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Polyamide Platinum Anticancer Complexes Designed to Target Specific DNA Sequences

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Two new platinum complexes, *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**) have been synthesized as proof-of-concept molecules in the design of agents that can specifically target genes in DNA. Coordinate covalent binding to DNA was demonstrated with electrospray ionization mass spectrometry. Using circular dichroism, these complexes were found to show greater DNA binding affinity to the target sequence: d(CAT**TGTCA**GAC)$_2$, than toward either d(GTC**TGTCA**ATG)$_2$, which contains different flanking sequences, or d(CAT**TGAGA**GAC)$_2$, which contains a double base pair mismatch sequence. **DJ1953-2** unwinds the DNA helix by around 13°, but neither metal complex significantly affects the DNA melting temperature. Unlike simple DNA minor groove binders, **DJ1953-2** is able to inhibit, in vitro, RNA synthesis. The cytotoxicity of both metal complexes in the L1210 murine leukaemia cell line was also determined, with **DJ1953-6** (34 μM) more active than **DJ1953-2** (>50 μM). These results demonstrate the potential of polyamide platinum complexes and provide the structural basis for designer agents that are able to recognize biologically relevant sequences and prevent DNA transcription and replication.

Introduction

Cancer continues to claim millions of lives worldwide, with an estimated 7.1 million people dying from the disease in 2002.[1] One leading anticancer agent, cisplatin, is useful in treating some human cancers but is limited by both its side-affects and the ability of some cancer cells to acquire a resistance to the drug.[2−4] In the search for more efficacious and less toxic anticancer agents, new drugs are being developed which can specifically target cancerous cells without affecting normal cells.

One specific target of designer drugs could be telomeres, specialized structures at the end of eukaryotic chromosomes that modulate chromosome replication,[5,6] usually comprising a G- or C-rich repeat sequence (TTAGGG in humans).[7] During cell division in normal cells, telomeres shorten in length due to the inability of DNA polymerase to replicate the ends of linear DNA.[8,9] In contrast, many cancerous cells show no net loss of telomere length upon cell division.[10,11] Accordingly, drugs which bind to telomere sequences in DNA or block telomerase activity could specifically target cancer cells. Another possible drug target is the cancer suppressor p53 protein, which regulates cell growth by either preventing or initiating programmed cell death.[12] About 50%

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of human cancers show genetic mutations in the p53 gene, preventing normal expression.[13,14] Since cancer is the unchecked proliferation of cells, p53's role is critical. Inactivation of p53 occurs by the binding of the protein coded by the murine double minute-2 gene (MDM2).[15] The gene is overexpressed in many human cancers and effectively inactivates p53's protein function.[16] By selectively binding to the MDM2 gene in DNA and blocking p53-MDM2 complex formation, novel inhibitors can reactivate the p53 pathway and program cell death in cancerous cells.[17]

Recently, polyamides have been developed that are capable of recognizing specific double-stranded DNA sequences.[18−20] DNA sequence recognition depends on the side-by-side amino acid pairings oriented N-to-C with respect to the 5′ → 3′ direction of the DNA duplex.[21−23] The three building blocks *N*-methylpyrrole (Py), *N*-methylimidazole (Im), and *N*-methyl-3-hydroxypyrrole (Hp) form four ring-pairings (Im/Py, Py/Im, Hp/Py, Py/Hp), which distinguish all four Watson−Crick base pairs.[24] These pairing rules therefore mean that, theoretically, polyamides can be synthesized that are able to recognize DNA sequences of any length.[25] Coupling the sequence specificity of polyamides to a platinum moiety should then allow the design of a range of agents that can target telomeres or mutant p53 genes in cancerous cells.

In this paper, we report the synthesis of two proof-of-concept molecules, *trans*-chlorodiamine[*N*-(6-aminoethyl)-4-[4-(*N*-methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxamido]-*N*-methylpyrrole-2-carboxamide]-platinum(II) chloride (**DJ1953-2**) and *trans*-chlorodiamine-[*N*-(6-aminohexyl)-4-[4-(*N*-methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxamido]-*N*-methylpyrrole-2-carboxamide]platinum(II) chloride (**DJ1953-6**), which contain a polyamide group capable of recognizing 5′-TGTCA-3′ DNA sequences (Figure 1), their DNA binding, and cytotoxicity in the L1210 murine leukaemia cell line. The results of this research provide the foundation in the design and synthesis of more complex agents that will be able to specifically target mutant p53/MDM2 genes or telomeres.

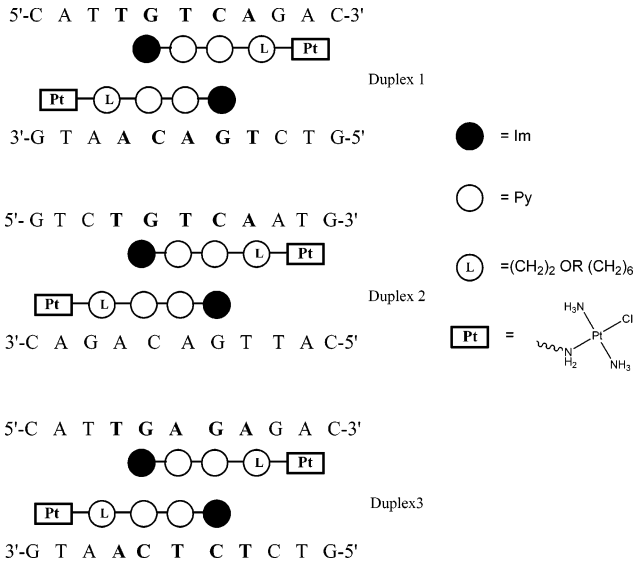

Figure 1. DNA base sequences of the three oligonucleotides studied, along with schematic binding models. The imidazole and pyrrole rings are shown as shaded and unshaded circles, respectively.

Experimental Section

Reagents. Di-*tert*-butyl dicarbonate, *N*-methylpyrrole-2-carboxylic acid, 1-hydroxy-1,2,3-benzotriazole (HOBt), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (EDCI), platinum(IV) oxide, DOWEX 550A OH anion-exchange resin, and transplatin were purchased from the Aldrich Chemical Co. *N*-methylimidazole-2-carboxylic acid was purchased from Bachem and *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium tetrafluoroborate (TBTU) from Auspep. pSP64 recombinant clone (pCC1) was donated by Suzanne Cutts, La Trobe University. pUC19 plasmid, SP6 RNA polymerase, >90% purity 15 U μL^{-1}, DTT, and optimized 5× transcription buffer was purchased from Promega. Ribonucleotide Set, 100 mM ATP, GTP, UTP, CTP solutions were purchased from Amersham Biosciences. RNA century marker was purchased from Ambion. Oligonucleotides were purchased from Geneworks, Melbourne, Australia. All other reagents and solvents used were of analytical or high purity grade.

General Methods. Reactions were monitored by thin-layer chromatography (TLC) using 0.25 mm thick precoated UV-sensitive silica gel plates. Compounds were visualized with short-wave ultraviolet light at 254 nm. Flash chromatography was carried out using Kieselgel 60 (230−400 mesh). NMR spectra were recorded on either a 300 Varian Unity*plus* or 400 MHz Varian Mercury NMR spectrometer at 25 °C using commercially available solvents referenced to TMS or internal standards.

DNA Melting. DNA melting profiles were acquired at 260 nm using a Cary 1E recording spectrophotometer equipped with a Peltier controlled cell holder and a cell length of 1 cm. The temperature gradient in all experiments was 0.5 °C min^{-1}. The oligonucleotides and metal complexes were combined in sodium phosphate buffer (10 mM), containing EDTA (1 mM) and NaCl (40 mM) adjusted to pH 7.0. The melting temperature (T_m) was taken to be the inflection point of the hyperchromic transition.

