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O-PROTECTED DERIVATIVES OF N-HYDROXYAMINO ACIDS

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Abstract—Various N-alkoxyamino acids were studied, and N-benzyloxyamino acids were chosen as the most suitable substrates for unambiguous synthesis of N-hydroxy peptides.

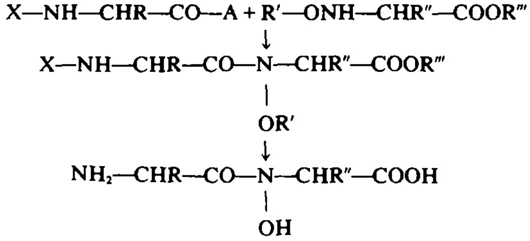
N-Hydroxyamino acids were obtained in the past century[1] but their derivatives have only recently been identified as constituents of natural products. The oxidized form of the peptide bond (the N-hydroxypeptide bond) is found chiefly in metabolites of microorganisms. Reviews of such hydroxamic acids have been given by Mikes,[2] Chimiak,[3] Neilands,[4] and recently by Meahr.[5] The biological role of these compounds is not clear; they act as growth factors, antibiotics, antitumour or fungistatic agents.[4] According to unconfirmed data, N-hydroxypeptides have been found in human and mouse tumours.[6]

In this situation, model compounds of the N-hydroxypeptide type are required to confirm the structures of natural products, to investigate their biological action, to adapt the methods of sequential analysis[7] and to study differences in enzymatic digestion.

N-Hydroxypeptides have so far not been obtained by the simple method of oxidizing the peptide bond. Several simple N-hydroxydipeptides are reported to have been obtained in two studies[8,9] by acylating free N-hydroxyglycine. The results of these studies did however, not ensure unambiguous synthesis, nor did they lead to pure N-hydroxypeptides with high yield.

Unambiguous synthesis of N-hydroxypeptide just as that of "normal" peptides must go through three stages: synthesis of adequately protected substrates, formation of the hydroxypeptide bond, and removal of the protecting group. Various methods have already been reported for obtaining N-hydroxyamino acid esters[10–12] but such substrates are not advantageous: we have recently found that acylation of N-hydroxaminoacid esters may lead to N- or O-acyl-N-hydroxyaminoacid esters, dependent on the activating method employed.[12] Moreover, N-hydroxyamino acids and their esters are inconvenient substrates because they have a free hydroxylamine group, and consequently have strong reducing properties.[11]

In view of this we have suggested[13] the following unambiguous path for the synthesis of N-hydroxypeptides, taking into account protection of the oxygen atom of the hydroxylamine function.



$$\begin{array}{c} X\text{—NH—CHR—CO—A} + R'\text{—ONH—CHR}''\text{—COOR}''' \\ \downarrow \\ X\text{—NH—CHR—CO—}\underset{\displaystyle OR'}{\underset{|}{N}}\text{—CHR}''\text{—COOR}''' \\ \downarrow \\ NH_2\text{—CHR—CO—}\underset{\displaystyle OH}{\underset{|}{N}}\text{—CHR}''\text{—COOH} \end{array}$$

where X—protect. groups, A—activating groups R,R′, R″, R‴ alkyl gr.

Since O-acyl-hydroxylamine derivatives are too reactive[14] and useless as blocked substrates, it seemed that only N-alkoxyamino acids could be key substrates for the procedure suggested. This synthetic route permits obtaining O-blocked substrates directly, without synthesis of N-hydroxyamino acids, which are not readily available and none of the methods for synthesis of N-hydroxyamino acids hitherto reported[3] has proved efficient; the method of obtaining them from a bromo acid has its limitations as mainly oximino acids are obtained.

We based the N-alkoxyamino acid synthesis presented here on the α-bromo acid aminolysis reaction, using various N-alkoxyamines. These "uncommon" amines were prepared by N-hydroxyphthalimide alkylation and removing the phthaloyl groups by hydrazinolysis.[15]

N-Alkoxyamino acid synthesis was effected in three variants: aminolysis, with twofold amine excess; with triethylamine as additional base with separation involving ion exchange resin.

With regard to the fact that the various alkyl groups differ in acidolysis rates, we prepared several types of N-alkoxyamino acids. Despite a number of reports already published on N-alkoxyamines, amino acid derivatives of this kind have so far been unknown.

N-Alkoxyamino acids are crystalline (apart from N-ethoxy and N-sec-butoxy alanines) colourless low-melting solids. Some of them decompose when stored for a long time. They are readily soluble in

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†Part of T. K.'s doctoral thesis (Gdańsk 1974).

