# NOTE

## SYNTHESIS OF (S)-$N^{\tau}$-[$D_3$] METHYLHISTIDINE (1)

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## SUMMARY

(S)-Histidine methyl ester was converted on a 20 g scale to its $N^{\alpha}$, $N^{\pi}$-cyclic urea by treatment with carbonyl diimidazole. The $N^{\tau}$ position was alkylated with [$D_3$]-methyl iodide and the product was hydrolyzed to give isomerically and isotopically pure $N^{\tau}$-[$D_3$] methylhistidine, without significant loss of optical activity.

Key words: deuterium, carbonyl diimidazole, (S)-5-Oxo-7-carbomethoxy- 5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidine, (S)-$N^{\tau}$-methylhistidine.

## INTRODUCTION

(S)-$N^{\tau}$-Methylhistidine, a unique amino acid synthesized by muscle and excreted into urine without recycling(2), has been used increasingly as a marker for protein catabolism(3,4). In connection with ongoing programs to expand the scope of stable isotope applications in clinical research, we have addressed the synthesis of its trideuteromethyl isotomer for use either as a tracer *in vivo* or as an isotopic dilution standard *in vitro*(5). Direct methylation of histidine itself with methyl iodide, as described in the review of Greenstein and Winitz(6), had proven to be an unsatisfactory approach, affording end product contaminated with $N^{\tau}$- and $N^{\pi}$-methylated analogs even after extensive chromatographic purification. This problem was circumvented by protecting the $N^{\alpha}$ and $N^{\pi}$ positions in a cyclic urea derivative while leaving the $N^{\tau}$ site available for exclusive methylation. Such an approach was first

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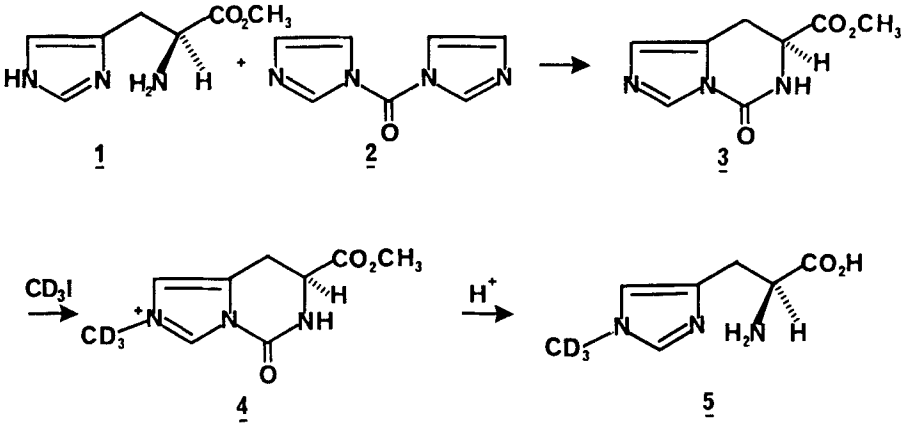
described by Durant, et al.(7) in their stereospecific synthesis of N-methylated histamine $H_2$-receptor antagonists and then applied by Knight(8) et al. to the radiolabeling of $N^{\tau}$-methylhistamine.

## RESULT AND DISCUSSION

(S)-Histidine methyl ester was treated with carbonyl diimidazole to produce the cyclic urea 3 in 60% yield. Compound 3 was then quaternized with




$[D_3]$methyl iodide to give $N^{\tau}$-methylated 4 in 60% yield. Hydrolysis of 4 was accomplished in boiling dil. hydrochloric acid, leaving $N^{\tau}$-$[D_3]$-methylhistidine (5) in 80% yield after final work-up. The optical purity of this material was at least 95% by comparison to the optical rotation of an authentic, unlabelled sample (Sigma Chemical Co.); and the isomeric and isotopic purities were high as determined by GC-MS analysis (Finnigan 4000) of the N-acetyl propylester (NAP) after separation on 3% SP-2300 at 250°C by the literature procedure(5). The methane chemical ionization (CI) ion chromatogram of $N^{\tau}$-trideuteromethyl-histidine NAP at the $[M+H]^+$ ion (m/z=257) showed a retention time of 2.2 minutes with a relative intensity of 100 in comparison to the ion at 2.9 minutes retention, showing a relative intensity of 1.2 and corresponding to the $N^{\pi}$-isotomer. An analysis of deuterium abundance of the ion clusters including

the parent ion under methane CI and the $[M-COOC_3H_7]^+$ ion (m/z = 169) under electron impact ionization demonstrated the product to be at least 96% $d_3$.

