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Studies on Hydroxy Amino Acids. IV.1) Syntheses of Several Peptides Containing Aziridinecarboxylic Acid Derived from the Corresponding Hydroxy Amino Acid Derivatives

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The β-elimination reaction of the *O*-tosyl β-hydroxy amino acid peptide derivative was thoroughly investigated. The *N*-tosyl-L-serylglycine ester and the *N*-tosyl-L-threonylglycine ester gave the *N*-tosyl-L-aziridinecarbonylglycine ester and the *N*-tosyl-3-methyl-L-aziridinecarbonylglycine ester as the resulting products respectively by the elimination reaction. On the other hand, the *N*-carbobenzoxyglycyl-L-serylglycine ester was not converted into the aziridine peptide derivative; only the dehydroalanine peptide was obtained. However, the *N*-tosyl-L-phenylalanyl-L-threonylglycine ester and the *N*-carbobenzoxyglycyl-L-threonylglycine ester were converted into the corresponding aziridine peptide, and the *N*-tosyl-L-phenylalanyl-3-methyl-L-aziridinecarbonylglycine ester and the *N*-carbobenzoxyglycyl-3-methyl-L-aziridinecarbonylglycine ester were obtained respectively. It was concluded that the peptides containing threonine were easily converted into the aziridine peptides and not the serine peptides by the β-elimination reaction.

The typical β-elimination reactions of the *N*-acylated hydroxy amino acid esters, such as serine or threonine derivatives, usually gave dehydroalanine derivatives as the resulting products. For example, the *N*-carbobenzoxy-*O*-tosyl-L-serine ethyl ester (IIe) was converted into the *N*-carbobenzoxydehydroalanine ester (IIIe) by the elimination reaction described by Photaki.2) The above result was also supported by the present investigation; that here is, the *N*-carbobenzoxyglycyl-*O*-tosyl-L-serine ethyl ester (IIf) and the *N*-carbobenzoxyglycyl-*O*-tosyl-L-threonine ethyl ester (IIk) were also converted into the corresponding *N*-carbobenzoxyglycyldehydroamino acid ester derivatives (IIIf, IIIk).

In the case of the *N*-alkylated serine ester, however, the aziridine ring is formed by the elimination reaction, even though the carboxyl group is replaced by the ester group. For example, the *N*-trityl-L-aziridinecarboxylic acid methyl ester (IVa) was obtained from the *N*-trityl-*O*-mesyl-L-serine methyl ester (IIa), as has been described by Smrt.3)

On the other hand, as has been described in a previous paper4), an aziridinecarboxylic acid derivative, *N*-tosyl-3-methyl-aziridinecarboxylic acid anilide(IVi), was obtained instead of the dehydroalanine derivative from the *N*,*O*-ditosyl-L-threonine anilide(IIi). We will use "Azyline" as the name of aziridinecarboxylic acid and "Azy" as an abbreviation of azyline.

In the present investigation, we have systematically tried to clarify the structural influences of the substituent group on the formation of the aziridine ring by the elimination reaction of the hydroxy amino acid peptide derivatives, as is shown in Table 1. As the first experiments, the substituent effects of the ester and amide groups of *N*-tosylated hydroxy amino acid in the elimination reaction were investigated. It was found that, the *N*,*O*-ditosyl-L-serine ethyl ester (IIb) was converted into the *N*-tosyldehydroalanine ester (IIIb), while the aziridine derivative was not obtained at all. On the other hand, *N*,*O*-ditosyl-L-serylglycine esters (IIc, IId), in which carboxyl groups of serine were protected by the amide group, gave only aziridine peptides derivatives (IVc, IVd).

In the case of the threonine derivatives, however, the *N*,*O*-ditosyl-L-threonine ethyl ester (IIh) was converted into both the *N*-tosyl-3-methyl-L-azylylglycine ester (IVh) and the *N*-tosyl-α-amino-crotonic acid ester (IIIh).

On the other hand, in the case of the amide derivative of threonine, the *N*-tosyl-3-methyl-L-azylylglycine ethyl ester (IVj) was obtained from the *N*,*O*-ditosyl-L-threonylglycine ethyl ester (IIj), as had been expected on the basis of the results of a previous investigation.

