Synthesis, Characterization, and Interaction with DNA of the Novel Metallointercalator Cationic Complex (2,2′:6′,2″-Terpyridine)methylplatinum(II)

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A series of salts of the type [Pt(terpy)Me]X (X = Cl, NO_3, PF_6, ClO_4, $B(C_6H_5)_4$) containing a new organometallic complex cation of platinum(II) with 2,2′:6′,2″-terpyridine (terpy) were synthesized and characterized by ^1H, ^{13}C, and ^{195}Pt NMR spectroscopy. No evidence for fluxionality of the terpyridine ligand was obtained, indicating that it is terdentate. The fourth position in the coordination plane is occupied by a methyl group. The resulting cation is unreactive toward substitution and is stable in aqueous solutions under mild conditions. The planarity and electron delocalization of the terpy moiety lead to extensive stacking interactions, forming dimers in dilute aqueous solution and larger aggregates when the concentration and/or the ionic strength increase. UV/vis and ^1H NMR spectra show characteristic dependencies on the concentration of the complex, the temperature, the solvent, and the ionic strength. Analysis of the absorption spectral data gives a value of 10 (±8) × 10^3 M^{-1} (T = 298 K; μ = 0.101 mol L^{-1}) for the dimerization equilibrium constant. Resonance light-scattering spectra, measured for the first time on a metal-containing noncyclic substrate, provide evidence for the tendency of the complex to form large self-aggregates even under low ionic strength conditions. The interaction of the cationic complex ion with calf thymus DNA was investigated by UV/vis, CD spectroscopy, resonance light-scattering, thermal denaturation, and gel electrophoresis mobility assays. At high r_f ratios the complex seems to form extended aggregates on the surface of the nucleic acid, but at lower r_f ratios evidence was obtained for intercalation.

Introduction

There has been considerable interest in the interactions of metal complexes with DNA and especially those factors that determine affinity and selectivity in binding.[1] Following the discovery of the antitumor activity of *cisplatin*, a great deal of effort was expended in determining the mechanism and specificity of this drug. It is now well established that *cisplatin* acts preferentially through covalent intrastrand binding, and a large number of *cisplatin* analogues have been developed with the intention of improving the clinical properties of this class of compounds.[2] A second binding mode for small molecules with nucleic acids is intercalation. This type of drug/nucleic acid interaction was first suggested by Lerman[3] in describing the binding to DNA of several small planar aromatic dyes, such as proflavin[4] and ethidium bromide[5], or clinical drugs, such as, for example, the antibiotic actinomycin D or daunomycin.[6] The intercalation process induces modifications of the structure and properties of DNA[7] and is considered as a preliminary step to mutagenesis for several substances.[8] The π-stacking interactions between the aromatic heterocyclic groups of base pairs and the aromatic moieties of an intercalating agent and hydrogen-bonding, electrostatic, and hydrophobic interactions all contribute to stabilizing the binding of these small flat molecules to the DNA helix. Metal complexes are particularly well suited for the study of these interactions because they offer the possibility of evaluating the effects of electronic and steric factors on the binding with the nucleic acids through systematic changes of the metal and the coordination sphere.

The first well-documented example of an intercalating metalloreagent was the complex (2-hydroxyethanethiolato)(2,2′:6′,2″-terpyridine)platinum(II), [Pt(terpy)(HET)]$^+$.[9−16] The choice of 2-hydroxyethanethiol as the monodentate ligand was dictated by the preference for a group which is not easily removed, in that the first tested complex, chloro(2,2′:6′,2″-terpyridine)-platinum(II),[12] gave a mixture of intercalation and covalent binding due to the presence of the labile chloride anion. The

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(1) (a) Barton, J. K. *Comments Inorg. Chem.* **1985**, *3*, 321−348. (b) Pyle, A. M.; Barton, J. K. In *Progress in Inorganic Chemistry: Bioinorganic Chemistry, Vol. 38*; Lippard, S. J., Ed.; John Wiley & Sons: New York, 1990; pp 413−475.
(2) (a) Lippard, S. J. *Pure Appl. Chem.* **1987**, *59*, 731. (b) Reedijk, J. *Pure Appl. Chem.* **1987**, *59*, 181. (c) Sherman, S. E.; Lippard, S. J. *Chem. Rev.* **1987**, *87*, 1153. (d) Nicolini, M., Ed. *Platinum and Other Metal Coordination Compounds in Cancer Chemiotherapy*; Martinus Nijhoff Publishing: Boston, MA, 1988. (e) Umapathy, P. *Coord. Chem. Rev.* **1989**, *95*, 129. (f) Lippard, S. J. *Coord. Chem. Rev.* **1991**, *59*, 731.
(3) Lerman, L. S. *J. Mol. Biol.* **1961**, *3*, 18−30.
(4) Lerman, L. S. *Proc. Natl. Acad. Sci. U.S.A.* **1963**, *49*, 94−102.
(5) Waring, M. J. *Biochim. Biophys. Acta* **1964**, *87*, 358−361. Bauer, W.; Vinograd, J. *J. Mol. Biol.* **1970**, *47*, 419−435.
(6) Hollstein, V. *Chem. Rev.* **1974**, *74*, 625−652.
(7) Long, E. C.; Barton, J. K. *Acc. Chem. Res.* **1990**, *23*, 271−273 and references therein.
(8) Saenger, W. *Principles of Nucleic Acid Structure*; Springer-Verlag: New York, 1984. Waring, M. J. *Annu. Rev. Biochem.* **1981**, *50*, 159−192.
(9) Jennette, K. W.; Lippard, S. J.; Vassiliades, G. A.; Bauer, W. R. *Proc. Natl. Acad. Sci. U.S.A.* **1974**, *71*, 3839−3843.
(10) Bond, P. J.; Langridge, R.; Jennette, K. W.; Lippard, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1975**, *72*, 4825−4829.
(11) Lippard, S. J.; Bond, P. J.; Wu, K. C.; Bauer, W. R. *Science* **1976**, *194*, 726−727.
(12) Howe-Grant, M.; Wu, K. C.; Bauer, W. R.; Lippard, S. J. *Biochemistry* **1976**, *15*, 4339−4346.
(13) Jennette, K. W.; Gill, J. T.; Sadownick, J. A.; Lippard, S. J. *J. Am. Chem. Soc.* **1976**, *98*, 6159−6168.
(14) Wong, Y.; Lippard, S. J. *J. Chem. Soc., Chem. Commun.* **1977**, 824−825.
(15) (a) Lippard, S. J. *Acc. Chem. Res.* **1978**, *11*, 211−217. (b) Barton, J. K.; Lippard, S. J. *Biochemistry* **1979**, *18*, 2661−2668. (c) Howe-Grant, M.; Lippard, S. J. *Biochemistry* **1979**, *18*, 5762−5769.

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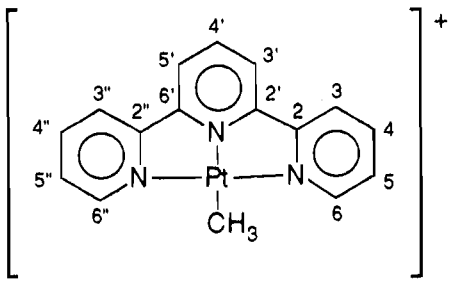
electron density of this square planar complex was used to obtain X-ray fiber diffraction evidence for the nearest-neighbor exclusion binding model.[10] Much effort has been devoted to the elucidation of the role of charge and of substituent groups in determining the non-covalent interactions of cationic platinum(II) complexes containing aromatic ligands such as 2,2′:6′,2″-terpyridine, 1,10-phenanthroline, and 2,2′-bipyridyl with the DNA duplex.[12] Recently, the use of chiral metal complexes, mainly of ruthenium(II) containing such ligands, attracted a great deal of interest due to the enantiomeric selectivity of the Δ isomer toward the right-handed DNA helix and the consequent applications in probing nucleic acid conformations.[1]

We profit from the presence in the literature of a clear and detailed characterization of the interactions of Pt complexes containing the terpy moiety with DNA to study the behavior of a strictly similar organometallic compound. The introduction of a Pt−C bond and the possibility of synthesizing complexes with organic moieties of suitable length and branching make these compounds promising in the design of bifunctional metallointercalators. This paper describes the synthesis of organometallic complexes of the type [Pt(terpy)(CH_3)]X (**I**)- and studies of their solution properties, as well as the interaction



(I)

of the platinum complex cation with nucleic acids.[17] This paper also reports the application of a recently described light-scattering technique (termed "resonance light-scattering")[18] to study the aggregation of the complex by itself and in the presence of nucleic acids. This technique has the advantage of being extremely sensitive and informative as to the nature of the aggregates. This is the first case of a metal-containing noncyclic substrate showing the resonance light-scattering (RLS) phenomenon. The properties of the complex [Pt(dien)(CH_3)]Cl have also been investigated, in order to permit a comparison with a system lacking the possibility of π-stacking interactions.

