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The Peptide Synthesis. I. Use of the *S*-Ethylmercapto Group for the Protection of the Thiol Function of Cysteine*[1]

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The *S*-ethylmercapto group was an useful protecting group for the thiol group of cysteine. This group was stable under the conditions of peptide synthesis and was simply split by thiophenol without any side reaction. Glutathione and oxytocin were synthesized using *S*-ethylmercapto-L-cysteine.

Many biologically-important peptides, such as glutathione, oxytocin, vasopressin, and insulin, contain one or several cysteine or cystine residues

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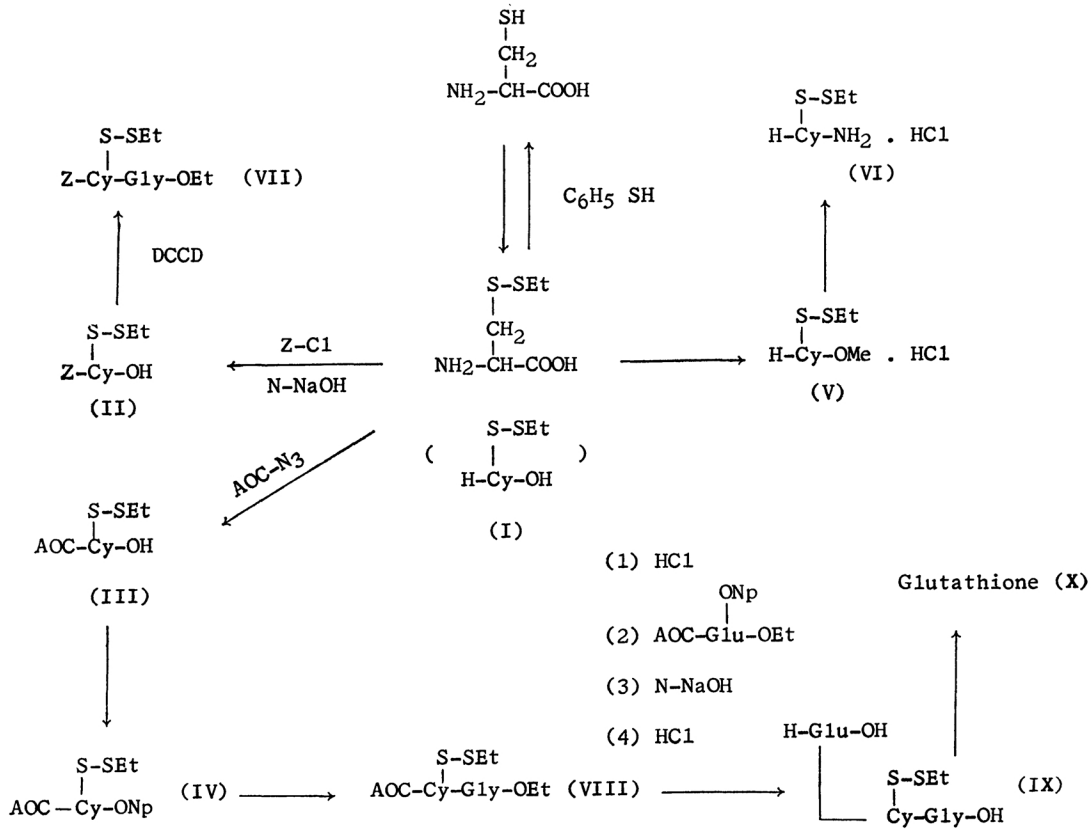

Fig. 1. The synthesis of glutathione and some *S*-ethylmercapto-L-cysteine derivatives.
Z=Carbobenzoxy; AOC=*t*-Amyloxycarboxyl; ONp=*p*-Nitrophenylester

in the molecule. Therefore, it is necessary to protect the thiol group of cysteine during the process of the synthesis of such peptides. A number of groups, such as benzyl,[1] its derivatives,[2,3] triphenylmethyl,[4] *t*-butyl,[5] carbobenzoxy,[6] some acyl derivatives,[7] and others,[8] have previously been proposed as protectors of the thiol group of cysteine. The most universal one among them is benzyl group,[1] which is very easy to introduce and which is stable under usual conditions of peptide synthesis. However, its removal requires a rather drastic treatment, which may give rise to side reactions, such as bond cleavage. As for the other protecting groups, some of them were too stable for them to be removed from the final products without causing a partial destruction of the peptide, while others were too labile to be used for the synthesis.

