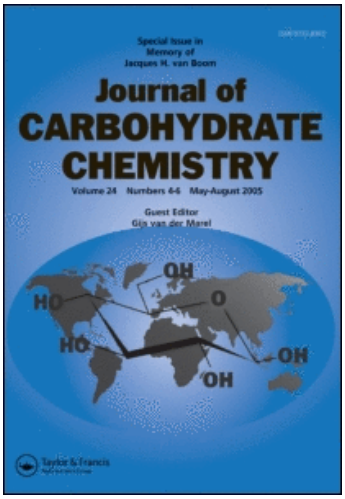

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Olefin Cross-Metathesis: A Powerful Tool for Constructing Vaccines Composed of Multimeric Antigens

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The preparation of biologically pertinent glycosylamino acids from *O*-pentenyl glycosides is described. The procedure involves sequential cross-metathesis reactions followed by hydrogenation. The generality and value of this procedure have been demonstrated by the preparation of peracetylated Gb3, GM2, and fucosyl GM1 glycosylamino acids, which are of potentially large value in the preparation of future anticancer vaccines.

Keywords Cross-metathesis, Glycosylamino acids, *O*-pentenyl glycosides, Grubbs catalyst, Isomerization

INTRODUCTION

The discovery of specific carbohydrate epitopes associated with transformed cells[1] has raised the possibility of developing active immunotherapeutic agents to fight human cancers, and much work has been done toward the realization of this goal. Carbohydrate antigens, usually attached through a linker domain to an immunogenic carrier protein, have been introduced into a host immune system and have been investigated for their ability to generate an immune response to circulating tumor cells and micrometastases.[2,3]

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Traditionally, one of the main drawbacks to this cell-free glycoconjugate vaccine approach is the limited availability of purified tumor-associated carbohydrate antigens to build the construct. Fortunately, the confluence of recent advances in synthetic methodology have allowed for the preparation of complex carbohydrate-based tumor antigens and for further evaluation of such fully synthetic antigens at the clinical level.

The design of fully synthetic, carbohydrate-based anticancer vaccines has been an area of ongoing and urgent interest in our laboratory. Our first vaccines to be evaluated in the clinic were monomeric. Thus, each vaccine construct was composed of a single carbohydrate antigen conjugated to a carrier protein.[4] During the course of our research program, we came to consider the possibility that multiple repeats, or clusters, of the carbohydrate on a peptide backbone might elicit a more robust immune response. Second-generation vaccines were designed to take advantage of the molecular architecture of mucins, which feature clusters of tumor-associated carbohydrate antigens, and several such clustered vaccines were synthesized. Among those prepared and evaluated in our laboratory were the synthetic trimeric antigen clusters of Tn, TF, and STn, which each contained the native mucin glycopeptide architecture (Fig. 1). These constructs were assembled according to our "cassette" approach and were demonstrated to be immunogenic.[5]

A series of phase I trials,[4] which established the immunogenicity and safety of these vaccines, did not take into consideration the degree of heterogeneity of carbohydrates expressed on transformed cell surfaces, even within a particular cancer type. This heterogeneity of the type and distribution of carbohydrate expression is dependent on the stage of cellular development.[6,7] In contemplating the design of highly potent, broadly effective vaccines in which several different antigens associated with a specific cancer type would be displayed on a single peptide backbone, we realized we would need to couple individual building blocks to create a molecular level analog of a mucin populated cell surface.

In designing this unimolecular multivalent vaccine, we decided to utilize non-natural amino acids as components of new peptide-linked vaccines.[8] We surmised that such unnatural linkages might also result in an enhanced immune response. Furthermore, we reasoned that the use of amino acids containing long, aliphatic side chains might serve to distance the glycosides from the peptide backbone, thus facilitating glycopeptide synthesis. With

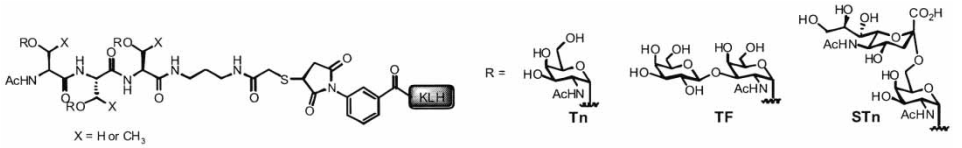


Figure 1: Structures of clustered vaccines.

these considerations in mind, we sought to prepare a unimolecular, trivalent vaccine in which non-natural amino acids were used as linkers to the three different carbohydrate domains (Tn, Lewis^y, and Globo-H). Preclinical evaluation of this construct has revealed promising levels of immunogenic response to each individual carbohydrate antigen.[9] Encouraged by these results, we sought to design and synthesize a multivalent construct that would target a particular cancer type, namely prostate cancer. Toward this goal, we successfully completed the total synthesis of a pentameric vaccine containing five known prostate tumor-associated antigens: Tn, TF, STn, Lewis^y, and Globo-H (Fig. 2). Early preclinical studies are currently underway with this construct, and preliminary results are encouraging.[10]

Concurrently, we hoped to revisit the concept employed in the design of the second-generation vaccines: that of "clustering," or installing multiple repeats of the same antigen on a peptide backbone. In this vein, our ultimate target in cancer vaccine preparation is a "cluster of clusters," in which each relevant antigen is displayed in triplicate along the peptide backbone. Hopefully, a synergistic effect of antigen clustering and of incorporating different antigen types onto a unimolecular construct could be realized. In increasing the complexity level of our goals, we had to first verify the enhanced immunogenicity of these individual clusters that employ non-natural amino acids as linkers.

