New Peptide Synthesis Using the Ozonolysate of 2-(1-Phthalimido)alkyl-5-phenyloxazoles

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It was proved that 2-(1-phthalimido)alkyl-5-phenyloxazoles **3** were useful synthetic intermediates for peptide synthesis, where the oxazole ring acted as not only the carboxyl protecting group but also the carboxyl activating group upon ozonolysis.

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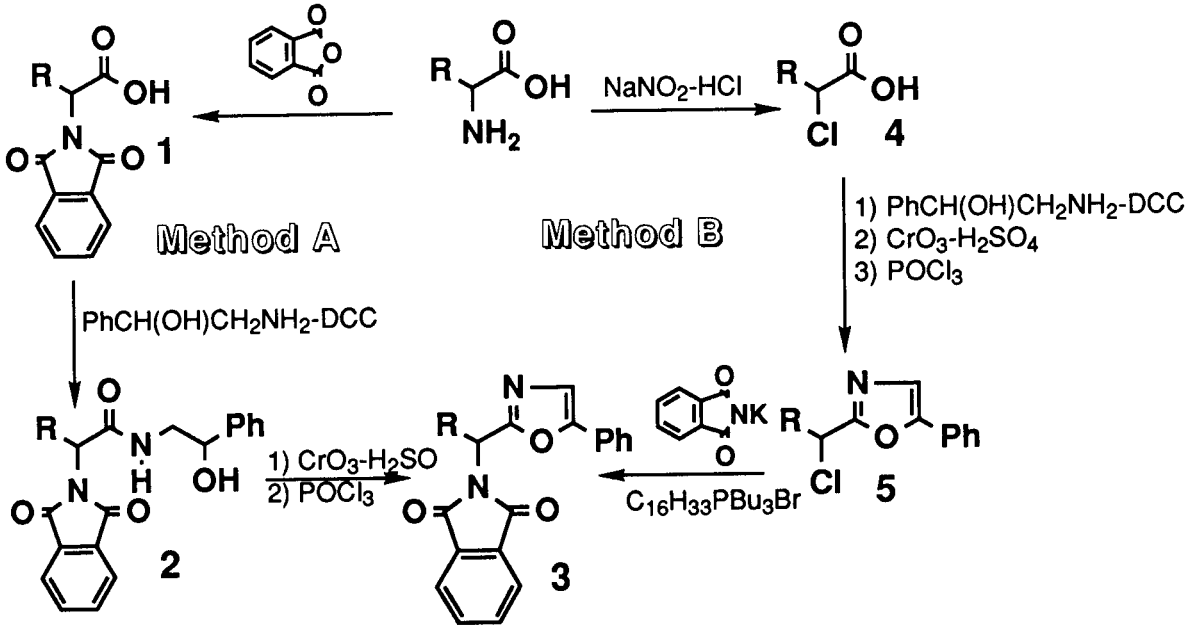
When the condensation reaction between the carboxyl group of one amino acid and the amino group of a different amino acid is performed for peptide synthesis, the interaction between the carboxyl group and the amino group of the same amino acid must be prevented. Generally protecting and activating the functional groups of an amino acid have played roles of the prevention of dimerization and the acceleration of peptide bond formation, respectively. However, few papers have appeared indicating that one functional derivative may act as both the protecting and the activating group except in the case of (4-methylthio)phenyl esters [1]. Recently we have investigated the chemical behaviours of oxazoles [2]. Although the oxazole ring is inert toward acids, bases, reductants, and oxidants, 2,5-disubstituted oxazoles are easily ozonolyzed into the corresponding acid anhydrides [3], which are the activated derivatives of the carboxyl group. From the facts, the oxazole ring must play both roles of protection and activation of the carboxyl group in peptide synthesis. Therefore the synthesis and the ozonolysis of 2-(1-phthalimido)alkyl-5-phenyloxazoles and the subsequent peptide bond formation will be discussed in this paper.