In the present investigation, we could establish that the ethylmercapto group can be an useful protecting group for the thiol function of cysteine. This group can very easily be introduced by usual methods of the synthesis of unsymmetrical disulfide, such as the treatment of cysteine with the ethyl thiosulfinic ethyl ester.[9] The crystalline *S*-ethylmercapto-L-cysteine obtained is surprisingly stable under most conditions of peptide synthesis, and no disproportionation or other abnormal reactions occur except when strong acids and bases are involved.

It is convertible in a nearly quantitative yield into its *N*-carbobenzoxy-, *N*-*t*-amyloxycarbonyl-,[10] or into *N*-*t*-butyloxycarbonyl - derivatives by

1) V. du Vigneaud, L. F. Audieth and H. S. Loring, *J. Am. Chem. Soc.*, **52**, 4500 (1930).
2) C. Berse, R. Boucher and L. Piche, *J. Org. Chem.*, **22**, 805 (1957).
3) S. Akabori, S. Sakakibara, Y. Shimonishi and Y. Nobuhara, This Bulletin, **37**, 433 (1964).
4) G. Amiard, R. Heymes and L. Velluz, *Bull. Soc. Chim. France*, **1956**, 698.
5) G. H. L. Nefkens, G. I. Tesser and R. J. F. Nivard, *Rec. trav. chim.*, **79**, 688 (1960).
6) A. Berger, J. Noguchi and E. Katchalski, *J. Am. Chem. Soc.*, **78**, 4483 (1956).
7) L. Zervas and I. Photaki, *ibid.*, **84**, 3887 (1962).
8) D. L. Ross, C. G. Skinner and W. Shive, *J. Med. Pharm. Chem.*, **3**, 519 (1961); St. Guttmann, *Helv. Chim. Acta*, **49**, 83 (1966).
9) S. Yurugi, *Yakugaku Zasshi (Tokyo)*, **74**, 513 (1954).
10) S. Sakakibara, M. Shin, M. Fujino, Y. Shimonishi, S. Inoue and N. Inukai, This Bulletin, **38**, 1522 (1965).