Table 1. N-alkoxyamino acids R–O–NH–CHR′–COOH

R	R′	m.p. °C	Yield %	Formula Mol. wt.	Calcd.			Found		
					C	H	N	C	H	N
Me	Me	96–8	100	$C_4H_9NO_3$ 119·12	40·33	7·61	11·76	40·60	7·67	11·54
i-Pro	i-Bu	113–5	15[b,c]	$C_9H_{19}NO_3$ 189·26	57·12	10·12	7·40	57·14	9·94	7·24
i-Pro	i-Bu	115–8[a]	19[b,c]	$C_9H_{19}NO_3$ 189·26	57·12	10·12	7·40	57·04	10·05	7·47
i-Pro	n-Bu	106–8	19[b,c]	$C_9H_{19}NO_3$ 189·26	57·12	10·12	7·40	57·51	9·81	7·52
s-Bu	Me	oil	72	$C_{17}H_{15}NO_3$ 161·20	52·15	9·38	8·69	52·05	9·30	7·72[d]
t-Bu	Me	63–5	96	$C_7H_{15}NO_3$ 161·20	52·15	9·38	8·69	51·90	9·39	8·68

[a] N-isopropoxy-D-leucine has $[\alpha]_D^{20} = +11{\cdot}6$ (c 1·6 EtOH), all other compounds are racemic.
[b] Isolated using Zeolite 225.
[c] Obtained by A. Kitowska.
[d] Structure confirmed by NMR.

Table 2. N-benzyloxyamino acids $C_6H_5CH_2$–ONH–CHR–COOH

R	m.p. °C	Conf.	Yield%	$[\alpha]_D^{20}$ (c2 EtOH)	Formula Mol. wt.	Calcd.			Found		
						C	H	N	C	H	N
H	116–7		92		$C_9H_{11}NO_3$ 181·19	59·66	6·12	7·73	59·71	6·10	7·80
Me	123–4	DL	95		$C_{10}H_{13}NO_3$	61·54	6·67	7·17	61·37	6·82	6·98
Me	113–4	L	81	−25·1	195·22				62·07	6·62	7·29
Me	114–5	D	54	+25·5					62·03	6·57	7·03
Et	118–9	DL	70		$C_{11}H_{15}NO_3$ 209·24	63·14	7·22	6·69	62·88	6·93	6·51
i–Pro	120–2	DL	80		$C_{12}H_{17}NO_3$	64·55	7·67	6·27	64·71	7·92	6·33
i-Pro	117–8	L	30	+22·05	223·27				64·87	7·70	6·17
i-Pro	118–20	D	25	−21·5					64·83	7·33	6·62
i-Bu	129–31	DL	51		$C_{13}H_{19}NO_3$	65·81	8·07	5·90	65·98	8·22	5·50
i-Bu	121–4	L	45	−23·0	237·30				66·00	8·00	5·60
i-Bu	122–4	D	34	+21·5					65·45	7·78	6·02
Bzl	136–8	DL	56		$C_{16}H_{17}NO_3$	70·83	6·31	5·16	71·10	6·44	4·98
Bzl	129–31	D	50	+10·5	271·32				70·95	6·09	4·94
H[a]	103–5		30		$C_9H_{10}N_2O_5$ 226·19	47·79	4·46	12·38	48·01	4·45	12·13
Me[a]	163–5	DL	90		$C_{10}H_{12}N_2O_5$ 240·21	50·00	5·04	11·62	50·31	4·83	11·67
i-Pro[a]	148–50	DL	40		$C_{12}H_{16}N_2O_5$ 268·27	53·72	6·01	10·44	54·08	5·93	10·68

[a] N-p-nitrobenzyloxyamino acids.

most organic solvents, except in petroleum ether and poorly soluble in water. The fact that they do not crystallize readily, makes synthesis sometimes difficult. In contrast, high-melting benzyloxy and p-nitro-benbenzyloxy amino acids crystallize readily.

The structures of N-alkyoxyamino acids have been confirmed by NMR spectroscopy. Selected NMR spectra are shown in Table 3. The presence of the benzyloxy group is shown by the methylene signlet at δ 4·85 (2H) and the aromatic signal at 7·55 (5H). The NH signal is broad and can be observed only in $CDCl_3$ at 4·5–6·5 ppm.

N-Alkoxyamino acids are somewhat similar in solubility to N-acylamino acids, but in contrast, their IR spectra do not show bands at 1730 cm^{-1}, typical of the carbonyl group, suggesting a zwitter-ion structure. In thin layer chromatography in solvent systems used for amino acids they have higher R_f values (Table 4). They give a positive ninhydrin reaction, the sensitivity of which is as low as for N-methylamino acids.

