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Expanding the pH Range of Metal−Nucleobase Complexes for Acid−Base Chemistry: Properties of Bis(guanine) Complexes of (bpy)Pt^{II} with Either Two Major or Major and Minor Tautomers Bonded Simultaneously

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Metal nucleobase complexes provide a potential for acid−base chemistry in the physiological pH range and consequently may contribute or be actively involved in catalytic reactions of nucleic acids, notably of RNAs. Expansion of the available pH range is achieved if additional ligands are involved, for example, an aqua ligand or a second nucleobase, and if relevant pK_a values are sufficiently close. Two bpy's (bpy = 2,2′-bipyridine) containing Pt^{II} complexes have been studied in this context: [Pt(bpy)(9-MeGH-*N7*)$_2$](NO$_3$)$_2$·H$_2$O (**2**) and Pt(bpy)(9-EtG-*N7*)(9-EtG-*N1*)·3H$_2$O (**3′**) (with 9-MeGH = 9-methylguanine; 9-EtGH = 9-ethylguanine). Relevant pK_a values, as determined by pD-dependent ^{1}H NMR spectroscopy in D$_2$O, of the neutral guanine ligands were found to be ca. 7.78 ± 0.01 and 8.38 ± 0.01 for **2**, yet 4.00 ± 0.03 and 7.7 ± 0.1 for **3′** (values converted to H$_2$O) for each of the two guanine ligands. These values suggest that complex **3′** provides a pH range of roughly 4−8 for potential acid−base chemistry, and furthermore that in favorable cases compounds with two ionizable ligands can function as an acid and a base simultaneously. X-ray crystal structures of both **2** and **3′** are presented and, in addition, that of [Pt(bpy)(9-EtGH-*N7*)$_2$](NO$_3$)$_2$·(9-EtGH)·5H$_2$O (**2′′**). Regarding the use of ^{1}H NMR spectroscopy for the determination of pK_a values, we note that chemical shifts referenced to sodium 3-(trimethylsilyl)propanesulfonate must be treated with caution when applying cationic complexes because of the possibility of ion pairing. It can lead to mistakes in chemical shift values.

Introduction

Functional groups of biomolecules with inherent pK_a values close to 7 or with pK_a values "shifted" into this range through environmental influences are prone to become engaged in acid−base catalysis.[1] The imidazole ring of a histidine residue in a protein is an example of the first case,[2] and cytosine or adenine moieties in ribozymes, displaying pK_a values of ca. 6−8, are typical examples of shifted pK_a values.[3] We have long been interested in effects of metal ions on pK_a shifts of nucleobases and have recently summarized available findings.[4] In brief, metal coordination to a neutral nucleobase in its major tautomeric form acidifies nucleobase protons, whereas metal binding to a deprotonated nucleobase causes an apparent increase in basicity. If the metalated nucleobase is present in a rare tautomeric structure, the macroscopic pK_a value is strongly perturbed. For example, the model nucleobase 1-methylcytosine in its aminooxo tautomer structure has a pK_a of close to 17 (deprotonation of exocyclic amino group),[5] yet an estimated pK_a of 12.7 in its rare iminooxo tautomer structure (with deprotonation of N3H).[6] If a metal is coordinated to the exocyclic N4 position of this minor tautomer, the pK_a approaches the value of the physiological pH![7]

We have been interested in strategies used by metal ions to expand the available pH range. This is the case, for example, if a metal ion carries *two* ligands engaged in acid−base equilibria. The addition of a second identical nucleobase bonded in an identical fashion to the metal ion usually does not have a large effect.[8,9] In fact, frequently the pK_{a1} and pK_{a2}

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(7) See, e.g.: (a) Sanz Miguel, P. J.; Lax, P.; Lippert, B. *J. Inorg. Biochem.* **2006**, *100*, 980. (b) Pichierri, F.; Holthenrich, D.; Zangrando, E.; Lippert, B.; Randaccio, L. *J. Biol. Inorg. Chem.* **1996**, *1*, 439.
(8) For guanine complexes (1:1, 2:1), see: Griesser, R.; Kampf, G.; Kapinos, L. E.; Komeda, S.; Lippert, B.; Reedijk, J.; Sigel, H. *Inorg. Chem.* **2003**, *42*, 32.
(9) For 1-methyluracil complexes of Pt^{II}, an effect of the stoichiometry (1:1, 2:1, 4:1) is seen: Holland, L.; Shen, W.-Z.; Micklitz, W.; Lippert, B. *Inorg. Chem.* **2007**, *46*, 11356.

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values are very close, and in titration curves, the two steps overlap. There are exceptions, however.[10]

Another way of expanding the pK_a range is to combine two different ligands, for example, a nucleobase and an aqua ligand. For example, *cis*-[Pt(NH_3)$_2$(9-MeGH-*N7*)(H_2O)]$^{2+}$ (9-MeGH = 9-methylguanine) has two pK_a values of 6.2 (aqua ligand) and 8.7 (guanine nucleobase),[11] hence providing an overlapping pH range.

Here, we report on yet another strategy to expand the pH range available for acid−base chemistry, namely, to bind two *identical* nucleobases in *two different* ways to a single metal. Two distinctly different pK_a values are to be expected if the nucleobases are bonded to the same metal ion as two different tautomers. To this end, we have synthesized and crystallized Pt(bpy)(9-EtG-*N7*)(9-EtG-*N1*) (**3′**) and have studied its acid−base equilibria. In the fully protonated state, [Pt(bpy)(9-EtGH-*N7*)(9-EtGH-*N1*)]$^{2+}$, the N7 bonded guanine adopts the preferred tautomeric structure, whereas the N1 bonded guanine, which carries the proton at N7, represents a rare tautomer structure ("metal-stabilized rare tautomer"[12]). We have previously described two Pt^{II} complexes containing the major and the minor tautomers of the model nucleobase 1-methylcytosine (1MeC) bonded simultaneously to the metal, *trans*-Pt(1-MeC-*N3*)(1-MeC-*N4*)X_2 (X = Cl, I).[13] As the relevant pK_a values of Pt(1-MeC-*N3*) (ca. 13) and Pt(1-MeC-*N4*) (ca. 7 − 8) differ by more than 5 log units, and because of the high pK_a of the N3-bonded cytosine, the idea of expanding the pH range for possible acid−base chemistry in biological systems is probably not reasonable with this combination of nucleobases.

