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Synthesis and characterisation of metallopolyamide complexes

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ABSTRACT

Platinum(II) and ruthenium(II)-based complexes that contain imidazole, pyrrole and β-alanine subunits, capable of recognising specific DNA base-pair sequences, have been synthesised. These polyamides, two platinum(II), $[Cl(NH_3)_2Pt\text{-}L_6\text{-}\beta\text{-}Ala\text{-}Py\text{-}L_4\text{-}Im]^+$ (HSP-2), and $[Cl(NH_3)_2Pt\text{-}L_6\text{-}\beta\text{-}Ala\text{-}PyPyPy\text{-}L_4\text{-}ImImIm]^+$ (HSP-6) and two ruthenium, Δ and Λ-[β-Ala-Py-L_4-Im-β-dpq-Ru(phen)$_2$]$^{2+}$ (Δ and Λ-RUP1), were designed to recognise DNA sequences up to seven base-pairs in length. They were obtained in good yield by a combination of solid and solution phase chemistries. The chirality of the ruthenium precursors Δ- and Λ-[Ru(phen)$_2$(phendo)]$^{2+}$ was conserved throughout the synthesis. Characterisation was achieved using NMR, UV–Vis and ESI-MS and CD for Δ- and Λ-RUP1. Cytotoxicity was not determined for HSP-2 or HSP-6, due to insolubility, however the IC_{50} values of Δ and Λ-RUP1 were confirmed to be greater than 40 μM (at an incubation time of 48 h). LD studies indicated that the ruthenium complexes interact with ct-DNA through a mixed binding mode, which is influenced by complex concentration and chirality.

1. Introduction

Cisplatin, carboplatin and oxaliplatin are used for the treatment of a variety of human cancers including head, neck, ovarian, testicular, lung and colorectal cancers [1]. When treatment by cisplatin is poorly tolerated, carboplatin is used, whereas oxaliplatin is used to treat specific cisplatin-resistant cancers [2]. Despite being efficient drugs, their use is restricted by dose limiting side effects, such as nephrotoxicity, neurotoxicity and ototoxicity, and intrinsic or acquired resistance [1,3]. There are several mechanisms which produce resistance such as decreased cellular uptake/increased drug efflux, increased drug deactivation arising from increased intracellular glutathione concentration and enhanced DNA adduct repair/tolerance [3a]. These limitations drive the researcher to search for new compounds with which to treat cancer. Producing metallodrugs that can target specific sequences of DNA and deliver therapeutic effect is an area of research at the interface of biology and chemistry that is proving to be a challenge.

Sequence selective polyamides, capable of targeting specific DNA sequences, have been synthesised over the past ten years. They comprise of heterocyclic groups linked by flexible bonds that rotate, and thus conform to the contour of the minor groove of DNA. This topological complementarity is stabilised by van der Waals interactions between the polyamide and the groove of DNA. When imidazole (Im) and/or pyrrole (Py) heterocyclic rings, β-alanine (β-ala) and/or amino butyric acid (L_4 linker) residues (Fig. 1) are combined in specific combinations they can target specific sequences of DNA [4]. The base-pairing rules that have been developed, relate to the anti-parallel association of the pyrrole–imidazole polyamide to their predicted target DNA sequences [4b]. Each pair of heterocyclic rings recognises a specific base-pair in DNA: Im/Py and Py/Im pairings distinguish between G:C and C:G base pairs respectively, while a combination of Py/Py, β-ala and the L_4 linker is selective for both A:T and T:A base pairs. The L_4 linker facilitates the formation of a hairpin by allowing it to fold and form dimers with itself. The hairpin polyamides have a distinct advantage in binding, achieving affinities and specificities comparable to DNA-binding proteins.

Previously we have synthesised *trans*-chlorodiammine[*N*-(2-aminoethyl)-4-[4-(*N*-methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxamido]-*N*-methylpyrrole-2-carboxamide]platinum(II) chloride (DJ1953-2) and *trans*-chlorodiammine-[*N*-(6-aminohexyl)-4-[4-(*N*-methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxamido]-*N*-methylpyrrole-2-carboxamide]platinum(II) chloride (DJ1953-6) (Fig. 2) [5]. Circular dichroism (CD) studies have shown that these complexes are capable of binding the DNA sequence d(CATTGTCAGAC)$_2$. Although neither complex affected DNA melting temperatures, DJ1953-2 unwound the helix by 13°. DJ1953-2 was also found to inhibit RNA synthesis *in vitro*. The cytotoxicity of both metal complexes in the murine leukaemia cell line (L1210) was also determined, with DJ1953-6 (34 μM) being more active than DJ1953-2 (>50 μM) [5].

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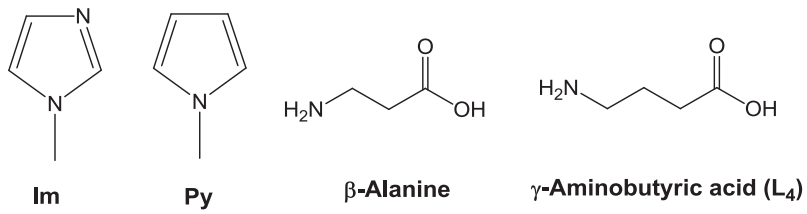

Fig. 1. The structures of the heterocyclic groups, Im and Py, together with β-alanine and γ-aminobutyric acid linker.

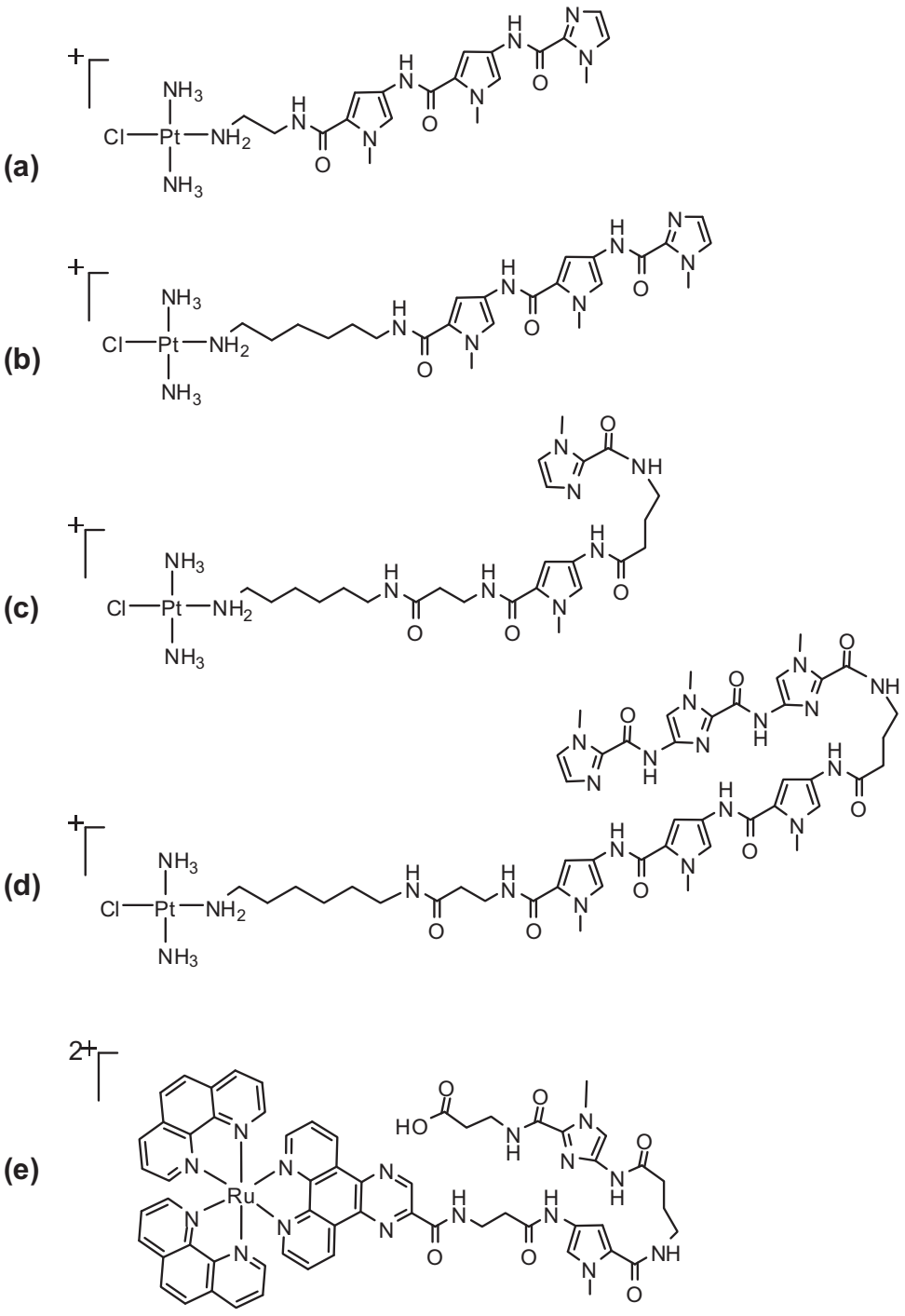

Fig. 2. The polyamide complexes DJ1953-2 (a), DJ1953-6 (b), HSP-2 (c), HSP-6 (d) and RUP1(e).

