# Functionalization of osmium arene anticancer complexes with (poly)arginine: effect on cellular uptake, internalization and cytotoxicity

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Attaching peptides to metallodrugs may result in improved biological properties of the complexes. The potential use of cell penetrating peptides (CPPs) as cell delivery vectors is attractive since directed cell uptake of (metallo)drugs remains a major challenge in anticancer drug design. In this work we report the synthesis of peptide conjugates of the organometallic $Os^{II}$ anticancer complex [($\eta^6$-biphenyl)Os(picolinate)Cl]) with different arginine (Arg) chain lengths. Complexes conjugated to $Arg_5$ or $Arg_8$ at the 5-position of the picoline ring, increase Os uptake

into A2780 human ovarian cancer cells by ca. 2x and 10x, respectively, whereas a single Arg had no effect. Furthermore, a 15-fold increase in binding of Os to DNA, a potential target for these complexes, was observed for $Arg_8$ compared to the $Arg_1$ conjugate. The $Arg_5$ and $Arg_8$ conjugates exhibited fast kinetics of binding to calf thymus DNA and an ability to precipitate DNA at very low concentrations. In serum-free medium, the $Arg_8$ complex was cytotoxic ($IC_{50}$ 33 μM) and appears to be a rare example of a bioactive organometallic peptide conjugate. Experiments on CHO cells deficient in DNA repair suggested that unrepaired DNA damage contributes to the cytotoxicity of the $Arg_5$ and $Arg_8$ conjugates. These studies demonstrate the potential for use of cell- and nucleus-penetrating peptides in targeting organometallic arene anticancer complexes.

[1]Abbreviations: ACN, acetonitrile; Arg, argentine; bip, biphenyl; CPP, cell-penetrating peptide; CT, calf thymus; DMF, dimethylformamide; DIPEA, N,N-diisopropylethylamine; FCS, foetal calf serum; FMOC, fluorenylmethyloxycarbonyl chloride; ICP-MS, inductively coupled plasma mass spectrometry; pbf, 2,2,4,6,7-pentamethyldihydrobenzofurane-5-sulfonyl; PBS, phosphate buffered saline, rHA, recombinant human albumin; RP-HPLC, reverse phase high performance liquid chromatography; SPPS, solid phase peptide synthesis; TBTU, O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate; TFA, trifluoracetic acid; TIS, triisopropylsilane

## INTRODUCTION

The efficient passage of drugs into cells remains a major hurdle in anticancer drug design. Many drug candidates fail because of insufficient cell uptake, requiring high quantities of drug administration which often leads to undesirable side-effects. In recent years, several cell penetrating peptides (CPPs) with potential for improving drug delivery into cells have been discovered *(1-2)*.CPPs have been shown to carry various molecules, such as fluorophores, doxorubicin and small proteins efficiently into cells *(3)*. The most studied CPPs are arginine-rich derivatives of the HIV Tat (RKKRRQRRR) and Antennapedia (RQIKIWFQNRRMKWKK) peptides *(4)*. The importance of guanidinium groups in these peptides was demonstrated by Rothbard and Wender *et al.* who systematically replaced these cationic groups with neutral alanine residues *(5)*. This led to a strong reduction of cell uptake, correlating directly with the number of arginines substituted. Furthermore, homopolymers of arginines have been shown to cross the cell membrane more efficiently than other cationic homopolymers (*e.g.* poly-lysine, poly-ornithine and poly-histidine) *(6)*. In the same study it was shown that the ability of polyarginines to penetrate cells increased in proportion to the number of arginines. High cell uptake was observed with chain lengths of eight to fifteen arginine residues. However, chain lengths longer than fifteen arginine residues were much less successful in penetrating cells. Interestingly, homopeptides consisting of seven to nine arginines have been reported to display higher cellular uptake compared to the Tat peptide itself *(7)*.

Transition metal complexes are being actively explored as novel chemotherapeutic agents *(8-11)*. Their facile uptake into cells is important in order to achieve their therapeutic effect. Several studies exploring cell uptake of metal peptide conjugates have been reported. For example, *Barton et al.* have reported rhodium and

ruthenium-octa-arginine conjugates that are efficiently taken up by the cell and do not alter the site-specificity of the complexes *(12)*. An interesting method for the delivery of therapeutics to the cell nucleus has been described by *Metzler-Nolte et al. (13)*. This involved attachment of the SV4-40T antigen nuclear localization signal (NLS) to the cobaltocenium ion ($[Co(Cp)_2]^+$). The cobaltocenium-NLS conjugate significantly accumulated in the nucleus of HepG2 cells.

Recently we and others have reported organometallic osmium(II) arene complexes with promising cancer cell cytotoxicity *(14-21)*. Some of these complexes have been shown to induce non-repairable damage to DNA by causing a large degree of DNA unwinding *(22)*. In this work, we have attached polyarginines with varying chain lengths to osmium arene complexes and studied their in vitro toxicity, cellular uptake, cell distribution, DNA and albumin protein binding. We have investigated the effect of peptide chain length on cell and nucleus penetration, and effect on the cytotoxicity. This appears to be the first study to show that conjugation of biomolecules such as CPPs to these types of organometallic arene complexes can greatly influence their biological properties.

## MATERIALS AND METHODS

**Materials.** Parent complex **1**, ([($\eta^6$-bip)Os(picolinate)Cl]), and precursor complex **2**, ([($\eta^6$-bip)Os(4-$CO_2$-picolinate)Cl]), were synthesized and characterized as previously described *(19)*. Solid-phase bound and protected arginine derivatives were purchased from Fluka, reaction syringes (5 mL) were bought from Multisyntech (Witten, Germany). TBTU (99.5% purity) and pipiridine (99% purity) from Sigma-Aldrich. The peptides $Arg_5$ and $Arg_8$ were purchased from Clonestar Peptide Services (Brno, Czech Republic). Recombinant human albumin (rHA) (batch A-1887, Lot

14H9319), supplied by Sigma, was dialyzed against 100 mM $NH_4HCO_3$, pH 7.9, and stored as a solution at 277 K. Calf thymus (CT) DNA (42 % G + C, mean molecular mass ca. 2 x $10^7$) was prepared and characterized as described previously *(23-24)*.

Tissue culture reagents were obtained from Invitrogen (Paisley, UK). The A2780 ovarian cancer cell line was obtained from the ECACC (European collection of cell culture, Salisbury, UK). The cells were maintained in RPMI 1640 and were supplemented with 10% foetal calf serum, 1% L-glutamine and 1% penicillin/streptomycin. All cells were grown at 310 K in a humidified atmosphere containing 5% $CO_2$.

**Synthesis of peptide-conjugates compounds 3–6.** The syntheses of compounds **3**–**6** were performed manually in a syringe equipped with a porous filter using Fmoc solid-phase procedures. Lysine was bound to Wang resin (50 mg, 20 μmol, loading 0.4-0.6 mmol/g), and also arginine was bound to Wang resin (40 mg, 20 μmol, loading 0.4-0.8 mmol/g). Arginine (FMOC protected and pbf side-chain protected, pbf= 2,2,4,6,7-pentamethyldihydrobenzofurane-5-sulfonyl), lysine (FMOC protected) and precursor complex **2** (e.g. [($\eta^6$-bip)Os(4-$CO_2$-pico)Cl]) were used. Synthesis cycle: (1) Fmoc deprotection using 3 mL of 20% piperidine solution in DMF for 10 min with shaking on a IKA Vibrax VXC basic shaker for 4 h at 500 g/min. (2) DMF wash five times with 2 mL DMF while shaking for about 1 min. (3) Coupling for 20 min while shaking using; the corresponding monomer (0.06 mmol, 3-fold excess), TBTU (0.058 mmol, 2.9-fold excess) and DIPEA (0.042 mL, 0.24 mmol, 12-fold excess, activation period of 1 min). (4) DMF wash 5 times with 2 mL DMF while shaking for about 1 min. Final cleavage: the resin was washed five times with 2 mL dichloromethane with 1 min shaking each wash. The syringes were dried in a vacuum dessicator for 2 h. The final deprotection and cleavage from the resin was

performed with a 2 mL mixture (v/v) of 95:2.5:2.5, TFA:$H_2O$:TIS (TIS = triisopropylsilane) for 2 h at ambient temperature. Work up: the cleavage solution and a 1 mL TFA wash were combined and poured into cold ether (10 mL, 280 K). The suspension was centrifuged (8000 rpm, 10 min), and washed twice with 10 mL of cold ether. The crude product was dissolved in water, filtered and purified by semi-preparative HPLC using gradient: 5% B > 40 % B in 40 min, A: $H_2O$ + 0.1 % TFA, B : ACN + 0.1 % TFA (ACN = acetonitrile and TFA = trifluoroacetic acid).

