# New Applications of the *n*-Pentenyl Glycoside Method in the Synthesis and Immunoconjugation of Fucosyl $GM_1$: A Highly Tumor-Specific Antigen Associated with Small Cell Lung Carcinoma

**Jennifer R. Allen[†] and Samuel J. Danishefsky*[,†,‡]**

*Contribution from the Laboratory of Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, New York 10021 (USA) and Department of Chemistry, Columbia University, Havemeyer Hall, New York, New York, 10027*

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**Abstract:** The synthesis of fucosyl $GM_1$ pentenyl glycoside **1b**, and its conjugation to carrier protein KLH to give **1c** is related. Bioconjugation of **1b** was realized using the pendant olefin contained in the reducing end *n*-pentenyl glycoside (NPG). The key step of the endeavor is a stereospecific [3 + 3] coupling reaction using our sulfonamido glycosidation protocol (see **23** + **12** → **15**). Pre-installation of the NPG was required for an optimal [3 + 3] coupling yield and to allow for smooth global deprotection. The synthesis and subsequent immunocharacterization served to confirm the assigned structure of the natural tumor antigen. Fully synthetic conjugate **1c** advances our program toward the goal of using a synthetic vaccine containing fucosyl $GM_1$ as a potential target for immune attack against small cell lung carcinoma.

## Introduction

It has been known for some time that certain glycolipids and glycoproteins, which are either not detectable or barely detectable in a particular normal tissue type by immunohistochemistry, are more commonly encountered in tumors of that tissue.[1] These high levels of expression on tumor cells will often result in an antibody response, apparently too weak or otherwise ineffective to provide requisite immunoprotection or immunorejection. Nonetheless, the idea that vaccination with noncell surface-bound chemically equivalent antigens could trigger a clinically useful active immune response is certainly very attractive. It provides the basis for using carbohydrates in the development of antitumor vaccines.[2]

Cancer carbohydrate antigens such as TF, Tn,[3] sTn, KH-1,[4] $Le^y$,[5] and Globo-H[6] are suitable targets for both active and passive immunotherapies because they have been carefully characterized as being overexpressed at the surface of malignant cells in a variety of cancers (breast, colon, prostate, ovarian, liver, small cell lung, and adenocarcinomas). In addition, they have been immunocharacterized by monoclonal antibodies and therefore have relevant serological markers available for immunological studies. Such studies suggest that patients immunized in an adjuvant setting with carbohydrate-based vaccines produce antibodies reactive with cancer cells and that the production of such antibodies prohibits tumor reoccurrence and correlates with a more favorable diagnosis.[7]

A major drawback in using carbohydrate epitopes, however, is that they are generally not readily available by isolation from natural sources. It therefore falls to the organic chemist to provide a workable program of synthesis such that the epitope of interest can be systematically studied in a favorable molecular context optimized to elicit an immunological response. The present state of the art in the field primarily relies on incorporation of the epitope into a carrier protein.[8] Several strategies for fashioning the linkage between carbohydrate and protein have been followed, although optimal spacer−linker combinations have not been determined. Nonetheless, successful synthesis of the entire construct provides a basis for beginning the immunological evaluation.

[†] Sloan-Kettering Institute for Cancer Research

[‡] Columbia University

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An ongoing project in our laboratory involves the development of anti-cancer vaccines that incorporate fully synthetic carbohydrate moieties.[9] Our strategy involves total synthesis of the carbohydrate epitope and its subsequent covalent bioconjugation to carrier protein. The vaccine constructs are then subjected to appropriate mouse immunization studies, with the ultimate goal of advancing to human clinical trials. This strategy has resulted in several fully synthetic tumor-associated carbohydrate-based vaccines which are at various stages of advanced preclinical and clinical processing. In fact, our Globo-H based vaccine is undergoing clinical evaluation for the treatment of prostate and breast carcinomas at the phase II level,[10] while a Lewis$^y$ antigen-based vaccine, already tested in ovarian cancer, is awaiting more extensive follow-up evaluation.[11]

We became interested in the fucosylated $GM_1$ ganglioside, **1a**, for a number of reasons. Nilsson et al. identified fucosyl $GM_1$ as a specific marker associated with small cell lung cancer (SCLC) cells.[12] These workers isolated the glycosphingolipid fucosyl $GM_1$ (**1a**) as the major ganglioside component contained in human SCLC tissue. Furthermore, monoclonal antibodies (F12) to the antigen serve to detect fucosyl $GM_1$ in tissues and serum of SCLC patients.[13] Immunohistochemistry studies have suggested that, due to its highly restricted distribution in normal tissues, fucosyl $GM_1$ could be an excellent target for immune attack against SCLC. *Remarkably, fucosyl $GM_1$ has thus far not been found on any other human cancer cell lines, indicating that it is very SCLC-specific*.[14]

The structural assignment of the carbohydrate moiety of the SCLC antigen was based on a combination of enzymatic and chemical degradations.[12a] While there was no particular reason to question this assignment, the development of a carbohydrate-based attack on SCLC could benefit from a definitive assignment of the linkage modes of the various monosaccharides, including the stereochemistry at each glycosidic attachment. No syntheses of this carbohydrate sector have appeared in the literature. Thus, our program directed to the preparation of an immunoofunctional antigen by chemical means (vide infra) would also encompass the goal of securing the structure assignment. We note that our synthetic program would allow for presentation of the hexasaccharide epitope independent of the ceramide to the F12 mAb to ensure that all specificity is directed at the carbohydrate sector. Furthermore, the construct must be so functionalized as to anticipate the need for its conjugation to the carrier protein in anticipation of building an effective antitumor vaccine.

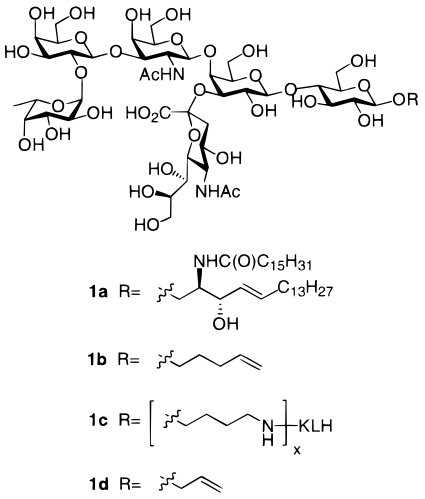


**Figure 1.** Fucosyl $GM_1$.

We report herein our synthesis of fucosyl $GM_1$ pentenyl glycoside, **1b**. The pendant olefin-containing glycoside provided three large advantages. It was used to facilitate [3 + 3] coupling, to enable global deprotection and to function as an access point for conjugation to the carrier protein keyhole limpet hemocyanin (KLH). We also report the initial immunocharacterization of **1b** (Figure 1).

## Synthetic Strategy and Results

Given that there were no reported total syntheses of the hexasaccharide moiety, at the outset of this program we were much influenced by our earlier ventures directed at Globo-H[15] and the pentasaccharide $GM_1$.[16] Those syntheses were highlighted by a remarkable late-stage convergent sulfonamido glycosidation reaction governed by a proximal hydroxyl-directing effect.[17] Because of the presence of potentially labile fucose and sialic acid moieties required for fucosyl $GM_1$, such a coupling was viewed as a particularly difficult merger. It was not without some concerns that we charted a course based on exposing our sulfonamido glycosidation format[18] to a new challenge. Our retrosynthetic analysis is shown in Scheme 1.