Accumulated experience in carbohydrate synthesis has allowed us to target more complex carbohydrate antigens than the antigens (Tn, TF, and STn) that

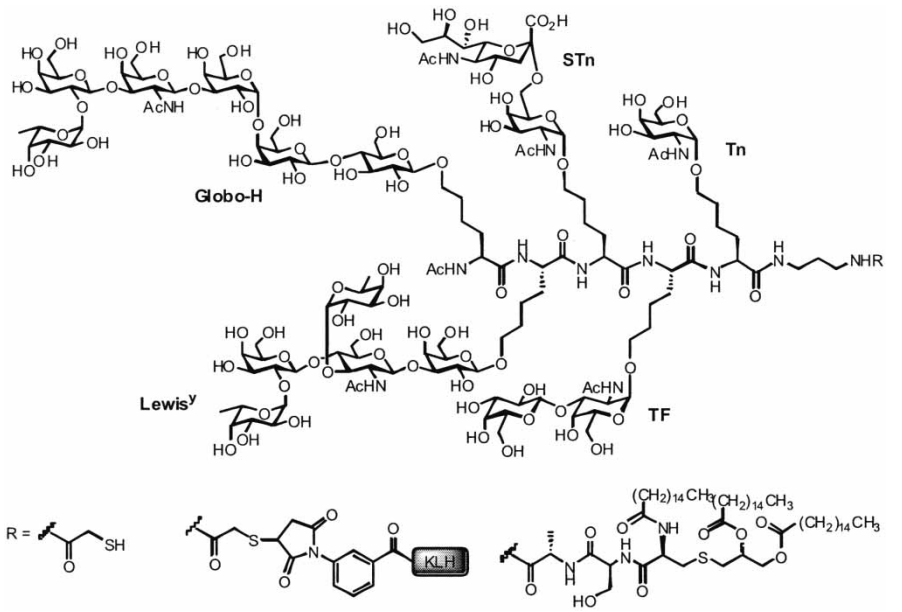


Figure 2: Multivalent vaccine designed for treatment of prostate cancer.

were studied earlier in the context of trimeric cancer vaccines. We were specifically interested in fucosyl GM1 ganglioside. In 1984, Nilsson and coworkers isolated the glycosphingolipid fucosyl GM1 and identified it as a specific marker associated with small cell lung cancer (SCLC) cells.[11,12] Immunohistochemistry studies suggested that, due to its highly restricted distribution in normal tissues, fucosyl GM1 could be an excellent target for immune attack against SCLC. Furthermore, at least for the moment, fucosyl GM1 has thus far not been found on any other human cancer cell lines.[5] Upon completion of the synthesis of fucosyl GM1,[13] preclinical studies demonstrated immunogenicity of our synthetic fucosyl GM1-KLH conjugate.[14] However, this antigen had not been tested in the triplicate settings.

In designing a putative fucosyl GM1 trimeric SCLC vaccine, we elected to extend the distance between the carbohydrate residues and the peptide backbone with a six-carbon linker because of the steric bulkiness of fucosyl GM1 (Fig. 3). We report herein the reevaluation of the cross-metathesis reaction for the preparation of fucosyl GM1 glycosylamino acid, a building block for the glycopeptide vaccine.

The broad utility of our modified procedure has been demonstrated through the preparation of the Gb3 and GM2 glycosylamino acids. Gb3 is a glycosphingolipid that has been shown to be overexpressed in Burkitt lymphoma cell lines, human ovarian cancer, human teratocarcinoma, human embryonal carcinoma, and other types of tumor cells.[15] Likewise, GM2 is expressed on the cell surface of a number of human cancers, including melanoma, sarcoma, and renal cancer.[16] Intriguingly, GM2-reactive antibodies are cytotoxic in vitro against $GM2^+$ human cancer cells.[17] Several clinical trials with GM2 derived from different sources are currently in progress, including large phase III trials in high-risk melanoma patients.

