

Organic synthesis in pursuit of immunology: Large-scale synthesis of peracetylated GM2 glycosylamino acid for preparation of a multiantigenic prostate cancer vaccine

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Abstract—We describe herein the stereocontrolled, large-scale synthesis of peracetylated GM2 glycosylamino acid. Key features of the synthesis include a newly modified [1 + 3] coupling reaction and an olefin cross-metathesis-hydrogenation sequence. The GM2 glycosylamino acid is now ready for incorporation into a hexavalent prostate cancer vaccine construct.

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1. Introduction

It has long been a goal of tumor immunologists to harness the power of the human immune system in the battle against cancer.¹ To this end, it is known that transformed cancer cells are distinguishable from normal cells in that they often express aberrant levels of carbohydrate antigens on their cell surfaces.² These carbohydrate epitopes, if appropriately introduced to the host immune system, could stimulate a B cell response, leading to the production of antibodies that would selectively target and hopefully eliminate circulating tumor cells and micrometastases.³ It should be noted that, because these carbohydrate antigens are often isolable in only very low levels, it falls to the synthetic chemist to design and execute routes to sufficient quantities of carbohydrate to allow for biological evaluation. Indeed, significant resources from our laboratory have been directed toward this broad goal.

This area of cancer research continues to be of particular ongoing interest to our laboratory.⁴ Recent advances in synthetic chemistry have allowed for the preparation of complex carbohydrate-based tumor antigens in quantities sufficient for significant biological evaluations. A

number of our fully synthetic carbohydrate constructs—usually attached through a linker domain to an immunogenic carrier protein—have been introduced into host immune systems and investigated in Phase I settings for their ability to generate an immune response.^{3,5} The results of these clinical investigations have been promising, in that the vaccines have been well tolerated and have met serological expectations.^{6,7} More advanced clinical trials using fully synthetic vaccines are being readied. The design of these first generation carbohydrate vaccines did not take into account the degree of heterogeneity of carbohydrates expressed on transformed cell surfaces. Even within a particular cancer type, there is a considerable amount of variation in the level and nature of cell-surface carbohydrates expressed.² With this consideration in mind, we have envisioned that by displaying several different antigens closely associated with a specific cancer on a single molecule (unimolecular multivalent construct), we could increase the percentage of cells to be targeted. We expect that if this, indeed, proves to be the case, then the effectiveness of later generation vaccines could well be improved.

At the outset of our investigation, we had to decide how to present multiple carbohydrate antigens in a single molecule. Inspired by the mode of presentation in natural mucins, we have come to favor inclusion of multiple tumor antigens into a single polypeptide construct.^{8,9} Subsequently, we focused on non-natural amino acids

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as components of our peptide-linked vaccines.¹⁰ We surmised that such unnatural linkages might result in an enhanced immune response. Furthermore, the use of amino acids containing long, aliphatic side chains may serve to distance the glycosides from the peptide backbone and facilitate glycopeptide synthesis.

A unimolecular trivalent vaccine (Fig. 1) employing these non-natural amino acids as linkers to carbohydrate domains (Tn, Lewis^x, and Globo-H) has been prepared and has undergone preliminary investigation.¹¹ Preclinical evaluation of this vaccine construct has shown promising levels of immunogenicity, with antibody responses observed against each individual carbohydrate antigen, demonstrating proof of principle for a unimolecular multivalent vaccine construct with a non-natural amino acid linker.

Encouraged by these results, we launched a program to synthesize a pentameric vaccine that would target

prostate cancer. Toward this end, we designed a multivalent vaccine conjugate containing five carbohydrate antigens known to be expressed in high levels on prostate cancer cell surfaces:² Tn, TF, STn, Lewis^x, and Globo-H (Fig. 2). We conjugated the vaccine separately to two different carrier proteins, KLH and Pam₃Cys.¹² The promising in vivo preclinical serological results of these pentavalent constructs will be reported shortly.¹³

As the next logical step in developing and evaluating the multivalent vaccine concept, we turned our attention to the design of a prostate cancer vaccine, in which a sixth, but potentially important carbohydrate antigen, would be incorporated. We hope to gain an incremental immunological advantage for our newest vaccine. This hexavalent construct would incorporate the antigens displayed on the pentavalent vaccine (Globo-H, Lewis^x, STn, TF, and Tn), as well as a sixth antigen, GM2. Several factors influenced our decision to target GM2. First,

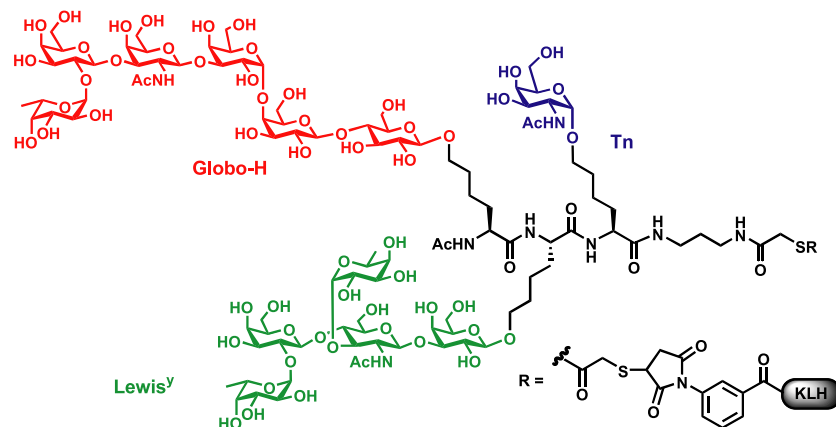


Figure 1. A unimolecular trivalent vaccine.

