Pentafluorophenyl Esters for the Temporary Protection of the α-Carboxy Group in Solid Phase Glycopeptide Synthesis

Morten Meldal and Knud J. Jensen

The Department of Chemistry, Carlsberg Laboratory, Gamle Carlsberg Vej 10, DK-2500 Valby, Copenhagen, Denmark

The pentafluorophenyl ester of fluoren-9-ylmethoxycarbonyl serine was found to be stable to glycosylation conditions and the purified glycosylation product was easily incorporated into a solid phase assembly of a nonapeptide analogue of the antifreeze glycopeptides.

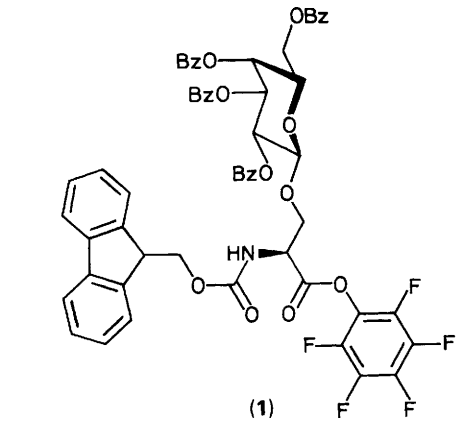
O-Glycosyl peptides have attracted much attention in recent years owing to their numerous functions in biological systems, and interest in access to synthetic model compounds is increasing. The direct glycosylation of protected peptides has not been found to be a successful approach to the preparation of *O*-glycopeptides.[1—3] The alternative route in which the protected and glycosylated hydroxy amino acids are used in a stepwise assembly of peptides, either on the solid phase or in solution, has been described by several authors as a reasonably reliable method.[1,2,4—20] The *O*-glycosidic bond to threonine or serine is, however, sensitive to acid, and treatment with strong base results in β-elimination leaving only a limited number of the synthetic strategies generally used in peptide synthesis available for the preparation of glycopeptides. The base labile fluoren-9-ylmethoxycarbonyl (Fmoc) protection of the α-amino group allows repetitive deprotection to be carried out with the mild organic base, morpholine.[6—8,10,12,15,17—19]

There have been a few reports on the synthesis of *O*-glycopeptides by solid phase techniques.[4,6—8,20] In general, these reports describe the use of the *in situ* generated hydroxybenzotriazole esters[4,7,8] or symmetrical anhydrides[6] of the *O*-glycosylated Fmoc amino acids in the coupling reaction. In these procedures a large excess of precious glycosyl amino acid is used to ensure complete acylation.

Active esters of *N*-hydroxysuccinimide,[10,17] 2- and 4-nitrophenol,[10,11,19,21] and pentachlorophenol[21] have been used previously for the protection of the carboxy group of amino acids during the glycosylation, and the active products have been purified by chromatography and used for peptide synthesis in solution. These esters, however, are not generally suitable for solid phase synthesis due to their low reactivity and the possibilities of side reactions,[22] although their reactivity may be enhanced by catalysis. For this reason the procedure used exclusively in the solid phase synthesis of *O*-glycopeptides has been to protect the carboxy function with more permanent protective groups which after a chromatographic purification procedure have been removed selectively. The free carboxy group has then been activated *in situ* for incorporation into glycopeptides, because of the high reactivities required for successful coupling reactions in solid phase synthesis. In the continuous flow version of the Fmoc-polyamide solid phase method, this reactivity has best been achieved by using pentafluorophenyl (Pfp) esters[23] or esters of 3,4-dihydro-3-hydroxy-4-oxo-1,2,3-benzotriazine (Dhbt).[24]

In this report we describe the successful application of the very active Pfp-esters for α-carboxyl protection during glycosylation and the use of these esters in solid phase synthesis.