There have been widespread efforts to utilise ruthenium complexes in order to investigate the principle involved in DNA recognition [6], act as artificial nucleases [7], and luminescent probes for DNA [8] and to examine electron transfer mediated by DNA [9]. Many investigations have focussed upon metallointercalators – that is, the insertion of a planar aromatic ring system between the stacked bases of DNA. These complexes are generally of the form $[Ru(L)_2(I_L)]^{2+}$, where L is a ligand such as 2,2′-bipyridine or 1,10-phenanthroline (phen), and I_L is a ligand capable of insertion between the base pairs of DNA, such as dipyrido[3,2-a:2′,3′-c]phenazine (dppz) or dipyrido[3,2-d:2′3′-f]quinoxaline (dpq) [10].

Here we report the solid phase synthesis and characterisation of four sequence selective hairpin metallopolyamides, two platinum(II) complexes – $[Cl(NH_3)_2Pt\text{-}L_6\text{-}\beta\text{-}Ala\text{-}Py\text{-}L_4\text{-}Im]^+$ (HSP-2), and $[Cl(NH_3)_2Pt\text{-}L_6\text{-}\beta\text{-}Ala\text{-}PyPyPy\text{-}L_4\text{-}ImImIm]^+$ (HSP-6) and two ruthenium complexes – Δ and Λ-[β-Ala-Py-L_4-Im-β-dpq-Ru(-phen)$_2$]$^{2+}$ (Δ and Λ-RUP1) (Fig. 2). The platinum metallopolyamides have the potential to covalently bind whereas the ruthenium metallopolyamides are expected to interact by a combination of intercalation and groove binding. The cytotoxicity, guanosine binding and interaction of Δ and Λ-RUP1 with ct-DNA have been investigated through linear dichroism (LD).

2. Experimental

2.1. Materials

N-Methyl-1H-imidazole-2-carboxylic acid was purchased from Bachem. (9H-fluoren-9-yl)methyl chloroformate-(Fmoc), γ-amino-butyric acid and Fmoc-β-alanine were purchased from Fluka. *N*-Methyl-1H-imidazole, *N*,*N*′-diisopropylethylamine (DIEA) and transplatin were purchased from Sigma–Aldrich. 2-Chloro-chlorotrityl resin, Fmoc-lysine-(Fmoc)-OH and 2-(1H-benzotriazole-1-YL)-1,1,3,3-tetramethyluroniumhexafluorophosphate (HBTU) were purchased from Auspep, Australia. Boc-L-2,4-diaminobutyric acid-(Fmoc) was purchased from PepTech. *N*-Methyl-1H-pyrrole was purchased from AlfaAesar. Potassium tetrachloroplatinate(II) and ruthenium(III) chloride hydrate were purchased from Precious Metals Online. Fmoc-protected amino-caproic acid (L_6) and Fmoc-γ-aminobutyric acid (L_4) were bought from Merck while isopropanol was bought from Baxter. Ethyl acetate and anhydrous methanol were purchased from Mallinckrodt Chemicals. ct-DNA was purchased from Invitrogen. Dowex Monosphere 550A (OH) was purchased from Sigma. All other solvents were of analytical grade and were used as received from Biolab. Silica gel 60 (0.003–0.040 mm) was bought from Silicycle (Canada). Dimethylsulfoxide (D6, 99.9%), acetonitrile (D3, 96–97%), acetone (D6, 99.9%), dimethylformamide (D7, 99.9%) and deuterium oxide (D2, 99.9%) were purchased from Cambridge Isotope Laboratories. The heterocyclic *N*-methylimidazole and *N*-methylpyrrole rings for the polyamide were synthesised then activated as previously described [11]. 1,10-Phenanthroline-5,6-dione (phendo) was synthesised using an adaptation of the method by Yanagida et al. [12] (0.88 g, 76%). ^{1}H NMR 400 MHz (d_6-DMSO): δ 9.05 (d, 2H, *J* = 3.23 Hz); 8.45 (d, 2H, *J* = 7.47 Hz); 7.74 (t, 2H, *J* = 5.88 Hz). Full method is in the Supplementary data. All other reagents and solvents were used as received unless otherwise stated.

2.2. Instruments and physical measurements

NMR spectra were obtained on a 300 MHz Varian Mercury or a 400 MHz Bruker Avance spectrometer, either in d_6-DMSO, d_7-DMF or D_2O, referenced internally to the solvent. NMR experiments were run at 35 °C for DMSO and 25 °C for D_2O/DMF unless otherwise stated. ^{195}Pt NMR were externally referenced to K_2PtCl_4 (−1631 ppm) [13].

Positive ion ESI mass spectra were acquired using a Micromass (Wyntheshawe, UK) Quattro Micro™ spectrometer equipped with a Z-spray probe. Solutions containing concentrations ranging between 10 and 50 μM were injected into the instruments at a flow rate of 10 μL min^{-1}. The source and desolvation temperatures were 150 and 120 °C, respectively. The capillary tip potential and cone voltage were 2500 and 50 V, respectively. Between 10 and 50 acquisitions were summed to obtain spectra, which were calibrated against a standard CsI solution (750 mM) over the same *m/z* range. CD and LD spectra were recorded on a Jasco J-810 spectropolarimeter at room temperature. Samples were collected between 190 and 600 nm. Machine assisted synthesis was performed on a Symphony Quartet 3.21 protein synthesiser on a 0.28 mmol scale. Each cycle of monomer addition involved a DCM wash (∼10 mL), a DMF wash (∼10 mL) and deprotection with 20% piperidine/DMF (∼10 mL). Activated acids were added manually to the reaction vessel at the end of a deprotection cycle. Once the required molecule had been constructed, the resin was washed with copious amounts of DMF and DCM and dried for 1 h under $N_{2(g)}$.

2.3. Synthesis of platinum(II) precursors

2.3.1. Synthesis of Pt-L_6-NHBoc

Transplatin (2.50 g, 8.33 mmol) and silver nitrate (1.27 g, 7.49 mmol) in DMF (100 mL) were stirred in the dark under $N_{2(g)}$ for 12 h. The mixture was filtered and added to a solution of *tert*-butyl-6-aminohexylcarbamate [14] (2.00 g, 9.26 mmol) in DMF (30 mL). The solution was stirred in the dark under $N_{2(g)}$ for 18 h. The solvent was removed under reduced pressure, acetone (5 mL) was added and the mixture was kept at 4 °C overnight. The precipitate that formed was filtered and re-suspended in methanol (70 mL). This mixture was filtered and the filtrate was evaporated under reduced pressure to obtain Pt-L_6-NH.Boc (2.74 g, 76%) as a yellow/white powder. ^{1}H NMR 300 MHz (d_2-D_2O): δ 2.93 (t, 2H, *J* = 6.1 Hz), 2.55 (bm, 2H), 1.57 (bm, 2H), 1.3 (bp, 6H). ^{195}Pt NMR 85 MHz (d_7-DMF): −2423 (s).

2.3.2. Synthesis of Pt-L_6-NH_2

Pt-L_6-NH.Boc (0.20 g, 0.39 mmol) was dissolved in methanol (8 mL) before HCl (4 M, 7 mL) was added and the mixture stirred for 48 h. The solvent was evaporated under reduced pressure to obtain a yellow solid. Salt conversion was carried out using Dowex-OH to obtain the product as a white solid (0.14 g, 87%). ^{1}H NMR 300 MHz (d_6-DMSO): δ 7.85 (s, 2H), 5.42 (s, 2H), 4.01(s, 6H), 2.75 (t, 2H, *J* = 6.1 Hz), 2.56 (bp, 2H), 1.56 (bp, 4H), 1.30 (bp, 4H); ^{195}Pt NMR 85 MHz (d_7-DMF): −2415 (bs).

2.4. Synthesis and resolution of ruthenium(II) precursors

2.4.1. Synthesis of [Ru(phen)$_2$Cl$_2$]

An adaptation of the method by Hiort was used [15] to obtain the black crystallised product (1.3 g, 26%). ^{1}H NMR 400 MHz (d_3-acetonitrile): δ 8.69 (d, 4H, *J* = 7.51 Hz); 8.31 (s, 4H, *J* = 7.51 Hz); 8.10 (d, 4H, *J* = 7.51 Hz), 7.62 (t, 4H, *J* = 5.53 Hz).

