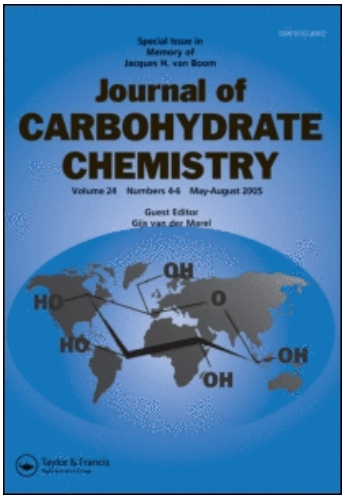

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SYNTHESIS OF β-D-(1→4)-SUBSTITUTED TREHALOSE OLIGOSACCHARIDES

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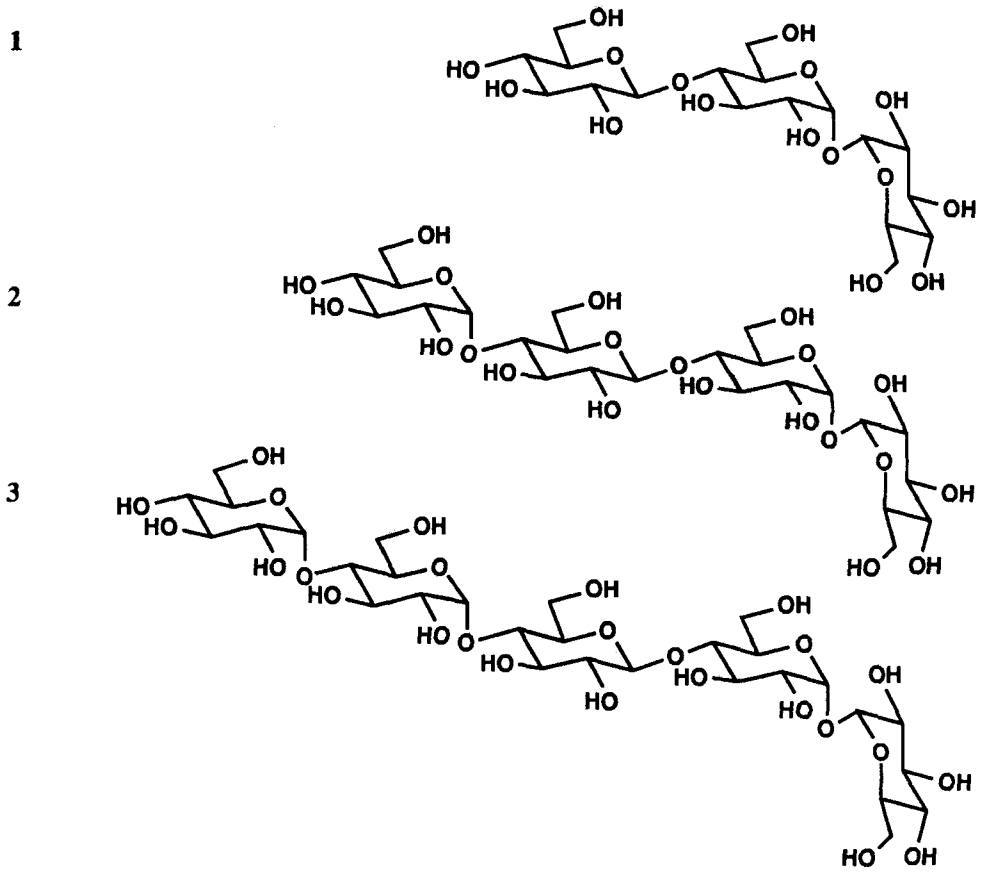
ABSTRACT

Glycosylation of 2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**5**) with α-D-glucopyranosyl, α-maltosyl, and α-maltotriosyl bromides **4**, **7**, and **8** afforded the β-D-(1→4)-substituted trehalose tri-, tetra-, and pentasaccharides **6**, **9**, and **10** which were fully characterized by ^{1}H NMR spectroscopy. Deprotection gave the free oligosaccharides **1**, **2**, and **3**.

INTRODUCTION

We have recently described the synthesis of α-D-(1→4)-substituted trehalose oligosaccharides[2,3] as substructures of Trestatin A.[4] These oligosaccharides, after sulfation, were tested for smooth muscle cell (SMC) antiproliferative activity, and it was demonstrated that a sulfated pentasaccharide is necessary to convey heparin-like antiproliferative activity.[5] The migration and proliferation of SMCs are important processes in the development of arteriosclerotic lesions,[6] and inhibition of these events are expected to lead to the treatment of arteriosclerotic disorders.

Surprisingly, the analogous β-D-(1→4)-substituted trehalose oligosaccharides were even more active.[5] Sulfated β-D-glucosyl-(1→4)-

