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Measurement of the Dissociation of Eu^{II}-Containing Cryptates Using Murexide

Chamika U. Lenora[‡], Richard J. Staples[†], Matthew J. Allen^{‡,*}

[‡]Department of Chemistry, Wayne State University, Detroit, Michigan 48202, United States.

[†]Department of Chemistry, Michigan State University, East Lansing, Michigan 48824, United States.

Abstract

Dissociation rates of five Eu^{II}-containing cryptates in water were measured using UV–visible spectroscopy and murexide at pH 6.5, 7, 7.5, 8, and 9. Murexide was used as a coordinating dye for Eu^{II}. Results for a known cryptate were within experimental error of the value obtained using other methods, and enabled measurement of other cryptates. This validation of the use of murexide to study the dissociation of Eu^{II}-containing cryptates enables use with other complexes of Eu^{II}.

Graphical Abstract

The dissociation of a series of Eu^{II}-containing cryptates has been studied using murexide as a metallochromic indicator.

INTRODUCTION

Eu^{II}-containing complexes have potential uses in catalysis,¹ luminescence applications,² and redox-responsive magnetic resonance imaging^{3,4} due to their redox, optical, and magnetic

* **Corresponding Author:** mallen@chem.wayne.edu.

Author Contributions

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ASSOCIATED CONTENT

Supporting information. The supporting information is available free of charge on the ACS publications website at DOI: Absorbance as a function of [Eu^{II}], Eu^{II}-murexide equilibrium, crystallography data, and ¹H- and ¹³C-NMR spectra.

Notes

The authors declare no competing financial interest.

properties. In many applications, kinetic inertness is a key parameter for the design of ligands. However, there are a limited number of reports describing measurement of the rates of dissociation of Eu^{II} -containing complexes.^{5–7} Part of the reason for the limited number of studies is that many of the methods used to measure dissociation rates of metal complexes are not conveniently compatible with Eu^{II} -containing complexes; consequently, there is a need for new methods to measure the kinetic parameters of Eu^{II} -containing complexes.

Techniques commonly used to measure dissociation rates of metal chelates include NMR-based methods,⁸ electrochemical techniques,⁵ relaxometric methods,⁹ and spectrophotometric methods (luminescence¹⁰ and UV–visible spectroscopy¹¹).¹² Direct NMR-based methods are not suitable for kinetic measurements of Eu^{II} -containing complexes due to NMR line broadening. Electrochemical techniques have been used to study the dissociation rates of some Eu^{II} -containing cryptates,^{5,6} but these methods require high concentrations (>10 mM) of samples rendering this technique impractical in many cases. Relaxometric methods can be influenced by the presence of different counter ions. Furthermore, luminescence spectroscopy is not always useful because not all Eu^{II} -containing complexes are luminescent and solvents, like water, often quench luminescence. Among the techniques mentioned above, UV–visible spectroscopy is the most widely used;¹² however, the overlap of the UV–visible absorption spectra of Eu^{II} -containing cryptates with uncomplexed Eu^{II} can limit the usefulness of UV–visible spectroscopy with respect to monitoring dissociation. Therefore, metallochromic indicators can be useful in measuring dissociation rates that are otherwise difficult to study. These indicators provide an optical response to changes in the concentration of complexed and uncomplexed metal ions.

Murexide (Figure 1) has been used as an indicator to monitor the formation and dissociation of alkali and alkaline earth cryptates.^{13,14} Furthermore, trivalent lanthanide and transition metal ions are known to coordinate with murexide.^{14,15} Solutions of murexide are deep-purple in color and turn to orange upon coordinating to metal ions, even in dilute (~0.2 mM) conditions.¹⁶ Because murexide binds to Sr^{II} and Ca^{II} and because Eu^{II} is intermediate in size between these two ions,¹⁷ we hypothesized that murexide would respond to uncomplexed Eu^{II} similarly to how it responds to alkaline earth metals, enabling kinetic and thermodynamic quantities, including dissociation rates that are of interest for many applications of Eu^{II} -containing complexes, to be calculated from the amount of uncomplexed Eu^{II} . Herein, we describe testing of the use of murexide to measure dissociation rates of Eu^{II} -containing cryptates.

