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The synthesis of deoxy-α-Gal epitope derivatives for the evaluation of an anti-α-Gal antibody binding

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Abstract

α-Gal epitopes (also termed as α-Gal) are carbohydrate structures bearing the α-D-Gal-(1→3)-β-D-Gal terminus **1** and are known to be the antigen responsible for antibody-mediated hyperacute rejection in xenotransplantation. Terminal 2-, 3-, 4-, and 6-deoxy-Gal derivatives of α-Gal were synthesized. Inhibition ELISA using mouse laminin was established to determine the binding affinity of the synthesized α-Gal derivatives. 4-Deoxy-α-Gal derivative **7** showed a significant reduction in antibody recognition. The IC_{50} value was 15-fold poorer than the standard α-Gal epitopes α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Glc-NHAc (**39**) and α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Glc-OBn (**40**). A similar observation was seen with 2-deoxy-α-Gal derivative **5**, whose IC_{50} value was nearly tenfold higher than the standards. Interestingly, substitution at the terminal 3-position resulted in only a fourfold decrease in antibody recognition, suggesting a possible point of future derivation. Finally, 6-deoxy-α-Gal derivative **8** exhibited similar antibody recognition to both α-Gal epitope **39** and α-Gal epitope **40**. This strongly suggests that derivatization at the 6-position can be accomplished without loss of antibody recognition. These findings can be utilized for the future design of other α-Gal derivatives.

Keywords: α-Gal epitope; Deoxy sugars; Xenotransplantation; Anti-α-Gal antibody

1. Introduction

Tissue and organ transplantation has become the preferred treatment for people suffering from organ failure.[1] Unfortunately the limited supply of suitable organs is a serious problem associated with this procedure. In 1999, roughly 72,000 Americans were on organ/tissue transplant waiting lists, with only approximately 21,000 transplantations carried out.[2]

One novel solution to the limited availability of organs for transplantation is the use of genetically engineered animal organs, an idea referred to as xenotransplantation.[3] The pig is considered the primary candidate factoring in ethical considerations, infectious disease concerns, and size.[4] Despite this promising proposal, human antibody-mediated hyperacute rejection poses a major obstacle. Investigation into the hyperacute rejection indicated that the initial steps involve the recognition of human natural anti-Gal antibody to carbohydrate epitopes bearing an α-D-Gal-(1→3)-β-D-Gal terminus **1** (termed as α-Gal).[5] Trisaccharides α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Glc-OR (**2**) and α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-GlcNAc-OR″ (**3**) and pentasaccharide α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-GlcNAc-(1→3)-β-D-Gal-(1→4)-β-D-Glc-OR‴ (**4**) have been identified as the major α-Gal epitopes responsible for the initiation of the immuonological response. These epitopes have been found to be expressed in most mammalian species with the exception of humans, apes and Old World primates (Fig. 1).[6] Conversely, anti-Gal antibodies (known as anti-α-Gal) are among the most abundant human natural antibodies, with concentrations of 1–2% of total serum IgG and 3–8% of total serum IgM.[7]

Several methods have been studied to prevent the hyperacute rejection and to prolong the xenograft cell survival through elimination or reduction of the interaction between α-Gal and anti-α-Gal.[8] These include depletion through immunoadsorption of anti-α-Gal an-

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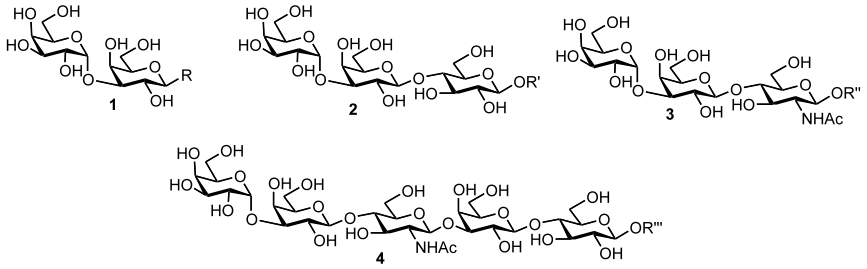


Fig. 1. Major α-Gal epitopes.

tibodies using α-Gal immobilized affinity columns[9] and infusion of synthetic α-Gal epitopes to inhibit binding of anti-α-Gal to xenograft cells.[10] One of the major factors limiting the application of these methods is the substantial amount of synthetic α-Gal oligosaccharide needed. Studies on porcine endothelial cells bearing the α-Gal epitope indicate that millimolar amounts of α-Gal epitope are needed to achieve 90% inhibition of anti-α-Gal binding.[11] At these concentrations, application of synthetic α-Gal epitopes cannot be considered practical. Therefore, the design of synthetic α-Gal mimics that exhibiting higher avidity becomes the next logical area to explore.

In order to design an effective α-Gal mimic, one must gain a better understanding of the interaction between anti-α-Gal antibody and α-Gal. One relatively effective way is the use of monodeoxy derivatives. A study conducted by Lemieux et al.[12] probed the interaction of monoclonal antibodies specific for Lewis-a (Le^a) human blood group determinant using eight monodeoxy derivatives. The rationale for using deoxy derivatives was based on earlier studies that indicated that clusters of polar groups provided key hydrophobic binding with the antibody.[13] Evidence suggested that oligosaccharides assume, if stereochemically possible, an intramolecular hydrogen binding conformation exposing key polar hydroxyl groups to the antibody binding site. Replacing a polar hydroxyl group with nonpolar hydrogen essentially eliminates any potential for hydrogen bonding which in turn affects antibody recognition.

With this approach in mind we decided to synthesize a series of monodeoxy derivatives **5**–**8** (Fig. 2) of α-Gal. Focus was placed on the crucial terminal galactose residue. ELISA would then be used to evaluate anti-α-Gal recognition of the deoxy α-Gal derivatives.

2. Results and discussion

Synthesis.—Lactose azide acceptor **9**[14] was employed for key glycosylations leading to the formation of the monodeoxy derivatives **5**, **6**, **7**, and **8**. The synthetically useful azido group at the anomeric position of lactose **9** was introduced for convenient transformation into other derivatives and glycoconjugates.[15] A stereoselective high-yielding glycosylation[16] between corresponding deoxy monosaccharide donors **10a**–**c** and lactose **9** (Scheme 1) was used in the synthesis of trisaccharide **6**, **7**, and **8**.

Synthesis of derivative **5** used a different approach starting with peracetylated galactal **12** that was transformed to 2-deoxy-2-iodo monosaccharide **13** (Scheme 2) in 62% yield.[17] This highly selective reaction proceeds by the addition of an iodine atom (I) to the C-2 position of the galactal analogous to azidonitration.[18] TMS–OTf was used to catalyze the glycosylation between donor **13** and lactose acceptor **9**. The resulting trisaccharide **14** was obtained in a modest 54% yield as a 90:10 α/β mixture after approximately 1.5 h at −30 °C. Variation in temperature and time did not appear to significantly improve the yield of the glycosylation. Treatment of trisaccharide **14** with Bu_3SnH, Et_3B and oxygen lead to the dehalogenated trisaccharide **15**. Finally, treatment with NaOMe in anhydrous MeOH lead to the desired 2-deoxy-α-Gal derivative **5**.

Originally in our synthetic approach for the synthesis of 3-deoxy-α-Gal derivative **6**, and the 4-deoxy α-Gal derivative **7**, we envisioned the use of a common inter-

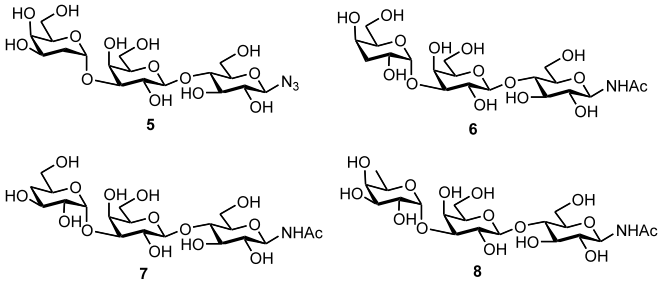
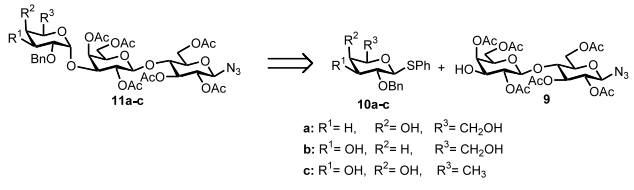
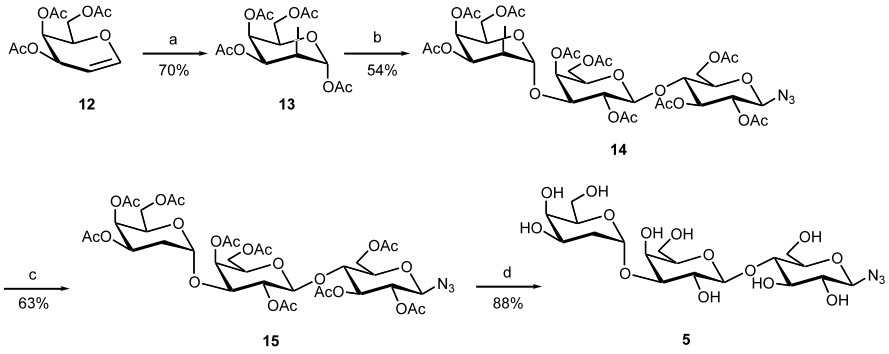


Fig. 2. Terminal monodeoxy-α-Gal derivatives.



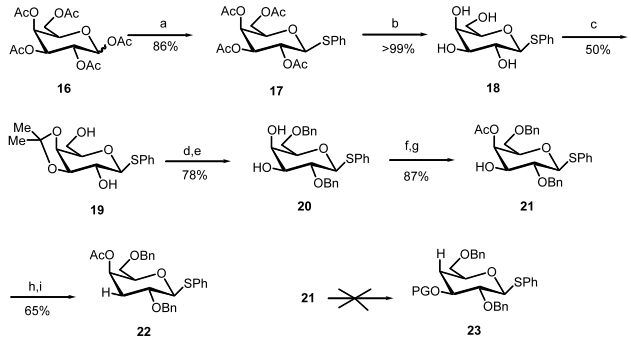
Scheme 1. Thioglycosylation.



