High sensitivity to transmission of attenuation in the metal activating effect of platinum on sequential H–D exchange at the four C(8) sites of inosines in tetrakis(inosine)platinum(II) chloride

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Hydrogen–deuterium exchange of the carbon-bound C(8)-H protons of the inosine residues in tetrakis(inosine)platinum(II) chloride, S, with Pt binding at N(7), was studied in aqueous buffer solutions at 60 °C by ^{1}H NMR spectroscopy. The kinetics at all four C(8) sites as a function of pD of the D_2O/OD^- medium was measured through the disappearance of the C(8)-H signal, which yielded the pseudo first-order rate constant for exchange, k_{obs}. Plots of k_{obs} *versus* [OD^-] showed curvature reminiscent of saturation type kinetics and indicative of competitive deprotonation of N(1)-H sites. In contrast, the analogous N(1)-methylated *cis*-bis(1-methylinosine)diammineplatinum(II) chloride leads to a linear k_{obs} *versus* [OD^-] plot. The potentiometrically determined macroscopic composite N(1)-H ionization constant was further dissected into the successive microscopic N(1)-H acidity constants of the four inosine residues of the complex S. The k_{obs} values were also deconvoluted into individual rate constants k_{ex} (*i.e.* k_0, k_1, k_2, k_3 for exchange of the successively deprotonated inosine moieties, S, S_1, S_2, S_3, it being assumed that S_4 where all four inosine ligands are deprotonated at N(1) is unreactive ("immunized") to exchange. The k_{ex} values show a progressive attenuation in Pt activation of H–D exchange along the series, k_0, k_1, k_2, k_3. The k_{ex} data thus generated, together with the deconvoluted individual pK_a values allow the construction of the plot, log k_{ex} [C(8)-H] *vs.* pK_a [N(H)-1]. Remarkably, this plot exhibits good linearity ($R^2 = 0.99$), which accords this as a linear free energy relationship (LFER). The large negative slope value (−2.3) of this LFER reflects the high sensitivity of transmission of electron density from the ionized N(1) *via* Pt and/or through space to the remaining C(8)-H sites. This is to our knowledge the first instance in which a LFER is generated through modulation of a structure in a single molecule. One can anticipate that this approach may lead to: (1) predicting N-H acidity; (2) C-H H–D exchange susceptibility in a range of metal–biomolecule complexes; (3) their carbon acidity.

Introduction

In continuation of our studies on metal ion–biomolecule interactions,[1–6] we report herein on hydrogen–deuterium exchange kinetics of the carbon-bound C(8)-H protons of inosine residues in tetrakis(inosine)platinum(II) chloride [$(ino)_4Pt(II)Cl_2$ or S, Pt-binding at N(7)]. Our aim is to probe the interplay of the metal ion activating effect on H–D exchange and the influence of deprotonation of a remote nitrogen site [N(1)]. Remarkably, our study reveals a high sensitivity to transmission of attenuation of the metal ion activating effect, as a novel structure–activity relationship (LFER).

The essentiality of metal ions, notably transition metal ions, in life processes is well-recognized,[7–10] even when specific biomolecular modes of action have not been fully elucidated. The great majority of enzymes that utilize nucleoside phosphate substrates also require a divalent metal ion;[11] incorporation of these ions into enzymes has been reviewed.[12] While heavy metal ions such as mercury exhibit toxicity,[13–16] on the other hand, platinum is the central atom in such widely-used chemotherapeutic agents as *cis*-platin.[17–21] The efficacy of *cis*-platin is thought to derive from the square-planar structure of the Pt(II) nucleoside complexes that may intercalate between DNA strands. Generally, effects, deleterious or beneficial, arise in part from binding of the metals (with or without displacement of other ligands) to donor (N,O,S) sites in five-membered heterocycles such as imidazole, thiazole, oxazole that are key moieties of many biomolecules, *e.g.* histidine, guanosine, among the many purines, nucleosides/nucleotides, nucleic acids *etc*.

A valuable handle for mechanistic studies is provided by the acidity of C(2)-H in thiazoles, imidazoles *etc.* and of the strictly analogous C(8)-H in purines, nucleic acids and so forth; this feature permits studies of isotopic H–D–T exchange under conditions where H^+ or a metal ion, M^{n+}, may play a catalytic role. Significantly, such heterocycles have pK_a values that allow deprotonation in the physiological pH range and, therefore, undergo isotopic exchange under mild conditions.

In principle, three separate pathways may each contribute to H–D isotopic exchange at a carbon site (C(2) in Scheme 1) in a five-membered heterocycle such as imidazole (bonded to

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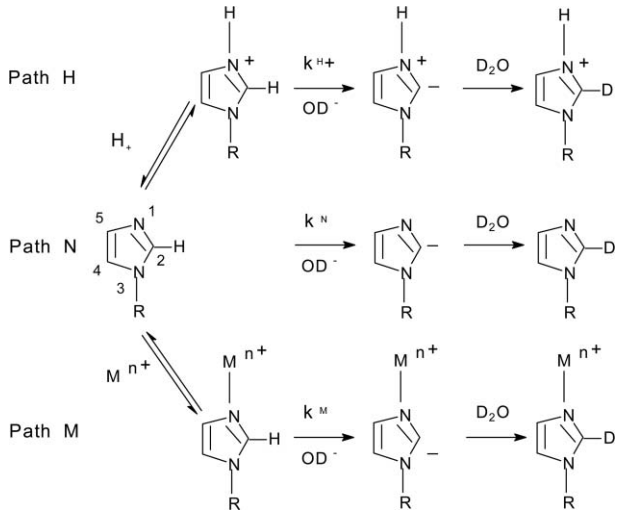


Scheme 1

an R = ribose group at N(3) in Scheme 1), the prototype for other biomolecular heterocycles such as inosine. In inosine, a six-membered heterocyclic ring is fused to the five-membered ring; this has been omitted from Scheme 1 for simplicity. The central pathway is Path N for the neutral heterocycle; it involves preferential abstraction of the C(2) proton,[22,23] analogous to C(8)-H in inosine, to give a carbanion that may then undergo rapid deuteration from the "deuterium pool" made up of the solvent deuterium oxide.

