Enhanced Cyclization of *N*-Benzyloxycarbonyl-*N*-substituted Dipeptide Methyl Esters with Ammonia

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Synopsis. Cyclization of *N,N'*-[*N*-(benzyloxycarbonyl)(Z)-iminobis(methylenecarbonyl)]bis[glycine] dimethyl ester to the hydantoin derivative took place easily under mild ammonolysis conditions. Replacement of Z by *p*-methoxybenzyloxycarbonyl also led to the cyclization. Influence of different substituent groups on the cyclization was studied with some *N*-Z-dipeptide methyl esters.

The benzyloxycarbonyl (Z) has been used frequently in peptide synthesis as an amino-protecting group. However, elimination of Z has been reported in some instances. For example, some *N*-Z-peptide esters give hydantoins or urea derivatives with the loss of benzyl alcohol by alkaline saponification, where glycine is next to N-terminal residue.[1-3] In addition, *N*-Z-dipeptide esters carrying glycine as C-terminus were converted to 3-(carbamoylmethyl)hydantoins, when the side chain of the N-terminal amino acid is rather large such as benzyl, 2-(methylthio)ethyl, or isobutyl group.[4,5] The present study concerns enhanced cyclization of *N*-Z- and *N*-*p*-methoxybenzyloxycarbonyl (Z(OMe))-*N*-substituted glycylglycine methyl esters with ammonia.

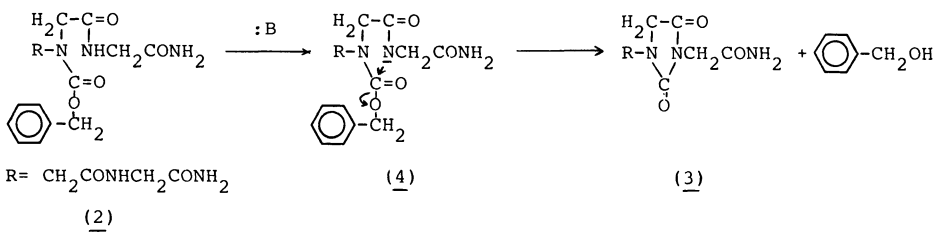
Results and Discussion

In an attempt to obtain *N,N'*-[*N*-Z-iminobis(methylenecarbonyl)]bis[glycinamide] (**2**), the corresponding dimethyl ester (**1**) was subjected to ammonolysis in methanol (MeOH) containing dry ammonia (ca. 6 M) (1 M=1 mol dm^{-3}) at room temperature for 24 h. Contrary to expectation, the product was soluble in water, ^{1}H and ^{13}C NMR spectra of which no longer showed signals characteristic of phenyl group. The amino acid analysis of the acid hydrolyzate revealed the presence of glycine and ammonia in a molar ratio of 1:2, and the absence of iminodiacetic acid (IDAA). The findings suggested that cyclization with a loss of Z group took place during the ammonolysis. The ^{1}H NMR spectrum was well consistent with a cyclic structure, 3-(carbamoylmethyl)-1-(carbamoylmethylaminocarbonylmethyl)hydantoin (**3**). The signals could be assigned as follows: A doublet at δ=3.66 is ascribed to NHC*H*$_2$ protons; three singlets at 3.94, 4.02, and 4.07 to the three CH$_2$, respectively; a pair of singlet at 7.08 and 7.35 to CONH$_2$; a pair of singlet at 7.22 and 7.56 to CONH$_2$; a triplet at 8.32 to NH.

For further identification of the product, the acid hydrolyzate was trimethylsilylated with *N,O*-bis(trimethylsilyl)(TMS)-trifluoroacetamide (BSTFA) and then analyzed by the gas chromatography-mass spectrometry (GC-MS). On GC, two peaks eluted: The first peak was identified as an *N,N,O*-tri-TMS derivative of glycine. In the mass spectrum of the second peak, the molecular (M^+) and M−15 (CH_3) ions were observed at *m/z* 360 and 345, respectively, indicating that this compound is the *O,O'*-di-TMS derivative of 1,3-bis(carboxymethyl)hydantoin (mol. wt.=360). Thus the ammonolysis product was confirmed not to be the linear diamide (**2**), but the cyclic amide (hydantoin derivative, **3**).

