Synthesis and evaluation of the in vitro DNA-binding properties of chiral *cis*-dichloro(pyridyloxazoline)platinum(II) complexes[1]

David W. Dodd, Heather E. Toews, Michael J. Trevail, Michael C. Jennings, Robert H.E. Hudson, and Nathan D. Jones

Abstract: A series of chiral cis-dichloro(pyridyloxazoline)platinum(II) and palladium(II) complexes were synthesized and their reactivity towards a defined sequence of single-stranded and double-stranded DNA was investigated in comparison to cisplatin. The compounds differed in the nature and absolute configuration of the substituent at the *C*4 position of the oxazoline ring. The DNA-binding ability of these compounds was evaluated by HPLC analysis, post metal exposure, of enzymatic digests of an undecamer duplex containing one putative metallation site. Polyacrylamide gel electrophoresis (PAGE) and thermal denaturation confirmed the results of the HPLC analysis, which showed that the stereochemistry and character of the substituent at the *C*4 position of the oxazoline ring had little effect on DNA binding, possibly due to the formation of monofunctional adducts.

Key words: cisplatin, chiral, pyridyloxazoline, DNA-binding studies, platinum, palladium.

Résumé : On a réalisé la synthèse d'une série de complexes chiraux des *cis*-dichloro(pyridyloxazoline)platine(II) et palladium(II) et on a étudié leur réactivité, par comparaison au cisplatine, vis-à-vis une séquence définie d'ADN à un brin et à deux brins. Les composés diffèrent dans la nature et la configuration absolue du substituant dans la position *C*4 du noyau oxazoline. La capacité de liaison à l'ADN de ces composés a été évaluée par une analyse par chromatographie liquide à haute performance (CLHP) à la suite de l'exposition aux métaux, de mélanges obtenus par digestion enzymatique d'un duplex undécamère contenant un site putatif de métallation. Une électrophorèse sur gel de polyacrylamide (EGPA) et une dénaturation thermique ont permis de confirmer les résultats de l'analyse de CLHP qui avait montré que la stéréochimie et le caractère du substituant en position *C*4 du noyau oxazoline n'ont que peu d'effet sur la fixation sur l'ADN, probablement en raison de la formation d'adduits monofonctionnels.

Mots-clés : cisplatine, chiral, pyridyloxazoline, études de fixation d'ADN, platine, palladium.

[Traduit par la Rédaction]

Introduction

The serendipitous discovery of the antiproliferative properties of *cis*-dichlorodiammineplatinum(II) (cisplatin) over 30 years ago led to its eventual use as a chemotherapeutic agent (1). Today, cisplatin remains one of the most widely used drugs for the treatment of solid cell tumours (2). Motivated by cisplatin's effectiveness, similar treatments have been pursued; however, despite vigorous research towards this end, only a handful of successful analogues have been developed (3). While complexes of other metals, such as titanium, rhenium, ruthenium, and gallium, have shown promise as potential antineoplastic agents, the literature of metal anticancer therapeutics is dominated by platinum complexes (4). The efficacy of cisplatin in the treatment of carcinomata is almost universally thought to be due to its ability to form intrastrand adducts between adjacent purines in DNA (2–5).

The design principles of cisplatin analogues were described two years after the first clinical trials: the complexes should be neutral and four-coordinate with two coordination sites occupied by labile leaving ligands and two occupied by inert am(m)ine ligand(s) in a cis geometry (6).

Once cisplatin enters the cell, the lower intracellular chloride concentration permits dissociation of the halides resulting in the formation of an aquated platinum centre. This cationic species reacts predominantly with the *N*7 of guanine or adenine nucleobases (5). The guanine adduct is thought to be stabilized through hydrogen bonding of an ammine proton with the carbonyl oxygen of *C*6 making deoxyguanosine dinucleotide (-dGpdG-) the preferred target sequence (7). Binding to DNA in this fashion causes a kinking of the double helix and a widening of the minor groove; this can inhibit transcription and lead to apoptosis by inducing the

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Dedicated to Prof. Dick Puddephatt on the occasion of his 65th birthday and in recognition of his outstanding contributions to chemistry.

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binding of high mobility group (HMG) box proteins (5). Although cisplatin is indeed an effective drug, there are problems associated with prolonged dosages, such as toxicity and acquired resistance. Therefore, the search continues for improved platinum-based therapies.

While several thousand cisplatin analogues have been synthesized and tested for biological activity (8), only a select few have been entered into clinical trials. This has led many in the scientific community to believe that the search for Pt(II) chemotherapeutics is futile. Discovery of a drug suitable for clinical trials is a rare occurrence in every field, yet failure in cisplatin analogue discovery draws inordinate attention. Perhaps the reason for the reluctance to continue in this area is due to the remarkable simplicity of cisplatin itself: even minor substitutions cause drastic changes in efficacy. Although the structure of cisplatin is simple, the mode of action certainly is not, and many questions remain unanswered (5).

