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Synthesis of Selenium-Containing Peptides*

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ABSTRACT: A general and convenient method for the synthesis of Se-benzyl-L-selenocysteine derivatives, including Se-benzyl-L-selenocysteine peptides, is described. The method involves the nucleophilic displacement of the *O*-tosyl moiety in *O*-tosylated serine derivatives and *O*-tosylated serine residues within peptides by sodium benzyl selenolate. The applicability of this method was demonstrated in the synthesis of the protected C-terminal tetrapeptide of selenooxytocin and selenoglutathione. This work suggests the feasibility of replacing serine residues with selenocysteine residues in other biologically significant peptides.

In connection with our investigations (Walter and Chan, 1967; Theodoropoulos *et al.*, 1967) concerning the replacement of sulfur by selenium moieties within biologically active peptides and proteins, it became apparent that a general method for the synthesis of optically active selenium-containing amino acids and peptides had to be developed. Adopting Painter's (1947) synthesis of Se-benzyl-DL-selenocysteine, Frank (1964a) recently prepared the methyl Se-benzyl-L-selenocysteinate. The carboxyl group of this ester was liberated hydrolytically by treatment with boiling aqueous hydrochloric acid, thus providing the Se-benzyl-L-selenocysteine. In view of the necessity for such drastic reaction conditions it is not surprising that we and others[1] were unable to repeat this preparation of Se-benzyl-L-selenocysteine with consistent results.

In contrast, a consistent and convenient procedure for the preparation of Se-benzyl-L-selenocysteine is provided by the nucleophilic displacement of the *O*-*p*-toluenesulfonate moiety of L-serine by the benzyl selenolate anion. This method has the additional advantages of operating under very mild reaction conditions and of being applicable to the synthesis of Se-benzyl-L-selenocysteine compounds which bear selectively removable protecting groups. It should be noted that the susceptibility of the *O*-*p*-toluenesulfonate group of *O*-tosylated derivatives toward nucleophiles was demonstrated in the course of the synthesis of L-cysteine derivatives and peptides (Zervas and Photaki, 1960; Photaki, 1963; Photaki and Bardakos, 1965; Zioudrou *et al.*, 1965).

In preliminary experiments we have found that benzyl *N*-carbobenzoxy-*O*-tosyl-L-serinate indeed re-

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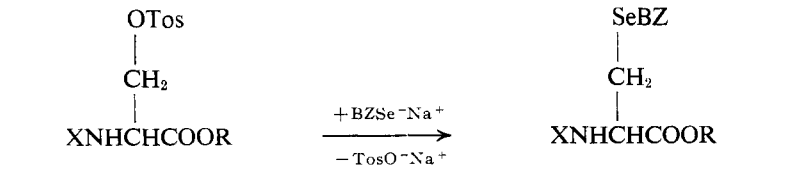
SCHEME I



Ia, X = Z; R = BZ
b, X = Z; R = Me
c, X = Z; R = DPM
d, X = BOC; R = BZLN
e, X = Z; R = BZLN
Tos = p-$CH_3C_6H_4SO_2$
BZ = $C_6H_5CH_2$
Z = $C_6H_5CH_2OCO$

IIa, X = Z; R = BZ
b, X = Z; R = Me
c, X = Z; R = DPM
d, X = BOC; R = BZLN
e, X = Z; R = BZLN
Me = CH_3
DPM = $(C_6H_5)_2CH$
BZLN = p-$NO_2C_6H_4CH_2$

