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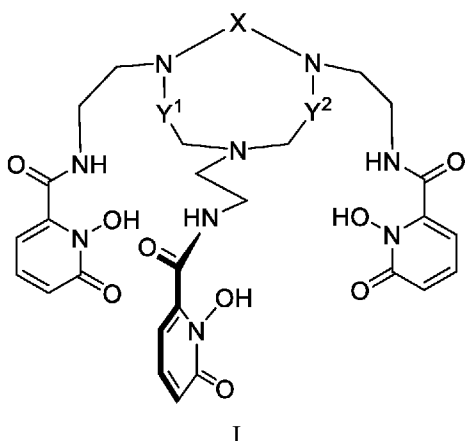
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(54) Title: LIGANDS AND COMPLEXES AND USES THEREOF



(57) Abstract: Disclosed herein are ligands of formula I or salts thereof, and rare earth metal complexes of the ligands of formula I or salts thereof. Also disclosed herein are devices and materials comprising ligands of formula I or salts thereof, or rare earth metal complexes of the ligands of formula I or salts thereof as well as methods of using the ligands, complexes, devices and materials for detecting ions such as phosphate, methods for removing ions such as phosphate from aqueous solutions or mixtures, and methods for treating hyperphosphatemia. Also disclosed herein are additional ligands and metal complexes thereof as well as uses for these ligands and metal complexes.

LIGANDS AND COMPLEXES AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATION

This application claims priority to United States Provisional Application Number
5 63/472,542 filed on 12 June 2023, which is incorporated by reference herein.

GOVERNMENT FUNDING

This invention was made with government support under DK124333 awarded by the
National Institutes of Health. The government has certain rights in the invention.

BACKGROUND OF THE INVENTION

Hyperphosphatemia is a condition whereby phosphate concentration in the blood reaches
levels above 1.46 mmol/L. It appears primarily in patients with chronic kidney diseases (CKD)
and those undergoing dialysis because the latter is inefficient at removing phosphate from the
15 blood (Desoi, C. A.; et al., *J. Am. Soc. Nephrol.* 1993, 4, 1214-1218). If left untreated, the
condition leads to vascular calcification, up to 200 mg/week, and thus, in the long term, increased
morbidity and mortality (Cupisti, A.; et al., *Int. J. Nephrol. Renovasc. Dis.* 2013, 193-205).
Hyperphosphatemia is currently managed by phosphate binders, oral salts such as CaCO_3 or
 $\text{La}_2(\text{CO}_3)_2$, or polymers such as Sevelamer®, that bind phosphate in the blood (G. London.; et al.
20 *Clin. Nephrol.* 2010, 74, 423-432; Zhang, C.; et al., *BMC Nephrol.* 2013, 14, 226; Steven
Fishbane; *Am. J. of Kidney Dis.* 2009, 55, 307-315). Beyond significant side effects and poor
patient compliance due to the high pill burden, phosphate binders have limited success rates. For
instance, in one study only 17% of patients taking $\text{La}_2(\text{CO}_3)_3$ achieved target blood phosphate
levels (Bhargava, R.; et al., *BMC Nephrol.* 2019, 20, 37).

25 An alternative to using oral phosphate binders is to directly scrub the blood from excess
phosphate. With the goal of developing a new paradigm to the treatment of hyperphosphatemia
via blood filtration, inorganic phosphate receptors capable of removing phosphate from complex
aqueous systems such as blood with high affinity and high selectivity over other endogenous
anions have been developed (Pierre, V. C.; et al., *Front. Chem.* 2022, 10, 821020). Due to their
30 high oxophilicity and lability, lanthanide complexes (Martinon, T. L. M.; et al., *Chem. Asian J.*
2022, 17, e202200495; Ramakrishnam Raju, M. V.; et al., *Chem. Soc. Rev.* 2020, 49, 1090-1108;
Harris, S. M.; et al., *Environ. Sci. Technol.* 2017, 51, 4549-4558) are better suited for this
application than other metals such as copper (Tobey, S. L.; et al., *J. Am. Chem. Soc.* 2003, 125,
4026-4027; Goswami, S.; et al., *Tetrahedron Lett.* 2010, 51, 6707-6710), zinc (Singh, R.; et al.,

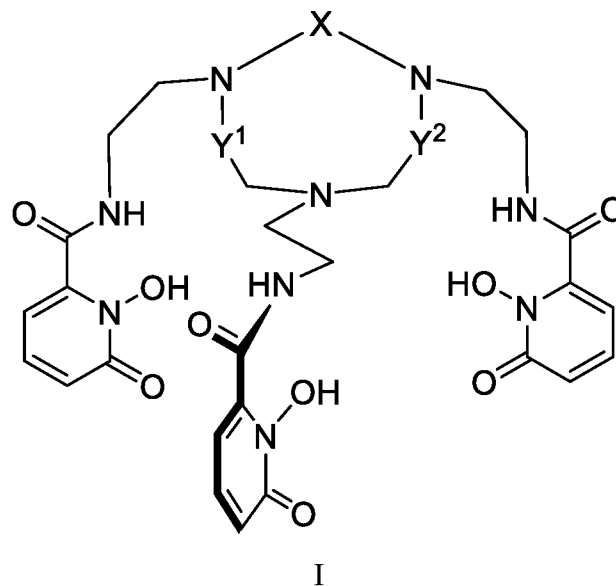
RSC Adv. 2016, 6, 112246-112252; Shi, B.; et. al., *Sens. Actuators B Chem.* 2014, 190, 555-561; Lee, H. N.; et al., *Org. Lett.* 2007, 9, 243-246), and iron (Huang, S.-Y.; et al., *JACS Au* 2022, 2, 1604-1609). The key to achieving both sensitivity and selectivity in a flexible lanthanide(III) receptor (Wilharm, R. K.; et al., *Inorg. Chem.* 2022, 61, 4130-4142) is to optimize the basicity of the chelating podand (Ramakrishnam Raju; et al., *Inorg. Chem.* 2019, 58, 15189-15201) and the geometry of the ligand (Huang, S.-Y.; et al., *Inorg. Chem.* 2020, 59, 4096-4108). Affinity for phosphate can then be fine-tuned without affecting selectivity by either changing the lanthanide ion (Wilharm, R. K.; et al., *Inorg. Chem.* 2021, 60, 15808-15817) or the charge of the complex (Huang, S.-Y.; et al., *Inorg. Chem.* 2019, 58, 16087-16099).

Although these phosphate receptors are intended for ex-vivo use while immobilized on a polymer support in conjunction with dialysis, for safety reasons no lanthanide ion must leach from the receptor during the procedure. Indeed, uncomplexed or “free” gadolinium(III) ions released by weak gadolinium-based MRI contrast agents have been linked to nephrogenic system fibrosis and brain deposits (Weller, A.; et al., *Pediatr. Nephrol.* 2014, 29, 1927-1937); similar toxicity is anticipated for the other lanthanide ions. Therefore, both the thermodynamic stability and the kinetic inertness of the complexes are important parameters to consider in the design of the receptor.

Accordingly, there is an ongoing need for ligands and metal complexes (e.g., rare earth metal complexes such as Eu^{III} complexes), that have one or more improved properties and/or are useful. Such ligands and metal complexes may be more stable (e.g., less leaching of metal) and/or may be useful for detecting ions (e.g., anions such as phosphate), for capturing and/or removing ions (e.g., anions such as phosphate) from aqueous solutions or mixtures (e.g., aqueous mixtures, blood, serum, waste water), and/or for treating hyperphosphatemia.

SUMMARY OF THE INVENTION

One embodiment provides a ligand of formula I or a salt thereof, or a rare earth metal complex of the ligand of formula I or a salt thereof or hydrate thereof,



wherein:

X is $-(CH_2)_2-$, $-(CH_2)_3-$, $-(CH_2)_pN(CH_2)_q-$

Y¹ is $-(CH_2)-$, or $-(CH_2)_2-$;

Y² is $-(CH_2)-$, or $-(CH_2)_2-$;

wherein Y¹ is $-(CH_2)_2-$ when X is $-(CH_2)_2-$ and Y² is $-(CH_2)-$;

p is 2 or 3;

q is 2 or 3; and wherein the compound of formula I is optionally substituted with one or more (e.g., 1, 2, or 3) linking groups.

One embodiment provides a material or device comprising one or more ligands or a salt thereof, or one or more metal complexes or a salt thereof or a hydrate thereof, as described herein.

One embodiment provides a method to detect or capture inorganic phosphate, comprising contacting the phosphate with a ligand or salt thereof, or a metal complex or a salt thereof or a hydrate thereof, or a material or device as described herein.

One embodiment provides a method to treat hyperphosphatemia in a mammal in need thereof comprising contacting the blood of the mammal in need thereof, with a ligand or salt thereof, or a metal complex or a salt thereof or a hydrate thereof, or a material or device as described herein.

One embodiment provides the use of a ligand or salt thereof, or a rare earth metal complex or a salt thereof, or a material or device as described herein for the treatment of hyperphosphatemia in a mammal in need thereof.

One embodiment provides the use of a ligand or salt thereof, or a rare earth metal complex or a salt thereof, or a material or device as described herein for medical therapy.

One embodiment provides the use of a ligand or salt thereof, or a rare earth metal complex or a salt thereof as described herein to prepare a device or material for the treatment of hyperphosphatemia.

One embodiment provides processes and intermediates disclosed herein that are useful for preparing a ligand of formula I or a salt thereof, or a metal complex of (e.g., a rare earth metal complex) of a ligand of formula I or a salt thereof.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 shows the chemical structures structure of the linear phosphate receptors Eu^{III} -3,3-Ser-HOPO (1), Eu^{III} -3,3-Gly-HOPO (2), and Eu^{III} -2,2-Li-HOPO (3) as well as the macrocyclic Eu^{III} -TACN-HOPO (4), Eu^{III} -TACD-HOPO (5), Eu^{III} -cyclen-HOPO (6), and Eu^{III} -cyclam-HOPO (7).

Figure 2 shows the time-delayed luminescence intensity of Eu^{III} -L-HOPO upon titration with $\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-$. The binding isotherms (solid lines) were fitted to a 1:1 stoichiometry for Eu^{III} -TACN-HOPO (4) or a 1:2 stoichiometry for Eu^{III} -cyclen-HOPO (6) and Eu^{III} -cyclam-HOPO (7) based on the results from the luminescence lifetimes studies. Experimental conditions: $[\text{Eu}^{\text{III}}\text{-L-HOPO}] = 7.35 \mu\text{M}$ in H_2O , $\text{pH} = 7.4$, $\lambda_{\text{ex}} = 334 \text{ nm}$, excitation and emission slit widths = 10 nm, I = integrated luminescence intensity from 550 to 750 nm, I_0 : integrated luminescence intensity in the absence of $[\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-]$, time delay = 0.1 ms. Error bars represent standard deviation, $n = 3$.

Figures 3A-3C show the selectivity of macrocyclic compounds. Figure 3A shows Eu^{III} -cyclen-HOPO, Figure 3B shows Eu^{III} -cyclam-HOPO, and Figure 3C shows Eu^{III} -TACN-HOPO for competing anions in water. White bars represent the time-delayed relative luminescence intensity after addition of 10 equiv of the appropriate anions (NaF , NaCl , NaBr , NaI , K_2SO_4 , NaHCO_3 , KNO_3 , Na_2AsO_4 , $\text{Na}_4\text{P}_2\text{O}_7$, $\text{NaC}_2\text{H}_3\text{O}_2$, $\text{Na}_3\text{C}_6\text{H}_5\text{O}_7$, and $\text{NaC}_3\text{H}_5\text{O}_3$). Dark grey bars represent the time-delayed relative luminescence intensity after subsequent addition of 10 eq. of phosphate. I = integrated luminescence intensity from 550 to 750 nm, I_0 = integrated luminescence intensity in absence of $[\text{HPO}_4^{2-}/\text{H}_2\text{PO}_4^-]$. Experimental conditions: $[\text{Eu}^{\text{III}}\text{-L-HOPO}] = 7.35 \mu\text{M}$ in H_2O , pH of 7.4, $T = 25^\circ\text{C}$, $\lambda_{\text{ex}} = 334\text{-}340 \text{ nm}$, excitation and emission slit widths = 10 nm, time delay = 0.1 ms. Error bars represent standard deviation, $n = 3$.

Figure 4 shows intensity time traces of ligand exchange of Eu^{III} -L-HOPO complexes with DTPA (diethylene triamine peracetic acid). I = integrated luminescence intensity from 550 to 750 nm in the presence of 5 equivalents of DTPA, I_0 = integrated luminescence intensity in absence of DTPA. Ligand exchange with Eu^{III} -Ser-HOPO (1) and Eu^{III} -TACN-HOPO (4) follows first order

kinetics, whereas ligand exchange with Eu^{III}-cyclam-HOPO (7) follows second order kinetics. Ligand exchange with Eu^{III}-cyclen-HOPO (6) does not fit either first or second order kinetics suggesting a more complex mechanism of ligand exchange. Experimental conditions: [Eu^{III}-L-HOPO] = 7.35 μ M in 0.1 M KCl, pH of 7.4, T = 25°C, λ_{ex} = 334-340 nm; excitation and emission slit widths = 10 nm, time delay = 0.1 ms.

Figures 5A-5C show excitation (black (left side)) and emission (grey (right side)) profiles of Eu^{III}-L-HOPO. Figure 5A shows Eu^{III}-cyclen-HOPO. Figure 5B shows Eu^{III}-cyclam-HOPO, and Figure 5C shows Eu^{III}-TACN-HOPO. Experimental conditions: [Eu^{III}-L-HOPO] = 7.35 μ M in H₂O, pH 7.4, T = 25°C; λ_{ex} , 334-340 nm, λ_{em} , 615 nm; excitation and emission slit widths, 10 nm; gate time, 5 ms; decay time, 0.02 ms; time delay, 0.1 ms.

Figure 6 shows time-delayed luminescence host-guest titration data of Eu^{III}-cyclen-HOPO to phosphate. Intensity was closely monitored between two samples of identical concentration; the non-aggregated sample having been left to equilibrate at 60°C overnight and sonicated prior to measurement. Experimental conditions: [Eu^{III}-cyclen-HOPO] = 7.35 μ M in H₂O, pH 7.4, T = 25°C; λ_{ex} , 334 nm; gate time, 5 ms; decay time, 0.02 ms; time delay, 0.1 ms.

Figure 7 shows luminescence lifetime of Eu^{III}-L-HOPO in presence or absence of 10 eq. phosphate: emission intensity decay of Eu^{III}-L-HOPO with variant mole fraction of H₂O (XH₂O) (left); extrapolation of lifetime in D₂O of Eu^{III}-L-HOPO at XH₂O = 0 (right). Experimental conditions: [Eu^{III}-L-HOPO] = 7.35 μ M in H₂O, pH 7.4, T = 25°C; λ_{ex} = 334-341 nm, λ_{em} = 615 nm; gate time 0.02 ms, excitation and emission slit widths, 10 nm, delay time, 0.1 ms.

Figures 8A-8C show time-dependent luminescence response of Eu^{III}-L-HOPO to phosphate. Figure 8A shows Eu^{III}-cyclen-HOPO; Figure 8B shows Eu^{III}-cyclam-HOPO; and Figure 8C shows Eu^{III}-TACN-HOPO. I: integrated luminescence intensity from 550-750 nm in the presence of 10 eq. of phosphate, I₀: integrated luminescence intensity in the absence of phosphate. *Experimental conditions*: [Eu^{III}-L-HOPO] = 7.35 μ M in H₂O, pH 7.4, T = 25°C; λ_{ex} , 334-340 nm; gate time, 5 ms; decay time, 0.02; time delay, 0.1 ms.

Figures 9A-9C show gradients of Eu^{III} complexes and coumarin utilized to determine relative quantum yield. Intensity of coumarin was integrated from 335-590 nm. Intensity of Eu^{III}-L-HOPO was integrated from a varying range to avoid Rayleigh scattering from the excitation wavelength. Figure 9A shows Eu^{III}-cyclen-HOPO (515-640 nm, 693-750 nm), Figure 9B shows Eu^{III}-cyclam-HOPO (515-635 nm, 685-750 nm), and Figure 9C shows Eu^{III}-TACN-HOPO (515-645 nm, 705-750 nm). Experimental conditions: [Eu^{III}-L-HOPO] = 7.35 μ M in H₂O, pH 7.4, T = 25°C; λ_{ex} Eu^{III}-L-HOPO, 334-338 nm; λ_{ex} coumarin, 311 nm; gate time, 0.1 ms.

Figure 10 shows the time-delayed luminescence response of Eu^{III} -cyclen-HOPO to 0.01 M HEPES in presence and absence of 10 eq. phosphate, intensity integrated from 550-750 nm.

Experimental conditions: $[\text{Eu}^{\text{III}}\text{-cyclen-HOPO}] = 7.35 \mu\text{M}$ in H_2O , pH 7.4, $T = 25^\circ\text{C}$; λ_{ex} , 334 nm; gate time, 5 ms; decay time, 0.02 ms; time delay, 0.1 ms.

Figure 11 shows the time-dependent luminescence competition titration data of Eu^{III} -cyclen-HOPO to DTPA approaching thermodynamic equilibrium KL in presence of 0.01 M HEPES. I: integrated luminescence intensity from 550-750 nm in the presence of 10 eq. of phosphate, I_0 : integrated luminescence intensity in the absence of phosphate. I_{eq} : the integrated luminescence intensity once thermodynamic equilibrium is reached. Plotted nonlinear fittings demonstrate the data fit to the respective decay kinetics. *Experimental conditions:* $[\text{Eu}^{\text{III}}\text{-cyclen-HOPO}] = 7.35 \mu\text{M}$ in 0.01 M HEPES, pH 7.4, $T = 25^\circ\text{C}$; λ_{ex} , 334 nm; gate time, 5 ms; decay time, 0.02 ms; time delay, 0.1 ms.

Figure 12 shows the time-delayed luminescence competition titration data of Eu^{III} -L-HOPO to DTPA approaching thermodynamic equilibrium KL unadjusted for I_{eq} : integrated luminescence intensity at equilibrium. I: integrated luminescence intensity from 550-750 nm in the presence of 10 eq. of phosphate, I_0 : integrated luminescence intensity in the absence of phosphate. *Experimental conditions:* $[\text{Eu}^{\text{III}}\text{-cyclen-HOPO}] = 7.35 \mu\text{M}$ in H_2O , pH 7.4, $T = 25^\circ\text{C}$; λ_{ex} , 334-380 nm; gate time, 5 ms; decay time, 0.02 ms; time delay, 0.1 ms.

Figures 13A-13D show the time-dependent luminescence competition titration data of Eu^{III} -L-HOPO to DTPA approaching thermodynamic equilibrium KL. Figure 13A shows Eu^{III} -Ser-HOPO, Figure 13B shows Eu^{III} -TACN-HOPO, Figure 13C shows Eu^{III} -cyclen-HOPO, Figure 13D shows Eu^{III} -cyclam-HOPO. I: integrated luminescence intensity from 550-750 nm in the presence of 10 eq. of phosphate, I_0 : integrated luminescence intensity in the absence of phosphate. I_{eq} : the integrated luminescence intensity once thermodynamic equilibrium is reached. The plotted nonlinear fittings demonstrate the data fit to the respective decay kinetics. *Experimental conditions:* $[\text{Eu}^{\text{III}}\text{-cyclen-HOPO}] = 7.35 \mu\text{M}$ in H_2O , pH 7.4, $T = 25^\circ\text{C}$; λ_{ex} , 334-340 nm; gate time, 5 ms; decay time, 0.02 ms; time delay, 0.1 ms.

Figure 14 shows the time-delayed luminescence competition titration data of Eu^{III} -L-HOPO to DTPA. I: integrated luminescence intensity from 550 nm to 750 nm in the presence of 1-15 eq. DTPA, I_0 : integrated luminescence intensity in the absence of DTPA. The 0-0.8 I/I_0 range is intensified for clarity. *Experimental conditions:* $[\text{Eu}^{\text{III}}\text{-L-HOPO}] = 7.35 \mu\text{M}$ in 0.1 M KCl, pH 7.4; $T = 25^\circ\text{C}$; λ_{ex} , 334-340 nm; gate time, 5 ms; decay time, 0.02; time delay, 0.1 ms. Error bars represent std. dev., $n = 3$.

DETAILED DESCRIPTION

The following definitions are used, unless otherwise described: halo or halogen is fluoro, chloro, bromo, or iodo. Alkyl and alkoxy, etc. denote both straight and branched groups but reference to an individual radical such as propyl embraces only the straight chain radical (a
5 branched chain isomer such as isopropyl being specifically referred to).

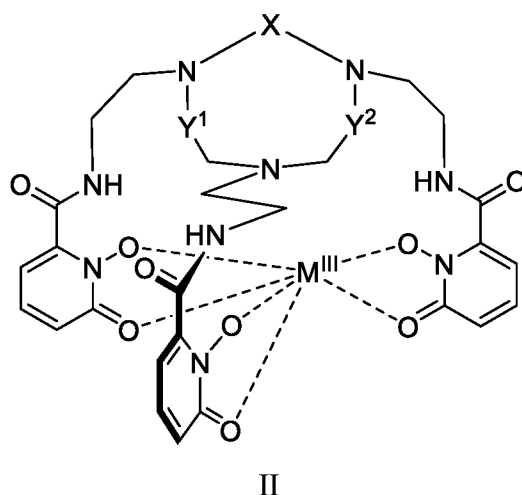
As described herein, the term linker refers to any molecular moiety that connects the ligand of formula I (or a complex thereof) to another entity such as a material or device. The term linker also includes linkers which include within the linker a reactive group that is useful to connect the ligand of formula I (or a complex thereof) to another entity such as a material or
10 device (e.g., linkers that include reactive groups wherein the reactive group is useful for covalently binding to the material or device). Examples of materials include polymers (e.g., a synthetic or natural polymer), hydrogels, membranes, nanoparticles, or other materials (e.g., solid material). Materials also include conducting polymers and conducting materials (e.g., metal or carbon materials). A device can be any device (e.g., electronic device, solid device) that the metal
15 complex can be attached (e.g., attached through a linker). The linker can be variable, provided it functions to connect the ligand of formula I to another molecular entity, so that both the ligand of formula I (or a complex thereof) and the other molecular entity can function as described herein. The linker can be covalently bonded to the ligand of formula I at any suitable atom such as a carbon or nitrogen atom. The linker can vary in length and atom composition (e.g., C, H, N, O, S,
20 halo, P) and for example can be branched or non-branched or saturated or unsaturated or a combination thereof. In one embodiment the linker connects the ligand of formula I (or a complex thereof) to another entity such as a material or device (e.g., through covalent bonds). In one embodiment the linker includes one or more reactive groups. The reactive group is useful for covalently bonding the remainder of the linker to a material or device. Examples of reactive
25 groups include amines, alcohols, thiols, amides, esters, carboxylic acids, aldehydes and epoxides.

In one embodiment the ligand of formula I (or a metal complex thereof) can be combined with one or more other materials without being bonded (e.g., covalently bonded) to the material(s). Thus, one embodiment provides a material comprising a ligand of formula I (or a metal complex thereof).

30 The term polymer includes any polymer suitable for linking to the metal complex described herein (e.g., synthetic or natural polymer). Examples include polyamides (including star polyamides), polyethyleneglycol, polyethylenimine, polysulfone, polyethersulfone, and conducting polymers (e.g., conducting polymers for electronic devices). The term hydrogel includes any hydrogel suitable for linking to the metal complex described herein. Examples

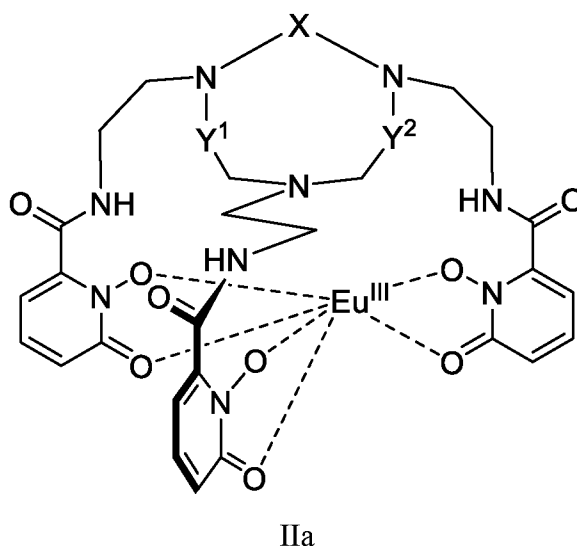
include crosslinked poly(N-isopropylacrylamide), crosslinked polyvinyl alcohol, PMA (polymethacrylate), PMMA (polymethylmethacrylate), PEMA (polyethylmethacrylate), and chitosan. The term membrane includes any membrane suitable for linking to the metal complex described herein. The term nanoparticle includes any nanoparticle suitable for linking to the metal complex described herein (e.g., metal-based, silica-based). Examples include gold nanoparticles, iron oxide nanoparticles, and silica nanoparticles. The term material includes any material suitable for linking to the metal complex (e.g., a solid material). Examples include carbon, porous carbon, gold, carbon nanotubes, CuO nanowires, and WO₃ nanowires.

One embodiment provides a rare earth metal complex of claim 1 or a salt thereof or a hydrate thereof, wherein the rare earth metal complex is a complex of formula II or salt thereof or hydrate thereof:



wherein the rare earth metal is M^{III}.

