Synthesis and DNA-Binding Studies of a Dinuclear Gadolinium(III)- Platinum(II) Complex

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Dedicated to Sir John "Kappa" Cornforth, FRS for his many outstanding contributions to Organic and Biological Chemistry.

Abstract

The synthesis and characterisation of a new dinuclear Gd(III)-Pt(II) complex (**1**·PF_6) containing a functionalised macrocyclic 1,4,7,10-tetraazacyclododecane-1,4,7-triacetic acid (DO3A) derivative linked to a Pt(II)-*terpy* (*terpy* = 2,2':6',2"-terpyridine) unit by means of a short thiolato linker are reported. The complex was synthesised in six steps from cyclen by means of a modular synthetic strategy. A preliminary DNA-binding study with calf-thymus DNA (ct-DNA) was performed on the Gd(III)-Pt(II) complex using Linear Dichroism (LD). The observed changes in the DNA LD signal in the presence of the metal complex is wholly consistent with an intercalative binding mode. Furthermore, an induced negative LD signal in the UV absorption region of the complex provides strong evidence of a strong DNA-binding interaction. The *in vitro* cytotoxicity of **1**·PF_6 towards a human glioblastoma cell line (T98G) was also determined.

Introduction

Gd(III) complexes are widely used in medicine. They are extensively used as diagnostic agents in magnetic resonance imaging (MRI) as water relaxation agents to improve the image contrast,[1-3] but Gd(III) complexes have more recently shown potential as therapeutic agents. The archetypal example of a therapeutic Gd(III) agent is Motexafin-Gd (MGd), which underwent Phase I/II clinical trials as a radiosensitiser in the treatment of lung tumour metastases to the brain by means of whole-brain radiotherapy until the FDA stopped further clinical trials with this agent in late 2007.[4-9] However, Gd(III) complexes can indeed offer intriguing new avenues for cancer research with respect to binary therapies. Notable examples of binary cancer therapies include neutron capture therapy (NCT)[10-14] and photon activation therapy (PAT).[7,9] Both GdNCT and GdPAT are dependent on the production of the high linear-energy transfer Auger Coster-Krönig (ACK) electrons which result from the thermal neutron capture or photon activation reactions of the *f*-block element (or more specifically, in the case of NCT, the non-radioactive and naturally-occurring ^{157}Gd isotope). Due to the extremely short path-length (*ca.* 12nm) exhibited by ACK electrons,[15] the development of tumour-selective Gd agents which are localised nearby important macromolecules such as DNA or sub-cellular organelles such as mitochondria is critical.[10-14] Recently, both sub-cellular components have been successfully targeted by Gd(III).[10,16] The first example of a Gd(III)-Pt(II) DNA metallointercalator which was found to selectively-deliver Gd(III) to A459 lung tumour cell nuclei was reported in 2010 by Crossley, *et al.*[10] Herein, we present the next generation of DNA metallointercalators which addresses three unfavourable key factors associated with the prototype Gd(III)-Pt(II) complex: i) improved hydrolytic stability by the introduction of the macrocyclic DO3A ligand instead of the acyclic DTPA (diethylenetriaminepentaacetato) ligand, ii) decreased cytotoxicity in the absence of external X-ray photons or thermal neutrons by reducing the number of $[Pt^{II}(terpy)]^{2+}$ (*terpy* = 2,2':6',2"-terpyridine) units from two to one, and iii) increased cellular uptake by lowering the overall charge of the complex from +2 to +1. The modular synthetic strategy reported herein will also allow for the preparation of a family of related potential Gd(III) agents for potential application in GdNCT and GdPAT.

Results and Discussion

Synthesis

The target complex **1**·PF_6 was successfully synthesised as outlined in Scheme 1.

<Insert Scheme 1 here>

The ^{t}Bu-protected macrocycle DO3A-^{t}Bu$_3$ was synthesised according to the method developed by Moore and Patterson.[17] The synthesis of the linker unit first involved the protection of the thiol group in cysteamine with a triphenylmethane (trityl) group, as described by Gale, *et al.*[18] The synthesis of *N*-(2-bromoacetyl)-triphenylmethyl-cysteamine was carried out by using a modified procedure developed by O'Neil, *et al.*[19] whereby the bromoacetylation was conducted at -78 °C in a dry-ice/acetone bath with very slow dropwise addition of trityl-cysteamine.