RESULTS AND DISCUSSION

To study the interaction of Eu^{II} with murexide in aqueous solution, we acquired UV–visible spectra of murexide in the presence and absence of Eu^{II} . These spectra show that the absorption maximum of murexide shifts from 525 to 485 nm upon the addition of EuCl_2 (Figure 2). Further, the difference in the maximum absorption shifted slightly to shorter wavelengths with increasing amounts of Eu^{II} . This type of spectral shift in murexide towards shorter wavelengths upon interacting with metal ions has been observed for alkaline-earth-metal–murexide interactions,¹⁸ transition-metal–murexide interactions¹⁹ and lanthanide–murexide interactions.¹⁴

In addition to a shift in wavelength, the absorption intensity decreased with increasing concentration of Eu^{II} (Figure 2), and solutions became colorless upon reaching ratios of Eu^{II} to murexide of 1:1 or greater. To confirm that the decrease in absorption was not due to reduction of murexide by Eu^{II} , we performed a control experiment that involved the oxidation of Eu^{II} to Eu^{III} . A mixture of EuCl_2 (10 mM, 60 μL , 1 equiv) and murexide (10 mM, 60 μL , 1 equiv) in 2-amino-2-hydroxymethyl-propane-1,3-diol buffer (100 mM, pH 7) that was colorless was opened to air to oxidize Eu^{II} to Eu^{III} . The color of the sample changed to orange in less than two minutes. To confirm that this color change was due to the interaction of Eu^{III} with murexide, we mixed $\text{EuCl}_3 \cdot 6\text{H}_2\text{O}$ and murexide in a 1:1 stoichiometry and observed the same color as with the Eu^{II} sample that was exposed to air. These results indicate that the interaction of Eu^{III} with murexide caused the orange color and that murexide was not degraded to a noticeable amount by Eu^{II} . Additionally, when Eu^{II} was mixed with murexide in water, there was no significant change (ANOVA single factor analysis, 95% confidence interval) in absorbance as a function of time indicating that the system reached equilibrium within minutes of mixing Eu^{II} with murexide and was stable for at least 50 minutes (Figure S2). After characterizing the spectroscopic changes to murexide upon interaction with Eu^{II} , we validated the use of murexide to measure dissociation rates by measuring the dissociation rates of two reported complexes, Eu1Cl_2 and Eu2Cl_2 (Figure 3).⁶

To measure the dissociation rate of Eu1Cl_2 , we selected conditions (0.5 M Me_4NClO_4) similar to the reported conditions used to determine a first-order rate constant with electrochemistry.⁶ To match the pH conditions used in the electrochemistry study, we adjusted the pH to 7 using 2-amino-2-hydroxymethyl-propane-1,3-diol. We measured the change in absorption at 485 nm as a function of time. The measured absorption values were used to calculate concentrations of Eu^{II} using a calibration curve, and a linear relationship between the natural log of cryptate concentration and time indicated that dissociation is first order with respect to Eu1Cl_2 (Figure 4), consistent with the report using electrochemistry.⁶ Therefore, the dissociation rate constant (k_d) was obtained as the slope of the plot in Figure 4, and the results are summarized in Table 1. The value of k_d obtained using murexide matches the reported k_d of Eu1Cl_2 obtained using electrochemistry.⁶

Additionally, conditional dissociation rates ($k_{d,\text{obs}}$) for Eu2Cl_2 were reported at different pH values in aqueous $\text{LiClO}_4/\text{HClO}_4$ (1 M),⁶ where the y-intercept of the linear fit of the plot of $k_{d,\text{obs}}$ versus $[\text{H}^+]$ was used to report the k_d as $1 \times 10^{-4} \text{ s}^{-1}$.⁶ However, when we mixed murexide with Eu2Cl_2 in $\text{LiClO}_4/\text{HClO}_4$ (1 M), the color of the murexide disappeared instantaneously at each pH value. These color-change observations were inconsistent with a k_d of $1 \times 10^{-4} \text{ s}^{-1}$.

To explore the cause of the rapid color change, we further studied Eu2Cl_2 in the solid state and in solution. Crystal structure analysis and proton relaxation enhancement measurements²⁰ revealed that Eu^{II} complexed with ligand **2** both in the solid state and in solution. The crystal structure of Eu2Cl_2 (Figure 5) shows Eu^{II} with a coordination number of nine in an eclipsed hula-hoop geometry. Seven of the nine sites are occupied by the five oxygen atoms and the two nitrogen atoms of the cryptand, and two adjacent coordination sites are occupied by water molecules. In solution, water-proton relaxation rate was measured as a function of the amount of ligand **2** added to a solution of Eu^{II} . The relaxation

rate decreased with the addition of **2** up to a 1:1 ligand-to-metal ratio (Figure 6), and after that point, the rate did not change (ANOVA single factor analysis, 95% confidence interval). Because ligand **2** binds to Eu^{II} in solution and the crystal structure reveals two adjacent coordinated water molecules, we envision that upon mixing of murexide with Eu₂Cl₂, one molecule of murexide replaces the two adjacent inner-sphere water molecules, binding to [Eu₂]²⁺ in a bidentate manner.