The other two pathways depict mechanisms whereby H–D exchange may be enhanced. In Path H, protonation of the nitrogen adjacent to the exchanging C–H site would be expected to polarize the C–H bond whereas after deprotonation, the carbanion formed would be of the ylide or dipole-stabilized type[22–24] that may also be resonance stabilized (Fig. 1).[22] Path H illustrates the proton-activating factor[25] (*paf*; quantitatively k_{H+}/k from Scheme 1) in H–D exchange in a host of heterocyclic biomolecules.[26] It is Path M, however, that is most pertinent to the present study. Here, complexation of a metal ion to the same nitrogen, adjacent to the site of isotopic exchange, acts to enhance the exchange and yields a resonance-stabilized ylide-type structure comparable to that arising from Path H (Fig. 1 for the case of inosine).[5] In analogy to *paf*, Path M defines the metal activating factor[26] (*maf*, defined by the rate constant ratio, k_M/k, Scheme 1) in isotopic exchange of these heterocycles. In general, the trend in ease of C–H exchange proceeds from the protonated species to the metallated and to the neutral substrate, in turn, *i.e.*, in terms of pathways, generally Path H is favored over Path M, which is favored over Path N.[6,26]

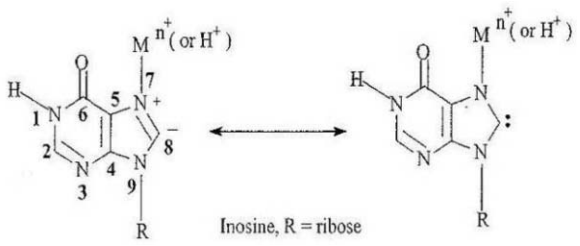
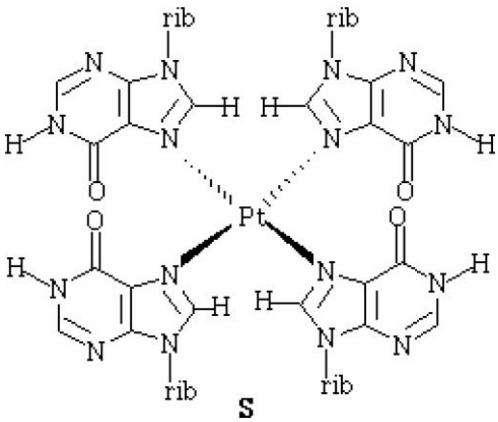


Fig. 1 Ylide-type carbanion (left) stabilized by contribution from carbenoid resonance form (right) in H–D exchange at C(8)-H in N(7)-metallated (or protonated) inosine (R = ribose). The inosine ring is numbered as shown in the complexes discussed in the text.

H–D exchange at C(2) of the parent imidazolium cation (Scheme 1, Path H; R = H) and of structurally similar azoles such as the *N*,*N*-dimethylimidazolium cation (Scheme 1, Path Me—for methyl—analogous to Path H, where R = CH_3 and the H at N(1) is replaced by CH_3) has recently been revisited by the Amyes–Richard research group.[24b] These results on the base compound for such exchanges will be considered in light of the current H–D exchange kinetic results and the progressive structural modulation required to transit from imidazole to the tetrakis(inosine)Pt(II) complex, S, including the respective effects of *paf* and *maf*.

The current study, then, focusses on H–D exchange at the C(8)-H site of the substitution-inert square-planar platinum complex, $[(ino)_4Pt(II)Cl_2]$, S, where the inosine rings are all bound to platinum *via* N(7) of each inosine ligand. The complex contains four C(8)-H exchange sites and also four N(1)-H sites, which may ionize depending on the pD of the medium. To distinguish between these various reactive centers, the inosine ligands have been arbitrarily labeled A through D, so that ino($CH_AN H_A$) refers to an inosine moiety labeled "A" where C(8)-H and



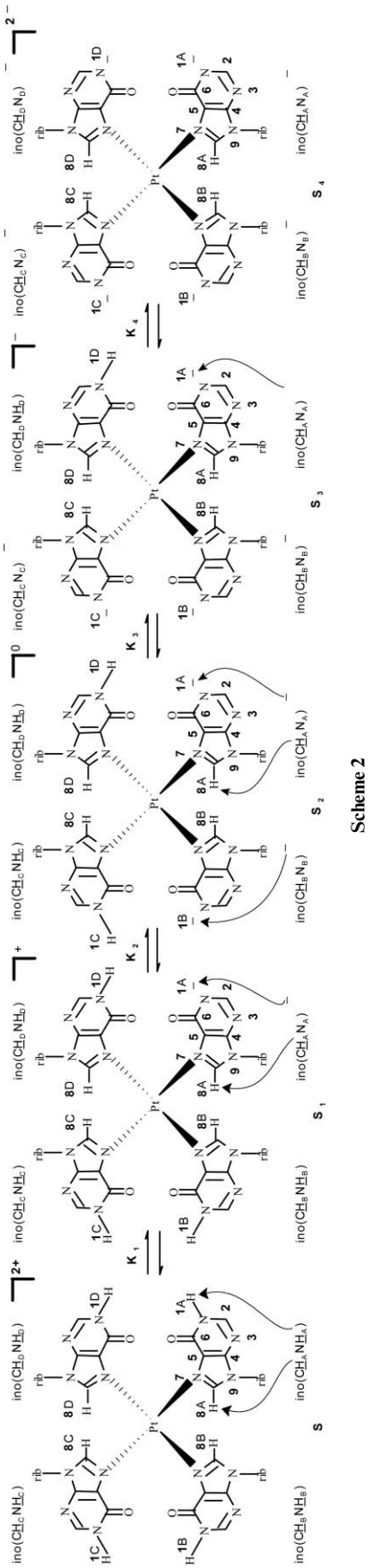
S

N(1)-H sites are still intact. Analogously, ino($CD_BN H_B$) indicates that in the inosine ring labeled "B" the C(8)-H has undergone H–D exchange. The kinetics of H–D exchange at all four C(8) sites, as a function of the pD of the D_2O/OD^- medium, were followed and the results are discussed herein with respect to deprotonation of the N(1)-H sites. Thus, the present study probes both the effect of platinum complexation at N(7) of the inosine ligands on C(8) H–D exchange and on N(1)-H ionization. Uniquely, the correlation between the two effects forms a novel linear free energy relationship (LFER) involving a single reactive substrate that quantifies the attenuation in the metal activating factor for platinum that results from sequential N(1)-H deprotonation.

Results

Kinetics of C(8)-H–D exchange

The kinetics of isotopic hydrogen exchange of the four C(8)-H protons in the $(ino)_4PtCl_2$ complex, S (Schemes 2 and 3), were conveniently measured by ^1H NMR spectrometry (60 °C, D_2O). The pD of the medium was maintained using deuterium-exchanged phosphate buffers.