Although it is unlikely that a simple substitution of the ammine ligands will yield a complex with activity surpassing that of cisplatin, more elaborate changes, such as variation in the σ-donation character of the ligand and the introduction of chirality, could yield complexes with novel reactivities in vitro and corresponding cytostatic effects in vivo.

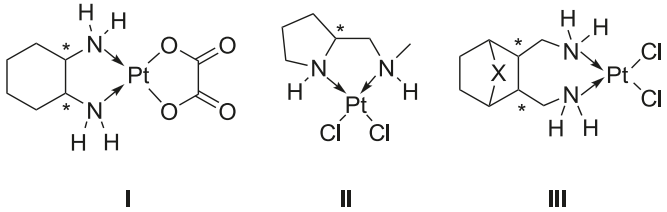
As DNA is a chiral molecule, binding of chiral platinum complexes results in diastereomeric adducts. Different binding affinities for enantiomeric complexes have been reported and (of no lesser importance) it has also been found that the diastereomeric adducts may be dealt with by cellular machinery in vastly different ways (9). For example, the *S,S*-enantiomer of (1,2-diaminocyclohexane)oxalatoplatinum(II) (Fig. 1, *S,S*-**I**) is significantly more mutagenic than the *R,R*-enantiomer, and consequently only the latter was entered into clinical trials (9, 10). Other examples of chiral DNA-binding Pt complexes that have been studied in detail include dichloro(*N*-methyl-2-aminomethylpyrrolidine)platinum(II) (Fig. 1, **II**) (10) and complexes involving 1,2-bis(aminomethyl)carbobicyclic ligands (Fig. 1, **III**) (11). Differences in reactivities of enantiomeric complexes towards DNA are rare (12).

Although hydrogen bonding involving coordinated NH groups has been considered necessary for cytostatic activity (13), there are examples of compounds lacking an NH proton that display significant antitumour effects (14, 15).

Pyridyloxazolines, derived from the condensation of a chiral β-amino alcohol with 2-cyanopyridine, are planar, bidentate ligands for platinum(II) and are necessarily bound cis (Fig. 2). The substituent at the α-position of the amino alcohol, *C*4 in the resultant oxazoline ring, can be varied greatly and inexpensively as the alcohols are obtained by the reduction of the corresponding enantiopure amino acids (16, 17).

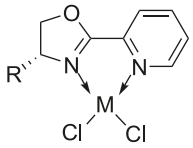
Pyridyloxazoline Pt(II) complexes have found use in enantioselective enyne cyclizations (18), and the unsubstituted complex **6** has shown some promise as an anticancer therapeutic, displaying significant toxicity towards rat brain tumour cell lines in vitro (19). The palladium analogues have not been used in DNA-binding studies, but have been investigated as asymmetric catalysts for the intermolecular asymmetric Heck reaction (16).

Fig. 1. Chiral platinum complexes **I**–**III** studied as cisplatin analogues (9–11). Chiral centres are denoted by asterisks. X=O, CH_2, CH_2CH_2.

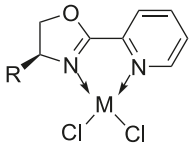


I **II** **III**

Fig. 2. Pyridyloxazoline platinum and palladium dichlorides employed in this study. The chiral centre is derived from the enantiomerically pure amino acid.



R-**1** R = iPr, M = Pt
R-**2** R = Bn, M = Pt
R-**3** R = Ph, M = Pt
R-**4** R = iPr, M = Pd



S-**1** R = iPr, M = Pt
S-**2** R = Bn, M = Pt
S-**3** R = Ph, M = Pt
S-**4** R = iPr, M = Pd
S-**5** R = sBu, M = Pt
6 R = H, M = Pt

In this work, binding of these complexes to a defined sequence of oligomeric double-stranded (ds) DNA containing one putative metallation site was investigated by HPLC analysis of enzymatic digests. The duplexes that were exposed to the metal complexes were also analyzed by thermal denaturation and polyacrylamide gel electrophoresis (PAGE). The difference in binding abilities between enantiomers and side-chain identity was investigated; the objective being to gain a better understanding of the structure–activity relationship of chiral platinum antineoplastic agents.

Experimental[3]

General

Pyridyloxazoline ligands (17) and the metal precursors $PdCl_2(PhCN)_2$ (20) and *cis*/*trans*-$PtCl_2(SMe_2)_2$ (21) were synthesized according to literature procedures and characterization data matched those of the known compounds. Platinum compounds **1**–**3**, **5**, and **6** were synthesized by a general procedure as outlined for *R*-**1**; spectral data for enantiomeric complexes were identical, as expected. Solution ^{1}H NMR spectra were collected using a Varian 400 spectrometer (400.09 MHz for ^{1}H and 100.61 MHz for ^{13}C) at RT in

[3] Supplementary data for this article are available on the journal Web site (canjchem.nrc.ca) or may be purchased from the Depository of Unpublished Data, Document Delivery, CISTI, National Research Council Canada, Ottawa, ON K1A 0R6, Canada. DUD 3818. For more information on obtaining material, refer to cisti-icist.nrc-cnrc.gc.ca/cms/unpub_e.shtml. CCDC 694283 contains the crystallographic data for this manuscript. These data can be obtained, free of charge, via www.ccdc.cam.ac.uk/conts/retrieving.html (Or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax +44 1223 336033; or deposit@ccdc.cam.ac.uk).

