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Modulation of Antibody Affinity by Synthetic Modifications of the Most Exposed Pyranose Residue of a Trisaccharide Epitope

David R. Bundle*[a] and Eva Eichler[b]

[a]*Department of Chemistry, University of Alberta, Edmonton, Alberta, T6G 2G2 Canada*
[b]*Institute of Biological Sciences, National Research Council of Canada, Ottawa, Ontario, K1A 0R6 Canada*

Abstract—When the *Salmonella* trisaccharide epitope, methyl 3-*O*-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-2-*O*-(α-D-galactopyranosyl)-α-D-mannopyranoside **12** is bound by a monoclonal antibody Se155.4, the 3,6-dideoxy-α-D-hexose is completely buried, while the galactopyranosyl mannopyranosyl units lay across the protein surface. Crystallography of an antibody complexed with **12** also shows that the galactose residue is the most exposed saccharide. A simplified strategy to synthesize **12** and analogues modified at the galactose residue is described. Monosaccharide building blocks containing benzyl ether and ester protecting groups were used for efficient assembly of trisaccharides that can be deprotected by a single hydrogenolysis step, or occasionally preceded by a transesterification stage. Glycosylation of methyl 2-*O*-benzoyl-4,6-di-*O*-benzyl-α-D-mannopyranoside **4** by 2,4-di-*O*-benzyl-3,6-dideoxy-D-*xylo*-hexopyranosyl chloride **8** affords after transesterification the disaccharide acceptor **10**. This disaccharide serves as a universal acceptor for glycosylation by glycosyl donors that lead, following facile deprotection, to the α- and β-D-*galacto*, α- and β-D-*gluco*, and 2-amino-2-deoxy-α-D-galacto trisaccharides **12**, **14**, **17**, **18** and **21**. Only a small change in binding energy $\Delta(\Delta G)$ occurs when the α-D-galactopyranosyl residue of **12** is replaced by either an α-D-glucopyranosyl **17** or a 2-amino-2-deoxy α-D-galactopyranosyl unit **21**. Whereas binding of the β-D-glucopyranose congener **18** was tolerated by the antibody, the β-D-galactopyranose analogue **14**, showed a 250 fold loss of affinity. These relative activities of the trisaccharide congeners are correlated with a solved crystal structure for the antibody complex with **12**.

Introduction

Recognition of oligosaccharide epitopes presented to the immune system as components of vaccines initiates an immune response which in the case of pathogenic bacteria provides protection against subsequent infection through the generation of immunological memory and circulating antibodies with fine specificity for the carbohydrate antigens.[1] Natural infection by bacteria such as *Salmonella* also induces circulating polysaccharide specific antibody, the detection of which by chemically defined glycoconjugates provides a sensitive diagnostic test for the bacterium and its serotype.[2] Antibodies that bind to certain tumour associated antigens with oligosaccharide epitopes may also aid in cancer therapies.[3,4] Although the fine stereochemical specificity and structural requirements of antibody–carbohydrate interactions are well appreciated,[5-10] only recently have detailed crystal structures of oligosaccharide–antibody complexes become available.[11-14] These studies suggest that the size of the binding site for carbohydrate antigens generally lies at the lower limit of previous estimates,[9,10] between 2 and 4 saccharide residues.

The several high resolution crystal structures now available for oligosaccharides complexed with the *Salmonella* monoclonal antibody Se155.4 show in all cases a binding site complementary to the trisaccharide epitope **12**. The 3,6-dideoxy-D-*xylo*-hexose residue (abequose) is completely buried in a shallow binding pocket while mannose and galactose lie on the surface of the variable domains.[11-13] Solution NMR and crystal structure data are consistent with two bound conformations of the epitope.[12,15] This ligand flexibility is essentially confined to torsional variation about the galactose to mannose linkage, since the abequose to mannose torsional angles adopt values close to the minimum energy conformer.[15] One factor in this flexibility is the role of a hydrogen bond between Abe *O*-2 and Gal *O*-2. In the trisaccharide **12**–Fab complex this hydrogen bond is present, while in the complex of **12** with a single chain antibody molecule, the bond is bridged by a bound water molecule[13,15] (Figure 1). These results suggest that in the complex exposed residues may adopt conformations that are modulated by water molecules. However, it seems unlikely that such exposed residues will compete successfully with bulk solvent to form strong hydrogen bonds to amino acids close to the periphery of the binding site.[12]

In order to undertake a comprehensive investigation of the molecular recognition of this epitope, we developed a general synthetic scheme that was used with minor modification for the assembly of trisaccharide analogues bearing functional group replacements in each of the three saccharide residues. Systematic variation of the configuration and stereochemistry of the exposed residue that substitutes *O*-2 of mannose in the *Salmonella* trisaccharide epitope has been undertaken to investigate the relative contributions of isomeric saccharide residues to the overall interaction energy. In this paper we report the central protection strategy to obtain the native ligand **12**, and the synthesis of trisaccharides modified at the galactopyranose residue. The relative binding energies determined by titration calorimetry are compared with crystal structure data.

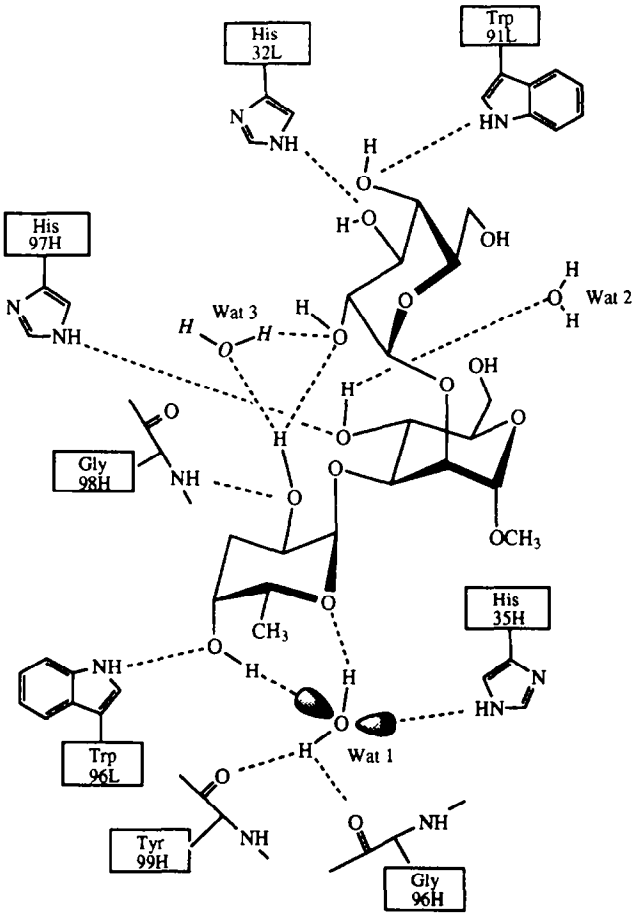
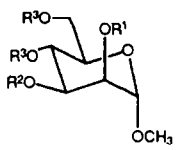


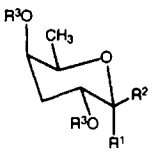
Figure 1. The hydrogen bonding map for trisaccharide–antibody interactions in the binding site, inferred from the protein crystal structures. The water molecule (Wat 3) shown in italics and positioned between Abe *O*-2 and Gal *O*-2 is present only when the direct hydrogen bond between these atoms is absent. This situation occurs in two solved crystal structures; trisaccharide **12** with single chain Fv, and a heptasaccharide with Fab.

Results and Discussion

Previous strategies for the synthesis of the branched trisaccharide **12** attached to a linking arm for subsequent glycoconjugate applications have utilized a mannose derivative protected by an acetal functionality.[16–18] In order to simplify deprotection strategies and avoid acid conditions toward which the 3,6-dideoxyhexose glycosidic linkage is particularly labile,[17,18] a mannose acceptor protected by benzyl ethers was selected. The readily available and crystalline methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside[19] **1** was benzylated to yield **2** which afforded the benzylated diol **3**. Regioselective benzoylation of **3** gave **4** by way of the 2,3-orthobenzoate intermediate.[20] Selective protection in this way provided for the introduction of abequose prior to introduction of the galactose residue (*c.f.* Refs 16–18).

