The Use of Crown Ethers in Peptide Chemistry. Part 2.[1] Syntheses of Dipeptide Complexes with Cyclic Polyether 18-Crown-6 and their Derivatisation with DMSO

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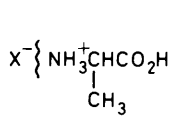
As part of a study the aim of which is to use crown compounds as non-covalent protecting groups in peptide synthesis, we have explored the reactivity of 18-crown-6-ether–dipeptide complexes with dicyclohexylcarbodi-imide (DCC) in dimethyl sulphoxide (DMSO). At a reactant concentration of *ca.* 0.02 mol dm^{-3} the DCC did not activate the dipeptide, and the *N*-acylurea derivative slowly formed. At concentrations of *ca.* 0.2 mol dm^{-3} the complexes proved to be unstable and reacted with the solvent to form a DMSO–peptide adduct. The reaction mechanism leading to the latter was elucidated and shown to involve an initial acid-catalysed addition of DMSO to DCC. The presence in solution of nucleophiles gave the peptide ester thus indicating DCC-mediated activation of the peptide carboxylic acid group. The results from this study were used to design the conditions necessary for an effective non-covalent protection of the amino group during peptide synthesis.

In a previous communication[1] we have described the synthesis and characterisation of amino acid complexes with crown ethers. The objective of the research was to study their behaviour in organic solvents in the presence of coupling reagents commonly employed in peptide synthesis. Using dicyclohexylcarbodi-imide (DCC) in acetonitrile or chloroform we have found that the alanine derivative (**1**) reacts to form oligomeric species. The mechanism of the reaction has been elucidated and shown to involve an intermediate (**2**) in which the carboxyl and NH_3^+ groups are simultaneously linked to DCC *via* a covalent and a hydrogen bond respectively. Thus activation of the carboxylic group and deprotection of the NH_3^+ can occur, providing the basis for formation of peptide linkages.

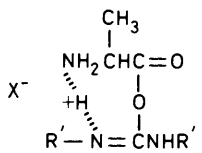
In this paper we have gone on to explore the role of solvent and reactant concentrations in order to find a method for controlling the formation of oligomeric species. We have synthesized glycylglycine complexes with 18-crown-6 (**3**) and studied their reactivity with DCC in dimethylsulphoxide (DMSO) solutions.

Both solvent and substrate were selected in order to avoid the destabilising effects of the hydrogen bond on the activated carboxy–DCC adduct (**2**). Thus the replacement of the amino acid with a dipeptide displaces the NH_3^+ from the site it occupies in structure (**2**). Furthermore, the use of DMSO, a solvent known for its donor properties, should favour solvent–solute hydrogen bonds over intramolecular ones.

All the reactions discussed in this paper have been studied by ^1H n.m.r. with the signal of incompletely deuteriated DMSO used as internal standard. Repetition of the reactions on a preparative scale afforded the products for their chemical and physical characterisation.



(**1a,b**)



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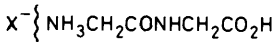
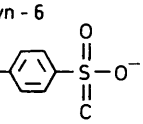


(**3a,b**)

{ = 18 - Crown - 6

a; X = 

b; X = Cl^-

$R' = C_6H_{11}$

i; R = $CH_2NHCOCH_2NH_3^+\{X^-$

ii; R = $CH_2NHCOCH_2\overset{+}{N}HS(CH_3)_2X^-$

Experimental

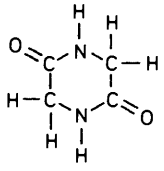
All reactions were followed by ^1H n.m.r. on a Varian XL300 spectrometer. [$^2H_6$]DMSO was supplied by Aldrich and stored over type 5A molecular sieve in an airtight container. DMSO was distilled under vacuum (31 °C; 0.5 mmHg) and kept in the dark over type 5A molecular sieve. DCC was distilled under vacuum (92 °C; 0.3 mmHg) and kept in a desiccator. All solvents used on the h.p.l.c. were of h.p.l.c. grade and filtered before use.

