Synthesis of disaccharide congeners of the *Trichinella spiralis* glycan and binding site mapping of two monoclonal antibodies

Ping Zhang, Judith Appleton, Chang-Chun Ling, and David R. Bundle

Abstract: The tetrasaccharide epitope, β-D-Tyv*p*(1→3)β-D-GalNAc*p*(1→4)[α-L-Fuc*p*(1→3)]β-D-GlcNAc*p* (**1**) is the major constituent of the *N*-glycan expressed on the cell surface of the parasite *Trichinella spiralis*. Two monoclonal antibodies (Mabs 9D4 and 18H1) that protect rats against infection by *T. spiralis* bind the terminal disaccharide epitope β-D-Tyv*p*(1→3)β-D-GalNAc*p* conjugated to BSA. The syntheses of disaccharide congeners containing mono-deoxy, mono-methyl, as well as modifications to replace the acetamido group are reported. These target disaccharides were assayed for binding to the protective MAbs. For each antibody different clusters of three hydroxyl groups, that include C-2 and C-4 of tyvelose and for 18H1, the GalNAc acetamido group, provide the key polar interactions with the antibody binding sites. Mapping of the sites by functional group replacement revealed a similar pattern of recognition for the dideoxyhexose by the two MAbs while each recognizes distinct surfaces of the GalNAc residue. Consequently although both antibodies bury the 4-OH of tyvelose, the principal contact surface occurs on opposite sides of the 3,6-dideoxyhexose.

Key words: β-tyveloside, 3,6-dideoxy-D-*arabino*-hexose, *Trichinella* carbohydrate antigen, antibody mapping, *Trichinella spiralis*, *N*-glycans, molecular recognition of carbohydrates, antigen topology, functional group replacement.

Résumé : Le tétrasaccharide épitope, β-D-Tyv*p*(1→3)β-D-GalNAc*p*(1→4)[α-L-Fuc*p*(1→3)]β-D-GlcNAc*p* (**1**) est un constituant majeur du *N*-glucane extrait de la surface de la cellule du parasite *Trichinella spiralis*. Deux anticorps monoclonaux (Mabs 9D4 et 18H1) qui protègent les rats contre les infections par le *T. spiralis* se lient au disaccharide terminal épitope β-D-Tyv*p*(1→3)β-D-GalNAc*p* conjugué au BSA. On rapporte les synthèses de congénères du disaccharide comportant des modifications de type monodésoxy- ou monométhyle ainsi que le remplacement du groupe acétamido. On a évalué les capacités de ces disaccharides à se lier aux MAbs protecteurs. Pour chacun des anticorps, différents ensembles de trois groupes hydroxyles incluant les C-2 et C-4 du tyvelose ainsi que le groupe acétamido GalNAc du 18H1 fournissent les interactions polaires clés avec les sites de liaison de l'anticorps. Une carte des sites obtenue par le remplacement des groupes fonctionnels a permis de réaliser qu'un patron semblable existe pour la reconnaissance du didésoxyhexose par les deux MAbs alors que chacun d'eux reconnaît des surfaces distinctes du résidu GalNAc. En conséquence, même si les deux anticorps ensevelissent le 4-OH du tyvelose, le principal contact de surface se produit sur les faces opposées du 3,6-didésoxyhexose.

Mots clés : β-tyveloside, 3,6-didésoxy-D-*arabino*-hexose, hydrate de carbone antigène du *Trichinella*, carte de l'anticorps, *Trichinella spiralis*, *N*-glycanes, reconnaissance moléculaire des hydrates de carbone, topologie des antigènes, remplacement d'un groupe fonctionnel.

[Traduit par la Rédaction]

Introduction

The causative agent of trichinosis a parasitic nematode, *Trichinella spiralis*, has an exceptionally large host range and is endemic in many carnivorous animals. The parasite establishes itself in the intestinal epithelia of the host animal, following consumption of infected tissue and causes acute muscle pain, fatigue, fever, and in severe cases, death (1). During invasion, *T. spiralis* secretes an array of glycoproteins that are key to successful colonization of the intestine by the parasite. Antibodies against the glycan portion of the glycoproteins can prevent parasitic invasion and cause expulsion of the *T. spiralis* larvae from the intestine (2, 3). Here the syntheses of congeners of the native, terminal disaccharide β-D-Tyv*p*(1→3)β-D-GalNAc*p* are reported. The inhibitory activities of these ligands are used to define the precise specificity and topology of the epitope's contact surface with the binding sites of two protective rat

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We dedicate this paper to the memory of Professor Raymond U. Lemieux.

P. Zhang, C.-C. Ling, and D.R. Bundle.[1] Department of Chemistry, The University of Alberta, Edmonton, AB T6G 2G2, Canada. **J. Appleton.** James A. Baker Institute, College of Veterinary Medicine, Cornell University, Ithaca, NY 14853, U.S.A.

[1]Corresponding author (e-mail: dave.bundle@ualberta.ca).

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monoclonal antibodies (MAbs) produced from lymphocytes of rats infected with *T. spiralis*.

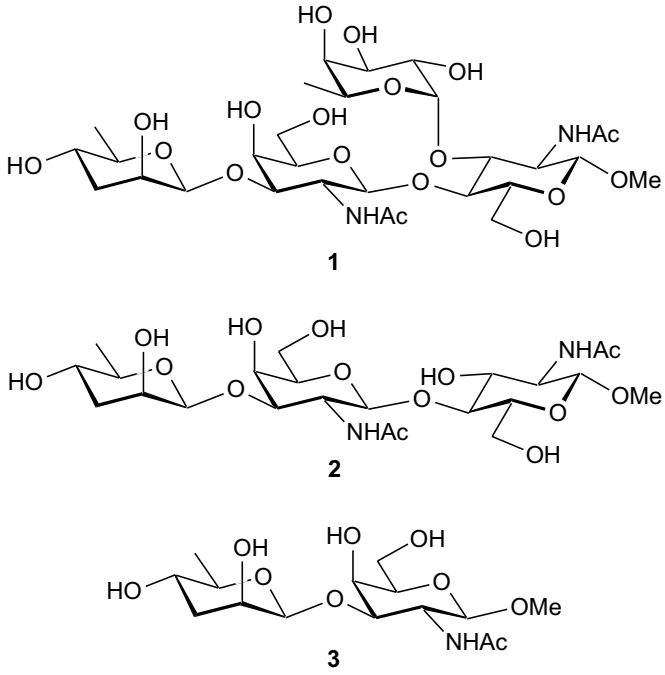
In common with many other nematodes (4), *Trichinella* secretes and displays on its cuticle a large number of proteins that display a rich repertoire of *N*-linked glycans (5, 6). Complex-type *N*-glycans in eukaryotes are characterized by the presence of variable numbers of antennae (usually two, three, or four, called bi-, tri-, and tetraantennary, respectively). In mammals the antennae stubs are usually elongated by the addition of β-Gal to give β-Gal(1→4)GlcNAc or "LacNAc". In a restricted number of mammalian glycoproteins, β-GalNAc is added to the GlcNAc stubs in place of β-Gal and thus they have β-GalNAc(1→4)GlcNAc or "LacdiNAc" antennae. Both types of antennae are found in vertebrates but LacdiNAc is the preferred antennae building block in many helminths including *Trichinella* (5, 6). The L1 stage of *T. spiralis* modifies LacdiNAc with phosphorylcholine moieties or by substitution with 3,6-dideoxy-D-*arabino*-hexose (D-tyvelose), a sugar normally confined to the cell wall lipopolysaccharide (LPS) of gram-negative bacteria. Both modifications create highly immunogenic epitopes.

Trichinella spiralis faces an aggressive immune response from its animal host, but avoids extinction by changing its expression of antigenic molecules as it changes life stages (7, 8) and by residing in intracellular habitats (9, 10). Antibody responses induced by L1 stage larvae in the intestine and in the muscle are largely directed at *N*-glycans unique to this stage (11–14). Glycans capped by tyvelose are the target of protective immunity against *T. spiralis* and antibodies specific for one of these glycans protect rats against reinfection with *T. spiralis* (3, 15, 16). The structure of the antennae of the protective antigen is shown (**1**, Fig. 1). The most striking and unusual feature is the presence of tyvelose as its β-pyranoside form rather than the α-anomer, which previously was the only naturally occurring form to have been observed in bacterial LPS antigens of pathogenic *Salmonella* (17).

Some monoclonal tyvelose-specific antibodies that bind the *T. spiralis* glycan provide protection to passively immunized rats (3, 5). Antibody 9D4 has high protective efficacy in rats and exhibits high affinity for tetrasaccharide **1**. Another tyvelose specific antibody 18H1, also shows poor protective efficacy (3, 15). It fails to cause expulsion of larvae from the intestines of rats, even though it exhibits most of the inhibitory effects shown by antibody 9D4.

No structural details of these antibody binding sites are available, although preliminary crystallographic data has been collected for MAb 9D4, and both antibody Fv regions have been partially sequenced (18). The 3,6-dideoxy arabinohexose was inferred to be linked to the *N*-glycans via the rare β-linkage based on binding studies with di- (16, 19), tri- (20, 21), and tetrasaccharides (22, 23). MAb 9D4 showed the highest affinity for tetrasaccharide **1**, while trisaccharide **2** had five times weaker affinity than the tetrasaccharide, and three times higher affinity than the disaccharide **3** (Table 1) (21, 24). This indicates the binding site of this antibody has contacts to all four sugars of the tetrasaccharide **1**, while the majority of its binding energy is focused around the dideoxyhexose containing disaccharide, an observation that has been found for other antibodies recognizing glycans containing dideoxyhexoses (25). MAb 18H1 unexpectedly recognized the trisaccharide **2** with an affinity comparable to that of the tetrasaccharide **1** and recognized the disaccharide **3** with six times higher affinity. This data is clearly inconsistent with earlier rationalization of the poor protective efficacy of MAb 18H1 that hypothesized it had been raised against a non-fucosylated linear arm of the glycan (16), and that the linear trisaccharide **2** was the preferred epitope. This would require that trisaccharide **2** be bound more tightly than the branched tetrasaccharide **1**. Consistent with the mapping data from the three different sized ligands (**1**–**3**), MAb 9D4 shows a higher affinity for its complementary ligand **1** than 18H1 shows for its smaller and complementary disaccharide epitope **3** (Table 1).

Fig. 1. The structure of the terminal tetrasaccharide **1** of the *N*-glycan expressed by the L1 stage larvae of the *T. spiralis* parasite, and its component terminal trisaccharide **2** and disaccharide **3**.



Chemical mapping by functional group replacement has proven to be a very successful tool to acquire information about carbohydrate-binding sites of antibodies (26–30), enzymes (31, 32), and lectins (26, 33). The strategy as devised by Lemieux and co-workers (34) involves the use of monodeoxy and mono-*O*-methyl congeners to identify the most important polar contacts between the carbohydrate epitope and protein. When the binding data of the modified epitopes with protein are combined with computer-assisted modeling, an overall picture of the binding site emerges, and the carbohydrate surface located in the site vs. that exposed to solvent water as well as the complementary interacting surfaces can be identified.

Results and discussion

The methyl glycoside of β-D-Tyv*p*(1→3)β-D-GalNAc*p* **3**, together with 11 other synthetic targets (Fig. 2) are used in this chemical mapping study. Disaccharides **4** and **5** are used to probe the importance of the 2-acetamido-2-deoxy group for binding, while analogs **6**–**8** contain modifications to probe the 4-position of GalNAc and disaccharides **9** and **10**,

Fig. 2. The structures of the synthetic disaccharide congeners **4**–**14** of the native disaccharide **3**.

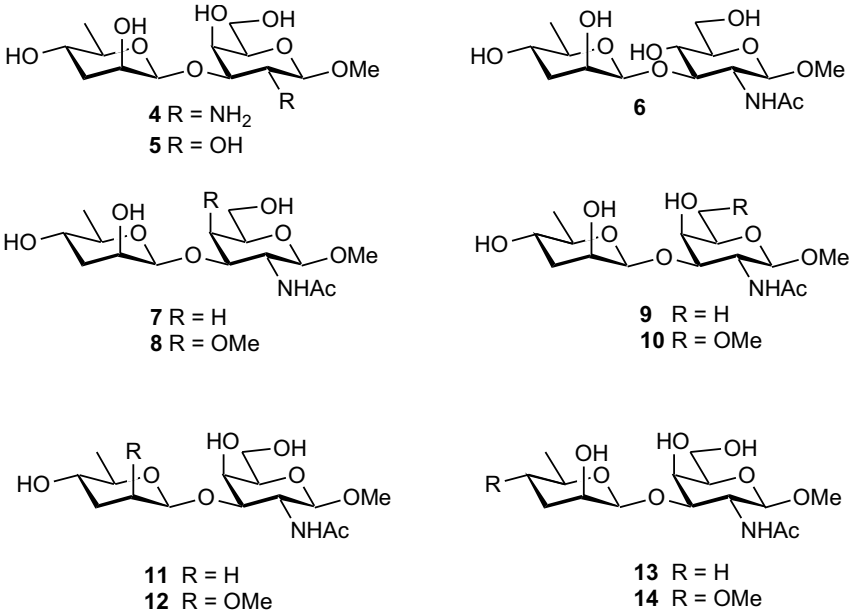


Table 1. Epitope size of the *Trichinella spiralis* glycan recognized by MAbs 9D4 and 18H1.

Oligosaccharide	IC_{50} (μM)[a]		Relative inhibitory power[b]	
inhibitor	9D4	18H1	9D4	18H1
Tetrasaccharide **1**	15	>500	100	< 9
Trisaccharide **2**	58	~480	26	~19
Disaccharide **3**	178	93	8	100

[a]Calculated from 50% inhibition data with an estimated accuracy of ~ ±5%.
[b]Inhibition potency of **1** or **3** set as 100.

the 6-position of GalNAc. Modifications were made to the tyvelose residue to investigate the contributions of the hydroxyl groups at the C-2′ and C-4′ positions. Compounds **11** and **12** are 2′-monodeoxy and 2′-*O*-monomethyl congeners while compounds **13** and **14** are 4′-monodeoxy and 4′-*O*-monomethyl analogues.

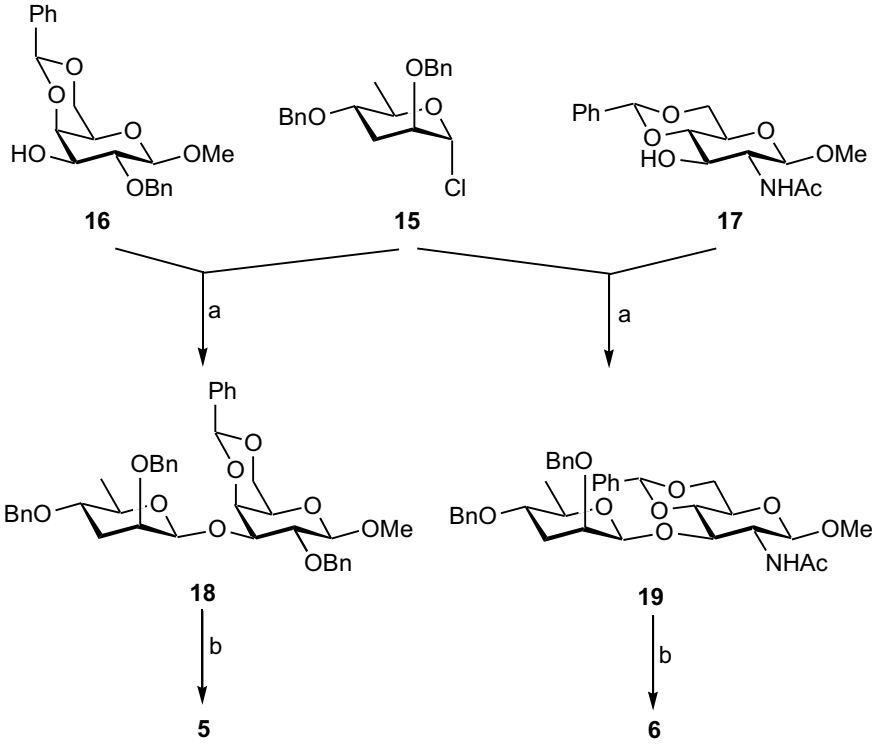
Initially, disaccharides **5** and **6** were prepared from an easily accessible tyvelose donor **15** (22) (Scheme 1), using insoluble silver zeolite as promoter. When reacting with galactosyl acceptor **16** (35) or glucosamine acceptor **17**, the disaccharides **18** and **19** were obtained in only poor yields (→**18** (15%), →**19** (26%)). These are typical and representative yields in the preparation of β-mannopyranosides using insoluble silver salt and halides as donors. Both the disaccharides **18** and **19** were deprotected by hydrogenation to give targeted compounds **5** (89%) and **6** (97%).

In our synthesis of several targets, differentiation between the hydroxyl group OH-4′ and OH-2′ of the tyvelose residue was essential. Obviously the donor **15** was not satisfactory since both positions are protected by benzyl groups. Even though the synthetic scheme seems short, the fact that the glycosylations proceeded in low yields also prevented us from obtaining final products in reasonable amounts, especially when an extended reaction sequence was involved. This necessitated an alternative route for creation of the β-tyveloside linkage.

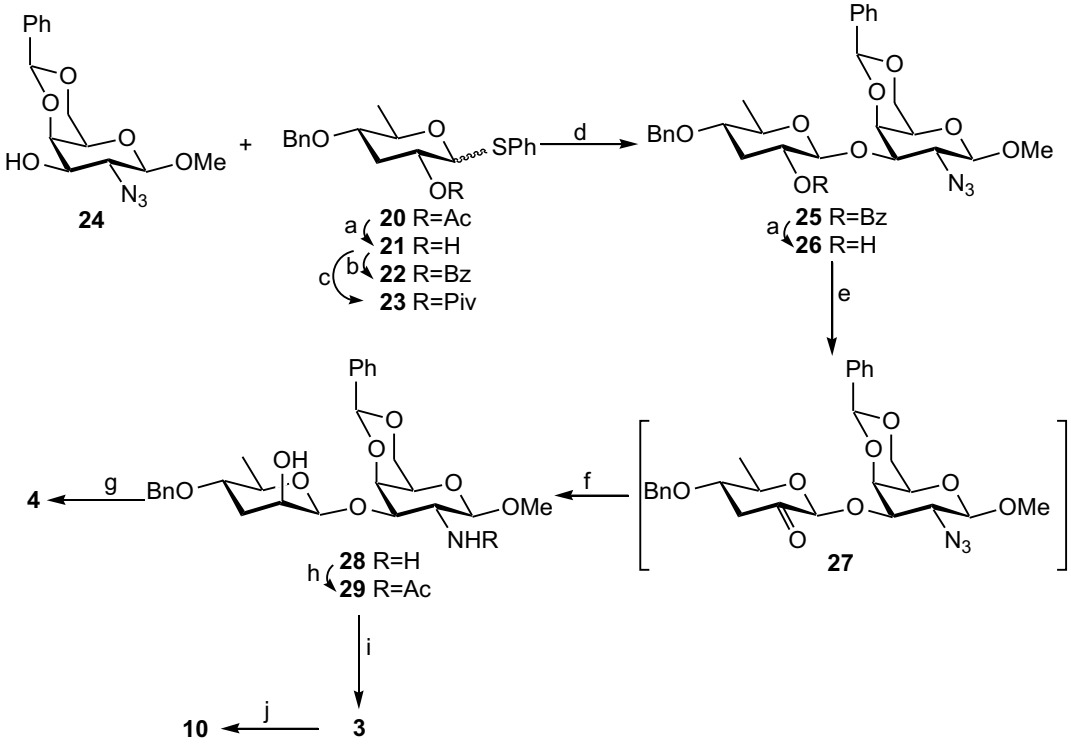
Use of a 3,6-dideoxy-D-ribohexopyranose (paratose) derivative as a precursor of the target tyvelosides became the strategy of choice, since the anomeric β-linkage is easily introduced by exploiting a C-2 participating group. The C-2 hydroxyl group can be inverted to its C-2 epimer through an oxidation–reduction sequence (36, 37). It was reported that by carefully choosing the reducing agent, the axial OH-2 epimer could be obtained in high yield. Recently, we published a new methodology to allow the preparation of 3,6-dideoxy-D-hexopyranoses (abequose and paratose) on a large scale and in high yields (38); furthermore, this methodology permits the facile introduction of different protecting groups at the C-2 and C-4 positions. For this purpose we employed a previously prepared paratose thioglycoside **20** as starting material (38).

