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Synthesis of Tn and Sialyl Tn Building Blocks for Solid Phase Glycopeptide Synthesis

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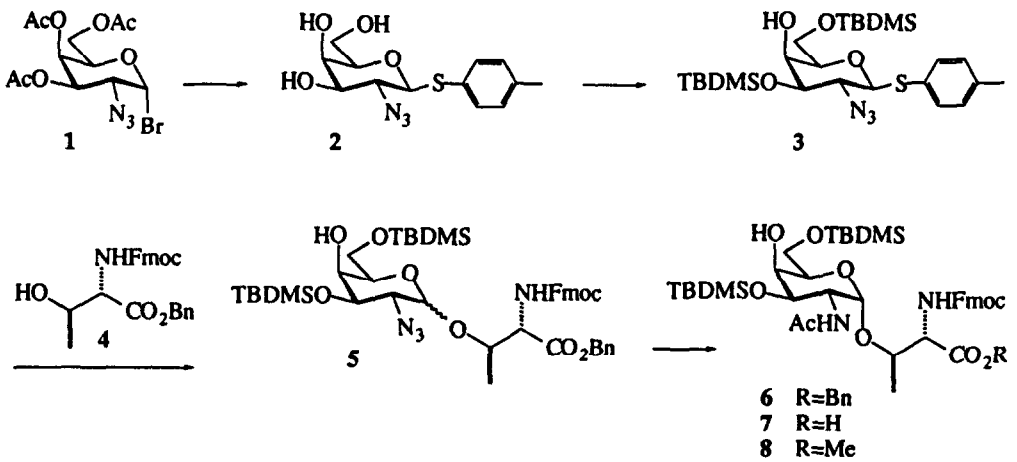
Abstract: Tn (GalNAcα1→*O*-Thr) and sialyl Tn (NeuAcα2→6GalNAcα1→*O*-Thr) building blocks for direct use in Fmoc solid phase glycopeptide synthesis have been prepared in 3 and 5 steps, respectively, from *p*-cresyl 2-azido-2-deoxy-3,6-di-*O*-*tert*-butyldimethylsilyl-1-thio-β-D-galacto-pyranoside (3). The silyl protective groups used for the GalNAc moiety of the Tn building block may be removed simultaneously with glycopeptide cleavage from the solid phase under acidic conditions.

The Tn (GalNAcα1→*O*-Ser/Thr) and sialyl Tn (NeuAcα2→6GalNAcα1→*O*-Ser/Thr) epitopes are *O*-linked tumour associated antigens present in glycoproteins on the surface of cancer cells[1]. These antigens also appear in mucins[2], as partial structures of saccharides present in glycophorin on human red blood cells[3], and on the HIV envelope glycoprotein gp120[4]. Several syntheses of the Tn antigen have been reported[5], whereas only a few reports describing synthesis of the sialyl Tn antigen[6,7,8] have appeared. Previous sialyl Tn building blocks are, however, not suitable for direct use in solid phase glycopeptide synthesis and we now present the synthesis of a building block which meets this criterion. An improved Tn building block which facilitates use in solid phase synthesis is also described.

Formation of the α-*O*-glycosidic bond between GalNAc and serine or threonine has predominantly been carried out using 2-azido-2-deoxy-glycosyl halides as glycosyl donors[5]. Thioglycosides allow activation under a variety of mild conditions but have been less extensively studied[9,10]. We therefore designed a synthetic route towards the Tn and sialyl Tn antigens based on thioglycoside methodology. The readily available azidobromide **1**[11,12] was converted in one pot to the *p*-cresylthio glycoside **2**[13] (68%) by treatment with *p*-thiocresol/NaOH in EtOH/$CHCl_3$. The triol **2** was treated with TBDMSCl[14] which gave the key 3,6-di-*O*-silylated glycosyl donor **3**[15] (99%). Acetylation of **3** confirmed that the unreactive HO-4 had not been silylated; a downfield ^1H NMR shift for H-4 from δ 3.83 ppm in **3** to 5.29 ppm was observed. NBS/QOTf[16] mediated glycosylation of N^{α}-Fmoc threonine benzyl ester **4** with **3** in CH_2Cl_2 gave the glycoside **5** (71%) as an inseparable mixture of α- and β-anomers. Reduction of the azido group in **5** was effected with AcSH[17] in pyridine and purification with normal phase HPLC gave the α-glycosylated threonine **6** (67%), as well as the corresponding β-glycoside

