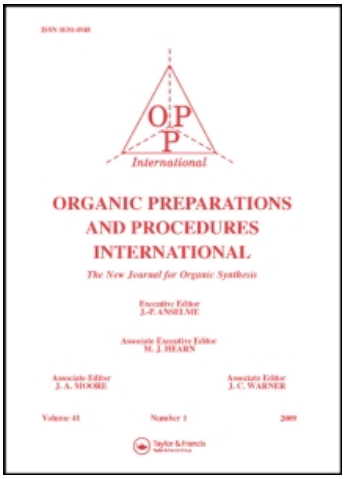

Organic Preparations and Procedures International
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t902189982

AN IMPROVED SYNTHESIS OF L-HISTIDINE BENZYL ESTER

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To cite this Article Felix, A. M. and Winter, D. P.(1970) 'AN IMPROVED SYNTHESIS OF L-HISTIDINE BENZYL ESTER', Organic Preparations and Procedures International, 2: 4, 255 — 258
To link to this Article: DOI: 10.1080/00304947009458624
URL: http://dx.doi.org/10.1080/00304947009458624

ORGANIC PREPARATIONS AND PROCEDURES, 2(4), 255-258 (1970)

AN IMPROVED SYNTHESIS OF L-HISTIDINE BENZYL ESTER

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A survey of the literature reveals that L-histidine methyl ester dihydrochloride is used frequently as the starting material for the synthesis of C-terminal histidine peptides. The alkaline conditions required to hydrolyze the methyl ester enhances the chance of racemization and may lead to side reactions. The benzyl ester protecting group for the C-terminus of peptides has found wide appeal owing to its ability to undergo cleavage with hydrogen bromide or via hydrogenolysis without racemization.

We would like to report a novel application of the procedure of Fasman and Sarin[1] for the synthesis of L-histidine benzyl ester. The previous method[2] requires a long reflux of L-histidine hydrochloride monohydrate with p-toluenesulfonic acid followed by careful isolation of L-histidine benzyl ester di-p-toluenesulfonate as the hydrate. In our hands the synthesis of this compound by the published method was consistently unsuccessful.

$$\text{BOC}^{3}\text{-His-OH} \longrightarrow \underset{\text{(I)}}{\text{BOC-His-OBz}} \longrightarrow \underset{\text{(II)}}{\text{H-His-OBz.2HCl}}$$

EXPERIMENTAL

N-t-butyloxycarbonyl-L-histidine benzyl ester (I). To a solution of 1.5g. (5.9 mmole) of N-t-butyloxycarbonyl-L-histidine[4] in 70 ml. of methanol is added, with stirring at room temperature, ethereal phenyldiazomethane[1]. The addition is followed

by thin layer chromatography (1:10; methanol:chloroform) until all of the acid is consumed. The reaction mixture is evaporated to dryness, the residual yellow oil is dissolved in 100 ml. of 2M citric acid and washed with ether (2x75 ml.). The aqueous solution is treated with 50% aqueous K_2CO_3 to pH 9 and extracted with ethyl acetate (3x100 ml.). The combined ethyl acetate fractions are washed with 100 ml. of saturated NaCl, dried over anhydrous $MgSO_4$ and evaporated to dryness. The resultant light amber oil is dried in vacuo, giving 1.95g.(98%) with $[\alpha]_D^{25}$-17.96° (C,1.5, methanol).

Anal. Calcd. for $C_{18}H_{23}N_3O_4$: C, 62.59; H, 6.71; N, 12.17. Found: C, 62.56; H, 6.75; N, 11.88.

L-Histidine Benzyl Ester Dihydrochloride (II). A solution of 1.9g (5.5 mmoles) of N-t-butyloxycarbonyl-L-histidine benzyl ester in 80 ml of 4 M HCl in dioxane[5,6] is stirred for 2 hours at room temperature. Anhydrous ether (60 ml.) is added and the resultant solution decanted from the precipitated product. The precipitate is triturated three times with anhydrous ether and dried in vacuo, giving 1.7g. (97%) of white hygroscopic solid with $[\alpha]_D^{25}$+6.54° (C, 2.7, methanol).

Anal. Calcd. for $C_{13}H_{15}N_3O_2$·2HCl: C, 49.07; H, 5.39; N, 13.21. Found: C, 49.32; H, 5.50; N, 13.01.

The compound is homogeneous by thin-layer chromatography on Silica-gel G in 3 solvent systems. Plates were developed with ninhydrin, R_f 0.60 (n-butanol-ethyl acetate-acetic acid-water, 1:1:1:1), R_f 0.65 (n-butanol-acetic acid-water-pyridine, 15:3:12:10), R_f 0.50 (n-butanol-acetic acid-water, 4:1:1).

The dihydrochloride 1.35 g. (4.2 mmole) is converted to the free base, L-histidine benzyl ester, by dissolving in

50 ml. of 50% aqueous K_2CO_3 and extracting with ethyl acetate (3 x 50 ml.). The combined ethyl acetate fractions are washed with saturated NaCl, dried over anhydrous $MgSO_4$ and evaporated to dryness. The resultant colorless oil is dried in vacuo, giving 684 mg. (66%). The free base is soluble in tetrahydrofuran, chloroform, dimethylformamide and ethyl acetate.

L-Histidine benzyl ester was shown to couple with N-benzyloxycarbonylglycine by either the mixed anhydride (ethyl-chloroformate in tetrahydrofuran) or azide (in ethyl acetate) methods[7]. Hydrogenation of the resultant N-benzyloxycarbonyl-glycyl-L-histidine benzyl ester in methanol with 5% Pd-$BaSO_4$ afforded glycyl-L-histidine[8].

Acknowledgement. The authors are indebted to the Physical Chemistry Department under the direction of Dr. P. Bommer for all of the physical spectra determinations and microanalyses.

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2. S.Akabori, S.Sakakibara and S.Shiina, J.Chem.Soc.Japan, 31, 784 (1958).
3. Abbreviations for protecting groups are: BOC, t-butyl-oxycarbonyl; Bz, benzyl; His, histidinyl.
4. Obtained commercially from Fox Chemical Co., Los Angeles, California.
5. G.W.Anderson and A.C.McGregor, J.Am.Chem.Soc., 79, 6180 (1957).
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7. Crude yields of 87% were obtained by either method. Crystallization from ethyl acetate-hexane gave analytically pure white amorphous product, mp 80-85°, $[\alpha]_D^{25}$-8.89° (C, 2.1,ethanol). Lit.[2] reports mp 99-100.2°, $[\alpha]_D^{25}$-6.8° (C, 2, ethanol).

8. The crude product was lyophilized from water to afford white amorphous product, mp 170°, $[\alpha]_D^{25}$ + 25° (C, 1.0, water). Lit.[2] for the hydrochloride reports mp 174-175°, $[\alpha]_D^{30}$ + 28.5° (C, 1.0, water) and[9] mp 175°, $[\alpha]_D^{25}$ + 25° (C, 1.0, water).

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(Received March 9, 1970)