bulk-diffusion coefficient of $1.64 \pm 0.02 \times 10^{-5}$ cm^2/s for a 0.01 *M* $AgNO_3$ solution is calculated. The hydrogen ion concentration has no noticeable effect on *D* over the p*H* range −0.48 to 7.5 but silver, sodium, iron, and sucrose quite an appreciable one; quite in accord with laws known to govern ionic diffusion processes.

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Use of N^{im}-*t*-Butyloxycarbonyl in the Synthesis of Poly-L-histidine

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N^{α},N^{im}-Di-*t*-butyloxycarbonyl-L-histidine, N^{α}-benzyloxycarbonyl-N^{im}-*t*-butyloxycarbonyl-L-histidine and poly-L-histidine have been synthesized. The role in peptide synthesis of *t*-butyloxycarbonyl as an imidazole-blocking group is discussed and compared to other currently available imidazole-blocking groups.

On a synthétisé les N^{α},N^{im}-di-*t*-butyloxycarbonyl L-histidine, N^{α}-benzyloxycarbonyl N^{im}-*t*-butyloxycarbonyl L-histidine et poly-L-histidine. On discute du rôle dans les synthèses peptidiques du groupe *t*-butyloxycarbonyl pour bloquer l'imidazole et on le compare avec celui que peuvent jouer d'autres groupes qui sont disponibles présentement pour remplir cette fonction.

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One of the main requirements associated with the synthesis of histidine-containing peptides has been the selection of a suitable protecting group for the imidazole nitrogen of the amino acid. A useful protecting group should satisfy a number of requirements such as ready availability, stability to the manipulations needed to purify the blocked peptides, stability to the removal conditions of some N and C protecting groups to assure conventional stepwise peptide synthesis, and the selective removal of the protective group under conditions that will not lead to racemization or to the destruction of labile peptide bonds. In addition, it is of great importance that the basicity of the imidazole nitrogen will be minimized while protected.

The benzyl group (1) has been used most often

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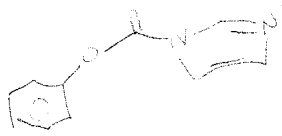


for the protection of the imidazole (im)[3] of histidine but it does not mask the basicity of the ring. In addition, the use of sodium in liquid ammonia for the removal of the benzyl group is drastic, leading in some cases to the cleavage of peptide bonds (2). Removal of the benzyl group from the imidazole group effected by catalytic hydrogenation is very slow, and impossible when sulfur-containing amino acids are present in the peptide. The im-benzyloxycarbonyl (Cbz) group (3) is very labile to basic conditions and to various nucleophilic reagents. Thus the reaction of N^{α},N^{im}-di-Cbz-L-histidine *N*-hydroxysuccinimide ester with L-alanine in a mixture of water and dioxane in the presence of two equivalents of $NaHCO_3$ led to the formation of dipeptide with a free imidazole group.[4] The new imidazole-protecting groups 2,2,2-trifluoro-1-benzyloxycarbonylaminoethyl (4) and 2,2,2-trifluoro-1-butyloxycarbonylaminoethyl (4) which are removed from imidazole by strong acids, the dinitrophenyl which is removed readily from imidazole by thiolysis (5), and the piperidinocarbonyl (6) which is labile to basic conditions and stable to acids, are most promising. However, little information on their use in peptide synthesis is available. The use of *t*-butyloxycarbonyl (*t*-Boc) as an imidazole-blocking group has been briefly described (7, 8). Some further properties of this blocking group have been investigated and its role as a blocking group is discussed.

N^{α},N^{im}-Di-*t*-Boc-L-histidine was obtained in 50% yield on reacting histidine with *t*-Boc azide in the presence of MgO (9). The im-*t*-butyloxycarbonyl (*im*-Boc) group could be removed by strong acids such as HBr in trifluoroacetic acid, HBr in acetic acid, or anhydrous HF, however it is stable to the action of HCl in dioxane. It would thus appear to be compatible with the *t*-Boc or nitrophenylsulfenyl α-amino protecting groups. In addition the *im*-Boc group was found to be much more stable towards alkali than *im*-Cbz group (3, 8). Thus when N^{α},N^{im}-di-*t*-Boc-L-histidine *N*-hydroxysuccinimide ester was coupled with L-alanyl-γ-benzyl-L-glutamic acid the corresponding di-Boc-tripeptide was obtained[4] in 80% yield. Moreover, the basicity of the imidazole ring was reduced markedly by its protection with the *t*-Boc group, since various derivatives such as N^{α},N^{im}-di-*t*-Boc-L-histidine or N^{α}-Cbz-N^{im}-*t*-Boc-L-histidine were not soluble in dilute aqueous acid solutions.

Poly-L-histidine has, in the past, been synthesized by polymerizing N^{im}-benzyl-L-histidine *N*-carboxylanhydride (10). The benzyl group was subsequently removed by treatment with sodium in liquid NH_3. We wish to report here the use of the new imidazole-protecting group, *im*-Boc, in the synthesis of this polymer.

