A Total Synthesis of the Methyl Glycoside of Ganglioside GM_1

Samit K. Bhattacharya[†] and Samuel J. Danishefsky*[,†,‡]

Department of Chemistry, Columbia University, Havemeyer Hall, New York, New York 10027, and The Laboratory for Bioorganic Chemistry, The Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021

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The total synthesis of the methyl glycoside of GM_1 (**1b**) has been accomplished. The key step in the synthesis involves the sulfonamidoglycosidation reaction, which is used to create a β-linkage leading to a GalNAc residue joined to the C4 hydroxyl group of a galactose unit of a C3 sialylated lactosyl moiety. The "proximal hydroxyl" directing effect, which has been postulated before, manifests in this context as well leading to the preponderant formation of the β-glycoside. Together with asialo GM_1 and other substructures, the GM_1 methyl glycoside has been submitted for biological assays as potential ligands for bacterial and viral infection sites.

Background

Bronchial infection by microorganisms, particularly *Pseudomonas aeruginosa*, is apparently responsible for the morbidity and mortality of victims of cystic fibrosis (CF). Al Awqati and co-workers have found that CF bronchial and pancreatic epithelia reversibly bind the microorganism *P. aeruginosa*.[1,2] Strong evidence was put forward to suggest that asialo GM_1 (**2a**, see Figure 1), which is bound through a hydrophobic attraction to the apical membrane of CF epithelia, is a likely binding site for *P. aeruginosa* and that its increased abundance contributes to bronchial bacterial invasion (Figure 1).

These findings stimulate interest in evaluating glycosides of the type **2b** where the core carbohydrate is retained, but the ceramide attachment is replaced by simple glycosidic linkages. The synthetic carbohydrate ligands in suitably bioavailable form could serve as "decoys" to prevent or clear up bacterial infection. The understanding of how glycolipid "ligands" interact with protein receptors in infectious bacteria could well benefit from an insight as to structure−activity relationships (SAR) of the carbohydrate domain. Efforts toward that goal have already led to a stereoselective synthetic route to asialo GM_1 and other simpler glycosides.[3] For a thorough investigation of whether these asialoglycosides can function as potential antagonists of bacterial invasion in keeping with the findings of Tuomanen,[4] Al Awqati,[1] and Krivan,[5] one needed access to sialylated glycosides mimicking GM_1 for the case at hand.

The target being a suitable analogue of GM_1 where the ceramide moiety would be substituted with a simpler alkyl group, we set out to synthesize the methyl glycoside applying the glycal methodology[6] that had been developed

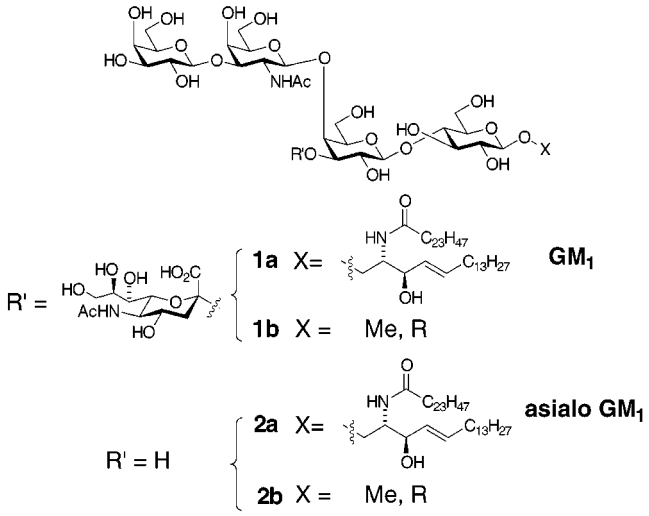

Figure 1.

in our laboratories hoping to apply the lessons learned in the asialo GM_1 synthesis.[3] GM_1 itself had also been synthesized previously by Ogawa,[7] Hasegawa,[8] and Schmidt.[9]

In our initial retrosynthetic analysis, drawing upon our synthesis of asialo GM_1, we decided to target the tetrasaccharide moiety of asialo GM_1 with a suitable protecting group at C3 of the internal galactose unit. This acceptor site would then be unmasked prior to coupling with the sialic acid moiety to provide the penultimate pentasaccharide glycal, which upon epoxidation and methanolysis would deliver the protected version of GM_1-methyl glycoside. Thus, we hoped to couple the ABCD glycal with a sialic acid unit (E) (Scheme 1).

The ABCD tetrasaccharide glycal would arise from the coupling of the disaccharide sectors AB and CD. These disaccharides would ultimately evolve from a combination of monocyclic glycals. In the case at hand, the

[†] Columbia University.
[‡] The Sloan-Kettering Institute for Cancer Research.

(1) Imundo, L.; Barasch, J.; Prince, A.; Al-Awqati, Q. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3019−3023.
(2) Barasch, J.; Kiss, B.; Prince, A.; Saiman, L.; Gruenert, D.; Al-Awqati, Q. *Nature* **1991**, *352*, 70−73. Barasch, J.; Al-Awqati, Q. *J. Cell. Sci. (Suppl.)* **1993**, *17*, 229−233.
(3) Kwon, O.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1998**, *120*, 1588.
(4) Cundell, D. R.; Tuomanen, E. *Microb. Pathog.* **1994**, *17*, 361−374.
(5) Krivan, H. C.; Roberts, D. D.; Ginsberg, V. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6157−6161.
(6) Danishefsky, S. J.; Bilodeau M. T. *Angew. Chem., Intl. Ed. Engl.* **1996**, *35*, 1380.
(7) Sugimoto, M.; Numata, M.; Koike, K.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1986**, *156*, C1.
(8) Hasegawa, A.; Nagahama, T.; Kiso, M. *Carbohydr. Res.* **1992**, *235*, C13. Hasegawa, A.; Ishida, H.; Nagahama, T.; Kiso, M. *J. Carbohydr. Chem.* **1993**, *12*, 703.
(9) Stauch, T.; Greilich, U.; Schmidt, R. R. *Liebigs Ann.* **1995**, 2101.

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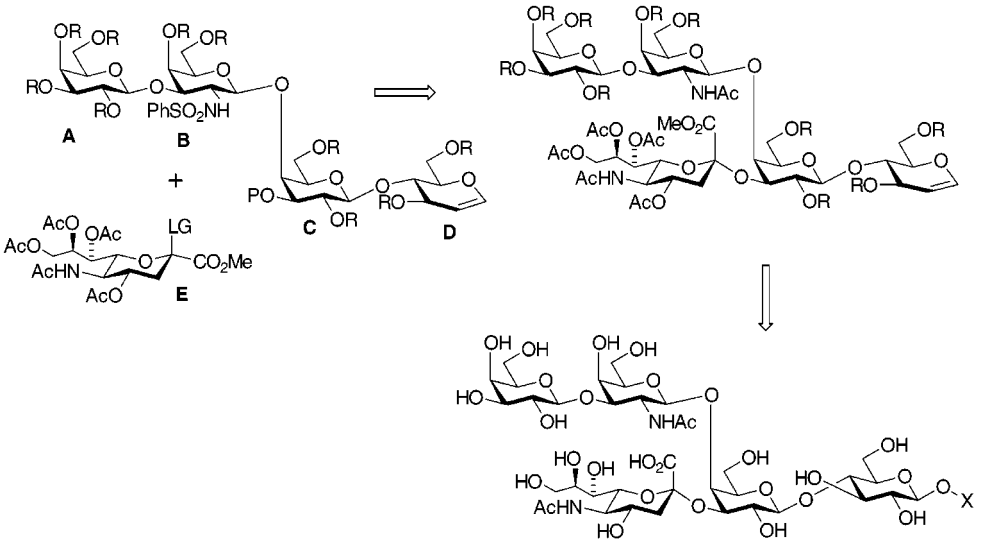
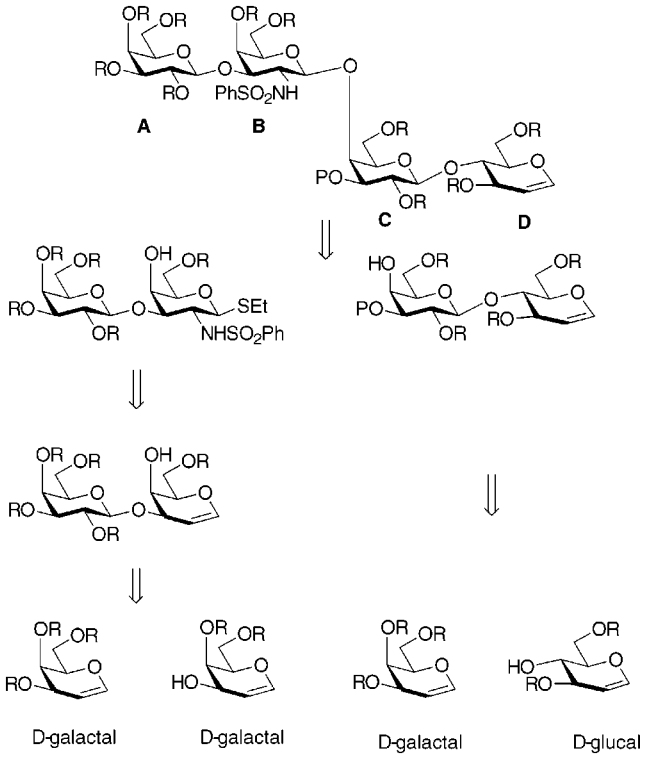
Scheme 1