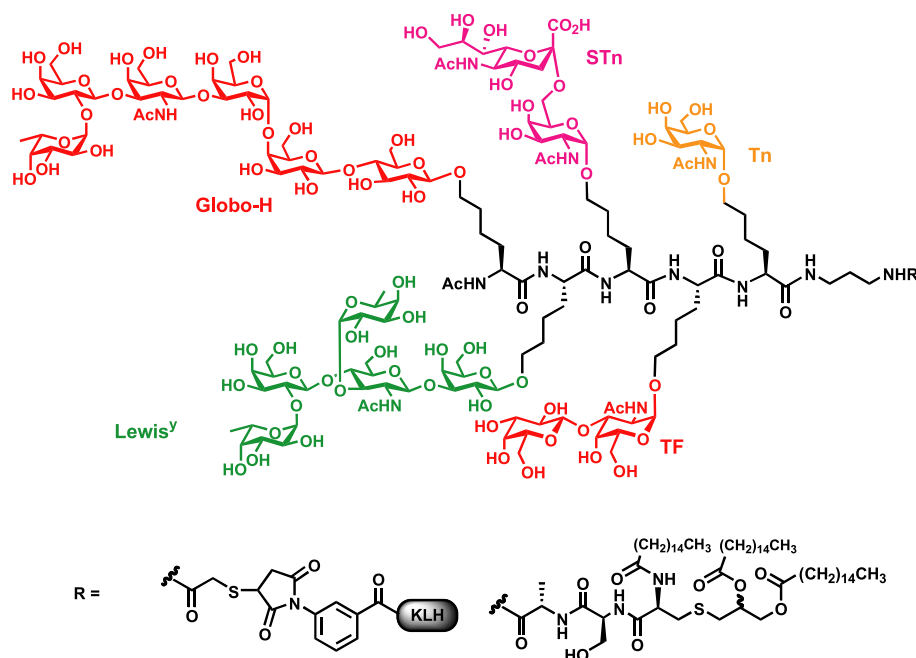


Figure 2. Pentavalent vaccine designed for use in prostate cancer.

this antigen is expressed on the cell surfaces of a number of human cancers, including prostate cancer.^{2a} In addition, GM2-induced antibodies have already been demonstrated to be active against human GM2⁺ tumor cells.¹⁴ Most importantly, human clinical trials have previously been conducted with GM2 alone (in the context of melanoma), and the results of these trials have suggested clinical advantages for the enhanced GM2 antibody.^{14b}

In designing our hexavalent construct, we decided to insert the sixth antigen, GM2, between STn and Lewis^y, due to its size (Fig. 3). The first challenge was then to synthesize the GM2 glycosylamino acid (**1**). We describe herein our synthesis of this compound.

In synthesizing our multivalent vaccines, we rely on the ‘cassette’ approach to vaccine synthesis, developed in our laboratory. According to this assembly logic, we first generate a pool of glycosylamino acids, which we then iteratively couple to fashion the spacer modified peptide backbone expressing the antigens in the desired sequence. We have developed a number of methods for the synthesis of the individual glycosylamino acid cassettes, which have been described in detail elsewhere.^{15–17} Each of these methods ultimately delivers an unnatural glycosylamino acid with a carbon linker between the anomeric oxygen and the α -carbon of the amino acid.

2. Results and discussion

Given that there were no reported syntheses of the GM2 glycosylamino acid at the outset of this program,¹⁸ we decided to follow the olefin metathesis-hydrogenation strategy, which we had successfully employed in our preparation of the fucosyl GM1 glycosylamino acid.¹⁹ Our synthesis would thus commence with the prepara-

tion of monosaccharide **2**²⁰ and trisaccharide **3**.²¹ Coupling of the two components should lead to tetrasaccharide **4**, which would undergo olefin cross-metathesis with **5**. Hydrogenation of the resultant olefin should give rise to the target glycosylamino acid **1** (see Scheme 1).

Though each building block (**2** and **3**), at some level, has been previously prepared in our laboratory, we were well aware of several difficulties which might present themselves in the synthesis of GM2 glycosylamino acid, including: (a) execution of a high-yielding, β -selective coupling between the galactosamine residue (**2**) and the less reactive 4- β -hydroxyl group of the pentenyl glycoside trisaccharide (**3**); (b) the presence of a labile sialic acid moiety; (c) the possibility of terminal olefin migration under cross-metathesis reaction conditions, which could result in the generation of undesired products.

Our earlier investigations had indicated that the presence of a free hydroxyl linkage at C₄ of donor **2** would be necessary to direct the formation of the requisite β -linkage in the coupling of **2** and **3**.^{21,22} Indeed, this turned out to be the case. Donor **2** favored β -glycosylation via sulfonamido participation under the guidance of the ‘proximal hydroxyl’ directing effect. Because the preparation of the monosaccharide **2** is relatively straightforward, we decided to use excess **2** in the coupling with our more complex trisaccharide acceptor, **3**, in an effort to maximize the reaction efficiency. The newly devised [1 + 3] coupling conditions, shown in Scheme 2, worked quite well on a multigram scale, ultimately, for instance, affording up to 4.6 g of the desired tetrasaccharide **6**.