2.4.2. Synthesis of [Ru(phen)$_2$phendo]Cl$_2$

An adaptation of the method by Hiort et al. [15] was used to synthesise this complex (0.8 g, 94%). ^{1}H NMR 400 MHz (d_6-acetone): δ 8.9 (d, 1H, *J* = 8.15 Hz); 8.84 (d, 1H, *J* = 8.15 Hz); 8.74 (d, 1H, *J* = 4.88 Hz); 8.68 (d, 1H, *J* = 7.87 Hz); 8.49 (q, 2H, *J* = 15.32, 6.38 Hz); 8.38 (q, 2H, *J* = 12.75, 7.36 Hz); 8.05 (q, 1H, *J* = 8.32, 3.08 Hz); 7.83 (q, 1H, *J* = 8.22, 2.98 Hz); 7.75 (q, 1H, *J* = 7.86, 2.24 Hz). Electronic spectra λ_{max} nm (ε mol^{-1} dm^{-3} cm^{-1}, 50%

CH_3CN in H_2O): 220 (68000), 260 (16794), 290 (25950), 435 (15996).

2.4.3. Resolution of $[Ru(phen)_2(phendo)](PF_6)_2$

The Δ- and Λ-isomers of $[Ru(phen)_2(phendo)](PF_6)_2$ were synthesised using the method by Hiort et al. [15].

Δ-$[Ru(phen)_2(phendo)](PF_6)_2$ (0.18 g, 39%). CD (H_2O:CH_3CN, 1.5×10^{-6} M, RT.): λ nm (Δε mdeg M cm): 256 (65.2), 267 (−113.2), 296 (−61.3), 410 (9.2), 460 (−7.1).

Λ-$[Ru(phen)_2(phendo)](PF_6)_2$ (0.10 g, 22%). CD (H_2O:CH_3CN, 1.5×10^{-6} M, RT.): λ nm (Δε mdeg M cm): 256 (−54.6), 267 (94.7), 296 (51.9), 410 (−7.8), 4604 (6.0).

2.5. Synthesis of HSP-2, HSP-6 and RUP1

2.5.1. Synthesis of polyamides (general method) [11a]

Fmoc-β-alanine-chlorotrityl resin was prepared by the addition of Fmoc-β-alanine-OH to 2-chloro-chlorotrityl resin. The next step of the reaction was undertaken by machine assisted protocol. The Fmoc-protected amino group of the β-alanine resin was removed by the addition of 20% piperidine in DMF (10 mL). This was followed by the addition of an activated monomer to the resin, allowing a mix time from 3.5 to 9 h depending upon the monomer used. The resin was filtered and washed with DMF (2×10 mL), DCM (10 mL) and DMF (10 mL). The amino group was deprotected and the cycle of addition, mixing and deprotection commenced and continued until the desired polyamide was formed.

2.5.2. Resin preparation

Fmoc-β-alanine-OH (0.15 g, 0.48 mmol) was added to DIEA (0.35 mL) in anhydrous DCM (4 mL) and the solution was stirred for 5 min. It was then added to a suspension of 2-chloro-chlorotrityl resin (0.50 g, 0.50 mmol) in anhydrous DCM (3 mL) and stirred gently for 5.5 h. MeOH (2.5 mL) was added and the suspension was stirred for a further 0.5 h. The solution was filtered and the solid resin was air dried for 12 h.

2.5.3. Activation of monomers and coupling (general method)

The monomer or linker of choice was dissolved in a mixture of DMF and NMP followed by the addition of the coupling agent, HBTU. DIEA was added and the solution was stirred for 10 min. The activated monomer was added manually to the resin and the suspension was mixed for any time between 3.5 - 9 h, whilst being aspirated with $N_{2(g)}$. The resin was filtered, washed with DMF (10 mL), treated with 20% piperidine in DMF (10 mL) and washed with DCM (2×10 mL) and DMF (3×10 mL). The specific monomers, activation conditions and coupling times for specific DNA base-pair sequences are shown in Tables 1–3.

2.5.4. Polyamide cleavage (general method)

Once prepared, the polyamides were cleaved from the resin by adding a solution of DCM (10 mL), trifluoroethanol (TFE, 2.4 mL) and acetic acid (1.2 mL) and shaken gently for 1.5 h. The resin was removed by filtration and washed with TFE:DCM (1:4, 6 mL) before it was subjected to the cleaving conditions twice more. The yellow/brown filtrate was collected and the solvent was removed under reduced pressure to ~2 mL. Cold diethyl ether (4 mL) was added and the mixture cooled to 4 °C for 1.5 h to precipitate a yellow solid. The solvent was decanted and water (3 mL) was added to the solid before the solution was lyophilised to yield the desired polyamide.

2.5.5. Synthesis of β-Ala-Py-L_4-Im

The polyamide was cleaved from the resin, as previously described, to obtain a pale yellow powder (0.08 g, 67%). ^{1}H NMR 300 MHz (d_6-DMSO): δ 9.74 (s, 1H), 8.41 (t, 1H, J = 5.8 Hz), 7.99 (t, 1H, J = 5.5 Hz), 7.31 (s, 1H), 7.07 (s, 1H), 6.95 (s, 1H), 6.62 (s, 1H), 3.92 (s, 3H), 3.75 (s, 3H), 3.34 (q, 2H, J = 7.0 Hz), 3.24 (q, 2H, J = 7.2 Hz), 2.42 (t, 2H, J = 7.2 Hz), 2.22 (t, 2H, J = 7.4 Hz), 1.77 (m, 2H, J = 7.1 Hz).

2.5.6. Synthesis of HSP-2

β-Ala-Py-L_4-Im (0.05 g, 0.12 mmol) in DMF (100 mL) was activated using HBTU (0.04 mg, 0.12 mmol) and DIEA (0.06 mL, 0.35 mmol). The solution was stirred for 5 min before Pt-L_6-NH_2 (0.025 mg, 0.07 mmol) was added and the mixture stirred overnight under $N_{2(g)}$. The solvent was removed under reduced pressure before the addition of acetone to precipitate the product as a slightly yellow coloured powder (0.04 mg, 72.9%). ^{1}H NMR 300 MHz (d_6-DMSO): δ 9.73 (s, 1H), 8.40 (t, 1H, J = 5.8 Hz), 7.92 (t, 1H, J = 5.5 Hz), 7.83 (t, 1H, J = 5.6 Hz), 7.31 (s, 1H), 7.07 (s, 1H), 6.96 (s, 1H), 6.61 (s, 1H), 5.41 (bp, 2H), 4.00 (s, 6H), 3.92 (s, 3H), 3.75 (s, 3H), 3.34 (q, 2H, J = 7.0 Hz), 3.24 (q, 2H, J = 7.2 Hz), 3.02 (q, 2H, J = 6.7 Hz), 2.50 (bm, 2H), 2.42 (t, 2H, J = 7.2 Hz), 2.22 (t, 2H, J = 7.4 Hz), 1.77 (p, 2H, J = 7.1 Hz); ^{195}Pt NMR 85 MHz (d_7-DMF): -2425 (bs). *Anal.* Calc. for $C_{24}H_{44}Cl_2N_{10}O_4Pt{\cdot}H_2O$: C, 35.12; H, 5.65; N, 17.07%; Found: C, 35.44; H, 5.64; N, 16.67%. ESI-MS Calc. for $C_{24}H_{44}ClN_{10}O_4Pt$ [M−Cl$^-$] 767.2 m/z; found 767.2 m/z. Electronic spectra λ_{max} nm (ε mol^{-1} dm^{-3} cm^{-1}, 50% CH_3CN in H_2O): 220 (68000), 260 (16794), 290 (25950), 435 (15996).

2.5.7. Synthesis of β-Ala-PyPyPy-L_4-ImImIm

β-Ala-PyPyPy-L_4-ImImIm was cleaved from the resin using the general procedure described for chlorotrityl resin polyamide cleavage. The procedure was carried out twice to obtain a pale yellow powder (0.20 g, 80%). ^{1}H NMR 300 MHz (d_6-DMSO): δ 10.00 (s, 1H), 9.84 (s, 1H), 9.83 (s, 1H), 9.78 (s, 1H), 9.57 (s, 1H), 8.30 (t, 1H, J = 5.8 Hz), 7.96 (t, 1H, J = 5.4 Hz), 7.63 (s, 1H), 7.51 (s, 1H), 7.43 (s, 1H), 7.21 (d, 1H, J = 1.4 Hz), 7.17 (d, 1H, J = 1.4 Hz), 7.15 (d, 1H, J = 1.4 Hz), 7.07 (s, 1H), 7.03 (d, 1H, J = 1.4 Hz), 6.88 (d, 1H, J = 1.6 Hz), 6.85 (d, 1H, J = 1.6 Hz), 4.03 (s, 3H), 4.00 (s, 3H), 3.96 (s, 3H), 3.84 (s, 3H), 3.83 (s, 3H), 3.80 (s, 3H), 3.39 (t, 2H, J = 7.0 Hz), 3.37 (q, 2H, J = 7.3 Hz), 2.47 (t, 2H, J = 7.1 Hz), 2.29 (t, 2H, J = 7.4 Hz), 1.82 (m, 2H, J = 7.1 Hz).