Experimental Section

Preparation of the Complexes. K_2PtCl_4 was obtained from Strem Chemical Co. and was separated from metallic Pt and K_2PtCl_6 by dissolving it in water and filtering. The 2,2′:6′,2″-terpyridine (terpy) ligand and tetramethyltin were received from Aldrich, and their purity was checked by ^1H NMR. Dimethyl sulfoxide was purified by liquid chromatography on alumina under argon and stored over molecular sieves. The solvents used were purified and dried by standard techniques. All the other reagents were of the highest commercial grade available and were used as received or were purified by distillation or recrystallization where necessary.

(a) ***trans*-[Pt(Me_2SO)$_2$(CH_3)Cl]** was prepared according to a published method[19] and was crystallized several times from dichloromethane/diethyl ether mixtures.

(b) [Pt(terpy)(CH_3)]Cl (1). A methanolic solution of terpy (70 mg; 0.3 mmol in 20 mL) was added dropwise to a stirred solution of *trans*-[Pt(Me_2SO)$_2$(CH_3)Cl] (120 mg; 0.3 mmol in 20 mL). The solution immediately turned orange, and fine crystals of the same color began to precipitate. After 30 min, the solid was separated from the solution, washed with several small portions of cold dichloromethane and diethyl ether, and dried in a desiccator over P_2O_5, yielding 120 mg of fine orange needles (yield 84%). Anal. Calcd for $C_{16}H_{14}N_3ClPt$: H, 2.95; C, 40.13; N, 8.78; Cl, 7.4. Found: H, 2.89; C, 40.3; N, 8.82; Cl, 7.2. FABMS *m/e*: 443, $[M^+]^+$; 428, $[M^+ - CH_3]^+$. ^1H NMR (0.01M in dmso-d_6): δ 8.85 (dd, 2H, $^3J_{HH}$ = 5.5 Hz, $^4J_{HH}$ = 1.1 Hz, $^3J_{PtH}$ = 51.0 Hz), 8.60 (m, 4H), 8.53 (t, 1H, $^3J_{HH}$ = 7.8 Hz), 8.43 (t, 2H, $^3J_{HH}$ = 7.7 Hz), 7.85 (t, 2H, $^3J_{HH}$ = 5.5 Hz, $^4J_{PtH}$ = 20.0 Hz), 1.07 (s, 3H, $^2J_{PtH}$ = 73.6 Hz). ^{13}C NMR (dmso-d_6): δ 159.0, 151.5 (4C, C_2 + $C_{2'}$), 150.9 (2C, $^2J_{PtC}$ = 34.0 Hz, C_6), 141.0 (1C, $C_{4'}$), 140.3 (2C, C_4), 128.6 (2C, $^3J_{PtC}$ = 43.4 Hz, C_5), 125.4 (2C, $^3J_{PtC}$ = 29.3 Hz, C_3), 123.6 (2C, $^3J_{PtC}$ = 11.7 Hz, $C_{3'}$), −5.7 (1C, $^1J_{PtC}$ = 794.5 Hz, CH_3). ^{195}Pt (dmso-d_6): δ −3221.

(c) [Pt(terpy)(CH_3)](NO_3) (2). This complex was prepared *in situ* and characterized in solution. A stoichiometric amount of silver nitrate dissolved in a small volume of dmso-d_6 was added dropwise to a solution of [Pt(terpy)(CH_3)]Cl in the same solvent. The precipitated AgCl was removed by centrifugation and filtration of the solution. ^1H NMR (0.01 M in dmso-d_6): δ 8.77 (dd, 2H, $^3J_{HH}$ = 5.5 Hz, $^4J_{HH}$ = 1.0 Hz, $^3J_{PtH}$ = 50.1 Hz), 8.55 (m, 4H), 8.49 (t, 1H, $^3J_{HH}$ = 7.7 Hz), 8.41 (t, 2H, $^3J_{HH}$ = 7.7 Hz), 7.82 (t, 2H, $^3J_{HH}$ = 5.5 Hz, $^4J_{PtH}$ = 20.0 Hz), 1.00 (s, 3H, $^2J_{PtH}$ = 73.7 Hz). ^{13}C NMR (dmso-d_6): δ 158.9, 151.4 (4C, C_2 + $C_{2'}$), 150.9 (2C, $^2J_{PtC}$ = 30.4 Hz, C_6), 140.9 (1C, $C_{4'}$), 140.3 (2C, C_4), 128.6 (2C, $^3J_{PtC}$ = 39.4 Hz, C_5), 125.3 (2C, $^3J_{PtC}$ = 29.3 Hz, C_3), 123.5 (2C, $^3J_{PtC}$ = 10.6 Hz, $C_{3'}$), −5.7 (1C, $^1J_{PtC}$ = 750.0 Hz, CH_3). ^{195}Pt (dmso-d_6): δ −3220.

(d) [Pt(terpy)(CH_3)](PF_6) (3). In a typical preparative procedure, a stoichiometric amount of KPF_6, dissolved in a small volume of methanol, was added to a hot methanolic solution of [Pt(terpy)(CH_3)]Cl. On standing, fine yellow-orange needles precipitated out of the solution. The precipitate was collected by filtration, washed with several portions of cold methanol, and dried under vacuum (yield 90%). Anal. Calcd for $C_{16}H_{14}N_3PF_6Pt$: H, 2.40; C, 32.66; N, 7.14. Found: H, 2.46; C, 32.1; N, 7.23. FABMS *m/e:* 443, $[M^+]^+$; 428, $[M^+ - CH_3]^+$. ^1H NMR (0.01 M in dmso-d_6): δ 8.74 (dd, 2H, $^3J_{HH}$ = 5.5 Hz, $^4J_{HH}$ = 1.1 Hz, $^3J_{PtH}$ = 50.4 Hz), 8.51 (m, 4H), 8.46 (t, 1H, $^3J_{HH}$ = 7.8 Hz), 8.40 (t, 2H, $^3J_{HH}$ = 7.7 Hz), 7.80 (t, 2H, $^3J_{HH}$ = 5.5 Hz, $^4J_{PtH}$ = 20.0 Hz), 0.96 (s, 3H, $^2J_{PtH}$ = 73.5 Hz). ^{13}C NMR (dmso-d_6): δ 159.0, 151.5 (4C, C_2 + $C_{2'}$), 150.9 (2C, $^2J_{PtC}$ = 30.5 Hz, C_6), 141.0 (1C, $C_{4'}$), 140.4 (2C, C_4), 128.7 (2C, $^3J_{PtC}$ = 43.4 Hz, C_5), 125.4 (2C, $^3J_{PtC}$ = 29.3 Hz, C_3), 123.6 (2C, $^3J_{PtC}$ = 10.6 Hz, $C_{3'}$), −5.7 (1C, $^1J_{PtC}$ = 794.6 Hz, CH_3). ^{195}Pt (dmso-d_6): δ −3220.

(e) [Pt(terpy)(CH_3)](ClO_4) (4). *Caution! Perchlorate salts of metal complexes are potentially explosive and should be handled with care.* This complex was prepared as reported for the hexafluorophosphate salt using $LiClO_4$ as the precipitating agent (yield 85%). Anal. Calcd for $C_{16}H_{14}N_3ClO_4Pt$: H, 2.60; C, 35.40; N, 7.74. Found: H, 2.56; C, 35.1; N, 7.63. FABMS *m/e:* 443, $[M^+]^+$; 428, $[M^+ - CH_3]^+$. ^1H NMR (0.01 M in dmso-d_6): δ 8.87 (dd, 2H, $^3J_{HH}$ = 5.5 Hz, $^4J_{HH}$ = 1.1 Hz, $^3J_{PtH}$ = 51.3 Hz), 8.60 (m, 4H), 8.54 (t, 1H, $^3J_{HH}$ = 7.8 Hz), 8.44 (t, 2H, $^3J_{HH}$ = 7.7 Hz), 7.85 (t, 2H, $^3J_{HH}$ = 5.5 Hz, $^4J_{PtH}$ = 20.0 Hz), 1.09 (s, 3H, $^2J_{PtH}$ = 73.1 Hz). ^{13}C NMR (dmso-d_6): δ 158.9, 151.4 (4C, C_2 + $C_{2'}$), 150.9 (2C, $^2J_{PtC}$ = 30.5 Hz, C_6), 141.0 (1C, $C_{4'}$), 140.3 (2C, C_4), 128.6 (2C, $^3J_{PtC}$ = 39.9 Hz, C_5), 125.3 (2C, $^3J_{PtC}$ = 28.2 Hz, C_3), 123.5 (2C, $^3J_{PtC}$ = 12.9 Hz, $C_{3'}$), −5.7 (1C, $^1J_{PtC}$ = 748.9 Hz, CH_3). ^{195}Pt (dmso-d_6): δ −3219.