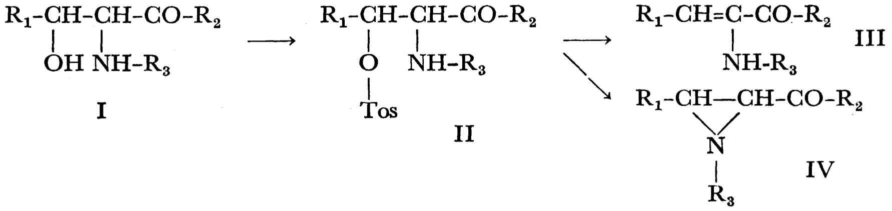

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TABLE 1. REACTION PRODUCTS BY THE β-ELIMINATION REACTION OF THE *O*-TOSYLATED HYDROXY AMINO ACID DERIVATIVES.[a]

I, II	Protecting groups			Products (Yield%)	
	R_1	R_2	R_3	III	IV
a	H	-OMe	Tri-	—	80.2[b]
b	H	-OEt	Tos-	90	—
c	H	-Gly-OEt	Tos-	—	80.6
d	H	-Gly-OMe	Tos-	—	80
e	H	-OEt	Z-	63[c]	—
f	H	-OEt	Z-Gly-	95	—
g	H	-Gly-OEt	Z-Gly-	67	—
h	CH_3	-OEt	Tos-	29	64
i	CH_3	-NH-C_6H_5	Tos-	—	65[d]
j	CH_3	-Gly-OEt	Tos-	—	96 (oil)
k	CH_3	-OEt	Z-Gly-	87.5	—
l	CH_3	-Gly-OBzl	Z-Gly-	—	80
m	CH_3	-Gly-OEt	Tos-L-Phe-	—	60

a) Elimination reactions were carried out in tetrahydrofuran solution in the presence of triethylamine, but some of the cases, benzene-methanol or acetonitrile was used as the solvent and *N*-ethylpiperidine was used as the base.
b) *N*-Tri-*O*-Mesyl-L-serine methyl ester is used as the starting material in literature.[3]
c) This value shows the yield of the free acid in literature.[4]
d) This result was published in the previous paper.[4]

As the next experiments, the substituent effects of the *N*-acyl masking group of the hydroxy amino acid amide derivatives were investigated. It was found that the *N*-carbobenzoxyglycyl-*O*-tosyl-L-serylglycine ethyl ester (IIg) was converted into the corresponding *N*-carbobenzoxyglycyldehydroalanylglycine ethyl ester (IIIg), but no aziridine derivatives were obtained. However, the *N*-carbobenzoxyglycyl-*O*-tosyl-L-threonylglycine benzyl ester (IIl) and the *N*-tosyl-L-phenylalanyl-L-threonylglycine ethyl ester (IIm) were converted into the *N*-carbobenzoxyglycyl-3-methyl-L-azylylglycine benzyl ester (IVl) and *N*-tosyl-L-phenylalanyl-3-methyl-L-azylylglycine ethyl ester (IVm). These were the first synthetic peptides in which aziridinecarboxylic acid was placed at the middle position in the peptide linkage.

On the basis of the above experiments, it was concluded that threonine peptides and their derivatives (R_1=-CH_3) are relatively more easily converted into the corresponding aziridine derivatives than the serine peptides derivatives (R_1=-H). The replacement of the ester group (R_2) of the hydroxy amino acid by an amide group, such as the anilide or glycine ester, increased the tendency of the ring formation. The aminoacyl group (R_3) did not promote the tendency of the ring formation; only aziridine peptides derivatives were synthesized in the case of threonine peptipdes (R_1=-CH_3).

It is a matter of course that the aziridine derivatives preserved their optical activity throughout the β-elimination reaction, unlike the dehydroamino acid derivatives, which did not show any optical activity at all. The X-ray crystallographic analysis of the compound, *N*-tosyl-L-azylylglycine, also supported the above results; that is, the original configuration on the α-carbon atom was retained during the elimination procedure. (Fig. 1). The results of the X-ray crystallographic analysis will be published in This Bulletin soon.

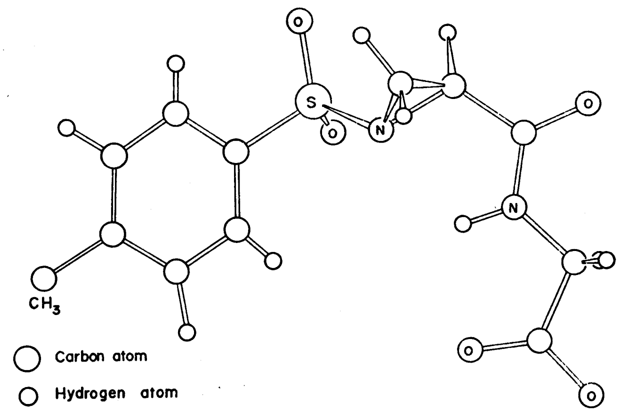

Fig. 1. The molecular structure of *N*-tosyl-L-azylylglycine as determined by X-ray analysis.

The *O*-tosylations of hydroxy amino acid derivatives were carried out in a dry pyridine solution with freshly-prepared *p*-toluenesulfonyl chloride at −5—−10°C. The *O*-tosylated products (II) thus obtained were gradually decomposed, especially in the solution, and were stored in a refrigerator.

The optimum conditions for a good yield of the aziridine derivative were also investigated by using the *N*,*O*-ditosyl-L-serylglycine ethyl ester (IIc). The results are summarized in Table 2.

