Metal-stabilized rare tautomers of nucleobases 4. On the question of adenine tautomerization by a coordinated platinum(II)

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Abstract

An attempt is described and critically asssessed to use the 'method of basicity measurements' for estimating the effect of Pt(II) electrophiles bound to the N7 position of the model nucleobase 9-methyladenine to shift the tautomer equilibrium from the preferred 6-amino to the rare 6-imino form. The question of tautomerization of an adenine nucleobase is of biological interest with regard to the now established preference for A→T transversions as a consequence of 5′ (ApG) adduct formation of the antitumor agent Cisplatin. For this purpose, a series of model nucleobase complexes of Pt(II) with 9-methyladenine (9-MeA), 1,9-dimethyladenine (1,9-DimeA) and 6,9-dimethyladenine (6,9-DimeA), and their protonated forms have been prepared and their UV spectra at various pH values recorded. In two cases, the compounds were characterized also by X-ray analysis, *cis*-[Cl_2Pt(1,9-DimeAH)(NH_3)]Cl (**2c**) and Cl_3Pt(6,9-DimeAH)·H_2O (**3a**). Based on the experimentally determined pK_a values of the platinated adeninium ligands, tautomer equilibrium constants K_T were calculated and found to be $10^{-4.5}$ for [Cl_3Pt(9-MeA)]$^-$ and $10^{-4.6}$ for *cis*-Cl_2Pt(NH_3)(9-MeA). These values compare with $10^{-4.8}$ for 9-MeA. The observed shifts in favor of the rare imino tautomer on Pt binding to N7 are considered too small to unambiguously explain the observed preferential A→T transversion on the basis of an initial mispair between a platinated adenine in its imino form and an adenine in its normal amino tautomer.

Introduction

Metal ions and metal coordination compounds in many cases have been found to be mutagenic or even involved in carcinogenesis [1]. Possible ways of interference of metal species with proper base pairing in DNA have been discussed [2]. It appears that at least three basic possibilities exist, which involve alterations either at the template, the substrate, and/or the polymerase level. As to non-complementary base pairing patterns which, if not repaired, result in mutagenic events, modern oligonucleotide chemistry in combination with X-ray crystallography [3] and sophisticated NMR spectroscopy [4] has been able to demonstrate the existence of base mismatches in duplex DNA. These mismatches involve either two neutral or one neutral and a protonated nucleobase or one of the two bases in a *syn* orientation. With one possible exception of a base pair between two thymines from two different DNA hairpin molecules [5], none of these odd base pairs includes a rare nucleobase tautomer, a finding which certainly is not unexpected. Similar structural data on mismatches between a metallated nucleobase and a 'wrong' base in a double-stranded oligonucleotide are not available at present. On the other hand, the melting behaviour of a G,G platinated duplex oligonucleotide with a wrong nucleobase across the platinated 5′-G has been studied [6], and there have been quantum mechanical approaches to this question [7]*.

Our attempts to model metal–nucleobase interactions that are potentially relevant to base-mispairing mechanisms, have provided some insight. Applying inert metal species such as Pt(II) or Pt(IV), we have reported on: (i) mispairing between two guanine nucleobases, brought about by metal binding to N7 of one base [8]; (ii) the stabilization of a rare cytosine tautomer in its

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*G denotes guanine. Other abbreviations used: A = adenine, T = thymine, 9-MeA = 9-methyladenine, 1,9-DimeA = 1,9-dimethyladenine, 1,6-DimeA = 1,6-dimethyladenine, 1-MeC = 1-methylcytosine, 1-MeU = 1-methyluracil anion, 1-MeT = 1-methylthymine anion.

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metal-complexed form [9]; (iii) a Pt-assisted tautomerization of uracil [10] and thymine nucleobases [11]. Moreover, (iv) the substitution of a hydrogen from a hydrogen bond between two nucleobases by a metal ion of suitable geometry (*trans*-a_2Pt(II) [12], Ag(I) [13]) provides another pathway for the stabilization of odd base pairs.

The motivation for the study described here came from work reported by Burnouf *et al.* [14], who determined the mutation spectrum of the antitumor agent *cis*-$(NH_3)_2PtCl_2$ (*cis*-DDP, Cisplatin), and found that the d(ApG) adduct of *cis*-DDP in DNA is at least five times more mutagenic than the d(GpG) adduct, which represents the major cross-link in platinated DNA [15]. There is a high mutation specificity for the 5′ base A, leading primarily (>80%) to A→T transversions. It is generally accepted [16] that the mutational pathway of the A→T transversion involves a pair between two adenines as the central mispairing step (Fig. 1). One of the two adenines is the rare imino tautomer of adenine (A*), the other one the normal amino tautomer in a *syn* conformation. Considering the geometry of the G(*anti*)·A(*anti*) mismatch [17], an A*(*anti*)·A(*anti*) pair would seem to be possible as well. Unlike for several other mismatches, which might be explained by a base-wobble as a consequence of steric distortion caused by a bulky mutagen [18], for example, it is difficult to explain base pairing between two adenines that maintain the *cis* orientation of the glycosidic bonds, without involving a rare tautomer structure of one of the two bases. The alternative would be no base pairing at all, similar to the situation in apurinic sites,where adenines are known to be preferentially inserted across such lesions [19].