Circular Dichroism (CD). CD measurements were recorded on a Jasco J-810 CD spectropolarimeter at room temperature using a cell length of 1 cm. Titrations were performed by incrementally adding aliquots of each platinum complex to a 2600 μL solution of 5 μM oligonucleotide. After each addition, an average CD spectrum from 240 to 400 nm (20 accumulations) was recorded. The concentration of each platinum complex ranged from 0 to 10

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μM. Solutions were made in sodium phosphate buffer (10 mM), containing EDTA (1 mM) and NaCl (40 mM) adjusted to pH 7.0.

DNA Unwinding. Unwinding of negatively supercoiled DNA was carried out as reported by Loskotova and Brabec.[26] The unwinding angle of the DNA as a result of metal complex binding, per platinum−DNA adduct, was calculated by determining the r_b value at which the complete transformation of the supercoiled to relaxed form of the plasmid was attained. The pUC19 plasmid was incubated with the metal complex (dissolved in H_2O) for 1 h at 37 °C. The samples were precipitated by ethanol and redissolved in Tris/acetate−EDTA buffer (0.04 M Tris/acetate and 1 mM EDTA, pH 7.0). Aliquots of each sample were then subjected to electrophoresis on a 1% agarose gel at 25 °C, at 80 V. The gels were stained with ethidium bromide (EtBr) and visualized with an electronic UV transilluminator (Ultra Lum) using Gel Doc (Biorad) and Quantity One software.

DNA Transcription Assay. The inhibition of RNA synthesis by **DJ1953-2** was assessed using a recombinant pSP64 derivative. Seven 1.5 mL microcentrifuge tubes were setup as follows, DNA template (2 μL, 150 ng μL^{-1}, final concentration 46.8 μM), NTP (0.5 μL of each, ATP, CTP, UTP, and GTP, 100 μM), 5× transcription buffer (4 μL), BSA (0.2 μL, 0.2 μg), and DTT (2 μL, 100 mM). **DJ1953-2** was titrated into the reaction tubes at 0, 10, 20, 30, 40, 60, and 80 μM concentrations and the volume made up to 20 μL with H_2O. Experiments were commenced after reacting for 12 h (dark, 4 °C) with the addition of 15 units of SP6 RNA polymerase. An RNA marker was prepared during the incubation (1 μL RNA century marker and 9 μL H_2O). Loading buffer was added to all tubes and heated to 90 °C for 10 min. The samples were loaded hot onto a 2% formaldehyde agarose gel in FA buffer and electrophoresed at 60 V for 3 h. The gel was visualized by staining with EtBr and analyzed using Gel Doc/Quantitiy One software.

Electrospray Ionization Mass Spectrometry. All mass spectra were acquired using a Micromass (Wyntheshaw, UK) Qtof 2 spectrometer with a Z-spray probe. Reactions were performed using solutions containing 25 μM duplex DNA and either 25 or 50 μM platinum complex in 0.1 M ammonium acetate that had been adjusted to pH 8.5. Spectra were obtained approximately 0 and 4 h after mixing. Samples were diluted with 0.1 M ammonium acetate (pH 8.5) so that the final concentration of oligonucleotide was 10 μM and then injected into the mass spectrometer using a Harvard model 22 syringe pump (Natick, MA) at a flow rate of 20 μL min^{-1}. Negative-ion ESI-MS were acquired using a probe tip potential of 2500 V, a cone voltage of 50 V, and the source block and desolvation temperatures set to 60 and 80 °C, respectively. The transport and aperture were set to 2.0 and 12.0, respectively. In most experiments, spectra were acquired over the range 500−3,000 *m*/*z*. Typically 50−70 scans were summed to obtain representative spectra. The data points were calibrated against a standard CsI solution (750 mM) over the same *m*/*z* range.

Synthesis of DJ1953-2. *N*-Methyl-4-nitropyrrole-2-carboxylic acid (**1**),[27] methyl *N*-methyl-4-nitropyrrole-2-carboxylate (**2**),[28] methyl-4-(*N*-methyl-4-nitropyrrole-2-carboxamido)-*N*-methylpyrrole-2-carboxylate (**3**),[27] 4-(*N*-methyl-4-nitropyrrole-2-carboxamido)-*N*-methylpyrrole-2-carboxylic acid (**3a**),[27] and *tert*-butyl-2-[4-(*N*-methyl-4-nitropyrrole-2-carboxamido)-*N*-methylpyrrole-2-carboxamido]ethylcarbamate (**5**)[29] were made as previously described.

***tert*-Butyl-2-aminoethylcarbamate (4).** A solution of di-*tert*-butyl dicarbonate (1.00 g, 4.58 mmol) in CH_2Cl_2 (12 mL) was added over a period of 2.5 h to a solution of the 1,2-diaminoethane (2.07 g, 35.61 mmol) in CH_2Cl_2 (12 mL), which was cooled in an ice bath. The mixture was stirred at room temperature for 24 h, and the solvent removed under reduced pressure. Water (20 mL) was added, and the mixture filtered. The filtrate was extracted with CH_2Cl_2, the organic layer dried (anhydrous $MgSO_4$), and the solvent removed to yield the product, which was dried under vacuum. The product was isolated as a clear oil (0.64 g, 87%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.93 (bs, 1H), 3.15 (q, 2H, J = 6.0, 10.8 Hz), 2.77 (t, 2H, J = 6.0 Hz), 1.42 (s, 9H), 1.41 (s, 2H).

***tert*-Butyl-2-{4-[4-(*N*-methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxamido]-*N*-methylpyrrole-2-carboxamido}ethylcarbamate (6).** To **5** (0.05 g, 0.12 mmol) in MeOH (20 mL) was added PtO_2 (0.01 g), and the solution stirred under H_2(g) (1 atm) for 26 h. The catalyst was filtered through Celite, DMF (3 mL) added, and MeOH was removed under vacuum. *N*-Methylimidazole-2-carboxylic acid **5a** (0.02 g, 0.15 mmol) was added followed by HOBt (0.03 g, 0.19 mmol), TBTU (0.06 g, 0.19 mmol), and Et_3N (0.17 mL, 1.24 mmol). The solution was stirred for 1 h, solvent removed under reduced pressure, and the residue purified by flash chromatography (3−5% MeOH/CH_2Cl_2), yielding the product (0.03 g, 52%) as a yellow solid, which was dried under vacuum. ^{1}H NMR (400 MHz, d_6-DMSO): δ 10.47 (s, 1H), 9.93 (s, 1H), 7.98 (t, 1H, J = 6.0 Hz), 7.39 (d, 1H, J = 1.2 Hz), 7.28 (d, 1H, J = 1.5 Hz), 7.18 (d, 1H, J = 1.5 Hz), 7.14 (d, 1H, J = 1.8 Hz), 7.03 (d, 1H, J = 1.2 Hz), 6.86 (bs, 2H), 3.98 (s, 3H), 3.82 (s, 3H), 3.78 (s, 3H), 3.18 (m, 2H), 3.04 (m, 2H), 1.37 (s, 9H).

***N*-(2-Aminoethyl)-4-[4-(*N*-methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxamido]-*N*-methylpyrrole-2-carboxamide (6a).** Compound **6** (0.03 g, 0.06 mmol) and TFA/CH_2Cl_2 (1:1, 2 mL) containing water (40 μL) were stirred at room temperature for 1.5 h. The solvent was removed under reduced pressure, and the residue stirred with MeOH-washed DOWEX 550A OH anion-exchange resin (0.05 g, 0.17 mmol). The solution was decanted and evaporated, $CHCl_3$ (5 mL) added, and the solid (0.02 g, 90%) collected and dried under vacuum. ^{1}H NMR (400 MHz, d_6-DMSO): δ 10.49 (s, 1H), 9.96 (s, 1H), 8.13 (t, 1H, J = 6.0 Hz), 7.71 (bs, 2H), 7.40 (d, 1H, J = 1.5 Hz), 7.28 (d, 1H, J = 1.5 Hz), 7.18 (d, 1H, J = 1.5 Hz), 7.15 (d, 1H, J = 1.8 Hz), 7.04 (d, 1H, J = 1.2 Hz), 6.98 (d, 1H J = 1.5 Hz), 3.98 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H), 3.5−3.2 (m, 2H), 2.92 (m, 2H).