As shown in Scheme 1, ring closure to **3** involves proton abstraction from nitrogen of the peptide bonds of **2**. Then, the resulting anion (**4**) attacks the carbonyl carbon of the Z group, giving **3** along with the benzyloxyde anion. The ring closure is completed when the benzyloxyde anion abstracts a proton from the conjugate acid BH^+ forming benzyl alcohol. The hydantoin ring is not opened by the excess of ammonia because of its weak basicity.[6] The time course of the ammonolysis at room temperature was followed by HPLC. Benzyl alcohol and **3** were the final products. In contrast, **2** was found to be an intermediate product, which disappeared completely in 8 h.

When Z(OMe) was used as an *N*-protecting group (**5**) instead of Z, the cyclization also took place, but that was not the case for *t*-butoxycarbonyl (Boc) group (**6**); in the latter instance, the *N*-protected linear diamide (**7**) was obtained. This difference can be explained in terms of the nature of the *N*-protecting groups. The phenyl group of Z and Z(OMe) stabilizes the benzyloxyde-type anion by electron-withdrawing, leading to the cyclization. In contrast, the *t*-butyl group of Boc makes the corresponding anion very unstable by electron-repelling, and it consequently prevents the cyclization.



Scheme 1. A possible mechanism for cyclization.

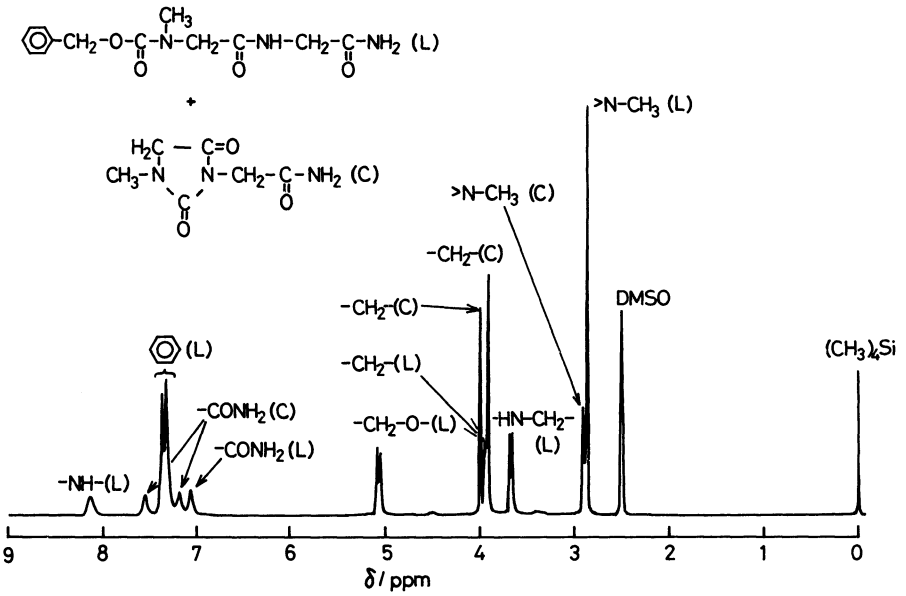


Fig. 1. ^{1}H NMR spectrum for the ammonolysis product of **9**.

Table 1. Results of the Ammonolysis of *N*-Z-dipeptide Methyl Esters

Parent ester	Ratio of the product	
	Linear amide	Cyclic amide
Z-DL-Ala-Gly-OMe (**8**)	100	—
Z-Sar-Gly-OMe (**9**)	54	46
Z-CEGly-Gly-OMe (**10**)	40	60
Z-CEAla-Gly-OMe (**11**)	8	92
Z-CEGly-DL-Ala-OMe (**12**)	100	—
Z(OMe)-CEGly-Gly-OMe (**13**)[a)]	60	40

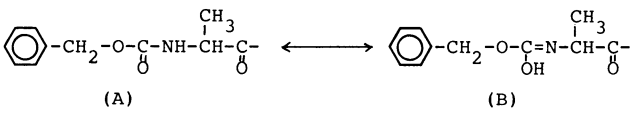
a) *N*-Z(OMe) derivative.

In order to investigate the influence of different substituent groups, some *N*-Z-dipeptide methyl esters were subjected to ammonolysis at room temperature for 24 h (Table 1). The NH of peptide bond was allowed to be free, because this NH plays an important role in the cyclization (Scheme 1). The ratio between linear and cyclic amides in the products was determined by the ^{1}H NMR spectroscopy.

