Tetrahedron Letters No. 18, pp 1523 - 1526, 1977. Pergamon Press. Printed in Great Britain.

A NEW, VERSATILE ACYL ACTIVATING REAGENT FOR PEPTIDE SYNTHESIS: ASYMMETRICALLY SELECTIVE PEPTIDE SYNTHESIS BY USE OF OPTICALLY ACTIVE 3-HYDROXYHYDANTOIN

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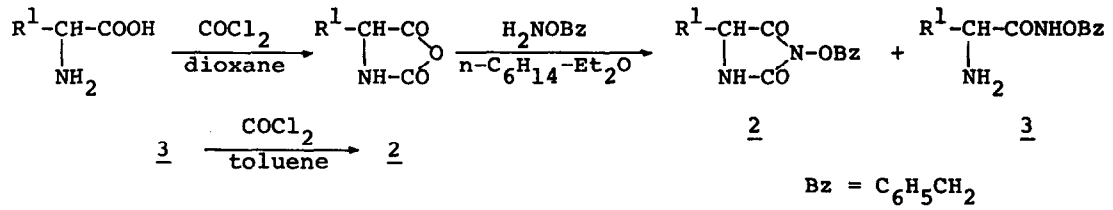
(Received in Japan 28 February 1977; received in UK for publication 22 March 1977)

There have been studied a number of acyl activating reagents containing N-hydroxyimide group represented by N-hydroxysuccinimide for peptide synthesis[1]. These compounds are employed extensively to prepare peptides quantitatively without racemization.

In this communication, we designed 3-hydroxyhydantoin 1 as a new acyl activating reagent containing N-hydroxyimide structure. Furthermore, since this reagent has an asymmetric center, the asymmetrically selective reaction would be expected when 3-hydroxyhydantoin ester of N-blocked α-amino acid is reacted with racemic α-amino acid ester.

The preparation of 3-benzyloxyhydantoin 2 was carried out as follows:



The N-caboxyanhydride(NCA) of α-amino acid was prepared by usual method. After the solution of NCA was added dropwise to a n-hexane-ether solution of twice as much benzyloxyamine as NCA by mole at 0°C, the mixture was stirred for a week at room temperature to produce a mixture of 2 and benzyloxyamide 3[2]. Amide 3 formed as a by-product was easily converted to 2 in good yield by treating with phosgene (Table 1).

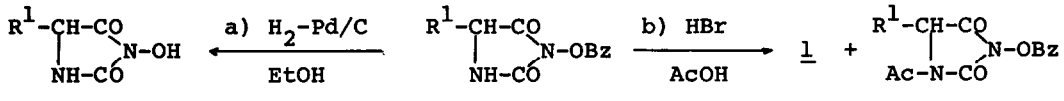
Debenzylation of 2 was carried out through the following two routes:

Table 1 Syntheses of 3-Benzyloxyhydantoin 2

R^1	Yield (%) 2	3	3 → 2	m.p. (°C)	I.R. (cm^{-1}) NH	C=O	N.M.R.(δ) NH
CH_3(L)[a]	34	42	89	109-10	3280	1760,1720	6.02[b]
CH_3(D,L)	32	49	86	112.5-3	3240	1780,1730,1720	6.12[b]
i-C_4H_9(L)	30	33	94	121-2	3180	1780,1720	8.44[c]

a) Types of optical isomer of starting α-amino acid were shown in ( ).
b) Measured in $CDCl_3$ c) Measured in DMSO-d_6

2 was a) treated with hydrogen (atmospheric pressure) in the presence of palladium/carbon, and b) reacted with hydrogen bromide in acetic acid.



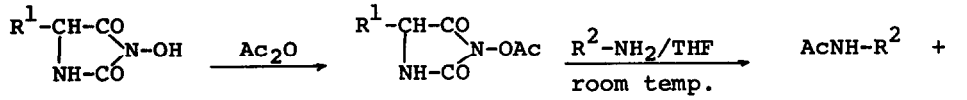
1 2 4

In the route b), 1-acetyl-3-benzyloxyhydantoin 4 besides 1 was formed, but in route a), 1 was prepared exclusively (Table 2).

