Published in final edited form as:

J Carbohydr Chem. 2014 September 1; 33(7-8): 381–394. doi:10.1080/07328303.2014.925913.

Synthesis of an Aminooxy Derivative of the Trisaccharide Globotriose Gb3

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Abstract

The synthesis of α -aminooxy trisaccharide moiety [α -D-Gal-(1,4)- β -D-Gal-(1,4)- β -D-Glc- α -aminooxy], related to the cell surface globotriaosylceramide (Gb3) receptor of the B subunit of the AB₅ Shiga toxin of *Shigella dysenteriae*, has been synthesized for the first time in 11 steps with a 15% overall isolated yield. A highlight of this work entails utilizing chemically compatible synthetic transformations, including those related to glycosylation, incorporative of the succinimidyl moiety as a precursor to the aminooxy Gb3 derivative. The fully deprotected trisaccharide aminooxy compound was reacted with a carbonyl compound leading to oxime formation in quantitative yield underscoring the importance for future glyco-conjugations.

Keywords

aminooxy sugar; Gb3 trisaccharide; Shiga toxin; glycosylation

Human oligosaccharides and glycoconjugates^[1–5] are well-known entities that play critical roles in maintaining homeostatic biological processes,^[6,7] however those that come from foreign sources (e.g. bacterial-based) or that are aberrant in nature have attracted a great deal of attention from the synthetic and biological communities for a sustained period of time due to their strong relationship in diseased states. Within the aforementioned classifications, glycosphingolipids^[8–12] are known to be naturally occurring bioactive molecules most often embedded in the membrane of all animal cells and even in some plant cells.^[13–16] Physiological functions of glycosphingolipids include mediating intercellular interactions as well as modulating the activity of some proteins in the plasma membrane. Accumulation of glycosphingolipids in the lysosome can result in glycosphingolipid storage diseases such as Gaucher's disease^[17,18] Fabry disease^[19,20] urinary-tract infections^[21] as well as a host of others complications including pediatric neurodegenerative disease^[22]

One detrimental glycolipid effect caused by Shiga toxin(s) is the loss of millions of human lives each year. In 1897 Kiyoshi Shiga first described the bacterial origin of dysentery caused by the bacillus *Shigella dysenteriae*. The Shiga toxins have two subunits: 1) unit A and 2) unit B and belong to the AB₅ toxin^[23,24] family of antigens. The cytotoxic enzyme unit A, an *N*-glycosidase which catalyzes rRNA depurination activity leading to cell death,

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is non-covalently linked with a non-toxic homopentameric cell-adhesion carrier B₅ unit. Each of these units have three binding sites for a specific glycolipid called globotriaosyl (Gb3), known as a P^k-trisaccharide.^[21,25] The B₅ unit mediates uptake and intracellular transport of the toxin through specific binding to the sugar domain of the Gb3 in the plasma membrane of cells. Gb3 also serves as a cell-surface receptor for Shiga-like toxins (SLTs).^[26–30] Not surprisingly, SLT vaccine development has been an important area of study from the early 1990's.^[31] Encouragingly, there are Shiga toxin vaccine candidates currently being pursued. One strategy utilizes live attenuated^[32] Shiga-toxin producing *E. coli* and another uses the carrier protein KLH conjugated to Shiga-toxin^[33]. In both of the aforementioned accounts, the vaccines have been able to produce active and functional IgG antibodies capable of neutralizing Shiga-toxin, however a successful verdict is still pending.

In noting the importance of these carbohydrate-protein binding interactions, numerous syntheses of Gb3 have been reported by prominent, synthetic carbohydrate groups.^[34–46] Those strategies have incorporated linkers at the anomeric position including the ceramide,^[46] *p*-aminophenyl(*p*AP),^[34] 6-(*p*-cinnamoylphenoxy)hexyl tether,^[35] and methyl glycosides.^[36] In an attempt to develop physiologically stable oxime bonds for sugar-sugar couplings for entirely carbohydrate-based vaccines, our approach, is to introduce the aminoxy precursor succinimidyl group at an early stage in the synthetic endeavour taking advantage of a flexible, functional group strategy amenable to derivitization.^[47] In utilizing chemically compatible reagents, the goal is to develop orthogonal reactions that are non-toxic, high yielding, and are robust toward pre-exposure of an aminoxy moiety for the synthesis of Gb3- α -ONH₂. Insofar as we are aware, Gb3- α -ONH₂ has never been previously synthesized and the benign nature and orthogonality of the reagents we have employed overcome some of the current challenges regarding succinimidyl lability.