The most satisfactory results were obtained when the solution of *N*,*O*-ditosyl derivatives in tetrahydrofuran was treated with 1.0—1.2 equivalent amounts of triethylamine at the refluxing temperature for a short time. Consequently, in the present investigation, tetrahydrofuran and triethylamine were mainly used as the solvent and the base respectively.

Table 2. The reaction condition and the yield of *N*-tosyl-L-azylylglycine ethyl ester (IVc)

Solvents[a]	Concentration of IIc (g/m*l*)	Bases[b]	Eq. amt. of bases	Reaction temp. (°C)	Reaction time	Yield (%)
THF	0.10	EP	1.0	38	10 hr	18
THF	0.10	EP	2.0	35	19 hr	15
THF	0.10	DEA	1.0	38	17 hr	18
THF	0.10	TEA	1.1	37	24 hr	27
CH_2Cl_2	0.16	TEA	1.2	37	16 hr	11
$CHCl_3$	0.16	TEA	1.1	37	16 hr	44
AcOEt	0.16	TEA	1.1	37	16 hr	46
MeCN	0.16	TEA	1.1	37	16 hr	50
THF	0.20	TEA	1.1	reflux	30 min	80
MeCN	0.16	TEA	1.0	reflux	30 min	81
DMSO	0.16	TEA	1.1	70	20 min	87

a) THF: tetrahydrofuran, DMSO: dimethyl sulfoxide. b) EP: *N*-ethylpiperidine, DEA: diethylamine, TEA: triethylamine.

Experimental

All the melting points are uncorrected. The NMR spectra were obtained with a Hitachi R-20B High Resolution NMR spectrometer, while the chemical shifts are given from TMS as the internal reference.

N-Tosyl-L-serine Ethyl Ester (Ib). Ib was prepared from *N*-tosyl-L-serine[5] (20.0 g, 77 mmol) by the method of Stevens[6] in a 92% (20.4 g) yield; mp 103—104°C. $[\alpha]_{\rm D}^{23}$ −8.2° (*c* 2.2, EtOH).

Found: C, 49.98; H, 5.96; N, 5.06; S, 11.45%. Calcd for $C_{12}H_{15}O_5N_5$: C, 50.16; H, 5.96; N, 4.88; S, 11.16%.

N-Tosyl-L-serylglycine Ethyl Ester (Ic). Into a solution of *N*-tosyl-L-serine hydrazide[5] (27.4 g, 0.10 mol) in acetic acid (11 m*l*) and N hydrochloric acid (200 m*l*), we added 3N sodium nitrite (50 m*l*), drop by drop, under cooling at −5—−10°C. After the solution had been stirred for 10 min, the product was extracted with ice-cold ethyl acetate (200 m*l*); the organic layer was neutralized with ice-cold saturated aqueous sodium bicarbonate and immediately cooled under −5°C. A solution of glycine ethyl ester hydrochloride[7] (41.9 g, 0.30 mol) and triethylamine (41.8 m*l*, 0.30 mol) in ethyl acetate (50 m*l*) was then added, drop by drop, to the azide solution. The stirring was continued for 4 hr at −5°C. After the reaction mixture had then been allowed to stand overnight in a refrigerator, the mixture was washed with N hydrochloric acid, N sodium bicarbonate, and water, and was dried over anhydrous sodium sulfate. The solution was concentrated under reduced pressure. The Ic produced was collected and recrystallized from ethyl acetate. The crystals were obtained in an 85% yield (29.3 g); mp 108—109°C. $[\alpha]_{\rm D}^{23}$ −24.3° (*c* 2.0, EtOH).

Found: C, 48.74; H, 5.75; N, 8.19; S, 9.16%. Calcd for $C_{14}H_{20}O_6N_2S$: C, 48.82; H, 5.85; N, 8.14; S, 9.31%.

N-Tosyl-L-serylglycine Methyl Ester (Id). Id was prepared from *N*-tosyl-L-serine hydrazide[5] (27.4 g, 0.10 mol) and the glycine methyl ester (from hydrochloride[8] (37.8 g, 0.30 mol)) by the above azide procedure in an 84% (28.9 g) yield; mp 107—108°C. $[\alpha]_{\rm D}^{23}$ −20.7° (*c* 2.0, EtOH).

Found: C, 47.07; H, 5.51; N, 8.45; S, 9.93%. Calcd for $C_{13}H_{18}O_6N_2S$: C, 47.26; H, 5.49; N, 8.48; S, 9.71%.

N-Carbobenzoxyglycyl-L-serine Ethyl Ester (If). *N*-Carbobenzoxyglycine hydrazide[9] (22.3 g, 0.10 mol) was coupled with the L-serine ethyl ester (from hydrochloride[10] (17.0 g, 0.10 mol)) in ethyl acetate by the above azide procedure. Recrystallization from hot ethyl acetate gave a pure material (24.8 g (76.4%)); mp 112.5°C. $[\alpha]_{\rm D}^{23}$ +17.0° (*c* 2.1, dioxane).

