Simple Convenient Synthesis of 1-Methylcytosine

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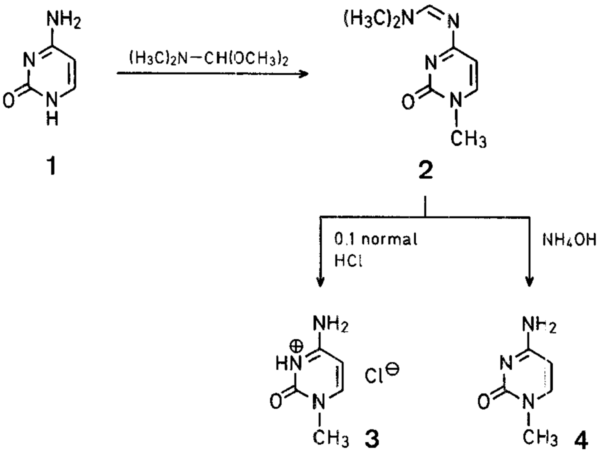
Methylation of heterocyclic base components of nucleic acids is a well documented reaction due to the chemical mutagenesis and carcinogenesis arising from various methylating agents[1,2,3] and to the detection and isolation of various methylated nucleic acid base residues from both RNA and DNA[4,5,6]. Nevertheless, the availability of several potential sites for such alkylation on these multi-nitrogen heterocycles sets a synthetic requirement for selectivity in the position of alkylation. Because of our experience with the 1-alkylation of cytosine[7] and the availability of 1-methylcytosine apparently only on custom order, we sought a better and more convenient way to synthesize 1-methylcytosine as first of a series. Interest has been generated in this compound, for example, because of its peptidyl transferase activity[8], its complementary base-pairing with 9-methylguanine[9], and the spectroscopic detection of the restricted rotation of its extranuclear amino function[10]. The published methods for the synthesis of 1-methylcytosine suffer from the problems of poor yield, multi-step sequence, and/or isomer separation: (a) rearrangement of 4-amino-2-methoxypyrimidine by pyrolysis[11]; (b) reaction of silver sulfate with 4-amino-2-methoxy-1-methylpyrimidinium iodide[11]; reaction of ammonia with 4-methoxy-1-methyl-2-oxo-1,2-dihydropyrimidine at 100 °C[12,13]; and direct methylation of cytosine[14-17].

We present here an efficient, simple, and convenient two-step synthesis of 1-methylcytosine starting with cytosine. Our method is based upon the finding[18,19,20] that *N*,*N*-dimethylformamide acetals[21] alkylate (or aralkylate) heterocyclic bases at amide nitrogens when no free amino groups are present, as in inosine, uridine, and thymidine, and form dimethylaminomethylene derivatives when an extranuclear amino function is present, as in adenosine and cytidine. Guanosine, which has adjacent amide and extranuclear amino functions reacts with *N*,*N*-dimethylformamide dimethyl acetal to give the N^2-dimethylaminomethylene derivative and with *N*,*N*-dimethylformamide dibenzyl acetal to give 1-benzylguanosine[22]. We were therefore encouraged to try the reaction with cytosine in the hope, since the amide and amino groups are sufficiently removed from each other, that we could expect both methylation at N-1 and functionalization at N-4.

This rationale was borne out by experiment. When cytosine (1) was caused to react with *N*,*N*-dimethylformamide dimethyl acetal (1,1-dimethoxy-*N*,*N*-dimethylmethanamine) with trifluoroacetic acid as a catalyst, N^4-(*N*,*N*-dimethylaminomethylene)-1-methylcytosine (2) was obtained in practically quantitative yield. Microanalyses, ^{1}H-N.M.R., ^{13}C-N.M.R., and mass spectral data provided identification. Compound 2 was hydrolyzed practically quantitatively either with 0.1 normal hydrochloric acid to 1-methylcytosine hydrochloride (3) or with concentrated ammonium hydroxide to 1-methylcytosine (4). The melting points and spectral data of salt 3 and base 4[11,12,23,24,25] were in agreement with the reported values. The method is applicable to large-scale preparation of 1-methylcytosine and is expected to be extendable to the synthesis of other 1-substituted cytosines.