RESULTS AND DISCUSSION

Our strategy for the construction of the glycopeptide vaccines requires the fashioning of a pool of glycosylamino acids. Glycosylamino acids are important

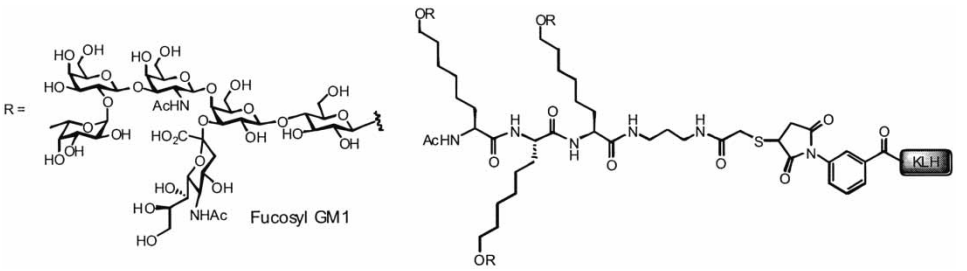


Figure 3: Design of fucosyl GM1 trimeric vaccine.

components of many biologically active compounds and have been prepared by several methods.[18] In our program, the amino acid functionality has been introduced through anomeric pentenyl groups via an ozonolysis-Horner-Emmons olefination-asymmetric hydrogenation protocol,[7] by direct glycosylation of carbohydrate donors with hydroxynorleucine in the presence of Lewis acid,[19,20] or by cross-metathesis reaction of *O*-allyl glycosides with Fmoc-L-allylglycine benzyl ester.[21] These methods ultimately deliver unnatural glycosylamino acids with a four-carbon linker between the anomeric oxygen and the α-carbon of the amino acid (Fig. 4).

Since we proposed to elongate the linker for the construction of trimeric fucosyl GM1 glycopeptide, we took advantage of the terminal olefin function of fucosyl GM1 pentenyl glycoside (**1**) as a convenient access point to couple this complex glycan to Fmoc-L-allylglycine benzyl ester (**2**) via cross-metathesis (Sch. 1). The powerful tool of cross-metathesis has been demonstrated through facile conversion of simple alkene precursors into functionalized olefins in numerous settings, including our own glycosylamino acid synthesis.[21] Furthermore, by utilizing this preformed, intact β-linkage we would obviate the difficulties surrounding the nonreliable stereochemistry of the glycosylation. Simultaneous reduction of the side-chain olefinic linkage and removal of the benzyl protecting group by catalytic hydrogenation would afford pure *N*-protected glycosylamino acid primed for incorporation into polypeptide vaccines.

The first experiment was carried out with a 10 fold excess of the amino acid (**2**) and 10 mol% Grubbs catalyst (**3**). Unlike earlier Grubbs catalysts, the more reactive catalyst (**3**) allowed for the rapid and complete consumption of **1**.

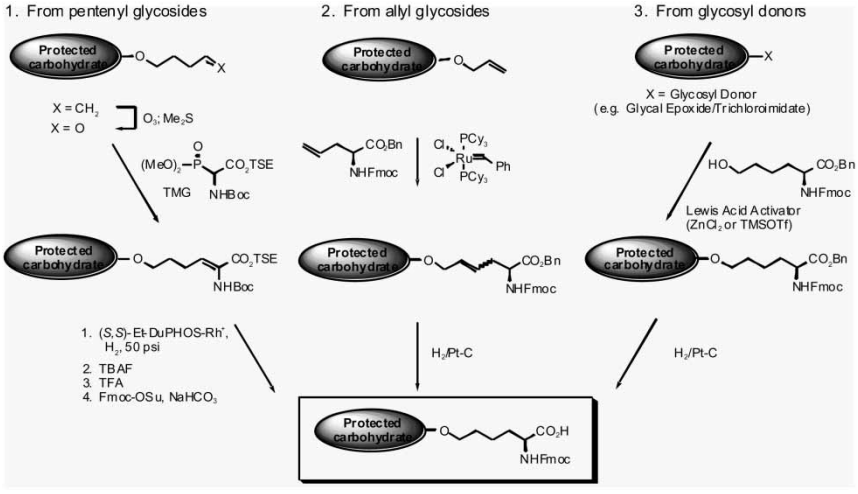
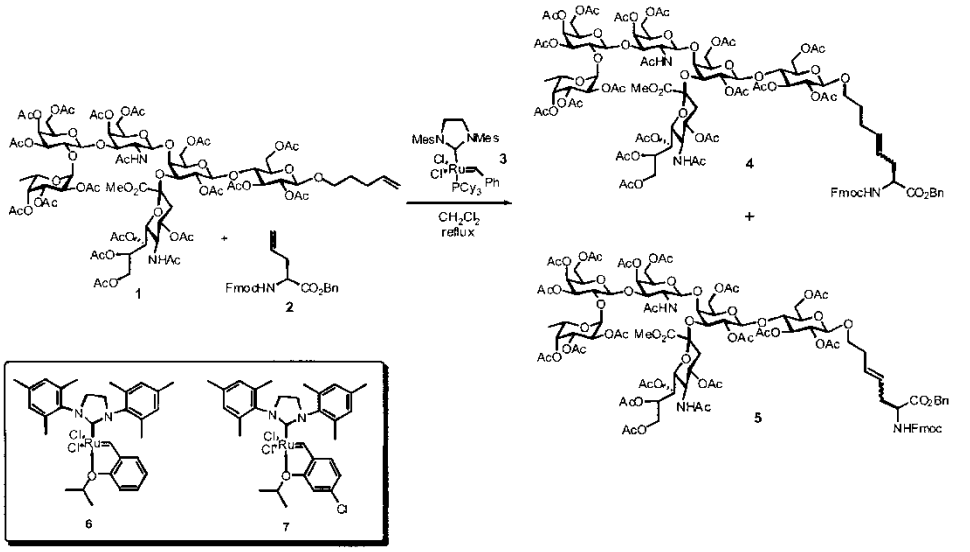


Figure 4: Methods used to install the amino acid functionality to carbohydrate domains.[22]



Scheme 1: Problematic cross-metathesis reaction conditions.