Since benzoyl and pivaloyl protecting groups are superior participating groups when compared to the corresponding acetate ester, we converted **20** to the 2-OH derivative **21** and then prepared the corresponding benzoylated and pivaloylated donors **22** and **23** (Scheme 2). In our preliminary investigation, it was found that **22** and **23** gave similar yields when reacted with 2-azido galactosyl acceptor **24** (39). Due to the relative ease with which the benzoate group can be removed, the benzoylated donor **22** was used for all glycosylations. Using *N*-iodosuccinimide – triflic acid as the activation conditions, the disaccharide **25** was obtained in 83% yield. After removal of benzoate (→**26**, 94%), the 2′-hydroxyl group was oxidized to the corresponding ketone **27** by DMSO–Ac_2O. The intermediate ketone **27** was not isolated but immediately reduced. When excess L-selectride was used as the reducing reagent, the corresponding β-tyveloside was obtained in excellent stereoselectivity (→**28**, 92%), no paratose derivative was detected by NMR,

Scheme 1. (*a*) Silver zeolite – MS 4 Å – CH_2Cl_2; (*b*) H_2–$Pd(OH)_2$/C–MeOH.

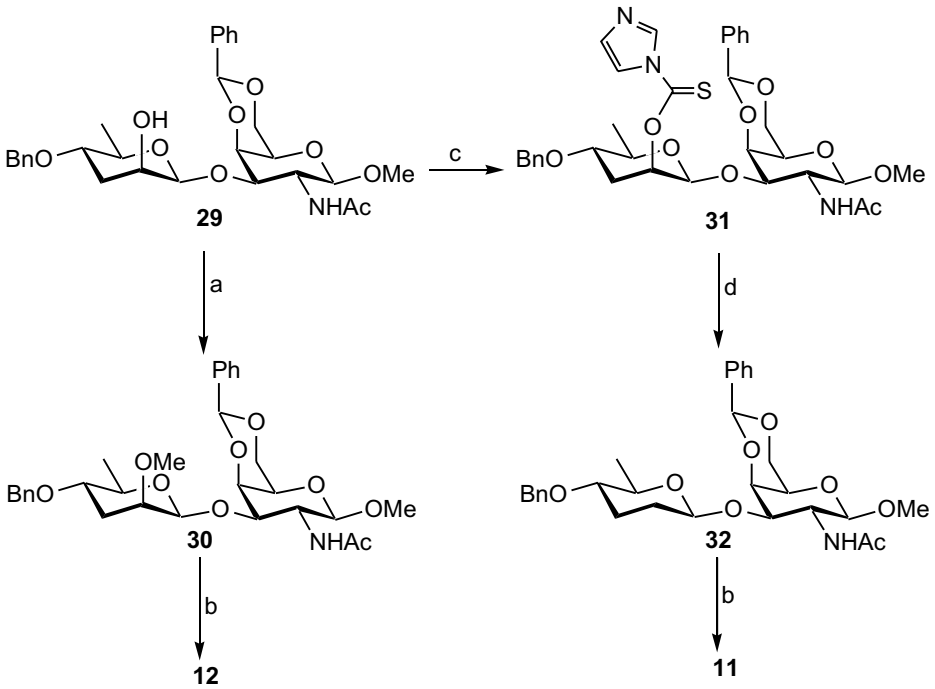


Scheme 2. (*a*) MeONa–MeOH; (*b*) BzCl–Py–CH_2Cl_2; (*c*) PivCl–Py–CH_2Cl_2; (*d*) NIS – TfOH – MS 4 Å – CH_2Cl_2; (*e*) DMSO–Ac_2O; (*f*) L-selectride–THF; (*g*) Na–NH_3 (liq.); (*h*) Ac_2O–MeOH; (*i*) H_2–$Pd(OH)_2$–MeOH; (*j*) (*i*) Bu_2SnO–MeOH, (*ii*) MeI–CsF–DMF.

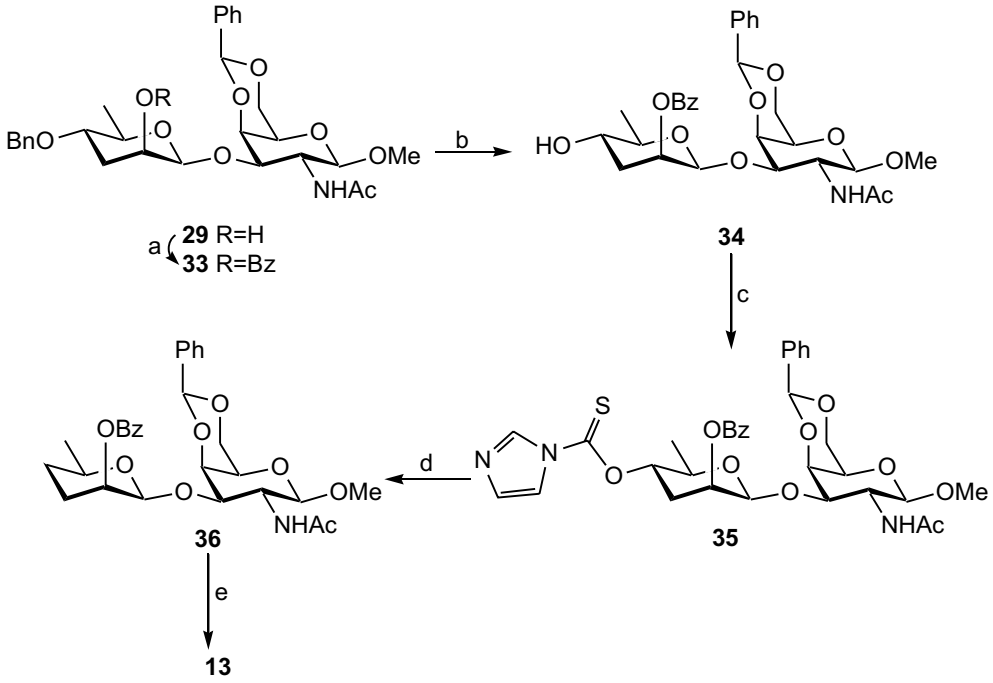


and the 2-azido group was simultaneously reduced to the amine. The amino disaccharide **4** (90%) was obtained from **28** following removal of benzyl and benzylidene groups by Birch reduction. The fully protected 2-amino disaccharide **28** was acetylated using acetic anhydride in methanol (→**29**, 93%), and the protecting groups were successfully removed by hydrogenation to afford the native disaccharide **3** (88%). When compound **3** was subjected to a dibutyltin-oxide-assisted

Scheme 3. (*a*) MeI–NaH–DMF; (*b*) H_2–$Pd(OH)_2$/C–MeOH; (*c*) Im_2CS–toluene, 90°C; (*d*) *n*-Bu_3SnH–AIBN–toluene, reflux.



Scheme 4. (*a*) BzCl–Py–CH_2Cl_2; (*b*) NH_4HCO_2–Pd/C–MeOH, reflux; (*c*) Im_2CS–toluene, 90°C; (*d*) *n*-Bu_3SnH–AIBN–toluene, reflux; (*e*) (*i*) MeONa–MeOH, (*ii*) H_2–$Pd(OH)_2$/C–MeOH.

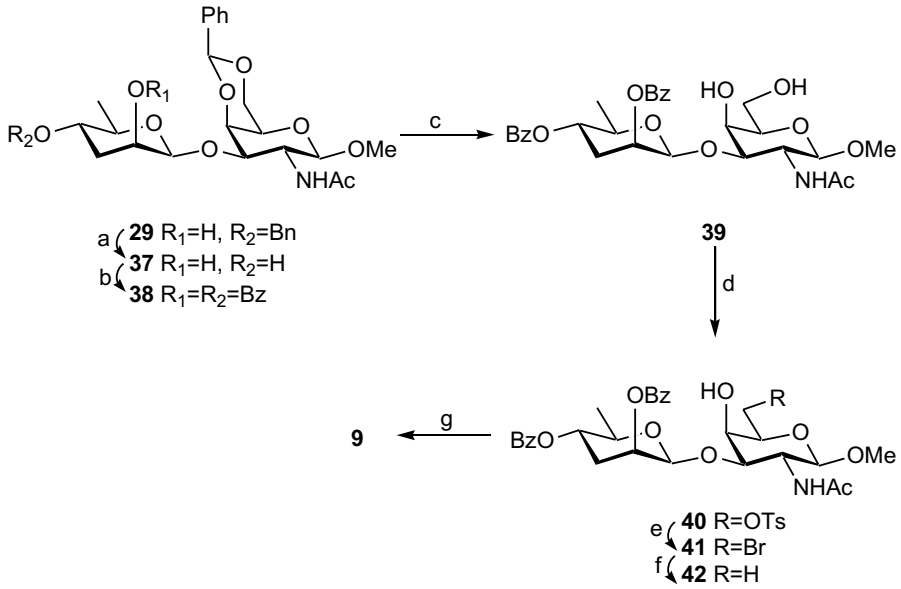


methylation, the corresponding 6-*O*-methyl ether derivative **10** was obtained in moderate yield (44%).

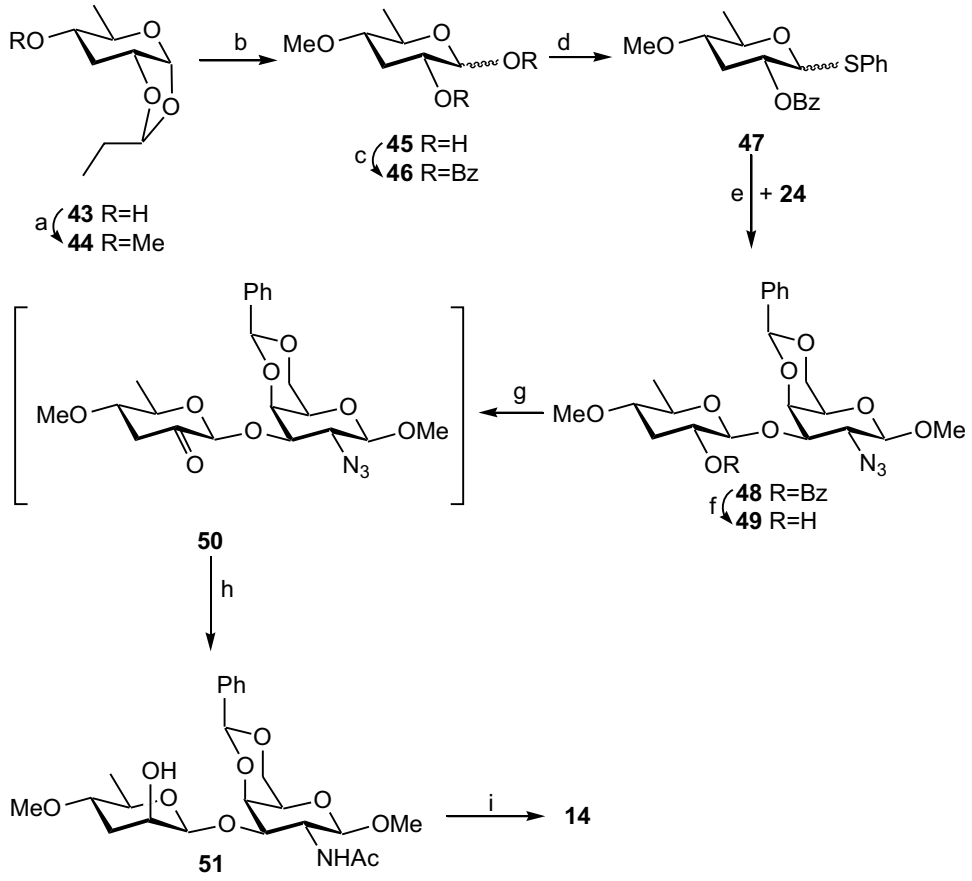
With compound **29** in hand, the 2′-hydroxyl group was methylated using sodium hydride (Scheme 3) and a controlled amount of methyl iodide as reagent, the methylated derivative **30** was obtained (37%). The poor yield reflected incomplete methylation which results from the conditions employed which are designed to avoid *N*-methylation. The benzyl and benzylidene were removed by hydrogenation to give the 2′-OMe disaccharide **12** (93% yield). To prepare the corresponding 2′-deoxgenated disaccharide **11**, **29** was transformed to **31** (98%) by reaction with 1,1′-thiocarbonyldiimidazole, and 2′-deoxygenation was realized after subjecting **31** to reaction with tributyltin hydride (→**32**, 95%) (40). After hydrogenation, the desired 2′-deoxy compound **11** was obtained in excellent yield (95%).

The 4′-deoxygenated disaccharide **13** was prepared from disaccharide **29** (Scheme 4). Benzoylation of **29** gave **33**

Scheme 5. (*a*) NH_4HCO_2–Pd/C–MeOH, reflux; (*b*) Bz_2O–Py–DMAP; (*c*) H_2–$Pd(OH)_2$/C–MeOH; (*d*) TsCl–Py; (*e*) LiBr–KI–DMF, 100°C; (*f*) *n*-Bu_3SnH–AIBN–toluene, reflux; (*g*) NaOMe–MeOH.

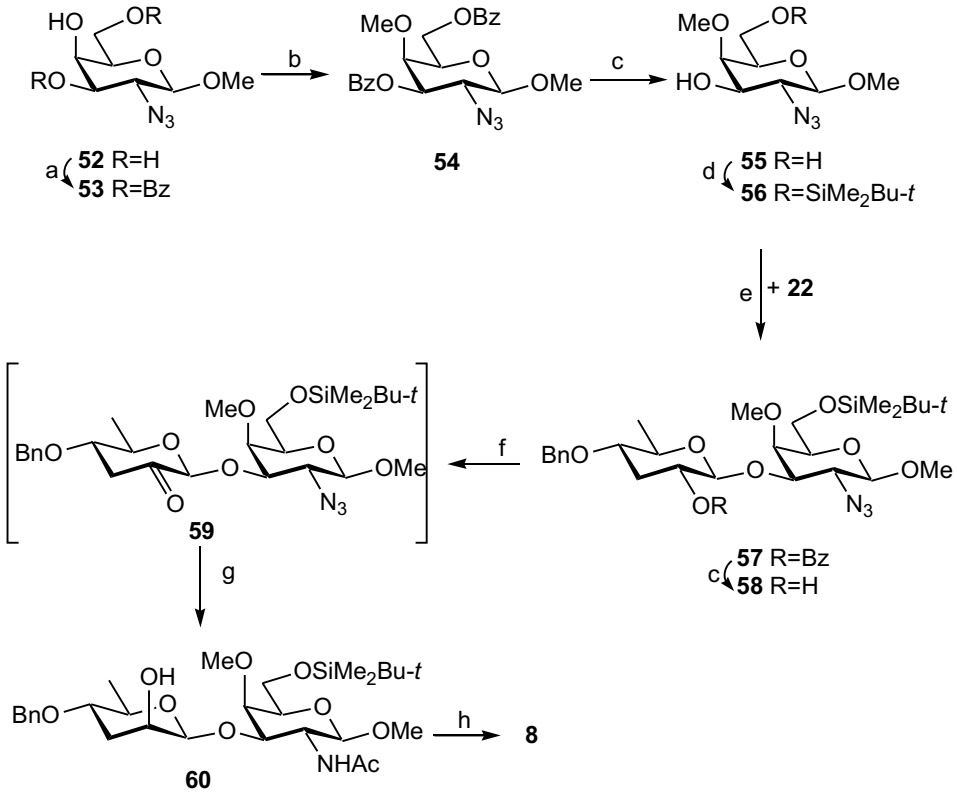


Scheme 6. (*a*) MeI–NaH–DMF; (*b*) 10% H_2SO_4 – THF, 65°C; (*c*) BzCl–Py–CH_2Cl_2; (*d*) Me_3SiSPh – TMSOTf – MS 4 Å – CH_2Cl_2; (*e*) NIS – TfOH – MS 4 Å – CH_2Cl_2; (*f*) MeONa–MeOH; (*g*) DMSO–Ac_2O; (*h*) L-selectride–THF; (*i*) H_2–$Pd(OH)_2$/C–MeOH.

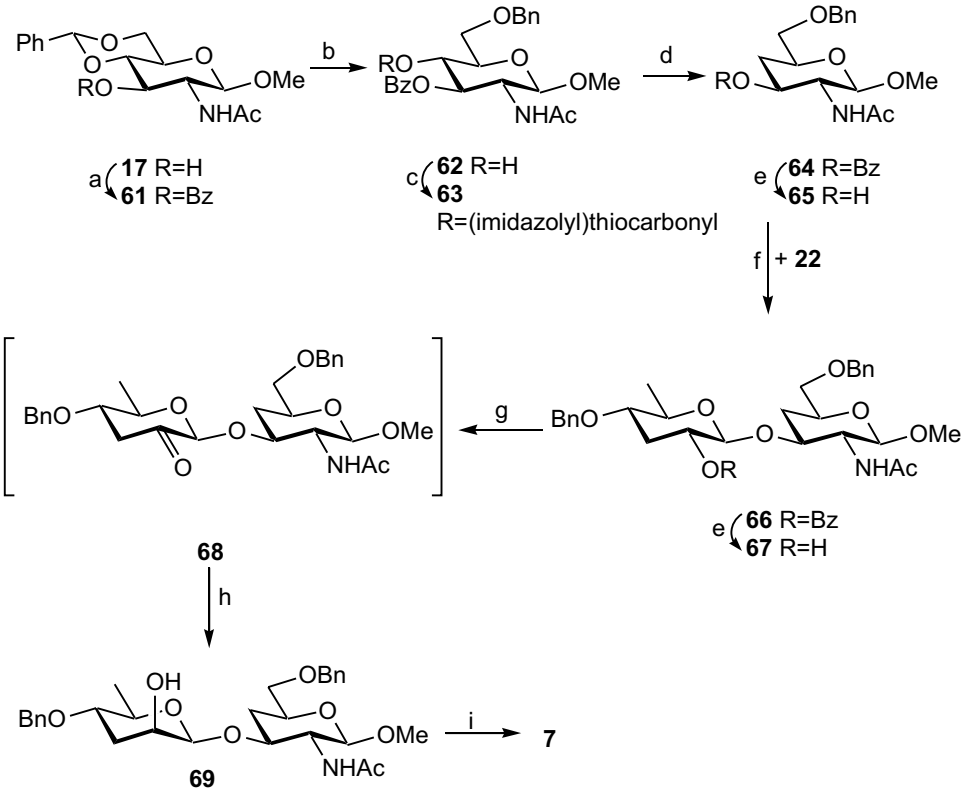


(87%), and the 4′-benzyl group was selectively removed by transfer hydrogenation in the presence of the benzylidene acetal using ammonium formate – Pd-C conditions (→**34**, 78%). The 4′-OH was deoxygenated via a similar sequence to that described for the synthesis of compound **32**, (→**35** (92%), →**36** (83%)), and the final disaccharide **13** was obtained after removing the protecting groups by Zemplen transesterification and hydrogenation (→**13**, 88%).

Scheme 7. (*a*) $(Bu_3Sn)_2O$–BzCl–toluene; (*b*) MeOTf–DTBMP–CH_2Cl_2, reflux; (*c*) NaOMe–MeOH; (*d*) *t*-$BuMe_2SiCl$–Py; (*e*) NIS – TfOH – MS 4 Å – CH_2Cl_2; (*f*) DMSO–Ac_2O; (*g*) (*i*) L-selectride–THF, (*ii*) Ac_2O–MeOH; (*h*) (*i*) H_2–$Pd(OH)_2$/C–MeOH, (*ii*) TBAF–THF.



Scheme 8. (*a*) BzCl–Py–CH_2Cl_2; (*b*) $NaBH_3CN$ – HCl – MS 4 Å –THF; (*c*) Im_2CS–toluene, 90°C; (*d*) *n*-Bu_3SnH–AIBN–toluene, reflux; (*e*) MeONa–MeOH; (*f*) NIS – TfOH – MS 4 Å – CH_2Cl_2; (*g*) DMSO–Ac_2O; (*h*) L-selectride–THF; (*i*) Na–NH_3.



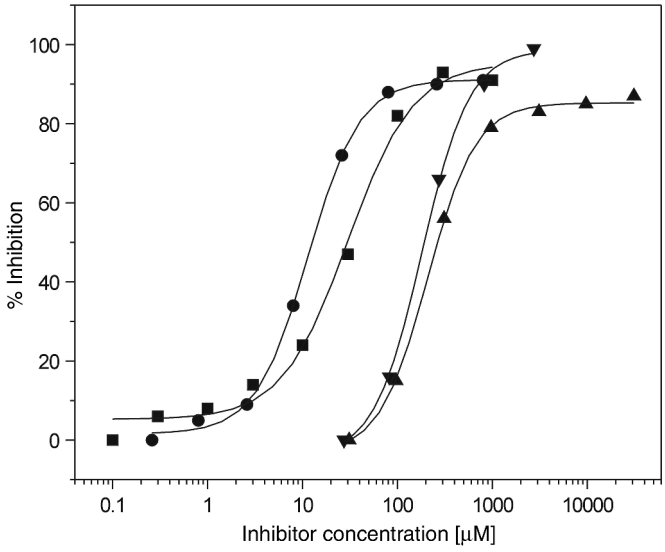
The synthesis of 6-deoxyGalNAc disaccharide **9** also started from compound **29** (Scheme 5). The benzyl ether was removed selectively in the presence of the benzylidene acetal in a manner similar to the conversion of **33** to **34** to give **37** (79%), together with **3** (11%) resulting from loss of the benzylidene acetal. The diol **37** was benzoylated (→**38**, 89%), and the acetal was removed under standard hydrogenation conditions using palladium hydroxide on charcoal as catalyst (→**39**, 89%). The 6-OH was reduced through a sequence of tosylation (→**40**), displacement by bromide to give **41**, and reduction using tributyltin hydride (→**42**). The intermediates **40**–**42** were not isolated, and after transesterification of the 2′,4′-dibenzoate **42**, the targeted compound **9** was obtained in moderate yield (49% from **39**).

