

N-Hydroxyamide-containing Heterocycles. New Effective Additives for Peptide
Synthesis by the Dicyclohexylcarbodiimide Method

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N-Hydroxyamide-containing heterocycles, 1-hydroxy-4,6-dimethyl-2(1H)-pyrimidinone and -5,6-dimethyl-2(1H)-pyrazinone, are prepared. Judging from the result of model dipeptide (Z-Ala-Phe-OMe) formation, these compounds are found to be new effective additives for peptide synthesis by the dicyclohexylcarbodiimide (DCC) method at the points of high yield and no detectable racemization.

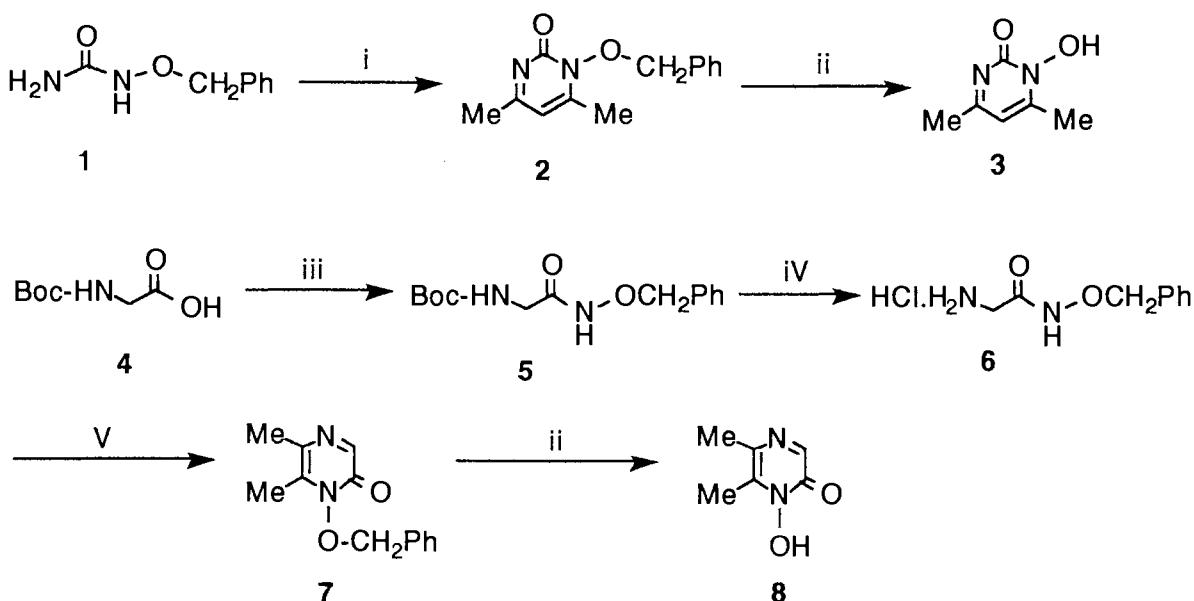
Recently the application of heterocycles to organic syntheses has received considerable attention.^{1,2)} However, the application of N-hydroxyamide-containing heterocycles has been limited. In 1987 Okuwaki and co-workers reported that 1-benzyloxy-2(1H)-pyrazinone derived from 1-hydroxy-2(1H)-pyrazinone and benzoyl chloride was treated with amines and aliphatic alcohols to give the corresponding N-benzoylamines and alkyl benzoates in high yields, respectively.³⁾ Therefore, the O-acyl derivative of N-hydroxyamide-containing heterocycles is regarded as a kind of active ester and should be applicable as the new mild acylating agent.

A variety of methods for peptide bond formation have been developed.⁴⁾ Among them the DCC-additive method is the most convenient because of its mild reaction conditions, easy operations, and suppression of racemization. N-Hydroxysuccinimide (HOSu)⁵⁾ and 1-hydroxybenzotriazole (HOBt)⁶⁾ are widely used as additives, but they have some disadvantages.⁴⁾ No paper on the application of N-hydroxyamide-containing diazines to peptide synthesis has been reported, to the best of our knowledge.

In this paper, we wish to describe the utilization of diazines such as 1-hydroxy-4,6-dimethyl-

2(1H)-pyrimidinone (**3**) and -5,6-dimethyl-2(1H)-pyrazinone (**8**) as new effective additives for peptide synthesis by the DCC method.

The synthetic procedures of N-hydroxyamide-containing heterocycles **3** and **8** are depicted in Scheme 1. The benzyl was used as the O-protecting group.



Scheme 1. Reagents and conditions: i) 2,4-Pentanedione/concd H_2SO_4 (reflux, 1 h, 46%); ii) $\text{H}_2/10\%$ Pd-C in MeOH (r.t., 20 min); iii) Isobutyl chloroformate/ $\text{Et}_3\text{N}/\text{NH}_2\text{OCH}_2\text{Ph}$ ($-15\text{ }^\circ\text{C}$ to room temp, overnight, 77%); iv) 4 M HCl in dioxane ($0\text{ }^\circ\text{C}$, 1 h, 83%); v) 2,3-Butanedione/5 M NaOH ($-30\text{ }^\circ\text{C}$ to room temp, overnight, 53%).

