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(S)-5-(p-Nitrobenzyl)-PCTA, a Promising Bifunctional Ligand with Advantageous Metal Ion Complexation Kinetics

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Abstract

A bifunctional version of PCTA (3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15),11,13-triene-3,6,9,-triacetic acid) that exhibits fast complexation kinetics with the trivalent lanthanide(III) ions was synthesized in reasonable yields starting from N, N', N''-tristosyl-(S)-2-(p-nitrobenzyl)-diethylenetriamine. pH-potentiometric studies showed that the basicities of p-nitrobenzyl-PCTA and the parent ligand PCTA were similar. The stability of M(NO₂-Bn-PCTA) (M = Mg²⁺, Ca²⁺, Cu²⁺, Zn²⁺) complexes was similar to that of the corresponding PCTA complexes while the stability of Ln³⁺ complexes of the bifunctional ligand is somewhat lower than that of PCTA chelates. The rate of complex formation of Ln(NO₂-Bn-PCTA) complexes was found to be quite similar to that of PCTA, a ligand known to exhibit the fastest formation rates among all lanthanide macrocyclic ligand complexes studied to date. The acid catalyzed decomplexation kinetic studies of the selected Ln(NO₂-Bn-PCTA) complexes showed that the kinetic inertness of the complexes was comparable to that of Ln(DOTA) chelates making the bifunctional ligand NO₂-Bn-PCTA suitable for labeling biological vectors with radioisotopes for nuclear medicine applications.

INTRODUCTION

The choice of chelating system for biomedical imaging and therapeutic applications is largely determined by the nature of the metal ion required for the given application. Several rare earth metal ions (lanthanides and yttrium) and main group III elements are frequently selected for diagnostic (¹¹¹In, ⁶⁷Ga, ⁶⁸Ga, ⁸⁶Y, ¹⁷⁷Lu) and/or therapeutic (⁹⁰Y, ¹⁷⁷Lu, ¹⁶⁶Ho, ¹⁴⁹Pm, ¹⁵³Sm) nuclear medicine applications.(1,2) A common characteristic feature of these metals is their tendency to exist as trivalent cations that form stable complexes in aqueous media with polyamino polycarboxylate type ligands such as the acyclic H₅DTPA (diethylenetriamine-N, N, N', N'', N'''-pentaacetic acid) and the macrocyclic H₄DOTA (1,4,7,10-tetraazacyclododecane-1,4,7,10-teraacetic acid) systems (Figure 1).

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Supporting Information Available. Detailed synthesis of the ligand, ¹H-¹H COSY, ¹³C APT and ¹³C-¹H HMQC NMR spectra of nitrobenzyl pycen, evaluation of kinetic data, the rate constants characterizing the rearrangement of the intermediates to the final products in formation reactions of Ce(NO₂-Bn-PCTA), Eu(NO₂-Bn-PCTA) and Yb(NO₂-Bn-PCTA) complexes, the *k_p* values for dissociation of Ce(NO₂-Bn-PCTA), Eu(NO₂-Bn-PCTA) and Yb(NO₂-Bn-PCTA) complexes. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Bifunctional derivatives of DOTA are increasingly used to radiolabel peptides, proteins and other biomolecules.(3) The success of DOTA-bifunctionals is largely due to their ability to form complexes with these metal ions with high thermodynamic stability and extraordinary kinetic inertness. However, slow complex formation kinetics with DOTA under mild conditions can be a disadvantage, especially when sensitive biological vectors are labeled or when short half-lived isotopes are used.(4,5) Therefore, macrocyclic ligands that complex radioisotopes faster than DOTA and still retain reasonably high kinetic inertness are very desirable. The acyclic octadentate DTPA and its bifunctional versions form complexes much faster than DOTA derivatives but these complexes have significantly lower kinetic inertness than the corresponding DOTA derivatives despite recent improvements achieved by substituting one of the flexible ethane-1,2-diamine units of DTPA by the more rigid propane-1,2-diamine (MX-DTPA) or cyclohexane-1,2-diamine bridge (CHX-DTPA) (Figure 1). (6)

Recently, a few attempts have been reported to overcome the problem of slow complex formation involving DOTA-based ligands (Figure 2). Brechbiel and co-workers have synthesized a new class of ligands that integrate a macrocyclic triazacyclononane-diacetic acid and acyclic iminodiacetic acid moiety (bimodal or “hybrid” ligands). When the two units are linked together through an ethylene bridge, the resulting ligand (NETA) was shown to have improved complexation kinetics (fast complexation with Y^{3+} , excellent serum stability and rapid clearance of the radiolanthanide chelates).(7) A bifunctional version of NETA has also been reported recently.(4,8)

Aime and colleagues synthesized an interesting cyclic heptadentate ligand, AAZTA.(9) The authors have not reported detailed equilibrium and kinetic data for this system but it was noted that the reaction of AAZTA with lanthanides was almost instantaneous.(9)

Our approach to a bifunctional macrocyclic chelator with improved metal ion complexation kinetics is based on the ligand H_3PCTA (3,6,9,15-tetraazabicyclo[9.3.1]pentadeca-1(15), 11,13-triene-3,6,9-triacetic acid, Figure 2). We recently reported that $PCTA$ forms complexes with lanthanide ions about an order of magnitude faster than DOTA.(10) Furthermore, the acid catalyzed dissociation rates of the $Ln(PCTA)$ complexes were found to be satisfactorily slow despite their somewhat low thermodynamic stabilities.(10) Here we report the synthesis of a bifunctional version of $PCTA$, (NO_2 -Bn- $PCTA$), the protonation constants of the ligand, the stability constants of the ligand with various metal ions and the formation and dissociation rates of some $Ln(NO_2$ -Bn- $PCTA)$ complexes.

EXPERIMENTAL SECTION

Synthesis

The detailed synthetic procedures for the bifunctional ligand (S)-5-(p-nitrobenzyl)- $PCTA$ (NO_2 -Bn- $PCTA$) is reported in the Supplementary Information. Analytically pure samples of the ligand used in the equilibrium and kinetics studies were obtained by preparative HPLC purification on Phenomenex® Luna 5u C18(2) column [elution with H_2O (0.038% HCl): MeCN (gradient elution from 0% MeCN to 50% MeCN in 30 minutes)].

Equilibrium studies

pH-potentiometric titrations were carried out with a Thermo Orion EA940 expandable ion analyzer using Thermo Orion semi-micro combination electrode 8103BN in a thermostated (at 25.0°C) vessel. A Metrohm DOSIMATE 665 autoburette (5 mL capacity) was used for base additions and 1.0 M KCl was used to maintain the ionic strength. All equilibrium measurements (direct titrations) were carried out in 10.00 mL sample volumes with magnetic stirring. During the titrations, argon gas was passed over the sample surface to maintain a CO_2 -free

environment. Standard buffers (Borax: 0.01 M, pH = 9.180 and KH-phthalate 0.05 M, pH = 4.005) were used to calibrate the electrode. The titrant, a carbonate-free KOH solution, was standardized against 0.05 M KH-phthalate solution by pH-potentiometry. The H^+ ion concentrations corresponding to the measured pH values were calculated by the method proposed by Irving et al.(11) The ion-product of water was established in a separate acid-base titration experiment ($pK_w=13.747$) and used in our calculations.