(f) [Pt(terpy)(CH_3)]($B(C_6H_5)_4$) (5). This complex was obtained in a manner analogous to that described for other complexes by precipitat-

(16) (a) Dewan, J. C.; Lippard, S. J.; Bauer, W. R. *J. Am. Chem. Soc.* **1980**, *102*, 858−860. (b) Wakelin, L. P. G.; McFadyen, W. D.; Walpole, A.; Roos, I. A. G. *Biochem. J.* **1984**, *222*, 203−215. (c) McFadyen, W. D.; Wakelin, L. P. G.; Roos, I. A. G.; Hillcoat, B. L. *Biochem. J.* **1986**, *238*, 757−763. (d) McFadyen, W. D.; Wakelin, L. P. G.; Roos, I. A. G.; Hillcoat, B. L. *Biochem. J.* **1987**, *242*, 177−183.

(17) A preliminary account of this work was presented during the First International Conference on Scaling Concepts and Complex Fluids, Copanello (CZ), Italy, July 4−7, 1994.

(18) Pasternack, R. F.; Bustamante, C.; Collings, P. J.; Giannetto, A.; Gibbs, E. J. *J. Am. Chem. Soc.* **1993**, *115*, 5393−9. Pasternack, R. F.; Schaefer, K. F.; Hambright, P. *Inorg. Chem.* **1994**, *33*, 2062−5.

(19) Eaborn, C.; Kundu, K.; Pidcock, A. J. *J. Chem. Soc., Dalton Trans.* **1981**, 933.

Table 1. ^{1}H NMR Data for terpy and [Pt(terpy)Me]X Complexes[a]

complex	no.	H_6	H_5	H_4	H_3	$H_{3'}$	$H_{4'}$	Me
terpy		8.70	7.45	7.98	8.58	8.42	8.06	
[Pt(terpy)Me]Cl	**1**	8.85 (51.0)[b]	7.85	8.43	8.60	8.60	8.53	1.07 (73.6)[c]
[Pt(terpy)Me]Cl[d]	**1**	8.98 (52.2)	7.32	8.39	8.48	8.48	8.45	1.23 (73.7)
[Pt(terpy)Me]Cl[e]	**1**	7.82 (50.0)	7.25	7.89	7.59	7.50	7.68	0.00 (74.0)
[Pt(terpy)Me](NO_3)	**2**	8.77 (50.1)	7.82	8.41	8.55	8.55	8.49	1.00 (73.7)
[Pt(terpy)Me](PF_6)	**3**	8.74 (50.4)	7.80	8.40	8.51	8.51	8.46	0.96 (73.5)
[Pt(terpy)Me](ClO_4)	**4**	8.87 (51.3)	7.85	8.44	8.60	8.60	8.54	1.09 (73.1)
[Pt(terpy)Me](BPh_4)	**5**	8.88 (50.9)	7.85	8.44	8.60	8.60	8.52	1.10 (74.0)

[a] At 308 K in dmso-d_6 as solvent (0.01 M). Spectra were recorded at 300.13 MHz, and chemical shifts are given in ppm from TMS as external standard. [b] $^3J_{PtH}$ in Hz. [c] $^2J_{PtH}$ in Hz. [d] In CD_3OD at 298 K (0.001 M). [e] In D_2O at 298 K (0.0032 M).

ing with $NaB(C_6H_5)_4$ (yield 95%). Anal. Calcd for $C_{40}H_{34}BN_3Pt$: H, 4.49; C, 63.0; N, 5.51. Found: H, 4.58; C, 62.1; N, 5.56. FABMS *m/e:* 443, $[M^+]^+$, 428, $[M^+ - CH_3]^+$. ^{1}H NMR (0.01 M in dmso-d_6): δ 8.88 (dd, 2H, $^3J_{HH}$ = 5.5 Hz, $^4J_{HH}$ = 1.1 Hz, $^3J_{PtH}$ = 50.9 Hz), 8.60 (m, 4H), 8.52 (t, 1H, $^3J_{HH}$ = 7.8 Hz), 8.44 (t, 2H, $^3J_{HH}$ = 7.7 Hz), 7.85 (t, 2H, $^3J_{HH}$ = 5.5 Hz, $^4J_{PtH}$ = 20.0 Hz), 7.17 (m, 8H), 6.91 (t, 8H, $^3J_{HH}$ = 7.2 Hz), 6.77 (t, 4H, $^3J_{HH}$ = 7.2 Hz), 1.10 (s, 3H, $^2J_{PtH}$ = 74.0 Hz). ^{13}C NMR (dmso-d_6): δ 163 (4C, $^1J_{BC}$ = 49.2 Hz, C_{quat} BPh_4), 158.9, 151.4 (4C, C_2 + $C_{2'}$), 150.8 (2C, $^2J_{PtC}$ = 32.1 Hz, C_6), 140.9 (1C, $C_{4'}$), 140.3 (2C, C_4), 135.2 (8C, C_{meta} BPh_4), 128.6 (2C, $^3J_{PtC}$ = 43.2 Hz, C_5), 125.3 (2C, $^3J_{PtC}$ = 29.5 Hz, C_3), 124.9 (8C, $^2J_{BC}$ = 2.3 Hz, C_{orto} BPh_4), 123.5 (2C, $^3J_{PtC}$ = 10.0 Hz, $C_{3'}$), 121.1 (4C, C_{para} BPh_4), −5.7 (1C, $^1J_{PtC}$ = 748.2, CH_3). ^{195}Pt (dmso-d_6): δ −3221.

(g) [Pt(dien)(CH_3)]Cl (6). This complex was prepared by adding dropwise a methanolic solution of dien (103 mg; 1 mmol) to a stirred solution of *trans*-[Pt(Me_2SO)$_2$(CH_3)Cl] (401 mg; 1 mmol). After 1 h, the yellowish solution was concentrated under vacuum, diethyl ether was added, and the mixture was cooled. The white precipitate was collected, washed with diethyl ether, and dried under vacuum (260 mg; yield 75%). ^{1}H NMR (dmso-d_6): δ 5.38 (br m, 1H, $^3J_{PtH}$ = 39.6 Hz, NH), 4.97 (br m, 4H, $^3J_{PtH}$ = 55.0 Hz, NH_2), 3.09 (m, 4H, $^3J_{PtH}$ = 66.5 Hz, CH_2), 2.73 (br m, 4H, $^3J_{PtH}$ = 34.0 Hz, CH_2), 0.14 (s, 3H, $^2J_{PtH}$ = 80.3 Hz, CH_3). ^{13}C NMR (dmso-d_6): δ 51.7 (2C, $^2J_{PtC}$ = 28.1 Hz, CH_2), 50.4 (2C, $^2J_{PtC}$ = 25.8 Hz, CH_2), −28.7 (1C, $^1J_{PtC}$ = 748.8 Hz, CH_3). ^{195}Pt (dmso-d_6): δ −3367.

Nucleic Acid Binding Studies. Water used in the experiment with nucleic acids was purified with a Millipore apparatus. The following buffer solutions were used in the present study: (A) phosphate 1 mM, 10 mM NaCl, pH 7.0; (B) phosphate 1 mM, NaCl 0.1 M, pH 7.0; (C) TBE, Tris 45 mM, H_3BO_3 45 mM, Na_2H_2EDTA 1 mM, pH 7.5; (D) phosphate 1 mM, pH 7.0. Calf thymus (ct) DNA (type I) was obtained from Sigma Chemical Co. and was purified using a literature method.[20] The concentration of a ct DNA stock solution was determined by using ϵ_{260} = 1.33 × 10^4 M^{-1} cm^{-1} (in molar base pairs).[20] pUC19 plasmid DNA was isolated from *E. coli* by using a Qiagen DNA purification kit. The amount of the supercoiled form was checked by gel electrophoresis on agarose. The preparation contained a 30% amount of the nicked relaxed form.

Instrumentation. pH measurements were obtained using an Orion combined glass electrode on a Radiometer pHM62 instrument. UV/vis electronic spectra were recorded on a Cary 219 or a Cary 2200 spectrophotometer. Circular dichroism spectra were recorded with an Aviv 62 DS spectrophotometer in 1.0 cm cuvettes and they are reported as ellipticities in millidegrees. The light-scattering measurements were performed on a SPEX F111 spectrofluorimeter employing a synchronous-scan protocol and right-angle geometry.[18] ^{1}H, ^{13}C, ^{195}Pt, and ^{35}Cl NMR spectra were obtained on a Bruker AMX-R 300 spectrometer equipped with a broad-band probe operating at 300.13, 75.5, 64.22, and 29.4 MHz, respectively. Sample solutions in D_2O (99.8%) were referenced to 3-(trimethylsilyl)propionate (TSP), while sample solutions in dmso-d_6 or chloroform-*d* were referenced to TMS. In each case, chemical shifts (δ) are reported in ppm downfield from TMS, and coupling constants in Hz. ^{195}Pt NMR spectra were obtained using a $\pi/2$ pulse of 7 μs and a relaxation delay of 1 s. Typically the spectral width was set at 62.5 kHz with 16K data points. Typical ^{35}Cl NMR acquisition parameters were a spectral width of 5 kHz with 4K data points, a $\pi/2$ pulse of 8 μs, and a relaxation delay of 1s. The FIDs were transformed after applying an exponential multiplication which introduced a 10 Hz line-broadening factor. The temperature within the probe was checked by the methanol or ethylene glycol method.[21] ^{195}Pt and ^{35}Cl chemical shifts were referenced to the absolute frequency scales by setting the TMS resonance exactly to 100 MHz.[22] ^{195}Pt chemical shifts were converted to the Na_2PtCl_6 scale by using the following expression: $\delta(Na_2PtCl_6) = \delta$(frequency scale) − 4533.[22]

The terpy ^{13}C NMR spectrum was assigned using a standard 2D heteronuclear correlation pulse sequence.