The deprotection sequence commenced with the removal of the silyl groups (Scheme 3). This step was followed by deacetylation, ester hydrolysis, debenzylation, and peracetylation, to ultimately provide lactone **7** in 54%

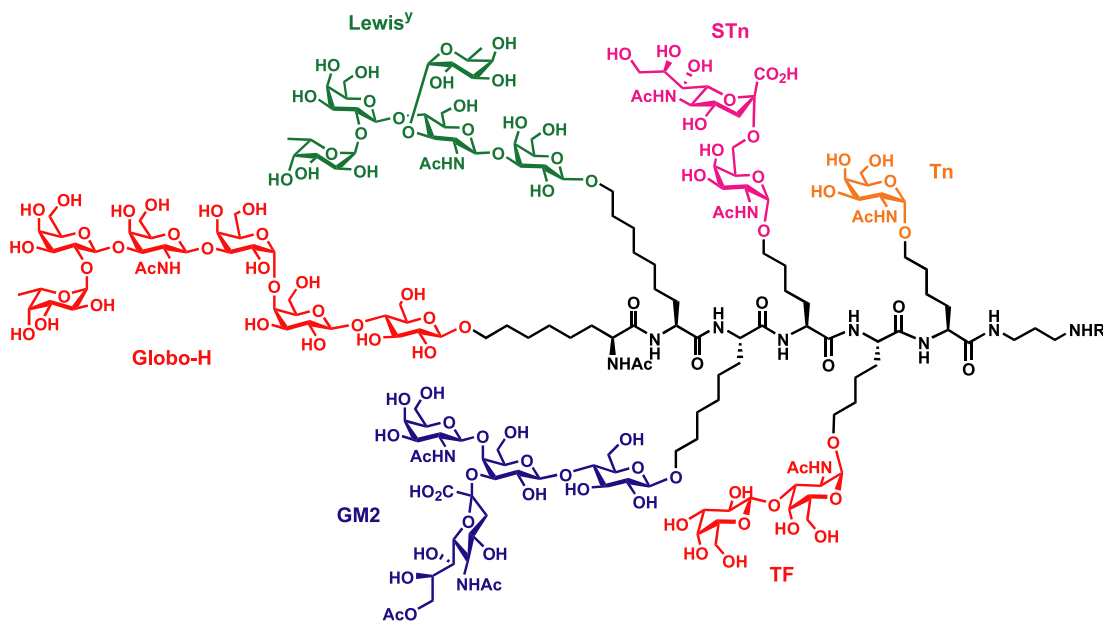
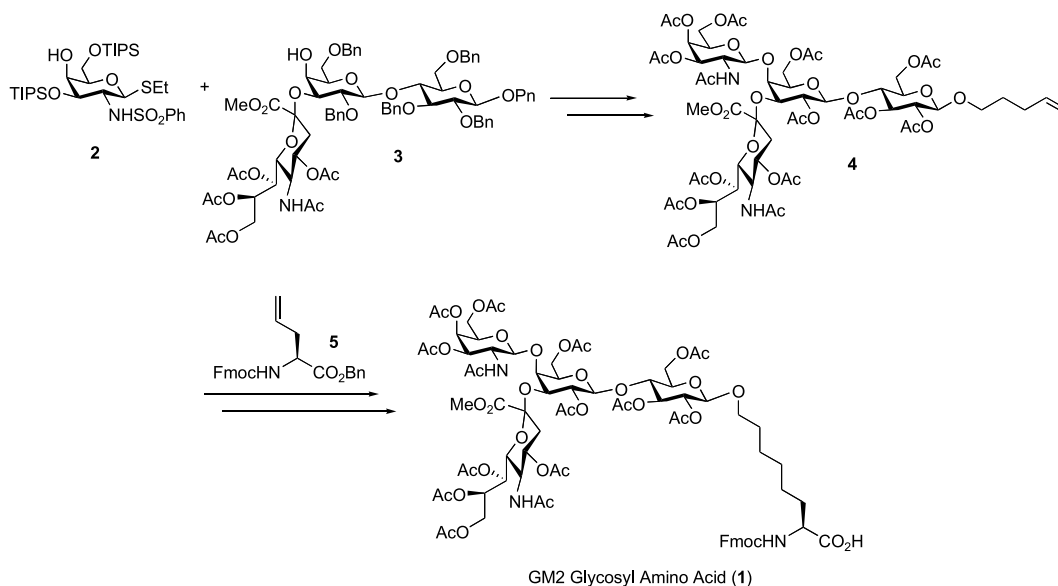
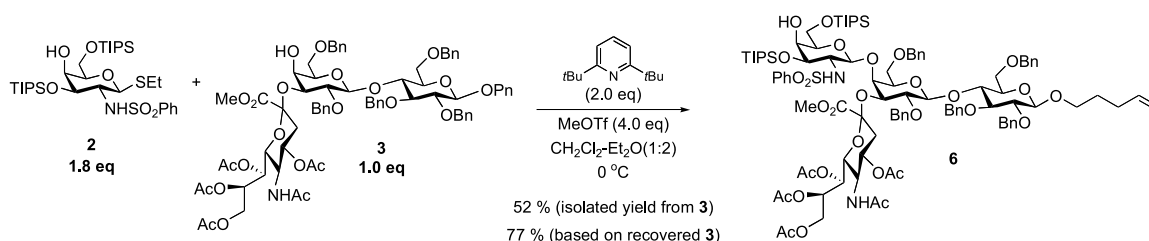