Stock solutions of $MgCl_2$, $CaCl_2$, $ZnCl_2$, $CuCl_2$, YCl_3 , $InCl_3$ and $LnCl_3$ were prepared from analytical grade salts (Aldrich and Sigma, 99.9%). The concentration of the stock solutions were determined by complexometric titration using standardized Na_2H_2EDTA solution in the presence of an appropriate end-point indicator (eriochromeblack-T for $MgCl_2$, and $ZnCl_2$, calconcarboxylic acid for $CaCl_2$, murexide for $CuCl_2$ and xylenol orange for YCl_3 , $InCl_3$ and $LnCl_3$ stock solutions).(12) The concentration of the ligand stock solution was determined by pH-potentiometry from the titration data obtained in the absence and in the presence of about 50-fold excess $CaCl_2$.

The protonation constants of the ligand were calculated from the data obtained by titrating 2.5 mM and 4.4 mM ligand solutions (total 506 data pairs) with standardized KOH solution (0.1713 M) in the pH range of 1.8–11.9. The protonation constants of the ligand ($\log K_i^H$) are defined as:

$$K_i^H = \frac{[H_iL]}{[H_{i-1}L][H^+]} \quad (1)$$

where $i = 1, 2, \dots, 5$ and $[H_{i-1}L]$ and $[H^+]$ are the equilibrium concentrations of the ligand ($i=1$), protonated forms of the ligand ($i=2, \dots, 5$) and hydrogen ions, respectively.

In the systems containing Mg^{2+} , Ca^{2+} , Zn^{2+} or Cu^{2+} , equilibrium in acidic media was reached rapidly enough to use a direct titration data for stability constant determinations. The titrations were carried out at 1:1 and 2:1 metal to ligand concentration ratio ($C_{Lig}=2.5$ mM). The data obtained at different concentration ratios were combined and fitted simultaneously. Even though the rate of complex formation of the $Ln(NO_2-Bn-PCTA)$ complexes is faster than that of the $Ln(DOTA)$ complexes, they are not fast enough to determine the stability of the complexes by direct titration. Owing to the slow formation reactions of $Y(NO_2-Bn-PCTA)$ In ($NO_2-Bn-PCTA$) and the $Ln(NO_2-Bn-PCTA)$ complexes, the “out-of-cell” technique was applied for the stability constant determinations. The same method was used earlier to determine the stability constants of some $Ln(PCTA)$ complexes and further details may be found elsewhere.(10,13)

The program PSEQUAD was used to process the titration data (calculation of the protonation and stability constants).(14) The reliability of the protonation and stability constants are characterized by the calculated standard deviation values shown in parenthesis and fitting parameter values (ΔV – is the difference between the experimental and the calculated titration curves expressed in mL of the titrant) given in the footnotes of the tables.

Formation kinetics

The rates of complex formation of $Ce(NO_2-Bn-PCTA)$, $Eu(NO_2-Bn-PCTA)$ and $Yb(NO_2-Bn-PCTA)$ were studied at 25°C and 1.0 M KCl ionic strength using an Applied Photophysics RX. 2000 rapid mixing accessory attached to a Cary 300 Bio UV-vis spectrophotometer. The formation reactions at low pH were sufficiently slow and were followed by conventional UV-vis spectroscopy. A typical concentration of PCTA was 0.2 mM while the concentration of $NO_2-Bn-PCTA$ was approximately 0.075 mM. The concentration of the metal ions was varied

in the range 4–40 mM (20× to 200× metal excess) when the saturation curves were studied while it was set to 200-fold metal excess for the pH dependent measurements. Yb(PCTA) complex formation was studied in the pH range 3.59–5.16 while in the studies involving the NO₂-Bn-PCTA ligand, the following pH ranges were used: pH = 3.93–5.69 for Ce³⁺ and Eu³⁺ and pH = 3.93–5.45 for Yb³⁺.

Both PCTA and NO₂-Bn-PCTA contain a convenient built-in chromophore (pyridine unit) and both the formation and dissociation kinetics studies were performed by following the changes in the $\pi \rightarrow \pi^*$ transition at 278 nm. At this wavelength the absorbance of the metal ions is either weak (Ce³⁺ and Eu³⁺) or zero (Y³⁺ and Yb³⁺). During the formation kinetic studies the non-coordinating buffers *N*-methylpiperazine (NMP, log K_2^H = 4.83) and dimethylpiperazine (DMP, log K_2^H = 4.18) were used to maintain the pH constant at a concentration of 0.05 M. (14)

Equation 2 was used to calculate the first order rate constants (k_{obs} for formation and k_d for dissociation), where A_0 , A_e and A_t are the absorbance values measured at the start of the reaction ($t=0$), at equilibrium and at time t , respectively. The data were fitted to Eqn 2 with the software Scientist® (Micromath) using a standard least-squares procedure.

$$A_t = A_e + (A_0 - A_e) \cdot e^{(-k_{obs} \cdot t)} \quad (2)$$

The relative error for fitting of the absorbance vs. time data was less than 1% while the calculated first order rate constants, k_{obs} and k_p , were reproduced to within $\pm 3\%$ as determined in 12–15 identical experiments (each point on the curves reflects an average of 12–15 identical kinetic runs) for formation and 3 experiments for the dissociation reactions.

Kinetics of Dissociation

The acid catalyzed dissociation kinetics of Ce(NO₂-Bn-PCTA), Eu(NO₂-Bn-PCTA) and Yb(NO₂-Bn-PCTA) complexes were measured under pseudo-first-order conditions by mixing the appropriate complexes with a large excess hydrochloric acid (0.25–2.0 M) while keeping the ionic strength constant at 3.0 M with added KCl [(H⁺+K⁺)Cl[−]]. A total of eight reactions was followed by direct spectrophotometry at 25°C until the conversion reached 80–100% in 0.075 mM complex solutions. Under these conditions changes of ~0.2 abs. units were detected throughout the dissociation reaction. In addition, the absorbance data, recorded as a function of time at 278 nm, showed no evidence of significant amplitude loss during the mixing of the components (usually 6–8 sec) and were fitted to Eqn. 2 as a single-exponential decay with excellent confidence.

RESULTS AND DISCUSSION

Ligand design and synthesis

The aromatic isothiocyanato group is one of the most commonly used reactive functionalities for bioconjugation. It readily reacts with primary amino groups of peptides or proteins to form a stable thiourea linkage under mild conditions. The isothiocyanato functionality can easily be synthesized by reducing the corresponding nitro derivative and reacting the resulting aniline with thiophosgene. Both the aromatic isothiocyanato and amino compounds are sensitive to hydrolysis and oxidation, respectively, and have limited shelf-lives so they are not suitable for extended equilibrium and kinetic measurements lasting several days (such as the “out-of-cell” technique). The nitro derivative, however, is stable at room temperature in aqueous solutions and can conveniently be used in these studies. Considering the parent ligand PCTA, the aromatic isothiocyanato function can be attached to three chemically distinct positions: one of

