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Original article

Synthesis, characterisation and *in vitro* cytotoxicity studies of a series of chiral platinum(II) complexes based on the 2-aminomethylpyrrolidine ligand: X-ray crystal structure of [$PtCl_2$(*R*-dimepyrr)] (*R*-dimepyrr = *N*-dimethyl-2(*R*)-aminomethylpyrrolidine)

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ABSTRACT

A series of platinum(II) complexes were synthesised based on the enantiomerically pure amino acid proline. Novel synthetic pathways were developed, adapted from standard peptide chemistry, to produce the 2-aminomethylpyrrolidine (pyrr) ligand and its derivatives with differing arrangements of methyl substituents at the exocyclic amine sites. The crystal structure of [$PtCl_2$(*R*-dimepyrr)] (*R*-dimepyrr = *N*,*N*-dimethyl-2(*R*)-aminomethylpyrrolidine) is reported and the five-membered ligand ring has been shown to be in an envelope conformation. Cytotoxicity studies were carried out on the ovarian cancer A2780 tumour cell line and its cisplatin-resistant variant, A2780cisR. Remarkably good activity was seen for several of the drugs when compared to cisplatin despite the addition of substantial steric bulk to the amine groups, and there was a lack of cross-resistance with cisplatin seen for some compounds.

1. Introduction

Platinum(II) anti-cancer agents are now arguably the most widely used chemotherapeutic agents in the world, and with their increasing use in combination therapies through emerging synergies with molecularly targeted agents, their place in the clinic is only set to strengthen [1–4]. However, one of the major problems preventing a more wide-spread use of platinum agents in anti-cancer therapy has been their severe side-effects, leading to a continuing effort on the part of researchers world-wide to discover new platinum analogues. Thousands of platinum(II) compounds have been synthesised and screened in the last 30 years since the discovery of cisplatin [5,6] and more than 28 have been clinically trialled, but few have reached the clinic [2,6].

Chiral diamine ligands enjoy a long history in the research towards cytotoxically active platinum(II) drugs [5,7]. The discovery by Burchenal et al. of the lack of cross-resistance of platinum(II) complexes containing the cyclohexane-1,2-diamine (chxn) ligand is evident from the high activity in cisplatin-resistant L1210 leukemia lines [8–10] and heralded the start of investigation into chiral diamines. The work done by Kidani et al. into the *trans*-1,2-chxn complexes [11–20] has culminated in the use of the drug oxaliplatin for the treatment of colorectal cancers [20]. Oxaliplatin is now one of the top ten selling drugs with world-wide sales in the 2005–2006 financial year of US$1.6 billion.

The synthesis and anti-tumour activity of the dichloroplatinum(II) complex of the 2(*S*)-aminomethylpyrrolidine (pyrr) ligand was first reported by Brunner et al. in 1986 [21] and more recently, Morikawa et al. described the synthesis of a series of ligands based on the 2-aminomethylpyrrolidine ligand [22–24]. Platinum(II) complexes of the 2-aminomethylpyrrolidine ligand were synthesised as both the dichloro and cyclobutane-1,1-dicarboxylate (CBDC) derivatives, with the *R*-enantiomer of the latter going on to Phase III clinical trials as the drug DWA-2114R and the *S*-enantiomer being rejected early on for reasons of toxicity [24–26]. Since the differences between the activity and toxicity of chiral complexes arises from interaction with chiral targets, they can provide valuable insights into the factors that control such interactions.

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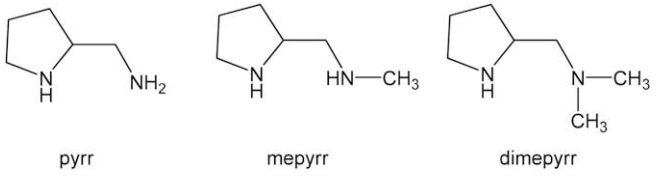
We report here the synthesis of a series of platinum(II) complexes of enantiomerically pure ligands based on the amino acid proline (Scheme 1). These chiral pyrrolidine ligands are methylated derivatives of the parent pyrr ligand that has only hydrogen atoms at three different substituent sites, two at an exocyclic nitrogen and one at an endocyclic nitrogen atom. These ligands have a five-membered ring containing a chiral (stereogenic) carbon atom, from which extends a methylene bridge to the exocyclic nitrogen atom. Both the exocyclic and endocyclic nitrogen atoms can become chiral upon coordination to the platinum(II) atom. The crystal structure of the platinum(II) complex containing one of these ligands is also reported here.

2. Experimental section

2.1. Instrumentation

Melting points were measured on a Gallenkamp digital melting point apparatus and are reported uncorrected. ^{1}H NMR spectroscopy of the ligands was carried out on a Bruker Avance 300 MHz spectrometer. ^{1}H and ^{13}C NMR spectroscopy of the platinum(II) complexes was carried out on a Bruker AMX 400 MHz spectrometer. All spectra were recorded using commercially available solvents (Merck) of 99.6% isotopic purity or better. The optical rotations of the ligands were measured at 589 nm using an Optical Activity POLAAR 2001 Dual Wavelength Polarimeter with a 1 dm cell at ambient temperature. Mass spectral analysis of the ligands was carried out on a Hewlett Packard 5989A Mass Spectrometer Engine and are presented as a chemical ionisation spectrum. The sample was introduced through the particle beam interface to the chemical ionisation source. The reagent gas used was methane, with a source temperature of 220 °C. The spectra were recorded by Dr. X.-M. Song. The circular dichroism measurements for the platinum(II) complexes were performed on a JASCO J-710 spectropolarimeter equipped with J-700 software for Windows. The spectra were recorded using approximately 10^{-3} M *N*,*N*-dimethylformamide solutions between 260 and 500 nm, with a sensitivity setting of 200 mdeg and a spectral band width of 1.0 nm. The instrument was calibrated prior to use with camphor sulfonate ($\lambda_{max} = 291.5$ nm, $\Theta = 188.7$ mdeg). Samples were placed in a cylindrical quartz cell (width 1 cm). Diffuse reflectance infrared Fourier transform spectra (DRIFTS) of the platinum(II) complexes were obtained on a BIO-RAD FTS-40 spectrophotometer with Win-IR Windows software. Potassium bromide was used for the background and matrix over the range of 400–4000 cm^{-1}. Solvents used were of laboratory grade and were used without further purification; THF was dried and stored over sodium wire. Due to the suspected carcinogenic nature of benzene, care was taken during its use, including use in a fumehood at all times. Proline was purchased from Aldrich in its pure *S*- and *R*-enantiomeric forms and was used as obtained. Microanalysis was carried out at the Research School of Chemistry, Australian National University, Canberra, for analysis of carbon, hydrogen and nitrogen. The absorbance of treated A2780 cancer cells was read in a SpectraCount, Packard microplate reader.



