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Reactions of L-α-Tosylamino-β-propiolactone. II.[1,2] A Novel Synthesis of L-Seryl Peptides

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Optically active α-tosylamino-β-propiolactone (III) was synthesized from N-tosyl-L-asparagine *via* L-2-tosylamino-3-aminopropionic acid. The β-propiolactone (III) reacted with amino acid esters or peptide esters in organic solvents to form L-seryl peptide esters in good yields. Racemization did not actually occur during the peptide coupling reaction.

Introduction

It has been known that the side reactions of serine such as β-elimination[4] and N→O acyl shifts[5] due to the reactivity of the hydroxyl group make it difficult to synthesize serine-containing peptides. Such tendency increases especially in the case of coupling for the seryl peptides in which serine exists on the N-terminal of the peptides. In order to avoid these side reactions, various attempts have been made to find a reversible protection for this group.

Among these, a proporsal for the intramolecular lactone formation with the hydroxyl and carboxyl groups of serine would be particularly noticable. Because, by the formation of lactone ring, there would be a good possibility of both protecting the hydroxyl group and activating the carboxyl group simultaneously during the coupling reaction. J.C. Sheehan, *et al.*[6] reported L-α-tritylamino-β-propiolactone which was synthesized from N-trityl-L-serine by the use of diisopropyl carbodiimide within 15% yield. Then they got N-trityl-L-seryl-L-alanine methyl ester from this lactone and L-alanine methyl ester in dioxane, however further investigation has not been published yet in detail.

In the present paper, a new approach for the synthesis of L-seryl peptides from L-α-tosylamino-β-propiolactone is described.

Results and Discussion

Optically active α-tosylamino-β-propiolactone (III) was synthesized from N-tosyl-L-asparagine (I) *via* L-2-tosylamino-3-aminopropionic acid (II). Diazotization of II was carried out in the aqueous solution of the organic acid at 0—5°, and III was obtained in 50—60% yield. It was found in our previous paper[7] that III reacted with amines to form the corre-

1) This work was presented at the 88th Anual Meeting of Pharmaceutical Society of Japan, Tokyo, Apr. 1968.
2) Part I: N. Yoneda, T. Fujii, M. Umeda, H. Yasuo, Y. Taguchi and K. Okumura, *Yakugaku Zasshi*, **89**, 98 (1969).
3) Location: *Kashima-cho, Higashi-yodogawa-ku, Osaka.*
4) R. Schwyzer, A. Costopanagiotis and P. Sieber, *Chimia*, **16**, 295 (1962).
5) F. Weygand and H. Rinuo, *Ber.*, **92**, 517 (1959).
6) J.C. Sheehan, K. Hasopacher and Y.L. Yeh, *J. Am. Chem. Soc.*, **81**, 6086 (1959); J.C. Sheehan, *Ann., New York Acad. Sci.*, **88**, 665 (1960).
7) N. Yoneda, T. Fujii, M. Umeda, H. Yasuo, T. Taguchi and K. Okumura, *Yakugaku Zasshi*, **89**, 98 (1969).

sponding amino acids or amides due to the ring opening at the position of (a) or (b) in the lactone, as shown in Chart 1.

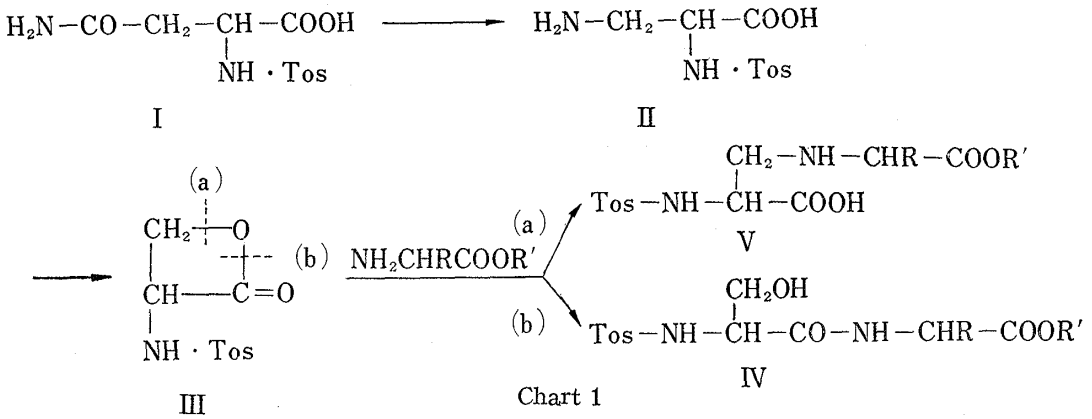

Chart 1

In the case of the synthesis of L-seryl peptides, which are corresponded to IV, it is required that the ring opening reaction should occur selectively at the position of (b).