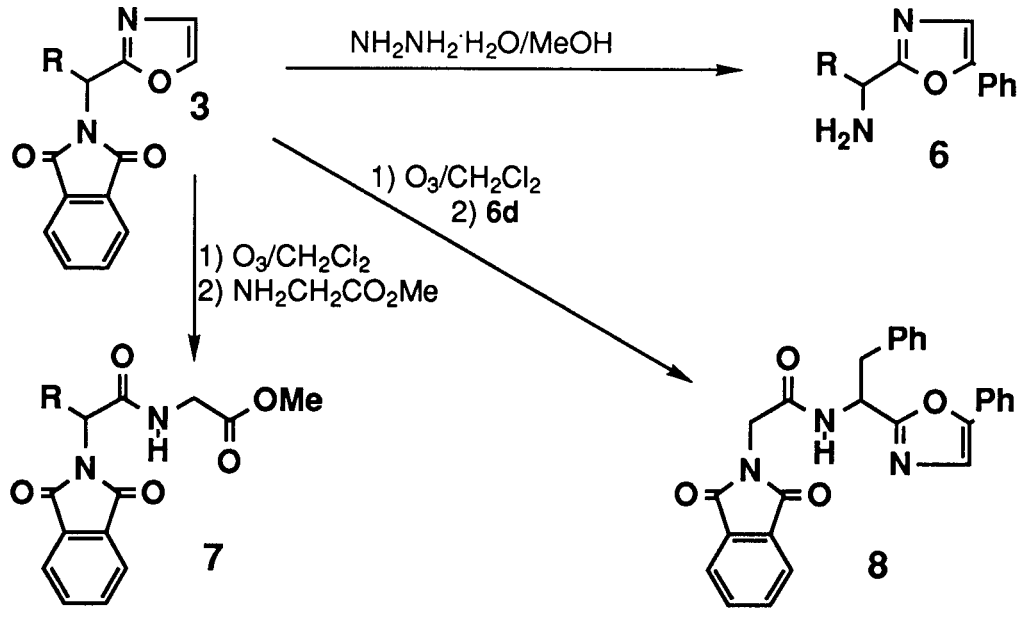
In method A, *S*-2-phthalimidopropionic acid (**1b**), which was easily prepared from L-alanine and phthalic anhydride in boiling benzene in the presence of triethylamine [4], was treated with 2-amino-1-phenylethanol in the presence of dicyclohexyl-carbodiimide (DCC), and subsequently oxidized to afford 2-[(1-phthalimido)propanamido]-1-phenylethanol (**2b**). The resulting **2b** was converted into 2-(2-phthalimido)ethyl-5-phenyloxazole (**3b**) by the treatment with chromic acid and then with phosphorus oxychloride. Similarly, 2-phthalimidomethyl- **3a**, 2-(2-methyl-1-phthalimido)propyl- **3c**, and 2-(2-phenyl-1-phthalimido)-ethyl-5-phenyloxazoles **3d** were prepared from phthalic anhydride and the corresponding L-amino acids such as glycine, L-valine and L-phenylalanine. In method A, an inversion reaction on the asymmetric center was not expected and thus the *S*-configuration should be retained.

In method B, *S*-2-chloropropionic acid (**4b**), which was

Scheme 1



Scheme 2



easily prepared from L-alanine by the diazotization [5], was converted into 2-(1-chloro)ethyl-5-phenyloxazole (**5b**) by treatment with 2-amino-1-phenylethanol. By treatment of **5b** with potassium phthalimide in boiling DMF in the presence of hexadecyltributylphosphonium bromide [6], **3b** was obtained. Similarly, **3a**, **3c**, and **3d** were prepared by method B from bromoacetic acid, L-valine, and L-phenylalanine, respectively. Since the $[\alpha]_D$ values of these products were observed to be positive while those of **3** made by Method A were negative values, compounds **3** (by method B) are proposed to have the *R*-configuration. From these results, the most favourable method was concluded to be method A, where **3** was prepared in higher total and optical yields under the conditions without any optimization. However, method B should occasionally be applicable for the preparation of **3** having a different configuration.