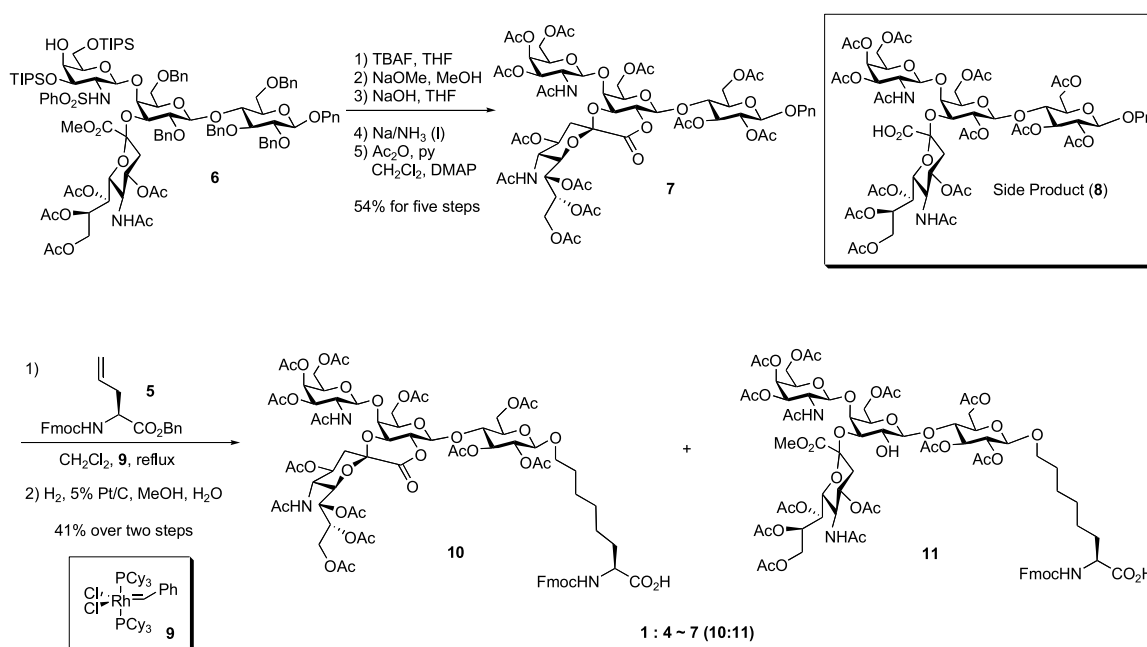
Figure 3. Proposed hexavalent vaccine for use in prostate cancer.



Scheme 1. Synthetic strategy toward GM2 glycosyl amino acid (1).



Scheme 2. Preparation of tetrasaccharide 6.



Scheme 3. Functionalization of tetrasaccharide 6.

overall yield. With the appropriately protected glycoside in hand, the stage was set to attempt the olefin metathesis-hydrogenation sequence. Thus, 7 was treated with

Fmoc-L-allylglycine benzyl ester (5, 7 equiv) and appropriate Grubbs' catalyst 9. The resultant inseparable mixture of unreacted 7 and olefin cross-metathesis products

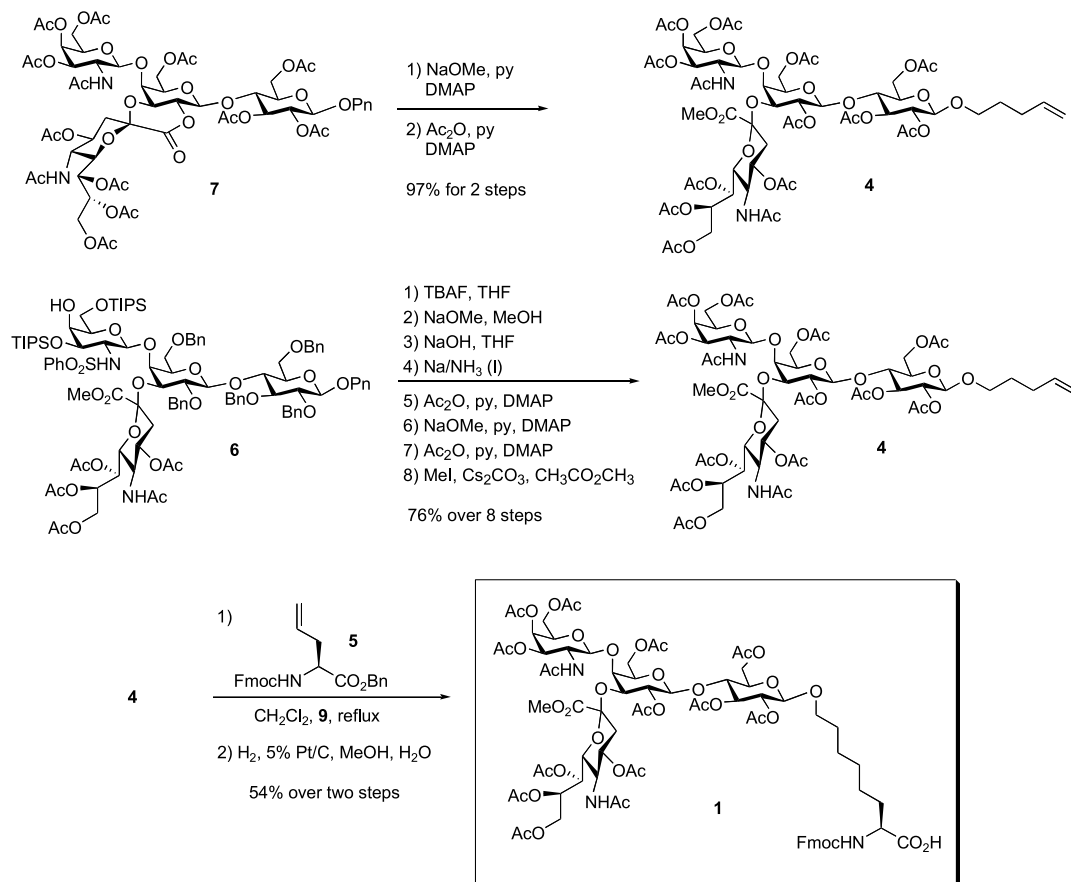
(*E*- and *Z*-isomers) was subjected to catalytic hydrogenation conditions. The side-chain olefinic linkage was reduced with concomitant removal of the benzyl protecting group to afford **10**. We were pleased to find that, in this case, our initial concerns regarding terminal olefin isomerization during olefin metathesis appeared to have been unwarranted. We did not observe any of the side products that would have resulted from olefin bond migration.¹⁶ However, under neutral conditions, we did obtain a mixture of the desired **10** and the undesired **11**, in which the lactone had been opened to reveal the free alcohol. We were concerned that the presence of this free alcohol may complicate the subsequent glycopeptide formation and we, therefore, sought to adjust our route slightly so that the tetrasaccharide would be fully acetate-protected before entering the metathesis-hydrogenation sequence. Furthermore, we had identified a major by-product of the deprotection–protection sequence leading from **6** to **7**. This by-product (**8**), the result of lactone cleavage, could not be retrieved from silica gel during the purification of **7**. It was hoped that our modified procedure might allow us to avoid the loss of valuable material associated with the formation of this side product.

