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Synthesis of Sialyl-Tn Antigen. Regioselective Sialylation of a Galactosamine Threonine Conjugate Unblocked in the Carbohydrate Portion

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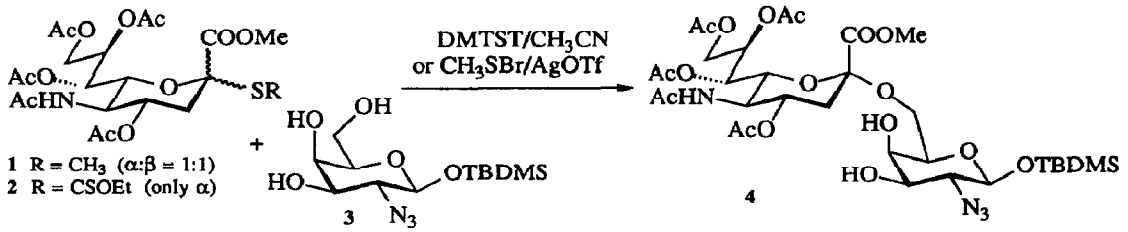
Abstract: Regioselective and stereocontrolled formation of the sialyl-Tn antigen conjugate **6** was achieved by reacting the NeuNAc-2-xanthate **2** with the GalNAc threonine acceptor **5** bearing three hydroxy groups. Complete deprotection afforded the sialyl-Tn antigen structure **11**.

Sialylation of natural glycoproteins plays a crucial part in biological selectivity and recognition[1]. The sialyl-Tn epitope (α-NeuNAc-(2->6)-α-GalNAc-(1->3)-L-Ser/Thr) has been identified on the surface of cancer cells and was described as a potential tumour-marker in cancer diagnosis[2]. Preparative access to sialyl-Tn glycopeptides of exactly specified structure is essential if the functions of these sialylated structures are to be elucidated. As glycopeptides isolated from biological sources often are microheterogeneous, synthetic glycopeptides are of particular interest.

We here report on a straightforward synthesis of a sialyl-Tn-building block during which protective group manipulations are reduced and the expensive *N*-acetylneuraminic acid moiety is introduced at a late step.

Model reactions of the *tert*-butyldimethylsilyl protected 2-azido galactoside **3**[3] with sialyl donors **1** and **2** showed that the activation of the xanthate **2**[4] by methylsulfenyl triflate gives the desired disaccharide **4** in higher yield (61 %) and stereoselectivity (α:β = 6.7:1) than the activation of the corresponding methyl 2-thiosialoside **1**[5] by dimethyl(methylthio)sulfonium triflate (49 %, α:β = 2.2:1) or methylsulfenyl triflate (50 %, α:β = 3.6:1).

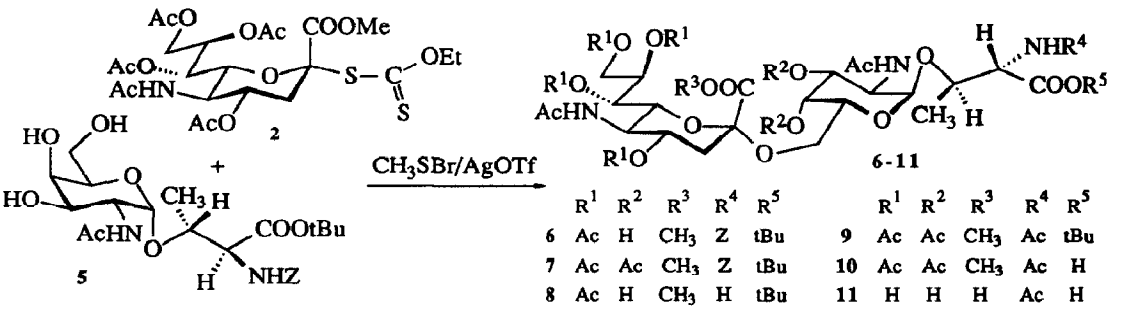


Reaction of the sialyl xanthate **2** with galactosamine threonine derivative **5**[6] was carried out using two equivalents of the donor and of methylsulfenyl triflate in CH_3CN/CH_2Cl_2 at -70 °C[7]. The glycosyl-threonine linkage as well as the *tert*-butyl ester proved to be stable under these conditions. A completely regioselective formation of the sialyl(2->6)galactosamine threonine conjugate was accomplished (71 %) in an anomeric ratio of α:β = 4:1. The desired α-anomer (sialyl-Tn) **6** was isolated in pure form by RP-HPLC in a yield of 36 %. As a byproduct the glycal of NeuNAc was formed[8]. Consideration of the ^{1}H-NMR spectroscopic data for **6** and for its *O*-acetylated derivative **7** confirmed that the (2->6)-linkage was formed exclusively.