Scheme 2

Table 1 Pseudo-first order rate constants, k_{obs}, for C(8)-H to C(8)-D exchange in tetrakis(inosine)platinum(II) chloride, S (pD 6.18–9.07, 60 °C)

pD[a,b]	10^6 [OD^-]/M[b]	k_{obs}/s^{-1}[c]	k_{calc}/s^{-1}[d]
6.18	0.024	1.09×10^{-4}	0.46×10^{-4}
6.82	0.105	1.26×10^{-4}	1.54×10^{-4}
6.92	0.132	1.87×10^{-4}	1.79×10^{-4}
7.76	0.912	3.36×10^{-4}	3.55×10^{-4}
8.05	1.78	3.96×10^{-4}	3.69×10^{-4}
8.76	9.12	3.49×10^{-4}	3.69×10^{-4}
8.87	11.7	4.09×10^{-4}	3.69×10^{-4}
9.07	18.6	3.65×10^{-4}	3.69×10^{-4}

[a] Room temperature measurements of pH were corrected to give these pD values (pD = pH + 0.4).[27] [b] Calculated from the value of pK_w (D_2O) = 13.8 at 60 °C.[28] [c] Pseudo-first order rate constants were determined at 60 °C. The values reported at pD 6.92 and 8.76 are average values. In comparable NMR kinetic studies, typical errors of ±10% have been reported,[24b] and provide a more conservative error estimate for the current work. The value obtained at pD 6.18 incorporates a larger experimental error, see text. [d] Calculated from eqn (2) using the values of the individual second-order rate constants from Table 2.

The rate of disappearance of the distinct C(8)-H singlet (δ 8.93 ppm in D_2O) was followed with time for not less than one half-life. Standard first-order plots [ln(area of C(8)-H peak/area of non-exchanging C(1′)-H of the N(9) ribose) *versus* time] were linear at each pD studied and yielded the observed pseudo-first order rate constants for exchange, k_{obs}, listed in Table 1.

Thus, the overall exchange process follows eqn (1):

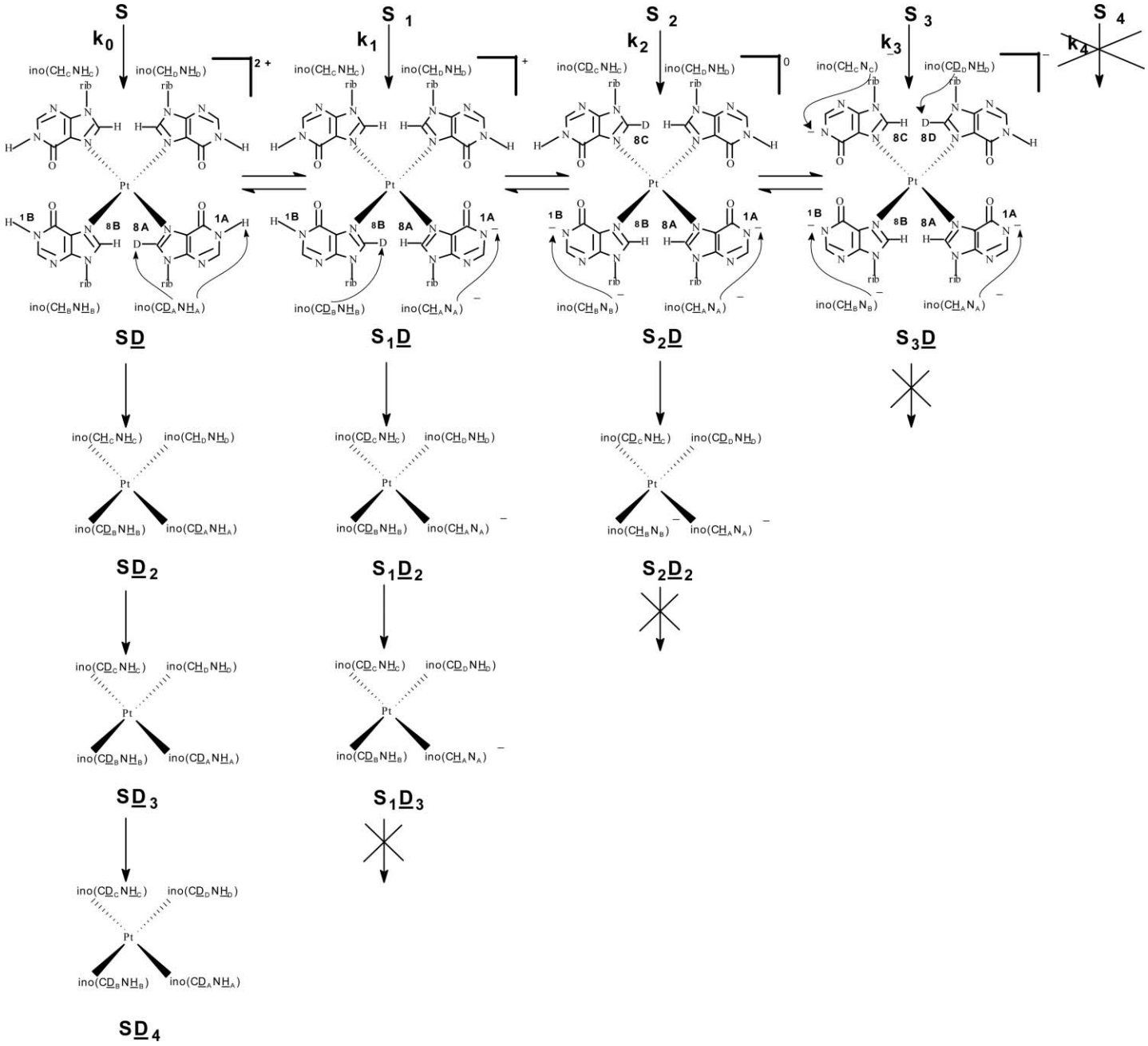
$$-\mathrm{d[C(8)\text{-}H]}/\mathrm{d}t = k_{obs}[S]_T \quad (1)$$

where k_{obs} is the pseudo-first order rate constant, compiled in Table 1, where the solutions were buffered and so the deuteroxide concentration relative to the total concentration of complex(es), $[S]_T$ is fixed.

