A Peptide Synthesis *via* Hydroxamic Acids

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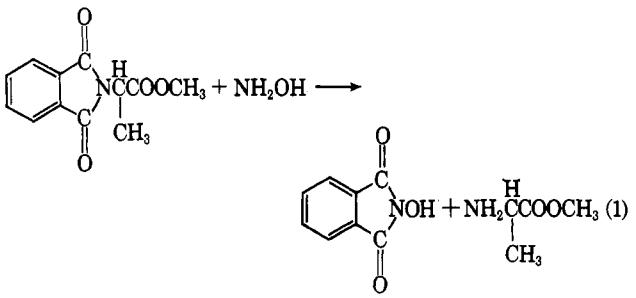
Hydroxamates of free and N-acylated amino acids, peptides, and proteins have been described in the literature and submitted to the Lossen rearrangement for purposes of characterization of the parent compounds.[1-3] Esters and other derivatives of carboxylic acids react with free hydroxylamine to yield the hydroxamic acids which can be easily identified by their color reaction with Fe^{+3} ions and which serve in turn as identification for the presence of esters and other derivatives of carboxylic acids. The ease and facility with which this reaction takes place affords a criterion for the "activation" of these groups towards attack by nucleophilic reactants.[4,5]

It is possible, however, to regard hydroxamic acids themselves as "activated" derivatives of carboxylic acids of the type RCOX and one might consequently contemplate the formation of an amide bond by attack of an amine on these compounds. We found only one citation in the literature for the formation of an amide from a hydroxamic acid, namely the formation of benzanilide by the reaction of aniline with benzhydroxamic acid.[6]

In order to investigate this type of reaction we prepared a number of hydroxamic acid derivatives of acylated amino acids and dipeptides which are listed in Table I. These compounds can be prepared by one of the following two methods. The corresponding ester is allowed to react with hydroxylamine in an alkaline methanol solution and the resultant hydroxamate salt is liberated with acid according to the general procedure of Hauser and Renfrow[7] (method A). Alternatively, the trialkylammonium salt of the free acid is treated with alkyl chloroformate to yield a mixed carbonic anhydride which in turn is decomposed by hydroxylamine to yield the corresponding hydroxamic acid[3] (method B). Both methods have been used in our work with fair yields. Method A, however, may be more suitable for a sequence of peptide syntheses, since the reaction of a hydroxamic acid with an amino acid ester yields a peptide ester, ready for further conversion into a hydroxamic acid. Both ethyl and methyl esters could be used for that purpose with good results. Since benzyl esters of amino acids are sometimes preferred in peptide syntheses, we used in one case (Table I, no. 5, N-benzyloxycarbonylglycylglycylhydroxamic acid) the corresponding benzyl ester as starting material, and in this case the yield was low. We also were unsuccessful in the preparation of hydroxamic acid derivatives of phthaloylamino acids. When methyl N-phthaloylalaninate reacted with hydroxylamine according to method A the resultant solution quickly turned yellow and then red. The usual work-up produced, in 88% yield, a substance with a melting point of 234° which gave no color reaction with Fe^{+3} ions and was identified as N-hydroxyphthalimide.[8,9] The reaction apparently went according to eq. 1. All hydroxamic acids prepared gave the characteristic



red color with Fe^{+3} ions and all except one could be obtained as white crystalline solids and characterized by melting points and elementary analysis. Only N-benzyloxycarbonylglycyl-DL-alanylhydroxamic acid could not be obtained in crystalline form and was used as isolated from the reaction mixture.

With these hydroxamic acids the anilides and amides in Table II and dipeptides and tripeptides in Table III were prepared. The reaction between the hydroxamic acid and the amine (or amino acid ester) was carried out in an inert solvent under reflux and stirring. The choice of solvents posed some difficulties. Inertness of the solvent was imperative since the reactants are prone to rearrangements and solvolysis. Completely inert solvents, however, were too nonpolar to have satisfactory solvent properties especially for the hydroxamic acids of acylated dipeptides. Among the varieties of solvents tried, dioxane gave consistently satisfactory results either with or without addition of ethanol to enhance solubility. Toluene proved a useful solvent as long as the hydroxamic acids dissolved in it to some extent during reflux. Some hydroxamic acids of acylated dipeptides proved to be insoluble in both these solvents and for these 1-butanol was used successfully. The reaction proceeds with evolution of ammonia (as tested with Nessler's reagent) which is apparently produced by decomposition of the liberated hydroxylamine at the reflux temperature of the re-

(1) S. Seifter, P. M. Gallop, S. Michaels, and E. Meilman, *J. Biol. Chem.*, **235**, 2613 (1960).