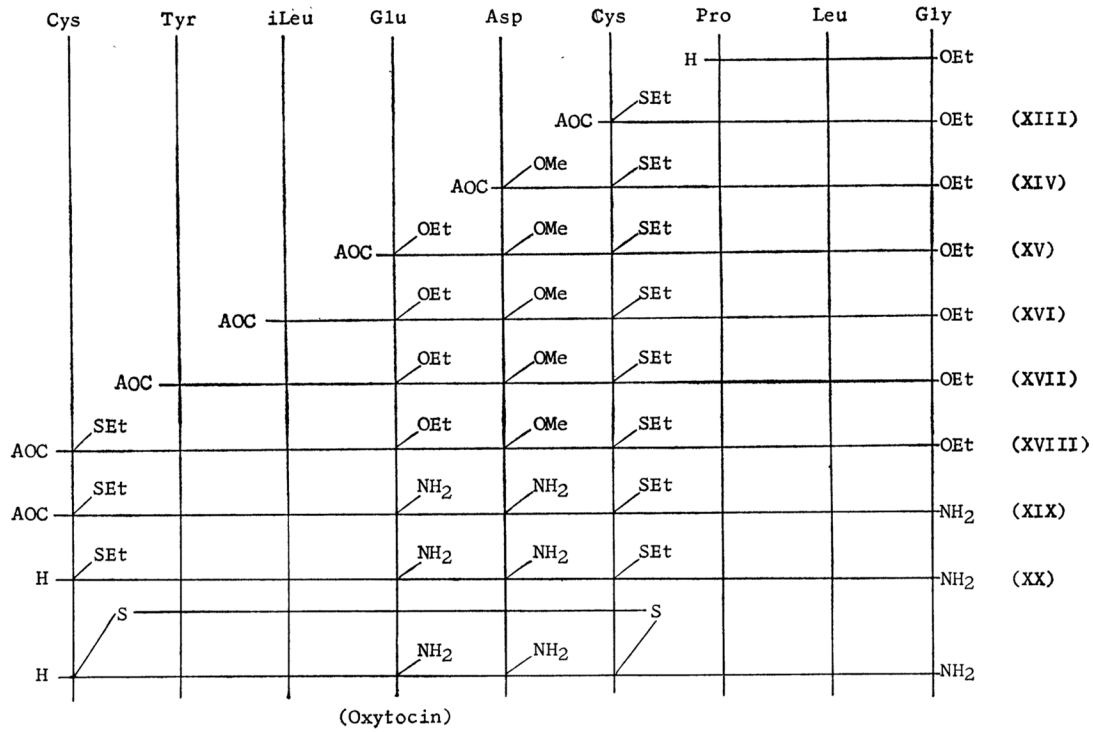

Fig. 2. The schematic diagram of the synthesis of oxytocin.
AOC=*t*-Amyloxycarbonyl

common methods.*[2] The derivatives can then be converted to activated esters or directly coupled with an amino acid derivative or a peptide derivative by means of mixed anhydride or *N*, *N'*-dicyclohexylcarbodiimide. The *N*-protecting group is removed from the peptide obtained and coupled with another *N*-protected amino acid or peptide by the usual methods.

The alkyl ester of *S*-ethylmercapto-L-cysteine is also easily synthesized, and it can be converted into its amide.

The *S*-ethylmercapto group can be cleft from the final product by treating it under relatively mild conditions with mercaptans of a high-"Reducing" potency, especialy thiophenol or thioglycollic acid, in a suitable solvent and preferably below 50°C.

Figure 1 depicts the synthesis of glutathione, an example of a peptide containing a cysteine residue, with the aim of elucidating the above-mentioned reactions.

The cleavage of the *S*-ethylmercapto group from γ-L-glutamyl-*S*-ethylmercapto-L-cysteinyl-glycine was performed with thiophenol or thioglycollic acid, after which the mixture was left overnight in water at 45°C.

Figure 2 depicts the synthesis of oxytocin, the wel-lknown hormone of the posterior pituitary gland.

This shows that larger peptides may also be synthesized by the use of the *S*-ethylmercapto group as the protector of the thiol group. As is indicated in Fig. 2, it was synthesized by the step-by-step elongation method, using *N*-*t*-amyloxycarbonyl amino acids. The *N*-*t*-amyloxycarbonyl group was removed by treatment with hydrogen chloride in methanol or dioxane, or with trifluoroacetic acid. The *N*-*t*-amyloxycarbonyl-nonapeptide ester (XVIII) was converted to the corresponding amide (XIX) by treating it with 25% ammonia in methanol in a sealed tube for two days at room temperature. The cleavage of the *S*-ethylmercapto groups of the nonapeptideamide (XX) was performed in the same way as in the case of glutathione synthesis, using thiophenol. The two free thiol groups in the molecule thus obtained are converted to a disulfide residue by aeration in an aqueous solution at pH 6.5. In this case, it is undesirable to use thioglycollic acid in stead of thiophenol for the reason present by Martin *et al.*[11] The oxytocin obtained showed a strong oxytocic activity when

*[2] In the case of the synthesis of *N*-formyl-*S*-ethylmercapto-L-cysteine (mp 134—135°C, $[\alpha]_D^{24}$ −45.9° (*c* 0.44, methanol)), *N*, *N'*-diformyl-L-cystine was formed as the by-product.

11) P. J. Martin and H. O. Schild, *Brit. J. Pharmacol.*, **25**, 418 (1965).

tested on the isolated uterus of a rat. No complication occurred during the synthesis with *S*-ethylmercapto-L-cysteine residues.

We are now attempting to apply the *S*-ethylmercapto-L-cysteine to solid-phase peptide synthesis.[12] This effort will be reported on in the following paper.

In conclusion, the *S*-ethylmercapto group can be considered to be a useful protecting group of the thiol residue of cysteine. The group is stable under the usual conditions of peptide synthesis, and it can be simply split in a suitable solvent without any side reaction.

