Synthesis of Three Tethered Trisaccharides to Probe Entropy Contributions in Carbohydrate–Protein Interactions

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Abstract. Crystal structure data show that the branched trisaccharide **1** constitutes the complete antigenic determinant of the *Salmonella* serogroup B antigen that is recognized by monoclonal antibody SE155.4. In an effort to characterize the entropic costs associated with immobilization of glycosidic torsional angles in the bound state, three distinct intramolecularly tethered analogues of this trisaccharide **2**–**4** have been synthesized. Two trisaccharides are tethered by a methylene acetal via the O-2 position of the 3,6-dideoxy-hexose, abequose to either O-2 of galactose (**2**) or O-4 of mannose (**3**). The third tether, α,α′-di-thio-*p*-xylene, spans the C-6 atoms of the mannose and galactose residues to create trisaccharide **4**. The acetal tethers of **2** and **3** span hydroxyl centers that are known to be involved in intramolecular sugar–sugar hydrogens bonds, but both trisaccharides are biologically inactive due to distorted conformations that cannot be accommodated in the antibody binding site. Trisaccharide **4** is active since both hydroxymethyl groups of galactose and mannose are solvent exposed in the bound state and the constrained conformation of **4** is virtually superimposable on the bound conformation of **1**. Despite the retained complimentary and significant reduction of torsional flexibility, trisaccharide **4** exhibits a $\Delta G^\circ = -7.6$ kcal mol^{-1} compared to $\Delta G^\circ = -7.1$ kcal mol^{-1} for **1**. The modest free energy gain for the tethered trisaccharide **4** arises from a small entropy gain ($T\Delta\Delta S = 0.3$ kcal mol^{-1}) and an even smaller enthalpic change ($\Delta\Delta H = -0.2$ kcal mol^{-1}).

INTRODUCTION

The crystal structure of the monoclonal antibody SE155.4 has been solved as both its Fab and single chain forms with various ligands at high resolution.[1–4] These data provide the atomic detail that is needed to address several issues related to the energetics of protein–sugar interactions. The principle objective of the syntheses reported here was an investigation of binding energy changes that result when trisaccharide **1** is pre-organized for binding by restriction of flexibility about the glycosidic linkages, i.e., the entropic loss when a protein binding site selects a single bound conformation. The resulting freezing or restriction of inter-saccharide bond rotamers that accompanies binding of unconstrained oligosaccharides has been estimated to carry a conformational entropy penalty as large as 1–2 kcal mol^{-1} per rotor.[5] Other estimates for freezing single bond rotamers are more conservative at ~0.6 kcal mol^{-1} per torsion.[6,7]

The binding site of the monoclonal antibody SE155.4 is dominated by aromatic amino acid residues and buries the 3,6-dideoxy-D-*xylo*-hexose (abequose, Abe) of the trisaccharide epitope **1**. The mannose and galactose residues are partially solvent exposed, although both are postulated to make hydrogen bonds to the antibody (Fig. 1).[1–3] To date, in all crystal structures,[1–3] the Abe–Man glycosidic linkage adopts a conformation close to that predicted by potential energy calculations.[2] Depending on the complex, the Gal–Man glycosidic linkage exists in one of two conformations, I and II (Fig. 2), which are related by a shift in the Φ/Ψ glycosidic torsional angles.[1,3,4] In the complex a hydrogen bond between Abe O-2 and Gal O-2 is either direct (conformation I)[1] or mediated by a water molecule (conformation II),[3] while the hydrogen bonding pattern to the galactose residue is also modified.[4]

In order to facilitate a comparison of the energetics of

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binding of tethered and native trisaccharides using titration microcalorimetry,[8] tethered trisaccharides **2**–**4** were synthesized. In the bound state or in solution, trisaccharide **1** has been shown to have hydrogen bonds between Abe O-2 and Gal O-2, and between Abe O-2 and Man O-4.[2] Trisaccharides **2** and **3** are each tethered by a methylene acetal in an attempt to simulate these conformations. Trisaccharide **4**, by contrast, is tethered between Gal C-6 and Man C-6, two relatively close and solvent-exposed atoms that are well removed from the protein surface, and hence reduce the possibility of protein–tether interactions. The activities of these and other tethered derivatives[9] have been assayed by solid phase binding and titration microcalorimetry.[8]

SYNTHESIS AND DISCUSSION

The design of the required synthons for the preparation of the tethered trisaccharides relied on thioglycosides as glycosylation donors since our previous experience suggested that this approach would give straightforward results.[10] In addition, the preparation of the desired tethered trisaccharides **2** and **3** required the synthesis of a common derivative of abequose **12** or **17** in which the OH-2 was selectively protected by a *p*-methoxybenzyl group, which could be selectively removed in order to introduce the methylene acetal tether. The synthesis of **2** and **3** required two other intermediates, **20** and **32**, in which the OH-2 of the galactose unit and OH-4 of the mannose residue were also selectively protected with the *p*-methoxybenzyl group. In the case of the tethered trisaccharide **4**, both the OH-6 groups of the mannose and galactose residues needed to be selectively protected and activated.

SYNTHESIS OF THE TETHERED TRISACCHARIDE 2

The presence of axial and equatorial hydroxyl groups in the 3,6-dideoxyhexosides **5** and **13**[10] suggests that there should be a significant difference in reactivity between these O-2 and O-4 positions. The axial hydroxyl group OH-2 of a 4,6-protected mannopyranoside is reported to undergo benzoylation or benzylation reactions more readily than the equatorial O-3, so that the O-2 benzoylated or benzylated derivatives were obtained as major products.[11]

Selective benzylation of the ethyl 1-thioglycoside of abequose was primarily carried out with the β-isomer **5**. Under mild conditions, using barium oxide-barium hydroxide octahydrate as base and benzyl bromide (1.1 equivalent) as reagent in an aprotic solvent, the reaction proceeded very slowly at 0 → 50 °C, and this approach was not pursued further. However, with sodium hydride as base, the reaction proceeded smoothly at 0 °C. After chromatography on silica gel, the 2-monobenzylated product **8** was isolated in 29% yield, along with the 2,4-dibenzylated compound **6** (17%) and 4-monobenzylated product **7** (6%) together with unreacted starting material **5** (25%).

To obtain the target abequose donor **12** in which the OH-2 was selectively protected by a *p*-methoxybenzyl group, we applied reaction conditions similar to those above with *p*-methoxybenzyl chloride. Similar results were observed, and the 2-*O*-*p*-methoxybenzylated product **11** was isolated in 33% yield, along with the 2,4-di-*O*-*p*-methoxybenzylated derivative **9** (26%) and 4-mono-*p*-methoxybenzylated **10** (14%). However, in this reaction no unreacted starting material **5** was recovered.

Our previous work[10] indicated that both α- and β-isomers of ethyl 3,6-dideoxy-1-thio-D-*xylo*-hexopyranoside react equally well during glycosylation. Here we used the α-isomer **17** as the glycosyl donor. Alkylation of **13** under conditions similar to those above gave the 2-*O*-*p*-methoxybenzylated product **16**, isolated in 27% yield, along with the 2,4-di-*O*-*p*-methoxybenzylated derivative **14** (23%) and 4-*O*-*p*-methoxybenzylated compound **15** (11%). The OH-4 hydroxyl groups in both the anomeric isomers **11** and **16** were subsequently benzylated, and the desired donors **12** (90%) and **17** (94%) were obtained in excellent yields.

The selective protection of the of ethyl 1-thiogalactopyranoside with a 2-*O*-*p*-methoxybenzyl group started with the acetal **18**.[12] Using aqueous fluoroboric acid in methanol at 0 °C,[12] the acetal groups were selectively removed in the presence of the acid-sensitive *p*-methoxybenzyl group. The triol **19** was isolated in 91% yield and perbenzylation afforded the desired donor **20** in 85% yield.

Glycosylation of the known mannopyranosyl acceptor **21**[13] by the abequose donor **12** proceeded smoothly in anhydrous dichloromethane using *N*-iodosuccinimide, and silver trifluoromethanesulfonate to give the desired disaccharide **22** in 64% yield. A similar yield was obtained with the corresponding α-isomer donor **17**. The 2-*O*-benzoate was then removed by transesterification (94%). Alcohol **23** was glycosylated with ethyl 1-thiogalactopyranoside **20** using methyl triflate as promoter in anhydrous dichloromethane, and the desired trisaccharide **24** was obtained in 94% yield. Subsequently, both the *p*-methoxybenzyl protecting groups were successfully removed by cerium ammonium nitrate in acetonitrile–water. The reaction proceeded rapidly at room temperature; and after chromatography on silica gel, the diol **25** was obtained in 73% yield.

Formation of the formaldehyde acetal and closure of the macrocyclic ring were completed in a one-pot reac-

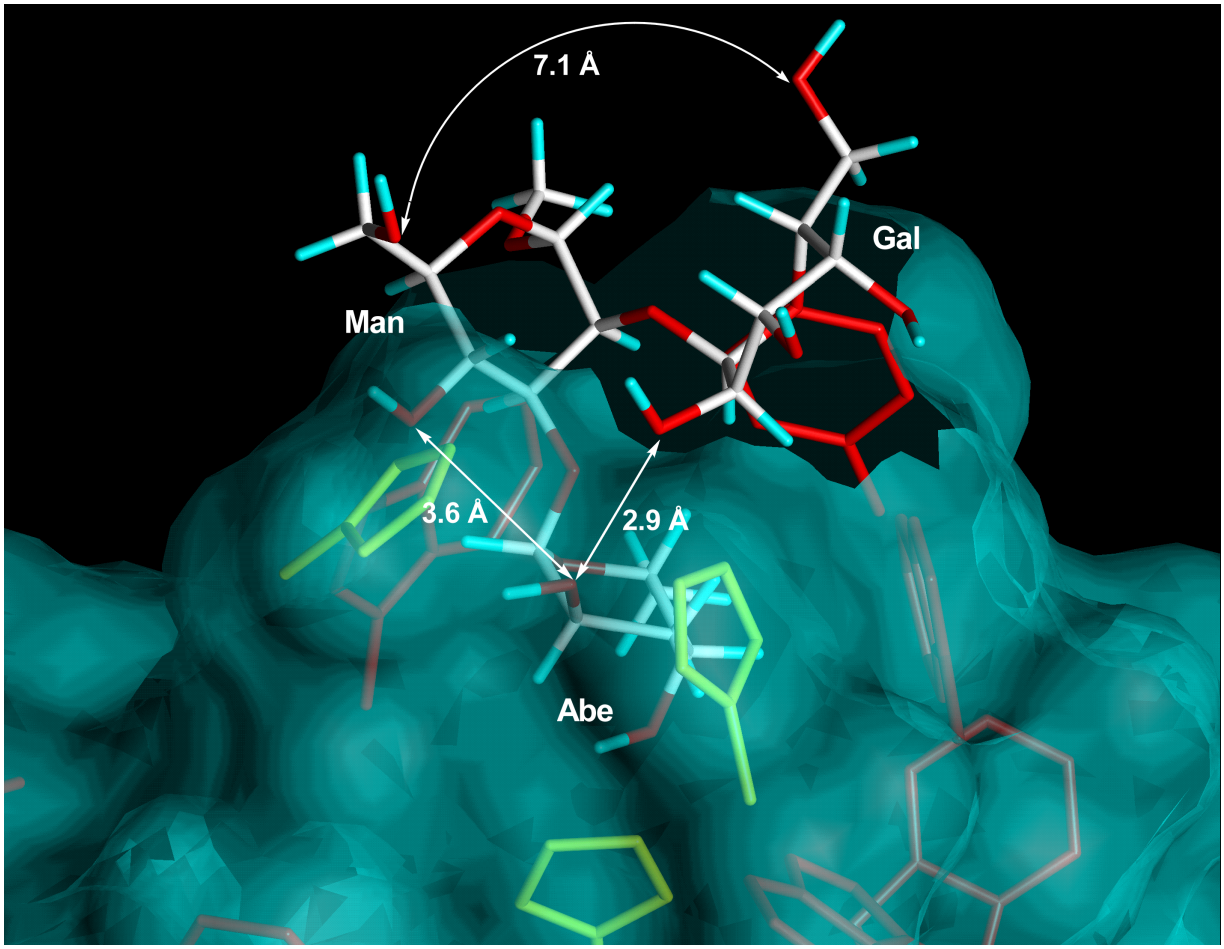
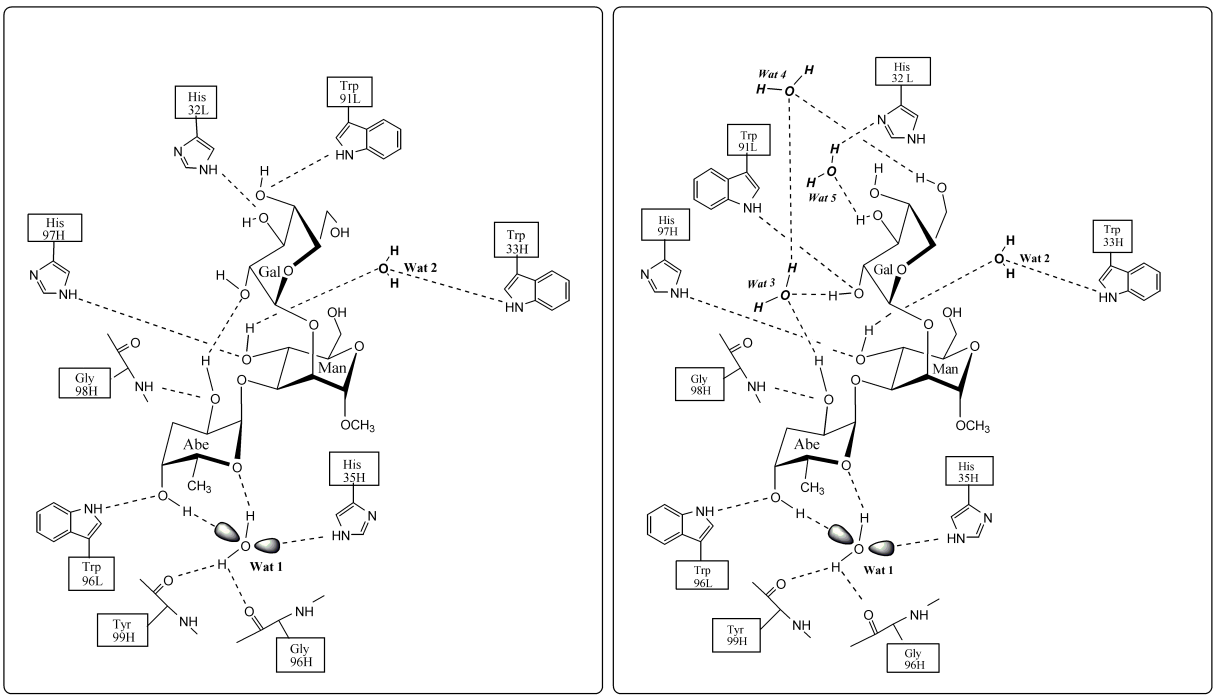


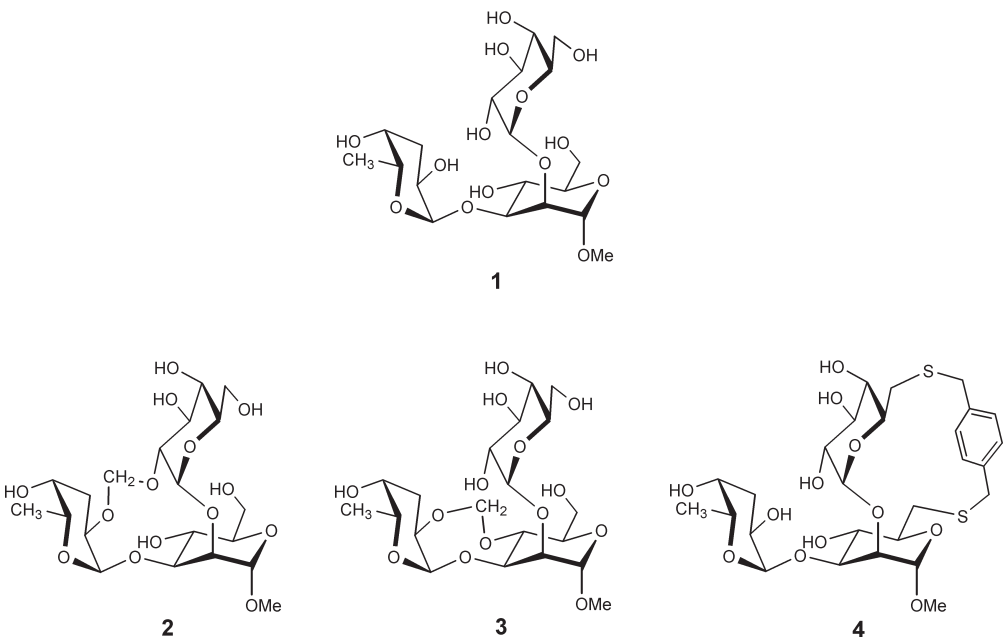
Fig. 1. Trisaccharide **1** complexed with antibody Se155.4. The abequose residue is buried in the binding site and the surfaces of both mannose and galactose make contact with the protein, while parts of mannose and most of the galactose residues are solvent-exposed. The interatomic distances for the two intramolecular sugar–sugar hydrogen bonds between Abe O-2 and Gal O-2 and Man O-4 are shown. Also shown is the interatomic distance between the 06 atoms of the Gal and Man hydroxymethyl groups.