However, we observed isomerization of the terminal olefin of **1**, which resulted in an inseparable mixture of four products (**4** and **5** as mixtures of *E* and *Z* isomers).[23–25] The reaction progress was followed by low-resolution mass spectrometry, which clearly showed the presence of **5**, although not the relative amounts of **4** and **5** (Fig. 5). We also attempted the metathesis with Hoveyda-Grubbs II catalyst[26] (**6**) and Zhan catalyst[27] (**7**), anticipating short reaction time, excellent conversion, and no isomerization of the terminal olefin. However, in each case, we obtained the same mixture of products. Following this impasse, we studied our cross-metathesis reaction in greater detail.

Initial studies were carried out with peracetyl β-*O*-pentenyl-lactose (**8**). No isomerization of **8** occurred with the use of Grubbs catalyst (**9**) and only the desired coupling product **10** was observed (Sch. 2, Eq. (1). However, the low conversion of this reaction (33%) was problematic. To prevent double-bond migration, we installed an additional methyl group on the terminal olefin of **8** by cross-metathesis with *trans*-2-butene to provide hexenyl glycoside **11**. Surprisingly, we again obtained a mixture of **10** and **12** upon cross-metathesis of **11** and **2** in the presence of catalyst **3** (Sch. 2, Eq. (2). We postulated the presence of an undesired pathway, wherein ruthenium carbene is first generated from homodimerization of **2** to **13**. The ruthenium carbene then transforms **11** to **8**, which is susceptible to isomerization to **14**. Therefore, to minimize the homodimerization of **2**, we "capped" it with a methyl group to provide **15**. In the event, **15** and **11**, each containing a methyl cap,[28] successfully underwent cross-metathesis to generate the desired **10** in 86% yield as the exclusive