Experimental*3

S-Ethylmercapto-L-cysteine (I). This compound was prepared from L-cysteine and the ethylthiosulfinic ethyl ester (Et-S-S-Et, with O above the second S) according to the procedure of Yurugi *et al.*[9] Mp 208°C, $[\alpha]_D^{15}$ −148.3° (*c* 0.3, N HCl). (*lit.* $[\alpha]_D^{15}$ −148.5° ±1.4° (*c* 0.3, N HCl)).

N-Carbobenzoxy-S-ethylmercapto-L-cysteine (II). A solution of I (0.9 g, 0.005 mol) in cold N NaOH (5 m*l*) was treated with carbobenzoxy chloride (0.9 g, 0.0053 mol) and N NaOH (5.3 m*l*) alternately under vigorous stirring below 0°C. The stirring was then continued for several more hours at room temperature. When the usual extraction procedures were followed, an oily material was obtained which was crystallized by adding petroleum ether. The crude product was recrystallized from ethylacetate and petroleum ether; wt 1.3 g (82.7%), mp 77—81°C, $[\alpha]_D^{24}$ −121.3° (*c* 1.0, methanol).

Found: C, 49.65; H, 5.45; N, 4.51%. Calcd for $C_{13}H_{17}O_4NS_2$: C, 49.50; H, 5.43; N, 4.44%.

N-t-Amyloxycarbonyl-S-ethylmercapto-L-cysteine (III). A mixture of I (15 g, 0.083 mol), triethylamine (35 m*l*, 0.25 mol), and *t*-amyloxycarbonylazide[13] (20 g, 0.128 mol) in a mixture of water (200 m*l*) and dioxane (300 m*l*) was allowed to react while being stirred at 45°C overnight. After the dioxane had been evaporated off *in vacuo*, the aqueous solution was washed with ether and acidified with 0.5 N HCl. The oily product which separated was then extracted with ethylacetate. The extracted solution was washed with H_2O and dried over anhydrous sodium sulfate. When the solvent had evaporated off, an oily product (III) remained; wt 20 g. Compound III was mixed with an equimolar amount of dicyclohexylamine in ethyl acetate. The precipitated crystals were collected by filtration and recrystallized from ethyl acetate; mp 124—128°C, $[\alpha]_D^{24}$ −45.8° (*c* 0.5, methanol).

Found: C, 58.20; H, 9.34; N, 5.94%. Calcd for $C_{23}H_{44}O_4N_2S_2$: C, 57.94; H, 9.30; N, 5.88%.

N-t-Amyloxycarbonyl-S-ethylmercapto-L-cysteine *p*-Nitrophenyl Ester (IV). Compound III (16.4 g, 0.0556 mol) and *p*-nitrophenol (7.75 g, 0.0556 mol) were dissolved in ethylacetate (150 m*l*) and then treated with *N*, *N'*-dicyclohexylcarbodiimide (11.5 g, 0.0556 mol) for 3 hr below 5°C. The precipitated dicyclohexylurea was filtered off, and the ethylacetate was evaporated off *in vacuo*. A solid residue was then recrystallized from ethylacetate and petroleum ether; wt 23 g (95%), mp 59—62°C, $[\alpha]_D^{23}$ −50.8° (*c* 0.8, methanol).

Found: C, 49.24; H, 5.89; N, 6.89%. Calcd for $C_{17}H_{24}O_6N_2S_2$: C, 49.04; H, 5.81; N, 6.73%.

S-Ethylmercapto-L-cysteine Methyl Ester Hydrochloride (V). Compound I was added to 10% HCl in methanol or to 3 equimolar amounts of thionylchloride in methanol and left overnight at room temperature. The solvent was then removed under reduced pressure, and the crude product was recrystallized from methanol and ether; mp 135—137°C, $[\alpha]_D^{24}$ −67.7° (*c* 0.5, methanol).

Found: C, 30.85; H, 6.18; N, 5.95%. Calcd for $C_6H_{13}NO_2S_2 \cdot HCl$: C, 31.09; H, 6.09; N, 6.04%.

