Acyclopyrimidine *C*-Nucleosides. Synthesis of Acyclopseudoisocytidine and its Derivatives

Chung K. Chu

Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy,
University of Georgia, Athens, GA 30602

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Acyclopseudouridine (IV), acyclopseudoisocytidine (VII), and their 1-methyl derivatives (V and VIII) were synthesized from 5-hydroxymethyluracil (I). Acyclopseudouridine (IV) was conveniently prepared from the condensation of 5-hydroxymethyluracil (I) with ethylene glycol under acidic condition. Compound IV also could be prepared from 5-chloromethyluracil, however, this procedure was found to be inferior to the direct condensation method. Methylation of IV with dimethylformamide dimethyl acetal gave 1,3-dimethylacyclopseudouridine (VI) which was subsequently converted to acyclopseudoisocytidine (VII). 1-Methyl derivatives V and VIII were obtained from the selective methylation of IV and VII, respectively. 5-Hydroxymethyluracil also reacted with 2-mercaptoethanol to give the sulfide derivative (IX).

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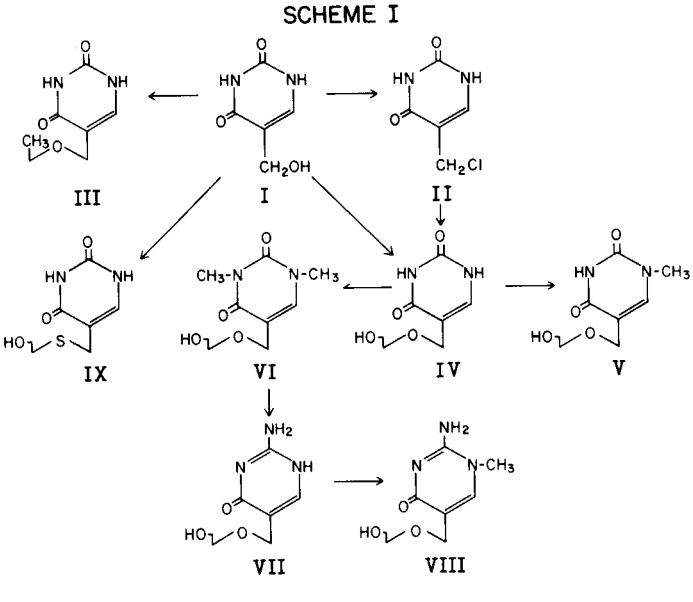
During the search of new anticancer agents, various substituted pyrimidine *C*-nucleosides related to pseudouridine have been synthesized [1-8]. One analog, pseudoisocytidine [5-(βD-ribofuranosyl)isocytosine] [9,10], was found to be a potent antileukemic agent *in vitro* as well as *in vivo*, and this was the first synthetic *C*-nucleoside for which any significant anticancer activities have been demonstrated. Interestingly, the compound was more active in ara-*C* resistant cell lines than in ara-*C* sensitive leukemic cell lines [9-10]. However, preclinical pharmacology followed by phase 1 clinical study showed that this compound causes hepatotoxicity in man [11]. Despite this fact, *C*-nucleosides continue to be a new class of compounds of potentially useful chemotherapeutic agents [12,13].

Since the successful development of acyclovir (Zovirax[R]) by Schaeffer and coworkers [14] as a clinically useful antiherpes agent, a number of pyrimidine [15-17] and purine [18,19] acyclonucleosides have been synthesized as potental antitumor and antiviral agents. This report deals with the synthesis of acyclopyrimidine *C*-nucleosides related to pseudoisocytidine and pseudouridine (Scheme I).

SCHEME I



The starting material, 5-hydroxymethyluracil (I) was prepared by a modification of the method of Cline and coworkers [20]. In this report 5-ethoxymethyluracil (III) was prepared from the reaction of 5-hydroxymethyluracil and ethanol with concentrated hydrochloric acid as a catalyst. This indicated facile formation of a primary carbonium ion from the acid-catalyzed dehydration of I. The similar reaction condition was utilized for the synthesis of 5-[(2-hydroxyethoxy)methyl]uracil(acyclopseudouridine, IV), which served as the starting material for other acyclopyrimidine *C*-nucleosides. Thus, a mixture of I and an excess of ethylene glycol was heated for a short period (5 minutes) until the mixture became near homogenous. Analysis by tlc indicated that prolonged heating produced side products. No attempts have been made for identification of these unknowns. This method not only required a short reaction time but also gave a better yield and cleaner product than that *via* 5-chloromethyluracil (II) [21].