The synthesis of 3,6-dideoxy-D-*xylo*-hexopyranose derivatives has been relatively inconvenient[16,21] when compared to the rather abbreviated routes to the *arabino*- or *ribo*- analogues.[22,23] However, a simple 6 step synthesis of **5** was recently reported from methyl α-D-glucopyranoside utilizing a high yield hydride reduction of methyl 3,4-anhydro-6-*O*-toluenesulphonyl-α-D-galactopyranoside.[24] Benzylation to give **6**[25] and acid hydrolysis to the hemiacetals **7a** and **7b** then provides easy access to the glycosyl halide **8** via *in situ* generation of Vilsmeier type reagents.[22]





1 $R^1, R^2 = C(CH_3)_2$, $R^3 = H$
2 $R^1, R^2 = C(CH_3)_2$, $R^3 = Bzl$
3 $R^1 = R^2 = H$, $R^3 = Bzl$
4 $R^1 = Bz$, $R^2 = H$, $R^3 = Bzl$

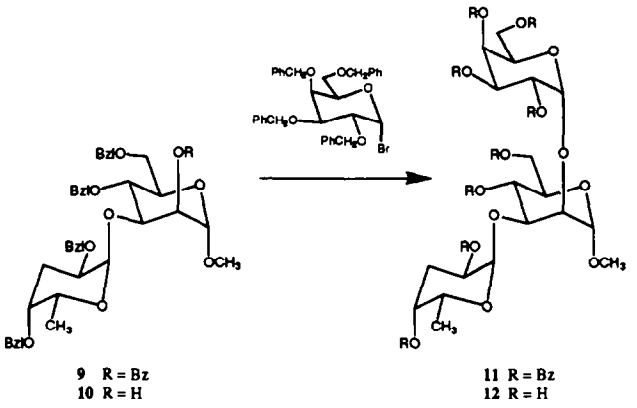
5 $R^1 = OCH_3$, $R^2 = R^3 = H$
6 $R^1 = OCH_3$, $R^2 = H$, $R^3 = Bzl$
7a $R^1 = OH$, $R^2 = H$, $R^3 = Bzl$
7b $R^1 = H$, $R^2 = OH$, $R^3 = Bzl$
8 $R^1 = Cl$, $R^2 = H$, $R^3 = Bzl$

The fully protected disaccharide **9** was obtained in 58 % yield by a Königs–Knorr reaction promoted by silver trifluoromethanesulphonate. The selectively protected acceptor **10** was isolated as a solid without chromatography following transesterfication of purified **9** or after chromatography following transesterification of crude **9**. This disaccharide served as a universal acceptor for a range of donors leading to the native trisaccharide epitope and four trisaccharide analogues.

Tetra-*O*-benzyl-α-D-galactose[26] was converted to the corresponding galactopyranosyl bromide by reaction with (bromomethylene)-dimethyliminium bromide generated *in situ* from oxalyl bromide and *N*,*N*-dimethylformamide (DMF).[22] Glycosylation of **10** using silver trifluoromethanesulphonate as promoter gave the perbenzylated trisaccharide **11** in 86 % yield, from which the deprotected trisaccharide **12** was obtained via a single hydrogenolysis step followed by gel filtration on a Biogel P-2 column. The protected trisaccharide containing a β-linked galactose residue was obtained by activation of ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside[27] by iodonium ions generated by *N*-iodosuccinimide (NIS)/trifluoromethanesulphonic acid[28,29] in the presence of **10**. The β-D-*galacto*-trisaccharide **13** was deprotected by transesterification and hydrogenolysis to yield **14**.

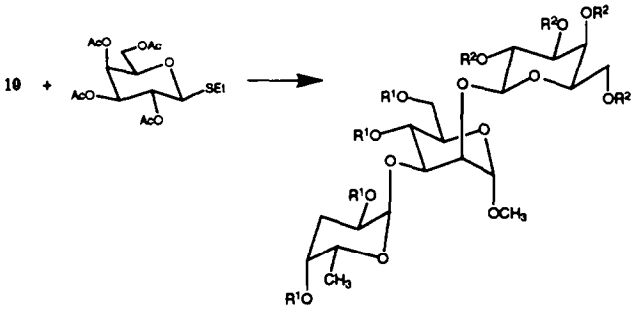
The corresponding α-D- and β-D-*gluco*-trisaccharides **15** and **16** were obtained from a single reaction, since under silver trifluoromethanesulphonate promoted glycosylation of **10** with tetra-*O*-benzyl-α-D-glucopyranosyl bromide[26] indifferent stereocontrol is exerted and the respective yields of **15** and **16** following chromatography were 45 % and 24 %. After hydrogenolysis the deprotected products **17** and **18** were purified by gel filtration.

As expected 2-azido-2-deoxy-3,4,6-tri-*O*-acetyl-β-D-galactopyranosyl chloride[30] gave stereoselectively and in good yield the trisaccharide **19**. In order to avoid $O \rightarrow N$ acetyl group migration, **19** was first transesterified to **20**. The benzyl protecting groups were then hydrogenolyzed with simultaneous generation of the amino functionality. The 2-amino-2-deoxy trisaccharide derivative **21** was isolated as its acetate salt. The ^1H and ^{13}C chemical shifts for the *H*-2 and *C*-2 resonances of **21** confirmed that no *N*-acetate formation had occurred.

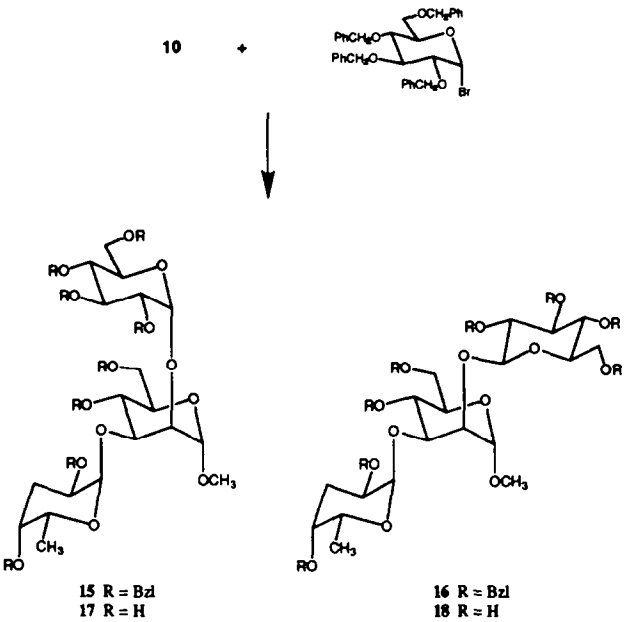


9 R = Bz
10 R = H

11 R = Bz
12 R = H

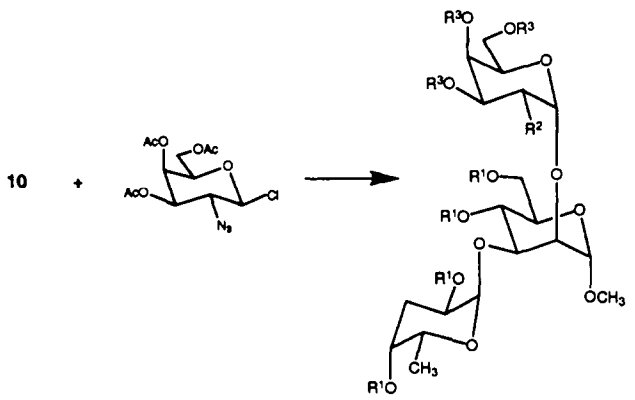


13 R^1 = Bzl, R^2 = Ac
14 R^1 = H, R^2 = H



15 R = Bzl
17 R = H

16 R = Bzl
18 R = H



19 R^1 = Bzl, R^2 = N_3, R^3 = Ac
20 R^1 = Bzl, R^2 = N_3, R^3 = H
21 R^1 = H, R^2 = NH_2, R^3 = H

The anomeric configuration of all glycosidic linkages was unambiguously assigned by heteronuclear single bond coupling constants observed for the anomeric carbon atoms[31] and supported by homonuclear $^3J_{1,2}$ values for the 1,2-*cis*-linked glycosides.

