DALTON
FULL PAPER

Synthesis and characterization of monodeoxynucleotide tethered platinum-(II) and -(IV) complexes †

Sumei Ren, Lisheng Cai * and Brent M. Segal

Department of Chemistry, The University of Illinois at Chicago, Chicago, Illinois 60607, USA

Received 6th November 1998, Accepted 25th February 1999

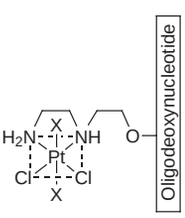
Improved syntheses of platinum-(II) and -(IV) complexes with pendant hydroxy groups have been elaborated. When the hydroxy groups of the platinum(II) complexes were subjected to phosphoramidite coupling agents to synthesize the corresponding phosphoramidites, intramolecular displacement of the chloride on the platinum by the incoming phosphoramidite group occurred. This generated chloro{*N*-2-[(2-cyanoethyl)(diisopropylamino)phosphinoxy]-ethylenediamine}platinum(II) chloride, which was characterized by a number of physical techniques including X-ray diffraction study. It crystallized from methanol–diethyl ether as white block crystals. Single crystal X-ray analysis revealed that the platinum adopts a four-co-ordinate square planar configuration, provided by the two amine nitrogens, one phosphorus, and one chloride ion. The co-ordination of the phosphoramidite group prevents further manipulation of this group to connect with other hydroxy groups. Intramolecular electron transfer reaction occurred when the platinum(IV) complexes were used in the same reaction. A new strategy has been developed to couple monodeoxynucleotides with the hydroxy groups of the tethered platinum-(II) and -(IV) complexes through the use of monodeoxynucleotide phosphoramidite agents. Every peak in the ^{1}H, ^{13}C-{^{1}H} and ^{195}Pt NMR spectra for all the compounds reported has been assigned through the combination of 1-D ^{1}H, ^{13}C-{^{1}H}, 2-D COSY, XHCORR (heteronuclear chemical shift correlation), HMQC (heteronuclear multiple quantum correlation), and the comparison of chemical shifts among analogs.

Introduction

cis-Diamminedichloroplatinum(II) (*cis*-DDP; *cis*-platin) is an effective anticancer drug routinely prescribed for testicular, ovarian, bladder, lung and other carcinomas.[1] The mechanism of its cytotoxicity toward cancer cells has been extensively studied, and its preferences in binding, *i.e.* to the amine functionality of adjacent purine sites in double stranded (ds) DNA, have been noted.[2] *In vitro*, approximately 65% of drug–DNA adducts formed occur at d(GpG) sites, and 25% at d(ApG) sites, minor products at d(GpNpG) or interstrand sites.[3] The product of the association at d(GpG) sites is also a square planar platinum(II) complex, where the original chloride ligands have been displaced by the N-7 amino groups of adjacent guanine bases, and where the ammonia ligands serve as hydrogen bond donors to the oxygen atoms of the intervening phosphodiester linkage and other hydrogen bond acceptor sites.[4]

Despite the availability of molecular details relevant to the *cis*-DDP binding and function, many questions of importance to its clinical utility and effectiveness remain: *cis*-DDP use is hindered by intrinsic and induced resistance, and mitigated by the general cytotoxicity of the drug. Recent investigations on *cis*-platin analogs focus on platinum complexes with carrier molecules as ligands[5] and complexes containing more than one platinum atom.[6]

We are interested in the use of the platinum center as a molecular fragment or moiety to recognize GG base pairs when connecting to an oligodeoxynucleotide (ODN). This new agent may recognize a specific sequence or gene with unprecedented affinity and efficiency, providing a mechanism for targeting platinum drugs to specific sites in the genome. For our initial studies, the ODN was chosen on the basis of its characteristics for sequence specific binding through mRNA and DNA duplex/triplex formation.[7] Three stages of this study are reported here: first, the improved synthesis and detailed characterization of the platinum-(II) and -(IV) complexes with tethered hydroxy group(s) (Scheme 1); second, coupling of the above



Scheme 1 Generic form of the compounds in this study.

platinum complexes with phosphoramidite agents; third, the synthesis of the tethered platinum complexes with monodeoxynucleotide(s).

Metal-containing oligonucleotides have been designed and synthesized for a variety of reasons including: (1) incorporation of photo- and redox-active transition metal ions to study the energy and electron-transfer processes through DNA;[8] (2) development of DNA hybridization probes and sensors;[9] (3) search for new modes of covalent attachment of metal complexes to DNA;[10] (4) mimic of endogenous nuclease activity that effects selective RNA hydrolysis;[11] (5) incorporation of platinum center in specific site of DNA or RNA for the mechanistic study of *cis*-DDP anticancer activity.[12] They are constructed *via* three major pathways: (a) the synthesis of a chelator-containing oligonucleotide followed by metal complexation;[7b,13] (b) the synthesis of an end-functionalized oligonucleotide to which a metal complex can be conjugated;[11b,14] and (c) the incorporation of metal-containing phosphoramidites or H-phosphonates.[11a,12,15] The first method depends on the selectivity between the chelator and the oligonucleotide backbone, the second on the specificity of the conjugation method and its

† *Supplementary data available*: COSY and XHCORR spectra of complex **1**, diagrams of possible stereoisomers. Available from BLDSC (No. SUP 57515, 5 pp.). See Instructions for Authors, 1999, Issue 1 (http://www.rsc.org/dalton).

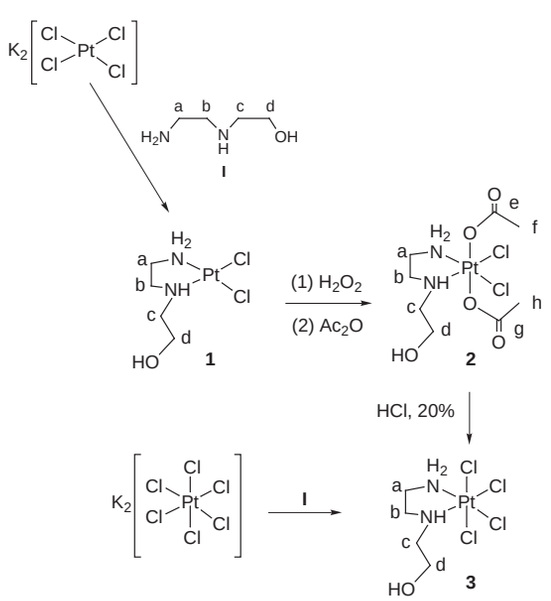
Table 1 The ^{1}H NMR chemical shifts of various compounds containing the dichloro[*N*-(2-hydroxyethyl)ethylenediamine]platinum fragment in DMSO-d_6 unless indicated otherwise

Compound	a	b	c	d	f	h	N–H or O–H
I[a]	2.62, m, 2 H	2.62, m, 2 H	2.78, m, 2 H	3.61, t, 2 H			2.50–2.90, br s
I	2.50, m, 2 H	2.50, m, 2 H	2.53, m, 2 H	3.42, t, 2 H			2.40–2.60, br s
1	2.28, m, 2 H	2.70, m, 1 H; 2.43, m, 1 H	3.25, m, 1 H; 3.00, m, 1 H	3.58, m, 1 H; 3.53, m, 1 H			6.05, br s, 1 H; 5.26, br s, 1 H; 5.02, br s, 1 H; 4.66, s, 1 H
2	2.81, m, 2 H	2.62, m, 1 H; 2.05, m, 1 H	3.78, m, 1 H; 2.90, m, 1 H	3.65, m, 2 H	1.96, s, 3 H	1.96, s, 3 H	10.48, br s, 1 H; 9.62, br s, 1 H; 8.05, br s, 1 H; 5.10, br s, 1 H
2[b]	2.81, m, 2 H	2.74, m, 1 H; 2.13, m, 1 H	3.78, m, 1 H; 3.15, m, 1 H	3.70, m, 2 H	1.96, s, 3 H	1.96, s, 3 H	10.19, br s, 1 H; 10.05, br s, 1 H; 9.28, br s, 1 H; 6.37, br s, 1 H
3	2.84, m, 2 H	2.79, m, 1 H; 2.47, m, 1 H	3.57, m, 1 H; 3.10, m, 1 H	3.76, t, 2 H			7.61, br s, 1 H; 7.48, br s, 1 H; 7.19, br s, 1 H; 5.72, s, 1 H
4	2.52, m, 2 H	3.03, m, 1 H; 2.91, m, 1 H	2.52, m, 1 H; 3.03, m, 1 H	3.87, m, 1 H; 4.27, m, 1 H			7.84, br s, 1 H; 5.64, br s, 1 H; 5.52, br s, 1 H
7	2.89, m, 1 H; 2.68, m, 1 H	2.89, m, 1 H; 2.68, m, 1 H	3.79, m, 2 H	3.97, m, 2 H	2.09, s, 3 H	1.95, s, 3 H	
8[c]	3.21, m, 1 H; 2.97, m, 1 H	2.43, m, 2 H	4.16, m, 2 H	4.21, m, 2 H			

[a] In chloroform. [b] In CD_3CN. [c] In D_2O.

compatibility with the metal complex and the third on the inertness of the metal complex toward the reaction conditions of the oxidation and deprotection during the oligonucleotide synthesis or the nature of the metal complex is not critical.

