Published in final edited form as:

Tetrahedron Lett. 2009 February 11; 50(6): 712–714. doi:10.1016/j.tetlet.2008.11.113.

Synthesis of Biotinylated Tumor-Associated Carbohydrate Antigens for Immunological Studies

Jianglong Zhu^a, Qian Wan^a, and Samuel J. Danishefsky^{a,b}

^a Laboratory for Bioorganic Chemistry, Sloan-Kettering Institute for Cancer Research, 1275 York Avenue, New York, NY 10065, USA

^b Department of Chemistry, Columbia University, 3000 Broadway, New York, NY 10027, USA

Abstract

Syntheses of several biotinylated human cancer-associated carbohydrate antigens for immunological studies are described.

The development of an effective paradigm for cancer immunotherapy is a worthy goal of synthetic chemists and immunologists. Over the past two decades, our laboratory¹ and others² have sought to develop broadly effective carbohydrate-based antitumor vaccines, which would stimulate the immune system to identify and eradicate micrometastatic cancer cells. This approach takes advantage of the fact that tumor cells display aberrant levels and types of cell surface carbohydrates, a feature which, if properly exploited, could make tumor cells distinguishable to the immune system. Recently, our efforts in this field culminated in the synthesis of a first-generation, unimolecular pentavalent construct, in which five prostate and breast cancer-associated carbohydrate antigens – Globo-H, Lewis^x, STn, TF, and Tn – were incorporated on a single peptide backbone.³ Subsequent covalent conjugation to keyhole limpet hemocyanin (KLH) or the immunogenic lipopeptide PamCys furnished fully synthetic carbohydrate-based vaccines. Preliminary immunological studies in mice revealed the KLH conjugate to be very effective in inducing immune responses. Fluorescent Activated Cell Sorter (FACS) assay analysis indicated that the antibodies induced by the KLH-conjugate react significantly with three cell lines expressing high levels of two or more of the corresponding antigens. The cumulative data suggest that the immunological properties of the individual antigens are preserved in these highly elaborate constructs. Encouraged by these findings, we hope to evaluate the central concept of the unimolecular multiantigenic vaccine in the setting of clinical trials.

Due to the observation that only minimal antibody response was induced against the Lewis^x ceramide by the first-generation construct, we decided to pursue the synthesis of a modified second-generation unimolecular pentavalent construct (**1**) and its corresponding KLH conjugate (**2**) (Figure 1).^{3b} In this construct, the Lewis^x antigen is replaced with the tetrasaccharide antigen, GM2. This particular antigen was selected for inclusion because it is overexpressed on the cell surfaces of a number of human carcinomas, including prostate and breast cancer.⁴ In addition, GM2-induced antibodies have previously been shown to be active against human GM2-positive cells, and human clinical trials conducted with GM2 alone have demonstrated a correlation between enhanced GM2 antibody levels and survival.

In previous studies of carbohydrate-based anticancer vaccines, we had prepared rather complex oligosaccharide-based sphingosinelipid (or ceramide) constructs for the purposes of antibody binding studies.⁵ However, the total synthesis of these carbohydrate-based ceramides proved to be quite challenging. With a range of synthetic carbohydrate-based glycosylamino acid building blocks in hand, we hope to develop an efficient protocol for the preparation of

biotinylated constructs, to be used in antibody purification and serological studies. As described herein, we sought to prepare biotinylated constructs, specifically tailored to assist in the biological and clinical evaluation of construct **1**. Since the discovery of avidin, streptavidin, and biotin, biotin-linked reagents have been extensively employed as important tools in the study of biochemical transformations, protein interactions, and protein and antibody purifications.⁶ Our synthetic strategy for the installation of a biotin functionality onto the protected carbohydrate, via a polyethylene glycol linker, is described in Figure 2. As shown, appendage of a Boc-protected diaminopropyl unit (**4**) onto the glycosylamino acid (**3**), with subsequent removal of the Boc protecting group, would provide the free amine **5**. Reaction of this primary amine with commercially available water-soluble NHS-PEO₄-biotin (**6**), followed by global deprotection, would afford the biotin-linked protected carbohydrate antigen, **7**.

As depicted in Scheme 1, Tn glycosylamino acid **8**⁷ was coupled with the Boc-protected diaminopropyl unit **4**, through the EDCI/HOBt protocol. Subsequent Fmoc (fluorenylmethyl carbamate) cleavage^{3b} and acetamide formation provided **9** in 82% yield over three steps. Following removal of the Boc functionality, the resultant primary amine was reacted under slightly basic conditions with the active ester functionality of NHS-PEO₄-Biotin **6**, to afford the corresponding biotin-linked peracetylated Tn antigen. Finally, global hydrolysis furnished the biotinylated Tn antigen **10** (92% yield over three steps).