the pyridine carbons, one of the acetate sidearms, or one of the carbon atoms of the ethylene bridges in the cyclen backbone. Each of these options has advantages and disadvantages. For example, functionalization of the pyridine moiety would be expected to have the least effect on the chelation properties of the ligand (the p-NO₂-benzyl substituent does not have a strong inductive or mesomeric effect and so it is not expected to significantly alter the electron density on the pyridine N-atom) while side-arm functionalization may have an adverse effect on the metal binding ability of the acetate pendant arms. We have chosen the ethylene bridge for functionalization because our assumption was that this would eliminate any unwanted interference with the metal binding and the rigidifying effect of macrocyclic backbone substitution may actually add to the kinetic inertness of the resulting complexes. In addition, the backbone functionalized PCTA would structurally be analogous to the nitro precursor of the most frequently used bifunctional ligand, p-isothiocyanatobenzyl-DOTA, and so comparison of the equilibrium and kinetic properties of these two ligands could provide further insight into the design of new bifunctional chelators. The synthesis of the new bifunctional ligand, (S)-5-(p-nitrobenzyl)-PCTA (NO₂-Bn-PCTA, **6**) is outlined in Scheme 1. *N, N', N''*-tritosyl (S)-2-(p-nitrobenzyl)-diethylenetriamine was prepared starting from L-nitrophenyl alanine following published procedures.⁽¹⁶⁾ The key step of the synthesis is the Richman-Atkins type cyclization of the disodium salt of *N, N', N''*-tritosyl-(S)-2-(p-nitrobenzyl)-diethylenetriamine (tosyl = p-toluenesulfonyl) **1** with 2,6-bis(chloromethyl)pyridine **2** in DMF giving about 55% yield of the tritosyl-nitrobenzyl-pyclen.⁽¹⁷⁾ It is worth noting that the analogous cyclization reaction between the disodium salt of ditosyl-nitrobenzyl ethylenediamine and the tetratosyl derivative of *N, N'*-bis(2-hydroxyethyl)-ethylenediamine failed to give the expected tetratosyl nitrobenzyl-cyclen due to side reactions involving the nitro group.⁽¹⁸⁾ Interestingly, in this case, the aromatic nitro group did not affect the cyclization reaction, probably because the benzyl-halide type bis(chloromethyl)pyridine is a more reactive alkylating agent than the O-tosyl derivative of *N, N'*-bis(2-hydroxyethyl)-ethylenediamine or diethanolamine. The tosyl groups of **3** were removed in concentrated sulfuric acid at 180 °C to afford (S)-5-(p-nitrobenzyl)-pyclen **4** in good yield. The ¹H NMR spectrum of this cyclic tetramine is quite complicated as all the protons are magnetically nonequivalent and correct assignment could only be achieved with the help of ¹H-¹H COSY, ¹³C APT and ¹³C-¹H HMQC NMR experiments (Figures S1-S3). Nitrobenzyl pyclen **4** was alkylated with *tert*-butyl bromoacetate in the presence of anhydrous potassium carbonate in acetonitrile to yield the *tert*-butyl ester **5**. The *tert*-butyl ester groups were subsequently cleaved in aqueous hydrochloric acid to afford the ligand **6** as the HCl salt in about 35% overall yield.

Protonation and stability constant determinations

The bifunctional ligand (S)-5-(p-nitrobenzyl)-PCTA has seven protonation sites and five protonation steps of these were detected in the pH range 1.8–11.8 when the ligand was titrated with strong base (KOH). The protonation constants obtained by pH-potentiometric measurements are presented in Table 1 along with the previously reported values for PCTA, DOTA and the bifunctional NO₂-Bn-DOTA.

The first and the second protonation constants can be assigned to nitrogen atoms of the macrocyclic ring, while the next 3 protonation steps occur most likely at the acetate pendant arms. The first protonation constant of PCTA and NO₂-Bn-PCTA are nearly identical but there is a noticeable difference in the second protonation constants (about 0.5 log K units). This was not unexpected as similar trend has been observed for the protonation constants of DOTA and p-NO₂-Bn-DOTA. The somewhat smaller log *K*₂ values found for the bifunctional versions is likely due to the conformational changes in the ethylene bridge of the macrocyclic ring induced by the p-nitrobenzyl substituent.⁽¹³⁾ Interestingly, in the case of NO₂-Bn-DOTA, both the first and the second protonation constants were affected while for NO₂-Bn-PCTA only the second protonation constant is affected. This may be explained by the considerably less conformational

flexibility of PCTA derivatives as compared to the DOTA-based ligands due to the rigidifying effect of the pyridine ring. Consequently, the extra strain introduced by the p-nitrobenzyl substituent will have a smaller effect on the pycen macrocycle. Differences in the protonation sequence of NO₂-Bn-PCTA and NO₂-Bn-DOTA may also play a role. According to ¹H-NMR studies, the most basic site in PCTA is the nitrogen atom opposite to the pyridine ring.(25) Addition of a second proton results in a rearrangement of the protonation sites to the two tertiary nitrogen atoms positioned *trans*- to each other and *cis* to the pyridine nitrogen.(25) In NO₂-Bn-PCTA, the p-nitrobenzyl substituent is attached next to a tertiary nitrogen atom positioned *cis* to the pyridine nitrogen and the extra strain induced at this nitrogen atom reduces its basicity. The total basicity ($\Sigma \log K_i^H$) of PCTA and NO₂-Bn-PCTA is similar, around 25.8–25.9. Although this value is reasonably favorable, it is about 3 log *K* units lower than that of the DO3A and 6 to 7 log *K* units lower than that of the DOTA and NO₂-Bn-DOTA. As a consequence, the thermodynamic stability constants for the complexes of PCTA-based ligands are expected to be somewhat lower than the corresponding DOTA-complexes.

The stability constants of the complexes of Mg²⁺, Ca²⁺, Zn²⁺ and Cu²⁺ ions with NO₂-Bn-PCTA were determined by pH-potentiometry in the presence of 1.0 M KCl as ionic background. The stability constants of these complexes along with the constants characterizing the stability of the M(DO3A), M(PCTA) and M(DOTA) complexes are listed in Table 2 (the stability constants of Mg²⁺, Ca²⁺, Zn²⁺ and Cu²⁺ complexes of NO₂-Bn-DOTA have not been reported yet). The pH-potentiometric titration data for both the 1:1 and 1:2 metal to ligand concentration ratios could be fitted well by assuming the formation of ML and MHL species only. The stability and protonation constants characterizing the formation of the Mg²⁺ and Ca(NO₂-Bn-PCTA) complexes are in excellent agreement with data obtained for the Mg²⁺ and Ca(PCTA) complexes. Unlike the Cu²⁺ and Zn(PCTA) systems, no evidence of formation of ternary hydroxo complexes, MLH₋₁, was found in titrations with these same metal ions and NO₂-Bn-PCTA, likely due to the higher stability of the latter complexes.

The data in Table 2 show that, unlike DOTA-based ligands, both PCTA and NO₂-Bn-PCTA form more stable complexes with Zn²⁺ than with Cu²⁺. This observation is in accordance with the results reported earlier by Delgado *et al.* on the selectivity of some pyridine containing macrocyclic ligands for Zn²⁺ over Cu²⁺.(26) Interestingly, the selectivity was observed exclusively for the 12-membered PCTA ligand. (26) This unexpected selectivity is likely due to the rigid nature of the pycen backbone and the slightly different type of interaction required (angle) between the pyridine nitrogen atom and Zn²⁺ and Cu²⁺ ions. This explanation is supported by the crystal structure of the [Cu(pycen)Br]ClO₄, which shows that the pyridine nitrogen — Cu²⁺ bond length is shorter than the secondary amine — Cu²⁺ bonds, while in the ([Zn(pycen)Cl])₂ZnCl₄ complex the length of the pyridine-N — Zn²⁺ bond is comparable to that of the secondary amine-Zn²⁺ bonds.(26,30)

The stability constants (log *K*_{ML}) of the complexes formed with some Ln³⁺, Y³⁺ and In³⁺ ions with NO₂-Bn-PCTA were determined by “out-of-cell” potentiometric titration technique because of their slow formation rates. There was evidence for the formation of MHL species only for the larger metal ions (Ce³⁺ and Nd³⁺, log *K*_{CeL}^H=2.17 and log *K*_{NdL}^H=1.93, respectively) while ternary hydroxo complex formation was not considered. The stability constants of NO₂-Bn-PCTA complexes with these trivalent ions are listed in Table 3 along with previously reported values for structurally related ligands.

