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SYNTHETIC VACCINES: I. SYNTHESIS OF MULTIVALENT Tn ANTIGEN CLUSTER-LYSYLLYSINE CONJUGATES[1]

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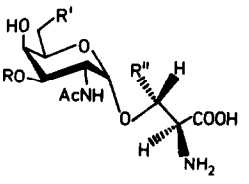
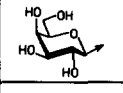
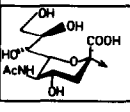
Abstract: Monomer, dimer, and trimer of Tn antigen (GalNAcα1→O-Ser) were coupled to L-lysyllysine to construct multivalent and clustering structures of Tn antigen. The method has made it possible to provide pertinent carbohydrate antigens for the development of synthetic vaccines against tumors.

T (Galβ1→3GalNAcα1→O-Ser/Thr)[2] (1), Tn (GalNAcα1→O-Ser/Thr)[3] (2), and sialosyl-Tn (NeuAcα2→6GalNAc-α1→O→Ser/Thr)[4] (3) structures have been identified as human tumor-associated carbohydrate antigens.[5] These antigens in normal tissue constitute the core structures of mucin-type glycoproteins, and are therefore present in a cryptic form, whereas those in most human carcinomas in which biosynthesis of carbohydrate chains is blocked are exposed at the surface. Thus, the expression of these antigens, particularly Tn and sialosyl-Tn, is highly specific to a variety of human cancers, and is essentially absent in normal tissues.[5] Chemical synthesis of Tn and sialosyl-Tn is, therefore, of great importance in development of anti-tumor vaccine for suppression of tumor growth.[5c] Syntheses of each carbohydrate antigen and of glycopeptides containing clusters of T and Tn have been accomplished by several groups[6]; in these cases new glycosidation methods, including in situ anomerization[6a] and trichloroacetimidate[6b] methods, and direct glycosidation in the presence of trifluoromethanesulfonic anhydride[6c], were successfully applied to prepare α-glycosyl aminoacids.

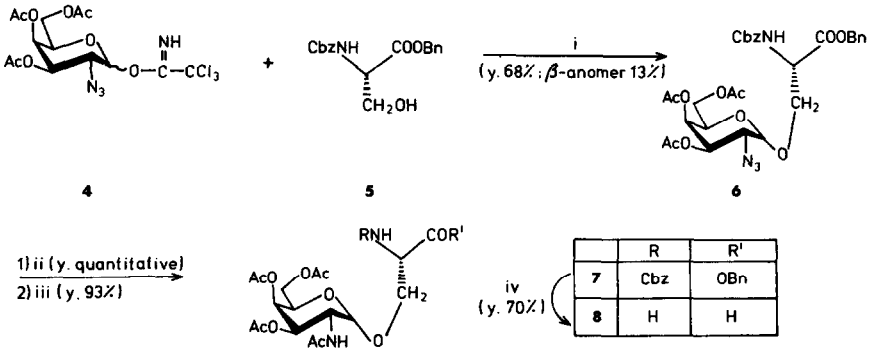
Since multivalent clustered Tn/sialosyl-Tn is of practical importance to induce efficient immune response, we report here the synthesis of Tn antigen-lysyllysine conjugates (**25**, **26**, and **27**) possessing multivalent and clustering structures. These structural arrangements seem to be important for recognition of carbohydrates by cells or binding proteins.[7] The synthetic antigens will be used as immunogens, after being covalently linked to synthetic or natural carrier molecules, to elicit host immune response against tumors.



Antigens	R	R'	R''
T		H	H / CH_3
Tn	H	H	H / CH_3
Sialyl-Tn	H		H / CH_3

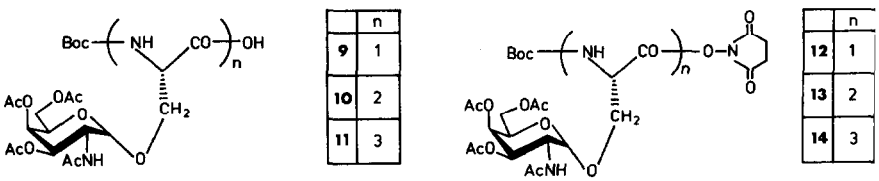
Trichloroacetimidate method[8] was employed to prepare α-glycosyl aminoacid. Glycosidation between glycosyl imidate **4**[8] (α/β= 1/1), readily available from D-galactose through azidonitration, and L-serine derivative **5**[9] in the presence of trimethylsilyl triflate (TMS-OTf) afforded exclusively the α-glycosyl aminoacid **6** (α/β= 5/1)

Scheme I



Reagents: i) TMS-OT_f, CH_2Cl_2, -15°; ii) H_2S, Pyr-H_2O (3/1); iii) Ac_2O-Pyr; iv) H_2 (1 atm), 10% Pd/C, MeOH.