Scheme 1.

2.2. Preparation of N-carbobenzoxy-proline-p-nitrophenol ester

The synthesis of the intermediate common to 2-aminomethyl pyrrolidine, *N*-methyl-2-aminomethylpyrrolidine, and *N*-dimethyl-2-aminomethylpyrrolidine was carried out as follows. Following the method of Berger et al. [27], benzyl chloroformate (17.39 g, 0.102 mol) and sodium hydroxide (2 M, 65 cm^3, Fluka) were added simultaneously over 15 min with vigorous stirring to an ice-cooled solution of proline (10.0 g, 0.087 mol) in sodium hydroxide (2 M, 44 cm^3). The solution was then extracted with diethyl ether (2×100 cm^3) and the aqueous layer retained and acidified to approximately pH 4 with 6 M HCl. The resulting solution was extracted with ethyl acetate (2×50 cm^3) and the organic layer washed with water, dried over anhydrous sodium sulfate and filtered, yielding *N*-carbobenzoxy-proline. This solution was used in the following step.

Following the method of Bodanszky and du Vigneaud [28], *p*-nitrophenol in 20% excess (14.52 g, 0.104 mol, Fluka) was added to a 0.2–0.5 M solution of *N*-carbobenzoxy-proline in ethyl acetate and the solution cooled to 0 °C. Dicyclohexylcarbodiimide (DCC) (17.95 g, 0.087 mol) was added and the solution stirred for half an hour; it was then allowed to equilibrate to room temperature and stirred for a further hour.

The solid dicyclohexylcarbodiimide urea that formed during the reaction was removed by suction filtration and washed with ethyl acetate. The combined washings and filtrate were taken to dryness by rotary evaporation. The resulting oil was redissolved in chloroform to precipitate further DCC urea that was again removed by suction filtration. The solution was cooled and the product precipitated by the slow addition of ethanol. The solid product was removed under suction and the process repeated to produce further crops. The crude product was further purified by recrystallisation from hot ethanol to obtain pure *N*-carbobenzoxy-proline-*p*-nitrophenol ester as fine white crystals. Yield: 17.68 g, 55% (*S*-enantiomer), 22.82 g, 71% (*R*-enantiomer). ^{1}H NMR: solvent DMSO-d_6, ppm; 1.98 (q, 2H); 2.36 (m, 2H); 3.52 (m, 2H); 4.62 (m, 1H); 5.14 (q, 2H); 7.28 (d, 5H); 7.36 (d, 5H); 8.30 (q, 2H). m.p.: (93 ± 1) °C.

2.3. Preparation of 2-aminomethylpyrrolidine (pyrr)

N-Carbobenzoxy-proline-*p*-nitrophenol ester (27.62 g, 0.075 mol) was dissolved in methanol (~800 cm^3) and cooled to 0 °C. The solution was saturated with dry ammonia gas, triethylamine (5 cm^3) was added, and the mixture left to stand at room temperature for 2 h. The solution was again cooled to 0 °C, saturated with dry ammonia gas and left to stand at room temperature for 2 h. This process was repeated a further four times and the solution was then refluxed for 2.5 h. The solvent was removed by rotary evaporation to give the product as a white solid contaminated with some yellow oil. The oil was removed by redissolving the residue in chloroform and mixing with neutral activated alumina (50 g) and filtering under suction through a pad of neutral activated alumina. The solvent was again removed by rotary evaporation. This process was repeated, removing as much of the residual *p*-nitrophenol as possible; the remainder was then removed using a short column of neutral activated alumina and eluting with chloroform. Yield: 16.76 g, 90% (*S*-enantiomer), 15.46 g, 83% (*R*-enantiomer).

N-Carbobenzoxy-proline-amide (16.76 g, 0.068 mol) was dissolved in methanol (200 cm^3), palladium on carbon catalyst was added and nitrogen was bubbled through the mixture for a few minutes to displace any oxygen. Hydrogen gas was gently bubbled through the solution until evolution of carbon dioxide ceased, as determined by passing the exit gas through a saturated calcium

hydroxide solution and monitoring the formation of insoluble calcium carbonate. The reaction was complete within 7 h. The Pd/C catalyst was removed by filtration under suction through a pad of celite and the solvent removed by rotary evaporation to leave the product as a white solid. The yield for this step was essentially quantitative.

