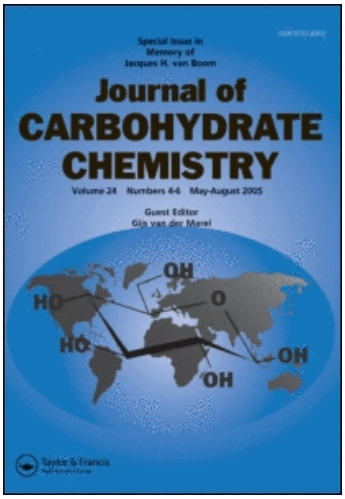

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SYNTHETIC α,β-(1→4)-GLUCAN OLIGOSACCHARIDES AS MODELS FOR HEPARAN SULFATE

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ABSTRACT

α,β-(1→4)-Glucans were devised as models for heparan sulfate with the simplifying assumptions that carboxyl-reduction and sulfation of heparan sulfate does not decrease the SMC antiproliferative activity and that *N*-sulfates in glucosamines can be replaced by *O*-sulfates. The target oligosaccharides were synthesized using maltosyl building blocks. Glycosylation of methyl 2,3,6,2',3',6'-hexa-*O*-benzyl-β-maltoside (**1**) with hepta-*O*-acetyl-α-maltosyl bromide (**2**) furnished tetrasaccharide **3** which was deprotected to α-D-Glc-(1→4)-β-D-Glc-(1→4)-α-D-Glc-(1→4)-β-D-Glc-(1→OCH_3) (**5**) or, alternatively, converted to the tetrasaccharide glycosyl acceptor (**8**) with one free hydroxyl function (4''''-OH). Further glycosylation with glucosyl or maltosyl bromide followed by deblocking gave the pentasaccharide [β-D-Glc-(1→4)-α-D-Glc-(1→4)]$_2$-β-D-Glc-(1→OCH_3) (**11**) and hexasaccharide [α-D-Glc-(1→4)-β-D-Glc-(1→4)]$_2$-α-D-Glc-(1→4)-β-D-Glc-(1→OCH_3) (**14**). The protected tetrasaccharide **3** and hexasaccharide **12** were fully characterized by ^1H and ^{13}C NMR spectroscopy. Assignments were possible using 1D TOCSY, T-ROESY, $^1H,^1H$ 2D COSY supplemented by ^1H-detected one-bond and multiple-bond $^1H,^{13}C$ 2D COSY experiments.

INTRODUCTION

Heparan sulfates isolated from endothelial cells[1] or smooth muscle cells (SMC)[2] were found to exhibit very high SMC antiproliferative activities and

are believed to be endogenous regulators of SMC growth,[3] an important process in the development of arteriosclerotic lesions.[4,5] Also the related glucosaminoglycan heparin, besides its clinically exploited anticoagulant properties, is an inhibitor of SMC growth,[6] although less potent than the heparan sulfates mentioned above.

The antiproliferative and antithrombin III mediated anticoagulant effects of heparin are not linked.[7] Thus, we could demonstrate that carboxyl-reduction of heparin followed by sulfation of primary hydroxyl groups to give so-called "CRS-heparin" (carboxyl-reduced sulfated heparin) restored the antiproliferative activity but abolished the anticoagulant activity of heparin.[8]

An investigation by Castellot et al.[9] on size-fractionated heparin had shown that dodecasaccharide fractions are required to obtain heparin-like antiproliferative activity. Oversulfation increased the activity and cut down the requirements for a heparin-like effect to approximately octasaccharide fractions. On the other hand, we have discovered highly sulfated tetrasaccharides with heparin-like activity.[10,11] It became thus conceivable that also relatively small substructures of heparan sulfates with high antiproliferative effect may exist. In this context, we describe here the synthesis of simplified heparan sulfate oligosaccharides for subsequent sulfation and biological characterization.

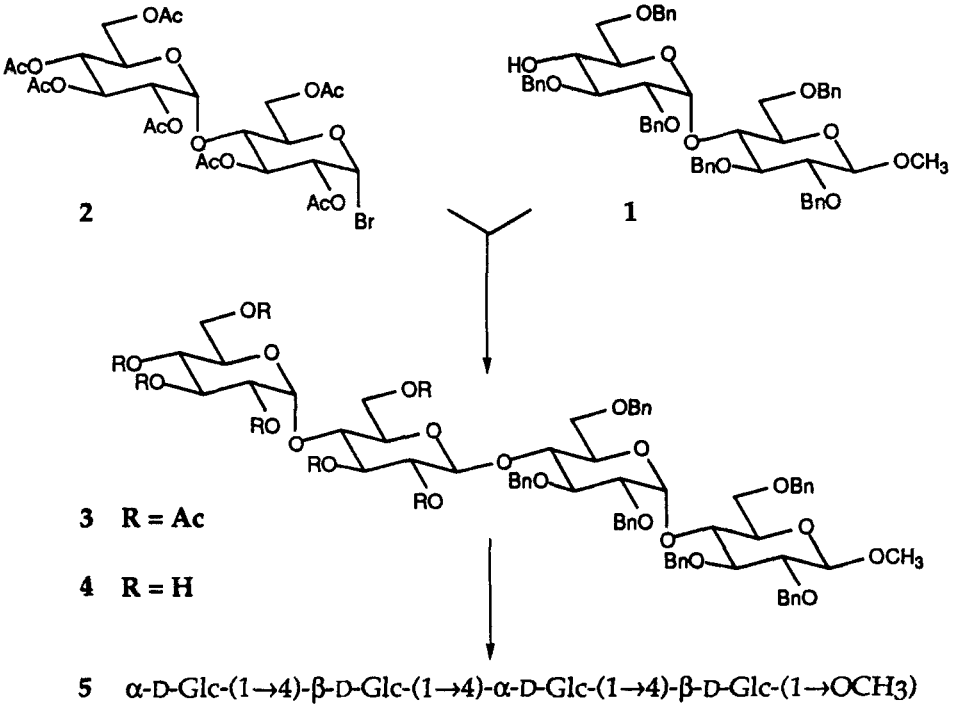
RESULTS AND DISCUSSION

While in heparin the major repeating unit is a substituted α-D-GlcN-(1→4)-α-L-IdoA, the analogous building block in heparan sulfate is α-D-GlcN-(1→4)-β-D-GlcA. To arrive at model saccharides of heparan sulfate that can be synthesized in a relatively straightforward manner we made the following simplifying assumptions: i) Carboxyl-reduction and sulfation of heparan sulfate does not decrease the SMC antiproliferative activity, in analogy to the high antiproliferative activity of CRS-heparin.[8] ii) *N*-Sulfates in glucosamines can be replaced by *O*-sulfates; this approach has been used successfully in the synthesis of simplified anticoagulant heparin saccharides.[12]

