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Synthesis, Characterization, and Structures of Indium In(DTPA-BA_2) and Yttrium Y(DTPA-BA_2)(CH_3OH) Complexes (BA = Benzylamine): Models for ^{111}In- and ^{90}Y-Labeled DTPA-Biomolecule Conjugates

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To explore structural differences in In^{3+}, Y^{3+}, and Lu^{3+} chelates, we prepared M(DTPA-BA_2) complexes (M = In, Y, and Lu; DTPA-BA_2 = N,N″-bis(benzylcarbamoylmethyl)diethylenetriamine-N,N′,N″-triacetic acid) by reacting the trisodium salt of DTPA-BA_2 with 1 equiv of metal chloride or nitrate. All three complexes have been characterized by elemental analysis, HPLC, IR, ES-MS, and NMR (^{1}H and ^{13}C) methods. ES-MS spectral and elemental analysis data are consistent with the proposed formula for M(DTPA-BA_2) (M = In, Y, and Lu) and have been confirmed by the X-ray crystal structures of both In(DTPA-BA_2)·$2H_2O$ and Y(DTPA-BA_2)(CH_3OH) complexes. By a reversed-phase HPLC method, it was found that In(DTPA-BA_2) is more hydrophilic than M(DTPA-BA_2) (M = Y and Lu), most likely due to the dissociation of the two carbonyl oxygen donors in solution. The X-ray crystal structure of In(DTPA-BA_2) revealed a rare example of an eight-coordinated In^{3+} complex with DTPA-BA_2 bonding to the In^{3+} in a distorted square antiprism coordination geometry. Both benzylamine groups are in the trans position relative to the acetate-chelating arm that is attached to the central N atom. The Y^{3+} in Y(DTPA-BA_2)(CH_3OH) is nine-coordinated with an octadentate DTPA-BA_2 and a methanol oxygen. The coordination geometry is best described as a tricapped trigonal prism. One benzylamine group is trans and the other cis to the acetate-chelating arm that is attached to the central N atom. All three M(DTPA-BA_2) complexes (M = In, Y, and Lu) exist as at least three isomers in solution (~10 mM), as shown by the presence of 6–8 overlapped ^{1}H NMR signals from the methylene hydrogens of the benzylamine groups. The coordinated DTPA-BA_2 remains rigid even at temperatures >85 °C. The exchange rate between different isomers in M(DTPA-BA_2) (M = In, Y, and Lu) is relatively slow at high concentrations (>1.0 mM), but it is fast due to the partial dissociation and rapid interconversion of different isomers at lower concentrations (~10 μM). It is not surprising that M(DTPA-BA_2) complexes (M = In, Y, and Lu) appear as a single peak in their respective HPLC chromatogram.

Introduction

There is great current interest in the ^{90}Y-labeled biomolecules, including monoclonal antibodies, antibody fragments, peptides, and peptidomimetics, as target-specific therapeutic radiopharmaceuticals for the treatment of cancers. Several papers have recently appeared covering a broad range of topics related to radiolabeled small biomolecules (BM) as therapeutic radiopharmaceuticals.[1−10] While the ^{90}Y-labeled bioconjugate is used for tumor radiotherapy, the corresponding ^{111}In-labeled bioconjugate is often used as a surrogate for imaging and dosimetry determination.[11−20] The advantage of using ^{111}In as an imaging surrogate for ^{90}Y is that $^{111}InCl_3$ is commercially available and has a half-life of $t_{1/2}$ = 2.8 days, which is almost identical to that of ^{90}Y ($t_{1/2}$ = 2.7 days). Although many ^{90}Y-labeled small peptides have been studied

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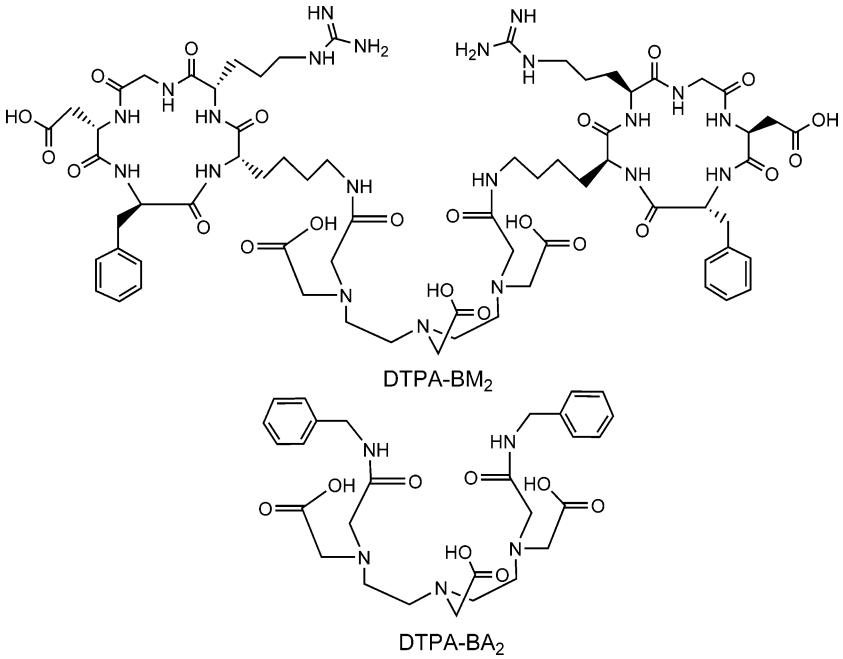

Figure 1. Structures of the DTPA-peptide conjugate DTPA-BM_2 (top) and its model compound DTPA-BA_2 (bottom).

for their therapeutic efficacy in tumor therapy,[11−21] very few studies have been directed toward understanding the differences between ^{90}Y- and ^{111}In-labeled BFC-BM (BFC = bifunctional chelator) conjugates with respect to their lipophilicity, structures, and biodistribution characteristics.

Recently, we initiated a series of studies on the radiochemistry of ^{90}Y- and ^{111}In-labeled DTPA-BM and DOTA-BM conjugates[22−26] and the coordination chemistry of In^{3+} and Y^{3+} with DTPA and DOTA derivatives.[27,28] These studies are aimed at exploring the differences between In^{3+} and Y^{3+} chelates[22−28] and how these differences influence the physical and biological properties of ^{90}Y- and ^{111}In-labeled DTPA- and DOTA-BM bioconjugates.[29] In our previous communications,[22,23] we reported the synthesis and characterization of the ^{111}In- and ^{90}Y-labeled DTPA-peptide conjugate (Figure 1, DTPA-BM_2). It was found that ^{111}In(DTPA-BM_2) is more hydrophilic than the corresponding ^{90}Y analogue, suggesting a different coordination sphere in the ^{111}In and ^{90}Y complexes of the same DTPA-BM_2 conjugate. The reversed-phase HPLC method for both ^{111}In(DTPA-BM_2) and ^{90}Y(DTPA-BM_2) complexes showed one radiometric peak in their respective HPLC chromatogram due to a rapid interconversion of different isomers.

