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# Novel Heterobimetallic Radiotheranostic: Preparation, Activity, and Biodistribution

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A novel $Ru^{II}$(arene) theranostic complex is presented. It is based on a 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid macrocycle bearing a triarylphosphine and can be tracked in vivo by using the $\gamma$ emission of $^{153}Sm$ atoms. Notably, the heteroditopic ligand can be selectively metalated with ruthenium at the phosphorus atom despite the presence of other functionalities that are prone to metal coordination. Subsequent labeling with radionuclides such as $^{153}Sm$ can then be performed easily. The resulting heterobimetallic complex exhibits favorable solubility and stability properties in biologically relevant media. It also shows in vitro cytotoxicity in line with that expected for this type of metallodrug, and is nontoxic to the organism as a whole. As a proof of concept, initial studies in healthy mice were performed to obtain information about the uptake, biodistribution, and excretion of the radiolabeled complex.

## Introduction

Metallic complexes have been at the forefront of cancer therapy for decades. Indeed, cisplatin and its derivatives are some of the most widely used anticancer drugs in the world, specifically against testicular and ovarian cancers.[1] This success has raised the profile of bioinorganic chemistry, which has, in recent years, seen the development of a plethora of metal complexes targeted toward therapeutic applications.[2] An assortment of complexes with different metals have proven to be active in a variety of biologically relevant assays, with the standout metals being platinum and ruthenium. In particular, the Ru(arene) complexes reported by Dyson, Sadler, and their respective co-workers have shown remarkable anticancer or anti-metastatic activities (complexes **I** and **II**; Figure 1).[3]

With the exception of cisplatin and related species, the majority of early biometallic (bioinorganic and bioorganometallic) research was centered on the discovery of complexes that displayed maximum activity. Such an approach led to the discovery of several very potent biometallics but meant that the thoughtful modification of these species was challenging. In the last five years, a shift has taken place in the field, with more emphasis being placed on understanding how these metallodrugs work. In this respect, theranostics have contributed richly to our understanding. Initially, theranostics, a neologism built from the contraction of the words therapy and diagnostic, were compounds that helped in the identification of a patient's disease in order to select the specific therapy required (this method was previously called personalized medicine). Today, chemists enlarge this field to compounds composed by both a therapeutic moiety and an imaging agent. This specificity allows researchers to follow the theranostic during its "life", which gives some clues on its mechanism of action. Clearly, in an investigation of the mechanism of action, virtually the entirety of all cellular components (proteins, DNA, RNA, etc.) in the whole body must be considered. This level of complexity seriously hampers our ability to fully comprehend how therapeutic metallic complexes work and, thus, it also hampers our attempts to make new, more efficient metallodrugs.[3e,4]

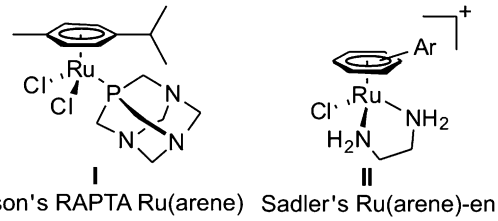


**Figure 1.** Examples of known ruthenium-based complexes with very promising anticancer activity.

Scintigraphic imaging of radionuclides has proven to be a valuable tool to gain a big-picture idea of what's happening in the body. It occurred to us that a ligand suitable for the simultaneous coordination of therapeutically relevant metals

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and radionuclides would allow the localization of the corresponding relevant metal complexes within the body. Chiefly, our major goal was to reveal the organ specificity, the uptake rate, and the clearance of these fascinating complexes.

Thus, we selected heterotopic ligand **1** (Figure 2). Phosphine complexes are among the most promising biometallics and, therefore, the ligand is based on a functionalized triphenylphosphine moiety.[5] In this manner, we hoped to produce Ru–phosphine complexes that share a resemblance in nature and, hopefully, in activity with known potential metallodrugs. On the other part of the ligand, we envisaged a 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid (DOTA) moiety, which would be used to complex the radionuclide. DOTA-derivative macrocycles have proven to be excellent ligands for a variety of radionuclides, such as $^{111}In^{III}$, $^{68}Ga^{III}$, and $^{153}Sm^{III}$ atoms, all of which have been used for imaging and/or therapy.[6] Similarly to the 1,3,5-triaza-7-phosphaadamantane (PTA) ligand used by Dyson and co-workers to construct RAPTA complexes, DOTA macrocycles and their metal complexes are known to be highly water soluble, and so we envisaged that the covalent attachment of such a moiety would impart a favorable solubility profile in biologically relevant media. In addition, we, among others, have successfully developed modified DOTA macrocycles that act as bifunctional chelating agents, and we were therefore optimistic that DOTA macrocycles could be ideal candidates to create the proposed heteronuclear complex.[7]

