Chlorimine Formation and Deamination of the Model Nucleobase 1-Methylcytosine

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Treatment of the model nucleobase 1-methylcytosine with chlorine in aqueous solution gives, depending on the reaction conditions, 5-chloro-1-methylcytosine (**3**) or its hemiprotonated form **2**, 5,5-dichloro-6-hydroxy-5,6-dihydro-4-chlorimino-1-methylpyrimidin-2-one (**5**), and 5,5-dichloro-6-hydroxy-5,6-dihydro-1-methyluracil (**4**). While substitution of H5 of 1-methylcytosine by chlorine giving **2** and **3**, and subsequent addition of hypochlorous acid to the 5,6-double bond is not unexpected, chlorination of the exocyclic amino group **5** and deamination to give **4** is novel. The X-ray structures of **4** and **5** are reported.

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While electrophilic halogen substitution at the C5 position of pyrimidine nucleobases and HOX (X = halogen) addition to the 5,6 double bond have been studied in great detail [1], halogenation reactions involving the exocyclic amino group of cytosines appear to have been investigated less intensively [2]. Patton *et al*, on the basis of mass spectra and reaction with potassium iodide and starch, suggested that hypochlorous acid converts cytosine bases into derivatives containing $-NHCl$ functions in the 4-position [2a]. Hydrolytic deamination of cytosines to the corresponding uracils (thymines), on the other hand, has been observed to occur under a number of circumstances, *e.g.* under strongly alkaline or even physiological conditions in the heat [3], on bisulfite treatment [4], or on uv irradiation in aqueous solution [5].

Our interest in chlorination reactions of nucleobases originated in our work of preparing models of *cis*-diammineplatinum(IV) interactions with DNA nucleobases. In the course of these studies we found that the usual way of preparing such complexes - oxidative addition of the dihalogen to the respective Pt(II) complex - simultaneously may lead to oxidation of the metal *and* halogenation of the nucleobase [6].

Depending on the conditions of the reaction, several products are isolated when 1-methylcytosine (**1**) is treated with chlorine in water (Figure 1). 5-Chloro-1-methylcytosine hemihydrochloride hemihydrate (**2**) is obtained on brief bubbling of chlorine gas through an aqueous solution of **1** and subsequent fast evaporation. We asume that **2** crystallizes in a similar fashion as related hemiprotonated cytosines in a centrosymmetric dimeric arrangement with three hydrogen bonds between O2, N3, and N4 [7]. Deprotonation of **2** by means of sodium hydroxide gives 5-chloro-1-methylcytosine monohydrate (**3**). The pK_a value of protonated **3**, which has been determined potentiometrically as 2.9 in water [8], reflects the expected increase in acidity as compared to protonated **1** (pK_a 4.5) [9].

A more prolonged exposure of **1** to chlorine gives 5,5-dichloro-6-hydroxy-5,6-dihydro-1-methyluracil (**4**) as well as other unidentified products. In **4**, the cytosine has undergone deamination to the uracil and in addition there has been substitution of the original proton at C5 by chlorine followed by hypochlorous acid addition to the 5.6 double bond.

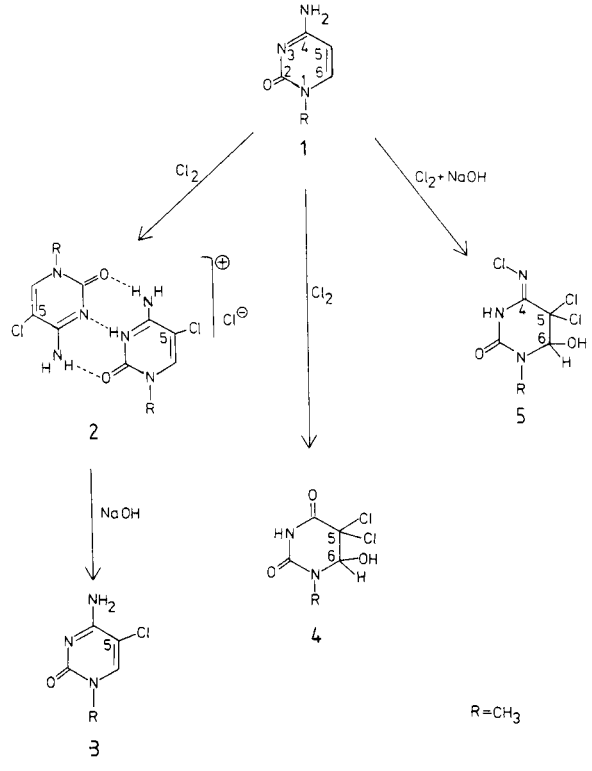

Figure 1. Schematic pathway of the preparation of the various derivatives of 1-methylcytosine.