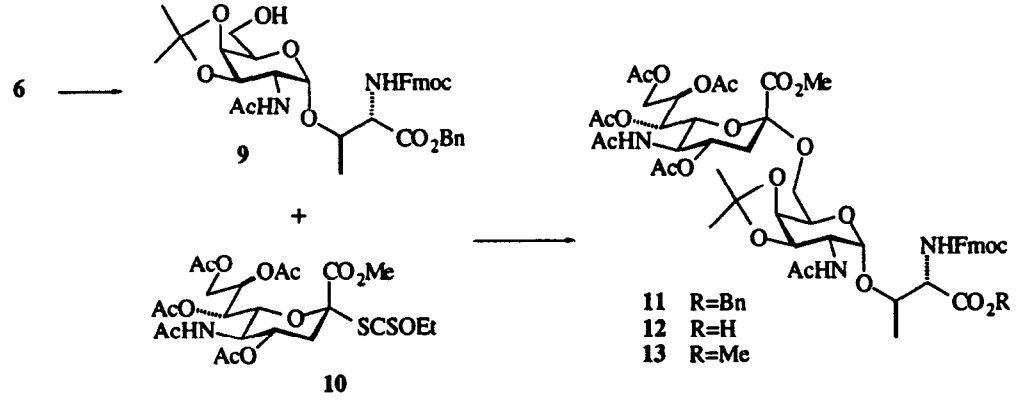
(13%). Hydrogenolysis of the benzyl ester in **6** with H_2-Pd/C gave the target Tn building block **7** (90%). Due to poor resolution in the ^{1}H-NMR spectrum of **7** the structural confirmation was instead made on the methyl ester **8**[15], obtained after treatment of **7** with $TMSCHN_2$[18]. The Tn building block **7** can be used directly in Fmoc solid phase glycopeptide synthesis, and the silyl groups used for protection of the GalNAc moiety may be removed under standard cleavage from the solid phase with TFA, without affecting the α-glycosidic bond. Silyl protection of the carbohydrate moiety thus avoids potential side reactions such as epimerisation of peptide stereocenters, β-elimination, and aspartimide formation, which may be encountered on basic deprotection of the *O*-acetylated Tn building blocks previously described in the literature[5].



Several methods commonly used for reduction of azido groups failed or gave poor yields when employed to the azide **5**. No conversion of **5** was obtained with $NiCl_2/NaBH_4$[19] or with H_2S/pyridine[20], and in the latter case addition of triethylamine caused cleavage of the Fmoc group. Reduction with the tertiary phosphines[21] trioctylphosphine and tributylphosphine, and subsequent acetylation, gave low yields of **6** and its β-anomer (50-70% and 40%, respectively). With AcSH, reduction and acetylation was either sluggish, or required heating at 50°C, but yields were still modest (~50%).

In the synthesis of the sialyl Tn building block, the TBDMS protective groups were first removed from **6** with HOAc/H_2O/THF and isopropylidenation[22] of the resulting triol then gave the glycosyl acceptor **9** (74%, two steps). The triol obtained after deprotection of **6** had a low solubility in organic media and could therefore not be sialylated directly. Instead, sialylation of **9** with the xanthate **10**[23] (1.2 eq.) under MSB/AgOTf promotion in CH_3CN/CH_2Cl_2 at -78°C[24] gave the fully protected sialyl Tn antigen **11** (32% isolated yield, 32% of **9** was recovered), together with the corresponding β-glycoside (~5%). The α-anomeric configuration of the sialic acid residue in **11** was established by determination of the coupling constant[25,26] between C-1' and H-$3'_{ax}$ (J=6.2 Hz). Since the sialylation of **9** proceeded with good stereoselectivity the need for a sialic acid donor which provides anchimeric assistance[7] from a substituent at C-3 was avoided, thereby limiting the number of synthetic steps. After hydrogenolysis of the benzyl ester in **11** with H_2-Pd/C, the target glycosylated amino acid **12** (87%) was obtained, and its structure was confirmed using the corresponding methyl ester **13**[15] as

described for **7**. The sialyl Tn building block **12** can be used directly in Fmoc solid phase synthesis while previous building blocks have drawbacks such as requiring exchange of the Thr-N^{α} protective group[6,8], or employing benzyl protective groups[7] for the carbohydrate moiety which must be removed under conditions that are incompatible with the presence of methionine and cysteine residues in the glycopeptide.



The use of the Tn and sialyl Tn building blocks **7** and **12** in solid phase glycopeptide synthesis is now being investigated.

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REFERENCES AND NOTES

1. Hakomori, S.-i. *Adv. Cancer Res.* **1989**, 52, 257-331.
2. Carraway, K. L.; Hull, S. R. *Glycobiology* **1991**, 1, 131-138.
3. Gooley, A. A.; Pisano, A.; Williams, K. L. *Trends Glycosci. Glycotechn.* **1994**, 6, 328-338.
4. Hansen, J.-E. S.; Clausen, H.; Nielsen, C.; Teglbjærg, L. S.; Hansen, L. H.; Nielsen, C. M.; Dabelsteen, E.; Mathiesen, L.; Hakomori, S.-i.; Nielsen, J. O. *J. Virol.* **1990**, 64, 2833-2840.
5. Nakahara, Y.; Iijima, H.; Ogawa, T. *Synthetic Oligosaccharides*, Kovác, P. Ed., ACS Symposium series 560, American Chemical Society, Washington DC, **1994**; pp. 249-266, and references therein.
6. Iijima, H.; Ogawa, T. *Carbohydr. Res.* **1988**, 172, 183-193.
7. Nakahara, Y.; Iijima, H.; Shibayama, S.; Ogawa, T. *Carbohydr. Res.* **1991**, 216, 211-225.
8. Liebe, B.; Kunz, H. *Tetrahedron Lett.* **1994**, 35, 8777-8780.
9. Paulsen, H.; Rauwald, W.; Weichert, U. *Liebigs Ann. Chem.* **1988**, 75-86.
10. Braun, P.; Waldmann, H; Kunz, H. *Synlett* **1992**, 39-40.
11. Lemieux, R. D.; Ratcliffe, R. M. *Can. J. Chem.* **1979**, 57, 1244-1251.

