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Phosphinyl and Phosphinothioyl Amino Acids and Peptides. VII. The Use of the Dimethylphosphinothioyl Group as a Thiol-protecting Group of Cysteine

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The use of the dimethylphosphinothioyl (Mpt) group for the protection of the thiol function in the cysteine side-chain was studied. *N,S*-Bis(Mpt)-L-cysteine was obtained directly by means of a Schotten-Baumann-type reaction of Mpt–Cl with L-cysteine. The *S*–Mpt group was stable under acidic conditions, except for HBr in acetic acid, and it could be removed by treatment with $AgNO_3$ or $Hg(OAc)_2$ in an aqueous medium. Glutathione was synthesized by utilizing the new *S*-protecting group.

The selection of proper protecting groups for various kinds of functional groups of amino acids is very important in peptide synthesis. Some of the common amino acids with side-chain functional groups can be incorporated into a peptide chain without protecting these groups, but the thiol function of cysteine should be protected up to the last stage of the synthesis because of its high nucleophilicity and its lability to air oxidation.

Recently we reported the use of a series of phosphinothioyl groups for the protection of the α-amino functions of amino acids by taking advantage of the well-known acid lability of the P–N bonds.[1-4] These groups could also be utilized for the protection of the phenolic hydroxyl group of tyrosine.[5] Since the P–O bond was stable under the usual acidic conditions encountered during the peptide synthesis, a good orthogonal relationship between the *N*- and *O*-phosphinothioyl groups was established. Since it seems that a similar advantage can be expected for the *S*-phosphinothioyl groups, we tried to use these groups for the protection of the thiol function of cysteine.

In an earlier paper of this series we have already described the preparation and some reactions of *S*-diphenylphosphinothioyl(Ppt) derivatives of L-cysteine and its ester.[2] In these studies it was found that the thiol function was phosphinothioylated preferentially rather than the amino function and that it was regenerated by alkaline hydrolysis. However, *N,S*-Bis(Ppt)-L-cysteine, which would be a key compound to be used in peptide synthesis, could be successfully prepared in only a low yield. We also found that the dimethylphosphinothioyl(Mpt) group was more acid-labile than the Ppt group when used as an amino-protecting group.[3] We have now tried to prepare the Mpt derivatives of L-cysteine.

Results and Discussion

When the L-cysteine methyl ester was treated with dimethylphosphinothioyl chloride (Mpt–Cl)[3] in the presence of *N,N*-diisopropylethylamine (DIEA), the *N,S*-Bis(Mpt)-L-cysteine methyl ester (**1**) was obtained in a 79% yield as a chromatographically homogeneous oil. Because of the high reactivity of the Mpt–Cl, a Schotten-Baumann-type reaction of L-cysteine with the Mpt–Cl could also be performed without difficulty to afford *N,S*-Bis(Mpt)-L-cysteine (**2**) in a 50% yield as its dicyclohexylamine (DCHA) salt. From **1** and **2**, the *N*-Mpt group could be removed selectively by treatment with HCl reagents to give the *S*-Mpt-L-cysteine methyl ester hydrochloride (**3**) and *S*-Mpt-L-cysteine hydrochloride (**4**) in quantitative and 89% yields respectively:

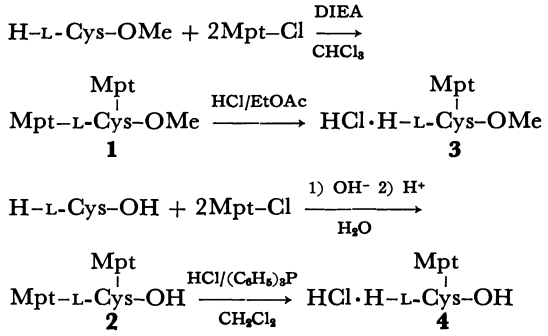


Table 1. Stability of the *S*-Mpt group

Compound	Reagent	Equiv.	Temp °C	Time h	Reaction[a]
Mpt-L-Cys(Mpt)-OMe	2 M† HCl/1 M Ph_3P/CH_2Cl_2	50	RT	24	None
	2 M HCl/EtOAc	50	RT	24	None
	1 M HCl/CH_3OH	50	RT	24	None
	2 M HCl/AcOH	50	RT	24	None
H-L-Cys(Mpt)-OH	1 M HCl/H_2O	100	RT	24	None[b]
	1 M HCl/H_2O	100	100	15 min	Partial
	6 M HCl/H_2O	120	110	20	Complete
	25% HBr/AcOH	100	RT	30 min	Partial
	TFA	100	RT	24	None

a) Detected by TLC. b) Determined by means of an amino-acid-analyzer. † 1 M=1 mol dm^{-3}.

