Pursuit of Optimal Carbohydrate-Based Anticancer Vaccines: Preparation of a Multiantigenic Unimolecular Glycopeptide Containing the Tn, MBr1, and Lewis^y Antigens

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Received July 27, 2000

Abstract: A novel preparation of nonnatural glycoamino acids starting from *n*-pentenyl glycosides is described. The approach involves a Horner−Emmons olefination with a suitably protected glycine-derived phosphonate, followed by catalytic asymmetric hydrogenation, which proceeds with excellent diastereomeric selectivity. The synthetic methodology was useful for the preparation of glycoamino acids containing the Tn antigen, the MBr1 antigen (Globo-H), the Le^y antigen, and lactose. These glycoamino acids can also serve as units for peptide synthesis. The synthesis of polyvalent glycopeptides containing three different antitumor antigens is described (**28** and **29**), and these have been prepared for conjugation to carrier protein in order to access the immunogenicity for tumor immunotherapy applications.

Introduction

Aberrant cell-surface glycosylation is often strongly associated with tumor progression and malignancy.[1] These changes in cell surface carbohydrate patterns presumably reflect incomplete or novel glycosylation in tumor masses. Trends in carbohydrate expression by different tumor types have been noted and reveal unusual abundances of such cell surface antigens as a function of the particular tumor. Indeed, oftentimes carbohydrate antigens can be rather specific to a certain type of tumor and are not overexpressed or recognized by the immune system in normal tissue.[2] The isolation and careful structural identification of specific carbohydrate antigens overexpressed in cancer cells has provided a framework for an attack using carbohydrate-based tumor immunotherapy.[3] These studies, however, have been severely complicated by the difficult availability of these antigens from natural sources. Obtaining even a milligram of tumor antigen from collections of cancerous tissues is a major undertaking. To assess the potential value of carbohydrate-based tumor antigens in medicine, it would be necessary to gain access to such agents through chemical synthesis.

There is a long-term history of vaccines derived from abundant bacterial polysaccharide antigens. These vaccines stimulate an immune response, including high titers of antibodies, which provide additional protection from subsequent bacterial infection.[4] Similarly, given the presence of tumor associated carbohydrate antigens on the surface of tumor cells, there is reason to hope that vaccination of cancer patients in an adjuvant setting could bring about similar results. The likely production of antibodies derived from defined carbohydrate-based tumor antigens, in the hope that they would provide protection against circulating tumor cells and micrometastases, has been a major consideration in their selection for anti-cancer vaccine construction and testing.[5] At the present time, several preclinical mouse and human clinical trials have demonstrated that carbohydrate-based cancer vaccines can induce antibody responses against tumor-associated antigens. These results suggest that tumor immunotherapy employing fully synthetic carbohydrate-based vaccines could hold promise as a means for a nontoxic treatment of human cancer.[6]

Our laboratory has actively been engaged in synthesizing complex oligosaccharides and glycoconjugates.[7] As a result of our advances in this area, we began a program some time ago to explore the chemical synthesis of tumor-associated carbohydrate antigens with the goal of investigating totally synthetic carbohydrate-based vaccines in laboratory and clinical settings.[8]

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10.1021/ja002779i CCC: $20.00
Published on Web 02/10/2001

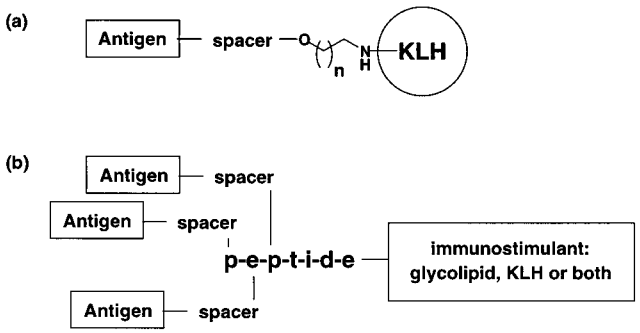

Figure 1. Glycoconjugate components of anticancer vaccines: (a) tumor antigen conjugated to carrier protein KLH via reductive alkylation of *n*-allyl ($n = 1$) or *n*-pentenyl ($n = 3$) glycosides; (b) glycopeptide-presented clustered antigens.

Our continuing chemical studies have focused on synthetic methodology of general applicability for the preparation of mimics of cell surface carbohydrates in the form of glycoproteins, glycolipids, and glycopeptides. This progress has allowed us to assemble a number of fully synthetic vaccine constructs for ongoing human clinical trials.

A variety of approaches have been adopted to elicit a more sustained immune response beyond that triggered by exposure to native synthetic antigens. These attempts include chemical modification of carbohydrates, additional administration of immunological adjuvants, and covalent attachment of carbohydrates to immunogenic protein carriers. Presently, we and others[9] are pursuing vaccine constructs containing the desired tumor antigen bioconjugated (through a spacer unit) to carrier protein keyhole limpet hemocyanin (KLH). These are administered in concert with a nonsynthetic immunoadjuvant (i.e., QS-21).[10] More specifically, we have investigated both glycolipid structures and glycopeptide structures conjugated to KLH (Figure 1b). Covalent modification of KLH with complex carbohydrates was accomplished via reductive amination of aldehydes derived from *n*-allyl and *n*-pentenyl glycosides (Figure 1a).[11] The disclosure herein focuses on new general methodology for the synthesis of glycoamino acids from their corresponding *n*-pentenyl glycosides and serves to provide important new options for vaccine development. The advances reported below have allowed for a unique assembly of a multiantigenic glycopeptide containing the tumor antigens Globo-H (MBr1 antigen), Lewis^y, and the Tn antigen.