Found: C, 55.36; H, 6.16; N, 8.59%. Calcd for $C_{15}H_{20}O_6N_2$: C, 55.55; H, 6.21; N, 8.64%.

N-Tosyl-L-threonine Ethyl Ester (Ih). Ih was prepared from *N*-tosyl-L-threonine[11] (109 g, 0.4 mol) by the method of Brenner[12] in a 98% (118 g) yield; mp 106°C. $[\alpha]_{\rm D}^{23}$ −4.4° (*c* 2.0, MeOH).

Found: C, 52.05; H, 6.55; N, 4.92; S, 10.52%. Calcd for $C_{13}H_{19}O_5NS$: C, 51.81; H, 6.35; N, 4.65; S, 10.64%.

N-Tosyl-L-threonylglycine Ethyl Ester (Ij). *N*-Tosyl-L-threonine hydrazide[12] (14.41 g, 50 mmol) was coupled with the glycine ethyl ester (from hydrochloride[7] (20.9 g, 150 mmol)) by the above azide procedure in an 84% (15.0 g) yield. Recrystallization from ethyl acetate–*n*-hexane gave a pure material; mp 116—116.5°C. $[\alpha]_{\rm D}^{23}$ −23.2° (*c* 2.0, MeOH).

Found: C, 50.02; H, 5.86; N, 7.91; S, 9.05%. Calcd for $C_{15}H_{22}O_6N_2S$: C, 50.26; H, 6.19; N, 7.82; S, 8.95%.

N-Carbobenzoxyglycyl-L-threonine Hydrazide. The *N*-carbobenzoxyglycyl-L-threonine ethyl ester[13] (30 g, 88 mmol) was dissolved in 80% hydrazine hydrate (37.3 g, 0.62 mol) in absolute methanol (40 m*l*). After the reaction mixture had been allowed to stand overnight in a refrigerator, a crude precipitate was collected and recrystallized from hot methanol. The yield was 27.9 g (97.5%); mp 172—173°C.

N-Carbobenzoxyglycyl-L-threonylglycine Benzyl Ester (Il). *N*-Carbobenzoxyglycyl-L-threonine hydrazide (27.4 g, 84 mmol) was coupled with the glycine benzyl ester (from tosylate[14] (26.4 g, 0.168 mol)) by the above azide procedure. Recrystallization from hot methanol gave a pure material;

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27.8 g (72%); mp 156—157°C. $[\alpha]_D^{23}$ −15.7° (*c* 2.0, dioxane).

Found: C, 60.35; H, 6.03; N, 9.34%. Calcd for $C_{23}H_{27}O_7N_3$: C, 60.38; H, 5.95; N, 9.19%.

N-*Tosyl*-L-*phenylalanyl*-L-*threonine Hydrazide*. The *N*-tosyl-L-phenylalanyl-L-threonine ethyl ester was prepared from *N*-tosyl-L-phenylalanine[15] and L-threonine ethyl ester hydrochloride[16] by the succinimide-ester method,[17] and was directly used for the next step without purification. The crude acyl peptide ester (15.9 g, 35 mmol) was treated with 80% hydrazine hydrate (43 m*l*) in methanol (20 m*l*) at room temperature for 44 hr. The crystals which appeared were collected and recrystallized from hot methanol in a 75% yield (from the ester) (11.4 g); mp 193°C.

Found: C, 55.48; H, 6.10; N, 12.62; S, 7.40%. Calcd for $C_{20}H_{26}O_5N_4S$: C, 55.28; H, 6.03; N, 12.89; S, 7.38%.

N-*Tosyl*-L-*phenylalnyl*-L-*threonylglycine Ethyl Ester (Im)*. *N*-Tosyl-L-phenylalanyl-L-threonine hydrazide (11.3 g, 26 mmol) was coupled with the glycine ethyl ester (from hydrochloride[7]) (10.5 g, 75 mmol) by the above azide procedure. The yield of the crude product was 63.5% (8.4 g). Recrystallization from hot ethyl acetate–*n*-hexane gave a pure material; mp 162—163°C. $[\alpha]_D^{23}$ −65.3° (*c* 1.0, EtOH).

Found: C, 57.20; H, 6.20; N, 8.44; S, 6.53%. Calcd for $C_{23}H_{31}O_7N_3S$: C, 57.01; H, 6.18; N, 8.31; S, 6.34%.