Although these results thus already presented good proof of the acid stability of the *S*-Mpt group, it was further tested under various kinds of acidic conditions; the results are shown in Table 1.

The *S*-Mpt group is stable toward dry hydrogen chloride in organic solvents and trifluoroacetic acid (TFA), although the group is partly cleaved by HBr in acetic acid, probably because of the presence of small amounts of bromine molecules. Under the strongly acidic conditions used for the hydrolysis of peptides prior to amino-acid analysis (6 M HCl, 110 °C 20 h), the P–S bond was also hydrolyzed.

Under the conditions of alkaline hydrolysis, the *S*-Mpt group was removable, while the removal reaction was sometimes accompanied by β-elimination to give dehydroalanine (*⊿*Ala) derivatives. For example, **4** was hydrolyzed completely within 2 h at room temperature by treatment with 4 molar equiv. of a 1 M NaOH solution to regenerate cysteine, but the *t*-butoxycarbonyl-(Boc)-L-alanyl-*S*-Mpt-L-cysteine methyl ester (**6**) gave a 2.5 : 1 mixture of Boc-L-alanyl-L-cysteine and Boc-L-alanyldehydroalanine:

Boc-L-Ala-L-Cys(Mpt)-OMe → Boc-L-Ala-L-Cys-OH (hydrolysis)
Boc-L-Ala-*⊿*Ala-OH (β-elimination)

The elimination reaction was found to occur also by means of the action of a tertiary base. If this side reaction is such that it can not be avoided in the presence of any tertiary base, the *S*-Mpt-L-cysteine residue will lose its merit as a synthetic unit. Therefore, the dependence of the *⊿*Ala formation upon the base strength was examined by using **6** as a model compound. The reaction was carried out in a sample tube for NMR spectroscopy measurements, and the yield of the Boc-L-alanyldehydroalanine methyl ester (**7**) produced was obtained from the intensity of the ester methyl signal of **7**. The

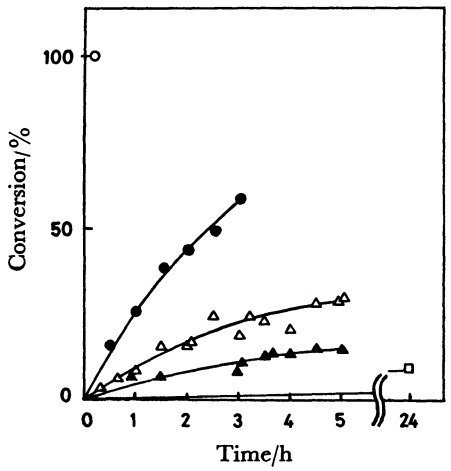


Fig. 1. Effects of the structure of tertiary amines on the dehydroalanine formation

Boc-L-Ala-L-Cys(Mpt)-OMe —Base, $CDCl_3$→ Boc-L-Ala-*⊿*Ala-OMe

(○: DBU, ●: triethylamine, △: tributylamine, ▲: 1,2,2,6,6-pentamethylpiperidine, □: DIEA)

Table 2. Removal of the *S*-Mpt group from H-L-Cys-(Mpt)-OH by $AgNO_3$ or $Hg(OAc)_2$ at 0 °C in H_2O

Reagent	Equiv.	Time h	Amino-acid ratio			
			Gly[a]	Cys	Cys(Mpt)	Unknown
$AgNO_3$	2	1	1	0.78	0	+
$AgNO_3$	3	1	1	0.95	0	+
$AgNO_3$	4	20 min	1	0.95	0	±
$AgNO_3$	4	30 min	1	1.10	0	−
$Hg(OAc)_2$	1	1	1	0.41	0.24	+
$Hg(OAc)_2$	2	1	1	0.77	0	+
$Hg(OAc)_2$	3	1	1	1.02	0	−

a) Internal standard.

