

# Synthesis and Characterization of New Transplatinum Complexes Containing Phosphane Groups – Cytotoxic Studies in Cisplatin-Resistant Cells

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In this paper we report the synthesis and characterization of novel *trans* platinum(ii) complexes of general formula *trans*-[PtCl<sub>2</sub>(L)(PPh<sub>3</sub>)], where L is NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub> (complex **1**), (*R*)-NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub> (complex **2**), (*S*)-NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub> (complex **3**) and NH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub> (complex **4**). The new complexes contain phosphane ligands *trans* to the amine group. X-ray crystal structure determinations of com-

plexes **1** and **3** confirmed their *trans* configurations. The cytotoxic activity of these complexes have been evaluated and compared with the activity of *trans* platinum complexes already viewed as potential drugs.

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## Introduction

Current platinum-based drug research is moving towards the development of new agents capable of overcoming the problem of acquired resistance to cisplatin in order to be active against a wider range of cancer types.<sup>[1,2]</sup> For decades, platinum complexes of *trans* geometry were not regarded as potential drugs because transplatin is an inactive isomer. Over the last ten years, however, transplatinum complexes have been developed as new drugs.<sup>[3,4]</sup> We have recently shown that *trans* amino complexes such as *trans*-[PtCl<sub>2</sub>(dimethylamine)(isopropylamine)] are able to circumvent *cis*-DDP resistance in tumour cells transformed by *ras* oncogenes (Pam212-*ras*), and are also able to kill these cells through apoptosis.<sup>[5]</sup> In addition, we found in the literature a wide range of active platinum complexes containing aminophosphane,<sup>[6,7]</sup> triphos ligand<sup>[8]</sup> and phosphorus derivative ligands.<sup>[9]</sup> Following these results we thought that the study of the effect of the phosphane in the *trans* configuration could help to extend understanding of the cytotoxic activity of *trans* platinum complexes.

In this paper we report the synthesis and characterization of *trans*-[PtCl<sub>2</sub>(L)(PPh<sub>3</sub>)] complexes, where L is NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub> (**1**), (*R*)-NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub> (**2**), (*S*)-NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub> (**3**) and NH<sub>2</sub>CH(CH<sub>3</sub>)<sub>2</sub> (**4**). As far as we know, these complexes are the first Pt complexes with phosphane groups and chiral amines in *trans* configurations. Moreover, they have chloride leaving groups,

which may facilitate comparison with some previously reported *trans* platinum complexes, such as *trans*-[PtCl<sub>2</sub>(dimethylamine)(isopropylamine)].<sup>[4]</sup>

## Results and Discussion

The new *trans*-Pt complexes with phosphane groups are prepared in three different steps. In the first step, K<sub>2</sub>PtCl<sub>4</sub> reacts with PPh<sub>3</sub> to produce the *cis* isomer. In a second step, direct treatment of the *cis* isomer with PtCl<sub>2</sub> affords the dimer complexes [(μ-Cl)PtCl(PPh<sub>3</sub>)]<sub>2</sub>. Further addition of the desired amine splits the chloro-bridged complex to afford the final *trans* complex *trans*-[PtCl<sub>2</sub>L(PPh<sub>3</sub>)]. A scheme of the synthesis pathway is depicted in Figure 1.

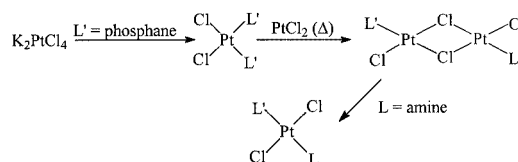


Figure 1. Chart of the synthesis pathway for the complexes **1**, **2**, **3** and **4**

The characterization of these complexes was carried out by IR and <sup>195</sup>Pt, <sup>31</sup>P, <sup>1</sup>H and <sup>13</sup>C NMR. The IR spectra show information in the low region in which ν(Pt–Cl) bands are detected.<sup>[10]</sup> As would be expected for a *trans*-Pt geometry, complexes **1**, **2** and **3** each show only one ν(Pt–Cl) band, at 346 cm<sup>-1</sup>.<sup>[11]</sup> Complex **4** also shows a single ν(Pt–Cl) band, but located at 344 cm<sup>-1</sup>. The ν(Pt–N) band in complexes **1**, **2**, **3** and **4** appears at 430

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$\text{cm}^{-1}$ . These  $\nu(\text{Pt}-\text{N})$  bands are lower than the  $\nu(\text{Pt}-\text{N})$  band found in *trans*-[PtCl<sub>2</sub>(dma)(ipa)],<sup>[5]</sup> probably due to the greater *trans* influence of the phosphorus atom, while the Pt–Cl stretching band is virtually independent of this substitution.<sup>[11]</sup> This effect is also observed in the X-ray structure discussed below.

The *cis*- and *trans*-platinum complexes can readily be distinguished by comparison of the  $^1J(^{31}\text{P}-^{195}\text{Pt})$  values, measured from the  $^{195}\text{Pt}$  satellites on the high-field and low-field doublets.<sup>[12]</sup> We found in the literature that complexes such as *cis*-[PtCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>] show a larger coupling constant than the *trans* isomer between  $^{195}\text{Pt}$  and  $^{31}\text{P}$ , but any decrease in the covalency of the Pt–P bond will also reduce the coupling.<sup>[11]</sup> Complexes **1**, **2**, **3** and **4**, in which the P atom is *trans* to the N atom, each show a value of 3600 Hz, normal for a  $^{31}\text{P}$  atom *trans* to an N donor.<sup>[7,11,13]</sup>

The structures of complexes **1** and **3** were determined by X-ray analysis. Selected bond lengths (Å) and angles (°) for complex **1** and **3** are summarized in Table 1 and Table 2, respectively.

Table 1. Selected bond lengths (Å) and angles (°) for complex **1**

Pt–Cl(1)	2.295(3)	Pt–Cl(2)	2.287(3)
Pt–P	2.243(2)	Pt–N	2.144(8)
P–C(5)	1.818(8)	P–C(11)	1.829(9)
P–C(17)	1.823(11)	N–C(1)	1.448(18)
Cl(1)–Pt–Cl(2)	172.72(9)	Cl(1)–Pt–P	96.26(9)
Cl(1)–Pt–N	87.0(3)	Cl(2)–Pt–P	90.06(9)
Cl(2)–Pt–N	87.0(3)	P–Pt–N	173.5(3)
Pt–P–C(5)	113.3(3)	Pt–P–C(11)	116.5(3)
Pt–P–C(17)	110.2(3)	Pt–N–C(1)	115.7(8)

