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Amphiphilic EuDOTA-tetraamide complexes form micelles with enhanced CEST sensitivity

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Abstract

The synthesis and characterization of four new DOTA-tetraamide ligands having variable alkyl chain lengths (C₁, C₁₂, C₁₄, and C₁₆) and their respective europium (III) complexes are reported. The three EuL complexes having long alkyl chains spontaneously form micelles of variable size. The critical micelle concentration differed for each complex (lower for the C₁₆ complex than the C₁₂ complex) while micelle size increased with increasing alkyl chain length. Chemical exchange saturation transfer (CEST) experiments showed that all four Eu(III) complexes display slow-to-intermediate water exchange kinetics. As expected, the CEST signals in these complexes decreased with increasing temperatures due to faster water exchange but, interestingly, the CEST signals for the C₁₄ and C₁₆ complexes approached a maximum near 25°C consistent with exchange limited CEST at or near room temperature. The water residence lifetimes obtained by fitting the CEST spectra to the Bloch equations increased in parallel with an increase in alkyl carbon chain-length. By comparisons with the monomethylamide complex, which served as control, the data illustrate that micelle formation serves to slow the rate of water exchange in these systems. The complex having the largest CEST effect per unit Eu(III) concentration (the C₁₆ analog) had a detection limit of 5.3 μM. This represents an approximate 250-fold increase in sensitivity relative to the monomethylamide control (detection limit ~1.3 mM). These features highlight the potential of using micelle-based systems such as these as paramagnetic chemical exchange saturation transfer (PARACEST) agents for molecular imaging by MRI.

Keywords

PARACEST agents; micelles; MRI

INTRODUCTION

Magnetic resonance imaging (MRI) has evolved into one of the most powerful non-invasive techniques in diagnostic medicine because it uses non-ionizing radiation and allows exquisite high resolution imaging of the human anatomy. In addition, the clinical value of

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Supporting Information

Plots of fluorescence intensity versus Eu³⁺ concentration, calculation of CMCs, full CEST spectra recorded at different saturation powers and different temperatures, detection limit plots and calculations, plot of natural log of *k*_{ex} versus reciprocal temperature and calculation of the activation energies for Eu-(1), Eu-(12), Eu-(14), and Eu-(16).

MRI has been significantly augmented by the availability of gadolinium-based T_1 agents as approved contrast media for enhancing soft tissue contrast by shortening the longitudinal relaxation times of water protons. While these agents are very effective at highlighting tissues with abnormal perfusion such as most tumors, current contrast agents provide no direct physiological or metabolic information. This need has stimulated a rich and active area of research devoted to the design of new MRI agents capable of producing water contrast by mechanisms other than relaxation.^[1]

Chemical exchange saturation transfer (CEST) agents represent an emerging class of contrast media that can alter image contrast by decreasing the total bulk water signal intensity. In a typical CEST experiment, the resonance of the labile protons on the CEST agent may be selectively saturated by using a specific radiofrequency (RF) pulse. Saturated magnetization is then transferred from the labile protons to the bulk water protons through a chemical exchange process that has to be maintained within the slow-to-intermediate regime. The result is a decrease in the bulk water signal intensity which translates into a negative contrast in an MR image.^[2]

Early versions of CEST agents consisted of low molecular weight diamagnetic compounds with $\Delta\omega$ values within 5 ppm of bulk water.^[3, 4] These small $\Delta\omega$ values not only restricted CEST to slow exchanging systems but also increased the possibility of directly saturating the bulk water signal. Hence, the discovery that certain paramagnetic lanthanide complexes with DOTA-tetraamide ligands induced large hyperfine shifts in the bound water protons and other proximate exchanging protons in the chelating ligand^[5] ultimately led to the development of a new class of CEST agents now referred to as paramagnetic chemical exchange saturation transfer (PARACEST) agents.

PARACEST agents possess a number of appealing features over their diamagnetic counterparts; for example, $\Delta\omega$ which can range from +500 ppm to -720 ppm depending upon which lanthanide is used.^[6] Eu^{3+} complexes of DOTA-tetraamide ligands have been the most widely studied PARACEST agents to date because these complexes display the slowest water exchange kinetics of all lanthanide complexes.^[7] In addition, this ligand architecture is easily modified to create agents that are sensitive to a variety of physiological parameters,^[8, 9] metalions,^[10] metabolites,^[11, 12] and enzymes.^[13-15] Nevertheless, first generation DIACEST and PARACEST agents are relatively insensitive, typically requiring millimolar concentrations to produce a detectable signal. The development of more sensitive PARACEST agents is critical to make them effective for molecular imaging of such biological processes. While a handful of sensitivity enhancement methods are possible, the most straightforward is to increase the number of exchanging groups (N) per agent. Attempts at improving sensitivity by increasing N have focused largely on macromolecular agents (both diamagnetic and paramagnetic)^[16-20] and nanoparticulate agents.^[21-24]

The use of nanoparticle platforms for sensitivity enhancement is attractive for CEST since such systems have the potential of delivering a high payload of exchangeable groups, either through surface conjugation or encapsulation of multiple small-molecule agents, to a target of interest. While this method has proven efficient in some cases,^[21, 24] other nanoparticulate systems have been shown to disrupt the CEST signal by rapid catalysis of the proton exchange from an encapsulated agent or by interference from a broad magnetization transfer-like signal which obscures CEST from paramagnetic exchange sites.^[23] These results emphasize the importance of choosing the proper nanoparticle composition and the physical location of the CEST agent in the nanoparticle. In this work, we report a series of amphiphilic PARACEST agents capable of assembling into micelles as an approach toward increasing N. Micelles have been widely studied as vehicles for drug delivery due to the possibility of controlling their structural characteristics through simple

changes in physicochemical properties such as size and surface charge.^[25] More recently, it has been shown that the T_1 sensitivity of Gd(III) can be amplified by choosing amphiphilic ligands capable of micelle formation. The T_1 enhancement in such complexes results from a combination of an increase in N plus the added benefit of restricted molecular motion.^[26-29] Given these proven advantages, a micellar platform is also attractive for PARACEST simply on the basis of an increase in N , the number of exchanging protons that can be saturated and then exchange into the large pool of bulk water. One unknown but critical factor for CEST are the water or proton exchange rates may be altered, either to an advantage or disadvantage for CEST, when a PARACEST complex is part of a micelle structure. Here, we address both questions by reporting the synthesis and characterization of four new PARACEST agents with variable alkyl carbon chain-lengths (Chart 1) and the influence of carbon chain-length on the CEST properties of these systems.