(2) P. M. Gallop, S. Seifter, M. Lukin, and E. Meilman, *ibid.*, **235**, 2619 (1960).

(3) T. Wieland and H. Fritz, *Ber.*, **86**, 1186 (1953).

(4) T. Wieland and D. Stimming, *Ann.*, **579**, 97 (1953).

(5) J. D. Raacke, *Biochim. Biophys. Acta*, **27**, 416 (1958).

(6) G. Minunni, *Gazz. chim. ital.*, **20**, 657 (1890), as cited in H. Yale, *Chem. Rev.*, **33**, 209 (1943).

(7) C. R. Hauser and W. B. Renfrow, Jr., "Organic Syntheses," Coll. Vol. II, A. M. Blat, Ed., John Wiley and Sons, Inc., New York, N. Y., 1943, p. 67.

(8) G. H. L. Nefkens, G. I Tesser, and R. J. F. Nivard, *Rec. trav. chim.*, **81**, 689 (1962).

(9) L. Cohn, *Ann.*, **205**, 295 (1880).

TABLE I

HYDROXAMIC ACIDS, RNHOH, PREPARED

R–	Method	M.p., °C.	Recrystg. solvent	Yield, %	Calcd.[e]	Found[e]
(1) Bz-gly-[a]	B	156–158	Ethyl acetate	68	C: 55.6	55.4
	A	159–160[b]			H: 5.2	5.1
	Ethyl ester			93	N: 14.4	14.0
(2) Bz-gly-gly-	B	177–179 dec.	Absolute EtOH,	77.2	C: 52.6	52.3
	A		*n*-BuOH		H: 5.2	5.5
	Ethyl ester			80.5	N: 16.7	16.4
(3) Bz-ala-	A	168	Acetonitrile	84.5	C: 57.7	57.7
	Methyl ester[c]				H: 5.8	6.0
					N: 13.4	13.2
(4) Cbze-gly-	B	118–120	Dioxane–toluene	70	C: 53.8	54.1
					H: 5.3	5.3
					N: 12.5	12.5
(5) Cbze-gly-gly-	A	153–154	EtOH	75	C: 51.2	51.1
	Ethyl ester				H: 5.3	5.0
	Benzyl ester			8.5	N: 14.9	15.1
(6) Cbze-ala-	A	127	Chloroform	66.5	C: 55.4	55.4
	Methyl ester[c]				H: 5.9	5.8
					N: 11.7	11.4
(7) Cbze-ala-gly-	B	140	Nitromethane	73.4	C: 52.9	52.7
					H: 5.8	6.0
					N: 14.2	13.7
(8) Cbze-gly-ala-	B[d]			77		

[a] Abbreviations used in these tables are Bz, Benzoyl-; gly, glycyl-; ala, alanyl-; Cbze, benzyloxycarbonyl-. [b] *Cf.* ref. 4. [c] *Cf.* Experimental section. [d] This substance could not be isolated in the pure form. [e] %.

TABLE II

ANILIDES AND AMIDES PREPARED FROM HYDROXAMIC ACIDS

$$R^*NHOH + NH_2R \longrightarrow R^*NHR$$

R*	Reaction time, hr.	Reaction solvent	Yield, %	M.p., °C.	Recrystg. solvent	Calcd.[d]	Found[d]
(A) R = C_6H_5—							
(1) Bz-ala-	27	Dioxane	31	174	EtOH–water	C: 71.6	71.6
				175[a]		H: 6.0	5.9
						N: 10.4	10.7
(2) Cbze-ala-	24	Dioxane	64	148	EtOH–water	C: 68.5	68.6
						H: 6.0	6.1
						N: 9.4	9.6
(3) Bz-gly-	7	Toluene	69	210	EtOH	C: 70.9	70.7
				208.5[b]		H: 5.5	5.6
						N: 11.0	10.9
(4) Cbze-gly-	7	Toluene	55	143	EtOH	C: 67.6	67.6
				146–147[c]		H: 5.6	5.9
						N: 9.9	9.6
(B) R = $C_6H_5CH_2$—							
(5) Bz-gly-	10	*t*-BuOH	50	162	EtOH	C: 71.6	71.5
						H: 6.0	5.8
						N: 10.4	10.3

