Reaction of 4-picoline(2,2′:6′,2″-terpyridine)platinum(II) with nucleosides

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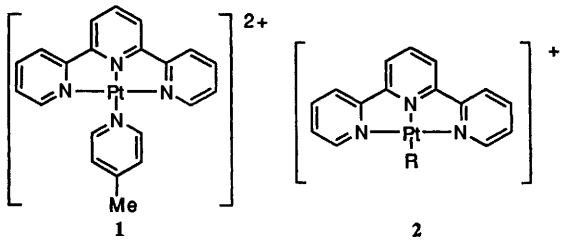
The four nucleosides found in DNA react with 4-picoline(2,2′:6′,2″-terpyridine)platinum(II). Guanosine and 2′-deoxyguanosine are platinated at N^7. Adenosine and 2′-deoxyadenosine are platinated at both N^1 and N^6 with loss of a proton and there is no evidence of monoplatinated intermediates. It is proposed that platination occurs initially at N^1, the most nucleophilic site, which leads to loss of a proton from the N^6 amino function and subsequent rapid platination at this site. This conclusion is supported by NMR and mass spectral evidence. 2′-Deoxycytidine is similarly platinated at N^3 and N^4 and a similar explanation is proposed. Platination of thymidine is very slow and the product(s) has not been characterised.

Introduction

4-Picoline(2,2′:6′,2″-terpyridine)platinum(II) **1** has recently been shown to be a potent intercalator of poly[d(A-T)$_2$], the equilibrium binding constant of 2×10^7 M^{-1} being greater than that of ethidium bromide, and more than two orders of magnitude greater than the corresponding monocationic complexes **2** (R = Cl or SCH_2CH_2OH). We have ascribed this increase in binding energy to the double positive charge on the intercalator. In the course of experiments to show that binding was due to intercalation, the binding constant was measured at increasing concentrations of sodium chloride. This experiment provided support for binding due to intercalation, but it also revealed that at high salt concentration *i.e.* above about 150 mM, the picoline ligand was displaced by chloride ion.[1] It has previously been observed that chloro(2,2′:6′,2″-terpyridine)-platinum(II) **2** (R = Cl) is 10^3 to 10^4 times more reactive to ligand substitution than chloro(diethylenetriamine)platinum(II).[2] A possible explanation of this phenomenon is that the terpyridine moiety can accept electron density from the metal ion into its π^* orbitals.[2,3] We now report that not only chloride ion will affect displacement of the picoline ligand but that the nucleosides, guanosine, 2′-deoxyguanosine, adenosine, 2′-deoxyadenosine and 2′-deoxycytidine are also effective nucleophiles under physiological conditions.



1

2

Results and discussion

During the course of our study of the interaction of **1** with DNA, it became apparent that the displacement of the picoline moiety presumably by a nucleobase of DNA was occurring. It had been suggested by Howe-Grant *et al.*[4] that chloro(2,2′:6′,2″-terpyridine)platinum(II) chloride **2** (R = Cl) reacts with DNA to form covalent Pt–DNA species although the sites of platination were not identified. ^{1}H NMR spectroscopy proved to be a useful technique to study these reactions because of the large chemical shift difference between the methyl resonance of free and complexed picoline in **1**. All the nucleosides studied (A, G, dA, dG, dC and T) were found to react with **1** to give platinated nucleosides. The reactions were slow, however, and only equilibrium mixtures of the platinated nucleosides and starting materials were obtained when equimolar amounts of **1** and nucleosides were incubated at 37 °C in the presence of sodium phosphate buffer at pH 5.5 for several days.

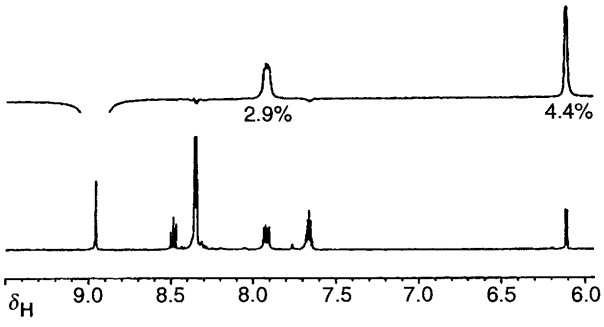


Fig. 1 NOE difference spectra (500 MHz, ^2H_2O) of N^7-Pt(terpy)-guanosine complex

