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HYDROXAMIC ACIDS AND THEIR DERIVATIVES—III*

PREPARATION OF ESTERS OF PIVALOHYDROXAMIC ACID AND THEIR USE IN PEPTIDE SYNTHESIS†

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Abstract—Reaction of N-protected amino acids with pivalonitrile oxide affords "active esters" which are useful in peptide syntheses.

IN PART I of this series[1] we reported the use of benzonitrile oxides for the synthesis of O-acylbenzohydroxamic acids. The latter were found to be "active esters" and could be coupled with amino acid esters to form the peptide bond.

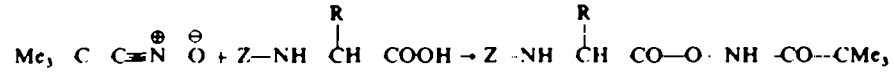
Following the previous results, two considerations motivated us to try an aliphatic nitrile oxide in place of substituted benzonitrile oxides: (i) there are reports suggesting that aliphatic nitrile oxides may be more reactive than their aromatic counterparts[2] and (ii) aliphatic hydroxamic acids are, at least partially, soluble in water; this was expected to be of great utility in the second stage.

In the actual choice of the aliphatic nitrile oxide, there was a third factor which impelled us to choose pivalonitrile oxide—we expected that the bulky t-butyl group might prevent, or at least retard, the familiar dimerization to furoxan.

Pivalaldoxime was chlorinated to the hydroxamoyl chloride; the nitrile oxide was generated *in situ*, as usual.[2]

Formation of active esters. Reaction of the nitrile oxide with N-protected amino acids at 0° gave very good yields of the following crystalline active esters (Table 1).

TABLE 1



R	Yield	m.p.
H	90%	127°
Ph CH_2—	90%	104–106°
Me_2CH	72%	129–130°
Me_2CH CH_2—	73%	106–107°

Z = Ph CH_2—O CO—.

* Part II: K. Nagarajan, S. Rajappa and V. S. Iyer, *Tetrahedron* **23**, 1049 (1967).

† Contribution No. 94 from CIBA Research Centre, Bombay.

[1] T. R. Govindachari, K. Nagarajan, S. Rajappa, A. S. Akerkar and V. S. Iyer, *Tetrahedron* **22**, 3367 (1966).

[2] cf: G. Zinner and H. Günther, *Chem. Ber.* **98**, 1353 (1965).

Peptide formation. Reaction of the active ester from N-benzyloxycarbonyl phenylalanine with ethyl glycinate at room temperature in DMF gave only a 62% yield of the dipeptide after 44 hr.

$$Z\cdot Phe\cdot O\cdot NH\cdot CO\cdot CMe_3 + H\cdot Gly\cdot OEt \rightarrow Z\cdot Phe\cdot Gly\cdot OEt + Me_3C\cdot CO\cdot NH\cdot OH$$

However, the use of ethyl glycinate hydrochloride in conjunction with sodium acetate[3] gave an 88·5% yield of the pure dipeptide in 4 hr at room temperature. Similarly, the following dipeptides were made (Table 2).

TABLE 2

Dipeptide	Yield	$[\alpha]_D$ found	$[\alpha]_D$ reported	Ref.
Z·Phe·Gly·OEt	88·5%	−17·4	−16·9	4
Z·Gly·Tyr·OEt	73·0%	+18·9	+19·2	5
Z·Leu·Leu·OMe	60·0%	−35·7	−35·3	6

Test for racemization. The Anderson test[7] was carried out in order to detect any propensity for racemization during the course of this reaction. This involved the following steps:

$$Z\cdot Gly\cdot Phe\cdot OH + Me_3C\cdot C\equiv\overset{\oplus}{N}\text{–}\overset{\ominus}{O} \rightarrow Z\cdot Gly\cdot Phe\cdot O\cdot NH\cdot CO\cdot CMe_3$$

$$\downarrow\ H\cdot Gly\cdot OEt$$

$$Z\cdot Gly\cdot Phe\cdot Gly\cdot OEt$$

In spite of the most careful fractional crystallization, no trace of racemic material could be found in the resultant tripeptide obtained in 78% yield.

Amino acids with side-chain functional groups

(i) Attempts to prepare Z·Ser·Gly·OEt using pivalonitrile oxide gave variable yields of impure product. The active ester of N-benzyloxycarbonyl serine could not be obtained crystalline.

(ii) Z·Glu·OH (OEt) gave a non-crystalline active ester, which on condensation with H·Ser·Gly·OEt gave a 58% yield of the tripeptide:

$$Z\cdot Glu(OEt)\cdot O\cdot NH\cdot CO\cdot CMe_3 + H\cdot Ser\cdot Gly\cdot OEt \rightarrow Z\cdot Glu(OEt)\cdot Ser\cdot Gly\cdot OEt$$

[3] cf. S. M. Beaumont, B. O. Handford, J. H. Jones and G. T. Young, *Chem. Commun.* 53 (1965); B. O. Handford, J. H. Jones, G. T. Young and T. F. N. Johnson, *J. Chem. Soc.* 6814 (1965).

