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Synthesis and properties of a functionalized heterobimetallic Re(I)–Gd(III) complex as a potential dual-contrast agent for molecular imaging

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ABSTRACT

In the design of dual-imaging probes, the first functionalized and neutral heterobimetallic Re(I)–Gd(III) complex, highly soluble in aqueous solutions, has been prepared. This system exhibits interesting photophysical properties (λ_{em} = 578 nm, ϕ = 1.4%) for optical imaging and substantial higher relaxivity (r_1 = 6.6 mM^{-1} s^{-1} at 0.47 T and 37 °C) than the clinically used MRI contrast agents. Moreover, this system incorporates an aromatic ester functionality suitable for bioconjugation.

Re(I) complexes of general formula $[ReX(CO)_3(N^N)]^+$ where N^N indicates a chelating α,α'-diimine ligand (such as 2,2′-bipyridine (bpy) or 2-pyridyltriazole (pyta)) and X a neutral monodentate ligand (azaheterocycle such as pyridine) are currently attracting much interest for their wide applications in bioimaging. These complexes have found uses as biological probes because of their kinetic inertness in many different chemical and biological environments, as well as their rich physico-chemical behavior and their general synthetic flexibility and modularity of the X component.

These complexes are well known as triplet metal-to-ligand charge transfer (^{3}MLCT) luminescent systems with useful photophysical properties.[1] They can luminesce at room temperature, with excitation and emission in the visible domain (350–600 nm) and exhibit long-lived emission (μs domain) which potentially can overcome the major drawback of organic fluorophores, that is the superposition of short-lived fluorescence of biological materials. As a consequence, these complexes are exploited in in vitro optical imaging, that is cellular imaging using fluorescence microscopy.[2] A wide range of such d^6 complexes have also been shown to be highly effective sensitizers for lanthanide(III) ions (Yb(III) and Nd(III) in particular) which open perspectives for optical bioimaging in the NIR domain (900–1200 nm).[3] Recently, it has been demonstrated that these metal tris-carbonyl species may be used as IR bio-imaging labels, thanks to their specific IR signal in the 1800–2000 cm^{-1} region, where biological samples are transparent.[4] Finally, the derivatization of the coligand X opens many avenues to bioconjugation or to the formation of hetero dinuclear complexes through the introduction of another chelating unit.[5,6]

On the other hand, the design of multi-imaging probes, allowing to combine strengths of several imaging modalities was intensified in recent years due to the emergence of molecular imaging sciences.[7] In this context, heterobimetallic complexes based on rhenium(I) architectures are promising dual-imaging probes, combining both optical imaging and other imaging modalities such as (i) magnetic resonance imaging (additional Gd(III) core),[6a,c] (ii) single photon emission computed tomography (additional ^{111}In or ^{99m}Tc core),[6b] (iii) positron emission tomography (additional ^{89}Y, ^{64}Cu or ^{68}Ga core).

In this Letter, we report the synthesis of a new bifunctional neutral probe for bimodal imaging, based on fluorescence (Re core) and magnetic resonance (Gd(III) core) imaging modalities (compound **1**, Scheme 1).[8] On one hand, this hybrid platform uses a

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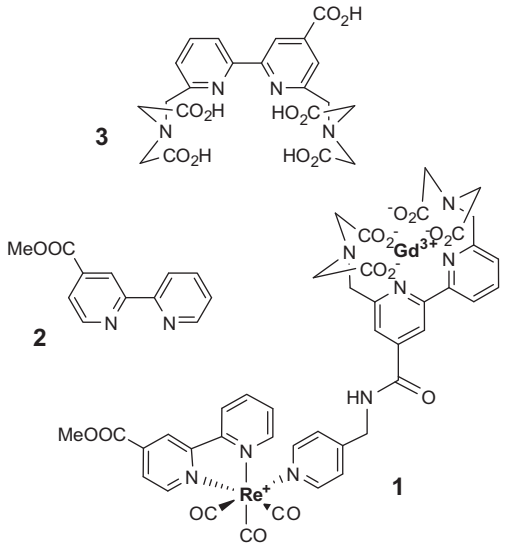
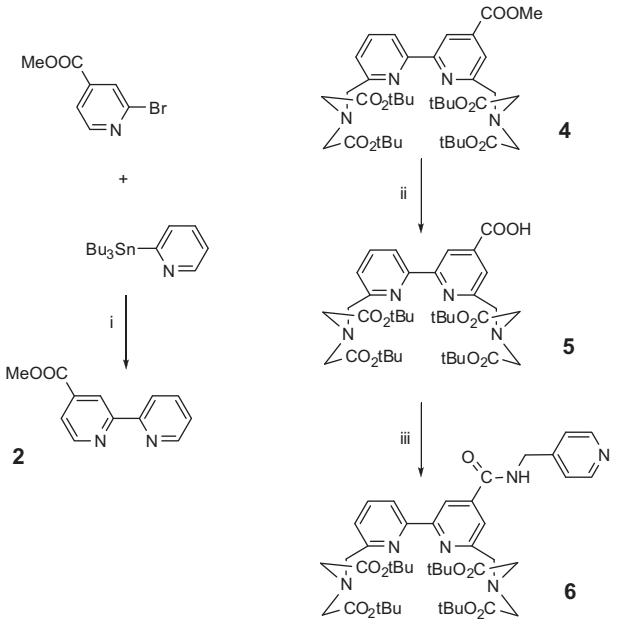


