Improved Method for the Synthesis of *o*-Glycosylated Fmoc Amino Acids to be used in Solid-phase Glycopeptide Synthesis (Fmoc = fluoren-9-ylmethoxycarbonyl)

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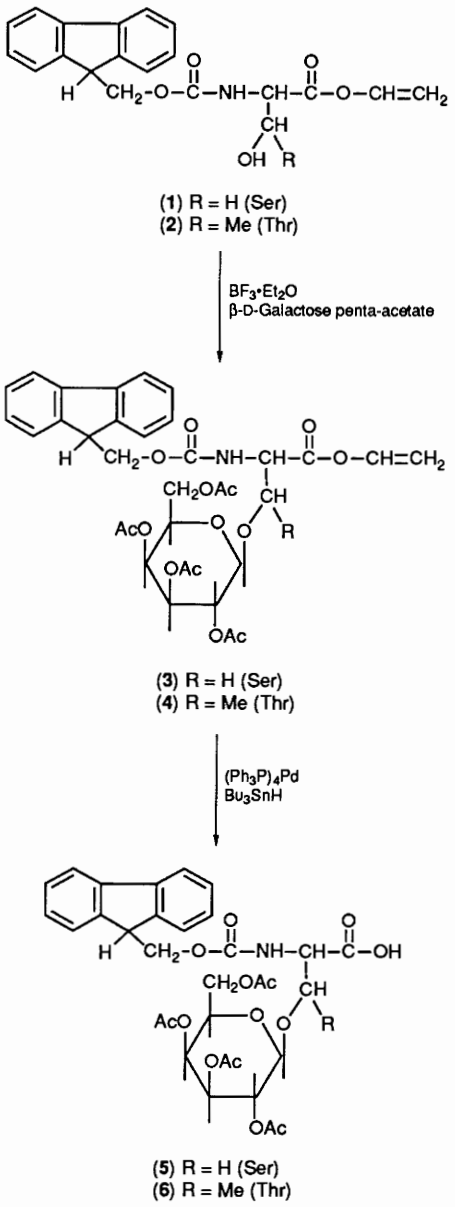
The building blocks O^1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-N^{α}-(fluoren-9-ylmethoxycarbonyl)serine (**5**) and O^1-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-N^{α}-(fluoren-9-ylmethoxycarbonyl)threonine (**6**) for use in solid-phase glycopeptide synthesis can be obtained *via* their allyl esters by mild treatment with tetrakis(triphenylphosphine)palladium(0) and tributyltin hydride with no Fmoc elimination or sugar cleavage or anomerization.

Solid-phase peptide synthesis (SPPS)[1,2] is the most useful approach for the rapid preparation of peptide analogues. Owing to the growing evidence of the important biological role of glycopeptides and glycoproteins, development of solid-phase glycopeptide synthesis (SPGPS) has been concentrating efforts of peptide chemists in recent years.[3—5] Improvements have been achieved concerning both the handle to which the first amino acid is coupled[6] and the preparation of the appropriate glycosylated building blocks.[4a,7] Owing to the sensitivity of the glycosidic bond



towards acids and bases[8] the Fmoc procedure[9] is the methodology of choice.[4—6] In the literature, the preparation of *O*-glycosylated N^{α}-fluoren-9-ylmethoxycarbonyl amino acids includes either the introduction of the Fmoc group in a final step after glycosylation of the amino acid *N*- and *C*-protected with hydrogen-sensitive groups[4b,5] or final cleavage of acid labile esters of the Fmoc *N*-protected glycosylated derivatives.[4a,7] Such routes are either too long or not fully compatible with the glycosidic bond. Other groups labile under mild conditions such as allyl esters have been successfully used in glycopeptide synthesis in solution[3,10] and would be more appropriate than the acid-labile esters mentioned as a second approach. Nevertheless, the use of this group together with Fmoc *N*-protection has been hampered by the presence of nucleophiles such as morpholine in the standard cleavage media. In the present work we propose the use of allyl esters as *C*-protecting groups and their hydrostannolytic cleavage.[11] We show that such conditions are fully compatible with both Fmoc *N*-protection and the glycosidic bond.

Table 1. Analytical data for compounds (**1**)—(**6**).

