Constrained H-Type 2 Blood Group Trisaccharide Synthesized in a Bioactive Conformation via Intramolecular Glycosylation

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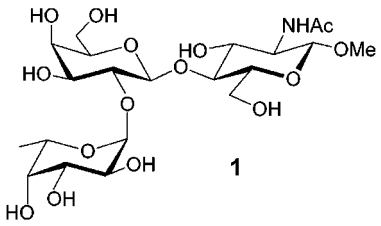
The methyl glycoside of the H-type 2 trisaccharide **1** was synthesized in a constrained, bioactive conformation via intramolecular aglycon delivery. Computer modeling of the crystal structure of the *Ulex europaeus* I lectin with a docked H-type 2 trisaccharide suggested that the disaccharide Gal*p*(1→4)Glc*p*NAc1→OCH_3 could be tethered in a bioactive conformation if Gal O-6 and GlcNAc O-3 are linked via a three-carbon tether. The ethyl 1-thiogalactopyranoside **13** was used to alkylate the methyl 2-acetamido-2-deoxy glucopyranoside **7**, and the resulting dimer was subjected to intramolecular glycosylation following protecting group manipulation. The tethered disaccharide **4** was glycosylated by the activated fucopyranosyl donor **3** to give the protected target molecule **17**. Solid-phase binding assays showed that the tethered trisaccharide **2** was 3-fold less active than native H-type 2 trisaccharide **1** when assayed against the *U. europaeus* I lectin, whereas it was 250 times less active when assayed with the *Psophocarpus tetragonolobus* II lectin. The observed activities are consistent with published models for H-trisaccharide interactions with *Ulex* and *Psophocarpus* lectins and provide further evidence that suggests reduction of oligosaccharide flexibility by intramolecular tethering provides no significant gain in binding energy.

Introduction

In the search for carbohydrate-based agonists,[1] the moderate free energy of sugar−protein interactions repeatedly confounds the discovery of high affinity carbohydrate ligands. In this study a constrained analogue of the human blood group trisaccharide **1** was synthesized by intramolecular glycosylation to yield the preordered trisaccharide **2**, which by reducing entropic losses might



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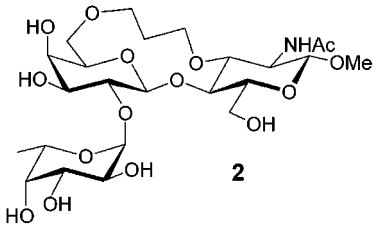

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be expected to show higher affinity. The H-type 2 epitope is a good model to probe the influence of oligosaccharide flexibility on the thermodynamics of oligosaccharide−lectin interactions, because the epitope is recognized by four different lectins, *Ulex europaeus* I,[2] *Psophocarpus tetragonolobus* II (winged bean lectin),[3] *Galactia tenuiflora*,[4] and *Erythrina corallodendron*.[5] Each lectin employs a distinct mode of binding and recognizes different topological features of the sugar epitope.[6,7]

The intrinsic affinity for oligosaccharide binding by proteins, such as antibodies and lectins, is generally characterized by association constants within the range 10^3-10^6 M^{-1}. For many lectin−oligosaccharide interactions, the modest affinity and free energy of interaction are characterized by a strong enthalpic contribution offset by unfavorable entropy.[8−13] It has been suggested that oligosaccharide conformational flexibility arising from motion about each interresidue glycosidic bond may account for most of the unfavorable entropy.[14] Some theoretical estimates place the entropic loss per immobilized rotamer as high as 0.6−2.0 kcal mol^{-1}.[14,15] Attempts to verify this hypothesis by synthesizing oli-

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gosaccharides that are constrained in a conformation preorganized for binding have failed to show substantially higher free energy of binding.[16−21] In two well studied cases,[17,18] the change in entropy was shown to be alternatively favorable[18] and unfavorable[17] but not decisive. Testing this hypothesis poses significant problems. The smaller of the two estimates for the energetic penalty associated with freezing rotamers seems to be the most reasonable figure and is based on freezing a bond that is free to equilibrate between three rotamers of equal energy. However, for the glycosidic bond, the *exo*-anomeric effect skews the rotameric population between two and not three rotamers. Consequently it seems unlikely that the entropy associated with this process would exceed 0.1−0.3 kcal mol^{-1} at 37 °C. Introduction of a tether reduces the range of allowed glycosidic torsional angles φ/ψ, but the tether itself is flexible as it forms part of a macrocyclic ring, which even for short tethers spans at least 10−13 bonds.[17,18] Recent work by Mammen et al.[22] suggests that previous estimates of torsional entropy are too high. Taken together these considerations predict that gains in the free energy of association from tethering oligosaccharides will be small.

The suggestion that glycosidic torsions are the major contributor to loss of conformational entropy on complexation remains an unproven hypothesis. An alternative interpretation of the thermodynamic data emphasizes that the *exo*-anomeric effect imposes conformational preferences on glycosidic linkages and stresses the importance of reordering solvent water about polyamphiphilic surfaces.[8,9]

Preorganization of an oligosaccharide epitope in its bound conformation poses several difficulties, especially in relation to the ability of the tether to constrain the oligosaccharide in a bioactive conformation. By selecting an oligosaccharide such as the human blood group H-type 2 epitope for such studies, it is possible to access a range of distinct lectin binding sites and increase the potential for identifying a recognition system in which entropic gains may be observed. Furthermore, the crystal structures of two lectins that bind H-antigen have been solved,[23,24] and with the exception of *E. corallodendron* lectin,[25] all of the lectin sites have been extensively mapped by epitope congeners,[6,26−29] thereby facilitating the design of tethered oligosaccharides.

U. europaeus I,[2] the primary lectin target of this synthetic study, binds to the H-type 2 human blood group determinant α-L-Fuc(1→2)-β-D-Gal(1→4)-β-D-GlcNAcOR.[6] The lectin is used clinically in the identification of blood group "O" individuals because it agglutinates human red blood cells that display the H-type 2 determinant as part of cell membrane glycolipids or glycoproteins. The crystal structure of the H-type 2 oligosaccharide-specific lectin from *U. europaeus* without bound saccharide has been solved.[23] Thermodynamic data from van't Hoff plots for the binding of H-type 2 by *Ulex* lectin show large favorable enthalpy opposed by large unfavorable entropy ($\Delta G° = -8$ kcal/mol, $\Delta H° = -29$ kcal/mol, $T\Delta S° = -21$ kcal/mol).[26] The size of the entropic penalty suggests that a conformational change may accompany the binding of the sugar.[14,30−34]

To study the magnitude and origin of the entropic penalty, we have synthesized a H-type 2 trisaccharide with a tether that bridges the Gal and GlcNAc monosaccharides. Although this tethered saccharide **2** has been designed primarily for binding with *U. europaeus* I lectin, the bioactivity of **1** and **2** are also determined with *P. tetragonolobus* II lectin (winged bean lectin),[3,35] another H-type 2-specific lectin. The activities will also eventually be measured for *G. tenuiflora*[4] and *E. corallodendron*,[5] lectins. In the search for affinity gains from preorganization of oligosaccharide epitopes, the H-type 2 epitope thus maximizes the probability for observing an improvement in $\Delta G°$ due to a decrease in the entropy penalty. The work has general and practical relevance to the search for high affinity carbohydrate-based agonists.

Results

The design of a tethered H-type 2 trisaccharide is based on a crystal structure of the *Ulex* lectin[23] and published studies of congener binding to three lectins, *U. europaeus* I, *G. tenuiflora*, and *P. tetragonobolus* II.[6,26−29] Although there is currently no reported structure for the complex with oligosaccharide, the *Ulex* lectin cocrystallizes with a molecule of 2-methyl-2,4-pentanediol, the precipitant used for protein crystallization, and this complex provides a reference point for saccharide docking. Inhibition data for a series of monodeoxy, monomethyl, and monodeoxyfluoro analogues of the H-type 2 trisaccharide have identified the functional groups essential for binding.[6,26−29] In the case of *U. europaeus* I, the O-2, O-3, and O-4 hydroxyl groups of fucose were essential. It was further concluded that these hydroxyls must be buried within the binding site of *Ulex*, while the hydroxyl group at the O-3 position of galactose is hydrogen bonded to the lectin at the periphery of the combining site. A pentose congener of H-type 2 in which the methyl group of fucose was replaced by hydrogen was also found to have a signifi-

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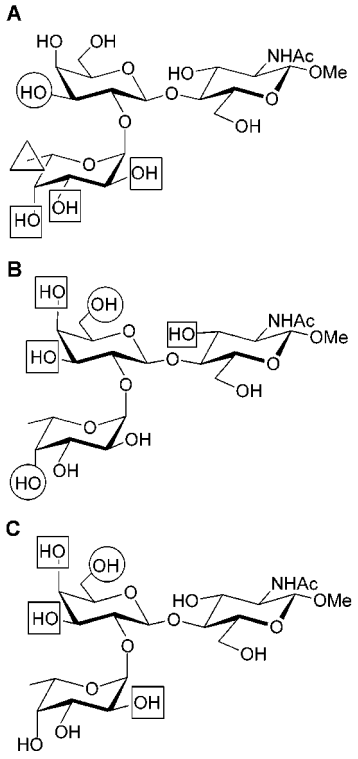

Figure 1. Conclusions of epitope mapping studies for H-type 2 trisaccharide with 3 lectins: (a) *Ulex europaeus* I, (b) *Psophocarpus tetragonolobus* II (winged bean lectin), and (c) *Galactia tenuiflora*. Three types of interactions are noted: (i) buried hydroxyl groups involved in hydrogen bonds deep within the binding site are identified within a square, (ii) essential hydrophobic interactions are identified by a triangle, and (iii) hydroxyl groups implicated in hydrogen bonds at the periphery of the binding site are identified by a circle.

cantly decreased activity, indicating that the methyl group fulfills an important hydrophobic interaction with the lectin.[26] The results of binding site mapping with trisaccharide congeners for the three H-type 2-specific lectins, *U. europaeus* I, *G. tenuiflora*, and *P. tetragonolobus* II are summarized (Figure 1).