[4] J. P. Greenstein and M. Winitz, *Chemistry of the Amino acids* Vol. 2; p. 1135. Wiley, N.Y. (1961).

[5] Ref. 4, p. 1131.

[6] Ref. 4, p. 1133.

[7] G. W. Anderson and F. M. Callahan, *J. Am. Chem. Soc.* **80**, 2902 (1958).

(iii) The reaction of N-benzyloxycarbonyl asparagine with pivalonitrile oxide had to be carried out in dioxan–chloroform because of the poor solubility of the acid. Even under these conditions, part of the acid was recovered. The active ester formed was immediately reacted with H·Ser·Gly·OEt in acetonitrile solution. The tripeptide directly crystallized out of the solution. The yield of Z·Asp(NH$_2$)·Ser·Gly·OEt was about 45% from the active ester.

Peptide formation in aqueous solution

(i) Reaction of N-benzyloxycarbonyl glycine active ester with ethyl glycinate hydrochloride and sodium acetate in aqueous dioxan for 4½ hr gave a 54% yield of the pure dipeptide.

(ii) In spite of numerous attempts under a variety of conditions the active ester did not react with free glycine in water. Either the active ester was recovered (in absence of added base) or a mixture of Z·Gly·OH and Z·Gly·Gly·OH was obtained (in presence of base).

CONCLUSION

It would thus appear that pivalohydroxamic acid active esters are useful for peptide synthesis. The method seems to compare favourably with existing ones for the synthesis of di- and tripeptides. In attempts to exploit this for larger peptides, we have come across interesting side-reactions which are reported in the accompanying paper.

EXPERIMENTAL

All amino acids used, except glycine, had the L-configuration.

Active ester with N-benzyloxycarbonylphenylalanine

α-Chloropivalaldoxime[2] (1·75 g; 0·013 mole) in dry $CHCl_3$ (20 ml) was cooled in ice–salt and treated with Et_3N (1·01 g; 0·01 mole). The mixture was shaken well for 1 min, and then a pre-cooled soln of N-benzyloxycarbonyl phenylalanine (3 g, 0·01 mole) in dry $CHCl_3$ (30 ml) was added with swirling. The mixture was left in the refrigerator for 24 hr. The $CHCl_3$ soln was then washed with water, sat. $NaHCO_3$aq and again with water. (As most of the active esters are soluble in Na_2CO_3aq, care should be taken to check the pH of the bicarbonate soln). The $CHCl_3$ soln was dried and the solvent removed *in vacuo* at a bath temp of 45°. The oily residue was dried thoroughly *in vacuo* and then triturated with ether petrol. The yield of solid active ester was 3·3–3·6 g (82·90%) depending on the age and purity of the reagent. A sample was recrystallized from AcOEt petrol to provide O-(N-benzyloxycarbonyl L-phenylalanyl) pivalohydroxamic acid, m.p. 104–106°. (Found: C, 66·25; H, 6·81. $C_{22}H_{26}O_5N_2$ requires: C, 66·31; H, 6·58%). IR (Nujol) band at 1780 cm^{-1}.

The following were similarly prepared:

TABLE 3

Compound	Formula	Analysis			
		Found		Calc.	
		C	H	C	H
Z·Gly·O·NH·CO·CMe$_3$	$C_{15}H_{20}O_5N_2$	58·82	6·37	58·43	6·54
Z·Val·O·NH·CO·CMe$_3$	$C_{18}H_{26}O_5N_2$	61·78	7·57	61·70	7·48
Z·Leu·O·NH·CO·CMe$_3$	$C_{19}H_{28}O_5N_2$	62·65	7·60	62·62	7·74

Some representative peptide syntheses

(i) *Z·Phe·Gly·OEt.* O-(N-Benzyloxycarbonylphenylalanyl) pivalohydroxamic acid (2·0 g) in acetonitrile (distilled over P_2O_5: 20 ml) was added to ethyl glycinate hydrochloride (0·7 g) in DMF (10 ml). Powdered $AcONa \cdot 3H_2O$ (0·7 g) was then added and the mixture stirred at room temp for 5 hr. The soln was then filtered and the solvents distilled off *in vacuo*. The residue was taken up in AcOEt, washed with water, ice-cold 0·5N NaOH, water, 1N HCl, and again with water. The organic layer was dried and evaporated to dryness *in vacuo*. The residue, on crystallization from AcOEt-petrol afforded Z·Phe·Gly·OEt (1·7 g), m.p. and mixed m.p. 107–109°; $[\alpha]_D$ −17·4° (*c*, 2 in EtOH). *Z·Leu·Leu·OMe* was made by a similar procedure in about 50% yield. Continuing the reaction for 40 hr gave a 60% yield, m.p. 93–94°, $[\alpha]_D^{24}$ −35·7° (*c*, 2 in EtOH). (Found: C, 64·17; H, 8·32. Calc. for $C_{21}H_{32}O_5N_2$: C, 64·26; H, 8·22%.)