**[($\eta^6$-bip)Os(pico-C(O)-Lys)Cl] (3).** $M_r$ ($C_{25}H_{27}ClN_4O_4Os$) = 673.2, ESI-MS (+ve): 640.1 [($\eta^6$-bip)Os(pico-C(O)-Lys)OH + H]$^+$, 320.6 [($\eta^6$-bip)Os(pico-C(O)-Lys)OH + 2H]$^{2+}$.

**[($\eta^6$-bip)Os(pico-C(O)-Arg)Cl] (4).** $M_r$ ($C_{25}H_{27}ClN_6O_4Os$) = 702.1, ESI-MS (+ve): 668.1 [($\eta^6$-bip)Os(pico-C(O)-Arg)]$^+$, 334.6 [($\eta^6$-bip)Os(pico-C(O)-Arg) + H]$^{2+}$.

**[($\eta^6$-bip)Os(pico-C(O)-(Arg)$_5$)Cl] (5).** $M_r$ ($C_{49}H_{74}ClN_{21}O_9Os$) = 1328.0, ESI-MS (+ve): 1292.5 [($\eta^6$-bip)Os(pico-C(O)-(Arg)$_5$)]$^+$, 645.8 [($\eta^6$-bip)Os(pico-C(O)-(Arg)$_5$) + H]$^{2+}$, 431.5 [($\eta^6$-bip)Os(pico-C(O)-(Arg)$_5$) + 2H]$^{3+}$.

**[($\eta^6$-bip)Os(pico-C(O)-(Arg)$_8$)Cl] (6).** $M_r$ ($C_{67}H_{66}ClN_{33}O_{12}Os$) = 1796.9, ESI-MS (+ve): 599.6 [($\eta^6$-bip)Os(pico-C(O)-(Arg)$_8$)Cl + 3H]$^{3+}$, 440.97 [($\eta^6$-bip)Os(pico-C(O)-(Arg)$_8$) + 4H]$^{4+}$, 352.98 [($\eta^6$-bip)Os(pico-C(O)-(Arg)$_8$) + 4H]$^{5+}$.

**ESI/HRMS.** Electrospray ionisation mass spectra were obtained on a Bruker MicroTOF spectrometer. Samples were prepared either in water and the cone voltage and source temperature varied depending on the sample. Data were processed using DataAnalysis 3.3 (Bruker Daltonics).

**RP-HPLC.** Purification of the complexes RP-HPLC was performed on a HP 1100 Series HPLC Systems (Agilent) using a semipreperative C-18 PLRP-S (7.5 mm x 300 mm, 300 Å/8 μm; polymer laboratories, UK) column.

***In vitro* growth inhibition assay.** The human ovarian A2780 cancer cells were plated in a 96-well plate at a density of 5000 cells/well in medium containing fetal calf serum (FCS). On day 3, the FCS-containing medium was removed and fresh FCS-free medium was supplied and the cells treated with $Os^{II}$ complexes, $Arg_5$ or $Arg_8$ at concentrations ranging from 0.5 $\mu$M to 200 $\mu$M. Cells were exposed to the complexes for 24 h, washed, supplied with fresh FCS containing medium, allowed to grow for three doubling times (72 h), and then the protein content measured (proportional to cell survival) using the sulforhodamine B (SRB) assay *(25)*.

The CHO-K1 cell line (wild type) and its mutant cell line MMC-2 were kindly supplied by Dr. M. Pirsel from the Cancer Research Institute, Slovak Academy of Sciences, Bratislava (Slovakia). These cells were grown in DMEM medium and the $IC_{50}$ values were determined in quadruplicate following the protocol given above.

**Drug uptake, DNA adduct formation and cytosol/nucleus fractionation.** A2780 Cells were plated at a density of $3\times10^6$ cells/100 mm for complexes **3**–**6** and $6\times10^6$ cells/100 mm for complex **2**, in a Petri dish in 12 mL of FCS-containing culture medium on day 1 (three dishes were prepared per compound tested and three untreated control plates). On day 2, the FCS-containing medium was removed and fresh FCS-free medium was supplied and the cells were exposed to the $Os^{II}$ arene complexes at a concentration of 35 μM. Stock solutions (0.2 mM) of osmium compounds were made up fresh in saline before being diluted in FCS-free medium to give a final concentration of 35 μM. After 24 h drug exposure, cells were trypsinised and the cell suspension counted. One-third of the cells was centrifuged, washed with PBS and stored at 253 K until analyzed for Os content. The remaining two-thirds was used for DNA extraction using the Nucleon genomic DNA extraction kit (GE healthcare, Amersham, UK) (BACC-1 protocol) and cytosol/nucleus fractionation

using the cell fractionation kit (Biovision, Mountain View, US) (fractionPREP$^{TM}$). All samples were stored at 253 K until analysis for Os content.

**ICP-MS analysis.** The whole cell pellets, DNA samples and cytosol/nucleus fractionation samples were digested as described below. To the cell pellet was added 0.8 mL and to the extracts 0.4 mL of freshly-distilled 72% $HNO_3$ and the samples were then transferred into wheaton v-vials® (Aldrich). The vials were heated in an oven at 373 K for 16 h. The vials were allowed to cool and then each sample was transferred to a volumetric tube. The vials were washed with doubly-deionised water (DDW) and diluted 10 times with DDW to obtain 7.2% $HNO_3$ sample solutions.

**ICP-MS Instrumentation and Calibration.** All ICP-MS analysis was carried out on an Agilent Technologies 7500 series ICP-MS machine. The settings for ICP-MS are listed in Table S1. All water used for ICP-MS analysis was purified using a USF Elgar UHQ instrument. The osmium Specpure plasma standard (Alfa Aesar, 1000 ppm in 5% HCl) was diluted with Milli-Q water to 20 ppm. The standards for calibration were freshly prepared by diluting this stock solution with 3% $HNO_3$. The concentrations used were 60, 20, 10, 5, 4, 2, 1, 0.4, 0.2 and 0.1 ppb. With the instrumentation settings listed in Table S1, the detection limit was typically 2 ppt (n=10), and sensitivity 6188 $^{189}$Os ion counts for 5 ppb of Os standard in helium mode and 10 ppt (n=10), and sensitivity 35900 $^{189}$Os ion counts for 5 ppb of Os standard in no gas mode.

**Albumin Binding.** The kinetic course of the reactions of recombinant human albumin (rHA) with complex **5** was followed with ICP-MS. Reactions were carried out in triplicate at 0, 0.25, 1, 2, 4, 8 and 24 h at 310 K. After the reaction time, the two fractions were separated by ultrafiltration using three cycles of centrifugation (30 min.

at 277 K, 2516 g) with a 30 kDa cutoff filter. Samples were diluted five times with doubly deionised water (DDW) and stored at 253 K for ICP-MS analysis.

**Metalation reactions in a cell-free medium.** CT DNA was incubated with $Os^{II}$ complex in 10 mM $NaClO_4$ (at pH 6) at 37 ℃ in the dark. The amount of the metal complex was determined by flameless atomic absorption spectrometry (FAAS). Absorption spectra were measured with a Beckman DU 7 4000 spectrophotometer equipped with a thermoelectrically controlled cell holder and quartz cells with the pathlength of 1 cm. The FAAS measurements were carried out on a Varian AA240Z Zeeman atomic absorption spectrometer equipped with a GTA 120 graphite tube atomizer.

