Prevention of Diketopiperazine Formation in Peptide Synthesis by a Simultaneous Deprotection–Coupling Procedure: Entrapment of Reactive Nucleophilic Species by *in situ* Acylation†

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Hydrogenolysis of Z-amino acid-D-Pro-OMe dipeptides in the presence of acetic acid results, almost quantitatively, in the formation of diketopiperazines, whereas in the presence of Boc- or 2-(trimethylsilyl)ethoxycarbonyl protected amino acid pentafluorophenyl or *N*-hydroxysuccinimidyl active esters, protected tripeptides are formed; a simultaneous deprotection/acylation methodology with potential utility for peptide synthesis thus results.

During the course of our work on the synthesis of analogues of the cyclic tetrapeptide HC-toxin (cyclo[-Aoe-D-Pro-Ala-D-Ala-],[1]† we observed that hydrogenolysis of the dipeptides Z-Asu(OBu^t)-D-Pro-OMe and Z-Ala-D-Pro-OMe in methanol and in the presence of acetic acid yielded, almost quantitatively, the cyclic dipeptides cyclo[-Asu(OBu^t)-D-Pro-] and cyclo[-Ala-D-Pro-] (Scheme 1).

Such diketopiperazine (DKP) formation is a historically well-precedented side-reaction in peptide synthesis.[2] The cyclisation arises from an intramolecular aminolysis and formation of a six-membered ring; it occurs most especially in L,D-dipeptides and is catalysed by carboxylic acids.[3] The cyclisation is also assisted by amino acids which readily form *cis*-amide bonds. DKP formation is thus a particular problem in dipeptides where one of the amino acids is glycine or proline (as above) or another imino acid.[2—5]

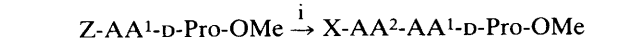
We reasoned that if the reactive free amine could be trapped by acylation prior to intramolecular aminolysis, DKP formation would be precluded. Furthermore, if the acylation reagent were a suitably protected and activated derivative of the next amino acid, a one-pot deprotection/coupling would result. We therefore required (Scheme 2) a hydrogenolysis-stable N-protecting group (X) combined with carboxy-activation (–OR) which would be sufficient to trap the reactive nucleophile, yet would not epimerise the amino acid under the reaction conditions. The active esters of *N*-hydroxysuccinimide (–OSu) and pentafluorophenol (–OPfp) appeared to meet these latter criteria,[6] but the choice of N-protection required greater consideration. All benzyl-derived carbamates were excluded, as were halogen-containing protecting groups because of the likelihood of side-reactions under the hydrogenation conditions. We thus turned to the perennial Boc-protection and also to the 2-(trimethylsilyl)ethoxycarbonyl (Teoc)-protecting group[7] because of our interest in this latter functionality[8] as an alternative acid-labile protecting group which is less sterically hindered.

Thus, hydrogenolysis of Z-Ala-D-Pro-OMe in dioxane (5—10 mM) in the presence of 10% Pd on C catalyst (30—40% by weight) and 1.25—1.5 equiv. of N-protected D-alanine active ester‡ for 2—2.5 h gave the results in Table 1, entries 1—4. Similarly, deprotection of Z-Asu(OBu^t)-D-Pro-OMe in the presence of Teoc-D-Ala-OPfp yielded the tripeptide Teoc-D-Ala-Asu(OBu^t)-D-Pro-OMe (Table 1, entry 5).

In a preliminary investigation of a solution-phase system more closely resembling the troublesome sequence studied by Gisin and Merrifield (solid phase),[3] hydrogenolysis of Z-D-Val-Pro-OMe under the same conditions as above, but in the presence of Boc- or Teoc-protected valine or proline active esters, gave comparable yields of target tripeptides (Table 1, entries 6—9).

A number of observations can be made from these results. Firstly, the acylating ability of the active ester is important. Pentafluorophenyl esters possess the highest reaction rates of all currently utilised active esters[10] and thus might be expected to be more efficient trapping agents than *N*-hydroxysuccinimidyl esters. Secondly, the reaction is highly dependent on

Scheme 1. i H_2, 10% Pd on C, AcOH (1.1 equiv.), MeOH, 3 h.



Scheme 2. i H_2, 10% Pd on C, X-AA^2-OR.

Table 1. Hydrogenolysis of Z-protected dipeptide esters in dioxane in the presence of 10% Pd on C catalyst and N-protected amino acid active esters.