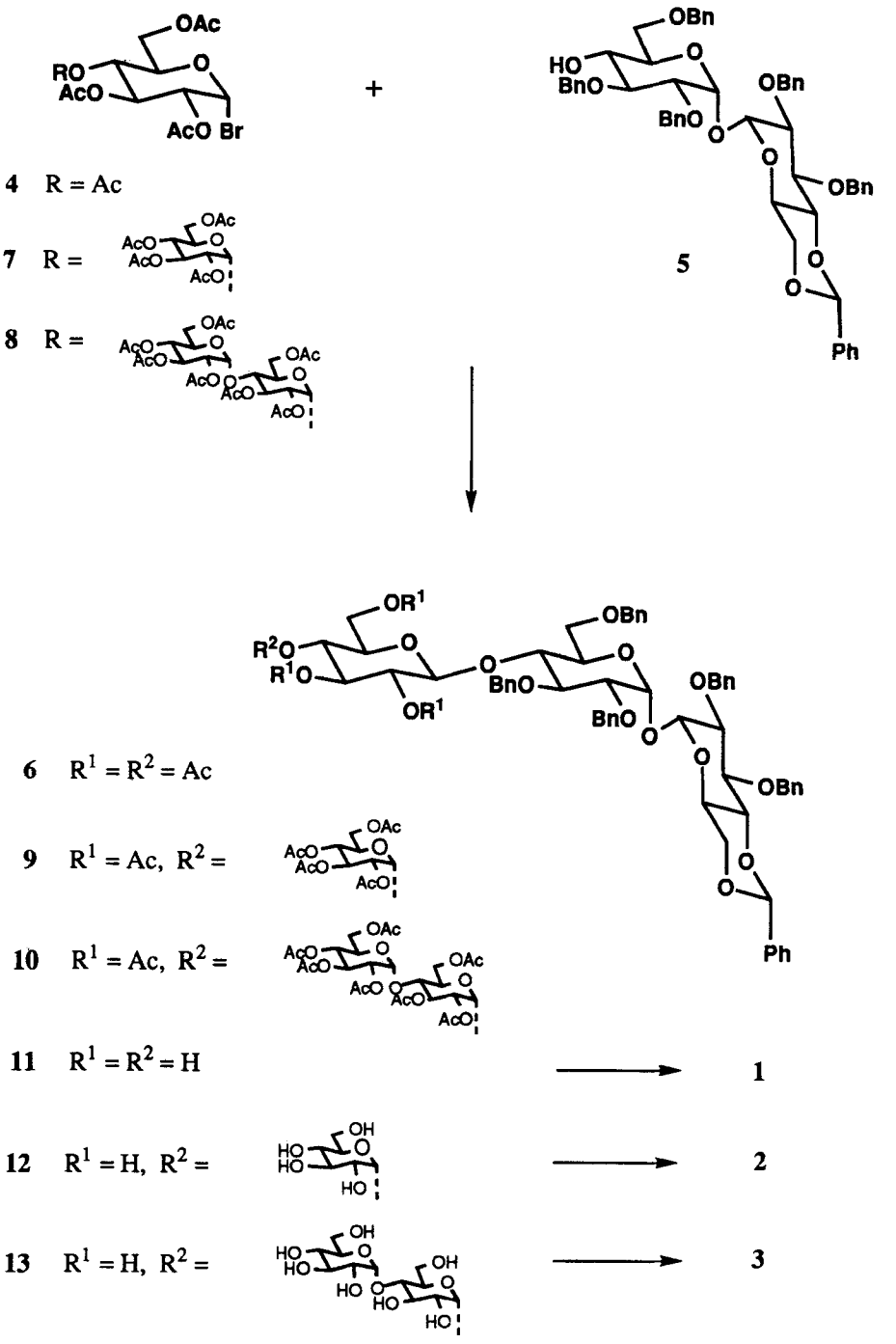


Scheme 1

trehalose (sulfated **1**) already displayed considerable antiproliferative activity, and sulfated β-maltosyl-(1→4)-trehalose (sulfated **2**) proved to be in the activity range of heparin. The sulfated β-maltotriosyl-(1→4)-trehalose (sulfated **3**) was only slightly more active than sulfated **2**. An enzymatic preparation of trisaccharide **1** had been reported.[7] In the following, the chemical synthesis of the β-D-(1→4)-substituted trehalose oligosaccharides is described in detail.

RESULTS AND DISCUSSION

Koenigs-Knorr glycosylation, with α-D-glucopyranosyl bromide (**4**) as glycosyl donor, of the established[2,3,8] trehalose glycosyl acceptor 2,3,6-tri-*O*-



Scheme 2

benzyl-α-D-glucopyranosyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**5**) using silver triflate[9]/tetramethylurea as catalyst/acid scavenger system, furnished the trisaccharide **6** in 64 % yield.[10] Since the glycosyl acceptor **5** is of low reactivity[2] a strong catalyst is required.[11] Thus, analogous glycosylations with the less reactive mercuric bromide/4 Å molecular sieves in dichloromethane or with mercuric cyanide in toluene/acetonitrile as solvent gave inferior yields of 37 % and 16 %, respectively.

In a block synthesis approach, Koenigs-Knorr glycosylation of **5** with hepta-*O*-acetyl-α-maltosyl bromide (**7**)[12] or deca-*O*-acetyl-α-maltotriosyl bromide (**8**)[13] under silver triflate catalysis gave the protected tetrasaccharide **9** and pentasaccharide **10** in yields of 90% and 52%, respectively. As expected, all glycosylation reactions proceeded stereoselectively due to the neighbouring group effect of the acetate in the 2-position of the glycosyl donor, and no trace of α-D-substituted oligosaccharide analogue was detected.

The structures of these oligosaccharides were confirmed by their ^{1}H NMR spectra, that were fully assigned using suitable NMR techniques such as 1D TOCSY, 1D or 2D ROESY and, in part, H,H-COSY. The β-D-nature of the newly formed glycosidic bonds was proven through their typical vicinal coupling constants, $J_{1'',2''}$ = 7.7 - 8.1 Hz. For trisaccharide **6**, a series of 1D TOCSY experiments with selective inversions of H-5, H-6b', and H-1'' gave the assignments of all ring protons, data for the benzylic methylene protons were taken from a 2D ROESY experiment. In the case of tetrasaccharide **9**, selective inversions of H-1'', H-5'', and H-1''' furnished the assignments in the maltosyl moiety of the molecule, whereas inversion of H-1/H-1' gave only partial information which was supplemented by 2D ROESY and H,H-COSY experiments. Selective inversions of H-1, H-1', H-5'', H-1''', and H-2'''' in 1D TOCSY experiments with **10** allowed assignments of all ring protons.

Transesterification of the fully protected oligosaccharides **6**, **9**, and **10** with sodium methoxide or carbonate furnished saccharides **11**, **12**, and **13** in excellent yields. The ^{1}H NMR assignments for **11** were obtained with the help of a COSY spectrum. In the ^{1}H NMR spectrum of **12** in chloroform even the combined application of 1D TOCSY and COSY did not lead to complete assignments due to accumulation of ca. 16 protons in the region of 3.7 - 3.4 ppm. In DMSO-d6, this part of the spectrum was slightly more spread, and assignments were made using 1D TOCSY and H,C-COSY. The spectra of pentasaccharide **13** were analyzed applying 1D TOCSY, 1D ROESY, H/H- COSY and H/C-COSY techniques.