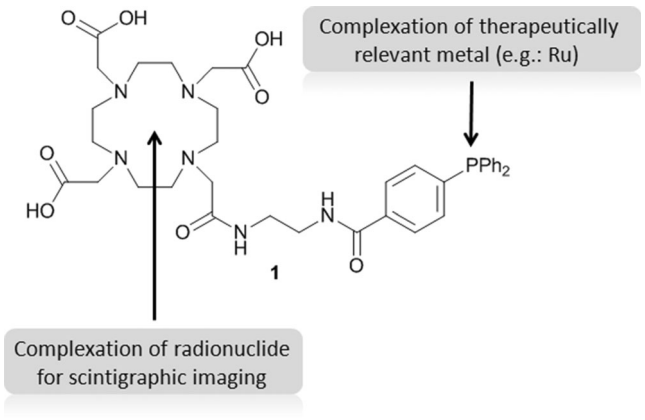


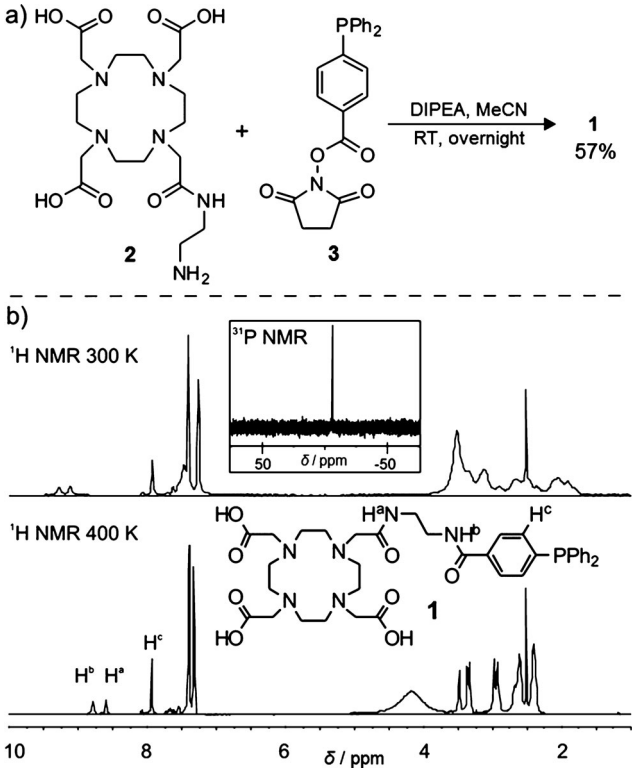

**Figure 2.** Proposed strategy for the heterobimetallic radiotheranostic agents.

## Results and Discussion

### Synthesis of the heterotopic ligand

The chemistry involved in the preparation of complexes resembling RAPTA is relatively sensitive, especially during and after rutheniation. Conversely, the chemistry of DOTA-like macrocycles can be broadly thought of as amino acid chemistry and appears, at first glance, to be incompatible with the delicate nature of $Ru^{II}$ complexes. Thus, we anticipated synthetic difficulties in obtaining the targeted heterobimetallic theranostic agent. Indeed, this was the case and among several envisioned strategies, only one allowed access to the desired product in satisfactory yield.

To minimize the synthetic complications, we coupled the phosphine ligand and the DOTA macrocycle at the last step of the synthesis of bifunctional heterotopic ligand **1**. This convergent strategy entailed an amide coupling between a DOTA macrocycle decorated with an amine arm, **2**, and the phosphine activated ester **3** (Scheme 1 a). Both coupling partners

