L-CYSTATHIONINE DERIVATIVES FOR THE SYNTHESIS OF PEPTIDES*

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The preparation is described of L-cystathionine derivatives with amino protecting groups, which can readily be cleaved acidolytically and permit a selective acylation of both amino groups, and of a derivative with carboxyls protected by different groups.

Some time ago[1] we described certain cystathionine** derivatives suitable for the synthesis of the so-called carba analogs of biologically active peptides, *i.e.* of such analogs in which cystine is replaced by cystathionine. The synthesis of additional compounds of this type[4] required that the series of derivatives *IIb* through *IIe* (ref.[1]) and *IIf* (ref.[5]) prepared earlier be complemented by derivatives with amino groups protected by groups which are cleaved more easily than the benzyloxycarbonyl group. In this study we describe the preparation of derivatives *IIj* through *III*, in which of most stable amino protecting group is the tert-butyloxycarbonyl group, and derivative *IIm* with the *p*-methoxybenzyloxycarbonyl group. Likewise, the alternately protected N^{α}- and $N^{\alpha'}$-amino group of derivatives *IIj* and *III* is important for the selective extension of the peptide chain at both amino termini of the cystathionine derivative. A similar possibility at the carboxyl terminus offer derivatives *IIg* and *IIh*.

The derivatives of α-amino-γ-bromobutyric acid were used to start with. Tert-butyloxycarbonyl derivative *Ib* was prepared from α-amino-γ-bromobutyric acid methyl ester hydrochloride[1] by the reaction of the corresponding isocyanate with tert-butanol, by acylation with tert-butyloxycarbonyl azide, and with the aid of 2-(tert-butyloxycarbonyloxyimino)-2-phenylacetonitrile[6]. The relatively low yields of all procedures are caused by the strong tendency of the starting ester to form a lactone ring both in weakly basic and neutral media. Similar observations were made also during the preparation of *p*-methoxybenzyloxycarbonyl derivative *Ic*.

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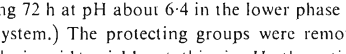
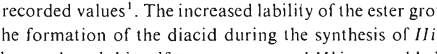
** The amino acids in this study are of L-configuration. The nomenclature and symbols of amino acids and their derivatives comply with the proposal published before[2]; see papers[1,3] for cystathionine derivatives.

The tert-butyloxycarbonyl derivative of cystathionine *IIj* was prepared by reduction of cystine by sodium in liquid ammonia and alkylation of sulfur *in situ* by N-tert-butyloxycarbonyl-α-amino-γ-bromobutyric acid methyl ester (*Ib*). The *p*-methoxybenzyloxycarbonyl derivative of cystathionine *IIm* was prepared in an analogous manner using *Ic*. Cystathionine derivative *III* which is an isomer of *IIj* described above, was synthetized from N-tert-butyloxycarbonylcysteine, obtained from N,N'-bis(tert-butyloxycarbonyl)cystine[7,8] by reduction by sodium in liquid ammonia, by alkylation with α-amino-γ-bromobutyric acid methyl ester hydrochloride. The product, which was purified by countercurrent distribution, shows increased lability even in weakly basic media. (The methyl ester was hydrolyzed to about 20% during 48 h and to 40% during 72 h at pH about 6·4 in the lower phase of the contercurrent distribution solvent system.) The protecting groups were removed from derivative *III* in boiling hydrochloric acid to yield cystathionine *IIa* the optical rotation of which corresponded to recorded values[1]. The increased lability of the ester group is probably responsible for the formation of the diacid during the synthesis of *IIi* by the action of sodium benzyloxycarbonyl thiosulfate on compound *III* in a weakly basic medium. When derivative *IIi* is synthetized from derivative *IIb* by acylation using 2-(tert-butyloxycarbonyloxyimino)-2-phenylacetonitrile the byproduct is not formed yet the small quantity of contaminants prevents dicyclohexylammonium salt *IIi* from crystallization (which is not the case with the chromatographically purified preparation). Similar difficulties were encountered also during the preparation of cystathionine derivative *IIk* by treatment of compound *III* with *o*-nitrobenzenesulfenyl chloride.