As seen, we dissected the hexasaccharide core of **2a** into two component trisaccharide structures, **3** and **4**, each containing a densely functionalized array. In the forward sense, the coupling of DEF donor **3** and ABC acceptor **4** would be the most complex union we have thus far attempted using the sulfonamido glycosidation protocol. Our previous results had indicated that the presence of a free hydroxyl at C4 of the reducing end galactose (see asterisk) in donor **3** would be necessary to direct the formation of the required $\beta$-linkage.[15,17] Successful coupling would lead to the hexasaccharide core containing both the fucose and sialic acid moieties. We initially set out to synthesize **1** via a hexasaccharide glycal of type **2a**. In accordance with earlier work in our laboratory, the flexible terminal glycal would, hopefully, allow for a late-stage installation of the ceramide side chain to ultimately generate glycolipid **1a**. Moreover, the glycal could be used to allow for production of allyl glycoside **1d**, whose terminal olefin could then serve as a linker group for bioconjugation. A functionalized glucopyranoside **2b** could also serve our purposes (vide infra).

(9) For a recent review of efforts continuing in our laboratory, see: Danishefsky, S. J.; Allen, J. R. *Angew. Chem., Int. Ed.* **1999**, in press and references therein.

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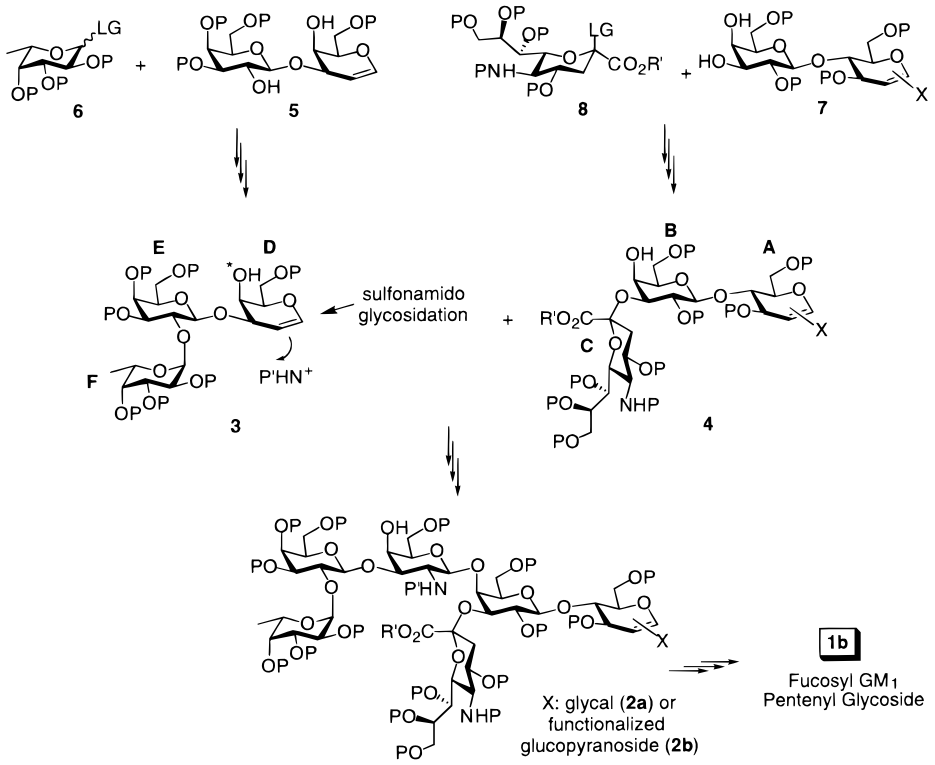
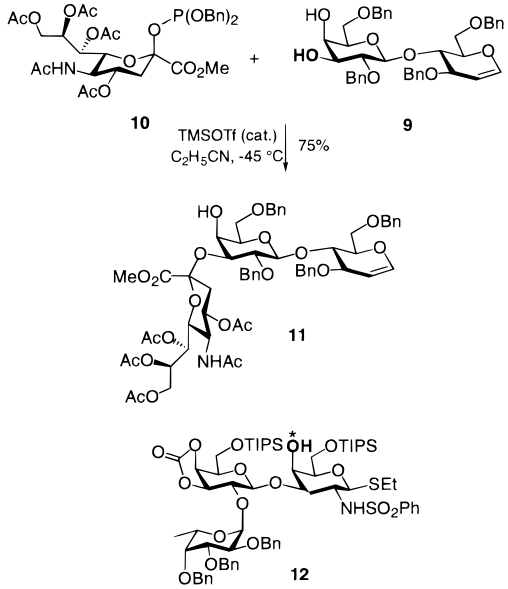
**Scheme 1**




In anticipation of the decisive [3 + 3] coupling, the trisaccharide acceptor **4** required a differentiated C4 acceptor site in the B ring. Thus, aside from the uncertainties associated with carrying potentially labile fucoside and sialoside linkages in the coupling step, there was another concern. *As seen, both the acceptor and donor moieties would be carrying potentially equally reactive C4 axial galactose hydroxyl groups.* Hence, there was a significant question as to the controllable sequencing of the donor and acceptor roles. It was conceivable that careful selection of protecting groups could serve to "de-activate" the C4 axial hydroxyl in the donor trisaccharide (**3**) toward nucleophilic attack, thus allowing for **4** to act as the requisite acceptor.[19] Such a strategy would presumably rely on steric and electronic arguments. In addition, inherent in the designed merger is that glycosidic activation of the donor **3** should also serve to de-activate its C4 hydroxyl group toward self-coupling and consequently permit the desired coupling. Since acceptor **4** also eventually joins to the sialic acid moiety, the ABC trisaccharide can further be dissected into bifunctionalized lactose derivative **7** and an appropriate sialic acid donor **8**. The synthesis of the glycal corresponding to **4** was worked out in the course of our synthesis of $GM_1$.[16] We hoped to prepare the donor corresponding to **3** by using methodology in our synthesis of the MBr1 antigen, Globo-H.[15] The DEF trisaccharide is constructed by selective fucosylation using fucosyl donor **6** on disaccharide **5**, itself available from two glycal building blocks. Thus, in short order we hoped to be in a position to examine the exciting possibility of constructing the core of fucosyl $GM_1$ by a highly convergent, but challenging, [3 + 3] coupling.

We begin our account (as shown in Scheme 2) with the synthesis of the ABC trisaccharide starting from the known protected lactal derivative, **9**.[17] Selective sialylation of the C3′ equatorial hydroxyl in **9** (see bold) proceeded smoothly with phosphite donor **10**[20] to yield the glycal **11** as the only observable isomer in 75% yield. We note that use of the chloro donor[21] and the thiomethyl donor[22] corresponding to **10** in coupling reactions with **9** proceeded in poor yields and with reduced anomeric selectivity. In addition, we employed propionitrile as the solvent because of the necessity to perform the reaction at low temperatures. Use of elevated reaction temperatures in acetonitrile as the solvent resulted in diminished anomeric selectivity, regioselectivity, and lower chemical yields.

**Scheme 2**




(19) Our synthesis of asialo $GM_1$ addresses a similar situation. See ref 17.