Following the method of Fenton et al. [29], proline-amide (8.52 g, 0.075 mol) was dissolved in dry tetrahydrofuran (THF, 250 cm^3) and the mixture cooled to 0 °C. Freshly crushed lithium aluminium hydride (14.16 g, 0.375 mol) was slowly added and the mixture was left to stir at 0 °C for 1 h then allowed to slowly come to room temperature. After refluxing for 48 h the reaction was quenched by adding a solution of water (24 g) in THF (200 cm^3), dropwise at first, until all reaction ceased. The resultant solid was filtered off under suction and the filter cake washed with hot THF (3 × 250 cm^3). The combined filtrate and washings were reduced to leave the crude product as a pale brown oil. This oil was further purified by vacuum distillation; the fraction at 75–95 °C (∼0.4 mm Hg) was collected, giving the pure 2-aminomethylpyrrolidine as a clear oil. Yield: 1.51 g, 20% (*S*-enantiomer), 2.27 g, 30% (*R*-enantiomer); *m*/*z* 100, calc. 100. ^{1}H NMR: solvent $CDCl_3$, ppm; 0.97 (d, 1H); 1.10 (d, 2H); 1.31 (m, 1H); 1.54 (s, H_2O); 1.76 (m, 1H); 2.65 (m, 1H); 2.91 (m, 2H); 3.05 (m, 1H). $[\alpha]_D$ −5.8° {−1.0°} (*c* = 1, methanol).

2.4. Preparation of N-methyl-2-aminomethylpyrrolidine (mepyrr)

Following the method of Fenton [30], *N*-carbobenzoxy-proline-*p*-nitrophenol ester (20.0 g, 0.054 mol) was dissolved in warm absolute ethanol (400 cm^3). Methylamine (alcohol 33%, 40 cm^3) and then triethylamine (5 cm^3) were added to the solution. The mixture was stirred at room temperature for 12 h and the solvent removed by rotary evaporation to leave a yellow oil contaminated with a bright orange solid. The oil was redissolved in chloroform and filtered to remove as much as possible of the solid contaminant. The filtrate was then taken to dryness and the displaced *p*-nitrophenol removed by running the solution through three columns of neutral activated alumina, eluting with chloroform, giving an essentially quantitative yield.

The benzyl chloroformate protecting group was removed as described above. The resulting proline-methyl-amide (7.63 g, 0.062 mol) in dry THF (250 cm^3) was reduced using freshly crushed $LiAlH_4$ (11.75 g, 0.395 mol) to give crude *N*-methyl-2-aminomethylpyrrolidine as a pale yellow oil. This was purified by Kugelrohr vacuum distillation with the fraction at 95–120 °C (∼0.4 mm Hg) collected to give the pure ligand as a clear oil. Yield: 2.02 g, 33% (*S*-enantiomer), 1.78 g, 29% (*R*-enantiomer); *m*/*z* 114, calc. 114. NMR: solvent $CDCl_3$, ppm; 1.32 (m, 1H); 1.74 (m, 3H); 1.92 (s, NH); 2.40 (s, 3H); 2.51 (t, 2H); 2.88 (m, 2H); 3.18 (m, 1H). $[\alpha]_D$ + 16.9° {−6.1°} (*c* = 1, methanol).

2.5. Preparation of N,N-dimethyl-2-aminomethylpyrrolidine (dimepyrr)

N-Carbobenzoxy-proline-*p*-nitrophenol ester (20.0 g, 0.054 mol) was dissolved in warm absolute ethanol (400 cm^3). Dimethylamine hydrochloride (5.30 g, 0.065 mol) was added, followed by triethylamine (15 cm^3, 0.108 mol). The mixture was stoppered and left to stir for 12 h, the solution was filtered, and the solvent removed by rotary evaporation. This afforded the product as a bright yellow solid that was redissolved in chloroform and eluted through two columns of neutral activated alumina to remove the displaced *p*-nitrophenol. The fractions were observed to contain large quantities of the solid DCC urea. This was found to be insoluble in 2-propanol, while the intended product was soluble, allowing the DCC urea to be removed by filtration under suction. Quantitative yields were achieved for this step. The compound was treated with hydrogen gas over Pd/C catalyst as before, having dissolved the dimethylamide product (15.86 g, 0.057 mol) in methanol (200 cm^3). An essentially quantitative yield was again obtained.

The dimethylamide product (7.26 g, 0.051 mol) was reduced as described earlier with $LiAlH_4$ (9.69 g, 0.256 mol) and the mixture refluxed for 48 h. The crude *N*-dimethyl-2-aminomethylpyrrolidine was obtained as a light yellow oil. This was purified by Kugelrohr distillation and the fraction at 90–125 °C (∼0.4 mm Hg) collected to give the pure product as a clear oil. Yield: 2.61 g, 38% (*S*-enantiomer), 2.13 g, 31% (*R*-enantiomer); *m*/*z* 128, calc. 128. ^{1}H NMR: solvent $CDCl_3$, ppm; 1.38 (m, 1H); 1.70 (m, 2H); 2.21 (s, 6H); 2.31 (t, 1H); 2.88 (m, 1H); 3.21 (m, 1H). $[\alpha]_D$ + 0.9° {−4.7°} (*c* = 1, methanol).

2.6. Preparation of the platinum(II) complexes

Using a procedure developed by Fenton [33] from a published method [34], *cis*-dichlorobis(dimethylsulfoxide)platinum(II) (0.422 g, 1 mmol) [35,36] was suspended in methanol (40 cm^3). The appropriate diamine ligand (1 mmol) in methanol (20 cm^3) was added and the mixture stirred at room temperature until all solids had dissolved, giving a clear, pale yellow solution. The solution was stirred for a further 1.5 h and the methanol removed by rotary evaporation at 40 °C. The residue was dissolved in water (20 cm^3) and excess lithium chloride added (∼0.5 g) and the solution gently heated on a steam bath until the volume had reduced to around 10 cm^3.

The products formed as fine yellow crystals that were collected at the pump and washed with a small amount of ice-cold water, followed by ethanol and then diethyl ether for drying. The crystals were air-dried for an hour, the aqueous washings and the mother liquor were retained for a second crop that was collected in the same manner. The yields of the complexes synthesised are given in Table 1 along with the results of the microanalyses.

