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Solid-Phase *N*-Glycopeptide Synthesis Using Allyl Side-Chain Protected Fmoc-Amino Acids[1]

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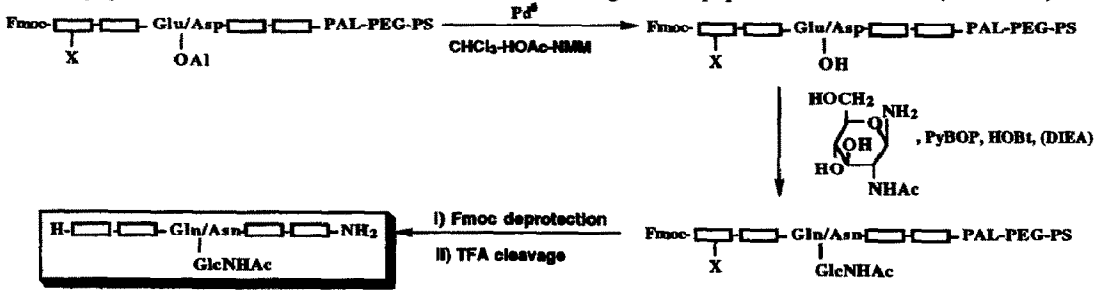
Abstract: *A method for the preparation of glycopeptides using a three-dimensional orthogonal solid-phase strategy (Fmoc/tBu/allyl) with selective allyl removal and direct coupling of glycosylamines to the carboxyl function while the peptide is attached to the resin is described.*

Interest in glycopeptides and glycoproteins has increased due to their involvement in important biological recognition phenomena and transport processes.[2] These complex natural products possess a challenge to synthesize due to the sensitivity of the glycosidic bonds to strong acids and bases. There are two general methods for the preparation of glycosylated peptides *via* solid-phase synthesis. The carbohydrate can be introduced *via* an *O*- or *N*-glycosylated amino acid[2b,3] or a post synthetic glycosylation of resin-bound peptide having free hydroxyl or carboxylic acid function.[4]

Using the latter method to prepare *N*-glycopeptides, it is mandatory to have a side-chain protecting group that is stable to stepwise elongation of the peptide and is removed before the glycosylation. Thus, a third level of orthogonality to the protection scheme would assist in the construction of these complex peptides. An allyl/Fmoc/*t*Bu protection scheme provides a mild and flexible orthogonal strategy. The potential of allyl chemistry was recognized in solid-phase peptide chemistry as a handle, which served to attach the growing peptide chain to the resin, to prepare glycopeptides containing sensitive *O*-glycosidic bonds.[5] The use of allyl side-chain protected Fmoc amino acids for the preparation of cyclic, branched and MAP peptides has been described.[6] Recently developed mild conditions to deprotect allyl functions by automation are compatible with classical Fmoc/*t*Bu-based methods, occur quantitatively, and avoid undesired side reactions.[7] Our strategy for the preparation of *N*-glycopeptides allows assembly of the peptide linked to the resin, followed by selective removal of the allyl ester of the carboxylic acid function, coupling of the glycosylamine while the peptide is still attached to the resin, and final cleavage of the peptide from the resin (Scheme 1).



Scheme 1

To demonstrate the effectiveness of this strategy, Ac-Asn(β-D-GlcNHAc)-Val-Phe-NH_2 and H-Tyr-Gln(β-D-GlcNHAc)-Gly-Phe-Pro-NH_2 were selected. Peptide elongation was carried out in the C→N direction on polyethylene glycol-polystyrene graft (PEG-PS) resin *via* a PAL linker on a Millipore 9050-Plus continuous-flow synthesizer.[8] Deprotection of the allyl ester was accomplished with $Pd(PPh_3)_4$ (3 equiv) in a solution of $CHCl_3$–HOAc–NMM (37:2:1) for 2 h at 25 °C and washing with a solution of DIEA (0.5% v/v) and sodium diethyldithiocarbamate (0.5% w/v) in DMF, followed by PyBOP/HOBt/DIEA-mediated

