A Novel Modification of Peptides by Amino-acid Insertion

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Summary A reaction sequence for the insertion of amino-acid residues into peptides has been developed using *N*-ethylacetamide and a Gly-Gly derivative as models

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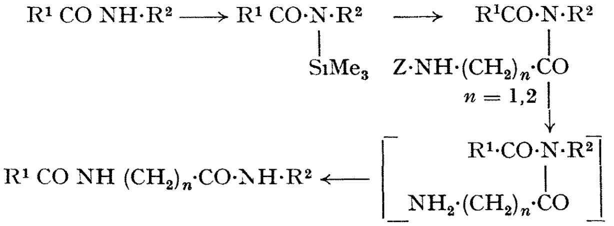
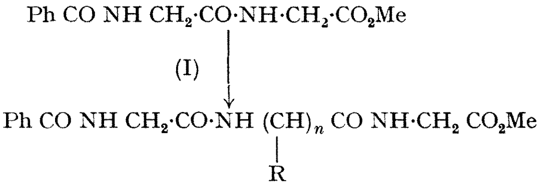
THERE is earlier evidence of intramolecular interactions of amides with hydroxy- or amino-groups in both linear and cyclic systems[1] This has led us to investigate the possibility of utilising such a reaction, under mild conditions, for the modification of peptides by the insertion of single amino-acid residues The following sequence has been studied



$$R^1\,CO\,NH\cdot R^2 \longrightarrow R^1\,CO\cdot\underset{\displaystyle SiMe_3}{N}\cdot R^2 \longrightarrow R^1CO\cdot\underset{\displaystyle Z\cdot NH\cdot(CH_2)_n\cdot CO}{N}\cdot R^2 \xrightarrow{n\,=\,1,2} \left[\,R^1\cdot CO\cdot\underset{\displaystyle NH_2\cdot(CH_2)_n\cdot CO}{N}\cdot R^2\right] \longrightarrow R^1\,CO\,NH\,(CH_2)_n\cdot CO\cdot NH\cdot R^2$$

Preliminary investigations with *N*-ethylacetamide established that such an insertion process could be achieved in high yield by mild reactions at room temperature Silylation of the amide (trimethylchlorosilane–triethylamine, 90% yield) was followed by acylation (80—90% yield) with the chloride of benzyloxycarbonyl glycine (or β-alanine) using similar conditions to those which have been applied to lactams[2] Deprotection of the *N*-acyl derivative by hydrogenolysis, gave the rearrangement product in excellent yield (85%)

When this reaction sequence was applied to the glycylglycine derivative (I), using glycine, L-alanine, L-tyrosine, or β-alanine for insertion, the corresponding tripeptides (II, III) were obtained The identities of these products

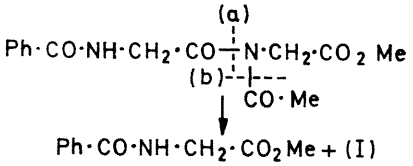


$$Ph\,CO\,NH\,CH_2\cdot CO\cdot NH\cdot CH_2\cdot CO_2Me \xrightarrow{(I)} Ph\,CO\,NH\,CH_2\cdot CO\cdot NH\,\underset{\displaystyle R}{(CH)_n}\,CO\,NH\cdot CH_2\,CO_2Me$$

(II) $n = 1$, R = H, Me, HO·Ph·CH_2·
(III) $n = 2$, R = H

were established by comparison with material synthesised by conventional methods In the case using L-alanine, the product Bz·Gly·L-Ala·Gly·OMe was shown to have the same rotation ($[\alpha]_D^{24}$ −44°, *c* 0·05, MeOH) as that of the same tripeptide derivative prepared by sequential coupling using dicyclohexylcarbodi-imide, evidently no significant degree of racemisation had occurred in the course of the insertion process

It is of interest that the acylation of the diamide (I) by this method took place preferentially at one amide bond. There was evidence of traces of the di-acyl derivative (and the corresponding tetrapeptide derived by insertion of two residues) but the main product was the mono-*N*-acyl derivative of the amide function linking the two glycine residues. This assignment of structure was supported by the identification of *N*-benzoylglycine methyl ester ('a' cleavage) and the peptide (I) ('b' cleavage) as major products of methanolysis of the *N*-acetylated peptide (IV).[3]



(IV)

$$\mathrm{Ph{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO}\overset{\text{(a)}}{\vdots}\underset{\overset{\text{(b)}\,\vdots}{\mathrm{CO{\cdot}Me}}}{\mathrm{N}}\mathrm{{\cdot}CH_2{\cdot}CO_2Me} \quad \text{(IV)}$$

$$\downarrow$$

$$\mathrm{Ph{\cdot}CO{\cdot}NH{\cdot}CH_2{\cdot}CO_2Me} + \text{(I)}$$

This indication of preferential insertion, and some earlier evidence of preferential silylation of peptides,[4] enhances the interest in the possibility of using this reaction sequence for the modification of polypeptides and proteins.

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