Platination of the Exocyclic Amino Group of the Adenine Nucleobase by Pt^{II} Migration

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Migration of coordinated Pt^{II} from the endocyclic N(1) site to the exocyclic nitrogen atom in 9-methyladenine occurs at high pH values. The resulting N(6)-platinated complex has been characterized by X-ray crystal structure analysis and by ^{1}H- and ^{195}Pt-NMR spectroscopy. The ^{1}H-NMR spectra show that the Pt^{II}(dien) unit at N(6) can adopt either a *syn* or an *anti* conformation with respect to the N(1) site, and that protonation of the ring nitrogen atoms N(1) and/or N(7) perturbs the *syn*/*anti* equilibrium.

The ability of Pt^{II} to form kinetically inert complexes with the base residues in DNA is crucial for the biological activity of various platinum anticancer drugs.[1][2] Although different Pt^{II} compounds show a clear preference for the guanine N(7) site followed by the adenine N(7) atom,[3] coordination to other sites cannot be excluded when assessing the active binding mode of Pt drugs. For example, the selectivity of the N(7) site most probably results from kinetic factors, since in 9-substituted purine nucleobases the Pt−N(1) bond seems to be thermodynamically more stable than the Pt−N(7) bond.[4] Because of the differing basicities of the purine nitrogen atoms, the distribution of Pt^{II} between the N(1) and N(7) sites can be affected by (de)protonation of the purine nucleobases.[3][5] When coordination to these sites is hindered, Pt^{II} binding to N(3) may be observed,[5][6] but only in very rare cases is platination of the exocyclic amino or oxo groups of 9-substituted purines observed.[5] For example, coordination of Pt^{II} to C(6)−NH_2 in adenosine and 2′-deoxyadenosine has been proposed with 4-picoline(2,2′:6,2′′-terpyridine)platinum(II), but only *after* the initial N(1) platination.[7] There are also a few reported cases of structurally characterized complexes exhibiting N(6) binding mode with other metal ions.[8] Hence, in the search for unusual Pt^{II} binding patterns we report here the preparation, X-ray crystal structure determination, and NMR-spectroscopic characterization of the model nucleobase 9-methyladenine (9-made) bearing a Pt^{II}(dien) unit (dien = diethylenetriamine) at N(6).

In general, metal-ion binding to the exocyclic amino group of the adenine moiety requires proton abstraction from the NH_2 group and it is the high pK_a value (ca. 17) of the NH_2 group which makes direct complexation at this site very difficult.[5][8] However, it is known that coordination of electrophilic Pt^{II} to the adenine N(1) and N(7) sites lowers the pK_a value of the exocyclic NH_2 group by ca. 4 log units.[5] In addition, the N(6) atom seems to be spatially quite close to Pt^{II} in N(1)-platinated adenine derivatives.[4b] These considerations prompted us to employ an indirect strategy to accomplish Pt^{II} binding to the N(6) site, i.e. via the N(1)-platinated species. Although the reaction of aquated Pt^{II}(dien) with an excess of 9-made initially gives the N(1)-bound complex as the minor species, a slow N(7) → N(1) isomerization takes place at elevated temperatures analogous to that found earlier with adenosine.[4b] The employment of [Pt(dien)(9-made-*N*1)]$(ClO_4)_2$ (**1**) as the starting material enables the facile preparation of the N(6)-platinated species, [Pt(dien)(9-made-*N*6)]$(ClO_4)_2$ (**2**), in strongly basic solution.

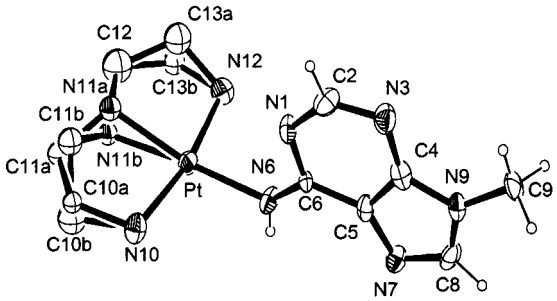

Figure 1. Structure of the cation of [Pt(dien)(9-made-*N*6)]$(ClO_4)_2$; selected interatomic distances [Å] and angles [°] with estimated deviations in parentheses: Pt−N(6) 2.010(18), Pt−N(10) 2.079(17), Pt−N(11a) 2.09(4), Pt−N(11b) 2.04(3), Pt−N(12) 2.081(18), N(6)−C(6) 1.30(3), N(6)−Pt−N(11a) 167.0(14), N(6)−Pt−N(11b) 171.5(12), N(6)−Pt−N(10) 98.8(8), N(6)−Pt−N(12) 92.8(8), N(10)−Pt−N(12) 168.3(6), C(6)−N(6)−Pt 128.9(13)

