Pergamon

Bioorganic & Medicinal Chemistry, Vol. 4, No. 7, pp 1007–1013, 1996

PII: S0968-0896(96)00095-8

0968-0896/96 $15.00 + 0.00

A Mass Spectrometric Investigation of the Reaction Between 4,4′-Vinylenedipyridine Bis[2,2′:6′,2″-terpyridine Platinum(II)] and the Self-Complementary Oligonucleotide d(CpGpTpApCpG)

Gordon Lowe,[a,*] James A. McCloskey,[b] Jinsong Ni[b] and Tirayut Vilaivan[a]

[a]*Dyson Perrins Laboratory, Oxford University, South Parks Road, Oxford OX1 3QY, U.K.*

[b]*Department of Medicinal Chemistry, 311A Skaggs Hall, University of Utah, Salt Lake City, UT 84112-1115, U.S.A.*

Abstract—4,4′-Vinylenedipyridine bis[2,2′:6′,2″-terpyridine platinum(II)], a potential intermolecular bis-intercalator of DNA, reacts slowly at Pt^{II}, the linker being displaced by nucleobases. Crystallization of 4,4′-vinylenedipyridine bis[2,2′:6′,2″-terpyridine platinum(II)] in the presence of the self-complementary oligonucleotide d(CpGpTpApCpG) gave a product which was analyzed by electrospray ionization mass spectrometry. Molecular mass measurements demonstrated ligation of one terpyridine Pt^{II}, which was shown by liquid chromatography–mass spectrometry to reside exclusively on deoxyguanosine. The terpyridine Pt^{II} was shown by tandem mass spectrometry to be attached to the 3′-terminal deoxyguanosine, consistent with N-7 substitution. Copyright © 1996 Elsevier Science Ltd

Introduction

4-Picolin 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 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). This increase in binding energy has been ascribed to the double-positive charge on the Pt^{II} complex.[1] Attempts to obtain crystals suitable for X-ray analysis of the complex of **1** with the self-complementary oligonucleotide d(CpGpTpApCpG) were unsuccessful. This oligonucleotide was chosen because of the availability of the high-resolution structure of its complex with daunomycin, an intercalator of DNA with antitumor activity.[2] Good crystals were obtained, however, from a solution of 4,4′-vinylenedipyridine bis[2,2′:6′,2″-terpyridine platinum(II)] (**3**) and the oligonucleotide d(CpGpTpApCpG), but the crystals did not diffract X-rays to high resolution.[3] From a series of NMR experiments, it became clear that some nucleosides, especially guanosine, were able to slowly displace 4-picoline from **1**. Although it is well established that purine bases, especially guanine, displace chloride ion from Pt^{II} derivatives, the best known example being *cis*platin {*cis*-[$Pt(NH_3)_2Cl_2$]},[4] we are not aware of any previous report of the substitution of an N-ligand at Pt^{II} by a nucleobase. Indeed, it has recently been reported that *cis*-[$Pt(NH_3)_2$(pyridine)Cl]$^+$ forms monofunctional adducts with DNA, the pyridine ligand being perfectly stable.[5] Since **3** had been prepared as a potential intermolecular bis-intercalator of DNA for probing DNA topology,[6,7] it was imperative to investigate this observation further. We now report a mass spectrometric investigation of the product formed during the crystallization of **3** with the self-complementary oligonucleotide d(CpGpTpApCpG).

Key words: platination, electrospray mass spectrometry, collision-induced dissociation.

Results and Discussion

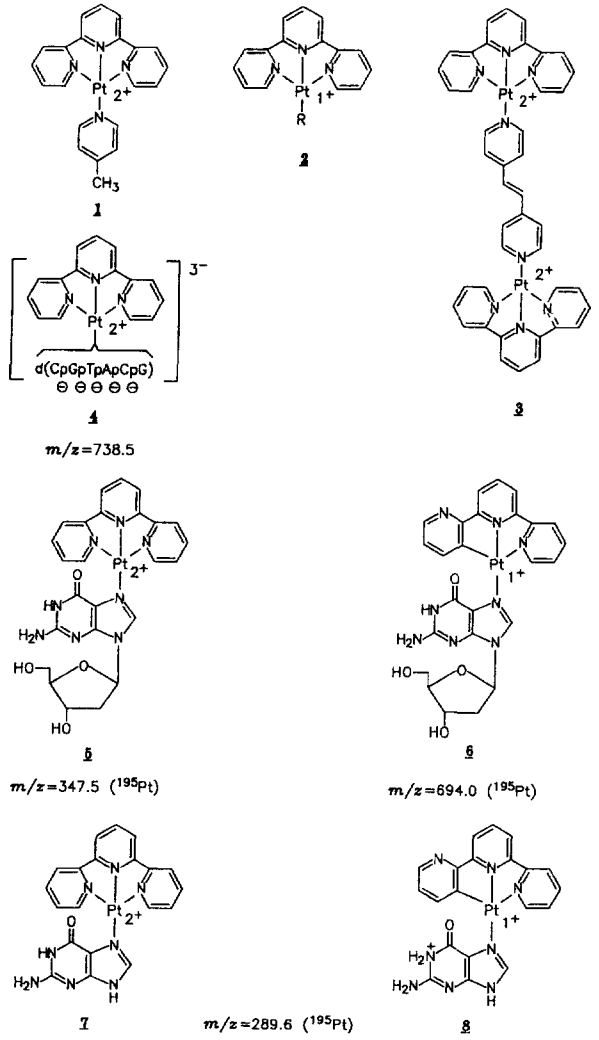
Mass spectrometry has evolved in recent years as a powerful technique for the structural characterization of oligonucleotides and their derivatives,[8,9] primarily due to advances in ionization methodology. FABMS was effectively applied in earlier studies for the characterization of platinated adducts of oligonucleotides and their constituents,[10–14] in some cases, coupled with collision-induced dissociation (CID).[15,16] However, for studies of oligonucleotides their polar nature resulted in practical limitations with regard to molecular size that could be efficiently transferred to the gas phase, resulting in lower quality data. Electrospray ionization has overcome a number of these obstacles,[17,18] and has been successfully coupled with liquid chromatography (LC–MS) for analysis of *cis*platin and related compounds,[19,20] platinated nucleosides, and small oligonucleotides.[21]