Initially it was planned to obtain the 4′-OMe disaccharide **14** by methylation of intermediate **34**. However, when **34** was methylated using methyl triflate and 2,6-di-*tert*-butyl-4-methylpyridine, no methylated product was isolated. It was then decided to use a 4-*O*-methylated paratose donor **47** as starting material (Scheme 6). The paratose thioglycoside **47** was obtained in several transformation steps. Thus, starting from compound **43** (38), the 4-OH was methylated (→**44**, 92%), and the 1,2-propylidene acetal was hydrolyzed and the diol **45** benzoylated (→**46**, 96%). The anomeric benzoate was converted to the mixture of anomeric thioglycosides **47** (89%) using $PhSSiMe_3$–TMSOTf as reagents. Disaccharide **48** was obtained in good yield (78%) after coupling **47** with acceptor **24** using *N*-iodosuccinimide – triflic acid as promoter. After removing the 2′-benzoate (→**49**, 94%), the 4′-*O*-methylated disaccharide **10** was obtained by oxidation to the keto compound **50** followed by reduction and deprotection (→**51** (76%), →**14** (94%)).

To prepare the 4-*O*-monomethylated disaccharide **8**, we decided to use compound **56** as an acceptor (Scheme 7). Compound **56** has a methyl group preinstalled at the 4-position, and was prepared starting from 2-azido galactopyranosyl derivative **52** (39). Thus, by heating a solution of triol **52** with bis(tributyltin)oxide, the 3,6-positions were benzoylated to give **53** in very good yield (86%) and selectivity. The 4-OH was methylated (→**54**, 94%) using methyl triflate as reagent in the presence of the bulky base, 2,6-di-*tert*-butyl-4-methylpyridine. The benzoates were removed (→**55**, 97%) and the primary hydroxyl group was selectively silylated using *tert*-butyldimethylsily chloride as reagent to give the acceptor **56** (84%). Glycosylation of **56** with donor **22** afforded the disaccharide **57** in 71% yield. After removing the 2′-*O*-benzoate (→**58**, 98%), the 2′-equatorial hydroxyl group was inverted to the corresponding 2′-axial configuration by an oxidation–reduction sequence as describe above (→**60**, 76%), and the disaccharide **8** (88%) was obtained following deprotection.

The synthesis of 4-mono-deoxygenated disaccharide **7** (Scheme 8) started with glucosamine alcohol **17**. The 3-hydroxyl group was protected as a benzoate (→**61**, 96%), and the 4,6-*O*-benzylidene acetal was regioselectively opened to afford the alcohol **62** in excellent yield (95%). The 4-OH position was deoxygenated through a two-step Barton–McCombie deoxygenation reaction (40) as describe above (→**63** (94%), →**64** (88%)). After removing the 3-*O*-benzoate (→**65**, 94%), the acceptor **65** was glycosylated by donor **22** to produce the disaccharide **66** in moderate yield (54%).

Fig. 3. Representative inhibition curves obtained with the monoclonal antibody 9D4 and the following disaccharides: disaccharide **10** (6-*O*-methyl GalNAc congener) (●), disaccharide **4** (2-amino GalNAc congener) (■), native disaccharide **3** (▼), disaccharide **5** (2-hydroxy GalNAc congener) (▲). Microtiter plates were coated with [β-D-Tyv(1→3)β-D-GalNAc]$_8$-BSA glycoconjugate and diluted monoclonal antibody solutions containing increasing amounts of inhibitor were added to the plate. Bound antibody was detected by a species-specific antibody–horseradish peroxidase conjugate and percentage inhibitions are expressed relative to wells that contained no inhibitor. Each point is the average of three values and the standard deviation on each point was ±5% or better.



Compound **66** was debenzoylated to give the alcohol **67** (91%). Oxidation gave the keto derivative **68**, which was reduced to give **69.** The protecting groups were finally removed by Birch reduction to afford the pure 4-deoxy-disaccharide **7** (overall 23% yield from **67**).

We have described the synthesis of a comprehensive set of disaccharide congeners **4**–**14** (Fig. 2) related to the *T. spiralis* epitope β-D-Tyv*p*(1→3)β-D-GalNAc*p* (**3**). The congeners are modified at each position bearing a hydroxyl or acetamido group. The ability of these disaccharides to inhibit binding of the monoclonal antibodies 9D4 and 18H1 to a β-D-Tyv*p*(1→3)β-D-GalNAc*p* BSA glyconconjugate (16) was assayed in a solid-phase ELISA format. Briefly the disaccharide conjugate was coated on ELISA plates and incubated with antibody in the presence of increasing amounts of inhibitor. Bound antibody was detected by a goat anti-rat IgG antibody and percentage inhibition was calculated relative to wells that contained no inhibitor. The structure activity data was used to infer a topological map of the epitope surface for each antibody binding site. The quality of the inhibition data obtained in this work is illustrated in Fig. 3 and the inhibitory activities for compounds **1**–**14** with both monoclonal antibodies 9D4 and 18H1 are recorded in Tables 1–3.

In designing the set of congeners we followed the approach pioneered by Lemieux and co-workers (34) for detecting intermolecular hydrogen bonds present in a protein–oligosaccharide complex. The inhibitory power of mono-deoxy and mono-*O*-methyl congeners identifies hydroxyl

Table 2. Inhibitory activities of disaccharides **3**–**14** with the IgG MAb 9D4.

Compound	IC_{50} (μM)[a]	Relative inhibitory power[b]
3	178	100
4	30	588
5	220	90
6	–ve	0
7	199	89
8	–ve	0
9	239	75
10	10	1780
11	190	94
12	20	890
13	–ve	0
14	–ve	0

[a]Calculated from 50% inhibition data with an estimated accuracy of ~ ±5%.
[b]Inhibition potency of **3** set as 100.

Table 3. Inhibitory activities of disaccharides **3**–**14** with the IgG MAb 18H1.

Compound	IC_{50} (μM)[a]	Relative inhibitory power[b]
3	93	100
4	–ve	0
5	–ve	0
6	60	150
7	68	137
8	127	75
9	112	80
10	45	200
11	370	25
12	18	500
13	–ve	0
14	–ve	0

[a]Calculated from 50% inhibition data with an estimated accuracy of ~ ±5%.
[b]Inhibition potency of **3** set as 100.

groups that are buried and involved in key polar interactions with the protein, as well as those hydroxyl groups exposed to solvent water. Hydroxyl groups that are positioned around the periphery of the complex may also be identified from the relative inhibitory power of monodeoxy and mono-*O*-methyl congeners thus providing a detailed topology of the interacting surface. Inactive monodeoxy and mono-*O*-methyl congeners pinpoint hydroxyl groups that are buried and involved in key polar interactions. When these modifications are made to solvent exposed hydroxyl groups only minor changes are registered in their binding activity. However, when mono-*O*-methylation or monodeoxygenation replaces hydroxyl groups that lie at the periphery of the protein–oligosaccharide complex large swings in activity for either one or both of the derivatives can be observed. Frequently the changes may result in enhanced binding energy. In general, binding at the periphery is indicated when the deoxy congener is active but the mono-*O*-methyl compound is inactive (41). However, if a hydroxyl at the periphery is an acceptor of a hydrogen bond the *O*-methyl derivative may be even more active than the deoxy congener (42, 43).

At first appearance the recognition of the dideoxyhexose residue by both antibodies is similar. The C-4 hydroxyl group is buried in the binding site and makes essential polar contacts. The hydroxyl group at C-2 appears to make much weaker polar contacts perhaps via a hydrogen bond either from the protein or via a water molecule. Mono-*O*-methyl disaccharide **12** is five to eight times more active than **3** with each antibody, whereas, with the antibody 9D4, the deoxy compound is almost as active as the native disaccharide **3**, while with 18H1 the same compound is fourfold less active. In both cases this suggests that this hydroxyl group resides close to the periphery of the binding site. When interacting with 18H1 **12** may either accept a hydrogen bond or the methyl group can make favourable contacts with the protein, and (or) displace a water molecule.

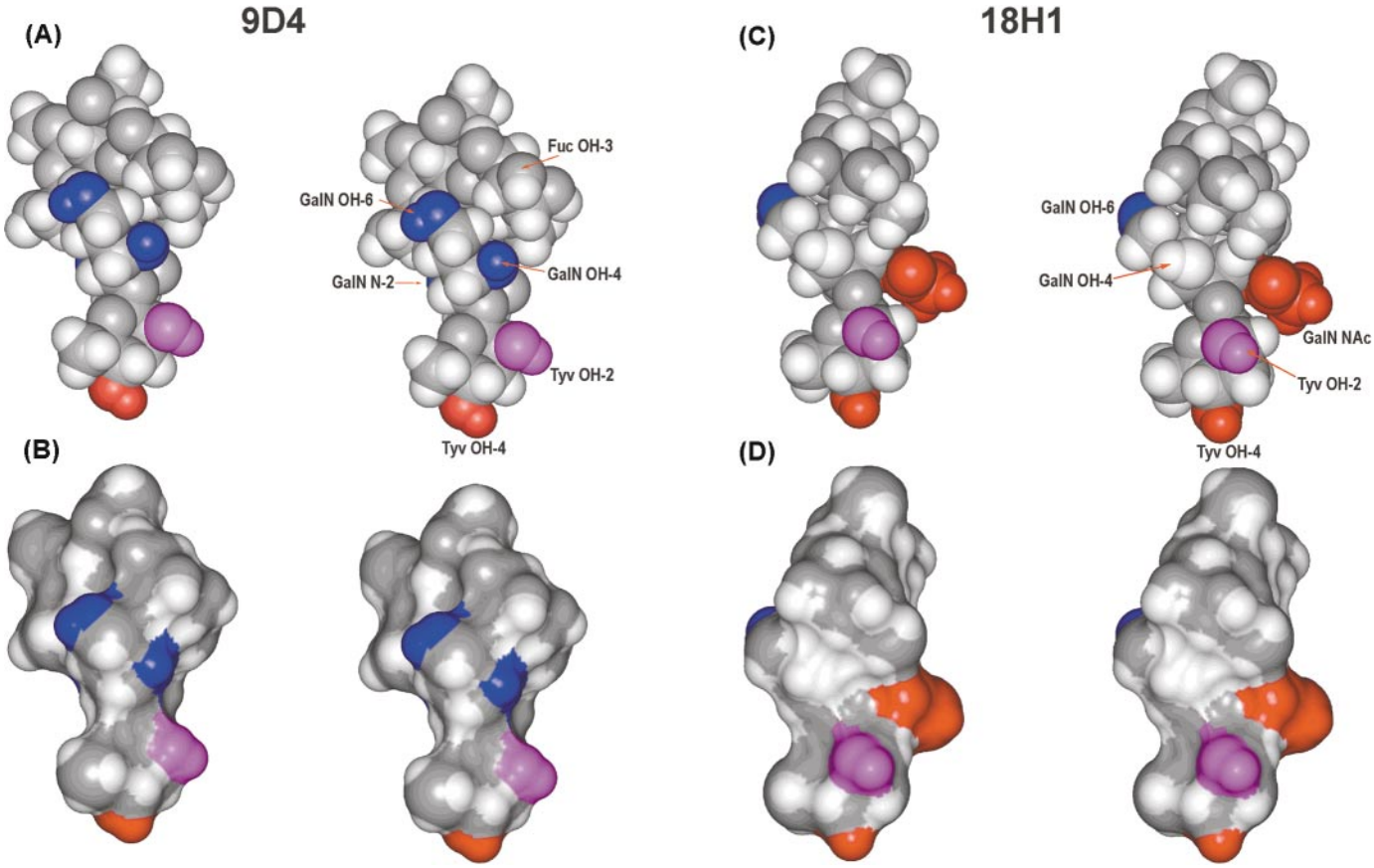
It is immediately obvious that the two monoclonal antibodies recognize the GalNAc residue via completely different surfaces. The 2-acetamido group is of major importance for MAb 18H1, since disaccharides modified at C-2 (**4** and **5**) are inactive. The C-4 hydroxyl group is clearly not involved in protein contacts since the C-4 epimer **6** is more active than **3**, and the 4-deoxy and mono-*O*-methyl disaccharides **7** and **8** have activities close to that of **3**. The C-6 hydroxyl must reside close to the periphery of the binding site since the methyl ether **10** is twice as active as **3**.

The recognition of GalNAc by MAb 9D4 is quite distinct. The acetamido group is not important to binding, since Gal may substitute for GalNAc without impact, and in fact the free amino disaccharide **5** is almost six times more active than **3**. Steric changes at C-4 are prohibitive since the C-4 epimer **6** is inactive, as is the mono-*O*-methyl disaccharide **8**. However, the 4-deoxy disaccharide **7** is almost unchanged compared to **3.** The 6-deoxy disaccharide **9** is only slightly less active than **3**, while the methyl ether **10** is 18 times more active than **3**. This suggests that both C-4 and C-6 hydroxyl group reside at the edge of the binding site and the C-6 *O*-methyl group is able to make a productive interaction at the periphery of the binding site via van der Waal contacts or by replacing a water molecule.

These data (Table 3) may be integrated with the initial inhibition data for tetra, tri, and disaccharides **1**–**3** (Table 1) and lead to the conclusion that the poorly protective MAb 18H1 only interacts with the terminal disaccharide of the *T. spiralis N*-glycan chains. There are no antibody contacts with the proximal branching fucose residue, since the underside of the Tyv and GalNAc residues contact the binding site leaving Tyv O-2 at the periphery and the major polar contacts being O-4 (Tyv) and the 2-acetamido group of GalNAc. This is consistent with the noninvolvement of GalNAc 4-OH. The key polar groups and those at the periphery of the binding site are shown in the stereo plots as CPK plots and as a Connolly surface (44) (Fig. 4).

While the recognition of Tyv by MAb 9D4 initially appeared to be similar to that by 18H1, the most deeply buried surface of the Tyv likely extends around the opposite face involving the C-6 methyl group and ring oxygen atoms. For 9D4 the involvement of its binding site with the GalNAc residue is almost the exact opposite of the situation with 18H1, and must involve the top face of the GalNAc residue. Whereas, the acetamido group can be replaced by OH without significant loss of binding, both O-4 and O-6 are close to the periphery of the site, and since the fucose residue is adjacent to these groups and protrudes from this upper face,

Fig. 4. Stereo drawings of the epitopes recognized by antibodies 9D4 and 18H1. Functional groups shown in red make crucial hydrogen bonds with the binding sites. Groups shown in pink are involved in contacts at the periphery of the binding site. Groups shaded blue are also located at the periphery. (*a*) Space-filling model showing the epitope residues Tyv O-4 and O-2 and GalNAc O-4 that create the contact surface for the 9D4 epitope. (*b*) Connolly surface of the 9D4 epitope. (*c*) Space-filling model showing the epitope residues Tyv O-4 and O-2 and GalNAc acetamido groups that create the contact surface for the 18H1 epitope. (*d*) Connolly surface of the 18H1 epitope.



its involvement in binding together with a minor contribution from the GlcNAc residue are readily appreciated (Fig. 4, Tables 1 and 2).

Investigation of a number of antibody binding sites by functional group replacement studies has shown that a small number of hydroxyl groups, and where appropriate, acetamido groups provide from two to four key polar contacts. Without these complex formation does not occur. The binding of *T. spiralis N*-glycans to antibody 18H1 conforms to this pattern, where O-4 of tyvelose and the acetamido group of GalNAc are involved in crucial polar interactions and to a lesser extent O-2 of tyvelose. Monoclonal 9D4 is quite different and its recognition of the disaccharide involves only one key polar contact, which is again O-4 of tyvelose. The tyvelose residue is recognized from the opposite face, which would require its 6-deoxy group to be buried and involved in hydrophobic contacts. Involvement of GalNAc O-4 and O-6 at the periphery is consistent with this, as is the ability to modify the acetamido function without penalty. The complete recognition surface of the carbohydrate epitope for 9D4 must extend to include the GlcNAc and Fuc residues since both trisaccharide **2** and tetrasaccharide **1** are more active inhibitors than the disaccharide **3** (Table 1). One or both of these residues may provide additional polar contacts. At this stage we would infer that this is most likely to be O-2 or O-3 of fucose.

The superior protective ability of MAb 9D4 correlates with the higher affinity for its larger tetrasaccharide target epitope, which is about 20 times the affinity of the poorly protective MAb 18H1 for its smaller disaccharide target epitope (Table 1) (21, 24). Although the key recognition element in both cases is the terminal disaccharide, the surface of the oligosaccharide epitope that is buried determines the degree to which the buried surface area can be extended. For MAb 9D4 the inclusion of the fucose residue can be contrasted with the restriction of MAb 18H1 to contacts that do not extend beyond the terminal disaccharide and involve polar contacts with O-4 and O-2 (Tyv) and NHAc (GalNAc) and possible non-polar interactions with this group. It appears that the additional specificity element and larger surface area of interaction lead to tighter binding and that this is essential for full bioactivity.

Experimental

General methods

Optical rotations were measured with a PerkinElmer 241 polarimeter for samples in a 10-cm cell at 22 ± 2°C and $[\alpha]_D$

values are given in units of 10^{-1} deg cm^2 g^{-1}. Analytical TLC was performed on plates of Silica Gel 60-F_{254} (Merck, Darmstadt) with detection by quenching of fluorescence and (or) by charring with 5% sulfuric acid in water. All commercial reagents were used as supplied and chromatography solvents were distilled prior to use. Column chromatography was performed on Silica Gel 60 (Silicycle, Quebec, Canada). ^{1}H NMR spectra were recorded on Varian Unity 300, 500, or 600 MHz. The first-order proton chemical shifts δ_H are referenced to either residual $CHCl_3$ (δ_H 7.24, $CDCl_3$) or residual CD_2HOD (δ_H 3.30, CD_3OD), or internal acetone (δ_H 2.225, D_2O). First order coupling constants are given in Hz. HMQC-NMR spectra were recorded on Varian spectrometers operating at 300, 500, or 600 MHz. The ^{13}C chemical shifts (δ_C) are referenced to internal $CDCl_3$ (δ_C 77.00, $CDCl_3$). Organic solutions were dried prior to concentration under vacuum at <40°C (bath). Final compounds were purified by reverse-phase chromatography performed on a Waters 600 HPLC system, using a Beckman semipreparative C-18 column (10 × 250 mm, 5 μ), and the products were detected with a Waters 2487 UV detector or a Waters 2410 refractive index monitor. Microanalyses and electrospray mass spectra were performed by the analytical services of this department. Reaction yields were not optimized.

Enzyme immunoassay

Inhibition assays were performed in triplicate in 96 well microtiter plates with the previously described rat monoclonal antibodies 18H1 (IgG_1) and 9D4 (IgG_{2c}) (3). Microtiter plates were incubated for 18 h at 4°C with a solution of [β-D-Tyv(1→3)β-D-GalNAc]$_8$-BSA glycoconjugate (100 μL/well) dissolved in 0.01 M phosphate-buffered saline solution at a concentration of 1 μg mL^{-1} (16). The plate was washed with PBST (5×) and blocked for 1 h at room temperature by incubation with 2% BSA in PBS (100 μL). After the plate had been washed with PBST (3×), antibody solution with or without inhibitor was added (100 μL) to the plate incubated for 18 h at room temperature. Monoclonal antibody 18H1 was used from ascites fluid at a final dilution of 5×10^{-5} and 9D4 at a dilution of 1.5×10^{-3}. The plate was washed with PBST (5×), and goat anti-rat IgG antibody conjugated to horse radish peroxidase (Kirkegaard and Perry Lab, Mandel, Guelph, ON) solution (100 μL) diluted (1:5000) in PBS was incubated for 1 h at room temperature. The plate was washed with PBST (5×), 3,3′,5,5′-tetramethylbenzidine (TMB, 100 μL) was added, and after 2 min the colour reaction was stopped by the addition of 1 M phosphoric acid (100 μL). Absorbance was read at 450 nm and percent inhibition was calculated using wells containing no inhibitor as the reference point. Triplicate samples typically exhibited OD values with a standard deviation of ±5% or better. Wells with OD values outside this error range were discarded and eliminated from the data analysis. Inhibition curves were drawn using the software package Origin 5.0 (OriginLab, Northampton, MA), and the IC_{50} value was computed by the software.

Methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2,4-di-*O*-benzyl-3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-β-D-galactopyranoside (18)

A mixture of acceptor **16** (35) (105 mg, 0.28 mmol), silver zeolite (300 mg), and 4 Å molecular sieves (300 mg) in anhyd CH_2Cl_2 (3 mL) was stirred for 30 min at room temperature, and cooled to −40°C. A solution of tyvelosyl chloride **15** (125 mg, 0.36 mmol, prepared by reacting the corresponding hemi-acetal with oxalyl chloride (22)) in anhyd CH_2Cl_2 (2.5 mL) was added dropwise and the mixture was slowly warmed to room temperature and stirred for 24 h. The mixture was filtered off, and the filtrate was concentrated. The disaccharide **18** was obtained by chromatography on silica gel using 15% EtOAc – toluene as eluent (29 mg, 15%), $[\alpha]_D$ + 27.3 (*c* 2.1, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.51–7.58 (m, 2H, Ar), 7.10–7.36 (m, 18H, Ar), 5.56 (s, 1H, PhCH), 4.83 (d, 1H, *J* = 11.2 Hz, Bn), 4.77 (d, 1H, *J* = 13.2 Hz, Bn), 4.75 ("s", 1H, H-1′), 4.68 (d, 1H, *J* = 13.2 Hz, Bn), 4.55 (d, 1H, *J* = 11.5 Hz, Bn), 4.27–4.37 (m, 4H, H-1, H-6a, H-4, Bn), 4.06 (dd, 1H, *J* = 1.6, 12.3 Hz, H-6b), 3.76–3.84 (m, 2H, H-2, H-3), 3.57 (s, 3H, OMe), 3.28–3.52 (m, 4H, H-5, H2′, H-4′, H-5′), 2.23 (d′t′, 1H, *J* = 4.0, 13.4 Hz, H-3e′), 1.31 (d, 3H, *J* = 6.1 Hz, H-6′), 1.18 (m, 1H, H-3a′). Anal. calcd. for $C_{41}H_{46}O_9$: C 72.12, H 6.79; found: C 72.19, H 6.49.

Methyl 3-*O*-(3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-β-D-galactopyranoside (5)

A mixture of disaccharide **18** (26 mg, 0.038 mmol) and 10% $Pd(OH)_2$ on charcoal (20 mg) in MeOH (10 mL) was hydrogenated overnight. The catalyst was filtered and the filtrate was concentrated, and the residue was purified by reverse phase HPLC using H_2O–MeOH (linear gradient 0 → 20%) as eluent to afford **5** (11 mg, 89%), $[\alpha]_D$ –21.2 (*c* 0.6, MeOH). ^{1}H NMR (CD_3OD) δ: 4.76 (d, 1H, *J* = 1.1 Hz, H-1′), 4.16 (m, 1H, H-1, high order), 4.03 (m, 1H, H-4), 3.96 (m, 1H, H-2′), 3.69–3.79 (m 2H, H-6a, H-6b), 3.59–3.61 (m, 2H, H-2, H-3), 3.45–3.57 (m, 6H, H-5, H-4′, OMe, H-4), 3.30 (dq, 1H, *J* = 6.1, 9.3 Hz, H-5′), 2.14 (ddd, 1H, *J* = 3.3, 4.6, 13.6 Hz, H-3e′), 1.55 (ddd, 1H, *J* = 2.9, 11.4, 13.6 Hz, H-3a′), 1.27 (d, 3H, *J* = 6.1 Hz, H-6′). HR-ES-MS *m/e* calcd. for $C_{13}H_{24}O_9$ (MNa^+): 347.13180; found: 347.131922.

Methyl 2-acetamido-3-*O*-(2,4-di-*O*-benzyl-3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (19)

A mixture of acceptor **17** (45) (88 mg, 0.27 mmol), silver zeolite (300 mg), and 4 Å molecular sieves (400 mg) in anhyd CH_2Cl_2 (3 mL) was stirred for 30 min at room temperature, and cooled to −40°C. A solution of tyvelosyl chloride **15** (173 mg, 0.50 mmol, prepared by reacting the corresponding hemi-acetal with oxalyl chloride (22)) in anhyd CH_2Cl_2 (2.5 mL) was added dropwise and the mixture was slowly warmed to room temperature and stirred for 24 h. The mixture was filtered off, and the filtrate was concentrated. The disaccharide **19** was obtained by chromatography on silica gel using 35% EtOAc – toluene as eluent (44 mg, 26%), $[\alpha]_D$ –0.8 (*c* 1.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.47–7.52 (m, 2H, Ar), 7.23–7.37 (m, 13H, Ar), 5.52 (s, 1H, PhCH), 5.45 (d, 1H, *J* = 7.1Hz, NH), 4.86 (d, 1H, *J* = 8.2 Hz, H-1), 4.70 (d, 1H, *J* = 1.2 Hz, H-1′), 4.64 (d, 1H, *J* = 12.5 Hz, Bn), 4.58 (d, 1H, *J* = 12.5 Hz, Bn), 4.38–4.50 (m, 3H, 2 × Bn, H-3), 4.32 (dd, 1H, *J* = 4.9, 10.4 Hz, H-6a), 3.76 (t, 1H, *J* = 10.2 Hz, H-4), 3.65 (t, 1H, *J* = 9.2 Hz, H-6b), 3.59 (m, 1H, H-2′), 3.47 (s, 3H, OMe), 3.36–3.53 (m, 3H, H-5, H-4′, H-5′), 3.13 (m, 1H, H-2), 2.27 (ddd, 1H,

J = 4.5, 4.5, 13.5 Hz, H-3e′), 1.74 (s, 3H, Ac), 1.43 (br m, 1H, H-3a′), 1.20 (d, 3H, *J* = 5.7 Hz, H-6′). Anal. calcd. for $C_{36}H_{43}O_9N$: C 68.23, H 6.84, N 2.21; found: C 68.01, H 6.81, N 2.19.

Methyl 2-acetamido-3-*O*-(3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-2-deoxy-β-D-glucopyranoside (6)

Disaccharide **19** (40 mg, 0.063 mmol) was deprotected in a similar fashion as compound **5** and compound **6** (22.4 mg, 97%) was purified by reverse-phase HPLC using H_2O–MeOH (linear gradient $0 \rightarrow 20\%$) as eluent. $[\alpha]_D$ –29.7 (*c* 0.7, MeOH). ^{1}H NMR (D_2O) δ: 4.63 (d, 1H, *J* = 0.9 Hz, H-1′), 4.50 (d, 1H, *J* = 8.6 Hz, H-1), 3.94 (dd, 1H, *J* = 2.3, 13.0 Hz, H-6a), 3.90 (m, 1H, H-2′), 3.80 (dd, 1H, *J* = 6.8, 10.4 Hz, H-2), 3.77 (dd, 1H, *J* = 5.5, 13.0 Hz, H-6b), 3.70 (dd, 1H, *J* = 9.0, 10.8 Hz, H-3), 3.57 (m, 1H, H-4′), 3.53 ("t", 1H, 10.8 Hz, H-4), 3.52 (s, 3H, OMe), 3.46–3.52 (m, 2H, H-5, H-5′), 2.19 (ddd, 1H, *J* = 3.7, 4.6, 13.9 Hz, H-3e′), 2.04 (s, 3H, Ac), 1.68 (ddd, 1H, *J* = 3.1, 11.5, 14.3 Hz, H-3a′), 1.29 (d, 3H, *J* = 6.2 Hz, H-6′). HR-ES-MS *m*/*e* calcd. for $C_{15}H_{27}O_9N$ (MNa^+): 388.15835; found: 388.158837.

Phenyl 4-*O*-benzyl-3,6-dideoxy-1-thio-α,β-D-ribo-hexopyranoside (21)

Thioglycoside **20** (38) (α/β: 37:73, 3.50 g, 9.40 mmol) was transesterified in anhyd MeOH with a catalytic amount of NaOMe. After 3 h, the mixture was neutralized with Amberlite IR-120 (H^+), and evaporated to dryness to give alcohol **21** (α/β: 37:73, 3.10 g, quantitative). ^{1}H NMR ($CDCl_3$) for β-anomer δ: 7.25–7.53 (m, 10H, Ar), 4.61 (d, 1H, *J* = 11.7 Hz, Bn), 4.46 (d, 1H, *J* = 11.7 Hz, Bn), 4.45 (d, 1H, *J* = 9.5 Hz, H-1), 3.40–3.49 (m, 2H, H-2, H-5), 3.15 (ddd, 1H, *J* = 4.4, 8.8,10.8 Hz, H-4), 2.63 (d′t′, 1H, *J* = 4.6, 12.3 Hz, H-3e), 1.49 (d′t′, 1H, *J* = 11.4, 11.4 Hz, H-3a), 1.34 (d, 1H, *J* = 6.3 Hz, H-6); α-anomer δ: 7.25–7.51 (m, 10H, Ar), 5.40 (d, 1H, *J* = 4.6 Hz, H-1), 4.64 (d, 1H, *J* = 11.5 Hz, Bn), 4.50 (d, 1H, *J* = 11.5 Hz, Bn), 4.12 (dq, 1H, *J* = 6.1, 9.2 Hz, H-5), 3.93 (d′t′, 1H, *J* = 4.6, 9.0, 11.5 Hz, H-2), 3.16 (m, 1H, H-4), 2.40 (dd′t′, 1H, *J* = 0.7, 4.2, 12.3 Hz, H-3e), 1.57 (d′t′, 1H, 11.7, 11.7 Hz, H-3a), 1.30 (d, 3H, *J* = 6.1 Hz, H-6). Anal. calcd. for $C_{19}H_{22}O_3S$: C 69.06, H 6.71; found: C 68.73, H 6.77.

Phenyl 2-*O*-benzoyl-4-*O*-benzyl-3,6-dideoxy-1-thio-α,β-D-ribo-hexopyranoside (22)

Benzoyl chloride (2.4 mL, 20.5 mmol) was added dropwise to an ice-cold solution of alcohol **21** (2.80 g, 8.47 mmol) in a mixture of anhyd CH_2Cl_2 (60 mL) and anhydrous pyridine (30 mL), and the mixture was stirred for 3 h. Methanol (2 mL) was added to quench the reaction, and the mixture was concentrated to dryness. The residue was dissolved in EtOAc (150 mL), and the organic solution was washed with 2 M HCl (1 × 50 mL), sat. $NaHCO_3$ (1 × 50 mL), and saturated brine (1 × 50mL), dried and evaporated. Chromatography on silica gel using 5% EtOAc – hexane as eluent gave the donor **22** as an inseparable mixture of anomers (α/β: 37:73, 3.39 g, 92% yield). ^{1}H NMR ($CDCl_3$) for β-anomer δ: 8.05 (m, 2 H, Bz), 7.57 (m, 1H, Bz), 7.42–7.49 (m, 4H, Ar), 7.20–7.37 (m, 8H, Ar), 4.91 (ddd, 1H, *J* = 4.9, 9.9, 10.9 Hz, H-2), 4.82 (d, 1H, *J* = 9.9 Hz, H-1), 4.62 (d, 1H, *J* = 11.5 Hz, Bn), 4.46 (d, 1H, *J* = 11.5 Hz, Bn), 3.52 (dq, 1H, *J* = 6.1, 9.0 Hz, H-5), 3.27 (ddd, 1H, *J* = 4.5, 9.0, 10.9 Hz, H-4), 2.84 (d′t′, 1H, *J* = 4.6, 11.9 Hz, H-3e), 1.66 (d′t′, 1H, *J* = 11.1, 11.1 Hz, H-3a), 1.39 (d, 1H, *J* = 6.1 Hz, H-6); α-anomer δ: 8.08 (m, 1H, Bz), 7.57 (m, 1H, Bz), 7.42–7.49 (m, 4H, Ar), 7.20–7.37 (m, 8H, Ar), 5.76 (d, 1H, *J* = 5.0 Hz, H-1), 5.29 (d′t′, 1H, *J* = 5.1, 10.1 Hz, H-2), 4.68 (d, 1H, *J* = 11.6 Hz, Bn), 4.51 (d, 1H, *J* = 11.6 Hz, Bn), 4.28 (dq, 1H, *J* = 6.1, 9.2 Hz, H-5), 3.30 (ddd, 1H, *J* = 4.6, 7.1, 11.0 Hz, H-4), 2.54 (dd′t′, 1H, *J* = 1.2, 4.6, 11.8 Hz, H-3e), 2.00 (d′t′, 1H, 12.0, 12.0 Hz, H-3a), 1.29 (d, 3H, *J* = 6.2 Hz, H-6). Anal. calcd. for $C_{26}H_{26}O_4S$: C 71.86, H 6.03; found: C 71.95, H 6.16.

Phenyl 4-*O*-benzyl-3,6-dideoxy-2-*O*-pivaloyl-1-thio-α,β-D-ribo-hexopyranoside (23)

The alcohol **21** (80 mg, 0.24 mmol) was reacted with pivaloyl chloride (89.4 μL, 0.73 mmol) in a similar fashion to afford the pivaloyl donor **23** (α/β: 37:73, 92 mg, 92%). ^{1}H NMR ($CDCl_3$) for β-anomer δ: 7.21–7.48 (m, 10H, Ar), 4.64–4.73 (m, 2H, H-1, H-2), 4.61 (d, 1H, *J* = 11.4 Hz, Bn), 4.43 (d, 1H, *J* = 11.4 Hz, Bn), 3.44 (dq, 1H, *J* = 6.1, 9.0 Hz, H-5), 3.20 (ddd, 1H, *J* = 4.5, 9.1, 10.9 Hz, H-4), 2.65 (d′t′, 1H, *J* = 4.5, 12.1 Hz, H-3e), 1.50 (m, 1H, H-3a), 1.34 (d, 1H, *J* = 6.1 Hz, H-6), 1.23 (s, 9H, Piv); α-anomer δ: 7.21–7.48 (m, 10H, Ar), 5.65 (d, 1H, *J* = 5.2 Hz, H-1), 4.96 (d′t′, 1H, *J* = 5.1, 10.1 Hz, H-2), 4.67 (d, 1H, *J* = 11.5 Hz, Bn), 4.48 (d, 1H, *J* = 11.5 Hz, Bn), 4.22 (dq, 1H, *J* = 6.1, 9.2 Hz, H-5), 3.23 (m, 1H, m, H-4), 2.36 (dd′t′, 1H, *J* = 1.2, 4.6, 12.0 Hz, H-3e), 1.84 (d′t′, 1H, *J* = 12.1, 12.1 Hz, H-3a), 1.26 (d, 3H, *J* = 6.2 Hz, H-6), 1.25 (s, 9H, Piv). Anal. calcd. for $C_{24}H_{30}O_4S$: C 69.53, H 7.29; found: C 69.33, H 7.52.

Methyl 2-azido-3-*O*-(2-*O*-benzoyl-4-*O*-benzyl-3,6-dideoxy-β-D-ribo-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (25)

A mixture of donor **22** (α/β: 37:73, 807 mg, 1.86 mmol), acceptor **24** (39) (519 mg, 1.70 mmol), and 4 Å molecular sieves (1.5 g) in anhyd CH_2Cl_2 (16 mL) was cooled to –60°C, and NIS (796 mg, 3.70 mmol) was added. After stirring for 1 h, TfOH (31 μL) was added dropwise, the reaction was continued for 3 h and warmed to –30°C. Et_3N (1.0 mL) was added to quench the reaction. The insoluble material was filtered off and washed with CH_2Cl_2. The organic solution was washed with a 1:1 mixture of aq $Na_2S_2O_3$ (10%) and $NaHCO_3$ (sat.) (1 × 50 mL), dried and concentrated. Disaccharide **25** was obtained by chromatography on silica gel using 5% EtOAc – toluene as eluent (891 mg, 83% yield), $[\alpha]_D$ –14.9 (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 8.08 (m, 2H, Bz), 7.56 (m, 1H, Bz), 7.20–7.51 (m, 12H, Ar), 5.52 (s, 1H, PhCH), 4.87–4.95 (m, 2H, H-1′, H-2′), 4.63 (d, 1H, *J* = 11.4 Hz, Bn), 4.45 (d, 1H, *J* = 11.4 Hz, Bn), 4.31 (dd, 1H, *J* = 1.5, 12.4 Hz, H-6a), 4.21–4.25 (m, 2H, H-1, H-4), 3.78 (dd, 1H, *J* = 8.0, 10.6 Hz, H-2), 3.50–3.59 (m, 5H, OMe, H-3, H-5′), 3.40 (m, 1H, H-5), 3.30 (ddd, 1H, *J* = 4.5, 9.1, 10.9 Hz, H-4′), 2.85 (d′t′, 1H, *J* = 4.5, 12.1 Hz, H-3e′), 1.59 (d′t′, 1H, *J* = 11.1, 11.1 Hz, H-3a′), 1.34 (d, 3H, *J* = 6.1 Hz, H-6′). Anal. calcd. for $C_{34}H_{37}O_9N_3$: C 64.65, H 5.90, N 6.65; found: C 64.47, H 5.61, N 6.29.

Methyl 2-azido-3-*O*-(4-*O*-benzyl-3,6-dideoxy-β-D-ribo-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (26)

Disaccharide **25** (550 mg, 0.811 mmol) was dissolved in anhyd MeOH (5 mL), and a solution of NaOMe in MeOH

(100 μL) was added. After 2 h, the solution was neutralized with Amberlite IR-120 (H^+) resin and filtered. The filtrate was concentrated and the disaccharide **26** was obtained by chromatography on silica gel using 20% EtOAc–toluene as eluent (432 mg, 94% yield), $[\alpha]_D$ +39.0 (*c* 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.53 (m, 2H, Ph), 7.26–7.38 (m, 8H, Ar), 5.53 (s, 1H, PhCH), 4.62 (d, 1H, *J* = 11.6 Hz, Bn), 4.45 (d, 1H, *J* = 11.6 Hz, Bn), 4.44 (d, 1H, *J* = 7.3 Hz, H-1′), 4.32 (dd, 1H, *J* = 1.6, 12.4 Hz, H-6a), 4.25 (dd, 1H, *J* = 0.5, 3.6 Hz, H-4), 4.22 (d, 1H, *J* = 8.0 Hz, H-1), 4.05 (dd, 1H, *J* = 1.7, 12.4 Hz, H-6b), 3.86 (dd, 1H, *J* = 8.0, 10.6 Hz, H-2), 3.58 (s, 2H, OMe), 3.42–2.56 (m, 3H, H-3, H-2′, H-5′), 3.40 (m, 1H, H-5), 3.30 (ddd, 1H, *J* = 4.4, 8.8, 10.9 Hz, H-4′), 2.50 (d′t′, 1H, *J* = 4.7, 12.3 Hz, H-3e′), 1.44 (d′t′, 1H, *J* = 11.7, 11.7 Hz, H-3a′), 1.29 (d, 3H, *J* = 6.2 Hz, H-6′). Anal. calcd. for $C_{27}H_{33}O_8N_3$: C 61.64, H 6.30, N 7.96; found: C 61.88, H 6.34, N 7.88.

Methyl 2-amino-3-*O*-(4-*O*-benzyl-3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (28)

The alcohol **26** (289 mg, 0.548 mmol) was dissolved in a mixture of anhyd DMSO (4 mL) and Ac_2O (2 mL) at 0°C. The reaction was left at ambient temperature overnight. After removing the solvents under reduced pressure, the mixture was filtered through a thin bed of silica gel using 40% EtOAc–hexane as eluent to give the intermediate ketone **27** (235 mg, 82% yield) which was divided in two parts for the preparation of both compound **28** and **29**.

A portion of **27** (40 mg, 0.076 mmol) was dissolved in anhyd THF (3 mL) at 0°C, a solution of L-selectride in THF (1.0 M, 300 μL, 0.3 mmol) was added dropwise and the reaction was continued for 1 h. Water (1 mL) was added and the reaction mixture was concentrated. The syrupy mixture was dissolved in CH_2Cl_2 (50 mL), washed with a 10% aqueous solution of $Na_2S_2O_3$ (20 mL) and saturated brine (20 mL), dried and concentrated. The mixture was purified by chromatography on silica gel using 5% MeOH–CH_2Cl_2 as eluent to afford the amine **28** (35 mg, 92% yield), $[\alpha]_D$ +40.2 (*c* 1.5, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.49 (m, 2H, Ar), 7.22–7.36 (m, 8H, Ar), 5.53 (s, 1H, PhCH), 4.76 (d, 1H, *J* = 1.0 Hz, H-1′), 4.59 (d, 1H, *J* = 11.5 Hz, Bn), 4.44 (d, 1H, *J* = 11.5 Hz, Bn), 4.31 (dd, 1H, *J* = 1.4, 12.4 Hz, H-6a), 4.25 ("d", 1H, *J* = 3.1 Hz, H-4), 4.12 (d, 1H, *J* = 7.8 Hz, H-1), 4.05 (dd, 1H, *J* = 1.7, 12.4 Hz, H-6b), 3.94 (m, 1H, H-2′), 3.65 (dd, 1H, *J* = 3.9, 10.5 Hz, H-3), 3.53 (s, 3H, OMe), 3.48 (m, 2H, H-4′ +H-5′), 3.41 (m, 1H, H-5), 3.26 (dd, 1H, *J* = 7.9, 10.4 Hz, H-2), 2.43 (d′t′, 1H, *J* = 3.9, 13.7 Hz, H-3e′), 1.52 (m, 1H, H-3a′), 1.32 (d, 3H, *J* = 5.7 Hz, H-6′). HR-ES-MS *m*/*e* calcd. for $C_{27}H_{35}O_8N$ (MH^+): 502.24409; found: 502.244418.