Entry	Active ester[a]	Yield of tripeptide[b]/%
1	Teoc-D-Ala-OSu	59[c]
2	Boc-D-Ala-OSu	None isolated
3	Teoc-D-Ala-OPfp	79[c]
4	Boc-D-Ala-OPfp	18[c]
5	Teoc-D-Ala-OPfp	72[d]
6	Teoc-Val-OPfp	79[e]
7	Boc-Val-OPfp	51[e]
8	Teoc-Pro-OPfp	80[e]
9	Boc-Pro-OPfp	23[e]

[a] The active esters were synthesised from Teoc-AA[8] and Boc-AA *via* DCC-mediated coupling of the acid to the hydroxy component in EtOAc. The Teoc-active esters were oils, soluble in hexane, which were used without further purification; the Boc-active esters were fully characterisable crystalline solids.[9] [b] All new compounds gave satisfactory spectral and analytical data. [c] Tripeptide = X-D-Ala-Ala-D-Pro-OMe (X = Teoc or Boc). [d] Tripeptide = X-D-Ala-Asu(OBu^t)-D-Pro-OMe. [e] Tripeptide = X-AA-D-Val-Pro-OMe (AA = valine or proline).

† Abbreviations used in the text follow IUPAC-IUB rules as described in *Pure Appl. Chem.*, 1984, **56**, 595. Additional abbreviations used: AA = amino acid; Aoe = L-2-amino-8-oxo-9,10-epoxydecanoic acid; Asu = L-2-aminosuberic acid; DKP = diketopiperazine (= 2,5-dioxopiperazine); –OSu = *N*-hydroxysuccinimidyl ester; –OPfp = pentafluorophenyl ester; Teoc = 2-(trimethylsilyl)ethoxycarbonyl; Fmoc = (9-fluorenyl)methoxycarbonyl; MeLeu = *N*-methyl-leucine; MeVal = *N*-methylvaline; DCC = dicyclohexylcarbodiimide.

‡ In our synthesis of HC-Toxin analogues D-Ala is the next amino acid.[1]

the substitution at the amino group, particularly the steric bulk of the protecting group and whether the carbamate N–H is secondary or tertiary; however, it appears to be less perturbed by side-chain β-substitution. (The higher yields obtained with Boc-Val-OPfp compared to Boc-Ala-OPfp may be due to the more prolonged existence of H_2N-D-Val-Pro-OMe compared to H_2N-Ala-D-Pro-OMe; similar dipeptides containing alanine have been observed in our laboratory to cyclise more readily than those with other hydrophobic amino acids.)[11]

It seems likely that the deprotection/acylation reaction and the competing cyclisation occur on the surface of the catalyst,§[6] and that unless the incoming electrophile has ready access to the adsorbed amine function, intramolecular reaction will predominate. Hence a sterically unhindered acylating agent will be better able to entrap the reactive species than a hindered one.¶ The Teoc-group, with its steric bulk ($-SiMe_3$) removed further from the carbamate by two methylenes, considerably reduces the steric hindrance to the amino acid carboxylate. Hence entrapment by the Teoc-protected amino acid active esters is facilitated relative to the corresponding Boc-protected derivatives.

Thus, we have demonstrated that carbamate-protected, and in particular *N*-Teoc amino acid, pentafluorophenyl esters can be used to circumvent an annoying and occasionally serious side reaction in peptide synthesis, that of cyclo-dipeptide or DKP formation.‖[13] The resultant simultaneous deprotection/coupling procedure involves minimal intermediate purification and a one-pot reaction; it is quick and convenient and has potential applicability not only in solution phase but also in solid phase peptide synthesis. In this respect it can be seen as a complementary procedure to the Sheppard Fmoc-AA-OPfp techique[10a] but using Merrifield resin and acid-labile N^{α}-amino protection. The methodology may also be useful for so-called 'difficult' couplings[14] in peptide synthesis and, in a wider sense, for the general entrapment of reactive nucleophilic species by acylation.

Received, 24th March 1987; Com. 377

§ Such a surface effect has been proposed by Schmidt *et al.* to account for the high cyclisation and low side product yields in their deprotection/cyclisation procedure.[6]

¶ Interference, attributable to the Boc-group, has been observed in our laboratory during the coupling of hindered N-protected amino acid mixed phosphinic-carboxylic anhydrides resulting in low yields and prolonged reaction times.[12]

‖ Attempts to use this procedure in the synthesis of models of the tetrapeptide 8—11 of the immunosuppressive agent cyclosporine A[5] starting from Z-MeLeu-MeVal-OMe and Teoc-L-Phe-OPfp, resulted only in DKP formation. In this case, the presence of two imino acids leads to unusually facile DKP formation, which is evidently favoured, kinetically, over peptide bond formation. This has been overcome by use of t-butyl ester protection.[13]

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