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Silyl Protection in the Solid-Phase Synthesis of N-linked Neoglycopeptides. One-step Deprotection of Fully Protected Neoglycopeptides.

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Abstract: Silyl protection of the saccharide part in a building block for solid-phase synthesis of N-linked neoglycopeptides is described. Per-O-(trimethylsilylated) 1-amino-1-deoxy-D-glucitol **3** was reacted selectively with N^{α}-Fmoc-Asp(Cl)-OPfp **1** and the resulting Pfp-ester **4** was incorporated into D-Ala[1] Peptide-T amide and a biologically active pentapeptide. The silyl protection allowed cleavage and complete and simultaneous deprotection of the resulting peptides.

The glycoprotein glycans play an important role in the cellular transport, as signals for processing and for intercellular communication.[1] With the increasing understanding of the importance of the glycan part it will become indispensable to have access to a large variety of well defined fragments of glycoproteins and neoglycopeptides for biological studies.

Both O- and N-linked glycopeptides have been most effectively synthesized by a building block strategy, in which glycosylated amino acids are activated and coupled to a peptide linked to a solid support.[2]

In solution synthesis of small glycopeptides benzyl protection of the saccharide has often been employed, the removal of which from the final product requires reductive cleavage. However, catalytic hydrogenation is incompatible with cysteine and methionine containing peptides and as a result often gives side-reactions with more complex structures. Consequently, the use of acyl protecting groups for the saccharide has prevailed in the solid phase synthesis of glycopeptides. The acetyl or benzoyl groups may be removed with various mild nucleophiles in transesterification or hydrolysis reactions and most frequently hydrazine or dilute methoxide in methanol have been used successfully. However, low solubility of the intermediates and products is recurrently a crucial problem, in particular with multiple techniques (e.g. MCPS).[3] Often heterogeneous and critically prolonged deprotection times are necessary even in methanol or water. In the synthesis of N-linked glycopeptides the problem may be avoided by omission of the saccharide protection, however, this strategy is not without problems.[2]

We have previously reported a general strategy employing acetyl or benzoyl protection of the saccharide in glycosylation of Fmoc-amino acid Pfp-esters and direct incorporation of the well characterized products in complex glycopeptides.[4,5] However, an attempt to apply this method to 1-amino-alditols[6] was not successful due to O $\rightarrow$ N acyl migration to the more basic primary amino group. This work extends the method to application of easily removable trimethylsilyl (TMS) protection of the saccharide allowing a single acid cleavage and deprotection step in the synthesis of N-linked neoglycopeptides. As a model study, we have synthesized an Fmoc-protected Pfp-ester activated asparagine building block carrying TMS protected D-glucitol. Treatment of 1-amino-1-deoxy-D-glucitol[7] **2** in pyridine with TMS-Cl at room temperature afforded pure syrupy **3** (3.0 g, 94%) after concentration *in vacuo* followed by concentration with dry n-pentane and filtration to remove

pyridine, hydrochloride. A mixture of one eqv. of **3** and two eqv. N-ethyl-morpholine in dry THF was added dropwise to a THF solution of one eqv. N^{α}-Fmoc-Asp(Cl)-OPfp[8] **1** at - 40°C. N-Ethyl-morpholine, hydrochloride precipitated instantaneously and the reaction mixture was allowed to reach room temperature. The hydrochloride was removed by filtration and the resulting filtrate was passed through a short column of dry silica gel (eluent hexane/ethylacetate, 7/1). The asparagine building block N^{α}-Fmoc-Asn(N^{γ}-[penta-O-TMS-1-deoxy-**D**-glucitol-1-yl])-OPfp[9] **4** was isolated as a syrup (4.8 g, 89%).

In order to investigate the stability of the TMS groups under the conditions used in Fmoc-based solid phase synthesis,[10] methyl tetra-O-TMS-α-**D**-*manno*pyranoside[11] was treated with DMF for 24 h, 20% piperidine in DMF for 1 h and 50% morpholine in DMF for 1 h. No decomposition was observed under any of these conditions according to ^{1}H NMR. However, treatment with 2% of the strong base DBU in DMF for 1 h gave several products according to ^{1}H NMR which were not analyzed further.