Electrospray ionization methodology was applied, therefore, in the present study for characterization of the product of reaction between **3** and d(CpGpTpApCpG). As discussed in the following sections, MS data permit establishment of the principal structural features of the adduct, although some details

of the ion structures and their mechanisms of formation have not been firmly established and warrant independent study.

Molecular mass analysis of the oligonucleotide–platinum complex

The electrospray mass spectrum of the product of crystallization, with NH_4^+ counter ion, is presented in Figure 1. As shown by ion assignments tabulated in Table 1, two components are clearly present, of M_r 2218.8 (component M) and 1792.3 (m). Mass spectrometer resolving power was set to provide nonresolved ion profiles of the four principal Pt isotopes (^{194}Pt, ^{195}Pt, ^{196}Pt, ^{198}Pt), and so the measured mass values are based on the atomic weight of Pt (195.09) rather than on monoisotopic values. Component M corresponds exactly in mass to the 2,2′:6′,2″-terpyridine Pt^{II}–d(CpGpTpApCpG) complex in which one ligand moiety has been added (error 0.0 Da), and component m represents the noncomplexed oligonucleotide (error 0.1 Da). The Pt-containing component (**4**) constitutes approximately 25% of the sample in terms of total ion current, but this value is taken as a lower limit because the extent to which m may be derived by gas-phase



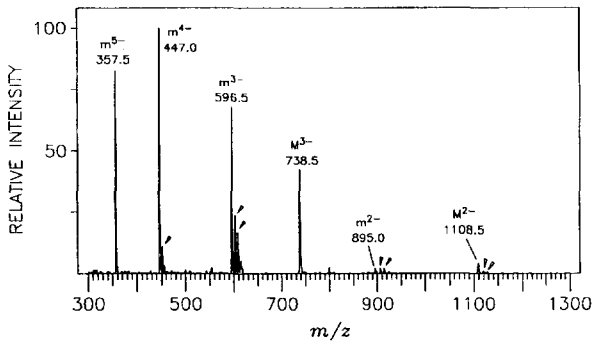


Figure 1. Negative ion electrospray ionization mass spectrum of the product of crystallization of d(CpGpTpApCpG) with 4,4′-vinylenedipyridine bis[2,2′:6′,2″-terpyridine Pt^{II}]. Wedges: alkali ion adduct peaks. Ion assignments for components M (Pt-containing) and m (non-Pt containing) are listed in Table 1.

dissociation of M is not known. The absence of ions representing two terpyridine adduct moieties (requiring $M_r = 2645$), for example at both dGs, suggests that reaction has occurred selectively at only one site.

It is of interest that the charge state assignments listed in Table 1 are supported by the observed pattern of alkali ion adduct formation to phosphate groups, a common feature of polynucleotide mass spectra, such as the Na^+ and K^+ adducts of *m/z* 447.0 and 596.5 in Figure 1. However, the *m/z* 357.5 peak is not accompanied by alkali adducts because at net charge of 5– the backbone is fully ionized so that no neutral phosphates are available for H^+/Na^+ or H^+/K^+ interchange. An analogous interpretation holds for the *m/z* 738.5 ion, in which a net charge of 3– is produced by a combination of five phosphates (charge 5–) and Pt^{II} (charge 2+). Thus, 3– represents the maximum charge state observed for component M, due to the presence of Pt^{2+} in the ion.

Characterization of deoxyguanosine as the sole site of derivatization

Experiments were conducted to establish the site of ligation in the oligonucleotide. The platinated oligonucleotide crystallization product was hydrolyzed enzymatically to deoxyribonucleosides and analyzed by LC–MS, which produced the chromatogram presented in Figure 2. In addition to the principal deoxynucleosides dC, dG, dT and dA, three minor peaks were observed. From the corresponding positive ion electro-

Table 1. Ion assignments for the negative ion electrospray mass spectrum shown in Figure 1

Molecular species	*m/z*	Ion charge (*z*)	M_r Obsd	M_r Calcd
M, platinum-containing (compound **4**)	738.5	3–	2218.8	2218.8
	1108.5	2–		
m, non-platinum-containing	357.5	5–	1792.3	1792.2
	447.0	4–		
	596.5	3–		
	895.0	2–		