Methyl 2-amino-3-*O*-(3,6-dideoxy-β-D-arabino-hexopyranosyl)-2-deoxy-β-D-galactopyranoside (4)

With the help of a dry ice – acetone condenser, liquid ammonia (25 mL) was collected in a flask containing the amine **28** (35 mg, 0.07 mmol). A small piece of sodium (100 mg) was added, and reaction was continued for 4 h. During this time, the reaction mixture remained dark blue. The condenser was removed and MeOH (0.5 mL) was added. The colour of the reaction mixture turned white and the ammonia was allowed to evaporate. After the evaporation of NH_3, the residue was purified by reverse-phase chromatography using a gradient of H_2O–MeOH (0 → 30%) as eluent to yield compound **4** (20.5 mg, 90% yield), $[\alpha]_D$ –7.1 (*c* 0.2, MeOH). ^1H NMR (D_2O) δ: 4.84 (d, 1H, *J* = 0.7 Hz, H-1′), 4.60 (d, 1H, *J* = 8.6 Hz, H-1), 4.28 ("d", 1H, *J* = 2.9 Hz, H-4), 4.10 (m, 1H, H-2′), 4.03 (dd, 1H, *J* = 2.9, 10.8 Hz, H-3), 3.84 (dd, 1H, *J* = 7.9, 11.7 Hz, H-6a), 3.78 (dd, 1H, *J* = 4.2, 11.7 Hz, H-6b), 3.76 (m, 1H, H-5), 3.61 (s, 3H, OMe), 3.58 (ddd, 1H, *J* = 4.8, 9.34, 11.5 Hz, H-4′), 3.48 (dq, 1H, *J* = 6.0, 9.3 Hz, H-5′), 3.32 (dd, 1H, *J* = 8.6, 10.6 Hz, H-2), 2.20 (ddd, 1H, *J* = 3.5, 4.6, 13.9 Hz, H-3e′), 1.69 (ddd, 1H, *J* = 2.9, 11.5, 14.1 Hz, H-3e′), 1.28 (d, 3H, *J* = 6.1 Hz, H-6′). HR-ES-MS *m*/*e* calcd. for $C_{13}H_{25}O_8N$ (MNa^+): 346.14779; found: 346.148284.

Methyl 2-acetamido-3-*O*-(4-*O*-benzyl-3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (29)

The intermediate ketone **27** (106 mg, 0.2 mmol) was dissolved in anhyd THF (3 mL) at 0°C, a solution of L-selectride in THF (1.0 M, 1.5 mL, 1.5 mmol) was added dropwise, and the reaction was continued at 0°C for 1 h. Water (0.3 mL) was added, after 10 min, Ac_2O (2 mL) was added dropwise, the reaction was continued for 30 min. After concentration, the mixture was dissolved in CH_2Cl_2 (100 mL), the organic solution was washed with a 10% aqueous solution of $Na_2S_2O_3$ (1 × 50 mL) and saturated brine (50 mL), dried, and evaporated. The mixture was purified by chromatography on silica gel using 2.5% MeOH–CH_2Cl_2 as eluent to afford compound **29** (102 mg, 93% yield), $[\alpha]_D$ +56.5 (*c* 0.6, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.46–7.52 (m, 2H, Ar), 7.22–7.36 (m, 8H, Ar), 5.95 (d, 1H, *J* = 7.0 Hz, NH), 5.52 (s, 1H, PhCH), 5.02 (d, 1H, *J* = 8.2 Hz, H-1), 4.73 (dd, 1H, *J* = 3.4, 11.1 Hz, H-3), 4.57 (d, 1H, *J* ~ 1 Hz, H-1′), 4.56 (d, 1H, *J* = 11.5 Hz, Bn), 4.42 (d, 1H, *J* = 11.5 Hz, Bn), 4.26–4.36 (m, 2H, H-6a, H-4), 4.04 (dd, 1H, *J* = 1.6, 12.4 Hz, H-6b), 3.83 (br m, 1H, H-2′), 3.51 (br, 4H, OMe, H-5), 3.38–3.50 (m, 3H, H-2, H-4′, H-5′), 2.33–2.39 (m, 2H, H-3e′, OH-2′), 1.85 (s, 3H, Ac), 1.38 (m, 1H, H-3a′), 1.30 (d, 3H, *J* = 5.6 Hz, H-6′). HR-ES-MS *m*/*e* calcd. for $C_{29}H_{37}O_9N$ (MNa^+): 566.23660; found: 566.236763.

Methyl 2-acetamido-3-*O*-(3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-2-deoxy-β-D-galactopyranoside (3)

Compound **29** (25 mg, 0.046 mmol) was deprotected in a similar fashion as compound **5** and disaccharide **3** (14.7 mg, 88% yield) was purified by reverse-phase HPLC using a gradient of H_2O–MeOH (0 → 30%) as eluent, $[\alpha]_D$ –32.4 (*c* 0.5, MeOH). ^1H NMR (D_2O) δ: 4.68 (d, 1H, *J* = 0.7 Hz, H-1′), 4.44 (d, 1H, *J* = 8.6 Hz, H-1), 4.12 ("d", 1H, *J* = 3.1 Hz, H-4), 4.01 (dd, 1H, *J* = 8.6, 10.8 Hz, H-2), 3.89 (m, 1H, H-2′), 3.89 (dd, 1H, *J* = 3.1, 10.8 Hz, H-3), 3.82 (dd, 1H, *J* = 7.9, 11.7 Hz, H-6a), 3.77 (dd, 1H, *J* = 4.2, 11.7 Hz, H-6b), 3.70 (m, 1H, H-5), 3.55 (ddd, 1H, *J* = 4.8, 9.3, 11.4 Hz, H-4′), 3.52 (s, 3H, OMe), 3.43 (dq, 1H, *J* = 6.2, 9.5 Hz, H-5′), 2.17 (ddd, 1H, *J* = 3.5, 4.8, 14.1 Hz, H-3e′), 2.02 (s, 3H, Ac), 1.65 (ddd, 1H, *J* = 3.1, 11.5, 14.3 Hz, H-3a′), 1.27 (d, 3H, *J* = 6.2 Hz, H-6′). HR-ES-MS *m*/*e* calcd. for $C_{15}H_{27}O_9N$ (MNa^+): 388.15835; found: 388.158854.

Methyl 2-acetamido-3-*O*-(3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-2-deoxy-6-*O*-methyl-β-D-galactopyranoside (10)

A mixture of tetraol **3** (31.6 mg, 0.0865 mmol) and dibutyltin oxide (26 mg, 0.104 mmol) in anhydrous methanol (4 mL) was refluxed for 2 h. The mixture was concentrated and coevaporated with toluene (2 × 15 mL), and dried under high vacuum. The mixture was dissolved in anhyd DMF (2 mL). CsF (40 mg, 0.259 mmol) and MeI (16 μL, 0.259 mmol) were added, and the reaction was heated to 50°C overnight. The solution was concentrated under reduced pressure and the desired 6-*O*-methyl disaccharide **10** was obtained by reverse-phase HPLC using a gradient of H_2O–MeOH (0 → 20%) as eluent (14.5 mg, 44% yield), $[\alpha]_D$ –34.8 (*c* 0.3, MeOH). Starting material **3** (18 mg) was also recovered. ^{1}H NMR (D_2O) δ: 4.68 ("s", 1H, H-1′), 4.45 (d, 1H, *J* = 8.6 Hz, H-1), 4.10 ("d", 1H, *J* = 3.2 Hz, H-4), 4.01 (dd, 1H, *J* = 8.6, 10.8 Hz, H-2), 3.89 (m, 1H, H-2′), 3.87 (dd, 1H, *J* = 3.1, 10.8 Hz, H-3), 3.83 (m, 1H, H-5), 3.72 (dd, 1H, *J* = 7.7, 10.8 Hz, H-6a), 3.68 (dd, 1H, *J* = 4.0, 11.0 Hz, H-6b), 3.55 (ddd, 1H, *J* = 4.6, 9.2, 11.4 Hz, H-4′), 3.52 (s, 3H, OMe), 3.44 (dq, 1H, *J* = 6.0, 9.3 Hz, H-5′), 3.42 (s, 3H, OMe), 2.17 (ddd, 1H, *J* = 3.7, 4.6, 13.9 Hz, H-3e′), 2.03 (s, 3H, Ac), 1.65 (ddd, 1H, *J* = 3.1, 11.5, 14.3 Hz, H-3a′), 1.28 (d, 3H, *J* = 6.2 Hz, H-6′). HR-ES-MS *m/e* calcd. for $C_{16}H_{29}O_9N$ (MNa^+): 402.17400; found: 402.174490.

Methyl 2-acetamido-3-*O*-(4-*O*-benzyl-3,6-dideoxy-2-*O*-methyl-β-D-*arabino*-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (30)

Alcohol **29** (42 mg, 0.077 mmol) was dissolved in anhyd DMF (1 mL) at 0°C, NaH (9 mg, 0.38 mmol) was added, and the mixture was stirred for 15 min. MeI (5.2 μL, 0.085 mmol) was added, and the reaction was continued for another 1 h. Methanol (100 μL) was added, and the mixture was concentrated. Chromatography on silica gel using 5% MeOH–CH_2Cl_2, gave the desired disaccharide **30** (16 mg, 37% yield), $[\alpha]_D$ +42.6 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.44–7.53 (m, 2H, Ar), 7.21–7.33 (m, 8H, Ar), 5.78 (d, 1H, *J* = 6.4 Hz, NH), 5.55 (s, 1H, PhCH), 5.02 (d, 1H, *J* = 8.2 Hz, H-1), 4.67 (br d, 1H, *J* ~ 1 Hz, H-1′), 4.59 (dd, 1H, *J* = 3.3, 11.1 Hz, H-3), 4.55 (d, 1H, *J* = 11.6 Hz, Bn), 4.44 (d, 1H, *J* = 11.6 Hz, Bn), 4.34 (d, 1H, *J* = 3.1 Hz, H-4), 4.30 (dd, 1H, *J* ~ 1, 12.4 Hz, H-6a), 4.06 (dd, 1H, *J* = 1.4, 12.3 Hz, H-6b), 3.35–3.58 (m, 5H, H-5′, H-2′, H-4′ H-2, H-5), 3.50 (s, 3H, OMe), 3.40 (s, 3H, OMe), 2.35 (ddd, 1H, *J* = 4.4, 4.4, 13.5 Hz, H-3e′), 1.93 (s, 3H, Ac), 1.43 (dd, 1H, *J* = 3.0, 10.3, 13.3 Hz, H-3a′), 1.31 (d, 3H, H-6′). Anal. calcd. for $C_{30}H_{39}O_9N$: C 64.62, H 7.05, N 2.51; found: C 64.32, H 7.15, N 2.59.

Methyl 2-acetamido-3-*O*-(3,6-dideoxy-2-*O*-methyl-β-D-*arabino*-hexopyranosyl)-2-deoxy-β-D-galactopyranoside (12)

Disaccharide **30** (16 mg) was deprotected in a similar fashion as compound **5** and disaccharide **12** (10.1 mg, 93% yield) was obtained by flash chromatography on reverse-phase silica gel followed by reverse-phase HPLC using a gradient of H_2O–MeOH (0 → 30%) as eluent. $[\alpha]_D$ –36.5 (*c* 0.2, MeOH). ^{1}H NMR (D_2O) δ: 4.73 ("s", 1H, H-1′), 4.44 (d, 1H, *J* = 8.6 Hz, H-1), 4.13 ("d", 1H, *J* = 3.1 Hz, H-4), 4.01 (dd, 1H, *J* = 8.6, 10.8 Hz, H-2), 3.83 (dd, 1H, *J* = 7.9, 12.1 Hz, H-6a), 3.81 (dd, 1H, *J* = 3.1, 10.8 Hz, H-3), 3.77 (dd, 1H, *J* = 4.2, 11.7 Hz, H-6b), 3.70 (m, 1H, H-5), 3.56 (m, 1H, H-2′), 3.52 (s, 3H, OMe), 3.42–3.48 (m, 2H, H-4′, H-5′), 3.41 (s, 3H, OMe), 2.37 (d′t′, 1H, *J* = 3.7, 14.1 Hz, H-3e′), 2.03 (s, 3H, Ac), 1.54 (ddd, 1H, *J* = 2.8, 11.2, 14.1 Hz, H-3a′), 1.26 (d, 3H, *J* = 5.7 Hz, H-6′). HR-ES-MS *m/e* calcd. for $C_{16}H_{29}O_9N$ (MNa^+): 402.17400; found: 402.174693.

Methyl 2-acetamido-3-*O*-(4-*O*-benzyl-3,6-dideoxy-2-*O*-imidazoylthiocarbonyl-β-D-*arabino*-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (31)

To a solution of disaccharide **29** (30 mg, 0.055 mmol) in anhydrous toluene (2.5 mL), was added 1,1′-thiocarbonyldiimidazole (27 mg, 0.15 mmol), and the mixture was heated to 90°C for 17 h. After cooling, the mixture was concentrated, and disaccharide **31** was obtained following chromatography on silica gel using 2% MeOH–CH_2Cl_2 as eluent (35.3 mg, 98% yield), $[\alpha]_D$ +21.5 (*c* 0.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 8.31 (s, 1H, imidazole), 7.20–7.44 (m, 11H, Ar), 6.71 (s, 1H, imidazole), 5.88 (d, 1H, *J* = 6.5 Hz, NH), 5.66 (m, 1H, H-2′), 5.51 (s, 1H, PhCH), 4.95 (d, 1H, *J* = 8.2 Hz, H-1), 4.87 ("s", 1H, H-1′), 4.75 (dd, 1H, *J* = 3.4, 11.1 Hz, H-3), 4.54 (d, 1H, *J* = 11.5 Hz, Bn), 4.42 (d, 1H, *J* = 11.5 Hz, Bn), 4.33 ("d", 1H, *J* = 3.3 Hz, H-4), 4.30 (dd, 1H, *J* = 1.2, 12.5 Hz, H-6a), 4.05 (dd, 1H, *J* = 1.4, 12.4 Hz, H-6b), 3.58 (m, 1H, H-5′), 3.50 (m, 4H, H-5, OMe), 3.40 (m, 1H, H-2), 3.25 (m, 1H, H-4), 2.63 (ddd, 1H, *J* = 3.9, 3.9, 14.5 Hz, H-3e′), 1.95 (s, 3H, Ac), 1.70 (ddd, 1H, *J* = 3.0, 11.1, 14.1 Hz, H-3a′), 1.34 (d, 3H, *J* = 6.1 Hz, H-6′). Anal. calcd. for $C_{33}H_{39}O_9N_3S$: C 60.63, H 6.01, N 6.43; found: C 60.37, H 6.13, N 6.34.

Methyl 2-acetamido-3-*O*-(4-*O*-benzyl-2,3,6-trideoxy-β-D-erythro-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (32)

To a refluxing solution of disaccharide **31** (35.3 mg, 0.054 mmol) in anhydrous toluene (2 mL) was added dropwise a solution of tributyltin hydride (45 μL, 0.16 mmol) in toluene (0.75 mL), and a catalytic amount of AIBN (~2 mg) was added, the reaction was refluxed for 8 h. After concentration, the mixture was chromatographed on silica gel using 2% MeOH–CH_2Cl_2 as eluent to give disaccharide **32** (27 mg, 95% yield), $[\alpha]_D$ +68.9 (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.46–7.52 (m, 2H, Ar), 7.20–7.38 (m, 8H, Ar), 5.85 (br, 1H, NH), 5.53 (s, 1H, PhCH), 5.06 (d, 1H, *J* = 8.2 Hz, H-1), 4.56–4.67 (m, 3H, H-3, Bn, H-1′), 4.43 (d, 1H, Bn), 4.33 (d, 1H, *J* = 3.2 Hz, H-4), 4.29 (dd, 1H, *J* ~ 1, 12.5 Hz, H-6a), 4.05 (dd, 1H, *J* = 1.4, 12.5 Hz, H-6b), 3.50 (m, 4H, OMe, H-5), 3.34–3.46 (m, 2H, H-2, H-5′), 3.04 (m, 1H, H-4′), 2.15 (m, 1H, H-3e′), 1.89 (s, 3H, Ac), 1.84 (m, 1H, H-3a′), 1.30–1.62 (m, 2H, H-2a′, H-2b′), 1.27 (d, 3H, *J* = 6.1 Hz, H-6′). Anal. calcd. for $C_{29}H_{37}O_8N$: C 66.02, H 7.07, N 2.66; found: C 66.38, H 7.30, N 2.68.

Methyl 2-acetamido-3-*O*-(2,3,6-trideoxy-β-D-erythro-hexopyranosyl)-2-deoxy-β-D-galactopyranoside (11)

Compound **32** (25 mg, 0.047 mmol) was deprotected in a similar fashion as compound **5** and disaccharide **11** (15.7 mg, 95% yield) was obtained by reverse-phase HPLC using a gradient of H_2O–MeOH (0 → 30%) as eluent. $[\alpha]_D$ –29.0 (*c* 0.2, MeOH). ^{1}H NMR (D_2O) δ: 4.69 (dd, 1H, *J* =

2.0, 9.5 Hz, H-1′), 4.44 (d, 1H, *J* = 8.6 Hz, H-1), 4.09 ("d", 1H, *J* = 3.3 Hz, H-4), 3.98 (dd, 1H, *J* = 8.8, 10.8 Hz, H-2), 3.83 (dd, 1H, *J* = 3.1, 10.8 Hz, H-3), 3.82 (dd, 1H, *J* = 8.1, 11.2 Hz, H-6a), 3.76 (dd, 1H, *J* = 4.4, 11.9 Hz, H-6b), 3.69 (m, 1H, H-5), 3.52 (s, 3H, OMe), 3.42 (dq, 1H, *J* = 6.2, 9.2 Hz, H-5′), 3.27 (m, 1H, H-4′), 2.04 (m, 1H, H-3e′), 2.03 (s, 3H, Ac), 1.84 (m, 1H, H-2e′), 1.45–1.59 (m, 2H, H-2a′, H-3a′), 1.26 (d, 3H, *J* = 6.2 Hz, H-6′). HR-ES-MS *m*/*e* calcd. for $C_{15}H_{27}O_8N$ (MNa^+): 372.16344; found: 372.163591.

Methyl 2-acetamido-3-*O*-(2-*O*-benzoyl-4-*O*-benzyl-3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (33)

Disaccharide **29** (50 mg, 0.092 mmol) was dissolved in a 1:1 mixture of pyridine–CH_2Cl_2 (3 mL) at –30°C, benzoyl chloride (12 μL, 0.101 mmol) was added and the reaction was stirred at ambient temperature for 7 h. TLC revealed that the starting material was not completely consumed. The reaction mixture was cooled to –30°C and more benzoyl chloride (8 μL, 0.068 mmol) was added. The reaction was allowed to warm to –10°C over 30 min, at which point TLC showed the starting material to be completely transformed. Methanol (200 μL) was added, and the mixture was concentrated under reduced pressure. Chromatography on silica gel using 2% MeOH–CH_2Cl_2 as eluent gave compound **33** (52 mg, 87% yield), $[\alpha]_D$ +44.3 (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.98 (m, 2H, Bz), 7.46 (m, 1H, Bz), 7.08–7.36 (m, 7H, Ar), 5.88 (d, 1H, *J* = 6.8 Hz, NH), 5.51 (s, 1H, PhCH), 5.34 (m, 1H, H-2′), 5.05 (d, 1H, *J* = 8.2 Hz, H-1), 4.81 (s, 1H, H-1′), 4.68 (dd, 1H, *J* = 3.4, 11.1 Hz, H-3), 4.55 (d, 1H, *J* = 11.5 Hz, Bn), 4.41 (d, 1H, *J* = 11.5 Hz, Bn), 4.33 (d, 1H, *J* = 3.1 Hz, H-4), 4.29 (dd, 1H, *J* = 1.2, 12.3 Hz, H-6a), 4.04 (dd, 1H, *J* = 1.6, 12.3 Hz, H-6b), 3.57 (m, 1H, H-5′), 3.48 (s, 1H, H-5′), 3.47 (s, 4H, OMe, H-5), 3.31–3.46 (m, 2H, H-4′, H-2), 2.46 (d′t′, 1H, *J* = 4.0,, 14.0 Hz, H-3e′), 1.95 (s, 3H, Ac), 1.70 (ddd, 1H, 3.2, 11.2, 14.1 Hz, H-3a′), 1.36 (d, 1H, *J* = 6.1 Hz, H-6′). Anal. calcd. for $C_{36}H_{41}O_{10}N$: C 66.76, H 6.38, N 2.16; found: C 66.38, H 6.28, N 2.05.

