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SYNTHESIS OF O-ACYLBENZOHYDROXAMIC ACIDS AND THEIR USE IN PEPTIDE SYNTHESIS*

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Abstract—The addition of N-protected amino acids to benzonitrile oxides yields activated esters, which on coupling with amino acid esters lead to peptides.

THE use of esters of hydroxylamine with N-protected amino acids is rapidly gaining currency in the synthesis of peptides. Both N,N-dialkyl[1,2] and N,N-diacyl[3,4] hydroxylamines have been employed for this purpose. There have been sporadic reports[5] of the use of a N-monoacylhydroxylamine (benzohydroxamic acid) to form an active ester with N-protected amino acids. The esterification is brought about by dicyclohexylcarbodiimide. We wish to report a simple synthesis of esters of benzohydroxamic acids with N-protected amino acids and their use in peptide synthesis.

For the synthesis of the active esters, we chose to study the addition of N-protected amino acids to benzonitrile oxides. The reaction has been recognized in principle,[6] but no systematic study has been published. The nitrile oxide, liberated *in situ* by triethylamine from an α-chlorobenzaldoxime, was reacted with the N-protected amino acid to give the desired ester. An IR spectrum of the reaction mixture showed initially a band at about 2300 cm^{-1} which disappeared with time, as the band due to the active ester at 1760–1780 cm^{-1} appeared. In one experiment α,*p*-dichlorobenzaldoxime was treated with aqueous sodium carbonate and the liberated nitrile oxide extracted into ether. The ethereal solution was now treated with *p*-chlorobenzoic acid to give O,N-bis-(*p*-chlorobenzoyl) hydroxylamine, identical with the known compound. These data provide suggestive, if not compelling, evidence that in the reaction of the protected amino acids with an α-chlorobenzaldoxime a benzonitrile oxide is implicated.[7]

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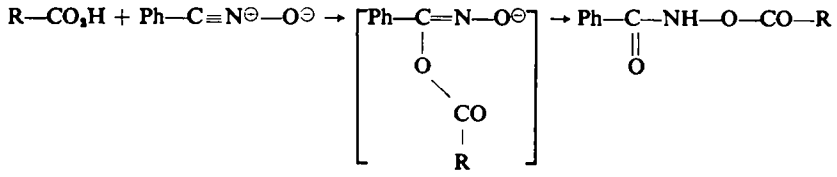
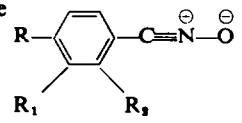
[1] S. M. Beaumont, B. O. Handford, J. H. Jones and G. T. Young, *Chemical Communications* (London), 53 (1965); B. O. Handford, J. H. Jones, G. T. Young and T. F. N. Johnson, *J. Chem. Soc.* 6814 (1965).
[2] S. Bittner, Y. Knobler and M. Frankel, *Tetrahedron Letters* 95 (1965).
[3] G. H. L. Nefkens, G. I. Tesser and R. J. F. Nivard, *Rec. Trav. Chim.* **81**, 683 (1962).
[4] G. W. Anderson, J. E. Zimmermann and F. M. Callahan, *J. Amer. Chem. Soc.* **86**, 1839 (1964).
[5] [a] J. Pless, *Peptides*, Proceedings of the Fifth European Symposium, 1962, p. 69. Pergamon Press, Oxford (1963). [b] M. Löw and L. Kisfaludy, Seventh European Peptide Symposium, Budapest, 1964; *Chem. Abstr.* **63**, 13404 (1965).
[6] Cf. P. Grünanger amd P. V. Finzi, *Chem. Abstr.* **54**, 3379 (1960).
[7] Cf. J. K. Sutherland and D. A. Widdowson, *J. Chem. Soc.* 4651 (1964).