To test if murexide displaced water bound to Eu₂Cl₂ in solution, we measured the change in relaxation rate of solutions of Eu₂Cl₂ as a function of amount of murexide (Figure 7). The results show that the relaxation rate decreased as the amount of murexide increased, consistent with what would be expected if murexide replaced the bound water. The formation of a ternary complex of murexide, Eu^{II}, and **2** suggests that murexide is not suitable to measure the dissociation of Eu^{II}-containing complexes with adjacent coordination sites available for bidentate ligands.

After validating the technique with reported complexes, the dissociation rates of three other Eu^{II}-containing cryptates were measured with murexide. The dissociation rates for Eu^{II} and these ligands, **3–5**, have not been reported. Ligands **3** and **4** were selected because they have been studied with Eu^{II} as potential MRI contrast agents,^{4,20} and ligand **5** was selected to establish the ability of murexide to differentiate small differences in dissociation rates, expected based on differences in the electronics of the ring among ligands **3–5**. The introduction of a methyl group onto the benzo ring was expected to slightly decrease dissociation rates due to the inductive donating effect of the methyl group. Cryptand **3** is commercially available, and the synthesis of cryptand **4** has been reported.⁴ Additionally, characterization of Eu^{II}-containing complexes of cryptands **3** and **4** has been reported.^{4,20} Cryptand **5** was synthesized in four steps starting from a commercially available catechol (Scheme 1). We studied the formation of Eu₅Cl₂ in solution using proton relaxation enhancement (Figure 8). The relaxation rate decreased with the addition of ligand up to a ligand-to-metal ratio of 1:1, and after that point, the rate did not change. This data is consistent with the formation of a 1:1 complex in solution. After characterization, we focused on studying the kinetic dissociation of Eu^{II}-containing cryptates of cryptands **1** and **3–5**.

The conditional dissociation rates of Eu^{II}-containing complexes Eu₁Cl₂, Eu₃Cl₂, Eu₄Cl₂, and Eu₅Cl₂ were measured at pH values of 6.5, 7.0, 7.5, 8.0, and 9.0 to include physiologically relevant values and to enable collection of enough data to obtain graphs of $k_{d,obs}$ versus [H⁺] to determine spontaneous dissociation rates (k_d) (equation 1)^{6,8}.

$$k_{d,obs} = k_d + k_H[H^+] \quad (1)$$

In equation 1, k_d is the spontaneous dissociation rate, and k_H is the acid-catalyzed dissociation rate. In the measurement of $k_{d,obs}$, pH values lower than 6.5 were not explored because murexide is not stable at low pH,²¹ and pH 9 was chosen as the upper limit because Eu^{II} precipitates as hydroxide above pH 9.⁶ For the dissociation experiment, Eu^{II}-containing complexes were prepared by mixing an aqueous solution of ligand (1.1 equiv) with an

aqueous solution of EuCl_2 (1.0 equiv). Dissociated Eu^{II} interacts with murexide as indicated in equation 2, and this interaction changes the absorption of murexide. Therefore, dissociation rates were measured using the change in absorption at 485 nm [constant ionic strength (0.5 M Me_4NClO_4) at 25 ± 0.5 °C] as a function of time.

In equation 2, **L** is a cryptand, **M** is murexide, and x is the number of murexide molecules bound to Eu^{II} . For clarity, water and counterions are not included in the equation. The dissociated Eu^{II} ion interacts with murexide, and the interaction of Eu with murexide inhibits back-complexation of cryptand with Eu^{II} . The validity of this experiment was supported by our data with Eu1Cl_2 that reproduced the dissociation rate of Eu1Cl_2 that was determined using electrochemistry.⁶ The conditional dissociation rates obtained for Eu^{II} -containing cryptates Eu1Cl_2 , Eu3Cl_2 , Eu4Cl_2 , and Eu5Cl_2 at five different pH values (Table 2) were used to obtain k_d values at 25 °C. The plots of $k_{d,\text{obs}}$ versus $[\text{H}^+]$ were fit to straight lines (Figure 9), and values of k_d were calculated as the y-intercepts of the lines (Table 3). The value of k_d obtained in this way for Eu1Cl_2 using murexide is in good agreement with the value reported from electrochemical methods ($\sim 5 \times 10^{-5} \text{ s}^{-1}$).⁶