Background

Substantial efforts in our laboratory have been dedicated to the chemical synthesis of glycopeptides carrying tumor-associated antigens.[12] Typically, naturally occurring *O*-glycopeptides and proteins are glycosylated through either anomerically attached serine or threonine with an α-glycosidic linkage. For example, mucin glycoproteins possess amino acid sequences with a very high percentage of serine and threonine residues wherein the first carbohydrate moiety is universally α-O-linked *N*-acetylgalactosamine.[13] The glycophorin family of α-O-linked carbohydrates, containing Tn, TF, STn, ST, and glycophorin, is a well-known and -studied class of tumor antigens that carry this unique linkage.[14] Much synthetic effort in this area has focused on the stereoselective construction of the naturally required α-glycosidic linkage.[15] Studies in our own laboratory, relying on a "cassette" modality rather than a maximally convergent approach, have been detailed elsewhere.[8,12,16]

The synthesis of more readily available and more chemically stable glycopeptide analogues has also been advanced in the literature.[17] For example, C-linked and S-linked oligosaccharides can offer increased stability to hydrolysis. Furthermore, these nonnatural linkages avoid problems associated with the instability of the *O*-glycosyl serine.[18] It appears that suitable glycopeptide mimics can simulate the activity of their native counterparts. In the case of enzyme inhibitors, unnatural linkages can even result in better therapeutic agents.[19] The question of whether glycopeptides derived from nonnatural amino acids and nonnatural linkages can be as immunogenic as their natural counterparts in an antitumor vaccine setting has never been systematically addressed. In fact, it may be anticipated that less-natural synthetic compounds may prove to be more immunogenic, because they are potentially more recognizable as "nonself" by the immune system.[12a]

We recently reported a second-generation synthesis of the MBr1 antigen (Globo-H)[20] and the total synthesis of the

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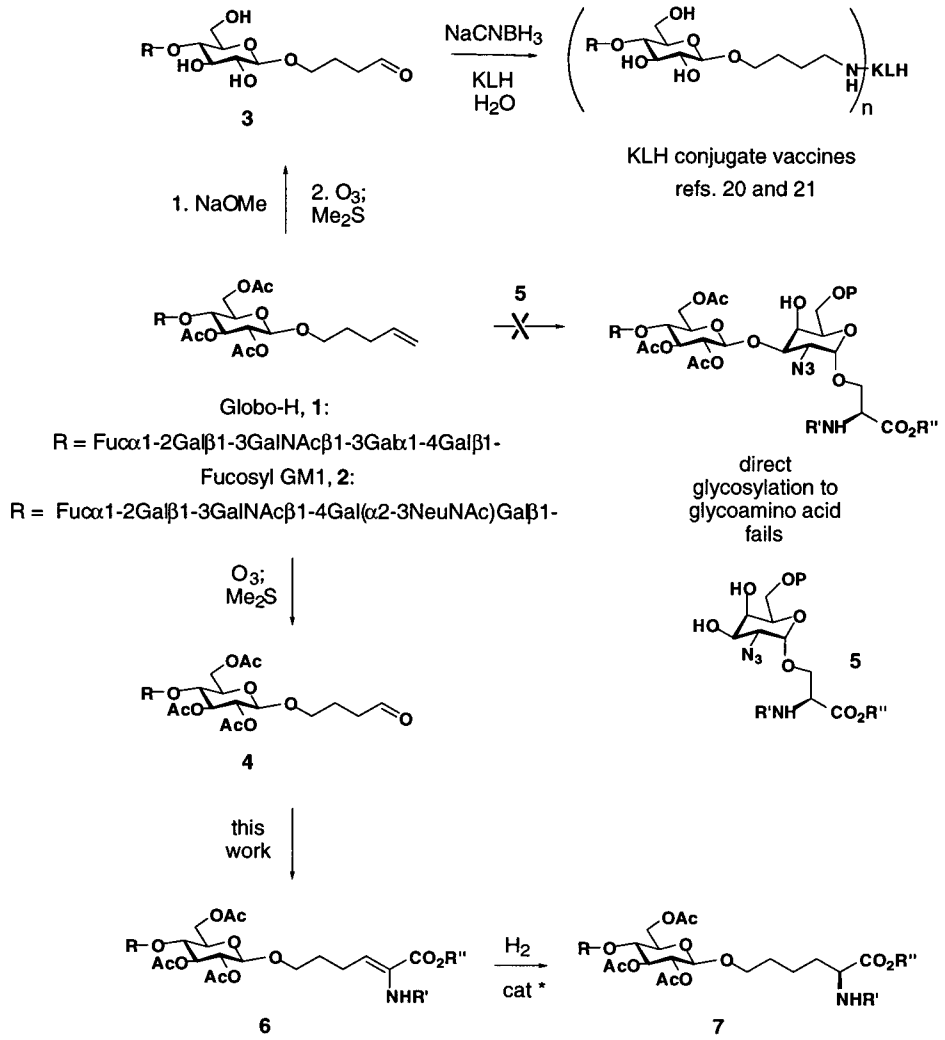

Figure 2. Previous immunoconjugation from NPG-derived tumor antigens **1** and **2** and newly developed glycoamino acid sequence.

fucosylated ganglioside of GM_1 (fucosyl GM_1),[21] each as its corresponding *n*-pentenyl glycoside (NPG). The (mAb) MBr1 antigen Globo-H was originally isolated and characterized by Hakomori et al. from the human breast cancer cell line MCF-7[22] and has subsequently been further characterized as being overexpressed in other types of carcinomas including colon, prostate, lung, ovary, and small cell lung cancers.[23] The first generation total synthesis of this antigen by our laboratory[24] has culminated in phase II and III human clinical trials using a fully synthetic Globo-H vaccine against prostate and breast cancer.[25] Fucosyl GM_1 has been identified as a highly specific marker associated with small cell lung cancer cells[26] and a vaccine containing this synthetic antigen is currently undergoing mouse immunization experiments aimed against small cell lung cancer.[21]

Incorporating the reducing end NPG in those earlier studies offered two benefits. The anomeric *n*-pentenyl glycoside linkage served as an efficient linker for immunoconjugation to carrier protein KLH (see top of Figure 2) and also provided some advantages in terms of synthetic convergency.[20,21] Furthermore, although unproven at the time of those studies, in the context of protected carbohydrates **1** and **2**, the NPG moieties might serve as useful donors for glycosylation, following the pioneering work of Fraser-Reid and associates.[27]

