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Platinum(II)-bis(9-methyladenine) complexes; N1→N6 migration of Pt(II) *vs* deamination of coordinated methyladenine†

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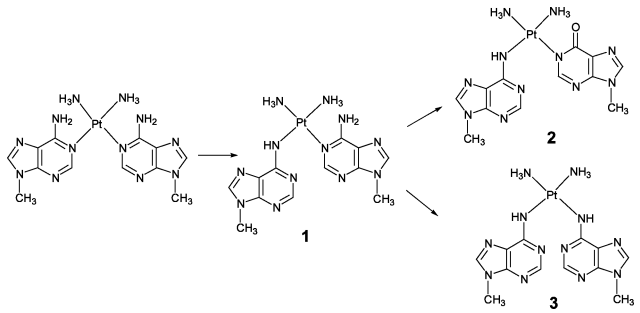
Stepwise migration of coordinated Pt(II) from the endocyclic N1 site to the exocyclic amino group occurs in the bis(9-methyladenine-N1) complex of *cis*-$Pt^{II}(NH_3)_2$ in basic solution, whereafter deamination of the 9-methyladenine still coordinated at N-1 competes with a second migration step.

Due to the general inertness of Pt(II) and Pt(IV) and the high thermodynamic stability of Pt–N bonds, the factors affecting the initial binding step of Pt to DNA are considered crucial for understanding the biological activity of anticarcinogenic Pt drugs.[1–3] In this respect, findings on relatively facile Pt–N bond rearrangements at the oligonucleotide level are of prime interest[4,5] since they may occur as soon as the platinated oligonucleotides are hybridised with their complementary ribo- or deoxyribonucleotide strands.[4] But the exact mechanism for this type of rearrangement is unknown rendering model studies in this field highly desirable. In some cases, Pt–N bond rearrangements have been reported in simple complexes within the nucleobase moiety[6] or within the auxiliary ligand.[7] A clear picture though, is not apparent regarding the factors controlling these migrations and they may even require the co-operation of other nucleophiles[5a] or involve Pt(IV) as an intermediate.[8]

Very recently, we have shown that in isomeric bis(9-methyladenine) complexes under basic conditions, coordinated Pt(II) undergoes an intramolecular N1→N6 or N7→N6 migration upon displacement of an NH_2 proton.[9] Subsequently, the product, *cis*-$[Pt(NH_3)_2(9\text{-made-N6})(9\text{-made-N7})]^{n+}$ (9-made = 9-methyladenine), undergoes a slow deamination reaction of the N7-bound 9-made to provide the corresponding hypoxanthine complex instead of a second migration step. Herein, we report that *cis*-$[Pt(NH_3)_2(9\text{-made-N1})_2]^{2+}$, also in aqueous alkali, first undergoes an intramolecular migration to give the N1,N6-bound species *cis*-$[Pt(NH_3)_2(9\text{-made-N1})(9\text{-made-N6})]^{n+}$ (**1**) which, after isolation, can then be slowly transformed into either the deaminated species *cis*-$[Pt(NH_3)_2(9\text{-made-N6})(9\text{-mhyp-N7})]^{n+}$ (**2**, 9-mhyp = 9-methylhypoxanthine), or the doubly-migrated species *cis*-$[Pt(NH_3)_2(9\text{-made-N6})_2]^{n+}$ (**3**, Scheme 1). The structural elucidations of **1–3** were based on a combination of NMR (symmetry arguments and by comparison to analogous complexes, see Table 1 and ESI†), MS, and chemical logic. In all cases, a ^{195}Pt NMR signal was observed at *ca.* −2,550 ±50 ppm, typical for an N_4-coordinated Pt species.[10]

Treatment of *cis*-$[Pt(NH_3)_2(9\text{-made-N1})_2]^{2+}$ (the ^1H NMR of which shows two interconverting sets of nucleobase signals[10]) with base produced **1**‡, for which the ^1H NMR displayed two pairs of interconverting sets of nucleobase signals in the ratio of *ca.* 2:1 for each of the interconverting sets. The exchange processes likely to be in effect are the oft-encountered restricted rotation about the Pt–N_{nucleo} bonds due to internal hydrogen bonding involving the non-complexed nitrogens of the nucleobases (*e.g.* N-1, N-6, or N-7) and suitable donor sources (*e.g.* NH_3), and/or the *syn*/*anti* interconversion about the N_6–C_6 bond in the adenine moiety.[9,10] Therefore, either two different bases were present in **1**, or a single base type was present but coordinated at different sites. But given the expectation based on our previous observations[6,9] and the ensuing results for **2** and **3** (*vide infra*), it was ascertained that the bis-complex where one 9-made ligand is coordinated at the N1 site and the other at the N6 site, was formed. From the assignment of H-8 (identifiable by its long-range correlation to the definitive C-5 which resonates in the vicinity of 120 ppm) in the N1-bound moiety



Scheme 1 Stepwise reaction of the starting material in basic solution, first to **1** and then onto either **2** or **3** (charges omitted for clarity).