To determine if murexide is able to differentiate the dissociation rate of an unsubstituted cryptate from a benzo-substituted cryptate, we compared the dissociation rate of Eu1Cl_2 with Eu3Cl_2 . The dissociation rate (k_d) of Eu3Cl_2 is 4.2× faster than the dissociation rate of Eu1Cl_2 . This difference of dissociation rates between Eu1Cl_2 and Eu3Cl_2 likely arises from the structural differences between the two ligands. Previous kinetic studies with alkaline earth metal complexes of ligands **1** and **3** revealed that the introduction of a benzo group increases dissociation rates.²² For example, the dissociation rate of $\text{Sr}^{\text{II}}\text{3}$ is 3.3× faster than $\text{Sr}^{\text{II}}\text{1}$ in water at 25 °C.²² Due to similar charge and size of Eu^{II} and Sr^{II} , we expected that the dissociation of Eu1Cl_2 and Eu3Cl_2 would follow the same trend as the analogous Sr^{II} -containing complexes, and our data is in line with that expectation. The dissociation-rate studies of Eu1Cl_2 and Eu3Cl_2 reveal that murexide is able to differentiate dissociation rates between unsubstituted and benzo-substituted cryptates.

To compare functionalized benzo-substituted cryptates Eu4Cl_2 and Eu5Cl_2 from unfunctionalized benzo-substituted cryptate Eu3Cl_2 , we measured the dissociation rates of Eu4Cl_2 and Eu5Cl_2 . The results (Table 3) show that the dissociation rate of Eu4Cl_2 is 2× times faster than the dissociation rate of Eu3Cl_2 . The data suggests that the electron-withdrawing nature of F has more influence on dissociation rate than the π -donating ability. The inductive electron-withdrawing nature of F decreases the electron donating ability of oxygens attached to the benzo ring, consistent with more rapid dissociation, and the π -donating ability of the F substituent would be expected to counteract the influence of inductive electron-withdrawing effect. There was no difference (Student t -test, $p = 0.6$) between the dissociation rates of Eu3Cl_2 and Eu5Cl_2 (Table 3), indicating that the inductive electron-donating effect of the methyl group did not increase the electron density of oxygen attached to benzo ring enough to lead to an observable change in dissociation rate using

murexide. The difference in dissociation rates between Eu4Cl_2 and Eu5Cl_2 indicates that the inductive electron withdrawing ability of F has a measurable influence on dissociation rate relative to the inductive donating effect of a methyl group.

CONCLUSIONS

We have described the use of UV–visible spectroscopy with murexide to study the dissociation rates of Eu^{II} -containing cryptates. The rates of dissociation of Eu^{II} -containing cryptates increase in the order of $\text{Eu1Cl}_2 < \text{Eu3Cl}_3 \approx \text{Eu5Cl}_2 < \text{Eu4Cl}_2$. The findings presented here demonstrate the utility of murexide in the measurement of the dissociation rates of Eu^{II} -containing cryptates, and these findings are likely to be useful in the characterization of many Eu^{II} -containing complexes.

EXPERIMENTAL DETAILS

General.

Commercially available chemicals were used without further purification unless otherwise stated. Water was purified using a PURELAB Ultra Mk2 water purification system (ELGA) and degassed under reduced pressure prior to use. Murexide was purchased from Sigma Aldrich. 5,6-(4-Fluorobenzo)-4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8.8.8]hexacos-5-ene (**4**) was synthesized following reported procedures.⁴

Flash chromatography was performed using silica gel 60, 230–400 mesh (EMD chemicals). Analytical thin-layer chromatography (TLC) was carried out on ASTM TLC plates precoated with silica gel 60 F₂₅₄ (250 μm layer thickness). TLC visualization was achieved using a UV lamp followed by charring with potassium permanganate stain (3 g KMnO_4 , 20 g K_2CO_3 , 5 mL 5% w/v aqueous NaOH, 300 mL H_2O).