All the three methods are not directly suitable for the construction of the generic compound outlined in Scheme 1. Platinum sources such as K_2PtCl_4 should react with both the nucleic bases and a chelator such as ethylenediamine, making method (a) impractical. Platinum(II) complexes such as **1** in Scheme 2 are subject to ligand substitution reactions in concentrated ammonia solution with prolonged heating, which is required for the deprotection of nucleotides, if the platinum fragment is attached before deprotection. Otherwise a specific connection method to join **1** with oligonucleotides needs to differentiate between the hydroxy group and amino group, and does not interfere with the platinum complex, requiring development of new strategies for method (b). Here we report our effort to develop synthetic strategies along the general method (c).



Scheme 2 Synthetic scheme for hydroxy group tethered platinum complexes.

Results and discussion

The first step in our program is the synthesis of a platinum complex with a pendant OH group to serve as an ODN tethering site. This assumes that the reactivity of the OH group is not dramatically changed by the metal fragment.

Dichloro[*N*-(2-hydroxyethyl)ethylenediamine]platinum(II) 1

The synthesis of this compound was reported as early as 1974.[16] It shows significant anticancer activity against Sarcoma-180 ascites tumour cells in mouse or cancer cell line L1210 and little toxicity to the host, not more effective than *cis*-DDP.[17] However, no spectroscopic characterization of this compound has been reported. After co-ordination to the platinum center, the central nitrogen of the ligand becomes a chiral center, and only one set of ^{13}C-{^{1}H} NMR signals is observed. The assignment of the peaks comes from the combination of 1-D ^{1}H, ^{13}C-{^{1}H}, and 2-D COSY, and XHCORR (heteronuclear chemical shift correlation) experiments (Tables 1 and 2 and Scheme 2). The ^{1}H 2-D COSY and ^{13}C-{^{1}H}–^{1}H XHCORR spectra are given in SUP 57515. The assignment began with the methylene bound to the hydroxy group, which gave the most downfield ^{13}C-{^{1}H} peak and non-exchangeable ^{1}H peaks. Two different sets of ^{1}H peaks were observed for this CH_2 group, suggesting that the rotation around the C–C single bond is somewhat restricted. This is consistent with the co-ordination of the hydroxyl group with the platinum center. Scalar coupling between methylene group c and d, or between a and b, was clearly observed in the ^{1}H 2-D COSY spectrum. The distinction between a and b was based on the scalar coupling of b with the proton on the central NH group, which couples with c. The two different sets of ^{1}H NMR peaks for each of groups a, b, and c apparently come from the central chiral amine center. The clearly prolonged relaxation time for the hydroxy proton allows the identification of its peak from those of the NH groups, whose

Table 2 The ^{13}C-{^1H} NMR chemical shifts of various compounds containing the dichloro[*N*-(2-hydroxyethyl)ethylenediamine]platinum fragment in DMSO-d_6 unless indicated otherwise

Compound	a	b	c	d	e	f	g	h
I[a]	41.4	51.6	52.0	60.3				
I	42.3	52.5	53.2	61.1				
1	47.4	56.9	55.3	59.2				
2	48.7	56.8	53.8	58.0	181.9	23.7	181.4	21.2
2[b]	48.8	56.9	54.0	58.1	182.9	24.2	182.5	24.1
3	48.1	57.2	55.8	57.8				
4	42.4	52.9	60.4	64.9				
7	47.3	57.0	56.3	59.4	172.9	23.0	172.9	22.6
8	49.3, 48.3, 47.2, 46.0, 45.7	57.2, 56.4, 54.7, 53.5, 51.4	55.3, 52.0,	63.6, 63.0, 61.8, 61.4, 61.1				

[a] In chloroform. [b] In CD_3CN.

relaxation time is shortened by the quadrupole moment of the abundant ^{14}N nucleus.[18]

The OH group is apparently weakly co-ordinated. The chemical shift of the CH_2O group is within the range for primary alcohols, not for ester groups. Reaction of complex **1** with acetic anhydride gives the corresponding acetate ester cleanly. All this information converges on the conclusion that there is an interaction between the hydroxy group and platinum(II) center but it may be quite weak. This suggests that reactions at the OH group should occur without much interference from Pt. However, under basic conditions, intramolecular displacement of the chloride by the alkoxide group has been reported.[19]

Diacetatodichloro[*N*-(2-hydroxyethyl)ethylenediamine]-platinum(IV) 2

Multiple attempts to oxidize platinum-(II) to -(IV) complexes by hydrogen peroxide in different solvents failed to generate the dichlorodihydroxy[*N*-(2-hydroxyethyl)ethylenediamine]-platinum(IV) complex as predicted by the synthesis of analogous compounds.[17b,20] Oxidation has only been realized through the combination of a controlled amount of hydrogen peroxide and acetic anhydride in acetic acid. For **2** there is no possibility for co-ordination of the hydroxy group with the platinum center. The difference of the ^1H and ^{13}C NMR spectra for the CH_2OH group of **1** and **2** confirms the conclusion drawn above that an interaction exists between the hydroxy group and platinum in compound **1**. A total of 6 isomers, including two diastereomers and two pairs of enantiomers (see SUP 57515), are possible from the platinum center for this compound. Combined with the central chiral amine center, a total of 12 isomers is possible. A single peak for each carbon in the ^{13}C-{^1H} NMR spectrum and a single set of peaks for each proton in the ^1H NMR spectrum (Tables 1 and 2) may imply that only one enantiomer pair exists in solution, as shown in Scheme 2.

Tetrachloro[*N*-(2-hydroxyethyl)ethylenediamine]platinum(IV) 3

This compound was first mentioned in 1983 in the screening of platinum compounds as anticancer agents,[17b] however no report about its synthesis or characterization has appeared. In this study it was synthesized through the substitution of the acetato group in **2** by chloride in 20% hydrochloric acid (Scheme 2 and Tables 1 and 2); also through the direct reaction of potassium hexachloroplatinate(IV) with 2-(2-aminoethylamino)ethanol in low yield upon prolonged refluxing in an aqueous solution. Most of the side product is a yellowish insoluble material.

When we tried an initial coupling reaction we were surprised to find displacement of a chloride accompanying linkage of the phosphoramidite with the tethering OH group. Detailed description of the reaction follows.

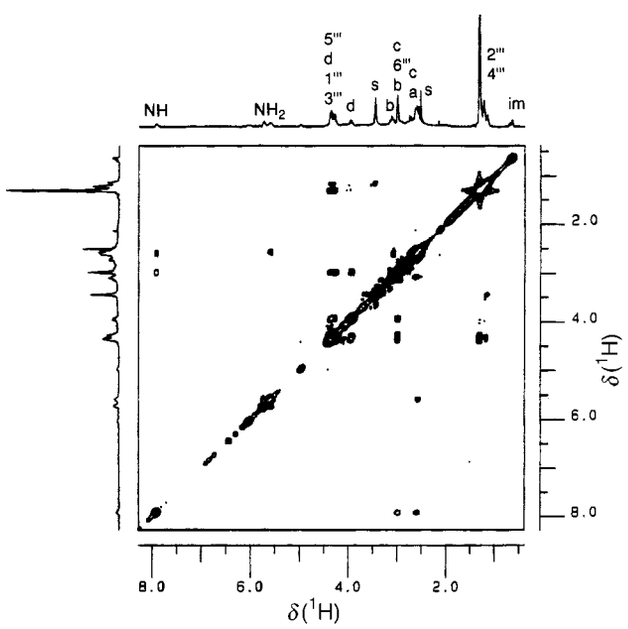
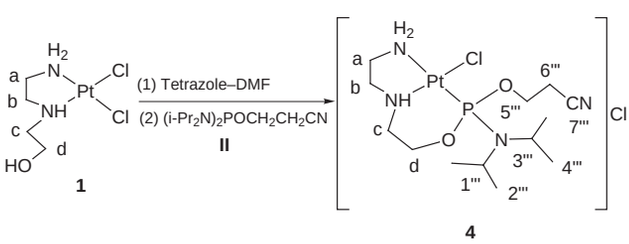


Fig. 1 The COSY NMR spectrum of compound **4**; im = impurity.

Chloro{*N*-2-[(2-cyanoethyl)(diisopropylamino)phosphinoxy]-ethylenediamine}platinum(II) chloride 4

The structure of complex **4** is shown on Scheme 3, and the



Scheme 3 Synthetic scheme for compound **4**.

interaction between the Pt and phosphite center is established through the observation of scalar coupling in both ^{195}Pt and ^{31}P NMR spectra, and later on confirmed by X-ray diffraction studies. A doublet in the ^{195}Pt NMR gives a coupling constant of 4911 Hz for $^1J_{Pt-P}$. In the ^{31}P NMR spectrum a central singlet at δ 81.1 with 2/3 of the intensity symmetrically flanked by a doublet, each peak of which has 1/6 of the total intensity, with a coupling constant of 4911 Hz. The COSY and XHCORR spectra are shown in Figs. 1 and 2. Labelling and assignments for this compound are summarized

Table 3 The ^{1}H NMR chemical shifts of various compounds containing the phosphoramidite fragment

Compound	1‴,3‴	2‴	4‴	5‴	6‴
4[a]	4.27, m, 4 H	1.23, s, 6 H	1.23, s, 6 H	4.27, m, 2 H	2.91, m, 2 H
III[b]	3.48, m, 2 H	1.13, s, 3 H; 1.11, s, 3 H	1.06, s, 3 H; 1.00, s, 3 H	3.62, m, 2 H	2.58, m, 1 H; 2.49, m, 1 H
7[a]				3.74, m, 1 H; 3.54, m, 1 H	3.00, m, 1 H; 2.76, m, 1 H
8[a]				3.21, m, 2 H	2.97, m, 2 H

[a] In DMSO-d_6. [b] In CD_3CN. [c] In D_2O.

