Reactivity of Aromatic *o*-Hydroxy Oximes. II. The Use of Esters of Aromatic *o*-Hydroxy Oximes in Peptide Synthesis

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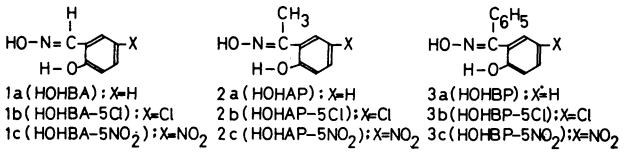
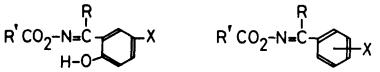
Synopsis. Esters of *N*-protected amino acids with *o*-hydroxybenzaldehyde oxime, *o*-hydroxyacetophenone oxime, *o*-hydroxybenzophenone oxime, and their 5-Cl and 5-NO_2 derivatives were prepared by several methods. Various dipeptide derivatives with high purity were obtained in good yield by use of these active ester reagents.

There are numerous reports on the use of oxime compounds and their derivatives for peptide synthesis.[1,2] The reactivity of oxime esters with an amine component is generally low.[2] Therefore, weak acid catalysts are often used to enhance the reactivity of oxime esters. We reported in the previous paper[3] that esters with *N*-(benzyloxycarbonyl)glycine (Z–Gly) of aromatic *o*-hydroxy oximes, *e.g.*, *o*-hydroxybenzaldehyde oxime (HOHBA), *o*-hydroxyacetophenone oxime (HOHAP), *o*-hydroxybenzophenone oxime (HOHBP), and their 5-Cl and 5-NO_2 derivatives of (*E*)-configuration, react rapidly with benzylamine without use of any acids in THF at 30 °C. In the present study, we report that some new esters of the above-mentioned oximes with various acylamino acids have been prepared and that these oxime esters are very effective for peptide synthesis.



1a(HOHBA): X=H 2a(HOHAP): X=H 3a(HOHBP): X=H
1b(HOHBA-5Cl): X=Cl 2b(HOHAP-5Cl): X=Cl 3b(HOHBP-5Cl): X=Cl
1c(HOHBA-5NO_2): X=NO_2 2c(HOHAP-5NO_2): X=NO_2 3c(HOHBP-5NO_2): X=NO_2



4: R'CO_2= amino acid residue 5: R'CO_2=amino acid residue

Results and Discussion

The active esters of *N*-protected amino acids were prepared from the amino acids and the oxime **1**, **2**, or **3** (A) by a direct condensation using dicyclohexylcarbodiimide (DCC), (B) with carbonic carboxylic anhydride, or (C) with acid chloride. Method (A) appears to be more useful than the others because of its mild reaction conditions.

Table 1. Oxime esters of amino acids

Ester[a]	Method	Yield %	Mp θ_m/°C	$[\alpha]_D^{24}$/° (*c* 1, DMF)
Boc-Gly-OHBA	(B)	68.0	123—126	—
Boc-Gly-OHAP	(A)	73.5	135—138	—
Z-Ala-OHBA	(A)	86.9	94—96	−19.7
Z-Ala-OHAP	(A)	66.2	99.5—100	−42.4
Boc-Ala-OHBA	(A)	82.0	100—102	−26.4[b]
Boc-Ala-OHAP	(A)	44.3	104—106	−67.1
Boc-Phe-OHBA	(A)	47.9	113—115.5	−2.9
Boc-Phe-OHAP	(A)	39.2	114.5—116.5	−21.0
Z-Phe-OHAP	(A)	68.6	116.5—118.5	−21.1
Nps-Phe-OHAP	(A)	87.7	132—134	−129.1
Nps-Phe-OHAP-5NO_2	(A)	57.0	145.5—148.5	−90.7
Z-Leu-OHAP	(A)	78.7	112—113.5	−36.5
Boc-Lys(Z)-OHAP	(A)	72.8	105—107	−32.9
Nps-Pro-OHAP	(A)	85.5	152.5—154	−193.5

a) Abbreviations were used in the same mode as in the previous paper[3] and all oxime esters were characterized by elemental analysis. b) In $CHCl_3$.

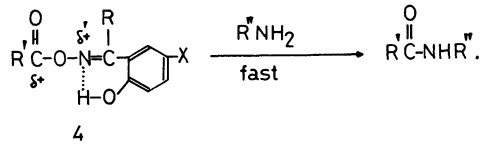
The active esters of the oximes are shown in Table 1 except those[3] of Z–Gly previously reported. Although the oximes themselves have a bifunctional nature, only the hydroxyimino group in the molecule is esterified with *N*-protected amino acids.[3] Therefore, it is not necessary to protect the phenolic hydroxyl group for the preparation of these esters. When **4** is prepared by these methods, alkaline washing of the reaction mixture cannot be applied in the case of the reaction with **1c**, **2c**, or **3c** because of the existence of *o*-hydroxyl group which is considerably acidic, and therefore the procedure for the isolation of **4** requires some considerations.