As expected, the stability of the Ln(NO₂-Bn-PCTA) complexes increases substantially from Ce³⁺ to Eu³⁺ then increases only gradually with decreasing ion size, maximizing at Lu³⁺. This trend is similar to that seen previously for complexes of macrocyclic ligands with a similar size and number of donor atoms (Ln(PCTA), Ln(DO3A), Ln(DOTA)).(10,31,32,35,36) Despite the similar basicities of PCTA and NO₂-Bn-PCTA, the stability of the Ln(III)

complexes formed with the bifunctional ligand are about 0.5–1 log K units lower than those of the parent ligand, PCTA. Since the basicities of these ligands are similar, this difference is likely due to the steric effect of the p-nitrobenzyl substituent. Although the thermodynamic stabilities of lanthanide complexes of NO₂-Bn-PCTA are somewhat lower than stability of the corresponding DOTA complexes, they remain reasonably favorable for potential *in vivo* applications.(37)

It is of interest to note that In³⁺ forms quite stable complexes with both PCTA and NO₂-Bn-PCTA (the log K_{ML} value is comparable to the value published for In(DOTA). However, for a more meaningful comparison, the available literature data on In³⁺-ligand stability constants should be analyzed carefully because these are often measured in the presence of Cl[−] containing electrolytes (e.g. KCl, NaCl and Me₄NCl) used to maintain the constant ionic strength during titrations. However, Cl[−] ions can form relatively stable complexes with In³⁺ ion with log β_1 , log β_2 and log β_3 values of 2.58, 3.84 and 4.20, respectively (25 °C, I = 3.0 M NaClO₄). (38) If the formation of InCl_{*x*}^{(3−*x*)+} species is not taken into account, then the resulting stability constants only represent the lower limits of stability and the actual stability constants are somewhat higher (with about 4 log K units). This appears to be the case for In(DOTA), as its stability was measured in the presence of 0.1M KCl and the formation of InCl_{*x*}^{(3−*x*)+} species was ignored by Clarke et al.(36), whereas it was accounted for in the calculated stabilities of In(PCTA) and In(NO₂-Bn-PCTA) here. In the pH range 1.5–2.5, the conditional stabilities of the In(PCTA) and In(NO₂-Bn-PCTA) complexes are relatively low and the Cl[−] ions can compete well with these ligands. Under the conditions employed in our study (I=1.0 M KCl) only the complexes InCl₂[−], InCl₃ and In(PCTA) are present in the equilibrium (there is no free In³⁺) and the stability of In-complexes listed in Table 3 were calculated with the use of the known log β_X values. It is worth noting that the stability of In(PCTA) published by Delgado et al. is 3.3 log K units lower than that measured here (Table 3).(26) The competitive Cl[−] complex formation cannot explain the discrepancy in this case since Delgado et al. used MeN₄NO₃ to set the ionic strength of the samples.(26) It is more likely that these differences originate from differences in the protonation constants of the ligand due to the different methods used to evaluate these constants.

Kinetics of Complex Formation

The formation kinetics of the complexes involving macrocyclic ligands are usually considerably slower than the corresponding complexes formed with multidentate, open-chain ligands.(39) Unlike the polycarboxylate ligands derived from linear polyamines, the corresponding polycarboxylate macrocyclic ligands tend to form relatively stable protonated “out-of-cage” complexes as intermediates which then rearrange slowly to the thermodynamically stable complex in a general base-catalyzed step.(40–42) Mono-, di- and occasionally triprotonated intermediates have been demonstrated by different spectroscopic techniques (UV-vis, luminescence, NMR, EXAFS etc).(43–53) The formation kinetics of Ln (III) complexes are most easily studied by UV-vis spectroscopy (Ce³⁺ and Eu³⁺) or relaxivity measurements (Gd³⁺). (43,46–49) Complexation of the smaller ionic radii Ln(III) ions that have no absorption in the UV-vis (Yb³⁺ or Lu³⁺) can be followed indirectly by UV-vis spectroscopy using an appropriate acid-base indicator to register slight pH changes during the course of the reaction.(43,46,54) Fortunately, PCTA and NO₂-Bn-PCTA have a convenient built-in chromophore arising from the $\pi \rightarrow \pi^*$ transition of the pyridine moiety ($\epsilon_{\max} = 1.25 \times 10^4$ dm³mol^{−1}cm^{−1}, $\lambda_{\max} = 269$ nm, pH=3.97, for NO₂-Bn-PCTA) that undergoes a bathochromic shift upon metal ion encapsulation ($\lambda_{\max} = 278$ nm) (Figure 3). As the bathochromic shift and the molar extinction coefficient are almost independent of which metal ion is encapsulated, this transition can be used to monitor complex formation for a large variety of metal ions. Here, formation kinetics were examined for three lanthanide ions, one of larger ionic radius (Ce³⁺

-103 pm), one of medium ionic radius (Eu^{3+} – 95 pm) and one of smaller ionic radius (Yb^{3+} – 86 pm) to compare the effect of metal ion size on the rates of complex formation.

The formation kinetics of $\text{Ln}(\text{NO}_2\text{-Bn-PCTA})$ ($\text{Ln} = \text{Ce}^{3+}$, Eu^{3+} and Yb^{3+}) were studied at different pH and metal ion concentrations (20 to 200-fold metal excess) under pseudo-first-order conditions. In the presence of excess Ln^{3+} , the rate of complex formation may be described as:

$$\frac{d[\text{LnL}]_t}{dt} = k_{\text{obs}}[\text{L}]_t \quad (3)$$

where $[\text{L}]_t$ is the total concentration of the $\text{NO}_2\text{-Bn-PCTA}$ (complexed and uncomplexed forms) and k_{obs} is a pseudo-first-order rate constant. Plots of k_{obs} versus Ln^{3+} ion concentration showed saturation kinetics at high Ln^{3+} concentration. The shape of these plots is characteristic of rapid formation of an intermediate that rearranges slowly to the final product in a slow, rate determining step. A similar mechanism has been reported for other $\text{Ln}(\text{III})$ complexes formed with macrocyclic polyaminopolycarboxylic acid ligands including $\text{Ln}(\text{NOTA})$, $\text{Ln}(\text{DOTA})^-$, $\text{Ln}(\text{TRITA})^-$ and $\text{Ln}(\text{PCTA})$.(10,43,46,49) The intermediate formed along the pathway to stable LnDOTA and LnPCTA complexes has been shown to be a diprotonated species while intermediate formed in the LnNOTA and LnDETA complexes is monoprotonated.(46,55) In each case, the number of protons in the intermediate reflects the basicity of the macrocyclic nitrogen donor atoms in these systems. Tetraazamacrocycles generally have two very basic nitrogen atoms while triaza macrocycles typically have one basic and one moderately basic nitrogen,(56) the difference between the 1st and 2nd protonation constants ($\log K_1^H - \log K_2^H$) is generally larger for triaza macrocycles (4.74–6.31 for NOTA) than for tetraaza-macrocyclic ligands ($\log K_1^H - \log K_2^H = 2.33\text{--}2.90$ for DOTA (the range of the protonation constant differences reflect the literature data reported for the given ligand)). The difference between the 1st and 2nd protonation constants of $\text{NO}_2\text{-Bn-PCTA}$ (4.59) is somewhat greater than the same difference in PCTA (3.99) and lies between the corresponding differences for DOTA , $\text{NO}_2\text{-Bn-DOTA}$ and NOTA . This comparison suggests that the $\text{Ln}(\text{NO}_2\text{-Bn-PCTA})$ intermediates will likely also be diprotonated. This was verified experimentally for the Yb^{3+} complex in weakly buffered solutions by a UV-vis spectroscopic method described earlier. (43,46,54) Changes in the absorbance of a sample containing an acid-base indicator (methyl orange) in weakly buffered solutions (0.01M DMP) were calibrated by the addition of a known amount of strong acid stock solution. Using this calibration curve the concentration (number of moles) of protons released immediately after mixing of the two components (ligand and metal-ion solution) and at the end of the complexation reaction was determined and compared. The components (ligand and metal ion solutions) were mixed at pH 3.95, a slight pH change was registered upon mixing followed by a more pronounced change in pH during the approach to equilibrium (~6 hours). From the volume of acid needed to return the pH to its original value, it was estimated that about 4 times more protons were released in the slow process than in the fast one. Since the ligand exists as $\text{H}_2.5\text{L}$ at pH ~4, the release of 0.5 eq. of H^+ upon mixing followed by the liberation of 2 eq. of H^+ after reaching equilibrium is consistent with formation of a diprotonated intermediate in $\text{Yb}(\text{NO}_2\text{-Bn-PCTA})$.

