Studies on 2-Aziridinecarboxylic Acid. II.[1] A Novel Synthesis of Threonine *O*-Peptide Derivatives *via* (2*S*,3*S*)-3-Methyl-2-aziridinecarboxylic Acid

Takumi TANAKA, Kiichiro NAKAJIMA, Toru MAEDA, Akihiro NAKAMURA, Noboru HAYASHI, and Kenji OKAWA*

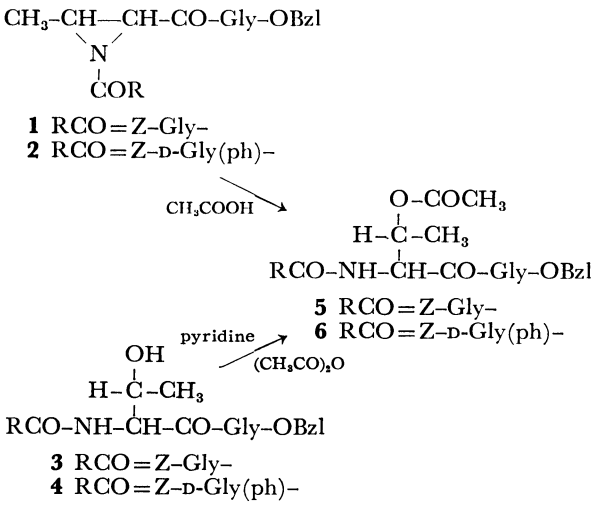
Department of Chemistry, Faculty of Science, Kwansei Gakuin University, Nishinomiya 662

(Received March 23, 1979)

A new synthesis of threonine *O*-peptides *via* the ring opening reaction of aziridine peptide with carboxylic acid has been investigated. Benzyl (2*S*,3*S*)-1-[*N*-(benzyloxycarbonyl)glycyl]-3-methyl-2-aziridinecarbonylglycinate was treated with several *N*-protected amino acids or dipeptides at their mixed melting point, and the corresponding *O*-aminoacyl or dipeptidyl esters of L-threonine peptide derivative were obtained in good yields without racemization.

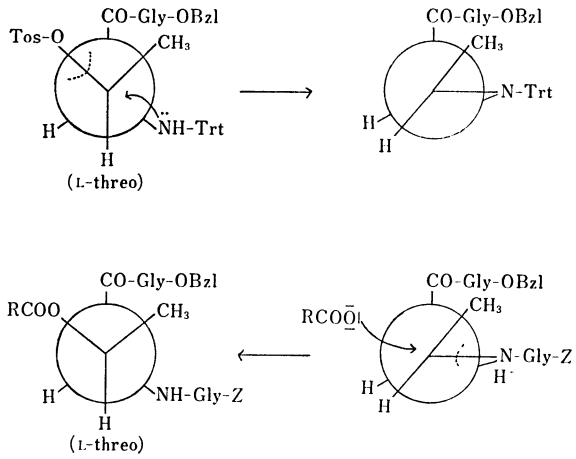
Depsipeptide represents a major part of the biological active cyclic peptides. Many studies on an effective synthetic method of these peptides have been carried out. So far total synthesis, except in some cases, has been accomplished by the usual cyclization under peptide bond formation after performance of ester linkage at the first step in the synthetic procedure. Synthetic approaches in this field are limited. It is therefore desirable to establish a convenient synthetic method for *O*-peptide without racemization.

In a previous paper,[2] it was reported that *N*-tosyl-*O*-acetyl-L-threoninanilide is obtained quantitatively *via* the ring opening reaction of (2*S*,3*S*)-1-tosyl-3-methyl-2-aziridinecarboxanilide by the action of acetic acid in the presence of BF_3.



Scheme 1.

The method was applied to aziridine peptides such as Z-Gly-3-Me-Azy-Gly-OBzl (**1**) and Z-D-Gly(ph)-3-Me-Azy-Gly-OBzl (**2**).[3] The corresponding *O*-acetylthreonine peptide derivatives (**5**, **6**) were easily obtained in the absence of any catalyst as shown in Scheme 1 and Table 1. On the other hand, authentic samples of **5** and **6** were prepared from Z-Gly(or D-Gly(ph))-L-Thr-Gly-OBzl (**3**, **4**) according to the usual *O*-acetylation procedure. The yields of the *O*-acetylthreonine peptide derivatives were equally good, no remarkable differences being observed between the two methods. The configuration of β-carbon atom of threonine residue was retained in its original threo form, double inversion being encountered through the formation and cleavage reaction of the aziridine ring (Scheme 2).



Scheme 2.