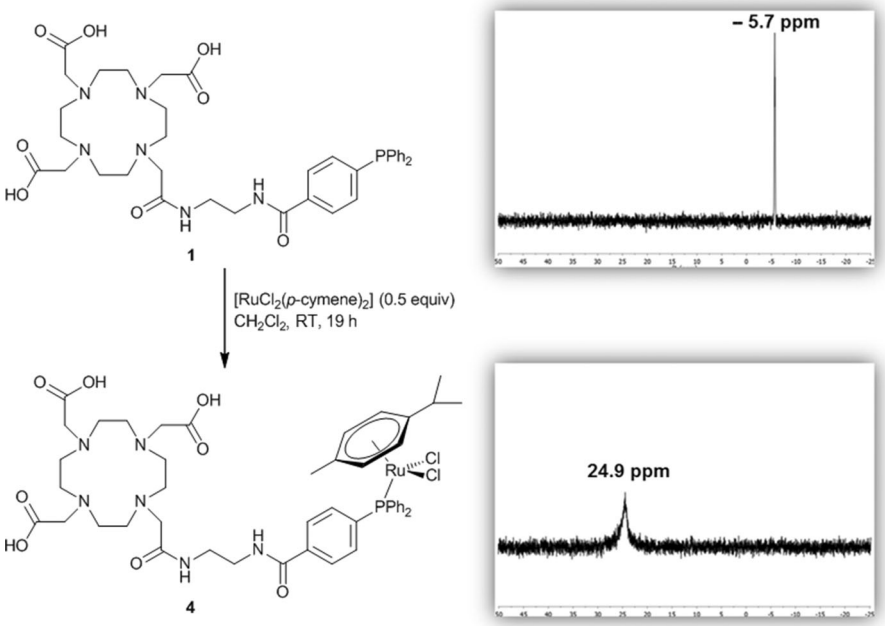



**Scheme 1.** a) Amide coupling to produce heteroditopic ligand **1**. *Reagents and conditions*: DIPEA, MeCN, RT, overnight. b) $^1$H and $^{31}P\{^1H\}$ NMR spectra (in $[D_6]$DMSO) at 300 K (top) and 400 K (bottom) of **1**; diagnostic protons are labeled.

were prepared by routes previously used in our laboratories.[8] These conditions provided the desired heterotopic ligand **1** in 57% yield and in sufficient quantities for the metalation studies and biological tests. The $^1$H NMR spectrum of heteroditopic ligand **1** displayed broad and poorly defined signals and was initially difficult to interpret. Such behavior is typical for DOTA derivatives and often hampers confident characterization. However, upon heating the sample to 400 K, the spectrum became well-resolved, and the key proton signals could be clearly observed and assigned (Scheme 1 b). The $^{31}P\{^1H\}$ NMR spectrum displayed a single signal in the expected region at $\delta = -5.7$ ppm.

### Selective complexation

The main synthetic challenge of this work was to succeed in selectively coordinating the Ru atom at the phosphine moiety



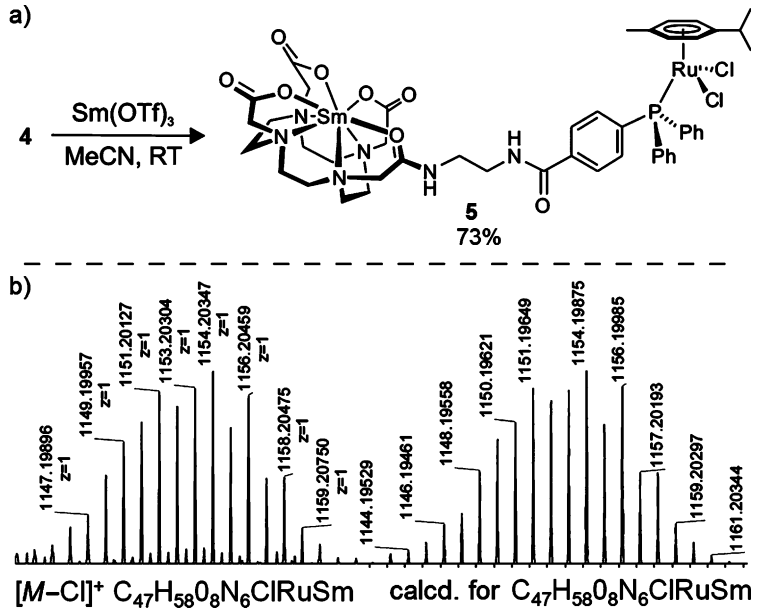

**Scheme 2.** Selective rutheniation of the phosphine moiety of heteroditopic ligand **1**. Insets on the right: $^{31}P\{^1H\}$ NMR (121.5 MHz, 300 K, $CDCl_3$) spectra of the free ligand **1** (top) and the ruthenium complex **4** (bottom).