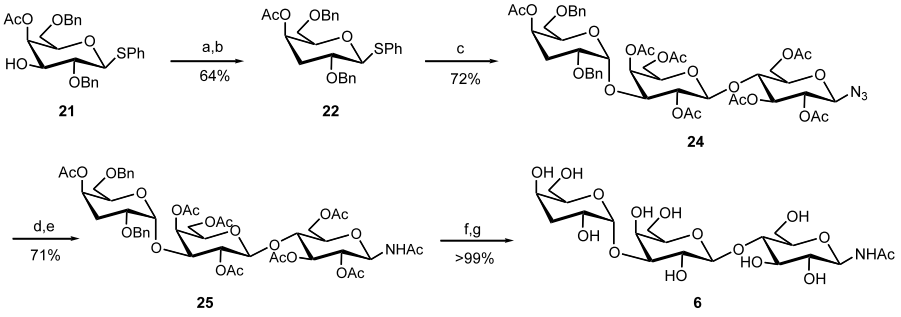
Scheme 2. (a) CAN, NaI, HOAc, MeCN, −15 °C to 25 °C; (b) TMSOTf, lactose **9**, 4 Å MS, CH_2Cl_2, −30 °C, 1.5 h; (c) Bu_3SnH, Et_3B, O_2, benzene, rt, 0.5 h; (d) anhyd MeOH, NaOMe, pH 9.

mediate **21** (Scheme 3). With just a few transformations we could synthesize both 3-deoxy-α-Gal thiophenyl donor **22** and 4-deoxy-α-Gal thiophenyl donor **23** from intermediate **21**. Starting with commercially available penta-*O*-acetyl-D-galactose (**16**), a glycosylation with thiophenol was carried out in the presence of a Lewis acid. Following deprotection, a selective acetonide protection of the C-3, C-4 diol gave rise to compound **19** in 50% yield.[19] Benzylation, followed by removal of the 3,4-*O*-isopropylidene moiety, gave compound **20**. Next triethyl orthoacetate was used to selectively protect the C-4 hydroxyl group after ring opening promoted by 80% HOAc resulting in intermediate **21**. Functionalization of the C-3 hydroxyl group with phenyl chlorothionocarbonate (phenoxythiocarbonyl chloride), employing 4-(dimethylamino)-pyridine (DMAP) as a catalyst, afforded a *O*-(phenoxythiocarbonyl) ester, which then underwent free-radical-mediated Barton–McCombie deoxygenation with tributylstannane (tributyltin hydride) with satisfactory conversion to the thiophenyl 3-deoxy sugar donor **22** (Scheme 4).[20] TfOH-catalyzed *N*-iodosuccinamide-mediated glycosylation of donor **22** and lactose azide **9** produced trisaccharide **24** in 72% yield. Next reduction of the azide was carried out using selective hydrogenation via Adams' catalyst,[21] followed by *N*-acylation of the free amino group using acetic anhydride and triethylamine, led to compound **25**. Final deprotection gave rise to 3-deoxy-α-Gal derivative **6**. We attempted to employ a similar reaction strategy for 4-deoxy-α-Gal derivative **7**; however, we encountered difficulties in finding a suitable protecting group that could be used in the synthesis of the thiophenyl 4-deoxy sugar donor **23**. Initially we attempted to produce the C-3 hydroxyl group using a variety of silyl protecting groups. Under mildly basic conditions we were able to successfully protect the hydroxyl group. Unfortunately, removal of the acetyl-protecting group at the C-4 position caused an extensive amount of intramolecular migration of the silyl group between the C-4 and the C-3 position, rendering this approach unacceptable. A variety of other protecting methods was attempted without any significant success. It was clear that the problem was due to the base-sensitive acetyl group on the C-4 position. Since it became obvious that the approach needed to be redesigned, the decision was made to synthesize the 4-deoxygalactose donor using a different synthetic route.

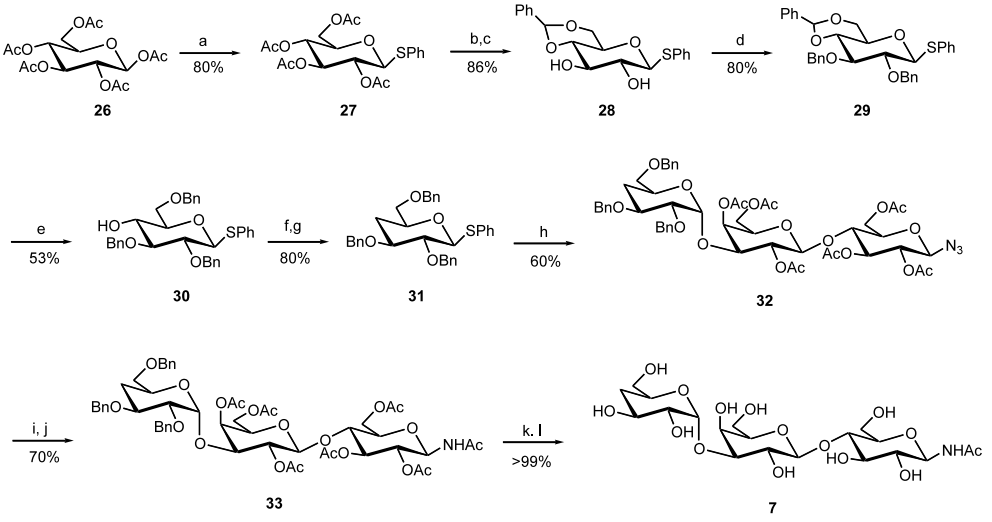
From commercially available penta-*O*-acetyl-D-glucose (**26**) (Scheme 5), glycosylation lead to the satisfactory formation of thioglycoside **27**. Base-catalyzed removal of the acetyl protecting groups led to the free thioglycoside **28**, which upon acid-catalyzed treatment with α,α-dimethoxytoluene lead to the selective 4,6-*O*-benzylidene thioglucoside **29**.[22] The free hydroxyl groups were then *O*-benzylated prior to selective reductive deprotection mediated by sodium cyanoborohydride, affording the 2,3,6-tri-*O*-benzylated thioglucoside **30**.[23] Treatment with phenyl chlorothionocarbonate, followed by free-radical deoxygenation, led to the 4-deoxythiophenyl donor **31**. TfOH-catalyzed glycosylation of donor **31** and lactose azide acceptor **9** gave trisaccharide **32** in relatively modest yield. Following the same protection and deprotection strategy resulted in 4-deoxy-α-Gal derivative **7**.



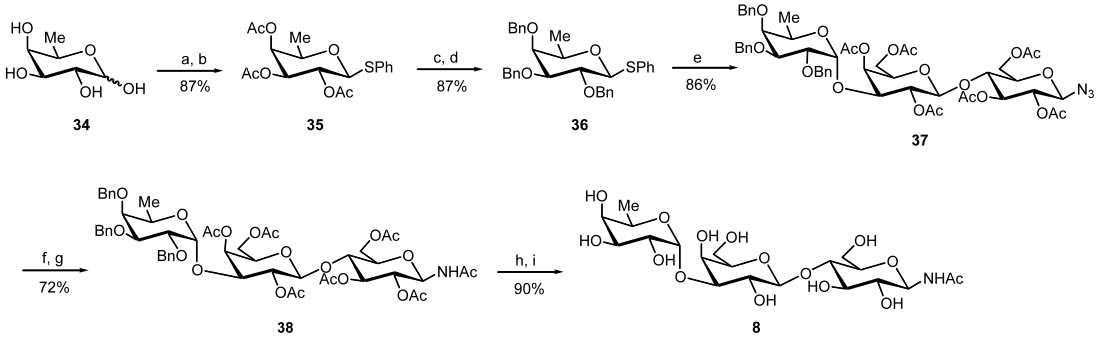
Scheme 3. (a) PhSH, $BF_3{\cdot}Et_2O$, CH_2Cl_2; (b) anhyd MeOH, NaOMe, pH 9; (c) 2,2-dimethoxypropane, TsOH, CH_2Cl_2; (d) BnBr, NaH, DMF; (e) 80% HOAc, 90 °C; (f) $MeC(OEt)_3$, TsOH, benzene; (g) 80% HOAc; (h) PTC–Cl, DMAP, $CHCl_2$, 16 h; (i) Bu_3SnH, AIBN, benzene, 2 h.



Scheme 4. (a) PTC–Cl, DMAP, CH_2Cl_2, 12 h; (b) AIBN, Bu_3SnH, benzene 1.25 h; (c) lactose **9**, TfOH, CH_2Cl_2; NIS (d) PtO_2, H_2, EtOH, 2 h; (e) CH_2Cl_2, Et_3N, Ac_2O, 0 °C, 2 h; (f) $Pd(OH)_2$–C, MeOH, H_2, 18 h; (g) MeOH, NaOMe, pH 9.



Scheme 5. (a) PhSh, BF_3·Et2O, CH_2Cl_2; (b) NaOMe, MeOH, pH 9; (c) α,α-dimethoxypropane, TsOH; MeCN, 18 h; (d) BnBr, NaH, DMF; (e) $NaCNBH_3$, HCl, Et_2O, THF; (f) PTC–Cl, DMAP, CH_2Cl_2, 12 h; (g) Bu_3SnH, AIBN, benzene 1 h; (h) lactose **9**, TfOH, NIS, CH_2Cl_2; (i) PtO_2, H_2, EtOH, 2 h; (j) CH_2Cl_2, Et_3N, Ac_2O, 0 °C, 2 h; (k) $Pd(OH)_2$–C, MeOH, H_2, 18 h; (l) NaOMe, MeOH, pH 9.



Scheme 6. (a) Ac_2O, DMAP, pyridine; (b) PhSH, CH_2Cl_2, BF_3 Et_2O; (c) NaOMe, MeOH, pH 9; (d) BnBr, DMF, NaH; (e) TfOH, lactose **9**, NIS, CH_2Cl_2; (f) Pt_2O, H_2, EtOH, 1 h; (g) Ac_2O, Et_3N; (h) Pd/C, H_2,18 h; (i) NaOMe, MeOH, pH 9.

The final terminal 6-deoxy-α-Gal derivative was synthesized starting from commercially available D-fucose **34** (Scheme 6). Using our established synthetic strategy[14] we were able produce thiophenyl donor **36** in four transformations with an overall yield of 76%. Glycosylation catalyzed by TfOH in the presence of lactose acceptor **9** lead to trisaccharide intermediate **37**. Following established protocol lead to the formation of 6-deoxy-α-Gal derivative **8**.

Binding avidity analysis of deoxy-α-Gal derivatives.— The evaluation of the binding affinities of synthesized deoxy-α-Gal derivatives **5**–**8** to human anti-α-Gal anti-

bodies was accomplished by inhibition enzyme-linked immunosorbent assay (ELISA).[24] Purified human (male, blood type AB) polyclonal anti-α-Gal antibody was used as the primary antibody, and mouse laminin was used as the natural source of α-Gal. Studies have suggested that anti-Gal antibodies are encoded by a small number of conserved germline genes[7b] demonstrating limited clonal diversity.[25] This, in general, may account for the specificity observed in recognizing the α-D-Gal-(1→3)-D-Gal moiety.[26] Therefore, it is not believed that there will be any significant difference in molecular recognition between the various anti-Gal antibodies clones for this evaluation. The concentrations of deoxy-α-Gal derivatives at 50% inhibition (IC_{50}) of anti-α-Gal antibody binding to α-Gal on the mouse laminin was measured and compared to standard compounds α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-GlcNHAc (**39**) and α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Glc-OBn (**40**). Substitution of the C-6 hydroxyl group with hydrogen did not significantly affect the inhibition activities of anti-α-Gal antibodies, compared to the standard compounds **39** and **40**. In contrast, removal of the polar group at the C-4 position drastically lowered inhibition nearly 15-fold. This result was expected since the C-4 hydroxyl group is a key polar group involved in anti-α-Gal antibody binding to α-Gal. Hydroxyl substitution at the C-3 position exhibited comparable inhibition with only a relatively small decrease in the IC_{50}. As expected substitution of the C-2 hydroxyl group also decreased anti-α-Gal antibody recognition. The effects were nearly tenfold as compared to the standard compounds **39** and **40**. These results indicate that the key protein–carbohydrate interaction between α-Gal and anti-α-Gal antibodies tolerates substitution at the C-6 and C-3 position of the terminal galactose residue of α-Gal. C-4 and C-2 substitution is detrimental to the binding between the protein and carbohydrate epitope (Table 1).