Since no major differences in the mechanism of complex formation of $\text{Ln}(\text{NO}_2\text{-Bn-PCTA})$ and $\text{Ln}(\text{PCTA})$ complexes could be detected, the raw data measured for the formation of $\text{Ln}(\text{NO}_2\text{-Bn-PCTA})$ complexes were treated the same way as the $\text{Ln}(\text{PCTA})$ complexes, the details of which will not be repeated here.(10) Briefly, the pseudo-first-order rate constants obtained at various pH and metal concentrations were fitted to Eqn. (4) (additional information can be found in electronic supporting information) and the k_r rate constants and the stability

constants of the intermediates, $K_{\text{Ln}(\text{H}_2\text{L})^*}$ were calculated as described for the $\text{Ln}(\text{PCTA})$ complexes.

$$k_{\text{obs}} = \frac{k_r \frac{K_{\text{Ln}(\text{H}_2\text{L})^*}}{\alpha_{\text{H}_2\text{L}}} [\text{Ln}^{3+}]}{1 + \frac{K_{\text{Ln}(\text{H}_2\text{L})^*}}{\alpha_{\text{H}_2\text{L}}} [\text{Ln}^{3+}]} \quad (4)$$

The stability constants of the Ce^{3+} and $\text{Eu}(\text{H}_2\text{NO}_2\text{-Bn-PCTA})$ intermediates determined from the kinetics data were 2.83 ± 0.16 and 2.90 ± 0.11 , respectively. The stability of the $\text{Yb}(\text{H}_2\text{PCTA})$ was also redetermined in the present study and found to be in excellent agreement with our previously published value (2.70 ± 0.08). The stability constants of the intermediates calculated from the kinetic data are lower than those obtained from pH-potentiometric titration for the Ce^{3+} , Eu^{3+} and $\text{Yb}(\text{H}_2\text{DOTA})^+$ complexes ($\log K = 4.5$, 4.3 and 4.2 respectively), consistent with differences in the structures expected for these intermediates. The nature of this intermediate has been thoroughly investigated for DOTA. Recent pH-potentiometry, luminescence and extended X-ray absorption fine structure (EXAFS) spectroscopy studies have indicated that the formation of $\text{Ln}(\text{DOTA})$ chelates actually involves two diprotonated intermediates, (51,52) the first of which is formed instantaneously where the central Ln^{3+} ion is coordinated to oxygens of each of the four carboxylate groups and five water molecules ($q = 5$). This intermediate rapidly converts into the more stable diprotonated species in which the metal ion is coordinated to four oxygens of the acetate sidearms, two N-atoms of the macrocyclic ring and three water molecules ($q = 3$). The stability of the more stable ($q = 3$) intermediate has been determined by both pH-potentiometry and spectrophotometry ($\log K = 4.5$, 4.3 , and 4.2 for Ce^{3+} , Eu^{3+} and Yb^{3+} , respectively, by pH-potentiometry). (43) For ligands with three acetate sidearms (PCTA, DO3A) the stability of the intermediate is expected to be lower. (57) There are no stability data available for the intermediates in $\text{Ln}(\text{DO3A})$ systems but q was found to be 4.6 for the $\text{Eu}(\text{DO3A})$ complex. (52) Data published for [10-[2,3-dihydroxy-1-(hydroxymethyl)propyl]-DO3A (DO3A-butriol) ($\log K = 2.4$, 2.5 , and 2.43 for the Ce^{3+} , Eu^{3+} and Yb^{3+} intermediates, respectively) suggest that only the acetate oxygens coordinate. (57) Considering the Eu^{3+} aqua ion as nine-coordinate, one can assume that the PCTA and $\text{NO}_2\text{-Bn-PCTA}$ ligands also coordinate to the metal by the three acetate oxygen donor atoms. This assumption is supported by the fact that the stability of the intermediates are higher than the stability of the monoacetate complexes and are very similar to the stability of Ln -dicarboxylic acid complexes. (15)

It is now generally accepted that the mechanism of complex formation for DOTA and DOTA-like ligands involves the rearrangement of the diprotonated intermediates through rapid deprotonation of the intermediate to a monoprotinated species in a fast equilibrium step. This is followed by the release of the second proton in a slow, rate determining step and the rapid structural rearrangement of the fully non-protonated complex to the final product. In accordance with this mechanism, the validity of general base catalysis has been confirmed in some cases. (10,24,57)

Eqn. (4) can further be simplified if the experimental conditions are chosen so that the conditional stability constant of the intermediate will be high ($K_{\text{Ln}(\text{H}_2\text{L})^*}^C = K_{\text{Ln}(\text{H}_2\text{L})^*}/\alpha_{\text{H}_2\text{L}}$) and the reaction will be pseudo-first-order ($C_{\text{Ln}} \geq 10C_{\text{L}}$). Under these conditions $K_{\text{Ln}(\text{H}_2\text{L})^*}^C \times [\text{Ln}]_{\text{tot}} \gg 1$ and thus 1 can be neglected in the denominator of Eqn. 4. This simplifies Eqn. 4 to $k_{\text{obs}} \approx k_r$, (k_r is the “saturation value” of the rate constant k_{obs} when the formation of the intermediate is complete). In other words, the k_r values can be approximated with k_{obs} if a large excess of Ln^{3+} is used over the ligand. The necessary excess of the Ln^{3+} to reach the $k_{\text{obs}} \approx$

k_r condition can be determined from the expression of stability constant of the accumulated intermediate (Eqn. 5):

$$K_{Ln(H_2L)^*} [Ln^{3+}] = \frac{[Ln(H_2L)^*]}{[H_2L]} \quad (5)$$

To ensure quantitative formation of the intermediate, the ratio $[Ln(H_2L)^*]/[H_2L]$ ([bound]/[unbound]) in Eqn. 5 should be $\gg 1$. The ratio [bound]/[unbound] will be equal to 1 when the ratio $[Ln^{3+}]/[H_2L] = 19$ (as calculated from the stability constant of the intermediate), so for a more detailed pH dependence study, the ratio $[Ln^{3+}]/[H_2L]$ was set to 200.