12. Broddefalk, J.; Nilsson, U.; Kihlberg, J. *J. Carbohydr. Chem.* **1994**, 13, 129-132.
13. Lüning, B.; Norberg, T.; Tejbrant, J. *Glycoconjugate J.* **1989**, 6, 5-19.
14. Halmos, T.; Montserret, R.; Filippi, J.; Antonakis, K. *Carbohydr. Res.* **1987**, 170, 57-69.
15. High resolution MS and selected ^{1}H NMR-data, (500 MHz, $CDCl_3$, δ=7.25 ppm):
3: HRMS $(M+H)^+$ 540.2747 calcd, 540.2751 obsd. NMR: δ 4.33 (d, 9.7 Hz, H-1), 3.90 (dd, 6.4 and 10.3 Hz, H-6), 3.83 (dd, 5.5 and 10.2 Hz, H-6), 3.83 (H-4), 3.52 (dd, 3.1 and 9.1 Hz, H-3), 3.47 (t, 9.4 Hz, H-2), 3.42 (bt, 5.9 Hz, H-5), 2.33 (s, cresyl-Me), 0.91 and 0.88 (2 s, 2 tBu), 0.16, 0.12, 0.09, and 0.08 (4 s, 4 MeSi).
8: HRMS $(M+H)^+$ 787.4022 calcd, 787.3997 obsd. NMR: δ 5.64 (d, 10.1 Hz, AcNH), 5.40 (d, 9.4 Hz, $^{\alpha}$NH), 4.74 (d, 3.7 Hz, H-1), 4.41 (dt, 3.6 and 10.3 Hz, H-2), 4.37 (dd, 2.3 and 9.5 Hz, H-α), 3.74 (s, CO_2Me), 3.69 (dd, 2.9 and 10.3 Hz, H-3), 2.02 (s, Ac), 1.32 (d, 6.3 Hz, H-γ).
13: $[\alpha]_D^{25}$ +34° (*c* 0.6, $CHCl_3$). HRMS $(M+H)^+$ 1072.4138 calcd, 1072.4136 obsd. NMR: δ 5.76 (d, 9.7 Hz, AcNH), 5.49 (d, 9.3 Hz, $^{\alpha}$NH), 5.37 (ddd, 2.8, 6.0 and 7.6 Hz, H-8'), 5.20 (d, 9.6 Hz, AcNH'), 4.90 (ddd, 4.8, 9.8 and 12.0 Hz, H-4'), 4.71 (d, 3.5 Hz, H-1), 4.34 (dd, 2.7 and 12.4 Hz, H-9'), 4.12 (dd, 5.9 and 12.4 Hz, H-9'), 3.79 (s, CO_2Me), 3.34 (s, CO_2Me), 2.60 (dd, 4.7 and 12.8 Hz, H-3'$_{eq}$), 1.98 (t, 12.8 Hz, H-3'$_{ax}$), 1.30 (d, 6.4 Hz, H-γ).
16. Fukase, K.; Hasuoka, A.; Kinoshita, I.; Aoki, Y.; Kusumoto, S. *Tetrahedron* **1995**, 51, 4923-4932.
17. Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem.* **1988**, 53, 1580-1582.
18. Hashimoto, N.; Aoyama, T.; Shiorri, T. *Chem. Pharm. Bull.* **1981**, 29, 1475-1478.
19. Heyns, K.; Feldmann, J.; Hadamczyk, D.; Schwentner, J.; Thiem, J. *Chem. Ber.* **1981**, 114, 232-239.
20. Adachi, T.; Yamada, Y.; Inoue, I.; Saneyoshi, M. *Synthesis* **1977**, 45-46.
21. Friedrich-Bochnitschek, S.; Waldman, H.; Kunz, H. *J. Org. Chem.* **1989**, 54, 751-756.
22. Catelani, G.; Colonna, F.; Marra, A. *Carbohydr. Res.* **1988**, 182, 297-300.
23. Marra, A.; Sinaÿ, P. *Carbohydr. Res.* **1989**, 187, 35-42.
24. Lönn, H.; Stenvall, K. *Tetrahedron Lett.* **1992**, 33, 115-116.
25. Hori, H.; Nakajima, T.; Nishida, Y.; Ohrui, H.; Meguro, H. *Tetrahedron Lett.* **1988**, 29, 6317-6320.
26. Ercegovic, T.; Magnusson, G. *J. Chem. Soc., Chem. Comm.* **1994**, 831-832.

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