Table 2. Selected bond lengths (Å) and angles (°) for complex **3**

Pt(1)–Cl(1)	2.287 (3)	Pt(2)–Cl(3)	2.312 (3)
Pt(1)–Cl(2)	2.291 (3)	Pt(2)–Cl(4)	2.294 (3)
Pt(1)–P(1)	2.261 (3)	Pt(2)–P(2)	2.211 (3)
Pt(1)–N(1)	2.096 (11)	Pt(2)–N(2)	2.180 (12)
Cl(1)–Pt(1)–Cl(2)	174.62 (13)	Cl(3)–Pt(2)–Cl(4)	169.99 (14)
N(1)–Pt(1)–Cl(1)	87.8 (3)	N(2)–Pt(2)–Cl(3)	84.5 (4)
N(1)–Pt(1)–Cl(2)	87.3 (3)	N(2)–Pt(2)–Cl(4)	85.5 (4)
N(1)–Pt(1)–P(1)	177.3 (3)	N(2)–Pt(2)–P(2)	174.7 (4)
P(1)–Pt(1)–Cl(1)	94.35 (11)	P(2)–Pt(2)–Cl(3)	99.18 (12)
P(1)–Pt(1)–Cl(2)	90.55 (11)	P(2)–Pt(2)–Cl(4)	90.72 (13)

A view of the complex **1**, *trans*-{PtCl<sub>2</sub>[NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>](PPh<sub>3</sub>)}, is shown in Figure 2. The space group for the complex **1** is centrosymmetric, so this compound is an equimolar mixture of the *S* and *R* enantiomers. The crystal structure of this compound consists of discrete molecules in which PPh<sub>3</sub> and NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub> groups are  $\sigma$  bonded to the platinum atom in *trans* configuration. The platinum atom has a square-planar environment with typical angles for square-planar platinum complexes.<sup>[4,11,14]</sup> The Pt–N distance is similar to that found in complexes with planar amines *trans* to the triethylphosphane ligand, while the Pt–P distance is slightly longer.<sup>[14]</sup> This difference is probably due to the steric effect of the different bulks and the different electronic effects of the

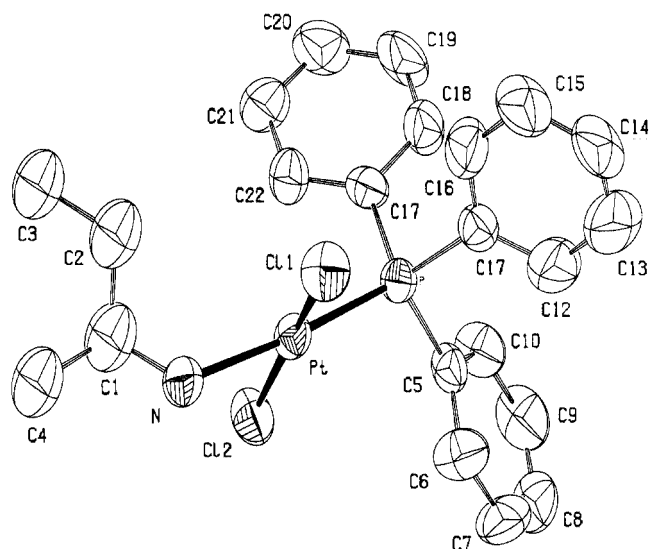


Figure 2. Ortep view of complex **1**

phenyl substituent in the phosphane group.<sup>[15]</sup> No significant differences in the Pt–Cl *trans* distances are found in comparison with *trans*-[PtCl<sub>2</sub>(benzoquinoline)(PEt<sub>3</sub>)]<sup>[14]</sup> and platinum complexes such as *trans*-[PtCl<sub>2</sub>(dma)(ipa)].<sup>[5]</sup> Only the Pt–N distance is significantly longer than those in the complexes mentioned, which is consistent with the greater *trans* influence of phosphane ligands relative to aliphatic amine ones.

The H22(C22)–Pt distance is fairly short [2.97(14) Å] and the distance H2(C2)–Pt is even shorter, in comparison with those found in the references (see Table 3).<sup>[12]</sup> The data reported in the literature also support the idea of a weak interaction between H and Pt atoms when such a distance is from 2.3 to 2.9 Å, which includes the distance here.<sup>[14]</sup>

Table 3. Distances (Pt, X) (Å) for complex **1**

<i>d</i> (Pt, X)	Atom X
3.064(13)	C(1)
3.344(9)	C(17)
3.374(13)	C(2)
3.401(8)	C(5)
3.457(12)	C(22)
3.469(8)	C(11)
2.4443	H(1A)
2.8679	H(2)
2.97(14)	H(22)
3.1334	H(1A)
3.1546	H(1)
3.31(6)	H(6)

A view of the complex **3**, which contains the enantiomerically pure amine moiety (*S*)-NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>, is shown in Figure 3. The crystal structure of this compound consists of two different discrete molecules in which PPh<sub>3</sub> and (*S*)-NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub> groups are  $\sigma$  bonded to platinum in a *trans* configuration. Although both molecules look like very similar, the main difference between them is the C–H...ring  $\pi$  interaction between C(214)–H(214) and