A plot of the pseudo-first order rate constants *versus* deuteroxide concentration did not yield a straight line as would be expected for a simple second-order process; the plot was curved in a manner reminiscent of saturation-type kinetics (Fig. 2). Such curvature has been taken to be indicative of the intervention of competitive deprotonation of the N(1)-H site(s), particularly in the higher pD region.[26,29–32] In contrast, *N*-methylated *cis*-bis(1-methylinosine)diammineplatinum(II) chloride [(1-MeIno)$_2$(NH_3)$_2$Pt(II)Cl_2] under the same conditions leads to a linear k_{obs} *versus* [OD^-] plot.[4,33a] Here, the N(1) site is methylated and cannot undergo comparable deprotonation,[29] which accounts for the difference in the two plots. Note at higher pH (pD) than the range studied here, a possible upturn in the rate for exchange could occur.[6,26]

The pseudo-first order rate constants (Table 1) can be dissected into second-order rate constants (Table 2) that represent four different overall exchange processes (Scheme 2), with the assumption that the complex in which all four N(1)-H sites are deprotonated, *i.e.*, S_4, is "immunized" against H–D exchange at C(8)-H (*vide infra*). The observed rate constant (k_{obs}) was deconvoluted into individual rate constants for exchange (k_0, k_1, k_2, k_3 and $k_4 = 0$) according to eqn (2):

$$k_{obs} = \frac{k_0[OD] + k_1K_1[OD]^2 + k_2K_1K_2[OD]^3 + k_3K_1K_2K_3[OD]^4}{1 + K_1[OD] + K_1K_2[OD]^2 + K_1K_2K_3[OD]^3 + K_1K_2K_3K_4[OD]^4} \quad (2)$$



Scheme 3

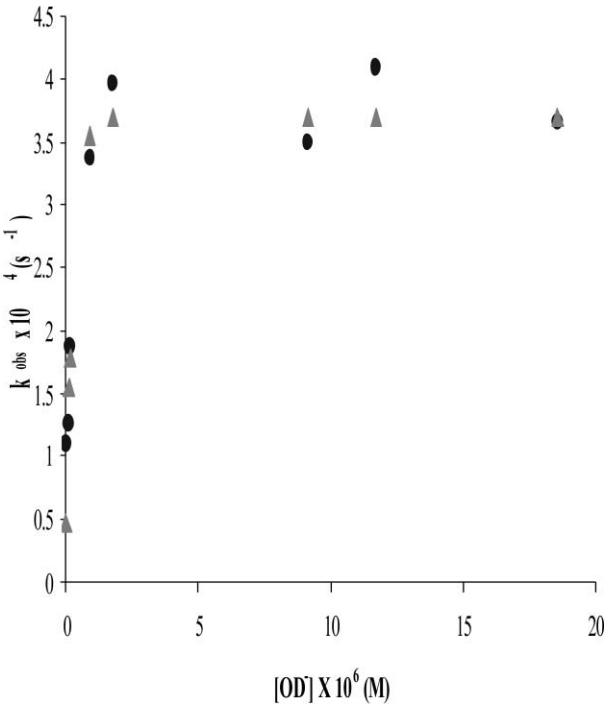


Fig. 2 Plots of k_{obs} *versus* deuteroxide ion concentration for C(8)-H isotopic exchange in complex S indicating saturation type behavior: experimental k_{obs} values, full circles; triangles, k_{obs} values calculated according to eqn (2).

Table 2 Microscopic rate constants for exchange (k_{ex}) at C(8)-H of S, S_1, S_2 and S_3 complexes (pD 6.18–9.07, 60 °C)

Complex[a]	k_{ex}[b]/M^{-1} s^{-1}	p[c]	k_{ex}/p (M^{-1} s^{-1})[d]
S	2.1×10^3	4	525 (k_0)
S_1	5.0×10^2	3	167 (k_1)
S_2	1.4×10^2	2	70 (k_2)
S_3	4.1×10^1	1	41 (k_3)

[a] Structures of the complexes are shown in abbreviated form in Schemes 2 and 3. [b] Second-order rate constant from exchange from the C(8) position from non-linear least squares treatment of eqn (2). [c] Represents the number of equivalent exchangeable sites. [d] Statistically-corrected microscopic second-order rate constants for C(H)-8 exchange.

The individual rate constants are designated: k_0 to k_3, where the subscript "0" indicates that no N(1)-H positions are ionized for k_0 and it increases in increments of one as each N(1)-H is successively deprotonated. Hence, k_0 represents the rate constant for deuterium exchange at the C(8)-H site of any of the four co-ordinated inosine moieties of the neutral complex, S, while k_4 for H–D exchange from S_4, where all four inosine ligands are deprotonated at N(1) is taken to have a value of zero in this treatment (*vide infra*). The ionization constants K_1 through K_4 are step-wise acidity constants for N(1)-H ionization obtained from the overlapping pK_a.[33b]

Application of eqn (2) involved the input of estimates for the microscopic C(8)-H–D exchange rate constants, k_0 to k_3, as well as the measured N(1)-H ionization constants. In the case of k_0, the second-order rate constant for exchange in the related $(1\text{-MeIno})_2(NH_3)_2Pt(II)Cl_2$ complex, where comparable N(1)-H ionization is not possible (see above),[33] provides an estimate for the value of k_0 as 2×10^3 M^{-1} s^{-1}. Similarly, on the basis of model compounds, the following initial values for the second-order rate constants were assigned: $k_1 = 1 \times 10^3$ M^{-1} s^{-1}, $k_2 = 5 \times 10^2$ M^{-1} s^{-1} and $k_3 = 1 \times 10^2$ M^{-1} s^{-1}.

Table 3 Microscopic N(H)-1 acidity constants (60 °C)

pK_a	p	q	p/q	$\log(p/q)$	$^a pK_a^{corr}$
7.07	4	1	4	0.60	7.67
7.68	3	2	1.5	0.17	7.85
8.21	2	3	0.67	−0.18	8.03
8.75	1	4	0.25	−0.60	8.15

[a] Corrected statistically for the number of equivalent acid sites, p, and for the equivalent base sites in the relevant conjugate base, q.

Input of the experimentally determined (deconvoluted) N(1)-H ionization constants (K_1, K_2, *etc.* from the relevant pK_a values given in Table 3) and the initial estimates for the second-order rate constants into eqn (2) was followed by iterative non-linear least squares treatment of the data[34] until the calculated k_{obs} values from eqn (2) were in reasonable agreement with the experimental k_{obs} values (Table 1) at the pD values (*i.e.*, [OD^-]) studied; the experimental k_{obs} values and those calculated from the microscopic rate constants obtained were in agreement typically to within 10%, with the exception of the value determined in the most acidic medium, which incorporates a significantly higher error. However, there was no trend in the differences between the calculated and experimental observed rate constants (Fig. 2). The microscopic rate constants ($k_{ex} = k_0$ to k_3) that emerge from this non-linear least squares fitting of eqn (2) to the observed rate constant values are listed in Table 2. These constants are then corrected statistically and the values are given in the same Table.