Fast atom bombardment mass spectrometry (FABMS) was performed in a glycerol matrix with a Kratos instrument equipped with a standard FAB source.

Melting temperature measurements on *ct*-DNA were obtained on a Perkin-Elmer Lambda 3A spectrophotometer, connected to a computer-controlled Peltier heating apparatus. The melting profiles were recorded using a linear temperature gradient of 0.5 °C/min.

Gel Electrophoresis Studies. In a typical procedure a series of samples containing 0.4 μg of pUC19 DNA in a TBE buffer were incubated for 30 min with different amounts of the platinum complex at 25 °C. DNA mobility assays were performed by gel electrophoresis through 1.2% agarose slab gels with a TBE running buffer. The experiments were run overnight (14 h; total course 85 mm) in the dark, with a voltage of 45−50 V. The gels were stained with an aqueous solution of ethidium bromide and photographed on Polaroid 667 film using a UV transilluminator.

Results

The chloride salt of this cationic organometallic square planar platinum(II) complex ion (**I**) was synthesized quite easily by the following reaction:

$$\textit{trans}\text{-[Pt(Me}_2\text{SO)}_2\text{(CH}_3\text{)Cl]} + \text{terpy} \rightarrow [\text{Pt(terpy)(CH}_3\text{)}]\text{Cl} + 2\text{Me}_2\text{SO} \quad (1)$$

The simultaneous presence in the starting complex of two sulfoxides[23] and a highly trans-activating methyl group[24] makes the substitution by the terdentate ligand very facile. The formation of the reaction product can be monitored by conventional spectrophotometry in the near-UV/vis region.

The chloride salt is quite soluble in dimethyl sulfoxide, water, alcoholic solvents, and acetone but only slightly soluble in chloroform and dichloromethane. UV/vis spectra show aqueous solutions of the complex to be stable for weeks. When the chloride salt of **I** is heated for 30 min in chloroform at 50 °C,

(20) Pasternack, R. F.; Gibbs, E. J.; Villafranca, J. J. *Biochemistry* **1983**, *22*, 2406.

(21) Van Geet, A. L. *Anal. Chem.* **1968**, *40*, 2227−2229. Van Geet, A. L. *Anal. Chem.* **1970**, *42*, 679−680.

(22) Pregosin, P. S. *Coord. Chem. Rev.* **1982**, *44*, 247. Pregosin, P. S. *Annu. Rep. NMR Spectrosc.* **1986**, *17*, 285−349. Lindman, B.; Forsen, S. In *NMR and the Periodic Table*; Harris, R. K., Mann, B. E., Eds.; Academic Press Inc.: London, 1978; Chapter 13, pp 421−438. Harris, R. K. In *NMR and the Periodic Table*; Harris, R. K., Mann, B. E., Eds.; Academic Press Inc.: London, 1978; Chapter 1, p 8.

(23) Romeo, R.; Lanza, S.; Minniti, D.; Tobe, M. L. *Inorg. Chem.* **1983**, *22*, 2006−2010.

(24) Wilkins, R. G. *Kinetics and Mechanism of Reactions of Transition Metal Complexes*; VCH: New York, 1991; p 237.

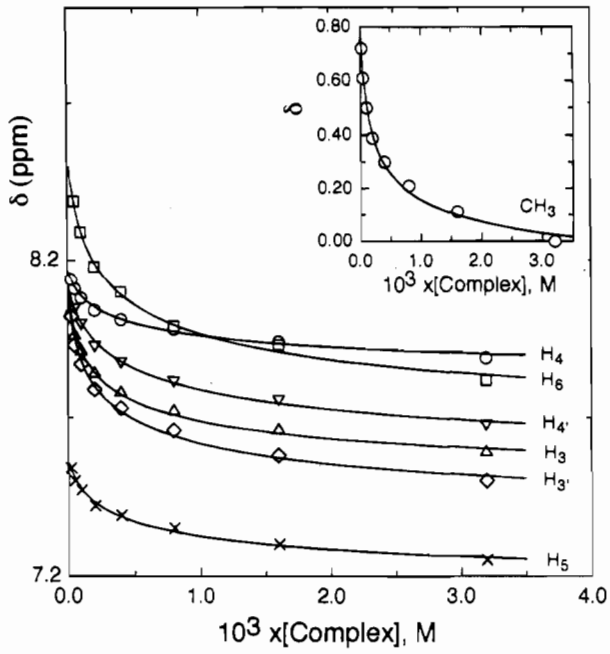


Figure 1. Concentration dependence of ^{1}H NMR resonances of [Pt(terpy)Me]Cl in D_2O at 298 K.

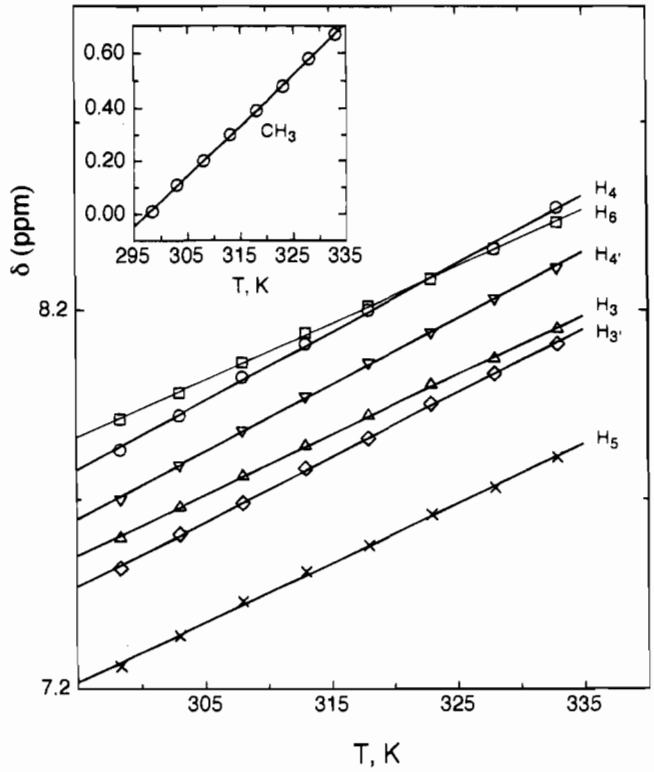


Figure 2. 300.13 MHz ^{1}H NMR chemical shift temperature dependence of [Pt(terpy)Me]Cl in D_2O ([complex] = 0.01 M).

a light red solution is obtained from which a red compound slowly precipitates. When the same compound is boiled in pyridine, the solution turns yellow and fine crystals of the same color slowly separate from the solution. UV/vis and ^{1}H NMR spectra clearly show that these apparently different compounds are in fact identical to the starting complex (*vide infra*).

The ^{1}H NMR spectrum of **I** in dmso-d_6 was assigned by following the numeration pattern shown above and could be made according to previously published results for analogous complexes.[13] The assignment was facilitated by the presence of large coupling constants associated with the isotropically abundant ^{195}Pt (33%, $I = {}^1/_2$). The signal at δ 1.07 is due to the methyl protons and shows the expected satellite bands with a coupling constant of $^2J_{PtH} = 73.6$ Hz, which is in the range of values reported in the literature for a methyl group trans to pyridine.[25] The aromatic region is characterized by four groups of lines. The most downfield shifted signal at δ 8.85, attributable to H_6, is a doublet coupled with H_5 ($^3J_{HH} = 5.5$ Hz) with satellite peaks due to ^{195}Pt coupling ($^3J_{PtH} = 51.0$ Hz). The signal at δ 7.85 is a triplet assignable to H_5 and is coupled with the platinum center ($^4J_{PtH} = 20$ Hz). The remaining multiplets centered at δ 8.60, 8.53, and 8.43 belong to the other aromatic ring protons. A collection of ^{1}H NMR data for the various complexes is reported in Table 1. The peaks in the spectra are concentration, temperature, and solvent dependent. Figure 1 shows the concentration dependence of the chemical shifts of [Pt(terpy)(CH_3)]Cl in aqueous solution. The methyl group is strongly affected by the ring current, and upon an increase in the concentration, it moves from δ 0.71 to δ 0. The effect of the temperature is the reverse, and as shown in Figure 2, it is possible to see a general shift to higher frequencies when the sample is heated from 298 to 333 K. The ionic strength, too, exerts a strong influence on the shape of the spectrum. The addition of small amounts of sodium chloride to the solution produces a large effect on the peaks with all of the signals moving to lower frequencies (e.g. the methyl group resonance moves from δ 0.02 to δ −0.42), while the line widths become very large. These effects are related to the extensive stacking of the complex in aqueous solution.[26] As expected, this manifestation of stacking is smaller in dilute, low ionic strength solution, at high temperatures and in a nonaqueous solvent.[13]

^{13}C NMR spectra of complexes **1**−**5** were assigned on the basis of the terpy carbon spectrum, the presence of coupling constants with ^{195}Pt, and the knowledge of the trans-influence effect on their size.[25] Unlike the proton spectra, the ^{13}C results do not display systematic chemical shift dependencies on concentration. ^{195}Pt NMR spectra show a single quite broad resonance independent of the nature of the counterion and centered at δ −3220.

