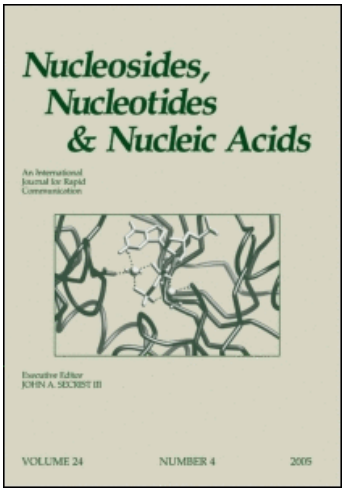

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Synthesis of 3, N^4-Dimethylcytidine

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SYNTHESIS OF 3,N^4-DIMETHYLCYTIDINE

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Abstract. Two procedures are described for the synthesis of 3,N^4-dimethylcytidine, a compound usually obtained in only trace quantities.

Monoalkylation of the pyrimidine ring in cytosine nucleosides occurs readily and nearly exclusively at the 3-position[1-3]. Very low yields of products arising from N-4 alkylation are observed[3]. N^4,N^4-bisalkylated cytidines are sometimes obtained in small amounts but the 3,N^4-bisalkylated product is rarely observed[3,4]. We have previously reported the use of fluoride ion[5] as a catalyst in preparing 3,N^4-dimethylcytidine. We recently needed this compound in quantity for biological testing and found the yields in the fluoride catalyzed reactions were highly variable. Apparently difficult-to-control changes in the quality of tetrabutylammonium fluoride (TBAF) had pronounced effects on the alkylation reaction. In this report we describe a new and direct route to 3,N^4-dimethylcytidine (3) as well as a more consistent TBAF catalyzed reaction.

For a direct route to compound 3 we decided to prepare both 3-methylcytidine (1) and N^4-methylcytidine (2). Compound 1 is readily available by the alkylaton of cytidine. Compound 2 was prepared by the method originally described by Fox et al.[6]. Both compounds 1 and 2 were subjected to a variety of conditions using methyl iodide, dimethyl sulfate and trimethyl phosphate as alkylating agents. None of the conditions investigated led to more than trace amounts of the desired 3

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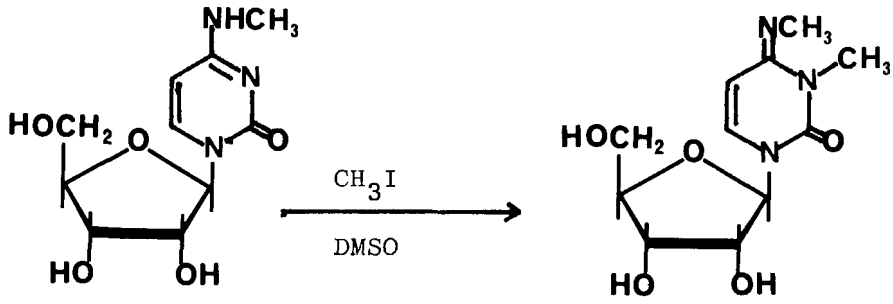
when 1 was the starting material. While similar results were obtained in the majority of the cases with 2, one set of conditions - a large excess of methyl iodide in DMSO - led to complete conversion (TLC) of 2 into 3.



2 3

The initial product of the methyl iodide alkylation of 2 is the hydroiodide salt of 3. The hydroiodide salt can be recovered by precipitation from the product mixture. However if the product is recovered by TLC the free base 3 is obtained. The ^{13}C-NMR spectra are summarized below in Table 1.

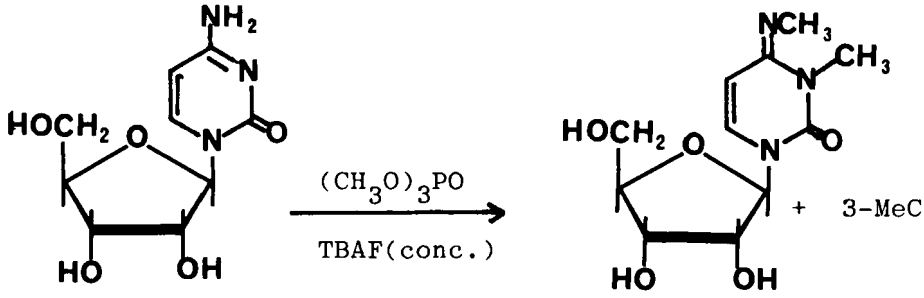
Table 1.

^{13}C NMR Data of Methylated Cytidines

		Compound			
Carbon	Cytidine	1	2	3	N^4,N^4-DiMeC
1'	89.29	90.59	89.13	90.75	89.29
2'	74.13	74.13	74.02	74.19	74.24
3'	69.60	68.58	69.44	68.31	69.22
4'	84.22	84.71	84.11	88.49	84.11
5'	60.81	59.62	60.65	59.41	60.43
2	155.81	107.72	155.54	147.39	144.62
4	165.68	158.83	163.85	157.43	163.14
5	94.15	94.04	94.63	91.40	91.34
6	141.67	141.41	140.11	142.43	141.46
3-Me		30.81		30.68	
4-Me			26.88	26.12	37.40
					37.18

*Recorded on a Brucker WH-90 Spectrometer using DMSO-d_6 as solvent. Chemical Shifts (δ) are in ppm relative to TMS.