Neutralization of an aqueous solution of **1** after a 15 minutes treatment with chlorine gas and crystallization of the resulting solution ($5 \ll pH \ll 7$) at 3° gives 5,5,-dichloro-6-hydroxy-5,6-dihydro-4-chlorimino-1-methylpyrimidin-2-one (**5**) in 50-60% yield. In moderately acidic solution ($pH \ll 3$), **5**, reacts to **4** and other unidentified products. This behavior suggests that **5** is at least *one* intermediate of the reaction **2**→**4**.

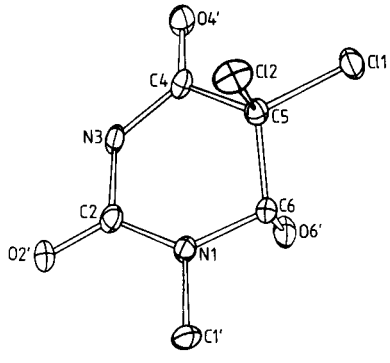

Figure 2. Molecular structure of compound **4**, derived from 1-methylcytosine through deamination and modification at the 5- and 6-positions.

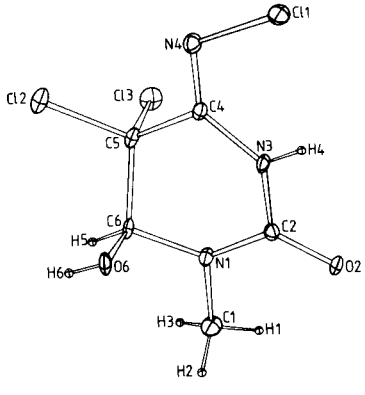

Figure 3. Molecular structure of the 4-chlorimino derivative **5**.

Crystal structures have been determined for **4** (Figure 2) and **5** (Figure 3). Positional parameters and temperature factors as well as interatomic distances and angles of both compounds are given in Tables 1-3. As can be seen from these data, with the exception of the groups in the 4-positions, the basic structures of **4** and **5** are very similar. Unlike the starting compound **1**, which is planar [10], rings **4** and **5** have lost planarity as a consequence of hypochlorous acid addition. The crystal structure of **5** clearly proves the existence of the chlorimino group at the 4-position and a proton at N3. Based on these results it seems likely that the previously reported 4-*N*-chlorocytosine [2a], formulated as chloramines with $-NHCl$ groups at C4, may indeed represent the chlorimine tautomers as well.

Table 1

Atom Coordinates of $C_5H_6Cl_2N_2O_3$ (**4**) and $C_5H_6Cl_3N_3O_2$ (**5**)

4

ATOM	X	Y	Z	U
N1	0.5966(2)	−0.1500(2)	0.3608(3)	0.034(3)
C1'	0.5921(3)	−0.2342(3)	0.4613(4)	0.043(3)
C2	0.5177(2)	−0.1372(3)	0.2751(3)	0.032(3)
O2'	0.4392(2)	−0.1866(2)	0.2831(3)	0.047(3)
N3	0.5289(2)	−0.0636(2)	0.1712(3)	0.035(3)
C4	0.6068(2)	0.0030(3)	0.1525(3)	0.030(3)
O4'	0.6152(2)	0.0579(2)	0.0541(3)	0.042(3)
C5	0.6813(3)	0.0027(3)	0.2730(3)	0.031(3)
Cl1	0.7996(1)	0.0524(1)	0.2213(1)	0.044(1)
Cl2	0.6305(1)	0.0829(1)	0.4023(1)	0.051(1)
C6	0.6956(2)	−0.1093(3)	0.3244(4)	0.030(3)
O6'	0.7356(2)	−0.1699(2)	0.2206(3)	0.038(2)