tion.[14] In the presence of excess of sodium hydride, the diol **25** was first converted to the disodium salt and followed by addition of dibromomethane. Presumably the intermediate bromomethyl derivative may be attacked by either another oxygen anion in the same molecule or by one from another molecule. Intramolecular attack would lead to the desired tethered product **26**, while the intermolecular attack would lead to undesired oligomeric byproducts. As the two hydroxyl groups in the same molecule are close to each other in low energy conformations, the desired tether should be the major component in the reaction mixture. The reaction was carried out by treating the diol **25** with 10 equiv of sodium hydride in anhydrous *N*,*N*-dimethylformamide and using only 1.3 equiv of dibromomethane. After 24 h, TLC indicated the presence of one major spot. Although NMR showed the presence of an unidentified impurity (~30%) together with the major compound, chromatography on silica gel using different eluents could not resolve the mixture. The pattern of the methylene acetal signals could not be identified since they were overlapped by the signals of benzyl protecting groups. However, comparison with the NMR spectrum of **25** suggested the formation of the desired product. The chemical shifts for the H-1 of the galactose unit shifted significantly downfield from 5.10 to 5.91 ppm, while the H-1s of abequose and mannose residues remained roughly the same. The H-3e resonance of the abequose unit shifted upfield from 2.16 to 1.93 ppm, while the H-3a resonance shifted downfield from 1.60 to 2.03 ppm, respectively. The H-6 of the abequose unit shifted upfield from 1.07 ppm to 0.86 ppm. These are strong indications that the methylene group was successfully incorporated at the two hydroxyl positions. The pres-

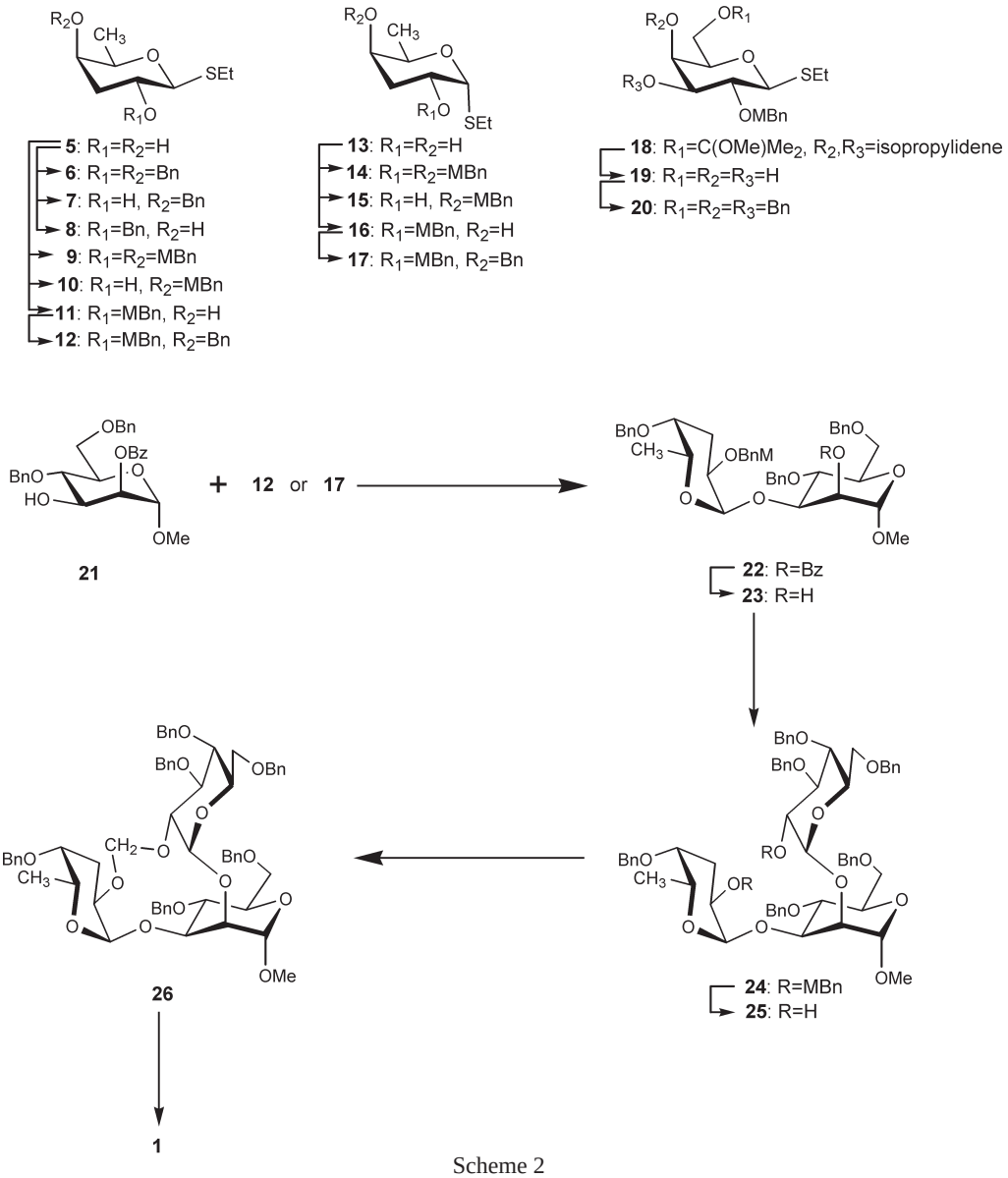


Hydrogen Bond Map for Bound Conformer I **Hydrogen Bond Map for Bound Conformer II**

Fig. 2. Hydrogen bonding maps for the solved crystal structures of trisaccharide **1** complexed with Fab[2] and with single chain Fv[3]. Conformation I is observed when **1** is complexed with Fab and has a directed Abe O-2 to Gal O-2 hydrogen bond. Bound conformation II is observed in the single chain Fv crystal structure and shows the same hydrogen bond, but in this case mediated via a bound water molecule.



Scheme 1

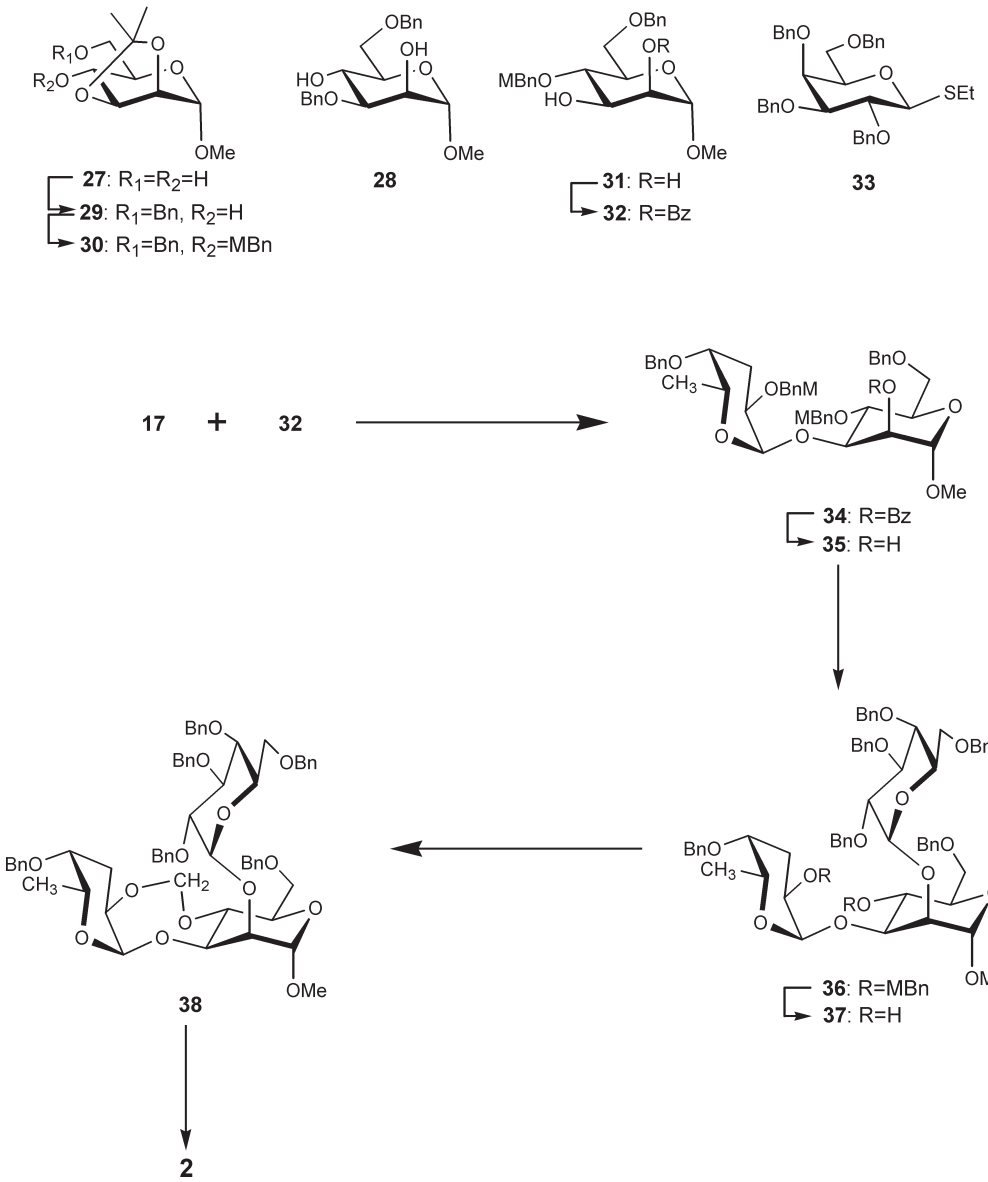


Scheme 2

ence of such a short methylene group in the center of the molecule alters significantly the conformation of the glycosidic linkages, leading to substantial chemical shift changes. The success in introducing the methylene group was finally proved after removing all the protecting groups. Hydrogenation in methanol solution over 5% palladium on charcoal gave the target tethered trisaccharide after purification by chromatography on silica gel, followed by reverse phase chromatography on a C18 column. The overall yield for the two steps was 15%. In addition to the three anomeric protons, the NMR spectrum showed clearly in the region 4.5 ppm to 5.5 ppm, two sets of doublets resonating at 5.12 ppm (J = 8.4 Hz) and 4.93 ppm (J = 8.4 Hz), which correspond to the two protons of the methylene tether. This was confirmed by a two-dimensional experiment (T-ROESY) that measured the through-space interaction and which clearly showed that one of these two protons interacts with H-2 of the galactose unit, while the other interacts with H-2 of the abequose unit.

SYNTHESIS OF THE TETHERED TRISACCHARIDE 3

The synthesis of tethered trisaccharide **3** started with the preparation of the mannose acceptor **32** selectively protected at the O-4 position. Using a known 2,3-*O*-isopropylidene mannopyranoside **27**[15] as starting material, the O-6 position was selectively benzylated using

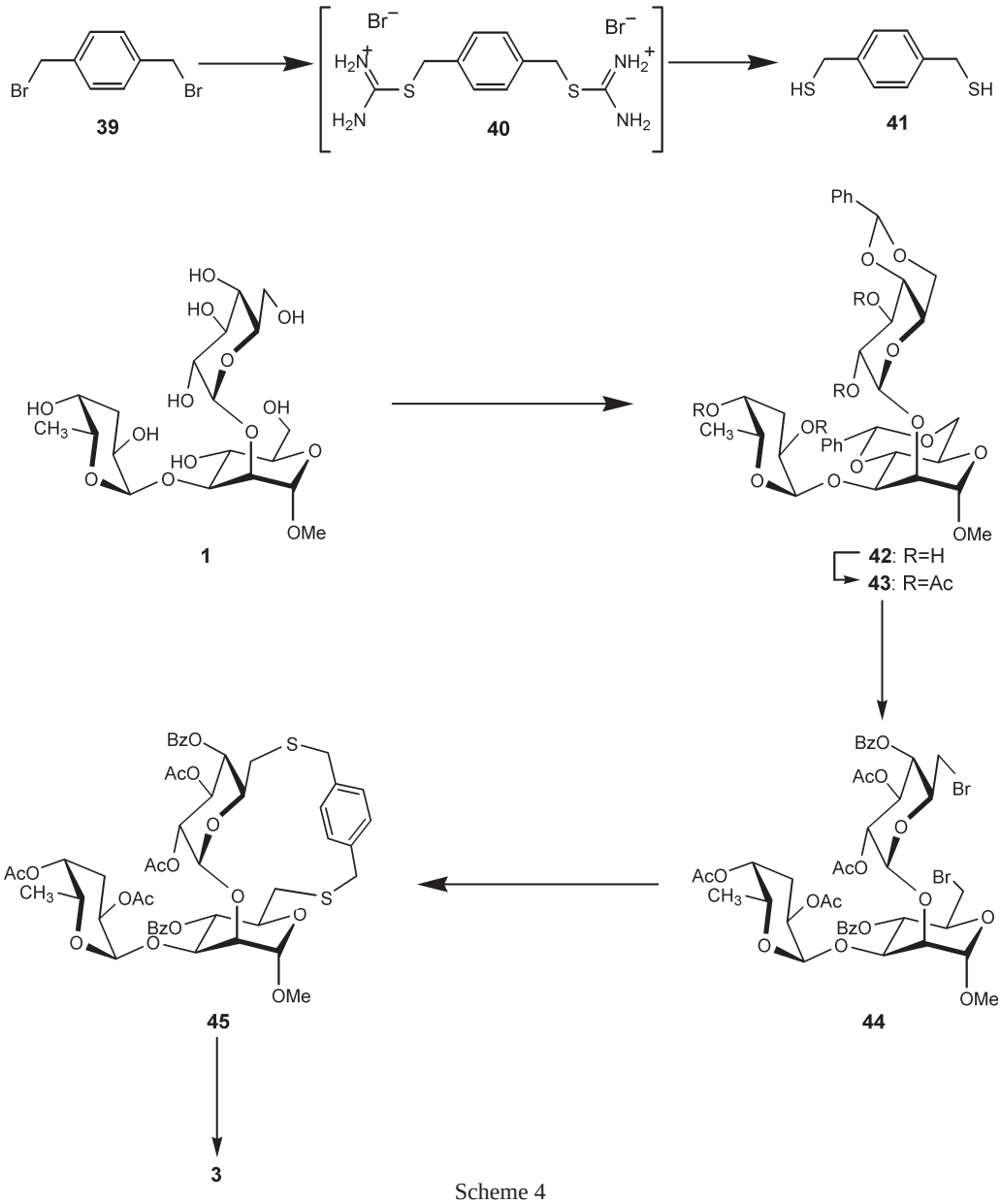


Scheme 3

di-*n*-butyltin oxide as activator and benzyl bromide as reagent.[16,17] The 6-*O*-monobenzylated product **29** was isolated in 54% yield. Interestingly, no 4-*O*-monobenzylated product was observed. Instead, a 3,6-di-*O*-benzylated derivative **28**[18] was obtained as the major side product in 17% yield. A possible mechanism to account for this observation involves reaction of a second molecule of dibutyltin oxide with the initially formed 4,6-stannylene acetal via coordination to O-3 of compound **27**, followed by hydrolysis of the activated acetonide to a transiently formed 2,3:4,6-di-*O*-stannylene acetal (Scheme 5). Selective benzylation at the 3 and 6 positions gives **28**.

The alcohol **29** was *p*-methoxybenzylated as above to afford **30** in 91% yield. In this case, the removal of the isopropylidene acetal in the presence of an acid-sensitive *p*-methoxybenzyl group proceeded more slowly than for the galactose derivative **19**. After 48 h reaction with aqueous fluoroboric acid in methanol, the 2,3-diol **31** was isolated in 81% yield. The diol was selectively benzoylated at O-2 through a 2-step reaction sequence using trimethyl orthobenzoate to form first the 2,3-cyclic orthoester, followed by regioselective opening in 80% aqueous acetic acid to give the desired acceptor **32** in 88% yield.