Treatment of lactone **7** under basic methanolysis conditions, followed by acetylation of the resultant hydroxyl group, gave rise to methyl ester **4** in 97% yield over

two steps (Scheme 4). We were ultimately able to develop an efficient eight-step, one-flask procedure to directly convert the saccharide coupled product, **6**, to the per-acetylated olefin-metathesis precursor, **4**, in 76% overall yield. It should be noted that the final methylation step allowed accrual of the side product **8** in the final global protection. This high-yielding sequence was also scalable, allowing us to prepare, for instance, ca. 0.5 g of per-acetylated GM2 glycoside **4** in a single trial. Finally, the olefin metathesis-hydrogenation sequence proceeded smoothly to afford the target acetate-protected GM2 glycosylamino acid, **1**, which is now ready for incorporation into the hexavalent prostate cancer vaccine construct.

3. Conclusion

In conclusion, a stereocontrolled, large-scale synthesis of GM2 glycosylamino acid has been achieved. Some of the key features of this route include a newly modified [1 + 3] coupling reaction and an optimized global deprotection–protection sequence to allow for the efficient preparation of the appropriately protected GM2 glycoside. Once again, olefin cross-metathesis has proven to be a powerful tool in the preparation of non-natural glycosylamino acids. The construction of the hexavalent vaccine and the results of immunological investigations will be reported in due course.



Scheme 4. Preparation of GM2 glycosylamino acid **1**.

4. Experimental

4.1. General methods

All reactions were carried out under argon with dry solvents, oven- or flame-dried glassware, and magnetic stirring. All solvents were of reagent grade or HPLC grade. Reactions were monitored by thin layer chromatography (TLC) using 0.25-mm E. Merck pre-coated silica gel plates. Flash chromatography was performed with the indicated solvents and E. Merck silica gel 60 (particle size 0.040–0.063 mm). Yields refer to chromatographically and spectroscopically pure compounds. Reagents were purchased from commercial suppliers and used without further purification.

^1H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 or a Bruker Avance DRX-500 MHz spectrometers in CDCl_3 [referenced to 7.26 ppm (δ) for ^1H NMR and 77.0 ppm for ^{13}C NMR] and CD_3OD [referenced to 3.30 ppm (δ) for ^1H NMR and 49.05 ppm for ^{13}C NMR]. Low resolution mass spectra (ionspray, a variation of electrospray) were acquired on a Perkin-Elmer Sciex API 100 spectrometer. High-resolution mass spectra (fast atom bombardment, FAB) were acquired on a Micromass 70-SE-4F spectrometer. Infrared spectra were obtained on a Perkin-Elmer 1600 FT-IR spectrophotometer with a NaCl plate. Optical rotations were measured with a Perkin-Elmer 241 polarimeter in the solvent indicated.

4.2. Tetrasaccharide, 6

The thioethyl donor **2** (2.365 g, 3.5 mmol) and acceptor **3** (2.567 g, 1.92 mmol) were combined, azeotroped with anhydrous toluene (4×125 mL), and placed under high vacuum for 16 h. The mixture was then dissolved in CH_2Cl_2 (20 mL) and Et_2O (40 mL), treated with freshly flame-dried 4 Å molecular sieves and 2,6-di-*tert*-butylpyridine, and then stirred at rt for 45 min. The mixture was cooled to 0 °C. Methyl triflate (0.87 mL, 7.69 mmol) was added in one portion, and the reaction was allowed to stir for an additional 5 h at 0 °C. The reaction was quenched by the addition of solid NaHCO_3 , filtered through Celite, washed with EtOAc, concentrated, and purified by flash column chromatography (1:1, 1:2, 1:3 hexanes/EtOAc) to give 1.94 g (52%) tetrasaccharide **6** and 0.84 g of recover trisaccharide acceptor **3**.