The X-ray crystal structure of **2** confirms Pt^{II} binding to the deprotonated amino group (Figure 1).[9] Comparison with **1** reveals that the N(1) → N(6) migration of Pt^{II} does not substantially change the nucleobase geometry. Since **2** was isolated at pH = 6, the nucleobase moiety of the dication is expected to bear a proton.[10] Unfortunately, unambiguous assignment of the protonation site was not forthcoming from the X-ray data. Although all the ring nitrogen atoms N(1), N(3), and N(7) display nonbonded contacts, only the N(1) site seems to act as a donor [N(1)···O(13a) 2.83(6) Å, N(1)···O(14b) 3.16(4) Å].[11] It is worth noting that the distance of 3.96 Å between N(1) and N(12) does not indicate an intramolecular H bond in *dicationic* **2**, even

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though this interaction may be present in the *syn* conformer of deprotonated **2** (vide infra). These findings suggest that it is the N(1) site that bears the required proton, as was also observed in the N(6)-bound Hg^{II} complex.[8b] In fact, both dicationic **2** and the Hg^{II} complex[8b] represent a metalated form of the rare imino tautomer of the adenine nucleobase. However, the internal ring angle C(2)−N(1)−C(6) does not unambiguously verify N(1) as the protonation site[8b] since the value of 122.8(16)° found for **2** has only increased by 2.2σ[12] from that in free 9-made,[13] viz. 118.7(1)°. For comparison, in the N(6)-bound Hg^{II} complex the C(2)−N(1)−C(6) angle [126(1)°] increases by 7.3σ upon N(1) protonation.[8b]

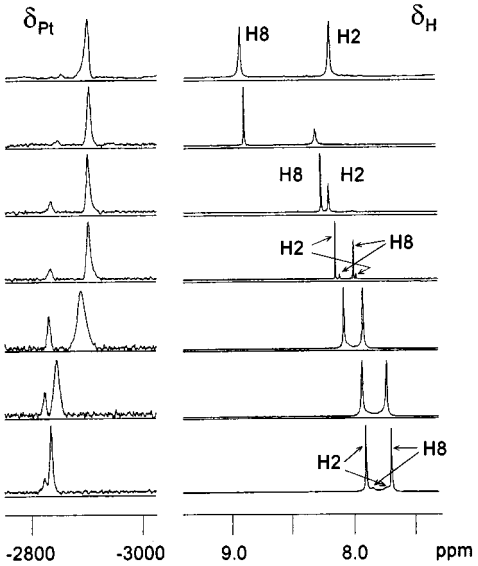

Figure 2. Sections of the ^{195}Pt- and ^{1}H-NMR spectra of **2** in D_2O/water solutions at different pH values (from bottom to top: 12.6, 8.5, 7.4, 6.0, 1.4, 0.6, ca. 0); the assignments of H(2) and H(8) are based on long-range C−H correlations (major conformer) and exchange correlations (minor conformer)

Both, ^{195}Pt- and ^{1}H-NMR spectra show pH-dependent dynamic processes for **2** (Figure 2),[14] which may be attributed predominantly to the restricted rotation about the C(6)−N(6) bond.[4b] Thus, the Pt^{II}(dien) unit may adopt either a *syn* or an *anti* orientation with respect to the N(1) site.[8b] In the deprotonated (monocationic) **2**, the major *syn* conformation is concomitant with an apparent H bond formed between dien−NH_2 and the N(1) site bearing a (partial) negative charge resulting in a stronger deshielding of H(2) relative to H(8). In the minor *anti* conformer, the reversal of the H(2) and H(8) chemical shifts may be attributed to H-bond formation between dien−NH_2 and the N(7) site. With decreasing pH value all of the ^{1}H signals shift downfield suggestive of an effect from both protonation and a shift in the conformational equilibrium. In the formation of dicationic **2**, N(1) protonation in the major species eliminates the H bonding from dien−NH_2 to N(1) and may cause a shift in the *syn* ⇌ *anti* equilibrium. As a consequence, both H(2) and H(8) are shifted downfield in the major species, the former due to protonation and the latter due to H bonding. Because two sets of aromatic protons are again detected for dicationic **2**, also with a reverse of the order of H(2) and H(8) chemical shifts in the minor conformer, there seems to be, in addition to the restricted rotation about the C(6)−N(6) bond, also a concomitant prototropic tautomeric equilibrium in effect between N(1) and N(7). The larger downfield shift of H(8) over H(2) in the minor species of the dicationic **2** suggests that in this case it is the N(7) site which is protonated with the Pt^{II}-(dien) unit in the *syn* conformation. This is implied by the comparable chemical shifts for H(2) in the monocationic, major species and in the dicationic, minor species, where in both cases H bonding can be in effect between dien−NH_2 and the N(1) site, and for H(8) in the monocationic, minor species and in the dicationic, major species where similarly in both cases H bonding can be in effect between dien−NH_2 and the N(7) site. Unfortunately, we were unable to independently confirm the proposed conformations. Further acidification down to pH < 0 results in a marked downfield shift only for H(8) suggesting that the second protonation occurs predominantly at N(7). Although the *syn* and *anti* conformers are no longer resolvable in the aromatic protons, the two species remain discernible in the ^{195}Pt-NMR spectra.