Acyclopseudouridine (IV) was readily identified by the pmr spectrum which showed benzylic type protons as a singlet (δ 4.10) and ethylene (-CH_2CH_2-) protons as multiplets (δ 3.35-3.55). This indicated the formation of an ether linkage. Presence of a uracil ring was confirmed by uv spectra at various *p*H's. Acyclonucleoside IV was then treated with dimethylformamide dimethyl acetal to obtain 1,3-dimethylated uracil derivative (VI) which served as a starting material for the preparation of acyclopseudoisocytidine (VII). The ring transformation of the uracil derivative (VI) to the isocytosine derivative (VII) was accomplished with free guanidine (6,8). However, isolation of the product was somewhat difficult due to the formation of a triazine (melamine) from the self condensation of guanidine. Fractional crystallization followed by the preparation of the hydrochloride salt afforded an analytical sam-

ple.

Since methylated bases occur in the DNA as well as in the RNA of all cellular organisms, it was of interest to prepare the methylated derivatives of IV and VII. Thus, 1-methylacyclopseudoisocytidine (VIII) and 1-methylpseudouridine (V) were obtained by the selective methylation of VII and IV with methyl iodide, respectively [5].

It was also of interest to prepare a sulfur analog of (IV), which could also be obtained from I utilizing mercaptoethanol instead of ethylene glycol. The identification of the compound IX was based on the pmr spectrum in which benzylic (δ 3.34) and methylene (-CH_2CH_2-) protons (δ 2.51) appeared at higher field than that of IV (δ 4.10 and 3.35-3.55, respectively) due to the shielding effects of sulfur.

None of the above compounds showed any significant inhibitory activity against L-1210 mouse leukemic cells or herpes virus 1 (except V) [22].

EXPERIMENTAL

Melting points were determined on a Thomas-Hoover capillary melting point apparatus and are uncorrected. The pmr spectra were recorded on a JEOL FX 90Q fourier transform spectrometer (90 MHz). Tetramethylsilane was the internal standard for organic solvents and sodium 3-(trimethylsilyl)-1-propane-1-sulfonate (DSS) was the internal standard for deuterium oxide; chemical shifts are reported in parts per million (δ), and signals are described as s (singlet), d (doublet), t (triplet), q (quartet), b (broad), m (multiplet). Ultraviolet spectra were recorded on a Bausch & Lomb Spectronic 2000 spectrometer. The tlc analysis was performed on Uniplates purchased from Analtech Co. or Pre-coated tlc sheets (Silica gel 60 F-254) by EM Laboratories, Inc. Elemental analysis were performed by Atlantic Microlab, Inc., Atlanta, GA.

5-Hydroxymethyluracil (I).

5-Hydroxymethyluracil was prepared by a modification of the method of Cline and co-workers [20]. After heating a mixture of uracil (36 g, 0.32 mole) and paraformaldehyde (12 g, 0.4 mole) at 50-60° in 500 ml of 0.42*N* potassium hydroxide (11.76 g) for three days, the resulting unknown precipitates (0.6 g) were filtered off and the filtrate was neutralized with concentrated hydrochloric acid, and the resulting white precipitates was filtered, washed with cold water, and then dried in a desicator to afford white powder (36.5 g) (literature 36.6 g).

5-[(2-Hydroxyethoxy)methyl]uracil (IV, Acyclopseudouridine).

Method A.