Figure 1 shows the ^{1}H NMR spectrum for the ammonolysis product of Z-Sar-Gly-OMe (**9**). The absence of singlet at δ=3.6 indicated that ammonolysis of the ester was completed under these conditions. From the comparison of the spectrum (Fig. 1) with that of authentic Z-Sar-Gly-NH_2, the signals marked as L were assigned to the linear amide. But one of the two signals of $CONH_2$ overlaps with phenyl protons of Z. The remaining signals marked as C were well consistent with the cyclic amide, 3-(carbamoylmethyl)-1-methylhydantoin (**14**) as follows: A singlet at δ=2.91 was ascribed to NCH_3 protons; a singlet at 3.91 to CH_2; a singlet at 3.99 to CH_2; a pair of singlets at 7.19 and 7.55 to $CONH_2$. In this instance, the ratio of the two amides was determined by comparing the integrated value of CH_2O protons at δ=5.04 and 5.08 (linear amide) with that of NCH_3 at δ=2.87 and 2.91 (both amides). The formation of **14** was also confirmed by GC-MS after trimethylsilylation of the hydrolyzate of the reaction mixture.

As can be seen in Fig. 1, the absorption of the phenyl and CH_2O protons appeared like a doublet, respectively, thouth they must be singlets normally. For example, these two appeared as singlets for Z-Gly-Gly-NH_2. However, we found that **9** also exhibited such a signal splitting in the 200 MHz spectrum. Therefore, it seems plausible to consider that the signal splitting is due to the presence of the two conformational isomers in a DMSO solution of *N*-Z-*N*-substituted glycylglycine methyl esters and amides. In the 60 MHz spectra, such a signal splitting was not observed because of low resolution.

Table 1 presents the results of the ammonolysis. As we expected, the cyclization was not observed for Z-Gly-Gly-OMe and Z-DL-Ala-Gly-OMe (**8**), which carry no *N*-substituent groups. On the other hand, *N*-Z-*N*-substituted dipeptide esters having glycine residue as C-terminus were generally prone to ring closure. For example, **9** gave a mixture of the linear and cyclic amides in a ratio of 54:46, while its isomer **8** yielded only the normal linear amide. This difference could be explained in terms of the resonance effect. The contribution of resonance structure B increases the electron density on the carbonyl carbon of Z, so



(A) (B)

that the amide anion cannot attack it (Scheme 1). In contrast, such resonance is no longer possible with *N*-Z-*N*-substituted dipeptide esters, the carbonyl carbons of which remain electron-lacking.

The cyclization seems to depend on the nature of the *N*-substituent groups. For example, the extent of the cyclization of Z-CEGly-Gly-OMe[7] (**10**) was higher than that of **9**. This finding could be explained in terms of the difference in electron-repelling ability of the two substituent groups, CH_2CH_2CN and CH_3; the latter repels electron more strongly than the former, increasing electron density of the carbonyl carbon of Z of **9**. The substitution on α-carbon of the N-terminal glycine enhanced the cyclization significantly, as shown for Z-CEAla-Gly-OMe[7] (**11**). In contrast, the substitution on α-carbon of the C-terminal glycine prevented the cyclization completely, as shown for Z-CEGly-DL-Ala-OMe (**12**). This finding indicates that glycine residue carrying a reactive amino group must be present as C-terminus for the cyclization, because the hydantoin is a secondary amide, the diacyl derivative of C-terminal amino acid.

As we can expect, the corresponding *N*-Z(OMe)-dipeptides were also found to cyclize under the ammonolysis conditions. In the instance of Z(OMe)-CEGly-Gly-OMe (**13**), the extent of the cyclization was comparable with that of the corresponding *N*-Z derivative (**10**).

In conclusion, a special care in ammonolysis of *N*-Z- and *N*-Z(OMe)-*N*-substituted dipeptide esters was suggested. Such cyclization, however, will provide a convenient method for the synthesis of 1,3-disubstituted hydantoins.

Experimental

All the melting points determined by a Yanagimoto micro-melting point apparatus were not corrected. ^1H NMR spectra were obtained by a JEOL FX-200 (FT 200 MHz) spectrometer using tetramethylsilane as an internal reference. GC-MS was carried out as previously reported[8] to give 20 eV spectra. HPLC was carried out by a JASCO 880 pump connected with a JASCO 875 UV monitor (column, Finepak SIL C_{18}).

***N*-Protected IDAAs.** Introduction of Z, Z(OMe), and *t*-Boc to IDAA was carried out using Z-Cl,[9] MZ-SDP,[9] and Boc-SDP,[9] respectively, according to the conventional methods.