Table 4 The ^{13}C-{^{1}H} NMR chemical shifts of various compounds containing the phosphoramidite fragment

Compound	1‴,3‴	2‴	4‴	5‴	6‴	7‴
4[a]	49.2, 49.1	24.1, 23.9	24.1, 23.9	62.5	19.9, 19.8	119.4
III[b]	43.9	25.0	25.0	59.6	21.0	119.4
7[a]				64.7	19.0	118.1
8[c]				65.8	23.3, 22.9	120.0

[a] In DMSO-d_6. [b] In CD_3CN. [c] In D_2O.

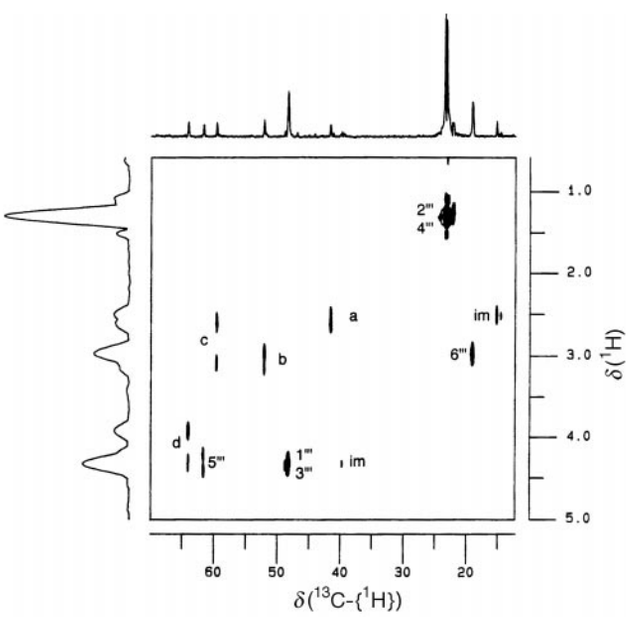


Fig. 2 The XHCORR NMR spectrum of compound **4**.

in Tables 1–4. Clear identification of the isopropyl groups was possible in the ^{13}C-{^{1}H} spectrum based on their distinctive intensities. Since the nitrogen center is adjacent to a chiral phosphorus center the two isopropyl groups have different chemical shifts in the ^{13}C-{^{1}H} spectrum, although identical peaks were observed in the ^{1}H NMR spectrum. Methylene group 6‴ has also two distinctive ^{13}C-{^{1}H} chemical shifts at δ 19.8 and 19.9, as determined by comparing with those of analogous compounds.[21] Scalar coupling in ^{1}H 2-D COSY allows the identification of 5‴ to be either δ 62.5 or 64.9. One of those two peaks would belong to methylene group d, which is also bound to an alkoxide group. Since d is within a six membered ring, the axial and equatorial hydrogens have quite different environments, requiring larger chemical shift separation of the two protons on this carbon in the ^{1}H NMR spectrum. So, 5‴ was assigned to be at δ 62.5 and d at δ 64.9. The assignment of b and c was based on analogous arguments. Somehow, the chemical shift of the methylene group a in the ^{13}C-{^{1}H} NMR is distinct from the other groups, and was assigned by comparing with those of analogous compounds such as **1**.

Table 5 Bond lengths (Å) and angles (°) for compound **4**

Pt(1)–N(2)	2.047(10)	Pt(1)–N(1)	2.113(9)
Pt(1)–P(1)	2.211(3)	Pt(1)–Cl(1)	2.300(4)
N(1)–C(1)	1.480(14)	C(1)–C(2)	1.470(13)
C(2)–N(2)	1.486(13)	N(2)–C(3)	1.495(13)
C(3)–C(4)	1.51(2)	C(4)–O(1)	1.43(2)
O(1)–P(1)	1.597(9)	P(1)–O(2)	1.613(9)
P(1)–N(3)	1.624(11)	O(2)–C(5)	1.408(14)
C(5)–C(6)	1.50(2)	C(6)–C(7)	1.43(2)
C(7)–N(4)	1.12(2)	N(3)–C(11)	1.45(2)
N(3)–C(8)	1.48(2)	C(8)–C(9)	1.54(2)
C(8)–C(10)	1.55(2)	C(11)–C(13)	1.42(2)
C(11)–C(12)	1.60(2)		
N(2)–Pt(1)–N(1)	81.8(4)	N(2)–Pt(1)–P(1)	94.4(3)
N(1)–Pt(1)–P(1)	173.7(3)	N(2)–Pt(1)–Cl(1)	171.9(3)
N(1)–Pt(1)–Cl(1)	91.0(3)	P(1)–Pt(1)–Cl(1)	93.03(14)
C(1)–N(1)–Pt(1)	107.2(6)	C(2)–C(1)–N(1)	106.8(9)
C(1)–C(2)–N(2)	108.8(9)	C(2)–N(2)–C(3)	110.6(9)
C(2)–N(2)–Pt(1)	109.4(7)	C(3)–N(2)–Pt(1)	118.2(7)
N(2)–C(3)–C(4)	112.3(10)	O(1)–C(4)–C(3)	113.1(11)
C(4)–O(1)–P(1)	118.2(7)	O(1)–P(1)–O(2)	98.7(5)
O(1)–P(1)–N(3)	107.1(5)	O(2)–P(1)–N(3)	104.6(6)
O(1)–P(1)–Pt(1)	111.0(4)	O(2)–P(1)–Pt(1)	113.8(3)
N(3)–P(1)–Pt(1)	119.4(4)	C(5)–O(2)–P(1)	121.2(9)
O(2)–C(5)–C(6)	111.1(13)	C(7)–C(6)–C(5)	113.0(12)
N(4)–C(7)–C(6)	178(2)	C(11)–N(3)–C(8)	117.6(11)
C(11)–N(3)–P(1)	123.2(10)	C(8)–N(3)–P(1)	118.6(9)
N(3)–C(8)–C(9)	110.9(12)	N(3)–C(8)–C(10)	109.3(12)
C(9)–C(8)–C(10)	113.0(13)	C(13)–C(11)–N(3)	119.9(13)
C(13)–C(11)–C(12)	106(2)	N(3)–C(11)–C(12)	109(2)

Structure of the platinum complex 4

Complex **4** crystallizes from methanol–diethyl ether as white rectangular blocks. Owing to the poor quality of the crystals only a limited amount of diffraction data was collected (up to 45°). Therefore, relatively large errors are anticipated. Significant bond distances and angles in the co-ordination sphere are presented in Table 5 and the ORTEP[22] diagram of the structure is shown in Fig. 3.

The platinum atom adopts a four-co-ordinate square planar geometry, provided by the two amino nitrogens, one phosphorus, and one chloride ion. The five membered ring formed by the ethylenediamine fragment and platinum adopts a half envelope conformation with carbon C(1) at the apex position. The six membered ring formed by the central nitrogen and phosphorus atom adopts a chair-like conformation. Both the phosphorus and central nitrogen atoms are asymmetric centers. In the unit cell two molecules exist as (N_S, P_R), the other two as (N_R, P_S). No molecules with the combination of (N_R, P_R) and (N_S, P_S) are observed. Atom N(3) adopts a trigonal planar configuration, being sp^2 hybridized. Probably the lone pair of electrons on this nitrogen interacts with the empty d orbital of the phosphorus. The interaction may also be strengthened by the co-ordination of the phosphorus with the platinum. The other chlorine atom (Cl2) free from the co-ordination sphere of Pt lies just above the central nitrogen atom N(2) with an internuclear distance of 3.1 Å, and with a bonding angle around the hydrogen atom on N(2) of 176°, consistent with hydrogen-bonding formation. Other

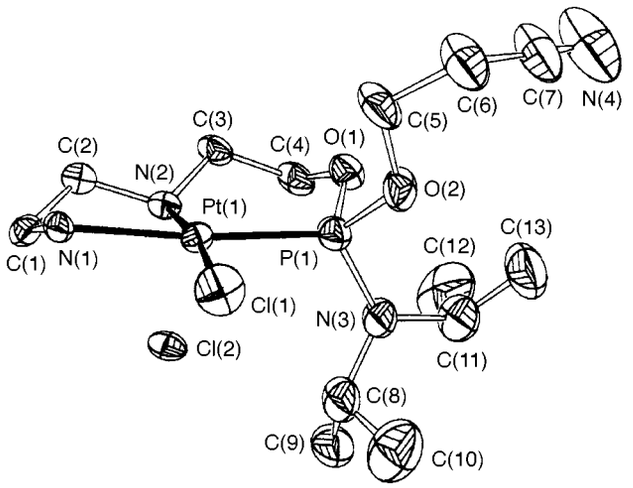


Fig. 3 Molecular structure of compound **4** with atomic numbering (ORTEP, 50% probability ellipsoids, hydrogen atoms omitted for clarity).

portions of the molecule have normal structures for the same type of compounds.