acts readily with sodium benzyl selenolate to yield benzyl *N*-carbobenzoxy-Se-benzyl-L-selenocysteinate in consistently high yields (approximately 90%). Considering the pronounced tendency of *O*-tosylserine derivatives to undergo β elimination in an alkaline medium (Photaki, 1963), it was desirable to establish that the optical activity had been retained during the above transformation reaction. Accordingly we attempted to convert benzyl *N*-carbobenzoxy-Se-benzyl-L-selenocysteinate to Se-benzyl-L-selenocysteine, which was previously prepared by two independent routes (Frank, 1964a; Walter and Chan, 1967). However, as had been anticipated, the elimination of the benzyl group protecting the carboxyl function did not proceed to completion during treatment of the benzyl *N*-carbobenzoxy-Se-benzyl-L-selenocysteinate with 3 N HBr in glacial acetic acid at room temperature for 3 hr;[2] in fact, benzyl Se-benzyl-L-selenocysteinate hydrobromide was isolated as the major reaction product. Because of this difficulty in liberating the carboxyl group, a new approach, involving a more acid-labile ester (Gazis *et al.*, 1966; Hiskey and Adams, 1965; Aboderin *et al.*, 1965; Stelakatos *et al.*, 1966), was initiated. Thus, *N*-carbobenzoxy-L-serine on treatment with diphenyldiazomethane (Miller, 1959) at 5° in ethyl acetate gave the diphenylmethyl *N*-carbobenzoxy-L-serinate, which in turn was allowed to react with tosyl chloride in anhydrous pyridine to produce the diphenylmethyl *N*-carbobenzoxy-*O*-tosyl-L-serinate. The latter compound was converted to the diphenylmethyl *N*-carbobenzoxy-Se-benzyl-L-selenocysteinate upon treatment with sodium benzyl selenolate. Removal of both the amino- and the carboxyl-protecting groups from this fully protected ester by treatment with 2 N HBr in glacial acetic acid at room temperature for 1 hr afforded the intermediary Se-benzyl-L-selenocysteine hydrobromide. Upon adjustment of the pH to 5.5 the hydrobromide salt was converted to the free base, Se-benzyl-L-selenocysteine, which exhibited an optical rotation comparable to that recorded previously for this compound obtained either by acid hydrolysis of methyl Se-benzyl-L-selenocysteinate (Frank, 1964a) or by resolution of *N*-acetyl-Se-benzyl-DL-selenocysteine with hog acylase (Walter and Chan, 1967).

Our next objective was to secure a Se-benzyl-L-selenocysteine derivative bearing amino- and carboxyl-protecting groups selected so as to permit the elimination or alteration of either one without affecting the other. Such an intermediate allows the attachment of a Se-benzyl-L-selenocysteine residue to either the amino or the carboxyl end of an amino acid or a peptide chain. For the preparation of this key intermediate *N*-carbobenzoxy-L-serine was selectively esterified with *p*-nitrobenzyltosylate (Theodoropoulos and Tsangaris, 1964) to yield *p*-nitrobenzyl *N*-carbobenzoxy-L-serinate. This ester was subsequently tosylated to provide *p*-nitrobenzyl *N*-carbobenzoxy-*O*-tosyl-L-serinate, which in turn was allowed to react with the benzyl selenolate anion, to give the versatile intermediate *p*-nitrobenzyl *N*-carbobenzoxy-Se-benzyl-L-selenocysteinate in high yield.

On the one hand, treatment of this selenium-containing ester with 2 N HBr in acetic acid eliminated selectively the *N*-carbobenzoxy group and by addition of ether the crystalline *p*-nitrobenzyl Se-benzyl-L-selenocysteinate hydrobromide was obtained. This nitrogen-deprotected ester constitutes a useful intermediate for the preparation of C-terminal L-selenocysteine peptides.