Synthesis of Tos–Glycylglycine Complex (**3a**).—To a solution of toluene-*p*-sulphonic acid (7.2 g, 3.8×10^{-2} mol) in H_2O–EtOH (50 cm^3, 1:1), was added glycylglycine (5 g, 3.8×10^{-2} mol). After the reaction had been stirred at room temperature the solvent was removed, then the dipeptide salt suspended in 50 cm^3 EtOH containing one equivalent of 18-crown-6 (10.0 g) and the suspension warmed until it became clear. The solution was then allowed to cool to room temperature and EtOAc was added dropwise until it became cloudy. The complex crystallised out with time (20 g, 93%). A similar method was used for the synthesis of (**3b**). From 5 g of glycylglycine dipeptide we obtained 13.1 g (80%) of the crystalline complex.

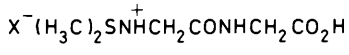
An example of the procedure for a reaction followed by ^1H

Table. N.m.r. and mass spectral data.

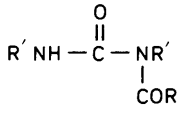
Compound	Diagnostic n.m.r. data/δ	Mass Spectral Data/*m*/*z*
(**5a**)	8.38 (t, 1 H, NHpept)	193 $[(CH_3)_2SNHCH_2CONHCH_2COOH]^+$
	6.47 (t, 1 H, NH-S)	148 $[(CH_3)_2NHCH_2CONHCH_2]^+$
	3.70 (m, 4 H, $C_\alpha H_2$'s)	135 $[(CH_3)_2SNHCH_2CONH_2]^+$
		90 $[(CH_3)_2SNHCH_2]^+$
(**5b**)	8.88 (t, 1 H, NHpept)	193 $[(CH_3)_2SNHCH_2CONHCH_2COOH]^+$
	7.40 (t, 1 H, NH-S)	177 $[(CH_3)_2SNHCH_2CONHCH_2COH]^+$
		133 $[(CH_3)_2SNHCH_2CONH]^+$
		90 $[(CH_3)_2SNCH_2]^+$
(**6i**)	8.60 (t, 1 H, NH cpx)	603 $[^{\ddagger}NH_3CH_2CONHCH_2CONRCONHR]^+$
	8.38 (d, 1 H, NH DCC)	339 $[NH_3CH_2CONHCH_2CONRCONHR]^+$
	4.35 (d, 2 H, $C_\alpha H_2$'s)	214 $[NH_3CH_2CONHCH_2CONR]^+$
(**6ii**)	not available	399 $[(CH_3)_2SNHCH_2CONHCH_2CONRCONHR]^+$
		274 $[(CH_3)_2SNHCH_2CONHCH_2CONHR]^+$
(**8a**)	8.78 (t, 1 H, NH-S)	290 $[(CH_3)_2SNHCH_2CONHCH_2COON(COCH_2)_2]^+$
	6.39 (t, 1 H, NHpept)	
	4.23 (d, 2 H, $C_\alpha H_2$'s)	
	2.70 (s, 4 H, CH_2's)	
(**8b**)	9.10 (t, 1 H, NH-S)	247 $[CH_3SNHCH_2CONHCH_2COONCOCH_2CH_2]^+$
	7.22 (t, 1 H, NHpept)	145 $[NH_2CH_2CONHCH_2COON]^+$
	4.27 (d, 2 H, $C_\alpha H_2$'s)	
	2.75 (s, 4 H, CH_2's)	
(**9a**)	9.00 (t, 1 H, NH est)	494 $[^{\ddagger}NH_3CH_2CONHCH_2COON(COCH_2)_2]^+$
	4.29 (d, 2 H, $C_\alpha H_2$'s)	230 $[NH_3CH_2CONHCH_2COON(COCH_2)_2]^+$
	2.71 (s, 4 H, CH_2's)	
(**9b**)	9.43 (t, 1 H, NH est)	as for (**9a**)
	4.36 (d, 2 H, $C_\alpha H_2$'s)	
	2.75 (s, 4 H, CH_2's)	



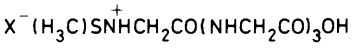
(**4**)



(**5a**;**b**)



(**6i**;**ii**)



(**7**)

n.m.r. is as follows. The purified dipeptide complex (**3a**) (57 mg, 1×10^{-4} mol, 1 equiv.) in 0.5 cm^3 dry $[^2H_6]$DMSO was treated with one equivalent of freshly distilled dicyclohexylcarbodiimide (DCC, 20.6 mg, 1 equiv.). The solution was quickly transferred to an n.m.r. tube and spectra taken every 5 min for the first hour.