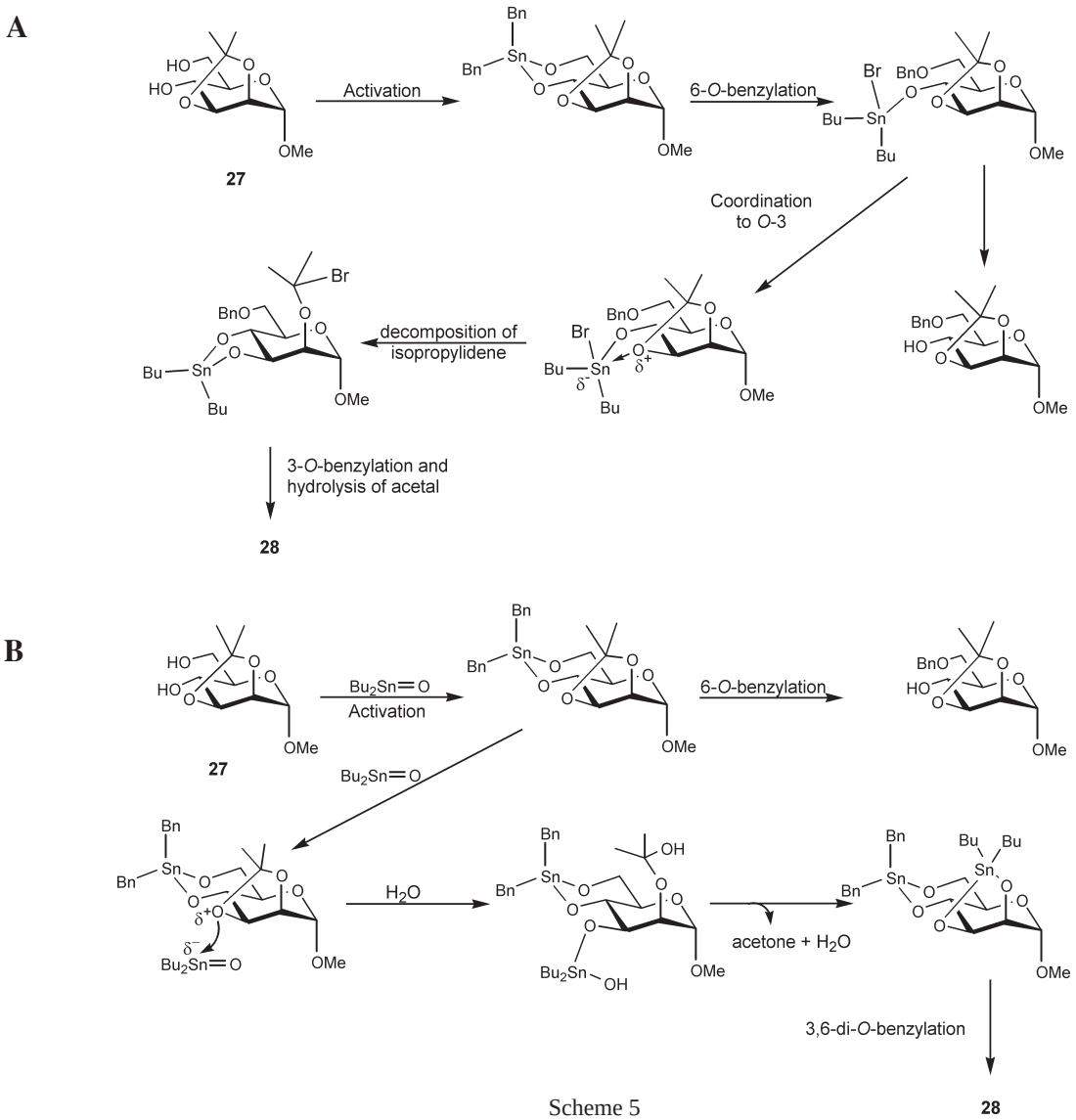
Glycosylation of the acceptor **32** by donors **12** or **17**, using methyl triflate as promoter, gave the disaccharide



Scheme 4

34 in 83% yield after chromatography. The benzoate of disaccharide **34** was first removed (→ **35**, 93%), and the resulting disaccharide acceptor **35** was glycosylated with the thiogalactoside **33**, using methyl triflate as activator. The target trisaccharide **36** was obtained in 83% yield. Both *p*-methoxybenzyl groups were successfully removed using the cerium ammonium nitrate method (→ **37**, 79%). By analogy with **25**, after reacting the disodium salt of **37** with dibromomethane, the tethered trisaccharide **38** was isolated in pure form in 27% yield. The NMR spectrum of the tethered trisaccharide **38** compared to the precursor **37**, exhibited marked changes in chemical shifts. The anomeric protons of the galactose unit and abequose unit experience downfield shifts from δ_H 5.17 and 5.05 ppm to 5.55 and 5.44 ppm. The final tethered trisaccharide **3** was obtained in 90% yield after the removal of the benzyl groups.

The NMR spectrum of the final trisaccharide **3** showed beside the three anomeric protons, two sets of doublets resonating at 4.93 ppm (J = 6.6 Hz) and 4.85 ppm (J = 6.6 Hz), which correspond to the two protons of the methylene tether. Two dimensional T-ROESY demonstrated that one of the tether protons correlates with the H-4, H-6a, and H-6b protons of the mannose unit, while the other tether proton correlates only with H-2 of the abequose unit.



Scheme 5

SYNTHESIS OF THE TETHERED TRISACCHARIDE 4

The synthesis of the tethered trisaccharide **4** required activation at the O-6-positions of both the galactose and mannose units. As there are only two primary positions in this trisaccharide structure, we chose to activate both O-6 positions in a single-pot reaction at the trisaccharide stage.

Using trisaccharide **1**[13] as starting material, two strategies were considered. The first concerned the use of a bulky trityl protecting group to selectively protect the primary positions, followed by protection of the remaining hydroxyl groups and selective removal of the trityl groups. The primary hydroxyl groups could then be selectively activated by sulfonates. However, our investigation showed that tritylation was not completely selective. Two major products were present in the reaction mixture, and this strategy was abandoned.

The second strategy involved the use of two benzylidene groups to protect the 4- and 6-positions of both the mannose and galactose units. The remaining hydroxyl groups could be protected using acetate esters. NBS opening of both the 4,6-benzylidene acetal[19,20] would lead simultaneously to an intermediate activated in both the mannose and galactose units. Thus, reaction with α,α-dimethoxytoluene gave the diacetal **42** in 54% yield, and the remaining hydroxyl groups were then acetylated (→ **43**, 87%). Selective opening of the acetals was achieved by reacting **43** with *N*-bromosuccinimide (3 equiv) in anhydrous carbon tetrachloride, and the dibromide **44** was obtained in 63% yield after chromatography.

Table 1
500 MHz ^{1}H NMR of the tethered trisaccharides[a] **2**, **3**, and **4**

		H1 (J_{12})	H2 (J_{12}, J_{23})	H3 (J_{23}, J_{34})	H4 (J_{34}, J_{45})	H5	H6 (J_{56})	H6′ ($J_{56'}$, $J_{66'}$)
2	Abe	5.90	3.94	2.12/1.95	3.90	4.19	1.13	
		(3.5)	(13.1,3.0)	(4.0)	(1.4)		(6.6)	
	Man	5.08	3.98	4.18	4.24	3.66	3.94	3.83
		(1.4)	(3.5)	(10.5)	(9.2)		(2.2)	(6.0)
	Gal	5.23	3.76	3.99	3.98	4.05	3.76	3.73
		(4.0)	(9.1)	(3.4)	(1.0)		(6.8)	(4.9)
	Tether: 5.12 (8.4), 4.93 (8.4); OMe: 3.40							
3	Abe	5.52	3.96	2.12/1.97	3.88	4.06	1.15	
		(4.0)	(12.1,5.6)	(3.0,3.7)	(0.9)		(6.6)	
	Man	4.96	4.24	3.98	4.06	3.73	3.86	3.77
		(1.8)	(3.0)	(9.7)	(9.7)		(2.0)	(6.0)
	Gal	5.28	3.77	3.92	3.98	4.01	3.77	3.76
		(4.0)	(10.3)	(3.3)	(1.1)		(7.4)	(4.8)
	Tether: 4.93 (6.6), 4.85 (6.6); OMe: 3.41							
4	Abe	5.30	3.96	1.95/1.93	3.82	3.98	1.13	
		(3.8)					(6.6)	
	Man	4.14	3.64	3.71	3.58	3.36	3.23	2.72
		(1.7)	(3.5)	(3.1)	(9.8)		(2.1,15.2)	(7.5,15.2)
	Gal	4.84	3.68	3.65	4.20	3.46	2.95	2.83
		(3.8)	(3.9,10.6)	(3.7,10.6)	(1.1,3.3)		(6.7,15.0)	(7.6,15.0)
	Tether: 7.49, 7.36, 7.24, 7.22, 3.87 (14.8), 3.81 (14.8), 3.79 (14.5), 3.76 (14.5); OMe: 3.39							

[a] The chemical shifts for trisaccharide **1** in D_2O are reported in ref 2.

Table 2
500 MHz ^{13}C NMR of the tethered trisaccharides[a] **2**, **3**, and **4**

		C1	C2	C3	C4	C5	C6	$J_{C1,H1}$
	Abe	95.0	75.8	32.4	69.6	67.4	16.5	174.5
2	Man	100.6	83.3	77.6	64.3	73.4	61.9	173.8
	Gal	102.6	79.8	68.5	70.4	72.2	62.6	171.6
	Tether:100.4; OMe: 55.8							
	Abe	100.1	71.5	32.7	68.8	68.5	16.8	169.4
3	Man	100.8	77.7	80.0	73.0	72.2	61.0	173.3
	Gal	101.5	69.9	70.5	70.4	72.7	62.5	173.3
	Tether: 94.7; OMe: 55.9							
	Abe	101.0	64.2	33.5	69.3	67.6	15.9	171.8
4	Man	99.9	81.8	78.2	70.2	72.6	34.0	173.1
	Gal	103.4	69.2	69.9	70.6	72.2	34.8	171.6
	Tether: 39.4, 39.0; OMe: 55.8							

[a] The chemical shifts for trisaccharide **1** in D_2O are reported in ref 2.

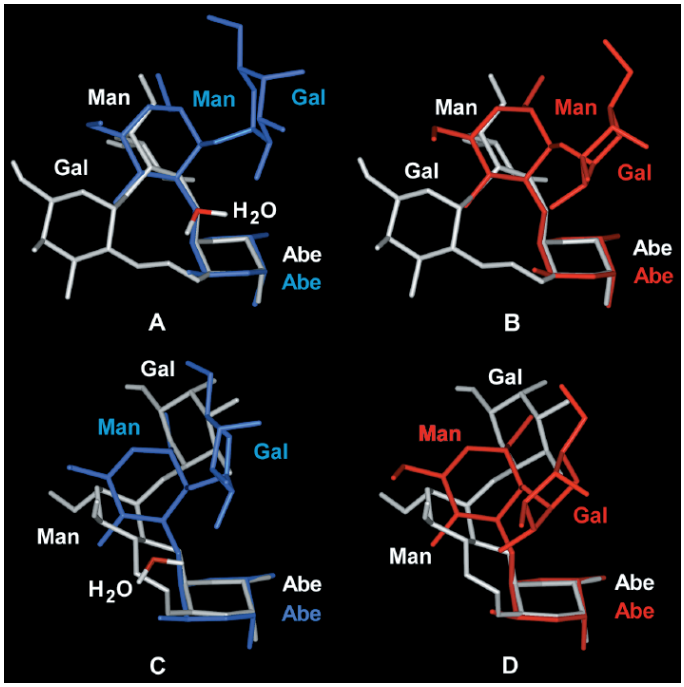


Fig. 3. A comparison of the two bound conformations of trisaccharide **1**, conformations I and II with the conformations adopted by the acetal-tethered trisaccharides **2** and **3**. In each case, the coordinates of the abequose residue were superimposed to display the relative orientations of the mannose and galactose residues in the tethered trisaccharides. (**A**) Conformation II of trisaccharide **1** (blue), superimposed on trisaccharide **2** (white). The bound water that mediates the hydrogen bond present in the bound conformation II is shown. (**B**) Conformation I of trisaccharide **1** (red), superimposed on trisaccharide **2** (white). (**C**) Conformation II of trisaccharide **1** (blue), superimposed on trisaccharide **3** (white). The bound water that mediates the hydrogen bond in the bound conformation II is shown. (**D**) Conformation I of trisaccharide **1** (red), superimposed on trisaccharide **3** (white).

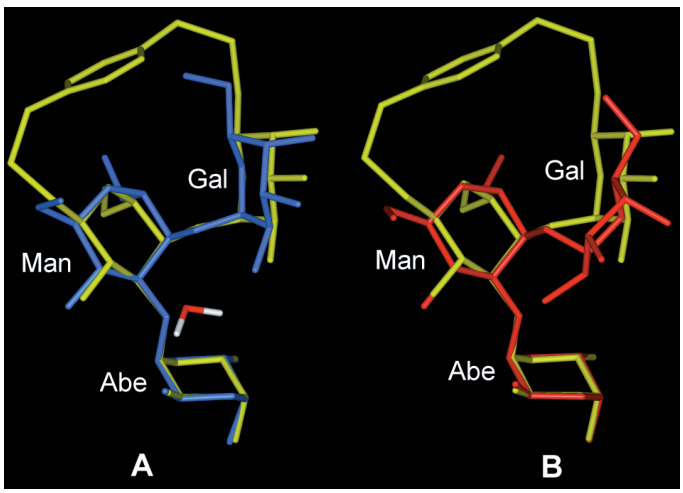


Fig. 4. The tethered trisaccharide **4** (yellow) superimposed on **A**, bound conformation II (blue) and **B**, bound conformation I (red) of trisaccharide **1**. The coordinates of the abequose residue were superimposed to display the relative orientations of the mannose and galactose residues in the tethered and non-tethered trisaccharides.

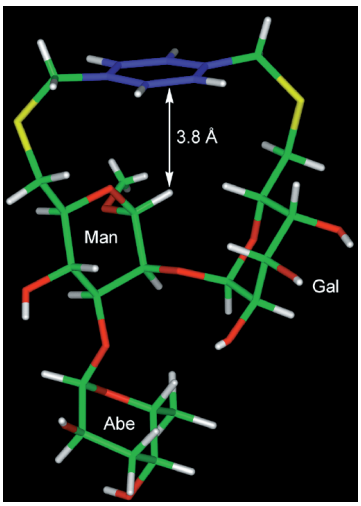


Fig. 5. The proximity of the Man H-1 proton to the center of the aromatic ring of the tether in trisaccharide **4** is illustrated and this shielding by the ring current accounts for the substantial upfield shift in the resonance of this proton.

The α,α′-di-thio-*p*-xylylene tether **41** was prepared by reacting the commercial α,α′-dibromo-*p*-xylene **39** with thiourea, followed by hydrolysis of the intermediate thiourea salts **40**. The dimercaptane **41** was converted to the dipotassium salt before use. The introduction of the α,α′-di-thio-*p*-xylylene tether and closure of the macrocyclic ring was carried out between the dipotassium salt of **41** and the dibromide **44**. The reaction proceeded in very low yield, presumably due to the large average distance between the two reaction sites. Consequently, the intermolecular reaction competes with the intramolecular ring closure. Nevertheless, this reaction scheme represents a short, direct route to the tethered product **45** (17%). The most significant NMR evidence for the formation of **45** was the presence of 4 sets of doublets at 2.93 ppm (J = 2.2 Hz, 15.0 Hz), 2.76 ppm (J = 8.1 Hz, 15.0 Hz), 2.67 ppm (J = 6.2 Hz, 15.2 Hz), and 2.49 ppm (J = 9.3 Hz, 15.0 Hz), corresponding to the H-6a (Man), H-6a (Gal), H-6b (Gal), and H-6b (Man) protons. The H-4 of the galactose unit shifted significantly downfield from 5.81 ppm to 6.23 ppm. The H-1 of the mannose unit shifted upfield by 1.85 ppm from 5.67 ppm to 3.82 ppm. This was considered as direct evidence of the presence of the tether in the molecule, as the anomeric proton should be located in the face of the aromatic ring of the tether. The resulting anisotropic effect of the aromatic ring causes an upfield chemical shift of this proton. After subjecting **45** to Zemplén transesterification conditions, all the protecting groups were removed, and the final tethered trisaccharide **4** was obtained by preparative HPLC on a reverse-phase C18 column.

The NMR spectrum of the final product **4** recorded at 600 MHz NMR was particularly interesting. Four sets of doublet or doublets corresponding to the H-6a (Man), H-6a (Gal), H-6b (Gal), and H-6b (Man) appear at 3.23, 2.95, 2.83, and 2.72 ppm, respectively. The anomeric proton, H-1, of the α-mannose unit resonates at unusually high field, 4.14 ppm, and the line shapes of the four aromatic protons are of special interest since all appear as broad resonances at 7.49, 7.36, 7.24, and 7.22 ppm. This is probably due to the slow rotation of the tether's aromatic ring. Since the aromatic ring is likely too close to the van der Waals surface of the trisaccharide to permit free rotation about either the S–CH_2 or CH_2–C_6H_4 bonds of the tether, the lines of the latter are broadened, while the motions of protons in other parts of the molecule are not constrained and show little if any linebroadening. The four benzyl protons of the tether resonate at 3.87 ppm, 3.81 ppm, 3.79 ppm, and 3.76 ppm, all of which appear as sharp doublets with J around 14.5 Hz (Table 1).