S-Ethylmercapto-L-cysteineamide Hydrochloride (VI). Compound V (2.3 g, 0.01 mol) was treated with 25% ammonia in methanol (30 m*l*) in a sealed tube for 2 days at room temperature. The solvent was then evaporated off, and the solid residue was recrystallized from methanol and ether; wt 1.9 g (88%), mp 170—175°C (150°C sinter).

Found: C, 28.05; H, 6.35; N, 13.31%. Calcd for $C_5H_{12}N_2OS_2 \cdot HCl$: C, 27.71; H, 6.05; N, 12.92%.

N-Carbobenzoxy-S-ethylmercapto-L-cysteinylglycin Ethyl Ester (VII). Compound II (0.3 g, 0.001 mol), the glycine ethyl ester HCl (0.15 g, 0.001 mol), and triethylamine (0.14 m*l*, 0.001 mol) were dissolved in dimethylformamide (3.5 m*l*), after which the solution was treated with *N*, *N'*-dicyclohexylcarbodiimide (0.21 g, 0.001 mol) at room temperature overnight. The dicyclohexylurea thus precipitated was filtered off. This filtrate was diluted with ethylacetate (50 m*l*), washed successively with H_2O, 5% sodium bicarbonate, and N HCl, and then dried over anhydrous sodium sulfate. The solvent was evaporated off *in vacuo*, and the solid residue was recrystallized from ethyl acetate and petroleum ether; wt 0.3 g (75%), mp 100—102°C, $[\alpha]_D^{24}$ −99.1° (*c* 0.5. meathnol).

Found: C, 50.69; H, 5.95; N, 6.83%. Calcd for $C_{17}H_{24}O_5N_2S_2$: C, 50.99; H, 6.04; N, 7.00%.

N-t-Amyloxycarbonyl-S-ethylmercapto-L-cysteinyl-glycine Ethyl Ester (VIII). A mixture of compound IV (2.1 g, 0.005 mol), the glycine ethyl ester HCl (0.7 g, 0.005 mol), and triethylamine (0.7 m*l*, 0.005 mol) in dimethylformamide (15 m*l*) was allowed to stand at room temperature overnight. Ethyl acetate (100 m*l*) was then added to the solution, and it was washed with H_2O, N NH_4OH, and 0.2 N HCl, and then dried over anhydrous sodium sulfate. The evaporation of the solvent left an oily product; wt 1.4 g (74%), $[\alpha]_D^{23}$ −70.5° (*c* 0.44, methanol).

Found: C, 47.51; H, 7.35; N, 7.32%. Calcd for $C_{15}H_{28}O_5N_2S_2$: C, 47.36; H, 7.42; N, 7.37%.

γ-L-Glutamyl-S-ethylmercapto-L-cysteinylglycine. (S-Ethylmercapto-glutathione) (IX). A solution of compound VIII (3.8 g, 0.01 mol) in 7% HCl in methanol was left for 1 hr at room temperature,

12) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963); R. B. Merrifield, *Science*, **150**, 178 (1965); *etc.*

*3 All the melting points given are uncorrected. Each reaction process was cheched by thin-layer chromatography. The *N-t*-amyloxycarbonyl amino acids and their derivatives were synthesized by Sakakibara's method.[10]

13) S. Sakakibara, "The 4th Symposium on Peptide Chemistry," Institute for Protein Research, Osaka University (1965), p. 24.

after which the solvent removed under reduced pressure. The oily residue was dissolved in dimethylformamide (10 m*l*) and neutralized with triethylamine (1.4 m*l*, 0.01 mol). The neutralized solution was allowed to react with the *N*-*t*-amyloxycarbonyl-L-glutamic acid-α-ethyl-γ-*p*-nitrophenyl ester (4.1 g, 0.01 mol) for 20 hr at room temperature. The reaction mixture was then diluted with ethyl acetate and washed in the usual way. The oily product which remained upon the evaporation of the solvent was saponified with N NaOH (18.5 m*l*) in methanol (100 m*l*) for 2 hr below 10°C. The solution was concentrated to 30 m*l* under reduced pressure at room temperature, and then 10% aqueous HCl (20 m*l*) was added and the mixture was stirred for 30 min. The pH of the solution was adjusted to 6 with sodium bicarbonate. White crystals were precipitated out immediately. The crystals thus obtained were recrystallized from hot water; wt 2.5 g, mp 200—201°C, $[\alpha]_D^{23}$ −91.5° (*c* 1.0, dimethylformamide).