In this manuscript, we report, in addition to the X-ray crystal structures of **2** and **3′** also that of a closely related compound, [Pt(bpy)(9-EtGH-*N7*)$_2$](NO_3)$_2$·(9-EtGH)·5H_2O (**2″**). However, we will not deal in detail with other topical aspects of $(bpy)Pt^{II}$ chemistry such as retromodels of cisplatin-DNA adducts using bpy or o-phen (o-phen = *ortho*-phenanthroline) ligands instead of $(NH_3)_2$,[14] mechanistic studies (e.g., cyclometalation[15]), or photophysical work,[16] to name a few.

Results and Discussion

Reactions of $(bpy)Pt^{II}$ with Guanine Model Nucleobases. Despite its wide use in coordination chemistry, there appear to be no X-ray structurally established cases of $(bpy)Pt^{II}$ complexes with nucleobases.[17] With the related $(bpy)Pd^{II}$, a limited number of examples with pyrimidine nucleobases exist.[18,19] Dinuclear motifs with stacking of two $(bpy)Pd^{II}$ units are a common feature among these compounds.

The reaction of Pt(bpy)Cl_2 and its aquated species with 9-methylguanine (9-MeGH) in water yielded two main compounds, [Pt(bpy)(9-MeGH-*N7*)Cl]Cl (**1**) and [Pt(bpy)(9-MeGH-*N7*)$_2$](NO_3)$_2$·H_2O (**2**). Compounds **1** and **2** were unambiguously identified by ^{1}H NMR spectroscopy and elemental analysis, and **2** in addition by single-crystal X-ray analysis. The ^{1}H NMR spectrum of **1** in D_2O, pD 4.92 (Figure 1), displays four highly diagnostic bpy resonances, namely, two H5 doublet-of-doublet-of-doublets centered at 7.47 and 7.75 as a consequence of two different ligands bonded to the $(bpy)Pt^{II}$ chelate,[20] a strongly downfield shifted H6′ doublet-of-doublet opposite the Cl ligand (c.f. Figure 1), and an upfield shifted H6 resonance. The H6′ resonance at the same time displays ill-resolved ^{195}Pt satellites ($^3J \sim 30$ Hz). The upfield shift of H6 is a consequence of roughly orthogonal orientation of the 9-MeGH-*N7* ligand and its ring current and is commonly observed in these kinds of compounds.[14d,20]

Reactions of [Pt(bpy)(9-MeGH-*N7*)(D_2O)]$^{2+}$, obtained in situ from **1** upon treatment with $AgNO_3$ (2 equiv) in D_2O, or of **1** directly with free 9-MeGH were carried out in D_2O at different pD values and followed by ^{1}H NMR spectroscopy. In an acidic medium, for example, pD 2.8, essentially [Pt(bpy)(9-MeGH-*N7*)$_2$]$^{2+}$ forms, as verified by a comparison of ^{1}H NMR spectra of an authentical sample of **2**. Reactions carried out at alkaline pD (ca. 8−10) gave more complicated spectra. Variations in pD of such samples allowed the identification of the guanine-H8 resonance of the bis(9-methylguanine) complex **2** and, in addition, of a species later assigned to Pt(bpy)(9-MeG-*N7*)(9-MeG-*N1*) (**3**), following the crystallographic characterization of the corresponding 9-ethylguanine complex Pt(bpy)(9-EtG-*N7*)(9-EtG-*N1*)·3H_2O (**3′**) (vide infra).

Crystal Structure Analysis and ^{1}H NMR Spectrum of [Pt(bpy)(9-MeGH-*N7*)$_2$](NO_3)$_2$·H_2O (2). A view of the cation of [Pt(bpy)(9-MeGH-*N7*)$_2$](NO_3)$_2$·H_2O (**2**) is given in Figure 2. Selected interatomic distances and angles are listed in Table 1. The two 9-MeGH bases are bonded to Pt via the N7 positions and adopt a *head−tail* arrangement. Consequently, the cation is chiral, with both enantiomers present in the crystal lattice. Pt−N distances are normal, but angles about the Pt show the expected variations as a consequence of the small bite angles of the bpy ligand. Surprisingly, Pt is out of the bpy plane by 0.15 Å, but well within the N_4 plane (0.01 Å). Both planes, bpy and the Pt−N_4, define an angle of 5.5°.

Dihedral angles between the Pt coordination planes and the planes of the two guanines are almost identical, 83.5° (9-MeGH_a) and 83.2° (9-MeGH_b), with an angle of 81.4° between the two guanine nucleobases. Thus, Pt−O6 apical contacts are similar for both guanines (Pt1···O6a, 3.525(4) Å; Pt1···O6b, 3.479(4) Å). Symmetry-related cations form dimers through bpy stacking (3.41Å; Supporting Information). In addition, 9-MeGH_a ligands form

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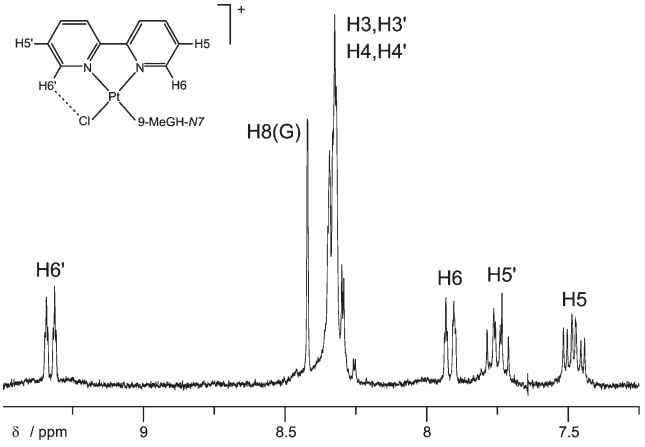

Figure 1. Low-field portion of ^{1}H NMR spectrum of **1** in D_2O, pD 4.92.