The reaction conditions to form IV selectively were investigated with varying solvents, temperatures, and reaction times. L-Phenylalanine methyl ester was first used as a model of amino acid esters, because the resultant products of both the peptide type (IV) and the amino acid type (V) were found to be quite favorable for crystallization, and the ratio of IV to V could be checked exactly. Results are shown in Table I and Fig. 1.

Table I. Reaction of III with H–Phe–OMe in Various Solvents

Solvents	Reaction condition	Product IV %	Product V %
AcOEt	25—30°/5 hr	76.0	16.8
AcOEt	40—45°/5 hr	76.0	19.0
AcOEt	40—45°/3 hr (AcOH 1 drop)	82.0	10.3
Dioxane	25—30°/5 hr	62.0	31.2
THF	25—30°/5 hr	76.2	23.8
CH_3CN	35—40°/5 hr	47.3	46.0
$CHCl_3$	45—50°/5 hr	52.0	32.1
THF–H_2O (1:1)	35—40°/5 hr	40.5	47.5
CH_3CN–H_2O (1:1)	35—40°/5 hr	37.3	50.0

IV: mp 131—133° $[\alpha]_D^{20}$ +60.34° (c=1, dioxane) *Anal.* Calcd. N, 6.66. Found. N, 6.43
V: mp 166—167° $[\alpha]_D^{20}$ −35.16° (c=1, 1N NaOH) *Anal.* Calcd. N, 6.66. Found. N, 6.63

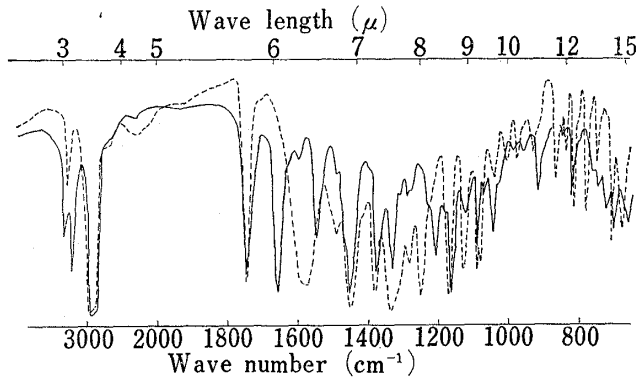

Fig. 1. IR Spectra of IV and V Type Compounds (in Nujol)

------ : V type compound R=⟨○⟩–CH_2–
——— : IV type compound R′=CH_3–

It was found that IV was obtained more than 76% yield in the solvent of ethyl acetate or tetrahydrofuran, however, the ratios of IV to V lowered in dioxane, chloroform and acetonitrile.

It is of interest to note that in the solvent systems of tetrahydrofuran–water (1:1) or acetonitrile–water (1:1), ring opening reaction at the position of (a) was found to be rather predominant. In our previous paper, III reacted with amines in water to form the corresponding amides predominantly, with opening

at the position of (b) in the lactone. This opposite result would be attributed to the differences of solubilities and basicities or reaction rates between amines and amino acid esters in aqueous solvent systems. As III is considered to be an active ester in the peptide coupling reaction, it should be better to carry out the reaction in anhydrous solvent systems as an usual way.

As the reaction rates were found to decrease relatively in comparison with those of amines, much more reaction times and reaction temperature were required in the peptide coupling reactions. However, the rate was catalytically accelerated by adding a small amount of acetic acid during the reaction.

From these results, ethyl acetate was mainly chosen as a reaction solvent in the experiments described later. Another merit of this solvent lies on the fact that it can be used for the extraction solvent as well as for the reaction solvent. Reaction mixture was directly washed with acidic and alkaline aqueous solutions successively to remove unreacted amino acid esters and V formed as a side reaction. Thus N-tosyl-L-seryl pepdide esters (IV) were easily isolated in good yield with high purity.

