Peptide Synthesis *via* *N*-Acylated Aziridinone.[1] II. The Reaction of *N*-Acylated Aziridinone and Its Use in Peptide Synthesis

Muneji Miyoshi

Department of Synthetic Chemistry, Research Laboratory of Applied Biochemistry, Tanabe Seiyaku Co., Ltd., Kashima-cho, Higashiyodogawa-ku, Osaka 532

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Optically active *N*-acylated aziridinone, which was synthesized by the dehydration of the corresponding L-acylamino acid, was treated with various nucleophiles, such as alcohol, amine, and water. The ring fission of the aziridinone took place exclusively at the carbonyl-nitrogen bond to give L-acylamino acid derivatives. The reaction was used successfully in the peptide synthesis, using an amino acid ester as a nucleophile. A retention of the optical activity was observed throughout the reaction.

Interest in aziridinone chemistry has increased since Baumgarten[2] first proved the existence of *N*-*t*-butyl-3-phenylaziridinone by spectral studies in 1961. Recently, a number of data on the physical and chemical properties of the aziridinone have been accumulated, and work on it is still in progress.

The aziridinone chemistry reported[3–8] thus far may be summarized as follows.

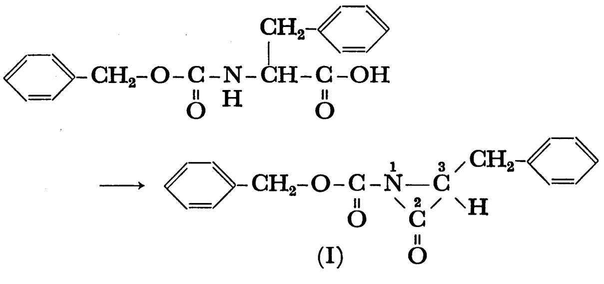
All of the aziridinones synthesized to date have the *N*-substituent, which is an electron-donating and a sterically bulky alkyl group. Among them, *N*-*t*-butyl and *N*-adamantyl-aziridinones were isolated in the pure form.

Among the substituents at *C*-3, the bulky alkyl group generally increases the stability of the ring, while the aryl group decreases the stability of the aziridinone under otherwise identical conditions. *C*-3 disubstituted aziridinones are more stable than *C*-3 monosubstituted ones under the same conditions. In general, the *N*-*t*-alkyl-aziridinones isolated and investigated are very reactive with nucleophiles to give the corresponding adducts by the fission of the three-membered ring. Depending on the nucleophiles, a high selectivity is observed in the type of ring opening. For example, reactions with proton-containing nucleophiles, such as water, alcohols, amines, and ethyl glycinate, lead exclusively to amides because of the fission at the alkyl-nitrogen bond. On the other hand, reactions with aprotic nucleophiles, such as sodium methoxide and potassium *t*-butoxide, give amino acid derivatives as the only product, corresponding to a ring-opening at the carbonyl-nitrogen bond.

In the previous paper,[9] the author reported a new method of synthesizing the optically-active *N*-acylated aziridinones, which have a hydrogen atom on the asymmetric carbon at *C*-3, from the corresponding L-acylamino acids. The present paper will describe the reactivity of the new *N*-acylated aziridinones and an approach to the peptide synthesis by the use of these compounds.

Results and Discussion

Reactions of 3-Benzyl-1-benzyloxycarbonylaziridin-2-one (I) with Various Nucleophiles. The 3-benzyl-1-benzyloxycarbonylaziridine-2-one (I) was synthesized by the dehydration of benzyloxycarbonyl-L-phenylalanine (Z–Phe), as had been described in detail previously.[9]



(I)

The crystals of I were dissolved in anhydrous tetrahydrofuran (THF), and all of the reactions with nucleophiles were carried out at -10—0 °C over a period of one hour under stirring. The nucleophiles used in this study were water, *t*-butyl alcohol, potassium *t*-butoxide, aniline, and benzylamine. The results are summarized

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[1] A part of this study has been reported in a preliminary communication. This Bulletin, **43**, 3321 (1970). Also presented at the 8th Symposium on Peptide Chemistry, Osaka, Nov. 1970, and at the 24th Annual Meeting of the Chemical Society of Japan, Osaka, April, 1971.

[2] H. E. Baumgarten, R. L. Zey, and U. Krolls, *J. Amer. Chem. Soc.*, **83**, 4469 (1961).

[3] H. E. Baumgarten, *ibid.*, **84**, 4975 (1962); H. E. Baumgarten and J. F. Fureholzer, *ibid.*, **85**, 3303 (1963); H. E. Baumgarten, R. D. Clark, and S. L. Endres, *Tetrahedron Lett.*, **1967**, 5033.

[4] J. C. Sheehan and I. Lengyel, *J. Amer. Chem. Soc.*, **86**, 1356 (1964); J. C. Sheehan and I. Lengyel, *J. Org. Chem.*, **31**, 4244 (1966); J. C. Sheehan and J. Beeson, *J. Amer. Chem. Soc.*, **89**, 362, 366 (1967); J. C. Sheehan and M. M. Nafissi-V, *ibid.*, **91**, 1176 (1969).

[5] I. Lengyel and D. B. Uliss, *Chem. Commun.*, **1968**, 1621; I. Lengyel and J. C. Sheehan, *Angew. Chem.*, **80**, 27, (1968).

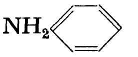
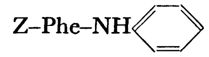
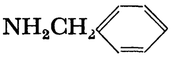
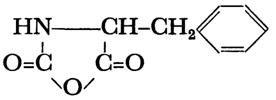
[6] K. Bott, *Angew. Chem.*, **79**, 943 (1967); K. Bott, *Tetrahedron Lett.*, **1968**, 3323; K, Boot. *Chem. Ber.*, **103**, 3850 (1970); K. Bott, *Ann. Chem.*, **755**, 58 (1972).