Design of Constrained H-type 2 Derivatives. To design tethered derivatives of H-type 2 trisaccharide preorganized in its bound conformation, the following criteria had to be fulfilled:

- The tether should not be attached to key polar groups that are involved in protein−carbohydrate interactions.
- The conformation of the bioactive epitope should be well approximated by tethering.
- When bound, the tether should not interfere sterically with the surface of the protein.

To ensure that the tether met the first requirement, reference was made to the epitope mapping studies[6,26−29] summarized in Figure 1.

Because the principle lectin of interest is the *Ulex* lectin, the binding topography of the H-type 2 epitope indicated that the O-3 position of *N*-acetylglucosamine and the O-6 position of galactose would be suitable sites for tethering; neither site was involved in obligatory sugar−protein interactions and both occupied solvent exposed position in the complex (compound **2**). Tethers at these positions could interfere with the protein surface of the winged bean lectin and very probably would cause problems for *Galactia*, which requires O-3 of GlcNAc for a buried hydrogen bond.[6]

Computer Modeling. The model of the tethered H-type 2 *Ulex* complex was developed in several stages. The coordinates for the *Ulex* structure with bound 2-methyl-2,4-pentanediol provided the starting point for positioning the ligand in the lectin site. Following an unpublished procedure of Lemieux (personal communication), coordinates for α-L-fucopyranose were used to dock fucose in a manner such that the O-3 and O-4 atoms of α-L-fucopyranose were overlaid on the two hydroxyl groups of the pentanediol. Because epitope mapping of the H-type 2 ligand established that O-3 and O-4 of fucose are essential to binding[6] and possess a synclinal relationship, this mode of docking seemed most consistent with the binding motifs of several published saccharide−lectin complexes.[12] Having docked the monosaccharide residue, the coordinates of this sugar provided a key feature onto which the more complex trisaccharide **1** and **2** could be overlaid.

The conformation of trisaccharide **1** was modeled using the GEGOP force field,[36] and the global minimum energy conformation was selected. Because most oligosaccharide−protein complexes reveal bound oligosaccharides in conformations close to their energy minima, the fucose residue of trisaccharide **1** in its global energy conformer was overlaid on the fucose residue that was docked in the pentanediol binding site. This gave a model of trisaccharide **1** docked with lectin (Figure 2). The approach was subsequently justified as transferred NOE studies (Milton and Bundle, unpublished results) are consistent with a bound H trisaccharide epitope in this low energy conformation.

Trisaccharide **1** in its low energy conformation was investigated for tethering. Molecular modeling revealed that a three-carbon linker between Gal O-6 and GlcNAc O-3 would create a tethered H-type structure **2**, which is effectively constrained to a narrow range of conformers that closely approximate the lowest energy forms of **1**. However, the tethered structure retains sufficient flexibility to adopt several low energy conformations that are similar to those of bound trisaccharide **1**. To confirm that the bound form of the tethered H-trisaccharide **2** could adopt realistic conformations while avoiding tether−protein contacts, energy minimizations for **2** were performed with the CVFF force field of the *Discover* software and then the minimized structure was docked to give a lectin−trisaccharide **2** complex. The docked complex was created by overlaying **2** on the bound form of the H-type 2 trisaccharide **1**.

The rationale for assuming that the pentanediol occupied the saccharide binding site of the *Ulex* lectin is based on several factors. Several crystal structures of legume lectins and their substrates show large sequence homologies including certain invariant amino acids that participate in binding site hydrogen bonding and hydrophobic interactions and others that are involved in the coordination of the calcium and manganese ions.[8,9,12,37] From the crystal structure of the *Ulex* lectin, it is found that the amino acids usually involved in coordinating the metal ions, asparagine and aspartic acid, are present and are in close vicinity to the ions.[23] The pentanediol binding site contains the amino acids asparagine, aspartic acid,

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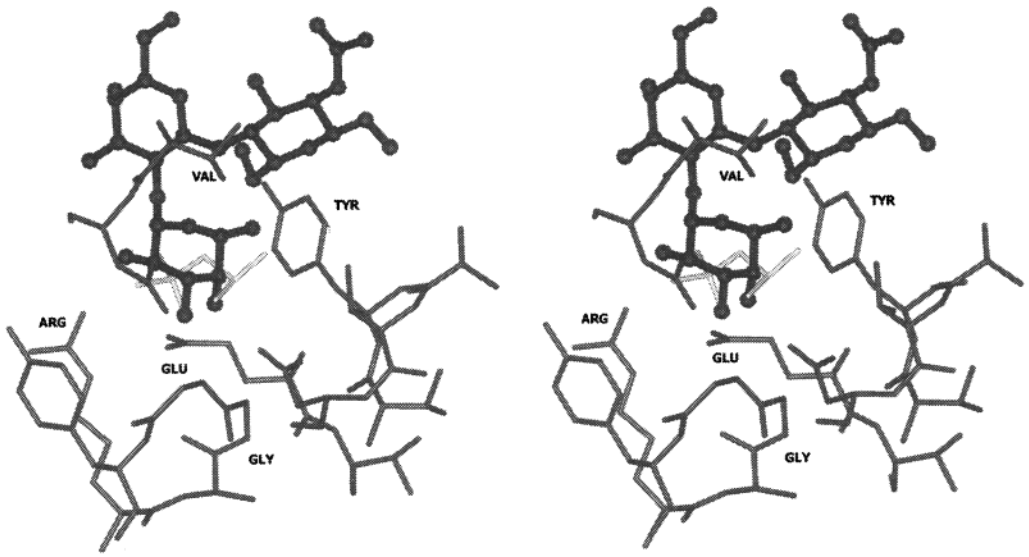

Figure 2. Computer modeling: stereo plot of the binding site amino acids (mid gray lines) of the *Ulex* lectin cocrystallized with a molecule of (*R*)-2,4-dihydroxy-2-methylpentane (light gray lines) (Delbaere et al., unpublished data). The 3- and 4-hydroxyl groups of the fucose residue of H-type 2 trisaccharide **1** (black lines) are superimposed over those of the dihydroxymethylpentane.

and tyrosine, those often involved in buried carbohydrate–lectin hydrogen bonding and hydrophobic interactions. In this context, *U. europaeus* I fits the general binding site motif of legume lectins.[12,37] In comparing the X-ray structure of *Ulex* with that of other legume lectins, the location of the 5-, 6-, and 7-stranded β-sheets and the metal ions are superimposeable. In addition, the binding site of 2-methyl-2,4-pentanediol is found to overlap with the binding site of the carbohydrate substrates.

Synthesis. Strategy. Thioglycosides are "versatile" glycosyl donors because they may be activated in various ways or readily converted into other glycosyl donors.[38] They also allow a number of protective group manipulations. Therefore, a thioglycoside was the donor of choice for the glycosylation reactions. Retrosynthetic analysis (Figure 3) suggested tethering the ethyl thiogalactoside **5** and the 2-acetamido-2-deoxy-glycopyranoside **7** via a 1,3-propanediol. The order of assembly of these three components may vary. Glycosylation of the two sugars followed by tethering of the disaccharide may be envisioned. A second route to the tethered disaccharide **4** would involve initial tethering of the two sugars with the 1,3-propanediol linker, followed by an intramolecular glycosylation. The second method of assembly was the method of choice because it has been demonstrated that intramolecular glycosylations generally proceed in high yield,[18,39–42] whereas the tethering of preassembled oligosaccharides and formation of large rings are low yield processes.[17] Computer modeling showed that even without neighboring group participation a tethered structure would favor the delivery of the galactopyranosyl group leading to β-glycoside formation in **4**. This preference may be attributed to a combination of ring strain and a steric

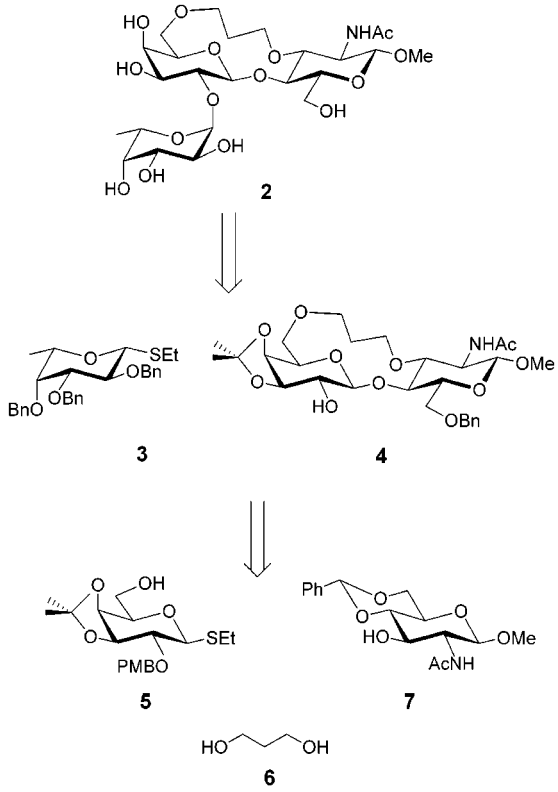

Figure 3. Retrosynthesis of the tethered H-type 2 trisaccharide **2**.