The rate constants k_r obtained under these conditions were found to be inversely proportional to the H^+ ion concentration (Eqn. 6) for all the metal ions and ligands studied in the present work (Figure 4). The k_{OH} values were obtained from the fitting of k_r values gained at different pH values to Equation 6 (Table 4).

$$k_r = \frac{k_H}{[H^+]} = k_{OH} [OH^-] \quad (6)$$

The rate of complex formation (rearrangement of the intermediates) increases with decreasing lanthanide-ion size. This decreasing tendency of the k_{OH} values along the lanthanide series is not surprising since it has been reported for numerous cyclen derivatives with acetate pendant arms, and can be explained by the higher charge density of the heavier lanthanides (the OH^- ion catalyzed deprotonation is faster for the metal ions with greater positive charge density). (42) The Ln(NOTA) complexes do not follow this trend and for some DOTA-tetra(amides) the opposite order of formation constants was recently reported. (46,61,62) For the latter complexes, the extremely slow water exchange that is typical of DOTA-tetraamide systems seems to be responsible for this unusual behaviour across the lanthanide series. (61,62)

Kinetics of Dissociation

The kinetic inertness of a complex will largely determine its fate *in vivo*. (63) Even if the thermodynamic stability of the complex is not very high, it is possible that it can safely be used for biomedical applications if the complex does not dissociate *in vivo*. The usefulness of acid catalyzed dissociation rates in predicting the fate of a complex *in vivo* was first noted by Wedeking when it was found that the extent of long term Gd-deposition in the whole body, liver and femur of mice was correlated well with the *ex vivo* acid catalyzed dissociation rates of various open chain and macrocyclic ^{153}Gd -labeled complexes. (64) The deposition of Gd was the lowest for Gd(DOTA) and this is in good agreement with its extremely slow proton assisted dissociation rate. (64) Generally, Ln(III) complexes of DOTA-like ligands is very slow under physiological conditions (63), probably due to their tightly packed and highly rigid structure. (45) Later it was realized that the *in vivo* dissociation mechanism of acyclic and macrocyclic lanthanide complexes is quite different. The dominant pathway for *in vivo* dissociation of Ln-chelates of DOTA-like ligands involves the acid catalyzed dissociation and the acid independent spontaneous dissociation. On the other hand, Ln-complexes of open chain ligands (EDTA, DTPA) dissociate *in vivo* through metal ion $[Zn(II), Cu(II)]$ catalyzed pathway, which is acid-independent above pH 4.5. (63) Therefore, the *in vitro* dissociation kinetic studies of macrocyclic chelates are focused on the acid catalyzed dissociation and from the kinetic data the k_1 and k_2 rate constants corresponding to acid-assisted dissociation of the mono and diprotonated complexes as well as the k_0 rate constant that describes the acid-independent spontaneous dissociation can be estimated. (43,45) Since at $pH > 2$, $Ln(NO_2-Bn-PCTA)$

complexes dissociate too slowly to collect kinetic data over a reasonable period of time, the acid catalyzed dissociation of Ln(NO₂-Bn-PCTA) complexes was examined at pH << 2, where the complexes are thermodynamically unstable. Under these experimental conditions, proton-assisted dissociation follows first-order kinetics with respect to the complex ([H⁺] >> [Ln(NO₂-Bn-PCTA)]) and the rate of the dissociation is directly proportional to the total concentration of the complex:

$$-\frac{d[\text{LnL}]_t}{dt} = k_d [\text{LnL}]_t \quad (7)$$

where k_d is a pseudo-first-order rate constant. Under the conditions employed in the current work (0.25 M HCl < C < 2.00 M HCl), complex dissociation proceeds via some combination of acid-assisted and spontaneous dissociation and consequently the total concentration of the complex will be a sum of equilibrium concentrations of the complex ([LnL]) and its different protonated forms ([LnH_xL] where x = 1, 2, ...)

The dependence of the pseudo-first order dissociation rate constants (k_d) on the H⁺ ion-concentration was measured for Ce³⁺, Eu³⁺ and Yb(NO₂-Bn-PCTA) (Figure 5, each point on this figure is an average of two identical kinetic runs). These data demonstrate that the rate of dissociation of the complexes is either proportional to the acid concentration (Ce and Yb) or shows a second-order dependence and can be described by Eqns. 8 and 9, respectively:

$$k_d = k_0 + k_1 \cdot [\text{H}^+] \quad (8)$$

$$k_d = k_0 + k_1 \cdot [\text{H}^+] + k_2 [\text{H}^+]^2 \quad (9)$$

where k_0 is a constant that describes the acid-independent dissociation (spontaneous dissociation) while k_1 and k_2 corresponds to acid-assisted dissociation of the protonated complexes. Ln³⁺ complexes of the parent PCTA behaved slightly differently, since Eu(PCTA) and Yb(PCTA) exhibited linear dependence at low acid concentrations while saturation type dependence was observed for Ce(PCTA), Y(PCTA) and Yb(PCTA) at higher acid concentration. This is likely a consequence of the lower thermodynamic stability of Ln(NO₂-Bn-PCTA) complexes. As a result, the mono- and diprotonated intermediates are also expected to have lower stability and the dissociation curves reach “saturation” at higher acidity beyond the range of acid concentration used in the study.

The acid catalyzed dissociation of NO₂-Bn-PCTA complexes very likely follows the same mechanism that accounts for the dissociation of DOTA complexes, with protonation of a carboxylate oxygen as the initial step. The protonated species then undergoes rearrangement while the proton is transferred from the acetate arm to a ring nitrogen atom. Electrostatic repulsion between the protonated nitrogen and the central metal ion in the macrocyclic cavity of ligand causes the formation of an “out-of-basket” complex which may then dissociate directly or can simultaneously follow a pathway that involves an attack by a second proton. Proton transfer and rearrangement of the complex occurs concurrently and either of these processes can be the rate limiting step. The rigidity of the macrocyclic ligand is responsible for the high kinetic inertness of these complexes.

In the physiologically relevant pH range the contribution of the spontaneous dissociation of the complex, characterized by the k_0 rate constant, may be significant. The value of k_0 can be estimated from the kinetic data (Eqns. 8) but normally its value is zero within the experimental

error because under the experimental conditions ($\text{pH} \ll 2$) the contribution of the spontaneous dissociation to the dissociation process is very small. In our studies fitting of dissociation kinetics data for the Eu^{3+} and Yb^{3+} complexes returned a small and negative value for k_0 . Similar conclusions were reached by other researchers who determined the rate constant characterizing the spontaneous dissociation of $\text{Gd}(\text{DOTA})$ from the kinetic data and the contribution of the acid-independent dissociation was neglected. (43,45) We have found a positive $k_0 = (4.0 \pm 0.3) \times 10^{-5} \text{ s}^{-1}$ with acceptable uncertainty for $\text{Ce}(\text{NO}_2\text{-Bn-PCTA})$. However, this value appears to be too high for spontaneous dissociation of this type of complexes. As reported earlier, (10) the rate of spontaneous dissociation can be predicted from acid-catalyzed dissociation rate data. By comparing data obtained for complexes of macrocyclic ligands of similar ring size, number and quality of donor atoms it was found that the rate of spontaneous dissociation is usually 4–6 orders of magnitude lower than the acid catalyzed dissociation rate constant, k_1 . (10) This approximation gives about 10^{-8} to 10^{-9} s^{-1} as the lower limit of the rate constant characterizing spontaneous dissociation of the $\text{Ln}(\text{NO}_2\text{-Bn-PCTA})$ complexes. In order to determine the k_0 value more precisely, the measurements must be performed at a pH range closer to 7. Preliminary data collected for $\text{Gd}(\text{PCTA})$ and $\text{Gd}(\text{NO}_2\text{-Bn-PCTA})$ in the pH range of 3.0–6.0 and in the presence of Zn^{2+} as scavenger metal ion indicate that a estimate for k_0 is about 10^{-8} to 10^{-9} s^{-1} . Moreover, a recent study on the transmetallation of $\text{Gd}(\text{PCTA})$ and its rigidified derivative, $\text{Gd}(\text{CHX-PCTA})$ as well as other cyclen based macrocyclic $\text{Gd}(\text{III})$ complexes in the presence of Zn^{2+} ions and at physiological pH showed no measureable dissociation of the complexes over the time period of 9000 minutes. (37,65)

