Synthesis of Mesoionic Triazolopyridine. III. Applications of *N*-Acyl Mesoionic Triazolopyridines as Acylating Reagents

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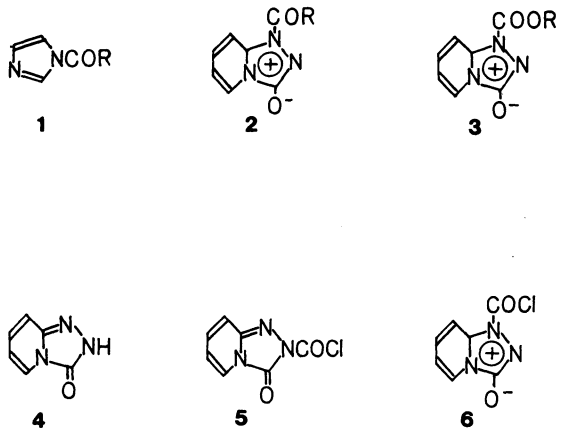
Utility of *N*-acyl mesoionic triazolopyridines as acylating reagents was investigated concerning with peptide synthesis. Several dipeptides and *N*-alkoxycarbonyl amino acids were prepared by use of these reagents.

N-Acylimidazoles(imidazolides, **1**) are widely used for organic synthesis[1] as acylating reagents because of their easy preparation and appropriate reactivity. Thus, imidazolides are readily prepared[2] from carboxylic acids under very mild conditions, and are utilized in a wide variety of synthetic processes, *e.g.*, amides[1,2] esters,[3] aldehydes,[4] ketones,[1] and C–C bond formations.[5] However, the instability of imidazolides on hydrolytic decomposition requires careful handling and delineates their appications. To remove this defect, other types of active amides are being developed.[6–8]

In the preceding paper[9] of this series, we described the synthesis and some properties of *N*-acyl and *N*-alkoxycarbonyl mesoionic triazolopyridines† (**2** and **3**). These compounds are readily synthesized from carboxylic acids or alcohols by using *N*-(chloroformyl)-triazolopyridines (**5** and **6**) under the very mild conditions. Preliminary study[9] revealed that the active amide of these types (**2** and **3**) was relatively stable toward hydrolytic decomposition but more active than corresponding imidazolide in the reaction with amines. These properties of the mesoionic azolide have indicated the potential of these compounds as acylating reagents. To examine the utility of the mesoionic azolides as acylating reagents, we have first applied them for a synthesis of amides. In this paper, we wish to describe the applications of these mesoionic azolides (**2** and **3**) for peptide synthesis including both of peptide-bond formation and introduction of urethane-type protecting groups to amine moieties of amino acids.



1 2 3 4 5 6

Fig. 1.

† Both classes of compounds are characterized by combination of *N*-acylazole(azolide)[1] with mosoionic ring. Hence, we wish to use a term "mesoionic azolide" for the compounds (**2** and **3**) in this paper.

Results and Discussion

The *N*-acyl mesoionic azolides (**2**) were applied for peptide-bond formation, and the urethane-type mesoionic azolides (**3**) were utilized for the introduction of amineprotecting groups.

Peptide-bond Formation. One of the major problem in peptide synthesis is racemization, which proceeds[10] *via* 5(4*H*)-oxazolone (**8**) formation at the activated carboxyl group of an *N*-acyl amino acid or acyl peptide (Scheme 1). To avoid this problem, the *N*-blocked amino acids (**9**) by urethane-type protecting groups, which suppress the oxazolone formation, are usually used for the coupling reactions of amino acids.

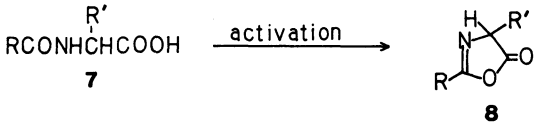
N^{α}-Benzyloxycarbonyl (Z) or N^{α}-(*t*-butoxycalbonyl) (Boc) amino acids reacted rapidly with **5** in the presence of one equimolor of tertiary amines in an aprotic solvent with evolution of gas. The reactions completed within 10 min at 4—25 °C, and the reaction mixture turned fluorescent yellow-green, indicating the formation of the mesoionic azolides (**2**). The products (**10**) were isolated easily by washing the reaction mixture with water, followed by removing the solvent *in vacuo*. However, in most cases, the reaction mixtures were used to the following coupling reactions without isolation of the mesoionic azolides. Reactions of the mesoionic azolides, such as **10**, with amino acid esters (**11**, R^3=alkyl) took place smoothly and generally completed within 1—3 h; in the case of sterically hindered amino acids, *e.g.*, isoleucine or valine, longer time (—15 h) was required to complete the reaction. According to the progress of the reaction, disappearance of the fluorescence of the reaction mixture and precipitation of the by-product (**4**) were found. These changes can be used to monitor the reaction. The by-product (**4**) is only slightly soluble in most of solvents. Therefore, it was almost separated by filtration, and the small amount of **4** remaining in the filtrate was completely removed by washing with 4% sodium hydrogencarbonate or 0.5 M copper(II) sulfate buffered with sodium citrate. Thus, desired dipeptide esters (**12**, R^3= alkyl) were isolated from the reaction mixture without any difficulties. The yields of the products were good.