results summarized in Fig. 1 show that the *S*-Mpt group is stable enough in the presence of such a bulky base as DIEA to allow the repeated treatments with this base which are necessary to neutralize acid salts of peptide amines containing the *S*-Mpt-L-cysteine residue during synthesis. It may also be noted that the *S*-Mpt-L-cysteine residue can be converted to the *⊿*Ala residue so readily by the action of 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) as to make *S*-Mpt-L-cysteine a new and easily available precursor of *⊿*Ala.

The removal of the *S*-Mpt group without any accompanying *⊿*Ala formation was accomplished by utilizing $AgNO_3$ or $Hg(OAc)_2$ as a catalyst for the hydrolysis. The removal conditions were studied by using **4** (neutralized by NH_4OH) as a substrate, since **4** was eluted from the standard amino-acid-analyzer column in the same position with glutamic acid and could be easily monitored. From these reactions it was found that an unexpected intermediate was produced. This intermediate, which could not be isolated, was also hydrolyzed by the use of the metallic reagents in excess, as is shown in Table 2. In order to elucidate the structure of the intermediate, the reaction was attempted by using a dipeptide model, which might produce a more stable intermediate and a dehydroalanine derivative. A starting dipeptide, H-L-Ala-L-Cys(Mpt)-OH (**9**), and the expected products, H-L-Ala-L-Cys-OH (**10**) and H-L-Ala-*⊿*Ala-OH (**11**), were prepared according to the procedures illustrated in Fig. 2.

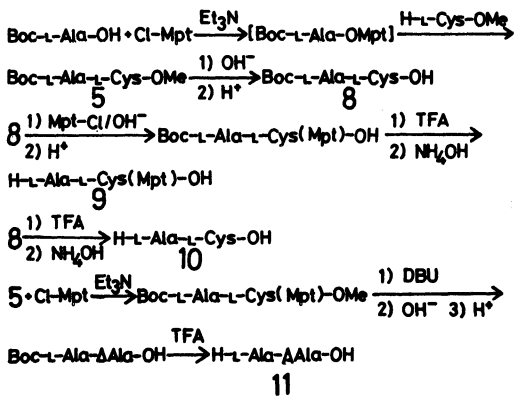
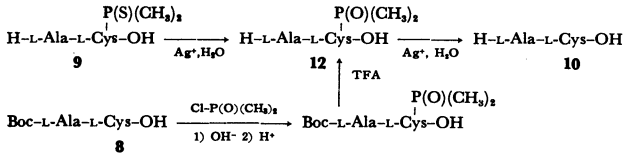


Fig. 2. Synthesis of model peptides.

Elution patterns from an amino-acid-analyzer column of a mixture of products from the reaction of **9** with $AgNO_3$ are shown in Fig. 3. Although the starting

material, **9** (elution volume=110 cm³), disappeared within 60 min at 0 °C upon the use of 2 molar equiv. of $AgNO_3$, the reaction was still incomplete, as was shown by the presence of an intermediate. When a 4 molar excess of the reagent was used, complete deprotection occurred to afford **10** (elution volume =115 cm³), without any accompanying dehydroalanine formation (elution volume of **11**=132 cm³). In order to elucidate the structure of the intermediate, it was isolated from a separate reaction mixture by ion-exchange chromatography to give a considerably pure solid material, which showed methyl-proton signals coupled with P, but different from those for the Mpt group. Based on this result, it was speculated that the structure of the compound was the *S*-dimethylphosphinyl (Dmp=$-P(O)(CH_3)_2$) derivative of **10**, H–L-Ala–L-Cys(Dmp)–OH (**12**), produced by the oxidative desulfurization of **9**:



This speculation was confirmed by analyzing a product prepared by the reaction of **8** with dimethylphosphinyl chloride, followed by *N*-deprotection by TFA. As expected, a peak appeared at the same position as the unknown product described above. This result does not necessarily mean that the removal of the *S*-Mpt group by $AgNO_3$ proceeds only through the intermediate formation of the Dmp derivative, but the consumption of $AgNO_3$ being more than the expected amount would be explained by this extra oxidation.