This study was conducted with the aim of exploring the possibility that a metal (Pt(II)) coordinated at the

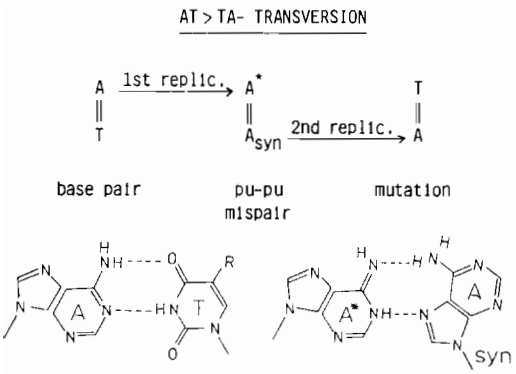


Fig. 1. Generally accepted mechanism for AT→TA transversion (top) and schematic views of normal A=T base pair (bottom left) and mispair between the rare adenine tautomer (A*) and the preferred adenine tautomer (A) in a *syn* orientation (bottom right).

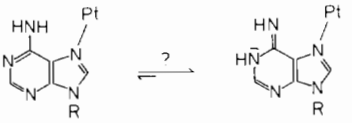


Fig. 2. Question: Does Pt coordination at N7 of adenine shift the tautomer equilibrium towards the imino form?

N7 site of the nucleobase 9-methyladenine (9-MeA) affects the tautomer equilibrium (Fig. 2).

Experimental

Preparation

9-MeA [20], (1,9-DimeAH)ClO_4 [21] and 6,9-DimeA [21] were prepared according to published methods.

Cl_3Pt(9-MeAH-*N7*) (**1a**) was prepared from K_2PtCl_4 and 9-MeA as described [22]. When the reaction was carried out in more dilute HCl (0.1 N) and at room temperature, a poorly soluble, salmon-pink material of composition [9-MeAH]$_2$[$PtCl_4$] (**1b**) was obtained in good yield within 1–2 h. *Anal.* Calc. for $(C_6H_8N_5)_2PtCl_4$ (**1b**): C, 22.62; H, 2.54; N, 21.98; Cl, 22.25. Found: C, 22.7; H, 2.4; N, 21.8; Cl, 22.3%.

Treatment of **1a** with an excess of NH_3 gives $[(NH_3)_3$Pt(9-MeA-*N7*)$]Cl_2 \cdot 2H_2O$ (**1c**) and, via $AgClO_4$, the perchlorate salt $[(NH_3)_3$Pt(9-MeA-*N7*)$](ClO_4)_2$ (**1d**) [23]. Careful addition of NH_3 (6 ml, 25%) to an aqueous suspension of **1a** (1.8 mmol in 15 ml) initially dissolves **1a** completely, but leads to a yellowish-white precipitate **1e** within 5 min. The yield is 90–95%. **1e** analyzes as Cl_2Pt(9-MeA-*N7*)(NH_3). *Anal.* Calc. for Cl_2Pt-$(C_6H_7N_5)(NH_3)$: C, 16.67; H, 2.34; N, 19.45; Cl, 16.40. Found: C, 16.6; H, 2.3; N, 19.2; Cl, 15.8%. In analogy to the structurally characterized corresponding 1,9-DimeAH complex **2c** we assume that **1e** has a *cis* geometry.

Other Pt(II) complexes containing N7 bound 9-MeA were [(dien)Pt(9-MeA-*N7*)$](NO_3)_2$ [24], *cis*- and *trans*-$[(NH_3)_2$Pt(1-MeC)(9-MeA-*N7*)$](ClO_4)_2$ [25] and *cis*-$[(NH_3)_2$Pt(9-MeA-*N7*)X$]ClO_4$ (X=1-MeU or 1-MeT) [26].

Cl_3Pt(1,9-DimeAH-*N7*)$\cdot H_2O$ (**2a**) was prepared as follows. (1,9-DimeAH)ClO_4 (3 mmol) was slightly warmed in HCl (0.1 M, 40 ml) until all material had dissolved and was then allowed to cool to 22 °C. K_2PtCl_4 (3 mmol) was added with stirring. After 5 min, when precipitation started, stirring was stopped. After 22 h, the yellow–tan precipitate (1.32 g, 91%) was filtered off, washed with water, methanol and ether and dried in air. *Anal.* Calc. for $Cl_3Pt(C_7H_{10}N_5)\cdot H_2O$ (**2a**): C, 17.38; H, 2.51; N, 14.48; Cl, 21.99. Found: C, 17.4; H, 2.1; N, 14.3; Cl, 21.5%.

cis-Cl_2Pt(1,9-DimeA-*N7*)(NH_3) (**2b**) was prepared by a similar method to that for **1e**. 700 mg of **2a** were suspended in 10 ml of water. After addition of 6 ml of 25% NH_3 (pH 11.4), a yellow–orange solution formed, the color of which faded to pale orange within minutes. After 15 min, the yellowish precipitate that formed (350 mg, 54%) was filtered off, washed twice with 2.5 ml water, then methanol and ether and finally dried in air. *Anal.* Calc. for $Cl_2Pt(C_7H_9N_5)(NH_3)$ (**2b**): C, 18.67; H, 2.69; N, 18.67; Cl, 15.76. Found: C, 18.5; H, 2.7; N, 18.8; Cl, 15.2%.

cis-[$Cl_2Pt(NH_3)$(1,9-DimeAH-*N7*)]Cl (**2c**) was prepared from **2b** (100 mg in 1.2 ml of 0.2 N HCl) and obtained as yellow single crystals in 40% yield upon slow evaporation. *Anal.* Calc. for $Cl_3Pt(C_7H_{10}N_5)(NH_3)$: C, 17.42; H, 2.72; N, 17.41; Cl, 22.03. Found: C, 17.4; H, 2.8; N, 17.3; Cl, 22.1%.

