O-acetyl-4-O-[3,6-di-O-acetyl-4-
O(2,3,4,6-tetra-O-acetyl-α-D-galactopyranosyl)2-O-[2-(p-nitrophenyloxycarbonyloxy)ethyl]-β-D-galactopyranosyl-β-D-glucopyranose (Kanda et al. 2005) (15, 220mg, 0.194mmol) in dry DCM (2 mL). The mixture was left at room temperature for 3 days. Then it was concentrated and chromatographed on silica gel using hexane-acetone (2:1-1:2 to provide the dimer 16 (148mg, 81%) %), [α]_D +54.0° (c 1.1, CHCl₃), ¹H-NMR (CDCl₃): δ: 6.21 (d, 2 H, J₁,₂,₃,₄ 1.2Hz, H-4”), 5.34 (dd, 2 H, J₂,₃” 11.1Hz, J₃”,₄” 3.4Hz, H-3”), 5.27 (t, 2 H, J₂,₃ = J₃,₄ 8.7Hz, H-2), 5.22-5.16 (m, 4 H, H-1, H-2”), 4.97 (d, 2 H, J₁”,₃” 3.5Hz, H-1”), 4.82 (t, 2 H, J₁,₂ 9.6Hz, H-2), 4.69 (dd, 2 H, J₂”,₃” 10.4Hz, J₃”,₄” 2.8Hz, H-3”), 4.49 (dd, 2 H, J₅,₆A 0.5Hz, J₆A,₆B 11.9Hz, H-6A), 4.41-4.36 (m, 4 H, H-5”, H-6A”), 4.32 (dd, 2 H, J₅,₆B 1.3Hz, H-5B), 4.27(d, 2 H, J₁,₂ 7.6Hz, H-1), 4.15-4.02 (m, 10 H, H-6B”, H-6A”, H-6B”, OCH₂), 3.99(d, 2 H, H-4”), 3.89-3.87 (m, 2H, OCH, 3.80-3.70 (m, 6H, H-4, H-5, OCH), 3.66 (t, 2 H, J₅,₆A = J₅,₆B 6.8Hz, H-5”), 3.41(dd, 2 H, H-2”), 3.20-3.10 (m, 4 H, NCH₂), 2.12, 2.09, 2.06, 2.05, 2.04, 2.03, 1.96 (8s, 60 H, Ac), 1.56-1.50 (m, 4 H, CH₂), 1.38-1.32 (m, 2 H, CH₂).

**Bis-[(1-acetamido-1-deoxy-4-O-[4-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranose)-2’-yloxy]ethyloxycarbamoyl--pentane (3)**

To a solution of 16 (134 mg, 0.064 mg) in dry methanol (2.5mL) methanolic NaOMe solution (1 N, 0.3 mL) and the mixture was left at room temperature overnight, then it was neutralized with Dowex 50W-X12(H⁺-form). The resin was filtered off and the filtrate was concentrated, then it was purified on HPLC and freeze-dried to give the deprotected dimer 3 (72 mg, 85%)%, [α]_D +45.0° (c 1.0, water), ¹H-NMR (D₂O): δ 4.97 (2d, 4 H, J₁,₂ 9.2 Hz, J₁”,₂” 4.0Hz, H-1, H-1”), 4.55 (d, 2H, J₁,₂ 7.8 Hz, H-1”), 4.35 (t, 2H,
$J_{5'',6a''}^7-J_{5'',6b''}^7-6.5$ Hz, H-5''), 4.24-4.18 (m, 4 H, CH$_2$O), 4.14-3.96 (m, 2 H, CH$_2$O), 3.96 (d, 2 H, $J_{3',4'}^7 3.2$ Hz, H-4'), 3.94 (d, 2 H, $J_{3'',4''}^7 3.4$ Hz, H-4''), 3.96-3.90 (m, 6 H, H-6a, H-5', H-3''), 3.87-3.80 (m, 6 H, H-6b, H-6a', H-2''), 3.78-3.66 (m, 12 H, H-3, H-4, H-3', H-6b', H-6a'', H-6b''), 3.64-3.60 (ddd, 2 H, $J_{1.8}$ Hz, $J_{4.4}$ Hz, $J_{9.5}$ Hz, H-5), 3.43 (m, 4H, H-2, H-2'), 3.06 (t, 4H, $J_{6.8}$ Hz, CH$_2$N), 2.08 (s, 6 H, NAc), 1.52 (m, 4 H, CH$_2$), 1.34 (m, 2 H, CH$_2$). HRMS-ES $m/z$ 689.24798 ([M+2Na]$^+$, C$_{51}$H$_{88}$N$_4$O$_{36}$Na$_2^+$ requires 1378.49627.