^1H -NMR, correlation spectroscopy (COSY), ^{13}C -NMR, distortionless enhancement by polarization transfer (DEPT), and heteronuclear multiple quantum coherence (HMQC) spectra were obtained using an MR400 (9.4 T) or V500 (11.7 T) spectrometer. Peaks in ^1H - and ^{13}C -NMR spectra were assigned using DEPT, COSY, and HMQC spectra. Chemical shifts are reported relative to residual solvent signals (CD_3CN : ^1H : δ 1.96, ^{13}C : δ 1.32; CDCl_3 : ^1H : δ 7.27, ^{13}C : δ 77.23; CD_3OD : ^1H : δ 3.34, ^{13}C : δ 49.86). ^1H -NMR data are assumed to be first order, and the apparent multiplicity is reported as “s” = singlet, “dd” = doublet of doublet, “t” = triplet, “m” = multiplet, and “brs” = broad singlet. Italicized elements are those that are responsible for the shifts. High-resolution electrospray ionization mass spectra (HRESIMS) were obtained using an electrospray time-of-flight high-resolution Waters Micromass LCT Premier XE mass spectrometer.

Concentrations of Eu were determined using energy-dispersive X-ray fluorescence (EDXF) spectroscopy with a Shimadzu EDX-7000 spectrometer in the Lumigen Instrument Center in the Department of Chemistry at Wayne State University. Calibration curves were generated using fluorescence intensity at 5.845 keV for 25–100 ppm or 100–1000 ppm concentration ranges (diluted from Sigma Aldrich ICP standard, Eu_2O_3 in aqueous nitric acid (2%) 1000 ppm).

UV–visible absorbance was measured using a Shimadzu UVmini-1240 spectrophotometer. Samples were prepared inside a wet glove box (water allowed, but not O₂) under an atmosphere of N₂. Samples were loaded into airtight cuvettes and sealed with paraffin wax prior to removal from the glove box.²³

Values of pH were measured using an Oakton pH 700 meter at ambient temperature.

X-ray quality crystals of EuCl₂ were obtained by slow evaporation in a wet glove box. EuCl₂ (51.0 mg, 0.229 mmol) and ligand **2** (88.8 mg, 0.267 mmol) were dissolved in methanol (0.8 mL) under an inert atmosphere. The resulting colorless solution was stirred for 3 h then filtered through a 0.2 µm hydrophilic filter (Millex–LG hydrophilic). Tetrahydrofuran vapor was diffused into the filtrate to obtain a white precipitate. The white precipitate was dissolved in acetone (0.3 mL), and the sample was allowed to slowly evaporate to yield colorless block-shaped crystals suitable for X-ray diffraction. A suitable crystal (0.20 × 0.13 × 0.08) mm³ was selected and mounted on a nylon loop with paratone oil on a Bruker APEX-II CCD diffractometer. The crystal was kept at 173(2) K during data collection. Using Olex2,²⁴ the structure was solved with the ShelXT structure solution program,²⁵ using the intrinsic-phasing solution method. The model was refined with version of XL²⁶ using least-squares minimization.

Preparation of buffers.

Buffers for measurements at pH 7, 7.5, 8, and 9 were prepared by dissolving 2-amino-2-hydroxymethyl-propane-1,3-diol (12.1 g, 0.100 mol) in water (90 mL). The pH of the solutions were adjusted to the desired pH with the addition of aqueous HCl (3 M). Additional water was added to bring the final volume of each buffer to 100.00 mL. Buffer for measurements at pH 6.5 was prepared by dissolving 3-morpholinopropane-1-sulfonic acid (21.0 g, 0.100 mol) in water (90 mL). The pH of the solution was adjusted to 6.5 with the addition of aqueous NaOH (1 M). Additional water was added to bring the final volume to 100.00 mL.