Table 1
ELISA assay results for deoxy-α-Gal derivatives

Compound	IC_{50} μM
α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Glc-NHAc (**39**)	1.2
α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Glc-OBn (**40**)	5.7
2-Deoxy-α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Glc-NHAc (**5**)	50.1
3-Deoxy-α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Glc-NHAc (**6**)	21.5
4-Deoxy-α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Glc-NHAc (**7**)	92
6-Deoxy-α-D-Gal-(1→3)-β-D-Gal-(1→4)-β-D-Glc-NHAc (**8**)	4.0

3. Conclusions

In summary, four monodeoxy derivatives of the α-Gal epitope were synthesized via facile carbohydrate transformations. High-yielding and stereoselective glycosylations were employed to incorporate the crucial α-D-Gal-(1→3)-Gal linkage. The derivatives were all assessed for biological activity using ELISA. The results indicate that removal of the hydroxyl group at the C-6 and the C-3 position does not appear to significantly decrease anti-α-Gal antibody recognition. This data then suggests the potential to attach a variety of functional groups at these positions generating a large array of α-Gal derivatives for use in further binding studies. Such derivatives will not only serve as tools for structure–binding experiments but may also prove to have practical applications in cell xenotransplantations.

4. Experimental

General.—^{1}H and ^{13}C NMR spectra were recorded on Varian 300 MHz, VXR400 and Unity 500 MHz spectrometers. Mass spectra (ESI, FAB, EI) were run on Kratos MS-80 and MS-50 instruments at Wayne State University. HRMS were run at the University of California–Riverside. Thin-layer chromatography (TLC) was conducted on precoated Whatman K6F Silica Gel 60 Å TLC plates with a fluorescent indicator. EM Science Silica Gel 60 (230–400 Mesh) was used for column chromatography. Optical absorptions for the ELISA assays were determined on a BioRad microplate reader model 550 at wavelengths of 650 and 450 nm. Type AB human serum, peroxidase-conjugated goat anti-human IgG antibody, and mouse laminin (a basement membrane glycoprotein containing 50–70 α-Gal epitopes per molecule) were purchased from commercial sources.

Compounds **39** and **40** were synthesized using previous protocols.[27] Lactose azide **9** was supplied from the previous synthesis.[14] Size-exclusion chromatography was performed on BioGel P2 resin and on Sephadex G-15 resin using distilled water as the eluent. Other reagents were obtained from commercial sources.

1,3,4,6-Tetra-O-acetyl-2-deoxy-2-iodo-α-D-talose (**13**).—3,4,6-Tri-*O*-acetyl-D-galactal (**12**, 1.00 g, 3.67 mmol) was dissolved in CH_3CN (20 mL) and cooled to −15 °C. CAN (5.23 g, 9.54 mmol) and glacial HOAc (2 mL) were added to the stirring mixture. Next NaI (715 mg, 4.77 mmol) in CH_3CN (10 mL) was added dropwise over approximately 30 min. The solution was then allowed to gradually warm to rt. After 4 h the reaction was complete, and the mixture was washed with 10% $Na_2S_2O_3$, satd aq $NaHCO_3$, and brine. The organic layer was then dried over anhyd Na_2SO_4, concentrated and purified by column chromatography (1:2

EtOAc–hexanes) to afford the deoxyiodotalose derivative **13** (1.18 g, 70%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 6.48 (s, 1 H), 5.41 (s, 1 H), 4.87 (broad s, 1 H), 4.34 (d, *J* 6.5 Hz, 1 H), 4.26 (s, 1 H), 4.17 (d, *J* 5 Hz, 2 H), 2.17 (s, 3 H), 2.12 (s, 3 H), 2.07 (s, 3 H), 2.02 (s, 3 H); ^{13}C NMR (125.68 MHz, $CDCl_3$): δ 170.6, 170.1, 169.7, 168.3, 96.2 (C-1), 69.2, 65.0, 61.6, 21.1, 21.0, 20.8, 19.1. FABMS(+): Calcd for $C_{14}H_{19}IKO_9$, *m*/*z* 496.97; Found, 497.01.

3,4,5-Tri-O-*acetyl-2-deoxy-2-iodo-α*-D-*talopyranosyl-(1→3)-2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl)-(1→4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranosyl azide* (**14**).—A suspension of iodotaloside **13** (500 mg, 1.09 mmol), acceptor **9** (743 mg, 1.20 mmol) and 4 Å MS (600 mg) was stirred at ambient temperature for 30 min before being cooled to −30 °C. Next TMS-OTf (0.049 mL, 0.273 mmol) was added dropwise. After 90 min the reaction was complete as determined by TLC, and it was quenched with Et_3N. The mixture was then filtered through a Celite pad, diluted with CH_2Cl_2 and washed. The diluted product was then dried, filtered and concentrated in vacuo. The crude product was then purified by column chromatography (1:1.5 EtOAc–hexanes) to afford compound **14** (600 mg, 54%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.47 (s, 1 H), 5.29 (d, *J* 3.0 Hz, 2 H), 5.14 (t, *J* 9.3 Hz, 1 H), 4.98 (dd, *J* 10.5, 7.8 Hz, 1 H), 4.79 (t, *J* 9.0 Hz, 1 H), 4.60–4.57 (m, 2 H), 4.43–4.34 (m, 2 H), 4.16–3.97 (m, 7 H), 3.82–3.63 (m, 4 H), 2.11 (s, 6 H), 2.06–1.98 (m, 21 H); ^{13}C NMR (75.46 MHz, $CDCl_3$): δ 170.7, 170.5, 170.4,170.1, 169.7, 169.5, 168.9, 101.1, 99.9, 87.8, 75.7, 74.9, 73.8, 72.6, 71.1, 70.1, 69.9, 67.5, 65.1, 65.0, 61.9, 61.7, 61.1, multiple 21.1–20.6, 19.1. ESIMS(+): Calcd for $C_{36}H_{48}IN_3NaO_{23}$, *m*/*z* 1040.16; Found, 1039.95.

3,4,5-Tri-O-*acetyl-2-deoxy-α*-D-lyxo-*hexopyranosyl-(1→3)-2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranosyl azide* (**15**).—To a mixture of compound **14** (360 mg, 0.35 mmol) and Bu_3SnH (0.24 mL, 0.885 mmol) in distilled benzene (5 mL) was added Et_3B (1 M in hexanes, 0.089 mL, 0.088 mmol). After 30 min the reaction was complete, and the solvent was removed in vacuo. The crude mixture was then diluted with EtOAc and worked up. The solvent was then dried over anhyd Na_2SO_4, filtered, concentrated and purified by column (1:1 EtOAc–hexanes) to afford compound **15** (200 mg, 63%). ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.34 (d, *J* 3.0 Hz, 1 H), 5.29 (d, *J* 2.0 Hz, 1 H), 5.21–5.17 (m, 2 H), 5.04 (dd *J* 10.0, 8.0 Hz, 1 H), 4.98 (dt, *J* 11.5, 3.8 Hz, 1 H), 4.84 (t,d, *J* 10.0, 1.0 Hz, 1 H), 4.62 (d, *J* 8.5 Hz, 1 H), 4.45 (d, *J* 12 Hz, 1 H) 4.40 (d, *J* 7.0 Hz, 1 H), 4.16–3.97 (m, 7 H), 3.83–3.76 (m, 3 H), 3.71–3.68 (m, 1 H), 2.14 (s, 3 H), 2.11–2.03 (m, 25 H), 1.94 (s, 3 H) 1.70–1.65 (C-2, m, 2 H); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 170.7, 170.6, 170.5,170.4, 170.1, 169.8, 169.7, 169.0, 101.3, 94.3, 87.8, 75.7, 75.0, 72.7, 72.4, 71.2, 70.9, 69.9, 67.5, 66.3, 65.9, 64.9, 62.2, 61.9, 61.3, 31.4 multiple 20.9–20.7. ESIMS(+): Calcd for $C_{36}H_{49}N_3NaO_{23}$, *m*/*z* 914.27; Found, 914.38.

2-Deoxy-α-D-lyxo-*hexopyranosyl-(1→3)-β*-D-*galactopyranosyl-(1→4)-β*-D-*glucopyranosyl azide* (**5**).—Compound **15** (160 mg, 0.18 mmol) was dissolved in anhyd MeOH, followed by the addition of NaOMe to pH 9. After 2 h, Dowex 50W × 2-100 (H^+) was added to neutralize the reaction mixture. The resin was then filtered and the filtrate was evaporated to afford **5** (90 mg, >95%). ^{1}H NMR (500 MHz, D_2O): δ 5.06 (broad, 1 H), 4.32 (d, 1 H), 3.89–3.42 (m, 17 H), 3.21 (broad, 1 H), 1.78–1.72 (m, 2 H); ^{13}C NMR (125.6 MHz, D_2O): δ 102.8, 93.4, 90.0, 77.9, 76.77, 75.6, 75.3, 74.5, 72.6, 71.0, 69.7, 67.6, 64.8, 64.1, 61.6, 61.1, 60.0, 31.3. HRFABMS(+): Calcd for $C_{18}H_{31}N_3NaO_{14}$, *m*/*z* 536.1704; Found, 536.1714.

Phenyl 1-thio-β-D-*galactopyranoside* (**18**).—Commercially available **16** (10.0 g, 25.7 mmol) was dissolved in anhyd CH_2Cl_2 (150 mL) and cooled to 0 °C. Thiophenol (3.43 mL, 33.4 mmol) was added to the mixture with stirring for 30 min. Then $BF_3{\cdot}Et_2O$ (9.77 mL, 77.0 mmol) was slowly injected into the mixture. After 4 h the reaction was complete, and the mixture was diluted and washed with satd aq $NaHCO_3$ and brine. After drying over anhyd Na_2SO_4, the solvent was removed in vacuo, and the crude product was purified by column chromatography (1:5 EtOAc–hexanes) to afford compound **17** (9.7 g, 86%). To a mixture of compound **17** (9.7 g, 22.0 mmol) in anhyd MeOH (100 mL), NaOMe powder was added in small portions to pH 9. After 2 h the solution was neutralized using Dowex 50W × 2-100 (H^+). The resin was then filtered off and the solvent was removed in vacuo to afford a powdery solid **18** (5.99 g, >99%). ^{1}H NMR (400 MHz, D_2O): δ 7.58–7.55 (m, 2 H), 7.42–7.35 (m, 3 H), 4.76 (d, *J* 9.6 Hz, 1 H, H-1), 3.97 (d, *J* 3.2 Hz, 1 H), 3.76–3.59 (m, 5 H); ^{13}C NMR (100.61 MHz, D_2O): δ 133.0, 131.4, 129.6, 88.3 (C-1), 79.3, 74.3, 69.5, 68.9, 61.2. FABMS(+): Calcd for $C_{12}H_{16}NaO_5S$, *m*/*z* 295.06; Found, 295.18.