of **1** and then in chelating the radionuclide within the attached DOTA cage without disturbing the phosphine–metal complex. Due to the practicalities of handling radioactive compounds and the short half-life of the radiometal, the chelation of the radionuclide must occur just before administration and is, thus, essentially the last step. With this in mind, we were pleased to find that rutheniation of **1** occurred solely at the phosphorus atom and could be achieved in good yield by simply adding the metal precursor to a solution of heteroditopic ligand **1** (Scheme 2). Despite the preference of ruthenium for the soft phosphorus atom, the selectivity of the metalation was not entirely expected. Given the sheer quantity of functionality displayed by the DOTA moiety, which is known to coordinate ruthenium, it is notable that the metal can discriminate between the phosphine and the tetraazamacrocycle. In the event, 0.5 equivalent of $[RuCl_2(p\text{-cymene})]_2$ was added to a solution of heteroditopic ligand **1** in chloroform. Simple precipitation followed by solvent washing provided the pure complex **4**. The complexation was monitored by $^{31}P\{^1H\}$ NMR spectroscopy (Scheme 2, inset on the right). A significant shift of the resonance was observed upon going from the free ligand (singlet at $\delta = -5.7$ ppm) to the complex (singlet at $\delta = 24.9$ ppm).

Initial metalation studies of the DOTA macrocycle were performed with cold samarium. This was done to obtain material for cytotoxicity evaluation and, importantly, to identify and characterize the analogous radioactive compound [$^{153}$Sm]**5**. The best results were obtained by simply stirring the DOTA–ruthenium-(arene) adduct with 1.2 equiv samarium trifluoromethanesulfonate in acetonitrile at room temperature (Scheme 3). Overnight, the reaction came to completion and produced the envisaged heterobimetallic theranostic agent **5**.

### Radiolabeling

After the successful production of complex **5**, we proceeded with the radiochemical labeling studies. By using HPLC analysis, the reaction kinetics of $^{153}$Sm with **4** was found to be dependent on temperature, pH value, and the metal/ligand molar ratio (M/L). We initially performed the labeling at room temperature with a 1:2 metal/ligand molar ratio and pH 5.5. Under these conditions, poor labeling yields were obtained, even after a 24 h reaction time. However, after only 10 min at 70 °C, the labeling yield was high, no colloidal species were found by ITLC-SG analysis, and no free $^{153}$Sm species were detected in the HPLC chromatogram ($t_R = 2.5$ min). As can be seen in Figure 3, one major radiochemical species ($t_R = 12.5$ min) representing ~85% of the total activity and two minor peaks ($t_R = 11.3$ and 13.4 min) were obtained. The major peak corresponds to the radiocomplex [$^{153}$Sm]**5** and its authenticity was established by comparing its chromatographic behavior with that of the inac-




**Scheme 3.** a) Metalation of the DOTA macrocycle under mild conditions gives theranostic agent **5**. b) The observed high-resolution mass spectrum (left) matches perfectly with the calculated spectrum (right) to confirm the identity of **5**.

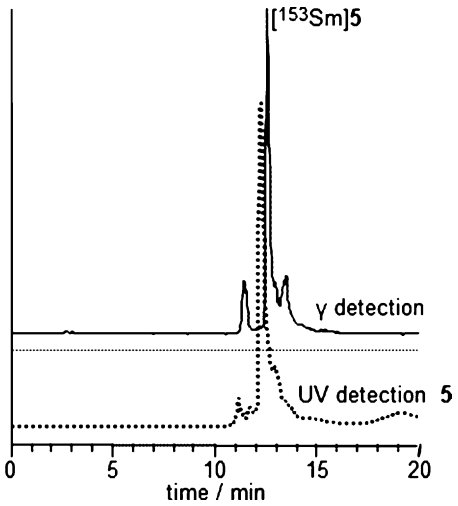



**Figure 3.** HPLC chromatograms of the nonradioactive complex **5** (UV detection at 254 nm; dotted trace; retention time ($t_R = 12.4$ min) and the radioactive analogue [$^{153}$Sm]**5** (γ detection; $t_R = 12.5$ min). Under the same conditions, the free $^{153}$Sm species has an $t_R$ value of 2.5 min.

tive analogue **5**, which was prepared previously (Figure 3). To evaluate the stability of the radioactive complex, the radiolabeling was done at 70 °C for 10 min as described above and then the complex was rapidly cooled and maintained at room temperature. Under these conditions, the preparation is stable for at least one day. The complex is also stable in physiological media (saline, 24 h, at 37 °C).