$[\alpha]_{\text{D}}^{20} = 5.69$ ($c = 5.71$, CHCl_3); IR (cm^{-1}): ν 3300, 2918, 2248, 1956, 1747, 1692, 1536, 1454, 1370, 1217, 1130, 913; ^1H NMR (CDCl_3 , 400 MHz): δ 7.71 (d, 2H, $J = 7.8$ Hz), 7.62 (d, 2H, $J = 7.6$ Hz), 7.48–7.18 (m, 24H), 5.82 (m, 1H), 5.35 (d, 1H, $J = 8.6$ Hz), 5.29 (d, 1H, $J = 9.7$ Hz), 5.17 (m, 3H), 5.09 (dt, 1H, $J = 5.4$, 10.9 Hz), 5.01 (d, 1H, $J = 17.2$ Hz), 4.96 (d, 1H, $J = 10.2$ Hz), 4.92–4.81 (3d, 3H), 4.77–4.66 (3d, 3H), 4.62 (d, 1H, $J = 12.2$ Hz), 4.45 (d, 1H, $J = 12.2$ Hz), 4.38–4.29 (3d, 3H), 4.21–3.68 (m, 19H), 3.90 (s, 3H), 3.56 (m, 2H), 3.39 (m, 4H), 2.30 (m, 2H), 2.17 (m, 3H), 2.04 (s, 3H), 2.01 (s, 3H), 1.99 (s, 3H), 1.91 (s, 3H), 1.90 (s, 3H), 1.74 (m, 2H), 1.73 (m, 2H) 1.02–0.79 (m, 42 H); ^{13}C NMR (CDCl_3 , 100 Hz): δ 170.7,

170.3, 169.8, 169.0, 168.9, 142.5, 139.2, 139.1, 138.9, 138.5, 138.1, 131.5, 129.5, 128.4, 128.3, 128.2, 128.1, 127.9, 127.8, 127.7, 127.4, 127.3, 127.1, 126.8, 114.8, 103.5, 103.4, 102.8, 99.6, 82.8, 81.7, 79.4, 77.7, 76.5, 75.9, 75.2, 75.1, 74.6, 74.2, 73.8, 73.24, 73.16, 73.0, 71.8, 69.5, 69.3, 69.2, 68.6, 68.4, 68.1, 66.3, 61.43, 61.35, 57.4, 53.2, 49.5, 34.8, 30.2, 28.9, 23.2, 20.8, 20.6, 20.5, 18.3, 18.1, 17.9, 12.8, 11.8. ESI-MS: m/z 1969.1 $[\text{M}+\text{Na}]^+$.

4.3. Lactone, 7

To a solution of the tetrasaccharide **6** (0.565 g, 0.29 mmol) in THF (10.0 mL) was added glacial AcOH (0.17 mL, 10.0 equiv) and TBAF (6.0 mL, 1.0 M in THF, 10.0 equiv). The reaction mixture was stirred at room temperature for 2 days and poured into ice-water (100 mL) followed by extraction with EtOAc (4×150 mL). The organic extracts were combined, dried over MgSO_4 , and concentrated. The resulting triol was dissolved in anhydrous MeOH (9.0 mL) and sodium methoxide was added (1.2 mL of a 25% solution in MeOH). The contents were stirred at room temperature for 3 days, and 3.8 mL water and 3.8 mL THF were added. Stirring at room temperature for an additional 2 days followed by neutralization with Dowex- H^+ , filtration with MeOH washing, and concentration afforded the crude material which was allowed to dry under high vacuum for 1 day. To a solution of sodium (0.70 g) in liquid NH_3 (80 mL) was added a solution of the resulting white solid in THF (12 mL), and the resulting mixture was stirred at -78 °C for 3 h. The reaction was quenched with anhydrous MeOH (20 mL), warmed to room temperature, and concentrated with a stream of dry argon. The residue was diluted with MeOH (80 mL) and treated with Dowex 50W×8-400 until pH was nearly 5–6. The mixture was filtered and concentrated to a white solid. The white solid was dissolved in pyridine (12.0 mL) and Ac_2O (6.0 mL) at room temperature. To the solution of tetrasaccharide was added DMAP (2.8 mg) and stirred for an additional 2 days. Concentration followed by purification by column chromatography (gradient elution 100% EtOAc \rightarrow 100/1, 80/1, 40/1 EtOAc/MeOH) gave lactone **7** (0.2135 g, 55% yield over five steps).

