SYNTHESIS OF [2-^{14}C]DEZAGUANINE MESYLATE (6-AMINO-1,5-DIHYDRO-4H-IMIDAZO[4,5-c]PYRIDIN-4-ONE-6-^{14}C METHANESULFONATE), A NEW ANTITUMOR AGENT

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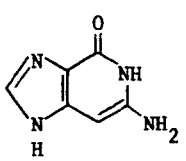
SUMMARY

Dezaguanine mesylate (CI-908 mesylate, 3-deazaguanine mesylate, 6-amino-1,5-dihydro-4H-imidazo[4,5-c]pyridin-4-one methanesulfonate, 7), a new antitumor agent, was labeled in the 2-position by use of a new synthesis to 3-deazaguanine. Thus, methyl 4-(hydroxymethyl)-1-(triphenylmethyl-1H-imidazole-5-carboxylate (10) was converted with thionyl chloride to the corresponding chloromethyl derivative 11, which was treated with [^{14}C]-potassium cyanide to afford the labeled penultimate intermediate, methyl 5-(cyanomethyl)-1H-imidazole-4-carboxylate (12). Ring closure of 12 with methanolic ammonia and subsequent salt formation provided [2-^{14}C]3-deazaguanine mesylate (13).

Key Words: 3-Deazaguanine mesylate, dezaguanine mesylate, [6-^{14}C]6-Amino-1,5-dihydroimidazo[4,5-c]pyridin-4-one methanesulfonate, ^{14}C, antitumor, methyl 5-(chloromethyl)-1H-imidazole-4-carboxylate, hydrochloride.

INTRODUCTION

3-Deazaguanine (1, dezaguanine, CI-908, 6-Amino-1,5-dihydro-4H-imidazo-[4,5-c]pyridin-4-one) (1), a broad spectrum guanine antimetabolite (2) first reported by Cook *et al.* (4) in 1975 and 1976 has exhibited sufficient preclinical antitumor activity, particularly against rat and mouse mammary adenocarcinomas, slow and fast growing mammary tumors in mice, and the human breast xenograft subrenal capsule implant system (5,6), to warrant Phase 1 clinical trials.



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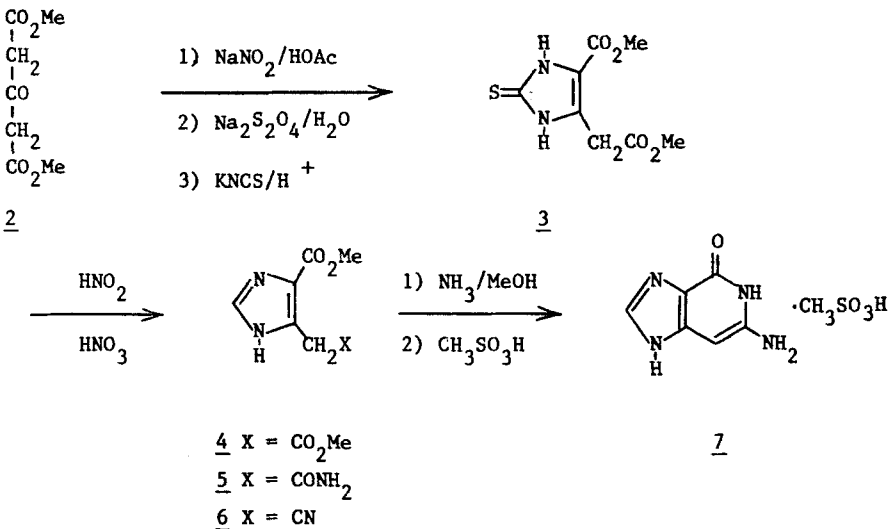
by Schwartz et al. (9), we felt that the tritium label was too labile for the desired in vivo studies and elected to prepare a ^{14}C-labeled compound.

RESULTS AND DISCUSSION

A review of the current process (4), and indeed the only known route to 3-deazaguanine, revealed that 3-deazaguanine originally was derived from dimethyl 1,3-acetonedicarboxylate and thiocyanate. Thus, the use of the available process for our radiochemical synthesis is not practical, since labeled acetonedicarboxylate is not readily available. A ten-fold excess of thiocyanic acid (as its potassium salt) is also required in this process. Moreover, these agents are used in an initial one pot reaction (see Scheme I) to provide the imidazole-2-thione 3 in approximately 20% yield.