## RESULTS

**Synthesis and characterization of peptide conjugates.** Unfunctionalized biphenyl $Os^{II}$ picolinate complex **1** and conjugation precursor *p*-carboxy picolinate complex **2** were synthesized as previously reported *(15-19)*. Complexes **3** and **4** containing a single lysine and arginine, respectively, and complexes **5** and **6**, conjugates with five and eight arginines (Figure 1), respectively, were synthesized using solid phase methods (SPPS) as illustrated in Figure 2. The Fmoc protected amino acids were activated and coupled to the resin with a TBTU/DIPEA mixture. In the final step, complex **2** was coupled to the resin with the same reaction mixture. Cleavage from the resin was performed with standard Fmoc cleavage conditions (95% TFA), since osmium complex **2** was found to be stable under these strongly acidic conditions. The final products were purified using reverse-phase semi-preparative HPLC.

Two peaks assignable to the aqua adduct and the parent chlorido compound were observed in the RP-HPLC chromatogram (substitution of Cl by $H_2O$). In addition, in all chromatograms of conjugates **3**–**6**, a small amount of compound **2** was observed in both its aqua and chloride parent forms (see Figure S1 for an example of a RP-HPLC trace for **6**). To ensure that the correct fractions had been isolated, the separated peaks were analysed using Micro-TOF mass spectrometry. Peaks belonging to several positively-charged species of the conjugates were observed in the mass spectra of all conjugates (see Figure 3 for **6**), due to the ease of protonation of the arginine under neutral aqueous conditions.

**Cytotoxicity.** We have previously found that osmium complex **1** exhibits promising activity in the A2780 human ovarian cancer cell line ($IC_{50}$ = 4.5 μM) *(15)*. First we investigated whether the peptide conjugates would retain this activity. Moderate cytotoxicity was observed for complexes **5** and **6** when tested in fetal-calf-serum (FCS)-free conditions during the 24 h drug exposure time. The cytotoxicity increases with increase in length of the Arg chain, with an $IC_{50}$ value of 71.5 μM ± 2.5 for $Arg_5$ compound **5**, and 32.9 μM ± 3 for $Arg_8$ compound **6**. The dose-response curves for **5** and **6** are shown in Figure 4. For control purposes, the $IC_{50}$ of cisplatin was also determined using the same assay (0.69 μM ± 0.15, Figure S2).

In contrast, under the same conditions, no growth inhibition of A2780 human ovarian cancer cells was observed for the $Arg_5$ and $Arg_8$ peptides alone (up to concentrations of 200 μM, i.e. $IC_{50}$ > 200 μM).

To assess the importance of DNA damage in the cytotoxic effects of Os-$Arg_5$ complex **5** and Os-$Arg_8$ complex **6**, their $IC_{50}$ values in the Chinese hamster ovary CHO-K1 cell line (wild type) and in its mutant cell line MMC-2 carrying the ERCC3/XPB mutation, were also determined (Table 1). ERCC3 is an ATP-dependent

DNA helicase that plays an essential role in nucleotide excision repair *(26)*. The complexes are more potent in MMC-2 cells (mutant) than in the parental WT cell line. The $IC_{50}$ values for Os-$Arg_5$ complex **5** and Os-$Arg_8$ complex **6** in CHO-K1 were significantly different (>1.7 x) from that in MMC-2, $p<0.05$

**Cellular uptake.** The cellular uptake of complexes **1**, **4-6** in the ovarian A2780 cancer cell line was compared to determine if the CPP-osmium conjugates show increased cellular uptake compared to unconjugated osmium complex **1**. Osmium uptake in A2780 cells was determined by ICP-MS after a 24 h exposure to the compounds in FCS-free medium. The highest intracellular levels of osmium were observed after exposure to the octarginine complex **6** (329.3±37.7 pmol Os/$10^6$ cells) followed by the pentaarginine compound **5** (73.4±4.7 pmol Os/$10^6$ cells), with the lowest osmium concentrations being found for the monoarginine complex **4** and parent complex **1** (37.8±4.2 pmol Os/$10^6$ and 37.2±4 pmol Os/$10^6$ cells, respectively, Figure 5).

**Cell distribution and DNA binding.** The osmium content in the nuclear and cytosolic fractions isolated from A2780 cells after 24 h exposure to compounds **1**, **4-6** in FCS-free medium was determined, and the results are shown in Figure 6. For complexes **4-6**, the sum of these fractions accounted for only about 40% of the whole cellular Os uptake, while for complex **1**, 80% of the whole cell uptake was accounted for.

The cell distribution for unfunctionalized osmium complex **1** is very similar to that of monoarginine complex **4**. The amount of osmium localizing in the cell nucleus increases with increasing arginine chain length (**6** > **5** > **4** > **1**, Figure 6A). This amounts to 11% (**1**), 22% (**4**), 32% (**5**) and 44% (**6**) of the total amount of osmium in

the fractions localizing in the cell nucleus, and 89% (**1**), 78% (**4**), 68% (**5**) and 56% (**6**) of osmium localizing in the cytosolic fractions (Figure 6B).

To investigate whether DNA is also a potential target for the osmium-bioconjugates, DNA was isolated from A2780 cells treated with complexes **4**–**6** and the levels of osmium on DNA were determined by ICP-MS (Figure 7). The osmium content of DNA followed the order **6** > **5** > **4** (3.86 ±0.56 pmol Os/$10^6$ for **6**, 0.87 ±0.27 pmol Os/$10^6$ cells for **5** and 0.26 ±0.08 pmol Os/$10^6$ cells for **4**).

**Binding to Albumin.** The protein albumin constitutes about 60% of serum. To investigate whether binding of the peptide conjugates to albumin was responsible for the inactivity of **3**–**6** when tested in FCS containing medium, complex **5** was incubated with recombinant human albumin (rHA) in a 1:1 mol ratio for 0.25, 1, 2, 4, 8 and 24 h in 100 mM ammonium bicarbonate buffer. The fractions containing bound and unbound **5** were isolated using molecular weight cut-off filters (30 kDa) and analysed by ICP-MS (Figure 8). Within 15 min of mixing., ca. 41% of complex **5** was bound to rHA. The amount of osmium bound to rHA increased slightly after 1 and 2 h (to ca. 43% and 48%, respectively). After 24 h about 15% of osmium complex **5** remained unbound (Figure 8).

**DNA binding in cell-free media.** The finding that the peptide conjugates are capable of delivering osmium to DNA in the cell nucleus prompted us to examine the binding of $Os^{II}$-arene peptide conjugates to DNA in a cell free medium. The rate of binding of complexes **1, 5** and **6** to CT DNA was determined at a $r_i$ value (the molar ratio of free metal complex to nucleotide-phosphates at the onset of incubation with DNA) of 0.01 in 10 mM $NaClO_4$ at 310 K in the dark. $Os^{II}$ arene complexes were incubated with CT DNA, aliquots were removed at various time intervals, rapidly cooled and precipitated out by addition of ethanol. The osmium content in the

supernatant was determined by flameless atomic absorption spectrometry (FAAS) (Figure 9). The rate of DNA binding increased with increase in length of the arginine chain, with a $t_{50\%}$ value (the time at which the binding reached 50%) of 114 ± 8 min for compound **1**, 21 ± 2 min for compound **5** and 15 ± 1 for compound **6**. Complexes **1**, **5-6** bound to CT DNA almost quantitatively (91-93%) after 48 hours.

The binding of complexes **1, 5** and **6** to CT DNA at various $r_i$ values was also examined. CT DNA (4 μg, for all samples) was incubated with different concentrations of $Os^{II}$ arene complex in the $r_i$ value range of 0–0.06 in different initial volumes (25 μl, 50 μl, 75 μl and 100 μl). After the incubation (10 mM $NaClO_4$ at 37 ℃ in the dark for 48 h) the volume of each sample was adjusted to 100 μL. The samples were subsequently centrifuged (14 000g, 20 min) and the absorbance of the supernatant was measured at 260 nm. The absorbance of CT DNA incubated with complex **1** was independent of $r_i$ in the range of $r_i$ values tested (not shown). In contrast, the absorbance was independent of the concentration of complex **5** or **6** only up to $r_i$ values of ca. 0.02 and 0.01, respectively. At higher $r_i$ values the absorbance started to decrease (Figure 10).