One embodiment provides a rare earth metal complex or a salt thereof of claim 1, wherein the rare earth metal complex is a Eu^{III} complex of formula IIa:



or salt thereof.

In one embodiment X is $-(\text{CH}_2)_2-$, $-(\text{CH}_2)_3-$, $-(\text{CH}_2)_2\text{N}(\text{CH}_2)_2-$, or $-(\text{CH}_2)_3\text{N}(\text{CH}_2)_2-$.

In one embodiment Y^1 is $-(\text{CH}_2)-$.

In one embodiment Y^1 is $-(\text{CH}_2)_2-$.

In one embodiment Y^2 is $-(\text{CH}_2)-$.

5 In one embodiment Y^2 is $-(\text{CH}_2)_2-$.

In one embodiment the linker comprises a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 2 to 50 carbon atoms wherein one or more of the carbon atoms is optionally replaced independently by $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}^a)-$, and wherein the chain is optionally substituted with one or more (e.g. 1, 2, 3, 4, 5 or more) substituents independently
10 selected from $(\text{C}_1\text{-C}_4)\text{alkyl}$, $(\text{C}_1\text{-C}_6)\text{alkoxy}$, oxo ($=\text{O}$), and halo, wherein each R^a is independently H or $(\text{C}_1\text{-C}_6)\text{alkyl}$.

In one embodiment the linker comprises a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 15 carbon atoms wherein one or more of the carbon atoms is optionally replaced independently by $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}^a)-$, and wherein the chain is
15 optionally substituted with one or more (e.g. 1, 2, 3, 4, 5 or more) substituents independently selected from $(\text{C}_1\text{-C}_4)\text{alkyl}$, $(\text{C}_1\text{-C}_6)\text{alkoxy}$, oxo ($=\text{O}$), and halo, wherein each R^a is independently H or $(\text{C}_1\text{-C}_6)\text{alkyl}$.

In one embodiment the linker comprises a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 10 carbon atoms wherein one or more of the
20 carbon atoms is optionally replaced independently by $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}^a)-$, and wherein the chain is optionally substituted with one or more (e.g. 1, 2, 3, 4, 5 or more) substituents independently selected from $(\text{C}_1\text{-C}_4)\text{alkyl}$, $(\text{C}_1\text{-C}_6)\text{alkoxy}$, oxo ($=\text{O}$), and halo, wherein each R^a is independently H or $(\text{C}_1\text{-C}_6)\text{alkyl}$.

In one embodiment the linker comprises a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 20 carbon atoms wherein one or more of the
25 carbon atoms is optionally replaced independently by $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}^a)-$, and wherein the chain is optionally substituted with one or more (e.g. 1, 2, 3, 4, 5 or more) substituents independently selected from $(\text{C}_1\text{-C}_4)\text{alkyl}$, $(\text{C}_1\text{-C}_6)\text{alkoxy}$, oxo ($=\text{O}$), and halo, wherein each R^a is independently H or $(\text{C}_1\text{-C}_6)\text{alkyl}$, wherein the linker includes one or more reactive groups.

30 In one embodiment the linker comprises a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 15 carbon atoms wherein one or more of the carbon atoms is optionally replaced independently by $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}^a)-$, and wherein the chain is optionally substituted with one or more (e.g. 1, 2, 3, 4, 5 or more) substituents independently

selected from (C₁-C₄)alkyl, (C₁-C₆)alkoxy, oxo (=O), and halo, wherein each R^a is independently H or (C₁-C₆)alkyl, wherein the linker includes one or more reactive groups.

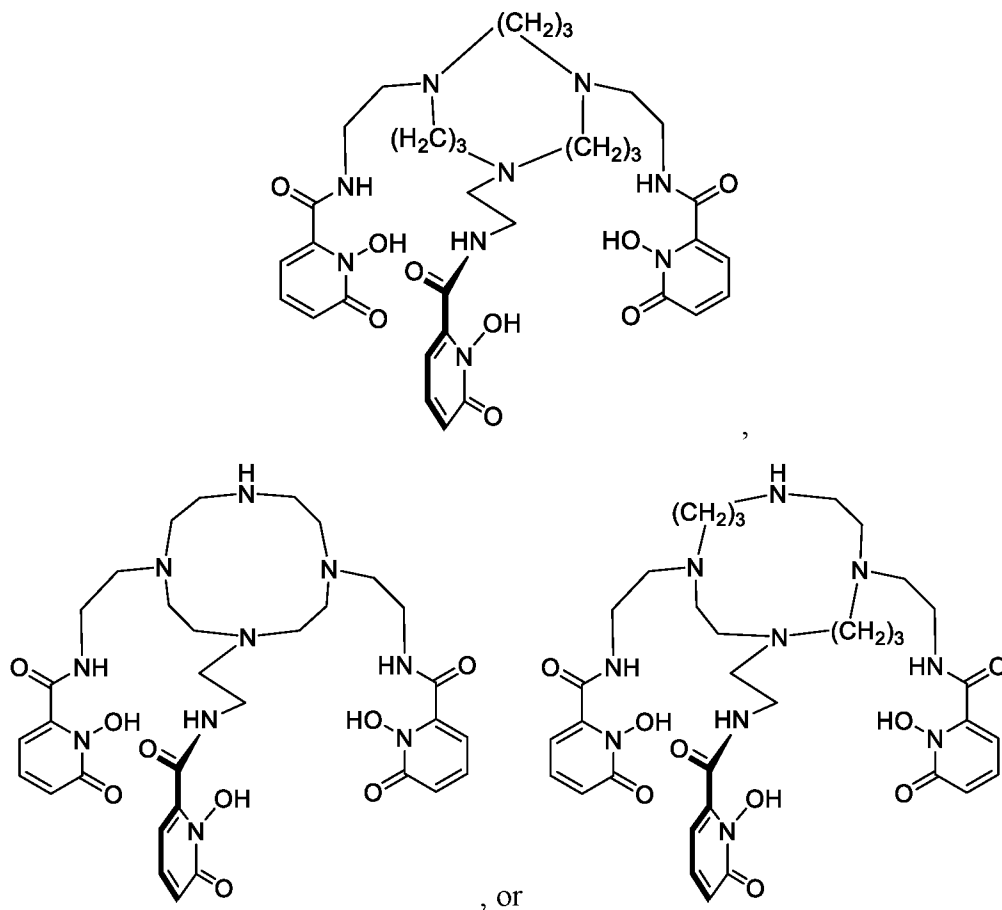
In one embodiment the linker comprises a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 10 carbon atoms wherein one or more of the carbon atoms is optionally replaced independently by -O-, -S-, -N(R^a)-, and wherein the chain is optionally substituted with one or more (e.g. 1, 2, 3, 4, 5 or more) substituents independently selected from (C₁-C₄)alkyl, (C₁-C₆)alkoxy, oxo (=O), and halo, wherein each R^a is independently H or (C₁-C₆)alkyl, wherein the linker includes one or more reactive groups.

For the six embodiments listed above, another six corresponding embodiments provide P in addition to -O-, -S-, -N(R^a)- so that one or more of the carbon atoms is optionally replaced independently by -O-, -S-, -N(R^a)-, and P.

In one embodiment the linker is a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 6 carbon atoms wherein one or more of the carbon atoms is optionally replaced by a N(R^a)-, wherein the R^a is H or (C₁-C₆)alkyl.

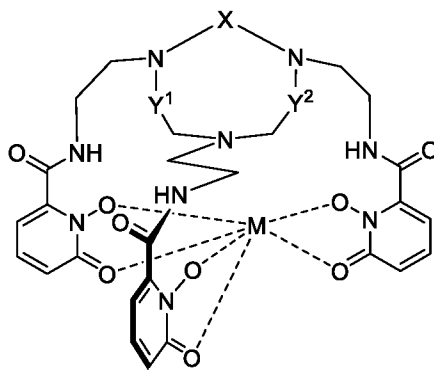
In one embodiment the linker is -(C₂-C₆)alkylNH₂ or -(C₂-C₆)alkylNH.

One embodiment provides a ligand or salt thereof that is



or a salt thereof.

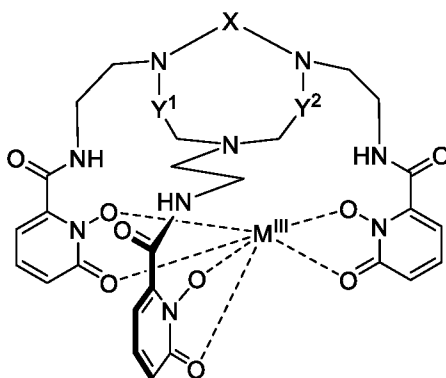
One embodiment provides a metal complex or a salt thereof that is metal complex is a complex of formula II or salt thereof:



II

5 wherein the M is a metal (e.g., a rare earth metal).

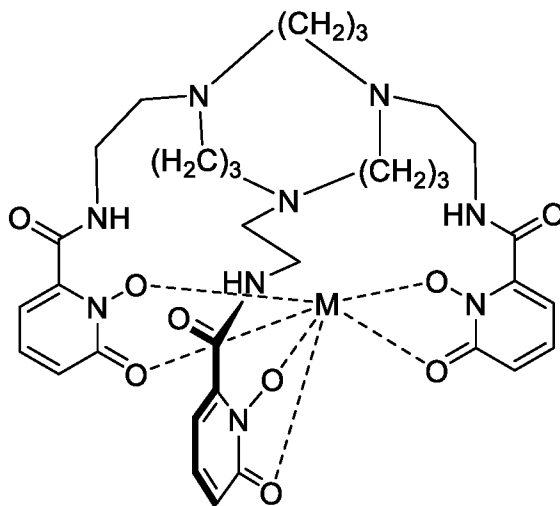
One embodiment provides a metal complex or a salt thereof that is metal complex is a complex of formula II or salt thereof:

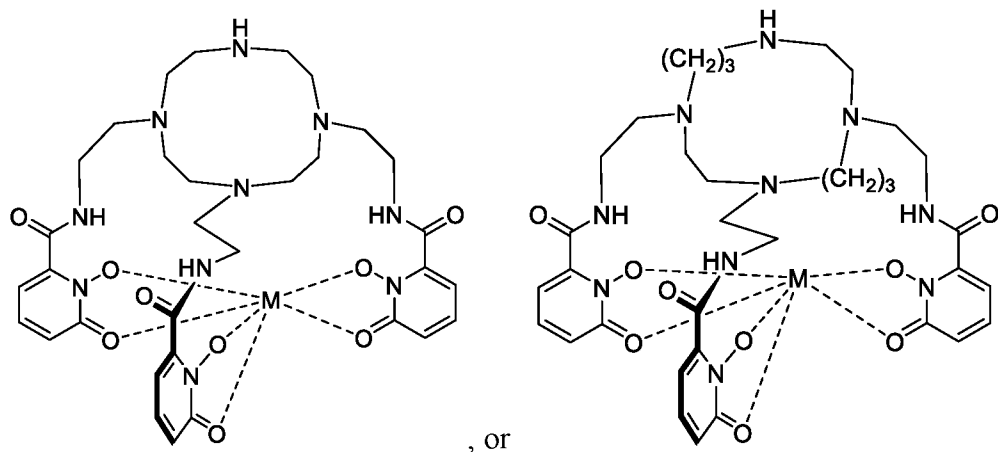


II

10 wherein the M^{III} is a metal (e.g., a rare earth metal).

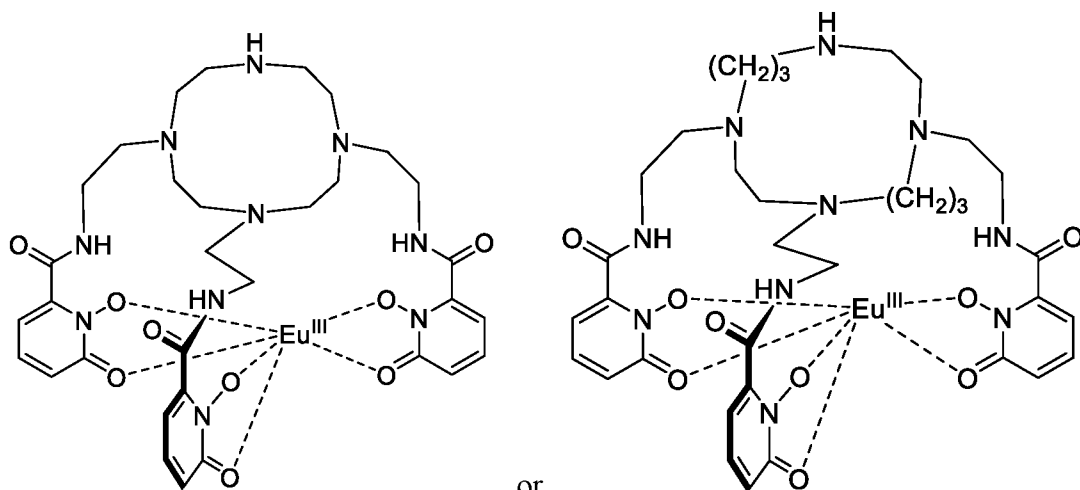
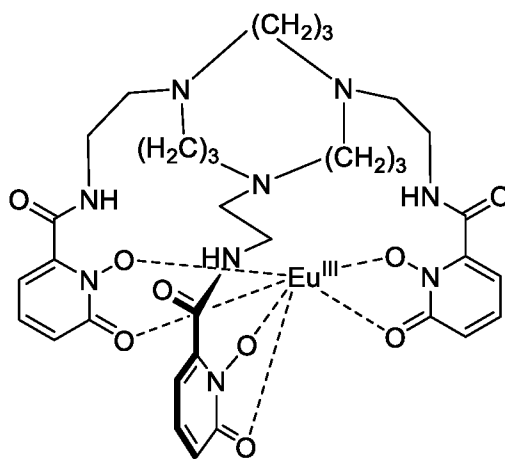
One embodiment provides a metal complex or a salt thereof that is





wherein the M is a metal (e.g., a rare earth metal, or a metal “M^{III}” or a rare earth metal M^{III}).

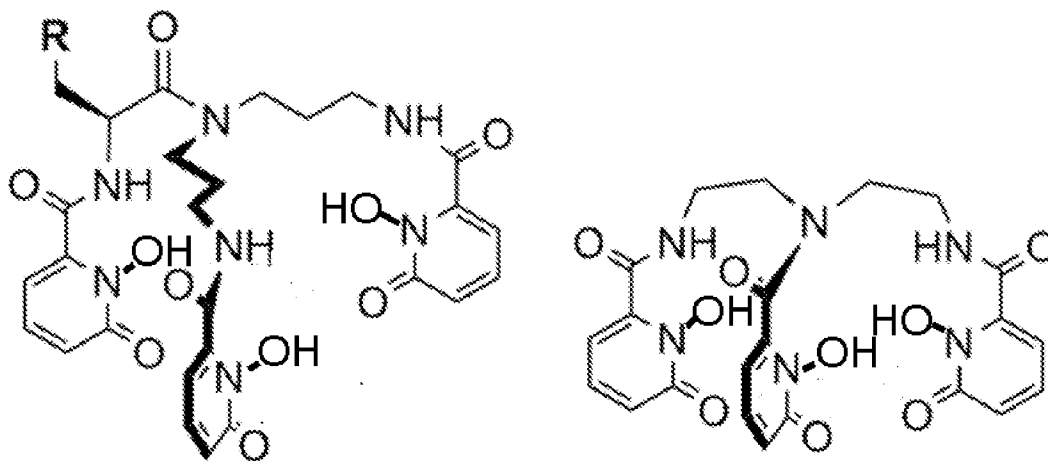
One embodiment provides a metal complex or a salt thereof that is



5

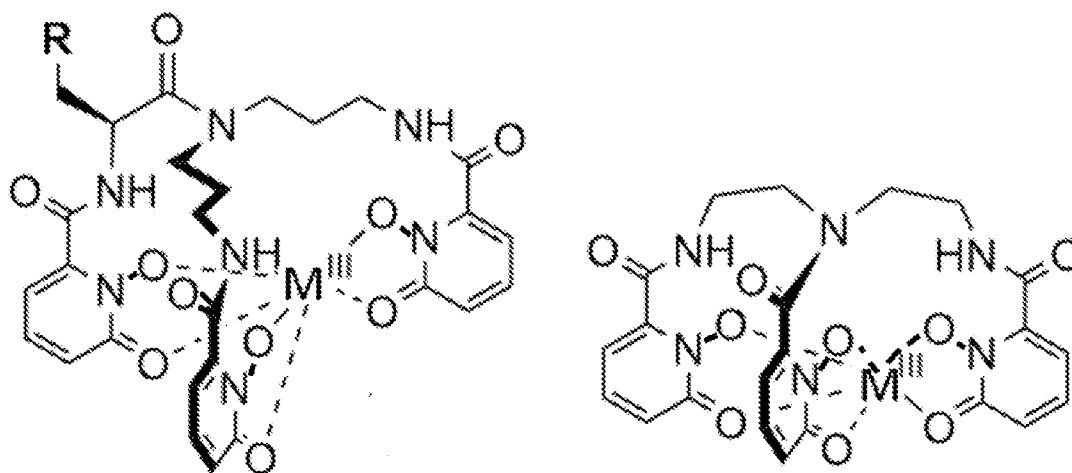
or a salt thereof.

One embodiment provides a ligand or salt thereof that is



wherein R is H or OH.

One embodiment provides a rare earth metal complex or salt thereof that is



3

5 wherein M is a rare earth metal and R is H or OH.

In one embodiment the rare earth metal is lanthanum(La), cerium(Ce), praseodymium(Pr), neodymium(Nd), samarium(Sm), europium(Eu), gadolinium(Gd), terbium(Tb), dysprosium(Dy), holmium(Ho), erbium(Er), thulium(Tm), ytterbium(Yb), lutetium(Lu), scandium(Sc), yttrium(Y) or promethium(Pm).

10 In one embodiment the rare earth metal is La^{III}, Ce^{III}, Pr^{III}, Nd^{III}, Sm^{III}, Eu^{III}, Gd^{III}, Tb^{III}, Dy^{III}, Ho^{III}, Er^{III}, Tm^{III}, Yb^{III}, Lu^{III}, Sc^{III}, Y^{III} or Pm^{III}.

In one embodiment the rare earth metal is Eu^{III}.

In one embodiment the metal complex is a metal complex or hydrate thereof.

One embodiment provides a material or device comprising one or more ligands or a salt thereof, or one or more complexes or a salt thereof, as described herein.

In one embodiment the material or device is a hydrogel, membrane, nanoparticle, or other material.

One embodiment provides a method to detect or capture inorganic phosphate, comprising contacting the phosphate with a ligand or salt thereof, or a complex or a salt thereof, or a material
5 or device as described herein. In one embodiment, the phosphate is selectively detected or captured in the presence of other anions. In one embodiment the other anions are selected from the group consisting of carbonate, nitrate, sulfate, halides, arsenate and pyrophosphate. In one embodiment the phosphate is contacted with a ligand or salt thereof, or a complex or a salt thereof, or a material or device, as a liquid sample at about neutral pH. In one embodiment the
10 liquid sample is sample is an aqueous sample. In one embodiment the phosphate is detected with fluorescence sensing by an indicator displacement assay. In one embodiment the phosphate is captured. In one embodiment the phosphate is captured from an aqueous mixture or solution.

One embodiment provides a method to treat hyperphosphatemia in a mammal in need thereof comprising contacting the blood of the mammal in need thereof, with a ligand or salt
15 thereof, or a complex or a salt thereof, or a material or device as described in herein. In one embodiment the mammal has kidney disease (e.g., chronic kidney disease).

One embodiment provides a device or material with a ligand or a rare earth metal complex of the ligand as described herein attached thereto.

One embodiment provides a device or material for sensing, detecting, and/or selectively
20 capturing phosphate from a mixture (e.g., aqueous mixture), water or blood with a ligand or a rare earth metal complex of the ligand as described herein attached thereto.

One embodiment provides a method for sensing, detecting, and/or selectively capturing phosphate from a mixture (e.g., an aqueous mixture), water or blood comprising contacting the device or material as described herein with the mixture (e.g., an aqueous mixture), water or blood,
25 wherein the device or material has a ligand or a rare earth metal complex of the ligand as described herein attached thereto.

One embodiment provides a device or material comprising one or more ligands or rare earth metal complexes of the ligands as described herein attached thereto.

One embodiment provides a device or material for sensing, detecting, and/or selectively
30 capturing phosphate from a mixture (e.g., an aqueous mixture), water or blood wherein the device comprises one or more ligands or a rare earth metal complexes of the ligands as described herein.

One embodiment provides a method for sensing, detecting, and/or selectively capturing phosphate from a mixture (e.g., aqueous mixture), water or blood comprising contacting the device or material as described herein with the mixture (e.g., an aqueous mixture), water or blood,

wherein the device or material comprises one or more ligands or a rare earth metal complexes of the ligands as described herein attached thereto.

The invention will now be illustrated by the following non-limiting example.

Ligand Design

Much has been learned on the kinetic inertness of lanthanide complexes intended for biomedical applications from gadolinium-based MRI contrast agents (Caravan, P.; et al., *Chem. Rev.* 1999, 99, 2293-2352; Thibon, A.; et al., *Anal. Bioanal. Chem.* 2009, 394, 107-120).

Macrocyclic complexes such as Gd-DOTA are significantly more kinetically inert than linear analogues such as Gd-DTPA even though they have similar thermodynamic stability (Cacheris, W. P.; et al., *Magn. Reson. Imaging* 1990, 8, 467-481). It was postulated that HOPO-based phosphate receptors could be rendered more kinetically inert by replacing the linear backbone with a macrocyclic one.

Importantly, has been previously determined that ligand geometry is one of the predominant parameters governing the affinity of tripodal, tris-HOPO complexes for phosphate. Minor differences in geometry arising from small modifications in the ligand cap causes substantial effect in the affinity of HOPO-based europium(III) complexes for phosphate (Huang, S.-Y.; et al., *Inorg. Chem.* 2020, 59, 4096-4108). Moreover, even though such changes also affect the coordination number of the europium(III) complex, there is no relationship between its number of inner-sphere water molecules (q) and its affinity for phosphate. Therefore, although it can be postulated that by replacing the linear cap of Eu^{III}-3,3-Ser-HOPO (1) for a macrocyclic one may increase its kinetic inertness, its effect on the affinity of the receptor for phosphate cannot be predetermined.

With this in mind, four different macrocyclic HOPO-based Eu^{III} complexes (Figure 1): Eu^{III}-TACN-HOPO (4), Eu^{III}-TACD-HOPO (5), Eu^{III}-cyclen-HOPO (6) and Eu^{III}-cyclam-HOPO (7) were designed and evaluated. Each comprise a different macrocyclic cap, which is expected to increase the inertness of the europium(III) complex and also to influence its affinity for anions. The tris-bidentate ligands ensure that the complexes have either 2 or 3 open coordination sites for water or anion coordination. Of note, in each case a small ethylene linker bridges the HOPO moieties to the cap. These linkers were incorporated for synthetic reasons: the syntheses of the analogous ligands without the linker were very poor yielding. Notably, similar tripodal TACN-based and tetrapodal cyclen-based HOPO complexes were previously reported by Raymond (Werner, E. J.; et al., *J. Am. Chem. Soc.* 2007, 129, 1870-1871) and Law (Dai, L.; et al., *Chem.*

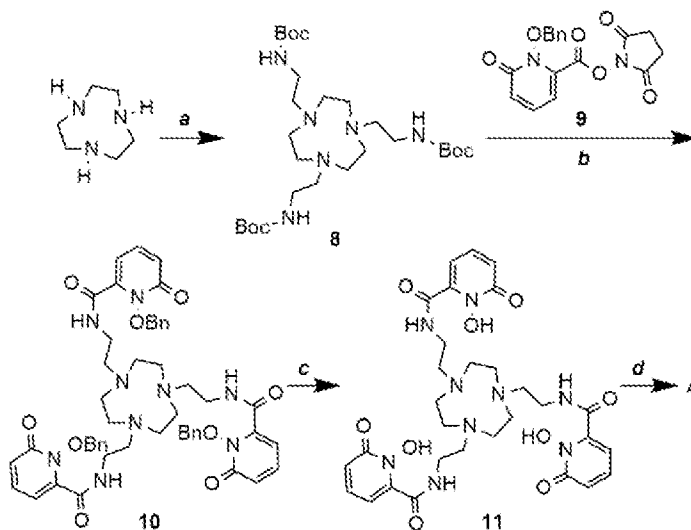
Sci. 2019, 10, 4550-4559; Arnedo-Sanchez, L.; et al., *ChemPlusChem* 2021, 86, 483-491), respectively, for imaging applications, although neither their kinetic inertness nor their affinity for anions were investigated.