5

ATOM	X	Y	Z	U
Cl1	0.7654(1)	0.6715(1)	0.3284(1)	0.025(1)
Cl2	0.7538(1)	0.4710(1)	0.9336(1)	0.030(1)
Cl3	0.6798(1)	0.3120(1)	0.6101(1)	0.025(1)
N1	0.9258(2)	0.2849(2)	0.5636(4)	0.015(2)
C1	0.9796(3)	0.1728(3)	0.5668(5)	0.022(3)
C2	0.9330(2)	0.3550(3)	0.3991(5)	0.014(2)
O2	0.9825(2)	0.3302(2)	0.2493(3)	0.018(2)
N3	0.8806(2)	0.4608(2)	0.4052(4)	0.015(2)
C4	0.8060(2)	0.4925(3)	0.5402(4)	0.014(2)
N4	0.7478(2)	0.5830(2)	0.5382(4)	0.019(2)
C5	0.7894(2)	0.4037(3)	0.7063(5)	0.016(3)
C6	0.8931(2)	0.3304(3)	0.7555(5)	0.015(3)
O6	0.9780(2)	0.3972(2)	0.8512(3)	0.018(2)
H1	0.9756(0)	0.1351(0)	0.4367(0)	0.040(0)
H2	1.0531(0)	0.1778(0)	0.6237(0)	0.040(0)
H3	0.9440(0)	0.1227(0)	0.6347(0)	0.040(0)
H4	0.8943(0)	0.5134(0)	0.3161(0)	0.040(0)
H5	0.8750(0)	0.2657(0)	0.8428(0)	0.040(0)
H6	0.9747(0)	0.3902(0)	0.9782(0)	0.040(0)

The C4-N4 bond length of 1.272(4) Å in **5** is significantly shorter than the 1.336(2) Å observed in **1** [10], and corresponds to a double bond. The N-Cl distance of 1.736(3) Å is within the range expected for a single bond of this type [11].

In conclusion, our results indicate a rather versatile reactivity pattern of 1-methylcytosine towards chlorine, exceeding that of uracil derivatives, for example. Apart from the substitution of H5 by chlorine, which gives **2** (**3**) most likely *via* an addition-elimination mechanism [1], and subsequent addition of hypochlorous acid to the 5,6 double bond of **2** (**3**), there is also chlorination of the exocyclic amino group to give **5**, and finally deamination to the uracil derivative **4**.

Table 2

Bond Distances (Å) and Angles (deg) of 5,5-dichloro-6-hydroxy-5,6-dihydro-1-methyluracil (**4**)

N1 - C1′	1.466(4)
N1 - C2	1.348(4)
C2 - O2′	1.220(4)
C2 - N3	1.398(4)
N3 - C4	1.353(4)
C4 - O4′	1.199(4)
C4 - C5	1.535(4)
C5 - Cl1	1.764(4)
C5 - C12	1.766(3)
C5 - C6	1.541(5)
C6 - O6′	1.385(4)
C6 - N1	1.454(4)
C1′ - N1 - C6	117.9(3)
C1′ - N1 - C2	118.4(3)
C6 - N1 - C2	120.0(3)
O2′ - C2 - N1	123.6(3)
O2′ - C2 - N3	119.4(3)
N1 - C2 - N3	117.0(3)
C2 - N3 - C4	127.6(3)
O4′ - C4 - N3	123.6(3)
O4′ - C4 - C5	123.9(3)
N3 - C4 - C5	112.5(3)
Cl1 - C5 - C4	110.4(2)
Cl1 - C5 - C6	109.0(2)
Cl1 - C5 - C12	109.2(2)
Cl2 - C5 - C4	107.7(2)
Cl2 - C5 - C6	111.2(2)
C4 - C5 - C6	109.4(3)
O6′ - C6 - C5	109.7(3)
O6′ - C6 - N1	108.6(3)
C5 - C6 - N1	107.9(3)

Table 3

Bond Distances (Å) and Angles (deg) of 5,5-dichloro-6-hydroxy-5,6-dihydro-4-chlorimino-1-methylpyrimidine-2-one (**5**).