The macrocycle DO3A-^{t}Bu$_3$ was then derivatised by using the trityl-protected thiol linker group under basic conditions in acetonitrile whilst maintaining the reaction temperature of the reaction mixture at 70°C for 5 hours. Under these optimised reaction conditions, the yield of the protected trityl-DO3A-^{t}Bu$_3$ (**2**) was significantly improved in comparison with that of the reported synthetic protocol.[20]

The global deprotection of **2** was carried out in a 1:1 solution of TFA/propanethiol in the presence of triethylsilane to yield the thiol-DO3A (**3**). Deprotection of a trityl group to a thiol functionality is often reversed by re-substitution of the trityl group, resulting in low yields of the desired product.[21] In order to improve the yield of the desired products, triethylsilane and excess propanethiol were used, acting as trityl group deactivator and *tert*-butyl scavenger, respectively.[21,22]

The final step of the synthesis involved the complexation to the two metal ions, Pt(II) and Gd(III). The chlorido ligand in [Pt(*terpy*)Cl]$^{+}$ is a reasonable leaving group and can be readily substituted by thiolato ligands in aqueous solution.[23] A one-pot synthesis employing both $GdCl_3$ and [Pt(*terpy*)Cl]Cl·2H$_2$O in the presence of the deprotected macrocycle **3** was successfully carried out to afford **1**·Cl. A metathesis reaction employing aqueous KPF_6 afforded the pure microcrystalline target complex **1**·PF_6.

Characterisation of 1·PF$_6$

High resolution ESI-MS was used to confirm the identity of **1**·PF_6. Due to several naturally-occurring isotopes of both Pt and Gd, the isotopic envelope is quite pronounced and the molecular ion peak is very distinctive. The theoretically calculated and experimentally determined ESI-MS (Figure S1) were in very good agreement with the most intense peak attributed to [M+H]$^{+}$ observed at *m/z* of 1044.16286 (Calc. *m/z* 1044.16155). The purity of

1·PF_6 was confirmed by elemental analysis and full details are given in the experimental section. Unfortunately, despite repeated attempts, crystals of **1**·PF_6 which were suitable for X-ray diffraction could not be obtained.

In Vitro Cytotoxicity Study

The *in vitro* cytotoxicity of **1**·PF_6 was assessed by means of a standard MTT assay protocol by using a human glioblastoma (T98G) cell line. The IC_{50} value was determined to be 31.3 ± 0.7 μM which demonstrates a significantly lower cytotoxicity compared to that of the prototype DNA-targeted Gd(III)-Pt(II) agent or MGd.[8,10] Interestingly, the relative cell viability in the presence of **1**·PF_6 did not significantly decrease further, even at the highest concentration (250 μM) a relative cell viability of 43.1 ± 0.5 % was observed (Figure S2). This unusual trend is possibly the result of strong non-covalent (π-π) stacking interactions between $\mathbf{1}^+$ cations in aqueous solution which might lead to a reduced cellular uptake at higher concentrations of complex due to the formation of dimers and higher-order aggregates, as has been observed previously with other Pt(II)-*terpy* complexes.[24]

Preliminary DNA-Binding Study

Linear Dichroism (LD) is a measure of the absorption difference between linearly and perpendicularly polarised light.[25-28] Unless the system in question has inherent anisotropy orientation, certain techniques are required to introduce anisotropy in samples in order to measure their LD spectrum. Orientation techniques include squeezed gel methods, electric field orientation and flow techniques. Large macromolecules such as DNA (>1000 base-pairs) can be easily oriented by the flow techniques using a flow couette cell, which was exclusively used in this study.[25]

The non-covalent binding interaction between **1**·PF_6 and calf-thymus (ct) DNA was investigated by recording the LD spectrum of a ct-DNA solution with increasing concentrations of **1**·PF_6. The concentration of ct-DNA was kept constant in order to avoid alterations in the LD signals due to viscosity changes. The experiments were carried out in phosphate saline buffer (1.0 mM PO_4^{3-} and 2.0 mM NaCl; pH 7.0). Separate solutions were made for each measurement in order to minimise the errors associated with a single solution being continuously titrated. The LD titration curves are presented in Figure 1.

