Inorg. Chem. **2003**, *42*, 8831–8837

Synthesis, Characterization, and X-ray Crystal Structure of In(DOTA-AA) (AA = *p*-Aminoanilide): A Model for ^{111}In-Labeled DOTA-Biomolecule Conjugates

Shuang Liu,* Zhengjie He, Wen-Yuan Hsieh, and Phillip E. Fanwick

Department of Industrial and Physical Pharmacy, School of Pharmacy, Purdue University, 575 Stadium Mall Drive, West Lafayette, Indiana 47907

Received August 21, 2003

This report describes the synthesis and structural characterization of the indium complex of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(*p*-aminoanilide) (DOTA-AA), a model compound for ^{111}In-labeled DOTA-biomolecule conjugates. In(DOTA-AA) was prepared by reacting DOTA-AA with 1 equiv of $InCl_3$ in 0.5 M ammonium acetate buffer (pH ∼ 6). It was characterized by spectroscopic methods (IR, ES-MS, and ^{1}H NMR), elemental analysis, and X-ray crystallography. For comparison purposes, we also prepared the complex Y(DOTA-AA). ES-MS and ^{1}H NMR data are consistent with the proposed structure. HPLC analysis using a reversed phase method shows that the retention time of In(DOTA-AA) is ∼2.0 min shorter than that of Y(DOTA-AA), demonstrating that In(DOTA-monoamide) is more hydrophilic than Y(DOTA-monoamide). In the solid state, In(DOTA-AA) has a twisted square antiprismatic coordination geometry with all eight donor atoms (N_4O_4) bonded to the In center. The average In–N and In–O distances are almost identical to those of Y–N and Y–O bonds found in Y(DOTA-D-Phe-NH_2) even though the ionic radius of Y^{3+} is much longer than that of In^{3+}. It seems that In^{3+} does not fit the coordination cavity of DOTA-AA perfectly. The ^{1}H NMR data clearly demonstrated that In(DOTA-AA) becomes fluxional at room temperature, most likely due to dissociation of the acetamide-oxygen, rotation of acetate chelating arms, and inversion of ethylenic groups of the macrocyclic ring. Results from this study and our previous studies (Liu, S.; Pietryka, J.; Ellars C. E.; Edwards D. S. *Bioconjugate Chem.* **2002**, *13*, 902–913) suggest that the In^{3+} complex of DOTA-monoamide in the solid state might be different from that in solution due to dissociation of the carbonyl-oxygen donor. Although Y^{3+} and In^{3+} complexes of DOTA-monoamide are both eight-coordinate in the solid state, the difference in their solution structures is most likely responsible for their difference in lipophilicity.

Introduction

There is a great current interest in the ^{90}Y-labeled bioconjugates as target-specific therapeutic radiopharmaceuticals for the treatment of cancers. Several reviews have appeared recently covering a broad range of topics related to radiolabeled small biomolecules (BMs) 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 the imaging surrogate for ^{90}Y is that $^{111}InCl_3$ is com-

* To whom correspondence should be addressed. E-mail: lius@pharmacy.purdue.edu. Phone: 765-494-0236. Fax: 765-496-3367.

(1) Reubi, J. C. *Q. J. Nucl. Med.* **1997**, *41*, 63.
(2) Volkert, W. A.; Hoffman, T. J. *Chem. Rev.* **1999**, *99*, 2269.
(3) Heeg, M. J.; Jurisson, S. *Acc. Chem. Res.* **1999**, *32*, 1053.
(4) Kwekkeboom, D.; Krenning, E. P.; de Jong, M. *J. Nucl. Med.* **2000**, *41*, 1704.
(5) Boerman, O. C.; Oyen, W. J. G.; Corstens, F. H. M. *Semin. Nucl. Med.* **2000**, *30*, 195.
(6) Liu, S.; Edwards, D. S. *Bioconjugate Chem.* **2001**, *12*, 7.
(7) Signore, A.; Annovazzi, A.; Chianelli, M.; Coretti, F.; Van de Wiele, C.; Watherhouse, R. N.; Scopinaro, F. *Eur. J. Nucl. Med.* **2001**, *28*, 1555.
(8) Liu, S.; Edwards, D. S. *Top. Curr. Chem.* **2002**, *222*, 259.
(9) Jong, M.; Kwekkeboom, D.; Valkema, R.; Krenning, E. P. *Eur. J. Nucl. Med.* **2003**, *30*, 463.
(10) Fichna, J.; Janecka, A. *Bioconjugate Chem.* **2003**, *14*, 3.
(11) Smith-Jones, P. M.; Stolz, B.; Albert, R.; Ruser, G.; Briner, U.; Maecke, H. R.; Bruns, C. *Nucl. Med. Biol.* **1998**, *25*, 181.
(12) Van Hagen, P. M.; Breeman, W. A. P.; Bernard, H. F.; Schaar, M.; Mooij, C. M.; Srinivasan, A.; Schmidt, M. A.; Krenning, E. P.; de Jong, M. *Int. J. Can. (Radiat. Oncol. Invest.)* **2000**, *8*, 186.
(13) De Jong, M.; Bakker, W. H.; Breeman, W. A. P.; Bernard, B. F.; Hofland, L. J.; Visser, T. J.; Srinivasan, A.; Schmidt, M.; Béhé, M.; Mäcke, H.; Krenning, E. P. *Int. J. Cancer.* **1998**, *75*, 406.
(14) De Jong, M.; Bakker, W. H.; Bernard, B. F.; Valkema, R.; Kwekkeboom, D. J.; Reubi, J.-C.; Srinivasan, A.; Schmidt, M.; Krenning, E. P. *J. Nucl. Med.* **1999**, *40*, 2081.