The deprotection of **11**, **12**, and **13** was completed by concomitant hydrogenolysis of the benzyl and benzylidene groups to give the target oligosaccharides **1**, **2**, and **3**, also in near quantitative yields.

EXPERIMENTAL

General Procedures. Solvents and reagents were bought from Fluka. Evaporation: *in vacuo*, conducted with Büchi rotary evaporator. TLC: precoated silica gel 60F-254 plates (Merck), detection by UV light (254 nm) and spraying with a 10% solution of concentrated sulfuric acid in methanol followed by heating. Specific rotations: Perkin-Elmer Polarimeter 241, measured at 20 °C. ^{1}H NMR: Bruker AM-400 (400 MHz) with Aspect 3000; ARX-400 (400 MHz) with ASPECT 1 station and z-gradient accessory kit with 10 Amps power amplifier for pulsed field z-gradient (PFG) experiments; chemical shifts in ppm relative to tetramethylsilane or sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl)-propionate as internal reference. Standard Bruker pulse programs were applied except for the 1D TOCSY experiment with MLEV-17 mixing sequence, and the 1D ROESY with 'chopped' 90° spin-lock of typically 0.6 s duration and 8% duty cycle. Selective 180° excitation was achieved in both cases by application of a sequence of DANTE pulses (between 800 and 2400 times 2 μs duration with interpulse delay of 70 μs). All experimental details and the general procedure of assignment of the ^{1}H signals based on these experiments were as described previously.[2,3]

2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (6). To a soln of trehalose glycosyl acceptor **5** (3.02 g, 3.43 mmol) and silver triflate (2.61 g, 10.2 mmol) in abs dichloromethane (30 mL) and tetramethylurea (2.5 mL) was added dropwise a soln of α-D-acetobromoglucose (**4**, 4.20 g, 10.2 mmol) in abs dichloromethane (60 mL) at -30 °C. The reaction mixture was stirred at this temperature for 2 h, at rt for 20 h, and then filtered through a pad of filter aid. The filtrate and dichloromethane washings were combined and washed with aq sodium bicarbonate soln and twice with ice water. The organic phases were dried over magnesium sulfate and concd. The residue was chromatographed on silica gel using toluene / ether 6:1 as eluent to furnish **6** (2.66 g, 64 %) as a syrup: $[\alpha]_D$ +53.0 ° (*c* 0.2, dioxane); ^{1}H NMR ($CDCl_3$, 400 MHz; 1D TOCSY, 2D ROESY) δ 7.51 - 7.22 (m, 30H, aromat), 5.54 (s, 1H, CHPh), 5.128 and 5.126 (2 d, 2H, H-1' and H-1), 5.02 (dd ~ t, $J_{4'',5''}$ = 9.9 Hz,

H-4''), 5.02, 4.79 (2 d, 2H, J_{gem} = 11.5 Hz, CH_2Ph), 5.01, 4.92 (2 d, 2H, J_{gem} ≈ 11.5 Hz, CH_2Ph), 4.94 (dd ~ t, $J_{3'',4''}$ ≈ 9 Hz, H-3''), 4.89 (dd ~ t, $J_{2'',3''}$ ≈ 9 Hz, H-2''), 4.74, 4.68 (2 d, 2H, J_{gem} ≈ 12 Hz, CH_2Ph), 4.73, 4.40 (2 d, 2H, J_{gem} = 12.0 Hz, CH_2Ph), 4.70, 4.64 (2 d, 2H, J_{gem} ≈ 12 Hz, CH_2Ph), 4.53 (d, $J_{1'',2''}$ = 7.7 Hz, H-1''), 4.27 (ddd ~ dt, H-5), 4.16 (dd ~ t, $J_{3,4}$ ≈ 9.6 Hz, H-3), 4.14 (dd, $J_{6a'',6b''}$ = 11.6 Hz, H-6a''), 4.09 (dd, $J_{5,6a}$ = 4.8 Hz, $J_{6a,6b}$ = 10.2 Hz, H-6a), 4.03 (ddd ~ m, H-5'), 3.93-3.89 (m, 2H, H-3', H-4'), 3.86 (dd, $J_{5'',6b''}$ = 2.4 Hz, H-6b''), 3.64 (dd ~ t, $J_{5,6b}$ ≈ 10.3 Hz, H-6b), 3.62 (2H, dd ~ t, $J_{4,5}$ ≈ 9.4 Hz, H-4 and dd, $J_{5',6a'}$ = 3 Hz, $J_{6a',6b'}$ = 11.0 Hz, H-6a'), 4.58 (dd, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 9.3, H-2), 3.53 (m, 1H, not first order, H-2'), 3.44 (dd, $J_{5',6b'}$ = 1.8 Hz, H-6b'), 3.28 (ddd, $J_{5'',6a''}$ = 4.0 Hz, H-5''), 2.00, 1.97, 1.94, 1.71 (4 s, 12H, OAc).

Anal. Calcd for $C_{68}H_{74}O_{20}$: C, 67.43; H, 6.16. Found: C, 67.22; H, 6.22.