Introduction of acetals between Abe O-2 and Gal O-2 or between Abe O-2 and Man O-4 to produce trisaccharides **2** or **3** is intended to simulate constraints that result from intramolecular hydrogen bonds in the bound state or DMSO solution.[2] The ^{13}C NMR spectra of **2** or **3** (Table 2) confirm the presence of acetals linked to the atoms in question, since both C-2 atoms of Abe and Gal show downfield shifts of ~10 ppm relative to **1**.[2] Similar shifts of slightly smaller magnitude ~7–8 ppm are observed when the acetal spans C-2 Abe and C-4 Man. Altered glycosylation shifts[21,22] (~5 ppm) are also observed for C-2 but not C-3 of mannose in compound **2**, while the anomeric carbon of Abe is shifted upfield by ~5 ppm. Based on published correlations of anomeric shift changes vs. torsional angle changes,[23] these observations would suggest that the Abe–Gal and the Gal–Man glycosidic linkages adopt unusual torsional angles. On the basis of the ^{13}C chemical shifts of **2** in D_2O, we would expect this tethered trisaccharide to be significantly distorted from the solution and bound conformations of **1**. Molecular modeling confirms this prediction (Fig. 3A,B). Large changes in chemical shifts are not seen when the ^{13}C shifts of **3** and **1** are compared, and molecular modeling confirms that the tethered trisaccharide **3** is less distorted than compound **2** (Fig. 3C,D). Since compound **4** contains thioether groups at C-6 of both mannose and galactose residues, the chemical shift changes in this compound relative to **1** must be treated more cautiously. However, a significant glycosylation shift of ~3 ppm can be seen on C-2 of mannose for compound **4**, implying that the ψ angle of the Gal–Man bond has shifted somewhat relative to the solution structure of **1**. In fact, molecular modeling shows that the conformation of **4** correlates well with bound conformer II (Fig. 4A), and the Gal–Man linkage is shifted some-

Table 3
Thermodynamics of antibody–oligosaccharide interaction (kcal mol^{-1}) for native **1** and tethered trisaccharide **4** at 25 ± 0.2 °C[a]

Ligand	K_A (M^{-1})	$\Delta G°$	$\Delta H°$	$T\Delta S°$
1	$1.60 \pm 0.28 \times 10^5$	–7.10 ± 0.10	–6.75 ± 0.38	0.34 ± 0.48
4	$3.85 \pm 0.05 \times 10^5$	–7.60 ± 0.01	–6.95 ± 0.05	0.65 ± 0.06

[a] taken from ref 8. Reproduced with permission from J. Am. Chem. Soc. 1998, *120*, 5317–5318. Copyright 1998 Am. Chem. Soc.

what relative to the bound conformer I (Fig. 4B). Both depictions show that the constrained trisaccharide **4** closely resembles both bound conformations of **1**. This model is supported by the NMR data showing linebroadening and the aromatic-ring-induced proton shift on H-1 of mannose, which is explained by the proximity of this anomeric proton to the center of the aromatic ring (Fig. 5).

The conformational models would predict that the trisaccharides **2** and **3** should be inactive since tethering has distorted the relative positions of both the mannose and galactose residues. Short tethers composed of sp^3 hybridized carbons are poorly disposed to simulate the linear geometry of intramolecular sugar–sugar hydrogen bonds. Conversely, longer tethers that span solvent-exposed groups of the epitope have allowed the bound conformation to be well simulated, while avoiding unfavorable protein–tether contacts. In this context, compound **4** would be predicted to be as active as **1**, and accurate thermodynamic data confirm this expectation (Table 3).[8] However, the same data show that there are only small differences for the entropy term when compound **1** and compound **4** are bound by antibody SE155.4. This implies that the entropic costs to binding the unconstrained trisaccharide **1** are not as large as would be estimated based on some literature predictions.[5] If this is so, our data lend support to the contention that the bound form of an oligosaccharide is selected from the most heavily populated conformational families, and as suggested by Lemieux[24] and supported by Chervenak and Toone,[25] the origins of the large enthalpy and entropy changes that occur when carbohydrates are bound by proteins originate from reordering of water about the new complex.

EXPERIMENTAL

Optical rotations were performed on a Perkin-Elmer 241 polarimeter at 22 ± 2 °C. Analytical TLC was employed on silica gel 60-F_{254} (E. Merck, Darmstadt) with detection by quenching of fluorescence and/or by charring with sulfuric acid. Iatrobeads refer to a beaded silica gel 6RS-8060 manufactured by Iatron Laboratories (Tokyo). All commercial reagents were used as supplied, and chromatography solvents were distilled prior to use. Column chromatography was performed on silica gel 60 (40–60 mesh). ^{1}H NMR spectra were recorded at 360 MHz (Brüker WM-360) and first-order proton chemical shifts δ_H are referenced to either internal $CHCl_3$ (δ_H 7.24, $CDCl_3$) or 0.1% (v/v) internal acetone (δ_H 2.225, D_2O). ^{13}C NMR spectra were recorded at 75.5 MHz (Brüker AM-300) and ^{13}C chemical shifts δ_C are referenced to internal $CHCl_3$ (δ_C 77.00, $CDCl_3$). Organic solutions were dried prior to concentration under vacuum at <40 °C (bath). Microanalyses and electrospray mass spectra were carried out by the analytical services of this Department.

Ethyl 2,4-di-O-*benzyl-3,6-dideoxy-1-thio*-β-D-xylo-*hexopyranoside* (**6**)[10], *ethyl 4*-O-*benzyl-3,6-dideoxy-1-thio*-β-D-xylo-*hexopyranoside* (**7**), *ethyl 2*-O-*benzyl-3,6-dideoxy-1-thio*-β-D-xylo-*hexopyranoside* (**8**). A solution of ethyl 3,6-dideoxy-1-thio-β-D-*xylo*-hexopyranoside (**5**) (53.2 mg, 0.28 mmol) in anhydrous DMF (2 mL) was cooled to 0 °C, sodium hydride (80% dispersion in mineral oil, 17.4 mg, 0.58 mmol) was added, and the solution was stirred for 10 min. Benzyl bromide (35.5 μL, 0.31 mmol) was added and the solution was stirred for 1 h at 0 °C. The reaction was quenched by addition of MeOH (0.5 mL), and the reaction mixture was diluted with ether (30 mL), washed with 5% brine (2 × 15 mL), dried over anhydrous Na_2SO_4, and evaporated under high vacuum. Chromatography on silica gel using 20% → 30% (v/v) EtOAc/pentane gave first the 2,4-dibenzylated compound **6** (18.0 mg, 17%), followed by the 4-benzylated compound **7** (4.8 mg, 6%), the 2-benzylated compound **8** (23.0 mg, 29%); and finally, unreacted starting material was recovered (13.2 mg, 25%).

Data for **7**: $[\alpha]_D^{22}$ –58.9° (*c* 0.4, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.24–7.35 (m, 5H, $C_6H_5CH_2$), 4.70 (d, 1H, *J* = 12.2 Hz, $C_6H_5CH_2$), 4.47 (d, 1H, *J* = 12.2 Hz, $C_6H_5CH_2$), 4.27 (d, 1H, *J* = 9.5 Hz, H-1), 3.80 (m, 1H, H-2), 3.62 (dq, 1H, *J* = 1.5 Hz, 6.5 Hz, H-5), 3.43 (m, 1H, H-4), 2.75 (m, 2H, SEt), 2.56 (m, 1H, H-3e), 2.31 (b s, 1H, OH-2), 1.48 (m, 1H, H-3a), 1.33 (t, 3H, *J* = 7.5 Hz, SEt), 1.27 (d, 3H, *J* = 6.5 Hz, H-6). High Res. ES-MS: 305.117887 (M + Na^+). Anal. Calcd for $C_{15}H_{22}O_3S$: C: 63.80%, H: 7.85%. Found: C: 63.44%, H: 7.90%.

Data for **8**: $[\alpha]_D^{22}$ –54.7° (*c* 0.3, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.23–7.37 (m, 5H, $C_6H_5CH_2$), 4.70 (d, 1H, *J* = 11.5 Hz, $C_6H_5CH_2$), 4.58 (d, 1H, *J* = 11.5 Hz, $C_6H_5CH_2$), 4.48 (d, 1H, *J* = 9.6 Hz, H-1), 3.71 (m, 1H, H-4), 3.63 (dq, 1H, H-5), 3.56 (m, 1H, H-2), 2.73 (m, 2H, SCH_2CH_3), 2.45 (m, 1H, H-3e), 1.62 (m, 1H, H-3a), 1.31 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3), 1.23 (d, 3H, *J* = 6.5 Hz, H-6). High Res. ES-MS: 305.117846 (M + Na^+). Anal. Calcd for $C_{25}H_{22}O_3S$: C: 63.80%. H: 7.85%. Found: C: 63.96%, H: 8.07%.

Ethyl 2,4-di-O-*(p-methoxybenzyl)-3,6-dideoxy-1-thio*-β-D-xylo-*hexopyranoside* (**9**), *ethyl 4*-O-*(p-methoxybenzyl)-3,6-dideoxy-1-thio*-β-D-xylo-*hexopyranoside* (**10**), *ethyl 2*-O-*(p-methoxybenzyl)-3,6-dideoxy-1-thio*-β-D-xylo-*hexopyranoside* (**11**). A solution of ethyl 3,6-dideoxy-1-thio-β-**D**-*xylo*-hexopyranoside (**5**) (442.0 mg 2.30 mmol) in anhydrous DMF (7 mL) was cooled to 0 °C, sodium hydride (80% dispersion in mineral oil, 145.0 mg, 4.80 mmol) was added, and the solution was stirred for 10 min. *p*-Methoxybenzyl chloride (370 μL, 2.73 mmol) was added, and the solution was stirred for 1 h at 0 °C. The reaction was quenched by addition of MeOH (2 mL), and the reaction mixture was diluted with CH_2Cl_2, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under high vacuum. Chromatography on silica gel using 30% (v/v) EtOAc/pentane gave first the di-*p*-methoxybenzylated compound **9** (256.8 mg, 26%), followed by the 4-*p*-methoxybenzylated compound **10** (97.4 mg, 14%), and 2-*p*-methoxybenzylated compound **11** (233.6 mg, 33%).

Data for **9**: $[\alpha]_D^{22}$ –53.2° (*c* 1.4, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.28 (d, 2H, *J* = 8.7 Hz, *o*-H-MeO$C_6H_5CH_2$), 7.18 (d, 2H, *J* = 8.7 Hz, *o*-H-MeO$C_6H_5CH_2$), 6.81–6.88 (m, 4H, *m*-H-MeO$C_6H_5CH_2$), 4.63 (d, 1H, *J* = 11.3 Hz, $MeOC_6H_5CH_2$), 4.48 (d, 1H, *J* = 12.0 Hz, $MeOC_6H_5CH_2$), 4.47 (d, 1H, *J* = 11.9 Hz, $MeOC_6H_5CH_2$), 4.42 (d, 1H, *J* = 9.4 Hz, H-1), 4.30 (d, 1H, *J* = 12.0 Hz, $MeOC_6H_5CH_2$), 3.79 (s, 3H, OMe), 3.78 (s, 3H, OMe), 3.50–3.60 (m, 2H, H-2 + H-5), 3.34 (m, 1H, H-4), 2.70 (m, 2H, SCH_2CH_3), 2.34 (m, 1H, H-3e), 1.40 (m, 1H, H-3a), 1.27 (t, 3H, *J* = 7.5 Hz, SCH_2CH_3), 1.19 (d, 3H, *J* = 6.5 Hz, H-6). High Res. ES-MS: 455.186155 (M + Na^+). Anal. Calcd for $C_{24}H_{32}O_5S$: C: 66.64%, H: 7.46%. Found: C: 66.74%, H: 7.53%.

Data for **10**: $[\alpha]_D^{22}$ –48.9° (*c* 0.5, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.25 (d, 2H, *J* = 8.7 Hz, *o*-H-MeO$C_6H_5CH_2$), 6.86 (d, 2H, *J* = 8.7 Hz, *m*-H-MeO$C_6H_5CH_2$), 4.64 (d, 1H, *J* = 11.9 Hz, MeO$C_6H_5CH_2$), 4.39 (d, 1H, *J* = 12.1 Hz, $MeOC_6H_5CH_2$), 4.24 (d, 1H, *J* = 9.5 Hz, H-1), 3.80 (s, 3H, OMe), 3.76 (m, 1H, H-2), 3.60 (dq, 1H, H-5), 3.41 (m, 1H, H-4), 2.74 (m, 2H, SCH_2CH_3), 2.53 (m, 1H, H-3e), 2.32 (d, 1H, *J* = 1.7 Hz, OH-2), 1.44 (m, 1H, H-3a), 1.30 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3), 1.26 (d, 3H, *J* = 6.6 Hz, H-6). High Res. ES-MS: 335.128655 (M + Na^+). Anal. Calcd for $C_{16}H_{24}O_4S$: C: 61.51%, H: 7.74%. Found: C: 61.84%, H: 7.85%.

Data for **11**: $[\alpha]_D^{22}$ –46.3° (*c* 1.1, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.27 (d, 2H, *J* = 8.6 Hz, *o*-H-MeO$C_6H_5CH_2$), 6.82 (d, 2H, *J* = 8.6 Hz, *m*-H-MeO$C_6H_5CH_2$), 4.62 (d, 1H, *J* = 11.1 Hz, $MeOC_6H_5CH_2$), 4.51 (d, 1H, *J* = 11.1 Hz, $MeOC_6H_5CH_2$), 4.46 (d, 1H, *J* = 9.6 Hz, H-1), 3.78 (s, 3H, OMe), 3.69 (m, 1H, H-4), 3.62 (m, 1H, H-5), 3.55 (m, 1H, H-2), 2.70 (m, 2H, SCH_2CH_3), 2.40 (m, 1H, H-3e), 1.96 (b s, 1H, OH-4), 1.60 (m, 1H, H-3a), 1.30 (t, 3H, *J* = 7.5 Hz, SCH_2CH_3), 1.24 (d, 3H, *J* = 6.5 Hz, H-6). High Res. ES-MS: 335.12957 (M + Na^+). Anal. Calcd for $C_{16}H_{24}O_4S$: C: 61.51%, H: 7.74%. Found: C: 61.74%, H: 7.98%.

Ethyl 4-O-*benzyl-2*-O-*(p-methoxybenzyl)-3,6-dideoxy-1-thio*-β-D-xylo-*hexopyranoside* (**12**). Sodium hydride (80% dispersion in mineral oil, 119.7 mg, 3.99 mmol) was added by portions to a solution containing compound **11** (417.0 mg, 1.33 mmol) and benzyl bromide (485 μL, 3.99 mmol) in anhydrous DMF (10 mL). The reaction was continued at room temperature for 1 h and quenched by addition of methanol (1.0 mL). The reaction mixture was diluted with EtOAc (75 mL), and washed with 5% brine (2 × 30 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated. The pure compound **12** (483.0 mg, 90%) was isolated by flash chromatography on silica gel using 8% (v/v) EtOAc/pentane. $[\alpha]_D^{22}$ +119.3° (*c* 1.7, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.23–7.34 (m, 7H, $C_6H_5CH_2$ + *o*-H-MeO$C_6H_5CH_2$), 6.85 (d, 2H, *J* = 8.7 Hz, *m*-H-MeO$C_6H_5CH_2$), 4.62 (d, 1H, *J* = 11.2 Hz, $MeOC_6H_5CH_2$), 4.52 (d, 1H, *J* = 12.2 Hz, $C_6H_5CH_2$), 4.47 (d, 1H, *J* = 11.3 Hz, $MeOC_6H_5CH_2$), 4.43 (d, 1H, *J* = 9.5 Hz, H-1), 4.37 (d, 1H, *J* = 12.2 Hz, $C_6H_5CH_2$), 3.77 (s, 3H, OMe), 3.51–3.62 (m, 2H, H-2 + H-5), 3.38 (m, 1H, H-4), 2.71 (m, 2H, SCH_2CH_3), 2.38 (m, 1H, H-3e), 1.42 (m, 1H, H-3a), 1.28 (t, 3H, *J* = 7.5 Hz, SCH_2CH_3), 1.24 (d, 3H, *J* = 6.4 Hz, H-6). High Res. ES-MS: 425.17656 (M + Na^+). Anal. Calcd for $C_{23}H_{30}O_4S$: C: 68.60%, H: 7.51%. Found: C: 68.55%, H: 7.64%.