***trans*-Chlorodiammine[*N*-(2-aminoethyl)-4-[4-(*N*-methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxamido]-*N*-methylpyrrole-2-carboxamide]platinum(II) Chloride (DJ1953-2).** Transplatin (0.10 g, 0.34 mmol) and **6a** (0.14 g, 0.34 mmol) in H_2O (45 mL) were refluxed for 24 h until the mixture dissolved. The solution was then cooled and filtered. The solvent was removed under reduced pressure, and MeOH (10 mL) added. The solid was removed, and the filtrate concentrated. CH_2Cl_2 was added (10 mL) and stirred for 30 min. The resulting precipitate (0.15 g, 63%) was collected and dried under vacuum. ^{1}H NMR (400 MHz, d_6-DMSO): δ 11.52 (s, 1H), 10.09 (s, 1H), 8.17 (t, 1H, J = 5.4 Hz), 7.53 (s, 1H), 7.44 (s, 1H), 7.29 (s, 1H), 7.19 (s, 1H), 7.17 (s, 1H), 7.14 (s, 1H), 7.04 (s, 1H), 6.97 (s, 1H), 4.01 (s, 6H), 3.91 (s, 3H), 3.82 (s, 6H), 2.93 (m, 4H); ESI-MS *m*/*z* calcd for $C_{19}H_{31}ClN_{10}O_3Pt$ $[M + H]^+$ 677.18, found 677.0

Synthesis of DJ1953-6. Methyl-4-(*N*-methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxylate (7). Compound **2**

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(0.10 g, 0.54 mmol) in MeOH (30 mL) and Pd/C (10%, 0.02 g) were stirred under H_2(g) (1 atm) for 1 h. The mixture was filtered through Celite, DMF (3 mL) was added, and the MeOH removed under reduced pressure. *N*-Methylimidazole-2-carboxylic acid **5a** (0.08 g, 0.65 mmol) was added followed by HOBt (0.11 g, 0.82 mmol), TBTU (0.26 g, 0.82 mmol), and Et_3N (0.38 mL, 2.71 mmol). The solution was stirred for 1 h, the solvent removed under reduced pressure, and the residue purified by flash chromatography (10% MeOH/CH_2Cl_2), yielding the product (0.09 g, 64%) as a cream-colored solid. ^{1}H NMR (400 MHz, d_6-DMSO): δ 10.55 (s, 1H), 7.53 (d, 1H, J = 2.1 Hz), 7.39 (d, 1H, J = 1.0 Hz), 7.03 (d, 1H, J = 1.0 Hz), 7.02 (d, 1H, J = 1.8 Hz), 3.97 (s, 3H), 3.83 (s, 3H), 3.72 (s, 3H).

4-(*N*-Methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxylic acid (7a). To **7** (0.07 g, 0.25 mmol) in THF/MeOH (1:1, 6 mL) was added LiOH (1 M, 6 mL), and the solution was stirred at 60 °C for 1.5 h. The organic solvents were removed under reduced pressure, and the solution acidified with HCl (1 M, pH 3). The solid (0.06 g, 96%) was collected and dried under vacuum. ^{1}H NMR (400 MHz, d_6-DMSO): δ 12.20 (bs, 1H), 10.48 (s, 1H), 7.45 (d, 1H, J = 1.8 Hz), 7.38 (s, 1H), 7.03 (s, 1H), 6.97 (d, 1H, J = 1.8 Hz), 3.97 (s, 3H), 3.81 (s, 3H).

Methyl-4-[4-(*N*-methylimidazole-2-carboxamide)-*N*-methylpyrrole-2-carboxamide]-*N*-methylpyrrole-2-carboxylate (8). A solution of **2** (0.07 g, 0.40 mmol) in MeOH (30 mL) and Pd/C (10%, 0.02 g) was stirred under H_2(g) (1 atm) for 1 h. The mixture was filtered through Celite, DMF (3 mL) was added, and the MeOH was removed under reduced pressure. **7a** (0.07 g, 0.27 mmol) was added followed by HOBt (0.05 g, 0.35 mmol), TBTU (0.11 g, 0.35 mmol), and Et_3N (0.30 mL, 1.30 mmol). The solution was stirred for 1 h, the solvent removed under reduced pressure, and the residue purified by flash chromatography (2% MeOH/CH_2Cl_2), yielding the product (0.09 g, 87%) as a light brown solid, which was dried under vacuum. ^{1}H NMR (400 MHz, d_6-DMSO): δ 7.38 (d, 1H, J = 2.1 Hz), 7.33 (d, 1H, J = 2.1 Hz), 7.26 (d, 1H, J = 1.0 Hz) 7.06 (d, 1H, J = 1.0 Hz), 6.95 (d, 1H, J = 2.1 Hz), 6.94 (d, 1H, J = 2.4 Hz), 4.06 (s, 3H), 3.93 (s, 3H), 3.90 (s, 3H), 3.80 (s, 3H).

4-[4-(*N*-Methylimidazole-2-carboxamide)-*N*-methylpyrrole-2-carboxamide]-*N*-methylpyrrole-2-carboxylic Acid (8a). To **8** (0.04 g, 0.12 mmol) in THF/MeOH (1:1, 2 mL) was added LiOH (1 M, 2 mL), and the solution stirred at 60 °C for 1.5 h. The solvent was evaporated, the solution cooled and acidified with HCl (1 M, pH 3). The solid (0.04 g, 94%) was collected and dried under vacuum. ^{1}H NMR (400 MHz, d_6-DMSO): δ 12.17 (bs, 1H), 10.49 (s, 1H), 9.93 (s, 1H), 7.42 (d, 1H, J = 2.1 Hz), 7.39 (d, 1H, J = 2.1 Hz), 7.27 (d, 1H, J = 1.0 Hz) 7.17 (d, 1H, J = 1.0 Hz), 7.03 (d, 1H, J = 2.1 Hz), 6.83 (d, 1H, J = 2.4 Hz), 3.98 (s, 3H), 3.83 (s, 3H), 3.81 (s, 3H).

***tert*-Butyl-6-aminohexylcarbamate (9).** A solution of di-*tert*-butyl-dicarbonate (1.00 g, 4.58 mmol) in CH_2Cl_2 (12 mL) was added over a period of 2.5 h to a solution of the 1,6-diaminohexane (4.14 g, 35.61 mmol) in CH_2Cl_2 (12 mL), which was cooled in an ice bath. The mixture was stirred at room temperature for 24 h, and the solvent removed under reduced pressure. Water (20 mL) was added, and the mixture filtered. The filtrate was extracted with CH_2Cl_2, the organic layer dried (anhydrous $MgSO_4$), and the solvent removed to yield the product, which was dried under vacuum. The product was isolated as an oil (2.57 g, 81%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.51 (bs, 1H), 3.05 (q, 2H, J = 6.3 Hz, J = 12.9 Hz), 2.66 (t, 2H, J = 6.9 Hz), 2.15 (s, 2 H), 1.40 (s, 9H), 1.29 (m, 8H).