Cl_3Pt(6,9-DimeAH-*N7*)·H_2O (**3a**) was prepared in analogy to **1a** and **2a** from 6,9-DimeA and K_2PtCl_4 (1 mmol each) in HCl (2.5 N, 10 ml) at 50 °C within several hours. Orange–yellow cubes or plates, yield 73%. *Anal.* Calc. for $Cl_3Pt(C_7H_{10}N_5)\cdot H_2O$ (**3a**): C, 17.38; H, 2.51; N, 14.48; Cl, 21.99. Found: C, 17.5; H, 2.5; N, 14.4; Cl, 22.2%.

cis-Cl_2Pt(6,9-DimeA)(NH_3 (**3b**) was obtained in analogy to **1e** and **2b** from **3b** (200 mg suspended in 4.5 ml of water; addition of 1.5 ml of 25% NH_3) as a yellowish-white material in 49% yield. *Anal.* Calc. for $Cl_2Pt(NH_3)(C_7H_9N_5)$: C, 18.84; H, 2.72; N, 18.84; Cl, 15.89. Found: C, 18.7; H, 2.7; N, 18.5; Cl, 15.8%.

X-ray crystallography

X-ray crystal structures were performed for **2c** and **3a** on a Philips-PW1100 single crystal diffractometer using graphite monochromated Mo Kα radiation ($\lambda = 0.71069$ Å) at room temperature. Details relevant to data collection and refinement are given in Table 1; atomic coordinates and isotropic temperature factors in Tables 2 (**2c**) and 3 (**3a**). Intensity data were corrected for Lorentz and polarization effects and, at a later stage, for absorption [27]. The coordinates of the Pt atoms were found in a three-dimensional Patterson map. The other non-hydrogen atoms were determined by subsequent ΔF syntheses; H atoms were ignored. Complex scattering factors for neutral atoms were taken from refs. 28 and 29. The SHELX program package was used for the calculations [30].

Spectroscopy

IR spectra were measured as KBr pellets using Perkin-Elmer spectrometers 577 and 783.

UV spectra were recorded on a Perkin-Elmer 55 in 1 cm cuvettes. pK_a values were determined spectrophotometrically using the Henderson–Hasselbalch relation

TABLE 1. Crystallographic data for *cis*-[$Cl_2Pt(NH_3)$(1,9-DimeAH)]Cl (**2c**) and Cl_3Pt(6,9-DimeAH)·H_2O (**3a**)

	2c	**3a**
Formula weight	482.67	483.66
Space group	*Pbca*	$P2_1/n$
a (Å)	22.699(12)	9.332(2)
b (Å)	10.667(5)	15.985(3)
c (Å)	11.343(7)	9.091(2)
α (°)		
β (°)		93.14(2)
γ (°)		
V (Å^3)	2746.48	1354.1
Z	8	4
D_{calc} (g cm^{-3})	2.335	2.373
Crystal size (mm)	0.5×0.5×0.4	0.2×0.2×0.2
μ (cm^{-1})	103.6	105
θ Range (°)	2–25	2–25
No. unique reflections	2418	2662
No. unique reflections used in calculations	2253 $F_o > 2\sigma F_o$	2501 $F_o > 2\sigma F_o$
No. parameters	154	154
R	0.053	0.030
R_w (F)	0.052	0.030 ($w^{-1} = \sigma^2(F) + 0.0004F^2$)

TABLE 2. Atomic coordinates and equivalent isotropic temperature factors (Å^2) for **2c**

Atom	x	y	z	U
Pt1	0.6101(1)	0.2169(1)	0.4401(1)	0.023(1)
Cl1	0.5495(1)	0.1671(3)	0.5981(2)	0.041(1)
Cl2	0.6370(1)	0.4023(2)	0.5250(2)	0.039(1)
Cl3	0.2470(1)	0.4963(2)	0.7234(2)	0.040(1)
N10	0.6633(4)	0.2570(8)	0.2992(8)	0.036(3)
N1	0.6678(5)	−0.2288(8)	0.4644(9)	0.040(3)
C1′	0.7105(6)	−0.2823(11)	0.5479(13)	0.052(5)
C2	0.6437(7)	−0.3059(10)	0.3772(11)	0.045(4)
N3	0.6068(5)	−0.2694(8)	0.2966(9)	0.041(3)
C4	0.5922(4)	−0.1464(10)	0.3037(9)	0.027(3)
C5	0.6120(5)	−0.0630(9)	0.3876(10)	0.029(3)
C6	0.6517(5)	−0.1037(9)	0.4749(10)	0.032(3)
N6′	0.6726(5)	−0.0331(9)	0.5590(8)	0.040(3)
N7	0.5895(3)	0.0547(7)	0.3615(7)	0.023(2)
C8	0.5559(5)	0.0383(9)	0.2650(9)	0.027(3)
N9	0.5571(4)	−0.0833(8)	0.2277(8)	0.031(3)
C9′	0.5252(6)	−0.1314(12)	0.1230(11)	0.047(4)

$$pK_a = pH - \log \frac{E_K - E}{E - E_B}$$

where E_K is the absorbance of the adeninium complex, E_B is the absorbance of the adenine complex, and E is the absorbance of a mixture of both species at a given pH and a given λ each. In order to suppress Cl^- solvolysis and also to keep the ionic strength constant, equiconcentrated solutions were usually measured in 0.1 M NaCl, except for strongly acidic solutions ($0 < pH < 1$), where HCl was applied. pH values were

TABLE 3. Atomic coordinates and equivalent isotropic temperature factors (Å^2) for **3a**