The preparation of Fmoc-Ser-O-Pfp has been described by Kisfaludy and Schön[25,26] and the analogous preparation of Fmoc-Ser-O-Dhbt is not complicated.[27] Fmoc-Ser-O-Dhbt is, however, only slightly soluble in the solvents used for glycosylation reactions, and the glycosylation is best carried out with Fmoc-Ser-O-Pfp in apolar solvents. When Fmoc-Ser-O-Pfp is prepared by a condensation reaction between Fmoc-Ser-OH, promoted by carbodiimides, urea byproducts are formed as seen by ^{1}H NMR and ^{13}C NMR spectroscopy. As with Fmoc-Ser(Bu^t)-O-Pfp these byproducts can not be removed by recrystallizing, but Fmoc-Ser-O-Pfp could be purified by chromatography on a column of dried (120 °C, 24 h) silica gel with dry solvents (33% ethyl acetate in light petroleum) to yield 54% of the pure Fmoc-Ser-O-Pfp with a melting point considerably higher (m.p. 144—145.5 °C) than reported (m.p. 125—130[25] and 124—126 °C[26]). This purification was found to be crucial and the procedure was also



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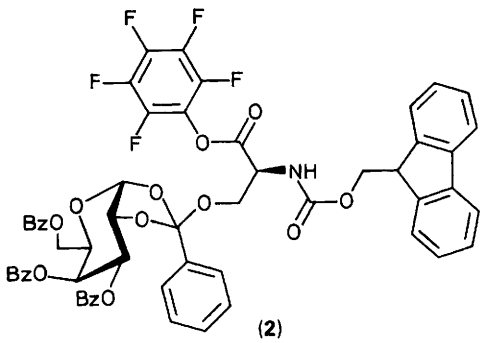

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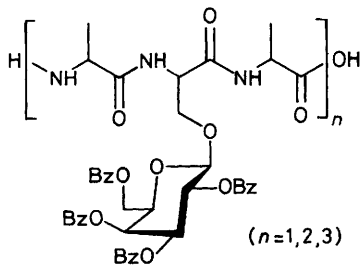

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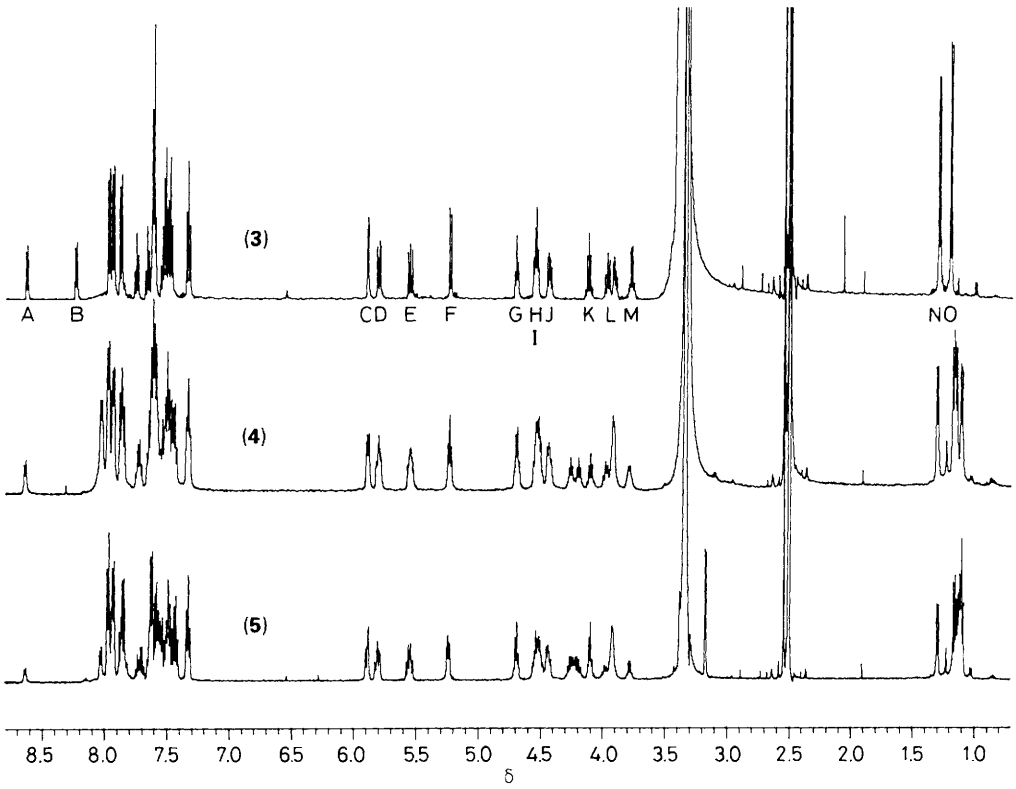

Figure 1. The ^{1}H NMR spectra of (**3**), (**4**), and (**5**) are shown together with the assignment of (**3**) [A, Ser NH; B, Ala-3 NH; C, H-4; D, H-3; E, H-2; F, H-1 (*J* 8.0 Hz); G, H-5; H, Ser H_α; I, H-6; J, H6′; K, Ala-3 H_α; L, Ser H_β; M, Ala-1-H_α; N, Ala-1 Me; O, Ala-3 Me].

applied to the successive purification of the glycosylated Fmoc-Ser-O-Pfp-ester.