10.1021/ic0349914 CCC: $25.00
Published on Web 11/26/2003

mercially 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). However, recent studies have shown differences between ^{90}Y- and ^{111}In-labeled antibodies and small peptides with respect to their biodistribution characteristics.[17,21−23] This causes some concerns about the validity of ^{111}In-labeled BFC−BM conjugates as imaging surrogates for their corresponding ^{90}Y analogues.

Although many ^{90}Y-labeled small peptides have been studied for their therapeutic efficacy in tumor therapy,[11−20] very few studies have been directed toward understanding the differences between the ^{90}Y- and ^{111}In-labeled BFC-BM conjugates with respect to their lipophilicity, structures, and biodistribution characteristics. Thus, we have initiated a series of studies on the radiochemistry of ^{90}Y- and ^{111}In-labeled DTPA- and DOTA-BM conjugates,[24−29] and the coordination chemistry of In^{3+} and Y^{3+} with DOTA-monoamide derivatives.[30] These studies are aimed at exploring the differences between In^{3+} and Y^{3+} chelates,[24−26,29] 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 communication,[30] we reported the synthesis of In^{3+} and Y^{3+} complexes of 1,4,7,10-tetraaza-4,7,10-tris-(carboxymethyl)-1-cyclododecylacetylbenzylamine (DOTA-BA, see Figure 1). Y(DOTA-BA) and In(DOTA-BA) were prepared as model compounds for the corresponding ^{90}Y- and ^{111}In-labeled DOTA-BM conjugates. By a reversed phase HPLC method, it was found that In(DOTA-BA) is more hydrophilic than Y(DOTA-BA). The NMR (^1H and ^{13}C) data clearly demonstrated that In(DOTA-BA) is fluxional at room temperature while Y(DOTA-BA) only becomes fluxional only at elevated temperatures (>50 °C), suggesting that In(DOTA-BA) and Y(DOTA-BA) might not have the same structure in the solution.[30]

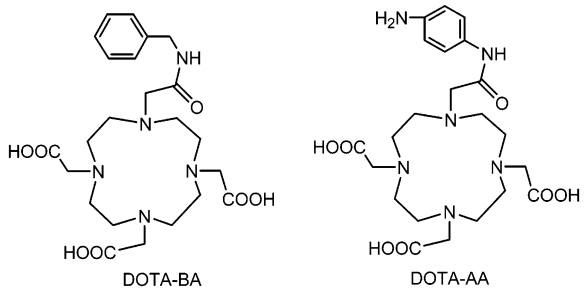

Figure 1. Structures of two model compounds: DOTA-BA and DOTA-AA.

Recently, Mäcke and co-workers described the synthesis and the crystal structure of Y(DOTA-D-Phe-NH_2). It was found that Y^{3+} is eight-coordinated in a square antiprismatic coordination geometry with four amine-nitrogen, one carbonyl-oxygen, and three carboxylate-oxygen atoms bonding to the metal center.[31] To further explore the structural differences between In^{3+} and Y^{3+} chelates, we prepared the In^{3+} complex of 1,4,7,10-tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(*p*-aminoanilide) (DOTA-AA). As a continuation of our previous studies,[24−30] we now present the synthesis and characterization of In(DOTA-AA). The goal of this study is to compare structures of Y(DOTA-D-Phe-NH_2) and In(DOTA-AA) in the solid state, and to understand the differences between solution and solid state structures of In(DOTA-AA).