The synthetic scheme for compound **4** is shown in Scheme 3. The reagent 2-cyanoethyltetraisopropylphosphorodiamidite gives the cleanest reaction in the presence of tetrazole, which functions as the catalyst for this coupling reaction and other analogous reactions. Two other common coupling agents used in DNA synthesis, tris(1,1,1,3,3,3-hexafluoropropyl) phosphite and 2-cyanoethyl diisopropylphosphoramidochloridite, give only mixtures or no reactions at all. Our initial design is to use the phosphoramidite group as a bridge to connect with mono- or oligo-deoxynucleotides. Co-ordination of the phosphorus in **4** dramatically changes the chemical properties of the phosphoramidite group. In this case no reaction with other alcohols is observed under a variety of conditions with tetrazole or 5-methylsulfanyltetrazole as the catalyst. Oxidation of **4** by H_2O_2, H_2O_2–AcOH, or H_2O_2–Ac_2O–AcOH gives a mixture as revealed by the appearance of multiplets in the ^{31}P NMR spectra.

Mechanistic implication

The formation of compound **4** in less than one hour has implications for factors that affect the rate of ligand substitution of platinum(II) complexes. Since no reaction is observed without tetrazole, substitution of the chloride by the phosphoramidite group is unlikely to be the first step in the overall transformation. The most reasonable assumption for the mechanism is that the hydroxy group is connected with the coupling agent first, and then intramolecular substitution of the chloride by the tethered phosphoramidite group occurs. In that sense, the half life of the intramolecular substitution should be less than 0.5 h, which is much shorter than that of the solvolysis of **1** in DMSO. This was measured to be about 3.5 h by ^{195}Pt NMR at room temperature.

Complexes 5–7

The structures and labelling of the complexes are shown in Scheme 4. The reaction of compound **III** with complex **1** and tetrazole as the catalyst generates **5** and **6** as revealed by a combination of NMR studies. The ^{31}P-$\{^1H\}$ NMR spectrum of **5** shows one doublet of multiplets with a coupling constant of 5197 Hz, while that of **6** shows a set of two peaks around δ 140 and 139. The chemical shift of the latter resembles those of free phosphite ligands. Since no reaction is observed without tetrazole, direct co-ordination without a covalent tether is unlikely. The coexistence of **5** and **6** is also deduced from the simultaneous observation of two sets of peaks located around

Table 6 The ^{195}Pt NMR chemical shifts for compounds in DMSO-d_6

Compound	Pt^{II}	Pt^{II}–P	Pt^{IV}
1	−2355		
2			1015.2
3			−286.5
4		−3936.2 ($^1J_{Pt-P}$ = 4911 Hz)	
5		−3661.3, −3676.5 ($^1J_{Pt-P}$ = 5479 Hz)	
6	−2350		
7			1034, 795, 760, 755, 580, 331 or 1008, 829, 741, 726, 520, 298[a]
8			448.0[a]

[a] In water–CH_3OH.

δ −3660 and −2350 respectively in the ^{195}Pt NMR spectrum. Since the chemical shift of the ^{195}Pt NMR signal is sensitive to the immediate co-ordination environment of the central platinum atom, comparison of the chemical shifts with those of the established compounds in Table 6 and discussion about "^{195}Pt NMR studies of the platinum complexes" below allow the assignment of the structures of **5** and **6** as shown in Scheme 4. Integration of the ^{195}Pt NMR signals allows the estimation of the relative proportion of **5** and **6** to be 35:65. Two isomers are observed for **5** with the relative ratio of about 2:1. The relative composition of **5** and **6** remains constant during the reaction time of 2–3 h. However, the number of NMR peaks in each region increases with time after this period, but the overall shape of the peaks remains.

Both complexes **5** and **6** are not very stable, changing to complicated mixtures if left in solution overnight or if exposed to air, probably because of the sensitivity of the phosphite group toward hydrolysis and oxidation.[23] They were not isolated. Instead, direct oxidation by H_2O_2 with acetic anhydride in acetic acid was performed to generate **7**. The upfield ^{31}P-$\{^1H\}$ chemical shift of **7** confirms the oxidation state of the phosphorus to be +5. The presence of platinum(IV) is indicated by the observation of ^{195}Pt signals around δ 300 to 1000 and by the absence of oxidation waves in cyclic voltammetry. A total of 4 isomers is expected based on the 2 stereochemical centers generated during the reaction. However 6 peaks are observed on a RP18 column in HPLC analysis, and ^{195}Pt NMR peaks from δ 330 to 1030. This means that more than one geometrical or stereochemical isomer was generated at the platinum center. A total of 6 isomers, including two diasteromers and two pairs of enantiomers, is possible from the platinum center (see SUP 57515). When this is combined with the two newly generated chiral centers, a total of 24 isomers is possible for **7**. No attempt was made to separate them and to identify their individual peaks.

It is apparent that the different isomers show identical NMR signals for those groups beyond the phosphate from the platinum center and for the two acetate groups on the platinum, since only one set of peaks was observed and assigned (Tables 1–4 and 7–10). However, multiple peaks belonging to each of the four methylene groups from a to d were observed, due to different isomers existing in solution.

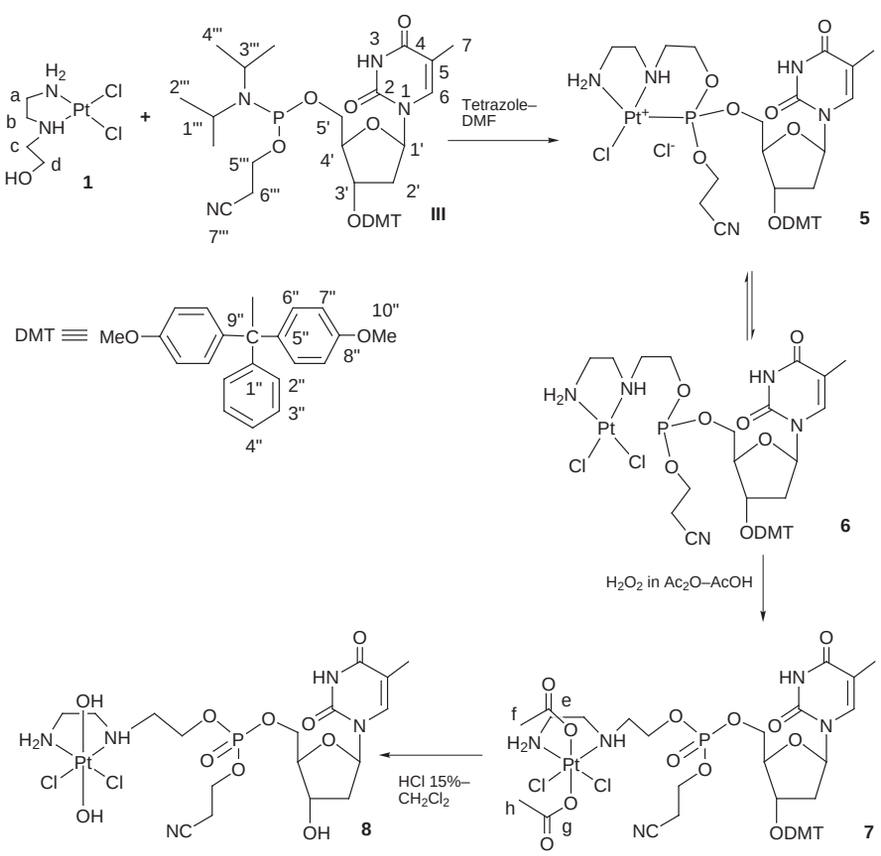
Complex 8

Multiple attempts have been tried to remove the protection group DMT from the 3′ position. Typical conditions for removing such a group failed in this case. Hydrolysis in the presence of 15% hydrochloric acid solution resulted in a deep red solution with minimum deprotection. Complete deprotection by the acid solution was observed when the reaction mixture was repeatedly extracted with CH_2Cl_2 to remove DMT–X

Table 7 The ^{1}H NMR chemical shifts of various compounds containing the thymine nucleoside fragment in DMSO-d_6 unless indicated otherwise

Compound	1′	2′	3′	4′	5′	5	6	7	N–H or O–H
III[a]	6.22, m, 1 H	1.63, m, 2 H	4.62, d, 1 H or 4.57, d, 1 H	4.27, s, 1 H or 3.99, s, 1 H	3.66, m, 2 H		7.23, s, 1 H	1.78, s, 3 H	9.10, br s
7[b]	6.24, m, 1 H	1.92, m, 1 H; 1.82, m, 1 H	4.36, m, 1 H or 4.31, m, 1 H	4.05, m, 1 H or 3.91, m, 1 H	4.24, m, 2 H		7.34, s, 1 H	1.76, s, 3 H; 1.74, s, 3 H	8.37, br s
8[c]	6.36, m, 1 H	2.13, m, 2 H	4.62, m, 1 H	3.97, m, 1 H	4.59, m, 2 H		7.74, s, 1 H		8.48, s; 7.97, s; 7.78, s

[a] In CD_3CN. [b] In DMSO-d_6. [c] In D_2O.



Scheme 4 Synthetic scheme for monodeoxynucleotide tethered platinum complexes.

compounds (X = Cl or OH). To our surprise, the acetate groups on the platinum are also hydrolyzed, supporting the conclusion that the chemical properties of the Pt–OH group resemble those of alcohols.[20]

The same number of isomers was anticipated for both complexes **7** and **8** (see SUP 57515). Their ^{1}H NMR spectra were quite similar for the corresponding sites (Tables 1–4, and 7 and 8). However, the ^{13}C-{^{1}H} signals for **8** are quite different from those of **7**. The multiple peaks belonging to each methylene group from a to d were spread out and severely overlapped, and each site from most of the sugar and heterobase portion also showed multiple peaks due to different isomers in solution. However, only one peak was observed on ^{195}Pt and ^{31}P-{^{1}H} NMR for **8**.