Ia, R^1 = Z, R^2 = CH_3; *Ib*, R^1 = Boc, R^2 = CH_3; *Ic*, R^1 = Z(OCH_3), R^2 = CH_3



IIa,	R^1 = H,	R^2 = H, R^3 = H,	R^4 = H;
IIb,	R^1 = Z,	R^2 = CH_3, R^3 = H,	R^4 = H;
IIc,	R^1 = Z,	R^2 = CH_3, R^3 = Nps,	R^4 = H;
IId,	R^1 = Z,	R^2 = CH_3, R^3 = Tos,	R^4 = H;
IIe,	R^1 = Z,	R^2 = H, R^3 = H,	R^4 = H;
IIf,	R^1 = Z,	R^2 = CH_3, R^3 = Z(OCH_3),	R^4 = H;
IIg,	R^1 = Z,	R^2 = CH_3, R^3 = H,	R^4 = t-C_4H_9
IIh,	R^1 = Z,	R^2 = H, R^3 = H,	R^4 = t-C_4H_9
IIi,	R^1 = Z,	R^2 = CH_3, R^3 = Boc,	R^4 = H
IIj,	R^1 = Boc,	R^2 = CH_3, R^3 = H,	R^4 = H
IIk,	R^1 = Nps,	R^2 = CH_3, R^3 = Boc,	R^4 = H;
III,	R^1 = H,	R^2 = CH_3, R^3 = Boc,	R^4 = H;
IIm,	R^1 = Z(OCH_3),	R^2 = CH_3, R^3 = H,	R^4 = H

Derivative *IIg* bearing an ester group readily split off by acidolysis was prepared by treatment of *IIb* with 2-methyl-1-propene. The cleavage of the tert-butyl ester group under the conditions of alkaline hydrolysis has been recorded in literature[9-13] several times earlier. In our experiments too compound *IIe* was formed in addition to derivative *IIh* when two equivalents of sodium hydroxide were used. When one base equivalent was used for the alkaline hydrolysis practically pure product *IIh* was obtained.

EXPERIMENTAL

The melting points were determined on a Kofler block and are not corrected. The samples for elemental analysis were dried 24 h at room temperature and 150 Pa; amorphous products, oils and foams, were dried 48 to 96 h at 40°C. Thin-layer chromatography was carried out on silica gel (Silufol, Kavalier) in the following solvent systems: 2-butanol–98% formic acid–water (75 : 13·5 : 11·5) (S1), 2-butanol–25% aqueous ammonia–water (85 : 7·5 : 7·5) (S2), 1-butanol–acetic acid–water (4 : 1 : 1) (S3), 1-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 6) (S4), n-heptane–tert-butanol–pyridine (5 : 1 : 1) (S5), 2% ethanol in benzene (S6), 1-butanol–acetic acid–ethyl acetate–water (1 : 1 : 1 : 1) (S7), 5% methanol in benzene (S8), and 20% methanol in benzene (S9). Paper electrophoresis was carried out in a wet chamber in 1M acetic acid (pH 2·4) and in pyridine acetate buffer (pH 5·7) on Whatman No 3MM paper 60 min at a potential gradient of 20 V/cm. The detection was effected by ninhydrin or chlorination. The reaction mixtures were evaporated in a vacuum rotary evaporator at bath temperatures of 30–40°C, using the vacuum of a water aspirator (at 150 Pa when mixtures containing dimethylformamide were evaporated). The IR spectra were measured in model UR 20 Zeiss Jena Spectrophotometer in chloroform unless stated otherwise.

Tert-butyloxycarbonyl-α-amino-γ-bromobutyric Acid Methyl Ester (*Ib*)