Table 2 Syntheses of 3-Hydroxyhydantoin 1

R^1	Yield (%) Route (a)	Route (b)	m.p. (°C)	I.R. (cm^{-1}) NH,OH	C=O
CH_3	97.8	82.5	163-4	3300,3170	1770,1725,1695
i-C_4H_9	100	92.7	165-6	3250-3100	1770,1730,1690

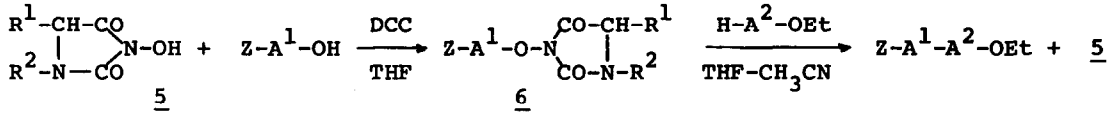
In order to see the effect of 1 on acyl activation, 1 was treated with acetic anhydride and the resulting 3-acetoxyhydantoin was reacted with amines. Corresponding amides were obtained in good yield as shown below.



1	Yield(%)	m.p.(°C)	Yield(%)	
R^1 = CH_3	94.1	111-2	89.7(R^2=C_6H_5)	63.7(R^2=C_6H_{11})
R^1 = i-C_4H_9	99.7	118-9	91.5(")	74.2(")

Next, the utility of 1 using as the active ester of α-amino acid was examined. Thus, 1 was reacted with N-blocked amino acids at 0°C for 6 hr in THF by use of N,N'-dicyclohexylcarbodiimide (DCC) as condensing agent. After N,N'-dicyclohexylurea was removed by filtration, ethyl glycinate (free base) in

THF-acetonitrile was added to the filtrate and stirred for 24 hr at 0°C. The reaction would proceed as depicted below (5, R^2=H, A^1=L-Ala,L-Leu, A^2=Gly).



5 6

R^1= CH_3, i-C_4H_9 R^2= H,Ac A^1= Gly, Ala, Leu A^2= Gly, Ala

The results are summarized in Table 3 . These data show that dipeptides are obtained in good yields holding the configuration of original asymmetric center.

Table 3 Preparation of Peptides Using 3-Hydroxyhydantoin

	Z-L-Ala-Gly-OEt			Z-L-Leu-Gly-OEt		
R^1	Yield(%)	m.p.(°C)	$[\alpha]_D^{a)}$	Yield(%)	m.p.(°C)	$[\alpha]_D^{a)}$
CH_3	88.5	98-9	-21.3	91.3	102-3	-27.1
i-C_4H_9	87.2	100	-21.4	87.2	103-4	-27.2
[lit]		99-100	-21.2[b)]		103-4	-27.2[c)]

a) c=1,at 25°C in EtOH, b) H.J.Pannemann, A.F.Max and J.F.Arens, Rec.Trav. Chim.,77,487(1958), c) G.W.Anderson, J.Blodinger and A.D.Welcher, J.Amer. Chem.Soc.,74,5309(1952).

In above procedure, if the active ester (6) bearing asymmetric center reacts predominantly with either L- or D-isomer of α-amino acid, highly optical active dipeptide should be obtainable from the racemates at a single effort. Thus, 3-hydroxyhydantoin ester of α-amino acid (6, A^1= Gly,L-Ala) were allowed to react with twice moles of L-,D- or D,L-ethyl alanate in THF-acetonitrile (1:1) at 0°C for 24 hr. The results are summarized in Table 4 . The yields of dipeptides obtained from L- ethyl alanate were superior to that from D-isomer. The Table also shows the reaction proceeds with high selectivity as deduced from the optical purity based on the specific rotation of dipeptides.