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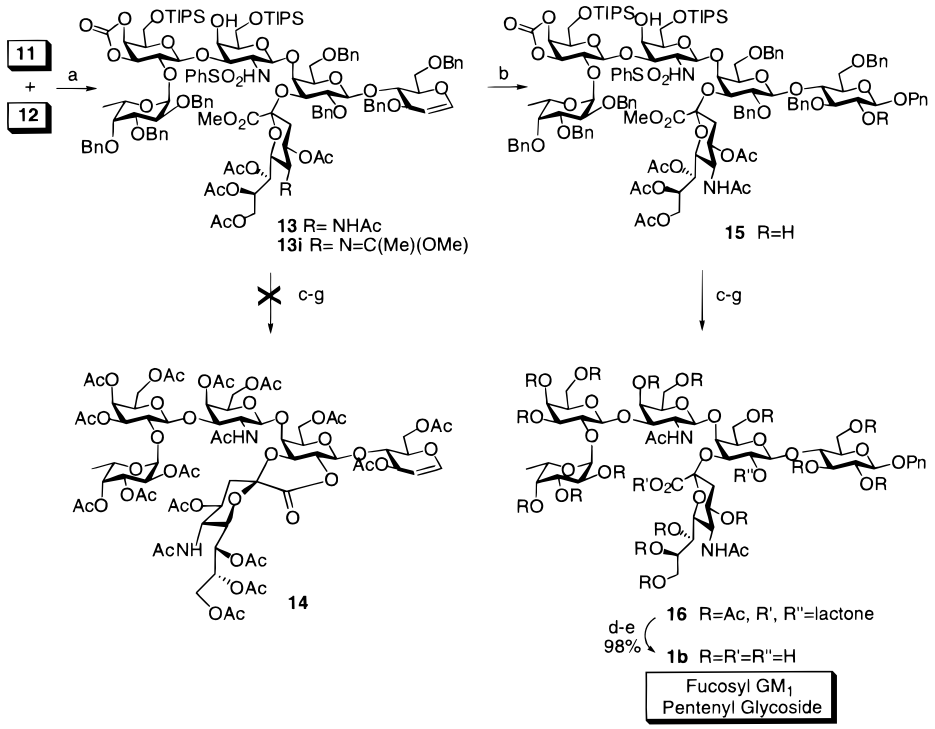
**Scheme 3**[a]




[a] Reagents: (a) MeOTf, $CH_2Cl_2$:$Et_2O$ (2:1), 0 °C, 23%; (b) (i) DMDO, $CH_2Cl_2$; (ii) PnOH, $ZnCl_2$, −78 °C, 65%; (c) TBAF, AcOH, THF; (d) NaOMe, MeOH; (e) NaOH, THF; (f) Na/$NH_3$, THF −78 °C, then MeOH; (g) $Ac_2O$, pyridine, DMAP, $CH_2Cl_2$, 46% five steps.

The key DEF trisaccharide was synthesized as previously described in our Globo-H synthesis.[15] The requisite thioethyl donor **12** is shown in Scheme 2. On the basis of previous experience, it was expected that this specific donor would favor $\beta$-glycosidation via sulfonamido participation under the close guidance of the "proximal hydroxyl" directing effect (see asterisk).[15,17] This presumption was in fact reduced to practice. In an experiment directed at "proof of principle", reaction of **12** with 5.0 equivalents of MeOTf [23] in the presence of **11** gave the desired hexasaccharide **13**, albeit in low yield (23%, Scheme 3).

Attempts to increase the efficiency in the [3 + 3] coupling met with only limited success. A significant complication was that the promoter (MeOTf), after prolonged reaction times or when used in a large excess, reacted with the *N*-acetamide contained on the sialic acid ring to give an imidate derivative, **13i**.[24] Besides **13i**, byproducts formed in the [3 + 3] coupling included degraded donor corresponding to **12** and the imidate version of recovered acceptor **11**, also formed by exposure to MeOTf. Not surprisingly, the imidates could be hydrolyzed back to the desired acetamides.[25] However, given the extra steps involved in such a protocol, we chose to attempt to avoid imidate formation by using a minimal amount of promoter (1.5−2.5 equiv). This attempted fine-tuning also adversely affected the coupling yield. Similarly, use of either a large excess of donor **12** or acceptor **11** did not significantly improve this step.

Nonetheless, with **13** in hand, we next attempted the removal of the protecting groups. Unfortunately, under all conditions attempted, we were unable to accomplish this goal. Desilylation of **13** with buffered TBAF followed by transesterification with sodium methoxide to remove the cyclic carbonate and ester-protecting groups cleanly produced the desired hexasaccharide containing nine hydroxyl groups. Saponification of the methyl ester could be effected with either NaOH/$H_2O$/MeOH, LiOH/$H_2O$/THF, or $K_2CO_3$/$H_2O$/MeOH. However, upon subjecting the resulting material to reduction with sodium in liquid ammonia and re-acetylation, the peracetate **14** was not obtained.[26] While the decomposition products were not characterized in detail, they seemed to lack the terminal glycal linkage. Deletion of the saponification step in the deprotection sequence, resulted in elimination of the sialic acid residue during the dissolving metal reduction phase. Thus, following this protocol, we were able to obtain a peracetylated pentasaccharide corresponding to essentially fucosyl asialo $GM_1$. Apparently, there are effects operating in the substrate for the dissolving metal reduction which precludes clean conversion to **14**.

In an effort to find a hexasaccharide which was suitable for global deprotection, we considered replacing the reducing end glycal. For vaccine development we required, in any case, a linker that could be modified to allow for conjugation to KLH. Previous syntheses in our carbohydrate-based vaccine program utilized the terminal olefin contained in an allyl glycoside (analogous to structure **1d**) as a linker for bioconjugation. As depicted in Figure 2, the allyl glycoside in those constructs was installed in a late-stage glycosidation by solvolysis of a glycal epoxide. Typically, the glycal was maintained through the global deprotection to the peracetate stage. In those investigations, it was demonstrated that epoxidation of the glycal, followed by treatment with allyl alcohol gave the corresponding allyl glycoside. Finally, removal of the ester-protecting groups yielded fully deprotected allyl glycoside poised for bioconjugation.[27]

(23) (a) Lonn, H. *Carbohydr. Res.* **1985**, *134*, 105. (b) Lonn, H. *J. Carbohydr. Chem.* **1987**, *6*, 301.

(24) See Experimental Section for characterization of this compound.

(25) Conditions used to effect this transformation were either $Bu_4NI$/Amberlyst $H^+$/acetone or $H_2O_2$/AcOH/$CH_2Cl_2$.

(26) We note that this is not a general result in using highly advanced glycals. For example, analogous deprotection steps have been accomplished with the hexasaccharide glycal corresponding to Globo-H and the non-asaccharide glycal corresponding to the KH-1 antigen (see ref 9 for a review). This appears to be the only case thus far where we have been unable to affect such conversions, pointing to perhaps the sensitivity of the sialic acid and glycal units in this particular structure.

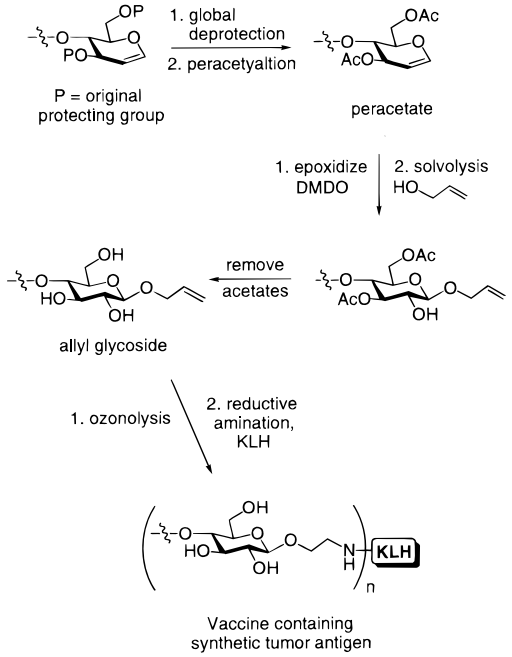



**Figure 2.** Solvolysis of glycal epoxides with allyl alcohol en route to KLH conjugation.

Through these previous studies, it was also determined that prefunctionalization to the allylic ether at the stage of original protection was not possible due to its instability under the necessary dissolving metal reduction conditions in the global deprotection sequence. The inability to affect the global deprotection of hexasaccharide **13** to peracetate **14** undermined application of this methodology to the synthesis of fucosyl $GM_1$ allyl glycoside, **1d**.

