Recognition of platinum(ii) amine complexes by nucleotides: role of phosphate and carbonyl groups in ([$^{15}N_3$]diethylenetriamine)-(guanosine 5′-monophosphate)platinum(ii)

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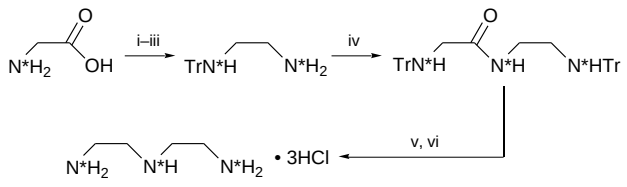
Two-dimensional [^{1}H,^{15}N] NMR studies show that in the model monofunctional DNA adduct [Pt([$^{15}N_3$]dien)(5′-GMP-*N*7)]$^{2+}$ [5′-GMP = guanosine 5′-monophosphate, dien = 1,5-diamino-3-azapentane (prepared by a novel synthesis from glycine)], stereospecific hydrogen-bonding interactions involving Pt–NH protons are promoted by deprotonation of both the 5′-phosphate group and N^{1}H of coordinated 5′-GMP.

A surprising recent finding is that monofunctional adducts of $[PtCl_2(NH_3)_2]$ (cisplatin) and $[Pt(NH_3)_2(H_2O)_2]^{2+}$ with one of the guanine bases in GG oligonucleotides can be very long lived.[1,2] It is therefore important to understand the factors which lead to stabilization of particular adducts between platinum am(m)ine complexes and DNA. Complexes of $[Pt(dien)]^{2+}$ with nucleotides have been widely studied since they represent stable monofunctional adducts, and the ability of $[Pt(dien)]^{2+}$ to introduce local denaturation of DNA has been demonstrated, for example by Brabec *et al*. using chemical probes.[3]

The NH groups on platinum am(m)ine ligands are thought to play a major role in determining the nature of the adducts *via* hydrogen-bonding interactions with DNA,[4] and we have shown previously that these can be investigated in solution using two-dimensional [^{1}H,^{15}N] NMR and ^{15}N-labelled ligands.[5] Use of this method with dien complexes is complicated by the lack of an efficient synthesis of dien which can be adapted to ^{15}N labelling. We report here briefly a novel efficient synthesis of [$^{15}N_3$]dien, and show that use of this allows an NMR study of hydrogen-bonding interactions in nucleotide complexes such as [Pt([$^{15}N_3$]dien)(5′-GMP-*N*7)]$^{2+}$. The NMR data implicate both the 5′-phosphate and C^6 carbonyl groups in pH-dependent stereospecific interactions with the Pt–NH_2 groups.

^{15}N-Labelled dien cannot readily be prepared by the Gabriel method[6] since [^{15}N]diethanolamine is required as a starting material. The preparation of this from ethylene oxide and excess $^{15}NH_3$ is uneconomic since the yield is low, the product cannot be readily separated from ethanolamine and triethanolamine, and the intermediate 2,2′-dichlorodiethylamine is unstable and difficult to handle. Therefore we have developed the new method shown in Scheme 1, which uses [^{15}N]glycine as starting material. The trityl group was used to protect the amino group throughout the synthesis. Amidation of [^{15}N]tritylglycine with $^{15}NH_3$ afforded [$^{15}N_2$]tritylglycinamide, which was then reduced to [$^{15}N_2$]monotritylethylenediamine by $LiAlH_4$. 1-([^{15}N]Tritylglycyl)-2-([$^{15}N_2$]trityl)ethylenediamine was obtained as a condensation product from [$^{15}N_2$]monotritylethylenediamine and [^{15}N]tritylglycine. After reduction and cleavage of the protecting group, the hydrochloride salt of [$^{15}N_3$]dien was obtained in 20% overall yield based on [^{15}N]glycine. Full details of the synthesis will be published elsewhere.



Scheme 1 *Reagents and conditions*: i, TrCl, Tr = Trityl, Pr^{i}OH–H_2O, room temp.; ii, ethyl chloroformate, $^{15}NH_3$, $CHCl_3$, room temp.; iii, $LiAlH_4$, thf, room temp.; iv, ethyl chloroformate, ^{15}N-tritylglycine, $CHCl_3$, room temp.; v, $LiAlH_4$, thf, 75 °C; vi, 4.8 m HCl, reflux

The platinum complex [Pt([$^{15}N_3$]dien)Cl]Cl was prepared according to the literature method for the unlabelled complex.[7] The two-dimensional [^{1}H,^{15}N] HSQC NMR spectrum of a fresh solution (*i.e*. prior to hydrolysis) of [Pt([$^{15}N_3$]dien)Cl]$^+$ **1** in H_2O–D_2O (9 : 1) showed three sets of cross-peaks at δ 5.23/−33.9, 5.02/−33.9 and 6.65/8.13 (see Fig. 1). On the basis of their shifts,[8] the former two peaks can be assigned to non-equivalent protons on the two NH_2 groups, and the latter to the NH group.

