Synthesis of ^{14}C-Labelled Etintidine Hydrochloride

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SUMMARY

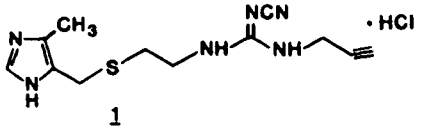
Two ^{14}C preparations of the H_2-antagonist etintidine hydrochloride are reported. In one, the label is introduced by reacting [1-^{14}C] propargylamine with the appropriate isothiourea, followed by hydrochloride formation. This afforded a material of 95% radiochemical purity whose specific activity was 1.5 mCi/mmol.

To obtain material labelled in an alternate position, uniformly labelled ^{14}C-cysteamine hydrochloride was reacted with 4-methyl-5-hydroxymethyl imidazole hydrochloride to produce 4-methyl-5-[(2-aminoethyl)thiomethyl]imidazole. This was converted in three additional steps to etintidine hydrochloride yielding material of 96% radiochemical purity and specific activity of 9.0 mCi/mmol.

Key Words: Etintidine, H_2-antagonist, [1-^{14}C]propargylamine, [U, ^{14}C]cysteamine hydrochloride.

INTRODUCTION

In connection with pharmacokinetic and metabolism studies related to the H_2-antagonist etintidine hydrochloride (1),[1] we required the preparation of ^{14}C labelled material. Based on metabolic studies[2,3] on the structurally related compound cimetidine (2),[4] and our desire for a relatively short synthetic scheme, the label was introduced as



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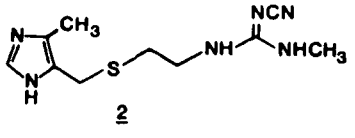

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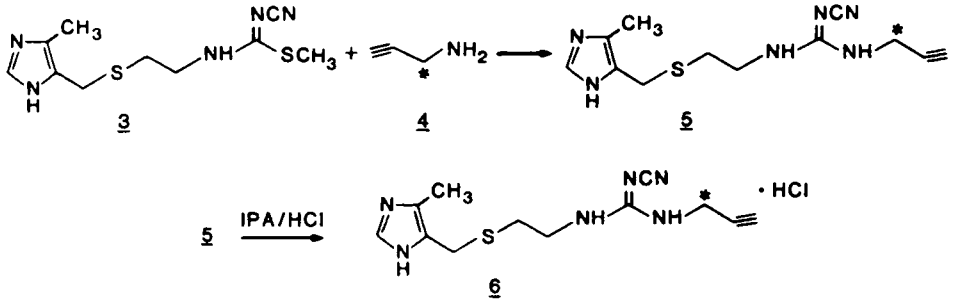
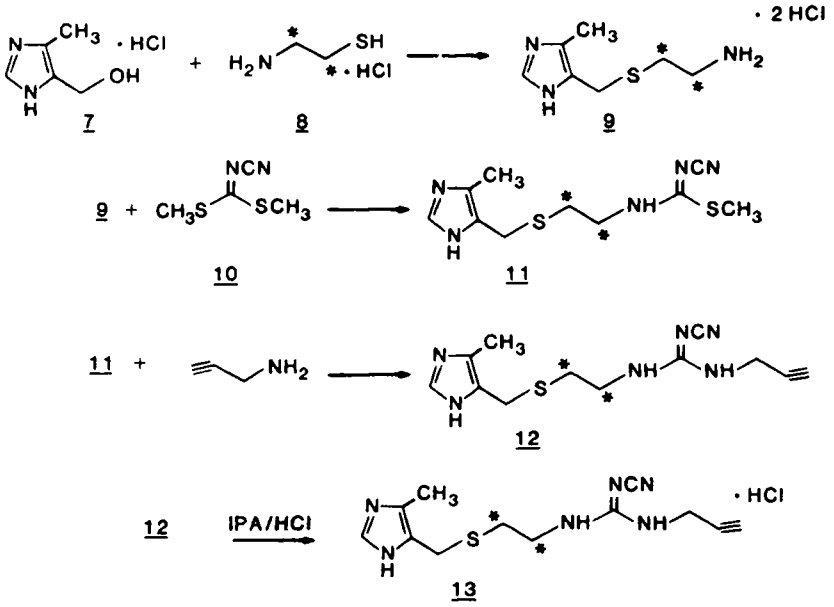
shown in Scheme 1. Alternatively, uniformly labelled cysteamine hydrochloride was utilized as shown in Scheme 2.