Methyl 2-acetamido-3-*O*-(2-*O*-benzoyl-3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (34)

A mixture of disaccharide **33** (52 mg, 0.08 mmol), HCO_2NH_4 (25 mg, 0.40 mmol) and 5% Pd-C (34 mg) in MeOH (10 mL) was heated to reflux for 1 h. The catalyst was filtered off and the filtrate was concentrated. Disaccharide **34** was obtained by chromatography on silica gel using 2% MeOH – CH_2Cl_2 as eluent (35.2 mg, 78% yield), $[\alpha]_D$ +32.6 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 7.98 (m, 2H, Bz), 7.45 (m, 1H, Bz), 7.07–7.34 (m, 7H, Ar), 6.08 (d, 1H, *J* = 7.1 Hz, NH), 5.50 (s, 1H, PhCH), 5.31 (m, 1H, H-2′), 5.00 (d, 1H, *J* = 8.2 Hz, H-1), 4.78 ("s", 1H, H-1′), 4.65 (dd, 1H, *J* = 3.3, 11.1 Hz, H-3), 4.10 (d, 1H, *J* = 3.3 Hz, H-4), 4.27 (dd, 1H, *J* ~ 1.0, 12.3 Hz, H-6a), 4.02 (dd, 1H, *J* = 1.3, 12.3 Hz, H-6b), 3.61 (m, 1H, H-4′), 3.46 (s, 4H, OMe, H-5), 3.34–3.45 (m, 2H, H-5′, H-2), 2.29 (d′t′, 1H, *J* = 5.1, 14.0 Hz, H-3e′), 1.96 (s, 3H, Ac), 1.66 (ddd, 1H, 3.2, 11.2, 14.1 Hz, H-3a′), 1.34 (d, 1H, *J* = 6.1 Hz, H-6′). Anal. calcd. for $C_{29}H_{35}O_{10}N$: C 62.47, H 6.33, N 2.51; found: C 62.26, H 6.34, N 2.33.

Methyl 2-acetamido-3-*O*-(2-*O*-benzoyl-3,6-dideoxy-4-*O*-imidazoylthiocarbonyl-β-D-*arabino*-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (35)

To a solution of disaccharide **34** (30 mg, 0.055 mmol) in anhydrous toluene (3 mL), was added 1,1′-thiocarbonyldiimidazole (35 mg, 0.18 mmol), and the mixture was heated to reflux for 8 h. After cooling, the mixture was concentrated, and the disaccharide **35** was obtained by chromatography on silica gel using 2.5 → 5% MeOH – CH_2Cl_2 as eluent (32.9 mg, 92% yield), $[\alpha]_D$ +5.2 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 8.29 (t, 1H, *J* = 0.8 Hz, imidazole), 8.03 (m, 2H, Bz), 7.56 (t, 1H, 1.6 Hz, imidazole), 7.51 (m, 1H, Bz), 7.13–7.38 (m, 7H, Ar), 7.03 (dd, 1H, *J* = 0.8, 1.6 Hz, imidazole), 6.00 (d, 1H, *J* = 7.1 Hz, NH), 5.56 (m, 1H, H-4′), 5.53 (s, 1H, PhCH), 5.43 (m, 1H, H-2′), 4.96 (d, 1H, *J* = 1.5 Hz, H-1′), 4.93 (d, 1H, *J* = 8.2 Hz, H-1), 4.69 (dd, 1H, *J* = 3.4, 11.2 Hz, H-3), 4.27–4.36 (m, 2H, H-4, H-6a), 4.06 (dd, 1H, *J* = 1.6, 12.4 Hz, H-6b), 3.94 (dq, 1H, *J* = 6.1, 9.0 Hz, H-5′), 3.53 (m, 1H, H-2), 3.49 (m, 1H, H-5), 3.48 (s, 3H, OMe), 2.67 (d′t′, 1H, *J* = 4.5, 13.8 Hz, H-3e′), 1.93 (s, 3H, Ac), 1.84 (ddd, 1H, 3.4, 10.5, 13.8 Hz, H-3a′), 1.37 (d, 1H, *J* = 6.2 Hz, H-6′). Anal. calcd. for $C_{33}H_{37}O_{10}N_3S$: C 59.36, H 5.59, N 6.29; found: C 59.01, H 5.33, N 6.24.

Methyl 2-acetamido-3-*O*-(2-*O*-benzoyl-3,4,6-trideoxy-β-D-threo-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (36)

To a refluxing solution of disaccharide **35** (32.9 mg, 0.061 mmol) in anhydrous toluene (2 mL) was added dropwise a solution of tributyltin hydride (41 μL, 0.15 mmol) in toluene (0.75 mL), and a catalytic amount of AIBN (~2 mg) was added, the reaction was continued to reflux for 3 h. After concentration, the mixture was chromatographed on silica gel using 2% MeOH – CH_2Cl_2 as eluent to give disaccharide **36** (22 mg, 83% yield), $[\alpha]_D$ +36.2 (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 8.01 (m, 2H, Bz), 7.46 (m, 1H, Bz), 7.09–7.36 (m, 7H, Ar), 5.85 (d, 1H, *J* = 6.9 Hz, NH), 5.53 (s, 1H, PhCH), 5.16 (m, 1H, H-2′), 5.03 (d, 1H, *J* = 8.2 Hz, H-1), 4.71 (d, 1H, *J* = 1.1 Hz, H-1′), 4.63 (dd, 1H, *J* = 3.4, 11.2 Hz, H-3), 4.35 (d, 1H, *J* = 3.3 Hz, H-4), 4.29 (dd, 1H, *J* = 1.5, 12.3 Hz, H-6a), 4.04 (dd, 1H, *J* = 1.4, 12.3 Hz, H-6b), 3.64 (m, 1H, H-5′), 3.47 (m, 4H, H-5+ OMe), 3.41 (m, 1H, H-2), 2.07 (m, 1H, H-3e′), 1.96 (s, 3H, Ac), 1.72 (m, 1H, H-3a′), 1.30–1.68 (m, 2H, H-4a′, H4e′), 1.27 (d, 3H, *J* = 6.2 Hz, H-6′). Anal. calcd. for $C_{29}H_{35}O_9N$: C 64.31, H 6.51, N 2.59; found: C 64.00, H 6.42, N 2.56.

Methyl 2-acetamido-3-*O*-(3,4,6-trideoxy-β-D-threo-hexopyranosyl)-2-deoxy-β-D-galactopyranoside (13)

Compound **36** (22 mg, 0.0406 mmol) was dissolved in anhyd MeOH (3 mL), and a solution of NaOMe in MeOH (1.5 M, 400 μL) was added. After 1 h, the mixture was neutralized with Amberlite IR-120 (H^+), the solution was filtered and evaporated to dryness. The residue was hydrogenated in a similar fashion as compound **5** and disaccharide **13** (12.5 mg, 88% yield) was purified by reverse-phase HPLC using a gradient of H_2O–MeOH (0 → 30%) as eluent. $[\alpha]_D$ –34 (*c* 0.5, MeOH). ^{1}H NMR (D_2O) δ: 4.60 (d, 1H, *J* = 0.9 Hz, H-1′), 4.45 (d, 1H, *J* = 8.6 Hz, H-1), 4.12 ("d", 1H, *J* = 3.1 Hz, H-4), 4.02 (dd, 1H, *J* = 8.6,

10.8 Hz, H-2), 3.87 (dd, 1H, *J* = 3.3, 10.8 Hz, H-3), 3.83 (dd, 1H, *J* = 7.9, 11.9 Hz, H-6a), 3.78 (dd, 1H, *J* = 4.2, 11.7 Hz, H-6b), 3.69–3.72 (m, 3H, H-2′, H-5′, H-5), 3.52 (s, 3H, OMe), 2.03 (s, 3H, Ac), 1.89 (m, 1H, H-3e′), 1.75 (m, 1H, H-3a′), 1.42–1.47 (m, 2H, H-4e′, H-4a′), 1.21 (d, 3H, *J* = 6.2 Hz, H-6′). HR-ES-MS *m/e* calcd. for $C_{15}H_{27}O_8N$ (MNa^+): 372.16344; found: 372.164136.

Methyl 2-acetamido-3-*O*-(3,6-dideoxy-β-D-arabino-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (37)

To a solution of disaccharide **29** (243 mg, 0.45 mmol) in MeOH (35 mL), was added 10% Pd-C (250 mg) and HCO_2NH_4 (243 mg, 3.86 mmol), and the mixture was heated to reflux for 1 h. The catalyst was filtered off, and the filtrate was concentrated. The syrupy mixture was purified by chromatography on silica gel using 10% → 20% MeOH – CH_2Cl_2 as eluent to give first disaccharide **37** (160 mg, 79% yield) and then disaccharide **1** (18 mg, 11% yield), $[\alpha]_D$ +30.3 (*c* 0.4, MeOH). ^{1}H NMR ($CDCl_3$) δ: 7.48 (m, 2H, Ph), 7.29–7.35 (m, 3H, Ph), 5.81 (br, 1H, NH), 5.53 (s, 1H, PhCH), 5.01 (d, 1H, *J* = 8.3 Hz, H-1), 4.77 (dd, 1H, *J* = 3.2, 11.1 Hz, H-3), 4.60 ("s", 1H, H-1′), 4.32 ("d", 1H, *J* = 3.2 Hz, H-4), 4.30 (dd, 1H, *J* = 1.0, 12.2 Hz, H-6a), 4.06 (dd, 1H, *J* = 1.1, 12.2 Hz, H-6b), 3.82 (m, 1H, H-2′), 3.65 (ddd, 1H, *J* = 4.4, 9.2, 10.8 Hz, H-4′), 3.51 (s, 4H, OMe, H-5), 3.44 (m, 1H, H-2), 3.30 (dq, 1H, *J* = 6.1, 9.0 Hz, H-5′), 2.24 (d′t′, 1H, *J* = 3.8, 14.1, H-3e′), 1.44 (ddd, 1H, *J* = 2.9, 11.0, 13.7 Hz, H-3a′), 1.31 (d, 3H, *J* = 6.1 Hz, H-6′). Anal. calcd. for $C_{22}H_{31}O_9N$: C 58.27, H 6.89, N 3.09; found: C 57.84, H 6.90, N 3.08.

Methyl 2-acetamido-3-*O*-(2,4-di-*O*-benzoyl-3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (38)

Benzoic anhydride (112 mg, 0.534 mmol) and DMAP (10 mg, 0.0818 mmol) were added to diol **37** (80.9 mg, 0.178 mmol) was dissolved in anhydrous pyridine (3 mL). After stirring for 18 h at ambient temperature, H_2O (0.5 mL) was added to destroy the excess anhydride, and the mixture was concentrated to dryness. Dibenzoate **38** was obtained by chromatography on silica gel using 3% MeOH – CH_2Cl_2 as eluent (105 mg, 89% yield), $[\alpha]_D$ +25.2 (*c* 0.8, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 8.03 (m, 2H, Bz), 7.98 (m, 2H, Bz), 7.12–7.60 (m, 11H, Ar), 5.78 (d, 1H, *J* = 7.0 Hz, NH), 5.54 (s, 1H, PhCH), 5.43 (m, 1H, H-2′), 5.08 (m, 1H, H-4′), 4.99 (d, 1H, *J* = 8.3 Hz, H-1), 4.91 (d, 1H, *J* = 1.3 Hz, H-1′), 4.70 (dd, 1H, *J* = 3.4, 11.2 Hz, H-3), 4.36 (d, 1H, *J* = 3.3 Hz, H-4), 4.30 (dd, 1H, *J* = 1.3, 12.4 Hz, H-6a), 4.06 (dd, 1H, *J* = 1.6, 12.4 Hz, H-6b), 3.81 (dq, 1H, *J* = 6.2, 9.0 Hz, H-5′), 3.49 (m, 1H, H-5), 3.48 (s, 3H, OMe), 3.47 (m, 1H, H-2), 2.54 (d′t′, 1H, *J* = 4.4, 13.9 Hz, H-3e′), 1.97 (s, 3H, Ac), 1.85 (ddd, 1H, *J* = 3.3, 10.5, 13.9 Hz, H-3a′),1.35 (d, 3H, *J* = 6.1 Hz, H-6′). Anal. calcd. for $C_{36}H_{39}O_{11}N$: C 65.35, H 5.94, N 2.12; found: C 65.55, H 5.93, N 1.95.

Methyl 2-acetamido-3-*O*-(2,4-di-*O*-benzoyl-3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-2-deoxy-β-D-galactopyranoside (39)

A solution of compound **38** (52 mg, 0.078 mmol) in MeOH (30 mL) was hydrogenated over 10% $Pd(OH)_2$ for 18 h. The catalyst was filtered off and the filtrate was concentrated. Diol **39** was purified by chromatography on silica gel using 5% MeOH – CH_2Cl_2 as eluent (40 mg, 89% yield), $[\alpha]_D$ –48.1 (*c* 0.3, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 8.05 (m, 2H, Bz), 7.98 (m, 2H, Bz), 7.53–7.61 (m, 2H, Bz), 7.40–7.49 (m, 4H, Bz), 5.94 (br, 1H, NH), 5.46 (m, 1H, H-2′), 5.10 (m, 1H, H-4′), 5.03 (d, 1H, *J* = 8.2 Hz, H-1), 4.86 (br s, 1H, H-1′), 4.62 (dd, 1H, *J* = 3.1, 10.6 Hz, H-3), 4.10 (d, 1H, *J* = 2.5 Hz, H-4), 3.94 (dd, 1H, *J* = 6.5, 11.6 Hz, H-6a), 3.75–3.88 (m, 2H, H-6b, H-5′), 3.62 (m, 1H, H-5), 3.47 (s, 3H, OMe), 3.05 (m, 1H, H-2), 2.55 (d′t′, 1H, *J* = 3.7, 14.1 Hz, H-3e′), 2.03 (s, 3H, Ac), 1.92 (ddd, 1H, *J* = 3.2, 10.5, 14.1 Hz, H-3a′),1.34 (d, 3H, *J* = 6.1 Hz, H-6′). Anal. calcd. for $C_{29}H_{35}O_{11}N$: C 60.72, H 6.15, N 2.44; found: C 60.38, H 6.05, N 2.66.

Methyl 2-acetamido-3-O-(3,6-dideoxy-β-D-arabino-hexopyranosyl)-2,6-dideoxy-β-D-galactopyranoside (9)

Tolunesulfonyl chloride (12.5 mg, 0.066 mmol) was added to a solution of compound **39** (34.7 mg, 0.06 mmol) in anhydrous pyridine (2 mL), and the reaction was stirred at room temperature for 3 h. Water (0.3 mL) was added to quench the reaction. The mixture was diluted with EtOAc (50 mL), washed with brine (1 × 25 mL), dried and concentrated. Lithium bromide (34.2 mg, 0.39 mmol) and KI (6 mg, 0.036 mmol) were added to a solution of the residue in DMF (1.5 mL), and the resulting solution was heated to 100°C for 1 h. The reaction mixture was diluted with EtOAc (75 mL), washed with brine (sat., 2 × 25 mL), dried and evaporated. Tributyltin hydride (330 μL, 0.12 mmol) and AIBN (5 mg, 0.03 mmol) were added to a refluxing solution of bromide **41** (25 mg, 0.04 mmol) in anhydrous toluene (2 mL). After 1 h, the reaction was cooled to room temperature and concentrated. The syrupy mixture was dissolved in anhyd MeOH (5 mL), and a solution of MeONa in MeOH (1.5 M, 50 μL) was added. After stirring for 1 h, the reaction was neutralized with Amberlite IR-120 (H^+) resin, filtered and concentrated. Disaccharide **9** was obtained by reverse-phase HPLC using a gradient of H_2O–MeOH (0 → 30%) as eluent (10.5 mg, 49% yield), $[\alpha]_D$ –38.4 (*c* 0.5, MeOH). ^{1}H NMR (D_2O) δ: 4.68 (d, 1H, *J* = 0.9 Hz, H-1′), 4.42 (d, 1H, *J* = 8.6 Hz, H-1), 3.97 (dd, 1H, *J* = 8.6, 10.8 Hz, H-2), 3.94 ("d", 1H, *J* = 3.1 Hz, H-4), 3.89 (m, 1H, H-2′), 3.86 (dd, 1H, *J* = 3.1, 10.8 Hz, H-3), 3.80 (dq, 1H, *J* = 0.7, 6.6 Hz, H-5), 3.55 (ddd, 1H, *J* = 4.8, 9.3, 11.5 Hz, H-4′), 3.49 (s, 3H, OMe), 3.43 (dq, 1H, *J* = 6.1, 9.3 Hz, H-5′), 2.17 (ddd, 1H, *J* = 3.5, 4.8, 14.1 Hz, H-3e′), 2.02 (s, 3H, Ac), 1.65 (ddd, 1H, *J* = 2.9, 11.5, 14.3 Hz, H-3e′), 1.29 (d, 3H, *J* = 6.4 Hz, H-6), 1.27 (d, 3H, *J* = 6.0 Hz, H-6′). HR-ES-MS *m/e* calcd. for $C_{15}H_{27}O_8N$ (MNa^+): 372.16344; found: 372.163372.

3,6-Dideoxy-4-*O*-methyl-1,2-*O*-propylidene-α-D-ribo-hexopyranose (44)

Sodium hydride (60% in mineral oil, 218 mg, 5.445 mmol) was added to a solution of compound **43** (38) (205 mg, 1.089 mmol) in anhyd DMF, followed after 10 min by MeI (136 μL, 2.178 mmol), and the reaction was stirred at ambient temperature for 2 h. MeOH (0.5 mL) was added to quench the reaction, and the reaction mixture was diluted with EtOAc (75 mL), washed with brine (sat., 2 × 30 mL),

dried and evaporated. The compound **44** was obtained by chromatography on silica gel using 20% EtOAc – hexane as eluent (201 mg, 92% yield), $[\alpha]_D$ +44.6 (*c* 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 5.30 (d, 1H, *J* = 5.2 Hz, H-1), 4.73 (t, 1H, *J* = 4.9 Hz, CH_3CH_2CH), 4.01 (m, 1H, H-2), 3.80 (dq, 1H, *J* = 6.1, 9.3 Hz, H-5), 3.31 (s, 3H, OMe), 3.08 (m, 1H, H-4), 2.17 (ddd, 1H, *J* = 1.7, 2.6, 15.5 Hz, H-3e), 1.86 (ddd, 1H, *J* = 3.6, 7.6, 15.9 Hz, H-3a), 1.77 (m, 2H, CH_3CH_2), 1.23 (d, 3H, *J* = 6.3 Hz, H-6), 0.98 (t, 3H, *J* = 7.6 hz, CH_3CH_2). Anal. calcd. for $C_{10}H_{18}O_4$: C 59.39, H 8.97; found: C 59.63, H 8.88.

1,2-Di-*O*-benzoyl-3,6-dideoxy-4-*O*-methyl-α,β-D-ribo-hexopyranose (46)

The 1,2-*O*-propylidene derivative **44** (112 mg, 0.55 mmol) was dissolved in THF (5 mL), and a 10% aqueous solution of H_2SO_4 (1 mL) was added, and the reaction was stirred at 65°C for 2 h. The mixture was diluted with CH_2Cl_2 (100 mL), washed with aq $NaHCO_3$ (sat., 2 × 30 mL), dried, and evaporated to afford the crude diol **45** that was used directly to the next step. Benzoyl chloride (314.9 μL, 1.21 mmol) was added to an ice-cold solution of the crude diol **45** in a mixture of anhyd CH_2Cl_2 (1 mL) and pyridine (0.5 mL), and the reaction was continued for 1 h. Methanol (0.5 mL) was added to quench the reaction. The reaction mixture was diluted with CH_2Cl_2 (50 mL), the solution extracted with H_2O, then dried, and evaporated. Dibenzoate **46** was obtained as a α,β-mixture by chromatography on silica gel using 10% EtOAc – hexane as eluent (α/β: 1:1, 197 mg, 96% yield). ^1H NMR (CD_3OD) for α-anomer δ: 8.10 (m, 2H, Bz), 7.90 (m, 2 H, Bz), 7.30–7.63 (m, 3H, Bz), 6.52 (d, 1H, *J* = 3.3 Hz, H-1), 5.27 (m, 1H, H-2), 3.89 (dq, 1H, *J* = 6.4, 9.3 Hz, H-5), 3.43 (s, 3H, OMe), 3.17 (m, 1H, H-4), 2.66 (dd′t′, 1H, *J* = 0.9, 4.4, 11.4 Hz, H-3e), 2.05 (d′t′, 11.5, 11.5 Hz, H-3a), 1.29 (d, 3H, *J* = 6.1 Hz, H-6); β-anomer δ: 8.02 (m, 2H, Bz), 7.96 (m, 2 H, Bz), 7.30–7.63 (m, 3H, Bz), 6.06 (d, 1H, *J* = 8.2 Hz, H-1), 5.24 (m, 1H, H-2), 3.67 (dq, 1H, *J* = 6.1, 9.0 Hz, H-5), 3.40 (s, 3H, OMe), 3.12 (m, 1H, H-4), 2.83 (d′t′, 1H, *J* = 4.9, 12.1 Hz, H-3e), 1.66 (d′t′, 11.9, 11.9 Hz, H-3a), 1.35 (d, 3H, *J* = 6.1 Hz, H-6). Anal. calcd. for $C_{21}H_{22}O_6$: C 68.10, H 5.99; found: C 68.43, H 5.95.