RESULTS AND DISCUSSION

Synthesis

Scheme 1 outlines the synthetic procedure for preparing the compounds used in this study. The mono-protected macrocycle **2** was synthesized according to previously published methods.^[32] Alkylation of **2** was carried out with **3a** or **3b** using standard conditions followed by purification using silica gel column chromatography. The CBz protecting group was then removed by hydrogenolysis in the presence of H_2 and palladium on carbon (10 %) to yield **5a** and **5b**, respectively. Subsequently, alkylation of **5a** or **5b** with an alkylbromoacetamide (**6a – d**) was performed and the crude products purified by column chromatography. The ethyl and *tert*-butyl ester groups were removed with LiOH or TFA, respectively, to yield the target ligands. Europium complexes with ligands 1, 12, 14 and 16 were prepared by combining stoichiometric quantities of each ligand and europium (III) chloride in water while maintaining the pH between 5.5 and 6. After 24 hrs, the complexation was complete as confirmed by the absence of non-complexed Eu^{3+} using xylenol orange titration. The resulting solutions were then lyophilized to yield the final compounds used in this study.

Based upon the crystal structure of EuDOTA-(glycine ethyl ester)₄,^[5] and previous studies on a number of other similar ligand systems,^[33, 34] the approximate solution geometry of the Eu(III) complexes prepared here was predicted to have the three glycinate arms oriented axially above the Eu^{3+} -OH₂ bond and the single alkylamide sidearm oriented equatorially (Figure 1). This model is based on the known tendency for smaller, polar amide arms to adopt an axial conformation due to lower steric hindrance, while bulkier, hydrophobic pendant arms, lacking an α -carbonyl group have a preference for an equatorial position.^[34] This suggested that Eu-(12), Eu-(14), and Eu-(16) would likely form micelles in solution with the paramagnetic center located on the micelle surface and the hydrophobic tails buried inside the micelle core.

Determination of critical micelle concentration

An interesting property exhibited by surfactants is their ability to self-assemble into micelles at concentrations above the critical micelle concentration (CMC). To test for micelle formation, fluorescence titrations of each complex were performed in the presence of 8-anilino-1-naphthalenesulfonic acid (ANS), an anionic dye that is weakly fluorescent in water but becomes highly fluorescent when located in a lipid environment.^[30] A large increase in the fluorescence intensity and a blue shift in the emission spectrum of ANS is indicative of micelle formation. Plots of fluorescence intensity *versus* EuL concentration showed in a clear break in linearity at the CMC for each species (see supporting information). The CMC determined from these plots for Eu-(12), Eu-(14), and Eu-(16) were 67 μ M, 48 μ M, and 26

μM , respectively, consistent with the anticipated decrease in CMC with increasing carbon chain length. An identical titration for Eu-(1) did not show a break or increase in fluorescence intensity over a similar concentration range, consistent with lack of micelle formation. The CMCs measured for these systems are quite low compared to other GdDOTA- and GdDTPA-based amphiphilic agents previously reported,^[26-28, 35] presumably due to the reduced polarity of the amide pendant arms compared to the acetate arms and hence their overall, greater hydrophobicity. These results suggest that Eu-(12), Eu-(14), and Eu-(16) have a greater chance of retaining a micelle structure in blood compared to previously reported gadolinium analogues, or perhaps will interact more strongly with hydrophobic regions of serum albumin and exhibit a longer vascular retention lifetime.^[36, 37]

Dynamic light scattering

Micelle size, as determined by dynamic light scattering (DLS) at two concentrations, was found to increase modestly as the alkyl carbon chain length increased from 12 to 16 carbons (3.7 – 5.5 nm, Figure 2). These diameters agreed closely to those estimated from simple theory,^[38] (equations 1 and 2, where l_c represents the critical hydrocarbon chain length, n is the number of carbon atoms in the hydrophobic tail, and R is the radius of the micelle). The slightly larger diameters observed by DLS reflect the fact that these values are a measure of the hydrodynamic diameter of the micelle and not the unsolvated micelle. Both theory and experiment illustrate that relatively small changes in the length of the hydrophobic chain (2 carbons) can result in observable differences in micelle size.

$$l_c \leq l_{\max} \approx (0.154 + 0.126n) \text{ nm} \quad (1)$$

$$R \leq l_c \quad (2)$$

Given these measured diameters, the aggregation number (M) of each micelle can be estimated using equations 3 and 4, where v corresponds to the hydrocarbon chain volume. The aggregation number is particularly important since it provides an estimate of how many exchanging groups are present per micelle.

$$v \approx (27.4 + 26.9n) \times 10^{-3} \text{ nm}^3 \quad (3)$$

$$M = 4\pi R^3 / 3v \quad (4)$$

Using the theoretical diameters, the values of M for Eu-(12), Eu-(14) and Eu-(16) were calculated to be 56, 74, and 94 molecules per micelle, respectively. This shows that PARACEST-based micelles can be prepared from 50-100 Eu(III) complexes per micelle depending upon alkyl chain length, a feature that may prove helpful in amplifying the CEST signal from a single, targeted micelle.

High resolution ^1H -NMR studies

High resolution ^1H NMR spectroscopy (Figure 3) was used to characterize the coordination geometries of the Eu^{3+} complexes in these micelles. The macrocyclic axial proton (H_4 proton) resonance appearing near 26 ppm indicates that both the monomeric and micellar complexes exist largely as square antiprismatic (SAP) structures.^[39] All proton resonances in the ^1H -NMR spectra of Eu-(12), Eu-(14), and Eu-(16) were broad in comparison to those observed for Eu-(1), consistent with less motion in the higher molecular weight micellar

species. Qualitatively, it was quite evident that all ^1H NMR linewidths increased in parallel with increasing alkyl carbon chain length. A resonance was not observed for a Eu^{3+} -bound water molecule in any of these spectra but, given that the chemical shift of the Eu^{3+} -shifted water resonance is consistently found 2-fold further downfield compared to the H_4 proton resonance in all EuDOTA-tetraamide complexes,^[40] one would anticipate finding a water exchange CEST peak near 52 ppm for each of these complexes if water exchange is sufficiently slow.

