SYNTHESIS OF TN AND SIALYL TN ANTIGEN–LIPID A ANALOG CONJUGATES FOR SYNTHETIC VACCINES

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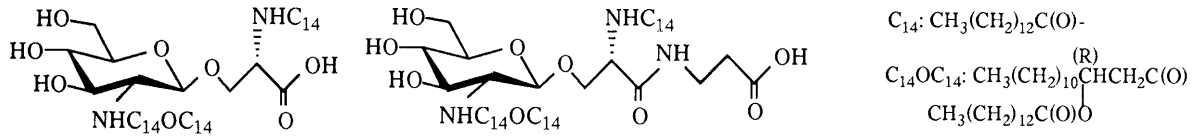
Conjugates of Tn and sialyl Tn antigen with *N*-teradecanoyl L-seryl-β-alanine-containing D-glucosamine derivatives structurally related to lipid A as a immunoadjuvant were synthesized for the development of totally synthetic vaccines against cancers or HIV.

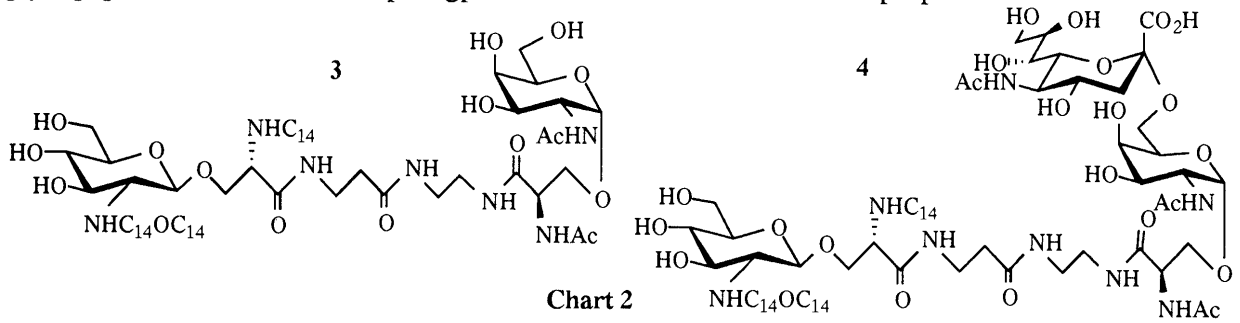
KEY WORDS Tn; sialyl Tn; lipid A analog; synthetic vaccine; immunoadjuvant

Lipid A is well known to be responsible for the expression of many biological activities, such as endotoxicity, adjuvanticity, antitumor activity, etc. by lipopolysaccharide (LPS) of gram-negative bacteria.[1] A large number of compounds related to lipid A partial structures have been synthesized with the aim of enhancing its potentially beneficial immunostimulatory properties.[2] We have already reported the synthesis and biological activities of *N*-acylated L-serine-, L-threonine-, or L-homoserine-containing D-glucosamine derivatives structurally similar to the lipid A disaccharide backbone.[3] As a result, it has been found that the *N*-tetradecanoyl L-serine-linked lipid A analog (**1**) exhibited potent mitogenic activity. In addition, we found that compound **2** introduced β-alanine into the compound **1** possessed the same structure as that of **1** (Chart 1).



1 **2** Chart 1

Recently, development of totally synthetic vaccines against cancers or HIV using synthetic immunoadjuvants, such as *N*-acetyl-muramyl-L-alanyl-D-isoglutamine (MDP) and lipopeptide analog, has been attempted.[4] We planned to develop completely synthetic vaccines which consist of this lipid A analog (**2**) as a synthetic immunoadjuvant, covalently coupled to a low-molecular-weight antigen. We selected Tn [α-D-GalNAc-(1→O)-Ser] and sialyl Tn [α-D-Neu5Ac-(2→6)-α-D-GalNAc-(1→O)-Ser] epitopes for antigens. Tn and sialyl Tn epitopes have been identified as tumour-associated carbohydrate antigens present in glycoproteins on the surface of cancer cells.[5] Further, Tn and sialyl Tn epitopes have been discovered on the envelope glycoprotein gp120 of the human immunodeficiency virus (HIV).[6] Synthetic cancer vaccines based on the Tn epitope, and Tn glycopeptides from the V3-loop of gp120 as HIV vaccines have been prepared.[7]