In this context, we describe, a synthesis of a Gb3 trisaccharide derivative noted as [α -D-Gal-(1,4)- β -D-Gal-(1,4)- β -D-Glc- α -aminoxy] (**1**) (Fig 1). The synthesis of Gb3- α -ONH₂ was achieved by sequential convergent glycosylations of a suitably protected monosaccharide (**2**) as well as disaccharide (**3**) that were prepared from commercially available reducing sugars using chemically compatible reaction conditions (Fig. 2). The use of *N*-hydroxysuccinimide (**4**) (NHS) was essential for installing the -ONH₂ functionality. Other similar types of NHS equivalents, such as *N*-hydroxyphthalimide (PhtOH)^[48–50] *N*-pentenoyl (NHPent)^[51–54] have also been used for the -ONH₂ functionality but due to our experience with NHS, others were not utilized.^[47,55]

Phenyl- β -D-galactopyranosyl-(1,4)-1-thio- β -D-glucopyranoside (**3**) was prepared from D-lactose following a literature procedure.^[46] Ethyl-2,3,4,6-tetra-*O*-benzyl-1-thio- β -D-galactopyranoside (**2**),^[56] derived from D-galactose and *N*-hydroxysuccinamide (**4**) were commercially available. Compound **3** was treated with benzaldehyde dimethyl acetal to protect the 4- and 6-hydroxy groups in the presence of camphor sulphonic acid to yield compound **5**, which was then benzylated using benzyl bromide and sodium hydride furnishing compound **6** in 81% overall isolated yield (for two steps) (Scheme 1). Stereoselective glycosylation of disaccharide donor **6** with *N*-hydroxy succinimide **4**^[57] (1.5 eq. due to low solubility in DCM) in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)^[58,59] yielded compound **7** in 90% as a

mixture of anomers (10:1, α/β) as determined from ^1H NMR. Structural confirmation of compound **7**, using spectral analysis, was assigned as follows: δ 5.56 (d, $J = 4.0$ Hz, 1 H, 1_{A}), 5.51 (s, 1 H, PhCH), 4.35 (d, $J = 7.9$ Hz, 1 H, 1_{B}) and 2.75 (s, 4 H, $-\text{C}(=\text{O})\text{CH}_2-\text{CH}_2-\text{C}(\text{O})$). The ^{13}C NMR data was as follows: δ 101.7 (C- 1_{A}), 103.2 (C- 1_{B}), 101.7 (PhCH), 171.1 (2 C, $-\text{C}(\text{O})\text{CH}_2-\text{CH}_2-\text{C}(\text{O})$), and 25.8 (2 C, $-\text{C}(=\text{O})\text{CH}_2-\text{CH}_2-\text{C}(\text{O})$). Successful 1,2-*cis*-glycosylation of the NHS derivative was supported by the coupling constant ($J = 4.0$ Hz) of the anomeric H-1 in compound **7**. Selective reductive ring opening of the benzylidene acetal of **7** was achieved by treatment with ethereal HCl and sodium cyanoborohydride in THF to produce disaccharide acceptor **8** with a 4-OH exposed as a single regioisomer in an overall isolated 69% yield.