CEST Studies

CEST spectra were collected on each complex (40 mM in water) at different saturation powers (9.4, 14.1, 18.8, and 23.5 μT) and different temperatures. Given that the CMC of all three amphiphilic compounds were in the 20-70 μM range, it would have been impossible to investigate the CEST properties of the monomeric forms of Eu-(12), Eu-(14), and Eu-(16) so it was assumed that the CEST properties of Eu-(1) would serve as an approximate model. Figure 4 shows the CEST spectra of Eu-(1), Eu-(12), Eu-(14), and Eu-(16) at 25°C. It is obvious that all four compounds exhibit a strong water exchange peak near 53 ppm as anticipated from the ^1H NMR studies. This observation suggests that changing the length of the hydrophobic chain had minimal influence on the ligand field around the Eu^{3+} in each of these complexes. One unanticipated but interesting observation was that the linewidths of the Eu^{3+} -bound water exchange and the bulk water peaks are more narrow in the spectrum of Eu-(16) than in the spectrum of Eu-(1) or Eu-(12) or Eu-(14). This reflects a difference in the water exchange kinetics in these complexes and suggests that packing of the EuL headgroups in the amphiphilic micellar systems does have an impact on the water exchange characteristics of these agents.

The water exchange CEST intensities of all four compounds were similar at 25°C but became well-differentiated upon warming the samples to 37°C. Here, the CEST spectra registered pronounced differences both in intensity and in shape (Figure 5). This behavior is typical of EuDOTA-tetraamide complexes^[9] and the differences observed here demonstrate qualitatively that the water exchange rates in these complexes falls in the order, $\text{Eu}-(1) > \text{Eu}-(12) > \text{Eu}-(14) > \text{Eu}-(16)$, with Eu-(16) being the slowest. The magnitude of CEST (M_s/M_o) at the optimal frequency at each temperature is plotted *versus* temperature in Figure 6. It is evident from these data that water exchange in Eu-(1) and Eu-(12) do not become exchange limited at the lowest temperature examined here (25°C) while water exchange in Eu-(14) and Eu-(16) do appear to begin approaching an exchange limit as the temperature nears 25°C. These results demonstrate that aggregation of a collection of Eu^{3+} chelates on a micelle surface is beneficial for CEST by slowing water exchange and thereby amplifying the CEST signal.

A fit of these CEST spectra to the Bloch equations modified for chemical exchange provided the water residence lifetimes (τ_m) summarized in Figure 7. From these data, the activation energies of the water exchange process were determined to be 69 kJ/mol, 82 kJ/mol, 86 kJ/mol, and 88 kJ/mol for Eu-(1), Eu-(12), Eu-(14), and Eu-(16) respectively by plotting the natural log of k_{ex} versus reciprocal temperature (supporting information). These quantitative data support the qualitative observations described above; water exchange slows down with an increase in alkyl chain length as reflected in the higher activation energy for water exchange. This result was somewhat unexpected because previous studies have shown that increasing the bulkiness of an amide substituent results in faster water exchange, presumably due to greater exposure of the coordination center to bulk water.^[34] Assuming a standard micelle model for these systems, this suggests that EuL headgroups are interacting with each other in a way that stabilizes the Eu^{3+} -water bond. The modest yet significant increase in bound water lifetime (τ_m) in micelles prepared from the C_{12} complex *versus* C_{16}

complex again suggests that a higher density of EuL headgroups as indicated by differences in aggregation numbers promotes slower water exchange.

CEST detection limits

Plots of CEST intensity (M_s/M_o for the water exchange peak) *versus* Eu^{3+} concentration are shown for Eu-(1) and Eu-(16) in Figure 8. These data were fit to an exponential function and the result was used to calculate the detection limit (DL) of each compound (defined as the concentration at which a 5% CEST signal is observed). The DL of Eu-(1) was estimated at 1300 μM while the DL of Eu-(16) was about 3-fold lower, estimated at 475 μM . Given that the latter concentration (475 μM) is well above the CMC of Eu-(16), these differences can only be ascribed to the improved water exchange characteristics of Eu-(16). Although it is yet to be determined whether the micelles formed by Eu-(16) are stable enough for *in vivo* use, given that there are ninety Eu-(16) monomers per micelle, the detection limit of each micelle is estimated at $\sim 5.3 \mu\text{M}$. This represents an approximate 250-fold sensitivity enhancement over Eu-(1).

CONCLUSIONS

Four new EuDOTA-tetraamide complexes having a single amide substituent with variable alkyl-carbon chain lengths were synthesized and characterized by dynamic light scattering, high resolution NMR and CEST spectroscopy. The three complexes having a single alkyl amide side-chain with 12-16 carbons all form micelles at relatively low concentrations. The CMCs determined with the aid of a fluorescence probe showed that as the alkyl chain length was increased, the CMC decreased as expected. DLS data confirmed the formation of micelles in solution, the size of which increased with an increase in alkyl carbon chain length. The water residence lifetimes in each complex (micelle) obtained by fitting the CEST data to the Bloch equations were also found to increase with an increase in alkyl carbon chain length. Eu-(16) has the most promising CEST features, the slowest water residence lifetime at 37°C, the lowest CMC, and the highest micelle aggregation number. The lowest detection limit for Eu-(16) was calculated to be 475 μM on a per Eu^{3+} basis and 5.3 μM on a per micelle basis. Thus, micelle formation affords a 250-fold enhancement in CEST sensitivity over a control compound having a single methylamide substituent.

EXPERIMENTAL SECTION

General Remarks

All reagents and solvents were purchased from commercial sources and used as received, unless otherwise stated. ^1H and ^{13}C NMR spectra were recorded on a Bruker AVANCE III 400 NMR spectrometer operating at 400.13 MHz and 100.03 MHz respectively. Mass spectra were obtained by HT Laboratories San Diego, California. Inductively coupled plasma measurements and elemental analyses were performed by Galbraith Laboratories Inc. Knoxville, Tennessee. Fluorescence experiments were performed using a Perkin Elmer LS 50B Luminescence spectrometer at 25°C. Particle size analysis was performed at 25°C using a Brookhaven 90Plus Particle Size Analyzer equipped with a 35 mW diode laser. Melting points were determined using a Fisher – Johns melting point apparatus (values uncorrected).

General procedure for the synthesis of the N-alkyl-bromoacetamides (3a – b, and 6a – d)

A solution of bromoacetyl bromide (1.2 equiv) in dichloromethane (20 mL) was added dropwise to a cooled solution (0°C) of the corresponding amine (1 equiv) and K_2CO_3 (3 equiv) in a mixture of dichloromethane (100 mL) and water (50 mL). The resulting solution was allowed to warm to room temperature and stirred for 16 h after which the organic layer

was washed with water (2 × 30 mL) and brine (1 × 30 mL), dried over Na₂SO₄, and concentrated *in vacuo*. The crude product was recrystallized either from ethyl acetate or a mixture of methanol and water to afford the corresponding bromoacetamide.

