Amino-acids and Peptides. Part XXXVII.[1] Trifluoroacetylation during the Coupling of t-Butoxycarbonylamino-acids with Peptide Esters in the Presence of Trifluoroacetate Anion

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The use of dicyclohexylcarbodi-imide to form peptide bonds in the presence of trifluoroacetate anion can cause substantial trifluoroacetylation of the amino-component. The addition of 1-hydroxybenzotriazole greatly reduces or eliminates this side-reaction.

It is common practice in peptide synthesis to prepare the amino-component by the action of trifluoroacetic acid on t-butoxycarbonyl-amino-acid or -peptide esters, to liberate the free amino-group by the addition of a tertiary amine, and to proceed with the coupling reaction in the presence of the trifluoroacetate so formed. We have found that in such cases trifluoroacetylation can be a major side-reaction, particularly when dicyclohexylcarbodi-imide is used to effect the coupling, the carboxy-component being activated in the presence of the trifluoroacetate. The possibility was envisaged earlier by Steglich and Weygand,[2] but no examples have, as far as we are aware, been reported, and we record our experience here. One of us (M. L.) earlier obtained evidence of formylation (and in a lesser degree, of acetylation) during the coupling of t-butoxycarbonylglycine pentachlorophenyl ester with prolyl-leucylglycine, in the presence of the respective anions, and extensive formylation under such conditions has been reported by Schnabel, Klostermeyer, and Berndt.[3]

Trifluoroacetylation during the coupling of peptide ester trifluoroacetates [a]

Expt.	Carboxy-component (mmol)	Amino-component (mmol)	Anion present (mmol)	Coupling reagent (mmol)	Reaction time	Crude [b] yield (%)	Trifluoroacetyl derivative [c] (yield (%)
1	Boc-Pro (4·0)	Pro-Gly-OPic (0·5) [d]	$CF_3 \cdot CO_2^-$ (1·0)	DCCI (2·5)	*e*		21% (F, 3·4%)
2	Boc-Pro (2·0)	Pro-Gly-OPic (0·25)	$Me_3C \cdot CO_2^-$ (0·5) Br^- (0·5)	DCCI (1·25)	*e*	85	(No pivaloyl derivative detected by t.l.c.)
3	Boc-Pro (2·0)	Pro-Gly-OPic (1·0)	$CF_3 \cdot CO_2^-$ (2·0)	DCCI (2·0) HOBt (2·0)	2 h	93	None detected by t.l.c.
4	Boc-Pro (0·75)	Pro-Gly-OPic (0·5)	$CF_3 \cdot CO_2^-$ (10·0)	DCCI (0·75) HOBt (0·75)	*e*	95	None detected by t.l.c.
5	Boc-Pro (1·5)	Pro-Gly-OPic (1·5)	$CF_3 \cdot CO_2^-$ (3·0)	DCCI (1·5)	*e*	85	5% (F, 0·8%)
6	Boc-Pro-OTcp (4·0)	Pro-Gly-OPic (1·0) [f]	$CF_3 \cdot CO_2^-$ (2·0)		4 weeks	69	None detected by t.l.c.
7	Boc-Gly (5·0)	Pro-Gly-OPic (2·0)	$CF_3 \cdot CO_2^-$ (20)	DCCI (3·0)	*e*	88	8% (F, 1·2%)
8	Boc-Leu (5·0)	Pro-Gly-OPic (2·0)	$CF_3 \cdot CO_2^-$ (20)	DCCI (3·0)	*e*	93	9% (F, 1·4%)
9	Boc-Phe (5·0)	Pro-Gly-OPic (2·0)	$CF_3 \cdot CO_2^-$ (20)	DCCI (3·0)	*e*	86	20% (F, 3·2%)
10	Boc-Pro (5·4)	Phe-Arg(NO_2)-OPic (2·17)	$CF_3 \cdot CO_2^-$ (4·34)	DCCI (4·6)	*e*		26% (isolated)
11	Boc-Pro (0·625)	Phe-Arg(NO_2)-OPic (0·25)	$Me_3C \cdot CO_2^-$ (1·0) Br^- (0·50)	DCCI (0·375)	*e*	89	(No pivaloyl derivative detected by t.l.c.)
12	Boc-Pro (14·2)	Phe-Arg(NO_2)-OPic (10·9)	$CF_3 \cdot CO_2^-$ (21·8)	DCCI (14·2) HOBt (14·2)	2 h	84	0·15% (t.l.c.) 0·1—0·2% (CF_3^+)
13	Boc-Pro-OTcp (3·75)	Phe-Arg(NO_2)-OPic (2·50)	$CF_3 \cdot CO_2^-$ (5·0)		4 days	97	0·15% (t.l.c.) CF_3^+, weak; F, 0%
14	Boc-Pro (6·35)	Cha-Arg(NO_2)-OPic (2·1) [d]	$CF_3 \cdot CO_2^-$ (4·2)	DCCI (4·45)	*e*		23% (isolated)
15	Boc-Pro (0·80)	Pro-Phe-OPic (0·32) [h]	$CF_3 \cdot CO_2^-$ (0·64)	DCCI (0·48)	*e*	87	5% (F, 0·61%)
16	Z-Pro (1·25)	Pro-Phe-OPic (0·50)	$CF_3 \cdot CO_2^-$ (1·0)	DCCI (0·75)	*e*	90	3% (F, 0·32%)
17	Boc-Pro (0·75)	Pro-Phe-OPic (0·50)	$CF_3 \cdot CO_2^-$ (1·0)	DCCI (0·75) HOBt (0·75)	2 h	84	None detected by t.l.c. or F analysis
18	Boc-Pro-OPy (0·75) [i]	Pro-Phe-OPic (0·50)	$CF_3 \cdot CO_2^-$ (1·0)		50 h		33% (F, 4·2%)
19	Boc-Pro-SPy (0·75) [j]	Pro-Phe-OPic (0·5)	$CF_3 \cdot CO_2^-$ (1·0)		50 h		26% (F, 3·25%)