***tert*-Butyl-6-{4-[4-(*N*-methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxamido]-*N*-methylpyrrole-2-carboxamido}-hexylcarbamate (10).** To a mixture of **8a** (0.08 g, 0.21 mmol), **9** (0.06 g, 0.27 mmol), HOBt (0.04 g, 0.31 mmol), and TBTU (0.01 g, 0.31 mmol) in DMF (5 mL) was added Et_3N (0.30 mL, 1.30 mmol), and the solution was stirred for 1 h. The solvent was removed under reduced pressure, and the residue purified by flash chromatography (10% MeOH/CH_2Cl_2), to yield the product (0.09 g, 79%) as a light brown solid, which was dried under vacuum. ^{1}H NMR (400 MHz, d_6-DMSO): δ 10.48 (s, 1H), 9.91 (s, 1H), 7.99 (t, 1H, J = 6.0 Hz) 7.39 (d, 1H, J = 1.2 Hz), 7.28 (d, 1H, J = 1.5 Hz), 7.17 (d, 1H, J = 1.5 Hz) 7.14 (d, 1H, J = 1.8 Hz), 7.04 (d, 1H, J = 1.2 Hz), 6.84 (d, 1H, J = 1.5 Hz), 6.79 (bs, 1H), 3.98 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H), 3.12 (m, 2H), 1.49 (m, 2H), 1.35 (s, 17H).

***N*-(6-Aminohexyl)-4-[4-(*N*-methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxamido]-*N*-methylpyrrole-2-carboxamide (10a).** To a solution of **10** (0.07 g, 0.13 mmol) in CH_2Cl_2 (2 mL) was added TFA/CH_2Cl_2 (1:1, 2 mL) containing H_2O (40 μL), and the solution was stirred for 1.5 h. The solvent was removed under reduced pressure, and the residue stirred with MeOH-washed DOWEX 550A OH anion-exchange resin (0.04 g, 0.13 mmol). The solution was decanted and evaporated, $CHCl_3$ (5 mL) added, and the solid (0.05 g, 90%) collected and dried under vacuum. ^{1}H NMR (400 MHz, d_6-DMSO): δ 10.49 (s, 1H), 9.91 (s, 1H), 8.01 (t, 1H, J = 6.0 Hz) 7.94 (s, 1H), 7.59 (bs, 2H) 7.41 (s, 1H), 7.27 (s, 1H), 7.15 (s, 1H), 7.05 (s, 1H), 6.89 (s, 1H), 3.98 (s, 3H), 3.83 (s, 3H), 3.78 (s, 3H), 2.76 (m, 2H), 1.49 (m, 2H), 1.29 (m, 8H).

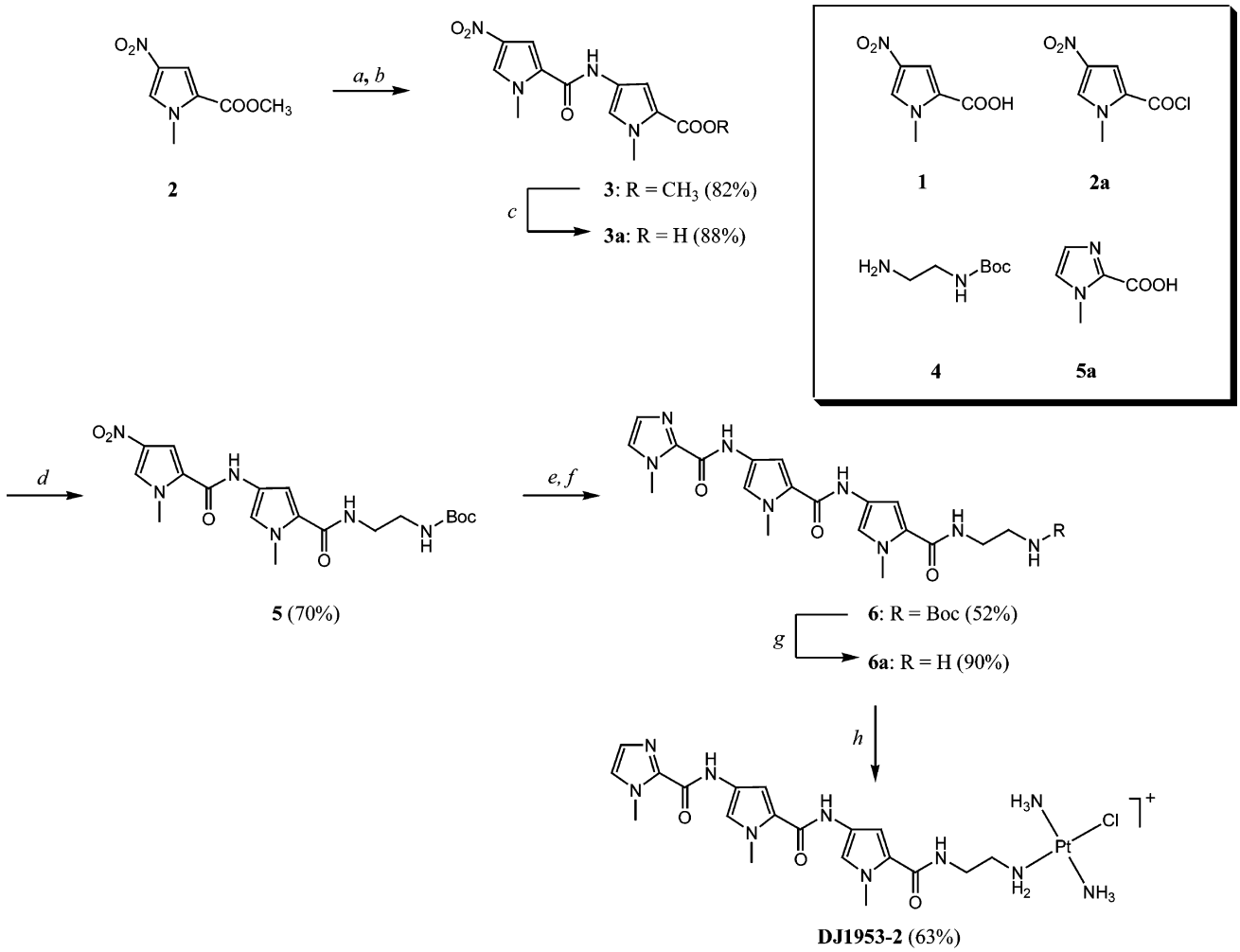
***trans*-Chlorodiammine[*N*-(6-aminohexyl)-4-[4-(*N*-methylimidazole-2-carboxamido)-*N*-methylpyrrole-2-carboxamido]-*N*-methylpyrrole-2-carboxamide]platinum(II) Chloride (DJ1953-6).** Transplatin (0.04 g, 0.13 mmol) and **10a** (0.06 g, 0.13 mmol) in H_2O (20 mL) were refluxed for 24 h until the mixture dissolved. The solution was then cooled and filtered. The solvent was removed under reduced pressure, MeOH (10 mL) added, and the solid removed. The filtrate was concentrated, CH_2Cl_2 added (10 mL) and stirred for 30 min. The resultant solid (0.09 g, 80%) was collected and dried under vacuum. ^{1}H NMR (400 MHz, d_6-DMSO): δ 11.42 (s, 1H), 10.05 (s, 1H), 8.03 (t, 1H, J = 5.4 Hz), 7.57 (d, 1H, J = 1.5 Hz), 7.44 (d, 1H, J = 1.5 Hz), 7.33 (d, 1H, J = 1.2 Hz), 7.28 (s, 1H), 7.16 (d, 1H, J = 1.5 Hz), 7.14 (d, 1H, J = 1.8 Hz), 7.11 (s, 1H), 6.89 (d, 1H, J = 1.8 Hz), 4.01 (s, 6H), 3.91 (s, 3H), 3.84 (s, 3H), 3.79 (s, 3H), 2.88 (m, 2H), 2.74 (m, 2H), 1.51 (m, 4H), 1.29 (m, 4H); ESI-MS m/z calcd for $C_{23}H_{39}ClN_{10}O_3Pt$ $[M + H]^+$ 733.25, found 733.0.