2,3,4,6- Tetra -*O* -acetyl-α-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (9). To a soln of trehalose glycosyl acceptor **5** (10.00 g, 11.35 mmol) and silver triflate (4.37 g, 17.0 mmol) in abs dichloromethane (60 mL) and tetramethylurea (2.1 mL, 18.2 mmol) was added dropwise a soln of α-maltosyl bromide[12] (**7**, 4.20 g, 10.2 mmol) in abs dichloromethane (50 mL) at 0 °C. After stirring at rt for 8 h silver triflate (1.09 g, 4.25 mmol), tetramethylurea (0.53 mL, 4.54 mmol), and bromide **7** (2.98 g, 4.25 mmol) were added, and stirring was continued for 18 h. The reaction mixture was worked up as described for **6**. Chromatography on silica gel using ethyl acetate / hexane 1:1 as eluent furnished pure **9** as a colourless syrup (15.41 g, 90.5%); $[\alpha]_D$ +84.0 ° (*c* 0.2, dioxane); ^1H NMR ($CDCl_3$, 400 MHz; 1D TOCSY, 2D ROESY, H,H-COSY) δ 5.54 (s, 1H, CHPh), 5.38 (dd, 1H, $J_{3''',4'''}$ = 9.8 Hz, H-3'''), 5.32 (d, 1H, $J_{1''',2'''}$ = 3.9 Hz, H-1'''), 5.13 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1), 5.12 (d, 1H, $J_{1',2'}$ = 4.1 Hz, H-1'), 5.06 (dd ~ t, 1H, $J_{4''',5'''}$ = 10.4 Hz, H-4'''), 5.01 (dd ~ t, 1H, $J_{3'',4''}$ ≈ 9.5 Hz, H-3''), 5.01, 4.73 (2 d, 2H, CH_2Ph), 5.00, 4.90 (2 d, 2H, J_{gem} = 11.2 Hz, CH_2Ph), 4.86 (dd, 1H, $J_{2''',3'''}$ = 10.7 Hz, H-2'''), 4.76, 4.39 (2 d, 2H, J_{gem} = 12.0 Hz, CH_2Ph), 4.73, 4.67 (2 d, 2H, CH_2Ph), 4.72 (dd ~ t, 1H, H-2''), 4.67, 4.61 (2 d, 2H, J_{gem} = 12.0 Hz, CH_2Ph), 4.48 (d, 1H, $J_{1'',2''}$ = 8.1 Hz, H-1''), 4.26 (ddd, 1H, $J_{5,6a}$ = 4.8 Hz, $J_{5,6b}$ = 10.2 Hz, H-5), 4.21 (dd, 1H, $J_{5''',6a'''}$ = 3.5 Hz, $J_{6a''',6b'''}$ = 12.6 Hz, H-6a'''), 4.11 (dd, 1H, H-6a''), 4.08 (dd, 1H, H-6a), 4.05 (dd, 1H, $J_{5'',6b''}$ = 4 Hz, $J_{6a'',6b''}$ = 12 Hz, H-6b''), 4.01 (ddd ~ br d, 1H, H-5'), 3.97 (dd, 1H, $J_{5''',6b'''}$ = 2.1 Hz, H-6b'''), ~3.89, ~3.85 (2 dd ~ t, 2H, H-3', H-4'), 3.87 (dd ~ t and ~ddd, 2H, H-4'', H-5'''), 3.64 (dd ~ t, 1H, $J_{6a,6b}$ ≈ 10.5 Hz, H-6b), 3.63 (dd, 1H, $J_{5',6a'}$ ≈ 3 Hz, H-6a'), 3.62 (dd ~ t, 1H, $J_{4,5}$ = 10.0 Hz, H-4), 3.57 (dd, 1H, $J_{2,3}$ =

9.4 Hz, H-2), 3.52 (dd, 1H, $J_{2',3'}$ = 9.3 Hz, H-2'), 3.47 (dd, 1H, J5',6b' ≈ 1.2 Hz, $J_{6a',6b'}$ ≈ 10.5 Hz, H-6b'), 3.13 (ddd, 1H, $J_{4'',5''}$ = 9.8 Hz, $J_{5'',6a''}$ = 2.5 Hz, H-5''), 2.10, 2.07, 2.05, 2.04, 2.02, 1.96, 1.92 (7 s, 21H, OAc).

Anal. Calcd for $C_{80}H_{90}O_{28}$: C, 64.08; H, 6.05. Found: C, 63.82; H, 6.05.