As an application of the Mpt group as a thiol-protecting group, the synthesis of glutathione was attempted according to the scheme illustrated in Fig. 4.

The removal of the *S*-Mpt group was performed either at the final stage (Method A) or before the removal of the other protecting groups (Method B); the latter gave a better yield.

Further applications of the *S*-Mpt-L-cysteine derivatives for the synthesis of cysteine and dehydroalanine containing peptides are now in progress.

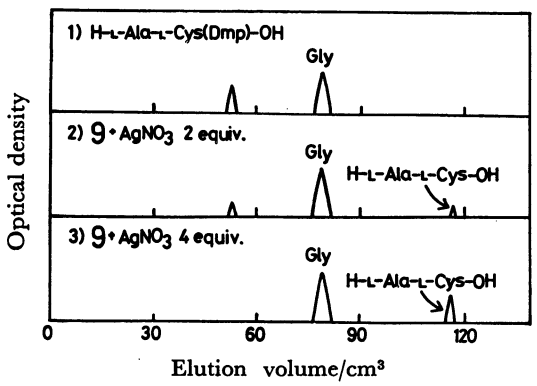


Fig. 3. Elution patterns of a model peptide, glycine (internal standard) and products from the deprotection reaction of H–L-Ala–L-Cys(Mpt)–OH by $AgNO_3$.

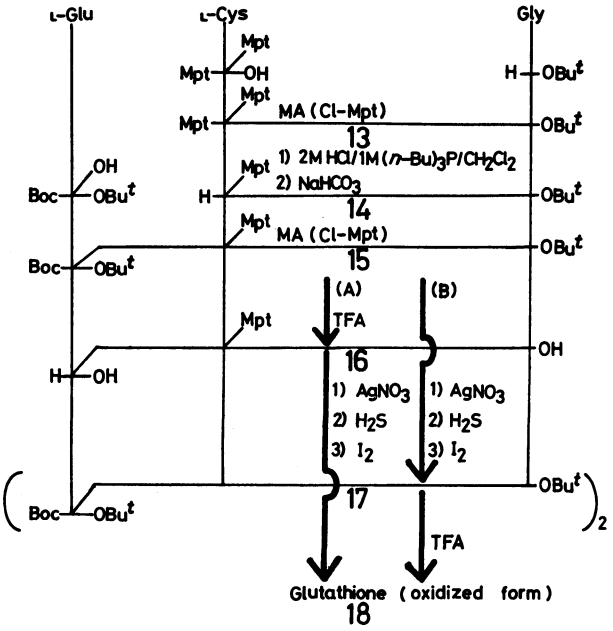


Fig. 4. Synthesis of glutathione.

Experimental

Thin-layer chromatography (TLC) was performed on silica gel plates (Merck $60F_{254}$) in the following solvent systems: dichloromethane (R_f^1), ether (R_f^2), ethyl acetate (R_f^3), chloroform–methanol–acetic acid (95 : 5 : 3, R_f^4), chloroform–methanol–acetic acid (85 : 25 : 20, R_f^5), chloroform–methanol–acetic acid–water (45 : 30 : 6 : 9, R_f^6), phenol–water (4 : 1 w/v, R_f^7), and 1-butanol–ethyl acetate–acetic acid–water (1 : 1 : 1 : 1, R_f^8). The products were detected on TLC plates using ultraviolet light, iodine vapor, ninhydrin, and the sodium nitroprusside reagent.