The contemplated next step in our progression to a more effective presentation of antigens in antitumor vaccines required an efficient and general way to incorporate these two antigens into glycopeptides. Initial efforts were directed at performing a direct glycosylation of donors **1** or **2** with cassette acceptor **5**, which contains the required serine linkage (Figure 2).[12b] Unfortunately, these [6 + 1] couplings failed and this approach was abandoned.[28]

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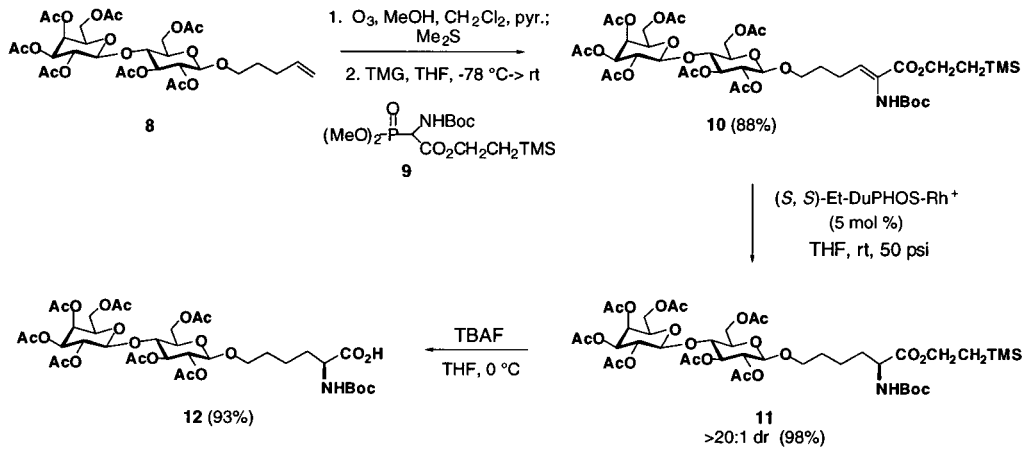
Scheme 1



Fortunately an alternative approach was suggested by the results of Toone and co-workers. These workers had described the catalytic asymmetric hydrogenation of glycosylated enamide esters, derived from *C*-allyl glycosides, to produce carbon linked glycosyl serines.[29] Application of an analogous transformation using an NPG as a starting material is suggested in Figure 2.[30] The new approach anticipated a Horner−Emmons olefination of the protected aldehyde **4** with a suitably protected glycine derived phosphonate to give enamide ester **6**. Subsequent catalytic asymmetric hydrogenation would hopefully yield diastereomerically pure glycoamino acid **7**, provided the necessary stereoselectivty could be achieved. We recognized that the resulting glycoamino acid construct would correspond to a nonnatural linkage between an oligosaccharide and amino acid. However, given the impasse reached in incorporating Globo-H into a conventional O-linked glycopeptide, the possibility of gaining access through the use of a NPG spacer domain was attractive. In addition, the study of the effect of these types of nonnatural linkages in a vaccine setting would allow for the assessment of inducing antibody production against artificial glycopeptides. Accordingly, it was toward these directions that we turned our attentions. Our results are reported in the discussion which follows.

Results and Discussion

Having previously accomplished the synthesis of **1** and **2**,[20,21] the possibility of a sequence consisting of suitable olefination followed by asymmetric hydrogenation starting with NPG precursors was addressed. We were conscious of the fact that in the hydrogenation reaction, the newly generated stereogenic center would be five atoms removed from other chirality in the molecule. Although this nonproximity might be favorable in terms of promoting the reduction reaction and might help in favoring high diastereoselectivity margins, we anticipated that it could complicate our analysis of the outcome and separation of the products by chromatographic means. Accordingly, as a starting point, we chose to examine peracetylated lactose derivatives as models.[31] The required lactose-derived enamide ester substrate was prepared according to Scheme 1. Ozonolysis of the NPG **8** [20] followed by reductive workup gave the corresponding aldehyde derivative. The crude aldehyde was then subjected to Horner−Emmons olefination using tetramethylguanidine and phosphonate **9**. Phosphonate **9**, with *N*-Boc and 2-(trimethylsilyl)ethyl ester (TSE) protection,[32] was chosen because of the need for the resulting glycoamino acids to be orthogonally suitable for peptide couplings in the presence of acetate carbohydrate protecting groups. The enamide ester **10** was obtained as a single geometric isomer in 88% yield for the 2-step procedure.

In the hydrogenation of similar C-glycosylated enamides, Toone had reported the use of chiral DuPHOS ligands as catalyst precursors[33] and had optimized the resulting diastereomeric excesses of the monosaccharide products with respect to ligand and solvent. Following their report, we found optimal conditions for asymmetric hydrogenation of enamide ester **10** to be as shown. We chose to use the *S*,*S* ligand isomer, which has been well-characterized in these types of systems to give the *S* isomer in the amino acid product.[34] The protected glycoamino acid was obtained in 98% yield and was determined to have been formed with a diastereomeric ratio (dr) > 20:1. Remarkably, the *t*-Boc protons are nearly baseline resolved and, in the asymmetric reaction, the minor isomer could not be detected by ^{1}H NMR. ^{13}C analysis also supports the conclusion that the minor isomer is not formed within the limits of NMR detection.[35] Hydrogenation of **10** with an achiral catalyst (Pd/C, MeOH) produced a 1:1 mixture of *R*- and *S*-configured **11**, providing a comparison for diastereomeric ratio determination. This reaction also indicates that chirality transfer to yield **11** occurs from the chiral