Equilibrium constants for ionization of successive N(1)-H sites in the four inosines of complex S

Potentiometric titration of S yielded a composite pK_a value for the complex (half equivalence point) of 7.93. This value could be separated into the four step-wise N(1)-H acidity constants using standard methods[33b,35,36] which were adjusted for temperature[37] to provide the values given in Table 3 and used as invariant inputs in the curve fitting[34] to eqn (2) for the kinetics of exchange. The initial microscopic acidity constants, corrected to 60 °C, were the values used in the kinetic treatment (in eqn (2)). However, for purposes of further discussion, these values were also statistically corrected for the number of N(1)-H acidic sites in the complex, p, and the equivalent base sites in the resultant conjugate base, q. Therefore, complex S has four acidic sites ($p = 4$) and its conjugate base has only one basic site ($q = 1$), the statistical correction factor (p/q) is four and the logarithm of this value is added to the initial pK_a for S to yield the statistically-corrected value.

In the following discussion, we consider the origin of the attenuation in rate constants for H–D exchange as a function of sequential N(1)-H ionization in the four inosine moieties of the complexes $S \rightarrow S_1 \rightarrow S_2 \rightarrow S_3 \rightarrow S_4$.

Discussion

1. Overview of sequential N(1)-H ionization and C(8)-H exchange in tetrakis(inosine)platinum(II) chloride complex.

1a. Principles. In the $(ino)_4Pt(II)$ system under study, where the basicity of the medium (pD) substantially overlaps the composite

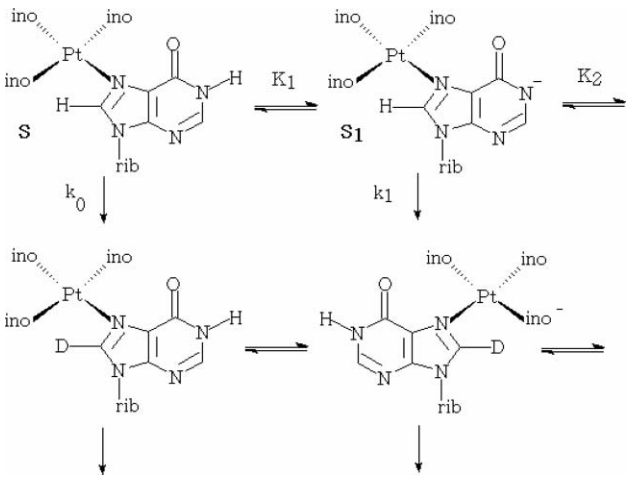
pK_a for the complex, four distinct N(1)-H ionized species may be expected to be present to some degree. Consequently, S in which none of the four inosine ligands has undergone ionization of the N(1) proton, S_1 in which one of these sites has ionized, S_2 where two of these sites have ionized and so forth up to S_4 in which all possible N(1) protons have ionized, may equilibrate. Thus, in solution all five species may be present simultaneously; the speciation is, of course, pH/pD dependent (*vide supra*). In terms of Scheme 2, the four inosine rings have been arbitrarily labeled from "A" to "D" and ionization of N(1)-H of the "A" inosine of S gives rise to the singly-deprotonated species designated S_1. The arbitrary nature of the designation of the inosine rings means that the structure shown as S_1 in Scheme 2 must be taken to represent any of the possible singly-deprotonated species. Therefore, deprotonation of N(1)-H of the "B", "C" or "D" rings is phenomenologically equivalent to deprotonation of the nitrogen of the "A" ring as depicted in Scheme 2. This caveat applies to all of the structures shown in Schemes 2 and 3. These equilibria are established in diffusion-controlled processes, contrasting with the rate processes to be considered below.

In reviewing the acid–base and metal ion complexation behaviour of nucleosides and related biomolecules,[38–43] Sigel and Griesser[44] considered the effects of complexation of platinum at N(7) of inosine on its N(1)-H acidity. Specifically, the pK_a difference (ΔpK_a) between N(1)-H of the inosine ligand of the diethylenetriamine (dien) inosine platinum complex ion [(dien)Pt(ino)$^{2+}$] and neutral inosine was found to be 1.52 ± 0.1.[45,46] Similar effects of Pt binding to N(7) on N(1)-H acidity were also reported for the related platinum 9-methylhypoxanthine complex [(dien)Pt(9-methylhypoxanthine)$^{2+}$], that differs from the inosine Pt complex only in the replacement of the N(9) ribose of the latter by a methyl group. This similarity emphasizes the lack of influence of the N(9) ribose on the N(1)-H acidity, while also indicating that this pK_a directly connects the effect of N(7) platination on N(1)-H ionization.

Comparison can be made to the neutral tetrakis(ino)$PtCl_2$, S, where the first deconvoluted pK_a (7.07; first column, first entry, Table 3) is the appropriate value; it represents deprotonation of a single N(1)-H site from any of the four inosine ligands. The N(1)-H pK_a for inosine at 34 °C,[47] corrected to 60 °C,[37] has a value of 8.65. Hence, $\Delta pK_a = pK_a(\text{inosine}) - pK_a(\text{S}) = 8.65 - 7.07 = 1.58$, a value well within the limits found by Sigel and coworkers. The acidifying effect of platinum for the first deprotonation of N(1)-H of S agrees with that found for other platinum complexes that contain a single structurally similar ligand and confirms the current assessment of the step-wise ionization constants in this tetrakis(inosine)platinum chloride system.

The H–D exchange process at C(8) of the inosine moieties is illustrated in Scheme 3. As shown generally in Scheme 1, C(8)-H → C(8)-D exchange occurs through slow rate-determining proton abstraction by base to give the resonance-stabilized ylide-like intermediate (Fig. 1), which is then rapidly deuterated through reaction with solvent D_2O. Since the species S through S_4 may all be present in solution to some extent, each may be expected to be a candidate for H–D exchange. However, while our kinetic treatment assumes S_4 is H–D exchange inert, access *via* equilibration (S_4, S_3, *etc.*) leads to other vertical exchange avenues so that SD_4 is the final product, as observed in the NMR experiments, *i.e.* S → S_1D → S_2D_2 → S_3D_3 → S_4D_4.