O-*Tosylation of Hydroxy Amino Acid Derivatives.*

N,O-*Ditosyl*-L-*serine Ethyl Ester (IIb)*. *p*-Toluenesulfonyl chloride (28.6 g, 105 mmol) in dry pyridine (45 m*l*) was stirred, drop by drop, into a solution of Ib (21.5 g, 75 mmol) in pyridine (150 m*l*) at −10°C over a 30-min period. The mixture was stirred for 1 hr at 0°C and then for an additional several hours at room temperature. The reaction mixture was poured into crushed ice (500 g) and allowed to stand overnight in a refrigerator. The precipitated products were dissolved in ethyl acetate, washed with water, and dried over anhydrous sodium sulfate. After the solution had been evaporated under reduced pressure, IIh was obtained as white crystals. Recrystallization from ethyl acetate-*n*-hexane gave pure crystals in a 52% (17 g) yield; mp 117—118°C. $[\alpha]_D^{23}$ +21.7° (*c* 1.0, MeOH).

Found: C, 51.64; H, 5.26; N, 3.04; S, 14.18%. Calcd for $C_{19}H_{23}O_7NS_2$: C, 51.68; H, 5.25; N, 3.17; S, 14.53%.

N,O-*Ditosyl*-L-*serylglycine Ethyl Ester (IIc)*. IIc was prepared from Ic (10.3 g, 30 mmol) and *p*-toluenesulfonyl chloride (11.4 g, 50 mmol) in a dry pyridine (30 m*l*) solution by the above tosylation procedure. The crude product was obtained in a 92% (13.8 g) yield. The crude materials were recrystallized from ether-ethyl acetate; mp 107—108°C. $[\alpha]_D^{23}$ −2.7° (*c* 2.2, EtOH).

Found: C, 50.51; H, 5.15; N, 5.68; S, 12.76%. Calcd for $C_{21}H_{26}O_8N_2S_2$: C, 50.59; H, 5.26; N, 5.62; S, 12.86%.

N,O-*Ditosyl*-L-*serylglycine Methyl Ester (IId)*. IId was prepared from Id (10.3 g, 30 mmol) and *p*-toluenesulfonyl chloride (11.4 g, 50 mmol) in a dry pyridine solution (30 m*l*) by the above tosylation procedure in an 85% (12.7 g) yield; mp 114—115°C. $[\alpha]_D^{23}$ −4.8° (*c* 2.0, $CHCl_3$).

Found: C, 49.58; H, 4.97; N, 5.61; S, 13.24%. Calcd for $C_{20}H_{24}O_8N_2S_2$: C, 49.58; H, 4.99; N, 5.78; S, 13.23%.

N-*Carbobenzoxyglycyl*-O-*tosyl*-L-*serine Ethyl Ester (IIf)*. IIf was prepared from If (3.2 g, 10 mmol) and *p*-toluenesulfonyl chloride (3.8 g, 20 mmol) in dry pyridine (10 m*l*) by the above tosylation procedure in a 71.3% (3.5 g) yield. Recrystallization from ethyl acetate–ether gave a pure material; mp 109°—110°C. $[\alpha]_D^{23}$ +22.5° (*c* 2.0, dioxane).

Found: C, 54.97; H, 5.40; N, 5.79; S, 6.58%. Calcd for $C_{22}H_{26}O_8N_2S$: C, 55.22; H, 5.48; N, 1.85; S, 6.70%.

N,O-*Ditosyl*-L-*threonine Ethyl Ester (IIh)*. IIh was prepared from Ih (15.1 g, 50 mmol) and *p*-toluenesulfonyl chloride (19.1 g, 100 mmol) in dry pyridine (45 m*l*) by the above tosylation procedure. Recrystallization from ethyl acetate–*n*-hexane gave a pure material in a 71% (16.1 g) yield; mp 118—119°C. $[\alpha]_D^{23}$ +20.3° (*c* 2.0. MeOH).

Found: C, 52.62; H, 5.41; N, 3.01; S, 14.20%. Calcd for $C_{20}H_{25}O_7NS_2$: C, 52.73; H, 5.53; N, 3.08; S, 14.08%.

N,O-*Ditosyl*-L-*threonylglycine Ethyl Ester (IIj)*. IIj was prepared from Ij (17.9 g, 50 mmol) and *p*-toluenesulfonyl chloride (19.0 g, 100 mmol) in dry pyridine (30 m*l*) by the aobve tosylation procedure. Recrystallization from ethyl acetate–petroleum ether gave a pure material in an 85% (22.2 g) yield; mp 122—122.5°C. $[\alpha]_D^{23}$ +18.1° (*c* 2.0, MeOH).

Found: C, 51.36; H, 5.45; N, 5.31; S, 12.26%. Calcd for $C_{22}H_{28}O_8N_2S_2$: C, 51.55; H, 5.51; N, 5.47; S, 12.51%.

N-*Carbobenzoxyglycyl*-O-*tosyl*-L-*threonine Ethyl Ester (IIk)*. IIk was prepared from the *N*-carbobenzoxyglycyl-L-threonine ethyl ester[13] (3.4 g, 10 mmol) and *p*-toluenesulfonyl chloride (3.8 g, 20 mmol) in dry pyridine (10 m*l*) by the above tosylation procedure. When the crude products were recrystallized from ether, fine crystals were obtained in a 71.4% (3.5 g) yield; mp 79—80°C. $[\alpha]_D^{23}$ +21.3° (*c* 1.0, EtOH).

