Peptide Synthesis *via* Oxidation-Reduction Condensation by the Use of Non-metallic Compound as a Mercaptan Scavenger

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We have recently shown[1] that peptides with a high optical purity are produced in excellent yields (as determined by the Young test[2]) by the use of di-*p*-anisylmercury as a mercaptan scavenger *via* the following oxidation-reduction condensation reaction:

$$\begin{aligned}&\text{Bz-L-Leu-OH} + \text{H-Gly-OEt} + Ph_3P + (o\text{-}O_2NC_6H_4S)_2\\&+(p\text{-}CH_3OC_6H_4)_2Hg \longrightarrow \text{Bz-L-Leu-Gly-OEt} + Ph_3P{=}O\\&+(o\text{-}O_2NC_6H_4S)_2Hg + 2\,p\text{-}CH_3OC_6H_5 \qquad (1)\end{aligned}$$

In recent years, the potential utility of the solid phase method in peptide synthesis has been extended. With respect to this problem, various non-metallic mercaptan scavengers were investigated in an attempt to purge the insoluble metal mercaptide in the above equation.

First, the reaction was tried by the use of olefins as mercaptan scavengers; they yielded soluble sulfides instead of metal compounds (Eq. (1)), and good results were obtained. When the above reaction was carried out by using 2,3-dihydropyran and zinc chloride, the *N*-benzoyl-L-leucylglycine ethyl ester (Bz-L-Leu-Gly-OEt) was obtained in a 92% yield; mp 147—152°C $[\alpha]^{20}_{D}$ $-32.9°$ (*c* 3.1, EtOH), L isomer 97%.[2] Another olefin, *n*-butyl vinyl ether, also gave a good result (90% yield, L isomer 95%).

Then, the reaction was carried out by using the sulfenate ester, which yields disulfide; a good result was obtained here also. When equimolar amounts of *N*-benzoyl-L-leucine, the *N*-*o*-nitrophenylsulfenylglycine ethyl ester, and triphenylphosphine were allowed to react in the presence of methyl 2,4-dinitrobenzenesulfenate in methylene chloride for 3 hr, Bz-L-Leu-Gly-OEt was obtained in a 92% yield; L isomer 92%.

Next, the reaction was tried in the absence of a mercaptan scavenger by using dipyridyl-2,2′-disulfide as a disulfide component, on the assumption that the 2-mercaptopyridine produced along with the peptide would isomerize to the stable thione form.[3] In a typical experiment, ethyl glycinate (5 mmol) and triphenylphosphine (5 mmol) in methylene chloride were added at room temperature to a stirred mixture of equimolar amounts of *N*-benzoyl-L-leucine and dipyridyl-2,2′-disulfide in methylene chloride. After stirring for 30 min, Bz-L-Leu-Gly-OEt was isolated by an ordinary procedure;[1] 1.46 g (91%); mp 148—152°C, $[\alpha]^{20}_{D}$ $-32.6°$ (*c* 3.1, EtOH), L isomer 96%.

Finally, it was found that the third process could be successfully applied to the solid phase method and the dipeptide thus prepared in a high yield. For example, 0.644 mmol of *t*-amyloxycarbonylglycine estrified to 2 g of the supporting cross-linked polystyrene resin[4] was deprotected by 2N hydrogen chloride in acetic acid, and the general procedure[4] of the solid phase method was followed. Coupling was carried out using a threefold excess of *t*-butyloxycarbonyl-L-leucine monohydrate, triphenylphosphine, and dipyridyl-2,2′-disulfide in methylene chloride for 6 hr. The peptide was liberated from the resin by bubbling in hydrogen bromide in trifluoroacetic acid; after it had been evaporated to dryness and water had been added, the resin was filtered off. The amount of the peptide in the filtrate was determined by the aminoacid analyser to be 0.64 mmol of Leu-Gly (99% yield) (calculated from the authentic sample) and the glycine peak was not observed. This solution was desalted by DEAE cellulose, and the peptide was precipitated by adding ethanol after the water had been evaporated *in vacuo* to a very small amount. Ninety-one milligrams of H-L-Leu-Gly-OH (76% yield) were isolated after drying over phosphorus pentoxide at 100°C for 2 hr, mp 235—240°C (dec.), $[\alpha]^{20}_{D}$ 86.5 (*c* 2, H_2O). Found: C, 51.07; H, 8.48; N, 15.11%. Calcd for $C_8H_{16}N_2O_3$: C, 51.05; H, 8.57; N, 14.88%. [lit,[5] mp 235—242°C (dec.), $[\alpha]^{20}_{D}$ 85.99 (*c* 2, H_2O)]. The amino-acid analysis of a 24-hr 6N HCl hydrolysate gave: Leu, 0.92; Gly, 0.95.

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4) G. R. Marshall and R. B. Merrifield, *Biochemistry*, **4**, 2394 (1965).

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