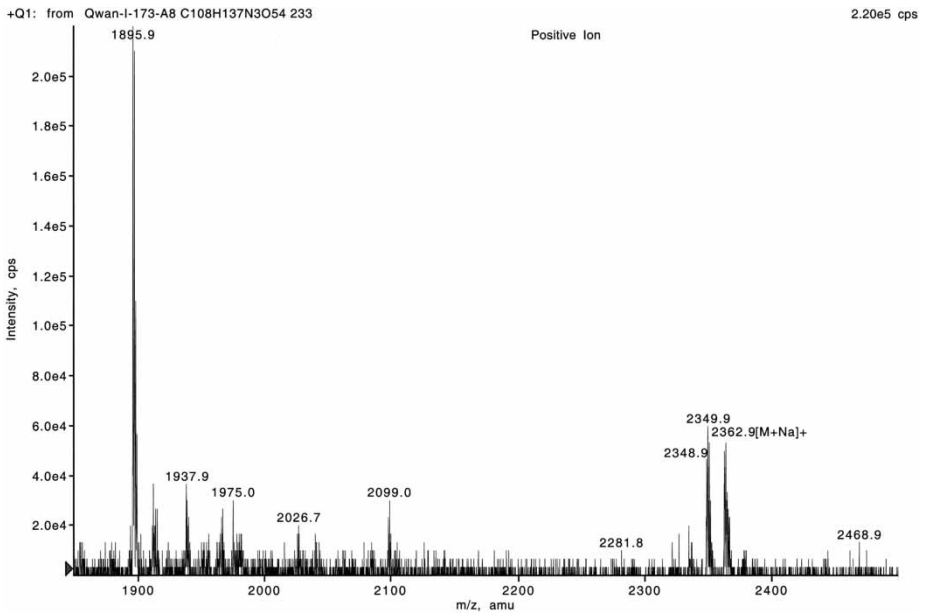


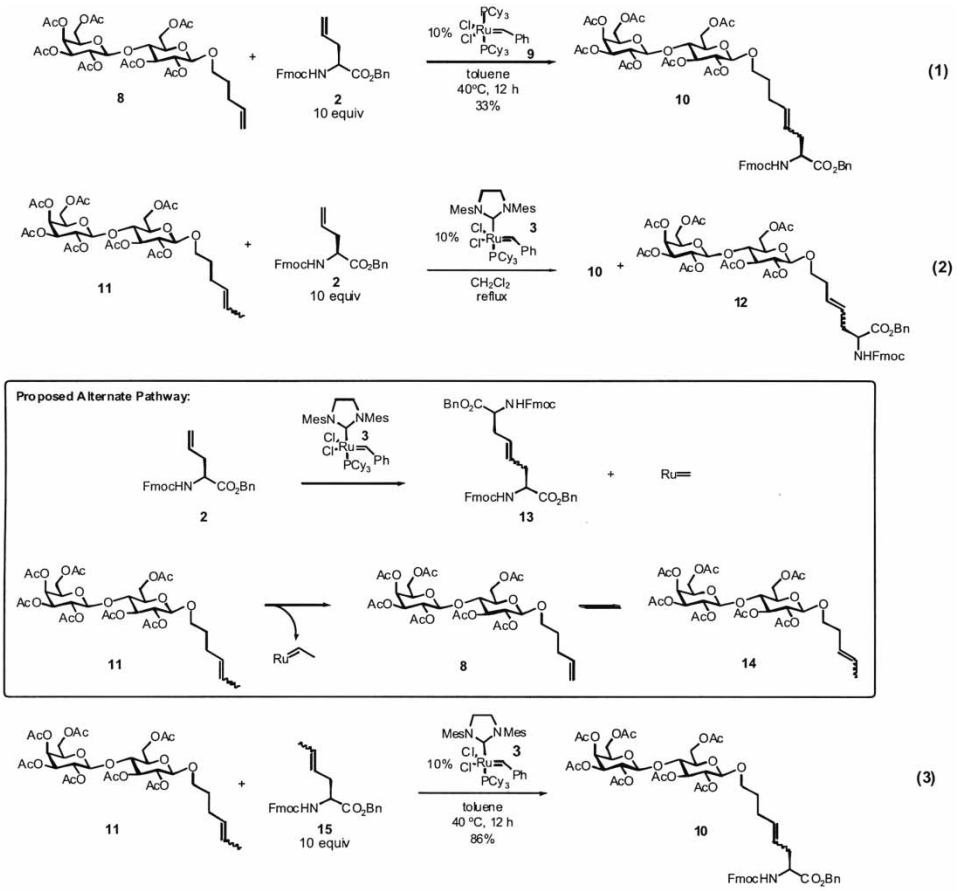
Figure 5: Low-resolution mass spectrum of cross-metathesis reaction crude products.

coupling product. None of the undesired product, **12**, was observed by low-resolution mass spectrometry.

Using this modified cross-metathesis reaction procedure, reactions were carried out with several *O*-pentenyl glycosides of differing levels of complexity. The product olefins, obtained as mixtures of *E* and *Z* isomers, were reduced by catalytic hydrogenation, with concomitant deblocking of the benzyl esters. The yields were determined for the three-step sequence starting from the *O*-pentenyl glycosides (Table 1). In addition to fucosyl GM1, we were able to gain synthetic access to the biologically interesting gangliosides, Gb3 and GM2, as peracetylated *O*-pentenyl glycosides.

As indicated from the results in Table 1, the three-step sequence conveniently allows for the preparation of glycosylamino acids in good overall yields. Although we have not established the nature of catalyst-derived ruthenium species responsible for the pentenyl isomerization, we have developed a simple preisomerization strategy (methyl cap) that effectively suppresses the undesired isomerization pathway while allowing progression of the metathesis pathway.

In conclusion, we have described a mild and efficient route to complex glycosylamino acids from pentenyl glycosides by a procedure of sequential cross-metathesis followed by hydrogenation. The resulting *N*-protected amino acids are equipped for incorporation into multivalent or clustered manifestations of carbohydrate-based cancer vaccines. Construction of the vaccines and the results of the immunologic investigations will be reported in due course.



Scheme 2: Optimization of cross-metathesis reaction substrates.

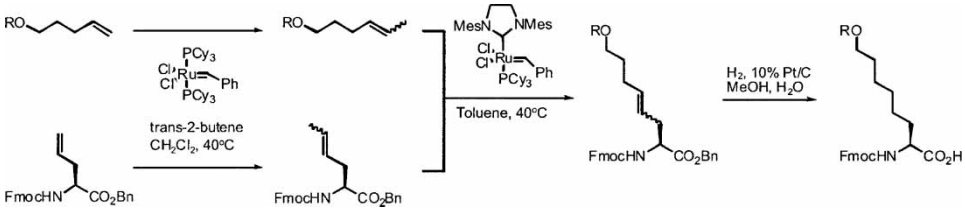
EXPERIMENTAL

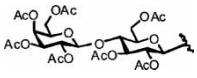
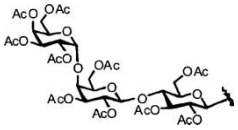
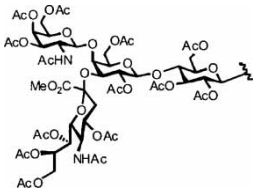
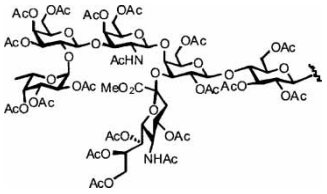
Materials and Methods

All reactions were carried out under argon with dry solvents, oven- or flame-dried glassware, and magnetic stirring. All solvents were reagent grade or HPLC grade. Reactions were monitored by thin layer chromatography (TLC) using 0.25-mm E. Merck precoated 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.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AMX-400 MHz or a Bruker Advance DRX-500 MHz spectrometer in $CDCl_3$ [referenced to

Table 1: Convenient three-step sequence for preparation of glycosylamino acids from pentenyl glycosides.