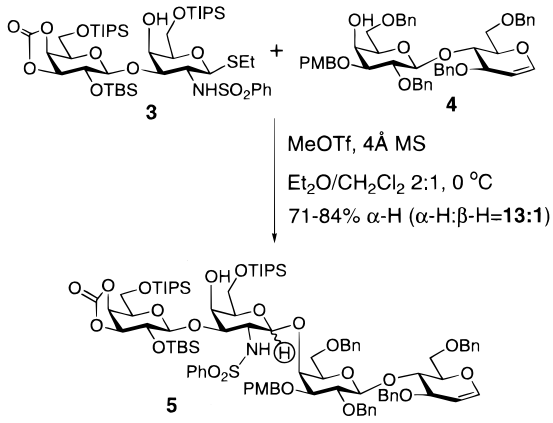
assembly would be comprised of three D-galactal units and one D-glucal unit (Scheme 2).

Scheme 2



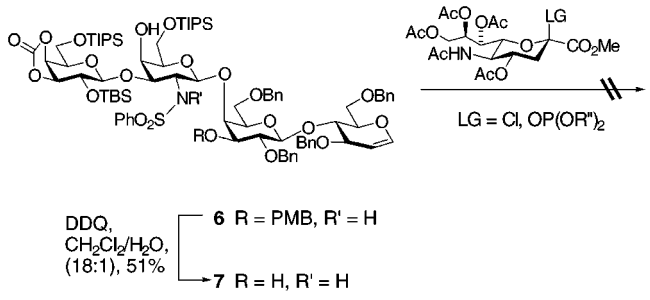
The synthesis of the azaglycosyl donor **3** corresponding to the AB sector disaccharide piece had been described before.[3] For the CD sector disaccharide, we chose to use the tetrabenzyl lactal compound **4**, with a PMB group masking the C3′ hydroxyl and the axial C4′ hydroxyl group available to function as a glycosyl acceptor site. This intermediate was prepared exactly as the similarly described C3′ benzyl protected lactal, in our asialo GM1 report. Coupling of **3** and **4** under mediation by methyl triflate under the conditions shown provided the desired β-glycoside **5** (Scheme 3), as well as a 6% yield of the α-anomer. The preponderance of the β-glycoside was now anticipated, on the basis of precedent.[3] It is apparently a consequence of intramolecular hydrogen bonding between the free C4 hydroxyl in the AB sector and the ring oxygen, thus leading to attenuation of the anomeric control in the coupling event.[3]

Scheme 3



The C4 PMB group was deprotected by action of DDQ (Scheme 4). When the resultant tetrasaccharide acceptor was submitted to glycosylation using sialic acid, choride, or phosphite based donors, the results were dismayingly unproductive. The tetrasaccharide was returned unreacted. The N-acetylated acceptor (**6**, R′ = Ac) also failed to react under such sialylation conditions. A similar failure had marked the Schmidt synthesis of GM_1. In that case, an azide had to be substituted in place of the acetamido group in the glycosyl acceptors.[9]

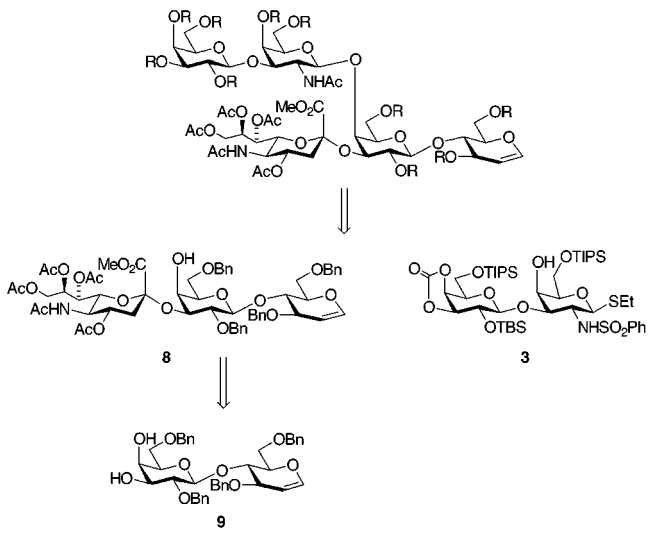
Scheme 4



Accordingly, we revised our retrosynthetic analysis with a view to introducing the sialyl unit at an earlier stage. With this in mind, we decided to target the trisaccharide **8** (which is an intermediate in the synthesis of GM_3)[10] and carry out the key sulfonamidoglycosidation with this trisaccharide as an acceptor. Of course, the reactivity of the axial C4 hydroxyl, with the neighboring sialyl group in place, in the context of the sulfonamidoglycosylation was an issue that had hitherto not been explored and could be cause for serious concern.

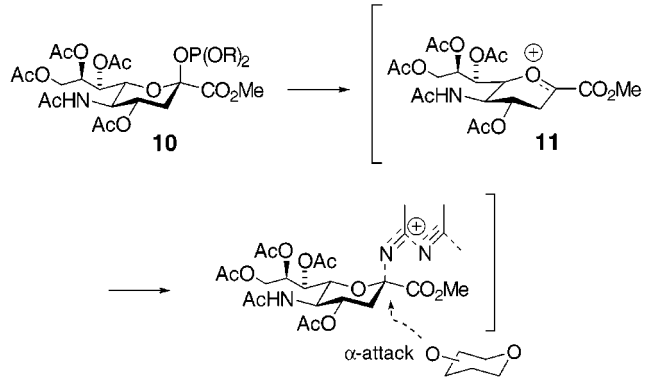
However, we were heartened by the work of Ogawa and Hasegawa, who had carried out similar [3 + 2] couplings.[7,8] Thus, in our revised strategy we planned to intersect with diol **9** (Scheme 5), which was expected to be coupled with a suitable sialyl donor.