<Insert Figure 1 here>

The addition of **1**·PF_6 to ct-DNA caused marked changes in the LD signal of the DNA in solution, consistent with significant structural changes in the ct-DNA when **1**·PF_6 was present. An important trend observed was the increase in the negativity of the LD signal at 260 nm, providing evidence that the ct-DNA was becoming more oriented in flow upon the addition of **1**·PF_6. This result is consistent with an intercalative binding mode by **1**·PF_6,[26] whereby the non-covalent insertion of the Pt(II)-*terpy* intercalator between the DNA base-pairs causes the ct-DNA to lengthen and stiffen. It should also be noted that an induced LD signal was observed in the 310-360 nm region. This negative signal was found to increase with increasing concentration of **1**·PF_6. The negative LD signal qualitatively provides an indication of the angle of binding of **1**·PF_6 to ct-DNA, indicating a perpendicular transition moment of the complex to the axis of polarisation. The signal in this range is most likely associated with an electronic transition within the *terpy* ligand ($\pi \rightarrow \pi^*$, 306-350 nm).[29] As $\pi \rightarrow \pi^*$ transition moments are polarised along the plane of an aromatic system, the LD spectra in Figure 1 shows that the *terpy* ligand of **1**·PF_6 must be co-planar with the ct-DNA bases and approximately perpendicular to the ct-DNA axis. These preliminary DNA binding studies provide strong evidence of an intercalative binding interaction between **1**·PF_6 and ct-DNA and, therefore, DNA intercalation is the dominant binding mode at a complex-to-base pair ratio of 1:1.

Conclusion

A new dinuclear Gd(III)-Pt(II) complex **1**·PF_6 was synthesised by means of a six-step synthesis starting from cyclen and it has been characterised by the use of high resolution ESI-MS and elemental analysis. The synthesis protocol developed in this work allows for the preparation of a family of potential Gd(III) agents for potential use in binary therapies. The *in vitro* cytotoxicity of **1**·PF_6 towards a human glioblastoma cell line (T98G) was also determined and it displayed a significantly reduced cytotoxicity compared to the previously-reported prototype,[10] a trinuclear Gd(III)-Pt(II) complex. Complex **1**·PF_6 was found to bind to ct-DNA by using the LD technique and the results are wholly consistent with a non-covalent binding, intercalative interaction. Investigations regarding the cellular uptake and biodistribution using synchrotron X-ray fluorescence (XRF) of **1**·PF_6 are planned. The exploration of the structure-activity relationships of this family of complexes represents a new avenue of research regarding GdNCT and GdPAT.

Experimental

Materials and Methods: Distilled water was used in all experiments requiring this solvent. Anhydrous CH_2Cl_2 was dried by freshly distilling from calcium hydride according to the procedures reported by Armarego and Chai.[30] All other solvents were used without prior purification. The macrocycle DO3A-^{t}Bu$_3$ was synthesised according to the method developed by Moore and Patterson.[17] The synthesis of triphenylmethyl cysteamine hydrochloride (Trityl-cysteamine hydrochloride) was carried out as described by Gale, *et al.*[18] All precursor chemicals were commercially available. Triethylenetetraamine (TETA) was purchased from Alfa Aesar and all other chemicals were purchased from the Sigma-Aldrich Chemical Company. An exceptions is [Pt(*terpy*)Cl]Cl·2H_2O which was prepared by the method described by Lowe and Vilaivan.[31] Human glioblastoma multiforme (T98G) cells were maintained as monolayers in minimum essential medium supplemented with 10% fetal bovine serum, penicillin (100 units/mL), streptomycin (100 μg/mL) and L-glutamine (2.5 mM), at 37 °C in a humidified 5% CO_2 atmosphere. All linear dichroism measurements were carried out at room temperature in 1.0×10^{-3} M phosphate buffer (pH 7) containing 2.0×10^{-3} M NaCl. Duplicates of each reading were taken. LD spectra of ct-DNA (2×10^{-4} M) were performed in the presence of **1**·PF_6 (ratio of complex:DNA ranged from 0 to 1, using increments of 0.125 except for one reading at 0.0625). The DNA in the samples was oriented by a flow couette cell (path length = 0.1 cm) rotating at a velocity generated by a 2 V power supply. The data were collected with 6 accumulations between 200 and 400 nm at a rate of 500 nm min^{-1} using a 1 nm spectral band width, 1 second response time, a step resolution of 0.5 nm and 5 L min^{-1} N_2 flow rate.