Glycosylamino Acid	R Glycoside	Yield (%) over Three Steps
16		74%
17 Gb3		57%
18 GM2		51%
19		53%

7.26 ppm (δ) for ^{1}H NMR and 77.0 ppm for ^{13}C NMR] and CD_3OD [referenced to 3.30 ppm (δ) for ^{1}H 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 an NaCl plate. Optical rotations were measured with a Perkin-Elmer 241 polarimeter in the solvent indicated.

Fmoc-L-But-2-enylglycine benzyl ester (15): (mixture of *E* and *Z:* 2.8/1): ^{1}H NMR (400 MHz, $CDCl_3$)^{TM}: 7.77 (d, 2H, $J = 7.5$ Hz), 7.62–7.50 (m, 2H),

7.53–7.30 (m, 9H), 5.68–5.62 (m, 0.2H), 5.54–5.45 (m, 0.8H), 5.38–5.13 (m, 3.9 H), 4.56–4.45 (m, 0.9H), 4.39 (d, 2H, $J = 7.2$ Hz), 4.25–4.20 (m, 1.1H), 2.70–2.46 (m, 2H), 1.63 (d, 2.2H, $J = 6.0$ Hz), 1.57 (d, 0.8H, $J = 6.4$ Hz). ESI-MS: m/z 464.2 $[M + Na]^+$.

General Procedure

To a solution of *O*-pentenyl glycoside or Fmoc-L-allyglycine benzyl ester in CH_2Cl_2 at -78°C was added liquid *trans*-2-butene, and then the Grubbs I catalyst (25% equiv) was added. The sealed reaction vessel was heated to 40°C for 3 hr. The mixture was purified by flash chromatography. A mixture of hexenyl glycoside, **15** (10 equiv.), and Grubbs II catalyst (10%) in toluene (carbohydrate 0.01 mmol, toluene 0.2 mL) under N_2 flow was heated at 40°C for 3 to 6 hr. Chromatography afforded the mixture of *E* and *Z* olefins. The catalytic hydrogenation and deprotection of the benzyl group were realized in presence of 10% Pt/C in MeOH/H_2O (15/1) under H_2 from 1 day to 5 days.

Peracetylated β-*O*-pentenyl-Gb3:[30] $[\alpha]_D^{24} = +60.0^\circ$ (*c* 1.0, $CHCl_3$); IR (cm^{-1}) γ: 2941, 1749, 1370, 1231, 1052. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.80–5.72 (m, 1H), 5.58 (s, 1H), 5.38 (dd, 1H, $J = 2.8$, 11.0 Hz), 5.21–5.15 (m, 2H), 5.09 (dd, 1H, $J = 8.0$, 10.4 Hz), 5.01–4.85 (m, 4H), 4.72 (d, 1H, $J = 10.7$ Hz), 4.51–4.40 (m, 5H), 4.18–4.07 (m, 4H), 4.00 (s, 1H), 3.86–3.73 (m, 3H), 3.63–3.59 (m, 1H), 3.50–3.44 (m, 1H), 2.16–1.97 (m, 32 H), 1.71–1.60 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 171.06, 170.87, 170.85, 170.84, 170.83, 170.82, 170.47, 170.08, 170.06, 169.91, 138.21, 115.45, 101.50, 100.94, 100.02, 76.93, 73.56, 73.21, 72.89, 72.22, 72.20, 69.68, 69.38, 69.24, 68.28, 68.04, 67.53, 67.47, 62.74, 61.72, 60.66, 30.22, 28.98, 21.32, 21.25, 21.12, 21.11, 21.10, 21.08, 21.03, 20.98, 20.90, 20.79. ESI-MS: m/z 1015.7 $[M + Na]^+$.

Peracetylated β-*O*-pentenyl-GM2:[31] $[\alpha]_D^{23} = -21.8^\circ$ (*c* 2.18, $CHCl_3$); IR (cm^{-1}) γ: 3384, 2957, 1747, 1684, 1665, 1540, 1435, 1370, 1230, 1167, 1129, and 1046. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 5.97 (m, 1 H), 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 $[M + Na]^+$.