N-(2-bromoacetyl)-glycine ethyl ester (3a)

[8] Colorless solid (13.1 g, 82 %). ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 6.99 (s br, 1H, NH), 4.17 (q, ³J_{H,H} = 7 Hz, 2H, OCH₂CH₃), 3.99 (d, ³J_{H,H} = 5.2 Hz, 2H, CH₂NH), 3.85 (s, 2H, CH₂Br), 1.23 (t, ³J_{H,H} = 7 Hz, 3H, OCH₂CH₃). ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 14.1 (OCH₂CH₃), 28.5 (CH₂Br), 41.9 (CH₂NH), 61.7 (OCH₂CH₃), 165.8 (C=O), 169.2 (C=O). m/z (ESMS ESI⁺): 225 (100 %, [M+H]⁺).

N-(2-bromoacetyl)-glycine t-butyl ester (3b)

Colorless crystalline solid (12.6 g, 84 %). ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 7.00 (s br, 1H, NH), 3.96 (d, ³J_{H,H} = 5.2 Hz, 2H, NHCH₂), 3.91 (s, 2H, BrCH₂), 1.48 (s, 9H, C(CH₃)₃). ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 28.0 (C(CH₃)₃), 28.7 (BrCH₂), 42.6 (NHCH₂), 82.8 (C(CH₃)₃), 165.6 (C=O), 168.3 (COO). MP: 90 – 94 °C.

2-bromo-N-methyl acetamide (6a)

Colorless crystalline solid (5.6 g, 49 %). ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 6.81 (s br, 1H, NH), 3.84 (s, 2H, BrCH₂), 2.82 (d, ³J_{H,H} = 4.9 Hz, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 26.9 (CH₃), 29.0 (BrCH₂), 166.3 (C=O). m/z (ESMS ESI⁺): 152 ([M+H]⁺, 100%). MP: 41 – 45 °C.

2-bromo-N-dodecyl acetamide (6b)

Colorless, crystalline solid (7.0 g, 85 %). ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 6.50 (s br, 1H, NH), 3.89 (s, 2H, BrCH₂), 3.30 (q, ³J_{H,H} = 7 Hz, 2H, NHCH₂), 1.56 (m, 2H, NH₂CH₂CH₂), 1.27 (s br, 18H, (CH₂)₉CH₃), 0.88 (t, ³J_{H,H} = 7 Hz, 3H, CH₂CH₃). ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 14.1 (CH₃), 22.7 (CH₂CH₃), 26.8 – 29.6 ((CH₂)₉CH₂CH₃), 31.9 (BrCH₂), 40.3 (NHCH₂), 165.1 (C=O). m/z (ESMS ESI⁺): 308 ([M+2H]⁺, 100%). MP: 51 – 55 °C.

2-bromo-N-tetradecyl acetamide (6c)

Colorless solid (7.2 g, 86 %). ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 6.40 (s br, 1H, NH), 3.81 (s, 2H, BrCH₂), 3.21 (q, ³J_{H,H} = 7 Hz, 2H, NHCH₂), 1.47 (m, 2H, NHCH₂CH₂), 1.19 (s, 22H, (CH₂)₉CH₃), 0.81 (t, ³J_{H,H} = 7 Hz, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 14.1 (CH₃), 22.7 (CH₂CH₃), 26.8 (NH(CH₂)₂CH₂), 29.3 – 29.7 ((CH₂)₉CH₂CH₃), 31.9 (NHCH₂CH₂), 38.1 (BrCH₂), 40.3 (NHCH₂), 165.1 (C=O). MP: 48 – 50 °C.

2-bromo-N-hexadecyl acetamide (6d)

Colorless solid (7.4 g, 99 %). ¹H NMR (400 MHz, CDCl₃, 25 °C): δ = 6.42 (s br, 1H, NH), 3.81 (s, 2H, BrCH₂), 3.20 (q, ³J_{H,H} = 7 Hz, 2H, NHCH₂), 1.46 (m, 2H, NHCH₂CH₂), 1.19 (s, 26H, (CH₂)₉CH₃), 0.81 (t, ³J_{H,H} = 7 Hz, 3H, CH₃). ¹³C NMR (100 MHz, CDCl₃, 25 °C): δ = 14.1 (CH₃), 22.7 (CH₂CH₃), 27.01 (NH(CH₂)₂CH₂), 29.2 – 29.7 ((CH₂)₁₁CH₂CH₃), 31.9 (NHCH₂CH₂), 39.3 (BrCH₂), 40.3 (NHCH₂), 165.1 (C=O). MP: 61 – 65 °C.

1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine ethyl ester), 10-benzyl carbamate (4a)

To a solution of compound **2** (4.3 g, 13.7 mmol) and K₂CO₃ (7.6 g, 54.9 mmol) in anhydrous acetonitrile (250 mL) was added compound **3a** (9.2 g, 41.1 mmol). The reaction mixture was heated to reflux (65 °C) for 18 h and the solvents were removed *in vacuo*. The

residue was dissolved in dichloromethane (100 mL) and washed with water (2×50 mL). The organic layer was dried over Na_2SO_4 , the solvents were removed in vacuo to yield a brown hygroscopic solid (9.3 g, 92 %) which was used without further purification. ^1H NMR (400MHz, CDCl_3 , 25°C): δ = 7.42 (s br, 3H, *NH*), 7.26 (m, 5H, Ph), 5.0 (s, 2H, OCH_2Ph), 4.09 (q, $^3J_{\text{H,H}} = 7$ Hz, 6H, CH_2CH_3), 3.87 and 3.91 (d br, $^3J_{\text{H,H}} = 5.2$ Hz, 6H, CH_2COO), 3.51 (s br, 6H, $\text{NCH}_2\text{C=O}$), 2.67 – 3.16 (s br, 16H, ring CH_2N), 1.19 (t, $^3J_{\text{H,H}} = 7$ Hz, 9H, CH_3). ^{13}C NMR (100MHz, CDCl_3 , 25°C): δ = 14.1 (CH_3), 40.8 (CH_2COO), 47.3 – 53.9 (ring CH_2N), 58.8 ($\text{CH}_2\text{C=O}$), 61.4 (CH_2CH_3), 67.4 (OCH_2Ph), 128.1 (4-Ph), 128.2 (3-Ph), 128.6 (2-Ph), 136.4 (1-Ph), 156.5 (NCOO), 170.0 (C=O), 171.0 (CH_2COO). *m/z* (ESMS ESI+): 737 ($[\text{M}+\text{H}]^+$, 100 %), 759 ($[\text{M}+\text{Na}]^+$, 28 %).