2.5.8. Synthesis of HSP-6

β-Ala-PyPyPy-L_4-ImImIm (0.07 g, 0.18 mmol), in DMF was activated using HBTU (0.03 mg, 0.07 mmol) and DIEA (0.04 mL, 0.22 mmol). The solution was stirred for 5 min before Pt-L_6-NH_2 (0.02 mg, 0.05 mmol) was added and the mixture stirred overnight under $N_{2(g)}$. The solvent was removed under reduced pressure before acetone was added to precipitate the product. HSP-6 (59 mg, 86.4%) was obtained as a slightly yellow coloured powder. ^{1}H NMR 300 MHz (d_6-DMSO): δ 9.98 (s, 1H), 9.84 (s, 1H), 9.83 (s,

Table 1
The activation conditions used for the monomers for the synthesis of β-Ala-Py-L_4-Im.

Monomer/linker	Mass (mg)	Moles (mmol)	DMF (mL)	NMP (mL)	HBTU (mg)	DIEA (mL)	Time (h)
PyE	86.97	0.24	3	3	83.28	0.12	3.5
Fmoc-γ-aminobutyric acid linker	87.21	0.27	3	3	83.28	0.12	4
ImE	78.08	0.22	4	2	83.28	0.12	5

Table 2
The activation conditions used for the monomers for the synthesis of β-Ala-PyPyPy-L_4-ImImIm.

Monomer/linker	Mass (mg)	Moles (mmol)	DMF (mL)	NMP (mL)	HBTU (mg)	DIEA (mL)	Time (h)
PyE	86.97	0.24	3	3	83.28	0.12	3.5
PyE	86.97	0.24	3	3	83.28	0.12	3.5
PyE	86.97	0.24	3	3	83.28	0.12	3.5
Fmoc-γ-aminobutyric acid linker	87.21	0.27	3	3	83.28	0.12	3.5
ImE	78.08	0.22	4	2	83.28	0.12	5
ImE	78.08	0.22	4	2	83.28	0.12	9
ImE	78.08	0.22	4	2	83.28	0.12	9

Table 3
The activation conditions used for the monomers for the synthesis of **Λ**- and -RUP1.

Monomer/linker	Mass (mg)	Moles (mmol)	DMF (mL)	NMP (mL)	HBTU (mg)	DIEA (mL)	Time (h)
PyE	86.97	0.24	3	3	83.28	0.12	3.5
Fmoc-γ-aminobutyric acid linker	87.21	0.27	3	3	83.28	0.12	4
ImE	78.08	0.22	4	2	83.28	0.12	4.5
Fmoc-β-alanine linker	74.72	0.24	4	2	83.28	0.12	4
Fmoc-DAP(Fmoc)-OH	131.65	0.25	3	3	83.28	0.12	7
Λ- or Δ-[Ru(phen)$_2$(phendo)](PF$_6$)$_2$	200.00	0.21	5	–	–	–	5

1H), 9.78 (s, 1H), 9.57 (s, 1H), 8.28 (t, 1H, J = 5.8 Hz), 7.93 (t, 1H, J = 5.4 Hz), 7.81 (t, 1H, J = 5.4 Hz), 7.61 (s, 1H), 7.49 (s, 1H), 7.42 (s, 1H), 7.21 (d, 1H, J = 1.4 Hz), 7.17 (d, 1H, J = 1.4 Hz), 7.15 (d, 1H, J = 1.4 Hz), 7.07 (s, 1H), 7.03 (d, 1H, J = 1.4 Hz), 6.87 (d, 1H, J = 1.6 Hz), 6.83 (d, 1H, J = 1.6 Hz), 5.19 (bp, 2H), 4.03 (s, 3H), 4.98 (s, 3H), 3.94 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.78 (s, 3H), 3.39 (t, 2H), 3.37 (q, 2H, J = 7.3 Hz), 3.02 (q, 2H, J = 7.1 Hz), 2.47 (t, 2H, J = 7.1 Hz), 2.29 (m, 4H, J = 7.4 Hz), 1.82 (p, 2H, J = 7.1 Hz); ^{195}Pt NMR 85 MHz (d_7-DMF): −2420 (bs). *Anal.* Calc. for $C_{46}H_{66}Cl_2N_{20}O_8Pt{\cdot}6H_2O{\cdot}2.5C_3H_7NO$: C, 40.57; H, 6.08; N, 19.90%; Found: C, 40.84; H, 5.76; N, 19.66%. ESI-MS Calc. for $C_{46}H_{66}ClN_{20}O_8Pt$ $[M-Cl^-]^+$ 1257.6 m/z; found 1257.4 m/z.

2.5.9. Synthesis of Δ- and -RUP1

Δ-RUP1: ^{1}H NMR 400 MHz (d_6-DMSO): δ 10.05 (d, 1H, J = 8.03 Hz); 9.86 (s, 1H); 9.81 (s, 1H); 9.58 (d, 1H, J = 8.81 Hz); 8.85 (s, 2H); 8.46 (s, 2H); 8.29 (s, 2H); 8.13 (s, 1H); 8.06 (s, 1H); 7.99 (s, 1H); 7.84 (s, 2H); 7.44 (s, 1H); 7.13 (s, 1H); 6.67 (s, 1H); 3.96 (s, 1H); 3.82 (s, 3H); 3.39 (s, 2H); 3.32 (s, 2H); 2.41 (s, 2H); 2.31 (s, 2H); 1.83 (s, 2H). ESI-MS Calc. for $C_{60}H_{52}N_{16}O_7Ru$ expected (m/z): 605.30; found (m/z): 605.12. CD (H_2O:CH_3CN, 1.5×10^{-6} M, RT.): λ nm ($\Delta\varepsilon$ mdeg M cm^{-1}): 256 (86.3), 267 (−156.3), 296 (−35.6), 410 (5.8), 460 (−6.7). Electronic spectra λ_{max} nm (ε mol^{-1} dm^{-3} cm^{-1}, 50% CH_3CN in H_2O): 263 (1.2×10^5), 444 (1.7×10^4).

Λ-RUP1: ^{1}H NMR 400 MHz (d_6-DMSO): δ 10.03 (d, 1H, J = 8.03 Hz), 9.87 (s, 1H), 9.78 (s, 1H), 9.60 (d, 1H, J = 7.81 Hz), 8.85 (d, 2H, J = 7.74 Hz), 8.46 (s, 2H), 8.31 (s, 2H), 8.26 (s, 1H), 8.13 (s, 1H), 8.00 (m, 2H), 7.84 (s, 2H), 7.47 (s, 1H), 7.13 (s, 1H), 6.69 (s, 1H), 3.95 (s, 1H), 3.83 (s, 3H), 2.95 (s, 2H), 2.83 (s, 2H), 2.49 (s, 2H), 2.31 (s, 2H), 1.84 (m, 2H). ESI-MS Calc. for $C_{60}H_{52}N_{16}O_7Ru$ expected (m/z): 605.30; Found (m/z): 605.12%. CD (H_2O:CH_3CN, 1.5×10^{-6} M, RT.): λ nm ($\Delta\varepsilon$ mdeg M cm^{-1}): 256 (−82.8), 267 (143.5), 296 (33.9), 410 (−5.6), 460 (6.4). Electronic spectra λ_{max} nm (ε mol^{-1} dm^{-3} cm^{-1}, 50% CH_3CN in H_2O): 263 (1.2×10^5), 444 (1.7×10^4).

2.6. Biological studies

2.6.1. Cytotoxicity

IC_{50} testing was conducted on the metallopolyamide complexes using the L1210 murine luekemia cell line are the results for growth inhibition studies of compounds using the Coulter Counting (CC) assay. These tests were conducted at the Andrew Durant drug testing facility at the Peter MacCullum Cancer Institute. The metallopolyamide complexes were dissolved in DMSO prior to use. L1210 cells were exposed to the complexes for 48 h and the experiments were carried out in duplicate.

2.6.2. LD studies of DNA binding affinity

Couette flow cell was used to measure the interactions between ct-DNA and the ruthenium complexes. Phosphate buffer (10 mM, pH 7) with sodium fluoride (20 mM) was used throughout the LD experiments. Sodium fluoride was chosen as it does not absorb at low wavelengths (<200 nm) like sodium chloride [16]. To calculate the concentration of ct-DNA, a molar extinction value at 260 nm of ε = 13 200 mol^{-1} cm^{-1} was used [17]. ct-DNA (700 μL, 50 μM) was placed in a Couette flow cell and the inner cylinder was rotated (2 V) to orient ct-DNA and a LD spectrum was recorded. Fifteen samples were prepared, with increasing metal complex and a constant ct-DNA concentration (as shown in Supplementary data), and allowed to equilibrate overnight at 25 °C. The LD of these samples both spinning and non-spinning was measured. The non-spinning measurement was subtracted from the spinning to correct for background noise. The LD spectrum of ct-DNA was subtracted from the sample spectrum to obtain a difference LD for each sample. All binding experiments were performed in duplicates.

