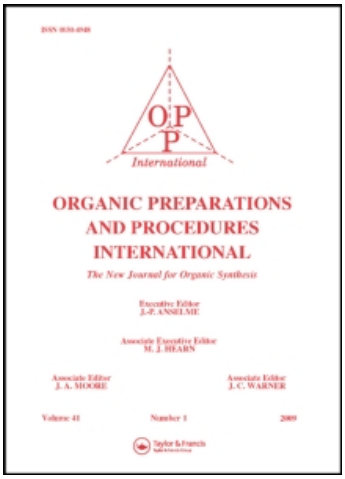

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SYNTHESIS OF *N*α-BENZYLOXYCARBONYL-L-HISTIDINE HYDRAZIDE: AN UNDESIRED SIDE-PRODUCT

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SYNTHESIS OF N^{α}-BENZYLOXYCARBONYL-L-HISTIDINE HYDRAZIDE: AN UNDESIRED SIDE-PRODUCT

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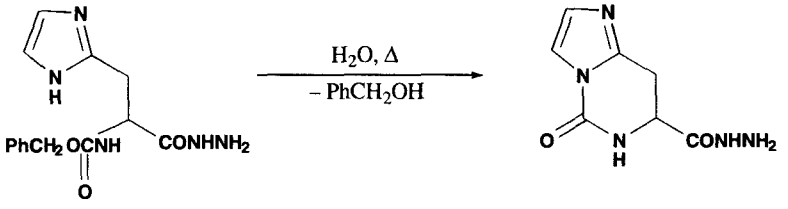
Histidine belongs to so-called "problematic" amino acids in peptide synthesis due to its imidazole ring complicating many synthesis procedures;[1-5] racemization of the histidine moiety is the main side-reaction.[3,5-7] Therefore, in peptide synthesis carried out in solution, histidine is generally incorporated with an N^{α}-urethane protection and using the azide coupling, both of which are known to cause little, if any,[6] racemization.[3] Because the existing protecting groups for the imidazole function are not quite satisfactory,[3,5] histidine as a rule has been incorporated into peptide chains, without blockage of its imidazole ring.[3,6,8] Z-His-$NHNH_2$[9] is one of the most frequently employed derivatives of this amino acid and is available at low cost and used for large-scale peptide production.[6] We needed Z-His-$NHNH_2$ for peptide synthesis in solution[8] and it was obtained and crystallized from water as in the original procedure.[1] The crystallization, however, was plagued by the formation of a side-product. We now report the nature of this side-reaction.

Both crude and purified Z-His-$NHNH_2$ upon reflux in water was converted into 2-oxo-1,3,8-triazabicyclo[4.3.0]-6,8-nonadien-4-carboxyhydrazide, whose structure was confirmed by elemental analysis, ^1H and ^{13}C NMR, FTIR and MS (including fragmentation pattern, meta-stable ions analysis and accurate mass measurements). The longer the heating the more of this product was formed. We thus recommend avoiding crystallization of Z-His-$NHNH_2$ from water, and only washing the crude compound with ethanol instead. This affords the homogenous Z-His-$NHNH_2$ as determined by TLC and HPLC and in higher yield than in the original procedure.[1] Good yield in the crystallization of crude Z-D-His-$NHNH_2$ from ethanol has been mentioned,[10] but without comment.

EXPERIMENTAL SECTION

Solvents purified according to usual methods[11] were stored over drying agents. Solvents were removed from reaction mixtures *in vacuo* on a rotary evaporator at a bath temperature not exceeding 30°. Reactions were monitored and the homogeneity of products was checked on silica gel plates (DC Alufolien Kieselgel 60 No 5553 Merck) in the following solvent systems: A = chloroform-methanol-dioxane-concd. ammonia (12:7:5:1), B = 2-butanone-pyridine-water-acetic acid (70:15:15:2), C = n-butanol-acetic acid-ethyl acetate-water (1:1:1:1). Spots were visualized with ninhydrin and chlorine-KI-tolidine reagent. Mps. were determined on a BOËTIUS heating block and are given uncorrected. HPLC analyses were carried out using "System Gold" for Methods Development consisting of a Model 126 programmable solvent module, a Model 168 diode array detector operating at $\lambda = 210$ nm, a Model 210A injector valve (all from Beckman) and PC 386SX (Wearnes) with "System Gold"

version 5.1 software for data collection and controller function. An Alltech Alltima, C_{18}, 5μ, 150 x 4.6 mm column, 0.1% trifluoroacetic acid-acetonitrile (84:16) as a mobile phase with a flow rate 1 mL/min were applied.