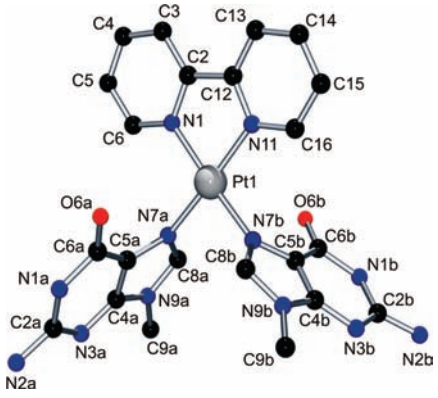

Figure 2. View of cation of **2** with atom numbering scheme.

pairs through 2-fold hydrogen bonds between N2 and N3 sites (2.977(7) Å) at the sugar edge, as frequently seen in solid-state structures of self pairs of N7 platinated guanine residues (Figure 3).[21] The Watson−Crick edges of 9-MeGH$_a$ ligands are blocked by a hydrogen-bonded nitrate anion. Similarly, the Watson−Crick edge of 9-MeGH$_b$ is hydrogen-bonded to the second nitrate anion. This situation is reminiscent to that previously reported for [Pt(en)(9-EtGH-*N7*)$_2$](NO$_3$)$_2$ (aq).[22] In addition, the water molecule of crystallization is hydrogen-bonded to the O6 site of the 9-MeGH$_a$ (2.916(7) Å) and to a nitrate anion (O1w(H)···O21, 2.887(6) Å).

The ^{1}H NMR spectrum of **2** is relatively simple (Supporting Information). The bpy resonances (D_2O, pD 3.6) occur at 7.58 (H5, t), 7.82 (H6, d), 8.45 (H3, d), and 8.39 (H4, t). As with [Pt(bpy)(9-MeGH-*N7*)Cl]Cl (**1**), the H6 resonance is at a high field, sensing the ring current of the guanine bases. Distances between H6 and 9-MeGH rings are 2.95 and 3.13 Å (in **2**) and 2.96 and 3.01 Å (**2′′**).

There are also two resonances of low intensity (CH_3 resonance at 3.78 ppm, bpy doublet centered at 7.96 ppm; pD 2.4), which neither fit to free 9-MeGH, [Pt(bpy)(9-MeGH-*N7*)Cl]$^+$, nor [Pt(bpy)(9-MeGH-*N7*)(D_2O)]$^{2+}$. Therefore, we tentatively assigned these resonances to a second rotamer of **2**, possibly the *head−head* rotamer of **2**.[23,24] However, none of the other expected resonances due to the minor rotamer are observed, presumably because of signal overlap.

The pD-dependent spectra of **2** in D_2O were recorded in order to determine the acidities of the two guanine ligands (Figure 4). Both the H8 resonance and the CH_3 resonance of the methyl group of the major rotamer were followed. Only a single pK_a value for deprotonation of the two guanine ligands was obtained, at 8.08 ± 0.01 (converted to water, c.f., Experimental Section; mean value of 8.09 for H8 resonance and 8.06 for CH_3 resonance). The value suggests two successive pK_a values of ≤7.78 and ≥8.38, which are virtually identical to those of *cis*-[Pt(NH$_3$)$_2$(9-EtGH-*N7*)$_2$]$^{2+}$.[8] This is somewhat surprising in that the bpy ligand in **2** could have been expected to have a marked acidifying effect on the guanine ligands due to its π-acceptor properties,[25] exceeding that of the ammonia ligands. The free nucleobase deprotonates with a pK_a of 9.56.[26]

We wish to point out a problem we faced during the NMR spectroscopic determination of the pK_a values of the guanine ligands in **2**. Initially, we obtained a titration curve which was analyzed in terms of two distinct deprotonation steps, giving pK_a values of 6.4 ± 0.2 and 8.25 ± 0.05. What struck us was the relatively minor change in chemical shifts of the first deprotonation step ($\Delta\delta$ for H8 ~ 0.04 ppm), which we interpreted in terms of formation of hemideprotonated guanine pairs,[27−29] which could have been expected to minimize the effect on H8 shifts. It was the critical comment of a reviewer, who suggested confirmation of the assumption by studying the concentration dependence as a function of pH, which led us to repeat all of the NMR experiments. They showed that our initial conclusions were erroneous. As it turned out, the anion of the reference used by us, sodium 3-(trimethylsilyl)propanesulfonate (TSP), was the source of the problem in that it obviously interacted with the cationic Pt complex. We detected this phenomenon by applying simultaneously a second standard, [NMe$_4$]BF$_4$, resonating at 3.18 ppm downfield from the methyl resonance of

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(24) We note that, for [Pt(bpy)L$_2$]$^{2+}$ (with L = substituted pyrimidine or pyridine ligands), a doubling of certain L resonances is indicative of the presence of *head−head* and *head−tail* rotamers, with no doubling of bpy resonances observed, however. See, e.g., ref 20 or: Ching, H. Y. V.; Clegg, J. K.; Rendina, L. M. *Dalton Trans.* **2007**, 2121.