## DISCUSSION

The aim of the study was to investigate the possibility of conjugating cell penetrating peptides (CPPs) to organometallic osmium(II) anticancer complexes and determine whether this would lead to increased cell and nuclear uptake into ovarian A2780 cancer cells. Also the ability of the conjugates to bind to DNA was assessed as this is the proposed target for the parent complex **1**. It should be noted that the parent picolinate complex itself (**1**) is taken up by cells without the attachment of a CPP *(27)*. The attachment of a CPP may, however, not only increase cellular uptake but also

improve delivery of the osmium complexes to the cell nucleus and the potential target site, DNA. With this goal, the well known CPP octa-arginine was attached to osmium arene complex **2** and its cell penetration and internalization capabilities compared to unfunctionalized complex **1**. Also osmium(II) arene peptide conjugates with either five arginines or a single arginine were prepared, in order to establish the role of the peptide chain length.

**Synthesis and Characterization.** Four osmium arene-peptide conjugates were synthesized using solid phase peptide synthetic (SPPS) methods. The carboxylate group on the 4-position of the chelating picolinate ligand of osmium arene complex **2** (Figure 1) provided a suitable functionality for coupling by SPPS. Cleavage from the resin and deprotection of the pbf side-chain protecting groups of arginine was achieved in strong acidic conditions of 95% TFA (see Figure 2 for the general synthetic procedure). The carboxylate derivative **2** was found to be very stable in such strong acidic conditions. This in combination with the good solubility of **2** in DMF and the strategically placed carboxylic acid functionality, made compound **2** a good candidate for peptide conjugation using SPPS.

The conjugates were purified using reverse-phase semi-preparative HPLC, and for all four compounds peaks assignable to the aqua adduct were visible in the chromatogram during separation indicating that hydrolysis of the osmium-chloride bond occurs rapidly (Figure S1). The side-chains of arginine and lysine amino acids are strongly basic and are therefore readily protonated and observed in neutral aqueous conditions by mass spectrometry (Figure 3). These multiple charges confer good aqueous solubility on the conjugates.

**Cancer Cell Cytoxicity.** Complexes **3**–**6** were inactive at concentrations of up to 150 μM when tested in media containing 10% foetal calf serum (FCS). Albumin is

the most abundant serum protein (~60%), and it seemed likely that the peptide conjugates were binding strongly to albumin resulting in the loss of activity. There are several reported cases where the ability of certain CPPs to penetrate cells in vitro was enhanced in the absence of serum in the media *(7, 28-29)*.

To test this hypothesis, the cells were treated with compounds for 24 h in a medium which was free of FCS. Cytotoxic activity was found for the pentaarginine conjugate **5** ($IC_{50}$ 71.5 ± 2.5 μM), and increasing the chain length from five to eight arginines (complex **6**) resulted in a more than two-fold increase in cytotoxicity ($IC_{50}$ 32.9 ± 3.0 μM , Figure 4). Complexes **3** and **4**, conjugated with one lysine and one arginine, respectively, were non-toxic up to concentrations of 400 μM, probably because the additional positive charge on the complex hinders uptake and one such amino acid is not sufficient to be recognized by the transport system. The $IC_{50}$ of well-known anticancer drug cisplatin (*cis*-$[PtCl_2NH_3)_2]$) was also determined under these conditions for control purposes and was found to be 0.69 ± 0.15 μM (average of three independent experiments), a value within the expected range for cisplatin in this cell line.

The moderate activity for the CPP-conjugates compared to the unconjugated complexes may be due to the cationic nature of the peptides. The positively-charged peptide chain may interact with other cellular components explaining the lower bioactivity of the conjugates. A quenching of activity on conjugation to peptides has been observed for other organometallic complexes as well *(30-31)*. However, the 2-fold increase on going from five to eight arginines is notable and complex **6** represents one of the most active organometallic peptide conjugates reported to date.

**Albumin Binding.** To investigate whether the presence of albumin (the most abundant blood plasma protein, ca. 0.6 mM) deactivates the peptide conjugates *in*

*vitro*, complex **5** was incubated with recombinant human albumin (rHA) in a 1:1 ratio and was monitored over time (Figure 8). About 41% of complex **5** reacted with rHA within 15 min. The amount of **5** bound to rHA increased steadily over time and after 24 h (the last time-point studied) ca. 85% of **5** was bound to albumin. These data show that the osmium pentaarginine conjugate **5** readily forms adducts with albumin. The same behavior would be expected for conjugate **6**, probably with even tighter binding. This may account for the inactivity of the conjugates in FCS-containing media. However, reversible binding to albumin can be advantageous for distributing drugs in the body as well as providing a reservoir to prolong the duration of action. The ability of serum albumin to bind drugs reversibly and to transport them has been well established *(32)*. It is important to note is that albumin binding also affects drug absorption, metabolism, distribution and excretion of the complexes *in vivo*.

**Cellular Uptake.** The effect of the peptide chain length on the cell uptake of the complexes was investigated in the ovarian A2780 cancer cell line. Uptake decreased in the order **6** >> **5** > **4** > **1** (Figure 5), the order of decreasing peptide chain length. The dramatic increase in cell uptake (4.5-fold) on lengthening the Arg chain from $Arg_5$ in complex **5** to $Arg_8$ in complex **6** is particularly striking and demonstrates the importance of the arginine chain length for the cell penetration capabilities of these organometallic-polyarginine conjugates. Interestingly, conjugation to $Arg_8$ increases cell uptake of compound **6** by ca. 10-fold compared to unfunctionalized complex **1** or monoarginine complex **4** (Figure 5). The fact that complex **4**, is taken up by cells to the same extent as unfunctionalized complex **1**, and yet is inactive in the ovarian cancer cell line, demonstrates that attachment of a single cationic amino acid is not a suitable strategy for improving the biological properties for these complexes.

**Cell Distribution and DNA Uptake.** The distribution of the peptide conjugates in isolated cell fractions (*e.g.* cytosol and nucleus) was determined for compounds **1**, **4**–**6** after a 24 h exposure time. The amount of osmium detected in the nuclear fraction dramatically increased with increasing conjugation: **1** < **4** < **5** < **6** (*e.g.* 11% for **1**, 23% for **4**, 32% for **5** and 44% for **6**, Figure 6) showing that increasing the arginine chain length not only increases cellular uptake but also increases cellular internalisation. Interestingly, almost half of the total amount of osmium from complex **6** was detected in the cell nucleus. This increase in uptake into the nucleus with the longer arginine chain length probably explains the higher cytotoxicity of **5** and **6**. Polyarginines and TAT-mediated delivery have been shown to increase cell internalisation with other cargos as well *(33)*.

Nuclear DNA is a potential target for $Os^{II}$ arene compounds *(22)*. The target may be defined as a site within the cell which is altered by the drug and whose modification leads to cell death. Several criteria are used to suggest that a certain type of lesion is the critical lesion which kills the tumor cells. One of the criteria used to suggest that DNA is the target is based on the observation that a drug exhibits a higher toxicity in cells which are deficient in DNA repair *(34)*. This is the case because the persistence of DNA lesions depends on the capacity of cells to repair the damage. The assay used in this work is based on introducing a point mutation in a gene coding one of the proteins necessary for the repair of DNA. Mutant Chinese hamster ovary CHO-K1 cells, MMC-2 carrying the ERCC3/XPB mutation, which are deficient in DNA repair, were significantly more sensitive to killing by complex **5** Os-$Arg_5$ and complex **6** Os-$Arg_8$ than were the wild type cells (Table 1). These results show that cells must remove or bypass DNA lesions if they are to survive treatment with Os-

$Arg_5$ or Os-$Arg_8$ and imply that unrepaired DNA damage contributes to their cytotoxicity.