Results

Metal Complex Synthesis. The synthesis of **DJ1953-2** began with the nitration of the commercially available *N*-methylpyrrole-2-carboxylic acid to give derivative **1**. The nitro acid was protected as its methyl ester (**2**), reduced by catalytic hydrogenation, and coupled with the acid chloride (**2a**), to give **3** in 82% yield (Scheme 1). Hydrolysis of the ester and reaction of **3a** with the Boc-protected diamine **4**, in the presence of EDCI as the coupling agent, afforded the nitro intermediate **5**. Final coupling with *N*-methylimidazole-2-carboxylic acid (**5a**), after reduction of **5** with platinum-(IV) oxide, gave the protected tripeptide **6** in a 52% yield. The free amine **6a**, isolated after removal of the Boc group with trifluoroacetic acid, was stirred with DOWEX OH anion-exchange resin and then reacted with transplatin to give the metal complex in 63% yield.

In the reduction of the nitro dipyrrole (**5**), the more reactive PtO_2 was the catalyst of choice. In a number of reactions,

Scheme 1[a]



[a] Reagents and conditions: (a) 10% Pd/C, $H_{2(g)}$, MeOH, 1 h; (b) **2a**, DIEA, THF, 30 min; (c) NaOH, EtOH, 80 °C; (d) **4**, EDCI/HOBt, DCM, 20 h; (e) PtO_2, $H_{2(g)}$, MeOH, 26 h; (f) **5a**, TBTU/HOBt, Et_3N, DMF, 1 h; (g) TFA/DCM, then DOWEX 550A OH anion-exchange resin; (h) H_2O, transplatin, 100 °C, 24 h.

10% palladium on carbon gave poor results (incomplete reaction, long reaction times) even at high pressures (50−60 psi) and elevated temperatures (40−55 °C). The free amine generated was also found to be extremely unstable and was reacted without isolation. Formation of its hydrochloride was unfeasible due to the inclusion of the acid-labile Boc group.

DJ1953-6 was obtained by the successive coupling of the amine component to the corresponding activated acid (Scheme 2). The TBTU-mediated reactions using the additive HOBt, in the presence of Et_3N, allowed for the rapid construction of the growing polyamide in high yields. Thus, the nitro ester (**2**) was reduced with Pd/C in MeOH and coupled to the in situ activated acid (**5a**) to give the amide (**7**). Repeated couplings afforded compounds **8** and **10** in 87% and 79% yields, respectively. Final deprotection and attachment to transplatin gave the metal complex in 80% yield. The final compounds and intermediates were characterized by NMR and mass spectrometry. In all cases, NMR chemical shifts were comparable to published values.[27,29,30]

DNA Melting. DNA melting studies were used to assess the impact of the platinum complexes on the thermal stability of DNA. Thermal denaturation experiments were conducted with both metal complexes and three oligonucleotides in 40 mM NaCl, which is comparable to the salt concentrations used for other platinum metal complexes.[31−34] Duplex **1** $d(CATTGTCAGAC)_2$ contains a central five-base-pair sequence which is the target site for **DJ1953-2** and **DJ1953-6**. Duplex **2** $d(GTCTGTCAATG)_2$ contains the same central binding site as duplex **1** but surrounded by different flanking sequences, while duplex **3** $d(CATGAGAGAC)_2$ contains a double-base-pair mismatch sequence. In the experiments, the amount of oligonucleotide was kept constant while the metal complex concentration ratio was varied with respect to the DNA. The results demonstrated there was no significant

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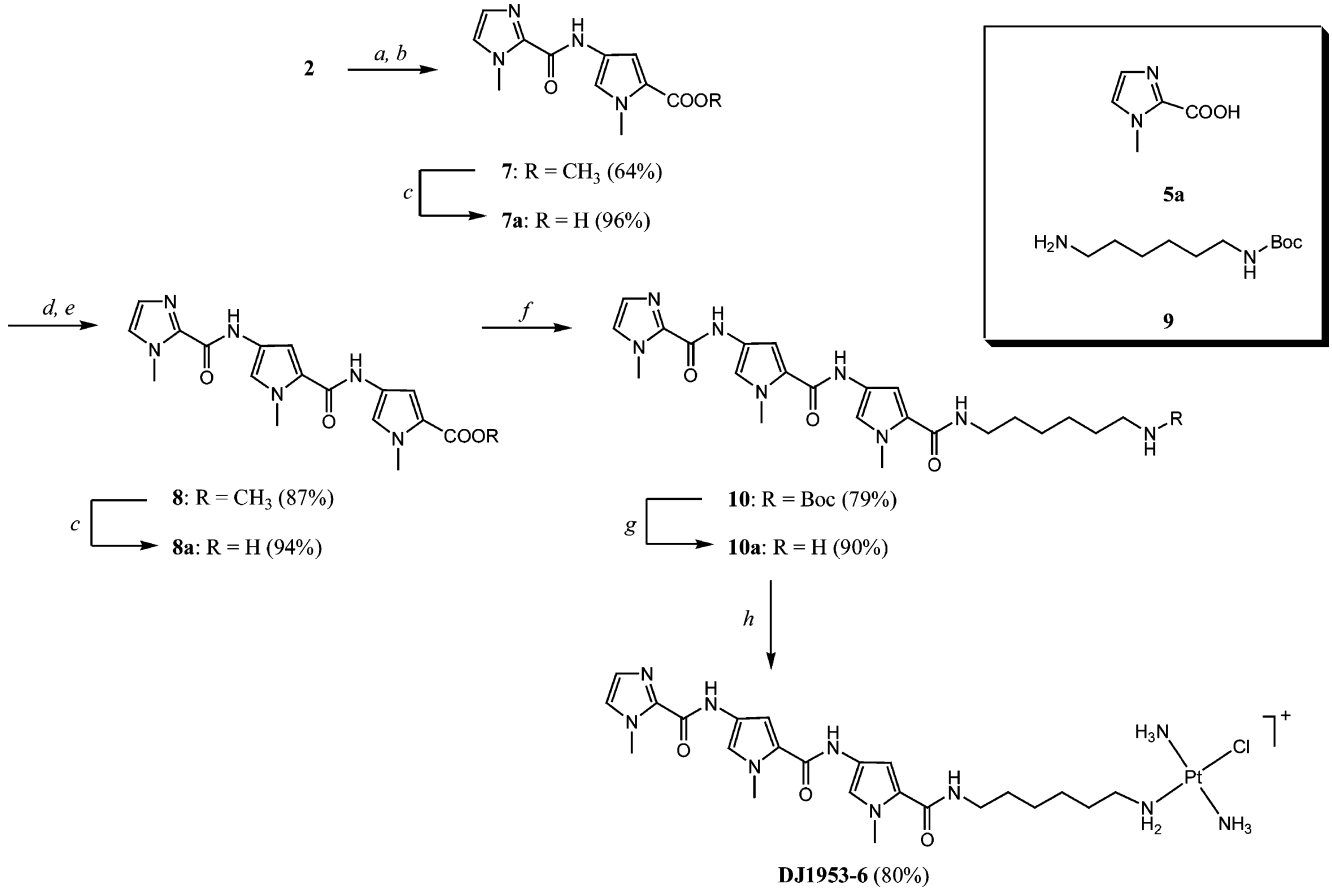
Scheme 2[a]



[a] Reagents and conditions: (a) 10% Pd/C, $H_{2(g)}$, MeOH, 1 h; (b) **5a**, TBTU/HOBt, Et_3N, DMF, 1 h; (c) LiOH, THF/MeOH, 60 °C, 1.5 h; (d) **2**, 10% Pd/C, $H_{2(g)}$, MeOH, 1 h; (e) TBTU/HOBt, Et_3N, DMF, 1 h; (f) **9**, TBTU/HOBt, Et_3N, DMF, 1 h; (g) TFA/DCM, then DOWEX 550A OH anion-exchange resin; (h) H_2O, transplatin, 100 °C, 24 h.