Experimental Section

Materials and Methods. Chemicals were purchased from Sigma Aldrich (St. Louis, MO) and were used as received. 1,4,7,10-Tetraazacyclododecane-1,4,7,10-tetraacetic acid mono(p-aminoanilide) (DOTA-AA) was purchased from Macrocyclics, Inc. (Dallas, TX). The NMR (^1H and $^1H-^1H$ COSY) data were obtained using a Bruker DRX 600 MHz FT NMR spectrometer, and chemical shifts as δ are reported in ppm relative to TMS. The infrared (IR) spectrum was recorded on a Perkin-Elmer FT-IR spectrometer. Mass spectral data of complexes In(DOTA-AA) and Y(DOTA-AA) 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, $\lambda = 254$ nm) and a Zorbax CN column (4.6 mm × 250 mm, 300 Å pore size). The flow rate was 1 mL/min with the mobile phase starting 90% of solvent A (10 mM ammonium acetate buffer, pH = 6.8) and 10% solvent B (acetonitrile) to 80% solvent A and 20% of solvent B at 20 min.

Synthesis of In(DOTA-AA). To a 5 mL vial were added DOTA-AA (150 mg, 0.30 mmol), anhydrous $InCl_3$ (68 mg, 0.309 mmol),

(15) De Jong, M.; Breeman, W. A. P.; Bakker, W. H.; Kooij, P. P. M.; Bernard, B. F.; Hofland, L. J.; Visser, T. J.; Srinivasan, A.; Schmidt, M. A.; Erion, J. L.; Bugaj, J. E.; Mäcke, H.; Krenning, E. P. *Anticancer Res.* **1998**, *58*, 437−441.
(16) Reubi, J. C.; Waser, B.; Schaer, J. C.; Laederach, U.; Erion, J.; Srinivasam, A.; Schmidt, M. A.; Bugaj, J. E. *Eur. J. Nucl. Med.* **1998**, *25*, 481.
(17) Behr, T. M.; Jenner, N.; Béhé, M.; Angerstein, C.; Gratz, S.; Raue, F.; Becker, W. *J. Nucl. Med.* **1999**, *40*, 1029.
(18) Behr, T. M.; Jenner, N.; Béhé, M.; Angerstein, C.; Gratz, S.; Raue, F.; Becker, W. *Clin. Cancer Res.* **1945**, *5*, 3124s.
(19) Kwekkeboom, D. J.; Bakker, W. H.; Kooj, P. P. M.; Erion, J.; Srinivasan, A.; de Jong, M.; Reubi, J.-C.; Krenning, E. P. *Eur. J. Nucl. Med.* **2000**, *27*, 1312.
(20) Janssen, M.; Oyen, W. J. G.; Massuger, L. F. A. G.; Frielink, C.; Dijkgraaf, I.; Edwards, D. S.; Rajopadyhe, M.; Corsten, F. H. M.; Boerman, O. C. *Cancer Res* **2002**, *62*, 6146.
(21) Carrasquillo, J. A.; White, J. D.; Paik, C. H.; Raubitschek, A.; Le, N.; Rotman, M.; Brechbiel, M. W.; Gansow, O. A.; Top, L. E.; Perentesis, P.; Reynolds, J. C.; Nelson, D. L.; Waldmann, T. A. *J. Nucl. Med.* **1999**, *40*, 268.
(22) Canera, L.; Kinuya, S.; Garmestani, K.; Brechbiel, M. W.; Wu, C.; Pai, L. H.; McMurry, T. J.; Gansow, O. A.; Pastan, I.; Paik, C. H.; Carrasquillo, J. A. *Eur. J. Nucl. Med.* **1994**, *21*, 640.
(23) Rösch, F.; Herzog, H.; Stolz, B.; Brockmann, J.; Köhle, M.; Mühlensiepen, H.; Marbach, P.; Müller-Gärtner, H.-W. *Eur. J. Nucl. Med.* **1999**, *26*, 358.
(24) Liu, S.; Cheung, E.; Rajopadyhe, M.; Williams, N. E.; Overoye K. L.; Edwards, D. S. *Bioconjugate Chem.* **2001**, *12*, 84.
(25) Liu, S.; Edwards, D. S. *Bioconjugate Chem.* **2001**, *12*, 630.
(26) Liu, S.; Cheung, E.; Rajopadyhe, M.; Ziegler, M. C.; Edwards, D. S. *Bioconjugate Chem.* **2001**, *12*, 559.
(27) Liu, S.; Edwards, D. S. *Bioconjugate Chem.* **2001**, *12*, 554.
(28) Liu, S.; Ellars, C.; Harris, T. D.; Edwards, D. S. *Bioconjugate Chem.*, in press.
(29) Onthank, D. C.; Liu, S.; Silva, P. J.; Barrett, J. A.; Harris, T. D.; Robinson, S. P.; Edwards, D. S. *Bioconjugate Chem.*, submitted.
(30) Liu, S.; Pietryka, J.; Ellars, C. E.; Edwards, D. S. *Bioconjugate Chem.* **2002**, *13*, 902.
(31) Heppler, A.; Froidevaux, S.; Mäcke, H. R.; Jermann, E.; Béhé, M.; Powell, P.; Hennig, M. *Chem. Eur. J.* **1999**, *5*, 1974.