On the other hand, the *p*-nitrobenzyl *N*-carbobenzoxy-Se-benzyl-L-selenocysteinate, upon mild treatment with hydrazine, afforded the hydrazide with physical properties identical with those obtained from the corresponding benzyl ester. To demonstrate the usefulness of the hydrazide in preparing N-terminal L-selenocysteine peptides, it was converted to the azide either with sodium nitrite or *t*-butyl nitrite (Honzl and Rudinger, 1961) in strongly acidic media. Coupling of this azide with L-prolyl-L-leucylglycinamide (Boisson-

[2] It is known that the benzyl ester group is removed by prolonged action of HBr–glacial acetic acid at room temperature (Häussler, 1960); however, owing to the instability of the selenium moiety the reaction time had to be limited.

nas *et al.*, 1955; Zaoral and Rudinger, 1955; Lutz *et al.*, 1959) gave the tetrapeptide, *N*-carbobenzoxy-Se-benzyl-L-selenocysteinyl-L-prolyl-L-leucylglycinamide with values for melting point and optical rotation somewhat higher than those reported previously (Frank, 1964b; Walter and du Vigneaud, 1965, 1966; Walter and Chan, 1967). Thus, this approach provides an alternative convenient method for preparing the C-terminal tetrapeptide sequence of selenium-containing oxytocin analogs.

The nucleophilic displacement of the *O*-tosyl moiety by the benzyl selenolate anion has been extended to *O*-tosylserine peptides, as illustrated in the synthesis of a protected selenoglutathione. As starting material for this synthesis we employed benzyl *N*-*t*-butyloxycarbonyl-L-serylglycinate which upon tosylation yielded benzyl *N*-*t*-butyloxycarbonyl-*O*-tosyl-L-serylglycinate. This ester was transformed to benzyl *N*-*t*-butyloxycarbonyl-Se-benzyl-L-selenocysteinylglycinate which was partially deprotected by treatment with trifluoroacetic acid and allowed to react with α-benzyl *N*-carbobenzoxy-L-glutamate (Sachs and Brand, 1953; Losse *et al.*, 1963) in the presence of *N*,*N*′-dicyclohexylcarbodiimide (Sheehan and Hess, 1955). The resulting protected selenoglutathione, benzyl *N*-carbobenzoxy-γ-L-glutamyl(α-benzyl ester)-Se-benzyl-L-selenocysteinylglycinate, was isolated in an over-all yield of 60%. The foregoing results suggest the feasibility of replacing serine residues with selenocysteine residues in other biologically significant peptides and proteins.

Experimental Section[3]

Benzyl N-Carbobenzoxy-O-tosyl-L-serinate (Ia) (Scheme I). To a solution of 6.6 g of benzyl *N*-carbobenzoxy-L-serinate in 10 ml of anhydrous pyridine, precooled to −10°, 4.5 g of tosyl chloride was added. After being allowed to stand for 3 hr at −10° the mixture was poured onto crushed ice. The reaction product precipitated as a syrup which solidified upon continued cooling. The solid was collected by filtration, dried over P_2O_5 *in vacuo*, and recrystallized from absolute ethanol; wt 7.5 g, mp 75.5–77°, $[\alpha]_D^{24}$ −7.41° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{25}H_{25}NO_7S$: C, 62.1; H, 5.21; N, 2.87. Found: C, 62.0; H, 5.34; N, 2.99.

Benzyl N-Carbobenzoxy-Se-benzyl-L-selenocysteinate (IIa). Benzylselenol (5.6 g) was dissolved in 10 ml of dimethylformamide and cooled to 0°. To the clear solution 1.4 g of sodium hydroxide dissolved in 3 ml of water was added and immediately afterwards 14.4 g of Ia dissolved in 40 ml of acetone. Instantaneously the sodium *p*-toluenesulfonate began to precipitate. The reaction flask was vigorously shaken during the next 2–3 min after which period the pH was adjusted to between 5 and 6 with dilute acetic acid. The solvent was evaporated to dryness in high vacuum and upon addition of ice-cold water the product solidified. It was recrystallized from ethanol–ethyl acetate (3:1); wt 12 g, mp 64–65°, $[\alpha]_D^{28}$ −39.2° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{25}H_{25}NO_4Se$: C, 62.2; H, 5.22; N, 2.90. Found: C, 62.4; H, 5.35; N, 3.10.