† Electronic supplementary information (ESI) available: general experimental details and NMR data for various Pt-coordinated nucleobase ligands. See http://www.rsc.org/suppdata/cc/b3/b315987f/

Table 1 ^1H, ^{13}C, and ^{195}Pt NMR data (δ/ppm) for **1–3** in $D_2O/D_2O–H_2O$

	C-2/H-2	C-8/H-8	C_{Me}/H_{Me}	C-4	C-5	C-6
1, N1	156.35	147.11	32.97	150.49	121.20	158.01
major sp	8.812	8.182	3.816	Pt –2,580	NH_3s 4.380,	4.434
1, N1	156.82	146.96	32.97	150.49	120.86	158.01
minor sp	8.776	8.196	3.828	Pt –2,504	NH_3s 4.252,	4.265
1, N6	147.46	145.29	33.15	147.26	123.25	157.25
major sp	8.196	8.068	3.783		–	
1, N6	147.64	145.79	33.15	150.02	122.05	154.51
minor sp	7.968	8.273	3.884		–	
2,	147.19	145.40	33.01[a]	147.19	123.8[b]	157.19
N6-made	8.140	7.952[c]	3.747[a]		Pt –2,562	
2,	145.4[b]	157.71	32.86[a,c]	145.5[b]	120.6[b]	not obs
N1-mhyp	7.97[c]	8.409	3.733[a]		–	
3	147.38	145.52	33.04	146.0[b]	123.69	157.3[c]
	8.086[c]	8.018[c]	3.787[c]		Pt –2,603	

[a] Signals (as pairs) interchangeable. [b] δ measured indirectly. [c] Broad.

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(adjudged by analogy), followed the allocation of all of the signals to one nucleobase or the other based on HMBC and HMQC experiments. The assigned values of the N6-coordinated 9-made and the N1-coordinated 9-made were found to compare extremely well to literature values for similarly-coordinated 9-mades, thus lending support to the assigned structure.

In the ^{1}H NMR spectrum of **2**, two sets of nucleobase signals were present (four equal-intensity aromatic signals and two equal-intensity methyl signals), but it was determined that these two sets were not exchanging despite some signals being clearly exchange-broadened. Therefore, either two different bases were present in the complex or a single base type was present but site-coordinated differently in each base. Analysis by ESI$^+$–MS provided a recognisable isotope pattern (for an ion containing one platinum atom and a number of carbon and nitrogen atoms) for the "molecular ion" which began at *m*/*z* 526 amu—a value which is consistent with an N6-bound (deprotonated) 9-made moiety as one coordinated nucleobase and a 9-mhyp moiety as the other coordinated nucleobase. Consistent with this, the $[M - NH_3]^+$ and $[M - (NH_3)_2]^+$ ions provided similar isotopic patterns at the appropriate lower masses. Product ion analysis of selected ions (*e.g.* 526, 527 amu) provided ions of 150 ($[9\text{-made} + H]^+$) and 151 ($[9\text{-mhyp} + H]^+$) amu in approximately equal intensities, *i.e.* 9-made and 9-mhyp were both cleaved off the selected ions in stoichiometric amounts, a result which is consistent with thiourea treatment[9] (*vide infra*). Therefore, based on these results and consideration of the reaction conditions, it was concluded that the structure of **2** must be *cis*-$[Pt(NH_3)_2(9\text{-mhyp-N1})(9\text{-made-N6})]^{n+}$.

The assignment of the H-8 signals was made by comparison to literature values to identify them as belonging to either a 9-mhyp moiety or an N6-bound 9-made moiety. From then followed the allocation of all of the signals to one nucleobase or the other based on HMBC and HMQC experiments (except the methyl C–H signal pairs which are interchangeable between the bases as insufficient resolution precluded their firm allocation). The assigned values of the N6-coordinated 9-made were found to compare extremely well to literature values for similarly-coordinated 9-mades (and **1**), thus lending further support to the assigned structure. The lack of appropriate literature values precluded the firm confirmation of an N1-coordinated 9-mhyp, but those available did permit the exclusion of an N7-bound 9-mhyp moiety.[11] ^{1}H NMR spectra were also acquired at various temperatures (8.8, 25, 50, and 75 °C), revealing that more than one dynamic process was in effect as evidenced by the sharpening and then re-broadening (over and beyond that caused by deterioration in the field homogeneity) of H-8 in the 9-made moiety. At 75 °C, **2** rapidly decomposed.