3. Results and discussion

3.1. Synthesis and characterisation of HSP-2

The synthesis of β-Ala-Py-L_4-Im (shown in Fig. 3) was achieved using Fmoc-β-alanine-chlorotrityl resin. The polyamide was cleaved from the resin using acetic acid and TFE before it was lyophilized to obtain a yellow powder (67%). HSP-2 was produced by combining the polyamide, β-Ala-Py-L_4-Im at Pt-L_6-NH_2 through solution phase chemistry. The product was obtained in a yield of 67.2% (see Fig. 4).

The β-alanine monomer attached to the resin was deprotected by the addition of 20% piperidine in DMF. This removed the Fmoc-protecting group, resulting in a free –NH_2. In a separate vessel, PyE was dissolved in DMF/NMP mixture (1:1 v/v) and activated by the addition of HBTU and DIEA. HBTU itself needs to be activated by the addition of DIEA [18]. This mixture was stirred for ~5 min. HBTU forms a temporary bond with PyE by displacing

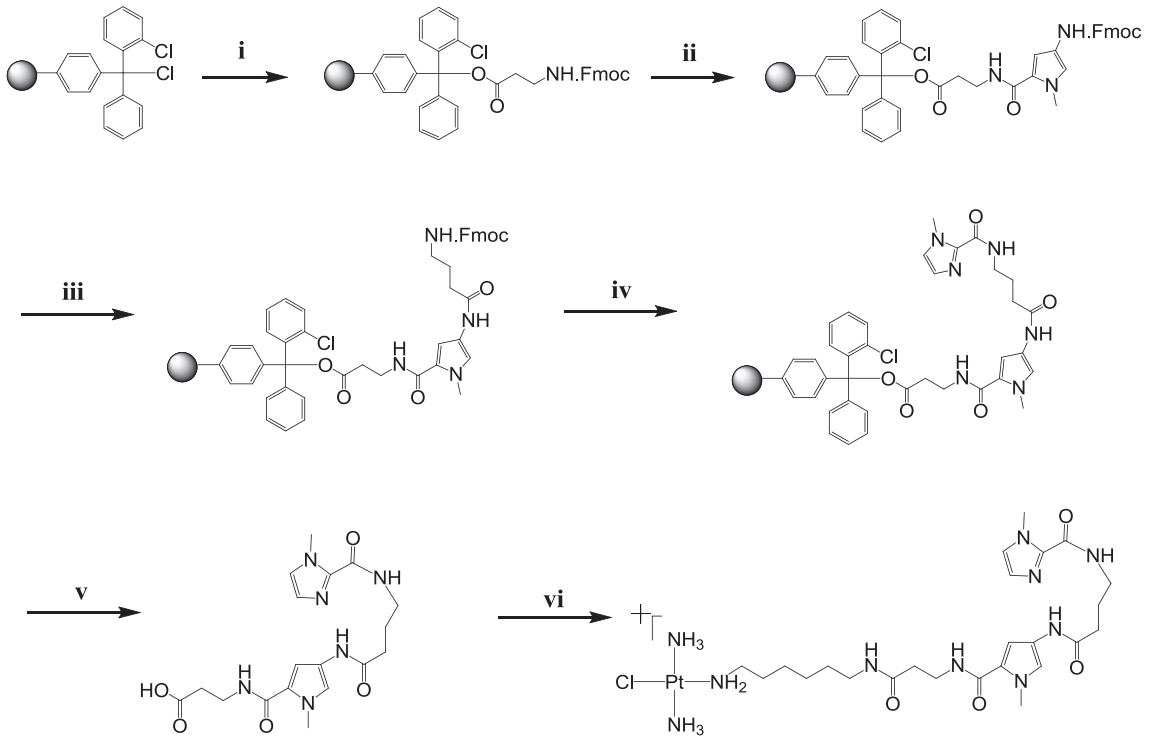

Fig. 3. The summary of the synthesis of HSP-2. The reaction conditions used were (i) DIEA, Fmoc-β-alanine-OH, 5 h; (ii) 20% piperidine in DMF, PyE, 3.5 h; (iii) 20% piperidine in DMF, Fmoc-γ-aminobutyric acid, 4 h; (iv) 20% piperidine in DMF, Im, 4.5 h; (v) TFE/acetic acid/DCM (2:1:7 v/v) mixture, 2 h; (vi) activated β-Ala-Py-L_4-Im was stirred with Pt-L_6-NH_2 overnight.

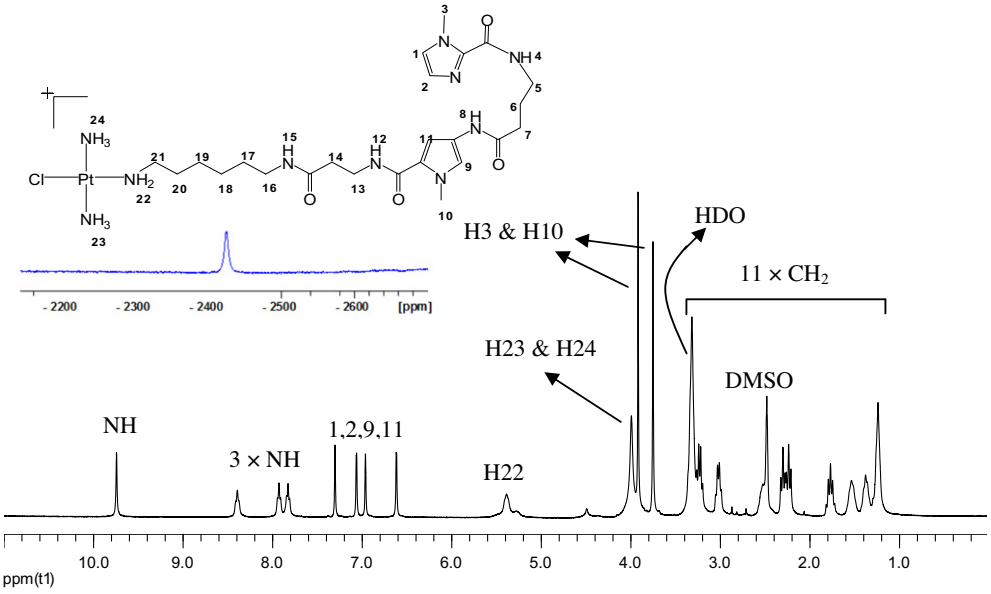

Fig. 4. The chemical structure of HSP-2 along with the ^{195}Pt and ^{1}H NMR spectrum in d_6-DMSO at 35 °C with labelled proton resonances.

the –OH group. The activated solution of PyE was added to the resin and the suspension was stirred for 3.5 h under $N_{2(g)}$. This ensured that most of the activated PyE would react with the β-alanine group on the resin. The free amine group on the β-alanine displaced the HBTU group to form a peptide bond between PyE and β-alanine.

One amide singlet, at 9.73 ppm, was observed for the proton labelled 8 in the ^{1}H NMR of HSP-2. Three amide triplets at 8.40, 7.92 and 7.83 ppm correspond to the protons labelled 4, 12 and 16, respectively. The four aromatic protons 1, 2, 9, and 11 were assigned to the four singlets at 7.31, 7.07, 6.96 and 6.61 ppm. The NH_2 directly coordinated to the platinum(II) centre was observed as a broad peak at 5.41 ppm, while the two platinum(II)-bound NH_3 groups were observed together as a broad singlet at 4.00 ppm. The two singlets at 3.92 and 3.75 ppm were assigned as the two methyl groups labelled 3 and 10. The eleven CH_2 groups were observed in the aliphatic region between 1.77 and 3.34 ppm. The peak of the platinum(II) was observed at −2425 ppm as a

broad singlet. ESI-MS gave a parent peak corresponding to the singly charged ion HSP-2^+ in aqueous solution. The purity of HSP-2 was assessed by elemental analysis; with the analysis indicating that HSP-2 contains one H_2O of hydration.

3.2. Synthesis and characterisation of HSP-6

3.2. Synthesis and characterisation of HSP-6

The synthesis of HSP-6 was achieved in an overall yield of 69.1% in a similar stepwise assembly as shown for HSP-2 in Fig. 3. The ^{1}H NMR spectrum of HSP-6 (Fig. 5) shows five amide singlets between 9.98 and 9.57 ppm which correspond to protons labelled 7, 17/21, 4, and 13, respectively. Three amide triplets at 8.28, 7.93 and 7.81 ppm correspond to 9, 25 and 38, respectively. The ten singlets/doublets between 7.61 and 6.83 ppm correspond to the ten aromatic protons. The terminal $-NH_2$ group is observed as a broad peak at 5.19 ppm while the six singlets between 4.03 and 3.78 ppm correspond to the $6 \times N-CH_3$ groups. The $2 \times -NH_3$ groups resonate at the same frequency as the $N-CH_3$ groups and therefore cannot be observed. The $11 \times -CH_2$ groups resonate in the aliphatic region between 3.39 and 1.82 ppm. The ^{195}Pt NMR spectrum of HSP-6 shows a single platinum resonance at −2420 ppm. The chemical shift is consistent with similar metal complexes with PtN_3Cl coordination spheres [4b,19]. Both HSP-2 and HSP-6 displayed poor solubility in aqueous solutions; as a result both complexes were dissolved in 1% DMSO in water/ACN (1:1, v/v) for the purposes of mass spectrometry. In the ESI mass spectrum of HSP-6, there is one prominent ion peak which occurs at *m/z* 1,257.4 and corresponds to the parent metal complex with the 1^+ charge. Fragmentation ions are also observed. The peak at *m/z* 993.4 can be assigned to the metal complex minus the *trans*-chlorodiamine platinum group.