The position of the label in the above schemes are in contrast to the location in ^{14}C cimetidine, namely in the 2 position of the imidazole ring.[3]



SCHEME 1. PROPARGYLAMINE ROUTE



SCHEME 2. CYSTEAMINE HYDROCHLORIDE ROUTE

RESULTS AND DISCUSSION

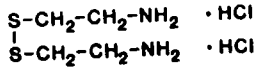
Propargylamine Route (Scheme 1)

It has been reported that to obtain a reasonable yield for the reaction of isothiourea 3 and propargylamine, 4, a large excess of the latter was required.[5] Since we were introducing the label *via* this compound, we needed to minimize that excess. Several cold runs demonstrated that at a mole ratio of 4:1 (4 to 3), a good yield was obtained in a reasonable time frame. Furthermore, the free base 5 did not require purification by column chromatography under these reaction conditions.[5]

During preliminary cold runs, the conversion to the hydrochloride salt occasionally resulted in obtaining a hydrated form of 6 (mp 94-96°C). This appeared to be related to the temperature at which the 6N HCl was added to the isopropyl alcohol slurry of free base 5. Room temperature consistently afforded anhydrous material whereas 0-5°C most often afforded the hydrate which was quite insoluble in the reaction mixture.

Cysteamine Hydrochloride Route (Scheme 2)

Since the radiochemical purity of the uniformly labelled cysteamine hydrochloride (8) used was only 57%, it was important to investigate optimum conditions for the coupling reaction (see Scheme 2). The single major impurity in 8 was the dimer 14, *ca* 18% as determined by TLC radioscan (IPA/EtOH/water/conc HCl 75:75:54:5; cellulose plates, I_2 development). A second major unknown impurity was present (11%) followed by numerous unknown impurities at the 1-5% level.



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Hence, several cold runs were conducted, deliberately contaminating 8 with 15% of 14, whereupon a reasonable workup procedure for the isolation of 9 in moderate yield was obtained (see Experimental). The labelled dihydrochloride 9 actually produced contained ca 3% of 14 as determined by quantitative TLC. To compensate for this, the amount of cyanocarbonate 10 was increased in the reaction with 9. The isothiourea obtained, 11, was comparable in purity to that used in the propargylamine route (Scheme 1). However, despite this high purity, the free base 12 obtained in the subsequent reaction had a somewhat lower radiochemical purity than expected (93%). It was not necessary to carry out column chromatography for purification since conversion to the hydrochloride afforded 13 in good radiochemical purity.

EXPERIMENTAL

Materials and Methods

Melting points are uncorrected.

The labelled propargylamine (3.8 mCi/mmol) was obtained from New England Nuclear, Boston, Massachusetts as a solution in methanol.

The labelled cysteamine hydrochloride (8.2 mCi/mmol) was obtained from Amersham Corp.

TLC was performed by spotting 4 µg (1 µl) in one or more of the following systems:

A. Chloroform/Methanol/Hexane/Conc NH_4OH (82:30:20:5) on silica gel GF plates.

B. Methanol/H_2O (90:10) on C_{18} plates.

C. Acetonitrile/Methanol/Conc NH_4OH (50:48:2) on silica gel GF plates.

HPLC was performed on a system equipped with a Model 8800 quarternary solvent delivery system from DuPont Instruments, a Model 710B autosampler and a reversed phase column (µBondapak C_{18}; 3.9 mm x 30 cm) from Waters Associates.

TLC and HPLC of all labelled compounds were identical to their unlabelled counterparts.

A Model SF 7733 variable wavelength UV detector (229 nm) from Kratos was used in conjunction with a Flo-One™ HS radioflow monitor from Radiomatic Instruments and Chemical Co., Inc. The UV detector and radioflow monitor were connected in series with column effluent passing from UV detector to the radioflow monitor. All column separations were accomplished by using a mixture of 0.02M sodium acetate/methanol/85% phosphoric acid (1000/100/1 by volume) as the mobile phase at a flow rate of 2 ml/min. Flo-Scint III (Radiomatic Instruments and Chemical Co., Inc.) was used as a flow through (4 ml/min) scintillation cocktail. This system was used for all radiochemical purity determinations reported.

