



(51) International Patent Classification:

A61K 49/06 (2006.01) A61K 51/04 (2006.01)
A61K 49/04 (2006.01) A61K 51/00 (2006.01)

(21) International Application Number:

PCT/US20 11/042061

(22) International Filing Date:

27 June 2011 (27.06.2011)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/359,333 28 June 2010 (28.06.2010) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK,

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(54) Title: AGENTS AND COMPOUNDS FOR IMAGING AND OTHER APPLICATIONS, AND METHODS OF USE AND SYNTHESIS THEREOF

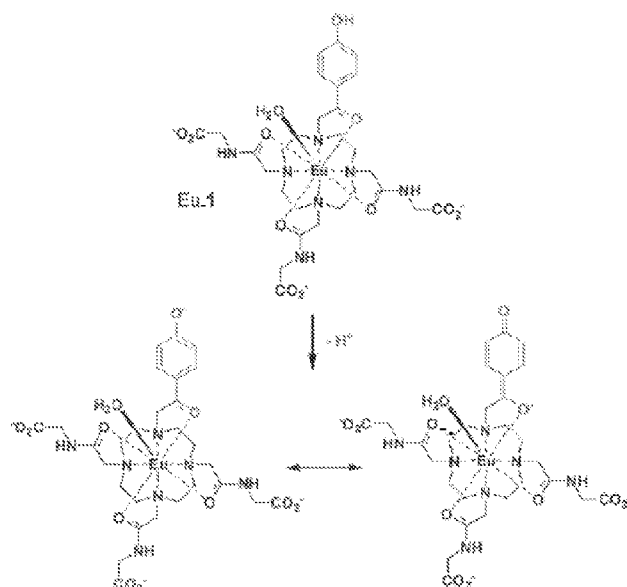


Figure 1

(57) Abstract: According to an illustrative embodiment, provided herein are paramagnetic chemical exchange saturation transfer MRI contrast agents having a tetraazacyclododecane ligand and a paramagnetic ion that provides a ratiometric imaging measurement that can be used to measure biological parameters including pH, temperature, reactive oxygen species, and specific enzymes, among others. The agents and compounds may also be usable in other applications. Methods of use and synthesis of the agents and compounds herein are also provided.

**AGENTS AND COMPOUNDS FOR IMAGING AND OTHER APPLICATIONS, AND
METHODS OF USE AND SYNTHESIS THEREOF**

TECHNICAL FIELD

5 **[0001]** The illustrative embodiments relate in general to the field of imaging agents and other agents, and specifically, to paramagnetic chemical exchange saturation transfer mechanism based magnetic resonance imaging (MRI) contrast agents.

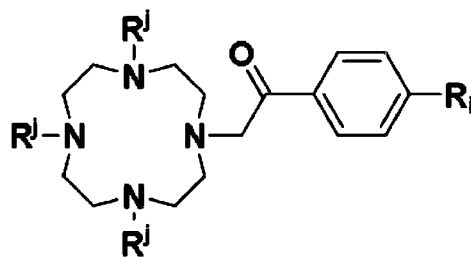
BACKGROUND

10 **[0002]** This invention was made with United States Government support under Contract No. 1R01EB004582 awarded by the National Institutes of Health. The United States Government has certain rights in this invention.

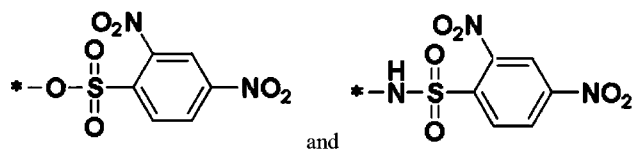
15 **[0003]** MRI is one of the most widely used, noninvasive imaging modalities in clinical medicine. An MRI system is a machine that may use magnetic field and pulses of radio wave energy to generate images of tissue and structures inside the body. A powerful magnetic field may be applied to the body to cause the hydrogen atoms in the body to become aligned with the direction of the magnetic field. Radio waves may then be briefly transmitted at the body to cause precession of protons within the patient based on the magnetic field conditions. Turning off the radio frequency energy may result in energy being released from the movement of the protons, which generates a signal that can be recorded by a computer. One reason for the popularity of MRI in clinical medicine is that image contrast arises from inherent differences in water proton densities and relaxation rates between various tissue components. For example, tissue that has the least hydrogen atoms (such as bones) may appear dark, while tissue that has many hydrogen atoms (such as fatty tissue) may appear much brighter. Even with these natural contrast differences between tissues, exogenous contrast agents that alter proton relaxation times may be used to enhance contrast between various tissue compartments. Current imaging agents
25 may have properties that render them less useful for imaging or determining certain parameters, such as a pH level, a presence of a highly reactive oxygen species, a biological metal ion concentration, an oxygen concentration, or a presence of enzyme activity, among others, or for performing other chemical or biological functions or applications.

SUMMARY

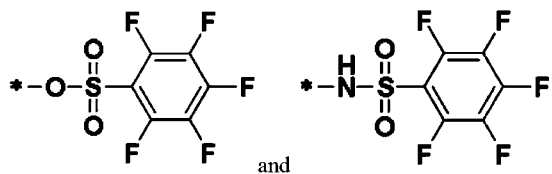
[0004] In one embodiment, a composition of matter includes a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including the formula:



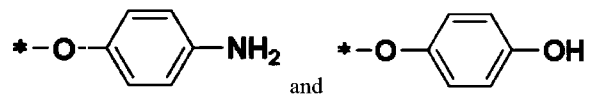
- 5 In this embodiment, **R_j** is selected from the group consisting of **R_i**, **R₂**, **R₃**, **R₄**, **R₅**, **R_e**, **R₇**, and **R_Λ**, **R₁** is selected from the group consisting of **OR'**, **O₂R'**, **SR'**, and **SOR'**, **R₂** is selected from the group consisting of **NHR'**, **CO₂R'**, **S₀₃(R')₂**, and **P₀₃(R')₂**, **R₃** is selected from the group consisting of **NO₂** and **C=O**, **R₄** is selected from the group consisting of:



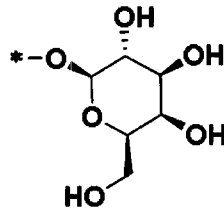
- 10 **R₅** is selected from the group consisting of:



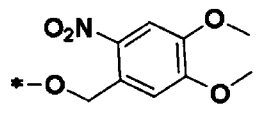
R₆ is selected from the group consisting of:



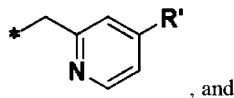
R₇ includes:



R_8 includes:

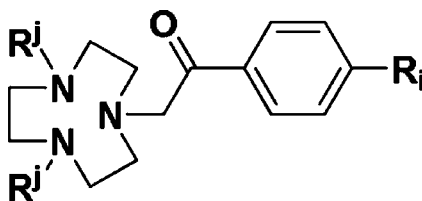


each R^j is selected from the group consisting of R^1 , R^2 , R^3 , R^4 , R^5 , and R^6 , and R^1 includes
 5 $CR'H-CONH-(CH_2)_n-CO_2-R'$. n is an integer, and $0 < n < 20$. R^2 includes $CR'H-CONH-(CH_2)_n-$
 $PO-(OR')_2$, R^3 includes $CR'H-COCH_2R'$, R^4 includes $CR'H-PO(OR')-(CH_2)_n-CO_2-R'$, R^5
 includes $CR'H-PO(OR')-R'$, R^6 includes:

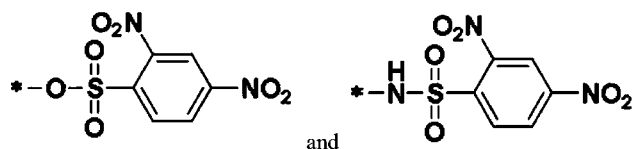


R' is selected from the group consisting of H, an alkyl group having 20 carbon atoms or less, a
 10 cycloalkyl group having 20 carbon atoms or less, and an alkyloxy group having 20 carbon atoms
 or less and 10 oxygen atoms or less.

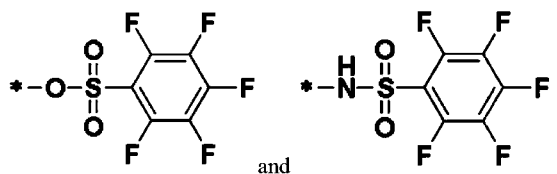
[0005] In another embodiment, a composition of matter includes a paramagnetic chemical
 exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including the
 formula:



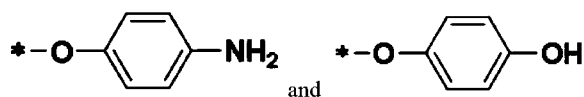
15 In this embodiment, R_i is selected from the group consisting of R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R^A ,
 R_1 is selected from the group consisting of OR' , O_2R' , SR' , and SOR' , R_2 is selected from the
 group consisting of NHR' , CO_2R' , $SO_3(R')_2$, and $PO_3(R')_2$, R_3 is selected from the group
 consisting of NO_2 and $C=O$, R_4 is selected from the group consisting of:



R₅ is selected from the group consisting of:

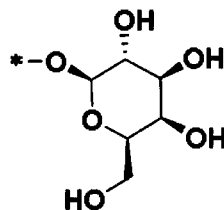


R₆ is selected from the group consisting of:

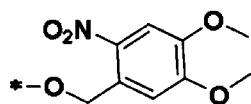


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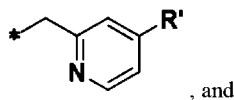
R₇ includes:



R₈ includes:

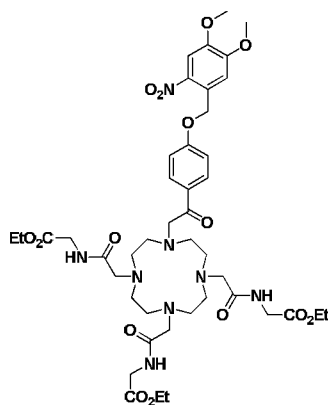


- 10 each R^j is selected from the group consisting of R¹, R², R³, R⁴, R⁵, and R⁶, and R¹ includes CR'H-CONH-(CH₂)_n-CO₂-R'. n is an integer, and 0 < n < 20. R² includes CR'H-CONH-(CH₂)_n-PO-(OR')₂, R³ includes CR'H-COCH₂R', R⁴ includes CR'H-PO(OR')-(CH₂)_n-CO₂-R', R⁵ includes CR'H-PO(OR')-R', R⁶ includes:

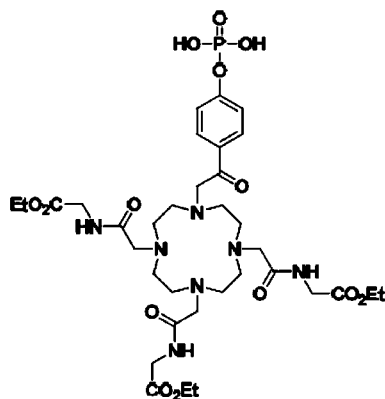


- 15 R' is selected from the group consisting of H, an alkyl group having 20 carbon atoms or less, a cycloalkyl group having 20 carbon atoms or less, and an alkyloxy group having 20 carbon atoms or less and 10 oxygen atoms or less.

[0006] In another embodiment, a composition of matter includes a light-sensitive contrast agent usable as a composition for a drug delivery system including the formula:

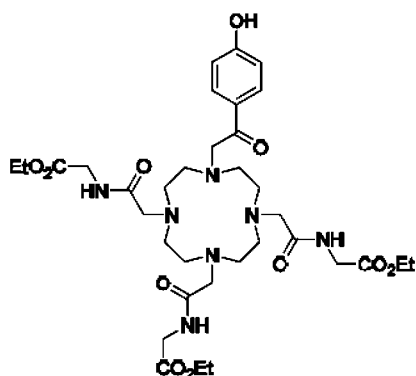


[0007] In one embodiment, a method of synthesizing a first compound having the formula:



5

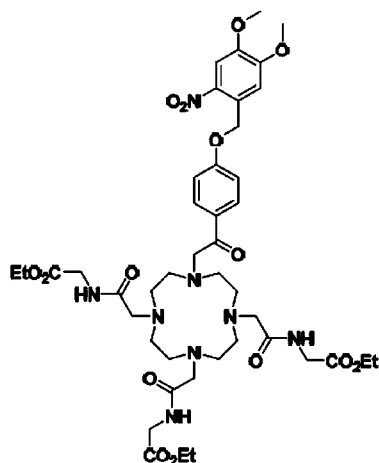
includes providing a second compound having the formula:



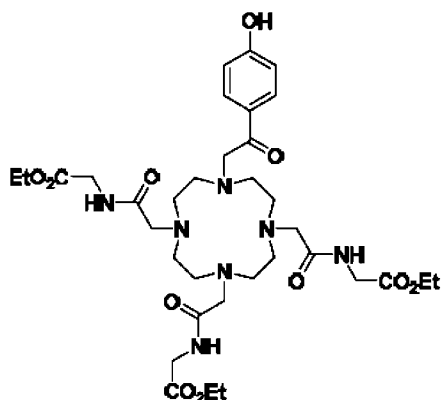
providing phosphoryl chloride, providing triethylamine, adding the second compound, the phosphoryl chloride, and the triethylamine to CH_2Cl_2 to form a solution, stirring the solution for a predetermined period of time, and extracting the solution with water. The water may form a

5 water layer. The method also includes evaporating the water layer to form a solid, and subjecting the solid to high-performance liquid chromatography to form a product containing the first compound.

[0008] In one embodiment, a method of synthesizing a first compound having the formula:

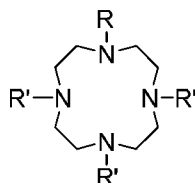


10 includes providing a second compound having the formula:



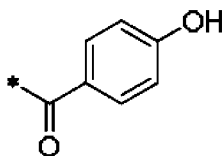
providing 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene, providing K_2CO_3 , adding the second compound, the 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene, and the K_2CO_3 to CH_3CN to form a mixture, stirring the mixture under N_2 for a predetermined period of time, filtering and evaporating the mixture until at least partially dried to form a crude compound, and purifying the crude compound using flash column chromatography on silica to form a solid containing the first compound.

[0009] In one embodiment, a composition of matter includes a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including the formula:



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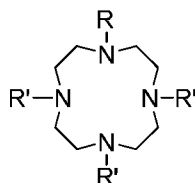
R may include:



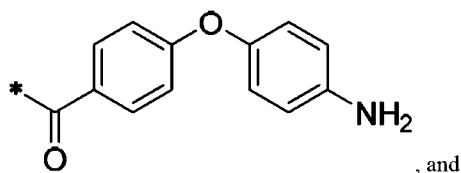
, and

each R' may be selected from the group consisting of $CH_2CONHCH_2COOH$,
 $CH_2CONHCH_2COOC_2H_5$, CH_2CONH_2 , $CH_2CONHCH_2PO(OC_2H_5)_2$, $CH_2CONHCH_2PO_3H_2$,
 $CH_2CONHCH_2PO(OC(CH_3)_2)_2$, $CH_2CONHCH_2PO(OCH_2CH_2CH_3)_2$,
 $CH_2CONHCH_2PO(OCH_2CH_2CH_2CH_3)_2$, and $CH_2CONHCH_2PO(OC(CH_3)_3)_2$.

[0010] In one embodiment, a composition of matter includes a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including the formula:

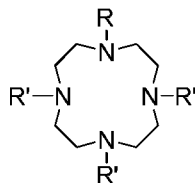


5 R may include:

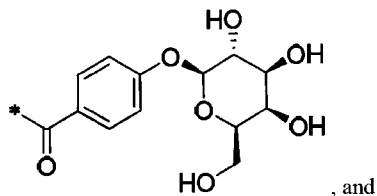


each R' may be selected from the group consisting of CH₂CONHCH₂COOH, CH₂CONHCH₂COOC₂H₅, CH₂CONH₂, CH₂CONHCH₂PO(OC₂H₅)₂, CH₂CONHCH₂P(O)₃H₂, CH₂CONHCH₂PO(OC(CH₃)₂)₂, CH₂CONHCH₂PO(OCH₂CH₂CH₃)₂,
 10 CH₂CONHCH₂PO(OCH₂CH₂CH₂CH₃)₂, and CH₂CONHCH₂PO(OC(CH₃)₃)₂.

[0011] In one embodiment, a composition of matter includes a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including the formula:



15 R may include:



each R' may be selected from the group consisting of CH₂CONHCH₂COOH, CH₂CONHCH₂COOC₂H₅, CH₂CONH₂, CH₂CONHCH₂PO(OC₂H₅)₂, CH₂CONHCH₂P(O)₃H₂, CH₂CONHCH₂PO(OC(CH₃)₂)₂, CH₂CONHCH₂PO(OCH₂CH₂CH₃)₂, and CH₂CONHCH₂PO(OC(CH₃)₃)₂.

CH₂CONHCH₂PO(OC(CH₃)₂)₂, CH₂CONHCH₂PO(OCH₂CH₂CH₃)₂,
 CH₂CONHCH₂PO(OCH₂CH₂CH₂CH₃)₂, and CH₂CONHCH₂PO(OC(CH₃)₃)₂.

[0012] In one embodiment, a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent for determining a chemical parameter including a
 5 europium(III) DOTA-tris(amide) complex includes four side chains, and one of the four side chains connects an aromatic group by a carbonyl bond.

[0013] In one embodiment, a method for determining one or more parameters includes obtaining a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including a europium(III) DOTA-tris(amide) complex including four side chains.
 10 One of the four side chains connects an aromatic group by a carbonyl bond. The paramagnetic chemical exchange saturation transfer MRI contrast agent is adapted to provide a ratiometric imaging measurement. The method also includes administering the paramagnetic chemical exchange saturation transfer MRI contrast agent to a patient, and detecting a signal in the patient that correlates to one or more parameters. The one or more parameters includes at least one of a
 15 pH level, a presence of a highly reactive oxygen species, a biological metal ion concentration, an oxygen concentration, a presence of enzyme activity, a temperature, a metabolite concentration, or an O₂ partial pressure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0014] Figure 1 illustrates a structure of the pH-responsive PARACEST agent, Eu-1 according to an illustrative embodiment;

[0015] Figure 2 illustrates the UV-vis spectra of Eu-1 recorded as a function of solution pH according to an illustrative embodiment;

[0016] Figure 3 is plot illustrating pH dependence of CEST spectra for Eu-1 (10 mM) recorded at 9.4 T and 298 K according to an illustrative embodiment;

[0017] Figure 4 illustrates CEST images of phantoms water (w) or 10 mM containing either water or Eu-1 adjusted to the indicated pH (9.4 T, 298 K) according to an illustrative embodiment;

[0018] Figure 5 illustrates a synthesis scheme for the preparation of Al according to an illustrative embodiment;

[0019] Figure 6 illustrates the proton equilibrium in Eu(III)-Al complex according to an illustrative embodiment;

[0020] Figure 7 is a plot illustrating pH dependence of CEST (scatter) and CEST fitting (line) spectra for Eu(III)-Al (30 mM) recorded at 9.4 T and 298 K according to an illustrative embodiment;

[0021] Figure 8 is a plot illustrating pH dependence of CEST peak position for Eu(III)-Al according to an illustrative embodiment;

[0022] Figure 9 is a plot illustrating pH dependence of CEST spectra for Eu(III)-Al (10 mM) recorded at 9.4 T and 298 K over the pH range 6.0 to 7.6 according to an illustrative embodiment;

[0023] Figure 10 is a plot illustrating pH dependence of the ratiometric plot by exploitation of the ratio of CEST intensity at 55 ppm to 50 ppm according to an illustrative embodiment;

[0024] Figure 11 illustrates the synthesis scheme for the preparation of Bl according to an illustrative embodiment;

[0025] Figure 12 illustrates the reaction of Eu(III)-Bl with hROS producing Eu(III)-Al according to an illustrative embodiment;

[0026] Figure 13 is a plot illustrating NaOCl concentration dependence of CEST spectra for Eu(III)-Bl (10 mM) recorded at 9.4 T and 298 K in 10 mM HEPES buffer according to an illustrative embodiment;

[0027] Figure 14 is a plot illustrating NaOCl concentration dependence of the ratiometric plot for Eu(III)-Bl by exploitation the ratio of CEST intensity at 54 ppm to 49 ppm according to an illustrative embodiment;

[0028] Figure 15 is a plot illustrating the CEST ratiometric responses (54 ppm / 49 ppm) of Eu(III)-B1 (10 mM) in the presence of 50 mM various hROS according to an illustrative embodiment;

[0029] Figure 16 illustrates a synthesis scheme for the preparation of CI according to an illustrative embodiment;

[0030] Figure 17 illustrates β -galactosidase catalyzed hydrolysis of Eu(III)-Cl producing Eu(III)-Al according to an illustrative embodiment;

[0031] Figure 18 is a plot illustrating time dependence of CEST spectra for Eu(III)-Cl (10 mM) in presence of 66 U β -galactosidase recorded at 9.4 T and 298 K in 10 mM Tris buffer according to an illustrative embodiment;

[0032] Figure 19 is a plot illustrating the time dependent ratiometric response (54 ppm / 48 ppm) of Eu(III)-Cl in presence of 66 U β -galactosidase recorded at 9.4 T and 298 K in 10 mM Tris buffer according to an illustrative embodiment;

[0033] Figures 20 and 21 illustrate chemical structures for Eu(III)-Al and Eu(III)-B1 and general structure transformation in response to the analytes according to an illustrative embodiment;

[0034] Figure 22 is a schematic of the preparation of a ligand according to an illustrative embodiment;

[0035] Figure 23 illustrates the $^1\text{H-NMR}$ spectra for Eu(III)-Al complex (20 mM) recorded in D_2O at 289K with a pD of (a) 5.0 and (b) 8.9 according to an illustrative embodiment;

[0036] Figure 24 is a graph of pD dependence of chemical shift of aromatic proton according to an illustrative embodiment;

[0037] Figures 25A and 25B are plots of pH dependence of UV-vis spectra for (a) Eu(III)-Al and (b) free ligand Al (20 μM) recorded in aqueous solution according to an illustrative embodiment;

[0038] Figure 26 is a plot of a normalized titration curve showing the increase in absorbance at 360 nm for Eu(III)-Al ($\blacksquare$) and at 340 nm for free ligand Al (**A**) as a function of increasing pH according to an illustrative embodiment;

[0039] Figure 27 is a schematic of the proton equilibrium of free ligand Al and resonance representation of the deprotonated Eu(III)-Al complexes according to an illustrative embodiment;

[0040] Figure 28 is a plot of the luminescent emission spectrum of Eu(III)-Al (20 μM) recorded in aqueous solution ($\lambda_{\text{ex}} = 310 \text{ nm}$) at pH 3.3 according to an illustrative embodiment;

[0041] Figure 29 is a plot of the pH dependence of luminescence intensity changes at 580 nm for Eu(III)-Al (20 uM) recorded in aqueous solution ($\lambda_{\text{ex}} = 310$ nm) according to an illustrative embodiment;

5 [0042] Figure 30 is an image of a Jablonski diagram of the Eu(III)-Al according to an illustrative embodiment showing that the proximity of the triplet energy level of the quinone group causes substantial back energy transfer;

[0043] Figure 31 is plot of pH dependence of CEST spectra for Eu(III)-Al recorded at 9.4 T and 310 K according to an illustrative embodiment, and Figure 32 is a corresponding pH ratiometric plot for Eu(III)-Al by exploitation the ratio of CEST intensity at 55 ppm to 46 ppm;

10 [0044] Figure 33 shows the UV-vis spectral changes of Eu(III)-Bl as a function of OCT concentration according to an illustrative embodiment;

[0045] Figures 34A and 34B are images of NaOCl concentration dependence of CEST spectra for Eu(III)-Bl recorded at 9.4 T and 298 K in 10 mM HEPES buffer and NaOCl concentration dependence of the ratiometric plot for Eu(III)-Bl by exploitation the ratio of CEST intensity at 54 ppm to 49 ppm according to an illustrative embodiment;

15 [0046] Figure 35 is a pH dependence of CEST ratiometric image according to an illustrative embodiment; and

[0047] Figure 36 shows a decomposition of Eu(III)-r to form Eu(III)-I by ROS according to an illustrative embodiment.

20

DETAILED DESCRIPTION

[0048] In the following detailed description of the illustrative embodiments, reference is made to the accompanying drawings that form a part hereof. These embodiments are described in sufficient detail to enable those skilled in the art to practice the embodiments, and it is understood that other embodiments may be utilized and that logical structural, mechanical, electrical, and chemical changes may be made without departing from the spirit or scope of the embodiments. To avoid detail not necessary to enable those skilled in the art to practice the embodiments described herein, the description may omit certain information known to those skilled in the art. The following detailed description is, therefore, not to be taken in a limiting sense, and the scope of the illustrative embodiments are defined only by the appended claims.

[0049] To facilitate the understanding of the illustrative embodiments, a number of terms are defined below. Terms defined herein have meanings as commonly understood by a person of ordinary skill in the areas relevant to the illustrative embodiments. Terms such as "a", "an" and "the" are not intended to refer to only a singular entity, but include the general class of which a specific example may be used for illustration. The terminology herein is used to describe specific embodiments of the disclosure, but their usage does not delimit the disclosure, except as outlined in the claims.

[0050] Magnetic resonance imaging (MRI) is one of the most versatile diagnostic tools for exploitation of intrinsic tissue and structural differences. The specificity and sensitivity of MRI may be further enhanced by the use of paramagnetic complexes or magnetic particles to shorten the water relaxation times (T_1 , T_2 , or T_2^*). Although Gd^{3+} -based T_1 -shortening contrast agents are widely used in clinical exams, an approach called chemical exchange saturation transfer (CEST) has been used to generate image contrast by taking advantage of slow-to-intermediate exchange conditions ($k_{ex} \leq \Delta\omega$) between the agent labile proton pool and bulk water pool. By presaturation of the agent labile proton pool, the saturated nucleus may spin then exchange with the larger bulk water proton pool, which results in a reduction in the total water signal detected by the MRI.

[0051] Paramagnetic versions of CEST agents, or PARACEST agents, may be used instead of Gd^{3+} -based imaging agents. Image contrast produced by a PARACEST agent may be switched "on" or "off" by application of frequency-selective radio frequency pulses. This feature may allow potential multiplexing of agents in a single study. Also, since contrast in these systems may be based on chemical exchange of either labile protons or water molecules, the agents may be extremely sensitive to exchange rates (k_{ex}). In particular, PARACEST agents may have exchangeable protons that are shifted well away from the bulk water resonance and this may be

desireable over diamagnetic CEST agents. The sensitivity to exchange rates may facilitate development of concentration-independent agents that respond to biological or physiological events (biologically responsive sensors). PARACEST sensors using a variety of design platforms may be utilized for measuring pH, temperature, Zn^{2+} , glucose, nitric oxide, phosphate esters, enzyme activity, and other parameters.

5 [0052] Since the CEST contrast may be highly sensitive and dependent on the exchanging rate (k_{ex}) of mobile protons, modulation of k_{ex} is normally used in the development of PARACEST sensor for detection of a variety of biological species such as glucose, lactate, nitric oxide, phosphate ester, and enzyme. PARACEST sensors may have a CEST signal that changes
10 intensity in response to external stimuli. This may require a separate measure of agent concentration (ratiometric imaging measurements) to obtain quantitative results. Some exceptions may include agents that use either a cocktail of agents or single agents having multiple weakly shifted -NH exchangeable protons for ratiometric imaging. The latter design feature may rely on exchange sites that are relatively close to the bulk water frequency (e.g., <15
15 ppm). Responsive PARACEST contrast agents may lack the capacity for ratiometric imaging measurements, which limits their potential use in vivo because the agent concentration must also be known.

[0053] The illustrative embodiments may provide a solution to the problems above, among others, by presenting, in one embodiment, a novel europium(III) DOTA-monoketonetris(amide)
20 complex comprising a single highly shifted water exchange peak whose frequency varies as a function of solution pH. Based on the observation that the CEST resonance frequency shifts in response to pH and that CEST ratiometric imaging may be used for taking direct measurements of pH, aspects of the illustrative embodiments may be converted to a general platform for imaging a variety of other biological parameters. Thus, the illustrative embodiments describe a
25 particular new class of molecules that act as sensors of biological and physiological parameters such as tissue acidity (pH), temperature, reactive oxygen species (ROS), and the presence of particular enzymes, and which may also be used in other clinical applications, including, but not limited to, drug delivery. The agents in the illustrative embodiments may provide a direct quantitative readout of a parameter of interest using a standard clinical imaging scanner. The
30 agents of the illustrative embodiments may be used clinically for diagnosis of a variety of diseases including cancer, diabetes, and heart disease, among others.

[0054] The Eu^{3+} -based PARACEST agents described in the illustrative embodiments may provide a concentration independent measure of pH by ratiometric CEST imaging. Thus, the agent may be used to measure pH by use of ratiometric CEST imaging without the need of a

second concentration marker as required with some existing PARACEST agents. With the Eu^{3+} -based PARACEST agent, ratiometric image data may be collected at CEST activation frequencies widely separated from the bulk water frequency, and the acid dissociation constant (pK_a) of the sensor may be suitable or nearly ideal for imaging pH over a range of interest for

5 detecting abnormal physiology.

[0055] In some cases, previously reported Eu^{3+} -based PARACEST agents may have a highly shifted water exchange peak that is independent of pH between 5 and 8. However, it has been observed that the chemical shift of the Eu^{3+} -bound water exchange peak may be altered considerably by varying the electron density on a single amide oxygen donor in these DOTA-tetraamide systems. This observation may be expanded to multifrequency PARACEST agents. The illustrative embodiments present a new type of complex, **Eu-1**, wherein one of the amide side chains may be replaced by a ketone oxygen donor that may be directly conjugated to an ionizable group, in this case a phenol unit. A non-limiting example structure of a pH-responsive PARACEST agent, **Eu-1**, as described in an illustrative embodiment, is shown in Figure 1. In

10 the illustrative embodiments, deprotonation (i.e., removal) of the phenolic proton may result in conjugation of the resulting quinone-like structure with the acetyl oxygen atom coordinated to the Eu^{3+} ion, as shown in the bottom of Figure 1.

[0056] Figure 2 illustrates the UV-vis spectra of **Eu-1** ($20\ \mu\text{M}$) recorded as a function of solution pH according to an illustrative embodiment. The arrows in Figure 2 indicate the direction of the absorbance changes with increasing pH in one embodiment, and the figure insert illustrates an example of the titration curve showing the increase in absorbance at 360 nm as a function of pH. The absorption spectrum of **Eu-1** in Figure 2 displays a bathochromic shift from 310 to 360 nm as the phenolic proton is removed, consistent with extended delocalization of the phenolate anion through the π system to form the quinone-like structure. This shift may place a more

25 negative charge on the carbonyl oxygen atom coordinated to the Eu^{3+} ion shown in the bottom of Figure 1. The pK_a derived from these optical data (6.7 ± 0.1) may be suitable or nearly ideal for pH measurements in biological systems. One may predict *a priori* that, in one embodiment, such a resonance structure may also alter the water exchange rate and potentially the frequency of the exchanging water molecule.