The results are summarized in Table 2. The yield of reaction products was always good and their purity was also quite satisfactory. The most significant advantage of our oxime ester method is that no addition of acid

Table 2. Synthesis of dipeptide derivatives[a] by using active esters **4**

Active ester[b]	Amine component	Yield[c] %	Mp θ_m/°C Observed	Mp θ_m/°C Lit	$[\alpha]_D^{24}$/° Observed (Lit, °C)	*c*/%	Solvent
Z-Gly-OHBA[3]	Gly-OEt	88.9	76—78	80—81[5]	—		
Z-Gly-OHAP[3]	Gly-OEt	77.5	76—78		—		
Z-Ala-OHBA	Gly-OEt	65.2	96—97	98—99[6]	−22.4(−21.3, 22)[6]	1	EtOH
Z-Ala-OHAP	Gly-OEt	72.0	95—96	97.5—98[7]	−24.3(−24.4, 20)[7]	1	EtOH
Z-Ala-OHBP[d]	Gly-OEt	48.8	97—98		−21.5	1	EtOH
Boc-Leu-OHBA[e]	Gly-OMe	35.0	129—130	128—131[8]	−21.2(−20.7, 24)[8]	1	EtOH
Boc-Leu-OHAP[e]	Gly-OMe	42.3	131—131.5		−21.0	1	EtOH
Z-Leu-OHBA	Gly-OEt	66.9	100—100.5	102—104[10]	−26.6(−27.5, 20)[10]	5	EtOH
Z-Leu-OHAP	Gly-OEt	50.8	101.5—102		−28.0	5	EtOH
Boc-Phe-OHBA	Gly-OEt	48.2	87—88	89.5—90[9]	−4.9 (−4.3, 25)[9]	2	EtOH
Boc-Phe-OHAP	Gly-OEt	56.4	88—90		−4.7	2	EtOH
Z-Phe-OHBA	Gly-OEt	36.2	109—109.5	111[6]	−15.7(−16.8, 22)[6]	2	EtOH
Z-Phe-OHAP	Gly-OEt	65.2	108.5—111		−14.4	2	EtOH
Z-Phe-OHAP-5Cl[d]	Gly-OEt	72.4	106—108		−15.5	2	EtOH
Boc-Lys(Z)-OHAP	Gly-OEt	83.3	61—63	68[11]	−12.1(−13.1, 25)[11]	1	MeOH

a) All dipeptide derivatives were characterized by elementary analysis. b) Abbreviations were used in the same mode as in the previous paper.[3] c) **4** coupled with an amine component. d) A viscous oil. e) A colorless oil.



4

catalyst is required for enhancing the reactivity of **4**, since the aminolysis accelerates by the favorable mechanism of intramolecular acid-catalysis.[3]

With this new coupling method, the reaction of **4** with the amine component can progress successfully without any addition of weak acid catalyst. To check the reactivity of **4**, the reaction of Z–Gly–OHAP (**4**; R=CH_3, X=H)[3] and Z–Gly–OAP (**5**; R=CH_3, X=H)[2] with ethyl glycinate (Gly–OEt) in THF at room temperature was carried out with a reaction time of 60 min. The yields of Z–Gly–Gly–OEt obtained were 75 and 35%, respectively. From the above instances, a period of one night may be regarded as a sufficient reaction time for the completion of the condensation. The dipeptide derivative from a reaction mixture is easily isolated by alkaline washing in the usual fashion, since the free oxime has an acidic *o*-hydroxyl group, especially with the 5-NO_2 derivatives. Because of high reactivity of **4** and easy availability of the peptide derivatives, these esters appear to be quite useful for peptide synthesis.

Experimental

The purity of products was tested by TLC.[3] The IR spectra of all compounds were determined by a JASCO A-102 spectrophotometer. The specific rotations were measured with a JASCO automatic polarimeter DIP-140. All the amino acids except Gly were of L-configuration. All the oximes and active esters **4** were prepared as described in the previous paper.[3] *N*-Benzyloxycarbonyl amino acids and *N*-(*t*-butoxycarbonyl) (Boc) amino acids were prepared in the usual way. *N*-(*o*-Nitrophenylsulphenyl) (Nps) amino acids were prepared as described by Zervas *et al.*[4]

Preparation of Dipeptides. Twenty mmol of *N*-protected amino acid oxime ester **4**, 20 mmol of hydrohalide of amino acid alkyl ester, and 20 mmol of triethylamine were dissolved in THF or DMF (about 150 ml). The mixture was stirred overnight at room temperature and then the solvent was removed. The crude products was dissolved in ethyl acetate. The solution was washed successively with 5% citric acid, 1 mol dm^{-3} $NaHCO_3$, and water, and dried over magnesium sulfate. The ethyl acetate was removed *in vacuo*. The resulting residue was triturated with petroleum ether to give a solid. The purified product was recrystallized from an appropriate solvent.

Comparison of Reactivity between Z–Gly–OHAP and Z–Gly–OAP. Triethylamine (1.4 ml, 10 mmol) was added to a suspension of Gly–OEt·HCl (1.40 g, 10 mmol) in THF (50 ml) with stirring. After 15 min, Z–Gly–OHAP (3.42 g, 10 mmol) was added to the solution at room temperature. After 60 min, a cold 2 mol dm^{-3} HCl was added to the mixture in order to terminate the coupling, and the mixture was worked up similarly to the above procedure. A recrystallization of crude product from ethyl acetate-petroleum ether gave Z–Gly–Gly–OEt: 2.21 g; mp 79.5—80.5 °C. The reaction of Z–Gly–OAP with Gly–OEt was carried out simultaneously under the same conditions with the result: 1.04 g, mp 76—78.5 °C.

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