[$PtCl_2$(*R*-pyrr)] was synthesised by both the above method and by the method of Morikawa et al. [24]. In the latter method, the diamine (0.050 g, 1 mmol) was added to a solution of potassium tetrachloroplatinate(II) (0.400 g, 1 mmol) in water (∼8 cm^3). The solution was stirred at room temperature for 3.5 h. A precipitate formed that was collected at the pump and washed with a small amount of ice-cold water and ethanol, and air-dried for 1 h to afford the complex as a pale yellow powder. Yield: 0.165 g (90%).

2.7. NMR characterisation

All complexes were characterised by NMR and the results are given below. The spectra of many of the complexes could not be unambiguously assigned due to their complexity arising from their formation as diastereomeric mixtures. This reveals a lack of sufficient steric bulk about the five-membered pyrrolidine ring to enforce a single specific chirality at the coordinating nitrogen atoms.

[$PtCl_2$(pyrr)]. A single set of signals was observed for these complexes. This is to be expected as the exocyclic nitrogen in this compound is not chiral, and so no possibility of diastereomers exists. ^{1}H NMR: solvent DMF-d_7, ppm; 1.80 (m, 2H); 1.91 (m, 1H); 2.06 (m, 1H); 2.59 (m, 1H); 2.68 (m, 1H); 3.07 (m, 1H); 3.33 (m, 2H); 5.46 (d, NH_2); 6.49 (s, NH). ^{13}C NMR: solvent DMF-d_7, ppm; 25.18 (CH_2); 25.25 (CH_2); 51.64 (CH_2); 52.18 (CH_2); 67.43 (CH).

[$PtCl_2$(mepyrr)]. Both enantiomers show evidence of diastereomers in both the complexity of the ^{1}H NMR spectrum and a second minor set of signals in the ^{13}C NMR spectra. *S*-enantiomer: ^{1}H NMR: solvent DMF-d_7, ppm; 1.76 (m, 2H); 1.97 (m, 2H); 2.61 (s, 3H); 2.71 (d, 2H); 2.98 (m, 1H); 3.12 (m, 1H); 3.28 (m, 1H); 5.94 (s, NH minor); 6.08 (s, NH major); 6.38 (s, NH minor); 6.47 (s, NH major).

Table 1
Synthetic results for the platinum(II) complexes.

Complex and molecular formula	Yield (%)	Calculated (%)			Found (%)		
		C	H	N	C	H	N
[$PtCl_2$(*S*-pyrr)], $C_5H_{12}N_2Cl_2Pt$	43	16.40	3.30	7.65	16.70	3.35	6.82
[$PtCl_2$(*R*-pyrr)], $C_5H_{12}N_2Cl_2Pt$	25	16.40	3.30	7.65	16.32	3.31	7.51
[$PtCl_2$(*S*-mepyrr)], $C_6H_{14}N_2Cl_2Pt$	100	18.96	3.71	7.37	18.89	3.64	7.21
[$PtCl_2$(*R*-mepyrr)], $C_6H_{14}N_2Cl_2Pt$	57	18.96	3.71	7.37	18.89	3.71	7.14
[$PtCl_2$(*S*-dimepyrr)], $C_7H_{16}N_2Cl_2Pt$	70	21.33	4.09	7.11	21.50	4.05	7.04
[$PtCl_2$(*R*-dimepyrr)], $C_7H_{16}N_2Cl_2Pt$	70	21.33	4.09	7.11	21.24	4.07	6.59

R-enantiomer. ^{1}H NMR: solvent DMF-d_7, ppm; 1.76 (m, 2H); 1.99 (m, 2H); 2.61 (s, 3H); 2.73 (s, 2H); 3.01 (m, 1H); 3.14 (m, 1H); 3.30 (m, 1H); 6.08 (s, NH); 6.47 (s, NH).

The ^{13}C spectrum shows that in both enantiomers, the signals due to the minor diastereomer are slightly less than half as intense as those from the major diastereomer. *S*-enantiomer: ^{13}C NMR: solvent DMF-d_7, ppm; 29.3 (CH_2, minor); 29.7 (CH_2, major); 29.9 (CH_2, major); 30.0 (CH_2, minor); 45.9 (CH_3); 55.5 (CH_2, minor); 56.2 (CH_2, major); 66.4 (CH_2, major); 67.8 (CH_2, minor); 68.8 (CH, major); 69.1 (CH, minor). *R*-enantiomer: ^{13}C NMR: solvent DMF-d_7, ppm; 29.3 (CH_2, minor); 29.7 (CH_2, major); 29.9 (CH_2, major); 30.0 (CH_2, minor); 45.9 (CH_3); 55.5 (CH_2, minor); 56.2 (CH_2, major); 66.3 (CH_2, major); 67.8 (CH_2, minor); 68.8 (CH, major); 69.1 (CH, minor).

[$PtCl_2$(dimepyrr)]. It was not possible to characterise this complex by ^{13}C and DEPT NMR spectroscopy as a sufficiently concentrated solution could not be produced in any of the available deuterated solvents. ^{1}H NMR: solvent DMF-d_7, ppm; 1.28 (s, 1H); 1.65 (m, 1H); 1.82 (m, 1H); 1.91 (m, 1H); 2.01 (m, 1H); 2.62 (dd, 1H); 2.85 (s, 6H); 3.15 (m, 1H); 3.27 (m, 1H); 3.85 (m, 1H); 6.40 (s, NH).