The rather complex heparan sulfate backbone could thus be reduced to a (1→4)-glucan with alternating α- and β-linkages (Scheme 1). A synthetic approach to oligosaccharides of this type is obvious, since with maltose a readily available α-D-linked disaccharide building block is at hand. β-Linkage

[→4)-α-D-GlcN-(1→4)-β-D-GlcA]$_n$	Heparan sulfate, constitutive backbone
i) ⇓	
[→4)-α-D-GlcN-(1→4)-β-D-Glc]$_n$	CRS-Heparan sulfate
ii) ⇓	
[→4)-α-D-Glc-(1→4)-β-D-Glc]$_n$	α,β-(1→4)-Glucan

Scheme 1



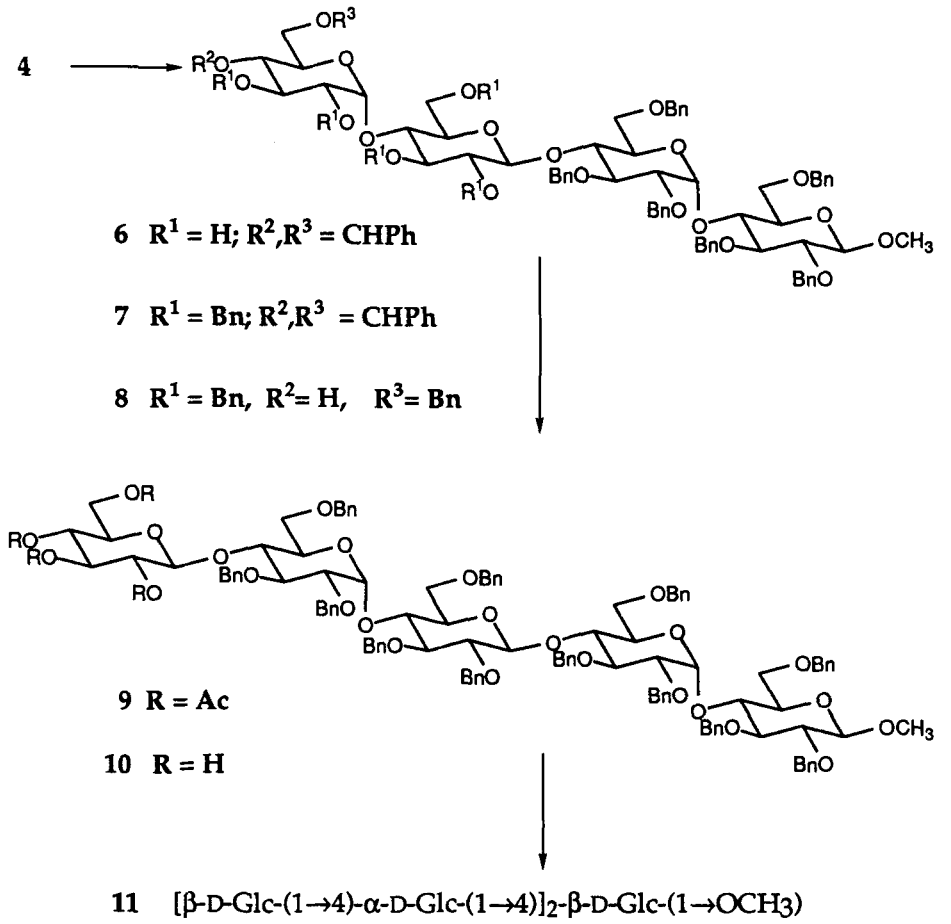
Scheme 2

of maltose units would thus build up the targeted (1→4)-glucan. For the termination at the reducing end we chose to use a methyl glycoside so that methyl β-maltoside[13] constitutes a disaccharide substructure. To synthetically extend this unit we prepared methyl 2,3,6,2',3',6'-hexa-*O*-benzyl-β-maltoside (**1**)[14] by reductive opening of the 4',6'-*O*-benzylidene precursor. This glycosyl acceptor was selected because the benzyl protective groups do not further decrease the inherently low reactivity of the 4'-hydroxyl group.[15,16]

Silver triflate mediated glycosylation[17] of **1** with hepta-*O*-acetyl-α-maltosyl bromide[18] (**2**) furnished the tetrasaccharide **3** in 64 % yield. Standard deacetylation of **3** with methanolate gave **4**, which was further deblocked by hydrogenolysis to give the free tetrasaccharide **5** in very good yield.

The protected tetrasaccharide **3** was fully analyzed by NMR spectroscopy. Since the proton signals of the four anomeric protons were sufficiently separated, nearly all protons could be readily assigned by a series of 1D TOCSY experiments. Upon inversion of the magnetization of the anomeric protons by a selective 180° DANTE pulse sequence, followed by an MLEV17 mixing sequence of increasing spin-locking duration in subsequent experiments, relatively simple sub-spectra of the protons of the four rings were obtained. Supplementary information on the sequence of the protons in the individual rings and their chemical shifts was derived from $^{1}H,^{1}H$ 2D COSY. The assignment of the sub-spectra to the individual pyranose rings was straightforward because the added maltosyl moiety is acetylated so that their proton signals are subjected to a characteristic downfield shift. In addition, these assignments were confirmed by 1D T-ROESY experiments. Excitation of protons H-1 and of H-1''' indicated, besides the expected intra-ring through-space connectivities such as H-1→OCH3, H-1→H-3, H-5, H-1'''→H-2''', additional inter-ring ROEs of medium to strong intensity such as H-1'→H-4, and H-1'''→H-4''. The typical coupling constant of $J_{1'',2''} = 8.0$ Hz proved the diaxial orientation of H-1''/H-2'' and thus the β-D-linkage of the newly formed glycosidic bond. The assignment of the ^{13}C signals was derived from $^{1}H,^{13}C$ 2D COSY experiments.