Methyl N-Carbobenzoxy-Se-benzyl-L-selenocysteinate (IIb).[4] Methyl *N*-carbobenzoxy-*O*-tosyl-L-serinate (15.4 g) (Photaki, 1963), in 28 ml of dimethylformamide, was treated with 6.13 g of benzylselenol in 12 ml of acetone containing 1.43 g of sodium hydroxide dissolved in 10 ml of water. The conversion product was isolated as described in the preceding section and crystallized from ethyl acetate–ligroin (bp 60–90°); wt 13.5 g, mp 73–74°, $[\alpha]_D^{24}$ −40.0° (*c* 3, dimethylformamide).

Anal. Calcd for $C_{19}H_{21}NO_4Se$: C, 56.2; H, 5.21; N, 3.45. Found: C, 56.1; H, 5.17; N, 3.33.

Diphenylmethyl N-Carbobenzoxy-L-serinate. To an ice-cold solution of 7.5 g of *N*-carbobenzoxy-L-serine in 50 ml of ethyl acetate 5.5 g of diphenyldiazomethane was added. Stirring was continued while the reaction mixture was allowed to warm up to room temperature. After 12 hr the ethyl acetate was removed *in vacuo* and the resulting residue was dissolved in 100 ml of chloroform. The organic phase was washed with three 50-ml portions of 5% sodium bicarbonate solution and subsequently with 30 ml of water. Following drying over anhydrous sodium sulfate the volume of the chloroform was decreased under reduced pressure. Upon addition of ligroin the ester crystallized in short needles; wt 10.0 g, mp 127°, $[\alpha]_D^{24}$ −13.6° (*c* 3, dimethylformamide).

Anal. Calcd for $C_{24}H_{23}NO_5$: C, 71.1; H, 5.68; N, 3.45. Found: C, 71.1; H, 5.77; N, 3.60.

Diphenylmethyl N-Carbobenzoxy-O-tosyl-L-serinate (Ic). To a cooled solution (−10°) of 7.0 g of diphenylmethyl *N*-carbobenzoxy-L-serinate in 20 ml of anhydrous pyridine, 4.5 g of tosyl chloride was added. After being kept for 3 hr at 0° the tosyl derivative was worked up according to the procedure described under benzyl *N*-carbobenzoxy-*O*-tosyl-L-serinate (Ia); wt 7.0 g, mp 83–84°, $[\alpha]_D^{23}$ −15.2° (*c* 3, dimethylformamide).

Anal. Calcd for $C_{31}H_{29}NO_7S$: C, 66.5; H, 5.18; N, 2.50. Found: C, 66.4; H, 5.13; N, 2.62.

Diphenylmethyl N-Carbobenzoxy-Se-benzyl-L-selenocysteinate (IIc). As in the procedure described for the preparation of IIa, 11.0 g of Ic in 40 ml of acetone was added to a solution of 4.0 g of benzylselenol in 10 ml of dimethylformamide containing 0.8 g of sodium hydroxide dissolved in 5 ml of water. The reaction mixture was worked up in the usual manner and the product was recrystallized from ethanol; wt 7.0 g, mp 66–67°, $[\alpha]_D^{24}$ −48.0° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{31}H_{29}NO_4Se$: C, 66.7; H, 5.23; N, 2.50. Found: C, 66.5; H, 5.37; N, 2.34.

[3] Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are corrected. The optical rotations were measured with a Carl Zeiss photoelectric precision polarimeter 0.005°. Elementary analyses were carried out by Galbraith Laboratories, Knoxville, Tenn. Prior to analysis the compounds were dried at 56° under high vacuum over P_2O_5.