3 **4** Chart 2

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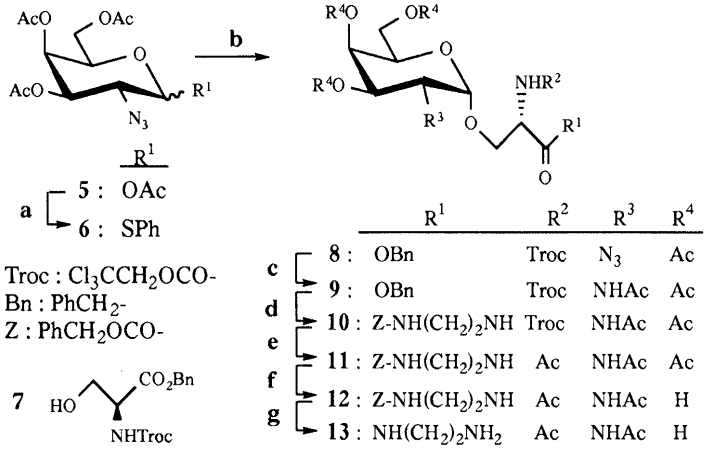
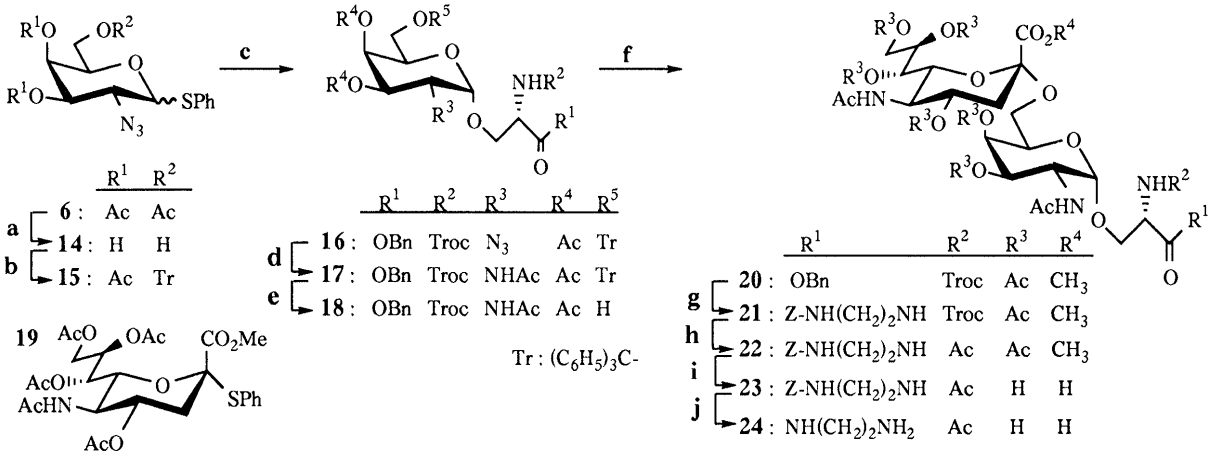
Here, we describe the synthesis of conjugates **3** and **4** of the Tn and sialyl Tn antigens with *N*-tetradecanoyl- L-seryl-β-alanine-containing D-glucosamine derivatives (**2**) structurally similar to lipid A, which were expected to induce antigen-specific immune responses (Chart 2).
Several syntheses of the Tn and sialyl Tn antigens have been reported.[8] We achieved the synthesis of the Tn and sialyl Tn antigen derivatives (**13**, **24**) by introducing ethylene diamine as a spacer by the routes shown in Charts 3 and 4.



a) PhSH, BF_3•Et_2O, CH_2Cl_2, rt, 4 h, 91%.
b) **7**, NBS, I_2, TBAOTf, Et_2O, rt, 2 h, 65%.
c) AcSH, pyridine, CH_2Cl_2, rt, 4 h, 68%.
d) 1) Pd-black, H_2, THF, rt, 10 h;
2) Z-NH$(CH_2)_2NH_2$•HCl, WSC•HCl, HOBt, Et_3N, DMF, rt, 20 h, 80%.
e) 1) Zn, AcOH, 40°C, 18 h;
2) Ac_2O, DMAP, pyridine, CH_2Cl_2, rt, 10 h, 65%.
f) 0.1N KOH, MeOH, 0°C, 2 h, 92%.
g) $Pd(OH)_2$, H_2, MeOH, rt, 12 h, quant.

Chart 3

First, the synthesis of the Tn antigen derivative (**13**) was carried out as follows. Treatment of **5**[3,c] with thiophenol (PhSH) in the presence of boron trifluoride etherate (BF_3OEt_2) gave thiophenylglycoside (**6**). Coupling of **6** and **7** with *N*-bromosuccinimide (NBS), iodine, and tetrabutylammonium trifluoromethanesulfonate (TBAOTf) as the promoter gave the α-glycoside (**8**).[9] The α–configuration of **8** was determined from the coupling constant value (3.6 Hz) of the signal due to the anomeric proton in the proton magnetic resonance (^{1}H-NMR) spectrum of **8**. Reduction of the azido group of **8** gave **9**. After removal of the benzyl group of **9**, a *N*-benzyloxycarbonyl-aminoethylamino (Z-EDA) group was introduced into the carboxyl group by the water-soluble carbodiimide (WSC)-1-hydroxy-1H-benzotriazole (HOBt) coupling method to give **10**. Cleavage of the 2,2,2-trichloroehoxycarbonyl (Troc) group of **10**, followed by acetylation using Ac_2O gave **11**. *O*-Deacylation of **11** with 0.1 N aqueous KOH afforded **12**. Finally, hydrogenolytic removal of the benzyloxycarbonyl group using palladium hydroxide gave the desired product **13**.