[a] E. Mohr and P. Stroschein, *Ber.*, **42**, 2521 (1909). [b] T. Curtius, *J. prakt. Chem.*, **52**, 257 (1895). [c] J. R. Vaughan and R. L. Osato, *J. Am. Chem. Soc.*, **73**, 5553 (1951). [d] %.

action.[10] Thus the reaction apparently proceeds according to eq. 2.

$$RC(=O)NHOH + NH_2R' \longrightarrow RC(=O)NHR' + NH_2OH \quad (2)$$

The peptides prepared thus from the corresponding hydroxamic acids are known and easy to identify. The only exception is ethyl N-benzyloxycarbonyl-glycyl-DL-alanylglycinate for which a melting point of 179° has been reported.[11] Our product melted at 139° and showed the correct elementary analysis. We did, however, prepare this tripeptide derivative by an unambiguous route (from N-benzyloxycarbonylglycyl-DL-alanine and ethyl glycinate *via* the mixed carbonic anhydride procedure) and the resultant tripeptide derivative, too, had a melting point of 139° and correct elementary analysis.

In conclusion, it is pointed out that the method, outlined above, has been used so far for the synthesis of small peptides from simple amino acids. It remains to be seen whether it can be used for the synthesis of larger peptides from amino acids with additional functional groups and especially whether optically active amino acids and peptides retain their activity in this

(10) T. Moeller "Inorganic Chemistry," John Wiley and Sons, Inc., New York, N. Y., 1952, p. 580.

(11) S. Goldschmidt and M. Wick, *Ann.*, **575**, 217 (1952).

TABLE III

PEPTIDES PREPARED FROM HYDROXAMIC ACIDS

	M.p., °C.	Yield, %	Reaction solvent	Reaction time, hr.	Recrystg. solvent		Calcd.[g]	Found[g]
(A) Dipeptides								
(1) Bz-gly-gly-OEt	115	62	Dioxane–EtOH	30	Water	C:	59.1	59.1
	117[a]	30	Toluene–EtOH	6		H:	6.1	6.4
		10	Acetic acid	20		N:	10.6	10.7
			Dimethylformamide	20				
		42	Toluene	6				
		43.5	Toluene	6[b]				
		32.5	*t*-BuOH	48				
(2) Cbze-gly-gly-OEt	78–79	50	Dioxane–EtOH	25	Water–EtOH	C:	57.2	57.0
	78–82[c]					H:	6.1	5.8
						N:	9.5	9.3
(3) Cbze-ala-gly-OEt	86–87	53	Dioxane	30	Petr. ether–chloroform	C:	58.5	58.4
	81[d]					H:	6.5	6.6
					EtOH–water	N:	9.1	9.0
(4) Bz-ala-gly-OEt	108	36	Dioxane	35	Water	C:	60.4	60.4
	108[e]					H:	6.5	6.4
						N:	10.1	10.3
(B) Tripeptides								
(5) Cbze-gly-gly-gly-OEt	164	80	Dioxane–EtOH	35[b]	Water	C:	54.8	55.0
	164–166[c]					H:	5.9	5.9
						N:	11.9	11.8
(6) Bz-gly-gly-gly-OEt	168	70.4	*n*-BuOH	18	Water	C:	56.1	56.4
	173[a]					H:	5.9	6.2
						N:	13.0	13.0
(7) Cbze-ala-gly-gly-OEt	110	76	Dioxane	5[b]	Water–EtOH	C:	55.9	56.0
	113[f]					H:	6.3	6.2
						N:	11.5	11.5
(8) Cbze-gly-ala-gly-OEt	139	73	Dioxane	7[b]	Water–EtOH	C:	55.9	55.6
	179[c]					H:	6.3	6.3
						N:	11.5	11.4