$[\alpha]_{\text{D}}^{22} = -24.4$ ($c = 1.02$, CHCl_3); IR (cm^{-1}): ν 3388, 2941, 2253, 1754, 1681, 1537, 1434, 1371, 1292, 1234, 1172, 1126, 1048, 918; ^1H NMR (CDCl_3 , 400 MHz): δ 6.38 (d, 1H, $J = 8.6$ Hz), 5.75 (m, 1H), 5.68 (d, 1H, $J = 16.4$ Hz), 5.50 (dt, 1H, $J = 5.3$, 10.8 Hz), 5.38 (d, 1H, $J = 2.9$ Hz), 5.21 (m, 5H), 5.00–4.87 (m, 3H), 4.58 (dd, 1H, $J = 7.3$, 10.8 Hz), 4.52–4.31 (m, 6H), 4.20 (m, 3H), 4.08 (m, 1H), 3.99 (m, 2H), 3.95–3.80 (m, 4H), 3.76 (d, 1H, $J = 10.7$ Hz), 3.66 (m, 2H), 3.46 (m, 1H), 2.46 (dd, 1H, $J = 5.3$, 13.4 Hz), 2.18 (s, 3H), 2.16 (s, 3H), 2.08 (s, 3H), 2.07 (s, 3H), 2.067 (s, 3H), 2.05 (s, 7H), 2.01 (s, 7H), 2.00 (s, 5H), 1.93 (s, 3H), 1.87 (s, 3H), 1.84 (m, 1H), 1.64 (m, 2H); ^{13}C NMR (CDCl_3 , 100 Hz): δ 171.1, 170.9, 170.7, 170.44, 170.39, 170.3, 170.1, 170.0, 169.4, 162.6, 137.7, 115.0, 100.5, 99.9, 98.6, 97.4, 77.2, 76.6, 75.0, 73.7, 73.0, 72.7, 72.6, 71.9, 71.7, 70.9, 70.2, 69.8, 69.2, 68.0, 67.4, 66.8, 63.2, 63.0,

62.1, 61.5, 51.0, 48.9, 38.4, 29.7, 28.5, 23.5, 23.1, 20.8, 20.7, 20.65, 20.59, 20.51, 20.46. ESI-MS: m/z 1371.2 $[M+Na]^+$.

4.4. Methyl ester, 4

To a solution of the tetrasaccharide **6** (0.9678 g, 0.497 mmol) in THF (10.0 mL) was added glacial AcOH (0.28 mL, 10.0 equiv) and TBAF (10.0 mL of 1.0 M in THF, 10.0 equiv). The reaction mixture was stirred at room temperature for 2 days and poured into ice-water (50 mL) followed by extraction with EtOAc. The organic extracts were dried over $MgSO_4$ and concentrated. The resulting triol was dissolved in anhydrous MeOH (20.0 mL) and sodium methoxide was added (2.4 mL of a 25% solution in MeOH). The contents were stirred at room temperature for 3 days, and then 8.0 mL water and 8.0 mL THF were added. Stirring at room temperature for an additional 2 days was followed by neutralization with Dowex- H^+ , filtration with MeOH washing, and concentration. The crude material was allowed to dry under high vacuum for 1 day. To a blue solution of sodium (0.70 g) in liquid NH_3 (80 mL) was added a solution of the resulting white solid in THF (30.0 mL), and the resulting mixture was stirred at $-78^\circ C$ for 2 h. The reaction was quenched by the addition of anhydrous MeOH (20 mL), warmed to room temperature, and concentrated with a stream of dry argon. The residue was diluted with MeOH (70 mL) and treated with Dowex 50wX8-400 until pH was nearly 5–6. The mixture was filtered and concentrated to a solid. The solid was dissolved in pyridine (20.0 mL) and Ac_2O (10.0 mL) at room temperature. To the solution of tetrasaccharide was added DMAP (3.8 mg) and stirred for an additional 2 days. The reaction mixture was cooled to $0^\circ C$, followed by treatment with MeOH. To this solution was added DMAP (4.2 mg) and stirred at room temperature for an additional 4 days. The reaction mixture was concentrated and co-evaporated with toluene (4 \times 100 mL). The residue was dissolved in pyridine (19.0 mL) and Ac_2O (9.0 mL) at room temperature. The mixture was stirred for 1 day and then concentrated. The residue was dissolved in MeOAc (40.0 mL) and MeI (1.0 mL). To the solution cesium carbonate (0.146 g) was added, stirred for 3 h, and diluted with methyl acetate (800 mL), the organic layer was washed with saturated NaCl-saturated NH_4Cl (1:1, 100 mL), saturated $NaHCO_3$ (100 mL), brine (100 mL), and dried over $MgSO_4$. Concentration followed by purification by column chromatography (gradient elution 100% EtOAc \rightarrow 100/1, 80/1, 60/1, 40/1 EtOAc/MeOH) gave methyl ester **4** (0.5354 g, 76% yield over eight steps, each step 97% yield).

$[\alpha]_D^{23} = -21.8$ ($c = 2.18$, $CHCl_3$); IR (cm^{-1}): ν 3384, 2957, 1747, 1684, 1665, 1540, 1435, 1370, 1230, 1167, 1129, 1046; 1H NMR (400 MHz, $CDCl_3$): δ 5.97 (m, 1H), 5.73–5.62 (m, 2H), 5.54 (d, 1H, $J = 10.0$ Hz), 5.45 (m, 1H), 5.28 (dd, 1H, $J = 1.7, 9.5$ Hz), 5.25 (d, 1H, $J = 2.9$ Hz), 5.07 (t, 1H, $J = 9.3$ Hz), 5.02 (d, 1H, $J = 8.2$ Hz), 4.88 (m, 3H), 4.76 (m, 2H), 4.53 (d, 1H, $J = 7.8$ Hz), 4.36 (m, 2H), 4.27 (dd, 1H, 1.9, 12.3 Hz), 4.13 (m, 2H), 4.01 (m, 4H), 3.89 (m, 2H), 3.77 (m,