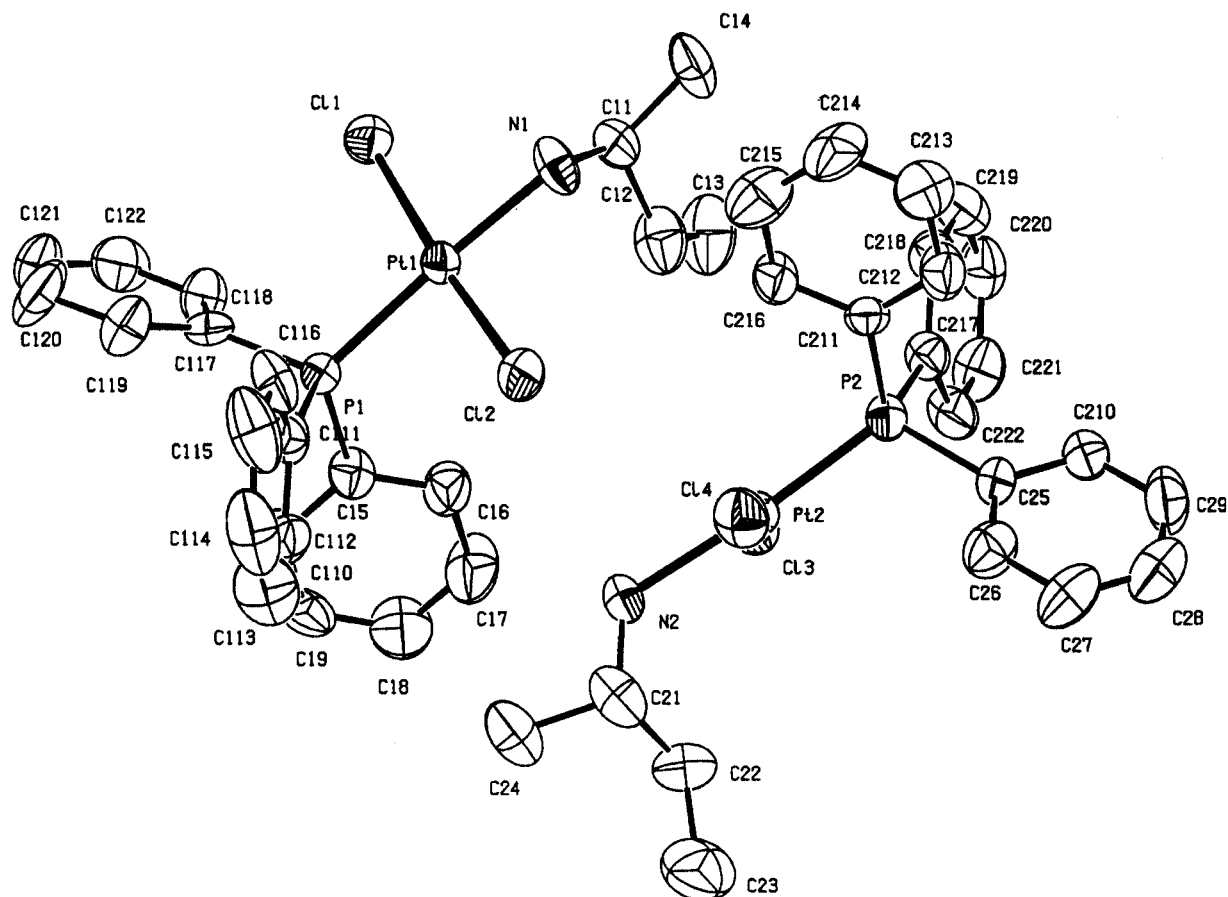


Figure 3. Ortep view of complex 3

the phenyl represented by C(25), C(26), C(27), C(28), C(29) and C(210) (symmetry code for phenyl ring:  $1 + x, y, z$ ). The distance from H(214) to the aromatic ring centre is 2.635 Å. This  $\pi$  interaction produces a short lengthening of the P(2)–C(25) bond [1.831(12) Å, while this distance is 1.811(12) Å in the equivalent bond in the other molecule] and a shortening of the Pt(2)–P(2) bond [2.211(3) Å, while this value is 2.261(3) Å in the other molecule]. This effect is reflected in the Pt(2)–N(2) distance, which is longer than Pt(1)–N(1) or other bond lengths described in the literature for similar complex such as *trans*[PtCl<sub>2</sub>(benzoquinoline)-(PEt<sub>3</sub>)],<sup>[14]</sup> These lengthening and shortening interactions could be interpreted in terms of a high *trans* influence in the molecule of Pt(2). The variation in Pt(1)–Cl(1) or Pt(2)–Cl(3) in both structures is related to the value of the Cl(1 or 3)–Pt(1 or 2)–P bond angles. The widening of this angle increases the lengthening of the Pt–Cl bond, so this variation is attributable to steric effects.

The H16(C16)–Pt(1) distance in complex **3a** is fairly short (2.9758 Å) and is similar to the equivalent distance found in complex **1**.

Complex **1** shows a weak hydrogen bond. The hydrogen bonds are: N–H(1A)⋯Cl(1) (symmetry code for Cl(1) =  $1 - x, -y, 1 - z$ ) with bond lengths N–H = 0.8594 Å, H⋯Cl = 2.6738 Å, and N⋯Cl = 3.496(9) Å. On the other hand, hydrogen bonding was not detected for complex **3**,

although the only difference with complex **1** is the chirality of the amine.

We determined the cytotoxic activities of complexes **1**, **2**, **3** and **4** against the Pam 212-*ras* line, which are cisplatin-resistant cells through overexpression of H-*ras* oncogene. The cytotoxicities of these complexes were evaluated by using the MTT cell survival assay. The IC<sub>50</sub> values were defined as the concentration of compound producing 50% of cell death. As shown in Figure 4, the lowest IC<sub>50</sub> values

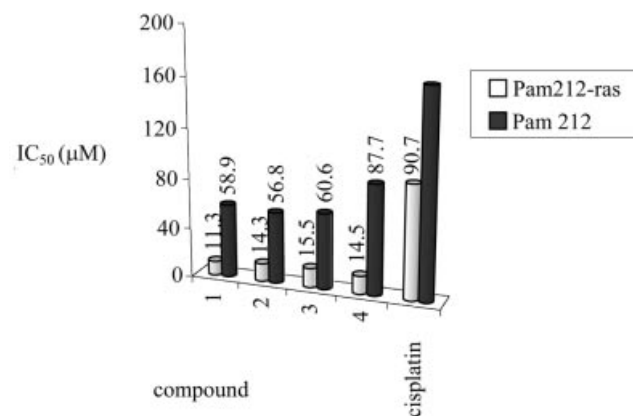


Figure 4. IC<sub>50</sub> values (μM) for cisplatin and *trans* phosphane platinum derivatives (**1**, **2**, **3** and **4**) in Pam 212 and Pam 212-*ras* cell lines

were obtained with the compounds prepared from the racemic and enantiomerically pure *R* ligand and the *S* isomer ( $IC_{50}$  values of 56.84, 58.9 and 60.66  $\mu M$ ). However, the isopropylamine derivative (compound **4**) seems to be less cytotoxic to Pam 212-*ras* cells than compounds **1**, **2** and **3** ( $IC_{50}$  value of 87.73  $\mu M$ ) although this compound is still more active than cisplatin ( $IC_{50}$  value of 164  $\mu M$ ).

In order to compare the molecular and cellular pharmacological properties of these complexes with those of *trans*-[Pt(dma)(ipa)Cl<sub>2</sub>], which is also active against cisplatin-resistant cell lines by induction of apoptotic cell death,<sup>[16]</sup> we analysed the status of the genomic DNA of Pam 212-*ras* cells after treatment with the novel *trans*-Pt complexes with phosphane ligands. It is known that the final biochemical hallmark of apoptosis is the digestion of genomic DNA into DNA fragments corresponding to multiples of the oligonucleosome unit ("DNA laddering").<sup>[17]</sup> In addition, it is also known that certain antitumor drugs may achieve a therapeutic index by selective killing of transformed cells without provoking severe toxicity of wild-type cells. Moreover, it has been proposed that this ability may be related with the induction of tumour cell death through apoptosis at drug concentrations lower than those needed to kill normal cells.<sup>[18]</sup>

Figure 5 shows the electrophoresis in agarose gel of genomic DNA extracted from Pam 212-*ras* cells treated for 24 hours with quantities of complexes **1**, **2**, **3** and **4** corresponding to their  $IC_{50}$  or  $2 \times IC_{50}$  values. The lanes are: 2 and 3 (complex **2**), 4 and 5 (complex **3**), 6 and 7 (complex **1**) and lane 8 (complex **4**). It may be observed in lanes 2 to 8 that the genomic DNA of Pam 212-*ras* cells appears as a continuous smear and not as a ladder of DNA bands, which would be indicative of apoptosis induction.<sup>[17]</sup> This leads us to believe that these new platinum complexes have modes of activity against Pam 212-*ras* cells different to those of the previously published isopropylamine derivatives, which induced DNA laddering in these cisplatin-resistant cell lines.<sup>[16]</sup> It is therefore likely that *trans*-Pt complexes with phosphane ligands may induce cell death through nec-