Scheme 1. Structures of compounds **1**–**3**.

functionalized 2,2′-bipyridine moiety (**2**, bpyCOOMe) which allows both a photosensitization of the rhenium center as well as bioconjugation by means of the aromatic ester functionality.[9] On the other hand, the chelating part of Gd(III) metal uses a 2,2′-bipyridine-based polyaminocarboxylate compound (**3**), which presents a promising ligand for MRI and optical imaging based on Ln(III) ions.[10] Moreover, an ^{111}In.**3** bioconjugate was recently used with success by some of us as a molecular nuclear imaging agent for the visualization of CCK2R positive tumours in small animals.[11]

The synthesis of starting materials, 4-functionalized 2,2′-bipyridine ligands **2** and **6** is depicted in Scheme 2.

Compound **2** was prepared through a Stille cross-coupling reaction between 2-(tri-*n*-butyl-stannyl)pyridine and methyl 2-bromopyridine-4-carboxylate in the presence of $Pd(P(Ph_3)_4)$ as the catalyst. Under microwave irradiation, this reaction was completed in 2 h and afforded the desired product in 72% yield after purification on alumina column. The fully protected bifunctional chelating agent **4** was synthesized in four steps from commercially available reagents as described previously (45% overall yield).[9] After a selective hydrolysis of the methyl ester group, the resulting carboxylic acid function was activated with a phosphonium-HOBt peptide coupling reagent (PyBOP). The formation of an amide bond between this in situ activated carboxylic function and the amino group of 4-picolylamine afforded the desired product **6** in 79% isolated yield.



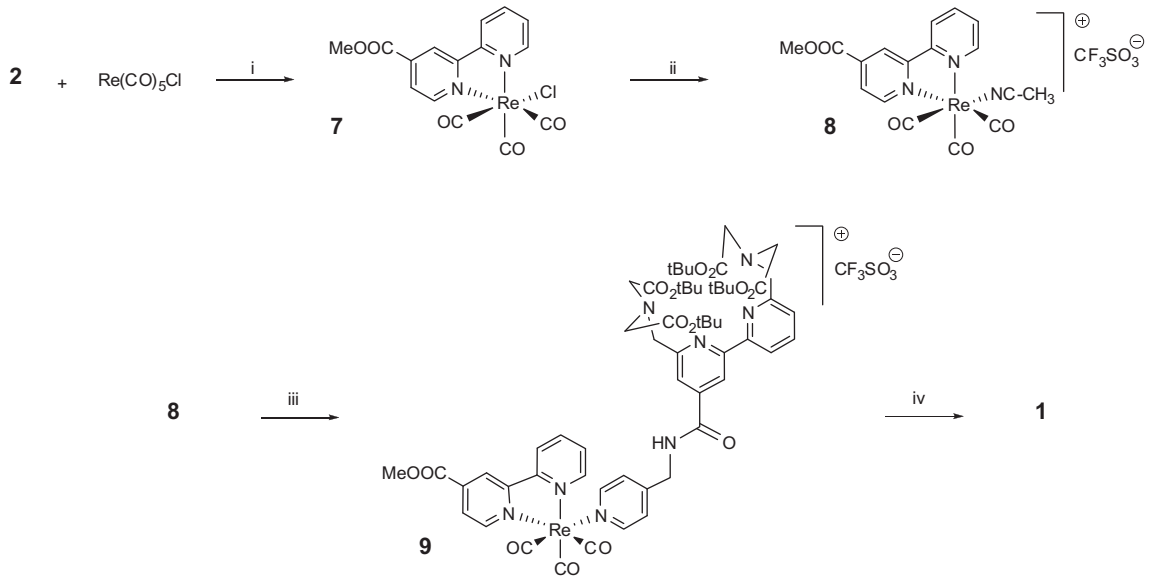
Scheme 2. Preparation of compounds **2** and **6**. Reagents and conditions: (i) $Pd(PPh_3)_4$ (0.2 equiv), CuBr (0.16 equiv), toluene, 140 °C (microwave 260 W), 2 h, 72%; (ii) K_2CO_3 (1 equiv), methanol/water (2:1), rt, 24 h, 89%; (iii) 4-picolylamine (1.3 equiv), PyBOP (1.6 equiv), iPr$_2$NEt (5 equiv), CH_2Cl_2, rt, 48 h, 79%.