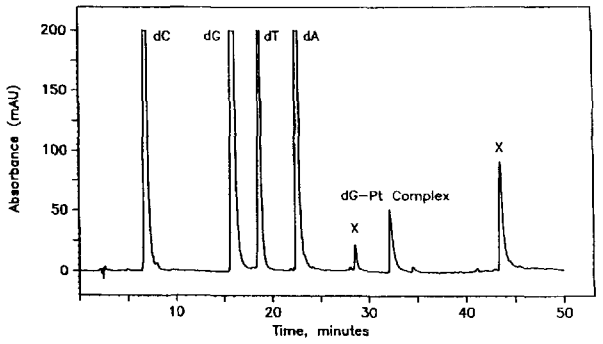


Figure 2. Reversed-phase HPLC chromatogram from LC–MS analysis of enzymatically digested d(CpGpTpApCpG)–terpyridine Pt^{II} complex. UV detection at 254 nm. X, impurities shown by their mass spectra not to be nucleosides or nucleotides. Mass spectrum acquired at 32.4 minutes is shown in Figure 3.

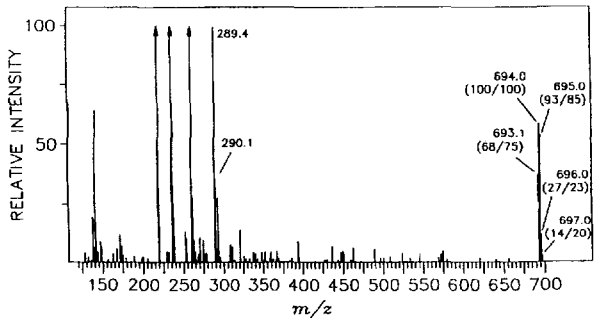


Figure 3. Positive ion electrospray mass spectrum acquired at 32.4 min in the LC–MS chromatogram shown in Figure 2. Values in parentheses are (experimental/theoretical) relative abundance values for ion composition $C_{25}H_{23}N_8O_4Pt$. Peaks below *m/z* 289 are due to background.

spray mass spectra acquired every 1 s during the analysis, it was determined that two components (denoted by X, Fig. 2) were non-nucleotide related impurities, while the 32.4 minute eluant contained platinum.

The mass spectrum acquired at 32.4 min is shown in Figure 3. Mass spectrometer resolving power was set at approximately unit resolution, permitting separation of isotopic species differing by one mass unit in the *m/z* 693–697 cluster of peaks. The isotopic pattern observed corresponds to a singly charged species containing one Pt atom plus the mass of dG (267 Da) plus 232 Da for the terpyridine ligand. The overall composition $C_{25}H_{23}N_8O_4Pt$ fits the observed experimental isotope pattern as shown by comparison of experimental and theoretical isotopic abundances in Figure 3. The mass values observed require that one hydrogen has been lost, consistent with structure **6** or an isomer such as **5** $-H^+$ (e.g., from N-1), as discussed further below. The partially resolved peaks at *m/z* 289.4–290.1 are assigned as the doubly charged nucleobase–Pt adduct **7**, which from the presently available data cannot be distinguished from **8**, although the structure **7** is much more likely. In either case the ion observed arises from loss of deoxyribose with retention of one hydrogen, the prevalent gas-phase dissociation pathway for nucleosides,[22] thus indicating guanine as the site of platination.

Further insights into the structure of the dG–terpyridine–Pt adduct were sought by examination of the deoxyguanosine terpyridine Pt^{II} adduct prepared separately and shown by ^1H NMR spectroscopy to have the structure **5**. The electrospray ionization mass spectrum (not shown) of the nucleoside adduct **5** exhibited the same singly charged mass and abundance pattern as in Figure 3 (*m/z* 693–697), and in addition an isotopic pattern of doubly charged ions at *m/z* 347–349 representing the guanine–terpyridine–Pt^{II} moiety. The *m/z* 347.5 ion (containing ^{195}Pt isotope) was mass-selected and dissociated, leaving no remaining precursor ion and producing the mass spectrum presented in Figure 4. Ion assignments are represented schematically in Figure 5.

The collision-induced dissociation mass spectrum shown in Figure 4 is relatively complex, but rational assignments can be made in support of ligation at N-7 of guanine by one terpyridine–Pt moiety. It is notable that a number of product ions occur at higher *m/z* values than the precursor ion, thus implying the presence of singly charged Pt. The assignments shown are facilitated by a combination of constraints arising from the charge of the ion, the accuracy with which the *m/z* is measured ($\sim \pm 0.2$ *m/z* units), and the presence or absence of Pt^{II}. As shown (Fig. 5), non-Pt containing singly charged ions (*m/z* 110, 117, 135, 152, 268) are observed which correspond to established products of dissociation of nucleosides,[23] and of protonated guanine[24] and its selectively ^{15}N-labeled analogues.[25] Pt-containing ions in Figure 5 have been categorized by the mass and charge of the terpyridine–Pt ligands R_1, R_2, and R_3.