[a] T. Curtius and R. Wustenfeld, *J. prakt. Chem.*, **70**, 78, 83 (1904). [b] In this experiment tributylamine instead of triethylamine was used to liberate the ethyl glycinate from its hydrochloride. [c] *Cf.* ref. 11. [d] See ref. 13. [e] Th. Curtius and C. F. van der Linden, *Ann.*, **70**, 153 (1904). [f] G. Schramm and H. Wissmann, *Ber.*, **91**, 1073 (1958). [g] %.

synthetic procedure. These problems are at present under investigation.

Experimental[12]

Methyl N-benzoyl-DL-alaninate was prepared by adding N-benzoyl-DL-alanine to an ethereal solution of diazomethane in the cold. The ether was then evaporated and the residue recrystallized from cyclohexane and melted at 82°.

Anal. Calcd. for $C_{11}H_{13}NO_3$: C, 63.8; H, 6.3; N, 6.8. Found: C, 63.9; H, 6.1; N, 6.8.

Methyl N-benzyloxycarbonyl-DL-alaninate was prepared as above from N-benzyloxycarbonyl-DL-alanine and diazomethane. It was recrystallized from water–ethanol (5:1) and melted at 46°.

Anal. Calcd. for $C_{12}H_{15}NO_4$: C, 60.8; H, 6.3; N, 5.9. Found: C, 60.9; H, 6.4; N, 5.8.

Methyl N-phthaloyl-DL-alaninate was prepared as above from N-phthaloyl-DL-alanine and diazomethane. It was recrystallized from water–methanol and melted at 67°.

Anal. Calcd. for $C_{12}H_{11}NO_4$: C, 61.9; H, 4.7; N, 6.0. Found: C, 61.8; H, 4.9; N, 6.4.

N-Benzyloxycarbonyl-DL-alanylhydroxamic Acid (Method A).—A 9.1-g. sample (0.0384 mole) of methyl N-benzyloxycarbonyl-DL-alaninate was added to a methanolic solution of hydroxylamine [prepared from 5.1 g. (0.0735 mole) of hydroxylamine hydrochloride and 5.8 g. (0.103 mole) of potassium hydroxide]. The resultant solution was left to stand at room temperature for 48 hr. after which time a strong color reaction with Fe^{+3} ions was obtained. The solution was then evaporated to dryness under vacuum at room temperature and the residual potassium salt dissolved in a minimum of dilute (5%) hydrochloric acid. The free acid precipitated upon cooling and was collected and air-dried to yield 6 g. (66%). It was twice recrystallized from chloroform and melted at 127° (for analysis see Table I).

N-Benzyloxycarbonyl-DL-alanylglycylhydroxamic Acid (Method B).—A 5.6-g. sample (0.02 mole) of N-benzyloxycarbonyl-DL-alanylglycine[13] was dispersed in 135 ml. of sodium-dried tetrahydrofuran and 2.02 g. (0.02 mole) of triethylamine added. The resultant solution was added dropwise to a cooled (ice-water bath) and stirred solution of 2.16 g. of ethyl chloroformate in 25 ml. of tetrahydrofuran during 45 min. After the addition, the solution was stirred and cooled for another 25 min. and the precipitate of triethylammonium chloride was then filtered. A solution of hydroxylamine [prepared from 1.4 g. (0.02 mole) of hydroxylamine hydrochloride and 0.8 g. (0.02 mole) of sodium hydroxide] in methanol was then added to the filtrate and the resultant solution concentrated by distillation at normal pressure to about one-tenth of its initial volume. The residue was cooled and diluted with ether. The precipitated product was then filtered and air-dried, yield 4.3 g. (73%). It was recrystallized from nitromethane and melted at 140° (for analysis see Table I).