Scheme 5



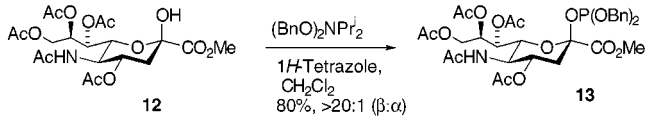
The advantages of a sialyl phosphite donor (**10**, Scheme 6) have been argued by Schmidt et al.[11] This arrangement works well in sialyl couplings compared to the classical chloride donors. However, in our hands, preparation of the desired β-sialyl phosphite was always associated with varying amounts of the α-phosphite anomer. This result, by itself, should not pose a problem, as this anomer should also lead to the common intermediate **11**. Trapping of this system by acetonitrile should give a nitrile−nitrilium conjugate (as postulated by Schmidt[11]), which would ultimately be attacked by a free hydroxyl of the acceptor giving rise to an α-linked sialyl-coupled product.

Scheme 6



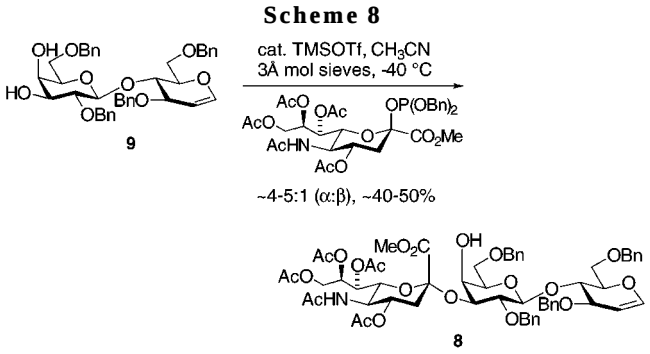
Studies carried out with the mixture of phosphites in our laboratories had shown that the α-anomer was less reactive and was obtained unreacted leading to complications in the isolation and purification of the resultant product of such couplings.[12] Wong et al. have put forward the advantages of a benzyl phosphite donor utilizing a dibenzyl phosphoramidite as starting material.[13] We were disappointed to find that, even with this method of preparation of the phosphite donor, mixtures of α- and β-anomers (albeit preponderant in the desired latter) resulted. Eventually, we discovered that use of the diisopropyl phosphoramidite as the source of the phosphite group provided, reproducibly, an ∼20:1 (β/α) mixture of the anomers in excellent yields (Scheme 7).

Scheme 7



Armed with a clean method to produce the sialyl phosphite, we carried out the sialylation of the disaccharide acceptor **9**. Although other byproducts also resulted, we obtained the desired trisaccharide **8** in ∼40−50% yields (Scheme 8).

Scheme 8



We now faced the crucial azaglycosidation reaction in the context of a sialyl-containing acceptor. In the event, much to our satisfaction, the reaction of the thioethyl donor **3** and the acceptor **8**, mediated by methyl triflate, provided the desired pentasaccharide glycal **14** (Scheme 9). The yields of **14** tended to be in the range of ∼40−50%. The process was further complicated by subsequent reaction of the acetamido group of this pentasaccharide leading to imino ether formation at that center. The imino ether **15** was rehabilitated by hydrolysis under nonaqueous conditions to the parent pentasaccharide, albeit in modest yields. The intramolecular hydrogen bonding effect of the C-4 hydroxyl in the thioethyl donor was manifested in this coupling as well,[3] leading to preponderant amounts of the β-isomer and, on occasions, negligible amounts of the α-isomer.

With the pentasaccharide glycal corresponding to GM_1 in hand, we focused on using the glycal to fashion the

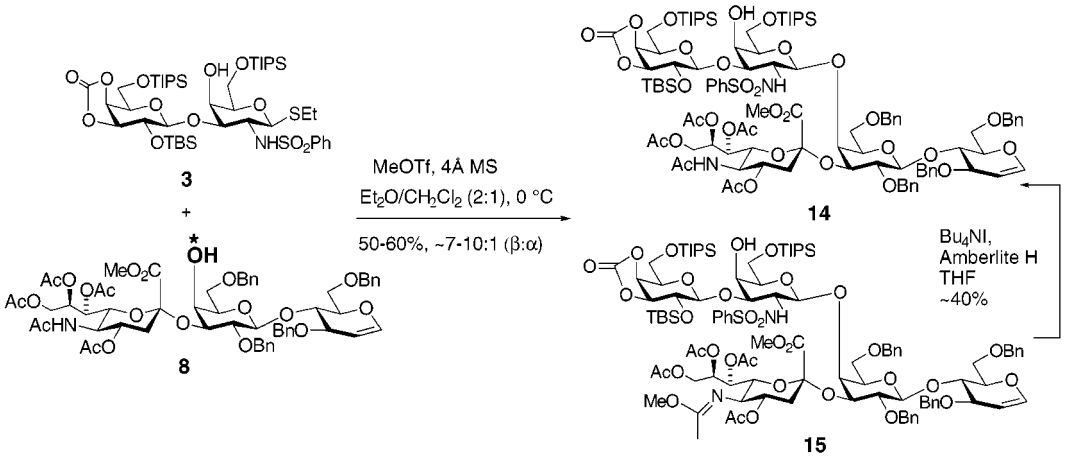
(10) For synthesis of GM_3 see: (a) Sugimoto, M.; Ogawa, T. *Glycoconjugate J.* **1985**, *2*, 5. (b) Murase, T.; Ishida, H.; Kiso, M.; Hasegawa, A. *Carbohydr. Res.* **1989**, *188*, 71. (c) Ito, Y.; Paulson, J. C. *J. Am. Chem. Soc.* **1993**, *115*, 1603.

(11) (a) Martin, T. J.; Schmidt, R. R. *Tetrahedron Lett.* **1992**, *33*, 6123. (b) Martin, T. J.; Brescelo, R.; Toepfer, A.; Schmidt, R. R. *Glycoconjugate J.* **1993**, *10*, 16.

(12) Sames, D. Unpublished results.

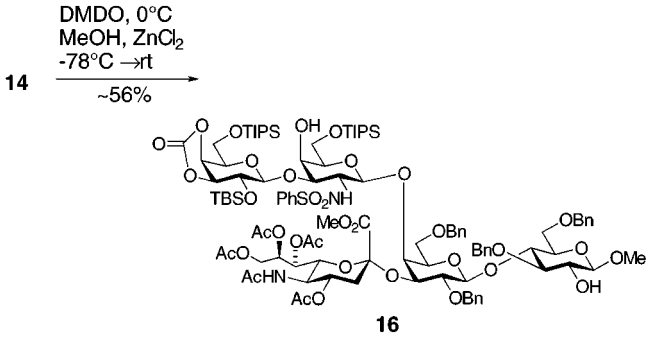
(13) Sim, M. M.; Kondo, H.; Wong, C.-H. *J. Am. Chem. Soc.* **1993**, *115*, 2260.

Scheme 9



desired methyl glycoside. To that end, the glycal **14** was treated with DMDO, and the resultant α-epoxide was subjected to methanolysis under mediation by anhydrous zinc chloride to afford a 56% yield of **16** (Scheme 10). It was critical to use $ZnCl_2$ to accelerate the opening of the epoxide. Prolonged treatment with methanol alone led to incomplete conversions and also deacetylations in the sialyl sector. Although, at this stage, the assignment of stereochemistry at glycoside **16** was based to a considerable extent on well-established analogies rather than on unambiguous data on the compounds themselves, *β*-linkages between the four saccharide units were corroborated by the observed coupling constants in the range of 9−11 Hz for all the four anomeric hydrogens (identified by HMQC analysis of the methyl glycoside **16**).

