Amino Acids and Peptides. XI.[1] Simple Preparation of N^{α}-Protected Histidine

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Since most N^{α}-protected histidines are water-soluble, their preparation and purification are troublesome. We have developed a simple and easy purification procedure for the preparation of N^{α}-protected histidines, such as Boc–His–OH, Z–His–OH and Z(OMe)–His–OH, using ion-exchange column chromatography.

Keywords histidine; N^{α}-protected histidine; *tert*-butoxycarbonylhistidine; *p*-methoxybenzyloxycarbonylhistidine; benzyloxycarbonylhistidine; ion-exchange chromatography

Most N^{α}-protected amino acids for peptide synthesis are soluble in various organic solvents, so that they can be purified easily by extraction with an organic solvent when they are prepared. However, N^{α}-protected histidines, such as N^{α}-*tert*-butoxycarbonylhistidine (Boc–His–OH),[2] N^{α}-benzyloxycarbonylhistidine (Z–His–OH)[3] and N^{α}-*p*-methoxybenzyloxycarbonylhistidine (Z(OMe)–His–OH),[4] which are important synthetic intermediates for both α-amino and imidazole protected histidine, can not be purified by extraction with organic solvents because of their high water-solubility. Thus the preparation of N^{α}-protected histidines is not easy, or at least is more complicated as compared with those of other N^{α}-protected amino acids. For example, Boc–His–OH has been prepared from H–His–OH *via* H–His–OMe according to route (A) shown in Fig. 1. This synthetic route takes much time and entails variable yields in each step depending on experimental skill. We have developed a simple purification method for the preparation of Boc–His–OH using ion-exchange resin as shown by route (B) in Fig. 1. H–His–OH was *tert*-butoxycarbonylated with 2-*tert*-butyloxycarbonylimino-2-phenylacetonitrile (Boc–ON)[5] in a mixture of dioxane and water. After evaporation of the solvent, the reaction mixture dissolved in water was washed with ethyl acetate and evaporated to dryness. The residue was dissolved in a mixture of pH 3.8, 0.1 M pyridinium acetate buffer and methanol (1 : 1) and the solution was applied to a Dowex 50 (H^+ form) column equilibrated with the same mixture. The column was developed with a pH gradient system of 50% methanol–0.1 M pyridinium acetate buffer from pH 3.8 to pH 5.8. The Boc group was stable to Dowex 50 resin equilibrated with 50% methanol–pyridinium acetate buffer, but unstable to the resin in 50% methanol–water. Boc–His–OH was easily isolated; diBoc–His–OH and H–His–OH were eluted into the fractions preceding and following the desired fraction, respectively.

Schaich *et al.*[4] reported the preparation of Z(OMe)–His–OH *via* its extraction with ethyl acetate at pH 4.5. We followed their procedure, but obtained Z(OMe)–His–OH only in a low yield of 45%. This low yield may have resulted from inadequate extraction of the product by that procedure. Yajima *et al.*[6] also prepared Z(OMe)–His–OH from histidine and *p*-methoxybenzyloxycarbonylazide [Z(OMe)–N_3].[7] They treated the reaction mixture with alkali to remove the Z(OMe) group on the imidazole ring and used the resulting mixture for tosylation on the imidazole ring without further purification. We purified the relevant mixture by Dowex 50 column chromatography as described for Boc–His–OH and obtained Z(OMe)–His–OH in an overall yield of 59%. The difference in yield between Boc–His–OH and Z(OMe)–His–OH is attributed to different reaction rates of Boc–ON and Z(OMe)–N_3.

Preparation of Z–His–OH was reported by Patchornik *et al.*[3] They carried out dibenzyloxycarbonylation of histidine followed by hydrolysis with alcoholic potassium hydroxide. We prepared Z–His–OH as follows: His was allowed to react with Z–Cl in a molar ratio of 1 to 1 and the resulting crude material was purified by Dowex 50 column chromatography as described above. The reaction yield was 63%.

N^{α}-9-Fluorenylmethyloxycarbonylhistidine (Fmoc–His–OH) could not be purified in the same manner because of its low solubility in the mixture of methanol and pyridinium acetate buffer. Purification was achieved by washing with water and methanol.

Experimental

Melting points are uncorrected. Solvent systems for ascending thin-layer chromatography (TLC) on Silica gel G (type 60, E. Merck) are indicated as follows: Rf^2 BuOH–pyridine–AcOH–H_2O (4 : 1 : 1 : 2).