Of all the nucleosides studied, guanosine and 2′-deoxyguanosine reacted with the Pt^{II} complex **1** in the most straightforward manner. The course of the reaction with guanosine is pH dependent. At pH ~ 6.5 or above several products were formed and the ^{1}H NMR spectrum was complicated. However, clean reaction occurs between pH 5.5–6. Isolation of the complex by gel filtration left it contaminated with significant amounts of starting materials. The identical Pt–G complex, however, was formed quantitatively by reacting guanosine and [Pt(terpy)-Cl]Cl **2** (R = Cl) in the presence of silver ion. ^{1}H NMR spectra of this yellow complex are shown in Fig. 1. The singlet at δ_H 8.95 was assigned to H^8 of the complexed guanosine. The relative integration suggests this is a 1 : 1 complex of guanosine and (2,2′:6′,2″-terpyridine)platinum(II) which is in agreement with mass spectral data. The large downfield shift of the H^8 resonance (~ 0.8 ppm from H^8 in guanosine) indicates binding of the (2,2′:6′,2″-terpyridine)platinum(II) at N^7 of guanosine as expected. The observed positive NOE enhancement of terpyridine-$H^{6,6''}$ signals at δ_H 7.92 (2.9%) upon irradiation at H^8 (Table 1) provides further supporting evidence for the structure **3** (X = OH). 2′-Deoxyguanosine reacts with **1** in a similar way giving a complex also with a large downfield shift

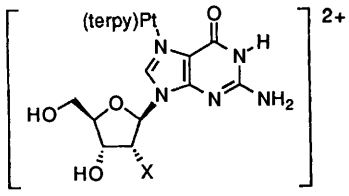
Table 1 Results of NOE experiments on platinum–nucleosides complexes. NOE difference spectra of the complexes were recorded in ^2H_2O at 500 MHz except for the 2′-deoxycytidine complex and irradiation of A-N^{6}H which were carried out in C^2H_3CN. Only selected enhancements were shown

	Proton irradiated	Observed NOE enhancement (%)		
		$H^{1'}$	H^8	$H^{6,6''}$ (N^7)
N^7-Pt(terpy)·G	H^8	4.4	—	2.9

	Proton irradiated	Observed NOE enhancement (%)				
		$H^{1'}$	H^2	H^8	$H^{6,6''}$ (N^1)	$H^{6,6''}$ (N^6)
N^1,N^6-[Pt(terpy)]$_2$·A	$H^{1'}$	—	0	2.3	0	0
	H^2	0	—	0	2.0	0
	H^8	4.5	0	0	0	0
	N^{6}H	0	0	0	0	12.6

	Proton irradiated	Observed NOE enhancement (%)				
		$H^{1'}$	H^5	H^6	$H^{6,6''}$ (N^3)	$H^{6,6''}$ (N^4)
N^3,N^4-[Pt(terpy)]$_2$·dC	$H^{1'}$	—	0	3.5	0	0
	H^5	0	—	10.2	0	0
	N^{4}H	0	15.5	0	0	11.8

of H^8, so the analogous structure **3** (X = H) is proposed. In both complexes no ^1H–^{195}Pt coupling was observed due to relaxation by chemical shift anisotropy which broadens the ^{195}Pt satellites. This is a well recognised phenomenon when high field NMR spectrometers are used.[5]



3

When adenosine reacted with 4-picoline(2,2′:6′,2″-terpyridine)platinum(II) **1** at pH 5.5 and 37 °C an orange–red product was obtained. The chemical shifts of both adenine CH protons in the complex changed significantly upon complexation. The singlet at δ_H 8.55 is assigned to the H^8 resonance since it was exchanged when heated in ^2H_2O at 85 °C for 6 h (Fig. 2).[5] The other singlet at lower field (δ_H 8.85) is, therefore, assigned to H^2. The large chemical shift difference (1.0 ppm downfield) of the H^2 proton compared with that of free adenosine provides good evidence that binding of platinum occurs at N^1 of adenine, although the proton–platinum coupling was again not observed. Coordination at N^3 was ruled out by analogy to several other transition metal–adenosine complexes.[6] The relative integration of terpyridine and adenine protons suggested that the ratio of (2,2′:6′,2″-terpyridine)platinum(II) to adenosine is 2:1. The assignment of terpyridine protons was assisted by a COSY experiment and this showed that both sets of proton resonances of each terpyridine system have very similar chemical shifts except the two sets of $H^{6,6''}$ resonances which are separated by 0.4 ppm suggesting a different environment of the two sets of $H^{6,6''}$. The more upfield $H^{6,6''}$ resonance appears at δ_H 8.05 which compares very well to other (2,2′:6′,2″ terpyridine)platinum(II) complexes bearing aromatic nitrogen ligands at the fourth coordination site such as **1** and **3**. This may be ascribed to the anisotropic effect of the aromatic fourth ligand, which, due to the steric requirement, has to rotate out of the plane of terpyridine ring system. The significantly higher chemical shift (δ_H 8.42) of the other terpyridine $H^{6,6''}$ suggests that the fourth ligand of this (2,2′:6′,2″ terpyridine)platinum(II) system is not a pyridine-type nitrogen ligand.