2,3,4,6-Tetra-*O*- acetyl-α-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*- acetyl -α-D-glucopyranosyl-(1→ 4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (10). To a soln of trehalose glycosyl acceptor **5** (4.00 g, 4.5 mmol) and silver triflate (1.74 g, 6.8 mmol) in abs dichloromethane (35 mL) and tetramethylurea (3.1 mL, 26.2 mmol) was added dropwise a soln of α-maltotriosyl bromide[13] (**8**, 6.67 g, 6.75 mmol) in abs dichloromethane (40 mL) at -30 °C. After stirring at that temperature for 1 h and at rt for 3 h, 4Å molecular sieves (ca. 1 g) and bromide **8** (3.4 g, 3.4 mmol) were added, and stirring was continued for 48 h. The reaction mixture was worked up as described for **6**. Chromatography on silica gel using ethyl acetate/hexane 3:2 as eluent furnished pure **10** as a colourless syrup (4.25 g, 52%); $[\alpha]_D$ +100.5 ° (*c* 0.2, dioxane); ^{1}H NMR ($CDCl_3$, 400 MHz; 1D TOCSY, 1D ROESY) δ 7.50 - 7.23 (m, 30H, aromat), 5.54 (s, 1H, CHPh), 5.45 (dd, 1H, $J_{3''',4'''}$ = 8.1 Hz, H-3'''), 5.43 (d, 1H, $J_{1'''',2''''}$ = 4.0 Hz, H-1''''), 5.38 (dd, 1H, $J_{3'''',4''''}$ = 9.5 Hz, H-3''''), 5.18 (d, 1H, $J_{1''',2'''}$ = 4.0 Hz, H-1'''), 5.13 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1), 5.12 (d, 1H, H-1'), 5.09 (dd ~ t, 1H, $J_{4'''',5''''}$ = 10.5 Hz, H-4''''), 5.00, 4.90 (2 d, 2H, J_{gem} = 10.5 Hz, CH_2Ph), 5.00, 4.72 (2 d, 2H, J_{gem} = 11.8 Hz, CH_2Ph), 4.97 (dd ~ t, 1H, $J_{3'',4''}$ = 8.7 Hz, H-3''), 4.88 (dd ~ t, 1H, $J_{2'''',3''''}$ = 10.5 Hz, H-2''''), 4.77, 4.37 (2 d, 2H, J_{gem} = 12.0 Hz, CH_2Ph), 4.73, 4.68 (2 d, 2H, J_{gem} ≈ 12.5 Hz, CH_2Ph), 4.74 (dd ~ t, 1H, $J_{2''',3'''}$ = 10.5 Hz, H-2'''), 4.69 (dd ~ t, 1H, $J_{2'',3''}$ = 9.5 Hz, H-2''), 4.68, 4.62 (2 d, 2H, J_{gem} = 12.5 Hz, CH_2Ph), 4.44 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1''), 4.43 (dd, 1H, $J_{5''',6a'''}$ = 2.0 Hz, $J_{6a''',6b'''}$ = 12.8 Hz, H-6a'''), 4.26 (ddd ~ dt and dd, 2H, $J_{4,5}$ = 10.0 Hz, H-5, $J_{5'''',6a''''}$ = 3.0 Hz, $J_{6a'''',6b''''}$ = 12.5 Hz, H-6a''''), 4.15 (dd ~ t, 1H, H-3), 4.13 (2 dd, 2H, $J_{5'',6a''}$ = 3 Hz, H-6a'', $J_{5''',6b'''}$ ≤ 3.0 Hz, H-6b'''), 4.08 (2 dd, 2H, $J_{5,6a}$ = 5.0 Hz, $J_{6a,6b}$ = 10,0 Hz, H-6a, $J_{5'',6b''}$ = 4 Hz, H-6b''), 4.06 (dd, 1H, $J_{5'''',6b''''}$ = 2.2 Hz, H-6b''''), 4.00 (ddd ~ dt, 1H, $J_{4',5'}$ = 9.5 Hz, H-5'), 3.95 (dd ~ t, 1H, H-4'''), 3.93 (ddd ~ dt, 1H, H-5''''), 3.91 (~ddd, 1H, H-5'''), 3.90 (dd ~ t, 1H, $J_{3,4}$ = 8.5 Hz, H-4'), 3.85 (dd ~ t, 1H, H-3'), 3.84 (dd ~ t, 1H, H-4''), 3.65 (dd, 1H, $J_{5',6a'}$ ≈ 2 Hz, H-6a'), 3.64 (dd ~ t, 1H, H-4), 3.62 (dd ~ t, 1H, $J_{5,6b}$ = 10.0 Hz, H-6b), 3.58 (dd, 1H, $J_{2,3}$ = 9.8 Hz, H-2), 3.54 (dd, 1H, $J_{1',2'}$ = 3.8 Hz, $J_{2',3'}$ = 9.2 Hz, H-2'), 3.47 (dd, 1H, $J_{5',6b'}$ = 1.5 Hz, $J_{6a',6b'}$ = 10,8 Hz, H-6b'), 3.15 (ddd ~ dt, 1H, $J_{4'',5''}$ = 9.8 Hz, H-5''), 2.15, 2.13, 2.10, 2.074, 2.070 , 2.05 , 2.02, 2.01, 1.99, 1.71 (10 s, 30H, OAc).

Anal. Calcd for $C_{92}H_{106}O_{36}$: C, 61.81; H, 5.98. Found: C, 62.10; H, 6.04.

β-D-Glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (11). A soln of trisaccharide **6** (2.63 g, 2.17 mmol) in methanol (100 mL) and cyclohexane (30 mL) was treated with a 2% soln of sodium methoxide in methanol for 1.5 h at rt. The soln was neutralized by addition of Amberlite IR 120 (H^+). The resin was filtered off, and the filtrate was concentrated. The residue was filtered over silica gel using ethyl acetate/methanol/water 95:1:1 as eluent to obtain pure **11** (2.26 g, 100%) as colourless syrup; $[\alpha]_D$ +90.1 ° (*c* 0.2, dioxane); ^1H NMR ($CDCl_3$, 400 MHz; 1D TOCSY, H,H-COSY) δ 7.51 - 7.49 (m, 2H, aromat), 7.41 - 7.25 (m, 28H, aromat), 5.56 (s, 1H, CHPh), 5.133 (d, 1H, $J_{1',2'}$ = 3.7 Hz, H-1'), 5.124 (d, 1H, H-1), 4.99, 4.87 (2 d, 2H, J_{gem} = 11.1 Hz, CH_2Ph), 4.95, 4.90 (2 d, 2H, J_{gem} = 11.2 Hz, CH_2Ph), 4.81, 4.65 (2 d, 2H, J_{gem} = 11.9 Hz, CH_2Ph), 4.72, 4.69 (2 d, 2H, J_{gem} = 11.9 Hz, CH_2Ph), 4.59, 4.43 (2 d, 2H, J_{gem}=12.0 Hz, CH_2Ph), 4.42 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1''), 4.26 (ddd ~ dt, 1H, $J_{4,5}$ ≈ 10 Hz, $J_{5,6a}$ = 4.9 Hz, H-5), 4.15 (dd ~ t, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 4.13 (ddd ~ dt, 1H, H-5'), 4.12 (dd, 1H, $J_{6a,6b}$ = 10.4 Hz, H-6a), 4.00 (dd ~ t, 1H, $J_{3',4'}$ = 9.0 Hz, H-3'), 3.94 (dd ~ t, 1H, $J_{4',5'}$ = 9.7 Hz, H-4'), 3.67 (dd ~ t, 1H, $J_{5,6b}$ ≈ 10 Hz, H-6b), 3.65 (dd ~ t, 1H, H-4), 3.64 (dd, 1H, $J_{5',6a'}$ ≈ 3 Hz, H-6a'), 3.61 (dd, 1H, $J_{1,2}$ = 3.8 Hz, $J_{2,3}$ = 9.5 Hz, H-2), 3.60 (ddd, 1H, $J_{5'',6a''}$ ≈ 3 Hz, H-6a''), 3.56 (dd, 1H, $J_{2',3'}$ = 9.3 Hz, H-2'), 3.44 (ddd ~ dt, 1H, $J_{3'',4''}$ ≈ 9 Hz, H-4''), 3.42 (ddd, 1H, H-6b''), 3.35 (dd, 1H, $J_{5',6b'}$ = 1.5 Hz, H-6b'), 3.31 (ddd ~ dt, 1H, H-3''), 3.22 (dd ~ t, 1H, H-2''), 3.20 (d, 1H, $J_{2'',2''\text{-OH}}$ = 2.4 Hz, 2''-OH), 3.01 (ddd ~ dt, 1H, $J_{4'',5''}$ ≈ 9 Hz, $J_{5'',6b''}$ = 5.5 Hz, H-5''), 2.93 (d, 1H, $J_{3'',3''\text{-OH}}$ = 1.8 Hz, 3''-OH), 2.59 (d, 1H, $J_{4'',4''\text{-OH}}$ = 3.0 Hz, 4''-OH), 1.81 (dd ~ t, 1H, $J_{6a'',6''\text{-OH}} \approx J_{6b'',6''\text{-OH}}$= 6.5 Hz, 6''-OH).