Regarding the fundamental properties of **3** for peptide synthesis, a methanol solution of **3b** was warmed at 50° for 48 hours but a significant amount of racemization was not observed. Even in the presence of triethylamine (5%), racemization proceeded quite slowly. From the fact that 2-(1-amino)ethyl-5-phenyloxazole (**6b**) was obtained by treatment of **3b** with hydrazine hydrate in boiling methanol, the oxazole ring was stable under these conditions of hydrazinolysis.

When an ozone-oxygen stream was bubbled at -78° into a methanol solution of **3b** made by method A, methyl *S*-2-phthalimidopropionate was obtained in 53% yield with an optical purity of 74% compared with an authentic sample. From these facts, the oxazole ring of **3b** seemed to

Table 1
Synthesis of 3 from the Corresponding Amino Acids

	R	Method A Total Yield	Method A $[\alpha]_D$	Method B Total Yield	Method B $[\alpha]_D$
3a	H	15%	—	12% [a]	—
3b	Me	43%	-19.29°	4%	+3.47°
3c	*i*-Pr	14%	-72.38°	4%	+36.40°
3d	$PhCH_2$	33%	-128.24°	4%	+4.81°

[a] Total yield from bromacetic acid.

Table 2
The Reaction of 3 and Glycine Methyl Ester

Reactant	R	Product	Yield	$[\alpha]_D$	$[\alpha]_D$ by DCC
3a	H	**7a**	71	—	—
3b	Me	**7b**	86	+0.20°	+1.07°
3c	*i*-Pr	**7c**	66	-13.46°	-14.86°
3d	$PhCH_2$	**7d**	99	-118.87°	-141.47°

be ozonolyzed to the acid anhydride, which was subsequently quenched with methanol to afford the methyl ester. Also the phthalyl group was shown to be a good *N*-protecting group toward the ozonolysis. Furthermore, **3b** made by method A was proved to be the *S*-configuration of moderately high optical purity.

Next **3b** was treated with excess ozone in dichloromethane at -78° followed by the addition of glycine methyl

ester. Methyl [2-(phthalimido)propanamido]acetate (**7b**) was obtained in 86% yield. As a result the oxazole ring was converted by ozonolysis into the *C*-terminal active form, of which was easily condensed with an amino group to form the peptide bond. Similarly, **3a**, **3c**, and **3d** were ozonolyzed and subsequently treated with glycine methyl ester to afford methyl (phthalimidoacetamido)acetate (**7a**), methyl (2-phthalimido-3-methylbutanamido)acetate (**7c**), and methyl (2-phthalimido-3-phenylpropanamido)acetate (**7d**), respectively. In order to compare the specific rotation, **7b-7d** were also prepared from the corresponding **1** and glycine methyl ester by the use of DCC.

The ozonolysate of **3a** was treated with **6d**, which was prepared by the hydrazinolysis of **3d**, to give *N*-[1-(5-phenyloxazol-2-yl)-2-phenyl]ethyl 2-(phthalimido)acetamide (**8**) in 86% yield. Since **8** was regarded as the protected derivative of glycylphenylalanine with phthalyl group on *N*- and with oxazole ring on *C*-terminals, **8** should be utilized to the further peptide synthesis by either ozonolysis or hydrazinolysis.

In conclusion, it has been shown that 2-(1-phthalimido)-alkyl-5-phenyloxazoles **3** are useful synthetic intermediates for peptide synthesis, where the oxazole ring acted not only as the carboxyl protecting group but also as the carboxyl activating group during ozonolysis.

EXPERIMENTAL

Melting points were uncorrected. The nmr spectra were obtained on a JEOL FX-100 (100 MHz) spectrometer in deuteriochloroform solution with tetramethylsilane as an internal standard. Specific Rotations were measured on a JASCO DIP-360 digital polarimeter. Elemental analyses were performed by a Perkin-Elmer Model 240 elemental analyzer.

Synthesis of 2-(1-Phthalimido)alkyl-5-phenyloxazole (**3**).

Method A.