The complex [Pt(dien)(CH_3)]Cl was prepared by following essentially the same synthetic procedure as for the terpy analog. It was completely characterized by ^{1}H, ^{13}C, and ^{195}Pt NMR spectroscopy. The ^{1}H NMR spectrum in dmso-d_6 shows a singlet at δ 0.14 with a ^{195}Pt coupling constant of 80.3 Hz for the methyl group. All the other multiplets in the spectrum are easily assignable and exhibit coupling to ^{195}Pt. The ^{13}C NMR spectrum consists of three resonances, showing coupling with the metal center, positioned at δ 51.7, 50.4, and −28.7 attributable to the methylene and to the methyl groups, respectively. The ^{195}Pt NMR resonance is centered at δ −3367, in a range near that of the terpy analogues. None of the spectra of this complex display any significant dependence on the concentration, temperature, or solvent composition.

Electronic Spectra. In aqueous solution, complex **1** obeys Beer's law at concentrations below 100 μM (no salt or buffer

(25) Appleton, T. G.; Clark, H. C.; Manzer, L. E. *Coord. Chem. Rev.* **1973**, *10*, 335−422.
(26) Johnson, C. E., Jr.; Bovey, F. A. *J. Chem. Phys.* **1958**, *29*, 1012−1014. Bovey, F. A. *Nuclear Magnetic Resonance Spectroscopy*; Academic: New York, 1969. Fischer, B. E.; Sigel, H. *J. Am. Chem. Soc.* **1980**, *102*, 2998−3008. Chaudhuri, P.; Sigel, H. *J. Am. Chem. Soc.* **1977**, *99*, 3142−3150. Mitchell, P. R.; Sigel, H. *J. Am. Chem. Soc.* **1978**, *100*, 1564−1570. Fukuda, Y.; Mitchell, P. R.; Sigel, H. *Helv. Chim. Acta* **1978**, *61*, 638−647. Scheller, K. H.; Hofstetter, F.; Mitchell, P. R.; Prijs, B.; Sigel, H. *J. Am. Chem. Soc.* **1981**, *103*, 247−260. Sigel, H.; Scheller, K. H.; Scheller-Krattiger, V.; Prijs, B. *J. Am. Chem. Soc.* **1986**, *108*, 4171−4178. Sigel, H. *Pure Appl. Chem.* **1989**, *61*, 923−932.

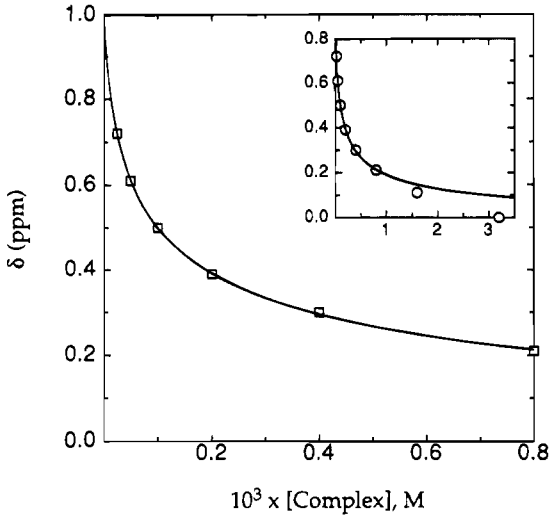


Figure 3. Concentration dependence of ^{1}H NMR chemical shift for the methyl group of [Pt(terpy)Me]Cl in D_2O at 298 K (see text for details).

added). Maxima in the UV/vis spectra are found at 397 nm (ϵ 1380 cm^{-1} M^{-1}), 331 (7890), 313 (8150), and 267 (20 090). When the concentration is increased, the dependence becomes nonlinear, even though the positions of most of the peaks remain unchanged. This pattern of behavior can be explained by the formation of aggregated species in solution as a consequence of stacking interactions between the planar aromatic moieties of the terpyridine ligands. The ionic strength influences the spectrum of the complex; i.e., an increase in NaCl concentration causes hypochromism of all the bands of the complex as well as a small bathochromic shift of the 397 nm band. Attempts to study the aggregation process from a kinetic point of view failed, probably because the relaxation process related to the association is too fast to be followed by the *T*-jump technique in the concentration range investigated.

A dimerization model appears to account for the aggregation at low concentration (<1 mM) and ionic strength. Therefore we can consider the following equilibrium:

$$2M \leftrightarrow D$$

with the equilibrium constant given by $K_{eq} = [D]/[M]^2$. The dimerization constant, K_d, and the molar extinction coefficients of the dimer, ϵ_D, and monomer, ϵ_M, are the parameters to be optimized in a nonlinear least-squares fitting procedure applied to the absorption data using a previously reported equation.[27] The dimerization constant for [Pt(terpy)(CH_3)]Cl in buffer B (μ = 0.101 M at 298 K) obtained by the method described above using the 331 nm band with $[Pt]_T$ ranging between 6 and 250 μM has a value of 10 (±8) × 10^3 M^{-1} (ϵ_M = 7770 ± 480 M^{-1} cm^{-1}; ϵ_D = 6790 ± 520 M^{-1} cm^{-1}). The value of ϵ_M obtained from this analysis is in excellent agreement with the value obtained at much lower concentration from the Beer's law analysis. The value of K_{eq} is somewhat smaller than the value estimated from ^{1}H NMR data. By applying a best fitting procedure to the equation reported by Angerman et al.[28] and using the chemical shifts of the methyl group, it is possible to derive K_{eq} = 26 (±1) × 10^3 M^{-1} at 298.2 K (six data points; δ_M = 1.01 ± 0.04 and δ_D = −0.05 ± 0.02, where δ_M and δ_D are the chemical shifts of the monomeric and dimeric species respectively; these values are not corrected for the ionic strength). As shown in the inset of Figure 3, a large deviation from a dimerization model is detectable in the higher concentration range. As expected, dynamic[29] and resonance light-scattering experiments are unable to show significant aggregation of the complex under the experimental conditions of the NMR measurements (no salt and low complex concentration), indicating that only the dimerization model is operative. On increasing salt and complex concentrations, a kinetic process, leading to large aggregates, becomes observable with UV/vis, NMR, and RLS techniques.

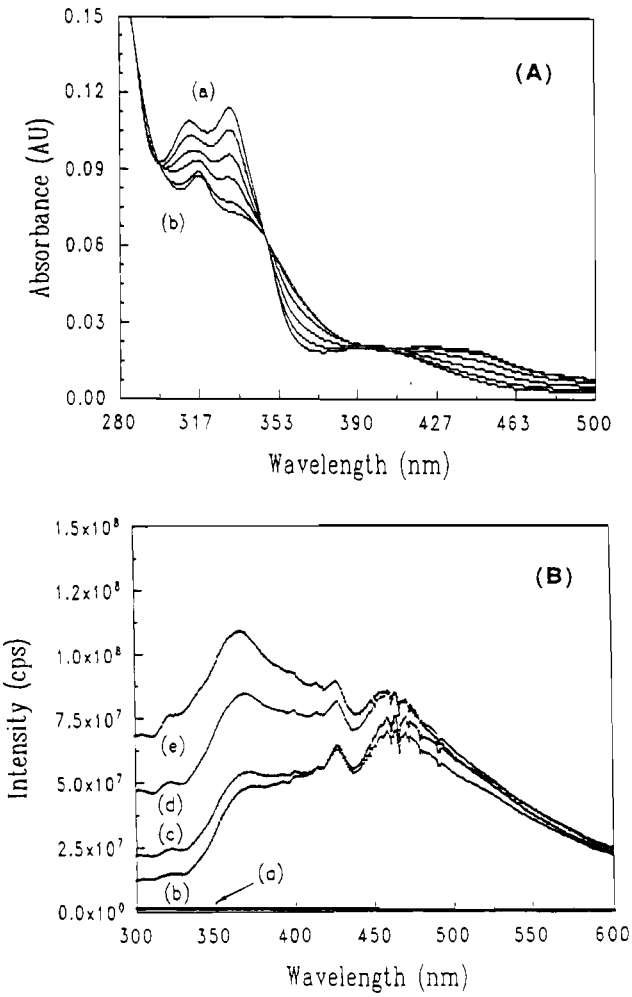


Figure 4. (A) Spectral changes associated with the addition of calf thymus DNA to a solution of [Pt(terpy)Me]Cl in buffer D at 25 °C. Experimental conditions: (a) [complex] = 12.5 μM; (b) [DNA] = 0.6, 1.8, 3.0, 3.6, 4.7 μM. (B) Resonance light−scattering profiles: (a) 1 mM phosphate buffer; (b) 12.5 μM [Pt(terpy)Me]Cl; after the addition of *ct*-DNA, (c)−(e) [DNA] = 0.6, 3.0, 4.7 μM.