[7] E. R. Talaty, A. E. Dupuy, Jr., and A. E. Cancienne, Jr., *J. Heterocycl. Chem.*, **4**, 657 (1967); E. R. Talaty and A. E. Dupuy, Jr., *Chem. Commun.*, **1968**, 790; E. R. Talay, A' E. Dupuy, Jr., and T. H. Golson, *ibid.*, **1969**, 49; E. R. Talay and C. M. Utermoehlen, *ibid.*, **1970**, 473; E. R. Talay, C. M. Utermoehlen, and L. H. Stekoll, *Synthesis*, **1971**, 543; E. R. Talay, and C. M. Utermoehlen, *Tetrahedron Lett.*, **1970**, 3321; E. R. Talaty, A. E. Dupuy, Jr., C. K. Johnson, T. P. Pirotte, W. A. Fletcher, and R. E. Thosmpson, *ibid.*, **1970**, 4435.

[8] A. H. J. Wang, I. C. Paul, E. R. Talaty, and A. E. Dupuy, Jr., *Chem. Commun.*, **1972**, 43.

[9] Part I. This Bulletin, **46**, 212 (1973).

Table 1. Reaction of **I** with nucleophiles

No.	Nucleophile	Product	Yield (%)	Mp (°C)	$[\alpha]_D^{20}$ (*c* 1)
1	*t*-BuOH	Z–Phe–OBu^t	83.3	81—82	−6.0(EtOH)
2	*t*-BuOK	Z–Phe–OBu^t	56.8	81—82	−6.0(EtOH)
3	 NH_2	 Z–Phe–NH	84.6	164—166	+30.0(THF)
4	 NH_2CH_2	 Z–Phe–NHCH$_2$	85.3	157—158	−12.0(THF)
5	H_2O	(Z–Phe)$_2$O	47.2	144—146	+14.6(Dioxane)
6	H_2/Pd		78.5	96—98	−110.4(THF)

in Table 1.

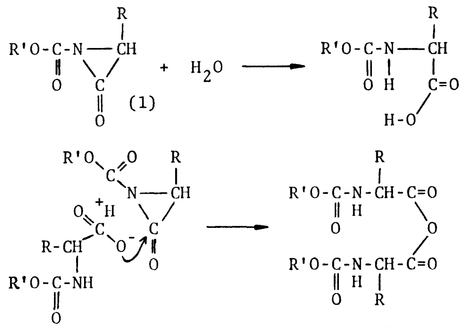
It is interesting to note that all of the products obtained are amino acid derivatives, corresponding to the fission of the carbonyl-nitrogen bond on the ring. The exclusive selectivity of this ring fission is observed even when *t*-butyl alcohol is used as a proton-containing nucleophile. The same product of benzyloxycarbonyl-L-phenylalanine *t*-butyl ester (Z–Phe–OBu^t) is also obtained by the use of potassium *t*-butoxide, which is an aprotic nucleophile.

The fact that the ring fission always takes place at the carbonyl-nitrogen bond, no matter what the species of the attacking nucleophiles, may be attributed to the lower electron density on the ring of I compared with that of *N*-*t*-alkyl-aziridinone.

As an urethan carbonyl group affects lone-pair electrons of the nitrogen atom so as to decrease the electron density, the protonation ability of the nitrogen atom on the ring as a Lewis-base would be diminished. Therefore, a nucleophile would selectively attack the carbonyl carbon, even if a proton donor were present in the reaction media. Further, the effect of the *N*,*N*-diacyl group should also be taken into consideration. The possibility of the selective ring fission at the carbonyl-nitrogen bond would be greatly increased because of the sensitive reactivity of the *N*-substituted diacyl group. The results of an MO calculation also support the above explanation of the observed ring fission; these data will be summarized later.

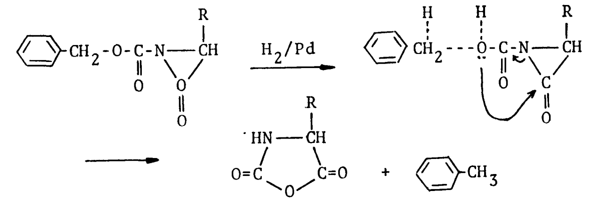
It is also interesting to note that the reaction of I with water leads to the formation of an intermolecular anhydride of Z–Phe (5), while catalytic hydrogenation over palladium-black in ether gives the corresponding intramolecular anhydride (6); the so-called "*N*-carboxy-α-amino acid anhydride" (NCA).

Wieland and Bernhard[10] reported that Z-amino acid reacted with phosgene ($COCl_2$) at 0 °C in the presence of tertiary amine to form the same intermolecular anhydride (5). In this study, I was directly treated with water to form Z–Phe in the first step; the formation of the anhydride (5) would occur *via* an intermolecular attack by the ionized carboxyl anion of the Z–Phe on the carbonyl carbon of the other molecule of the aziridinone.



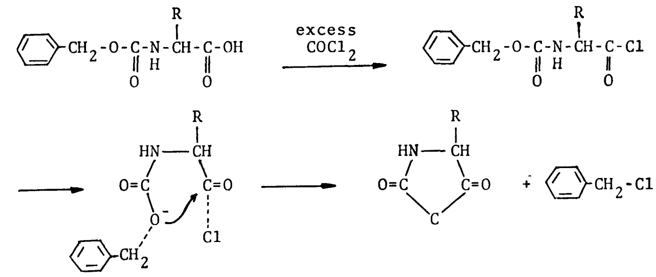
(5)

On the contrary, hydrogenolysis takes place at the benzyl-oxygen bond to form toluene and a carbamic acid residue, when catalytic hydrogenation is carried out in anhydrous ether. Therefore, the fast intramolecular attack by the carboxyl group on the adjacent carbonyl carbon in the same molecule must then take place before the decarboxylation of the carbamic acid occurs.



(6)

The proposed mechanism is partially similar to that of the NCA formation reaction commonly employed. In this case, the intramolecular attack by the carboxyl group of the carbamic acid on the activated carbonyl group in the same molecule takes place under anhydrous conditions at 40—60 °C.



It should also be noted that the retention of the full optical activity is observed in every reaction. This observation suggests two significant facts. The first is that I has a full optical activity and that racemization does not occur during the ring-formation reaction. The

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[10] T. Wieland and H. Bernhard, *Ann. Chem.*, **572**, 190 (1951).