Found: C, 55.93; H, 5.74; N, 5.70; S, 6.43%. Calcd for $C_{23}H_{28}O_8N_2S$: C, 56.08; H, 5.73; N, 5.69; S, 6.51%.

N-*Carbobenzoxyglycyl*-O-*tosyl*-L-*threonylglycine Benzyl Ester (IIl)*. IIl was prepared from Il (4.6 g, 10 mmol) and *p*-toluenesulfonyl chloride (3.8 g, 20 mmol) in dry pyridine (25 m*l*) by the above tosylation procedure. Recrystallization from ethyl acetate–ether gave a pure material in a 77.6% (4.8 g) yield; mp 115—116°C. $[\alpha]_D^{23}$ −11.8° (*c* 1.0, THF).

Found: C, 58.83; H, 5.41; N, 6.78; S, 4.95%. Calcd for $C_{30}H_{34}O_9N_3S$: C, 58.83; H, 5.59; N, 6.86; S, 5.23%.

N-*Tosyl*-L-*phenylalanyl*-O-*tosyl*-L-*threonylglycine Ethyl Ester (IIm)*. IIm was obtained from Im (7.8 g, 15.5 mmol) and *p*-toluenesulfonyl chloride (5.9 g, 31 mmol) in dry pyridine (24 m*l*) by the above tosylation procedure. Recrystallization from ethanol–ether gave a pure material in a 52% (5.3 g) yield; mp 121.3—122.5°C. $[\alpha]_D^{23}$ +7.9° (*c* 1.0, THF).

Found: C, 56.44; H, 5.69; N, 6.33; S, 9.47%. Calcd for $C_{31}H_{37}O_9N_3S_2$: C, 56.43; H, 5.65; N, 6.37; S, 9.72%.

β-Eliminations of O-*tosylated Hydroxy Amino Acid Derivatives.* N-*Tosyldehydroalanine Ethyl Ester(IIIb)*. A solution of IIb (2.2 g, 5 mmol) in tetrahydrofuran (18 m*l*) and triethylamine (0.7 m*l*, 5 mmol) was stirred for 9 hr at 35°C and then for 18 hr at room temperature. The reaction mixture was evaporated under reduced pressure, and the residue was dissolved in benzene; this benzene solution was washed with water three times, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residual oily material was crystallized by scratching and then recrystallized from ether–petroleum ether; a pure material was obtained in a 90% (1.2 g) yield; mp 73—75.5°C.

Found: C, 52.69; H, 5.44; N, 5.40; S, 12.12%. Calcd for $C_{12}H_{15}O_4NS$: C, 53.51; H, 5.61; N, 5.20; S, 11.91%. NMR (DMSO-d_6): δ 5.33 ppm (1H(s) CH_2=), 5.52 ppm (1H(s) CH_2=).

N-*Tosyl*-L-*azylylglycine Ethyl Ester (IVc)*. A solution of IIc (5.0 g, 10 mmol) and triethylamine (1.4 m*l*, 10 mmol) in tetrahydrofuran (30 m*l*) was refluxed for 30 min. After the removal of the solvent, water was added to the residue.

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15) E. A. Popenoe and V. du Vigneaud, *J. Amer. Chem. Soc.*, **76**, 6202 (1954).

16) D. F. Elliott, *J. Chem. Soc.*, **1949**, 589.

17) G. W. Anderson, J. E. Zimmerman, and F. M. Callakan, *J. Amer. Chem. Soc.*, **86** 1839 (1964).

The reddish-brown mass was then dissolved in chloroform. The solution was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residue was recrystallized from chloroform-ether in an 80.6% (2.6 g) yield; mp 147—148°C. $[\alpha]_D^{23}$ −59.1° (*c* 2.3, dioxane).

Found: C, 51.76; H, 5.74; N, 8.58; S, 9.82%. Calcd for $C_{14}H_{18}O_5N_2S$: C, 51.52; H, 5.56; N, 8.58; S, 9.84%. NMR ($CDCl_3$): δ 2.43 ppm (1H(d), J_{trans}=4.2 Hz Azy; $-CH_2-$, 2.77 ppm (1H(d), J_{cis}=7.7 Hz Azy; $-CH_2-$), 3.32 ppm (1H(q), J_{trans}=4.2 Hz, J_{cis}=7.7 Hz Azy; α-proton).

N-*Tosyl*-L-*azylylglycine Methyl Ester (IVd).* IVd was obtained from IId (4.8 g, 10 mmol) and triethylamine (1.4 m*l*, 10 mmol) in tetrahydrofuran (40 m*l*) by the above elimination reaction procedure (IVc). Recrystallization from chloroform–ether gave a pure material in an 80% (2.6 g) yield; mp 116—117°C. $[\alpha]_D^{23}$ −55.7° (*c* 2.0, dioxane).