With these building blocks in our hands, the dinuclear Re/Gd complex **1** was prepared in a four step synthesis in an overall yield of 18% (Scheme 3). Complexation to the tricarbonylrhenium core was achieved by refluxing a methanolic solution of the bpyCOOMe ligand **2** with $[Re(CO)_5Cl]$. Chloride abstraction of the resulting new *fac*-$[Re(CO)_3$(bpyCOOMe)Cl] complex, **7**, was achieved by AgOTf in acetonitrile at 60 °C yielding the corresponding Re-bound acetonitrile molecule, **8**. The substitution of the metal-bound acetonitrile molecule by pyridine derivative **6** took place over 1 h under microwave irradiation and led to Re(I) complex **9** in 56% isolated yield. Cleavage of the *tert*-butyl ester protecting groups was then accomplished using trifluoroacetic acid at room temperature, and the subsequent treatment of the tetracid compound with 1 equiv of $GdCl_3$ in water yields the neutral dinuclear complex Re/Gd **1**.

Due to the paramagnetic nature of the gadolinium part, the purity of this dinuclear complex **1** was established on the basis of the RP-UPLC chromatogram and its structure determined by IR and mass spectrometric analyses.[12] This complex is soluble in water (> 0.01 M) and its fluorescence emission and paramagnetic properties in aerated water solutions remained unchanged for several days at room temperature, highlighting its kinetic inertness in this medium.

UV–vis and preliminary fluorescence studies of the dinuclear complex **1** were undertaken at room temperature in aerated Tris buffer solution at pH 7.4 (Fig. 1). The electronic absorption spectrum of **1** exhibits intense absorptions centred at 247 and 315 nm (ε = 27,000 and 19,000 M^{-1} cm^{-1}, respectively) which are assigned to intraligand transitions [π-π^* (bpyCOOMe and py-bpy moieties)] because similar absorptions are observed for the uncoordinated ligands. The lower-energy absorption shoulders at ca., 340–430 nm with extinction coefficients in the order of 2×10^3 M^{-1} cm^{-1} are assigned to spin-allowed metal-to-ligand charge-transfer (MLCT) [dπ(Re) → π^*(bpyCOOMe ligand)] transitions. Excitation of dinuclear complex at λ_{exc} >350 nm gives rise to a sizeable fluorescence emission at 578 nm which is assigned to originate from a ^{3}MLCT [dπ(Re) → π^*(bpyCOOMe ligand)] excited state on the basis of previous spectroscopic studies of [Re(py)(CO_3)bpy] complexes.[5] The complex **1** displays a quantum yield of 1.4%, sufficiently large for detection in fluorescence microscopy.[4] It has to be noted that photophysical properties of Re(I) complexes in aqueous solutions are poorly reported in the literature.[13]

In order to assess the potential of this Re/Gd complex to act as MRI contrast agent, we estimated its relaxometric properties at body temperature (310 K) with proton larmor frequencies of 20 and 60 MHz which correspond to magnetic fields operating currently in clinical imaging (0.2–3T). The proton longitudinal relaxivities r_1 of compound **1** in H_2O at physiological pH were equal to 6.6 and 6.0 mM^{-1} s^{-1}, at 20 and 60 MHz, respectively. These values are 1.7–1.9 times larger than those found in mono-aqua contrast agents $[Gd(DTPA)(H_2O)]^{2-}$ or $[Gd(DOTA)(H_2O)]^-$ which are currently used in clinical practice.[14] They are also larger than those found in some diaqua Gd(III) complexes such as DO3A derivatives.[15] Interestingly, no substantial reduction of r_1 value was observed when **1** was incubated in phosphate-buffered saline solution. This behavior suggests that no significant displacement of water molecule from the Gd(III) coordination sphere in **1** occurs