TABLE 2. REACTION OF *N*-ACYLATED AZIRIDINONES DERIVED FROM ACYL AMINO ACID

No.	Acyl amino acid	Nucleophile	Product	Yield (%)	Mp (°C)	$[\alpha]_D^{20}$ (c 1)
7	Z-Val	MeOH	Z-Val-OMe	81.9	54—55	−18.9(MeOH)
8	Z-Ala	H_2O	$(Z\text{-}Ala)_2O$	45.2	120—122	−11.0(THF)
9	Z-Val	H_2O	$(Z\text{-}Val)_2O$	45.6	96—98	+10.7(THF)
10	Br-Z-Phe	H_2O	$(Br\text{-}Z\text{-}Phe)_2O$	51.1	147—148.5	+2.0(THF)
11	Cl-Z-Phe	H_2O	$(Cl\text{-}Z\text{-}Phe)_2O$	47.8	149—150	−3.0(THF)
12	Tos-Phe	MeOH	Tos-Phe-OMe	52.5	91—93	+3.1(MeOH)

second is that the ring-opening reaction of I with various nucleophiles also proceeds without racemization.

Reaction of N-*Acylated Aziridinone with Nucleophiles.* From the subsequent study, it was found that the isolation of I synthesized is not always necessary for the sake of the successive reaction with nucleophiles; the reaction was performed directly by the use of the I without isolation before use. Thus, the reaction procedure may not only be simplified but may also be applied to other *N*-acylated aziridinones which are not isolated in the crystalline form. The results are summarized in Table 2.

Reaction of N-*Acylated Aziridinones with Amino Acid Derivatives.* The crystal of I was dissolved in chloroform, and into this solution was added a solution

TABLE 3. REACTION OF *N*-ACYLATED AZIRIDINONES WITH AMINO ACID DERIVATIVES

No	Z-AA	Nucleophile	Product
13	Z-Phe[a]	H-Gly-OEt	Z-Phe-Gly-OEt
14	Z-Ala	H-Gly-OEt	Z-Ala-Gly-OEt
15	Z-Val	H-Gly-OEt	Z-Val-Gly-OEt
16	Z-Leu	H-Gly-OEt	Z-Leu-Gly-OEt
17	Z-Ile	H-Gly-OEt	Z-Ile-Gly-OEt
18	Z-Met	H-Gly-OEt	Z-Met-Gly-OEt
19	Z-(Ph)Gly	H-Gly-OEt	Z-(Ph)Gly-Gly-OEt
20	Z-Phe	H-Ile-OEt	Z-Phe-Ile-OEt
21	Z-Phe	H-Val(Me)-OMe[b]	Z-Phe-Val(Me)-OMe
22	Z-Phe	H-Ile-OH	Z-Phe-Ile-OH
23	Z-Phe	H-Val-OH	Z-Phe-Val-OH
24	Z-Phe	H-Ile-Gly-OMe	Z-Phe-Ile-Gly-OMe
25	Z-Thr	H-Phe-OMe	Z-Thr-Phe-OMe
26	Z-Trp	H-Met-OMe	Z-Trp-Met-OMe
27	Z-Azp(Bzl)	H-Phe-OMe	Z-Azp(Bzl)-Phe-OMe
28	Z-Lys(Tos)	H-Gly-OEt	Z-Lys(Tos)-Gly-OEt
29	Tos-Phe	H-Gly-OEt	Tos-Phe-Gly-OEt

No	Yield (%)	Mp (°C)	$[\alpha]_D^{20}$ (c 1)	Lit.[18,19]	Found (%)			Calcd (%)		
					C	H	N	C	H	N
13	83.8	109—111	−16.8[c]	−17[c]	65.63	6.37	7.38	65.61	6.29	7.29
14	71.1	99—101	−22.2[c]	−18	58.54	6.49	9.10	58.43	6.54	9.09
15	81.7	162—164	−27.0[c]	−31[d]	60.58	7.17	8.41	60.70	7.19	8.33
16	63.8	102—104	−25.5[c]	−27[c]	61.92	7.37	8.17	61.70	7.48	8.00
17	64.5	155—156	−25.6[c]	−24[d]	61.47	7.28	7.83	61.70	7.48	8.00
18	68.0	92—93	−15.5[c]	−20[f]	55.30	6.53	7.43	55.43	6.56	7.61
19	55.6	142—144	+32.5[c]		65.28	6.18	7.59	64.85	5.99	7.56
20	62.5	98—99	−10.0[d]	−10[c]	67.72	7.23	6.29	68.16	7.32	6.36
21	81.0	79—81	−70.0[d]		67.78	6.93	6.60	67.58	7.09	6.57
22	48.5	122—124	+5.1[e]		65.38	7.08	6.95	66.97	6.84	6.79
23	38.8	411—143	−3.4[e]		66.13	6.55	7.04	66.31	6.58	7.03
24	71.8	188—189	−31.0[d]	−30[d]	64.74	6.94	8.53	64.85	6.49	8.73
25	55.8	103—105	+6.1[f]		63.87	6.32	6.60	64.06	5.87	6.79
26	77.9	137—138	−24.0[d]		61.90	6.16	8.89	62.10	6.05	8.69
27	72.4	114—116	−14.8[f]	−15[f]	67.01	5.68	5.52	67.17	5.83	5.40
28	66.7	151—152	−4.8[g]	−4.7	56.98	6.42	8.21	57.79	6.49	8.09
29	49.4	105—107	+27.9[c]		59.34	5.97	7.00	59.40	5.98	6.93

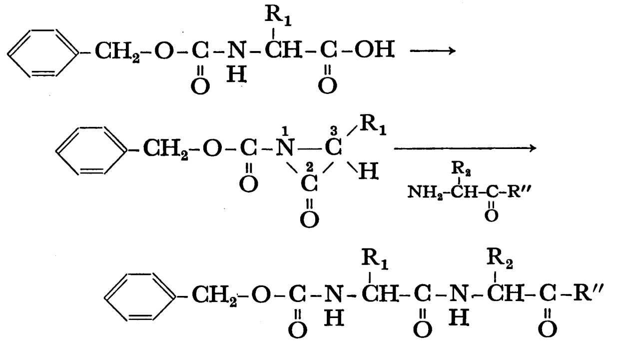
a) Crystal of (I) was used. b) *N*-Methyl valine methyl ester, reacted overnight. c) In EtOH. d) In MeOH. e) In THF. f) In DMF. g) In $CHCl_3$.

of ethyl glycinate at -10—-20 °C under stirring for one hr. The reaction proceeded selectively to form an optically-active peptide by the fission of the carbonyl-nitrogen bond, as had been expected.