Anal. Calcd for $C_{60}H_{66}O_{16}$: C, 69.08; H, 6.28. Found: C, 68.86; H, 6.34.

α-D-Glucopyranosyl-(1 →4)-β-D-glucopyranosyl-(1 →4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (12). A soln of tetrasaccharide **9** (14.3 g, 9.54 mmol) in methanol (150 mL) and cyclohexane (40 mL) was treated with a 2% soln of sodium methoxide in methanol (60 mL) for 30 min at rt. The soln was neutralized by addition of Amberlite IR 120 (H^+). The resin was filtered off, and the filtrate was concentrated. The residue was filtered over silica gel using ethyl acetate/methanol/water 95:1:1 as eluent to obtain pure **12** (11.35 g, 99%) as colourless syrup; $[\alpha]_D$ +108.4 ° (*c* 0.2, dioxane); ^1H NMR ($CDCl_3$, 400 MHz; 1D TOCSY, H,H-COSY) δ 7.49 - 7.47 (m, 2H, aromat), 7.38 - 7.18 (m, 28H, aromat), 5.53 (s, 1H, CHPh), 5.36 (br s, 1H, H-1'''), 5.22 (br s, 1H, OH), 5.11 (d, 1H, $J_{1',2'}$ =

3.6 Hz, H-1'), 5.07 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1), 5.03 (d ~ br s, 1H, H-1'''), 4.92, 4.81 (2 d, 2H, J_{gem} = 11.0 Hz, CH_2Ph), 4.90, 4.87 (2 d, 2H, J_{gem} = 11.6 Hz, CH_2Ph), 4.73, 4.59 (2 d, 2H, J_{gem} = 12.0 Hz, CH_2Ph), 4.67, 4.65 (2 d, 2H, J_{gem} = 12.0 Hz, CH_2Ph), 4.53, 4.44 (2 d, 2H, J_{gem} = 12.2 Hz, CH_2Ph), 4.37 (d, 1H, $J_{1'',2''}$ = 7.6 Hz, H-1''), 4.23 (ddd ~ dt, 1H, $J_{5,6a}$ = 5.0 Hz, $J_{5,6b}$ = 10.0 Hz, H-5), 4.12 (~ ddd, 1H, H-5'), 4.11 (dd ~ t, 1H, $J_{3,4}$ ≈ 8.5 Hz, H-3), 4.08 (dd, 1H, $J_{6a,6b}$ ≈ 10 Hz, H-6a), 3.95 (dd ~ t, 1H, ΣJ = 19.0 Hz, H-3'), 3.90 (dd ~ t, 1H, ΣJ = 18.0 Hz, H-4'), ~3.69 (~dd, H-6a'), 3.68 (dd ~ t, 1H, H-4'''), ~3.64 (H-6b), 3.61 (dd ~ t, 1H, $J_{4,5}$ = 10.0 Hz, H-3), 3.55 (dd, 1H, $J_{2,3}$ ≈ 9 Hz, H-2), 3.54 (~dd, 2H, H-2', H-2'''), 3.48 (br dd ~ t, 1H, H-3''), 3.47 (ddd ~ dt, 1H, H-3'''), 3.39 (dd ~ br d, 1H, $J_{6a',6b'}$ = 10.8 Hz, H-6b'), 3.26 (dd ~ t, 1H, $J_{2'',3''}$ = 8.2 Hz, H-2''), 2.95 (d, 1H, $J_{3''',3'''\text{-OH}}$ ≈ 7 Hz, 3'''-OH); ^1H NMR (DMSO-d_6, 400 MHz; 1D TOCSY, H,C-COSY) δ 7.46 - 7.24 (m, 30H, aromat), 5.67 (s, 1H, CHPh), 5.23, 5.24 (2 d, 2H, H-1, H-1'), 5.02 (d, 1H, $J_{1''',2'''}$ = 3.8 Hz, H-1'''), 5.00, 4.68 (2 d, 2H, J_{gem} = 11.2 Hz, C-3'-CH_2Ph), 4.80, 4.75 (2 d, 2H, J_{gem} = 11.8 Hz, C-3-CH_2Ph), 4.69 (s, 2H, C-2-CH_2Ph), 4.68, 4.63 (2 d, 2H, J_{gem} = 11.8 Hz, C-2'-CH_2Ph), 4.48 (s, 2H, C-6'-CH_2Ph), 4.38 (d, 1H, $J_{1'',2''}$ = 7.8 Hz, H-1''), 4.07 (m, 2H, H-5, H-5'), 4.05 (br d, 1H, H-6a'), 3.90 (dd, 1H, $J_{5,6a}$ ≈ 2 Hz, $J_{6a,6b}$ ≈ 10 Hz, H-6a), 3.89 (2 dd ~ t, 2H, H-3, H-4'), 3.80 (dd ~ t, 1H, ΣJ = 18.6 Hz, H-3'), 3.72 (2 dd ~ t, 2H, H-4, H-6b), 3.65 (dd, 1H, H-6a''), 3.63 (dd, 1H, H-6a'''), 3.55 (dd, 1H, H-2), 3.64 (br d, H-6b'), 3.54 (dd, 1H, H-2'), 3.53 (br d, 1H, H-6b'), 3.48 (ddd ~ dt, 1H, H-5'''), 3.53 (dd, 1H, H-6b'''), 3.38 (dd ~ t, 1H, $J_{3''',4'''}$ = 9.6 Hz, H-3'''), 3.40 - 3.33 (m, 2H, H-3'', H-4''), 3.26 (dd, 1H, $J_{2''',3'''}$ = 9.6 Hz, H-2'''), 3.07 (dd, 1H, H-2''), 3.06 (dd ~ t, 1H, H-4'''), 3.03 (ddd ~ br d, 1H, H-5''); ^{13}C NMR (DMSO-d_6, 100 MHz; H,C-COSY) δ 139.36, 138.94, 138.37, 138.29, 138.09, 137.68 (6C, quaternary aromatic C), 102.64 (C-1''), 100.83 (C-1'''), 100.23 (benzylidene C), 93.83, 93.01 (C-1, C-1'), 81.11 (C-4), 79.64 (C-3'''), 79.54 (C-3'), 78.75 (C-2'), 78.42 (C-2), 78.00 (C-3), 76.42 (C-4''), 76.08 (C-4'), 75.54 (C-5''), 74.04, 73.95 (2C, CH_2Ph), 73.47 (C-5'''), 73.38 (C-3''), 73.21 (C-2'''), 72.57 (2-CH_2Ph), 72.34 (CH_2Ph), 72.25 (2'-CH_2Ph), 70.46 (C-5'), 69.84 (C-4'''), 68.11 (C-6), 68.01 (C-6'), 62.70 (C-5), 60.74 (C-6''), 60.51 (C-6''').