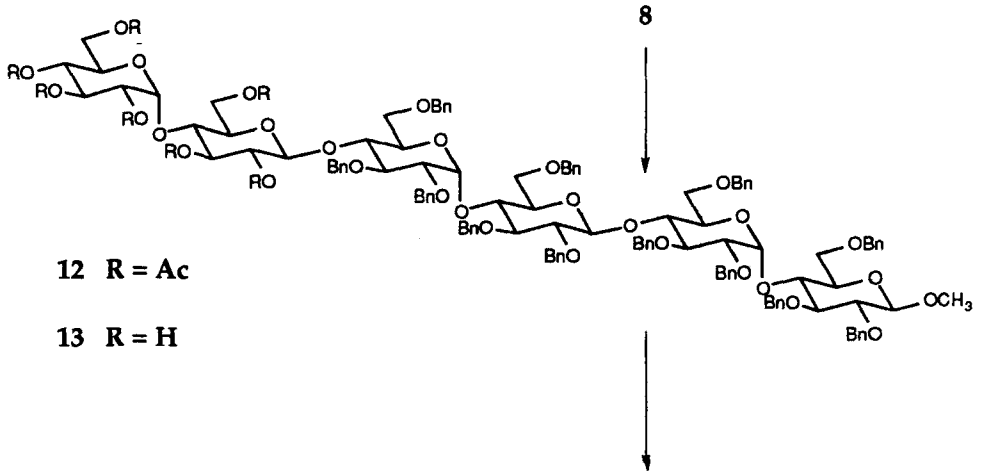
To further extend the glycosidic structure we repeated the protective group strategy that was employed in the construction of glycosyl acceptor **1**. Thus, tetrasaccharide **4** was selectively protected in the 4''',6'''-positions by benzylidenation with benzaldehyde and zinc chloride to give **6** in good yield (79 %). The remaining hydroxyl groups were benzylated with sodium hydride and benzyl chloride in dimethyl sulfoxide to furnish **7** in a yield of



Scheme 3

67 %. The benzylidene acetal of **7** was opened regioselectively with sodium cyanoborohydride in the presence of hydrochloric acid in diethyl ether as described by Garegg et al.[19] to produce **8** in good yield (82 %). This compound has a single free hydroxyl function (4''''-OH) ready for glycosylation and was used as a glycosyl acceptor in the following reactions.

Koenigs-Knorr glucosylation of tetrasaccharide acceptor **8** with tetra-*O*-acetyl-α-D-glucosyl bromide and silver triflate[17] as promoter furnished pentasaccharide **9**. The rather moderate yield of 57 % in this reaction reflects the low reactivity of the 4'''-OH group of **8** compared to the 4'-OH group of **1**. Standard deprotection with methanolate to give **10** followed by



14 [α-D-Glc-(1→4)-β-D-Glc-(1→4)]$_2$-α-D-Glc-(1→4)-β-D-Glc-(1→OCH$_3$)

Scheme 4

hydrogenation furnished the free pentasaccharide **11** in virtually quantitative yield. The correct β-stereochemistry at the newly formed anomeric center C-1'''' was evident from the ^{1}H NMR spectrum displaying the presence of three anomeric protons (H-1, H-1'', H-1'''') with the typical coupling constants for diaxial protons.

Silver triflate mediated glycosylation of tetrasaccharide **8** with maltosyl bromide **2** gave hexasaccharide **12**, again in moderate yield (63 %). Deprotection as discussed above led to the partially and then fully deblocked hexasaccharides **13** and **14**, respectively.

The protected hexasaccharide **12** could be completely analyzed by NMR spectroscopy despite the considerable complexity of the spectrum. Since in the beginning three of the six anomeric protons (H-1, H-1'' and H-1'''') were not unambiguously identified in the ^{1}H NMR due to severe overlap, their approximate chemical shifts were extracted from a one-bond ^{1}H,^{13}C 2D correlation experiment. In the ^{13}C NMR spectrum, the signals of the anomeric carbons absorb between 95.73 and 104.52 ppm, well separated from all other signals. The ^{1}H shifts of their directly attached protons were then readily accessible from the position of the cross peaks in the 2D spectrum. This considerably simplified their identification in the ^{1}H spectrum. Starting from the anomeric protons, the subsequent 1D TOCSY experiments generated

a set of six sub-spectra of the different rings and most proton signals, except H-6, H-6a', H-6a''' and H-6'''', could be identified. The assignment of the sub-spectra to the individual pyranose rings was evident for the acetylated maltosyl moiety because of the characteristic downfield shifts. These assignments were confirmed and others additionally established by some 1D T-ROESY experiments showing medium to strong inter-ring ROEs H-1'→H-4, H-1'''→H-4'', H-1''''→H-4''', and H-1'''''→H-4''''. The linking of the pyranoses and hence the assignment of the sub-spectra to the six pyranose rings could also be deduced from corresponding cross peaks in the multiple-bond $^1H,^{13}C$ 2D COSY experiment caused by three-bond $^1H,^{13}C$ couplings. Thus, the first ring was identified by a cross peak C-1/OCH_3. Clearly identified were also cross peaks C-4/H-1', C-1'/H-4, C-1''/H-4', C-4''/H-1''', C-1''''/H-4''', C-4'''/H-1'''', C-4''''/H-1''''', C-1''''/H-4''''. The typical coupling constant of $J_{1'''',2''''} = 8.1$ Hz proved the diaxial orientation of H-1''''/H-2'''' and thus the β-D-linkage of the newly formed glycosidic bond. Further confirmation of the assignment of some protons was provided by the $^1H,^1H$ 2D COSY experiment. Some of the coupling constants could not be measured in the 400 MHz ^1H NMR spectrum because of strongly overlapping signals. These data were taken from a 600 MHz spectrum.

In conclusion, three α,β-(1→4)-glucan oligosaccharides, namely tetrasaccharide **5**, pentasaccharide **11**, and hexasaccharide **14**, were effectively prepared in a block synthesis approach.