Ethyl 2,4-di-O-*(p-methoxybenzyl)-3,6-dideoxy-1-thio*-α-D-xylo-*hexopyranoside* (**14**), *ethyl 4*-O-*(p-methoxybenzyl)-3,6-dideoxy-1-thio*-α-D-xylo-*hexopyranoside* (**15**), *ethyl 2*-O-*(p-methoxybenzyl)-3,6-dideoxy-1-thio*-α-D-xylo-*hexopyranoside* (**16**). A solution of ethyl 2-*O*-3,6-dideoxy-1-thio-α-D-*xylo*-hexopyranoside (**13**) (862.3 mg 4.49 mmol) in anhydrous DMF (15 mL) was cooled to 0 °C, sodium hydride (80% dispersion in mineral oil, 282.9 mg, 9.43 mmol) was added, and the solution was stirred for 10 min. *p*-Methoxybenzyl chloride (683.0 μL, 4.94 mmol) was added and the solution was stirred for 1 h at 0 °C. The reaction was quenched by addition of MeOH (3 mL), the reaction mixture was diluted with CH_2Cl_2, washed with H_2O, dried over anhydrous Na_2SO_4, and evaporated under high vacuum. Chromatography on silica gel using 30% (v/v) EtOAc/pentane gave first the di-*p*-methoxybenzylated compound **14** (450.0 mg, 23%), followed by the 4-*p*-methoxybenzylated compound **15** (150.0 mg, 11%), and 2-*p*-methoxybenzylated compound **16** (377.8 mg, 27%). Unreacted starting material (210.0 mg, 24%) was also recovered.

Data for **14**: $[\alpha]_D^{22}$ +83.5° (*c* 0.34, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.30 (d, 2H, *J* = 8.6 Hz, *o*-H-MeO$C_6H_5CH_2$), 7.21 (d, 2H, *J* = 8.6 Hz, *o*-H-MeO$C_6H_5CH_2$), 6.85 (m, 4H, *m*-H-MeO$C_6H_5CH_2$), 5.47 (d, 1H, *J* = 5.0 Hz, H-1), 4.58 (d, 1H, *J* = 11.4 Hz, $MeOC_6H_5CH_2$), 4.50 (d, 1H, *J* = 11.8 Hz, $MeOC_6H_5CH_2$), 4.40 (d, 1H, *J* = 11.4 Hz, $MeOC_6H_5CH_2$), 4.32 (d, 1H, *J* = 11.8 Hz, $MeOC_6H_5CH_2$), 4.20 (dq, 1H, H-5), 4.03 (m, 1H, H-2), 3.78 (s, 3H, OMe), 3.73 (m, 1H, H-4), 2.57 (m, 2H, SCH_2CH_3), 2.04 (m, 1H, H-3e), 1.86 (m, 1H, H-3a), 1.62 (d, 1H, *J* = 6.8 Hz, OH-4), 1.29 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3), 1.17 (d, 3H, *J* = 6.6 Hz, H-6). High Res. ES-MS: 455.185844 (M + Na^+). Anal. Calcd for $C_{24}H_{32}O_5S$: C: 66.64%, H: 7.46%. Found: C: 66.27%, H: 7.46%.

Data for **15**: $[\alpha]_D^{22}$ +117° (*c* 0.5, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.26 (d, 2H, *J* = 8.7 Hz, *o*-H-MeO$C_6H_5CH_2$), 6.86 (d, 2H, *J* = 8.6 Hz, *m*-H-MeO$C_6H_5CH_2$), 5.28 (d, 1H, *J* = 5.0 Hz, H-1), 4.61 (d, 1H, *J* = 11.7 Hz, $MeOC_6H_5CH_2$), 4.37 (d, 1H, *J* = 11.8 Hz, $MeOC_6H_5CH_2$), 4.24 (m, 1H, H-2), 4.11 (dq, 1H, H-5), 3.79 (s, 3H, OMe), 3.38 (m, 1H, H-4), 2.65 (m, 2H, SCH_2CH_3), 2.19 (m, 1H, H-3e), 1.45 (m, 1H, H-3a), 1.29 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3), 1.17 (d, 3H, *J* = 6.6 Hz, H-6). High Res. ES-MS: 335.12486 (M + Na^+). Anal. Calcd for $C_{16}H_{24}O_4S$: C: 61.51%, H: 7.74%. Found: C: 61.78%, H: 7.89%.

Data for **16**: $[\alpha]_D^{22}$ +190.5° (*c* 0.8, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.26 (d, 2H, *J* = 8.5 Hz, *o*-H-MeO$C_6H_5CH_2$), 6.85 (d, 2H, *J* = 8.6 Hz, *m*-H-MeO$C_6H_5CH_2$), 5.42 (d, 1H, *J* = 5.1 Hz, H-1), 4.56 (d, 1H, *J* = 11.4 Hz, $MeOC_6H_5CH_2$), 4.41 (d, 1H, *J* = 11.4 Hz, $MeOC_6H_5CH_2$), 4.29 (dq, 1H, *J* = 1.1 Hz, 6.6 Hz, H-5), 4.03 (m, 1H, H-2), 3.78 (s, 3H, OMe), 3.73 (m, 1H, H-4), 2.57 (m, 2H, SCH_2CH_3), 2.04 (m, 1H, H-3e), 1.86 (m, 1H, H-3a), 1.62 (d, 1H, *J* = 6.8 Hz, OH-4), 1.29 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3), 1.17 (d, 3H, *J* = 6.6 Hz, H-6). High Res. ES-MS: 335.12944 (M + Na^+). Anal. Calcd for $C_{16}H_{24}O_4S$: C: 61.51%, H: 7.74%. Found: C: 61.70%, H: 7.79%.

Ethyl 4-O-*benzyl-2*-O-*(p-methoxybenzyl)-3,6-dideoxy-1-thio*-α-D-xylo-*hexopyranoside* (**17**). Sodium hydride (80% dispersion in mineral oil, 154.0 mg, 5.10 mmol) was added by portions to a solution containing compound **16** (320.7 mg, 1.03 mmol) and benzyl bromide (375 μL, 3.09 mmol) in anhydrous DMF (12 mL). The reaction was continued at room temperature for 1 h and quenched by addition of methanol (0.5 mL). The reaction mixture was diluted with EtOAc (75 mL), and washed with 5% brine (2 × 30 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated. The pure compound **17** (388.0 mg, 94%) was isolated by flash chromatography on silica gel using 8% (v/v) EtOAc/pentane. $[\alpha]_D^{22}$ +119.3° (*c* 1.7, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.24–7.36 (m, 7H, $C_6H_5CH_2$ + *o*-H-MeO$C_6H_5CH_2$), 6.85 (d, 2H, *J* = 8.6 Hz, *m*-H-MeO$C_6H_5CH_2$), 5.46 (d, 1H, *J* = 5.0 Hz, H-1), 4.59 (d, 1H, *J* = 12.1 Hz, MeO$C_6H_5CH_2$), 4.58 (d, 1H, *J* = 11.4 Hz, $C_6H_5CH_2$), 4.39 (d, 2H, *J* ≈ 11.5 Hz, ArCH_2–), 4.21 (dq, 1H, H-5), 4.09 (m, 1H, H-2), 3.78 (s, 3H, OMe), 3.43 (m, 1H, H-4), 2.56 (m, 2H, SCH_2CH_3), 2.12 (m, 1H, H-3e), 1.73 (m, 1H, H-3a), 1.28 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3), 1.17 (d, 3H, *J* = 6.6 Hz, H-6). High Res. ES-MS: 425.17696 (M + Na^+). Anal. Calcd for $C_{23}H_{30}O_4S$: C: 68.60%, H: 7.51%. Found: C: 68.20%, H: 7.57%.

Ethyl 3,4-di-O-*isopropylidene-2*-O-(p-*methoxybenzyl)-6*-O-*(2-methoxy-2-propyl)-1-thio*-β-D-*galactopyranoside* (**18**). A solution of ethyl 3,4-di-*O*-isopropylidene-6-*O*-(2-methoxy-2-propyl)-1-thio-β-D-galactopyranoside (5.55 g 16.5 mmol) in anhydrous DMF (20 mL) was treated with NaH (80% dispersion in mineral oil, 2.48 g, 82.7 mmol) at 0 °C for 20 min, *p*-methoxybenzyl chloride (5.0 mL, 36.9 mmol) was added dropwise and the reaction was continued for 5 h while the temperature was allowed to rise slowly to room temperature. The reaction was quenched by addition of methanol (5.0 mL), the reaction mixture was diluted with CH_2Cl_2, washed with H_2O, and concentrated. Chromatography of the residue on silica gel using 20/80/1 EtOAc/pentane/Et_3N as eluent afforded pure compound **18** (3.43 g) in 46% yield. $[\alpha]_D^{22}$ –4.8° (*c* 0.5, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.33 (d, 2H, *J* = 8.7 Hz, *o*-H-MeO$C_6H_5CH_2$), 6.88 (d, 1H, *J* = 8.7 Hz, *m*-H-MeO$C_6H_5CH_2$), 4.74 (d, 1H, *J* = 11.2 Hz, MeO$C_6H_5CH_2$), 4.64 (d, 1H, *J* = 11.2 Hz, MeO$C_6H_5CH_2$), 4.46 (d, 1H, *J* = 9.7 Hz, H-1), 4.28 (dd, 1H, *J* = 2.0 Hz, 5.6 Hz, H-4), 4.22 (t, 1H, *J* = 5.7 Hz, H-3), 3.92 (m, 1H, H-5), 3.77 (s, 3H, OMe), 3.58 (m, 2H, H-6a + H-6b), 3.37 (dd, 1H, *J* = 6.3 Hz, 9.7 Hz, H-2), 3.17 (s, 3H, Me_2C(O*Me*)-), 2.66 (m, 2H, SCH_2CH_3), 1.41 (s, 3H, Me), 1.30 (s, 3H, Me), 1.28 (s, 6H, Me_2C(OMe)-), 1.24 (t, 3H, *J* = 7.5 Hz, SCH_2CH_3). High Res. ES-MS: 479.207941 (M + Na^+). Anal. Calcd for $C_{23}H_{36}O_7S$: C: 60.50%, H: 7.95%. Found: C: 60.85%, H: 7.72%.

Ethyl 2-O-(p-methoxybenzyl)-1-thio-β-D-*galactopyranoside* (**19**). A solution of **18** (1.34 g, 2.90 mmol) in MeOH (15 mL) was treated with a solution of HBF_4 (48% aqueous, 134 μL) at 0 °C overnight, NEt_3 (0.5 mL) was added and the solution was concentrated under high vacuum. The pure compound **19** (0.92 g, 91%) was isolated by chromatography on silica gel using 3% (v/v) MeOH/CH_2Cl_2 as eluent. $[\alpha]_D^{22}$ + 12.7° (*c* 1.1, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.32 (d, 2H, *J* = 8.6 Hz, *o*-H-MeO$C_6H_5CH_2$), 6.88 (d, 2H, *J* = 8.7 Hz, *m*-H-MeO$C_6H_5CH_2$), 4.88 (d, 1H, *J* = 10.8 Hz, MeO$C_6H_5CH_2$), 4.59 (d, 1H, *J* = 10.8 Hz, MeO$C_6H_5CH_2$), 4.41 (d, 1H, *J* = 9.5 Hz, H-1), 4.00 (dd, 1H, *J* = 0.9 Hz, 3.2Hz, H-4), 3.92 (dd, 1H, *J* = 6.1 Hz, 11.9 Hz, H-6a), 3.82 (dd, 1H, *J* = 4.3 Hz, 11.9 Hz, H-6b), 3.79 (s, 3H, OMe), 3.58 (dd, 1H, *J* = 3.3 Hz, 8.9 Hz, H-3), 3.49 (m, 2H, H-2 + H-5), 2.77 (m, 2H, SCH_2CH_3), 1.32 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3). High Res. ES-MS: 367.119072 (M + Na^+). Anal. Calcd for $C_{16}H_{24}O_6S$: C: 55.80%, H: 7.02%. Found: C: 55.59%, H: 7.06%.

Ethyl 3,4,6-tri-O-*benzyl-2*-O-*(p-methoxybenzyl)-1-thio*-β-D-*galactopyranoside* (**20**). Sodium hydride (80% dispersion in mineral oil, 435 mg 14.5 mmol) was added by portions to a cold solution of compound **19** (1.0 g 2.9 mmol) and benzyl bromide (3.17 mL, 8.7 mmol) in anhydrous DMF (12 mL) at 0 °C. The reaction was continued for 4 h while the temperature was allowed to rise to room temperature. The reaction was quenched by addition of methanol (1.0 mL). The solution was diluted with EtOAc (80 mL), washed with 5% (2 × 30 mL), dried over anhydrous Na_2SO_4, and concentrated. The pure compound **20** (1.52 g, 85%) was obtained by flash chromatography on silica gel using 5% (v/v) EtOAc/toluene as eluent. $[\alpha]_D^{22}$ + 2.4° (*c* 1.0, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.24–7.38 (m, 17H, $C_6H_5CH_2$ + *o*-H-MeO$C_6H_5CH_2$), 6.85 (d, 1H, *J* = 8.6 Hz, *m*-H-MeO$C_6H_5CH_2$), 4.97 (d, 1H, *J* = 11.7 Hz, ArCH_2–), 4.40–4.86 (m, 8H, H-1 + ArCH_2–), 3.95 (b d, 1H, *J* = 2.5 Hz, H-4), 3.83 (t, 1H, *J* = 9.5 Hz, H-2), 3.79 (s, 3H, OMe), 3.55–3.61 (m, 4H, H-3 + H-5 + H-6a + H-6b), 2.74 (m, 2H, SCH_2CH_3), 1.30 (t, 3H, *J* = 7.4 Hz, SCH_2CH_3). High Res. ES-MS: 637.26022 (M + Na^+). Anal. Calcd for $C_{37}H_{42}O_6S$: C: 72.28%, H: 6.89%. Found: C: 72.22%, H: 7.13%.

Methyl 2-O-*benzoyl-4,6-di*-O-*benzyl-3*-O-*(2′*-O-*(p-methoxybenzyl)-4′*-O-*benzyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)*-α-D-*mannopyranoside* (**22**). A mixture containing acceptor **21** (200.8 mg 0.42 mmol), donor **12** or **17** (337 mg, 0.84 mmol), and molecular sieve 4Å (1.0 g) was dried under high vacuum for 2 h, CH_2Cl_2 (10 mL) was added, the mixture was protected under argon, and stirred for 1 h at 0 °C. *N*-iodosuccinimide (248.7 mg,1.05 mmol) was added, a few crystals of silver trifluoromethanesulfonate were added, and the reaction was continued for 15 min. Et_3N (1 mL) was added to quench the reaction, the solids were filtered off through a thin bed of Celite, and the residues washed with more CH_2Cl_2. The combined organic solution was washed with a 1:1 mixture of saturated aqueous $NaHCO_3$ and 10% aqueous $Na_2S_2O_3$, dried over anhydrous Na_2SO_4, and concentrated. Chromatography on silica gel using 8% (v/v) EtOAc/toluene gave pure disaccharide **22** (220 mg, 64%). $[\alpha]_D^{22}$ + 17.1° (*c* 0.6, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 8.04 (m, 2H, *o*-H-C_6H_5CO), 7.54 (m, 1H, *p*-H-C_6H_5CO), 7.11–7.39 (m, 17H, *m*-H-C_6H_5CO + MeO$C_6H_5CH_2$), 7.05 (d, 1H, *J* = 8.6 Hz, *m*-H-MeO$C_6H_5CH_2$), 6.70 (d, 1H, *J* = 8.7 Hz, *o*-H-MeO$C_6H_5CH_2$), 5.38 (dd, 1H, *J* = 2.0 Hz, 3.1 Hz, H-2), 5.12–5.18 (m, 2H, H-1′ + ArCH_2–), 4.85 (d, 1H, *J* = 1.8 Hz, H-1), 4.66 (d, 1H, *J* = 12.0 Hz, ArCH_2–), 4.56 (d, 1H, *J* = 11.3 Hz, ArCH_2–), 4.50 (d, 1H, *J* = 12.1 Hz, ArCH_2–), 4.46 (d, 1H, *J* = 12.4 Hz, ArCH_2–), 4.28 – 4.38 (m, 4H, H-3 + ArCH_2–), 4.14 (t,

3H, *J* = 9.6 Hz, H-4), 3.91 (dq, 1H, H-5′), 3.71–3.88 (m, 7H, OMe + *Me*OC$_6$H$_5$CH$_2$ + H-2′ +H-5 + H-6a + H-6b + OMe + *Me*OC$_6$H$_5$CH$_2$), 3.40 (s, 3H, OMe), 3.30 (br, 1H, H-4′), 1.98 (m, 1H, H-3e′), 1.76 (m, 1H, H-3a′), 1.05 (d, 3H, *J* = 6.6 Hz, H-6′). High Res. ES-MS: 841.356686 (M + Na$^+$). Anal. Calcd for $C_{49}H_{54}O_{11}$: C: 71.86%, H: 6.65%. Found: C: 71.84%, H: 6.83 %.