[0057] Figure 3 illustrates the pH dependence of CEST spectra for **Eu-1** recorded at 9.4 T and 298 K according to an illustrative embodiment. The figure insert provides an expanded view of the water exchange peak as a function of pH. ($[\text{Eu}^{3+}] = 10\ \text{mM}$, $B_1 = 14.1\ \mu\text{T}$, and saturation time = 2 s.) The CEST spectra of **Eu-1** recorded at five different pH values in Figure 3 show a change in exchange frequency from pH 6.0 to 7.6. The pK_a of **Eu-1** derived from the CEST data

may be 6.5 ± 0.1 , which may be nearly identical to the value determined optically. One example CEST feature of having an unusually large change in exchange frequency suggests it may be possible to image pH directly using **Eu-1** and ratiometric CEST imaging. For example, the ratio of CEST intensities after activation of **Eu-1** at 55 versus 49 ppm may be nearly linear between

5 pH 6.0 and 7.6 and independent of **Eu-1** concentration.

[0058] Other combinations of activation frequencies may give similar results. **Eu-1** may offer several advantages over previously reported ratiometric pH indicators. For example, the pH measurement may be made using a single reagent rather than a cocktail of agents. By way of further example, the exchange peak in **Eu-1** may be shifted well away from the frequency of

10 solvent protons, so the **Eu-1** agent may be activated without concern about partial off-resonance saturation of bulk water protons.

[0059] Figure 4 illustrates various non-limiting example images of a phantom containing either water (w) or 10 mM **Eu-1** adjusted to the indicated pH (9.4 T, 298 K), wherein image (a) shows proton density images, image (b) shows ratio water intensities after activation at 54 versus 47

15 ppm, and image (c) shows calculated pH values as determined by ratiometric CEST imaging in one embodiment. To demonstrate the simplicity of using this **Eu-1** agent for imaging pH by MRI, CEST images of a phantom prepared from five **Eu-1** samples adjusted to different pH values (plus a control sample lacking **Eu-1**) are collected at two presaturation frequencies, 54 and 47 ppm. The CEST intensity ratio in these two images according to one embodiment is

20 shown in Figure 4(b) as a color map. The sample containing water alone may show nearly perfect cancellation, while the CEST ratio in samples of **Eu-1** may vary from 0.43 (sample at pH 6.0) to 2.32 (sample at pH 7.6). Using these ratios and a calibration curve, the pH values derived from the CEST images as illustrated in Figure 4(c) may match those values measured by use of a pH electrode as illustrated in Figure 4(a).

25 **[0060]** The bound water lifetimes (τ_M) of the protonated and deprotonated **Eu-1** species may be determined by fitting CEST spectra recorded at pH 5.0 and 8.0, respectively, to the Bloch equations. This fitting procedure may give τ_M values of 239 μ s at pH 5.0 and 120 μ s at pH 8.0. This result may be considered to be consistent with the expected increase in water exchange rate as the acetyl oxygen donor atom gains a more negative charge at the higher pH value. The

30 width of the water exchange peak in **Eu-1** may broaden somewhat at high pH values, again consistent with faster water exchange. Water exchange in **Eu-1** may be even faster at 310 K as expected (τ_M may be found to be 123 and 45 μ s for the protonated versus deprotonated species at these same two pH values) while the frequency shifts in the bound water exchange peak may be

similar to those found at 298 K. This indicates that **Eu-1** may also be effective for ratiometric CEST imaging of pH at physiological temperatures as well.

[0061] Eu(III)-Al may be used to image pH by MRI using ratiometric CEST principles. Deprotonation of a single phenolic proton between pH 6 and 7.6 may result in an ~5 ppm shift in the water exchange CEST peak which may be detected by MRI. In one embodiment, ratiometric imaging may be achieved by collecting two CEST images at two slightly different activation frequencies providing a direct readout of solution pH without the need of a concentration marker.

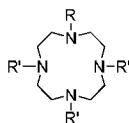
[0062] The use of PARACEST contrast agents may facilitate the development of various kinds of responsive agents. A structurally novel complex Eu(III)-Al, according to an illustrative embodiment, may be used to image pH by using ratiometric CEST principles. Deprotonation of a phenolic proton may result in an approximate 5 ppm downfield shift in the water exchange CEST resonance frequency over pH 6.0 ~ 7.6. In one example, this shift may enable the elimination of the need of a concentration marker required in the regular ratiometric CEST imaging by following two CEST images of single bound water CEST resonance frequency at two slightly different activation frequencies. Meanwhile, Eu(III)-Al may emit phenol-sensitized luminescence. The luminescence may be switched "off" with the light-promoted deprotonation of the phenolic proton, which may open up the possibility of pH imaging by luminescence. Furthermore, the Eu(III)-Al may be used as a basic platform in the development of other types of responsive imaging agents as well. This may be demonstrated in Eu(III)-B 1, which may be built by replacing the phenolic proton in Eu(III)-Al with *para*-aminophenyl group. Eu(III)-B 1 may irreversibly be degraded by some reactive oxygen species (ROS) such as OCl⁻ and ·OH to Eu(III)-Al. Still, downfield shift in the bound water CEST peak accompanying this structural transformation may render the ratiometric measure of hROS concentration without considering the use of concentration marker.

[0063] Thus, in an illustrative embodiment, PARACEST-based MRI contrast agents for *ex vivo*, *in vitro*, or *in vivo* determination of chemical parameters of diagnostic interest such as pH, highly reactive oxygen species (hROS), biological metal ion concentrations, or enzyme activity, among others, may be created. Deprotonation or cleavage of a specific chemical bond in the agent may result in the downfield shift of CEST resonance frequency which may be used by ratiometric imaging of the above-mentioned parameters by MRI. The PARACEST-based MRI contrast agents described in the illustrative embodiments may have a tetraazacyclododecane ligand and a paramagnetic ion that provides a ratiometric imaging measurement.

[0064] In some examples, CEST effects may be made independently of the absolute concentration of the contrast agent by using a ratiometric method. The ratiometric method may be applied to systems with two different pools of mobile protons, either present in the same molecule or provided by two different contrast agents, which may be activated sequentially and selectively. Like the ratiometric method, a shift in the water proton resonance frequency (PRF) method may also be independent of agent concentration. The PRF method may be used for MRI thermometry owing, at least in part, to the strong temperature dependence of the chemical shift of bound water in Eu(III)-DOTA-tetraamide complexes.

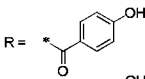
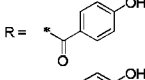
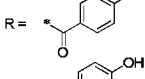
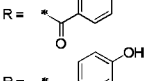
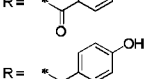
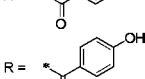
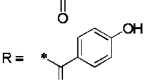
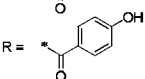
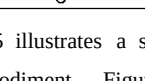
[0065] It may be demonstrated that the bound water resonance in some Eu(III)-DOTA-tetraamide complexes may vary considerably due to the variation of local coordination environment by the introduction of different amide side-chains, from which multi-frequency PARACEST contrast agents may be developed. In one example, PRF-based ratiometric sensors may be built in which the coordination environment changes are strong enough to shift the bound water resonance frequency of Eu(III)-DOTA-amide complexes by external stimuli.

[0066] The illustrative embodiments may include a pH responsive PARACEST agent that includes a tetraazacyclododecane ligand that may have a general formula:



wherein non-limiting examples of pendent arms R and R' are illustrated in TABLE 1.

[0067] TABLE 1. Chemical structure of ligand A according to an illustrative embodiment:

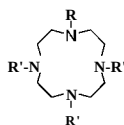
$R =$ 	$R' =$ $^*-\text{CH}_2\text{CONHCH}_2\text{COOH}$	Ligand A1
$R =$ 	$R' =$ $^*-\text{CH}_2\text{CONHCH}_2\text{COOC}_2\text{H}_5$	Ligand A2
$R =$ 	$R' =$ $^*-\text{CH}_2\text{CONH}_2$	Ligand A3
$R =$ 	$R' =$ $^*-\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OC}_2\text{H}_5)_2$	Ligand A4
$R =$ 	$R' =$ $^*-\text{CH}_2\text{CONHCH}_2\text{PO}_3\text{H}_2$	Ligand A5
$R =$ 	$R' =$ $^*-\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OC}(\text{CH}_3)_2)_2$	Ligand A6
$R =$ 	$R' =$ $^*-\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OCH}_2\text{CH}_2\text{CH}_3)_2$	Ligand A7
$R =$ 	$R' =$ $^*-\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2$	Ligand A8
$R =$ 	$R' =$ $^*-\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OC}(\text{CH}_3)_2)_2$	Ligand A9

[0068] Figure 5 illustrates a synthesis scheme for the preparation of A1 according to one illustrative embodiment. Figure 6 illustrates the proton equilibrium in Eu(III)-A1 complex according to one illustrative embodiment. Figure 7 is a plot illustrating pH dependence of CEST (scatter) and CEST fitting (line) spectra for Eu(III)-A1 recorded at 9.4 T and 298 K according to one illustrative embodiment (e.g., $[\text{Eu}^{3+}] = 30 \text{ mM}$, $B_1 = 14.1 \text{ } \mu\text{T}$, sat. time = 2 s). Figure 8 is a plot illustrating pH dependence of CEST peak position for Eu(III)-A1 according to one illustrative embodiment. Figure 9 is a plot illustrating pH dependence of CEST spectra for Eu(III)-A1 recorded at 9.4 T and 298 K over the pH range 6.0 to 7.6 according to one illustrative embodiment (e.g., $[\text{Eu}^{3+}] = 10 \text{ mM}$, $B_1 = 14.1 \text{ } \mu\text{T}$, sat. time = 2 s). Figure 10 is a plot illustrating pH dependence of the ratiometric plot by exploitation of the ratio of CEST intensity at 55 ppm to 50 ppm according to one illustrative embodiment.

[0069] For example, UV-vis pH titration results may demonstrate the proton equilibrium for the Eu(III)-A1 as shown in Figure 6. The acidity of phenol moiety may be greatly enhanced due, at least in part, to the extensive electron conjugation of phenolate by forming a quinone-like resonance structure for the deprotonated form of Eu(III)-A1. Eu(III) may experience a much stronger ligand field due to an increased electron donation to the metal center upon the phenol deprotonation, which in turn may result in not only a weakening of the metal-water coordination and accelerating water exchange, but also an increase in dipolar NMR shift of complex and

downfield shift of the CEST resonance frequency. The pH dependence of CEST profiles for Eu(III)-A1 may confirm the above expectation (Figure 7). The CEST profiles may show pH dependence of not only changes in CEST intensity (1-M/Mo) but also in CEST resonance frequency. The pH dependence of CEST resonance frequency (Figure 8) may correlate with the ligand field of complex. Specifically, the CEST exchange frequency may shift from 50 ppm at pH 4.5 to 55 ppm at pH 8.4, a shift that is large enough for a ratiometric determination of pH.

[0070] Another embodiment of PARACEST agents that are responsive to the highly reactive oxygen species (hROS) may be built on the pH responsive agent platform. The embodiment of hROS agents comprising a tetraazacyclododecane ligand may have a general formula as follows:



wherein non-limiting examples of pendent arms R and R' are illustrated in TABLE 2.

[0071] TABLE 2. Chemical structure of ligand B according to an illustrative embodiment:

R =	R' = $^*-CH_2CONHCH_2COOH$	Ligand B1
R =	R' = $^*-CH_2CONHCH_2COOC_2H_5$	Ligand B2
R =	R' = $^*-CH_2CONH_2$	Ligand B3
R =	R' = $^*-CH_2CONHCH_2PO(OC_2H_5)_2$	Ligand B4
R =	R' = $^*-CH_2CONHCH_2PO_3H_2$	Ligand B5
R =	R' = $^*-CH_2CONHCH_2PO(OC(CH_3)_2)_2$	Ligand B6
R =	R' = $^*-CH_2CONHCH_2PO(OCH_2CH_2CH_3)_2$	Ligand B7
R =	R' = $^*-CH_2CONHCH_2PO(OCH_2CH_2CH_2CH_3)_2$	Ligand B8
R =	R' = $^*-CH_2CONHCH_2PO(OC(CH_3)_3)_2$	Ligand B9

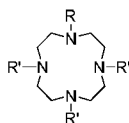
[0072] Figure 11 illustrates a synthesis scheme for the preparation of B1 according to an illustrative embodiment. Figure 12 illustrates a reaction of Eu(III)-B1 with hROS producing Eu(III)-A1 according to an illustrative embodiment. Figure 13 is a plot illustrating NaOCl

concentration dependence of CEST spectra for Eu(III)-Bl recorded at 9.4 T and 298 K in 10 mM HEPES buffer according to an illustrative embodiment (e.g., $[\text{Eu}^{3+}] = 10 \text{ mM}$, $\text{pH} = 7.5$, $B_1 = 14.1 \text{ } \mu\text{T}$, sat. time = 2 s). Figure 14 is a plot illustrating NaOCl concentration dependence of the ratiometric plot for Eu(III)-Bl by exploitation of the ratio of CEST intensity at 54 ppm to 49 ppm according to an illustrative embodiment. Figure 15 is a plot illustrating CEST ratiometric responses (54 ppm /49 ppm) of Eu(III)-Bl (10 mM) in the presence of 50 mM various hROS according to an illustrative embodiment.

[0073] Highly reactive oxygen species (hROS) such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), and hydroxyl radical ($\text{HO}\cdot$) may be mediators for the pathological conditions of various diseases. Colorimetric, chemiluminescent, or fluorescence-based assays may be used to measure cell-derived hROS. Various fluorescence hROS sensors may be used to meet the different assay requirements and a variety of design mechanisms such as the formation of endoperoxide, deprotection, or O-dearylated may be employed to create the new sensors.

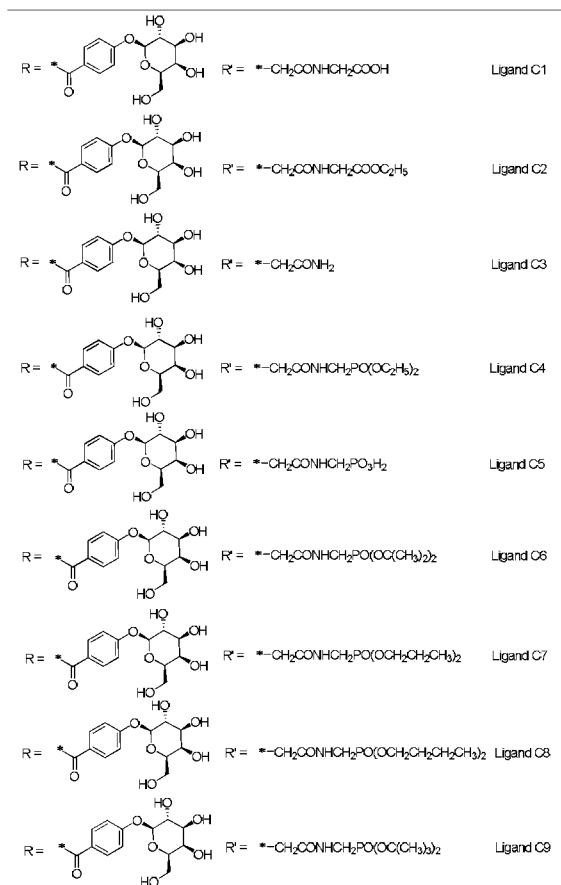
[0074] MRI may have high spatial resolution and the ability to extract, simultaneously, physiological and anatomical information of soft tissue. An example of the hROS responsive PARACEST agent present in the illustrative embodiments is shown in Figure 12. The agent may be O-dearylated upon reaction with hROS to yield a deprotonated pH responsive agent. This may enhance the ligand field experienced by the Eu(III) metal center, which in turn may downfield shift the CEST resonance frequency. As shown in Figure 13, the CEST peak around 49 ppm may decrease with the appearance of the new CEST peak around 54 ppm with the addition of NaOCl. Again, a ratiometric determination of hROS using CEST is possible.

[0075] Another embodiment of PARACEST agents in the illustrative embodiments are β -galactosidase responsive agents, which may be designed on the pH responsive agent platform. The embodiment of β -galactosidase responsive agents comprising a tetraazacyclododecane ligand may have a general formula as follows:



wherein non-limiting examples of pendent arms R and R' are illustrated in TABLE 3.

[0076] TABLE 3. Chemical structure of ligand C according to an illustrative embodiment:



[0077] Figure 16 illustrates a synthesis scheme for the preparation of CI according to an illustrative embodiment. Figure 17 illustrates a β -galactosidase catalyzed hydrolysis of Eu(III)-Cl producing Eu(III)-Al according to an illustrative embodiment. Figure 18 is a plot illustrating time dependence of CEST spectra for Eu(III)-Cl in the presence of 66 U β -galactosidase recorded at 9.4 T and 298 K in 10 mM Tris buffer according to an illustrative embodiment (e.g., $[\text{Eu}^{3+}] = 10 \text{ mM}$, $\text{pH} = 7.4$, $B_1 = 14.1 \text{ } \mu\text{T}$, sat. time = 2 s.).

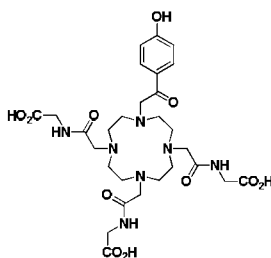
[0078] The transformation of a large amount of the enzyme responsive PARACEST agents may be realized through multiple enzyme-catalyzed cycles. Therefore, in one embodiment, PARACEST detection of enzyme activity may be possible even at very low enzyme concentrations.

[0079] The structure of a β -galactosidase responsive agent is shown in Figure 17 according to an illustrative embodiment. The β -D-galactopyranoside-phenyl linkage may be hydrolyzed by the

enzyme; β -galactosidase may yield the pH responsive agent; β -galactosidase catalysis removal of yff-D-galactopyranoside may produce the pH responsive agent. As shown in Figure 18, in the absence of β -galactosidase, the CEST spectrum for Eu(III)-Cl may exhibit a typical profile that may be characteristic of the regular complexes. But, in the presence of β -galactosidase, the CEST profile may change with a little decrease of the CEST resonance frequency at 48 ppm and the appearance of a shoulder in the 52-55 ppm range over time. The ratiometric CEST intensity ratio (54 ppm / 49 ppm) may also experience a change from the initial value of 0.18 to 0.35 after four hours of reaction time in the presence of the enzyme (Figure 19). Thus, the β -galactosidase concentration and β -galactosidase catalyzed kinetics may be evaluated by using the Eu(III)-Cl.

- 10 **[0080]** Non-limiting examples of a contrast agent prepared in accordance with the illustrative embodiments are presented below for illustrative purposes and do not limit the scope of the claimed embodiments.

[0081] Example preparation of N,N',N''-[[10-[2-(4-hydroxyphenyl)-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]tris(1-oxo-2,1-ethanediyl)]tris-glycine (A1).



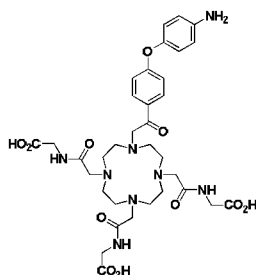
- 15 **[0082]** Ligand A1 may be prepared according to Figure 5 in one embodiment. 1-(4-(benzyloxy)phenyl)ethanone (6) and N,N',N''-[1,4,7,10-tetraazacyclododecane-1,4,7-triyl]tris(1-oxo-2,1-ethanediyl)]tris-glycine, 1,1',1''-triethyl ester (4) may be synthesized by, e.g., established procedures.

- 20 **[0083]** 2-bromo-1-[4-(phenylmethoxy)phenyl]-ethanone (5). To a solution of compound (6) (4.5 g, 20 mmol) in dichloromethane/MeOH (250 mL/100 mL) may be added $(C_4H_9)_4N^+Br_3^-$ (10.1 g, 21 mmol). The mixtures may be stirred at room temperature until a decoloration of the orange solution. The solvent may then be removed under vacuum. The resulting solid may be dissolved in water and extracted with ether (3x50 mL). The ether layer may be dried with Na_2SO_4 and evaporated under reduced pressure to give a raw product. The pure product may be obtained as colorless needles after recrystallization from hexane/ethyl acetate (4.5g, 75%). (e.g., Mp = 82-84 °C. 1H NMR (400 MHz, $CDCl_3$), δ 4.40 (2H, s, $BrCH_2$), 5.16 (2H, s, $PhCH_2$), 7.05 (2H, d, $^3J_{HH} = 8.0$ Hz, Ph), 7.37-7.46 (5H, m, Ph), 7.98 (2H, d, $^3J_{HH} = 8.0$ Hz, Ph)).

- [0084]** N,N',N''-[[10-[2-[4-(benzyloxy)phenyl]-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]tris(l-oxo-2,l-ethanediy)]tris-glycine, I,Γ,Γ'-triethyl ester (3). Compound (4) (5.5 g, 9.1 mmol), compound (5) (3.0 g, 9.9 mmol) and NaHC(3/4) (0.86 g, 10 mmol) may be added in acetonitrile (150 mL) and heated at 65 °C under N₂ for 24 hours with stirring. The reaction mixture may be cooled to room temperature and filtered. The filtrate may be evaporated to give a foam-like solid. The crude compound may be purified by flash column chromatography on silica, eluting first with CH₂Cl₂ and then with CftCt/methanol (95:5 v/v), to afford a pale yellow solid (6.8 g, 90%). (e.g., Mp = 110-112 °C. R_f = 0.85 (15% MeOH in chloroform, neutral A₁₀). 3/4 NMR (400 MHz, CDCl₃), 1.06 (6H, s br, C_H₃), 1.22 (3H, t, ³J_{H-H} = 8.0 Hz, C_H₃), 1.80-2.30 (16H, br, ring C_H₂N), 3.32 (6H, s br, NCH₂C=O), 3.84 (2H, s br, NCH₂C=O), 3.91 (8H, s br, NHCH₂CO₂ and OCH₂CH₃), 4.10 (4H, m, OCH₂CH₃), 5.09 (2H, s, PhCH₂), 6.95 (2H, d, ³J_{H-H} = 8.0 Hz, Ph), 7.32-7.41 (5H, m, Ph), 7.88 (2H, d, ³J_{H-H} = 8.0 Hz, Ph), 8.1 (3H, s, NH)).
- [0085]** N,N',N''-[[10-[2-(4-hydroxyphenyl)-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]tris(l-oxo-2,l-ethanediy)]tris-glycine, I,Γ,Γ'-triethyl ester (2). Compound 3 (4.15 g, 5 mmol) may be dissolved in ethanol (50 mL), and 10% palladium on carbon (0.3 g) may be added. The reaction mixture may then be shaken on a Parr hydrogenator under a 3/4 pressure of 40 psi at room temperature for 12 h. The reaction may be filtered, and the solvents may be removed under reduced pressure to afford a colorless solid (3.50 g, 95%). (e.g., R_f = 0.70 (15% MeOH in chloroform, neutral A₁₀). 3/4 NMR (400 MHz, D₂O), 1.10 (6H, t, ³J_{H-H} = 8.0 Hz, CH₃), 1.19 (3H, t, ³J_{H-H} = 8.0 Hz, CH₃), 3.0-3.5 (16H, br, ring CH₂N), 3.77 (6H, s br, NCH₂C=O), 3.84 (2H, s br, NCH₂C=O), 3.93 (6H, s br, NHCH₂O), 4.12 (6H, m, OCH₂CH₃), 6.91 (2H, d, ³J_{H-H} = 8.0 Hz, Ph), 7.79 (2H, d, ³J_{H-H} = 8.0 Hz, Ph)).
- [0086]** N,N',N''-[[10-[2-(4-hydroxyphenyl)-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]tris(l-oxo-2,l-ethanediy)]tris-glycine (1). Compound 2 (0.185 g, 0.25 mmol) may be added into a mixture of LiOH (0.2 M, 5.8 mL) and THF (5 mL), and stirred at 0 °C for 2 hours, and then may be stirred at room temperature. The saponification reaction may be monitored by NMR. Upon the disappearance of ethyl ester groups, THF may be evaporated under reduced pressure. The resulting solution may be adjusted to pH 2.0 by addition of HCl and lyophilized to dryness giving the title compound as a pale yellow solid, which may be purified by preparative HPLC to give pure ligand A1 as a white hygroscopic powder (136 mg, 83%). (e.g., 3/4 NMR (400 MHz, D₂O, pD = 10.0), 2.61 (4H, s br, ring CH₂N), 2.67 (4H, s br, ring CH₂N), 2.90 (4H, s br, NCH₂C=O), 3.15-3.20 (12H, m br, NCH₂C=O, ring CH₂N), 3.53 (4H, s br,

NHCH₂C=O), 3.71 (2H, s br, NHCH₂C=O), 6.46 (2H, d, $\nu_{\text{H-H}} = 8.0$ Hz, Ph), 7.65 (2H, d, $\nu_{\text{H-H}} = 8.0$ Hz, Ph)).

[0087] Example preparation of N,N',N''-[[10-[2-[4-(4-aminophenoxy)phenyl]-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]tris(1-oxo-2,1-ethanediy)]tris-glycine (B1).



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[0088] Ligand B1 may be prepared according to Figure 11 according to one illustrative embodiment. 1-(4-(4-nitrophenoxy)phenyl)ethanone (7) may be synthesized by, e.g., established procedures.

[0089] 2-bromo-1-(4-(4-nitrophenoxy)phenyl)ethanone (8). To a solution of compound (7) (6.1 g, 24 mmol) in dichloromethane/MeOH (250 mL/100 mL) may be added (C₄H₉)N⁺Br₃⁻ (12.0 g, 25 mmol). The mixtures may be stirred at room temperature until a decoloration of the orange solution. The solvent may then be removed under vacuum. The resulting solid may be dissolved in water and extracted with chloroform (3x50 mL). The organic layer may be dried with Na₂SO₄ and evaporated under reduced pressure to give a raw product. The pure product may be obtained as yellow needles after recrystallization from hexane/ethyl acetate (6.8g, 85%). (e.g., Mp = 91-93 °C. ¹H NMR (400 MHz, CDCl₃), δ 4.44 (2H, s, BrCH₂), 7.13-7.17 (2H, m, Ph), 8.07 (2H, d, $\nu_{\text{H-H}} = 8.0$ Hz, Ph), 8.27 (2H, d, $\nu_{\text{H-H}} = 8.0$ Hz, Ph)).

[0090] N,N',N''-[[10-[2-[4-(4-nitrophenoxy)phenyl]-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]tris(1-oxo-2,1-ethanediy)]tris-glycine, I, Γ , Γ' -triethyl ester (9). Compound (4) (5.3 g, 8.8 mmol), compound (8) (3.0 g, 9.0 mmol) and K₂CO₃ (2.2 g, 16 mmol) may be added in acetonitrile (150 mL) and may be stirred at room temperature under N₂ for 24 hours. The reaction mixture may then be filtered. The filtrate may be evaporated to give a foam-like solid. The crude compound may be purified by flash column chromatography on silica, eluting first with CH₂Cl₂ and then with CH₂Cl₂/methanol (95:5 v/v), to afford a yellow solid (6.4 g, 85%). (e.g., Mp = 94-96 °C. $R_f = 0.85$ (15% MeOH in chloroform, neutral A₁₀). ¹H NMR (400 MHz, CDCl₃), 1.05 (6H, s br, CH₃), 1.15 (3H, t, $\nu_{\text{H-H}} = 8.0$ Hz, CH₃), 2.30-2.90 (16H, br, ring CH₂N), 3.21-3.27 (4H, br, NCH₂C=O), 3.79-3.90 (10H, d br, NCH₂C=O and

NHCH₂CO₂), 4.04 (6H, m, OCH₂CH₃), 7.02 (4H, m, Ph), 7.84 (1H, s br, NH), 7.92 (2H, d, ³J_{H-H} = 8.0 Hz, Ph), 8.04 (2H, s br, NH), 8.16 (2H, d, ³J_{H-H} = 8.0 Hz, Ph)).

[0091] N,N',N''-[[10-[2-[4-(4-aminophenoxy)phenyl]-2-oxoethyl]-1,4,7,10-

tetraazacyclododecane-1,4,7-triyl]tris(1-oxo-2,1-ethanediyl)]tris-glycine, 1,1',1''-triethyl ester

5 (10). Compound (9) (2.5 g, 2.9 mmol) may be dissolved in ethanol (50 mL), and 10% palladium on carbon (0.1 g) may be added. The reaction mixture may then be shaken on a Parr hydrogenator under a H₂ pressure of 40 psi at room temperature for 12 h. The reaction may be filtered, and the solvents may be removed under reduced pressure to afford a colorless solid (2.25 g, 95%). (e.g., R_f = 0.75 (15% MeOH in chloroform, neutral A₁₀ 3). Mp = 119-121 °C.

10 ³/4 NMR (400 MHz, CDCl₃), 1.10 (6H, s br, CH₃), 1.23 (3H, t, ³J_{H-H} = 8.0 Hz, CH₃), 2.20-2.90 (16H, br, ring CH₂N), 3.26-3.31 (4H, br, NCH₂C=O), 3.85-3.92 (10H, d br, NCH₂C=O and NHCH₂CO₂), 4.08 (6H, m, OCH₂CH₃), 6.70 (2H, d, ³J_{H-H} = 8.0 Hz, Ph), 6.81 (2H, d, ³J_{H-H} = 8.0 Hz, Ph), 6.86 (2H, d, ³J_{H-H} = 8.0 Hz, Ph), 7.84 (2H, d, ³J_{H-H} = 8.0 Hz, Ph), 7.90 (1H, m br, NH), 8.03 (3H, m br, NH)).