2 3

4 X = CO_2Me
5 X = $CONH_2$
6 X = CN

7

SCHEME I

Radiolabeled 3-deazaguanine methanesulfonic acid salt (mesylate) (7) was desired for initial clinical trials and further biochemical studies. Although a tritiated sample of 3-deazaguanine was used for earlier biochemical studies

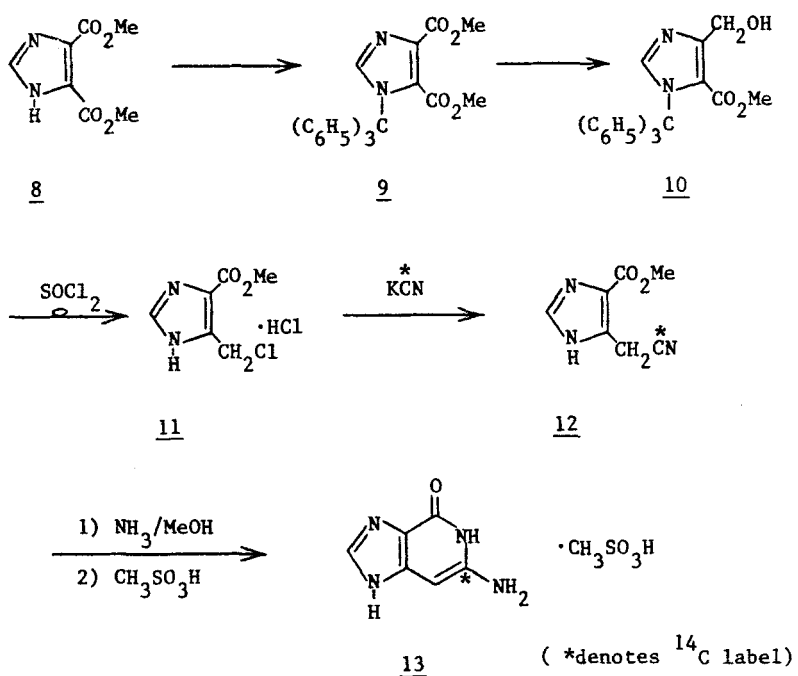
A ^{14}C-labeled imidazole-2-thione thus obtained must then be taken through a five-step sequence to 3-deazaguanine mesylate (7). Because of the difficulties of carrying a ^{14}C-labeled material through at least six steps and the likelihood of a very low overall yield, we sought alternative routes to [^{14}C]3-deazaguanine which would allow the incorporation of the label in the molecule at a later stage.

One potential alternative route to 3-deazaguanine which we initially considered, was that reported by de Bode and Salemink (10) in 1974 in an early unsuccessful attempt to prepare 3-deazaguanine from formic acid ring-closure procedures on certain diaminopyridine derivatives. Acylated 3-deazaguanines resulting from these cyclizations could not be deprotected to provide the desired heterocycle. Formulation studies have shown that 3-deazaguanine is somewhat unstable in acidic or basic solutions (7). Thus, strenuous conditions required to remove acyl groups from 3-deazaguanine preclude the synthesis of a ^{14}C-labeled derivative of 3-deazaguanine by these procedures.

We have elected to utilize the well known chemistry of the final steps of the original process to 3-deazaguanine in devising a synthesis of ^{14}C-labeled 3-deazaguanine and sought to prepare the penultimate imidazole intermediate 6 in one radiochemical step. Thus, only two radiochemical reactions would be required to synthesize [^{14}C]3-deazaguanine. New chemistry would be required to prepare a ^{14}C-labeled 6, but its subsequent cyclization with ammonia is well known and proceeds in high yield to 3-deazaguanine. Furthermore, the penultimate imidazole intermediate 6 is a valuable compound in that it is the convergent intermediate to prodrugs, nucleosides, and nucleotides of 3-deazaguanine such as 7- or 9-(tetrahydropyranyl)-3-deazaguanine (11), 2'-deoxy-3-deazaguanosine (3), 7- or 9-β-D-ribofuranosyl-3-deazaguanine (4), and 3-deazaguanylic acid (4), which all have significant biological activity and may at some later date be desired in their ^{14}C-labeled form.

Our plan was to synthesize the unknown methyl 5-(chloromethyl)-1H-imidazole-4-carboxylate (11) and convert it to 12 by reaction with potassium [^{14}C]cyanide, thereby incorporating the label into the nitrile carbon (Scheme II).

Kavadias et al. (12) have recently described the synthesis of methyl 4-(hydroxymethyl)-1-(triphenylmethyl)-1H-imidazole-5-carboxylate (10) from commercially available dimethyl 1H-imidazole-4,5-dicarboxylate (8, Scheme II).



SCHEME II

We have resynthesized 10 according to their procedure and subsequently converted it with thionyl chloride to obtain the novel, crystalline methyl 5-(chloromethyl)-1H-imidazole-4-carboxylate hydrochloride (11) in 88% yield.