Peracetylated β-*O*-pentenyl fucosyl GM1:[32] $[\alpha]_D^{24} = -40.4^\circ$ (c 1.0, $CHCl_3$); IR (cm^{-1}) γ: 3382, 2969, 1747, 1690, 1371, 1232, 1166, 1131, 1058. ^1H NMR (400 MHz, $CDCl_3$) δ: 7.16 (d, 1H, $J = 6.1$ Hz), 5.80–5.67 (m, 1H), 5.60 (ddd, 1H, $J = 2.5$, 6.0, 9.0 Hz), 5.44 (d, 1H, $J = 3.5$ Hz), 5.39–5.28 (m, 4H), 5.24–5.10 (m, 4H), 5.05–4.88 (m, 6H), 4.82 (dd, 1H, $J = 8.1$, 9.4 Hz), 4.75 (ddd, 1H, $J = 4.2$, 10.5, 12.4 Hz), 4.67 (d, 1H, $J = 7.8$ Hz), 4.51 (d, 1H, $J = 7.8$ Hz), 4.67 (dd, 1H, $J = 6.4$, 13.1 Hz), 4.39 (d, 1H, $J = 7.9$ Hz), 4.22–3.70 (m, 18 H), 3.79 (s, 3H), 3.57 (m. 2H), 3.50–3.40 (m, 2H), 3.02 (m, 1H), 2.82 (dd, 1H, $J = 4.3$, 13.0 Hz), 2.19 (s, 3H), 2.17–2.07 (m, 12H), 2.06–1.9 (m, 48H), 1.81 (s, 3H), 1.7–1.54 (m, 3H), 1.21 (s, 3H), 1.11 (d, 3H, $J = 6.5$ Hz). ^{13}C NMR (100 MHz, $CDCl_3$) δ: 174.13, 171.58, 171.49, 170.98, 170.93, 170.91, 170.84, 170.80, 170.73, 170.71, 170.56, 170.18, 170.16, 170.05, 169.94, 169.74, 168.71, 138.26, 115.45, 102.59, 100.99, 100.94, 99.27, 97.80, 94.89, 77.64, 76.21, 74.24, 74.10, 73.89, 73.80, 73.36, 72.98, 72.53, 72.38, 72.29, 71.84, 71.46, 70.88, 70.70, 70.31, 69.90, 69.67, 67.49, 65.32, 63.81, 62.97, 61.10, 56.00, 54.21, 53.05, 49.81, 30.26, 29.69, 29.00, 24.01, 23.55, 21.85, 21.28, 21.52, 21.22, 21.19, 21.17, 21.07, 20.97, 20.96, 20.88, 16.44. ESI-MS: m/z 1963.9 $[M + Na]^+$.

Peracetylated lactose glycosylamino acid (16):[33] $[\alpha]_D^{24} = -10^\circ$ (c 1.0, $CHCl_3$); IR (cm^{-1}) γ: 2936, 1752, 1369, 1228, 1053. ^1H NMR (400 MHz, MeOD) δ: 7.80 (d, 2H, $J = 7.5$ Hz), 7.70–7.55 (m, 2H), 7.38 (t, 2H, $J = 7.4$ Hz), 7.30 (t, 2H, $J = 7.4$ Hz), 5.34 (d, 1H, $J = 3.3$ Hz), 5.16 (t, 1H, $J = 9.3$ Hz), 5.09 (dd, 1H, $J = 3.4$, 10.3 Hz), 5.01 (dd, 1H, $J = 7.8$, 10.3 Hz), 4.80 (dd, 1H, $J = 8.0$, 9.5 Hz), 4.66 (d, 1H, $J = 7.9$ Hz), 4.53 (d, 1H, $J = 8.0$ Hz), 4.65 (dd, 1H, $J = 1.5$, 12.0 Hz), 4.40–4.25 (m, 2H), 4.21 (t, 1H, $J = 6.7$ Hz), 4.15–4.05 (m, 5H), 3.90–3.56 (m, 3H), 3.46 (td, $J = 9.7$, 6.5 Hz), 2.12 (s, 3H), 2.08 (s, 3H), 2.04 (s, 3H), 2.03 (s, 6H), 1.92 (s, 3H), 1.86 (s, 3H), 1.60–1.45 (br, 2H), 1.4–1.27 (br, 6H). ^{13}C NMR (100 MHz, MeOD) δ: 172.77, 172.41, 172.33, 172.15, 171.82, 171.69, 171.55, 145.80, 145.60, 143.00, 129.23, 128.61, 126.70, 121.38, 102.47, 102.07, 78.09, 74.90, 74.27, 73.62, 72.99, 72.15, 71.30, 71.10, 70.99, 68.99, 68.25, 64.01, 62.68, 56.45, 34.50, 32.50, 32.08, 30.89, 30.31, 29.94, 27.25, 21.54, 21.17, 21.13, 21.00, 21.88. ESI-MS: m/z 1016.6 $[M + H]^+$.

Peracetylated Gb3 glycosylamino acid (17): $[\alpha]_D^{24} = +32.4^\circ$ (c 0.5, $CHCl_3$); IR (cm^{-1}) γ: 2936, 1753, 1370, 1232, 1052. ^1H NMR (400 MHz, MeOD) δ: 7.70 (d, 2H, $J = 7.5$ Hz), 7.60–7.50 (m, 2H), 7.29 (t, 2H, $J = 7.4$ Hz), 7.21 (t, 2H, $J = 7.4$ Hz), 5.43 (m, 1 H), 5.29 (dd, 1H, $J = 3.3$, 11.0 Hz), 5.09 (dd, 1H, $J = 3.5$, 11.0 Hz), 5.07 (t, 1H, $J = 9.3$ Hz), 5.00 (dd, 1H, $J = 7.8$, 10.7 Hz), 4.94