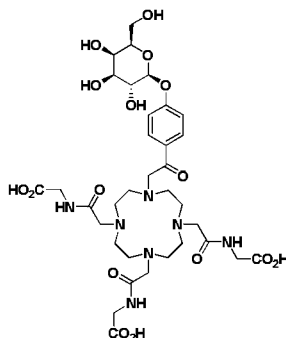
15 **[0092]** N,N',N''-[[10-[2-[4-(4-aminophenoxy)phenyl]-2-oxoethyl]-1,4,7,10-

tetraazacyclododecane-1,4,7-triyl]tris(1-oxo-2,1-ethanediyl)]tris-glycine (BI). Compound (2)

(0.5 g, 0.6 mmol) may be added into a mixture of LiOH (0.2 M, 14 mL) and THF (5 mL), and may be stirred at 0 °C for 2 hours, and then may be stirred at room temperature. The saponification reaction may be monitored by NMR. Upon the disappearance of ethyl ester

20 groups, THF may be evaporated under reduced pressure. The resulting solution may be adjusted to pH 2.0 by addition of HCl and lyophilized to dryness giving the title compound as pale yellow solid in quantitative yield. (e.g., ¹H NMR (400 MHz, D₂O, pD = 10.0), 2.72-2.78 (12H, d br, ring CH₂N), 2.96 (4H, s br, ring CH₂N), 3.25-3.32 (8H, d br, NCH₂C=O, ring CH₂N), 3.58 (4H, s br, NHCH₂C=O), 3.67 (2H, s br, NHCH₂C=O), 6.82 (2H, d, ³J_{H-H} = 8.0 Hz, Ph), 6.91 (2H, d, ³J_{H-H} = 8.0 Hz, Ph), 6.97 (2H, d, ³J_{H-H} = 8.0 Hz, Ph), 7.85 (2H, d, ³J_{H-H} = 8.0 Hz, Ph)).

25 **[0093]** Example preparation of N,N',N''-[[10-[2-[4-(4-D-galactopyranoside)phenyl]-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]tris(1-oxo-2,1-ethanediyl)]tris-glycine (CI).



[0094] Ligand CI may be prepared according to Figure 16 according to an illustrative embodiment. N,N',N''-[[10-[2-[4-((2',3',4',6'-tetra-O-acetyl- α -D-galactopyranoside))phenyl]-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]tris(l-oxo-2,l-ethanediyl)]tris-glycine,

- 5 I,I',I''-triethyl ester (11). Compound (2) (2.2 g, 3.0 mmol), 2,3,4,6-tetra-O-acetyl- α -D-galactopyranosyl bromide (3.6 g, 9.0 mmol) and $C_5H_5CO_3$ (3.25 g, 10 mmol) may be added in dry DMF (10 mL) and the mixture may be stirred under N_2 for 12 hours. The reaction mixture may then be filtered and evaporated to dryness. The crude compound may be purified by flash column chromatography on silica, eluting first with CH_2Cl_2 and then with CF_3CH_2OH (90:10 v/v), to afford yellow solid (1.9 g, 60%). (e.g., Mp = 128-130 °C. R_f = 0.65 (15% MeOH in chloroform, neutral Al_2O_3). 1H NMR (400 MHz, $CDCl_3$), 1.05 (6H, s br, CH_3), 1.18 (3H, t, $^3J_{H-H}$ = 8.0 Hz, CH_3), 1.95 (3H, s, CH_3), 1.99 (3H, s, CH_3), 2.02 (3H, s, CH_3), 2.11 (3H, s, CH_3), 2.20-2.80 (12H, br, ring CH_2N), 3.26-3.31 (4H, br, $NCH_2C=O$), 3.80-3.87 (10H, d br, $NCH_2C=O$ and $NHCH_2CO_2$), 4.02-4.12 (8H, m, OCH_2CH_3 and OCH_2CH), 5.06-5.10 (2H, m, CH) 5.40-5.45 (3H, m, CH), 6.94 (2H, d, $^3J_{H-H}$ = 8.0 Hz, Ph), 6.85 (2H, d, $^3J_{H-H}$ = 8.0 Hz, Ph), 7.92-8.05 (3H, m, NH)).
- 15

- [0095] N,N',N''-[[10-[2-[4-(α -D-galactopyranoside)phenyl]-2-oxoethyl]-1,4,7,10-tetraazacyclododecane-1,4,7-triyl]tris(l-oxo-2,l-ethanediyl)]tris-glycine (CI). Compound (11) (0.185 g, 0.25 mmol) may be added into a mixture solution of water (5 mL) and CH_3CN (5 mL) and may be stirred at 0 °C. The pH of the solution may be adjusted to 12 and may be maintained at this value by continuous addition of 1N NaOH until it remains constant. Then the resulting solution may be adjusted to pH 3.0 by addition of HCl and lyophilized to dryness giving the title compound as pale yellow solid in quantitative yield. (e.g., 1H NMR (400 MHz, D_2O), 2.50-3.60 (24H, m br, ring CH_2N and $NCH_2C=O$), 3.63-3.88 (11H, m, $NHCH_2C=O$, CH and $CHCFb$), 5.05 (1H, d, $^3J_{H-H}$ = 8.0 Hz, CH), 7.09 (2H, d, $^3J_{H-H}$ = 8.0 Hz, Ph), 7.79 (2H, d, $^3J_{H-H}$ = 8.0 Hz, Ph)).
- 20
- 25

[0096] Example preparation of Europium(III)-ligand complexes.

[0097] Ligand Al, B1 or C1 (0.015 mmol) may be dissolved in water (2 mL). A stock solution of europium triflate (0.014 mmol) may be added into the above solution. The mixture may be adjusted to a pH around 6.0 by careful addition of NaOH. The pH of reaction mixture may be maintained at around 6.0 and may be stirred at 70 °C for 12 hours. Then the excess of free Eu^{3+} may be checked with xylenol orange. If there is excess of free Eu^{3+} in the solution, more ligand (1 mg) may be added, the pH of solution may be tuned to 6.0, and stirring may occur for another 12 hours and the excess of free Eu^{3+} may be checked. If no free Eu^{3+} is detected, the aqueous solution may be lyophilized to give the complex as white powder. The complexes may be used without further purification.

10 [0098] NMR method. ^1H and ^{13}C NMR spectra may be recorded on, e.g., a Bruker AVANCE III 400 NMR spectrometer operating at 400.13 and 100.62 MHz, respectively. CEST spectra of complexes in pure water may also be recorded on the same spectrometer operating at 400.13 MHz. Pre-saturation pulses of 2 s duration may be applied at four saturation powers of 9.4, 14.1, 18.8, and 23.5 μT . The CEST spectra may be fitted to the Bloch equations with 3-pool
15 model by use of a nonlinear fitting algorithm written in, e.g., MATLAB[®] 7 (Mathworks Inc., Natick, MA).

[0099] pH responsive PARACEST contrast agent. Figure 7 shows the pH dependence of CEST profiles for Eu(III)-Al at 298 K according to an illustrative embodiment. The CEST profiles show pH dependence of not only the CEST intensity ($1-M_s/M_0$) changes but also CEST
20 resonance frequency changes. CEST intensities may increase from 28% at pH 2.5 with a CEST resonance frequency of 46.1 ppm to a maximum intensity of 55% at pH 4.5 with a CEST resonance frequency of 50.0 ppm. The CEST intensities may level off over the pH range of 5 to 6. Then CEST intensities may decrease gradually to 51% at pH 7.6 but with a significant CEST resonance frequency increment to 54.7 ppm. At last, the CEST intensities may decrease to 28%
25 at pH 8.4 with a CEST resonance frequency of 55.1 ppm. The corresponding ^1H at different pH, which may be determined by fitting modified Bloch equations to the CEST spectra using a non-linear fitting algorithm written in MATLAB[®], may correlate well with the CEST intensity changes. τ_m of 150 to 200 μs over the pH range of 4.5 to 7.6 may be close to the optimal exchange rate for CEST producing. At the same time, the pH dependence of CEST frequencies
30 (Figure 8) may correlate well with the ligand field of complex. In particular, a sigmoidal curve may be found by fitting the CEST frequencies over the pH range of 4.5 to 8.4, which gives a pK_a of 6.5. Therefore, more pH data points may be measured over the pH range of 6.0 to 7.0 (Figure 9). The CEST frequency may shift, from 49 ppm to 53 ppm, over this small pH range but it may still be large enough for a ratiometric determination of pH using CEST, in one non-limiting

example. A linear calibration curve may be obtained when the CEST intensity ratio 55 ppm / 50 ppm is plotted as a function of pH (Figure 10). This may indicate that this particular agent may be most effective around pH 7.0, so it may be a suitable chemical choice for physiologic pH measurements.

- 5 **[00100]** hROS responsive PARACEST contrast agent. Neutrophils are a population of circulating blood cells for defending against pathogenic microorganisms. PMA and fatty acids may activate the NADPH oxidase in neutrophils to generate $O_2^{\cdot -}$. Then $O_2^{\cdot -}$ may be converted to $\frac{3}{4} O_3$ and $\frac{3}{4} O_4$, which are thought to be precursors of more potent oxidizing agents, such as HO, OCl⁻, and $\frac{3}{4} O_3^{\cdot -}$. These highly reactive oxygen species may play key roles in killing bacteria.
- 10 **[00101]** The reactivity of Eu(III)-B1 complex with potent hROS OCl⁻ was examined in HEPES buffer with an agent concentration of 10 mM. As shown in the CEST titration spectra at 298K (Figure 13), with the addition of NaOCl up to 10 mM, which may be 1 molar equivalence relative to sensor, there may be very little changes in the CEST peaks. But with the further addition of NaOCl up to 3 molar equivalences, a significant decrease in the CEST peak around
- 15 49 ppm may be observed with the appearance of a new CEST peak around 54 ppm. The CEST peak at 49 ppm may disappear completely after the concentration of NaOCl is up to 10 molar equivalences. These spectral changes may make it possible to use the ratiometric determination of OCl⁻ using CEST. A good linear calibration curve may be obtained when the CEST intensity ratio 54 ppm / 49 ppm was plotted as a function of pH (Figure 14). Similar titrations may also
- 20 be performed on other peroxides such as H_2O_2 , $HO^{\cdot}$, $O_2^{\cdot -}$, and $ONOO^{\cdot -}$. Based on the ratiometric value (54 ppm / 49 ppm) from each titration (Figure 15), only $HO^{\cdot}$ may show considerable reactivity with agent complex, in one example. There is almost no ratiometric value change for $O_2^{\cdot -}$, H_2O_2 , and $ONOO^{\cdot -}$ titrations. OCl⁻ may be evaluated by the new PARACEST agent, in one embodiment.
- 25 **[00102]** β -galactosidase responsive PARACEST contrast agent. In one embodiment, β -galactosidase catalyzed hydrolysis of Eu(III)-Cl may be performed under conditions as follows. A small solution of β -galactosidase in Tris buffer (pH 7.4, 10 mM) may be added to a solution of the agent (500 μ L, 10 mM) in Tris buffer (pH 7.4, 10 mM) to get 66 units of β -galactosidase in solution mixtures. The mixtures may be incubated at 37 °C. The CEST of mixtures may then
- 30 be recorded at different times. Figure 18 displays the CEST spectral changes for Eu(III)-Cl in the absence and presence of 66 U β -galactosidase according to an illustrative embodiment. In the absence of β -galactosidase, the CEST resonance frequency for Eu(III)-Cl may be at 48 ppm. Although no CEST resonance frequency shift may be observed in the presence of the β -galactosidase, the shape of the CEST spectrum may change significantly over time.

Specifically, CEST intensity at 48 ppm may decrease with the appearance of a shoulder in the 52-55 ppm range, which may be considered to be consistent with the CEST resonance frequency of the expected hydrolyzed product. Still, ratiometric determination of β -galactosidase may be possible using CEST. The CEST intensity ratio (54 ppm / 49 ppm) for the complex only spectrum may be 0.18. After four hours, the value for the complex-enzyme mixtures may increase to 0.35 (Figure 19). The β -galactosidase concentration and β -galactosidase catalyzed kinetic may be evaluated by using the Eu(III)-Cl.

[00103] If a single CEST resonance frequency shifts considerably in response to the analytes, the CEST ratiometric measurement may be achieved by following the shifting of signal resonance frequency, an approach that may be analogous to those used in ratiometric optical sensors. However, in terms of CEST, there may be a lack of concrete means to shift the mobile proton resonance frequency of the agent in response to the analytes. For instance, the CEST intensity and resonance frequency arising from the coordinated water molecule of Eu(III) DOTA-tetramide complexes may be independent of pH over 5-8, in a non-limiting example. Nevertheless, the bound water CEST properties in Eu(III) DOTA-tetramide and its derivatives may be considerably modulated by varying the electron donating ability of amide side chains to central metals, from which multi-frequency PARACEST agents may be subsequently developed. Thus, identification of a system is possible whose CEST resonance frequency position may be regulated if the electronic donation properties of the ligand side chain were altered to a significant extent in response to the external stimulus.

[00104] Figure 20 is an image of the structure of the Eu(III)-A1 and Eu(III)-B1 according to an illustrative embodiment. The agent Eu(III)-A1 (Figure 20) is a complex that may show the CEST resonance frequency shifting with pH. Structurally, Eu(III)-A1 may be built on a trade-off between modulating electronic donation properties of side chain and meanwhile maintaining the slow water exchange for CEST generation by replacing only one of the glycinate side arm in Eu(III)-DOTA-(gly)₄ with acetyl-phenol unit, in one non-limiting example. The phenolate electron may be transferred to Eu-carbonyl coordination over the aromatic bridge, thereby regulating the CEST properties. Meanwhile, the optical imaging modality may be introduced to and combined with the PARACEST imaging modality by utilizing the energy transfer ability of phenol subunit. In that case, the optical imaging modality may compensate the MR imaging modality for its low sensitivity, in one example. In one embodiment, luminescence of Eu(III) complexes may be a tool for sensing biological parameters due to their unique photophysical properties such as long wavelength emission and long-lived excited states, based on which the

time-resolved luminescent measurements may be scarcely affected by shorter-lived autofluorescence present *in vivo* and interferences associated with Rayleigh scattering.

[00105] Ligand may be prepared according to the synthesis route shown in Figure 22 according to illustrative embodiments. The bromination of compound S6 and S6' with
 5 brominating agent TBA·Br₃ may give side arm S5 and S5' in good yields. Then the side arms may be alkylated with compound 4 in acetonitrile with sodium hydrogen carbonate as a base affording macrocyclic intermediate 3 and 3', respectively. The consequent hydrogenolysis step over a palladium on carbon catalyst may offer ester-contained compounds in fairly high yield. Finally, the ethyl ester groups may be hydrolyzed under basic condition giving ligand A1 and 1',
 10 which may be characterized on the basis of ¹H and ¹³C NMR, MS, and elemental analysis. Complexations may be performed in water by mixing stoichiometric amounts of Eu(triflate)₃ and ligands. The complexes may be fully formed within 12 hours at 70 °C.

[00106] Figure 23 is an image of the ¹H-NMR spectra of Eu(III)-A1 complex (20 mM) recorded in D₂O at 289K with a pD of (a) 5.0 and (b) 8.9 according to an illustrative
 15 embodiment. Resonance frequency from HDO is marked by asterisk. The ¹H-NMR spectrum of Eu(III)-A1 in D₂O may be complicated due to the lack of molecular symmetry at pD 5.0 (a).

[00107] Especially in the upfield region, resonance frequencies may be broad. Broader singlet at 7.20 ppm and sharper singlet at 6.59 ppm may be assigned as aromatic protons based on COSY spectral analysis, meaning the dipolar NMR chemical shift effects from metal center
 20 on aromatic protons may be fairly small. In aqueous solutions, Ln(III) complexes of DOTA-tetraamide and its derivatives may normally exist in the form of two inter-converting coordination isomers: square anti-prism (SAP) and twisted square antiprism (TSAP). The axial protons of macrocyclic backbone may be found between 24 and 36 ppm in the SAP isomers and between 5 and 12 ppm in the TSAP isomers for Eu(III) complexes. Based on this information,
 25 three singlet resonance frequencies integrated with an intensity ratio of 1 : 2 : 1 between 20 and 30 ppm may be from axial protons of a descent SAP isomer. Very weak signals from the descent TSAP isomer, less than 5%, may be observed between 7 and 9 ppm indicating Eu(III)-A1 may exist predominantly as the descent SAP isomer in the solution.

[00108] Well-defined resonance frequencies may be observed after the pD rises to 8.9. In
 30 one example, an expected number of 27 aliphatic C-H protons according to the structure may be matched by the observed number of resonance frequencies (Figure 23(b)). At the same time, the axial protons may split into four singlet resonance frequencies with an integral intensity ratio of 1 : 1 : 1 : 1 and three of them may shift more than 5 ppm to downfield relative to those at pD 5.0, and may indicate significant changes in the local structure and coordination environment of

Eu(III) center. On the contrary, the aromatic protons may shift to upfield, which may be due to the increased shielding effects with the deprotonation of phenolic proton. In one non-limiting example, little upfield shifts of the aromatic protons may be observed over the pD 2.9 ~ 6.0. After that, very quick upfield shifts may be found until pD 8.3, in a non-limiting example.

5 [00109] Figure 24 is a graph of pD dependence on the chemical shift of an aromatic proton according to an illustrative embodiment. In particular, the broader singlet resonance frequency at 7.20 ppm at pD 5.0 may be merged into solvent residue resonance frequency above pD 8.3. The shifts of the sharper singlet aromatic resonance frequency as a function of pD may give a sigmoidal curve (Figure 24). In one example, this result may be consistent with a simple
 10 proton equilibrium, from which the $pK^{\wedge*}$ of equilibrium in D_2O may be determined using standard least-squares fitting technique. H_2O equivalent pK_a^H may be calculated to be 7.0 ± 0.1 by using the equation ($pK^H = 0.929 \times pK^* + 0.42$) for conversion of protonation constants measured in D_2O into the corresponding constants in H_2O .

[00110] Figure 25A and 25B are plots of pH dependence of UV-vis spectra for (a)
 15 Eu(III)-Al and (b) free ligand Al (20 uM) recorded in an aqueous solution according to an illustrative embodiment. Arrow indicates the direction of changes as the pH increases. The protonation equilibrium of Eu(III)-Al was further examined through spectrophotometric titrations in the aqueous solution. Figure 25A shows the UV-vis spectral changes of Eu(III)-Al as a function of pH, and the absorbance spectra of Eu(III)-Al may be highly pH dependent.
 20 Under an acidic solution, $\pi-\pi^*$ transition of the aromatic system may give an absorbance band with λ_{max} at 310 nm. This transition band may begin to drop gradually when the solution pH is raised to 5.2, and a new charger transfer band with λ_{max} at 360 nm may appear with further pH increments. The full disappearance of $\pi-\pi^*$ transition may be observed above pH 7.7 accompanying no further rise in the charger transfer band. Figure 26 is a plot of a normalized
 25 titration curve showing the increase in absorbance at 360 nm for Eu(III)-Al ($\blacksquare$) and at 340 nm for free ligand Al (**A**) as a function of increasing pH according to an illustrative embodiment. Symbols may represent experimental data, and lines may represent fitted data to simple sigmoidal function. There may exist three well-defined isobestic points at 238, 262, and 328 nm and the titration profile of pH versus absorbance intensities at 360 nm in Figure 26 may exhibit a
 30 sigmoidal curve supporting a simple phenol proton equilibrium. A pK_a of 6.7 ± 0.1 from the UV-vis titrations may be close to that from the above NMR titrations.

[00111] UV-vis pH titrations may be carried out on free ligand 1. Free ligand may display a $\pi-\pi^*$ transition with λ_{max} at 275 nm under acidic conditions and a charger transfer with λ_{max} at 340 nm under basic conditions (Figure 25B), both of which may experience a

hypsochromic shift with respect to the corresponding Eu(III) complex (Figure 25A). In one non-limiting example, no well-defined isobestic point may be observed during the entire pH titration suggesting, e.g., the complication of proton equilibrium. The proton equilibrium associated with a charger transfer process may be evaluated. A pK_a of 7.7 ± 0.1 may be found by fitting the increase of charger transfer intensities, which may be one unit larger than that of the corresponding Eu(III) complex. Such results may be explained by the resonance effects of deprotonated Eu(III)-Al complex (Figure 27). Figure 27 is a schematic of the proton equilibrium of free ligand Al and resonance representation of the deprotonated Eu(III)-Al complexes according to an illustrative embodiment. With respect to free ligand, the extent of electron conjugation and the easiness of proton deprotonation may be dramatically enhanced, in one example, by forming a quinone-like resonance structure for the deprotonated Eu(III)-Al, by which the excess of electrons on the *para* ketone subunit may be consumed by forming a strong Eu(III)-oxygen coordination band, so it may be the presence of Eu(III) that facilitates the deprotonation of phenolic proton to give a relatively low pK_a of equilibrium.

[00112] In a non-limiting example, the above NMR and absorbance spectral studies may reveal the reversible structure changes and, in turn, extensive electron derealization in complex controlled by pH. Eu(III) may experience much stronger ligand fields due to an increased ligand electron donation to the metal center upon the phenolic deprotonation. This may be detected as a change in Eu(III) emission intensity and CEST property.

[00113] Figure 28 is a plot of the luminescent emission spectrum of Eu(III)-Al (20 μ M) recorded in aqueous solution ($\lambda_{exc} = 310$ nm) at pH 3.3 according to an illustrative embodiment. Luminescence studies were carried out on Eu(III)-Al by excitation of the phenol sensitizer at 310 nm to determine the antenna effect of phenol group and pH responsive properties of luminescence. The luminescent spectrum of Eu(III)-Al may display emission bands characteristic of the Eu(III) complexes at pH 3.3 (Figure 28); specifically, there may exist three electron-dipolar-allowed transitions $\Delta J = 0$ (580 nm), $\Delta J = 2$ (616 nm), and $\Delta J = 4$ (688, 692, 695 and 700 nm), and one magnetic-dipolar-allowed transition $\Delta J = 1$ (590 and 594 nm). In one example, the strong intensity of $\Delta J = 0$ transition relative to that of structural similar Eu(III) complexes may suggest the high asymmetry of Eu(III)-Al. The most intensive emission bands may be from $\Delta J = 2$ and $\Delta J = 4$ transitions, which may be sensitive to coordination environment. All emission transitions may be particularly sensitive to pH, in one example. The emission may be switched "off" above pH 6.0. The decrease in emission intensity may be paralleled with a decrease in the luminescent lifetime. At pH 2.8, two lifetimes $\tau_1 = 500$ μ s (-90%) and $\tau_2 = 380$ μ s (-10%) may be found from the lifetime fitting. Due to the better overlapping, in one non-

limiting example, between the excited state (5Do) of europium with vibronic levels of OH oscillators than those with OD oscillators, longer lifetimes may be measured in D_2O with $\tau_f = 2670 \mu s$ (-90%) and $\tau_d = 1080 \mu s$ (-10%). The hydration state, $q = \ln(k_{H_2O} - k_{D_2O} - C_{HOH} - 3 \times C_{NH})$, measured, in one example, with a corrected Horrocks' method by subtracting a contribution of $C_{HOH} = 0.25$ for water oscillator and $C_{NH} = 0.075$ for each of three amide oscillators may be calculated to be 1.4 for both species. Considering that the contribution factor from phenol unit (an unknown value) may not be taken in account during the calculation, it may be predicted that the real q value may be smaller than 1.4 or close to 1.0. Endeavors to determine the q value at high pH may fail, in one example, because of a sharp drop of lifetimes with pH, the complex luminescent lifetimes may be smaller than 100 μs above pH 5, which may be too short to be measured accurately with the available instrumentation.

[00114] Figure 29 is a plot of the pH dependence of luminescence intensity changes at 580 nm for Eu(III)-Al (20 μM) recorded in aqueous solution ($\lambda_{exc} = 310$ nm) according to an illustrative embodiment. As is evident from the titration profile of Eu(III) emission intensity versus pH shown in Figure 29, a sigmoidal curve may be in accordance with simple proton equilibrium, but a low pK^a of 4.6 may be found from the fitting. Compared with those from NMR and UV-vis titrations, the low pK^a from the luminescent pH titrations may be explained by the occurrence of the excited-state intermolecular proton transfer (ESPT) from the proton donor (phenol proton) to acceptor (water). The acidities of phenol and its derivatives may be greatly enhanced in the excited states due to the redistribution of the oxygen electronic densities upon excitation. For example, a $pK^{excited}$ of 3.6 in excited state for phenol may be much lower than the pK^{ground} of 10.6 in the ground state. A consequence for the existence of the ESPT processes may be a complication in the determination of the proton dissociation constants with fluorescence titrations because of a dependence of fluorescence on the acid-base chemistry of the excited state as well as the ground state. In some case, the "inflection region" from the fluorescence titration curve may extend over the entire pH interval between the ground and excited state pK_a values, and the resulting $pK^{apparent}$ from fluorescence titration may be a joint result of the pK^{ground} and the $pK^{excited}$, lying just between them. $pK^{apparent}$ from luminescence titrations may be much lower than pK^{ground} from UV-vis titrations, in one example.

[00115] Figure 30 is an image of a Jablonski diagram of the Eu(III)-Al according to an illustrative embodiment, which shows that the proximity of the triplet energy level of the quinone group may cause substantial back energy transfer. Structure change may occur with the deprotonation of phenolic proton, which may account for the significant "on-off" switching in luminescence with the raising of pH. For an efficient energy transfer from the sensitizer to

Eu(III) ${}^3\text{F}_4$ (17240 cm^{-1}) level, the triplet energy of an aromatic chromophore may be at least above 22000 cm^{-1} (Figure 30); otherwise, the back energy transfer from the excited ${}^3\text{F}_4$ level to chromophore may quench the luminescence. In comparison with a high triplet energy of 28000 cm^{-1} for phenol group, the quinone group may have an triplet energy around 18300 cm^{-1} , which may be smaller than the threshold value for efficient energy transfer. As a consequence, the small energy difference between the quinone like moiety and the Eu(III) excited state ${}^3\text{F}_4$ may tend to result in the occurrence of a significant back energy transfer process at the expense of Eu(III) emission.

[00116] In one non-limiting example, an increased ligand electron donation to the Eu(III) metal center may result in not only a weakening of the metal-water coordination and accelerating water exchange but also an increase in Eu(III) ligand field strength and, consequently, dipolar NMR shift of ligand. From the pH dependence studies on the chemical shifts of the axial ring protons (e.g., Figure 23), which may be used as a reliable probe of the local magnetic anisotropy, the downfield shifting of axial protons may be indicative of an increase in the ligand field strength with the deprotonation of phenolic proton, and bound water resonance may shift to downfield due to ligand field induced enhancement in the dipolar NMR shift ability of Eu(III).

[00117] Figure 8 is a plot of pH dependence of bound water CEST resonance frequency for Eu(III)-Al recorded at 9.4 T and 298 K according to an illustrative embodiment ($[\text{Eu}^{3+}] = 30$ mM, $B_1 = 14.1$ μT , and sat. time = 2 s). Symbols may represent experimental data, and lines may represent fitted data to a simple sigmoidal function. In one non-limiting example, bound water CEST resonance frequencies may dramatically shift to downfield with the raising of pH. Specifically, five ppm downfield shift in CEST resonance frequency may be observed over the pH 4.5-8.4 at 298 K (Figure 8), whereby a pK_a of 6.5 may be found by sigmoidal fitting to the data.

[00118] In an illustrative embodiment, a ratiometric measurement may be performed by following a single proton pool with a larger chemical shift difference ($\Delta\omega \approx 50$ ppm) rather than following multiple proton pools from a cocktail of agents. The results of τ_M (μs) and δ (ppm) from the fitting of the CEST spectra at 298 and 310 K.

PH	τ_M (μ s) of bound water		δ (ppm) of bound water	
	298 K	310 K	298 K	310 K
6.0	200	70	50.7	46.7
6.4	218	93	51.4	47.4
6.8	169	54	52.7	48.7
7.2	154	47	53.4	49.3
7.6	145	44	54.4	50.0

[00119] The water residence lifetime τ_m at different pH determined by fitting modified Bloch equations may correlates well with the CEST intensity and profile changes, in one embodiment. τ_m of 200 μ s at pH 6.0 may increase a little to 218 μ s at pH 6.4 and then may steadily decline to 145 μ s at pH 7.6, which may indicate that the Eu-water coordination gets weaker with the delocalization of the phenolate electron to the metal center, thus accelerating water exchange rate. Although the CEST profile may broaden and CEST resonance frequency may shift to upfield considerably at 310 K (Figure 31), the CEST resonance frequency shifting magnitude with pH may be almost the same as that at 298 K, so the ratiometric method may be feasible and efficient at this temperature (Figure 32). A sharp decline in τ_m relative to those at 298 K, 70 and 44 μ s at pH 6.0 and 7.6, respectively, may explain these dramatic changes in CEST spectra.

[00120] Eu(III)-Al as a platform for the design of hROS responsive agents. The illustrative embodiments may show how the optical and PARACEST modality may be integrated into a single agent for pH sensing purpose and how the PARACEST agent may ratiometrically be made to sense the pH without the need of a concentration marker. Eu(III)-Al may be employed as a platform for building more responsive agents. The illustrative embodiments may demonstrate Eu(III)-Bl herein as part of a program to develop PARACEST agent for potentially sensing the highly reactive oxygen species (hROS).

[00121] hROS are reactive molecule oxygen such as hydrogen peroxide (H_2O_2) or hydroxyl radicals (HO), and may play key role in mediating many pathogenic processes caused by diseases including carcinogenesis, inflammation, and ischemia-reperfusion injury, among others. hROS may form as a natural by-product of the normal metabolism of oxygen. For example, in neutrophilic leukocytes, much of the superoxide may be dismutated to H_2O_2 and the latter may be transformed by myeloperoxidase into hypochlorite, OCl^- , another potent cytotoxic species. Consequently, the detection of ROS may be used to understand the biological processes like oxidative stress which eventually leads to cell death. Methods including chemiluminescence and fluorescence may be used to measure cell-related ROS production and

accumulation, but these established assays may suffer from an inherent drawback of low spatial resolution. This may be compensated by the MRI based assays.

[00122] Aryloxyphenols or aryloxyaniline may be O-dearylated by hROS such as HO, reactive intermediates of peroxidase, and cytochrome P450, based on which several irreversible
5 fluorescence probes for ROS may be developed. Likewise, hROS responsive PARACEST agent may be built if the phenolic hydroxyl proton is replaced with para-aniline (Figure 36). In one example, Eu(III)-1' may be converted into Eu(III)-1 after the O-dearylation reaction, and then the ratiometric CEST response may be observed as long as a proper condition is selected.

[00123] The liROS-induced O-dearylation reaction was investigated through
10 spectrophotometric titrations with potent hROS OC1^- in aqueous HEPES buffer solution (pH = 7.6). Figure 33 shows the UV-vis spectral changes of Eu(III)-B1 as a function of OCT concentration according to an illustrative embodiment. UV-vis changes of Eu(III)-B1 (10 μM) may occur with the addition of NaOCl in HEPES buffer at pH 7.6 and 310 K. An absorbance band around 310 nm may decrease gradually with the appearance of a new absorbance band
15 around 360 nm. The new absorbance band may happen to be in the same position as that of deprotonated Eu(III)-A1 (Figure 25A), which may support the occurrence of O-dearylation reaction displayed in Figure 36. In one example, a large excess of OC1^- may be needed to push this irreversible reaction forward. In addition, no luminescence may be detected in Eu(III)-B1, which may be ascribed to the fact that the HOMO level of aniline moiety may be high enough to
20 induce the photoinduced electron transfer quench of the acetyl-aryloxy antenna adjacent to the Eu(III) metal center.

