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Note

Conformation-dependent *O*-glycosylation of threonine-containing dipeptides

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In the synthesis of glycopeptides as possible models in the structural studies of glycoproteins, two possible strategies can be considered, involving chain elongation of a suitably protected glycosylated amino acid and glycosylation of the desired peptide backbone. The latter simplifies the synthetic procedures after glycosylation, but problems of stereospecificity and lower yields could hamper its practicability for longer peptides. However, dipeptides and possibly tripeptides should not drastically affect the reactivity of a glycosylation site and condensation of such small glycopeptides would be eminently suitable for glycopeptides with repeating subunits, for example, antifreeze glycoproteins where the threonine in the Ala-Thr-Ala unit is *O*-glycosylically linked to a disaccharide[1].

We have investigated the efficiency of glycosylation with increasing length of the peptide, using 3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-β-D-galactopyranosyl chloride[2] under Helferich conditions[3]. The results are as shown in Table I.

Condensation of the tripeptide, *N*-(benzyloxycarbonyl)-L-alanyl-L-threonyl-L-alanine methyl ester (Z-Ala-Thr-Ala-OMe) failed under the various conditions attempted, indicating the limitation on peptide length imposed by this procedure. The dipeptides show more clearly the steric problems involved. When there is an amino acid residue on the carbonyl side of the Thr residue, there seems to be no effect on the reactivity of the Thr hydroxyl-group to form the glycopeptide. The yields are similar to those obtained on glycosylating *N*-(benzyloxycarbonyl)-L-threonine methyl ester (Z-Thr-OMe); if an amino acid is on the amine side of the Thr residue, as with the tripeptide, reaction does not proceed.

Although this is a limited study using only one synthetic method, the results are supported by those of Lacombe and Pavia[4]. In the condensation of *N*-(benzyloxycarbonyl)-L-threonyl-L-glycine benzyl ester and *N*-(benzyloxycarbonyl)-L-glycyl-L-threonine benzyl ester with 2,3,4,6-tetra-*O*-benzyl-D-galactopyranose using the trifluoromethanesulfonic anhydride method, the higher yield (85%) was

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TABLE I

YIELD OF *O*-GLYCOPEPTIDES FROM THE REACTION OF 3,4,6-TRI-*O*-ACETYL-2-AZIDO-2-DEOXY-β-D-GALACTOPYRANOSYL CHLORIDE WITH THREONINE-CONTAINING PEPTIDES

Threonine-containing peptides[a]	*Scale (mmol)*	*Yield*[b] *(mg)*	*Yield (%)*
Z-Thr-OMe	1	287	49.4
	1	267	46.0
Z-Thr-Ala-OMe	1	293	45.0
	1	313	48.0
Z-Thr-Pro-OMe	1	300	44.3
	1	288	42.5
Z-Ala-Thr-OMe	1	—	—
Z-Ala-Thr-Ala-OMe	1	—	—

[a]2 Equiv. of peptide used. [b]Calculated after flash chromatography.

found for the peptide in which the glycine was on the carbonyl side of the Thr residue. The yield was only 40% for the dipeptide having the opposite order[4]. Different reaction conditions and the use of a different amino acid adjacent to the Thr residue indicated some common steric problems involved in the glycosylation of small threonine-containing peptides. Also, all the glycopeptides synthesised reflect the high stereoselectivity of the reaction which gave only the α anomer.

On consideration of CPK molecular models, this effect is not difficult to understand. The most stable conformation[5] of the glycosidic bond in α-glycopeptides is when the threonine H^{β} is in close proximity to H-1. In such an orientation, there is little or no steric interaction of the sugar and the peptides in 3-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-*N*-(benzyloxycarbonyl)-L-threonine methyl ester, 3-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-*N*-(benzyloxycarbonyl)-L-threonyl-L-alanine methyl ester, and 3-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-*N*-(benzyloxycarbonyl)-L-threonyl-L-proline methyl ester, but, with 3-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-*N*-(benzyloxycarbonyl)-L-alanyl-L-threonine methyl ester and 3-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-*N*-(benzyloxycarbonyl)-L-alanyl-L-threonyl-L-alanine methyl ester, steric interactions are possible. This is especially so when the Ala methyl group faces away from the Thr side-chain. Large steric interactions arise with the benzyl of the benzyloxycarbonyl group and AcO-4 of the sugar. Such a conformation is likely for antifreeze glycoproteins, considering the hydrophilic nature of the Thr side-chain and the hydrophobic Ala side-chain[6,7].

These preliminary results indicate the impracticability of glycosylating threonine-containing peptides as a general method in the synthesis of glycopeptides. Also, the steric interactions for the most part were due to the protecting groups on both the sugar and the peptide. This stresses the importance of selecting suitable protecting-groups in the conformational studies of small glycopeptides.

EXPERIMENTAL

All peptides were synthesised by standard liquid-phase methods, using dicyclohexylcarbodiimide–1-hydroxybenzotriazole as the coupling reagent. Each was recrystallised from ethyl acetate–light petroleum and identified by t.l.c. and n.m.r. spectroscopy.

The *O*-glycopeptides were synthesised by using the general conditions described by Ferrari and Pavia[3]. The product was purified by flash chromatography with chloroform–ether (9:1), and identified by t.l.c. and n.m.r. spectroscopy. There was only one isomer formed in each reaction, as clearly shown by a single ^{13}C resonance for C-1 in the 100-p.p.m. region; this was determined to be the α anomer[8]. For the synthesis of 3-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-*N*-(benzyloxycarbonyl)-L-alanyl-L-threonine methyl ester and 3-*O*-(3,4,6-tri-*O*-acetyl-2-azido-2-deoxy-α-D-galactopyranosyl)-*N*-(benzyloxycarbonyl)-L-alanyl-L-threonyl-L-alanine methyl ester, an equimolar mixture of peptide and glycosyl halide was monitored by t.l.c. during 48 h. Purification and analysis of all products showed no *O*-glycosylated peptides.

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