Synthetic Vaccines: Synthesis of a Dimeric Tn Antigen–Lipopeptide Conjugate That Elicits Immune Responses against Tn-Expressing Glycoproteins

Tatsushi Toyokuni,*[1a,b] Barbara Dean,[1a] Shaopei Cai,[1a] Diane Boivin,[1a] Sen-itiroh Hakomori,[1a,c] and Anil K. Singhal*[1a,c]

The Biomembrane Institute
201 Elliott Avenue West, Seattle, Washington 98119
Departments of Chemistry and Pathobiology
University of Washington, Seattle, Washington 98195

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Incomplete glycosylation has frequently been described in various experimental and human cancer cells. This results in the accumulation of the core-region structures including Tn (GalNAcα1→*O*-Ser/Thr),[2,3] sialosyl Tn (NeuAcα2→6GalNAcα1→*O*-Ser/Thr),[3-5] and T (Galβ1→3GalNAcα1→*O*-Ser/Thr)[2,3] antigens. These antigens in normal cells are cryptic since they are further elongated to construct complex oligosaccharide chains, whereas those in most human carcinomas are exposed at the surface due to a block in carbohydrate chain elongation. Thus, the expression of these antigens is highly specific to cancer cells and is essentially absent in normal cells.[6]

We have been involved in development of synthetic vaccines based on tumor-associated carbohydrate antigens for the active specific immunotherapy of cancer.[7,8] We have recently shown that immunization of mice with either desialylated ovine submaxillary mucin (A-OSM),[9a] which predominantly expresses the Tn antigen (>96% GalNAc by sugar analysis),[9] or synthetic dimeric Tn antigen (di-Tn) coupled to protein carriers (keyhole limpet hemocyanin (KLH) or ovine serum albumin)[10] effectively provides protection in mice against a challenge by highly metastatic TA3-Ha murine mammary adenocarcinoma,[11] which show strong expression of Tn antigen.[12] Ideally, synthetic vaccines should elicit a strong immune response without the aid of macromolecular carriers or adjuvants, which then would eliminate irrelevant determinants and ambiguity in composition and structure.[13] In this communication, we report that synthetic di-Tn coupled to tripalmitoyl-*S*-glycerylcysteinylserine (P_3CS)[14,15] (Figure 1) is a completely synthetic, low-molecular-weight, carrier-free immunogen that elicits immune responses against Tn-expressing glycoproteins. To our knowledge, this is the first example that a synthetic, small carbohydrate antigen can generate an immune response against a tumor-associated carbohydrate antigen without the use of a macromolecular carrier or an adjuvant.

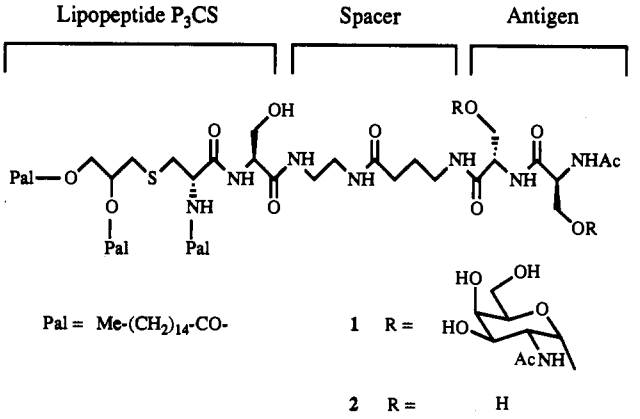

Figure 1. Structure of dimeric Tn antigen–lipopeptide conjugate.

The assembly of the di-Tn[16] **8**, suitable for coupling to the functionalized P_3CS **14**, is shown in Scheme 1. As a spacer, 4-aminobutyric acid was introduced to the properly protected Tn antigen **3**,[16d] by the *N*-hydroxysuccinimide (NHS) ester method (**3** → **4** → **5**). The amino group of **5** was unmasked by acidolysis to give the amine, whose condensation with the NHS ester **4** yielded the dimer **6**. Sequential acidolysis, capping with Ac_2O

* Address correspondence to these authors at The Biomembrane Institute.

(1) (a) The Biomembrane Institute. (b) Department of Chemistry, University of Washington. (c) Department of Pathobiology, University of Washington.

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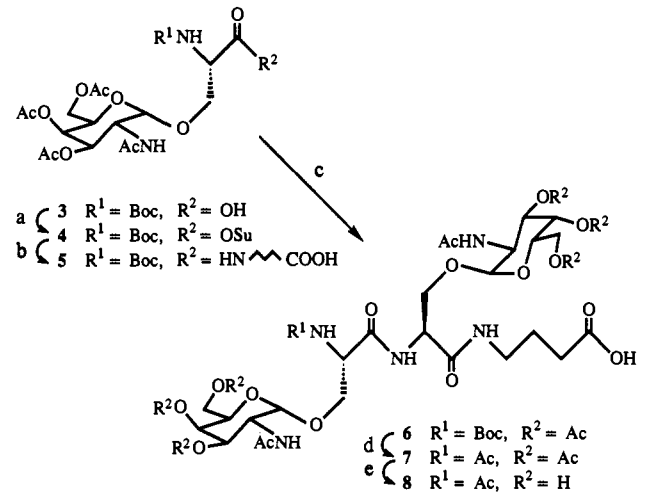
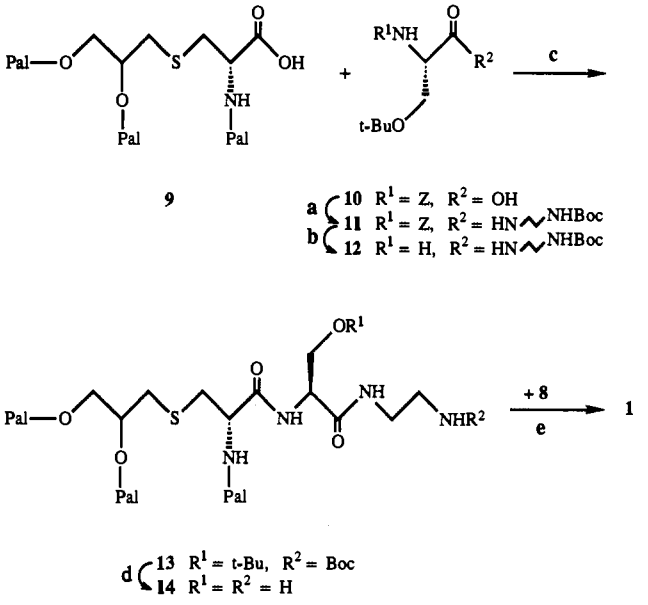
Scheme 1. Synthesis of Dimeric Tn Antigen **8**[a]