[00124] Figure 34A shows NaOCl concentration dependence of CEST spectra for Eu(III)-B1 recorded at 9.4 T and 298 K in 10 mM HEPES buffer according to an illustrative embodiment. Figure 34B shows NaOCl concentration dependence of the ratiometric plot for
25 Eu(III)-B1 by exploitation of the ratio of CEST intensity at 54 ppm to 49 ppm according to an illustrative embodiment (e.g., $[\text{Eu}^{3+}] = 10 \text{ mM}$, pH = 7.5, $B_1 = 14.1 \mu\text{T}$, and sat. time = 2 s). The reactivity of Eu(III)-B1 complex with OC1^- was examined by CEST titration in HEPES buffer with an agent concentration of 10 mM at 298 K as shown in Figure 34A. In one example, with the addition of NaOCl up to 10 mM, one molar equivalence relative to the agent, there may be
30 few changes in the CEST resonance frequency. But with the further addition of NaOCl of up to three molar equivalences, a significant decrease in the CEST resonance frequency around 49 ppm may be observed with the appearance of a new CEST resonance frequency around 54 ppm. The CEST resonance frequency at 49 ppm may disappear completely after the concentration of

NaOCl is up to 10 equivalents. Still, these spectral changes may allow for ratiometrically determining the OCl without considering the concentration marker.

[00125] In one embodiment, a structurally novel Eu(III)-Al complex may be synthesized and characterized using a range of spectroscopic techniques. Eu(III)-Al may show pH controlled "off-on" luminescence, which may be potentially used as *in vitro* optical imaging agent for studies of acidic organelles such as lysosomes and endosomes of live cells. In one example, the NMR experiments demonstrate the local coordination environment of Eu(III) may significantly be modulated by the deprotonation of phenolic proton resulting in the downfield shift in the bound water exchange CEST resonance frequency. This shift may enable the elimination of the need of a concentration marker required in the regular ratiometric CEST imaging. Furthermore, the Eu(III)-Al may be employed as a basic platform in the development of other types of responsive imaging agents as well.

[00126] ^1H , ^{13}C NMR and CEST spectra may be recorded, e.g., on a Bruker AVANCE III 400 NMR spectrometer. A pre-saturation pulse of 2 s may be applied at saturation powers of 14.1 μT during the CEST acquisitions. CEST imaging may be recorded on, e.g., Varian 9.4 T small animal imaging system. pH values of the samples for CEST may be maintained by MES or HEPES buffers (5 mM). The CEST spectra may be fitted to the Bloch equations with 3-pool model by use of a nonlinear fitting algorithm written in, e.g., MATLAB[®] 7 (Mathworks Inc., Natick, MA). Melting points may be determined on, e.g., a Fisher-Johns melting point apparatus without correction.

[00127] The pH of titration samples may be measured with, e.g., a Denver Instrument UltraBasic UB-5 pH meter and the pH may be adjusted by addition of concentrated solutions of KOH or HCl. The D_2O solutions of KOD or DCl may be used to adjust the pD for the titration performed in D_2O . pD values in D_2O solutions may be calculated by adding a constant of 0.4 to the pH*, which may be a direct reading in a H_2O -calibrated pH meter. pK_A of equilibrium may be determined using standard least-squares fitting technique from the corresponding titration data. pK^* calculated directly from pH* in D_2O may be transferred into H_2O equivalent pK^A by using equation $\text{pK}^\text{A} = 0.929 \times \text{pH}^* + 0.42$.

[00128] Ultraviolet absorbance spectra may be recorded using, e.g., Varian Cary 300 Bio UV/Vis spectrophotometer equipped with thermostatted cell holders. Luminescent spectra and lifetime measurements may be recorded on, e.g., an Edinburgh Instruments FL/FS900CDT fluorometer equipped with a 450 W xenon arc lamp and a 100 W μi 920H flash lamp. Full emission spectra may be recorded from 525-725 nm using a 0.5 nm step size.

[00129] Responsive PARACEST agents may have a CEST signal that changes intensity in response to external stimuli but these may require a separate measure of agent concentration. Some exceptions may be agents that incorporate a cocktail of agents with weakly shifted -NH exchangeable protons for ratiometric imaging, but this design may have the exchange site relatively close to the bulk water frequency (typically < 15 ppm away). This problem, among others, is addressed by presenting in the illustrative embodiments a novel europium(III) DOTA-monoketone-trisamide complex having a highly shifted water exchange CEST peak (50-55 ppm) that may switch frequency as a function of solution pH. In one example, this single agent may be used for a direct readout of pH by ratiometric CEST imaging.

[00130] Eu^{3+} -based PARACEST agents may have a highly shifted water exchange peak that is independent of pH between 5 and 8.4. In one example, the chemical shift of the Eu^{3+} -bound water exchange peak may be altered considerably by varying the electron density on even a single amide oxygen donor in these DOTA-tetraamide systems. This observation may be expanded to multi-frequency.

[00131] Referring back to Figure 2, a graph of the pH dependence of the UV-vis spectrum of Eu(III)-Al (20 μM) recorded in aqueous solution is shown according to an illustrative embodiment. In one example, the absorption spectrum of Eu(III)-Al may show not only hyperchromic effect but also bathochromic shift from 310 nm to 360 nm with increasing pH as the phenolic proton is removed. This may be considered to be consistent with extended derealization of the phenolate anion through the π system to form a quinone-like structure which places considerable negative charge on the carbonyl oxygen atom coordinated to the Eu^{3+} ion. The pK_a derived from these optical data was 6.7 ± 0.1 , and usable for a biological pH sensor. In one example, such a resonance structure may alter the water exchange rate and potentially the frequency of the exchanging water molecule. CEST spectra of Eu(III)-Al at five pH values may show a surprisingly large change in chemical shift with increasing pH. The pK_a derived from the CEST data may be 6.5 ± 0.1 , which may be identical to that derived from absorption data (Figure 2).

[00132] In an illustrative embodiment, Eu(III)-Al may be used as a direct readout of pH by collecting two different CEST images and using the ratio as a concentration independent measure of pH. A plot of the ratio of CEST intensity at 55 ppm *versus* 49 ppm may be linear over the pH range 6.0 to 7.6. Using an illustrative embodiment, a measurement may be performed using a single reagent compared to a cocktail of agents. Also, the exchange peak may be ~50 ppm downfield of water so it may be activated without concern about off-resonance saturation of the bulk water resonance itself.

[00133] To demonstrate using this agent for imaging pH by MRI, CEST images of a phantom prepared from five Eu(III)-Al samples adjusted to different pH values (plus a water control) were collected by subtracting the water intensity measured in "on" images (presaturation pulse set to either +54 or +47 ppm) from the water intensity measured in the corresponding "off" images (presaturation pulse set to either -54 or -47 ppm). The ratio of these two CEST images is shown in Figure 35 as a map and as 3D surface plots. Figure 35 is an image of the pH dependence of ratiometric CEST according to an illustrative embodiment. Both images show nearly perfect cancellation of the pure water sample while the CEST ratio in the samples varied from 0.43 (pH 6.0 sample) to 2.32 (pH 7.6 sample), a substantial dynamic range.

10 Using these ratios and the prior calibration curve, the pH values determined by CEST imaging and as reported by a pH electrode may be identical.

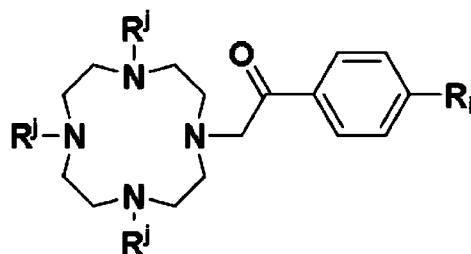
[00134] The width of the water exchange peak in Eu(III)-Al may broaden somewhat at high pH values, which may be consistent with faster water exchange. This may be quantified by fitting each CEST spectrum to the Bloch equations. The bound water lifetime, τ_m , may be -200 μ s at pH 6.0 and -145 μ s at pH 7.6. This result may be consistent with the expected increase in water exchange rate as the acetyl oxygen donor atom gains more negative charge. Water exchange may be even faster in this complex at 310K (τ_m varies from 70 and 44 μ s over this same pH range) but the frequency shifts in the bound water exchange peak may be about the same as that seen at 298 K. This indicates that Eu(III)-Al may also be usable for ratiometric CEST imaging of pH at more physiological temperatures.

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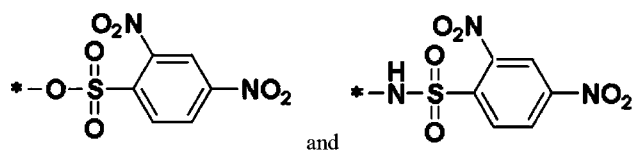
[00135] In an illustrative embodiment, a novel Eu³⁺-based PARACEST agent is reported for imaging pH using ratiometric CEST imaging principles. In one embodiment, ratiometric imaging may be performed using two slightly different activation frequencies, both well away from the bulk water frequency. Also, the pK_a of this system may be such that the largest changes in CEST may occur between pH 6 and 7.6, and usable for sensing physiological pH.

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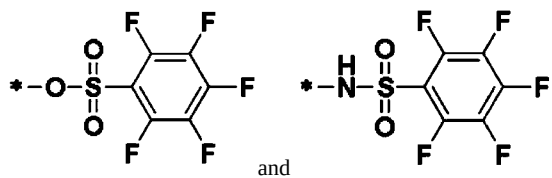
[00136] Illustrative embodiments are provided herein that may be useful in MRI imaging and other applications. In one embodiment, a composition of matter includes a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including the formula:



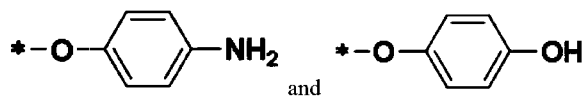
In this embodiment, R_1 is selected from the group consisting of R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 , R_1 is selected from the group consisting of OR' , O_2R' , SR' , and SOR' , R_2 is selected from the group consisting of NHR' , CO_2R' , $SO_3(R')_2$, and $PO_3(R')_2$, R_3 is selected from the group consisting of NO_2 and $C=O$, R_4 is selected from the group consisting of:



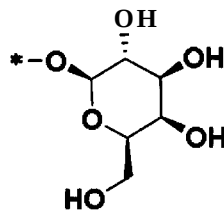
R_5 is selected from the group consisting of:



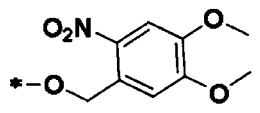
R_6 is selected from the group consisting of:



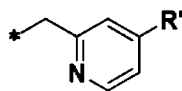
R_7 includes:



R_8 includes:



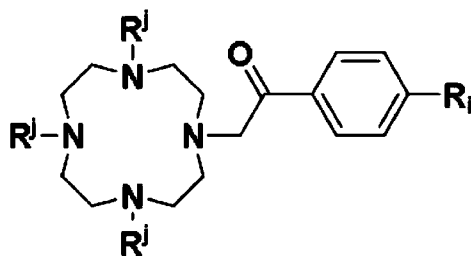
each R^j is selected from the group consisting of R^1 , R^2 , R^3 , R^4 , R^5 , and R^6 , and R^1 includes $CR'H-CONH-(CH_2)_n-CO_2-R'$. n is an integer, and $0 < n < 20$. R^2 includes $CR'H-CONH-(CH_2)_n-PO-(OR')_2$, R^3 includes $CR'H-COCH_2R'$, R^4 includes $CR'H-PO(OR')-(CH_2)_n-CO_2-R'$, R^5 includes $CR'H-PO(OR')-R'$, R^6 includes:



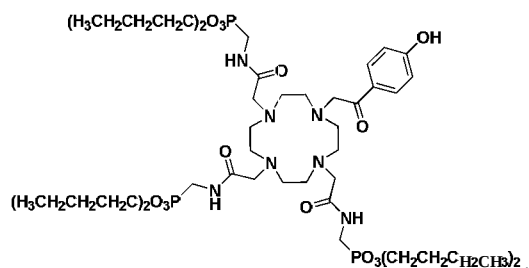
, and

R' is selected from the group consisting of H, an alkyl group having 20 carbon atoms or less, a cycloalkyl group having 20 carbon atoms or less, and an alkyloxy group having 20 carbon atoms or less and 10 oxygen atoms or less. This embodiment may also include a paramagnetic metal ion coordinated to the composition of matter to form the paramagnetic chemical exchange saturation transfer MRI contrast agent. The paramagnetic metal may be selected from the group consisting of Eu^{3+} , Tb^{3+} , Dy^{3+} , Ho^{3+} , Pr^{3+} , Nd^{3+} , Sm^{3+} , Er^{3+} , Tm^{3+} , Fe^{2+} , Fe^{3+} , Mn^{2+} , Co^{2+} , Ni^{2+} , V^{2+} , Mo^{3+} , and Cr^{3+} .

[00137] In one embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent may include a tetraazacyclododecane ligand. In one embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent may consist of the formula:

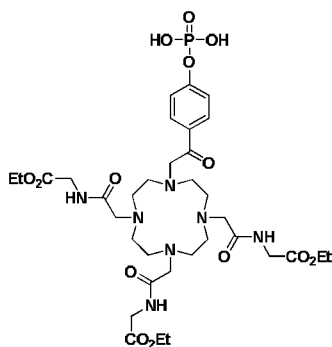


In one embodiment, each R_j group in the paramagnetic chemical exchange saturation transfer MRI contrast agent may be the same. In one embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent may include the formula:



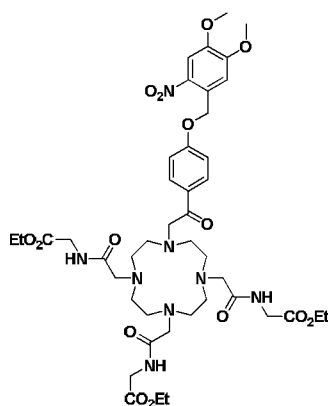
In one embodiment, a method for determining pH may use magnetic resonance imaging with the paramagnetic chemical exchange saturation transfer MRI contrast agent having the above formula.

- 5 **[00138]** In one embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent may include the formula:

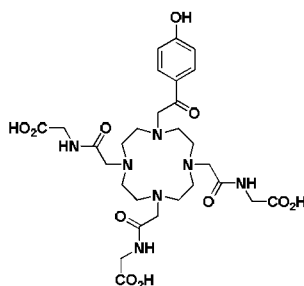


In one embodiment, a method for determining enzymatic activity may use magnetic resonance imaging with the paramagnetic chemical exchange saturation transfer MRI contrast agent having the above formula.

- 10 **[00139]** In one embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent may include the formula:



[00140] In one embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent may include the formula:



- 5 In one embodiment, a paramagnetic ion may be associated with the paramagnetic chemical exchange saturation transfer MRI contrast agent having the above formula, among others, and the paramagnetic ion may be selected from the group consisting of iron (II) (high spin), iron (III), cobalt (II), nickel (II), praseodymium (III), neodymium (III), dysprosium (III), erbium (III), terbium (III), holmium (III), thulium (III), ytterbium (III), and europium (III), and a physiological acceptable salt thereof.

[00141] In one embodiment, the composition of matter may include a single paramagnetic complex compound endowed with a metal bound water. In another embodiment, the R_i may be removable by a presence of a chemical parameter, and the chemical parameter may be at least one of a predetermined pH level, a highly reactive oxygen species, or an enzyme.

- 15 **[00142]** In one embodiment, a method for determining a chemical parameter using the paramagnetic chemical exchange saturation transfer MRI contrast agent is provided, and the chemical parameter may be at least one of pH, a presence of a highly reactive oxygen species, or

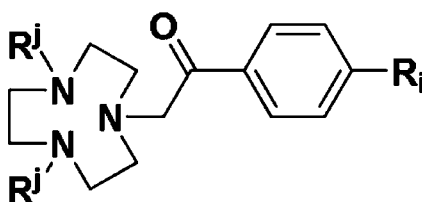
a presence of enzyme activity. In this embodiment, the method may use ratiometric chemical exchange saturation transfer imaging to determine the chemical parameter.

[00143] In one embodiment, a method for determining a chemical parameter using the paramagnetic chemical exchange saturation transfer MRI contrast agent is provided; the chemical parameter may be at least one of pH, a presence of a highly reactive oxygen species, or a presence of enzyme activity, and the chemical parameter may be determined *in vivo* in at least one of a body, organ, fluid, or tissue of a human or animal.

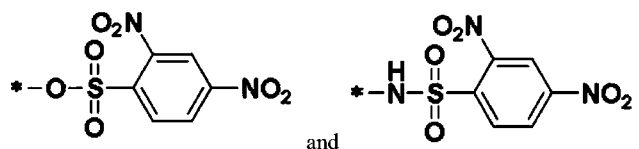
[00144] In one embodiment, a method for determining a chemical parameter using the paramagnetic chemical exchange saturation transfer MRI contrast agent is provided; the chemical parameter may be at least one of pH, a presence of a highly reactive oxygen species, or a presence of enzyme activity, and the chemical parameter may be determined either *in vitro* or *ex vivo*. In one embodiment, a method for delivering a drug into a patient using the composition of matter is provided.

[00145] In one embodiment, the composition of matter may be labeled with a radionuclide to form a radionuclide-labeled contrast agent. The radionuclide may include at least one of Bi-212, Bi-213, Pb-203, Cu-64, Cu-67, Ga-66, Ga-67, Ga-68, Lu-177, In-111, In-113, Y-86, Y-90, Dy-162, Dy-165, Dy-167, Ho-166, Pr-142, Pr-143, Pm-149 or Tb-149. In one embodiment, an imaging method may include delivering the radionuclide-labeled contrast agent into a patient, and imaging a portion of the patient containing the radionuclide-labeled contrast agent using positron emission tomography.

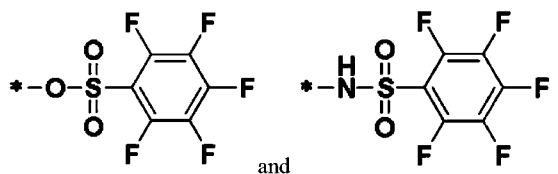
[00146] In one embodiment, a composition of matter includes a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including the formula:



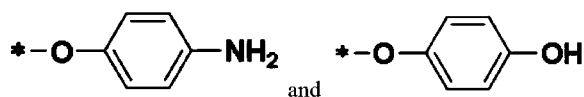
In this embodiment, R_i is selected from the group consisting of R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 . R_1 is selected from the group consisting of OR' , O_2R' , SR' , and SOR' , R_2 is selected from the group consisting of NHR' , CO_2R' , $SO_3(R')_2$, and $PO_3(R')_2$, R_3 is selected from the group consisting of NO_2 and $C=O$, R_4 is selected from the group consisting of:



R₅ is selected from the group consisting of:

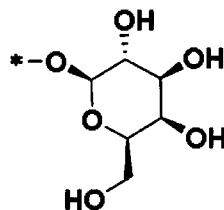


R₆ is selected from the group consisting of:

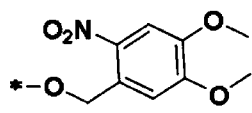


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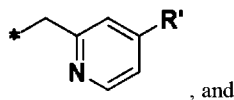
R₇ includes:



R₈ includes:

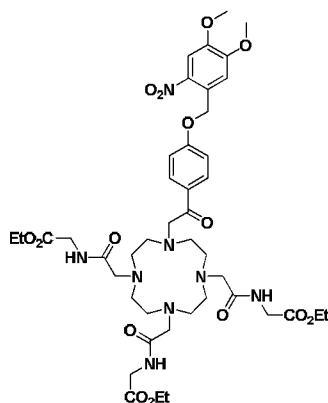


- 10 each R^j is selected from the group consisting of R¹, R², R³, R⁴, R⁵, and R⁶, and R¹ includes CR'H-CONH-(CH₂)_n-C(=O)-R'. n is an integer, and 0<n<20. R² includes CR'H-CONH-(CH₂)_n-PO-(OR')₂, R³ includes CR'H-COCH₂R', R⁴ includes CR'H-PO(OR')-(CH₂)_n-C(=O)-R', R⁵ includes CR'H-PO(OR')-R', R⁶ includes:



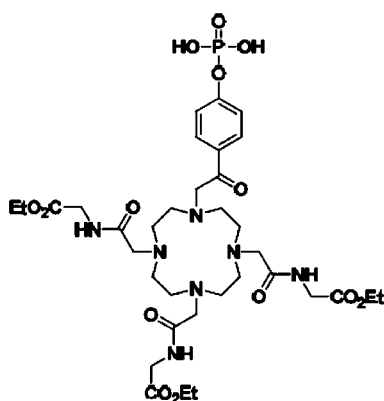
- 15 R' is selected from the group consisting of H, an alkyl group having 20 carbon atoms or less, a cycloalkyl group having 20 carbon atoms or less, and an alkyloxy group having 20 carbon atoms or less and 10 oxygen atoms or less.

[00147] In another embodiment, a composition of matter includes a light-sensitive contrast agent usable as a composition for a drug delivery system (e.g., micelle, liposome, etc.) including the formula:

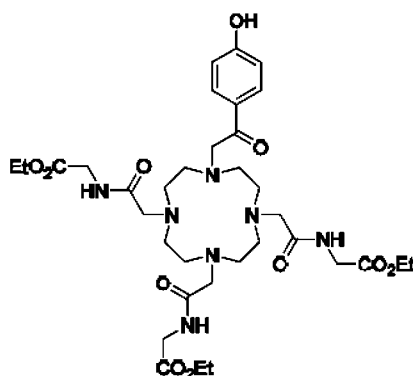


- 5 In one embodiment, a method for delivering a drug to a patient includes combining the light-sensitive contrast agent with the drug to form a combined light-sensitive drug delivery system, delivering the combined light-sensitive drug delivery system into the patient, and exposing the combined light-sensitive drug delivery system to electromagnetic radiation to release the drug into the patient. In one embodiment, the method also includes detecting a concentration of the
- 10 drug in the patient. In another embodiment, the electromagnetic radiation may include at least one of ultraviolet or near-infrared radiation.

[00148] In one embodiment, a method of synthesizing a first compound having the formula:



- 15 includes providing a second compound having the formula:

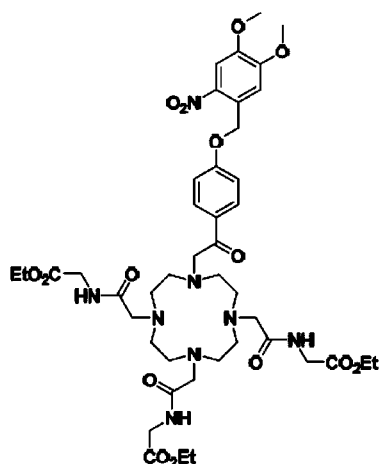


providing phosphoryl chloride, providing triethylamine, adding the second compound, the phosphoryl chloride, and the triethylamine to CH_2Cl_2 to form a solution, stirring the solution for a predetermined period of time, and extracting the solution with water. The water may form a

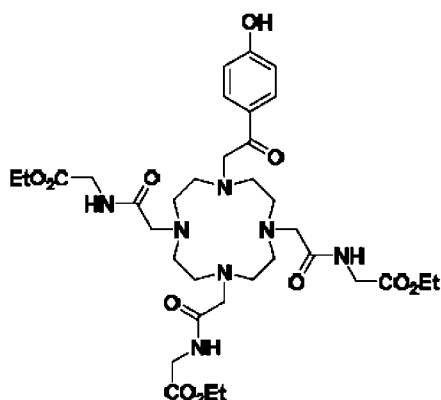
5 water layer. The method also includes evaporating the water layer to form a solid, and subjecting the solid to high-performance liquid chromatography to form a product containing the first compound. In another embodiment, providing the second compound includes providing one molar equivalence of the second compound, providing the phosphoryl chloride includes providing four molar equivalences of the phosphoryl chloride, and providing the triethylamine

10 includes providing three molar equivalences of the triethylamine. In another embodiment, stirring the solution for the predetermined period of time includes stirring the solution for approximately 24 hours, and the solid is a yellow solid.

[00149] In one embodiment, a method of synthesizing a first compound having the formula:



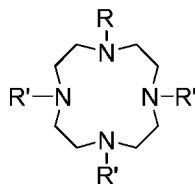
includes providing a second compound having the formula:



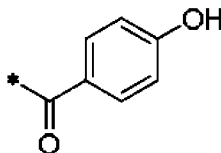
- providing 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene, providing K_2CO_3 , adding the second
- 5 compound, the 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene, and the K_2CO_3 to CH_3CN to form a mixture, stirring the mixture under N_2 for a predetermined period of time, filtering and evaporating the mixture until at least partially dried to form a crude compound, and purifying the crude compound using flash column chromatography on silica to form a solid containing the first compound. In another embodiment, providing the second compound includes providing
- 10 one molar equivalence of the second compound, providing the 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene includes providing one molar equivalence of the 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene, and providing the K_2CO_3 includes providing one molar equivalence of the K_2CO_3 . In another embodiment, stirring the mixture under N_2 for the predetermined

period of time includes stirring the mixture under N₂ for approximately 12 hours, and the solid is a yellow solid.

[00150] In one embodiment, a composition of matter includes a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including the formula:

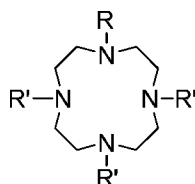


R may include:

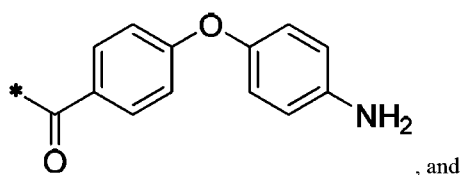


, and

- each R' may be selected from the group consisting of CH₂CONHCH₂COOH, CH₂CONHCH₂COOC₂H₅, CH₂CONH₂, CH₂CONHCH₂PO(OC₂H₅)₂, CH₂CONHCH₂P(O)₃H₂, CH₂CONHCH₂PO(OC(CH₃)₂)₂, CH₂CONHCH₂PO(OCH₂CH₂CH₃)₂, CH₂CONHCH₂PO(OCH₂CH₂CH₂CH₃)₂, and CH₂CONHCH₂PO(OC(CH₃)₃)₂. In one embodiment, a method for obtaining a ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent to determine at least one of a pH level, a presence of a highly reactive oxygen species, a biological metal ion concentration, or a presence of enzyme activity is provided. In this method, obtaining the ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent may be performed either *in vitro* or *ex vivo*. In another embodiment, obtaining the ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent may be performed *in vivo*. In another embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent is associated with a europium (III). In another embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent produces a frequency shift in a chemical exchange saturation transfer exchange peak.
- [00151]** In one embodiment, a composition of matter includes a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including the formula:



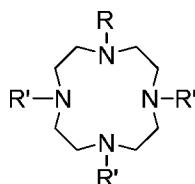
R may include:



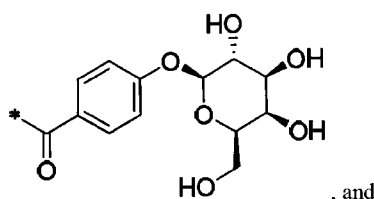
, and

- 5 each R' may be selected from the group consisting of $\text{CH}_2\text{CONHCH}_2\text{COOH}$, $\text{CH}_2\text{CONHCH}_2\text{COOC}_2\text{H}_5$, CH_2CONH_2 , $\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OC}_2\text{H}_5)_2$, $\text{CH}_2\text{CONHCH}_2\text{PO}_3\text{H}_2$, $\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OC}(\text{CH}_3)_2)_2$, $\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OCH}_2\text{CH}_2\text{CH}_3)_2$, $\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OCH}_2\text{CH}_2\text{CH}_2\text{CH}_3)_2$, and $\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OC}(\text{CH}_3)_3)_2$. In one embodiment, a method for obtaining a ratiometric imaging measurement using the paramagnetic
- 10 chemical exchange saturation transfer MRI contrast agent to determine at least one of a pH level, a presence of a highly reactive oxygen species, a biological metal ion concentration, an oxygen concentration, or a presence of enzyme activity is provided. In one embodiment, obtaining the ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent may be performed either *in vitro* or *ex vivo*. In another
- 15 embodiment, obtaining the ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent may be performed *in vivo*. In another embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent is associated with a europium (III). In another embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent produces a frequency shift in a chemical exchange
- 20 saturation transfer exchange peak.

[00152] In one embodiment, a composition of matter includes a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including the formula:



R may include:



, and

- 5 each R' may be selected from the group consisting of $\text{CH}_2\text{CONHCH}_2\text{COOH}$, $\text{CH}_2\text{CONHCH}_2\text{COOC}_2\text{H}_5$, CH_2CONH_2 , $\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OC}_2\text{H}_5)_2$, $\text{CH}_2\text{CONHCH}_2\text{PO}_3\text{H}_2$, $\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OC}(\text{CH}_3)_2)_2$, $\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OCH}_2\text{CH}_2\text{CH}_3)_2$, $\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OCH}_2\text{CH}_2\text{CH}_3)_2$, and $\text{CH}_2\text{CONHCH}_2\text{PO}(\text{OC}(\text{CH}_3)_3)_2$. In one embodiment, a method for obtaining a ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent to determine at least one of a pH level, a presence of a highly reactive oxygen species, a biological metal ion concentration, or a presence of enzyme activity is provided. In another embodiment, obtaining the ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent may be performed either *in vitro* or *ex vivo*. In another embodiment, obtaining the ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent may be performed *in vivo*. In another embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent is associated with a europium (III). In another embodiment, the paramagnetic chemical exchange saturation transfer MRI contrast agent produces a frequency shift in a chemical exchange saturation transfer exchange peak.

- 20 **[00153]** In one embodiment, a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent for determining a chemical parameter including a europium(III) DOTA-tris(amide) complex includes four side chains, and one of the four side chains connects an aromatic group by a carbonyl bond. In another embodiment, the europium(III) DOTA-tris(amide) complex consists of four side chains. In another embodiment, the one of the four side chains connects the aromatic group by $-\text{CH}_2-\text{CO}-$. In another

embodiment, the chemical parameter is at least one of a pH level, a presence of a highly reactive oxygen species, a biological metal ion concentration, an oxygen concentration, or a presence of enzyme activity.