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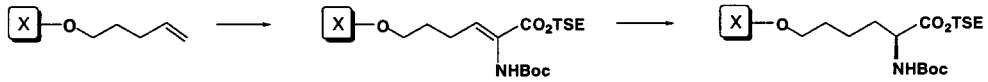
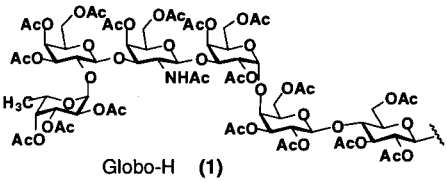
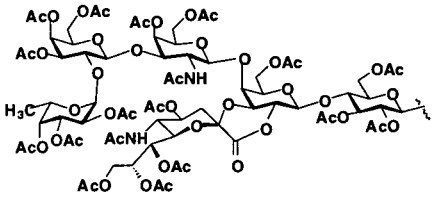
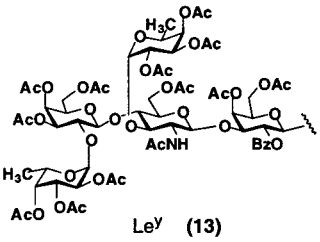
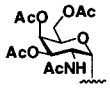
(35) In all cases, the diastereomeric ratios were determined by ^{1}H NMR (500 MHz).

Table 1. Olefination/Asymmetric Hydrogenation of a Variety of Tumor Antigen NPGs



X =	Conditions: (1) O_3, CH_2Cl_2, MeOH, pyr.; then Me_2S; (2) TMG, **9**, THF, -78 °C product, yield	Conditions: (*S*, *S*)-Et-DuPHOS-Rh^+ H_2, 50 psi, THF product, yield, dr
 Globo-H **(1)**	**15,** 72%	**19**, 98%, >20:1
 Fucosyl GM_1 **(2)**	**16**,10-22%	**20**, 93%, >20:1
 Le^y **(13)**	**17,** 85%	**21**, 99%, >20:1
 **(14)** α-Tn antigen	**18**, 75%	**22**, 99%, >20:1

ligand and not carbohydrate-derived substrate control. A final step to be performed prior to moving to synthesis and assembly of tumor antigens was that of demonstrating deprotectability of the blocking groups contained in the amino acid side chain. In the event, reaction of **11** with TBAF in THF gave acid **12**, suitably prepared for peptide coupling, in 93% isolated yield.

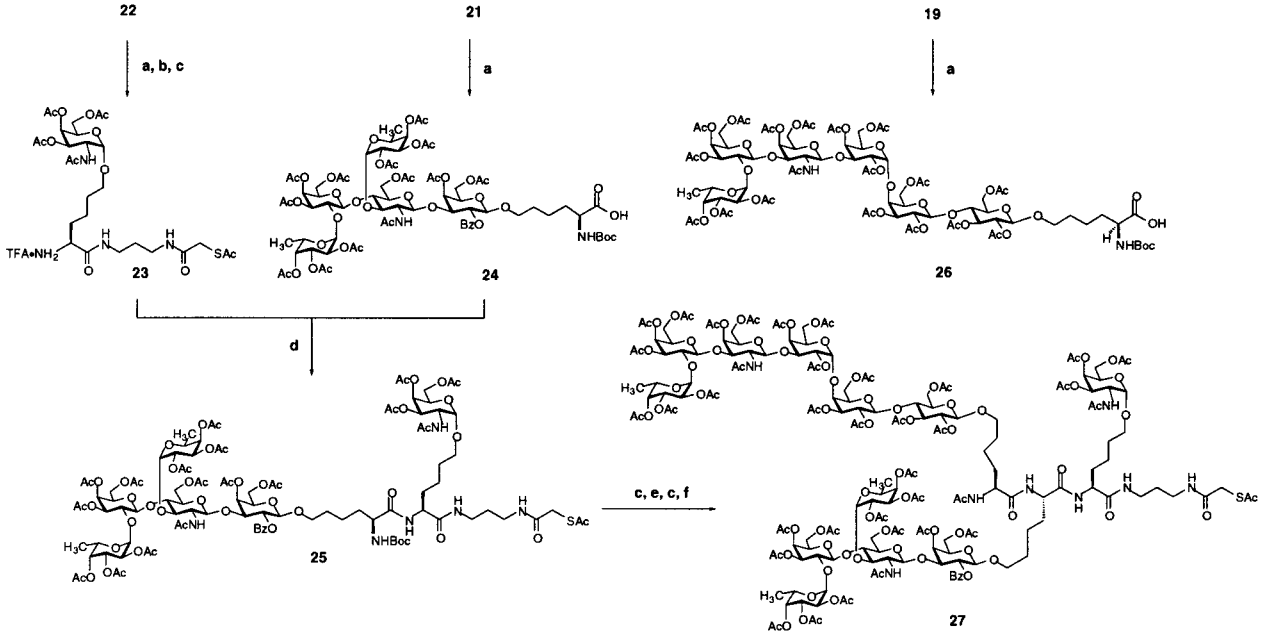
With the methodology demonstrated in the lactose model, we moved to our more advanced hexasaccharides **1** and **2** as well as to other antigens of interest. As shown in Table 1, olefination of the peracetylated *n*-pentenyl glycoside of Globo-H **1** under the same conditions as those used in Scheme 1 provided the corresponding enamide ester **15** in 72% yield as a single isomer. We were disappointed to find that a similar reaction with fucosyl GM_1 hexasaccharide **2** provided **16** in a very low yield (10−22%). Although we do not have an explanation for this result, we note that **2** is the only oligosaccharide on which we have attempted the olefination in the presence of a sialic acid residue. However, in both cases, through use of the (*S*, *S*)-Et-DuPHOS−Rh^+ catalyst system, the hydrogenation of **15** and **16** proceeded in excellent yield, producing **19** and **20** as single diastereomers, as determined by ^1H NMR analysis. Compounds **19** and **20** represent the first examples of synthetic glycoamino acids containing the complex oligosaccharides Globo-H and fucosyl GM_1.