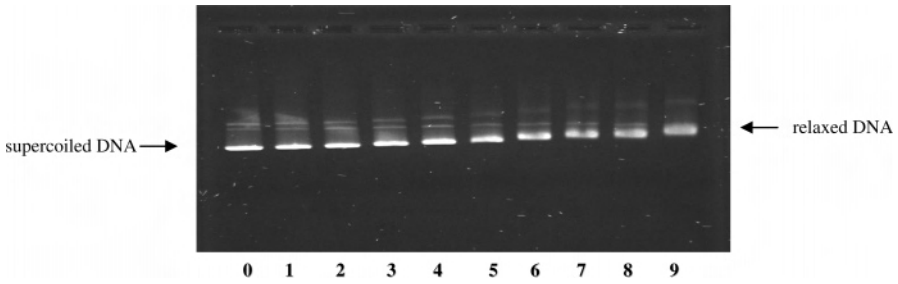


Figure 2. Unwinding of supercoiled pUC19 plasmid DNA by **DJ1953-2**. Lanes: **0** control $r_b = 0$; **1** $r_b = 0.008$; **2** $r_b = 0.\ 0.016$; **3** $r_b = 0.025$; **4** $r_b = 0.033$; **5** $r_b = 0.041$; **6** $r_b = 0.049$; **7** $r_b = 0.057$; **8** $r_b = 0.066$; **9** $r_b = 0.074$. The coalescence point (r_b) denotes the bound metal complex-to-nucleotide ratio.

change in the T_m of any of the three oligonucleotides with either metal complex.

DNA Unwinding. Unwinding of the DNA double helix is signaled by a decrease in the supercoiled density of closed circular DNA, and the degree of unwinding is determined by the rate of the DNA's migration through an agarose gel.[35] Figure 2 shows the results of gel electrophoresis on supercoiled pUC19 DNA after reaction with **DJ1953-2** at drug-to-nucleotide ratios of between 0.008 and 0.074. A general trend can be seen where migration is impeded with increasing drug concentration, consistent with an increased unwinding of DNA helix to the relaxed form. At the drug-to-nucleotide ratio of 0.074, the DNA is at maximum relaxation and corresponds to a 13° unwinding of the helix.

DNA Transcription Assay. The ability of the platinum complexes to block RNA transcription was examined using SP6 RNA polymerase and the recombinant pSP64 derivative in transcription assays. Figure 3 shows the increasing inhibition of the 335 base full length RNA transcript with increasing **DJ1953-2** concentration. Complete inhibition of RNA synthesis is observed in lane 8 at a metal complex concentration of 80 μM, and 50% inhibition of synthesis is observed at a concentration of 46.5 μM. Importantly, the parent compound, distamycin, is unable to block the elongation of growing RNA chains by DNA-dependent RNA polymerases.[36]

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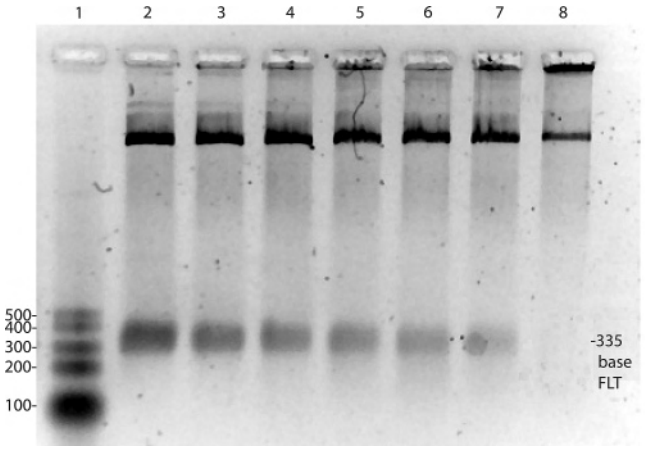

Figure 3. Inhibition of RNA synthesis on the recombinant pSP64 derivative modified by **DJ1953-2**. Lanes: **1** 100−500 base RNA marker; **2** 0; **3** 10; **4** 20; **5** 30; **6** 40; **7** 60; **8** 80 μM of **DJ1953-2**. The inhibition of the full-length transcript (FLT) can be seen at 335 bases (lane **8**).

ESI-MS DNA Binding. The reactivity of the platinum complexes toward DNA was examined through ESI-MS experiments. DNA binding profiles were measured on the reaction of both platinum complexes with DNA containing their target sequence. Spectra were obtained immediately after and 4 h after the addition of metal complex. At 0 h, no ions demonstrating oligonucleotide binding were seen. After 4 h, however, the formation of platinum−DNA adducts was observed through the molecular ions 1938.2 and 1947.6 *m*/*z* for **DJ1953-2** and **DJ1953-6**, respectively, corresponding to one monofunctional coordinate covalent adduct (complex + one oligonucleotide strand − chloride). This result demonstrates that the metal complexes form coordinate covalent adducts with DNA.

Circular Dichroism. CD spectropolarimetry was used to determine the equilibrium constant and binding strength of the platinum complexes. CD titrations were carried out by incremental addition of **DJ1953-2** and **DJ1953-6** into a fixed concentration of DNA, resulting in changes to the DNA spectra. Preferential binding was also quantified by varying the sequence of the oligonucleotides (target, mismatch, and flanking bases). CD and ICD spectra obtained for **DJ1953-2** are shown in Figure 4.

In the oligonucleotides studied, the characteristic positive and negative bands for B-DNA are observed at 280 and 245 nm, respectively. Compounds **DJ1953-2** and **DJ1953-6** bind to the oligonucleotides, as shown by the appearance of DNA-induced ligand CD bands at about 300−360 nm. The positive induction at ~320 nm is due to the UV absorption $\pi \rightarrow \pi^*$ transition of the polyamide binding in the minor groove of the platinum−DNA complex.[37−39] These DNA-induced ligand CD bands directly correlate the interactions of the metal complex with DNA, as the platinum complexes themselves do not exhibit any CD spectra. Titration of each platinum complex with duplex **1** (target site) produced an intense positive band at 321 nm. Titration with duplex **2** (flanking sequence change) gave a less pronounced positive band; however, addition of either metal complex to duplex **3** (mismatch site) produced weaker induction bands at 321 nm. The presence of the negative and positive bands of DNA at ~245 and 280 nm, respectively, suggests that the conformation of DNA in the platinum−DNA complexes remained in the B-form. The result correlates well with crystallography and NMR evidence, which shows DNA remains in the B-form when bound by distamycin and netropsin.[40,41]

Titration curves at 320 nm extracted from the CD spectra were then used to determine the equilibrium binding constants (*K*). Determination of *K* was obtained directly from the experimental data using a nonlinear least-squares fit of a simple equilibrium model, using eq 1:[42]

$$\frac{\epsilon}{\alpha} = \frac{1}{2}\left\{\frac{1}{K} + L_T + \frac{B_T}{n} \pm \sqrt{\left(\frac{1}{K} + L_T + \frac{B_T}{n}\right)^2 - \frac{4B_T L_T}{n}}\right\} \quad (1)$$

where ϵ is the extinction coefficient in mol^{-1} dm^3 cm^{-1}, L_T is the total number of ligands [L_F (free ligand) + L_B (bound ligand)], B_T is the total number of bases, *n* is the number of bases per binding site, and α is a function of wavelength. The *K* values derived by this method are presented in Table 1. Apparent binding affinities of both **DJ1953-2** and **DJ1953-6**, in general, follow the order: target site > flanking sequence change > mismatch site. The only exception is **DJ1953-6** to its mismatch sequence, where the level of binding is similar to that for the target sequence.