Although the Y^{3+} and Lu^{3+} complexes of DTPA-BA_2 (N,N″-bis(benzylcarbamoylmethyl)diethylenetriamine-N,N′,N″-triacetic acid) have been reported,[30,31] there is very little information available for its In^{3+} complex. To further explore the structural differences in the In^{3+}, Y^{3+}, and Lu^{3+} complexes, we prepared the In(DTPA-BA_2) complex. For the purpose of comparison, we also prepared M(DTPA-BA_2) complexes (M = Y and Lu). As a continuation of our interest in the coordination chemistry of In^{3+}, Y^{3+}, and lanthanide metals with DTPA and DOTA derivatives,[22−28] we now present the synthesis and characterization of M(DTPA-BA_2) complexes (M = In, Y, and Lu). The objective of this study

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is to compare their solid-state structures and solution characteristics.

Experimental Section

Materials and Methods. Chemicals were purchased from Sigma Aldrich (St. Louis, MO) and were used as received. DTPA-BA_2 was prepared as its trisodium salt according to the literature method.[31] The NMR (^{1}H, ^{13}C, and ^{1}H−^{1}H COSY) data were obtained using a Bruker DRX 300 MHz FT NMR spectrometer, and chemical shifts (δ) are reported in parts per million relative to TMS. Infrared (IR) spectra were recorded on a Perkin-Elmer FT-IR spectrometer. Mass spectral data were collected using both positive and negative modes on a Finnigan LCQ classic mass spectrometer (School of Pharmacy, Purdue University). Elemental analysis was performed by Dr. H. Daniel Lee using a Perkin-Elmer Series III analyzer (Department of Chemistry, Purdue University). The HPLC method used a LabAlliance semi-prep HPLC system with a LabAlliance UV−vis detector (model 500, λ = 254 nm) and a Zorbax CN column (4.6 × 250 mm, 300 Å pore size). The flow rate was 1 mL/min, with the mobile phase being isocratic for the first 5 min using 90% solvent A (25 mM ammonium acetate buffer, pH = 6.8) and 10% solvent B (acetonitrile), followed by a gradient starting from 90% solvent A and 10% solvent B at 5 min to 60% solvent A and 40% solvent B at 20 min.

Synthesis of In(DTPA-BA_2). To a 10 mL vial were added trisodium salt of DTPA-BA_2 (191 mg, 0.30 mmol) and anhydrous $InCl_3$ (66 mg, 0.30 mmol) in 5 mL of water and 3 mL of methanol. The pH was adjusted to 6.0−7.0, if necessary. The mixture was heated at 80−90 °C for 30 min. The reaction mixture was filtered. Slow evaporation of the solvents afforded the product as a white solid, which was then recrystallized from water to give crystals suitable for X-ray crystallography. The solid was separated and dried under vacuum overnight before being submitted for elemental analysis. The yield was 130 mg (∼62%). A sample was analyzed by HPLC (purity >99%), and the retention time was 15.0 min. IR (KBr, cm^{-1}): 1629.14 (s, $\upsilon_{C=O}$) and 3435.20 (bs, υ_{O-H}). MS (ESI, positive mode): m/z = 684.11 for $[C_{28}H_{35}InN_5O_8]^+$. MS (ESI, negative mode): m/z = 682.11 for $[C_{28}H_{33}InN_5O_8]^-$. ^{1}H NMR (D_2O, 25 °C): δ 1.45−2.80 (m, 18H, CH_2N, CH_2CO_2, and CH_2CONH), 3.75−4.50 (m, 4H, CH_2Ph), 7.00−7.40 (m, 10H, aromatic). Anal. Calcd for $C_{28}H_{34}InN_5O_8 \cdot H_2O$: C, 47.93; H, 5.14; N, 9.98. Found: C, 48.22; H, 5.22; N, 9.75.

Synthesis of Y(DTPA-BA_2). To a 10 mL vial were added trisodium salt of DTPA-BA_2 (191 mg, 0.30 mmol) and $Y(NO_3)_3 \cdot 4H_2O$ (104 mg, 0.30 mmol) in 3 mL of water and 5 mL of methanol. The pH was adjusted to 6.0−7.0. The mixture was heated at 80−90 °C for 10 min. Slow evaporation of the solvent afforded the expected product as a white solid. The solid was separated and dried under vacuum overnight before being submitted for elemental analysis. Recrystallization from the methanol produced crystals suitable for X-ray crystallography. The yield was 126 mg (∼65%). A sample was analyzed by HPLC (purity >99%), and the retention time was 16.1 min. IR (KBr, cm^{-1}): 1629.10 (s, $\upsilon_{C=O}$) and 3412.60 (bs, υ_{O-H}). MS (ESI, positive mode): m/z = 658.17 for $[C_{28}H_{35}YN_5O_8]^+$. MS (ESI, negative mode): m/z = 656.17 for $[C_{28}H_{33}YN_5O_8]^-$. ^{1}H NMR (D_2O, 25 °C): δ 2.00−3.80 (m, 18H, CH_2N, CH_2CO_2, and CH_2CONH), 3.80−4.50 (m, 4H, CH_2Ph), 7.00−7.30 (m, 10H, aromatic). Anal. Calcd for $C_{28}H_{34}YN_5O_8 \cdot (H_2O)(CH_3OH)(NaNO_3)_{0.5}$: C, 46.05; H, 5.57; N, 10.60. Found: C, 46.44; H, 5.38; N, 10.27.

Synthesis of Lu(DTPA-BA_2). Lu(DTPA-BA_2) was prepared using the same procedure as that for Y(DTPA-BA_2), except that $Lu(CH_3CO_2)_3 \cdot H_2O$ was used for the synthesis. The product was isolated as a white solid. The solid was dried under vacuum overnight. The yield was 170 mg (∼76%). The sample was analyzed by HPLC (purity >99%), and the retention time was 16.1 min. IR (KBr, cm^{-1}): 1620.56 (s, $\upsilon_{C=O}$) and 3435.04 (bs, υ_{O-H}). MS (ESI, positive mode): m/z = 743.97 for $[C_{28}H_{34}LuN_5O_8]^+$. MS (ESI, negative mode): m/z = 742.5 for $[C_{28}H_{34}LuN_5O_8]^-$. ^{1}H NMR (D_2O, 25 °C): δ 2.00−3.80 (m, 18H, CH_2N, CH_2CO_2, and CH_2CONH), 3.80−4.40 (m, 4H, CH_2Ph), 7.00−7.30 (m, 10H, aromatic). Anal. Calcd for $C_{28}H_{38}LuN_5O_8 \cdot 2H_2O$: C, 43.14; H, 4.91; N, 8.98. Found: C, 43.46; H, 5.05; N, 8.94.