The above results prompted us to attempt effective preparation of *O*-peptides which might be used as an important intermediate of depsipeptide synthesis. A preliminary test using Z-Gly-OH to Z-Gly-3-Me-Azy-Gly-OBzl (**1**) with BF_3 in CH_2Cl_2 at 25 °C, gave low yield (about 20%). The aziridine ring showed unexpected resistance against Z-Gly-OH. However, the reaction proceeded with greater facility under drastic

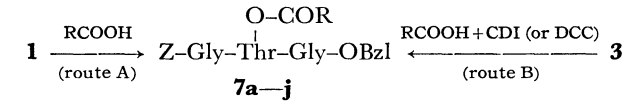
Table 1. Formation of *O*-acetylthreonine peptide derivatives (**5**, **6**)

Compd	Starting compd	Reaction temp/°C	Yield/%	Mp/°C	$[\alpha]_D^{23}$ a)
5	**1**	25	82.5	133—134	+10.5
	3	0	85.7	134—135	+ 9.7
6	**2**	25	88.8	235—236	+18.5
	4	0	80.4	235—236	+18.9

a) *c* 1.0 in DMF.

Table 2. Formation of threonine *O*-peptide derivatives (**7a—j**)

Compd	RCOOH	Route	Reagent	Reaction temp/°C	Yield/%	Mp/°C	$[\alpha]_D^{23}$
7a	Z-Gly-OH	A	—	110	97.0	135—136	+10.3(*c* 1.0, DMF)
		B	CDI	0	21.6	136—137	+11.9(*c* 0.4, DMF)
7b	Boc-Leu-OH	A	—	85	90.8	powder	−19.7(*c* 1.0, MeOH)
		B	CDI	0	16.0	powder	−19.6(*c* 0.8, MeOH)
7c	Boc-Pro-OH	A	—	125	92.3	powder	−26.2(*c* 0.85, MeOH)
7d	Boc-Phe-OH	A	—	100	73.9	100—102	− 6.2(*c* 0.93, MeOH)
7e	Z-Ser-OH	A	—	96	84.5	123—125	−12.3(*c* 0.97, MeOH)
7f	Boc-Met(O)-OH	A	—	100	95.5	powder	− 9.2(*c* 1.05, MeOH)
7g	Boc-Glu(OBzl)-OH	A	—	100	82.7	powder	−10.7(*c* 1.07, MeOH)
7h	Z-MeVal-OH	A	—	110	93.0	117—118.5	+ 36.8(*c* 0.98, MeOH)
7i	Boc-Leu-Leu-OH	A	—	115	80.0	powder	−26.8(*c* 0.91, MeOH)
		B	CDI	0	17.6	powder	−20.2(*c* 0.62, MeOH)
		B	DCC	−10	1.5	powder	−24.0(*c* 0.88, MeOH)
7j	Boc-Pro-Sar-OH	A	—	85	92.0	95—96	−23.1(*c* 0.5, MeOH)



Scheme 3.

conditions, *i.e.*, at the mixed melting point of the two reactants in the absence of catalyst or solvent as shown in Scheme 3 (*via* route A). Several *N*-protected amino acids and dipeptides were used as the carboxylic acid component. All the reactions through the ring opening (*via* route A) were accomplished in a short time (2—4 h), threonine *O*-peptide derivatives (**7a—j**) being obtained in good yields. The yields and physical characteristics of **7a—j** are given in Table 2.

Special attention was paid to racemization during the course of heating, but none was observed: **7i** (*via* route A); $[\alpha]_D^{23}$ −26.8° (*c* 0.91, MeOH), ($[\alpha]_D^{23}$ −27.4° (*c* 0.9, MeOH)).[4] Partial racemization might occur by the usual method (*via* route B), using activating reagent (CDI or DCC), during the course of direct introduction of *N*-protected dipeptide.

From the results, it was concluded that the new method would become a convenient means for the synthesis of biological active depsipeptides.

Experimental

All the melting points are uncorrected. The optical rotations were measured on a Parkin-Elmer 141 Polarimeter. Purity of the synthetic compounds was confirmed by thin layer chromatography on silica gel G.

Synthesis of O-*Acetylthreonine Peptide Derivatives. Z-Gly-*L-*Thr(OAc)-Gly-OBzl (5).* *From 1*: Z-Gly-3-Me-Azy-Gly-OBzl (**1**)[3b] (220 mg, 0.5 mmol) was dissolved in acetic acid (5 ml) and the solution was left to stand at room temperature for 3 days. After removal of acetic acid, the crystals obtained were collected and recrystallized from ethyl acetate–ether–hexane (206 mg). Found: C, 59.78; H, 5.36; N, 8.39%. Calcd for $C_{25}H_{29}O_8N_3$: C, 60.11; H, 5.85; N, 8.41%.