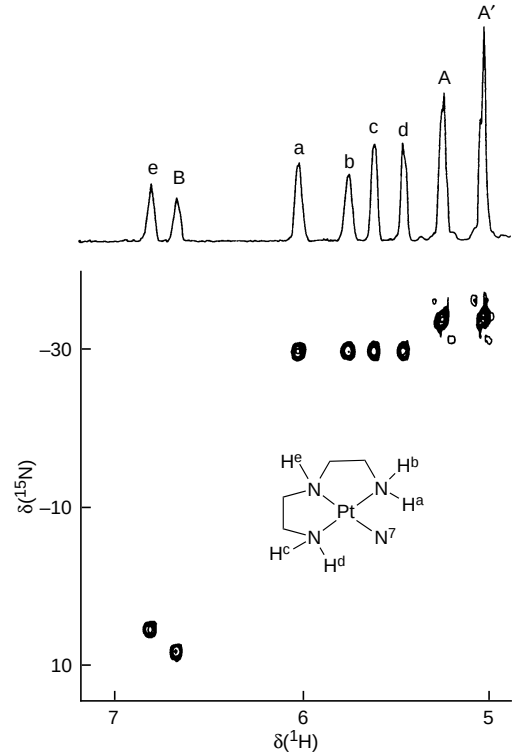


Fig. 1 Reaction of [Pt([$^{15}N_3$]dien)Cl]$^+$ **1** with 5′-GMP. This two-dimensional [^{1}H,^{15}N] HSQC NMR spectrum was recorded 1.2 h after mixing and shows NH_2 and NH peaks for complex **1** labelled **A**, **A′** and **B**, respectively, and for the GMP adduct **2** labelled **a**–**e** (all five NH protons are non-equivalent). After 12 h the reaction was complete and only peaks for **2** were observable. The large downfield shift of peak **a** is notable. ^{195}Pt satellites in both the ^{1}H and ^{15}N dimensions are evident for complex **1** but not for **2** (satellites broaden with increases in molecular size and chemical shift anisotropy). *Conditions*: mol ratio Pt : GMP 0.8 : 1 (5 mm), H_2O–D_2O (9 : 1), pH 6.83, 298 K.

As an initial model for DNA binding, we studied the adduct formed from the reaction of 5′-GMP with **1** (5 mm, 1 : 1 mol ratio) at pH 6.8, 298 K. The two-dimensional spectrum after 1.2 h of reaction is shown in Fig. 1, where peaks assignable to the starting material **1** and to the product [Pt([$^{15}N_3$]dien)(GMP-*N*^{7})]$^{2+}$ **2** are well resolved. Most notable is the presence of five cross-peaks for **2** showing that all the NH protons are magnetically non-equivalent. This might be expected in view of the chirality of the bound nucleotide. In order to understand the origin of the low-field shifts of the peaks for **2**, their pH dependence was studied over the range pH 3–11. There was little change in the ^{1}H,^{15}N shift of peak **e** assigned to the central NH group over the range pH 3–9.5, but above this pH it broadened and disappeared, probably due to an increased exchange rate. The behaviour of the NH_2 peaks **a**, **b**, **c** and **d** is shown in Fig. 2. All four peaks show two-step titrations with pK_a values of 5.58 and 8.54 (averages for peaks **a** and **c**). The shift changes for peaks **a** and **c** are much larger than those for peaks **b** and **d**.

The two pK_a values can be associated with deprotonation of the 5′-phosphate (5.58) and the N^{1}H group (8.54) of coordinated 5′-GMP in **2**. For comparison, the phosphate pK_a value for [Pt(en)(5′-GMP)$_2$]$^{2+}$ is 0.38 units lower than the value for free 5′-GMP (6.20) indicative of the presence of Pt–NH···ℑ-phosphate hydrogen bonding.[5] There are previous reports that the pK_a of N^{1}H of guanine (*ca*. 9.5) is lowered by *ca*. 0.9–1.3 when guanine derivatives are coordinated to Pt^{II} *via* N^7.[9] The large low-field PtNH$_2$ ^{1}H NMR shifts observed for complex **2** (peaks **a** and **c**) on deprotonation of the N^{1}H group of bound GMP are intriguing since these PtNH$_2$ protons appear to be far away from N^1. This can be explained by an increase in the strength of hydrogen bonding between PtNH$_2$ and the C^6 carbonyl group due to an increase in electron density at C^{6}O on deprotonation of N^1. It is assumed that there is free rotation about Pt–N^7 and therefore both NH$_2$ groups are involved in hydrogen bonding. Keto–enol tautomerism could increase the negative charge on C^{6}O, as illustrated in the structure presented for complex **2**. Such hydrogen bonding involving C^{6}O has been found to play a role in determining the structure of Pt am(m)ine nucleotide complexes in the crystalline state, occurring for example in [Pt(dien){AGA-*N*^{7}(2)}],[10] but evidence for its presence in solution has previously proved difficult to obtain. The present data appear to provide evidence for such interactions at pH values close to those of biological relevance. Related NH···carbonyl interactions have been reported to play a major role in the stabilization of ternary complexes of zinc(ii) cyclen complexes with thymine derivatives.[11]

The methods described here should also readily allow studies of chelate ring-opening reactions of [Pt([$^{15}N_3$]dien)]$^{2+}$ complexes. These have been the subject of much recent interest.[12] It is notable from our preliminary work that complex **2** showed no evidence for ring opening at pH 3.