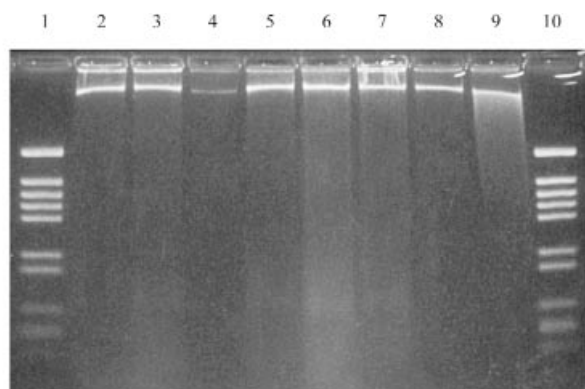


Figure 5. Agarose gel electrophoresis of genomic DNA extracted from Pam 212-*ras* cells treated with quantities corresponding to the  $IC_{50}$  values of: complex **2** (lanes 2 and 3); compound **3** (lanes 4 and 5); racemic complex **1** (lanes 6 and 7); complex **4** (lane 8); and *cis*-DDP, lane 9; – lanes 1 and 10: controls of  $\Phi_{29}$ -HindIII digested DNA

rotic pathways and/or unfinished apoptotic processes.<sup>[19]</sup> As reported previously, treatment of Pam 212-*ras* cells with a quantity of *cis*-DDP corresponding to the  $IC_{50}$  value did not produce a DNA ladder either, in agreement with the fact that cisplatin is only able to kill this resistant cell line through necrosis and at high drug concentrations.<sup>[16]</sup>

On the other hand, DNA-binding experiments of complexes **1**, **2**, **3** and **4** with pBR322 plasmid were carried out to determine the molar ratio of bound Pt per nucleotide ( $r_b$ ) at an input molar ratio of Pt per nucleotide ( $r_i$ ) of 0.1. Total reflection X-ray fluorescence (TXRF) data showed that binding of compounds **1** to **4** to pBR322 is much lower than that of cisplatin (see Table 4). In fact at  $r_i = 0.1$ , the  $r_b$  for cisplatin was 0.09, while the  $r_b$  values for compounds **1**, **2**, **3** and **4** were 0.002, 0.008, 0.009 and 0.002, respectively, so the binding of complexes **1–4** to pBR322 plasmid DNA is 10 to 45 times lower than that of cisplatin.

Table 4. TXRF data for binding of compounds **1** to **4** to pBR322 plasmid DNA.  $r_i$  = input molar ratio of Pt per nucleotide;  $r_b$  = molar ratio of bound Pt per nucleotide

	$r_i$	$r_b$
Cisplatin	0.1	0.09
Complex <b>1</b> (racemic)	0.1	0.002
Complex <b>2</b> (chiral <i>R</i> )	0.1	0.008
Complex <b>3</b> (chiral <i>S</i> )	0.1	0.009
Complex <b>4</b> (isopropylamine)	0.1	0.002

Further experiments with pBR322 showed that complexes **1–4** do not alter the electrophoretic mobility either of the oc (open circular) form or of the ccc (covalently closed circular) form of plasmid DNA (see Figure 6). In contrast, we have previously reported that, at  $r_i = 0.1$ , both *cis*-DDP and *trans*-[Pt(dma)(ipa)Cl<sub>2</sub>] decreases the mobility of the ccc form and increases in the mobility of the oc form in pBR322 DNA.<sup>[5]</sup> Consequently, the electrophoretic results, in combination with the  $r_b$  data obtained by TXRF, suggest that complexes **1–4** do not induce relevant structural changes in the double helix, due to their low levels of binding to DNA.

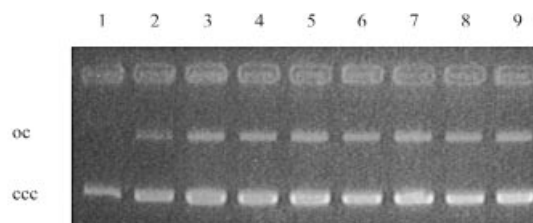


Figure 6. Agarose gel electrophoresis of pBR322 plasmid treated with the *trans* phosphane platinum complexes; lane 1: DNA of control plasmid; lanes 2 to 9, plasmid DNA incubated with complexes **4**, **1**, **2** and **3** at  $r_i = 0.05$  and 0.1 respectively; oc = open circular DNA form; ccc = covalently closed circular DNA form



## Conclusions

Replacement of the two amine groups of transplatin by aliphatic amine and phosphane groups results in circumvention of cisplatin resistance. Moreover, these new *trans*-Pt complexes probably achieve this circumvention in a different way from the *trans*-platinum complexes with mixed aliphatic amines that we have previously reported.<sup>[5,16]</sup> In fact, the *trans*-Pt complexes with phosphane groups do not provoke DNA laddering, which is the final hallmark of the apoptotic process. Overall, the results indicate that, relative to the extent of platinum binding to DNA, these new *trans*-Pt complexes are disproportionately more potent than cisplatin in the induction of cytotoxicity in Pam 212-*ras* cells. As recently suggested for the circumvention of cisplatin resistance by the antitumor drug oxaliplatin, it is likely that the induction of cell death by necrotic pathways and/or unfinished apoptotic programs, possibly enhanced by a contribution of targets other than DNA (such as proteins), may be an important factor in the mechanisms of action of complexes **1–4**. We think that the hydrophobic PPh<sub>3</sub> ligand in complexes **1–4** might also, similarly to the 1,2-diaminocyclohexane ligand in oxaliplatin, direct drug reactivity toward cellular proteins with sulfhydryl groups in hydrophobic pockets that may be poorly reactive with *cis*-DDP, which is polarized.<sup>[20]</sup>

## Experimental Section

Infrared spectra were recorded in Nujol mulls on CsI windows and KBr pellets in the 4000–200 cm<sup>−1</sup> range with a Perkin–Elmer Model 283 spectrophotometer. NMR spectra were recorded on a Bruker AMX 300 (300 MHz) spectrometer in [D<sub>6</sub>]acetone solution. Elemental analysis were performed on a Perkin–Elmer 2400 Series II microanalyzer.

**Structural Determination and Refinement of the Complex 1:** A prismatic crystal (0.1 × 0.1 × 0.2 mm) was selected and mounted on a MAR345 diffractometer with image plate detector. Unit cell parameters were determined from automatic centring of 5289 reflections (3 < θ < 3.1°) and refined by the least-squares method. Intensities were collected with graphite monochromatized Mo-*K*α radiation. 5329 reflections were measured in the range 2.42 ≤ θ ≤ 24.99, 3170 of which were non-equivalent by symmetry [*R*<sub>int</sub>(on *I*) = 0.021]. 2896 reflections were assumed as observed on application of the condition *I* > 2σ(*I*). Lorentz-polarization and absorption corrections were made. The structure was solved by direct methods, with the SHELXS computer program<sup>[21]</sup> and refined by the full-matrix, least-squares method, with the SHELX97 computer program,<sup>[21]</sup> using 3170 reflections (very negative intensities were not assumed). The function minimized was Σ*w* ||*F*<sub>o</sub>|<sup>2</sup> − |*F*<sub>c</sub>|<sup>2</sup>|<sup>2</sup>, where *w* = [σ<sup>2</sup>(*I*) + (0.0723 *P*)<sup>2</sup> + 4.1899*P*]<sup>−1</sup>, and *P* = (|*F*<sub>o</sub>|<sup>2</sup> + 2|*F*<sub>c</sub>|<sup>2</sup>)/3, *f*, *f*' and *f*'' were taken from the International Tables of X-ray Crystallography.<sup>[22]</sup> Ten H atoms were located from a difference synthesis and refined with an overall isotropic temperature factor, and sixteen H atoms were computed and refined with a riding model, with an isotropic temperature factor equal to 1.2 times the equivalent temperature factor of the linked atom. The final *R*(on *F*) factor was 0.042, *wR*(on |*F*<sub>c</sub>|<sup>2</sup>) = 0.107 and goodness of fit = 1.082 for all observed reflections. The number of refined para-

meters was 286. Max. shift/esd = 0.00, Mean shift/esd = 0.00. Max. and min. peaks in final difference synthesis was 0.848 and −0.874 e·Å<sup>−3</sup>, respectively. The details of the data collected and the structural analyses are summarized in Table 5.