N,S-Bis(dimethylphosphinothioyl)-L-cysteine Methyl Ester (Mpt-L-Cys(Mpt)-OMe) (1). A solution of Mpt–Cl[3] (1.29 g, 10 mmol) in 10 cm³ of chloroform was added to a suspension of HCl·H–L-Cys–OMe (0.858 g, 5 mmol) in 10 cm³ of chloroform and 2.1 cm³ (15 mmol) of *N,N*-diisopropylethylamine at 0 °C. After having been stirred for 3 h at 0 °C, the solution was evaporated *in vacuo*. The residue was dissolved in ethyl acetate. The solution was washed successively with water, an ice-cold 5% citric acid solution, water, a 5% sodium hydrogencarbonate solution, water, and a saturated sodium chloride solution, dried over anhydrous sodium sulfate, and evaporated. The oily residue was dissolved in dichloromethane and placed in a silica-gel column (2×10 cm). Subsequent elution with dichloromethane gave **1** as colorless oil; 1.26 g (79%). $[\alpha]_D^{24}$ −13.0° (*c* 1, ethanol); R_f^1 0.60, R_f^2 0.76. Found: C, 29.91; H, 6.30; N, 4.36%. Calcd for $C_8H_{19}NO_2P_2S_3$: C, 30.09; H, 6.00; N, 4.39%.

N,S-Bis(dimethylphosphinothioyl)-L-cysteine Dicyclohexylamine Salt (Mpt-L-Cys(Mpt)-OH·DCHA) (2). L-Cys (2.24 g, 20 mmol) was dissolved in a 1 M sodium hydroxide solution (20 cm³), and then Mpt–Cl (5.65 g, 44 mmol) in ether (40 cm³) was added, drop by drop, at 0 °C. While the mixture was being stirred vigorously, the 1 M sodium hydroxide solution was added carefully, at a rate chosen so as to maintain the pH of the solution between 9.0 and 9.5, until no drop in the pH was observed. Then the solution was extracted twice with ethyl acetate. The aqueous layer was acidified (pH 3—4) with solid citric acid at 0 °C and then extracted with ethyl acetate several times. The organic layer was washed with

water, dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The oily residue was dissolved in ethyl acetate and treated with DCHA (4 cm³, 20 mmol). DCHA salt was collected by filtration, washed with ethyl acetate and ether, and dried. Subsequent recrystallization from ethanol gave **2** as colorless crystals; 5.0 g (50%). mp 150—151 °C; $[\alpha]_D^{24}$ −6.2° (*c* 1, methanol); R_f^4 0.35, R_f^5 0.88. Found: C, 46.34; H, 8.14; N, 5.91; P, 11.97%. Calcd for $C_{19}H_{40}N_2O_2P_2S_3 \cdot 1/2\ H_2O$: C, 46.04, H, 8.34; N, 5.65; P, 12.49%.

S-Dimethylphosphinothioyl-L-cysteine Methyl Ester Hydrochloride (H-L-Cys(Mpt)-OMe·HCl) (3). Compound **1** (9.65 g, 30 mmol) was stirred with 2.2 M HCl in ethyl acetate at room temperature for 2.5 h. The solvent was then removed *in vacuo*, and the residue was dissolved in methanol. The solution was treated with Amberlyst A-21 resin to neutralize it and to remove a small amount of dimethylphosphinothioic acid (Mpt–OH), and then filtered. The filtrate was treated with an equimolar amount of HCl in methanol and evaporated to give a chromatographically homogeneous oil; 8.19 g (quantitative). R_f^4 0.14, R_f^5 0.58.

S-Dimethylphosphinothioyl-L-cysteine Hydrochloride (H-L-Cys(Mpt)-OH·HCl) (4). Compound **2** (4.87 g, 10 mmol) was separated between a 5% citric acid solution and ethyl acetate. The ethyl acetate layer was washed with water, dried, and evaporated. The residue was dissolved in methanol (4 cm³) and dichloromethane (30 cm³), 2 M HCl/1 M Ph_3P/CH_2Cl_2[3] (15 cm³) was added at 0 °C, and the mixture was kept standing at room temperature for 3 h. The crystalline precipitate was then collected by filtration, washed with CH_2Cl_2, and dried over P_2O_5; 2.22 g (89%). An analytical sample was recrystallized from methanol–CH_2Cl_2–ether; mp 157—158 °C (decomp); $[\alpha]_D^{24}$ −18.4° (*c* 1, methanol); R_f^5 0.21. Found: C, 24.13; H, 5.54; N, 5.53; P, 12.61%. Calcd for $C_5H_{13}NO_2PS_2Cl$: C, 24.09; H, 5.26; N, 5.61; P, 12.40%.