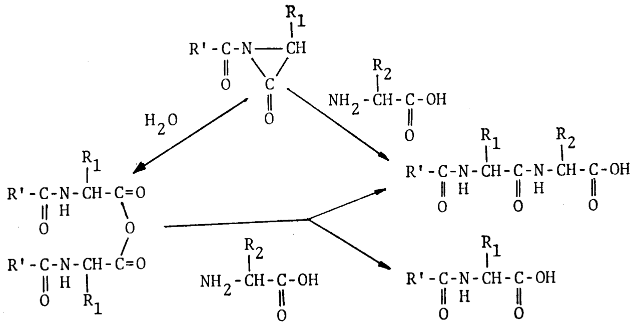
An amino acid ester hydrochloride dissolved in chloroform ($CHCl_3$) was neutralized with an equivalent triethylamine (TEA) and used for further experiments without removal of triethylamine hydrochloride (TEA–HCl) formed in the solution.

Various 3-substituted-1-benzyloxycarbonyl-aziridin-2-ones were prepared from the corresponding benzyloxycarbonyl-L-amino acid (Z–AA) in the same manner as had been described earlier.[9] Into the reaction mixture of the aziridinone was directly added a solution of amino acid ester at -10—-20 °C under stirring; the reaction was then allowed to continue for one hr.

Prior to the reaction, a functional group in the side chain of Z–AA was protected by a protecting group commonly used in peptide chemistry. It was found that the reaction also proceeded selectively by the fission of the carbonyl-nitrogen bond to form an optically-active peptide. The results are summarized in Table 3.



When free amino acid was used as a nucleophile, it was dissolved in 2 N sodium hydroxide and the coupling reaction was carried out in an aqueous solvent system. In this case, free amino acid would react with either of the aziridinone and the anhydride resulting from the decomposition of the aziridinone by the excess water, as is shown in the next scheme.

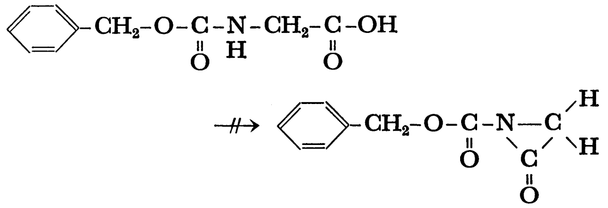


The substituent at *C*-3 in the 3-substituted-1-benzyloxycarbonyl-aziridin-2-one corresponds to the side chain (R_1) of amino acid. As is shown in Table 3, no distinct difference in the yields of the peptides was observed among the reaction of ethyl glycinate with the aziridinones derived from alanine, valine, leucine, and isoleucine. In fact, each of the reactions seems to proceed with little difference in the tlc. The variety of the yields observed in the reactions seems rather to be caused by the experimental conditions.

In most conventional peptide coupling methods, however, it is well known that the yield of a desired peptide is still unsatisfactory with an amino acid containing a bulky alkyl side chain. It should be noted that, in this coupling method, the bulkiness of the side chain (R_1) of amino acid does not have any substantial effect on the yields of the peptides.

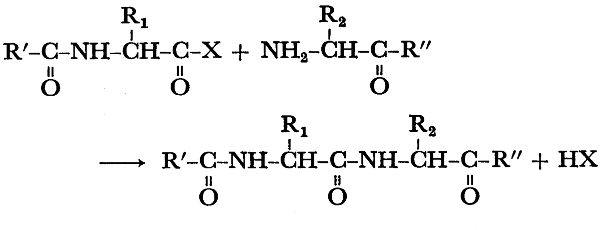
As has been mentioned earlier, a bulky alkyl group at *C*-3 in the *N*-*t*-alkyl-aziridinone generally increases the stability of the ring under otherwise identical conditions. In the formation of the aziridinone, the bulkiness of R_1 would bring about a good result by diminishing the decomposition of the extremely reactive ring. Such a contribution of the bulky R_1 to the formation of the ring would offset its lower reactivity in the subsequent ring opening reaction with an amino acid ester due to the steric effect of R_1.

The corresponding aziridinone derived from benzyloxycarbonyl-glycine (Z–Gly, R_1=H) was, however, too unstable to be isolated. The product resulting from the subsequent reaction with ethyl glycinate could not be identified, though Wieland and Heinke[11] reported that *N,N'*-dibenzyloxycarbonyl-glycylglycine was obtained from the reaction of Z–Gly with ethyl glycinate by the use of their "phosphorus oxychloride method."



In the case of an imino acid such as proline, the *N*-substituted-aziridinone cannot be formed and the coupling method in the present study can not be used.

A Characteristic of the New Peptide Coupling Method. A peptide coupling is generally carried out by two consecutive procedures; first introducing an electron-withdrawing group to the carboxyl group of acyl amino acid in order to activate the carbonyl carbon, and then, reacting it with the amino group of the other amino acid and peptide, as is shown in the next scheme:



$$\mathrm{R'\text{-}\underset{\underset{O}{\|}}{C}\text{-}NH\text{-}\overset{\overset{R_1}{|}}{C}H\text{-}\underset{\underset{O}{\|}}{C}\text{-}X + NH_2\text{-}\overset{\overset{R_2}{|}}{C}H\text{-}\underset{\underset{O}{\|}}{C}\text{-}R''}$$

$$\longrightarrow \mathrm{R'\text{-}\underset{\underset{O}{\|}}{C}\text{-}NH\text{-}\overset{\overset{R_1}{|}}{C}H\text{-}\underset{\underset{O}{\|}}{C}\text{-}NH\text{-}\overset{\overset{R_2}{|}}{C}H\text{-}\underset{\underset{O}{\|}}{C}\text{-}R'' + HX}$$

An activating group (X) should be eliminated before the nucleophilic substitution by an amino group to the carbonyl carbon is accomplished. The HX formed subsequently in the reaction media must be removed in order for the reaction to proceed completely. The reactivity is influenced by the ease of the elimination of the activating group (X), and the reaction proceeds *via* either an elimination reaction mechanism or a substitution reaction mechanism, depending on the

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[11] T. Wieland and B. Heinke, *Ann. Chem.*, **599**, 70 (1956).