Titration calorimetry was used to determine association constants and binding enthalpies for the 5 deprotected trisaccharides with purified Se155.4 antibody.[12] This data then provides the Gibbs free energy and entropy.[32,33] The data is expressed as relative energy changes for stereoisomers **14**, **17**, and **18** and for the functional group replacement (OH → NH_2) **21** using the native ligand **12** as reference (Table 1). All four analogues are weaker binders but it is clear that **17** and **21** differ only slightly in activity. Since the Gal *C*-2 substituent acts as the proton acceptor group, the hydrogen bond between Abe *O*-2 and Gal *O*-2 (Figure 1) would be weakened by the presence of the 2-amino group which is expected to be protonated at the neutral pH of the assay. The hydrogen bond between Gal *O*-4 and Trp 91L (light chain residue #91) inferred from the crystallography data[11] (Figure 1) is also seen to be of minor consequence to complex stability since the *C*-4 epimer **17** displays high activity. Both the β-linked *galacto*- and *gluco*-trisaccharides **14** and **18** are significantly less active than the native ligand. While the altered anomeric configuration of the *gluco*- analogue **18** is tolerated, a dramatic effect is induced by the *galacto*-congener **14**. Uncharacteristically large changes in enthalpy and entropy are observed compared to the other ligands. In fact, it is the very large magnitude of the enthalpy for **14** that enables the accurate measurement of such weak binding by titration microcalorimetry. The experiment was reproduced with new batches of antibody and ligand and gave similar results within experimental uncertainty. The source of the huge enthalpy is puzzling. An enthalpy of this magnitude seems unlikely to stem from direct interactions with the neutral sugar moiety and without structural information definitive conclusions cannot be reached. Comparison with **18** indicates that the axial 4-OH group of **14** must play a crucial role and it is probable that the significantly different orientation of a β-Gal as opposed to an α-Gal residue results in new saccharide–protein contacts. These could induce mutually unfavourable conformational changes which result in the formation of strong ionic interactions for the peptide chain in a micro environment with a low dielectric constant. The very large entropy term signifies major losses of motional freedom.[12]

These data suggest that exposed surface residues such as the galactose unit of trisaccharide do not form strong hydrogen bonds with the antibody binding site. It is also seen that the inter-saccharide hydrogen bond is not a major determinant of bound oligosaccharide conformation as was originally proposed.[11] This is consistent with recent results that show the orientation of the exposed residue is determined in part by the participation of bound water molecules in the oligosaccharide complex.[13,15]

Table 1. Thermodynamics of antibody binding to modified trisaccharides[a]

Inhibitor		Relative Activity	Δ(ΔG)	Δ(ΔH) kJmol^{-1}	-Δ(TΔS)
αGal[Abe]Man	**12**	100.0	-	-	-
βGal[Abe]Man	**14**	0.4	+14.0	-82.7	+96.6
αGlc[Abe]Man	**17**	62.0	+1.3	-17.7	+18.4
βGlc[Abe]Man	**18**	7.0	+4.9	+8.4	-3.5
αGalNH$_2$[Abe]Man	**21**	62.0	+1.2	-6.4	+7.6

[a]Thermodynamic data taken from ref 12.

Experimental

General methods

Optical rotations were measured at 589 nm at 20 °C unless otherwise indicated. Column chromatography employed silica gel 60 (0.040–0.063 mm). The NMR spectra were recorded on Bruker AM-200, AM-500 and AMX-600 spectrometers. Unless otherwise indicated, ^{1}H NMR spectra were recorded at 500 and 600 MHz and ^{13}C NMR data were recorded at 50 MHz at ambient temperature. Chemical shifts were referenced as follows: solutions in $CDCl_3$ (internal standard, for ^{1}H: residual $CHCl_3$ δ_H 7.24; for ^{13}C: $CDCl_3$ δ_c 77.00), and D_2O (internal 1.0 % v/v acetone δ_H 2.225, δ_c 31.07). Carbon chemical shifts and first order proton chemical shifts and coupling constants were obtained from one-dimensional ^{13}C- and ^{1}H-NMR spectra. Assignments of proton and carbon resonances were based on COSY and ^{1}H–^{13}C correlated HMQC experiments. The ^{1}H–^{13}C correlated HMQC experiments were carried out without carbon-decoupling, permitting the measurement of the C–H coupling constants for the anomeric carbons in the F-2 dimension.

Titration microcalorimetry was performed with purified Se155.4 antibody according to previously reported protocols.[12,32,33]

Methyl 4,6-di-O-benzyl-3,4-O-isopropylidene-α-D-mannopyranoside (2)

A solution of methyl 3,4-*O*-isopropylidene-α-D-mannopyranoside[19] **1** (10.00 g, 42.7 mmol) in DMF (50 mL) was added dropwise to a solution of benzyl bromide (15.10 mL, 127 mmol), DMF (150 mL) and sodium hydride (3.10 g, 130 mmol) cooled to 0 °C. After stirring the reaction mixture overnight at room temperature, excess sodium hydride was quenched with methanol and the resulting solution was concentrated to a syrup. The residue was then dispersed in water (200 mL), extracted with ethyl acetate (2 × 300 mL) and the combined organic fractions were dried over $MgSO_4$. Removal of the solvent yielded an oil which was purified by chromatography (9:1, hexane: EtOAc) to give **2** (15.9 g, 90 % yield), [α] +42.7 ° (*c* 1.1, chloroform). ^{1}H NMR ($CDCl_3$): δ 7.36–7.25 (m, 10H, Ph), 4.96 (d, $J_{1,2}$ = 0.9 Hz, H-1), 4.85 (d, 1H, *J* = 11.7 Hz, CH_2Ph), 4.75 (d, 1H, *J* = 12.1, CH_2Ph), 4.54 (d, 1H, *J* = 12.1 Hz, CH_2Ph), 4.53 (d, 1H, *J* = 11.7 Hz, CH_2Ph), 4.30 (dd, 1H, $J_{3,4}$ = 6.9 Hz, H-3), 4.13 (dd, 1H, $J_{2,3}$ = 6.9, H-2), 3.80–3.51 (m, 4H, H-4, 5, 6), 3.39 (s, 3H, OMe); ^{13}C NMR ($CDCl_3$): δ 138.1, 128.2–127.5 (Ph), 109.3 (*C*Me$_2$), 98.2 (C-1), 78.6 (C-4), 75.8 (2C, C-2, C-3), 69.3 (C-6), 66.3 (C-5), 54.8 (OMe), 27.9, 26.3 (C*Me*$_2$). Anal. calcd for $C_{24}H_{30}O_6$: C, 69.54; H, 7.29. Found: C, 69.47; H, 7.38.

Methyl 4,6-di-O-benzyl-α-D-mannopyranoside (3)

Compound **2** (7.20 g, 17.4mmol) was added to an aqueous 90 % trifluoroacetic acid solution (55 mL) and the resulting mixture was stirred for 15 min at room temperature. The solution was then concentrated to a syrup and co-evaporated with toluene (3 × 150 mL). Recrystallization from diethyl ether/hexane gave **3** (5.00 g). Chromatography of the mother liquor (31:10:4, hexane:EtOAc:MeOH) yielded a further 1.00 g of **3** (92 % yield), mp 101–102 °C, [α] +75.0 ° (*c* 0.9, chloroform). ^{1}H NMR ($CDCl_3$): δ 7.38–7.17 (m, 10H, Ph), 4.78 (d, 1H, *J* = 11.0 Hz, CH_2Ph), 4.70 (s, 1H, H-1), 4.65 (d, 1H, *J* = 11.8 Hz, CH_2Ph), 4.54 (d, 1H, *J* = 11.0 Hz, CH_2Ph), 4.52 (d, 1H, *J* = 11.8 Hz, CH_2Ph), 3.95–3.68 (m, 6H), 3.36 (s, 3H, OMe), 3.00 (br s, 2H, 2,3-OH). Anal. calcd for $C_{21}H_{26}O_6$: C, 67.36; H, 6.70. Found: C, 67.16; H, 7.18.

