Formation and Reactions of 2-Substituted 4,4-Dimethyl-5(4*H*)-oxazolones.[1] The 5(4*H*)-Oxazolone as a Stable Intermediate in Peptide Bond Formation

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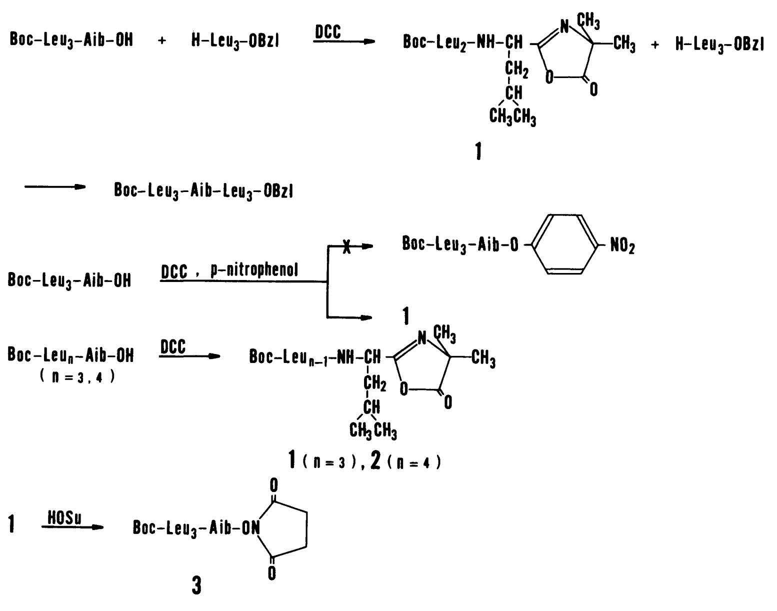
The reaction of Boc–Leu$_3$–Aib–OH with H–Leu$_3$–OBzl using DCC as a coupling reagent proceeded *via* the exclusive 2-substituted 4,4-dimethyl-5(4*H*)-oxazolone formation followed by a slow aminolysis. Similar treatment of Boc–Leu$_3$–Aib–OH with DCC in the presence of *p*-nitrophenol did not produce a *p*-nitrophenyl ester, but produced the 2-substituted 4,4-dimethyl-5(4*H*)-oxazolone (**1**) in a high yield within 20 min. The high potential for the oxazolone formation of Boc–Leu$_n$–Aib–OH (n=3 and 4) is apparently attributed to the conformational effect of C-terminal Aib residues, namely, the restriction of the backbone dihedral angles ϕ and ψ of Aib residues. Ring-opening reactions of oxazolones with hydroxy compounds were easily monitored by following the disappearance of the intense oxazolone peak at 1820 cm^{-1}. The oxazolone (**1**) reacted faster with HOSu than H–Leu$_3$–OBzl, followed by the slow aminolysis of the succinimide ester produced. In contrast, HOBt did not react with the oxazolones derived from Boc–Leu$_n$–Aib–OH (n=3 and 4), but strongly catalyzed the aminolysis of the oxazolones in dichloromethane to form peptide bonds in nearly quantitative yields. A little catalytic effect of acetic acid on the oxazolone aminolysis indicated that the oxazolone aminolysis in the presence of HOBt did not proceed through a simple acid catalytic pathway, but proceeded through a biphilic (electrophilic–nucleophilic) pathway.

The most common route to racemization during peptide bond formation involves a 5(4*H*)-oxazolone-mediated mechanism and, in many circumstances, a 5(4*H*)-oxazolone can be detected directly when an acylamino acid or peptide acid is converted to an activated derivative. Thus, the racemization and ring-opening reactions of 5(4*H*)-oxazolones with nucleophiles have been widely studied by many workers.[2] The role of 5(4*H*)-oxazolones in peptide synthesis has been reviewed by Kemp[3] and Benoiton[4] and, in their reviews, they considered the following issues: (a) the evidence for the intermediacy of 5(4*H*)-oxazolones in peptide coupling reactions, (b) the nature of the catalysis for the rate-determining ring closure, (c) the chemical behaviors of 5(4*H*)-oxazolones, and (d) the effect of substituents on the ease of 5(4*H*)-oxazolone formation. However, little attempt has been made concerning the possibility of using 5(4*H*)-oxazolones derived from peptides as coupling reagents because of lability of C$^\alpha$ hydrogen atoms.[2]