The active esters from the reaction of carbobenzoxyphenylalanine with various nuclear substituted benzonitrile oxides (generated *in situ* from the α-chlorobenzaldoxime with triethylamine) under conditions generally employed are listed in Table 1. *p*-Chlorobenzonitrile oxide was best suited for the preparation of active esters. It would be difficult to correlate the yields with nuclear substitution as several factors may be involved which would influence not only the rate of addition, but also the stability of the products. Thus nuclear substitutents may be expected to affect (i) the stability of the nitrile oxide, its propensity for dimerization and reactivity towards acids, and (ii) the ease of Lossen rearrangement of the O-acylbenzohydroxamates.[8]

TABLE 1

Nitrile oxide 	Yield % of active ester
$R = R_1 = R_2 = H$	45·5
$R = Cl$; $R_1 = R_2 = H$	72·5
$R = R_2 = Cl$; $R_1 = H$	26·6
$R = R_1 = Cl$; $R_2 = H$	41·25
$R = R_2 = H$; $R_1 = NO_2$	37·8

In one experiment using 2,4-dichlorobenzonitrile oxide, the only product isolated was bis(2,4-dichlorophenyl) urea, formed *via* Lossen rearrangment. This side-reaction however, was not present to any significant extent in the case of *p*-chlorobenzonitrile oxide. An IR check on the reaction mixture itself showed no trace of isocyanate, and no urea was detectable in the product.

Table 2 records the properties of esters of *p*-chlorobenzohydroxamic acid with five N-protected amino acids. Three of these were obtained crystalline. The other two were not crystallizable, but were pure enough for the next step. In the case of N-carbobenzoxyglycine, the active ester with *p*-chlorobenzohydroxamic acid was also

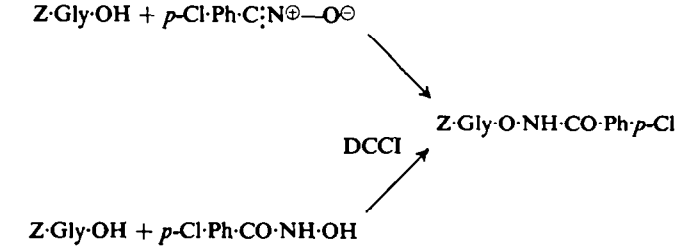
TABLE 2

Active ester	State	$[\alpha]^{\circ}_D$*	Yield %
Z·Gly·O·NH·CO·Ph·*p*-Cl	Crystalline	—	65
Z·Phe·O·NH·CO·Ph·*p*-Cl	Crystalline	−47·3	72·5
Z.Val·O·NH·CO·Ph·*p*-Cl	Froth	—	(100)
Z·Pro·O·NH·CO·Ph·*p*-Cl	Gum	—	(85)
Bz·Leu·O·NH·CO·Ph·*p*-Cl	Crystalline	−62·1	70

* *c*, 2 in AcOEt.

[8] D. C. Berndt and H. Shechter, *J. Org. Chem.* **29**, 916 (1964).

obtained using DCCI for coupling. The products by the two methods were identical:



Peptide formation. Coupling of the active esters with ethyl glycinate gave the protected dipeptides in about 80% yield. The products were comparable to those reported in the literature. (Table 3).

TABLE 3

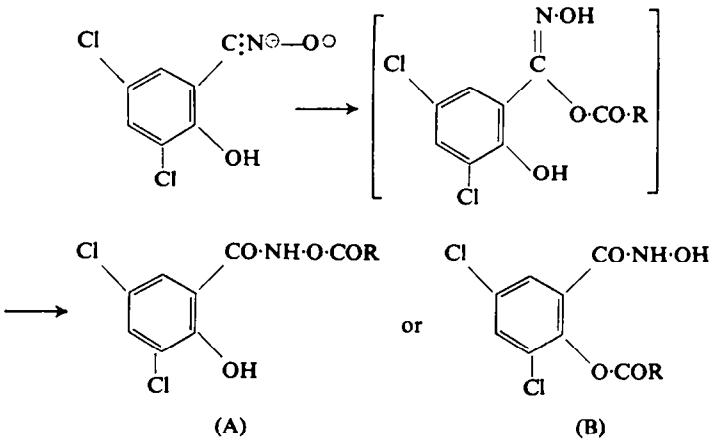
Peptide	% Yield of crystallized product		M.p.°C		$[\alpha]_D$°	
	Coupling stage only	Overall	Observed	Reported	Observed	Reported
Z·Gly·Gly·OEt	74·8	48·6	79–80	80–81[9]		
Z·Phe·Gly·OEt	74	53·6	108–110	109–110[10]	−17·5[a]	−16·9[11]
Z·Val·Gly·OEt	—	43·2	166	166[12]	−5·7[b]	−6·0[13]
H·Pro·Gly·OH	—	29·0	230–232 (decomp)	236 (decomp)	−21·07[c]	−19·8[14]
Bz·Leu·Gly·OEt	75	53·0	153–155	156·5–157	−33·6[d]	−34·0[15]

[a] *c*, 2 in EtOH; [b] *c*, 1 in EtOAc; [c] *c*, 4 in water; [d] *c*, 3 in EtOH.