a) NaOMe, MeOH, rt, 8 h, 95%; b) 1)TrCl, DMAP, pyridine, DMF, 70°C, 10 h; 2) Ac_2O, rt, 4 h, 81%; c) **7**, NBS, I_2, TBAOTf, Et_2O, rt, 2 h, 70%; d) AcSH, pyridine, CH_2Cl_2, rt, 8 h, 69%; e) 80%AcOH, 60°C, 6 h, 71%;f) **19**, NBS, I_2, TBAOTf, CH_3CN,- 40°C, 4 h, 55%; g) 1) Pd-black, H_2, MeOH-THF, rt, 36 h; 2) Z-NH$(CH_2)_2NH_2$•HCl, WSC•HCl, HOBt, Et_3N, DMF, rt, 18 h, 64%; h) 1) Zn, AcOH, 40°C, 18 h; 2) Ac_2O, DMAP, pyridine, CH_2Cl_2, rt, 16 h, 73%; i) 0.1N KOH, MeOH, rt, 2 h, 99%; j) $Pd(OH)_2$, H_2, MeOH, rt, 2 h, quant.

Chart 4

Next, the sialyl Tn antigen derivative (**24**) was synthesized as follows. Methanolysis of **6** in the presence of NaOMe gave **14**. A triphenylmethyl (Tr) group was selectively introduced into the 6-hydroxy group of **14**, and subsequent acetylation of the remaining hydroxy group gave **15**. As described for **8**, glycosylation of **7** and **15** gave the α-glycoside (**16**). After reduction of the azido group of **16**, removal of the Tr group gave **18**. As described for **8** and **16**, glycosylation of **18** and sialosyl donor **19** gave the α-glycoside (**20**). The procedure as described for the preparation of **13** from **9** provided the desired product **24** from **20**.

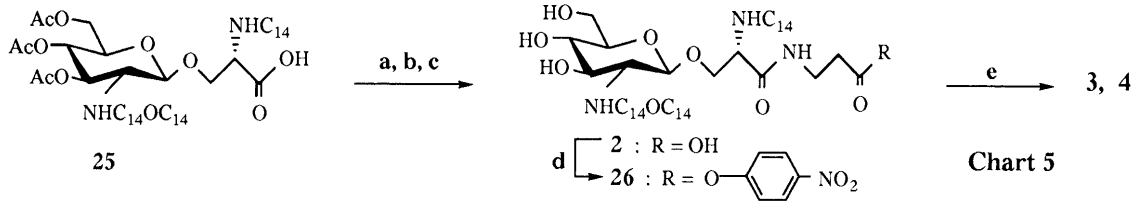


Chart 5

a) H-β-Ala-OBn•TosOH, WSC•HCl, HOBt, DMF, rt, 20 h, 74%; b) Pd-black, H_2, rt, 8 h, 89%; c) conc.NH_4OH, MeOH-THF, rt, 20 h, 79%; d) *p*-nitophenol, WSC•HCl, rt, 8 h, 55%; e) **13** or **24**, NMM, rt, 4 d, 21% (**3**), 43% (**4**).

The Tn antigen derivative (**13**) and sialyl Tn antigen derivative (**24**)-lipid A analog (**2**) conjugates were constructed as shown in Chart 5. β-Alanine benzyl ester was introduced into the carboxyl group of **25**,[3a] and subsequent catalytic hydrogenolysis using palladium-black gave lipid A analog (**2**). Finally, compound **2** was coupled to **13** and **24** by the *p*-nitrophenyl ester method to give conjugates **3**[10] and **4**,[11] respectively, after purification by chromatography on a silica gel column and Sephadex LH-20, followed by lyophilization from H_2O.

Studies on the biological activites of conjugates **3** and **4** are in progress.

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10) $[\alpha]_D^{25}$+3.3° (c=0.28, $CHCl_3$:MeOH=2:3); FAB-MS (m/z): 1381[(M+Na)$^+$ for $C_{69}H_{127}N_7O_{19}$].
11) $[\alpha]_D^{25}$ -7.2° (c=0.20, $CHCl_3$:MeOH=2:3); FAB-MS (m/z):1694[(M+Na)$^+$ for $C_{80}H_{144}NaN_8O_{27}$].

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