Pergamon

Tetrahedron Letters 43 (2002) 6107–6110

TETRAHEDRON LETTERS

Construction of carbohydrate-based antitumor vaccines: synthesis of glycosyl amino acids by olefin cross-metathesis

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Received 27 June 2002; accepted 3 July 2002

Abstract—The synthesis of biologically relevant glycosyl amino acids from corresponding *O*-allyl glycosides is described. The procedure involves a cross-metathesis reaction with Fmoc-L-allylglycine benzyl ester, followed by reduction of the resulting olefin via catalytic hydrogenation, with the concomitant release of the free acid. This method has also been applied to the breast and prostate cancer antigen Globo-H, to afford a hexasaccharide glycosyl amino acid that has been previously incorporated in a polyvalent antitumor vaccine. © 2002 Published by Elsevier Science Ltd.

The presence of differentially-expressed glycoforms on surfaces of malignant cells offers the exciting promise of active immunotherapeutic intervention against human cancers.[1] These carbohydrate epitopes can be potentially incorporated into suitably designed vaccines, thereby recruiting the vast assets of the immune system in combatting cancer. As it has been difficult to isolate purified samples of tumor antigenic glycoconjugates in required amounts, total chemical synthesis has been an important source of material for evaluation of their biological efficacy. We have been involved in various synthetic and subsequent preclinical and clinical studies with a number of tumor-associated carbohydrate antigens.[2] The promising nature of the results thus far has

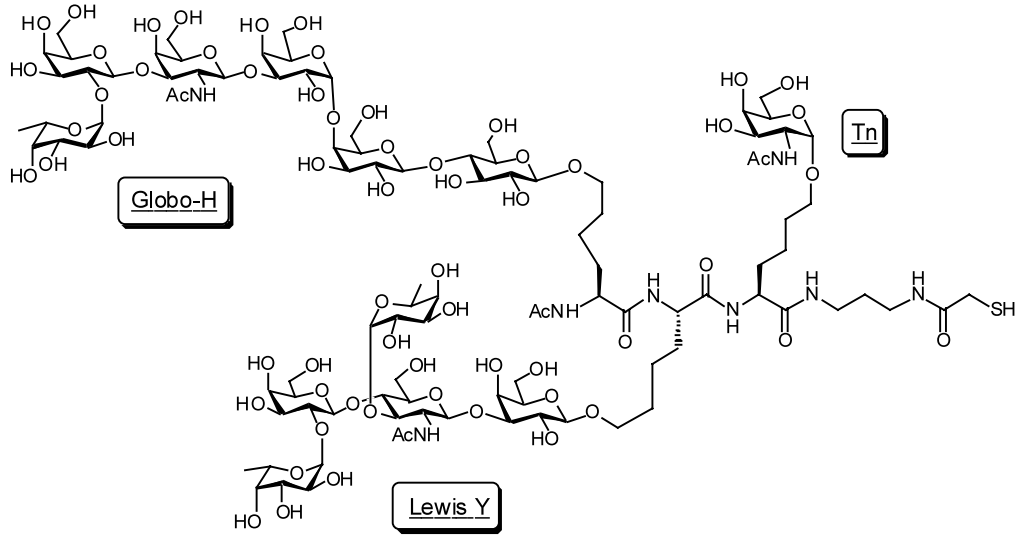

Figure 1. Unimolecular trivalent cancer pro-vaccine, comprising the Globo-H, Lewis Y and Tn antigens, linked by peptide coupling of the corresponding non-natural glycosyl amino acids.

Keywords: glycosyl amino acids; cross-metathesis; allyl glycosides; carbohydrate-based antitumor vaccines.

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0040-4039/02/$ - see front matter © 2002 Published by Elsevier Science Ltd.
PII: S0040-4039(02)01336-9

spurred the search for optimal modes of vaccine design, especially with respect to antigen presentation and conjugation.

From our investigations, we have come to favor incorporation of the tumor antigens into polypeptide constructs, including clustered domains that simulate mucins, commonly expressed at the cell surface in transformed tissue.[3] We are also evaluating the concept of polyvalency. In principle, polyvalency might provoke an immunological response to a higher percentage of tumor cells *with a single chemical entity* bearing multiple antigens (Fig. 1).[4] These glycosylated peptides are subsequently attached to immunogenic carrier proteins (e.g. KLH), before vaccination in an adjuvant setting. For the construction of these goal structures, the synthetic oligosaccharides were converted into *O*-linked glycosyl amino acids, suitable for peptidic coupling.