*Bis-([1-acetamido-1-deoxy-2,3,6,3',4',6'-hexa-O-acetyl-β-D-lactose-2' -\text{yloxy}]ethyl)oxycarbamoyl-propane (18).*

To a solution of 1-acetamido-1-deoxy-2,3,6-tri-O-acetyl-4-O-(3,4,6-tri-O-acetyl-2-O-\{2-(p-nitrophenyloxycarbonyloxy)ethyl\}-β-D-galactopyranosyl)-β-D-glucopyranose (Kitov et al. 2003) (17, 1 g, 1.18 mmol) and triethylamine (120 mg, 1.18 mmol) in DCM (10 mL) a solution of 1,3-diaminopropane (40 mg, 0.54 mmol) in DCM (2 mL) was added. The mixture was stirred for 2 h then concentrated and chromatographed on silica gel with hexane-acetone (2:1-1:1) to give dimer 18 (670 mg, 83%), [α]$_D$ +18.0° (c 1.2, CHCl$_3$), $^1$H-NMR (CDCl$_3$): δ 6.23 (d, 2H, $J_{1,NH}$ 9.2 Hz, NHAc), 5.77 (t, 2H, $J_{CH2,NH}$ 5.9 Hz, NH), 5.29 (dd, 2H, $J_{3',4'}$ 3.5 Hz, $J_{4',5'}$ 0.8 Hz, H-4'), 5.27 (m, 2H, H-3), 5.22 (t, $J_{1,2}$ 9.4 Hz, H-1), 4.82 (dd, 2H, $J_{2',3'}$ 10.1 Hz, H-3'), 4.81 (t, 2H, H-2), 4.48 (d, 2H, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.32 (broad d, 2H, H-6b), 4.26 (d, 2H, $J_{1',2'}$ 7.7 Hz, H-1'), 4.13-4.10 (m, 2H, CH$_2$O), 4.08 (dd, 2H, $J_{5',6'6''}$ 6.2 Hz, $J_{6',a,6'b}$ 11.1 Hz, H-6'a), 4.02 (dd, 2H, $J_{5',6'b}$ 7.3 Hz, H-6'b), 4.04-3.98 (m, 2H, CH$_2$O), 3.82 (ddd, 2H, H-5'), 3.79-3.74 (m, 4H, H-4, H-5), 3.70 (m, 2H, H-5), 3.35 (dd, 2H, H-2'), 3.20 (m, 4H, CH$_2$N), 2.10, 2.09, 2.04, 2.03, 2.02, 1.98, 1.97 (7s, 42 H, Ac), 1.69 (m, 2H, CH$_2$).
Bis-([1-acetamido-1-deoxy-β-D-lactose-2’-yloxy]ethyl)oxycarbamoyl-propane (19).