Z-IDAA: Yield 73%; mp 82—84 °C. Anal. ($C_{12}H_{13}NO_6$) C, H, N.

Z(OMe)-IDAA: Yield 85%; mp 121—122 °C. Anal. ($C_{13}H_{15}NO_7$) H, C, N.

***t*-Boc-IDAA:** Yield 65%; mp 117—120 °C. Anal. ($C_9H_{15}NO_6$) C, H, N.

***N*-Protected IDAA Disuccinimido Esters.** These were prepared by the dicyclohexylcarbodiimide method.

Z-IDAA Disuccinimido Ester: Yield 93%; mp 178—181 °C. Anal. ($C_{20}H_{19}N_3O_{10}$) C, H, N.

Z(OMe)-IDAA Disuccinimido Ester: Yield 64%; mp 201—203 °C. Found: C, 51.03; H, 4.44; N, 8.96%. Calcd for $C_{21}H_{21}N_3O_{11}$: C, 51.33; H, 4.31; N, 8.55%.

***t*-Boc-IDAA Disuccinimido Ester:** Yield 96%; mp 171—174 °C. Anal. ($C_{17}H_{21}N_3O_{10}$) C, H, N.

***N,N'*-[*N*-Protected Iminobis(methylenecarbonyl)]bis[glycine] Dimethyl Esters.** These were prepared by coupling of the *N*-protected IDAA disuccinimido ester with glycine methyl ester.

Z-N($CH_2CONHCH_2COOCH_3$)$_2$ (1): Yield 51%; mp 89—92 °C. Anal. ($C_{18}H_{23}N_3O_8$) C, H, N.

Z(OMe)-N($CH_2CONHCH_2COOCH_3$)$_2$ (5): Yield 53%; mp 82—84 °C. Anal. ($C_{19}H_{25}N_3O_9$) C, H, N.

***t*-Boc-N($CH_2CONHCH_2COOCH_3$)$_2$ (6):** Yield 50%; mp 120—123 °C. Anal. ($C_{15}H_{25}N_3O_8$) C, H, N.

Ammonolysis of 1, 5, and 6. The *N*-protected ester (1 g) was dissolved in MeOH (5 ml) and then 50 ml of MeOH containing dry NH_3 (ca. 6 M) was added. After standing at room temperature for 24 h, the solution was evaporated to dryness and then the residue was recrystallized. The time course of **1** was followed with HPLC by analyzing the aliquots of the reaction mixture at appropriate intervals.

Ammonolysis of 1: Yield 56%; mp 209—211 °C. Anal. ($C_9H_{13}N_5O_5$ (cyclic amide)) C, H, N.

Ammonolysis of 5: Yield 65%; mp 207—209 °C. Found: C, 39.98; H, 4.80; N, 25.34%. Calcd for $C_9H_{13}N_5O_5$ (cyclic amide): C, 39.86; H, 4.83; N, 25.82%.

Ammonolysis of 6: Yield 90%; mp 87—90 °C. Anal. ($C_{13}H_{23}N_5O_6$ (linear amide)) C, H, N. The amino acid analysis of the hydrolyzate revealed the presence of glycine and IDAA in a molar ratio of 2:1.

***N*-Z- and *N*-Z(OMe)-dipeptide Methyl Esters.** Compounds **8—13** were prepared by the conventional method of peptide synthesis. The elemental analyses were well agreed with the calculated values except **12**. Melting points were as follows: Z-DL-Ala-Gly-OMe (**8**), 74—76 °C; Z-Sar-Gly-OMe (**9**), 74—77 °C; Z-CEGly-Gly-OMe (**10**), 60—63 °C; Z-CEAla-Gly-OMe (**11**), 103—105 °C; Z-CEGly-DL-Ala-OMe (**12**), oil; Z(OMe)-CEGly-Gly-OMe (**13**), 83—85 °C.

Ammonolysis of *N*-Z- and *N*-Z(OMe)-dipeptide Methyl Esters. The ester (1 g) was dissolved in MeOH (6 ml) and then 50 ml of MeOH containing dry NH_3 (ca. 6 M) was added. After ammonolysis at room temperature for 24 h, the solution was evaporated to dryness with a rotary evaporator. The residue was dried in vacuum. A part of the residue was dissolved in DMSO-d_6 and ^1H NMR spectra were obtained.

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