$CDCl_3$ (unless otherwise noted) and referenced to residual ^1H or ^{13}C in the deuterated solvent. Coupling constants are given in Hz. Elemental analyses were conducted by Chemisar Laboratories Inc., Guelph, ON. Denaturing polyacrylamide gels were cast and run according to literature procedures (22). The resultant gels were documented by colour digital photography over fluorescent TLC plates illuminated at 258 nm. For clarity, the image presented has been rendered in grayscale, inverted and contrast/brightness adjusted in Adobe Photoshop.

Optical rotations were recorded on an Atago Polax-2L polarimeter at a concentration of 1 g /100 mL at 589 nm in CH_2Cl_2 solution.

Stock solutions of the complexes (40 μmol/L) were prepared by dissolution in a minimum amount of DMF (<0.5 mL) followed by dilution with Milli-Q deionized water to 100 mL and stored at RT in the dark for no longer than 2 weeks. DNA was purchased from the University of Calgary DNA Services and used without further purification.

Syntheses

*Dichloro-2-[(4**R**)-4-**iso**-propyl-4,5-dihydro-1,3-oxazol-2-yl]pyridineplatinum(II) (**R**-1)*

The title complex was synthesized by the addition of *cis*/*trans*-$PtCl_2(SMe_2)_2$ (0.23 g, 0.60 mmol) to 5 mL of 1,2-dichloroethane containing a slight excess of pyridyl-oxazoline ligand (0.14 g, 0.72 mmol). The resultant yellow solution was heated to reflux for 4 h over which time it darkened in colour. The solvent was removed in vacuo and the remaining oil triturated with Et_2O (10 mL). Vacuum filtration gave 0.27 g (94%) of *R*-**1** as an orange/yellow powder. [α] = –65.0°. ^1H NMR δ: 9.51 (m, 1H, py), 8.17 (m, 1H, py), 7.76 (m, 1H, py), 7.63 (m, 1H, py), 5.00 (m, 1H, CH_2), 4.86 (m, 1H, CH), 4.71 (m, 1H, CH_2), 2.99 (m, 1H, CH), 0.98 (d, 3H, CH_3, ^3J = 7.1), 0.83 (d, 3H, CH_3, ^3J = 6.9). $^{13}C\{^1H\}$ NMR δ: 173.2, 149.7, 145.0, 139.4, 129.4, 126.6, 73.3, 68.2, 28.9, 18.9, 14.1. HRMS (ESI) calcd. for (M + Na) $C_{11}H_{14}N_2Cl_2ONaPt$: 477.0008; found: 477.0015. Anal. calcd. for $C_{11}H_{14}N_2Cl_2OPt$: C, 28.96; H, 3.09; N, 6.14%. Found: C, 28.90; H, 3.10; N, 6.21%.

*Dichloro-2-[(4**S**)-4-**iso**-propyl-4,5-dihydro-1,3-oxazol-2-yl]pyridineplatinum(II) (**S**-1)*

Yield: 94%. [α] = 70.0°. (ESI) calcd. for (M + Na) $C_{11}H_{14}N_2Cl_2ONaPt$: 477.0008; found: 476.9996. Orange plates suitable for X-ray diffraction analysis were provided by slow diffusion of Et_2O into a CH_2Cl_2 solution of the title complex.

*Dichloro-2-[(4**R**)-4-benzyl-4,5-dihydro-1,3-oxazol-2-yl]pyridineplatinum(II) (**R**-2)*

Yield: 72%. ^1H NMR δ: 9.63 (m, 1H, py), 8.15 (m, 1H, py), 7.67 (m, 1H, py), 7.39 (m, 1H, py), 7.27 (m, 5H, Ar), 5.01 (m, 1H, CH_2), 4.93 (m, 1H, CH), 4.85 (m, 1H, CH_2), 3.66 (dd, 1H, CH_2, ^2J = 13.9, ^3J = 10.7), 3.11 (dd, 1H, CH_2, ^2J = 13.9, ^3J = 5.8). $^{13}C\{^1H\}$ NMR δ: 173.9, 150.1, 145.3, 139.7, 135.9, 130.2, 129.5, 129.2, 127.6, 126.8, 64.5, 39.8. HRMS (ESI) calcd. for (M + Na) $C_{15}H_{14}N_2Cl_2ONaPt$: 526.0029; found: 526.0008. Anal. calcd. for $C_{15}H_{14}N_2Cl_2OPt$: C, 35.73; H, 2.80; N, 5.56%. Found: C, 36.02; H, 2.76; N, 5.54%.