Instrumentation: ^{1}H NMR spectra were recorded at 300 K on either a Bruker AVANCE 200 spectrometer at 200 MHz or a Bruker AVANCE 300 spectrometer at 300 MHz. All $^{13}C\{^1H\}$ NMR spectra were recorded at 300 K on a Bruker AVANCE 300 spectrometer at 75 MHz. All NMR signals (δ) are reported in ppm. ^{1}H and $^{13}C\{^1H\}$ NMR spectra were referenced to tetramethylsilane (TMS) at 0 ppm or their residual solvent peaks. Low resolution ESI-MS were recorded on a Finnigan LCQ mass spectrometer. High resolution ESI-FT-ICR-MS data were recorded on a Bruker 7.0 T mass spectrometer. Cell viability was determined by measuring the absorbance at 600 nm using a Victor3V microplate reader (PerkinElmer). Linear Dichroism (LD) studies were performed on a Jasco J-710 spectropolarimeter. Elemental analyses were performed by the Campbell Microanalytical Laboratory, Otago, New Zealand.

***Biological Assays*:** The *in vitro* cytotoxicity of **1**·PF_6 was assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay.[32,33] Briefly, cells were harvested with trypsin (0.1% *v/v*), and cell pellets were isolated by centrifugation. Cells were then re-suspended to a single cell suspension, cell numbers were counted using a haemocytometer (Weber), and then cells were seeded (density 1×10^4 cells per well) in growth medium (100 μL) using 96-well plates and were allowed to adhere overnight at 37 °C. Cells were incubated at 37 °C in a humidified atmosphere of 5% CO_2 in the presence of **1**·PF_6 or the vehicle (control). The stock solution of **1**·PF_6 (10 mM) was made up in Milli-Q water and filter-sterilised prior to use. The maximum concentration (MaxC) of 250 μM (and further serial dilutions) were diluted with complete minimum essential medium. Milli-Q water was used for the vehicle control, reflecting the amount of water used for MaxC. Serial dilutions of **1**·PF_6 were added to wells with 6 repeats per concentration. Maximum concentration (MaxC) for the experiments was 250 μM and minimum concentration was 1.95 μM ($N = 4$). After 72 h, MTT solution in phosphate-buffered saline (PBS; 30 μL, 0.17% *w/v*) was added and the incubation was continued. After a further 4 h, the culture medium and excess MTT solution were removed and the resulting MTT–formazan crystals dissolved by addition of 200 μL DMSO. Cell viability was determined by measuring the absorbance at 600 nm and all readings were blank corrected before they were normalised to wells containing the absolute control (cells only), and the level of MTT was expressed relative to the corresponding treated cells as % viability. A vehicle control was used to investigate the influence of the vehicle on the cells and no significant changes were observed when compared to the absolute control. The corresponding IC_{50} value for **1**·PF_6 was determined at the dose required to induce a 50% decrease in cell viability. All experiments were conducted in quadruplicate and the IC_{50} value is reported with a standard error.

Synthesis of N-(2-bromoacetyl)-triphenylmethyl-cysteamine: The compound was synthesised by using a modified procedure reported by O'Neil *et al.*[19] Trityl cysteamine hydrochloride (1.50 g, 4.21 mmol) and triethylamine were dissolved in CH_2Cl_2 (10 mL). The solution was added dropwise to a stirred solution of bromoacetyl bromide (0.4 mL, *d* 2.32, 4.59 mmol) in CH_2Cl_2 (4 mL) at -78 °C. The reaction mixture was then allowed to warm to room temperature and it was then quenched with water (15 mL). CH_2Cl_2 (20 mL) was added and the organic layer was extracted and washed with 1 M HCl (1 × 20 mL), water (1 × 20 mL), $NaHCO_3$ (saturated, 1 × 20 mL) and brine (1 × 20 mL). The organic layer was dried over Na_2SO_4 and concentrated to a volume of 10 mL. Addition of *n*-hexane (45 mL) resulted

in the formation of colourless crystals that were filtered off and washed with cold *n*-hexane (3 × 5 mL) to yield the desired product (1.55 g, 84 %). ^{1}H NMR 200 MHz ($CDCl_3$) δ 7.52-7.40 (m, 6H, phenyl***H***), 7.41-7.21 (m, 12H, phenyl***H***), 6.56 (s, 1H, N***H***), 3.81 (s, 2H, C***H***$_2$Br), 3.11 (q, 2H, $^{3}J_{HH}$ = 6.2 Hz, C***H***$_2$NHC=O), 2.43 (t, 2H, $^{3}J_{HH}$ = 6.4 Hz, C***H***$_2$SCPh$_3$).