Table 5. Crystal data and structure refinement for *trans*-[PtCl<sub>2</sub>(NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>)(PPh<sub>3</sub>)]

Identification code	Complex <b>1</b>
Empirical formula	C <sub>22</sub> H <sub>26</sub> Cl <sub>2</sub> NPt
Molecular mass	601.40
Temperature	293(2) K
Wavelength	0.71069 Å
Crystal system, space group	Triclinic, <i>P</i> $\bar{1}$
Unit cell dimensions	<i>a</i> = 9.7310(10) Å, <i>α</i> = 94.08° <i>b</i> = 11.0710(10) Å <i>β</i> = 106.8260(10)° <i>c</i> = 12.0930(10) Å, <i>γ</i> = 112.17°
Volume	1130.68(18) Å <sup>3</sup>
<i>Z</i>	2
Calculated density	1.766 Mg/m <sup>3</sup>
Absorption coefficient	6.519 mm <sup>−1</sup>
<i>F</i> (000)	584
Crystal size	0.1 × 0.1 × 0.2 mm
Theta range for data collection	2.42 to 24.99 °
Index ranges	0 ≤ <i>h</i> ≤ 11, −13 ≤ <i>k</i> ≤ 12, −14 ≤ <i>l</i> ≤ 13
Reflections collected/unique	5329/3170 [ <i>R</i> <sub>int</sub> = 0.0216]
Refinement method	Full-matrix, least-squares on <i>F</i> <sup>2</sup>
Data/restraints/parameters	3170/18/286
Goodness-of-fit on <i>F</i> <sup>2</sup>	1.084
Final <i>R</i> indices [ <i>I</i> > 2σ( <i>I</i> )]	<i>R</i> 1 = 0.0425, <i>wR</i> 2 = 0.1074
<i>R</i> indices (all data)	<i>R</i> 1 = 0.0463, <i>wR</i> 2 = 0.1117
Largest diff. peak and hole	0.848 and −0.874 e·Å <sup>−3</sup>

**Structural Determination and Refinement of the Complex 3:** A prismatic crystal (0.1 × 0.1 × 0.2 mm) was selected and mounted on a MAR345 diffractometer with a image plate detector. Unit cell parameters were determined from automatic centring of 9961 reflections (3 < θ < 31°) and refined by the least-squares method. Intensities were collected with graphite monochromatized Mo-*K*α radiation. 16358 reflections were measured in the range 2.40 ≤ θ ≤ 25.01, 3747 of which were non-equivalent by symmetry [*R*<sub>int</sub>(on *I*) = 0.038]. 3460 reflections were assumed as observed, by application of the condition *I* > 2σ(*I*). Lorentz-polarization and absorption corrections were made.

The structure was solved by direct methods, with the SHELXS computer program<sup>[21]</sup> and refined by the full-matrix, least-squares method with the SHELX97 computer program,<sup>[21]</sup> using 3747 reflections (very negative intensities were not assumed). The function minimized was Σ*w* ||*F*<sub>o</sub>|<sup>2</sup> − |*F*<sub>c</sub>|<sup>2</sup>|<sup>2</sup>, where *w* = [σ<sup>2</sup>(*I*) + (0.0214 *P*)<sup>2</sup> + 11.378*P*]<sup>−1</sup>, and *P* = (|*F*<sub>o</sub>|<sup>2</sup> + 2|*F*<sub>c</sub>|<sup>2</sup>)/3, *f*, *f*' and *f*'' were taken from the International Tables of X-ray Crystallography.<sup>[22]</sup> The chirality of the structure was defined from the Flack coefficient, which is equal to 0.035(12) for the given results.<sup>[23]</sup> All H atoms were computed and refined with a riding model, with an isotropic temperature factor equal to 1.2 times the equivalent temperature factor of the radiator, which are linked. The final *R*(on *F*) factor was 0.030, *wR*(on |*F*<sub>c</sub>|<sup>2</sup>) = 0.065 and goodness of fit = 1.083 for all observed reflections. The number of refined parameters was 487. Max. shift/esd = 0.00, Mean shift/esd = 0.00. Max. and min. peaks in final difference synthesis was 0.601 and −0.676 e·Å<sup>−3</sup>,

respectively. The details of the data collected and the structural analyses are summarized in Table 6.

Table 6. Crystal data and structure refinement for *trans*-[PtCl<sub>2</sub>(S-NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>)(PPh<sub>3</sub>)]

Identification code	Complex <b>3</b>
Empirical formula	C <sub>22</sub> H <sub>26</sub> Cl <sub>2</sub> NPPt
Molecular mass	601.40
Temperature	293(2) K
Wavelength	0.71069 Å
Crystal system, space group	Monoclinic, <i>P</i> 2 <sub>1</sub>
Unit cell dimensions	<i>a</i> = 9.2170(10) Å <i>α</i> = 90° <i>b</i> = 13.7940(10) Å <i>β</i> = 95.107(10)° <i>c</i> = 17.8690(10) Å <i>γ</i> = 90°
Volume	2262.8(3) Å <sup>3</sup>
<i>Z</i>	4
Calculated density	1.765 Mg/m <sup>3</sup>
Absorption coefficient	6.515 mm <sup>-1</sup>
<i>F</i> (000)	1168
Crystal size	0.1 × 0.1 × 0.2 mm
Theta range for data collection	2.40 to 25.01°
Index ranges	0 ≤ <i>h</i> ≤ 10, 0 ≤ <i>k</i> ≤ 16, −21 ≤ <i>l</i> ≤ 21
Reflections collected/unique	16358/3747 [ <i>R</i> <sub>int</sub> = 0.0385]
Refinement method	Full-matrix, least-squares on <i>F</i> <sup>2</sup>
Data/restraints/parameters	3747/ 1/487
Goodness-of-fit on <i>F</i> <sup>2</sup>	1.083
Final <i>R</i> indices [ <i>I</i> > 2σ( <i>I</i> )]	<i>R</i> <sub>1</sub> = 0.0300, <i>wR</i> <sub>2</sub> = 0.0654
<i>R</i> indices (all data)	<i>R</i> <sub>1</sub> = 0.0344, <i>wR</i> <sub>2</sub> = 0.0676
Absolute structure parameter	0.035 (12)
Largest diff. peak and hole	0.601 and −0.676 e <sup>−</sup> Å <sup>−3</sup>