Glycosyl amino acids are important components of many biologically active compounds, and have been prepared by several techniques.[5] In our program, this has been accomplished either through direct glycosylation of carbohydrate donors with Ser/Thr derivatives, mimicking the presentation on natural mucins,[3,4b] or through anomeric pentenyl groups via an ozonolysis-Wittig-asymmetric hydrogenation protocol.[4a] This latter method ultimately delivers non-natural glycosyl amino acids, with a four-carbon linker between the anomeric oxygen and the α-carbon of the amino acid (see Fig. 1). Preclinical evaluation of such a multivalent vaccine construct has shown promising levels of immunogenic response, with responses observed to each individual carbohydrate antigen.[6]

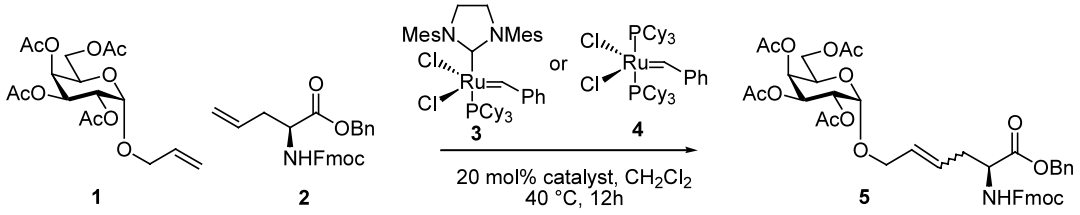
As part of our earlier vaccine design, we took advantage of an anomeric allylic function, readily available from corresponding carbohydrate donors, as a convenient handle to conjugate complex fully synthetic glycans to immunogenic carrier proteins.[2] We have been seeking to gain access to other useful functional groups from the allyl moiety, especially amino acids, suitable for incorporation of these allylic glycosides into our current new-generation vaccines. Analysis of potential modalities for advancing the terminal olefin for incorporation into glycopeptide constructs led us to consider application of the powerful technique of olefin cross-metathesis. The ability to derivatize double bonds via the cross-metathesis reaction has been the subject of a number of elegant studies.[7] We decided to examine the reaction of allylic glycosides of fully synthetic oligosaccharides with the commercially available amino acid, L-allylglycine.[8] In this letter, we report on the success of this approach.[9] Following reduction of the side-chain olefinic linkages by catalytic hydrogenation, pure *N*-protected glycosyl amino acids are obtained in good yield, ready for incorporation into model polypeptide vaccines.

Initial studies were carried out with peracetyl α-*O*-allyl-D-galactose (**1**) and Fmoc-L-allylglycine benzyl ester (**2**) (Table 1). With a twofold excess of the amino acid, we obtained a 26% yield of the desired product (**5**) with the catalyst **3**,[10] along with dimers of both the substrates. Switching to catalyst **4**[11] led to an increased yield of product (49%, entry 2), which was further optimized to 70% by increasing the amount of allyl amino acid (entry 3).[12] This led to a marked decrease in the unproductive dimerization of the allyl glycoside, while increasing the metathesis reaction between the two olefins. We selected these conditions for further study.

Using these reaction conditions with the catalyst **4** in the presence of an excess of allylglycine, the cross-metathesis with different simple and complex *O*-allyl glycosides was accomplished (Table 2). The yields were uniformly in the 60–70% range, with the excess allylglycine **2** being easily separable by silica gel chromatography. The substrates included the hexasaccharide breast and prostate cancer antigen Globo-H (**6c**, entry 3), the synthesis of which had been initially reported by us in 1995[13a,13b] and is currently in Phase II clinical trials against breast cancer.[13c]

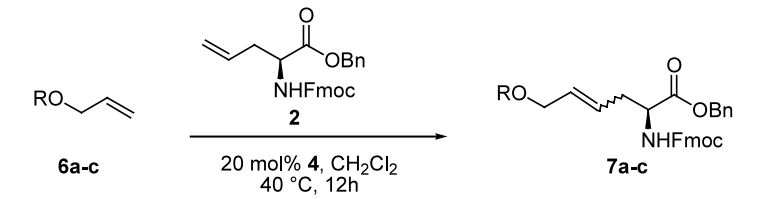
The product olefins, obtained as mixtures of *E*:*Z* isomers, were reduced by catalytic hydrogenation, with concomitant deblocking of the benzyl esters, revealing the *N*-protected free acids in excellent yields ready for peptide coupling (e.g. Globo-H in Scheme 1). It is interesting to note that the metathesis-reduction protocol with **6c** affords the same Globo-H amino acid used in the synthesis of the multivalent vaccine described in