Synthesis of tri-tert-butyl-2,2',2''-(10-(2-oxo-2-((2-(tritylthio)ethyl)amino)ethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetate (2): A mixture of *N*-(2-bromoacetyl)-triphenylmethyl-cysteamine (435 mg, 1.22 mmol), DO3A-^{t}Bu$_3$·HBr (720 mg, 1.21 mmol) and K_2CO_3 in MeCN (20 mL) was heated at 70 °C for 5 h. The mixture was allowed to cool to room temperature and KBr was removed by filtration. The filtrate was evaporated to dryness and the residue dissolved in CH_2Cl_2 (30 mL). The solution was washed with water (30 mL), $NaHCO_3$ (saturated, 1 x 30 mL) and brine (1 x 30 mL). The organic layer was dried over Na_2SO_4 and then concentrated under reduced pressure to approximately 10 mL. To this solution was added diethyl ether (65 mL) and the product was allowed to crystallise out of solution over 3 days. Filtration yielded product **2** as colourless crystals (732 mg, 69 %). ^{1}H NMR 300 MHz ($CDCl_3$): δ 8.56 (t, 1H, N***H***, $^{3}J_{HH}$ = 5.6 Hz), 7.38 (m, 6H, phenyl***H***), 7.27 (m, 6H, phenyl***H***), 7.17 (m, 3H, phenyl***H***), 3.66-1.72 (bm, 28H, CH_2), 1.48 (s, 9H, CH_3), 1.42 (s, 18H, CH_3). ^{13}C{^{1}H} NMR 75 MHz ($CDCl_3$): δ 172.4 (C=O), 171.7 (C=O), 144.9 (C_qSCPh$_3$), 129.6 (phenyl), 127.9 (phenyl), 126.6 (phenyl), 81.8 (C), 66.4 (CH_2), 56.2 (CH_2), 55.7 (CH_2), 38.0 (CH_2), 31.9 (CH_2), 28.0 (CH_3), 28.0 (CH_3). ESI-MS: *m/z* 874.20 $[M+H]^+$; 896.20 $[M+Na]^+$.

Synthesis of 2,2',2''-(10-(2-((2-mercaptoethyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (3): The protected macrocycle **2** (350 mg, 0.40 mmol), was added to a solution of propanethiol (4 mL), and triethylsilane (0.15 mL, *d* 0.733 0.94 mmol). To this solution was added TFA (4 mL) dropwise with stirring and the reaction mixture was allowed to stir overnight at room temperature. The volatiles were removed *in vacuo*, and the remaining colourless solid was partitioned between water (30 mL) and chloroform (30 mL). The aqueous layer was collected and washed further with chloroform (2×20 mL). The solution was then concentrated to a volume of approximately 1 mL under reduced pressure. The solution was then lyophilised overnight to yield the deprotected macrocycle **3** as a very hygroscopic, colourless solid in quantitative yield (184

mg). ^{1}H NMR 300 MHz (D_2O): δ 4.16-2.70 (bm, 28H, CH_2). ESI-FT-ICR-MS for $[M+H]^+$: Calc. 464.21735; Found 464.21796.

Synthesis of (2,2',2''-(10-(2-((2-mercaptoethyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetato-N,N',N'',N''',O,O',O'')-gadolinium(III)-2,2':6',2''-terpyridineplatinum(II) hexafluorophosphate (1·PF$_6$): [Pt(*terpy*)Cl]Cl·2H_2O (45.9 mg, 0.09 mmol) and $GdCl_3 \cdot 6H_2O$ (25.7 mg, 0.07 mmol) were dissolved in water (5 mL). To this solution was added the deprotected macrocycle **3** (90.1 mg, 0.19 mmol), in water (5 mL) in a dropwise manner. An immediate colour change from orange to red-purple was observed. The reaction was allowed to stir overnight at room temperature. The reaction mixture was concentrated *in vacuo* and acetone was added to precipitate **1**·Cl as a purple solid. The solid was washed with acetone (2 × 30 mL) and then dried *in vacuo*. The purple solid was dissolved in a minimum amount of water and a saturated solution of KPF_6 was added dropwise to afford a purple precipitate. The solid was filtered off and washed with water (2 × 5 mL). Recrystallisation of the solid from water afforded **1**·PF_6 (67.5 mg, 66 %). Anal. Calc. for $C_{33}H_{40}F_6GdN_8O_7PPtS \cdot 6H_2O$: C 30.53, H 4.04, N 8.63. Found: C 30.65, H 3.80, N 8.38 %. ESI-FT-ICR-MS for $[M\text{-}PF_6]^+$: Calc. 1044.16155. Found 1044.16288.