Dimethyl 2,2'-((4-methyl-1,2-phenylene)bis(oxy))diacetate (6): A mixture of 4-methylbenzene-1,2-diol (1.00 g, 8.07 mmol, 1 equiv), acetone (50 mL), potassium carbonate (6.20 g, 40.3 mmol, 5 equiv), and methylbromoacetate (10.0 mL, 106 mmol, 13.1 equiv) was heated at reflux for 8 h, cooled to ambient temperature, and filtered. Solvent was removed under reduced pressure to obtain a yellow oil. The oil was dissolved in ethyl acetate (40 mL) and washed with water (3 × 20 mL). The organic layer was dried over anhydrous magnesium sulfate and filtered. Solvent was removed under reduced pressure before purification was performed using silica gel chromatography (3:1 hexanes/ethyl acetate) to yield 1.95 g (90%) of **6** as a pale-yellow oil. ¹H NMR (400 MHz, CDCl₃, δ): 2.28 (s, 3H, CH₃), 3.80 (s, 3H, CH₃), 3.81 (s, 3H, CH₃), 4.70 (s, 2H, CH₂), 4.72 (s, 2H, CH₂), 6.68–6.85 (m, 3H, CH); ¹³C NMR (101 MHz, CDCl₃, δ): 21.1 (CH₃), 52.4 (CH₃), 66.8 (CH₂), 67.2 (CH₂), 116.0 (CH), 116.5 (CH), 123.1 (CH), 132.8, 146.0, 148.0, 169.8, 169.9; TLC: *R*_f = 0.18 (3:1 hexanes/ethyl acetate); HRESIMS (*m/z*): [M + H]⁺ calcd for, C₁₃H₁₇O₆, 269.1026; found, 269.1014.

2,2'-(4-Methyl-1,2-phenylene)diacetic acid (7): To a solution of **6** (0.874 g, 3.26 mmol, 1 equiv) in water (25 mL) was added Dowex resin (200 mesh, 50W×8, hydrogen

form, 0.456 g). The mixture was heated at reflux for 36 h. The resulting mixture was filtered while hot, and the solvent in the filtrate was removed under reduced pressure to obtain 0.752 g (96%) of **7** as an off-white solid. ^1H NMR (400 MHz, CD_3OD , δ): 2.28 (s, 3H, CH_3), 4.68 (s, 3H, CH_3), 4.71 (s, 3H, CH_3), 5.06 (brs, 2H, OH), 6.73–6.91 (m, 3H, CH); ^{13}C NMR (101 MHz, CD_3OD , δ): 22.0 (CH_3), 68.3 (CH_2), 68.8 (CH_2), 118.1 (CH), 118.6 (CH), 124.8 (CH), 134.7, 148.3, 150.4, 173.9, 174.0; HRESIMS (m/z): $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{11}\text{H}_{12}\text{O}_6\text{Na}$, 263.0532; found, 263.0536.

5,6-(4-Methylbenzo)-4,7,13,16,20,23-hexaoxa-1,10-diazabicyclo[8.8.8]hexacosane-2,9-dione (8):

To diacid **7** (0.571 g, 2.34 mmol, 1 equiv) was added thionyl chloride (10.0 mL, 0.140 mmol, 60 equiv) under an atmosphere of Ar. The mixture was heated at reflux until the mixture became a clear, yellow solution. The reaction mixture was concentrated under reduced pressure to obtain a yellow oil that was dissolved in anhydrous toluene (50 mL) under an atmosphere of Ar. A separate solution of 4,13-diaza-18-crown-6-ether (0.613 g, 2.34 mmol, 1 equiv), triethylamine (3.0 mL, 22 mmol, 9.4 equiv), and anhydrous dichloromethane (7 mL) in anhydrous toluene (40 mL) was prepared. Both solutions were simultaneously added dropwise over 1 h to a flask containing anhydrous toluene (300 mL) at 0 °C under an atmosphere of Ar. The resulting reaction mixture was stirred for 12 h while warming to ambient temperature. Solvent was removed under reduced pressure before purification was performed using silica gel chromatography (10:1 dichloromethane/methanol) to yield 0.590 g (54%) of **8** as a white solid. ^1H NMR (400 MHz, CD_3CN , δ): 2.16–2.31 (m, 3H, CH_3), 2.70–2.90 (m, 2H, CH_2), 3.25–3.80 (m, 20H, CH_2), 4.02–4.25 (m, 2H, CH_2), 4.57–4.96 (m, 2H, CH_2), 5.22–5.55 (m, 2H, CH_2), 6.62–7.02 (m, 3H, CH); ^{13}C NMR (101 MHz, CD_3CN , δ): 20.8 (CH_3), 48.5 (CH_2), 48.6 (CH_2), 48.7 (CH_2), 48.9 (CH_2), 49.1 (CH_2), 49.2 (CH_2), 49.9 (CH_2), 55.5 (CH_2), 67.9 (CH_2), 68.2 (CH_2), 69.8 (CH_2), 69.9 (CH_2), 70.1 (CH_2), 70.2 (CH_2), 71.5 (CH_2), 71.9 (CH_2), 117.2 (CH), 117.8 (CH), 122.6 (CH), 123.3 (CH), 132.1, 133.1, 146.8, 147.1, 148.8, 149.3, 168.9, 169.67, 169.74; TLC: R_f = 0.27 (10:1 dichloromethane/methanol); HRESIMS (m/z): $[\text{M} + \text{Na}]^+$ calcd for $\text{C}_{23}\text{H}_{34}\text{N}_2\text{O}_8\text{Na}$, 489.2213; found, 489.2219.

