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Unexpected Shift to a 4-Imino Tautomer Upon N⁴-Acylation of 5-Methylcytosin-1-yl Nucleoside Analogues

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UNEXPECTED SHIFT TO A 4-IMINO TAUTOMER UPON N⁴-ACYLATION OF 5-METHYLCYTOSIN-1-YL NUCLEOSIDE ANALOGUES.

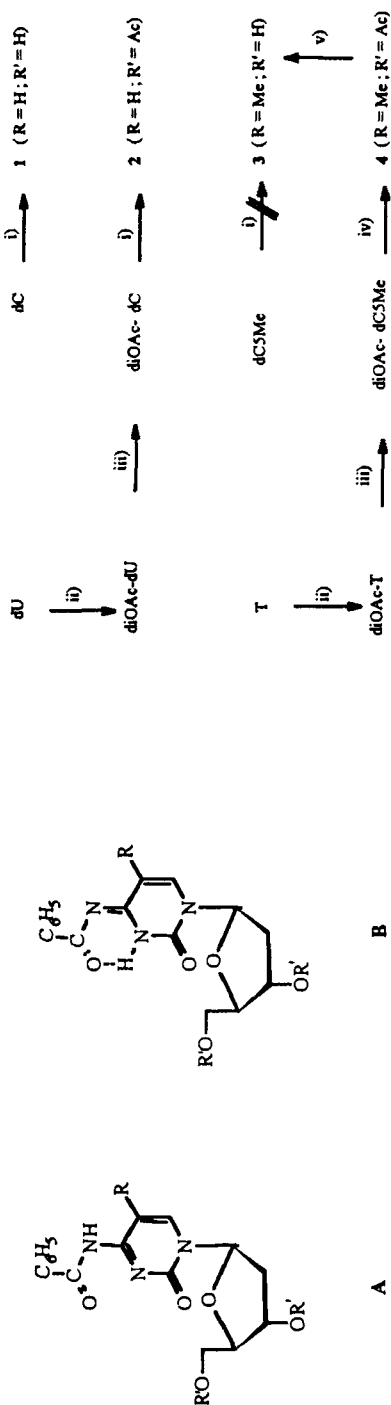
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ABSTRACT : In contrast with the behaviour of 5-unsubstituted cytosine nucleoside analogues, 5-methylcytosine derivatives show upon N⁴-benzoylation, commonly used as base protection in oligonucleotide synthesis, a tautomeric change of the base moiety from a 4-amino- into a 4-imino isomer. In the latter form, which is easily diagnosticized by ¹³C NMR and confirmed by X-ray data, the compounds seem to be hydrolytically less stable.

Base protection is a prerequisite for incorporation of nucleosides in oligonucleotides. Cytosine bases are generally protected with a benzoyl group in the N⁴-position of the 4-amino tautomer of the cytosin-1-yl base (A). When this protection scheme was applied to 5-methyl-cytosin-1-yl nucleoside analogues, we obtained substantially lower yields of the reaction, and we observed unexpected but significant differences in the C¹³-spectrum in comparison with that of the N-acylated 5-unsubstituted analogue. In order to find an explanation of the seemingly anomalous and hitherto unnoticed shifts in the carbon spectra, we repeated the N-acylation experiments with the more easily available natural deoxycytidine and 5-methyldeoxycytidine nucleosides and with their 3',5'-di-O-acetylated derivatives, and studied the structure of these compounds.

2'-Deoxycytidine (dC) and 5-methyl-2'-deoxycytidine (dC5Me) were obtained as commercial products, and the other compounds were prepared as indicated in scheme 1. All compounds were thoroughly purified by column chromatography on silica gel and characterized by mass and nmr spectroscopy. Nmr spectra were all taken in



Scheme 1.

TABLE 1 : ¹³C Shifts for the heterocyclic base carbons as measured in DMSO-_d₆.