Scheme 10

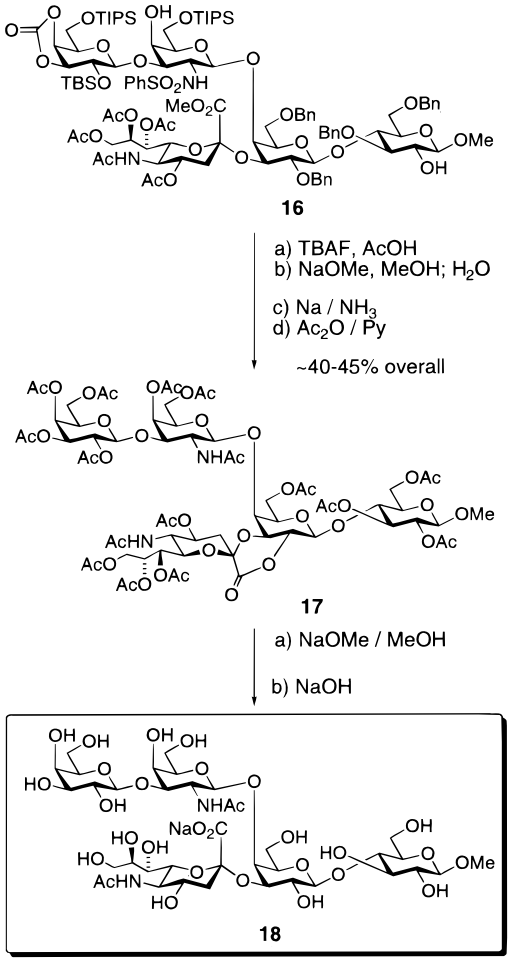


The concluding deprotection sequence commenced with desilylation. This step was followed by deacetylation and ester hydrolysis, debenzylation, and peracetylation to provide a lactone **17** in ~40−45% yield. Finally, all the *O*-acyl bonds were cleaved and after saponification, the GM_1 methyl glycoside (**18**) was isolated as its sodium salt (Scheme 11).

Our assignment of the structure of **18** was based on the NMR analysis of the intermediates en route to the final structure. The structure of **18** is also supported by the agreement of its high-resolution mass spectrum with theory (TOF-MS ES+ calcd for $C_{38}H_{63}N_2O_{29}Na_2$ 1057.3312, found 1057.3352).

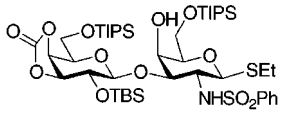
In summary, the total synthesis of the target system **18** has been accomplished. The key azaglycosidation coupling step uniting disaccharide **3** with the sialyl-containing acceptor **8** is promoted by methyl triflate. This capability, along with our previously accomplished synthesis of the asialo methyl glycoside (see structure **2b**) can now be exploited to allow for pinning down of the role of sialic acid residues in preventing attachment of bacterial receptors to cell surface carbohdyrate ligands.

Scheme 11



It is not clear whether the single methyl glycoside in a structure such as asialo target system **2b** will permit bacterial adherence or whether clustered structures more akin to the architecture of cell surfaces will be necessary. In any case, given the ability to synthesize either **2b** or **18**, the role of the sialic acid in modulating the ligand receptor recognition can be established in a rigorous way.

Experimental Procedures[14]

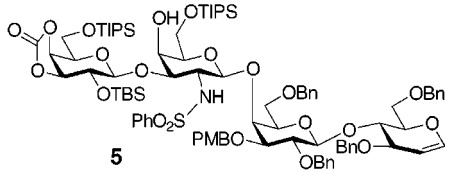


3

Modified Procedure for Preparation of Thioglycoside 3. The glycal (250 mg, 0.33 mmol) was azeotropically dried with anhydrous benzene and placed under vacuum overnight and thereafter placed under Ar. Freshly dried 4 Å molecular sieves (300 mg) and benzenesulfonamide (52 mg) were then added to the glycal in a glovebox. CH_2Cl_2 (4 mL) was added, and the solution was stirred at 0 °C for ∼10 min. Iodonium bis(*sym*-collidine)perchlorate (IDCP) (201 mg, 0.43 mmol) that had been weighed in the dark in a glovebox was added quickly all at once to the reaction mixture and the resultant solution stirred at 0 °C in the dark (reaction vessel covered with Al foil). TLC analysis after ∼10 min revealed that most of the starting material had been consumed. The reaction was stirred for a further 15 min at 0 °C and then diluted with ether and filtered through a glass-sintered funnel (pore size M) in the dark. The filtrate was then washed with aqueous $Na_2S_2O_3$ solution once followed by water and then vigorously extracted with aqueous $CuSO_4$ solution (×4), washed with water again, and dried over anhydrous Na_2SO_4, and the solvents were evaporated at a bath temperature of 5−10 °C. The crude material was taken up in CH_2Cl_2 and then filtered through a short pipet column of anhydrous Na_2SO_4, and the filtrate was evaporated and then placed under vacuum for 30 min.

An oven-dried vial (Chemglass) was cooled under Ar and then charged with anhydrous DMF (0.5 mL) and EtSH (122 μL, 1.65 mmol). The solution was cooled to ∼−40 °C, and a solution of LHMDS in THF (1.0 M, 1 mL) was added followed by addition of a solution of the crude iodosulfonamide (prepared above) in anhydrous DMF (3 mL) via cannula. The solution was stirred at −40 °C for ∼1 h and then slowly warmed to −10 °C in another 1 h. The reaction mixture was then diluted with EtOAc at this temperature and then quickly added to ice followed by addition of saturated aqueous NH_4Cl solution. The aqueous portion was then re-extracted with EtOAc, and the combined organics were washed with brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude material was chromatographed with EtOAc−hexanes (5 → 15%) to provide the desired thioglycoside **3** (129 mg, 40%).

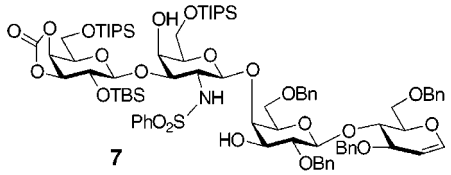
For spectroscopic data see ref 3.



5

Preparation of the Tetrasaccharide 5. The thioglycoside **3** (183 mg, 0.19 mmol) and the alcohol **4** (142 mg, 0.19 mmol) were weighed in an oven-dried flask and were then azeotropically dried using anhydrous benzene and thereafter placed under vacuum overnight. To this mixture were added freshly activated 4 Å molecular sieves (1.6 g) followed by a mixture of CH_2Cl_2 (1.5 mL) and ether (3 mL). The solution was stirred for about 10 min at room temperature before being cooled to 0 °C. After ∼15 min, MeOTf (0.11 mL, 0.97 mmol) was added dropwise and the solution allowed to stir for ∼12 h in a Cryocool bath temperature of ∼0−5 °C. TLC at this point showed some starting material left that did not disappear over time. Therefore, Et_3N (0.4 mL) was added to quench the reaction and the reaction mixture diluted with ether. After filtration through a Celite bed and evaporation of the filtrate, the resulting material was chromatographed using EtOAc−hexane (5 → 20%) to yield the desired major tetrasaccharide **5** (198 mg, 62% based on thioglycoside, 89% based on recovered alcohol **4**). The ratio of **5** (β linkage) and its anomeric isomer (α-linkage) was found to be 14:1 (isolated).