Following the difficulties we encountered, it was recognized that that the well-known pentenyl glycoside linkage, thoroughly and elegantly developed by Fraser-Reid and associates,[28] might be used to advantage in this context. *n*-Pentenyl glycosides (NPG) have been widely employed as substrates for a variety of reactions occurring at the anomeric center of oligosaccharides. NPGs are stable to a range of reaction conditions and reagents, but are readily activated for glycosidation reactions by treatment with a halogen oxidant (Figure 3). As a result of their stability and the neutral conditions required for their activation, pentenyl glycosides have been demonstrated to be valuable linkages for mechanistic and synthetic studies. However, a terminal pentenyl group could also provide a valuable handle for bioconjugation. *This linkage, in contrast to the aforementioned allyl glycoside, should be stable under the deprotection conditions we planned to apply, in particular the reductive phase of the global deprotection sequence.* Although pentenyl glycosides have not previously been used for immunoconjugation, it seemed reasonable to suppose that the four-carbon aldehyde derived from **1b** might be suitable for this purpose.

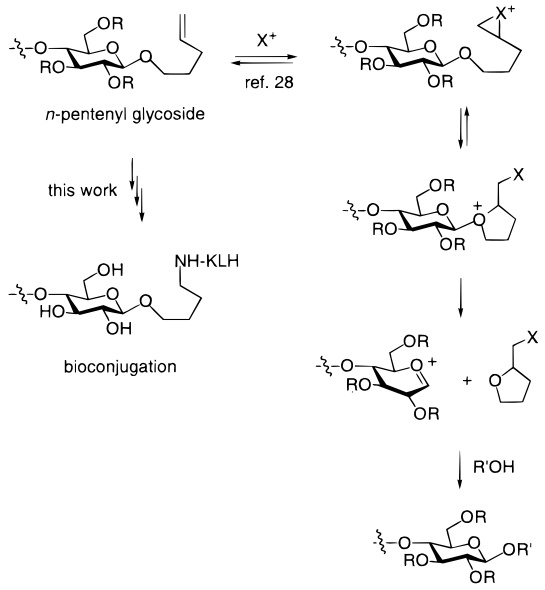



**Figure 3.** *n*-Pentenyl glycosides (NPG) as glycosidic donors.

In our earliest attempts at reduction to practice, glycal **13** was subjected to epoxidation under standard procedures with 3,3-dimethyldioxirane (Scheme 3). Reaction with pentenyl alcohol and anhydrous zinc chloride[29] afforded the glycoside **15** in 65% yield. Indeed, with the pentenyl glycoside in place, global deprotection of **15** was possible. The sequence shown in Scheme 3 furnished the peracetylated hexasaccharide lactone **16** in 46% yield ( five steps). Removal of the acetates with sodium methoxide followed by saponification of the resulting methyl ester yielded the target, fucosyl $GM_1$ pentenyl glycoside, **1b**. Our assignment of structure **1b** is based on $^1$H and $^{13}$C NMR analysis of **1b**, in conjunction with characterization of intermediates en route to the final structure, and is supported by high-resolution mass spectrometry.

As noted at the outset, our goal was not only to carry out a total synthesis of a bioconjugatable analogue of fucosyl $GM_1$ but also to produce significant quantities of this epitope for potential preclinical, and eventually clinical evaluation. Clearly, the low [3 + 3] coupling yield would undercut this goal and would thereby hamper the progress on biological evaluation.

Accordingly we explored the possibility that the pivotal [3 + 3] coupling step might be more effective with a glycoside rather than with a glycal at the "reducing end" of the acceptor. Ideally, this type of modification might allow for a more efficient total synthesis in terms of convergency, while still allowing for smooth global deprotection. As shown in Scheme 4, we first focused on pentenyl lactoside. For this purpose, lactose octaacetate was converted to the known bromide **17**.[30] Reaction of this compound with pentenyl alcohol under promotion by silver carbonate delivered the desired pentenyl glycoside, **18**, on 100 g scale.[31] An analogous coupling to produce **17** using silver triflate as promoter resulted in only a 17% yield of the desired product. Removal of the acetates yielded lactoside **19**. Again we engaged the C3′ and C4′ hydroxyl groups, this time as the dimethyl ketal **20**. This reaction, as currently conducted, is accompanied by formation of minor amounts of 4, 6-acetonide.[32,33] Presumably the 4,6-isomer can be recycled into the scheme, although at this stage we have not done so. However, in light of the subsequent results (vide infra), the NPG method

(27) The methodology described was applied in the synthesis of allyl glycosides corresponding to the Globo-H, KH-I, Lewis$^y$, and $N_3$ antigens. See ref 9 for a review of this methodology as applied to carbohydrate-based vaccines.

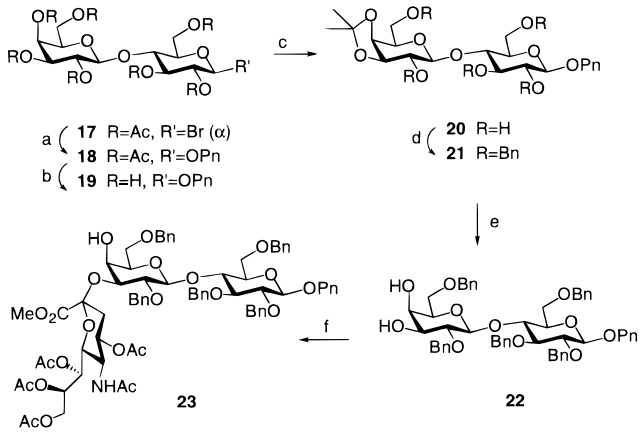
(28) For a review of NPGs, see: (a) Fraser-Reid, B. O.; Udodong, U. E.; Zufan, W.; Ottosson, H.; Merritt, R.; Rao, S.; Roberts, C.; Madsen, R. *Synlett* **1992**, 927. See also: (b) Udodong, U. E.; Madsen, R.; Roberts, C.; Fraser-Reid, B. O. *J. Am. Chem. Soc.* **1993**, *115*, 7886. (c) Merritt, J. R.; Fraser-Reid, B. O. *J. Am. Chem. Soc.* **1994**, *116*, 8334. (d) Fraser-Reid, B.; Wu, Z.; Udodong, U. E.; Ottosson, H. *J. Org. Chem.* **1990**, *55*, 6068. (e) Mootoo, D. R.; Date, V.; Fraser-Reid, B. O. *J. Am. Chem. Soc.* **1988**, *110*, 2662. (f) Mootoo, D. R.; Konradsson, P.; Fraser-Reid, B. O. *J. Am. Chem. Soc.* **1989**, *111*, 8540 and references therein.

(29) Gordon, D.; Danishefsky, S. J. *Carbohydrate Res.* **1990**, *206*, 361.

(30) (a) Reithal, Y. *J. Am. Chem. Soc.* **1952**, *74*, 4210. (b) Dasgupta, F., Anderson, L. *Carbohydr. Res.* **1994**, *264*, 155.

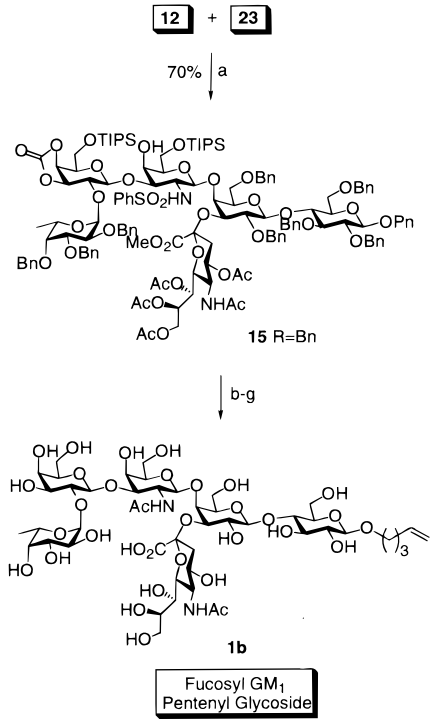
(31) Rodriguez, E. B.; Stick, R. V. *Aust. J. Chem.* **1990**, *43*, 665.