5 RESULTS AND DISCUSSION

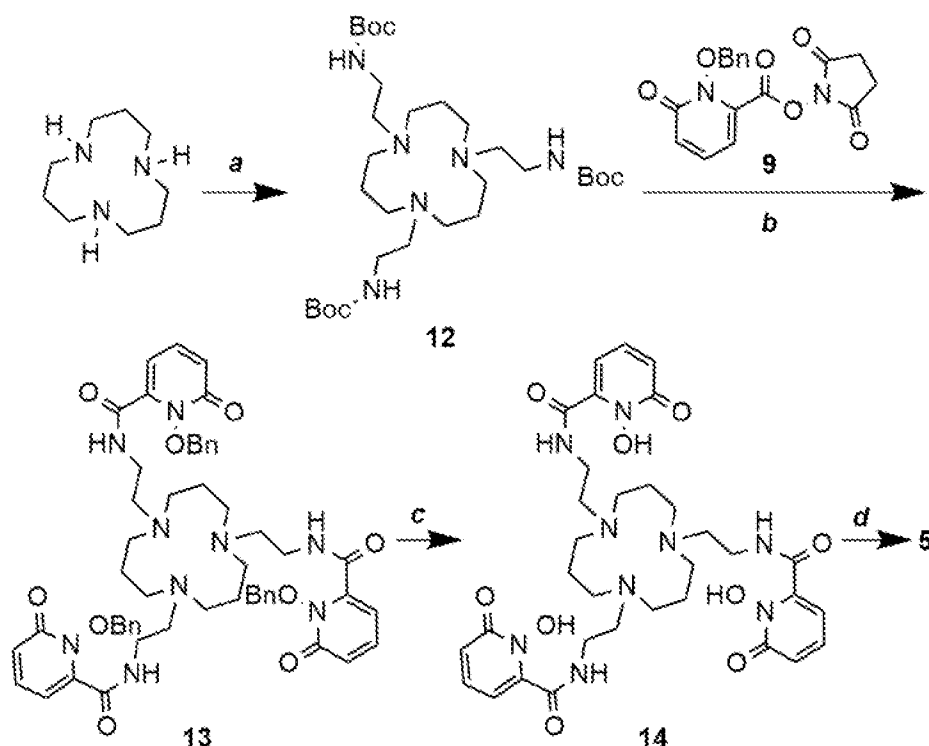
Synthesis

The two complexes comprising a cyclotriaza ligand cap, Eu^{III}-TACN-HOPO (4) and Eu^{III}-TACD-HOPO (5), were synthesized according to Schemes 1 and 2, respectively. In a first step, all the cyclic secondary amines were *N*-alkylated with BOC-protected 2-bromoethylamine to yield the extended caps 8 and 12, respectively. Deprotection with trifluoroacetic acid yielded the primary amines that were then coupled with HOPO(Bn)-Su (9) to yield the protected ligands TACN-HOPO-Bn (10) and TACD-HOPO-Bn (13), respectively. The *N*-hydroxysuccinimide-activated and benzyl-protected 1,2-hydroxypyridinone moiety HOPO(Bn)-Su (9) was previously synthesized according to literature procedures (Guérard, F.; et al., *Dalton Trans.* 2017, 46, 4749-4758). Deprotection of 10 and 13 under acidic conditions yielded the free ligand TACN-HOPO (11) and TACD-HOPO (14) that were subsequently complexed with EuCl₃·6H₂O in the presence of pyridine to yield the final receptors 4 and 5. Importantly, since these complexes are kinetically inert, their formation requires reflux in methanol for extended periods of time (5 days).

Scheme 1. Synthesis of Eu^{III}-TACN-HOPO (4).^a



^a Reagents and conditions: a) BOC-NH-CH₂-CH₂-Br, Cs₂CO₃, CH₃CN, 80°C, 48 h; b) i: CH₂Cl₂, TFA, 0°C to rt; ii: HOPO(Bn)-OSu (9), CH₃CN/H₂O (1/1 v), K₂CO₃, 12 h; c) HCl/AcOH (1/1 v), 48 h; d) EuCl₃·6H₂O, MeOH, pyridine, 65°C, 60 h.

Scheme 2. Synthesis of Eu^{III}-TACD-HOPO (5).^a

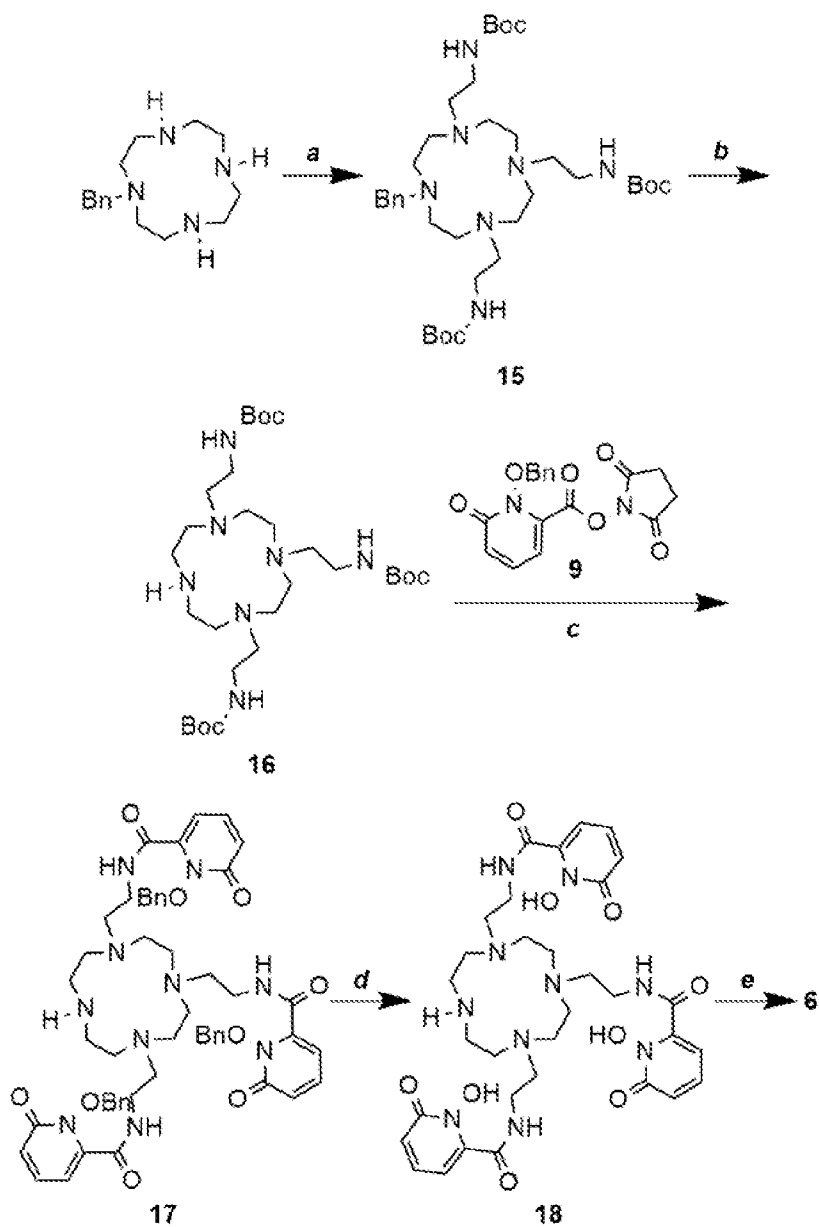
^a Reagents and conditions: a) BOC-NH-CH₂-CH₂-Br, Cs₂CO₃, DMF, 80°C, 48 h; b) i: CH₂Cl₂, TFA, 0°C to rt; ii: HOPO(Bn)-OSu (9), CH₃CN/H₂O (1/1 v), K₂CO₃, 12 h; c) HCl/AcOH (1/1 v), 48 h; d) EuCl₃·6H₂O, MeOH, pyridine, 65°C, 60 h.

The two complexes comprising a tetraaza ligand cap, Eu^{III}-cyclen-HOPO (6) and Eu^{III}-cyclam-HOPO (7), were synthesized according to Schemes 3 and 4, respectively. Since only three HOPO podands are to be conjugated on a tetraaza ligand cap, the synthesis of the cyclen derivative starts with mono-benzyl protected ligand cap (Massue, J.; et al., *Tetrahedron Lett.* 2007, 48, 8052-8055). *N*-alkylation of the unprotected secondary amine with BOC-protected 2-bromoethylamine yielded the extended cap 16. Benzyl deprotection of the cyclen cap with Pd/C, followed by BOC deprotection of the primary amine under acidic condition enabled conjugation of the HOPO(Bn)-Su (9) moiety to yield the protected ligands cyclen-HOPO-Bn (17). This approach exploits the poor reactivity of the cyclen's nitrogen compared to the primary amines of the arms. Deprotection under acidic conditions yielded the free ligand cyclen-HOPO (18) that was subsequently complexed with EuCl₃·6H₂O in the presence of pyridine in refluxing methanol for 5 days to yield the final complex Eu^{III}-cyclen-HOPO (6). The cyclam derivative follows a similar scheme except that the cyclam cap did not require mono-protection prior to its *N*-alkylation. The cyclam is poorly reactive, so that only three of its four secondary amines could be coupled with BOC-protected 2-bromoethylamine to yield the extended cap 19. Deprotection under acidic condition yielded the extended cap that was immediately coupled with HOPO(Bn)-Su (9) moiety

to yield the protected ligands cyclam-HOPO-Bn (20). Deprotection under acidic conditions yielded the free ligand cyclam-HOPO (21) that was subsequently complexed with $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ to yield the final complex Eu^{III} -cyclam-HOPO (7).

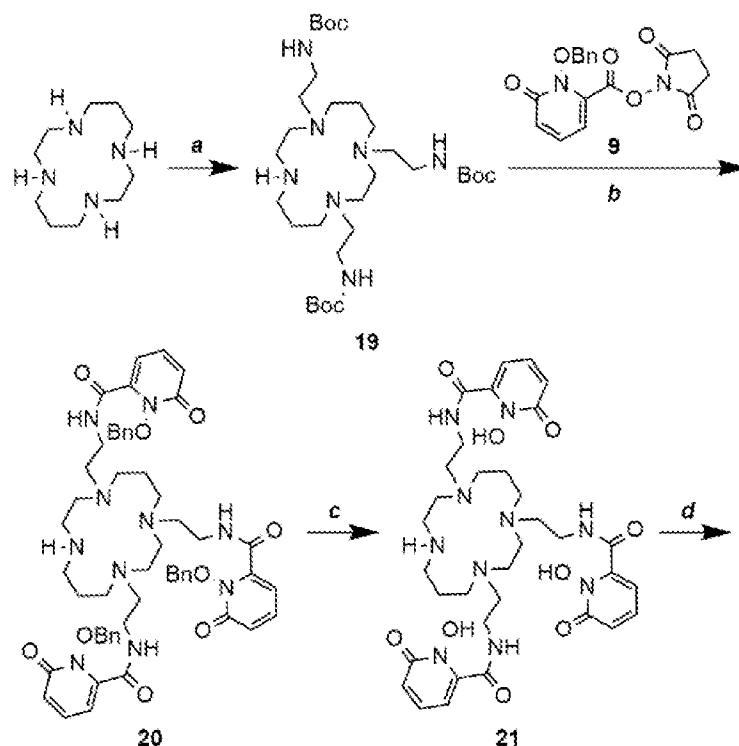
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Scheme 3. Synthesis of Eu^{III} -cyclen-HOPO (6).^a



^a Reagents and conditions: a) $\text{Boc-NH-CH}_2\text{-CH}_2\text{-Br}$, Cs_2CO_3 , DMF, 80°C , 48 h; b) H_2 (g), 10 wt% Pd/C, MeOH, rt, 12 h; c) i: CH_2Cl_2 , TFA, 0°C to rt; ii: HOPO(Bn)-OSu (9), $\text{CH}_3\text{CN}/\text{H}_2\text{O}$ (1/1 v/v), K_2CO_3 , 12 h; d) HCl/AcOH (1/1 v/v), 2 day; e) $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$, MeOH, pyridine, 65°C , 60 h.

10

Scheme 4. Synthesis of Eu^{III}-cyclam-HOPO (7).^a

^a Reagents and conditions: a) BOC-NH-CH₂-CH₂-Br, Cs₂CO₃, CH₃CN, 80°C, 48 h; b) i: CH₂Cl₂, TFA, 0°C to rt; ii: HOPO(Bn)-OSu (9), CH₃CN/H₂O (1/1 v), K₂CO₃, 12 h; c) HCl/AcOH (1/1 v), 48 h; EuCl₃·6H₂O, MeOH, pyridine, 65°C, 60 h.

Affinity of lanthanide complexes for phosphate

1,2-Hydroxypyridine is an excellent sensitizer of europium(III). Moreover, both the luminescence intensity and the lifetime of Eu^{III} complexes are highly dependent on the number of inner-sphere water molecules. As phosphate or other anions coordinate Eu^{III}, they displace the inner-sphere water molecules, resulting in an increase in both the luminescence intensity and the lifetimes of the excited states of the Eu^{III} center. Together, these characteristics not only facilitate the study of anion recognition by aqueous Eu^{III} complexes of 1,2-HOPO with open coordination sites, but essentially render any such complex a luminescent probe (Martinon, T. L. M.; et al., *Chem. Asian J.* 2022, 17, e202200495; Thibon, A.; et al., *Anal. Bioanal. Chem.* 2009, 394, 107-120).

The affinity of the macrocyclic HOPO complexes 4 – 7 for phosphate were thus initially studied by monitoring the europium-centered emission by time-gated luminescence spectroscopy with time. Unfortunately, the solubility of Eu^{III}-TACD-HOPO (5) in water at neutral pH was so poor that this complex could not be studied. Eu^{III}-cyclen-HOPO (6) also tends to aggregate in neutral water with time due to the hydrophobic nature of the ethylene groups of the cyclen

backbone. This tendency of cyclen-based lanthanide complexes to form supramolecular assemblies due to hydrophobic interactions was exploited successfully by Ferrauto and Gianolio to develop high-relaxivity gadolinium(III) MRI contrast agents (Di Gregorio, E.; et al., *Chem. Sci.* 2021, 12, 1368-1377). However, in this case, similar aggregation mutes the luminescence response of the Eu^{III}-cyclen-HOPO (6) receptor. Any solution of macrocycle complexes thus must be freshly prepared before study (Figure 6).

The number of inner-sphere water molecule of each receptor in the presence and absence of 10 equivalents of phosphate can be determined from the luminescence lifetime of the complexes in H₂O and D₂O according to the classical Parker-Horrocks equation (Dickins, R. S.; et al., *Chem. Commun.* 1996, 697-698; Beeby, A.; et al., *J. Chem. Soc., Perkin Trans 2* 1999, 493-504; Supkowski, R. M.; et al., *Inorganica Chim. Acta* 2002, 340, 44-48):

$$q = 1.2[(1/\tau_{H_2O} - 1/\tau_{D_2O}) - 0.25 - 0.0075x] \quad (1)$$

where τ_{H_2O} and τ_{D_2O} are the luminescence lifetimes of the Eu^{III} in H₂O and D₂O, respectively, and x is the number of N-H oscillators in close proximity to the Eu^{III} ion. The luminescence lifetime in D₂O was extrapolated from serial dilution of an aqueous solution by D₂O (see Figure 7).

The luminescence lifetimes and corresponding number of inner-sphere water molecules for each complex in the absence and presence of phosphate are reported in Table 1. As can be seen from these results, even though all complexes use tripodal tris-bidentate ligands, their coordination number differs. Whereas Eu^{III}-TACN-HOPO (4) and Eu^{III}-cyclam-HOPO (7) bind three inner-sphere water molecules and are thus nine coordinate, Eu^{III}-cyclen-HOPO (6) is eight-coordinate with two inner-sphere water molecules. Of note, the q value of Eu^{III}-TACN-HOPO (4) matches that previously determined by Werner and Raymond (Guérard, F.; et al., *Dalton Trans.* 2017, 46, 4749-4758). The observations that Eu^{III} complexes of tris-tripodal HOPO ligands can have either eight or nine coordinate ground states are consistent with previous reports (Pierre, V. C.; et al., *Front. Chem.* 2022, 10, 821020; Ramakrishnam Raju, M. V.; et al., *Chem. Soc. Rev.* 2020, 49, 1090-1108; Pierre, V. C.; et al., *Inorg. Chem.* 2006, 45, 8355-8364; Pierre, V. C.; et al., *J. Am. Chem. Soc.* 2006, 128, 5344-5345). Because the eight and nine coordination states of this class of complexes are very close in energy, minor modifications in the ligand cap are sufficient to favor one or the other ground-state coordination number.

Table 1. Luminescence lifetime and number of inner-sphere water molecules (q) of Eu^{III}-L-HOPO receptors in the absence and presence of 10 eq. HPO₄²⁻/H₂PO₄⁻.^a

Receptor	$\tau_{\text{H}_2\text{O}}$ (ms)	$\tau_{\text{D}_2\text{O}}$ (ms)	q	Δq
Eu ^{III} -TACN-HOPO (4)	0.19	0.39	3.0	1.0
Eu ^{III} -TACN-HOPO (4) + 10 P _i	0.24	0.45	2.0	
Eu ^{III} -cyclen-HOPO (6)	0.25	0.46	2.0	2.0
Eu ^{III} -cyclen-HOPO (6) + 10 P _i	0.40	0.44	0	
Eu ^{III} -cyclam-HOPO (7)	0.22	0.54	2.9	2.1
Eu ^{III} -cyclam-HOPO (7) + 10 P _i	0.39	0.59	0.8	

^a Experimental conditions: [Eu^{III}-L-HOPO] = 7.35 μ M in H₂O, pH 7.4, T = 25°C; λ_{ex} = 334-341 nm, λ_{em} = 615 nm; delay time, 0.1 ms.

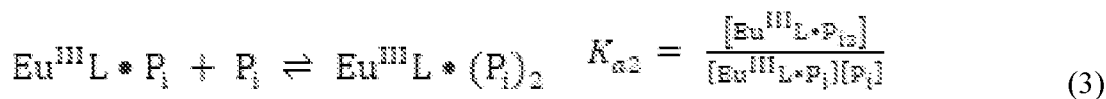
5 All three complexes bind phosphate. Unlike the linear receptors exemplified by Eu^{III}-3,3-Ser-HOPO (1), the macrocycle complexes, particularly the tetra-aza complexes, bind phosphate with significantly slower kinetics. Eu^{III}-3,3-cyclen-HOPO (6), for instance, requires an hour to reach equilibrium (Figure 8). These kinetic studies enable us to ensure that all titrations and solutions studies discussed hereafter were performed at thermodynamic equilibrium. In each case,
10 every measurement—luminescence intensity or lifetime— was performed at least 1 hour after addition of an anion.

Although all three complexes bind phosphate, they do not do so with the same stoichiometry. Phosphate is known to coordinate lanthanide ion in a mono-dentate manner (Dickins, R. S.; et al., *J. Am. Chem. Soc.* 2002, 124, 12697-12705). Both Eu^{III}-cyclen-HOPO (6)
15 and Eu^{III}-cyclam-HOPO (7) lose two inner-sphere water molecules in the presence of 10 equivalents of phosphate and can thus be inferred to bind two phosphate anions, resulting in the formation of Eu^{III}L·Pi₂ complexes with a 1:2 stoichiometry. On the other hand, only one of the three inner-sphere water molecules of Eu^{III}-TACN-HOPO (4) is displaced by water, resulting in the formation of a Eu^{III}L·Pi ternary complex with a 1:1 stoichiometry. As is apparent from these
20 results, there is no relationship between the number of open coordination sites on the receptor and its affinity for phosphate. Eu^{III}-cyclen-HOPO (6), which has only two inner-sphere water molecules, is the only receptor that has high affinity for phosphate such that the anion displaces all its inner-sphere water molecules. On the other hand, both Eu^{III}-TACN-HOPO (4) and Eu^{III}-cyclam-HOPO (5), which are nine coordinates, retain either 2 or 1 of their inner-sphere water
25 molecules in the presence of excess phosphate, respectively. The same lack of relationship between number of inner-sphere water molecules and affinity for coordinating anions with linear

analogues has been previously demonstrated (Huang, S.-Y.; et al., *Inorg. Chem.* 2020, 59, 4096-4108; Pierre, V. C.; et al., *Inorg. Chem.* 2006, 45, 8355-8364; Pierre, V. C.; et al., *J. Am. Chem. Soc.* 2006, 128, 5344-5345). The lack of predictability of these results highlights some of the difficulties in designing lanthanide based receptors for anions.

Displacement of the inner-sphere water molecules by phosphate also increases the luminescence intensity of the Eu^{III} complex, a characteristic that enables rapid determination of the affinity of each receptor for the anion. The titrations of each Eu^{III} complex with phosphate in water at neutral pH and room temperature are shown in Figure 2. Based on kinetic studies, in each case, measurements were performed at least 60 min after addition of the anion so as to ensure that thermodynamic equilibrium is reached.

Based on the stoichiometry determined from the luminescence lifetimes studies, those titrations were fitted to either 1:2 or 1:1 equilibria. The stepwise (K) and cumulative (β) association constants correspond to the following equilibria where $\text{Eu}^{\text{III}}\text{L}$ corresponds to europium(III) receptors (Figure 1); phosphate is abbreviated as P_i (charges are omitted for clarity). Do note that at neutral pH, phosphate is present as a near 1:1 ratio of H_2PO_4^- and HPO_4^{2-} .



The 1:1 and 1:2 equilibria were fitted to equations 5 and 6, respectively, as described by Thordarson (Thordarson, P., *Chem. Soc. Rev.* 2011, 40, 1305-1323.) where I and I_0 are the integrated intensity of the receptor in the presence and absence of phosphate, respectively and K_1 and K_2 are the first and second association constants, respectively. The resulting derived constants (a_0 , a_1 and a_2) after simplifying the proportionality constant are determined through fitting, of which a_0 is equal to the I/I_0 prior to introduction of the guest.

$$\frac{I}{I_0} = \frac{a_1 K_1 [\text{P}_i] + a_0}{1 + K_1 [\text{P}_i]} \quad (5)$$

$$\frac{I}{I_0} = \frac{a_2 K_1 K_2 [\text{P}_i]^2 + a_1 K_1 [\text{P}_i] + a_0}{1 + K_1 K_2 [\text{P}_i]^2 + K_1 [\text{P}_i]} \quad (6)$$

The stepwise and overall association constants of the three Eu^{III} receptors for phosphate are given in Table 2. All three complexes have similar first association constants. The tetraaza derivatives 6 and 7 differentiate themselves from the triaza derivative (4) in that they can also coordinate a second phosphate anion. The second association constant of Eu^{III}-cyclen-HOPO (6) for Pi is approximately an order of magnitude higher than that of Eu^{III}-cyclam-HOPO (7). The cyclen derivative 6 has an overall association constant, β , for phosphate fifty times higher than the cyclam analog and five order of magnitudes higher than the TACN one. Its high affinity for phosphate makes it a promising receptor for the treatment of hyperphosphatemia. The affinity for phosphate of Eu^{III}-cyclen-HOPO (6) is comparable to that of Eu^{III}-2,2-Li-HOPO (3), a HOPO-based Eu^{III} receptor previously reported, that also binds the anion in a 1:2 stoichiometry (Huang, S.-Y.; et al., *Inorg. Chem.* 2020, 59, 4096-4108). Although it is sufficiently high for medical applications, the overall affinity for Pi (β) remains noticeably lower than that of Eu^{III}-Gly-HOPO (2), which binds phosphate with a 1:3 stoichiometry (Huang, S.-Y.; et al., *Inorg. Chem.* 2020, 59, 4096-4108). It is, however, noticeably higher than that of other receptors that function in water at neutral pH (Ramakrishnam Raju, M. V.; et al., *Chem. Soc. Rev.* 2020, 49, 1090-1108).

Table 2. Stepwise (K_a) and cumulative (β) association constants of Eu^{III} receptors for phosphate and quantum yields (Φ_{EuL}) of Eu^{III}-L-HOPO in the absence of any anion.^a

Receptor	K_{a1}	K_{a2}	$\text{Log } \beta_{1:1,1:2}$	Φ_{EuL}
Eu ^{III} -TACN-HOPO (4)	$4.0 \times 10^4 \text{ M}^{-1}$	-	$4.0 \times 10^4 \text{ M}^{-1}$	1.0%
Eu ^{III} -cyclen-HOPO (6)	$5.8 \times 10^4 \text{ M}^{-1}$	$7.4 \times 10^4 \text{ M}^{-1}$	$4.9 \times 10^9 \text{ M}^{-1}$	1.0%
Eu ^{III} -cyclam-HOPO (7)	$5.0 \times 10^4 \text{ M}^{-1}$	$8.5 \times 10^3 \text{ M}^{-1}$	$4.2 \times 10^8 \text{ M}^{-1}$	0.8%

^a Experimental conditions: [Eu^{III}-L-HOPO] = 7.35 μM in H₂O, pH of 7.4; T = 25°C; λ_{ex} , 334-340 nm; time delay, 0.1 ms.

It has been previously determined that for the linear analogues, there is a relationship between the quantum yield (Φ_{EuL}) of HOPO-based Eu^{III} complexes and their affinity for phosphate. Eu^{III}-2,2-Li-HOPO (3) and Eu^{III}-Gly-HOPO (2) had significantly lower quantum yield than their counterparts that do not bind phosphate, even after accounting for difference in hydration numbers. This suggest that phosphate coordination requires minor destabilization of the complex. If this destabilization occurs by slightly altering the coordination of Eu^{III} by 1,2-HOPO, this also affects the efficiency of 1,2-HOPO to sensitize europium(III), thereby decreasing Φ_{EuL} .

(Pierre, V. C.; et al., *Front. Chem.* 2022, 10, 82102; Huang, S.-Y.; et al., *Inorg. Chem.* 2020, 59, 4096-4108).