A mixture of 5-hydroxymethyluracil I (10 g, 0.07 mole), ethylene glycol (70 ml, dried over 3A° molecular sieves) and concentrated hydrochloric acid (1.2 ml) was heated until the mixture became a near homogenous solution (about 4-5 minutes), and then the reaction mixture was immediately filtered to remove a small amount of undissolved starting material. The filtrate was cooled in an ice-bath during which the mixture solidified. Addition of acetone (50 ml), trituration, and then filtration gave a white solid (10.5 g, 81%) which was recrystallized from acetone-methanol (1:1) to afford white crystals, IV, mp 245-250°; uv: λ max 258 nm (*p*H 1-7), 283 (*p*H 13); ^{1}H nmr (DMSO-d_6): 3.35-3.55 (m, 4H, CH_2CH_2), 4.10 (s, 2H, CH_2), 7.42 (d, 1H, H-6, J = 5.4 Hz, collapsed to a singlet with D_2O), 10.85 (broad d, 1H, N_1-H, J = 5.4 Hz exchangeable), 11.01 (b, 1H, N_3-H, exchangeable).

Anal. Calcd. for $C_7H_{10}N_2O_4$: C, 45.16; H 5.38; N, 15.05. Found: C, 45.18; H, 5.42; N, 15.04.

Method B.

A mixture of II [21] (1.285 g, 0.01 mole), ethylene glycol (0.93 g, 0.015 mole), pyridine (1 ml) and *N,N*-dimethylformamide (15 ml) was stirred for 24 hours and then the solvent was evaporated to near dryness. To the mixture acetone (10 ml) was added, triturated and filtration yielded a white solid III (0.85 g, 46%) which contains a small amount of 5-hydroxymethyluracil (I).

5-[2-(Hydroxyethoxy)methyl]-1-methyluracil (V, 1-Methylacyclopseudouridine).

A mixture of IV (1.86 g, 0.01 mole), hexamethyldisilazane (HMDS, 30 ml) and ammonium sulfate (100 mg) was refluxed until the mixture became a homogenous solution (*ca.* 2.5 hours). Stirring was continued for another hour. Excess HMDS was then removed *in vacuo* and the residue was dissolved in dry acetonitrile (30 ml). Methyl iodide (2 ml) was added to the solution and the mixture was stirred for 24 hours. The solvent and an excess methyl iodide were removed *in vacuo*. The residue was dissolved in methanol (100 ml) and neutralized with Ambelite IR-45 (OH^-). The mixture was filtered, ethanol (10 ml) was added, and the mixture was left overnight at room temperature, during which time some white crystals precipitated. Further precipitation was induced by scratching and cooling in an ice-bath. The combined yield was 1.1 g (55%). Recrystallization from ethanol gave an analytical sample, mp 259-261°; uv: λ max 267 nm (*p*H 1-7), 264 (*p*H 13); ^{1}H nmr (deuterium oxide): 3.39 (s, 3H, CH_3), 3.6-3.8 (m, 4H, CH_2CH_2), 4.31 (s, 2H, CH_2), 7.77 (s, 1H, H-6).

Anal. Calcd. for $C_8H_{12}N_2O_4$: C, 48.00; H, 6.00; N, 14.00. Found: C, 47.72; H, 5.76; N, 14.27.

1,3-Dimethyl-5-[(2-hydroxyethoxy)methyl]uracil (VI, 1,3-Dimethylacyclopseudouridine).

A mixture of IV (3.0 g) and dimethylformamide dimethylacetal [6] was refluxed for 25 minutes during which the suspension became a solution. The mixture was then concentrated *in vacuo* to obtain a syrup, which contained four minor spots on tlc along with a major compound (Rf = 0.65, chloroform methanol = 10/1). The syrup, however, was suitable for the next reaction. An analytical sample was obtained from preparative thin-layer chromatography on silica gel using the same solvent system as above, mp 82-83°; uv: λ max 265 nm (*p*H 1-13); ^{1}H nmr (deuteriochloroform): 3.45 (s, 3H, CH_3), 3.42 (s, 3H, CH_3), 3.42-3.74 (m, 4H, CH_2CH_2), 4.33 (d, 2H, CH_2, J = 0.88 Hz), 7.32 (t, 1H, H-6, J = 0.88 Hz).

Anal. Calcd. for $C_9H_{14}N_2O_4$: C, 50.47; H, 6.54; N, 13.08. Found: C, 50.29; H, 6.60; N, 13.04.

5-[(2-Hydroxyethoxy)methyl]isocytosine (VII, Acyclopseudoisocytidine).