In the ^{1}H NMR spectrum of **3**, only one set of nucleobase signals was present, albeit for exchanged-broadened signals, but nevertheless suggestive of the presence of only one base type, and same-site coordinated bases at that. The MS analysis was more complicated though as a clearly recognisable isotope pattern corresponding to an ion containing one platinum atom and additional carbon and nitrogen atoms could not be discerned for the molecular ion. The isotope pattern started at *m*/*z* 525 amu, as it should for a structure pertaining to $\{cis\text{-}[Pt(NH_3)_2(9\text{-made-N6})_2] + H\}^+$ (*i.e.* each adenine unit is deprotonated for complexation to the platinum atom but an additional H^+ is, by necessity, incorporated somewhere in the complex to yield an overall charge of +1); however, the ion intensities diverged from that expected, notably the ions alluding to the isotopes ^{194}Pt (smaller in intensity), [^{196}Pt + 1] (greater in intensity), and [^{198}Pt + 1] (greater in intensity). Due to this latter ion, it thus appeared that the pattern extended to higher mass then it otherwise should. This conundrum was resolved by the realisation that two isotope patterns were overlapped, *i.e.* two platinum-containing species were present in solution, or at least present in the gaseous phase, and which differed by one mass unit. The $[M + H - NH_3]^+$ and $[M + H - (NH_3)_2]^+$ ions also provided similarly distorted isotopic patterns. Thus, degradation of the sample was occurring upon standing and/or during development of the gaseous ions in the ESI source. MS analysis of an aged sample provided a spectrum that lacked the ion of *m*/*z* 525 amu altogether, and furthermore, displayed a standard-looking isotope pattern beginning at *m*/*z* 526 amu, thereby confirming that degradation did indeed occur upon standing in addition to any degradation that may occur in the ESI source. Product ion analysis was most illuminating: whilst ions of *m*/*z* 526 and 527 amu provided daughter ions at *m*/*z* values of 150 and 151 amu in approximately equal intensities, and therefore alluding to both 9-made and 9-mhyp being produced in equal amounts, the ion of *m*/*z* 525 amu, however, essentially only provided a daughter ion of *m*/*z* 150 amu, thus inferring that 9-made alone emanated from this ion, a result which is also consistent with thiourea treatment[9] (*vide infra*). Thus, based on these results and consideration of both the reaction conditions and the structure of **2**, it was concluded that the structure of **3** must be *cis*-$[Pt(NH_3)_2(9\text{-made-N6})_2]$.

The NMR signal assignments started from the HMBC correlations of the methyl protons to identify both C-4 and C-8 as the exchange broadening precluded the observation of any aromatic proton correlations, and together with the HMQC spectrum (for which all correlations were present), left only the assignment of C-6 and C-5 (widely separated) by default. The assigned values were in excellent agreement with the literature values for similarly coordinated 9-mades (and also **1** and **2**), thus lending further support to the assigned structure.

The slow decomposition of **3** at 25 °C was also evident by ^{1}H NMR as indicated by the appearance of additional signals in both the aromatic and methyl regions of the spectrum. Thus measurements at higher temperature were not performed whilst measurements at 6 °C only served to exacerbate the exchange broadening.

Notes and references

‡ *Synthetic procedure.* Treatment of *cis*-$[Pt(NH_3)_2(9\text{-made-N1})_2]^{2+}$ (as the diperchlorate)[10] in 10 mL of 0.05 M NaOH for 2 h at 65 °C afforded a solution of **1** (purity *ca.* 85%), which was passed through a preparative RP-18 column[9] to yield a chromatographically pure solution of **1**. This solution was further treated in 3 mL of 0.8 M NaOH for 4 h at 85 °C to provide a solution containing **2** (*ca.* 45%) and **3** (*ca.* 30%) as the main components. The reaction mixture was neutralised with perchloric acid and fractionated with an RP-18 column using 30% methanol in aqueous 0.1 M $NaClO_4$ as eluent. Refractionation of the combined fractions of **2** (and similarly for **3**) with 30% methanol in water was employed to remove excess electrolyte and to finally yield chromatographically pure (> 90%) solutions of **2** (and **3**). Compounds **1–3** were found to be extremely soluble in water at pH 6 irrespective of the counterion, whilst **2** and **3** tended to precipitate out at higher pHs. Treatment of a sample of **2** with thiourea provided three HPLC-detectable end products in approximately stoichiometric amounts, *viz.* $[Pt(tu)_4]^{2+}$, 9-made, and 9-mhyp, whilst **3** gave only $[Pt(tu)_4]^{2+}$ and 9-made, in a ratio of *ca.* 1:2. The end products were identified by co-elution with authentic samples.[9]

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