An identical method was used when *N*-hydroxysuccinimide (NSu, 115 mg, 1 equiv.) was added to the initial reaction solution.

The larger scale reactions were done in DMSO on the bench with no stirring of the solutions in an attempt to duplicate the conditions used for the n.m.r. analysis. An example of this is as follows. The dipeptide complex (**3a**) (1.14 g, 2×10^{-3} mol) was dissolved in dry DMSO (10 cm^3) and DCC added (412.6 mg, 1 equiv.). After 18 h at room temperature the solvent was removed under reduced pressure and the residue treated with chloroform which precipitated DCU (278 mg, 62%). Upon repetition of this treatment virtually all DCU could be recovered. After removal of the solvent the oily residue was treated with acetonitrile. Upon standing at room temperature a gummy material formed; this was separated from the supernatant, dried and shown by n.m.r. and mass spectrometry to be the DMSO–dipeptide adduct (**5a**) (566 mg, 78%) (see Table). Further purification of the product was not possible due to its extreme instability.

Results

(a) *Tos–Glycylglycine Complex* (**3a**) (c 0.02 mol dm^{-3}).—The complex (**3a**) (1×10^{-5} mol) was dissolved in $[^2H_6]$DMSO (0.5 cm^3) containing DCC (1 equiv.). The solution was quickly transferred to an n.m.r. tube and the reaction monitored at 300 MHz. Within the first 20 h there was no detectable consumption of DCC and only after 25 h two signals of identical intensity, a triplet δ 8.60 and a doublet at δ 8.38, indicated the presence in solution of a new species. The ratio of the latter to the starting material was 3:5 and did not vary during the following 70 h.

The reaction was then repeated on a larger scale and the product isolated by h.p.l.c. A base peak at *m*/*z* 214 in the mass spectrum and the presence of only one, intense n.O.e. at the N–C–N carbon of DCC upon irradiation of the NH doublet at δ 8.38, indicated that the structure of the product was that of the *N*-acyl urea derivative (**6**). DCU and *N*-acyl urea accounted for 90% of the DCC used in the reaction. Repetition of the latter in the presence of NSu (1 equiv.) (see text below) did not afford any dipeptide ester and identical results to those described above were obtained. We therefore concluded that, in the conditions used for the reaction, activation of the carboxylic group by DCC did not occur.

(b) *Tos–Glycylglycine complex* (**3a**) (c 0.2 mol dm^{-3}).—In an attempt to promote activation of the dipeptide by DCC we increased tenfold the reagent concentrations. Precipitation of DCU immediately followed the addition of DCC (1 equiv.) to a $[^2H_6]$DMSO solution containing (**3a**) (1×10^{-4} mol). However the first dipeptide product formed only after 20 min, as indicated by the appearance of two triplets at δ 8.38 and 6.47. The concentration of this new product increased in the

following hour, although at a rate slower than that of DCC consumption. The formation of the product ceased as the last of the DCC was consumed and resumed upon addition of a second equivalent of the coupling reagent. When this was also consumed, there was about 20% of the starting dipeptide left in solution the rest having been converted into the dioxopiperazine derivative (**4**) (less than 10%) and a major product of unknown structure (about 70%). The latter was isolated upon repetition of the reaction on a preparative scale, and shown by n.m.r. and mass spectrometry to be the dipeptide–DMSO adduct (**5a**).

In order to evaluate the role, if any, of the complex counter-ion in the formation of (**5a**), we repeated the above reactions with the tosylate ion substituted for the chloride ion in the glycylglycine complex.