Scheme II



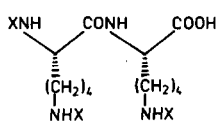
monomeric **8** $\xrightarrow{i\ (y.\ 98\%)}$ **9** $\xrightarrow{ii\ (y.\ 85\%)}$ **12**

dimeric **12** + **8** $\xrightarrow{iii\ (y.\ 85\%)}$ **10** $\xrightarrow{ii\ (y.\ 90\%)}$ **13**

trimeric **13** + **8** $\xrightarrow{iii\ (y.\ 75\%)}$ **11** $\xrightarrow{ii\ (y.\ 60\%)}$ **14**

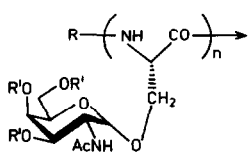
Reagents: i) Boc_2O, Et_3N, MeOH; ii) NHS, EDC, DMF, iii) Et_3N, DMF.

Scheme III



X: H L-Lysyllysine **15**

X:



	n	R	R'
16 **17** **18**	1 2 3	Boc	Ac
19 **20** **21**	1 2 3	H	Ac
22 **23** **24**	1 2 3	Ac	Ac
25 **26** **27**	1 2 3	H	H

12 + **15** $\xrightarrow{i\ (y.\ 80\%)}$ **16** $\xrightarrow{ii\ (y.\ 70\%)}$ **19** $\xrightarrow{iii\ (y.\ 90\%)}$ **22** $\xrightarrow{iv\ (y.\ 92\%)}$ **25**

13 + **15** $\xrightarrow{i\ (y.\ 63\%)}$ **17** $\xrightarrow{ii\ (y.\ 65\%)}$ **20** $\xrightarrow{iii\ (y.\ 84\%)}$ **23** $\xrightarrow{iv\ (y.\ 80\%)}$ **26**

14 + **15** $\xrightarrow{i\ (y.\ 55\%)}$ **18** $\xrightarrow{ii\ (y.\ 60\%)}$ **21** $\xrightarrow{iii\ (y.\ 85\%)}$ **24** $\xrightarrow{iv\ (y.\ 85\%)}$ **27**

Reagents: i) Et_3N, DMF-H_2O; ii) HCOOH, rt, 2h; iii) Ac_2O, Et_3N, MeOH; iv) 10% Et_3N, MeOH-H_2O.

^{1}H-NMR ($CDCl_3$) for **6**: $\delta_{H\text{-}1}$ 4.88 (d, J=3.5 Hz); for the β-anomer: $\delta_{H\text{-}1}$ 4.28 (d, J=8.0 Hz), $[\alpha]_D$ for **6**: +92.6° (c 2.7, $CHCl_3$), lit.[6a] +73.5° (c 1.55, $CDCl_3$); for the β-anomer: -2.67° (c 0.5, $CHCl_3$)), regardless of the anomeric configuration of glycosyl donor **4** (Scheme I). The α- and β-anomers were easily separated by flash column chromatography (silica, hexane-EtOAc 3/2). Selective reduction of the azido group in **6** by H_2S in aqueous pyridine,[10] followed by acetylation, afforded the protected Tn antigen **7**.[6a] Subsequent hydrogenolysis provided, after purification by Bio-Gel® P-2 column chromatography with water as the eluent, the key intermediate **8** ($[\alpha]_D$ +83.2° (c 0.05, methanol), lit.[6a] $[\alpha]_D$ +93° (c 0.5, methanol)).

