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POLYETHYLENE GLYCOL BOUND BENZYL- AND FLUORENYL DERIVATIVES AS SOLUBILIZING SIDE-CHAIN PROTECTING GROUPS IN PEPTIDE SYNTHESIS

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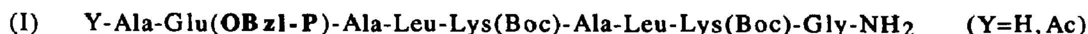
ABSTRACT: The covalent attachment of polyethylene glycol (PEG) to commonly used protecting groups and their use in peptide synthesis was examined. PEG bound to fluorenyl- and benzyl- type side chain protection for Lys and Glu results in increased solubility of hydrophobic peptides due to a distruption of β-sheet conformations. PEG-protected peptides can be subject to HPLC purification and further used in convergent strategies for the synthesis of large peptides.

Polyethylene glycol (PEG) has been previously introduced as solubilising C-terminal protecting group in stepwise peptide synthesis ('liquid-phase-method')[1]. Recently, the N-terminal fixation of PEG to carrier bound peptides in Solid Phase Peptide Synthesis (SPPS) has been proposed[2]. The attachment of PEG blocks to amino acid side chains in the centre of oligopeptides results in a disruption of β-sheet conformations, which is considered to be the major source of aggregation and low solubility of hydrophobic peptides[3]. Consequently, the attachment of PEG to commonly used amino acid protecting groups should represent an attractive tool for overcoming the problem of low solubility of fully protected peptides during both SPPS and in solution synthesis. In this work, we describe the chemical synthesis of PEG side-chain protecting groups of the fluorenyl, benzyl- and carboxylated-type for Glu and Lys and their subsequent incorporation into peptides.

The synthesis of the PEG compounds and their introduction in N^{α}-Fmoc or Boc protected Glu and Lys derivatives are shown in Scheme 1. The PEG_{550} carboxylic acid[4] reacts with the functionalized side-chain protecting groups **2** and **6** to result in the PEG derivatives **3** and **7**, respectively, in high yields[5]. The subsequent introduction of **3** and **7** as side-chain protection of Glu **4**,**8** and Lys **5** was achieved in overall yields of 60-80%. The chemical stability of the PEG-protected amino acid derivatives **4**,**5**,**8** proved to be comparable to their conventionally protected form, since the Bzl-P and Cbo-P protecting groups in **4** and **5** respectively, were removed by strong acid (10%TFMSA/TFA, 60min), whereas Fm-P in compound **8** was readily cleaved by piperidine (1N in CH_2Cl_2, 30min).