[00154] In one embodiment, a method for determining one or more parameters includes
5 obtaining a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent including a europium(III) DOTA-tris(amide) complex including four side chains. One of the four side chains connects an aromatic group by a carbonyl bond. The paramagnetic chemical exchange saturation transfer MRI contrast agent is adapted to provide a ratiometric imaging measurement. The method also includes administering the paramagnetic
10 chemical exchange saturation transfer MRI contrast agent to a patient, and detecting a signal in the patient that correlates to one or more parameters. The one or more parameters includes at least one of a pH level, a presence of a highly reactive oxygen species, a biological metal ion concentration, an oxygen concentration, a presence of enzyme activity, a temperature, a metabolite concentration, or an $\frac{3}{4}$ partial pressure. In another embodiment, the one of the four
15 side chains connects the aromatic group by $-\text{CH}_2-\text{CO}-$.

[00155] It is contemplated that any embodiment discussed in this specification may be implemented with respect to any method, kit, reagent, or composition of the illustrative embodiments, and vice versa. Furthermore, compositions of the invention may be used to achieve methods of the illustrative embodiments.

[00156] It will be understood that particular embodiments described herein are shown by
20 way of illustration and not as limitations of the embodiments. The principal features of the illustrative embodiments may be employed in various embodiments without departing from the scope of the embodiments. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, numerous equivalents to the specific procedures
25 described herein. Such equivalents are considered to be within the scope of the illustrative embodiments and are covered by the claims.

[00157] The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one." The use of the
30 term "or" in the claims is used to mean "and/or" unless explicitly indicated to refer to alternatives only or the alternatives are mutually exclusive, although the disclosure supports a definition that refers to only alternatives and "and/or." Throughout this application, the term "about" is used to indicate that a value includes the inherent variation of error for the device, the

method being employed to determine the value, or the variation that exists among the study subjects.

[00158] As used in this specification and claim(s), the words "comprising" (and any form of comprising, such as "comprise" and "comprises"), "having" (and any form of having, such as
5 "have" and "has"), "including" (and any form of including, such as "includes" and "include") or "containing" (and any form of containing, such as "contains" and "contain") are inclusive or open-ended and do not exclude additional, unrecited elements or method steps.

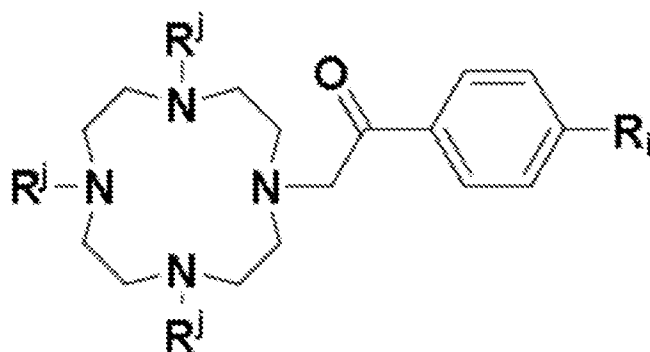
[00159] The term "or combinations thereof" as used herein refers to all permutations and combinations of the listed items preceding the term. For example, "A, B, C, or combinations
10 thereof" is intended to include at least one of: A, B, C, AB, AC, BC, or ABC, and if order is important in a particular context, also BA, CA, CB, CBA, BCA, ACB, BAC, or CAB. Continuing with this example, expressly included are combinations that contain repeats of one or more item or term, such as BB, AAA, MB, BBC, AAABCCCC, CBBAAA, CABABB, and so forth. The skilled artisan will understand that typically there is no limit on the number of
15 items or terms in any combination, unless otherwise apparent from the context.

[00160] All of the compositions and/or methods disclosed and claimed herein may be made and executed without undue experimentation in light of the present disclosure. While the compositions and methods of the illustrative embodiments have been described in terms of preferred embodiments, it will be apparent to those of skill in the art that variations may be
20 applied to the compositions and/or methods and in the steps or in the sequence of steps of the method described herein without departing from the concept, spirit and scope of the embodiments. All such similar substitutes and modifications apparent to those skilled in the art are deemed to be within the spirit, scope and concept of the illustrative embodiments as defined by the appended claims.

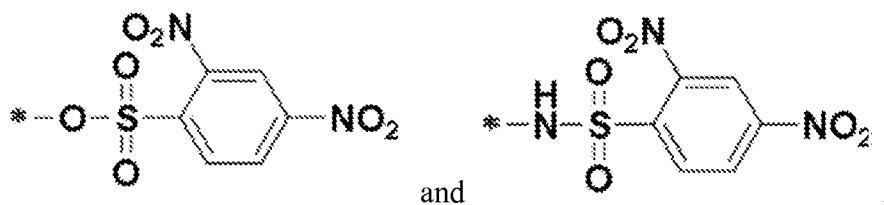
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What is claimed is:

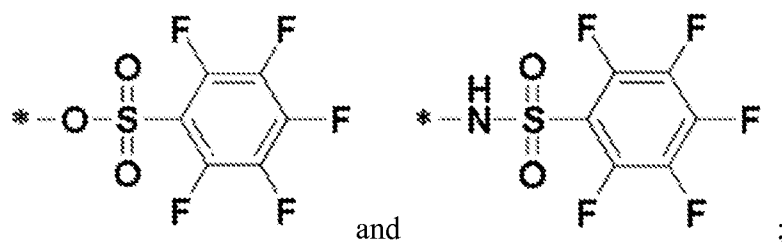
1. A composition of matter comprising:
a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI)
contrast agent comprising the formula:



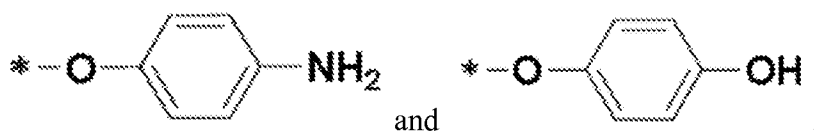
- 5 ;
- wherein R_1 is selected from the group consisting of R_1 , R_2 , R_3 , R_4 , R_5 , R_6 , R_7 , and R_8 ;
 wherein R_1 is selected from the group consisting of OR' , O_2R' , SR' , and SOR' ;
 wherein R_2 is selected from the group consisting of NHR' , CO_2R' , $SO_3(R')_2$, and
 10 $PO_3(R')_2$;
 wherein R_3 is selected from the group consisting of NO_2 and $C=O$;
 wherein R_4 is selected from the group consisting of:



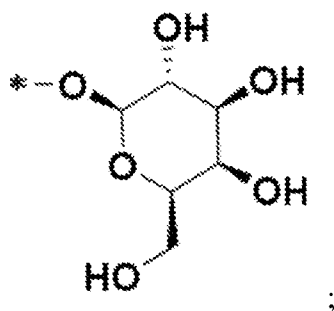
wherein R_5 is selected from the group consisting of:



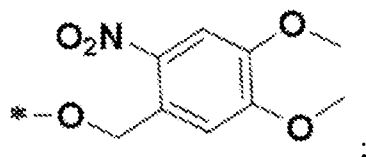
- 15 wherein R_6 is selected from the group consisting of:



wherein R_7 comprises:



wherein R_8 comprises:



wherein each R^j is selected from the group consisting of R^1 , R^2 , R^3 , R^4 , R^5 , and R^6 ;

5 wherein R^1 comprises $CR'H-CONH-(CH_2)_n-CO_2-R'$, wherein n is an integer, and wherein $0 < n < 20$;

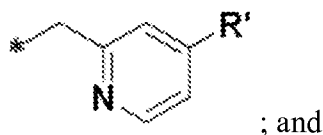
wherein R^2 comprises $CR'H-CONH-(CH_2)_n-PO-(OR')_2$;

wherein R^3 comprises $CR'H-COCH_2R'$;

wherein R^4 comprises $CR'H-PO(OR')-(CH_2)_n-CO_2-R'$;

10 wherein R^5 comprises $CR'H-PO(OR')-R'$;

wherein R^6 comprises:



15 wherein R' is selected from the group consisting of H, an alkyl group having 20 carbon atoms or less, a cycloalkyl group having 20 carbon atoms or less, and an alkyloxy group having 20 carbon atoms or less and 10 oxygen atoms or less.

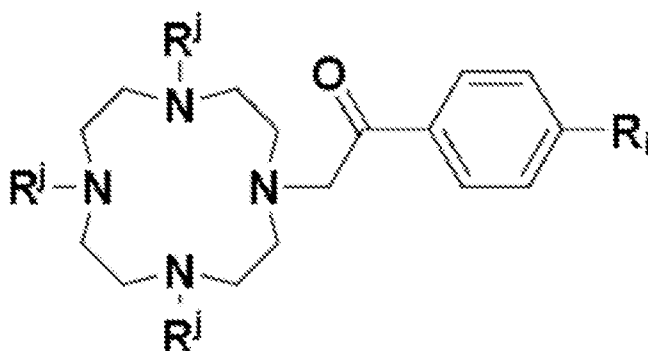
2. The composition of matter of claim 1, further comprising:

a paramagnetic metal ion coordinated to the composition of matter to form the paramagnetic chemical exchange saturation transfer MRI contrast agent, wherein the paramagnetic metal is selected from the group consisting of Eu^{3+} , Tb^{3+} , Dy^{3+} , Ho^{3+} , Pr^{3+} , Nd^{3+} , Sm^{3+} , Er^{3+} , Tm^{3+} , Fe^{2+} , Fe^{3+} , Mn^{2+} , Co^{2+} , Ni^{2+} , V^{2+} , Mo^{3+} , and Cr^{3+} .

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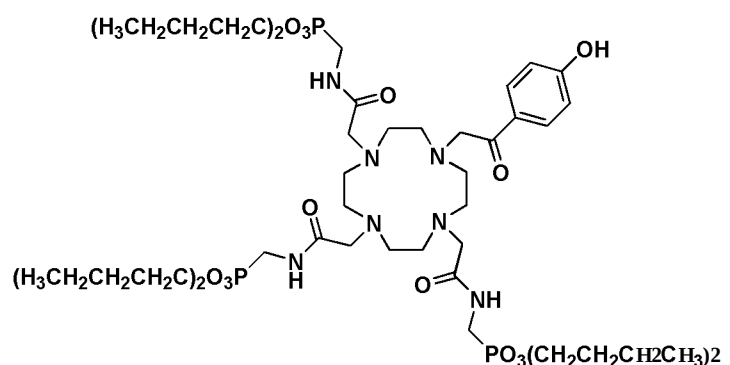
3. The composition of matter of claim 1, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent comprises a tetraazacyclododecane ligand.

4. The composition of matter of claim 1, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent consists of the formula:



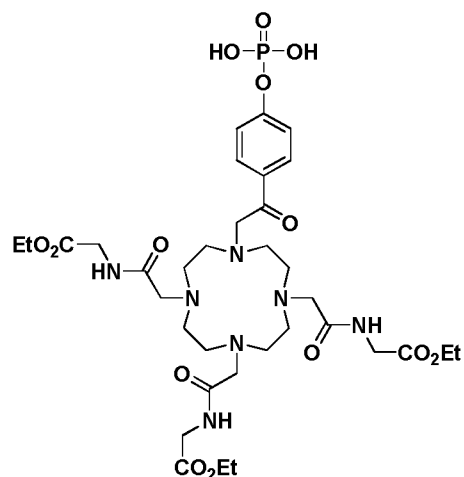
5. The composition of matter of claim 1, wherein each R_j group in the paramagnetic chemical exchange saturation transfer MRI contrast agent is the same.

6. The composition of matter of claim 1, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent comprises the formula:



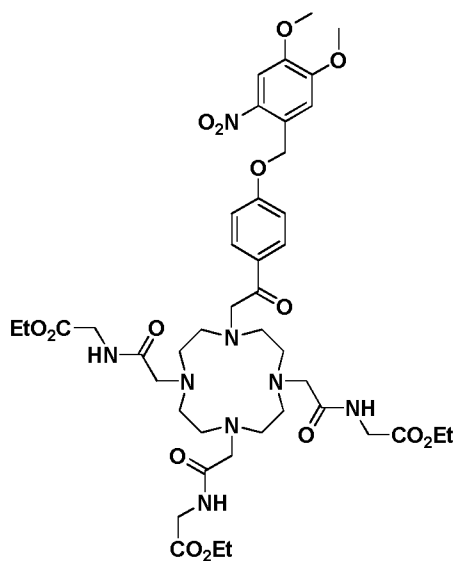
7. A method for determining pH using magnetic resonance imaging with the paramagnetic chemical exchange saturation transfer MRI contrast agent of claim 6.

8. The composition of matter of claim 1, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent comprises the formula:

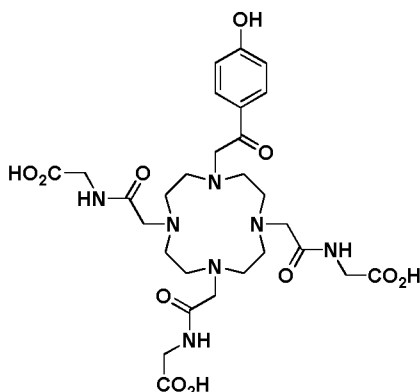


9. A method for determining enzymatic activity using magnetic resonance imaging with the paramagnetic chemical exchange saturation transfer MRI contrast agent of claim 8.

10. The composition of matter of claim 1, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent comprises the formula:



11. The composition of matter of claim 1, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent comprises the formula:



12. The composition of matter of claim 11, wherein a paramagnetic ion is associated with the paramagnetic chemical exchange saturation transfer MRI contrast agent and the paramagnetic ion is selected from the group consisting of iron (II) (high spin), iron (III), cobalt (II), nickel (II), praseodymium (III), neodymium (III), dysprosium (III), erbium (III), terbium (III), holmium (III), thulium (III), ytterbium (III), and europium (III), and a physiological acceptable salt thereof.

13. The composition of matter of claim 1, further comprising:
a single paramagnetic complex compound endowed with a metal bound water.

14. The composition of matter of claim 1, wherein the Ri is removable by a presence of a chemical parameter, wherein the chemical parameter is at least one of a predetermined pH level, a highly reactive oxygen species, or an enzyme.

15. A method for determining a chemical parameter using the composition of matter of claim 1, wherein the chemical parameter is at least one of pH, a presence of a highly reactive oxygen species, or a presence of enzyme activity.

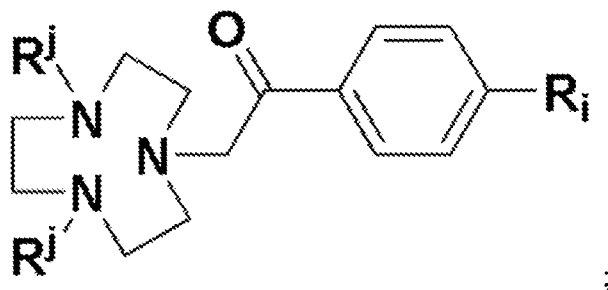
16. The method of claim 15, wherein the method uses ratiometric chemical exchange saturation transfer imaging to determine the chemical parameter.

17. A method for determining a chemical parameter using the composition of matter of claim 1, wherein the chemical parameter is at least one of pH, a presence of a highly reactive oxygen species, or a presence of enzyme activity, and wherein the chemical parameter is determined *in vivo* in at least one of a body, organ, fluid, or tissue of a human or animal.

18. A method for determining a chemical parameter using the composition of matter of claim 1, wherein the chemical parameter is at least one of pH, a presence of a highly reactive oxygen species, or a presence of enzyme activity, and wherein the chemical parameter is determined either *in vitro* or *ex vivo*.
- 5 19. A method for delivering a drug into a patient using the composition of matter of claim 1.
20. The composition of matter of claim 1, the composition of matter labeled with a radionuclide to form a radionuclide-labeled contrast agent, the radionuclide comprising at least one of Bi-212, Bi-213, Pb-203, Cu-64, Cu-67, Ga-66, Ga-67, Ga-68, Lu-177, In-111, In-113, Y-86, Y-90, Dy-162, Dy-165, Dy-167, Ho-166, Pr-142, Pr-143, Pm-149 or Tb-149.
- 10 21. An imaging method comprising:
delivering the radionuclide-labeled contrast agent of claim 20 into a patient; and
imaging a portion of the patient containing the radionuclide-labeled contrast agent using positron emission tomography.

22. A composition of matter comprising:

a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent comprising the formula:



;

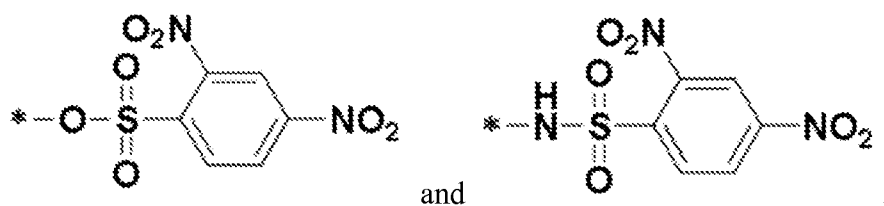
5 wherein Ri is selected from the group consisting of Ri, R₂, R₃, R₄, R₅, R₆, R₇, and R₈;

wherein Ri is selected from the group consisting of OR', O₂R', SR', and SOR';

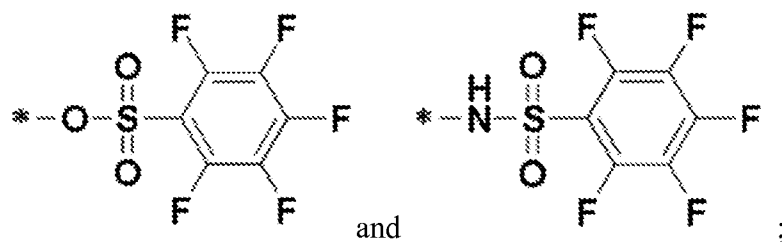
wherein R₂ is selected from the group consisting of NHR', CO₂R', SO₃(R')₂, and PO₃(R')₂;

wherein R₃ is selected from the group consisting of NO₂ and C=O;

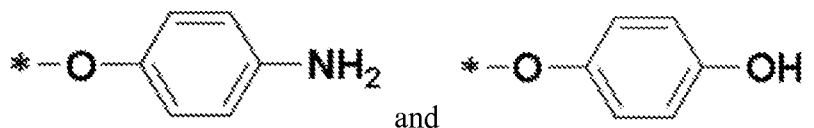
10 wherein R₄ is selected from the group consisting of:



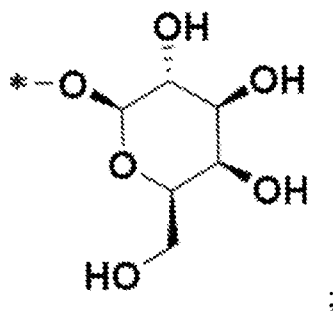
wherein R₅ is selected from the group consisting of:



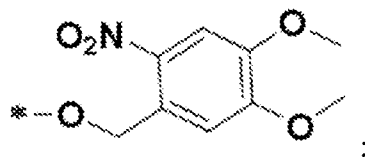
wherein R₆ is selected from the group consisting of:



15 wherein R₇ comprises:



wherein R_8 comprises:



wherein each R^j is selected from the group consisting of R^1 , R^2 , R^3 , R^4 , R^5 , and R^6 ;

5 wherein R^1 comprises $CR'H-CONH-(CH_2)_n-CO_2-R'$, wherein n is an integer, and wherein $0 < n < 20$;

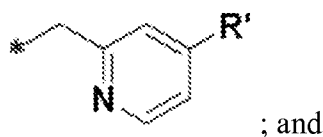
wherein R^2 comprises $CR'H-CONH-(CH_2)_n-PO-(OR')_2$;

wherein R^3 comprises $CR'H-COCH_2R'$;

wherein R^4 comprises $CR'H-PO(OR')-(CH_2)_n-CO_2-R'$;

10 wherein R^5 comprises $CR'H-PO(OR')-R'$;

wherein R^6 comprises:

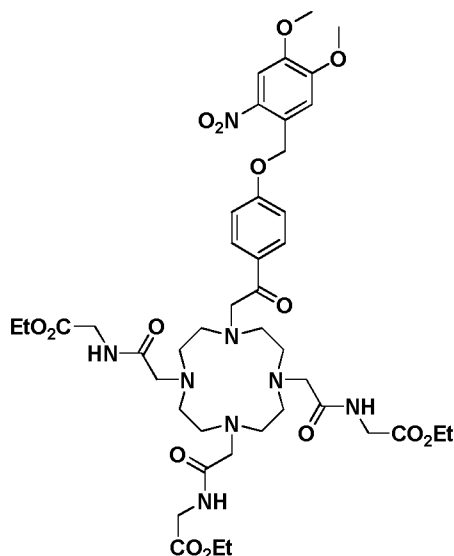


; and

wherein R' is selected from the group consisting of H, an alkyl group having 20 carbon atoms or less, a cycloalkyl group having 20 carbon atoms or less, and an alkyloxy group having
15 20 carbon atoms or less and 10 oxygen atoms or less.

23. A composition of matter comprising:

a light-sensitive contrast agent usable as a composition for a drug delivery system comprising the formula:



24. A method for delivering a drug to a patient, the method comprising:

combining the light-sensitive contrast agent of claim 23 with the drug to form a combined light-sensitive drug delivery system;

delivering the combined light-sensitive drug delivery system into the patient; and

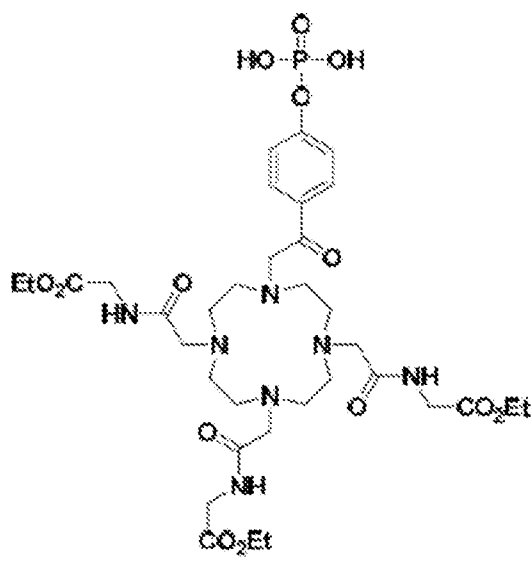
exposing the combined light-sensitive drug delivery system to electromagnetic radiation to release the drug into the patient.

25. The method of claim 24, further comprising:

detecting a concentration of the drug in the patient.

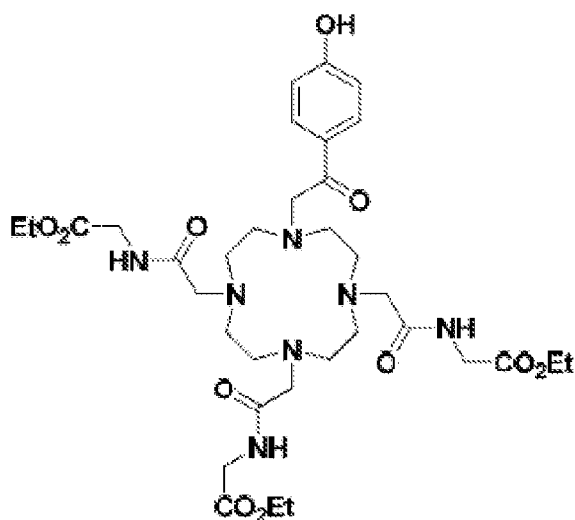
26. The method of claim 24, wherein the electromagnetic radiation comprises at least one of ultraviolet or near-infrared radiation.

27. A method of synthesizing a first compound having the formula:



the method comprising:

providing a second compound having the formula:



providing phosphoryl chloride;

providing triethylamine;

adding the second compound, the phosphoryl chloride, and the triethylamine to CH_2Cl_2 to form a solution;

10 stirring the solution for a predetermined period of time;

extracting the solution with water, the water forming a water layer;

evaporating the water layer to form a solid; and

subjecting the solid to high-performance liquid chromatography to form a product containing the first compound.

28. The method of claim 27, wherein providing the second compound comprises providing one molar equivalence of the second compound;

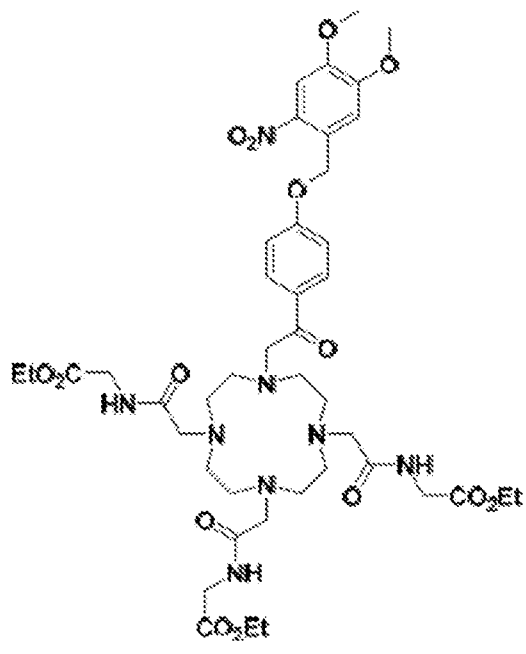
wherein providing the phosphoryl chloride comprises providing four molar equivalences of the phosphoryl chloride; and

5 wherein providing the triethylamine comprises providing three molar equivalences of the triethylamine.

29. The method of claim 28, wherein stirring the solution for the predetermined period of time comprises stirring the solution for approximately 24 hours; and

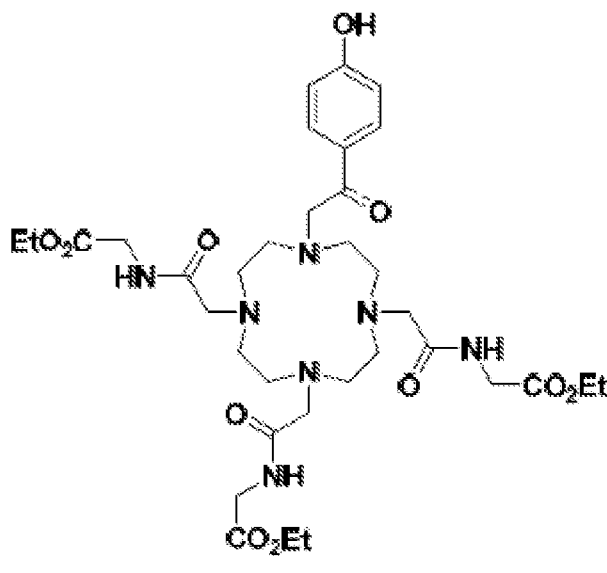
wherein the solid is a yellow solid.

10 30. A method of synthesizing a first compound having the formula:



the method comprising:

providing a second compound having the formula:



providing 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene;

providing K_2CO_3 ;

5 adding the second compound, the 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene, and the K_2CO_3 to CH_3CN to form a mixture;

stirring the mixture under N_2 for a predetermined period of time;

filtering and evaporating the mixture until at least partially dried to form a crude compound; and

10 purifying the crude compound using flash column chromatography on silica to form a solid containing the first compound.

31. The method of claim 30, wherein providing the second compound comprises providing one molar equivalence of the second compound;

wherein providing the 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene comprises providing one molar equivalence of the 1-(bromomethyl)-4,5-dimethoxy-2-nitrobenzene; and

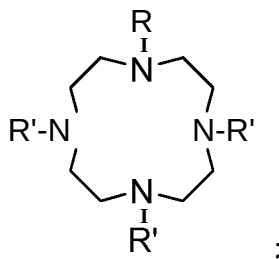
15 wherein providing the K_2CO_3 comprises providing one molar equivalence of the K_2CO_3 .

32. The method of claim 31, wherein stirring the mixture under N_2 for the predetermined period of time comprises stirring the mixture under N_2 for approximately 12 hours; and

wherein the solid is a yellow solid.

33. A composition of matter comprising:

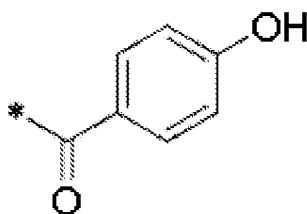
a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent comprising the formula:



;

5

wherein R comprises:



; and

wherein each R' is selected from the group consisting of CH₂CONHCH₂COOH, CH₂CONHCH₂COOC₂H₅, CH₂CONH₂, CH₂CONHCH₂PO(OC₂H₅)₂, CH₂CONHCH₂PO₃H₂,
 10 CH₂CONHCH₂PO(OC(CH₃)₂)₂, CH₂CONHCH₂PO(OCH₂CH₂CH₃)₂,
 CH₂CONHCH₂PO(OCH₂CH₂CH₂CH₃)₂, and CH₂CONHCH₂PO(OC(CH₃)₃)₂.

34. A method for obtaining a ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent of claim 33 to determine at least one of a pH level, a presence of a highly reactive oxygen species, a biological metal ion
 15 concentration, or a presence of enzyme activity.

35. The method of claim 34, wherein obtaining the ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent is performed either *in vitro* or *ex vivo*.

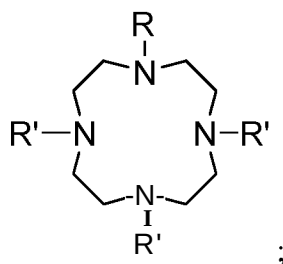
36. The method of claim 34, wherein obtaining the ratiometric imaging measurement using
 20 the paramagnetic chemical exchange saturation transfer MRI contrast agent is performed *in vivo*.

37. The composition of matter of claim 33, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent is associated with a europium (III).

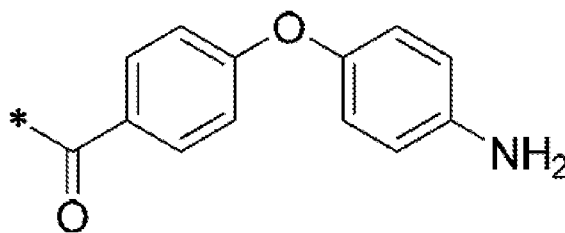
38. The composition of matter of claim 33, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent produces a frequency shift in a chemical exchange saturation transfer exchange peak.

39. A composition of matter comprising:

5 a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent comprising the formula:



wherein R comprises:



; and

wherein each R' is selected from the group consisting of CH₂CONHCH₂COOH, CH₂CONHCH₂COOC₂H₅, CH₂CONH₂, CH₂CONHCH₂PO(OC₂H₅)₂, CH₂CONHCH₂PO₃H₂, CH₂CONHCH₂PO(OC(CH₃)₂)₂, CH₂CONHCH₂PO(OCH₂CH₂CH₃)₂, CH₂CONHCH₂PO(OCH₂CH₂CH₂CH₃)₂, and CH₂CONHCH₂PO(OC(CH₃)₃)₂.

15 40. A method for obtaining a ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent of claim 39 to determine at least one of a pH level, a presence of a highly reactive oxygen species, a biological metal ion concentration, an oxygen concentration, or a presence of enzyme activity.

