The Safety Catch Principle in Solid Phase Peptide Synthesis

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Summary *N*-Protected amino-acid active esters react readily with polystyrenesulphonamide yielding, after deprotection, the poly(aminoacylsulphonamides): these polymers are suitable for solid phase peptide synthesis, the completed peptide being cleaved from the resin by methylation and hydrolysis or aminolysis.

THE stability of the resin–peptide bond is a critical feature of Merrifield's solid phase peptide synthesis.[1,2] This bond must withstand the repeated acidic cleavage of amino-protecting groups, and yet at the end of the synthesis be itself cleaved under acceptably mild conditions. It seemed that this was an ideal situation in which to apply the 'safety catch principle', *i.e.* that an otherwise stable bond is eventually labilised at the appropriate moment by a specific chemical modification. Such a safety catch was discerned in the reactions of acylsulphonamides (I).[3,4] These compounds are resistant to alkaline hydrolysis because basic attack ionises the acidic NH group (pK_a *ca.* 2·5), but the *N*-methyl derivatives (II) (obtained by the action of diazomethane) are rapidly cleaved under very mild conditions.

$$\underset{\text{(I)}}{RCO{\cdot}NH{\cdot}SO_2Ar} \xrightarrow{CH_2N_2} RCO{\cdot}NMe{\cdot}SO_2Ar \xrightarrow{OH^-} \underset{\text{(II)}}{RCO_2^-} + MeNH{\cdot}SO_2Ar$$

We have developed a new solid phase system for the synthesis of peptides which utilises the acylsulphonamide bond for the attachment of the peptide to the resin.†

† Marshall and Liener[5] have recently described an oxidative method for labilising a peptide–resin bond.

Within certain limitations, this system allows the preparation of terminal and side chain protected peptides, as well as their amides and hydrazides. Such derivatives are sometimes not accessible by the usual Merrifield procedure.

Fully sulphonated styrene–2% divinylbenzene copolymer (Bio-Rad AG 50W-X2) was converted, by treatment with chlorosulphonic acid and then aqueous ammonia, into the polysulphonamide. About 7% of the sulphonic acid groups were inaccessible to these reagents and remained unchanged; their presence did not interfere with the subsequent steps. Treatment of the sulphonamide resin in dimethylformamide with the 2,4,5-trichlorophenyl esters of various t-butoxycarbonyl- or benzyloxycarbonyl-amino-acids (2 equiv. based on total sulphonamide) and triethylamine (1 equiv. based on sulphonamide + sulphonic acid) gave up to 25% incorporation of the amino-acid (*ca.* 1 mmol/g) as the acylsulphonamides. Further acylation of the resin sulphonamide seemed to be inhibited by electrostatic effects within the resin matrix, ionisation of the more acidic[4] acylsulphonamide groups inhibiting ionisation (and hence acylation) of the residual sulphonamide. This effect was also observed in only partially sulphonated resins. In practice, acylation of the resin was usually restricted to 0·2—0·7 mmol/g (usually $<0{\cdot}25$ mmol/g for benzyloxycarbonyl derivatives‡) by adjustment of the activated ester and base concentrations, *e.g.*, 0·2 equiv. of t-butoxycarbonylglycine 2,4,5-trichlorophenyl ester and 1 equiv. of triethylamine gave resin containing 0·70 mmol/g of glycine after reaction for 48 h at room temperature.

The acylsulphonamide bond is completely stable to the acidic conditions (trifluoroacetic acid or HBr–AcOH, respectively) used for the removal of t-butoxycarbonyl and benzyloxycarbonyl protecting groups. After acidic treatment, the resins were washed well with chloroform, chloroform–5% triethylamine, chloroform, chloroform–1% acetic acid, chloroform, and then acetone, leaving the amino-acylsulphonamide in the zwitterionic form (III). Addition of the second and successive amino-acid residues was carried out using trichlorophenyl ester derivatives as before,

$$\overset{+}{N}H_3\cdot CHR\cdot CO\cdot \overset{-}{N}\cdot SO_2\text{–polystyrene}$$

(III)

but in the presence of the weak base 2,6-lutidine rather than triethylamine. Under these conditions, no evidence could be obtained for the acylation of the initially unchanged free sulphonamide, whereas reaction with the primary amino-group was usually complete with 3 equiv. of active ester within 24 h.§

For the removal of the completed acylpeptide from the resin, the acylsulphonamide bonds were first labilised by *N*-methylation with diazomethane in ether–acetone.¶ As expected, the protected peptide could then be easily removed from the resin by alkaline hydrolysis (1 equiv. 0·5N-NaOH), by aminolysis (an excess of 0·5N-NH_3 in dioxan), or by hydrazinolysis (3 equiv. methanolic hydrazine).

The method has been tested by the preparation of a number of peptide derivatives, *e.g.* (figures given in parenthesis are the yields before and after purification by crystallisation or precipitation and are based on the first residue on the resin): Z·Ala·Leu·Ala·Gly·OH (67, 51%); BOC·Thr·Ala·Ile·Gly·Val·Gly·OH (88, 57%); Z·Gln·Thr·Ala·Ile·Gly·Val·Gly·OH (80, 62%); BOC·Met·Leu·Gly·OH (92, 75%); BOC·Met·Leu·Gly·NH·NH_2 (75, 68%); Z·Val·Gly·NH_2 (81% after purification); Z·Val·Gly·NH·NH_2 (75% after purification). t-Butoxycarbonylamino-acid derivatives were generally preferable to benzyloxycarbonyl derivatives. Some limitations have been encountered, notably in partial racemisation of *C*-terminal residues and in transpeptidation reactions of ω-esters of aspartyl and glutamyl peptides. Thus benzyloxycarbonyl-L-leucyl-L-alanine contained 2·5% of the LD diastereoisomer, and benzyloxycarbonylglycyl-L-alanine hydrazide contained 0·4% of the D-enantiomer. During the alkaline hydrolysis step, serious $\alpha \rightarrow \omega$ isomerisation occurred with ω-benzyl esters of aspartyl and glutamyl peptides. Using the corresponding t-butyl esters, however, we detected no isomerisation in the preparation of α-glutamylglycine, although α-aspartylglycine was accompanied by 4·5% of its β-isomer. The aspartylglycine sequence is probably the most severe test of the transpeptidation reaction,[7] and it is unlikely to be serious with other aspartyl sequences. The use of side chain t-butyl esters will, however, require combination with very acid-labile protecting groups, *e.g.* the *p*-diphenylisopropoxycarbonyl group,[8] as has already been suggested in other variants of the Merrifield procedure.[9]

These limitations mean that the present method is not likely to play a major role in peptide methodology, but we believe that it can be practically very useful for preparation of a fairly wide variety of oligopeptide sequences.

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‡ Otherwise cleavage of the benzyloxycarbonyl group was sometimes incomplete.
§ Reactions with active ester derivatives of valine and isoleucine were extended to 48 h.
¶ Lower yields of detached peptide were obtained if this methylation was carried out in more polar solvents, presumably because of competitive *O*-methylation.[6]

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