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Table 1. Selected Interatomic Distances [Å] and Angles [deg] for Compounds **2**, **2′′**, and **3′**[a]

		Compound **2**	
Pt1−N1	1.983(6)	N1−Pt1−N11	81.5(2)
Pt1−N11	1.997(6)	N1−Pt1−N7a	95.9(2)
Pt1−N7a	2.006(5)	N11−Pt1−N7b	94.9(2)
Pt1−N1b	2.006(5)	N7a−Pt1-N7b	87.6(2)
N2a(H)···N3a$^{(i)}$	2.977(7)	N11−Pt1−N7a	177.4(2)
O1w(H)···O6a	2.916(7)	N1−Pt1−N7b	176.3(2)
		C2a−N1a−C6a	126.7(5)
		C2b−N1b−C6b	125.8(5)
		Compound **2′′**	
Pt1−N1	2.045(9)	N1−Pt1−N11	80.8(4)
Pt1−N11	1.989(9)	N11−Pt1−N7a	96.0(4)
Pt1−N7a	2.030(8)	N1−Pt1−N7b	95.2(4)
Pt1−N7b	2.006(9)	N7a−Pt1-N7b	87.9(4)
N1c(H)···O6a$^{(ii)}$	2.660(13)	N1−Pt1−N7a	176.6(4)
N2a(H)···O6c$^{(iii)}$	2.931(13)	N11−Pt1−N7b	175.2(4)
N2c(H)···O6b	2.940(14)	C2a−N1a−C6a	124.6(10)
		C2b−N1b−C6b	124.8(10)
		Compound **3′**	
Pt1−N1	2.001(8)	N1−Pt1−N11	80.4(4)
Pt1−N11	1.979(8)	N11−Pt1−N7a	95.8(4)
Pt1−N7a	1.999(8)	N1−Pt1−N1b	96.1(3)
Pt1−N1b	2.017(8)	N7a−Pt1-N1b	87.8(3)
N2b(H)···O6a	2.953(10)	N1−Pt1−N7a	175.9(3)
N2b(H)···O6a$^{(iv)}$	2.974(11)	N11−Pt1−N1b	176.1(3)
N1a···(H)O1w	2.853(13)	C2a−N1a−C6a	119.3(9)
N3b···(H)O1w$^{(iv)}$	2.940(13)	C2b−N1b−C6b	119.8(10)

[a] Symmetry equivalences: (i) $-x+1, -y+1, -z+1$; (ii) $x, -y+1/2, z-1/2$; (iii) $-x, y-1/2, -z-1/2$; (iv) $-x-1, -y+1, -z+1$.

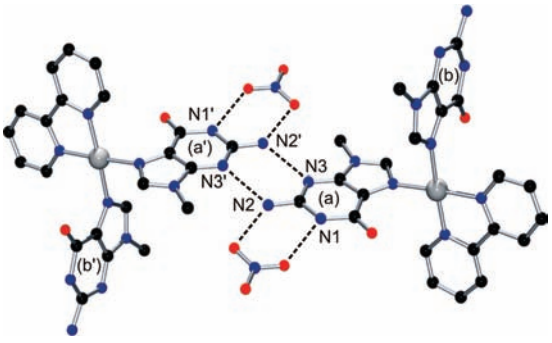

Figure 3. Details of intermolecular hydrogen bonding between 9-MeGH$_a$ ligands and with nitrate anions in **2**. Hydrogen bonding takes place also between N1H and N2H sites of 9-MeGH$_b$ and the second nitrate anion (not shown).

TSP. We found that low concentrations of TSP caused upfield shifts of its methyl resonances, whereas high concentrations of TSP kept the resonance approximately constant.[30] Changes in chemical shifts were as large as 0.05 ppm when varying the amount of TSP by a factor of 6. Our misleading initial results were thus the consequence of samples which contained varying amounts of TSP. We had previously noted that the TSP anion could interact with cationic metallacalix[4]arenes in a typical host−guest manner,[31] thereby causing considerable upfield shifts of the Si(Me)$_3$ signal of TSP. However, we had

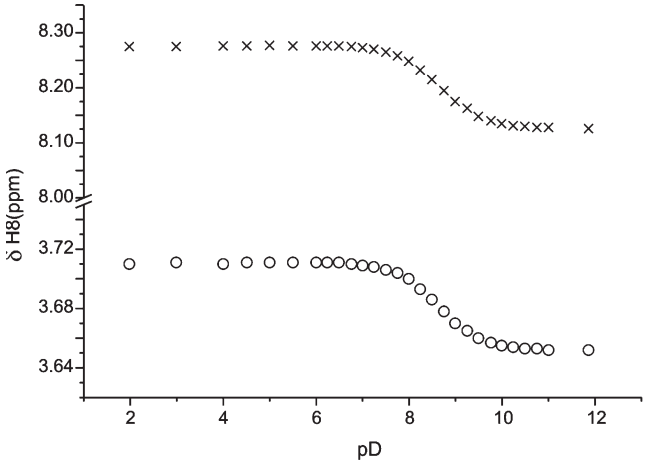

Figure 4. pD dependence of CH_3 (○) and H8 (×) resonances of the 9-MeGH-*N7* ligands in **2**.

not expected this to also happen with compounds of a type similar to **2**. As a consequence of our findings, we propose always double-checking the usefulness of TSP as a standard in ^{1}H NMR spectroscopy by use of a second, cationic reference such as $[NMe_4]^+$ when studying chemical shifts of cationic metal complexes.

[Pt(bpy)(9-EtGH-*N7*)$_2$](NO$_3$)$_2$ (2′) and [Pt(bpy)(9-EtGH-*N7*)$_2$](NO$_3$)$_2$·9EtGH·5H$_2$O (2′′). The reaction of (bpy)Pt^{II} with 9-ethylguanine instead of 9-methylguanine and with an excess of 9-EtGH over (bpy)Pt^{II} (3:1), carried out on an NMR scale in D_2O, pD 1.3, revealed the formation of the bis(9-ethylguanine) complex **2′**.

(30) With fast exchange between relatively weakly bonded and free TSP anions, the effect on chemical shift of the reference is minimized.

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As with **2**, a bpy signal (d) of low intensity (~5% of a doublet at 7.86 ppm) is observed at 8.00 ppm, which tentatively is also attributed to a second rotamer. Consistent with its composition, only a single H5-bpy resonance of the proper relative intensity is seen at 7.60 ppm. From the NMR sample, yellow crystals formed in low yield, which were isolated and proved to be a 9-EtGH adduct of **2′**, $[Pt(bpy)(9\text{-EtGH-}N7)_2](NO_3)_2\cdot 9EtGH\cdot 5H_2O$ (**2″**). In **2″**, the two guanines likewise adopt a *head−tail* arrangement (Supporting Information). Overall, the cation of **2″** is very similar to that of **2**. Salient structural features are listed in Table 1. None of these are unusual.