The quantum yields of all three water-soluble macrocyclic complexes were determined by comparison with a coumarin reference according to equation 7 (Williams, A. T. R.; et al., *Analyst* 1983, 108, 1067-1071):

$$\Phi_{\text{EuL}} = \Phi_r * \frac{m_s}{m_r} * \left(\frac{n_r}{n_s}\right)^2 \quad (7)$$

where Φ_r denotes the quantum yield of coumarin, m_s and m_r the luminescence emission of the sample and reference, respectively, and n_s and n_r the refractive indexes of the solutions containing the sample and reference, respectively. The measured quantum yields are reported in Table 2 and Figure 9. Interestingly, the relationship noted above also holds for these macrocyclic analogues but to a lesser extent. The quantum yields of all three complexes are lower than those of linear analogues with equal number of inner-sphere water molecules that do not bind phosphate (Huang, S.-Y.; et al., *Inorg. Chem.* 2020, 59, 4096-4108). Of note, they are noticeably lower than those observed by Wong and Law for the octadentate analog (Dai, L.; et al., *Chem. Sci.* 2019, 10, 4550-4559). This suggests that phosphate coordination requires minor destabilization of the complex, even for macrocyclic analogues.

Selectivity of receptors

While affinity for phosphate in water at neutral pH is critical to the translation of the receptor for the treatment of hyperphosphatemia, its selectivity against endogenous anions is just as important. The selectivity of all three macrocyclic receptors against anions of physiological significance are shown in Figure 3. Except for citrate, the luminescence intensity of each receptor does not increase in the presence of excess competing anions (white bars). Moreover, the luminescence intensity increase of the receptor upon addition of phosphate is independent of the presence of the competing anion (grey bars), which indicates that those competing anions do not interfere with phosphate coordination. As expected, none of the receptors bind chloride, which is present at 98-106 mM concentration in blood, or any other halide. Importantly, the receptors also do not have any affinity for bicarbonate, nor does bicarbonate affect coordination to phosphate. This selectivity for phosphate over bicarbonate is difficult to achieve for lanthanide-based receptors and probes, but it is crucial for the intended application (Martinon, T. L. M.; et al., *Chem. Asian J.* 2022, 17, e202200495; Thibon, A.; et al., *Anal. Bioanal. Chem.* 2009, 394, 107-120; Cacheris, W. P.; et al., *Magn. Reson. Imaging* 1990, 8, 467-481). Indeed, bicarbonate is present at much higher concentration in the blood (23-30 mM for HCO_3^- vs 0.8-1.45 mM for Pi),

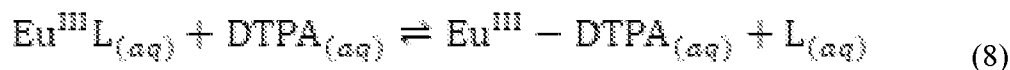
and 19.1% of patients with CKD, stages 4-5, suffer from low carbonate levels < 22 mmol/L (Eustace, J. A.; et al., *Kidney Int.* 2004, 65, 1031-1040). Removing excess phosphate from blood without further decreasing bicarbonate levels is thus important. It should be noted, however, that there is no selectivity between orthophosphate and pyrophosphate, which is typical of receptors who operate through direct phosphate coordination binding mode (Martinon, T. L. M.; et al., *Chem. Asian J.* 2022, 17, e202200495). This is not detrimental, given as excess orthophosphate should also be removed from the blood of hyperphosphatemic patients.

Interestingly, the selectivities of these macrocyclic complexes are a little worse than their linear analogues, with some affinity for bidentate anions such as pyrophosphate and citrate. This affinity for bidentate anions could be due to the placement of the open (water-filled) coordination sites on the Eu^{III} cis to each other. This cis organization has been observed in the crystal structures of macrocyclic lanthanide complexes bearing two open coordination sites such as that of Gd-DO3A (Chang, C. A.; et al., *Inorg. Chem.* 1993, 32, 3501-3508). This cis orientation positions the open coordination sites closer to each other such that coordination of pyrophosphate and citrate becomes possible. On the other hand, the open coordination sites in the tripodal tris-bidentate complexes such as Ln-TREN-MAM are much further from each other, which would disfavor pyrophosphate and citrate coordination (Wilharm, R. K.; et al., *Inorg. Chem.* 2022, 61, 4130-4142).

Unusually, all three receptors are highly selective for phosphate over arsenate, making them unique among both organic and inorganic receptors. Indeed, since orthophosphate and arsenate have the same geometry and similar basicity, no other receptor that function in water at neutral pH has been able to achieve this selectivity (Ramakrishnam Raju, M. V.; et al., *Chem. Soc. Rev.* 2020, 49, 1090-1108). A last note should be made on buffers. The results noted above were performed in water at neutral pH in the absence of organic buffers. Good's buffers such as HEPES are known to bind some metal ions and anions (Jordan, J. H.; et al., *J. Am. Chem. Soc.* 2021, 143, 18605-18616). Increasing number of reports highlight the effect that common buffers such as HEPES have on the apparent selectivity and affinity of inorganic luminescent probes and MRI contrast agent (Babel, L.; et al., *Chem.* 2020, 2, 193-202; Ferreira, C. M. H.; et al., *RSC Adv.* 2015, 5, 30989-31003; Mandal, P.; et al., *J. Biol. Inorg. Chem.* 2022, 27, 249-260; Major, J. L.; et al., *Proc. Natl. Acad. Sci. U.S.A.* 2007, 104, 13881-13886). Such an effect was also observed (Figure 10).

Kinetic inertness of lanthanide complexes

The macrocyclic HOPO receptors were designed to not only bind phosphate, but to have improved kinetic inertness over their linear analogues. As discussed above, macrocyclic MRI contrast agents were observed to be less toxic and to transmetallate *in vivo* with physiological ions to a lesser extent than their linear analogues (Radbruch, A.; et al., *Invest Radiol* 2019, 54, 531-536). The kinetic inertness of the macrocyclic receptors 1, 4, 6, and the linear analog 7 were determined by monitoring the ligand exchange of the Eu^{III} complexes in the presence of a five-fold excess of diethylenetriaminepentaacetate (DTPA) in water at neutral pH by time-gated luminescence spectroscopy (Eq. 8).



DTPA was chosen for these studies since the ligand forms stable lanthanide complexes (thereby making it a strong competitor) and because it is a labile ligand that undergoes transmetallation rapidly. Advantageously, whereas 1,2-HOPO is an excellent sensitizer for Eu^{III}, DTPA is not. The equilibrium can thus be readily monitored via the Eu^{III}-centered luminescence sensitized at 334 nm. The half-life, $t_{1/2}$, or time required for half of the Eu^{III}-L-HOPO complexes to transmetallate to DTPA are reported in Table 3. This data corresponds to the time half of the signal have decayed. The linear analog, Eu^{III}-Ser-HOPO (1) was included in the study as a comparison. HEPES was not utilized in these studies, as it was found to affect the rate of transmetallation (Figure 11).

Table 3. Conditional stability constants, $\text{Log } K_{\text{EuL}}^c$ and half-life of transmetallation with DTPA ($t_{1/2}$)^a

Receptor	$\text{Log } K_{\text{EuL}}^c$	$t_{1/2}$
Eu ^{III} -DTPA	17.86	
Eu ^{III} -Ser-HOPO (1)	17.88	21 sec
Eu ^{III} -TACN-HOPO (4)	17.83	2.5 min
Eu ^{III} -Cyclen-HOPO (6)	17.96	8.0 min
Eu ^{III} -Cyclam-HOPO (7)	17.80	26 sec

^a Experimental conditions: [Eu^{III}-L-HOPO] = 7.35 μM in 0.1 M KCl (aq), pH = 7.4, T = 25°C, λ_{ex} = 334-340 nm, λ_{em} = 615 nm, delay time = 0.1 ms. The $t_{1/2}$, time at which half of the transmetallation occurred, were determined from graphical interpolation of experimental data shown in Figure 4.

As anticipated, the parent linear receptor Eu^{III}-Ser-HOPO (1) is labile and undergoes ligand exchange with DTPA very rapidly, with a half-life of ca. half a minute. Thermodynamic equilibrium of ligand exchange for this complex is reached within 5 min (Figure 4). As hypothesized, the macrocyclic analogues are more kinetically inert, but with significant differences. Eu^{III}-cyclam-HOPO (7) also displays rapid ligand exchange with DTPA, with a half-life comparable to the linear complex. The cyclam is nonetheless more inert than the linear analogue: 30 min are required for equilibrium and full ligand exchange to occur. Eu^{III}-TACN-HOPO (4) is even more inert, with a half-life of 2.5 min, and requiring an hour to reach equilibrium. Eu^{III}-cyclen-HOPO (6) is the most inert of all the complexes, with the longest $t_{1/2}$. In this case, ligand exchange requires at least 2 hours to reach equilibrium.

Noticeably, the kinetics of ligand exchange with DTPA mirror those of phosphate coordination (Figure 13). Indeed, Eu^{III}-Ser-HOPO (1) binds phosphate significantly faster than Eu^{III}-cyclen-HOPO (6). This similarity in trends suggests that ligand exchange with DTPA likely begins with coordination of the Eu^{III} center by one of the carboxylate arms via displacement of the inner-sphere water molecules. Although a complete determination of the mechanism of ligand exchange of these macrocyclic complexes is beyond the scope of this study, it is worth noting that not all four complexes follow the same kinetics of ligand exchange, and therefore the same mechanism. Indeed, Eu^{III}-TACN-HOPO (4) and Eu^{III}-Ser-HOPO (1) both follow 1st order kinetics, where the rate of ligand exchange can be fitted to equation 9:

$$[A] = [A_0]e^{(-k_L t)} \quad (9)$$

where $[A]$ = the concentration of complex as it progresses towards equilibrium, $[A_0]$ = the starting concentration of complex, t = time, and k_L = the rate constant of trans-chelation reaction of the Eu^{III}L complex with DTPA. Eu^{III}-cyclam-HOPO (7) on the other hand, follows a second order kinetics which can be fitted to equation 10:

$$[A] = [A_0]/(1 + [A_0]k_L t) \quad (10)$$

Interestingly, the data for Eu^{III}-cyclen-HOPO (6) does not fit to either a 1st order or a 2nd order kinetics, which suggests that, in this case, ligand exchange occurs via multiple competing mechanisms.

Stability of lanthanide complexes

Once the kinetics of ligand exchange with DTPA has been determined, including the time needed to reach equilibrium, the conditional stability constant K^c_{EuL} of each europium(III) complex at pH 7.4 can also be measured by direct competition with DTPA. These equilibria were

directly monitored by UV-visible spectroscopy using the luminescence spectroscopy using the free and complexed HOPO ligands as references. As depicted in equation 12, because this conditional equilibrium constant essentially reflects the competition between the metal ion and protons for the ligand, $K_{\text{EuL}}^{\text{c}}$ is a function of pH:

$$K_{\text{EuL}}^{\text{c}} = K_{\text{EuL}} / \alpha_{\text{H}} \quad (11)$$

Where

$$\alpha_{\text{H}} = 1 + K_1[\text{H}^+] + K_1 K_2 [\text{H}^+]^2 + \dots + K_1 K_2 \dots K_n [\text{H}^+]^n \quad (12)$$

In equation 12, $K_1 K_2 \dots$ are the protonation constants of the ligand L (where L is the fully deprotonated ligand) (Caravan, P.; et al., *Chem. Rev.* 1999, 99, 2293-2352; Caravan, P.; et al., *Chem. Rev.* 1999, 99, 2293-2352). The conditional stability constant of Eu^{III} -DTPA at pH 7.4 calculated from the published formation constant of Eu^{III} -DTPA (K_{EuDTPA}) and the protonation constants of DTPA is given in Table 3. The conditional stability ($K_{\text{EuL}}^{\text{c}}$) of the Eu^{III} -HOPO complexes can then be calculated from the same spectrophotometric competition data used above and from the conditional stability constant of the competing Eu^{III} -DTPA, $K_{\text{EuDTPA}}^{\text{c}}$, under the specific conditions considered according to equation 13:

$$K_{\text{EuL}}^{\text{c}} = K_{\text{EuDTPA}}^{\text{c}} \frac{[\text{DTPA}]_{\text{t}}}{[\text{L}]_{\text{t}}} \quad (13)$$

where $[\text{DTPA}]_{\text{t}}$ and $[\text{L}]_{\text{t}}$ are the total (free and metal-bound) concentrations of the competing DTPA and HOPO ligands, respectively.

The conditional stability constant, $K_{\text{EuL}}^{\text{c}}$, of complexes 1, 4, 6, and 7 are given in Table 3. All four complexes show similar stability, with Log $K_{\text{EuL}}^{\text{c}}$ ranging between 17.83 and 17. The stability of Eu^{III} -TACN-HOPO (4) matches that previously determined by Raymond for the Gd^{III} analog (Werner, E. J.; et al., *J. Am. Chem. Soc.* 2007, 129, 1870-1871). These values are comparable to that of Eu^{III} -DTPA ($\log K_{\text{EuDTPA}} = 17.86$) (Moeller, T.; et al., *J. Inorg. Nucl. Chem.* 1962, 24, 499-510) and to those of other macrocyclic gadolinium-based MRI contrast agents (Caravan, P.; et al., *Chem. Rev.* 1999, 99, 2293-2352). Increasing the kinetic inertness of highly-stable HOPO-based Eu^{III} phosphate receptors was thus successfully achieved.

Conclusion

Macrocyclic tris-bidentate Eu^{III} complexes bearing three 1,2-hydroxypyridinonate have lower solubility in water than their linear analogues such that only three of the four complexes could be evaluated. One of these complexes— Eu^{III} -cyclen-HOPO (6)—is eight coordinate whereas the other two— Eu^{III} -TACN-HOPO (4) and Eu^{III} -cyclam-HOPO (7)—are nine coordinate. This difference highlight the closeness in energy of the two coordination geometries.

Unlike their linear counterparts, all three macrocyclic complexes bind orthophosphate, albeit with different affinities. As previously observed with the linear analogues (Huang, S.-Y.; et al., *Inorg. Chem.* 2020, 59, 4096-4108; Pierre, V. C.; et al., *Inorg. Chem.* 2006, 45, 8355-8364; Pierre, V. C.; et al., *J. Am. Chem. Soc.* 2006, 128, 5344-5345), there is no relationship between the number of inner-sphere water molecules (or open coordination sites) and the affinity of the lanthanide complex for anions. Eu^{III}-cyclen-HOPO (6), which only has two inner-sphere water molecules, has higher affinity for phosphate than both Eu^{III}-TACN-HOPO (4) and Eu^{III}-cyclam-HOPO (7), which have a q = 3. Although the affinities of the macrocyclic complexes for phosphate is lower than the parent Eu^{III}-Ser-HOPO (1), they remain sufficient for medical applications of phosphate sequestration. Importantly, all macrocyclics are selective over other competing anions, notably HCO₃⁻ and Cl⁻, two anions present in higher concentrations in the blood.

The macrocyclic tripodal complexes Eu^{III}-TACN-HOPO (4), Eu^{III}-cyclen-HOPO (6), and Eu^{III}-cyclam-HOPO (7) all have thermodynamic stability comparable to the linear analog Eu^{III}-Ser-HOPO (1), with log K_c = 17.8±0.1. Replacement of a linear cap with a macrocyclic one therefore does not affect the high stability of these complexes, which is necessary for biomedical applications. The macrocyclic ligand caps do, however, affect the kinetic lability of the europium(III) complex and the rate at which it exchanges ligand with DTPA, but not to the same extent. Whereas 30 min is required for DTPA to completely exchange Eu^{III} with cyclam-HOPO (7), 1 hour is required for TACN-HOPO (4) and 2 hours for cyclen-HOPO (6). In comparison, complete ligand exchange between Eu^{III}-Ser-HOPO (1) and DTPA occurs in less than 5 min. The combination of high affinity for phosphate in water at neutral pH, high selectivity over bicarbonate and chloride, high stability and high kinetic inertness render Eu^{III}-cyclen-HOPO a promising candidate for the treatment of hyperphosphatemia via sequestration of excess phosphate in blood via membrane-immobilized receptors.

EXPERIMENTAL

General Considerations.

Unless stated otherwise, all chemicals were purchased from commercial suppliers and used without further purification. Deuterated solvents were obtained from Cambridge Isotope Laboratories (Tewksbury, MA). Distilled water was further purified by a Millipore Simplicity UV system (resistivity of 18×10⁶ Ω). All organic extracts were dried over anhydrous MgSO₄(s). Flash chromatography was performed on Merck Silica Gel. ¹H NMR and ¹³C NMR spectra were recorded on a Bruker Advance III 400 instrument at 400 and 100 MHz, respectively, or a Bruker

Advance III AV 500 instrument at 500 and 125 MHz, respectively, at the LeClaire-Dow instrumentation facility of the Department of Chemistry of the University of Minnesota. The residual solvent peaks were used as internal references. Data for ^1H NMR are recorded as follows: chemical shift (δ , parts per million), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; br, broad; m, multiplet), coupling constant (Hz), integration. Data for ^{13}C NMR are recorded as follows: chemical shift (δ , parts per million). Low-resolution (LR) and high-resolution (HR) electrospray ionization time-of-flight mass spectrometry (ESI/TOF-MS) data were recorded on a Bruker BioTOF I instrument at the LeClaire-Dow instrumentation facility of the Department of Chemistry of the University of Minnesota. Ultraviolet–visible spectra were recorded on a Varian Cary 100 Bio Spectrophotometer. Data were collected over the range of 250–400 nm. Luminescence data were acquired on a Varian Cary Eclipse fluorescence spectrophotometer using a quartz cell with a path length of 1 cm and a chamber volume of 500 μL . Time-gated luminescent spectra were recorded with a time delay of 0.1 ms and a gate time of 5 ms. Unless otherwise stated, sample solutions were allowed to equilibrate for 10 min before measurement of their luminescence spectra, as it has been demonstrated that this time was sufficient to achieve thermodynamic equilibrium (Figure 8). Luminescence data were processed with Scilab 6.0.2 and QtiPlot 0.9.8.9 software. All pH measurements were performed using a Thermo Scientific Ag/AgCl refillable probe and a Thermo Orion 3 Benchtop pH meter.

Synthesis.

The benzyl-protected HOPO hydroxysuccinimide activated analogue 9 were synthesized according published procedures with successful synthesis established by ^1H NMR, ^{13}C NMR and ESI-MS (Guérard, F.; et al., *Dalton Trans.* 2017, 46, 4749-4758). The linear receptor Eu^{III} -Ser-HOPO (1) was previously synthesized according to published procedures with successful synthesis established by ^1H NMR, ^{13}C NMR and ESI-MS (Huang, S.-Y.; et al., *Inorg. Chem.* 2019, 58, 16087-16099).

Preparation of tri-tert-butyl ((1,4,7-triazonane-1,4,7-triyl)tris(ethane-2,1-diyl))tricarbamate (8).

Cs_2CO_3 (7.56 g, 23.2 mmol) was added to a stirred solution of TACN (500. mg, 3.86 mmol) in dry CH_3CN (50 mL) under N_2 (g) atmosphere at room temperature. The resulting suspension was stirred at room temperature for 20 min followed by addition of *tert*-butyl (2-(bromoamino)ethyl)carbamate (2.73 g, 12.2 mmol). The reaction mixture was stirred at 80°C for 2 days, then cooled to room temperature. The reaction mixture was filter and the residue further washed with EtOAc (40 mL). The combined filtrates were concentrated under reduced pressure.

The crude product was purified by column chromatography on neutral alumina using 0 to 1.5% CH₃OH in CH₂Cl₂ as an eluent to yield the desired compound as a colorless sticky-solid (1.56 g, 72%). ¹H NMR (400 MHz, CDCl₃): δ 5.58 (br, 1H), 3.28-3.19 (m, 6H), 2.70-2.64 (m, 18H), 1.43 (s, 27H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 156.1, 79.1, 57.7, 56.7, 38.8, 28.6 ppm. ESI-

LRMS. Calcd for C₂₇H₅₅N₆O₆ [M+H]⁺ : m/z 559.42. Found: m/z 559.24.

Preparation of N,N',N''-((1,4,7-Triazonane-1,4,7-triyl)tris(ethane-2,1-diyl))tris(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamide) (TACN-HOPO-OBn, 10).

Trifluoroacetic acid (TFA, 5 mL) was added to a stirred solution of the BOC protected ligand cap 8 (500 mg, 0.895 mmol) in CH₂Cl₂ (10 mL) cooled to 0°C. The reaction mixture was stirred at room temperature for 4 h, after which excess solvent and acid were removed under reduced pressure. The resulting TFA salt was used without further purification. This TFA salt was then dissolved in milli-Q water (20 mL). A solution of HOPO(Bn)-OSu (9, 1.01 g, 2.95 mmol) in CH₃CN (20 mL) was then added to the reaction mixture, followed by K₂CO₃ (620 mg, 4.47 mmol). The reaction mixture was stirred for 12 h at room temperature. The homogenous reaction mixture was extracted with ethyl acetate (4 × 40 mL) and the combined organic phases were washed with 10% NaHCO₃ (2 × 30 mL) and brine (30 mL). The organic phase dried over anhydrous MgSO₄ (s) and concentrated under reduced pressure. The crude product was purified by flash column chromatography over neutral alumina, eluting with 1% methanol/99% CH₂Cl₂ to yield 10 as a viscous foaming liquid (650 mg, 77.3%). ¹H NMR (400 MHz, CDCl₃): δ 7.55-7.52 (m, 3H), 7.48-7.46 (m, 3H), 7.39-7.29 (m, 15H), 6.73-6.71 (m, 1H), 6.42-6.33 (m, 3H), 5.36-5.30 (br, 6H), 3.29-3.25 (m, 6H), 2.52-2.17 (m, 18H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 158.7, 142.9, 138.3, 133.4, 130.8, 129.5, 128.6, 123.9, 79.5, 56.5, 56.2, 53.5, 38.0 ppm. ESI-LRMS. Calcd for C₅₁H₅₈N₉O₉ [M+H]⁺ : m/z 940.44. Found: 940.35.

Preparation of N,N',N''-((1,4,7-Triazonane-1,4,7-triyl)tris(ethane-2,1-diyl))tris(1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamide) (TACN-HOPO, 11).

The benzyl-protected TACN-HOPO ligand 10 (100 mg, 0.106 mmol) was dissolved in a 1:1 mixture of glacial acetic acid (2.5 mL) and HCl (2.5 mL). The reaction mixture was stirred at room temperature for 2 days. The volatiles were then removed under reduced pressure. The crude product was further co-evaporated with methanol (3 × 15 mL) and triturated with diethyl ether (2 × 20 mL). The crude product was further purified by C₁₈-reverse phase column chromatography using 20% acetonitrile/80% CH₂Cl₂ water to afford the TACN-HOPO ligand as a white powder (60 mg, 84%). ¹H NMR (400 MHz, CD₃OD): δ 7.31 (dd, *J*₁ = 8 Hz, *J*₂ = 4 Hz, 3H), 6.72 (dd, *J*₁ =

8 Hz, $J_2 = 4$ Hz, 3H), 6.60 (dd, $J_1 = 8$ Hz, $J_2 = 4$ Hz, 3H), 3.63 (t, $J = 4$ Hz, 6H), 3.14 (t, $J = 4$ Hz, 6H), 3.07 (br, 12H) ppm. ^{13}C NMR (100 MHz, $(\text{CD}_3)_2\text{SO}$): δ 160.9, 159.2, 141.5, 134.6, 118.6, 105.2, 55.4, 51.4, 36.6 ppm. ESI-LRMS. Calcd for $\text{C}_{30}\text{H}_{40}\text{N}_9\text{O}_9$ $[\text{M}+\text{H}]^+$: m/z 670.29. Found: m/z 670.18.

5

Preparation of Eu^{III} -TACN-HOPO (4).

The deprotected TACN-HOPO ligand 11 (50 mg, 0.074 mmol) and $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ (27 mg, 0.074 mmol) were dissolved in methanol (10 mL). Pyridine (120 μL , 1.49 mmol) was subsequently added to the reaction mixture, which was stirred at 65 °C for 5 days. The reaction mixture was cooled to room temperature and the volatiles were removed under reduced pressure. The complex was then triturated with diethyl ether (3×10 mL), filtered, dissolved in mQ water (5 mL) and lyophilized to yield 4 as a beige powder (55 mg, 90%). ESI-LRMS. Calcd for $\text{C}_{30}\text{H}_{37}\text{EuN}_9\text{O}_9$ $[\text{M}+\text{H}]^+$: m/z 820.19. Found: m/z 820.12

15 Preparation of tri-*tert*-butyl ((1,5,9-triazacyclododecane-1,5,9-triyl)tris(ethane-2,1-diyl))tricarbamate (12)

Cs_2CO_3 (2.28 g, 7.00 mmol) was added to a stirred solution of TACD (200 mg, 1.16 mmol) in dry dimethylformamide (10 mL) under N_2 (g) atmosphere at room temperature. The resulting mixture was stirred at room temperature for 20 min, after which *tert*-butyl (2-(bromoamino)ethyl)carbamate (825 mg, 3.67 mmol) was added. The resulting reaction mixture was heated at 80°C for 2 days. The reaction mixture was then cooled to room temperature and filtered. The residue was washed with ethylacetate (40 mL), and the combined organic phases were concentrated under reduced pressure. The crude material was further purified by column chromatography on neutral alumina using 0 to 1.5% CH_3OH in CH_2Cl_2 to obtain the protected ligand cap 12 as a colorless sticky-solid (390 mg, 56.0%). ^1H NMR (400 MHz, CDCl_3): δ 5.28 (br), 3.23-3.18 (m., 3H), 2.50-2.44 (m, 18H), 1.64 (t, $J = 8$ Hz, 6H), 1.42 (s, 27H) ppm. ^{13}C NMR (100 MHz, CDCl_3): δ 156.0, 70.0, 53.1, 49.8, 37.7, 28.5, 22.2 ppm. ESI-LRMS. Calcd for $\text{C}_{30}\text{H}_{61}\text{N}_6\text{O}_6$ $[\text{M}+\text{H}]^+$: m/z 601.47. Found: m/z 601.38.