Atom	*x*	*y*	*z*	*U*
Pt1	0.0568(1)	0.1523(1)	1.0429(1)	0.029(1)
Cl1	0.0141(1)	0.2611(1)	0.8797(2)	0.046(1)
Cl2	−0.1455(1)	0.1856(1)	1.1612(1)	0.046(1)
Cl3	0.1069(2)	0.0454(1)	1.2048(2)	0.058(1)
N1	0.5537(5)	0.2482(3)	0.9516(6)	0.039(1)
C2	0.6345(6)	0.1937(4)	0.8731(8)	0.045(2)
N3	0.5881(5)	0.1237(3)	0.8195(7)	0.048(1)
C4	0.4496(6)	0.1073(3)	0.8542(7)	0.038(1)
C5	0.3614(6)	0.1568(3)	0.9288(6)	0.033(1)
C6	0.4141(6)	0.2347(3)	0.9771(6)	0.034(1)
N6′	0.3368(5)	0.2917(3)	1.0421(5)	0.039(1)
C6′	0.3896(8)	0.3775(4)	1.0799(9)	0.055(2)
N7	0.2305(5)	0.1172(3)	0.9373(5)	0.034(1)
C8	0.2431(7)	0.0429(3)	0.8700(7)	0.044(2)
N9	0.3772(5)	0.0346(3)	0.8184(6)	0.044(1)
C9′	0.4297(9)	−0.0378(4)	0.7336(10)	0.067(3)
O10	0.2499(6)	0.6213(3)	0.9924(7)	0.071(2)

varied by adding 0.1 M HCl or NaOH using a variopipette. pH values were measured by use of a standardized glass electrode. Only those spectra that showed strictly isosbestic behaviour were used for the calculations of pK_a. Usually three to four values of pK_a were determined at different wavelengths and averaged.

Results

Effects of Pt on 9-MeA: qualitative considerations

The effect of a Pt electrophile on a neutral nucleobase via an endocyclic nitrogen is to withdraw electron density from the heterocycle. As a consequence, the acidity of N–H or NH_2 groups increases, while the basicity of unprotonated N or O groups simultaneously decreases. This situation is in contrast to various Ru(II) complexes with heterocyclic ligands, where π backbonding from the metal to the heterocycle can effectively increase the ligand basicity [31], unless there is strong competition with other ligands at the metal such as CN^-, for example [32]. The effect of Pt(II) is related to the distance between the metal and the respective functional group, even though it has not been quantified as in the case of purine and pyrimidine complexes of $(NH_3)_5Ru(III)$ [33]. The relative magnitudes of both effects of Pt(II) have an influence on the tautomer equilibrium of the metalated nucleobase. As to 9-substituted adenines, Pt is expected (i) to increase the $N(6)H_2$ acidity and (ii) to decrease the basicity of N1, possibly also that of N3. The decrease in N1 basicity in N7 platinated 9-MeA complexes can be measured in aqueous solution under the assumption that N3 is no site of protonation (Table 4). The decrease in basicity (or increase in acidity of the corresponding 9-MeAH acid [34]) is between 2 and 3 log units, depending on the charge of the complex and the other ligands present. In contrast, the acidification of $N(6)H_2$ as a consequence of N7 Pt binding has never been measured due to the high p$K_a \approx 16.7$ of this group [35] and the only moderate acidifying effect of the Pt. Only on N1 Pt binding or platination at N1 and N7 is there a substantial decrease in pK_a to *c.* 13–14 and 10–11, respectively [24]. Qualitatively, an increase in NH_2 acidity is evident from the substantial downfield shift of the 9-MeA amino resonance in the ^{1}H NMR spectrum (DMSO or DMF) on Pt binding to N7, however [26a].

TABLE 4. pK_a values of N(1)H of 9-MeAH and of N7 platinated 9-MeAH$^+$ complexes

Species	pK_a[a]	Method[b]	Reference
9-MeAH	4.3	UV	34
	4.5	NMR	24
Cl_3Pt(9-MeAH) (**1a**)	2.5	UV	[c]
cis-$[Cl_2Pt(NH_3)$(9-MeAH)$]^+$ (**1e**)	1.9	UV	[c]
cis-$[(NH_3)_2Pt$(1-MeT)(9-MeAH)$]^{2+}$	2.2	NMR	26
cis-$[(NH_3)_2Pt$(1-MeU)(9-MeAH)$]^{2+}$	2.4	NMR	26
cis-$[(NH_3)_2Pt$(1-MeC)(9-MeAH)$]^{3+}$	1.6	NMR	25
$[(NH_3)_3Pt$(9-MeAH)$]^{3+}$	1.55	NMR	23
[(dien)Pt(9-MeAH)]$^{3+}$	1.9	NMR	24
	1.5	UV	24

[a]pK_a values ±0.05. [b]Referenced to H_2O as solvent. [c]This work.

Estimating tautomer distribution

Equilibria of two tautomers present in largely different ratio, e.g. $(10^{-4}$–$10^{-5})$: 1 as in the case of the naturally occurring nucleobases, are difficult to study. Of all physical methods available, the indirect 'method of basicity measurements' [36] has gained the widest application in estimating the equilibrium constant K_T of two heterocyclic tautomers A and B. According to this method

$$K_T = \frac{[A]}{[B]} \quad (1)$$

K_T is derived from the experimentally determined K_1 value for the protonation processes of the two tautomers in equilibrium, and the sum of the two individual constants K_A and K_B. Since the latter cannot be

$$K_1 = K_A + K_B \quad (2)$$

obtained experimentally, their values are estimated from compounds of fixed structure with the tautomerizable hydrogen replaced by a methyl group, hence $K_A = f_A K_{MeA}$ and $K_B = f_B K_{MeB}$*. Under the simplifying assumption, that the methyl group has no or at most

*K_{MeA} does not stand for methyladenine here.

a very small effect on the ionization constant ($f_A = f_B = 1$), two eqns. (3) and (4) are usually utilized, provided the difference between K_1 and the constant for the

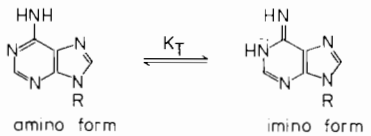
$$K_T = \frac{K_A}{K_1 - K_A} \quad (3)$$

$$K_T = \frac{K_1}{K_B} - 1 \quad (4)$$

alkylated species is sufficiently large. Frequently it becomes evident that the use of one of the two equations leads to a result without physical meaning.