DNA Binding Studies. (a) Electronic and Resonance Light-Scattering Spectra. The cationic complex ion [Pt(terpy)(CH_3)]$^+$ shows intense absorption in the visible region due to metal-to-ligand charge-transfer bands. These bands are perturbed in the presence of nucleic acids. Typically, the interaction of a intercalating reagent with DNA is characterized by following the hypochromism and bathochromic shift associated with the binding of the complex to the duplex.[30] The effect of addition of *ct*-DNA to a solution of complex **1** in buffer D is displayed in Figures 4 and 5. The spectral evidence shows two different processes occurring in different ranges of the formal

(27) Pasternack, R. F. *Ann. N.Y. Acad. Sci.* **1973**, *206*, 614−630.
(28) Angerman, N. S.; Victor, T. A.; Bell, C. L.; Danyluk, S. S. *Biochemistry* **1972**, *11*, 2402−2411.
(29) Mallamace, F.; Micali, N.; Monsú Scolaro, L.; Romeo, R. In preparation.
(30) Bloomfield, V. A.; Crothers, D. M.; Tinoco, I., Jr. *Physical Chemistry of Nucleic Acids*; Harper and Row: New York, 1974; p 432.

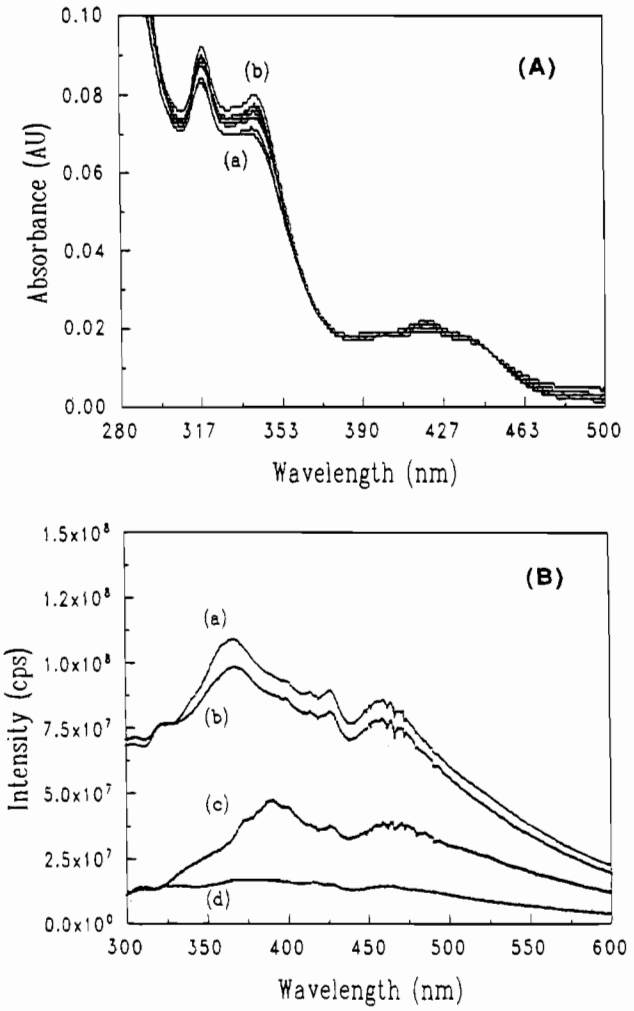


Figure 5. (A) Spectral changes associated with the addition of calf thymus DNA to a solution of [Pt(terpy)Me]Cl in buffer D at 25 °C. Experimental conditions: (a) [complex] = 12.5 μM; (b) [DNA] = 27.6, 36.2, 44.4, 63.7, 81.4, 112.7, 139.5 μM. (B) Resonance light-scattering profiles of 12.5 μM [Pt(terpy)Me]Cl after the addition of *ct*-DNA: (a)–(d) [DNA] = 4.7, 8.5, 44.4, 81.4 μM.

Pt/DNA ratio r_f (i.e., $r_f = [Pt]_{total}/[DNA]_{total}$). When $r_f > 1$, the spectral change in the UV/vis region (Figure 4A) shows the occurrence of a process with well-defined isosbestic points. The two bands at 313 and 331 nm display hypochromicity and very slight bathochromic shifts, while the 397 nm band shifts to 421 nm with hyperchromicity. The resonance light-scattering spectrum of complex **1** in the same buffer (no DNA) consists of a quite broad feature with maxima occurring at about 360 and 460 nm. Upon addition of DNA, the scattering intensity increases (Figure 4B), concomitant with the decrease in the absorption spectra. At $0.045 < r_f < 1$, the absorption spectra show a second process with different isosbestic points (Figure 5A). The amount of hypochromism and bathochromic shift (5 nm for the 313 nm band and 10 nm for the 331 nm band) is indicative of extensive interaction between base pairs and the aromatic moiety of the terpy ligand.[30] The corresponding resonance light-scattering profiles show a steep decrease in intensity but remain above the buffer reference spectrum throughout (Figure 5B).

(b) CD Spectra. A solution of complex **1** in buffer A does not exhibit any CD signal because of the symmetry of the molecule. As shown in Figure 6, circular dichroism spectra of [Pt(terpy)(CH_3)]Cl in the phosphate buffer A in the presence of *ct*-DNA ($r_f = 0.1$) display two positive features at about 340 and 400 nm and a negative one at 315 nm. These induced bands

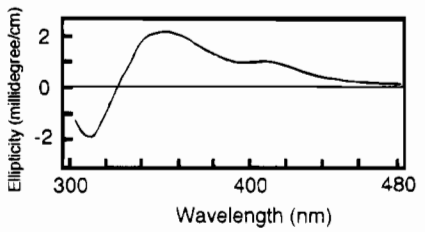


Figure 6. CD spectra of [Pt(terpy)Me]Cl in the presence of *ct*-DNA. Experimental conditions: [complex] = 0.05 mM; [DNA] = 1 mM in buffer A at 298 K.

in the CD spectra, arising from the coupling between the dipole transition moment of the nucleobases and of the ligand aromatic moiety, are indicative of the intercalative interaction of the platinum(II) complex with the chiral double helix of the nucleic acid.[7] When $r_f > 1$ ($[Pt]_T = 12.5$ μM), the CD spectra display no detectable bands in the visible range. Any attempt to obtain spectra in this regime of r_f ratios failed because, upon an increase in the concentration of the complex, precipitation of the DNA/complex adduct occurred.

(c) Thermal Denaturation of Nucleic Acids. DNA melting profiles were obtained at an r_f ratio 0.1 and at different ionic strength values. The interaction between complex **1** and DNA stabilizes the duplex toward thermal denaturation. A decrease in the ionic strength from 10 to 1 mM leads to an increase in the melting temperature (ΔT_m) of calf thymus DNA from 1.5 to 7.8 °C.

(d) Gel Electrophoresis on Plasmidial DNA. Gel electrophoresis of circular plasmidial DNA is a well-established method of measuring the degree of supercoiling.[31] Lippard et al. employed this technique[32] to determine the unwinding angle of supercoiled DNA for a series of platinum(II) complexes. The method is based on the measurement of the r_b (the bound complex to nucleotide ratio) at the coalescence point, i.e. when the closed circular (form I) and the nicked relaxed (form II) DNA co-migrate. Figure 7 shows that, at a formal drug-to-nucleotide ratio (r_f) of 0.070, the closed circular form band of pUC 19 starts to decrease its migration rate, approaching that of the nicked relaxed band. A complete analysis of the electrophoretic pattern was prevented because of precipitation of the adduct between DNA and the complex at the ionic strength required by the experiment.

(e) [Pt(dien)(CH_3)]Cl. Addition of nucleic acids to a solution of this complex in phosphate buffer does not affect its electronic spectrum nor is there any indication of an induced CD signal. The melting temperature of *ct*-DNA remains unaltered.