30 Preparation of N,N',N'' -((1,5,9-triazacyclododecane-1,5,9-triyl)tris(ethane-2,1-diyl))tris(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamide) (TACD-HOPO-OBn, 13).

Trifluoroacetic acid (TFA, 5 mL) was added to a stirred solution of ethylene-BOC protected ligand cap 12 (200 mg, 0.333 mmol) in CH_2Cl_2 (10 mL) at 0°C. The reaction mixture was stirred at room temperature for 4 h, after which excess solvent and acid were removed under

reduced pressure. The resulting TFA salt was used without further purification. This deprotected ligand cap was then dissolved in mQ water (20 mL). A solution of HOPO(Bn)-OSu (9, 376 mg, 1.09 mmol) in CH₃CN (20 mL) was then added to the reaction mixture, followed by K₂CO₃ (230 mg, 1.66 mmol). The reaction mixture was stirred for 12 h at room temperature. The homogenous reaction mixture extracted with ethylacetate (4 × 40 mL). The combined organic phases were washed with 10% NaHCO₃ (2 × 30 mL), brine (30 mL) dried over anhydrous MgSO₄ (s), and concentrated under reduced pressure. The crude product was purified by flash column chromatography over neutral alumina, eluting with 1% methanol/99% CH₂Cl₂ to yield the protected ligand 13 as a viscous foaming liquid (205 mg, 62.7%). ¹H NMR (400 MHz, CDCl₃): δ 7.49-7.47 z(m, 6H), 7.37-7.33 (m, 10 H), 7.32-7.31 (m, 2H), 7.29 (br, 1H), 6.72 (dd, *J*₁ = 8 Hz, *J*₂ = 4 Hz, 3H), 6.42 (dd, *J*₁ = 8 Hz, *J*₂ = 4 Hz, 3H), 5.29 (br, 6H), 3.32-3.21 (m, 6H), 2.35 (br, 6H), 2.18 (br, 12H), 1.23 (t, *J* = 8 Hz, 6H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 160.0, 158.6, 142.6, 138.2, 133.3, 130.4, 129.5, 128.6, 124.0, 106.1, 79.4, 52.0, 43.9, 37.1, 21.5 ppm. ESI-LRMS. Calcd for C₅₄H₆₄N₉O₉ [M+H]⁺ : m/z 982.48. Found: m/z 982.50

Preparation of N,N',N''-((1,5,9-Triazacyclododecane-1,5,9-triyl)tris(ethane-2,1-diyl))tris(1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamide) (TACD-HOPO, 14).

The benzyl-protected TACN-HOPO ligand 13 (0.10 g, 0.10 mmol) was dissolved in a 1:1 mixture of glacial acetic acid (2.5 mL) and HCl (2.5 mL). The reaction mixture was stirred at room temperature for 2 days. The solvents were then removed under reduced pressure. The crude product was further co-evaporated with methanol (3 × 15 mL) and further triturated diethyl ether (2 × 20 mL). The crude product was further purified by C₁₈-reverse phase column chromatography 20% acetonitrile/80% CH₂Cl₂ water to yield the 14 ligand as white powder (60 mg, 84%). ¹H NMR (400 MHz, CD₃OD): δ 7.19 (t, *J* = 8 Hz, 3H), 6.76-6.74 (m, 3H), 6.51-6.49 (m, 3H), 3.59 (br, 6H), 3.01 (br, 18H), 1.88 (br, 6H) ppm. ¹³C NMR (100 MHz, CD₃CN): δ 163.1, 161.9, 139.6, 134.9, 119.6, 110.1, 53.6, 49.9, 36.1, 20.5 ppm. ESI-LRMS. Calcd for C₃₃H₄₆N₉O₉ [M+H]⁺ : m/z 712.34. Found: m/z 712.30.

Preparation of Eu^{III}-TACD-HOPO (5).

The TACD-HOPO ligand 14 (50 mg, 0.070 mmol) and EuCl₃·6H₂O (25 mg, 0.070 mmol) were dissolved in methanol (10 mL). Pyridine (113 μL, 1.40 mmol) was subsequently added to the reaction mixture, which was stirred at 65 °C for 5 days. The reaction mixture was cooled to room temperature and the volatiles removed under reduced pressure. The product was further triturated with diethyl ether (3 × 10 mL), dissolved in mQ water (5 mL) and lyophilized to yield

the Eu^{III} complex 5 as a beige powder (45 mg, 75%). ESI-LRMS. Calcd for C₃₃H₄₃EuN₉O₉ [M+H]⁺: m/z 862.23. Found: m/z 862.24.

Preparation of tert-butyl (2-(7-benzyl-4,10-bis(2-((tert-butoxycarbonyl)amino)ethyl)-1,4,7,10-tetraazacyclododecan-1-yl)ethyl)carbamate (15).

Cs₂CO₃ (3.72 g, 11.4 mmol) was added to a stirred solution of mono(Bn)-cyclen (500 mg, 1.90 mmol) in dry CH₃CN (50 mL) under N₂ (g) atmosphere at room temperature. The reaction mixture was stirred at room temperature for 20 min before the addition of *tert*-butyl (2-(bromoamino)ethyl)carbamate (1.34 g, 6.00 mmol). The resulting reaction mixture was stirred at 80°C for 2 days. The reaction mixture was then cooled to room temperature, filtered and the residue washed with ethyl acetate (40 mL). The organic phase was then concentrated under reduced pressure and the crude material purified by column-chromatography on neutral alumina using 0 to 1.5% CH₃OH in CH₂Cl₂ to obtain the protected ligand 15 as a colorless sticky-solid (810 mg, 61.7%). ¹H NMR (400 MHz, CDCl₃): δ 7.30-7.26 (m, 5H), 6.10-6.05 (m, 2H), 3.53-3.49 (m, 2H), 3.19-3.16 (m, 6H), 2.63-2.06 (m, 22H), 1.44 (s, 27H) ppm. ESI-LRMS. Calcd for C₃₆H₆₆N₇O₆ [M+H]⁺: m/z 692.50. Found: m/z 692.48

Preparation of tert-butyl (2-(4,10-bis(2-((tert-butoxycarbonyl)amino)ethyl)-1,4,7,10-tetraazacyclododecan-1-yl)ethyl)carbamate (16).

The protected ligand 15 (600 mg, 0.867 mmol) was dissolved in anhydrous methanol (30 mL) and the solution was degassed before addition of 10 wt% Pd/C (100 mg). The suspension was further stirred under H₂ (g) (4 atm) in a Parr hydrogenator. The reaction mixture was further filtered over celite, and the residue washed with methanol (30 mL). The combined organic phases were concentrated under reduced pressure. The crude material was purified by column chromatography on neutral alumina using 0 to 1.5% CH₃OH in CH₂Cl₂ to obtain the desired deprotected 16 as a colorless sticky-solid (450 mg, 86.2%). ¹H NMR (400 MHz, CDCl₃): δ 10.31 (br, 2H), 6.04 (br, 1H), 5.52 (br, 1H), 3.26-3.19 (m, 6H), 3.00 (br, 4H), 2.89-2.87 (m, 4H), 2.72-2.59 (m, 14H), 1.42 (s, 27H) ppm. ¹³C NMR (100 MHz, CDCl₃): δ 156.2, 79.3, 56.6, 51.4, 49.3, 46.3, 38.5, 28.5 ppm. ESI-LRMS. Calcd for C₂₉H₆₀N₇O₆ [M+H]⁺: m/z 602.46. Found: m/z 602.41.

Preparation of N,N',N''-((1,4,7,10-Tetraazacyclododecane-1,4,7-triyl)tris(ethane-2,1-diyl))tris(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamide) (Cyclen-HOPO-OBn, 17).

TFA (5 mL) was added to a stirred solution of the ethylene-BOC protected **backbone 16** (400 mg, 0.665 mmol) in CH₂Cl₂ (10 mL) at 0°C. The reaction mixture was then stirred at room temperature for 4 h, after which excess solvent and acid were removed under reduced pressure. The resulting TFA salt was used without further purification. A solution of

HOPO(Bn)-OSu (**9**, 750 mg, 2.19 mmol) in CH₃CN (5 mL) followed by K₂CO₃ (460 mg, 3.32 mmol) were then added to a solution of the TFA salt of the ligand cap in mQ water (10 mL). The reaction mixture was stirred for 12 h at room temperature. The homogenous reaction mixture extracted with ethylacetate (4 × 40 mL). The combined organic phases were washed with 10% NaHCO₃ (2 × 30 mL), brine (30 mL), dried over anhydrous MgSO₄ (s), and concentrated under reduced pressure. The crude product was purified by flash column chromatography over silica, eluting with 1% methanol/99% CH₂Cl₂ to yield the protected ligand **17** as a viscous foaming liquid (490 mg, 74.9%). ¹H NMR (400 MHz, CDCl₃): δ 9.93 (br, 2H), 8.26 (br, 2H), 7.56-7.53 (m, 4H), 7.49-7.46 (m, 2H), 7.41-7.28 (m, 12H), 6.73 (dd, *J*₁ = 8 Hz, *J*₂ = 8 Hz, 1H), 6.63 (dd, *J*₁ = 4 Hz, *J*₂ = 4 Hz, 2H), 6.41-6.39 (m, 2H), 6.12-6.10 (m, 1H), 5.38 (br, 6H), 3.25 (br, 6H), 2.82-2.54 (m, 12H), 2.31-2.13 (m, 6H), 1.95-1.78 (m, 4H) ppm. ESI-LRMS. Calcd for C₅₃H₆₃N₁₀O₉ [M+H]⁺: m/z 983.47. Found: m/z 983.55.

Preparation of N,N',N''-((1,4,7,10-Tetraazacyclododecane-1,4,7-triyl)tris(ethane-2,1-diyl))tris(1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamide) (cyclen-HOPO, **18**).

The benzyl-protected cyclen-HOPO ligand **17** (100 mg, 0.0102 mmol) was dissolved in a 1:1 mixture of glacial acetic acid (2.5 mL) and HCl (2.5 mL). The reaction mixture was stirred at room temperature for 2 days. The volatiles were removed under reduced pressure. The crude product was further co-evaporated with methanol (3 × 15 mL) and triturated with diethyl ether (2 × 20 L). The crude product further purified by C₁₈-reverse phase chromatography 20% acetonitrile/80% CH₂Cl₂ water to yield **18** as white powder (62 mg, 86%). ¹H NMR (400 MHz, CD₃OD): δ 7.56-7.51 (m, 3H, b,b',b''), 6.91-6.89 (m, 1H), 6.83-6.77 (m, 3H), 6.69-6.67 (m, 2H), 3.93 (t, *J* = 8 Hz, 2H), 3.70 (t, *J* = 4 Hz, 2H), 3.60-3.57 (m, 8H), 3.53-3.30 (m, 4H), 3.18 (t, *J* = 4 Hz, 4H), 3.05 (br, 4H), 2.88 (t, *J* = 8 Hz, 4H) ppm. ¹³C NMR (100 MHz, CD₃OD): δ 163.9, 162.9, 160.3, 160.2, 141.4, 140.2, 139.4, 138.9, 121.6, 121.2, 110.3, 109.1, 55.4, 52.9, 47.9, 44.1, 36.0 ppm. ESI-LRMS. Calcd for C₅₃H₆₃N₁₀O₉ [M+H]⁺: m/z 713.34. Found: 713.33.

Preparation of Eu^{III}- Cyclen-HOPO (**6**).

The cyclen-HOPO ligand **18** (50 mg, 0.070 mmol) and EuCl₃·6H₂O (25 mg, 0.070 mmol) were dissolved in methanol (10 mL). Pyridine (113 μL, 1.40 mmol) was subsequently added to

the reaction mixture, which was stirred at 65 °C for 5 days. The reaction mixture was cooled to room temperature and the volatiles removed under reduced pressure. The product was triturated with diethyl ether (3 × 10 mL), dissolved in mQ water (5 mL) and lyophilized to yield the Eu^{III} complex as a beige powder (45 mg, 75%). ESI-LRMS. Calcd for C₃₂H₄₂EuN₁₀O₉ [M+H]⁺ : m/z 863.23. Found: m/z 863.27.

Preparation of di-*tert*-butyl ((4-(2-((*tert*-butoxycarbonyl)amino)ethyl)-1,4,8,11-tetraazacyclotetradecane-1,8-diyl)bis(ethane-2,1-diyl))dicarbamate (19).

Cs₂CO₃ (4.87 g, 15.0 mmol) was added to a stirred solution of cyclam (500 mg, 2.49 mmol) in dry CH₃CN (50 mL) under N₂ (g) atmosphere at room temperature. The resulting suspension was stirred at room temperature for 20 min, after which *tert*-butyl (2-(bromoamino)ethyl)carbamate (1.76 g, 7.86 mmol) was added. The resulting reaction mixture was heated at 80°C for 2 days. The reaction mixture was then cooled to room temperature, filtered, and the residues washed with ethyl acetate (40 mL). The organic phases were combined and concentrated under reduced pressure. The crude product was purified by column-chromatography on neutral alumina using 0 to 1.5% CH₃OH in CH₂Cl₂ to yield the protected ligand cap 19 as a colorless sticky-solid (950 mg, 60.6%). ¹H NMR (400 MHz, CDCl₃): δ 5.57 (br, 1H), 5.31 (br, 1H), 5.09 (br, 1H), 3.30-3.24 (m, 6H), 3.08-3.00 (m, 6H), 2.67-2.43 (m, 16H), 2.06 (br, 2H), 1.76-1.72 (m, 2H), 1.42 (s, 27H) ppm. ESI-LRMS. Calcd for C₃₁H₆₄N₇O₆ [M+H]⁺ : m/z 630.49. Found: m/z 630.48.

Preparation of N,N',N''-((1,4,8,11-Tetraazacyclotetradecane-1,4,8-triyl)tris(ethane-2,1-diyl))tris(1-(benzyloxy)-6-oxo-1,6-dihydropyridine-2-carboxamide) (Cyclam-HOPO-OBn, 20).

TFA (5 mL) was added to a stirred solution of ethylene-BOC protected backbone 19 (450 mg, 0.714 mmol) in CH₂Cl₂ (10 mL) at 0°C. The reaction mixture was then allowed to stir at room temperature for 4 h, after which the volatiles were removed under reduced pressure. The resulting TFA salt of the ligand cap was used without further purification. A solution of HOPO(Bn)-OSu (**9**, 807 mg, 2.35 mmol) in CH₃CN (20 mL) and K₂CO₃ (493 mg, 3.57 mmol) were then added to a solution of the TFA salt of the ligand cap in mQ water (20 mL). The reaction mixture was stirred for 12 h at room temperature. The reaction mixture extracted with ethyl acetate (4 × 40 mL). The organic phases were combined, washed with 10% NaHCO₃ (2 × 30 mL) and brine (30 mL), dried over anhydrous MgSO₄ (s), and concentrated under reduced pressure. The crude product was purified by flash column chromatography over neutral alumina, eluting with 1% methanol/99% CH₂Cl₂ to yield the protected ligand 20 as a viscous foaming liquid (450

mg, 62.3%). ^1H NMR (400 MHz, CDCl_3): δ 7.74 (br, 1H), 7.55-7.48 (m, 6H), 7.43 (br, 1H), 7.39-7.36 (m, 4H), 7.32-7.28 (m, 6H), 7.25-7.21 (m, 2H), 6.74-6.72 (m, 2H), 6.63-6.61 (m, 1H), 6.51-6.44 (m, 2H), 6.22 (br, 1H), 5.38-5.34 (m, 6H, h), 3.38-2.72 (m, 12H), 2.24-2.16 (m, 12H), 1.83 (br, 6H), 1.33-1.25 (m, 2H) ppm. ESI-LRMS. Calcd for $\text{C}_{55}\text{H}_{67}\text{N}_{10}\text{O}_9$ $[\text{M}+\text{H}]^+$: m/z 1011.50.

5 Found: m/z 1011.62.

Preparation of $\text{N,N',N''}-((1,4,8,11\text{-Tetraazacyclotetradecane-1,4,8-triyl})\text{tris(ethane-2,1-diyl)})\text{tris(1-hydroxy-6-oxo-1,6-dihydropyridine-2-carboxamide)}$ (cyclam-HOPO, 21).

The benzyl-protected cyclam-HOPO ligand 20 (100 mg, 0.0989 mmol) was dissolved in a
10 1:1 mixture of glacial acetic acid (2.5 mL) and HCl (2.5 mL). The reaction mixture was stirred at room temperature for 2 days. The volatile were removed under reduced pressure. The crude product was further co-evaporated with methanol (3×15 mL) and triturated diethyl ether (2×20 mL). The crude product was further purified by C_{18} -reverse phase column chromatography using 20% acetonitrile/80% CH_2Cl_2 water to yield the cyclam-HOPO ligand as white powder (65 mg,
15 89%). ^1H NMR (400 MHz, CD_3OD): δ 7.55-7.50 (m, 3H), 6.83-6.75 (m, 6H), 3.84 (t, $J = 6$ Hz, 2H), 3.68-3.63 (m, 6H), 3.52-3.41 (m, 10H), 3.17-3.02 (m, 10H), 2.20 (br, 4H) ppm ^{13}C NMR (100 MHz, CD_3OD): δ 163.7, 163.1, 163.0, 160.4, 160.3×2 , 141.6, 141.4×2 , 139.0×2 , 138.7, 121.3, 121.1, 109.4, 109.2, 54.5, 54.2, 52.8, 51.5, 50.0, 46.1, 44.2, 37.7, 36.9, 36.1, 23.1 ppm. ESI-LRMS. Calcd for $\text{C}_{34}\text{H}_{49}\text{N}_{10}\text{O}_9$ $[\text{M}+\text{H}]^+$: m/z 741.36. Found: m/z 741.38.

20

Preparation of Eu^{III} -cyclam-HOPO (7).

The cyclam-HOPO ligand 21 (50 mg, 0.067 mmol) and $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ (24 mg, 0.067 mmol) were dissolved in methanol (10 mL). Pyridine (108 μL , 1.35 mmol) was subsequently added to the reaction mixture, which was stirred at 65 $^\circ\text{C}$ for 5 days. The reaction mixture was cooled
25 down to room temperature and further evaporated under reduced pressure. The product was further triturated with diethyl ether (3×10 mL), dissolved in MQ water (5 mL) and lyophilized to yield the Eu^{III} complex as a beige powder (50 mg, 83%). ESI-LRMS. Calcd for $\text{C}_{34}\text{H}_{46}\text{EuN}_{10}\text{O}_9$ $[\text{M}+\text{H}]^+$: m/z 891.26. Found: m/z 891.35.

30 Determination of the number of inner-sphere water molecules, q.

The number of inner-sphere water molecules were determined by luminescence spectroscopy following published procedure (Pierre, V; et al., Royal Society of Chemistry, 2017; pp 142-149), using the modified Horrocks equation (Dickins, R. S.; et al, *Chem. Commun.* 1996, 697-698; Beeby, A.; et al., *J. Chem. Soc., Perkin Trans 2* 1999, 493-504; Supkowski, R. M.; et

al., *Inorganica Chim. Acta* 2002, 340, 44-48). Samples were either independently prepared in different ratios of H₂O/D₂O (4, 7) or were prepared through serial dilution with D₂O (6) to alleviate error from aggregation (Figure 7).

5 Affinity of Eu^{III}L complexes for phosphate.

Batch titrations conducted to determine the binding affinity (K_a) of each Eu^{III}-L-HOPO complex for phosphate were performed by generating two aqueous solutions: one of the Eu^{III} complex and one of the anion at pH 7.4). Appropriate volumes of each solution were added and diluted with H₂O to the desired concentrations. Each sample was allowed to equilibrate for at least
10 2 hours for phosphate to ensure that thermodynamic equilibrium was reached. For each titration, 8-10 points were collected between 1.47 μ M – 184 μ M of the anion alongside 7.35 μ M of Eu^{III}-L-HOPO .

Quantum Yield.

15 Relative quantum yield was measured according to the reported procedure (Würth, C.; et al., *Nat. Protoc.* 2013, 8, 1535-1550) using coumarin as a reference (0.32%) (Taniguchi, M.; et al., *Photochem. Photobiol.* 2018, 94, 290-327).

Selectivity.

20 Responses to anions were evaluated against 10 equivalents of the following solutions: NaF, NaCl, NaBr, NaI, K₂SO₄, NaHCO₃, KNO₃, Na₂AsO₄, Na₄P₂O₇, NaC₂H₃O₂, Na₃C₆H₅O₇, and NaC₃H₅O₃ dissolved to 10 mM stocks and pH adjusted to 7.4 using NaOH or HCl as necessary. Sample solutions were allowed to equilibrate for 3 hours before measurement of their time-gated luminescence spectra. Every data point was measured in triplicate from three independently
25 prepared samples.

Kinetics of transmetallation with DTPA.

The inertness of the Eu^{III} complexes were determined by monitoring the luminescence spectra of each Eu^{III} complex before and continuously after addition of 5 equivalents of
30 diethylenetriaminepentaacetic acid (DTPA). Samples were prepared from stock solution of concentrated complexes and ligands both at pH = 7.4 and T=25°C. A solution of DTPA (5 equivalents) was added a solution of the Eu^{III} complex (7.35 μ M) in KCl (0.1 M). The time-gated luminescence was measured continuously over time after addition of DTPA, beginning at intervals of 1 min. The concentrations of free and complexed ligand in each solution were

determined from reference solutions of the pure free ligand and Eu^{III} complex. Thermodynamic equilibrium was reached after no significant changes in the spectra were observed for at least 20 min.

5 Stability of lanthanide complexes.

The stability of the Eu^{III}-L-HOPO complexes were determined by competition with DTPA following a procedure previously reported (Doble, D. M. J.; et al., *Inorg. Chem.* 2003, 42, 4930-4937). The equilibria were monitored by luminescence spectroscopy. Samples were prepared from stock solutions of concentrated complexes (pH = 7.4) equilibrated at 60°C for 24 h.

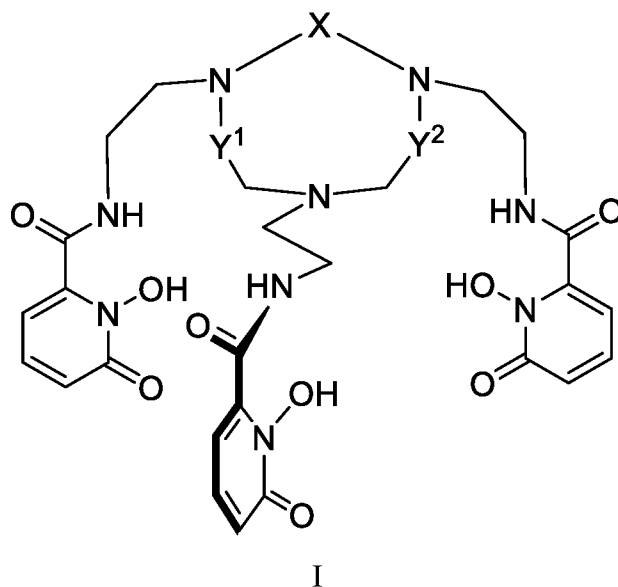
10 Samples containing known concentrations of each Eu^{III} complex (7.35 μM) with KCl (0.1 M) were added to solutions containing 1-10 equivalents DTPA. Upon addition of DTPA, the time-gated luminescence (delay time of 0.1 ms, gate time excitation slit width = 10 nm, emission slit width = 10 nm, T = 25°C) was measured after 3 hours to ensure thermodynamic equilibrium was reached. The concentrations of free and complexed ligand in each solution were determined from
15 reference solutions of the pure free ligand and Eu^{III} complex. The logK_c were determined as previously published from the reported stability constants for DTPA with Eu^{III} ions (Moeller, T.; et al., *J. Inorg. Nucl. Chem.* 1962, 24, 499-510), the complex's hydrolysis constants (Brown, P. L.; et al., Scandium, Yttrium and the Lanthanide Metals. In *Hydrolysis of Metal Ions*; John Wiley & Sons, Incorporated: Weinheim, Germany, 2016; pp 5103c-5107y) and the protonation constants
20 of DTPA (Pniok, M., Kubíček, V., Havlíčková, J., Kotek, J., Sabatie-Gogová, A., Plutnar, J., Huclier-Markai, S.; Hermann, P. Thermodynamic and Kinetic Study of Scandium(III) Complexes of DTPA and DOTA: A Step Toward Scandium Radiopharmaceuticals. *Chem. Eur. J.* 2014, 20, 7944-7955).