As in the case of **2**, the Watson−Crick edges (N1,N2) of the coordinated guanines in **2″** are blocked by nitrate counteranions. Other relevant hydrogen-bond interactions are those involving coordinated and free guanines: N2a(H)···O6c, 2.931(13) Å; O6a···N1c(H), 2.660(13) Å; and O6b···N2c(H), 2.940(14) Å.

The most interesting feature of **2″** is the stacking interactions between $(bpy)Pt^{II}$ and the free 9-EtGH (Figure 5). These interactions extend along the *c* axis, in which $(bpy)Pt^{II}$ and the free 9-EtGH are alternatively disposed (ca. 3.5 Å), thereby producing infinite columns. The view perpendicular to the guanine/bpy planes is given in Figure 6. Each $(bpy)Pt^{II}$ entity can be considered as formally intercalated between two guanine nucleobases or vice versa. Stacking interactions between (bpy)M (M = transition metal ion) and nucleotides, either inter-[32] or intramolecularly,[33] have previously been observed.

Compound **2″** is one of a few examples of model nucleobase complexes of Pt^{II} containing both coordinated and free nucleobases in the same compound. Another example is *cis*-$[Pt(NH_3)_2(1\text{-MeC-}N3)_2](NO_3)_2\cdot$ 1-MeC (with 1-MeC = 1-methylcytosine), in which the free cytosine nucleobase is stacked with a coordinated one and additionally hydrogen-bonded to counteranions and to another free nucleobase.[34]

X-Ray Crystal Structure of Pt(bpy)(9-EtG-*N7*)(9-EtG-*N1*)·3H₂O (3′). $[Pt(bpy)(H_2O)_2]^{2+}$, obtained from Pt(bpy)Cl_2 and $AgNO_3$ (2 equiv), was reacted with 9-EtGH initially without the pH adjusted and later with KOH added to reach pH 9.7. A view of the neutral complex **3′** and details of the packing are given in Figures 7 and 8, and in the Supporting Information. Selected structural features are listed in Table 1, and Table 2 contains data collection and refinement parameters for **2**, **2″**, and **3′**.

The two 9-ethylguaninato ligands in **3′** are bonded to $(bpy)Pt^{II}$ through N7 (9-EtG_a) and N1 (9-EtG_b), respectively. The O6 atoms of the nucleobases are at different sides of the PtN_4 coordination plane. The geometry of the (bpy)Pt^{II} moiety is normal, as are the Pt−N distances to the guanine bases: Pt1−N7a, 1.999(8) Å; Pt1−N1b, 2.017(8) Å. They do not differ significantly for the two linkage isomers. The N7-coordinated base is deprotonated at N1a. This site

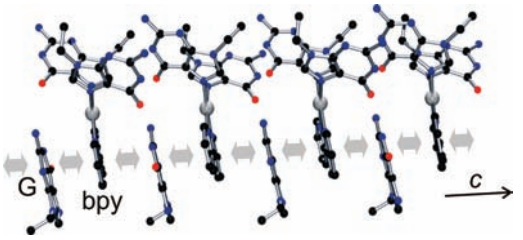

Figure 5. Directionality of the π-stacking interactions between $(bpy)Pt^{II}$ and free 9-EtGH along the *c* axis in **2″**.

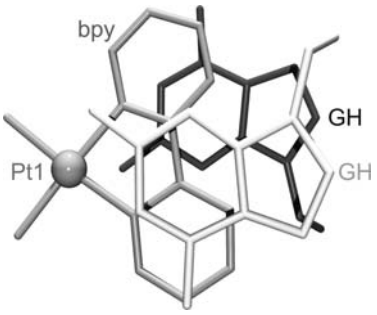

Figure 6. Top view of a $(bpy)Pt^{II}$ intercalation between two guanine nucleobases in **2″**.

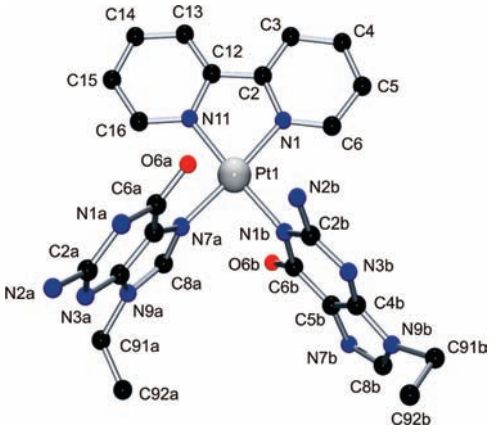

Figure 7. Molecular structure of the neutral complex Pt(bpy)(9-MeG-*N1*)(9-MeG-*N7*) (**3′**) with atom numbering scheme.

exhibits an internal ring angle of 119.3(9)°, clearly smaller in comparison to free guanine[35] or to other N1-protonated purine bases showing similar features.[36] The corresponding angle (C2b−N1b−C6b) for the N7-coordinated guanine is 119.8(10)°. Regarding the geometrical arrangement of the bases, dihedral angles of the bases relative to the Pt coordination plane are 84.4(2)° for 9-EtG_a and 81.9(2)° for 9-EtG_b. Angles involving Pt and the bonding site are almost identical in the case of 9-EtG_a (C8a−N7a−Pt, 126.3(8)° and C5a−N7a−Pt, 127.4(7)°) but differ considerably for 9-EtG_a (C2b−N1b−Pt, 122.5(7)° and C6b−N1b−Pt, 117.7(8)°), resulting in a marked inclination of this base.