Methyl 2-O-benzoyl-4,6-di-O-benzyl-α-D-mannopyranoside (4)

To a solution of triethylorthobenzoate (1.30 mL, 5.74 mmol) and *p*-toluenesulfonic acid (60mg) in DMF (25 mL), was added **3** (1.50 g, 4.01 mmol) and the resulting mixture was stirred for 5 h at 85 °C. Triethylamine (10 mL) was added and the mixture was concentrated to a syrup and dissolved in 80 % aqueous glacial acetic acid solution (100 mL). After stirring for 30 min at room temperature the mixture was concentrated and co-evaporated with toluene (2 × 200 mL). Chromatography (6:1, hexane:EtOAc) provided **4** (1.00 g, 52 %), [α] –17.0 ° (*c* 0.6, chloroform). ^{1}H NMR ($CDCl_3$): δ 8.15 (m, 1H, Ph), 7.70–7.30 (m, 14H, Ph), 5.48 (dd, 1H, $J_{2,3}$ = 3.1 Hz, H-2), 4.96 (d, 1H, *J* = 11.1 Hz, CH_2Ph), 4.97 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 4.87 (d, 1H, *J* = 12.0 Hz, 1H, CH_2Ph), 4.75 (d, 1H, *J* = 11.1 Hz, CH_2Ph), 4.67 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.38 (ddd, 1H, $J_{3,4}$ = 9.3 Hz, H-3), 4.13 (dd, 1H, $J_{4,5}$ = 9.3 Hz, H-4), 4.05–3.88 (m, 3H, H-5, H-6), 3.50 (s, OMe), 2.63 (d, 1H, 3-OH). Anal. calcd for $C_{28}H_{30}O_7$: C, 70.28; H, 6.32. Found: C, 70.04; H, 6.23.

Methyl 2,4-di-O-benzyl-3,6-dideoxy-α-D-xylo-hexopyranoside (6)

A solution of methyl 3,6-dideoxy-α-D-*xylo*-hexopyranoside[24] **5** (0.76 g, 4.69 mmol) in DMF (5 mL) was added to a suspension of sodium hydride (0.34 g, 14.2 mmol) in DMF (15 mL) at 0 °C and benzyl bromide (1.70 mL, 14.3 mmol) was added dropwise. The stirred mixture was allowed to reach room temperature overnight under nitrogen. Excess sodium hydride was then quenched with methanol and the solution was concentrated under reduced pressure. The residue was dissolved with CH_2Cl_2 (100 mL), washed with water (2 × 100 mL), dried over $MgSO_4$ and evaporated under reduced pressure. The resulting oil was purified by chromatography (25:1, hexane:EtOAc) to afford **6** 1.40 g (93 %); [α] +19.3 ° (*c* 1.5, chloroform), lit.[25] +18 °. ^{1}H NMR ($CDCl_3$): δ 7.50–7.20 (m, 10H, Ph), 4.79 (d, $J_{1,2}$ = 3.4 Hz, H-1), 4.70–4.62 (m, 3H, CH_2Ph), 4.46 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.03–3.88 (m, 2H, H-2, H-5), 3.49 (br s, 4H, H-4, OMe), 2.22 (ddd, $J_{3eq,3ax}$ = 12.8, $J_{2,3eq}$ = 4.0, $J_{3eq,4}$ = 4.0 Hz, H-3eq), 1.93 (ddd, $J_{2,3ax}$ = 12.8, $J_{3ax,4}$ = 2.8 Hz, H-3ax), 1.24 (d, 3H, $J_{5,6}$ = 6.3 Hz, H-6). Anal. calcd for $C_{21}H_{26}O_4$: C, 73.66; H, 7.65. Found: C, 73.36; H, 7.54.

2,4-Di-O-benzyl-3,6-dideoxy-D-xylo-hexopyranose (7a and 7b)

A solution of **6** (1.40 g, 4.09 mmol), in aqueous 80 % acetic acid (32 mL) and a 1 M aqueous hydrochloric acid solution (8 mL) was warmed to 85 °C and stirred for 2 h. The mixture was concentrated and the residue was co-evaporated with toluene (2 × 100 mL). The resulting syrup was purified by chromatography (7:3, hexane:EtOAc) to yield a mixture of the α,β isomers **7a** and **7b** (63 %, 0.84 g); ^{13}C NMR ($CDCl_3$): δ 138.6, 138.2, 138.1, 137.9 (Ph), 128.3–127.5 (Ph), 99.0 (C-1β), 90.7 (C-1α), 75.2, 75.0, 74.4, 73.7, 72.6 (C, C-4, -2α/β), 71.2 (CH_2Ph), 70.9, 66.1 (C-5α/β), 32.4, 27.0 (C-3α/β), 16.7, 16.3 (C-6α/β). Anal. calcd for $C_{20}H_{24}O_4$: C, 73.15; H, 7.37. Found: C, 72.95; H, 7.30.

2,4-Di-O-benzyl-3,6-dideoxy-α-D-xylo-hexopyranosyl chloride (8)

A solution of **7a/b** (0.68 g, 2.1 mmol), DMF (0.06 mL) and CH_2Cl_2 (15 mL) was treated dropwise with oxalyl chloride (0.56 mL, 6.3 mmol). After stirring for 3 h at room temperature the solution was concentrated under reduced pressure, diluted with a 1:1 CH_2Cl_2:hexane solution and filtered through glass-wool. The filtrate was then evaporated, dried for 1 h under vacuum, diluted with CH_2Cl_2 and stirred over 4 Å molecular sieves for 2 h. The glycosyl halide **8** (at least 95 % pure, judged from its ^{1}H NMR spectrum[34]) was obtained in greater than 90 % yield and due to limited stability it was used immediately in the glycosylation step.

Methyl 3-O-(2',4'-di-O-benzyl-3',6'-dideoxy-α-D-xylo-hexopyranosyl)-2-O-benzoyl-4,6-di-O-benzyl-α-D-mannopyranoside (9)

Activated, powdered molecular sieves 4 Å (700 mg) were suspended in a solution of **4** (70 mg, 0.146 mmol) dissolved in CH_2Cl_2 (3 mL) and the suspension was stirred under a N_2 atmosphere for 2 h. Silver trifluoromethanesulphonate (60 mg, 0.225 mmol) and 1,1,3,3-tetramethylurea (60 μL, 0.45 mmol) were added and the suspension was cooled to –45 °C. A CH_2Cl_2 solution of freshly prepared **8** (70.5 mg, 0.2 mmol) containing suspended molecular sieves (700 mg) (2 mL) was added and the mixture was allowed to come to room temperature overnight. TLC indicated little reaction and the reaction temperature was raised to 30 °C after further addition of silver trifluoromethanesulphonate (30 mg, 0.115 mmol). Trisaccharide formation was complete after 18 h. Triethylamine was added and the suspension was filtered through Celite. The filtrate was concentrated and the residue chromatographed (17:3, hexane:EtOAc) to yield **9** as a glass (98 mg, 58 %), [α][25] +28.8 °(*c*, 0.5, chloroform). ^{1}H NMR ($CDCl_3$): δ 8.2–7.1 (m, 25H, Ph), 5.42 (dd, 1H, $J_{2,3}$ = 2.9 Hz, H-2), 5.20 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1'), 5.16 (d, 1H, *J* = 11.3 Hz, CH_2Ph), 4.88 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.68 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.59 (d, 1H, *J* = 11.3 Hz, CH_2Ph), 4.52 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.49 (d, 1H, *J* = 12.1 Hz, CH_2Ph), 4.40 (d, 1H, *J* = 12.4 Hz, CH_2Ph), 4.37 (d, 1H, *J* = 12.4 Hz, CH_2Ph), 4.35 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3), 4.31 (d, 1H, *J* = 12.1 Hz, CH_2Ph), 4.17 (dd, 1H, $J_{4,5}$ = 9.4 Hz, H-4), 3.96 (m ,1H, H-5'), 3.90–3.81 (m, 3H, H-5, H-6, H-2'), 3.76 (bd, 1H, $J_{5,6}$ = 9.4 Hz, H-6), 3.42 (s, 3H, OMe), 3.31 (bs, 1H, H-4), 2.03 (m, 1H, H-3'eq), 1.81 (m, 1H, H-3'ax), 1.08 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6'); ^{13}C NMR (125 M Hz, $CDCl_3$): δ 99.49, 98.41 (C-1, C-1'). Anal. calcd for $C_{48}H_{52}O_{10}$: C, 73.01; H, 6.64. Found: C, 73.01; H, 6.59.