The acid-catalyzed dissociation rate constants for some $\text{Ln}(\text{NO}_2\text{-Bn-PCTA})$ complexes are listed and compared to those of other macrocyclic complexes in Table 5. Interestingly, when compared to the parent ligand, PCTA, the introduction of the p-nitrobenzyl substituent did not affect adversely the kinetic inertness of the $\text{Ln}(\text{NO}_2\text{-Bn-PCTA})$ complexes. Even though the thermodynamic stability of $\text{Ln}(\text{NO}_2\text{-Bn-PCTA})$ is slightly lower than the corresponding PCTA complexes, their kinetic inertness towards acid catalyzed dissociation is somewhat higher. This is likely due to the rigidifying effect of the p-nitrobenzyl substituent attached to the cyclen backbone. A similar trend was observed for open-chain and macrocyclic ligands when their structures were rigidified. For example, the half-life of $\text{Gd}(\text{cyclohexyl DOTA})$ in 1M HCl is 50 h compared to 23 h of $\text{Gd}(\text{DOTA})$. (67) The improvement in kinetic inertness is the most pronounced for the Ce^{3+} complex where the rate constant k_1 was found to be at least an order of magnitude lower than it is for the $\text{Ce}(\text{PCTA})$. Furthermore, $\text{Ce}(\text{NO}_2\text{-Bn-PCTA})$ was found to be even more inert towards acid-assisted dissociation than $\text{Ce}(\text{DOTA})$.

CONCLUSIONS

The novel bifunctional ligand p-nitrobenzyl-PCTA was synthesized in reasonable yields. The total basicity of p-nitrobenzyl-PCTA was very similar to that of PCTA and about 6 orders of magnitude less than the basicity of DOTA. As expected, the stability of the complexes of $\text{M}(\text{NO}_2\text{-Bn-PCTA})$ ($\text{M} = \text{Mg}^{2+}, \text{Ca}^{2+}, \text{Cu}^{2+}, \text{Zn}^{2+}$) is very similar to that of the corresponding PCTA complexes. The stability of the Ln complexes of this new bifunctional ligand is slightly lower ($\Delta \log K = 0.5\text{--}1.0$), than that of the parent ligand but the trend of the stability constants across the Ln^{3+} series remains unaffected. The stabilities of $\text{In}(\text{PCTA})$ and $\text{In}(\text{NO}_2\text{-Bn-PCTA})$ were found to be quite high ($\log K \sim 24$), about 3.3 log K units higher than previously reported for $\text{In}(\text{PCTA})$. This difference is a consequence of competitive complex formation between the Cl^- and In^{3+} . As expected, the formation of $\text{Ln}(\text{NO}_2\text{-Bn-PCTA})$ complexes proceeds through a diprotonated intermediate and the rate constant characterizing the OH^- ion catalyzed deprotonation and rearrangement of the intermediate was found to be comparable to that of PCTA complexes. The kinetic inertness of selected $\text{Ln}(\text{NO}_2\text{-Bn-PCTA})$ ($\text{Ln} = \text{Ce}, \text{Eu}, \text{Yb}$) complexes was studied by measuring the rate constant characterizing the acid-catalyzed

dissociation and was found to be higher than the corresponding PCTA chelates probably due to the rigidifying effect of the p-nitrobenzyl substituent. In summary, structural modification of the pycnen backbone with a p-nitrobenzyl substituent does not have an unfavorable influence on either the thermodynamic or kinetic properties of PCTA. The bifunctional ligand forms complexes about an order of magnitude faster than DOTA while the kinetic inertness of the Ln(NO₂-Bn-PCTA) complexes are comparable to that of DOTA chelates. The rapid complex formation kinetics combined with satisfactory thermodynamic stability and excellent kinetic inertness of the complexes renders this bifunctional ligand particularly suitable for nuclear medicine applications when rapid complex formation is required under mild conditions. In addition, the pyridine moiety is a good sensitizer for luminescent lanthanides such as Eu(III) and Tb(III) and using time gating imaging techniques the lanthanide emission can be easily separated from the background tissue fluorescence because of the much longer luminescent lifetime (milliseconds) of these ions. (68) Thus, p-nitrobenzyl-PCTA could also be used in the design and construction of *in vivo* bimodal (nuclear/optical or MRI/optical) imaging probes. (69,70)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
DTPA and DOTA and two bifunctional derivatives commonly used in nuclear medicine.

Figure 2.
Structure of NETA, AAZTA, PCTA and NO₂-Bn-PCTA.

Figure 3.

Absorbance changes in UV region during the formation of $\text{Ce}(\text{NO}_2\text{-Bn-PCTA})$ after mixing Ce^{3+} and the ligand at 10:1 metal to ligand concentration ratio at $\text{pH}=4.20$ (in 0.04 M DMP , $I=1.0\text{ M KCl}$ and $C_{\text{Lig}}=6\times 10^{-5}\text{ M}$), the curves represent the UV-spectrum of 1) 40 mM buffer, 2) $6\times 10^{-4}\text{ M CeCl}_3$ in DMP buffer, 3) $6\times 10^{-5}\text{ M}$ ligand in DMP buffer, 4) immediately after mixing the Ce^{3+} with the ligand ("0" min), 5) after 2 min, 6) after 7 min, 7) after 12 min and 8) after 70 min (equilibrium).

Figure 4. Formation rates of Ce(NO₂-Bn-PCTA) (blue diamonds), Eu(NO₂-Bn-PCTA) (green triangles), Yb(NO₂-Bn-PCTA) (purple circles) and Yb(PCTA) (red circles) vs. OH⁻ ion concentration (I=1.0 M KCl, t=25 °C).

Figure 5. Dependence of the dissociation rate constants (k_d) on the H^+ ion concentration in the dissociation of $\text{Ce}(\text{NO}_2\text{-Bn-PCTA})$ (blue diamonds), $\text{Eu}(\text{NO}_2\text{-Bn-PCTA})$ (green triangles), $\text{Yb}(\text{NO}_2\text{-Bn-PCTA})$ (purple circles) complexes at 25°C .

Scheme 1.
Synthesis of the bifunctional ligand, $\text{NO}_2\text{-Bn-PCTA}$.

Table 1
Protonation Constants of DO3A, PCTA, **NO₂-Bn-PCTA**, DOTA and **NO₂-Bn-DOTA** ligands (t = 25 °C).

Ligand	DO3A	PCTA	NO ₂ -Bn-PCTA	DOTA	NO ₂ -Bn-DOTA
I	0.1 M Me ₄ NCI ^{a,b}	1.0 M KCl ^c , 0.1 M Me ₄ NNO ₃ ^d	1.0 M KCl ^e	0.1 M KNO ₃ , 0.1 M Me ₄ NCI ^{f,g}	1.0 M Me ₄ NCI ^h
log K ₁ ^H	11.59 ^a , 12.46 ^b	11.36 ^c , 10.90 ^d	11.29 (0.01)	12.09 ^f , 12.60 ^g	10.93
log K ₂ ^H	9.24 ^a , 9.49 ^b	7.35 ^c , 7.11 ^d	6.70 (0.02)	9.76 ^f , 9.70 ^g	9.14
log K ₃ ^H	4.43 ^a , 4.26 ^b	3.83 ^c , 3.88 ^d	3.96 (0.03)	4.56 ^f , 4.50 ^g	4.44
log K ₄ ^H	3.48 ^a , 3.51 ^b	2.12 ^c , 2.27 ^d	2.08 (0.04)	4.09 ^f , 4.14 ^g	4.14
log K ₅ ^H	– ^a , 1.97 ^b	1.29 ^c , – ^d	1.82 (0.03)	– ^f , 2.32 ^g	2.33
Σlog K _i H	28.74 ^a , 29.36 ^b	25.96 ^c , 24.16 ^d	25.84	30.45 ^f , 33.26 ^g	32.43

^a reference (19),
^b reference (20),
^c reference (10),
^d reference(21,22),
^e ΔV= 9.74·10^{–3} for 506 data pairs,
^f reference (23),
^g reference (24),
^h the log K₆^H for this ligand is 1.40, reference (13).