Z-His-NHNH$_2$

2-Oxo-1,3,8-triazabicyclo[4.3.0]-6,8-nonadien-4-carboxyhydrazide

N$^{\alpha}$-Benzyloxycarbonyl-L-histidine Hydrazide (Z-His-NHNH$_2$).- The crude hydrazide produced according to Ref. 1. was washed with ethanol to remove hydrazine and was obtained in 57% yield, mp. 170.5-172.5° (lit.[1], yield 38-48%, mp. 171-173°). TLC: R_f(A): 0.46, R_f(B): 0.70, R_f(C): 0.70. HPLC: tR = 4.22 min, 100% purity.

2-Oxo-1,3,8-triazabicyclo[4.3.0]-6,8-nonadien-4-carboxyhydrazide.- Z-His-NHNH$_2$ (2.125 g, 7 mmol) was dissolved in boiling water (50 mL) and heated at reflux for 24 hrs. The water was evaporated, methanol (50 mL) added and the resulting precipitate collected and washed with ether to give 1.30 g (96%) of the product. Mp. 216-218°. TLC: R_f(A): 0.26, R_f(B): 0.45, R_f(C): 0.36. HPLC: tR = 1.30 min, 100% purity.

Anal. Calcd. for $C_7H_9N_5O_2$: C, 43.07; H, 4.65; N, 35.88. Found: C, 42.96; H, 4.54; N, 35.74

^{1}H NMR [$(CD_3)_2SO$] (Varian 200 MHz): δ 2.74 (C^5H, dd <14.9> <7.1> 1H), 2.96 (C^5H, dd <14.9> <4.2> 1H), 4.20 (C^4H, m <7.1> <4.2> <1.0> 1H), 6.81, 8.02 (C^7H, C^9H, s, s, 1H, 1H), 7.56 (N^3H, d <1.0> 1H).

^{13}C NMR [$(CD_3)_2SO$] (Varian 200 MHz): δ 29.44 (C^5), 55.00 (C^4), 116.65, 116.78 (C^7), 132.43 (C^6), 134.95 (C^9), 156.80 (C^2), 172.22 (*C*ONHNH$_2$).

FTIR (KBr) (Philips Analytical PU9800) cm^{-1}: 3462-ν_{as}(NH_2), 3240-ν_{sym}(NH_2), 3322-ν(NH hydrazide), 3109-ν_s(:C-H), 2935-ν_{as}(CH_2), 2835-ν_{sym}(CH_2), 1780-ν(C=O oxo), 1716-ν(C=O hydrazide), 1612-ν(C=N), 1591, 1529-AII), 1465-ν_s(C-C in plane ring), 838-ω(C-H), 632-ω (imidazole ring).

MS (AMD Intectra's AMD 604) m/z (%): 195 (M^+; 47), 179 (4), 167 (7), 164 (28), 136 (25), 118 (5), 110 (11), 109 (7), 108 (8), 93 (4), 82 (100), 81 (87), 54 (13).

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9. Abbreviations used: Boc = *t*-butoxycarbonyl, Z = benzyloxycarbonyl, His = histidine.

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THE LESS FAVORED 1-DERIVATIVES OF DIMETHYLDIHYDROPYRENE BY FORMYLATION

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Dimethyldihydropyrene (**1**) is an interesting bridged [14]annulene which is an excellent probe for ring current effects.[1,2] Access to 1-substituted derivatives has been limited however, because electrophilic substitution, while extremely facile, proceeds at the 2-position.[3-5] For example, nitration, acetylation and benzoylation give 99%, 93% and 84% of the corresponding 2-derivatives, with no