Scheme 3. Synthesis of dinuclear complex **1**. Reagents and conditions: (i) $Re(CO)_5Cl$ (1.2 equiv), MeOH, 65 °C; overnight, 91%; (ii) AgOTf (1.2 equiv), THF-CH_3CN, overnight, reflux, 100%; (iii) **6** (2.5 equiv), THF, 105 °C (microwave 155 W), 1 h, 56%; (iv) (a) CF_3COOH (300 equiv), CH_2Cl_2, rt, 24 h; then, (b) $GdCl_3$ $6H_2O$, H_2O (pH 5-6), rt, overnight, 36% (two steps).

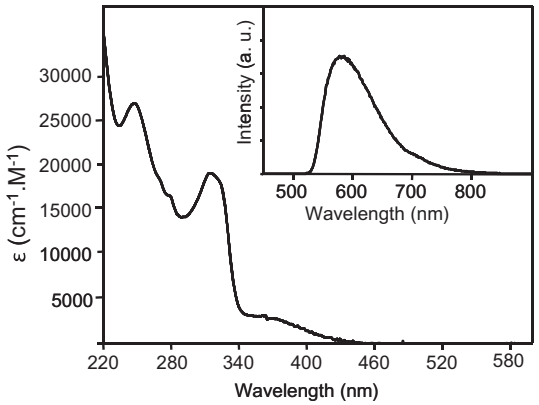


Figure 1. Absorption and emission (inset, λ_{exc} = 365 nm) of dinuclear complex **1** in 50 mM tris buffer (pH 7.4) at 298 K.

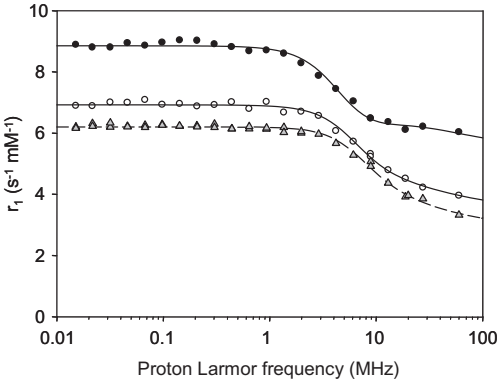


Figure 2. ^1H NMRD relaxivity profile of dinuclear complex **1** (closed circles), Gd **3** (open circles) and Gd-DTPA (closed triangles) in water at 310 K. The lines are drawn to guide the eyes.

in the presence of phosphate anion.[16] We have also investigated the relaxometric properties of **1** on a larger range of magnetic fields (0.01–60 MHz). The resulting NMRD relaxivity profile of **1** at 310 K (Fig. 2) revealed a high relaxivity at all magnetic fields and has the typical shape for a low-molecular-weight Gd complex. These values are larger over the whole magnetic field range than those of the parent complex Gd.**3**. This is probably the result of the increase in the molecular weight of **1** with a concomitant slower tumbling in solution, that is an increase in the rotational correlation time (τ_R).

Finally, no significant binding of the dinuclear complex **1** with Human Serum Albumin (HSA) was detected. In the presence of 4% HSA, relaxivity value r_1 of 7.4 mM^{-1} s^{-1} was observed at 20 MHz and 310 K. Such weak increase (<15%) of the r_1 value does not indicate a significant interaction of the complex with HSA.[17] This result is promising, since a molecular imaging agent must specifically bind to its target while avoiding non-specific interferences with blood macromolecules such HSA.

In conclusion, a trifunctional system combining a tridentate donor set for complexation of a $\{Re(CO)_3\}$ moiety, an octadentate donor set for complexation of a Gd(III) ion and an aromatic ester group suitable for a subsequent bioconjugation has been conveniently prepared. This system is highly soluble in water and aqueous buffer solutions, retains exploitable ^{3}MLCT emission characteristics of the Re(I) moiety for fluorescence imaging and displays a high relaxivity of the Gd(III) component promising for MRI. This hybrid system once bioconjugated with a given targeting vector will exhibit a unique biodistribution profile for two imaging probes. It should be useful for in vivo detection of pathologies via MRI and for supporting surgical guidance via intraoperative fluorescence imaging.