EXPERIMENTAL

General Procedures. Solvents and reagents were bought from Fluka. Evaporation: *in vacuo*, conducted with a 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. ^1H NMR: Bruker AC 250 (250 MHz), AM-400 (400 MHz) with Aspect 3000, ARX-400 (400 MHz) with ASPECT station 1 and z-gradient accessory kit with 10 Amps power amplifier for pulsed field z-gradient (PFG) experiments, AMX2-600 (600 MHz) with ASPECT station 1; chemical shifts in ppm relative to tetramethylsilane or sodium 2,2,3,3-tetradeutero-3-(trimethylsilyl)-propionate as internal standard. Standard Bruker pulse programs were applied for the PFG $^1H,^1H$ 2D COSY and the ^1H-detected

multiple-bond $^1H,^{13}C$ HMQC correlation experiments (pulse sequences COSYGS and INV4GSLRLP). The one-bond PFG $^1H,^{13}C$ HSQC experiment was as described recently.[20] Pulse sequence and experimental conditions for the 1D TOCSY and 1D T-ROESY experiments with $(180°_x - 180°_{-x})_n$ spin-lock of 0.6 s duration (n = 2400) and selective excitation by a sequence of DANTE pulses were essentially as described before.[16,21]

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (3). To a soln of glycosyl acceptor **1**[14] (5.3 g, 5.90 mmol) and acetobromomaltose **2**[18] (6.62 g, 8.85 mmol) in abs dichloromethane (85 mL) was added tetramethylurea (2.05 g, 17.7 mmol) and silver triflate (2.26 g, 8.8 mmol) at -10 °C. The reaction mixture was stirred at rt for 1 h and at 30-35 °C for 5 h, and then filtered through a pad of filter aid. The filtrate and dichloromethane washings were combined and washed twice with aq sodium bicarbonate soln. The organic phases were dried over magnesium sulfate and concentrated. The residue was chromatographed on silica gel using ethyl acetate/ hexane 1:2 and 2:3 as eluents to furnish **3** (5.7 g, 64 %) as a colourless foam: $[\alpha]_D$ +52.8 ° (*c* 0.5, chloroform); MS (ionspray) *m/z* 1538.8 (3%, $[M + NH_4]^+$); ^1H NMR ($CDCl_3$, 400 MHz; 1D TOCSY, 1D ROESY, $^1H,^{13}C$ 2D COSY) δ 7.42 - 7.00 (m, 30H, aromat), 5.68 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1'), 5.38 (dd ~ t, 1H, $J_{3''',4'''}$ = 9.8 Hz, H-3'''), 5.30 (d, 1H, $J_{1''',2'''}$ = 4.0 Hz, H-1'''), 5.05 (dd ~ t, 1H, $J_{4''',5'''}$ = 9.6 Hz, H-4'''), 4.97 (dd ~ t, 1H, $J_{3'',4''}$ = 8.4 Hz, H-3''), 4.96, 4.59 (2d, 2H, J_{gem} = 11.5 Hz, C-3'-OCH_2Ph), 4.93, 4.74 (2d, 2H, J_{gem} = 11.2 Hz, C-3-OCH_2Ph), 4.87, 4.61 (2d, 2H, J_{gem} = 11.0 Hz, C-2-OCH_2Ph), 4.86 (dd, 1H, $J_{2''',3'''}$ = 11.2 Hz, H-2'''), 4.71, 4.23 (2d, 2H, J_{gem} = 12.0 Hz, C-6'-OCH_2Ph), 4.69 (dd, 1H, $J_{2'',3''}$ = 9.6 Hz, H-2''), 4.61, 4.50 (2d, 2H, J_{gem} = 12.0 Hz, C-6-OCH_2Ph), 4.53, 4.44 (2d, 2H, J_{gem} = 11.8 Hz, C-2'-OCH_2Ph), 4.40 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1''), 4.31 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1), 4.21 (dd, 1H, $J_{5''',6a'''}$ = 3.5 Hz, $J_{6a''',6b'''}$ = 12.5 Hz, H-6a'''), 4.10 (dd, 1H, $J_{5'',6a''}$ = 2.9 Hz, $J_{6a'',6b''}$ = 11.8 Hz, H-6a''), 4.07 (dd ~ t, 1H, $J_{4,5}$ = 9.3 Hz, H-4), 4.00 (dd, 1H, $J_{5'',6b''}$ = 4.1 Hz, H-6b''), 3.97 (dd, 1H, $J_{5''',6b'''}$ = 2.4 Hz, H-6b'''), 3.87 (ddd ~ dt, 1H, H-5'''), 3.86 (dd ~ t, 1H, $J_{4',5'}$ = 8.5 Hz, H-4'), 3.82 (2H, dd ~ t, $J_{4'',5''}$ ≈ 9.6 Hz, H-4''; dd, H-6a), 3.77 (dd ~ t, 1H, $J_{3,4}$ = 8.7 Hz, H-3), 3.74 (2H, dd, $J_{5,6b}$ = 2.0 Hz, $J_{6a,6b}$ = 11.8 Hz, H-6b; dd, $J_{3',4'}$ = 8.5 Hz, H-3'), 3.66 (ddd ~ br d, 1H, H-5'), 3.62 (dd, 1H, $J_{5',6a'}$ = 2.5 Hz, $J_{6a',6b'}$ = 10.8 Hz, H-6a'), 3.56 (s, 3H, OCH_3), 3.53 (ddd, 1H, $J_{5,6a}$ = 3.5 Hz, H-5), 3.47 (dd, 1H, $J_{2,3}$ = 8.8 Hz, H-2), 3.42 (2H, dd, $J_{5',6b'}$ ≈ 1.5 Hz, H-6b'; dd, $J_{2',3'}$= 9.6 Hz, H-2'), 3.13 (ddd ~ dt, 1H, H-5''), 2.10, 2.06, 2.03,

2.01, 1.96, 1.89, 1.84 (7 s, 21H, Ac); ^{13}C NMR ($CDCl_3$, 100 MHz; ^1H,^{13}C 2D COSY) δ 170.40 (3C, C=O), 170.13, 169.83, 169.45, 169.31 (4C, C=O), 139.40, 138.62 (2C, quat. C arom.), 138.25 (2C, quat. C arom.), 137.84, 137.66 (2C, quat. C arom.), 128.67 - 126.66 (aromatic CH), 104.49 (C-1), 99.35 (C-1''), 96.52 (C-1'), 95.62 (C-1'''), 84.62 (C-3), 82.36 (C-2), 79.90 (C-3'), 78.15 (C-2'), 76.90 (C-4'), 75.86 (C-3''), 74.75 (C-5), 74.68, 74.49, 74.01 (3C, CH_2Ph), 73.65 (2C, CH_2Ph), 73.42 (1C, CH_2Ph), 73.04 (C-4''), 72.63 (C-2''), 72.18 (C-4), 71.47 (C-5''), 70.45 (C-5'), 69.95 (C-2'''), 69.19 (C-3'''), 68.72 (C-6), 68.42 (C-5'''), 67.85 (C-4'''), 67.49 (C-6'), 63.04 (C-6''), 61.29 (C-6'''), 56.79 (OCH_3), 20.83 (1C, Ac), 20.57 (2C, Ac), 20.50 (4C, Ac).