### Biological experiments

Notably, owing to the metalated DOTA macrocycle, complex **5** is highly water soluble. As discussed above, it is also stable in biologically relevant media. These properties, in line with the structural similarity of heterobimetallic theranostic **5** to known potential metallodrugs, especially RAPTA-type Ru(arene) species, encouraged us to proceed with biological evaluation.

The antiproliferative properties of **5** and its nonmetalated phosphine derivative **1** were assayed by monitoring cell growth inhibition. Cytotoxic activity was determined on the cisplatin-resistant human ovarian cancer (A2780cisR) cell line, after a 72 h exposure to the compounds. From the experimental data, $IC_{50}$ values were calculated. Interestingly, the activity against cisplatin-resistant ovarian carcinoma A2780cisR cells was significantly higher for Ru$^{II}$(arene) **5** than for phosphine **1** (176 μM versus >300 μM). Notably, for RAPTA-type metallodrugs, low $IC_{50}$ values are not generally observed. Instead, anticancer activity is usually expressed as an overall decrease in tumor count and size. Thus, the relatively high $IC_{50}$ value of 176 μM observed here is in line with that expected for this type of metallodrug.[3e]

Finally, we treated several healthy CD-1 mice with theranostic **5** at doses similar to those that would be used in the biodistribution study of the radioactive analogue. All of the mice tested survived this treatment, which showed that **5** does not have acute toxicity to the organism as a whole. The same could not be said for ligand **1**. All of the mice treated with **1** died more or less immediately after administration. This toxicity can be assigned to the nonmetalated DOTA macrocycle, which can chelate the body's cations.[9] The apparent nontoxicity of **5** confirms the in vivo stability of the DOTA–Sm complex. These results are critically important because they show that we had succeeded in preparing a stable Ru$^{II}$(arene) complex that was faithful to known metallodrugs and that could also be tracked within the body.

Hoping to gain, for the first time, an idea of the uptake, distribution, and excretion of Ru$^{II}$(arene)-based metallodrugs in the body, we carried out biodistribution studies of [$^{153}$Sm]**5** in healthy CD-1 mice. Results were obtained 1 h after administration and are summarized for the most relevant organs in Figure 4. The uptake in the tissues was calculated and expressed as a percentage of the injected radioactivity dose per gram of tissue (%ID g$^{-1}$).

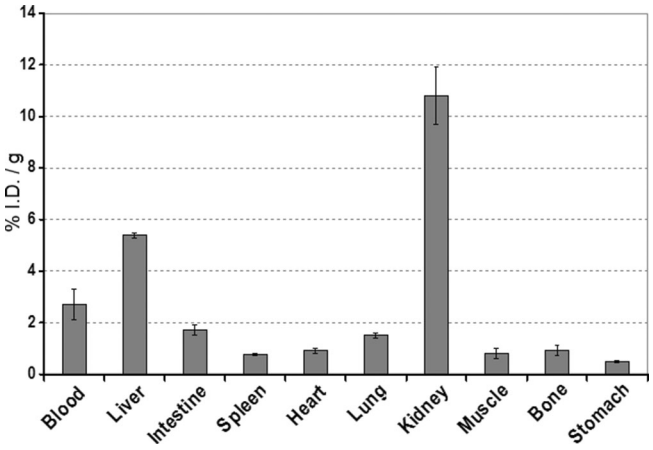



**Figure 4.** Biodistribution of the $^{153}$Sm-labeled Ru(arene) complex **5** at 1 h after administration to healthy CD-1 mice.

The most notable features of the biodistribution study of [$^{153}$Sm]**5** are the relatively slow clearance from blood ((2.7 ± 0.6) %ID g$^{-1}$), a fast clearance from soft tissues such as muscle ((0.8 ± 0.2) %ID g$^{-1}$), and no relevant uptake in the main organs, except those related with the excretory paths (liver, intestines, and kidneys). The overall rate of radioactivity excretion was relatively low (<40 %).