Found: C, 38.90; H, 5.78; N, 11.65%. Calcd for $C_{12}H_{21}N_3O_6S_2$: C, 39.22; H, 5.76; N, 11.44%.

Glutathione (X). Thiophenol or thioglycollic acid (0.5 m*l*) was added to a solution of compound IX (0.5 g) in water (10 m*l*). After the solution had been allowed to stand for 15 hr at 45°C, the reaction mixture was washed with ethylacetate. The aqueous solution was concentrated to 1 m*l* under reduced pressure and seeded with a crystal of glutathione. The crystals which then appeared in the solution were collected by filtration and recrystallized from H_2O and alcohol. The yield was nearly quantitative. Mp 187—190°C, $[\alpha]_D^{23}$ −19.1° (*c* 1.0, H_2O).

***N*-*t*-Amyloxycarbonyl-L-leucyl-glycine Ethyl Ester (XI).** A mixture of glycine ethyl ester hydrochloride (1.4 g, 0.01 mol), triethylamine (1.4 m*l*, 0.01 mol), and the *N*-*t*-amyloxycarbonyl-L-leucine *p*-nitrophenyl ester (3.7 g, 0.01 mol) in dimethylformamide (35 m*l*) was left at room temperature overnight. Ethylacetate (200 m*l*) was then added to the solution, and it was washed successively with H_2O, N NH_4OH, 0.2 N HCl, and H_2O, and dried over anhydrous sodium sulfate, after which the solvent was evaporated off. The oily product which remained was gradually crystallized at room temperature, and crude crystals were recrystallized from ethylacetate and petroleum ether; wt 2.9 g (88%), mp 45—54°C, $[\alpha]_D^{23}$ −28.1° (*c* 1.0, methanol).

Found: C, 58.30; H, 9.07; N, 8.28%. Calcd for $C_{16}H_{30}O_5N_2$: C, 58.16; H, 9.15; N, 8.45%.

***N*-*t*-Amyloxycarbonyl-L-prolyl-L-leucyl-glycine Ethyl Ester (XII).** Compound XI (6 g, 0.018 mol) was treated with 10% HCl in methanol for 1 hr at room temperature. The solvent was then evaporated off *in vacuo* at room temperature. The oily residue was dissolved in dimethylformamide (70 m*l*), and a triethylamine (2.8 m*l*, 0.02 mol) and the *N*-*t*-amyloxycarbonyl-L-proline *p*-nitrophenyl ester (6.3 g, 0.018 mol) were added to the solution. After 20 hr at room temperature, the solution was diluted with ethyl acetate (300 m*l*) and treated as has been described above. The crude crystals obtained were recrystallized from ethyl acetate and petroleum ether; wt 5 g (65%), mp 87—91°C, $[\alpha]_D^{23}$ −3.63° (*c* 1.0, methanol).

Found: C, 59.47; H, 8.72; N, 9.98%. Calcd for $C_{21}H_{37}O_6N_3$: C, 58.85; H, 8.94; N, 9.81%.

***N*-*t*-Amyloxycarbonyl-*S*-ethylmercapto-L-cysteinyl-L-prolyl-L-leucyl-glycine Ethyl Ester (XIII).** Compound XII (9.2 g, 0.0215 mol) was added to 2 N HCl in ethanol (30 m*l*) and left for 30 min at room temperature. The solvent was then evaporated off *in vacuo*. A solution of the oily residue in dimethylformamide (100 m*l*) was neutralized with triethylamine (3.6 m*l*, 0.0257 mol). Compound IV (9.4 g, 0.0216 mol) was added to the solution and allowed to react at room temperature overnight. The reaction mixture was then diluted with ethyl acetate (300 m*l*). The diluted solution was washed successively with H_2O, N NH_4OH, 0.25 N HCl, and H_2O, and dried over anhydrous sodium sulfate. The solution was then concentrated to give an oily product; wt 11.6 g (74.5%).