Table II. N-Tos-L-Ser-Peptide Esters

No.	Esters	Method	Yield (%)	mp (°C) (solv. of recrystaln.)	$[\alpha]_D^{20}$ ($c=1$)
1	Gly-OEt	B	42.0	105—107 (EtOH–ether)	−12.95° (a)
2	Ala-OEt	B	40.0	119—122 (EtOH–ether)	− 4.4° (b)
3	Val-OMe	A	69.0	125—127 (AcOEt–pet-ether)	− 4.58° (a)
4	Leu-OEt	A	71.4	97—99 (AcOEt–pet-ether)	−29.3° (b)
5	Phe-OMe	A	76.5	131—133 (AcOEt–ether)	+60.34° (a)
6	Trp-OMe	A	44.3	198—200 (MeOH)	+14.77° (c)
7	Met-OMe	A	65.6	118—120 (MeOH–AcOEt)	−23.30° (c)
8	(S·bzl)Cys-OMe	A	80.0	97—98 (MeOH–ether)	+ 7.44° (a)
9	Asp-$(OMe)_2$	A	60.3	102—104 (MeOH–ether)	− 6.28° (a)
10	(δZ)Orn-OMe	A	58.5	140—143 (AcOEt–pet-ether)	−19.16° (c)
11	Leu-Leu-OEt	A	55.4	149—153 (AcOEt–pet-ether)	−48.14° (c)
12	Gly-Phe-OMe	A	60.2	149—151 (AcOEt–pet-ether)	−50.63° (c)
13	Pro–Phe–OMe	C	66.7	96—98 (AcOEt–pet-ether)	−38.6° (c)

No.	Formula	Elementary analysis (%) Calcd. C	H	N	S	Found C	H	N	S
1	$C_{14}H_{20}O_6N_2S$	48.83	5.86	8.14		48.70	5.87	8.04	
2	$C_{15}H_{22}O_6N_2S$	50.27	6.19	7.82		50.43	5.96	7.85	
3	$C_{16}H_{24}O_6N_2S$	51.60	6.50	7.52		51.37	6.41	7.42	
4	$C_{18}H_{28}O_6N_2S$	53.99	7.05	7.00		53.60	6.98	6.77	
5	$C_{20}H_{24}O_6N_2S$	57.13	5.75	6.66		56.52	5.76	6.43	
6	$C_{22}H_{25}O_6N_3S$	57.51	5.48	9.15		57.05	5.54	8.85	
7	$C_{16}H_{24}O_6N_2S_2$	47.52	5.98	6.93	15.82	47.43	6.06	6.78	15.78
8	$C_{21}H_{26}O_6N_2S_2$	54.07	5.62	6.01	13.72	53.57	5.69	5.75	13.53
9	$C_{16}H_{22}O_8N_2S$	47.76	5.51	6.96		48.54	5.51	6.76	
10	$C_{24}H_{31}O_8N_3S$	55.27	5.99	8.06		54.84	6.03	7.81	
11	$C_{24}H_{39}O_7N_3S$	56.12	7.65	8.18		55.08	7.53	7.82	
12	$C_{22}H_{29}O_7N_3S$	55.11	6.10	8.76		55.44	5.95	8.30	
13	$C_{25}H_{31}O_7N_3S$	58.01	6.04	8.12		58.11	6.09	7.83	

(a) dioxane (b) EtOH (c) MeOH
method A: Amino acid ester hydrochlorides or peptide ester hydrochlorides were used as free bases.
method B: Triethylamine hydrochloride formed was present during the reaction.
method C: Peptide ester was used as a free base in tetrahydrofuran.

Various amino acid esters or peptide esters were treated with III in this manner. The results are summerized in Table II.

The ratios of IV to V and the total yields were observed to be nearly constant in both cases where IV in ethyl acetate was added to the ethyl acetate solution of the amino acid ester, or the latter was added to the former.

Check of Racemization

Though various peptide intermediates containing serine have been synthesized as the partial fragments of the biologically active peptide, most of the N-tosyl-L-seryl peptide esters (IV) afforded above are unknown compounds. Therefore examination of racemization was carried out as follows.

(1) Carbobenzoxy-L-seryl-L-phenylalanine benzyl ester was synthesized *via* both the azide and dicyclohexyl carbodiimide (DCCD) methods, which was permitted by the catalytic hydrogenation in the presence of palladium black to form L-seryl-L-phenylalanine. This route may be considered the most safety method against racemization, because any alkaline treatment was excluded.