5,6-(4-Methylbenzo)-4,7,13,16,21,24-hexaoxa-1,10-

diazabicyclo[8.8.8]hexacos-5-ene (5): To diamide **8** (0.549 g, 1.17 mmol, 1 equiv) was added borane-tetrahydrofuran complex (1.0 M in tetrahydrofuran, 35.0 mL, 35.0 mmol, 30 equiv) under an atmosphere of Ar. The resulting solution was heated at reflux for 23 h before the reaction was allowed to cool to ambient temperature. To the reaction was added aqueous hydrochloric acid (3.0 M, 0.050 L, 0.015 mol, 13 equiv) over 10 min, and the resulting white, turbid mixture was heated at reflux for 3 h to produce a clear, colorless solution that was cooled to ambient temperature. The pH of the resulting solution was adjusted to 11 with the addition of concentrated aqueous ammonium hydroxide (20 mL) before the solvent was removed under reduced pressure to afford a white solid. Purification was performed using silica gel chromatography (10:1 dichloromethane/methanol) to yield a white, oily solid that was dissolved in a concentrated aqueous solution of cesium carbonate and extracted with diethyl ether (4 × 20 mL). The diethyl ether layers were combined, dried over anhydrous magnesium sulfate, and filtered. Solvent was removed under reduced pressure to yield 0.332 g (65%) of **5** as a pale-yellow oil. ^1H NMR (400 MHz, CDCl_3 , δ):

2.23 (s, 3H, CH_3), 2.70 (brs, 8H, CH_2), 2.89 (dd, 4H, $J = 13.4, 5.9$ Hz, CH_2), 3.45–3.67 (m, 16H, CH_2), 4.06 (t, 4H, $J = 5.6$ Hz, CH_2), 6.60–6.78 (m, 3H, CH); ^{13}C NMR (101 MHz, CDCl_3 , δ): 20.9 (CH_3), 55.1 (CH_2), 55.2 (CH_2), 55.8 (CH_2), 55.9 (CH_2), 62.8 (CH_2), 68.2 (CH_2), 68.8 (CH_2), 70.06 (CH_2), 70.13 (CH_2), 70.8 (CH_2), 70.9 (CH_2), 115.9 (CH), 116.4 (CH), 121.7 (CH), 131.3, 147.0, 149.2; TLC: $R_f = 0.17$ (10:1 dichloromethane/methanol); HRESIMS (m/z): $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{23}\text{H}_{39}\text{N}_2\text{O}_6$, 439.2809; found, 439.2787.

Dissociation rate measurements.

Metal complexes for kinetic measurements were prepared inside a wet glove box under an atmosphere of N_2 . Aqueous solutions of EuCl_2 (0.150 mL, 10.0 mM) and ligand (**1** = 22.0 μL , 75.0 mM; **2** = 17 μL , 95 mM; **3** = 21.0 μL , 80.0 mM; **4** = 21.0 μL , 78.0 mM; or **5** = 16.0 μL , 102 mM) were added to water (1000 μL). The resulting solutions were stirred at ambient temperature under an inert atmosphere for 4 h before the addition of aqueous buffer (300 μL , 1.00 M) and tetramethylammonium chloride (300 μL , 5.00 M). A freshly prepared solution (36.0 μL , 25.0 mM) of murexide in water was added to the reaction mixture, and the final volume was brought to 3.00 mL with the addition of water. Samples were immediately sealed with paraffin wax,²³ and measurements were performed outside the glove box. Dissociation was monitored by measuring the change in the absorption with time in the range of 485–505 nm. The measured absorption values were used to calculate the concentration of Eu using a calibration curve. The calibration curve was prepared by measuring the absorption of murexide (0.2 mM) in the presence of varying amounts of EuCl_2 (0.01–0.18 mM). The concentrations of Eu obtained from the calibration curve were used as the concentrations of Eu^{II} -containing cryptates.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Structure of murexide.