[4] This compound was prepared in this laboratory by Dr. W. Gordon.

p-Nitrobenzyl N-t-Butyloxycarbonyl-L-serinate. To a mixture of 4.2 g of *N-t*-butyloxycarbonyl-L-serine and 2.8 ml of triethylamine in 20 ml of acetone 6.0 g of *p*-nitrobenzyltosylate was added. The resulting clear solution was kept at 40° for 2 hr and subsequently stored at room temperature overnight. After this period the solvent was removed under reduced pressure and the resulting residue was dissolved in ethyl acetate. After being washed with 5% sodium bicarbonate solution and water the organic layer was dried and finally evaporated. The product crystallized from ethyl acetate–ligroin (1:2); wt 4.2 g, mp 100–103°, $[\alpha]_D^{28}$ −6.81° (*c* 1.4, chloroform).

Anal. Calcd for $C_{15}H_{20}N_2O_7$: C, 52.9; H, 5.93; N, 8.23. Found: C, 52.9; H, 6.02; N, 8.06.

p-Nitrobenzyl N-t-Butyloxycarbonyl-O-tosyl-L-serinate (Id). *p*-Nitrobenzyl *N-t*-butyloxycarbonyl-L-serinate (2.0 g) was allowed to react with 1.3 g of tosyl chloride in 5 ml of anhydrous pyridine at −10°. Isolation by the procedure described under Ia gave 2.0 g of the tosylated ester. The product was recrystallized from ethanol; wt 1.5 g, mp 115–116°, $[\alpha]_D^{23}$ +13.8° (*c* 2, chloroform).

Anal. Calcd for $C_{22}H_{26}N_2O_9S$: N, 5.93; Found: N, 5.89.

p-Nitrobenzyl N-t-Butyloxycarbonyl-Se-benzyl-L-selenocysteinate (IId). Treatment of a solution of 2.8 g of Id in 20 ml of acetone with 1.0 g of benzylselenol dissolved in 7 ml of dimethylformamide in the presence of 0.3 g of sodium hydroxide in 2 ml of water yielded after the usual procedure as described for IIa, 1.9 g of product; mp 99–102°, $[\alpha]_D^{23}$ −18.3° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{22}H_{26}N_2O_6Se$: C, 55.3; H, 5.49; N, 5.86. Found: C, 54.8; H, 5.62; N, 5.67.

p-Nitrobenzyl N-Carbobenzoxy-L-serinate. A solution, comprising 20 ml of acetone and 4.2 ml of triethylamine containing 9.0 g of *p*-nitrobenzyltosylate, was refluxed. After 1 hr the solvent was removed under reduced pressure and the residue was dissolved in 50 ml of ethyl acetate. The organic layer was washed with two 30-ml portions of 5% sodium bicarbonate solution and with two 30-ml portions of water. After being dried over anhydrous sodium sulfate the solvent was evaporated *in vacuo* and the remaining residue was crystallized from ethyl acetate–ligroin; wt 8.0 g, mp 114–115°, $[\alpha]_D^{23}$ −13.5° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{18}H_{18}N_2O_7$: C, 57.8; H, 4.81; N, 7.48. Found: C, 57.6; H, 4.76; N, 7.39.

p-Nitrobenzyl N-Carbobenzoxy-O-tosyl-L-serinate (Ie). To 10 ml of precooled pyridine (−10°) 7.0 g of *p*-nitrobenzyl *N*-carbobenzoxy-L-serinate and 4.5 g of tosyl chloride were added. The reaction mixture was stored for the next 3 hr at −10° and then worked up as described earlier. The product was obtained after crystallization from ethanol; wt 7.0 g, mp 108–110°, $[\alpha]_D^{28}$ −6.56° (*c* 3, dimethylformamide).