Table 1. Selected Crystallographic Data for In(DTPA-BA_2)·$2H_2O$ and Y(DTPA-BA_2)(CH_3OH)·CH_3OH Complexes

formula	$C_{28}H_{38}InN_5O_{10}$	$C_{30}H_{42}N_5O_{10}Y$
fw	719.46	721.60
space group	*pbcn*	*P*121/*c*1
a (Å)	17.9189(3)	10.4880(2)
b (Å)	15.5385(3)	30.0779(8)
c (Å)	29.5594(6)	10.8938(3)
α (deg)	90	90
β (deg)	90	103.0320
β (deg)	90	90
V (Å^3)	8230.3(3)	3348.01(14)
Z	8	4
d_{calc} (g/cm^3)	1.151	1.161
T (K)	150	150
crystal dimensions (mm^3)	0.48 × 0.30 × 0.13	0.40 × 0.35 × 0.30
radiation (*λ*, Å)	Mo Kα (0.71073)	Mo Kα (0.71073)
transmission factors	0.704−0.927	0.704−0.927
R	0.049[a]	0.048[a]
R_w	0.142[b]	0.138[b]

[a] $R = \sum||F_o| - |F_c||/\sum|F_o|$, for $F_o^2 > 2\sigma(F_o^2)$. [b] $R_w = [\sum w(|F_o^2| - |F_c^2|)^2/\sum w|F_o^2|^2]^{1/2}$.

X-ray Crystallographic Analysis. Crystallographic data for In(DTPA-BA_2)·$2H_2O$ and Y(DTPA-BA_2)(CH_3OH)·CH_3OH were collected on a Nonius Kappa CCD diffractometer. Selected crystallographic data are listed in Table 1. Crystals were mounted on a glass fiber in a random orientation. Preliminary examination and data collection were performed using graphite monochromated Mo Kα radiation (λ = 0.71073 Å). Cell constants and an orientation matrix for data collection were obtained from a least-squares refinement, using the setting angles (θ) in the range of 2−27° for both In(DTPA-BA_2)·$2H_2O$ and Y(DTPA-BA_2)(CH_3OH)·CH_3OH. For the In(DTPA-BA_2)·$2H_2O$ complex, a total of 9799 reflections were collected, of which 6248 were unique. For the Y(DTPA-BA_2)(CH_3OH)·CH_3OH complex, a total of 7944 reflections were collected, and 6053 reflections were unique. Lorentz and polarization corrections were applied to the data. A linear absorption coefficient is 9.2 cm^{-1} for Mo Kα radiation. An empirical correction was applied using the SCALEPACK program.[32] The structure was solved using the structure solution program PATTY in DIRDIF99[33] and was refined on an AlphaServer 2100 using SHELXL97.[34] Crystallographic drawings were produced using the program ORTEP.

Results

Syntheses of M(DTPA-BA_2) Complexes (M = In, Y, and Lu). We prepared M(DTPA-BA_2) complexes (M = In,

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Y, and Lu) by reacting the trisodium salt of DTPA-BA_2 with 1 equiv of metal chloride or nitrate at pH > 6.0. M(DTPA-BA_2) complexes (M = In, Y, and Lu) were isolated from the reaction mixture and dried under vacuum overnight before being submitted for elemental analysis. Recrystallization from methanol or a 1:1 (v/v) mixture of water and methanol afforded crystals that were suitable for X-ray crystallography for the In(DTPA-BA_2)·$2H_2O$ and Y(DTPA-BA_2)(CH_3OH)·CH_3OH complexes.

Characterization of M(DTPA-BA_2) Complexes (M = In, Y, and Lu). M(DTPA-BA_2) complexes (M = In, Y, and Lu) have been characterized by elemental analysis, HPLC, IR, ES-MS, and NMR (^1H and ^{13}C) methods. The IR spectra of M(DTPA-BA_2) complexes (M = In, Y, and Lu) show strong and broad bands at ~3430 cm^{-1}, due to crystallization of the solvent (water or methanol) molecules, and strong bands at ~1630 cm^{-1}, due to the coordinated carboxylate and carbonyl groups. Upon coordination, stretching frequencies corresponding to the carboxylate ($\upsilon_{C=O}$ ~ 1656 cm^{-1}) groups undergo a "red shift" (25−30 cm^{-1}). The ES-MS spectral and elemental analysis data are completely consistent with the proposed formula for M(DTPA-BA_2) complexes (M = In, Y, and Lu).

A reversed-phase HPLC method was used for determining the relative lipophilicity of M(DTPA-BA_2) complexes (M = In, Y, and Lu). The pH of the ammonium acetate buffer is ~6.8. Figure 2 shows the typical HPLC chromatograms of M(DTPA-BA_2) (M = In, Y, and Lu), all of which show a single peak in the region of interest. Retention times of M(DTPA-BA_2) (M = Y and Lu) are almost identical. However, the HPLC retention time of In(DTPA-BA_2) is ~1.0 min shorter than that of Y(DTPA-BA_2) or Lu(DTPA-BA_2). Changing the pH from 6.5 to 8.5 did slightly change the HPLC retention times with no significant change in their HPLC profiles. These results are consistent with our previous observations that both ^{111}In(DTPA-BM_2) and ^{90}Y(DTPA-BM_2) [BM = c(RGDfK)] showed only a single peak in their HPLC chromatograms, with ^{111}In(DTPA-BM_2) being more hydrophilic than ^{90}Y(DTPA-BM_2).[22,23]