## EXPERIMENTAL

General: NMR were taken with a Varian EM360A Spectrometer. Optical rotation was measured using a Rudolph-62 polarimeter.

(S)-5-Oxo-7-carbomethoxy-5,6,7,8-tetrahydroimidazo[1,5-c]pyrimidine (3): (S)-Histidine methyl ester dihydrochloride (24g, 0.1 mole) was dissolved in 250 ml of methanol followed by 2 eq of sodium methoxide in 100 ml of methanol. After 1 hour, the solvent was evaporated under reduced pressure and the free base extracted into 300 ml of tetrahydrofuran chloroform (2:1). Carbonyl diimidazole (16g, 0.1 mole) in an equal volume of the 2:1 solvent mixture was added and the suspension stirred for 1 hour. Partial removal of solvent, again under reduced pressure, to a final volume of approximately 50 ml afforded a copious precipitate. Suction filtration followed by washes with cold tetrahydrofuran left 11 g (61%) of crystalline product. NMR ($CDCl_3$): 3.1-3.4 (m, 8-$CH_2$), 3.8 (s, $OCH_3$), 4.3 (t, J=6 Hz, 7-CH), 6.8 (s, 1-CH), 6.9 (bs, NH) and 8.1 ppm (s, 3-CH).

(S)-2-[$D_3$]Methyl-5-Oxo-7-carbomethoxyimidazo[1,5-c]pyrimidineiodide (4): Compound 3 was mixed with [$D_3$]methyl iodide (99 mol% D, 15g, 0.1 mole) and 100 ml of N,N-dimethylformamide in a sealed pressure glass vessel. The vessel was heated at 85°C for 24 hours. After chilling to room-temperature, ether (700 ml) was added, and the product settled out as an oily solid. Recrystallization from methanol-ether gave 11g (58%) of product 4. NMR ($D_2O$): 3.3 (d, J=5 Hz, 8-$CH_2$), 3.6 (s, $OCH_3$), 4.5 (t, J=5 Hz, 7-CH), 7.2 (s, 1-CH) and 9.2 ppm (s, 3-CH).

(S)-N$^{\tau}$-[$D_3$]methylhistidine (5): Compound 4 was heated in 100 ml of 6N HCl for 12 hours. The solvent and acid were evaporated under reduced pressure and the

residue was dissolved in 50 ml of water. After adjusting the pH to 7.5 with triethylamine, the solution was decolorized with activated charcoal and concentrated to a volume of 30 ml. After addition of 100 ml of ethanol, the precipitated crude amino acid was recrystallized from water-ethanol to give 4.6 g (82% yield) of 5 mp 240-242°C (dec.). TLC: Rf=0.33 single spot, on silica gel eluted by 10% conc. $NH_4OH$ in methanol. NMR ($D_2O$): 3.1 (d, J=6 Hz, β -$CH_2$), 4.0 (d, J=5 Hz, α -CH), 7.1 (s, ring CH) and 7.9 ppm (s, NCHN). $[\alpha]_D^{25^o}$ = -24.5° (c=5, $H_2O$); Sigma standard, $[\alpha]_D^{25^o}$ = -24.8 (c=5, $H_2O$); reported(9), $[\alpha]_D^{25^o}$ = -24.7 (c=2.8, $H_2O$)(10).

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10. Note should be made that optical rotations in distilled water of $N^{\tau}$ -methyl histidines may give spurious results as a function, presumably, of variations in pH between analytical trials. We have noted $[\alpha]_D^{25^o}$ values as low as -13 ± 1° both for the synthetic and authentic (S) materials in addition to the values reported here.