Recently we have demonstrated the usefulness of a novel strategy for solubility improvement in helical oligopeptides based on the restriction of the values of the backbone dihedral angles ϕ and ψ of Aib residues,[5] and the implication of the new findings for the study of artificial proteins containing Aib residues was discussed on the basis of the stabilizing efficacy of Aib residues on helical regions of proteins.[5–7] In the study of Aib-containing peptides,[5–7] we also found that the peptide containing an Aib residue at a C-terminal had high potential for the formation of 2-substituted 4,4-dimethyl-5(4*H*)-oxazolone and the oxazolone had low reactivity. Aib-containing peptides such as alamethicin, suzukacillin, and their peptide fragments have widely been studied mainly on their conformation.[8,9] But little information of the reactivity of an Aib residue is available.[8] Our attempts in this paper are directed towards elucidating the reactivity of a 2-substituted 4,4-dimethyl-5(4*H*)-oxazolone and exploring the possibility of using the 5(4*H*)-oxazolone as a coupling reagent, where the 5(4*H*)-oxazolone is free from racemization due to having a structure in which a labile C$^\alpha$ hydrogen atom of Ala is substituted with a methyl group.

Results

C-Terminal Aib residues are highly hindered[8] and the reaction of Boc–Leu$_3$–Aib–OH with H–Leu$_3$–OBzl using DCC as a coupling reagent proceeds *via* the exclusive 5(4*H*)-oxazolone formation followed by a slow aminolysis (Scheme 1). The characteristic IR absorption at 1820 cm^{-1} allows sensitive detection of the 5(4*H*)-oxazolone species. The IR spectrum of the reaction mixture in dichloromethane is shown in Fig. 1a. Similar treatment of Boc–Leu$_3$–Aib–OH with DCC in the presence of *p*-nitrophenol does not produce the *p*-nitrophenyl ester, but produces the oxazolone (**1**) in a high yield within 20 min (Scheme 1, Fig. 1b). The intense oxazolone peak at 1820 cm^{-1} indicates that the reaction is almost completed within 20 min, while the absence of the carbonyl band of *p*-nitrophenyl ester at about 1760 cm^{-1} indicates that the mixture does not contain the *p*-nitrophenyl ester (Fig. 1b). This is in remarkable contrast with the result that the same treatment of usual peptide acids produces exclusively corresponding *p*-nitrophenyl



Scheme 1.