In summary of the above, one can combine Schemes 2 and 3 in an abbreviated fashion as Scheme 4 which highlights both the horizontal [N(1)-H ionization] and vertical [C(8)-H exchange] processes outlined.



Scheme 4

1b. Electrostatic considerations. As a first approximation, the abstraction of a C(8) proton from the various species by deuteroxide may be considered on the basis of electrostatic attraction or repulsion. In the case of S, the $(ino)_4Pt(II)$ complex bears an overall 2+ charge and electrostatic attraction should favor approach of OD^-. As more N(1)-H centres ionize, in turn (S → S_1 → S_2 → S_3 → S_4), the overall charge on the complex becomes progressively more negative and, consequently, abstraction by OD^- becomes progressively less favorable. In fact, when species S_4 is reached, it is reasonable to presume that now the electrostatic repulsion between OD^- and this doubly-negatively charged complex raises the energy barrier such that this process makes a negligible contribution to the overall rate (k_{obs}) of exchange.

Electrostatic considerations will dictate that approach of OD^- to a potential C(8)-H exchange site should be less favorable for an inosine ring that itself already bears a negative charge as a result of N(1)-H ionization. Therefore, in Scheme 3, the exchange process for S_1, in which the N(1)-H of the A ring has ionized, leads to the structure S_1D; the C(8)-H of this A ring is effectively immunized against exchange. Instead, exchange occurs at C(8)-H of any other inosine ring; in Scheme 3, we have depicted arbitrarily the B ring as having undergone exchange (*vide supra*).

Thus, the N(1)-H ionization exerts both a localized and overall effect (*i.e.*, immunization of a given ring as compared to overall electrostatic repulsion) on susceptibility to exchange. Concomitantly, this would progressively raise the activation barriers to exchange in turn for each species. The barrier to exchange at S_4 is the highest of that for any of the species and it is a necessary assumption of our treatment of the kinetic data that S_4 is, for practical purposes, treated as inert to exchange under our reaction conditions according to the kinetic Scheme 3 (*vide infra*).

1c. Development of kinetic Scheme 3. In developing Scheme 3, it was assumed that if the N(1)-H site was ionized, the (A) ring was immunized against isotopic exchange at C(8). Therefore, the rate constants after statistical correction represent the rate constant

for exchange from the complex in which the platinum can exert its full metal activating factor (*maf*) on the exchange (k_0), as well as the rate constants for exchange at the positions in the complexed inosine rings not containing an ionized N(1)-H site. This rate constant, k_0, is itself a composite one, in that it represents the exchange in going from S to S*D* (vertical processes in Scheme 3), as well as that for conversion of S*D* into SD_2 and so forth. While these individual rate constants may be different, they should differ primarily as a result of an isotope effect that is assumed to be small. In brief, the rate constants, k_1 to k_3, reflect the influence of the ionized N(1) sites in mitigating *maf* attributed to N(7)-Pt. These constants are still composite in nature, including k_{ex} at all exchangeable sites in the initial substrate, *i.e.* the rate constants for the sequential exchange from $S_1 \rightarrow S_1D \rightarrow S_1D_2 \rightarrow S_1D_3$ are included in the single rate coefficient, k_1. Similar arguments apply to the constants, k_2 and k_3 (Scheme 3).

In consideration of Scheme 3 it can be seen that partially deuterated species such as S_1D, where C(8)-H of the B ring has been shown to have exchanged, can equilibrate with S*D*, where C(8)-H of the A ring can now exchange, since N(1)-H is no longer ionized, effectively leading to SD_2. In this way, through a combination of these horizontal equilibria and the vertical H–D exchange processes (Scheme 4), each inosine ring may ultimately be deuterated at C(8). In similar fashion, any of the N(1) ionized species will eventually end up as SD_4. In fact, experimentally, the NMR spectra show progressive decrease in the C(8)-H signal until it can no longer be observed and deuteration is complete.

Fruitful comparisons arise between the second-order rate constants for deprotonation at C(2) for the "parent azoles" like imidazole and the second-order rate constant for exchange at C(8) for S, k_0, where the constant has been statistically corrected (Table 3). The Amyes–Richard group[24b] recently re-examined the kinetics of exchange in the parent imidazole and measured a rate constant for deprotonation at C(2) by deuteroxide of 36.9 M^{-1} s^{-1} at 25 °C, where the nitrogen adjacent to the nascent carbanionic centre is protonated (*cf.* Path H in Scheme 1, R = H). Replacement of the activating proton by a methyl group, as well as a methylation of the other nitrogen site, yields *N*,*N*-dimethylimidazolium cation (DMI); the deprotonation rate constant here is less than a magnitude larger (247 M^{-1} s^{-1} at the same temperature) than that of the imidazolium cation.[24b] This order parallels that of the rate constant ratios that define *paf* and the corresponding methyl activating factor.[4] Note, however, that generally, *paf* is significantly greater than the *metal* activating factor (*maf*).[4,6] Benzo fusion to the DMI ring (to give DMBI) adds a further enhancement in the rate constant (5.71×10^3 M^{-1} s^{-1} at 25 °C).[24b]

Correction of the imidazolium rate constant to the temperature of the current study, 60 °C, using the activation energy reported by Noszal and Rabenstein (21.4 kcal mol^{-1}),[38] gives a value of 1.64×10^3 M^{-1} s^{-1} which is, as expected on the basis of comparison of typical *paf* and *maf* reactivity ratios,[4,6] greater than k_0 for S (5.25×10^2 M^{-1} s^{-1}). On the other hand, based on the kinetic result for DMBI the difference in reactivity may be lower than these values suggest, in that the N(7)-bound inosine moiety is structurally more similar to the DMBI cation than to the parent imidazolium cation.

In the next section, the *maf* for the tetrakis(inosine)Pt(II) complexes and the effect of N(1) deprotonation will be discussed in combination.

2. Linear free energy relationship: N(1) ionization attenuates Pt activation

It is clear from the foregoing kinetic results in the tetrakis(inosine)Pt(II) system that coordination of platinum at N(7) of the inosine moieties activates all the C(8)-H purine positions to deuterium exchange. The full *maf* is seen in exchange involving the unionized complex S, where the second-order rate constant for H–D exchange, k_0 (525 M^{-1} s^{-1}), is the largest of the set (Table 2). Equally clear, deprotonation at N(1) partly mitigates the activating effect of Pt on the remaining C(8) sites. Hence, in S_1 where N(1)-H of the A ring taken to be equivalent to the same site in any inosine ring, is deprotonated (Scheme 3), C(8)-H of rings B, C and D all become less responsive to H–D exchange. As a result, k_1 is now 167 M^{-1} s^{-1}, a decrease of 68% in the rate constant for C(8)-H exchange compared to k_0. It is plausible to propose that this progressive attenuation in Pt activation of exchange arises from a combination of through-space and through-bond transmission of electron density. As we have shown above (*i.e.* ΔpK_a argument), the N(1)-H acidity is clearly coupled to platinum binding at N(7) and it is reasonable to presume that the increased electron density at N(1) attendant upon ionization would have a corresponding effect on N(7)-Pt.