1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine ethyl ester) (5a)

Compound **4a** (5.7 g, 7.8 mmol) was dissolved in ethanol (75 mL), and 10 % palladium on carbon (0.6 g) was added. The mixture was shaken on a Parr hydrogenator under a hydrogen pressure of 55 psi at room temperature for 48 h. The reaction was filtered, and the solvents were removed in vacuo to afford the title compound as a tan oil (4.6 g, 99 %). ^1H NMR (400MHz, CDCl_3 , 25°C): δ = 7.71 (s br, 3H, *NH*), 4.10 (q, $^3J_{\text{H,H}} = 7$ Hz, 6H, CH_2CH_3), 3.84 and 3.92 (d br, $^3J_{\text{H,H}} = 5.2$ Hz, 6H, CH_2COO), 3.17 and 3.20 (s, 6H, $\text{NCH}_2\text{C=O}$), 2.60 – 2.80 (s br, 16H, ring CH_2N), 1.20 (t, $^3J_{\text{H,H}} = 7$ Hz, 9H, CH_3). ^{13}C NMR (100MHz, CDCl_3 , 25°C): δ = 14.1 (CH_3), 40.9 (CH_2COO), 51.7 – 57.4 (ring CH_2N), 58.3 and 59.8 ($\text{NCH}_2\text{C=O}$), 61.4 (CH_2CH_3), 170.1 and 170.2 (C=O), 171.6 and 171.9 (CH_2COO). *m/z* (ESMS ESI+): 601 ($[\text{M}+\text{H}]^+$, 100 %).

1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine t-butyl ester), 10-benzyl carbamate (4b)

A procedure analogous to that from compound **4a** was followed. Off-white solid (90 %). ^1H NMR (400MHz, CDCl_3 , 25°C): δ = 7.52 (t br, $^3J_{\text{H,H}} = 5.6$ Hz, 1H, *NH*), 7.37 (m, 5H, Ph), 7.34 (t br, $^3J_{\text{H,H}} = 5.6$ Hz, 2H, *NH*), 5.1 (s, 2H, OCH_2Ph), 3.87 and 3.92 (d br, $^3J_{\text{H,H}} = 5.6$ Hz, 6H, CH_2COO), 3.56 (s br, 6H, $\text{NCH}_2\text{C=O}$), 2.70 – 3.23 (s br, 16H, ring CH_2N), 1.46 (s, 27H, $\text{C}(\text{CH}_3)_3$). ^{13}C NMR (100MHz, CDCl_3 , 25°C): δ = 28.1 ($\text{C}(\text{CH}_3)_3$), 41.7 (CH_2COO), 53.9 (ring CH_2N), 58.9 ($\text{CH}_2\text{C=O}$), 67.3 (OCH_2Ph), 82.1 ($\text{C}(\text{CH}_3)_3$), 128.1 (4-Ph), 128.2 (3-Ph), 128.6 (2-Ph), 136.5 (1-Ph), 156.5 (NCOO), 169.1 (C=O), 171.1 (CH_2COO). *m/z* (ESMS ESI+): 821 ($[\text{M}+\text{H}]^+$, 100 %).

1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine t-butyl ester) (5b)

A procedure analogous to that of compound **5a** was followed. Tan hygroscopic solid (Quantitative Yield). ^1H NMR (400MHz, CDCl_3 , 25°C): δ = 9.10 (s br, 1H, *NH*), 8.23 (s br, 2H, *NH*), 3.82 (m br, 6H, CH_2COO), 3.72 (m br, 6H, $\text{NCH}_2\text{C=O}$), 3.04 – 3.37 (s br, 16H, ring CH_2N), 1.37 (s, 27H, $\text{C}(\text{CH}_3)_3$). ^{13}C NMR (100MHz, CDCl_3 , 25°C): δ = 28.1 ($\text{C}(\text{CH}_3)_3$), 41.9 (CH_2COO), 49.3 – 52.6 (ring CH_2N), 57.0 ($\text{CH}_2\text{C=O}$), 81.9 ($\text{C}(\text{CH}_3)_3$), 169.4 (C=O), 171.5 (CH_2COO). *m/z* (ESMS ESI+): 686 ($[\text{M}+\text{H}]^+$, 100 %).

1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine ethyl ester), 10-(N-methyl acetamide) (7a)

Compound **5a** (2.3 g, 3.84 mmol) was added to a mixture of compound **6a** (0.59 g, 3.84 mmol) and K_2CO_3 (1.1 g, 7.7 mmol) in anhydrous acetonitrile (90 mL) and the reaction was heated to reflux (60°C) for 6 h. The solvent was removed in vacuo and the resulting residue was taken up in dichloromethane and washed with water (2×30 mL) and brine (1×30 mL). The dichloromethane layer was dried with Na_2SO_4 , the solvent was removed in vacuo to yield the titled compound as a brown hygroscopic solid (1.25 g, 48 %) which was used without further purification. ^1H NMR (400MHz, CDCl_3 , 25°C): δ = 7.60, 7.53, and

6.99 (s br, 4H, NH), 4.10 (q, $^3J_{\text{H,H}} = 7$ Hz, 6H, CH_2CH_3), 3.93 (d, $^3J_{\text{H,H}} = 5.2$ Hz, 6H, CH_2COO), 3.03 – 3.07 (m, 8H, $\text{NCH}_2\text{C}=\text{O}$), 2.65 (s br, 3H, CH_3), 2.62 (s br, 16H, ring CH_2N), 1.21 (t, $J_{\text{H,H}} = 7$ Hz, 9H, CH_2CH_3). ^{13}C NMR (100MHz, CDCl_3 , 25°C): $\delta = 14.1$ (CH_2CH_3), 25.8 (CH_3), 40.7 (CH_2COO), 53.1 – 53.4 (ring CH_2N), 58.9, and 59.1 ($\text{NCH}_2\text{C}=\text{O}$), 61.4 (CH_2CH_3), 170.1 ($\text{C}=\text{ONH}$), 171.5 (COO). m/z (ESMS ESI+): 674 ($[\text{M} + \text{H}]^+$, 100 %), 696 ($[\text{M} + \text{Na}]^+$, 20 %). Anal. Found C = 46.3 %, H = 6.7 %, N = 14.7 %. $\text{C}_{50}\text{H}_{94}\text{N}_8\text{O}_{10} \cdot \text{KCl}$ requires C = 46.6 %, H = 7.0 %, N = 14.9 %.