Table 4. Electron densities at HOMO and LUMO

	HOMO				LUMO			
	A		B		A		B	
	N_1	O_4	N_1	O_4	C_2	C_3	C_2	C_3
S	0	0	0	0.0013	0	0.0001	0.0002	0.0002
Px	0	0	0.2465	0.0093	0.0001	0.0001	0.0010	0.0034
Py	0	0	0.0333	0.1255	0.0002	0.0001	0.0004	0.0017
Pz	0.0008	0	0.1102	0.0035	1.2701	0.0097	1.9694	0.0087
Total	0.0008	0	0.3900	0.1396	1.2704	0.0100	1.9710	0.0140

reaction conditions.

On the contrary, the *N*-acylated aziridinone does not involve any leaving group in the molecule. The acylated nitrogen atom, which is a member of the skeleton of amino acid itself, plays an effective role as a kind of electron-withdrawing group. Thus, the activation of the carbonyl-carbon depends largely upon the lower electron density on the ring as well as upon the extremely strained structure of the hetero three-membered ring, and the peptide coupling proceeds *via* a ring-opening addition reaction mechanism.

The retention of the full optical activity observed in the resulting peptides is caused by the following factors:

1) The reaction proceeds *via* a ring-opening addition reaction mechanism and no leaving group is eliminated during the reaction.

2) The addition reaction takes place rapidly at −10—−20 °C within an hour because of the reactivity of the aziridinone.

3) No bases other than the amino acid ester itself are present in the reaction media, and no base-catalyzed abstraction of a proton on the asymmetric carbon takes place during the coupling reaction.

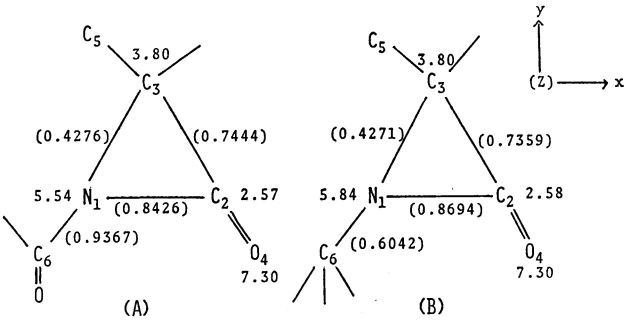
Thus, the new coupling method *via* *N*-acylated aziridinone will serve as a useful technique for peptide synthesis.

MO Calculation. An MO calculation of two types of aziridinone was carried out by the extended Hückel method[12–14] in order to find a clue to the explanation of the experimental results in the nucleophilic ring-opening reaction of *N*-acylated aziridinone.

1-Benzyloxycarbonyl-3-methyl-aziridinone (**A**) and 1-*t*-butyl-3-methyl-aziridinone (**B**) were chosen as the models of the *N*-acylated- and *N*-*t*-alkyl-aziridinones respectively.

For the input data of the bond length and the bond angles of the aziridinone ring, the X-ray analysis data of 1,3-diadamantyl-aziridinone reported by Wang *et al.*[8] were used; that is, the configuration at the nitrogen is pyramidal (the nitrogen atom lying 0.534 Å from the plane defined by its three substituents), and the adamantyl groups are *trans* to each other.

The calculated bond populations and atomic populations are shown in Scheme 1.



Scheme 1. Bond populations and atomic populations of the Aziridinone (A) and (B).
(A): 1-carbobenzoxy-3-methyl-aziridinone
(B): 1-*t*-butyl-3-methyl-aziridinone

A remarkable difference between (**A**) and (**B**) was observed, especially in the populations of N_1. The bond population of N_1–C_2 in (**A**) was smaller than that in (**B**) (0.8426; 0.8694) because of the increased interaction of N_1–C_6 (0.9367; 0.6042). A less atomic population of N_1 (5.54; 5.84) was also obtained, showing that the total electron density on the ring of (**A**) was much reduced compared to that of (**B**).

The AO populations of N_1 show that the decrease in the total charge of N_1 in (**A**) is mainly governed by the charge on the *Px*-orbital, corresponding to the reduced contribution of the N_1–C_2 bond.

AO Populations of N_1

Orbital	*S*	*Px*	*Py*	*Pz*	Total
(**A**)	1.4161	1.1460	1.3623	1.6252	5.5496
(**B**)	1.4975	1.3185	1.3979	1.6294	5.8433

In the nucleophilic ring opening reaction of the aziridinone, the position of the ring fission depends largely upon the nature of the attacking nucleophile as well as on the electronic structure of the ring, in which both HOMO and LUMO play a significant role.

The calculated electron densities at HOMO are shown in Table 4.

In the case of (**A**), the lone pairs scarcely exist on either N_1 or O_4. Under such circumstances, protonation does not occur even when a proton donor is present in the reaction system.

On the contrary, both N_1 and O_4 in (**B**) have enough lone pairs at HOMO to protonate if a proton donor is

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[12] R. Hoffmann, *J. Chem. Phys.*, **39**, 1397 (1963); R. S. Mulliken, *ibid.*, **23**, 1833 (1955); R. S. Mulliken, *ibid.*, **23**, 1841 (1955).

[13] T. Yonezawa, K. Shimizu, *Yuki Gosei Kagaku Kyokai Shi*, **26**, 719 (1968).

[14] The resonance integrals (H_{rs}) were evaluated by the Mulliken approximation, where S_{rs} represents the overlap integral between AOs

$$H_{rs} = \frac{1.75}{2}(H_{rr}+H_{ss})\cdot S_{rs}$$

MO: molecular orbital AO: atomic orbital HOMO: highest occupied MO LUMO: lowest unoccupied MO.

present. It is noted that, in the electron density of N_1 in (**B**), the contribution of the Px-orbital is the largest, showing that the lone pair of N_1 at HOMO would partially delocalize with the carbonyl oxygen (O_4).

The electron densities at LUMO in Table 4 clearly show that the interaction of an attacking nucleophile with C_2 is the largest and that the addition of a nucleophile takes place selectively at the C_2 position if a proton donor is absent. In spite of the decisive difference on the total electron density between C_2 and C_3 in (**B**), the AO electron densities of both Px- and Py-orbitals of C_3 at LUMO are greater than those of C_2. The possibility of the nucleophilic attack on C_3 in (**B**) seems, accordingly, to be greater than that in (**A**) if an attacking nucleophile comes from near the direction of the x or y-axis.