## Conclusions

We have successfully synthesized a Ru$^{II}$(arene) complex inspired by known therapeutic metal complexes. In contrast to known variants, the presented complex consists of a pendant DOTA derivative that can chelate radionuclides, which allows its location within the body to be determined. The evident complications in mixing the relatively sensitive nature of Ru$^{II}$ organometallic complexes with the relatively harsh chemistry and nature of DOTA macrocycles were tackled and overcome. It is of synthetic note that the heteroditopic ligand **1** can be easily rutheniated selectively at the phosphorus atom, despite

the array of functionalities prone to metal coordination on the adjacent DOTA cage. Subsequent labeling with radionuclides occurs without problem. The prepared $Ru^{II}$(arene) complex shows favorable stability and solubility profiles in biologically relevant media. Pleasingly, it shows in vitro cytotoxicity in line with that expected for this type of metallodrug and, just as importantly, it is nontoxic to the organism as a whole. The radiolabeled complex was tracked in the body to provide information about drug uptake, biodistribution, and excretion. This ability to obtain a big-picture idea of the location of the presented theranostic sets this work apart from the rest of the field. As metallotherapeutics develop, a thorough understanding of how biological activity is achieved will be imperative to designing and improving future metallodrugs. Thus, we believe strategies such as that shown here will be essential to the further development of this research field.

## Experimental Section

### *Synthetic procedures*

**General information**: All solvents were dried and distilled under argon before use. The *p*-cymene–ruthenium dimer was obtained by following the procedure of Bennett and Smith.[10] All other reagents were commercially available and used as received (DOTA-derivative **A** was provided by Chematech). The analyses were performed at the "Plateforme d'Analyses Chimiques et de Synthèse Moléculaire de l'Université de Bourgogne". The identity and purity ($\geq$95%) of the complexes were unambiguously established by using high-resolution mass spectrometry and multinuclear NMR spectroscopy. The exact masses of the complexes were obtained on a Thermo LTQ Orbitrap XL ESI-MS mass spectrometer. $^{1}$H (300.13, 500.13, or 600.23 MHz), $^{13}$C (75.5, 125.8, or 150.9 MHz), and $^{31}$P (121.5, 202.5, or 242.9 MHz) NMR spectra were recorded on Bruker 300 Avance III, Bruker 500 Avance III, or Bruker 600 Avance II spectrometers. Chemical shifts ($\delta$) are quoted in parts per million relative to tetramethylsilane (TMS; $^{1}$H and $^{13}$C), by using the residual protonated solvent ($^{1}$H) or the deuterated solvent ($^{13}$C) as an internal standard. Alternatively, 85% $H_3PO_4$ ($^{31}$P) was used as an external standard. The coupling constants are reported in Hertz. All aromatic positions (*ortho*, *meta*, and *para*) are defined by using phosphorus as the main group element. Infrared spectra were recorded on a Bruker Vector 22 FT-IR spectrophotometer (transmission mode) with 1% sample mixed with potassium bromide. The melting points were determined on a BÜCHI Melting Point B-545 instrument.

**2,2′,2″-(10-(2-((2-(4-(diphenylphosphino)benzamido)ethyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid (1)**: Compound **3** (0.6 g, 1.34 mmol) was dissolved in freshly distilled MeCN (30 mL) under argon. Diisopropylethylamine (DIPEA; 2.3 mL, 13.44 mmol) was then added. The reaction was stirred at room temperature for 1 h. Compound **2** (0.54 g, 1.34 mmol) was added and the solution was stirred at room temperature overnight. After evaporation of the solvent, the resultant solid was purified by silica gel column chromatography (eluent: $CHCl_3$/MeOH/25% $NH_3{\cdot}H_2O$ (6:3:1)) to afford the desired product as a white solid (0.56 g, 57%). $^{1}$H NMR (300 MHz, MeOD): $\delta$ = 2.0–3.56 (br m, 24H; cyclen), 7.29–7.84 ppm (m, 14H; $CH_{ar}$), NH signal not observed; $^{31}$P{$^{1}$H} NMR (121.5 MHz, $CDCl_3$): $\delta$ = −5.7 ppm; $^{13}$C{$^{1}$H} NMR (75.5 MHz, $D_2O$): $\delta$ = 48.3, 52.5, 56.4, 59.6, 127.6, 127.7, 128.8, 128.9, 129.2, 132.6, 132.9, 133.2, 133.5, 134.7, 136.0, 136.1, 140.2, 140.4, 166.0, 173.2, 175.3 ppm; HRMS (ESI): *m/z* calcd for $[C_{37}H_{47}N_6O_8P-2H+K]^-$: 771.26790; found: 771.26539.