5: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, J = 7.6 Hz, 2H), 7.41−7.18 (m, 27H), 6.89 (d, J = 8.3 Hz, 2H), 6.51 (d, J = 6.2 Hz, 1H), 5.64 (d, J = 4.0 Hz, 1H), 5.19 (s, 1H), 5.07 (d, J = 9.3 Hz, 1H), 4.95 (m, 1H), 4.88 (dd, J = 17.9, 10.7 Hz, 1H), 4.76 (d, J = 10.5 Hz, 1H), 4.64 (m, 2H), 4.58−4.44 (m, 5H), 4.41 (d, J = 6.4 Hz, 1H), 4.35 (m, 2H), 4.22 (m, 2H), 4.12 (br unresolved m, 1H), 4.07 (br unresolved m, 1H), 3.94 (m, 2H), 3.84 (m, 2H), 3.79 (s, 3H), 3.75 (m, 2H), 3.64 (dd, J = 10.5, 4.1 Hz, 1H), 3.57 (dd, J = 9.0, 4.8 Hz, 1H), 3.50 (m,1H), 3.44 (m, 2H), 3.38−3.27 (m, 3H), 3.22 (m 1H), 3.17 (d, J = 9.7 Hz, 1H), 3.03 (br s, 1H), 1.06, 1.05 and 1.02 (all s and overlapping m, 42H), 0.91 (s, 9H), 0.28 (s, 3H),0.19 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 183.8, 159.7, 156.1, 144.7, 138.5, 138.2, 130.0, 129.2, 128.3, 128.1, 128.0, 127.7, 127.4, 114.0, 102.8, 101.0, 99.3, 81.5, 80.9, 79.7, 75.7, 75.4, 75.2, 75.0, 74.3, 73.6, 73.2, 72.7, 70.9, 69.7, 69.3, 68.6, 67.8, 65.7, 61.8, 60.9, 56.0, 55.1, 29.7, 25.6, 17.9, 11.9, −4.5, −5.5. (36 unresolved); IR (ν_{max} cm^{-1}) 3529, 3234, 2931, 2891, 2862, 1793, 1509, 1460; $[\alpha]^{25}_D$ = −17.3° (*c* 1.5, $CHCl_3$); HRMS (FAB) calcd for $[C_{91}H_{129}NO_{22}SSi_3K]^+$ 1742.7655, found 1742.7672.

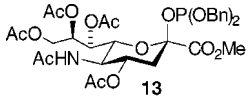


7

Preparation of Tetrasaccharide 7. The tetrasaccharide **5** (35 mg, 0.02 mmol) was taken up in CH_2Cl_2 (1 mL) and H_2O (0.06 mL). To this stirred solution was added DDQ (12 mg, 0.051 mmol). The reaction was monitored by TLC analysis and after ∼2 h, although a little starting material remained, the reaction was quenched by addition of saturated aqueous $NaHCO_3$ solution. The reaction mixture was extracted with CH_2Cl_2, and the organics were washed with water and dried over Na_2SO_4. The crude material was chromatographed with EtOAc−hexane (10 → 25%) to provide the alcohol **7** (16 mg, 51%).

7: ^{1}H NMR (500 MHz, C_6D_6) δ 7.90 (d, J = 7.7 Hz, 2H), 7.42−7.19 (m, 23H), 6.49 (d, J = 6.1 Hz, 1H), 5.62 (br unresolved m, 1H), 5.4 (s, 1H), 5.06 (d, J = 8.9 Hz, 1H), 4.99 (d, J = 10.7 Hz, 1H), 4.95 (m, 1H), 4.66 (d, J = 8.9 Hz, 1H), 4.63−4.32 (m, 10H), 4.25 (dd, J = 8.7, 5.4 Hz, 1H), 4.17 (br unresolved m, 1H), 4.08 (br unresolved m, 1H), 4.01 (br unresolved m, 1H), 3.94 (t, J = 8.7 Hz, 1H), 3.88 (br unresolved m, 1H), 3.86−3.79 (m, 3H), 3.76 (t, J = 9.4 Hz, 1H), 3.66 (dd, J = 10.5, 3.9 Hz, 1H), 3.61−3.51 (m, 4H), 3.46 (m, 1H), 3.38 (m, 4H), 3.01 (br s, 1H), 2.84 (br s, 1H), 1.05, 1.04 and 1.01 (all s and overlapping m, 42H) 0.89 (s, 9H), 0.26 (s, 3H),0.19 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155.9, 144.6, 138.3, 138.2, 138.1, 132.8, 129.2, 128.6, 128.4, 128.1, 128.0, 127.8, 127.7, 127.6, 127.5, 127.4, 102.7, 102.3, 101.2, 99.2, 81.7, 79.6, 75.7, 75.6, 75.4, 75.0, 73.8, 73.6, 73.4, 73.2, 72.8, 70.6, 69.8, 69.6, 68.8, 67.8, 65.8, 61.8, 60.9, 56.3, 29.7, 25.6, 17.9, 17.7, 11.9, −4.5, −5.3. (36 unresolved); IR (ν_{max} cm^{-1}) 3539, 3234, 2931, 2862, 1793, 1648, 1465; $[\alpha]^{22}_D$ = −19.9° (*c* 0.99, $CHCl_3$); HRMS (FAB) calcd for $[C_{83}H_{121}NO_{21}SSi_3K]^+$ 1622.7097, found 1622.7045.

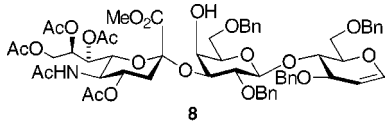
(14) **General Experimental Methods.** All commercial materials were used without purification unless otherwise noted. The following solvents were distilled under positive pressure of dry argon: tetrahydrofuran (THF), diethyl ether (Et_2O) from sodium−benzophenone ketyl, methylene chloride (CH_2Cl_2) and toluene (C_6H_5-CH_3) from CaH_2. All the reactions were performed under an inert (Ar) atmosphere. Analytical thin-layer chromatography was performed using E. Merck silica 60-F254 coated 0.25 mm plates. Compounds were visualized by dipping the plates in a cerium sulfate−ammonium molybdate solution followed by heating. Flash column chromatography was performed using the indicated solvent on E. Merck silica gel 60 (40−63 μm or 10−40 μm).



13

Preparation of the Sialyl Phosphite 13. The tetraacetate-NANA methyl ester **12** (200 mg, 0.41 mmol) was taken up in THF (6 mL). The 1*H*-tetrazole (120 mg, 1.71 mmol) was added, and to this stirred solution was added the phosphorimidite (0.32 mL, 0.94 mmol) in a dropwise fashion. The reaction mixture turned cloudy with the addition of the phosphorimidite. TLC analysis upon completion of addition showed almost complete consumption of the starting material. The reaction mixture was stirred for a further 5−10 min before being diluted with CH_2Cl_2 and then quenched with ice-cold 0.3 M aqueous HCl. The resultant solution was washed with saturated aqueous. $NaHCO_3$ solution and ice−water and dried over anhydrous Na_2SO_4. After concentration in vacuo, the material so obtained was chromatographed with EtOAc−hexanes (80%) to yield the desired β-anomeric phosphite **13** in pure form (201 mg, 67%) and a mixture of anomeric phosphites (20 mg, 7%) to reflect a combined ratio of ∼20:1 of the β/α anomers.

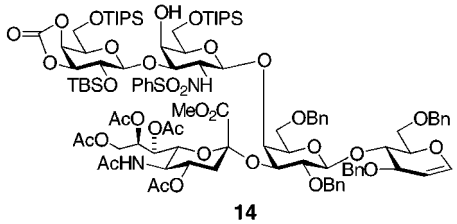
For spectroscopic data see ref 13.



8

Preparation of the Sialylated Trisaccharide 8. The diol **9** (260 mg) was azeotropically dried with anhydrous benzene and evacuated overnight. This was put under Ar, freshly dried 4 Å molecular sieves (400 mg) were added (in a glovebox) followed by anhydrous CH_3CN (5 mL), and the resultant suspension was stirred at room temperature for 30 min. The phosphite **13** (261 mg, 0.355 mmol) was separately taken up in anhydrous CH_3CN (3 mL) and added via cannula to the diol solution, and the resultant solution was allowed to stir at room temperature for a further 30 min. The reaction mixture was then cooled to ∼−40 °C and stirred for ∼15 min before addition of TMSOTf (10 μL, 0.056 mmol). After being stirred for ∼40 min at −40 → −35 °C, the reaction mixture was quenched by addition of saturated aqueous $NaHCO_3$, warmed to room temperature. The reaction mixture was then added to EtOAc and washed with saturated aqueous $NaHCO_3$, and the organics were dried over anhydrous Na_2SO_4. The resultant material was chromatographed with EtOAc−chloroform (70%) to give a total trisaccharide−product yield of 62% with the desired trisaccharide **8** as the major isomer (41%). Occasionally one of the byproducts—the sialic acid−glycal unit formed by the elimination of the anomeric phosphite, which coelutes with the mixture of trisaccaharide products—was separated by a gel filtration with Sephadex LH-120 using hexanes/CH_2Cl_2/MeOH (4:2:1) as eluent followed by a normal chromatography to separate the different isomers.