*Dichloro-2-[(4**S**)-4-benzyl-4,5-dihydro-1,3-oxazol-2-yl]pyridineplatinum(II) (**S**-2)*

Yield: 68%. HRMS (ESI) calcd. for (M + Na) $C_{15}H_{14}N_2Cl_2ONaPt$: 526.0029; found: 526.0041. Anal. calcd. for $C_{15}H_{14}N_2Cl_2OPt$: C, 35.73; H, 2.80; N, 5.56%. Found: C, 36.03; H, 3.06; N, 5.52%.

*Dichloro-2-[(4R)-4-phenyl-4,5-dihydro-1,3-oxazol-2-yl]pyridineplatinum(II) (**R**-3)*

Yield: 94%. ^1H NMR δ: 9.45 (m, 1H, py), 8.20 (m, 1H, py), 7.86 (m, 1H, py), 7.62 (m, 1H, py), 7.38 (m, 5H, Ph), 5.88 (dd, 1H, CH_2, ^2J = 10.2, ^3J = 4.9), 5.46 (dd, 1H, CH, ^3J = 4.9, ^3J = 3.8), 4.92 (dd, 1H, CH_2, ^2J = 10.2, ^3J = 3.8). $^{13}C\{^1H\}$ NMR (CD_2Cl_2): δ: 174.3, 150.0, 149.6, 144.8, 139.5, 139.1, 130.3, 129.9, 129.1, 127.1, 126.8, 126.3, 81.1, 66.4. HRMS (ESI) calcd. for (M + Na) $C_{14}H_{12}N_2Cl_2ONaPt$: 511.9872; found: 511.9881. Anal. calcd. for $C_{14}H_{12}N_2Cl_2OPt$: C, 34.30; H, 2.47; N, 5.71%. Found: C, 34.53; H, 2.72; N, 5.52%.

*Dichloro-2-[(4**S**)-4-phenyl-4,5-dihydro-1,3-oxazol-2-yl]pyridineplatinum(II) (**S**-3)*

Yield: 92%. HRMS (ESI) calcd. for (M + Na) $C_{14}H_{12}N_2ONaCl_2Pt$: 511.9872; found: 511.9869. Anal. calcd. for $C_{14}H_{12}N_2Cl_2OPt$: C, 34.30; H, 2.47; N, 5.71%. Found: C, 34.56; H, 2.75; N, 5.73%.

*Dichloro-2-[(4**R**)-4-**iso**-propyl-4,5-dihydro-1,3-oxazol-2-yl]pyridinepalladium(II) (**R**-4)*

The title complex was synthesized as described in the literature. Characterization data matched those of the known compound (17).

*Dichloro-2-[(4**S**)-4-**iso**-propyl-4,5-dihydro-1,3-oxazol-2-yl]pyridinepalladium(II) (**S**-4)*

The title complex was made in the same way as *R*-**4**. Spectroscopic data matched those of the enantiomeric compound.

*Dichloro-2-[(4**S**)-4-**sec**-butyl-4,5-dihydro-1,3-oxazol-2-yl]pyridineplatinum(II) (5)*

Yield: 84%. ^{1}HNMR δ: 9.26 (d, 1H, py, ^3J = 5.5), 7.8 (dd, 1H, py, ^3J = 5.5, ^3J = 7.8), 7.76 (d, 1H, py, ^3J = 7.6), 7.56 (dd, 1H, py, ^3J = 7.8, ^3J = 7.6), 5.13 (m, 1H, CH_2), 4.87 (m, 1H, CH), 4.78 (m, 1H, CH_2), 2.75 (m, 1H, CH), 1.29 (m, 2H, CH_2), 1.00 (t, 3H, CH_3, ^3J = 7.4), 0.78 (d, 3H, CH_3, ^3J = 6.8). $^{13}C\{^1H\}$ NMR δ: 173.2, 149.8, 145.0, 139.3, 129.3, 126.5, 73.3, 66.9, 35.5, 26.5, 18.0, 11.9. HRMS (ESI) calcd. for (M + Na) $C_{12}H_{16}N_2ONaCl_2Pt$: 492.0185; found: 492.0177.

Dichloro-2-[4,5-dihydro-1,3-oxazol-2-yl]pyridineplatinum(II) (6)

The title compound has been previously made by a different route (19). Following the procedure for *R*-**1**, **6** was obtained in 72% yield. The limited solubility of this complex did not allow for the measurement of NMR spectral data. Anal. calcd. for $C_8H_8N_2Cl_2OPt$: C, 23.20; H, 1.95; N, 6.76%. Found: C, 23.50; H, 1.89; N, 6.66%.

Crystallography

An orange plate was cut loose and then mounted on a glass fibre. Data were collected at low temperature (–123 °C) on a Nonius Kappa-CCD area detector diffractometer with COLLECT (Nonius B.V., 1997–2002). The unit cell parameters were calculated and refined from the full data set. Crystal cell refinement and data reduction were carried out using HKL2000 DENZO-SMN (23). The absorption correction was applied using HKL2000 DENZO-SMN (SCALEPACK). The crystal data and refinement parameters of *S*-**1** are listed in Table 1.