Boc–His–OH H–His–OH·HCl·H_2O (5 g, 24 mmol) dissolved in a mixture of H_2O (20 ml) and Et_3N (4.9 ml, 36 mmol) and Boc–ON (6.4 g, 26 mmol) dissolved in dioxane (20 ml) were combined and the mixture was stirred at pH 8 for 5 h. The solvent was evaporated off and the residue was

(A) the usual method [Handford *et al.*[2]]

$$\text{H–His–OH·HCl} \xrightarrow{(1)} \text{H–His–OMe·HCl} \xrightarrow{(2)} \text{Boc–His–OMe} \xrightarrow{(3)} \text{Boc–His–OH}$$

(1) H_2SO_4·MeOH (2) Boc–N_3 (3) OH^-

(B) the simple preparation method

$$\text{H–His–OH·HCl} \xrightarrow[\text{2) ion-exchange column chromatography}]{\text{1) Boc–ON}} \text{Boc–His–OH}$$

Fig. 1. Preparation of Boc–His–OH

dissolved in H_2O. The aqueous solution was washed with ether and concentrated. The residue was dissolved in 200 ml of a mixture of MeOH and 0.1 M pyridinium acetate buffer (pH 3.8) and applied to a Dowex 50 column (×2, H^+, 5.3×28 cm) equilibrated with the same mixture. The column was developed with a pH gradient from 3.8 to 5.8 in the same mixture. Fractions of 12 ml were collected and checked by means of the ninhydrin and Pauly tests.[8] Three products were found in the eluates; the first having Rf^2 0.73 in fractions 101—131 might be Boc-His(Boc)-OH, the second having Rf^2 0.35 in fractions 136—184 was the desired material, and the third having Rf^2 0.11 in fractions 204—227 was histidine. Fractions 136—184 were pooled and evaporated down. The residue was dried at 40 °C in a desiccator and precipitated from MeOH/ether. Yield 4.62 g (76%), mp 203—205 °C (lit.[2] 191—191.5 °C), Rf^2 0.35, $[\alpha]_D^{21}$ −15.2° (c=1.0, DMF) (lit.[2] −10.6°, c=1.0, DMF). *Anal.* Calcd for $C_{11}H_{17}N_3O_4$: C, 51.7; H, 6.7; N, 16.5. Found: C, 51.8; H, 6.8; N, 16.5.

Z(OMe)-His-OH H-His-OH·HCl·H_2O (5 g, 24 mmol) and Z(OMe)-N_3 (5.7 g, 28 mmol) were reacted in the usual manner[7] and the product was purified in the same way as described above. A Dowex column (5×27 cm) was used and fractions of 12 ml each were collected. Fractions 190—215 (Rf^2 0.52) were pooled and the solvent was evaporated off. The residue was dried at 40 °C in a desiccator and precipitated from MeOH/petroleum ether. Yield 5.02 g (66%), mp 55—58 °C (lit.[4] 50—51 °C), Rf^2 0.52, $[\alpha]_D^{23}$ +5.6° (c=1.0, AcOH) (lit.[4] +2.7°, c=1.0, AcOH). *Anal.* Calcd for $C_{15}H_{17}N_3O_5 \cdot 1/2\,H_2O$: C, 54.9; H, 5.5; N, 12.8. Found: C, 54.8; H, 5.4; N, 12.8.

Z-His-OH H-His-OH·HCl·H_2O (2 g, 9.54 mmol) was benzyloxycarbonylated with Z-Cl (1.63 g, 9.54 mmol) and 2 N NaOH (14.31 ml) under ice-cooling for 3 h. The reaction mixture was washed with ether, neutralized with AcOH, and evaporated down. The residue was dissolved in 100 ml of a mixture of MeOH and 0.1 M pyridinium acetate buffer (pH 3.8) and the solution was applied to a Dowex 50 column (3.5×36 cm). The column was developed in the same way described above. Fractions of 15 ml were collected and the desired material was eluted in fractions 97—106. The solvent was evaporated off and the residue was dried at 40 °C in a desiccator. Yield 1.83 g (63%), mp 158—161 °C (lit.[3] 166—167 °C), Rf^2 0.58, $[\alpha]_D^{23}$ −20.1° (c=1.0, 6 N HCl) (lit.[3] −25.0°, c=1.0, 6 N HCl). *Anal.* Calcd for $C_{14}H_{15}N_3O_4$: C, 58.1; H, 5.2; N, 14.5. Found: C, 58.1; H, 5.2; N, 14.5.

Fmoc-His-OH Fmoc-Cl (1 g, 3.87 mmol) dissolved in dioxane (12 ml) was added to a solution of H-His-OH·HCl·H_2O (810 mg, 3.87 mmol) in 10% $NaHCO_3$ (11.5 ml, 10.8 mmol) and the mixture was stirred for 5 h in an ice-bath. The solvent was evaporated off and the residue was dissolved in H_2O. The aqueous solution was washed with ether and adjusted to pH 4 with AcOH. The resulting precipitate was collected and washed with H_2O and hot MeOH. Yield 906 mg (62%), mp 171—174 °C, Rf^2 0.66, $[\alpha]_D^{26}$ −8.1° (c=1.0, HCOOH), $[\alpha]_D^{26}$ −3.2° (c=1.0, AcOH). *Anal.* Calcd for $C_{21}H_{19}N_3O_4 \cdot H_2O$: C, 63.8; H, 5.4; N, 10.6. Found: C, 64.0; H, 5.5; N, 10.2.

References and Notes

1) *a*) Standard abbreviations for amino acids, protecting groups, and peptides are used [*Eur. J. Biochem.*, **138**, 9 (1984)]; other abbreviations include DMF=dimethylformamide, N^{im}=nitrogen of imidazole; *b*) Part X: M. Maeda, K. Kawasaki, J. Watanabe, and H. Kaneto, *Chem. Pharm. Bull.*, **37**, 826 (1989).
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