Anal. Calcd for $C_{25}H_{24}N_2O_9S$: C, 56.8; H, 4.58; N, 5.30. Found: C, 56.9; H, 4.71; N, 5.32.

p-Nitrobenzyl N-Carbobenzoxy-Se-benzyl-L-selenocysteinate (IIe). Analogously to the procedure described for the preparation of IIa 5.3 g of Ie in 40 ml of acetone was added to a solution of 2.0 g of benzylselenol in 10 ml of dimethylformamide containing 0.4 g of sodium hydroxide dissolved in 3 ml of water. The reaction mixture was worked up according to the earlier description and the product was secured after recrystallization from ethyl acetate–ethanol (1:3); wt 4.0 g, mp 89–90.5°, $[\alpha]_D^{22}$ −34.1° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{25}H_{24}N_2O_6Se$: C, 56.9; H, 4.59; N, 5.31. Found: C, 57.1; H, 4.46; N, 5.48.

p-Nitrobenzyl Se-benzyl-L-selenocysteinate Hydrobromide. The protected ester IIe (2.4 g) was stirred for 0.5 hr with 10 ml of 2 N HBr in glacial acetic acid. During this period most of the hydrobromide precipitated. The precipitation was completed by the addition of 200 ml of anhydrous ether. The solid material was collected by filtration, repeatedly washed with ether, and finally recrystallized from isopropyl alcohol–dimethylformamide–ether (4:1:5); wt 1.5 g, mp 154–155°, $[\alpha]_D^{23}$ −11.1° (*c* 0.5, 95% ethanol).

Anal. Calcd for $C_{17}H_{18}N_2O_4Se \cdot HBr$: C, 43.1; H, 4.04; N, 5.90. Found: C, 43.3; H, 3.99; N, 5.81.

Se-benzyl-L-selenocysteine. Compound Ic (1.0 g) was dissolved in 3 ml of glacial acetic acid and treated with 5 ml of 3.3 N HBr (w/w) in glacial acetic acid. After 1 hr the hydrobromide was precipitated with 100 ml of ether, washed five times with 100-ml portions of ether, and then dried *in vacuo;* wt 0.56 g, mp 178–180° dec. A solution of 0.3 g of the hydrobromide in 3 ml of 1 N sodium hydroxide was titrated with 2 N hydrochloric acid until a pH of 5.5 was reached. The crystalline precipitate was isolated by filtration, washed with 2 ml of ice-cold water, and dried over P_2O_5 *in vacuo;* wt 0.115 g, mp 175–178° dec (the material started to soften at 173°), $[\alpha]_D^{23}$ +36.4° (*c* 2, 1 N sodium hydroxide); [lit. (Frank, 1964a) mp 191–192°, $[\alpha]_D^{23}$ +35.6° (*c* 2, 1 N sodium hydroxide) and (Walter and Chan, 1967) mp 183–184°, $[\alpha]_D^{24}$ +35.2° (*c* 2, 1 N sodium hydroxide)].

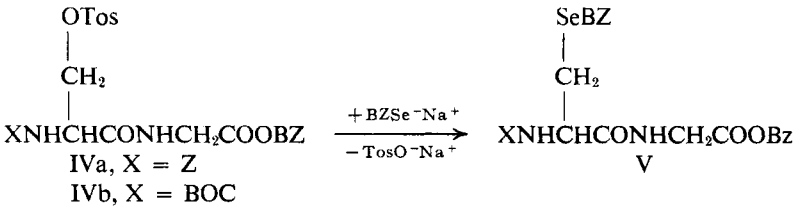
N-Carbobenzoxy-Se-benzyl-L-selenocysteine Hydrazide (III). A solution of 15.0 g of benzyl *N*-carbobenzoxy-Se-benzyl-L-selenocysteinate in 50 ml of methanol–dimethylformamide (2:1) was allowed to react overnight with 10 ml of hydrazine hydrate. The reaction was stopped by the addition of 300 ml of water. The precipitate, collected by filtration, washed with water, and finally with ether, crystallized from ethyl acetate–ligroin (bp 30–60°); wt 12.0 g, mp 137–139°, $[\alpha]_D^{22}$ −20.2° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{18}H_{21}N_3O_3Se$: C, 53.2; H, 5.23; N, 10.3. Found: C, 53.8; H, 5.39; N, 10.3.