**Scheme 4**[a]




[a] Reagents: (a) $Ag_2CO_3$, cat. $I_2$, PnOH, $CH_2Cl_2$, 75%; (b) NaOMe, MeOH; (c) acetone, cat. PPTS, 44% two steps; (d) BnBr, NaH, DMF; 84%; (e) 80% AcOH: $H_2O$, 90%; (f) **10**, TMSOTf, EtCN, molecular sieves, −40 °C, 77%.

**Scheme 5**[a]




[a] Reagents: (a) MeOTf, $CH_2Cl_2$:$Et_2O$, 0 °C, 70%; (b) TBAF, AcOH, THF; (c) NaOMe, MeOH; (d) NaOH, THF; (e) Na/$NH_3$, THF −78 °C, then MeOH; (f) $Ac_2O$, pyridine, DMAP, $CH_2Cl_2$, 45% five steps, (g) steps c−d, 96%.

is well-justified even with this inconvenience. Perbenzylation of **20** give **21** followed by actetonide removal with aqueous acetic acid yielded the desired AB acceptor **22**. Sialylation using phosphite donor **10** (see Scheme 2) proceeded in comparable yield to give the trisaccharide acceptor, **23**.

In the event, coupling of donor **12** with a 2.0 molar excess of the acceptor **23** containing the pentenyl linker proceeded with MeOTf promotion (1.5 equiv × 2) in excellent (70%) yield (see Scheme 5). It will be recalled that the previous [3 + 3] coupling using the glycal acceptor **11** proceeded in low yield (23%, Scheme 3). Moreover, subsequent transformations were required

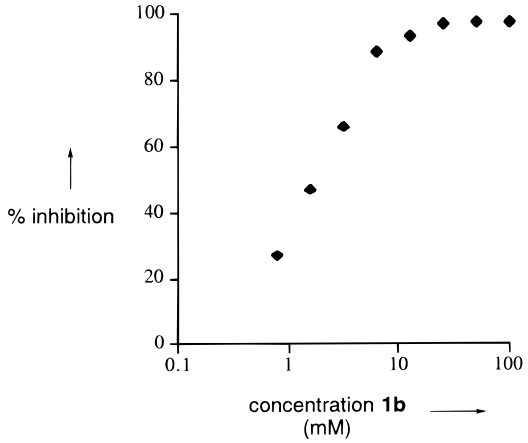
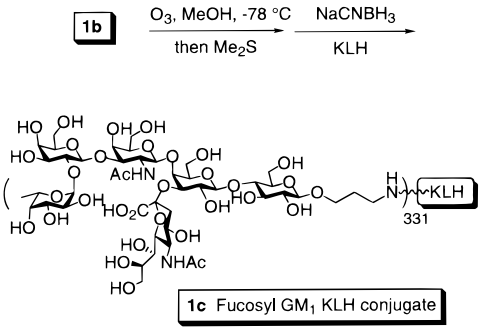



**Figure 4.** Inhibition of F12 antibody with synthetic 1b.

**Scheme 6**




to reach **15** (R = H). Formation of compound **15** (R = Bn) by the direct [3 + 3] coupling route presented in Scheme 5 represents a significant improvement in overall chemical yield and processing. Thus, these results demonstrate that in this particular merger, replacement of the glycal functionality by a more stable glycosidic protecting group does indeed improve the efficiency of the coupling step. Global deprotection under conditions identical to those in Scheme 3 yielded the characterized hexasaccharide, **1b**.

Since we were confident of the structure of our fully synthetic **1b**, we could use the construct assembled in the laboratory to verify the assignment of the carbohydrate sector of the natural tumor antigen. At the immune characterization level, synthetic **1b** was shown to bind to monoclonal antibody F12 in ELISA and immune thin-layer chromatography assays. Inhibition studies revealed that preincubation of F12 with **1b** completely inhibits reactivity of natural fucosyl $GM_1$, **1a**, with the antibody (Figure 4).[34] Clearly the synthetic fucosyl $GM_1$ pentenyl glycoside provides the antigenic epitope with which F12 reacts on SCLC cells.

Attention was then directed to the final of our outlined goals. We subjected synthetic **1b** to conjugation to carrier protein KLH, as shown in Scheme 6. The protocol[35] started with ozonolysis, thereby producing the uncharacterized aldehyde derivative. This step was followed by coupling to KLH by using reductive amination under the agency of sodium cyanoborohydride. Presumably coupling of the carbohydrate had occurred with the ϵ-amino group of lysine residues in the KLH. Hydrolytic carbohydrate analysis revealed approximately 331 carbohydrate residues per molecule of KLH.[36] Mouse immunization studies with this fully synthetic vaccine, **1c**, will be reported separately.

(32) Catelani, G.; Colonna, F.; Marra, A. *Carbohydr. Res.* **1988**, *182*, 297.

(33) See Supporting Information for details.

(34) Inhibition studies were performed with increasing concentrations of **1b** and were analyzed by ELISA.

(35) (a) Bernstein, M. A.; Hall, L. D. *Carbohydr. Res.* **1980**, *78*, C1. (b) Lemieux, R. U. *Chem. Soc. Rev.* **1978**, *7*, 423.

## Conclusions

The goals of chemical synthesis, bioconjugation, and immunocharacterization of the SCLC-specific fucosyl $GM_1$ have been realized. The approach to this hexasaccharide relies on a highly convergent [3 + 3] coupling reaction between the known donor **12** and acceptor **23** which carries with it a pentenyl linker for bioconjugation. The use of a pentenyl glycoside-protecting group at the reducing end of **1** offers clear advantages. We observed an increase in chemical yield in the crucial coupling using acceptor **23**, with the more stable glycosidic linkage, as compared to trisaccharide glycal **11**. The NPG modification allowed for a rather more efficient synthesis of potential conjugation precursors. Happily, it was demonstrated that the pentenyl linker serves as well as the allyl linker for conjugation purposes.[37] We are actively pursuing the efficacy of pentenyl glycosides in an analogous synthesis of the MBr1 antigen Globo-H. Reports on this work will be reported in due course.[38]

We have conducted the studies reported herein with the expectation that patients immunized in an adjuvant setting with fully synthetic vaccine conjugate **1c** might produce antibodies reactive with SCLC cells and that the production of such antibodies might mitigate against tumor spread which might have with it a more favorable prognosis. The preclinical results toward this goal are underway and will be disclosed when they become available.