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References

(1) Caravan, P. *Chem. Soc. Rev.* **2006**, *35*, 512.

(2) Reedijk, J. *Curr. Opin. Chem. Biol.* **1999**, *3*, 236.

(3) Zhang, Z.; Nair, S. A.; McMurry, T. J. *Curr. Med. Chem.* **2005**, *12*, 751.

(4) Forouzannia, A.; Richards, G. M.; Khuntia, D.; Mehta, M. P. *Expert Rev. Anticancer Ther.* **2007**, *7*, 785.

(5) De Stasio, G.; Rajesh, D.; Ford, J. M.; Daniels, M. J.; Erhardt, R. J.; Frazer, B. H.; Tyliszczak, T.; Gilles, M. K.; Conhaim, R. L.; Howard, S. P.; Fowler, J. F.; Esteve, F.; Mehta, M. P. *Clin. Cancer Res.* **2006**, *12*, 206.

(6) Mehta, M. P.; Shapiro, W. R.; Phan, S. C.; Gervais, R.; Carrie, C.; Chabot, P.; Patchell, R. A.; Glantz, M. J.; Recht, L.; Langer, C.; Sur, R. K.; Roa, W. H.; Mahe,

M. A.; Fortin, A.; Nieder, C.; Meyers, C. A.; Smith, J. A.; Miller, R. A.; Renschler, M. F. *Int. J. Radiat. Oncol. Biol. Phys.* **2009**, *73*, 1069.

(7) Miller, R. A.; Woodburn, K. W.; Fan, Q.; Lee, I.; Miles, D.; Duran, G.; Sikic, B.; Magda, D. *Clin. Cancer Res.* **2001**, 3215.

(8) Mody, T. D. and Sessler, J. L. *Porphyrins Phthalocyanines* **2001**, *5*, 134.

(9) William Jr., W. N.; Zinner, R. G.; Karp, D. D.; Oh, Y. W.; Glisson, B. S.; Phan, S.-C.; Stewart, D. J. *J. Thorac. Oncol.* **2007**, 745.

(10) Crossley, E. L.; Aitken, J. B.; Vogt, S.; Harris, H. H.; Rendina, L. M. *Angew. Chem., Int. Ed.* **2010**, *49*, 1231.

(11) De Stasio, G.; Casalbore, P.; Pallini, R.; Gilbert, B.; Sanita, F.; Ciotti, M. T.; Rosi, G.; Festinesi, A.; Larocca, L. M.; Rinelli, A.; Perret, D.; Mogk, D. W.; Perfetti, P.; Mehta, M. P.; Mercanti, D. *Cancer Res.* **2001**, *61*, 4272.

(12) Crossley, E. L.; Ching, H. Y. V.; Ioppolo, J. A.; Rendina, L. M. in *Bioinorganic Medicinal Chemistry,* Alessio, E. ed., Wiley-VCH, 2011, pp. 285-305.

(13) Issa, F; Ioppolo, J. A.; Rendina, L. M. in *Comprehensive Inorganic Chemistry II*, Reedijk, J.; Poeppelmeier, K. eds., Vol. 3 Oxford: Elsevier; 2013, pp. 877-900.

(14) Salt, C.; Lennox, A. J.; Takagaki, M.; Maguire, J. A.; Hosmane, N. S. *Russ. Chem. Bull.* **2004**, *53*, 1871.

(15) Goorley, T.; Nikjoo, H. *Radiat. Res.* **2000**, *154*, 556.

(16) Morrison, D. E.; Aitkin, J. B.; de Jonge, M. D.; Ioppolo, J. A.; Harris, H. H.; Rendina, L. M. *Chem. Commun.* **2014**, *50*, 2252.