When the mesoionic azolide (**10a**) was treated with sodium salts of amino acids (**11**, R^3=Na) in aqueous solution, dipeptides (**12**, R^3=H) were obtained in good yield. The yield of 93% for *N*-Z-glycylphenylalanine is obviously superior than those of other methods including imidazolide method; the yields of 55—66% were reported.[2,8,11] It seemed that this good yield could be ascribed to the stability of the mesoionic azolide toward hydrolytic decomposition. The conditions of the coupl-

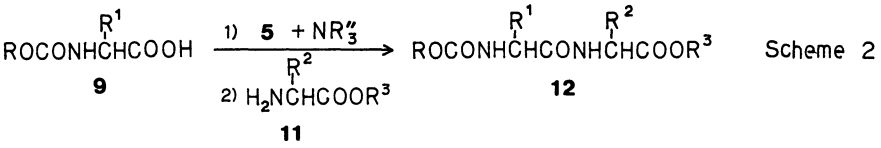
TABLE 1. PREPARATION OF DIPEPTIDES. REACTION CONDITIONS AND YIELDS

Run	Synthesized dipeptide	Reaction conditions			Yield/%[a]
		Temp/°C	Time/h	Solvent	
1	Z-Ile-Gly-OEt[b]	r.t.[d]	3	$CHCl_3$	96
2	Z-Ile-Leu-OEt[b]	r.t.	15	$CHCl_3$	73
3	Z-Arg(NO_2)-Pro-OBzl[b]	r.t.	3	$CHCl_3$	82
4	Z-pyro-Glu-His-OBzl[c]	r.t.	2	$CHCl_3$	87
5	Boc-Pro-Gly-OEt[c]	r.t.	0.5	$CHCl_3$	99
6	Z-Gly-Ala-OH[b]	4	3	Me_2CO-H_2O	91
7	Z-Gly-Phe-OH[b]	r.t.	0.5	Me_2CO-H_2O	93

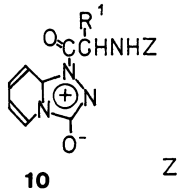
a) Isolated yield. b) Isolated mesoionic azolide was used. c) The coupling was carried out without isolation of the mesoionic azolide. d) Room temperature.



Scheme 1

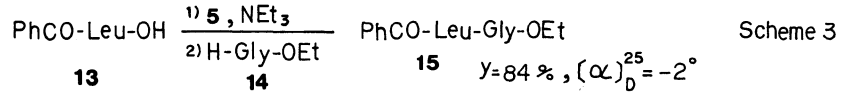


Scheme 2



a R^1:H-
b " :$C_2H_5(CH_3)CH$-
c " :NO_2-N=C(NH_2)NH$(CH_2)_3$-

Fig. 2.



Scheme 3

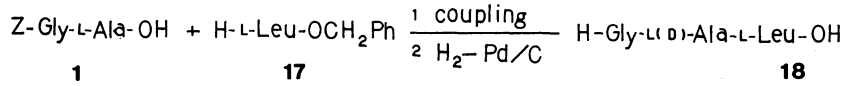


Fig. 3.

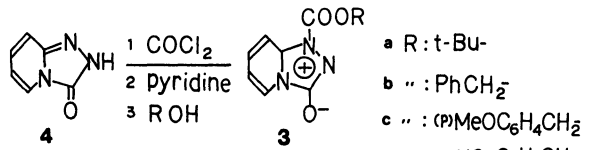
ing reaction and the yields of dipeptides are summarized in Table 1. The physical properties of the dipeptides are in agreement with those of the samples reported in literature.[2,24-26]

When *N*-benzoyl-L-leucine (**13**) was treated with **5**, instead of *N*-Boc or *N*-*Z* amino acid, relatively weak fluorescence of the reaction mixture and precipitation of **4** were found. These facts suggest the formation of oxazolone (**8**) instead of mesoionic azolide such as **10**. The following treatment of this reaction mixture with glycine ethyl ester (**14**) gave the dipeptide (**15**) with loss of optical activity. This peptide-bond formation seemed to proceed via oxazolone, which causes the racemization. To determine exact degree of the racemization in this method, Izumiya test[12] was achieved. The results are shown in Table 2. The racemization was minimized to 6.7% when the reaction was carried out in THF and in lower temperature (−20 °C). Further attempts to overcome the racemization were not achieved, since polypeptides are generally synthesized by stepwise condensation using an N^{α}-protected amino acid with a urethane-type protecting group, and practical methods[13,14] for fragment condensation now became available.