a) via *Isocyanate*: Phosgene was introduced into a suspension of α-amino-γ-bromobutyric acid methyl ester hydrochloride[1] (14 g) in dioxane (200 ml) at 50°C for 1 h until the ester was completely solubilized. Nitrogen was then passed 15 min through the solution, dioxane was distilled off, and the residue was dissolved in toluene (200 ml). The solution was treated at 120°C 20 min with phosgene and subsequently 10 min with nitrogen. The solution was decanted off from the brown oil which had separated, toluene was evaporated, and the residue was distilled (b.p. 85–90°C at 30 Pa). The yield was 7·89 g (69%) of the isocyanate. Tert-butanol (11·4 ml) and N-ethylpiperidine (4·7 ml) were added to the dry residue. The mixture was allowed to stand 36 h at room temperature, then taken to dryness, the residue dissolved in ethyl acetate, shaken with a 20% solution of citric acid and water, dried by sodium sulfate and evaporated. The dry residue was placed onto a silica gel column (2·5 × 15 cm; particle size 5–30 μ) in benzene. The individual fractions were identified by thin-layer chromatography on silica gel (S6). The pure product was isolated from fractions emerging between 400 and 900 ml, evaporated and crystallized under light petroleum. The yield was 4·16 g (23%) of a product of m.p. 39–41°C; R_F 0·77 (S1), 0·66 (S2), 0·73 (S4), 0·44 (S6). For $C_{10}H_{18}BrNO_4$ (296·2) calculated: 40·55% C, 6·13% H, 4·73% N; found: 42·08% C, 6·37% H, 4·86% N. (The differences in the analytical values are obviously due to the presence of tert-butyloxycarbonyl-α-aminobutyrolactone.) $[\alpha]_D^{25}$ −37·0° (*c* 0·5; methanol). IR spectrum: 1743 cm^{-1} (s), 1440 cm^{-1} (m), ($COOCH_3$); 3440 cm^{-1} (m), 1713 cm^{-1} (s), 1505 cm^{-1} (s), (OCO—NH); 1394 cm^{-1} (w), 1368 cm^{-1} (m), ($(CH_3)_3C$).

b) via *Tert-butyloxycarbonyl azide*: A solution of α-amino-γ-bromobutyric acid methyl ester hydrochloride (4·6 g) in dioxane (50 ml) and 1M-$NaHCO_3$ (20 ml) was treated with tert-butyloxycarbonyl azide (5·6 ml). The pH of the reaction mixture was kept constant at pH 7·0 by 1M-NaOH for 7 days. Two additional 2·8 ml portions of tert-butyloxycarbonyl azide were added to the reaction mixture after 48 and 96 h. Lastly, dioxane was evaporated and the product was extracted with ethyl acetate; the ethyl acetate extract was shaken with HSO_4^- buffer (pH 2), water, 0·5M-$NaHCO_3$ and water, dried by sodium sulfate and azeotropically (benzene). The dry residue was purified by chromatography on the silica gel column under the conditions described under *a*). The yield was 2·7 g (45%) of m.p. 39–42°C, chromategraphically identical with the product obtained as described under *a*).

c) via 2-(*tert-butyloxycarbonyloxyimino*)-2-*phenylacetonitrile*[6]: To a solution of α-amino-γ-bromobutyric acid methyl ester hydrochloride (0·92 g) in dioxane (3 ml) and water (3 ml) were added triethylamine (1·4 ml) and 2-(tert-butyloxycarbonyloxyimino)-2-phenylacetonitrile (1·1 g). The mixture was stirred 3 h at room temperature, dioxane was evaporated off and the residue was extracted with ethyl acetate. The ethyl acetate extract was shaken with 0·1M-H_2SO_4, water, 0·5M-$NaHCO_3$, water, dried by sodium sulfate and evaporated. Light petroleum (100 ml) was added to the dry residue, heated to boiling point and decanted off from the insoluble residue (2-hydroxyimino-2-phenylacetonitrile). The light petroleum solution was stored at −20°C; after 14 days light petroleum was again separated from the crystals which had formed and evaporated. The dry residue was purified by chromatography on a silica gel column (2 × 20 cm; particle size 30–60 μ; benzene with 2% of ethanol as eluent). Fractions emerging in 40 to 90 ml were pooled, evaporated, and again purified on a silica gel column (2 × 36 cm; particle size 30–60 μ; benzene with 20% of methanol as eluent). Detection was effected by thin-layer chromatography (S9) on silica gel. The pure product was isolated from fractions emerging between 55 and 65 ml. The yield was 0·19 g (16%) of a product identical with that prepared as described under *a*).

N^{α}-*p*-Methoxybenzyloxycarbonyl-α-amino-γ-bromobutyric Acid Methyl Ester (*Ic*)