1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine ethyl ester), 10-(N-dodecyl acetamide) (7b)

A procedure analogous to that for compound **7a** was followed. The residue was precipitated from ethyl acetate to afford the title compound as a tan solid (2.0 g, 93 %). ^1H -NMR (400MHz, CDCl_3 , 25°C): $\delta = 8.30$ – 7.07 (s br, 4H, NH), 4.04 (q, $^3J_{\text{H,H}} = 7$ Hz, 6H, CH_2CH_3), 3.89 (s br, 6H, CH_2COO), 3.21 (m, 2H, NHCH_2CH_2), 3.09 (s, 8H, $\text{NCH}_2\text{C}=\text{O}$), 2.43 – 2.65 (s br, 16H, ring CH_2N), 1.39 (m, 2H, NHCH_2CH_2), 1.19 (s br, 18H, $((\text{CH}_2)_9\text{CH}_3)$), 1.17 (t, $^3J_{\text{H,H}} = 7$ Hz, 9H, OCH_2CH_3), 0.81 (t, $^3J_{\text{H,H}} = 7$ Hz, 3H, CH_3). ^{13}C NMR (100MHz, CDCl_3 , 25°C): $\delta = 14.1$ (CH_3), 22.6 (CH_2CH_3), 27.1 ($\text{NH}(\text{CH}_2)_2\text{CH}_2$), 29.3 – 29.6 ($((\text{CH}_2)_7\text{CH}_3)$), 31.9 (NHCH_2CH_2), 39.6 (NHCH_2CH_2), 41.2 (CH_2COO), 50.6 (ring CH_2N), 57.3 and 57.6 ($\text{NCH}_2\text{C}=\text{O}$), 61.3 (OCH_2CH_3), 169.9 ($\text{C}=\text{ONH}(\text{CH}_2)_{11}\text{CH}_3$), 171.1 ($\text{C}=\text{ONHCH}_2\text{COO}$), 172.2 (CH_2COO). m/z (ESMS ESI+): 850 ($[\text{M} + \text{Na}]^+$, 100 %). MP: $121 - 124^\circ\text{C}$. Anal. Found C = 53.4 %, H = 8.3 %, N = 12.1 %. $\text{C}_{40}\text{H}_{74}\text{N}_8\text{O}_{10} \cdot \text{H}_2\text{O} \cdot \text{NaCl}$ requires C = 53.2 %, H = 8.5 %, N = 12.4 %.

1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine ethyl ester), 10-(N-tetradecyl acetamide) (7c)

A procedure analogous to that for compound **7a** was followed. The residue was purified by column chromatography over silica gel $R_f = 0.4$ (CH_2Cl_2 : MeOH, 100:10) to afford the title compound as a tan solid (2.2 g, 63 %). ^1H NMR (400MHz, MeOD, 25°C): $\delta = 4.16$ (q, $^3J_{\text{H,H}} = 7$ Hz, 6H, CH_2CH_3), 3.93 (s br, 6H, CH_2COO), 3.20 (m, 2H, NHCH_2CH_2), 3.15 (s, 8H, $\text{NCH}_2\text{C}=\text{O}$), 2.43 – 2.70 (s br, 16H, ring CH_2N), 1.48 (m, 2H, NHCH_2CH_2), 1.29 (s br, 22H, $(\text{CH}_2)_{11}\text{CH}_3$), 1.26 (t, $^3J_{\text{H,H}} = 7$ Hz, 9H, OCH_2CH_3), 0.90 (t, $^3J_{\text{H,H}} = 7$ Hz, 3H, CH_3). ^{13}C NMR (100MHz, MeOD, 25°C): $\delta = 14.4$ and 14.6 (CH_3), 23.7 (CH_2CH_3), 28.1 ($\text{NH}(\text{CH}_2)_2\text{CH}_2$), 30.4 – 30.8 ($((\text{CH}_2)_9\text{CH}_3)$), 33.1 (NHCH_2CH_2), 40.4 (NHCH_2CH_2), 42.0 (CH_2COO), 51.7 (ring CH_2N), 58.2 and 58.3 ($\text{NCH}_2\text{C}=\text{O}$), 62.3 (OCH_2CH_3), 171.5 ($\text{C}=\text{ONH}(\text{CH}_2)_{13}\text{CH}_3$), 173.3 ($\text{C}=\text{ONHCH}_2\text{COO}$), 174.3 (CH_2COO). m/z (ESMS ESI+): 878 ($[\text{M} + \text{Na}]^+$, 100%). MP: $142 - 144^\circ\text{C}$. Anal. Found C = 53.8 %, H = 8.4 %, N = 11.6 %. $\text{C}_{42}\text{H}_{78}\text{N}_8\text{O}_{10} \cdot \text{H}_2\text{O} \cdot \text{CH}_2\text{Cl}_2$ requires C = 53.9 %, H = 8.6 %, N = 11.7 %.

1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine t-butyl ester), 10-(N-hexadecyl acetamide) (7d)

A procedure analogous to that for compound **7a** was followed. Tan hygroscopic solid. ^1H NMR (400MHz, CDCl_3 , 25°C): $\delta = 7.60$ (s br, 2H, NH), 7.53 (s br, 1H, NH), 6.93 (s br, 1H, NH), 3.88 (s br, 6H, CH_2COO), 3.19 (m, 2H, NHCH_2CH_2), 3.12 (m, 6H, $\text{NCH}_2\text{C}=\text{O}$), 2.73 (s br, 16H, ring CH_2N), 1.45 (s, 27H, $\text{C}(\text{CH}_3)_3$), 1.24 (s br, 26H, $((\text{CH}_2)_{13}\text{CH}_3)$), 0.87 (t, $^3J_{\text{H,H}} = 7$ Hz, 3H, CH_3). ^{13}C NMR (100MHz, CDCl_3 , 25°C): $\delta = 14.1$ (CH_3), 22.7 (CH_2CH_3), 27.1 ($\text{NH}(\text{CH}_2)_2\text{CH}_2$), 28.1 ($\text{C}(\text{CH}_3)_3$), 29.3 – 29.8 ($((\text{CH}_2)_{11}\text{CH}_3)$), 31.9 (NHCH_2CH_2), 39.2 (NHCH_2CH_2), 41.7 (CH_2COO), 53.2 (ring CH_2N), 59.3 ($\text{CH}_2\text{C}=\text{O}$), 81.9 ($\text{C}(\text{CH}_3)_3$), 169.2 ($\text{C}=\text{O}$), 171.3 (CH_2COO). m/z (ESMS ESI+): 967 ($[\text{M} + \text{H}]^+$, 98 %), 989 ($[\text{M} + \text{Na}]^+$, 100 %). Anal. Found C = 61.9 %, H = 9.2 %, N = 11.0 %. $\text{C}_{50}\text{H}_{94}\text{N}_8\text{O}_{10} \cdot 0.5\text{CH}_3\text{OH}$ requires C = 61.7 %, H = 9.8 %, N = 11.4 %.