mismatch between 6-*O*-benzyl and 2′-*O*-*p*-methoxybenzyl groups. Other groups have reported that "prearranged" molecules favor the formation of one glycosidic form, depending on the relative configuration of the tethered donor and acceptor.[18,39]

A number of strategies were considered for tethering two monosaccharides prior to intramolecular glycosylation. Monosaccharide 3-hydroxypropyl ethers could be employed to displace sulfonate esters from the second monosaccharide residue. However, as depicted in routes 1 and 2 (Figure 4) such reactions would most likely lead to elimination products, because even primary sulfonates

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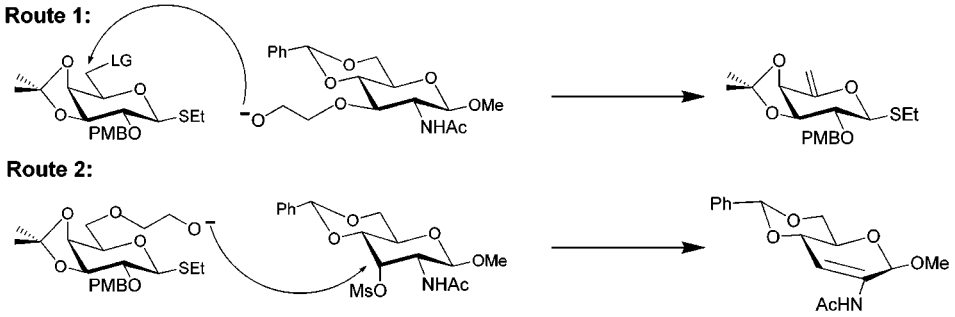


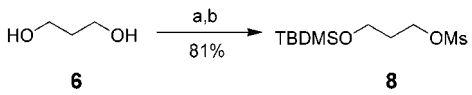
Figure 4. Potential problems in tethering the galactose and *N*-acetylglucosamine monosaccharides via displacement reactions.

of galactose are notoriously resistant to displacement reactions. At a secondary ring carbon, displacement is even more disfavored and complicated by inversion of configuration. Consequently, the tethered molecule **14** was approached by sequential substitution reactions using a monosaccharide alkoxide as the nucleophile, with the sulfonate leaving group attached to the tether moiety (Schemes 2 and 3).

Subsequent steps involved the selective deprotection of the O-2 position of galactose, followed by glycosylation with fucopyranosyl donor **3** and deprotection, would then lead to the desired tethered trisaccharide **2**.

Preparation of the Linker. The linker synthon **8** was prepared in a one-pot synthesis. Treatment of 1,3-propanediol with 1 equiv of sodium hydride in THF (Scheme 1) followed by addition of *tert*-butyldiphenylsilyl

Scheme 1[a]



[a] (a) NaH, THF, TBDMSCl; (b) Et_3N, MsCl.

chloride[43] gave the silyl ether, which was not isolated but reacted with methanesulfonyl chloride and triethylamine. This gave the desired linker **8** in 81% yield. Preparation of the linker in two distinct steps lead to a lower overall yield.

Preparation of the Galactose Donor. To synthesize a galactose donor, with two different groups at the O-2 and O-6 position, mixed acetal **10** was used (Scheme 2). This mixed acetal allowed the introduction of a *p*-methoxybenzyl group at the O-2 position of galactose; selective removal of the mixed acetal under mild conditions of hydrolysis then gave monosaccharide **5**. Reaction of the monosaccharide alkoxide generated from **5** with the linker **8** gave **11**, from which the TBDMS protecting group was removed with TBAF in THF. The resulting alcohol **12** was treated with triethylamine and methanesulfonyl chloride to give the desired galactose donor **13**.

Assembly of the Tethered Disaccharide. Tether coupling of the sugars was executed in dry THF. Once the alkoxide of **7** was formed and after the addition of methanesulfonate **13**, a few drops of DMSO were added, and the desired linked product **14** was obtained in 83% yield (Scheme 3). DMSO facilitates a faster reaction presumably by dissociation of the sodium alkoxide ion pair of **7**. Reductive opening of the benzylidene ring with sodium cyanoborohydride and hydrochloric acid in ether and THF gave compound **15**. A poor yield was obtained from the sodium cyanoborohydride reaction as a result of the acid sensitivity of the *p*-methoxybenzyl group and the ability of the acetonide to undergo reductive ring opening. Intermediate **15**, possessing both a donor site and an acceptor site, is set up to undergo an intramolecular glycosylation. Its structure, as well as that of the acetylated derivative, was verified by one-dimensional ^1H NMR, two-dimensional GCOSY, and HMQC experiments.

Two methods of activation were tried for the intramolecular glycosylation of **15**. The first method used NIS and silver triflate and gave a yield of 35%. However, this was accompanied by partial iodination of the *p*-methoxybenzyl group, a fatal side reaction because the iodinated *p*-methoxybenzyl group was found to be stable to DDQ oxidation. The second method of glycosylation using methyltriflate and 2,6-di-*tert*-butyl-4-methylpyridine (DTBMP) resulted in a higher yield of 64%, and a β/α ratio of 8:1 was observed.

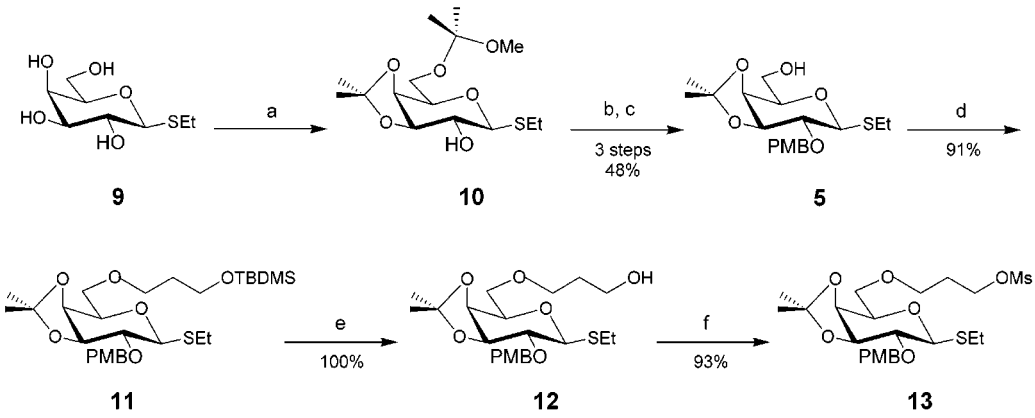
Synthesis and Deprotection of the Tethered Trisaccharide. Tethered disaccharide **16** was subjected to selective deprotection with DDQ to give **4** in 68% yield (Scheme 4), accompanied by partial loss of the isopropylidene acetal. The disaccharide acceptor **4** was glycosylated by the fucose thioglycoside **3**, which was activated by dimethyl(methylthio)sulfonium triflate (DMTST). This gave the tethered trisaccharide **17** in 55% yield. The protected tethered trisaccharide **17** was then deprotected by hydrolysis of the acetal, followed by hydrogenolysis of the benzyl groups to give the tethered H-type 2 trisaccharide **2** in 90% yield over two steps.

Biological Activity. The biological activity of the tethered trisaccharide **2** relative to that of the native trisaccharide **1** was determined by solid-phase immunoassay for the *U. europaeus* I and *P. tetragonolobus* II lectins. Affinity purified lectin adsorbed to microtiter plates was allowed to compete for biotin-labeled H-type 2 glycoconjugate in the presence and absence of the inhibitor **2**. In this assay format the affinity constant of an intermediate affinity ligand is well approximated by the inverse of the IC_{50} value.[44] The tethered trisaccharide **2** was a strong inhibitor of glycoconjugate binding to the *Ulex* lectin; however, the untethered H-type 2 ligand **1** exhibited binding that was 3 times higher (Figure 5a). In sharp contrast, trisaccharide **1** was 250 times more active than **2** when each was assayed for binding to *P. tetragonolobus* lectin (Figure 5b).

(43) McDougal, P. G.; Rico, J. G.; Oh, Y.-I.; Condon, B. D. *J. Org. Chem.* **1986**, *51*, 3388−3390.