N^{α}-*p*-Methoxybenzyloxycarbonyl azide (4·25 g) was added to a solution of α-amino-γ-bromobutyric acid methyl ester hydrochloride (4·6 g) in a mixture of dioxane (50 ml), water (10 ml), and 0·5M-$NaHCO_3$. The pH of the reaction mixture was maintained 48 h at 7·5 by 1M-NaOH. Another 1·0 g portion of *p*-methoxybenzyloxycarbonyl azide was added to the reaction mixture after 12 and 24 h. Finally dioxane was evaporated and the product was extracted with ethyl acetate; the ethyl acetate extract was shaken stepwise with 0·5M-$NaHCO_3$, water, 0·25M-H_2SO_4, and water, then dried by sodium sulfate and evaporated. The dry residue was purified chromatographically an a silica gel column (2·5 × 33 cm; particle size 30–60 μ; benzene with 2% of ethanol as eluent). The individual fractions were detected by thin-layer chromatography on silica gel (S6). The product was isolated from fractions eluted in 180 to 260 ml. The yield was 1·24 g (17·5%) of a oily product. $[\alpha]_D^{25}$ −39·4° (*c* 0·5; methanol); R_F 0·30 (S5), 0·60 (S6). IR spectrum: 3 435 cm^{-1} (m), 1725 cm^{-1} (s), 1515 cm^{-1} (s), (O—CO—NH); 1 742 cm^{-1} (s), 1 441 cm^{-1} (m), ($COOCH_3$); 2845 cm^{-1} (w), 1 250 cm^{-1} (s) + aromatic bands (CH_2O-Ar); contaminated with azide 2110 cm^{-1}.

Besides *p*-methoxybenzyloxycarbonyl azide (below 180 ml) and *p*-methoxybenzyl alcohol (from 400 ml up) was isolated as byproduct (from 500 ml up) N-*p*-methoxybenzyloxycarbonyl-α-aminobutyrolactone, m.p. 114–115°C. $[\alpha]_D^{25}$ −35·0° (*c* 0·2; methanol). For $C_{13}H_{15}NO_5$ (265·3) calculated: 58·86% C, 5·70% H, 5·28% N; found: 58·87% C, 5·68% H, 5·44% N. IR spectrum: 2845 cm^{-1} (m), 1250 cm^{-1} (vs), + aromatic bands (CHO_3—Ar); 3430 cm^{-1} (m), 1723 cm^{-1} (vs), 1713 cm^{-1} (sh), 1518 cm^{-1} (vs), (O—CO—NH); 1785 cm^{-1} (vs), 1175 cm^{-1} (vs), (five-membered lactone ring).

N$^{\alpha'}$-Benzyloxycarbonyl-cystathionine α-Tert-butyl Ester α'-Methyl Ester (*IIg*)

Sulfuric acid (0·25 ml) and isobutylene (25 ml) were added to a suspension of derivative *IIb* (1·0 g) in dichloromethane (25 ml). The mixture was shaken in a sealed-off pressure vessel at room temperature for 4 days (*IIb* is entirely solubilized in 20 min). The solution was then taken to dryness the dry residue was dissolved in ethyl acetate, the solution was shaken with 0·5M-$NaHCO_3$, water, dried by sodium sulfate, and again taken to dryness. The dry residue was triturated with light petroleum (the light petroleum extracts were discarded) and applied onto a silica gel column (1·5 × 13 cm; particle size 30–60 μ, eluent n-heptane–tert-butanol–pyridine, 5 : 1 : 1). The fractions were detected by thin-layer chromatography on silica gel (S5). The pure product was isolated from fractions emerging in 30 to 56 ml; after evaporation an oily product was obtained. Yield: 0·67 g (58%); $[\alpha]_D^{25}$ +2·4° (*c* 0·5 benzene), −23·4° (*c* 0·34, methanol); $E_{2,4}^{Gly}$ 0·94; $E_{5,7}^{His}$ 0·52; R_F 0·49 (S2), 0·20 (S5), 0·30 (S6). For $C_{20}H_{30}N_2O_6S$ (426·5) calculated: 56·32% C, 7·09% H, 6·57% N; found: 56·59% C, 6·97% H, 6·43% N. IR spectrum: 3435 cm^{-1} (m), 1715 cm^{-1} (sh), 1515 cm^{-1} (m), (O—CO—NH); 1728 cm^{-1} (s), 1156 cm^{-1} (s), (COOR); 1441 cm^{-1} (sh), ($COOCH_3$); 1396 cm^{-1} (w), 1371 cm^{-1} (m), ($(CH_2)_3C$).

Hydrolysis of Derivative *IIg* by Excess of Sodium Hydroxide

1M-NaOH (6 ml) was added to a solution of derivative *IIg* (1·3 g) in methanol (20 ml), the mixture was set aside for 1 h at room temperature, methanol was evaporated and the residue was placed onto a Dowex 50W-X4 column (H^+-form; 40 ml). The column was washed with water and the product was eluted by 10% aqueous pyridine solution. The effluents were taken to dryness, dried azeotropically (benzene), and reprecipitated from methanol and ether. The yield was 0·7 g of a product which according to paper chromatography and electrophoresis represented a mixture of several compounds: R_F 0·52 and 0·30 (S1), 0·16 and 0·00 (S2), 0·57 and 0·12 (S4); $E_{2,4}^{Gly}$ 0·88, 0·29, 0·15; $E_{5,7}^{Glu}$ 0·00 and 0·75.