N-(t-Butoxycarbonyl)-L-alanyl-L-cysteine Methyl Ester (Boc-L-Ala-L-Cys-OMe) (5). Mpt–Cl (2.57 g, 20 mmol) was added to an ice-cold, stirred solution of Boc-L-Ala-OH (3.78 g, 20 mmol) and triethylamine (2.80 cm³, 20 mmol) in 20 cm³ of chloroform, after which the mixture was stirred for 1 h.[6] To this solution, a mixture of HCl·H-L-Cys-OMe (3.43 g, 20 mmol) and triethylamine (5.6 cm³, 40 mmol) in 80 cm³ of chloroform was added in several portions at 0 °C. After having been kept stirring for 1 h at this temperature, the solution was washed in the usual way, dried, and evaporated *in vacuo*. The solidified residue was washed with ether and recrystallized from ethyl acetate–petroleum ether to give colorless crystals; 3.62 g (59%). mp 110—112 °C; $[\alpha]_D^{26}$ −35.0 °C (*c* 1, ethanol); R_f^2 0.16, R_f^3 0.62. Found: C, 46.74; H, 7.10; N, 9.29%. Calcd for $C_{12}H_{22}N_2O_5S$: C, 47.04; H, 7.24; N, 9.11%.

N-(t-Butoxycarbonyl)-L-alanyl-S-dimethylphosphinothioyl-L-cysteine Methyl Ester (Boc-L-Ala-L-Cys(Mpt)-OMe) (6). To an ice-cold solution of Boc-L-Ala-L-Cys-OMe (1.53 g, 5 mmol) and *N,N*-diisopropylethylamine (0.871 cm³, 5 mmol) in 5 cm³ of chloroform, Mpt–Cl (0.643 g, 5 mmol) was added, after which the mixture was stirred continuously for 1 h at 0 °C and for 3 h at room temperature. The solution was washed in the usual manner, dried over anhydrous sodium sulfate, and evaporated *in vacuo*. The solid residue was recrystallized from ethyl acetate–petroleum ether to give colorless crystals; 1.69 g (85%). mp 106—108 °C; $[\alpha]_D^{24}$ −28.5° (*c* 1, ethanol); R_f^2 0.38, R_f^3 0.72. Found: C, 42.04; H, 6.81; N, 7.18%. Calcd for $C_{14}H_{27}N_2O_5PS_2$: C, 42.20; H, 6.83; N, 7.03%.

N,S-Bis(dimethylphosphinothioyl)-L-cysteinylglycine t-Butyl Ester (Mpt-L-Cys(Mpt)-Gly-OBu^t) (13). Compound **2** (803 mg, 1.65 mmol) was dissolved in 4 cm³ of chloroform and treated with Mpt–Cl (212 mg, 1.65 mmol) at 0 °C. To this solution a solution of the glycine *t*-butyl ester (217 mg, 1.65 mmol) and *N,N*-diisopropylethylamine (0.29 cm³, 1.65 mmol) in 2 cm³ of chloroform was added, after which the solution was stirred for 2 h at 0 °C. After the solvent had been changed to ethyl acetate, the solution was washed with water, an ice-cold 5% citric acid solution, water, a 5% sodium hydrogencarbonate solution, water, and a saturated sodium chloride solution, and dried. After the removal of the solvent *in vacuo*, an oily residue was dissolved in dichloromethane and the solution was placed in a silica-gel column (1.7×20 cm). Subsequent elution by ether gave colorless crystals; 629 mg (91%). An analytical sample was recrystallized from ethyl acetate–petroleum ether; mp 110—112 °C; $[\alpha]_D^{20}$ −24° (*c* 1, methanol); R_f^2 0.17, R_f^3 0.54. Found: C, 37.03; H, 6.84; N, 6.45%. Calcd for $C_{13}H_{28}N_2O_3P_2S_3$: C, 37.31; H, 6.74; N, 6.69%.