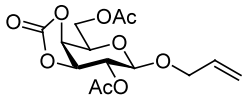
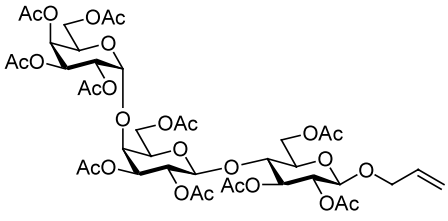
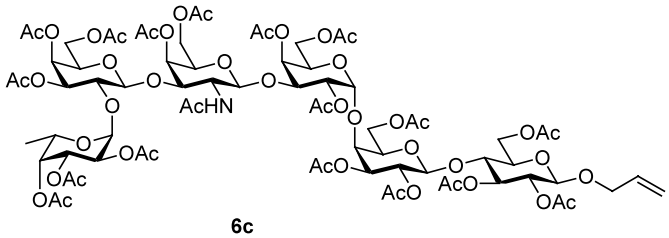
Table 1. Optimization of cross-metathesis reaction conditions with different catalysts and equivalents of L-allylglycine

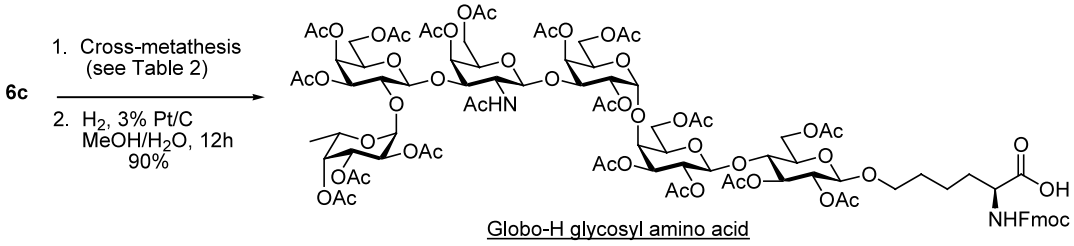


Entry	Equiv. of **2**	Catalyst	Yield (%)
1	2.0	**3**	26
2	2.0	**4**	49
3	5.0	**4**	70

Table 2. Cross-metathesis reaction of simple and complex allyl glycosides



Entry	Allyl Glycoside	Yield (%)
1.	 **6a**	66
2.	 **6b**	70
3.	 **6c**	69



Scheme 1. Globo-H glycosyl amino acid, obtained by cross-metathesis–reduction protocol.

Fig. 1 by our previous methodology, but avoids any late-stage manipulations required for generating the stereogenic α-amino center.

In conclusion, we describe a mild and efficient route to complex glycosyl amino acids from allyl glycosides by a cross-metathesis procedure. Catalytic hydrogenation afforded *N*-protected amino acids, ready for incorporation into polyvalent and clustered manifestations of carbohydrate-based cancer vaccines. This method offers advantages based on commercial starting materials and the lack of technically demanding reactions. It has been extended to encompass the breast tumor antigen for incorporation into glyco-polypeptides which mimic the cancer cell surface. Advanced applications of this method to the treatment of human cancers by immunotherapy is the subject of current investigations in the laboratory.

Acknowledgements

This work was supported by the National Institutes of Health (grant number: CA-28824). Postdoctoral fellowship support is acknowledged by D.M.C. (Natural Science and Engineering Research Council of Canada: PDF-230654-2000 and Alberta Heritage Foundation for Medical Research: 199901330).

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12. Experimental procedure: Peracetyl α-*O*-allyl-D-galactose (**1**, 185 mg, 0.47 mmol) was mixed with Fmoc-L-allylglycine-OBn (**2**, 1.01 g, 2.38 mmol, 5.0 equiv.) in CH_2Cl_2 (3 mL) and treated with catalyst **4** (39 mg, 0.047 mmol, 0.1 equiv.). The reaction was heated to reflux. After 6 h, another 0.1 equiv. of catalyst **4** was further added. The reaction was cooled to rt after 12 h (total) and purified by silica gel chromatography (40% EtOAc/hexanes) to afford metathesis product **5** (250 mg, 70%), along with 392 mg (39% of 5.0 equiv.) recovered **2** and 20 mg (11%) recovered **1**.
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