Application of this method to adenosine (pK_a of adoH$^+$, 3.65) and 1-methyladenosine (pK_a of 1-MeadoH, 8.25) gives $K_T = 10^{-4.6}$ for the tautomer equilibrium between the imino and amino forms [37] (Scheme 1). This value is subject to slight variations, depending on the pK_a values used, e.g. $K_T = 10^{-4.9}$ with pK_a of 1-MeadoH of 8.55 [38].

For 9-methyladenine we obtain $K_T = 10^{-4.8}$ using pK_a values of 4.3 for 9-MeAH$^+$ and 9.1 for 1,9-DimeAH$^+$. The alternative way to determine K_T from 9-MeA and 6,9-DimeA (pK_a of 6,9-DimeAH$^+$ is 3.8) leads to $K_T = -0.37$, which is physically meaningless, hence proves that the simplification $f_B = 1$ is not valid here (see also below).



Scheme 1.

Example 5-bromouracil

The frequency of spontaneous base substitution mutations in DNA (10^{-9}–10^{-12} per base pair synthesized) is considered to be related to the occurrence of the rare tautomer of the respective nucleobase [16]. The mutagenic activity of the nucleobase analogue 5-bromouracil, which causes transitions via mispairing with guanine, is generally ascribed to a shift in the tautomer equilibrium towards the 4-hydroxo, 2-oxo tautomer as compared to thymine [39].

Application of the 'method of basicity measurements' to 1-methyluracil and 1-methyl-5-bromouracil gives K_T values of *c.* 10^4 and $10^{3.3}$, respectively [40]*. This difference corresponds to a five-fold higher concentration of the rare tautomer or an increase from 0.1 to 0.5%. Although this trend seems to support the hypothesis mentioned above, the introduction of 5-bromouracil into a polynucleotide might change this picture in either direction (see also 'Discussion').

Tautomer equilibria of platinated adenines

In order to utilize the 'method of basicity measurements' for N7-platinated, 9-methylated adenines (Fig. 3), the following compounds were prepared and their pK_a values determined using pH-dependent UV spectra: Cl_3Pt(9-MeAH) (**1a**), *cis*-Cl_2Pt(9-MeA)(NH_3) (**1e**); Cl_3Pt(1,9-DimeAH)·H_2O (**2a**), *cis*-Cl_2Pt(1,9-DimeA)(NH_3) (**2b**) and its protonated form **2c**; Cl_3Pt(6,9-DimeAH)·H_2O (**3a**) and *cis*-Cl_2Pt(6,9-DimeA)(NH_3) (**3b**). It is known that 1-methyladenosine [41] and 1-alkyl-9-methyladenines [21] undergo Dimroth rearrangements to the 9-alkyl-9-methyladenines, and this fact has indeed been utilized to prepare 1,9-DimeA (see 'Experimental'). The UV spectra of 1,9-DimeA and its complexes did not provide any evidence for this rearrangement to occur within the time of the measurements. However, attempts to prepare additional Pt complexes of 1,9-DimeA applying alkaline conditions were unsuccessful as yet, possibly due to methyl group migration. pK_a values are listed in Table 5. pH dependent spectra of a representative example (**2b, 2c**) are given in Fig. 4. The acidifying effect of the Pt entities is similar in all three adenine systems, e.g. pK_a in Cl_3Pt(LH) is 1.8 (L = 9-MeA), 2.1 (L = 1,9-DimeA); 2.0 (L = 6,9-DimeA) and pK_a in *cis*-[Cl_2Pt(LH)(NH_3)]$^+$ = 2.4 (L = 9-MeA), 2.6 (L = 1,9-DimeA), *c.* 2.7 (L = 6,9-DimeA).

When spectra of corresponding complexes, e.g. **1e, 2b** and **3b**, in their protonated forms are compared, it is evident that those of the 9-MeAH$^+$ and 1,9-DimeAH$^+$ species, **1e** and **2b**, are almost superimposable at equal molar concentration ($\lambda_{max} = 262$ nm), while the 6,9-DimeAH$^+$ species (**3b**: $\lambda_{max} = 270$ nm, sh at *c.* 283 nm) displays a markedly different spectrum. Thus, protonation at N1 and methylation at this site affect the π-electron system of 9-MeA to a similar extent.

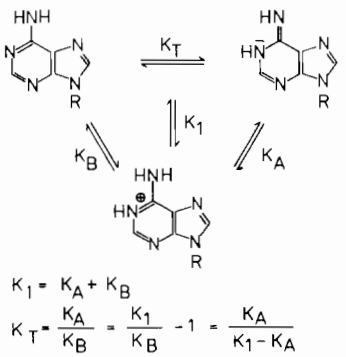


Fig. 3. Schematic representation of the 'method of basicity measurements' applied to N7 platinated adenines. K_A and K_B are the acidity constants of the corresponding Pt complexes of 1,9-DimeAH$^+$ and 6,9-DimeAH$^+$.

*Estimation of K_T by a different method [B] gives a larger difference in K_T values.