A part of the product was resolved preparatively (electrophoretically under the conditions described for analytical electrophoresis, in 1M acetic acid; the separation period was prolonged to 2 h). Strips cut off from the margins and the middle part of the electropherogram were used for the detection. The individual zones were eluted by dimethylformamide, the solvent was evaporated off and the material reprecipitated from methanol and ether. Three amounts of material were obtained; amount A, R_F-values 0·52 (S1), 0·16 (S2), 0·57 (S4); $E_{2,4}^{Gly}$ 0·88, $E_{5,7}^{His}$ 0·00; m.p. 85–88°C, identical with product *IIh*. The R_F-value of amount B was 0·30 (S1), 0·00 (S2), 0·12 (S4); $E_{2,4}^{Gly}$ 0·29; $E_{5,7}^{Glu}$ 0·77. The m.p. after recrystallization from water was 192–198°C; it corresponds to an authentic sample of derivative *IIe* in chromatographic and electrophoretic behavior and by the mixed melting point. Amount C, the R_F-value of which was 0·17 (S1), 0·16 (S3), $E_{2,4}^{Gly}$ 0·15; $E_{5,7}^{Glu}$ 0·72 and m.p. was 119–123°C, was obtained in a small quantity only and corresponds most likely to the sulfoxide of derivative *IIe*. It gave a positive test for sulfoxides[14] and its IR spectrum was the following; 1712 cm^{-1}, 1525 cm^{-1} (O—CO—NH); 700 cm^{-1} (C_6H_5); 2400–3400 cm^{-1}, 1645 cm^{-1}, 1525 cm^{-1}, 1400 cm^{-1} (Zwitter ion), 1030 cm^{-1} (SO). No protons of the tert-butyl group were revealed in the ^{1}H-NMR spectrum.

N$^{\alpha'}$-Benzyloxycarbonylcystathionine α-Tert-butyl Ester (*IIh*)

A solution of derivative *IIg* (0·22 g) in methanol (4 ml) was treated with 0·5M-NaOH (1·1 ml); after 40 min the pH of the mixture was adjusted to pH 6·0 (approximately) by 20% citric acid. Methanol was evaporated at room temperature and the dry residue was applied onto a column of Dowex 50W-X4 (H^+-form, 15 ml). The column was washed with water and the product was

eluted by 10% aqueous pyridine. The effluents were lyophilized and the material reprecipitated from methanol and ether. The yield was 50 mg (24%) of a product of m.p. 84–87°C. $[\alpha]_D^{25}$ −4·9° (*c* 0·2; methanol); $E_{2.4}^{Gly}$ 0·88; $E_{5.7}^{His}$ 0·00; R_F 0·52 (S1), 0·16 (S2), 0·44 (S3), 0·57 (S4). For $C_{19}H_{28}N_2O_6S.1\ H_2O$ (430·5) calculated: 53·01% C, 7·02% H, 6·51% N; found: 52·92% C, 6·52% H, 6·50% N.

Nᵅ-Tert-butyloxycarbonyl-Nᵅ′-benzyloxycarbonylcystathionine α′-Methyl Ester Dicyclohexylammonium Salt (*IIi*.DCHA)

a) *From benzyloxycarbonyl methyl ester* IIb. Triethylamine (0·42 ml) and 2-(tert-butyloxycarbonyloxyimino)-2-phenylacetonitrile[6] (0·55 g) were added to a solution of *IIb* (0·75 g) in water (4 ml) and dioxane (4 ml). The mixture was stirred 3 1/2 h, then diluted with water (10 ml) and extracted several times with ethyl acetate. The aqueous layer was acidified with a 5% solution of citric acid and the product was extracted with ethyl acetate. The ethyl acetate extract was shaken with water, dried by sodium sulfate and the solvent evaporated. The dry residue was dissolved in benzene, dicyclohexylamine (0·4 ml) was added and the solution was taken to dryness. The product was obtained either in the form of a foam or it was pulverized by triturationwith ether; the latter product was stable in the open air after drying in a desiccator. Yield 0·60 g (46%); $[\alpha]_D^{26}$ −13·7° (*c* 0·5; methanol). For $C_{33}H_{53}N_3O_8S$ (651·9) calculated: 60·80% C, 8·20% H, 6·45% N; found: 60·92% C, 8·33% H, 6·57% N. $E_{2.4}^{Gly}$ 0·00; $E_{5.7}^{Asp}$ 0·62; R_F 0·79 (S1), 0·73 (S3), 0·59 (S4), 0·82 (S7), 0·69 (S9). (The *E* and R_F-values are given after the release of the product from the dicyclohexylammonium salt.)