TABLE III. Properties of L-Seryl-L-phenylalanine Synthesized *via* Azide, DCCD and β-Lactone Methods

Synthetic method	Azide	DCCD	β-Lactone
Starting material	Z-L-Ser-$NHNH_2$	Z-Ser-OH	CH_2—O CH —C=O NH-Tos
Intermediate	Z-L-Ser-L-Phe-oBzl mp 129—130° $[\alpha]_D^{20}$ −8.2 (c=1, DMF)		Tos-L-Ser-L-Phe-OMe mp 131—133° $[\alpha]_D$ +60.34 (c=1, dioxane) Tos-L-Ser-L-Phe-OH mp 127—129° $[\alpha]_D^{20}$ −13.45 (c=1, 1N NaOH)
L-Seryl-L-phenylalanine	mp 226—227° $[\alpha]_D^{20}$ a) +53.8 Rf b) 0.72 N% 11.22	mp 225—226° $[\alpha]_D^{20}$ +54.2 Rf 0.72 N% 11.23	mp 223—224° $[\alpha]_D^{20}$ +56.2 Rf 0.72 N% 11.25

a) $[\alpha]_D^{20}$ was measured in AcOH, c=1. b) solvent system: BuOH–AcOH–H_2O (4:4:1)

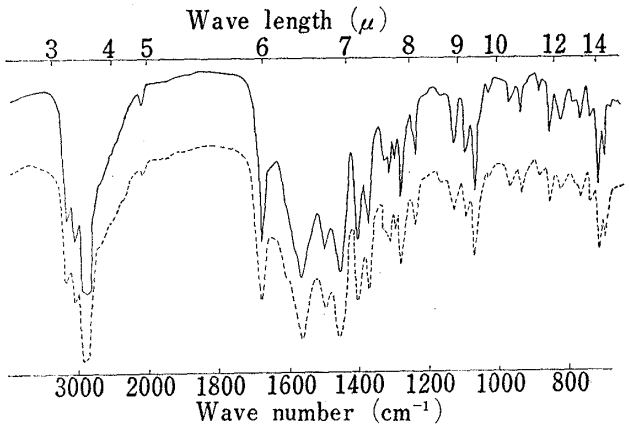

Fig. 2. IR Spectra of H-Ser-Phe-OH (in Nujol)

—— : H-Ser-Phe-OH (from azide method)
------ : H-Ser-Phe-OH (from β-lactone method)

On the other hand, N-tosyl-L-seryl-L-phenylalanine methyl ester (IV) was treated with alkaline hydrolysis and with metallic sodium reduction in liquid ammonia to get the corresponding free dipeptide.

Properties of the dipeptide afforded from the latter treatment were found to agree with those of the former satisfactorily. Results are shown in Table III and Fig 2.

G. T. Young[8] reported that triethylamine hydrochloride presented in the reaction mixture often caused

8) G.T. Young, "Peptides. Proceedings of the Fifth European Symposium," Pergamon Press. Oxford, 1962, p. 119.

reacemization due to the Cl-ion effect during the peptide coupling *via* the active ester method. However, few differences were observed between the reaction methods of A or B treated in this experiments.

(2) Enzymatic analysis of each dipeptides was carried out by hog kideny D-amino acid oxidase after acid hydrolysis. Results showed that D-amino acids detected were below 0.75% in each case.

(3) N-Tosyl-L-seryl peptide hydrazides may be useful intermediates in the case of elongation of the seryl peptides by the use of fragment condensation. Then N-tosyl-L-seryl-L-tyrosine hydrazide, which was listed in the previous report,[9] was also synthesized from IV $\left(R=CH_2-\langle\bigcirc\rangle-OH\right)$ by the treatment of hydrazine hydrate with a convensional manner. mp of the compound agreed with that of the literature, however, the value of $[\alpha]_D$ was not cited in it.

From the results mentioned above, it may be concluded that racemization does not occur actually during the coupling reaction of III with amino acid esters or peptide esters, and that III would offer a novel route for the seryl peptide syntheses as an useful starting material.

Experimental

L-α-Tosylamino-β-propiolactone (III)——L-2-Tosylamino-3-aminopropionic acid (II) (13.4 g, 0.05 mole) and citric acid (30 g) were dissolved in water (200 ml). The aqueous solution (100 ml) of sodium nitrite (10.4 g, 0.15 mole) was poured gradually under passing through the solution from the bottom of the flask at 0—5° with stirring for 2 hr. Stirring was continued for additional 3 hr at 5°, then the crystal formed was collected by filtration. The crude product was dissolved in ethyl acetate, washed with 5% sodium bicarbonate and water successively, and dried over sodium sulfate. Evaporation of the solvent under reduced pressure afforded the fine needle crystals, which were recrystallized from chloroform. 6.7 g, 56% mp 127—129°. $[\alpha]_D^{25}-14.4°$ ($c=1$, dioxane).