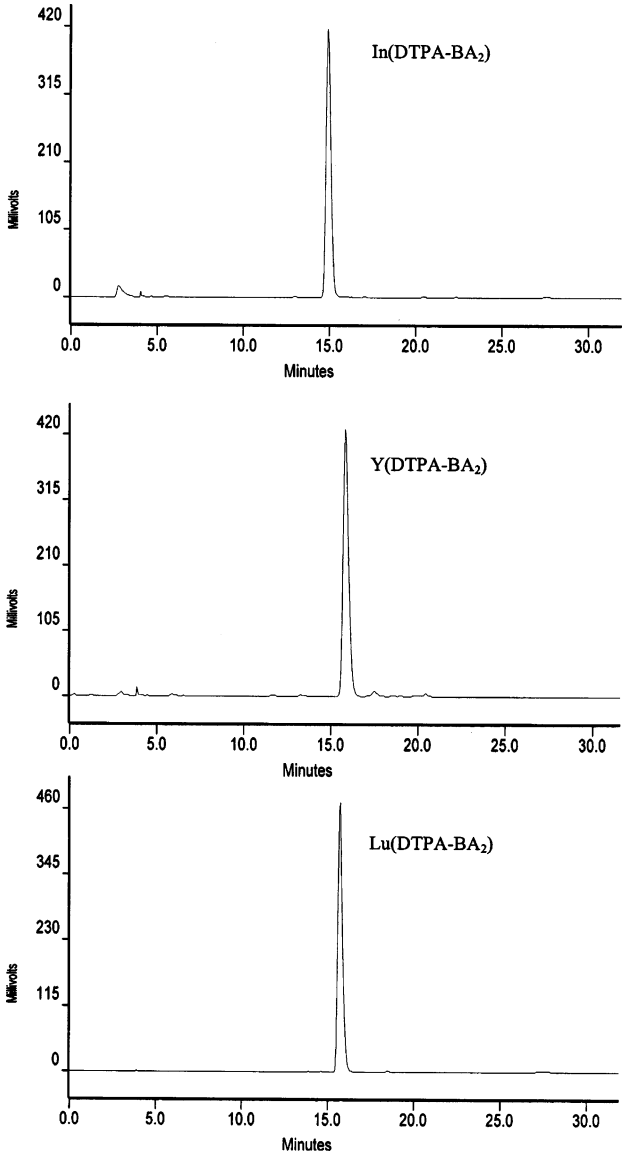

Figure 2. Typical HPLC chromatograms of In(DTPA-BA_2) (top), Y(DTPA-BA_2) (middle), and Lu(DTPA-BA_2) (bottom).

^1H and ^{13}C NMR Spectra of M(DTPA-BA_2) Complexes (M = In, Y, and Lu). (1) Room Temperature ^1H NMR. Figure 3 shows the room temperature ^1H NMR spectra of M(DTPA-BA_2) complexes (M = In, Y, and Lu) in D_2O (pH = 6.0−6.5). At room temperature, all three complexes show complicated and overlapped NMR patterns in the aliphatic region. However, we were able to identify three groups of NMR signals: 6−8 ^1H signals from the benzylic methylene hydrogens at 3.80−4.50 ppm, multiple ^1H signals from the methylene hydrogens of five acetate-chelating arms at 2.90−3.80 ppm, and several overlapped ^1H signals from the hydrogens of the two ethylene bridges at 1.50−2.90 ppm.

(2) Room Temperature ^{13}C NMR. Table 3 summarizes the room temperature ^{13}C NMR data for the M(DTPA-BA_2) complexes (M = In, Y, and Lu) in D_2O (pH = 6.0−6.5) using DMSO-d_6 as the internal standard. Figure 4 shows the room temperature ^{13}C NMR spectra from the carboxylate and carbonyl carbons for M(DTPA-BA_2) (M = In, Y, and Lu). ^{13}C NMR signals for the M(DTPA-BA_2) complexes (M = Y and Lu) are low due to their poor water solubility, but overlapped multiple peaks are clearly seen at room temperature. In general, ^{13}C NMR signals from carboxylate carbons are well-separated from those due to carbonyl carbons for all three complexes. There are three ^{13}C signals at 172−174 ppm from two carbonyl carbons and two overlapped ^{13}C signals at 177−178 ppm from the three coordinated carboxylate carbons in In(DTPA-BA_2). For M(DTPA-BA_2) (M = Y and Lu), there are four ^{13}C signals at 176−179 ppm from two carbonyl carbons and at least 6−7 overlapped ^{13}C signals at 181−183 ppm from three coordinated carboxylate carbons.

(3) Variable Temperature ^1H NMR. Figures SI−SIII show the variable temperature ^1H NMR (500 MHz) spectra of M(DTPA-BA_2) complexes (M = In, Y, and Lu) in D_2O (pH = 6.0−6.5). As the temperature increases, all three complexes start to show a broadening of peaks in the aliphatic region at 65 °C. Theoretically, ^1H NMR signals due to the methylene hydrogens of two benzylamine groups

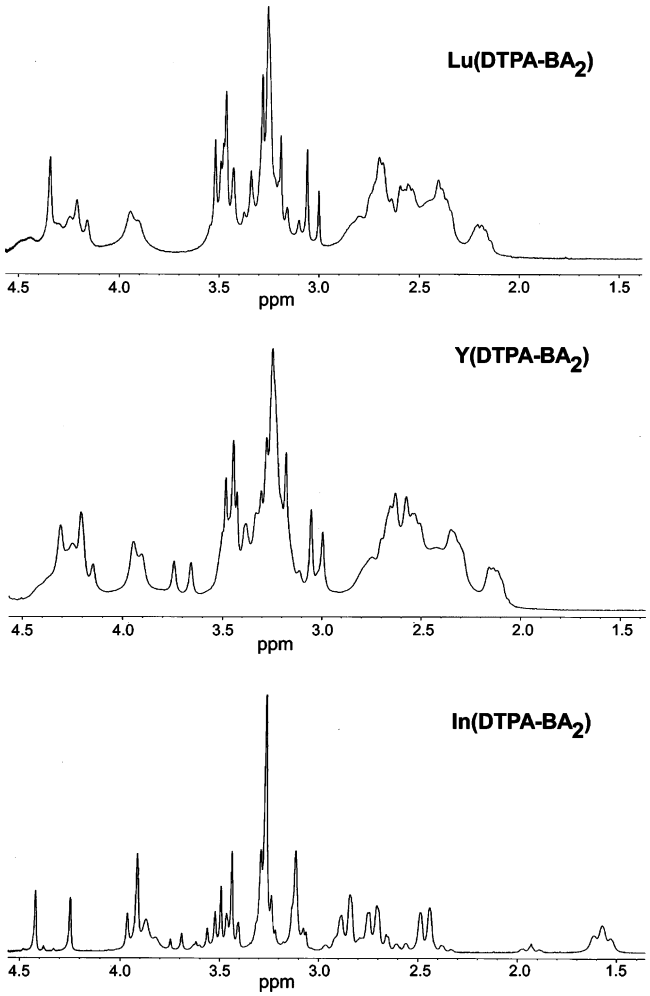

Figure 3. Aliphatic region of ^{1}H NMR (300 MHz) spectra of In(DTPA-BA_2) (bottom), Y(DTPA-BA_2) (middle), and Lu(DTPA-BA_2) (top).

Table 2. Selected Bond Distances for In(DTPA-BA_2)·$2H_2O$ and Y(DTPA-BA_2)(CH_3OH)·CH_3OH Complexes

atom 1	atom 2	distance (Å)	atom 1	atom 2	distance (Å)
In	O(171)	2.163(3)	Y	O(171)	2.3021(18)
In	O(111)	2.185(3)	Y	O(111)	2.3061(18)
In	O(19)	2.264(3)	Y	O(111)	2.3324(17)
In	O(141)	2.280(3)	Y	O(31)	2.401(2)
In	O(9)	2.355(3)	Y	O(9)	2.4040(18)
In	N(14)	2.366(3)	Y	O(19)	2.4236(17)
In	N(11)	2.407(3)	Y	N(14)	2.607(2)
In	N(17)	2.461(3)	Y	N(17)	2.655(2)
			Y	N(11)	2.762(2)

should appear as a singlet if these complexes become completely fluxional at higher temperatures, but we were unable to obtain the coalescent point for all three complexes even at 85 °C.