(d, 1H, $J = 3.5$ Hz), 4.85 (dd, 1H, $J = 2.6$, 10.7 Hz), 4.72 (t, 1H, $J = 9.7$ Hz), 4.57 (d, 1H, $J = 7.8$ Hz), 4.46 (d, 1H, $J = 8.1$ Hz), 4.43–4.38 (m, 2H), 4.35 (dd, 1H, $J = 7.2$, 11.3 Hz), 4.32–4.26 (m, 2H), 4.15–3.92 (m, 7H), 3.88 (t, 1H, $J = 6.4$ Hz), 3.74–3.67 (m, 2H), 3.63–3.58 (m, 1H), 3.54–3.25 (m, 1H), 3.43–3.35 (m, 1H), 2.03 (s, 3H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.95 (s, 6H), 1.94 (s, 3H), 1.92 (s, 3H), 1.89 (s, 3H), 1.86 (s, 3H), 1.80–1.67 (br, 1H), 1.65–1.14 (br, 3H), 1.32–1.28 (br, 7H). ^{13}C NMR (125 MHz, MeOD) δ: 172.78, 172.72, 172.50, 172.35, 171.79, 171.72, 171.58, 145.81, 145.65, 143.02, 129.24, 128.62, 126.69, 121.38, 102.49, 102.15, 101.16, 79.22, 78.03, 74.85, 74.47, 74.33, 73.88, 73.57, 71.92, 71.30, 71.00, 70.26, 69.71, 69.30, 68.87, 68.26, 64.16, 64.05, 62.19, 56.46, 33.40, 32.79, 32.51, 31.18, 30.89, 30.28, 29.95, 27.33, 27.27, 21.63, 21.43, 21.17, 21.11, 20.97. ESI-MS: m/z 1326.5 $[M + Na]^+$.

Peracetylated GM2 glycosylamino acid (18): $[\alpha]_D^{27} = -19.38^\circ$ (c 1.26, $CHCl_3$); IR (cm^{-1}) γ: 3364, 2939, 1747 1681, 1556, 1538, 1454, 1434, 1371, 1229, 1169, 1126, 1046. ^{1}H 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 $[M + H]^+$.

Peracetylated Fucosyl GM1 glycosylamino acid (19): $[\alpha]_D^{24} = -30.2^\circ$ (c 1.0, $CHCl_3$); IR (cm^{-1}) γ: 2931, 1747, 1370, 1231, 1047. ^{1}H NMR (500 MHz, MeOD) δ: 7.79 (d, 2H, $J = 7.5$ Hz), 7.66 (m, 2H), 7.38 (t, 2H, $J = 7.4$ Hz), 7.30 (t, 2H, $J = 7.4$ Hz), 5.61 (m, 1H), 5.48 (dd, 2H, $J = 3.1$, 9,5 Hz), 5.39 (dd, 1 H, $J = 2.3$, 9.7 Hz), 5.25 (m, 3H), 5.14–5.09 (m, 2H), 5.06 (dd, 2H, $J = 3.5$, 10.0 Hz), 4.94 (d, 1H, $J = 8.3$ Hz), 4.91 (dd, 1H, $J = 3.9$, 11.0 Hz), 4.84–4.77 (m, 1H), 4.66 (d, 1H, $J = 7.6$ Hz), 4.65 (d, 1H, $J = 7.9$ Hz), 4.56 (d, 1H,

$J = 8.0$ Hz), 4.52 (m, 1H), 4.47 (d, 1H, $J = 10.4$ Hz), 4.40–4.30 (m, 4H), 4.29–4.16 (m, 4H), 4.15–3.83 (m, 11H), 3.89 (s, 3H), 3.82–3.76 (m, 1H), 3.73–3.65 (m, 3H), 3.56 (m, 1H), 3.51–3.44 (m, 1H), 3.13 (dd, 1H, $J = 8.3$, 11.1 Hz), 2.85 (dd, 1H, $J = 4.7$, 13.0 Hz), 2.27 (s, 3H), 2.17 (s, 3H), 2.15 (s, 3H), 2.14 (s, 3H), 2.12 (s, 6H), 2.11 (s, 3H), 2.07 (s, 3H), 2.06 (s, 3H), 2.05 (s, 3H), 2.02 (s, 6H), 2.018 (s, 3H), 2.015 (s, 3H), 2.0 (s, 3H), 1.98 (s, 2H), 1.97 (s, 3H), 1.95 (s, 3H), 1.94 (s, 3H), 1.81 (s, 3H), 1.70–1.50 (br, 6H), 1.40–1.25 (br, 4H), 1.17 (d, 3H, $J = 6.5$ Hz). ^{13}C NMR (125 MHz, MeOD) δ: 175.90, 173.97, 172.82, 172.77, 172.74, 172.64, 172.59, 172.46, 172.43, 172.07, 171.87, 171.77, 171.50, 170.10, 145.82, 145.66, 143.02, 129.24, 128.62, 126.70, 121.37, 102.93, 102.84, 102.03, 101.22, 99.08, 97.71, 78.15, 75.40, 73.66, 71.30, 71.00, 69.24, 68.24, 66.55, 65.41, 64.22, 63.02, 56.47, 54.21, 32.51, 30.29, 29.95, 27.26, 21.63, 21.40, 21.30, 21.18, 21.14, 21.05, 21.01, 20.96, 20.92, 16.67. ESI-MS: m/z 2252.9 $[M + H]^+$.

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