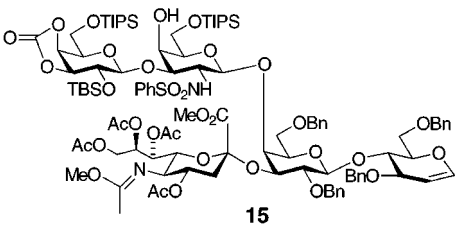
8: ^{1}H NMR (500 MHz, C_6D_6) δ 7.25 (m, 20H), 6.42 (d, J = 6.3 Hz, 1H), 5.38 (m, 1H), 5.28 (dd, J = 8, 1.5 Hz, 1H), 5.19 (d, J = 9.4 Hz, 1H), 4.86 (m, 2H), 4.69 (q, J = 11.6 Hz, 2H), 4.64 (d, J = 7.7 Hz, 1H), 4.58 (s, 2H), 4.45 (m, 4H), 4.28 (m, 2H), 4.11 (m, 3H), 4.0 (m, 2H), 3.95 (dd, J = 12.4, 5.9 Hz, 1H), 3.8−3.69 (m, 3H), 3.75 (s, 3H), 3.61 (m, 3H), 3.5 (dd, J = 9.2, 8.0 Hz, 1H), 2.71 (br s, 1H), 2.50 (dd, J = 13, 4.6 Hz, 1H), 2.08 (s, 3H), 1.99 (m, 1H), 1.97 (s, 3H), 1.91 (s, 3H), 1.85 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.8, 170.6, 170.2, 170.0, 168.5, 160.1, 144.4, 138.9, 138.7, 138.1, 128.3, 128.1, 127.9, 127.6, 127.3, 109.6, 102.6, 99.4, 98.2, 75.8, 75.7, 74.8, 73.5, 73.2, 72.7, 72.6, 71.9, 70.2, 69.0, 68.7, 68.1, 68.0, 67.2, 62.3, 53.4, 53.0, 49.3, 36.7, 29.7, 23.2,21.1, 20.8, 20.7, 20.6 (16 unresolved); IR (ν_{max} cm^{-1}) 3357, 3031, 2923, 1746, 1663, 1541, 1454; $[\alpha]^{20}_D$ = −15.2° (*c* 1.03, $CHCl_3$); HRMS (FAB) calcd for $[C_{60}H_{71}NO_{21}K]^+$ 1180.4156, found 1180.4194.



14

Preparation of the Pentasaccharide 14. The thioglycoside **3** (118 mg, 0.12 mmol) and the alcohol **8** (138 mg, 0.12 mmol) were weighed in an oven-dried flask and were then azeotropically dried using anhydrous benzene and thereafter placed under vacuum overnight. To this mixture were added freshly activated 4 Å molecular sieves (300 mg) followed by a mixture of CH_2Cl_2 (1 mL) and ether (2 mL). The solution was stirred for about 10 min at room temperature before being cooled to 0 °C. After ∼15 min, MeOTf (20 μL, 0.18 mmol) was added dropwise and the solution allowed to stir for ∼24 h in a Cryocool bath temperature of ∼5 °C. TLC at this point showed around 40% product formation. A further equivalent of MeOTf was then added and the resulting solution allowed to stir for another 12 h. Although TLC analysis showed starting material to be still present, since side products (evinced by other spots) were increasing, the reaction was terminated by addition of Et_3N (0.4 mL) and the reaction mixture was diluted with ether. After filtration through a Celite bed and evaporation of the filtrate, the resulting material was chromatographed using a gradient eluant. With EtOAc−hexanes (15 → 20%), the imino ether **15** (34 mg, 14%) eluted first. This was followed by EtOAc−hexanes (40 → 70%) to give the desired pentasaccharide **14**, as a >14:1 mixture (114 mg, 54% based on thioglycoside) and as a 3:1 mixture of isomers (14 mg, 6%) and finally EtOAc−hexanes (70 → 100%) to provide unreacted acceptor **8** (30 mg, 22%). The combined yield of pentasaccharide products was thus ∼60% based on thioglycoside and 76% based on recovered acceptor trisaccharide.

14: ^{1}H NMR (500 MHz, C_6D_6) δ 8.2, (dd, J = 8.4, 1.2 Hz, 2H), 7.5 (d, J = 7.1 Hz, 2H), 7.4−6.95 (m, 21H), 6.36 (d, J = 6.3 Hz, 1H), 5.89 (d, J = 3.6 Hz, 1H), 5.85 (br unresolved m, 1H), 5.59 (m, 1H), 5.41 (dd, J = 9.4, 1.9 Hz, 1H), 5.1 (m, 2H), 4.9 (dt, J = 10.8, 6.4 Hz, 1H), 4.9 (m, 3H), 4.79 (d, J = 9.1 Hz, 1H), 4.61−4.50 (m, 5H), 4.49−3.91 (m, 23H), 4.02 (s, 3H), 3.84 (m, 2H), 3.7 (d, J = 2.7 Hz, 1H), 3.6 (dd, J = 8.0, 7.7 Hz, 1H), 3.48 (m, 1H), 3.37 (m, 1H), 2.56 (dd, J = 14.0, 5.1 Hz, 1H), 2.37 (dd, J = 13.7, 12.0 Hz, 1H), 2.21 (s, 3H), 1.89 (s, 3H), 1.85 (s, 3H), 1.64 (s, 3H), 1.52 (s, 3H), 1.28 (m, 6H), 1.20−1.10 (all s, 36 H), 0.90 (s, 9H), 0.39 (s, 3H),0.16 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 170.6, 170.4, 170.2, 169.7, 169.2, 167.9, 160.1, 155.8, 144.5, 138.4, 138.1, 132.7, 129.3, 128.3, 128.2, 128.1, 127.9, 127.7, 127.6, 127.5, 127.3, 102.7, 102.4, 102.1, 101.5, 99.3, 99.3, 81.9, 78.1, 77.6, 75.6, 75.3, 75.2, 74.6, 73.5, 73.4, 73.3, 72.9, 72.6, 72.2, 70.9, 70.1, 69.6, 68.9, 68.7, 67.9, 67.9, 66.6, 65.8, 61.6, 60.9, 56.5, 53.2, 49.3, 35.7, 31.9, 29.6, 25.6, 23.2, 22.6, 20.9, 20.7, 20.7, 20.6, 17.9, 17.7, 14.1, 11.9, 11.8, −4.7, −5.3. (34 unresolved); IR (ν_{max} cm^{-1}) 3546, 3306, 3064, 3031, 2940, 2866, 1793, 1748, 1670, 1541, 1497; $[\alpha]^{20}_D$ = −26.3° (*c* 1.5, $CHCl_3$); HRMS (FAB) calcd for $[C_{103}H_{148}N_2O_{33}SSi_3K]^+$ 2095.8630, found 2095.8728.