Anal. Calcd for $C_{81}H_{94}O_{28}$: C, 64.19; H, 6.25. Found: C, 64.22; H, 6.22.

Methyl *O*-(α-D-Glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (4). To a soln of **3** (5.4 g, 3.5 mmol) in diethyl ether (27 mL) and methanol (108 mL) was a added a soln of sodium methanolate (2.7 mL of 2 g Na/ 100 mL methanol) at rt. The reaction mixture was kept for 16 h at rt, neutralized with Amberlite IR 120 (H^+) and filtered. After addition of a few drops of triethylamine, the filtrate and methanol washings were concentrated. The residue was chromatographed on silica gel using ethyl acetate/ methanol/ water 96:2:2 and 95:2.5:2.5 as eluents to obtain pure **4** (3.33 g, 78 %) as a colourless foam: $[\alpha]_D$ +63.6 ° (*c* 0.5, chloroform); MS (ionspray) *m/z* 1238.8 (17%, $[M + NH_4]^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.29 - 7.07 (m, 30H, aromat), 5.61 (d, 1H, $J_{1',2'}$ = 3.3 Hz, H-1'), 5.30 (br s, 1H, H-1'''), 3.55 (s, 3H, OCH_3).

Anal. Calcd for $C_{67}H_{80}O_{21}$: C, 65.89; H, 6.60. Found: C, 65.92; H, 6.39.

Methyl *O*-(α-D-Glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl-(1→4)-*O*-(α-D-glucopyranosyl)-(1→4)-β-D-glucopyranoside (5). A soln of **4** (780 mg, 0.64 mmol) in ethanol (30 mL) and water (10 mL) was hydrogenated in the presence of 10 % palladium on charcoal (305 mg) at 1.1 bar for 4 h. The reaction mixture was filtered through a pad of filter aid and washed with ethanol/ water 1:1. After addition of a few drops of triethylamine the filtrate was concentrated. The aqueous residue was lyophilized to obtain pure **5** (435 mg) as an amorphous colourless powder in quantitative yield; $[\alpha]_D$ +92.0 ° (*c* 0.2, water); MS (ionspray) *m/z* 703.4 (60%, $[M + NH_4]^+$); ^1H NMR (D_2O, 400 MHz) δ 5.61 (d, 2H, J = 3.6 Hz, H-1', H-1'''), 5.53 (d, 1H, $J_{1'',2''}$ = 7.9 Hz, H-1''), 5.40 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.58 (s, 3H, OCH_3).

Anal. Calcd for $C_{25}H_{44}O_{21}$: C, 44.12; H, 6.52. Found: C, 39.99; H, 6.59.

Methyl *O*-[4,6-*O*-(*R*)-Benzylidene-α-D-glucopyranosyl]-(1→4)-*O*-(β-D-glucopyranosyl)-(1→ 4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→ 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (6). A suspension of **4** (2.36 g, 1.93 mmol) in benzaldehyde (50 mL) was stirred in the presence of zinc chloride (10 g) for 18 h at rt and then poured into a stirred mixture of pentane (500 mL) and ice (100 g). The organic phase was decanted, and the aqueous phase was treated two more times with pentane to remove benzaldehyde. The aqueous phase was then extracted with ethyl acetate. The extracts were washed with brine, dried over magnesium sulfate, and concentrated. The residue was chromatographed on silica gel using ethyl acetate/ hexane 2:1 and ethyl acetate as eluents to furnish pure **6** (1.99 g, 79 %) as a foam; $[\alpha]_D$ +68.0 ° (*c* 0.1, chloroform); MS (ionspray) *m/z* 1327.6 (15%, $[M + NH_4]^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.49-7.47 (m, 2H, aromat), 7.38-7.24 (m, 26H, aromat), 7.19-7.08 (m, 7H, aromat), 5.68 (d, 1H, $J_{1',2'}$ = 3.8 Hz, H-1'), 5.50 (s, 1H, CHPh), 5.05 (d, 1H, $J_{1''',2'''}$ = 3.7 Hz, H-1'''), 4.96, 4.72 (2 d, 2H, J_{gem} = 11.6 Hz, CH_2Ph), 4.89, 4.61 (2 d, 2H, J_{gem} = 11.0 Hz, CH_2Ph), 4.86, 4.82 (2 d, 2H, J_{gem} = 11.6 Hz, CH_2Ph), 4.59, 4.55 (2 d, 2H, J_{gem} = 12.1 Hz, CH_2Ph), 4.55, 4.50 (2 d, 2H, J_{gem} = 11.8 Hz, CH_2Ph), 4.55, 4.34 (2 d, 2H, J_{gem} = 11.1 Hz, CH_2Ph), 4.38 (d, 1H, J = 7.7 Hz, H-1 or H-1''), 4.33 (d, 1H, J = 8.0 Hz, H-1'' or H-1), 3.58 (s, 3H, OCH_3).

Anal. Calcd for $C_{74}H_{84}O_{21}$: C, 67.88; H, 6.46. Found: C, 67.68; H, 6.53.

Methyl *O*-[2,3-Di-*O*-benzyl-4,6-*O*-(*R*)-benzylidene-α-D-glucopyranosyl]-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (7). A soln of **6** (1.97 g, 1.50 mmol) in Me_2SO (5 mL) was added dropwise within 10 min to a slurry of sodium hydride (0.60 g, 15 mmol, 60 % in mineral oil, washed with hexane) in Me_2SO (18 mL). After stirring for 90 min at rt benzyl chloride (2.1 g, 16.5 mmol) in Me_2SO (3 mL) was added dropwise within 10 min at rt. Stirring was continued for 3 h. The reaction mixture was then poured on ice/water and extracted twice with ethyl acetate. The organic phases were washed with cold water and brine, dried over magnesium sulfate, and concentrated. The residue was chromatographed on silica gel using ethyl acetate/ hexane 1:4 and 1:3 as eluent to give **7** (1.76 g, 67 %) as a foam; $[\alpha]_D$ +35.0 ° (*c* 0.2, chloroform); MS (FAB) *m/z* 1782.8 (100%, $[M + Na]^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.50-7.47 (m, 2H, aromat), 7.38-7.11 (m, 58H, aromat), 5.67 (d, 1H, $J_{1',2'}$ = 3.9 Hz, H-1'), 5.64 (d, 1H, $J_{1''',2'''}$ = 3.8 Hz, H-1'''), 5.52 (s, 1H, CHPh), 4.35 (d, 1H, J = 7.9 Hz, H-1 or H-1''), 4.30 (d, 1H, J ≈ 7.8 Hz, H-1'' or H-1), 3.55 (s, 3H, OCH_3).