Stereoselective glycosylation of disaccharide acceptor **8** with thioglycoside derivative **2**^[56] in the presence of (NIS) and TMSOTf furnished trisaccharide **9** in 57% yield as a mixture of anomers (20:1, α/β). Spectral ^1H NMR data analysis confirmed the trisaccharide formation of the 1,2-*cis* glycosidic linkage: δ 5.51 (d, $J = 4.0$ Hz, 1 H, 1_{A}), 5.07 (d, $J = 3.5$ Hz, 1 H, 1_{C}), 4.33 (d, $J = 7.74$ Hz, 1 H, 1_{B}), 2.75 (s, 4 H, $-\text{C}(=\text{O})\text{CH}_2-\text{CH}_2-\text{C}(\text{O})$). The ^{13}C NMR data was as follows: δ 171.1 (2 C, $-\text{C}(\text{O})\text{CH}_2-\text{CH}_2-\text{C}(\text{O})$), 103.2 (C- 1_{B}), 101.7 (C- 1_{A}), 101.1 (C- 1_{C}), 25.9 (2 C, $-\text{C}(=\text{O})\text{CH}_2-\text{CH}_2-\text{C}(\text{O})$). Both 1,2-*cis* glycosidic linkages were confirmed through the examination of coupling constants ($J = 4.0$ Hz) of the H-1 of compound **8** and ($J = 3.5$ Hz) of the H-1 of compound **4**.

In the final deprotection phase, we first utilized Pearlman's catalyst^[60] for benzyl group removal to obtain compound **10** followed by treatment with hydrazine hydrate^[21] for deprotection of the succinimide group to furnish the final product **1**. After completion as noted by TLC, two by-products^[57,61] were also characterized, namely acetohydrazide and tetrahydropyridazine-3,6-dione that are notorious to interfere with product purification. In noting the later to be true when using silica gel chromatography, these two by-products were eventually removed using a Bio-gel P-2 column with water as the eluent giving ultra-pure **1**.

In order to test the reactivity of the aminoxy **1**, we elected to utilize a simple carbonyl compound in the form of a ketone. As our group has been conjugating to carbonyl aldehydes, such as those noted in our previous published work,^[47,55] we were eager to utilize a carbonyl ketone for three reasons: 1) firstly as a means to validate that oxime formation would occur as readily with carbonyl ketones as our experience indicated it would with carbonyl aldehydes, 2) for future development of novel oxime protecting groups and 3) for future studies on the hydrolysis of oxime bonds. Thus 2-propanone was used for the oxime forming reaction which ultimately gave rise to compound **11** in quantitative yield.

In conclusion, a convenient, stereocontrolled synthesis of Gb3- α -ONH₂ was accomplished using a succinimidyl group at the anomeric position of lactose which remained intact until the final step of the synthesis. The succinimidyl group was transformed into an aminoxy moiety after global deprotection which readily allows for selective conjugation to carbonyl compounds. The aminoxy Gb3 was also reacted with 2-propanone to form an oxime link with which marks our initial attempts at developing new oxime protecting groups and for determining stability.

Experimental Section

General Experimental Methods

^1H , ^{13}C , 2D COSY and HMQC nuclear magnetic resonance spectra were recorded on Bruker 600 (^1H NMR-600 MHz; ^{13}C NMR 150) at ambient temperature with CDCl_3 , D_2O as solvent and TMS as internal reference unless otherwise stated. Chemical shifts are reported in δ ppm. Data for ^1H NMR are reported as follows: chemical shift, integration, multiplicity (s = singlet, d = doublet, dd = doublet of doublet, t = triplet, q = quartet, m = multiplet) and coupling constants in Hertz. All ^{13}C NMR spectra were recorded with complete proton decoupling. Low resolution mass spectra (LRMS) were acquired on a Waters Acuity Premiere XE TOF LC-MS using electrospray ionization.

All reactions were carried out in oven-dried glassware under an argon atmosphere unless otherwise noted. Reactions were monitored by thin layer chromatography over silica gel coated TLC plates. TLC was visualized by warming ceric sulphate (2% $\text{Ce}(\text{SO}_4)_2$ in 2 N H_2SO_4)-sprayed plates on a hot plate. Column chromatography was performed on silica gel 60 (0.040–0.063 mm). All chemicals were of commercial grade and were used without further purification. Anhydrous solvents were obtained from Aldrich and EMD. Yields refer to chromatographically and spectroscopically pure material unless otherwise noted.