NOE difference spectra of the adenosine complex were also obtained. The positive NOE enhancement of the higher field terpyridine-$H^{6,6''}$ (2%) upon irradiation at adenine-H^2 (Table 1) suggests that this (2,2′:6′,2″ terpyridine)platinum(II) is attached to adenine-N^1 supporting the conclusion based on chemical shift evidence. The other (2,2′:6′,2″-terpyridine)platinum(II) $H^{6,6''}$ did not show any enhancement when adenosine

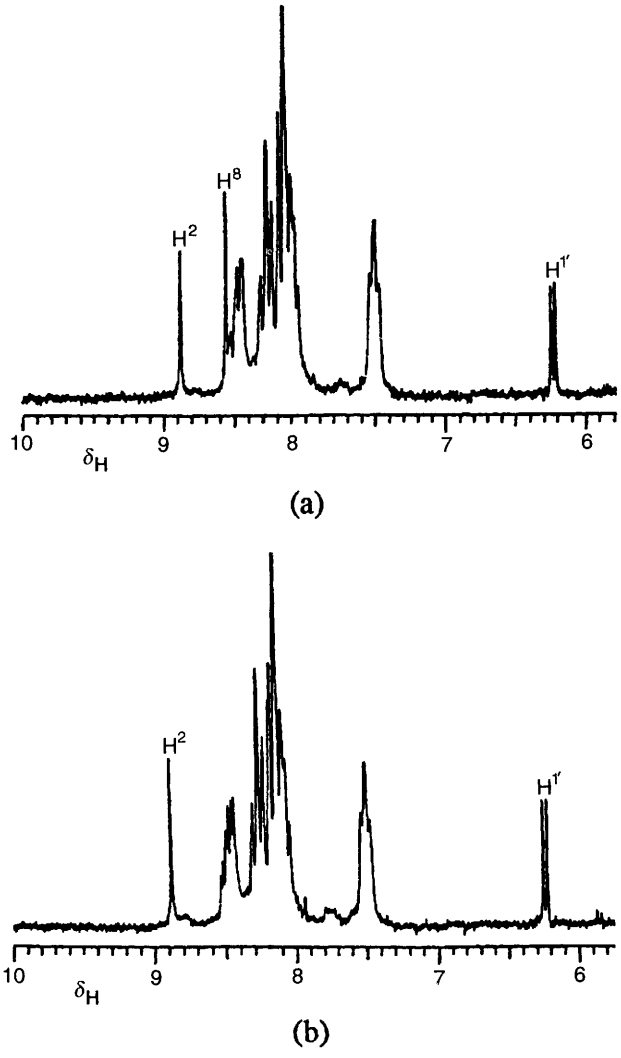
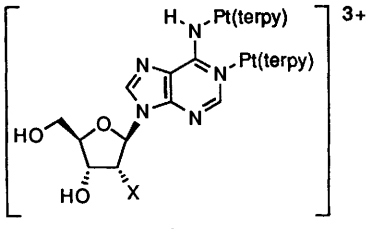


Fig. 2 Part of ^1H NMR spectra (200 MHz, ^2H_2O) of N^1,N^6-[Pt(terpy)]$_2$–adenosine complex (a) before (b) after deuterium exchange

H^2, H^8 and $H^{1'}$ were irradiated, suggesting that complexation does not occur at N^3 or N^7. This leaves only adenine N^6 for the second platination site which seems improbable at first since the adenine exocyclic amino group is a poor nucleophile due to the extensive delocalisation of the lone pair electrons on the heterocyclic ring. However, the electrospray mass spectrum of the complex revealed the molecular ion to be triply charged and with one mass unit less than the calculated mass of the expected quadruply charged complex, which suggested loss of a proton and hence the structure **4** (X = OH) for the complex. Although the proton loss could occur in the mass spectrometer, it is also possible that N^6 of adenosine may actually lose a proton because of platination at N^1, which would greatly increase the nucleophilicity of the former and allow it to react with the second (2,2′:6′,2″-terpyridine)platinum(II) rapidly. In order to confirm platination at N^6, NOE studies of the complex in aprotic solvents were undertaken to avoid the proton exchange. Initial attempts to synthesise this bis[(2,2′:6′,2″-terpyridine)platinum(II)] complex on a large scale from a reaction between the chloro Pt^{II} complex **2** (R = Cl) and adenosine in the presence of silver ion gave a complex mixture. Recognising the likely importance of deprotonation at N^6, the reaction was repeated in the presence of a base to facilitate proton abstraction. Indeed, upon addition of potassium carbonate to the reaction mixture, the bis[(2,2′:6′,2″-terpyridine)platinio(II)]-adenosine complex was obtained as the major product which was isolated as its hexafluorophosphate salt. Addition of the base is not necessary to induce proton abstraction in the reaction of **1** with adenosine probably because the displaced picoline would act as a base. ^{1}H NMR of the complex in C^2H_3CN clearly showed the adenine N^6H proton integrated for one proton. A subsequent NOE experiment in C^2H_3CN in which the N^6H was irradiated showed a positive enhancement (12.6%) of the lower field terpyridine $H^{6,6''}$ which confirmed the N^6 binding of the second (2,2′:6′,2″-terpyridine)platinum(II) (Table 1). 2′-Deoxyadenosine reacts with **1** or **2** (R = Cl) in a similar manner giving a bis-platinated complex with an identical ^{1}H NMR spectrum in the aromatic region. A NOESY experiment on this compound confirmed the same N^1, N^6 binding mode **4** (X = H) as in the adenosine complex discussed above.