The ability of the peptide conjugates **4**–**6** to deliver osmium to DNA follows the order **6** >> **5** > **4** (Figure 7). Octaarginine complex **6** delivers over 4-fold more Os to DNA compared to complex **5** and 15-fold more compared to mono-arginine-conjugated complex **4**. The overall increase in charge with increase in arginine chain length may result in more rapid and stronger binding to negatively-charged DNA, although the present experiments do not provide information on whether both Os and the ligands reach DNA. The high amount of DNA binding for **6** is not accompanied by potent cytotoxicity. Possibly the arginine chain hinders direct base (guanine) coordination of Os or the induction of DNA conformational changes resulting in weaker DNA binding and so lower cytotoxicity of the conjugate. However, the increase in nuclear uptake resulting in increased DNA binding shows promise for the future use of CPPs for targeting organometallic osmium complexes to DNA.

**DNA Binding.** The kinetics of DNA binding of the conjugates compared to the parent compound show that the rate of binding increases with increase in arginine chain length, with pentaarginine compound **5** binding over 5-fold faster and octaarginine compound **6** almost 8-fold faster compared to the parent compound. All complexes also bind quantitatively to DNA. This behavior can be attributed to the high positive charge of the complexes facilitating rapid binding to negatively-charged DNA.

The incubation of complexes **1**, **5** and **6** at different concentrations (*i.e.* $r_i$ values in the range of 0–0.06) with CT DNA showed that, in contrast to the parent compound, conjugates **5** and **6** showed a difference in absorbance at higher $r_i$ values. A plausible explanation for this observation is that complexes **5** and **6** precipitate DNA at higher

values of $r_i$, and that this capability to precipitate DNA increases with increasing length of the arginine chain.

The DNA interactions of parent complex **1** have been studied previously. This compound distorts DNA by intercalation of the biphenyl arene between DNA bases causing a large degree of DNA unwinding *(22)*. It is likely that the peptide conjugates form different interactions with DNA due to the cationic nature of the arginine chain. The fast kinetics of DNA binding of the conjugates as well as their ability to precipitate DNA at very low concentrations indicate a different mechanism of interaction with DNA compared to the parent compound, possibly explaining the difference in cytotoxicity between the conjugates and the parent compound.

## CONCLUSIONS

In this work we report the synthesis of osmium arene peptide conjugates by solid phase peptide synthesis (SPPS). The osmium arene parent complex (**2**) is very stable under aqueous and acidic conditions making it an ideal candidate for SPPS.

There is a correlation between longer arginine chain lengths and the cell uptake, nuclear uptake, DNA binding and cytotoxicity of the conjugates following the order **6** (eight arginine residues) >> **5** (five arginine residues) > **4** (one arginine) ~ **1** (unfunctionalized). In particular, osmium arene complexes **5** and **6** showed cytotoxicity in the ovarian cancer cell line that increased 2-fold going from five arginine residues (**5**) to eight arginine residues (**6**). Furthermore, osmium octaarginine conjugate (**6**) shows a 10-fold increase in cellular uptake and an over 15-fold increase in DNA binding compared to monoarginine compound **4**. The high nuclear uptake for **6** is likely related to the observed high DNA binding and higher cytotoxicity for this compound compared to the other conjugates. It is likely that DNA is also the target

for the conjugates as experiments on CHO cells deficient in DNA repair suggested that unrepaired DNA damage contributes to the cytotoxicity of Os-Arg5 (**5**) and Os-Arg8 (**6**) complexes. The conjugates are less cytotoxic compared to the parent compound. This is likely due to the cationic nature of the peptide which can bind to other cellular components, as well as differences in binding to DNA. Indeed, the rates of DNA binding of the conjugates were 5- to 8-fold faster compared to the parent compound, and also the conjugates were able to precipitate DNA at very low concentrations, indicating a different interaction with DNA.

The large increase in cell uptake, nuclear localization and DNA binding of the osmium conjugate **6** compared to the parent (unfunctionalized) complex shows that attaching a CPP introduces desirable features into this type of complex.

ACKNOWLEDGEMENT

We thank Dr. Lijiang Song (University of Warwick) for help with MS. This research was supported an HEIF award, by EPSRC grant no EP/H500308/1, ERC grant no. 247450 (BIOINCMED) and Science City (ERDF/AWM). This research was also supported by the Grant Agency of the CR (P301/10/0598) and by the Academy of Sciences of the CR (M200040901). The authors also acknowledge their participation in the EU COST Action D39 which enabled them to exchange regularly the most recent ideas in the field of anticancer metallodrugs with several European colleagues.

**Supporting Information Available:** Instrumental settings for ICP-MS (Table S1). RP-HPLC trace for the semipreparative purification of **6** (Figure S1). Dose-

response curve for cisplatin towards human ovarian A2780 cancer cells (Figure S2). This material is available free of charge via the Internet at http://pubs.acs.org.

LITERATURE CITED

(1) Vivès, E., (2005) Present and future of cell-penetrating peptide mediated delivery systems: "is the Trojan horse too wild to go only to Troy?". *J. Control Release 109,* 77-85.

(2) Vivès, E., Schmidt, J., Pèlegrin, A., (2008) Cell-penetrating and cell-targeting peptides in drug delivery. *Biochim. Biophys. Acta 1786,* 126-138.

(3) Futaki, S., (2002) Arginine-rich peptides: potential for intracellular delivery of macromolecules and the mystery of the translocation mechanisms. *Int. J. Pharm. 245,* 1-7.

(4) Magzoub, M. and Graslund, A., (2004) Cell-penetrating peptides: from inception to application. *Q. Rev. Biophys. 37,* 147-195.

(5) Wright, L. R., Rothbard, J. B., Wender, P. A., (2003) Guanidinium rich peptide transporters and drug delivery. *Curr. Protein Pept. Sci. 4,* 105-124.

(6) Mitchell, D. J., Kim, D. T., Steinman, L., Fathman, C. G., and Rothbard, J. B., (2000) Polyarginine enters cells more efficiently than other polycationic homopolymers. *J. Pept. Res. 56,* 318-325.

(7) Wender, P. A., Mitchell, D. J., Steinman, L., Fathman, C. G., and Rothbard, J. B., (2000) The design, synthesis, and evaluation of molecules that enable or enhance cellular uptake: peptoid molecular transporters. *Proc. Natl. Acad. Sci. U S A, 97,* 13003-13008.

(8) Dyson, P. J. and Sava, G., (2006) Metal-based antitumour drugs in the post genomic era. *Dalton Trans.* 1929-1933.

(9) Melchart, M., Habtemariam, A., Parsons, S., Moggach, S. A., and Sadler, P. J., (2006) Ruthenium(II) arene complexes containing four- and five-membered monoanionic O,O-chelate rings. *Inorg. Chim. Acta, 359,* 3020-3028.

(10) Berners-Price, S. J. and Filipovska, A., (2008) The design of gold-based, mitochondria-targeted chemotherapeutics. *Aust. J. Chem. 61,* 661-668.

(11) Jakupec, M. A., Galanski, M., Arion, V. B., Hartinger, C. G., and Keppler, B. K., (2008) Antitumour metal compounds: more than theme and variations. *Dalton Trans.* 183-194.

(12) Brunner, J. and Barton, J. K., (2006) Targeting DNA mismatches with rhodium intercalators functionalized with a cell-penetrating peptide. *Biochemistry, 45,* 12295-12302.

(13) Noor, F., Wustholz, A., Kinscherf, R, and Metzler-Nolte, N., (2005) A cobaltocenium-peptide bioconjugate shows enhanced cellular uptake and directed nuclear delivery. *Angew. Chem. Int. Ed. Engl. 44,* 2429-2432.

(14) Cebrian-Losantos, B., Krokhin, A. A., Stepanenko, I. N., Eichinger R., Jakupec, M. A., Arion, V. B., and Keppler, B. K., (2007) Osmium NAMI-A analogues: Synthesis, structural and spectroscopic characterization, and antiproliferative properties. *Inorg. Chem. 46,* 5023-5033.

(15) Peacock, A. F. A., Parsons, S., and Sadler, P. J., (2007) Tuning the hydrolytic aqueous chemistry of osmium arene complexes with N,O-chelating ligands to achieve cancer cell cytotoxicity. *J. Am. Chem. Soc. 129,* 3348-3357.

(16) Maksimoska, J., Williams, D. S., Atilla-Gokcumen, G. E., Smalley, K. S., Carroll, P. J., Webster, R. D., Filippakopoulos, P., Knapp, S., Herlyn, M., and Meggers, E., (2008) Similar biological activities of two isostructural ruthenium and osmium complexes. *Chem-Eur. J. 14,* 4816-4822.