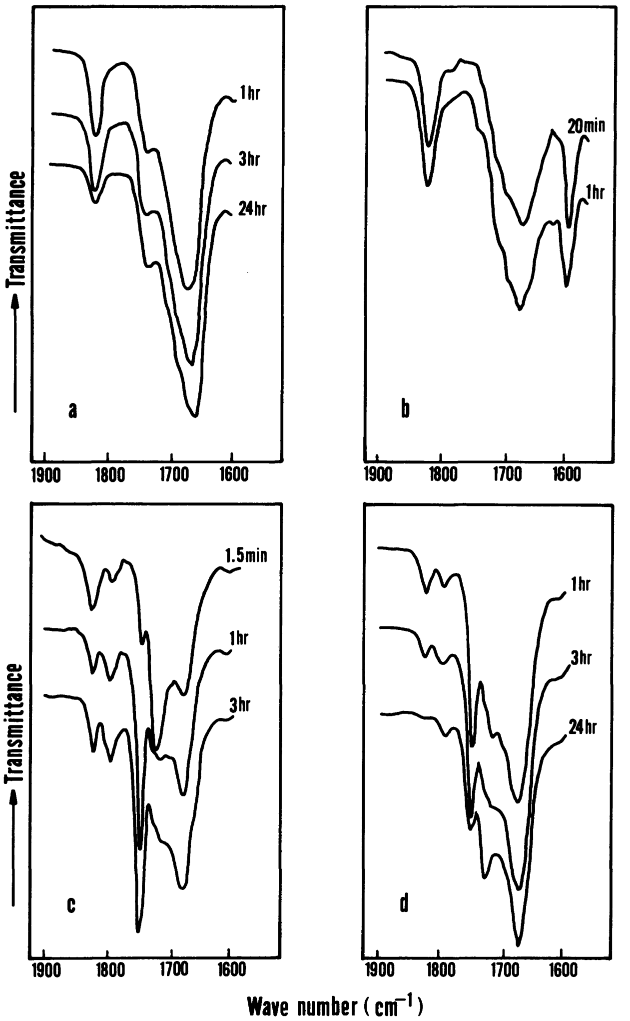


Fig. 1. Monitoring of the 5(4*H*)-oxazolone formation and the reaction of 5(4*H*)-oxazolone (**1**) with various nucleophiles by IR absorption spectra. The nucleophiles used are in the following: a, H–Leu$_3$–OBzl; b, *p*-nitrophenol; c, HOSu; d, HOSu, and H–Leu$_3$–OBzl.

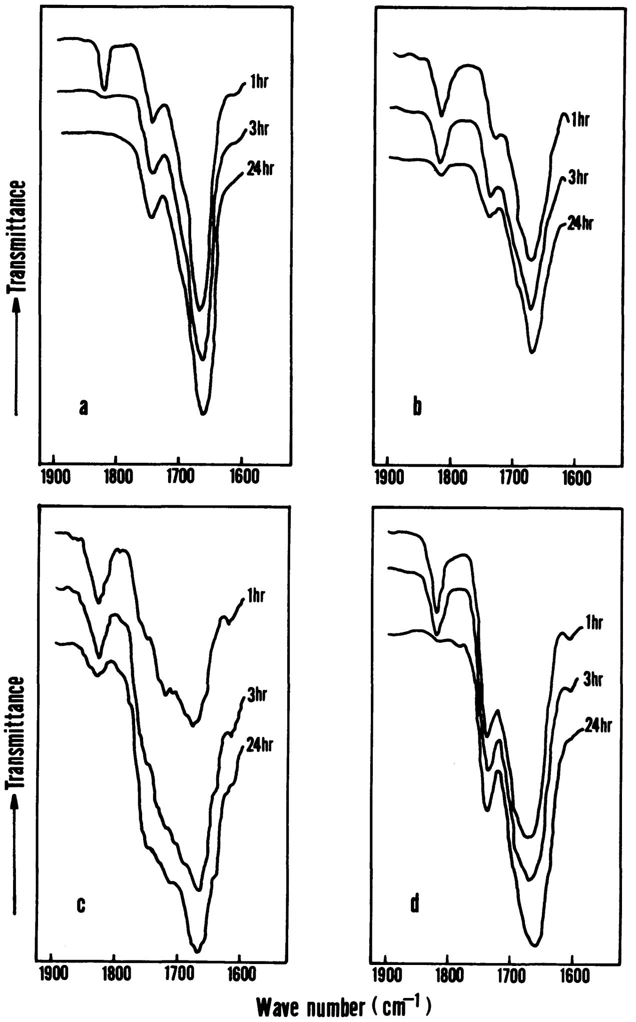


Fig. 2. Monitoring of the catalytic aminolysis of the oxazolone (**1**) with H–Leu$_3$–OBzl by IR absorption spectra. The catalysts used are in the following: a, HOBt; b, *p*-nitrophenol; c, acetic acid; d, imidazole.