[a] Further details are given in the Experimental section. Abbreviations follow the Tentative Rules of the I.U.P.A.C. Commission (reprinted in the Chemical Society Specialist Report on Amino-acids, Peptides, and Proteins, Vol. 4, 1972). Other abbreviations are: Cha = β-cyclohexylalanine; DCCI = dicyclohexylcarbodi-imide; HOBt = 1-hydroxybenzotriazole; Pic = 4-picolyl; Py = 2-pyridyl; SPy = 2-pyridylthio; Tcp = 2,4,5-trichlorophenyl. Optically active amino-acids are of the L-series. The solvent for the reactions was dimethylformamide, except in Experiments 10 and 14 (see Experimental section). [b] The percentage yield is given only for those products containing relatively small amounts of trifluoroacetyl derivative. [c] When the fluorine analysis alone is given, the percentage yield of trifluoroacetyl derivative is calculated from this figure. [d] Part XXXVI (ref. 1). [e] The reaction mixture was left overnight. [f] This is the experiment described in Part XXXVI,[1] for the preparation of authentic protected tripeptide ester. [g] D. J. Schafer, G. T. Young, D. F. Elliott, and R. Wade, *J. Chem. Soc.* (*C*), 1971, 46. [h] R. Garner and G. T. Young, *J. Chem. Soc.* (*C*), 1971, 50. [i] Ref. 5. We thank Dr. Morley for the sample. [j] Ref. 4.

The results are summarised in the Table. In two cases (Experiments 10 and 14) the trifluoroacetylpeptide ester was isolated (after decomposition of the t-butoxycarbonylpeptide ester by means of trifluoroacetic acid) and fully characterised; the molal percentages of trifluoroacetyl derivative in the whole isolated products were 24·5 and 38%, respectively. In other cases, the

[1] Part XXXVI, G. A. Fletcher and G. T. Young, *J.C.S., Perkin I*, 1972, 1867.

[2] F. Weygand and W. Steglich, *Z. Naturforsch.*, 1959, **14b**, 472.

[3] E. Schnabel, H. Klostermeyer, and H. Berndt, *Annalen*, 1971, **749**, 90.

amount of by-product was estimated by the fluorine content, with confirmation from semi-quantitative t.l.c., and (in two cases) by mass spectrometry, the intensity of the peak at *m/e* 69 (CF_3^+) being compared with that from known mixtures of product and by-product. In most cases considerable excess of the carboxy-component and of dicyclohexylcarbodi-imide over the amino-component was used, and this appears to favour trifluoroacetylation, but Experiment 5 shows that the use of stoicheiometric proportions of the reactants does not eliminate the side-reaction. It is to be expected that a hindered amino-component will encourage trifluoroacetylation, but Experiments 10 and 14 show that it is not necessary for a proline residue to be *N*-terminal. Similarly, the carboxy-component may be leucine or phenylalanine or even glycine. Benzyloxycarbonyl can replace t-butoxycarbonyl (Experiment 16). The corresponding 2,4,5-trichlorophenyl esters gave little or no by-product (Experiments 6 and 13) but the highly reactive 2-pyridyl esters [4,5] and 2-pyridyl thioesters [4] gave substantial amounts (Experiments 18 and 19); indeed, this side-reaction was first encountered in this laboratory in the coupling of t-butoxycarbonyl-L-proline 2-pyridyl thioester with L-leucylglycine 4-picolyl ester.[6]