The synthesis of poly-L-histidine is summarized in Scheme 1: N^{im}-*t*-Boc-L-histidine *N*-carboxylanhydride hydrochloride (**2**), prepared from N^{im}-*t*-Boc-N^{α}-Cbz-L-histidine (**1**), was converted to the free base **3** and polymerized in dioxane, using triethylamine as initiator. The blocked polymer, poly-N^{im}-*t*-Boc-L-histidine (**4**), with an average molecular weight of 21 000 as determined by its sedimentation and diffusion constants in dimethylformamide, assuming a partial specific volume ($\bar{v}$) of 0.75, was converted to poly-L-histidine hydrobromide (**5**) with HBr in trifluoroacetic acid.

From these preliminary studies one can conclude that the *t*-Boc blocking group on the imidazole ring has several advantages: (*i*) availability, (*ii*) ease of synthesis, (*iii*) ease of removal with no chance of racemization since carried out under anhydrous HBr treatment, (*iv*) the insolubility of *im*-Boc histidine peptides in aqueous acid solution indicates decreased basicity which results in higher yields in peptide synthesis illustrated by the large poly-L-histidine molecule synthesized (average degree of polymerization is approximately 100 compared to 50 obtained when the imidazole-blocking group was benzyl (10), and (*v*) stable to methods of purification.

Experimental

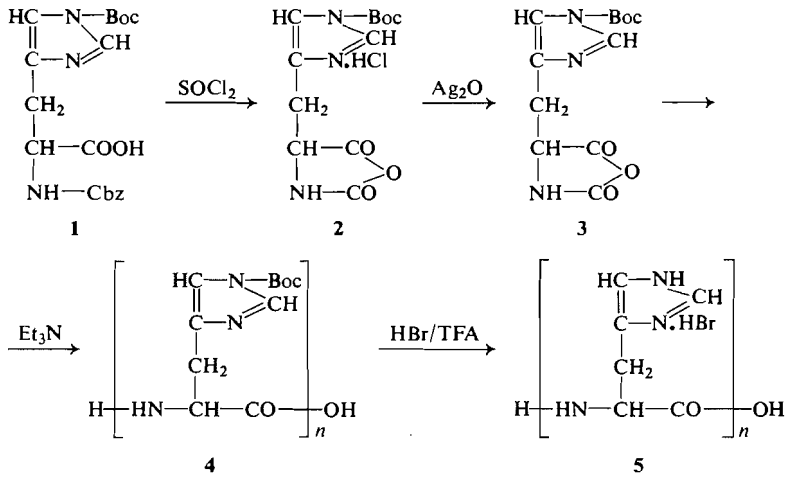
All melting points are uncorrected. Prior to analysis the compounds were dried at room temperature *in vacuo* over phosphorus pentoxide and NaOH.

N^{α},N^{im}-Di-t-butyloxycarbonyl-L-histidine

Histidine hydrochloride (31.5 g, 0.164 mol) was dissolved in 480 ml of water and added to a solution of *t*-butyloxycarbonyl azide (70 g, 0.49 mol) in 480 ml of dioxane. Magnesium oxide (24 g, 0.62 mol) was then added and the mixture was stirred for 4 days at room temperature. The excess MgO was filtered and the solution was evaporated in high *vacuo* (~25 °C). The oily residue obtained was dissolved in 100 ml of water, cooled

[3]Abbreviations: imidazole, im; benzyloxycarbonyl, Cbz; *t*-butyloxycarbonyl, *t*-Boc; im-*t*-butyloxycarbonyl, *im*-Boc.

[4]H. J. Goren, M. Fridkin, and E. Katchalski. Unpublished data.



Boc = $(CH_3)_3COCO-$; Cbz = $C_6H_5CH_2OCO-$

SCHEME 1

to 0 °C, and acidified with 50% aqueous citric acid. The oily product was extracted into ethyl acetate, the organic solution dried over Na_2SO_4, filtered, and evaporated *in vacuo* to give a colorless amorphous solid; 29 g (50% yield). All attempts to crystallize the product were unsuccessful; m.p. 70° (starts to soften slowly at 53°); $[\alpha]_D^{23}$ +13.9 (*c* 1.1, methanol).

Anal. Calcd. for $C_{16}H_{25}N_3O_6$: C, 54.07; H, 7.09; N, 11.83. Found: C, 54.77; H, 7.37; N, 11.85.

Neutralization equivalent Calcd.: 355. Found: 349, determined by anhydrous titration with sodium methoxide (11); 365, determined by anhydrous titration with perchloric in acetic acid (12).

N^{im}-t-Butyloxycarbonyl-N^{α}-benzyloxycarbonyl-L-histidine

Compound **1** was prepared similarly to the above compound on reaction of N^{α}-benzyloxycarbonyl-L-histidine (3) with *t*-butyloxycarbonyl azide in presence of MgO. Yield of amorphous solid 50%; m.p. 80° (starts to soften slowly at 55°); $[\alpha]_D^{23}$ +16.5 (*c* 1, methanol).

Anal. Calcd. for $C_{19}H_{23}N_3O_6$: C, 58.60; H, 5.95; N, 10.79. Found: C, 59.29; H, 5.75; N, 11.13.

Neutralization equivalent Calcd.: 389. Found: 380 (12) and 383 (11).