2-Phthalimidoalkanoic acids (70 mmoles), which were prepared according to the method of Bose [4], were added to a THF solution of DCC at 0°, and stirred for 30 minutes at room temperature. To the reaction mixture was added 2-amino-1-phenylethanol (77 mmoles) in THF at 0°, and stood overnight at room temperature. The product was extracted with dichloromethane under basic conditions, dried over anhydrous magnesium sulfate, and concentrated. The residue was purified by column chromatography on silica gel with benzene-ethyl acetate mixture to give **2**. Compound **2** (5 mmoles) in acetone (15 ml) was oxidized for 17 hours at room temperature with chromic acid solution which was prepared from chromium trioxide (10 mmoles), sulfuric acid (20 ml) and water (20 ml). The reaction mixture was extracted with dichloromethane under basic conditions. The organic layer was washed with water, dried over anhydrous magnesium sulfate, and concentrated. The residue was added to phosphorus oxychloride (13 mmoles) in benzene (30 ml), and the mixture was refluxed for 4 hours. After the usual work up, **3** was purified by column chromatography on silica gel with a benzene-ethyl acetate mixture or by distillation at reduced pressure.

Method B.

2-Chloroalkanoic acid **4** (5 mmoles), which was prepared from corresponding amino acid according to the method of Fu [5] was treated with 2-amino-1-phenylethanol to give **5** as described by Method A. The mixture of **5** (1 mmole), potassium phthalimide (1.25 mmoles) and hexadecyltributylphosphonium bromide (0.1 mmole) in DMF (10 ml) was heated for 2 hours at 100°. The mixture was poured into water and extracted with dichloromethane, dried over anhydrous magnesium sulfate, and concentrated. The purification of the product was carried out in the same manner.

(1-Phthalimido)methyl-5-phenyloxazole (**3a**).

This compound showes mp 132-134° (from aqueous ethanol); ^{1}H-nmr (deuteriochloroform): δ 5.05 (2H, s), 7.2-7.9 (10H, m); ^{13}C-nmr (deuteriochloroform): δ 34.8 (t), 122.1 (d), 123.7 (d), 124.3 (d), 128.6 (d), 128.9 (d), 132.0 (s), 134.3 (d), 152.1 (s), 167.3 (s).

Anal. Calcd. for $C_{18}H_{12}N_2O_3$: C, 71.05; H, 3.97; N, 9.21. Found: C, 70.60; H, 3.98; N, 9.21.

(1-Phthalimido)ethyl-5-phenyloxazole (**3b**).

This compound showes mp 119-121° (from aqueous ethanol); ^{1}H-nmr (deuteriochloroform): δ 1.98 (3H, d), 5.67 (1H, q), 7.3-7.9 (10H, m); ^{13}C-nmr (deuteriochloroform): δ 16.3 (q), 43.7 (d), 122.2 (d), 123.6 (d), 124.3 (d), 128.4 (d), 128.8 (d), 131.9 (s), 134.2 (d), 152.0 (s), 161.0 (s), 169.3 (s), 181.2 (s).

Anal. Calcd. for $C_{19}H_{14}N_2O_3$: C, 71.69; H, 4.43; N, 8.80. Found: C, 71.63; H, 4.46; N, 8.77.

(1-Phthalimido-2-methyl)propyl-5-phenyloxazole (**3c**).

This compound had ^{1}H-nmr (deuteriochloroform): δ 1.02 (3H, d), 1.18 (3H, d), 3.0-3.35 (1H, m), 5.20 (1H, d), 7.3-7.9 (10H, m); ^{13}C-nmr (deuteriochloroform): δ 17.2 (q), 17.9 (q), 28.6 (d), 54.5 (d), 122.0 (d), 123.6 (d), 124.3 (d), 128.5 (d), 128.8 (d). 131.7 (s), 134.2 (d), 151.6 (s), 160.3 (s), 167.6 (s), 181.2 (s).

Anal. Calcd. for $C_{21}H_{18}N_2O_3$: C, 72.82; H, 5.24; N, 8.09. Found: C, 72.70; H, 5.29; N, 8.02.

(1-Phthalimido-2-phenyl)ethyl-5-phenyloxazole (**3d**).