S-Dimethylphosphinothioyl-L-cysteinylglycine t-Butyl Ester (H-L-Cys(Mpt)-Gly-OBu^t) (14). Compound **13** (1.88 g, 4.49 mmol) was treated with 2 M HCl in dichloromethane containing 1 M of tributylphosphine (13.45 cm³). After having been stirred at 0 °C for 2.5 h and at room temperature for 6.5 h, the solution was evaporated. The residue was separated between water and ethyl acetate. The aqueous layer was washed with ethyl acetate, the pH was adjusted to 9 by adding solid sodium hydrogencarbonate, and extracted several times with ethyl acetate. The combined extracts were washed with water, dried over anhydrous sodium sulfate, and evaporated. The oily residue was purified by passing its ethyl acetate solution through a short silica-gel column (3×12 cm) to give a chromatographically homogeneous oil; 1.45 g (99%). R_f^4 0.21, R_f^5 0.68.

N-(t-Butoxycarbonyl)-L-γ-glutamyl-S-dimethylphosphinothioyl-L-cysteinylglycine Di-t-butyl Ester

$$\left(\begin{array}{l} \text{Boc-L-Glu-OBu}^t \\ \text{L-Cys(Mpt)-Gly-OBu}^t \end{array}\right) \textbf{(15)}.$$

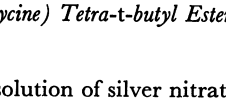
To an ice-cold solution of Boc-L-Glu-OBu^t·DCHA (737 mg, 1.52 mmol) in 3 cm³ of chloroform, Mpt–Cl (195 mg, 1.52 mmol) was added in several portions, after which the solution was stirred at 0 °C for 1 h. To this solution a mixture of Compound **14** (547 mg, 1.68 mmol) and *N,N*-diisopropylethylamine (0.29 cm³, 1.68 mmol) in 10 cm³ of chloroform was added. After having been stirred at 0 °C for 2 h, the solution was washed in the usual manner, dried, and evaporated *in vacuo*. The residue was purified by silica-gel column chromatography. After the elution of a small amount of symmetric dimethylphosphinothioic anhydride (Mpt–O–Mpt), Compound **15** was obtained as an amorphous solid by eluting with CH_2Cl_2 and then with CH_2Cl_2–methanol (9/1, v/v); 840 mg (90%). $[\alpha]_D^{20}$ −40° (*c* 1, methanol); R_f^2 0.25, R_f^3 0.74. Found: C, 48.77; H, 7.70; N, 6.54%. Calcd for $C_{25}H_{46}O_8N_3S_2P \cdot 1/2\ CH_3OH$: C, 48.79; H, 7.71; N, 6.69%.

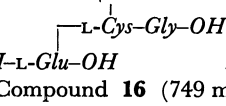
L-γ-Glutamyl-S-dimethylphosphinothioyl-L-cysteinylglycine

$$\left(\begin{array}{l} \text{H-L-Glu-OH} \\ \text{L-Cys(Mpt)-Gly-OH} \end{array}\right) \textbf{(16)}.$$

Trifluoroacetic acid (16.55 cm³) was added to an ice-cold solution of Compound **15** (1.093 g, 1.79 mmol) in 9 cm³ of CH_2Cl_2 containing anisole (3.57 cm³). After having been kept standing at room temperature overnight, the solution was evaporated. The residue was separated between water and ether, and the organic layer was extracted several times with water. The combined aqueous layer and extracts were evaporated *in vacuo*. Then the solid residue was dissolved in water, and the solution was placed in a column (1.2×51 cm) of Dowex 1-X2 ion-exchange resin (acetate form). The column was eluted with 8% acetic acid. The desired fractions (172—277 cm³) were pooled and evaporated *in vacuo* to give **16** as colorless crystals; 0.629 g (85%). An analytical sample was recrystallized from hot

water; mp 202—203 °C; $[\alpha]_D^{20}$ −23° (*c* 1, H_2O); R_f^6 0.23, R_f^8 0.42. Found: C, 34.89; H, 5.42; N, 10.08%. Calcd for $C_{12}H_{22}O_6N_3PS_2 \cdot 2/3\ H_2O$: C, 35.03; H, 5.72; N, 10.21%.