N-Hydroxyphthalimide.—A 13-g. sample (0.0558 mole) of methyl N-phthaloyl-DL-alaninate was added to a solution of hydroxylamine [prepared from 11.8 g. (0.17 mole) of hydroxylamine hydrochloride and 11.2 g. (0.2 mole) of potassium hydroxide] in 50 ml. of methanol. The resultant solution was left to stand for 48 hr. during which time it turned a deep red (color of N-hydroxyphthalimide anion) and deposited red crystals. The solution was concentrated *in vacuo* and the resultant slurry acidified with dilute hydrochloric acid. The resultant precipitate was filtered and air-dried, yield 8 g. (88%). It was recrystallized from ethanol and melted at 234°, lit.[14] 230°.

Anal. Calcd. for $C_8H_5NO_3$: C, 58.9; H, 8.6; N, 3.1. Found: C, 60.0; H, 8.3; N, 3.1.

Ethyl N-Benzyloxycarbonyl-DL-alanylglycylglycinate.—A 5-g. sample (0.017 mole) of N-benzyloxycarbonyl-DL-alanylglycyl-hydroxamic acid was dispersed with 2.8 g. (0.02 mole) of ethyl

(12) All melting points are uncorrected.

(13) S. Goldschmidt and H. Lautenschlager, *Ann.*, **580**, 68 (1953).

(14) G. H. L. Nefkens and G. I. Tesser, *J. Am. Chem. Soc.*, **83**, 1263 (1961).

glycinate hydrochloride and 3.7 g. (0.02 mole) of tributylamine in 50 ml. of dry dioxane and refluxed with stirring for 5 hr. During this time the initial reactants dissolved slowly and a new precipitate was formed. The progress of the reaction could be followed by the evolution of ammonia and by the gradual decline in intensity of the color reaction of the solution with Fe^{+3} ions. After 5 hr. this test was very weak and the solution was then evaporated to dryness under reduced pressure. The residue was dissolved in hot water, filtered, and left to crystallize in the ice box overnight. A 4.7-g. sample (76%) of product was obtained which was recrystallized from ethanol–water (1:1) and melted at 110°, lit. 113° (for analysis see Table III).

Ethyl N-benzyloxycarbonylglycyl-DL-alanylglycinate was prepared as above from 5.9 g. (0.02 mole) of crude N-benzyloxycarbonylglycyl-DL-alanylhydroxamic acid, 3.0 g. (0.022 mole) of ethyl glycinate hydrochloride, and 4 g. (0.022 mole) of tributylamine. A 5.3-g. sample (73%) of dry product was obtained which was recrystallized from ethanol–water (1:4) and melted at 139° (for analysis see Table III).

Ethyl N-Benzyloxycarbonylglycyl-DL-alanylglycinate (*via* Mixed Carbonic Anhydride).—A 5.6-g. sample (0.02 mole) of N-benzyloxycarbonylglycyl-DL-alanine[11] was dispersed in 145 ml. of sodium-dried tetrahydrofuran and 2.02 g. (0.02 mole) of triethylamine added. The resultant solution was added dropwise to a cooled (ice–water bath) and stirred solution of 2.16 g. of ethyl chloroformate in 25 ml. of tetrahydrofuran during a period of 45 min. After the addition the solution was stirred and cooled for another 25 min. and the precipitate of triethylammonium chloride was then filtered. A cooled solution of 2.8 g. (0.02 mole) of ethyl glycinate hydrochloride and 0.8 g. (0.02 mole) of sodium hydroxide in a minimum amount of water was then added to the filtrate and the resultant clear solution stirred and concentrated by distillation under normal pressure to approximately one-tenth its initial volume. The rest of the solvent was then evaporated under reduced pressure and the residue washed with 30 ml. of 10% sodium carbonate solution and then recrystallized from ethanol–water (1:4). Five grams (68.5%) of white crystalline product was obtained which melted at 139°.

Anal. Calcd. for $C_{17}H_{23}N_3O_6$: C, 55.9; H, 6.3; N, 11.5. Found: C, 55.9; H, 6.0; N, 11.4.