The glycosylation of Fmoc-Ser-O-Pfp with tetrabenzoyl-α-D-galactopyranosyl bromide in dichloromethane was carried out at −30 °C. The reaction was promoted by silver trifluoromethanesulphonate and a reaction time of 3 h was found necessary for the completion of the glycosylation. Then 2,4,6-collidine was added to the reaction mixture, which was allowed to warm up to room temperature and purified by chromatography by eluting (28% dry ethyl acetate in light petroleum) on dried silica gel to yield 74% of Fmoc-[Bz_4-β-D-Gal*p*-(1→3)-]Ser-O-Pfp (**1**) (Bz = benzoyl). Short reaction times as reported by Norberg *et al.*[28] and by Bock *et al.*[29] for less demanding glycosylations were insufficient and led to the formation of large amounts of the orthoester (**2**) after the addition of collidine. When the allyl ester of Fmoc-Ser-OH was used as the glycosyl acceptor the expected β-glycoside was immediately formed.[27] The long reaction time observed with the Pfp-ester may be attributed to steric hindrance. At the low temperature no byproducts could be observed and the extended reaction times presented no problems. The contact with 2,4,6-collidine did not result in any racemisation of the α-carbon according to 500 MHz ^{1}H NMR and 125.8 MHz ^{13}C NMR spectroscopy in which the diastereoisomers are expected to yield two different sets of chemical shifts. This result was confirmed in the spectra of the oligopeptide products.

Fmoc-[Bz_4-β-D-Gal*p*-(1→3)-]Ser-O-Pfp (**1**) was used in the assembly of antifreeze glycopeptide analogues, H{Ala-[Bz_4-β-D-Gal*p*-(1→3)-]Ser-Ala-}$_n$-OH, (*n* = 1, 2, and 3) (**3**)—(**5**), on 500 mg Macrosorb SPR-250 resin by the continuous flow method.[30] The first amino acid was coupled to a hydroxymethylphenoxyacetamide linker *via* the symmetrical anhydride by catalysis with 4-*N*,*N*-dimethylaminopyridine (DMAP). Deprotection of the α-amino group was effected by a 30 min treatment with 50% morpholine in dimethylformamide (DMF) throughout the synthesis. All alanines were added as Dhbt esters (2.5 equiv.), with 30 min reaction times. The Fmoc-[Bz_4-β-D-Gal*p*-(1→3)-]Ser-O-Pfp (**1**) (2 equiv.), was coupled with Dhbt-OH as an auxiliary nucleophile added to the column prior to recirculation of the ester. By visual inspection of the yellow colour which had developed in the resin column[31] the coupling reaction was found to be quantitative after 2.5 h reaction time, but the column was left overnight. Resin samples were withdrawn at the tripeptide (80 mg) and the hexapeptide (250 mg) stage.

The *O*-glycopeptides were cleaved off the resin with 95% trifluoroacetic acid (TFA) which did not lead to any detectable cleavage of the *O*-glycosidic bond according to HPLC. The crude peptides were purified by reversed phase HPLC to yield the tripeptide (**3**) (7 mg), the hexapeptide (**4**) (33 mg), and the nonapeptide (**5**) (44 mg). The stucture of (**1**)—(**5**) was confirmed by 500 MHz 1D and 2D NMR spectroscopy. In Figure 1 the assigned 1D ^{1}H NMR spectra of all three glycosyl peptides are shown. The proton chemical shift of the glycosyl units does not vary significantly. In a phase sensitive 2D-COSY spectrum all the expected correlations between α-protons and the backbone amide protons were observed. The complete deprotection of the glycosylpeptides is currently under investigation.

The approach to the synthesis of *O*-glycopeptides described

in this work can be considered a simple and valuable alternative to the known methods in solid phase glycopeptide synthesis.

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