3.3. Synthesis and characterisation of Δ- and Λ-RUP1

$[Ru(phen)_2(phendo)]^{2+}$ was chosen as the ruthenium precursor because the resolution of this complex had already been reported by Hiort et al. [15]. It was also essential that a ruthenium(II) intermediate that included a phendo ligand be used (resolved) to attach

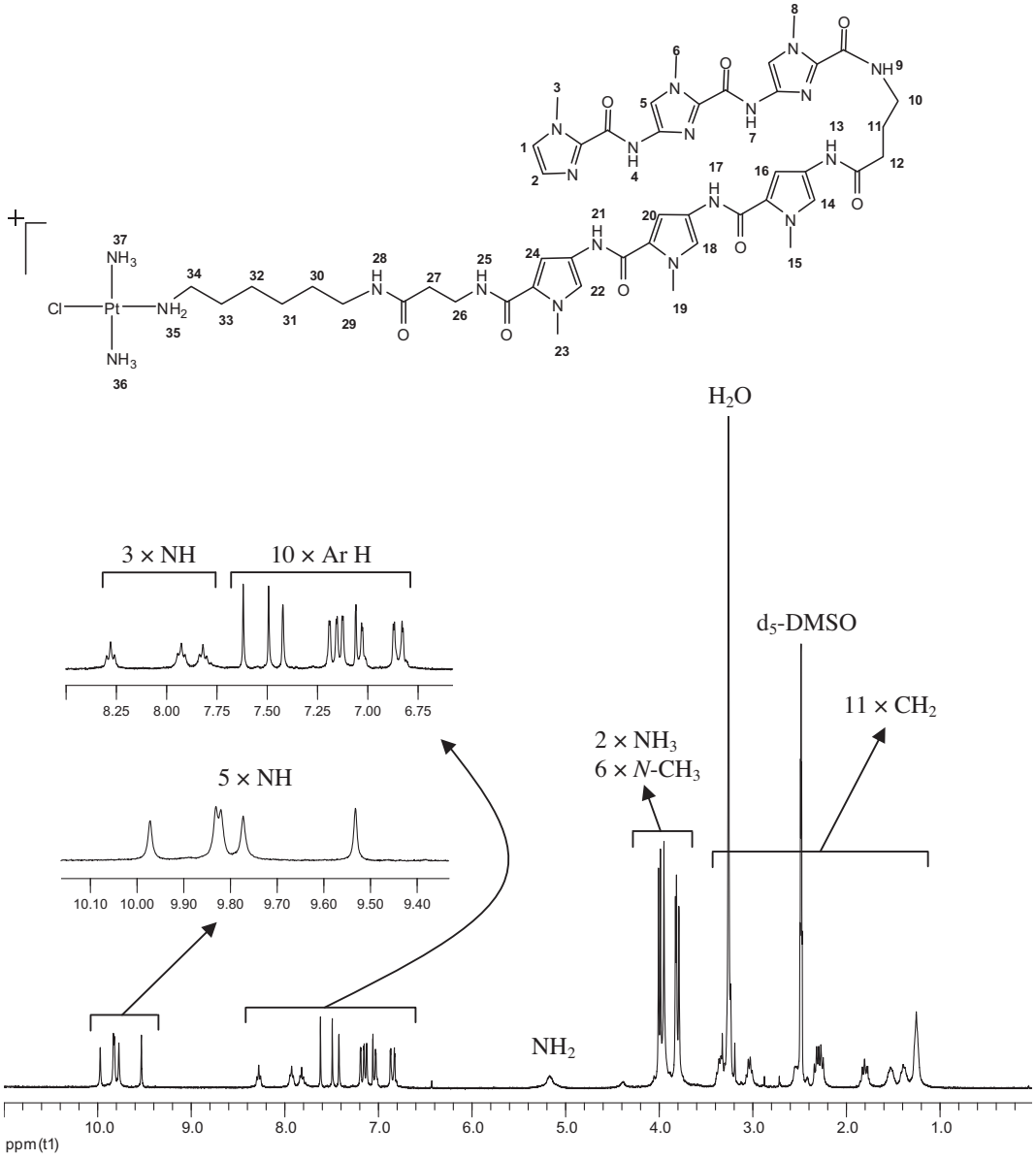

Fig. 5. The ^{1}H NMR spectrum of HSP-6 (Fig. 4.5) in d_6-DMSO at 35 °C.

a polyamide chain utilising solid phase synthesis [20]. The Δ and Λ-[Ru(phen)$_2$(phendo)]$^{2+}$ complexes isolated using the published method were in this instance of greater enantiomeric purity that had previously reported [15]. RUP1 was synthesised by solid phase chemistry using the chlorotrityl resin in a stepwise procedure as shown in Fig. 6. The synthesis was initiated with the preparation of the resin.

The monomers/linkers, Fmoc-γ-aminobutyric acid linker, ImE, Fmoc-β-alanine-OH and Fmoc-DAP-(Fmoc)-OH were added to the resin in a similar manner, after being activated with HBTU after the previous attached monomer/linker had been deprotected. The final step saw the addition of either Λ- or Δ-[Ru(phen)$_2$(phendo)](PF$_6$)$_2$ to the resin. Once the desired polyamide metal complex was synthesised, the resin was thoroughly washed with DCM, DMF, diethylether, H_2O, acetone and EtOH, to remove all unreacted materials, and dried under $N_{2(g)}$. The complex was then cleaved from the resin by the addition of TFE/acetic acid/DCM (2:1:7, v/v) mixture. The orange product was characterised by ^{1}H NMR, NOESY and ESI/MS.

From the ^{1}H NMR in Fig. 7 it can be seen that H4 and H7, and H5 and H6 are in the same chemical environment and correspond to the two signals; a doublet at 8.84 ppm (H4 and H7) and a singlet 8.46 ppm (H5 and H6). The methyl groups on the Im and Py rings resonate as singlets at 3.95 and 3.82 ppm respectively. The methyl