Interestingly, as shown most clearly by the structural relationships between *m/z* 427 and 214, change in oxidation state of Pt from 2+ to 1+ must be accompanied by a loss of hydrogen from the terpyridine moiety, evidently as a gas-phase ionic reaction. We speculate that this conversion can be accommodated by

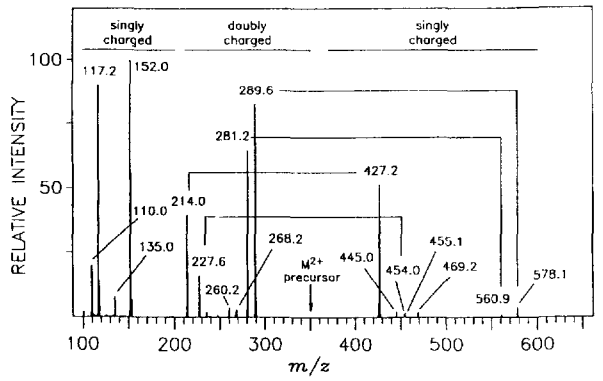


Figure 4. Positive ion collision-induced dissociation mass spectrum of double charged molecular ion of *m/z* 347.5 (designated M^{2+}) of deoxyguanosine terpyridine Pt^{II} complex. Connecting lines denote pairs of ions having *m/z* values corresponding to charge state relationships resulting from the loss of a proton, probably by a 'roll-over' 3-metallation mechanism as shown in Figure 6.

the 'roll-over' 3-metallation mechanism depicted in Figure 6. This mechanism is known to occur thermally in 2,2′-dipyridine Pt^{II} complexes.[26] Furthermore, this roll-over 3-metallation mechanism accounts for the relationship between three other pairs of ions (as marked in Fig. 5): 281.2–560.9, 289.6–578.1, and 227.6–454.0. In the latter three cases, the charged Pt ligand substitutes for a proton in ions normally produced by CID of protonated guanine:[24,25] *m/z* 135, 152 (Fig. 5), and 28 (protonated HCN).

A third class of Pt-ligand ions (see R_3 in Fig. 5) are the relatively minor singly charged species *m/z* 445, 455, and 469. Here also, the constraints of charge and mass suggest the simple structures given in Figure 5. The origins of these ions are not known, but it is reasonable to assume that Pt^{II} reduction occurs by electron transfer from argon collision gas: $Pt^{2+} + Ar \rightarrow Pt^{+} + Ar^{+}$. This process is energetically feasible because the ionization potential of Ar is relatively low, 15.8 eV.[27]

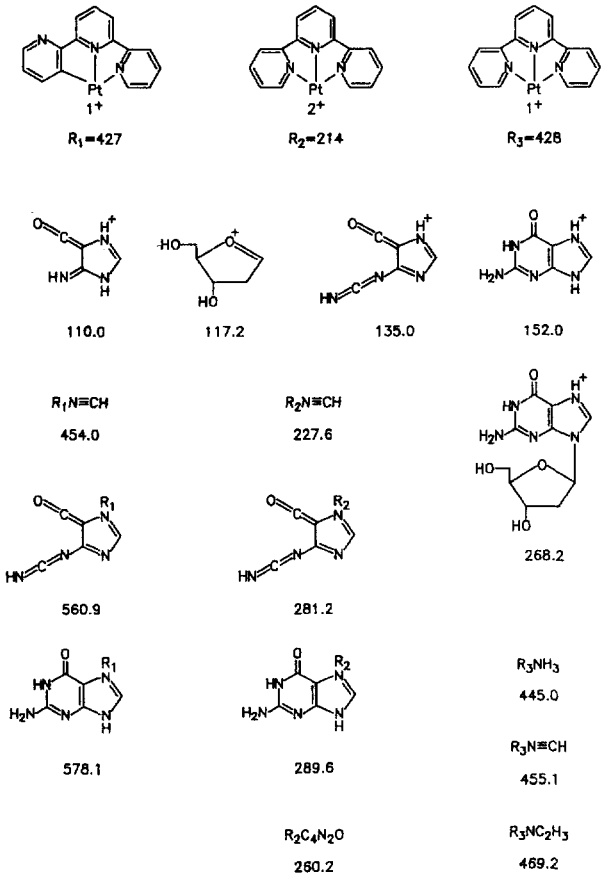


Figure 5. Schematic of structure assignments for ions in the mass spectrum shown in Figure 4. Sites of protonation are shown as N-7 for convenience.

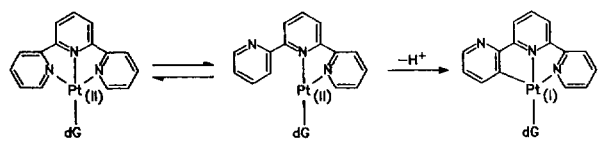


Figure 6. Proposed mechanism for the gas-phase generation of single charged terpyridine Pt^{I} complexes of deoxyguanosine.

Sequence analysis of the terpyridine–Pt–oligonucleotide complex

The triply charged molecular ion of the terpyridine–Pt–oligonucleotide complex (*m/z* 738.5 in Fig. 1) was collisionally activated and dissociated, leading to the mass spectrum shown in Figure 7. The ion selected contains one Pt ligand; its position in the sequence was established from mass ladders built from phosphate backbone cleavage reactions[28] as described below.