Discussion

Reaction 1 offers an easy synthetic route to the title complex cation. We failed in our attempts to grow single crystals suitable for X-ray analysis; these species form needles having a large internal cavity which are unsuitable for this analysis. As reported by Morgan et al. for the complex [Pt(terpy)Cl]Cl,[33] different crystal modifications exist which are characterized by different colors. This behavior is also shown by the complex [Pt(bipy)Cl_2] (red and yellow forms) and is due to different alignments of the bipyridyl moieties in the solid state packing.[34] The formation of stacks in the solid state is a well-documented phenomenon in the chemistry of d^8 square planar complexes[35]

(31) Bauer, W. R. *Annu. Rev. Biophys. Bioeng.* **1978**, *7*, 287.
(32) Keck, M. V.; Lippard, S. J. *J. Am. Chem. Soc.* **1992**, *114*, 3386–3390.
(33) Morgan, G. T.; Burstall, F. H. *J. Chem. Soc.* **1934**, 1498–1500.
(34) Osborn, R. S.; Rogers, D. *J. Chem. Soc., Dalton Trans.* **1974**, 1002.
(35) Miller, J. S.; Epstein, A. J. *Prog. Inorg. Chem.* **1976**, *20*, 1.

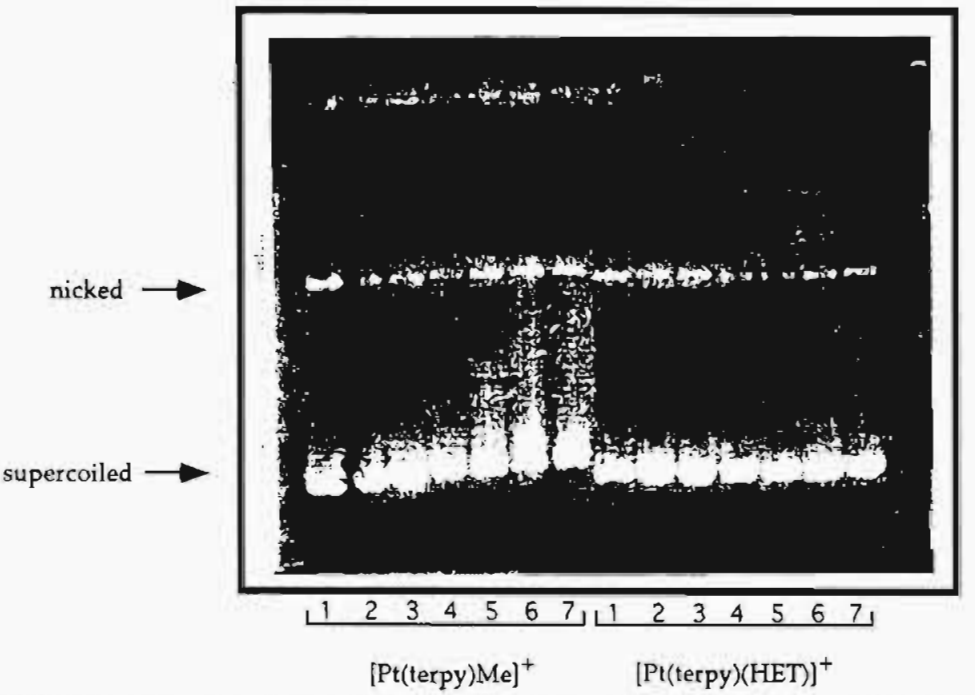
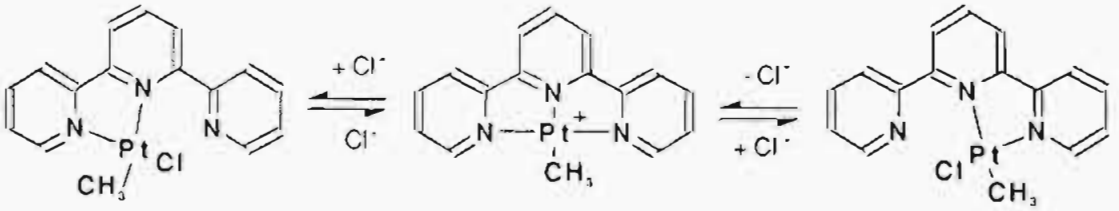


Figure 7. Gel electrophoresis in 1.2% agarose gels of pUC 19 DNA incubated with different amounts of [Pt(terpy)Me]Cl and [Pt(terpy)(HET)]-(NO_3) in TBE running buffer at 25 °C for 30 min. Lanes: (1) $r_f = 0$; (2) $r_f = 0.017$; (3) $r_f = 0.035$; (4) $r_f = 0.052$; (5) $r_f = 0.070$; (6) $r_f = 0.078$; (7) $r_f = 0.087$.

Scheme 1



and is a property closely related to their capability to intercalate into nucleic acids.[15a]

The ligand considered here, 2,2′:6′,2″-terpyridine, can bind to a metal ion acting as a mono-, bi-, or terdentate ligand, but on the basis of the well-established "chelate effect", it might be expected that terpy prefers the last possibility. However, Abel et al.[36] recently showed that terpy displays a fluxional behavior, giving a bidentate coordination oscillating between different binding sites in complexes of Pt(IV), Re(I), and W(0). Therefore, we wished to clarify the nature of the platinum(II) species in solution. The marked dependence of the chemical shifts on concentration, temperature, and solvent could perhaps be interpreted as due to an equilibrium process, fast on the NMR time scale, in which terpy acts as a bidentate ligand with the fourth coordination site occupied by chloride (or solvent), as shown in Scheme 1.

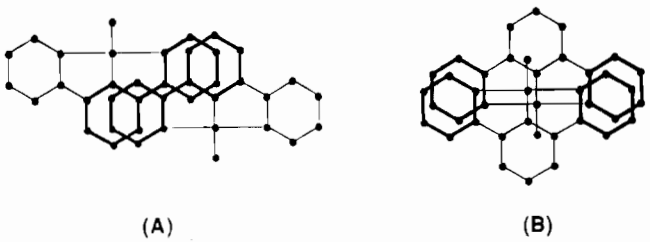
However, the existence of such an equilibrium would give rise to several effects, none of which are observed: (i) the $^3J_{PtH}$ of H_6 on the pyridine ring would have an averaged value of 25 Hz or even less due to the fast opening−closing of the chelate ring; (ii) these coupling constants $^3J_{PtH}$ would be dependent on the nature of the counterion; (iii) ^{195}Pt chemical shift, which is known to be strongly dependent on slight changes in the coordination sphere,[22] would show a significant shift on varying the counterion; also the line widths would be severely affected in the case of a fast exchange; (iv) finally, the ^{35}Cl line widths of the chloride complex (1), compared to those of a sample of a nonexchanging chloride such as the tetrabutylammonium salt, would be greatly affected by the exchange.

Table 1 clearly shows that the values of $^3J_{PtH}$ for different complexes are almost identical (50.7 ± 0.6 Hz) and these values are basically unaltered upon passing to methanol or water ($^3J_{PtH}$ = 52.2 and 50.0 Hz, respectively). Again the ^{195}Pt chemical shifts are unaffected by changing the anion, and the line widths of the resonances are broadened by quadrupolar relaxation due to the presence of three nitrogen atoms in the coordination sphere ($\Delta\nu_{1/2}$ = 300 ± 30 Hz). ^{35}Cl NMR spectra of separate solutions of complex **1** and of tetrabutylammonium chloride in dimethyl sulfoxide showed a single resonance at δ −143 (for frequency scale, see Experimental Section), with negligible differences in line width (16.6 and 19.8 Hz, respectively). All these findings are consistent with the view that terpy behaves as a terdentate ligand, and therefore a fluxional equilibrium can be excluded as being responsible for the observed phenomena in solution.

The cationic platinum(II) complex ion $[Pt(terpy)(CH_3)]^+$ shows a considerable tendency to stack in solution, giving a dimer or more extended polymers, according to the experimental conditions. A dimerization model fits the UV/vis data well over a range of low concentrations (6×10^{-6}−2.5×10^{-4} M) at μ

(36) Abel, E. W.; Long, N. J.; Orrell, K. G.; Osborne, A. G.; Pain, H. M.; Sik, V. *J. Chem. Soc., Chem. Commun.* **1992**, 303. Abel, E. W.; Dimitrov, V. S.; Long, N. J.; Orrell, K. G.; Osborne, A. G.; Sik, V.; Hursthouse, M. B.; Mazid, M. A. *J. Chem. Soc., Dalton Trans.* **1993**, 291−298. Abel, E. W.; Dimitrov, V. S.; Long, N. J.; Orrell, K. G.; Osborne, A. G.; Pain, H. M.; Sik, V.; Hursthouse, M. B.; Mazid, M. A. *J. Chem. Soc., Dalton Trans.* **1993**, 597−603.

Chart 1



(A) (B)

< 0.1 M. At higher concentrations or at higher ionic strengths, deviations occur. ^{1}H NMR evidence also supports this behavior. The shift to lower frequencies of the resonances in the NMR spectra is a well-documented phenomenon and is due to the effect of the ring currents of the different interacting aromatic moieties.[26] The values of the equilibrium constants measured with the two techniques are in quite good agreement (K_{eq} = (26 ± 1) × 10^3 M^{-1} from NMR experiments vs K_{eq} = (10 ± 8) × 10^3 M^{-1} from UV/vis data).