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Thermolysis of Azidoformates in Aromatic Compounds. A Synthesis of 1*H*-Azepin-1-yl Carboxylates

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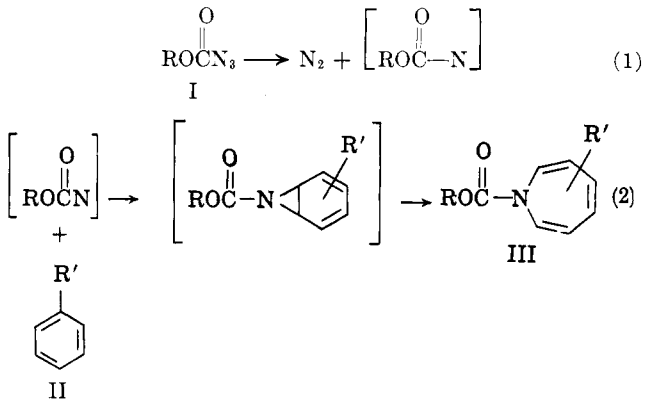
Two recent reports[1,2] have described the photolysis of ethyl azidoformate to yield carbethoxynitrene, and its subsequent reaction with benzene to yield N-carbethoxyazepine. As part of a study of the chemistry of azidoformates, we have found that simple thermolysis of ethyl and phenyl azidoformates in aromatic compounds also generates the intermediate nitrenes, as evidenced by the isolation of 1*H*-azepin-1-yl carboxylates.

Ethyl azidoformate was synthesized by a published procedure[3] and used for thermolysis without distillation. Phenyl azidoformate[4] was obtained from the reaction of phenyl chloroformate with sodium azide in acetone. The undistilled product from this reaction was found to contain some diphenyl carbonate. However, the use of this crude phenyl azidoformate for thermolysis did not appear to affect the reaction adversely. The presence of unchanged chloroformates in either of these azidoformates was deleterious to the thermolytic reaction. The undistilled azidoformates were generally used for thermolysis because explosions have been encountered when they have been heated excessively on distillation.

Ethyl azidoformate was thermolyzed in dry benzene solution (1–2% by weight) at 125° to yield N-carbethoxyazepine in about 40% yield. Although no exhaustive study of reaction conditions has been performed to maximize the yield, reaction times of 1–2 hr. and concentrations as described above have given satisfactory results. Removal of the benzene yielded a residue from which the azepine derivative could be isolated by distillation. However, better yields were obtained when the residue was chromatographed prior to being distilled. N-Carbethoxyazepine (III, R = $-C_2H_5$, R′ = H)



R = $-C_2H_5$, $-C_6H_5$; R′ = –H, $-CH_3$, –Cl, $-C_6H_5$

is a red-orange liquid stable indefinitely at 0° in sealed glass ampoules. Evidence for its structure was obtained from elemental, infrared, and ultraviolet analyses and from an examination of its nuclear magnetic resonance spectrum (see below). In addition, it was catalytically hydrogenated to N-carbethoxyhexamethylenimine; its properties were identical with those of an authentic sample. Similarly, ethyl azidoformate has been thermolyzed in toluene, chlorobenzene, and biphenyl to yield the respective 1*H*-azepin-1-yl carboxylates (III, R = $-C_2H_5$; R′ = CH_3, –Cl, $-C_6H_5$) of unknown isomeric composition.

Solutions of phenyl azidoformate in benzene (about 1% by weight) were heated at 125° for 2 hr. No study was made of the optimum temperature and time for this reaction, but solutions of higher concentration (about 10% by weight) were found to give noticeably larger amounts of tars. N-Carbophenoxyazepine (III, R = $-C_6H_5$, R′ = H) was isolated as bright yellow crystals by chromatography and sublimation. It is stable when stored in sealed ampoules in a refrigerator. Evidence for its structure was obtained by elemental and spectral analyses. Catalytic hydrogenation of N-carbophenoxyazepine yielded N-carbophenoxyhexamethylenimine which was identical with an authentic sample.

(1) K. Hafner and C. Konig, *Angew. Chem.*, **75**, 89 (1963).

(2) R. S. Berry, D. Cornell, and W. Lwowski, *J. Am. Chem. Soc.*, **85**, 1199 (1963); W. Lwowski, T. Maricich, and T. Mattingly, *ibid.*, **85**, 1200 (1963).

(3) M. Forster and H. Fierz, *J. Chem. Soc.*, (1908) 81.

(4) G. Smolinsky, E. Wasserman, and W. A. Yager, *J. Am. Chem. Soc.*, **84**, 3220 (1962).