Similarly, *p*-nitrobenzyl *N*-carbobenzoxy-Se-benzyl-L-selenocysteine was converted in 90% yield to the hydrazide which exhibited identical physical properties.

Benzyl N-Carbobenzoxy-O-tosyl-L-serylglycinate (IVa) (Scheme II). Benzyl *N*-carbobenzoxy-L-serylglycinate (4.0 g) (Fruton, 1942) was treated with 2.5 g of tosyl chloride in 10 ml of dry pyridine at −10°. The reaction mixture was worked up in the usual manner. The crude product was recrystallized from 20 ml of ethanol; wt 4.6 g, mp 87–89°, $[\alpha]_D^{23}$ +15.6° (*c* 2, chloroform).

SCHEME II



Anal. Calcd for $C_{27}H_{28}N_2O_8S$: C, 60.0; H, 5.22; N, 5.18. Found: C, 60.1; H, 5.36; N, 5.13.

*Benzyl N-Carbobenzoxy-Se-benzyl-*L*-selenocysteinylglycinate* (*Va*). A mixture, consisting of 1.5 g of benzylselenol and 0.4 g of sodium hydroxide dissolved in 2 ml of water, was added to dimethylformamide (5 ml at −10°) and immediately afterwards a solution of 4.5 g of IVa in 15 ml of acetone. After a reaction period of 2 min the product was isolated as described earlier. The solid material thus obtained crystallized from ethyl acetate–ligroin (bp 60–90°) (1:4); wt 3.2 g, mp 91–93°, $[\alpha]_D^{25}$ −28.9° (*c* 2, 95% ethanol).

Anal. Calcd for $C_{27}H_{28}N_2O_5Se$: C, 60.1; H, 5.23; N, 5.19. Found: C, 60.0; H, 5.16; N, 5.32.

*Benzyl N-t-Butyloxycarbonyl-*L*-serylglycinate.* An ice-cold suspension of 9.15 g of benzyl glycinate *p*-toluenesulfonate in methylene chloride was neutralized with 4.2 ml of triethylamine and allowed to react with *N-t*-butyloxycarbonyl-L-serine in the presence of 6.0 g of *N,N'*-dicyclohexylcarbodiimide. After 24-hr reaction time the *N,N'*-dicyclohexylurea was removed by filtration. The methylene chloride, following washing with three 30-ml portions of 5% sodium bicarbonate solution and two 30-ml portions of water, was dried over anhydrous sodium sulfate and finally evaporated. The syrupy residue thus obtained solidified upon trituration with ligroin. The solid product was recrystallized from ethyl acetate–ligroin; wt 7.2 g, mp 77–79°, $[\alpha]_D^{27}$ −13.0° (*c* 3, methanol).

Anal. Calcd for $C_{17}H_{24}N_2O_6$: C, 57.9; H, 6.87; N, 7.95. Found: C, 57.8; H, 7.02; N, 7.91.

*Benzyl N-t-Butyloxycarbonyl-O-tosyl-*L*-serylglycinate* (*IVb*). To a cooled solution (−10°) of 3.5 g of benzyl *N-t*-butyloxycarbonyl-L-serylglycinate in 10 ml of dry pyridine 3.8 g of tosyl chloride was added. After being kept for 3 hr at −10° the tosylated dipeptide was worked up according to the procedure described earlier. The product was recrystallized from ethanol with slight heating; wt 3.8 g, mp 101–103°, $[\alpha]_D^{23}$ +15.1° (*c* 2, chloroform).

Anal. Calcd for $C_{24}H_{30}N_2O_8S$: C, 57.1; H, 5.59; N, 5.55. Found: C, 56.8; H, 5.83; N, 5.63.