Reflection of our strategy

Among the three general strategies to synthesize metal-containing oligonucleotides, we selected to develop method (c) to construct our platinum-based complexes. Phosphitylation of **1** generated **4** as described above. The intramolecular coordination of the phosphoramidite group dramatically reduces its activity to such an extent that no reaction is observed for any alcohol under a variety of conditions including different derivatives of tetrazole as the catalyst, making this reaction not useful in the construction of our complexes. However, such coordinative ability is much smaller for the phosphite group than that for phosphoramidite, making **5** and **6** exist in equilibrium. Oxidation of the phosphite shifts the equilibrium to generate a single product **7**. Analogous oxidation of **4** on prolonged heating resulted in a mixture of products instead. Attempted phosphitylation of **3** under the same conditions as for **1** resulted in a mixture in a variety of conditions; presumably electron transfer reactions occurred among the reactants. Extension of this strategy to other conjugates between platinum and oligonucleotides is anticipated.

Electrochemical studies of the platinum complexes

All the oxidation and reduction reactions of the platinum-(II) or -(IV) complexes are irreversible. The values listed in Table 11 correspond to those of the maxima or minima of oxidation and reduction waves in cyclic voltammograms. Complex **1** shows only one oxidation wave at 1.1 V; no reduction wave is observed within the range 0 to −1.7 V. It looks like only the platinum center is available for oxidation. Complexes **2** and **3** only show

Table 8 The ^{13}C-{^{1}H} NMR chemical shifts of various compounds containing the thymine nucleoside fragment in DMSO-d_6 unless indicated otherwise

Compound	1′	2′	3′	4′	5′	2	4	5	6	7
III[a]	88.3	40.1	75.9	85.7	64.7	164.7	151.5	111.3	136.5	12.8
7[b]	86.8	38.1	74.5	83.5	62.6	163.5	150.5	110.0	135.7	12.0
8[c]	86.3	39.2, 39.1, 39.0, 38.7	71.7, 71.5, 71.3, 71.2	85.6, 85.4	64.6	167.1	152.3	112.3, 112.2	138.2, 137.9, 137.8	12.4, 12.3

[a] In CD_3CN. [b] In DMSO-d_6. [c] In D_2O.

Table 9 The ^{1}H NMR chemical shifts of various compounds containing the DMT fragment in $CDCl_3$ unless indicated otherwise

	Compound	
Peak	**III**[a]	**7**[b]
2″	7.45, m, 2 H	7.42, m, 2 H
3″	7.32, m, 2 H	7.35, m, 2 H
4″	7.32, m, 1 H	7.29, m, 1 H
6″	7.32, d, 4 H	7.29, d, 4 H
7″	6.87, d, 4 H	6.92, d, 4 H
10″	3.75, s, 3 H	3.74, s, 3 H

[a] In CD_3CN. [b] In DMSO-d_6.

Table 10 The ^{13}C-{^{1}H} NMR chemical shifts of various compounds containing the DMT fragment in $CDCl_3$ unless indicated otherwise

	Compound	
Peak	**III**[a]	**7**[b]
1″	146.6	145.0
2″	129.1	128.0
3″	129.1	127.8
4″	128.1	127.0
5″	137.3	135.7
6″	131.3	129.9
7″	114.4	113.4
8″	160.0	158.3
9″	86.4	83.8
10″	56.0	55.1

[a] In CD_3CN. [b] In DMSO-d_6.

reduction waves at −1.0 and −0.55 V; no oxidation wave is observed within the range 0–1.6 V, consistent with the reduction of Pt^{IV} to either Pt^{III} or Pt^{II}. Complex **4** shows both an oxidation wave at 1.34 and reduction waves at −0.58 and −1.14 V, respectively. This is consistent with the structure of the compound having a Pt–P bond. The phosphoramidite group as a ligand is known to stabilize low oxidation states of platinum. Most probably, the two reduction waves observed correspond to the couples Pt^{2+}–Pt^{+} and Pt^{+}–Pt^{0}. The intrinsic instability of the Pt^{+} makes the processes irreversible. The coupling agent 2-cyanoethyltetraisopropylphosphorodiamidite itself only gives an oxidation wave at 1.10 V, while the corresponding phosphate, which was obtained by oxidation of the coupling agent in CH_2Cl_2 with H_2O_2, does not show any redox activity within the range −1.7 to 1.6 V. Two reduction waves (−0.77 and −1.46 V) and their corresponding two re-oxidation waves (−1.03 and −0.61 V) have been observed for **7**. The corresponding phosphate without platinum, which was obtained by the oxidation of **III** in CH_2Cl_2 with H_2O_2, does not show any redox activity within the range of −1.7 to 1.6 V. The redox waves for **7** have been assigned to the couples Pt^{4+}–Pt^{3+} and Pt^{3+}–Pt^{2+}. The reduction potentials for the platinum(IV) complexes are within the range of −0.5 to −1.5 V, while the oxidation potentials for the platinum(II) complexes are within the range 1.0–1.4 V. These results show that the reduction of platinum(IV) complexes is feasible under physiological conditions.[24]

Table 11 Redox waves for the compounds from cyclic voltammograms measured in CH_3CN with 0.1 M tetrabutylammonium hexafluorophosphate as electrolyte

Compound	Pt^{II}	P	Pt^{IV}
1	Ox: 1.08		
2			Re: −1.00
3			Re: −0.55
4	Ox: 1.34		Re: −1.14
			Re–Ox: −0.58
7			Re: −0.77, −1.46
			Re–Ox: −1.03, −0.61
II		Ox: 1.10	
III		Ox: 1.20	

Ox = Oxidation, Re = reduction, Re–Ox = reoxidation after initial reduction.

^{195}Pt NMR studies of the platinum complexes

The ^{195}Pt NMR signal(s) help to establish the co-ordination environment, oxidation state of platinum, the interaction of platinum with phosphorus, and the stereoisomerism in some cases. The chemical shifts of *cis*- and *trans*-$\{Pt(NH_3)_2\}^{2+}$ monofunctional adducts $[Pt^{II}N_3Cl]$ of nucleobases are from δ −2030 to −2360, bifunctional adducts $[Pt^{II}N_4]$ from δ −2430 to −2870,[25,26] $Pt^{II}N_2SCl$ from δ −3120 to −3190,[27] $Pt^{II}N_2Cl_2$ from δ −2200 to −2300,[28] $Pt^{II}N_2O_2$ from δ −1620 to −1980,[28,29] $Pt^{II}N_3O_2$ from δ −2310 to −2350,[30] $Pt^{IV}N_3O_2Cl$ from δ +360 to +370,[30] $Pt^{IV}N_2O_2Cl_2$ from δ +600 to +1120,[28] and $Pt^{IV}N_2Cl_4$ from δ −80 to −250.[28] The ^{195}Pt NMR signals for the compounds of this study are summarized in Table 6. All the $Pt^{II}N_2Cl_2$ centers give ^{195}Pt NMR chemical shifts around δ −2350, the $Pt^{IV}N_2O_2Cl_2$ from δ +300 to +1050, and the one with $Pt^{IV}N_2Cl_4$ gives δ −287. The platinum complexes with a platinum–phosphoramidite bond $Pt^{II}N_2PCl$ give ^{195}Pt NMR chemical shifts from δ −3650 to −3950, the highest upfield chemical shift among the platinum(II) complexes reported. Except for compound **4**, no platinum complex having the co-ordination sphere $Pt^{II}N_2PCl$ has been reported where crystal structure and ^{31}P and ^{195}Pt NMR studies have been conducted at the same time to allow the comparison and correlation intended here. However, evaluation of platinum(II) phosphoramidite or phosphite complexes reported, where such studies have been conducted, reveals two features which are useful for structural deduction from NMR studies. They are the one-bond scalar coupling constant J_{Pt-P} and the chemical shifts of co-ordinated phosphorus(III) and Pt^{II}. A qualitative trend of the average Pt–P bond distance *vs.* $^{1}J_{Pt-P}$ in platinum(II) phosphoramidite or phosphite complexes has been established: Pt–P 2.26–2.34 Å *vs.* $^{1}J_{Pt-P}$ 3000–4300 Hz; Pt–P 2.15–2.22 Å *vs.* $^{1}J_{Pt-P}$ 5500–6300 Hz.[31] The parameters for **4**, Pt–P 2.21 Å *vs.* $^{1}J_{Pt-P}$ 4911 Hz, fall nicely within the range. The chemical shifts of co-ordinated phosphoramidite or phosphite are shifted upfield as compared with that of free phosphoramidite or phosphite, with individual values depending on the substituents. Values ranging from δ 77 to 126 have been reported for co-ordinated phosphoramidite,[31e–f] and δ 50 to 130 for co-ordinated phosphite.[31b,c,h–j,32] This is in contrast with the chemical shifts of free phosphoramidite, δ 140–160, and of phosphite, δ 115–150.[31h,i,33] Platinum

complexes with the co-ordination sphere $Pt^{II}P_2Cl_2$ give ^{195}Pt NMR chemical shifts from δ −4300 to −4400,[32c] and $Pt^{II}P_2I_2$, of δ −3500.[31f] From the limited amount of ^{195}Pt NMR chemical shifts, it seems that phosphorus-based ligands shift the chemical shift upfield, consistent with the observation for **4**. The ^{31}P-{^1H} and ^{195}Pt chemical shifts and coupling constants for **5** and **6** fall nicely within the above ranges.