(17) Renfrew, A. K., Phillips, A. D., Egger, A. E., Hartinger, C. G., Bosquain, S. S., Nazarov, A. A., Keppler, B. K., Gonsalvi, L., Peruzzini, M., and Dyson, P. J.,

(2009) Influence of Structural Variation on the Anticancer Activity of RAPTA-Type Complexes: ptn versus pta. *Organometallics 28,* 1165-1172.

(18) van Rijt, S. H., Hebden, A. J., Amaresekera T., Deeth R. J., Clarkson G. J., Parsons S., McGowan P. C., and Sadler, P. J., (2009) Amide Linkage Isomerism As an Activity Switch for Organometallic Osmium and Ruthenium Anticancer Complexes. *J. Med. Chem. 52,* 7753-7764.

(19) van Rijt, S. H., Peacock, A. F. A., Johnstone, R. D., Parsons, S., and Sadler, P. J., (2009) Organometallic Osmium(II) Arene Anticancer Complexes Containing Picolinate Derivatives. *Inorg. Chem. 48,* 1753-1762.

(20) Barry, N. P. E., Edafe, F., Dyson, P. J., and Therrien, B., (2010) Anticancer activity of osmium metalla-rectangles. *Dalton Trans. 39,* 2816-2820.

(21) Hanif, M., Nazarov, A. A., Hartinger, C. G., Kandioller, W., Jakupec, M. A., Arion, V. B., Dyson, P. J., and Keppler, B. K., (2010) Osmium(II)-versus ruthenium(II)-arene carbohydrate-based anticancer compounds: similarities and differences. *Dalton Trans. 39,* 7345-7352.

(22) Kostrhunova, H., Florian, J., Novakova, O., Peacock, A. F., Sadler P. J., and Brabec V., (2008) DNA interactions of monofunctional organometallic osmium(II) antitumor complexes in cell-free media. *J. Med. Chem. 51,* 3635-3643.

(23) Brabec, V. and Palecek, E., (1970) The influence of salts and pH on polarographic currents produced by denatured DNA. *Biophysik, 6,* 290-300.

(24) Brabec, V. and Palecek, E., (1976) Interaction of nucleic acids with electrically charged surfaces. II. Conformational changes in double-helical polynucleotides. *Biophys. Chem. 4,* 79-92.

(25) Skehan, P., Storeng, R., Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warren, J. T., Bokesch, H., Kenney, S., and Boyd, M. R., (1990) New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst. 82*, 1107-1112.

(26) Hall, H., Gursky, J., Nicodemou, A., Rybanská, I., Kimlíčková, E., and Piršel, M., (2006) Characterization of ERCC3 mutations in the Chinese hamster ovary 27-1, UV24 and MMC-2 cell lines. *Mutat. Res-Fund. Mol. M., 593*, 177-186.

(27) van Rijt, S. H., Mukherjee, A., Pizarro, A. M., and Sadler, P. J., (2010) Cytotoxicity, Hydrophobicity, Uptake, and Distribution of Osmium(II) Anticancer Complexes in Ovarian Cancer Cells. *J. Med. Chem. 53*, 840-849.

(28) Gammon, S. T., Villalobos, V. M., Prior, J. L., Sharma, V., and Piwnica-Worms, D., (2003) Quantitative analysis of permeation peptide complexes labeled with Technetium-99m: chiral and sequence-specific effects on net cell uptake. *Bioconjug. Chem. 14*, 368-376.

(29) Youngblood, D. S., Hatlevig, S. A., Hassinger, J. N., Iversen, P. L., and Moulton, H. M., (2007) Stability of cell-penetrating peptide-morpholino oligomer conjugates in human serum and in cells. *Bioconjug. Chem. 18*, 50-60.

(30) Metzler-Nolte, N., (2001) Labeling of Biomolecules for Medicinal Applications-Bioorganometallic Chemistry at Its Best. *Angew. Chem. Int. Ed. Engl. 40*, 1040-1043.

(31) Splith, K., Neundorf, I., Peindy N'Dongo, H. W., Vasylyeva, V., Merz, K., and Schatzschneider, U., (2010) Influence of the metal complex-to-peptide linker on the synthesis and properties of bioactive $CpMn(CO)_3$ peptide conjugates. *Dalton Trans. 39*, 2536-2545.

(32) Bertucci, C. and Domenici, E., (2002) Reversible and covalent binding of drugs to human serum albumin: methodological approaches and physiological relevance. *Curr. Med. Chem. 9,* 1463-1481.

(33) Dietz, G. P. H. and Bahr, M., (2004) Delivery of bioactive molecules into the cell: The Trojan horse approach. *Mol. Cell Neurosci. 27,* 85-131.

(34) Johnson, N. P., Butour, J. L., Villani, G., Wimmer, F. L., Defais, M., Pierson, V., and Brabec, V., (1989) Metal antitumor compounds: The mechanism of action of platinum complexes. *Prog. Clin. Biochem. Med. 10,* 1-24.

**Table 1**. $IC_{50}$ values (μM) for complexes **5 (**Os-$Arg_5$) and **6 (**Os-$Arg_8$)

| Complex | $IC_{50}$ / μM[a,b] | |
|---|---|---|
| | K1 cells | MMC-2 cells |
| **5** (Os-$Arg_5$) | > 60 | 36.2 ± 3.5[c] |
| **6** (Os-$Arg_8$) | 44.0 ± 3.1 | 24.3 ± 3.3[c] |

[a] Drug-treatment period was 24 h.

[b] Results are expressed as mean ±SD for four independent samples.

[c] Significantly different from the $IC_{50}$ values obtained for K1 cells, $p<0.05$.