(17) Moore, D. A.; Patterson, L. *Org. Synth.* **2008**, *85*, 10.

(18) Gale, E. M.; Patra, A. K.; Harrop, T. C. *Inorg. Chem.* **2009**, *48*, 5620.

(19) O'Neil, J. P.; Wilson, S. R.; Katzenellenbogen, J. A. *Inorg. Chem.* **1994**, *33*, 319.

(20) Wängler, C.; Wängler, B.; Eisenhut, M.; Haberkorn, U.; Mier, W. *Bioorg. Med. Chem.* **2008**, *16*, 2606.

(21) Raghunand, N.; Guntle, G. P.; Gokhale, V.; Nichol, G. S.; Mash, E. A.; Jagadish, B. *J. Med. Chem.* **2010**, *53*, 6747.

(22) Pearson, D. A.; Blanchette, M.; Baker, M. L.; Guindon, C. A. *Tetrahedron Lett.* **1989**, *30*, 2739.

(23) Lippard, S. J. *Acc. Chem. Res.* **1978**, *11*, 211.

(24) Todd, J. A.; Rendina, L. M. *Inorg. Chem.* **2002**, *41*, 3331.

(25) Rodger, A.; Norden, B. *Circular Dichroism & Linear Dichroism*; Oxford: University Press Oxford, 1997.

(26) Eriksson, M.; Nordén, B.; Jonathan B. Chaires, M. J. W. In *Methods in Enzymology*; Vol. 340 Academic Press; 2001, p 68.

(27) Rajendra, J.; Baxendale, M.; Dit Rap, L. G.; Rodger, A. *J. Am. Chem. Soc.* **2004**, *126*, 11182.

(28) Rajendra, J.; Rodger, A. *Chem. Eur. J.* **2005**, *11*, 4841.

(29) Bailey, J. A.; Hill, M. G.; Marsh, R. E.; Miskowski, V. M.; Schaefer, W. P.; Gray, H. B. *Inorg. Chem.* **1995**, *34*, 4591.

(30) Armarego, W. L. F.; Chai, C. *Purification of Laboratory Chemicals,* 5th Edition, 2003.

(31) G. Lowe; Vilaivan, T. *J. Chem. Res.* **1996**, 386.

(32) Hall, M. D.; Amjadi, S.; Zhang, M.; Beale, P. J.; Hambley, T. W. *J. Inorg. Biochem.* **2004**, *98*, 1614.

(33) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55.

Captions to Scheme and Figure

Scheme 1. Synthesis of **1**·PF_6.

Figure 1. LD spectra of ct-DNA (200 μM bp^{-1}) at 25 °C (phosphate saline buffer: PO_4^{3-}, 1.0 mM; NaCl, 2.0 mM; pH 7.0) in the presence of **1**·PF_6 (r_b = 0-1.0, 0-200 μM).

