

Selective Aggregation of a Platinum–Gadolinium Complex Within a Tumor-Cell Nucleus**

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Gadolinium(III) complexes are widely used in magnetic resonance imaging (MRI) as water relaxation agents to improve image contrast.^[1–3] Therapeutic gadolinium-containing agents are also known in which the metal complex enhances tumor response to chemotherapeutics such as cisplatin,^[4] or, more commonly, acts as a radiosensitizer in the treatment of diseases, such as cancer.^[5–11] Gadolinium may also play an important role in therapeutic techniques, such as synchrotron stereotactic radiotherapy (SSR), in which the selective delivery of gadolinium to the cell nucleus would significantly enhance the efficacy of the treatment.^[6] Indeed, De Stasio and co-workers have demonstrated that motexafin-Gd, a gadolinium(III) complex of the pentadentate texaphyrin ligand, was accumulated by approximately 90 % of glioblastoma cell nuclei in vitro, and its potential exploitation as a GdSSR agent is warranted.^[6]

In recent years, gadolinium complexes have also been explored as potential agents in an experimental anti-cancer treatment known as gadolinium neutron-capture therapy (GdNCT),^[12–14] which is closely related to the well-established boron neutron-capture therapy (BNCT).^[15–17] GdNCT utilizes the non-radioactive ¹⁵⁷Gd isotope (natural abundance 15.7 %) in a highly effective thermal neutron-capture reaction to destroy tumor cells. ¹⁵⁷Gd possesses the largest effective nuclear cross-section of all naturally-occurring elements (2.55×10^5 barns); this value is approximately 66 times greater than that of the ¹⁰B nucleus. ¹⁵⁷Gd undergoes neutron capture to give the products of internal conversion, accompanying Auger and Coster–Kronig (ACK) electron emission and 7.94 MeV of energy. However, the very limited range of ACK electrons means that the gadolinium complex must be localized in close proximity to critical cellular components, such as the cell nucleus, if the neutron capture reaction is to be exploited effectively. The use of gadolinium(III) complexes as potential GdNCT delivery agents to brain tumors has been described,^[18–21] although the feasibility of using archetypal MRI agents such as Gd-DTPA (DTPA = diethylenetriamine-pentaacetic acid) in a clinical context for GdNCT is considered unlikely owing to the limited number of tumor-cell nuclei that have been shown to incorporate gadolinium. Indeed, the number of gadolinium compounds reported to date that have a capacity to aggregate selectively in tumor-cell nuclei, for example, is very limited,^[6] and the search for new types of gadolinium(III) complexes with high nuclear affinity has recently been proposed.^[21]

Herein, we present a new Pt-Gd complex that can effectively target the nuclei of tumor cells by means of a functionalized dtpa ligand linked to two {Pt^{II}(terpy)} (terpy = 2,2':6',2''-terpyridine) units that have the capacity to bind DNA in an intercalative manner.^[22–24] Based on prior work with analogous Pt–Ln complexes (Ln = La, Nd, Eu), which were designed to act as luminescent probes for DNA recognition,^[24] we reasoned that the related Pt-Gd species **1** would have the capacity to deliver gadolinium to this important biomolecule. In this work, we report the first unequivocal example of gadolinium delivery to a tumor-cell nucleus by a platinum complex.

Complex **1** was prepared in good yield by a similar manner to that described for the analogous Pt–Ln species (Ln = La, Nd, Eu; Scheme 1).^[24] The convenient one-pot synthesis of **1** demonstrates the high affinity of the soft Pt^{II} and hard Gd^{III} cations for the soft and hard Lewis bases (S and N/O, respectively) that are present in the functionalized DTPA ligand. The purple Pt-Gd complex has excellent solubility and stability in aqueous solution, and no evidence was found for the loss of Pt^{II} or Gd^{III} ions from **1**, even after 24 h of standing in a buffered pH 7.4 solution at room temperature.