(c) *HCl–Glycylglycine Complex* (**3b**) (c 0.02 mol dm^{-3}).—At a complex concentration of 0.02 mol dm^{-3}, there was repetition of the results obtained with (**3a**), namely: (*i*) No activation of the carboxylic acid group occurred; (*ii*) DCC was not consumed during the first 2 h; (*iii*) *N*-acyl urea derivative was the only product detected in solution; and (*iv*) conversion to the *N*-acyl urea was over after 20 h.

(d) *HCl–Glycylglycine Complex* (**3b**) (c 0.2 mol dm^{-3}).—In this reaction, despite careful drying of both [$^2H_6$]DMSO and complex, the spectrum of (**3b**) showed the presence of water at δ 3.45. Unlike the tosylate case, addition of DCC (1 equiv.) did not produce any dipeptide derivative, despite hydration of DCC to DCU during the first 1 h. As the last of the DCC was consumed, two triplets of identical intensity appeared at δ 8.88 and 7.40. This new compound was later identified as the same dipeptide–DMSO adduct (**5b**) seen in the case of the tosylate complex. There was no further change in the spectrum until a second equivalent of DCC was added. This induced precipitation of DCU, this time paralleled by a decrease of the starting peptide complex and an increase in the intensity of (**5b**). At the end of the reaction, (**5b**) accounted for about 20% of the starting material. Other products of the reaction were the diketopiperazine (**4**), the *N*-acyl urea derivative (**6**) and the tetrapeptide–DMSO adduct (**7**).

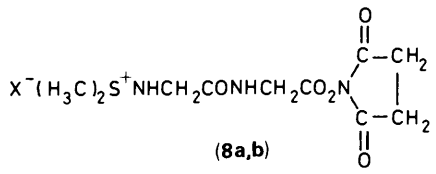
In an attempt to trap the activated carboxylic group which we believed was involved in the synthesis of (**5a**, **b**), the reactions were repeated with the nucleophile *N*-hydroxysuccinimide (NSu) (1 equiv.).

(e) *Tos–Glycylglycine Complex* (**3a**) *with NSu* (1 *equiv.*) (c 0.02 mol dm^{-3}).—The reactants appeared to be stable towards each other over the entire reaction period. There was no formation of active ester (within the n.m.r. detection limits) and only *N*-acyl urea was being formed at a rate which was similar to that observed in absence of NSu [see above (a)].

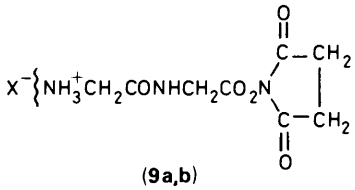
(f) *Tos–Glycylglycine Complex* (**3a**) *with NSu* (1 *equiv.*) (c 0.2 mol dm^{-3}).—Within the first 5 min of addition of DCC to a [$^2H_6$]DMSO solution containing the complex (**3a**) (1 equiv.) and NSu (1 equiv.), a triplet and a singlet appeared at δ 9.00 and 2.71 respectively. The intensity of these signals continued to increase during the following 45 min, during which time DCU was also forming at an identical rate. After 1 h, two triplets at δ 8.39 and 6.38 appeared in the n.m.r. spectrum and their intensity increased steadily during the next 60 min. Finally a third dipeptide derivative began to form after an additional hour of reaction (triplets at δ 8.75 and 6.39). The three products of the reaction were later isolated and identified by mass spectrometry as (*i*) the succinimide ester of the complex (**9a**) (*m*/*z* 494, 18%); (*ii*) the dipeptide–DMSO adduct (**5a**) (*m*/*z* 193, 17%); and (*iii*) the succinimide ester of adduct (**5a**), (**8a**) (*m*/*z* 290, 15%). After 16 h there was still 37% of starting material left in solution.

(g) *HCl–Glycylglycine Complex* (**3b**) *with NSu* (1 *equiv.*) (c 0.02 mol dm^{-3}).—This reaction was similar to that with the tosylate complex [see above (e)], except for the slow formation of DCU during the first 10 h.