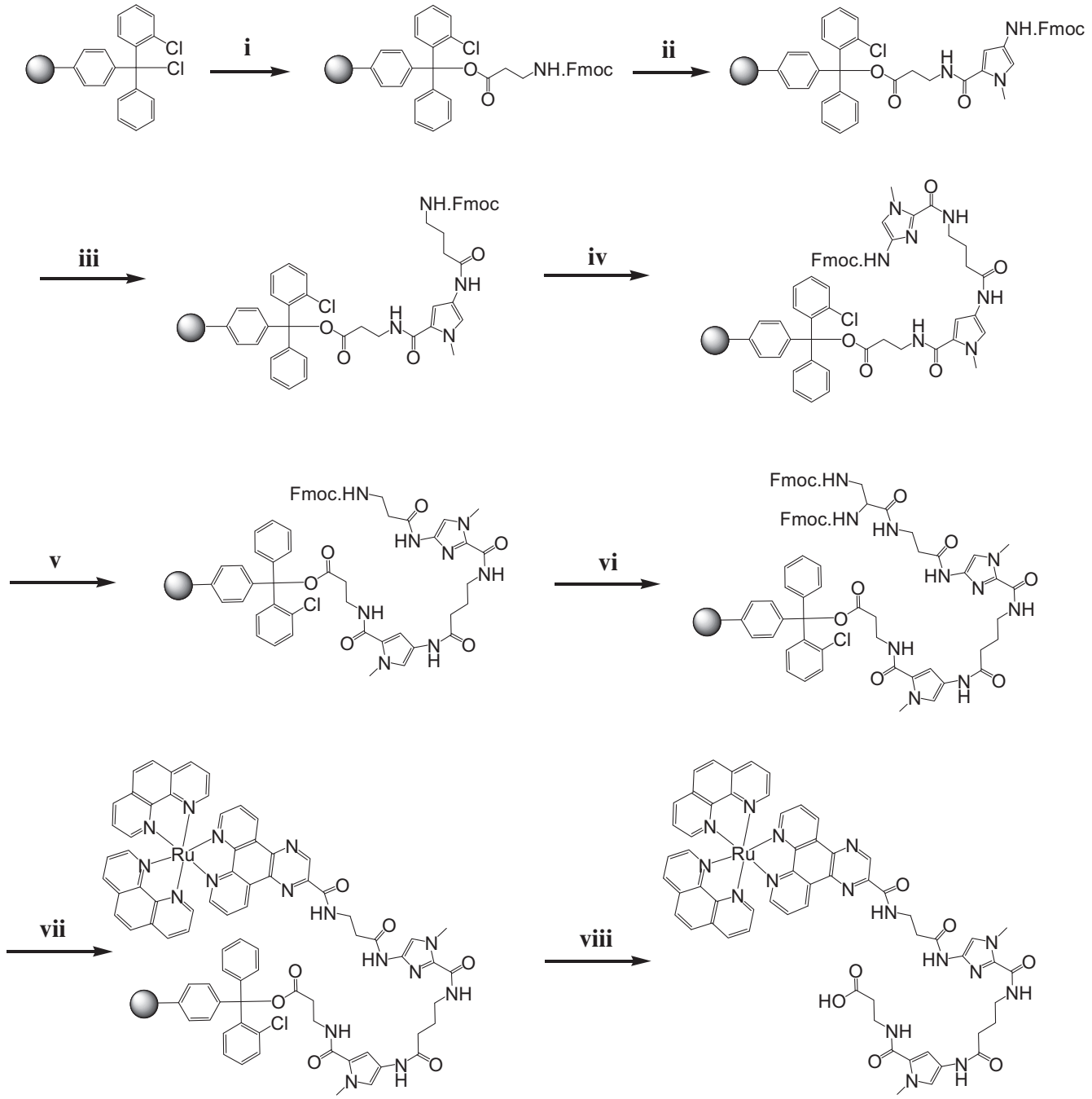

Fig. 6. The summary of the synthesis of Λ- and Δ-RUP1. The reaction conditions used were (i) DIEA, Fmoc-β-alanine-OH, 5 h; (ii) 20% piperidine in DMF, PyE, 3.5 h; (iii) 20% piperidine in DMF, Fmoc-γ-aminobutyric acid, 4 h; (iv) 20% piperidine in DMF, ImE, 4.5 h; (v) 20% piperidine in DMF, Fmoc-β-alanine-OH, 4 h; (vi) 20% piperidine in DMF, Fmoc-DAP-(Fmoc)-OH, 7 h; (vii) 20% piperidine in DMF, Δ- or Λ-[Ru(phen)$_2$(phendo)](PF$_6$)$_2$, 5 h; (viii) TFE/acetic acid/DCM (2:1:7 v/v) mixture, 2 h.

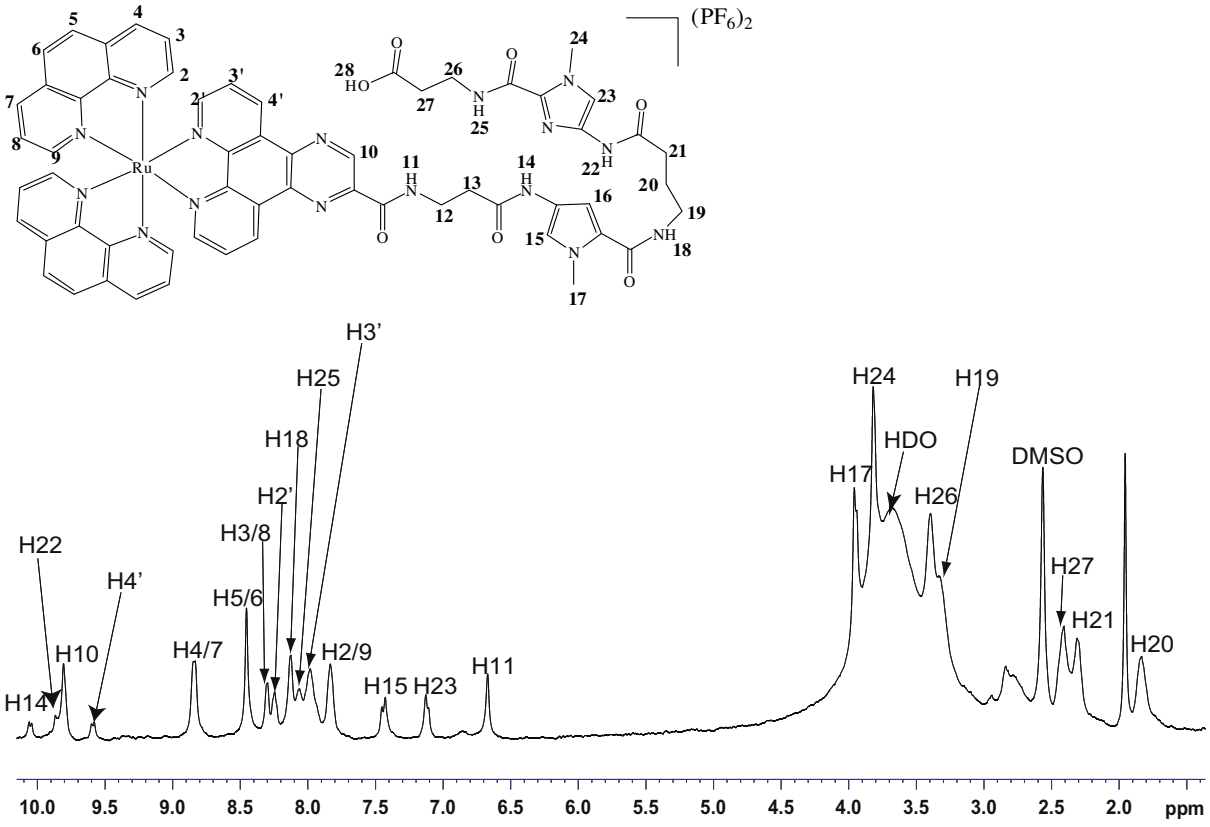

Fig. 7. The chemical structure of Λ-RUP1 along with the ^{1}H NMR spectrum in d_6-DMSO at 35 °C with labelled proton resonances.

singlets give a NOE signal (Supplementary data) to protons H15 and H23 (at 7.4 and 7.1 ppm), which correspond to the aromatic protons on the Im and Py rings respectively. Δ-RUP1 was characterised using ^{1}H NMR and NOESY and is identical to the Λ-isomer (Supplementary data).

ESI/MS experiments for the Λ- and Δ-RUP1 isomers showed that both complexes contain the parent ions carrying a 2+ charge of 605.3 (*m/z*), which was consistent with the theoretical value calculated (605.12 (*m/z*). When the UV spectra of RUP1 was compared to $[Ru(phen)_2(dpq)]^{2+}$ (Fig. 8), it was evident that that both complexes have similar transitions, with a broad metal centred transition at 450 nm, a sharp n–π^* transition at 265 nm with a shoulder at approximately 300 nm [21]. The contribution of the polyamide to these transitions was made evident from a difference UV spectra, produced by subtracting the UV of $[Ru(phen)_2(dpq)]^{2+}$ from that of RUP1. The method used to obtain the extinction coefficient for RUP1 is shown in the Supplementary data **(Figure S3 and Table 1).

The chirality of the metal precursors, Δ- or Λ-$[Ru(phen)_2(phendo)]^{2+}$ was conserved and is apparent in the CD of the products Δ- and Λ-RUP1. As the structure of either Δ- or Λ-$[Ru(phen)_2(dpq)]^{2+}$ make up the metal complexes component of the polyamide, their CD spectra were compared with to Δ- and Λ-RUP1. Slight changes in the wavelengths of the peaks were observed but more significantly there are changes in the intensities of these peaks which can be attributed to the influence of the polyamide (Fig. 9).

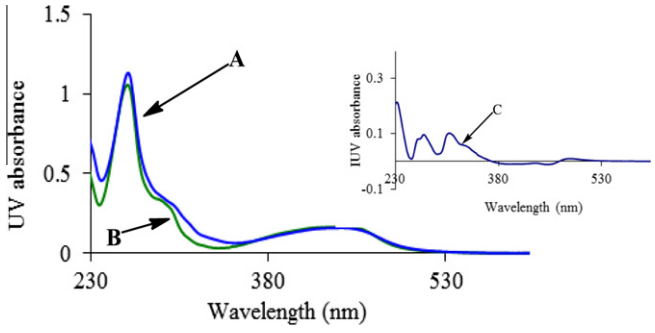

Fig. 8. The UV–Vis spectrum of RUP1 (A) and *rac*-$[Ru(phen)_2(dpq)]^{2+}$ (B) the insert shows a spectrum where UV (B) is subtracted from UV (A) this represents the polyamide contribution to the spectrum (C) in H_2O:acetonitrile (1:1 v/v) mixture at room temperature. The concentration of both complexes was 10 μM.

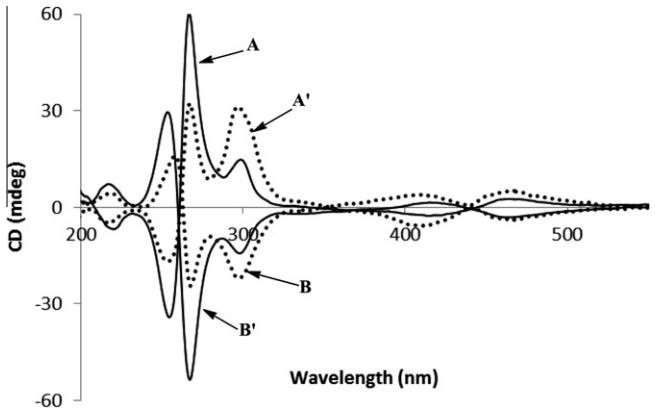

Fig. 9. The CD spectrum of Λ-RUP1 (A) and Δ-RUP1 (B), in comparison to Λ-$[Ru(phen)_2(phendo)]^{2+}$ (A′) and Δ-$[Ru(phen)_2(phendo)]^{2+}$ (B□) at 10 μM in H_2O:acetonitrile (1:1 v/v) mixture.