glycosylation,[9] then N^{α}-Fmoc removal, and cleavage of the peptide from the resin with TFA–H_2O (9:1). Analysis of the crude products by HPLC (Fig. 1) indicated that both glycopeptides were obtained in good yields with no evidence of non-glycosylated peptides. For the Asn-containing peptide (Fig. 1a), the major impurity (12%) was identified as the aspartimide. Presumably, this by-product was formed during the PyBOP/HOBt/DIEA-mediated coupling of the sugar to the peptide. Therefore, omission of DIEA from the coupling and the use of an excess of sugar as a source of base (10 equiv) reduced the level of impurity to 7%.

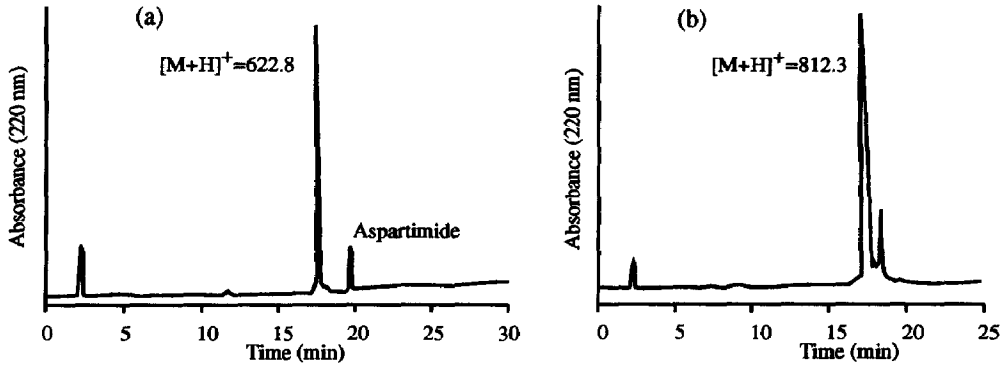


Figure 1. HPLC chromatograms of crude peptides directly after cleavage: a) Ac-Asn(β-D-GlcNHAc)-Val-Phe-NH_2; b) H-Tyr-Gln(β-D-GlcNHAc)-Gly-Phe-Pro-NH_2; Analysis conditions: C_{18}, linear gradient over 20 min of CH_3CN/0.1% TFA and H_2O/0.1% TFA from 2:98 to 1:1, flow rate 1.0 mL/min. Mass spectra were recorded on Waters prototype laser desorption-time-of-flight instrument.

To conclude, the use of an allyl/Fmoc/*t*Bu protection scheme was used as a method for the preparation of *N*-glycopeptides, thus increasing their availability for the exploration of biological processes. Removal of DIEA in the coupling of the glycoside to the peptide reduced the amount of aspartimide formation.

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References and Notes

1) Abbreviations used are as follows: Al, allyl; DIEA, *N,N*-diisopropylethylamine; Fmoc, 9-fluorenylmethyloxycarbonyl; GlcNHAc, 2-acetamido-1-amino-1,2-dideoxy-β-D-glucopyranose (*N*-acetyl-D-glucosamine); HOBt, 1-hydroxybenzotriazole; MAP, multiple antigenic peptide; NMM, *N*-methylmorpholine; PAL, 5-(4-Fmoc-aminomethyl-3,5-dimethoxyphenoxy)valeric acid; PEG, polyethylene glycol; PyBOP, benzotriazol-1-yloxytripyrrolidinophosphonium hexafluorophosphate; PS, polystyrene.

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9.) A typical glycosylation experiment was carried out as follows: Peptide resin (~ 100 mg) was suspended in DMF (1 mL), following which PyBOP (5 or 10 equiv), HOBt (5 or 10 equiv), 2-acetamido-1-amino-1,2-dideoxy-β-D-glucopyranose (5 or 10 equiv) and DIEA (10 equiv for reactions using 5 equiv of PyBOP and none for reactions using 10 equiv of PyBOP) in DMSO (1 mL) were added and the mixture was allowed to stand for 2 h at 25 °C.

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