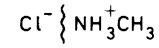
(h) *HCl–Glycylglycine Complex* (**3b**) *with NSu* (1 *equiv.*) (c 0.2 mol dm^{-3}).—In the reaction of DCC with complex (**3b**) in the presence of NSu (1 equiv.) (*c* 0.2 mol dm^{-3}), the results were similar to those obtained with the Tos complex (**3a**). One exception was the formation of DCU which proceeded at a rate faster than that of NSu ester (**9b**). As the intensity of the ester



(**8a**,**b**)



(**9a**,**b**)



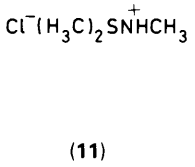
(**10**)

signals began to steady, two other products appeared in the spectrum. These were later identified as the dipeptide–DMSO adduct free acid (**5b**) and its succinimide ester derivative (**8b**). When NSu (2 equiv.) was used the reaction gave the same products, though the active esters were produced to a greater extent.

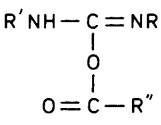
(i) *HCl–Methylamine Complex* (**10**).—To elucidate the part played by the carboxylic group in the reaction mechanism leading to the formation of adducts (**5a**, **b**), a series of reactions were carried out using the crown ether complex of methylamine hydrochloride (**10**). Addition of DCC (1 equiv.) to a solution of (**10**) (0.2 mol dm^{-3}) in [$^2H_6$]DMSO, did not induce formation of DCU or methylamine complex derivatives. However, within 10 min of the addition of acetic acid (1 equiv.) a quartet at δ 6.83 and a doublet at δ 2.67 appeared in the n.m.r. spectrum. After 26 h, this was the major component in the reaction solution and later was shown to be the DMSO–methylamine adduct (**11**). Similar results were obtained with catalytic amounts of acetic acid, though the formation of (**11**) occurred at a slower rate.

Discussion

One of the objectives of this study was to find experimental conditions, under which activation of the carboxylic group of the amino acid or peptide complexes with crown ether would occur without polymerisation.[1] Since this phenomenon appeared to be dependent on the apolar nature of the reaction solvents, the use of DMSO was thought to prevent the formation of these oligomers. Indeed, in the reactions at higher reactant concentrations the oligomerisation process was largely suppressed, although the peptide complexes proved to be unstable in these conditions due to a reaction between the amino group and the solvent DMSO. However, when the reactions were conducted in the presence of a nucleophile (NSu), there was formation of esters, showing that activation of the carboxylic acid group had been achieved. It was felt then, that the elucidation of the mechanisms involved in these reactions could be used to design those experimental conditions under which peptide-bond formation, without side reactions like those encountered in previous studies, is possible using



(11)



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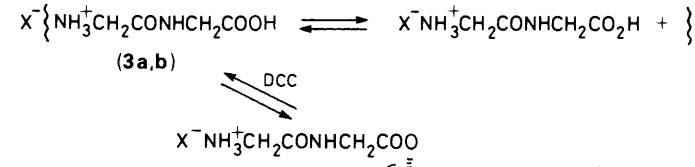
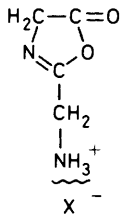
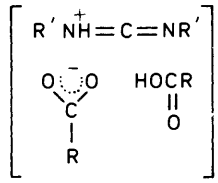


(13i;ii)



(14a,b)



(15)

amino acid protected with crown compounds. It is postulated[2] that amino acids react in solution with DCC to form the highly reactive *O*-acyl isourea (**12**). This then either condenses with a second molecule of amino acid to form the carboxy anhydride (**13**) or, in the case of *N*-acyl amino acids, rearranges to the cyclic oxazolone (**14**). All these compounds are highly reactive towards nucleophiles, whereas the *N*-acyl urea (**6**), which is thought to derive from (**12**), is quite stable. When we attempted a mechanistic explanation of the phenomena seen in the results section, the following facts were also considered: (*i*) Peptide or amino acid complexes of the type described here are indefinitely stable in DMSO solution;* (*ii*) at low concentration, activation of the peptide carboxy group does not take place as indicated by the lack of any of the products characteristic of this process; (*iii*) at higher peptide concentration the carboxy group reacts with DCC to yield an activated species, as shown by the formation of the succinimide ester; (*iv*) the formation of the DMSO adduct (**5a**, **b**) is catalysed by the carboxy group; (*v*) DMSO does not react with DCC in the presence of either catalytic or larger amounts of 18-crown-6;† (*vi*) finally there is no DCU precipitation from DMSO solutions containing DCC, 18-crown-6 and dry HCl.‡