25 All publications, patents, and patent documents are incorporated by reference herein, as though individually incorporated by reference. The document, Martinon, T.L.M.; et al., Kinetically Inert Macrocyclic Europium (III) Receptors for Phosphate, *Inorg. Chem.* 2023, 62, 10064-10076, is incorporated by reference herein. The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be
30 understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

CLAIMS

We claim:

1. A ligand of formula I or a salt thereof, or a rare earth metal complex of the ligand of formula I or a salt thereof,



wherein:

X is $-(CH_2)_2-$, $-(CH_2)_3-$, $-(CH_2)_pN(CH_2)_q-$

Y^1 is $-(CH_2)-$, or $-(CH_2)_2-$;

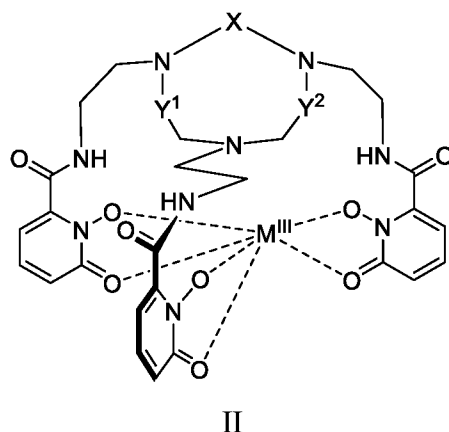
Y^2 is $-(CH_2)-$, or $-(CH_2)_2-$;

wherein Y^1 is $-(CH_2)_2-$ when X is $-(CH_2)_2-$ and Y^2 is $-(CH_2)-$;

p is 2 or 3;

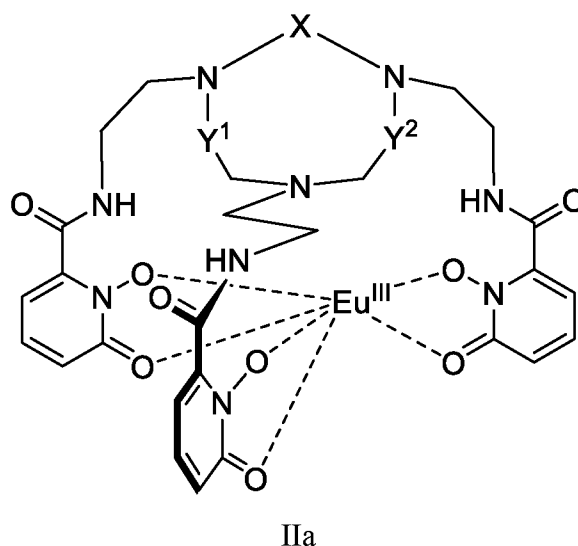
q is 2 or 3; and wherein the compound of formula I is optionally substituted with one or more linking groups.

2. The rare earth metal complex of claim 1 or a salt thereof, wherein the rare earth metal complex is a complex of formula II or salt thereof:



wherein the rare earth metal is M^{III} .

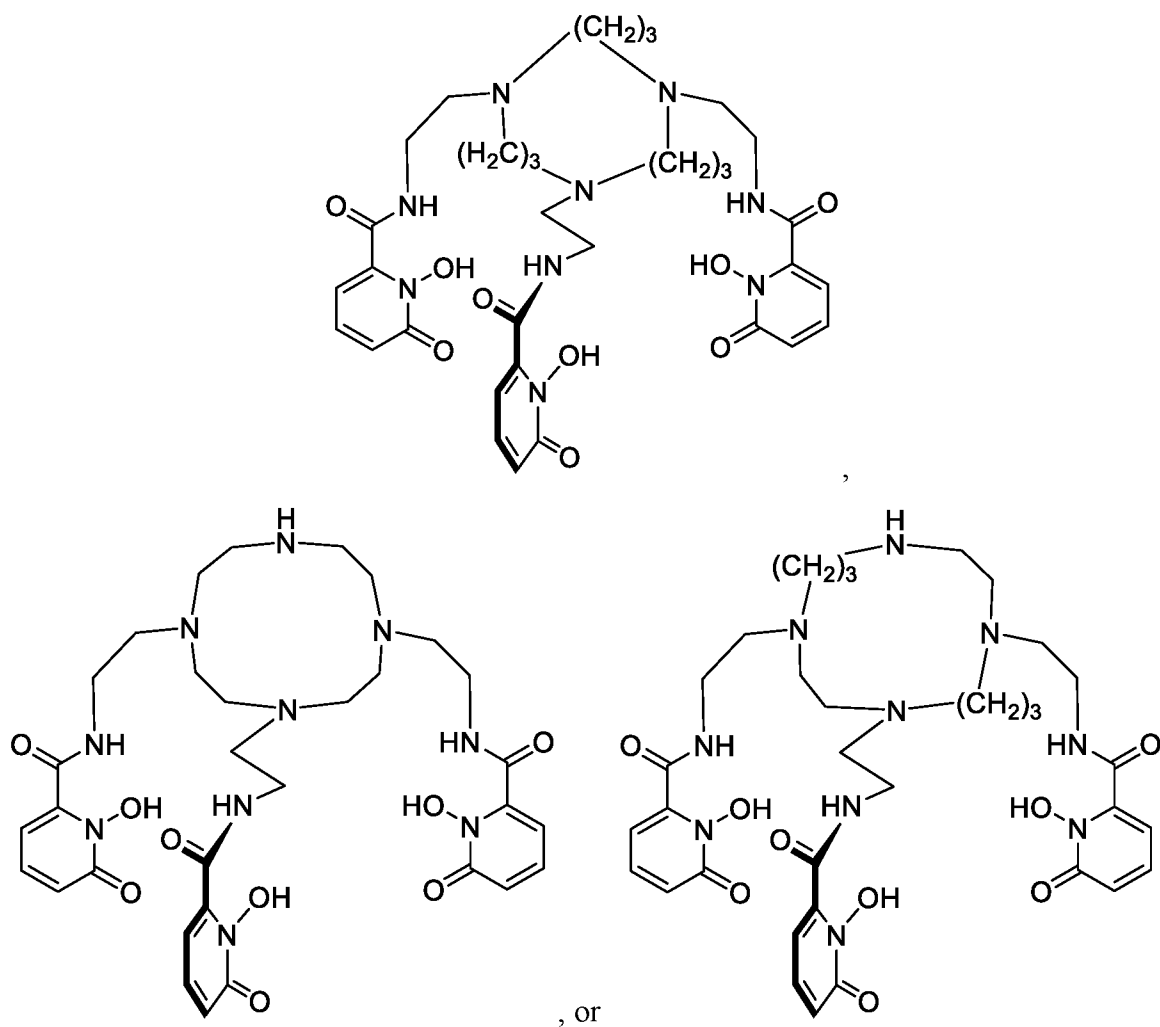
3. The rare earth metal complex or a salt thereof of claim 1, wherein the rare earth metal complex is a Eu^{III} complex of formula IIa:



or salt thereof.

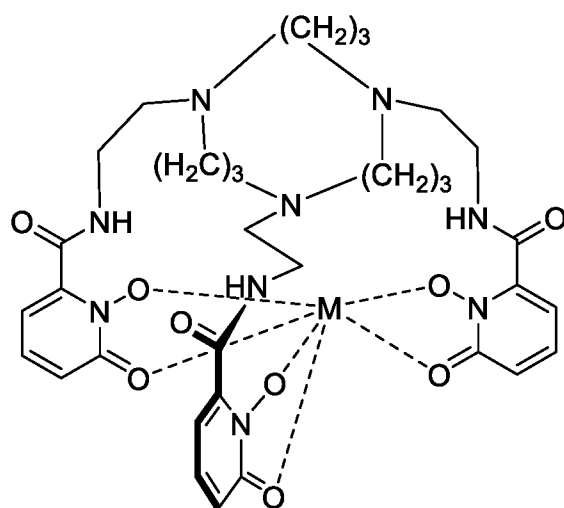
4. The ligand or salt thereof or rare earth metal complex or a salt thereof of any one of claims 1-3, wherein X is $-(\text{CH}_2)_2-$, $-(\text{CH}_2)_3-$, $-(\text{CH}_2)_2\text{N}(\text{CH}_2)_2-$, or $-(\text{CH}_2)_3\text{N}(\text{CH}_2)_2-$.
5. The ligand or salt thereof or rare earth metal complex or a salt thereof of any one of claims 1-4, wherein Y^1 is $-(\text{CH}_2)-$.
6. The ligand or salt thereof or rare earth metal complex or a salt thereof of any one of claims 1-4, wherein Y^1 is $-(\text{CH}_2)_2-$.
7. The ligand or salt thereof or rare earth metal complex or a salt thereof of any one of claims 1-6, wherein Y^2 is $-(\text{CH}_2)-$.
8. The ligand or salt thereof or rare earth metal complex or a salt thereof of any one of claims 1-6, wherein Y^2 is $-(\text{CH}_2)_2-$.
9. The ligand or salt thereof or rare earth metal complex of any one of claims 1-8, wherein the linker comprises a branched or unbranched, saturated or unsaturated, hydrocarbon chain, having from 1 to 10 carbon atoms wherein one or more of the carbon atoms is optionally replaced independently by $-\text{O}-$, $-\text{S}-$, $-\text{N}(\text{R}^a)-$, and wherein the chain is optionally substituted with one or more (e.g. 1, 2, 3, 4, 5 or more) substituents independently selected from (C_1-C_4) alkyl, (C_1-C_6) alkoxy, oxo ($=\text{O}$), and halo, wherein each R^a is independently H or (C_1-C_6) alkyl.

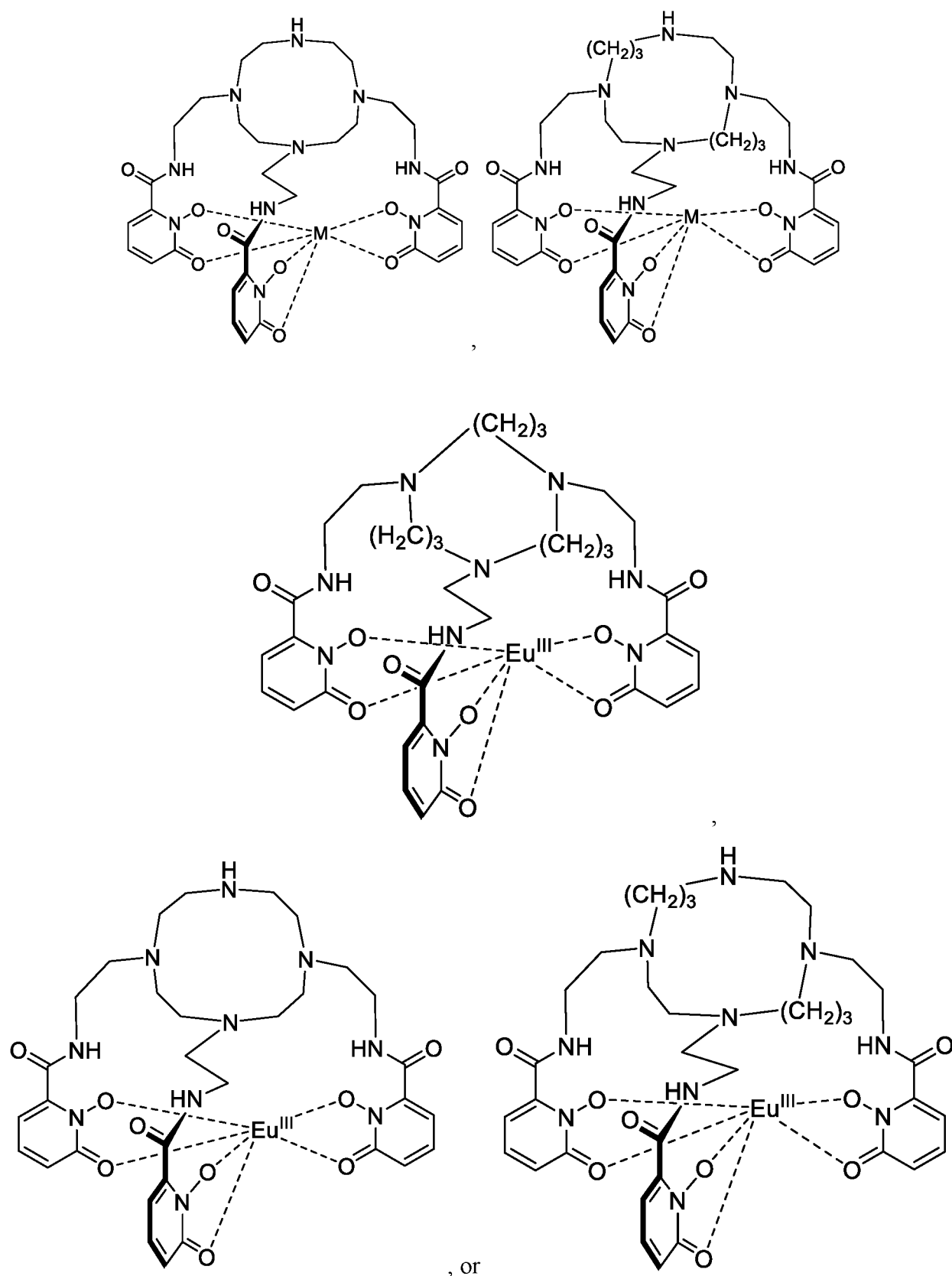
10. The ligand or salt thereof of claim 1 that is



or a salt thereof.

11. The rare earth metal complex or a salt thereof of claim 1 that is

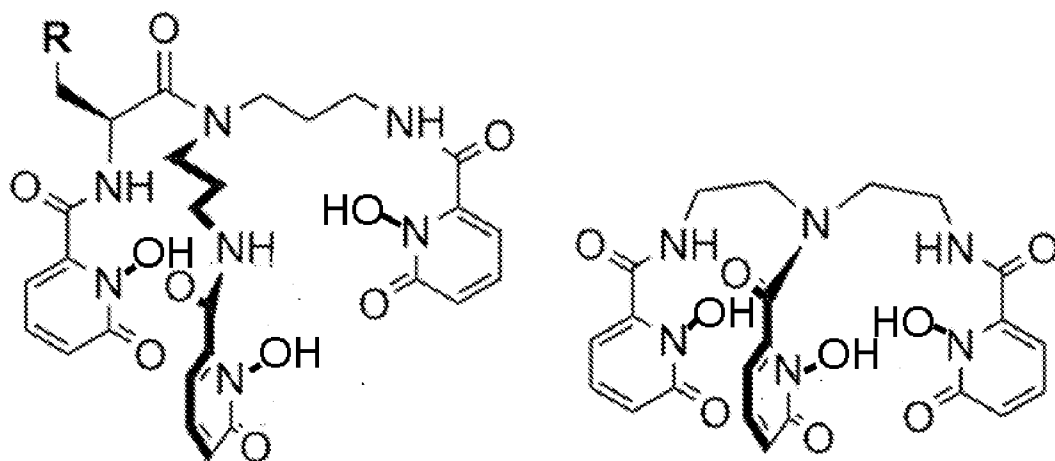




or a salt thereof,

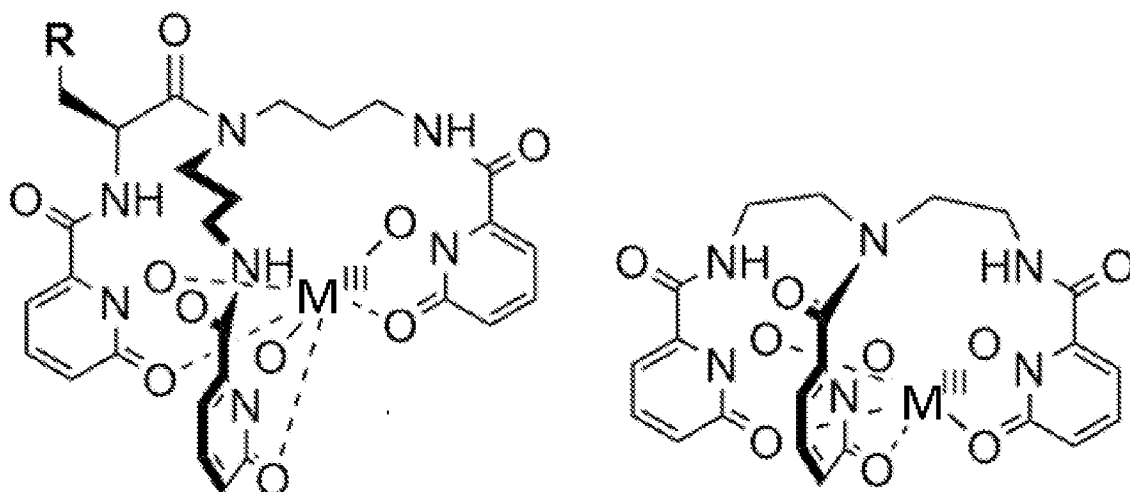
wherein the M is a rare earth metal or M is M^{III} wherein M^{III} is a rare earth metal.

12. A ligand or salt thereof that is



wherein R is H or OH.

13. A rare earth metal complex or salt thereof that is



wherein M is a rare earth metal and R is H or OH.

14. The rare earth metal complex or a salt thereof of claim 13, wherein the rare earth metal is Eu^{III} .

15. A material or device comprising one or more ligands or a salt thereof, or one or more rare earth metal complex or a salt thereof, as described in any one of claims 1-14.

16. The material or device of claim 15, wherein the material or device is a hydrogel, membrane, nanoparticle, polymer, conducting polymer, conducting material (e.g., metal or carbon), or other material.

17. A method to detect or capture inorganic phosphate, comprising contacting the phosphate with a ligand or salt thereof, or a rare earth metal complex or a salt thereof, or a material or device as described in any one of claims 1-16.
18. The method of claim 17, wherein the phosphate is selectively detected or captured in the presence of other anions.
19. The method of claim 18, wherein the other anions are selected from the group consisting of carbonate, nitrate, sulfate, halides, arsenate and pyrophosphate.
20. The method of any one of claims 17-19, wherein the phosphate is contacted with the with a ligand or salt thereof, or a rare earth metal complex or a salt thereof, or a material or device, as a liquid sample at about neutral pH.
21. The method of claim 18, wherein the liquid sample is sample is an aqueous sample.
22. The method of any one of claims 17-21, wherein the phosphate is detected with fluorescence sensing by an indicator displacement assay.
23. The method of any one of claims 17-21, wherein the phosphate is captured.
24. The method of claim 23, wherein the phosphate is captured from an aqueous mixture or solution.
25. A method to treat hyperphosphatemia in a mammal in need thereof comprising contacting the blood of the mammal in need thereof, with a ligand or salt thereof, or a rare earth metal complex or a salt thereof, or a material or device as described in any one of claims 1-16.
26. The method of claim 25, wherein the mammal has kidney disease (e.g., chronic, advanced, acute or advanced/acute kidney disease).
27. The use of a ligand or salt thereof, or a rare earth metal complex or a salt thereof, or a material or device as described in any one of claims 1-16 for the treatment of hyperphosphatemia in a mammal in need thereof.

28. The use of a ligand or salt thereof, or a rare earth metal complex or a salt thereof, or a material or device as described in any one of claims 1-16 for medical therapy.

29. The use of a ligand or salt thereof, or a rare earth metal complex or a salt thereof as described in any one of claims 1-14 to prepare a device or material for the treatment of hyperphosphatemia.

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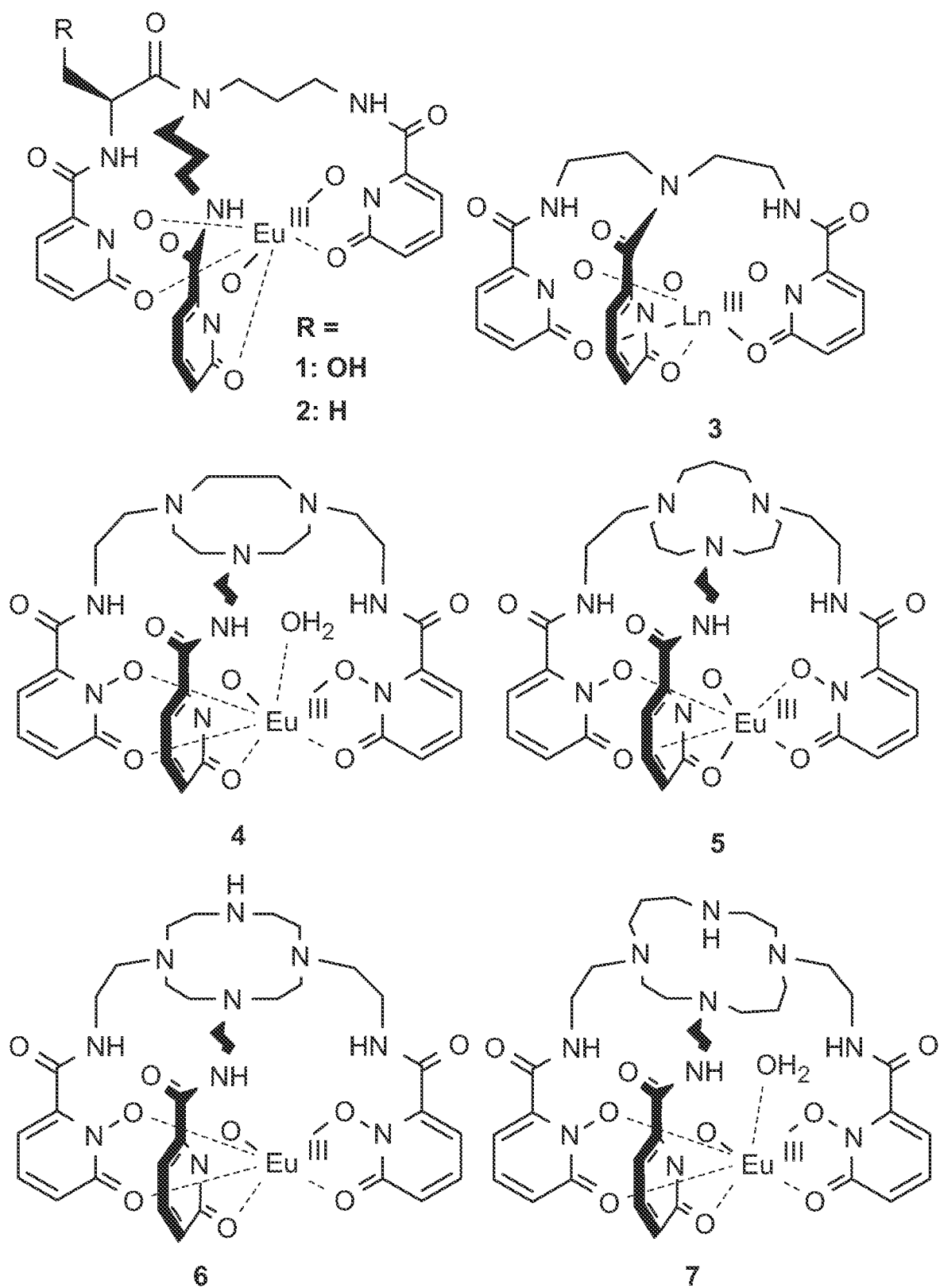


Figure 1

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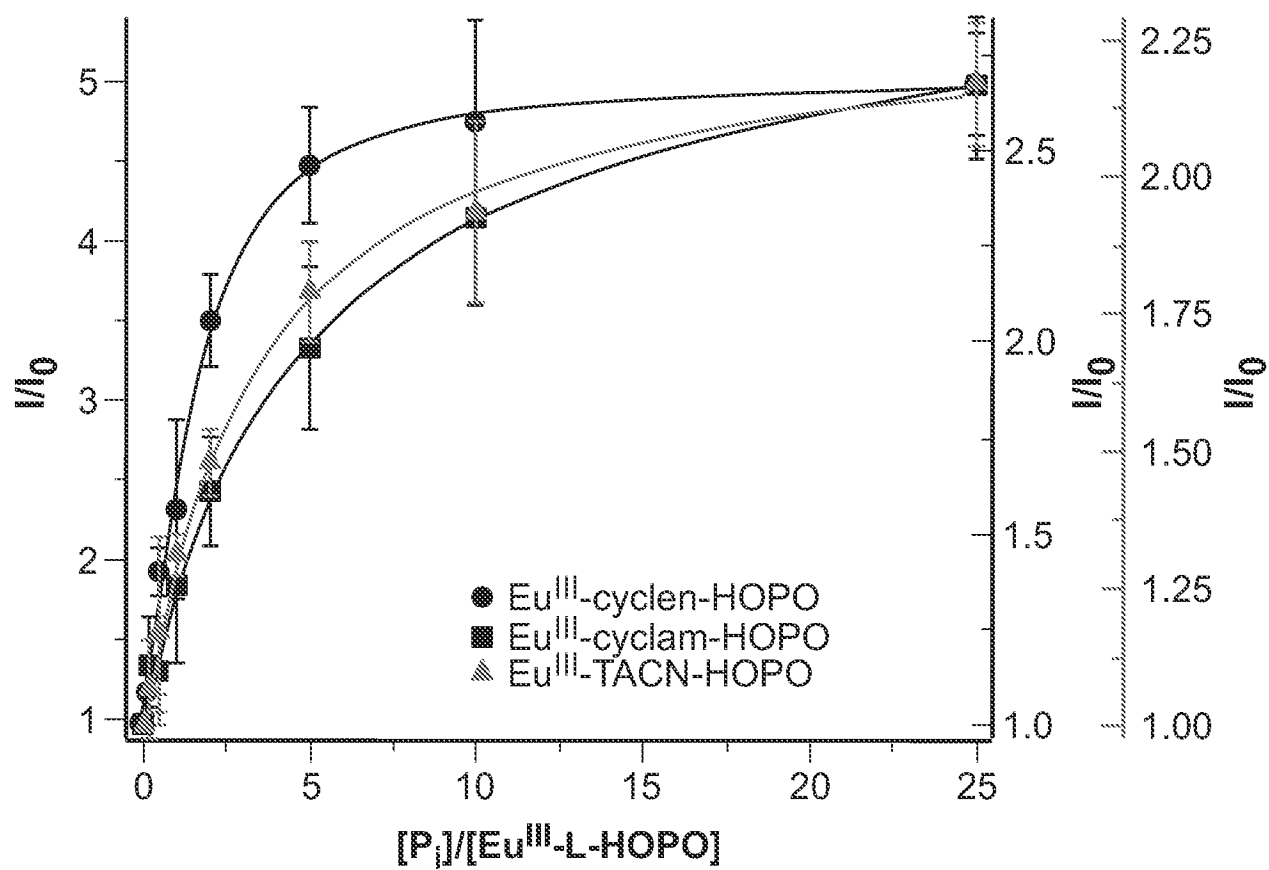