***N*-*t*-Amyloxycarbonyl-β-methyl-L-aspartyl-*S*-ethylmercapto-L-cysteinyl-L-prolyl-L-leucyl-glycine Ethyl Ester (XIV).** Compound XIII (11.6 g, 0.0196 mol) was treated with trifluoroacetic acid (16.2 g) for 1.5 hr at room temperature. The excess trifluoroacetic acid was then evaporated off *in vacuo* at room temperature, and the oily residue was dissolved in dimethylformamide (70 m*l*). The solution was neutralized with triethylamine (2.8 m*l*), 0.02 mol), and the *N*-*t*-amyloxycarbonyl-β-methyl-L-aspartic acid *p*-nitrophenyl ester (7.5 g, 0.0196 mol) was added to the solution. After the solution had been kept for 15 hr at room temperature, the reaction mixture was treated as has been described above. An oily product was obtained; wt 9 g (64%).

***N*-*t*-Amyloxycarbonyl-γ-ethyl-L-glutamyl-β-methyl-L-aspartyl-*S*-ethylmercapto-L-cysteinyl-L-prolyl-L-leucyl-glycine Ethyl Ester (XV).** Compound XIV (9.0 g, 0.0125 mol) was added to trifluoro acetic acid (10 m*l*) and kept at room temperature for 1.5 hr until the production of CO_2 gas stopped. After the excess trifluoroacetic acid had been evaporated off under reduced pressure, the oily residue was dissolved in dimethylformamide, and triethylamine (2 m*l*, 0.0143 mol) and the *N*-*t*-amyloxycarbonyl-γ-ethyl-L-glutamic acid *p*-nitrophenyl ester (7.5 g, 0.0196 mol) were added to the solution. After the solution had then been left for 20 hr, the reaction mixture was diluted with ethylacetate (300 m*l*). The diluted solution was treated as has been described above. An oily product was thus obtained; wt 9 g (81.5%).

***N*-*t*-Amyloxycarbonyl-L-isoleucyl-γ-ethyl-L-glutamyl-β-methyl-L-aspartyl-*S*-ethylmercapto-L-cysteinyl-L-prolyl-L-leucyl-glycine Ethyl Ester (XVI).** Compound XV (8.9 g, 0.01 mol) was treated with trifluoroacetic acid (15 m*l*) as has been described above. The oily residue was dissolved in dimethylformamide (70 m*l*), and the solution was neutralized with triethylamine (15 m*l*, 0.0107 mol), then *N*-*t*-amyloxycarbonyl-L-isoleucine *p*-nitrophenyl ester (3.8 g, 0.0104 mol) was added to the solution. After the mixture had been left for 20 hr, the reaction mixture was diluted with ethylacetate. The diluted solution was washed successively with H_2O, N NH_4OH, 0.25 N HCl, and H_2O, and then dried over anhydrous sodium sulfate. The dried solution was concentrated to a syrup which was then solidified by trituration with ether. Crude crystals were recrystallized from ethyl acetate and petroleum ether; wt 7 g (70%), mp 159—162°C, $[\alpha]_D^{24}$ −83.5° (*c* 0.6, methanol).

Found: C, 53.20; H, 7.64; N, 10.14%. Calcd for

$C_{44}H_{75}O_{14}N_7S_2$: C, 53.37; H, 7.64; N, 9.90%.