As regards possible through-space transfer of electron density to the central Pt of the complex, it is clear that delocalization of the N(1) charge into the adjacent amide-like carbonyl could place negative charge density in closer spatial proximity to Pt. This through-space effect is depicted in Fig. 3.

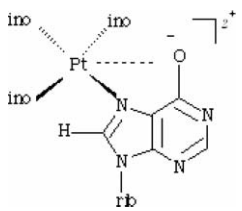


Fig. 3 Proposed delocalization of negative charge from an ionized N(1) site to an adjacent carbonyl and transfer of electron density to Pt *via* a through-space mode.

On going to S_2, where two N(1)-H sites are now deprotonated, the increased negative charge further mitigates the activating effect of Pt on the two remaining exchangeable C(8)-H sites in rings C and D (Scheme 3). This gives a further decrease (58%) in H–D exchange from S_2 relative to S_1. Transmission of the attenuation in *maf* continues as we proceed to S_3 (41% decrease). Finally, in this treatment, S_4 becomes fully immunized to H–D exchange.

Although it is an assumption of the kinetic analysis (*vide infra*) that S_4 is immunized against H–D exchange at C(8), the analysis can be extended to S_4 as an exchange-susceptible species. Calculation of $\Delta G^{\ddagger}$ *via* the Eyring equation,[48] using k_0 to k_3 values, gives a line ($R^2 = 0.977$) on plotting the activation free energies against the number of non-ionized positions with an intercept (corresponding to $n = 0$ for S_4) of 17.5 kcal mol^{-1} and an estimated second-order rate constant for C(8)-H exchange of 15.0 M^{-1} s^{-1}. Therefore, there is a decline in second-order exchange rate constants that occurs in decreasing increments in going down the series: from S $\rightarrow$ S_1 ($k_0 = 525$), $S_1 \rightarrow S_2$ ($k_1 = 167$), $S_2 \rightarrow S_3$ ($k_2 = 70$), $S_3 \rightarrow S_4$ ($k_3 = 41$) and, now, S_4 (estimated $k_4 = 15$).

Thus, $k_4/k_0 \times 100\% = 2.9\%$, *i.e.* within experimental error, which would further justify the assumption in our treatment.

The measure of the attenuation of Pt activation is quantified *via* the plot (Fig. 4) depicting the dependence of log k_{ex} (Table 2) on pK_a for the step-wise N(1)-H acidity constant K_1 through K_4 (Table 3). It is remarkable that this plot that relates deuterium isotopic exchange at C(8)-H sites remote from the ionized N(1) site shows good linearity ($R^2 = 0.99$). The relationship between log k for exchange and the pK_a values for N(1)-H ionization constitutes a linear free energy relationship (LFER). The slopes of such LFERs are normally taken to be a measure of sensitivity of the process to systematic modulation of electronic and structural effects. In this case, the large and negative slope (−2.3) reflects the high sensitivity of the effect of transmission of electron density from N(1), following deprotonation, *via* platinum and/or through space to the remaining C(8)-H sites. This follows from the significant *maf* afforded by Pt for exchange in these complexes. While *maf* is fully expressed in the unionized platinum complex, S, it is reasonable that the observed progressive mitigation of its effect through N(1)-H ionization will result as one moves to S_1 and then to S_2 and finally to S_3.

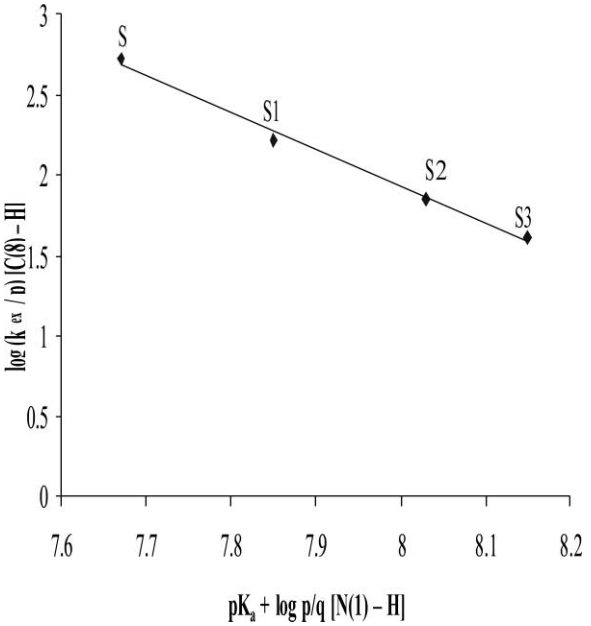


Fig. 4 Logarithmic plots of C(8)-H isotopic exchange (k_{obs}) in complex S and the successively N(1) deprotonated forms (S_1, S_2, S_3) *versus* N(1)-H pK_a, both statistically corrected (Tables 2 and 3).

Conclusions

To our knowledge, this tetrakis(inosine)platinum(II)/OD^- system represents the first instance in which such a large attenuation in *maf* has been demonstrated. Uniquely, this has been achieved through progressive modulation through pK_a (pD) changes of a single molecule, $(ino)_4Pt(II)Cl_2$. The outstanding feature of the current LFER (Fig. 4) is that such a seemingly minor change as ionizing N(1)-H remote from exchangeable C(8)-H in this very large molecule, manifests itself so visibly. Without platinum, the system reverts to inosine alone; no *maf* is possible. Clearly, the Pt atom of the series of complexes must play a major role in transmission of attenuation of *maf* to the C(8)-H centres caused by successive N(1)-H ionizations. Our study exhibits that Pt acts as conductor of any through-bond (or in the manner of Fig. 3, any through-space) transfer of electron density to the remaining C(8)-H reaction centers.