The simple dimerization model fails to fit the data at concentrations greater than 1 mM. At high ionic strength the line widths become so broad that it is quite difficult to resolve the NMR spectra. This phenomenon can be explained by the formation of high molecular weight species. These entities in solution have oblate shapes and are highly charged, so the different rotational correlation times and the anisotropy of the system could be responsible for the observed line broadening.[28]

The resonance light-scattering technique has proved to be a very useful and sensitive method for investigating highly aggregated species, particularly in the case of metal-free and complexed porphyrins, where the high values of the extinction coefficients of these compounds in the Soret region (ϵ of the order of 10^5 M^{-1} cm^{-1}) and the extensive electronic coupling between interacting chromophores are exploited.[18] Here, too, in a system characterized by much lower extinction coefficients (ϵ of the order of 10^4 M^{-1} cm^{-1}), RLS still gives satisfactory indications of the presence of extended aggregates (Figure 4B, spectrum b). Preliminary dynamic light-scattering experiments at various ionic strengths indicate the formation of very large assemblies (>0.1 μm).[29]

Although it is difficult, on the basis of the available evidence, to describe the structure that these highly aggregated species can assume in solution, it is possible to make a tentative assignment for the dimer consistent with the present chemical shift concentration dependence. Lippard et al.[13] have shown in an X-ray structure determination the presence of different stacking interactions of [Pt(terpy)(HET)]$^+$, giving a partially stacked head-to-tail (Chart 1, structure **A**) and a head-to-tail structure (Chart 1, structure **B**). The latter is quite common in platinum(II) and palladium(II) complexes containing the terpy ligand, while the former is reminiscent of the ethidium bromide monohydrate structure.[37] The head-to-tail structure **B** should exhibit a large variation of the methyl group chemical shift and discrete changes of the other resonances of the aromatic rings relative to the monomer. On the other hand, the partially stacked form, **A**, could show a detectable chemical shift dependence of the aromatic ring protons and only a small effect on the methyl group. The observation of a large chemical shift change (from about +1 to −0.5 ppm at high ionic strength) especially for the methyl resonance would suggest that structure **B** is the predominant form in solution.

The chemical shifts are observed to move to lower frequencies with increasing temperature. Such a dependence indicates that the π-stacking interactions between two terpyridyl moieties are weakened at high temperature. This observation is not surprising because negative values for the enthalpy of formation of such weakly bonded species are reported for platinum complexes containing 2,2′-bipyridyl,[38] purine bases, and acridine based dyes.[39]

Only in extremely dilute solutions can the platinum(II) complex be considered to be a monomer. The tendency to form dimers and larger aggregates in solution is a severe complication to a conventional analysis of the binding to DNA using electronic spectra such as those in Figure 4A and Figure 5A.[40] However, the results of RLS experiments help to clarify the picture of the steps involved in the interaction between **1** and DNA. The increase in intensity of the RLS spectra in Figure 4B, matched by a corresponding decrease of the optical density seen in Figure 4A, indicates that the addition of low concentrations of nucleic acids causes the distribution of the complex between its free self-aggregated form in solution and on the backbone of the DNA. Similar aggregates, in which the complex is bound on the outside of the duplex, giving extensive stacking interactions, have been postulated to explain the unusual behavior in the cooperative binding of [Pt(terpy)(HET)]$^+$ with synthetic double-stranded RNA[15b] in the range of high r_f values. In our system the phenomenon is at its peak at an r_f ratio of about 1, after which a completely reversed pattern of behavior is observed in the RLS and electronic spectra of Figure 5, indicating the beginning of the intercalative process. In this range of r_f ratios, the CD spectra, the gel electrophoretic mobility assay, and the thermal stabilization toward the denaturation all point to the conclusion that the organoplatinum complex intercalates between adjacent base pairs. At least at these low drug load conditions, intercalation is the dominant form of interaction. At this stage, however, a quantitative treatment of the spectroscopic data to obtain binding constants is prevented by the lack of a suitable model of the interaction between large aggregates and nucleic acids.

Summing up, the aggregation of cationic species is a general phenomenon which plays an important role in interpreting their solution properties. To appreciate more fully the factors involved in the interactions of these compounds with nucleic acids, it is necessary to take into account the possible formation of these assemblies. The presence of a hydrophobic methyl group instead of the more hydrophilic hydroxyethanethiolate group increases remarkably the self-aggregating properties of the terpy platinum(II) moiety. Studies with alkyl chains having variable lengths and bearing different substituents are being undertaken to investigate the impact of these features on the aggregation and binding properties.

Concluding Remarks

Square planar platinum(II) complexes of the type [Pt(terpy)-Me]X were particularly designed to have the following characteristics: (i) they must be soluble and thermally stable in water; (ii) in the cationic complex ion all the coordination sites must be blocked so as to make the metal inert to nucleophilic substitution; (iii) one of the coordinated ligands, the methyl

(37) Subramanian, E.; Trotter, J.; Bugg, C. E. *J. Cryst. Mol. Struct.* **1971**, *1*, 3.

(38) Yamauchi, O.; Odani, A.; Shimata, R.; Ishiguro, S. *Recl. Trav. Chim. Pay-Bas* **1987**, *106*, 196−197. Odani, A.; Shimata, R.; Masuda, K.; Yamauchi, O. *Inorg. Chem.* **1991**, *30*, 2133−2138.

(39) Leng, M.; Felsenfeld, G. *J. Mol. Biol.* **1966**, *15*, 455. Brahms, J.; Michelson, A. M.; Van Holde, K. E. *J. Mol. Biol.* **1966**, *15*, 467. Blears, D. J.; Danyluk, S. S. *J. Am. Chem. Soc.* **1967**, *89*, 21.

(40) Any attempt to analyze the spectroscopic data using the Scatchard analysis (Scatchard, G. *Ann. N.Y. Acad. Sci.* **1949**, *51*, 660) or the McGhee and von Hippel equation failed (McGhee, J. D.; von Hippel, P. H. *J. Mol. Biol.* **1974**, *86*, 469).

group in this case, must be a good probe of the changes of the electronic density induced by self-interactions or external interactions. Interest in the synthesis and characterization of this type of square planar compound is very high, as shown by recent reports on palladium(II) analogs, prepared with a much more complicated procedure than that used here for platinum(II).[41] Studies of intercalation with DNA of organometallic compounds are rare.[42] Thus, our aim was to include an organometallic species in the list of planar compounds tested for intercalation, having in mind the possible comparison with a well-established pattern of behavior coming from the detailed studies carried out by Lippard and co-workers on very similar coordination compounds, such as $[Pt(terpy)(HET)]^+$.

The most interesting feature of this study is the clear evidence of a marked tendency to self-aggregation of the complex ion $[Pt(terpy)Me]^+$, far greater than that of the corresponding $[Pt(terpy)(HET)]^+$. This is shown by the combination of spectroscopic techniques, among which RLS has proved to be extremely useful to show the formation of large assemblies of chromophores. Our results proved also for the first time that this technique is highly informative even for systems lacking the excellent absorption properties of the porphyrins.

It is difficult to envisage the way through which large complex aggregates interact with DNA but two distinct steps can be distinguished at high and at low drug load conditions, respectively. In the first one, there is a preliminary reversible distribution of aggregates on the surface of the nucleic acid followed by a proper intercalative process in which the complex ion is thought to interact with DNA in the usual way. At low r_f ratios the results od CD spectroscopy, gel electrophoresis and thermal denaturation all point toward intercalative interaction.

The question that begs an answer is whether this remarkable tendency to self-aggregation of a +1-charged platinum(II) complex is peculiar to compound **1** or whether it is a general feature of organometallic species, because of the strong σ-donor Pt−C bond and/or the hydrophobicity of the organic moiety. The possibility of performing a fine tuning of these properties by varying the substituent groups makes the synthesis and the study of the solution behavior of these compounds very interesting.

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Supplementary Material Available: Tables SI and SII, reporting the chemical shift concentration and temperature dependences (2 pages). Ordering information is given on any current masthead page.

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(41) Rulke, R. E.; Han, I. M.; Elsevier, C. J.; Vrieze, K.; van Leeuwen, P. W. N. M.; Roobeek, C. F.; Zoutberg, M. C.; Wang, Y. F.; Stam, C. H. *Inorg. Chim. Acta* **1990**, *169*, 5−8. Markies, B. A.; Wijkens, P.; Boersma, J.; Kooijman, H.; Veldman, N.; Spek, A. L.; van Koten, G. *Organometallics* **1994**, *13*, 3244−3258.

(42) Higgins, J. D., III; Neely, L.; Fricker, S. *J. Inorg. Biochem.* **1993**, *49*, 149−156.