N,N′-[*Bis*[N-(*t-butoxycarbonyl*)-L-*γ-glutamyl*]-L-*cystinyl*]*bis*-(*glycine*) *Tetra*-t-*butyl Ester*  (**17**). A solution of silver nitrate (340 mg, 2 mmol) in water (5 cm³) was added to a solution of Compound **15** (306 mg, 0.5 mmol) in methanol (10 cm³) containing pyridine (0.16 cm³, 2 mmol) at 0 °C. The mixture was stirred at 0 °C for 1 h. H_2S gas was then bubbled through the solution at 0 °C for 1 h. The precipitate was removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was dissolved in ethyl acetate, and the solution was washed with water, dried and evaporated. The residue was dissolved in CH_2Cl_2, and the solution was placed in a column (0.6×25 cm) of silica gel which was then eluted with ether. The eluates were combined and evaporated *in vacuo* to give a colorless oil; 261 mg (100%). R_f^2 0.31, R_f^3 0.76. This material was dissolved in methanol (5 cm³) containing pyridine (0.09 cm³, 1.1 mmol). To this a 0.1 M I_2–KI solution in 90% ethanol (2.63 cm³) was added at 0 °C. After this solution had been stirred for 1 h, the organic solvents were removed. The residue was dissolved in ethyl acetate, and the solution was washed with water and a sodium thiosulfate solution, dried, and evaporated. The residue was dissolved in CH_2Cl_2, and the solution was placed in a column (0.6×25 cm) of silica gel which was then eluted with CH_2Cl_2, followed by ether. The eluates were combined and evaporated *in vacuo* to give **17** as colorless crystals; 242 mg (93%). mp 102—107 °C; $[\alpha]_D^{20}$ −73° (*c* 1, methanol); R_f^2 0.42, R_f^3 0.84. Found: C, 53.12; H, 7.85; N, 8.05%. Calcd for $C_{46}H_{80}O_{16}N_6S_2$: C, 53.26; H, 7.77; N, 8.10%.

Glutathione (Oxidized Form)  (**18**). *Method A*: To a solution of Compound **16** (749 mg, 1.63 mmol) in water (70 cm³) containing pyridine (0.58 cm³, 7.15 mmol), 7.15 cm³ of 1 M silver nitrate solution was added at 0 °C. The mixture was then stirred at 0 °C for 1 h. H_2S gas was bubbled through the solution at 0 °C for 1 h. The precipitate was removed by filtration, and the filtrate was evaporated *in vacuo*. The residue was dissolved in a small volume of water and methanol, after which the solution was adjusted to pH 7 with pyridine at 0 °C and treated with iodine (232 mg, 1.83 mmol) in methanol (10 cm³). After the removal of the methanol *in vacuo*, the solution was placed in a column (1.2×61 cm) of Dowex 1-X2 ion-exchange resin (acetate form). The column was eluted with water (200 cm³) and then 10 % acetic acid. The desired fractions were colcollected, evaporated *in vacuo*, and dried over P_2O_5 to give **18** as colorless crystals; 270 mg (52%).

Method B: To a solution of Compound **17** (1.017 g, 0.98 mmol) in CH_2Cl_2 (20 cm³) containing anisole (5.11 cm³), trifluoroacetic acid (36.23 cm³) was added at room temperature. After having been kept standing overnight, the solvent was evaporated *in vacuo*; the residue was dissolved in water and placed in a column of Dowex 1-X2 ion-exchange resin (acetate form). Elution as described in Method A gave colorless crystals; 602 mg (97%). An analytical sample was recrystallized from water–ethanol; mp 178—180 °C; $[\alpha]_D^{20}$ −105° (*c* 1, H_2O) (lit,[7] mp 175—195 °C; $[\alpha]_D^{27}$ −92.8° (*c* 1.2, H_2O), lit,[8] $[\alpha]_D$ −94° (*c* 1, H_2O)); R_f^7 0.03, R_f^8 0.13. Found: C, 37.89; H, 5.70; N, 13.03%. Calcd for $C_{20}H_{32}N_6O_{12}S_2 \cdot H_2O$: C, 38.09; H, 5.43; N, 13.33%.

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