2.8. Crystallography

Crystals suitable for X-ray crystallography were obtained by recrystallisation of the [$PtCl_2$(*R*-dimepyrr)] complex from hot DMF followed by slow evaporation. The crystals were mounted on glass fibres with cyanoacrylate resin. Data was collected using an Enraf Nonius CAD4 four-circle diffractometer, employing graphite monochromated Mo Kα radiation. Cell constants were determined by a least-squares fit to the setting angles of 25 independent reflections. Lorentz, polarisation and absorption corrections were applied using teXsan software [37]. The structure was solved by direct methods using SHELXS-86 [38] and refined using least-squares methods with teXsan. Hydrogen atoms were included at calculated sites with fixed isotropic thermal parameters. Non-hydrogen atoms were refined anisotropically. All other calculations were performed using teXsan. The ORTEP plot [39] (30% thermal ellipsoids) of the complex is shown in Fig. 1.

2.9. Molecular modelling

Molecular mechanics modelling was performed on a Silicon Graphics Indigo workstation. The graphics program HyperChem [40] was used for construction of the models, with strain energy minimisation carried out using MOMEC-SG [41] with previously reported force fields [42]. Models of the dichloroplatinum(II) complexes were minimised until the root mean squared (r.m.s.) shift in all positional coordinates was less than or equal to 0.001 Å.

2.10. Cytotoxicity assays

In vitro cytotoxicity assays were performed using cisplatin as a standard. Activity of the complexes was measured on the human ovarian cancer cell line A2780 and the cisplatin-resistant variant A2780cisR. Cells were grown as monolayers in RPMI 1640 culture media (Trace Bioscience) supplemented with 10% foetal calf serum (FCS) and 2 mM glutamine and maintained in an humidified incubator at 37 °C in 5% CO_2. The cells were maintained in exponential growth phase by subculturing routinely with trypsin–EDTA. Cell counts were performed using a haemocytometer counter (Weber), with 8×10^4 and 6×10^4 A2780 and A2780cisR cells respectively seeded into each well of flat-bottom 96-well plates (Corning). Cells were allowed to attach overnight.

The complex and cisplatin stock solutions were made up fresh in RPMI 1640 media at concentrations of 0.5 mM. The cells were exposed to duplicate serial dilutions of the drug in tissue culture for 72 h, at a range of concentrations from 0 to 100 μM. The viability of the cells following treatment was determined using the MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyl bromide; thiazolyl blue) cytotoxicity assay [43]. MTT (10 μl of 5 mg/mL in growth medium) was added to each well and incubated for a further 4 h. The culture medium was removed from each well, and DMSO (150 μL, Sigma) added. The plate was shaken for 5 s and the absorbance measured immediately at 540 nm in a microplate reader. Activity of the complexes was measured in terms of IC_{50} (μM) values, determined as the drug concentration that reduced the absorbance to 50% of that in untreated control wells.

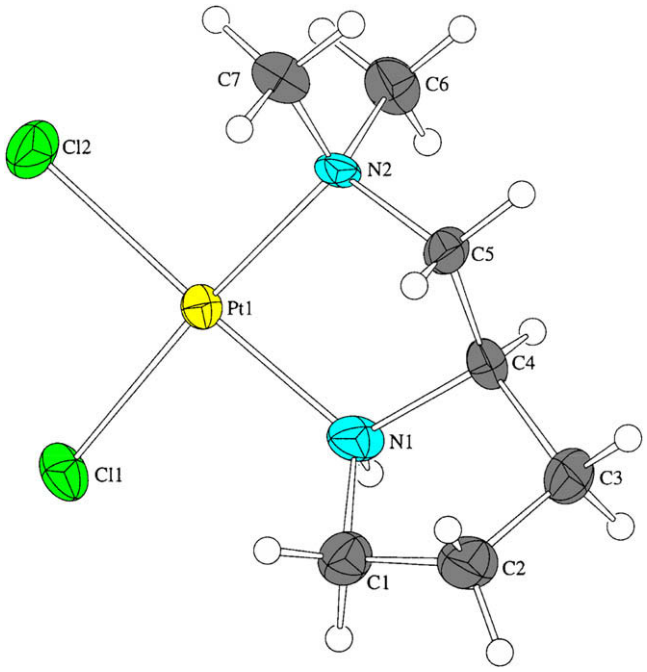


Fig. 1. ORTEP plot for [$PtCl_2$(*R*-dimepyrr)].

3. Results and discussion

3.1. Syntheses

A series of enantiomerically pure ligands derived from the naturally occurring amino acid proline were produced using the optically active forms of proline as precursors. It is essential that the complexes are of high enantiomeric and chemical purity for them to be meaningful probes of Pt/DNA interactions and this was confirmed by CD, IR and NMR spectroscopies, and polarimetry. The pyrr ligand was previously prepared from the naturally occurring α-amino acid *S*-proline [44,45] and Morikawa and colleagues detailed a synthetic method based on catalytic hydrogenation of 2-pyrrolcarboxaldoxime [22]. Substituted diamines were obtained by

catalytic hydrogenation of the appropriate 2-alkylaminomethylpyrrole [23]. In both cases, the ligands were obtained as racemic mixtures and were used without resolution into their separate enantiomers in the synthesis of the platinum(II) complexes and the subsequent biological studies. Later methods published by the same workers were based on synthetic procedures using the chiral precursor, the amino acid proline [24], with the pure optical isomers being used in studies from that point onwards.