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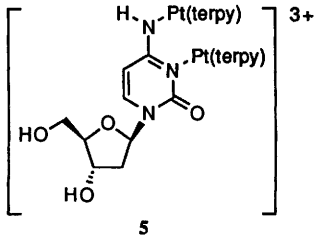
The reaction of the Pt(II) complex **1** with 2′-deoxycytidine under similar conditions also proceeded readily and the product crystallised from the reaction mixture on cooling as red microneedles. The ^{1}H NMR and electrospray mass spectra again suggested that this is a triply charged bis-platinated species with loss of one proton. The first binding site was tentatively assigned as N^3 of cytosine since this is the most nucleophilic site and all platinum–cytidine complexes reported to date were found to bind at this site.[6] The second binding site is most likely to be N^4 analogous to the adenosine complex giving the structure **5** for this bis[(2,2′:6′,2″-terpyridine)platinum(II)] complex. The binding at N^4 was confirmed by an NOE experiment in [$^2H_7$]DMF where the exchange of the 2′-deoxycytidine N^4H protons is slow. At room temperature the N^4H resonance was obscured by the terpyridine resonances but it was shifted upfield to a convenient position at 50 °C. Irradiation of this proton caused a positive NOE enhancement of cytosine-H^5 and one pair of terpyridine-$H^{6,6''}$ which provides conclusive evidence for N^4 binding. A NOE experiment of the same compound with hexafluorophosphate as counterion in C^2H_3CN gave similar results (Table 1) although in this solvent the spectrum is better resolved and the N^4H proton can be clearly seen at room temperature which also integrates as one proton. The 0.1 ppm chemical shift difference of the two sets of terpyridine $H^{6,6''}$ resonances in the 2′-deoxycytidine complex is less pronounced than in the adenosine complex and this is consistent with the less aromatic character of cytosine compared to adenine. Interestingly, the same product was also obtained by treatment of chloro(2,2′:6′,2″-terpyridine)platinum(II) chloride **2** (R = Cl) with one equivalent of the nucleoside in the presence of silver ion but in the absence of base, followed by precipitation with sodium tetrafluoroborate. This reflects the more acidic exocyclic NH protons of N^3-platinated deoxycytidine compared to N^1-platinated adenosine and 2′-deoxyadenosine.



5

Reactions of **1** with thymidine proceeded very slowly and in a much poorer yield. The product has not been isolated and attempts to synthesise this complex by reaction of the chloro complex **2** (R = Cl) with thymidine in the presence of silver ions resulted in a number of products.

The platination of N^7 in guanosine and 2′-deoxyguanosine is in accord with other studies of platinum–nucleoside complexes.[6] Although chloro(diethylenetriamine)platinum(II) has been reported to react with adenosine at N^1 and N^7 and with cytidine at N^3,[7] different binding patterns, *i.e.* N^1,N^6 for adenosine and 2′-deoxyadenosine and N^3,N^4 for deoxycytidine, were found for (2,2′:6′,2″-terpyridine)platinum(II) in this study. The absence of monoplatinated species even in the presence of an excess of nucleosides implies that the rate of attachment of the second positively charged (2,2′:6′,2″-terpyridine)platinum(II) to the monoplatinated nucleoside complexes is faster than the first. Furthermore, they do not follow the expected trend where transition metals prefer binding at heterocyclic nitrogens, not exocyclic amino nitrogens ($N^7 \sim N^1 \gg N^6$,N^3 for adenosine and $N^3 > O^2 \gg N^4$ for cytidine). Clearly proton loss at the exocyclic amino group, induced by platination at the heterocyclic nitrogen adjacent to it, increases its nucleophilicity and makes platination at this site very much more favourable. In cytidine complexes, coordination of platinum to N^4 with loss of a proton has been occasionally observed in addition to coordination at N^3.[6]

The rather unusual binding mode observed in adenosine, 2′-deoxyadenosine and 2′-deoxycytidine could be rationalised by an increase in acidity of the exocyclic amino groups upon coordination of the first (2,2′:6′,2″-terpyridine)platinum(II) at N^1 of adenosine and 2′-deoxyadenosine or N^3 of 2′-deoxycytidine and the proton loss probably immediately follows. It is interesting that the proton abstraction is possible even under the slightly acidic conditions (pH 5.5) where the reactions of the Pt^{II} complex **1** and the nucleosides were carried out. A second (2,2′:6′,2″-terpyridine)platinum(II) may already be in close proximity due to the tendency of (2,2′:6′,2″-terpyridine)platinum(II) to stack in aqueous solution,[8] and thus coordinate with the negatively charged nitrogen at a rate faster than the initial platination. The π–π and d^8–d^8 interactions resulting from stacking of the two platinum–terpyridine systems may also be another important factor in stabilising this