X-ray Crystal Structures of In(DTPA-BA_2) and Y(DTPA-BA_2)(CH_3OH). Figure 5 shows the ORTEP view of the In(DTPA-BA_2) structure. Crystallization water and hydrogen atoms are omitted for the purpose of clarity. Selected crystallographic data are listed in Table 1. There are eight In(DTPA-BA_2) molecules in each unit cell, along with two crystallization water molecules surrounding each In(DTPA-BA_2) molecule. DTPA-BA_2 acts as an octadentate ligand with all eight donor atoms (three amine nitrogens, three carboxylate oxygens, and two carbonyl oxygens) bonded to In^{3+}. Both benzylamine groups are in the trans

Table 3. Room Temperature ^{13}C NMR (300 MHz) Data for M(DTPA-BA_2) Complexes (M = In, Y, and Lu) in D_2O (pH = 6.0−6.5)

compound	aliphatic carbons	aromatic carbons	carboxylate/ carbonyl carbons
In(DTPA-BA_2)	44.385, 44.572	127.695, 128.291	171.358, 171.732, 171.899
		128.457, 129.319 137.066, 137.525 137.736	175.952, 176.199
Y(DTPA-BA_2)	44.852, 45.127	128.544, 129.171	176.410, 177.214, 177.560
	52.205, 53.860	129.330, 129.521	178.064, 181.098, 181.425
	57.310, 57.512	130.535, 130.622	181.500, 181.735, 182.039
	58.129, 59.530 62.403, 64.336 67.084	138.598, 138.650 138.924, 139.250	182.513
Lu(DTPA-BA_2)	44.867, 45.155	128.544, 129.171	176.799, 178.033, 178.628
	57.342, 53.860	129.330, 129.521	181.594, 181.908, 182.035
	57.410, 59.931	130.535, 130.622	182.098, 182.255, 182.438
	60.054, 62.555 67.506	138.598, 138.650 138.924, 139.250	182.575

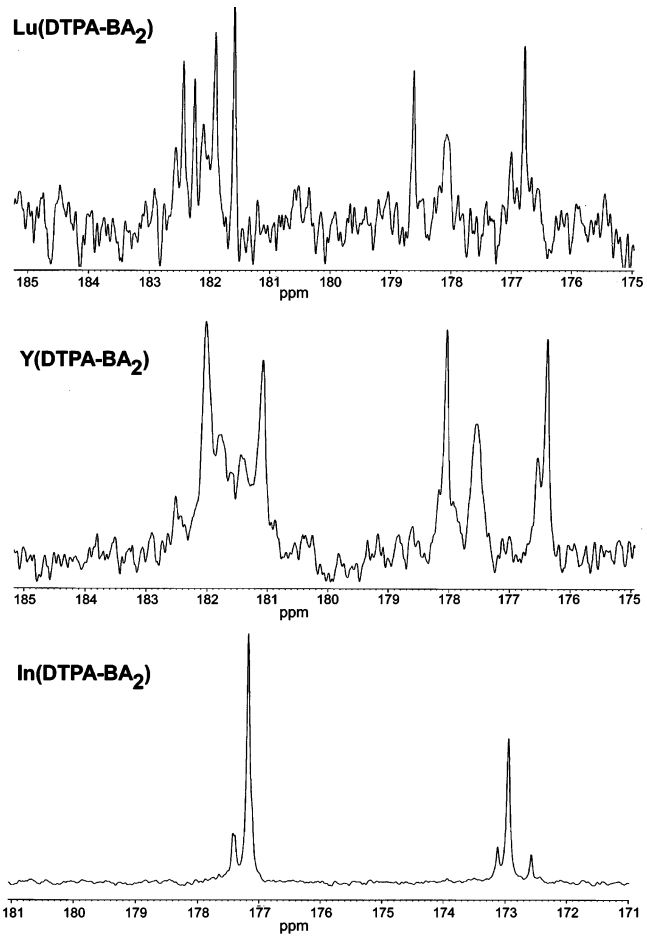

Figure 4. ^{13}C NMR spectra of carboxylate and carbonyl carbons for In-(DTPA-BA_2) (bottom), Y(DTPA-BA_2) (middle), and Lu(DTPA-BA_2) (top).

position relative to the carboxylate group that is attached to the central N. The coordination geometry is best described as the distorted square antiprism, with O(9), O(19), N(17), and O(171) occupying one square plane and N(11), N(14),

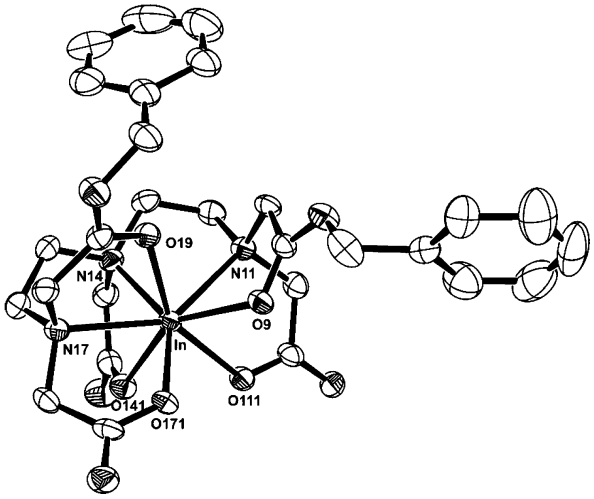

Figure 5. ORTEP drawing of In(DTPA-BA_2) (ellipsoids are at 50% probability). Crystallization water and hydrogen atoms are omitted for the purpose of clarity.

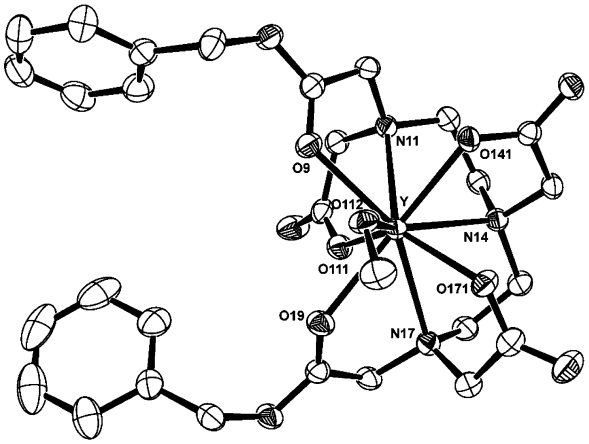

Figure 6. ORTEP drawing of Y(DTPA-BA_2)(CH_3OH) (ellipsoids are at 50% probability). Crystallization methanol and hydrogen atoms are omitted for the purpose of clarity.