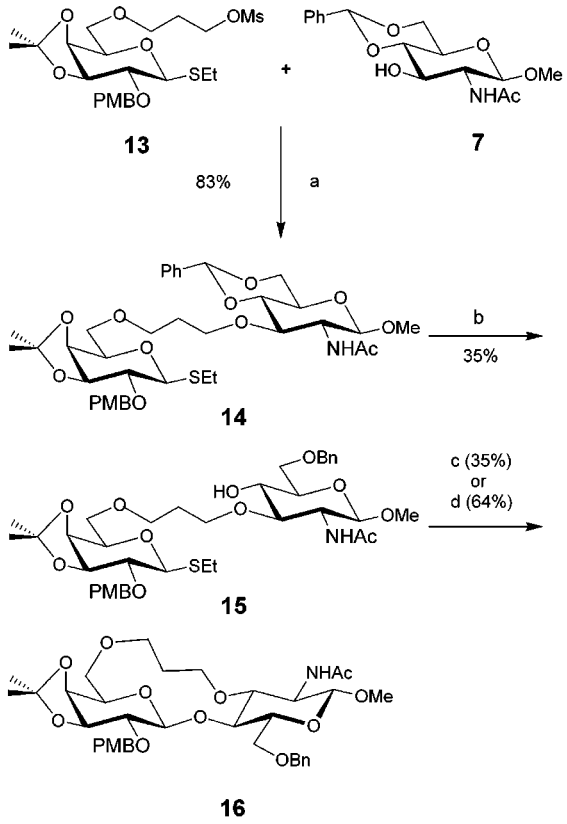
(44) Meikle, P. J.; Young, N. M.; Bundle, D. R. *J. Immunol. Methods* **1990**, *132*, 255−261.

Scheme 2[a]



[a] (a) $(CH_3)_2C(OCH_3)_2$, TsOH; (b) PMBCl, NaH, DMF; (c) H^+; (d) NaH, $TBDMSO(CH_2)_3OMs$, THF; (e) TBAF, THF; (f) MsCl, Et_3N.

Scheme 3[a]



[a] (a) NaH, THF, DMSO; (b) $NaCNBH_3$, HCl in ether, 3 Å MS, THF; (c) AgOTf, NIS, 4 Å MS, CH_2Cl_2; (d) MeOTf, 4 Å MS, DTBMP, CH_2Cl_2.

Conclusion

The synthesis of a tethered H-type 2 trisaccharide **2** has been accomplished via an intramolecular glycosylation reaction that utilized the tether to control the stereoselectivity of galactose−glucosamine glycosidic bond formation.

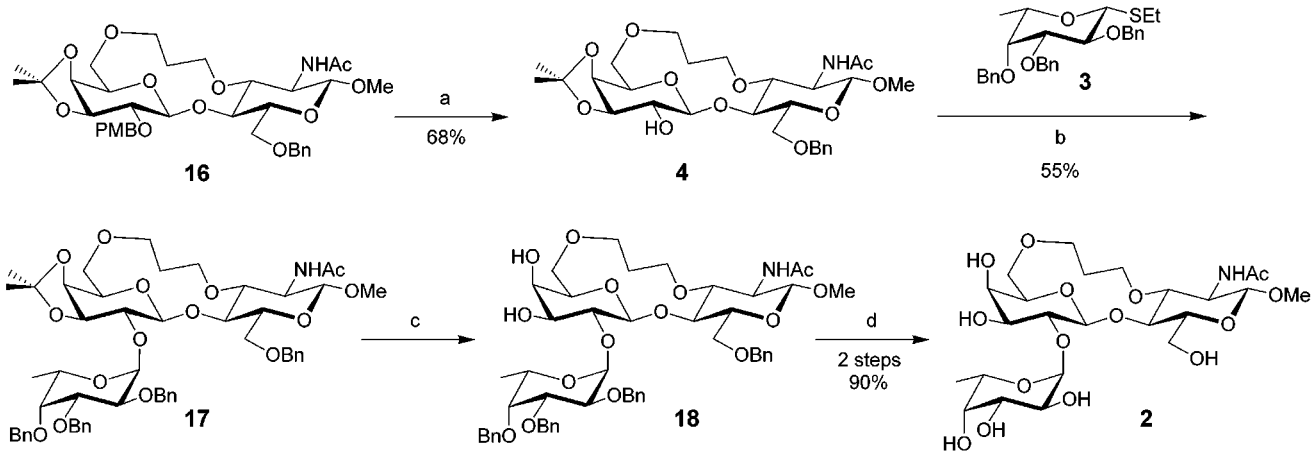
Ulex lectin is classified with the group of lectins that bind L-fucose; however, it binds the H-type 2 trisaccharide 900-fold tighter than L-fucose,[12] which indicates a protein−oligosaccharide contact surface that extends beyond the fucose residue. Epitope mapping by Lemieux et al. concluded that fucose and portions of the galactose residue make the most important polar contacts with *Ulex* protein. Binding of the tethered target molecule **2** to *Ulex* lectin showed decreased affinity, $\Delta(\Delta G°) = +0.6$ kcal mol^{-1} relative to H-type 2 trisaccharide **1**. The preservation of the binding energy suggests tethering has provided a bioactive conformation for **2** and is consistent with the molecular modeling and the topology of the recognition surface proposed by Lemieux.[6] In these studies the possible involvement of the O-6′ hydroxyl group in hydrogen bonding to solvent water at the periphery of the *Ulex* binding site was proposed, and the small loss of binding energy for the tethered trisaccharide **2** most likely arises from the functional group alterations at this center. The strong inhibitory power of **2** also provides support for the computer generated model for *Ulex* lectin complexed with the H-type 2 trisaccharide (Lemieux personal communication, Figure 2).

The dramatic reduction of inhibitory power for **2** with the *P. tetragonolobus* lectin ($\Delta(\Delta G°) = +3.3$ kcal mol^{-1}) points to substantial destabilization of the ligand−protein complex. Lemieux's model for the topology of the binding surface of **1** with the lectin proposed that O-6 of galactose is involved in hydrogen bonding at the periphery of the binding site.[6] Nevertheless, it should be noted that epitope mapping established that substitution of a 6-*O*-methyl galactose residue for galactose resulted in a $\Delta(\Delta G°) = +0.1$ kcal mol^{-1}. This would suggest that there are no immediate steric or electronic problems in alkylation of this hydroxyl group. Introduction of the tether may result in several changes that could account for the loss of binding energy. These would include changes in the rotamer distribution of the hydroxymethyl group, adverse affects on an extended network of hydrogen bonds that involve structured water molecules, or a simple steric clash between the tether and protein.

These data highlight the difficulty in designing a tether that can effectively constrain an oligosaccharide in a bioactive conformation while avoiding unfavorable protein contacts. Apparently, even modification of hydroxyl groups that are thought to lie at the periphery of the wing bean lectin binding site and which should be involved in relatively weak hydrogen bonds may cause sufficient unfavorable interactions to destabilize the complex by ca.+3 kcal mol^{-1}.

Interactions in the *Ulex* lectin site, which is the main focus of this study, are more promising. The solid-phase assays reported here show that the free energy of binding changes by only 0.6 kcal mol^{-1}. Published data from van't

Scheme 4[a]



[a] (a) DDQ, CH_2Cl_2, H_2O; (b) DMTST, 4 Å MS, DTBMP, CH_2Cl_2; (c) 90% AcOH 65 °C; (d) H_2, $Pd(OH)_2/C$, EtOH.

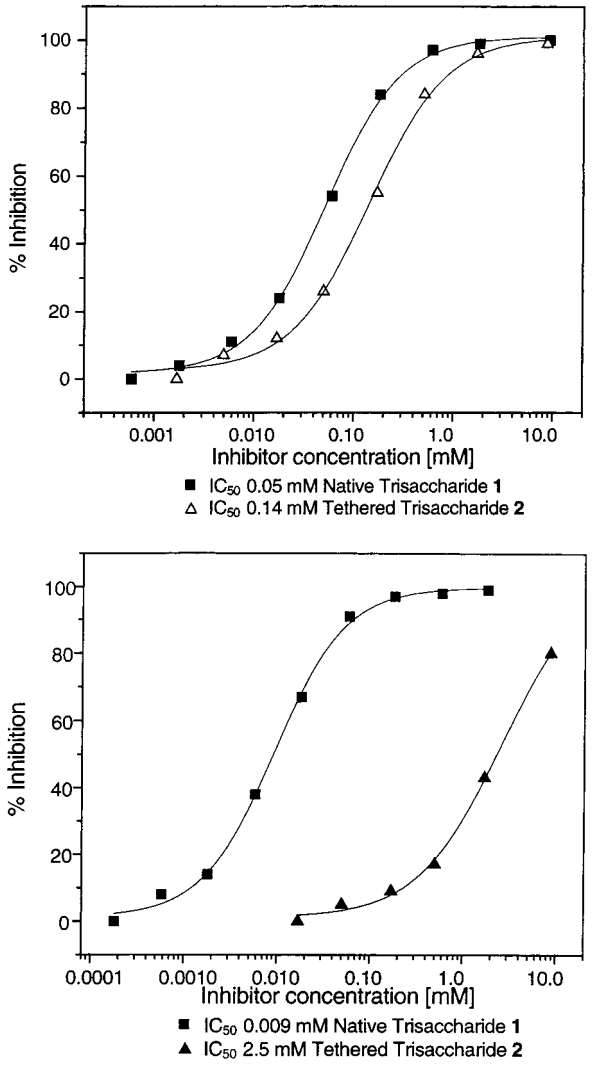


Figure 5. Biological activity of the tethered H-type 2 trisaccharide **2** relative to the native H-type 2 trisaccharide **1** assayed with the lectins (a) *Ulex europaeus* I and (b) *Psophocarpus tetragonolobus* II winged bean lectin.

Hoff plots[26] show a strong enthalpic contribution to binding ($\Delta H° = 29$ kcal mol^{-1}). Consequently titration calorimetry, which depends on a large enthalpic term for its sensitivity, should be able to provide useful data to determine the effect of tethering on the entropy of binding. These measurements combined with conformational studies of the solution conformation of the bound trisaccharide are the subject of further studies.