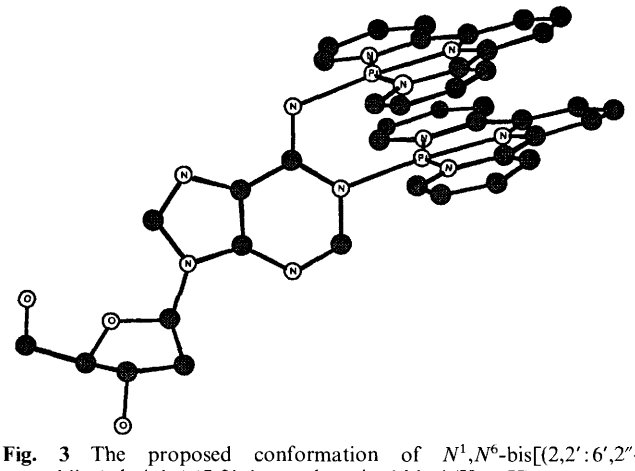


Fig. 3 The proposed conformation of N^1,N^6-bis[(2,2′:6′,2″-terpyridine)platinio(II)]-2′-deoxyadenosin-6-ide **4** (X = H)

mode of binding. The significance of such stacking of two (2,2′:6′,2″-terpyridine)platinum(II) groups is further emphasised by the two published crystal structures of bis[(2,2′:6′,2″-terpyridine)platinio(II)]guanidine complexes[9,10] which clearly show that the two (2,2′:6′,2″-terpyridine)platinum(II) groups stack on top of each other with close contact of the two platinum centres and the two terpyridine systems. The analogous structure of the Pt(terpy)–N–C–N–Pt(terpy) motif in $[Pt(terpy)]_2$–guanidine, N^3,N^4-$[Pt(terpy)]_2$–dC **5**, N^1,N^6-$[Pt(terpy)]_2$–A **4** (X = OH) and N^1,N^6-$[Pt(terpy)]_2$–dA **4** (X = H) is evident from molecular models. The electronic absorption spectra of the red 2′-deoxycytidine complex **5** and 2′-deoxyadenosine complex **4** (X = H) showed, in addition to the charge-transfer bands in the region 300–350 nm and the aromatic ligand bands in the region 200–300 nm, broad absorption bands at 495 (ε 2400 dm^3 mol^{-1} cm^{-1}) and 477 (ε 2800 dm^3 mol^{-1} cm^{-1}) nm respectively. Since similar absorption bands at 480 nm of similar molar extinction coefficient are characteristic for the $[Pt(terpy)]_2$–guanidine complex and its derivatives and were attributed to the Pt–Pt and/or ligand π–π interactions,[9] the presence of such bands in bis(2,2′,6′,2″-terpyridine)platinum(II)–nucleoside complexes are consistent with the stacked conformation depicted in Fig. 3. The large positive NOE enhancement observed at cytosine H^5 (15.5%) on irradiation at N^4H of the 2′-deoxycytidine complex **5** provides further supporting evidence for the stacked conformation (Table 1). Unfortunately a NOESY experiment on the 2′-deoxyadenosine complex **4** (X = H) did not provide information regarding the stacking of the two (2,2′:6′,2″-terpyridine)-platinum systems due to the very similar chemical shifts of the terpyridine protons. Further crystallographic studies are needed to formally confirm this.

It has been known for some time that chloro(2,2′:6′,2″-terpyridine)platinum(II) chloride **2** (R = Cl) reacts slowly with DNA,[4] and more recently it has been established that the platinum–triamine complexes *cis*-$[Pt(NH_3)_2$(4-picoline)Cl]Cl and *cis*-$[Pt(NH_3)_2$(4-Br-pyridine)Cl]Cl platinate DNA and block DNA replication *in vitro*.[11] It was demonstrated that these latter complexes were only monosubstituted by nucleobases and that the pyridine ligand remained coordinated to platinum(II). Interestingly they are nearly as active as cisplatin in blocking DNA replication and it appears that the aromatic ligand is essential for antitumour activity, since the related triamine complexes $[Pt(dien)Cl]^+$ and $[Pt(NH_3)_3Cl]^+$ also form monofunctional lesions in DNA but are ineffective as antitumour agents. The finding that (2,2′:6′,2″-terpyridine)platinum(II) binds to nucleosides at specific positions has significant implications for its interaction with DNA. As the adenine N^1,N^6 and cytosine N^3,N^4 are involved in Watson–Crick base pairing in DNA duplexes, these positions are not available for platination thus guanine N^7 is the only position likely to be attacked by (2,2′:6′,2″-terpyridine)platinum(II) which explains the specificity of binding of (2,2′:6′,2″-terpyridine)platinum(II) to DNA.[12]

Experimental

Gel filtration chromatography was performed on Sephadex G-10 or Sephadex G-15 (Sigma) using deionised water as eluent. Fractions were collected and freeze dried on a VirTis Freezemobile 5 SL Freezedrier.