Melting points were determined on a Büchi capillary melting point apparatus and are uncorrected. ^{1}H-N.M.R. spectra were recorded on a Varian EM-390 spectrometer operating at 90 MHz. ^{13}C-N.M.R. spectrum was obtained on a Jeol FX-60 Fourier Transform instrument, operating at 15.03 MHz and the values are reported in ppm from tetramethylsilane. Mass spectra were run on a Varian MAT CH-5 low resolution spectrometer coupled with a 620i computer and a STATOS recorder. U.V. absorption spectra were obtained on a Beckman Acta MVI spectrometer.

N^4-(*N*,*N*-Dimethylaminomethylene)-1-methylcytosine (2):

A mixture of cytosine (1; 1.5 g, 13.5 mmol) and *N*,*N*-dimethylformamide dimethyl acetal (20 ml, 150 mmol) is heated to boiling under nitrogen and trifluoroacetic acid (0.1 ml) is cautiously introduced through a serum cap. The mixture is heated under reflux for an additional 15 h, the solution cooled in an ice-bath, and the white solid that separates is collected by filtration and recrystallized from benzene/chloroform as silky white needles of 2; yield: 2.38 g (98%); m.p. 203–204 °C.

$C_8H_{12}N_4O$	calc.	C 53.32	H 6.71	N 31.09
(180.2)	found	53.21	6.84	31.34

M.S. (15 eV): *m/e* (relative intensity %) = 180 (M$^+$, 100); 165 (M$^+$ − CH$_3$, 6); 136 (94); 125 (3); 73 (12).

^{1}H-N.M.R. (DMSO-d_6): $\delta = 3.00$ (s, 3H, CH$_3$ of side-chain); 3.13 (s, 3H, CH$_3$ of side-chain); 3.28 (s, 3H, CH$_3$ at N-1); 5.83 (d, 1H, $J = 7.0$ Hz, H-5); 7.73 (d, 1H, $J = 7.0$ Hz, H-6); 8.53 ppm (s, 1H, =CH− of side-chain).

^{13}C-N.M.R. (CDCl$_3$): $\delta = 35.1$ (CH$_3$ of side-chain); 37.9 (CH$_3$ of side-chain); 41.3 (CH$_3$ at N-1); 102.5 (C-5); 146.8 (C-6); 157.6 (C-2); 158.2 (=C− of side-chain); 172.1 ppm (C-4).

1-Methylcytosine Hydrochloride (3):

A mixture of 2 (90 mg, 0.5 mmol) and 0.1 normal hydrochloric acid (10 ml) is stirred at room temperature for 12 h. (The reaction is monitored by the U.V. absorption of the reaction mixture, which shows the gradual disappearance of absorption above $\lambda_{max} = 300$ nm and appearance of absorption at $\lambda_{max} = \sim 280$ nm.) The clear solution is evaporated to dryness and the residue is coevaporated with anhydrous toluene (2 × 10 ml). The white solid obtained is recrystallized from methanol/ethyl acetate as colorless needles of 3; yield: 78 mg (97%); m.p. 278–279 °C (dec.) [Lit.[23], m.p. 285–300 °C (dec.)].

The ^{1}H-N.M.R.[25] and U.V.[23] spectral data of 3 were in agreement with the literature values.

$C_5H_8ClN_3O$	calc.	C 37.16	H 4.99	N 26.00
(161.6)	found	37.15	4.85	25.89

1-Methylcytosine (4):

A mixture of 2 (90 mg, 0.5 mmol) and concentrated ammonium hydroxide solution (10 ml) is stirred at room temperature for 10 h and then refluxed on a steam bath for 1 h to drive off excess ammonia. The solution is evaporated to dryness and the solid residue is recrystallized from methanol/petroleum ether as colorless prisms of 4; yield: 61 mg (98%); m.p. 301–302 °C (dec.) [Lit.[11,12], m.p. 300–303 °C (dec.)].

^{1}H-N.M.R.[25] and U.V.[23,24] spectral data of 4 were identical with the values reported in the literature.

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