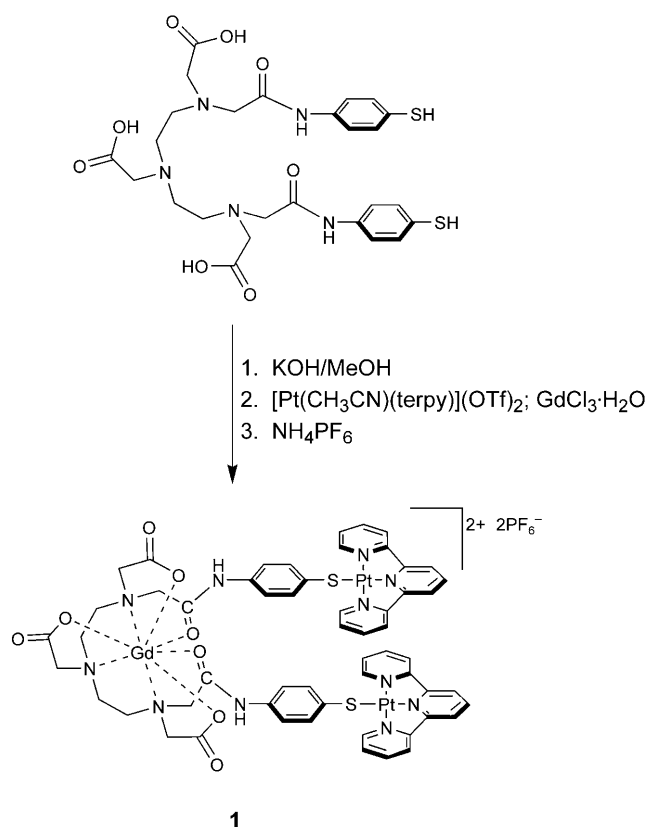
Preliminary DNA thermal denaturation (DNA melting) experiments involving calf-thymus DNA were performed on **1** at pH 7.4 (Supporting Information, Figure S1). There exists a significant difference in the melting temperatures between the free- and drug-treated DNA samples ($\Delta T_m = 4.5 \pm 0.5^\circ\text{C}$),

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Supporting information for this article, including experimental details, DNA melting plots, tables of ICP-MS data and elemental area densities, optical micrographs, scattered X-ray maps (XS), and XRF elemental distribution maps, is available on the WWW under <http://dx.doi.org/10.1002/anie.200902309>.



Scheme 1. The synthesis of **1**.

and the DNA-binding propensity of the complex follows that seen for the related Pt–Nd species^[24] and other dinuclear $\{\text{Pt}^{\text{II}}(\text{terpy})\}$ species.^[25–27]

Cellular uptake of **1** was determined by means of ICP–MS analyses of A549 human lung carcinoma cells and human aortic endothelial cells exposed to $5\ \mu\text{M}$ of the complex for 24 h. The data (Supporting Information, Tables S1 and S2) indicate that the tumor cells have a capacity to accumulate **1** by at least one order of magnitude higher than that of human endothelial cells, indicating that some tumor-selective uptake mechanism is operating for the complex. Tumor selectivity for other $\{\text{Pt}^{\text{II}}(\text{terpy})\}$ species is known^[28,29] and depends upon ligand-exchange processes involving either the transport of $\{\text{Pt}(\text{terpy})\}^{2+}$ units to tumor cells by human serum albumin^[28] or selenolate–thiolate ligand exchange in upregulated human thioredoxin reductase.^[29] Both of these mechanisms are inconsistent with the experimentally determined 2:1 molar ratio of platinum to gadolinium in both the tumor and normal cell lines. Indeed, the structural integrity of the complex appears to be maintained throughout the cell uptake process over a 24 h period, which is strongly supported by the ICP–MS data. It should be noted that robust, organometallic $\{\text{Pt}^{\text{II}}(\text{terpy})\}$ complexes bearing glycosylated acetylide and aryl-acetylide ligands show a significant degree of selectivity towards a series of human tumor-cell lines, with an approximate five-fold increase in cytotoxicity being observed when compared to a human kidney cell line.^[30]