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12. *Selected characterization data for compounds* ***6***, ***8***, ***9*** *and* ***1***. Compound **6**. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 8.71 (d, J = 1.4 Hz, 1H), 8.43 (d, J = 6.0 Hz, 2H), 8.24 (dd, J = 0.5, 7.8 Hz, 1H), 8.13 (t, J = 5.9 Hz, 1H), 8.07 (d, J = 1.4 Hz, 1H), 7.71 (t, J = 7.8 Hz, 1H), 7.47 (dd, J = 0.5, 7.8 Hz, 1H), 7.21 (d, J = 6.0 Hz, 2H), 4.62 (d, J = 5.9 Hz, 2H), 4.10 (s, 2H), 4.00 (s, 2H), 3.44 (s, 8H), 1.36 (s, 36H). MS (ESI)$^+$ m/z: 805.5 (100%) $[M+H]^+$. Anal. Calcd for $C_{43}H_{60}N_6O_9$: C, 64.16; H, 7.51; N, 10.44. Found: C, 63.91; H, 7.47; N, 10.12. Compound **8**. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 9.08 (dd, J = 0.5, 5.7 Hz, 1H), 8.99 (d J = 0.9 Hz, 1H), 8.96 (dd, J = 0.8, 5.5 Hz, 1H), 8.66 (d, J = 8.2 Hz, 1H), 8.30 (td, J = 8, 1.5 Hz, 1H), 8.15 (dd, J = 1.6, 5.7, 1H), 7.71 (ddd, J = 1.1, 5.5, 7.6 Hz, 1H), 4.06 (s, 3H), 2.25 (s, 3H); IR (ν_{max}, cm^{-1}) 2039 and 1924 (br) (C≡O), 1734 (C=O); HRMS (DCI, CH4)$^+$ m/z Calcd for $[C_{18}H_{13}N_3O_8F_3SRe–CH_3CN]^+$ = 631.9640. Found 631.9697. Anal. Calcd for $C_{18}H_{13}F_3N_3O_8ReS{\cdot}OEt_2$: C, 35.29; H, 3.10; N, 5.61. Found: C, 35.03; H, 3.02; N, 5.44. Compound **9**. UPLC (Acquity BEH C18 column, 1.7 μM, 100 Å, 50 × 2.1 mm, gradient: $HCOONH_4$ 10 mM pH 4/CH_3CN + 10% $HCOONH_4$ 100 mM pH 4 80/20 to 0/100 in 7 min; flow: 0.6 mL/min): t_R = 5.07 min. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) = 9.23 (d, J = 5.4 Hz, 1H), 9.11 (dd, J = 0.8, 5.4 Hz, 1H), 8.94 (d, J = 0.7 Hz, 1H), 8.69 (d, J = 1.3 Hz, 1H), 8.63 (d, J = 8.1 Hz, 1H), 8.32 (d, J = 7.8 Hz, 1H), 8.27 (d, J = 7.5 Hz, 1H), 8.22 (dd, J = 1.5, 5.7 Hz, 1H), 8.05–7.99 (m, 3H), 7.83–7.73 (m, 2H), 7.58 (d, J = 7.5 Hz, 1H), 7.35 (d, J = 6.3 Hz, 2H), 4.58 (br s, 2H), 4.13(s, 2H), 4.08 (s, 2H), 4.04 (s, 3H), 3.52 (s, 4H), 3.50 (s, 4H), 1.44 (s, 18H), 1.43 (s, 18H); ^{19}F NMR (300 MHz, $CDCl_3$) δ (ppm) = −78.3; HRMS (ESI$^+$) m/z Calcd for $[C_{59}H_{70}N_8O_{17}F_3SRe–CF_3SO_3^-]^+$ 1287.4541. Found 1287.4591.Dinuclear complex **1**: UPLC (Acquity BEH C18 column, 1.7 μM, 100 Å, 50 × 2.1 mm, gradient: $HCOONH_4$ 10 mM pH 4/CH_3CN + 10% $HCOONH_4$ 100 mM pH 4 80/20 to 0/100 in 7 min; flow: 0.6 mL/min): t_R = 1.59 min; HRMS (ESI)$^+$ Calcd for $[C_{42}H_{34}N_8O_{14}ReGd + H]^+$ = 1220.1071. Found 1220.1099;
IR (ν_{max}, cm^{-1}) 2027 and 1914 (br) (C≡O), 1686 (C=O), 1614 (COO^-); UV–vis (Tris buffer pH 7.4 50 mM): λ_{max} (ε, M^{-1} cm^{-1}) = 247 (27,000), 315 (19,000), 367 (2400). The absence of free Gd(III) ion in **1** was verified using a classic test with an arsenazo indicator solution.
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