**Dichloro[($\eta^6$-*p*-cymene)(2,2′,2″-(10-(2-((2-(4-(diphenylphosphino)-benzamido)ethyl)amino)-2-oxoethyl)-1,4,7,10-tetraazacyclododecane-1,4,7-triyl)triacetic acid)-ruthenium(II)] (4)**: Compound **1** (100 mg, 0.136 mmol) and $[RuCl_2(p\text{-cymene})]_2$ (41.6 mg, 0.068 mmol) were dissolved in freshly distilled $CH_2Cl_2$ (3.5 mL) under argon. The solution was stirred at room temperature for 19 h. The solution was concentrated under reduced pressure to a volume of 1 mL. Freshly distilled pentane (5 mL) was added to precipitate the product as an orange powder. The powder was filtrated under an inert atmosphere and washed with degassed acetone (2×2 mL) to afford the desired product as an orange-red solid (0.106 g, 78%). $^{1}$H NMR (300.13 MHz, $D_2O$): $\delta$ = 1.09 (d, 6H, $^{3}J$ = 6.9 Hz; $CHMe_2$), 1.87 (s, 3H; Me of *p*-cymene), 2.82 (br m, 1H; C*H*$Me_2$ (*p*-cymene)), 1.95–3.87 (br m, 24H; cyclen), 5.19 (d, 2H, $^{3}J$ = 5.9 Hz; $CH_{Ar}$ of *p*-cymene), 5.33 (d, 2H, $^{3}J$ = 5.9 Hz; $CH_{Ar}$ of *p*-cymene), 7.45–7.96 ppm (m, 14H; $CH_{ar}$), NH signal not observed; $^{31}$P{$^{1}$H} NMR (121.5 MHz, $CDCl_3$): $\delta$ = 24.9 ppm; $^{13}$C{$^{1}$H} NMR (75 MHz, $CDCl_3$): $\delta$ = 124.3–128.2 (m), 58.2 (s), 52.6 (m), 48.4 (m), 30.3 (s), 22.4 (s), 22.0 (m), 18.4 (s), 17.9 ppm (m); HRMS (ESI): *m/z* calcd for $[C_{47}H_{61}Cl_2N_6O_8PRu-2H+K]^-$: 1077.21951; found: 1077.21750.

**Dichloro[($\eta^6$-*p*-cymene)(samarium-2,2′,2″-(10-(2-((2-(4-(diphenyl-phosphino)benzamido)ethyl)amino)-2-oxoethyl)-1,4,7,10-tetraa-zacyclododecane-1,4,7-triyl)triacetate)-ruthenium(II)] (5)**: $Sm(OTf)_3$ (22 mg, 0.038 mmol) dissolved in dry MeCN (10 mL) was added to a solution of **4** (35 mg, 0.032 mmol) in dry MeCN (10 mL). After 36 h, the resulting mixture was filtered and the solid was washed with dry *n*-hexane. The desired product was obtained as a bright orange powder after having been dried under vacuum (32 mg, 73%). $^{1}$H NMR (300.13 MHz, DMSO): $\delta$ = 0.94 (d, 6H, $^{3}J$ = 6.9 Hz; $CHMe_2$),1.76 (s, 3H; Me of *p*-cymene), 1.05–3.25 (br m, 25H; cyclen + C*H*$Me_2$ (*p*-cymene)), 5.25 (d, 2H, $^{3}J$ = 5.4 Hz; $CH_{ar}$ of *p*-cymene), 5.32 (d, 2H, $^{3}J$ = 5.4 Hz; $CH_{Ar}$ of *p*-cymene), 7.05–7.95(m, 14H; $CH_{Ar}$), 8.85 ppm (br s, 2H; *H*$NCH_2CH_2NH$); $^{31}$P{$^{1}$H} NMR (121.5 MHz, $CDCl_3$): $\delta$ = 24.3 ppm; HRMS (ESI): *m/z* calcd for $[C_{48}H_{61}Cl_2N_6O_8PRuSm-Cl]^-$: 1154.19830; found: 1154.20347.

### *Synthesis of radiolabeled complexes*

$^{153}$Sm ($T_{1/2}$ = 46.8 h; $\beta^-$ = 0.67 MeV, 34%; 0.71 MeV, 44%; 0.81 MeV, 21%; $\gamma$ = 0.103 MeV, 38%) was produced by irradiating isotopically enriched $^{152}Sm(NO_3)_3$ at the ITN Portuguese Research Reactor. The nitrate targets were prepared by dissolving $^{152}Sm_2O_3$ with $HNO_3$ and then evaporating to dryness. Irradiation was performed at 1 MW, thermal neutron flux of $\sim 0.8\times10^{13}$ neutron cm$^{-2}$ s$^{-1}$, and epithermal neutron flux of $\sim 2\times10^{11}$ neutron cm$^{-2}$ s$^{-1}$. The specific activity after 2 h of irradiation and at end of bombardment was 185 MBq mg$^{-1}$ for $^{153}$Sm. After irradiation, the targets were reconstituted with water to produce stock solutions for the synthesis of the complexes. The $^{153}$Sm activity was measured in an ionization chamber (Aloka Curiemeter IGC-3).