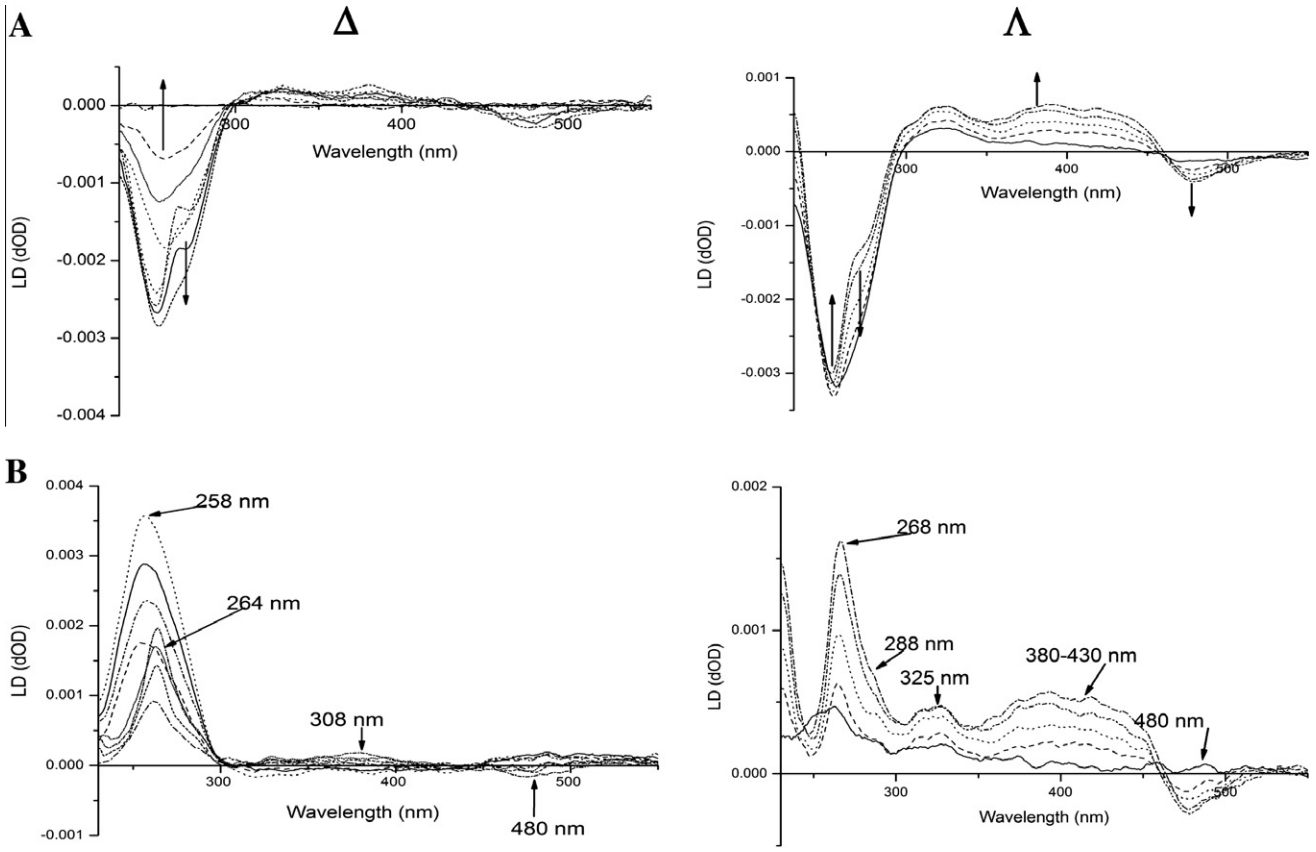

Fig. 10. (A) LD spectra of LD-DNA titration with Δ-RUP1 (left) and Λ-RUP1 (right). (B) LD spectra where the DNA spectrum has been subtracted from each LD-DNA titration spectrum of Δ-RUP1 (left) and Λ-RUP1 (right).The wavelength (nm) is plotted on the *X*-axis, while the MC complex concentration (M) on the *Y*- and the LD (dOD) on the *Z*-axis respectively.

3.4. Biological studies

3.4.1. In vitro testing. *In vitro* testing was conducted using L1210 murine leukaemia cells however HSP-2 and HSP-6 were not sufficiently soluble, even when dissolved in a small amount of DMSO or DMF, and precipitated out of the aqueous medium used for assays. As a result of this, the IC_{50} values of the platinum metallopolyamide complexes could not be determined. Ruthenium(II) complexes are not largely known for their cytotoxicity [22]. However, these tests were performed to evaluate the effect of the sequence selective 'tail' part of the molecule on overall cytotoxicity. Neither Δ nor Λ-RUP1 exhibited cytotoxicity with IC_{50} values >40 μM required to inhibit cell growth.

3.4.2. LD studies of DNA binding affinity. Previously, a ^{1}H NMR study conducted by Greguric et al. [23] reported that the Δ-[Ru(phen)$_2$(dpq)]$^{2+}$ complex interacted with a hexanucleotide through the minor groove by intercalation. Linear dichroism studies conducted by Lincoln et al. [24] indicated that the analogous [Ru(phen)$_2$(dppz)]$^{2+}$ complex intercalated within the DNA base pairs. The LD spectra of ct-DNA with the Δ- and Λ-[Ru(phen)$_2$(dpq)]$^{2+}$ and Δ- and Λ-isomers of RUP1 were measured and the resulting spectra compared. The initial LD spectrum of DNA shows a distinctive negative peak at 260 nm which is indicative of the π–π^* transitions of the DNA. Changes in the LD spectra that are produced upon the titration of Δ-[Ru(phen)$_2$(dpq)]$^{2+}$ into DNA shows that the negative intensity below 300 nm becomes more positive. Whereas the positive peak at 380 nm and the negative peak at 480 nm, due to MLCT of the metal complex, grow in intensity with increasing concentration (Supplementary data Fig. S4). The LD spectra of the titration of Λ-[Ru(phen)$_2$(dpq)]$^{2+}$ with DNA shows similar signals but with greater intensity at 380 and 480 nm (Supplementary data Fig. S4).

The LD transitions for Δ-RUP1 when bound to DNA, shows a decrease in intensity at 258 nm and a small positive at 264 nm which could indicate groove binding (Fig. 10A and B, left). Two small LD bands in the visible region are also evident with both the positive band centered at 380 nm and the negative band at 480 nm of similar magnitude like Δ-[Ru(phen)$_2$(dpq)]$^{2+}$. The difference LD shown in Fig. 10B, clearly shows the relative changes. When bound to DNA, Λ-RUP1 also shows a decrease in intensity in the LD spectrum at 268 nm with a shoulder at 288 nm and a positive signal at 325 nm (Fig. 10A and B, right). The signals due to MLCT of Λ-RUP1 grow in intensity with increasing concentration and are larger than seen for Δ-RUP1. Moreover the broad positive band at 380–430 nm is larger in intensity relative to the negative band at 480 nm. This evidence would suggest that groove binding is the mode of interaction for both Δ- and Λ-RUP1.

4. Conclusion

Four metallopolyamide complexes, two platinum(II) complexes – [Cl(NH$_3$)$_2$Pt-L$_6$-β-Ala-Py-L$_4$-Im]$^+$ (HSP-2) and [Cl(NH$_3$)$_2$Pt-L$_6$-β-Ala-PyPyPy-L$_4$-ImImIm]$^+$ (HSP-6), along with two ruthenium enantiomers, Δ and Λ-[β-Ala-Py-L$_4$-Im-β-dpq-Ru(phen)$_2$]$^{2+}$ (Δ and Λ-RUP1), were successfully synthesised in good yield by a combination of solid and solution phase chemistries. Characterisation was achieved using NMR and ESI-MS. CD for Δ- and Λ-RUP1. Confirmed that the chirality of the ruthenium precursors Δ- and Λ-[Ru(phen)$_2$(phendo)]$^{2+}$ was conserved throughout the synthesis. Cytotoxicity and binding interactions were not determined for

HSP-2 or HSP-6, which although isolated, were poorly soluble in aqueous solution inhibiting testing. Since the IC_{50} value of Δ and Λ-RUP1 were greater than 40 μM they have potential to be used as DNA probes. LD studies indicated that the ruthenium complexes interact with ct-DNA through a mixed binding mode, which is influenced by complex concentration and chirality.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ica.2012.06.013.

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