### Synthesis and Characterization

***cis*-[PtCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>]:** A PPh<sub>3</sub> (1.263 g, 4.82 mmol) solution in ethanol (2.5 mL) was added to K<sub>2</sub>PtCl<sub>4</sub> (1.0 g, 2.4 mmol) solution in water (2.5 mL) and the mixture was stirred in darkness at room temperature for 24 h. The product, a yellow pale precipitate, was collected and washed with water, ethanol and diethyl ether. The solid was then washed with chloroform, and a white precipitate (*cis* isomer) and a yellow solution (*trans* isomer) were obtained. The yellow solution produced a white precipitate on storage in chloroform. This isomerization of yellow *trans*-[PtCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>] to its white *cis* isomer is catalysed by light irradiation.<sup>[24]</sup>

White solid, yield: 98% (1.859 g). *v*(Pt–Cl): 317 cm<sup>−1</sup> and 292 cm<sup>−1</sup>, *v*(Pt–P): 464 cm<sup>−1</sup> and 445 cm<sup>−1</sup>. C<sub>36</sub>H<sub>30</sub>Cl<sub>2</sub>P<sub>2</sub>Pt (790.6): calcd. C 54.60, H 3.82; found C 54.05, H 3.73. <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) (ppm): 7.49 (m, 6 H, *ortho*); 7.32 (m, 3 H, *para*), 7.25 (m, 6 H, *meta*) ppm. <sup>31</sup>P NMR (300 MHz, CDCl<sub>3</sub>, 25 °C) (ppm): 14.81 (*J*<sub>P–Pt</sub> = 3673 Hz)

**[(μ-Cl)PtCl(PPh<sub>3</sub>)<sub>2</sub>]:** This synthesis was based on F. R. Hartley's method<sup>[25]</sup> and also on R. J. Goodfellow's method.<sup>[26]</sup> Goodfellow's method, with some modifications, affords the highest yield.

The complex *cis*-[PtCl<sub>2</sub>(PPh<sub>3</sub>)<sub>2</sub>] (0.38 mmol, 0.300 g) and PtCl<sub>2</sub> (0.4 mmol, 0.106 g) were finely ground with naphthalene in a mortar. The mixture was transferred into a flask. The mixture was then heated at reflux with stirring at 150 °C for 15 min. After cooling, the solid mixture was transferred to a Soxhlet thimble and washed with light petroleum to remove the naphthalene. The crude product was then extracted with dichloromethane to obtain a yellow-orange solution, which was taken to dryness under reduced pressure. The

solid was partially dissolved in chloroform to obtain an orange precipitate and a yellow solution. When the yellow solution was warmed in chloroform it turned orange, with precipitation of the orange isomer (*trans* isomer).

Deep orange solid, yield: 98% (0.394 g). *v*(Pt–Cl<sub>μ</sub>): 321 and 260 cm<sup>−1</sup>. *v*(Pt–Cl<sub>T</sub>): 355 cm<sup>−1</sup>. C<sub>36</sub>H<sub>30</sub>Cl<sub>4</sub>P<sub>2</sub>Pt<sub>2</sub> (1056.6): calcd. C 40.93, H 2.86, Cl 13.47; found C 40.96, H 2.79, Cl 13.36. <sup>1</sup>H NMR (300 MHz [D<sub>3</sub>]acetonitrile, 25 °C) (ppm): 7.71 (m, 6H), 7.67 (m, 6H), 7.46 (m, 3H). <sup>31</sup>P NMR (300 MHz [D<sub>3</sub>]acetonitrile, 25 °C) (ppm): 3.60 (*J*<sub>P–Pt</sub> = 4100.26 Hz)

***trans*-[PtCl<sub>2</sub>L(PPh<sub>3</sub>)]:** Complexes **1**, **2**, **3** and **4** were obtained Chatt's method, with variations.<sup>[27]</sup> [(μ-Cl)PtCl(PPh<sub>3</sub>)<sub>2</sub>] (0.142 mmol, 150 mg) was partially dissolved in acetone, together with the stoichiometry quantity of amine ligand (L), at room temperature. A solution was immediately formed, and this was generally accompanied by a lightening in colour. The acetone was removed under reduced pressure. Diethyl ether was added and the *trans*-[PtCl<sub>2</sub>L(PPh<sub>3</sub>)] was dissolved. The solution was filtered to remove the insoluble impurities. The *trans*-[PtCl<sub>2</sub>L(PPh<sub>3</sub>)] solution in diethyl ether produced crystals or a yellow precipitate on storage.

Yellow compounds, yields: 72% (complex **1**, 0.123 g), 61% (complex **2**, 0.104 g) and 50% (complex **3**, 0.085 g). *v*(Pt–Cl): 346 cm<sup>−1</sup>, *v*(Pt–P): 449 cm<sup>−1</sup>, *v*(Pt–N): 430 cm<sup>−1</sup>. C<sub>22</sub>H<sub>26</sub>Cl<sub>2</sub>NPPt (601.4): calcd. C 43.0, H 4.35, N 2.33; found C 43.76, H 4.22, N 2.24. <sup>1</sup>H NMR (300 MHz [D<sub>6</sub>]acetone, 25 °C) (ppm): 7.70 (m, 6 H, *ortho*) 7.44 (m, 9 H, *meta* and *para*), 3.9 (b.s. 2 H, NH<sub>2</sub>), 3.36 (sp, 1 H), 1.93 (m, 1 H), 1.60 (m, [NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>], 1 H, [NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>]), 1.40 (d, 3 H, [NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>]), 1.00 (t, 3 H, [NH<sub>2</sub>CH(CH<sub>3</sub>)CH<sub>2</sub>CH<sub>3</sub>]) ppm. <sup>31</sup>P NMR (300 MHz, [D<sub>6</sub>]acetone, 25 °C) (ppm): 11.96 (*J*<sub>P–Pt</sub> = 3600 Hz) ppm. <sup>14</sup>C NMR (300 MHz, [D<sub>6</sub>]acetone, 25 °C) (ppm): 135.56 (*C*<sub>meta</sub>), 131.47 (*C*<sub>para</sub>), 130.37 (*C*<sub>ipso</sub>), 128.64 (*C*<sub>ortho</sub>), 52.33 (C–NH<sub>2</sub>), 31.50 (CH<sub>2</sub>), 21.32 [CH(CH<sub>3</sub>)], 10.44 [CH<sub>2</sub>(CH<sub>3</sub>)]. [*α*]<sub>D</sub><sup>20</sup> = −48.4 (complex **2**), [*α*]<sub>D</sub><sup>20</sup> = +48.4 (complex **3**)