b) *From tert-butyloxycarbonyl methyl ester IIi*: Derivative *IIi* (0·67 g) and sodium benzyloxycarbonyl thiosulfate[15] (0·81 g) were dissolved in 0·5M-$NaHCO_3$ (40 ml). The mixture was stirred at room temperature and the pH was maintained at pH 8·3 by 1M-NaOH for 2 h. Subsequently the mixture was acidified by 6M-HCl to pH 3·0 and the product was extracted with ether. The ether extract was shaken with water, dried by sodium sulfate and evaporated. The dry residue was a mixture of two products (R_F 0·69 and 0·49 in system S9); $E_{5.7}^{Asp}$ 0·62 and 0·87). The mixture was chromatographed on a 2 × 30 cm silica gel (particle size 30–60 μ) column in benzene and 5% methanol. The individual fractions (10 ml) were analyzed by thin-layer chromatography (S9). The pure product was isolated from fractions emerging between 110 and 160 ml as an oil after evaporation of the solvent. Yield 0·28 g (30%): $[\alpha]_D^{25}$ −23·7° (*c* 0·5; methanol). The R_F and E-values were the same as those obtained with the product prepared as described under *a*). For $C_{21}H_{30}N_2O_8S$ (470·6) calculated: 53·60% C, 6·43% H, 5·95% N; found: 54·01% C, 6·52% H, 5·81% N. IR spectrum: 1740 cm^{-1} (s), 1440 cm^{-1} (m), ($COOCH_3$), 1722 cm^{-1} (s), 1418 cm^{-1} (w), 2400–3300 cm^{-1} (w, b), (COOH); 3435 cm^{-1} (m), 1713 cm^{-1} (s), 1510 cm^{-1} (s), (O—CO—NH); 1394 cm^{-1} (w), 1370 cm^{-1} (m), ($(CH_3)_3C$); 703 cm^{-1} (w) ($C_6H_5CH_2$). Derivative *IIi* (0·20 g) was dissolved in methanol and dicyclohexylamine (0·1 ml) and light petroleum were added to the solution. The product crystallized overnight in the cold room, was filtered off, and washed with light petroleum. Yield 0·16 g (58%) of a product of m.p. 100 to 104°C. $[\alpha]_D^{25}$ −14·5° (*c* 0·5; methanol). For $C_{33}H_{53}N_3O_8S$ (651·9) calculated: 60·80% C, 8·20% H, 6·45% N; found: 60·41% C, 7·99% H, 6·34% N. The byproduct obtained by the chromatographic purification was not examined in more detail. Its electrophoretic mobility shows that it represents most likely the diacid.

Nᵅ′-Tert-butyloxycarbonylcystathionine α′-Methyl Ester (*IIj*)

Cystine (0·72 g) was reduced by sodium in liquid ammonia. The blue color of the solution was removed by the addition of ammonium acetate and ester *Ib* (2·7 g) was added to the reaction

mixture. Ammonia was removed by lyophilization and the residue was dissolved in 0·1M-HCl (50 ml) at 0°C (the pH of the solution must be acidic). The solution was extracted with ether (ester *Ib* could be again isolated from the ethereal extracts), evacuated, and the residue applied to a column of Dowex 50W-X2 (H^+-form, 100 ml). The column was washed with water and the product was eluted by 10% aqueous pyridine. The eluate was acidified to pH 4·5—5·5 by acetic acid, concentrated to a small volume, and lyophilized. The lyophilisate was reprecipitated from methanol and ether. The yield was 1·70 g (84% in terms of cystine) of a product m.p. 155—160°C; recrystallization from water (3 ml) with the admixture of pyridine (0·1 ml) afforded 1·12 g (55%) of a product of m.p. 160—165°C. $E_{2.4}^{Gly}$ 0·35; $E_{5.7}^{His}$ 0·00. $[\alpha]_D^{25}$ —43·6° (*c* 0·5; methanol). For $C_{13}H_{24}N_2O_6S.0{\cdot}5\ H_2O$ (345·4) calculated: 45·20% C, 7·30% H, 8·11% N; found: 44·90% C, 7·03% H, 7·89% N.