Phenyl-(2,3-di-O-benzyl-4,6-O-benzylidene- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzyl-1 thio- β -D-glucopyranoside (6)

To a solution of compound **3** (2 g, 4.60 mmol) in anhydrous DMF (20 mL) were added benzaldehyde dimethyl acetal (0.84 g, 5.52 mmol), camphor sulfonic acid and the reaction mixture was allowed to stir overnight at room temperature. The reaction was quenched with triethylamine and solvents were removed under reduced pressure. The crude material **5** was dissolved in CH_2Cl_2 (50 mL) and the organic layer was washed 3 $\times$ with water, dried using Na_2SO_4 , concentrated and dried under vacuum. To a solution of the dried product in anhydrous DMF (30 mL) were added powdered NaH (at 60% w/w dispersion in mineral oil) (1.8 g, 45 mmol), benzyl bromide (3.8 mL, 32 mmol), and tetrabutylammonium bromide (200 mg, 0.62 mmol) and the reaction mixture was allowed to stir briskly at rt for 4 h. The reaction was quenched by adding satd. NH_4Cl and concentrated under reduced pressure. The crude mass was dissolved in CH_2Cl_2 (150 mL) and the organic layer was washed with water, dried using Na_2SO_4 , and finally concentrated. The crude product was purified over SiO_2 using hexane-EtOAc (7:1) as eluant to give pure compound **6** (3.28 g, 81%).

^1H NMR (600 MHz, CDCl_3)— δ 7.61–7.21 (m, 35 H, Ar-H), 5.50 (s, 1 H, PhCH), 5.29 (d J = 10.4 Hz, 1 H, PhCH₂), 4.89–4.76 (m, 7 H, PhCH₂), 4.69 (d, J = 9.8 Hz, 1 H, 1_A), 4.60–4.58 (d, J = 11.9 Hz, 1 H, PhCH₂), 4.53 (d, J = 7.9 Hz, 1 H, 1_B), 4.40 (1 H, PhCH₂), 4.30–4.26 (m, 1 H, 6_B), 4.09–4.08 (d, J = 3.7 Hz, 1 H, 4_B), 4.06 (t, $J_{1/2, 2/3}$ = 9.6 Hz, 1 H, 4_A), 3.97–3.95 (dd, $J_{1/2, 1, 3}$ = 3.9, 11.0 Hz, 1 H, 6_A), 3.91–3.89 (m, 1 H, 6_B), 3.84–3.79 (m, 2 H, 6_A, 2_B), 3.70 (t, J = 8.9 Hz, 1 H, 3_A), 3.52 (t, J = 9.9 Hz, 1 H, 2_A), 3.48–3.46 (dd, $J_{1/2, 1, 3}$ = 3.7, 9.6 Hz, 1 H, 3_B), 3.4–3.40 (m, 1 H, 5_A), 3.04 (m, 1 H, 5_B)

¹³C NMR (150 MHz, CDCl₃)— δ 139.0-126.8 (Ar-C), 103.1 (1_B), 101.6 (PhCH), 87.6 (1_A), 85.3 (3_A), 80.3 (2_A), 79.9 (3_B), 79.6 (5_A), 79.1 (2_B), 77.4 (4_A), 76.3 (OBn), 75.6 (OBn), 73.9 (4_B), 73.2 (OBn), 71.9 (OBn), 69.2 (6_B), 68.6 (6_A), 66.7 (5_B). ESI-MS (LR MS) [C₆₀H₆₀O₁₀SNa]⁺ found 995.9

Succinamidyl- (2,3-di-O- benzyl-4,6-O-benzylidene- β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzyl- β -D-glucopyranoside (7)

To a solution of compound **6** (3.28 g, 3.71 mmol) and compound **4** (*N*-hydroxysuccinamide) (1.28 g, 11.12 mmol) in anhydrous CH₂Cl₂ (50 mL) was added MS-4 $\text{\AA}$ (2.00 g,) and the reaction mixture was allowed to stir at rt for 1 h under argon. The reaction mixture was cooled to -20 °C and *N*-iodosuccinimide (NIS; 1.00 g, 4.45 mmol) and TMSOTf (80 μ L) were then added. After stirring at the same temperature for 3 h, the reaction mixture was quenched with saturated NaHCO₃ and sodium thiosulfate, filtered through a pad of celite, and finally washed with CH₂Cl₂ (100 mL). The organic layer was then washed 3 $\times$ with aqueous Na₂SO₄ and water in succession, dried using Na₂SO₄, and concentrated under reduced pressure to give the crude product, which was purified over SiO₂ using hexane-EtOAc (5:1) as the eluant to furnish pure α anomeric product **7** (2.96 g, 81%).