Scheme 1

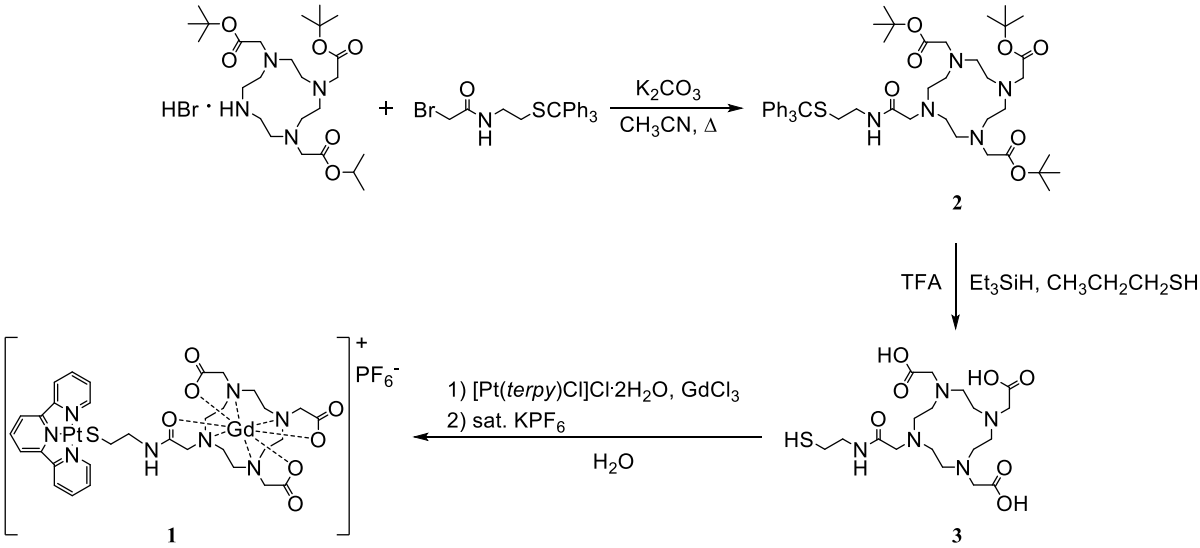
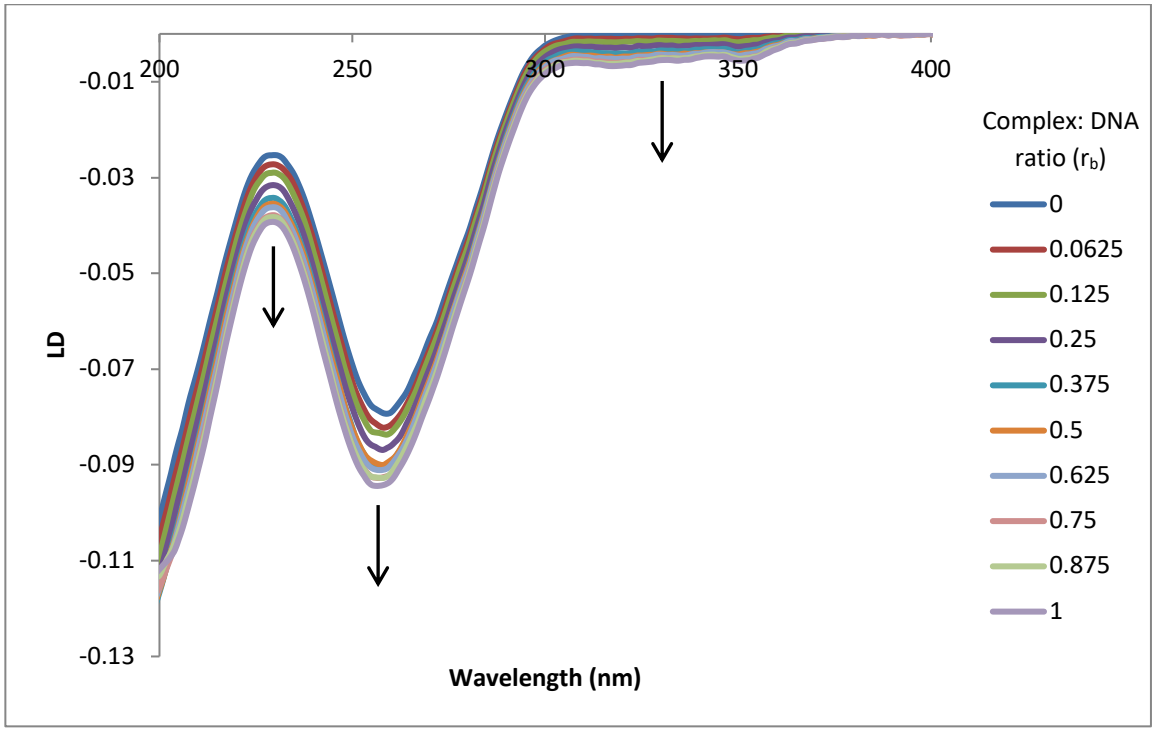

HBr · HN
Br
SCPh3
K2CO3
CH3CN, Δ
Ph3CS
2
TFA
Et3SiH, CH3CH2CH2SH
HS
HO
OH
3
1) [Pt(terpy)Cl]Cl·2H2O, GdCl3
2) sat. KPF6
H2O
PF6-
Gd
Pt
1

Figure 1


200
250
300
350
400
-0.01
-0.03
-0.05
-0.07
-0.09
-0.11
-0.13
LD
Wavelength (nm)
Complex: DNA ratio (r_b)
0
0.0625
0.125
0.25
0.375
0.5
0.625
0.75
0.875
1

Graphical Abstract

This paper reports the synthesis, characterisation, DNA-binding and *in vitro* cytotoxicity of a new dinuclear Gd(III)-Pt(II) complex (**1**·PF_6).