Experimental Section

All commercial reagents were used as supplied. Solvents used in reactions were dried according to standard methods.[45] Molecular sieves used in the experiments were flame-dried and then cooled under high vacuum immediately prior to use. TLC was performed on silica gel 60-F_{254} plates (E. Merck, Darmstadt) with detection by charring with 5% sulfuric acid in ethanol. Column chromatography was performed on silica gel 60 (E. Merck 40−60 μM, Darmstadt) with redistilled solvents.

Optical rotations were measured with a Perkin-Elmer 241 polarimeter at 22 °C. ^{1}H NMR spectra were recorded at 300, 500, or 600 MHz and are referenced to internal $CHCl_3$ (δ 7.24 ppm, $CDCl_3$) or to 0.1% external acetone (δ 2.225 ppm, D_2O). ^{13}C NMR spectra (HMQC or APT) were recorded at 75, 120, or 150 MHz and are referenced to internal $CHCl_3$ (δ 77.0 ppm, $CDCl_3$) or to 0.1% external acetone (δ 31.07 ppm, D_2O). Coupling constants are expressed in Hz. Assignments of resonances for the target compound **2** were made by two-dimensional homonuclear and heteronuclear shift correlation experiments (GCOSY and HMQC). Verification of the position of glycosidic linkages were made by homonuclear 2D offset compensated rotating-frame nuclear Overhauser effect (ROESY) experiments. Microanalyses were carried out by the analytical service at this department, and all samples submitted for elemental analyses were dried overnight under vacuum with phosphorus pentoxide or Drierite at 56 °C (refluxing acetone). Electrospray high-resolution mass spectra (ES HRMS) of all samples were recorded on a Micromass ZabSpec Hybrid Sector-TOF mass spectrometer.

Computational studies were performed using software from Biosym/MSI (San Diego). Minimization calculations for the tethered disaccharide **2** were done with the *Discover* program, using the CVFF force field, and graphical displays were printed out from the *Insight II* molecular modeling program.

Rigid body potential energy calculations were performed for the H-type 2 trisaccharide **1** using the CVFF force field, as well as the program GEGOP[36] that is based upon the HSEA force field.[46] Monosaccharide coordinates were generated from X-ray or neutron diffraction data. Potential energy maps (ϕ,ψ) were calculated for each glycosidic linkage at 5° intervals. For

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(46) Thøgersen, H.; Lemieux, R. U.; Bock, K.; Meyer, B. *Can. J. Chem.* **1982**, *60*, 44−57.

a given linkage, the map was obtained by choosing the minimum energy for each point (rigid body relaxed map).

Solid-phase immunoassay (EIA) were performed in duplicate as described below using a variant of previously described methods.[44,47] *U. europaeus* I lectin was purified by affinity chromatography on a fucose−agarose column (Sigma). Pure lectin was eluted by 1% fucose in PBS buffer. *Psophocarpus tetragonolobus* II lectin was purified on a lactose affinity column and eluted with 1% lactose in PBS buffer. The dialyzed lectin solutions were used to coat EIA plates. Biotin-labeled H-type 2 BSA glycoconjugate[48] was allowed to bind to solid-phase lectin in the presence and absence of inhibitors.

Affinity purified lectin dissolved in phosphate buffered saline (PBS) (1 μg/mL) was coated on 96-well ELISA plates overnight at 4°. The plate was washed five times with PBS containing Tween 20 (0.05% v/v), blocked with bovine serum albumin (BSA) (Sigma) 2.0% in PBS for 1 h, and then washed three times with PBS containing Tween 20 (PBST). A glycoconjugate consisting of H-type 2 trisaccharide conjugated to BSA and biotinylated (0.02−0.3 μg/mL) was mixed with inhibitor at concentrations in the range 0.1 nM to 10 mM. The duplicate mixtures were added to the coated microtiter plate and incubated at room temperature for 18 h. Then the plate was washed five times with PBST, and streptavidin horseradish peroxidase was added and incubated for 1 h at room temperature. This step was followed by washing five times with PBST. TMB horseradish peroxidase substrate was added, and after 2 min the color reaction was stopped with 1 M phosphoric acid. Absorbance was read at 450 nm, and percent inhibition was calculated using wells containing no inhibitor as the reference. The data were plotted with Origin software (Microcal Software, Northampton, MA).

1-*O*-*tert*-Butyldimethylsilyloxy-3-*O*-methanesulfonyloxy-propane (**8**). Diol **6** (3.06 g, 40.3 mmol) was added dropwise to a solution of NaH (0.967 g, 40.3 mmol) in dry THF (60 mL) purged with argon. After the mixture stirred for 1 h, TBDMSCl (6.06 g, 40.3 mmol) was added portionwise, and the reaction was stirred overnight. Triethylamine (8.3 mL, 61.4 mmol) was added followed by slow addition of MsCl (5 mL, 56.0 mmol) in dry THF (5 mL). After 90 min, the reaction was diluted with ether; washed with water, 1 M NaOH, and brine; dried over anhydrous Na_2SO_4; and concentrated. The residue was chromatographed (10:1 hexanes−EtOAc) to give a clear yellow liquid (8.83 g, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.29 (t, 2H, MsOCH_2), 3.67 (t, 2H, TBDMSOCH_2), 2.94 (s, 3H, CH_3SO$_3$), 1.88 (quintet, 2H, $CH_2CH_2CH_2$), 0.88 (s, 9H, *t*Bu), 0.05 (s, 6H, $(CH_3)_2$Si). ^{13}C NMR (75 MHz, $CDCl_3$): δ 67.2, 58.4, 37.2, 32.2, 25.9, −5.4. Anal. Calcd for $C_{10}H_{24}O_4SSi$ (268.46): C, 44.74; H, 9.01; S, 11.94. Found: C, 44.61; H, 9.22.

Ethyl 3,4-*O*-Isopropylidene-2-*O*-*p*-methoxybenzyl-1-thio-β-D-galactopyranoside (**5**). To a mixture of thioglycoside **9** (7.86 g, 20.0 mmol) and 2,2-dimethoxypropane (80 mL) was added toluenesulfonic acid (20 mg). The reaction mixture was stirred overnight, neutralized with triethylamine, concentrated, and evaporated twice with toluene. The residue was diluted with CH_2Cl_2, washed with brine, dried with anhydrous Na_2SO_4, and concentrated. The dried syrup was dissolved in DMF (70 mL) and purged with argon. To the ice-cooled solution was added NaH (0.58 g, 24.2 mmol) portionwise. After stirring for 45 min, *p*-methoxybenzyl chloride (4.10 mL, 30.3 mmol) was added dropwise. After 2 h, another 0.5 equiv of NaH and *p*-methoxybenzyl chloride were added to the reaction mixture. After an additional 2 h, the reaction was complete, and diethylamine (1.5 mL) was added to the mixture to destroy excess *p*-methoxybenzyl chloride. The reaction was then quenched with EtOAc containing residual water, diluted with CH_2Cl_2, washed with $NaHCO_3$ and water, and then stirred with 1 M HCl until the mixed acetal (R_f 0.79; 1:1 hexanes−EtOAc) had been completely hydrolyzed to **5** (R_f 0.35). The organic layer was then washed with water, $NaHCO_3$, and brine and finally dried over anhydrous Na_2SO_4. After removal of the solvent, the crude syrup was chromatographed on silica (5:2 hexanes−EtOAc containing 5% triethylamine) to give **5** that crystallized from ether (3.65 g, 48%) mp 90−91 °C. $[\alpha]_D$ −2.9° (*c* 2.8, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 (d, 2H, *J* 8.8, $CH_3OC_6H_4$), 6.85 (d, 2H, $CH_3OC_6H_4$), 4.75 (d, 1H, J_{gem} 11.0, $C_6H_4CH_2O$), 4.67 (d, 1H, $C_6H_4CH_2O$), 4.40 (d, 1H, $J_{1,2}$ 9.5, H-1), 4.22 (t, 1H, $J_{2,3}$ 6.1, $J_{3,4}$ 5.9, H-3), 4.17 (dd, 1H, $J_{4,5}$ 2.0, H-4), 3.88−3.96 (m, 1H, H-6a or H-6b), 3.74−3.80 (m, 5H, H-5, H-6a or H-6b, $CH_3OC_6H_4$), 3.42 (dd, 1H, H-2), 2.70 (m, 2H, SCH_2CH_3), 1.43, 1.33 (2s, 3H each, CCH_3), 1.28 (t, 3H, *J* 7.4, SCH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.3, 130.3, 130.0, 129.9, 113.8, 113.7, 109.9, 83.8, 79.6, 78.8, 75.7, 74.0, 73.3, 69.4, 55.3, 27.9, 26.4, 24.8, 15.0. Anal. Calcd for $C_{19}H_{28}O_6S$ (384.50): C, 59.35; H, 7.34; S, 8.34. Found: C, 59.30; H, 7.41.