Conclusion

We have improved the synthetic procedures for the synthesis of some hydroxy group tethered platinum-(II) and -(IV) complexes. Phosphoramidite coupling agents were shown to react with the tethered hydroxy groups of the platinum complexes. However, intramolecular displacement of the chloride by the incoming phosphoramidite group prevents further manipulation of the latter to connect with other hydroxy group(s) such as those of oligodeoxynucleotides to synthesize ODN-tethered platinum complexes. A new strategy has been developed to couple monodeoxynucleotides with the hydroxy group(s) of the tethered platinum(II) complexes through the use of the monodeoxynucleotide phosphoramidite agents. Every peak in the ^1H and ^{13}C-{^1H} NMR spectra of all the compounds reported in this study has been assigned through the combination of 1-D ^1H, ^{13}C-{^1H}, 2-D COSY, XHCORR, HMQC (heteronuclear multiple quantum correlation), and the comparison of chemical shifts among analogs. Extension of the strategy to other oligodeoxynucleotides and evaluation of the anticancer activities of these compounds are in progress.

Experimental

Materials, methods, and instrumentation

Potassium tetrachloroplatinate (K_2PtCl_4) and potassium hexachloroplatinate (K_2PtCl_6) were purchased from Sausville Chemical Company (Garfield, NJ), 2-cyanoethyl tetraisopropylphosphoramidite, tris(1,1,1,3,3,3-hexafluoropropyl) phosphite, 2-cyanoethyl diisopropylphosphoramidochloridite, tetrazole, 5-methylthiotetrazole, 2-(2-aminoethylamino)ethanol, $FeCp_2^+PF_6^-$, and $Bu_4N^+PF_6^-$ from Aldrich (Milwaukee, WI), H_3PO_4 (85%), hydrogen peroxide, acetic acid, dimethyl sulfoxide (DMSO), dimethylformamide (DMF), acetonitrile and methylene chloride, from Fisher Scientific (Pittsburgh, PA), acetic anhydride from Mallinckrodt Chemical (Paris, KY), hydrochloric acid from EM Science (Gibbstown, NJ), compound **III** from Glen Research (Sterling, VA) and deuteriated dimethyl sulfoxide, water, methanol and dimethylformamide from Cambridge Isotope Company (Cambridge, MA). All chemicals were used as received unless otherwise stated.

Air-sensitive materials were handled either in a Vacuum Atmospheres glove-box or by using standard Schlenk techniques under dinitrogen. Solvents used in the glove-box were purified by standard methods. Elemental analyses were performed by Galbraith Laboratories, Inc. (Knoxville, TN, USA), Midwest Microlab (Indianapolis, IN, USA), and H. Kolbe Mikroanalytisches Laboratorium (Mülheim, Germany).

NMR measurements

All platinum(II) complexes undergo solvolysis at various speeds in DMSO solutions. Minimum time exposure is required when conducting NMR studies in this solvent. Owing to solubility limitations of some platinum(II) complexes, use of this solvent sometimes is unavoidable. The 1-D ^1H and ^{13}C-{^1H} NMR spectra were obtained on either a Bruker AC 200 or 400 spectrometer, and the chemical shifts referenced to the residual solvent peaks. Standard parameters were used. The 1-D ^{31}P-{^1H} NMR spectra were obtained on a Varian 300 spectrometer, and the chemical shifts referenced to external 85% phosphoric acid in water. The 1-D ^{195}Pt NMR spectra were also obtained on a Varian 300 spectrometer, and the chemical shifts referenced to *cis*-DDP in DMSO-d_6 at δ −2124, which was calibrated by using K_2PtCl_4 in water (δ −1623 relative to Na_2PtCl_6) as an external standard. Typical parameters were: frequency 64.224–64.414 MHz (varied according to the windows used for different compounds), spectral width 100 000 Hz, acquisition time 6 ms, relaxation delay 0 s, pulse width 14 μs, temperature 23 °C, decoupler off, line broadening 150 Hz, and FT size 1024. The 2-D ^1H COSY experiments were performed on a Bruker AC 400 spectrometer. Typical parameters were: data size 1024 × 256, dummy scan 2, magnitude processing, relaxation delay 1 s, evolution time 3 μs, 90° pulse width 6.5 μs, scan number 16. Typical parameters for 2-D XHCORR experiments were: data size 2048 × 256, dummy scan 2, magnitude processing, relaxation delay 0 s, evolution time 3 μs, mixing time 3.3 ms, increment dwelling time of ^1H, scan number 128. Typical parameters for 2-D HMQC experiments were: 2048 × 512, dummy scan 4, phase sensitive processing, relaxation delay 1.5 s, evolution time 6 μs, mixing time 3 ms, increment dewelling time of ^1H, scan number 32.

Electrochemical measurements

Electrochemical measurements were performed using an EG&G Model 283 potentiostat under a dinitrogen atmosphere. For cyclic voltammetry experiments, a solution for each compound at 1–10 mM containing 0.1 M Bu_4NPF_6 supporting electrolyte and a platinum micro disc working electrode were used. Potentials were determined *vs*. an AgCl–NaCl reference electrode. The stability of the reference electrode was checked by performing the same experiment with added $FeCp_2^+PF_6^-$ ($E^\circ_{1/2} = 0.454$ V).

Preparations

Dichloro[*N*-(2-hydroxyethyl)ethylenediamine]platinum(II) 1.[16] Potassium tetrachloroplatinate (200 mg, 0.482 mmol) was placed in a 20 mL vial. Dilute hydrochloric acid solution (4.0 mL, concentrated HCl solution : deionized water = 3 : 100) was added to form a pink solution. 2-(2-Aminoethylamino)ethanol from a graduated cylinder was added drop by drop with a pipette until the pH reached 8.0, as monitored by pH paper. The amount added was calculated; 70% more by volume was then added. The solution was stirred for 30 min. During this time the color changed from pink to pale yellow, and a lot of yellow precipitate appeared. The solution was allowed to stand for about 30 min until it became almost colorless. The solid was filtered through a fine-filter paper, washed by 3 × 1 mL cold water and 3 × 1 mL ether, then dried to give 152 mg of complex **1**. Yield 85%. Electrospray MS: *m*/*z* 413, $[M + CH_3CN]^+$, 10; 376, $[M + CH_3CN - Cl]^+$, 3; and 335, $[M - Cl]^+$, 10% (Found: C, 12.83; H, 3.25; Cl, 19.21; N, 7.60; Pt, 52.57. Calc. for $C_4H_{12}Cl_2N_2OPt$: C, 12.98; H, 3.27; Cl, 19.16; N, 7.57; Pt, 52.70%).

Diacetatodichloro[*N*-(2-hydroxyethyl)ethylenediamine]platinum(IV) 2. Complex **1** (100 mg, 0.270 mmol) was suspended in 1.5 mL acetic acid. A total of 9.3 equivalents of acetic anhydride was added in one portion (2.56*W*, where *W* is the weight of **1**). The mixture was then stirred for one minute, 1.5 equivalents of H_2O_2 solution (30%, 0.445*W*) were added in one portion and the solution was stirred for one more hour or until it became almost clear. Methanol (1 mL) was added to quench the reaction, and the mixture was filtered. The solvent was removed, and the solid was washed with diethyl ether to get the product **2** as a yellow powder (95 mg, 80%). Electrospray MS: *m*/*z* 429, $[M - OAc]^+$; 411, $(M - OAc - H_2O]^+$; 392, $[M - OAc - Cl]^+$; 385, $[M - OAc - CH_2CH_2OH]^+$; 369, $[M - 2OAc]^+$; and 333, $[M - 2OAc - HCl^+]$. FAB MS: *m*/*z* 487, M^+; 453 $[M - Cl]^+$; 427, $[M - OAc]^+$; 393, $[M - OAc - Cl]^+$; 333, $[M - 2OAc - HCl]^+$; 309, 275, 195, Pt^+ (Found: C, 19.61; H,

3.79; Cl, 14.50; N, 5.69; Pt, 39.78. Calc. for $C_8H_{18}Cl_2N_2O_5Pt$: C, 19.68; H, 3.72; Cl, 14.52; N, 5.74; Pt, 39.95%).

Tetrachloro[*N*-(2-hydroxyethyl)ethylenediamine]platinum(IV) 3. Complex **2** (165 mg, 0.445 mmol) was suspended in 20% HCl aqueous solution and stirred at room temperature for 8 h. After the insoluble material was filtered off, the solvent was removed and the resulting solid washed by diethyl ether to afford the product **3** (80 mg, 41%). Electrospray MS: *m*/*z* 411, $[M - CH_2OH]^+$; 369, $[M - 2Cl]^+$; and 333, $[M - 3Cl]^+$ (Found: C, 11.13; H, 2.78; Cl, 31.98; N, 6.50; Pt, 44.55. Calc. for $C_4H_{12}Cl_4N_2OPt$: C, 10.89; H, 2.74; Cl, 32.14; N, 6.35; Pt, 44.22%).

Alternatively K_2PtCl_6 (20 mg, 0.0411 mmol) was suspended in 2 mL of water. The solution was heated at 70 °C until the compound was completely dissolved. 2-(2-Aminoethylamino)-ethanol (4.7 mg, 0.0452 mmol) was added in one portion. The mixture was heated at 70 °C overnight. After removal of the solvent, the solid was washed with diethyl ether, and extracted with CH_3OH. The product formed upon removal of CH_3OH was characterized by NMR study in DMSO-d_6.