Methyl 4,6-di-O-*benzyl-3*-O-*(2′*-O-*(*p-*methoxybenzyl)-4′*-O-*benzyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)*-α-D-*mannopyranoside* (**23**). Disaccharide **22** (170 mg, 0.208 mmol) was dissolved in anhydrous methanol (5 mL), a solution of sodium methoxide in anhydrous methanol (200 µL, 1.27 M) was added, and the mixture was stirred for 2 h at room temperature. When the starting material was consumed, the solution was neutralized with Amberlite IR-120 (H$^+$). The resin was filtered off and the solution was concentrated under high vacuum. The alcohol **23** was purified by flash chromatography using 25% (v/v) EtOAc/toluene as eluent (139 mg, 94%). $[\alpha]_D^{22}$ + 66.8° (*c* 0.5, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.10–7.34 (m, 15H, *C$_6$H$_5$*CH$_2$), 7.08 (d, 2H, *J* = 8.6 Hz, *o*-H-MeO*C$_6$H$_5$*CH$_2$), 6.72 (d, 2H, *J* = 8.7 Hz, *m*-H-*C$_6$H$_5$*CH$_2$), 5.16 (d, 1H, *J* = 3.4 Hz, H-1′), 5.04 (d, 1H, *J* = 11.3 Hz, Ar*CH$_2$*–), 4.74 (d, 1H, *J* = 1.6 Hz, H-1), 4.60 (d, 1H, *J* = 12.2 Hz, Ar*CH$_2$*–), 4.53 (d, 1H, *J* = 12.0 Hz, Ar*CH$_2$*–), 4.50 (d, 1H, *J* = 12.1 Hz, Ar*CH$_2$*–), 4.44 (d, 1H, *J* = 11.2 Hz, Ar*CH$_2$*–), 4.39 (d, 1H, *J* = 11.9 Hz, Ar*CH$_2$*–), 4.36 (d, 1H, *J* = 12.1 Hz, Ar*CH$_2$*–), 4.32 (d, 1H, *J* = 12.1 Hz, Ar*CH$_2$*–), 4.03–4.11 (m, 2H, H-3 + H-5′), 3.98 (dd, 1H, *J* = 1.8 Hz, 3.34 Hz, H-2), 3.86 (t, 1H, *J* = 9.3 Hz, H-4), 3.64–3.85 (m, 8H, H-2′ + H-5 + H-6a + H-6b + OMe+*Me*OC$_6$H$_5$CH$_2$), 3.45 (br. 1H, H-4′), 3.36 (s, 3H, OMe), 2.12 (m, 1H, H-3e′), 1.87 (m, 1H, H-3a′), 1.15 (d, 3H, *J* = 6.6 Hz, H-6′). High Res. ES-MS: 737.331267 (M + Na$^+$). Anal. Calcd for $C_{42}H_{50}O_{10}$: C: 70.57%, H: 7.05%. Found: C: 70.39%, H: 7.09%.

Methyl 4,6-di-O-*benzyl-2*-O-*(3″,4″,6″-tri*-O-*benzyl-2″*-O-*(*p-*methoxybenzyl)*-α-D-*galactopyranosyl)-3*-O-*(2′*-O-*(*p-*methoxybenzyl)-4′*-O-*benzyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)*-α-D-*mannopyranoside* (**24**). A mixture of disaccharide **23** (226.6 mg, 0.317 mmol), donor **20** (487.9 mg, 0.79 mmol), and molecular sieve 4Å (1.0 g) was dried under high vacuum for 2 h, CH_2Cl_2 (10 mL) was added, and the mixture was stirred for 1 h at 0 °C under argon. 2,6-Di-*tert*-butyl-4-methylpyridine (200.0 mg, 0.95 mmol) and methyl trifluoromethanesulfonate (101 µL, 0.857 mmol) were added at 0 °C. After 30 min, the solid was filtered off through a thin bed of Celite, and washed with more CH_2Cl_2 (80 mL). The combined organic solution was washed with H_2O (1 × 20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated. The trisaccharide **24** was obtained by chromatography using 5% (v/v) EtOAc/toluene as eluent (378.6 mg, 94%). $[\alpha]_D^{22}$ + 47° (*c* 0.5, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.08–7.34 (m, 32H, *o*-H-MeO*C$_6$H$_5$*CH$_2$ + C$_6$H$_5$), 6.91 (d, 2H, *J* = 8.6 Hz, *o*-H-MeO*C$_6$H$_5$*CH$_2$), 6.71 (d, 2H, *J* = 8.7 Hz, *m*-H-MeO*C$_6$H$_5$*CH$_2$), 6.61 (d, 2H, *J* = 8.7 Hz, *m*-H-MeO*C$_6$H$_5$*CH$_2$), 5.53 (d, 1H, *J* = 3.6 Hz, H-1″), 5.19 (d, 1H, *J* = 3.2 Hz, H-1′), 5.00 (d, 1H, *J* = 12.4 Hz, Ar*CH$_2$*–), 4.93 (d, 1H, *J* = 11.2 Hz, Ar*CH$_2$*–), 4.91 (d, 1H, *J* = 11.4 Hz, Ar*CH$_2$*–), 4.77 (d, 1H, *J* = 1.8 Hz, H-1), 4.73 (d, 1H, *J* = 11.8 Hz, Ar*CH$_2$*–), 4.63 (d, 2H, *J* = 11.7 Hz, Ar*CH$_2$*–), 4.57 (d, 1H, *J* = 11.8 Hz, Ar*CH$_2$*–), 4.54 (d, 1H, *J* = 11.4 Hz, Ar*CH$_2$*–), 4.47 (d, 1H, *J* = 11.9 Hz, Ar*CH$_2$*–), 4.45 (d, 1H, *J* = 12.0 Hz, Ar*CH$_2$*–), 4.40 (d, 2H, *J* = 11.2 Hz, Ar*CH$_2$*–), 4.35 (d, 1H, *J* = 11.7 Hz, Ar*CH$_2$*–), 3.91 – 4.30 (m, 10 H, 3 × Ar*CH$_2$*– + H-3 + H-2 + H-5″ + H-5′ + H-4 + H-3″ + H-2″), 3.87 (d, 1H, *J* = 2.0 Hz, H-4″), 3.62–3.80 (m, 10H, H-5 +H-2′ + H-6a + H-6b + 2 × OMe+*Me*OC$_6$H$_5$CH$_2$), 3.53 (dd, 1H, *J* = 6.5 Hz, 9.8 Hz, H-6a″), 3.46 (dd, 1H, *J* = 6.1 Hz, 9.8 Hz, H-6b″), 3.24 (s, 3H, OMe), 3.14 (br, 1H, H-4′), 1.94 (m, 1H, H-3e′), 1.70 (m, 1H, H-3a′), 1.07 (d, 3H, *J* = 6.5 Hz, H-6′). High Res. ES-MS: 1289.581045 (M + Na$^+$).

Methyl 4,6-di-O-*benzyl-2*-O-*(3″,4″,6″-tri*-O-*benzyl*-α-D-*galactopyranosyl)-3*-O-*(4′*-O-*benzyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)*-α-D-*mannopyranoside* (**25**). A solution of trisaccharide **24** (346.5 mg, 0.273 mmol) in CH_3CN (15 mL) was treated with water (1.0 mL) and ceric ammonium nitrate (750 mg 1.36 mmol) for 1.5 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water (3 × 30 mL), dried over anhydrous Na_2SO_4, and evaporated. The crude material was chromatographed on silica gel using 25% (v/v) EtOAc/toluene to give **25** (203.6 mg, 73%). $[\alpha]_D^{22}$ + 57.6° (*c* 0.8, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.17–7.36 (m, 30H, $C_6H_5CH_2$–), 5.16 (d, 1H, *J* = 3.6 Hz, H-1′), 5.10 (d, 1H, *J* = 4.0 Hz, H-1″), 4.86 (d, 1H, *J* = 11.4 Hz, Ar*CH$_2$*–), 4.77 (d, 1H, *J* = 1.4 Hz, H-1), 4.74 (d, 1H, *J* = 10.6 Hz, Ar*CH$_2$*–), 4.38–4.70 (m, 10H, Ar*CH$_2$*–), 4.09–4.18 (m, 3H, H-3 + H-2″ + H-5″), 3.95 (m, 1H, H-2′), 3.77–3.91 (m, 5H, H-2 + H-4 + H-5′ + H-3″ + H-4″), 3.66–3.74 (m, 2H, H-5 + H-6a), 3.60 (dd, 1H, *J* = 2.6 Hz, 10 Hz, H-6b), 3.57 (dd, 1H, *J* = 6.5 Hz, 9.5 Hz, H-6a″), 3.49 (dd, 1H, *J* = 6.1 Hz, 9.6 Hz, H-6b″), 3.39 (br, 1H, H-4′), 3.21 (s, 3H, OMe), 2.16 (m, 1H, H-3e′), 1.60 (m, 1H, H-3a′), 1.13 (d, 3H, *J* = 6.5 Hz, H-6′). High Res. ES-MS: 1049.467115 (M + Na$^+$). Anal. Calcd for $C_{61}H_{69}O_{14}$: C: 71.33%, H: 6.87%. Found: C: 70.68%, H: 6.86%.

Methyl 4,6-di-O-*benzyl-2*-O-*(3″,4″,6″-tri*-O-*benzyl*-α-D-*galactopyranosyl)-3*-O-*(4′*-O-*benzyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)-2′,2″-di*-O-*methylene*-α-D-*mannopyranoside* (**26**). A solution of the trisaccharide diol **25** (107 mg, 0.104 mmol) in anhydrous DMF (3 mL) was treated with sodium hydride (80% dispersion in mineral oil, 25 mg, 1.04 mmol) for 10 min., and dibromomethane (10 µl, 0.142 mmol) was added dropwise. The reaction was stirred at room temperature for 24 hr, methanol (0.5 mL) was added to quench the reaction, and the reaction mixture was concentrated under high vacuum. Chromatography using 5% (v/v) EtOAc/toluene as eluent could not purify the trisaccharide **26**, the impure fractions (40.0 mg, containing ~30% of another unidentified product) of the compound was collected and deprotected. ^{1}H NMR (360 MHz, $CDCl_3$): selected data: δ 5.91 (d, 1H, *J* = 2.6 Hz, H-1″), 5.16 (d, 1H, *J* = 3.7 Hz, H-1′), 4.94 (d, 1H, *J* [a] 1 Hz, H-1), 3.10 (s, 3H, OMe), 2.03 (m, 1H, H-3a′), 1.93 (m, 1H, H-3e′), 0.86 (d, 3H, *J* = 6.5 Hz, H-6′).

Methyl 2-O-*(*α-D-*galactopyranosyl)-3*-O-*(3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)-2′,2″-di*-O-*methylene*-α-D-*mannopyranoside* (**2**). To a solution of the impure protected and

tethered trisaccharide **26** (33 mg) and 5% Pd on charcoal (35 mg) in methanol (25 mL) was added 3 drops of acetic acid, and the resulting mixture was hydrogenated overnight. The catalyst was filtered off through a Millipore membrane filter (0.25 micron), and the filtrate was concentrated under high vacuum. The crude material was first purified by silica gel chromatography using 10 → 20% (v/v) MeOH/CH_2Cl_2 as eluent, followed by reversed phase silica gel chromatography (C18) using 10 → 15% MeOH/H_2O as eluent to give the pure, tethered trisaccharide **2** (8.0 mg) which was lyophilized. $[\alpha]_D^{22}$ + 25.3° (*c* 0.3, H_2O). ^1H and ^{13}C NMR (500 MHz, D_2O): See Tables 1 and 2. High Res. ES-MS: 521.184860 (M + Na^+).

Methyl 3,6-di-O-*benzyl*-α-D-*mannopyranoside* (**28**) *and Methyl 2,3*-O-*isopropylidene-6*-O-*benzyl*-α-D-*mannopyranoside* (**29**). A mixture of methyl 2,3-di-*O*-isopropylidene-α-D-mannopyranoside (**27**) (5.00 g, 21.4 mmol) and di-*n*-butyltin oxide (7.50 g, 30.1 mmol) in toluene (250 mL) was refluxed for 1.5 h, with a Dean–Stark tube and the water generated during the reaction was removed. The solution was cooled and evaporated to dryness under reduced pressure. The resulting syrupy material was dissolved in anhydrous DMF (60 mL), benzyl bromide (8.8 mL, 74.0 mmol) and cesium fluoride (6.50 g, 42.8 mmol) were added, and the reaction was stirred at room temperature for 3 h. The mixture was filtered through a thin bed of silica gel using EtOAc as eluent, the clear organic solution was concentrated, and the mixture was purified by chromatography on silica gel using 25% (v/v) EtOAc/pentane to afford first: **29** (3.70 g, 54%); followed by **28** (1.18 g, 17%).

Data for **28**: $[\alpha]_D^{22}$ + 26° (*c* 1.5, $CHCl_3$). ^1H NMR (360 MHz, $CDCl_3$): δ 7.24–7.36 (m, 10H, $C_6H_5CH_2$), 4.76 (d, 1H, *J* = 1.6 Hz, H-1), 4.70 (d, 1H, *J* = 11.6 Hz, $C_6H_5CH_2$), 4.63 (d, 1H, *J* = 11.6 Hz, $C_6H_5CH_2$), 4.61 (d, 1H, *J* = 12.0 Hz, $C_6H_5CH_2$), 4.56 (d, 1H, *J* = 12.0 Hz, $C_6H_5CH_2$), 3.98 (dd, 1H, *J* = 1.6 Hz, 3.3 Hz, H-2), 3.90 (t, 1H, *J* = 9.1 Hz, H-4), 3.68–3.77 (m, 3H, H-5 + H-6a + H-6b), 3.66 (dd, 1H, *J* = 3.3 Hz, 9.2 Hz, H-3), 3.36 (s, 3H, OMe). High Res. FAB MS: 397.0 (M + Na^+). Anal. Calcd for $C_{21}H_{26}O_6$: C: 67.36%, H: 7.00%. Found: C: 66.60%, H: 6.97%.

Data for **29**: $[\alpha]_D^{22}$ + 8.3° (*c* 1.9, $CHCl_3$). ^1H NMR (360 MHz, $CDCl_3$): δ 7.17–7.25 (m, 5H, $C_6H_5CH_2$), 4.84 (s, 1H, H-1), 4.55 (d, 1H, *J* = 12.1 Hz, $C_6H_5CH_2$), 4.48 (d, 1H, *J* = 12.1 Hz, $C_6H_5CH_2$), 4.02 (br s, 2H, H-2 + H-3), 3.61–3.66 (m, 4H, H-4 + H-5 + H-6 + H-6′), 3.30 (s, 3H, OMe), 1.42 (s, 3H, Me), 1.25 (s, 3H, Me). High Res. ES-MS: 347.147751 (M + Na^+). Anal. Calcd for $C_{17}H_{24}O_6$: C: 62.95%, H: 7.46%. Found: C: 62.94%, H: 7.55%.

Methyl 2,3-O-*isopropylidene-6*-O-*benzyl-4*-O-(p-*methoxybenzyl)*-α-D-*mannopyranoside* (**30**). The alcohol (**29**) (1.60 g, 4.96 mmol) was dissolved in anhydrous DMF (15 mL) and the solution was cooled in an ice bath. Sodium hydride (80% dispersion in mineral oil, 445 mg, 14.8 mmol) was added by portions, the mixture was stirred for 20 min. *p*-Methoxybenzyl chloride (1.4 mL, 9.90 mmol) was added dropwise, the reaction was continued at 0 °C for 2 h. MeOH (0.5 mL) was added to quench the reaction. The mixture was diluted with EtOAc (100 mL), and the organic solution was washed with 5% brine (3 × 30 mL), dried over anhydrous Na_2SO_4, filtered, and evaporated. Chromatography on silica gel using 7% (v/v) EtOAc/toluene yielded compound **30** (2.0 g, 91%). $[\alpha]_D^{22}$ + 40.5° (*c* 1.1, $CHCl_3$). ^1H NMR (360 MHz, $CDCl_3$): δ 7.24–7.34 (m, 5 H, $C_6H_5CH_2$), 7.16 (d, 2H, *J* = 8.5 Hz, *o*-H-MeO$C_6H_5CH_2$), 6.80 (d, 2H, *J* = 8.6 Hz, *m*-H-MeO$C_6H_5CH_2$), 4.92 (s, 1H, H-1), 4.76 (d, 1H, *J* = 11.1 Hz, ArCH_2–), 4.61 (d, 1H, *J* = 12.2 Hz, ArCH_2–), 4.53 (d, 1H, *J* = 12.2 Hz, ArCH_2–), 4.45 (d, 1H, *J* = 11.1 Hz, ArCH_2–), 4.27 ('t', 1H, *J* [a] 6.4 Hz, H-3), 4.11 (d, 1H, *J* = 5.9 Hz, H-2), 3.77 (s, 3H, MeO$C_6H_5CH_2$+OMe), 3.61 – 3.75 (m, 3H, H-5 + H6a + H-6b), 3.52 (dd, 1H, *J* = 7.0 Hz, 9.9 Hz, H-4), 3.37 (s, 3H, OMe), 1.50 (s, 3H, Me), 1.35 (s, 3H, Me). High Res. ES-MS: 467.20449 (M + Na^+). Anal. Calcd for $C_{25}H_{32}O_7$: C: 67.55%, H: 7.26%. Found: C: 67.35%, H: 7.24%.