## Experimental Section[39]

**Trisaccharide 23.** The phosphite donor **10** (1.0 g, 1.35 mmmol) and lactosyl acceptor **22** (2.5 g, 2.90 mmol) were combined, azeotroped with anhydrous benzene, and placed under high vacuum for 2 h. The mixture was dissolved in anhydrous $CH_3CH_2CN$ (distilled from $CaH_2$), freshly activated 4 Å molecular sieves were added, and the reaction was cooled to −40 °C. A portion of TMSOTf (0.1 equiv, 27 $\mu$L) was added, and the reaction was allowed to stir at −40 °C overnight. The reaction was then warmed to −30 °C, and another 0.1 equiv of TMSOTf was added. Upon stirring for an additional 2 h at −30 °C, the reaction was quenched by the addition of solid $NaHCO_3$ and was filtered through a plug of Celite with the aid of EtOAc. The organic layer was washed with saturated $NaHCO_3$ (2 × 400 mL) and dried over $MgSO_4$. Evaporation of the organic layer gave a cloudy oil which was subjected to flash column chromatography using careful gradient elution in order to recover acceptor and product trisaccharide (20% EtOAc/hexanes → 75% EtOAc/hexanes). The product (1.35 g, 75%) was obtained as a white foam, and 0.95 g of starting acceptor was recovered: $[\alpha]^{22}_D$ 2.38° (*c* 1.30, $CHCl_3$); IR (film $CHCl_3$) 3106, 2866, 1744, 1689, 1368, 1222, 1055 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 7.40−7.17 (m, 25H), 5.79 (m, 1H), 5.38 (m, 1H), 5.27 (dd, 1H, $J$ = 8.0, 2.0 Hz), 5.08 (d, 1H, $J$ = 10.0 Hz), 4.95 (m, 3H), 4.86 (d, 1H, $J$ = 10.9 Hz), 4.75 (d, 1H, $J$ = 5.7 Hz), 4.72 (d, 1H, $J$ = 10.8 Hz), 4.68 (d, 1H, $J$ = 11.0 Hz), 4.56 (d, 1H, $J$ = 11.9 Hz), 4.54 (d, 1H, $J$ = 7.6 Hz), 4.44 (d, 1H, $J$ = 12.2 Hz), 4.39 (m, 1H), 4.32−4.25 (m, 3H), 4.06−3.88 (m, 6H), 3.79 (m, 2H), 3.72 (s, 3H), 3.65 (m, 3H), 3.54−3.44 (m, 5H), 3.35 (m, 2H), 2.66 (d, OH, 1H, $J$ = 3.3 Hz), 2.47 (dd, 1H, $J$ = 13.0, 4.7 Hz), 2.12 (m, 2H), 2.06 (s, 3H), 2.02 (m, 1H), 1.98 (s, 3H), 1.95 (s, 3H),1.85 (s, 3H), 1.83 (s, 3H), 1.71 (s, 3H) ; $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 170.77, 170.53, 170.23, 169.92, 169.87, 168.32, 139.09, 138.90, 138.61, 138.45, 138.34, 138.05, 128.27, 128.21, 137.99, 127.51, 127.42, 127.11, 114.81, 103.48, 102.29, 98.32, 82.90, 81.80, 78.37, 76.50, 76.30, 75.31, 75.01, 74.89, 74.82, 73.23, 72.97, 72.66, 72.37, 69.16, 69.03, 68.69, 68.43, 68.36, 67.81, 67.08, 62.21, 52.99, 49.17, 36.41, 30.17, 28.89, 23.11, 21.08, 20.77, 60.67, 60.47; HRMS (FAB) calcd for $C_{72}H_{87}NO_{23}Na$ (M + $Na^+$) 1356.5566, found 1356.5557.

**Hexasaccharide 15 (R = Bn).** The thioethyl donor **12** (311 mg, 0.243 mmol) and acceptor **23** (627 mg, 0.487 mmol) were combined, azeotroped with anhydrous benzene (5 × 5 mL), and placed under high vacuum for 5 h. The mixture was then dissolved in 1.6 mL of $CH_2Cl_2$ and 3.2 mL of $Et_2O$ (0.05 M total), treated with freshly prepared 4 Å molecular sieves, and cooled to 0 °C. Methyl triflate (1.5 equiv, 41 $\mu$L) was added in one portion, and the reaction stirred at 0 °C overnight. In the morning, another 20 $\mu$L of MeOTf was added, and the reaction was allowed to stir for an additional 2 h at 5 °C. The reaction was quenched by the addition of solid $NaHCO_3$, filtered through Celite with EtOAc, concentrated, and purified by flash column chromatography (gradient elution 25% EtOAc/hexanes → 50% → 75% EtOAc/hexanes) to give 437 mg (70%) of hexasaccharide as a white foam and 300 mg of recovered trisaccharide acceptor: $[\alpha]^{22}_D$ −29.4° (*c* 3.25, $CHCl_3$); IR (film $CHCl_3$) 3285, 3028, 2940, 2865, 1794, 1749, 1690, 1220, 1090 $cm^{-1}$; $^1H$ NMR ($CDCl_3$, 400 MHz) $\delta$ 7.74 (d, 2H, $J$ = 7.5 Hz), 7.34−7.08 (m, 43H), 5.75 (m, 1H), 5.52 (d, 1H, $J$ = 4.7 Hz), 5.29 (app s, 1H), 5.23 (dd, 1H, $J$ = 9.5, 1.4 Hz), 5.15 (m, 1H), 5.02 (d, 1H, $J$ = 9.8 Hz)4.97−4.87 (m, 5H), 4.84 (d, 1H, $J$ = 10.9 Hz), 4.81−4.70 (m, 5H), 4.63 (d, 1H, $J$ = 11.6 Hz), 4.57 (m, 3H), 4.44 (d, 1H, $J$ = 7.2 Hz), 4.40 (d, 1H, $J$ = 12.2 Hz), 4.30 (d, 1H, $J$ = 7.8 Hz), 4.10 (m, 2H), 3.98−3.81 (m, 12H), 3.82 (s, 3H), 3.78−3.68 (m, 7H), 3.64−3.45 (m, 8H), 3.27 (m, 3H), 3.17 (dd, 1H), 2.80 (d, OH, 1H, $J$ = 2.1 Hz), 2.19 (dd, 1H, $J$ = 13.0, 4.5 Hz), 2.10 (m, 3H), 2.01 (s, 3H), 1.92 (s, 3H), 1.88 (s, 3H), 1.82 (s, 3H), 1.81 (s, 3H), 1.68 (m, 2H), 1.08 (d, 3H, $J$ = 5.4 Hz), 1.00−0.92 (m, 42H); $^{13}C$ NMR ($CDCl_3$, 100 MHz) $\delta$ 170.61, 170.34, 170.26, 169.66, 167.78, 155.48, 138.95, 138.65, 138.63, 138.56, 138.42, 138.38, 138.27, 138.05, 132.17, 129.02, 128.59, 128.46, 128.18, 128.05, 127.91, 127.63, 127.51, 127.24, 127.09, 114.80, 103.42, 102.76, 102.45, 100.16, 99.58, 98.76, 82.87, 81.53, 79.06, 77.32, 77.24, 77.16, 75.12, 75.07, 74.95, 74.80, 73.92, 73.27, 73.04, 72.93, 72.19, 69.23, 69.14, 69.09, 67.89, 67.53, 61.76, 61.58, 61.12, 56.39, 53.60, 49.19, 35.36, 30.17, 28.89, 23.13, 20.97, 20.75, 20.62, 20.53, 17.85, 17.53, 17.33, 16.72, 11.80, 11.74; HRMS (FAB) calcd for $C_{136}H_{178}N_2O_{39}SSi_2$ (M + $Na^+$) 2574.1163, found 2574.1130.