Methyl 3-O-(2,4-di-O-benzyl-3,6-dideoxy-α-D-xylo-hexopyranoside)-4,6-di-O-benzyl-α-D-mannopyranoside (10)

Method A: disaccharide **9** (2.6 g) was dissolved in methanol (150 mL) and 1 M sodium methoxide (7 mL) was added. After 18 h a further 2 mL of base was added and after 24 h the reaction mixture was worked up. The solution was neutralized with Amberlite (IR-120 H^+) ion exchange resin and the solid residue after filtration and concentration was triturated with hexane. The solid product **10** (2.03 g, 90 %) was pure by TLC, ^{1}H NMR and elemental analysis.

Method B: the crude disaccharide **9** was filtered through a small silica gel column, treated directly with saturated ammonia in methanol solution and the desired compound **10** was then isolated in 50 % yield by chromatography (3:1, hexane:EtOAc), [α] +83.0 ° (*c* 1.2, chloroform). ^{1}H NMR ($CDCl_3$): δ 7.38–7.03 (m, 20H, Ph), 5.18 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1'), 5.04 (d, 1H, *J* = 11.2 Hz, CH_2Ph), 4.77 (d, 1H, $J_{1,2}$ = 1.9 Hz, H-1), 4.61 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.53 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.47 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.60 (d, 1H, *J* = 11.2 Hz, CH_2Ph), 4.39 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.37 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.09 (dd, 1H, $J_{2,3}$ = 3.5, Hz, H-3), 4.08 (dq, 1H, $J_{4,5}$ = 1.8 Hz, H-5'), 4.00 (br s, 1H, H-2), 3.88 (dd, 1H, $J_{3,4}$ = 9.4, $J_{4,5}$ = 9.4 Hz, H-4), 3.87 (ddd, 1H, $J_{2,3ax}$ = 13.0, $J_{2,3eq}$ = 3.5 Hz, H-2'), 3.78 (ddd, 1H, $J_{5,6a}$ = 4.1, $J_{5,6b}$ = 2.0 Hz, H-5), 3.72 (dd, 1H, $J_{6a,6b}$ = 10.5 Hz, H-6a), 3.68 (dd,

1H, H-6b), 3.44 (br s, 1H, H-4'), 3.38 (s, 3H, OMe) 2.39 (br s, 1H, 2-OH), 2.12 (ddd, 1H, $J_{3eq,3ax}$ = 13.0, $J_{2,3eq}$ = 3.5, $J_{3eq,4}$ = 3.5 Hz, H-3'eq), 1.90 (ddd, 1H, $J_{2,3ax}$ = 13.0 Hz, $J_{3ax,4}$ = 2.5 Hz, H-3'ax) 1.18 (d, 3H, $J_{6,5}$ = 6.5 Hz, H-6'); ^{13}C NMR ($CDCl_3$): δ 100.3 ($^1J_{C,H}$ = 169 Hz, C-1), 98.5 ($^1J_{C,H}$ = 168 Hz, C-1'). Anal. calcd for $C_{41}H_{48}O_9$: C, 71.91; H, 7.06. Found: C, 72.04; H, 7.06.

*Methyl 2-O-(2,3,4,6-tetra-O-benzyl-α-D-galactopyranosyl)-3-O-(2,4-di-O-benzyl-3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-4,6-di-O-benzyl-α-D-mannopyranoside (11)*

Activated 4 Å molecular sieves (1 g) were added to a solution of **10** (90 mg, 0.132 mmol) in dry CH_2Cl_2 (distilled from P_2O_5) (2 mL) and the suspension stirred for 2 h. 1,1,3,3-Tetramethylurea (42 μL, 0.34 mmol) was added, followed by silver trifluoromethanesulphonate (60 mg, 0.23 mmol). This mixture was stirred for a few minutes, then cooled to –45 °C under N_2. A suspension of 2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl chloride[22] (145 mg, 0.26 mmol) and 4 Å molecular sieves in CH_2Cl_2 (1 mL) was added dropwise from a syringe and the mixture was allowed to warm to room temperature. TLC after 16 h showed the reaction to be incomplete and more silver trifluoromethanesulphonate (60 mg) and 1,1,3,3-tetramethylurea (42 μL) were added to the reaction mixture which was then heated to 30 °C. Reaction was complete in 1.5 h. Triethylamine (0.2 mL) was added to neutralize the reaction, and the solids were filtered off through Celite. The filtrate was evaporated and the residue chromatographed (4:1, hexane:EtOAc) to give **11** (137 mg, 86 %) of syrup, $[\alpha]^{25}$ +97.8 ° (*c* 0.5, chloroform). ^{1}H NMR ($CDCl_3$) (500 M Hz): δ 7.6–7.1 (m, 40H, 8-Ph), 5.62 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1"), 5.24 (d, 1H, $J_{1,2}$ = 3.0 Hz, H-1'), 5.08 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 5.00 (d, 1H, *J* = 11.7 Hz, C*H*$_2$Ph), 4.97 (d, 1H, *J* = 11.4 Hz, CH$_2$-Ph), 4.83 (d, 1H, $J_{1,2}$ = 1.3 Hz, H-1), 4.78 (d, 1H, *J* = 11.9 Hz, C*H*$_2$Ph), 4.77 (d, 1H, *J* = 11.8 Hz, C*H*$_2$Ph), 4.69 (d, 1H, *J* = 12.1 Hz, C*H*$_2$Ph), 4.63 (d, 1H, *J* = 11.5 Hz, C*H*$_2$Ph), 4.60 (d, 1H, *J* = 11.3 Hz, C*H*$_2$Ph), 4.53 (d, 1H, *J* = 11.6 Hz, C*H*$_2$Ph), 4.52 (d, 1H, *J* = 12.2 Hz, C*H*$_2$Ph), 4.46 (d, 1H, *J* = 11.7 Hz, C*H*$_2$Ph), 4.42 (d, 1H, *J* = 12.0 Hz, C*H*$_2$Ph), 4.39 (d, 1H, *J* = 11.8 Hz, C*H*$_2$Ph), 4.43–4.31 (m, 2H, H-3, C*H*$_2$Ph), 4.26 (d, 1H, *J* = 12.1 Hz, C*H*$_2$Ph), 4.23 (d, 1H, *J* = 12.1 Hz, C*H*$_2$Ph), 4.16–4.12 (m, 3H, H-2", H-5", H-4), 4.09 (d, 1H, H-2), 4.04–4.01 (m, 2H, H-5', H-3"), 3.94 (bs, 1H, H-4"), 3.79–3.76 (m, 3H, H-2', H-5, H-6), 3.70 (bd, 1H, $J_{5,6}$ = 9.2 Hz, H-6), 3.59 (dd, 1H, $J_{5,6}$ = 9.7 Hz, H-6"), 3.54 (dd, 1H, $J_{5,6}$ 6.1 Hz, H-6"), 3.30 (s, 3H, -OMe), 3.15 (bs, 1H, H-4'), 2.07 (m, 1H, H-3'eq), 1.85 (m, 1H, H-3'ax), 1.22 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6'). ^{13}C NMR (125 M Hz): δ 99.94 ($J_{C,H}$ = 172.4 Hz, C-1), 99.09 ($J_{C,H}$ = 171.6 Hz, C-1'), 97.80 ($J_{C,H}$ = 170.9 Hz, C-1"). Anal. calcd for $C_{75}H_{82}O_{14}$: C, 74.60; H, 6.84. Found: C, 74.30; H, 6.62.