Such a side-reaction could of course be countered by the use of the free dipeptide ester or of a salt having a much less nucleophilic anion. In a recent synthesis of bradykinin [7] we used hydrogen chloride in dioxan to remove t-butoxycarbonyl groups and coupled, for example, t-butoxycarbonyl-L-proline with L-prolylglycyl-L-phenylalanyl-*O*-benzyl-L-seryl-L-prolyl-phenylalanyl-*N*(ω)-nitro-L-arginine 4-picolyl ester by means of dicyclohexylcarbodi-imide satisfactorily. But in some cases such deprotection reactions have been found to be incomplete, because of the precipitation of the hydrochloride of the unchanged t-butoxycarbonylpeptide 4-picolyl ester.[8] An alternative would be to replace the trifluoroacetate anion by a less nucleophilic anion such as pivalate, and in Experiments 2 and 11 no pivaloylation was detected. However, we have found that in our model reactions the dicyclohexylcarbodi-imide–1-hydroxybenzotriazole coupling procedure of König and Geiger [9] very nearly eliminates this side reaction; in Experiments 3, 4, 12, and 17 only traces (*ca.* 0·15%) of trifluoroacetyl derivatives were detected in the product, even when (Experiment 4) a large excess of triethylammonium trifluoroacetate was present during the reaction.

EXPERIMENTAL

The general instructions in Part XXXVI [1] apply. The general synthetic procedures described there were used for the removal of the t-butoxycarbonyl group by means of trifluoroacetic acid, for the liberation of the amino-component by means of triethylamine, and for coupling; all protected peptide 4-picolyl esters were isolated by the citric acid extraction procedure described there. In each case except Experiment 10 (described below) the coupling solvent was dimethylformamide. The usual procedure for the liberation of the amino-component from its trifluoroacetate provides 2 molar proportions of trifluoroacetate anion. Authentic t-butoxycarbonyl-L-prolyl-L-prolylglycine 4-picolyl ester and t-butoxycarbonyl-L-prolyl-β-cyclohexyl-L-alanyl-*N*(ω)-nitro-L-arginine 4-picolyl ester are described in Part XXXVI,[1] and t-butoxycarbonyl-L-prolyl-L-phenylalanyl-*N*(ω)-nitro-L-arginine 4-picolyl ester is described in Part XXXII.[7] t-Butoxycarbonyl-L-prolyl-L-prolyl-L-phenylalanine 4-picolyl ester is new and is described below. Other coupling products were contaminated with the trifluoroacetyl by-product and were not purified. Further details of certain Experiments follow:

Experiments 2 *and* 11. The t-butoxycarbonyldipeptide ester was treated with 2·8N-hydrogen bromide in acetic acid for 25 min, the resulting dihydrobromide was precipitated by ether, the free dipeptide ester was liberated by triethylamine in dimethylformamide, and the triethylammonium pivalate was added. The absence of pivaloyldipeptide ester in the product was confirmed by treating it with trifluoroacetic acid to destroy the t-butoxycarbonyl derivative; again t.l.c. revealed only one component in each case.

Experiment 10. t-Butoxycarbonyl-L-proline (5·4 mmol) was coupled with L-phenylalanyl-*N*(ω)-nitro-L-arginine 4-picolyl ester [7] (2·17 mmol; liberated *in situ* from the trifluoroacetate by triethylamine in the usual way) by means of dicyclohexylcarbodi-imide (4·6 mmol) in a mixture of dichloromethane (10 ml) and dimethylformamide (2 ml) at room temperature. After removal of the dicyclohexylurea, the filtrate was evaporated to dryness. The residue was dissolved in ethyl acetate; the solution was washed with aqueous sodium hydrogen carbonate, dried, and evaporated. The residue was triturated with ether, giving the product (1·27 g) which t.l.c. showed to contain two main components [R_F 0·31 and 0·39 (G3), the second spot being ninhydrin-negative]. The t-butoxycarbonyl group was removed in the usual way by means of trifluoroacetic acid, giving the product (1·569 g), a portion (1·04 g) of which was dissolved in ethyl acetate and aqueous 4% sodium hydrogen carbonate. The ethyl acetate layer was washed with water, dried, and evaporated; the residue (0·204 g, 0·367 mmol, 26%) was recrystallised from 50% ethanol, giving *trifluoroacetyl-L-phenylalanyl-N(ω)-nitro-L-arginine 4-picolyl ester* (0·124 g) of m.p. 118—121°; R_F 0·25 (E4); 0·71 (G1); 0·72 (H) (Found: C, 49·8; H, 4·7; N, 17·35. $C_{23}H_{26}F_3N_7O_6$ requires C, 49·8; H, 4·75; N, 17·7%). The sodium hydrogen carbonate extract was saturated with brine and extracted with a mixture of n-butanol (50 ml), ethyl acetate (40 ml), and dichloromethane (10 ml); the extract was washed (brine), dried, and evaporated, giving amorphous prolylphenylalanyl-*N*(ω)-nitroarginine 4-picolyl ester (0·626 g, 1·13 mmol) (identical on t.l.c. with a sample prepared from the bis-trifluoroacetate). The total isolated product therefore contained 24·5 mol % of trifluoroacetyl dipeptide ester.