Anal. Calcd for $C_{109}H_{114}O_{21}$: C, 74.38; H, 6.52. Found: C, 74.22; H, 6.58.

Methyl *O*-(2,3,6-Tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-benzyl -α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (8). To a soln of 7 (1.7 g, 0.96 mmol) in abs tetrahydrofuran (17 mL) were added 3Å molecular sieves (0.85 g) at 0 °C followed by sodium cyanoborohydride (0.94 g, 12.6 mmol) and a few crystals of methyl orange. Hydrogen chloride in diethyl ether (18 mL of 1.3 m soln, 23.4 mmol) was added dropwise to the milky reaction mixture within 30 min. After stirring for 1 h at 0 °C the orange-red reaction mixture was poured into sodium bicarbonate soln, and tetrahydrofuran was evaporated under reduced pressure. The aqueous residue was extracted with ethyl acetate. The organic phases were washed with brine, dried over magnesium sulfate, and concentrated. The residue was chromatographed on silica gel using ethyl acetate/ hexane 1:3 as eluent to give **8** (1.38 g, 82 %) as a foam; $[\alpha]_D$ +30.0 ° (*c* 0.1, chloroform); MS (FAB) *m/z* 1800.7 (100%, $[M + K]^+$), 1760.8 (25%, $[M + H]^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.31-7.09 (m, 60H, aromat), 5.67 (d, 1H, $J_{1',2'}$ = 3.9 Hz, H-1'), 5.62 (d, 1H, $J_{1''',2'''}$ = 3.5 Hz, H-1'''), 4.37 (d, 1H, $J_{1'',2''} \approx 8$ Hz, H-1''), 4.31 (d, 1H, $J_{1,2} \approx 7.8$ Hz, H-1), 3.55 (s, 3H, OCH_3), 2.42 (br s, 1H, 4'''-OH).

Anal. Calcd for $C_{109}H_{116}O_{21}$: C, 74.30; H, 6.64. Found: C, 74.10; H, 6.71.

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→ 4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→ 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (9). To a soln of glycosyl acceptor **8** (0.67 g, 0.38 mmol) and acetobromoglucose (0.32 g, 0.77 mmol) in abs dichloromethane (8 mL) was added tetramethylurea (0.17 g, 1.5 mmol) and silver triflate (193 mg, 0.75 mmol) at -10 °C. The reaction mixture was stirred at 30-35 °C for 7 h, and then filtered through a pad of filter aid. The filtrate and dichloromethane washings were combined and washed twice with aq sodium bicarbonate soln. The organic phases were dried over magnesium sulfate and concentrated. The residue was chromatographed on silica gel using ethyl acetate/ hexane 1:3 and 1:2 as eluents to furnish **9** (0.45 g, 57 %) as a colourless foam: $[\alpha]_D$ +26.0 ° (*c* 0.2, chloroform); MS (FAB) *m/z* 2113.7 (47%, $[M + Na]^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.40-7.07 (m, 60H, aromat), 5.68 (d, 1H, $J_{1',2'}$ = 3.9 Hz, H-1'), 5.63 (d, 1H, $J_{1''',2'''}$ = 3.8 Hz, H-1'''), 3.55 (s, 3H, OCH_3), 2.00, 1.97, 1.90, 1.81 (4 s, 12H, Ac).

Anal. Calcd for $C_{123}H_{134}O_{30}$: C, 70.60; H, 6.45. Found: C, 70.30; H, 6.54.

Methyl *O*-(β-D-Glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→ 4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→ 4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)- 2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (10). To a soln of **9** (0.43 g, 0.205 mmol) in tetrahydrofuran (2 mL) and methanol (10 mL) was a added a soln of sodium methanolate (0.45 mL of 2.0 g Na/ 100 mL methanol) at rt. The reaction mixture was kept for 16 h at rt, neutralized with Amberlite IR 120 (H^+) and filtered. After addition of a few drops of triethylamine, the filtrate and methanol washings were concentrated. The residue was chromatographed on silica gel using ethyl acetate as eluent to obtain pure **10** (0.39 g, 96 %) as a colourless foam: $[\alpha]_D$ +45.0 ° (*c* 0.2, chloroform); MS (FAB) *m/z* 1946.7 (100%, $[M + Na]^+$); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.32-7.07 (m, 60H, aromat), 5.67 (d, 1H, $J_{1',2'}$ = 3.9 Hz, H-1'), 5.64 (d, 1H, $J_{1''',2'''}$ = 3.7 Hz, H-1'''), 3.55 (s, 3H, OCH_3), 2.63 (br s, 1H, OH), 2.50 (br s, 1H, OH), 1.70 (br s, 2H, OH).

Anal. Calcd for $C_{115}H_{126}O_{26}$: C, 71.78; H, 6.60. Found: C, 71.96; H, 6.54.

Methyl *O*-(β-D-Glucopyranosyl)-(1→4)-*O*-(α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1 → 4)-*O*-(α-D-glucopyranosyl)-(1→ 4)- β-D-glucopyranoside (11). A soln of **10** (365 mg, 0.19 mmol) in ethanol (15 mL) and water (3 mL) was hydrogenated in the presence of 10 % palladium on charcoal (300 mg) at 1.1 bar for 20 h. The reaction mixture was filtered through a pad of filter aid and washed with ethanol water 1:1. After addition of a few drops of triethylamine the filtrate was concentrated. The aqueous residue (ca 5 mL) was lyophilized to obtain pure **11** (160 mg) as an amorphous colourless powder in quantitative yield; $[\alpha]_D$ +84.0 ° (*c* 0.1, water); MS (ionspray) *m/z* 865.4 (100%, $[M + Na]^+$), 843.4 (15%, $[M + H]^+$); ^1H NMR (D_2O, 400 MHz) δ 5.42, 5.41 (2 d, 2H, J ≈ 3.9 Hz, J ≈ 3.8 Hz, H-1', H-1'''), 4.54 (d, 1H, $J_{1'',2''}$ ≈ 7.6 Hz, H-1''), 4.52 (d, 1H, $J_{1'''',2''''}$ ≈ 7.8 Hz, H-1''''), 4.40 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.58 (s, 3H, OCH_3).