General Procedure (Method A)——An amino acid ester hydrochloride or a peptide ester hydrochloride (0.06—0.07 mole) was neutralized with sodium bicarbonate in the minimum volume of water, and extracted well with ethyl acetate (300 ml) three times. The extract layer was washed with a saturated sodium chloride aqueous solution and dried over sodium sulfate. The aqueous layer was checked by the thin-layer chromatography using Silica gel G (solvent system: chloroform–methanol–acetic acid (95 : 5 : 3)) to confirm how much the free base was lost. If a considerable amount of the free base was remained in the aqueous layer, the base extracted was used with correction. However, in the case of L-phenylalanine methyl ester hydrochloride, loss of the free base was negligible. L-α-Tosylamino-β-propiolactone (III) (12.05 g, 0.05 mole) in ethyl acetate (100 ml) was then added slowly to the ethyl acetate solution of the free base with stirring at 40—50° during 30 min, and stirring was continued for additional 3 hr at room temperature. The reaction in progress was checked by the thin-layer chromatography using Silica gel G in the same solvent system as above. *Rf* values, which were detected by iodine or hydrobromic acid and ninhydrin were as follows, III; 0.75, IV; 0.70, V; 0.50; free base; 0.05. The reaction mixture was washed with 1N hydrochloric acid, 5% sodium bicarbonate and water successively, and dried over sodium sulfate. After removal of the solvent *in vacuo*, the crude product was recrystallized from the suitable solvent system listed in Table II.

General Procedure (Method B)——An amino acid ester hydrochloride or a peptide ester hydrochloride (0.055 mole) in ethyl acetate was neutralized with triethylamine (8 ml, 0.055 mole). To the solution, III (12.05 g, 0.05 mole) in ethyl acetate was added with stirring at 40—50°, then the stirring was continued for 3 hr at room temperature. Reaction mixture was treated in the same way as described above.

General Procedure (Method C)——An amino acid ester hydrochloride or a peptide ester hydrochloride (0.06—0.07 mole) was neutralized with sodium bicarbonate in the minimum volume of water, and was extracted well with ether (300 ml) three times. The ether-layer was washed with saturated sodium chloride aqueous solution and dried over sodium sulfate.

The ether was evaporated under reduced pressure at lower temperature, and tetrahydrofuran was added to transfer the solvent. III (12.05 g, 0.05 mole) in tetrahydrofuran (100 ml) was then added to the tetrahydrofuran solution of the free base with stirring at 40—50 °, and the reaction was carried out as described above.

Ethyl acetate (600 ml) was then added to the reaction mixture and the mixture was washed with N hydrochloric acid, 5% sodium bicarbonate and water successively, and dried over sodium sulfate.

9) K. Hofmann, A. Jöhl, E. Furlenmeier and Heini Kappeler, *J. Am. Chem. Soc.*, **79**, 1636 (1957).

Evaporation of the solvent *in vacuo* left the crude product, which was recrystallized from the suitable solvent system.

L-Seryl-L-phenylalanine——1-a) N-Tosyl-L-seryl-L-phenylalanine: N-Tosyl-L-seryl-L-phenylalanine methyl ester (IV, R=$-CH_2-$ ) (4.2 g, 0.01 mole) was treated with N sodium hydroxide (40 ml) at 10° for 1.5 hr. After extracting with ethyl acetate, the aqueous layer was acidified with N hydrochloric acid, and the crude product precipitated was collected by filtration, and washed well with water. The crude product was recrystallized from methanol. 3.7 g, 91.1%. mp 127—129°. $[\alpha]_D^{20}$ −13.45 (c=1, 1N NaOH). *Anal.* Calcd. for $C_{19}H_{22}O_6N_2S$: N, 6.89. Found: N, 7.02.

1-b) L-Seryl-L-phenylalanine: N-Tosyl-L-seryl-L-phenylalanine (2.5 g, 0.006 mole) was dissolved in liquid ammonia (100 ml), and sliced metallic soda (1.5 g) was added portionwise with stirring at −50——60°. Blue color was maintained for 30 min, then ammonium acetate was added and liquid ammonia was concentrated to dryness below 0° and 2N hydrochloric acid was added to the residue to neutralization. After removal of colored substances by the extraction with ethyl acetate, methanol was added to the aqueous layer (1:1) and allowed to stand overnight in an ice-box.