Routine ^1H NMR spectra were recorded at 200 MHz on a Varian Gemini 200 spectrometer. High field NMR spectra (500 MHz) and other special NMR experiments (COSY, NOE, NOESY) were performed on a Bruker AMX500 spectrometer. ^2H_2O (99.9 atom%, Fluorochem Ltd.), $[^2H_7]$DMF (99.5 atom%, Aldrich) or C^2H_3CN (99.5 atom%, Aldrich) were used as solvents for NMR studies. Chemical shifts (δ_H) are quoted in ppm relative to tetramethylsilane and internally referenced to the residual protonated solvent resonances except in ^2H_2O where sodium 2,2-dimethyl-2-silapentane-5-sulfonate (DSS) was used as internal reference. Low resolution mass spectra were obtained on a V.G. Biotech-BioQ mass spectrometer. Mass values are quoted in Daltons (m/z) with only the molecular ion and major fragments being quoted.

Chloro(2,2′:6′,2″-terpyridine)platinum(II) chloride dihydrate was obtained from Aldrich. The 4-picoline Pt^{II} complex **1** tetrafluoroborate was synthesised as previously reported.[1] Adenosine and guanosine were purchased from Aldrich and all other nucleosides from Sigma. 2′-Deoxycytidine hydrochloride was treated with Amberlite IRA-400 anion exchange resin (OH^- form, Sigma) to remove the chloride ion. All other chemicals were used without further purification. Deionised water was used for all experiments.

^1H NMR studies of reactions of 1 and nucleosides

Solutions of 4-picoline(2,2′:6′,2″-terpyridine)platinum(II) tetrafluoroborate **1** (6.9 mg, 10 μmol) and the nucleosides (A, G, dA, dG, dC, T) (10 μmol) in deionised water (600 μl) containing sodium phosphate buffer pH 5.5 (0.5 M; 100 μl) were incubated at 37 °C for 3 days. The solutions were freeze dried and the residues were taken up in ^2H_2O (600 μl) containing DSS in ^2H_2O (20 mM; 10 μl) and $Na_2{}^2HPO_4$ in ^2H_2O (0.5 M; 100 μl) was added to each sample in order to keep the apparent pH between 7–8. The ^1H NMR spectra were then recorded at 200 MHz.

Isolation and characterisation of N^7-[(2,2′:6′,2″-terpyridine)-platinio(II)]guanosine complex 3 (X = OH)

Method A from picoline complex 1. 4-Picoline complex **1** (13.9 mg, 20 μmol) and guanosine (28.5 mg, 100 μmol) were dissolved in deionised water (1.8 ml). A sodium phosphate buffer pH 5.5 (0.5 M; 200 μl) was added and the solution was incubated at 37 °C for 10 days. The complex was purified on Sephadex G-10. ^1H NMR of the product after gel filtration on a second column indicated that it was a platinated guanosine but contaminated with significant amounts of starting materials.

Method B from chloro complex 2 (R = Cl). A solution of [Pt(terpy)Cl]Cl·$2H_2O$ **2** (R = Cl) (26.7 mg, 50 μmol) and silver nitrate (17 mg, 100 μmol) in deionised water (1.0 μl) was heated at 70–80 °C in a water bath for 2 h. The AgCl precipitate was centrifuged off, the solution added to guanosine (15 mg, 52 μmol) and the solution heated for another 2 h at 70–80 °C. After centrifugation, the yellow solution was freeze-dried to give the nearly pure complex (contaminated by trace of guanosine) in quantitative yield; δ_H(500 MHz, ^2H_2O) 3.82 (1 H, dd, J 12.8, 4.1 Hz, CH^aH^{b}OH), 3.91 (1 H, dd, J 12.7, 2.9 Hz, CH^aH^bOH), 4.28 (1 H, m, $H^{4'}$), 4.43 (1 H, t, J 4.8 Hz, $H^{3'}$), 4.82 (1 H, t, J 5.0 Hz, $H^{2'}$), 6.10 (1 H, d, J 4.8 Hz, $H^{1'}$), 7.65 (2 H, m, terpy $H^{5,5''}$), 7.92 (2 H, dd, J 11.5, 5.6 Hz, terpy $H^{6,6''}$), 8.32–8.36 (6 H, m, terpy $H^{3,3''}$, $H^{4,4''}$ and $H^{3',5'}$), 8.49 (1 H, t, J 5.0 Hz, terpy

$H^{4'}$), 8.95 (1 H, s, guanine-H^8); *m/z* (ES MS) 355.78 (M^{2+}, 100%).