Chain extensions of primary and secondary alkyl halides via cyanide displacements are useful, well known reactions (13). However, activated halides such as allylic and benzylic halides are likely to undergo hydrolysis to the alcohol rather than displacement by cyanide (14). Several modifications have been described which tend to minimize the hydrolysis

reaction, most notable being the use of potassium cyanide in the presence of 18-crown-6 ether (15). Heterocyclic benzylic halides such as our requisite imidazole intermediate 11 would appear to be quite reactive towards cyanide and solvolysis reactions. Interestingly, a number of chain extensions of chloromethylimidazoles to the corresponding cyanomethylimidazoles have appeared in the literature in histamine analog research as early as 1911 (16). The yields of the reactions are generally about 50%, which is acceptable for our purposes. However, a five- to ten-fold excess of potassium cyanide is usually employed. In our model reactions of 11 with one equivalent of potassium cyanide, a very low yield of 6 was obtained along with much decomposition. Methyl 5-(hydroxymethyl)-1H-imidazole-4-carboxylate, the likely hydrolysis product, could not be detected via TLC and may have decomposed further (17). Utilizing two equivalents of potassium cyanide provided a corresponding increase in the yield of 6 (23%). We have explored a variety of conditions (solvents, temperature, time, bases, and potassium cyanide equivalents) and particularly anhydrous conditions without a significant increase in the yield of 6. Catalysis of the cyanide displacement reaction by 18-crown-6 ether in acetonitrile, seemingly optimum conditions according to Zubrick *et al.* (15), did not appear to afford a significant advantage. Two equivalents of potassium cyanide appear to compete more favorably with hydrolysis or polymerization reactions. The fact that the increase of potassium cyanide to 5.5 equivalents resulted in lower yields than two equivalents indicated that potassium cyanide may catalyze the hydrolysis of the ester function (18,19).

Although our yields of 6 from model studies were low, we have elected to continue this approach for the synthesis of [2-^{14}C]3-deazaguanine because of the urgent need for it in Phase 1 clinical studies. Furthermore, the process employs only two equivalents of potassium [^{14}C]cyanide rather than large excesses, involves an imidazole precursor 11 which was readily synthesized, and allows easy purification of the reaction product, penultimate

intermediate 12, via column chromatography procedures albeit in low yield.

Treatment of 11 with two equivalents of potassium [^{14}C]cyanide in acetonitrile at 5°C provided a 26% yield (13% radiochemical yield) of the labeled penultimate imidazole intermediate 12 after silica gel chromatography. Imidazole 12 was cyclized in methanolic ammonia at 130° for seven days (4) to afford [2-^{14}C]3-deazaguanine in 67% yield after crystallization. The [2-^{14}C]3-deazaguanine in methanol was treated with methanesulfonic acid to afford 5.119 mCi of the desired [2-^{14}C]3-deazaguanine mesylate (13) at a specific activity of 37.14 mCi/mg (9.47 mCi/mmol). The radiochemical purity via TLC and HPLC was greater than 98%.

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. ^{1}H NMR spectra were determined on a Varian XL-300 (300 MHz) spectrometer. Chemical shifts were reported in δ (ppm) downfield from tetramethylsilane. Liquid scintillation counting was performed with a Packard 4530 liquid scintillation counter using Beckman Ready-Solv MP or Mallinckrodt Handifluro liquid scintillation cocktail.

Thin layer chromatography (TLC) was performed on Whatman $KC_{18}F$ plates (0.2 mm). The final compound 13 was analyzed using the TLC systems described as follows: CH_3CN:THF:H_2O (50:25:25) + 0.1 g HOAc + 0.1 g $NaHCO_3$, R_f = 0.52; MeOH:0.1 M NaOAc (1:1), R_f = 0.6; CH_3CN:0.5 M NaCl (1:4), R_f = 0.62. The TLC plates were radiochemically analyzed using a Berthold LB 2832 automatic TLC linear analyzer. High pressure liquid chromatography (HPLC) was performed using a Spectra Physics SP8700 solvent delivery system, a Kratos Spectroflow 773 variable wavelength UV detector, a Hewlett-Packard 3390A integrator and a United Technologies Packard Tri-Carb RAM 7500 radioactivity monitor. The column used was an Alltech C_{18}, 5 μ, 4.6 cm ID x 15 cm; the solvent system was CH_3CN:0.005 M Pic B-8:HOAc [20:80:1] with a flow rate of 1 ml/min and detection at 254 nm, T_r = 5.04 min.