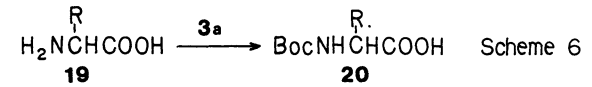
TABLE 2. IZUMIYA TEST

Run	Activation conditions			Tripeptide	
	Temp °C	Time min	Solvent	Yield %	Extent of racemization %
1	0	20	$CHCl_3$	90	16.3
2	−20	10	THF	97	6.7

Protection of Amino Function by Using the Mesoionic Azolide (3). Since benzyloxycarbonyl fragment was introduced as an amino-protecting group in peptide synthesis, a number of analogous groups with a variety of

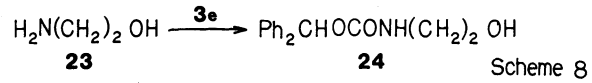


Scheme 5

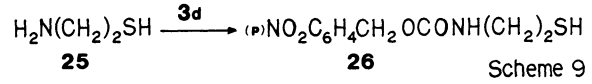


Scheme 6

Scheme 7



Scheme 8



Scheme 9

Fig. 4.

the deblocking conditions have been utilized, *e.g.*, *t*-butoxycarbonyl (Boc),[15] *p*-methoxybenzyloxycarbonyl (PMZ),[15,16] *p*-nitrobenzyloxycarbonyl (PNZ),[17] benzhydryloxycarbonyl (Bhoc),[18] trichloroethoxycarbonyl (Troc),[19] and the others. Introduction of these blocking groups to amino acids has been done by using corresponding chloride or azide. However, the former procedure suffers from the instability of the reagents and the later cases have a fear of explosion in the synthesis of the reagent. To overcome these defects, some of reagents[20,21] have been developed in connection with the synthesis of *N*-Boc amino acid, which are most widely used for peptide synthesis.

Table 3. Urethane-type mesoionic azolides (3)

	R	Yield/%	Mp θ_m/°C
3a	*t*-Bu-	87	112—114
3b	$PhCH_2$-	90	140
3c	*p*-$MeOC_6H_4CH_2$-	82	127—130
3d	*p*-$NO_2C_6H_4CH_2$-	92	230
3e	Ph_2CH-	95	147—149
3f	Cl_3CCH_2-	88	140

The urethane-type mesoionic azolides (**3**) are obtained in good yield from a variety of alcohols by using **6**, or more conveniently by subsequent treatment of **4** with phosgene, pyridine and appropriate alcohols, (Fig. 4) and (Table 3). These mesoionic azolides were highly crystalline solids and could be stored in the air at room temperature for several months without any practical decomposition. It was found these reagents are applicable to the introduction of a variety of urethane-type *N*-protecting groups. Reactions of mesoionic *N*-Boc triazolopyridine (**3a**) with sodium salts of amino acids in aqueous acetone took place smoothly at room temperature, and generally completed in about 1—5 h, and the yields of the *N*-Boc amino acids were good (80—100%) (Table 4). The other urethane-type protecting groups were also easily introduced by using corresponding mesoionic azolide (**3**). For example mesoionic *N*-*Z*-triazolopyridine (**3b**) reacted with glycine sodium salt under the same conditions to give *N*-(benzyloxycarbonyl)glycine in 86% yield. In case of amino acid, like phenylglycine which has low solubility in aqueous solution, precipitation of the amino acid frequently occurred during the course of the eraction. This causes lowering the yields of the products. This problem was overcome by controlling the pH (10.2—10.5) of the reaction mixture or by exchanging the solvent from aqueous acetone to aqueous pyridine. Another problem in this procedure is the competitive conversion of the mesoionic azolide (**3**) into less reactive nonmesoionic N^2-acyl analogs.[9] However, this problem was overcome using excess of the reagent (1.2—2.0 molar equivalent). By using these conditions, several *N*-(alkoxycarbonyl)-

Table 4. Boc-amino acids. yields and reaction conditions

Boc-Amino acid[a]	Reagent mol	Solvent	Reaction time/h	Yield %	Mp θ_m/°C	Ref θ_m/°C
Boc-L-Trp-OH	1.4	A	6	90	137—139	137—138[21]
Boc-L-Glu-OH	1.4	A	3	82	113—114	110—112[23]
Boc-L-Ile-OH·DCHA	1.4	A	4	86	124—126	123—125[21]
Boc-L-Met-OH·DCHA	1.4	A	4	92	136—138	137—139[21]
Boc-L-Arg(NO_2)-OH	1.4	A	5	85	116(decomp)	115—116(decomp)[21]
Boc-L-Pro-OH	1.1	B	2	88	133—134	132—133[21]
Boc-L-Phe-OH	1.4	C	4	77	87—89	84—86[21]
Boc-L-Tyr-OH·DCHA	1.4	A	4	81	198(decomp)	202(decomp)[21]
Boc-L-Val-OH·DCHA	1.4	A	3	89	138—139	
Boc-L-Ser-OH·DCHA	1.4	A	4	71	136—138	140—142[23]
Boc-D-Phg-OH	2.0	A	4	77	85—88	87—89.5[21]
Boc-D-Phg-OH	2.0	A(pH 10.5)	2	84	86—88	

a) Each of amino acids (10 mmol) was dissolved in 10 ml of 1 M sodium hydroxide, then organic solvents were added. A: Acetone (10 ml)-pyridine (2 ml), B: no additive, C: dioxane (10 ml)-pyridine (2 ml).

phenylglycines (**22a**, **c**, **f**) were obtained in satisfactory yields. These results show that the mesoionic azolides (**3**) are useful reagents for the introduction of a variety of urethane-type protecting groups. These reactions of **3** with amino acids proceed in aqueous solution under mild conditions, and the yields of the *N*-protected amino acids are satisfactory. These good results are probably attributed to the feature of the mesoionic azolide. Thus, both of polar structure of the mesoionic ring and higher reactivity toward amines compared with water are favorable for reaction in aqueous solution. Alternative example of the highly selective reactivity of the mesoionic azolide were found in the following experiments. The reaction of **3e** with 2-aminoethanol (**23**) gave *N*-Boc-2-aminoethanol (**24**) in 96% yield (Scheme 8). More interesting result was obtained in the case of 2-aminoethanethiol (**25**). The reaction of **3d** with **25** gave *N*-protected product (**26**) in good yield (Scheme 9). It was reported[22] that **26** was hardly prepared by direct *N*-acylation of **25** since *N*- and *S*-acylations compete in the reaction.