1-Hydroxy-4,6-dimethyl-2(1H)-pyrimidinone (**3**)⁷ was obtained by reaction of N-benzyloxyurea (**1**) with 2,4-pentanedione and subsequent hydrogenation of the resulting 1-benzyloxy-4,6-dimethyl-2(1H)-pyrimidinone (**2**). N-t-Butyloxycarbonylglycine (**4**) was treated with O-benzyl-hydroxylamine by means of the mixed anhydride method to give the corresponding amide (**5**). After deprotection of tert-butyloxycarbonyl group of compound **5**, the resulting glycine N-benzyloxyamide (**6**) was allowed to react with 2,3-butanedione to afford 1-benzyloxy-5,6-dimethyl-2(1H)-pyrazinone (**7**). The hydrogenation of compound **7** with 10% Pd-C gave 1-hydroxy-5,6-dimethyl-2(1H)-pyrazinone (**8**).⁷ The prolonged reduction of compounds **2** and **7** caused the overreduction. Both compounds **3** and **8** are highly soluble in water, which is one of the most important properties as additives for peptide synthesis.

The usefulness of diazines **3** and **8** as additives was investigated by coupling reaction of Z-Ala-OH and H-Phe-OMe. In this case, the occurrence of racemization during the coupling

reaction was easily estimated by means of ^1H NMR according to the method reported by Weinstein et al.^{8,9)} and Kawasaki et al.;¹⁰⁾ the methyl proton signals of D-Ala and L-Ala separately appeared at δ 1.28 and 1.33 ppm, respectively.

Table 1. Coupling yields of Z-Ala-Phe-OMe by the DCC-additive method

Z-Ala-OH	+	H-Phe-OMe	DCC-additive	Z-Ala-Phe-OMe
Additive	None	HOBr	3	8
Yield/%	53	78	83	85

A typical procedure for coupling of Z-Ala-OH and H-Phe-OMe is as follows. To a mixture of Z-Ala-OH (1.15 g, 5 mmol), HCl-H-Phe-OMe (1.08 g, 5 mmol), N-methylmorpholine (505 mg, 5 mmol), and 1-hydroxy-5,6-dimethyl-2(1H)-pyrazinone (**8**, 700 mg, 5 mmol) in THF (20 ml) was added DCC (1.03 g, 5 mmol) at -5 °C. The mixture was stirred for 3 h at 0 °C and then 20 h at room temperature before the removal of the resulting N,N'-dicyclohexyl-urea by filtration. After usual workup, recrystallization from AcOEt-petroleum ether afforded Z-Ala-Phe-OMe 10-12) in 85% yield. Coupling yields of the dipeptide with other additives are summarized in Table 1. From the point of yield, both diazines **3** and **8** are superior to the most conventional HOBr as additives. ^1H NMR was measured in order to estimate the occurrence of racemization. The methyl proton signal of Ala of Z-Ala-Phe-OMe¹²⁾ prepared by the DCC-compound **8** method is shown in Fig. 1. In case of diazine **3**, the same spectrum was recorded. (not shown here) No signal was observed at δ 1.28 ppm, indicating that a measurable racemization did not occur during the coupling reaction. Similarly the coupling reaction of Z-Phe-OH and H-Ala-OMe in the presence of diazines **3** and **8** by the DCC method afforded Z-Phe-Ala-OMe¹³⁾ in 81 and 86% yields, respectively.

It is concluded from these results that diazines **3** and **8** are effective new additives for peptide synthesis by the DCC method.

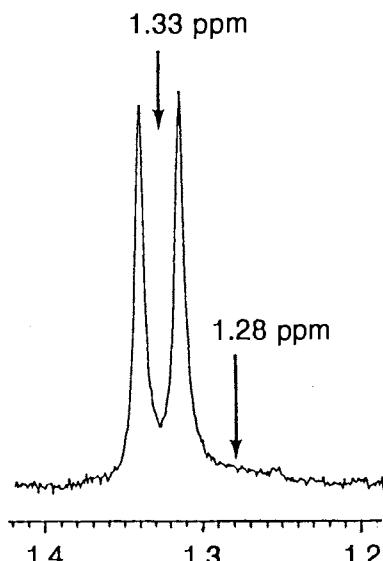


Fig. 1. ^1H NMR spectrum of the methyl signal of Ala in Z-Ala-Phe-OMe prepared by the DCC-compound **8** method.

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- 7) Compound 3: Mp 179-181 °C, pKa 6.1, hydroxamic acid test (+), IR (KBr): 3350 (broad) and 1720 cm⁻¹; ¹H NMR (CDCl₃): 2.30 (3H, s), 2.45 (3H, s), 6.15 (1H, s), and 8.30 ppm (1H, br s). Anal. Found: C, 51.29; H, 5.72; N, 19.94%. Calcd for C₆H₈N₂O₂·0.5H₂O: C, 51.10; H, 5.75; N, 19.87%. Compound 7: Mp 118-121 °C, IR (KBr): 1650, 740, and 690 cm⁻¹; ¹H NMR (CDCl₃): 2.18 (3H, s), 2.27 (3H, s), 5.29 (2H, s), 7.44 (5H, m), and 8.14 ppm (1H, s). Anal. Found: C, 67.75; H, 6.17; N, 11.84%. Calcd for C₁₃H₁₄N₂O₂: C, 67.83; H, 6.13; N, 12.18%. Compound 8: Mp 147-149 °C (lit¹⁴) mp 145-149 °C), pKa 4.8, hydroxamic acid test (+).
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- 12) Synthetic Z-Ala-Phe-OMe: Mp 100-102 °C (lit¹¹) mp 99-100 °C, [α]_D²² -14.9 ° (c 1.0, EtOH) {lit¹⁰ [α]_D²⁶ -10.4 ° (c 1.0, EtOH)}
- 13) Synthetic Z-Phe-Ala-OMe: Mp 128-129 °C (lit¹⁵) mp 130-131 °C, [α]_D²⁹ -24 ° (c 1.0, MeOH).
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