Figure 2. UV-visible spectra of aqueous solutions of murexide with EuCl_2 in water (pH 7); [murexide] = 0.2 mM, [EuCl_2] = 0 (■), 0.1 (— —), 0.12 (- · -), 0.15 (- · · -), 0.16 (·· ·), and 0.18 mM (—).

Figure 3.
Cryptands used for dissociation studies.

Figure 4.
Representative plot of $\ln[\text{Eu1Cl}_2]$ versus time.

Figure 5.

Crystal structure of Eu_2Cl_2 (R -factor = 0.0309, resolution = 0.75 Å). Thermal ellipsoids are drawn at the 50% probability level. Hydrogen atoms and outer-sphere chloride counter anions are omitted for clarity. Grey = C, red = O, blue = N, and green = Eu. Crystallographic data for this structure has been deposited at the Cambridge Crystallographic Data Centre under deposition number CCDC 1826380.

Figure 6. Water proton relaxation rates ($R_{1\text{obs}}$) of aqueous solutions of EuCl_2 (1 mM) upon addition of different concentrations of ligand **2** at 37 °C and 60 MHz. Error bars represent the standard error of the mean of three independently prepared samples.

Figure 7.

Water proton relaxation rate ($R_{1\text{obs}}$) of aqueous solutions of Eu2Cl_2 (0.7 mM) upon addition of murexide at 37 °C and 60 MHz. Error bars represent the standard error of the mean of three independently prepared samples.

Figure 8.

Water proton relaxation rates ($R_{1\text{obs}}$) of aqueous solutions of EuCl_2 (1 mM) upon addition of different concentrations of ligand **5** at 37 °C and 60 MHz. Error bars represent the standard error of the mean of three independently prepared samples.

Figure 9. Dissociation rates of Eu^{II}-containing cryptates of ligands **1** and **3–5** as a function of [H⁺], where ○ = Eu**1**Cl₂; △ = Eu**3**Cl₂; □ = Eu**4**Cl₂; and ◇ = Eu**5**Cl₂. Error bars represent the standard error of the mean of three independently prepared samples.

Scheme 1.
Synthesis of ligand **5**

Table 1.Dissociation of Eu1Cl₂ (0.5 mM) at 25 °C.

Electrolyte (0.5 M)	Ba(NO ₃) ₂ (mM)	murexide (mM)	$k_{\text{d+obs}} (\times 10^{-5} \text{ s}^{-1})$	pH
Et ₄ NClO ₄	33	0	3.0 ^a	7
Me ₄ NCl ₄	0	0.2	3.0 ± 0.3 ^b	7

^a from reference 6^b mean ± standard error of the mean of three independently prepared samples

Table 2.

Conditional dissociation rates ($\times 10^{-5} \text{ s}^{-1}$)^{*} of Eu^{II}-containing cryptates at different pH values at 25 ± 0.5 °C.^{**}

Complex	$k_{d,obs}$ (pH 6.5)	$k_{d,obs}$ (pH 7)	$k_{d,obs}$ (pH 7.5)	$k_{d,obs}$ (pH 8)	$k_{d,obs}$ (pH 9)
Eu ^{II} 1	6.4 ± 0.5	3.04 ± 0.3	2.4 ± 0.5	1.7 ± 0.2	1.4 ± 0.12
Eu ^{II} 3	14.5 ± 0.4	9.3 ± 0.7	7.4 ± 0.4	6.7 ± 0.3	6.5 ± 0.2
Eu ^{II} 4	24.6 ± 0.2	18.3 ± 0.7	15.2 ± 0.3	14.1 ± 0.7	12.8 ± 0.1
Eu ^{II} 5	12.4 ± 0.8	7.7 ± 0.2	7.2 ± 0.2	6.6 ± 0.2	6.0 ± 0.3

^{*} Values are reported as the mean $\pm$ the standard error of the mean of three independently prepared samples.

^{**} Ionic strength = 0.5 M.

Table 3.Rates of dissociation of Eu^{II}-containing cryptates.

Complex	$k_d (\times 10^{-5} \text{ s}^{-1})$
Eu1Cl ₂	1.58 ± 0.04
Eu3Cl ₂	6.6 ± 0.3
Eu4Cl ₂	13.4 ± 0.4
Eu5Cl ₂	6.2 ± 0.2