As described above, *N*-Boc and *N*-*Z* amino acids were converted readily to the mesoionic azolide (**2**) under very mild conditions by using the reagent (**5**). Coupling reactions of these mesoionic azolides with amino acid esters or amino acid salts proceeded smoothly yielding dipeptides. The yields were satisfactory even in the reaction being conducted in aqueous solution. A variety of urethane-type mesoionic azolides were also utilized successfully for the specific introduction of urethane-type protecting groups to the amino moieties of several amines having another functional groups. Remarkable selective reactivity of these mesoionic azolides (**2** and **3**) toward amino moiety was found in the experiments described above. The results revealed the utility of these mesoionic azolides (**2** and **3**) as acylating reagents, and indicate possible use for the acylation of complicated molecules.

Experimental

Melting points were determined on a Yazawa hot-stage apparatus and were uncorrected. IR, UV, and NMR spectra were recorded on JASCO A102, Hitachi 200-20 and Varian EM 360L spectrometers respectively.

N-(Chloroformyl)triazolopyridines (**5** and **6**) were prepared in the manner previously reported.[9]

*1-[N-(Benzyloxycarbonyl)glycyl]-1,2,4-triazolo[4,3-a]pyridinium-3-olate (**10a**).* To a solution of *N*-(benzyloxycarbonyl)-glycine (6.27 g, 30 mmol) in chloroform (80 ml) were successively added triethylamine (4.20 ml, 30 mmol) and 5.91 g (30 mmol) of 2-chloroformyl-1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one (**5**). After the mixture had been stirred at room temperature for 1 h, the precipitate formed was collected by filtration to give 8.23 g (84%) of **10a**. The filtrate was washed twice with cold water, dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was crystallized from chloroform–ether to give second crop of **10a** (328 mg, 3.3%); mp 173—176 °C. UV (THF) λ_{max}: 375 nm. IR (Nujol): 1720, 1695 cm^{-1} (C=O). Found: C, 58.65; H, 4.13; N, 17.05%. Calcd for $C_{16}H_{14}N_4O_4$: C, 58.89; H, 4.32; N, 17.17%.

*1-(N-Benzyloxycarbonyl-L-isoleucyl)-1,2,4-triazolo[4,3-a]pyridinium-3-olate (**10b**).* To a solution of *N*-benzyloxycarbonyl-L-isoleucine (7.96 g, 30 mmol) in chloroform (100 ml) was added triethylamine (4.2 ml, 30 mmol), and the mixture was cooled to 4 °C, then 5.91 g (30 mmol) of **5** was added. After having been stirred for 1 h, at room temperature, the reaction mixture was washed twice with cold water, dried over anhydro us sodium sulfate, and evaporated *in vacuo*. The residue was crystallized from chloroform–ether giving pure **10b** (10.83 g, 95%); mp 165—170 °C. UV (THF) λ_{max}: 374 nm. Found: C, 62.92; H, 5.84; N, 14.65%. Calcd for $C_{20}H_{22}N_4O_4$: C, 62.81; H, 5.80; N, 14.65%.

*1-(N^{a}-Benzyloxycarbonyl-N^{g}-nitro-L-arginyl)-1,2,4-triazolo[4,3-a]pyridinium-3-olate (**10c**).* A solution of N^{a}-benzyloxycarbonyl-N^{g}-nitro-L-arginine (7.06 g, 20 mmol) and triethylamine (2.80 ml, 20 mmol) in dichloromethane (30 ml) was added dropwise into a suspension of **5** (4.00 g, 20 mmol) in dichloromethane (50 ml) at a temperature between 4 °C to 7 °C with stirring. The mixture was stirred for 1 h at 4 °C, then allowed to stand at room temperature for 2 h. The crystalline precipitate was collected by filtration, washed with chloroform, dried *in vacuo* to give **10c** (8.08 g, 85.5%). The filtrate and the washing were combined, washed twice with cold water, dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was crystallized from chloroform–ether to give second crop of **10c** (1.381 g, 14.5%); mp 120 °C. UV (CH_2Cl_2) λ_{max}: 378 nm. Found: C, 49.24; H, 4.96; N, 22.68%. Calcd for $C_{20}H_{22}N_8O_6 \cdot H_2O$: C, 49.16; H, 4.95; N, 22.95%.