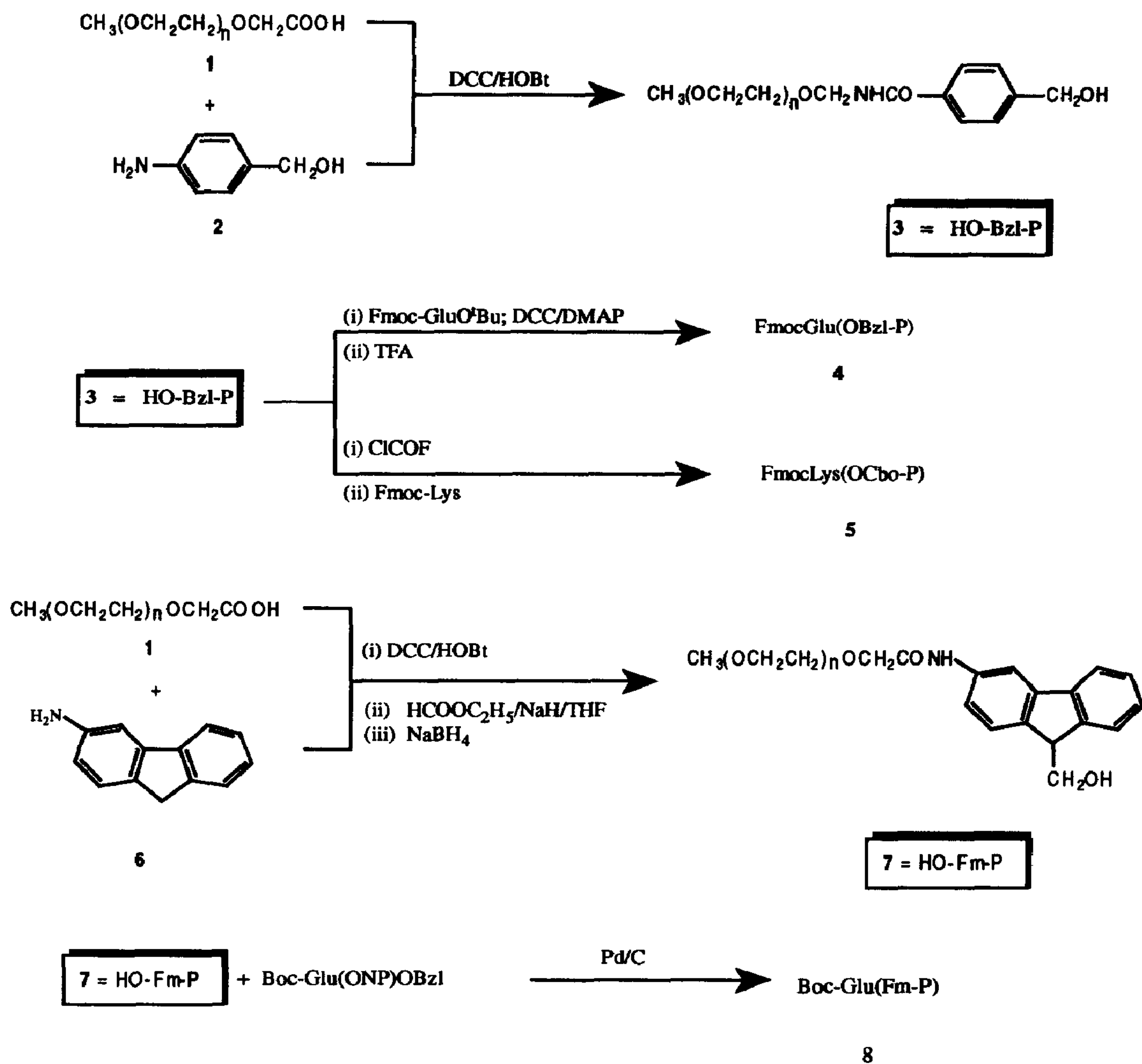
In order to evaluate the propensity of the PEG derivatives for their use in SPPS[6], the model peptide (I)



(I) Y-Ala-Glu(**OBzl-P**)-Ala-Leu-Lys(Boc)-Ala-Leu-Lys(Boc)-Gly-NH_2 (Y=H, Ac)

was built up applying standard Fmoc-strategy[7] on an acid labile-resin[8], since it was shown that the Fmoc/OtBu strategy is fully compatible with the PEG system[2].

Scheme 1. Synthesis of PEG protected amino acids



During the coupling reactions, no significant difference in the coupling kinetics of the PEG-Glu derivative or subsequent residues compared to their PEG-free analogues was observed. The fully protected nonapeptide (I) and its shorter chain analogues were analyzed by RP-HPLC, following cleavage from the resin. As depicted in Fig. 1, all peptides elute as individual sharp peak; most notably, the PEG chain did not level off the differences in the physico-chemical properties of the homologues, as exemplified by the separation of the N-

terminally acetylated nonapeptide 9' from the truncated sequences 8' or 7'. Interestingly, the presence of an N-terminal charge (peptides 7,8,9,) exerts a much stronger effect upon the retention time of the peptide than does the PEG chain, as seen from the differences in retention times of peaks 9 and 9' (Fig. 1) . Consequently, peptides protected by PEG can be readily purified by standard HPLC conditions and show increased solvation characteristics when incorporated into SPPS.

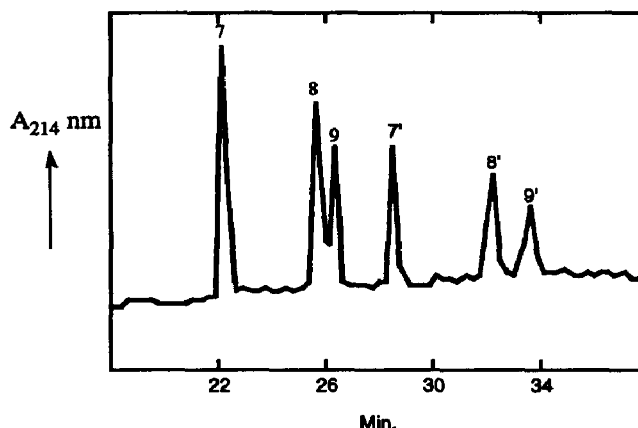


Figure 1. RP-HPLC (C_{18}) of model nonapeptide (I) 9 and its truncated sequences 8 and 7. 9' corresponds to N-terminally acetylated analogues and its shortened sequences 8' and 7'.