**Compound 1b.** To a solution of the hexasaccharide (130 mg, 0.0509 mmol) in THF (2.0 mL) was added glacial AcOH (10.0 equiv, 29 $\mu$L) and TBAF (1.0 M THF, 10.0 equiv, 0.509 mL). The reaction stirred at room temperature overnight and was poured into ice water and extracted with EtOAc (3 × 50 mL). The organic extracts were washed with saturated $NaHCO_3$ (50 mL) and brine (50 mL), dried over $MgSO_4$, and concentrated to an oil which was purified through a short plug of silica gel with EtOAc. The resulting triol was dissolved in anhydrous MeOH (2.5 mL), and sodium methoxide was added (0.250 mL of a 25% solution in MeOH). The reaction stirred at room temperature for 18 h, and then 0.5 mL of THF and 0.5 mL of $H_2O$ were added. Stirring at room temperature for an additional 24 h was followed by neutralization with Dowex-$H^+$, filtration with MeOH washings, and concentration. The crude material was allowed to dry under high vacuum for 1 day. To the resulting white solid was added THF (0.5 mL) and condensed liquid $NH_3$ (∼10 mL) at −78 °C. Sodium (∼50 mg) was added and the resulting blue solution stirred at −78 °C for 1.5 h. The reaction was quenched with anhydrous MeOH (∼5 mL), brought to room temperature, and concentrated with a stream of dry $N_2$ to a volume of ∼2 mL. The reaction was neutralized with Dowex-$H^+$, filtered with MeOH washings, and concentrated to a white solid. The white solid was dissolved in 1.0 mL of pyridine and 1.0 mL of $CH_2Cl_2$ and cooled to 0 °C. A crystal of DMAP was added followed by addition of acetic anhydride (1.0 mL). The ice bath was removed, and the reaction stirred at room temperature overnight. Concentration followed by purification by flash column chromatography (gradient elution 75% EtOAc/hexanes → 100% EtOAc → 5% MeOH/EtOAc) gave 44 mg (46%) of **16** as a white solid: $^1H$ NMR (MeOH, 400 MHz) $\delta$ 8.02 (d, 1H, $J$ = 9.9 Hz), 7.87 (d, 1H, $J$ = 9.2 Hz), 5.76 (m, 1H), 5.49 (m, 1H), 5.39 (d, 1H, $J$ = 2.9 Hz), 5.34−5.31 (m, 2H), 5.22 (d, 1H, $J$ = 3.4 Hz), 5.19 (d, 1H, $J$ = 4.1 Hz), 5.17 (d, 1H, $J$ = 3.5 Hz), 5.12−5.05 (m, 3H), 4.97 (dd,

(36) For a typical procedure, see: (a) Lloyd, K. O.; Savage, A. *Glycoconjugate J.* **1991**, *8*, 439. (b) Hardy, M. R.; Townsend, R. R. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 3289.

(37) The yields of carbohydrate conjugates are similar for both the allyl linker and the pentenyl linker. The incorporation of ∼300 carbohydrates per molecule of carrier protein is what was expected based on results using the allyl glycoside of Globo-H.

(38) Allen, J. R.; Allen, J. G.; Zhang, X.-F.; Williams, L. J.; Zatorski, A.; Danishefsky, S. J. *Eur. J. Chem.*, in press.

(39) Please see attached Supporting Information for general experimental procedures.

1H, $J = 16.8$, 1.7 Hz), 4.91 (dd, 1H, $J = 10.0$, 1.7 Hz), 4.81−4.75 (m, 3H), 4.65−4.60 (m, 2H), 4.52 (d, 1H, $J = 7.9$ Hz), 4.48−4.44 (m, 2H), 4.37 (dd, 1H, $J = 10.0$, 2.5 Hz), 4.28 (dd, 1H, $J = 12.5$, 2.4 Hz), 4.22−4.18 (m, 2H), 4.14−3.99 (m, 9H), 3.96−3.92 (m, 2H), 3.89 (d, 1H, $J = 2.9$ Hz), 3.88−3.77 (m, 4H), 3.72−3.62 (m, 3H), 3.51−3.45 (m, 1H), 2.74 (dd, 1H, $J = 11.3$, 4.5 Hz), 2.19 (s, 3H), 2.13 (s, 3H), 2.11 (s, 3H), 2.10 (s, 3H), 2.09 (s, 3H), 2.08 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 2.00 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 0.91 (s, 3H), 0.180 (s, 3H), 1.61 (m, 2H), 1.14 (d, 3H, $J = 6.4$ Hz), 3 protons buried beneath acetates (2 Pn, 1 C3ax); $^{13}$C NMR (MeOH, 100 MHz) $\delta$ 174.64, 173.64, 172.98, 172.89, 172.63, 172.56, 172.48, 172.44, 172.34, 172.27, 172.04, 171.99, 171.76, 171.73, 171.62, 171.35, 171.25, 139.23, 115.47, 104.62, 103.26, 101.86, 101.63, 100.78, 97.31, 78.22, 76.53, 75.08, 74.69, 74.29, 73.91, 73.53, 72.94, 72.71, 72.56, 72.16, 72.06, 71.89, 71.74, 70.19, 69.87, 69.33, 69.11, 68.92, 65.96, 65.65, 63.68, 63.52, 62.69, 54.01, 53.09, 50.60, 40.19, 31.09, 29.96, 24.17, 24.06, 22.73, 21.76, 21.59, 21.46, 21.20, 21.06, 20.89, 20.75, 20.63, 20.55, 16.52.

The peracetate (40 mg) was dissolved in anhydrous MeOH (2.0 mL), and 150 $\mu$L of sodium methoxide was added (25% solution in MeOH). The reaction stirred at room temperature for 18 h, and then 0.5 mL of THF and 0.5 mL of $H_2O$ were added. The reaction stirred for another 24 h at room temperature. Neutralization with Dowex-$H^+$ (pH ~6−7) was followed by filtration with MeOH washings, concentration, and purification using P-2 Gel ($H_2O$ elutent) to yield 24 mg (96%) of a white solid: IR 3346, 2940, 2882, 1657, 1620, 1376, 1069 cm$^{-1}$; $^1$H NMR ($D_2O$, 400 MHz) $\delta$ 5.86 (m, 1H), 5.18 (d, 1H, $J = 4.0$ Hz), 5.04 (dd, 1H, $J = 17.22$, 1.7 Hz), 4.97 (dd, 1H, $J = 10.6$ Hz), 4.63 (d, 1H, $J = 7.6$ Hz), 4.57 (d, 1H, $J = 7.7$), 4.46 (d, 1H, $J = 7.9$ Hz), 4.43 (d, 1H, $J = 8.1$ Hz), 4.15 (m, 1H), 4.09−4.02 (m, 3H), 3.94−3.84 (m, 5H), 3.80−3.63 (m, 18H), 3.60−3.53 (m, 6H), 3.47 (dd, 1H, $J = 10.3$, 1.8), 3.32 (t, 1H), 3.26 (t, 2H), 2.62 (dd, 1H, $J = 13.4$, 4.3 Hz), 2.09 (m, 2H), 1.98 (s, 6H), 1.86 (m, 1H), 1.67 (m, 2H), 1.15 (d, 3H, $J = 6.5$ Hz); $^{13}$C NMR ($D_2O$, 100 MHz) $\delta$ 176.29, 175.43, 175.16, 139.97, 115.99, 104.38, 103.77, 103.30, 103.22, 102.25, 100.35, 79.67, 78.12, 77.65, 77.03, 76.06, 75.94, 75.62, 75.44, 75.24, 74.85, 74.19, 74.01, 73.45, 73.01, 71.15, 70.72, 70.32, 69.87, 69.64, 69.25, 67.93, 64.01, 62.29, 62.07, 61.63, 61.29, 52.79, 52.70, 50.04, 38.45, 30.53, 29.17, 23.89, 23.23, 16.53; HRMS (FAB) calcd for $C_{48}H_{79}N_2O_{33}Na_2$ [M − H + 2Na]$^+$ 1257.4360, found 1257.4337.