A further check for possible racemization in the two-stage peptide synthesis was made using the sensitive Young test.[15] N-Benzoyl-L-leucine was added to *p*-chlorobenzonitrile oxide to yield the active ester, m.p. 156–157°, $[\alpha]_D$ −62·1°, which on reaction with ethylglycinate afforded in 84% yield crude N-benzoyl-L-leucylglycine ethyl ester of m.p. 142–145°, $[\alpha]_D$ −34·7°, and giving correct analytical values. Recrystallization gave the protected dipeptide of m.p. 153–155°, $[\alpha]_D$ −33·6°. The observed rotations for the crude and purified products were in excellent agreement with those reported for the one by the azide procedure.

As an extension of this series, it was decided to investigate the addition of carboxylic acids to the known 3,5-dichloro-2-hydroxybenzonitrile oxide.[16] *A priori* rearrangement can proceed in two directions to give structure A or B, both useful for peptide synthesis, as A is a O-acylhydroxamic acid and B has a strong resemblance to a Kemp–Woodward active ester.[17]

[9] J. P. Greenstein and M. Winitz, *Chemistry of the Amino Acids*, Vol. 2. p. 1128. J. Wiley (1961).
[10] G. W. Anderson and R. W. Young, *J. Amer. Chem. Soc.* **74**, 5307 (1952).
[11] Ref. 9. p. 1135.
[12] Ref. 9. p. 1138.
[13] F. Bergel and J. A. Stock, *J. Chem. Soc.* 3658 (1960).
[14] H. N. Rydon and P. W. G. Smith, *J. Chem. Soc.* 3642 (1956).
[15] M. W. Williams and G. T. Young, *J. Chem. Soc.* 881 (1963).
[16] R. H. Wiley and B. J. Wakefield, *J. Org. Chem.* **25**, 546 (1960).
[17] D. S. Kemp and R. B. Woodward, *Tetrahedron* **21**, 3019 (1965).



Carbobenzoxyglycine was added to the nitrile oxide generated *in situ*, and the progress of the reaction was monitored by IR. Disappearance of the nitrile oxide band at 2300 cm^{-1} was complete after about 20 hr at room temperature, and correspondingly, the active ester band appeared at 1780–1800 cm^{-1}. However, during work-up of the solution, the colour rapidly deepened, and the only compound which could be isolated was a deep-yellow substance in very low yield. This did not have the properties expected for the active ester. So in another experiment, the reaction was allowed to proceed for 24 hr and then, without attempting to isolate the active ester, ethylglycinate was added to the solution and left at room temperature for 3 days. This gave the dipeptide in 24% yield.

It would thus appear that addition of N-protected amino acids of aromatic nitrile oxides provides an easy synthesis of activated esters. The resultant O-acylhydroxamic acids couple with amino acid esters to give dipeptides in reasonable yields with no impairment of optical activity. However, this two-stage peptide synthesis does not appear to offer any marked advantages over some of the better existing methods.

EXPERIMENTAL

M.ps are uncorrected. The benzaldoximes were prepared by standard methods. Chlorination of these in chf soln gave the corresponding α-chlorobenzaldoximes.[16-18]

Addition of N-protected amino acids to benzonitrile oxides. A soln of α,p-dichlorobenzaldoxime (1·9 g) in dry chf (15 ml) was cooled in an ice-bath and treated with triethylamine (1 g), followed by a soln of N-carbobenzoxy-L-phenylalanine (3 g) in dry chf (20 ml). The mixture was kept for 24–28 hr. at room temp in the dark. The soln was then washed with water, $NaHCO_3$aq, and once more with water, dried, and the solvent removed under red. press. at a bath temp below 40°. Crystallization of the residue from AcOEt–petrol gave *O-(N-carbobenzoxyphenylalanyl)-4-chlorobenzohydroxamic acid*, m.p. 144–145°. A further quantity of this substance could be obtained by filtering the mother liquor through a short column of silica gel, total yield, 3·25g. IR (KBr) peaks at 1805, 1690 and 1670 cm^{-1}. (Found: C, 63·77; H, 4·46. $C_{24}H_{21}O_5N_2Cl$ requires: C, 63·64; H, 4·67%.)