*Methyl 2-O-α-D-galactopyranosyl-3-O-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-α-D-mannopyranoside (12)*

Hydrogenolysis of **11** (105 mg, 0.087 mmol) in glacial acetic acid (10 mL) over 5 % palladium on carbon (Pd/C) (100 mg) and column chromatography of the concentrated residue first on CHELEX 100 (BioRad), then BioGel P-2 (BioRad) using water as an eluant afforded 28.5 mg, 67 % of **12** [α] +134.8 ° (*c* 0.4, water). ^{1}H NMR (D_2O) (600 MHz): δ 5.143 (d, 1H, $J_{1,2}$ = 4.0 Hz, H-1") 5.098 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1') 5.065 (d, 1H, $J_{1,2}$ = 1.1 Hz, H-1), 4.098 (H-5'), 4.062 (H-5"), 4.022 (H-2'), 3.992 (H-4"), 3.97 (H-2, H-3), 3.950 (H-4), 3.905 (H-3"), 3.903 (H-6), 3.864 (H-4'), 3.786 (H-6), 3.782 (H-2"), 3.738 (H-6"), 3.652 (H-5), 3.415 (OMe), 1.976, 1.976 (H-3'eq, H3'ax), 1.180 (d, 3H, $J_{5,6}$ = 6.7 Hz, H-6'); ^{13}C NMR (125 MHz, D_2O): δ 101.68 (C-1"), 100.83 (C-1'), 99.40 (C-1), 78.73 (C-3), 78.54 (C-2), 73.72 (C-5), 71.52 (C-5"), 69.69 (C-3"), 69.03 (C-4"), 68.77 (C-2"), 67.51 (C-4'), 66.53 (C-4), 66.17 (C-5'), 63.28 (C-2'), 60.96 (C-6), 60.77 (C-6"), 54.24 (OMe), 34.87 (C-3'), 16.77 (C-6'). Anal. calcd for $C_{19}H_{34}O_{14}$; C, 46.91; H, 7.05; O, 46.06. Found: C, 46.78; H, 7.05.

*Methyl 2-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-3-O-(2,4-di-O-benzyl-3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-4,6-di-O-benzyl-α-D-mannopyranoside (13)*

Ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside[27] (108 mg, 0.275 mmol), disaccharide **10** (171 mg, 0.25 mmol) and freshly activated, powdered 4 Å molecular sieves (600 mg) were suspended in dry CH_2Cl_2 (10 mL) and the mixture was stirred under N_2 atmosphere for 2 h.

N-Iodosuccinimide (140 mg, 0.625 mmol) was added to the stirred suspension, followed by 0.17 mL aliquots of 0.15 M trifluoromethanesulphonic acid solution. The optimum yield of trisaccharide as judged by TLC was reached after the third aliquot. The reaction mixture was quenched by addition of triethylamine (0.5 mL). More CH_2Cl_2 was added, the suspension was filtered through Celite, washed with saturated sodium bicarbonate and 10 % sodium thiosulphate solution, dried over magnesium sulphate and concentrated. Column chromatography (4:1, hexane:EtOAc) afforded **13** (131 mg, 52 %) of [α] +27.7° (*c* 0.87, chloroform). ^{1}H NMR ($CDCl_3$) (500 MHz): δ 7.3–7.1 (m, 20H, 4 × CH_2*Ph*), 5.57 (d, 1H, $J_{3,4}$ = 3.0 Hz, H-4"), 5.42 (dd, 1H, $J_{2,3}$ = 10.3 Hz, H-2"), 5.29 (d, 1H, $J_{1,2}$ = 2.8 Hz, H-1'), 5.25 (d, 1H, *J* = 11.3 Hz, CH_2*Ph*), 5.20 (dd, 1H, $J_{3,4}$ = 10.5 Hz, H-3"), 4.89 (d, 1H, $J_{1,2}$ = 1.8 Hz, H-1), 4.80 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1"), 4.72–4.50 (m, 5H, 5 × CH_2*Ph*), 4.45 (dd, 1H, H-5'), 4.32 (dd, 1H, $J_{3,4}$ = 8.6 Hz, H-3), 4.28 (dd, 1H, $J_{5,6}$ = 7.2 Hz, H-6"), 4.20 (bs, 1H, H-2a), 4.15 (dd, 1H, *J* = 5.9 and 11.1 Hz, H-6"), 4.05–3.95 (m, 3H, H-5", H-2' and H-5), 3.87 (t, 1H, $J_{4,5}$ = 8.9 Hz, H-4), 3.82 (m, 2H, H-6), 3.62 (bs, 1H, H-4'), 3.55 (bs, 3H, -OMe), 2.1–1.9 (m, 14H, H-3'eq, H-3'ax and 4-OAc), 1.15 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6'). ^{13}C NMR (125 MHz): δ 99.21 ($^1J_{C,H}$ = 158 Hz, C-1"), 98.79 ($^1J_{C,H}$ = 167 Hz, C-1), 97.69 ($^1J_{C,H}$ = 167 Hz, C-1'). Anal. calcd for $C_{55}H_{66}O_{18}$: C, 65.08; H, 6.55. Found: C, 64.66; H, 6.62.

*Methyl 2-O-β-D-galactopyranosyl-3-O-(3,6-dideoxy-α-D-*xylo*-hexopyranosyl)-α-D-mannopyranoside (14)*

Trisaccharide **13** (130 mg, 0.128 mmol) was dissolved in

anhydrous methanol (10 mL), 1 M sodium methoxide (0.25 mL) was added and the mixture was stirred at room temperature overnight.

Neutralization with Amberlite (IR-120 H^+) ion exchange resin and subsequent chromatography (100:1, EtOAc: methanol) gave pure deacetylated trisaccharide. Debenzylation in glacial acetic acid (10 mL) over 10 % Pd/C (90 mg) and hydrogen for 18 h gave crude **14** after filtration through Celite. The concentrated crude residue was purified by chromatography (15:3:2, EtOAc:methanol: water) followed by gel filtration on a Bio-Gel P-2 column (1.6 × 95 cm) using water as eluant. Freeze drying of the pure fractions gave **14** (20 mg, 33 % yield) [α] + 58.6 ° (*c* 1.0, water). ^{1}H NMR (D_2O) (500 MHz): δ 5.17 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1'), 4.88 (s, 1H, H-1), 4.45 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1"), 4.17 (q, 1H, H-5'), 4.14 (bs, 1H, H-2), 4.05–3.85 (m, 7H, H-2', H-3, H-4", H-4, H-4', H-6" and H-6"), 3.76 (dd, 1H, *J* = 3.8 and 9.3 Hz, H-6), 3.68–3.63 (m, 4H, H-6, H-5, H-5" and H-3"), 3.55 (ddd, 1H, H-2"), 3.43 (s, 3H, -OMe), 2.09 (ddd, 1H, H-3'eq), 1.97 (m, 1H, H-3'ax), 1.15 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6'); ^{13}C NMR (125 MHz): δ 103.22 ($^1J_{C,H}$ 161.0 Hz, C-1"), 100.58 ($^1J_{C,H}$ = 173.3 Hz, C-1'), 99.60 ($^1J_{C,H}$ 172.2 Hz, C-1). Anal. calcd for $C_{19}H_{34}O_{14}$: C, 46.91; H, 7.05. Found: C, 46.61; H, 7.03.

Methyl 2-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-3-O-(2,4-di-O-benzyl-3,6-dideoxy-α-D-xylo-hexopyranosyl)-4,6-di-O-benzyl-α-D-mannopyranoside (15) and methyl 2-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-3-O-(2,4-di-O-benzyl-3,6-dideoxy-α-D-xylo-hexopyranosyl)-4,6-di-O-benzyl-α-D-mannopyranoside (16)

Oxalyl bromide (42 μL, 0.75 mmol) was added dropwise from a syringe to a stirred solution of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose[35] (135 mg, 0.25 mmol) in CH_2Cl_2 (5 mL) containing DMF (20 μL, 0.25 mmol) under N_2. After 2 h at room temperature, the solution was concentrated. Solids precipitated when the residue was dissolved in 1:1 hexane:EtOAc were filtered off through freshly dried silica gel. The filtrate was concentrated, dissolved in CH_2Cl_2 (5 mL) and then activated molecular sieves 4 Å (1 g) were added. This mixture was then stirred for 20 min before addition to the glycosylation reaction.

Disaccharide **10** (85 mg, 0.125 mmol) was dissolved in CH_2Cl_2 (5 mL) and activated molecular sieves 4 Å (1 g) were added. This suspension was stirred for 2 h. 1,1,3,3-Tetramethylurea (40 μL, 0.32 mmol) was added, followed by silver trifluoromethanesulphonate (85 mg, 0.32 mmol). This was stirred for 5 min, then while under N_2 atm, the solution was cooled to –45 °C. The glucosyl bromide was added dropwise from a syringe and the mixture was allowed to warm to room temperature over several hours. TLC after 16 h showed unreacted **10**, and another portion of silver trifluoromethanesulphonate (42 mg, 0.16 mmol) was added. After another 24 h all of the disaccharide acceptor **10** was consumed, and triethylamine (0.2 mL) was added to terminate the reaction. Solids were filtered off through Celite, and the filtrate was evaporated. Chromatography (7:1, hexane:EtOAc) gave 68 mg of **15** (45 %) and 36 mg of β isomer **16** (24 %).