Following the six-step procedure described in Scheme 1, we prepared a variety of biotinylated carbohydrate antigens from their corresponding glycosylamino acids. As shown in Table 1, a variety of monovalent biotinylated antigen constructs – incorporating the TF,⁷ STn,⁷ GM2,⁸ and Globo-H3b⁹ carbohydrates – were accessed in very good overall yield (68–73% over six steps, entries 1–4). Moreover, we have also prepared the biotinylated unimolecular pentavalent antigen **15** from the corresponding pentavalent construct, bearing an N-Boc-protected diaminopropyl moiety (AcHN–GloboH–GM2–STn–TF–Tn–CONH–(CH₂)₃–NHBoc) in reasonable isolated yield over three steps (entry 5).

In summary, we have described herein the preparation of a number of complex biotinylated human cancer-associated carbohydrate antigens. These synthetic biotinylated carbohydrate constructs are being employed in the preclinical evaluation of pentavalent construct **1**. We ultimately hope to commence Phase I clinical trials of **1** at Memorial Sloan-Kettering Cancer Center in the near future.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the NIH (CA28824 to S.J.D.). We thank Dr. George Sukenick, Ms. Hui Fang, and Ms. Sylvi Rusli of the Sloan-Kettering Institute's NMR core facility for mass spectral and NMR spectroscopic analysis, and Ms. Rebecca Wilson for valuable help in editing the manuscript.

References

1. Ouerfelli O, Warren JD, Wilson RM, Danishefsky SJ. *Exp Rev Vaccine* 2005;4:677.
2. (a) Liakatos A, Kunz H. *Curr Opin Mol Ther* 2007;9:35. [PubMed: 17330400] (b) Ingale S, Wolfert MA, Gaekwad J, Buskas T, Boons GJ. *Nat Chem Biol* 2007;3:663. [PubMed: 17767155]
3. (a) Keding SJ, Danishefsky SJ. *Proc Natl Acad Sci USA* 2004;101:11937. [PubMed: 15280546] (b) Ragupathi G, Koide F, Livingston PO, Cho YS, Atsushi E, Wan Q, Spassova MK, Keding SJ, Allen J, Ouerfelli O, Wilson RM, Danishefsky SJ. *J Am Chem Soc* 2006;128:2715. [PubMed: 16492059]

4. (a) Livingston PO, Natoli EJ, Calves MJ, Stockert E, Oettgen HF, Old LJ. *Proc Natl Acad Sci USA* 1987;84:2911. [PubMed: 3472242] (b) Livingston PO, Wong GY, Adluri S, Tao Y, Padavan M, Parente R, Hanlon C, Calves MJ, Helling F, Ritter G. *J Clin Oncol* 1994;12:1036. [PubMed: 8164027]
5. (a) Park TK, Kim IJ, Hu S, Bilodeau MT, Randolph JT, Kwon O, Danishefsky SJ. *J Am Chem Soc* 1996;118:11488. (b) Bilodeau MT, Park TK, Hu S, Randolph JT, Danishefsky SJ, Livingston PO, Zhang S. *J Am Chem Soc* 1995;117:7840.
6. (a) Savage MD. *BioMethods* 1996;7:1. (b) Liu XY, Kamo N, Miyake J. *Recent Res Dev Biophys Chem* 2001;2:71. (c) Lue RYP, Chen GYJ, Zhu Q, Lesaichere ML, Yao SQ. *Met Mol Biol* 2004;264:85. (d) Laitinen OH, Airenne KJ, Raety JK, Wirth T, Ylae-Herttuala S. *Lett Drug Des Discovery* 2005;2:124.
7. Keding SJ, Endo A, Danishefsky SJ. *Tetrahedron* 2003;59:7023.
8. Cho YS, Wan Q, Danishefsky SJ. *Bioorg Med Chem Lett* 2005;13:5259.
9. Zhu J, Wan Q, Yang G, Ouerfelli O, Danishefsky SJ. *Heterocycles*. 2008 submitted for publication.

Figure 1.

A second-generation unimolecular pentavalent vaccine construct containing Globo-H, GM2, STn, TF and Tn

Figure 2.
General strategy toward biotin-linked carbohydrate antigens.

Scheme 1.

Synthesis of biotinylated Tn antigen **10**. (a) EDCI, HOBT, DMF/ CH_2Cl_2 , **4**; (b) Et_2NH , DMF; (c) Ac_2O , 82% for three steps; (d) $\text{CF}_3\text{CO}_2\text{H}$, CH_2Cl_2 ; (e) DMF, satd NaHCO_3 , **6**; (f) NaOH , MeOH , 92% for three steps.

Syntheses of biotinylated TF, STn, GM2, Globo-H and unimolecular pentavalent antigens

entry	biotinylated antigen	yield ^a
1	<p>11</p>	73%
2	<p>12</p>	73%
3	<p>13</p>	70%
4	<p>14</p>	68%

15 was prepared from the corresponding pentavalent construct bearing N-Boc-protected diaminopropyl moiety (AcHN-GloboH-GM2-STn-TF-Tn-CONH-(CH₂)₃-NHBoc) in 26% isolated yield for three steps