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Synthesis of biotinylated tumor-associated carbohydrate antigens for immunological studies

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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 laboratory[1] and others[2] 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^y, 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^y 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**) (Fig. 1).[3b] In this construct, the Lewis^y 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 cancers.[4] In addition, GM2-induced antibodies had previously been shown to be active against human GM2-positive cells, and human clinical trials conducted

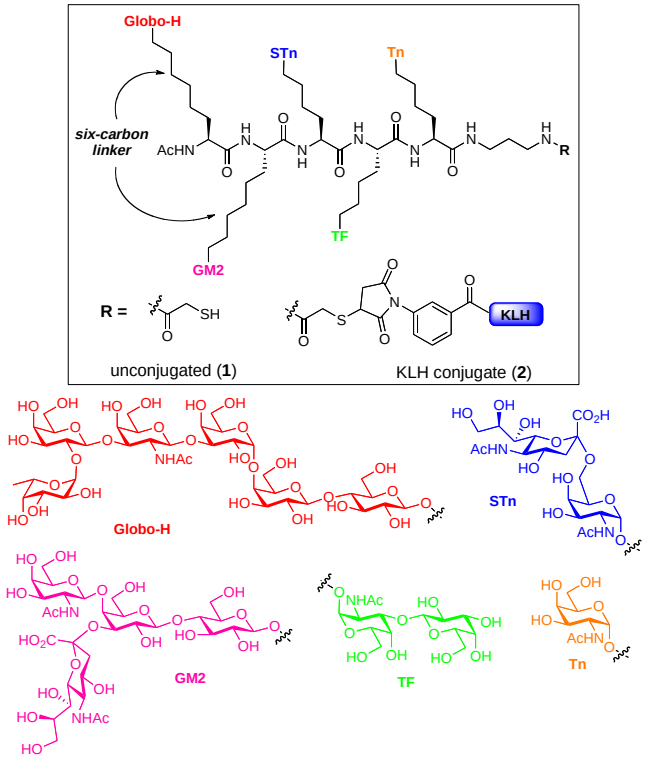

Figure 1. A second-generation unimolecular pentavalent vaccine construct containing Globo-H, GM2, STn, TF, and Tn.

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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 sphigosinelipid (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–PEO_4–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 reacted under slightly basic conditions with the active ester functionality of NHS–PEO_4–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-H[3b,9] 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–$(CH_2)_3$–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.

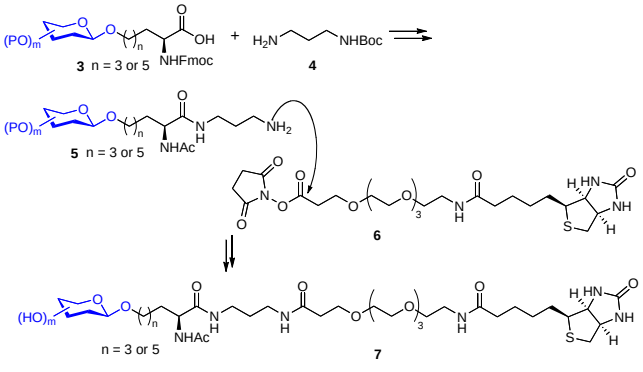
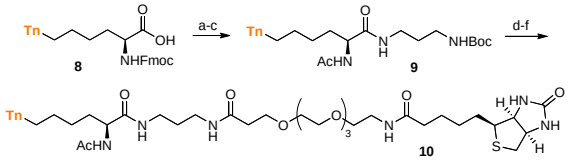
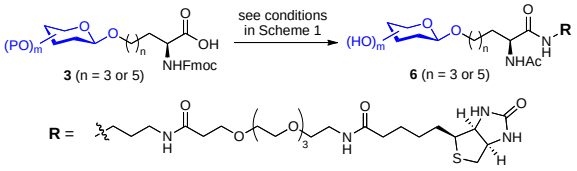


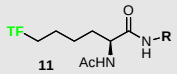
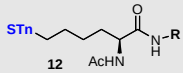
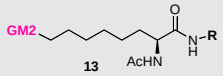
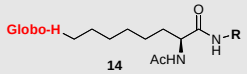
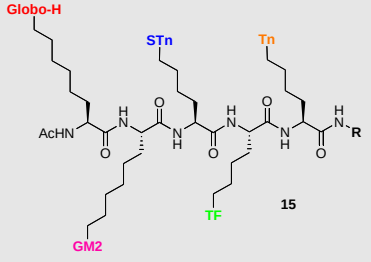
Figure 2. General strategy toward biotin-linked carbohydrate antigens.



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) CF_3CO_2H, CH_2Cl_2; (e) DMF, satd $NaHCO_3$, **6**; (f) NaOH, MeOH, 92% for three steps.

Table 1
Syntheses of biotinylated TF, STn, GM2, Globo-H and unimolecular pentavalent antigens



Entry	Biotinylated antigen	Yield[a] (%)
1		73
2		73
3		70
4		68
5		26[b]

[a] Isolated yield over six steps.
[b] Compound **15** was prepared from the corresponding pentavalent construct bearing *N*-Boc-protected diaminopropyl moiety (AcHN–GloboH–GM2–STn–TF–Tn–CONH–$(CH_2)_3$–NHBoc) in 26% isolated yield for three steps.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2008.11.113.

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