TABLE 5. pK_a values of dimethylated adenines and their Pt complexes and K_T values

Species	pK_a[a]	Reference	K_T[b]
9-MeAH$^+$	4.3	34	$10^{-4.8}$
1,9-DimeAH$^+$	9.1	[c]	
	9.1	21	
6,9-DimeAH$^+$	3.8	[c]	
Cl_3Pt(9-MeAH) (**1a**)	2.5	[c]	$10^{-4.5}$
Cl_3Pt(1,9-DimeAH) (**2a**)	7.0	[c]	
Cl_3Pt(6,9-DimeAH) (**3a**)	1.8	[c]	
cis-[Cl_2Pt(9-MeAH)(NH_3)]$^+$ (**1e**)	1.9	[c]	$10^{-4.6}$
cis-[Cl_2Pt(1,9-DimeAH)(NH_3)]$^+$ (**2c**)	6.5	[c]	
cis-[Cl_2Pt(6,9-DimeAH)(NH_3)]$^+$ (**3b**)	≈1.1	[d]	

[a]pK_a values correspond to pK_1 (9-MeAH compounds) and pK_a or pK_B (DimeAH compounds) used in eqns. (3) and (4); values ±0.05. [b]K_T defined as c[minor tautomer]/c[major tautomer]. [c]This work. [d]Estimated only.

Application of eqns. (3) and (4) to the platinated adenines reveals that physically meaningful K_T values are obtained only when pK_a values of 1,9-DimeA analogues of the 9-MeA compounds are used. With 6,9-DimeA analogues, negative K_T values are obtained. K_T is $10^{-4.5}$ and $10^{-4.6}$ for [Cl_3Pt(9-MeA)]$^-$ and *cis*-Cl_2Pt(9-MeA)(NH_3), respectively, with K_T defined as the ratio between concentration of minor to major tautomers. These results will be further discussed below.

Characterization of 1,9- and 1,6-DimeAH complexes

In order to unambiguously establish the composition of representative examples of the dimethylated adenine complexes and also to confirm the general way of preparation of Cl_3Pt(LH) complexes from K_2PtCl_4 and LH = substituted adeninium and of *cis*-$Cl_2Pt(NH_3)L$ from Cl_3Pt(LH) and NH_3, X-ray crystal structure analyses were carried out for *cis*-[Cl_2Pt(1,9-DimeAH)(NH_3)]Cl (**2c**) and Cl_3Pt(6,9-DimeAH)·H_2O (**3a**). The cation of **2c** and the neutral molecule **3a** are depicted in Figs. 5 and 6. Selected interatomic distances and angles of both compounds are listed in Tables 6 and 7.

The *cis*-geometry of **2c** confirmed expectations of a higher kinetic *trans* effect of Cl over N. We assume *cis* geometries also for **1e** and **2b** for the same reason. Pt–Cl distances in **2c** (2.321(3) and 2.283(3) Å) are significantly (9σ) different and possibly the consequence of the higher structural *trans* influence of NH_3 over the cationic adeninium ligand. Pt is markedly (0.24 Å) non-planar with the adeninium ring, a feature also observed in Pt(II) complexes containing neutral purine nucleobases, for example [42]. The 1,9-DimeAH plane and the Pt coordination plane are at a 76.9° angle. The relatively large errors of the structure determination of **2c** do not justify any detailed comparison of nucleobase bond lengths and angles with those of the ligand [1,9-DimeAH]Cl [43] or a Co(III) complex [44].

Except for the presence of a methyl substituent at N6, the structure of **3a** bears a close resemblance with that of the 9-MeA analogue reported by Terzis *et al.* [45]. In **3a**, the CH_3 group adopts the usual *trans* conformation relative to the C5–C6 bond, as in the case of the free ligand 6,9-DimeA [46] and related adenines [46b, 47], pointing away from the Cl_3Pt entity at N7. Although protons were not localized in **3a**, we can safely assume that it is the N1 site rather than

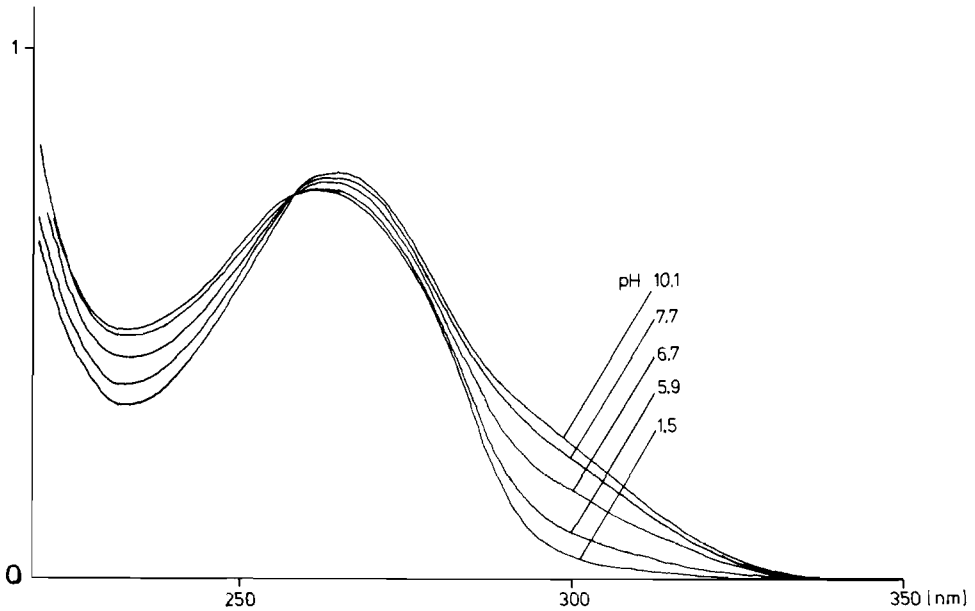


Fig. 4. pH dependent UV spectra of **2c** ($C_{Pt} = 1.2 \times 10^{-4}$ mol/l) against 0.1 M NaCl.