We also performed such transformations on two other clinically promising antigens to generate their corresponding glycoamino acids. Thus, Lewis^y (Le^y) oligosaccharide has been identified as an important antigen for eliciting antibodies against colon, liver, prostate, and ovarian carcinomas.[36] We have previously synthesized both a Le^y−KLH conjugate vaccine[37] and a clustered Le^y glycopeptide (of natural α-O-linked configuration) glycoconjugate attached to either a glycolipid or KLH.[12a] Our synthetic constructs have been used in initiating human clinical trials against ovarian cancer.[38] A Le^y-derived glycoamino acid synthesized by the procedure detailed herein would provide an opportunity to experimentally determine if the presence of the α-O-linkage was, indeed, critical for

(36) (a) Lloyd, K. O. *Am. J. Clin. Path.* **1987**, *87*, 129. (b) Lloyd, K. O. *Cancer Biol.* **1991**, *2*, 421. (c) Yin, B. W.; Finstad, C. L.; Kitamura, K.; Federici, M. G.; Welshinger, M.; Kudryashov, V.; Hoskins, W. J.; Welt, S.; Lloyd, K. O. *Int. J. Cancer* **1996**, *65*, 406.

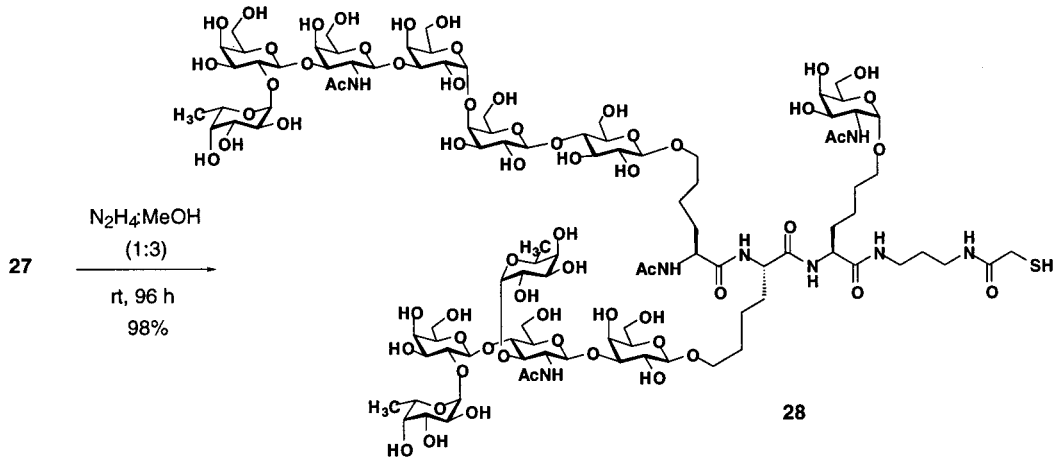
(37) Danishefsky, S. J.; Behar, V.; Randolph, J. T.; Lloyd, K. O. *J. Am. Chem. Soc.* **1995**, *117*, 5701.

Scheme 2[a]



[a]Conditions: (a) TBAF, THF; (b) $AcSCH_2C(O)(CH_2)_3NH_2$, BOP reagent, iPr$_2$NEt, 54%, 2 steps; (c) TFA, CH_2Cl_2; (d) BOP reagent, iPr$_2$NEt, 86%, 2 steps; (e) 26, BOP reagent, iPr$_2$NEt, 64%, 2 steps; (f) Ac_2O, Et_3N, cat. DMAP, 95%, 2 steps

Scheme 3



immunogenicity in these vaccines. We, as well as others,[39] have also synthesized and studied clustered glycopeptides containing the Tn antigen and have evaluated the antibody response to vaccination of mice.[12b] The Tn antigen is also found naturally α-O-linked through serine or threonine and is commonly synthesized in that fashion. A direct comparison of an NPG derived glycoamino acid with the O-linked system would now be possible.

The results starting with both Le^y *n*-pentenyl glycoside **13**[12a] and the α-linked *n*-pentenyl glycoside of the Tn antigen **14** (GalNAc) are presented in Table 1.[40] The pentasaccharide **13** was available as an intermediate in the synthesis of Le^y glycopeptide cluster and, consequently, illustrates the potential advantage of this strategy. Thus, if immunogenicity is retained in the artificial constructs, these NPG-derived glycoamino acids offer a much shorter synthetic route to vaccine glycoconjugates than do their native counterparts. Olefination of **13** and **14** was uneventful, and enamide esters **17** and **18** were obtained in 85% and 75% yields respectively, again as single isomers. Asymmetric hydrogenation, as before, also produced diastereomerically pure glycoamino acids **21** and **22** in excellent yields.

With glycoamino acids **19**−**22** in hand, we were ready to proceed to the peptide assembly phase. Immunological studies indicate that, in general, the clustering of antigens in glycopeptides results in a more therapeutically useful immune response than with singly glycosylated peptides.[6f,9d,12a] Accordingly, we considered a new and exciting possibility. To date, the clustering of antigens has been accomplished with the same antigen across the peptide backbone. We set out to investigate the synthesis of a single peptide *that has three different antigens in a clustered format.*

The rationale for constructing such a polyvalent vaccine candidate draws from the potential to provide a more protective

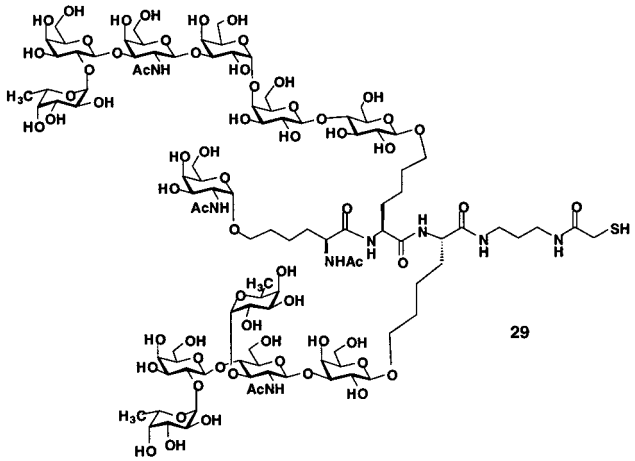
(38) (a) Kudryashov, V.; Kim, H. M.; Ragupathi, G.; Danishefsky, S. J.; Livingston, P. O.; Lloyd, K. O. *Cancer Immunol. Immunother.* **1998**, *45*, 281. (b) Sabbatini, P.; Kudryashov, V.; Danishefsky, S. J.; Livingston, P. O.; Ragupathi, G.; Bornmann, W.; Spassova, M.; Zatorski, A.; Spriggs, D.; Aghajanian, C.; Soignet, S.; Peyton, M.; O'Flaherty, C.; Curtin, J.; Lloyd, K. O. *Int. J. Cancer* **2000**, *87*, 79.