Ethyl 3,4-*O*-Isopropylidene-2-*O*-*p*-methoxybenzyl-6-*O*-(3′-*O*-*tert*-butyldimethylsilyloxypropyl)-1-thio-β-D-galactopyranoside (**11**). NaH (25 mg, 1.0 mmol) was added to a solution of alcohol **5** (378 mg, 1.0 mmol) in dry THF (4 mL). After the mixture stirred for 30 min at 60 °C, a solution of 1-*O*-*tert*-butyldimethylsilyloxy-3-*O*-methanesulfonyloxy-propane (360 mg, 1.0 mmol) in dry THF (1 mL) was added dropwise. The reaction mixture was stirred at 60 °C for 3 h, and then 1 equiv of both NaH and linker were added. The reaction mixture was allowed to concentrate by evaporation of solvent to approximately one-third of its original volume. TLC indicated complete conversion of the alcohol. The reaction was quenched with EtOAc containing residual water; diluted with CH_2Cl_2; washed with water, $NaHCO_3$, and brine; and then dried over anhydrous Na_2SO_4. After concentration, column chromatography of the crude material (8:1 hexanes−EtOAc) gave **11** (501 mg, 91%) as an oil. $[\alpha]_D$ −8.8° (*c* 14.7, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.32 (d, 2H, *J* 8.4, $CH_3OC_6H_4$), 6.84 (d, 2H, $CH_3OC_6H_4$), 4.75 (d, 1H, J_{gem} 11.0, $C_6H_4CH_2O$), 4.66 (d, 1H, $C_6H_4CH_2O$), 4.38 (d, 1H, $J_{1,2}$ 9.7, H-1), 4.16 (m, 2H, H-3, H-4), 3.81 (H-5), 3.77 (s, C$H_3OC_6H_4$), 3.66 (H_{link}-1), 3.60−3.70 (H-6a, H-6b), 3.55 (m, 2H, H_{link}-3), 3.40 (dd, 1H, $J_{2,3}$ 6.1, H-2), 2.69 (m, 2H, SCH_2CH_3), 1.75 (quintet, 2H, H_{link}-2), 1.42, 1.32 (2s, 3H each, CCH_3), 1.28 (t, 3H, *J* 7.5, SCH_2CH_3), 0.86 (s, 9H, $(CH_3)_3$CSi), 0.02 (s, 6H, $(CH_3)_2$Si). ^{13}C NMR (300 MHz, $CDCl_3$): δ 130.0, 113.8, 83.6 ($^1J_{C,H}$ 152.2), 79.2, 78.4, 75.1, 73.6, 72.9, 69.7, 67.9, 59.7, 55.1, 32.5, 27.5, 26.0, 25.6, 24.4, 14.8, −5.6. Anal. Calcd for $C_{28}H_{48}O_7SSi$ (556.85): C, 60.39; H, 8.69; S, 5.76. Found: C, 60.37; H, 8.81.

Ethyl 3,4-*O*-Isopropylidene-2-*O*-*p*-methoxybenzyl-6-*O*-(3′-hydroxypropyl)-1-thio-β-D-galactopyranoside (**12**). Compound **11** (218 mg, 0.4 mmol) was stirred for 1 h with a solution of TBAF in THF (1 M, 5 mL). The reaction mixture was evaporated, diluted with CH_2Cl_2, washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The crude syrup was chromatographed (1:1 hexanes−EtOAc) to give **12** (172 mg, 100%) as an oil. $[\alpha]_D$ −9.7° (*c* 3.2, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.31 (d, 2H, *J* 8.7, $CH_3OC_6H_4$), 6.83 (d, 2H, $CH_3OC_6H_4$), 4.73 (d, 1H, J_{gem} 11.1, $C_6H_4CH_2O$), 4.65 (d, 1H, $C_6H_4CH_2O$), 4.38 (d, 1H, $J_{1,2}$ 9.6, H-1), 4.17 (H-3), 4.13 (H-4), 3.83 (m, 1H, H-5), 3.76 (s, C$H_3OC_6H_4$), 3.74, 3.68 (H_{link}-1, H_{link}-3), 3.66−3.74 (H-6a, H-6b), 3.40 (dd, 1H, $J_{2,3}$ 6.0, H-2), 2.58−2.80 (m, 2H, SCH_2CH_3), 1.79 (m, 2H, H_{link}-2), 1.40, 1.32 (2s, 3H each, CCH_3), 1.27 (t, 3H, *J* 7.4, SCH_2CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 159.3, 130.0, 113.7, 129.9, 110.0, 83.7 ($^1J_{C,H}$ 152.2), 79.6, 78.6, 75.4, 73.9, 73.2, 70.3, 70.9, 61.9, 55.3, 31.8, 27.9, 26.3, 24.6, 14.9. Anal. Calcd for $C_{22}H_{34}O_7S$ (442.58): C, 59.70; H, 7.74; S, 7.25. Found: C, 59.51; H, 7.90; S, 7.18.

Ethyl 3,4-*O*-Isopropylidene-2-*O*-*p*-methoxybenzyl-6-*O*-(3′-*O*-methanesulfonyloxypropyl)-1-thio-β-D-galactopyranoside (**13**). Triethylamine (0.11 mL, 0.8 mmol) was added to a solution of alcohol **12** (237 mg, 0.5 mmol) in dry CH_2Cl_2 (4 mL). Methanesulfonyl chloride (0.07 mL, 0.9 mmol) in CH_2Cl_2 (1 mL) was added dropwise. After stirring for 2 h at room temperature, the solution was diluted with CH_2Cl_2; washed with water, 1 M NaOH, water, and brine; and dried over anhydrous Na_2SO_4. Evaporation and chromatography (3:2 hexanes−EtOAc) gave **13** (263 mg, 93%) as a white solid. $[\alpha]_D$

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−6.6° (*c* 28.8, $CHCl_3$). ^1H NMR (300 MHz, $CDCl_3$): δ 7.32 (d, 2H, *J* 8.7, $CH_3OC_6H_4$), 6.85 (d, 2H, $CH_3OC_6H_4$), 4.74 (d, 1H, J_{gem} 11.0, $C_6H_4CH_2O$), 4.66 (d, 1H, $C_6H_4CH_2O$), 4.38 (d, 1H, $J_{1,2}$ 9.6, H-1), 4.31 (t, 2H, *J* 6.2, H_{link}-3), 4.17 ($J_{2,3}$ 6.0, $J_{3,4}$ 5.8, H-3), 4.15 ($J_{4,5}$ 2.0, H-4), 3.83 (ddd, 1H, $J_{5,6ab}$ 5.1 & 6.9, H-5), 3.77 (s, 3H, $CH_3OC_6H_4$), 3.64−3.70 (m, 2H, H-6a, H-6b), 3.52−3.64 (m, 2H, H_{link}-1), 3.40 (dd, 1H, $J_{2,3}$ 6.0, H-2), 2.98 (s, 3H, MsO), 2.58−2.78 (m, 2H, SCH_2CH_3), 1.98 (quintet, 2H, *J* 6.13, H_{link}-2), 1.42, 1.33 (2s, 3H each, CCH_3), 1.28 (t, 3H, *J* 7.4, SCH_2CH_3). ^{13}C NMR (300 MHz, $CDCl_3$): δ 129.6, 113.5, 83.5, 79.3, 78.4, 75.2, 73.6, 72.9, 69.9, 66.8, 66.3, 55.0, 37.0, 29.1, 27.5, 25.9, 24.4, 14.6. Anal. Calcd for $C_{23}H_{36}O_9S_2$ (520.67): C, 53.06; H, 6.97; S, 12.32. Found: C, 52.92; H, 7.07; S, 12.59.

Ethyl 3,4-*O*-Isopropylidene-2-*O*-*p*-methoxybenzyl-6-*O*-(3′-*O*-(methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-*β*-D-glucopyranos-3-oxy)propyl)-1-thio-*β*-D-galactopyranoside (14). Sodium hydride (9.7 mg, 0.4 mmol) was added to a solution of glucopyranoside **7** (135 mg, 0.4 mmol) in dry THF (3 mL). The mixture was stirred for 30 min at 60 °C. The methanesulfonate **13** (146 mg, 0.3 mmol) in dry THF (2 mL) was added, and the reaction was stirred at 60 °C for 20 min. Dry Me_2SO (0.5 mL) was added, and after an additional 2−4 h of heating at 60 °C, the reaction was complete. The cooled reaction mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ and brine, and dried over Na_2SO_4. The concentrated residue was chromatographed (2:1 hexane−acetone) and gave **14** (174 mg, 83%) as a white solid. $[\alpha]_D$ 17.5° (*c* 5.1, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 6.84−7.50 (9H, ArH), 5.93 (d, 1H, *J* 8.0, N*H*Ac), 5.51 (s, 1H, PhC*H*O), 4.70−4.82 (m, 2H, J_{gem} 11.4, $J_{1,2}$ 8.3, $C_6H_4CH_2O$ and H-1), 4.65 (d,1H, $C_6H_4CH_2O$), 4.36 ($J_{1',2'}$ 9.6, H′-1), 4.16 (t, 1H, $J_{2',3'}$ ∼6.0, $J_{3',4'}$ ∼6.0, H′-3),4.09 (dd, 1H, $J_{4',5'}$ 1.9, H′-4), 3.98 ($J_{3,4}$ ∼9.6, H-3), 3.94 (m, 2H, H_{link}-1), 3.76 (H-5), 3.72−3.82 (m, 4H, $CH_3OC_6H_4$ and H′-5), 3.60−3.70 (H-6a, H-6b), 3.59−3.79 (H′-6a, H′-6b), 3.56 (H-4), 3.50−3.70 (H_{link}-3), 3.50 (s, OCH_3), 3.50 (H-2), 3.40 (H′-2), 2.56−2.80 (m, 2H, SCH_2CH_3), 2.01 (s, 3H, $NHCOCH_3$), 1.66−1.82 (m, 2H, H_{link}-2), 1.39, 1.30 (2s, 3H each, CCH_3), 1.26 (t, 3H, *J* 7.3, SCH_2CH_3). ^{13}C NMR (300 MHz, $CDCl_3$): δ 129.7, 113.6, 128.8, 128.0, 125.8, 101.7, 101.0, 83.8, 82.3, 79.4, 78.3, 77.5, 75.5, 73.6, 72.8, 69.9, 68.9, 68.5, 68.9, 67.7, 65.7, 55.1, 56.9, 30.1, 27.6, 26.1, 23.5, 24.8, 14.5. Anal. Calcd for $C_{38}H_{53}NO_{12}S$ (747.92): C, 61.03; H, 7.14; N, 1.87; S, 4.29. Found: C, 61.36; H, 7.28; N, 1.85.

