A New Method for the Protection of the Sulfhydryl Group during Peptide Synthesis

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The protection of the thiol group in the cysteine residue is of great importance in the field of peptide synthesis. Glutathione, oxytocin and vasopressin have been synthesized by many investigators, and the *S*-benzyl group has generally been used to protect the thiol during the procedures. Recently, however, in connection with the synthesis of insulin, several new *S*-protecting groups have been developed. Some of them, however, were too stable to be split from the final products without causing partial destruction of the peptide, and some of them were too labile to allow preparation of the peptide in a good yield.

In the present investigation, the *p*-methoxybenzyl (anisyl) group was found to be valuable for masking the thiol; that is, the *S*-*p*-methoxybenzyl group was easily cleaved by treatment with boiling trifluoroacetic acid, although it was not affected by hydrogen bromide, as is shown below. Moreover, the synthesis of *S*-*p*-methoxybenzyl-L-cysteine (I) was as easy as that of the *S*-benzyl derivative. Freshly-prepared L-cysteine was treated with *p*-methoxybenzyl chloride in liquid ammonia to form I, which was recrystallized from water; colorless plates were obtained in a 78% yield; m. p. 198~199°C (decomp.), $[\alpha]_D^{25}$ +22.6° (*c* 1.02, N NaOH). Found: C, 54.50; H, 6.33; N, 5.89; S, 13.05. Calcd. for $C_{11}H_{15}O_3NS$: C, 54.75; H, 6.26; N, 5.81; S, 13.29%. After the treatment of I with anhydrous trifluoroacetic acid containing phenol or anisol, followed by oxidation with iodine solution, L-cystine was recovered in a 84% yield; it had the same optical activity as the starting L-cystine. Compound I was submitted to reaction with carbobenzoxy chloride in a basic condition to give *N*-carbobenzoxy-*S*-*p*-methoxybenzyl-L-cysteine (II), which was recrystallized from toluene as colorless needles; yield, 76%; m.p. 66~67°C, $[\alpha]_D^{24}$ −42.1° (*c* 2.0, acetone). Found: C, 60.73; H, 5.80; N, 3.55, S, 8.46. Calcd. for $C_{19}H_{21}O_5NS$: C, 60.78; H, 5.64; N, 3.73; S, 8.54%. The dicyclohexylamine salt of II was recrystallized from ethanolether as fine colorless needles; m. p. 148~149°C, $[\alpha]_D^{27}$ −8.4° (*c* 2.22, EtOH). Found: C, 66.85; H, 7.90; N, 4.94; S, 5.60. Calcd. for $C_{31}H_{44}O_5N_2S$: C, 66.87; H, 7.97; N, 5.03; S, 5.76%. When II was treated with hydrogen bromide in acetic acid, *S*-*p*-methoxybenzyl-L-cysteine was recovered in a 77% yield; this had the same melting point as I and gave one spot on a paper chromatogram.

Compound II was coupled with glycine ethyl ester in the presence of dicyclohexylcarbodiimide in anhydrous acetonirile. Carbobenzoxy-*S*-*p*-methoxybenzyl-L-cysteinyl-glycine

ethyl ester (III) was obtained as fine colorless needles after recrystallization from ethyl acetate-petroleum benzine (81%); m.p. 87.5~89.5°C, $[\alpha]_D^{27}$ −31.1° (*c* 3.15, EtOH). Found: C, 59.89; H, 6.20; N, 5.95; S, 7.07. Calcd. for $C_{23}H_{28}O_6N_2S$: C, 59.98; H, 6.13; N, 6.08; S, 6.96%. Substance III was hydrolyzed in dioxane, using N sodium hydroxide, to give carbobenzoxy-*S*-*p*-methoxybenzyl-L-cysteinyl-glycine (IV), which was recrystallized from ethyl acetate-petroleum benzine as fine colorless needles (69%); m. p. 132~133.5°C, $[\alpha]_D^{24}$ −35.0° (*c* 3.14, EtOH). Found: C, 58.57; H, 5.42; N, 6.34; S, 7.35. Calcd. for $C_{21}H_{24}O_6N_2S$: C, 58.32; H, 5.59; N, 6.48; S, 7.41%. The treatment of IV with hydrogen bromide, followed by neutralization with aqueous sodium acetate, gave *S*-*p*-methoxybenzyl-L-cysteinyl-glycine (V) in a 83% yield; m.p. 164~164.5°C, (decomp.), $[\alpha]_D^{25}$ +29.3° (*c* 2.00, N NaOH). Found: C, 52.52; H, 6.02; N, 9.35; S, 10.47. Calcd. for $C_{13}H_{18}O_4N_2S$: C, 52.33; H, 6.08; N, 9.39; S, 10.75%. Compound V was refluxed with anhydrous trifluoroacetic acid containing phenol. The cysteinyl-glycine thus formed was oxidized by an iodine solution to L-cystinyl-diglycine (VI), which was purified by the procedure descrived by Weygand.[1] Yield, 48%; $[\alpha]_D^{28}$ −68.9° (*c* 0.944, water), reported[2] $[\alpha]_D^{27}$ −67.5° (*c* 1, water). Found: C, 33.91; H, 5.37; N, 16.10; S, 17.59. Calcd. for $C_{10}H_{18}O_6N_4S_2$: C, 33.89; H, 5.12; N, 15.81; S, 18.10%. Detailed results and the application of the procedure will be published elsewhere.

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2) H. S. Loring and V. du Vigneaud, *J. Biol. Chem.*, **111**, 385 (1935).