(39) See (a) Tokoyuni, T.; Hakomori, S.; Singhal, A. K. *Bioorg. Med. Chem.* **1994**, *11*, 1119. (b) Bay, S.; Lo-Man, R.; Osinaga, E.; Nakada, H.; Leclerc, C.; Cantacuzene, D. *J. Pept. Res.* **1997**, *49*, 620. (c) Liebe, B.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1997**. (d) Kunz, H.; Birnbach, S.; Wering, P. *Carbohydr. Res.* **1990**, *202*, 207 and references therein.

immune response using vaccination. Complement-mediated lysis of cells is initiated by antibody binding to antigen.[41] The effectiveness of the elimination of pathogens is, therefore, related to antibody concentration, and a polyvalent structure could be anticipated to provide more antibody density than a monovalent immunizing agent. In addition, with respect to cancer, increasing the number of tumor epitopes (antigens) involved in a vaccination protocol should result in a broader degree of immune protection against multiple cancers.[42] Therefore, in the context of carbohydrate-based antitumor vaccines, a polyvalent, multiantigenic attack against cancer warrants experimental consideration. Chemical synthesis provides an opportunity to merge multiple antigens into a single therapeutic agent. In terms of vaccine development, it would obviously be advantageous to develop a single molecular vaccine. The incorporation of individually synthesized tumor antigens into a single peptide to yield a unimolecular vaccine of defined chemical structure was envisioned.

Recognizing that the low yields obtained for the synthesis of **20** would seriously complicate the preparation of significant quantities of material for potential immunological study, and that fucosyl GM_1 is, in any case, extremely specific for only small cell lung cancer,[43] we deleted it from our ensemble and chose to construct a peptide containing the Tn antigen, Lewis^y antigen, and the MBr1 antigen (Globo-H). Toward this goal, we began by modifying the C-terminus of our eventual glycopeptide to include a conjugation handle for carrier protein KLH. The mercaptoacetamide unit has proven to be effective for this purpose.[44] As shown in Scheme 2, the Tn glycoamino acid **22** was treated with TBAF to reveal the corresponding carboxylic acid. Coupling with a diamino spacer terminating in a protected mercaptoacetamide ($AcSCH_2C(O)(CH_2)_3NH_2$[45]) under the agency of the BOP reagent (benzotriazol-1-oxytris-(dimethylamino)phosphonium hexafluorophosphate[46]), gave the corresponding amide in 54% yield for the 2 steps. Removal of the N-terminal Boc group gave amine **23** as its trifluoroacetate salt. The next antigen, Le^y, was prepared for coupling by reaction of **21** with TBAF to give acid **24**. Coupling of amine **23** with Le^y acid **24**, again with the BOP promoter, gave the Tn−Le^y dipeptide **25** in 86% yield. Last, Globo-H glycoamino acid **19** was treated with TBAF to give its corresponding acid **26**. Removal of the Boc protecting group in **25**, followed by coupling with acid **26** gave the Tn−Le^y−Globo-H tripeptide in 64% yield. Finally, the N-terminal Boc group was removed and the resulting anime capped as its acetate to give tripeptide **27** in 95% yield (Scheme 2). With all components in place, the ester and thioester protecting groups were removed with hydrazine in degassed methanol to give the fully deprotected glycopeptide **28** (Scheme 3) in excellent yield. To the best of our knowledge, this represents the first fully deprotected multiantigenic glycopeptide, containing three different tumor antigens, to be chemically synthesized.[47]

Construct **28** is being used to address several questions, including the immunogenic performance of the nonnatural four-carbon spacer between the peptide and oligosaccharide antigen. In addition, the power of chemical synthesis allows us to address the question of whether the order of antigens on the peptide affects their individual immunogenicity. Toward this end, following an analogous sequence of peptide synthesis, fully deprotected glycopeptide **29** was also synthesized.[48] This construct has Tn at the N-terminus, Le^y at the C-terminus, and Globo-H in the central position. Conjugation to KLH and immunological studies with **29**, as well as a direct immunological comparison of **28** and **29,** are underway and will be reported in due course.



29

Summary

A novel preparation of nonnatural glycoamino acids starting from *n*-pentenyl glycosides has been achieved with a variety of oligosaccharide tumor antigens. With the exception of poor results obtained in the olefination of fucosyl GM_1, the synthetic methodology appears to be general and was useful for the preparation of glycoamino acids containing the Tn antigen, the MBr1 antigen (Globo-H), the Le^y antigen, and lactose. These glycoamino acids can also serve as units for peptide synthesis. The synthesis of polyvalent glycopeptides containing three different antitumor antigens was accomplished (**28** and **29**) and has been prepared for conjugation to a carrier protein in order to access the immunogenicity for tumor immunotherapy applications. In addition, it has not escaped our attention that monovalent clusters of these antigens (for example, Globo-H and fucosyl GM_1) might also be therapeutically useful. Those results will be forthcoming and will also be disclosed in due course.