O(141), and O(111) occupying the other square plane. The In atom is deeply buried in the coordination cavity of DTPA-BA_2, with a distance of ∼1.29 Å from In to the square plane O(9)−O(19)−N(17)−O(171) and ∼1.24 Å from In to the square plane N(11)−N(14)−O(141)−O(111).

Table 2 lists selected In−N and In−O bond distances in the coordination sphere. The average In−N bond length is 2.411(3) Å. The average bond distance between In^{3+} and three carboxylate oxygen atoms is 2.213(3) Å. The average In−O (carbonyl) bond distance is 2.310(3) Å and is only slightly longer than those of In−O (carboxylate) bonds (varying from 2.163(3) to 2.280(3) Å). Both In−N and In−O bond distances in In(DTPA-BA_2) are very close to those (In−N = 2.430(2) Å and In−O = 2.254(2) Å) found in In-(DOTA-AA),[31] which also has a distorted square antiprismatic coordination geometry, with DOTA-AA being octadentate. However, the In−N and In−O bond distances in In(DTPA-BA_2) are ∼0.07 Å longer than those (2.1578(7)−2.202(7) Å for In−O bonds and 2.314(8)−2.395(8) Å for In−N bonds) in In(DO3A).[35] These In−O and In−N bond differences in In(DTPA-BA_2) and In(DO3A) may be caused by the changes in the coordination number.

Figure 6 shows the ORTEP view of the Y(DTPA-BA_2)(CH_3OH) structure. Crystallization methanol and hydrogen atoms are omitted for the purpose of clarity. The selected crystallographic data are listed in Table 1. There are eight Y(DTPA-BA_2)(CH_3OH) molecules in each unit cell, along with a crystallization methanol for each Y(DTPA-BA_2)(CH_3OH) molecule. DTPA-BA_2 coordinates to Y^{3+} as an octadentate ligand using all of its eight donor atoms (three amine nitrogens, three carboxylate oxygens, and two carbonyl oxygens), while the methanol oxygen occupies the remaining coordination site. The structure of Y(DTPA-BA_2)(CH_3OH) is almost identical to those of Y(DTPA-BA_2)(H_2O)[30] and Lu-(DTPA-BA_2)(H_2O),[31] except that the coordinated water is replaced by the methanol molecule during the recrystallization process. One benzylamine group is in the trans position, while the other benzylamine group is in the cis position relative to the carboxylate group that is attached to the central N atom. The coordination geometry is best described as a tricapped trigonal prism. The two triangular faces of the prism are constituted by O(9), O(19), and O(111) and N(14), O(141), and O(171), while N(11), N(17), and the coordinated methanol O(112) are in the three capping positions with respect to the three rectangular faces.

Table 2 lists selected Y−N and Y−O bond distances in the coordination sphere. The average Y−N bond length is 2.675(2) Å. The average bond distance between Y^{3+} and the three carboxylate oxygen atoms is 2.3135(18) Å. The average Y−O (carbonyl) bond distance is 2.402(2) Å and is only slightly longer than those of the Y−O (carboxylate) bonds (varying from 2.3021(18) to 2.3324(17) Å) because the electronic delocalization within the CO−NH moiety also gives a significant "negative" charge on the carbonyl oxygen atom.[30] The Y−O (methanol) bond length is 2.4236(17) Å. Both of the Y−N and Y−O bond distances in Y(DTPA-BA_2)(CH_3OH) are very close to those found in Y(DTPA-BA_2)(H_2O)[30] and Lu(DTPA-BA_2)(H_2O).[31] The elongation and variation of Y−N bonds demonstrate the relative weakness and lability of the less-polar Y−N bond in the strongly ionic yttrium complex.[30]

Discussion

In^{3+}, Y^{3+}, and Lu^{3+} are trivalent cations. The main difference is their size. As a result, their complexes of DOTA and DTPA derivatives often show different coordination chemistry with respect to their coordination number and solution properties. For example, Y^{3+} has an ionic radius of 1.02 Å,[36] which fits perfectly into the cavity of DOTA derivatives. In^{3+} has an ionic radius of 0.92 Å, which is smaller than that of Y^{3+}. The coordination number for In^{3+} is typically six or seven.[37−41] As a matter of fact, the

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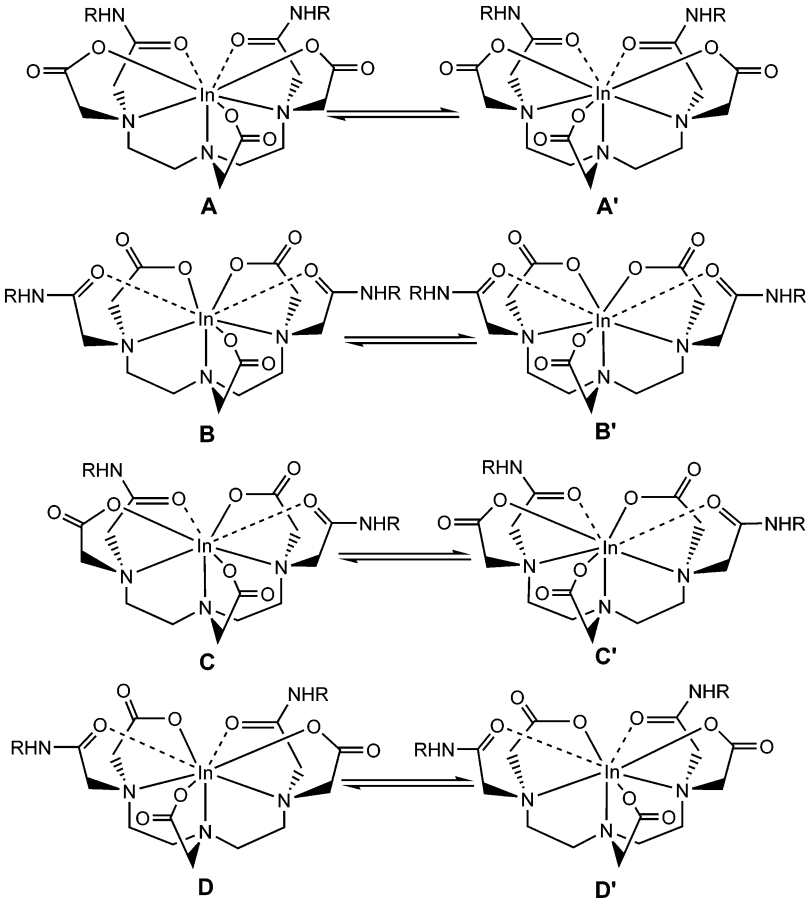

Figure 7. Possible isomers for In(DTPA-BA_2) in solution due to relative orientations of the two benzylamine groups relative to the carboxylate group attached to the central N atom.