When these observations were combined with those from the literature[3-5] for the mechanism of carboxy activation by DCC in polar solvent, the results presented in this paper were readily explained. Thus DeTar and co-workers[3] have demonstrated that DCC and carboxylic acids react in solution to form ionic pairs of the type shown in structure (**15**). Here, a second molecule of the acid component forms a hydrogen bond with the ionic pair thus facilitating the formation of the reactive anhydride. In our case, at a peptide concentration of 0.2 mol dm^{-3}, the formation of structure (**15**) is followed by the reaction with one of the nucleophiles present in solution. These are H_2O, DMSO, $-NH_2$, and NSu. The different pathways are discussed separately hereafter. (*a*) H_2O: The water is present as an impurity, either from the highly hygroscopic solvent or from the peptide complex. In solution it is presumably solvating the counter-ion, and this solvation will be greater for the smaller

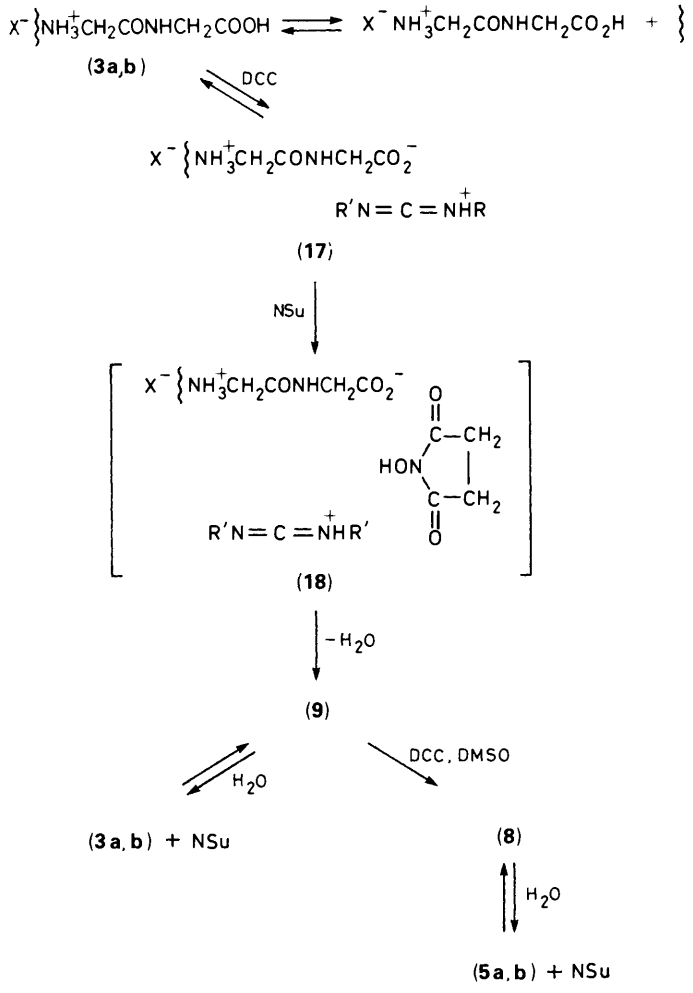
Scheme 1.