Found: C, 49.95; H, 5.23; N, 8.76; S, 10.49%. Calcd for $C_{13}H_{16}O_5N_2S$: C, 49.99; H, 5.16; N, 8.97; S, 10.27%.

N-*Carbobenzoxyglycyldehydroalanine Ethyl Ester (IIIf).* IIIf was prepared from IIf (2.4 g, 5 mmol) and triethylamine (1.4 m*l*, 10 mmol) in tetrahydrofuran (20 m*l*) by the above elimination reaction procedure (IVc). Recrystallization from ethyl acetate–ether gave a pure material in a 95% (1.5 g) yield; mp 81—81.5°C.

Found: C, 58.93; H, 5.96; N, 9.15%. Calcd for $C_{15}H_{18}O_5N_2$: C, 58.82; H, 5.92; N, 9.15%.

NMR ($CDCl_3$): δ 5.87 ppm (1H(d), J_{gem}=1.2 Hz, CH_2=), 6.53 ppm (1H(s), CH_2=).

N-*Carbobenzoxyglycyldehydroalanylglycine Ethyl Ester (IIIg).* A solution of the *N*-carbobenzoxyglycyl-*O*-tosyl-L-serylglycine ethyl ester[18] (5.4 g, 10 mmol) and *N*-ethylpiperidine (1.1 g, 10 mmol) in acetonitrile was incubated at 37°C for 24 hr. The solvent was then removed under reduced pressure, and the residue was dissolved in ethyl acetate. The solution was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The resulting product was crystallized from ethyl acetate–ether. IIIg was thus obtained in a 67% (2.4 g) yield; mp 130—132°C.

Found: C, 56.07; H, 5.87; N, 11.53%. Calcd for $C_{17}H_{21}O_6N_3$: C, 56.19; H, 5.83; N, 11.56%.

NMR (DMSO-d_6): δ 5.58 ppm (1H(*q*), *J*=0.7, *J*=1.1 Hz, CH_2=), 6.25 ppm (1H(d), *J*=0.7 Hz, CH_2=).

N-*Tosyl*-α-*amino-crotonic Acid Ethyl Ester (IIIh).* N-*Tosyl-3-methyl*-L-*azyline Ethyl Ester (IVh).* Into a solution of IIh (9.1 g, 20 mmol) in benzene–methanol (50 : 1 v/v, 51 m*l*), we added *N*-ethylpiperidine (2.76 m*l*, 20 mmol). The solution was refluxed for 8.5 hr and then allowed to stand overnight. The reaction mixture was washed with water three times, dried over anhydrous sodium sulfate, and concentrated under reduced pressure; when the crystals thus produced were filtered off, IIIh (1.23 g) was obtained. The mother solution was chromatographed on silica gel (150 g) with benzene-chloroform (1 : 1 v/v) to yield the reaction products, IIIh and IVh respectively. The R_f values of IIIh and IVh were 0.24 (0.42 g) and 0.46 (3.62 g) respectively. The collected crystals of IIIh amounted to 1.65 g (29%); they were recrystallized from ethyl acetate–*n*-hexane; mp 93—94°C.

Found: C, 55.37; H, 6.00; N, 4.89; S, 11.23%. Calcd for $C_{13}H_{17}O_4NS$: C, 55.10; H, 6.05; N, 4.94; S, 11.32%.

NMR ($CDCl_3$): δ 2.02 ppm (3H(d), *J*=7.2 Hz, CH_3-CH=), 6.93 ppm (1H(q), *J*=7.2 Hz, CH_3-CH=). IVh was obtained in a 64% (3.62 g) yield; mp 56—56.5°C. $[\alpha]_D^{23}$ −40.2° (*c* 2.0, MeOH).

Found: C, 55.12; H, 6.01; N, 4.88; S, 11.52%. Calcd for $C_{13}H_{17}O_4NS$: C, 55.10; H, 6.05; N, 4.94; S, 11.32%.

NMR ($CDCl_3$): δ 1.31 ppm (3H(d), *J*=5.5 Hz Azy; CH_3-CH-), 3.13 ppm (1H(q), *J*=5.5, J_{cis}=7.5 Hz Azy; CH_3-CH-), 3.36 ppm (1H(d), *J*=7.5 Hz Azy; α-proton).

N-*Tosyl-3-methyl*-L-*azylylglycine Ethyl Ester (IVj).* The solution of triethylamine (2.8 m*l*, 20 mmol) and IIj (10.3 g, 20 mmol) in tetrahydrofuran was incubated at 50°C for 25 hr. The solvent was removed under reduced pressure, and the residue was dissolved in ethyl acetate. The solution was washed with water and dried over anhydrous sodium sulfate. After the evaporation of the solvent under reduced pressure, an oily product (IVj) was obtained in a 96% (7.0 g) yield. All efforts at crystallization failed.