The reflection data and systematic absences were consistent with a monoclinic space group: $P2_1$. The SHELXTL/PC V6.14 for Windows NT (Sheldrick, G.M., 2001) suite of programs was used to solve the structure by direct methods. Subsequent difference Fourier syntheses allowed the remaining atoms to be located. There were two independent molecules in the asymmetric unit. For this crystal, twinning was also an issue; it is discussed below. Only the heavy atoms (Pt, Cl) were refined anisotropically, as some of the lighter atoms became non-positive definite when they were allowed to refine freely. The hydrogen atom positions were calculated geometrically and were included as riding on their respective carbon atoms. The largest residue electron density peak (0.877 e/Å^3) was associated with one of platinum atoms. Full-matrix least-squares refinement on F^2 gave R_1 = 3.21 for 2σ data and wR_2 = 6.66 for all data (GooF = 1.021).

The structure was solved readily, but the initial refinement stalled at R = 12.9%. Further, the light atoms did not refine successfully with an anisotropic model. There were signs of non-merohedral twinning in the *E* statistics and the Fobs values were consistently higher than the *F* calculations. WinGX (24) was used to "detwin" the data. ROTAX (25) found the Twin Law, which turned out to be a 180° rotation about the direct lattice direction (100). "Make HKLF5" was used to generate the detwinned file used in further refinement.

The structure refinement improved considerably with better values for R_1, wR_2, GooF, standard uncertainties, and residual electron density. The final R_1 value came down to 3.21%. The final solution, however, could still not be refined completely anisotropically, and the light atoms were left isotropic in the refinement. The constant for the Twin Law refined to a value of 0.47309. All these points served to confirm the correct Twin Law was chosen.

Table 1. X-ray crystallographic structural parameters for compound *S*-**1**.

Crystal	*S*-**1**
Empirical formula	$C_{11}H_{14}Cl_2N_2OPt$
Formula mass	456.23
Colour, habit	Orange, plates
Crystal dimensions (mm)	0.17 × 0.15 × 0.10
Crystal system	Monoclinic
Space group	$P2_1$
Z	4
a (Å)	6.7455(3)
b (Å)	20.2782(9)
c (Å)	9.5172(4)
α (°)	90
β (°)	93.10(2)
γ (°)	90
Collection ranges	$-4 \le h \le 4$, $-24 \le k \le 24$, $-11 \le l \le 11$
T (K)	150(2)
V (Å^3)	1299.93(10)
$D_{calcd.}$ (Mg m^{-3})	2.331
Radiation	Mo Kα (λ = 0.71073 Å)
Absorption coeff. (μ) (mm^{-1})	11.189
Absorption correction	Semi-empirical from equivalents
F(000)	856
θ range for data collection (°)	2.94 to 25.03
Observed reflections	11811
Independent reflections	2550 (R_{int} = 0.0760)
Data, restraints, parameters	2550, 37, 171
Goodness-of-fit on F^2	1.023
Final R indices ($l > 2\sigma(l)$)	R_1 = 0.0321, wR_2 = 0.0664
R indices (all data)	R_1 = 0.0395, wR_2 = 0.0693
Absolute structure parameter	–0.009(18)
Largest diff. peak, hole (e, Å^3)	0.889, –0.702

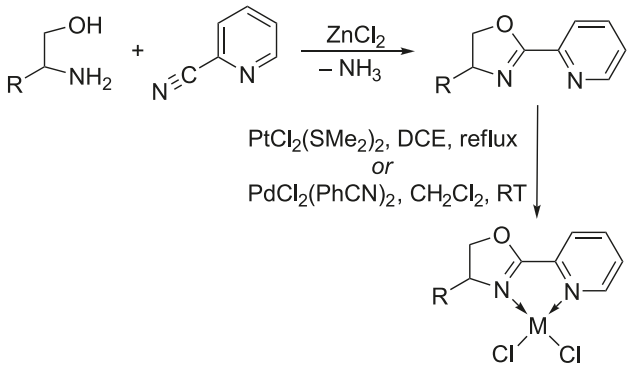
Nuclease digest studies

Duplex DNA was prepared by heating a 1:1 mixture of the complementary sequences in 10 mmol/L $NaClO_4$ to 90 °C and allowing the solution to come slowly to RT. The solution was then cooled to 4 °C for 2 h. Both single- and double-stranded DNA (2 μmol/L) were reacted with the complexes in a 1:1 molar ratio in 200 μL of 10 mmol/L $NaClO_4$ for 48 h in the dark. The buffer was then adjusted to 50 mmol/L Tris, 20 mmol/L $MgCl_2$, pH 7.5. Bovine serum albumin (BSA) was added to a final concentration of 0.02 μg/mL, and the DNA was digested sequentially with bovine pancreas DNase I (Sigma, 36 U for 4 h), nuclease P1 (Sigma, 10 U for 18 h), and alkaline phosphatase (Promega, 20 U for 4 h). The samples were then heated to 90 °C for 2 min to denature the enzymes and centrifuged for 10 min at 10 000 g. The supernatant was analyzed at 254 nm by RP–HPLC on a Varian Microsorb 100 Å C18 column using a flow rate of 1 mL min^{-1} and a solvent system of 100:0 0.1 mol/L aqueous [Et_3NH][OAc] (pH 6.5):MeCN from 0–5 min followed by a linear ramp to 90:10 over a period of 20 min. Nucleoside peaks were identified by co-injection of standards. The areas under the peaks were found by integration and analyzed in relation to one another. These digests and the HPLC analyses thereof were carried out in triplicate.