Phenyl 2-*O*-benzoyl-3,6-dideoxy-4-*O*-methyl-1-thio-α,β-D-ribo-hexopyranoside (47)

A solution of **46** (185 mg, 0.50 mmol) in anhydrous toluene (3 mL) was stirred with molecular sieves 4 Å (400 mg) for 30 min at 0°C. Me_3SiSPh (244 μL, 1.25 mmol) was added, followed by TMSOTf (97 μL, 0.5 mmol). Reaction was continued at room temperature overnight. Triethylamine (0.5 mL) was added, and the insoluble material was filtered off. After evaporation, the thioglycoside **47** was obtained by chromatography of the residue on silica gel using 5% EtOAc – hexane as eluent (α/β 4:6, 160 mg, 89%). ^1H NMR ($CDCl_3$) for α-anomer δ: 8.09 (m, 2H, Bz), 7.57 (m, 1H, Bz), 7.45 (m, 2H, Bz), 7.17–7.28 (m, 5H, Ph), 5.75 (d, 1H, *J* = 5.1 Hz, H-1), 5.29 (d′t′, 1H, *J* = 5.1, 12.4 Hz, H-2), 4.18 (dq, 1H, *J* = 6.2, 9.2 Hz, H-5), 3.40 (s, 3H, OMe), 3.07 (ddd, 1H, *J* = 4.5, 9.3, 10.9 Hz, H-4), 2.52 (dd′t′, 1H, *J* = 0.7, 4.5, 11.9 Hz, H-3e), 1.87 (d′t′, 1H, *J* = 12.1, 12.1 Hz, H-3a), 1.26 (d, 3H, *J* = 6.2 Hz, H-6); β-anomer δ: 8.04 (m, 2H, Bz), 7.57 (m, 1H, Bz), 7.46 (m, 2H, Bz), 7.26 (m, 5H, Ph), 4.90 (d′t′, 1H, *J* = 4.8, 9.9 Hz, H-21), 4.80 (d, 1H, *J* = 9.9 Hz, H-1), 3.42 (dq, 1H, *J* = 6.2, 9.2 Hz, H-5), 3.36 (s, 3H, OMe), 3.02 (m, 1H, H-4), 2.82 (d′t′, 1H, *J* = 4.5, 12.0 Hz, H-3e), 1.53 (d′t′, 1H, *J* = 12.1, 12.1 Hz, H-3a), 1.36 (d, 3H, *J* = 6.2 Hz, H-6). Anal. calcd. for $C_{20}H_{22}O_4S$: C 67.02, H 6.19; found: C 67.34, H 6.16.

Methyl 2-azido-3-*O*-(2-*O*-benzoyl-3,6-dideoxy-4-*O*-methyl-β-D-ribo-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (48)

A mixture containing thioglycoside **47** (140 mg, 0.40 mmol), alcohol **24** (39) (95 mg, 0.31 mmol) and 4 Å molecular sieves (500 mg) in CH_2Cl_2 was stirred for 30 min and *N*-iodosuccinimide (146 mg, 0.62 mmol) was added. After the reaction mixture had been cooled to –60°C, TfOH (6 μL, 0.067 mmol) was added, and the reaction was continued for 3 h. Triethylamine (0.5 mL) was added to quench the reaction, and disaccharide **48** was obtained in a similar fashion to that described above (134.7 mg, 78% yield), $[\alpha]_D$ –14.7 (*c* 0.8, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 8.02–8.08 (m, 2 H, Bz), 7.20–7.57 (m, 8H, Ar), 5.50 (s, 1H, PhCH), 4.82–4.96 (m, 2H, H-1′ + H-2′), 4.30 (dd, 1H, *J* = 1.6, 12.4 Hz, H-6a), 4.23 (br d, 1H, *J* = 3.1 Hz, H-4), 4.21 (d, 1H, *J* = 8.0 Hz, H-1), 4.04 (dd, 1H, *J* = 1.6, 12.4 Hz, H-6b), 3.77 (dd, 1H, *J* = 8.0, 10.6 Hz, H-2), 3.55 (s, 3H, OMe), 3.51 (dd, 1H, *J* = 3.5, 10.6 Hz, H-3), 3.43 (dq, 1H, *J* = 6.1, 9.0 Hz, H-5′), 3.39 (m, 1H, H-5), 3.35 (s, 3H, OMe), 3.04 (ddd, 1H, *J* = 4.5, 9.0, 10.8 Hz, H-4′), 2.79 (d′t′,1H, *J* = 4.4, 12.1 Hz, H-3e′), 1.45 (d′t′, 1H, *J* = ~11.5, 11.5 Hz, H-3a′), 1.31 (d, 3H, *J* = 6.1 Hz, H-6′). Anal. calcd. for $C_{28}H_{33}O_9N_3$: C 60.53, H 5.99, N 7.56; found: C 60.64, H 6.00, N 7.32.

Methyl 2-azido-3-*O*-(3,6-dideoxy-4-*O*-methyl-β-D-ribo-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (49)

Compound **48** (140 mg, 0.25 mmol) was transesterified as described above to give the disaccharide **49** (107 mg, 94% yield), $[\alpha]_D$ +22.7 (*c* 1.1, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.51 (m, 2H, Ph), 7.31–7.38 (m, 3H, Ph), 5.52 (s, 1H, PhCH), 4.43 (d, 1H, *J* = 7.3 Hz, H-1′), 4.31 (dd, 1H, *J* = 1.5, 12.4 Hz, H-6a), 4.24 ("d", 1H, *J* = 3.4 Hz, H-4), 4.22 (d, 1H, *J* = 8.0 Hz, H-1), 4.04 (dd, 1H, *J* = 1.7, 12.4 Hz, H-6b), 3.85 (dd, 1H, *J* = 8.1, 10.5 Hz, H-2), 3.58 (s, 3H, OMe), 3.53 (dd, 1H, *J* = 3.5, 10.6 Hz, H-3), 3.52 (dq, 1H, *J* = 6.1, 9.0 Hz, H-2′), 3.32–3.42 (m, 5H, H-5, H-5′, OMe), 3.35 (s, 3H, OMe), 2.92 (ddd, 1H, *J* = 4.4, 8.8, 10.9 Hz, H-4′), 2.49 (d′t′, 1H, *J* = 4.7, 12.3 Hz, H-3e′), 1.36 (d′t′, 1H, *J* ~ 11.5, 11.5 Hz, H-3a′), 1.28 (d, 3H, *J* = 6.1 Hz, H-6′). Anal. calcd. for $C_{21}H_{29}O_8N_3$: C 55.87, H 6.48, N 9.31; found: C 55.81, H 6.36, N 9.40.

Methyl 2- acetamido-3-*O*-(3,6-dideoxy-4-*O*-methyl-β-D-*arabino*-hexopyranosyl)-4,6-*O*-benzylidene-2-deoxy-β-D-galactopyranoside (51)

The disaccharide **49** (96.3 mg, 0.21 mmol) was oxidized by a mixture of Ac_2O (1 mL) and anhydrous DMSO (2 mL) at room temperature for 18 h to give the intermediate ketone **50**, after purification by column chromatography on silica gel using 50% EtOAc – hexane as eluent. Ketone **50** (66.2 mg, 0.147 mmol) was dissolved in anhyd THF

(2.5 mL) at 0°C, a solution of L-selectride in THF (1 M, 500 μL, 0.50 mmol) was added, and the reaction was continued for 1 h. Water (0.5 mL) was added to quench the reaction, and Ac_2O (0.5 mL) was added. After stirring for 30 min, the reaction mixture was concentrated to dryness. Disaccharide **51** was obtained by chromatography on silica gel using 5% MeOH – CH_2Cl_2 as eluent (52.6 mg, 76% yield), $[\alpha]_D$ +28.3 (*c* 0.7, $CHCl_3$). ^{1}H NMR (CD_3OD) δ: 7.45–7.51 (m, 2 H, Ph), 7.28–7.35 (m, 3H, Ph), 5.97 (d, 1H, *J* = 4.6 Hz, NH), 5.51 (s, 1H, PhCH), 5.01 (d, 1H, *J* = 8.2 Hz, H-1), 4.73 (dd, 1H, *J* = 3.4, 11.3 Hz, H-3), 4.56 (br "s", 1H, H-1′), 4.26–4.36 (m, 2H, H-6a, H-4), 4.04 (dd, 1H, *J* = 1.5, 12.4 Hz, H-6b), 3.81 (m, 1H, H-2′), 3.28–3.56 (m, 9H, 2 × OMe, H-5, H-2, H-5′), 3.18 (m, 1H, H-4′), 2.32 (d′t′, 1H, *J* = 4.1, 13.6 Hz, H-3e′), 1.84 (s, 3H, Ac), 1.22–1.34 (m, 4H, H-6′, H3a′). Anal. calcd. for $C_{23}H_{33}O_9N$: C 59.09, H 7.12, N 3.00; found: C 59.19, H 7.11, N 2.99.

Methyl 2-acetamido-3-*O*-(3,6-dideoxy-4-*O*-methyl-β-D-*arabino*-hexopyranosyl)-2-deoxy-β-D-galactopyranoside (14)

Compound **51** (42 mg, 0.09 mmol) was hydrogenated in a similar fashion as compound **5** and disaccharide **14** (32 mg, 94% yield) was obtained by reverse-phase HPLC using a gradient of H_2O–MeOH (0 → 30%) as eluent. $[\alpha]_D$ –24.5 (*c* 0.4, MeOH). ^{1}H NMR (D_2O) δ: 4.69 ("s", 1H, H-1′), 4.44 (d, 1H, *J* = 8.6 Hz, H-1), 4.12 ("d", 1H, *J* = 3.1 Hz, H-4), 4.01 (dd, 1H, *J* = 8.2, 10.6 Hz, H-2), 3.91 (m, 1H, H-2′), 3.86 (dd, 1H, *J* = 3.3, 10.8 Hz, H-3), 3.82 (dd, 1H, *J* = 7.9, 11.9 Hz, H-6a), 3.77 (dd, 1H, *J* = 4.2, 11.7 Hz, H-6b), 3.70 (m, 1H, H-5), 3.52 (s, 3H, OMe), 3.49 (dq, 1H, *J* = 6.2, 9.2 Hz, H-5′), 3.39 (s, 3H, OMe), 3.25 (ddd, 1H, *J* = 4.6, 9.3, 11.2 Hz, H-4′), 2.38 (d′t′, 1H, *J* = 4.2, 13.9 Hz, H-3e′), 2.02 (s, 3H, Ac), 1.54 (ddd, 1H, *J* = 2.9, 11.4, 14.1 Hz, H-3a′), 1.29 (d, 3H, *J* = 6.2 Hz, H-6′). HR-ES-MS *m*/*e* calcd. for $C_{16}H_{29}O_9N$ (MNa^+): 402.17400; found: 402.173637.

Methyl 2-azido-3,6-di-*O*-benzoyl-2-deoxy-β-D-galactopyranoside (53)

A solution of triol **52** (39) (860 mg, 3.9 mmol) and bis(tributyltin)oxide in anhydrous toluene (60 mL) was refluxed for 4.5 h with azeotropic removal of water. The solution was cooled to room temperature, and BzCl (1.0 mL, 8.97 mmol) was added. After stirring for 2.5 h, the mixture was diluted with EtOAc (75 mL), washed with brine (1 × 30 mL), dried and evaporated. Dibenzoate **53** was obtained by chromatography on silica gel using 15% EtOAc – hexane as eluent (1.46 g, 86% yield), $[\alpha]_D$ +29.8 (*c* 0.5, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 8.08 (m, 2H, Bz), 8.01 (m, 2H, Bz), 7.53–7.63 (m, 2H, Bz), 7.39–7.49 (m, 4H, Bz), 4.98 (dd, 1H, *J* = 3.2, 10.8 Hz, H-3), 4.66 (dd, 1H, *J* = 6.8, 11.4 Hz, H-6a), 4.51 (dd, 1H, *J* = 6.2, 11.3 Hz, H-6b), 4.34 (d, 1H, *J* = 8.0 Hz, H-1), 4.20 ("d", 1H, *J* = 2.7 Hz, H-4), 3.93 (dd, 1H, *J* = 8.0, 10.8 Hz, H-2), 3.91 (m, 1H, H-5), 3.61 (s, 3H, OMe), 2.53 (br s, 1H, OH-4). Anal. calcd. for $C_{21}H_{21}O_7N_3$: C 59.01, H 4.95, N 9.83; found: C 58.75, H 4.85, N 9.60.

Methyl 2-azido-3,6-di-*O*-benzoyl-2-deoxy-4-*O*-methyl-β-D-galactopyranoside (54)

A solution of dibenzoate **53** (416 mg, 0.973 mmol), MeOTf (330 μL, 2.91 mmol), and 2,6-di-*tert*-butyl-4-methylpyridine in anhyd CH_2Cl_2 (15 mL) was refluxed for 4 h. The mixture was diluted with EtOAc (75 mL), washed with H_2O (1 × 30 mL), dried and evaporated to dryness. The protected glycoside **54** was obtained by chromatography on silica gel using 15% EtOAc – hexane as eluent (401 mg, 94% yield), $[\alpha]_D$ –15.4 (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 8.11 (m, 2H, Bz), 8.02 (m, 2H, Bz), 7.53–7.64 (m, 2H, Bz), 7.40–7.52 (m, 4H, Bz), 5.00 (dd, 1H, *J* = 2.9, 10.8 Hz, H-3), 4.58 (dd, 1H, *J* = 6.4, 11.1 Hz, H-6a), 4.49 (dd, 1H, *J* = 6.8, 11.1 Hz, H-6b), 4.31 (d, 1H, *J* = 8.0 Hz, H-1), 3.82 (dd, 1H, *J* = 8.1, 10.7 Hz, H-2), 3.91 (m, 1H, H-5), 3.82 (br d, 1H, *J* = 2.9 Hz, H-4), 3.59 (s, 3H, OMe), 3.50 (s, 3H, OMe). Anal. calcd. for $C_{22}H_{23}O_7N_3$: C 59.86, H 5.25, N 9.52; found: C 59.83, H 5.31, N 9.28.

Methyl 2-azido-2-deoxy-4-*O*-methyl-β-D-galactopyranoside (55)

Dibenzoate **54** (350 mg, 0.79 mmol) was debenzoylated as described above to give the selectively methylated monosaccharide **55** (179 mg, 97% yield), $[\alpha]_D$ –12.2 (*c* 0.7, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 4.15 (m, 1H, H-1, high order), 3.94 (dd, 1H, *J* = 7.4, 11.2 Hz, H-6a), 3.94 (dd, 1H, *J* = 7.4, 11.2 Hz, H-6a), 3.77 (dd, 1H, *J* = 4.9, 11.2Hz, H-6b), 3.56 (s, 3H, OMe), 3.55 (s, 3H, OMe), 3.45–3.51 (m, 4H, H-2, H-3, H-4, H-5). Anal. calcd. for $C_8H_{15}O_5N_3$: C 41.20, H 6.48, N 18.02; found: C 41.53, H 6.53, N 18.33.

Methyl 2-azido-6-*O*-tert-butyldimethylsilyl-2-deoxy-4-*O*-methyl-β-D-galactopyranoside (56)

Diol **55** (108 mg, 0.46 mmol) was dissolved in anhydrous pyridine (4 mL), *t*-$BuMe_2SiCl$ (98 mg, 0.65 mmol) was added, and the reaction was stirred at ambient temperature for 3 h. Water (0.3 mL) was added and the mixture was concentrated. The syrup was chromatographed on silica gel using 15% EtOAc – hexane as eluent to yield **56** (135.1 mg, 84% yield), $[\alpha]_D$ –5.0 (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 4.11 (m, 1H, H-1, high order), 3.79 (dd, 1H, *J* = 8.6, 9.6 Hz, H-6a), 3.73 (dd, 1H, *J* = 5.6, 9.8 Hz, H-6a), 3.61 (br, 1H, H-4), 3.59 (s, 3H, OMe), 3.52 (s, 3H, OMe), 3.37–3.47 (m, 3H, H-2, H-3, H-5), 0.88 (s, 9H, *tert*-BuSi), 0.06 (s, 6H, $SiMe_2$). Anal. calcd. for $C_{14}H_{29}O_5N_3Si$: C 48.39, H 8.41, N 12.09; found: C 48.70, H 8.59, N 11.73.

Methyl 2-azido-3-*O*-(2-*O*-benzoyl-4-*O*-benzyl-3,6-dideoxy-β-D-ribo-hexopyranosyl)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-methyl-β-D-galactopyranoside (57)

A solution of acceptor **56** (40 mg, 0.115 mmol), thioglycoside **22** (55 mg, 0.127 mmol), and 4 Å molecular sieves (500 mg) in anhyd CH_2Cl_2 (3 mL) was stirred for 1 h, cooled to –40°C and NIS (54 mg, 0.23 mmol) and TfOH (4 μL) were added. After 1 h, the reaction was quenched with Et_3N. The mixture was diluted with EtOAc (75 mL), filtered, washed with a 10% aqueous solution of $Na_2S_2O_3$, dried and evaporated. Disaccharide **57** was obtained by chromatography on silica gel using 8% EtOAc – hexane as eluent (55 mg, 71%), $[\alpha]_D$ –3.4 (*c* 0.6, $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ: 8.07 (m, 2H, Bz), 7.53 (m, 1H, Bz), 7.42 (m, 2H, Bz), 7.24–7.38 (m, 5H, Bn), 4.90 (m, 1H, H-2′), 4.77 (d, 1H, *J* = 7.9 Hz, H-1′), 4.63 (d, 1H, *J* = 11.5 Hz, Bn), 4.45 (d, 1H, *J* = 11.5 Hz, Bn), 4.10 (d, 1H, *J* = 7.9 Hz, H-1), 3.73–3.83 (m, 2H, H-6a, H-4), 3.66 (dd, 1H, *J* = 5.3, 9.4 Hz, H-6b), 3.45–3.63 (m, 8H, 2 × OMe, H-5′, H-2), 3.35–3.45 (m, 2H, H-3, H-5), 3.26 (m, 1H, H-4′), 2.80 (d′t′, 1H, *J* = 4.8, 12.0 Hz,

H-3e′), 1.60 (d′t′, 1H, *J* = 11.5, 11.5 Hz, H-3a′), 1.30 (d, 3H, *J* = 6.1 Hz, H-6′), 0.88 (s, 9H, *t*-BuSi), 0.06 (s, 6H, Me_2Si). Anal. calcd. for $C_{34}H_{49}O_9N_3Si$: C 60.78, H 7.35, N 6.25; found: C 60.57, H 7.17, N 5.83.

Methyl 2-azido-3-*O*-(4-*O*-benzyl-3,6-dideoxy-β-D-ribo-hexopyranosyl)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-methyl-β-D-galactopyranoside (58)

A solution of **57** (50 mg, 0.0744 mmol) in anhyd MeOH (5 mL) at 0°C was de-acylated by addition of a solution of NaOMe in MeOH (1.5 M, 200 μL). After stirring for 5 h at 0°C, the mixture was neutralized with Amberlite IR-120 (H^+) resin and evaporated. The residue was chromatographed on silica gel using 15% EtOAc – hexane as eluent to give **58** (41.5 mg, 98% yield), $[\alpha]_D$ –11.6 (*c* 0.7, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.25–7.36 (m, 5H, Bn), 4.63 (d, 1H, *J* = 11.6 Hz, Bn), 4.45 (d, 1H, *J* = 11.6 Hz, Bn), 4.35 (d, 1H, *J* = 7.5 Hz, H-1′), 4.11 (d, 1H, *J* = 7.9 Hz, H-1), 3.74–3.82 (m, 2H, H-6a, H-4), 3.65–3.73 (m, 2H, H-3, H-6b), 3.54 (s, 3H, OMe), 3.36–3.53 (m, 7H, OMe, H-2′, H-5′, H-2, H-5), 3.13 (m, 1H, H-4′), 2.53 (d′t′, 1H, *J* = 4.7, 12.2 Hz, H-3e′), 1.47 (d′t′, 1H, *J* = 11.8, 11.8 Hz, H-3a′), 1.33 (d, 3H, *J* = 6.1 Hz, H-6′), 0.87 (s, 9H, *t*-BuSi), 0.05 (s, 6H, Me_2Si). Anal. calcd. for $C_{27}H_{45}O_8N_3Si$: C 57.12, H 7.99, N 7.40; found: C 57.00, H 7.83, N 7.45.