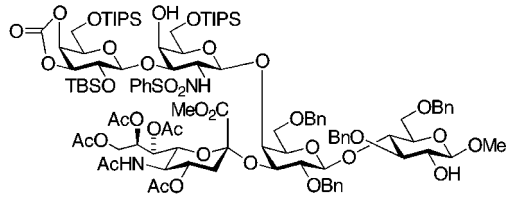


15

Data for the imino ether 15: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.87 (d, J = 7.5 Hz, 2H), 7.41−7.17 (m, 23H), 6.52 (d, J = 6.3 Hz, 1H), (5.51 (d, J = 4.2 Hz, 1H), 5.37 (s, 1H), 5.19 (m,

2H), 5.09 (d, J = 9.1 Hz, 1H), 4.99 (t, J = 5.1 Hz, 1H), 4.82 (m, 1H), 4.79 (d, J = 10.1 Hz, 1H), 4.65 (m, 2H), 4.56−4.42 (m, 8H), 4.37 (q, J = 11.6 Hz, 2H), 4.24 (m, 2H), 4.14 (br unresolved m, 1H), 4.06 (br unresolved m, 2H), 4.03 (d, J = 3.9 Hz, 1H), 4.01 (br unresolved m, 1H), 3.97 (s, 3H), 3.95 (m, 2H), 3.89 (dd, J = 9.7, 3.7 Hz, 1H), 3.87−3.61 (m, 11H), 3.58 (br s, 3H), 3.46 (m, 2H), 3.37 (t, J = 5.5 Hz, 1H), 3.26 (dd, J = 9.6, 7.9 Hz, 1H), 2.96 (m, 2H), 2.49 (dd, J = 13.5, 4.6 Hz, 1H), 2.1 (s, 3H), 1.96 (s, 3H), 1.95 (m partly hidden, 1H), 1.81 (s, 3H), 1.80 (s, 3H), 1.05, 1.04, 0.99 and 0.98 (all s and overlapping m, 42 H), 0.89 (s, 9H), 0.23 (s, 3H),0.18 (s, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.4, 169.3, 169.1, 168.9, 167.9, 164.8, 160.3, 155.8, 144.5, 138.6, 138.3, 138.1, 132.7, 129.2, 128.8, 128.3, 127.9, 127.7, 127.6, 127.4, 102.6, 102.4, 101.5, 99.7, 99.0, 81.8, 78.2, 75.6, 75.4, 74.2, 73.6, 73.5, 73.3, 72.8, 72.6, 70.3, 70.1, 69.6, 68.7, 68.4, 68.0, 67.9, 67.6, 65.8, 61.6, 60.9, 57.0, 56.6, 53.7, 52.3, 29.7, 25.6, 20.9, 20.6, 20.4, 17.9, 17.7, 15.4, 11.9, 11.8, −4.7, −5.3. (44 unresolved); IR (ν_{max} cm^{-1}) 3540, 3301, 3064, 3031, 2942, 2866, 1794, 1751, 1679, 1650, 1456; HRMS (FAB) calcd for $[C_{104}H_{150}N_2O_{33}SSi_3K]^+$ 2109.8787, found 2109.8745.

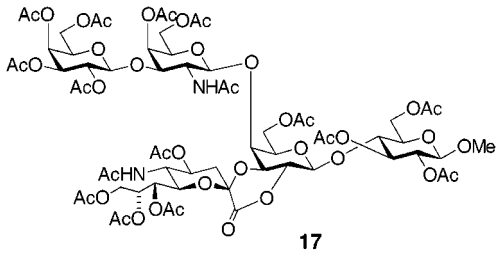
Conversion of the Imino Ether 15 to the Pentasaccharide 14. The imino ether **15** (∼20 mg, 0.001 mmol) was taken up in THF (0.5 mL). Tetrabutylammonium iodide (TBAI) (∼5 mg) was added followed by Amberlite IR-120 (prewashed with THF, ∼14 mg). The reaction was monitored by TLC analysis, more TBAI and Amberlite IR-120 were added, and the reaction mixture was allowed to stir overnight. Although starting material remained, on account of an increase of byproducts formation, the reaction was worked up by dilution with EtOAc, the Amberlite resin was filtered off, and the organics were washed first with saturated aqueous $NaHCO_3$ and then with aqueous $Na_2S_2O_3$ until the yellow color was discharged. After a further brine wash and drying over anydrous Na_2SO_4, the solvents were evaporated. The crude material was chromatographed with EtOAc−hexanes (25 → 60%) to provide the pentasaccharide **14** (8 mg, 40%).



16

Preparation of the Methyl Glycoside 16. The pentasaccharide **14** (61 mg, 0.03 mmol) was azeotropically dried with anhydrous benzene and put under vacuum overnight and then put under Ar. Freshly dried 4 Å molecular sieves (190 mg) were added (in a glovebox) followed by CH_2Cl_2 (2 mL), and the resulting suspension was allowed to stir at 0 °C for ∼20 min. A solution of DMDO/acetone (titrated immediately before addition, 0.75 mL, 0.045 mmol) was added and the solution stirred for 30 min at 0 °C. The solvent was then removed by a positive pressure of Ar followed by evacuation for ∼30 min. Anhydrous MeOH (2 mL) was then added to the material at 0 °C and the stirred solution cooled to −78 °C. THF (2 mL) was added at this point followed by a solution of $ZnCl_2$ (45 μL, 0.045 mmol). After 30 min at −78 °C, the reaction mixture was allowed to warm to room temperature over the course of 5 h and then stirred for an additional 30 min at room temperature before diluting with EtOAc and quenching with pH 7.0 buffer. The reaction mixture was extracted with EtOAc followed by salting out with NaCl (s), washed with brine, and dried over anhydrous Na_2SO_4. After evaporation of solvents, the crude material was chromatographed through a short column of SiO_2 using hexane/EtOAc/acetone (30:20:8 → 30:20:10 → 30:20:15) to provide the desired methyl glycoside **16** (54 mg, 86%).

16: ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.84 (d, J = 7.6 Hz, 2H), 7.35−7.10 (m, 23H), 5.6 (d, J = 3.7 Hz, 1H), 5.29 (dd, J = 9.5, 1.3 Hz, 1H), 5.2 (m, 2H), 5.15 (d, J = 10 Hz, 1H), 5.06 (d, J = 9 Hz, 1H), 4.92 (dd, J = 11.4, 4.8 Hz, 1H), 4.83 (q, J = 11.6 Hz, 2H), 4.8 (overlapping d, J = 10.3 Hz, 1H), 4.6 (m, 3H), 4.48 (m, 2H), 4.39 (m, 2H), 4.2 (m, 5H), 4.5−3.83 (m, 6H), 4.92 (overlapping s, 3H), 3.78 (m, 8H), 3.65 (dd, J = 8.7, 5.1 Hz, 1H), 3.60 (m, 1H), 3.53 (s, 3H), 3.46 (m, 4H), 3.33 (m, 1H), 3.25 (m, 1H), 2.83 (br s, 1H), 2.61 (s, 1H), 2.51 (br s, 1H), 2.30 (dd, J = 13.4, 4.8 Hz, 1H), 2.19 (d, J = 12.5 Hz, 1H), 2.10 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.88 (s, 3H), 1.87 (s, 3H), 1.01 (m, 6H), 1.04, 1.03, 0.99, 0.98 (all s, 36 H), 0.85 (s, 9H), 0.16 (s, 3H), 0.13 (s, 3H); ^{13}C NMR [125 MHz, $CDCl_3$] δ 170.6, 170.4, 169.7, 169.3, 167.7, 160.1, 155.6, 138.4, 137.9, 132.5, 129.1, 128.4, 128.3, 128.1, 128.0, 127.7, 127.6, 127.5, 127.2, 103.4, 102.9, 102.4, 102.1, 99.7, 83.0, 82.7, 79.4, 78.4, 76.0, 75.8, 75.5, 75.2, 74.4, 73.7, 73.4, 73.2, 72.6, 72.3, 69.3, 69.1, 68.8, 68.1, 67.9, 66.5, 65.7, 61.7, 61.5, 61.1, 56.9, 56.4, 53.8, 53.3, 49.2, 35.5, 29.7, 29.3, 25.5, 23.2, 20.9, 20.8, 20.7, 20.6, 17.9, 17.6, 11.9, 11.8, −4.8, −5.3. (36 unresolved); IR (ν_{max} cm^{-1}) 3546, 3294, 2941, 2866, 2959, 1794, 1750, 1686, 1455; $[\alpha]^{20}_D = -16.4°$ (c 2.7, $CHCl_3$); HRMS (FAB) calcd for $[C_{104}H_{152}N_2O_{35}SSi_3]^+$ 2127.9100, found 2127.9060.