Ethyl 3,4-*O*-Isopropylidene-2-*O*-*p*-methoxybenzyl-6-*O*-(3′-*O*-(methyl 2-acetamido-6-*O*-benzyl-2-deoxy-*β*-D-glucopyranos-3-oxy)propyl)-1-thio-*β*-D-galactopyranoside (15). Dry THF (5 mL) was added to a mixture of compound **14** (34 mg, 0.05 mmol), sodium cyanoborohydride (30 mg, 0.5 mmol), dry powdered 3 Å molecular sieves, and methyl orange (1 mg). The suspension was purged with argon. A freshly made saturated solution of HCl in dry ether was added dropwise to the reaction mixture until the evolution of gases ceased and the indicator turned bright pink. A further 2-fold volume of saturated HCl in ether was added, and the reaction was left to stir for 30 min. The reaction mixture was diluted with CH_2Cl_2, filtered through Celite, washed with $NaHCO_3$ and brine, and dried over Na_2SO_4. After solvent evaporation, column chromatography of the crude material (2:1 toluene−acetone) gave a white solid **15** (11.9 mg, 35%). $[\alpha]_D$ −12.1° (*c* 1.9, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 6.84−7.32 (9H, ArH), 5.77 (d, 1H, *J* 8.0, N*H*Ac), 4.73 (d, 1H, J_{gem} 11.0, $CH_3OC_6H_4CH_2O$), 4.69 (d, 1H, $J_{1,2}$ 8.2, H-1), 4.65 (d, 1H, $CH_3OC_6H_4CH_2O$), 4.59 (d, 1H, J_{gem} 12.1, $PhCH_2O$), 4.57 (d, 1H, $PhCH_2O$), 4.36 (d, 1H, $J_{1',2'}$ 9.5, H′-1), 4.15 (t, 1H, $J_{3',4'}$ ∼6.0, H′-3), 4.11 (dd, 1H, $J_{4',5'}$ 1.8, H′-4), 3.81 (ddd, 1H, $J_{5',6'a/b}$ 6.0, H′-5), 3.80 (H-6a, H-6b), 3.77 ($CH_3OC_6H_4$), 3.75, 3.61 (H_{link}-1, H_{link}-3), 3.73 (H-3), 3.66 (H′-6a, H′-6b), 3.50 (H-4), 3.48 (H-5), 3.46 (s, CH_3O), 3.39 (dd, 1H, $J_{2',3'}$ 6.4, H′-2), 3.29 (quartet, 1H, $J_{2,3}$ ∼8.2, H-2), 2.60−2.78 (m, 2H, SCH_2CH_3), 1.97 (s, 3H, $NHCOCH_3$), 1.70−1.83 (m, 2H, H_{link}-2), 1.40, 1.31 (2 s, 3H each, CCH_3), 1.28 (t, 3H, *J* 7.4, SCH_2CH_3). ^{13}C NMR (500 Hz, $CDCl_3$): δ 129.8, 113.5, 127.2, 100.9 ($^1J_{C,H}$ 162.8), 83.7 ($^1J_{C',H'}$ 154.8), 81.2, 79.7, 78.4, 75.2, 74.2, 74.0, 73.7, 73.2, 71.8, 70.2, 68.7, 68.5, 56.6, 56.5, 55.2, 29.56, 27.7, 26.2, 24.6, 23.3, 15.1. Anal. Calcd for $C_{38}H_{55}NO_{12}S$ (749.93): C, 60.86; H, 7.39; N, 1.87; S, 4.28. Found: C, 61.07; H, 7.60; N, 1.95; S, 4.23. The acetylated form of **15** provided a characteristic NMR spectrum showing a downfield shift of the 4-OH signal (4.88 ppm).

Methyl 2-Acetamido-6-*O*-benzyl-2-deoxy-4-*O*-(3,4-*O*-isopropylidene-2-*O*- *p*-methoxybenzyl-*β*-D-galactopyranosyl)-3,6′-di-*O*-(propan-1,3-diyl)-*β*-D-glucopyranoside (16). **Method 1.** To a dry mixture of starting material **15** (287 mg, 0.4 mmol) and powdered 4 Å molecular sieves purged with argon was added dry CH_2Cl_2 (17 mL). Once the reaction flask was covered with aluminum foil, NIS (129 mg, 0.6 mmol) and silver triflate (57 mg, 0.2 mmol) were added. After 30 min, the reaction mixture was diluted with CH_2Cl_2, and the molecular sieves were filtered off. The filtrate was washed with $Na_2S_2O_3$, $NaHCO_3$, and brine and dried. The concentrated material was chromatographed (2:1 toluene−acetone) to give impure **16** as a white solid that contained the iodonated and noniodonated *p*-methoxybenzyl group (90.2 mg, 35%).

Method 2. To a dry mixture of starting material **15** (97 mg, 0.1 mmol), DTBMP (93.1 mg, 0.5 mmol), and powdered 4 Å molecular sieves purged with argon was added dry CH_2Cl_2 (10 mL). Once the reaction had been stirred for 15 min, methyl triflate (44 μL, 0.4 mmol) was added. After 45 min, the reaction mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed with $NaHCO_3$, 0.5 M HCl, $NaHCO_3$, and brine; dried; and concentrated. The crude material was chromatographed (2:1 toluene−acetone) to give **16** (44 mg, 50%) as a white solid. The α-anomer of **16** was also isolated (5.6 mg, 7%). ^1H NMR (500 MHz, $CDCl_3$): δ 6.82−7.32 (9H, ArH), 5.25 (d, 1H, *J* 9.2, N*H*Ac), 4.69, 4.58 (2 d, 1H each, J_{gem} 11.1, $CH_3OC_6H_4CH_2O$), 4.43, 4.36 (2 d, 1H each, J_{gem} 12.1, $PhCH_2O$), 4.28 (d, 1H, $J_{1,2}$ 7.9, H-1), 4.20 (quartet, 1H, $J_{2,3}$ ∼8.5, H-2), 4.14 (d, 1H, $J_{1',2'}$ 8.4, H′-1), 4.03 (t, 1H, $J_{3,4}$ ∼6.3, H′-3), 3.96 (t, 1H, $J_{3,4}$ 9.5, $J_{4,5}$ 9.5, H-4), 3.90 (dd, 1H, $J_{3',4'}$ 5.5, $J_{4',5'}$ 2.0, H′-4), 3.84 (dd, 1H, $J_{5,6a,}$ 4.3, $J_{6a,6b}$ 10.5, H-6a), 3.77 (s, $CH_3OC_6H_4$), 3.76 (H′-5), 3.73 (H-6b), 3.73, 3.42 (H_{link}-1, H_{link}-3), 3.68 (H-3), 3.65−3.80 (H′-6a, H′-6b), 3.46 (s, 3H, OCH_3), 3.40−3.45 (m, 1H, H-5), 3.37 (dd, 1H, $J_{2',3'}$ 7.2, H′-2), 1.99 (s, 3H, $NHCOCH_3$), 1.60−1.76 (m, 2H, H_{link}-2), 1.35, 1.27 (2 s, 3H each, CCH_3). ^{13}C NMR (500 Hz, $CDCl_3$): δ 129.7, 113.4, 128.2, 127.6, 104.3 ($^1J_{C',H'}$ 158.7), 102.4 ($^1J_{C,H}$ 158.7), 80.2, 79.4, 73.9, 75.2, 73.5, 72.8, 72.8, 78.73, 50.96, 74.9, 68.9, 68.1, 56.9, 56.86, 55.0, 56.3, 30.5, 27.5, 26.1, 23.4. ES HRMS: *m*/*z* 710.3152 $[M + Na]^+ \pm 0.1$ mDa and 688.3 $[M + H]^+$ ($C_{36}H_{49}NO_{12}$ requires *m*/*z* 687.80).