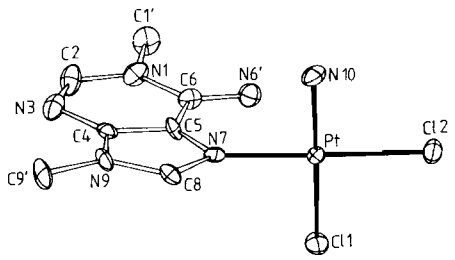


Fig. 5. Molecular cation *cis*-$[Cl_2Pt(NH_3)(1,9\text{-DimeAH})]^+$ of **2c**.

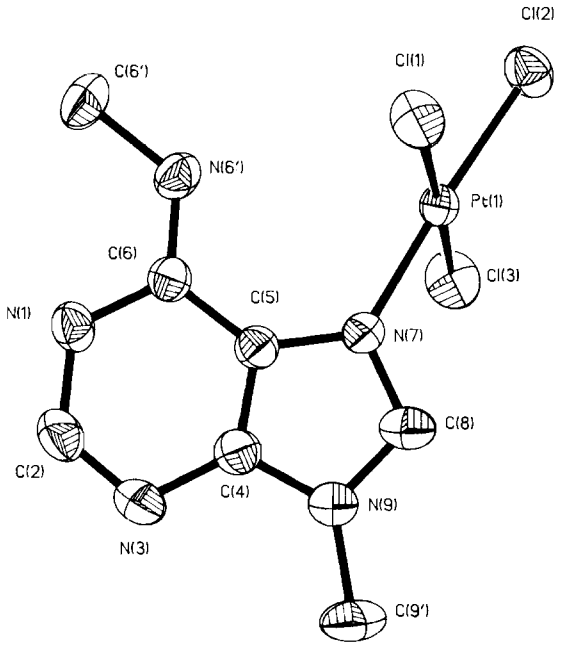


Fig. 6. The molecule $Cl_3Pt(6,9\text{-DimeAH})$ from **3a**.

N3 that carries the acidic proton: The criteria established for N1 protonated adenines [48] are clearly met. Specifically, the internal ring angle at N1, C2–N1–C6, of 124.4(6)° is markedly larger than that at N3, C2–N3–C4, which is 112.4(6)°, and close to values observed in 6-$MeAH^+$ [46], with the hydrogen located. Likewise, the expected lengthening of N1–C6 and shortening of C6–N6 are confirmed in **3a**. Pt–Cl distances are in the range found in other $PtCl_3$(heterocycle) compounds [45, 46–53]. We note that one of the Pt–Cl distances, to Cl1, is significantly (7.5σ) longer than the two others. Consistent with expectations [49], it is not the Cl *trans* to the protonated nucleobase.

As with **2c**, Pt is not coplanar with the 6,9-$DimeAH^+$ ring (deviation 0.12 Å). Both N6' (−0.12 Å) and C6' (−0.28 Å) are also markedly out of the best plane through the endocyclic atoms of the adeninium. The angle between the Pt coordination plane and the nucleobase plane is 66.9°. Except for a 2.81 Å hydrogen bond between N(1)H and the water molecule O10, there are no short H bonds in **3a**.

TABLE 6. Selected interatomic distances (Å), angles (°) and close contacts of **2c**

Pt1–Cl1	2.321(3)	Cl1–Pt1–Cl2	91.8(1)
Pt1–Cl2	2.883(3)	Cl1–Pt1–N10	178.8(3)
Pt1–N10	2.049(10)	Cl1–Pt1–N7	90.4(3)
N1–C1'	1.47(2)	Cl2–Pt1–N10	89.4(3)
N1–C2	1.40(2)	Cl2–Pt1–N7	177.7(3)
C2–N3	1.30(2)	N10–Pt1–N7	88.4(4)
N3–C4	1.36(1)		
C4–C5	1.38(2)		
C4–N9	1.35(1)		
C5–C6	1.41(2)		
C5–N7	1.39(1)		
C6–N1	1.39(1)		
C6–N6'	1.31(2)		
N7–C8	1.35(1)		
C8–N9	1.36(1)		
N9–C9'	1.48(2)		
Close contacts (<3.4 Å)			
N10–Cl3[1]	3.34	Pt1–N10–Cl3[1]	125
N10–Cl3[2]	3.31	Pt1–N10–Cl3[2]	103
N6'–Cl3[3]	3.09	C6–N6'–Cl3[3]	149

Symmetry operations: [1] = 1−x, 1−y, 1−z; [2] = 0.5+x, 0.5−y, 1−z; [3] = 1−x, −0.5+y, 1.5−z.

TABLE 7. Selected interatomic distances (Å), angles (°) and close contacts of **3a**

Pt1–Cl1	2.307(2)	Cl1–Pt1–Cl2	90.5(1)
Pt1–Cl2	2.286(2)	Cl1–Pt1–Cl3	178.1(1)
Pt1–Cl3	2.286(2)	Cl1–Pt1–N7	91.1(1)
Pt1–N7	2.008(5)	Cl2–Pt1–Cl3	90.9(1)
N1–C2	1.377(9)	Cl2–Pt1–N7	177.1(1)
C2–N3	1.286(9)	Cl3–Pt1–N7	87.5(2)
N3–C4	1.373(8)	C6–N1–C2	123.2(5)
C4–C5	1.351(9)	N1–C2–N3	124.4(6)
C5–C6	1.401(8)	C2–N3–C4	112.4(6)
C6–N6'	1.322(8)	N3–C4–C5	127.6(6)
N6'–C6'	1.492(9)	N3–C4–N9	124.4(6)
C6–N1	1.354(8)	C5–C4–N9	108.0(5)
C5–N7	1.382(8)	C4–C5–C6	117.7(6)
N7–C8	1.343(8)	C4–C5–N7	108.9(5)
C8–N9	1.367(9)	C6–C5–N7	133.3(6)
N9–C9'	1.488(10)	C5–C6–N1	114.4(5)
N9–C4	1.374(8)	C5–C6–N6'	124.2(5)
		N1–C6–N6'	121.4(5)
		C6–N6'–C6'	123.8(6)
		C5–N7–C8	106.3(5)
		C5–N7–Pt1	129.6(4)
		C8–N7–Pt1	123.9(4)
		N7–C8–N9	110.3(5)
		C8–N9–C4	106.5(5)
		C8–N9–C9'	125.9(6)
		C4–N9–C9'	127.5(6)
Close contacts			
Cl1–O10[1]	3.36	Pt1–Cl1–O10[1]	108
N1–O10[2]	2.81	C2–N1–O10[2]	101
Cl3–O10[3]	3.23	Pt1–Cl3–O10[3]	109
Cl2–N1[4]	3.32	Pt1–Cl2–N1[4]	90