On the other hand, in a proton-containing reaction system, the protonation of (**B**) will occur, prior to the attack of the nucleophile, at the position of either N_1 or O_4, since both of them have a reactive lone pair at HOMO.

In this case, the reaction may proceed in a different way, as has been reported previously.[3–7]

Although more quantitative claculations will be required to discuss further in detail, the calculated data presented above may reasonably explain the experimental results observed in the present study.

Experimental

The melting points are uncorrected. Thin layer chromatography (tlc) was performed with silica gel G (Merck), and the spots were detected by ninhydrin on heating at 100 °C after developing in the buffer solution ($CHCl_3$:AcOEt:AcOH, 85:15:3). The IR spectra were obtained using a Shimadzu IR-27G apparatus. The NMR spectra were recorded at 60 MHz in $CDCl_3$ with TMS as the internal standard using a Hitachi R-20A apparatus. The optical rotations were measured with a Yanagimoto OR-100 apparatus. THF, ether, and petroleum ether used throughout this experiment were dried over sodium metal wire after distillation. The $COCl_2$ gas was absorbed on dry ether to prepare the $COCl_2$–ether solution and was stored in a refrigerator. The TEA was purified by distillation before use.

*Benzyloxycarbonyl-*L*-phenylalanine* t*-Butyl Ester (1) and (2).* (a) Crystals of 3-benzyl-1-benzyloxycarbonylaziridin-2-one (1) (2.81 g, 0.01 mol) were dissolved in 30 ml of THF. Into this solution 15 ml of *t*-butanol was added with stirring at −10 °C. Subsequently, a 3 ml portion of pyridine was added to the reaction mixture. A ninhydrin positive spot at R_f 0.9 on tlc decreased, and at R_f 0.8 a new spot appeared; the latter detected by HBr/ninhydrin (hydrobromic acid must be sprayed over the plate prior to the detection by ninhydrin). After 1 hr, ethyl acetate (100 ml) was added to the reaction mixture, which was then washed with 5% sodium bicarbonate, 1M hydrochloric acid, and water successively, and dried over magnesium sulfate. The organic layer was evaporated under reduced pressure, and the residue was crystallized with ethyl acetate and petroleum ether. Recrystallization from the same solvents afforded 2.96 g (83.3%). Mp 81—82 °C, $[\alpha]_D^{20}$ −6.0° (*c* 1, EtOH). IR: $\nu_{C=O}$ 1745, 1695 cm^{-1}; ν_{NH} 3380 cm^{-1}. Found: C, 71.29; H, 6.80; N. 3.77%. Calcd for $C_{21}H_{25}NO_4$: C, 70.90; H, 7.09; N, 3.94%. Lit,[16] $[\alpha]_D^{20}$ −4.4° (*c* 2, EtOH).

(b) Freshly-prepared potassium *t*-butoxide (15 g, containing excess *t*-butanol) suspended in 50 ml of THF was stirred into a solution of I (2.81 g, 0.01 mol) in 30 ml of THF at −10 °C, and the reaction was allowed to continue for 1 hr at −10—0 °C. Then, ethyl acetate (150 ml) was added to the reaction mixture, and it was worked up in the same manner as had been described above. The crystals, 2.02 g (56.8%), were identified with those afforded in (a).

*Benzyloxycarbonyl-*L*-phenylalanine Anilide (3).* Into a solution of I (2.81 g, 0.01 mol) in THF (30 ml), 10 ml of aniline was added with stirring at −10 °C. After 1 hr, ethyl acetate (100 ml) was added; the reaction mixture was then washed with 1M hydrochloric acid, 5% sodium bicarbonate and water successively, and dried over magnesium sulfate. Evaporation *in vacuo* gave the product, which was subsequently recrystallized from ethyl acetate and petroleum ether. Yield, 3.16 g (84.6%), mp 164—166 °C, $[\alpha]_D^{20}$ +30.0° (*c* 1, THF). IR: $\nu_{C=O}$ 1690, 1655, 1540 cm^{-1}; ν_{NH} 3300 cm^{-1}. Found: C, 73.65; H, 5.92; N, 7.53%. Calcd for $C_{23}H_{22}N_2O_3$: C, 73.78; H, 5.92; N, 7.48%.

*Benzyloxycarbonyl-*L*-phenylalanine Benzylamide (4).* Into a solution of I (2.81 g, 0.01 mol) in THF (30 ml), 10 ml of benzylamine was added with stirring at −10 °C; stirring was then continued for 1 hr at −10—0 °C. After ethyl acetate (100 ml) had then been added, the reaction mixture was worked up in the way described in (3). Recrystallization from ethyl acetate gave 3.31 g (85.3%). Mp 157—158 °C, $[\alpha]_D^{20}$ −12.0° (*c* 1, DMF). IR: $\nu_{C=O}$ 1685, 1645, 1540 cm^{-1}; ν_{NH} 3300 cm^{-1}. Found: C, 74.40; H, 6.21; N, 7.34%. Calcd for $C_{24}H_{24}N_2O_3$: C, 74.20; H, 6.23; N, 7.21%.

*Benzyloxycarbonyl-*L*-phenylalanine Anhydride (5).* I (2.81 g, 0.01 mol) was dissolved in a mixture of THF (20 ml) and ethyl acetate (50 ml) at −10 °C; then 10 ml of water was stirred and the reaction temperature was allowed to rise to −5—0 °C over a 30 min period. The tlc showed two spots of R_f 0.7 and R_f 0.5 (Z–Phe) which were detected by HBr/ninhydrin respectively. The reaction mixture was washed twice with ice-cold 2% sodium bicarbonate and dried over magnesium sulfate. Evaporation *in vacuo* gave the product, which was subsequently recrystallized from ethyl acetate. 1.37 g (47.2%), mp 144—146 °C, $[\alpha]_D^{20}$ +14.6° (*c* 1, dioxane). IR: $\nu_{C=O}$ 1830, 1755, 1695 cm^{-1}; ν_{NH} 3300 cm^{-1}. Found: C, 70.15; H, 5.66; N, 4.76%. Calcd for $C_{34}H_{32}N_2O_7$: C, 70.33; H, 5.56; N, 4.83%. Lit,[16] mp 139—140 °C, $[\alpha]_D^{20}$ +19.7° (*c* 1, CH_2Cl_2).