4H), 3.74 (s, 3H), 3.51 (m, 2H), 3.43–3.30 (m, 3H), 2.70 (dd, 1H, $J = 3.5, 12.5$ Hz), 2.20 (s, 1H), 2.11 (s, 3H), 2.03 (s, 6H), 1.99 (s, 3H), 1.98 (s, 6H), 1.97 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.91 (s, 3H), 1.89 (s, 3H), 1.86 (s, 3H), 1.75 (s, 3H), 1.70–1.49 (m, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.16, 170.51, 170.33, 170.30, 170.21, 170.19, 170.11, 170.06, 169.95, 169.82, 169.43, 169.38, 169.28, 168.00, 165.60, 137.57, 114.80, 100.31, 100.26, 98.97, 97.25, 75.77, 73.24, 73.10, 72.61, 72.40, 71.90, 71.55, 71.48, 69.88, 69.35, 69.22, 68.98, 68.50, 67.42, 66.73, 66.62, 63.09, 62.08, 61.28, 52.58, 48.88, 36.97, 30.52, 29.57, 28.33, 23.21, 22.82, 21.17, 20.62, 20.53, 20.47, 20.41, 20.35, 19.53, 18.71. ESI-MS: m/z 1445.5 $[M+Na]^+$; HRMS (FAB) calcd for $C_{61}H_{87}N_2O_{36}$ $[M+H]^+$, 1423.5093; found, 1423.5039.

4.5. GM2 glycosylamino acid, 1

A mixture of methyl ester **4** (191.6 mg), allyl glycine **5** (304.1 mg, 5.3 equiv), and Grubbs I catalyst **9** (15.6 mg, 0.14 equiv) in CH_2Cl_2 (0.70 mL) under an argon stream was stirred at $45^\circ C$. After 24 h, catalyst **9** (14.6 mg) and CH_2Cl_2 (0.55 mL) was added, and the mixture was heated for one more day. Concentration followed by purification by column chromatography (gradient elution 100% EtOAc \rightarrow 70/1, 30/1, 20/1 EtOAc/MeOH) gave a mixture of **4** and desired products. The residue was dissolved in MeOH (15.0 mL) and water (1.0 mL), and 18.8 mg of 10% Pt/C was added. The mixture was stirred under an atmosphere of H_2 for 4 days. The reaction mixture was then filtered through Celite washed with MeOH, concentrated and then purified by column chromatography (gradient elution 100% EtOAc \rightarrow 9/1, 85/15 $CHCl_3$ /MeOH) to afford **1** (126.2 mg, 54% over two steps).

$[\alpha]_D^{27} = -19.3$ ($c = 1.26$, $CHCl_3$); IR (cm^{-1}): ν 3364, 2939, 1747, 1681, 1556, 1538, 1454, 1434, 1371, 1229, 1169, 1126, 1046. 1H NMR (500 MHz, MeOD): δ 7.78 (d, 2H, $J = 7.5$ Hz), 7.66–7.58 (m, 2H), 7.37 (t, 2H, $J = 7.4$ Hz), 7.29 (t, 2H, $J = 7.4$ Hz), 5.59 (t, 1H, $J = 3.5$ Hz), 5.57 (m, 1H), 5.38 (d, 1H, $J = 10.1$ Hz), 5.35 (d, 1H, $J = 3.1$ Hz), 5.11 (t, 1H, $J = 9.3$ Hz), 5.01 (d, 1H, $J = 9.3$ Hz), 4.97 (dd, 1H, $J = 8.1, 10.0$ Hz), 4.78 (t, 1H, $J = 8.8$ Hz), 4.65 (d, 1H, $J = 7.9$ Hz), 4.50 (m, 2H), 4.39–4.24 (m, 5H), 4.20 (t, 1H, $J = 6.7$ Hz), 4.13 (dd, 2H, $J = 6.3, 11.3$ Hz), 4.06 (m, 3H), 3.97 (m, 3H), 3.90 (s, 3H), 3.84 (t, 1H, $J = 9.4$ Hz), 3.77 (m, 1H), 3.71 (t, 1H, $J = 5.8$ Hz), 3.64–3.56 (m, 3H), 3.44 (m, 1H), 2.84 (dd, 1H, $J = 4.3, 12.7$ Hz), 2.24 (s, 3H), 2.15 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H), 2.06 (s, 6H), 2.04 (s, 3H), 2.02 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.965 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.82 (s, 3H), 1.81 (m, 1H), 1.63 (t, 2H, $J = 12.4$ Hz), 1.50 (m, 2H), 1.27 (m, 6H). ^{13}C NMR (125 MHz, MeOD): δ 174.06, 173.54, 172.35, 172.34, 172.29, 172.12, 172.08, 171.93, 171.70, 171.64, 171.50, 171.31, 171.07, 169.72, 158.55, 145.41, 145.24, 142.59, 128.87, 128.24, 126.32, 126.28, 121.02, 102.15, 101.64, 101.18, 98.84, 77.43, 74.87, 74.82, 74.36, 73.77, 73.26, 72.95, 72.64, 71.56, 71.08, 70.90, 70.66, 70.59, 69.74, 68.76, 68.30, 67.83, 64.80, 63.61, 63.52, 63.00, 53.88, 53.29, 49.94, 48.46, 38.46, 33.20, 30.50, 30.01, 26.92, 26.88, 23.32, 22.78, 22.12,

21.71, 21.62, 21.16, 21.03, 20.94, 20.90, 20.84, 20.76, 20.67, 20.63, 20.59, 18.02. ESI-MS: m/z 1734.8 $[M+H]^+$; HRMS (FAB) calcd for $C_{79}H_{104}N_3O_{40}$ $[M+H]^+$, 1734.6228; found, 1734.6196.

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