The clusters of protected Tn antigens were prepared by stepwise addition of **8** at the C-terminal of the activated serine unit as shown in Scheme II. The acid-labile tert-butyloxycarbonyl (Boc) group was chosen for protection of the amino group, and the carboxyl group was activated by conversion into its N-hydroxysuccinimide (NHS) esters. Treatment of **8** with di-tert-butyl dicarbonate (Boc_2O) in the presence of Et_3N afforded **9**[11] ($[\alpha]_D$ +79.1° (c 0.05, methanol), high-resolution fast atom bombardment mass spectrum (HRFABMS) 535.2139 ($C_{22}H_{34}N_2$+H, Δ0.00)), which was then converted to its NHS ester **12** by esterification with NHS in dry DMF containing 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC). The peptide linkage was formed in the presence of EDC in dry DMF containing Et_3N, and hence coupling between **12** and **8** yielded, after purification by Sephadex® LH-20 column chromatography with methanol as the eluent, dimer **10** ($[\alpha]_D$ +113° (c 4.24, $CHCl_3$), HRFABMS 951.3560 ($C_{39}H_{58}N_4O_{23}$+H, Δ+0.001)). After conversion of **10** to the active ester **13**, analogous coupling with **8** gave trimer **11** ($[\alpha]_D$ +122° (c 0.1, $CHCl_3$), HRFABMS 1389.4849 ($C_{56}H_{82}N_6O_{33}$+Na, Δ-0.003)), which was subsequently esterified with NHS to give **14**.

L-Lysyllysine (**15**)[9] is suitable for a backbone which constructs multivalent structures,[12] since its three amino groups (one α- and two ϵ-amino groups) can be utilized to attach three Tn-antigen clusters and one carboxyl group being available for direct coupling to carrier molecules. Addition of **15** in water containing Et_3N to a solution of NHS ester **12** (3.5 molar excess) in DMF, followed by purification by LH-20 column chromatography with methanol as the eluent, afforded high yield of monomeric conjugate **16** ($[\alpha]_D$ +80.0° (c 2.6, $CHCl_3$)). Acidolysis of the Boc group in **16** with HCOOH afforded the corresponding amino derivative **19** without any detectable cleavage at the O-glycosidic linkage.[13] In order to eliminate the cationic nature of an amino group, which gives rise to highly charged antigen-system, the amino group of serine was modified by N-acetylation with Ac_2O in methanol to give **22**. Subsequent basic hydrolysis of **22** with Et_3N in aqueous methanol, after purification by P-2 column chromatography with water as the eluent, gave monomeric Tn antigen-lysyllysine conjugate **25** ($[\alpha]_D$ +74.5° (c 0.5, H_2O), LRFABMS 1293 ($C_{51}H_{86}N_{10}O_{27}$+Na)). During the de-O-acetylation no products from β-elimination was detected.[14]

Using similar methodology, coupling of **13** to **15** afforded dimeric conjugate **17** ($[\alpha]_D$ +121.3° (c 0.1, $CDCl_3$)) and coupling of **14** to **15** yielded trimeric conjugate **18** ($[\alpha]_D$ +119° (c 0.1, $CDCl_3$)). Sequential acidolysis, N-acetylation, and basic hydrolysis converted **17** to dimeric Tn antigen-lysyllysine conjugate **26** ($[\alpha]_D$ +98.6° (c 0.1, H_2O), LRFABMS 2141 ($C_{84}H_{140}N_6O_{48}$+H)) (through **20** and **23**) and **18** to trimeric Tn antigen-lysyllysine conjugate **27** ($[\alpha]_D$ +101° (c 0.05, H_2O), LRFABMS 3033 ($C_{117}H_{194}N_{22}O_{69}$+Na)) (through **21** and **24**) (Scheme III).

An efficient presentation of properly arranged synthetic carbohydrate antigens (e.g., **25**, **26**, and **27**) to the immune system needs to be designed such that these antigens will be recognized by B and T lymphocytes without coupling to any macromolecular carriers,[15] resulting in elimination of irrelevant determinants and ambiguity of the vaccines. Such studies are currently underway in this laboratory.

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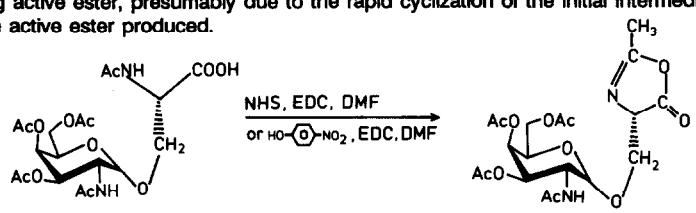
11. When acetamide derivative **A** was treated with NHS or p- nitrophenol in dry DMF containing EDC, oxazoline derivative **B** (LRFABMS 459 ($C_{19}H_{26}N_2O_{11}$ + H)) was isolated in ≈70% yield, instead of the corresponding active ester, presumably due to the rapid cyclization of the initial intermediate formed with EDC or of the active ester produced.



A **B**

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