N^{im}-t-Butyloxycarbonyl-L-histidine N-Carboxylanhydride Hydrochloride (2)

To a solution of N^{im}-*t*-butyloxycarbonyl-N^{α}-benzyloxycarbonyl-L-histidine (**1**) (1.1 g, 2.8 mmol) in 10 ml of dioxane, thionyl chloride (0.64 ml, 8.5 mmol) was added. The mixture was then stirred at room temperature when the anhydride hydrochloride started to precipitate from the reaction mixture after 30 min. Stirring was effected for an additional 4 h. The product was filtered, and washed with dioxane and ether. It was then suspended in dry acetone, stirred for 15 min, and absolute ether was added. Compound **2** was filtered, washed with ether, and dried *in vacuo* over P_2O_5 and NaOH. Yield 0.80 g (90%); m.p. 260–265° dec. (evolution of CO_2 starts slowly at 200 °C); $[\alpha]_D^{25}$ −34.0 (*c* 1.1, dimethylformamide).

Anal. Calcd. for $C_{12}H_{16}O_5N_3Cl$: C, 45.36; H, 5.08; N, 13.23; Cl, 11.5. Found: C, 44.71; H, 4.81; N, 13.41; Cl, 10.97.

Mol. Wt. Calcd.: 317.6. Found: 330 (11), assuming the presence of two titrable groups per molecule.

Poly-N^{im}-t-butyloxycarbonyl-L-histidine (4)

The anhydride **2** (1 g, 2.5 mmol) was dissolved in dry acetone (75 ml) and dry silver oxide (1 g) was added. The mixture was stirred until it showed negative in a chloride test (~15 min) and then the AgCl and excess of Ag_2O was filtered, washed with acetone, and the combined filtrate and washings were evaporated in high *vacuo* (~30 °C) to yield the neutral anhydride **3**. The oily anhydride **3** was dissolved in absolute dioxane (10 ml) and triethylamine (0.004 ml, 28.8 μmol) was added as an initiator. The polymerization proceeded at room temperature with evolution of CO_2 and a slight precipitate of polymer **4** appeared. After 4 days absolute ether (50 ml) was added, and the polymer which was thus precipitated was filtered, washed with ether, and dried *in vacuo* over P_2O_5 and NaOH. Yield 0.47 g (80%); $[\alpha]_D^{25}$ −8.2 (*c* 0.88, dimethylformamide).

Anal. Calcd. for $(C_{11}H_{15}N_2O_3)_n$: C, 55.68; H, 6.37; N, 17.71. Found: C, 55.63; H, 6.46; N, 17.65.

Neutralization equivalent Calcd.: 237. Found: 242 (12).

The polymer **4** obtained as above is soluble in chloroform, dimethylformamide, acetic acid, 50% aqueous acetic acid, and methanol.

Poly-L-histidine Hydrobromide (5)

Poly-N^{im}-*t*-butyloxycarbonyl-L-histidine (0.24 g, 1 mmol) was dissolved in anhydrous trifluoroacetic acid (10 ml) and HBr gas was passed through the solution for 90 min. The trifluoroacetic acid was removed in high *vacuo* (30° C) and the residue was triturated with dry ether, ethyl acetate and petroleum–ether (b.p. 30–60 °C) and dried *in vacuo* over NaOH and P_2O_5. Yield 0.2 g (90%); $[\alpha]_D^{25}$ −19.3 (*c* 0.6, water).

Anal. Calcd. for $(C_6H_8N_3OBr)_n \cdot n/2\ H_2O$: C, 31.73; H, 4.00; N, 18.50. Found: C, 31.45; H, 4.10; N, 18.00.

Neutralization equivalent Calcd.: 227. Found: 218, determined by titration of the bromide ions (13).

N^{im}-t-Butyloxycarbonyl-L-histidine Dihydrochloride

N^{α},N^{im}-Di-*t*-butyloxycarbonyl-L-histidine was dissolved in 2 *N* HCl in dioxane and after 5–10 min a white precipitate was formed. After another 45 min the mixture was triturated with absolute ether, the precipitate filtered, washed with ether, and dried over NaOH and P_2O_5 *in vacuo*: yield 87%.

Anal. Calcd. for $C_{11}H_{19}N_3O_4Cl_2$: N, 12.80; Cl, 21.65. Found: N, 12.83; Cl, 21.21.

Mol. Wt. Calcd.: 328. Found: 330 (11) assuming the presence of three titrable groups per molecule, and 336 (13) assuming the presence of two titrable groups per molecule.

On reactions of N^{α},N^{im}-di-*t*-butyloxycarbonyl-L-histidine with HBr in TFA or in acetic acid the dihydrobromides of histidine were obtained as shown by paper electrophoresis and nonaqueous titrations.

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Erratum: Synthesis of *p-O*-(Tetra-*O*-acetyl-α-D-glucopyranosyl)-penicillin G

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On page 3099 in the right hand column, the title and compound **7** should be "*p-O*-(tetra-*O*-acetyl-α-D-glucopyranosyl)-hydroxy-penicillin G". The word "hydroxy" is absent in the article.