4-Benzyl-2,5-oxazolidinedione (6). I (2.81 g, 0.01 mol) was dissolved in 100 ml of ether at 0 °C. After adding 1 g of 5% palladium-charcoal to the solution, catalytic hydrogenation was carried out under bubbling hydrogen gas at room temperature over a period of 30 min. Tlc showed a new spot at R_f 0.8, which was detected by ninhydrin. After the filtration of the catalyst, the ether was evaporated under reduced pressure on an ice bath. To the residue ether and petroleum ether were added; the solution was then stored in a refrigerator. Recrystallization from the same solvents yielded 1.50 g (78.5%); mp 96—98 °C (decomp.). $[\alpha]_D^{20}$ −110.4° (*c* 1, THF). IR: $\nu_{C=O}$ 1855, 1760 cm^{-1}; ν_{HN} 3250 cm^{-1}. NMR ($CDCl_3$, δ): 7.25 (m, 5H), 6.37 (bs, 1H), 4.54 (q, 1H), 3.10 (dd, 2H). Found: C, 63.51; H, 4.80; N, 7.20%. Calcd for $C_{10}H_9NO_3$: C, 62.82; H, 4.75; N, 7.33%.

*Benzyloxycarbonyl-*L*-valine Methyl Ester (7).* To a solution of benzyloxycarbonyl-L-valine (25.1 g, 0.1 mol) in 200 ml of THF, $COCl_2$–ether solution (50 ml, containing 0.1 mol of

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[15] G. W. Anderson and F. M. Callahan, *J. Amer. Chem. Soc.*, **82**, 3359 (1960).

[16] E. Wünsch and G. Wendlberger, *Chem. Ber.*, **100**, 160 (1967).

$COCl_2$) was added at −20 °C. TEA (14 ml, 0.1 mol) in 30 ml of ether was then stirred drop by drop to the solution over a period of 30 min at −20 °C. After 30 min, TEA (14 ml, 0.1 mol) in 30 ml of ether was added, drop by drop, to the reaction mixture again. After an additional 30 min of stirring at −20 °C, 100 ml of methanol was added to the reaction mixture; the reaction temperature was then allowed to rise to −5—−10 °C with stirring for 1 hr. Ethyl acetate (500 ml) was added to the reaction mixture, it was washed with 5% sodium bicarbonate, 1 N hydrochloric acid, and water successively, and the organic layer was dried over magnesium sulfate. The evaporation to the solvent under reduced pressure afforded the product, which was subsequently recrystallized from ethyl acetate and petroleum ether. 21.7 g (81.9%). Mp 54—56 °C, $[\alpha]_D^{20}$ −18.9° (*c* 1, MeOH). IR: $\nu_{C=O}$ 1745, 1690 cm^{-1}; ν_{NH} 3350 cm^{-1}. Found: C, 63.59; H, 7.46; N, 5.47%. Calcd for $C_{14}H_{19}NO_4$: C, 63.38; H, 7.22; N, 5.28%. Lit,[17] mp 56—57 °C, $[\alpha]_D$ −16.0° (*c* 3.1, EtOH).

General Procedure for the Anhydrides (8—11). Benzyloxycarbonyl-L-amino acid or *p*-halogeno-benzyloxycarbonyl-L-amino acid (0.1 mol) was dissolved in 200—300 ml of THF. To the solution, $COCl_2$–ether (50 ml, containing 0.1 mol of $COCl_2$) was added at −20—−30 °C. TEA (14 ml, 0.1 mol) in 30 ml of ether was stirred, drop by drop, into the solution during 30 min at the same temperature. After 30 min, TEA (14 ml, 0.1 mol) in 30 ml of ether was added again in the same way as above. After an additional 30 mins' stirring, 600 ml of ethyl acetate was added; the reaction mixture was then washed with 100 ml of ice-cold water. The washing of the organic layer with ice-cold water was repeated several times. Then the organic layer was washed twice more with ice-cold 2% sodium bicarbonate, and it was dried over magnesium sulfate. The evaporation of the solvent *in vacuo* gave the product, which was recrystallized from ethyl acetate and petroleum ether. IR: $\nu_{C=O}$ 1830—1810; 1760—1750, 1700—1685 cm^{-1}; ν_{NH} 3350—3300 cm^{-1}.

Benzyloxycarbonyl-L-alanine Anhydride (8). Mp 120—122 °C, $[\alpha]_D^{20}$ −11.0° (*c* 1, THF). Found: C, 60.41; H, 5.71; N, 6.29%. Calcd for $C_{22}H_{24}N_2O_7$: C, 61.67; H, 5.65; N, 6.54%.

Benzyloxycarbonyl-L-valine Anhydride (9). Mp 96—98 °C, $[\alpha]_D^{20}$ +10.7° (*c* 1, THF). Found: C, 64.53; H, 6.64; N, 5.78%. Calcd for $C_{26}H_{32}N_2O_7$: C, 64.45; H, 6.66; N, 5.78%.

p-Bromo-benzyloxycarbonyl-L-phenylalanine Anhydride (10). Mp 147—148.5 °C, $[\alpha]_D^{20}$ +2.0° (*c* 1, THF). Found: C, 55.30; H, 4.09; N, 3.79; Br, 21.64%. Calcd for $C_{34}H_{30}N_2O_7Br_2$: C, 54.57; H, 4.08; N, 3.68; Br, 20.29%.

p-Chloro-benzyloxycarbonyl-L-phenylalanine Anhydride (11). Mp 149—150 °C, $[\alpha]_D^{20}$ −3.0° (*c* 1, THF). Found: C, 62.67; H, 4.95; N, 4.30; Cl, 10.73%. Calcd for $C_{34}H_{30}N_2O_7Cl_2$: C, 62.72; H, 4.60; N, 4.33; Cl, 10.25%.

p-Toluenesulfonyl-L-phenylalanine Methyl Ester (12). To a solution of *p*-toluenesulfonyl-L-phenylalanine (31.9 g, 0.1 mol) in 300 ml of THF, $COCl_2$–ether solution (50 ml, containing 0.1 mol of $COCl_2$) was added at −20 °C. TEA (14 ml, 0.1 mol) in 30 ml of ether was then added, drop by drop, to the solution at −20—−30 °C. After 30 min, TEA (14 ml, 0.1 mol) in 30 ml of ether was added, drop by drop, to the reaction mixture again, and it was worked up in the same manner as had been described in (7). 17.5 g (52.5%). Mp 91—93 °C, $[\alpha]_D^{20}$ +3.1° (*c* 1, MeOH). IR: $\nu_{C=O}$ 1730 cm^{-1}; ν_{NH} 3270 cm^{-1}. Found: C, 60.89; H, 5.79; N, 4.36%. Calcd for $C_{17}H_{19}NO_4S$: C, 61.25; H, 5.75; N, 4.20%.