Phenyl 3,4-O-*isopropylidene-1-thio-β*-D-*galactopyranoside* (**19**).—Compound **18** (5.99 g, 22.0 mmol) was dissolved in a 1:1 mixture of dry CH_2Cl_2 and dimethoxypropane (30 mL). $TsOH{\cdot}H_2O$ (245 mg) was then added to the solution. After 3 h the reaction was concentrated in vacuo and diluted with $CHCl_3$. The solution was washed with satd aq $NaHCO_3$ and brine, then dried over Na_2SO_4. The solvent was then removed in vacuo. Next 5% HOAc (10 mL) was added. After 30 min the solution was extracted with $CHCl_3$ (3 × 20 mL) and washed with satd aq $NaHCO_3$ and brine. The organic layer was then dried over Na_2SO_4 and concentrated. Purification by column chromatography (2:1 EtOAc–hexanes) afforded compound **19** (3.43 g, 50.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53–7.50 (m, 2 H), 7.33–7.26 (m, 3 H), 4.46 (d, *J* 10.4 Hz, 1 H, H-1), 4.17 (dd, *J* 5.6, 2.0 Hz, 1 H), 4.13-4.08 (m, 1 H),

3.99–3.94 (dd, *J* 11.2, 7.2 Hz, 1 H), 3.88–3.85 (m, 1 H), 3.79 (dd, *J* 11.2, 3.6, 1 H), 3.56 (dd, *J* 10.4, 7.2 Hz, 1 H), 1.41 (s, 3 H), 1.32 (s, 3 H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 132.6, 132.2, 129.3, 128.3, 110.7, 87.9 (C-1), 79.5, 74.1, 71.7, 62.8, 28.6, 26.6. FABMS(+): Calcd for $C_{15}H_{20}NaO_5S$ *m*/*z* 335; Found 335.

Phenyl 2,6-di-O-*benzyl-1-thio-β*-D-*galactopyranoside* (**20**).—In a flame-dried flask equipped with a dropping funnel under nitrogen compound **19** (3.35 g, 10.73 mmol) was dissolved in dry DMF (25 mL) and cooled to 0 °C. NaH (3.43 g, in 60% oil dispersion, 8 equiv) was added in small portions over 1 h. Afterwards benzyl bromide (10.21 mL, 85.8 mmol) was added dropwise to the stirring solution over a period of 1 h. The resulting mixture was allowed to stir overnight at ambient temperature. MeOH (10 mL) was added to quench the excess NaH. The mixture was extracted with $CHCl_3$ (3 × 60 mL), and the extract was washed with satd aq $NaHCO_3$, and brine. The organic layer was then dried over Na_2SO_4 and concentrated in vacuo. Column chromatography (1:2 EtOAc–hexanes) of the residue afforded the benzylated intermediate (4.55 g, 86.0%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.58–7.55 (m, 2 H), 7.45–7.22 (m, 13 H), 4.83 (d, *J* 11.6 Hz, 1 H), 4.70 (d, *J* 12.0 Hz, 1 H), 4.67 (d, *J* 10.0 Hz, 1 H, H-1), 4.61 (d, *J* 12.0 Hz, 1 H), 4.54 (d, *J* 12.0 Hz, 1 H), 4.27 (m, 1 H), 4.27 (dd, *J* 6.0, 1.6 Hz, 1 H), 3.96 (m, 1 H), 3.84–3.77 (m, 2 H), 3.54 (dd, *J* 9.6, 6 Hz, 1 H), 1.42 (s, 3 H), 1.37 (s, 3 H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 138.5, 138.1, 134.2, 132.0, 129.0, 128.6, 128.5, 128.4, 127.9, 127.5, 110.3, 86.5 (C-1), 79.8, 78.5, 76.0, 74.1, 73.8, 73.7, 69.9, 28.0, 26.6. FABMS(+): Calcd for $C_{29}H_{32}KO_5S$, *m*/*z* 531; Found, 531. The benzylated intermediate (4.55 g, 9.24 mmol) was dissolved in 80% HOAc (40 mL) and heated to 80 °C. After 4 h the reaction was complete, and the solution was allowed to cool to rt. The solution was then concentrated in vacuo, and the residue was dissolved in $CHCl_3$ (30 mL). After workup, the organic layer was dried, and the solvent was removed to afford compound **20** (3.82 g, 91.0%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60–7.57 (m, 2 H), 7.39–7.25 (m, 13 H), 4.94 (d, *J* 10.8 Hz, 1 H), 4.70, (d, *J* 10.2 Hz, 1 H), 4.63 (d, *J* 9.6 Hz, 1 H, H-1), 4.03 (d, *J* 1.8 Hz, 1 H), 3.80 (s, 1 H), 3.67–3.58 (m, 3 H); ^{13}C NMR (75.46 MHz, $CDCl_3$): δ 138.3, 137.9, 134.2, 131.9, 129.2, 128.8, 128.7, 128.6, 128.3, 128.1, 128.0, 127.7, 87.8 (C-1), 78.4, 75.6, 75.3, 74.0, 69.9, 69.8. FABMS(+): Calcd for $C_{26}H_{28}KO_5S$, *m*/*z* 491; Found, 491.

Phenyl 4-O-*acetyl-2,6-di*-O-*benzyl-1-thio-β*-D-*galactopyranoside* (**21**).—Compound **20** (3.82 g, 8.44 mmol) was added to a mixture of dry benzene (50 mL), triethyl orthoacetate (30 mL), and TsOH·H_2O (60 mg). After approximately 1 h, Et_3N (5 mL) was added, and the solution was concentrated in vacuo. The residue was then dissolved in 60% HOAc (60 mL) and stirred at rt for 20 min. The mixture was then concentrated in vacuo and extracted with CH_2Cl_2. The extracts were washed with satd aq $NaHCO_3$ and water, and the organic layer was dried over Na_2SO_4. The solvent was removed in vacuo, and the crude residue was purified by column chromatography (1:3 EtOAc–hexanes) to afford compound **21** (3.40 g, 87.2%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.60–7.57 (m, 2 H), 7.40–7.25 (m, 13 H), 5.38 (d, *J* 2.0 Hz, 1 H), 4.93 (d, *J* 10.8 Hz, 1 H), 4.68 (d, *J* 9.3 Hz, 1 H, H-1), 4.67 (d, *J* 10.5 Hz, 1 H), 4.54 (d, *J* 11.7 Hz, 1 H), 4.43 (d, *J* 11.7 Hz, 1 H), 3.86–3.76 (m, 2 H), 3.63–3.50 (m, 3 H), 2.08 (s, 3 H); ^{13}C NMR (75.46 MHz, $CDCl_3$): δ 171.4, 138.1, 137.9, 134.1, 134.1, 131.8, 129.2, 128.8, 128.6, 128.5, 128.3, 128.1, 128.0, 127.7, 87.9 (C-1), 78.2, 76.3, 75.7, 74.1, 73.8, 70.5, 68.6, 21.1; FABMS(+): Calcd for $C_{28}H_{30}NaO_6S$, *m*/*z* 517; Found, 517.

Phenyl 4-O-*acetyl-2,6-di*-O-*benzyl-3-deoxy-1-thio-β*-D-xylo-*hexopyranoside* (**22**).—To the mixture of **21** (1.109 g, 2.24 mmol) dissolved in dry CH_2Cl_2 (50 mL) was added phenyl chlorothionocarbonate (PTC–Cl, 0.93 mL, 6.7 mmol) and cooled to 0 °C. Then DMAP (2.46 g, 20.2 mmol) was added in small portions. The solution was stirred overnight at ambient temperature. The mixture was then diluted with CH_2Cl_2 (50 mL) and washed with satd aq $NaHCO_3$, brine and phosphate buffer solution (pH 7.4). The organic layer was then dried over Na_2SO_4 and concentrated in vacuo. The crude residue was then dissolved in dry benzene (80 mL) under Ar, and Bu_3SnH (6.03 mL, 22.4 mmol) was added. The reaction mixture was then heated to 80 °C, followed by the addition of AIBN. After nearly 2 h the reaction was concentrated in vacuo, and the residue was dissolved in CH_3CN (50 mL). Next the diluted product mixture was extracted several times with hexanes. The CH_3CN layer was then concentrated in vacuo, and the residue was purified by flash column chromatography (8:1 EtOAc–hexanes) to afford compound **22** (0.74 g, 64.3%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.60–7.57 (m, 2 H), 7.35–7.24 (m, 13 H), 5.14 (d, *J* 1.6 Hz, 1 H), 4.78 (d, *J* 9.2 Hz, 1 H, H-1), 4.71 (d, *J* 11.6 Hz, 1 H), 4.58 (d, *J* 11.2 Hz, 1 H), 4.55 (d, *J* 11.6 Hz, 1 H), 4.42 (d, *J* 12.0 Hz, 1 H), 3.82–3.79 (m, 1 H), 3.65–3.52 (m, 3 H), 1.98 (s, 3 H) 1.85 (C-3, m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 170.4, 138.1, 137.9, 134.3, 131.7, 129.0, 128.6, 128.3, 128.1, 128.0, 127.9, 127.4, 89.6 (C-1), 78.1, 73.7, 72.7, 71.8, 68.8, 68.3, 35.2, 21.2. FABMS(+): Calcd for $C_{28}H_{30}KO_5S$, *m*/*z* 517; Found, 517.

4-O-*Acetyl-2,6-di*-O-*benzyl-3-deoxy-α*-D-xylo-*hexopyranosyl-(1→3)-2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranosyl azide* (**24**).—A suspension of acceptor **9** (0.388 g, 0.627 mmol), donor **22** (0.300 g, 0.570 mmol), 4 Å MS (1.2 g), and anhyd CH_2Cl_2 (8 mL) was stirred for 1 h at ambient temperature under Ar. The mixture was then

cooled to −78 °C and *N*-iodosuccinamide (190 mg, 0.845 mmol) was added. Next triflic acid (25.0 μL, 0.31 mmol) was added slowly to the mixture. After 1 h, all of the donor was consumed, and the reaction mixture was diluted with CH_2Cl_2 (20 mL) and filtered through a Celite pad. The filtrate was then washed with satd aq $NaHCO_3$, 10% $Na_2S_2O_3$ and dried over Na_2SO_4. The solvent was then removed in vacuo, and the crude residue was purified by flash column chromatography (1:2 EtOAc–hexanes) to afford trisaccharide **24** (407 mg, 72.4%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.35–7.26 (m, 10 H), 5.47 (d, *J* 2.8 Hz, 1 H), 5.19 (t, *J* 8.4 Hz, 1 H), 5.11 (dd, *J* 10.0, 8.0 Hz, 1 H), 5.05 (d, *J* 2.8 Hz, 1 H), 4.84 (t, *J* 9.2 Hz, 1 H), 4.62–4.43 (m, 6 H), 4.36 (d, *J* 8.0 Hz, 1 H), 4.14–4.08 (m, 2 H), 4.05 (d, *J* 6.4 Hz, 2 H), 3.96 (m, 1 H), 3.88–3.63 (m, 6 H), 3.46–3.39 (m, 2 H), 2.09 (s, 3 H), 2.08 (s, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 2.01 (s, 3 H), 1.94 (C-3, m, 2 H),1.90 (s, 3 H), 1.87 (s, 3 H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 170.6, 170.5, 170.2, 169.8, 169.7, 128.6, 128.5, 128.0, 127.9, 127.8, 101.3 (C-1′), 93.4, (C-1″), 87.8 (C-1), 75.7, 75.0, 73.5, 72.8, 72.6, 71.2, 71.1, 70.6, 70.4, 68.6, 68.5, 68.2, 64.6, 62.1, 61.5, 28.3, 21.1, 21.0, 20.9, 20.9, 20.8, 20.6. ESIMS(+): Calcd for $C_{46}H_{57}N_3NaO_{21}$, *m*/*z* 1010.34; Found, 1010.47.