*Benzyl N-t-Butyloxycarbonyl-Se-benzyl-*L*-selenocysteinylglycinate* (*Vb*). To precooled dimethylformamide (10 ml at −10°) a mixture, consisting of 6.7 g of benzylselenol and 1.4 g of sodium hydroxide dissolved in 3 ml of water, was added and immediately afterwards a solution of 14.0 g of IVb in 40 ml of acetone. After a reaction period of 3 min the product was isolated as described earlier. The solid thus obtained was recrystallized from ethyl acetate–ligroin (2:1); wt 11.5 g, mp 90–92°, $[\alpha]_D^{24}$ −27.5° (*c* 2, methanol).

Anal. Calcd for $C_{24}H_{30}N_2O_5Se$: C, 56.5; H, 5.95; N, 5.94. Found: C, 56.2; H, 5.84; N, 5.66.

*N-Carbobenzoxy-Se-benzyl-*L*-selenocysteinyl-*L*-prolyl-*L*-leucylglycinamide.* To a cold solution (−20°) of III (2.0 g) in 10 ml of dimethylformamide, 1 ml of concentrated hydrochloric acid and 0.5 g of *t*-butyl nitrite (or 0.35 g of sodium nitrite dissolved in 3 ml of water) were added. Following a reaction period of 3 min the temperature was lowered to −40°. The reaction mixture was neutralized with triethylamine (approximately 1 ml) and immediately thereafter the tripeptide, L-prolyl-L-leucylglycinamide (1.41 g), was added while cooling was terminated and stirring was continued for the next 24 hr. The product was precipitated by the addition of dilute acetic acid, collected by filtration, washed with dilute acetic acid, dried over P_2O_5 *in vacuo*, and finally recrystallized from ethanol–water (7:3); wt 1.5 g, mp 163–164°, $[\alpha]_D^{24}$ −54.2° (*c* 2, dimethylformamide); [lit. (Frank, 1964b) mp 154–156°, $[\alpha]_D^{25}$ −48.1° (*c* 0.7, dimethylformamide) and (Walter and du Vigneaud, 1965, 1966; Walter and Chan, 1967) mp 162–163°, $[\alpha]_D^{18}$ −52.3° (*c* 2, dimethylformamide)]. Upon descending chromatography performed on Whatman No. 1 paper in the solvent system chloroform–cyclohexane (7:3) a single spot was obtained with an R_F of 0.87 (Walter and Chan, 1967).

*Benzyl N-Carbobenzoxy-γ-*L*-glutamyl(α-benzyl Ester)-Se-benzyl-*L*-selenocysteinylglycinate.* Compound Vb (2.5 g) was treated with trifluoroacetic acid for 0.5 hr at room temperature. The oily residue obtained after evaporation of the solvent was washed by decantation with three 25-ml portions of precooled ligroin (−30°). After being dried for 3 days *in vacuo* over NaOH and P_2O_5, the residue was dissolved in 20 ml of methylene dichloride and allowed to react with 2.7 g of α-benzyl *N*-carbobenzoxy-L-glutamate in the presence of 1.0 g of *N,N'*-dicyclodihexylcarbodiimide. The *N,N'*-dicyclohexylurea was removed by filtration after 24 hr and the filtrate was diluted by the addition of 30 ml of methylene chloride. The organic layer was washed with two 30-ml portions of cold 1% sodium bicarbonate solution and two 30-ml portions of water. After being dried with anhydrous sodium sulfate the solvent was evaporated and the solid residue thus obtained was crystallized from ethyl acetate–ligroin; wt 1.8 g, mp 148–150°, $[\alpha]_D^{24}$ −26.1° (*c* 1, dimethylformamide). A sample

recrystallized from ethanol exhibited a melting point of 161–163°, $[\alpha]_D^{21}$ −26.6° (*c* 2, dimethylformamide).

Anal. Calcd for $C_{39}H_{41}N_3O_8Se$: C, 61.7; H, 5.44; N, 5.54. Found: C, 61.5; H, 5.60; N, 5.78.

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