Recrystallization from water and methanol (1:2) afforded colorless flat crystals. 1.0 g, 66.7%. mp 223—224°. $[\alpha]_D^{20}$ +56.2 (c=1, AcOH). *Anal.* Calcd. for $C_{12}H_{16}O_5N_2$: N, 11.15. Found: N, 11.25. *Rf* 0.72. (solvent system: BuOH–AcOH–H_2O (4:1:1).

N-Carbobenzoxy-L-seryl-L-phenylalanine Benzyl Ester——2-a) N-Carbobenzoxy-L-serine hydrazide[10] (5.1 g, 0.02 mole) was dissolved in dimethylformamide (200 ml) and chloroform (50 ml), and 6N hydrochloric acid (10 ml) was added at −10°. An aqueous solution of $NaNO_2$ (1.38 g, 0.02 mole) was added dropwise to the mixture with stirring at −10° during 30 min. After 20 min, L-phenylalanine benzyl ester (5.1 g, 0.02 mole, as a free base) in chloroform (150 ml) and triethylamine (11 ml) were added gradually. Stirring was continued for one hour at −5——10° and for additional 2 hr at room temperature, while the reaction mixture was maintained in slightly alkaline state with adding a small amount of triethylamine, if necessary. Then chloroform (100 ml) was added and the reaction mixture was washed with 1N hydrochloric acid, 5% sodium bicarbonate and water successively, and dried over sodium sulfate. Evaporation of the solvent left the crude product, which was recrystallized from ethyl acetate and petroleum ether (3:1). 8.3 g, 87%, mp 129—130°. $[\alpha]_D^{20}$ −8.2 (c=2, DMF) (lit.[11] mp 121—122°. $[\alpha]_D$ was not cited).

2-b) L-Seryl-L-phenylalanine: N-Carbobenzoxy-L-seryl-L-phenylalanine benzyl ester (4.8 g, 0.01 mole) was dissolved in aqueous ethanol (300 ml) and catalytic hydrogenation was carried out with bubbling hydrogen gas in the presence of palladium balck at room temperature for 5 hr. *Rf* values of the thin-layer chromatography in the same solvent system described above showed the completion of the reaction. *Rf* starting material; 0.70, product; 0.0.

After removal of the catalyst, methanol was evaporated *in vacuo*, and the crude product was recrystallized from water and methanol (1:1). 2.1 g, 84%. mp 226—227°. $[\alpha]_D^{20}$ +53.8 (c=1, AcOH). *Anal.* Calcd. for $C_{12}H_{16}O_5N_2$: N, 11.15. Found: N, 11.22. *Rf* 0.72 (solvent system: BuOH–AcOH–H_2O (4:1:1)).

Enzymatic Analyses——Each of the L-seryl-L-phenylalanine (50.453 mg, 200 μmole) synthesized *via* azide, DCCD or β-lactone method respectively, was hydrolyzed by 10 ml of hydrochloric acid (bp 107°) in a sealed tube at 110° for 24 hr. After evaporation of the hydrochloric acid solution completely, the residue was dissolved in water to total volume of 5 ml. 1.5 ml of the solution (60 μmole) was digested by the use of hog kidney D-amino acid oxidase and the volume of oxygen consumed was measured by Warburg manometer at 37°, adjusted to pH 8.3. Results are as follows.

D-amino acid contamined	*via* azide method	0.45 μmole (0.75%)
	via DCCD method	0.3 μmole (0.3%)
	via β-lactone method	0

N-Tosyl-L-seryl-L-tyrosine Hydrazide——N-Tosyl-L-seryl-L-tyrosine methyl ester (IV R=$-CH_2-$  -OH) (1.1 g, 0.0025 mole) was dissolved in ethanol (10 ml) and hydrazine hydrate (0.4 g) was added with stirring at 10°. Stirring was continued for 5 hr at room temperature, then water was added and the crude product was collected by filtration, which was washed well with water. Recrystallization from ethanol–water (1:1) afforded the needles. 0.75 g, 68%. mp 200—202° (lit.[6] mp 200—202°). *Anal.* Calcd. for $C_{19}H_{24}O_6N_4S$: N, 12.81. Found: N, 12.71.

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10) J.S. Fruton, *J. Biol. Chem.*, **146**, 463 (1942).
11) H. Determann, *Ann.*, **670**, 141 (1963).