The complex [$^{153}$Sm]**5** was prepared by dissolving **4** (4 mg) in MeOH or DMSO (90 μL) and then adding water (500 μL) and an adequate amount of $^{153}$Sm solution (30 μL) to achieve a 1:2 metal/ligand molar ratio. The final pH value was ~5.5 and the final ligand concentration was 6.3 mM. The radiolabeling efficiency and reaction kinetics of the radiocomplexes were determined by RP-HPLC and by ITLC-SG analysis by using silica gel (ITLC-SG) strips (Polygram, Macherey–Nagel) with MeOH/$H_2O$/conc. aq. $NH_3$ (2:4:0.2) and MeOH/6 M HCl (95:5) as the eluents. The radioactive distribu-

tion on the ITLC-SG strips was detected by using a Berthold LB 505 γ detector coupled to a radiochromatogram scanner.

HPLC analyses of the inactive and radioactive complexes were achieved on a reverse-phase EC-Nucleosil $C_{18}$ column (250×4 mm, 10 μm) eluted at a flow rate of 1.0 mL min$^{-1}$ with 0.1% trifluoroacetic acid (TFA) aqueous solution (A) and 0.1% TFA in MeCN (B) as the eluents and with the following linear gradient: 0–3 min, 100%A/0%B; 3–18 min, 100%A/0%B→0%A/100%B; 18–20 min, 0%A/100%B.

### *Cytotoxicity assays*

*Cell culture*: The human ovarian cancer cell line A2870cisR (resistant to cisplatin; ECACC, UK) was grown in RPMI 1640 culture medium (Invitrogen) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin at 37 °C in a humidified atmosphere of 95% of air and 5% $CO_2$ (Heraeus, Germany).

*Cytotoxicity assay*: The cytotoxicity of compounds **1** and **5** against the cell line was evaluated by using a colorimetric method based on the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), which is reduced by viable cells to yield purple formazan crystals. Cells were seeded in 96-well plates at a density of $1\times10^4$–$1.5\times10^4$ cells per well in culture medium (200 μL) and were left to incubate overnight for optimal adherence. After careful removal of the medium, a dilution series of the compounds (200 μL; stock solutions prepared fresh in DMSO) in medium were added and incubation was performed at 37 °C and 5% $CO_2$ for 72 h. The percentage of DMSO in the cell culture medium did not exceed 1%. At the end of the incubation period, the compounds were removed and the cells were incubated with MTT solution (200 μL; 500 μg mL$^{-1}$). After 3–4 h at 37 °C and 5% $CO_2$, the medium was removed and the purple formazan crystals were dissolved in DMSO (200 μL) by shaking. The cell viability was evaluated by measurement of the absorbance at 570 nm by using a plate spectrophotometer (Power Wave Xs, Bio-Tek). The cell viability was calculated by dividing the absorbance of each well by that of the control wells (cells treated with medium containing 1% DMSO). Each experiment was repeated at least two times and each point was determined in at least six replicates.

### *Biodistribution and in vivo stability studies*

Biodistribution of complex [$^{153}$Sm]**5** was evaluated in CD-1 mice (randomly bred, obtained from IFFA, CREDO, Spain) weighing ~20–25 g. Animals were intravenously injected in the tail vein with the test complex (2.9–5.5 MBq) diluted in 0.9% NaCl (100 μL). Mice were sacrificed by cervical dislocation at 1 h after injection. The dose administered and the radioactivity in the sacrificed animals was measured by using a dose calibrator (Curiemeter IGC-3, Aloka, Tokyo, Japan). The difference between the radioactivity in the injected and sacrificed animals was assumed to be due to excretion. Tissues of interest were dissected, rinsed to remove excess blood, and weighed, and their radioactivity was measured by using a γ counter (LB2111, Berthold, Germany). The uptake in the tissues was calculated and expressed as a percentage of the injected radioactivity dose per gram of tissue. For blood, bone, and muscle, the total activity was estimated by assuming that these organs constitute 6, 10, and 40% of the total body weight, respectively.

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