Upon dissociation, the majority of M^{3-} ions undergo loss of the terpyridine Pt^{II}; therefore, decomposition produces ions which bear no adduct sequence information and which exhibit the same *m/z* values as those derived from the collisional spectrum (not shown) of the corresponding non-platinated oligonucleotide, *m/z* 596.5 (Table 1). Non-platinated ions are also formed, denoted as m^{3-}, m^{4-}, and m^{5-} in Figure 7. The m^{5-} species corresponds simply to loss of the terpyridine Pt^{II} ligand, although this assignment cannot be distinguished from the base-loss product $(a-T)^{2-}$, as denoted in Figure 7. The *m/z* value of the m^{4-} ion requires net transfer of one proton from the terpyridine ligand to the oligonucleotide, i.e:

$$[\text{oligo}^{5-}\text{Pt}^{2+}\text{ligand}]^{3-} \rightarrow [\text{oligo}^{5-}\text{H}^{+}]^{4-} + [\text{Pt}^{2+}\text{ligand} - \text{H}^{-}]^{+}$$

m/z 738.5 *m/z* 447.0

Similarly, the *m/z* value of the m^{3-} ion (596.5) mandates a two proton transfer upon loss of the ligand, resulting in a net charge of 3− for the resulting ion.

The location of the dG–Pt adduct in the sequence was established as the 3′-terminus, based on the mass assignments shown in Figure 8. Two series of the ions

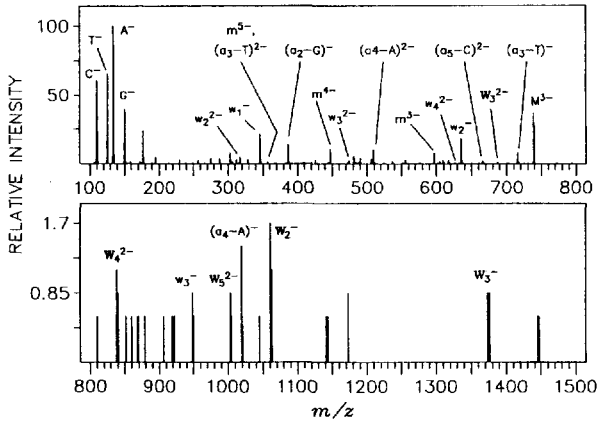


Figure 7. Negative ion collision-induced dissociation mass spectrum of the *m/z* 738.5 ion (designated M^{3-}) observed in Figure 1. Molecular ion notations such as M^{3-} refer to $(M-3H)^{3-}$ where M is the neutral platinated oligonucleotide. Ion nomenclature is taken from ref 28. Designations of M and W denote Pt-containing ions; m, w, and similar notations refer to non-Pt-containing ions. C, A, T, and G denote nucleobase anions.

are used for determination of sequence, the w series (arising from C3′–O cleavage containing the 3′ terminus), building a mass ladder in the 3′→5′ direction, and the a − base series (arising from C3′–O cleavage with base loss at that residue, and containing the 5′ terminus), building a mass ladder in the 5′→3′ direction.[28,29] The Pt-containing series of ions (denoted as W series) are recognized by their characteristic peak shapes due to unresolved clusters of Pt isotopes. No Pt-containing ions are observed in any of the a − base ion series, which together represent residues 5′-CGTAC-3′, a result that is consistent with modification on the 3′-terminal G. Mass values of the W series shows the required mass for the terpyridine–Pt ligand (428 Da) on all four residues represented, starting with the smallest W series ion observed, W_2^- (*m/z* 1061.6) representing the terminal platinated dinucleotide d(pCpG)-3′. The *m/z* difference between W_4 and W_5 ions is 164.7, where $z=2$, indicating unmodified dG (theoretical *m/z* difference 164.6), thus excluding the second G nucleotide as the site of ligation. Interestingly, the ion charge patterns in the W series compared with the non-platinated w series are unusual, reflecting the presence of Pt^{2+} on the 3′-terminus. For example, W_1^- should be the most abundant member of the ion series[24,29] (as is w_1^-), but W_1 is not observed because the net charge will be positive: pdG^- plus $Pt^{2+}=1+$. Similarly, the W_2 cleavage should yield a neutral product ($pdCpdG^{2-}$ plus Pt^{2+}), but a monocharged ion is produced at *m/z* 1061.6. This observation implies that two charges reside on one phosphate, which would be relatively unfavorable. However, either direct electrostatic charge stabilization of phosphate by Pt^{2+}, or phosphate neutralization by H^+ transferred from the terpyridine ligand (see Fig. 6) would explain the occurrence of this ion. A similar concept in which phosphate oxygen anion forms a bridge to Pt^+ has been advanced in studies involving FAB ionization and CID of tetranucleotide–Pt complexes.[10]

This work supports earlier conclusions[10,15,16] that collision-induced dissociation is a useful technique for structural studies of platinated nucleosides and nucleotides, although it is recognized that several issues could profit from more detailed investigation. For example, the reason for the absence of doubly charged molecular ions in the electrospray LC–MS spectrum in Figure 3 is unclear, in view of the presence of such ions (different instrument and conditions) in the direct infusion electrospray mass spectrum (not shown) that served as precursor to the mass spectrum in Figure 4. In addition, further experiments, outside the scope of the present study, would be useful to delineate the mechanistic and structural details of assignments in Figure 7.