Anal. Calcd for $C_{66}H_{76}O_{21}$: C, 65.77; H, 6.36. Found: C, 65.44; H, 6.47.

α-D-Glucopyranosyl-(1 →4)-α-D-glucopyranosyl-(1 →4)-β-D-glucopyranosyl-(1→4)-2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl 2,3-Di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (13). A soln of pentasaccharide **10** (3.46 g, 1.94 mmol) in methanol (45 mL) was stirred in the presence of anhydrous sodium carbonate for 24 h at rt. The reaction mixture was filtered over a pad of filter aid. The filtrate was neutralized by addition of Amberlite IR 120 (H^+).

The resin was filtered off, and the filtrate was concentrated. The residue was filtered over silica gel using ethyl acetate/methanol/water 80:15:5 as eluent to obtain pure **1** (2.29 g, 86%) as colourless syrup; $[\alpha]_D$ +120.5 ° (*c* 0.2, dioxane); ^{1}H NMR (DMSO-d_6, 400 MHz; 1D TOCSY, 1D ROESY, H,H-COSY, H,C-COSY) δ 7.44 - 7.24 (m, 30H, aromat), 5.67 (s, 1H, CHPh), 5.52 (d, 1H, $J_{3'',3''\text{-OH}} \approx 3.5$ Hz, H-3''-OH), 5.51 (d, 1H, 2'''-OH), 5.47 (2 d, 2H, 2''''-OH; $J_{3''',3'''\text{-OH}} \approx 3.5$ Hz, 3'''-OH), 5.32 (d, 1H, $J_{2'',2''\text{-OH}} = 5.2$ Hz, H-2''-OH), (5.23 (br d, 2H, H-1, H-1'), 5.03 (d, 1H, $J_{1''',2'''} = 3.8$ Hz, H-1'''), 5.00 (d, 1H, H-1''''), 4.99, 4.68 (2 d, 2H, $J_{gem} = 11.0$ Hz, C-3'-CH_2Ph), 4.90 (d, 1H, H-4''''-OH), 4.88 (d, 1H, H-3''''-OH), 4.79, 4.74 (2 d, 2H, $J_{gem} = 11.8$ Hz, C-3-CH_2Ph), 4.68 (s, 2H, C-2-CH_2Ph), 4.67, 4.63 (2 d, 2H, $J_{gem} = 11.5$ Hz, C-2'-CH_2Ph), 4.51 (dd ~ t, 1H, $J_{6''',6'''\text{-OH}} = 5.6$ Hz, H-6'''-OH), 4.54 (dd ~ t, 1H, $J_{6'''',6''''\text{-OH}} = 5.5$ Hz, H-6''''-OH), 4.48 (s, 2H, C-6'-CH_2Ph), 4.38 (d, 1H, $J_{1'',2''} = 7.8$ Hz, H-1''), 4.22 (dd ~ t, 1H, $J_{6'',6''\text{-OH}} = 5.4$ Hz, H-6''-OH), 4.09 (ddd ~ dd, 1H, H-5'), 4.08 (ddd, 1H, H-5), 4.07 (~dd, 1H, H-6a'), 3.90 (ddd ~ dt, 1H, H-6a), 3.88 (2 dd ~ t, 2H, $J_{3,4} \approx 8.8$ Hz, H-3, H-4'), 3.80 (ddd ~ dt, 1H, $J_{3',4'} = 8.2$ Hz, H-3'), 3.73 (ddd ~ dt, 1H, H-6b), 3.72 (dd ~ t, 1H, H-4), ~3.65 (1H, H-6a''''), 3.64 (ddd ~ dt, 1H, H-3'''), ~3.62 (2H, H-6a'', H-6a'''), 3.61 (dd, 1H, $J_{1,2} = 3.8$ Hz, $J_{2,3} \approx 9.8$ Hz, H-2), 3.60 (1H, H-6b''''), ~3.58 (~br d, 1H, H-5''''), 3.56 (dd ~ t, 1H, H-4'''), 3.54 (dd, 1H, $J_{1',2'} = 3.7$ Hz, $J_{2,3} = 9.8$ Hz, H-2'), 3.53 (dd, 1H, H-6b'), ~3.52 (1H, H-5'''), ~3.51 (1H, H-6b''), ~3.50 (1H, H-6b'''), 3.40 (ddd, 1H, $J_{3'''',4''''} \approx 9.5$ Hz, $J_{3'''',3''''\text{-OH}} = 5.0$ Hz, H-3''''), 3.37 (~dt, 1H, H-3''), 3.36 (dd ~ t, 1H, H-4''), 3.30 (ddd, 1H, $J_{2''',3'''} = 10.0$ Hz, $J_{2''',2'''\text{-OH}} = 6.2$ Hz, H-2'''), 3.24 (ddd, 1H, $J_{1'''',2''''} = 3.9$ Hz, $J_{2'''',3''''} = 10.0$ Hz, $J_{2'''',2''''\text{-OH}} = 6.2$ Hz, H-2''''), 3.08 (ddd ~ dt, 1H, $J_{4'''',4''''\text{-OH}} \approx 5.7$ Hz, H-4''''), ~3.06 (ddd ~ br q, 1H, H-2''), ~3.03 (1H, H-5''); ^{13}C NMR (DMSO-d_6, 100 MHz; H,C-COSY) δ 139.21, 138.78, 138.21, 138.13, 137.93, 137.53 (6C, quaternary aromatic C), 102.45 (C-1''), 100.80 (C-1'''), 100.45 (C-1''''), 100.08 (benzylidene C), 93.68, 92.86 (C-1, C-1'), 80.96 (C-4), 79.59 (C-3''''), 79.48 (C-3'), 78.59 (C-2'), 78.28 (C-2), 77.85 (C-3), 76.36 (C-4''), 75.96 (C-4'), 75.39 (C-5''), 73.88 (C-3'-CH_2Ph), 73.81 (C-3-CH_2Ph), 73.42 (C-2''''), 73.33 (C-2''), 73.23 (C-5'''), 73.10 (C-3''), 72.42 (C3'''), 72.43 (CH_2Ph), 72.18 (CH_2Ph), 72.11 (CH_2Ph), 71.90 (C-2'''), 71.61 (C-5''''), 70.29 (C-4''''), 69.82 (C-5'), 67.97 (C-6), 67.85 (C-6'), 62.55 (C-5), 60.72 (C-6'''), 60.45 (C-6''), 60.17 (C-6'''').