4-O-*Acetyl-2,6-di*-O-*benzyl-3-deoxy*-α-D-xylo-*hexopyranosyl)-(1→3)-2,4,6-tri*-O-*acetyl*-β-D-*galactopyranosy)-(1→4)*-N-*acetyl-2,3,6-tri*-O-*acetyl*-β-D-*glucopyranosylamine* (**25**).—Trisaccharide **24** (350 mg, 0.334 mmol) in EtOH was agitated with PtO_2 (Adams' catalyst, 20 mg) under a hydrogen (50 lb/in^2) atmosphere for 2 h. After completion, the PtO_2 was filtered, and the filtrate was concentrated in vacuo. The residue was then dissolved in dry CH_2Cl_2 (10 mL) with Et_3N (1 mL) and Ac_2O (0.3 mL) at 0 °C. The mixture was warmed to rt and stirred for 2 h. The solution was then diluted with CH_2Cl_2 (10 mL) and washed with 0.1 M HCl, satd aq $NaHCO_3$, and brine. The organic layer was then dried over Na_2SO_4 and concentrated in vacuo. The crude reside was then purified by column chromatography (1:1 EtOAc–hexanes) to afford compound **25** (238 mg, 71.2%).^{1}H NMR (500 MHz, $CDCl_3$): δ 7.31–7.22 (m, 10 H), 5.45 (d, *J* 3.5 Hz, 1 H), 5.24 (t, *J* 9.5 Hz, 1 H), 5.18–5.09 (m, 2 H), 5.01 (broad s, 1 H), 4.79 (d, *J* 9 Hz, 1 H), 4.60–4.32 (m, 6 H), 4.11–3.92 (m, 4 H), 3.87 (dd, *J* 10.5, 3.5 Hz, 1 H), 3.73–3.68 (m, 3 H), 3.64 (dt, *J* 12.0, 3.5 Hz, 1 H), 3.45–3.35 (m, 4 H), 2.06 (s, 3 H), 2.05 (s, 3 H), 2.02 (s, 3 H), 2.01 (s, 3 H), 1.99 (s, 3 H), 1.95 (s, 3 H), 1.93 (C-3, m, 2 H), 1.87 (s, 3 H), 1.84 (s, 3 H); ^{13}C NMR (125.6 MHz, $CDCl_3$): δ 171.5, 170.6, 170.57, 170.5, 170.2, 169.5, 168.9, 138.2, 138.1, 128.6, 128.5, 128.4, 128.0, 127.9, 127.8, 127.80, 127.7, 100.9 (C-1′), 93.4 (C-1″), 78.2 (C-1), 75.7, 74.7, 73.5, 72.7, 72.6, 71.2, 71.1, 71.05, 70.5, 70.4, 68.6, 68.3, 68.2, 64.7, 62.3, 61.5, 28.6 (C-3), 21.1, 21.0, 20.9, 20.9, 20.8, 20.7, 20.6, 20.5. ESIMS(+): Calcd for $C_{48}H_{61}NNaO_{22}$, *m*/*z* 1026.36; Found, 1026.31.

3-Deoxy-α-D-xylo-*hexopyranosyl-(1→3)*-β-D-*galactopyranosyl-(1→4)*-N-*acetyl*-β-D-*glucopyranosylamine* (**6**).—$Pd(OH)_2$/C (10% wt, 30 mg) was added to a mixture of **25** (238 mg, 0.237 mmol) in MeOH (30 mL). (**Caution!** Extreme fire hazard.) The suspension was charged with hydrogen (50 lb/in^2) and agitated overnight. The mixture was then filtered, and the filtrate was evaporated in vacuo. The residue was then dissolved in anhyd MeOH, followed by the addition of NaOMe powder to pH 9. After 2 h, Dowex 50W × 2-100 (H^+) was added to neutralize the mixture. The resin was then filtered, and the filtrate was evaporated to afford **6** in quantitative yield (125 mg). Further purification was done by gel filtration (Sephadex 25). ^{1}H NMR (500 MHz, D_2O): δ 4.88 (d, *J* 3.0 Hz, 1 H), 4.79 (d, *J* 9.5 Hz, 1 H), 4.36 (d, *J* 7.5 Hz, 1 H), 4.00–3.89 (m, 4 H), 3.75 (d, *J* 12 Hz, 1 H), 3.69–46 (m, 11 H), 3.26 (t, *J* 7.0 Hz, 1 H), 1.88 (s, 3 H) 1.85 (C-3, m, 2 H); ^{13}C NMR (125.6 MHz, D_2O): δ 175.6, 102.9, 94.7, 79.2, 78.1, 77.1, 76.4, 75.2, 71.5, 70.6, 69.7, 67.0, 65.0, 63.6, 63.4, 61.3, 61.1, 60.0, 32.9, 23.4. ESIMS (+): *m*/*z* Calcd 552.19, Found 552.04. HRFABMS(+): Calcd for $C_{20}H_{35}NNaO_{15}$, *m*/*z* 552.1904; Found, 522.1914.

Phenyl 2,3,4,6-tetra-O-*acetyl-1-thio*-β-D-*glucopyranoside* (**27**).—Compound **26** (15.0 g, 38.43 mmol) was dissolved in anhyd CH_2Cl_2 (150 mL) and cooled to 0 °C. Then thiophenol (11.83 mL, 115.29 mmol) was added. After 30 min $BF_3{\cdot}Et_2O$ (6.34 mL, 50.00 mmol) was slowly injected into the mixture. After 4 h the reaction was complete as monitored by TLC and diluted with CH_2Cl_2 (100 mL) and washed with satd aq $NaHCO_3$ and brine. After drying over Na_2SO_4, the solvent was removed in vacuo. Purification by column chromatography (1:5 EtOAc–hexanes) afforded compound **27** (13.33 g, 79%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.50–7.46 (m, 2 H), 7.32–7.25 (m, 3 H), 5.25–5.18 (m, 1 H), 5.06–4.93 (m, 2 H), C-1 4.69 (d, *J* 10.2 Hz, 1 H), 4.26–4.13 (m, 2 H), 3.74–3.68 (m, 1 H), 2.08 (s, 3 H), 2.07 (s, 3 H), 2.01 (s, 3 H), 1.98 (s, 3 H). ^{13}C NMR (75.46 MHz, $CDCl_3$): δ 170.8, 170.4, 169.6, 169.5, 133.3, 131.8, 129.1, 128.6, C-1 85.9, 75.9, 74.1, 70.1, 68.4, 62.3, 20.9, 20.8. FABMS(+): Calcd for $C_{20}H_{24}NaO_9S$, *m*/*z* 463; Found, 463.

Phenyl 4,6-O-*benzylidene-1-thio*-β-D-*glucopyranoside* (**28**).—To a mixture of compound **27** (13.00 g, 29.5 mmol) in anhyd MeOH (150 mL), solid NaOMe was added in small portions to pH 9. After 2 h the solution was neutralized using Dowex 50W × 2-100 (H^+). The resin was then filtered off, and the solvent removed in vacuo to afford a powdery solid (8.03 g). The powdery solid was dissolved in a solution of CH_3CN (300 mL) and α,α-dimethoxytoluene (6.6 mL 44.1 mmol). To the suspension was added TsOH (120 mg). The reaction mixture stirred overnight and was neutralized with Et_3N (2 mL). The slurry was then concentrated and

purified by column (20:1 $CHCl_3$–MeOH) to afford compound **28** (6.85 g, 86%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.45–7.36 (m, 4 H), 7.26–7.20 (m, 6 H) 5.42 (s, 1 H), C-1 4.55 (d, *J* 9.6 Hz, 1 H), 4.24 (dd, *J* 10.4, 4.0 Hz, 1 H), 3.77 (s, broad, 2 H), 3.69–3.60 (m, 2 H), 3.42–3.23 (m, 3 H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 137.1, 132.7, 129.3, 129.1, 128.3, 128.2, 125.4, 101.9, (C-1) 88.7, 80.4, 74.7, 72.9, 70.5, 68.7, 49.5, 49.3, 49.1, 48.9, 48.4; ESIMS(+) Calcd for $C_{19}H_{20}NaO_5S$, *m*/*z* 383.89; Found, 382.96.

Phenyl 2,3-di-O-*benzyl-4,6*-O-*benzylidene-1-thio-β*-D-*glucopyranoside* (**29**).—In a flame-dried flask equipped with a dropping funnel under nitrogen compound **28** (6.80 g, 18.86 mmol) was dissolved in dry DMF (60 mL) and cooled to 0 °C. NaH (6.1 g, in 60% oil dispersion, 8 equiv) was added in small portions over 1 h. Benzyl bromide (18.08 mL, 152.0 mmol) was then added dropwise to the stirring mixture over a period of 1 h, which was allowed to stir overnight at ambient temperature. MeOH (30 mL) was used to quench the excess NaH. The reaction mixture was then was poured over ice-water. The mixture was extracted with $CHCl_3$ (3 × 60 mL), and the extract was washed with satd aq $NaHCO_3$, and brine. The organic layer was then dried over Na_2SO_4 and concentrated in vacuo. Flash column chromatography (1:2 EtOAc–hexanes) afforded compound **29** (8.21 g, 80.0%). ^1H NMR (500 MHz, $CDCl_3$): δ 7.56–7.50 (m, 4 H), 7.41–7.30 (m, 16 H), 5.61 (s, 1 H), 4.96 (d, *J* 11.0 Hz, 1 H), 4.89–4.77 (m, 4 H), 4.13 (dd, *J* 10.5, 5.0 Hz, 1 H), 3.88–3.80 (m, 2 H), 3.73 (t, *J* 9.0 Hz, 1 H) 3.56–3.47(m, 2 H); ^{13}C NMR (125.7 MHz, $CDCl_3$): δ 138.5, 138.2, 137.4, 132.6, 129.2, 129.2, 128.6, 128.5, 128.4, 128.3, 128.1, 128.0, 126.2, 101.3, 88.5, 83.2, 81.7, 80.6, 76.1, 75.5, 70.4, 68.9. ESIMS(+) Calcd for $C_{33}H_{32}O_5SNa^+$, *m*/*z* 563.18; Found 563.00.