The following active esters were prepared in a similar manner, starting from the suitable acid and α-chlorobenzaldoxime (Table 4).

Condensation of N-carbobenzoxyglycine with p-chlorobenzohydroxamic acid. A mixture of N-carbobenzoxyglycine (0·52 g) and *p*-chlorobenzohydroxamic acid (0·43 g) in acetonitrile (20 ml) and DMF (10 ml) was cooled in ice–salt, and treated with DCCI (0·6 g) with stirring. The reaction mixture was left overnight, filtered and the filtrate evaporated to dryness *in vacuo*. The residue was

[18] British Pat. No. 949,371 [*Chem. Abstr.* **60**, 11949 (1964)].

TABLE 4

Active ester	M.p.	Formula	Analyses			
			Found		Calc.	
			C	H	C	H
Z·Phe·O·NH·CO·Ph	134–135°	$C_{24}H_{22}O_5N_2$	68·65	5·61	68·89	5·30
Z·Phe·O·NH·CO·Ph(*o*-Cl, *p*-Cl)	151–153°	$C_{24}H_{20}O_5N_2Cl_2$	59·53	4·16	59·15	4·14
Z·Phe·O·NH·CO·Ph(*m*-Cl, *p*-Cl)	145°	$C_{24}H_{20}O_5N_2Cl_2$	59·15	4·26	59·15	4·14
Z·Phe·O·NH·CO·Ph·*m*-NO_2	148°	$C_{24}H_{21}O_7N_3$	62·22	4·52	62·20	4·57
Z·Gly·O·NH·CO·Ph·*p*-Cl	173–174°	$C_{17}H_{15}O_5N_2Cl$	56·43	3·93	56·28	4·17
Z·Val·O·NH·CO·Ph·*p*-Cl	Froth					
Z·Pro·O·NH·CO·Ph·*p*-Cl	Gum					
Bz·Leu·O·NH·CO·Ph·*p*-Cl	156–157°	$C_{20}H_{21}O_4N_2Cl$	61·56	5·42	61·77	5·44

taken in AcOEt washed with water, $NaHCO_3$aq, and again with water. After drying the soln, the solvent was distilled off under red. press. The residual solid was crystallized from AcOEt–petrol to afford O-(N-carbobenzoxyglycly) *p*-chlorobenzohydroxamic acid (0·7 g), m.p. 173–174°, undepressed by admixture with the sample obtained by the nitrile oxide method.

Addition of p-*chlorobenzoic acid to* p-*chlorobenzonitrile oxide.* A soln of α,4-dichlorobenzaldoxime (1·9 g) in cold ether was shaken thoroughly with an ice-cold Na_2CO_3aq (0·53 g) in a separatory funnel. After a few min. the aq layer was tapped off, the ether layer washed once with water and dried ($CaCl_2$). This was then added to an ether soln of *p*-chlorobenzoic acid (1·6 g) and the whole refluxed for 1 hr. The mixture was left at room temp for 2 more hr, then washed with $NaHCO_3$aq and water, dried and concentrated. The crystals that formed were filtered and washed with ether–petrol, to give O,N-bis(*p*-chlorobenzoyl) hydroxylamine, m.p. 172–174° (0·65 g), identical with the product obtained by the Schotten-Baumann procedure.[19]