Experimental Section

General Procedure for Olefination, 15. The preparation of enamide **15** (Globo-H) is representative of this procedure. The *n*-pentenyl glycoside **1** (58 mg, 0.0322 mmol) was dissolved in 10:10:1 MeOH:CH_2Cl_2:pyridine (3 mL, typically 0.05−0.01 M) and cooled to −78 °C. A stream of dry ozone was passed through the reaction mixture until a pale blue color persisted. The ozone source was removed and the reaction was stirred at −78 °C for an additional 15 min, at which time a stream of dry nitrogen was applied to remove excess ozone. Dimethyl sulfide (50 equivs, 0.118 mL) was added to the cooled

(40) 1-*O*-PentGalNAc **14** was prepared from the corresponding trichloroacetimidate donor, itself available from azidonitration of the corresponding glycal, with low anomeric selectivity. See Supporting Information for experimental procedures and spectra.

(41) Tomlinson, S. *Curr. Opin. Immunol.* **1993**, *5*, 83.

(42) For vaccine strategies, see: Woodland, D. L.; Cole, G. A.; Doherty, P. C. In *Concepts in Vaccine Development*; Kaufmann, S. H., Ed.; Walter de Gruyter & Co.: Berlin, **1996**.

(43) Zhang, S.; Cordon-Cardo, C.; Zhang, H. S.; Reutter, V. E.; Adluri, S.; Hamilton, W. B.; Lloyd, K. O.; Livingston, P. O. *Int. J. Cancer* **1997**, *73*, 42.

(44) Brugghe, H. R. *Int. J. Pept. Res.* **1994**, *43*, 166.

(45) Prepared by reaction of commercially available *tert*-butyl *N*-(3-aminopropyl)-carbamate with (*S*)-acetylthioglycolic acid pentafluorophenyl ester (SAMA-Opfp), followed by treatment with trifluoroacetic acid.

(46) (a) Castro, B.; Dormoy, J. R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219. (b) Dormoy, J. R.; Castro, B. *Tetrahedron Lett.* **1979**, 3321. (c) Dormoy, J. R.; Castro, B. *Tetrahedron* **1981**, *37* 3699.

(47) Williams, L. J.; Harris, C. R.; Glunz, P. W.; Danishefsky, S. J. *Tetrahedron Lett.* **2000**, *41*, 9505.

(48) See supplementary material for spectra of intermediates.

mixture, the cooling bath was removed and the reaction was allowed to stir at room temperature for 4 h. The reaction was diluted with CH_2Cl_2 (10 mL), washed with water (50 mL), and back-extracted with additional CH_2Cl_2 (2 × 10 mL). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated. The crude aldehyde was typically not purified, but was azeotroped dry with anhydrous benzene (3 × 3 mL) and was used directly in the next step.

Phosphonate **9** (1.20 equivs, 14 mg) was dissolved in anhydrous THF (0.3 mL), cooled to −78 °C and tetramethyl guanidine (TMG) (1.25 equivs, 0.005 mL) was added dropwise. The reaction stirred at −78 for 30 min, followed by addition of the crude aldehyde (0.0322 mmol) in additional THF (2 × 0.3 mL, typically 0.1−0.02 M total reaction volume). The reaction was allowed to stir to room temperature overnight (10−15h), was extracted with EtOAc (10 mL), washed with 0.05 M aqueous HCl (50 mL), and back-extracted with additional EtOAc (2 × 10 mL). (Note: All of the TMG must be removed prior to asymmetric hydrogenation.) The combined organic layer was dried over $MgSO_4$, concentrated, and purified by flash column chromatography (75% EtOAc/hexanes → 100% EtOAc) to yield the desired enamide ester **15** as a single isomer. 72%, white foam; R_f 0.85 (100% EtOAc); IR ($CDCl_3$ film) 3373, 2956, 2951, 1748, 1370, 1069 cm^{-1}; ^1H NMR ($CDCl_3$, 500 MHz) δ 6.65 (d, 1H, J = 6.4 Hz), 6.44 (m, 1H), 6.07 (bs, 1H), 5.56 (d, 1H, J = 3.1 Hz), 5.44 (d, 1H, J = 3.4 Hz), 5.37 (d, 1H, J = 3.3 Hz), 5.27 (dd, 1H, J = 10.9, 3.0 Hz), 5.22 (d, 1H, J = 2.6 Hz), 5.20−5.17 (m, 2H), 5.15 (d, 1H, J = 2.1 Hz), 5.13 (d, 1H, J = 4.9 Hz), 5.09 (dd, 1H, J = 10.7, 7.3 Hz), 5.03 (dd, 1H, J = 11.1, 3.3 Hz), 4.96 (dd, 1H, J = 9.6, 3.5 Hz), 4.92 (dd, 1H, J = 11.2, 3.4 Hz), 4.85 (dd, 1H, J = 9.6, 8.0 Hz), 4.73 (dd, 1H, J = 10.9, 2.5 Hz), 4.50−4.38 (m, 6H), 4.34 (t, 1H, J = 6.2 Hz), 4.26−4.21 (m, 3H), 4.16−4.02 (m, 8H), 3.98 (d, 1H, J = 2.0 Hz), 3.94 (t, 1H, J = 6.4 Hz), 3.86−3.72 (m, 6H), 3.60−3.57 (m, 1H), 3.48−3.46 (m, 1H), 2.94−2.89 (m, 1H), 2.17−2.14 (m, 1H), 2.11−2.08 (m, 1H), 2.04 (s, 3H), 2.038 (s, 3H), 2.033 (s, 6H), 1.99 (s, 3H), 1.98 (s, 3H), 1.97 (s, 3H), 1.967 (s, 3H), 1.962 (s, 3H), 1.95 (s, 3H), 1.92 (s, 9H), 1.89 (s, 3H), 1.857 (s, 3H), 1.854 (s, 3H), 1.78 (s, 3H), 1.63−1.59 (m, 2H), 1.34 (s, 9H), 1.04 (d, 3H, J = 6.5 Hz), 0.93−0.90 (m, 2H), −0.06 (s, 9H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 172.20, 171.44, 170.94, 170.65, 170.52, 170.48, 170.44, 170.36, 170.29, 170.21, 170.17, 169.97, 169.63, 169.49, 169.31, 168.85, 164.78, 153.19, 134.70, 126.80, 102.35, 101.99, 101.26, 100.25, 99.12, 998.66, 94.21, 80.24, 76.88, 75.98, 73.61, 73.36, 73.08, 72.80, 72.56, 72.37, 71.81, 71.68, 71.46, 71.28, 70.78, 70.69, 70.67, 70.37, 70.06, 70.01, 68.92, 68.82, 67.99, 67.95, 67.54, 67.28, 66.94, 64.42, 62.14, 61.67, 61.29, 61.09, 60.92, 56.16, 28.12, 27.98, 24.52, 23.80, 23.03, 20.81, 20.73, 20.70, 20.68, 20.64, 20.60, 20.59, 20.54, 20.46, 20.40, 17.37, 17.24, 15.85, 15.48, 14.01, −1.58; HRMS (FAB) cald. for $C_{88}H_{128}N_2O_{51}SiNa$, 2079.7145; found, 2079.7174.