Chloro{*N*-2-[(2-cyanoethyl)(diisopropylamino)phosphinoxy]-ethylenediamine}platinum(II) chloride 4. Complex **1** (100 mg, 0.270 mmol) was dissolved in 2 mL of DMF. 2-Cyanoethyltetraisopropylphosphoramidite (86.5 μl, 0.270 mmol) and 5-methylsulfanyltetrazole (28.2 mg, 0.243 mmol) were added sequentially in one portion. The reaction mixture was stirred at room temperature (RT) for 1.0 h. The solvent was removed under vacuum, and the solid washed with diethyl ether to afford a yellow powder. The solid was then extracted with 100 mL CH_3CN to get rid of a white solid. Removal of the solvent generated an oily solid, which was washed with diethyl ether to give a very light yellow powder **4** (110 mg, 71%). ^{31}P-{^1H} NMR in DMSO-d_6: δ 81.1 (s), 81.1 (d, $^2J_{Pt-P}$ = 4911 Hz). Electrospray MS: *m*/*z* 614, $[M + CH_3CN]^+$; 535, $[M - Cl]^+$; 498, $[M - 2Cl]^+$; 435, $[M - Cl - N^iPr_2]^+$, theoretical for $C_7H_{15}ClN_3O_2PPt$ (Found: C, 26.10; H, 5.13; Cl, 11.64; N, 11.64; Pt, 32.46. Calc. for $C_{13}H_{27}Cl_2N_4O_2PPt$: C, 27.38; H, 5.12; Cl, 12.43; N, 9.82; Pt, 34.20%).

Complexes 5 and 6. Complex **1** (25 mg, 0.0676 mmol) was dissolved in 1.0 mL DMF. Compound **III** (50 mg, 0.0671 mmol) and 5-methylsulfanyl tetrazole (6.3 mg, 0.0541 mmol) were added sequentially in one portion. The reaction was stirred at RT for 2–3 h, or until just completed as checked by ^{31}P-{^1H} NMR. A small amount of insoluble material was removed by filtration, the solvent was removed under vacuum, and the resulting solid washed with diethyl ether to get a yellow solid **5** and **6** (55 mg, 74%). ^{31}P-{^1H} NMR in DMF: δ 73.5 (s), 73.2 (s), 72.8 (s), 72.5 (s), 72.6 (dm, $^2J_{Pt-P}$ = 5197 Hz) for **5**, and 139 (s), 140 (s) for **6**.

Complex 7. Complex **1** (30 mg, 0.0811 mmol) was dissolved in 1.0 mL DMF. Compound **III** (60 mg, 0.0806 mmol) and 5-methylsulfanyl tetrazole (8.4 mg, 0.0716 mmol) were added sequentially in one portion. The reaction was stirred at RT for 2–3 h, or until just completed as checked by ^{31}P-{^1H} NMR. Acetic acid (0.550 g, 9.17 mmol), acetic anhydride (0.130 g, 1.27 mmol), and 30% H_2O_2 (30 mg, 0.203 mmol) were added sequentially in one portion. The mixture was stirred for 15–30 min until the reaction was complete as checked by ^{31}P-{^1H} NMR. The DMF solution was poured into a large quantity of diethyl ether, and an oily solid precipitated. When the diethyl ether solution was almost clear it was decanted. The oily solid was dried under vacuum, and the solid was washed with diethyl ether to afford the product **7** (50 mg, 54% based on **III**). ^{31}P-{^1H} NMR in DMSO-d_6: δ 0.599 (s). Electrospray MS: *m*/*z* 1074, $[M - Ph]^+$; 976, $[M - Ph - OAc - Cl]^+$; 871, $[M - Ph - OAc - Cl - C_6H_4OCH_3]^+$; 806, $[M - DMT - Cl - CH_3CO + CH_3CN]^+$; 527, [3′-DMT − deoxythymidine − OH]$^+$; 453, $[OCH_2CH_2NHCH_2CH_2NH_2Pt(OAc)_2Cl]^+$; and 413, $[OCH_2CH_2NHCH_2CH_2NH_2Pt(OOC)Cl_2]^+$ (Found: C, 44.29; H, 4.87; Cl, 5.11; N, 7.31. Calc. for $C_{42}H_{53}Cl_2N_5O_{14}PPt$: C, 43.90; H, 4.66; Cl, 6.17; N, 6.10%).

Table 12 Crystal data and structure refinement for compound **4**

Empirical formula	$C_{13}H_{27}Cl_2N_4O_2PPt$
M	568.35
Crystal habit	White
Crystal system	Monoclinic
Space group	$P2_1/c$
a/Å	16.7474(4)
b/Å	14.1010(3)
c/Å	8.7566(3)
β/°	95.747(2)
V/Å^3	2057.52(10)
Z	4
D_c/Mg m^{-3}	1.835
μ/mm^{-1}	7.169
F(000)	1104
Total reflections	8522
Independent reflections	2680 [*R*(int) = 0.1129]
Final *R*1, *wR*2 [$I > 2\sigma(I)$]	0.0469, 0.0678
(all data)	0.0991, 0.0843
Goodness of fit on F^2	0.985
Data/restraints/parameters	2666/84/208
Maximum and minimum difference peaks/e Å^{-3}	0.816 and −0.951

Complex 8. Complex **7** (40 mg, 0.0348 mmol) was dissolved in 2 mL 15% HCl in water solution, CH_2Cl_2 (3 mL) was added and the mixture stirred for 15 min. The CH_2Cl_2 was removed and fresh added. The process was repeated two more times until no DMT–OH spot was observable on TLC. The aqueous solution was separated, neutralized to pH 7 and then filtered. Upon removal of the solvent to about 2 mL some white precipitate appeared and was discarded. After all solvent was removed a pale yellow solid resulted, which was extracted with DMF to get rid of a residual amount of NaCl. Removal of DMF afforded the product **8** (18 mg, 61%). ^{31}P-{^1H} NMR in water–CH_3OH (1:1), δ 0.918. Electrospray MS: *m*/*z* 761, $[M + H]^+$; 735, $[M + H - CH_3CN]^+$; 719, $[M - CH_2CN]^+$ (Found: C, 26.61; H, 3.99; Cl, 9.60; N, 10.27. Calc. for $C_{17}H_{30}Cl_2N_5O_{10}PPt$: C, 26.81; H, 3.97; Cl, 9.60; N, 9.19%).

Collection and reduction of X-ray data for complex 4

The crystal of complex **4** was transferred to a Siemens SMART/CCD three circle (χ fixed at 54.65°) diffractometer equipped with a cold stream of N_2 gas and a graphite-monochromated Mo-Kα radiation source (λ 0.71073 Å). Data collection was performed at −60 °C. A total of 1104 frames of two-dimensional diffraction images were collected, each of which was measured for 10 s. Crystallographic data are summarized in Table 12.

The raw data frames were processed to produce conventional intensity data by the program SAINT.[34] An initial background was determined from the first 12° of data. Integration was performed with constant spot sizes of 1.75° in the detector plane and 0.9° in ω. Absorption corrections were performed using the program SADABS.[35] The intensity data were also corrected for Lorentz-polarization effects.

Solution and refinement. The structure was solved by direct methods and standard Fourier-difference techniques. Four independent platinum molecules were found in the asymmetric unit cell. All final refinements were completed using SHELXL 93.[35] Refinement was carried out on F^2 for all reflections except for those with either very negative *F* or reflections flagged for potential systematic errors, normally for reflections besides strong ones.

CCDC reference number 186/1377.

Acknowledgements

Support from Department of Chemistry, The University of Illinois at Chicago is gratefully acknowledged.