Methyl 2-acetamido-3-*O*-(4-*O*-benzyl-3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-methyl-β-D-galactopyranoside (60)

Alcohol **58** (55 mg, 0.097 mmol) was oxidized using DMSO (2 mL) and Ac_2O (1 mL) as described above. The intermediate ketone **59** was reduced using a solution of L-selectride in THF (1.0 M, 200 μL) and acetylated using Ac_2O as described above to give the disaccharide **60** (43 mg, 76% yield), $[\alpha]_D$ +4.0 (*c* 0.6, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.22–7.36 (m, 5H, Bn), 6.05 (d, 1H, *J* = 6.9 Hz, NH), 4.85 (d, 1H, *J* = 8.2 Hz, H-1), 4.54–4.66 (m, 3H, H-3, H-1′, Bn), 4.43 (d, 1H, *J* = 11.5 Hz, Bn), 3.91 (br d, 1H, *J* = 2.7 Hz, H-4), 3.87 (m, 1H, H-2′), 3.62–3.80 (m, 2H, H-6a, H-6b), 3.37–3.60 (m, 9H, 2 × OMe, H-5, H-4′, H-5′), 3.25 (m, 1H, H-2), 2.85 (br s, 1H, OH-2′), 2.41 (d′t′, 1H, *J* = 3.1, 13.6 Hz, H-3e′), 1.49 (m, 1H, H-3a′), 1.28 (d, 3H, *J* = 5.2 Hz, H-6′), 0.88 (s, 9H, *t*-BuSi), 0.05 (s, 6H, Me_2Si). Anal. calcd. for $C_{29}H_{49}O_9NSi$: C 59.66, H 8.46, N 2.40; found: C 59.96, H 8.78, N 2.27.

Methyl 2-acetamido-3-*O*-(3,6-dideoxy-β-D-arabino-hexopyranosyl)-2-deoxy-4-*O*-methyl-β-D-galactopyranoside (8)

Compound **60** (35 mg, 0.06 mmol) was hydrogenated in a similar fashion as compound **5**. After evaporation, the residue was dissolved in anhyd THF (2 mL), a solution of TBAF in THF (1.0 M, 50 μL) was added and reaction was continued for 5 h. After concentration, disaccharide **8** was obtained by reverse-phase HPLC using a gradient of H_2O–MeOH (0 → 30%) as eluent (20 mg, 88% yield), $[\alpha]_D$ –47.6 (*c* 0.2, MeOH). ^1H NMR (D_2O) δ: 4.62 (d, 1H, *J* = 0.6 Hz, H-1′), 4.41 (d, 1H, *J* = 8.6 Hz, H-1), 3.97 (dd, 1H, *J* = 8.4, 10.8 Hz, H-2), 3.87 (dd, 1H, *J* = 3.1, 11.0 Hz, H-3), 3.86 (m, 2H, H-2′, H-3), 3.82 ("d", 1H, J = 3.1 Hz, H-4), 3.77–3.81 (m, 2H, H-6a, H-6b), 3.67 (m, 1H, H-5), 3.55 (m, 1H, H-4′), 3.50 (s, 3H, OMe), 3.42 (dq, 1H, *J* = 6.2, 9.2 Hz, H-5′), 2.17 (ddd, 1H, *J* = 3.5, 4.8, 13.9 Hz, H-3e′), 2.02 (s, 3H, Ac), 1.65 (ddd, 1H, *J* = 3.1, 11.7, 14.3 Hz, H-3a′), 1.29 (d, 3H, *J* = 6.2 Hz, H-6′). HR-ES-MS *m*/*e* calcd. for $C_{16}H_{29}O_9N$ (MH^+): 402.17400; found: 402.174200.

Methyl 2-acetamido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (61)

Benzoyl chloride (646 μL, 5.57 mmol) was added dropwise to an ice-cold solution of alcohol **17** (45) (1.5 g, 4.6 mmol) in a mixture of anhyd CH_2Cl_2 (10 mL) and pyridine (8 mL), and the mixture was stirred at room temperature for 5 h. Methanol (1 mL) was added, the mixture was concentrated and dissolved in CH_2Cl_2 (100 mL), washed with 5% $NaHCO_3$ (2 × 50 mL), saturated brine (1 × 50 mL), dried and evaporated. The benzoylated acetal **61** was obtained by chromatography on silica gel using 5% MeOH–CH_2Cl_2 as eluent (1.90 g, 96%), $[\alpha]_D$ –99.2 (*c* 0.8, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 8.03 (m, 2H, Bz), 7.56 (m, 1H, Bz), 7.43 (m, 2H, Bz), 7.40 (m, 2H, Ph), 7.26–7.32 (m, 3H, Ph), 6.05 (d, 1H, *J* = 9.5 Hz, NH), 5.58 (t, 1H, *J* = 9.8 Hz, H-3), 5.53 (s, 1H, PhCH), 4.47 (d, 1H, *J* = 8.4 Hz, H-1), 4.37 (dd, 1H, *J* = 5.0, 10.5 Hz, H-6a), 4.32 (d′t′, 1H, *J* = 10.0 Hz, 10.0 Hz, H-2), 3.86 (t, 1H, *J* = 9.4 Hz, H-4), 3.83 (t, 1H, *J* = 10.3 Hz, H-6b), 3.67 (m, 1H, H-5), 3.42 (s, 3H, OMe), 1.89 (s, 3H, Ac). Anal. calcd. for $C_{23}H_{25}O_7N$: C 64.63, H 5.90, N 3.28; found: C 64.56, H 5.88, N 3.21.

Methyl 2-acetamido-3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-β-D-glucopyranoside (62)

To a mixture containing compound **61** (1.0 g, 2.34 mmol), $NaBH_3CN$ (1.8 g, 28 mmol), and 4 Å molecular sieves (1.5 g) in anhyd THF (20 mL), was added a trace amount of methyl orange (~1 mg). A saturated solution of HCl in anhydrous ether (30 mL) was added dropwise until the color of the reaction became slightly pink. The reaction was continued for 2 h, the mixture was diluted with EtOAc (50 mL), and filtered. After concentration, the residue was dissolved in EtOAc (120 mL), and the solution was washed with 2 M HCl (2 × 50 mL), 10% $NaHCO_3$ aqueous solution (2 × 50 mL), dried, and evaporated. The alcohol **62** was obtained by chromatography on silica gel using 7.5% MeOH – CH_2Cl_2 as eluent (950 mg, 95%), $[\alpha]_D$ –13.2 (*c* 0.3, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 8.03 (m, 2H, Bz), 7.57 (m, 1H, Bz), 7.44 (m, 2H, Bz), 7.40 (m, 2H, Ph), 7.25–7.36 (m, 5H, Ph), 5.56 (d, 1H, *J* = 9.2 Hz, NH), 5.27 (dd, 1H, *J* = 9.0, 10.7 Hz, H-3), 4.64 (d, 1H, *J* = 11.9 Hz, Bn), 4.58 (d, 1H, *J* = 11.9 Hz, Bn), 4.48 (d, 1H, *J* = 8.3 Hz, H-1), 4.11 (d′t′, 1H, *J* = 8.8, 10.7 Hz, H-2), 3.90 (t, 1H, *J* = 9.3 Hz, H-4), 3.86 (dd, 1H, *J* = 4.9, 10.3 Hz, H-6a), 3.83 (dd, 1H, *J* = 4.8, 10.3 Hz, H-6b), 3.62 (m, 1H, H-5), 3.50 (s, 3H, OMe), 1.86 (s, 3H, Ac). Anal. calcd. for $C_{23}H_{27}O_7N$: C 64.32, H 6.34, N 3.26; found: C 64.16, H 6.12, N 3.01.

Methyl 2-acetamido-3-*O*-benzoyl-6-*O*-benzyl-2-deoxy-4-imidazoylthiocarbonyl-β-D-glucopyranoside (63)

1,1′-Thiocarbonyldiimidazole (538 mg, 2.72 mmol) was added to a solution of compound **62** (585 mg, 1.36 mmol) in anhydrous toluene (10 mL) and the mixture was heated to 90°C overnight. The solution was diluted with EtOAc (75 mL), washed with brine (1 × 25 mL), dried and concentrated to dryness to give **63** (691 mg, 94%), $[\alpha]_D$ –21.0 (*c*

0.8, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 8.13 (s, 1H, imidazole), 7.90 (m, 2H, Bz), 7.62 (s, 1H, imidazole), 7.51 (m, 1H, Bz), 7.17–7.40 (m, 7H, Ar), 7.02 (s, 1H, imidazole), 6.08 (t, 1H, *J* = 9.5 Hz, H-4), 5.86 (d, 1H, *J* = 8.8 Hz, NH), 5.78 (t, 1H, *J* = 10.5 Hz, H-3), 4.76 (d, 1H, *J* = 8.4 Hz, H-1), 4.46 (s, 2H, Bn), 4.08 (m, 1H, H-2), 3.96 (m, 1H, H-5), 3.71 (dd, 1H, *J* = 3.8, 10.6 Hz, H-6a), 3.65 (dd, 1H, *J* = 5.0, 10,8 Hz, H-6b), 3.54 (s, 3H, OMe), 1.86 (s, 3H, Ac), Anal. calcd. for $C_{27}H_{29}O_7N_3S$: C 60.10, H 5.42, N 7.79; found: C 60.21, H 5.33, N 7.72.

Methyl 2-acetamido-3-*O*-benzoyl-6-*O*-benzyl-2,4-dideoxy-β-D-xylo-hexopyranoside (64)

A solution of **63** (321.4 mg, 0.60 mmol) in anhydrous toluene (15 mL) was heated to reflux, a solution of tributyltin hydride (535 μL, 1.93 mmol) in anhydrous toluene (5 mL) and a catalytic amount of AIBN (~10 mg) were added. After 4 h, the deoxygenation was complete. Compound **64** was obtained by chromatography on silica gel using 3% MeOH – CH_2Cl_2 as eluent (217 mg, 88% yield), $[\alpha]_D$ –8.2 (*c* 0.7, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.99 (m, 2H, Bz), 7.54 (m 1H, Bz), 7.41 (m, 2H, Bz), 7.24–7.35 (m, 5H, Bn), 5.42 (br, 1H, NH), 4.57 (s, 2H, Bn), 4.43 (d, 1H *J* = 8.4 Hz, H-1), 4.02 (d′t′, 1H, *J* = 8.9, 10.3 Hz, H-2), 3.80 (m, 1H, H-5), 3.63 (dd, 1H, *J* = 5.7, 10.2 Hz, H-6a), 3.54 (dd, 1H, *J* = 4.6, 10.2 Hz, H-6b), 3.50 (s, 3H, Ome), 2.22 (ddd, 1H, *J* = 1.8, 5.1, 12.6 Hz, H-4e), 1.87 (s, 3H, Ac), 1.72 (d′t′, 1H, *J* = 11.5, 11.5 Hz, H-3a). Anal. calcd. for $C_{23}H_{27}O_6N$: C 66.81, H 6.58, N 3.39; found: C 66.59, H 6.41, N 3.02.

Methyl 2-acetamido-6-*O*-benzyl-2,4-dideoxy-β-D-xylo-hexopyranoside (65)

A solution of NaOMe in MeOH (1.5 M, 200 μL) was added to a solution of compound **64** (400 mg, 0.967 mmol) in anhyd MeOH (50 mL), and the reaction was stirred at room temperature for 2 h. After neutralization with Amberlite IR-120 (H^+), the organic solution was evaporated, and the acceptor **65** was obtained by chromatography on silica gel using 5% MeOH – CH_2Cl_2 as eluent (280 mg, 94% yield), $[\alpha]_D$ –63.3 (*c* 0.5, $CHCl_3$). ^1H NMR ($CDCl_3$) δ: 7.23–7.34 (m, 5H, Bn), 6.08 (d, 1H, *J* = 4.6 Hz, NH), 4.57 (d, 1H, *J* = 12.1 Hz, Bn), 4.52 (d, 1H, *J* = 12.1 Hz, Bn), 4.21 (d, 1H, *J* = 8.1 Hz, H-1), 3.77 (m, 1H, H-3), 3.65 (m, 1H, H-5), 3.58 (dd, 1H, *J* = 6.0, 10.3 Hz, H-6a), 3.51 (dd, 1H, *J* = 4.3, 10.2 Hz, H-6b), 3.48 (s, 3H, OMe), 3.30 (m, 1H, H-2), 2.05 (m, 1H, H-4e), 2.02 (s, 3H, Ac), 1.46 (d′t′, 1H, *J* = 11.6, 11.6 Hz, H-4a). Anal. calcd. for $C_{16}H_{23}O_5N$: C 62.12, H 7.49, N 4.53; found: C 61.87, H 7.37, N 4.29.

Methyl 2-acetamido-3-*O*-(2-*O*-benzoyl-4-*O*-benzyl-3,6-dideoxy-β-D-ribo-hexopyranosyl)-6-*O*-benzyl-2,4-dideoxy-β-D-xylo-hexopyranoside (66)

N-Iodosuccinimide (125 mg, 0.52 mmol) was added to a mixture of donor **22** (230 mg, 0.52 mmol), acceptor **65** (82 mg, 0.26 mmol), and 4 Å molecular sieves (350 mg) in anhyd CH_2Cl_2 (5 mL) The reaction was cooled to –60°C, and TfOH (5.0 μL) was added. After 3 h, the reaction was neutralized with Et_3N (0.5 mL), diluted with EtOAc (75 mL), and filtered. The organic solution was washed with a 10% aqueous solution of $Na_2S_2O_3$ (1 × 30 mL), and the mixture was purified by repeated chromatography on silica gel using 5% MeOH – CH_2Cl_2 to afford the disaccharide **66** (91 mg, 54% yield) in pure form, $[\alpha]_D$ +38.2 (*c* 0.5, $CHCl_3$). ^1H NMR (CDCl3) δ: 8.10 (m, 2H, Bz), 7.57 (m, 1H, Bz), 7.44 (m, 2H, Bz), 7.25–7.33 (m, 10H, Bn), 5.41 (d, 1H, *J* = 6.7 Hz, NH), 4.96 (m, 1H, H-2′), 4.90 (d, 1H, *J* = 8.2 Hz, H-1), 4.64 (d, 1H, *J* = 7.6 Hz, H-1′), 4.61 (d, 1H, *J* = 11.5 Hz, Bn), 4.57 (d, 1H, *J* = 12.1 Hz, Bn), 4.53 (d, 1H, *J* = 12.1 Hz, Bn), 4.46 (m, 1H, H-3), 4.45 (d, 1H, *J* = 11.7 Hz, Bn), 3.72 (m, 1H, H-5), 3.56 (dd, 1H, *J* = 5.9, 10.1 Hz, H-6a), 3.45–3.55 (m, 2H, H-5′, H-6b), 3.43 (s, 3H, OMe), 3.24 (m, 1H, H-4′), 2.76 (m, 1H, H-2), 2.65 (d′t′, 1H, *J* = 4.9, 12.0 Hz, H-3e′), 2.24 (m, 1H, H-4e), 1.62 (d′t′, 1H, *J* = 11.7, 11.7 Hz, H-3a′), 1.56 (s, 3H, Ac), 1.50 (d′t′, 1H, *J* = 11.6, 11.6 Hz, H-4a), 1.30 (d, 3H, *J* = 6.1 Hz, H-6′). HR-ES-MS *m*/*e* calcd. for $C_{36}H_{43}O_9N$ (MNa^+): 656.28355; found: 656.283345.

Methyl 2-acetamido-3-*O*-(4-*O*-benzyl-3,6-dideoxy-β-D-ribo-hexopyranosyl)-6-*O*-benzyl-2,4-dideoxy-β-D-xylo-hexopyranoside (67)

Disaccharide **66** (70 mg, 0.11 mmol) was dissolved in anhyd MeOH (15 mL), and a solution of NaOMe in MeOH (1.5 M, 200 μL) was added, the reaction was stirred at room temperature overnight. After neutralization with Amberlite IR-120 (H^+), the mixture was concentrated to dryness. The alcohol **67** was obtained (53 mg, 91% yield) by chromatography on silica gel using 7.5% MeOH – CH_2Cl_2 as eluent, $[\alpha]_D$ +3.3 (*c* 0.6, $CHCl_3$). ^1H NMR (CDCl3) δ: 7.22–7.34 (m, 10H, Bn), 5.58 (d, 1H, *J* = 7.9 Hz, NH), 4.57 (d, 1H, *J* = 11.3 Hz, Bn), 4.56 (d, 1H, *J* = 12.1 Hz, Bn), 4.53 (d, 1H, *J* = 12.1 Hz, Bn), 4.43 (d, 1H, *J* = 9.3 Hz, H-1), 4.41 (d, 1H, *J* = 11.8 Hz, Bn), 4.21 (d, 1H, *J* = 7.5 Hz, H-1′), 3.88 (m, 1H, H-3), 3.68 (m, 1H, H-5), 3.59 (dd, 1H, *J* = 6.0 Hz, 10.1 Hz, H-6a), 3.45–3.55 (m, 5H, H-2, H-6b, OMe), 3.34–3.42 (m, 2H, H-2′, H-5′), 3.10 (m, 1H, H-4′), 2.46 (d′t′, 1H, *J* = 4.6, 12.2 Hz, H-3e′), 2.19 (ddd, 1H, *J* = 1.8, 5.2, 13.4 Hz, H-4e), 1.99 (s, 3H, Ac), 1.57 (d′t′, 1H, *J* = 11.5, 11.4 Hz, H-4a), 1.38 (d′t′, 1H, *J* = 11.6, 11.6 Hz, H-3a′), 1.25 (d, 3H, *J* = 6.1 Hz, H-6′). HR-ES-MS *m*/*e* calcd. for $C_{29}H_{39}O_8N$ (MNa^+): 552.25734; found: 552.257502.

Methyl 2-acetamido-3-*O*-(3,6-dideoxy-β-D-*arabino*-hexopyranosyl)-2,4-dideoxy-β-D-xylo-hexopyranoside (7)

A solution of alcohol **67** (25 mg, 0.047 mmol) in anhyd DMSO (2 mL) and Ac_2O (1 mL) was stirred at 0°C for 30 min, and the reaction was left at room temperature overnight. After evaporation, the residue was partitioned in a mixture of EtOAc – 10% aqueous NaCl solution, and the organic solution was evaporated and dissolved in anhyd THF (3 mL) at 0°C. A solution of L-selectride in THF (1.0 M, 200 μL, 0.20 mmol) was added and the reaction was left at 0°C for 1 h. Water (0.3 mL) was added and the mixture was diluted with EtOAc (50 mL), washed with $Na_2S_2O_3$, brine, dried and concentrated to give crude **69**. Liquid ammonia (25 mL) was collected in a flask containing compound **69** (15 mg, 0.028 mmol) equipped with a dry ice condenser. Sodium (50 mg, 0.97 mmol) was added to this solution, and reaction was continued for 4 h. Methanol (0.5 mL) was added, and the condenser was removed to allow ammonia to evaporate. The residue was dissolved in H_2O (3 mL), and neutralized with a saturated aqueous solution of NH_4Cl and

evaporated. The desired disaccharide **7** was obtained by reverse-phase HPLC using a gradient of H_2O–MeOH (0 → 30%) as eluent (4.0 mg, 23%), $[\alpha]_D$ –55.5 (*c* 0.4, MeOH). ^1H NMR (D_2O) δ: 4.67 ("s", 1H, H-1′), 4.40 (d, 1H, *J* = 8.6 Hz, H-1), 3.89 (ddd, 1H, *J* = 5.1, 10.6, 10.6 Hz, H-3), 3.84 (m, 1H, H-2′), 3.63–3.72 (m, 3H, H-5, H-6a, H-6b), 3.62 (dd, 1H, *J* = 8.6, 10.1 Hz, H-2), 3.56 (ddd, 1H, *J* = 4.6, 9.3, 11.4 Hz, H-4′), 3.51 (s, 3H, OMe), 3.43 (dq, 1H, *J* = 6.1, 9.3 Hz, H-5′), 2.13–2.18 (m, 2H, H-3e′, H-4e), 2.02 (s, 3H, Ac), 1.64 (ddd, 1H, *J* = 3.1, 11.5, 14.3 Hz, H-3a′), 1.50 (d′t′, 1H, *J* = 11.5, 11.5 Hz, H-4a), 1.27 (d, 3H, *J* = 6.2 Hz, H-6′). HR-ES-MS *m/e* calcd. for $C_{15}H_{27}O_8N$ (MNa^+): 372.16344; found: 372.163817.

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