1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine), 10-(N-methyl acetamide) (1)

Compound **7a** (1.2 g, 1.82 mmol) was added to a LiOH solution (0.2 M, 36 mL) at 0°C. The solution was allowed to warm up to room temperature and stirred for 16 h. The resulting sample was lyophilized to afford the title compound in quantitative yield. ¹H NMR (400 MHz, D₂O, 25°C): δ = 3.64 and 3.59 (s br, 6H, CH₂COO), 2.99 – 3.09 (s br, 8H, NCH₂C=O) 2.39 and 2.59 (s br, 16H, ring CH₂N), 2.55 (s, 3H, CH₃). ¹³C NMR (100MHz, D₂O, 25°C): δ = 25.7 (CH₃), 43.2 (CH₂COO), 50.4 (ring CH₂N), 56.2 – 57.7 (NCH₂C=O), 173.3 (C=ONHCH₃), 173.9 (C=ONHCH₂COO) 176.8 (COO). m/z (ESMS ESI+): 589 ([M + H]⁺, 100 %). Elemental analysis was not performed due to the high salt content.

1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine), 10-(N-dodecyl acetamide) (12)

A procedure analogous to that for compound **1** was followed using compound **7b**. ¹H NMR (400MHz, D₂O, 25°C): δ = 3.73 and 3.69 (s br, 6H, CH₂COO), 3.22 (s, 8H, NCH₂C=O) 3.10 (m, 2H, NHCH₂CH₂), 2.54 – 2.70 (s br, 16H, ring CH₂N), 1.43 (m, 2H, NHCH₂CH₂), 1.22 (s br, 18H, ((CH₂)₉CH₃), 0.81 (t, ³J_{H,H} = 7 Hz, 3H, CH₃). ¹³C NMR (100MHz, D₂O, 25°C): δ = 13.9 (CH₃), 22.5 (CH₂CH₃), 26.8 (NH(CH₂)₂CH₂), 29.0 – 29.6 ((CH₂)₇CH₃), 31.8 (NHCH₂CH₂), 39.3 (NHCH₂CH₂), 43.2 and 43.5 (CH₂COO), 50.3 (ring CH₂N), 57.0 and 56.6 (NCH₂C=O), 171.8 (C=ONH(CH₂)₁₁CH₃), 173.4 (C=ONHCH₂COO) 176.1 (COO). m/z (ESMS, ESI-): 741 ([M-H]⁻, 100 %). Elemental analysis was not performed due to the high salt content.

1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine), 10-(N-tetradecyl acetamide) (14)

A procedure analogous to that for compound **1** was followed using compound **7c**. ¹H NMR (400MHz, D₂O, 25°C): δ = 3.71 and 3.64 (s br, 6H, CH₂COO), 3.21 (s, 8H, NCH₂C=O) 3.12 (m, 2H, NHCH₂CH₂), 2.58 (s br, 16H, ring CH₂N), 1.40 (m, 2H, NHCH₂CH₂), 1.19 (s br, 22H, ((CH₂)₁₁CH₃), 0.80 (t, ³J_{H,H} = 7 Hz, 3H, CH₃). ¹³C NMR (100MHz, D₂O, 25°C): δ = 14.0 (CH₃), 22.6 (CH₂CH₃), 26.9 (NH(CH₂)₂CH₂), 29.1 – 29.8 ((CH₂)₉CH₃), 31.9 (NHCH₂CH₂), 39.1 (NHCH₂CH₂), 43.1 and 43.4 (CH₂COO), 51.1 (ring CH₂N), 57.3 and 56.3 (NCH₂C=O), 171.4 (C=ONH(CH₂)₁₃CH₃), 173.1 (C=ONHCH₂COO) 176.1 (COO). m/z (ESMS, ESI-): 769 ([M-H]⁻, 100 %). Elemental analysis was not performed due to the high salt content.

1,4,7, 10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine), 10-(N-hexadecyl acetamide) (16)

Compound **7d** was suspended in TFA and stirred for 24 hrs at room temperature. The TFA was removed in vacuo and the residue was washed with ether, and dissolved in water. The resulting sample was lyophilized to afford the title compound in quantitative yield. ¹H NMR (400MHz, D₂O, 25°C): δ = 3.71 and 3.64 (s br, 6H, CH₂COO), 3.21 (s, 8H, NCH₂C=O) 3.12 (m, 2H, NHCH₂CH₂), 2.58 (s br, 16H, ring CH₂N), 1.40 (m, 2H, NHCH₂CH₂), 1.19 (s br, 26H, ((CH₂)₁₃CH₃) 0.80 (t, ³J_{H,H} = 7 Hz, 3H, CH₃). ¹³C NMR (100MHz, D₂O, 25°C): δ = 14.0 (CH₃), 22.7 (CH₂CH₃), 26.9 (NH(CH₂)₂CH₂), 29.2 – 29.9 ((CH₂)₁₁CH₃), 31.9 (NHCH₂CH₂), 39.1 (NHCH₂CH₂), 43.5 (CH₂COO), 51.2 (ring CH₂N), 57.4 (NCH₂C=O), 171.2 (C=ONH(CH₂)₁₅CH₃), 173.1 (C=ONHCH₂COO) 176.1 (COO). m/z (ESMS ESI-): 797 ([M-H]⁻, 100 %). Elemental analysis was not performed due to the high salt content.

General procedure for the formation of the europium(III) complexes

To a solution of EuCl₃ (0.9 equiv) in water, was added the corresponding ligand (1 equiv) and the mixture was stirred at 50 °C for 18 hr while the pH was maintained at 5.5 – 6. The

absence of excess metal was confirmed with the aid of a xylenol orange test after which the solution was lyophilized to yield the respective complexes as tan solids.

Europium (III) 1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine), 10-(N-methyl acetamide) [Eu-(1)]

$^1\text{H-NMR}$ (400MHz, D_2O , 25°C): δ = 26.4 (d br), -2.52 (t br), -4.85 (d br), -5.9 (d br), -8.25 (t, br), -10.9 (s br), -13.3 (t br). m/z (ESMS ESI+): 739 ($[\text{M}-2\text{H}]^+$, 100 %). Free ligand was present at less than 20 % as estimated by NMR.

