An Enzyme-scissile Linker for Solid-phase Peptide Synthesis

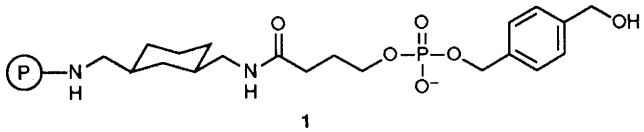
Donald T. Elmore,*[a] David J. S. Guthrie,[b] Andrew D. Wallace[c] and Stephen R. E. Bates[d]

[a] *Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, UK*
[b] *School of Biology and Biochemistry, Queen's University of Belfast, Northern Ireland BT9 BL*
[c] *Istituto di Recherche di Biologie Molecolare, Via Pontina KM30.600, 00040 Pomezia (Roma), Italy*
[d] *Applied Biosystems Ltd., 7 Kingsland Grange, Woolston, Warrington, Cheshire, UK*

We describe a new linker that contains a phosphodiester moiety for solid-phase peptide synthesis; after completion of the cycle of coupling and deprotection steps, the phosphodiester can be cleaved with phosphodiesterase.

Most methods for detaching peptides from the matrix used for solid-phase peptide synthesis involve acidolysis. If the α-amino group of each amino acid residue introduced into the synthetic peptide is protected by an acid-labile group, there is a real risk that a substantial fraction of the peptide will be detached by the end of the cycle of coupling and deprotection steps. This is one reason for the popularity of a base-labile group such as fluoren-9-ylmethoxycarbonyl (Fmoc). Some methods for detaching peptides from matrices without resorting to exposure to acids have been reported,[1–9] but enzymes have not previously been used for this purpose. The methodology described herein provides a procedure for deferring peptide detachment until after all protecting groups have been removed. Alternatively, convergent solid-phase peptide synthesis involves the assembly of peptide fragments of a larger molecule and detachment from the matrix with protecting groups on side chains intact ready for fragment coupling.[10] Photolabile linkers have been used for this purpose. The enzyme-cleavable linker reported here provides an alternative procedure. We have synthesized the linker **1** that is cleavable by a phosphodiesterase.

1,3-Bis(aminomethyl)cyclohexane was allowed to react with γ-butyrolactone to give 1-*N*-(4′-hydroxy)butanoylaminomethyl-3-aminomethylcyclohexane as a viscous oil. Pepsyn K was allowed to react with an excess of the latter in *N*,*N*-dimethylformamide (DMF) with stirring at 30 °C for four days. (A polyacrylamide resin, of which pepsyn K is an example, is required for use with enzymes in aqueous solution.) The derivatized resin was next phosphorylated with an equimolar amount of 4-nitrophenyl phosphorodichloridate in dry DMF–pyridine with agitation during 27 h. The dark coloured mixture was filtered and washed with DMF–pyridine to give a yellow solid. The product was next allowed to react with an excess of 1,4-bis(hydroxymethyl)benzene in DMF–pyridine (1 : 1) during 40 h. Finally, the 4-nitrophenyl ester group was removed by suspending the resin in a mixture of DMF, piperidine and water (1 : 1 : 1 v/v) for 3 h. The resin **1** was washed with water and dried over P_2O_5. It was found by digestion with phosphomonoesterase that about 8% of the total phosphate content was present as a monoester owing to incomplete reaction at the penultimate stage. When the resin **1** was digested first with bovine spleen phosphodiesterase at pH 5.7 and then with alkaline phosphomonoesterase, 0.175 mmol (g resin)$^{-1}$ of inorganic phosphate was liberated corresponding to a potential content of 0.3 mmol (g resin)$^{-1}$ based on the ester content of the original pepsyn K.



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β-Casomorphin was synthesized on the phosphodiester matrix using symmetrical anhydrides of α-*N*-Fmoc amino acids. The side chain of Tyr was protected as the *tert*-butyl ether. The Fmoc group was removed by treatment with DMF–piperidine (4 : 1) for 3 min. The *O*-Bu^t group was removed with CF_3CO_2H in presence of 0.5% PhSMe (this additive may be unnecessary in the light of the work by Johnson and Sheppard[9]). The peptide was detached from the resin by digestion with calf spleen phosphodiesterase in the presence of iodoacetamide to inhibit irreversibly a small amount of a thiol proteinase present as an impurity in the phosphodiesterase. The peptide ester was treated with HBr in $MeCO_2H$ (33% w/v) and then was indistinguishable by HPLC from an authentic specimen of β-casomorphin. Leu-

enkephalin (53%) was synthesized and identified by an analogous procedure. A collagenase substrate, Fmoc-Ala-Pro-Gly-Leu-Ala-Gly-OH (83%) was similarly synthesized. The improved yield in this latter example is believed to be due to an extended exposure to phosphodiesterase (7 days instead of 1 day) in the cleavage step.

Should the *p*-hydroxymethylbenzyl ester of a peptide be required, for example as an enzyme substrate, any residual phosphate ester on the peptide can be removed by treatment with alkaline phosphomonoesterase after detachment of the peptide derivative from the matrix with phosphodiesterase. Finally, it is likely that the presence of a charged group in the linker may decrease the risk of the assembled peptide folding back on the linker and resin thereby possibly sterically impeding some coupling steps.

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