Benzyloxycarbonyl-L-phenylalanyl-glycine Ethyl Ester (13). To a solution of ethyl glycinate hydrochloride (1.4 g, 0.01 mol) in 30 ml of $CHCl_3$, TEA (1.4 ml, 0.01 mol) was added for neutralization. Crystals of I (2.81 g, 0.01 mol) were dissolved in 20 ml of THF at −10 °C. Into this solution of I, the $CHCl_3$ solution of ethyl glycinate was added at −10—−20 °C; the reaction was then allowed to continue for 1 hr with stirring at the same temperature. After adding 40 ml of $CHCl_3$ to the reaction mixture, it was washed with 5% sodium bicarbonate, 1 N hydrochloric acid and water successively, and dried over magnesium sulfate. The evaporation of the solvent under reduced pressure afforded the product, which was recrystallized from ethyl acetate and petroleum ether. Yield, 3.22 g (83.8%), mp 109—111 °C, $[\alpha]_D^{20}$ −16.8° (*c* 1, EtOH). IR: $\nu_{C=O}$ 1760, 1695, 1655, 1540 cm^{-1}; ν_{NH} 3300 cm^{-1}.

General Procedure of the Reaction of 3-Substituted-1-benzyloxycarbonylaziridin-2-one with Amino Acid Ester. L-Amino acid ester hydrochloride (0.05 mol) was dissolved in 150—200 ml of $CHCl_3$, which was then neutralized with TEA (7 ml, 0.05 mol) at 0 °C. To a solution of benzyloxycarbonyl-L-amino acid (0.05 mol) in 100—200 ml of THF, a $COCl_2$–ether solution (25 ml, containing 0.05 mol of $COCl_2$) was added at −20—−30 °C. TEA (7 ml, 0.05 mol) in 15 ml of ether was stirred, drop by drop, into the solution over a period of 30 min at −20—−30 °C. After 30 min, TEA (7 ml, 0.05 mol) in 15 ml of ether was added again to the reaction mixture in the same way. After an additional 30 min, into the reaction mixture, a $CHCl_3$ solution of the L-amino acid ester was then added at −20 °C; the reaction proceeded for 1 hr at −10—−20 °C. A 400 ml portion of $CHCl_3$ was then added to the reaction mixture; it was washd with 5% sodium bicaronate, 1 N hydrochloric acid, and water successively, and dried over magnesium sulfate. The organic layer was evaporated under reduced pressure, then the crude product was crystallized with an appropriate solvent system.

Among the compounds listed in Table 3, there are some exceptions. In the case of Z–Phe–Val(Me)–OMe (21), considerable amounts of the *N*-methyl valine methyl ester still remained in the reaction mixture after 1 hr; they were detected by ninhydrin on tlc. The reaction was continued ovrenight with stirring at 0—−10 °C. In the case of Z–Phe–Ile–Gly–OMe (24), the dipeptide ester was reacted with the aziridinone; the reaction proceeded in the same manner as had been described in the other cases of amino acid esters.

General Procedure of the Reaction of 3-Substituted-1-benzyloxycarbonylaziridin-2-one with Free Amino Acid. To a solution of benzyloxycarbonyl-L-amino acid (0.05 mol) in THF (200 ml), $COCl_2$–ether (25 ml, containing 0.05 mol of $COCl_2$) was added at −20—−30 °C. TEA (7 ml, 0.05 mol) in ether (15 ml) was then stirred, drop by drop, into the solution over a 30 min period at the same temperature. After 30 min, TEA (7 ml, 0.05 mol) in ether (15 ml) was again added to the reaction mixture in the same manner as above. After 30 min, to the reaction mixture, a solution of L-amino acid (0.05 mol) in 2M sodium hydroxide was added at −10 °C; the reaction was then continued overnight with stirring at 0—−5 °C. After adding ethyl acetate (600 ml), the reaction mixture was washed with 1 N hydrochloric acid and water, and then dried over magnesium sulfate. Evaporation *in vacuo* gave a mixture of Z-dipeptide and Z-amino acid. Crystallization was repeated with an appropriate solvent system.

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[18] G. R. Pettit, "Synthetic Peptides," Vol. I and II. Van Nostrand Reinhold Company, New York. (1970 and 1971).

[19] J. P. Greenstein and M. Winitz, "Chemistry of the Amino Acids" Vol. 2, John Wiley & Sons Inc., New York (1971).

p-*Toluenesulfonyl*-L-*phenylalanyl-glycine Ethyl Ester (29).* To a solution of *p*-toluenesulfonyl-L-phenylalanine (31.9 g, 0.1 mol) in THF (200 ml), a $COCl_2$–ether solution (50 ml, containing 0.1 mol of $COCl_2$) was added at −20 °C. TEA (14 ml, 0.1 mol) in ether (30 ml) was added, drop by drop, to the solution at −20—−30 °C. After 30 min, TEA (14 ml, 0.1 mol) in ether (30 ml) was again added to the reaction mixture in the same way as above. Ethyl glycinate hydrochloride (14 g, 0.1 mol) dissolved in $CHCl_3$ (300 ml) was neutralized with TEA (14 ml, 0.1 mol); then the $CHCl_3$ solution was stirred into the reaction mixture at −20 °C. The reaction was allowed to continue overnight at 0—10 °C. 600 ml of $CHCl_3$ was added to the reaction mixture, which was then worked up in the same manner as had been described as the General Procedure. Recrystallization from ethyl acetate afforded 19.9 g (49.4%).

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