Anal. Calcd for $C_{31}H_{54}O_{26}$: C, 44.18; H, 6.46. Found: C, 44.23; H, 6.55.

Methyl *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-glucopyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (12). To a soln of glycosyl acceptor **8** (0.67 g, 0.38 mmol) and acetobromomaltose[18] (0.54 g, 0.77 mmol) in abs dichloromethane (8 mL) was added tetramethylurea (1.86 g, 1.5 mmol) and silver triflate (193 mg, 0.75 mmol) at -10 °C. The reaction mixture was stirred at 30-35 °C for 7 h, and then filtered through a

pad of filter aid. The filtrate and dichloromethane washings were combined and washed twice with aq sodium bicarbonate soln. The organic phases were dried over magnesium sulfate and concentrated. The residue was chromatographed on silica gel using ethyl acetate/ hexane 1:2 and 1:1 as eluents to furnish **12** (0.57 g, 63 %) as a colourless foam: $[\alpha]_D$ +49.0 ° (*c* 0.2, chloroform); MS (FAB) *m/z* 2403.0 (60%, $[M + Na]^+$); ^1H NMR ($CDCl_3$, 400 MHz and 600 MHz; 1D TOCSY, 1D T-ROESY, $^1H,^1H$ 2D COSY, $^1H,^{13}C$ 2D COSY) δ 7.45 - 7.03 (m, 60H, aromat), 5.66 (d, 1H, $J_{1',2'}$ = 3.9 Hz, H-1'), 5.62 (d, 1H, $J_{1''',2'''}$ = 3.9 Hz, H-1'''), 5.39 (dd ~ t, 1H, $J_{3'''',4''''}$ = 9.6 Hz, H-3''''), 5.30 (d, 1H, $J_{1'''',2''''}$ = 4.0 Hz, H-1''''), 5.05 (dd ~ t, 1H, $J_{4'''',5''''}$ = 9.6 Hz, H-4''''), 5.01, 4.65 (2d, 2H, J_{gem} = 11.8 Hz, C-3'-OCH_2Ph), 4.98, 4.61 (2d, 2H, J_{gem} = 11.5 Hz, C-3'''-OCH_2Ph), 4.96 (dd ~ t, 1H, $J_{3'''',4''''}$ = 8.8 Hz, H-3''''), 4.89, 4.72 (2d, 2H, J_{gem} = 11.4 Hz, C-3-OCH_2Ph), 4.86, 4.59 (2d, 2H, J_{gem} = 11.0 Hz, C-2-OCH_2Ph), 4.86 (dd, 1H, $J_{2'''',3''''}$ = 10.4 Hz, H-2''''), 4.79, 4.55 (2d, 2H, J_{gem} = 11.0 Hz, C-2''-OCH_2Ph), 4.76, 4.74 (2d, 2H, J_{gem} = 11.3 Hz, C-3''-OCH_2Ph), 4.70, 4.20 (2d, 2H, J_{gem} = 11.9 Hz, C-6'''-OCH_2Ph), 4.69 (dd, 1H, $J_{2'''',3''''}$ = 9.2 Hz, H-2''''), 4.62, 4.45 (2d, 2H, J_{gem} = 12.0 Hz, C-2'''-OCH_2Ph), 4.59, 4.42 (2d, 2H, J_{gem} = 11.5 Hz, C-2'-OCH_2Ph), 4.56, 4.21 (2d, 2H, J_{gem} = 12.0 Hz, C-6'-OCH_2Ph), 4.40, 4.31 (2d, 2H, J_{gem} = 12.0 Hz, C-6-OCH_2Ph), 4.30 (2H, s, C-6''-OCH_2Ph and d, $J_{1,2}$ = 7.6 Hz, H-1), 4.37 (d, 1H, $J_{1'''',2''''}$ = 8.1 Hz, H-1''''), 4.20 (dd, 1H, H-6a''''), 4.31 (d, 1H, $J_{1'',2''}$ = 8.0 Hz, H-1''), 4.12 (dd, 1H, $J_{5'''',6a''''}$ = 2.5 Hz, $J_{6a'''',6b''''}$ = 12.0 Hz, H-6a''''), 4.04 (dd ~ t, 1H, $J_{4,5}$ ≈ 9.0 Hz, H-4), 4.01 (dd, 1H, H-6b''''), 4.00 (dd ~ t, 1H, $J_{4',5'}$ = 9.8 Hz, H-4'), 3.97 (dd, 1H, $J_{5'''',6b''''}$ = 2.0 Hz, $J_{6a'''',6b''''}$ = 12.0 Hz, H-6b''''), 3.94 (dd ~ t, 1H, H-4''), 3.88 (ddd, 1H, $J_{5'''',6a''''}$ = 3.6 Hz, H-5''''), 3.87 (dd, 1H, $J_{4'''',5''''}$ ≈ 9.6 Hz, H-4''''), 3.82 (dd ~ t, 1H, H-4'''), 3.81 (dd ~ t, 1H, $J_{3',4'}$ = 8.2 Hz, H-3'), 3.77 (dd, 1H, $J_{5,6a}$ = 2.2 Hz, $J_{6a,6b}$ = 11.5 Hz, H-6a), 3.76 (dd ~ t, 2H, $J_{3,4}$ ≈ 9.0 Hz, H-3; $J_{3''',4'''}$ ≈ 8.7 Hz, H-3'''), 3.75 (1H, H-5'), 3.73 (dd, 1H, $J_{5',6a'}$ = 2.5 Hz, $J_{6a',6b'}$ = 11.5 Hz, H-6a'), 3.72 (dd, 1H, $J_{5,6b}$ = 2.0 Hz, H-6b), 3.65 (dd, 1H, $J_{5'',6a''}$ = 1.7 Hz, H-6a''), 3.62 (ddd ~ br d, 1H, $J_{5''',6b'''}$ = 1.7 Hz, H-5'''), 3.60 (dd, 1H, $J_{5''',6a'''}$ = 2.5 Hz, $J_{6a''',6b'''}$ = 10.9 Hz, H-6a'''), 3.59 (dd, 1H, $J_{6a'',6b''}$ = 12.0 Hz, H-6b''), 3.55 (ddd ~ dt, 1H, H-5), 3.54 (s, 3H, OCH_3), 3.47 (dd, $J_{3'',4''}$ = 8.0 Hz, H-3''), 3.45 (dd ~ t, 1H, $J_{2,3}$ ≈ 8.2 Hz, H-2), 3.44 (dd, $J_{2',3'}$= 9.8 Hz, H-2'), 3.42 (dd, $J_{2''',3'''}$= 9.5 Hz, H-2'''), 3.38 (2dd ~ br d, 2H, H-6b', H-6b'''), 3.34 (dd ~ t, $J_{2'',3''}$= 9.3 Hz, H-2''), 3.23 (ddd, 1H, $J_{4'',5''}$ = 9.8 Hz, $J_{5'',6b''}$ = 4.0 Hz, H-5''), 3.16 (ddd ~ dt, 1H, $J_{4'''',5''''}$ = 9.5 Hz, $J_{5'''',6b''''}$ = 4.5 Hz, H-5''''), 2.10, 2.06, 2.03, 2.01, 1.96, 1.89, 1.81 (7 s, 21H, Ac); ^{13}C NMR ($CDCl_3$, 100 MHz; DEPT-135, $^1H,^{13}C$ 2D COSY) δ 170.51 (3C, C=O at C-2'''', C-6'''', C-6''''), 170.24