Figure 2

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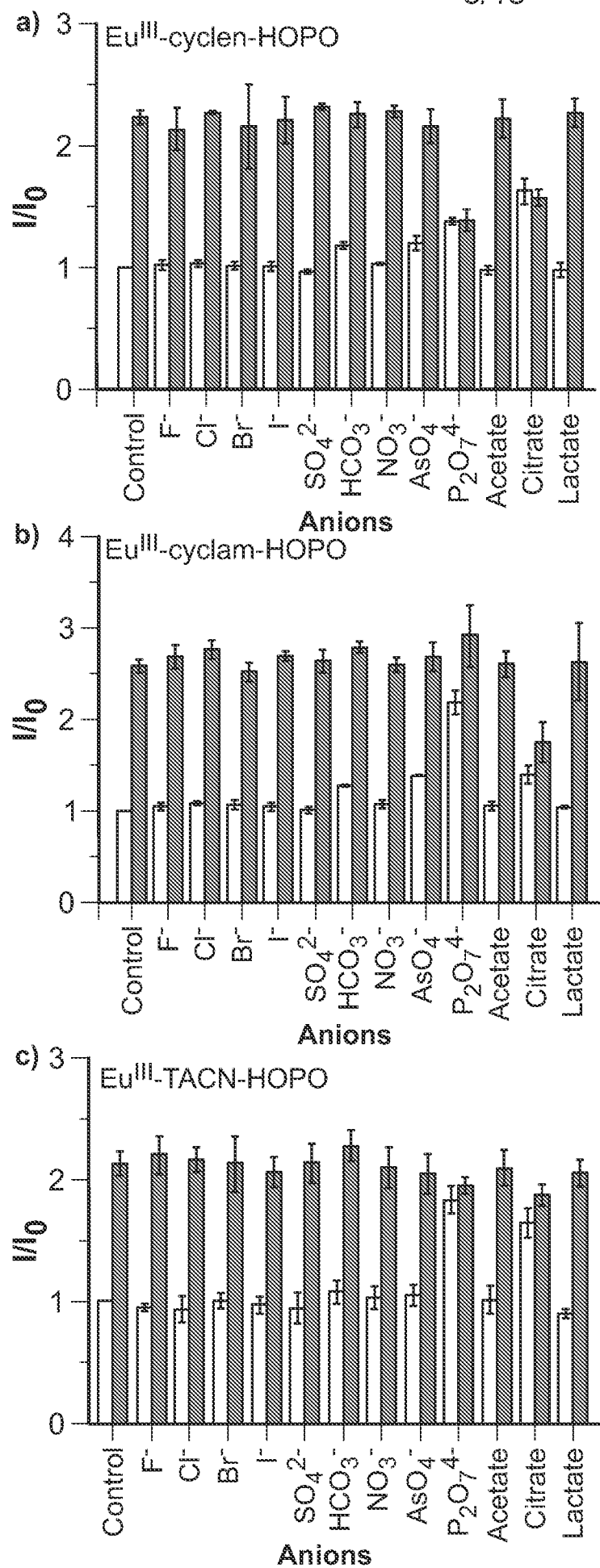


Figure 3

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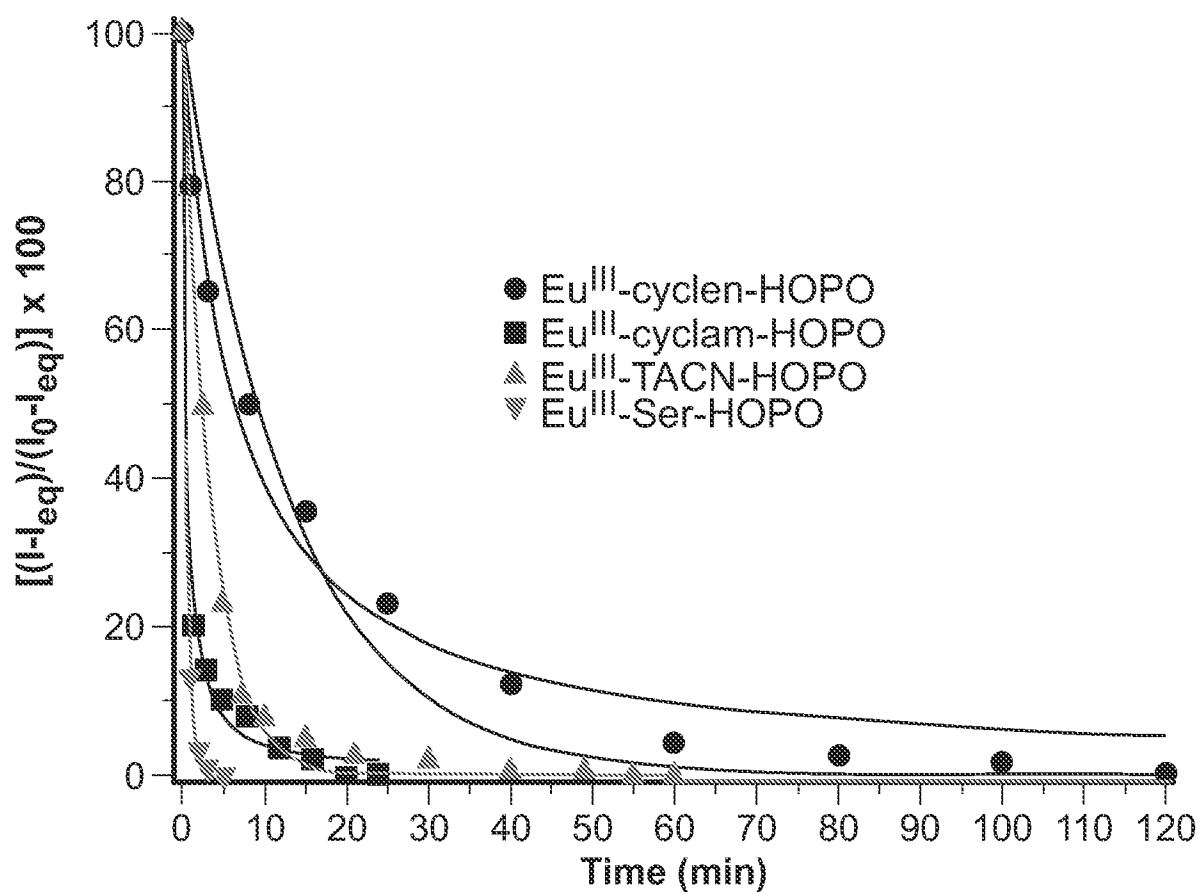


Figure 4

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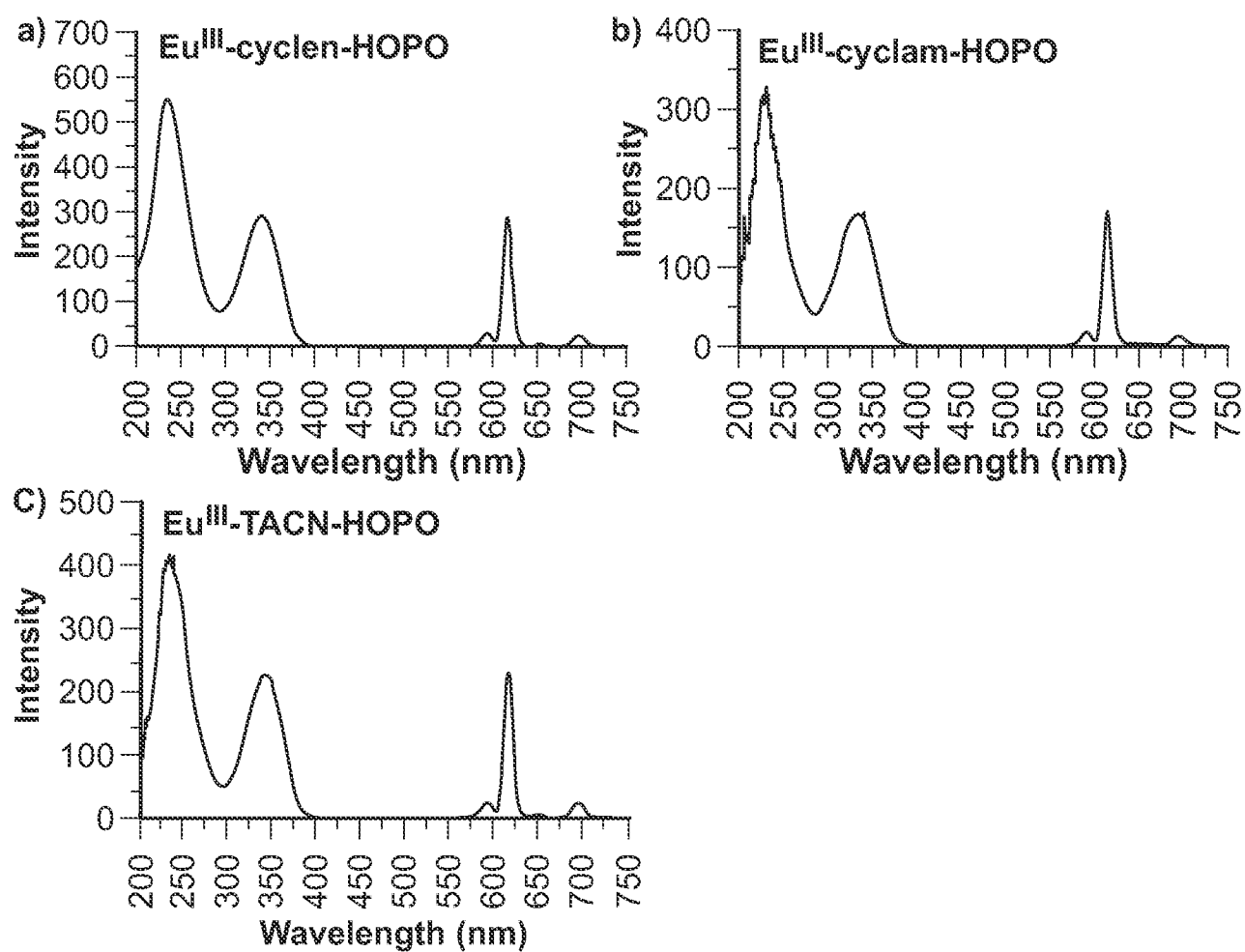


Figure 5A-5C

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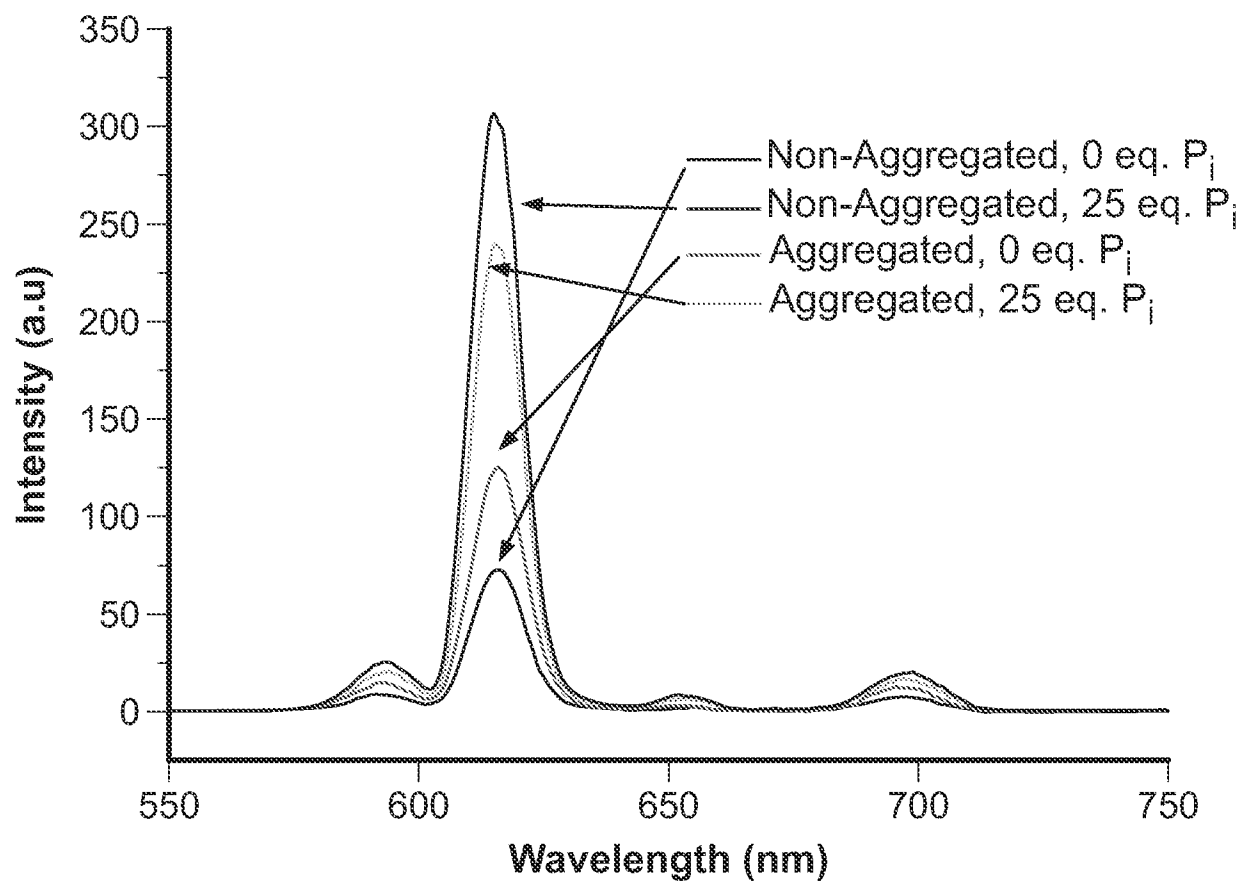


Figure 6

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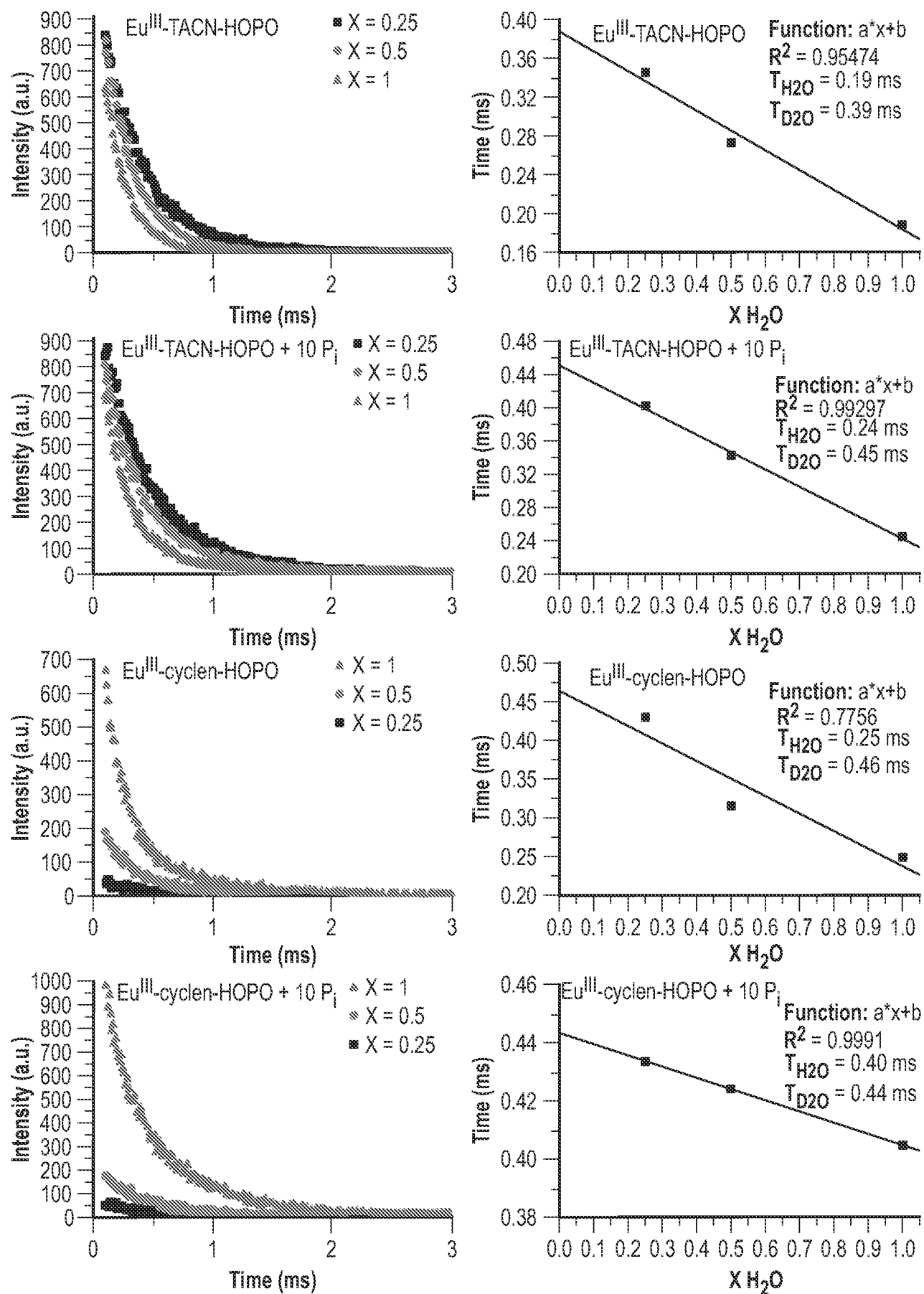


Figure 7

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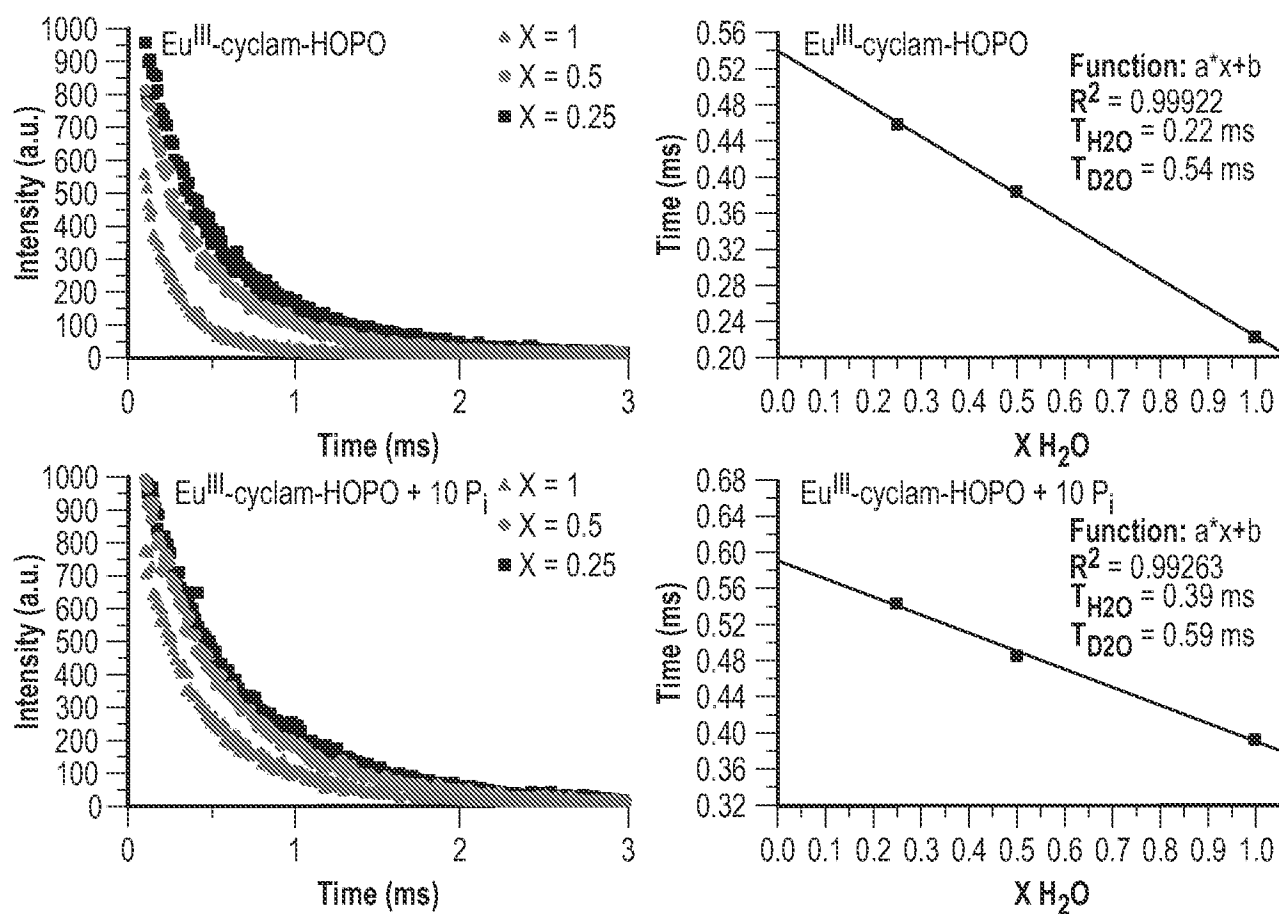
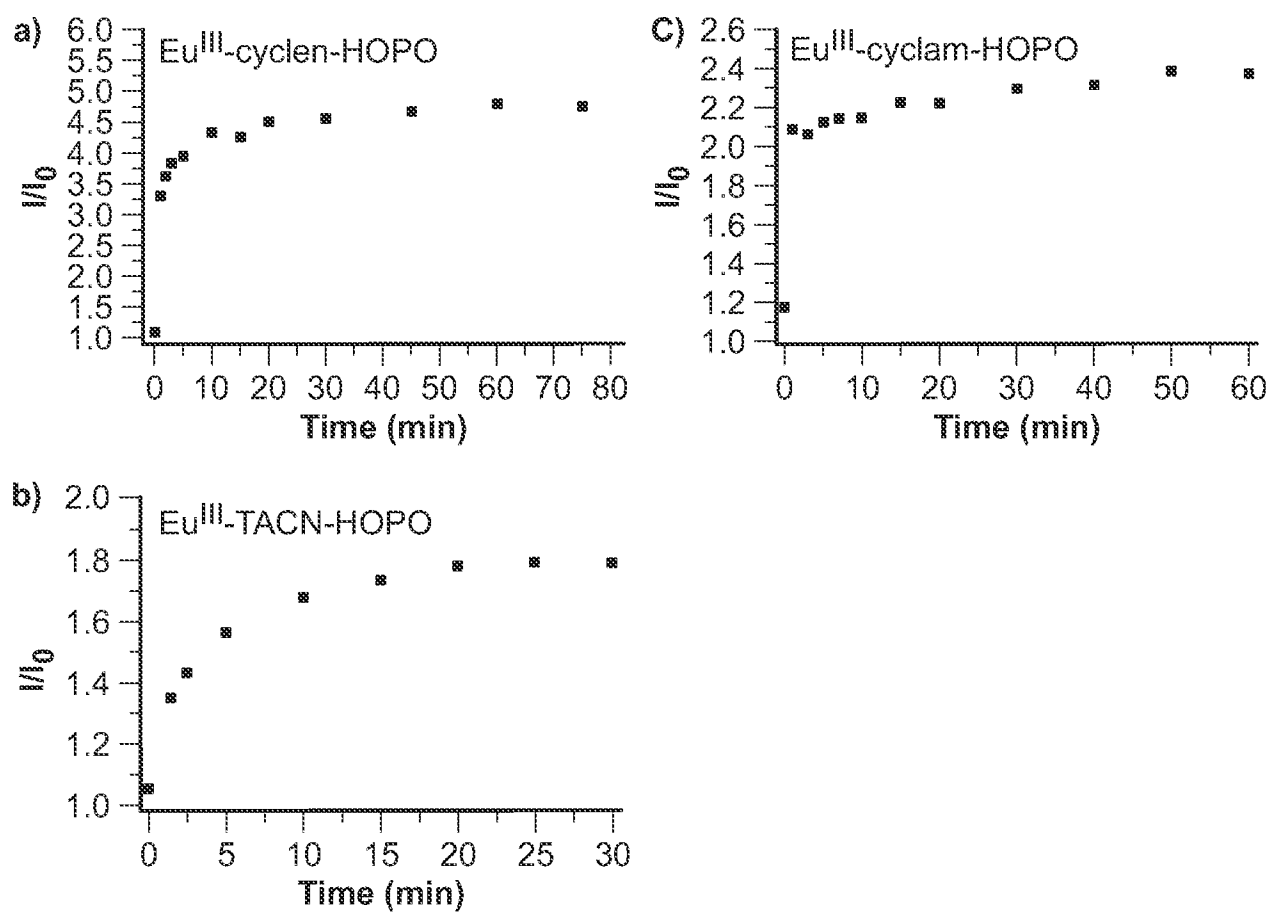


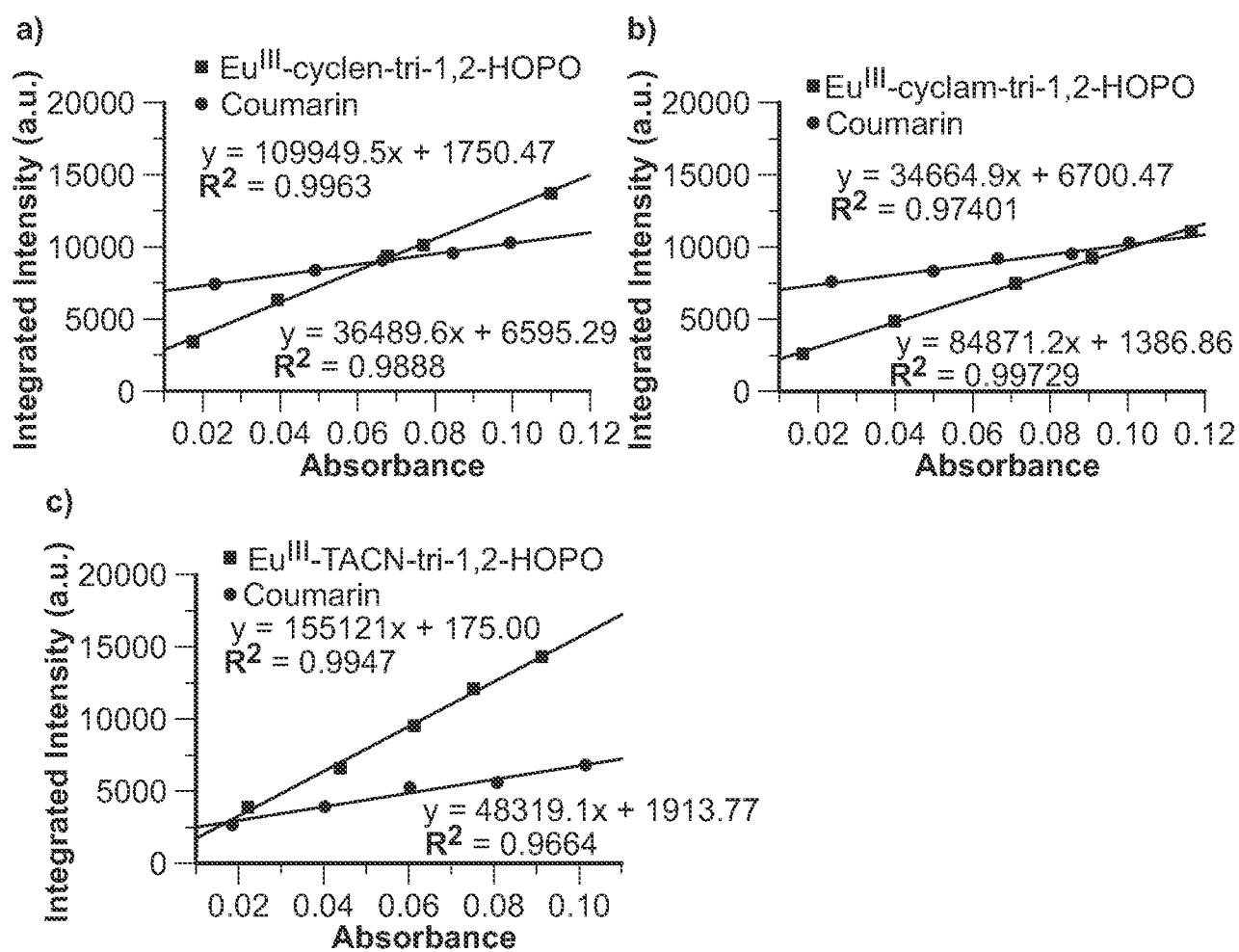
Figure 7 (Cont.)