Phenyl 2,3,6-tri-O-*benzyl-1-thio-β*-D-*glucopyranoside* (**30**).—Compound **29** (1.00 g, 1.85 mmol) was dissolved in a suspension of anhyd THF (30 mL) and 4 Å MS (1.00 g). To the stirring mixture was added HCl in Et_2O until the evolution of gas ceased. After 20 min the reaction was complete as monitored by TLC. The suspension was diluted with CH_2Cl_2 and filtered through a Celite pad. After workup the organic layer was dried over anhyd Na_2SO_4, filtered and concentrated in vacuo. Purification by column (1:3 EtOAc–hexanes) afforded compound **30** (530 mg, 53%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.59–7.25 (m, 20 H), 4.94 (d, *J* 2.4 Hz, 1 H), 4.92 (s, 1 H), 4.82–4.68 (m, 3 H), 4.59 (d, *J* 3.2 Hz, 2 H), 3.83–3.74 (m, 2 H), 3.70–3.65 (m, 1 H), 3.59–3.48 (m, 3 H), 2.61 (d, *J* 1.6 Hz, 1 H); ^{13}C (100.61 MHz, $CDCl_3$): δ 138.6, 138.1, 138.16, 134.0, 132.1, 129.3, 129.2, 128.9, 128.7, 128.6, 128.5, 128.2, 128.0, 127.9, 127.7, 87.9, 86.3, 80.7, 78.3, 75.7, 75.6, 73.9, 71.9, 70.6. ESIMS(+): Calcd for $C_{33}H_{34}KO_5S$, *m*/*z* 581.18; Found, 581.29.

Phenyl 2,3,6-tri-O-*benzyl-4-deoxy-1-thio-β*-D-xylo-*hexopyranoside* (**31**).—To the solution of **30** (500 mg, 0.921 mmol) dissolved in dry CH_2Cl_2 (50 mL) was added DMAP (1.01 g, 8.28 mmol) in small portions. The mixture was cooled to 0 °C, followed by the addition of phenyl chlorothionocarbonate (0.382 mL, 2.76 mmol). The solution stirred overnight at ambient temperature. The mixture was then diluted with CH_2Cl_2 (50 mL) and washed with satd aq $NaHCO_3$, brine and phosphate buffer solution (pH 7.4). The organic layer was then dried over Na_2SO_4 and concentrated in vacuo. The crude residue was then dissolved in dry benzene (20 mL) and charged with Ar. Bu_3SnH (1.982 mL, 7.37 mmol) was added, followed by gradual warming of the mixture to 80 °C. A catalytic amount of AIBN (15 mg) was added to the stirring reaction mixture. After approximately 1 h, the reaction was complete, and the mixture was cooled and concentrated in vacuo. The residue was then diluted with CH_3CN (50 mL) and extracted several times with hexanes. The CH_3CN layer was then concentrated in vacuo, and the residue was dissolved in CH_2Cl_2. The solution was then washed with 0.1 M HCl, $NaHCO_3$, and brine. Finally the solution was dried over Na_2SO_4 filtered and purified by column chromatography (1:5 EtOAc–hexanes) to afford compound **31** (400 mg, 82%). ^1H NMR (400 MHz, $CDCl_3$): δ 7.59–7.25 (m, 20 H), 4.96–4.53 (m, 7 H), 3.79–3.38 (m, 5 H) 1.71–1.62(m, 2 H); ^{13}C NMR (100.61 MHz, $CDCl_3$): δ 138.49, 138.28,137.9, 132.04, 129.89, 129.19, 129.08, 128.69, 128.65, 128.63, 128.54, 128.23, 128.05, 127.98, 127.76, 127.54, 87.9, 81.2, 80.63, 75.8, 75.4, 73.7, 72.6, 72.2, 33.9. ESIMS(+): Calcd for $C_{33}H_{34}NaO_4S$, *m*/*z* 549.21; Found, 549.03.

2,3,6-Tri-O-*benzyl-4-deoxy-α*-D-xylo-*hexopyranosyl)-(1→3)-2,4,6-tri*-O-*acetyl-β*-D-*galactopyranosyl-(1→4)-2,3,6-tri*-O-*acetyl-β*-D-*glucopyranosyl azide* (**32**).—A suspension of acceptor **8** (0.514 g, 0.835 mmol), donor **31** (0.400 g, 0.759 mmol), 4 Å MS (500 mg), and anhyd CH_2Cl_2 (6 mL) was stirred for 1 h at ambient temperature under Ar. The mixture was then cooled to −30 °C, and *N*-iodosuccinamide (196 mg) was added. Next triflic acid (12.0 μL, 0.121 mmol) was added slowly to the mixture. After 2 h, all of the acceptor was consumed, and the reaction mixture was diluted with CH_2Cl_2 (20 mL) and filtered through a Celite pad. The filtrate was then washed with satd aq $NaHCO_3$, 10% $Na_2S_2O_3$ and dried over Na_2SO_4. The solvent was then removed in vacuo, and the crude residue was purified by column chromatography (1:2 EtOAc–hexanes) to afford trisaccharide **32** (472 mg, 60.0%). ^1H NMR (400.1 MHz, $CDCl_3$): δ 7.40–7.24 (m, 15 H), 5.45 (d, *J* 3.6 Hz, 1 H), 5.18 (t, *J* 8.8 Hz, 2 H), 5.11–5.06 (m, 2 H), 4.84 (t, *J* 9.6 Hz, 1 H), 4.70 (m, 2 H), 4.65–4.60 (m, 3 H), 4.55 (s, 2 H), 4.44 (d, *J* 12.4 Hz, 1 H), 4.33 (d, *J* 7.2 Hz, 1 H), 4.12–3.98 (m 3 H), 3.87–3.67 (m, 5 H), 3.47–3.38 (m, 3 H), 2.09 (s, 3 H), 2.07 (s, 3 H), 2.06 (s,

3 H), 2.00 (s, 3 H), 1.91 (s, 3 H), 1.85 (m, 2 H), 1.80 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6, 170.5, 170.4, 169.8, 169.7, 168.9, 138.8, 138.7, 138.2, 128.6, 128.5, 128.4, 128.0, 127.9, 127.78, 127.75, 127.70, 101.4, 94.8, 87.9, 80.3, 75.7, 75.0, 74.5, 73.5, 72.7, 72.73, 72.4, 72.3, 71.2, 71.1, 70.5, 67.5, 64.6, 62.1, 61.4, 34.1 (C-4), 21.0 (m). ESIMS(+): Calcd for $C_{51}H_{61}N_3NaO_{20}$, m/z 1058.38; Found, 1058.48.

2,3,6-Tri-O-*benzyl*-*4-deoxy*-α-D-xylo-*hexopyranosyl*-*(1→3)*-*2,4,6-tri*-O-*acetyl*-β-D-*galactopyranosyl*-*(1→4)*-N-*acetyl*-*2,3,6-tri*-O-*acetyl*-β-D-*glucopyranosylamine* (**33**).—Trisaccharide **32** (470 mg, 0.476 mmol) in EtOH was agitated with PtO_2 (10 mg) under an atmosphere of hydrogen (50 lb/in^2) for 1 h. After the reaction was complete, the Pt was filtered and the filtrate was concentrated in vacuo. The residue was then dissolved in dry CH_2Cl_2 (30 mL) with Et_3N (5.6 mL) and Ac_2O (1.34 mL) at 0 °C. The mixture was then warmed to rt and stirred for 2 h. The solution was then diluted with CH_2Cl_2 (25 mL) and washed with 0.1 M HCl, satd aq $NaHCO_3$, and brine. The organic layer was then dried over Na_2SO_4 and concentrated in vacuo. The crude reside was then purified by column chromatography (1:1 EtOAc–hexanes) affording compound **33** (334 mg, 70%). ^{1}H NMR (400.1 MHz, $CDCl_3$): δ 7.40–7.24 (m, 15 H), 6.39 (broad s, 1 H), 5.45 (d, J 3.2 Hz, 1 H), 5.25 (m, 1 H), 5.18 (t, J 8.8 Hz, 1 H), 5.11–5.06 (m, 2 H), 4.80 (t, J 8.8 Hz, 1 H), 4.70–4.56 (m, 5 H), 4.53 (s, 2 H), 4.37 (d, J 11.2 Hz, 1 H), 4.30 (d, J 7.2 Hz, 1 H), 4.12–3.97 (m 3 H), 3.87–3.66 (m, 5 H), 3.43–3.33 (m, 3 H), 2.06 (s, 3 H), 2.04 (s, 3 H), 2.02 (s, 3 H), 1.99 (s, 3 H), 1.95 (s, 3 H), 1.89 (s, 3 H), 1.88 (m, 2 H), 1.79 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.5, 170.6, 170.5, 170.4, 169.8, 169.7, 168.9, 138.8, 138.7, 138.2, 128.6, 128.5, 128.4, 128.0, 127.9, 127.78, 127.75, 127.70, 101.4, 94.8, 80.3, 78.2, 75.7, 75.0, 74.5, 73.5, 72.7, 72.73, 72.4, 72.3, 71.2, 71.1, 70.5, 67.5, 64.6, 62.1, 61.4, 34.1 (C-4), 23.5, 21.0 (m). ESIMS(+): Calcd for $C_{53}H_{65}NNaO_{21}$, m/z 1074.39; Found, 1074.38.

4-Deoxy-α-D-xylo-*hexopyranosyl*-*(1→3)*-β-D-*galactopyranosyl*-*(1→4)*-N-*acetyl*-β-D-*glucopyranosylamine* (**7**).—$Pd(OH)_2$/C (10% wt, 30 mg) was added to a mixture of **33** (330 mg, 0.328 mmol) in MeOH (30 mL). (**Caution!** Extreme fire hazard.) The suspension was charged with hydrogen (50 lb/in^2) and agitated overnight. The mixture was then filtered, and the filtrate was evaporated in vacuo. The residue was then dissolved in anhyd MeOH, followed by the addition of NaOMe powder to pH 9. After 2 h, Dowex 50W × 2-100 (H^+) was added to neutralize the mixture. The resin was then filtered, and the filtrate was evaporated to afford **6** in quantitative yield (60 mg). Further purification was carried out by gel filtration (Sephadex 25). ^{1}H NMR (500 Hz, $CDCl_3$): δ 4.96 (d, J 3.5 Hz, 1 H), 4.81 (d, J 9.0 Hz, 1 H), 4.36 (d, J 8.0 Hz, 1 H) 4.05 (m, 1 H), 4.00 (d, J 3.5 Hz, 1 H), 3.89–3.83 (m, 1 H), 3.77 (d, J 12.5 Hz, 1 H) 3.65–3.48 (m, 10 H), 3.43 (m, 1 H), 3.34 (dd, J 10.0, 3.5 Hz, 1 H), 3.29 (m, 1 H), 1.91 (s, 3 H), 1.85 (m, 2 H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.6, 102.9, 96.1, 79.2, 78.1, 77.3, 76.4, 75.2, 73.2, 71.5, 71.1, 69.6, 68.9, 67.1, 65.0, 63.6, 61.1, 60.0, 34.1 (C-4), 22.2. FAB-HRMS(+): Calcd for $C_{20}H_{35}NaO_{15}$, m/z 552.1904; Found, 552.1927.