Isolation and characterisation of N^1,N^6-bis[(2,2′:6′,2″-terpyridine)platinio(II)]adenosin-6-ide 4 (X = OH) and N^1,N^6-bis[(2,2′:6′,2″-terpyridine)platinio(II)]-2′-deoxyadenosin-6-ide 4 (X = H) complexes

Method A from picoline complex 1. 4-Picoline complex **1** (13.9 mg, 20 μmol) and adenosine (26.8 mg, 100 μmol) were dissolved in deionised water (1.8 ml). Sodium phosphate buffer pH 5.5 (0.5 M, 200 μl) was added and the solution was incubated at 37 °C for 10 days. The deep orange solution was first purified on a column of Sephadex G-15 followed by chromatography on a column of Sephadex G-10. The orange fractions were collected and freeze dried to give the product as a red powder (~5 mg) δ_H(500 MHz, ^2H_2O) 3.96 (2 H, m, CH_2OH), 4.39 (1 H, m, $H^{4'}$), 4.50 (1 H, dd, *J* 5.6, 3.5 Hz, $H^{3'}$), 4.90 (1 H, dd, *J* 6.0, 5.5 Hz, $H^{2'}$), 6.23 (1 H, d, *J* 6.2 Hz, $H^{1'}$), 7.50 (4 H, 2 × t, terpy$_1$-$H^{5,5''}$ and terpy$_2$-$H^{5,5''}$), 8.05 (2 H, dd, *J* 10.1, 4.6 Hz, terpy$_1$-$H^{6,6''}$), 8.10 (4 H, 2 × d, terpy$_1$-$H^{3,3''}$ and terpy$_2$-$H^{3,3''}$), 8.15 (4 H, 2 × d, terpy$_1$-$H^{3',5'}$ and terpy$_2$-$H^{3',5'}$), 8.25 (4 H, 2 × t, terpy$_1$-$H^{4,4''}$ and terpy$_2$-$H^{4,4''}$), 8.42 (2 H, br m, terpy$_2$-$H^{6,6''}$), 8.48 (2 H, 2 × t, terpy$_1$-$H^{4'}$ and terpy$_2$-$H^{4'}$), 8.55 (1 H, s, adenine-H^8), 8.85 (1 H, s, adenine-H^2).

Method B from chloro complex 2 (R = Cl). A solution of [Pt(terpy)Cl]Cl·2H_2O **2** (R = Cl) (53.5 mg, 100 μmol) and silver nitrate (35.0 mg, 200 μmol) was heated at 70–80 °C in a water bath for 30 min and the AgCl precipitate was centrifuged off. To the solution were added adenosine (28 mg, 100 μmol) and potassium carbonate (14 mg, 100 mmol) and the solution was heated for another 2 h at 70–80 °C. After centrifugation, the deep red solution was neutralised by addition of NaH_2PO_4 and the complex precipitated with ammonium hexafluorophosphate. The orange precipitate was filtered off, washed with water and air dried. Re-precipitation of the hexafluorophosphate salt from acetonitrile–ether gave the complex as an orange–brown powder (64.6 mg, 84%), δ_H(200 MHz, C^2H_3CN) 3.65–3.95 (4 H, m, CH_2OH and 2 × OH), 4.20 (1 H, m, $H^{4'}$), 4.35 (1 H, br m, OH), 4.65 (1 H, dd, *J* 10.5, 4.0 Hz, $H^{3'}$), 4.80 (1 H, m, $H^{2'}$), 6.00 (1 H, d, *J* 6.6 Hz, $H^{1'}$), 7.08 (1 H, br s, adenine N^6H), 7.40 (4 H, m, 2 × terpy-$H^{5,5''}$), 7.80–8.05 (10 H, m, terpy$_1$-$H^{6,6''}$, 2 × terpy-$H^{3,3''}$, 2 × terpy-$H^{3',5'}$), 8.15 (4 H, m, 2 × terpy-$H^{4,4''}$), 8.31 (1 H, s, adenine-H^8), 8.30–8.42 (4 H, m, terpy$_2$-$H^{6,6''}$ and 2 × terpy-$H^{4'}$), 8.60 (1 H, s, adenine-H^2); *m/z* (ES MS) 374.3 (M^{3+}, 100%), 347.8 {[M − Pt(terpy) + H]$^{2+}$}, 330.2 {[M − ribose]$^{3+}$}. Similarly the 2′-deoxyadenosine complex was synthesised on the same scale, following counterion exchange and re-precipitation (69.5 mg, 90%), λ_{max}(CH_3CN)/nm 248 (ε/dm^3 mol^{-1} cm^{-1} 53 000), 274sh (54 000), 332 (18 000), 346sh (17 000) and 477sh (2800); δ_H(500 MHz, C^2H_3CN) 2.50 and 2.88 (2 H, 2 × m, 2 × $H^{2'}$), 3.50 (1 H, br m, OH), 3.75 (2 H, m, CH_2OH), 4.10 (1 H, m, $H^{4'}$), 4.43 (1 H, m, $H^{3'}$), 4.62 (1 H, br m, OH), 6.50 (1 H, dd, *J* 8.3, 5.9 Hz, $H^{1'}$), 7.02 (1 H, br s, adenine N^6H), 7.40 (4 H, m, 2 × terpy-$H^{5,5''}$), 7.80–8.05 (10 H, m, terpy$_1$-$H^{6,6''}$, 2 × terpy-$H^{3,3''}$, 2 × terpy-$H^{3',5'}$), 8.15 (4 H, m, 2 × terpy-$H^{4,4''}$), 8.30 (1 H, s, adenine-H^8), 8.30–8.40 (4 H, m, terpy$_2$-$H^{6,6''}$ and 2 × terpy-$H^{4'}$), 8.60 (1 H, s, adenine-H^2); *m/z* (ES MS) 369.0 (M^{3+}, 100%), 339.8 {[M − Pt(terpy) + H]$^{2+}$}, 330.2 {[M − deoxyribose]$^{3+}$}.