In an earlier synthesis[9] of a sialyl-Tn glycopeptide a 3-phenylthio neuraminic acid donor was used to facilitate a stereoselective sialylation. This elegant concept, however, demands additional synthetic steps.

An alternative approach[10] involved the sialylation of a trihydroxy galactosamine acceptor ($\alpha:\beta$ = 5:1), prior to the coupling of the obtained disaccharide to a serine derivative ($\alpha:\beta$ = 3:2).

The regioselective sialylation of the *O*-glycosyl amino acid acceptor described here proceeds with useful stereoselectivity, requires only minimum protecting group manipulations and offers therefore the possibility of introducing additional saccharide moieties and protecting groups in the galactosamine part of **6**.



	R^1	R^2	R^3	R^4	R^5		R^1	R^2	R^3	R^4	R^5
6	Ac	H	CH_3	Z	tBu	**9**	Ac	Ac	CH_3	Ac	tBu
7	Ac	Ac	CH_3	Z	tBu	**10**	Ac	Ac	CH_3	Ac	H
8	Ac	H	CH_3	H	tBu	**11**	H	H	H	Ac	H

Deprotection of **6** was achieved by hydrogenolytic removal of the benzyloxycarbonyl group (Pd-C (10 %) H_2, CH_3OH) to give **8** (92 %), subsequent acetylation using Ac_2O, DMAP, pyridine (**9**, 68 %), cleavage of the *tert*-butyl ester with formic acid (**10**, quantitative) and final Zemplén transesterification by using 1n NaOH in methanol gave **11**[11] (71 %) which was purified by size-exclusion chromatography on Sephadex G 15.

The synthesis of the sialyl-Tn antigen reported here provides an efficient access to this biologically interesting structure and to numerous of its selectively protected derivatives.

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4. The donor **2** was prepared as described by Marra, A.; Sinaÿ, P., *Carbohydr. Res.* **1989**, *187*, 35-42 from the peracetylated *N*-acetylneuraminic methyl ester ((1) Kuhn, R.; Lutz, P.; MacDonald, D. L., *Chem. Ber.* **1966**, *99*, 611, (2) Ac_2O, pyridine).
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6. To prepare **5**, 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactosyl bromide was reacted with benzyloxycarbonyl threonine *tert*-butyl ester (G. Braum, Dissertation, Universität Mainz 1991) in analogy to Paulsen, H.; Hölck, J.-P., *Carbohydr. Res.* **1982**, *109*, 89-107 (51 %). Reaction with thioacetic acid (70 %) and treatment with sodium methoxide (1 %) in methanol afforded glycosyl acceptor **5** (93 %).
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11. Analytical data for **11**: $[\alpha]_D^{22}$ = 46.8 (c = 0.5, H_2O); 100.6 MHz-^{13}C-NMR (D_2O) δ 175.1, 174.7, 174.2 (CO), 99.1 (C-1), 77.4 (β-C), 72.6, 71.8, 69.9, 68.6, 68.4, 68.3, 67.9 (C-3, C-4, C-5, C-4', C-6', C-7', C-8'), 63.7, 62.7 (C-6, C-9'), 51.9, 50.0 (C-2, C-5'), 40.2 (C-3'), 22.3, 22.1, 22.0 ($\underline{C}H_3CO$), 18.1 (CH_3); 400 MHz-^{1}H-NMR (D_2O) δ 3.85-3.83 (m, H-5', H-8'), 3.67 (m, 1H, H-4'), 3.53 (m, 1H, H-7'), 2.67 (dd, 1H, $J_{3'a,3'e}$ 12.3 Hz, $J_{3'e,4'}$ 4.4 Hz, H-3'e), 2.09, 2.02, 1.99 (s, 9H, CH_3CO), 1.63 (t, 1H, $J_{3'a,3'e}$ 12.1 Hz, H-3'a), 1.25 (d, 3H, J 4.6 Hz, CH_3); FAB-mass spectrum (positive ion): m/z 656.7 $(M+H)^+$ (Calc.: 656.2).

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