$N^{\alpha'}$-*o*-Nitrobenzenesulfenyl-N^{α}-Tert-butyloxycarbonyl-cystathionine α'-Methyl Ester Dicyclohexylammonium Salt (*IIk*.DCHA)

Derivative *III* (0·67 g) and triethylamine (0·8 ml) in dichloromethane (10 ml) were stirred with *o*-nitrobenzenesulfenyl chloride (0·40 g) for 2 h at room temperature. The resulting solution was extracted with 0·25M-H_2SO_4 and water, dried by sodium sulfate, the solvent evaporated and azeotropically dried (benzene). The dry residue represented a mixture of compounds (R_F 0·97, 0·69 and 0·53 (S9)). It was chromatographed on a column (2 × 35 cm) of silica gel (particle size 30—60 μ) in benzene containing 20% of methanol. The individual fractions were collected according to visual detection and their purity was checked by thin-layer chromatography (S9). The fractions which contained a product of R_F 0·69% (S9) were taken to dryness and dried *in vacuo* at 40°C and 150 Pa. The yield was 0·14 g (15%) of a product of m.p. 48—50°C. $[\alpha]_D^{25}$ —39·7° (*c* 0·17; methanol). R_F 0·60 (S3), 0·74 (S7), 0·69 (S9). For $C_{19}H_{27}N_3O_8S_2$ (489·6) calculated: 46·61% C, 5·56% H, 8·58% N; found: 46·99% C, 5·34% H, 8·12% N. IR spectrum: 1395 cm^{-1} (w), 1370 cm^{-1} (m), ($(CH_3)_3C$); 1738 cm^{-1} (sh), 1440 cm^{-1} (sh), ($COOCH_3$); 3440 cm^{-1} (m); 1713 cm^{-1} (s), 1515 cm^{-1} (s), (O—CO—NH); 2400—3300 cm^{-1} (m, b), 1725 cm^{-1} (sh), 1420 cm^{-1} (m), (COOH); 1596 cm^{-1} (m), 1569 cm^{-1} (m), (*o*-$NO_2C_6H_4S$—NH). Derivative *IIk* (0·10 g) was dissolved in benzene and dicyclohexylamine (0·04 ml) and light petroleum were added to the solution. The oily product which had separated was decanted, triturated with light petroleum, and finally dissolved in ether and evaporated. The yield was 0·13 g (95%) of a foam melting at 58—60°C. $[\alpha]_D^{25}$ —17·6° (*c* 0·2; methanol). For $C_{31}H_{50}N_4O_8S$ (670·9) calculated: 55·70% C, 7·51% H, 8·35% N; found: 55·41% C, 7·46% H, 8·22% N.

N^{α}-Tert-butyloxycarbonylcystathionine α'-Methyl Ester (*III*)

N,N'-Bis(tert-butyloxycarbonyl)cystine[7,8] (13·2 g, 30 mM) was reduced by sodium in liquid ammonia (300 ml). The solution was decolorized by ammonium chloride, α-amino-γ-bromobutyric acid methyl ester hydrochloride (20·8 g; 90 mM) was added and the solution was lyophilized (vacuum of a water aspirator). The lyophilisate was dissolved in 0·5M-$NaHCO_3$ (250 ml) and extracted with ether. Subsequently the solution was acidified to pH 3·0 by 1M-HCl, evacuated and filtered through a column of Dowex 50W-X4 (H^+-form, 1000 ml). The column was washed with water and the product was displaced by 10% solution of pyridine (cooled to 0°C). The effluents were acidified by acetic acid to pH approx. 6·0 and evaporated. The dry residue was reprecipitated from methanol by ether. The yield was 16·6 g (84%) of a crude product contaminated mostly with the diacid. The product was purified by contercurrent distribution. It was dissolved in 75 ml of the lower phase of the solvent system butanol–benzene–pyridine–0·1% acetic acid (6 : 2 : 1 : 9) and placed in tube 2 to 4 of the all-glass instrument for countercurrent distribu-