Further, it is well known that the acyl activating reagents of bifunctional N-hydroxy derivatives are used effectively as additives in DCC method[3]. So, the THF solution of DCC was added dropwise to the THF-acetonitrile (1:1) solution of N-blocked amino acid, D,L-ethyl alanate and hydantoin 5 at 0°C, followed by stirring for 24 hr. The results were included in Table 4 .

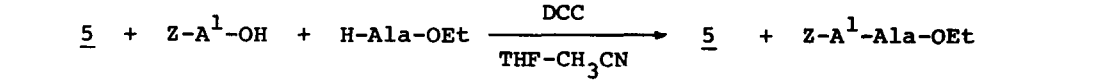


Table 4 Asymmetrically Selective Peptide Synthesis Using 3-Hydroxyhydantoin 1[a)]

Substituent R^1	Substituent R^2	$[\alpha]_D^{a)}$ of 5	Isomer of H-Ala-OEt	Z-Gly-Ala-OEt Y(%)	Z-Gly-Ala-OEt $[\alpha]_D$	Z-Gly-Ala-OEt e.e.(%)[b)]	Z-Ala-Ala-OEt Y(%)	Z-Ala-Ala-OEt $[\alpha]_D$	Z-Ala-Ala-OEt e.e.(%)[b)]
CH_3	H	-36.0	L	79.3	-27.1		86.7	-42.4	
			D	75.2	+26.9		76.9	+ 4.8	
			D,L	86.8	0	0	81.8	-38.1	81.8
	(as additive)		D,L	83.7	0	0	94.0	-30.7	50.5
$i\text{-}C_4H_9$	H	-43.3	L	95.6	-27.0		99.4	-42.4	
			D	72.2	+27.0		68.2	+ 4.8	
			D,L	86.2	- 3.5	12.8	84.4	-44.4	100
	(as additive)		D,L	81.7	0	0	93.1	-38.9	85.2
CH_3	Ac	+19.2	D,L				83.4	-31.9	55.8
	(as additive)		D,L				82.4	-30.6	50.0
$i\text{-}C_4H_9$	Ac	+10.3	D,L	78.5	-24.6	91.1	99.8	-44.2	100
	(as additive)		D,L	62.2	-17.4	64.2	46.9	-33.3	61.4

a) C=1, at 25°C in EtOH; b) e.e.(enantiomeric excess) was calculated by using $[\alpha]_D^{25}$ of Z-Ala-Ala-OEt(L-L;-42.4,L-D;+4.8) and Z-Gly-Ala-OEt(L;-27.0,D;27.0).

As for the selectivity, 5-isobutylhydantoin derivative was superior to 5-methyl derivative, 1-acetylhydantoin derivative[4] superior to 1-unsubstituted derivative and active ester method superior to the method used as additive. In the case of 5-isobutylhydantoin derivative having no substituent on 1-position, the optical purity was 100% in Z-Ala-Ala-OEt but 12.8% in Z-Gly-Ala-OEt by active ester method. By using 1-acetyl-3-hydroxy-5-isobutylhydantoin, the excellent optical purities of dipeptides were obtained in both Z-Ala-Ala-OEt(100%) and Z-Gly-Ala-OEt(91.1%) on active ester method. Especially, high efficiency in the latter(6, A^1=Gly) suggests the selectivity should be ascribed not to the asymmetry of amino acid but to that of 5-C in hydantoin.

REFERENCES AND NOTE

1. G.W. Anderson, J.E. Zimmermann and F.M. Callahan, J. Amer. Chem. Soc., 86 1839(1964) and so on.
2. L. Call, Monatsh. Chem., 101, 228(1970).
3. F. Weygand, D. Hoffmann and E. Wünsch, Z. Naturforsch., 21b, 426(1966).
4. 1-Acetyl-3-hydroxyhydantoins (6, R^1=CH_3, $i\text{-}C_4H_9$, R^2=Ac) were successfully prepared by acetylation($AcOH/H_2SO_4$, 75°C, 2 hr or AcCl/NaH-THF, 0°C, 7 hr) of corresponding 2 followed by debenzylation(H_2-Pd/C in ethanol).