N1 - C1	1.462(4)	C1 - N1 - C6	118.5(2)
N1 - C2	1.352(4)	C1 - N1 - C2	119.1(3)
C2 - O2	1.227(4)	C6 - N1 - C2	120.2(2)
C2 - N3	1.392(4)	N1 - C1 - H1	114.6
N3 - C4	1.374(4)	N1 - C1 - H2	111.3
C4 - N4	1.272(4)	N1 - C1 - H3	111.0
N4 - Cl1	1.736(3)	H1 - C1- H2	110.1
C4 - C5	1.521(4)	H1 - C1 - H3	98.9
C5 - Cl2	1.760(3)	H2 - C1 - H3	110.4
C5 - Cl3	1.783(3)	O2 - C2 - N1	123.8(3)
C5 - C6	1.540(4)	O2 - C2 - N3	119.6(3)
C6 - O6	1.398(4)	N1 - C2 - N3	116.5(3)
C6 - N1	1.443(4)	H4 - N3 - C2	119.1
C1 - H1	1.948	H4 - N3 - C4	114.6
C1 - H2	0.944	C2 - N3 - C4	126.3(2)
C1 - H3	0.872	N4 - C4 - N3	128.5(3)
N3 - H4	0.868	N4 - C4 - C5	117.2(3)
C6 - H5	0.980	N3 - C4 - C5	114.2(3)
O6 - H6	0.832	Cl1 - N4 - C4	112.9(2)
		Cl2 - C5 - Cl3	108.8(2)
		Cl2 - C5 - C4	110.5(2)
		Cl2 - C5 - C6	109.8(2)
		Cl3 - C5 - C4	107.8(2)
		Cl3 - C5 - C6	108.7(2)
		C4 - C5 - C6	111.1(2)
		O6 - C6 - H5	111.8
		O6 - C6 - C5	110.5(2)
		O6 - C6 - N1	109.3(2)
		H5 - C6 - C5	108.6
		H5 - C6 - N1	108.2
		C5 - O6 - N1	108.4(2)
		H6 - O6 - C6	106.3

EXPERIMENTAL

The uv spectra were recorded on a Cary 17D spectrometer, ^{1}H nmr spectra on a Jeol JNM-FX 60 FT spectrometer (deuterium oxide, tetramethylammonium tetrafluoroborate internal standard, shifts relative to sodium 3-(trimethylsilyl)propanesulfonate 3.187 ppm from standard). Reported pD values were obtained by adding 0.4 to the meter reading. Elemental analyses were carried out in the analytical chemistry laboratory of the Institute of Inorganic Chemistry at the Technical University Munich. 1-Methylcytosine was purchased from Vega Biochemicals.

5-Chloro-1-methylcytosine Hemihydrochloride Hemihydrate (**2**).

Through a solution of 1-methylcytosine (300 mg, 2.4 mmoles) in 5 ml of water, chlorine gas was bubbled in a low stream for 3-4 minutes. The solution was then immediately evaporated to dryness. Recrystallization of the product from water (60°) gave 320 mg of **2** (yield 65%); uv (water, *p*H 1): λ_1 (log ϵ) 298 nm (4.00), λ_2 (log ϵ) 217 nm (4.08); ^{1}H nmr (deuterium oxide, pD 3.2): δ 3.44 (CH_3), 8.07 (H6).

Anal. Calcd. for $C_5H_6ClN_3O{\cdot}0.5HCl{\cdot}\ 0.5H_2O$: C, 32.1; H, 4.1; N, 22.5; Cl, 28.5. Found: C, 32.0; H, 4.1; N, 22.5; Cl, 29.0.

5-Chloro-1-methylcytosine (**3**).

Compound **2** was dissolved in water (300 mg in 20 ml) and the solution (*p*H 2.7) brought to *p*H 7.8 by means of 0.1 *N* sodium hydroxide. Evaporation of the solution in air gave 200 mg of **3** (yield 70%). Colorless, transparent needles, loosing water and transparency in air; uv (water, *p*H 8): λ_1 (log ϵ) 287 nm (3.86), λ_2 (log ϵ) 217 nm (4.11); ^{1}H nmr (deuterium oxide, pD 5.2): δ 3.40 (CH_3), 7.87 (H6).