		Me	C-5	C-6	C-2	C-4	N-CO	Other
dC	(A)	-	94.87	142.10	154.84	164.92	-	-
dC-NBz	(1A)	-	96.27	145.13	154.57	163.16	167.77	133.5,132.9,128.7 (4x)
dC5Me	(A)	13.35	101.27	138.35	155.23	165.36	-	-
dC5Me-NBz	(3B)	13.32	109.86	139.64	148.65	160.22	176.85	136.6,132.6,129.4,128.4
diOAc-dC	(A)	-	94.72	140.94	155.18	165.86	-	-
diOAc-dC-NBz	(2A)	-	96.69	145.04	154.42	163.40	167.71	133.3,132.9,128.6 (4x)
diOAc-dC5Me	(A)	13.32	102.03	138.00	155.18	165.64	-	-
diOAc-dC5Me-NBz	(4B)	13.19	110.50	138.70	147.89	159.22	178.59	136.7,132.8,129.6,128.6

dimethylsulfoxide-_d₆ ($\delta_C = 39.6$ ppm and $\delta_H = 2.50$ ppm) using a Varian Gemini 200 or Unity 500 apparatus.

Spectroscopic analysis of the N-benzoyl-5-methyl-cytosine nucleoside revealed some striking differences upon benzoylation compared to the normal deoxycytidine pair. In the UV-spectrum a larger bathochromic shift (from 279 nm to 260/331 nm for dC5Me compared with 270 nm to 259/305 nm for dC) was observed upon benzoylation. Whereas in the ¹H NMR spectrum almost no significant changes were found compared with the 5-unsubstituted analogue, in the ¹³C NMR spectrum the benzoyl-CO and C5 signal showed a marked downfield shift, while C2, C4 and C6 all shifted upfield with respect to the 5-unsubstituted N-benzoyl-dC (TABLE 1), pointing to a change in the skeleton of the heterocyclic base. It should be noted also that the spectra showed no significant changes upon heating to 55°C or when using deuteriochloroform as solvent. As expected the same changes were observed upon comparison of the di-O-acetylated derivatives (2 and 4).

All these observations led us to the hypothesis of a different tautomer of the base moiety of the N⁴-benzoylated nucleoside as shown by the 4-imino isomer **B**. An X-ray analysis of **3B** confirmed the proposed structure (FIG 1 and TABLE 2).

This unusual and hitherto unmentioned structural change possibly originates from the steric hindrance and base-strengthening effect of the 5-methyl group, together with the stabilizing effect of the intramolecular H-bond between CO and N³H atoms.

In conclusion, when using 5-methyl-cytidine or analogues in oligonucleotides, one should be aware of this tautomeric change upon base-protection, which may have as a consequence that the compound behaves differently with respect to the normally used basic or acid hydrolytic conditions. The tautomeric change is easily diagnosticized by

TABLE 2 : Bond lengths for the base from X-ray data (see fig 1).

	(3B)	$\Delta d(C-Nbz)^{(2)}$
N3-C4	1.359	1.312
C4-N7	1.324	1.392
N7-C8	1.377	1.375
C8-O8	1.234	1.220
N1-C2	1.384	1.392
C2-O2	1.200	1.243
C2-N3	1.372	1.347
C4-C5	1.438	1.407
C5-C6	1.334	1.358
C6-N1	1.359	1.348
N3-H	0.860	-

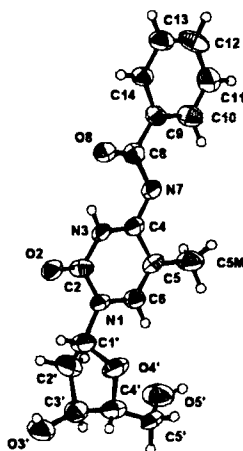


FIG. 1

^{13}C NMR spectroscopy. Further acylation experiments with other acyl groups are in progress to investigate the generality of this phenomenon.

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