[a] Reagents and conditions: (a) NHS, EtN=C=N(CH$_2$)$_3$NMe$_2$, CH$_2$Cl$_2$, 40 min; (b) H$_2$N(CH$_2$)$_3$COOH, EtN(*i*-Pr)$_2$, DMF–H$_2$O, 30 min, 89% from **3**; (c) (1) TFA, 10 min, (2) **4**, EtN(*i*-Pr)$_2$, DMF, 2 h, 70% from **5**; (d) (1) TFA, 10 min, (2) Ac$_2$O, C$_6$H$_5$N, 1 h, 92%; (e) 1 M aqueous NaOH, MeOH–H$_2$O, 15 min, 91%.

Scheme 2. Construction of Dimeric Tn Antigen–Lipopeptide Conjugate **1**[a]



[a] Reagents and conditions: (a) (1) HOBt, (*i*-Pr)$_2$N=C=N(*i*-Pr)$_2$, DMF, 30 min, (2) H$_2$N(CH$_2$)$_2$NHBoc, 1 h, 89%; (b) 10% Pd/C, H$_2$, MeOH, quantitative; (c) (1) activation of **9** (HOBt, (*i*-Pr)$_2$N=C=N(*i*-Pr)$_2$, CH$_2$Cl$_2$, 30 min), (2) coupling to **12** (1.5 h, 83%); (d) TFA, 1 h, quantitative; (e) (1) activation of **8** (NHS, EtN=C=N(CH$_2$)$_3$NMe$_2$, DMF, 4 h), (2) coupling to **14** (EtN(*i*-Pr)$_2$, DMF, 1 h, 57%).

(→**7**), and saponification furnished **8** in 52% overall yield from **3**.

Scheme 2 summarizes the construction of the di-Tn–P$_3$CS conjugate **1**. A linker was installed at the carboxyl group of the serine derivative **10** to generate amino functionality, which allows coupling to the carboxyl group of **8**, by attachment of mono-*N*-Boc-ethylenediamine[17] (→**11**). After hydrogenolysis, the resulting amine **12** was joined to the lipoamino acid P$_3$C–OH[18,19] (**9**) by the 1-hydroxybenzotriazole (HOBt) method. Acidolysis of the product **13** to the amine **14**, followed by coupling to **8** by the NHS ester method, completed the conjugation yielding **1**.

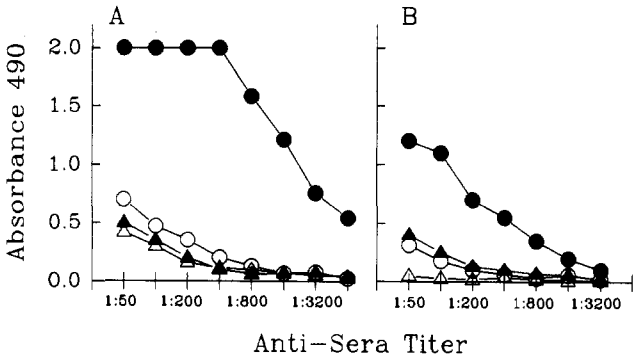


Figure 2. Serum anti-Tn IgM (A) and IgG (B) titers in mice immunized with either **1** (●), **2** (▲), **14** (○), or Intralipid (△). All compounds were dissolved in 1:1 Intralipid[21]–PBS at a concentration of 0.5 mg/mL. Mice were immunized twice (one week apart) with 100 μg of antigen subcutaneously at the base of the tail and at the neck. Seven days after the second immunization, sera were titered against A-OSM which predominantly expresses the Tn antigen[9] in an enzyme-linked immunosorbant assay (EL ISA).

The conjugate **1** was examined for its ability to stimulate Tn antigen specific immune response in mice. Mice immunized with **1** showed high anti-Tn antibody titers (binding against A-OSM). Interestingly, this immunization generated not only high IgM antibody response (Figure 2A) but also measurable IgG anti-Tn response (Figure 2B). This is significant since carbohydrate antigens are thought to stimulate B cells in the absence of any helper T cell enlistment and produce only IgM antibody response. None of the control groups, immunized with **2**,[20] **14**, and Intralipid,[21] showed any significant anti-Tn antibody response. Similar high anti-Tn binding was seen against di-Tn coupled to ovine serum albumin (data will be published elsewhere). It is possible that the lipopeptide is able to enhance the uptake of Tn antigen by the appropriate antigen presenting cells for increased immune response. Studies are underway to determine the effect of this antigen directly on the stimulation of T cells.

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Supplementary Material Available: ^{1}H NMR spectra for compounds **1**, **2**, **4–8**, and **11–14** and experimental procedures (17 pages). This material is contained in many libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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