Compound **4** was used in a solid phase assembly of two modified biologically active peptides. **D**-Ala^1 Peptide-T amide is a potential inhibitor of HIV virus binding to T-cells[12] and several glycosylated analogs have already been synthesized.[8,13,14] The pentapeptide Asn-Leu-Gly-Val-Cys(Acm) has shown to be biologically active[15] but suffer as a therapeutic agent from poor solubility in physiological fluids.

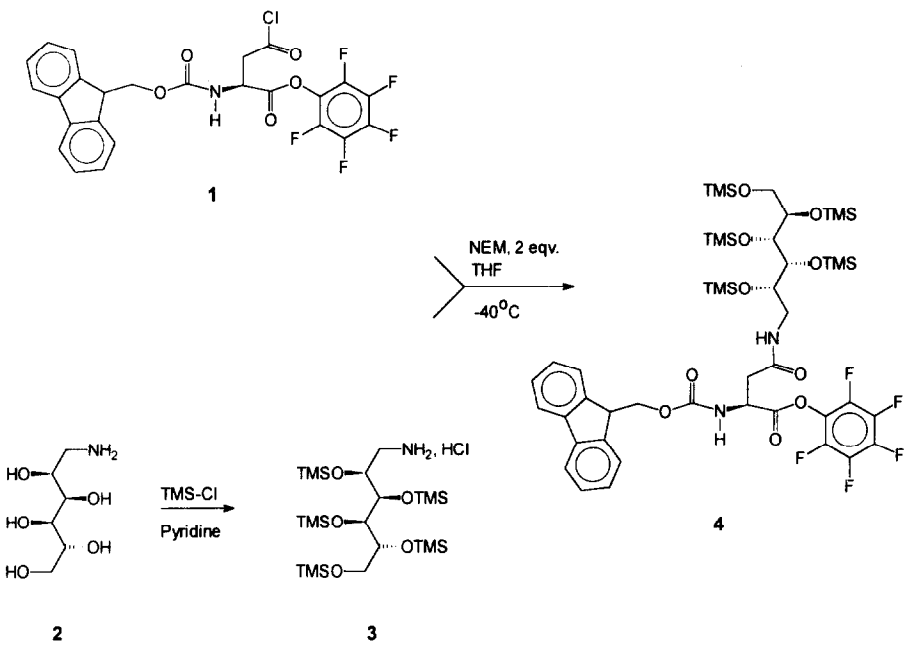

Figure 1. Synthesis of building block **4**.

The modified neoglycopeptides **D**-Ala-Ser-Thr-Thr-Thr-Asn(D-glucitol)-Tyr-Thr-NH_2 **5** and Asn(D-glucitol)-Leu-Gly-Val-Cys(Acm) **6** were synthesized manually on PEGA-resin[16] (0.07 mmol/g) using N^{α}-Fmoc amino acid Pfp or Dhbt-esters. **D**-Alanine was coupled as the free acid by the TBTU procedure.[17] Fmoc groups were removed with 20% piperidine in DMF. The peptides were cleaved from the resin with 92% TFA, 3 % anisole, 1 % EDT, 1 % thioanisole and 3 % water with simultaneous and quantitative removal of the TMS groups. HPLC purification afforded **5**[18] (14 mg, 33%) and **6**[19] (11 mg, 43%). The neoglycopeptides **5** and **6** were subjected to 1D and 2D NMR spectroscopy and all protons and carbons could be assigned unambiguously. Comparing chemical shifts and coupling constants with data from the unglycosylated parent peptides only very minor differences were observed. The introduction of hydroxyl groups enhanced the water solubility of the modified pentapeptide **6**.