To a solution of 18 (500 mg, 0.336 mmol) in dry MeOH (10 mL) a solution of NaOMe (1 M, 1 mL) was added. After stirring for 16 h at room temperature the mixture was neutralized by Dowex 50W-X12 (H⁻-form). The resin was filtered out, the supernatant concentrated and dried to give deprotected dimer 19 (315 mg, 93%), \([\alpha]\)D −7.8° (c 0.65, water), \(^1\)H-NMR (D₂O): \(\delta\) 4.97 (d, 2 H, \(J_{1,2}\) 9.2 Hz, H-1), 4.50 (d, 2 H, \(J_{1',2'}\) 7.9 Hz, H-1’), 4.22 (broad t, 4H, \(\frac{3}{3} J_{3,4} 4.4\) Hz, CH₂O), 4.07-4.02 (m, 2 H, CH₂O), 3.96-3.91 (m, 6H, H-4’, H-5’, CH₂O), 3.82-3.77 (m, 4 H, H-6a, H-3’), 3.74 (dd, 2H, \(J_{5,6a}\) 3.9 Hz, \(J_{6a,6b}\) 11.9 Hz, H-6b), 3.72-3.67 (m, 8H, H-4, H-6’a, H-6’b), 3.64 (ddd, 2H, J 2.0 Hz, J 4.7 Hz, J 6.7 Hz, H-5), 3.45-3.40 (m, 4H, H-2, H-2’), 3.18 (t, 4H, \(\frac{3}{3} J_{6.8} 6.8\) Hz, CH₂N), 2.08 (s, 6H, NAc), 1.72 (m, 2H, CH₂).

Bis-([1-acetamido-1-deoxy-4-O-[4-O-(α-D-galactopyranosyl)-β-D-galactopyranosyl]-β-D-glucopyranose)-2’-yloxy]ethyl)oxycarbamoyl-propane (4).

To a solution of 19 (60 mg, 0.061 mmol) in water (3.28 mL) HEPES buffer (0.98 mL, 1 M, 10 mM MnCl₂, 0.8 mg/mL BSA, pH 8), DTT solution (0.22 mL, 100 mM) and alkaline phosphatase (30 µL) UDP-Glc (100 mg, 0.164 mmol) was added followed by the enzyme α(1-4)-GalT/UDP-4’-epimerase (Kitov et al. 2003) (0.436 mL). The reaction was incubated at 37°C for 2 days then chromatographed using HPLC (C-18) (0-50% MeOH). The product 4 (73 mg, 91%) was collected, concentrated and lyophilized from water. \(^1\)H-NMR indicated 100% conversion, \([\alpha]\)D +48.3° (c 0.35, water), \(^1\)H-NMR (D₂O): \(\delta\) 4.98 (d, 2 H, \(J_{1,2}\) 9.1 Hz, H-1), 4.97 (s, 2H, H-1’’), 4.55 (d, 2 H, \(J_{1',2'}\) 7.8 Hz, H-1’), 4.35 (t, 2 H, \(J_{5',6'}\)~\(J_{5',6b}\)~6.5 Hz, H-5’’), 4.22 (broad t, 4H, \(\frac{3}{3} J_{3.6} 3.6\) Hz, CH₂O), 4.09-4.04 (m, 2 H,
CH₂O), 4.05 (d, 2H, J₃',₄':3.2 Hz, H-4’), 4.04 (dd, 2H, J₃",₄":3.2 Hz, J₄",₅":0.9 Hz, H-4’’),
3.97-3.90 (m, 8H, H-6a, H-5’, H-3’’, CH₂O), 3.86-3.80 (m, 6 H, H-6b, H-6’a, H-2’’),
3.77-3.67 (m, 12H, H-3, H-4, H-3’, H-6’b, H-6’’a, H-6’’b), 3.64-3.62 (ddd, 2H, J 1.8 Hz,
J 4.4 Hz, J 6.5 Hz, H-5), 3.43 (m, 4H, H-2, H-2’), 3.17 (t, 4H, ³J 6.7 Hz, CH₂N), 2.08 (s,
6H, NAc), 1.70 (m, 2H, CH₂). HRMS-ES m/z 1327.47588, C₄₉H₈₄N₄O₃₆Na⁺ requires
1327.47575.

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