General Procedure for Asymmetric Hydrogenation. Under an inert deoxygenated atmosphere, [(COD)Rh−((*S*, *S*)-Et-DuPHOS)]$^+$OTf$^-$ (0.005 mmol, 5 mol %) and the desired enamide ester (0.100 mmol) were dissolved in deoxygenated anhydrous THF (10 mL, 0.01 M) in a Fischer−Porter tube. The reaction vessel was pressurized with 50 psi of H_2 after three vacuum/H_2 cycles and stirred at 25 °C for 24−36 h or until the reaction turned from light orange to brown in color. The vessel was depressurized, and the mixture was concentrated and purified through a short plug of silica gel to yield the glycoamino acid.

General Procedure for *N*-Boc Deprotection. The desired glycoamino acid (0.100 mmol) was dissolved in CH_2Cl_2 (3.0 mL) with stirring. Trifluoroacetic acid (TFA) (3.0 mL) was added dropwise, and the reaction was stirred at room temperature for 1 h. The mixture was then concentrated with a stream of dry N_2 and azeotroped with anhydrous benzene (2 × 5 mL) to give the crude amine as its TFA salt, which was typically used without further purification.

General Procedure for TSE Ester Deprotection. The desired glycoamino acid (0.100 mmol) was dissolved in THF (1.0−3.0 mL) and cooled to 0 °C. A 1.0 M solution of TBAF in THF (0.250 mmol, 2.5 equivs) was added dropwise, the ice bath was removed, and the reaction was stirred at room temperature for 1−2 h, as judged by TLC. (Note: prolonged reaction times, i.e., >10 h, may result in deacetylation.) The reaction mixture was diluted with CH_2Cl_2 (10 mL), washed with 0.05M aqueous HCl (50 mL), and back-extracted with additional CH_2Cl_2 (2 × 10 mL). The combined organic layer was dried over anhydrous Mg_2SO_4 and concentrated. The crude acid was typically used without further purification. Acid **12**: ^1H NMR ($CDCl_3$, 400 MHz) δ 5.22 (d, 1H, J = 2.8 Hz), 5.07 (t, 1H, J = 9.3 Hz), 4.98 (dd, 1H, J = 10.4, 5.9 Hz), 4.84 (dd, 1H, J = 10.4, 3.5 Hz), 4.75 (dd, 1H, J = 9.5, 8.0 Hz), 4.42−4.35 (m, 2H), 4.34−4.31 (m, 1H), 4.15−4.14 (m, 1H), 4.03−3.94 (m, 4H), 3.77−3.65 (m, 5H), 3.49−3.45 (m, 1H), 3.37−3.33 (m, 1H), 3.10−3.07 (m, 1H), 2.03 (s, 3H), 2.00 (s, 3H), 1.96 (s, 3H), 1.94 (s, 3H), 1.92 (s, 3H), 1.84 (s, 3H), 1.51−1.46 (m, 4H), 1.32 (s, 9H).

General Procedure for BOP Reagent-Promoted Peptide Coupling. The desired amine and acid (equimolar amounts) were azeotroped together with anhydrous benzene and dried under high vacuum. The mixture was dissolved in CH_2Cl_2 (0.1−0.05M), BOP reagent (1.25 equivs) was added, and the solution was cooled to 0 °C over 15 min. A dropwise addition of Hunig's base (15 equivs) was followed by removal of the ice bath. The reaction was stirred at room temperature for 2−4 h, as judged by TLC. Concentration of the reaction mixture was followed by purification by flash column chromatography. In cases where byproduct HMPA was difficult to remove, the peptide was subjected to sephadex purification (LH-20, MeOH).

Acknowledgment. In this paper, we honor major discoveries from the laboratories of Professors Bertram Fraser Reid and Horst Kunz that enabled the progress described herein. The authors thank Dr. John G. Allen for investigating the coupling of **1** with **5** and Xu-Feng Zhang and Andrzej Zatorski for their assistance in the preparation of **1**. This work was supported by the National Institutes of Health (Grant Numbers AI16943/CA28824). Postdoctoral Fellowship support is gratefully acknowledged by J.R.A. (NIH F32 GM 19578) and C.R.H. (ACS PF-98-173-001). We gratefully acknowledge Dr. George Sukenick of the Sloan−Kettering Institute's NMR Core Facility (Grant Number CA-08748) for NMR and mass spectral analyses.

Supporting Information Available: Tabulated ^1H and ^{13}C NMR spectra, IR and mass spectral data for all compounds; detailed experimental procedures for the preparation of peptides **25**, **27**, and **28**; ^1H and ^{13}C NMR spectra for compounds **14**, **15**, **17**−**19**, **21**, **22**, **25**, and **28**; ^1H NMR spectra for compounds **16**, **27**, **29** and for nonnumbered intermediates en route to **28** and **29**; and synthetic procedures for the preparation of **14** (PDF). This material is available free of charge via the Internet at http://www.pubs.acs.org.

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