References

1 D. B. Zamble and S. J. Lippard, *Trends Biochem. Sci.*, 1995, **20**, 435; A. Pasini and F. Zunino, *Angew. Chem.*, *Int. Ed. Engl.*, 1987, **26**, 615.
2 P. C. Hydes and M. J. H. Russell, *Cancer Metastasis Rev.*, 1988, **7**, 67; D. Lemaire, M.-H. Fouchet and J. Kozelka, *J. Inorg. Biochem.*, 1994, **53**, 261; S. J. Lippard, *Metals in Medicine in Bioinorganic Chemistry*, University Science Books, Mill Valley, CA, 1994, ch. 9, pp. 505–583.
3 A. M. J. Fichtinger-Schepman, J. L. Van der Veer, J. H. J. den Hartog, P. H. M. Lohman and J. Reedijk, *Biochemistry*, 1985, **24**, 707.
4 P. M. Takahara, C. A. Frederick and S. J. Lippard, *J. Am. Chem. Soc.*, 1996, **118**, 12309.
5 G. Bérubé, Y. He, S. Groleau, A. Séné, H.-M. Thérien and M. Caron, *Inorg. Chim. Acta*, 1997, **262**, 139; N. Nagao, T. Kobayashi, T. Takayama, Y. Koike, Y. Ono, T. Watanabe, T. Mikami, M. Suzuki, T. Matumoto and M. Watabe, *Inorg. Chem.*, 1997, **36**, 4195.
6 P. Amo-Ochoa, V. M. González, J. M. Pérez, J. R. Masaguer, C. Alonso and C. Navarro-Ranninger, *J. Inorg. Biochem.*, 1996, **64**, 287.
7 (*a*) P. Dervan, *Proc. Natl. Acad. Sci. U.S.A.*, 1995, **92**, 10389; (*b*) G. B. Dreyer and P. B. Dervan, *Proc. Natl. Acad. Sci. U.S.A.*, 1985, **82**, 968; (*c*) E. E. Baird and P. B. Dervan, *J. Am. Chem. Soc.*, 1996, **118**, 6141; (*d*) M. E. Parks, E. E. Baird and P. B. Dervan, *J. Am. Chem. Soc.*, 1996, **118**, 6147; (*e*) M. E. Parks, E. E. Baird and P. B. Dervan, *J. Am. Chem. Soc.*, 1996, **118**, 6153; (*f*) J. W. Trauger, E. E. Baird, M. Mrksich and P. B. Dervan, *J. Am. Chem. Soc.*, 1996, **118**, 6160.
8 C. J. Murphy, M. R. Arkin, Y. Jenkins, N. D. Ghatlia, S. H. Bossmann, N. J. Turro and J. K. Barton, *Science*, 1993, **262**, 1025; T. J. Meade and J. F. Kayyem, *Angew. Chem.*, *Int. Ed. Engl.*, 1995, **34**, 352; P. J. Dandliker, R. E. Holmlin and J. K. Barton, *Science*, 1997, **275**, 1465; E. Meggers, D. Kusch and B. Giese, *Helv. Chim. Acta*, 1997, **80**, 640; T. L. Netzel, *J. Chem. Educ.*, 1997, **74**, 646; U. Diederichen, *Angew. Chem.*, *Int. Ed. Engl.*, 1997, **36**, 2317.
9 (*a*) W. Bannwarth, D. Schmidt, R. L. Stallard, C. Hornung, R. Knorr and F. Müller, *Helv. Chem. Acta*, 1988, **71**, 2085; (*b*) W. Bannwarth and D. Schmidt, *Tetrahedron Lett.*, 1989, **30**, 1513; (*c*) W. Bannwarth, W. Pfleiderer and F. Müller, *Helv. Chim. Acta*, 1991, **74**, 1991; (*d*) J. Telser, K. A. Cruickshank, K. S. Schanze and T. L. Netzel, *J. Am. Chem. Soc.*, 1989, **111**, 7221; (*e*) P. G. Sammes and G. Yahioglu, *Nat. Prod. Rep.*, 1996, **13**, 1; (*f*) T. Ihara, M. Nakayama, M. Murata, K. Nakano and M. Maeda, *Chem. Commun.*, 1997, 1609.
10 L. Jacquet, J. H. Davies, A. K. Mesmaeker and J. M. Kelly, *J. Am. Chem. Soc.*, 1997, **119**, 11763.
11 (*a*) D. Magda, S. Crofts, A. Lin, D. Miles, M. Wright and J. Sessler, *J. Am. Chem. Soc.*, 1997, **119**, 2293; (*b*) D. Magda, R. A. Miller, J. L. Sessler and B. L. Iverson, *J. Am. Chem. Soc.*, 1994, **116**, 7439; (*c*) C. B. Chen and D. S. Sigman, *J. Am. Chem. Soc.*, 1988, **110**, 6570.
12 R. Manchanda, S. U. Dunham and S. J. Lippard, *J. Am. Chem. Soc.*, 1996, **118**, 5144.
13 J. K. Bashkin, E. I. Frolova and U. Sampath, *J. Am. Chem. Soc.*, 1994, **116**, 5981; K. Matsumura, M. Endo and M. Komiyama, *J. Chem. Soc.*, *Chem. Commun.*, 1994, 2019.
14 J. Hall, D. Hüsken, U. Pieles, H. E. Moser and R. Häner, *Chem. Biol.*, 1994, **1**, 185; J. Hall, D. Hüsken and R. Häner *Nucleic Acids Res.*, 1996, **24**, 3522.
15 J. Schliepe, U. Berghoff, B. Lippert and D. Cech, *Angew. Chem.*, *Int. Ed. Engl.*, 1996, **35**, 646; R. C. Mucic, M. K. Herrlien, C. A. Mirkin and R. L. Letsinger, *Chem. Commun.*, 1996, 555.
16 G. W. Watt and J. S. Thompson, Jr., *J. Inorg. Nucl. Chem.*, 1974, **36**, 1075.
17 (*a*) B. K. Chakraborty, N. Biswas, K. Choudhury, R. K. Neogy and B. S. Sarma, *Chemotherapy* (*Basel*), 1985, **31**, 55; B. D. Sarma, S. K. Daley and R. K. Elespuru, *Chem.-Biol. Interact.*, 1983, **46**, 219.
18 G. J. Jenks, *J. Chem. Phys.*, 1971, **54**, 658; D. Rogers and M. T. Rogers, *J. Magn. Reson.*, 1972, **7**, 30.
19 V. B. Ukraintsev, S. B. Yakovlev and Y. N. Kukushkin, *J. Gen. Chem. USSR*, 1985, **55**, 1082.
20 C. M. Giandomenico, M. J. Abram, B. A. Murrer, J. F. Vollano, M. I. Rheinheimer, S. B. Wyer, G. E. Bossard and J. D. Higgins, III, *Inorg. Chem.*, 1995, **34**, 1015.
21 C. J. Pouchert and J. Behnke, *The Aldrich Library of ^{13}C and ^{1}H FT NMR Spectra*, 1st edn., Aldrich Chemical Company, Inc., Milwaukee, WI, 1993, vols. 1–3.
22 C. K. Johnson, ORTEP, Report ORNL-5138, Oak Ridge National Laboratory, Oak Ridge, TN, 1978.
23 Y. Hayakawa and M. Kataoka, *J. Am. Chem. Soc.*, 1997, **119**, 11758.
24 S. Choi, C. Filotto, M. Bisanzo, S. Delaney, D. Lagasee, J. L. Whiteworth, A. Jusko, C. Li, N. A. Wood, J. Willingham, A. Schwenker and K. Spaulding, *Inorg. Chem.*, 1998, **37**, 2500; L. Stryer, *Biochemistry*, W. H. Freeman, New York, 4th edn., 1995, p. 532.
25 D. P. Bancroft, C. A. Lepre and S. J. Lippard, *J. Am. Chem. Soc.*, 1990, **112**, 6860.
26 A. R. Khokhar and G. J. Lumetta, *J. Coord. Chem.*, 1992, **26**, 251.
27 S. Shamsuddin, S. Al-Baker, Z. H. Siddik and A. R. Khokhar, *Inorg. Chim. Acta*, 1996, **241**, 101.
28 S. Shamsuddin, I. Takahashi, Z. H. Siddik and A. R. Khokhar, *J. Inorg. Biochem.*, 1996, **61**, 291; P. Amo-Ochoa, V. M. González, J. M. Pérez, J. R. Masaguer, C. Alonso and C. Navarro-Ranninger, *J. Inorg. Biochem.*, 1996, **64**, 287.
29 A. R. Khokhar, S. Al-Baker, T. Brown and R. Perez-Soler, *J. Med. Chem.*, 1991, **34**, 325.
30 Q. Xu and A. R. Khokhar, *J. Inorg. Biochem.*, 1992, **48**, 217.
31 (*a*) Q.-B. Bao, S. J. Geib, A. L. Rheingold and T. B. Brill, *Inorg. Chem.*, 1987, **26**, 3453; (*b*) A. Crispini, K. N. Harrison, A. G. Orpen, P. G. Pringle and J. R. Wheatcroft, *J. Chem. Soc.*, *Dalton Trans.*, 1996, 1069; (*c*) E. E. Nifantyev, E. N. Rasadkina, L. K. Vasyanina, V. K. Belsky and A. I. Stash, *J. Organomet. Chem.*, 1997, **529**, 171; (*d*) K. S. Coleman, J. H. Holloway, E. G. Hope, D. R. Russell, G. C. Saunders and M. J. Atherton, *Polyhedron*, 1995, **14**, 2107; (*e*) V. S. Reddy, K. V. Katti and C. L. Baarnes, *Chem. Ber.*, 1994, **127**, 1355; (*f*) A. J. Arduengo, III, C. A. Steward and F. Davidson, *J. Am. Chem. Soc.*, 1986, **108**, 322; (*g*) S. D. Pastor, R. K. Rodebaugh, P. A. Odorisio, B. Pugin, G. Rihs and A. Togni, *Helv. Chim. Acta*, 1991, **74**, 1175; (*h*) E. N. Rasadkina, N. S. Magomedova, V. K. Bel'skii and E. E. Nifant'ev, *J. Gen. Chem. USSR.*, 1995, **65**, 182; (*i*) E. E. Nifant'ev, E. N. Rasadkina, T. A. Batalova, A. R. Bekker, A. I. Stash and V. K. Belskii, *J. Gen. Chem. USSR*, 1996, **66**, 1081; (*j*) D. E. Berry, G. W. Bushnell and K. R. Dixon, *Inorg. Chem.*, 1983, **22**, 1962; (*k*) A. N. Caldwell, L. Manojloviç-Muir and K. Muir, *J. Chem. Soc.*, *Dalton Trans.*, 1977, 2265.
32 Q.-B. Bao and T. B. Brill, *Inorg. Chem.*, 1987, **26**, 3447; J. M. Bevilacqua and R. Eisenberg, *Inorg. Chem.*, 1994, **33**, 2913; A. Albinati, P. S. Pregosin and H. Rüegger, *Inorg. Chem.*, 1984, **23**, 3223.
33 J. M. Solar, R. D. Rogers and W. R. Mason, *Inorg. Chem.*, 1984, **23**, 373.
34 SAINT, Siemens Industrial Automation, Inc., Madison, WI, 1995.
35 SADABS, G. M. Sheldrick, SHELXL93, Program for the Refinement of Crystal Structures, University of Göttingen, 1994.

Paper 8/08705I