A solution of sodium ethoxide [prepared from 1.61 g (0.07 mole) of metallic sodium and 40 ml of absolute ethanol] and guanidine hydrochloride (6.75 g, 0.07 mole) was stirred for 15 minutes at room temperature. The resulting white precipitate was filtered off and the filtrate was concentrated *in vacuo* to a syrup. Acyclonucleoside VI (2.0 g, 0.009 mole) was added and the mixture was heated at 90-95° for one hour under nitrogen.

The reaction mixture was cooled, dissolved in water (20 ml), and the solution was applied to an Ambelite IRC-50 (COOH) (2.5 × 30 cm) column. The column was washed with water until no uv absorbing material was eluted . The combined eluents were evaporated *in vacuo*. The resulting solid was triturated with ethanol, and the remaining solid (melamine) was filtered off. To the filtrate, hydrogen chloride gas was briefly introduced. The resulting colorless crystals were collected (265 mg). Recrystallization from ethanol gave an analytical sample as a hydrochloride salt, mp 272-276° dec; uv: λ max 286 nm (*p*H 7), shoulder 268 (*p*H 7), 259 (*p*H 1), 275 (*p*H 13); ^{1}H nmr (DMSO-d_6): 3.34-3.56 (m, 4H, CH_2CH_2), 4.15 (S, 2H, CH_2), 5.81-6.97 (b, 4H, NH_2 and NH, exchangeable).

Anal.. Calcd. for $C_7H_{12}ClN_3O_2$: C, 40.88; H, 5.84; Cl, 17.27; N, 20.44. Found: C, 40.83; H, 5.84; Cl, 17.15; N, 20.40.

5-[(2-Hydroxyethoxy)methyl]-1-methylisocytosine(VIII, 1-Methylacyclopseudoisocytidine).

A mixture of VII (free base, 710 mg, 0.0042 mole), HMDS (15 ml) and ammonium sulfate (50 mg) was refluxed until the suspension became a solution. Excess HMDS was then removed *in vacuo* and the residual

syrup was dissolved in dry acetonitrile (10 ml). Methyl iodide (1 ml) was added, and the mixture was stirred at room temperature for 48 hours. The mixture was then concentrated *in vacuo* and triturated with methanol (4 ml). Filtration gave a solid, which was redissolved in warm methanol. The solution was cooled to room temperature and treated with hydrogen chloride. The solvent was then partially evaporated *in vacuo*, and the resulting amorphorus crystals were collected by filtration to afford 350 mg (38%), mp 202-203° dec; uv: λ max 261 nm (*p*H 7-13), 264 (*p*H 262); ^{1}H nmr (DMSO-d_6): 3.34 (s, 3H, CH_3), 3.40-3.51 (m, 4H, CH_2CH_2), 4.10 (s, 2H, CH_2), 7.33 (S, 1H, H-6).

Anal. Calcd. for $C_8H_{14}ClN_3O_3$: C, 40.76; H, 5.95; Cl, 15.08; N, 17.83. Found: C, 40.64; H, 6.02; Cl, 15.24; N, 17.52.

5-[(2-Hydroxyethylthio)methyl]uracil (IX).

A mixture of 5-hydroxymethyluracil (1.0 g), 2-mercaptoethanol (10 ml) and concentrated hydrochloric acid (2 drops) was refluxed for 3 minutes until the suspension became a near solution. The mixture was then cooled immediately in an ice-bath. The resulting white solid was filtered and recrystallization from methanol afforded white crystals, mp 198-200° dec; uv: λ max 264 nm (*p*H 1-7), 290 (*p*H 13); ^{1}H nmr (DMSO-d_6): 2.51 (t, 2H, -CH_2-S, J = 7.4 Hz), 3.52 (t, 2H, O-CH_2, J = 7.4 Hz), 3.34 (s, 2H, CH_2), 7.40 (d, 1H, H-6, J = 5.9 Hz, collapsed to a singlet with deuterium oxide), 10.78 (broad d, 1H, N_1-H, J = 5.9 Hz, exchangeable), 11.11 (b, 1H, N_3-H, exchangeable).

Anal. Calcd. for $C_7H_{10}N_2O_3S$: C, 41.58; H, 4.95; N, 13.86; S, 15.84. Found: C, 41.68; H, 5.03; N, 13.81; S, 15.80.

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