Comparison of the acid-base properties of these compounds revealed higher acidity in the carboxyl group than in amino and N-hydroxyamino acids[16] and depressed basicity of the amine function (see Table 5).

Table 3. NMR of selected N-alkoxyamino acids R–O–NH–CHR′–COOH

Compound[a]	CH of amino acid residue				CH of alkoxy group		
	$C_\alpha H$	$C_\beta H$	$C_\gamma H$	$C_\delta H$	–CH–O–	others	
BzlO-Gly[b]	3·8 (s, 2H)				4·9 (s, 2H)	7·6 (s, 5H)	
BzlO-Ala[b]	3·82 (q, 1H)	1·3 (d, 3H)			4·85 (s, 2H)	7·5 (s, 5H)	
BzlO-But[c]	3·50 (t, 1H)	1·52 (q, 2H)	0·88 (t, 3H)		4·8 (s, 2H)	7·25 (s, 5H)	
BzlO-Val[c]	3·33 (d, 1H)	1·75 (m, 1H)	0·87 (d, 6H)		4·62 (s, 2H)	7·25 (s, 5H)	
BzlO-Leu[b]	3·7 (t, 1H)	1·50 (q, 2H)	1·87 (m, 1H)	1·05 (d,[d] 6H)	4·85 (s, 2H)	7·55 (s, 5H)	
BzlO-Phe[b]	4·0 (t, 1H)	3·0 (d, 2H)	7·47 (s, 5H)		4·85 (s, 2H)	7·55 (s, 5H)	
p-NO$_2$BzlO-Gly[b]	3·77 (s, 2H)				5·0 (s, 2H)	7·8 (d, 2H) 8·4 (d, 2H)	and
s-BuO-Ala[c]	3·67 (m, 1H)[d]	1·2 (d, 3H)			3·67 (m, 1H)[d]	1·37 (m, 2H) 1·1 (d, 3H)	0·8 (t, 3H)
t-BuO-Ala[b]	3·80 (q, 1H)	1·89 (d, 3H)				1·3 (s, 9H)	
i-ProO-Leu[b]	3·6 (t, 1H)	1·40 (t, 2H)	1·8 (m, 1H)	1·1 (d, 6H)	3·9 (m, 1H	1·25 (d, 6H)	
i-ProO-NLeu[c]	3·5 (m, 1H)		1·3 (m, 6H)	0·8 (t, 3H)	3·8 (m, 1H)	1·0 (d, 6H)	

[a] Standard abbreviations for amino acid residues and protecting group according to [21].

[b] In DMSO.

[c] In CDCl$_3$; broad signal of NH at 4·5–6·5 ppm.

[d] Signals of –CHO– and –CHN– overlap.

Table 4. Comparison of R_f values of N-alkoxyamino acids

	S_1	S_2
N-methoxy-alanine	0·66	0·55
N-s-butoxy-alanine	0·82	0·75
N-t-butoxy-alanine	0·84	0·82
N-benzyloxy-alanine	0·88	0·83
N-p-nitrobenzyloxy-alanine	0·87	0·82
N-hydroxy-alanine	0·45	0·02
Alanine	0·32	0·00
N-benzyloxy-glycine	0·87	0·75
N-hydroxy-glycine	0·35	0·01
Glycine	0·21	0·00
N-benzyloxy-valine	0·91	0·76
N-hydroxy-valine	0·58	0·06
Valine	0·46	0·02
N-benzyloxy-phenylalanine	0·90	0·81
N-hydroxy-phenylalanine	0·65	0·21
Phenylalanine	0·52	0·09
N-benzyloxy-leucine	0·88	0·82
Leucine	0·49	0·05
N-benzyloxy-aminobutyric ac.	0·91	0·77
N-hydroxy- -aminobutyric ac.	0·59	0·08

S_1: Solv. syst. n-BuOH-AcOH-H_2O (4:1:1).
S_2: Solv. syst. $CHCl_3$-MeOH-AcOH (85:10:5).

Table 5. Dissociation constans[a] of N-alkoxyamino acids

	pK_1	pK_2
N-methoxy-alanine	1·42	4·26
N-s-butoxy-alanine	1·06	3·37
N-t-butoxy-alanine	1·09	3·60
N-benzyloxy-alanine	1·57	4·23
N-hydroxy-amino acid[b]	2·1–2·3	5·7–5·8
Alanine[c]	2·34	9·96
Metoxyamine[c]		4·51
Ethylamine[c]		10·82
Acetic acid[c]	4·70	

[a] In water + 5% methanol at 25°C.
[b] According to Spencer.[16]
[c] Lit. data.