41. The method of claim 40, wherein obtaining the ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent is performed either *in vitro* or *ex vivo*.

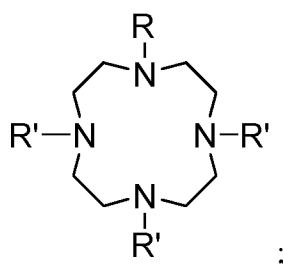
42. The method of claim 40, wherein obtaining the ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent is performed *in vivo*.

43. The composition of matter of claim 39, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent is associated with a europium (III).

44. The composition of matter of claim 39, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent produces a frequency shift in a chemical exchange saturation transfer exchange peak.

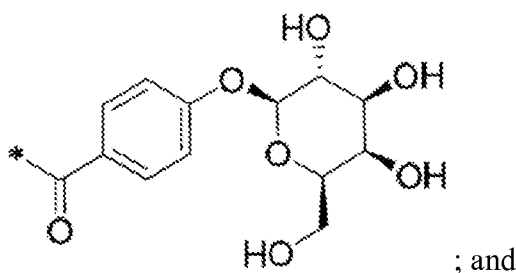
45. A composition of matter comprising:

a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent comprising the formula:



10

wherein R comprises:



wherein each R' is selected from the group consisting of CH₂CONHCH₂COOH, CH₂CONHCH₂COOC₂H₅, CH₂CONH₂, CH₂CONHCH₂PO(OC₂H₅)₂, CH₂CONHCH₂P(O)₃H₂, CH₂CONHCH₂PO(OC(CH₃)₂)₂, CH₂CONHCH₂PO(OCH₂CH₂CH₃)₂, CH₂CONHCH₂PO(OCH₂CH₂CH₂CH₃)₂, and CH₂CONHCH₂PO(OC(CH₃)₃)₂.

15

46. A method for obtaining a ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent of claim 45 to determine at least one of a pH level, a presence of a highly reactive oxygen species, a biological metal ion concentration, or a presence of enzyme activity.

20

47. The method of claim 46, wherein obtaining the ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent is performed either *in vitro* or *ex vivo*.

48. The method of claim 46, wherein obtaining the ratiometric imaging measurement using the paramagnetic chemical exchange saturation transfer MRI contrast agent is performed *in vivo*.

49. The composition of matter of claim 45, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent is associated with a europium (III).

5 50. The composition of matter of claim 45, wherein the paramagnetic chemical exchange saturation transfer MRI contrast agent produces a frequency shift in a chemical exchange saturation transfer exchange peak.

51. A paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent for determining a chemical parameter comprising a europium(III) DOTA-
10 tris(amide) complex comprising four side chains, one of the four side chains connecting an aromatic group by a carbonyl bond.

52. The paramagnetic chemical exchange saturation transfer MRI contrast agent of claim 51, wherein the europium(III) DOTA-tris(amide) complex consists of four side chains.

53. The paramagnetic chemical exchange saturation transfer MRI contrast agent of claim 51,
15 wherein the one of the four side chains connects the aromatic group by $-\text{CH}_2-\text{CO}-$.

54. The paramagnetic chemical exchange saturation transfer MRI contrast agent of claim 51, wherein the chemical parameter is at least one of a pH level, a presence of a highly reactive oxygen species, a biological metal ion concentration, an oxygen concentration, or a presence of enzyme activity.

55. A method for determining one or more parameters comprising:

obtaining a paramagnetic chemical exchange saturation transfer magnetic resonance imaging (MRI) contrast agent comprising a europium(III) DOTA-tris(amide) complex comprising four side chains, one of the four side chains connecting an aromatic group by a carbonyl bond, the paramagnetic chemical exchange saturation transfer MRI contrast agent adapted to provide a ratiometric imaging measurement;

administering the paramagnetic chemical exchange saturation transfer MRI contrast agent to a patient; and

detecting a signal in the patient that correlates to one or more parameters, the one or more parameters comprising at least one of a pH level, a presence of a highly reactive oxygen species, a biological metal ion concentration, an oxygen concentration, a presence of enzyme activity, a temperature, a metabolite concentration, or an O₂ partial pressure.

56. The method of claim 55, wherein the one of the four side chains connects the aromatic group by -CH₂-CO-.

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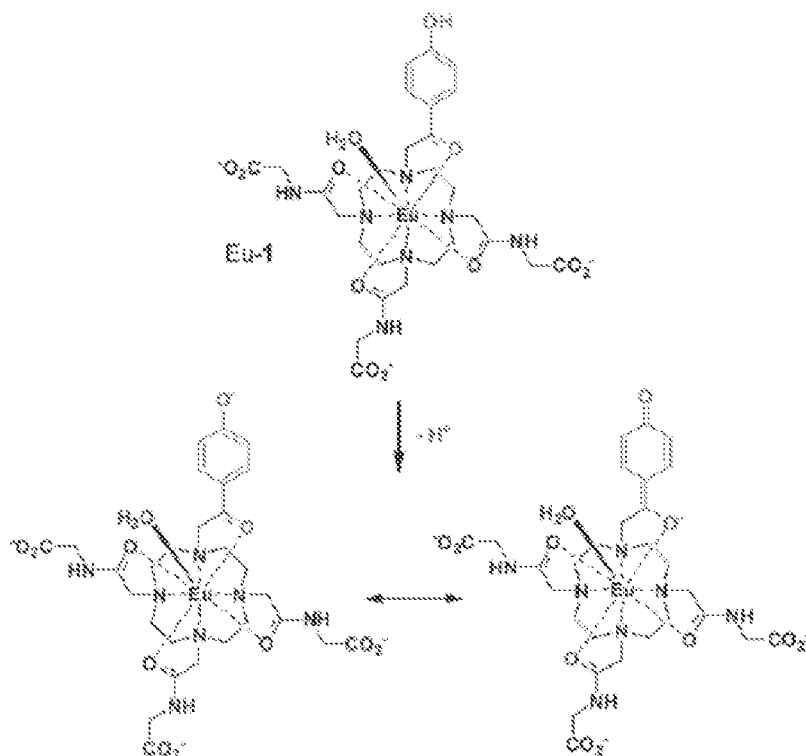


Figure 1

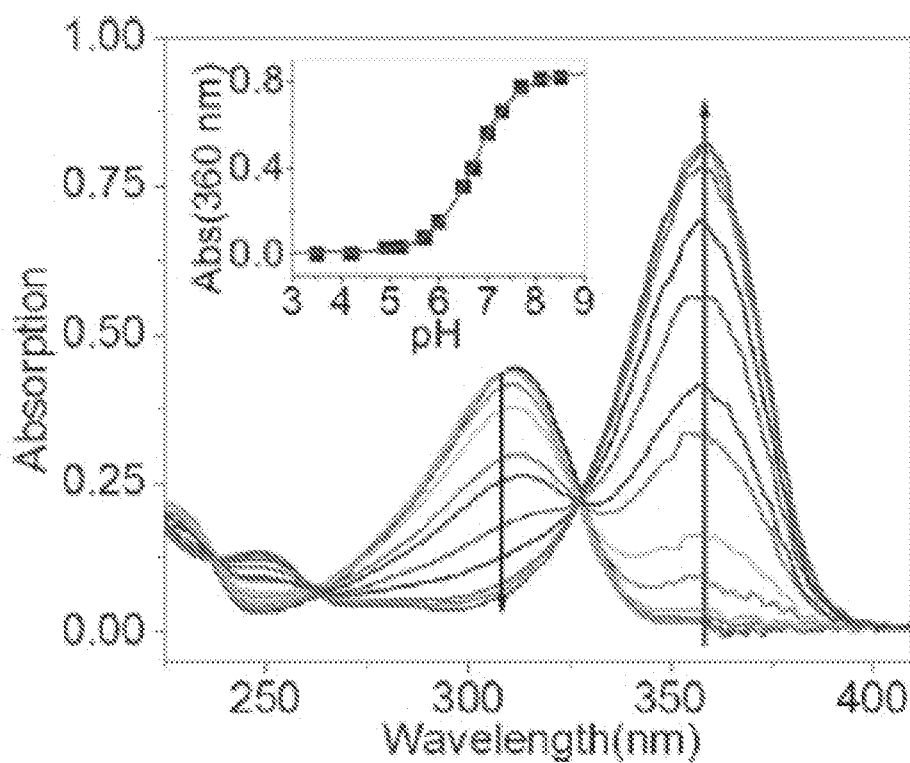


Figure 2

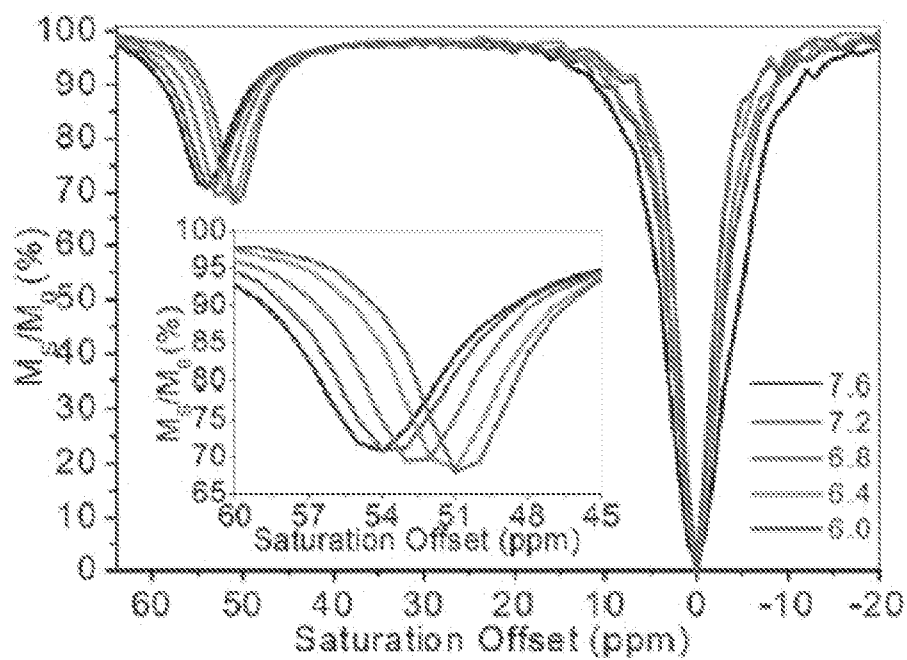


Figure 3

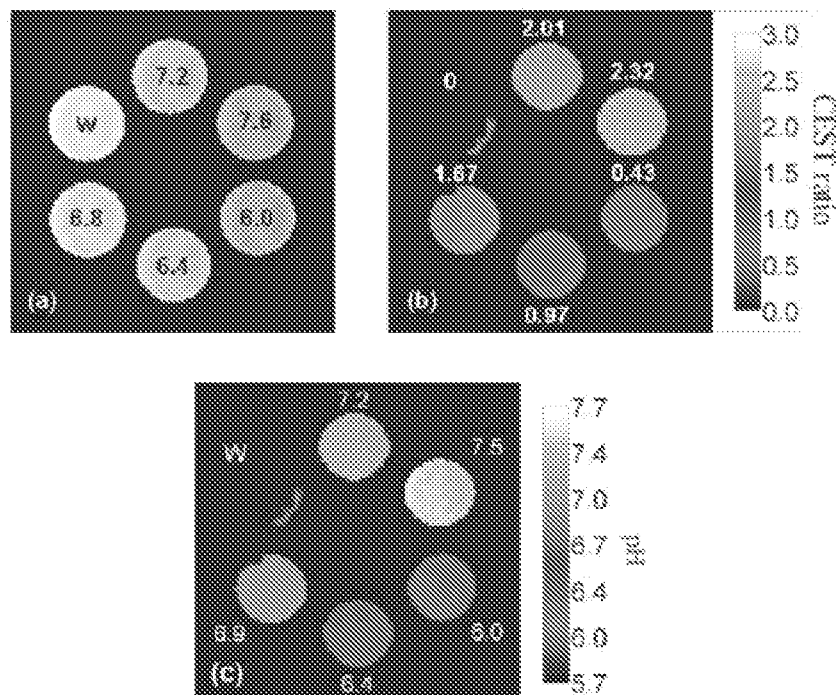


Figure 4

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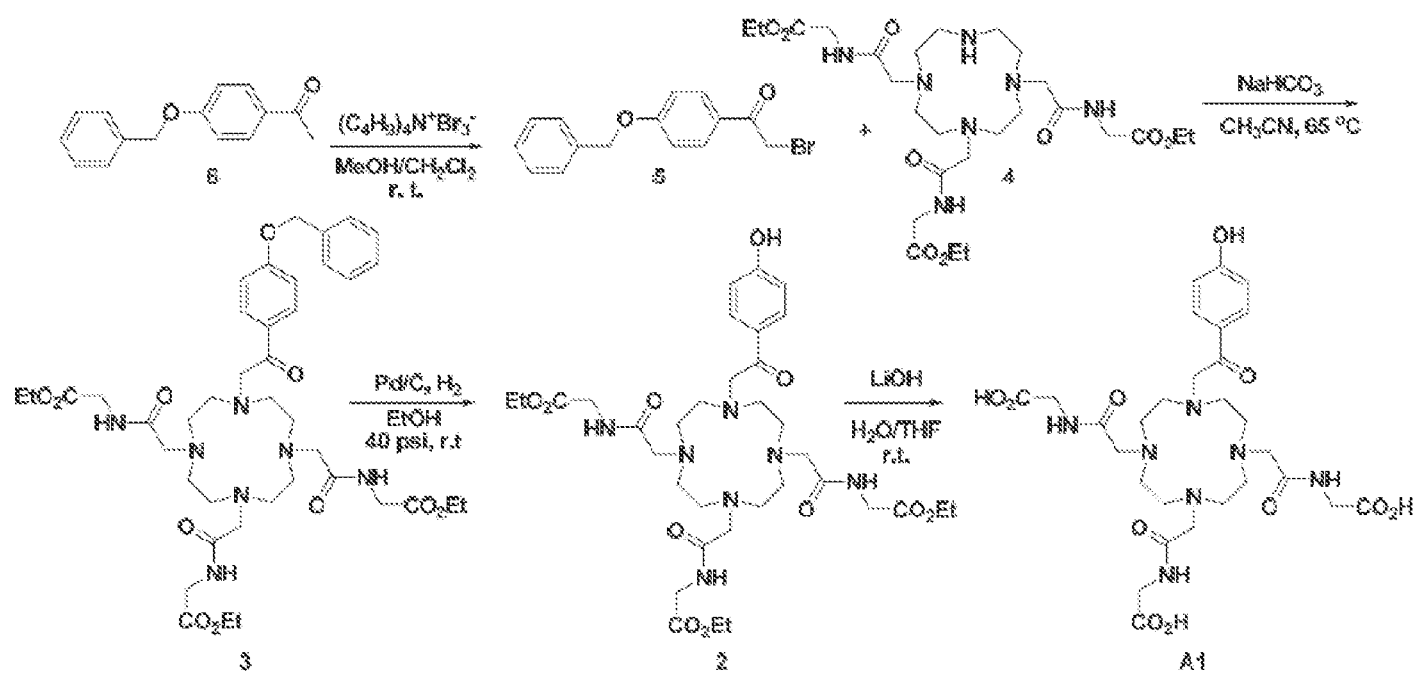


Figure 5

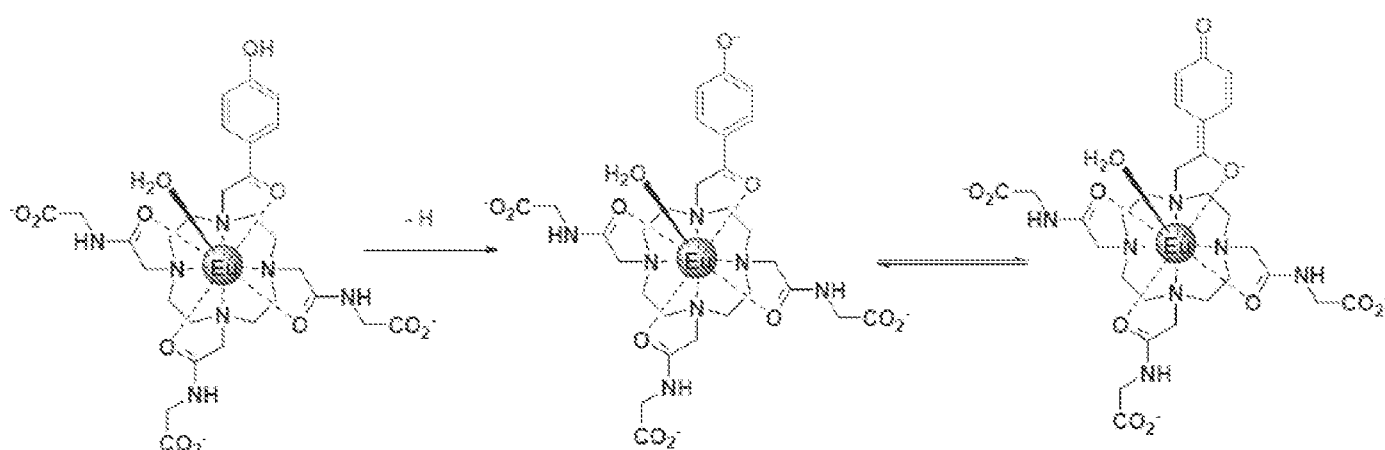
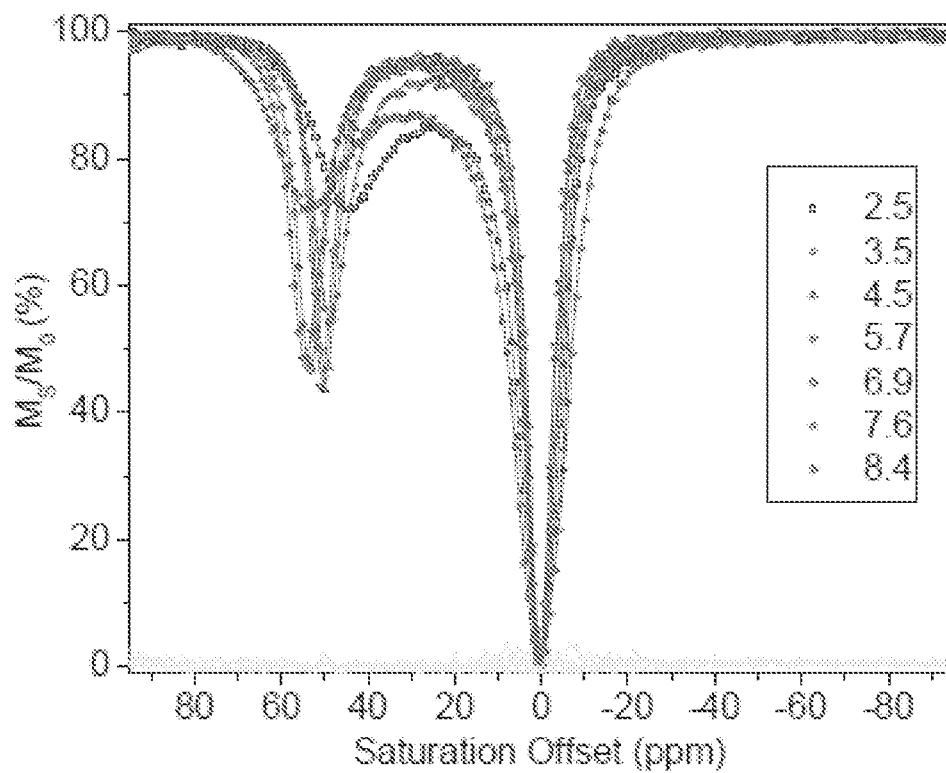
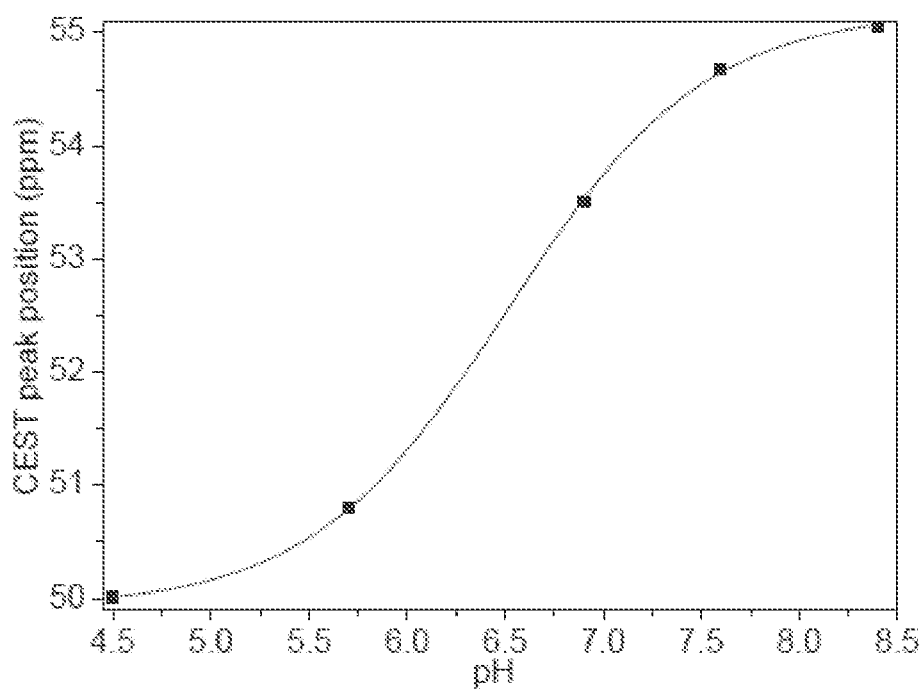
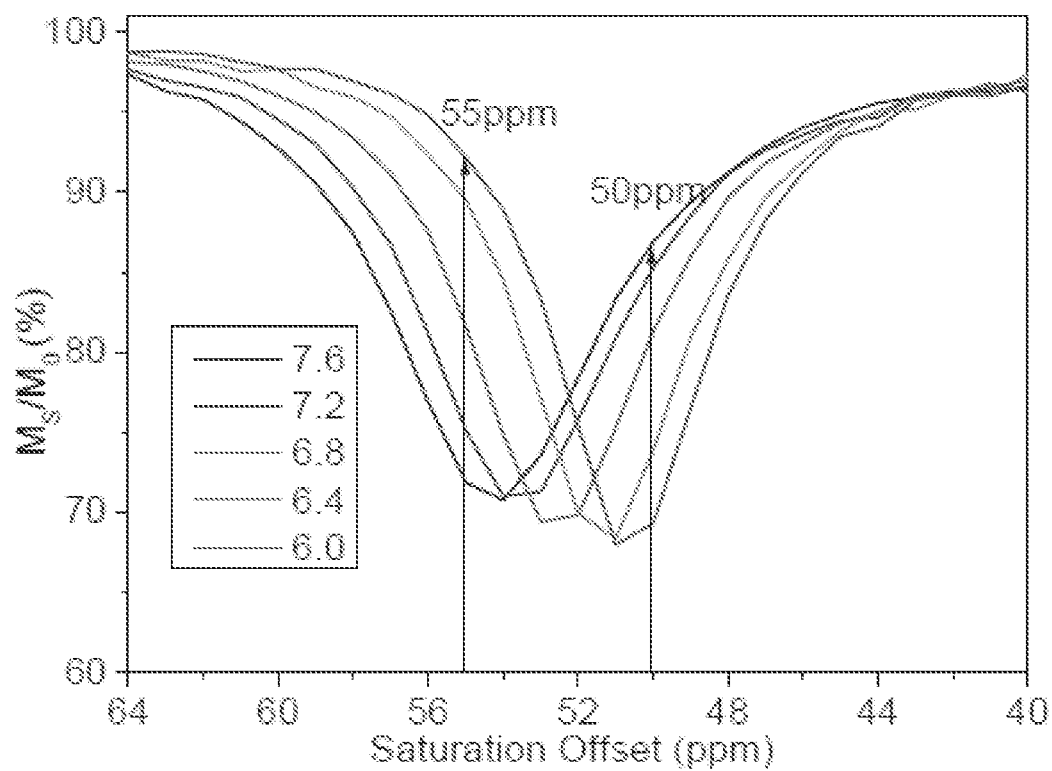
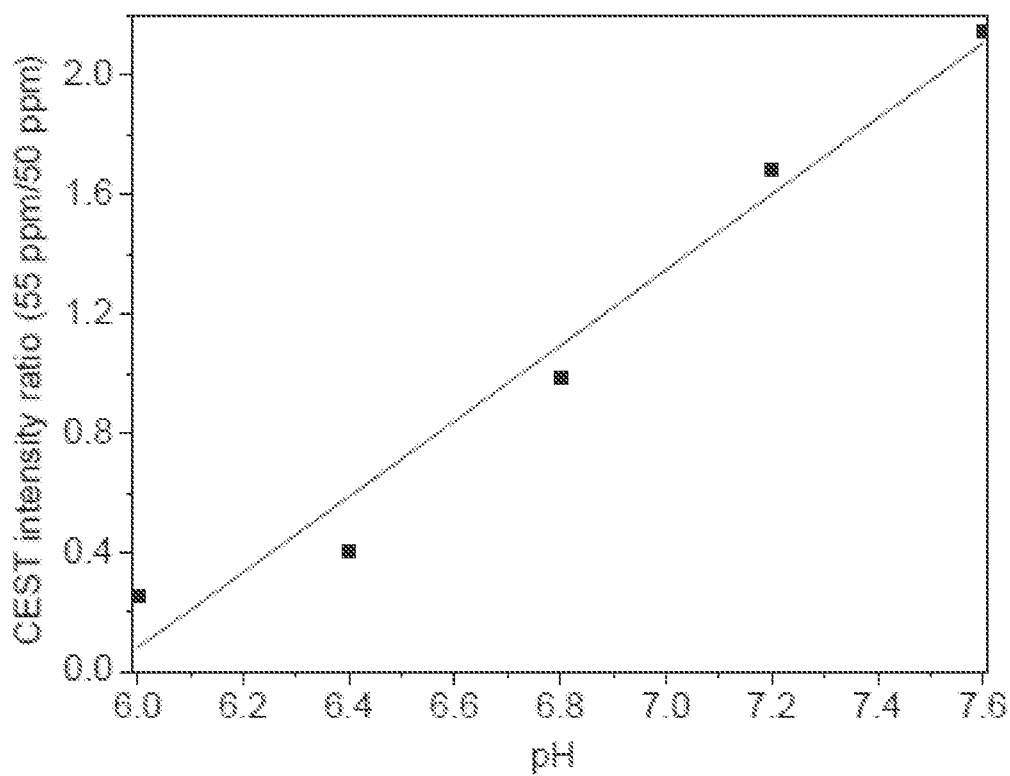


Figure 6

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**Figure 7****Figure 8**

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**Figure 9****Figure 10**

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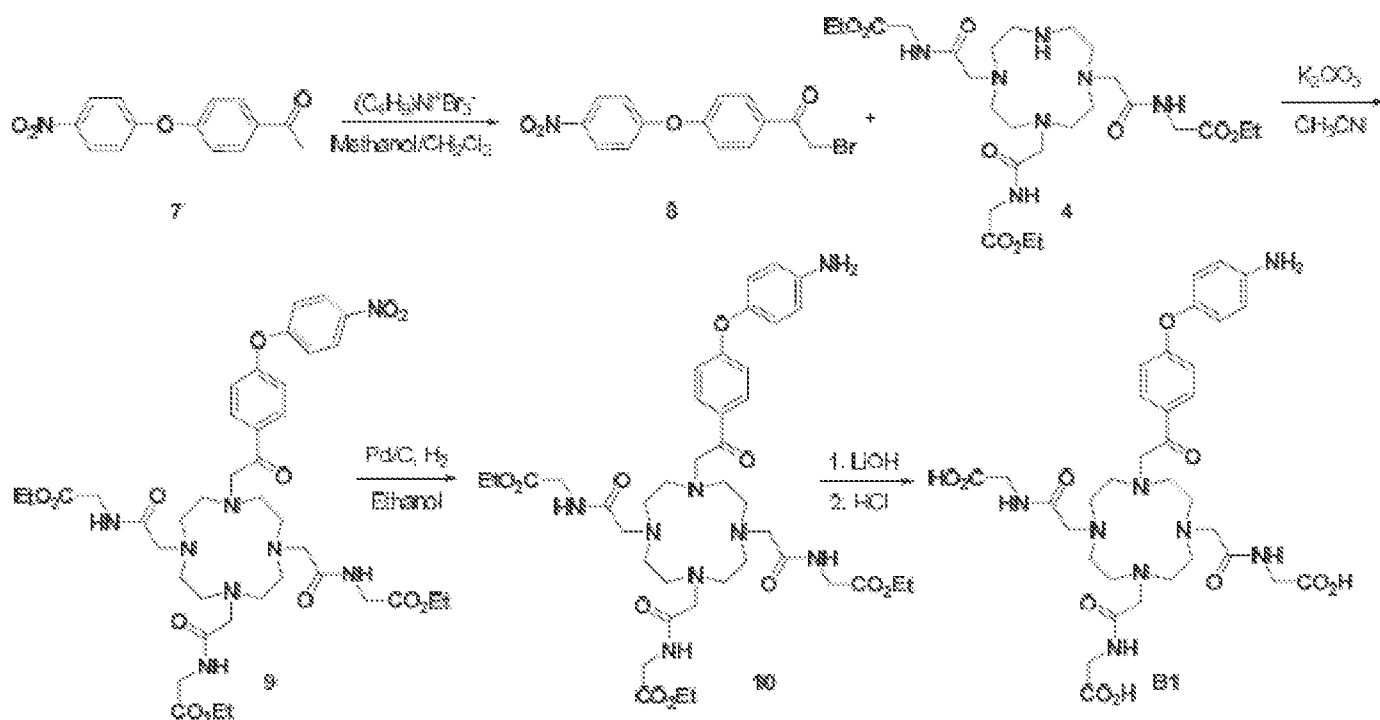