esters of peptides. Treatment of Boc–Leu$_n$–Aib–OH (n=3 and 4) with DCC in the absence of nucleophiles begins to precipitate *N,N'*-dicyclohexylurea almost immediately and produces the corresponding 5(4*H*)-oxazolones in high yields (Scheme 1). Treatment of Boc–Leu$_3$–Aib–OH with acetic anhydride also produced the oxazolone (**1**). Ring-opening reactions of oxazolones with hydroxy compounds were easily monitored by following the disappearance of the intense oxazolone peak at 1820 cm^{-1}. The hydroxy compounds examined were in the following: *p*-nitrophenol, *o*-nitrophenol, pentachlorophenol, 2,4,6-trichlorophenol, 8-quinolinol, HOBt, and HOSu. For the hydroxy compounds, only HOSu reacted with the oxazolones at room temperature in dichloromethane to produce the succinimide ester (**3**) (Scheme 1, Fig. 1c). Even in the presence of amino component peptides such as H–Leu$_3$–OBzl and H–Leu$_n$–Aib–OBzl (n=3 and 4), the oxazolone (**1**) reacted faster with HOSu than the amino component peptides, followed by the slow aminolysis of the ester (**3**) (Fig. 1d). In contrast, HOBt does not react with the oxazolone under the same conditions, but strongly catalyzes the aminolysis of the oxazolone (**1**) in dichloromethane to form a peptide bond in a nearly quantitative yield. The catalytic effect of HOBt on the aminolysis were easily monitored by following the disappearance of the intense oxazolone peak at 1820 cm^{-1} (Fig. 2a). The reaction was also monitored by HPLC on a gel filtration column (Fig. 3). Other hydroxy compounds showed a little

Table 1. The coupling yields of the catalytic aminolysis of the oxazolones (**1**) and (**2**) with various amino component peptides in the presence of HOBt

Oxazolone	Product	Yield/%
1	Boc-Leu$_3$-Aib-Leu$_3$-OBzl	98
1	Boc-(Leu$_3$-Aib)$_2$-OBzl	97
1	Boc-(Leu$_3$-Aib)$_3$-OBzl	97
2	Boc-Leu$_4$-Aib-Leu$_4$-OBzl	95
2	Boc-(Leu$_4$-Aib)$_2$-OBzl	95
2	Boc-(Leu$_4$-Aib)$_3$-OBzl	93

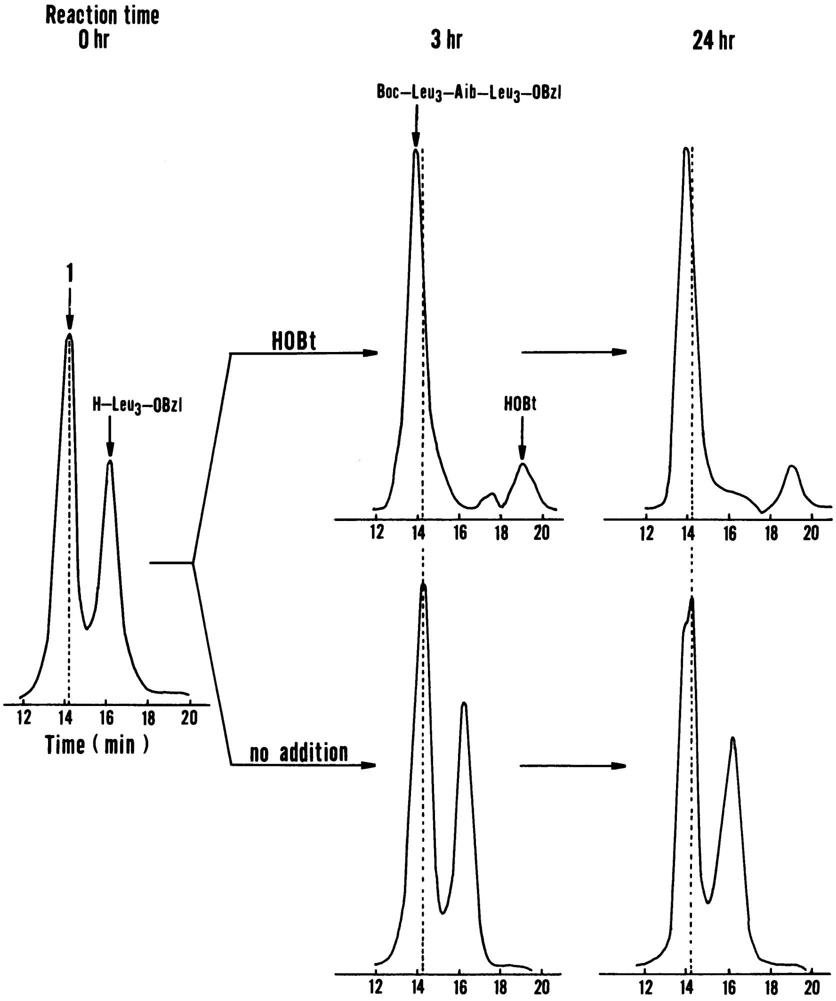


Fig. 3. Monitoring by HPLC of catalytic aminolysis of the oxazolone (**1**) with H–Leu$_3$–OBzl in the presence of HOBt.