N-*Tosyl-3-methyl*-L-*azylylglycine.* A solution of IVj (7.0 g) in acetonitrile (15 m*l*) and water (15 m*l*) was treated with N sodium hydroxide (22 m*l*) at 5°C for 1 hr and then allowed to stand at room temperature for 4 hr. After the acetonitrile had been removed under reduced pressure, the reaction mixture was acidified with 1 M citric acid (22 m*l*). The crystals which appeared were collected (6.0 g, 96%) and recrystallized from ethyl acetate–*n*-hexane (5.6 g); mp 138—139°C. $[\alpha]_D^{23}$ −52.3° (*c* 2.1, MeOH).

Found: C, 50.20; H, 4.97; N, 8.78; S, 10.29%. Calcd for $C_{13}H_{16}O_5N_2S$: C, 49.99; H, 5.16; N, 8.97; S, 10.26%.

NMR ($CDCl_3$): δ 1.23 ppm (3H(d), *J*=5.5 Hz Azy; CH_3-CH-), 3.02 ppm (1H(q), *J*=5.5, J_{cis}=7.5 Hz Azy; CH_3-CH-), 3.40 ppm (1H(d), J_{cis}=7.5 Hz Azy; α-proton).

N-*Carbobenzoxyglycyl*-α-*amino-crotonic Acid Ethyl Ester (IIIk).* A solution of IIk (3.5 g, 7.1 mmol) in tetrahydrofuran (15 m*l*) was treated with triethylamine (1.0 m*l*, 7.1 mmol) at 60°C for 4 hr, after which the solution was allowed to stand at room temperature for 15.5 hr. The solvent was then removed under reduced pressure; oily products were obtained and crystallized by the addition of *n*-hexane. IIIk was obtained in an 87.5% (2.1 g) yield; mp 86—88°C.

Found: C, 58.50; H, 6.33; N, 8.37%. Calcd for $C_{16}H_{20}O_5N_2\cdot 1/2\,H_2O$: C, 58.35; H, 6.43; N, 8.50%.

NMR ($CDCl_3$): δ 1.73 ppm (3H(d), *J*=6.8 Hz, CH_3-CH=), 6.79 ppm (1H(q), *J*=6.8 Hz, CH_3-CH=).

N-*Carbobenzoxyglycyl-3-methyl*-L-*azylylglycine Benzyl Ester (IVl).* A solution of IIIl (610 mg, 1 mmol) and triethylamine (0.28 m*l*, 2 mmol) in absolute tetrahydrofuran (10 m*l*) was refluxed for 20.5 hr. The solvent was then removed under reduced pressure, and the residue was dissolved in ethyl acetate. The solution was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residual oil was crystallized by the addition of isopropyl ether. Recrystallization from acetone–isopropyl ether gave pure material in an 80% (350 mg) yield; mp 114—116°C. $[\alpha]_D^{23}$ −35.7° (*c* 1.0, THF).

Found: C, 62.66; H, 5.71; N, 9.68%. Calcd for $C_{23}H_{25}O_6N_3$: C, 62.86; H, 5.73; N, 9.56%.

NMR (DMSO-d_6): δ 1.01 ppm (3H(d), *J*=6.3 Hz Azy; CH_3-CH-), 4.49 ppm (1H(m), Azy; CH_3-CH-), 4.80 ppm (1H(d), J_{cis}=11.9 Hz Azy; α-proton).

N-*Tosyl*-L-*phenylalanyl-3-methyl*-L-*azylylglycine Ethyl Ester (IVm).* A solution of IIIm (1.30 g, 2 mmol) and triethylamine (0.28 m*l*, 2 mmol) in tetrahydrofuran (8 m*l*) was incubated at 40—50°C for 53 hr. The solvent was then removed under reduced pressure, and the residue was dissolved in ethyl acetate. The solution was washed with water, dried over anhydrous sodium sulfate, and concentrated under reduced pressure. The residual oily material

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[18] I. Photaki and V. Bardakos, *ibid.*, **87** 3489 (1965).

was chromatographed on silica gel (140 g) with chloroform–ethyl acetate (1 : 1 v/v) to yield a pure product. IVm was obtained as crystals from ethyl acetate–*n*-hexane in a 60% (0.59 g) yield; mp 118—121.5°C. $[\alpha]_D^{23}$ −52.0° (*c* 1.0, MeOH).

Found: C, 58.85; H, 5.84; N, 8.57; S, 6.86%. Calcd for $C_{24}H_{29}O_6N_3S$: C, 59.12; H, 6.00; N, 8.62; S, 6.58%.

NMR ($CDCl_3$): δ 1.16 ppm (3H(d), J=6.0 Hz Azy; CH_3–$\underset{|}{C}$H–), 4.20 ppm (1H(m) Azy; CH_3–$\underset{|}{C}$H–), 4.32 ppm (1H(d), J_{cis}=10.2 Hz Azy; α-proton).

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