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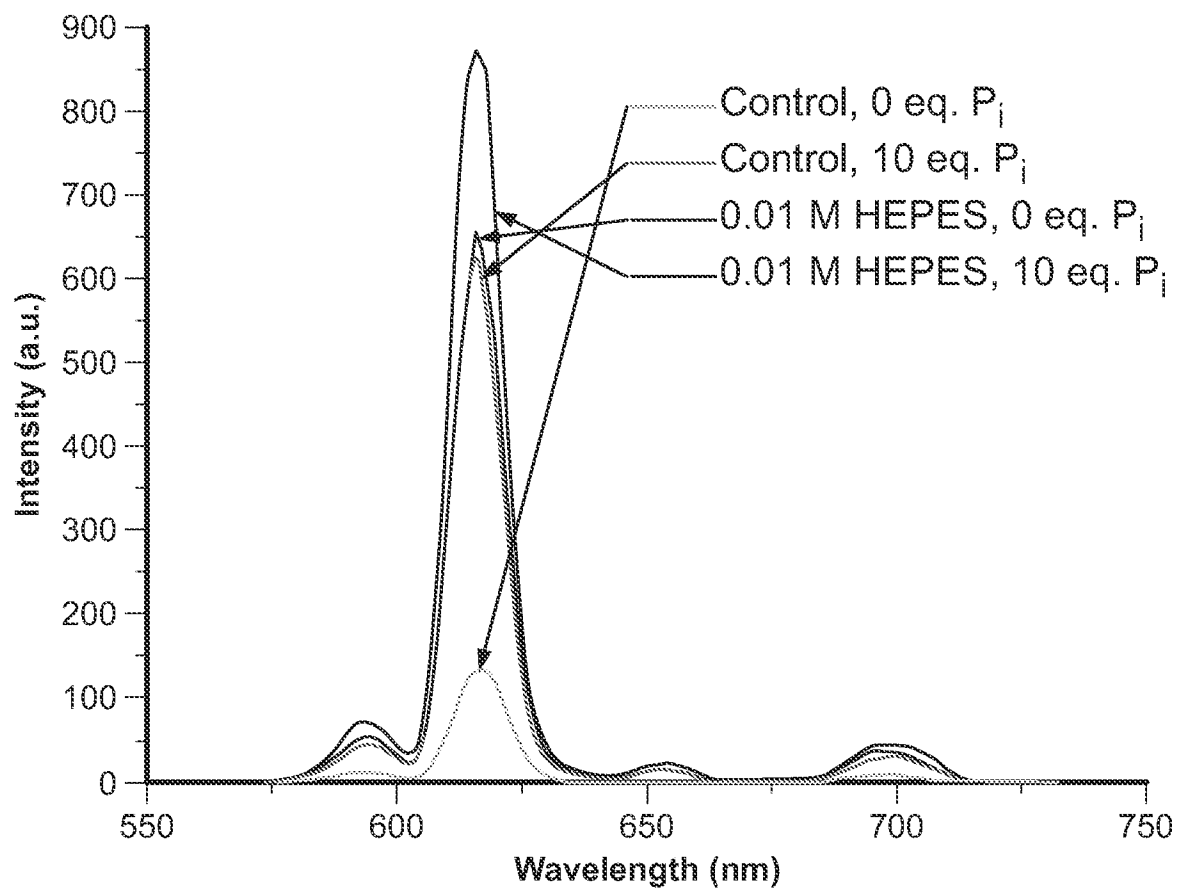
Figures 8A-8C

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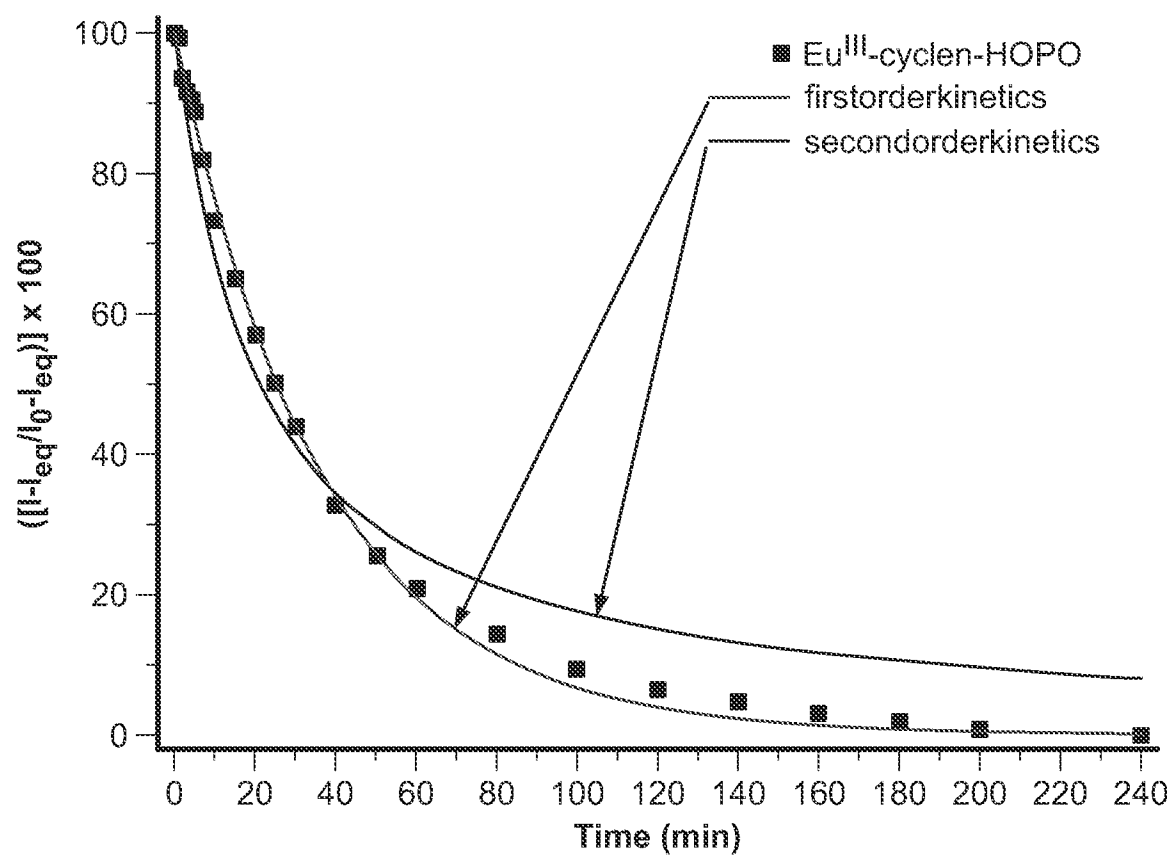
Figures 9A-9C

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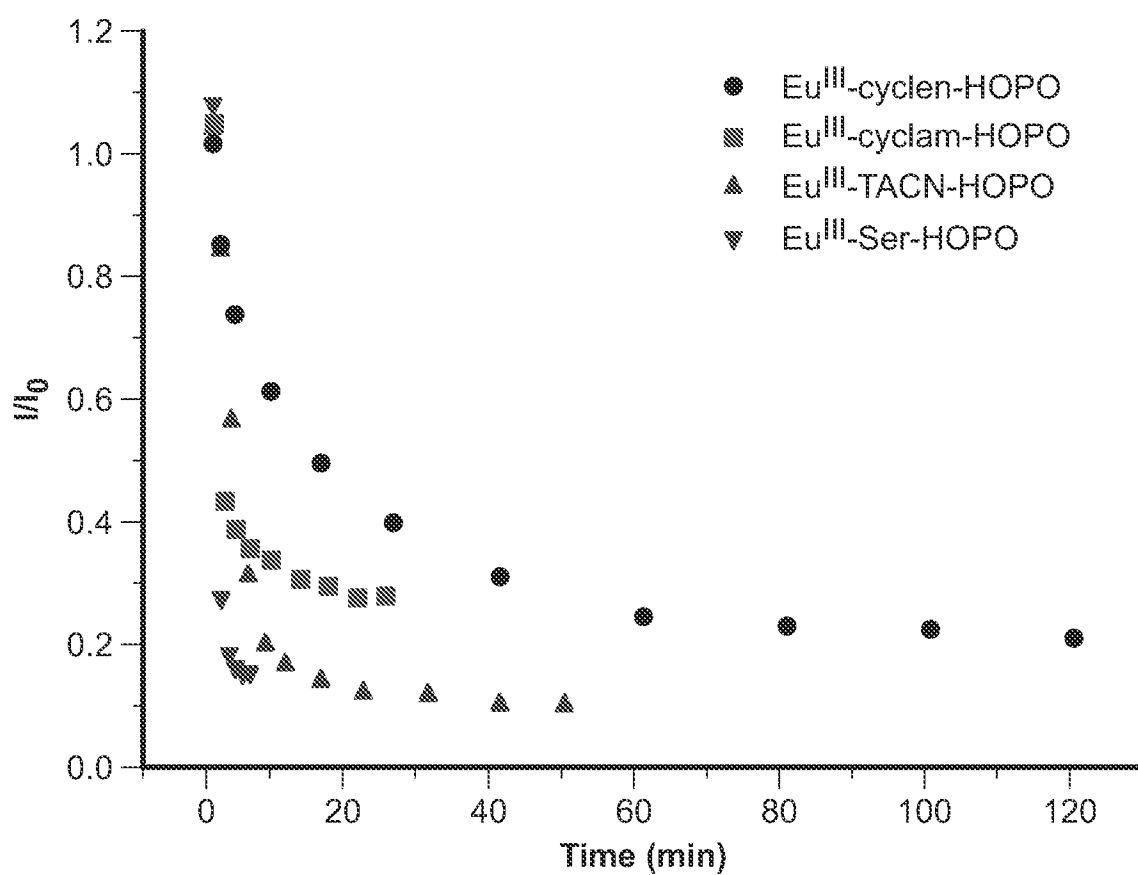
Figures 10

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Figures 11

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Figures 12

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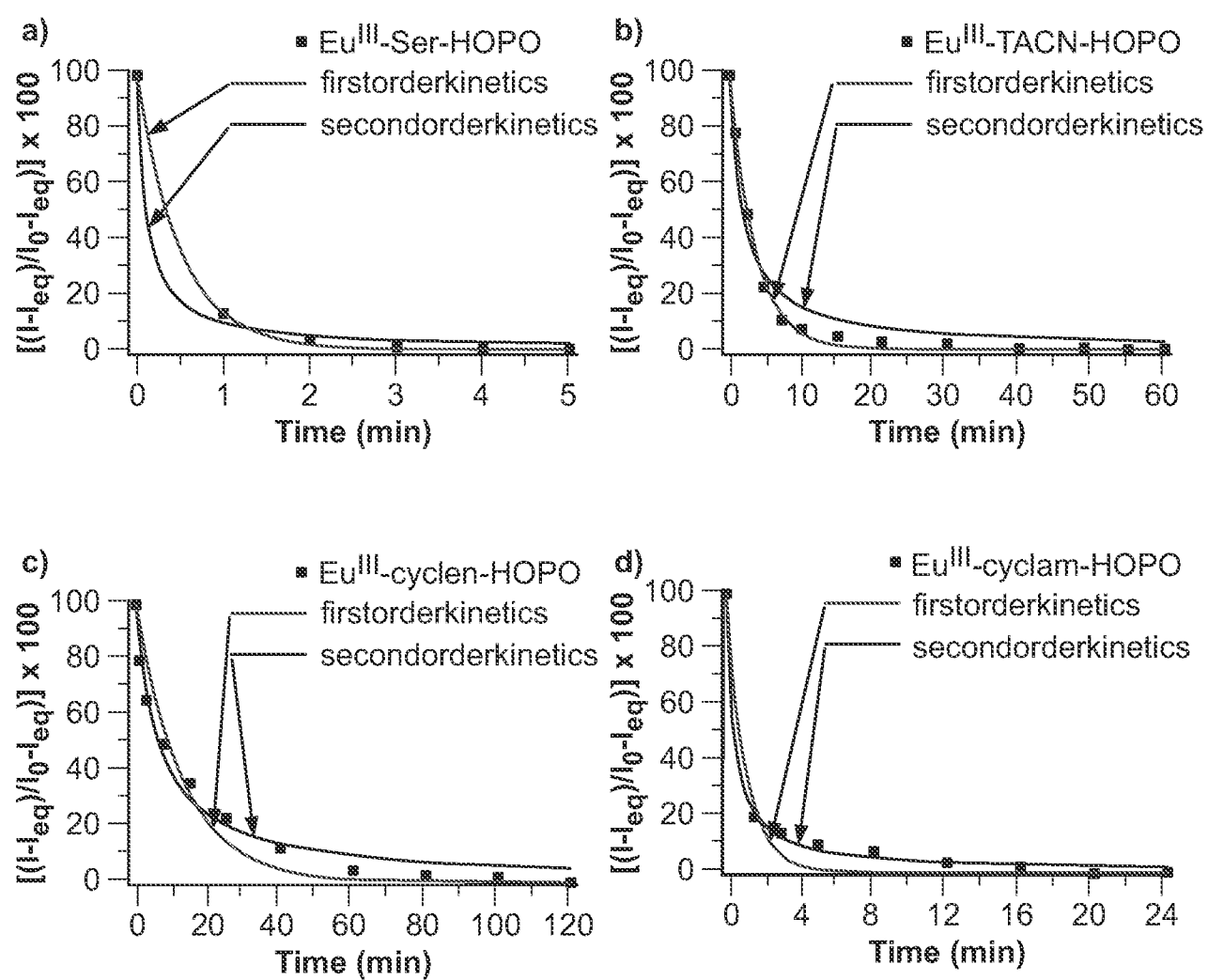


Figure 13A-13D

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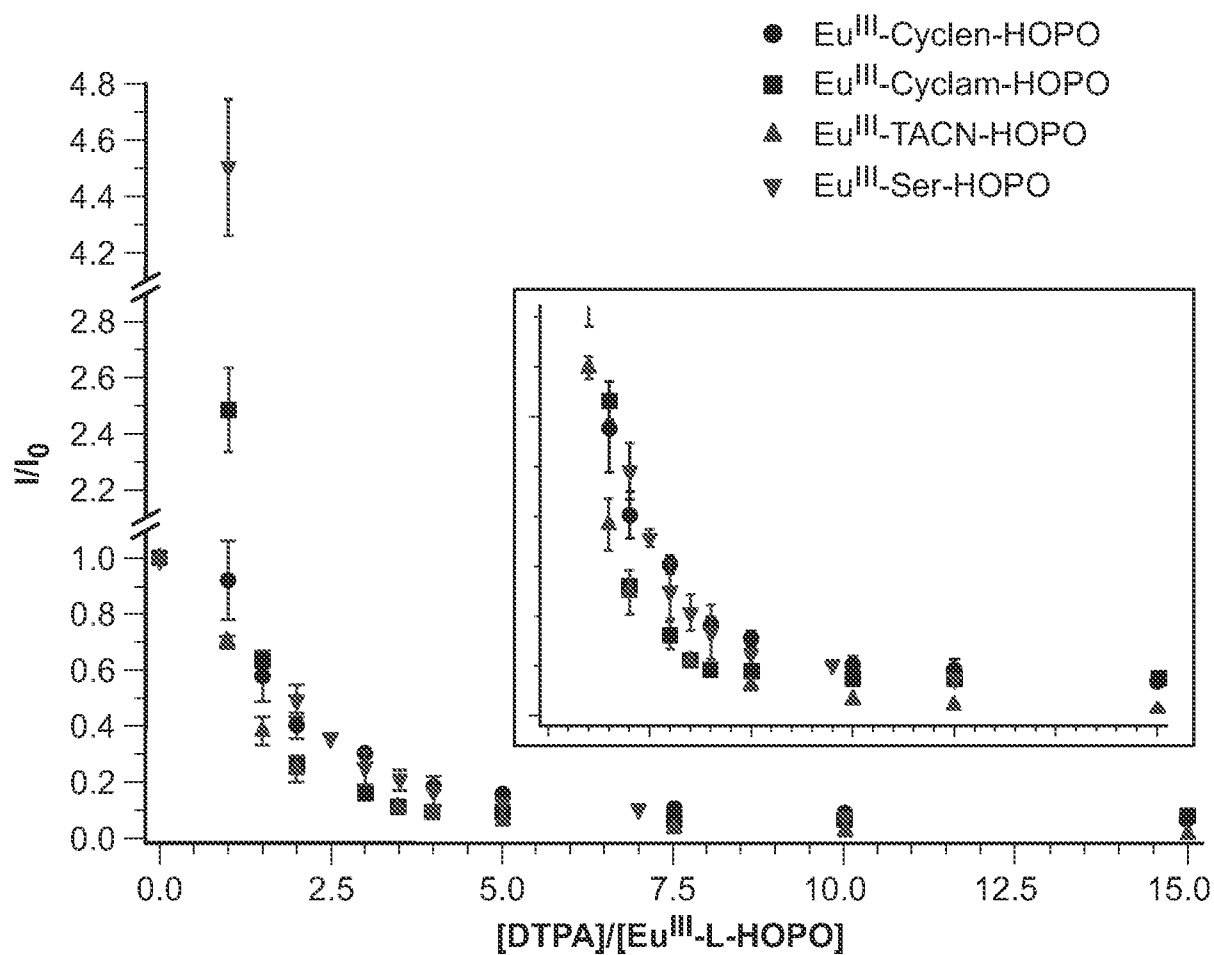


Figure 14

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US2024/033623

A. CLASSIFICATION OF SUBJECT MATTER

IPC: **C07C 229/12** (2023.01); **C07D 213/89** (2023.01); **C07C 211/65** (2023.01); **A61P 7/00** (2023.01); **C02F 1/68** (2023.01)
CPC: **C07C 229/12**; **C07D 213/89**; **C07C 211/65**; **A61P 7/00**; **C02F 1/683**; **C02F 2101/105**

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

See Search History Document

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

See Search History Document

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

See Search History Document

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 2018/0273406 A1 (REGENTS OF THE UNIVERSITY OF MINNESOTA) 27 September 2018 (27.09.2018) entire document	1, 4
A	US 5,741,912 A (RAMALINGAM et al.) 21 April 1998 (21.04.1998) entire document	1, 4
A	WO 2018/097871 A2 (THE REGENTS OF THE UNIVERSITY OF CALIFORNIA et al.) 31 May 2018 (31.05.2018) entire document	1, 4



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents:

“A” document defining the general state of the art which is not considered to be of particular relevance
“D” document cited by the applicant in the international application
“E” earlier application or patent but published on or after the international filing date
“L” document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
“O” document referring to an oral disclosure, use, exhibition or other means
“P” document published prior to the international filing date but later than the priority date claimed

“T” later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

“X” document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

“Y” document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

“&” document member of the same patent family

Date of the actual completion of the international search

17 August 2024 (17.08.2024)

Date of mailing of the international search report

27 September 2024 (27.09.2024)

Name and mailing address of the ISA/US

**Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450, Alexandria, VA 22313-1450**

Facsimile No. **571-273-8300**

Authorized officer

**MATOS
TAINA**

Telephone No. **571-272-4300**

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: **5-9, 15-29**
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined, the appropriate additional examination fees need to be paid.

Group I+: claims 1-4 and 10-14 are drawn to ligands of formula I or a salt thereof, or rare earth metal complexes of the ligand of formula I or a salt thereof.

The first invention of Group I+ is restricted to a ligand of formula I or salt thereof wherein X is $-(CH_2)_2-$; Y1 is $-(CH_2)_2-$; Y2 is $-(CH_2)-$; p is absent; q is absent; and wherein the compound of formula I is unsubstituted with one or more linking groups. The first named invention has been selected based on the guidance set forth in section 10.54 of the PCT International Search and Preliminary Examination Guidelines. Specifically, the first named invention was selected based on the first listed element for each of the variables presented in the claims (X, Y1, Y2, p, q – claim 1). It is believed that claims 1 and 4 read on this first named invention and thus these claims will be searched without fee to the extent that they read on the above embodiment.

Applicant is invited to elect additional formula(e) for each additional compound to be searched in a specific combination by paying an additional fee for each set of election. Each additional elected formula(e) requires the selection of a single definition for each compound variable. An exemplary election would be a ligand of formula I or salt thereof wherein X is $-(CH_2)_3-$; Y1 is $-(CH_2)_2-$; Y2 is $-(CH_2)-$; p is absent; q is absent; and wherein the compound of formula I is unsubstituted with one or more linking groups. Additional formula(e) will be searched upon the payment of additional fees. Applicants must specify the claims that read on any additional elected inventions. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the “+” group(s) will result in only the first claimed invention to be searched/examined.

The inventions listed in Groups I+ do not relate to a single general inventive concept under PCT Rule 13.1, because under PCT Rule 13.2 they lack the same or corresponding special technical features for the following reasons:

The Groups I+ formulae do not share a significant structural element requiring the selection of alternatives for the compound variables, X, Y1, Y2, p, q, and accordingly these groups lack unity a priori.

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

Additionally, even if Groups I+ were considered to share the technical features of a ligand having the core structure of formula I or a salt thereof, or a rare earth metal complex having the core structure of formula I or a salt thereof, these shared technical features do not represent a contribution over the prior art as disclosed by US 2018/0273406 A1 to Regents of the University of Minnesota (hereinafter, "Minnesota").

Minnesota teaches a ligand having the core structure of formula I or a salt thereof, or a rare earth metal complex having the core structure of formula I or a salt thereof (Para. [0005], a ligand or a rare earth metal complex of the ligand ... wherein the ligand is selected from the group consisting of the shown compounds and combinations thereof, wherein R is selected from the group consisting of ... 1-hydroxy-2-pyridinone).

The inventions listed in Groups I+ therefore lack unity under Rule 13 because they do not share a same or corresponding special technical feature.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **1, 4**

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.