***N*-*t*-Amyloxycarbonyl-L-tyrosyl-L-isoleucyl-γ-ethyl-L-glutamyl-β-methyl-L-aspartyl-*S*-ethylmercapto-L-cysteinyl-L-prolyl-L-leucyl-glycine Ethyl Ester (XVII).** Compound XVI (1.5 g, 0.0015 mol) was let stand in 7% HCl in methanol (12 m*l*) for 1 hr at room temperature. A dry ether was then added to the solution, and the white crystals thus precipitated were collected by filtration. *N*, *N'*-Dicyclohexylcarbodiimide (0.31 g, 0.0015 mol) was added to a mixture of the crystals obtained, triethylamine (0.21 m*l*, 0.0015 mol), and *N*-*t*-amyloxycarbonyl-L-tyrosine (0.45 g, 0.0015 mol) in dimethylformamide (6 m*l*). This solution was kept for 15 hr at room temperature. The dicyclohexylurea thus precipitated was filtered off, and the filtrate was diluted with ethylacetate (50 m*l*). The diluted solution was washed as has been described above and dried over anhydrous sodium sulfate. The dried solution was then concentrated to a syrup which was solidified by trituration with ether. The product was reprecipitated from ethyl acetate and ether; wt 1.13 g (65%), mp 137—140°C, $[\alpha]_D^{24}$ −73.3° (*c* 0.6, methanol).

Found: C, 54.89; H, 7.53; N, 9.83%. Calcd for $C_{53}H_{84}N_8O_{16}S_2$: C, 55.19; H, 7.34; N, 9.72%.

***N*-*t*-Amyloxycarbonyl-*S*-ethylmercapto-L-cysteinyl-L-tyrosyl-L-isoleucyl-γ-ethyl-L-glutamyl-β-methyl-L-aspartyl-*S*-ethyl-mercapto-L-cysteinyl-L-prolyl-L-leucyl-glycine Ethyl Ester (XVIII).** Compound XVII (0.315 g, 0.000275 mol) was treated with trifluoroacetic acid (1 m*l*) for 45 min at room temperature, after which ether (20 m*l*) was added to the solution. The precipitated crystals were collected by filtration and washed with dry ether. The crystals thus obtained were dissolved in dimethylformamide (4 m*l*) and neutralized with triethylamine (0.04 m*l*). Compound IV (0.14 g, 0.000274 mol) was added to the neutralized solution, and it was kept at room temperature overnight. The reaction mixture was diluted with ethylacetate (100 m*l*). The solution was washed as has been described above, and the solution was concentrated to a syrup, then this was reprecipitated twice from ethylacetate and ether as an amorphous powder; wt 0.26 g (72%), mp 163—174°C, $[\alpha]_D^{24}$ −80.1° (*c* 0.6, methanol).

Found: C, 52.54; H, 7.25; N, 10.07%. Calcd for $C_{58}H_{93}N_9O_{17}S_4$: C, 52.89; H, 7.12; N, 9.57%.

***N*-*t*-Amyloxycarbonyl-*S*-ethylmercapto-L-cysteinyl-L-tyrosyl-L-isoleucyl-L-glutaminyl-L-asparaginyl-*S*-ethylmercapto-L-cysteinyl-L-prolyl-L-leucyl-glycinamide (XIX).** A solution of XVIII (101 mg) in 25% ammonia in methanol (20 m*l*) was left for 2 days, after which the solution was concentrated to a syrup. This syrup was dissolved in ethylacetate, washed with 0.2 N HCl and H_2O, and dried over anhydrous sodium sulfate. The dried solution was concentrated, and the solid residue was reprecipitated with methanol and ether; wt 91 mg, mp 110—115°C $[\alpha]_D^{23}$ −48.4° (*c* 0.5 methanol).

Found: C, 50.06; H, 7.34; N, 14.80%. Calcd for $C_{53}H_{89}N_{13}O_{14}S_4$ (ammonium salt): C, 50.56; H, 7.12; N, 14.45%.

Oxytocin. Compound XIX (7 mg) was treated with 7% HCl in methanol (0.1 m*l*) for 40 min at room temperature. Dry ether was then added to the solution, and the solid thus precipitated (7 mg) (XX) was collected by filtration. This solid (7 mg) was dissolved in water (10 m*l*). Thiophenol (0.1 m*l*) was added to the aqueous solution, and the solution was kept for 12 hr at 45°C under stirring, after which the reaction mixture was washed with ethylacetate. The pH was adjusted to 6.5 with diluted aqueous ammonia, and carbon dioxide-free air was bubbled through the solution until the nitroprusside-test showed negative. Then the solution was acidified to pH 4 with acetic acid, and the small amount of insoluble material was filtered off. The final solution showed a strong oxytocin activity, as determined by rat-uterine contractive activity.

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