Since in the final stage of N-hydroxypeptide synthesis all protecting groups, including the alkyl group in the oxygen atom, have to be removed, it was necessary to make an initial selection of appropriate acyldolytic factors and to choose one type of N-alkoxyamino acid. Model tests of N–O bond splitting by means of trifluoroacetic acid, conducted on various amines, showed considerable stability of the N–O system, and heating for many hours gave only evidence of the N-acylating process.

In similar experiences with N-alkoxyamino acids, the cleaving process proceeded also with difficulty, whatever the alkyl structure (methyl, s-butyl, t-butyl- benzyl). Hydrogen bromide in acetic acid, which is commonly used in peptide chemistry, removes the protecting groups only at elevated temperatures, giving N-hydroxyamino acids. These results agree with those of Isowa[17] on N-ω-benzyloxy derivatives.

The best reagent, as found in initial tests, proved to be the recently-introduced boron trifluoracetate[18] which removes the benzyl group within an hour in good yield.

Finally, for further syntheses benzyloxyamino acids were chosen, in consideration of the favourable physical properties of benzyloxylamino aicds, particularly their high m.ps good crystallizibility, poor solubility in water, absence of marked differences in the cleaving process of the various alkyl groups, and relative availability.

Using the Testa method[19] optically active α-bromo acids were obtained, several of which we transformed into N-benzyloxyaminoacids of the L and D series. These compounds served for the synthesis of methyl, t-butyl and benzyl esters and for that of N-benzyloxy peptides. This will be discussed in a further report.

EXPERIMENTAL

M.ps are uncorrected. Optical rotations were measured on a Hilger–Watts polarimeter. Dissociation constants were determined potentiometrically. All pH titrations were carried out at 25 ± 0·1°C. The pH-meter (LBST-7 Eureka, Poland) was standardized against a potassium hydrogen phthalate and borax buffer. Ionic strength from 10^{-3} to 10^{-2}. NMR spectra were determined with a Tesla-Brno BS 487 apparatus at 80 MHz. Chromatograms were run on thin layers of silica gel (Kieselgel G, Merck).

N-alkoxyamino acids (general procedure)

0·1 Mole of α-bromoacid was added to a solution of 0·4 mole of N-alkoxyamine in 200 ml of anhydrous ethanol (or dioxane in the case of N-p-nitrobenzyloxyamine). The reaction mixture was allowed to stand at room temperature for two weeks. This solution was acidified (pH 4) using methanolic or ethereal hydrogen chloride, and evaporated to dryness under reduced pressure. Then 100 ml of anhydrous ether was added and the unreacted hydrochloride of the N-alkoxyamine was filtered off. The solid was washed with dry ether. The combined ether solvents were evaporated and unreacted α-bromoacid was removed by several extractions using petrol ether (b.p. 40–50°C). N-Alkoxy amino acid was crystallized from ethyl ether-petroleum or ethanol-water. Yields and m.ps are shown in Table 1. The reaction mixture may also be introduced on a Zeolite 225 (H^+) column previously washed with water to make the eluates bromoacid-free, and the N-alkoxy amino acid eluted with 3% aqueous ammonia. The eluates, evaporated to dryness, to afford the product.

N-alkoxylamino acids (procedure with tert base)

0·1 Mole of α-bromoacid was added a solution of 0·2 mole of N-alkoxyamine and 0·2 mole of triethylamine in 200 ml of ethanol (or dioxane). Conditions of reaction and separation procedure were as previously described. Yields by this modification were generally 20% lower.

N-benzyloxyamino acids

Proportions of reagents and solvent and reaction conditions were as described in general procedure. In the cases of benzyloxyleucine, -valine and -phenylalanine, the reaction mixture was refluxed for 8 h. Ethanol was then evaporated under reduced pressure and the residue was poured into 10% aqueous solution of sodium carbonate. The unreacted excess benzyloxyamine was extracted with ether (3 × 100 ml). After acidification (pH 3) of the water solution, the benzyloxyamino acid is precipitated. The crude product was separated, washed with water and recrystallized from ethanol-water.

Cleavage experiments

3 Millimoles of N-benzyloxyamine and 10 millimoles of boron trifluoroacetate in 2 ml trifluoroacetic acid was allowed to stand for 1 h at 0°C. The amount of hydroxylamine was determined iodometrically.[20] Total yield of the reaction was 80%. Similarly 1 millimole of benzyloxy-α-aminobutyric acid (or benzyloxy-valine) and 3·9 millimole of boron trifluoroacetate in 2 ml of trifluoroacetic acid was allowed to stand as above. The amount of released N-hydroxyamino acid, determined according to,[11] was 75%.

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