Trifluoroacetyl-L-phenylalanyl-*N*(ω)-nitro-L-arginine 4-picolyl ester was also prepared by dissolution of t-butoxycarbonyl-L-phenylalanyl-*N*(ω)-nitro-L-arginine 4-picolyl ester (0·45 mmol) in trifluoroacetic acid (1·0 ml) and (after

[4] K. Lloyd and G. T. Young, *J. Chem. Soc.* (*C*), 1971, 2890.
[5] A. S. Dutta and J. S. Morley, *J. Chem. Soc.* (*C*), 1971, 2896.
[6] K. Lloyd, D.Phil. Thesis, Oxford University, 1969.
[7] D. J. Schafer, G. T. Young, D. F. Elliott, and R. Wade, *J. Chem. Soc.* (*C*), 1971, 46.
[8] M. Löw, unpublished work.
[9] W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788.

45 min) adding triethylamine (9·0 mmol), dimethylformamide (1·5 ml), and dicyclohexylcarbodi-imide (2·25 mmol). Next day the product was isolated by the citric acid procedure,[1] giving a 57% yield of trifluoroacetyldipeptide ester, identical with the material reported above.

Experiment 14. The procedure described for Experiment 10 gave crude product (1·15 g) from which was obtained 0·488 mmol of trifluoroacetyldipeptide ester and 0·788 mmol of prolyl-β-cyclohexylalanyl-*N*(ω)-nitroarginine 4-picolyl ester; the molal proportion of trifluoroacetyl by-product was therefore 38%, and the yield of by-product was 23%. *Trifluoroacetyl-β-cyclohexyl-*L*-alanyl-*N(ω)*-nitro-*L*-arginine 4-picolyl* ester had m.p 107—110°, R_F 0·24 (E4); 0·62 (G3) (Found: C, 49·8; H, 5·8; F, 10·3; N, 17·3. $C_{23}H_{32}F_3N_7O_6$ requires C, 49·5; H, 5·6; F, 10·2; N, 17·6%).

Experiment 17. *t-Butoxycarbonyl-*L*-prolyl-*L*-prolyl-*L*-phenylalanine 4-picolyl ester* had $[\alpha]_D^{20}$ −62° (*c* 1·0 in EtOAc); R_F 0·62 (E4); 0·70 (G3) (Found: C, 63·9; H, 6·9; N, 10·0. $C_{30}H_{38}N_4O_6,H_2O$ requires C, 63·7; H, 7·0; N, 9·9%).

Experiments 18 *and* 19, In these experiments the excess of active ester was destroyed at the end of the reaction by the addition of water.[4]

Mass Spectrometric Detection of Trifluoroacetyl Derivatives (By R. T. Aplin).—Spectra were recorded on an A.E.I. MS9 spectrometer set to give a resolution of a minimum of 2500 (*i.e.* 400 p.p.m.) and the background spectrum was recorded from *m/e* 65 to 75. The sample was then introduced and the spectrum was recorded over the same mass range. The correct assignment of the CF_3^+ ion in the *m/e* 69 multiplet was achieved by peak enhancement with ' heptacosa ' (perfluorotributylamine) or (better) with ethyl trifluoroacetate. Common ions at *m/e* 69 are CF_3, 68·9952; C_3H_3NO, 69·0215; C_4H_5O, 69·0340; C_4H_7N, 69·0578; C_5H_9, 69·0704. The limit of sensitivity of detection was established by means of synthetic mixtures of trifluoroacetyl derivative and pure product; in each case, 0·1—0·2% could be detected.

Part of this work was carried out by Dr. M. Löw, of the Gedeon Richter Chemical Works, Budapest, Hungary, while in Oxford as a British Council visitor in 1970, and part by G. A. F., while holding an S.R.C.–C.A.P.S. studentship.

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