Phenyl 2,3,4-tri-O-*acetyl*-*1-thio*-β-D-*fucopyranoside* (**35**).—To a solution of compound **34** (1.00 g, 6.09 mmol) in pyridine (30 mL), Ac_2O(10 mL) and DMAP (20 mg) were added. After 6 h the mixture was concentrated in vacuo. The crude residue was then diluted with $CHCl_3$ (50 mL) and washed with 0.1 M HCl, water, satd aq $NaHCO_3$, and brine. After drying over Na_2SO_4 the solvent was removed in vacuo, and the crude residue was dissolved in anhyd CH_2Cl_2 and cooled to 0 °C. Then thiolphenol (0.7 mL, 6.82 mmol) was added. After 30 min $BF_3{\cdot}Et_2O$ (2.5 mL, 20.0 mmol) was slowly injected into the mixture. After 4 h the reaction mixture was diluted with CH_2Cl_2 (50 mL) and washed with satd aq $NaHCO_3$ and brine. After drying over Na_2SO_4 the solvent was removed in vacuo. Purification by column chromatography (1:5 EtOAc–hexanes) afforded compound **35** (2.02 g, 87.1%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50 (m, 2 H), 7.30 (m, 3 H), 5.28–5.18 (m, 2 H), 5.04 (dd, J 9.6, 3.2, 1 H), 4.68 (d, J 10.8, 1 H, H-1), 3.82 (q, J 6.3, 1 H) 2.13 (s, 3 H), 2.07 (s, 3 H), 1.95 (s, 3 H), 1.24 (d, J 6.4 Hz, 3 H, C-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.3, 169.7, 132.5, 129.0, 128.1, 86.6 (C-1), 73.3, 72.6, 70.5, 67.5, 21.0, 20.8, 20.6, 16.4 (C-6). ESIMS(+): Calcd for $C_{18}H_{22}NaO_7S$, m/z 405.10; Found, 404.99.

Phenyl 2,3,4-tri-O-*benzyl*-*1-thio*-β-D-*fucopyranoside* (**36**).—To a mixture of compound **35** (1.80 g, 4.71 mmol) in anhyd MeOH (30 mL), NaOMe powder was added in small portions to pH 9. After 2 h the solution was neutralized using Dowex 50W × 2-100 (H^+). The resin was then filtered off, and the solvent was removed in vacuo. The residue (1.00 g, 3.90 mmol) was dissolved in anhyd DMF and placed into a flame-dried flask equipped with a dropping funnel and maintained under nitrogen. The solution was cooled to 0 °C, followed by the addition of NaH (920 mg, in 60% oil dispersion, 7 equiv) over a period of 1 h. Benzyl bromide (3.0 mL, 25.2 mmol) was then slowly added dropwise to the stirring solution. The resulting mixture was allowed to stir overnight at ambient temperature. MeOH (3 mL) was then added to quench the excess NaH. The mixture was extracted with $CHCl_3$ (3 × 30 mL), and the extract was washed with satd aq $NaHCO_3$, and brine. The organic layer was then dried over Na_2SO_4 and concentrated in vacuo. Column chromatography (1:2 EtOAc–hexanes) of the residue afforded compound **36** (1.78 g, 86.8%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.67–7.24 (m, 20 H), 5.07 (d, J 11.7 Hz, 1 H), 4.83–4.75 (m, 3 H), 4.70 (d, J 9.3 Hz, 1 H, H-1), 4.63 (d, J 10.2 Hz, 1 H),

3.98 (m, 1 H), 3.68–3.56 (m, 4 H), 1.32 (d, *J* 6.3 Hz, 3 H, H-6); ^{13}C NMR (75.46 MHz, $CDCl_3$): δ 138.8, 138.4, 134.4, 131.6, 128.8, 128.5, 128.4, 128.2, 128.0, 127.8, 127.6, 127.0, 87.6 (C-1), 84.5, 76.6, 75.6, 74.6, 72.8, 72.1, 17.4 (C-6). ESIMS(+): Calcd for $C_{33}H_{34}NaO_4S$, *m*/*z* 549.21; Found, 549.05.

2,3,4-Tri-O-*benzyl*-α-D-*fucopyranosyl*-(1→3)-*2,4,6-tri*-O-*acetyl*-β-D-*galactopyranosyl*-(1→4)-*2,3,6-tri*-O-*acetyl*-β-D-*glucopyranosyl azide* (**37**).—A suspension of acceptor **9** (0.66 g, 1.06 mmol), donor **36** (0.47 g, 0.89 mmol), 4 Å MS (1.5 g), and anhyd CH_2Cl_2 (10 mL) was stirred for 1 h at ambient temperature under Ar. The mixture was then cooled to −78 °C, and *N*-iodosuccinamide (250 mg) was added. Next triflic acid (29.0 μL, 0.36 mmol) was added slowly to the mixture. After 1 h, the donor was consumed, and the reaction mixture was diluted with CH_2Cl_2 (20 mL) and filtered through a Celite pad. The filtrate was then washed with satd aq $NaHCO_3$, 10% $Na_2S_2O_3$, brine and dried over Na_2SO_4. The solvent was then removed in vacuo, and the crude residue was purified by column chromatography (1:2 EtOAc–hexanes) to afford trisaccharide **37** (790 mg, 85.7%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.36–7.24 (m, 15 H), 5.43 (d, *J* 2.7 Hz, 1 H), 5.20 (t, *J* 9.0 Hz, 1 H), 5.06 (m, 1 H), 4.97 (m, 2 H), 4.87–4.81 (m, 2 H), 4.75–4.70 (m, 2 H), 4.66–4.58 (m, 3 H), 4.46–4.42 (m, 2 H) 4.37 (d, *J* 7.8 Hz, 1 H), 4.13 (dd, *J* 12.6, 4.8 Hz, 1 H), 4.05 (d, *J* 6.6 Hz, 2 H), 3.89 (dd, *J* 10.2, 3.6 Hz, 1 H), 3.81–3.73 (m, 5 H), 3.57 (s, 1 H), 2.11 (s, 3 H), 2.07–2.06 (m, 6 H), 2.02 (s, 3 H), 1.98 (s, 3 H), 1.88 (s, 3 H), 1.09 (d, *J* 6.6 Hz, 3 H, H-6); ^{13}C NMR (75.45 MHz, $CDCl_3$): δ 170.1, 170.0, 169.7, 169.4, 169.2, 168.7, 138.7, 138.6, 138.4, 138.2, 128.3, 128.2, 128.0, 127.8, 127.5, 127.4, 127.3, 101.1 (C-1′), 96.0 (C-1″), 87.7 (C-1), 78.6, 78.1, 75.8, 75.2, 74.8, 74.8, 73.5, 73.3, 72.4, 71.1, 70.9, 70.6, 70.4, 67.2, 65.3, 61.9, 61.3, 20.4, 20.2 (m), 20.1, 16.1 (C-6). ESIMS(+): Calcd for $C_{51}H_{61}N_3NaO_{20}$, *m*/*z* 1058.37; Found, 1058.47.

2,3,4-Tri-O-*benzyl*-α-D-*fucopyranosyl*-(1→3)-*2,4,6-tri*-O-*acetyl*-β-D-*galactopyranosyl*-(1→4)-N-*acetyl*-*2,3,6-tri*-O-*acetyl*-β-D-*glucopyranosylamine* (**38**).—Trisaccharide **37** (350 mg, 0.34 mmol) in EtOH was agitated with PtO_2 (20 mg), under an atmosphere of hydrogen (50 lb/in^2) for 2 h. The Pt was then filtered, and the filtrate was concentrated in vacuo. The residue was then dissolved in dry CH_2Cl_2 (10 mL) with Et_3N (1 mL) and Ac_2O (0.3 mL) at 0 °C. The mixture was then warmed to rt and stirred for 2 h. The solution was diluted with CH_2Cl_2 (10 mL) and washed with 0.1 M HCl, satd aq $NaHCO_3$, and brine. The organic layer was then dried over Na_2SO_4 and concentrated in vacuo. The crude reside was purified by column chromatography (1:1 EtOAc–hexanes) to afford compound **38** (260 mg, 72%). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.22 (m, 15 H), 6.39 (d, *J* 8.8 Hz, 1 H), 5.41 (d, *J* 3.2 Hz, 1 H), 5.24 (m, 1 H), 5.16 (t, *J* 8.8 Hz, 1 H), 5.06 (m, 1 H), 4.97 (m, 2 H), 4.83–4.77 (m, 2 H), 4.72–4.56 (m, 4 H), 4.34 (d, *J* 10.8 Hz, 1 H), 4.29 (d, *J* 8.0 Hz, 1 H), 4.14–3.93 (m, 6 H), 3.45 (s, 1 H), 2.07 (s, 3 H), 2.05 (s, 3 H), 2.01 (s, 3 H), 1.99 (s, 3 H), 1.94 (s, 3 H), 1.90 (s, 3 H), 1.82 (s, 3 H), 1.07 (d, *J* 6.4 Hz, 3 H, H-6); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 171.4, 170.6, 170.3, 169.6, 169.4, 169.2, 168.7, 138.9, 138.8, 138.6, 128.6, 128.4, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6, 101.1 (C-1′), 95.9 (C-1″), 78.7, 78.3, 78.2, 75.9, 75.6, 75.0, 74.6, 73.8, 73.7, 73.5, 72.5, 71.3, 71.0, 70.7, 67.3, 65.4, 62.3, 61.6, 23.5, 20 (m), 16.1 (C-6). ESIMS(+): Calcd for $C_{53}H_{65}NNaO_{21}$, *m*/*z* 1074.39; Found, 1074.47.

α-D-*Fucopyranosyl*-(1→3)-β-D-*galactopyranosyl*-(1→4)-N-*acetyl*-β-D-*glucopyranosylamine* (**8**).—$Pd(OH)_2$/C (10% wt, 30 mg) was added to trisaccharide **38** (297 mg, 0.287 mmol) dissolved in MeOH (30 mL). (**Caution!** Extreme fire hazard.) The suspension was charged with hydrogen (50 lb/in^2) and agitated overnight. The mixture was then filtered, and the filtrate was evaporated in vacuo. The residue was then dissolved in anhyd MeOH, followed by the addition of NaOMe powder to pH 9. After 2 h, Dowex 50W × 2-100 (H^+) was added to neutralize the mixture. The resin was then filtered and the filtrate was evaporated to afford **4** after purification by gel filtration (Sephadex 25) (136 mg, 89.7%). ^{1}H NMR (500 MHz, D_2O): δ 4.89 (d, *J* 4.0 Hz, 1 H), 4.82 (d, *J* 10.0 Hz, 1 H), 4.36 (d, *J* 7.5 Hz, 1 H), 4.16 (q, *J* 7.5 Hz, 1 H), 3.83–3.68 (m, 2 H) 3.67–3.47 (m, 11 H), 3.30–3.26 (m, 1 H), 1.91 (s, 3 H), 1.04 (d, *J* 6.5 Hz, 3 H); ^{13}C NMR (125.6 MHz, D_2O): δ 175.6, 103.0, 96.1, 79.2, 78.1, 77.9, 76.4, 76.3, 75.2, 75.1, 72.0, 71.5, 69.6, 68.1, 67.0, 65.3, 61.1, 59.9, 22.2, 15.3 (C-6). HRFABMS(+): Calcd for $C_{20}H_{35}NNaO_{15}$, 552.1904; Found, 552.1925.