Experimental

Materials and methods

Chloro-2,2′:6′,2″-terpyridine platinum(II) chloride dihydrate, *trans*-1,2-bis-(4-pyridyl)ethylene and all other chemicals were of the highest purity grade obtained from Aldrich and used as received.

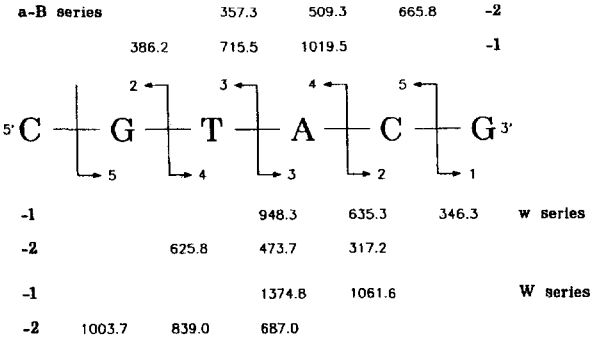


Figure 8. Mass ladder representation of sequence-determining ions from the mass spectrum shown in Figure 7. Mass/charge values are tabulated for Pt-containing ions with charges of 1− and 2− that contain the 3′-terminus (W series) and non-Pt-containing ions that contain the 3′ terminus (w series) and 5′-terminus (a−B series).

The oligonucleotide d(CpGpTpApCpG) was synthesized on an automated Applied Biosystems DNA synthesizer (Model 380B) using phosphoramidite chemistry by Mrs V. H. Cooper (Oxford Center for Molecular Sciences). The crude fully deprotected oligonucleotide was purified by reversed-phase HPLC (Waters 990 HPLC systems, Waters μBondapak C-18 semipreparative column 0.78×30 cm, P/N 84176, 0.1 M aqueous triethylammonium acetate/acetonitrile gradient).

Synthesis of *trans*-4,4′-vinylidenedipyridine bis[(2,2′:6′,2″-terpyridine) platinum(II)] nitrate (3). A solution of chloro-2,2′:6′,2″-terpyridine platinum(II) dihydrate (200 mg, 37.4 μmol) and *trans*-bis-(4-pyridyl)-ethylene (30 mg, 17 μmol) in 20% aqueous methanol (10 mL) was heated at reflux for 15 min. A solution of silver nitrate (127 mg, 74.8 μmol) in 3 mL of water was added and the solution was returned to reflux for a further 15 min. Solid sodium nitrate (5 g) was added to the suspension while hot, and the silver chloride precipitate was removed by filtration through Celite and the filter cake washed well with water. The filtrate was concentrated on a rotary evaporator until a yellow solid began to precipitate, then another 10 mL of saturated aqueous sodium nitrate was added, and the solution was kept at 4 °C overnight. The solid was filtered and washed with cold water and dried to give the crude product (217 mg). Recrystallization twice from water gave yellow needles (95 mg, 45%): mp >200 °C; ^{1}H NMR (500 MHz, D_2O): δ_H 7.62 (4H, m, 2×terpy $H_{4,4''}$), 7.78 (4H, d, $J=5.5$ Hz, 2×terpy $H_{3,3''}$), 7.79 (2H, s, 2×vinylic-H), 8.04 (4H, d, $J=6.8$ Hz, 2×py $H_{3,5}$), 8.28–8.33 (12H, m, 2×terpy $H_{6,6''}$, $H_{5,5''}$ and $H_{3',5'}$), 8.42 (2H, t, $J=8$ Hz, 2×terpy $H_{4'}$), and 9.06 (4H, d, $J=6.7$ Hz, 2×py $H_{2,6}$); λ_{max} (H_2O) nm: 239 (ε dm^3 mol^{-1} cm^{-1}, 9.5×10^4), 271 (7.0×10^4), 293 (5.9×10^4), 324 (5.2×10^4), 339 (5.3×10^4); *m/z* (ESI): 305.2 ($[M-Pt(terpy)]^{2+}$, 29%); 259.7 (M^{4+}, 100%).

Crystallization of the self-complementary d(CpGpTpApCpG) with 3. An aqueous solution containing

d(CpGpTpApCpG) (0.8–1.2 mM ds DNA), **3** (0.8–1.2 mM), $Mg(NO_3)_2$ (10–15 mM), and sodium cacodylate buffer (100 mM, pH 6.5) was equilibrated with 2-methyl-2,4-pentanediol (up to 20% in 30 mM sodium cacodylate buffer) by the hanging-drop method (5 μL drop size) at room temperature. Bright-yellow crystals formed after one month which were collected by centrifugation.

Preparation of oligonucleotide terpyridine Pt^{II} complex 4 for analysis by mass spectrometry. The magnesium ions in the crystals of the oligonucleotide terpyridine Pt^{II} complex **4** were replaced by ammonium ions as follows.[30] The precipitate and crystals from the crystallization experiment were collected by centrifugation and redissolved in deionized water (~40 nmol in 50 μL) and a 10 M aqueous solution of ammonium acetate (100 μL) was added. Absolute ethanol (350 μL) was added and the solution kept at −20 °C for 2 h. The yellow precipitate formed was collected by centrifugation and washed with cold 70% ethanol and redissolved in deionized water (100 μL). The final concentration of the oligonucleotide solution was approximately 300 pmol μL^{-1}.