(C=O at C-3''''), 169.95 (C=O at C-3'''''), 169.54 (C=O at C-2''''', 169.41 (C=O at C-4'''''), 139.47 (quat. arom. C at C-3'''), 138.39 (quat. arom. C at C-3'), 138.76, 138.72, 138.66 (3C, quat. arom. C), 138.39 (2C, quat. arom. C), 138.34, 138.19, 138.13 (3C, quat. arom. C), 137.73 (quat. arom. C at C-6'''), 137.69 (quat. arom. C at C-6'), 104.52 (C-1), 102.18 (C-1''), 99.39 (C-1''''), 96.96 (C-1'''), 96.91 (C-1'), 95.73 (C-1'''''), 84.73 (C-3), 84.65 (C-3''), 82.71 (C-2''), 82.40 (C-2), 80.32 (C-3'), 80.01 (C-3'''), 78.53 (C-2'), 78.33 (C-2'''), 77.01 (C-4'''), 75.92 (C-3''''), 75.82 (C-4'), 75.13 (C-5''), 75.04(CH_2Ph), 74.80 (2C, C-5 and CH_2Ph), 74.57, 74.71, 74.16, 74.05 (4C, CH_2Ph), 73.72 (2C, CH_2Ph), 73.59, 73.53, 73.48, 73.43 (4C, CH_2Ph), 73.17 (C-4''), 73.15 (C-4''''), 72.87 (C-4), 72.69 (C-2''''), 71.57 (C-5''''), 71.07 (C-5'), 70.51 (C-5'''), 70.07 (C-2'''''), 69.29 (C-3'''''), 69.23 (C-6), 68.89 (C-6''), 68.53 (C-5'''''), 67.96 (C-4'''''), 67.72 (C-6'), 67.51 (C-6'''), 63.12 (C-6''''), 61.39 (C-6'''''), 56.89 (OCH_3), 20.94 (1C, Ac at C-3''''), 20.67 (2C, Ac), 20.60 (3C, Ac), 20.58 (1C, Ac).

Anal. Calcd for $C_{135}H_{150}O_{38}$: C, 68.10; H, 6.35. Found: C, 67.85; H, 6.39.

Methyl *O*-(α-D-Glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→4)-(2,3,6-tri-*O*-benzyl-α-D-gluco-pyranosyl)-(1→4)-*O*-(2,3,6-tri-*O*-benzyl-β-D-gluco-pyranosyl)-(1→ 4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-β-D-glucopyranoside (13). To a soln of **12** (0.54 g, 0.227 mmol) in tetrahydrofuran (2 mL) and methanol (10 mL) was a added a soln of sodium methanolate (0.5 mL of 2.0 g Na/ 100 mL methanol) at rt. The reaction mixture was kept for 16 h at rt, neutralized with Amberlite IR 120 (H^+) and filtered. After addition of a few drops of triethylamine, the filtrate and methanol washings were concentrated. The residue was chromatographed on silica gel using ethyl acetate/ methanol/ water 96 : 2 : 2 as eluent to obtain pure **13** (455 mg, 95 %) as a colourless foam: $[\alpha]_D$ +59.5 ° (*c* 0.2, chloroform); MS (FAB) *m/z* 2108.7 (100%, $[M + Na]^+$); ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.27-7.07 (m, 60H, aromat), 5.66 (d, 1H, $J_{1',2'}$ = 3.5 Hz, H-1'), 5.62 (d, 1H, $J_{1''',2'''}$ = 3.0 Hz, H-1'''), 5.62 (br s, 1H, H-1'''''), 3.55 (s, 3H, OCH_3).

Anal. Calcd for $C_{121}H_{136}O_{31}$: C, 69.66; H, 6.57. Found: C, 69.55; H, 6.62.

Methyl *O*-(α-D-Glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl)-(1→4)-(α-D-glucopyranosyl)-(1→4)-*O*-(β-D-glucopyranosyl-(1→4)-*O*-(α-D-glucopyranosyl-(1→4)-β-D-glucopyranoside (14). A soln of **13** (430 mg, 0.206 mmol) in ethanol (14 mL) and water (2 mL) was hydrogenated in the presence of 10 % palladium on charcoal (300 mg) at 1.1 bar for 16 h. To avoid precipitation of the product more water was added with a syringe during the reaction. The reaction mixture was filtered through a pad of filter aid and washed with ethanol water 1:1. After addition of a few drops of triethylamine the filtrate

was concentrated. The aqueous residue (ca. 10 mL) was lyophilized to obtain pure **14** (210 mg) as an amorphous colourless powder in quantitative yield; $[\alpha]_D$ +110.0 ° (*c* 0.1, water); MS (ionspray) *m/z* 1027.6 (100%, $[M + Na]^+$), 843.4 (15%, $[M + H]^+$); ^1H NMR (D_2O, 400 MHz) δ 5.42 (~d, 3H, H-1', H-1''', H-1''''), 4.54 (d, 2H, J = 7.9 Hz, H-1'',H-1''''), 4.40 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1), 3.58 (s, 3H, OCH_3).

Anal. Calcd for $C_{37}H_{64}O_{31}$: C, 44.22; H, 6.42. Found: C, 44.28; H, 6.52.

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