Compound	HPLC[a] t_R/min	HPLC[a] k	FAB MS:[b] $M+1, M+23$	^{13}C NMR (δ)[c] (galactose C-1 shifts)
(**1**)	22.6	7.7	368.4, 390.5	
(**2**)	23.6	8.1	382.4, 404.3	
(**3**)	26.0	9.0	698.7, 720.7	101.6
(**4**)	26.7	9.3	712.7, 734.7	99.5
(**5**)	22.7	7.7	658.6, 680.5	101.8
(**6**)	23.5	8.0	672.7, 694.7	100.4

[a] HPLC C_{18} column (10 μm, 0.4 × 25 cm) eluted with 0.1% aqueous TFA–0.1% TFA in MeCN, linear gradient mode 10—100% organic solvent over 30 min at a flow rate of 0.9 ml/min. λ = 215 nm. [b] FAB MS: Samples dissolved in glycerol and bombarded with 8 keV xenon atoms and the ions produced accelerated through 8 kV. [c] ^{13}C NMR: 50 MHz, $CDCl_3$. No α-anomer C-1 signals (3—6 ppm upfield) were detected.

Compounds (**1**) and (**2**) were obtained from Fmoc-Ser-OH and Fmoc-Thr-OH respectively, using the caesium salt[12] and allyl bromide. Glycosylation of (**1**) and (**2**) was performed using the neighbouring assisted procedure[13] with $BF_3{\cdot}Et_2O$ as Lewis acid catalyst.[14] A solution of (**1**) or (**2**) (2.7 mmol) in CH_2Cl_2 was cooled to 0 °C, $BF_3{\cdot}Et_2O$ (13.6 mmol) was added dropwise, then β-D-galactose penta-acetate (8.1 mmol) was added in three portions at different times. The temperature was kept below 10 °C for a total reaction time of 6 h. After purification by reverse-phase silica gel medium pressure LC (MPLC) under the conditions stated below, compounds (**3**) and (**4**) were obtained with overall yields of 47% (0.89 g) and 65% (1.26 g), respectively. The final deprotection was carried out by successively adding $(Ph_3P)_4Pd$ (16 mg) and Bu_3SnH (227 μl) to a solution of (**3**) (0.71 mmol) or (**4**) (0.70 mmol) in CH_2Cl_2 (25 ml).[11] This reaction proceeded smoothly at room temperature to give the final building blocks (**5**) and (**6**) in quantitative yields within 30 min as ascertained by analytical HPLC using the elution conditions given below. After solvent evaporation, the resulting tributyltin derivatives were dissolved in ethyl acetate and the organic solutions washed with 1 M HCl and water, and dried over Na_2SO_4. After solvent elimination, compounds (**5**) and (**6**) were also purified by reverse-phase liquid chromatography with recoveries of 85% (397 mg) and 87% (413 mg), respectively. Purification conditions were: 4 × 1 g load of (**3**) and (**4**), 0.5 g load of (**5**) and (**6**); Lichroprep C_{18} column (40—63 μm; 2.5 × 30 cm) eluted under isocratic conditions with 0.05% aqueous TFA–0.05% TFA in MeCN (2 : 3) at a flow rate of 17 ml/min (TFA = CF_3CO_2H). Compounds (**3**)—(**6**) were found to be homogeneous by analytical HPLC, C_{18} column (10 μm; 0.4 × 25 cm) eluted with 0.1% aqueous TFA–0.1% TFA in MeCN, gradient mode 10—100% organic solvent over 30 min at a flow rate of 0.9 ml/min with detection at different wavelengths, and analytical TLC, compounds (**3**) and (**4**): di-isopropyl ether/acetic acid (87 : 13) R_f 0.32, hexane/ethyl acetate (1 : 4) R_f 0.53; compounds (**5**) and (**6**): hexane/ethyl acetate/acetic acid (17 : 80 : 3) R_f 0.33. Compounds (**3**)—(**6**) showed correct FAB mass spectra. The β-configuration of compounds (**3**) and (**4**) was fully preserved in (**5**) and (**6**) as ascertained by ^{13}C NMR spectroscopy. Relevant analytical data for (**3**)—(**6**) are summarized in Table 1.

The stability of Fmoc-Ser-OH and Fmoc-Thr-OH under hydrostannolytic conditions had previously been proved by analytical HPLC under the conditions given above, and TLC ($CHCl_3$/MeOH/acetic acid, 95 : 5 : 3) with ninhydrin detection.

After 4 h treatment no free amines were detected. Moreover, during hydrostannolytic elimination of the allyl group no cleavage of the glycosidic bond was detected by HPLC.

In conclusion, the hydrostannolytic cleavage of the allyl ester in the absence of other stronger nucleophiles has been shown to be fully compatible with Fmoc *N*-protection and the β-glycosidic bond. In consequence, we propose a very short reaction pathway without any strong acidic or basic treatment for the preparation of *O*-glycosylated building blocks to be used in Fmoc-based solid-phase glycopeptide synthesis. The preparation of other similar building blocks by the same procedure as well as the synthesis of new glycosyl neuropeptides using the glycosylated amino acids described here is under way.

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