Solid Phase Synthesis of Mono- and Di-saccharide-containing Glycopeptides

Björn Lüning,*[a] Thomas Norberg,[b] and Jan Tejbrant[a]

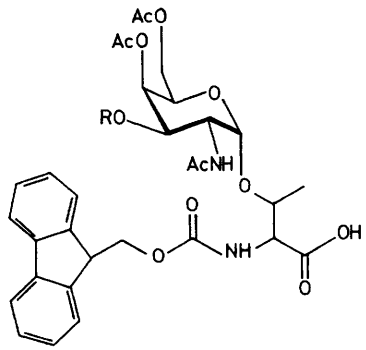
[a] *Department of Organic Chemistry, University of Stockholm, Stockholm, Sweden*
[b] *Organic Synthesis Department, BioCarb AB, Lund, Sweden*

Derivatives of Fmoc-threonine (Fmoc = fluoren-9-ylmethoxycarbonyl) with *O*-glycosidically peracetylated β-D-Gal*p*-(1 → 3)-α-D-GalNAc*p* or α-D-GalNAc*p* chains have been used in a solid phase synthesis of the oncofetal fibronectin sequence VTHPGY (benzyl protection was used on histidine and tyrosine); a super-acid-sensitive resin was used, which enabled the isolation of the protected glycopeptide after synthesis, a feature that substantially facilitated verification of the structure by n.m.r. and m.s.

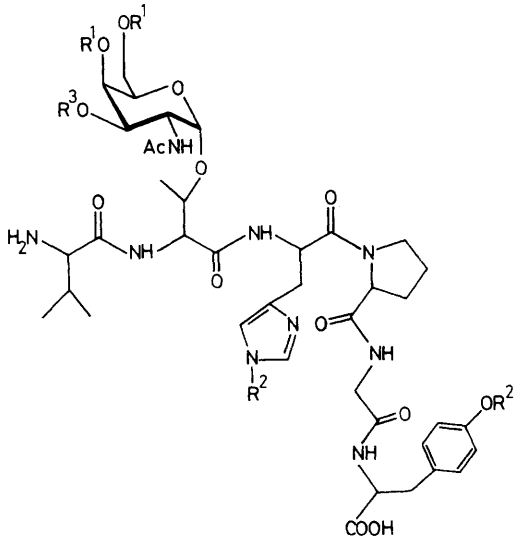
Glycosylation of peptides and proteins is an important post-translational process. Development of efficient techniques for the chemical synthesis of glycopeptides is therefore of considerable interest. Chemical glycosylation of suitably protected peptides and proteins has been reported for a few cases,[1—4] and the alternative synthetic route, using protected glycosylated amino acids in solution or solid phase peptide synthesis, is still early in its development.[5—7] We have successfully used the latter approach to synthesize the glycosylated hexapeptides (**5**) and (**6**).

Protecting groups requiring strongly acidic conditions for removal cannot be used in a glycopeptide synthesis because of the sensitivity of the glycosidic bonds in the oligosaccharide chains. However, base-sensitive Fmoc-protection (Fmoc = fluoren-9-yl-methoxycarbonyl) of the α-amino groups in combination with a handle to the solid phase that can be cleaved by weak acid, constitutes a possible protocol for glycopeptide synthesis. The conventional t-butyl side chain protection used with Fmoc-chemistry, which requires strong acid for removal, was replaced in this investigation by benzyl groups which could be removed by hydrogenation. The hydroxy groups of the carbohydrate moiety were protected as acetates, which could be removed easily by brief treatment with methanol/methoxide ion without β-elimination or racemization. Glycosylation of the threonine moiety was achieved[8] in an eight-step synthesis using Fmoc-threonine phenacyl ester, which after glycosylation was de-esterified with zinc in acetic acid to give the free acids (**1**) and (**2**).

As a target molecule, the *O*-glycosylated oncofetal sequence of fibronectin VTHPGY[9] was chosen. The following general conditions were employed during peptide synthesis: proline and valine were coupled as symmetrical anhydrides (2—4 times excess), and glycine, histidine, and glycosylated threonine as their 1-hydroxybenzotriazole esters (2—4 times excess). Attempts to couple glycine as a symmetrical anhydride resulted in almost quantitative double incorporation. The Fmoc groups were deprotected with 50% piperidine in dimethylformamide (DMF) before carbohydrate was incorporated, then with 50% morpholine in DMF.



(**1**) R = Ac
(**2**) R = tetra-*O*-acetyl-β-D-galactopyranosyl



(3) R^1 = Ac, R^2 = $PhCH_2$, R^3 = Ac
(4) R^1 = Ac, R^2 = $PhCH_2$, R^3 = tetra-*O*-acetyl-β-D-galactopyranosyl
(5) R^1 = H, R^2 = H, R^3 = H
(6) R^1 = H, R^2 = H, R^3 = β-D-galactopyranosyl

The synthesis was carried out as follows: starting with Fmoc-*O*-benzyltyrosine coupled to Sasrin resin (Bachem AG), Fmoc-glycine, Fmoc-proline, and Fmoc-*N*-benzylhistidine (Bachem AG) were successively coupled by a standard protocol,[10] then peracetylated Fmoc-*O*-(α-D-GalNAc*p*)-threonine[8] (**1**) (coupling time 48 h) was coupled, and finally Fmoc-valine was coupled by the standard protocol to give resin-bound, protected (**3**). Using the same protocol, resin-bound, protected (**4**) was synthesized using peracetylated Fmoc-*O*-(β-D-Gal*p*-(1→3)-α-D-GalNAc*p*)-threonine[8] (**2**). The Fmoc group was removed while the peptides were still bound to the resin. Final deprotection from the resin was made with 1% trifluoroacetic acid in dichloromethane, giving (**3**) [fast atom bombardment (f.a.b.) m.s. $M^+ + 1 = 1184$], 53 mg (87%) and (**4**) (f.a.b. m.s. $M^+ + 1 = 1470$), 78 mg (106%).

N.m.r. phase-sensitive DQF COSY spectra (CD_3OD) of (**3**) and (**4**) showed cross-peaks which enabled the assignment of all hydrogens in the amino acid residues, and in the carbohydrate moieties and the protective groups.

Treatment of (**3**) and (**4**) with 0.1 M methoxide in methanol for 20 min at ambient temperature removed the acetate groups, to give the peptides benzylated on histidine and tyrosine. The reactions were monitored by t.l.c.; after reaction completion only single spots were observed. Hydrogenolysis with 10% Pd/C at 55—60 psi and ambient temperature for 72 h gave the free glycopeptides. The yield of (**5**) was 17.0 mg (40%, f.a.b. m.s. $M^+ + 1 = 876$) and that of (**6**) was 21.6 mg (38%, f.a.b. m.s. $M^+ + 1 = 1038$). The n.m.r. spectra were in agreement with the postulated structures. Full experimental details for the synthesis of (**5**) and (**6**) will be published in the near future.[11]

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