Furthermore, both the *head−tail* orientation of the bases and their near-perpendicularity (89.3(2)°) entail an intramolecular hydrogen bond involving both nucleobases: N2b(H)···O6a, 2.953(10) Å. As shown in Figure 8, molecules of compound **3′** are organized such that they form pairs, which are stabilized by four cyclic hydrogen bonds, namely, by two intramolecular N2b(H)···O6a

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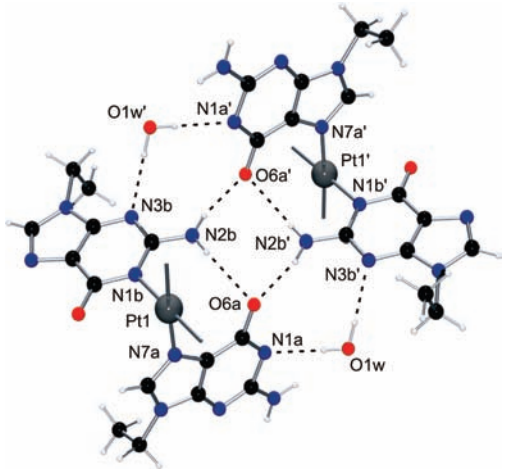


Figure 8. Closeup view of the hydrogen-bonding motif in **3′**. Bipyridine ligands are omitted for clarity.

(vide supra) and two intermolecular (N2b(H)···O6a ($-x-1, -y+1, -z+1$), 2.974(11) Å) ones. In addition, two water molecules reinforce this pairing scheme, with hydrogen bonds to the guanine bases at both sides of the pair: N1a···(H)O1w, 2.853(13) Å; N3b···(H)O1w ($-x-1, -y+1, -z+1$), 2.940(13) Å.

The association pattern between two cations of **3′** can be considered that of a diplatinated guanine quartet. The four bases are, however, not planar as in the famous G_4 quartets found in tetra-stranded nucleic acids[37] but, rather, form a chairlike arrangement as far as the four guanine molecules are concerned. Superficially, the pairing pattern seen in **3′** can be derived from a hydrogen-bonded dimer ("GG3"[38]) seen in ribbonlike assemblies of guanines[39] as well as in CG·G triplets,[40] followed by dimer formation via $N(2)H_2$ and O6 hydrogen bonds, nucleobase deprotonation, and metal coordination (Scheme 1). Because of the cis geometry about Pt, the bases are no longer coplanar, of course. The four guanine bases forming the quartet structure are enclosed by two pairs of stacked bpy ligands (Supporting Information).

Solution Equilibria of 3′. In aqueous solution, **3′** exists in different protonation states, depending on pH (Scheme 2). Relevant pK_a values were determined by pD-dependent ^{1}H NMR spectroscopy in D_2O (Figure 9). A typical spectrum, recorded at pD 2.0, is given in Figure 10.

As can be seen from Figure 9, both deprotonation/protonation steps are "felt" by the respective other guanine ligand. The pK_a values in D_2O are 8.3 ± 0.1 for the N7-bonded 9-MeGH and 4.51 ± 0.03 for the N1-bonded one, corresponding to 7.7 ± 0.1 and 4.00 ± 0.03 in H_2O. Values for the 9-MeG-*N1* linkage isomer compare well with those of bis(9-methylguanine-*N1*) complexes of (en)

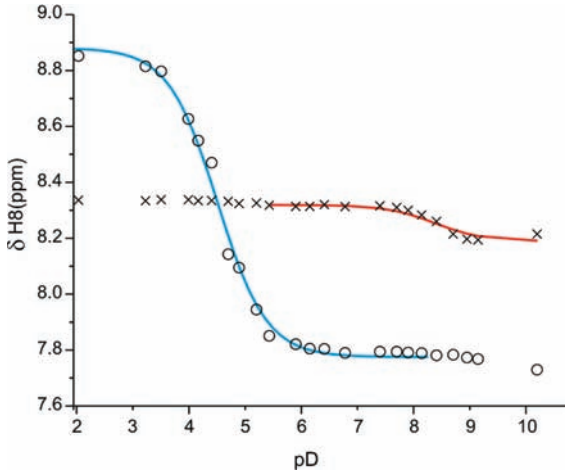


Figure 9. pD dependence of H8 resonances of the two differently bonded 9-ethylguanine ligands in **3′**: 9-EtG(H)-*N1* (○) and 9-EtG(H)-*N7* (×).

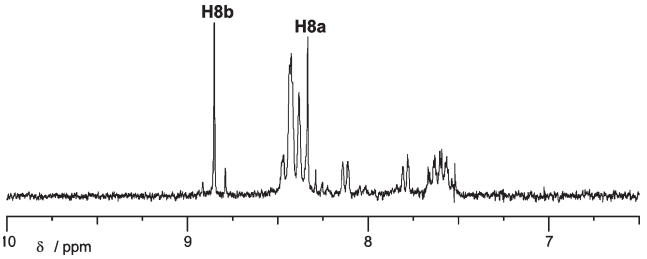
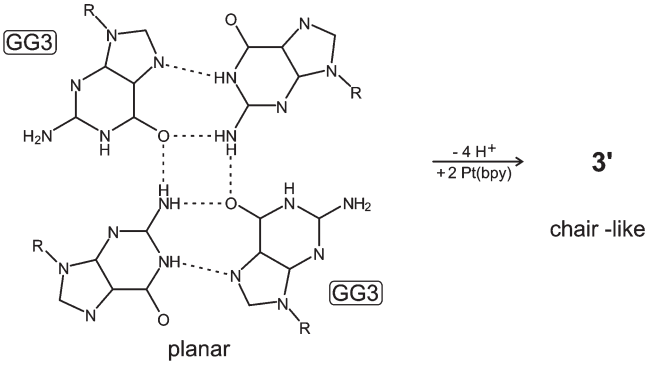
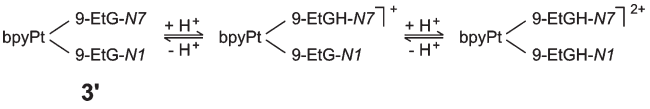


Figure 10. Low-field region of ^{1}H NMR spectrum of **3′** in D_2O, pD 2.0. The intense H8 resonance of 9-MeGH-*N1* at low field is readily identified at this pD.