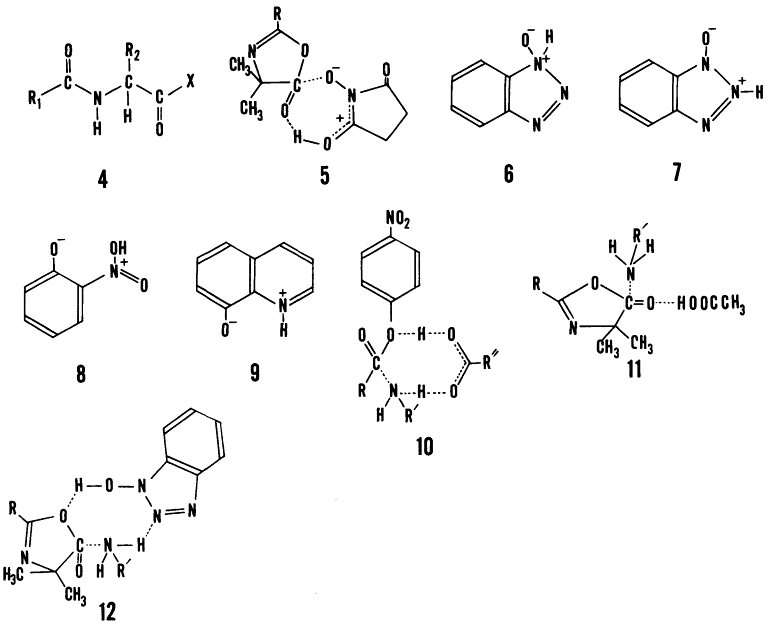
catalytic effect on the aminolysis of the oxazolone (**1**) with H-Leu$_3$-OBzl (Fig. 2b). Acetic acid and imidazole also catalyze the aminolysis of the oxazolone (**1**) (Fig. 2c and 2d). The catalytic effect was in the following order: HOBt $\gg$ imidazole>acetic acid, *p*-nitrophenol, pentachlorophenol, *o*-nitrophenol, 2,4,6-trichlorophenol>8-quinolinol. The aminolysis of the oxazolones (**1**) and (**2**) with various amino component peptides is practically catalyzed by HOBt in dichloromethane to form peptide bonds in nearly quantitative yields. (Table 1).

Discussion

In the reaction of Boc-Leu$_n$-Aib-OH with DCC in the presence of nucleophiles except for HOSu, the exclusive formation of 5(4*H*)-oxazolones is very surprising. In a series of C-terminal-activated derivatives of peptides (the structure **4** in Scheme 2), the effect of substituents R$_1$ and R$_2$ on the ease of 5(4*H*)-oxazolone formation has been reported by Young.[10] If one assumes that 5(4*H*)-oxazolone formation occurs by a base-catalyzed path, then one expects the acidity of the amide at R$_1$ to be the major determinant, in accord with the simple acyl order, CF$_3$CO>C$_6$H$_5$CO>CH$_3$CO. The effect of R$_2$ on the ease of 5(4*H*)-oxazolone formation is difficult to rationalize due to the complexity of the situation. In the case of the reaction of Boc-Leu$_n$-Aib-OH with DCC, the ease of the 2-substituted 4,4-dimethyl-5(4*H*)-oxazolone formation is apparently attributed to the conformational effect of C-terminal Aib residues, namely, the extreme restriction of the backbone dihedral angles ϕ and ψ of Aib residues.[9a,9f,11] In term of activation entropy, the conformational restriction is clearly favorable for the intramolecular cyclization, the oxazolone formation. On the other hand, the low reactivity of the oxazolone with nucleophiles results from steric hindrance of both methyl groups on the oxazolone.