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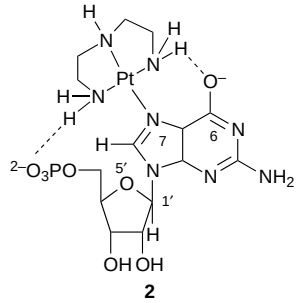

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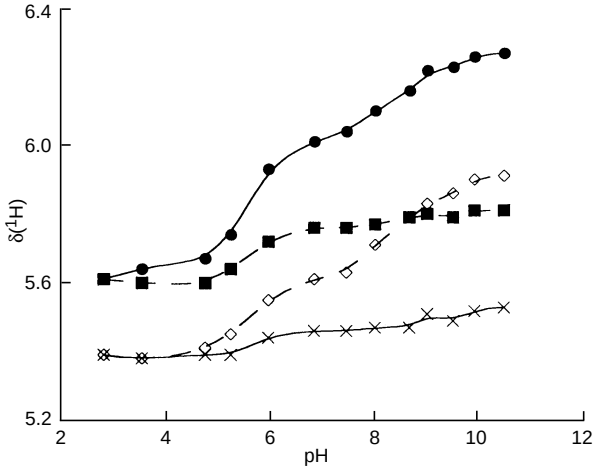

Fig. 2 Dependence of the NH ^{1}H NMR chemical shifts of complex **2** [Pt([$^{15}N_3$]dien)(5′-GMP-*N*^{7})]$^{2+}$ on pH; peak **a** (●), **b** (■) **c** (◇), **d** (×). The large downfield shifts of two of the resonances between pH 5 and 7 can be associated with deprotonation of the 5′-phosphate group, and between 7 and 9 with deprotonation of N^{1}H of GMP. Little shift of peak **e** (Fig. 1) was observed over this pH range.

Footnote

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References

1 F. Gonnet, F. Reeder, J. Kozelka and J.-C. Chottard, *Inorg. Chem*., 1996, **35**, 1653; F. Gonnet, F. Reeder, J. Kozelka and J.-C. Chottard, *Chem. Eur. J*., 1996, **2**, 1068.
2 K. J. Barnham, S. J. Berners-Price, T. A. Frenkiel, U. Frey and P. J. Sadler, *Angew. Int., Ed. Ed. Engl*., 1995, **34**, 1874; S. J. Berners-Price, K. J. Barnham, U. Frey and P. J. Sadler, *Chem. Eur. J*., 1996, **2**, 1283.
3 V. Brabec, V. Boudny and Z. Balcarova, *Biochemistry*, 1994, **33**, 1316.
4 J. Reedijk, *Inorg. Chim. Acta*, 1992, **198–200**, 873.
5 S. J. Berners-Price, J. D. Ranford, U. Frey and P. J. Sadler, *J. Am. Chem. Soc*., 1993, **115**, 9285; S. J. Berners-Price, J. D. Ranford and P. J. Sadler, *Inorg. Chem*., 1994, **33**, 5842.
6 F. G. Mann, *J. Chem. Soc*., 1934, 461.
7 G. Annibale, M. Brandolisio and B. Pitteri, *Polyhedron*, 1995, **14**, 451.
8 S. J. Berners-Price and P. J. Sadler, *Coord. Chem. Rev*., 1996, **151**, 1.
9 K. Inagaki and Y. Kidani, *J. Inorg. Biochem*., 1979, **11**, 39; J.-C. Chottard, J.-P. Girault, G. Chottard, J.-Y. Lallemand and D. Mansuy, *J. Am. Chem. Soc*., 1980, **102**, 5565; J.-P. Girault, G. Chottard, J.-Y. Lallemand and J.-C. Chottard, *Biochemistry*, 1982, **21**, 1355; G. Schröder, B. Lippert, M. Sabat, C. J. L. Lock, R. Faggiani, B. Song and H. Sigel, *J. Chem. Soc., Dalton Trans*., 1995, 3767.
10 G. Admiraal, M. Alink, C. Altona, F. J. Dijt, C. J. van Garderen, R. A. G. de Graff and J. Reedijk, *J. Am. Chem. Soc*., 1992, **114**, 930.
11 M. Shionoya, E. Kimura and M. Shiro, *J. Am. Chem. Soc*., 1993, **115**, 6730; M. Shionoya, T. Ikeda, E. Kimura and M. Shiro, *J. Am. Chem. Soc*., 1994, **116**, 3848.
12 See for example, M. Mikola, J. Vihanto and J. Arpalahti, *J. Chem. Soc., Chem. Commun*., 1995, 1759.

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