*Reaction of **10b** with H–Gly–OEt.* To a solution of glycine ethyl ester (356 mg, 3.15 mmol) in chloroform (10 ml) was added **10b** (1.15 g, 3 mmol), and the mixture was stirred at room temperature for 3 h. The reaction mixture was filtered to remove the by-product (**4**), and the filtrate was washed successively with 2% hydrochloric acid, water, 4% sodium hydrogencarbonate, and water, then dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was crystallized from methanol to give *N*-benzyloxycarbonyl-L-isoleucylglycine ethyl ester (1.01 g, 96%); mp 155—156 °C; $[\alpha]_D^{25}$ −27° (c 0.9, CH_3OH). Lit[24] mp 155 °C; $[\alpha]_D$ −26° (*c* 1, CH_3OH). Found: C, 61.67; H, 7.63; N, 8.26%. Calcd for $C_{18}H_{26}N_2O_5$: C, 61.70; H, 7.48; N, 7.99%.

*Reaction of **10b** with H–L-Leu–OEt.* Triethylamine (0.70 ml, 5 mmol) was added to a solution of leucine ethyl ester hydrochloride (1.00 g, 5 mmol) in chloroform (10 ml), then **10b** (1.91 g, 5 mmol) was added. After having been stirred at room temperature for 15 h, the reaction mixture was worked up in the same manner as described in the preceding experiment. Crystallization from ether gave *N*-benzyloxycarbonyl-L-isoleucyl-L-leucine ethyl ester (1.433 g, 71%); mp 116—117 °C; $[\alpha]_D^{25}$ −43.5° (*c* 0.8, CH_3OH). Found: C, 64.69; H, 8.44; N, 6.72%. Calcd for $C_{22}H_{34}N_2O_5$: C, 64.99; H, 8.43; N, 6.89%.

*Reaction of **10c** with H–L-Pro–OBzl.* To a suspension of L-proline benzyl ester hydrochloride (2.64 g, 11 mmol) in chloroform (20 ml) was added triethylamine (1.54 ml, 11 mmol), and the mixture was stirred for 10 min, then **10c** (4.88 g, 10 mmol) was added. After having been stirred at room temperature for 2 h, the reaction mixture was worked up in the same manner as described in the preceding experiment. Crystallization from ethanol–ether gave N^{a}-benzyloxycarbonyl-N^{g}-nitro-L-arginyl-L-proline benzyl ester (4.33 g, 82%); mp 145—146 °C; $[\alpha]_D^{22}$ −59° (*c* 1, CH_3OH). Lit,[25] $[\alpha]_D$ −61° (*c* 1.1, CH_3OH). Found: C, 57.49; H, 6.09; N, 15.46%. Calcd for $C_{26}H_{32}N_6O_7$: C, 57.77; H, 5.97; N, 15.55%.

*Coupling of Z-L-pyro-Glu-OH with H-L-His-OBzl by Use of **5**.* To a solution of *N*-benzyloxycarbonyl-L-pyroglutamic acid (526 mg, 2 mmol) in chloroform (10 ml) were added triethylamine (0.28 ml, 2 mmol) and **5** (396 mg, 2 mmol), and the

mixture was stirred at room temperature for 30 min, then L-histidine benzyl ester (490 mg, 2 mmol) was added. After having been stirred at room temperature for 2 h, the reaction mixture was filtered to remove the by-product (**4**). The filtrate was washed successively with water, 2% hydrochloric acid, 4% sodium hydrogencarbonate and water, then dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was crystallized from ethyl acetate to give *N*-benzyloxycarbonyl-L-pyroglutamyl-L-histidine benzyl ester (852 mg, 87%); mp 125—126 °C; $[\alpha]_D^{22}$ −40° (*c* 1.2, CH_3OH). Found: C, 63.40; H, 5.33; N, 11.33%. Calcd for $C_{19}H_{22}N_4O_5$: C, 63.68; H, 5.34; N, 11.42%.

Coupling of Boc-L-Pro-OH with H-Gly-OEt by Use of 5. To a stirred solution of Boc-L-proline (5.37 g, 25 mmol) in chloroform (50 ml) were added successively *N*-methylmorpholine (2.74 ml, 25 mmol) and **5** (4.95 g, 25 mmol) at a temperature below 10 °C, and the mixture was stirred at room temperature for 40 min, then glycine ethyl ester (2.83 g, 27.5 mmol) was added. After having been stirred at room temperature for 30 min, the reaction mixture was filtered to remove the by-product (**4**). The filtrate was washed successively with water, 0.5 M citric acid, water, 4% sodium hydrogencarbonate and water, then dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The oily residue was chromatographically pure *N*-Boc-L-prolylglycine ethyl ester (7.43 g, 99%). Found: C, 56.23; H, 8.22; N, 9.15%. Calcd for $C_{14}H_{24}N_2O_5$: C, 55.97; H, 8.05; N, 9.32%.