Results and discussion

The modular nature of the pyridyloxazoline ligand scaffold, combined with its brief synthesis from readily available enantiopure starting materials and facile metal coordination, permitted the rapid development of small libraries of enantiomeric Pt(II) and Pd(II) complexes. The pyridyloxazoline ligands were made by the $ZnCl_2$-catalyzed coupling of 2-cyanopyridine with the β-amino alcohols, valinol, phenylalaninol, phenylglycinol, isoleucinol, and ethanolamine, respectively, using the procedure developed by Bolm et al. (Scheme 1) (17). The amino alcohols, except

Scheme 1. Synthesis of dichloro(pyridyloxazoline)metal(II) complexes.



for ethanolamine, were themselves readily made by $NaBH_4/I_2$-reduction of both enantiomers of the corresponding amino acids, during which time no racemization occurs (26). The metal(II) complexes were made in good to excellent yields by the reaction of slight molar excesses of the ligands either with *cis*/*trans*-$PtCl_2(SMe_2)_2$ or *trans*-$PdCl_2(PhCN)_2$ in refluxing 1,2-dichloroethane or CH_2Cl_2 at RT. The complexes were yellow–orange, microcrystalline solids that were freely soluble in polar organic solvents, such as $CHCl_3$ and CH_2Cl_2, and insoluble in Et_2O and hexanes. All complexes were also sufficiently soluble in H_2O to allow DNA-binding studies.

The molecular structure of *S*-**1** is shown in Fig. 3; it is isostructural with the known analogous palladium derivative (16). As expected, the metal adopts an approximately square-planar coordination geometry, and the pyridyloxazoline ligand is roughly planar except for the *iso*-propyl substituent at the 4-position of the oxazoline ring, which projects prominently from one face of the complex and makes a torsion angle with it of ~62 °. The ligand has a "bite angle" of 79.711(4)°, which is nearly identical to that found for the palladium analogue, but unlike in that compound, whose M–oxazoline bond (2.017(5) Å) is significantly shorter than its M–pyridine bond (2.048(6) Å), there is no difference in the two M–N distances, which are both 2.015(11, 12) Å in *S*-**1**. However, there is, as in the palladium analogue, a significant difference in the M–Cl distances: in complexes of both metals, the M–Cl bond trans to the oxazoline ring is longer than that trans to the pyridine ring, which consistently suggests that oxazoline is a stronger field ligand than pyridine, and implies strong contribution from the resonance form that places a formal negative charge on the N atom of the free oxazoline.

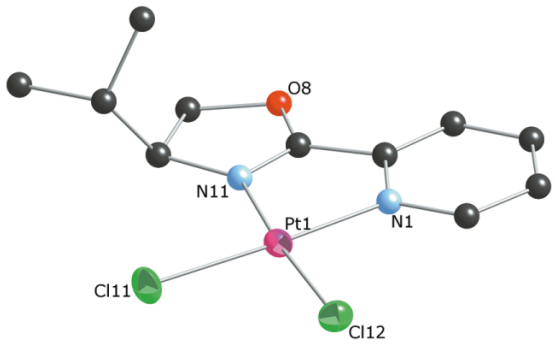
The particular undecamer sequence chosen for the present studies (shown below) has been used by Lippard and coworkers to obtain a solution NMR structure of a platinated duplex and is known to adopt the typical B-form of DNA in the absence of platination (27). The putative metallation site is underscored.

Top: strand-5′-CTCTCGGTCTC-3′

Bottom: strand-3′-GAGAGCCAGAG-5′

After incubation of the ds DNA test sequence with complexes **1**–**6** and complete digestion of the oligonucleotides,

Fig. 3. Partial ORTEP representation of the molecular structure of *S*-**1**, with H atoms omitted for clarity. Thermal ellipsoids for Pt and Cl atoms are shown at 50 % probablility; all other atoms are refined isotropically (see Experimental section). Selected bond distances (Å) and angles (°), with estimated standard deviations in parentheses: Pt1–N1 2.015(11), Pt1–N11 2.015(12), Pt1–Cl11 2.2869(3), Pt1–Cl12 2.298(4), N1–Pt1–N11 79.711(4), Pt1–N11–C10–C12 61.629(19).