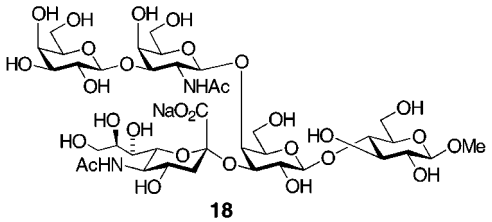


17

Preparation of Lactone 17. Tetrabutylammonium fluoride (TBAF) (1 M in THF, 0.51 mL, 20 equiv) was added to the methyl glycoside **16** (54 mg, 0.026 mmol) in THF (0.5 mL). The mixture was stirred for 48 h at room temperature and then concentrated. To the residue was added NaOMe (2% solution in MeOH, 1 mL) and the solution stirred at room temperature for another 48 h. To the reaction mixture were added 1 mL of water and 1 mL of THF and the mixture stirred for an additional 24 h at room temperature. The reaction mixture was cooled to 0 °C, and the pH was adjusted to ∼8−9 using Dowex 50X8-400 ion-exchange resin. The resin was removed by filtration, and the filtrate was concentrated to dryness. The residue was then dissolved in THF (2 mL). Meanwhile a two-necked flask fitted with a dry ice condenser was cooled to −78 °C, and about 15 mL of liquid NH_3 was collected. Na (ca. 4 × 4 × 4 mm) was added to it, and to the blue solution was added the solution in THF. The reaction was stirred under reflux of NH_3 for ∼30 min. The reaction was quenched with MeOH (5 mL), and the ammonia was evaporated. The residual mixture was treated with Dowex 50X8-400 ion-exchange resin to pH 10 and then concentrated in vacuo. The residue was taken up in pyridine (anhydrous, 2 mL), Ac_2O (2 mL) was added, and the resultant mixture was stirred for 48 h at room temperature. The mixture was concentrated in vacuo, and the residue was chromatographed first with MeOH−CH_2Cl_2 (4 → 5%) followed by EtOAc−CH_2Cl_2−MeOH (80:20:2 → 80:20:3 → 80:17:3 → 80:16:4 → 80:15:5) to yield the peracetate−lactone **17** as a pure compound (15 mg, 37%) and as a mixture of isomers (5 mg, 12%).

17: ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.45 (br unresolved m, 1H), 5.48 (m, 2H), 5.38 (d, J = 3.2 Hz, 1H), 5.29 (d, J = 2.8 Hz, 1H), 5.22 (m, 3H), 5.05 (d, J = 7.9 Hz, 1H), 5.03 (dd, J = 10.3, 7.9 Hz, 1H), 4.92 (dd, J = 10.4, 3.3 Hz, 1H), 4.88 (dd, J = 9.4, 8.1, 1H), 4.74 (d, J = 7.8 Hz, 1H), 4.56 (dd, J = 10.5, 7.5 Hz, 1H), 4.51 (d, J = 10.7 Hz, 2H), 4.39 (m, 5H), 4.22−4.0 (m, 7H), 3.94 (dd, J = 12.0, 7.5 Hz, 1H), 3.9−3.75 (m, 4H), 3.72 (dd, J = 10.6, 2.5 Hz, 1H), 3.68 (m, 1H), 3.62 (m, 1H), 3.52 (m, 1H), 3.46 (s, 3H), 2.46 (dd, J = 13.5, 5.4 Hz, 1H), 2.16 (s, 3H), 2.12 (s, 6H), 2.09 (s, 6H), 2.07 (s, 9H), 2.05 (s, 3H), 2.03 (s, 6H), 2.02 (s, 3H), 2.00 (s, 6H), 1.96 (s, 3H), 1.87 (s, 3H), 1.82 (dd, J = 13.1, 11.8 Hz, 1H); ^{13}C NMR (125 MHz, $CDCl_3$) δ 171.9, 171.1, 170.9, 170.4, 170.3, 170.2, 169.6, 169.5, 163.1, 101.4, 100.3, 99.9, 98.9, 97.9, 75.2, 74.3, 73.8, 73.0, 72.9, 72.7, 72.0, 71.8, 71.4, 70.9, 70.3, 69.2, 68.9, 68.8, 68.4, 68.1, 66.6, 63.5,

63.0, 62.5, 62.0, 60.6, 57.0, 54.5, 52.2, 49.3, 38.0, 29.7, 25.1, 24.0, 23.1, 20.9, 20.8, 20.7, 20.2 (17 unresolved); IR (ν_{max} cm^{-1}) 3584, 3390, 2937, 1748, 1673, 1537, 1435;HRMS (FAB) calcd for $[C_{66}H_{90}N_2O_{42}K]^+$ 1621.4605, found 1621.4552.



18

Synthesis of GM_1 Methyl Glycoside (18). To the peracetate **17** (15 mg, 0.01 mmol) were added MeOH (1 mL) and solid NaOMe (~ 5 mg), and the solution was stirred overnight at room temperature. To this was added 1 mL of water, and the mixture was stirred for an additional 24 h at room temperature. The reaction mixture was cooled to 0 °C, and the pH was adjusted to ~8−9 by treatment with Dowex 50X8-400 ion-exchange resin. The resin was removed by filtration and the filtrate concentrated and dried by lyophilization. The residue was subjected to Bio-Gel P2 (fine, 45−90 μm) column chromatography eluting with water to afford the sodium salt of GM_1 methyl glycoside **18** (8 mg, ~80%) after removal of water via lyophilization.

18: ^{1}H NMR (500 MHz, CD_3OD-D_2O) δ 4.87 (d, J = 8.6 Hz, 1H), 4.44 (dd, J = 8.0,7.6 Hz, 2H), 4.23(d, J = 7.9 Hz, 1H), 4.15 (m, 2H), 4.03 (m, 2H), 3.92−3.80 (m, 6H), 3.80−3.65 (m, 11H), 3.58−3.37 (m, 10H), 3.52 (s, 3H), 2.71 (dd, J = 12.7, 4.7 Hz, 1H), 2.01 (s, 3H), 1.99 (s, 3H), 1.92 (t, J = 11.9 Hz, 1H); ^{13}C NMR (125 MHz, CD_3OD-D_2O) δ 176.0, 175.3, 175.1, 106.2, 104.9, 104.4, 103.9, 103.2, 82.4, 80.7, 78.8, 76.3, 76.2, 76.1, 75.7, 75.4, 74.8, 74.3, 74.2, 73.4, 72.3, 71.2, 70.1, 69.6, 69.4, 64.8, 62.7, 62.3, 61.7, 61.6, 57.7, 53.5, 52.5, 38.3, 23.8, 22.8 (2 unresolved); $[\alpha]^{25}_D$ = +19.9° (c 0.25, MeOH−H_2O); HRMS (TOF, ES$^+$) calcd for $[C_{38}H_{63}N_2O_{29}Na_2]^+$ 1057.3312, found 1057.3552.

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Supporting Information Available: Experimental procedure for compound **4** and spectral data for all new compounds are included. This information is available free of charge via the Internet at http://pubs.acs.org.

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