From 3: Acetic anhydride (708 mg, 4 mmol) was added with stirring to a solution of Z-Gly-L-Thr-Gly-OBzl (**3**)[5] (458 mg, 1 mmol) in pyridine (5 ml) at 0 °C. After being stirred for 12 h, the reaction mixture was concentrated *in vacuo*. The residue was dissolved in ethyl acetate and the solution was washed with 10% citric acid, 1 M sodium hydrogencarbonate and water, dried over Na_2SO_4, and concentrated *in vacuo*. The residual product was crystallized from ethyl acetate–ether–hexane (428 mg). Found: C, 59.96; H, 5.73; N, 8.36%. The results are summarized in Table 1.

*Z-*D*-Gly(ph)-*L*-Thr(OAc)-Gly-OBzl (6).* *From 2*: Compound **6** was obtained in the same way as described above from Z-D-Gly(ph)-3-Me-Azy-Gly-OBzl (**2**)[3b] (515 mg, 1 mmol) and acetic acid (3 ml). Compound **6** was crystallized from ethyl acetate (511 mg). Found: C, 63.09; H, 5.86; N, 7.16%. Calcd for $C_{31}H_{33}O_8N_3 \cdot H_2O$: C, 62.72; H, 5.94; N, 7.08%.

From 4: Compound **6** was obtained in the same way as described above from Z-D-Gly(ph)-L-Thr-Gly-OBzl (**4**)[6] (267 mg, 0.5 mmol) and acetic anhydride (100 mg, 1 mmol) in pyridine (3 ml). Compound **6** was crystallized from ethyl acetate–ether (231 mg). Found: C, 62.67; H, 5.87; N, 7.01 %. The results are summarized in Table 1.

Z-Gly—

Synthesis of Threonine O-*Peptide Derivatives. Z-Gly-*L-*Thr-Gly-OBzl (7a).* Via *Route A*: A mixture of **1** (45 mg, 0.1 mmol) and Z-Gly-OH (45 mg, 0.22 mmol) was heated at 110 °C in an oil bath. After being heated for 2 h, the reaction mixture was dissolved in ethyl acetate. The solution was washed with 1 M sodium hydrogencarbonate and water, dried over Na_2SO_4, and concentrated *in vacuo*. The residual product was crystallized from ethyl acetate–ether–hexane (63 mg). Found: C, 61.07; H, 5.43; N, 8.52%. Calcd for $C_{33}H_{36}O_{10}N_4$: C, 61.10; H, 5.59; N, 8.64%.

Via *Route B*: CDI (194 mg, 1.2 mmol) was added with stirring to a solution of Z-Gly-OH (250 mg, 1.2 mmol) in THF (5 ml) at −10 °C. After 1 h, a solution of **3** (320 mg, 0.7 mmol) in dioxane (5 ml) was added to the reaction mixture with stirring at 0 °C. After being stirred for 4 h, the solvent was removed *in vacuo*. The residual product was dissolved in ethyl acetate and the solution was washed with 1 M sodium hydrogencarbonate and water, dried over Na_2SO_4, and concentrated *in vacuo*. The residual product was purified by column chromatography on silica gel in benzene–ethyl acetate (1: 1 v/v). Compound **7a** was crystallized from ethyl acetate–ether–hexane (99 mg). Found: C, 60.95; H, 5.54; N, 8.54%. The results are summarized in Table 2.

*Boc-*L*-Leu—*

*Z-Gly-*L*-Thr-Gly-OBzl (7b).* Via *Route A*: A mixture of **1** (104 mg, 0.24 mmol) and Boc-L-Leu-OH (166 mg, 0.72 mmol) was heated at 85 °C for 2 h, and then worked up as described for **7a**. The residual oily product was purified by

column chromatography on silica gel in $CHCl_3$–ethyl acetate (1: 1 v/v) to give an amorphous powder (146 mg). Found: C, 59.97; H, 6.92; N, 8.31%. Calcd for $C_{36}H_{46}O_{10}N_4 \cdot H_2O$: C, 60.66; H, 6.79; N, 7.86%.