Synthesis of 2,2′:6′,2″-terpyridine platinum(II) 2′-deoxyguanosine (5). A solution of chloro-2,2′:6′,2″-terpyridine platinum(II) dihydrate (25 mg, 48 μmol) and silver nitrate (16 mg, 95 μmol) in deionized water (1.0 mL) was heated at 70–80 °C in a water bath for 30 min. The silver chloride was removed by centrifugation and the solution added to 2′-deoxyguanosine (14 mg, 50 μmol) and the solution heated for a further 2 h at 70–80 °C. After centrifugation, the clear-yellow solution was freeze-dried to give 2,2′:6′,2″-terpyridine platinum(II) 2′-deoxyguanosine (**5**) as its nitrate salt in quantitative yield; ^1H NMR (200 MHz, D_2O): δ_H 2.48 and 2.70 (2H, m, CH_2OH), 3.62 (2H, m, $2 \times H_{2'}$), 3.98 (1H, m, $H_{4'}$), 6.30 (1H, t, $J = 6.8$ Hz, $H_{1'}$), 7.48 (2H, m, terpy $H_{5,5''}$), 7.72 (1H, d, $J = 5.8$ Hz, terpy $H_{6,6''}$), 8.12–8.23 (6H, m, terpy $H_{3,3''}$, $H_{4,4''}$, and $H_{3',5''}$), 8.29 (1H, dd, J = 6.3, 9.5 Hz, terpy $H_{4'}$), and 8.75 (1H, s, H_8). The $H_{3'}$ resonance was obscured by the solvent peak.

Enzymatic digestion and electrospray ionization liquid chromatography–mass spectrometry for nucleoside analysis. 2,2′:6′,2″-Terpyridine platinum(II)–d(CpGpTpApCpGp) (**4**) (4 μg) was digested with nuclease P1 (Sigma; one unit), venom phosphodiesterase I (Sigma; 0.02 unit) and alkaline phosphatase (Calbiochem.; one unit) as described,[31] except that P1 digestion was carried out overnight and the initial heat-denaturing step was omitted. The final volume was 15 μL. The LC–MS analysis of 10 μL of digest was accomplished using a Hewlett–Packard 1090 liquid chromatograph coupled to a Fisons Quattro II mass spectrometer (Manchester, U.K.). The digest was injected into a Supelco LC-18DB column (1 × 30 mm) and eluted at 100 μL min^{-1} using a gradient containing 5 mM ammonium acetate, pH 6.0, and 40% (aq) acetonitrile. Mass spectrometer conditions were as follows: ion source temperature 200 °C, needle voltage +3500 V, cone voltage +25 V and mass scan range *m/z* 100–725. Procedures for the analysis of data from LC–MS analysis of nucleosides in enzymatic digests of nucleic acids have been earlier detailed.[32]

Positive and negative ion mode electrospray MS and MS/MS for oligonucleotide analysis. The electrospray mass spectrum of **3** was determined on a VG Biotech Bio-Q mass spectrometer. Other oligonucleotide mass spectra were acquired using a Perkin–Elmer–Sciex API III+ triple quadrupole mass spectrometer (Thornhill, ON, Canada) equipped with an electrospray ionization source. In general, 20 pmol μL^{-1} concentration of sample in 50% isopropyl alcohol aqueous solution was infused at 1.5 μL min^{-1} flow rate. In the negative ion mode, the ES ionization voltage was kept at −3300 V and orifice voltage of −50 V was used. During data acquisition, the scan step size was maintained at 0.10 and 10 scans were accumulated in the range *m/z* 300–2000. In the negative ion collision-induced dissociation of **4**, the triply charged ion (*m/z* 738.5) was selected as the precursor ion by MS1 for collisional activation with argon gas at collision energy 45 eV (E_{lab}) and collision cell pressure 2.5×10^{15} atoms cm^{-2}. In the positive ion mode collision-induced dissociation of 2,2′:6′,2″-terpyridine platinum(II)–dG (**5**), the double charged ion (*m/z* 347.5) was selected as the precursor ion by MS1 for collisional activation with argon gas at collision energy 40 eV (E_{lab}) and collision cell pressure 2.5×10^{15} atoms cm^{-2}. The electrospray probe tip was maintained at +4100 V and the orifice voltage was set at +40 V.

Acknowledgements

The authors thank Dr Pamela F. Crain for oligonucleotide hydrolysis and LC–MS analysis, S. C. Pomerantz for preparation of figures, and Dr R. T. Aplin and Mrs E. McGuinness for the mass spectrum and the NMR spectra, respectively, of **3**. This work was supported by a DPST research scholarship from the Thai Government (to T.V.), and by grant GM21584 from the National Institute of General Medical Sciences (to J.A.M.).

References

1. McCoubrey, A.; Latham, H. C.; Cook, P. R.; Rodger, A.; Lowe, G. *FEBS Lett.* **1996**, *380*, 73.

2. Quigley, G. J.; Wang, A. H.-J.; Ughetto, G.; van der Marel, G.; van Boom, J. H.; Rich, A. *Proc. Natl. Acad. Sci. U.S.A.* **1980**, *77*, 7204.