Methyl 6-O-*benzyl-4*-O-*(*p-*methoxybenzyl)*-α-D-*mannopyranoside* (**31**). A solution of compound **30** (650 mg, 1.46 mmol) in methanol (8 mL) was cooled to 0 °C, HBF_4 (48% aqueous, 65 µl) was added, the mixture was stirred for 24 h while it rose to ambient temperature. Another aliquot of HBF_4 (48% aqueous, 65 µL) was added, and the reaction was continued for a further 24 h. NEt_3 (2 mL) was added, the solution was evaporated under high vacuum. Chromatography on silica gel using 5% (v/v) MeOH/CH_2Cl_2 as eluent afforded the desired diol **31** (479 mg, 81%). $[\alpha]_D^{22}$ + 69.1° (*c* 0.8, $CHCl_3$). ^1H NMR (360 MHz, $CDCl_3$): δ 7.24–7.37 (m, 5H, $C_6H_5CH_2$), 7.14 (d, 1H, *J* = 8.7 Hz, *o*-H-MeO$C_6H_5CH_2$), 6.81 (d, 1H, *J* = 8.7 Hz, *m*-H-MeO$C_6H_5CH_2$), 4.71 (d, 1H, *J* = 0.7 Hz, H-1), 4.65 (d, 1H, *J* = 11.8 Hz, ArCH_2–),), 4.62 (d, 1H, *J* = 10.3 Hz, ArCH_2–), 4.53 (d, 1H, *J* = 12.1 Hz, ArCH_2–), 4.48 (d, 1H, *J* = 11.0 Hz, ArCH_2–), 3.66 – 3.87 (m, 9H, H-2 + H-3 + H-4 + H-5 + H-6a + H-6b + OMe+*Me*O$C_6H_5CH_2$), 3.33 (s, 3H, OMe). High Res. ES-MS: 427.17303 (M + Na^+). Anal. Calcd for $C_{22}H_{28}O_7$: C: 65.33%, H: 6.98%. Found: C: 65.00%, H: 6.98%.

Methyl 2-O-*benzoyl-6*-O-*benzyl-4*-O-*(*p-*methoxybenzyl)*-α-D-*mannopyranoside* (**32**). A solution of diol **31** (631.4 mg, 1.56 mmol), trimethyl orthobenzoate (821 µL, 4.86 mmol) and camphorsulphonic acid (70 mg) in $CHCl_3$ (50 mL) was concentrated on a Rotavap under reduced pressure to ~15 mL, another portion of $CHCl_3$ (50 mL) was added and the resulting solution was concentrated to ~15 mL again. A third portion of $CHCl_3$ (50 mL) was added and the same process was repeated. TLC exam showed that the starting material had disappeared. Et_3N (2.5 mL) was added and the mixture was evaporated to dryness under high vacuum. The syrupy mixture was dissolved in 80% (v/v) HOAc/H_2O (30 mL), and the reaction was continued at room temperature for 1 h. The solution was evaporated to dryness. After chromatography on silica gel using 30% (v/v) EtOAc/pentane as eluent, the compound **32** was obtained as a colorless foam (702 mg, 88%). $[\alpha]_D^{22}$ –2.0° (*c* 1.8, $CHCl_3$). ^1H NMR (360 MHz, $CDCl_3$): δ 8.03 (m, 2H, *o*-H-C_6H_5CO), 7.55 (m, 1H, *p*-H-C_6H_5CO), 7.28–7.58 (m, 7H, *m*-H-C_6H_5CO + $C_6H_5CH_2$), 7.18 (d, 2H, *J* = 6.6 Hz, *o*-H-$C_6H_5CH_2$), 6.80 (d, 2H, *J* = 6.6 Hz, *m*-H-$C_6H_5CH_2$), 5.34 (1H, dd, *J* =1.8 Hz, 3.4 Hz, H-2), 4.84 (d, 1H, *J* = 1.7 Hz, H-1), 4.75

(d, 1H, *J* = 12.0 Hz, Ar*CH*$_2$–), 4.71 (d, 1H, *J* = 10.9 Hz, Ar*CH*$_2$–), 4.58 (d, 1H, *J* = 10.8 Hz, Ar*CH*$_2$–), 4.57 (d, 1H, *J* = 12.0 Hz, Ar*CH*$_2$–), 4.21 (dd, 1H, *J* = 3.4 Hz, 9.4 Hz, H-3), 3.97 (t, 1H, *J* = 9.4 Hz, H-4), 3.89 (dd, 1H, *J* = 4.1 Hz, 11.1 Hz, H-6a), 3.77–3.81 (m, 2H, H-5 + H-6b), 3.75 (s, 3H, OMe + *Me*OC$_6$H$_5$CH$_2$), 3.38 (s, 3H, OMe). High Res. ES-MS: 531.19945 (M + Na$^+$). Anal. Calcd for $C_{29}H_{32}O_8$: C: 68.50%, H: 6.34%. Found: C: 68.85%, H: 6.16%.

Methyl 2-O-*benzoyl-6*-O-*benzyl-4*-O-(p-*methoxybenzyl)-3*-O-*(2′*-O-(p-*methoxybenzyl)-4′*-O-*benzyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)*-α-D-*mannopyranoside* (**34**). A mixture containing acceptor **32** (223 mg, 0.438 mmol), donor **12** or **17** (264 mg, 0.675 mmol), and molecular sieve 4Å (0.5 g) was dried under high vacuum for 2 h. CH_2Cl_2 (10 mL) was added, the mixture was protected under argon, and stirred for 1 h at 0 °C. 2,6 Di-*tert*-butyl-4-methylpyridine (273 mg, 1.30 mmol) and methyl trifluoromethanesulfonate (137 μL, 1.17 mmol) were added, and the reaction was continued for 30 min. Et_3N (0.5 mL) was added to quench the reaction, the solids were filtered off through a thin bed of Celite, and washed with more CH_2Cl_2. The combined organic solution was evaporated, and chromatography on silica gel using 8% (v/v) EtOAc/toluene gave pure disaccharide **34** (309 mg, 83%). $[\alpha]_D^{22}$ + 22.4° (*c* 0.5, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 8.03 (m, 2H, *o*-H-*C*$_6$*H*$_5$CO), 7.54 (m, 1H, *p*-H-*C*$_6$*H*$_5$CO), 7.21–7.37 (m, 12 H, *C*$_6$*H*$_5$CH$_2$ + *m*-H-*C*$_6$*H*$_5$CO), 7.10 (m, 4H, *o*-H-MeO*C*$_6$*H*$_5$CH$_2$), 6.73 (m, 4H, *m*-H-MeO*C*$_6$*H*$_5$CH$_2$), 5.37 (dd, 1H, *J* = 2.0 Hz, 2.9 Hz, H-2), 5.13 (d, 1H, *J* = 3.1 Hz, H-1′), 5.04 (d, 1H, *J* = 10.7 Hz, Ar*CH*$_2$–), 4.84 (d, 1H, *J* = 1.6 Hz, H-1), 4.65 (d, 1H, *J* = 12.0 Hz, Ar*CH*$_2$–), 4.27 – 4.51 (m, 7H, Ar*CH*$_2$– + H-3), 4.11 (t, 1H, *J* = 9.5 Hz, H-4), 3.90 (m, 1H, H-5′), 3.70–3.84 (m, 10H, 2 × OMe *Me*OC$_6$H$_5$CH$_2$+ H-2′ + H-5 +H-6a + H-6b), 2.00 (m, 1H, H-3e′), 1.76 (m, 1H, H-3a′), 1.03 (d, 3H, *J* = 6.6 Hz, H-6′). High Res. ES-MS: 871.366398 (M + Na$^+$). Anal. Calcd for $C_{50}H_{56}O_{12}$: C: 70.74%, H: 6.65%. Found: C: 70.55%, H: 6.88%.

Methyl 6-O-*benzyl-4*-O-*(p-methoxybenzyl)-3*-O-*(2′*-O-(p-*methoxybenzyl)-4′*-O-*benzyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)*-α-D-*mannopyranoside* (**35**). Disaccharide **34** (200 mg, 0.24 mmol) was dissolved in anhydrous methanol (10 mL), a solution of sodium methoxide in anhydrous methanol (1 mL, 1.27 M) was added, and the mixture was stirred for 2 h at room temperature. When the starting material was consumed, the solution was neutralized with Amberlite IR-120 (H$^+$). The resin was filtered off and the solution was concentrated under high vacuum. The alcohol **23** was purified by flash chromatography using 25% (v/v) EtOAc/toluene as eluent (164 mg, 93%). $[\alpha]_D^{22}$ + 59.9° (*c* 0.8, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.25–7.33 (m, 10H, *C*$_6$*H*$_5$CH$_2$), 7.12 (d, 2H, *J* = 8.6 Hz, *m*-H-MeO*C*$_6$*H*$_5$CH$_2$), 7.02 (d, 2H, *J* = 8.6 Hz, *m*-H-MeO*C*$_6$*H*$_5$CH$_2$), 6.70 – 6.75 (m, 4H, *o*-H-MeO*C*$_6$*H*$_5$CH$_2$), 5.15 (d, 1H, *J* = 3.4 Hz, H-1′), 4.93 (d, 1H, *J* = 10.6 Hz, Ar*CH*$_2$–), 4.73 (d, 1H, *J* = 1.5 Hz, H-1), 4.60 (d, 1H, *J* = 12.2 Hz, Ar*CH*$_2$–), 4.53 (d, 1H, *J* = 12.0 Hz, Ar*CH*$_2$–), 4.49 (d, 1H, *J* = 12.3 Hz, Ar*CH*$_2$–), 4.43 (d, 1H, *J* = 11.8 Hz, Ar*CH*$_2$–), 4.37 (d, 2H, *J* ≈ 11.0 Hz, Ar*CH*$_2$–), 4.36 (d, 1H, *J* = 10.6 Hz, Ar*CH*$_2$–), 4.02–4.09 (m, 2H, H-3 + H-5′), 3.97 (dd, 1H, *J* = 1.7 Hz, 3.3 Hz, H-2), 3.80–3.88 (m, 2H, H-2′ + H-4), 3.74 (s, 3H, OMe+MeOC$_6$H$_5$*CH*$_2$), 3.73 (s, 3H, OMe-MeOC$_6$H$_5$*CH*$_2$), 3.63–3.72 (m, 3H, H-5 + H-6a + H-6b), 3.45 (br., 1H, H-4′), 3.35 (s, 3H, OMe), 2.12 (m, 1H, H-3e′), 1.87 (m, 1H, H-3a′), 1.16 (d, 3H, *J* = 6.6 Hz, H-6′). Anal. Calcd for $C_{43}H_{52}O_{11}$: C: 69.33%, H: 7.04%. Found: C: 69.11%, H: 7.25%.

Methyl 6-O-*benzyl-4*-O-(p-*methoxybenzyl)-2*-O-*(2″,3″, 4″,6″-tetra*-O-*benzyl*-α-D-*galactopyranosyl)-3*-O-*(2′*-O-(p-*methoxybenzyl)-4′*-O-*benzyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)*-α-D-*mannopyranoside* (**36**). A mixture of disaccharide **35** (177 mg, 0.237 mmol), donor **33** (347 mg, 0.593 mmol), and molecular sieve 4Å (600 mg) was dried under high vacuum for 2 h, CH_2Cl_2 (8 mL) was added, and the mixture was stirred for 1 h at 0 °C under argon, followed by the addition of 2,6 di-*tert*-butyl-4-methylpyridine (149 mg, 0.73 mmol) and methyl trifluoromethanesulfonate (81.5 μL, 0.71 mmol). After 30 min, the reaction was quenched by addition of Et_3N (1 mL). The solid was filtered off through a thin bed of Celite, and washed with CH_2Cl_2. The combined organic solution was evaporated to dryness. The trisaccharide **36** was obtained by chromatography using 5% (v/v) EtOAc/toluene as eluent (249 mg, 83%). $[\alpha]_D^{22}$ + 57.5° (c 0.9, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.14–7.45 (m, 3H, *C*$_6$*H*$_5$CH$_2$), 7.04 (d, 2H, *J* = 8.6 Hz, MeO*C*$_6$*H*$_5$CH$_2$), 7.00 (d, 2H, *J* = 8.6 Hz, MeO*C*$_6$*H*$_5$CH$_2$), 6.75 (d, 2H, *J* = 8.6 Hz, MeO*C*$_6$*H*$_5$CH$_2$), 6.67 (d, 2H, *J* = 8.6 Hz, MeO*C*$_6$*H*$_5$CH$_2$), 5.56 (d, 1H, *J* = 3.6 Hz, H-1″), 5.20 (d, 1H, *J* = 3.3 Hz, H-1′), 5.01 (d, 1H, *J* = 12.0 Hz, Ar*CH*$_2$–), 4.91–4.95 (m, 2H, Ar*CH*$_2$–), 4.80 (d, 1H, *J* = 1.8 Hz, H-1′), 4.75 (d, 1H, J = 11.8 Hz, Ar*CH*$_2$–), 4.64 (d, 1H, *J* = 12.0 Hz, Ar*CH*$_2$–), 4.59 (d, 1H, *J* = 11.8 Hz, Ar*CH*$_2$–), 4.57 (d, 1H, *J* = 11.5 Hz, Ar*CH*$_2$–), 4.50 (d, 1H, *J* = 11.8 Hz, Ar*CH*$_2$–), 4.48 (d, 1H, *J* = 12.0 Hz, Ar*CH*$_2$–), 4.42 (d, 2H, *J* = 11.8 Hz, Ar*CH*$_2$–), 4.21 – 4.32 (m, 5H, H-3 + Ar*CH*$_2$–), 3.97–4.16 (m, 6H, H-2 + H-5″ + H-2″ + H-5′ + H-4 + H-3″), 3.90 (d, 1H, *J* = 2.0 Hz, H-4″), 3.63–3.82 (m, 10 H, 2 × OMe + *Me*OC$_6$H$_5$CH$_2$ + H-5 + H-6a + H-6b + H-2′), 3.55 (dd, 1H, *J* = 6.5 Hz, 9.8 Hz, H-6a″), 3.49 (dd, 1H, *J* = 6.1 Hz, 9.7 Hz, H-6b″), 3.25 (s, 3H, OMe), 3.11 (br. 1H, H-4′), 1.95 (m, 1H, H-3e′), 1.71 (m, 1H, H-3a′), 1.09 (d, 3H, *J* = 6.5 Hz, H-6′). High Res. ES-MS: 1289.57662 (M + Na$^+$).

Methyl 6-O-*benzyl-2*-O-*(2″,3″,4″,6″-tetra*-O-*benzyl*-α-D-*galactopyranosyl)-3*-O-*(4′*-O-*benzyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)*-α-D-*mannopyranoside* (**37**). A solution of trisaccharide **36** (136 mg, 0.107 mmol) in CH_3CN (5 mL) was treated with water (400 μL) and ceric ammonium nitrate (300 mg, 0.549 mmol) for 1.5 h at room temperature. The reaction mixture was diluted with CH_2Cl_2 (50 mL), washed with water (2 × 20 mL), dried over anhydrous Na_2SO_4, and evaporated. The crude material was chromatographed on silica gel using 25% (v/v) EtOAc/toluene to give **37** (86.9 mg, 79%). $[\alpha]_D^{22}$ + 40.3° (*c* 0.6, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.17–7.34 (m, 30H, *C*$_6$*H*$_5$CH$_2$), 5.17 (d, 1H, *J* = 3.6 Hz, H-1″), 5.05 (d, 1H, *J* = 3.6 Hz, H-1′), 4.89 (d, 1H, *J* = 11.5 Hz, Ph*CH*$_2$–), 4.84 (d, 1H, *J* = 11.8 Hz, Ph*CH*$_2$–), 4.82 (d, 1H, *J* ≈ 1 Hz, H-1), 4.70 (d, *J* = 12.0 Hz, Ph*CH*$_2$–), 4.49–4.67 (m, 6H, Ph*CH*$_2$–), 4.47 (d, 1H, *J* = 11.8 Hz, PhCH$_2$–), 4.40 (d, 1H, *J* = 11.9 Hz, Ph*CH*$_2$–), 4.21 (d, 1H, *J* = 12.1 Hz, Ph*CH*$_2$–), 4.15 (t, 1H, *J* = 9.5 Hz, H-4), 4.03–4.10 (m, 2H, H-2″ + H-5″),

3.88–3.99 (m, 6H, H-2 + H-3 + H-2′ + H-5′ + H-3″ + H-4″), 3.67–3.83 (m, 3H, H-5 + H-6a + H-6b), 3.52 (dd, 1H, *J* = 6.5 Hz, 9.6 Hz, H-6a″), 3.45 (dd, 1H, *J* = 6.1 Hz, 9.6 Hz, H-6b″), 3.19 (s, 3H, OMe), 2.94 (br, 1H, H-4′), 1.95 (m, 1H, H-3e′), 1.50 (m, 1H, H-3a′), 1.07 (d, 1H, *J* = 6.5 Hz, H-6′). High Res. ES-MS: 1049.467065 (M + Na^+). Anal. Calcd for $C_{61}H_{69}O_{14}$: C: 71.39%, H: 6.78%. Found: C: 70.94%, H: 6.81%.