***trans*-[PtCl<sub>2</sub>(ipa)(PPh<sub>3</sub>)]:** Yellowish compound. complex **4**. Yield: 90% (0.150 g). *v*(Pt–Cl): 344 cm<sup>−1</sup>, *v*(Pt–P): 453 cm<sup>−1</sup> and 428 cm<sup>−1</sup>, *v*(Pt–N): 430 cm<sup>−1</sup>. C<sub>21</sub>H<sub>24</sub>Cl<sub>2</sub>NPPt (587.4): calcd. C 42.94, H 4.12, N 2.38; found C 42.80, H 4.08, N 2.43. <sup>1</sup>H NMR (300 MHz [D<sub>6</sub>]acetone, 25 °C) (ppm): 7.70 (m, 6 H, *C*<sub>ortho</sub>), 7.44 (m, 9 H, *meta* and *para*), 3.97 (b.s., 2 H, NH<sub>2</sub>), 3.59 (hp, 1 H, CH), 1.39 (d, 6 H, CH<sub>3</sub>, *J*<sub>H</sub> = 7 Hz). <sup>31</sup>P NMR (300 MHz [D<sub>6</sub>]acetone, 25 °C) (ppm): 11.92 (*J*<sub>P–Pt</sub> = 3600 Hz). <sup>14</sup>C NMR (300 MHz [D<sub>6</sub>]acetone, 25 °C) (ppm): 24.5 (CH<sub>3</sub>), 47.1 (CH), 128.6 (*C*<sub>ortho</sub>), 130.4 (*C*<sub>ipso</sub>), 131.4 (*C*<sub>para</sub>), 135.5 (*C*<sub>meta</sub>) ppm. <sup>195</sup>Pt NMR (300 MHz [D<sub>6</sub>]acetone, 25 °C) (ppm): −3792.7 (*J*<sub>P–Pt</sub> = 3608 Hz).

### Biochemical Probes

**Biologicals and Drugs:** 100 mm culture and microwell plates were obtained from NUNC LON (Roskilde, Denmark). MTT was purchased from Sigma Co., FCS was supplied by GIBCO-BRL, and ethanol was obtained from Merck. *cis*-Diamminedichloroplatinum(II), cisplatin or *cis*-DDP, was synthesized from K<sub>2</sub>PtCl<sub>4</sub> supplied by Johnson Matthey. *cis*-DDP was dissolved in NaClO<sub>4</sub> (10 mM). The *trans* platinum phosphane complexes were dissolved in acetone as 1 mg/ml solutions. These solutions were freshly prepared before use.

**Cell Lines and Culture Conditions:** Pam 212, a normal cell line of murine keratinocytes, and Pam 212-*ras*, a transformed murine keratinocytes cell line overexpressing the H-*ras* oncogene, were cultured in DMEM (Dulbecco modified Eagle's medium), supplemented with FCS (foetal calf serum, 10%), glutamine (2 mM), peni-

cillin (100 units/mL) and streptomycin (100 µg/mL) at 37 °C in an atmosphere of 95% air and 5% CO<sub>2</sub>. All cultures were passaged twice weekly, showing a doubling time between 16–24 hours.

**Cytotoxicity Assays:** Cell survival was evaluated by use of a system based on the tetrazolium compound MTT, which is reduced by living cells to yield a soluble formazan product that can be detected colorimetrically.<sup>[28]</sup> Cells were plated in 96-well sterile plates at a density of 10<sup>4</sup> cells/well in 100 µL of medium and were incubated for 3–4 h. The compounds were added to final concentrations from 0 to 200 µM in a volume of 100 µL/well. Twenty-four hours later, 50 µL of a MTT solution (1/5 in culture medium) freshly diluted to a concentration of 1 mg/mL were pipetted into each well, and the plate was incubated for 5 h at 37 °C in a humidified 5% CO<sub>2</sub> atmosphere. After the periods specified, the cell viability was evaluated by measurement of the absorbance at 520 nm, by use of a Whittaker Microplate Reader 2001. IC<sub>50</sub> values (compound concentration that produces 50% of cell growth inhibition) were calculated from curves constructed by plotting (%) cell survival versus drug concentration. The therapeutic index (T.I.) of every compound was calculated as the ratio of the IC<sub>50</sub> in normal cells versus the IC<sub>50</sub> in transformed cells. In control experiments it was observed that 10% acetone did not have any effect on cell growth. This was the highest percentage of acetone present in the cell cultures after addition of 200 µM of the compounds. All experiments were made in quadruplicate.

**DNA Fragmentation Assay:** Pam212-ras cells (5 × 10<sup>5</sup> cells/mL) were plated in 100 mm sterile dishes. The cells were treated with the compounds for 24 h to a final concentration of the IC<sub>50</sub> under the conditions described above.<sup>[28]</sup> The fraction of detached cells was collected by centrifugation of the culture media and washed twice with PBS. The cell pellet was disrupted with 700 µL of lysis buffer (150 mM Tris, tris(hydroxymethyl)aminomethane, pH 8.0; 100 mM NaCl; 100 mM EDTA, ethylenediamine tetracetate). The fraction of non-detached cells was also washed twice with BPS and lysed by addition of 700 µL of lysis buffer to the plate. Both cell fractions were joined and the whole cell lysate was treated with proteinase K (500 µg/mL) for 2 h at 55 °C. Afterwards, samples were exposed to RNase A (50 µg/mL) for 16 h at 37 °C. The DNA was first extracted with phenol, followed by phenol/chloroform/isoamyl alcohol (25:24:1) and a chloroform/isoamyl alcohol (24:1) phase. Subsequently, the DNA was precipitated overnight at –20 °C in 2.5 volumes of cold 100% ethanol/150 mM potassium acetate. After centrifugation at 12,000 rpm for 15 min to recover the precipitated DNA, the supernatant was discarded and the pellet was washed with 70% ethanol. Samples were dried on a SAVANT Speed Vac Concentrator and then resuspended in distilled water. The DNA concentration was calculated by determining the OD<sub>260</sub>. Electrophoresis of DNA (10 µg/well) was performed for 16 h at 75 V in 1.8% agarose gel with TAE (40 mM Tris-acetate, 1 mM EDTA, pH 8.0) as running buffer. The bands were viewed by ethidium bromide staining for 16 h and UV transillumination. DNA bands were analysed by laser densitometry with a Molecular Dynamics densitometer.

**Determination of Platinum Binding to pBR322 Plasmid DNA by TXRF:** pBR322 DNA aliquots (50 µg/mL) were incubated with the platinum compounds in a buffer solution containing 50 mM NaCl, 10 mM Tris.HCl (pH 7.4) and EDTA (0.1 mM) at *r*<sub>i</sub> = 0.1 (input molar ratio of Pt per nucleotide). Incubations were performed in the dark at 37 °C. After 24 hours of incubation, the DNA of the samples was precipitated with 0.1 volumes of sodium acetate (pH 4.7) and 2.5 volumes of absolute ethanol to eliminate unchanged drug. The DNA content in each sample was measured by UV spec-

trophotometry at 260 nm in a Shimadzu UV-240 spectrophotometer, and platinum bound to DNA was determined by total reflection X-ray fluorescence (TXRF). Experiments were carried out in triplicate.