In the ring-opening reactions of (4*S*)-2-phenyl-4-benzyloxazolone with α nucleophiles (species containing two adjacent nucleophilic centers), biphilic (electrophilic–nucleophilic) pathways have been estimated.[12] HOSu has also been viewed as proceeding by a concerted mechanism involving nucleophilic–electrophilic attack of an anionic form (the transition state **5** in Scheme 2).[12] The high reactivity of HOSu with the oxazolone (**1**) derived from Boc-Leu$_3$-Aib-OH can be understood as involving a biphilic pathway through the transition state **5** (Scheme 2). No reaction of HOBt with the oxazolone (**1**) may be attributed to no contribution of the zwitterionic structures **6** and **7** (Scheme 2) due to weak basicity of the nitrogen atoms. No reaction of 8-quinolinol and *o*-nitrophenol with the oxazolone (**1**) may also be attributed to no contribution of the zwitterionic structures **8** and **9** (Scheme 2).

Catalytic effects of HOBt on the oxazolone aminolysis is quite significant. The pK_a value of HOBt (pK_a=4.00) is close to that of acetic acid (pK_a=4.05) and HOSu (pK_a=4.05). In the detailed studies on the acid catalysis of *p*-nitrophenyl ester aminolysis, Nakamizo proposed a bifunctional concerted mechanism that involves the 8-membered transition state **10** (Scheme 2).[13] A different transition state was also visualized by Muzalewski.[14] A little catalytic effect of acetic acid on the oxazolone aminolysis indicates that the oxazolone aminolysis in the presence of HOBt does not proceed through the



Scheme 2.

simple acid catalytic pathway (the transition state **11** in Scheme 2), but proceed through the biphilic pathway (the transition state **12** in Scheme 2).

Peptides having an α,α-disubstituted α-amino acid residue at C-terminal are expected to have high potential for the oxazolone formation due to the restriction of the backbone dihedral angles ϕ and ψ of the amino acid residue. The study for the reactivity of α,α-disubstituted α-amino acid residues will be in progress.

Experimental

General. The uncorrected capillary melting points will be reported. Analytical HPLC of the peptides was performed on a column packed with an acrylonitrile-divinylbenzene-styrene copolymer (7.5X500 mm) using a mini micro pump NSP-800-5DX (Nihon Seimitsu Kagaku) and a refractive index detector model RI-3H (Japan Analytical Industry). The operating conditions of HPLC were the following: Column with exclusion limit of 5000; solvent, DMF; flow rate, 1 ml/min; chart speed, 0.5 cm/min; temperature, room temperature. The IR spectra in the solid state were recorded for nujor mulls with a JASCO model DS-701G spectrometer. Those in solution were recorded employing 0.025 mm pathlength cells with potassium bromide windows. The concentration of the solution was kept near 4.0×10^{-2} M[†].

*Preparation of Oxazolones from Boc-Leu$_n$-Aib-OH (*n=3 *and 4) Using DCC as a Dehydration Reagent.* Boc-Leu$_n$-Aib-OH (5 mmol) divided to five portions was added at 30 min intervals to an ice-chilled stirred solution of DCC (5 mmol) in 20 ml of dichloromethane. Precipitation of *N,N'*-dicyclohexylurea began almost immediately. After 1 h the solution was filtered and the dichloromethane was removed at room temperature under reduced pressure to give a solid material. The solid product was recrystallized from the appropriate solvents. The oxazolone (**1**), from ethyl acetate/hexane (1/5); yield, 80%; mp 161—163 °C.

Found: C, 60.91; H, 9.26; N, 10.63%. Calcd for $C_{27}H_{48}N_4O_6$ $0.5H_2O$: C, 60.76; H, 9.25; N, 10.50%.

The oxazolone (**2**), from tetrahydrofuran/hexane (1/3); yield, 83%; mp 205—207 °C.

Found: C, 62.05; H, 9.75; N, 11.09%. Calcd for $C_{33}H_{59}N_5O_7$ $0.5H_2O$: C, 61.98; H, 9.18; N, 10.62%.