¹H NMR (600 MHz, CDCl₃)— δ 7.58-7.16 (m, 30 H, Ar-H), 5.56 (d, *J* = 4.0 Hz, 1 H, 1_A), 5.51 (s, 1 H, PhCH), 5.23 (d, *J* = 10.4 Hz, 1 H, PhCH₂), 5.02 (d, *J* = 11.3 Hz, 1 H, PhCH₂), 5.03-4.77 (m, 6 H, PhCH₂), 4.76-4.75 (m, 1 H, 5_A), 4.52 (d, *J* = 11.9 Hz, 1 H, PhCH₂), 4.35 (d, *J* = 7.9 Hz, 1 H, 1_B), 4.30-4.27 (dd, *J*_{1/2,1,3} = 1.2, 12.4 Hz, 1 H, 6_B), 4.25 (d, *J* = 11.9 Hz, 1 H, PhCH₂), 4.13-4.11 (dd, *J*_{1/2,1,3} = 2.3, 11.2 Hz, 1 H, 6_A), 4.08-4.05 (m, 3 H, 3_A, 4_A, 4_B), 3.92-3.90 (dd, *J*_{1/2,1,3} = 1.7, 12.4 Hz, 1 H, 6_B), 3.83-3.80 (dd, *J*_{1/2,1,3} = 7.9, 9.6 Hz, 1 H, 2_B), 3.76-3.73 (m, 1 H, 2_A), 3.52-3.50 (dd, *J*_{1/2,1,3} = 1.9, 11.2 Hz, 1 H, 6_A), 3.34-3.32 (dd, *J*_{1/2,1,3} = 7.9, 9.6 Hz, 1 H, 3_B), 2.96 (s, 1 H, 5_B), 2.75 (s, 4 H, -C(=O)CH₂-CH₂-C(O)).

¹³C NMR (150 MHz, CDCl₃)— δ 171.1 (2 C, -C(O)CH₂-CH₂-C(O)), 139.3-126.9 (Ar-C), 103.2 (1_B), 101.7 (PhCH), 101.7 (1_A), 79.8 (3_A), 78.7 (3_B), 78.1 (2_A), 77.0 (4_A), 76.4 (OBn), 75.9 (OBn), 74.0 (4_B), 73.4 (OBn), 73.2 (OBn), 72.6 (5_A), 72.0 (OBn), 69.3 (6_B), 68.0 (6_A), 66.6 (5_B), 25.8 (2 C, -C(=O)CH₂-CH₂-C(O)). ESI-MS (LR MS) [C₅₈H₅₉NO₁₃Na]⁺ found 1000.2

Succinamidyl - (2,3,6-tri-O- benzyl - α -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzyl- β -D-glucopyranoside (8)

To a solution of compound **7** (2.96 g, 3.02 mmol) in THF (10 mL) sodium cyanoborohydride (0.95 g, 15.13 mmol) was added and stirred for 1 h under an atmosphere of argon, then the reaction mixture was cooled to ice temperature and treated with ethereal HCl until gas evolution ceased. The reaction mixture stirred for 15 mins in the same temperature then slowly warmed to room temperature and allowed to stir for 1 h further. After completion, the reaction mixture was filtered through a pad of celite. The filtrate was concentrated and purified on a silica gel column using hexane/EtOAc (2:1) as an eluent to give pure **8** (2.04 g, 69%).