Cytotoxicity. The influence of polymethylene chain length on cytotoxicity was examined against L1210 murine leukemia cells. **DJ1953-6** was more active against the cell line with an IC_{50} of 34 μM than **DJ1953-2** (>50 μM) and is much more active than distamycin (133 μM)[43] but less than cisplatin (0.5 μM).

Discussion

Attachment of a sequence-specific polyamide to a monofunctional platinum moiety produces a complex with synergistic effects. Experiments measuring the thermal stability of DNA upon binding by either metal complex (**DJ1953-2** or **DJ1953-6**) demonstrated that the complexes have no net effect on helix stability, with no significant increase or decrease in melting temperature. The measured differences in melting temperature between the target site binding by the metal complex and that of the mismatch site were compared. These results are in contrast to the parent compounds distamycin and netropsin which are known to stabilize double-stranded DNA and increase its melting

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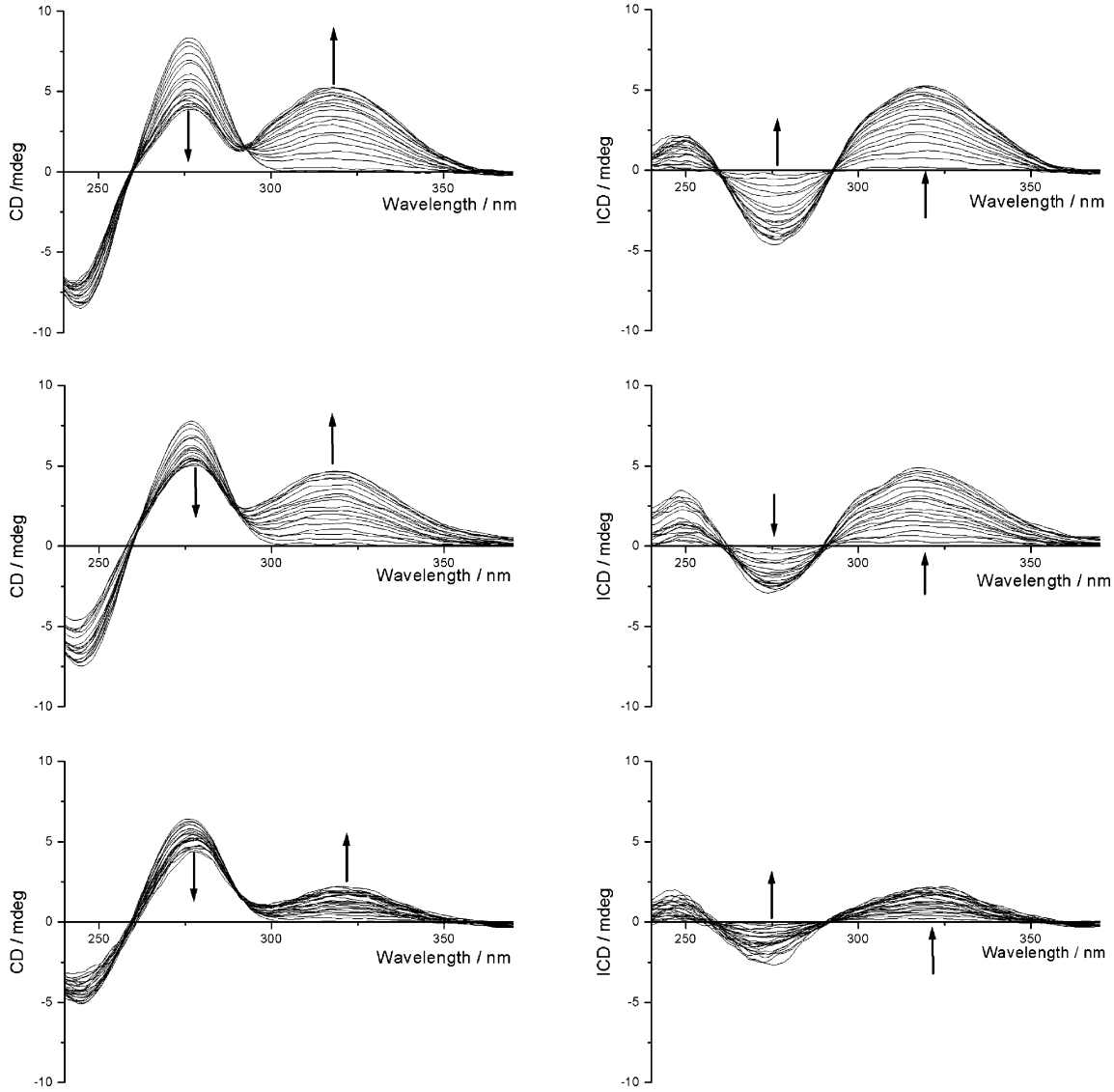

Figure 4. Circular dichroism and induced circular dichroism spectra at different binding ratios of **DJ1953-2**, with the three oligonucleotides; (top) duplex **1** d(CAT**TGTCA**GAC)$_2$-the target site; (middle) duplex **2** d(GTC**TGTCA**ATG)$_2$-flanking sequence change and (bottom) duplex **3** d(CAT**TGAGA**GAC)$_2$-mismatch site. The data were normalized by subtracting the unbound oligonucleotide spectrum.

Table 1. Equilibrium Binding Constants, *K*, Determined by the Least Squares Method[a]

	K (M^{-1})		
complex	d(CATTGTCAGAC)$_2$	d(GTCTGTCAATG)$_2$	d(CATTGAGAGAC)$_2$
DJ1953-2	1.2 ($\pm$0.1) $\times$ 10^6	5.6 ($\pm$0.2) $\times$ 10^5	3.0 ($\pm$0.1) $\times$ 10^5
DJ1953-6	2.4 ($\pm$0.1) $\times$ 10^6	4.4 ($\pm$0.2) $\times$ 10^5	1.3 ($\pm$0.1) $\times$ 10^6

[a] *K* values represent initial DNA recognition and preassociation of the metal complexes before coordinate covalent bond formation and were determined immediately after the addition of each metal complex to the oligonucleotides.

temperature.[44−46] One explanation may be due to the size of the complex. Previously it has been shown that at least four heterocyclic rings are required to increase the melting temperature of DNA.[47] Another explanation may be that, while the polyamide arm does stabilize the double helix, this effect may be negated upon binding by the platinum moiety, which may act to destabilize the helix. Some multinuclear platinum complexes, which form coordinate covalent bonds with DNA, have been shown under some conditions to decrease the melting temperature of DNA.[34]

Previously it has been shown that small molecules, such as the clinical agent cisplatin,[48] when bound to DNA result

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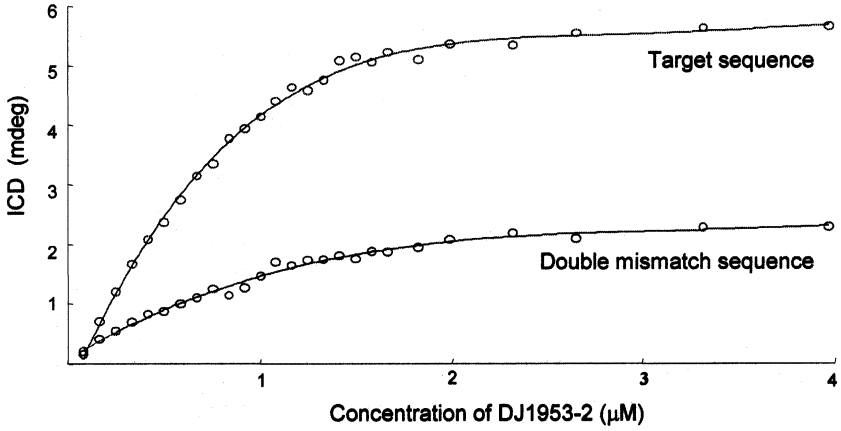