Scheme 1



Scheme 2



Pt^{II} and *trans*-a_2Pt^{II} (a = NH_3 or CH_3NH_2),[41] as well as 9-ethylguanine complexes of *cis*-$(NH_3)_2Pt^{II}$ and (dien)Pt^{II} with N1 coordination.[42] Protonation of the 9-MeG-*N1* ligand in **3′** causes a large (> 1 ppm) downfield shift of the H8 resonance, which makes this resonance particularly well-observed below pD 4, as there is no overlap

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(41) For 2:1 complexes of $enPt^{II}$ and *trans*-$(NH_3)_2Pt^{II}$, pK_a values are around 4 (mean value for two steps).
(42) van der Veer, J. L.; van der Elst, H.; Reedijk, J. *Inorg. Chem.* **1987**, *26*, 1536.

Table 2. Crystallographic Data for Compounds **2**, **2″**, and **3′**

compound	**2**	**2″**	**3′**
formula	$C_{22}H_{24}N_{14}O_9Pt$	$C_{31}H_{45}N_{19}O_{14}Pt$	$C_{24}H_{30}N_{12}O_5Pt$
fw (g mol^{-1})	823.64	1102.95	761.69
cryst color and habit	yellow prisms	yellow hexagons	yellow cubes
cryst syst	monoclinic	monoclinic	monoclinic
space group	*P*21/*c*	*P*21/*c*	*P*21/*c*
a (Å)	15.2631(4)	13.962(3)	12.182(2)
b (Å)	12.0657(4)	22.934(5)	24.942(5)
c (Å)	15.5578(5)	14.125(3)	10.094(2)
β (deg)	100.314(2)	92.37(3)	96.23(3)
Z	4	4	4
V (Å^3)	2818.83(15)	4519.0(16)	3048.9(10)
ρ_{calcd} (g cm^{-1})	1.941	1.621	1.659
μ (Mo Kα) (mm^{-1})	5.057	3.188	4.656
F(000)	1616	2216	1504
θ range (deg)	3.20 - 27.60	2.28 - 27.94	2.19 - 25.06
no. reflns collected	6336	9077	5351
no. reflns observed $I > 2\sigma(I)$	3558	2805	2392
no. params refined	415	556	374
R_1 (obs. data)	0.0426	0.0588	0.0470
wR_2 (obs. data)	0.0606	0.1526	0.1353
goodness-of-fit, *S*	1.005	0.844	0.860
residual ρ_{max}, ρ_{min} (e Å^{-3})	1.701, −1.344	−0.731, 0.113	1.150, −0.729

with bpy resonances (Figure 10). Similarly, the ethyl groups of the two guanine ligands are clearly distinguishable at low pD. The H5 and H5′ resonances of the bpy overlap at ca. 7.6 ppm.

Concluding Remarks

One way of perturbing nucleobase pK_a values in DNA or RNA, thereby making nucleobases potentially susceptible to acid−base catalysis, is metal coordination. In the case of metal complexes of neutral nucleobases, downward pK_a shifts are the rule. Because of its normal pK_a value of 9−10 for N1(H) and its known affinity for metal ions through the N7 position, guanine is to be considered a major candidate for such scenarios. Pt^{II}−am(m)ine entities at N7 typically lower the pK_a of guanine bases to ca. 7.8−8.5 (Table 3).[8,43] Of particular interest are metal compounds containing two ionizable ligands, because two protons can be liberated or accepted, either in two distinctly different pH ranges or in an overlapping one. In the latter case, the metal compound can act at the same time as an acid and a base−an ideal situation for catalysis.

We do not wish to overemphasize the particular pK_a values of the Pt^{II} complexes listed in Table 3 in terms of their potential as acid−base catalysts in nucleic acid chemistry, simply because of the fact that the local microenvironment can further modulate the pK_a values, sometimes quite substantially. What the data in Table 3 reveal, however, is that, by combining two ionizable ligands, the pH range of interest can be expanded significantly. This is true, in particular, when metal complexes of rare nucleobase tautomers are included in the considerations.

Experimental Section

Preparations. $PtCl_2$(bpy) was prepared as described in the literature.[44] K_2PtCl_4, 2,2'-bipyridine, 9-methylguanine (9-MeGH), and 9-ethylguanine (9-EtGH) were of commercial origin.

Preparation of [Pt(bpy)(9-MeGH-*N7*)Cl]Cl·0.5H_2O (1). Pt(bpy)Cl_2 (844.5 mg, 2.00 mmol) and 9-MeGH (3.40 mg, 2.00 mmol) were suspended in water (100 mL), and the mixture was stirred for 48 h at 60 °C. The resulting suspension was filtered, and the undissolved educts were removed. In the filtrate, yellow needles of **1** formed within three days at 4 °C. The yield was 134.5 mg (11%). Anal. calcd for hemihydrated **1**, $C_{16}H_{16}N_7O_{1.5}Cl_2Pt$: C, 32.22; H, 2.70; N, 16.44. Found: C, 32.0; H, 3.2; N, 16.6.

Preparation of [Pt(bpy)(9-MeGH-*N7*)$_2$](NO_3)$_2$·H_2O (2). $AgNO_3$ (169.8 mg, 1 mmol) was added to a suspension of Pt(bpy)Cl_2 (197.1 mg, 0.47 mmol) in 50 mL of water. The mixture was stirred at 40 °C in the dark for 24 h. After filtration of AgCl, 9-methylguanine (247.7 mg, 1.42 mmol) was added to the filtrate, and the mixture was allowed to stir for three days. The resulting suspension was filtered, and the solution was concentrated to 10 mL. Upon slow evaporation at room temperature, yellow crystals of **2** formed. The yield was 57 mg (15%). Anal. calcd for $C_{22}H_{24}N_{14}O_9Pt$: C, 32.05; H, 3.25; N, 23.85. Found: C, 32.1; H, 2.9; N, 23.8.