HPLC analyses were carried out. Nucleoside peaks were identified by the coinjection of standards, and integrated peak-area ratios were calculated. The ratios of nucleosides were calculated and analyzed as they are independent of reaction volume and concentration, are not influenced by volume of sample used for HPLC analysis, and eliminate errors associated with pipetting technique. Binding of cisplatin analogues to a ds DNA sequence is consistent with a marked decrease in dG/dC and a slight decrease in dA/dC because dG and dA are known to coordinate metal complexes. This dramatically shifts their retention times in RP–HPLC (28), although for the present work we did not conclusively identify the peaks belonging to metal–nucleoside adducts. The nucleoside peak-area ratios for the control digests conformed to the stoichiometry of the nucleosides within the DNA strands, given their relative absorptivities (extinction coefficients were obtained from ref. 29). The dG/dA ratio can be used as a measure of binding selectivity for dG over dA.

Results are shown in Fig. 4 for 1 equiv. of complex per duplex DNA. From the ratios of dG/dC, it can be seen that the pyridyloxazoline complexes tested are less reactive towards dG than cisplatin. From comparison with the control experiment, the composition of nucleosides is reduced in dG by ~40% and dA by ~8% upon exposure to cisplatin. Higher degrees of platination may be obtained by reaction at elevated temperatures with an excess of platinum salt (28) or through the use of bis-DMF/NO_3^- compounds (27). The low thermal stability of our test sequence precluded us from the use of the former and we avoided the latter as we wished not for complete saturation, but to determine the differences in binding between enantiomeric complexes. In all digests, dC/dT remains approximately constant implying that these bases are not reactive towards the platinum complexes, as expected (data not shown). Enantiomeric complexes showed no discernable patterns in their DNA-binding ability. In the case where R = *i*Pr [*R*-**1**: Δ(dG) = –32%; *S*-**1**: Δ(dG) = –23%], the *S* enantiomer is less reactive towards dG, but in the case where R = Ph [*R*-**2**: Δ(dG) = –16%; *S*-**2**: Δ(dG) = –23%], it is the *R* enantiomer that is less reactive. In the case

Fig. 4. Ratios of peak area for enzymatic digests of the undecamer duplex after exposure to no Pt containing compound (0), cisplatin (cisPt), and compounds **1**–**6**. Error bars are +/– 1 standard deviation.

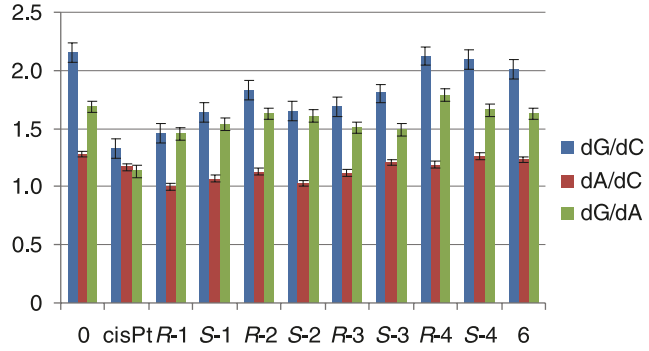
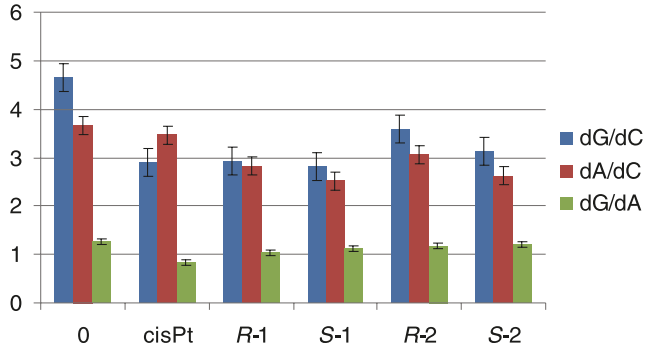


Fig. 5. Ratio of peak areas for the enzymatic digests of the bottom strand undecamer after exposure to no Pt containing compound (0), cisplatin (cisPt), and compounds **1** and **2**. Error bars are +/– 1 standard deviation.

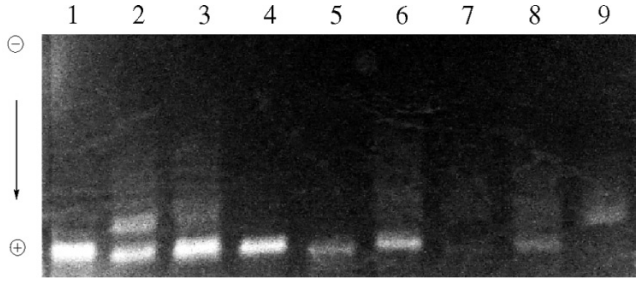


of R = Bn [*R*-**3**: Δ(dG) = –22%, *S*-**3**: Δ(dG) = –16%], there is little difference due to stereochemistry (Fig. 4). The unsubstituted ligand complex **6** (R = H) was the least reactive of the platinum complexes [Δ(dG) = –7%]; this was somewhat surprising as it was thought the decreased steric constraints would allow for greater DNA binding. It has been shown formerly that this compound has antineoplastic effects on cancer cells in vitro (19). The lack of binding in our study implies that this compound may have a different biological target and (or) mode of action. Both palladium complexes tested showed no significant deviations from the negative control [*R*-**4**: Δ(dG) = –1.4%; *S*-**4**: Δ(dG) = –3%]. Palladium complexes in a cis geometry are known to have lower binding affinities towards DNA than *cis*-dichloroplatinum compounds; however, the comparable trans-complexes often offer cytostatic effects surpassing that of cisplatin (30).