Via *Route B*: CDI (1.49 g, 9.20 mmol) was added with stirring to a solution of Boc–L-Leu–OH (1.94 g, 8.37 mmol) in THF (7 ml) at −10 °C. After 1 h, compound **3** (2.74 g, 6.0 mmol) in DMF (5 ml) was added to the reaction mixture with stirring at 0 °C. After being stirred for 3 days, it was worked up as described for **7a**. The residual product was purified by column chromatography on silica gel in $CHCl_3$–ethyl acetate (1: 1 v/v) to give an amorphous powder (646 mg). Found: C, 60.51; H, 7.13; N, 8.44%. The results are summarized in Table 2.

Boc–L-Pro–

*Z–Gly–L-Thr–Gly–OBzl (**7c**).* Via *Route A*: A mixture of **1** (659 mg, 1.5 mmol) and Boc–L-Pro–OH (1.29 mg, 6.0 mmol) was heated at 125 °C for 3 h, and then worked up as described for **7a**. The residual product was purified by column chromatography on silica gel in $CHCl_3$–ethyl acetate (1: 1 v/v) to give an amorphous powder (906 mg). Found: C, 59.66; H, 6.56; N, 8.19%. Calcd for $C_{33}H_{42}O_{10}N_4 \cdot 1/2H_2O$: C, 59.72; H, 6.53; N, 8.44%. The results are summarized in Table 2.

Boc–L-Phe–

*Z–Gly–L-Thr–Gly–OBzl (**7d**).* Via *Route A*: A mixture of **1** (220 mg, 0.5 mmol) and Boc–L-Phe–OH (531 mg, 2 mmol) was heated at 100 °C for 3 h, and then worked up as described for **7a**. Compound **7d** was crystallized from ethyl acetate–ether–hexane (260 mg). Found: C, 61.15; H, 6.35; N, 7.38 %. Calcd for $C_{37}H_{44}O_{10}N_4 \cdot H_2O$: C, 61.48; H, 6.42; N, 7.75 %. The results are summarized in Table 2.

Z–L-Ser–

*Z–Gly–L-Thr–Gly–OBzl (**7e**).* Via *Route A*: A mixture of **1** (800 mg, 1.82 mmol) and Z–L-Ser–OH (1.74 g, 7.28 mmol) was heated at 96 °C for 4 h, and then worked up as described for **7a**. Compound **7e** was crystallized from hot ethyl acetate (1.05 g). Found: C, 59.01; H, 5.64; N, 8.05%. Calcd for $C_{34}H_{38}O_{11}N_4 \cdot 1/2H_2O$: C, 59.36; H, 5.72; N, 8.51%. The results are summarized in Table 2.

Boc–L-Met(O)–

*Z–Gly–L-Thr–Gly–OBzl (**7f**).* Via *Route A*: A mixture of **1** (800 mg, 1.82 mmol) and Boc–L-Met(O)–OH (1.93 g, 7.28 mmol) was heated at 100 °C for 4 h, and then worked up as described for **7a**. The residual product was purified by column chromatography on silica gel in ethyl acetate–MeOH–$CHCl_3$ (1: 1: 8 v/v) to give an amorphous powder (1.22 g). Found: C, 55.11; H, 6.26; N, 7.73; S, 4.31%. Calcd for $C_{33}H_{43}O_{11}N_4S \cdot 1/2H_2O$: C, 55.53; H, 6.35; N, 7.85; S, 4.49%. The results are summarized in Table 2.

Boc–L-Glu(OBzl)–

*Z–Gly–L-Thr–Gly–OBzl (**7g**).* Via *Route A*: A mixture of **1** (300 mg, 0.68 mmol) and Boc–L-Glu(OBzl)–OH (922 mg, 2.73 mmol) was heated at 100 °C for 4 h, and then worked up as described for **7a**. The residual product was purified by column chromatography on silica gel in $CHCl_3$–ethyl acetate (1: 1 v/v) to give an amorphous powder (439 mg). Found: C, 61.72; H, 6.30; N, 7.35%. Calcd for $C_{40}H_{48}O_{12}N_4$: C, 61.84; H, 6.23; N, 7.21%. The results are summarized in Table 2.

Z–L-MeVal–

*Z–Gly–L-Thr–Gly–OBzl (**7h**).* Via *Route A*: A mixture of **1** (300 mg, 0.68 mmol) and Z–L-MeVal–OH (724 mg, 2.73 mmol) was heated at 110 °C for 4 h, and then worked up as described for **7a**. Compound **7h** was crystallized from ethyl acetate–ether–hexane (446 mg). Found: C, 63.11; H, 6.85; N, 8.02%. Calcd for $C_{37}H_{44}O_{10}N_4$: C, 63.05; H, 6.29; N, 7.95%. The results are summarized in Table 2.