Preparation of [Pt(bpy)(9-EtGH-*N7*)$_2$](NO_3)$_2$·9EtGH·5H_2O (2″). Pt(bpy)Cl_2 (12.52 mg, 0.030 mmol) and $AgNO_3$ (9.97 mg, 0.059 mmol) were suspended in D_2O (0.6 mL), stirred in the dark (12 h, 60 °C), and filtered. After addition of 9-EtGH (15.9 mg, 0.089 mmol) to the clear filtrate, the pD was adjusted to 0.8 (using 1 M DNO_3) and stirred at 60 °C for three days. The yellow precipitate formed by then was eliminated by centrifugation. From the resulting solution, yellow crystals of **2″** were collected in low yield after one day and characterized by X-ray crystallography.

Preparation of Pt(bpy)(9-EtG-*N7*)(9-EtG-*N1*)·3H_2O (3′). An aqueous suspension (15 mL) of $PtCl_2$(bpy) (205 mg, 0.486 mmol) and $AgNO_3$ (165 mg, 0.972 mmol) was stirred for 15 h at 40 °C with daylight excluded. AgCl was removed, and 9-EtGH (197 mg, 1.1 mmol) was added to the filtrate, which was allowed to stir for one day at 40 °C. The pH of the solution was then adjusted to 9.4 with KOH (1 M), and the solution was concentrated to 5 mL. One day later, a large amount of yellow precipitate was recovered. Subsequent recrystallization from H_2O gave yellow crystals of **3′**. The isolated yield was 39 mg (10.5%). Anal. calcd for $C_{24}H_{28}N_{12}O_4Pt$ (dihydrate): C, 38.76; H, 3.79; N, 22.60. Found: C, 38.4; H, 3.6; N, 22.6. X-ray crystallography showed the compound to contain three H_2O molecules.

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Table 3. pK_a Values (in H_2O) of Guanine Ligands

compound	pK_a	ΔpK_a[a]	ref
9-MeGH	9.56 ± 0.05		21
9-EtGH	9.57 ± 0.05		8
$[Pt(dien)(9\text{-EtGH-}N7)_2]^{2+}$	8.35 ± 0.20		8
cis-$[Pt(NH_3)_2(9\text{-EtGH-}N7)_2]^{2+}$	7.76 ± 0.09; 8.36 ± 0.09	0.6	8
trans-$[Pt(NH_3)_2(9\text{-EtGH-}N7)_2]^{2+}$	8.01 ± 0.01; 8.81 ± 0.01	0.8	36
$[Pt(bpy)(9\text{-MeGH-}N7)_2]^{2+}$ (**2**)	7.78 ± 0.01; 8.38 ± 0.01	0.6	this work
cis-$[Pt(NH_3)_2(H_2O)(9\text{-MeGH-}N7)_2]^{2+}$	6.2 ± 0.1; 8.7 ± 0.1	2.5	11
trans-$[Pt(NH_3)_2(H_2O)(9\text{-MeGH-}N7)_2]^{2+}$	5.27 ± 0.05; 8.87 ± 0.06	3.6	11
$[Pt(bpy)(9\text{-EtGH-}N1)(9\text{-EtGH-}N7)]^{2+}$ (**3′**)	4.00 ± 0.03; 7.7 ± 0.1	3.7	this work

[a] Difference of pK_a values of two ionizable ligands

Instrumentation. ^{1}H NMR spectra were recorded on Varian Mercury 200 FT NMR and Bruker DRX 400 instruments with TSP and tetramethylammonium tetrafluoroborate ($[NMe_4]^+BF_4^-$) as internal references. When TSP was used as the sole reference compound (δ = 0 for $Si(CH_3)_3$ signal), reliable shift data were obtained when it was present "in excess", namely, at least in a Pt/TSP ratio of 7:1. We are aware that the stronger the cation−TSP interaction is, the less suitable TSP is as an internal standard. pD values of NMR samples were determined by means of a glass electrode, after the addition of NaOD or DNO_3 to the respective sample. Elemental (C, H, N) analysis data were obtained on a Leco CHNS-932 instrument.

Determination of pK_a Values. The pK_a values were determined by pH-dependent ^{1}H NMR spectroscopy. Variation of the chemical shift of the guaninic H(8) and methyl protons were annotated at the different pD stages. The pD values were obtained by adding 0.4 to the pH meter reading (uncorrected pH*). The graphs (pD versus chemical shift) were evaluated with a nonlinear least-squares fit according to the Newton−Gauss method.[45] The obtained acidity constants (for D_2O) were transformed to values valid for H_2O.[46]

X-Ray Crystallography. Table 2 contains data collection and refinement parameters for **2**, **2″**, and **3′**. Data collection was performed on an Enraf−Nonius Kappa CCD diffractometer (compounds **2″** and **3′**) and on an Oxford Diffraction Sapphire2 CCD diffractometer (compound **2**), both equipped with Mo Kα radiation (λ = 0.71069 Å). Data reduction and cell refinement were carried out using DENZO and SCALE-PACK programs.[47] All of the structures were solved by standard Patterson methods and refined by full-matrix least-squares methods based on F^2 using the SHELXL-97[48] and WinGX[49] programs. All non-hydrogen atoms were refined anisotropically, and all of the hydrogen atoms were included in geometrically calculated positions and refined with isotropic displacement parameters according to the riding model.

Acknowledgment. This work was supported by the Deutsche Forschungsgemeinschaft (DFG). P.B.-B. thanks the Foundation Ramón Areces (Spain) for a postdoctoral fellowship. We thank Prof. W. Sheldrick and Ms. M. Winter (Ruhr-University of Bochum, Germany) for data collection of compound **2**.

Supporting Information Available: CIF files of X-ray crystal structure refinement data for $[Pt(bpy)(9\text{-MeGH-}N7)_2](NO_3)_2\cdot H_2O$ (**2**), $[Pt(bpy)(9\text{-EtGH-}N7)_2](NO_3)_2\cdot(9\text{-EtGH})\cdot 5H_2O$ (**2″**), and $Pt(bpy)(9\text{-EtG-}N7)(9\text{-EtG-}N1)\cdot 3H_2O$ (**3′**); ^{1}H NMR spectrum of **2′**; views of **2″**; and packing details of **2** and **3′**. This material is available free of charge via the Internet at http://pubs.acs.org.

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