The propensity for **1** to bind to DNA and also selectively accumulate within cancer cells in an intact manner prompted

us to investigate its uptake and bio-distribution within individual A549 human lung carcinoma cells and also its effect on endogenous elements by means of synchrotron XRF (X-ray fluorescence) imaging methods. Figure 1 shows ele-

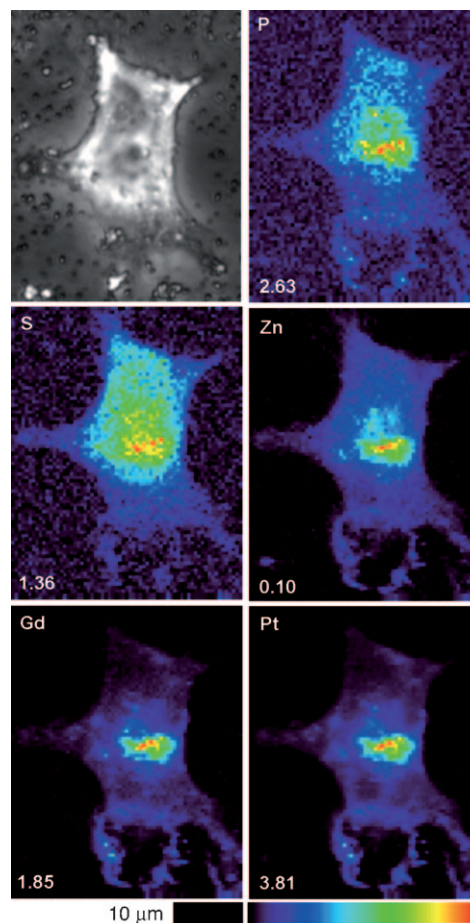


Figure 1. Optical micrograph (top left) and XRF elemental distribution maps for P, S, Zn, Gd, and Pt of a single A549 human lung carcinoma cell. Cells were treated with $5\ \mu\text{M}$ of **1** for 24 h. Maximal area densities are given in $\mu\text{g cm}^{-2}$ for each element at the bottom of each map.

mental distribution maps for a single A549 cancer cell treated with $5\ \mu\text{M}$ of **1** for 24 h. The most significant result is that high levels of both gadolinium and platinum are co-localized with the intense region in the zinc and phosphorus maps, which is assumed to define the nucleus. These results are consistent with the cellular uptake of **1** followed by its accumulation in the nucleus, and the same result was observed in all five cells measured (Supporting Information, Figures S2–S6). The fact that the gadolinium and platinum maps are essentially identical indicates that **1** remains largely intact throughout the uptake and accumulation processes, which is consistent with the results of the ICP–MS experiments (see above). Further support is given by the fact that the XRF-determined molar ratio of platinum to gadolinium in the cell matches the stoichiometry of the complex within experimental error (2:1; Supporting Information, Table S3).

XRF quantitation of intracellular elemental contents reveals an unsurprising statistically significant increase of

both platinum and gadolinium compared to control cells, where these elements exist at low background levels (Supporting Information, Table S3). Of greater interest is that potassium levels increase approximately ten-fold when cells are treated with **1**. Although most of the complex is localized in the nucleus of the cells, there is a visible and statistically significant increase in the levels in the non-nuclear region (Figure 1 and Supporting Information, Table S5). This result may account for the observed increase in intracellular potassium by an activating interaction of **1** with Na⁺/K⁺ ATPase,^[31] or by direct interaction of **1** with potassium ion channels that effectively trap K⁺ in the cell.^[32] Alternatively, **1** may indirectly downregulate potassium ion channel expression by targeting chromosomal DNA, but evidence to support any of these three hypotheses is absent.

In conclusion, we have demonstrated for the first time that a DNA–metalointercalator complex possesses the capacity to deliver gadolinium selectively to tumor-cell nuclei. Complex **1** not only remains intact during the cell uptake process but it also selectively targets the cell nuclei, presumably as a consequence of the intercalating platinum(II)-terpy moieties. We are in the process of synthesizing other gadolinium agents that have the capacity to target DNA, and GdNCT and GdSSR experiments with selected derivatives are planned. The results of this work will be reported in due course.

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