Boc–L-Leu–L-Leu–

*Z–Gly–L-Thr–Gly–OBzl (**7i**).* Via *Route A*: A mixture of **1** (300 mg, 0.68 mmol) and Boc–L-Leu–L-Leu–OH (941 mg, 2.73 mmol) was heated at 115 °C for 4 h, and then worked up as described for **7a**. The residual product was purified by column chromatography on silica gel in $CHCl_3$–ethyl acetate (1: 1 v/v) to give an amorphous powder (426 mg). Found: C, 60.70; H, 7.50; N, 8.30%. Calcd for $C_{40}H_{57}O_{11}N_5 \cdot 1/2H_2O$: C, 60.59; H, 7.37; N, 8.83%.

Via *Route B*: CDI (135 mg, 0.83 mmol) was added with stirring to a solution of Boc–L-Leu–L-Leu–OH (236 mg, 0.69 mmol) in THF (3 ml) at −10 °C. After 95 min, compound **3** (158 mg, 0.35 mmol) in THF (3 ml) was added to the reaction mixture with stirring at 0 °C. After 7 days, it was worked up as described for **7a**. The residual product was purified by column chromatography on silica gel in $CHCl_3$–ethyl acetate (1: 1 v/v) to give an amorphous powder (48 mg). Found: C, 61.12; H, 7.67; N, 8.87%. Calcd for $C_{40}H_{57}O_{11}N_5$: C, 61.28; H, 7.33; N, 8.93%.

Via *Route B*: Compound **3** (915 mg, 2 mmol) and Boc–L-Leu–L-Leu–OH (689 mg, 2 mmol) were combined with DCC (413 mg, 2.2 mmol) in absolute pyridine (8 ml) at −10 °C for 3 days. Isolation and washing procedure gave an oil, which was purified by column chromatography on silica gel in $CHCl_3$–ethyl acetate (1: 1 v/v) to give an amorphous powder (23.6 mg). Found: C, 58.67; H, 7.12; N, 7.74%. Calcd for $C_{40}H_{57}O_{11}N_5 \cdot H_2O$: C, 59.91; H, 7.42; N, 8.73%. The results are summarized in Table 2.

Boc–L-Pro–Sar–

*Z–Gly–L-Thr–Gly–OBzl (**7j**).* Via *Route A*: A mixture of **1** (55 mg, 0.13 mmol) and Boc–L-Pro–Sar–OH (143 mg, 0.5 mmol) was heated at 85 °C for 4 h, and then worked up as described for **7a**. Compound **7j** was crystallized from $CHCl_3$–ether–hexane (83.3 mg). Found: C, 58.27; H, 6.50; N, 9.15%. Calcd for $C_{36}H_{47}O_{11}N_5 \cdot H_2O$: C, 58.27; H, 6.64; N, 9.42%. The results are summarized in Table 2.

References

1) Part I; K. Nakajima, F. Takai, T. Tanaka, and K. Okawa, *Bull. Chem. Soc. Jpn.*, **51**, 1577 (1978).

2) K. Okawa, T. Kinutani, and K. Sakai, *Bull. Chem. Soc. Jpn.*, **41**, 1353 (1968).

3) a) Abbreviations according to IUPAC-IUB commission, *J. Biol. Chem.*, **247**, 977 (1972), are used. Z: benzyloxycarbonyl, Boc: *t*-butoxycarbonyl, Bzl: benzyl ester, CDI: *N,N'*-Carbonyldiimidazole, DCC: dicyclohexylcarbodiimide Gly(ph): α-phenylglycine. "Azyline" is used as a name of an 2-aziridinecarboxylic acid, "Azy" being its abbreviation. 3-Me–Azy: 3-methyl-2-aziridinecarboxylic acid. b) K. Okawa, K. Nakajima, T. Tanaka, and Y. Kawana, *Chem. Lett.*, **1975**, 591.

4) The standard sample of **7i** was prepared in the following way: deprotection of Boc group of **7b** by TFA, followed by coupling with Boc–L-Leu–OH using DCC: an amorphous powder, $[\alpha]_D^{23}$ −27.4° (*c* 0.9, MeOH). Found: C, 60.44; H, 7.33; N, 9.02%. Calcd for $C_{40}H_{57}O_{11}N_5 \cdot 1/2H_2O$: C, 60.59; H, 7.37; N, 8.83%.

5) Y. Nakagawa, T. Tsuno, K. Nakajima, M. Iwai, H. Kawai, and K. Okawa, *Bull. Chem. Soc. Jpn.*, **45**, 1162 (1972).

6) K. Nakajima, H. Kawai, M. Takai, and K. Okawa, *Bull. Chem. Soc. Jpn.*, **50**, 917 (1977).