Isolation and characterisation of N^3,$N^{4'}$-bis[(2,2′:6′,2″-terpyridine)platinio(II)]-2′-deoxycytidin-4-ide complex 5

Method A from picoline complex 1. As for the guanosine and adenosine complex starting from [Pt(terpy)(4-picoline)]$(BF_4)_2$ **1** (13.9 mg, 20 μmol) and 2′-deoxycytidine (22.6 mg, 100 μmol). The product which precipitated from the solution upon addition of a saturated aqueous solution of sodium tetrafluoroborate, was collected by centrifugation and recrystallised from water (5 mg, 37%), mp > 200 °C (Found: C, 34.0; H, 2.1; N, 8.8. $C_{39}H_{34}N_9O_4Pt_2B_3F_{12}$·0.3$NaBF_4$ requires C, 34.0, H, 2.5, N, 9.1%); δ_H(200 MHz, ^2H_2O) 2.52 (2 H, m, $H^{2'}$), 3.88 (2 H, m, CH_2OH), 4.12 (1 H, m, $H^{4'}$), 4.55 (1 H, m, $H^{3'}$), 6.42 (1 H, t, *J* 6.7 Hz, $H^{1'}$), 6.60 (1 H, d, *J* 7.8 Hz, cytosine-H^5), 7.50–7.56 (4 H, m, 2 × terpy-$H^{5,5''}$), 7.82 (1 H, d, *J* 7.8 Hz, cytosine-H^6), 8.0–8.5 (18 H, m, terpy-H); *m/z* (ES MS) 360.95 (M^{3+}, 100%).

Method B from chloro complex 2 (R = Cl). A solution of [Pt(terpy)Cl]Cl·2H_2O **2** (R = Cl) (26.7 mg, 50 μmol) and silver nitrate (17 mg, 100 μmol) in deionised water (1.0 ml) was heated at 70–80 °C in a water bath for 2 h. The AgCl precipitate was centrifuged off, the solution added to 2′-deoxycytidine (11.8 mg, 52 μmol), and the solution heated for another 2 h at 70–80 °C. After centrifugation, the product was precipitated by addition of sodium tetrafluoroborate and recrystallised from water to give the complex as red microneedles (25 mg, 70%). Counterion exchange with ammonium hexafluorophosphate in water provided the red hexafluorophosphate in quantitative yield, λ_{max}(CH_3CN)/nm 245 (ε/dm^3 mol^{-1} cm^{-1} 54 000), 274sh (48 000), 327 (15 000), 343sh (14 000) and 495sh (2400); δ_H(500 MHz, C^2H_3CN) 2.34 (m, 2 H, 2 × $H^{2'}$), 3.22 and 3.35 (2 H, 2 × br s, 2 × OH), 3.78 (1 H, dd, *J* 12.0, 3.6 Hz, CH^aH^bOH), 3.84 (1 H, dd, *J* 12.0, 3.3 Hz, CH^aH^bOH), 3.96 (1 H, dd, *J* 11.2, 3.6 Hz, $H^{4'}$), 4.43 (1 H, m, $H^{3'}$), 6.26 (1 H, t, *J* 6.3 Hz, $H^{1'}$), 6.37 (1 H, d, *J* 7.8 Hz, cytosine-H^5), 6.84 (1 H, br s, cytosine-N^6H), 7.44–7.54 (4 H, m, 2 × terpy-$H^{5,5''}$), 7.88–8.00 (9 H, m, 2 × terpy-$H^{3,3''}$, 2 × terpy-$H^{3',5'}$ and cytosine-H^6), 8.13–8.21 (6 H, m, 2 × terpy-$H^{4,4''}$ and terpy$_1$-$H^{6,6''}$), 8.27 (2 H, ddd, *J* 7.3, 5.5 and 1.3 Hz, terpy$_2$-$H^{6,6''}$), 8.33 and 8.38 (2 H, 2 × t, *J* 8.1 and 8.2 Hz, 2 × terpy-$H^{4'}$).

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