Methyl 4-(hydroxymethyl)-1-(triphenylmethyl)-1H-imidazole-5-carboxylate (10) (12)

To a stirred solution of 12.0 g (28 mmol) of dimethyl 1-(triphenylmethyl)-1H-imidazole-4,5-dicarboxylate (9) in 400 ml anhydrous tetrahydrofuran was added 1.84 g (84 mmol) of lithium borohydride in one portion. The solution was protected with a Drierite tube and stirred overnight (20 hr) at room temperature. The almost clear solution was poured into 3 ℓ water and mechanically stirred until the precipitate solidified. The material was separated by filtration, washed with water, dissolved in 300 ml methylene chloride, and again washed with water and saturated sodium chloride solution. The methylene chloride solution was dried over $MgSO_4$ and concentrated in vacuo to a colorless foam weighing 10 g. Trituration with boiling toluene effected crystallization and gave 7.20 g (64%) 10, mp 146-9° [lit (12) mp 162-3°] of sufficient purity for the subsequent chlorination reaction. The presence of the 5-hydroxymethyl isomer indicated by TLC was not discernible by NMR ($CDCl_3$) δ 3.17 (s, 3H, 5-CO_2CH_3), 4.83 (s, 2H, 4-CH_2OH), 7.1-7.4 (m, 15H, 1-$(C_6H_5)_3$, 7.55 (s, 1H, 2-H).

Methyl 5-(Chloromethyl)-1H-imidazole-4-carboxylate (11)

A solution of 3.0 g (7.53 mmol) 10 in 30 ml thionyl chloride was refluxed for one hour, and then the excess thionyl chloride was removed in vacuo followed by repeated addition/evaporation of toluene and absolute ethanol. The semisolid residue was crystallized from ethanol-ether to give 1.39 g (88%) of 11: mp 234-40° (dec.); NMR ($DMSO-d_6$) δ 3.87 (s, 3H, 5(4)-CO_2CH_3), 4.97 (s, 2H, 4(5)-CH_2Cl), 8.88 (s, 1H, 2-H).

Anal Calcd for $C_6H_8N_2O_2Cl_2$ (211.05): C, 34.15; H, 3.82; N, 13.27; Cl, 33.60. Found: C, 34.25; H, 3.87; N, 13.20; Cl, 33.65.

Methyl 5-[(cyano-^{14}C)methyl]-1H-imidazole-4-carboxylate (12)

A mixture of potassium [^{14}C]cyanide (124 mCi, specific activity 10 mCi/mmol) in H_2O (4 ml), tetrabutylammonium hydrogen sulfate (212 mg, 0.62 mmol) and CH_3CN (5 ml) was stirred at room temperature. To the resulting mixture was added 11 (1.307 g, 6.2 mmol) in H_2O (2 ml) and CH_3CN (15 ml) dropwise at 3°C. The temperature was allowed to return to room

temperature, and the biphasic reaction mixture was stirred overnight. Sodium bicarbonate (1.04 g, 12.4 mmol) was added to the reaction mixture, and the solvent was removed in vacuo at 40°C resulting in a pale yellow solid. Treatment of the solid with boiling ethyl acetate yielded 23 mCi which was 69% radiochemically pure. Removal of the solvent followed by purification by column chromatography (silica gel, acetone) resulted in 15.8 mCi of radiochemically pure product 12 (236 mg).

6-Amino-1,5-dihydro-4H-imidazo[4,5-c]-pyridin-4-one-6-^{14}C methanesulfonate (13)

The above-labeled nitrile 12 in MeOH (30 ml) in a stainless steel bomb was treated with anhydrous NH_3 introduced by bubbling for 10 min. The sealed bomb was heated at 130°C for 7 days. After the solvent was removed in vacuo at 40°C, the residue was dissolved in boiling H_2O, decolorized with Norit, and filtered. Storage at room temperature for 18 hr and then 5°C for 24 hr produced a crystal mass. The slurry of product was cooled in an ice bath and filtered to give [^{14}C]3-dezaguanine free base (10.7 mCi, 160 mg, $\geq$ 99% radiochemically pure) after vacuum drying at 100°C.

A portion of methanesulfonic acid (104 mg, 1.08 mmol) was added to a slurry of the above free base (10.25 mCi, 154 mg, 1.03 mmol) in MeOH (4 ml) resulting in momentary dissolution of the solids followed by precipitation. The slurry was stirred at room temperature for 2 hr, filtered, and dried in vacuo at 100°C for 20 hr to give 139 mg of the mesylate salt 13 (5.119 mCi, 50% radiochemical yield with a specific activity of 9.47 mCi/mmol as the mesylate hemi-hydrate). The IR and NMR are identical to reference unlabeled 3-dezaguanine mesylate 7, mp 271-273°C (reference standard 275-278°C).

Anal Calcd for $C_7H_{10}N_4O_4S \cdot 0.5\ H_2O$: C, 32.94; H, 4.31; N, 21.96. Found: C, 33.00; H, 4.28; N, 21.95.

REFERENCES AND NOTES

1. Dezaguanine mesylate is the nonproprietary name approved by the United States Adopted Names Council. Note the removal of the "a" from the common name, 3-deazaguanine.

2. 3-Deazaguanine and its prodrugs have significant antitumor, antiviral, antibacterial, and antiparasitic activities; see R. H. Revankar et al. (3) for references cited.

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