tion (Steady State Distribution Machine, Quickfit and Quartz Ltd., Stone, Staffordshire, England). The number of transfers of the upper phase was 210. Immediately after the last transfer the contents of tubes 40 to 75 were removed, acidified to pH approx. 4·0 by acetic acid and evaporated. (The rapid removal and acidification of the solution of the product is necessary since the methyl ester is hydrolyzed in the given solvent system. The decomposition of the product and of the admixtures was determined in an experiment with a small quantity of the product; the detection was effected by electrophoresis of the contents of the tubes in 1M-acetic acid.) The purified product was reprecipitated from methanol by ether. Yield 11·8 g (52%), m.p. 185–186°C; $[\alpha]_D^{25}$ +37·2° (*c* 0·5; methanol); $E_{2,4}^{Gly}$ 0·86; $E_{5,7}^{His}$ 0·00. For $C_{13}H_{24}N_2O_6S$ (336·4) calculated: 46·40% C, 7·19% H, 8·33% N; found: 46·44% C, 7·15% H, 8·45% N.

Cystathionine (*IIa*)

Ester *III* (0·4 g) was refluxed with azeotropic hydrochloric acid (10 ml) for 1 h. The solution was taken to dryness, excess hydrochloric acid was removed by repeated evaporation with water and the dry residue was dissolved in water (2 ml); the pH was adjusted to 6·0 by concentrated ammonia (in a pH-stat). After several hours of standing at 0°C the product was filtered off and washed with water. Yield 0·2 g (83%); $[\alpha]_D^{25}$ +22·5° (*c* 0·5; 1M-HCl). The chromatography, electrophoresis, and ORD curve were identical with those obtained with an authentic sample prepared earlier[1]. Recorded data: $[\alpha]_D^{25}$ +23·7 (*c* 1, 1M-HCl) (ref.[16–18]) and +22·7° (*c* 0·5, 1M-HCl)[1].

$N^{\alpha'}$-*p*-Methoxybenzyloxycarbonylcystathionine α'-Methyl Ester (*IIm*)

Cystine (0·24 g) was reduced by sodium in liquid ammonia. The blue color of the solution was removed by the addition of ammonium chloride and ester *Ic* (1·1 g, dissolved in ether) was added to the mixture. Ammonia was removed by lyophilization and the residue was dissolved in 0·1M-HCl (20 ml; the pH of the solution must be acidic). The solution was extracted with ether, evacuated, and applied to a column of Dowex 50W-X4 (H^+-form; 50 ml). The column was washed with water and the product was eluted by 10% aqueous pyridine. The eluate was acidified to pH 4·5–5·5 by acetic acid and lyophilized. The lyophilisate was dissolved in methanol (8 ml), the insoluble amount (cystine) was centrifuged off, and the supernatant was evaporated. The dry residue (the desired product contaminated with the diacid and cystine) was purified by countercurrent distribution. It was dissolved in 10 ml of the lower phase of the solvent system butanol–benzene–pyridine–0·1% acetic acid (6 : 2 : 1 : 9) and placed in the first tube of an all-glass instrument for countercurrent distribution (an instrument manufactured in the glass blowers' shop of this Institute, 20 tubes for 10 ml of lower and 10 ml of upper phase). The number of transfers of the upper phase was 20, the contents of the tubes were removed, acidified by 1 ml of acetic acid and detected by paper electrophoresis. The contents of tubes 11 to 18 were pooled, concentrated, and lyophilized. The lyophilisate was reprecipitated from methanol and ether. The yield was 75 mg (9·5%) of a product of m.p. 158–162°C. $E_{2,4}^{Gly}$ 0·30; $E_{5,7}^{His}$ 0·00; R_F 0·14 (S2), 0·33 (S3), 0·61 (S4). $[\alpha]_D^{25}$ −48·7° (*c* 0·2; methanol). For $C_{17}H_{24}N_2O_7S.0{\cdot}5\ H_2O$ (409·5) calculated: 49·89% C, 6·15% H, 6·85% N; found: 50·02% C, 5·84% H, 6·85% N.

The elemental analyses were carried out in the Analytical Department of this Institute (Head Dr J. Horáček). The optical rotation measurements were carried out by Mrs Z. Ledvinová. The IR *spectra were measured by Mrs K. Matoušková and Mr P. Formánek and interpreted by Dr J. Smolíková. The* ^{1}H-NMR *spectrum was measured by Dr P. Trška.*

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