In(DTPA-BA_2)·$2H_2O$complex described in this report represents a rare example of an eight-coordinated In^{3+} complex.[28,42−44]

In the solid state, DTPA-BA_2 bonds to In^{3+} as an octadentate chelator using two carbonyl oxygen, three amine nitrogen, and three acetate oxygen donor atoms, and only the trans−trans isomer was observed in the solid state (Figure 6). In aqueous solution, however, the two weakly bonded carbonyl oxygen atoms (Figure 7) may become dissociated due to the small size of In^{3+}. There are eight possible isomers in solution due to the relative orientations of the two benzylamine groups relative to the carboxylate group that is attached to the central nitrogen and due to the different spatial arrangement of eight N_3O_5 donor atoms. If the In(DTPA-BA_2) structure was rigid, one would expect at least four peaks in the HPLC chromatogram considering the fact that enantiomers cannot be separated by the HPLC method used in this study. However, the HPLC chromatogram of In(DTPA-BA_2) shows only a single peak in the region of interest, which is consistent with the presence of a single peak for ^{111}In-(DTPA-BM_2) [BM = c(RGDfK)][23] and suggests that In-(DTPA-BA_2) undergoes a rapid dissociation of carbonyl oxygen donors and interconversion between different isomers at very low concentrations (~10 μM). This assumption is consistent with the hydrophilicity of In(DTPA-BA_2) being higher than that of M(DTPA-BA_2) (M = Y and Lu) since the dissociated carbonyl oxygen atoms are able to form more hydrogen bonds with the surrounding water molecules.

In general, the yttrium and lanthanide metal complexes of the DTPA analogues assume distorted tricapped trigonal prism (TTP) geometries.[45] In the TTP arrangement, the neutral donor atoms with longer bond lengths will prefer to occupy face capping positions rather than a prismatic corner. However, since it is not possible for all three amine nitrogen donors to occupy capping positions, the central nitrogen atom tends to occupy a prismatic corner, while the two terminal amine nitrogen atoms occupy capping positions. Because of distortion, the actual coordination geometry is between TTP and monocapped square antiprism (CSAP).[45] The Y(DTPA-BA_2)(CH_3OH) structure is almost identical to those of Y(DTPA-BA_2)(H_2O)[30] and Lu(DTPA-BA_2)(H_2O),[31] except that the coordinated water is replaced by methanol. Due to their large size, both Y^{3+} and Lu^{3+} are, most likely, nine-coordinated in aqueous solution (Figure 8). Therefore, it is not unexpected that Y(DTPA-BA_2) and Lu(DTPA-BA_2) have

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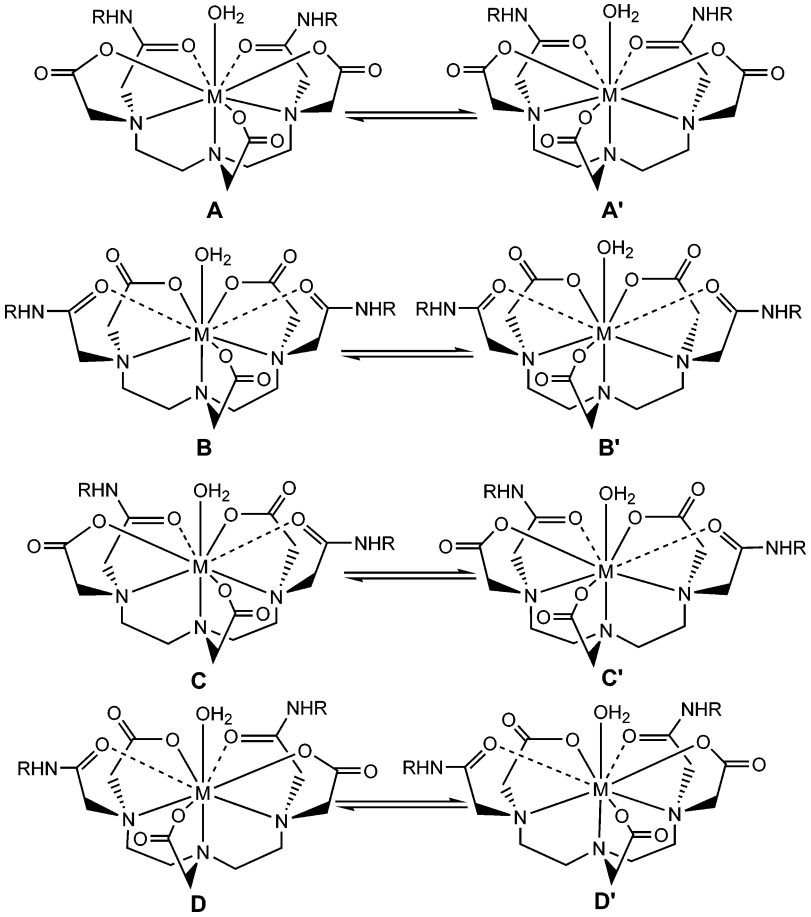

Figure 8. Possible isomers for M(DTPA-BA_2) complexes (M = Y and Lu) in solution.

almost identical HPLC retention times. The presence of a single peak in their HPLC chromatograms also suggests that M(DTPA-BA_2) complexes (M = Y and Lu) undergo a rapid dissociation of carbonyl oxygen donors and interconversion between different isomers at very low concentrations (~10 μM).

In recent years, much attention has been given to paramagnetic lanthanide metal chelates for their potential application as contrast agents for magnetic resonance imaging (MRI).[46−53] Parker and co-workers[30] reported the X-ray crystal structure of Y(DTPA-BA_2)(H_2O), but details of its NMR spectral data were not presented. Aime and co-workers[31] reported both the solid-state structure and ^{13}C NMR spectrum of Lu(DTPA-BA_2)(H_2O). It was found that there are at least three different isomers for Lu(DTPA-BA_2) in solution. In this study, we used both HPLC and NMR methods to characterize the solution properties of M(DTPA-BA_2) complexes (M = In, Y, and Lu). If there were only one isomer, as found in the solid state, one would expect only a singlet in D_2O from the methylene hydrogens of the benzylamine groups in In(DTPA-BA_2), due to their trans−trans orientation, and two singlets (in D_2O) from those in M(DTPA-BA_2) (M = Y and Lu), due to the trans−cis orientation of the benzylamine groups relative to the acetate-chelating arm that is attached to the central N atom. It is quite clear that all three complexes exist in aqueous solution in at least 3 different isomeric forms, as shown by the presence of 6−8 overlapped ^1H NMR signals (at 3.8−4.5 ppm) due to the methylene hydrogens of the two benzylamine groups. These isomers remain in solution even at 85 °C (Figures SI−SIII), suggesting that the rate of exchange among these isomers is still relatively slow on the NMR time scale.