chloride ion than for the relatively stable tosylate. The transport of water towards protonated DCC will be more efficient in the case of the chloride ion, hence explaining the higher rate of DCU formation seen for this ion (Scheme 3). (*b*) $-NH_2$: This is available from one of the acid–base equilibria shown in Scheme 1, and can react to form the tetrapeptide when water has been consumed. (*c*) DMSO: The activation of DMSO by DCC in the presence of carboxylic acids has been shown.[5] Here the DMSO–DCC adduct (**19**) reacts with $-NH_2$ to form the product (**5**) (see Scheme 1). (*d*) NSu: An alternative pathway is offered to the activated DCC–COOH complex by the presence in solution of the nucleophile NSu. The reaction which leads to the formation of the ester is faster than any of the others described above. Water is therefore still present in solution and available to hydrolyse the newly formed ester bond. An excess of NSu acts upon this equilibrium to yield more ester. At lower reactant concentrations the protonation of DCC by the carboxylic group does not take place as indicated by the absence of any of the above reactions. Presumably, stabilisation of the carboxylic group by DMSO prevents any reaction with the coupling agent. The formation of the *N*-acyl urea derivative must therefore be from an independent mechanism, and we believe that (**6**) is formed by nucleophilic attack of the DCC nitrogen on the carboxylic C=O.

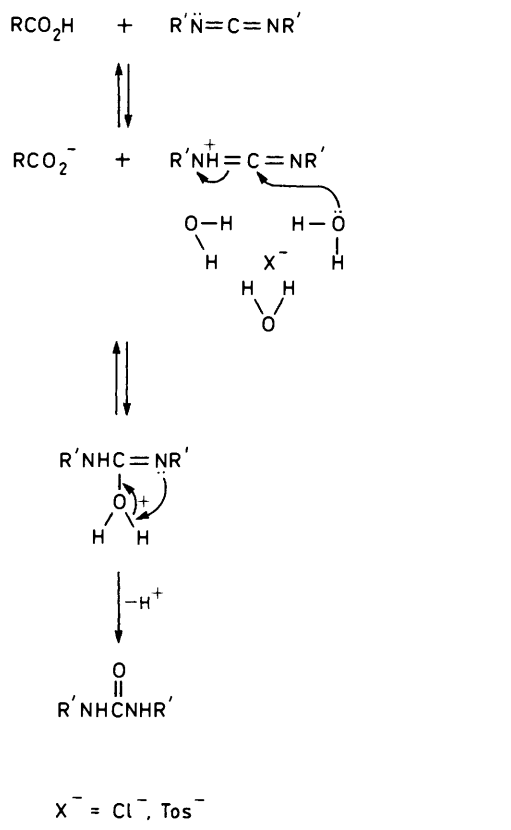
* Authors observations.

† The authors tried this reaction to ensure that activation of the DMSO was not by 18-crown-6, as shown by D. Marji, *J. Chem. Soc., Chem. Commun.*, 1987, 7.

‡ This reaction was done to ensure that the activation of DMSO by 18-crown-6 was not acid catalysed.



Scheme 2.



Scheme 3.

Conclusions

In this paper we have described the reactions the dipeptide complexes (**3a,b**) undertake when solubilised in DMSO solutions containing DCC. The different pathways which operate at higher peptide concentrations are summarised in Schemes 1 and 2. Two acid–base equilibria, where the base is DCC and the acid components are NH_3^+ and COOH, exist simultaneously in solution. Depending on the presence of a nucleophile such as NSu the ionic pair (**17**) is 'activated' to the complex (**18**) which in turn becomes the peptide ester (**9**). In the absence of NSu only the pathway leading to the DMSO–peptide derivative operates. As a corollary of this study, we have found that activation of the carboxy group to the *O*-acylisourea does not occur in DMSO solution at a peptide concentration up to 0.2 mol dm^{-3}. Finally, although by using DMSO we have been able to avoid the polymerisation that occurs in $CHCl_3$ or MeCN, the reaction between the peptide amino group and DMSO defied one of the objectives of this study, that is protection of amino acids with crown ethers during peptide synthesis. However, the replacement of DMSO with a solvent of equal solvating properties, but less prone to react with DCC, should, in principle, prevent this, and other, unwanted 'side reactions'. Thus, in preliminary experiments conducted using *N,N*-dimethylformamide as solvent, we have found that the synthesis of small peptides is possible starting from the amino acid complexes.[6] This study is currently under development and in particular we are trying to optimise the experimental conditions so that a method for stepwise synthesis using a non-covalent blocking group can be proposed.

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Received 29*th September* 1988; *Paper* 8/03875I