**TXRF Measurements:** The analysis by TXRF was performed with a Seifert Extra-II spectrometer (Seifert, Ahrensburg, Germany). TXRF determinations were carried out by a previously reported procedure.<sup>[29]</sup> Briefly, a 100 µL sample of the DNA precipitated from the solutions of pBR322 incubated with the platinum complexes was introduced into a 2 mL test tube. This solution was standardised with 100 ng/mL vanadium [Merck (Darmstadt, Germany) ICP vanadium standard solution]. Afterwards, the sample was introduced into a high-purity nitrogen flow concentrator at a temperature of 70 °C until the volume had been reduced five times. An aliquot of 5 µL was then taken, deposited on a clean quartz reflector and dried on a ceramic plate at a temperature of 50 °C. The entire process was carried out in a laminate flow chamber (Model A-100). The samples were analysed by following the X-ray molybdenum line under working conditions of 50 kV and 20 mA with a live-time of 1000 s and a dead time of 35%. Spectra were recorded between 0 and 20 keV. The following 15 elements were analysed simultaneously: P, S, K, Ca, V, Fe, Cu, Zn, As, Br, Rb, Sr, Ni, Mn and Pt, in order to obtain a correct deconvolution of profiles associated with the general spectrum. The Pt line was used for Pt quantification. The analytical sensitivity of the TXRF measurements was 0.3 to 22.4 ng Pt in a solution volume of 100 µL, with repeatability between 2 and 8% (the number of determinations was *n* = 3).

**Gel Electrophoresis of Drug:pBR322 Complexes:** pBR322 DNA aliquots (50 µg/mL) were incubated with the platinum compounds in a buffer solution containing 50 mM NaCl, 10 mM Tris.HCl (pH 7.4) and EDTA (0.1 mM) at several *r*<sub>i</sub> values (0.5 and 0.1). Incubations were performed in the dark at 37 °C. 20 µL aliquots of the drug:DNA complexes containing 1 µg of DNA were subjected to 1.5% agarose gel electrophoresis for 16 h at 25 V in TAE buffer (Tris-acetate 40 mM, EDTA 2 mM pH 8.0). The DNA was stained in the same buffer containing ethidium bromide (0.5 µg/mL). The gels were photographed with a MP-4 Polaroid camera with a 665 Polaroid film and an orange filter.

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- [1] B. Lippert, *Cisplatin*, Wiley-VCH, Weinheim, **1999**.
- [2] L. Kelland, N. Farrell, *Platinum-Based Drugs in Cancer Therapy*, Humana Press, **2000**.
- [3] M. V. Beusichem, N. Farrell, *Inorg. Chem.* **1992**, *31*, 934–939.
- [4] M. Coluccia, A. Nassi, F. Loseto, A. Boccarelli, M. A. Marigliò, D. Giordano, F. P. Intini, P. Caputo, G. Natile, *J. Med. Chem.* **1993**, *36*, 510–512.
- [5] J. M. Pérez, E. I. Montero, A. M. Gonzalez, X. Solans, M. Font-Bardia, M. A. Fuertes, C. Alonso, C. Navarro-Ranninger, *J. Med. Chem.* **2000**, *43*, 2411–2418.
- [6] K. Neplechová, J. Kasparová, O. Vraná, O. Nováková, A. Habtemariam, B. Watchman, P. J. Sadler, V. Brabec, *Mol. Pharm.* **1999**, *56*, 20–30.
- [7] N. Margiotta, A. Habtemariam, P. J. Sadler, *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 1185–1187.
- [8] P. Sevillano, A. Habtemariam, S. Parsons, A. Castiñeiras, M. E. García, P. J. Sadler, *J. Chem. Soc., Dalton Trans.* **1999**, *16*, 2861–2870.

- [9] W. Henderson, S. R. Alley, *Inorg. Chim. Acta* **2001**, 322, 106–112.
- [10] *Comprehensive Coordination Chemistry*, V5. Late Transition Elements, Eds.: G. Wilkinson, R. D. Gillard, J. A. McCleverty, Pergamon Press, **1987**.
- [11] T. G. Appleton, H. C. Clark, L. E. Manzer, *Coord. Chem. Rev.* **1973**, 10, 335–422.
- [12] P. S. Pregosin. In *Phosphorus-31 NMR Spectroscopy in Stereochemical analysis*. Eds.: J. G. Verkade, L. D. Louis, VCH Publishers, New York, pp. 465, **1987**.
- [13] A. D. Burrows, M. F. Mahon, M. T. Palmer, *J. Chem. Soc., Dalton Trans.* **2000**, 20, 3615–3619.
- [14] A. Albinati, P. S. Pregosin, F. Wombacher, *Inorg. Chem.* **1990**, 29, 1812–1817.
- [15] M. F. C. Guedes da Silva, E. M. P. R. P. Branco, Y. Wang, J. J. R. Fraústo da Silva, A. J. L. Pombeiro, R. Bertani, R. A. Michelin, M. Mozón, F. Benetollo, G. Bombieri, *J. Organomet. Chem.* **1995**, 490, 89–99.
- [16] E. I. Montero, S. Díaz, A. M. González-Vadillo, J. M. Pérez, C. Alonso, C. Navarro-Ranninger, *J. Med. Chem.* **1999**, 42, 4264–4268.
- [17] A. G. Quiroga, J. M. Pérez, E. I. Montero, D. W. West, C. Alonso, C. Navarro-Ranninger, *J. Inorg. Biochem.* **1999**, 75, 293–301.
- [18] S. W. Lowe, H. E. Ruley, T. Jacks, D. E. Housman, *Cell*. **1993**, 74, 957–967.
- [19] V. M. González, M. A. Fuertes, C. Alonso, J. M. Pérez, *Mol. Pharmacol.* **2001**, 59, 657–663.
- [20] E. Raymond, S. Faivre, S. Chaney, J. Woynarowski, E. Cvitkovic, *Mol. Cancer Ther.* **2002**, 1, 227–235.
- [21] Sheldrick, G. M., A computer program for determination of crystal structure. University of Göttingen, Germany. **1997**
- [22] International Tables of X-ray Crystallography, Ed. Kynoch press, Vol. IV, pp 99–100 and 149, **1974**.
- [23] H. D. Flack, *Acta Crystallogr., Sect. A* **1983**, 39, 876–881.
- [24] R. D. Gillard, M. F. Pilbrow, *J. Chem. Soc., Dalton Trans.* **1974**, 21, 2320–2325.
- [25] F. R. Hartley, G. W. Searle, *Inorg. Chem.* **1973**, 12, 1949–1951.
- [26] R. J. Goodfellow, L. M. Venanzi, *J. Chem. Soc.* **1965**, 7533–7534.
- [27] J. Chatt, L. M. Venanzi, *J. Chem. Soc.* **1955**, 3858–3864.
- [28] M. C. Alley, D. A. Scudiero, A. Monks, M. L. Hursey, M. J. Czerwinski, D. L. Fine, B. J. Abbott, J. G. Mayo, R. H. Shoemaker, M. R. Boyd, *Cancer Res.* **1998**, 48, 589–601.
- [29] R. Fernández-Ruiz, J. D. Tornero, V. M. González, C. Alonso, *Analyst* **1999**, 124, 583–585.

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