**Glycal Hexasaccharide 13.** The thioethyl donor **12** (120 mg, 0.0938 mmol) and acceptor **11** (122 mg, 0.108 mmol) were combined, azeotroped with anhydrous benzene (5 × 5 mL), and placed under high vacuum overnight. The mixture was dissolved in a 2:1 mixture of $Et_2O$:$CH_2Cl_2$ (2.7 mL total), 4 Å molecular sieves were added and the mixture stirred at room temperature for 1 h. The reaction was cooled to 0 °C, and 1.0 equiv of MeOTf (0.020 mL) was added. After 4 h at 0 °C another equivalent of MeOTf was added (0.020 mL), and the reaction continued to stir for another 4 h at 10 °C. The reaction was quenched with solid $NaHCO_3$, filtered through Celite with additional EtOAc (100 mL), and concentrated. The resulting mixture was purified by flash column chromatography to give 50 mg (23%) of the hexasaccharide glycal **13** and 85 mg of starting acceptor, **11**: $R_f$ 0.35 (66% EtOAc/hexanes); $^1$H NMR (500 MHz, $C_6D_6$) $\delta$ 8.31 (d, 2H), 7.62 (d, 2H), 7.52 (m, 4H), 7.45 (d, 2H), 7.40−7.15 (m, 31H), 6.47 (d, 1H, $J = 6.3$ Hz), 6.28 (apparent s, 1H), 6.09 (d, 1H, $J = 3.8$ Hz), 5.72 (m, 1H), 5.55 (dd, 1H, $J = 9.3$, 1.2 Hz), 5.51 (d, 1H, $J = 3.5$ Hz), 5.22 (d, 1H, $J = 10.8$ Hz), 5.15 (s, 1H), 5.13−5.06 (m, 3H), 5.05 (d, 1H, $J = 8.1$ Hz), 5.02 (m, 1H), 4.98 (d, 1H, $J = 10.8$ Hz), 4.85 (d, 1H, $J = 10.6$ Hz), 4.82 (d, 1H, $J = 9.4$ Hz), 4.73−4.66 (m, 8H), 4.55−4.34 (m, 10H), 4.38−4.32 (m, 5H), 4.30 (d, 1H), 4.18 (s, 3H), 4.21−4.12 (m, 6H), 4.06 (m, 2H), 3.99 (m, 4H), 3.85 (d, 1H), 3.74 (dd, 1H), 3.61 (m, 2H), 3.52 (t, 1H), 2.63 (dd, 1H, $J = 13.9$, 5.0), 2.48 (dd, 1H, $J = 13.4$ Hz), 2.35 (s, 3H), 2.01 (s, 3H), 1.98 (s, 3H), 1.72 (s, 3H), 1.64 (s, 3H), 1.57 (d, 3H, $J = 6.3$), 1.31−1.20 (m, 42H); $^{13}$C NMR (100 MHz, $CDCl_3$) $\delta$ 169.71, 169.39, 169.18, 168.70, 168.12, 166.99, 154.75, 143.47, 137.81, 137.71, 137.51, 137.42, 137.07, 131.65, 128.25, 127.52, 128.32, 127.26, 127.23, 127.19, 127.10, 126.98, 126.91, 126.83, 126.73, 126.62, 126.53, 126.36, 126.29, 101.67, 101.35, 98.69, 98.32, 98.26, 97.33, 80.48, 78.05, 77.06, 76.20, 75.50, 74.64, 74.22, 73.87, 73.49, 72.90, 72.38, 72.26, 71.93, 71.47, 71.20, 70.34, 70.17, 69.99, 69.13, 68.62, 68.10, 67.92, 67.01, 66.88, 66.68, 65.52, 60.92, 60.61, 55.51, 52.59, 48.31, 34.87, 28.68, 22.19, 19.95, 19.77, 19.68, 19.59, 16.93, 16.88, 15.79, 10.86, 10.78; HRMS (FAB) calcd for $C_{124}H_{162}N_2O_{37}Si_2$-SNa [M + Na]$^+$ 2382.0013, found 2382.0001.

**Imido-hexasaccharide 13i.** Performing the above reaction with 10 equiv of MeOTf added in one portion, under otherwise identical conditions, yields 28% of the following compound, which is much less polar than the parent N-acetylated hexasaccharide **13**. $R_f$ 0.35 (25% EtOAc/hexanes); $^1$H NMR (500 MHz, $C_6D_6$) $\delta$ 8.31 (d, 2H), 7.66 (d, 2H), 7.53 (t, 4H), 7.48 (d, 2H), 7.42−7.16 (m, 31H), 6.46 (d, 1H), 6.21 (app s, 1H), 6.15 (d, 1H, $J = 4.3$ Hz), 5.81 (d, 1H, $J = 9.2$ Hz), 5.72 (dt, 1H, $J = 12.8$, 2.4 Hz), 5.40 (m, 1H), 5.38 (d, 1H, $J = 3.5$ Hz), 5.20 (d, 1H, $J = 10.2$ Hz), 5.12 (t, 2H), 5.00 (m, 3H), 4.84 (d, 1H, J-6.2 Hz), 4.81 (d, 1H, $J = 4.5$ Hz), 4.73 (m, 2H), 4.70 (m, 2H), 4.67 (d, 1H, $J = 2.6$ Hz), 4.65 (m, 1H), 4.59 (m, 3H), 4.53−4.46 (m, 6H), 4.40 (m, 5H), 4.36 (d, 1H, $J = 3.1$ Hz), 4.30 (d, 1H, $J = 3.4$ Hz), 4.26 (m, 3H), 4.23 (app s, 1H), 4.20 (m, 3H), 4.11 (m, 2H), 4.04 (d, 1H, $J = 5.9$ Hz), 3.99 (s, 3H), 3.92 (d, 1H, $J = 3.2$ Hz), 3.87 (d, 1H, $J = 2.9$ Hz), 3.82 (d, 1H, $J = 6.5$ Hz), 3.70 (m, 1H), 3.64 (s, 3H), 3.60 (d, 1H), 3.28 (t, 1H), 2.94 (dd, 1H, $J = 13.7$, 4.5 Hz), 2.36 (t, 1H, $J = 13.3$ Hz), 2.14 (s, 3H), 1.91 (s, 3H), 1.83 (s, 3H), 1.81 (s, 3H), 1.60 (s, 3H), 1.53 (d, 3H, $J = 6.5$ Hz), 1.32−1.23 (m, 42H); $^{13}$C NMR (100 MHz, $CHCl_3$) $\delta$ 170.43, 169.30, 169.20, 168.98, 168.03, 164.74, 155.82, 144.74, 139.09, 138.75, 138.52, 138.48, 138.40, 138.39, 138.25, 138.17, 132.56, 129.22, 128.85, 128.39, 128.35, 128.30, 128.25, 128.01, 127.79, 127.71, 127.60, 127.55, 127.50, 127.48, 127.34, 102.57, 102.24, 99.69, 99.11, 98.25, 81.35, 79.09, 87.22, 75.64, 75.40, 74.90, 74.60, 74.15, 73.95, 73.50, 73.33, 72.94, 72.84, 72.52, 71.37, 71.17, 70.47, 70.17, 69.66, 69.05, 68.47, 68.11, 67.96, 67.71, 67.55, 61.91, 61.54, 61.05, 57.70, 56.50, 53.65, 52.75, 31.94, 29.71, 21.70, 20.97, 20.89, 20.64, 20.46, 20.44, 17.57, 16.81, 15.38, 14.13, 11.89, 11.80; LRMS (FAB) $C_{125}H_{164}N_2O_{37}SSi_2Na$ 2373 [M + Na]$^+$.

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**Supporting Information Available:** Experimental procedures for compounds **18**, **20**, **21**, and **22**, $^1$H and $^{13}$C spectra for compounds **13**, **15**, **16**, **18**, **20**−**23**, **1b** and $^1$H spectra for **13i** and **19** are available (PDF). This information is available free of charge via the Internet at http://pubs.acs.org.

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