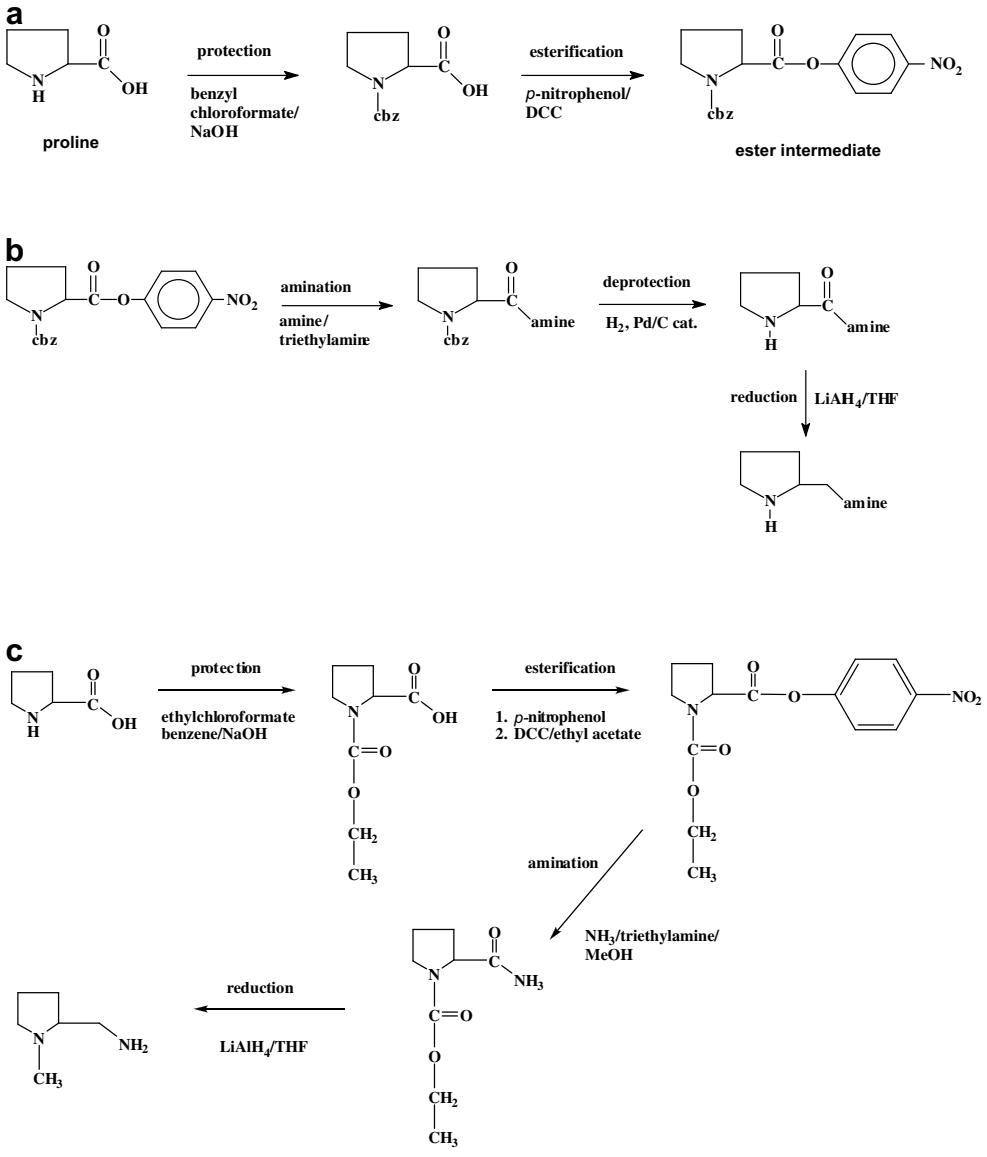
The synthetic schemes used here are derived from standard peptide chemistry, involving protection of the pyrrolidine nitrogen, formation of a suitable leaving group on the acid residue of the amino acid, substitution reaction and deprotection to form a chiral amide (Scheme 2). Subsequent reduction of the amide produced the appropriate diamine.

3.1.1. *N*-Carbobenzoxy-*S*-proline-*p*-nitrophenol ester

Synthesis of this compound involved protection of the secondary nitrogen amine with benzochloroformate, that upon coupling is known as the carbobenzoxy protecting group, cbz. The leaving group *p*-nitrophenol was attached to the carboxyl group of the amino acid residue using dicyclocarbohexyldiimide as a coupling agent which has the effect of eliminating water from the reaction and forcing the equilibrium towards the product side, forming dicyclocarbohexyl urea as a by-product (Scheme 2(a)).

3.1.2. *N*-Substituted aminomethylpyrrolidines

Detailed in Scheme 2(b) is the general synthetic scheme followed for the synthesis of 2(*S*)-aminomethylpyrrolidine and the subsequent ligands in the series. Taking a methanol or ethanol solution of cbz-*p*-NO_2 ester, triethylamine was used as a non-substituting, volatile organic base during the addition of the required amine. For synthesis of the parent amine, gaseous ammonia was needed, and multiple cycles of bubbling the gas through the solution until saturation followed by heating were carried out, with the yield for these ligands, not surprisingly, lower than in the other substituted amines. Removal of the carbobenzoxy protecting group was achieved by bubbling hydrogen gas through methanol solutions of the amides over a palladium on charcoal catalyst. This method is known to produce a cleaner product than an alternative method that employs HBr in acetic acid [46]. The



Scheme 2.

resultant amides were then aggressively reduced to the diamines with lithium aluminium hydride and the diamines purified by Kugelrohr vacuum distillation.

3.1.3. Platinum(II) complex synthesis

The preparation of the platinum(II) complexes first involved the synthesis of the platinum(II) intermediate, *cis*-[$PtCl_2(DMSO)_2$], with which the ligand was reacted. The diamine pyrrolidine ligands were coordinated to the platinum(II) ion by reaction with equivalent amounts of the *cis*-[$PtCl_2(DMSO)_2$] complex in methanol. Complete conversion from the chloro–DMSO–platinum(II) intermediate to the dichloroplatinum(II) product following addition of lithium chloride was monitored by infrared (IR) spectroscopy. The absence of peaks attributed to the DMSO group provided conclusive evidence of formation of only the dichloro product.

The [$PtCl_2$(*R*-pyrr)] complex was initially synthesised *via* the *cis*-[$PtCl_2(DMSO)_2$] intermediate. A subsequent attempt at synthesising this complex presented difficulties in the precipitation of the complex and the method of Morikawa et al. [24] was used instead. This latter method, in this case, gave a pure product, in approximately 35% yield.