¹H NMR (600 MHz, CDCl₃)— δ 7.53-7.23 (Ar-H), 5.56 (d, J = 4.0 Hz, 1 H, 1_A), 5.06-5.01 (m, 2 H, PhCH₂), 4.88-4.76 (m, 6 H, PhCH₂), 4.75-4.74 (m, 1 H, 5_A), 4.57-4.55 (m, 2 H, PhCH₂), 4.47-4.45 (d, J = 12.0 Hz, 1 H, PhCH₂), 4.35-4.34 (d, J = 7.8 Hz, 1 H, 1_B), 4.34-4.33 (d, J = 11.9 Hz, 1 H, PhCH₂), 4.09-3.98 (m, 4 H, 4_A, 4_B, 6_{A1}, 3_A), 3.76-3.74 (dd, $J_{1/2,1,3}$ = 7.0, 9.8 Hz, 1 H, 6_{B1}), 4.71-3.69 (dd, $J_{1/2,1,3}$ = 4.0, 9.8 Hz, 1 H, 2_A), 3.66-3.63 (dd, $J_{1/2,1,3}$ = 8.0, 9.1 Hz, 1 H, 2_B), 3.60-3.57 (m, 1 H, 6_{B2}), 3.56-3.54 (m, 1 H, 6_{A2}), 3.36-3.33 (m, 2 H, 3_B), 2.73 (s, 4 H, -C(=O)CH₂-CH₂-C(O)).

¹³C NMR (150 MHz, CDCl₃)— δ 171.0 (2 C, -C(O)CH₂-CH₂-C(O)), 139.2-127.4 (Ar-C), 102.6 (1_B), 101.4 (1_A), 81.1 (3_B), 79.5 (3_A), 79.1 (2_B), 77.8 (2_A), 75.7 (OBn), 75.6 (OBn), 75.5 (4_A), 73.6 (OBn), 73.2 (OBn), 73.1 (OBn), 73.0 (5_B), 72.4 (5_A), 72.1 (OBn), 68.6 (6_B), 67.5 (6_A), 66.3 (4_B), 25.4 (2 C-C(O)CH₂-CH₂-C(O)). ESI-MS (LR MS) [C₅₈H₆₁NO₁₃Na]⁺ found 1002.9.

Succinamidyl - (2,3,4,6-tetra-O-benzyl - α -D-galactopyranosyl) -(1 $\rightarrow$ 4)- (2,3,6-tri-O-benzyl - β -D-galactopyranosyl)-(1 $\rightarrow$ 4)-2,3,6-tri-O-benzyl- β -D-glucopyranoside (9)

To a solution of compound **8** (2.04 g, 2.08 mmol) and compound **2** (1.58 g, 2.70 mmol) in anhydrous CH₂Cl₂ (40 mL) was added MS-4 $\text{\AA}$ (1.00 g) and the reaction mixture was allowed to stir at rt for 1 h under argon. The vessel was then cooled to -10 °C and *N*-iodosuccinimide (NIS; 0.73 g, 3.24 mmol) and TMSOTf (40 μ L) were added. After stirring at a constant temperature for 4 h, the reaction mixture was quenched with saturated NaHCO₃ and sodium thiosulfate, filtered through a pad of celite, and washed 3 $\times$ with CH₂Cl₂ (100 mL). The organic layer was washed with aq. Na₂SO₄ and water in succession, dried using Na₂SO₄, and then concentrated under reduced pressure to give the crude product. Purification ensued over SiO₂ using hexane-EtOAc (2:1) as the eluent to furnish pure **9** (1.69 g, 54%).