Isolation of polyclonal anti-α-Gal antibody from human serum.—Polyclonal anti-Gal antibody was isolated from commercially available human male type AB serum using an α-Gal (immobilized trisaccharide [C-Gal-(1→3)-β-(1→4)-β-GlcNAc] on Sepharose beads) affinity chromatography column.[9a,9b] The human serum was heated at 56 °C in a water bath in order to inactivate human complement. After 30 min the serum was cooled to ambient temperature and passed through the column to allow for binding between the anti-Gal antibody and the immobilized α-Gal epitope. After extensive washing with phosphate-buffered saline (PBS, pH 7.4), the bound anti-Gal antibody was eluted with glycine·HCL buffer (pH 2.8). The elute containing the antibodies, as monitored by UV spectroscopy (280 nm), was immediately adjusted to pH 7.2 using 0.1 M NaOH. The resulting antibody solution was stored as frozen aliquots (about 200 μg/mL) in PBS buffer.

ELISA inhibition assay with mouse laminin.—An ELISA assay was conducted using mouse laminin, as the solid-phase immobilized antigen. The purified human anti-Gal antibody was initially incubated at vary-

ing concentrations of the terminal deoxy α-Gal derivatives for 2 h at rt with gentle shaking. An aliquote (50 μL) of the mixture was then added to each microtiter plate (Immulon 4) well which was pre-coated with mouse laminin (50 μL/well of 10 μg/mL in 0.1 N Na_2CO_3–$NaHCO_3$ buffer, pH 9.5). After a 1.5-h incubation period at rt, unbound antibodies were washed out with PBS-tween (pH 7.4 + 0.2% Tween). Next a secondary (1/1000 peroxidase-conjugated goat anti-human IgG; 50 μL/well) antibody was introduced and allowed to incubate for approximately 1 h. After extensive washing using PBS-tween buffer solution, the substrate TMB (9:1 3,3′5,5′-tetramethylbenzidine–H_2O_2; 100 μL/well) was added. Enzymatic oxidation stained each well in varying intensities of blue, absorption was measured at 650 nm. The oxidation was quenched by the addition of 1 N H_2SO_4 (100 μL/well). Optical absorption was measured at 450 nm. PBS with secondary antibody was used as background control. Purified anti-Gal antibody with secondary antibody was positive control as maximum staining (0% inhibition). The percent inhibition was calculated using the following equation:

$$(M - S)/(M - B) = \%\ \text{inhibition}.$$

S refers to the OD_{450} reading of the sample at different concentrations of terminal deoxy α-Gal derivatives **5**, **6**, **7**, and **8**, *B* is the OD_{450} reading of the background, and *M* is the OD_{450} reading of the maximum staining. IC_{50} values were calculated from plot of the % inhibition versus the concentration of the inhibitors.

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References

1. Lafferty, K. J. *Transplant Proc*. **1999**, *31*, 11S–13S.
2. *2000 Annual Report of the U.S. Scientific Registry for Transplant Recipients and the Organ Procurement and Transplantation Network: Transplant Data*: 1990–1999. US Department of Health and Human Services, Health Resources and Services Administration, Office of Special Programs, Division of Transplantation, Rockville, MD; United Network for Organ Sharing, Richmond, VA.
3. Lanza, R. P.; Cooper, D. K. C.; Chick, L. C. *Sci. Am*. **1997**, 53–59.
4. Evans, R. W. In *Xenotransplantation*; Platt, J. L., Ed.; ASM Press: Washington, DC, 2001; pp 29–51.
5. (a) Galili, U. *Sci. Med*. **1998**, *5*, 28–37;
   (b) Cooper, D. K. C. *Clin. Trans*. **1992**, *6*, 178–183;
   (c) Cooper, D. K. C.; Good, A. H.; Koren, E.; Oriol, R.; Malcolm, A. J.; Ippolito, R. M.; Neethling, F. A.; Ye, Y.; Romano, E.; Zhudi, N. *Transplant Immunol*. **1993**, *1*, 198–205.
6. (a) Cooper, D. K. C.; Koren, E.; Oriol, R. *Immunol. Rev*. **1994**, *141*, 31–58;
   (b) Sandrin, M. S.; Vaughan, H. A.; Mckenzie, I. F. C. *Transplant Rev*. **1994**, *8*, 134–149;
   (c) Samuelsson, B. E.; Rydberg, L.; Breimer, M. E.; Backer, A.; Gustavsson, M.; Holgersson, J.; Karlsson, E.; Uyterwaal, A.-C.; Cairns, T.; Welsh, K. *Immunol. Rev*. **1994**, *141*, 151–168;
   (d) Galili, U. *Springer Semin. Immunopathol*. **1993**, *15*, 155–171;
   (e) Galili, U. *Immunol. Ser*. **1992**, *55*, 355–373;
   (f) Galili, U. *Blood Cells* **1988**, *14*, 205–220;
   (g) Hamadeh, R.; Galili, U.; Zhou, P.; Griffiss, J. *Clin. Diagn. Lab. Immunol*. **1995**, *2*, 125–131.
7. (a) Galili, U.; Anaraki, F.; Thall, A.; Hill-Black, C.; Radic, M. *Blood* **1993**, *82*, 2485–2493;
   (b) McMorrow, I. M.; Comrack, C. A.; Sachs, D. H.; DerSimonian, H. *Transplantation* **1997**, *64*, 501–510.
8. Galili, U. *Immunol. Today* **1993**, *14*, 480–482.
9. (a) Xu, Y.; Lorf, T.; Sablinski, T.; Gianello, P.; Bailin, M.; Monroy, R.; Kozlowski, T.; Awwad, M.; Cooper, D. K. C.; Sachs, D. H. *Transplantation* **1998**, *65*, 172–179;
   (b) Taniguchi, S.; Neethling, F. A.; Korchagina, E. Y.; Bovin, N.; Ye, Y.; Kobayashi, T.; Niekrasz, M.; Li, S.; Koren, E.; Oriol, R.; Cooper, D. K. C. *Transplantation* **1996**, *62*, 1379–1384;
   (c) Kroshus, T. J.; Bolman, R. M.; Dalmasso, A. P. *Transplantation* **1996**, *62*, 5–12;
   (d) Leventhal, J. R.; John, R.; Fryer, J. P.; Witson, J. C.; Derlich, J.-M.; Remiszewski, J.; Dalmasso, A. P.; Matas, A. J.; Bolman, R. M., III *Transplantation* **1995**, *59*, 294–300.
10. (a) Nagosaka, T.; Kobayashi, T.; Muramatsu, H.; Fujimoto, H.; Muramatsu, T.; Tokagi, H. *Biochem. Biophys. Res. Commun*. **1997**, *232*, 731–736;
    (b) Simon, P. M.; Neethling, F. A.; Taniguchi, S.; Goode, P. L.; Zopf, D.; Hancock, W. W.; Cooper, D. K. C. *Transplantation* **1998**, *65*, 346–353;
    (c) Rieben, R.; von Allmen, E.; Korchagina, E. Y.; Nydegger, W.; Neethling, F. A.; Kujundzic, M.; Koren, E.; Bovin, N.; Cooper, D. K. C. *Xenotransplantation* **1995**, *2*, 98–106;
    (d) Li, S.; Neethling, F.; Taniguchi, S.; Yeh, J.-C.; Kobayashi, T.; Ye, Y.; Koren, E.; Cummings, R. D.; Cooper, D. K. C. *Transplantation* **1996**, *62*, 1324–1331;
    (e) Lambrigts, D.; Sachs, D. H.; Cooper, D. K. S. *Transplantation* **1998**, *66*, 547–561.
11. Galili, U.; Matta, K. L. *Transplantation* **1996**, *62*, 256–262.
12. Lemieux, R. U.; Hindsgaul, O.; Bird, P.; Narasimhan, S. *Carbohydr. Res*. **1988**, *178*, 293–305.
13. (a) Lemieux, R. U. Frontiers in Chemistry. In *Proceedings of the 28th IUPAC Congress*; Laidler, K. J., Ed.; Pergamon Press: Oxford, 1982;
    (b) Lemieux, R. U. In *Proceedings of the VIIth International Symposium on Medicinal Chemistry*; 1984; Vol. 1, pp 39–351.
14. Zhang, W.; Wang, J.; Li, J.; Yu, L.; Wang, P. G. *J. Carbohydr. Chem*. **1999**, *18*, 1009–1017.
15. Roy, R.; Laferriere, C. A. *J. Chem. Soc., Chem. Commun*. **1990**, 1709–1711.
16. Norber, T. In *Modern Methods in Carbohydrate Synthesis*; Khan, S. H.; O'Neill, R. A., Eds.; Harwood Academic: Netherlands, 1996; p 82.
17. Roush, W. R.; Narayan, S. *Org. Lett*. **1999**, *1*, 899–902.

18. Trahanovsky, W. S.; Robbins, M. D. *J. Am. Chem. Soc.* **1971**, *93*, 5256–5258.
19. Lemieux, R. U.; Driguez, H. *J. Am. Chem. Soc.* **1975**, *97*, 4069–4075.
20. (a) Barton, D. H. R.; Blundell, P.; Dorchak, J.; Jang, D. O.; Jaszberenyi, J. C. *Tetrahedron* **1991**, *47*, 8969–8984;
(b) Barton, D. H. R.; Jang, D. O.; Jaszberenyi, J. C. *Tetrahedron* **1993**, *49*, 2793–2804;
(c) Robins, M. J.; Wilson, J. S. Regiospecific and Stereoselective 2′-Deoxygenation of a Ribonucleoside. In *Nucleic Acid Chemistry Part* 2; Townsend, L. B.; Tipson, R. S., Eds.; Wiley: New York, 1991; pp 194–200.
21. Garg, H. G.; Jeanloz, R. W. *Adv. Carbohydr. Chem. Biochem.* **1985**, *45*, 135–201.
22. Jansson, K.; Ahlfors, S.; Frejd, T.; Kihlberg, J.; Magnusson, G. *J. Org. Chem.* **1988**, *53*, 5629–5647.
23. Garegg, P. J.; Hultberg, H.; Wallin, S. A. *Carbohydr. Res.* **1982**, *108*, 97–101.
24. (a) Galili, U.; Ron, M.; Sharon, R. *J. Immunol. Methods* **1992**, *151*, 117–122;
(b) Galili, U.; LaTemple, D. C.; Radic, M. Z. *Transplantation* **1998**, *65*, 1129–1132.
25. Parker, W.; Lateef, J.; Everett, M. L.; Platt, J. L. *Glycobiology* **1996**, *6*, 499–506.
26. Galili, U.; Buehler, J.; Shohet, S. B.; Macher, B. A. *J. Exp. Med.* **1987**, *165*, 693–704.
27. (a) Wang, J.; Chen, X.; Zhang, W.; Zacharek, S.; Chen, Y.; Wang, P. G. *J. Am. Chem. Soc.* **1999**, *121*, 8174–8181;
(b) Fang, J.; Chen, X.; Zhang, W.; Wang, J.; Andreana, P.; Wang, P. G. *J. Org. Chem.* **1999**, *64*, 4089–4094;
(c) Andreana, P., Wang, J., Wang, P. G. unpublished results.