3.2. Characterisation of the platinum complexes

The complexes were characterised for chemical and optical purity using ^{1}H, ^{13}C and DEPT NMR and CD spectroscopy respectively. In all cases the solvent used for these analyses was DMF (for CD spectra) or DMF-d_7. The DMF signals in the ^{1}H spectra, due to an isotropic impurity, appear at 2.79, 2.94 and 7.90 ppm; in the ^{13}C NMR spectra two multiplets appear at 30 and 35 ppm. The resonances due to these impurities were used as internal calibrants. DMF was chosen as the solvent due to the high solubility of the complexes in it, and it has been found that the chloride ligands did not exchange with DMF [47].

The NMR spectra of the complex in which the exocyclic amine was chiral produced a ^{13}C NMR spectrum that had two sets of signals and consequently could not be assigned unambiguously. The multiple signals indicate the formation of diastereomers which implies a lack of sufficient steric bulk about the five-membered pyrrolidine ring to enforce a single chirality at the coordinating nitrogen atoms. Complexes that lacked chirality at the exocyclic nitrogen atom were always able to be fully assigned because the ^{13}C NMR spectra of these complexes showed only a single set of signals.

The CD spectra of the complexes confirmed the enantiomeric purity of the complexes. The four sets of enantiomers produced spectra with the same qualitative shape with bands in approximately the same positions.

3.3. Crystallography

The crystal structure of the platinum(II) complex [$PtCl_2$(*R*-dimepyrr)] was determined and is represented in the ORTEP diagram shown in Fig. 1. Crystallographic data and associated collection parameters for the structure appear in Table 2. Bond lengths, bond angles and torsion angles are presented in Tables S1–S3. Lists of the atomic positional coordinates and thermal parameters of hydrogen and non-hydrogen atoms and details of least-squares planes calculations, are included in Tables S4–S7. Observed and calculated structure factors and intermolecular distances are available on request.

The [$PtCl_2$(*R*-mepyrr)] complex has a square-planar geometry about the platinum(II) atom, with *cis* chloro ligands and nitrogen donor atoms. Both the chloro ligands and nitrogen donor atoms deviate only slightly (0.004–0.005 Å) from the platinum coordination plane (defined by the platinum and the four donor atoms).

Table 2
Crystal data for [$PtCl_2$(*R*-dimepyrr)].

Crystal	[$PtCl_2$(*R*-dimepyrr)]
Formula weight *M*	394.22
Formula	$PtC_7H_{16}N_2Cl_2$
Crystal system	Orthorhombic
Space group	$P2_12_12_1$
a (Å)	8.309(5)
b (Å)	8.665(5)
c (Å)	15.198(8)
V (Å^3)	1066(2)
D_{calc} (g cm^{-3})	2.456
F(000)	736
Z	4
λ (Å)	0.71073
μ (mm^{-1})	13.564
T_{max} (mm^{-1})	0.456
T_{min} (mm^{-1})	0.264
Temperature (°C)	22.0
θ_{max}	27.46
Reflections collected	1963
Observations	1688
Range of *hkl*	$-1 \rightarrow 10$; $-1 \rightarrow 11$; $-1 \rightarrow 19$
Maximum shift	0.0073
R (F_0)	0.037
R_w	0.032

Examination of the angles about the platinum atom shows constraints caused by the bidentate ligand leading to a compression of the N(2)–Pt–N(1) angle to 84.3°. The strain has been borne slightly more by the exocyclic side of the ligand, with the Cl(2)–Pt–N(2) angle having opened up to 93.3°. This strain is also reflected in the Pt–N bond lengths. The exocyclic amine Pt–N(2) bond length is an unusually long 2.066(8) Å, while the endocyclic amine Pt–N(1) bond length is a more normal 2.02(1) Å. The crystal structure of (−)-(*R*)-[2-(aminomethyl)pyrrolidine](1,1-cyclobutanedicarboxylato)platinum-(II) monohydrate (DWA-2114R), containing the parent ligand has a much shorter Pt–N (exocyclic) bond length of 1.998(5) Å, while the Pt–N (endocyclic) bond length, at 2.019(4) Å, is similar to that found here [48]. The five-membered ring of the pyrrolidine ligand adopts an envelope conformation with the C(2) atom deviating by more than 0.3 Å from the approximately planar moiety comprising the four atoms N(1), C(1), C(4) and C(3). The C(4) carbon atom is a stereogenic centre, and here has *R* chirality. The endocyclic nitrogen, N(1), is also chiral, and it too has *R* chirality.

3.4. Molecular modelling of the Pt(II) complexes

The pyrrolidine system contains three potential chiral sites: the chiral carbon, plus the endocyclic and exocyclic nitrogen atoms that may gain a chirality upon coordination to the platinum(II) atom, depending on the nature of their amine substituent(s). It was found for each enantiomer that the most energetically favourable structure was one where the chirality of the carbon and the endocyclic nitrogen atoms are the same, as is illustrated by the mepyrr complex (Table 3) and supported by the crystal structure of the dimepyrr complex.

Table 3
Minimised strain energies for the Pt(II) complexes of [$PtCl_2$mepyrr], where *Xyz* describes *X* carbon chirality, *y* exocyclic nitrogen and *z* endocyclic nitrogen chiralities.

S-Enantiomer	*R*-Enantiomer	Total strain energy (kJ mol^{-1})
Sss	*Rrr*	51.0
Srs	*Rsr*	52.8
Srr	*Rss*	65.6
Ssr	*Rrs*	67.3

Table 4
Total strain energies for the pyrrolidine platinum(II) dichloro complexes.