Compound **15** α-isomer gave $[\alpha]^{25}$ + 69.1 ° (*c* 0.8, chloroform). ^{1}H NMR ($CDCl_3$): δ 5.75 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1b), 5.38 (d, 1H, $J_{1,2}$ = 3.2 Hz, H-1c), 5.20 (d, 1H, *J* = 11.9 Hz, CH_2Ph), 5.05 (d, 1H, *J* = 11.8 Hz, CH_2Ph), 5.00 (d, 1H, *J* = 10.8 Hz, CH_2Ph), 4.95 (d, 1H, *J* = 10.9 Hz, CH_2Ph), 4.87 (d, 1H, $J_{1,2}$ = 1.6 Hz, H-1), 4.82 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.77 (d, 1H, *J* = 10.3 Hz, CH_2Ph), 4.75 (d, 1H, *J* = 11.1 Hz, CH_2Ph), 4.69 (d, 1H, *J* = 12.1 Hz, CH_2Ph), 4.66–4.55 (m, 3H, 3-CH_2Ph, 4.51 (d, 1H, *J* = 12.0 Hz, CH_2Ph, 4.50 (d, 1H, *J* = 11.6 Hz, CH_2Ph), 4.44 (dd, 1H, $J_{3,4}$ = 9.7 Hz, H-3), 4.36 (d, 1H, *J* = 12.5 Hz, CH_2Ph), 4.35 (d, 1H, *J* = 12.0 Hz, CH_2Ph), 4.25 (m, 2H, H-4 and CH_2Ph), 4.16 (m, 2H, H-2 and H-3"), 4.08 (m, 1H, H-5'), 4.00 (m, 1H, H-5"), 3.90–3.72 (m, 8H, H-2', H-5, H-6, H-2", H-4", H-6"), 3.43 (s, 3H, -OMe), 3.37 (bs, 1H, H-4'), 2.08 (m, 1H, H-3'eq), 1.84 (m, 1H, H-3'ax), 1.24 (d, 1H, $J_{5,6}$ = 6.6 Hz, H-6'); ^{13}C NMR (125 MHz): δ 99.92 ($^1J_{C,H}$ = 172.7 Hz, C-1), 98.81 ($^1J_{C,H}$ = 173.3 Hz, C-1'), 96.77 ($^1J_{C,H}$ = 172.7 Hz, C-1"). Anal. calcd for $C_{75}H_{82}O_{14}$: C, 74.60; H, 6.84. Found: C, 74.30; H, 6.92.

Compound **16** β-isomer gave $[\alpha]^{25}$ +37.1 ° (*c* 0.5, chloroform). ^{1}H NMR ($CDCl_3$): δ 7.4–7.2 (m, 40H, 8-Ph), 5.3 (d, 1H, $J_{1,2}$ = 2.2 Hz, H-1'), 5.25–5.18 (m, 2H, 2-CH_2Ph), 5.02 (d, 1H, *J* = 10.9 Hz, CH_2Ph), 4.95 (bs, 1H, H-1), 4.88 (d, 1H, *J* = 10.7 Hz, CH_2Ph), 4.84 (d, 1H, *J* = 10.9 Hz, CH_2Ph), 4.65 (d, 1H, *J* = 10.5 Hz, CH_2Ph), 4.60 (d, 1H, $J_{1,2}$ = 7.8 Hz, H-1"), 4.56–4.37 (m, 10H, 9-CH_2Ph and H-5'), 4.32 (dd, 1H, $J_{2,3}$ = 3.5 and $J_{3,4}$ = 9.3 Hz, H-3), 4.22 (d, 1H, *J* = 12.3 Hz, CH_2Ph), 4.17 (bt, 1H, H-2), 4.09 (bt, 1H, $J_{4,5}$ = 9.9 Hz, H-4), 3.90–3.88 (m, 2H, H-2' and H-5), 3.74–3.60 (m, 7H, H-6, H-4", H-5", H-6", H-2"), 3.49–3.47 (m, 4H, H-3" and OMe), 3.42 (bs, 1H, H-4'), 2.15–2.06 (m, 2H, H-3'eq and H-3'ax), 1.23 (d, 3H, $J_{5,6}$ = 6.5 Hz, H-6'); ^{13}C NMR (125 MHz): δ 101.80 ((1C, $^1J_{C,H}$ = 157.08 Hz, C-1"), 99.00 (1C, $^1J_{C,H}$ = 171.89 Hz, C-1'), 98.08 (1C, $^1J_{C,H}$ = 169.93 Hz, C1). Anal. calcd for $C_{75}H_{82}O_4$: C, 74.60; H, 6.84. Found: C, 74.44; H, 6.84.

Methyl 2-O-α-D-glucopyranosyl-3-O-(3,6-dideoxy-α-D-xylo-hexopyranosyl)-α-D-mannopyranoside (17)

Hydrogenolysis of **15** (78 mg, 0.065 mmol) in glacial acetic acid (10 mL) using 5 % Pd/C (80 mg), and subsequent gel filtration on a Bio-Gel P-2 column (1.6 × 95 cm) afforded **17** (26 mg, 83 %) [α] +142.3 ° (*c* 0.1, water). ^{1}H NMR (D_2O): δ 5.15 (d, 1H, $J_{1,2}$ = 3.6 Hz, H-1"), 5.10 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1'), 5.03 (bs, 1H, H-1), 4.11 (q, 1H, H-5), 4.05–3.71 (m, 11H, H-2', H-2, H-6", H-4c, H6', H-5", H-4, H-3" and H-5), 3.67 (t, 1H, H3a), 3.52 (dd, 1H, $J_{2,3}$ = 3.7 and $J_{3,4}$ = 9.9 Hz, H-2b), 3.42 (s, 3H, -OMe), 3.4 (t, 1H, *J* = 9.4 Hz, H-4"), 2.03–1.97 (m, 2H, H-3'eq and H-3'ax), 1.2 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6'); ^{13}C NMR (125 HMHz): δ 101.72 ($^1J_{C,H}$ = 171.3 Hz, C-1"), 101.5 ($^1J_{C,H}$ = 171.3 Hz, C-1'), 100.5 ($^1J_{C,H}$ = 173.9 Hz, C-1). Anal. calcd

for $C_{19}H_{34}O_{14}$: C, 46.91; H, 7.05. Found: C, 46.62; H, 6.94.

Methyl 2-O-β-D-glucopyranosyl-3-O-(3,6-dideoxy-α-D-xylo-hexopyranosyl)-α-D-mannopyranoside (18)

Hydrogenolysis of **16** (85 mg 0.07 mmol) in 10 mL of glacial acetic acid over 5 % Pd/C (85 mg), followed by gel filtration on a Bio-Gel P-2 column using water as an eluant, afforded **18** (29.2 mg, 86 %), [α] +51.6 ° (*c* 0.4, water). ^{1}H NMR (D_2O): δ 5.15 (s, 1H, H-1'), 4.87 (s, 1H, H-1), 4.52 (d, 1H, $J_{1,2}$ = 7.7 Hz, H-1"), 4.21 (bd, 1H, H-5'), 4.14 (s, 1H, H-2), 4.03–3.94 (m, 2H, H-2', H-3), 3.92–3.77 (m, 5H, H-4, H-4', H-6, and H-6"), 3.74 (bd, 1H, H-6"), 3.66 (bd, 1H, $J_{4,5}$ = 8.5 Hz, H-5), 3.5–3.38 (m, 6H, H-3", H-4", H-5" and OMe), 3.3 (d, 1H, $J_{2,3}$ = 8.0 Hz, H2"), 2.17 (d, 1H, H-3'eq), 1.95 (bt, 1H, H-3'ax), 1.2 (d, 3H, $J_{5,6}$ = 6.1 Hz, H-6'); ^{13}C NMR (125 MHz); δ 102.40 ($^1J_{C,H}$ = 163.0, C-1"), 100.38 ($^1J_{C,H}$ = 172.1, C-1'), 99.25 ($^1J_{C,H}$ = 172.1 C-1). Anal. calcd for $C_{19}H_{34}O_{14}$: C, 46.91; H, 7.05. Found: C, 46.58; H, 7.11.