*Reaction of **10a** with H-Ala-OH.* To a solution of L-alanine (1.60 g, 18 mmol) in a mixture of 1 M sodium hydroxide (18 ml) and acetone (18 ml) was added **10a** (4.89 g, 15 mmol) at 4 °C. The mixture was stirred at 4 °C for 3 h, evaporated *in vacuo* to remove the acetone, and filtered to remove by-product. The filtrate was washed with ethyl acetate, acidified by adding 1 M hydrochloric acid, and extracted with ethyl acetate (×2). The ethyl acetate solution was washed successively with a 1 : 1 mixture of 0.5 M copper(II) sulfate and 1/6 M sodium citrate, and with water, dried over anhydrous sodium sulfate, and concentrated *in vacuo* giving a crystalline product. The product was collected by filtration, washed with small amount of ethyl acetate, and dried. *N*-(Benzyloxycarbonyl)glycyl-L-alanine (3.864 g, 91%) was obtained: mp 117 °C; $[\alpha]_D^{22}$ −9.7° (*c* 3, C_2H_5OH). Lit,[26]: mp 119.5 °C; $[\alpha]_D$ −9.8° (*c* 2.8, C_2H_5OH). found: C, 55.98; H, 5.60; N, 9.97%. Calcd for $C_{13}H_{16}N_2O_5$: C, 55.68; H, 5.75; N, 9.99%.

*Reaction of **10a** with H-L-Phe-OH.* To a solution of L-phenylalanine (2.48 g, 15 mmol) in a mixture of 1 M sodium hydroxide (15 ml) and acetone (15 ml) was added **10a** (4.89 g, 10 mmol). The mixture was stirred at room temperature for 30 min, then allowed to stand overnight in a refrigerator. The crystalline by-product (**4**) was removed by filtration. Sodium hydrogencarbonate (4%, 10 ml) was added to the filtrate. The mixture was washed with ethyl acetate, acidified by adding 1 M hydrochloric acid (17 ml), and then extracted with ethyl acetate. The ethyl acetate extract was washed with 1 : 1 mixture of 0.5 M copper(II) sulfate and 1/6 M sodium citrate, and with water, then dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was crystallized from ether to give *N*-(benzyloxycarbonyl)glycyl-L-phenylalanine (5.02 g, 93%): mp 124—125.5 °C; $[\alpha]_D^{22}$ +40° (*c* 1, C_2H_5OH). Lit,[2]: mp 126.5 °C; $[\alpha]_D$ +40° (C_2H_5OH). Found: C, 64.11; H, 5.55; N, 7.96%. Calcd for $C_{19}H_{20}N_2O_5$: C, 64.03; H, 5.66; N, 7.86%.

Coupling of Bz-Leu-OH with H-Gly-OEt by Use of 5. A solution of *N*-benzoyl-L-leucine (1.175 g, 5 mmol) and triethylamine (1.40 g, 5 mmol) in chloroform (5 ml) was added dropwise with stirring into a suspension of **5** (1.0 g, 5 mmol) in chloroform at −15 °C. Gas evolution, followed by precipitation of insoluble material was found. The mixture was stirred at −10 °C for 1 h, and glycine ethyl ester (515 mg, 5 mmol) was added. After having been stirred at −10 °C for 30 min, then at 4 °C for 3 h, the reaction mixture was filtered. The filtrate was washed with water, dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was crystallized from ether to give *N*-(benzoyl) leucylglycine ethyl ester (1.342 g, 84%); mp 144—147 °C; $[\alpha]_D^{22}$ −2° (*c* 1, C_2H_5OH): the value of $[\alpha]_D^{20}$ −34.0° (*c* 3.1, C_2H_5OH) was reported[10] for *N*-benzoyl-L-leucylglycine ethyl ester. Found: C, 63.88; H, 7.41; N, 9.03%. Calcd for $C_{17}H_{24}N_2O_4$: C, 63.73; H, 7.55; N, 8.75%.

Izumiya Test. Z-Gly-L-Ala-OH and authentic samples of H-Gly-L-Ala-L-Leu-OH and H-Gly-D-Ala-L-Leu-OH were prepared by the methods in the literature.[12] The dipeptide (Z-Gly-L-Ala-OH) was treated with **5** under the condotions summarized in Table 2, and then allowed to react with L-leucine benzyl ester at room temperature overnight to give tripeptide. After hydrogenation to remove the protecting group, the tripeptide (**18**) were analyzed according to the procedure in the literature.[12]

*General Procedure for Synthesis of the Urethane-type Mesoionic Azolide (3). Synthesis of **3a**:* Finely powdered 1,2,4-triazolo[4,3-*a*]pyridin-3(2*H*)-one[23] (40.5 g, 0.3 mol) was suspended in ethanol-free chloroform (1.2 l) prepared by passing through an alumina column, and a solution of phosgene (33 g, 0.33 mol) in THF (150 ml) was added with stirring at a temperature below 10 °C. The mixture was stirred at 4 °C for 45 min, pyridine (53.5 ml, 0.66 mol) was added. After the mixture had been stirred for 30 min at 4 °C, *t*-butyl alcohol (50 ml) was added, and resultant mixture was stirred at room temperature for 40 min. The reaction mixture was washed with cold water (×3), dried over anhydrous sodium sulfate, and concentrated *in vacuo* giving crystalline product, and ether was added slowly to complete the crystallization. The product (**3a**) was collected by filtration, washed with ether, and dried *in vacuo*. Yield; 77.0 g (87% as a one-half molar chloroform adduct). UV (CH_2Cl_2) λ_{max}: 363 nm. IR (Nujol): 1757, 1722 cm^{-1} (C=O). Found: C, 46.75; H, 4.52; N, 14.52%. Calcd for $C_{11}H_{13}N_3O_3 \cdot 1/2\ CHCl_3$: C, 46.82; H, 4.61; N, 14.25%.