This compound had mp 118-120° (from ethanol); ^{1}H-nmr (deuteriochloroform): δ 3.85 (2H, d), 5.84 (1H, t), 7.2-7.8 (15H, m); ^{13}C-nmr (deuteriochloroform): δ 35.6 (t), 49.4 (d), 1.22.1 (d), 123.5 (d), 124.3 (d), 127.0 (d), 128.6 (d), 128.8 (d), 129.0 (d), 131.5 (s), 134.1 (d), 136.4 (s), 151.9 (s), 160.1 (s), 167.3 (s), 181.2 (s).

Anal. Calcd. for $C_{25}H_{18}N_2O_3$: C, 76.13; H, 4.60; N, 7.10. Found: C, 76.10; H, 4.63; N, 7.09.

Racemization of **3b**.

A solution of **3b** in methanol was stirred at 50°C, and the specific rotation was measured with the interval. After 48 hours, racemization proceeded only 1%. Similarly, the specific rotation of **3b** in methanol containing 5% triethylamine decreased 19% at 50° during 48 hours.

Hydrazinolysis of **3**.

A mixture of **3** (1.6 mmoles) and hydrazine hydrate (4.8 mmoles) in methanol (15 ml) was refluxed overnight. The reaction mixture was filtered and the filtrate was concentrated. The residue was purified by column chromatography on silica gel with benzene-ethyl acetate.

(1-Amino)ethyl-5-phenyloxazole (**6b**).

This compound had ^{1}H-nmr (deuteriochloroform): δ 1.55 (3H, d), 2.4 (2H, broad s), 4.25 (1H, q), 7.2-7.8 (6H, m). The obtained oil

was solidified as the hydrochloride salt by the addition of hydrochloric acid in methanol, mp 195-197°.

Anal. Calcd. for $C_{11}H_{12}N_2O$·HCl: C, 58.80; H, 5.83; N, 12.47. Found: C, 58.49; H, 5.85; N, 12.23.

The Ozonolysis of **3b** in Methanol.

The ozone-oxygen stream was bubbled to the solution of **3b** (1 mmole) in methanol (20 ml) at -78°. When the reaction mixture changed blue, bubbling was stopped and excess ozone was released by bubbling in argon. After 1 hour at room temperature, the reaction mixture was diluted with water, extracted with dichloromethane. The organic layer was washed with aqueous sodium hydroxide, dried over anhydrous magnesium sulfate, and concentrated. Compound **6b** was purified by column chromatography on silica gel with benzene-ethyl acetate mixture. $[\alpha]_D$ -21.78°; ^{1}H-nmr (deuteriochloroform): δ 1.7 (3H, d), 2.3 (3H, s), 5.1 (1H, q), 7.9-8.1 (4H, m). Compound **6b** was also prepared *S*-2-phthalimido-propionic acid by the action of diazomethane; $[\alpha]_D$ -29.39°.

The Peptide Synthesis Using the Ozonolysate of **3**.

Ozonolysis of **3** (1 mmole) was performed in dichloromethane (20 ml) by the same method described above. Then glycine methyl ester (1 mmole) and triethylamine (120 mg) in dichloromethane (6 ml) was added at -78°. The reaction mixture was allowed to warm to room temperature for 2 hours and then was poured into water, extracted with dichloromethane under basic conditions, dried over anhydrous magnesium sulfate, and concentrated. The product **7** was purified by column chromatography on silica gel with benzene-ethyl acetate mixture.

Methyl [(Phthalimido)acetamido]acetate (**7a**).

This compound showes mp 182-184° (from benzene-hexane); ^{1}H-nmr (deuteriochloroform): δ 3.80 (3H, s), 4.12 (2H, d), 4.47 (2H, s), 6.7 (1H, broad s), 7.7-8.1 (4H, m).

Methyl [2-(Phthalimido)propanamido]acetate (**7b**).