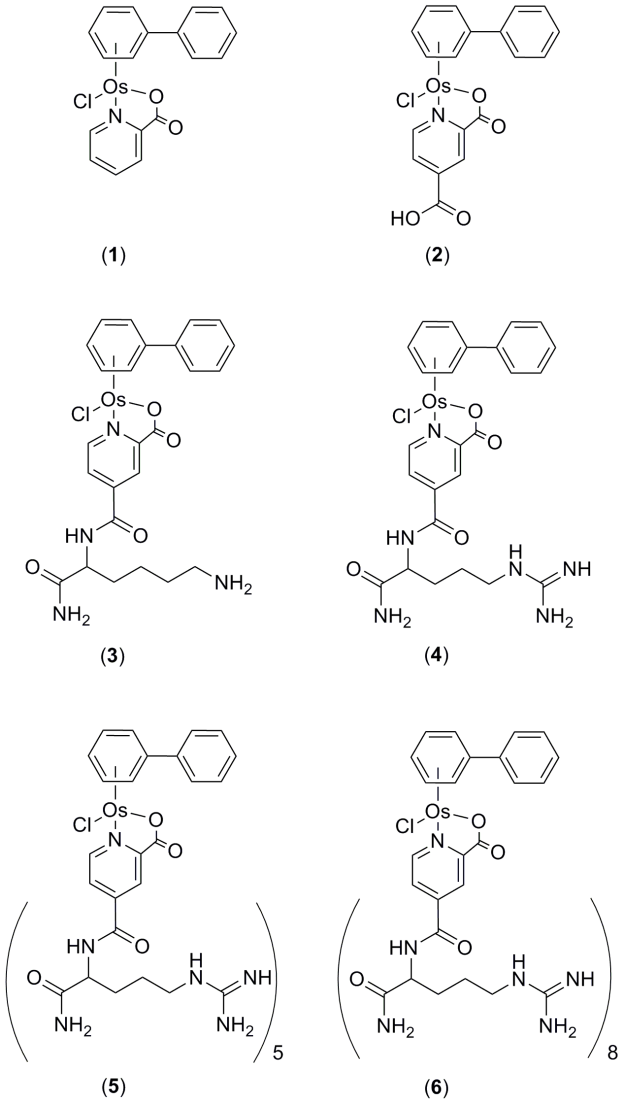


**Figure 1.** Chemical structures of the organometallic $Os^{II}$- peptide conjugates studied in this work.

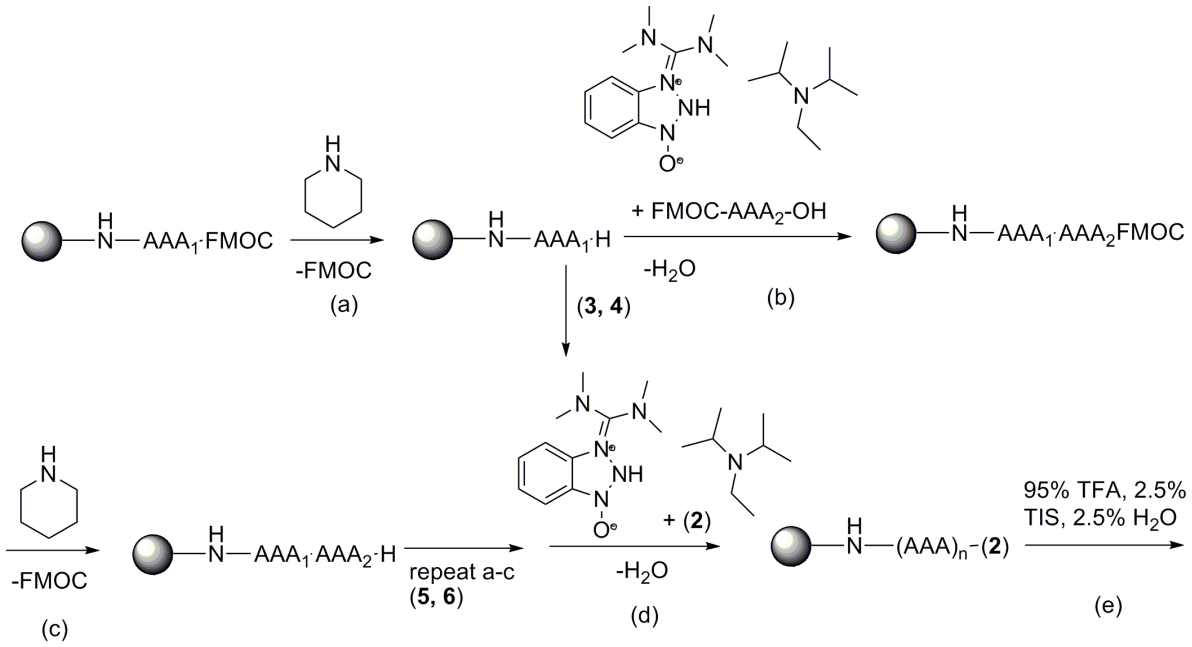


**Figure 2.** Strategy for the synthesis of conjugates **3-6** following the steps (a) FMOC-deprotection with a 20% piperidine solution in DMF, (b) Coupling using a TBTU, DIPEA, $AAA_1$ mixture in DMF, where $AAA_1$ = Lys for **3** and Arg for **4-6,** (c) FMOC-deprotection with a 20% piperidine solution in DMF, (d) Coupling using a TBTU, DIPEA, arginine/(**2**) mixture in DMF (e) Resin cleavage with a 95% TFA, 2.5% TIS, 2.5% $H_2O$ mixture.

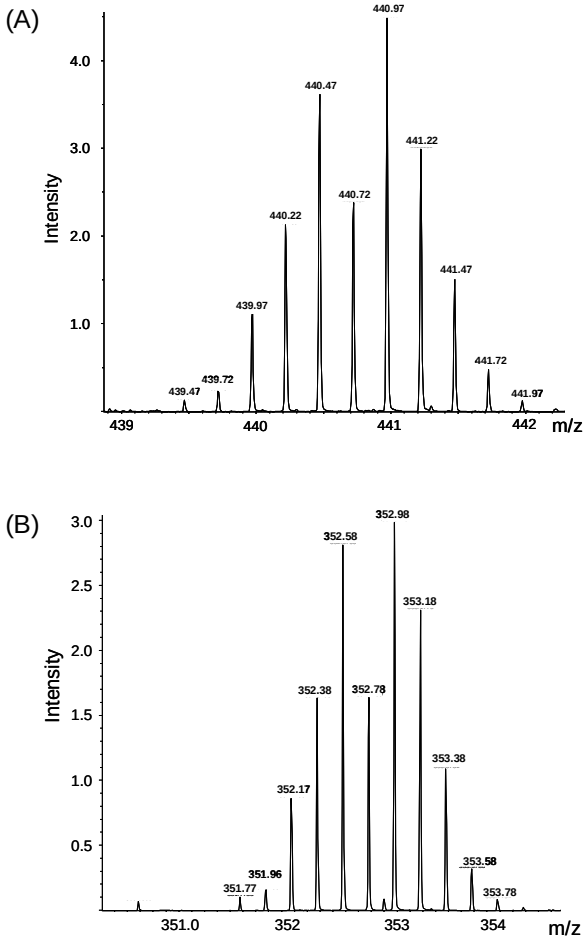


**Figure 3.** Mass spectra of purified compound **6** showing (A) $[(\eta^6\text{-bip})Os(pico\text{-}C(O)\text{-}(Arg)_8) + 4H]^{5+}$ and (B) $[(\eta^6\text{-bip})Os(pico\text{-}C(O)\text{-}(Arg)_8) + 3H]^{4+}$.

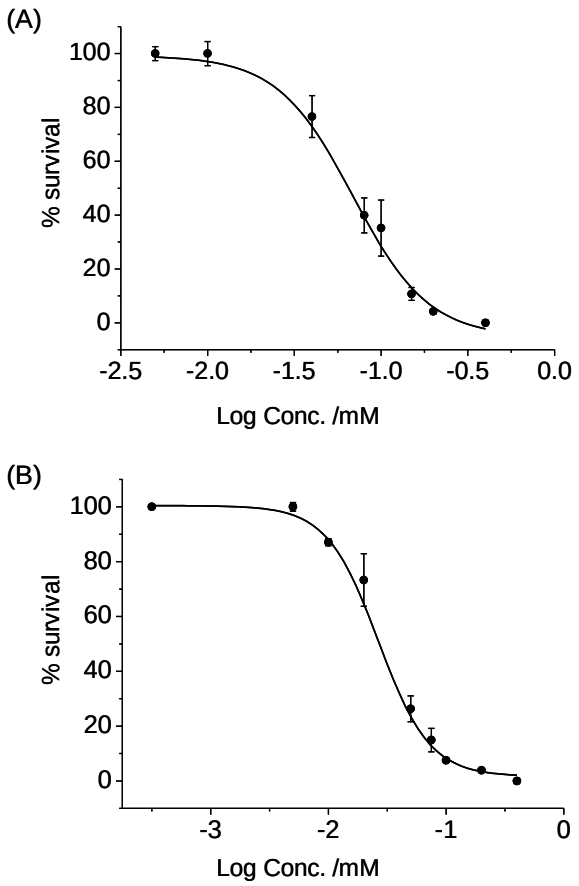


**Figure 4.** Dose-responds curves for (A) [($\eta^6$-bip)Os(pico-C(O)-(Arg)$_5$)Cl] (**5**) and (B) [($\eta^6$-bip)Os(pico-C(O)-(Arg)$_8$)Cl] (**6**) towards human ovarian A2780 cancer cells. The $IC_{50}$ values (concentrations that inhibit cell growth by 50%) obtained from these curves are 71.5 μM ± 2.5 for **5** and 32.9 μM ± 3 for **6**.

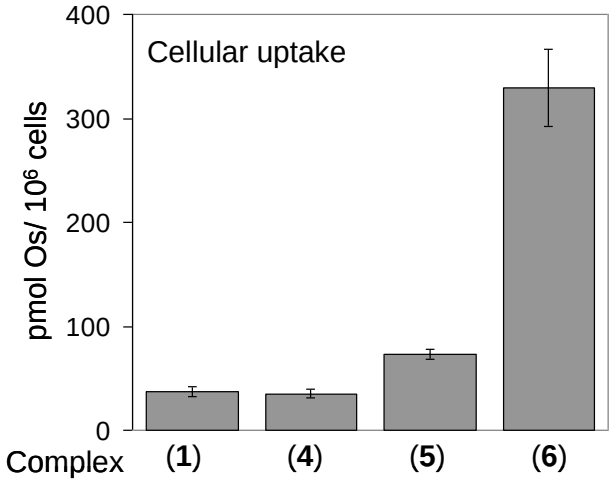


**Figure 5.** Cellular osmium concentrations determined in A2780 cells after exposure to 35 μM of complexes **1**, **4-6** for 24 h. Results are mean of 3 independent samples and are expressed as mean ±SD.