S-Enantiomer	*R*-Enantiomer	Total strain energy (kJ mol^{-1})
S-Pyrr	*R*-Pyrr	48.6
Sss-Mepyrr	*Rrr*-Mepyrr	51.0
Srs-Mepyrr	*Rsr*-Mepyrr	52.8
S-Dimepyrr	*R*-Dimepyrr	55.0

It is important to note that the difference between the total strain energies of the two lower energy diastereomers, which differ only in the chirality of the exocyclic nitrogen atom, is minimal indicating that they can be expected to form in similar amounts. Indeed, the ^{13}C NMR spectra showed the presence of two species, and one formed in excess over the other by a ratio of approximately 4:3. The total strain energies of each of the pyrrolidine platinum(II) complexes with the preferred combination of C(4) and endocyclic amine configurations are presented in Table 4 and reveal that in the case with a chiral exocyclic amine group (mepyrr) there is almost equal probability of each diastereomer forming.

The molecular models of the *R*-enantiomers of the complexes are presented in Fig. S2. The complexes illustrated in these molecular models possess a square-planar geometry about the platinum(II) atom, with the five-membered pyrrolidine ring adopting a stable envelope conformation. The angles about the platinum atom vary by little throughout the series: $(85.3 \pm 1)^\circ$ about the chelate ligand; $(91.5 \pm 1.7)^\circ$ on the exocyclic side of the complex; $(89.1 \pm 2.3)^\circ$ between the chloro atoms; and $(89.3 \pm 3.4)^\circ$ about the endocyclic side of the complex. The constraint placed on the complex by the chelate ligand, causing a compression of the angle between the two coordinating nitrogen atoms, is compensated for by an opening up of the angle on the exocyclic side of the ligand. The angles about the platinum atom of the crystal structure of [$PtCl_2$(*R*-dimepyrr)] are within the range of those measured from the molecular models. Overlaying the molecular model with the crystal structure of [$PtCl_2$(*R*-dimepyrr)] shows good agreement between the two structures (Fig. S3).

3.5. Cytotoxicity studies

The platinum(II) compound DWA-2114R ([Pt(CBDC)(*S*-pyrr)]) has previously demonstrated high activity against ovarian cancer in Phase III trials. The choice of an ovarian cancer cell line in this experiment is therefore most relevant. In order to probe any possible cross-resistance to cisplatin, a variant of this cell line which is resistant to cisplatin was also used. The IC_{50} values obtained for cisplatin are the same as those obtained in previous experiments, within error margins (Table 5).

Both enantiomers of the parent compound, [$PtCl_2$(pyrr)], showed activity similar to cisplatin in the A2780 cell line (within error margins). Almost identical activity was seen on the addition of a single methyl group at the exocyclic amine site as in [$PtCl_2$(mepyrr)], but a substantial decrease in activity was seen on increasing the steric bulk further at the exocyclic amine site by the addition of two methyl groups, as in the [$PtCl_2$(dimepyrr)] compounds. This change also gives rise to enantioselectivity in the degree of cross-resistance with cisplatin.

For all compounds, higher drug concentrations were required in the cisplatin-resistant cell lines, a degree of cross-resistance with-cisplatin. For the parent [$PtCl_2$(pyrr)] compound and the *R*-enantiomers of [$PtCl_2$(dimepyrr)] there was only a slight increase, implying reduced cross-resistance.

Table 5
Inhibition of growth (IC_{50}) of A2780 human cancer cell lines determined by the MTT growth inhibition assay. Standard deviations are in parentheses.

Drug	A2780, IC_{50} (μM)	A2780cisR, IC_{50} (μM)	Resistance factor ratios
Cisplatin	3.1 (1.1)	7.1 (3.2)	2.3
S-Pyrr	3.2 (1.6)	4.1 (0.5)	1.3
R-Pyrr	3.1 (0.6)	5.8 (1.3)	1.9
S-Mepyrr	3.5 (0.3)	7.6 (2.7)	2.2
R-Mepyrr	4.4 (0.6)	13.4 (3.0)	3.0
S-Dimepyrr	10.0 (1.4)	49.0 (9.0)	4.9
R-Dimepyrr	7.2 (2.8)	8.0 (1.6)	1.1

4. Conclusions

This series of compounds was designed to provide control over the number and orientation of the amine protons in the expectation that this would determine the number of possible hydrogen bonds between the complex and DNA and influence the activity of the compounds. The *R* configuration at the endocyclic amine allows for hydrogen bond formation, but the *S* does not [5]. However, the chirality at this group does not influence the cytotoxicity except in the bulkiest compounds, and then only marginally. Therefore, hydrogen bonding between the complex and the DNA does not appear to be a major determinant of activity in this series.

In ligands in which the exocyclic nitrogen atom had two different substituents, chirality was also induced at this atom upon its coordination to platinum. However, molecular mechanics studies revealed that the complexes are likely to form with both exocyclic nitrogen atom chiralities and this was confirmed by ^{13}C NMR studies. This finding implies that there is insufficient bulk and/or rigidity in the carbon backbone of the ligand to enforce a specific chirality onto the exocyclic nitrogen atom. The presence of diastereomers means that the 2-aminomethylpyrrolidine system fails to meet the criteria for the design of chiral drugs or chiral probes of drug/DNA interactions.

Cytotoxicity studies against ovarian cancer A2780 cell line and its cisplatin-resistant variant A2780cisR showed good activity for all compounds in comparison to cisplatin and a lack of cross-resistance to cisplatin in some of them. Thus, the amino-methylpyrolidine framework can be extensively modified without loss of activity allowing for tuning of the pharmacological properties. Therefore, this series of complexes is worthy of further investigation.

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Appendix. Supplementary data

Tables of crystal data, positional and thermal parameters, bond lengths and angles, torsion angles and least square planes. Supplementary data associated with this article can be found in the online version, at doi:10.1016/j.ejmech.2008.12.022.

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