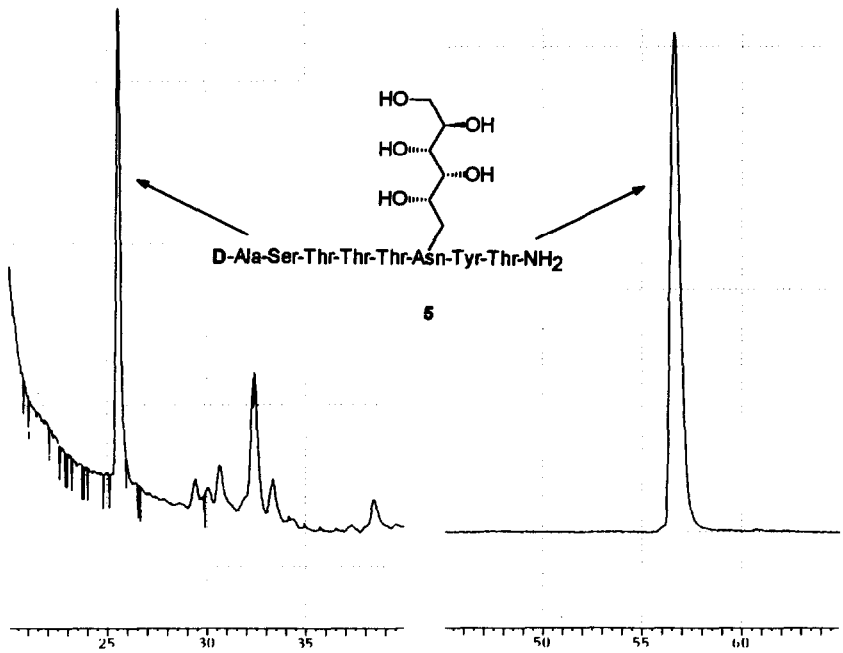

Figure 2. HPLC profiles of crude (left) and purified (right) neoglycopeptide **5**. The peak with retention time 32.5 min contains no peptide and corresponds to residual scavenger.

In this report a new protection strategy in the solid phase synthesis of N-linked neoglycopeptides has been described. The TMS group is easily introduced, stable to the conditions used in Fmoc-based solid phase peptide synthesis and allows simultaneous cleavage and complete deprotection of the resulting peptide. We are currently investigating the use of the TMS group in the protection of larger carbohydrate moieties. The building block **4** may, in particular, be used to increase the hydrophilicity and thereby the water solubility of peptides.[20]

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7. 1-Amino-1-deoxy-D-glucitol **2** was a gift from Dr. H. Röper, Cerestar, Gruppo Feruzzi.

8. Christiansen-Brams, I.; Meldal, M.; Bock, K. *J. Chem. Soc., Perkin Trans. 1*; in press. Compound **1** was prepared in a single step from commercially available N^{α}-Fmoc-Asp(OBu^t)-OPfp by treatment with TFA and thionyl chloride.

9. Compound **4**: FAB-MS, m/z 1045 (M + H$^+$). $C_{46}H_{69}F_5N_2Si_5O_{10}$ requires 1044.37. Elemental analysis: Found C 52.57, H 6.34, N 2.57%. Required C 52.85, H 6.65, N 2.68%. 500 MHz ^{1}H-NMR in $CDCl_3$, δ ppm (J Hz): D-glucitol 3.55 (2 protons, H1 and H1'), 3.92 (3 protons) and 3.70 (1 proton) (H2, H3, H4 and H5), 3.80 (H6, J_{56} 3, $J_{66'}$ 10.5), 3.53 (H6', $J_{56'}$ 7.5), Asn 6.40 (2 protons, N^{α}H and N^{γ}H), 5.04 (H^{α}), 3.15 (H^{β}, $J_{\alpha\beta}$ 4.5, $J_{\beta\beta'}$ 16), 2.88 ($H^{\beta'}$, $J_{\alpha\beta'}$ 4), Fmoc 4.29 (H9), 4.52 (CH_2, J_{9H} 7, $J_{HH'}$ 10.5), 4.38 (CH_2', $J_{9H'}$ 7.5). 125.77 MHz ^{13}C-NMR in $CDCl_3$, δ ppm: D-glucitol 42.4 (C1), 76.4, 75.7, 75.3 and 71.3 (C2, C3, C4 and C5), 63.8 (C6), Asn 50.6 (C^{α}), 37.6 (C^{β}), Fmoc 47.0 (C9), 67.6 (CH_2), Pfp 136.8-142.

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18. Compound **5**: FAB-MS, m/z 1021 (M + H$^+$). $C_{41}H_{68}N_{10}O_{20}$ requires 1020.46.

19. Compound **6**: FAB-MS, m/z 739 (M + H$^+$). $C_{29}H_{54}N_8O_{12}S$ requires 738.36.

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