Two mechanistic explanations may be given for the adenine N(1) → N(6) isomerization, both of which require deprotonation of the C(6)−NH_2 group. First, the migration of Pt may be analogous to the Dimroth rearrangement, in which an alkyl group migrates from a heterocyclic nitrogen atom to an α-amino or α-imino group.[15] Alternatively, the imino group at C6 may directly attack Pt^{II} in the starting compound resulting in a 5-coordinate intermediate, where cleavage of the expectedly weaker Pt−N(1) bond gives the N(6)-bound species. It is worth noting that in **1** the distance between N(6) and Pt^{II} is 3.19(1) Å, very similar to that found in the corresponding adenosine complex, viz. 3.25 Å (unit A) and 3.16 Å (unit B).[4b] At this stage both explanations are feasible, although the proposed simultaneous binding of Pt to the endocyclic and exocyclic N atoms in pyrimidine complexes[16] lends support to the latter explanation. We note, however, that the bond rearrangement in the adenine moiety must still be mechanistically different from that in the pyrimidine complex, since the latter occurs in acidic solution via Pt^{IV}, while the adenine N(1) → N(6) migration proceeds in strongly basic solution without any detectable redox reaction

Experimental Section

[Pt(dien)(9-made-*N*1)](ClO$_4$)$_2$ (1): A mixture of aquated Pt^{II}(dien) {1.0 mmol, prepared by treating [PtI(dien)]I with 1.9 equiv. of $AgClO_4$[17]} and 9-made[18] (2.0 mmol) in 15 mL of water was stirred for 7 d at 75−85°C. According to HPLC analysis the initial N(1)/N(7) ratio of 2:3 increased to 2:1 during this treatment. After concentration to ca. 5 mL, the pH of the solution was adjusted to 1.5 using 1.0 M $HClO_4$. The addition of $NaClO_4$ (4 mmol) afforded 460 mg of crystalline product. Recrystallization from 0.1 M $HClO_4$ gave clear prisms of [Pt(dien)(9-made-*N*1)](ClO$_4$)$_2$ (**1**). Based on HPLC analysis, the product was analytically pure. The crystals were washed with cold water and air-dried; yield 220 mg (45% from

Pt). The N(1)-binding mode in **1** was confirmed by X-ray crystal structure analysis.[19][20]

[Pt(dien)(9-made-*N*6)](ClO$_4$)$_2$ (2): For the preparation of **2**, 71 mg (0.11 mmol) of **1** was dissolved in 5 mL of 0.1 M NaOH. The solution (pH = 12.8) was stirred for 3 h at 60−65°C after which the pH value was adjusted to 6.5 by using 1 M $HClO_4$. The solution was concentrated to ca. 0.5 mL, and left at 4°C to afford [Pt(dien)(9-made-*N*6)](ClO$_4$)$_2$ (**2**) as thick, clear plates or prisms; yield 55 mg (77%). Satisfactory elemental analysis (C, H, N) was obtained. − $C_{10}H_{20}Cl_2N_8O_8Pt$ (646.33): calcd. C 18.58, H 3.12, N 17.34; found C 18.65, H 3.18, N 17.21.