*Preparation of Oxazolones from Boc-Leu$_n$-Aib-OH (*n=3 *and 4) Using Acetic Anhydride as a Dehydration Reagent.* A solution of a peptide (5 mmol) in acetic anhydride (20 ml) was heated at 110—120 °C for 15 min and then evaporated under reduced pressure. Last traces of acetic anhydride were removed by addition of toluene (10 ml) and evaporation, and the residual oxazolone recrystallized from the appropriate solvents. The yields of the oxazolones (**1**) and (**2**) were 95 and 78%, respectively.

*IR Absorption Monitoring of the 5(4*H*)-Oxazolone Formation by the Reaction of Boc-Leu$_3$-Aib-OH with DCC in the Presence of Nucleophiles (H-Leu$_3$-OBzl,* p*-Nitrophenol, HOSu, and HOSu + H-Leu$_3$-OBzl).* The formation of the 2-substituted 4,4-dimethyl-5(4*H*)-oxazolone (**1**) by the reaction of Boc-Leu$_3$-Aib-OH with DCC in the presence of nucleophiles were monitored by following the appearance of the intense oxazolone peak at 1820 cm^{-1}. The concentrations of Boc-Leu$_3$-Aib-OH, DCC, and nucleophiles were 4.0×10^{-1} M, respectively. The results were shown in Fig. 1.

Reactions of Oxazolones with Hydroxy Compounds. Ring-opening reactions of oxazolones with hydroxy compounds were carried out at room temperature in dichloromethane and monitored by following the disappearance of the intense oxazolone peak at 1820 cm^{-1}. The concentrations of the oxazolone and hydroxy compounds were 4.0×10^{-2} M, respectively, in the ring-opening studies.

Preparation of Boc-Leu$_3$-Aib-OH N*-Hydroxysuccinimide Ester.* To a solution of the oxazolone (**1**) from Boc-Leu$_3$-Aib-OH (1 mmol) in 10 ml of dichloromethane, *N*-hydroxysuccinimide (127 mg, 1.1 mmol) was added in one portion and the reaction was allowed to stir for 8 h at room temperature. The solvent was evaporated at room temperature under reduced pressure and 50 ml of water was added to dissolved unreacted *N*-hydroxysuccinimide. Crude product (530 mg, 73%), showing a single peak on HPLC, was obtained after filtration and washing with cold ether (to remove oxazolone). The product was recrystallized from ethyl acetate/hexane (1/2); yield, 87%; mp 133—137 °C.

Found: C, 56.16; H, 8.43; N, 10.40%. Calcd for $C_{31}H_{53}N_5O_9$ H_2O: C, 56.60; H, 8.43; N, 10.65%.

Peptide Bond Formation by the Reactions of the Oxazolone (1) with Amino Component Peptides. Ring-opening reactions of the oxazolone (**1**) with amino component peptides were carried out at room temperature in dichloromethane and monitored by following the disappearance of the intense oxazolone peak at 1820 cm^{-1}. The reactions were also monitored by HPLC on a gel filtration column. The concentrations of the oxazolone and amino component peptides were 4.0×10^{-2} M, respectively, in the ring-opening studies.

Catalytic Effects of Hydroxy Compounds on Peptide Bond Formation by the Reactions of the Oxazolone (1) with Amino Component Peptides. Accelerating effects of hydroxy compounds on the aminolysis of the oxazolone (**1**) were studied at room temperature in dichloromethane and monitored by following the disappearance of the intense oxazolone peak at 1820 cm^{-1}. The concentrations of the oxazolone, amino component peptide, and hydroxy compounds were 4.0×10^{-2} M, respectively, in the aminolysis. The reaction was also monitored by HPLC on a gel filtration column.

Preparation of Aib-containing Peptides Using the Oxazolones (1) and (2) as Coupling Reagents. A oxazolone (1.0 mmol) was added to a stirred mixture of an amino component peptide hydrochloride salt (1.0 mmol), *N*-methylmorpholine (1.0 mmol), and HOBt (1.0 mmol) in dichloromethane (20 ml). The reaction mixture was allowed to stir for 12 h at room temperature and then washed with water, 10% citric acid, and 5% sodium hydrogencarbonate, dried over sodium sulfate, and concentrated under reduced pressure. IR absorption spectra of crude products were free from the absorption band at 1820 cm^{-1} and in good agreement with those of authentic samples.[5,6)]

[†] 1 M=1 mol dm^{-3}.

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