This compound has mp 124-126° (from benzene-hexane); ^{1}H-nmr (deuteriochloroform): δ 1.73 (3H, d), 3.73 (3H, s), 4.06 (2H, d), 4.98 (1H, q), 6.7 (1H, broad s), 7.7-7.9 (4H, m); ^{13}C-nmr (deuteriochloroform): δ 15.2 (q), 41.5 (t), 49.3 (d), 52.4 (q), 123.6 (d), 131.8 (s), 134.3 (d), 167.7 (s), 169.4 (s), 170.1 (s).

Anal. Calcd. for $C_{14}H_{14}N_2O_5$: C, 57.93; H, 4.86; N, 9.65. Found: C, 57.67; H, 4.83; N, 9.77.

Methyl [2-Phthalimido)-3-methylbutanamido]acetate (**7c**).

This compound had ^{1}H-nmr (deuteriochloroform): δ 0.87 (3H, d), 1.13 (3H, d), 2.9 (1H, oct), 3.72 (3H, s), 4.04 (2H, AB-q), 4.48 (2H, d), 7.7-7.9 (4H, m); ^{13}C-nmr (deuteriochloroform): δ 19.5 (q), 19.7 (q), 27.7 (d), 41.2 (t), 52.3 (q), 62.9 (d), 123.7 (d), 131.4 (s), 134.4 (d), 168.4 (s), 169.1 (s), 169.9 (s).

Anal. Calcd. for $C_{16}H_{18}N_2O_5$: C, 60.37; H, 5.70; N, 8.80. Found: C, 60.39; H, 5.55; N, 8.51.

Methyl [2-(Phthalimido)-3-phenylpropanamido]acetate (**7d**).

This compound had mp 108-110° (from benzene-hexane); ^{1}H-nmr (deuteriochloroform); δ 3.57 (2H, d), 3.71 (3H, s), 4.05 (2H, d), 5.17 (1H, t), 6.8 (1H, broad s), 7.15 (5H, s), 7.3-7.8 (4H, m); ^{13}C-nmr (deuteriochloroform): δ 34.8 (t), 41.5 (t), 52.4 (q), 55.7 (d), 123.6 (d), 127.0 (d), 128.6 (d), 128.9 (d), 131.4 (s), 134.3 (d), 136.5 (s), 167.9 (s), 168.7 (s), 170.0 (s).

Anal. Calcd. for $C_{20}H_{18}N_2O_5$; C, 65.57; H, 4.95; N, 7.65. Found: C, 65.64; H, 4.93; N, 7.64.

Preparation of **7** by DCC.

The mixture of 2-phthalimidoalkanoic acid (**1**) (2 mmoles) and DCC (2 mmoles) in THF (5 ml) was stirred at room temperature for 30 minutes. To the reaction mixture was added glycine methyl ester (2 mmoles) and triethylamine (2.5 mmoles) in THF (5 ml), and stirred for 17 hours at room temperature. After work up, the residue was purified by column chromatography on silica gel with benzene-ethyl acetate mixture.

N-[1-(5-phenyloxazol-2-yl)-2-phenyl]ethyl-2-(phthalimido)acetamide (**8**).

This compound was prepared by treatment of the ozonolysate of **3a** with **6d** at -78° for 2 hours. The mixture was poured into water and extracted with dichloromethane under basic conditions. The residue was purified by recrystallization from benzene-hexane mixture, mp 203-204°; $[\alpha]_D$ -60.52°; ^{1}H-nmr (deuteriochloroform): δ 1.26 (1H, s), 3.31 (2H, d), 4.4 (2H, d), 5.4 (1H, q), 7.0-8.0 (15H, m); ^{13}C-nmr (deuteriochloroform): δ 39.8 (t), 40.6 (t), 49.2 (d), 121.4 (d), 123.6 (d), 124.3 (d), 127.0 (d), 127.5 (d), 128.5 (d), 128.6 (d), 128.9 (d), 129.4 (d), 132.1 (s), 134.1 (d), 135.6 (s), 151.8 (s), 162.2 (s), 166.0 (s), 167.7 (s).

Anal. Calcd. for $C_{27}H_{21}N_3O_4$·0.2H_2O: C, 71.26; H, 4.74; N, 9.23. Found: C, 71.05; H, 4.66; N, 9.36.

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