Symmetry operations: [1] = −x, 1−y, 2−z; [2] = 1−x, 1−y, 2−z; [3] = 0.5−x, −0.5+y, 2.5−z; [4] = −0.5+x, 0.5−y, −0.5+z.

Positions of the IR bands in the 1500–1700 cm^{-1} region proved to be of diagnostic value as far as the protonation state of the various adenines was concerned. For example, the two intense 9-MeA bands at 1675 and 1600 cm^{-1}, which are assigned to $\delta(NH_2)$ and $\nu_{ring}+\delta(NH_2)$ modes [54], respectively, occur at *c.* 1645 and 1590 cm^{-1} in N7 platinated species of neutral 9-MeA, but at 1680 and 1610 cm^{-1} in platinated 9-MeAH. Similarly, in complexes containing protonated 1,9-DimeA (**2a, 2c**) the δNH_2 modes occur at higher wavenumbers (1670, 1680 cm^{-1}) than in the complex with neutral 1,9-DimeA (**2b**: 1645 cm^{-1}), and the same is true for the IR bands around 1600 (1,9-DimeAH$^+$ complexes) and 1560 (1,9-DimeA complexes) cm^{-1}. Finally, also the 6,9-dimethyladenine compounds displayed these differences in the IR, viz. 1630 and 1595 cm^{-1} in **3b**, but 1690 and 1620 cm^{-1} in **3a**.

Discussion

The spectrum of mutations caused by *cis*-DDP [14, 55] has recently been shown to be dominated by A→T transversions, resulting from binding of the *cis*$(NH_3)_2$Pt(II) moiety to N7 of a 5′-adenine base and N7 of guanine in a d(ApG) sequence [14]. It means that in the central base mispairing step, an adenine nucleobase is put across the 5′ base of the template strand in the newly synthesized DNA strand. Following the generally accepted picture of A→T transversion mutations [16] and assuming that pairing between the 'wrong' bases takes place, the adenine in the template strand should adopt a rare imino tautomer structure, thereby allowing mispairing with an adenine in the new strand. Based on this concept, we have applied the 'method of basicity measurements' for estimation of tautomer equilibria to N7-platinated adenines. We believe that this is the first approach of this kind using Pt or any other metal. At the outset of the work it was hoped that the study might yield a clear shift in the tautomer equilibrium of a N9-substituted adenine upon N7 platination, even though we did not employ *cis*-$(NH_3)_2$Pt(II) model nucleobase complexes, and specifically not the mixed adenine, guanine adduct. Both preparative (1,9-DimeA complexes) and spectroscopic difficulties (overlapping UV bands of mixed nucleobase compounds) were responsible for this limitation. Moreover, there appears to be not just an electronic effect of the metal responsible for mutagenicity in the case of Pt (lower mutagenicity of monofunctional dienPt(II) compounds), but possibly also a steric one.

Our data on $[Cl_3PtL]^-$ and *cis*-$Cl_2Pt(NH_3)L$ (L=9-MeA) complexes reveal at most a trend towards a shift in tautomer equilibrium. Differences in K_T are 0.2 to 0.3 log units, corresponding to factors of 1.6–2. Clearly, these values are not to be considered significant, even though values in the case of 5-bromouracil are only slightly higher.

The crucial question – "Is adenine opposite to the (5′-ApG)Pt lesion introduced at any time of the replication process and, except for the observed mutation frequency, efficiently repaired, or is the wrong base adenine introduced with a rare frequency, corresponding to the mutation frequency after repair?" – cannot be answered at present. The second possibility would point towards a mechanism involving a rare tautomer in the platinated template, as outlined above, while the first possibility could be related to steric distortion, with the platinated adenine possibly functioning as an apurinic site [19]. Preliminary data [56] on the ApG adduct of *cis*-DDP in a nonanucleotide duplex indicate a severe distortion of the base pair between the platinated A and T. On the other hand, molecular mechanics calculations of *cis*-DDP binding to an ApG fragment in a tetranucleotide duplex seem to indicate no drastic sterical changes [57]. It should be interesting to find out, how introduction of A instead of T in the complementary strand affects the structure and distortion of DNA.

A crucial problem in estimating the effect of metal binding on the tautomer distribution of a nucleobase, which limits the usefulness of any model study, has not been addressed as yet. It relates to the question of 'medium' of the nucleobase. It is well established for heterocycles in general and for adenines specifically [38], that the dielectric constant of the medium has a major influence on the tautomer equilibrium. Thus, a change in K_T measured in aqueous solution may or may not be relevant to native DNA, depending on the environment, that is accessibility of water or protein, respectively. In the case of steric distortion caused by *cis*-DDP, this aspect may be of prime importance.

Supplementary material

Observed and calculated structure factors, anisotropic thermal factors, equations of planes and deviations of atoms can be obtained from the Fachinformationszentrum Karlsruhe, D-7514 Eggenstein-Leopoldshafen 2 under CSD 56032 on request. Requests should be accompanied by the complete literature citation.

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