Figure 11

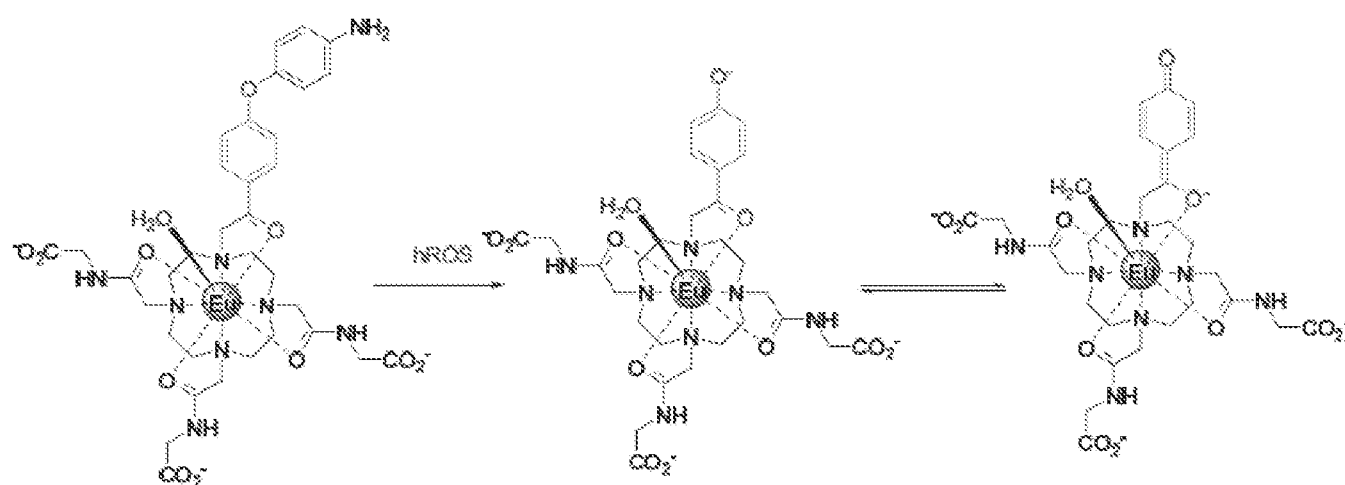
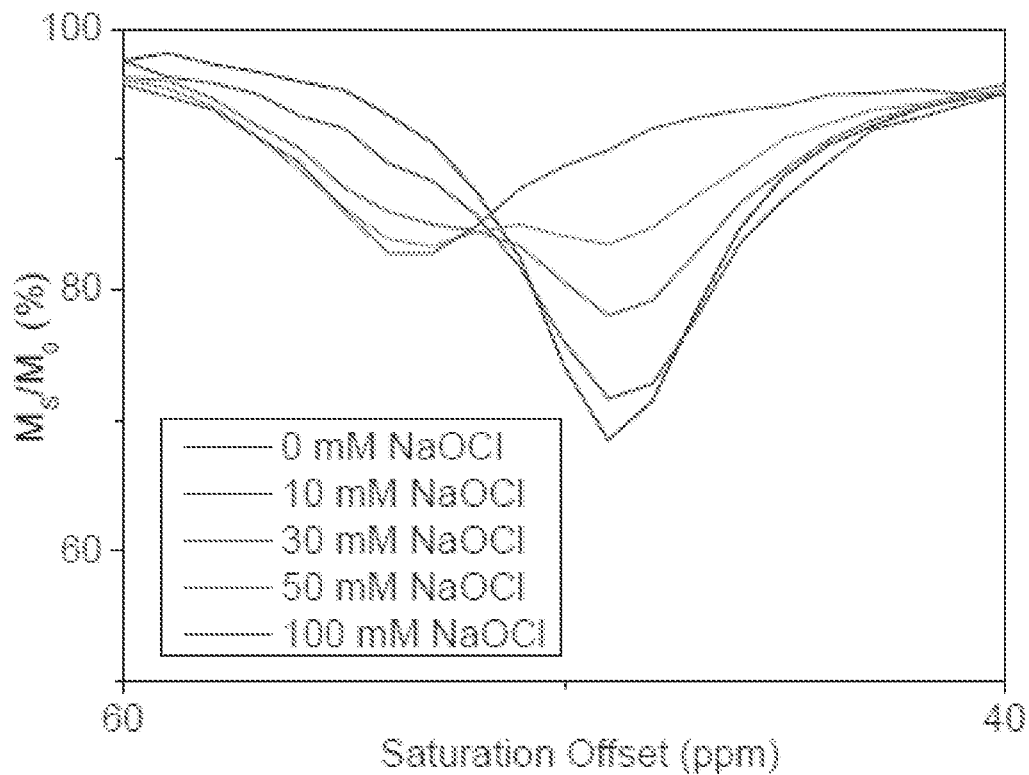
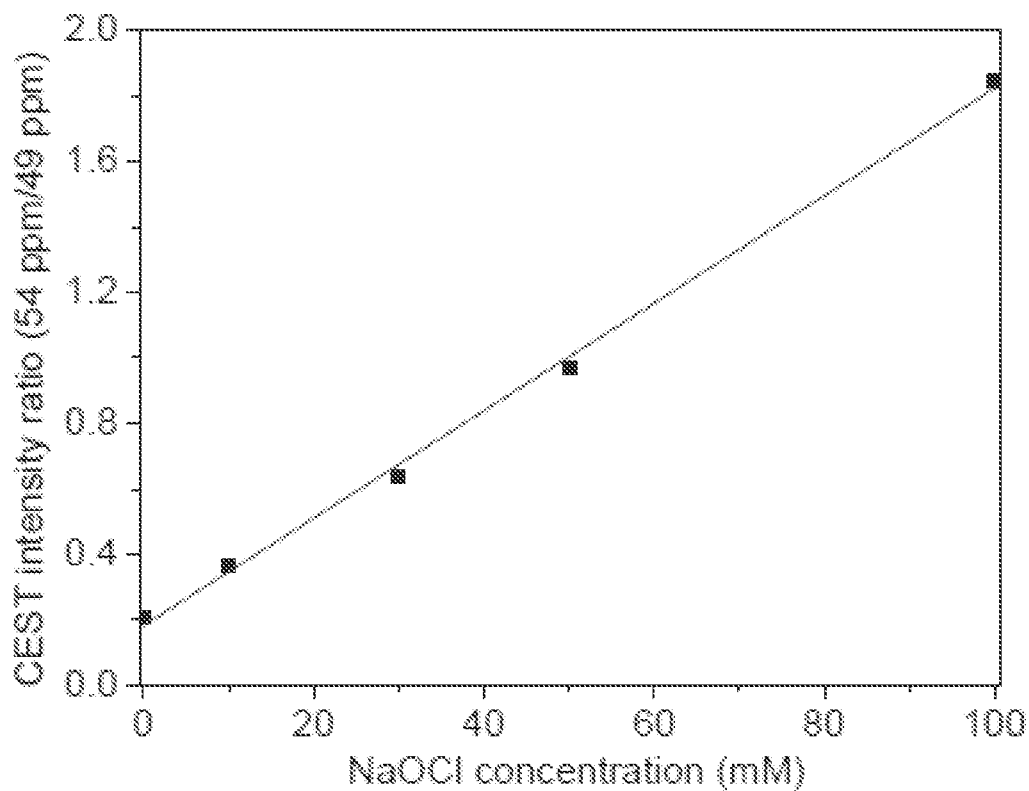


Figure 12

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**Figure 13****Figure 14**

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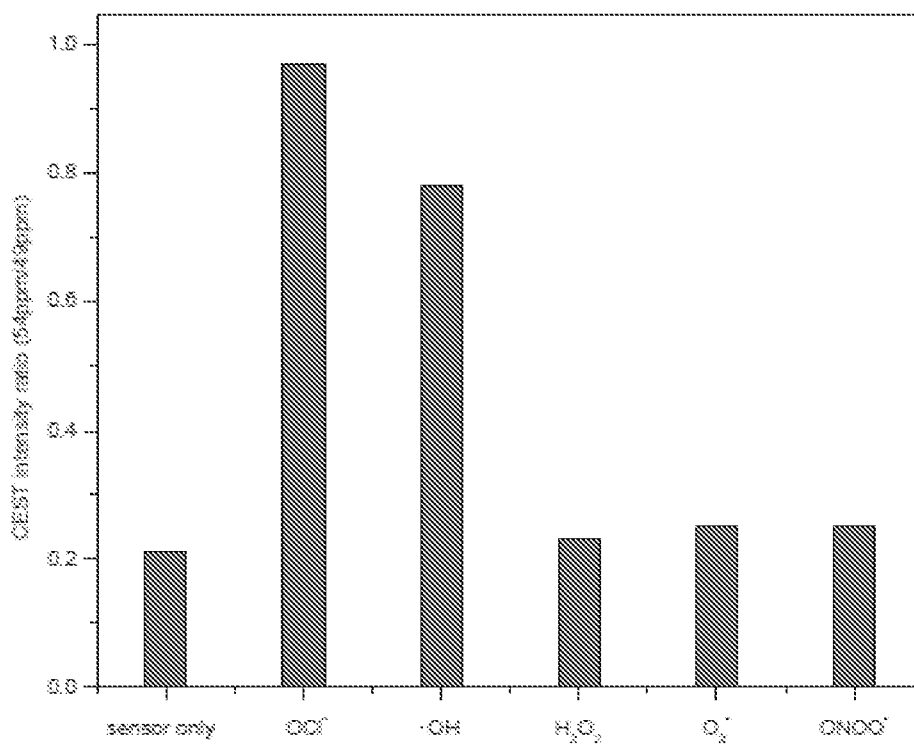


Figure 15

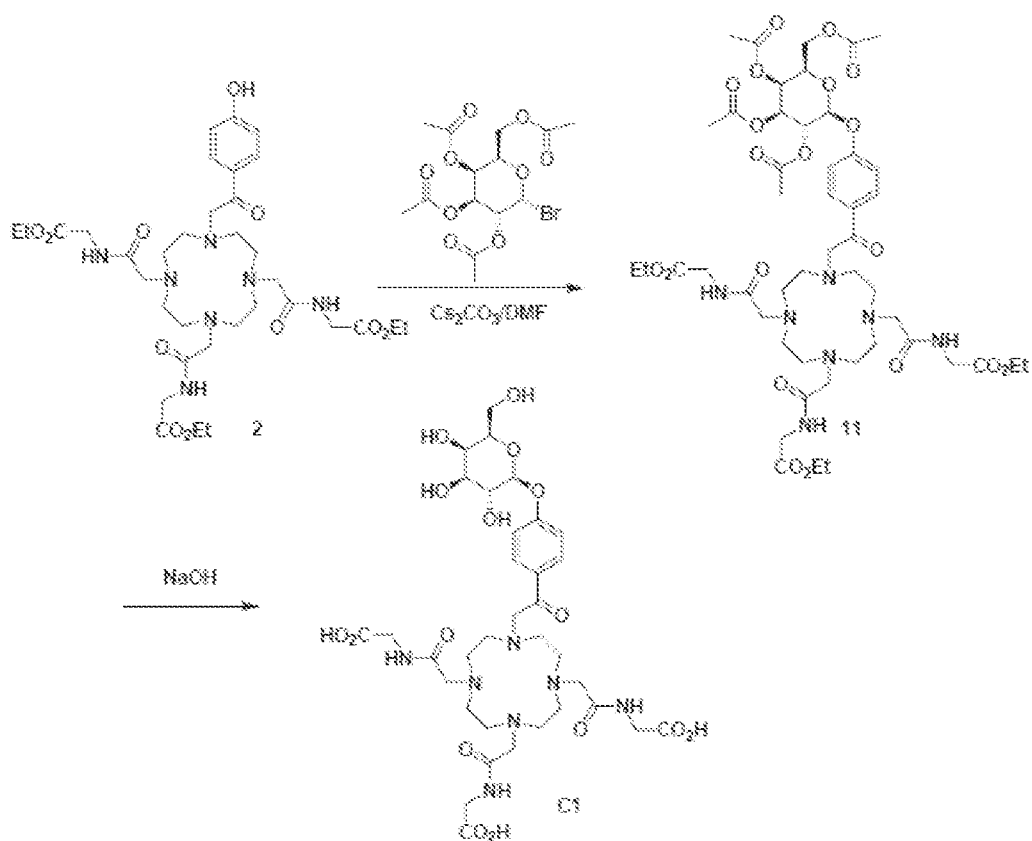


Figure 16

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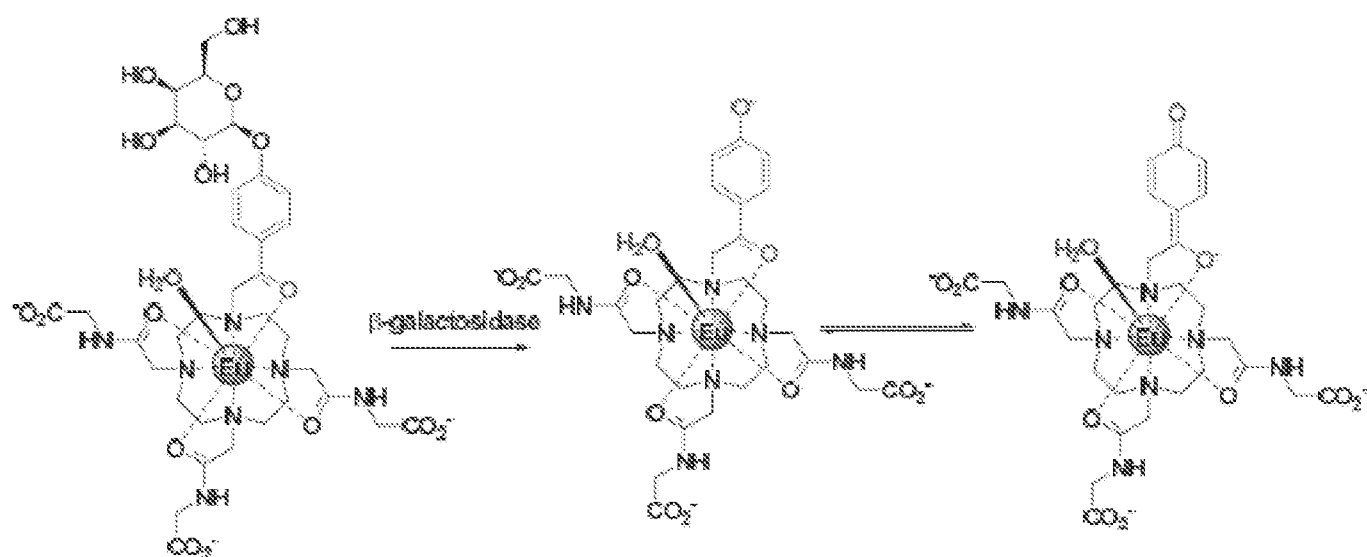


Figure 17

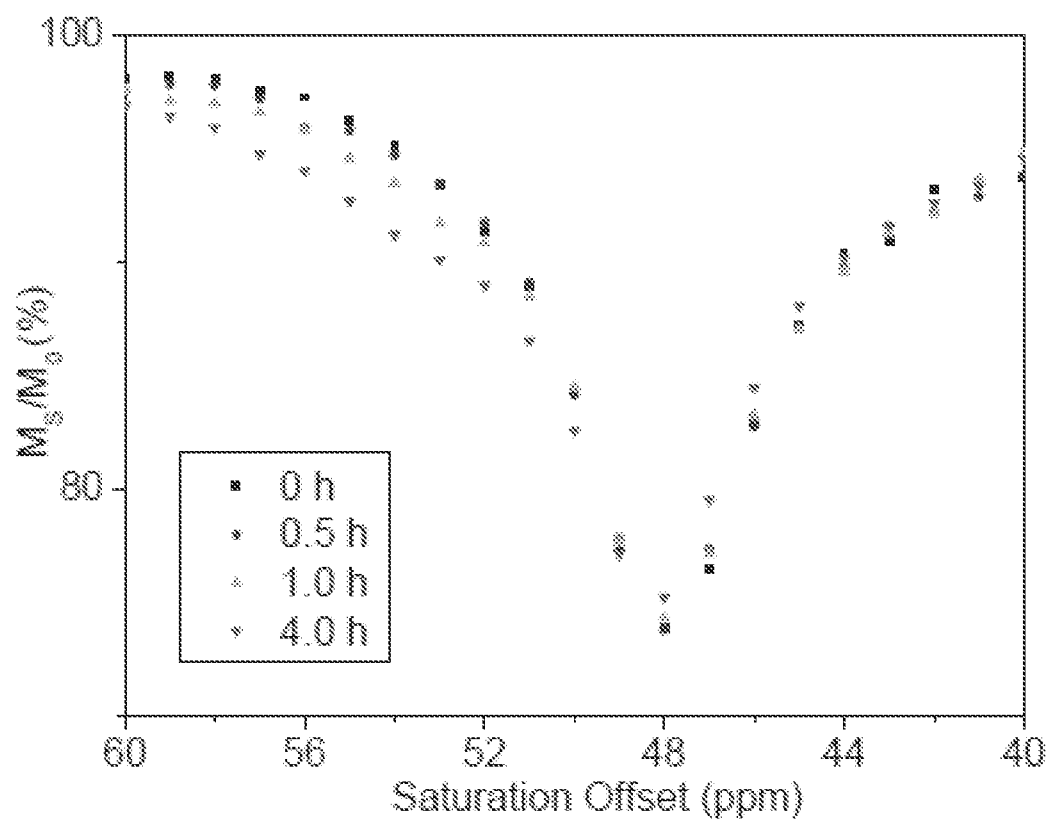


Figure 18

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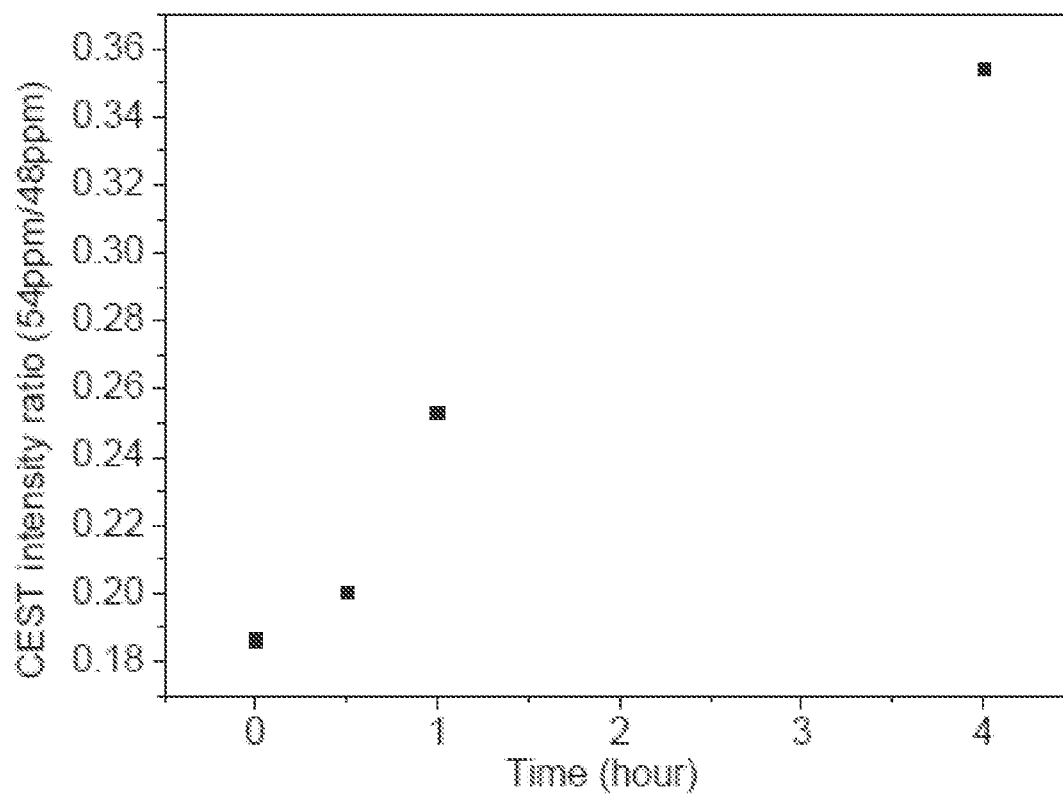


Figure 19

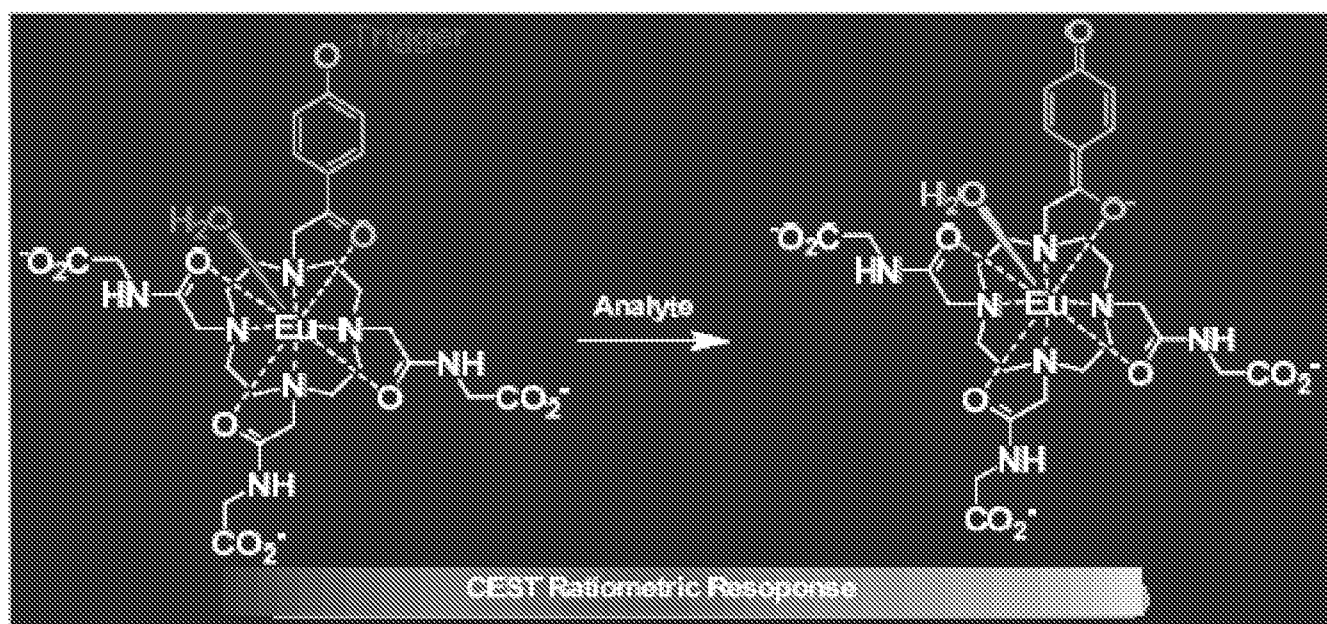


Figure 20

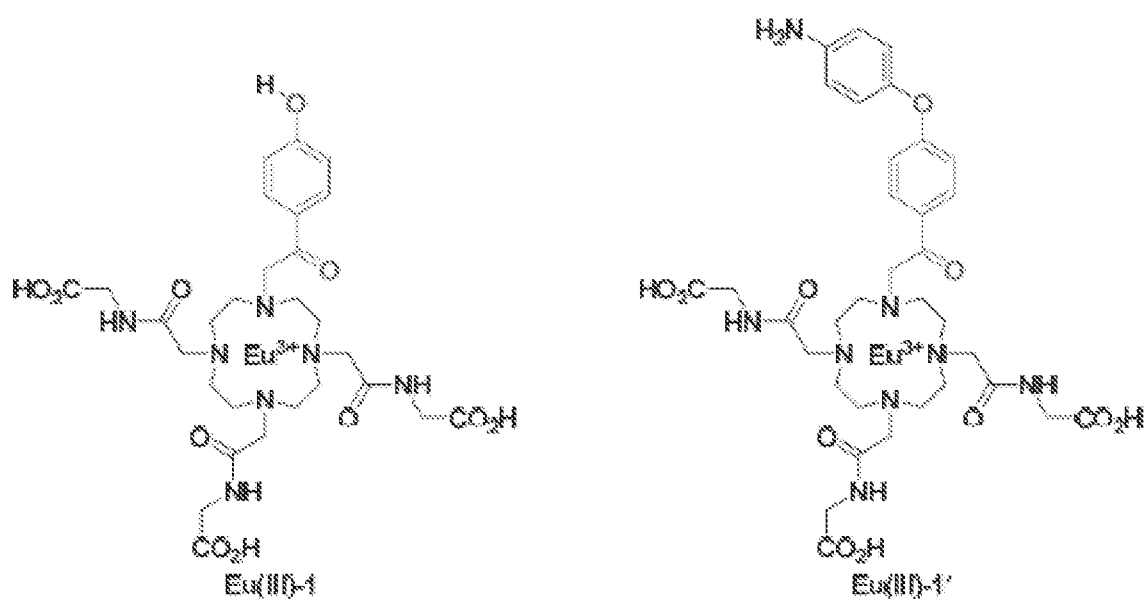


Figure 21

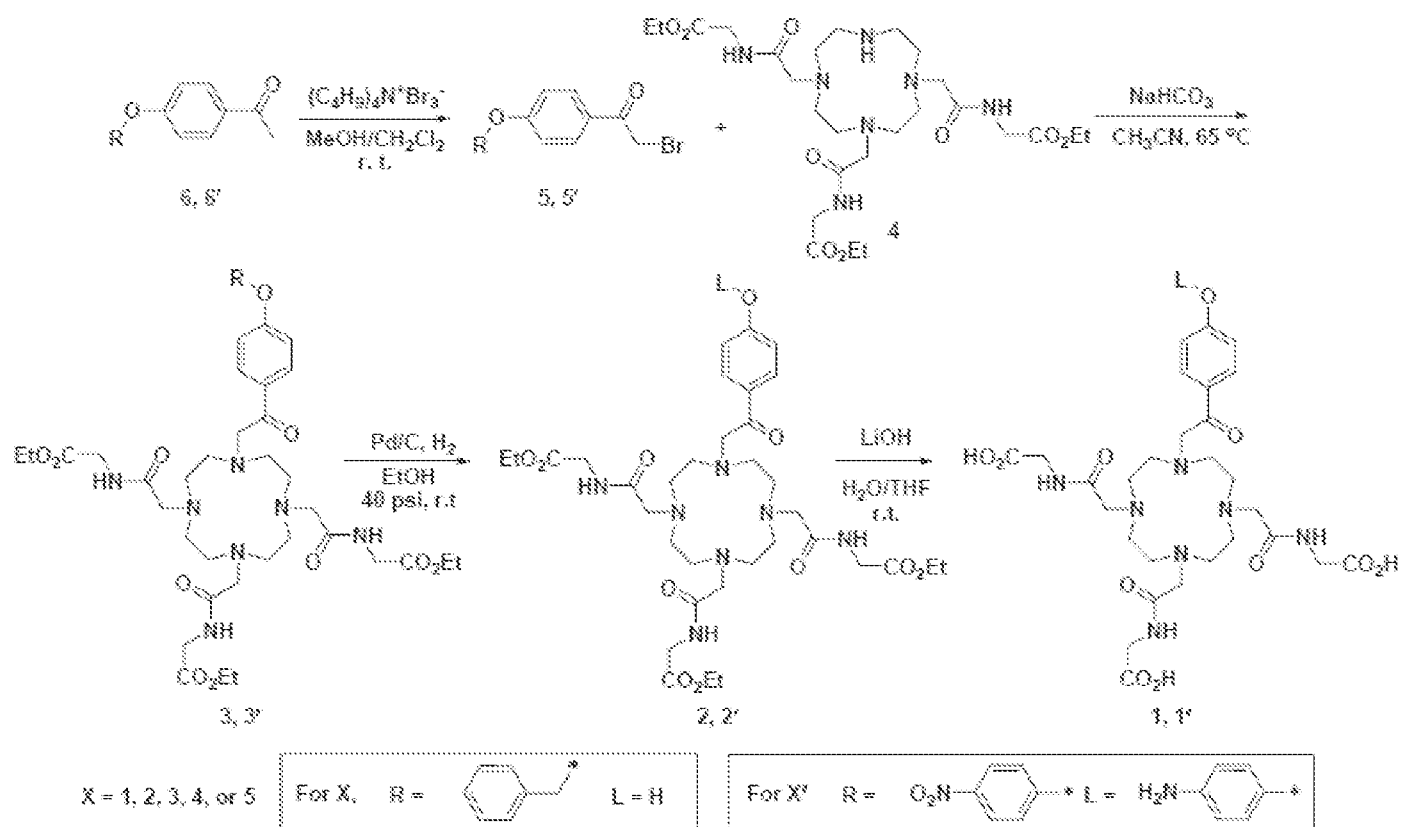


Figure 22

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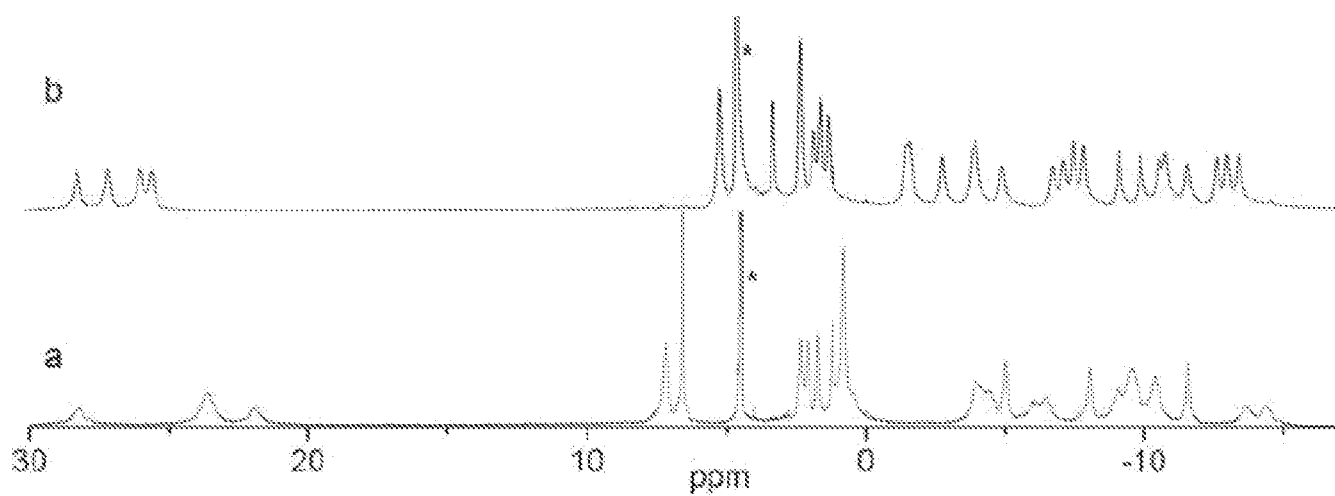