[^{14}C]Etintidine Free Base(5)

In a 15 ml 3-neck reaction flask equipped with a reflux condenser, glass stopper, rubber septum, and stir bar was added 3, (404.18 mg, 1.5 mmol). The ^{14}C-labelled propargylamine solution 4, (164.4 mg in 0.48 ml methanol, 2.99 mmol) was added to the reaction flask followed by unlabelled propargylamine (166.1 mg in 0.52 ml methanol, 3.01 mmol). The ampoule containing the labelled propargylamine was rinsed with methanol (4 x 0.25 ml), and the washings added to the reaction mixture to make a final concentration of propargylamine in methanol of 3 mmol/ml. The reaction was then refluxed 20.5 hr.[5] The solvent and excess propargylamine were removed by distillation under reduced pressure to give an amber colored gum-like residue. This residue was diluted with warm (60°C) isopropanol (0.8 ml) and the solution was cooled to room temperature. The resulting slurry was diluted with hexane (0.4 ml) and stirred at 0°C for 2 hr. The product was collected by filtration, washed with cold (5°C) i-PrOH/hexane 2:3 (0.5 ml), and dried *in vacuo* (dessicator) for 16 hr to afford 5 (252.9 mg, 0.92 mmol, 61% yield, mp 148-150°C, lit[1] 150-152°C).

[^{14}C] Etintidine Hydrochloride (6)

Etintidine free base 5, (229.4 mg, 0.81 mmol) was slurried in i-PrOH (0.6 ml) and 6N HCl (0.14 ml, 0.81 meq) was added slowly. The slurry went into solution after 2 min, and was allowed to stir at room temperature for 10 min. The solution was treated with charcoal (30 mg), stirred for 5 min, then filtered over Super-Cel. The filter cake was washed with i-PrOH/acetone 1:5 (0.4 ml). The resulting filtrate was diluted with acetone (3.5 ml) and stirred at room temperature. After 2 hr, the resulting slurry was stirred at 0°C for an additional 2 hr. The product was isolated by filtration, washed with cold (5°C) acetone (0.5 ml) and dried *in vacuo* (dessicator) for 16 hr to afford 6 (180.3 mg, 0.58 mmol, 71.5% yield, mp 158-160°C). TLC: System A, 1 spot R_f 0.55; System B, 1 spot R_f 0.78.

An IR spectrum corresponded to that of authentic etintidine hydrochloride. The specific activity of (6) was determined to be 1.5 mCi/mmol. HPLC analysis showed the radiochemical purity to be 95%.

[(2-Amino[U-^{14}C-ethyl])thiomethyl]-5-methylimidazole Dihydrochloride (9)

A 25 ml 3 neck flask equipped with a reflux condenser, glass stopper, rubber septum, and stir bar was charged with 4-methyl-5-imidazolemethanol hydrochloride 7, (603.3 mg, 4.06 mmol) and labelled cysteamine hydrochloride (8, 464.0 mg, 4.06 mmol) in glacial acetic acid (2.0 ml). The clear solution was refluxed for 5 hr, allowed to cool and then diluted with isopropanol (15 ml). The mixture was cooled at 0°C for 5 hr, and crystals of 9 precipitated accompanied by a tacky residue. The product was decanted from the residue and was filtered, washed with cold (5°C) isopropanol (2.0 ml), and dried on the filter under N_2 *in vacuo* to yield 3 (484.2 mg, 1.98 mmol, 49% yield, mp 171-175°C). TLC: System C, three spots, major spot R_f 0.48.