Future work to explore this LFER approach would involve systematic replacement of the central Pt by other metals. In this regard, Sigel and coworkers have found that the pK_a value that quantifies the metal effect on N(1)-H acidity is similar for Pd and Pt complexes.[45] Therefore, it is reasonable to anticipate a similar LFER to emerge from a study of the tetrakis(inosine)palladium complex. Equally, such work could involve not only inosine, but other purine or related ligand structures that contain both ionizable and exchangeable sites remote from one another. The LFER found here, consequently, may become a tool not only for investigating, but also predicting either N-H acidity or C-H H–D exchange susceptibility in a range of metal–biomolecule complexes and, in turn, carbon acidity in these complexes.

Experimental

Materials and instruments

Potassium tetrachloroplatinate (K_2PtCl_4; >99.9%) was purchased from Johnson Matthey, while inosine (99%), $Na_2HPO_4 \cdot H_2O$(99.9%) and NaH_2PO_4 (99.9%) were obtained from Sigma. NaOH and KOH pellets were purchased from BDH (Analar grade). Deuterium oxide (99.9 atom%) used for spectroscopic and kinetic studies was available commercially from Matheson Inc. All other reagents and solvents were obtained from commercial sources and used without further purification.

Products were characterized by melting point (uncorrected; Thomas-Hoover capillary melting point apparatus), ^{1}H NMR spectroscopy and elemental analysis (Canadian Microanalytical Services, Delta, B.C.) ^{1}H NMR spectra were recorded using a Bruker ACF-200 NMR spectrometer, operating at 200.1 MHz; chemical shifts (δ) are given in parts per million and the numbering cited below follows that given for inosine in the text above. Measurements of pH were made using either Acumet-90 (Fisher Scientific) or Φ 71 (Beckman) pH meters. The pH meters were calibrated as described below.

Preparation of Pt(II) complexes

cis-Dichlorobis(inosine)platinum(II) chloride, $[Pt(ino)_2Cl_2]Cl_2$

Inosine (0.536 g, 2.00 mmol) was added to distilled water (*ca.* 5 mL) and heated until the solid was fully dissolved, at which point a filtered aqueous solution (*ca.* 5 mL) of K_2PtCl_4 (0.415 g, 1.00 mmol) was added. The reddish-colored mixture was allowed to stand for 2 days at room temperature during which time the color changed to yellow. This solution was evaporated to dryness (*in vacuo*), the residue was partly re-dissolved in DMF (5 mL), filtered and excess ethanol (50 mL) was added to the filtrate to precipitate the crude product. The precipitate was filtered, washed, in turn, with copious amounts of dry ethanol and anhydrous diethyl ether and then dried *in vacuo* to yield 0.386 g (48%) of

a pale yellow powder. The ^{1}H NMR spectrum (D_2O–TSP) was in good agreement with that reported in the literature.[49]

^{13}C NMR (D_2O–TSP, ribose portion), δ: 90.38 (C-1′), 75.61 (C-2′), 70.67 (C-3′), 86.33 (C-4′), 61.76 (C-5′).

Tetrakis(inosine)platinum(II) chloride pentahydrate, $[Pt(ino)_4]Cl_2 \cdot 5H_2O$

To a solution of $[Pt(ino)_2Cl_2]Cl_2$ (0.10 g, 0.12 mmol) in water (*ca.* 20 mL) was added inosine (0.0668 g, 0.250 mmol); the resultant mixture was stirred and heated at 60 °C for 3 days. The solution was allowed to cool to room temperature, filtered and excess acetone (400 mL) was added to the filtrate. The white solid obtained was washed with copious amounts of acetone and anhydrous diethyl ether and dried *in vacuo* to yield 0.132 g (88%) of the crude tetrakis(inosine) complex. The complex was purified by redissolution in a minimum of distilled water followed by dropwise addition of acetone to precipitate 0.110 g (72% of crude) of colorless needles. Mp 188–190 °C (d).

^{1}H NMR (D_2O–TSP), δ: 8.93 (H-8, s), 8.23 (H-2, s), 6.09 (H-1′,d), 4.59 (H-2′, t), 4.36 (H-3′,t), 4.24 (H-4′, br q), 3.83 (H-5′,5″, m); in good agreement with reported literature.[49]

Anal. Calc. for $C_{40}H_{58}N_{16}O_{25}Cl_2Pt$, %: C (33.85), H (4.09), N (15.80).

Found %: C (32.98), H (3.79), N (15.62).

Kinetic determination of isotopic exchange

Measurements of isotopic C(8)-H exchange in the $(ino)_4PtCl_2$ complex were performed in aqueous phosphate buffers (pD 6–9) at 60 °C in deuterium oxide by monitoring the disappearance of the C(8)-H signal by ^{1}H NMR spectroscopy. The phosphate salts used in preparation of the buffer solutions were initially exchanged with deuterium by dissolution in a large excess of D_2O and subsequent removal of the solvent *in vacuo*. The buffer solutions were prepared from these isolated deuterated salts inside an Ar-filled glove box, in 10 mL volumetric flasks according to standard recipes.[50] The pH values in D_2O were measured at room temperature using a pH meter calibrated with standard aqueous (H_2O) buffers (BDH) of pH 4.0, 7.0 and 10.0, and are uncorrected for solvent isotope effects. The corresponding pD values were calculated by adding 0.4 to the pH meter readings.[27] The resultant pD values were corrected for temperature.[28]

Kinetic measurements required the dissolution of *ca.* 10 mg of the complex in 0.5 mL of the appropriate buffer solution followed by rapid shaking and the insertion of the tube into the variable temperature NMR probe with probe temperature equilibrated at 60 °C. The adjustment of operating parameters was as described previously.[4] Spectra were acquired as FIDs at preset intervals using the NMR kinetic program ASREACT1H.AU.[51] FIDs were Fourier transformed and degree of exchange at each time was obtained from the ratio of the electronically-determined peak area of the exchanging C(8)-H to that of the non-exchanging ribose C(1′)-H. The pseudo-first order rate constants for C(8) H–D exchange (k_{obs}) were determined from the negative slope of the plot of the logarithm of the ratio of the C-H8 : C-H1′ peak areas *versus* time, *i.e.* $k_{obs} = -2.303 \times$ slope. The microscopic rate constants for C(8)-H–D exchange from the four equivalent C(8) positions of the tetrakis(inosine) complex (k_0, k_1, k_2, k_3, defined by Scheme 3) were determined from iterative non-linear fit of the experimental acid dissociation constants (K_1, K_2, K_3, K_4, defined by Scheme 2) to eqn 2 (see above). The resultant microscopic rate constants were corrected statistically, as the acid dissociation constants also had been.

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