¹H NMR (600 MHz, CDCl₃)— δ 7.49-7.14 (m, 50 H, Ar-H), 5.51 (d, J = 4.0 Hz, 1 H, 1_A), 5.13 (J = 10.9 Hz, 1 H, PhCH₂), 5.07 (d, J = 3.5 Hz, 1 H, 1_C), 4.94 (J = 11.4 Hz, 1 H, PhCH₂), 4.89 (J = 11.1 Hz, 1 H, PhCH₂), 4.85-4.70 (m, 7 H, PhCH₂), 4.70-4.69 (d, J = 9.9 Hz, 1 H, 5_A), 4.56-4.45 (m, 5 H, PhCH₂), 4.40-4.37 (m, 1 H, 5_C), 4.33 (d, J = 7.74 Hz, 1 H, 1_B), 4.34-4.31 (m, 2 H, PhCH₂), 4.27-4.25 (d, J = 11.9 Hz, 1 H, PhCH₂), 4.21-4.19 (dd, $J_{1/2,1,3}$ = 8.4, 9.6 Hz, 1 H, 6_{B1}), 4.15-4.10 (m, 3 H, 6_{B1}, 2 PhCH₂), 4.07 (brs, 1 H, 2_C), 4.05-4.00 (m, 4 H, 4_A, 4_B, 4_C, 3_A), 3.99-3.96 (t, J = 9.3 Hz, 1 H, 3_A), 3.67-3.64 (m, 2 H, 2_A, 2_B), 3.57-3.55 (dd, $J_{1/2,1,3}$ = 5.3, 9.6 Hz, 1 H, 6_{B2}), 3.53-3.50 (t, J = 9.0 Hz, 1 H, 6_{C1}), 3.49-3.47 (m, 1 H, 6_A), 3.30-3.28 (m, 1 H, 5_B), 3.23-3.21 (dd, $J_{1/2,1,3}$ = 4.7, 8.6 Hz, 1 H, 3_B), 3.20-3.18 (q, 1 H, 6_C), 2.75 (s, 4 H, -C(=O)CH₂-CH₂-C(O)).

¹³C NMR (150 MHz, CDCl₃)— δ 171.1 (2 C, -C(O)CH₂-CH₂-C(O)), 139.5-127.5 (Ar-C), 103.2 (1_B), 101.7 (1_A), 101.1 (1_C), 81.9 (3_B), 79.7 (3_A), 79.7 (3_C), 79.3 (2_B), 77.9 (2_A), 76.9 (2_C), 76.5 (4_C), 76.0 (OBn), 75.9 (OBn), 75.5 (4_A), 75.2 (OBn), 75.1 (4_B), 74.0 (OBn), 73.7 (5_B), 73.5 (OBn), 73.4 (OBn), 73.3 (OBn), 72.7 (OBn), 72.6 (5_A), 72.4, (OBn), 69.7 (5_C), 68.2 (6_B), 68.1 (6_C), 67.8 (6_A), 25.9 (2 C-C(=O)CH₂-CH₂-C(O)). ESI-MS (LR MS) [C₉₂H₉₅NO₁₈Na]⁺ found 1525.4.

Succinamidyl - (α -D-galactopyranosyl) -(1 $\rightarrow$ 4)- (β -D-galactopyranosyl)-(1 $\rightarrow$ 4) - β -D-glucopyranoside (**10**)

To a solution of compound **9** (1.69 g, 1.12 mmol) in CH₃OH (30 mL) was added 20% Pd(OH)₂-C (200 mg) and mixture was allowed to stir at rt under a positive pressure of hydrogen for 10 h. The reaction mixture was filtered through a pad of celite and then washed with CH₃OH-H₂O (60 mL; 1:3 v/v). The combined filtrate was evaporated under reduced pressure to furnish compound **10**, which was purified through a Sephadex LH-20 column using H₂O as an eluent to give pure compound **10** (514 mg, 76%).

¹H NMR (600 MHz, CDCl₃)— δ 5.63 (d, J = 4.0 Hz, 1 H, 1_A), 5.15 (J = 3.6 Hz, 1 H, 1_C), 4.72 (J = 7.7 Hz, 1 H, 1_B), 4.62 (d, J = 10.2 Hz, 1 H, 5_A), 4.53 (m, 1 H, 5_B), 4.23-4.21 (m, 2 H, A₄, B₄), 4.15-4.09 (m, 4 H, C₃, C₅, A₆, C₆), 4.05-3.95 (m, 4 H, A₂, A₃, C₂, B₆, C₆), 3.94-3.89 (m, 4 H, B₃, C₄, A₆, B₆), 3.80-3.78 (m, 1 H, B₂), 3.00 (s, 4 H, -C(=O)CH₂-CH₂-C(O)).