The TBAF catalyzed alkylations of cytidine were investigated and it was found that the only consistent procedures for the preparation of 3 involved very concentrated solutions. For example when cytidine was dissolved in TBAF-THF with trimethylphosphate and the solvents were concentrated to a very viscous state, conversion of cytidine to 3 occurred in 2 to 7 days at room temperature. The desired product 3 was typically obtained in ~ 45% yield while 3-methylcytidine accounted for the remaining material.



3

When the reagents in the TBAF reaction were concentrated to a small but non-viscous volume, and the solution was heated at 60-70°C, nearly complete conversion to 3 was observed after 2 to 3 days. Once again the only other product observed was 3-methylcytidine.

Experimental

General Methods. Thin-layer chromatography data were recorded from Merck Kieselgel 60F 254 analytical sheets. Preparative thick layer plates (20 x 20 cm) were prepared using a 1 mm thick coating of Merck Kieselgel 60F 254. Descending paper chromatography was performed using Whatman 3 mm paper. The solvent system used was Solvent A: isopropanol-ammonium hydroxide-water (7:1:2 V/V/V).

Melting points are uncorrected and were measured on a Fisher-John melting point apparatus. UV spectra were recorded on a Carey 17 spectrophotometer. ^{1}H NMR spectra were recorded on a Varian T-60A and XL-200 spectrometers using D_2O as solvent. ^{13}C spectra were recorded on a Brucker WH-90 spectrometer using DMSO-d_6 as solvent.

Tetrabutylammonium fluoride in THF (0.68 M soln) was prepared as previously described. 3-Methylcytidine[1], N^4-methylcytidine[6] and N^4,N^4-

dimethylcytidine[8,9] were prepared according to published procedures and their properties, for comparison purposes are listed in Tables 1 to 3.

Synthesis of 3,N^4-Dimethylcytidine (3) From N^4-Methylcytidine and Methyl Iodide

Compound 2 (604 mg, 2.4 mmole) was dissolved in dry DMSO (15 ml). Methyl iodide (9 ml, 145 mmole) was added and the solution was stirred for 24 h at room temperature. TLC indicated 3 as the only nucleoside component of the solution.

Solvents were removed at reduced pressure and the purple residue was extracted with water. The aqueous solution was filtered from some insoluble material and lyophilized. The residue was dissolved in a minimum volume of DMSO and applied to twelve TLC plates which were then developed in 95% ethanol containing 1% NH_4OH. The product band was eluted with ethanol. After evaporation of ethanol and further coevaporation with absolute ethanol a solid residue was obtained. This powder was dried under vacuum and 268 mg (42%) of compound 3 was obtained (mp 202-203°(dec)). The product was identical to authentic material and to properties previously described[3,5]. The UV and NMR data are summarized in Tables 2 and 3 below.

The product 3 could be isolated from the reaction mixture as its hydroiodide salt in near quantitative yields. The hydroiodide salt had mp 194-199°C, UV properties identical to 3 but the ^{13}C spectrum of the free base showed the 3-methyl at 35.35 and the N^4-methyl at 28.22 ppm.

Table 2

Physical Properties of Methylated Cytidines

Compound	UV λmax (nm)			R_f*		mp
	pH1	pH7	pH13	Paper	TLC	°C
cytidine	279	270	271	0.52	0.37	215-216 (dec)
1	277	277	267	0.72	0.51	198-202 (dec)
2	281	270	272	0.69	0.38	178-180
N^4,N^4-DiMeC	286	277	278	0.68	0.40	+
3	284	283	276	0.82	0.56	202-203 (dec)

*Both paper chromatography and TLC in solvent A.
+Not obtained as a true solid.

Table 3

^{1}H NMR Data of Methylated Cytidines

Compound	δ(ppm)				
	5	6	1'	3-Me	N^4-Me
cytidine	d,5.95 $J_{5,6}$=7.52	d,7.74 $J_{5,6}$=7.52	d,5.80 $J_{1',2'}$=3.85		
1	d,6.22 $J_{5,6}$=8.01	d,8.04 $J_{5,6}$=8.01	d,5.81 $J_{1',2'}$=3.07	s,3.42	
2	d,5.90 $J_{5,6}$=7.57	d,7.60 $J_{5,6}$=7.57	d,5.83 $J_{1',2'}$=4.15		s,2.81
N^4,N^4-DiMeC	d,6.12 $J_{5,6}$=7.92	d,7.71 $J_{5,6}$=7.92	d,5.80 $J_{1',2'}$=4.34		s,3.03
3	d,6.26 $J_{5,6}$=8.20	d,8.10 $J_{5,6}$=8.20	d,5.74 $J_{1',2'}$=3.07	s,3.33	s,2.99

The hydroiodide salt could be removed by TLC chromatography as described above. The recovery was generally 40-50% with large losses experienced on silica gel chromatography of the polar material.

Conversion of Cytidine to 3

A. To cytidine (1 mmole) was added TBAF in THF (19 eq/mmole of cytidine) and trimethylphosphate (3 ml/mmole of cytidine). The mixture was concentrated to a gel-like state and stored in this manner for 2 to 7 days. The mixture consisted of 3-methylcytidine (54%) and 3,N^4,dimethylcytidine (46%). These products were separable in solvent A.

B. Cytidine (1 mmole) was added to TBAF in THF (19 eq) and the solvent was removed at reduced pressure. Trimethylphosphate (3 ml/mmole of cytidine) was added and the solution was heated at 60-70°C for 3 days. TLC indicated the mixture contained the desired 3 as well as some 1 in a ratio of 95:5. Compound 3 could be isolated pure by TLC chromatography in chloroform-ethanol (3:7) containing 1% NH_4OH.

Acknowledgement

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