Methyl 2-O-(2-azido-2-deoxy-3,4,6-tri-O-acetyl-α-D-galactopyranosyl)-3-O-(2,4-di-O-benzyl-3,6-dideoxy-α-D-xylo-hexopyranosyl)-4,6-di-O-benzyl-α-D-mannopyranoside (19)

Disaccharide **10** (250 mg, 0.36 mmol) dissolved in dry CH_2Cl_2 (20 mL) (distilled from P_2O_5), and a mixture of powdered, freshly activated 4 Å molecular sieves (2 g), silver trifluoromethanesulphonate (210 mg, 0.82 mmol) and 1,1,3,3-tetramethylurea (145 μL, 1.22 mmol) were stirred for 1 h. A previously prepared suspension of 2-azido -2- deoxy - 3,4,6 - tri- *O*-acetyl-β-D-galactopyranosyl chloride[30] (350 mg, 1 mmol) in CH_2Cl_2 (15 mL) and 4 Å molecular sieves (3 g) were added from a syringe at room temperature. After stirring at room temperature for 1 h, the reaction mixture was heated to 36 °C and left under N_2 overnight. Additional silver trifluoromethanesulphonate (210 mg, 0.82 mmol) was added, and after 8 h there was an approximate 40 % conversion to product . A third portion of silver trifluoromethanesulphonate (210 mg, 0.82 mmol) was added and the reaction was continued at 30 °C for 72 h. Triethylamine (1 mL) was added to the mixture, followed by CH_2Cl_2 and filtration through Celite. The concentrated filtrate was evaporated and the residue was chromatographed (3:1, EtOAc:hexane) to give starting disaccharide **10** (45 mg, 21 %), and **19** (253 mg, 70 %), [α] +129.4 ° (*c* 0.5, chloroform). ^{1}H NMR ($CDCl_3$): δ 7.4–7.1 (m, 20H, -Ph), 5.51 (bs, 1H, H-4"), 5.48 (d, 1H, $J_{3,4}$ = 3.2 Hz, H-3"), 5.26 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1"), 5.24 (d, 1H, $J_{1,2}$ = 3.1 Hz, H-1'), 5.11 (d, 1H, *J* = 11.5 Hz, C*H*$_2$Ph), 4.91 (bs, 1H, H-1), 4.63–4.57 (m, 3H, C*H*$_2$Ph), 4.54 (b, 1H, $J_{5,6}$ = 6.3 Hz, H-5"), 4.50–4.40 (m, 4H, C*H*$_2$Ph), 4.27 (dd, 1H, $J_{2,3}$ = 2.7 and $J_{3,4}$ = 9.5 Hz, H-3), 4.19 (dq, 1H, H-5'), 4.17 (dd, 1H, $J_{5,6}$ = 5.5 Hz, H-6"), 4.14 (dd, 1H, H-6"), 4.08–3.98 (m, 2H, H-4, H-2), 3.9 (ddd, 1H, H-2'), 3.81 (m, 1H, H-5), 3.69 (bd, 2H, $J_{5,6}$ = 3.3 Hz), 3.65 (dd, 1H, $J_{2,3}$ = 10.4 Hz, H-2"), 3.51 (bs, 1H, H-4'), 3.44 (s, 3H, -OMe), 2.25–2.0 (m, 11H, 3-OAc, H-3'eq, H-3'ax), 1.24 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6'). Anal. calcd for $C_{53}H_{63}N_3O_6$: C, 63.78; H, 6.36; N, 4.21. Found C, 63.27; H, 6.22; N, 4.19.

Methyl 2-O-2-azido-2-deoxy-α-D-galactopyranosyl)-3-O-(2,4- di- O- benzyl- 3,6- dideoxy-α-D-xylo-hexopyranosyl)-4,6-di-O-benzyl-α-D-mannopyranoside (20)

Methanolic 1 M sodium methoxide (1.2 mL) was added to a solution of **19** (145 mg, 0.81 mmol) in dry methanol. After 16 h stirring at room temperature, the solution was neutralized (Amberlite IR-120 H^+ resin), filtered and concentrated. Chromatography (EtOAc) gave **20** (105 mg, 83 %), [α] + 167.8 ° (*c* 0.7, chloroform). ^{1}H NMR data ($CDCl_3$): δ 5.19 (d, 1H, $J_{1,2}$ = 3.0 Hz, H-1'), 5.15 (d, 1H, *J* = 11.7 Hz, C*H*$_2$Ph), 5.04 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1"), 4.93 (d, 1H, $J_{1,2}$ = 1.7 Hz, H-1), 4.55 (d, 1H, *J* = 12 Hz, C*H*$_2$Ph), 4.54 (d, 1H, *J* = 11.6 Hz, C*H*$_2$Ph), 4.48–4.38 (m, 5H, C*H*$_2$Ph), 4.23–4.20 (m, 2H, H-5', H-3), 4.12–4.08 (m, 2H, H-4, H-5"), 3.94 (dd, 1H, $J_{2,3}$ = 10.5 Hz, H-3"), 3.91 (bs, 1H, H-2), 3.86 (dt, 1H, H-2"), 3.75–3.62 (m, 5H, H-5, H-6", H-6), 3.60 (bs, 1H, H-4"), 3.50 (bs, 1H, H-4'), 3.43 (dd, 1H, H-2'), 3.37 (s, 3H, -OMe), 2.17–2.0 (m, 2H, H-3'eq, H-3'ax), 1.19 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6'); ^{13}C NMR (125 MHz): δ 100.0 (C-1"), 99.9 (C-1'), 99.1 (C-1). Anal. calcd for $C_{47}H_{57}N_3O_{13}$: C, 64.74; H, 6.59; N, 4.82. Found C, 65.08; H, 6.58; N, 4.70.

Methyl 2-O-(2-amino-2-deoxy-α-D-galactopyranosyl)-3-O-(3,6- di- deoxy- α- D- xylo-hexopyranosyl)-α-D-mannopyranoside (21)

Trisaccharide **20** (105 mg, 0.12 mmol) dissolved in acetic acid (15 mL) was hydrogenated over 10 % Pd/C (100 mg) for 18 h. Reaction was not complete, and hydrogenation was continued for 6 h after additional catalyst (100 mg) was added. The catalyst was filtered off through Celite. The filtrate was concentrated, and chromatographed on a small column (15:7:3, EtOAc:MeOH:H_2O). A Bio-Gel P-2 column (1.6 × 90 cm) eluted with 0.1 M ammonium acetate achieved the final purification to give **21** (70 %), [α] +112.2 ° (*c* 0.6, methanol). ^{1}H NMR (D_2O): δ 5.36 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1"), 5.23 (d, 1H, $J_{1,2}$ = 3.4 Hz, H-1'), 5.01 (bs, 1H, H-1), 4.1–3.92 (m, 8H), 3.90 (bs, 2H, H-6"), 3.81 (dd, 1H, $J_{5,6}$ = 5.6 Hz, H-5"), 3.75 (d, 2H), 3.68 (m, 1H, H-4"), 3.37 (bs, 3H, -OMe), 3.25 (dd, 1H, $J_{2,3}$ = 10.6 Hz, H-2"), 2.00 (m, 1H, H-3'eq), 1.92 (m, 4H, H-3'ax, -OAc), 1.2 (d, 3H, $J_{5,6}$ = 6.6 Hz, H-6'). ^{13}C NMR (125.76 MHz): δ 100.17 (2C, $^1J_{C,H}$ = 173.8 Hz, C-1, $^1J_{C,H}$ = 173.2 Hz, C-1'), 99.65 ($^1J_{C,H}$ = 173.8 Hz, C-1"). Anal. calcd for $C_{19}H_{35}NO_{13}$. CH_3COOH: C, 46.23; H, 7.21; N, 2.57. Found C, 45.80; H, 7.36; N, 2.41.

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