3. Subramanya, H. S.; Wigley, D. B. Personal communication.

4. Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153.

5. Hollis, L. S.; Sundquist, W. I.; Burstyn, J. N.; Heiger-Bernays, W. J.; Bellon, S. F.; Ahmed, K. J.; Amundsen, A. R.; Stern, E. W.; Lippard, S. J. *Cancer Res.* **1991**, *51*, 1866.

6. Mullins, S. T.; Annan, N. K.; Cook, P. R.; Lowe, G. *Biochemistry* **1992**, *31*, 842.

7. Annan, N. K.; Cook, P. R.; Mullins, S. T.; Lowe, G. *Nucl. Acids Res.* **1992**, *20*, 983.

8. Limbach, P. A.; Crain, P. F.; McCloskey, J. A. *Curr. Opin. Biotechnol.* **1995**, *6*, 96.

9. Crain, P. F. In *Electrospray Ionization Mass Spectrometry: Fundamentals Instrumentation and Applications*; Cole, R. B., Ed.; Wiley: New York, 1996, in press.

10. Costello, C. E.; Comess, K. M.; Plaziak, A. S.; Bancroft, D. P.; Lippard, S. J. *Int. J. Mass Spectrom. Ion Processes* **1992**, *122*, 255.

11. Puzo, C.; Promé, J. C.; Macquet, J. P.; Lewis, I. A. S. *Biomed. Mass Spectrom.* **1982**, *9*, 552.

12. Siegel, M. M.; Bitha, P.; Child, R. G.; Hlavka, J. J.; Lin Y. I.; Chang, T. T. *Biomed. Mass Spectrom.* **1986**, *13*, 25.

13. Lepre, C. A.; Chassot, L.; Costello, C. E.; Lippard, S. J. *Biochemistry* **1990**, *29*, 811.

14. Volchenskova, I. I.; Shalimov, S. A.; Keisevich, L. V.; Pokrovskii, V. A.; Kopytin, S. N. *J. Struct. Chem.* **1994**, *35*, 528.

15. Martin, III, L. B.; Schreiner, A. F.; van Breemen, R. B. *Anal. Biochem.* **1991**, *193*, 6.

16. Comess, K. M.; Costello, C. E.; Lippard, S. J. *Biochemistry* **1990**, *29*, 2102.

17. Fenn, J. B.; Mann, M.; Ming, C. K.; Wong, S. F.; Whitehouse, C. M. *Science* **1989**, *246*, 64.

18. Covey, T. R.; Bonner, R. F.; Shushan, B. I.; Henion, J. D. *Rapid Commun. Mass Spectrom.* **1988**, *2*, 249.

19. Poon, G. K.; Mistry, P.; Lewis, S. *Biol. Mass Spectrom.* **1991**, *20*, 687.

20. Ehrsson, H.; Wallin I. B.; Andersson, A. S.; Edlund, P. O. *Anal. Chem.* **1995**, *67*, 3608.

21. Da Col, R.; Silvestro, L.; Baiocchi, C.; Giacosa, D.; Viano, I. *J. Chromatogr.* **1993**, *633*, 119; Wickham, G.; Iannitti, P.; Boschenok, J.; Sheil, M. M. *J. Mass Spect. Rapid Commun. Mass Spectrom.* **1995**, S197.

22. McCloskey, J. A. *Acc. Chem. Res.* **1991**, *24*, 81.

23. Crow, F. W.; Tomer, K. B.; Gross, M. L.; Bergstrom, D. E. *Anal. Biochem.* **1984**, *139*, 243.

24. Crain, P. F.; Gregson, J. M.; McCloskey, J. A.; Nelson, C. C.; Peltier, J. M.; Philips, D. R.; Pomerantz, S. C.; Reddy, D. M. In *Mass Spectrometry in the Biological Sciences*; Burlingame, A. L.; Carr, S. A., Eds.; Humana: Clifton, NJ, 1996; pp 497–517.

25. Gregson, J. M. Ph.D. Thesis, University of Utah, 1994, pp 18–68.

26. Skapki, A. C.; Sutcliffe, V. F.; Young, G. B. *J. Chem. Soc., Chem. Commun.* **1985**, 609.

27. *Handbook of Chemistry and Physics,* 64 ed.; Weast, R. C., Ed.; CRC: Boca Raton, 1984; pp E63–64.

28. McLuckey, S. A.; Habibi-Goudarzi, S. *J. Am. Chem. Soc.* **1993**, *115*, 12085.

29. McLuckey, S. A.; Van Berkel, G. J.; Glish, G. L. *J. Am. Soc. Mass Spectrom.* **1992**, *3*, 60.

30. Limbach, P. A.; Crain, P. F.; McCloskey, J. A. *J. Am. Soc. Mass Spectrom.* **1995**, *6*, 27.

31. Crain, P. F. *Methods Enzymol.* **1990**, *193*, 782.

32. Pomerantz, S. C.; McCloskey, J. A. *Methods Enzymol.* **1990**, *293*, 796.

(*Received in U.S.A.* 25 *January* 1996; *accepted* 14 *March* 1996)