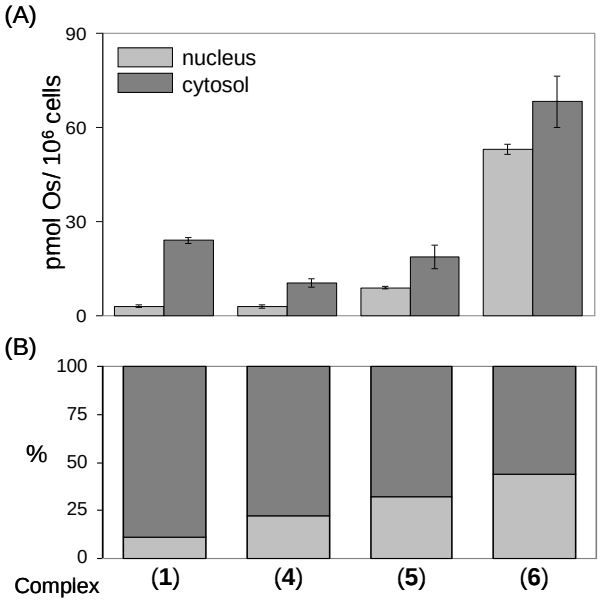


**Figure 6.** (A) Osmium concentrations in nucleus and cytosol fractions (pmol/$10^6$ cells) in A2780 ovarian cells after 24 h exposure to 35 μM of **1, 4**–**6**. Results are the mean of three independent experiments and are expressed as mean ± SD. (B) Percentage localizing in the cell fractions was calculated on the basis that their sum is 100%. For complexes **4**–**6** the total amount of Os in the cell fractions was only 40% of that detected for whole cellular uptake due to loss during the cell fractionation. For complex **1**, 80% was accounted for. For the latter it was possible to use many more cells in the assay.

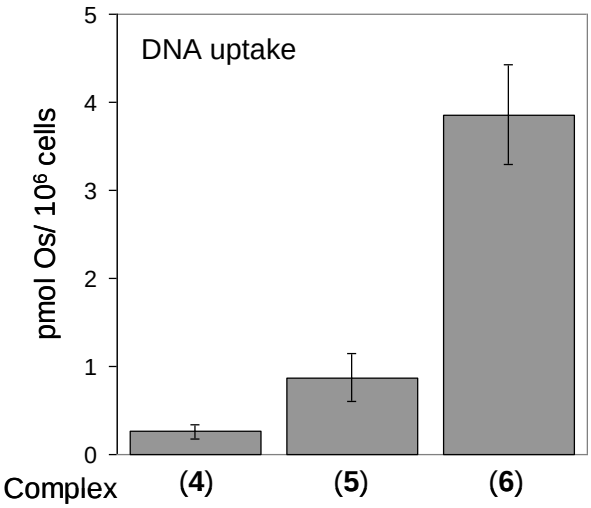


**Figure 7.** Osmium content of DNA from A2780 cells exposed to 35 μM of complexes **4-6** for 24 h. Results are expressed as mean ±SD for three independent samples.

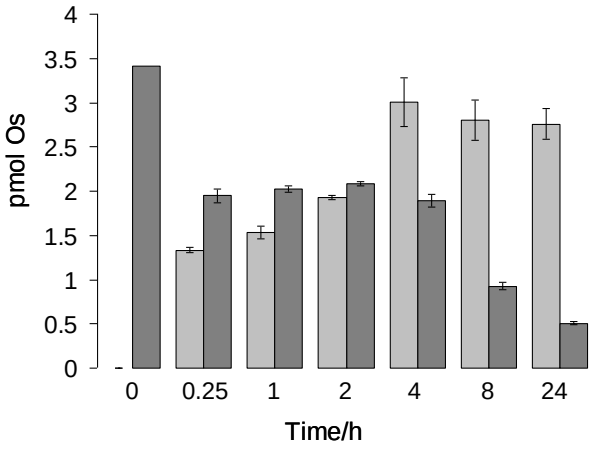


**Figure 8.** Time dependence for binding of $Arg_5$ complex **5** to rHA. The light grey bars represent Os bound to albumin and the dark grey bars represent unbound compound **5**.

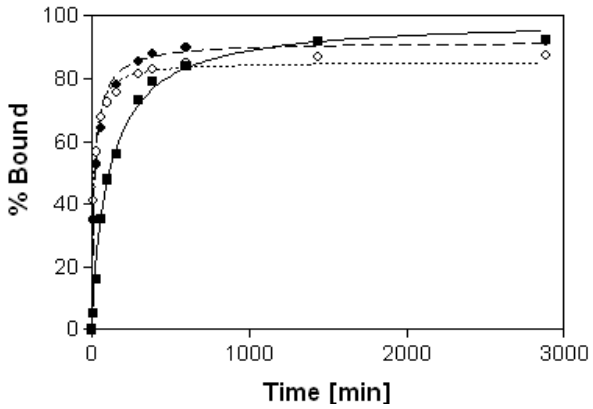


**Figure 9.** Kinetics of the binding of complexes **1** (■), **5** (◊) and **6** (♦) to CT DNA in 10 mM $NaClO_4$ (pH 6) at 310 K. The concentration of DNA was $9.4x10^{-5}$ M (related to the monomeric nucleotide content) and $r_i$ was 0.01.

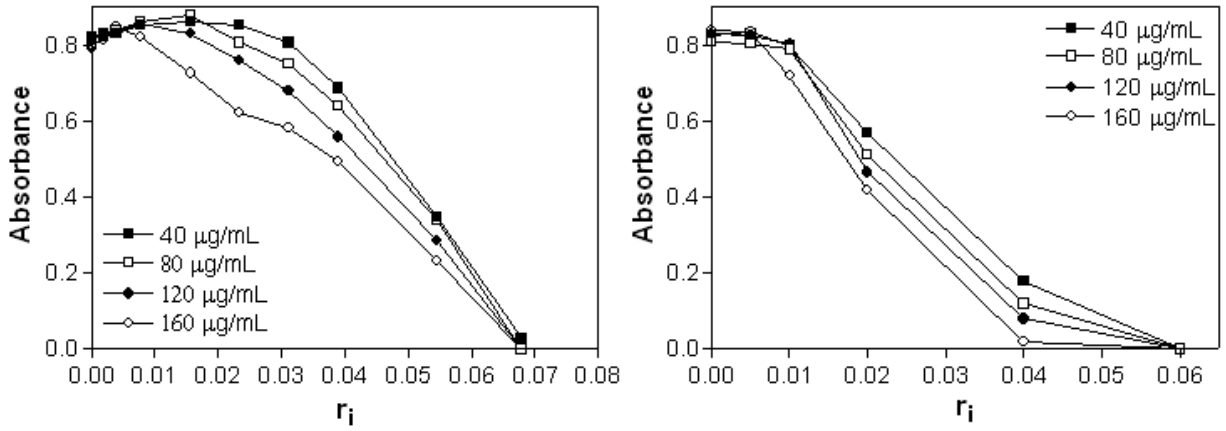


**Figure 10.** Dependencies of absorbance at 260 nm of the solutions of CT DNA modified by complexes **5** and **6** on $r_i$. For other details, see the text.

**TOC graphic**

Tagging an anticancer organometallic arene $Os^{II}$ anticancer complex with 5 or 8 arginine residues greatly increases cellular, nuclear and DNA uptake in A2780 human ovarian cancer cells. The $Arg_8$ complex is cytotoxic and a rare example of a bioactive organometallic peptide conjugate, demonstrating the potential for use of cell- and nucleus-penetrating peptides in targeting organometallic arene anticancer complexes.