Other mesoionic azolides (**3**) were prepared in the same manner by using appropriate alcohols instead of *t*-butyl alcohol. In some cases, the products were crystallized off from the reaction mixture during the course of the reaction, hence they could be collected by filtration. Result was summarized in Table 3. The physical data of **3** were as follows:

a) **3b**; mp 140 °C, Physical properties of this compound were identical to those of authentic samples.[9]

b) **3c**; mp 127—130 °C. UV (CH_2Cl_2) λ_{max} 363 nm. NMR ($CDCl_3$–CD_3OD); δ=8.53 (m, 1, H-5), 8.5—7.9 (m, 2, H-7 and H-8), 7.45 (m, 1, H-6), 7.42 (d, J=9 Hz, 2, aromatic H), 6.88 (d, J=9 Hz, 2, aromatic H), 5.42 (s, 2, $PhC\underline{H}_2$), 3.81 (s, 3, OCH_3). Found: C, 59.93; H, 4.54; N, 14.31%. Calcd for $C_{15}H_{13}N_3O_4$: C, 60.20; H, 4.38; N, 14.04%.

c) **3d**; mp 230 °C (decomp). UV (CH_2Cl_2) λ_{max} 363 nm. Found: C, 56.65; H, 3.01; N, 16.38%. Calcd for $C_{16}H_{10}N_4O_5$: C, 56.80; H, 2.98; N, 16.56%.

d) **3e**; mp 147—149 °C. UV (CH_2Cl_2)λ_{max} 263, 363 nm. Found: C, 68.59; H, 4.56; N, 12.23%. Calcd for $C_{20}H_{15}N_3O_3$ 1/4 H_2O: C, 68.65; H, 4.46; N, 12.01%.

e) **3f**; mp 140 °C. UV (CH_2Cl_2) λ_{max} 364 nm. NMR ($CDCl_3$–CD_3OD); δ=8.67 (m, 1, H-5), 8.6—8.1 (m, 2, H-7 and H-8), 7.61 (m, 1, H-6), 5.18 (s, 2, $Cl_3CC\underline{H}_2$). Found: C, 35.03; H, 1.99; N, 13.66; Cl, 34.13%. Calcd for $C_9H_6N_3$-

O_3Cl_3: C, 34.82; H, 1.95; N, 13.53; Cl, 34.25%.

General Procedure of Synthesis of N-*Boc Amino Acids.* *a) A Typical Procedure*: To a solution of L-tryptophan (2.04 g, 10 mmol) in a mixture of 1 M sodium hydroxide (10 ml), acetone (10 ml) and pyridine (2 ml), **3a** (4.14 g, 14 mmol as 1/2 $CHCl_3$ adduct) was added, and the mixture was stirred at room temperature for 3 h then evaporated *in vacuo* to remove the acetone, and the residual aqueous solution was allowed to stand overnight in a refrigerator. Crystalline by-product (**4**) was removed by filtration, and the filtrate was acidified with 0.5 M citric acid to pH 3, then extracted with ethyl acetate. The ethyl acetate solution was washed with a 1 : 1 mixture of 0.5 M copper(II) sulfate and 1/6 M sodium citrate, and with water, then dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was crystallized from ethyl acetate–hexane to give *N*-Boc-L-tryptophan (2.74 g, 90%); mp 137—139 °C. Found: C, 62.97; H, 6.40; N, 9.35%. Calcd for $C_{16}H_{20}N_2O_4$: C, 63.14; H, 6.62; N, 9.20%. Other *N*-Boc amino acids were synthesized in the almost same manner, under the conditions summarized in Table 4; *N*-Boc-glutamic acid was extracted with 1-butanol instead of ethyl acetate.

b) By pH Control: To a solution of D-phenylglycine (3.03 g, 20 mmol) in a mixture of 1 M sodium hydroxide (20 ml) and acetone (20 ml) was added **3a** (11.80 g, 40 mmol as 1/2 $CHCl_3$ adduct), and the mixture was stirred at room temperature for 30 min. pH of the reaction mixture was kept around 10.5 by adding 2 M sodium hydroxide. The reaction mixture was stirred for additional 1.5 h without the pH control, and then evaporated *in vacuo* to remove the acetone. The resultant aqueous solution was allowed to stand for 1 h in a refrigerator, then filtered to remove the by-product (**4**). The filtrate was worked up in the same manner as described in preceding experiment. Crystallization from hexane gave *N*-Boc-D-phenylglycine (4.21 g, 84%); mp 86—88 °C; lit,[21] 87—89.5 °C. $[\alpha]_D^{22}$ −148° (*c* 1, C_2H_5OH); lit,[23] $[\alpha]_D$ +150° (*c* 1, C_2H_5OH) for *N*-Boc-L-phenylglycine.