The dramatic increase in solubility was further demonstrated by the SPPS of the host-guest peptide (II)

(II) Ac-$[Ala]_y$-Glu(X)-$[Ala]_4$-NH_2

Whereas the PEG-protected peptide (X=OBzl-P) could be readily built up to the tetradecapeptide (y=9), the corresponding conventionally protected analogue (X=OBzl) proved only to be accessible up to the decapeptide (y=5). The rational behind this result stems from the preferred conformation of these peptides as revealed by the CD spectra (Fig. 2). While the Glu-benzyl protected peptide adopts a β-sheet conformation starting from y=4, the PEG-protected and Glu-deprotected analogues rather show partially helical conformations. Obviously, the incorporation of one single PEG block prevents the onset of an intermolecularly associated β-sheet conformation, resulting in a dramatic increase in the solvation and coupling kinetics of the growing peptide chain. We conclude, that PEG side-chain protecting groups represent a valuable tool for the chemical preparation of large peptide chains.

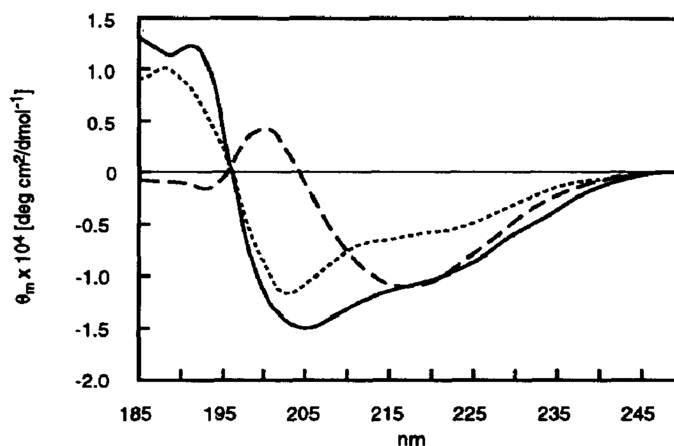


Figure 2. CD spectra of host - guest peptides Ac[Ala]$_4$-Glu(X)-[Ala]$_4$-NH$_2$ (1mg ml-1 TFE), (——) : X = (OBzl-P); (— —) : X = (OBzl); (----) : X = (OH)

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5. (a) Synthetic procedure to **HO-Bzl-P** 3 : see ref 3(a) .
   (b) Synthetic procedure to **HO-Fm-P** 7 : (i) 17.9g (30.4 mmol) of **1** (M_w = 550) was added to a mixture of 4.1g (30.4 mmol) HOBt and 3.8g (30.11 mmol) of DICI in 25ml dichloromethane (DCM). 5g (27.6 mmol) fluorenyl-2-amine **6** in 25ml DMF was added, and the mixture was stirred overnight at rt. The solvent was evaporated, the residue was taken up in DCM, washed with bicarbonate solution, and purified by silica gel chromatography (DCM) to give 15.2g (19.7 mmol) of intermediate compound (71%, oil). (ii) 15g (19.4 mmol) of this compound was dissolved in 100ml THF, and 3.8g (95 mmol) of NaH- dispersion (60%) was added with caution. The suspension was stirred slowly and 5ml (62.5 mmol) ethylformate in 5ml THF was added. The reaction mixture was heated under reflux for 1h, during which the colour changed to dark green. The reaction mixture was cooled to rt, put on 150g ice water and acidified with conc. acetic acid to pH 4. The water phase was extracted into DCM and purified by silica gel chromatography (DCM/MeOH 10/1) to give 9.7g (12.1 mmol) of oily product (62%). (iii) 6.8g (85 mmol) of the oil was dissolved in 100ml DCM and 1.3g (34 mmol) $NaBH_4$ was added. The solution was stirred overnight at rt, washed with 1N HCl, and dried over Na_2SO_4 to give 4.8g (5.6 mmol) of **7** (66%, oil). Product **7** was fully characterized by ^{1}H NMR, IR and mass spectrometry.
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