N-Cyano-N'-[2-[(5-methyl-1H-imidazol-4-yl)methylthio](U-^{14}C-ethyl)]-S-methyl isothiourea (11)

To a 15 ml 3 neck flask charged with methanolic NaOH (170.3 mg, 4.26 mmol in 5 ml MeOH) was added 4-[(2-amino[U-^{14}C-ethyl])-thiomethyl]-5-methylimidazole dihydrochloride 9 (483.2 mg, 1.98 mmol). The resulting cloudy solution was stirred for 30 min, and evaporated at 50°C under reduced pressure. The residue was diluted with isopropanol (4.0 ml), stirred at 0°C for 1 hr, and filtered to remove the sodium chloride. The filtrate, containing the free base of 9 was charged with dimethylcyano-dithioiminocarbonate (10, 298.3 mg, 2.04 mmol) and the mixture stirred overnight. The resulting precipitate was cooled at 0°C for 1.5 hr, and the product 11 was isolated by filtration, washed with cold (5°C) isopropanol (1.0 ml) and dried under N_2 *in vacuo* to yield crude 11 (307.6 mg, 58% yield, mp 147-148°C). This was recrystallized from isopropanol (1.8 ml) to yield 11 (226.6 mg, 0.84 mmol, 74% recovery, mp 147-148°C). TLC: System A, R_f 0.53; System C, R_f 0.82. HPLC analysis indicated a radiochemical purity of 97.5%. The specific activity was determined at this point to be 9.2 mCi/mmol.

^{14}C-Etintidine Free Base (12)

A 15 ml 3 neck flask was charged with 11 (223.3 mg, 0.83 mmol) and methanol (2.0 ml). Propargylamine (227 µl, 3.32 mmol) was added and the mixture stirred and heated to reflux for 20.5 hr. The solvent and excess amine were removed by vacuum distillation. The residue was dissolved in warm (60°C) isopropanol (0.5 ml) and the resulting solution was cooled to room temperature. The slurry was diluted with hexane (0.2 ml) and stirred at 0°C for 2 hr. The product was collected by filtration, washed with cold (5°C) isopropanol (0.3 ml), and dried under N_2 *in vacuo* to afford 12 (116.15 mg, 0.42 mmol, 51% yield, mp 141-143°C, lit[1]

150-152°C). TLC: System A, three spots, major spot R_f 0.57; System C, three spots, major spot R_f 0.61. HPLC analysis indicated a radiochemical purity of 93%.

^{14}C-Etintidine Hydrochloride (13)

Etintidine free base (12 114.2 mg, 0.41 mmol) was slurried in isopropanol (0.6 ml) in a 10 ml one neck flask at room temperature and 6.0N HCl (70 µl, 0.42 meq) was added slowly. The resulting solution was stirred for 10 min, treated with charcoal (10 mg), stirred for 5 min, and then filtered over Supercel and magnesium sulfate. The filter cake was washed with isopropanol/acetone 1:5 (0.4 ml). The filtrate and washings were diluted with acetone (3.8 ml) and stirred for 3 hr at 0°C. After dilution with 0.5 ml of hexane, precipitation of 13 occurred. The slurry was stirred at 0°C for 2 hr, then isolated by filtration, washed with cold (5°C) acetone (1.0 ml), and dried under N_2 in vacuo to afford 13 (70.96 mg, 0.23 mmol, 55% yield, mp 154- 156°C). TLC: System A, 1 spot, R_f 0.60; System B, 1 spot, R_f 0.78.

HPLC analysis indicated a radiochemical purity of 96.4%. The specific activity of 13 was determined to be 9.0 mCi/mmol.

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REFERENCES

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2. S. C. Mitchell, J. R. Idle and R. L. Smith - Xenobiotica 12 (5): 283 (1982)

3. D. C. Taylor, P. R. Cresswell and D. C. Bartlett - Drug Metabolism and Disposition 6 (1): 21 (1978).

4. Drugs of the Future 1 (1): 13 (1976).

5. In US Patent #4,112,234, September 5, 1978 (R. Crenshaw, G. Luke) it was reported that a nitrogen sweep of the reaction mixture was necessary to remove the generated methyl mercaptan. This would then prevent formation of mercaptan adducts of etintidine via addition to the triple bond. The presence of these adducts necessitated purification of the product by column chromatography to obtain crystalline material. In our work, due to the scale employed, the nitrogen sweep was not necessary to avoid chromatography both in this route as well as the route shown in Scheme 2.