N-*(p-Methoxybenzyloxycarbonyl)*-D-*phenylglycine.* D-Phenylglycine (3.03 g, 20 mmol) was treated with **3c** (8.95 g, 30 mmol) in the same manner as described before in the preparation of *N*-Boc-D-phenylglycine. Crystallization of the crude product from ethyl acetate–hexane gave desired product (5.16 g, 82%); mp 130—132 °C; $[\alpha]_D^{22}$ −102° (*c* 1, CH_3OH). NMR (DMSO-d_6–$CDCl_3$); δ=7.92 (d, J=8 Hz, 1, NH), 7.34 (s, 5, C_6H_5), 7.25 (d, J=9 Hz, 2, aromatic H), 6.84 (d, J=9 Hz, 2, aromatic H), 5.13 (d, J=8 Hz, 1, PhC<u>H</u>), 4.94 (s, 2, PhC<u>H</u>$_2$), 3.72 (s, 3, OCH_3). Found: C, 43.22; H, 8.91; N, 7.23%. Calcd for $C_{17}H_{17}NO_5$: C, 43.06; H, 8.78; N, 7.18%.

N-*(Benzyloxycarbonyl)glycine.* To a solution of glycine (375 mg, 5 mmol) in 1 M sodium hydroxide (5 ml) was added **3b** (1.48 g, 5.5 mmol), and the mixture was stirred at room temperature for 1 h and then allowed to stand for 3 h in a refrigerator. The reaction solution was filtered to remove the by-product (**4**). The filtrate was washed twice with ethyl acetate, acidified by adding 1 M hydrochloric acid (6 ml) and extracted with ethyl acetate. The ethyl acetate solution was washed twice water, dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was extracted with ether, and the extract was evaporated *in vacuo*. The residue was crystallized from ether–petroleum ether to give desired compound (898 mg, 86%); mp 120—121 °C. Found: C, 57.25; H, 5.34; N, 6.75%. Calcd for $C_{10}H_{11}NO_4$: C, 57.40; H, 5.30; N, 6.70%.

N-*Trichloroethoxycarbonyl*-D-*phenylglycine (22f).* To a solution of D-phenylglycine (1.51 g, 10 mmol) in a mixture of 1 M sodium hydroxide (10 ml), acetone (10 ml) and pyridine (2 ml) was added **3f** (3.5 g, 11 mmol), and the mixture was stirred at 4 °C for 2 h, then evaporated *in vacuo* to remove the acetone. The residual aqueous solution was allowed to stand overnight in a refrigerator, and then filtered to remove the by-product. The filtrate was washed with ethyl acetate, acidified with 1 M hydrochloric acid to pH 2.5, and extracted with ethyl acetate. The ethyl acetate solution was washed with a 1 : 1 mixture of 0.5 M copper(II) sulfate and 1/6 M sodium citrate, and with water, then dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The residue was crystallized from ether–petroleum ether to give desired product (3.10 g, 93%); mp 140—141.5 °C; $[\alpha]_D^{22}$ −105° (*c* 1.2, CH_3OH). Lit[21]: mp 141—143 °C. Found: C, 40.43; H, 3.15; N, 4.36; Cl, 31.91%. Calcd for $C_{11}H_{10}NO_4Cl_3$: C, 40.45; H, 3.09; N, 4.29; Cl; 32.56%.

Reaction of 3e with 2-Aminoethanol. To a solution of 2-aminoethanol (1.22 g, 20 mmol) in dichloromethane (30 ml) was added **3e** (6.0 g, 17.4 mmol), and the mixture was stirred at room temperature for 20 min, then filtered to remove the by-product (**4**). The filtrate was washed successively with 0.5 M citric acid, water, 5% sodium hydrogencarbonate, and water, then dried over anhydrous sodium sulfate, and concentrated giving crystalline product which was collected by filtration. 4.50 g (95.6%) of 2-(benzhydryloxycarbonylamino)ethanol was collected; mp 66—67 °C. NMR ($CDCl_3$); δ=7.36 (s, 10, C_6H_5), 6.84 (s, 1, Ph_2C<u>H</u>), 5.68 (t, J=5 Hz, 1, NH), 3.5—3.0 (m, 5, CH_2 and OH). Found: C, 71.25; H, 6.36; N, 5.25%. Calcd for $C_{16}H_{17}NO_3$: C, 70.83; H, 6.32; N, 5.16%.

Reaction of 3d with 2-Aminoethanethiol. To a suspension of 2-aminoethanethiol hydrochloride (1.25 g, 11 mmol) in dichloromethane was added triethylamine (1.4 ml, 10 mmol), and the mixture was stirred at room temperature for 30 min under nitrogen atmosphere, then **3d** (3.14 g, 10 mmol) was added. After having been stirred at room temperature for 30 min, the reaction mixture was filtered, and the filtrate was washed successively with water, 0.5 M citric acid, water, 4% sodium hydrogencarbonate and water. The chloroform solution was dried over anhydrous sodium sulfate, and evaporated *in vacuo* to give a solid, which was washed with diisopropyl ether to give 2-(*p*-nitrobenzyloxycarbonylamino)ethanethiol (1.950 g, 81%); mp 66—68 °C. NMR ($CDCl_3$); δ=8.14 (d, J=8.5 Hz, 2, aromatic H), 7.47 (d, J=8.5 Hz, 2, aromatic H), 5.61 (broad-s, 1, NH), 5.19 (s, 2, PhC<u>H</u>$_2$), 3.46 (t, J=7 Hz, 2, NC<u>H</u>$_2$), 2.68 (m, 2, SC<u>H</u>$_2$), 1.44 (t, J=8 Hz, 1, SH).

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