Figure 5. Binding of **DJ1953-2** to its target and double mismatch sequence. Titration curves were obtained at 320 nm.

in an unwinding of the helix by up to 13°. To some extent the ability of a complex to unwind DNA is correlated to its cytotoxicity; transplatin, the trans isomer of cisplatin only unwinds DNA by 9° and is not cytotoxic.[49] To assess the platinum complexes' functionality of binding, DNA unwinding experiments were conducted with **DJ1953-2**, which was found to unwind DNA by 13°. The parent compounds, distamycin and netropsin, which only groove bind, do not unwind supercoiled DNA by a significant amount.[26] In addition, the monofunctional platinum complexes *trans*-$[Pt(NH_3)_2(N8\text{-}Etd)Cl]^{2+}$, (Etd = ethidium), $[Pt(NH_3)_3Cl]^+$, and $[Pt(dien)Cl]^+$ have also been shown to unwind DNA by only 8° and 6°, respectively.[50] The results of this experiment therefore indicate that the polyamide component of **DJ1953-2** may act cooperatively with the platinum moiety to unwind the DNA helix. It is also possible that the complex is binding as a quasi-dinuclear platinum complex. Two **DJ1953-2** molecules may bind at the same time, with the polyamide groups of each metal complex forming an antiparallel dimer in the DNA minor groove. The formation of dimers is well established with polyamides;[21] therefore, it is likely that two metal complex molecules are acting together, and the two monofunctional platinum groups produce a DNA binding effect (like DNA unwinding) similar to a dinuclear platinum complex. Many of the multinuclear complexes reported by Farrell and co-workers, such as BBR3005, BBR3571, and BBR3464, unwind DNA by 14°, 12°, and 14°, respectively,[51,52] consistent with the unwinding caused by **DJ1953-2**.

Polyamides designed to target adjacent binding sites of transcription factors involved in HIV type 1 RNA synthesis specifically inhibit transcription in vitro.[53] The transcription factors bind to DNA in the minor groove and are likely to be inhibited by the minor-groove-binding polyamides.[54] Notably, one of the polyamide compounds of Dervan and co-workers, which bound its target sequence, was unable to inhibit transcription.[53] The result suggested that transcription of DNA can occur with a polyamide bound in the minor groove and that polyamides are only inhibitory to transcription when the compound interferes with the DNA binding activity of a required transcription factor. From transcription assays, it was observed that **DJ1953-2** can inhibit DNA transcription, again an unusual result given that many monofunctional platinum complexes and native polyamides are unable to terminate RNA synthesis;[55] the clinically ineffective compounds, $[PtCl(dien)]^+$ and $[PtCl(NH_3)_3]^+$, do not inhibit replication and transcription. These results are also consistent with a cooperative effect between the polyamide arm and the platinum moiety and/or a cooperative effect between two **DJ1953-2** complexes, acting as a quasi-dinuclear complex. Previously it has been shown that the multinuclear platinum complexes BBR3499 and BBR3464 are very efficient at preventing DNA/RNA transcription.[31,56]

The assessment of DNA conformational changes by CD demonstrated that **DJ1953-2** and **DJ1953-6** preferentially bind to selective sequences of DNA. **DJ1953-2** shows only a ~2.1-fold greater affinity for the target site [$K = (1.2 \pm 0.1) \times 10^6\ M^{-1}$] relative to the flanking sequence change site [$K = (5.6 \pm 0.2) \times 10^5\ M^{-1}$]. The complex, however, exhibits a 4-fold preference for its target site versus the mismatch site [$K = (3.0 \pm 0.1) \times 10^5\ M^{-1}$].

The difference in DNA affinity correlates well with the recognition of the side-by-side amino acid pairings. An Im/Py pair is specific for G.C (hydrogen bond between N3 of the imidazole ring and N2 of guanine), whereas a Py/Im pair targets a C.G base pair.[29,57] A Py/Py pair is partially degenerate and can bind A.T or T.A base pairs.[58] As illustrated in Figure 5, the negative Py/Im pairing of the complex in the mismatch site results in a loss of DNA affinity. The imidazole on the cytosine side of the base pair

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is unable to form a hydrogen bond with guanine's exocyclic amine, which points into the minor groove.[51,52]

DJ1953-6 binds its target sequence with a ~5.5-fold greater affinity [$K = (2.4 \pm 0.1) \times 10^6\ M^{-1}$] than its flanking sequence change site [$K = (4.4 \pm 0.2) \times 10^5\ M^{-1}$]. Only a 1.8-fold decrease in ligand binding is observed toward the mismatch site [$K = (1.3 \pm 0.1) \times 10^6\ M^{-1}$]. The complex does not strongly discriminate between its target and mismatch site. Although an increase in its binding affinity is observed, it also reduces its binding selectivity for the match site relative to a mismatch site. Interestingly, **DJ1953-6** shows a higher binding affinity compared to **DJ1953-2**, particularly with the mismatched sequence. As the only difference between the two metal complexes is the linker length, this result indicates that the linker affects binding affinity but probably not sequence selectivity.

The CD spectral characteristics of **DJ1953-2** and **DJ1953-6** are also comparable to those observed by the isomers of cisplatin tethered to distamycin [Pt-DIST(R) and Pt-DIST-(S)]. Covalent binding to ct-DNA by both of these chiral complexes also resulted in the appearance of a CD band centered at ~322 nm, which was indicative of both coordination and noncovalent binding.[26] The presence of an isoelliptic point at 289 nm reflected only one distamycin moiety binding per DNA adduct which gave rise to the induced CD band at ~322 nm.[59] Changes induced on ct-DNA, such as the loss of intensity in the positive band at around 280 nm and a decrease in the negative band at 245 nm, were distinctly different from those observed by untargeted cisplatin[60] or distamycin.[61,62] Although **DJ1953-2** and **DJ1953-6** are unable to induce irreversible DNA conformational changes (multinuclear complexes are able to induce B-to-A and B-to-Z DNA transitions), they do show the isoelliptic point at around 289 nm and display the same modified CD bands at 245 and 280 nm.

Despite the ability of **DJ1953-2** and **DJ1953-6** to unwind DNA and prevent transcription, these complexes do not show equivalent anticancer activity with IC_{50} values of >50 and 34 μM (48 h assay), respectively. These results show that at least **DJ1953-6** is more cytotoxic than distamycin (IC_{50}, 133 μM; 48 h assay),[43] indicating a significant effect by the platinum moiety. The minimal cytotoxic profiles observed may be attributed to two factors: reduced drug uptake and limited cellular accumulation or coordination of the complex in the minor groove. The polyamide segment binds in the minor groove of DNA, whereas the platinum complex forms adducts primarily in the DNA major groove. For these metal complexes, the binding of the polyamide arm may dominate and prevent platinum adduct formation. This hypothesis is supported by the cytotoxicity profile of the metal complexes, where the short polymethylene chain of **DJ1953-2** (two-carbon) results in little cytotoxicity. By tethering the platinum moiety to a longer chain, as in **DJ1953-6**, an improvement in biological activity occurs. If correct, the cytotoxicity of these platinum complexes may be altered by changing the chain length.

Conclusions

Two proof-of-concept compounds have been synthesized that combine a platinum moiety, capable of coordinate covalent bond formation with DNA, to a polyamide capable of recognizing specific DNA sequences. These metal complexes show greater binding affinity for the target sequence compared to the mismatch sequence. Both complexes can form coordinate covalent bonds with DNA but appear to form dimers in the DNA minor groove, acting as quasi-multinuclear platinum complexes.

While the cytotoxicity of either metal complex was not better than cisplatin, they are more active than the parent, naturally occurring distamycin polyamide, and provide the structural basis for a new series of extended polyamides with either mono- or dinuclear platinum complexes that can directly target specific double-stranded DNA sequences.

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