Acknowledgments

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[1] S. L. Bruhn, J. H. Toney, S. J. Lippard in *Progress in Inorganic Chemistry*, vol. 38 (Ed.: S. J. Lippard), Wiley, New York, **1990**, p. 477−516.
[2] J. Reedijk, *Chem Commun*. **1996**, 801−806.
[3] J. Arpalahti in *Metal Ions in Biological Systems*, vol. 32 (Eds.: A. Sigel, H. Sigel), Marcel Dekker, New York, **1996**, p. 379−395.
[4] [4a] R. B. Martin in *Platinum, Gold and Other Metal Chemotherapeutic Agents* (Ed.: S. J. Lippard), ACS Symposium Series 209, American Chemical Society, Washington DC, **1983**, p. 231−244. − [4b] J. Arpalahti, K. D. Klika, R. Sillanpää, R. Kivekäs, *J. Chem. Soc., Dalton Trans.* **1998**, 1397−1402.
[5] B. Lippert in *Progress in Inorganic Chemistry*, vol. 37 (Ed.: S. J. Lippard), Wiley, New York, **1989**, p. 1−97.
[6] C. Meiser, B. Song, E. Freisinger, M. Peilert, H. Sigel, B. Lippert, *Chem. Eur. J.* **1997**, *3*, 388−398.
[7] G. Lowe, T. Vilaivan, *J. Chem. Soc., Perkin Trans. 1* **1996**, 1499−1503.
[8] [8a] L. Y. Kuo, M. G. Kanatzidis, M. Sabat, A. L. Tipton, T. J. Marks, *J. Am. Chem. Soc*. **1991**, *113*, 9027−9045. − [8b] F. Zamora, M. Kunsman, M. Sabat, B. Lippert, *Inorg. Chem*. **1997**, *36*, 1583−1587. − [8c] See also: M. J. Clarke, *J. Am Chem. Soc*. **1978**, *100*, 5068−5075.
[9] Crystal structure analysis of **2**: $C_{10}H_{20}Cl_2N_8O_8Pt$ (M_r = 646.33), orthorhombic, space group *Pna*21 (no. 33), a = 18.652(2), b = 11.3199(12), c = 9.4299(7) Å, V = 1991.0(4) Å^3, Z = 4, $\rho_{calcd.}$ = 2.156 g cm^{-3}, F(000) = 1248; Rigaku AFC5S diffractometer, ambient temperature, Mo-K_α radiation (λ = 0.71069 Å), μ = 7.373 mm^{-1}, ω/2θ scan technique with 4.2° ≤ 2θ ≤ 50.04°, absorption correction: ψ scan (T_{min}/T_{max} = 0.51/1.00), 1874 measured reflections and 1873 observed reflections with $I > 2\sigma(I)$ (248 parameters) gave $R1$ = 0.0426 and $wR2$ = 0.0915, $R1$ = 0.0838 and $wR2$ = 0.1033 (all data), max/min residual electron density: 0.732/−1.185 eÅ^{-3}. The structure was solved by standard Patterson and difference Fourier methods and refined by full-matrix least-squares calculations on F^2 by employing SHELXL-97.[21] In the base moiety, the NH hydrogen atom was found from the electron density map and was refined, whilst the CH_3 and ring hydrogen atoms are at calculated positions. The dien hydrogen atoms were not included due to the disorder of the dien ligand. The perchlorate oxygen atoms also suffered disordering.[20]
[10] p$K_{\alpha1}$ = 1.2 ± 0.1, p$K_{\alpha2}$ = 7.65 ± 0.05 determined at 298.2 K in 0.1 M $NaClO_4$ using the potentiostatic technique described earlier, see: H. Lönnberg, J. Arpalahti, *Inorg. Chim. Acta* **1980**, *55*, 39−42.
[11] Shortest nonbonded contacts for N(3) and N(7) in **2**: N(10)···N(3^{i}) 3.31(3) Å, N(11a)···N(7^{ii}) 3.44(5) Å; symmetry operations: i) 0.5 − x, y − 0.5, z − 0.5, ii) 0.5 − x, 0.5 + y, z − 0.5.
[12] σ defined as $\sigma = (\sigma_1^2 + \sigma_2^2)^{1/2}$ with σ_1 and σ_2 being the errors in angles that are compared.[8b]
[13] T. J. Kistenmacher, M. Rossi, *Acta Crystallogr*. **1977**, *B33*, 253−257.
[14] The minor species is well resolved only at pD = 6 {broad signals at pD = 12: δ = 7.72 [H(2)], 7.88 [H(8)]; very broad, partially unresolved signals at pD = 8.5 and 7.4}. The NMR measurements were carried out as previously described.[4b]
[15] T. Fujii, T. Itaya, *Heterocycles* **1998**, *48*, 359−390.
[16] [16a] F. Pichierri, D. Holtenrich, E. Zangrando, B. Lippert, L. Randaccio, *J. Biol. Inorg. Chem*. **1996**, *1*, 439−445. − [16b] J. Müller, E. Zangrando, N. Pahlke, E. Freisinger, L. Randaccio, B. Lippert, *Chem. Eur. J.* **1998**, *4*, 397−405.
[17] J. Arpalahti, P. Lehikoinen, *Inorg. Chem*. **1990**, *29*, 2564−2567.
[18] H. Lönnberg, J. Ylikoski, J. Arpalahti, E. Ottoila, A. Vesala, *Acta Chem. Scand.* **1985**, *A39*, 171−180.
[19] F. Schwarz, B. Lippert, H. Schöllhorn, U. Thewalt, *Inorg. Chim. Acta* **1990**, *176*, 113−121.
[20] Crystallographic data (excluding structure factors) for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication no. CCDC-113725 (**1**) and -113726 (**2**). Copies of the data can be obtained free of charge on application to CCDC, 12 Union Road Cambridge, CB2 1EZ, UK [Fax: + 44-1223/336-033; E-mail: deposit@ccdc.cam.ac.uk].
[21] G. M. Sheldrick, *SHELXL 97, Program for the Refinement of Crystal Structures*, University of Göttingen, **1997**.

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