With respect to reactivity toward deoxyadenosine nucleosides in the context of duplex DNA, a parallel to the reactivity observed towards dG was found. For compound **1**, the *R*-enantiomer [Δ(dA) = –22%] was more reactive than the *S*-enantiomer [(Δ(dA) = –16%]; whereas for compound **2**, *R*-**2** [(Δ(dA) = –12%] was less reactive than *S*-**2** [(Δ(dA) = –19%]. For compound **3**, the reactivity of the *R*-stereoisomer [(Δ(dA) = –13%] was greater than that of the *S* [(Δ(dA) = –5%]. The loss of dA caused by compounds *R*-**4**, *S*-**4**, and **6** was modest at Δ(dA) = –7%, –1.5%, and –4%, respectively.

In the single-stranded digests, the lack of secondary structure should make the stereochemistry of the complexes less relevant. The complexes exhibited reactivity different from what was observed in the case of the duplex. In the reaction with the bottom strand (5′-GAGACCGAGAG-3′), where the only adjacent purines are dApdG, *R*-**1**, *S*-**1**, and *S*-**2** showed reactivity towards dG comparable to that of cisplatin, Δ(dG) = –37%, –39%, –33%, and –37%, respectively, Fig. 5. All complexes showed greater reactivity towards dA than cisplatin. Whereas cisplatin caused the loss of approximately 5% of dA, compounds *R*-**1**, *S*-**1**, *R*-**2**, and *S*-**2** reacted to a significantly greater extent causing the loss of 23%, 31%, 16%, and 29%, respectively. Decreased selectivity for dG is possibly due to the lack of an available NH proton. Of course, other electronic factors cannot be dismissed. Digests of the top strand (5′-CTCTCGGTCTC-3′) yielded very similar results to the duplex digestions where all compounds bound to dG less efficiently than cisplatin (data not shown).

Fig. 6. Denaturing polyacrylamide gel electrophoresis (PAGE) (negative exposure) of top strand (TS), bottom strand (BS), and double stranded (ds) DNA subjected to 1 equiv. of either *R*-**1**, cisplatin or no Pt. Lanes: 1: ds DNA, negative control; 2: ds DNA + cisplatin; 3: ds DNA + *R*-**1**; 4: BS, negative control; 5: TS, negative control; 6: BS + *R*-**1**; 7: TS + *R*-**1**; 8: BS + cisplatin; 9: TS + cisplatin.



Thermal denaturation of platinated duplexes showed that cisplatin lowered the melting temperature (T_m) of the duplex, which is consistent with the formation of 1,2-intrastrand adducts (the stability of the duplex is compromised to the extent that a ΔT_m of –20 °C can be observed) (2, 31), while the pyridyloxazoline complexes decreased the T_m only very slightly (ΔT_m = 1–3 °C). This indicates that substantial duplex deformation is not occurring and implies that 1,2 adducts are likely not formed.

Denaturing polyacrylamide gel electrophoresis (PAGE) of the platinated ds and ss DNA again confirmed the generally low reactivity of the compounds. However, DNA platination is revealed by electrophoretic analysis of the reaction mixtures employing the most reactive compound used in this study (*R*-**1**), (Fig. 6). The accumulation of positive charge, which occurs after platination of the sequence, results in lower mobility of the oligonucleotide within the gel matrix.

A significant shifting of the bands in the lanes containing cisplatin was observed. The lanes containing the pyridyloxazoline complex *R*-**1** either showed one band identical to the control (no platinum) or two bands, the top band (corresponding to a platinated ss sequence) appearing very faintly.

It is possible that, if left for longer periods of time, the compounds tested may show differences in binding between enantiomers, as the diastereomeric adducts may be resolved towards the thermodynamic product. It was however found that the ratios after 36 h or 48 h of incubation were not substantially different.

In conclusion, the pyridyloxazoline complexes of Pt and Pd tested in this study likely did not form 1,2-adducts with DNA, but had a higher propensity towards the formation of complexes with dA than cisplatin. It was also found that the nature and absolute configuration of the substituent at *C*4 of the oxazoline ring had no systematic or simple and predictable effect on reactivity. The previous work demonstrating the antineoplastic effects of **6** (19) may imply that monofunctional adducts formed are pharmacologically relevant. In vivo or in vitro biological assays may, in turn, highlight whether the chirality of these complexes is pertinent in regards to their efficacy as anticancer agents.

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