Methyl 2-Acetamido-6-*O*-benzyl-2-deoxy-4-*O*-(3,4-*O*-isopropylidene-*β*-D-galactopyranosyl)-3,6′-di-*O*-(propan-1,3-diyl)-*β*-D-glucopyranoside (4). To compound **16** (36 mg, 0.05 mmol) dissolved in CH_2Cl_2 (1 mL) was added water (0.06 mL) and DDQ (17 mg, 0.08 mmol). The mixture was stirred at room temperature for 1 h, and another 1.5 equiv of DDQ (17 mg, 0.08 mmol) was added. After 1 h the reaction was quenched with a saturated aqueous $NaHCO_3$ solution. The mixture was diluted with CH_2Cl_2, washed with $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated. The crude material was chromatographed (1:1 toluene−acetone) and gave **4** (20 mg, 67%) as a white solid. $[\alpha]_D$ 6.8° (*c* 3.7, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$): δ 7.26−7.36 (5H, Ph), 5.28 (d, 1H, *J* 9.0, N*H*), 4.65 (d, 1H, J_{gem} 12.1, $PhCH_2O$), 4.55 (d, 1H, $PhCH_2O$), 4.33 (d, 1H, $J_{1,2}$ 7.9, H-1), 4.12 (d, 1H, $J_{1',2'}$ 8.4, H′-1), 4.06 (quartet, 1H, $J_{2,3}$ ∼9.3, H-2), 3.94 (t, $J_{3,4}$ ∼5.5, H′-3), 3.88 (H′-4), 3.87 (H-6a), 3.86 (H-4), 3.79 (H-6b), 3.73, 3.68, 3.63, 3.58 (H_{link}-1, H_{link}-3, H′-6a, H′-6b), 3.67 (H-3), 3.53 (ddd, 1H, $J_{H,OH}$ 2.5, $J_{2',3'}$ 6.5, H′-2), 3.45 (H-5, CH_3O), 1.99 (s, 3H, $NHCOCH_3$), 1.60−1.78 (m, 2H, H_{link}-2), 1.56, 1.47 (2 s, 3H each, CCH_3). ^{13}C NMR (500 MHz, $CDCl_3$): δ 128.0, 127.9, 105.0 ($^1J_{C',H'}$ 153.1), 102.3 ($^1J_{C,H}$ 163.2), 79.9, 79.2, 77.6, 74.1, 73.7, 73.6, 73.0, 69.3, 68.3, 66.2, 59.3, 56.4, 52.3, 30.7, 27.9, 26.1, 23.5. Anal. Calcd for $C_{28}H_{41}NO_{11}$ (567.64): C, 59.25; H, 7.28; N, 2.47. Found: C, 59.56; H, 7.57; N, 2.42.

Methyl 2-Acetamido-6-*O*-benzyl-2-deoxy-4-*O*-(3,4-*O*-isopropylidene-2-*O*-(2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl)-*β*-D-galactopyranosyl)-3,6′-di-*O*-(propan-1,3-diyl)-*β*-D-glucopyranoside (17). A mixture of disaccharide **4** (50 mg, 0.09 mmol) and thioglycoside **3** (44 mg, 0.09 mmol) in dry CH_2Cl_2 (2 mL) was prepared and purged with argon. The solution of

sugars **3** and **4** was transferred via cannula to a mixture of DMTST (60 mg, 0.2 mmol), DTBMP (57 mg, 0.3 mmol), and 4 Å molecular sieves in dry CH_2Cl_2 (2 mL) at −68 °C purged with argon. The reaction was allowed to warm to room temperature. After stirring for 2 h the reaction was diluted with CH_2Cl_2, washed with $NaHCO_3$ and brine, dried over Na_2SO_4, and concentrated. The crude material was chromatographed (3:2 hexane−acetone) and gave **17** (47.6 mg, 55%) as a white solid. ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.26−7.43 (m, 20H, Ph), 5.59 (d, 1H, $J_{1'',2''}$ 3.8, H″-1), 5.25 (d, 1H, J 9.2, N*H*), 4.92, 4.61 (2 d, 2H, J_{gem} 11.6, PhCH_2O), 4.77, 4.61 (2 d, 2H, J_{gem} 11.9, PhCH_2O) 4.78, 4.74 (2 d, 2H, J_{gem} 11.6, PhCH_2O) 4.52, 4.48 (2 d, 2H, J_{gem} 12.0, PhCH_2O), 4.26 (d, 1H, $J_{1,2}$ 7.9, H-1), 4.18 (quartet, 1H, $J_{2,3}$ 9.6, H-2), 4.11 (d, $J_{1',2'}$ 8.6, H′-1), 4.09 ($J_{3',4'}$ 6.6, H′-3), 4.05 (dd, $J_{2,3}$ 10.1, H″-2), 3.95 (quartet, 1H, H″-5), 3.89 (t, 1H, $J_{3,4}$ 9.2, $J_{4,5}$ 9.3, H-4), 3.85 (dd, 1H, $J_{4,5}$ 5.5, H′-4), 3.82 (dd, 1H, $J_{3'',4''}$ 2.6, H″-3), 3.78 (H-6a, H-6b), 3.75 (H′-2), 3.70 (H′-5), 3.69−3.76 (H_{link}-1, H_{link}-3), 3.67 (H-3), 3.62 (d, 1H, $J_{4'',5''}$ 2.3, H″-4), 3.47 (s, 3H, CH_3O), 3.34−3.40 (m, 1H, H′-6a), 3.25 (m, 1H, H-5), 1.99 (s, 3H, NHCOCH_3), 1.64−1.74 (m, 2H, H_{link}-2), 1.44, 1.28 (2 s, 3H each, CCH_3), 1.09 (d, 3H, $J_{5'',6''}$ 6.4, H″-6). ^{13}C NMR (500 MHz, $CDCl_3$): δ 126.0−130.0, 102.5 ($^1J_{C',H'}$ 156.6), 102.3 ($^1J_{C,H}$ 159.0), 95.6 ($^1J_{C'',H''}$ 172.6), 80.5, 78.9, 78.3, 77.3, 76.1, 76.0, 75.1, 74.52 74.1, 73.6, 73.2, 72.5, 68.3, 68.0, 66.1, 56.3, 56.2, 50.4, 30.0, 27.6, 26.2, 23.1, 16.4. ES HRMS: *m*/*z* 1006.4577 [M + Na]$^+$ ± 1.2 mDa and 984.5 [M + H]$^+$ ($C_{55}H_{69}NO_{15}$ requires *m*/*z* 984.17).

Methyl 2-Acetamido-2-deoxy-4-*O*-(2-*O*-(α-L-fucopyranosyl)-β-D-galactopyranosyl)-3,6′-di-*O*-(propan-1,3-diyl)-β-D-glucopyranoside (2). The protected, tethered trisaccharide **17** (7 mg, 0.007 mmol) was dissolved in 90% acetic acid and heated at 65 °C for 6 h. The solvents were evaporated, and the residue coevaporated with toluene. The partially protected trisaccharide **18** (R_f 0.13 (2:3 hexane−acetone) was then dissolved in ethanol (10 mL), to which 20% palladium hydroxide on charcoal was added (10 mg). The mixture was hydrogenated for 18 h under an atmosphere of hydrogen to give **2** as a white solid. The suspension was filtered and concentrated, and the crude material was purified by HPLC (water−methanol gradient 0−10%) to give **2** (3.6 mg, 90%). $[\alpha]_D$ −88.7° (*c* 2.3, H_2O). R_f 0.41 (14:6:1 CH_2Cl_2−methanol−water). ^{1}H NMR (600 MHz, D_2O): δ 5.39 (d, 1H, $J_{1'',2''}$ 2.0, H″-1), 4.46 (d, 1H, $J_{1',2'}$ 8.1, H′-1), 4.45 (d, 1H, $J_{1,2}$ 8.2, H-1), 4.18 (quartet, 1H, H″-5), 4.13 (dd, 1H, $J_{2,3}$ 11.0, H-2), 4.09 (t, $J_{3,4}$ 9.5, $J_{4,5}$ 9.5, H-4), 4.07 (dd, $J_{6a,5}$ 1.8, J_{gem} 11.7, H-6a), 3.87 (H′-6a), 3.87 and 3.44 (H_{link}-1), 3.86 (H′-3), 3.85 (H-6b), 3.84 (H″-4), 3.82 (H″-2), 3.81 (H″-3), 3.79 (H′-5), 3.78 (H′-4), 3.78 and 3.65 (H_{link}-3), 3.77 (H-3), 3.71 (dd, $J_{2',3'}$ 9.5, H′-2), 3.70 (H′-6b), 3.52 (s, 3H, OCH_3), 3.45 (H-5), 2.04 (s, 3H, NHCOCH_3), 1.72−1.79 (m, 2H, H_{link}-2), 1.25 (d, 3H, $J_{5'',6''}$ 6.8, H″-6). ^{13}C NMR (125 MHz, D_2O): δ 175.1 (C═O, NHAc), 104.0 (C-1, $^1J_{C,H}$ 160.7), 103.0 (C′-1, $^1J_{C',H'}$ 161.7), 100.1 (C″-1, $^1J_{C'',H''}$ 175.2), 79.0 (C-3), 76.4 (C-5), 76.1 (C′-2), 75.6 (C′-5), 75.0 (C′-3), 74.4 (C-4), 72.6, 70.4, 69.9 (C′-4), 69.4 (C′-6), 69.4 (CH_2, C_{link}-3), 69.0 (C″-2, C″-3, C″-4), 67.7 (C″-5), 61.2 (C-6), 58.1 (CH_3O), 57.7 (CH_2, C_{link}-1), 51.2 (C-2), 29.5 (CH_2, C_{link}-2), 22.9 (NHCO*C*H_3), 16.1 (C″-6). ES HRMS: *m*/*z* 606.2373 [M + Na]$^+$ ± 0.1 mDa and 584.3 [M + H]$^+$ ($C_{24}H_{41}NO_{15}$ requires *m*/*z* 583.60).

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