(ii) *Z·Gly·Tyr·OEt.* A mixture of O-(N-benzyloxycarbonyl glycyl) pivalohydroxamic acid (1·5 g), ethyl tyrosinate hydrochloride (1·23 g) and AcONa (0·7 g) in acetonitrile (15 ml) and DMF (15 ml) was stirred as before for 4½ hr. The soln was filtered and evaporated to dryness *in vacuo*. The residue was taken up in AcOEt and washed with sat. $NaHCO_3$ aq, followed by water. The washings were repeated until such time that the aqueous soln gave no colour with $FeCl_3$ aq. The organic layer was then dried and evaporated to dryness. Crystallization from AcOEt-petrol gave *Z·Gly·Tyr·OEt* (1·45 g), m.p. 123–124°, $[\alpha]_D$ +18·9° (*c*, 5 in EtOH).

(iii) *Z·Asp(NH₂)·Ser·Gly·OEt.* (a) The active ester: α-Chloropivalaldoxime (0·95 g) was dissolved in dry $CHCl_3$ (30 ml) and cooled in ice salt. Et_3N (0·5 g) was added with shaking. To this mixture was added a soln of N-benzyloxycarbonylasparagine (1·33 g) in dioxan (90 ml). The soln was left in the refrigerator for 24–48 hr. The solvent was removed *in vacuo*, the residue taken up in $CHCl_3$, washed with water, $NaHCO_3$ aq and again with water. The $CHCl_3$ soln was dried and the solvent removed under reduced press. The residual oily active ester (1·25 g) had an IR band at 1780 cm^{-1} and was used as such for the next step.

The bicarbonate washings were combined and acidified with conc HCl. About 400 mg of N-benzyloxycarbonylasparagine was recovered.

(b) Z·Ser·Gly·OEt (0·8 g) in MeOH (40 ml) and AcOH (0·15 ml) was hydrogenolysed as usual to give the acetic acid salt of H·Ser·Gly·OEt (0·65 g).

(c) The Tripeptide: A mixture of the active ester (0·9 g) and AcOH·H·Ser·Gly·OEt (0·65 g) in dry acetonitrile (60 ml) was stirred at room temp for 24–36 hr. The tripeptide crystallized out of the reaction soln. This was filtered off to give 0·47 g of *Z·Asp(NH₂)·Ser·Gly·OEt*, m.p. 213–214°. Recrystallization from MeOH raised the m.p. to 224–226°. (Found: C, 51·86; H, 6·35. Calc. for $C_{19}H_{26}O_8N_4$: C, 52·05; H, 5·98%.)[8]

(iv) *Z·Glu(OEt)·Ser·Gly·OEt.* N-Benzyloxycarbonyl-γ-ethyl glutamic acid (3·1 g) was converted as usual to the active ester. Without purification, this was mixed with AcOH·H·Ser·Gly·OEt (from 2·8 g Z·Ser·Gly·OEt, as in the previous experiment) in 50 ml acetonitrile and stirred overnight at room temp. Working up as usual gave 2·4 g of the tripeptide, m.p. 145–147°. $[\alpha]_D^{22}$ −2·5° (*c*, 2 in EtOAc). (Found: C, 54·82; H, 6·67. Calc. for $C_{22}H_{31}O_9N_3$: C, 54·88; H, 6·49%.)

(v) *Z·Gly·Phe·Gly·OEt* (Anderson test). Z·Gly·Phe·OH was made in two steps via the ester using the pivalohydroxamic acid method in an overall yield of 84%. This acid (2·5 g) was reacted as usual in $CHCl_3$ soln with α-chloropivalaldoxime (1·23 g) and Et_3N (0·76 g). The active ester (3·1 g) was obtained as a gum by the usual work-up.

The active ester in acetonitrile (20 ml) was stirred with ethyl glycinate hydrochloride (0·95 g) in DMF (15 ml) together with AcONa (0·95 g) for 4 hr at room temp. The usual work-up gave 2·41 g of Z·Gly·Phe·Gly·OEt (78% after fractional crystallization in an attempt to detect any racemization. No racemic material was isolated), m.p. 117–119°. $[\alpha]_D$ −13·05 (*c*, 2·1 in EtOH).

[8] R. F. Fischer and R. R. Whetston, *J. Am. Chem. Soc.* 77, 750 (1955), have reported m.p. 139–140° for a "hydrate" of this tripeptide. However, our compound had the correct analytical and spectral data: IR (Nujol) bands at 1735, 1710, 1690 (Sh) and 1645 cm^{-1}. NMR (DMSO-d_6) signals corresponding to both the N-benzyloxycarbonyl and the terminal ethyl ester groups.

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