Figure 23

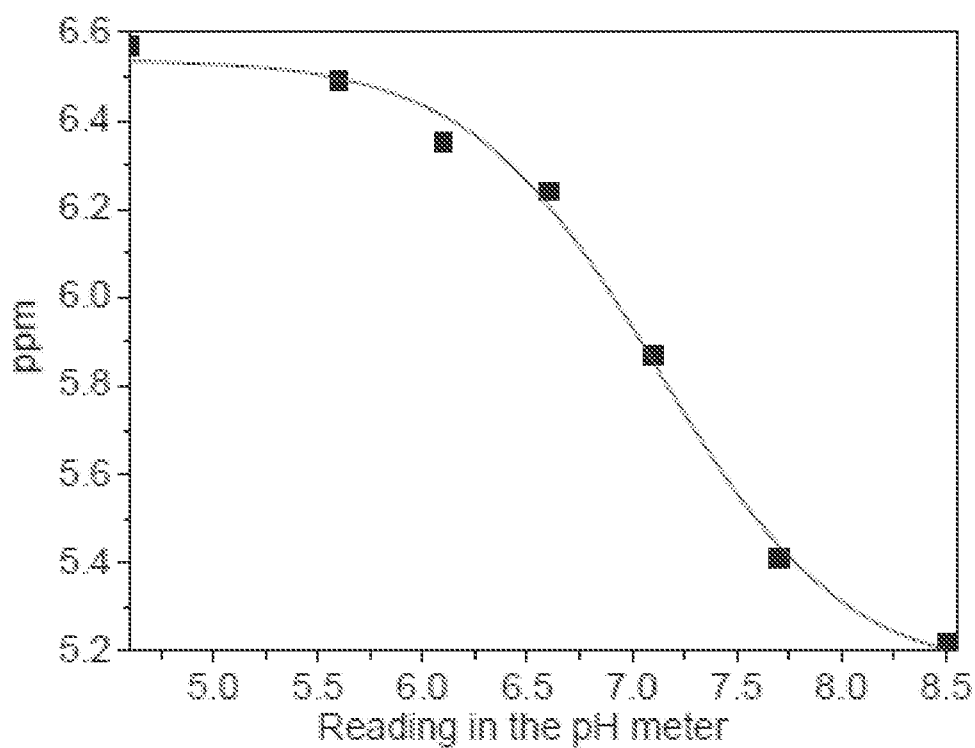


Figure 24

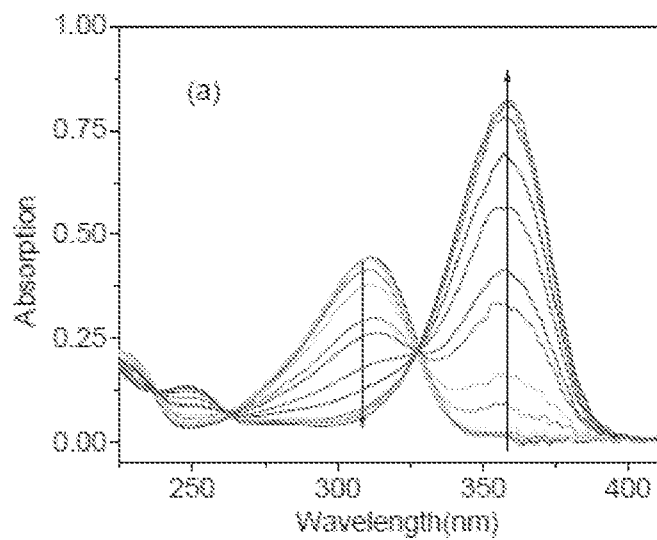


Figure 25A

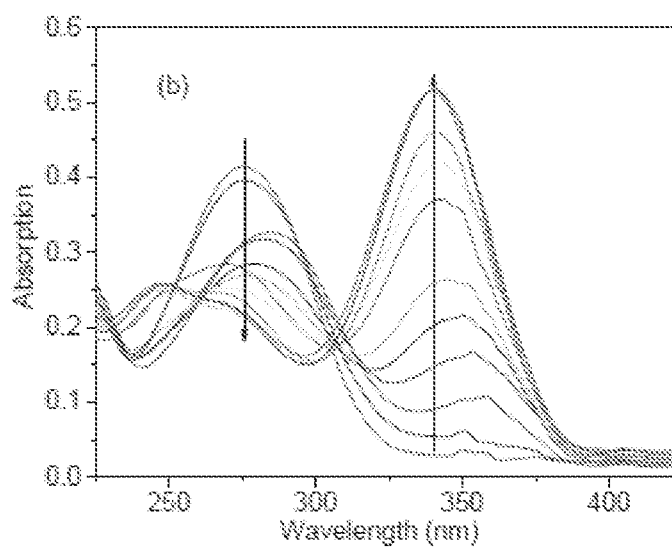


Figure 25B

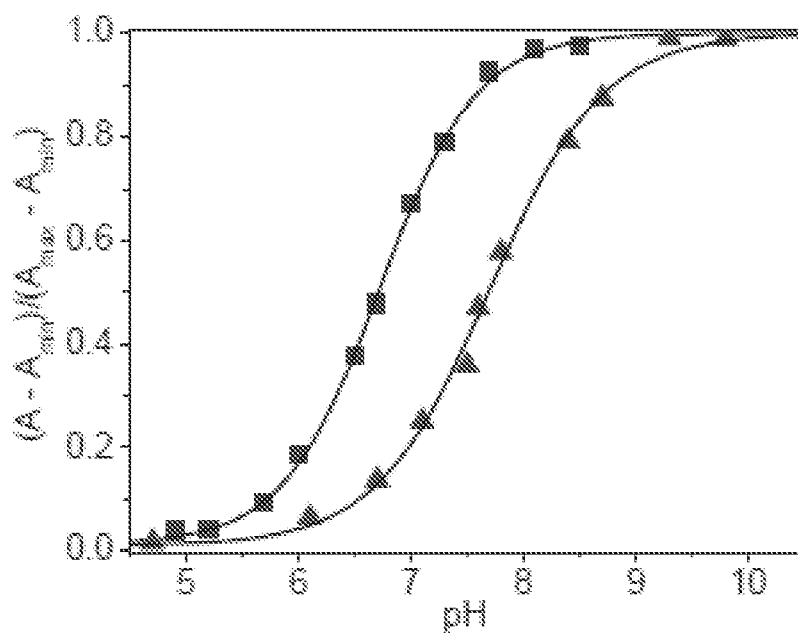


Figure 26

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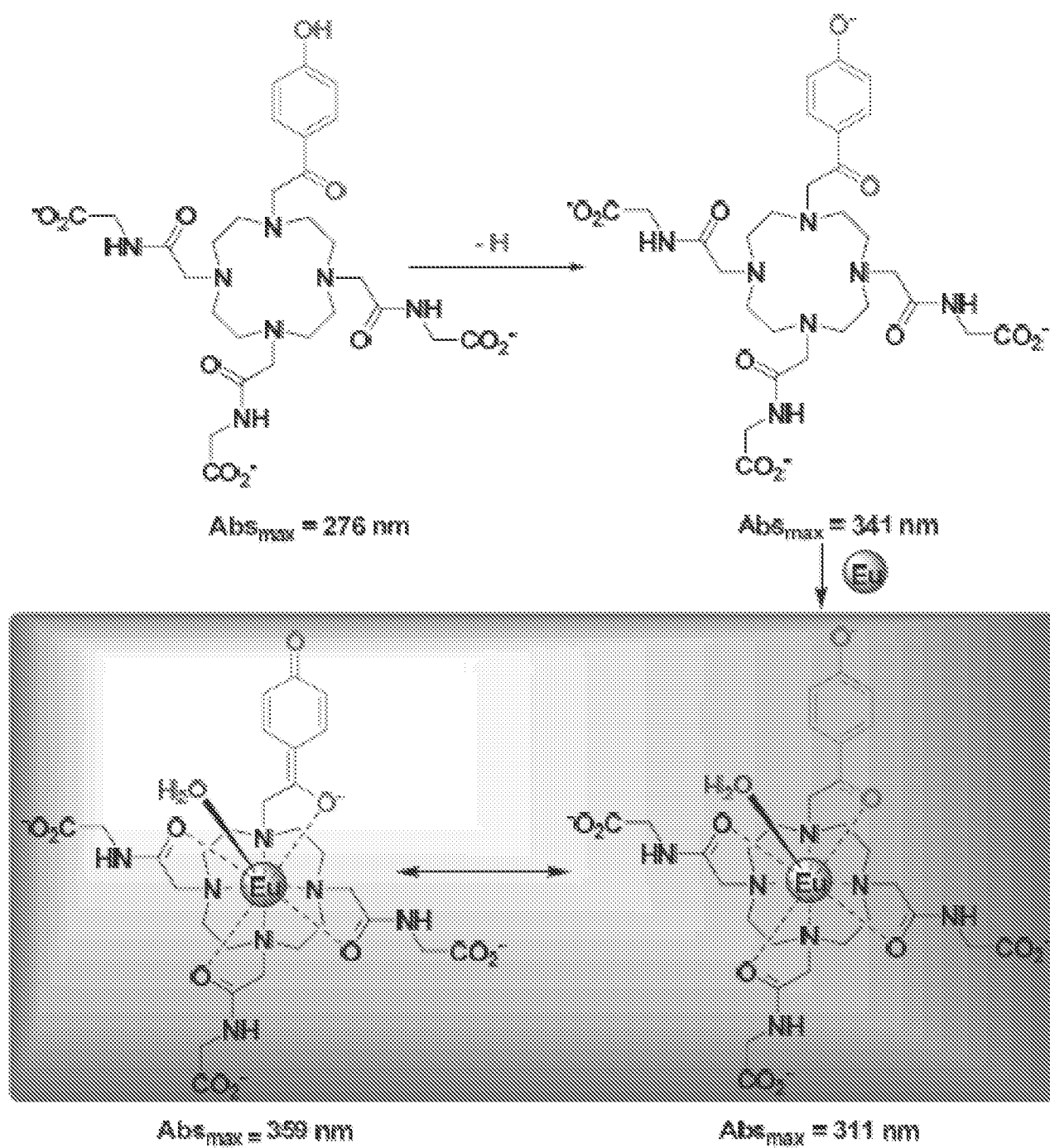
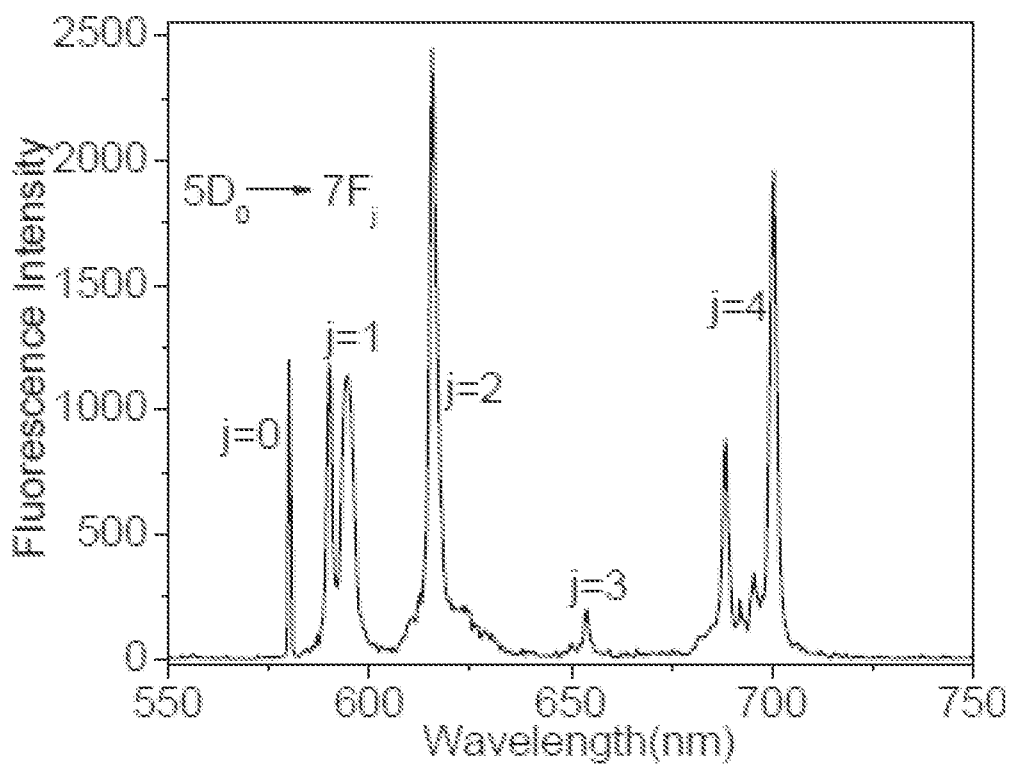
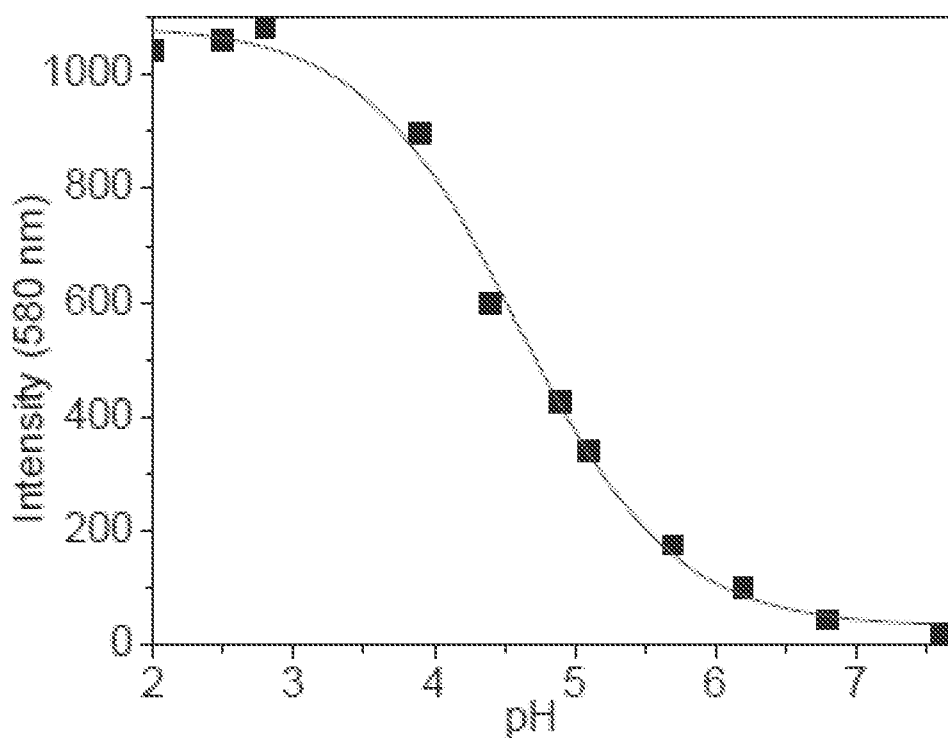


Figure 27

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**Figure 28****Figure 29**

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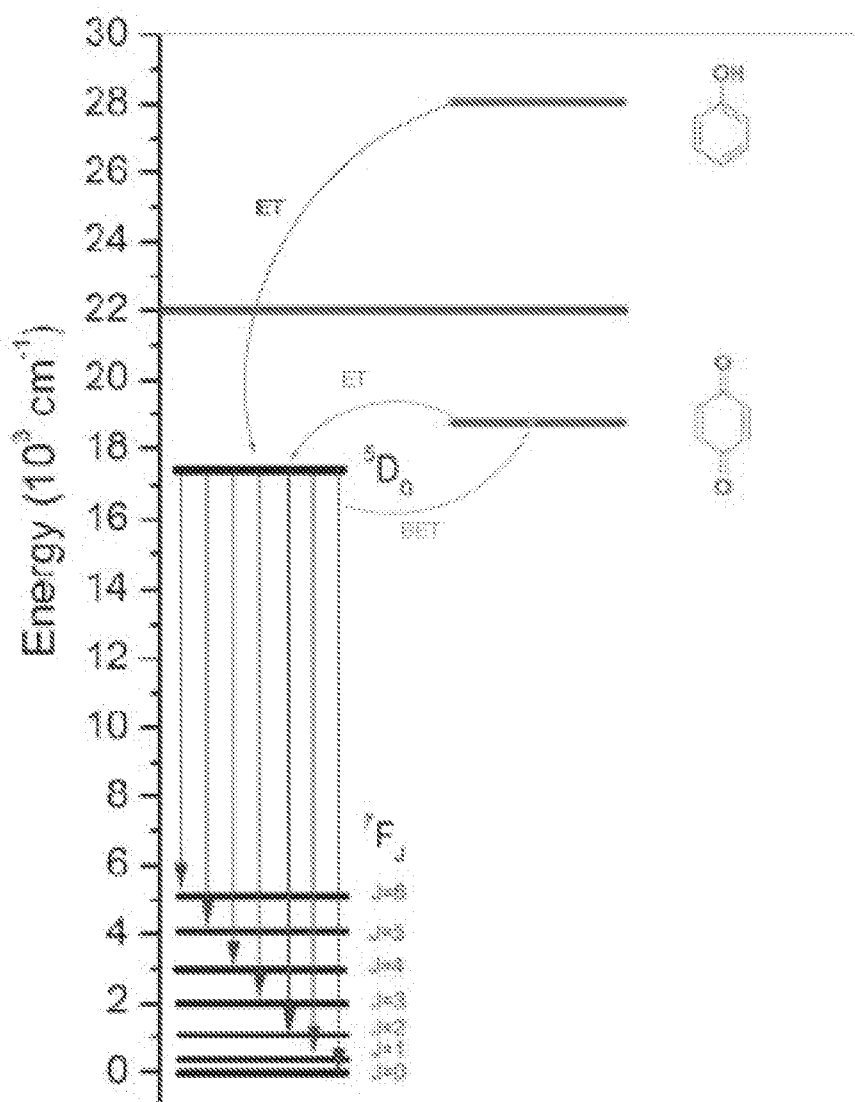


Figure 30

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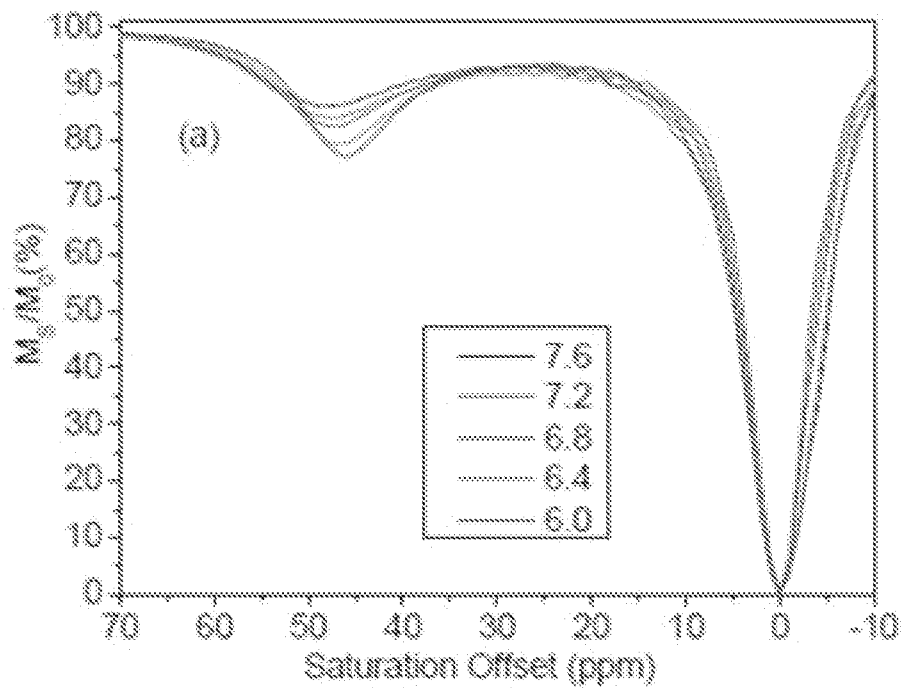


Figure 31

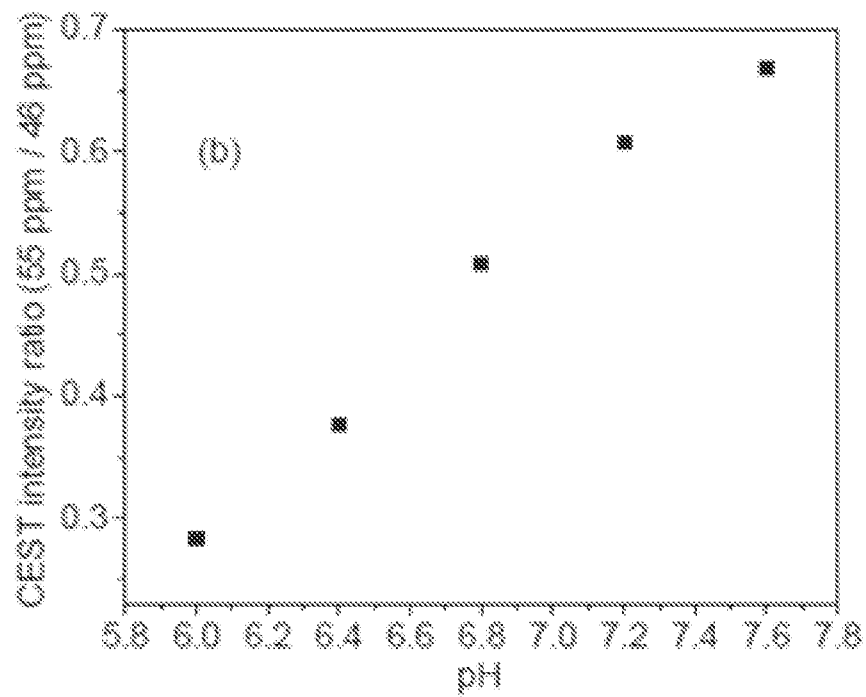


Figure 32

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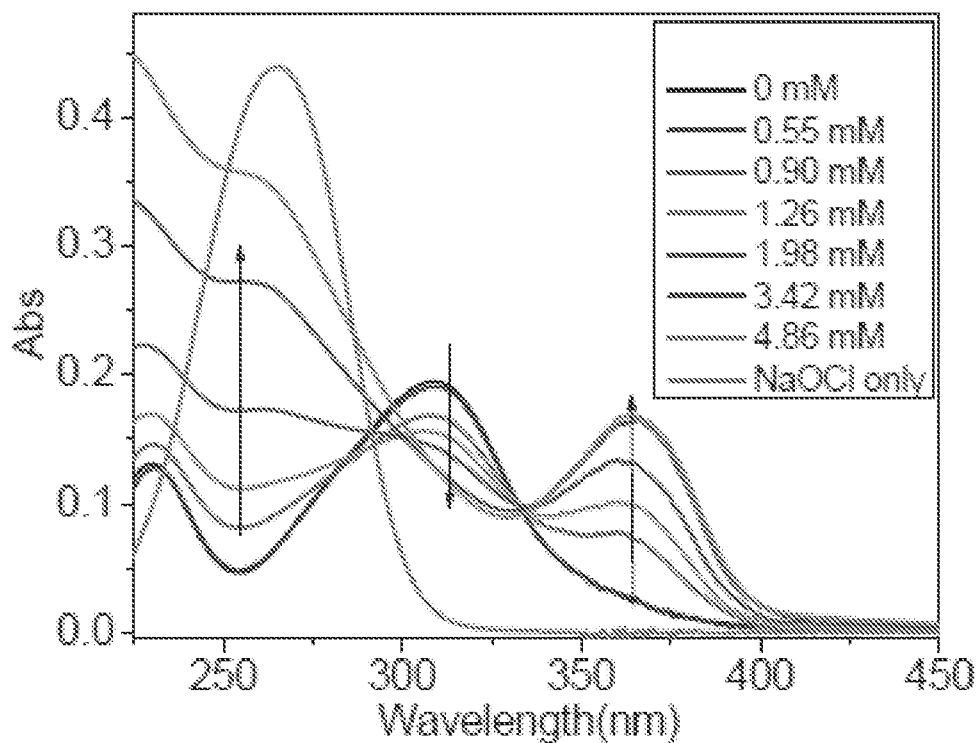


Figure 33

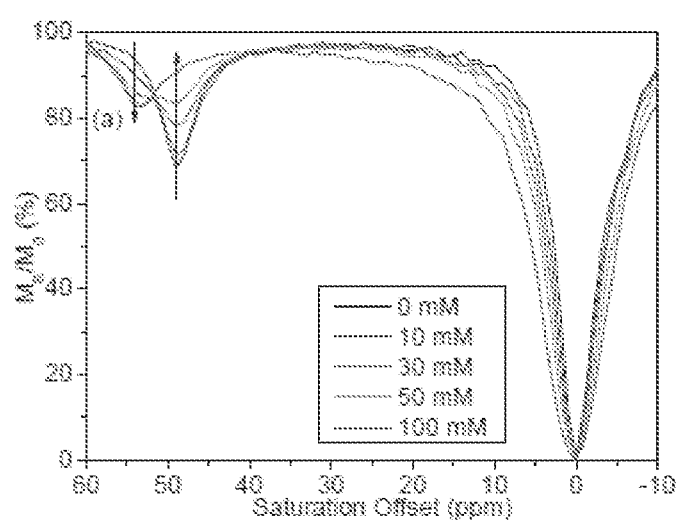


Figure 34A

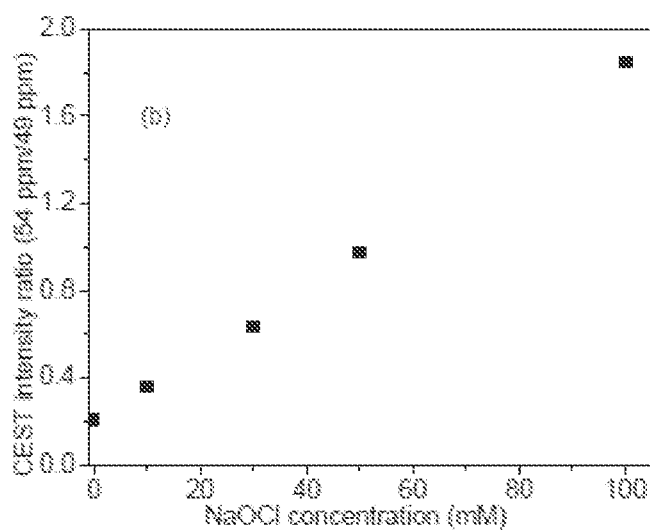


Figure 34B

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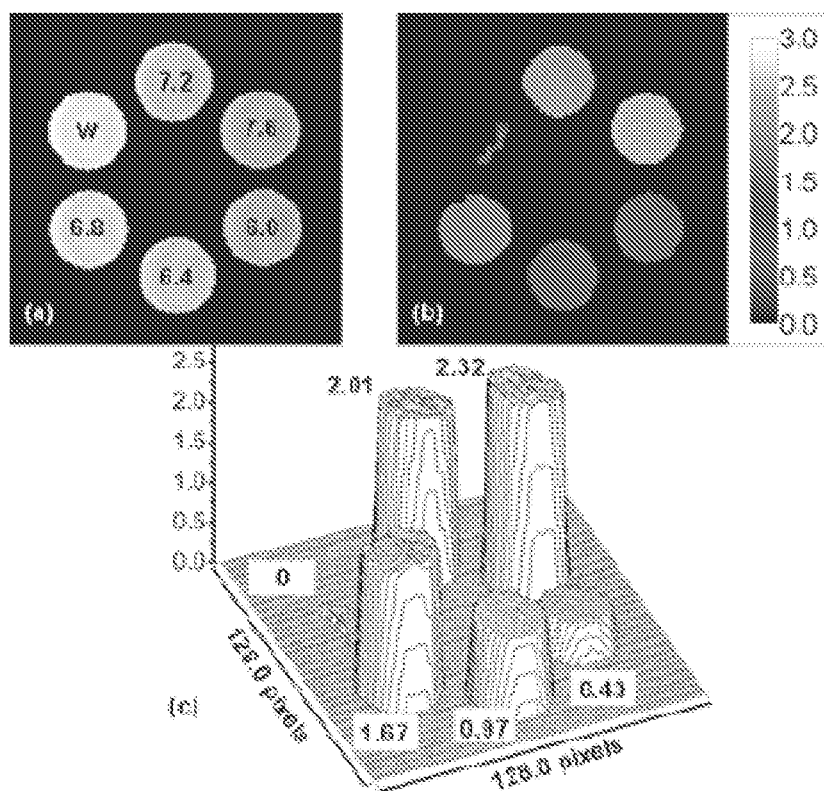


Figure 35

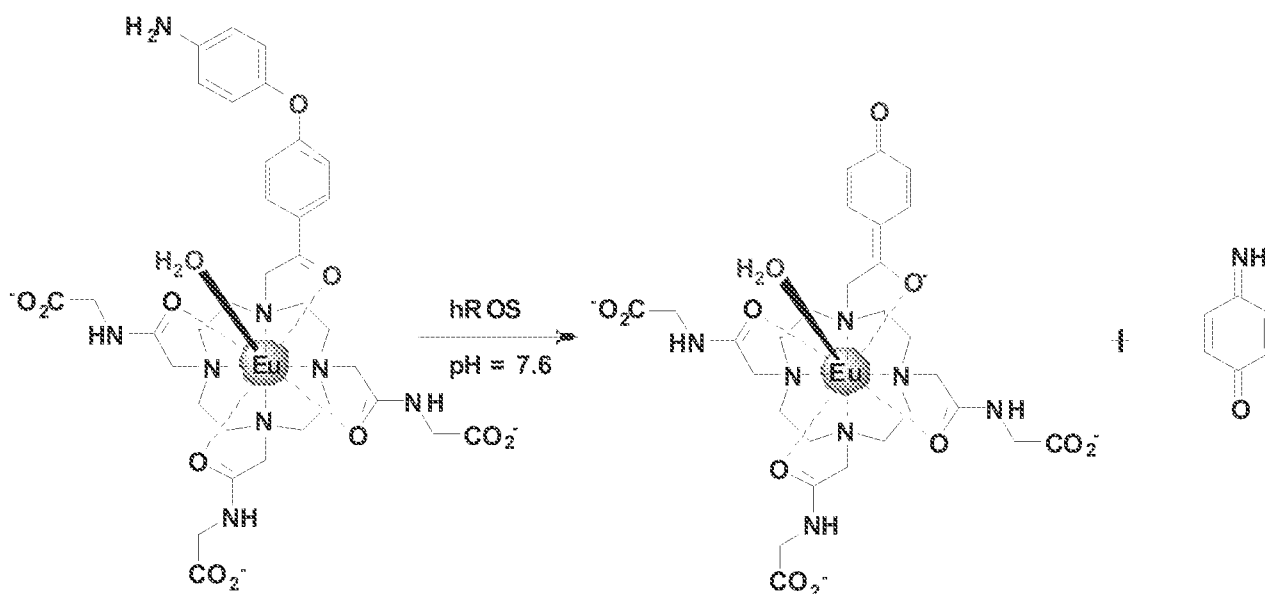


Figure 36