Anal. Calcd for $C_{72}H_{86}O_{26}$: C, 63.24; H, 6.34. Found: C, 62.91; H, 6.40.

β-D-Glucopyranosyl-(1→4)-α-D-glucopyranosyl α-D-Glucopyranoside (1). A soln of **11** (2.3 g, 2.2 mmol) in ethanol (120 mL) and water (30 mL) was reacted with hydrogen in the presence of 10% palladium-on-carbon for 2 h at

rt. The catalyst was removed by filtration over a pad of filter aid and filtration over a thin pad of silica gel. Concentration of the filtrate and washings furnished pure **14** (1.08 g, 97%) as colourless syrup; $[\alpha]_D$ +121.9 ° (*c* 0.2, water); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 4.87, 4.85 (2 d, 2H, J = 3.4 Hz, J = 3.3 Hz, H-1, H-1'), 4.24 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1'').

Anal. Calcd for $C_{18}H_{32}O_{16}$: C, 42.86; H, 6.39. Found: C, 42.54; H, 6.47.

α-D-Glucopyranosyl-(1 → 4)-β-D-glucopyranosyl -(1 → 4)-α-D-glucopyranosyl α-D-Glucopyranoside (2). A soln of **12** (2.64 g, 2.19 mmol) in ethanol (120 mL) and water (40 mL) was reacted with hydrogen in the presence of 10% palladium-on-carbon for 2 h at rt. The catalyst was removed by filtration over a pad of filter aid and filtration over a thin pad of silica gel. Concentration of the filtrate and washings furnished pure **2** (1.46 g, 100%) as colourless syrup; $[\alpha]_D$ +150.0 ° (*c* 0.2, water); ^{1}H NMR (DMSO-d_6, 400 MHz) δ 5.02 (d, 1H, $J_{1''',2'''}$ = 3.7 Hz, H-1'''), 4.87, 4.85 (2 d, 2H, both J = 3.5 Hz, H-1, H-1'), 4.30 (d, 1H, $J_{1'',2''}$ = 7.8 Hz, H-1'').

Anal. Calcd for $C_{24}H_{42}O_{21}$: C, 43.25; H, 6.35. Found: C, 43.28; H, 6.41.

α-D-Glucopyranosyl-(1 → 4)-α-D-glucopyranosyl-(1 → 4)-β-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl α-D-Glucopyranoside (3). A soln of **13** (2.00 g, 2.46 mmol) in ethanol (200 mL) and water (40 mL) was reacted with hydrogen in the presence of 5% palladium-on-carbon for 4 h at rt. The catalyst was removed by filtration over a pad of filter aid. The filtrate was concentrated and chromatographed over Sephadex LH 20 using water as eluent. Product fractions were concentrated and lyophilyzed to give pure amorphous **2** (1.18 g, 98%); $[\alpha]_D$ +172.5 ° (*c* 0.2, water); ^{1}H NMR (D_2O, 400 MHz) δ 5.41 (d, 2H, J ≈ 3.5 Hz, H-1''', H-1''''), 5.20 (d, 1H, $J_{1',2'}$ ≈ 3.7 Hz, H-1'), 5.19 (d, 1H, $J_{1,2}$ ≈ 3.2 Hz, H-1), 4.55 (d, 1H, $J_{1'',2''}$ = 7.9 Hz, H-1'').

Anal. Calcd for $C_{30}H_{52}O_{26}$: C, 43.48; H, 6.32. Found: C, 43.33; H, 6.39.

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