Although the M(DTPA-BA_2) complexes (M = In, Y, and Lu) show 6−8 overlapped ^1H signals from the methylene hydrogens of the two benzylamine groups in the region of 3.8−4.5 ppm, the chemical shifts and ^{13}C NMR patterns of the carboxylate and carbonyl carbon atoms in In(DTPA-BA_2) are significantly different from those in M(DTPA-BA_2) (M = Y and Lu). For example, there are three ^{13}C signals at 172−174 ppm from the two carbonyl carbons and two overlapped ^{13}C signals at 177−178 ppm from the three coordinated carboxylate carbons in In(DTPA-BA_2), while

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M(DTPA-BA_2) complexes (M = Y and Lu) show four ^{13}C signals at 176−179 ppm from two carbonyl carbons and 6−7 overlapped ^{13}C signals at 181−183 ppm from three coordinated carboxylate carbons. These data suggest that carbonyl oxygen donors in In(DTPA-BA_2) might become dissociated in solution, which makes ^{13}C NMR signals from carbonyl carbons more difficult to separate. This may also explain that the differences in the ^1H NMR patterns between In(DTPA-BA_2) and M(DTPA-BA_2) complexes (M = Y and Lu) are due to the methylene hydrogens of the two benzylamine groups.

There are two types of interconversion between different isomers of M(DTPA-BA_2) complexes (M = Y and Lu). The first involves the "wagging" of the diethylenetriamine backbone and the "shuffling" of the two NO_2 donor sets. This type of movement often occurs at low temperatures. The end result is the equilibrium between **A** and **A′**, **B** and **B′**, **C** and **C′**, or **D** and **D′** (Figure 8). The second type of interconversion involves inversion at the terminal amine nitrogen atoms. This type of inversion often requires simultaneous dissociation of all three terminal NO_2 donors and occurs only at high temperatures. The end result of this movement is the rapid exchange between **A**, **B**, **C**, and **D**. In theory, ^1H NMR signals due to the methylene hydrogens of the two benzylamine groups should appear as a singlet if the coordinated DTPA-BA_2 becomes completely fluxional. The absence of a coalescence point in the VT ^1H NMR spectra (Figures SII and SIII) of M(DTPA-BA_2) (M = Y and Lu) suggests that the coordinated DTPA-BA_2 remains firmly bonded to the metal center even at 85 °C. These results are completely consistent with those observed for Lu(DTPA-BA_2) in D_2O.[31]

It is important to note that the pH value in solutions containing the lanthanide complexes of DTPA derivatives has a significant impact on the rate of interconversion between different isomers.[54,55] For HPLC analyses of M(DTPA-BA_2) (M = In, Y, and Lu), the 25 mM ammonium acetate buffer was used as the mobile phase, and the pH was ~6.8, which was very close to the pH value in their corresponding NMR samples (pH = 6.0−6.5). Since the two isomers of In(DTPA-BM) and Y(DTPA-BM) were well-separated,[22,23] the isomers in the M(DTPA-BA_2) complexes (M = In, Y, and Lu) should also be separable using the same ammonium acetate buffer (pH = 6.8) as was used in the mobile phase. The observation of a single peak in the HPLC chromatograms of M(DTPA-BA_2) (M = In, Y, and Lu) strongly suggests a rapid interconversion between different isomers, and their different HPLC and NMR properties are not caused by their slight pH variation in the HPLC mobile phase and NMR samples.

Another factor influencing the solution behavior is the concentration of a metal complex. For HPLC analysis, a small sample (<5 μg) is injected into the HPLC system. The concentration of each compound is only ~10 μM, at which the dissociation of carbonyl oxygen atoms may become favored due to the fact that the thermodynamic stability of M(DTPA-BA_2) (M = In, Y, and Lu) is relatively lower than that of M(DTPA-MA) (M = In, Y, and Lu; MA = monoamide).[52] This assumption is supported by our previous observations that ^{111}In(DTPA-BM_2) and ^{90}Y(DTPA-BM_2) showed a single radiometric peak, while ^{111}In(DTPA-BM) and ^{90}Y(DTPA-BM) exhibited two radiometric peaks in their HPLC chromatograms under almost identical chromatographic conditions.[22,23] At the tracer (^{111}In and ^{90}Y) level, the concentration of each radiometal complex is extremely low (<1.0 nM). For NMR analysis, however, the concentration for all three complexes exceeds 5 mg/mL (~10 mM), at which it is much easier for the metal complex to remain intact. Therefore, it is reasonable to believe that the solution properties of M(DTPA-BA_2) (M = In, Y, and Lu) are, most likely, concentration-dependent.

Conclusions

In this report, we present the synthesis and structural characterization of M(DTPA-BA_2) complexes (M = In, Y, and Lu). All three complexes have been characterized by elemental analysis, HPLC, IR, ES-MS, and NMR methods. ES-MS spectral and elemental analysis data are completely consistent with the proposed formula for M(DTPA-BA_2) complexes (M = In, Y, and Lu) and have been confirmed by the X-ray crystal structures of both In(DTPA-BA_2)·$2H_2O$ and Y(DTPA-BA_2)(CH_3OH). Using a reversed-phase HPLC method, we found that In(DTPA-BA_2) is more hydrophilic than M(DTPA-BA_2) (M = Y and Lu); most likely, this is due to the dissociation of two carbonyl oxygen donors in solution. The X-ray crystal structure of In(DTPA-BA_2)·$2H_2O$ revealed an octadentate DTPA-BA_2 with all eight donor atoms bonding to In^{3+} in a distorted square antiprismatic coordination sphere. Both benzylamine groups are in the trans position relative to the acetate-chelating arm that is attached to N(14). In the solid state, the Y^{3+} in Y(DTPA-BA_2)(CH_3OH) is nine-coordinated in a tricapped trigonal prismatic coordination geometry with an octadentate DTPA-BA_2 and a methanol oxygen. One benzylamine group is trans and the other cis to the acetate-chelating arm that is attached to the central N atom. The NMR spectral data show that all three M(DTPA-BA_2) complexes (M = In, Y, and Lu) exist in solution (~10 mM) as at least 3 isomeric forms. At much lower concentrations (~10 μM), the exchange rate between the different isomers of M(DTPA-BA_2) (M = In, Y, and Lu) becomes relatively fast so that only an average signal is detected. That may explain why M(DTPA-BA_2) complexes (M = In, Y, and Lu) appear as a single peak in their respective HPLC chromatogram.

Supporting Information Available: X-ray crystallographic files are in CIF format for the reported structures. Variable temperature ^1H NMR spectra of M(DTPA-BA_2) (M = In, Y, and Lu) in D_2O (pH = 6.0−6.5) are in Microsoft word format (Figures SI−SIII). This material is available free of charge via the Internet at http://pubs.acs.org.

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