Anal. Calcd. for $C_5H_6ClN_3O{\cdot}H_2O$: N, 23.7; Cl, 19.9. Found: N, 23.9; Cl, 19.4. After 1 day in air: Calcd. for $C_5H_6ClN_3O{\cdot}0.25H_2O$: C, 36.6; H, 4.0; N, 25.6; Cl, 21.6. Found: C, 36.6; H, 4.0; N, 25.5; Cl, 21.7.

5,5-Dichloro-6-hydroxy-5,6-dihydro-1-methyluracil (**4**).

Through a solution of 1-methylcytosine (500 mg in 20 ml water) chlorine gas was bubbled for 10 minutes and the solution brought to dryness by rotary evaporation. The colorless precipitate was redissolved in 5 ml water and from the solution (*p*H 1-2) within 3 days at 3° 100 mg of **2** precipitated. After several days of slow evaporation, 200 mg of large, transparent cubes of **4** were removed (yield 22%). On complete evaporation to dryness a mixture of various products was obtained which was not analyzed; uv (water, *p*H 3) $\lambda < 200$ nm; ^{1}H nmr (deuterium oxide, pD 3.6): δ 3.15 (CH_3), 5.43 (H6).

Anal. Calcd. for $C_5H_6Cl_2N_2O_3$: C, 28.2; H, 2.9; N, 13.1; Cl, 33.3. Found: C, 27.8; H, 2.9; N, 13.4; Cl, 32.9.

5,5-Dichloro-6-hydroxy-5,6-dihydro-4-chlorimino-1-methylpyrimidin-2-one (**5**).

Through a solution of 1-methylcytosine (630 mg in 30 ml water) chlorine gas was bubbled for 15 minutes. Excess chlorine was then removed from the solution by a 5-minutes treatment at a rotavapor. Then 0.2 *N* sodium hydroxide was added to the solution to bring the *p*H to 6.7, the solution was concentrated to 15 ml volume by rotary evaporation and finally filtered. Occasionally more base had to be added at this stage to maintain the *p*H between 6 and 7. Slow evaporation of the solution at 3° gave 600 mg of a product which, according to the ir consisted of about 90% of **5**. Recrystallization from boiling water (20 ml) gave 200 mg of

crystalline **5** after 1 day at 3°; uv (water, *p*H 6); λ max (log ε) 222 nm (3.98); ^{1}H nmr (deuterium oxide, pD 3.5): δ 3.15 (CH_3), 5.41 (H6); (dimethylformamide-d_7): δ 3.10 (CH_3), 5.36 (H6, d, J = 5.4 Hz), 8.17 (OH, d), 10.55 (NH).

Anal. Calcd. for $C_5H_6Cl_3N_3O_2$: C, 24.4; H, 2.5; N, 17.1; O, 13.0; Cl, 43.1. Found: C, 24.7; H, 2.6; N, 17.6; O, 13.1; Cl, 42.7.

Crystallography.

Crystal data were taken at room temperature (**4**) and −100°C (**5**), respectively, on a Phillips PW-1100 diffractometer using graphite-monochromated Mo Kα radiation (λ = 0.71069 Å). The crystal data of **4** are as follows: $C_5H_6Cl_2N_2O_3$, formula weight 213.01, orthorhombic, space group Pbca, a = 13.215 (4), b = 12.898 (4), c = 9.777 (3) Å, U = 1666.5 Å^3, d_{obsd} = 1.69, d_{calcd} = 1.698 g cm^{-3}, Z = 8. The crystal data of **5** are as follows: $C_5H_6Cl_3N_3O_2$, formula weight 246.46, monoclinic, space group $P2_1/n$, a = 12.265 (3), b = 11.641 (2), c = 6.484 (1) Å, β = 95.38 (2)°, U = 921.7 Å^3, d_{obsd} = 1.73, d_{calcd} = 1.776 g cm^{-3}, Z = 4. Intensity data were collected using a $\theta/2\theta$ technique (θ_{max} 25°). 1456(**4**) and 1610 (**5**) independent reflections were measured, and 1453 (**4**) and 1578 (**5**) reflections with $F_o > 2\sigma(F_o)$ were used for the calculations. The structures were solved using direct methods. With **4**, all atoms except hydrogens, which were not localized, were refined anisotropically: R = 0.065, R_w = 0.083. With **5**, hydrogens were localized but not refined: R = 0.046, R_w = 0.052.

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