Europium (III) 1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine), 10-(N-dodecyl acetamide) [Eu-(12)]

$^1\text{H-NMR}$ (400MHz, D_2O , 25°C): δ = 25.7 (d br), -2.35 (t br), -4.35 (s br), -5.85 (d br), -8.33 (d br), -10.5 (d br), -12.5 (d br), -13.1 (d br). m/z (ESMS ESI+): 893 ($[\text{M}-2\text{H}]^+$, 100 %). Free ligand was present at less than 20 % as estimated by NMR.

Europium (III) 1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine), 10-(N-tetradecyl acetamide) [Eu-(14)]

$^1\text{H-NMR}$ (400MHz, D_2O , 25°C): δ = 25.7 (s br), -2.30 (t br), -4.11 (s br), 5.52 (d br), -7.9 (m br), -10.1 (m br), -12.5 (d br). m/z (ESMS ESI+): 921 ($[\text{M}-2\text{H}]^+$, 100 %). Free ligand was present at less than 20 % as estimated by NMR.

Europium (III) 1,4,7,10-tetraazacyclododecane-1,4,7-tris(2-acetamido-glycine), 10-(N-hexadecyl acetamide) [Eu-(16)]

$^1\text{H-NMR}$ (400MHz, D_2O , 25°C): δ = 25.7 (s br), -2.51 (t br), -4.04 (m br), -8.23 (d br), -10.5 (t br), -12.8 (d br). m/z (ESMS ESI+): 949 ($[\text{M}-2\text{H}]^+$, 100 %). Free ligand was present at less than 20 % as estimated by NMR.

Fluorescence studies

Critical micelle concentrations (CMC) were determined by fluorescence spectroscopy using a well established procedure.^[30] Emission spectra were recorded at room temperature using a 1.0 cm quartz cell, an excitation wavelength of 350 nm, scan rate of 200 nm/min, and excitation and emission slit widths of 10 nm and 5 nm respectively. 8-Anilino-1-naphthalenesulfonic acid (ANS) was used as the fluorescent probe. Aqueous stock solutions of ANS and the europium complexes were prepared using borate buffer (pH 8.5). Fluorescence titrations were performed by adding small aliquots of the amphiphile stock solution to 1 mL of 20 μM ANS with the appropriate corrections made for dilution. The CMC values were determined by plotting the relative fluorescence intensity of ANS at 480 nm, upon excitation at 350 nm as a function of europium (III) concentration.

Dynamic Light Scattering (DLS)

DLS was used to characterize micelle size. Samples were prepared by dissolving the appropriate amount of each agent in DI water to yield Eu^{3+} concentrations of 5 mM and 10 mM. 200 μL aliquots were transferred into plastic cuvettes with 1 cm path lengths, and analyzed at a pH of 7.3 (MES buffer) and 25°C .

CEST Experiments

CEST spectra were generated by applying a frequency-selective saturation pulse in 1 ppm increments over a 200 ppm frequency range, and plotting the residual bulk water signal intensity (M_s/M_0) as a function of saturation frequency. CEST studies were carried out at 25°C and pH 7.4 (MES buffer) using four different B_1 power levels (9.4, 14.1, 18.8, and

23.5 μ T), and a presaturation time of 6 s. The CEST properties of each sample were also investigated as a function of temperature using a B_1 of 14.1 μ T, pH 7.4, and a 6 s presaturation time.

Fitting CEST spectra to obtain bound water lifetimes

The water residence lifetime (τ_m) of each complex was evaluated by fitting the CEST spectra collected at multiple B_1 's to a three pool model (Eu³⁺-bound water, ligand amide protons, and bulk water) based on the numerical solutions to the Bloch equations.^[31] The T_1 and T_2 of bulk water (determined experimentally), the bound water and amide proton concentrations, presaturation time (in seconds) and saturation power (in Hz) were all included in the fitting procedure.

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Figure 1. Hyperchem molecular model illustrating the relative sizes of the Eu-(16) head-group and the C₁₆ hydrophobic tail. Black = carbon, blue = nitrogen, red = oxygen, white = hydrogen, central white = europium.

Figure 2.

DLS data showing the average diameters of micelles formed from Eu-(12) (unfilled), Eu-(14) (vertical), and Eu-(16) (diagonal) measured at two different Eu^{3+} concentrations (5 and 10 mM), pH 7, 25°C. The diameters estimated from basic theory (eqns (2) and (3)) are shown in comparison.

Figure 3. High resolution ^1H -NMR spectra of Eu-(1) (a), Eu-(12) (b), Eu-(14) (c), and Eu-(16) (d) recorded in D_2O at 25°C and 400 MHz.

Figure 4. CEST spectra of 40 mM Eu-1 (red squares), Eu-12 (blue circles), Eu-14 (magenta upward triangles), and Eu-16 (green downward triangles) recorded at 25°C, and pH 7.3. Saturation power = 14.1 μ T, and irradiation time = 6 s.

Figure 5.

CEST spectra of Eu-(1) (red squares), Eu-(12) (blue circles), Eu-(14) (magenta upward triangles), and Eu-(16) (green downward triangles) recorded at 37°C, and pH 7.3. $[Eu^{3+}] = 40$ mM, saturation power = 14.1 μ T, and irradiation time = 6 s.

Figure 6. Plot of magnitude of CEST effect at 14.1 μ T as a function of temperature for Eu-(1) (squares), Eu-(12) (circles), Eu-(14) (upward triangles), and Eu-(16) (downward triangles). $[\text{Eu}^{3+}] = 40 \text{ mM}$, and irradiation time = 6 s.

Figure 7.

Plot of water residence lifetimes (τ_m) as a function of temperature for Eu-(1) (squares), Eu-(12) (circles), Eu-(14) (upward triangles), and Eu-(16) (downward triangles). CEST spectra of 40 mM aqueous samples were recorded at pH 7, and 25 $^{\circ}C$ using a saturation power of 14.1 μT and irradiation time of 6 s (supporting information). τ_m values were obtained by fitting CEST spectra to the Bloch equations modified for exchange.^[31]

Figure 8. Plots of CEST signal as a function of Eu^{3+} concentration for Eu-(1) (squares) and Eu-(16) (circles). The points were fit to exponential functions (solid lines) and the resulting equations were used to estimate the detection limit of each PARACEST agent.

Scheme 1.

Synthetic scheme for the ligands and Eu^{3+} complexes examined in this study.

Chart 1.
General structure of PARACEST agents in this study.