Methyl 6-O-*benzyl-2*-O-*(2″,3″,4″,6″-tetra*-O-*benzyl*-α-D-*galactopyranosyl)-3*-O-*(4′*-O-*benzyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)-4,2′-di*-O-*methylene*-α-D-*mannopyranoside* (**38**). A solution of trisaccharide diol **37** (132 mg, 0.129 mmol) in anhydrous DMF (3 mL) was treated with sodium hydride (80% dispersion in mineral oil, 15 mg, 0.625 mmol) for 10 min, dibromomethane (18 µL, 0.258 mmol) was added dropwise, and the reaction was stirred at room temperature for 18 h. Methanol (1 mL) was added to quench the reaction. The reaction mixture was concentrated under high vacuum and purified by chromatography on silica gel using 5% (v/v) EtOAc/toluene to give the tethered trisaccharide **38** (37 mg, 27% yield). $[\alpha]_D^{22}$ + 31.1° (*c* 1.0, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 712–7.40 (m, 30H, $C_6H_5CH_2$), 5.55 (d, 1H, *J* = 3.6 Hz, H-1″), 5.44 (d, 1H, *J* = 3.6 Hz, H-1′), 4.93 (d, 1H, *J* = 11.8 Hz, PhCH_2–), 4.92 (d, 1H, *J* = 11.4 Hz, PhCH_2–), 4.79 (d, 1H, *J* ≈ 1 Hz, H-1), 4.77 (d, 1H, *J* = 12.1 Hz, PhCH_2–), 4.34–4.66 (m, 11H, –CHaHb– + PhCH_2–), 4.17–4.23 (m, 2H, H-2 + H-4), 4.09 (dd, 1H, *J* = 3.6 Hz, 10.6 Hz, H-3″), 3.94–4.02 (m, 3H, H-2 + H-3″ + H-5″), 3.87 (br d, 1H, *J* ≈ 1.7 Hz, H-4″), 3.81 (dq, 1H, H-2′), 3.67–3.80 (m, 3H, H-2′ + H-5 + H-6a), 3.54–3.62 (m, 2H, H-6b + H-6a″), 3.47 (dd, 1H, *J* = 6.6 Hz, 9.6 Hz, H-6b″), 3.24 (s, 3H, OMe), 3.05 (br, 1H, H-4′), 2.06 (m, 1H, H-3e′), 1.69 (m, 1H, H-3a′), 1.18 (d, 1H, *J* = 6.5 Hz, H-6′). FAB MS: 1061.3 (M + Na^+).

Methyl 2-O-*(*α-D-*galactopyranosyl)-3*-O-*(3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)-4,2′-di*-O-*methylene*-α-D-*mannopyranoside* (**3**). To a solution of the tethered trisaccharide **38** (44 mg, 0.042 mmol) and 5% Pd on charcoal (53 mg) in methanol (30 mL) was added acetic acid (10 mL), the resulting mixture was hydrogenated overnight. The mixture was filtered through a Millipore membrane filter (0.25 micron), and the organic solution was concentrated under high vacuum. The crude material was first purified by silica gel chromatography using 10 → 20% (v/v) MeOH/CH_2Cl_2 as eluent, followed by reversed phase silica gel chromatography (C18) using 10 → 15% MeOH/H_2O as eluent to give pure tethered trisaccharide **3** (19 mg, 90 %) which was lyophilized. $[\alpha]_D^{22}$ + 78.3° (*c* 0.3, H_2O). ^{1}H and ^{13}C NMR (500 MHz, D_2O): See Tables 1 and 2. ES-MS: 521.18665 (M + Na^+). Anal. Calcd for $C_{20}H_{34}O_{14}$: C: 67.55%, H: 7.26%; Found: C: 67.35%, H: 7.24%.

1,4-benzene-di-methanethiol (**41**). Thiourea (634 mg, 8.3 mmol) was added to a solution of α,α′-dibromo-*p*-xylene (1.0 g, 3.8 mmol) in acetone (20 mL). The mixture was heated to reflux for 1 h, and then cooled to room temperature. A precipitate of **40** was filtered off, and dried under high vacuum. The solid was dissolved in 2M NaOH (20 mL), the mixture was heated to reflux under argon for 2 h, and the solution was neutralized to pH ~2 with 2M HCl. The dimercaptane **41** was extracted into CH_2Cl_2, the organic solution was washed with H_2O (2 × 25 mL), dried over anhydrous Na_2SO_4, and evaporated. The residue (540 mg, 84%) was sufficiently pure and used without any further purification. ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.26 (s, 4H, –C_6H_4–), 3.71 (d, 4H, *J* = 7.5 Hz, –CH_2–), 1.74 (t, 2H, *J* = 7.5 Hz, –SH). High RES. ES-MS: 170.02221 (M + Na^+). Anal. Calcd for $C_8H_{10}S_2$: C: 56.43%, H: 5.92%. Found: C: 56.57%, H: 5.85%.

Methyl 4,6-O-*benzylidene-2*-O-*(4″,6″*-O-*benzylidene*-α-D-*galactopyranosyl)-3*-O-*(3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)*-α-D-*mannopyranoside* (**42**). Benzaldehyde dimethyl acetal (842 µL, 5.50 mmol) and camphorsulphonic acid (500 mg, 2.15 mmol) was added to a mixture of trisaccharide **1** (372 mg, 0.77 mmol) in anhydrous DMF (6 mL). The mixture was stirred at room temperature for 18 h, then Et_3N (1 mL) was added to quench the reaction, and the mixture was evaporated to dryness under reduced pressure. Compound **42** was obtained as colorless foam by chromatography on silica gel using 4% (v/v) MeOH/CH_2Cl_2 as eluent (275 mg, 54%). $[\alpha]_D^{22}$ + 52.5° (*c* 1.3, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.29–7.49 (m, 10H, C_6H_5), 5.59 (s, 1H, C_6H_5CH), 5.54 (s, 1H, C_6H_5CH), 5.28 (d, 1H, *J* = 2.0 Hz, H-1″), 5.26 (d, 1H, *J* = 3.7 Hz, H-1′), 4.72 (d, 1H, *J* = 1.5 Hz, H-1), 4.31 (dd, 1H, *J* = 3.3 Hz, 9.9 Hz, H-3), 4.20–4.29 (m, 3H, H-4″ + H-6a + H-6a″), 4.14 (t, 1H, *J* = 9.7 Hz, H-6b), 4.08 (dd, 1H, *J* = 1.6 Hz, 12.6 Hz, H-6b″), 3.98–4.02 (m, 3H, H-2″ + H-3″ + H-2), 3.87–3.96 (m, 2H, H-2′ + H-5′), 3.83 (br, 1H, H-5″), 3.73–3.82 (m, 2H, H-4 + H-5), 3.70 (br, 1H, H-4′), 3.38 (s, 3H, OMe), 2.04 (m, 1H, H-3e′), 1.76 (m, 1H, H-3a′), 1.17 (d, 3H, *J* = 6.6 Hz, H-6′). High Res. ES-MS: 685.24310 (M + Na^+). Anal. Calcd for $C_{33}H_{42}O_{14}$: C: 59.81%, H: 6.39%. Found: C: 58.64%, H: 6.61%.

Methyl 4,6-O-*benzylidene-2*-O-*(2″,3″-di*-O-*acetyl-4″,6″*-O-*benzylidene*-α-D-*galactopyranosyl)-3*-O-*(2′,4′-di*-O-*acetyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)-a*-D-*mannopyranoside* (**43**). Trisaccharide **42** (178 mg, 0.27 mmol) was treated with a 1:1 mixture of Ac_2O/pyridine (6 mL) at room temperature for 18 h, and the mixture was evaporated to dryness under reduced pressure. The acetylated trisaccharide **43** was purified by chromatography on silica gel using 25% → 30% (v/v) EtOAc/toluene as eluent (195 mg, 87%). $[\alpha]_D^{22}$ + 102.6° (*c* 1.0, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 7.50 (m, 2H, C_6H_5), 7.31–7.41 (m, 8H, C_6H_5), 5.25 (m, 3H, H-1″ + Ph*CH*), 5.42 (dd, 1H, *J* = 3.4 Hz, 10.9 Hz, H-2″), 5.36 (dd, 1H, *J* = 3.1 Hz, 10.9 Hz, H-3″), 5.04–5.11 (m, 2H, H-1′ + H-2′), 5.00 (br, 1H, H-4′), 4.64 (d, 1H, *J* = 1.6 Hz, H-1), 4.52 (1H, dd, *J* = 0.9 Hz, 3.1 Hz, H-4″), 4.27 (dd, 1H, *J* = 1.3 Hz, 12.6 Hz, H-6a), 4.25 (dd, 1H, *J* = 2.7Hz, 10.3 Hz, H-3), 4.22 (dd, 1H, *J* = 4.4 Hz, 9.9 Hz, H-6a″), 4.05–4.13 (m, 2H, H-6b + H-6b″), 4.00 ('t', 1H, *J* ≈ 2.2 Hz, H-2), 3.94 (dq, 1H, H-5′), 3.87 (t, 1H, *J* = 10.3 Hz, H-4), 3.73–3.83 (m, 2H, H-5 + H-5″), 3.38 (s, 3H, OMe), 2.11 (m, 1H, H-3e′), 2.10 (s, 3H, OAc), 2.09 (s, 3H, OAc), 2.08 (s, 3H, OAc), 1.88 (m, 1H, H-3a′), 1.44 (s, 3H, OAc), 1.10 (d, 1H, *J* = 6.5 Hz, H-6′). High Res. ES-MS: 853.28975 (M + Na^+). Anal. Calcd for $C_{41}H_{50}O_{18}$: C: 59.27%, H: 6.07%. Found: C: 59.14%, H: 6.24%.

Methyl 4-O-*benzoyl-6-bromo-6-deoxy-2*-O-*(2″,3″-di*-O-*acetyl-4″*-O-*benzoyl-6″-bromo-6″-deoxy*-α-D-*galacto-pyranosyl)-3*-O-*(2′,4′-di*-O-*acetyl-3′,6′-dideoxy*-α-D-xylo-

hexopyranosyl)-α-D-*mannopyranoside* (**44**). A mixture containing compound **43** (275 mg, 0.33 mmol), *N*-bromosuccinimide (176 mg, 0.59 mmol) and barium carbonate (262 mg, 1.32 mmol) in anhydrous carbon tetrachloride (10 mL) was heated to reflux for 3 h under the an atmosphere of argon. The solid was filtered off and washed with more EtOAc. The organic solution was evaporated to dryness under reduced pressure and the crude material was purified by chromatography on silica gel using 15% (v/v) EtOAc/toluene to afford the dibromido derivative of trisaccharide **44** (205 mg, 63%) as colorless foam. $[\alpha]_D^{22}$ + 122.5° (*c* 0.4, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 8.09 (m, 2H, *o*-H-C_6H_5CO), 7.95 (m, 2H, *o*-H-C_6H_5CO), 7.56–7.65 (m, 2H, *p*-H-C_6H_5CO), 7.41–7.53 (m, 4H, *m*-H-C_6H_5CO), 5.81 ('d', 1H, *J* ≈ 2.9 Hz, H-4″), 5.67 (d, 1H, *J* = 3.8 Hz, H-1″), 5.53 (t, 1H, *J* = 9.9 Hz, H-4), 5.49 (dd, 1H, *J* = 3.3 Hz, 10.9 Hz, H-3″), 5.21 (dd, 1G, *J* = 3.8 Hz, 10.9 Hz, H-2″), 5.05 (br, 1H, H-4′), 5.01 (d, 1H, *J* = 3.5 Hz, H-1′), 4.94 (m, 2H, H-1 + H-2′), 4.42 (dd, 1H, *J* = 2.4 Hz, 9.9 Hz, H-3), 4.38 (m, 1H, H-5″), 4.13 ('t', 1H, *J* ≈ 2.1 Hz, H-2), 4.01 (dq, 1H, H-5′), 3.95 (m, 1H, H-5), 3.49 (s, 3H, OMe), 3.38–3.48 (m, 4H, H-6a + H-6b + H-6a″ + H-6b″), 2.18 (s, 3H, OAc), 2.08 (m, 4H, OAc + H-3e′), 1.97 (s, 3H, OAc), 1.89 (m, 1H, H-3a′), 1.50 (s, 3H, OAc), 1.15 (d, 3H, *J* = 6.4 Hz, H-6′). High Res. ES-MS: 1011.10930 (M + Na^+). Anal. Calcd for $C_{41}H_{48}O_{18}Br_2$: C: 49.81%, H: 4.89%. Found: C: 50.44%, H: 4.97%.

Methyl 4-O-*benzoyl-6-thio-6-deoxy-2*-O-*(2″,3″-di*-O-*acetyl-4″*-O-*benzoyl-6″-thio-6″-deoxy*-α-D-*galacto-pyranosyl)-3*-O-*(2′,4′-di*-O-*acetyl-3′,6′-dideoxy*-α-D-xylo-*hexopyranosyl)-6,6″-(1,4-benzene di methylene)*-α-D-*mannopyranoside* (**45**). The dimercaptane **41** (482.1 mg, 2.83 mmol) was dissolved in anhydrous THF (8 mL) under argon, potassium *tert*-butoxide (668.8 mg, 5.66 mmol) was added, and the mixture was stirred for 30 min. Anhydrous ether (20 mL) was added, the mixture was stirred for 10 min, the yellowish precipitate was filtered, washed with more anhydrous ether (30 mL), and dried under high vacuum (650 mg).

The trisaccharide **44** (18.0 mg, 0.018 mmol) was dissolved in anhydrous DMF (1.0 mL) under argon, the dipotassium salt of **41** (21.0 mg, 0.085 mmol) and potassium iodide (5 mg, 0.030 mmol) were added, and the mixture was heated to 50 °C for 2 h. More dipotassium salt of **41** (27.4 mg, 0.11 mmol) was added, and the reaction was continued overnight at 50 °C. The mixture was concentrated and purified by repeated chromatography on silica gel using 15% (v/v) EtOAc/toluene as eluent. The product which has a R_f of 0.49 (30% AcOEt/toluene) was isolated (3.0 mg, 17%). $[\alpha]_D^{22}$ + 81.4° (*c* 0.2, $CHCl_3$). ^{1}H NMR (360 MHz, $CDCl_3$): δ 8.08 (m, 2H, *o*-H-C_6H_5CO), 7.91 (m, 2H, *o*-H-C_6H_5CO), 7.05–7.65 (m, 10H, *m*-H-C_6H_5CO + *p*-H-C_6H_5CO + –$CH_2C_6H_4CH_2$–), 6.23 ('d', 1H, *J* ≈ 2.9 Hz, H-4″), 5.32 (t, 1H, *J* = 9.9 Hz, H-4), 5.23 (d, 1H, *J* = 4.0 Hz, H-1″), 5.15 (dd, 1H, *J* = 2.9 Hz, 10.9 Hz, H-3″), 5.00 (dd, 1H, *J* = 3.9 Hz, 11.0 Hz, H-2″), 4.86–4.97 (m, 3H, H-1′ + H-2′ + H-4′), 4.18 (dd, 1H, *J* = 2.4 Hz, 10.0 Hz, H-3), 3.40–3.89 (m, 9H, H-1 + H-2 + H-3 + H-5 + H-5″ + –$CH_2C_6H_4CH_2$–), 3.39 (s, 3H, OMe), 2.93 (dd, 1H, *J* = 2.2 Hz, 15.0 Hz, H-6a), 2.76 (dd, 1H, *J* = 8.1 Hz, 15.0 Hz, H-6a″), 2.67 (dd, 1H, *J* = 6.2 Hz, 15.2 Hz, H-6b″), 2.49 (dd, 1H, *J* = 9.3 Hz, 15.0 Hz, H-6b), 2.03–2.10 (m, 10H, OAc + H-3a′), 1.83 (m, 1H, H-3e′), 1.35 (s, 3H, OAc), 1.08 (d, 3H, *J* = 6.6 Hz, H-6′). High Res. ES-MS: 1019.28002 (M + Na^+).

Methyl 6-thio-6-deoxy-2-O-*(6″-thio-6″-deoxy*-α-D-*galactopyranosyl)-3*-O-*(3′,6′-dideoxy*-α-D-*xylo-hexopyranosyl)-6,6″-(1,4-benzene di methylene)*-α-D-*mannopyranoside* (**4**). The protected trisaccharide 45 (14.0 mg) was dissolved in anhydrous MeOH (3 mL), a solution of NaOMe in anhydrous MeOH (1.27 M, 0.5 mL) was added, and the reaction was continued for 18 h. The mixture was neutralized with a few drop of HOAc, and the solution was concentrated to dryness under reduced pressure. The crude material was purified by preparative HPLC on reversed phase silica gel (C18) using 0% → 80% (v/v) MeOH/H_2O as eluent to afforded the tethered trisaccharide 4 in pure form (7.0 mg, 80%) which was lyophilized. $[\alpha]_D^{22}$ + 10.8° (c 0.2, H_2O). ^{1}H and ^{13}C NMR (500 MHz, D_2O): See Tables 1 and 2. High Res. ES-MS: 643.18556 (M + Na^+).

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