Peptide formation using the active esters

(i) N-*Carbobenzoxyphenylalanyl glycine ethyl ester.* A soln of ethyl glycinate hydrochloride (0·7 g) in DMF (15 ml) was treated with Et_3N (0·5 g). The precipitated hydrochloride was filtered and washed with a further quantity of DMF. The combined filtrate was added to a soln of O-(N-carbobenzoxy-phenylalanyl)-*p*-chlorobenzohydroxamic acid (2·25 g) in DMF (10 ml). The mixture was left at room temp for 24 hr, and then the solvent distilled off *in vacuo*. The residue was taken up in AcOEt, washed successively with water, ice-cold 0·5N NaOH, water, ice-cold 0·5N HCl and water. The AcOEt layer was dried and the solvent removed under red. press. Crystallization of the residue from AcOEt–petrol afforded the dipeptide (1·4 g) m.p. 108–110°, undepressed by admixture with an authentic specimen. The IR spectra of the two samples were superposable. (Found: C, 65·92; H, 6·44. Calc. for $C_{21}H_{24}O_5N_2$: C, 65·61; H, 6·29%.)

(ii) N-*Carbobenzoxyglycyl glycine ethyl ester.* Ethyl glycinate hydrochloride (0·7 g) was converted to the free base as before and added to O-(N-carbobenzoxyglycyl)-*p*-chlorobenzohydroxamic acid (1·81 g) in DMF. The mixture was left for 40 hr at room temp and yielded the dipepitde (1·1 g), m.p. 79–80°, undepressed by admixture with an authentic specimen.

(iii) N-*Carbobenzoxyvalyl glycine ethyl ester.* The carbobenzoxyvaline active ester (crude froth; 4·05 g) was similarly treated with ethyl glycinate (from 1·4 g of hydrochloride) in DMF and left for 3 days yielding N-carbobenzoxyvalyl glycine ethyl ester (1·45 g), m.p. 166°. (Found: C, 60·82; H, 7·40. Calc. for $C_{17}H_{24}O_5N_2$: C, 60·70; H, 7·19%.)

(iv) N-*Benzoylleucyl glycine ethyl ester.* O-(N-Benzoylleucyl)-*p*-chlorobenzohydroxamic acid

[19] *Beilstein*, Vol. IX, p. 341.

(1·95 g) reacted with ethyl glycinate (from 0·7 g of hydrochloride) in DMF soln (48 hr) yielding 1·35 g, m.p. 142–145°. Recrystallization from AcOEt–petrol gave the dipeptide, m.p. 153–155°. (Found: C, 63·55; H, 7·44. Calc. for $C_{17}H_{24}O_4N_2$: C, 63·73; H, 7·55%.)

(v) *Prolylglycine.* Carbobenzoyxprolyl active ester (crude gum; 3·4 g) was coupled with ethyl glycinate in DMF (80 hr) to yield the crude N-carbobenzoxyprolyl glycine ethyl ester (2·5 g).

The ester in acetone (8 mγ) was treated with N NaOH (16 ml) for 1 hr at room temp, the acetone was then removed *in vacuo* the soln cooled and acidified with 2N HCl. The liberated acid was extracted in AcOEt. The organic layer was dried and the solvent removed, leaving the acid as a gum (2·0 g).

The above carbobenzoxyprolylglycine was hydrogenolysed in presence of 10% Pd–C to yield the crystalline prolylglycine (0·5 g), m.p. 230–232° (d). (Found: C, 48·64; H, 7·15. Calc. for $C_7H_{12}O_3N_2$: C, 48·83; H, 7·03%.)

3,5-*Dichloro-2-hydroxybenzonitrile oxide for peptide formation*

Triethylamine (1·01 g) was added to a soln of N-carbobenzoxyglycine (2·1 g) in dry THF (20 ml). To this soln was added with ice-cooling and stirring, α,3,5-trichloro-2-hydroxybenzaldoxime (2·4 g) in 15 ml THF. The mixture was stirred overnight at room temp. Then a soln of ethyl glycinate (from 1·4 g of hydrochloride) in DMF (20 ml) was added. The mixture was let stand at room temp for 3 days. It was then evaporated to dryness *in vacuo*; the residue taken in AcOEt, finally yielded crude N-carbobenzoxyglycyl glycine ethyl ester (0·7 g), m.p. 78–80°, undepressed by admixture with an authentic specimen.

Acknowledgement—We thank Dr. S. Selvavinayakam and his associates for the analyses and IR spectra.