¹³C NMR (150 MHz, CDCl₃)— δ 175.1 (2 C, -C(=O)CH₂-CH₂-C(O)), 103.4 (1_B), 103.3 (1_A), 100.5 (1_C), 77.3 (B₄), 75.4 (C₄), 78.3 (C₅), 72.7 (A₅), 72.4 (B₃), 71.2 (B₂), 71.1 (2 C, A₄, B₅), 70.6 (A₃), 69.4 (A₂), 69.2 (C₂), 68.8 (C₃), 25.6 (2 C-C(=O)CH₂-CH₂-C(O)). ESI-MS (LR MS) [C₂₂H₃₅NO₁₈Na]⁺ found 623.9.

Aminoxy- (α -D-galactopyranosyl) -(1 $\rightarrow$ 4)- (β -D-galactopyranosyl)-(1 $\rightarrow$ 4) - β -D-glucopyranoside (**1**)

To a solution of compound **10** (514 mg, 0.85 mmol) in H₂O (20 mL) was added hydrazine hydrate and the mixture was allowed to stir at rt for 4 h. After completion of the reaction (TLC solvent (R_f 0.3) [4:3:1, n-butanol:H₂O:AcOH]), H₂O was evaporated under reduced pressure to furnish compound **1**, which was purified through a P-2 biogel column using H₂O as the eluent to give pure compound **1** (302 mg, 68%).

¹H NMR (600 MHz, CDCl₃)— δ 4.98 (d, J = 4.0 Hz, 1 H, 1_A), 4.92 (J = 3.9 Hz, 1 H, 1_C), 4.49 (J = 7.8 Hz, 1 H, 1_B), 4.34-4.32 (m, 2 H, 4_A, 4_B), 3.92-3.87 (m, 4 H, C₅, A₆, C₆, A₅), 3.82-3.79 (m, 3 H, C₂, A₆, B₆), 3.77-3.74 (m, 1 H, C₄), 3.73-3.71 (m, 1 H, A₃, B₃), 3.67-3.64 (m, 3 H, B₆, C₆, C₅), 3.62-3.60 (m, 1 H, A₂), 3.57-3.54 (m, 1 H, B₂).

¹³C NMR (150 MHz, CDCl₃)— δ 103.1 (1_B), 101.2 (1_A), 100.2 (1_C), 78.3 (C₅), 77.3 (B₄), 75.4 (C₄), 72.1 (A₃), 71.5 (B₃), 70.8 (B₂), 70.7 (A₂), 70.5 (C₂), 70.4 (A₄), 68.9 (B₅), 68.5 (C₃). ESI-MS (LR MS) [C₁₈H₃₃NO₁₆Na]⁺ found 542.0.

Acknowledgments

This work was supported by the National Institute of Health (NCI - R01 CA156661-01). S.G. and P.R.A. would like to acknowledge Dr. Yong Wah Kim (University of Toledo) for NMR assistance.

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Figure 1.

The Gb3- α -ONH₂ (1) as an aminooxy sugar for oxime-forming conjugation.

2 **3** **4**

Figure 2.
Compounds used in the synthesis of target trisaccharide Gb3- α -ONH₂.

Scheme 1.

Reagents: (a) benzaldehyde dimethyl acetal, DMF, rt, overnight, 89% isolated yield. (b) Benzyl bromide, NaH, TBAB (cat.), DMF, rt, 4 h, 91% overall isolated yield. (c) *N*-iodosuccinimide (NIS), trimethylsilyl trifluoromethanesulfonate, 4Å MS, CH₂Cl₂, -20 °C, 1 h, 81% isolated yield. (d) Etheral HCl, sodium cyanoborohydride, anhydrous THF, argon, 69% isolated yield.

Scheme 2.

Reagents: (a) *N*-iodosuccinimide, trimethylsilyl trifluoromethanesulfonic, MS 4Å, CH₂Cl₂, –20 °C, 1 h, 54% isolated yield. (b) H₂, 20% Pd(OH)₂-C, rt, 8 h, 76% isolated yield. (c) hydrazine monohydrate, MeOH:H₂O (1:1), rt, 3 h, 68% isolated yield.

Scheme 3. Reagents: (a) 2-propanone, H_2O , rt.