Table 2

Stability constants of the complexes formed with Mg^{2+} , Ca^{2+} , Cu^{2+} , and Zn^{2+} ions ($I=1.0$ M KCl, $t=25$ °C).

M^{2+}	Equilibrium	PCTA	$NO_2-Bn-PCTA$	$DO3A^c$	$DOTA^g$
Mg^{2+}	$[ML]/[M][L]$	$11.82^a, 12.35^b$	$12.39 (0.01)$	11.3^d	11.92
	$[MHL]/[M][H]$	$3.70^a, 3.82^b$	$3.52 (0.07)$	—	4.09
Ca^{2+}	$[ML]/[M][L]$	$12.38^a, 12.72^b$	$12.72 (0.01)$	$13.39, 11.74^e$	$17.23, 17.22^e$
	$[MHL]/[M][H]$	$3.66^a, 3.79^b$	$3.66 (0.03)$	—	3.54
Cu^{2+}	$[ML]/[M][L]$	$17.79^a, 18.79^b$	$19.11 (0.08)$	$21.65, 22.87^e$	$22.25, 22.44^e$
	$[MHL]/[M][H]$	$4.03^a, 3.58^b$	$3.67 (0.05)$	$3.86, 3.2^e, f$	$3.78, 4.31^e$
	$[ML]/[ML(OH)][H]$	$10.3^a, 11.28^b$	—	—	—
Zn^{2+}	$[ML]/[M][L]$	$18.22^a, 20.48^b$	$21.36 (0.11)$	$21.87, 19.26^e$	$21.10^h, 20.52^e$
	$[MHL]/[M][H]$	$3.64^a, 3.10^b$	$3.02 (0.03)$	$3.27^f, 3.77^e$	$4.18^h, 4.35^e$
	$[ML]/[ML(OH)][H]$	$9.4^a, 12.31^b$	—	11.44^e	—

^a reference (26),

^b reference (10),

^c reference (20),

^d reference (27),

^e reference (28),

^f the second protonation constant of $Cu(DO3A)$ is $\log K = 2.8$, for $Zn(DO3A)$ $\log K = 2.81$, for $Zn(DOTA)$ $\log K = 3.49$ and for $Cu(DOTA)$ $\log K = 3.52$ ($MHL + H = MH_2L$),

^g reference (29),

^h reference (23).

Table 3
Stability constants of ML type complexes formed with Y^{3+} , In^{3+} and some Ln^{3+} ions ($I=1.0$ M KCl, $t=25$ °C).

M^{3+}	DO3A ^a	PCTA ^d	NO ₂ -Bn-PCTA	DOTA ^h	p-NO ₂ -Bn-DOTA ^k
Ce ³⁺	19.7	18.15	17.89 (0.12)	23.39	—
Nd ³⁺	—	20.15	18.74 (0.04)	22.99	—
Eu ³⁺	20.6 ^b	20.26, 20.9–21.1 ^e	19.02 (0.09)	23.45	—
Gd ³⁺	21.0	20.39, 21.0 ^f	19.42 (0.07)	24.0 ⁱ , 24.67, 25.3 ^j	24.2
Ho ³⁺	—	20.24	19.66 (0.06)	24.54	—
Yb ³⁺	—	20.63	—	25.00	—
Lu ³⁺	23.0	—	19.85 (0.09)	25.41	24.9
Y ³⁺	21.1 ^c	20.28	18.78 (0.09)	24.9 ^j	24.5
In ³⁺	—	21.42 ^g 24.7 (0.1)	23.91 (0.09)	23.9 ^j	—

^a reference (31,32),

^b determined by luminescence reference (33,34),

^c (19),

^d reference (10),

^e reference (21,22),

^f reference (25),

^g $I = 0.1$ mol/dm³ Me₄NNO₃ from reference (26),

^h reference (35),

ⁱ $I = 0.1$ M KCl from reference (36),

^j reference (19),

^k reference (13).

Table 4

Rate constants, k_{OH} , characterizing rearrangement of the intermediates in Ce(NO₂-Bn-PCTA), Eu(NO₂-Bn-PCTA), and Yb(NO₂-Bn-PCTA) (I=1.0 M KCl, 25 °C).

	Ce ³⁺	Eu ³⁺ k_{OH} (M ⁻¹ s ⁻¹)	Y ³⁺	Yb ³⁺
DO3A ^a	–	2.1×10 ^{7b}	–	–
PCTA ^c	9.7×10 ⁷	1.74×10 ⁸	1.13×10 ⁸	1.11×10 ⁹
NO₂-Bn-PCTA	(1.01±0.01)×10⁸	(1.45±0.03)×10⁸	–	(1.28±0.03)×10^{9d}
DOTA ^e	3.5×10 ⁶ , 1.16×10 ^{6f} , 2.7×10 ^{6g}	1.1×10 ⁷ , 7.2×10 ^{6h}	5.9×10 ^{6b,i} , 6.4×10 ^{5h}	(5.59±0.10)×10⁸ 4.1×10 ⁷ , 9.3×10 ^{7g}

^a reference (58),

^b data corresponds to Gd(III) complex,

^c reference (10),

^d re-measured with stopped-flow method in the current study,

^e reference (43),

^f reference (59),

^g reference (24),

^h reference (44),

ⁱ from reference (45),

^j reference (60).

Table 5

Comparison of acid-assisted dissociation rate constants (k_1 , M⁻¹s⁻¹) for Ce³⁺, Eu³⁺ and Yb(NO₂-Bn-PCTA) complexes (t=25 °C and I =1.0 M KCl). Data for some other complexes formed with macrocyclic ligands are also listed for comparison.

	Ce ³⁺	Gd ³⁺	Yb ³⁺
DO3A ^a	1.12×10 ⁻¹	2.7×10 ⁻² , 1.17×10 ^{-2b}	2.8×10 ⁻²
PCTA ^d	9.6×10 ^{-4f}	5.2×10 ^{-2c} 5.1×10 ^{-4e} 1.1×10 ^{-3c,f}	3.9×10 ⁻⁴ 2.8×10 ^{-4f}
NO ₂ -Bn-PCTA	(4.77±0.22)×10 ⁻⁵	(1.75±0.06)×10 ^{-4g}	(2.78±0.03)×10 ⁻⁴
DOTA ^h	8×10 ⁻⁴ⁱ	2.0×10 ^{-5j} , 8.4×10 ^{-6k} , 1.4×10 ^{-5l}	–

^a calculated from “saturation kinetics” from reference (32),

^b reference (66),

^c data corresponds to YL complex,

^d reference (10),

^e data corresponds to EuL complex,

^f calculated from “saturation kinetics” obtained in a wide concentration range,

^g second-order dependence on H⁺ ion concentration with third-order rate constant (5.7±0.4)×10⁻⁵ M⁻²s⁻¹ was also observed,

^h reference (47),

ⁱ second-order dependence on H⁺ ion concentration with third-order rate constant 2.0 ×10⁻³ M⁻²s⁻¹ was also observed (47),

^j t= 37 °C reference (43),

^k reference (45),

^l data corresponds to EuL (43).