Table 1. Selected Crystallographic Data for In(DOTA-AA)·4H_2O

formula	$C_{22}H_{39}InN_6O_{11}$
fw	678.41
space group	$P2_1/n$ (No. 14)
a, Å	9.3412(2)
b, Å	23.4757(7)
c, Å	13.0486(4)
β, deg	108.5963(15)
V, Å^3	2712.04(13)
Z	4
d_{calc}, g/cm^3	1.661
T, K	150
crystal dimensions, mm^3	0.38 × 0.35 × 0.33
radiation (λ, Å)	Mo Kα (0.71073)
transm factors	0.54−0.74
R	0.036
R_w	0.072

and 0.5 mL of ammonium acetate buffer (0.5 M, pH = 6.0). The mixture was heated at 100 °C for 30 min. The reaction mixture was filtered, and the filtrate was transferred into a clean 5 mL vial. Slow evaporation of the solvent afforded the product as light pink crystals suitable for X-ray crystallography. The solid was separated by filtration and was dried under vacuum overnight before being submitted for elemental analysis. The yield was 137 mg (∼65%). A sample was analyzed by HPLC (purity >99%), and the retention time was 8.8 min. IR (cm^{-1}): 1624.7 (s, $\nu_{C=O}$) and 3433.2 (bs, ν_{O-H}). MS (ESI, positive mode): m/z = 607.2 for $[M + H]^+$ ($[C_{22}H_{32}InN_6O_7]^+$). MS (ESI, negative model): m/z = 605.2 for $[M - H]^-$ ($[C_{22}H_{30}InN_6O_7]^-$). ^{1}H NMR (90% H_2O/10% D_2O; 65 °C): 3.1−3.6 (m, 16 H, NCH_2CH_2N); 3.72 (AB quartet, 4H, CH_2CO_2); 3.74 (s, 2H, CH_2CO_2); 4.08 (s, 2H, CH_2CONH), 7.45 (d, 2H, aromatic, J_{HH} = 6.5 Hz) and 7.68 (d, 2H, aromatic, J_{HH} = 6.5 Hz). Anal. Calcd for $C_{22}H_{31}InN_6O_7$·4H_2O: C, 38.87; H, 5.80; N, 12.34. Found: C, 38.95; H, 5.79; N, 12.39.

Synthesis of Y(DOTA-AA). To a 5 mL vial were added DOTA-AA (15 mg, 0.03 mmol), $Y(NO_3)_3$·6H_2O (35 mg, 0.09 mmol), and 1.5 mL ammonium acetate buffer (0.5 M, pH = 6.8). The mixture was heated at 100 °C for 30 min. After filtration, the product was separated from the reaction mixture by HPLC. Collected fractions were combined and lyophilized to give a white powder. The yield was 11.5 mg. The sample was analyzed by HPLC (purity >98%), and the retention time was 10.8 min. MS (ESI, positive mode): m/z = 581.2 for $[M + H]^+$ ($[C_{22}H_{32}YN_6O_7]^+$). MS (ESI, negative model): m/z = 579.2 for $[M - H]^-$ ($[C_{22}H_{30}YN_6O_7]^-$). ^{1}H (90% H_2O/10% D_2O; 5 °C): 2.0−3.4 (b, 16 H, NCH_2CH_2N); 2.85 and 3.08 (AB quartet, 2H, CH_2CO_2); 2.90 and 3.32 (AB quartet, 4H, CH_2CO_2); 3.35 and 3.54 (AB quartet, 2H, CH_2CONH); 7.05 (d, 2H, aromatic, J_{HH} = 6.5 Hz), 7.35 (d, 2H, aromatic, J_{HH} = 6.5 Hz) and 10.7 (s, 1H, CONH).

X-ray Crystallographic Analysis. Crystallographic data for In(DOTA-AA)·4H_2O 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 least-squares refinement, using the setting angles in the range $1° < \theta < 27°$. A total of 17859 reflections were collected of which 6101 were unique. Lorentz and polarization corrections were applied to the data. A linear absorption coefficient is 9.2/cm for Mo Kα radiation. An empirical correction was applied using the program SCALEPACK.[32] The structure was solved using the structure solution program PATTY in DIRDIF99[33] and was refined on a AlphaServer 2100 using SHELXL97.[34] Crystallographic drawing were produced using the program ORTEP.

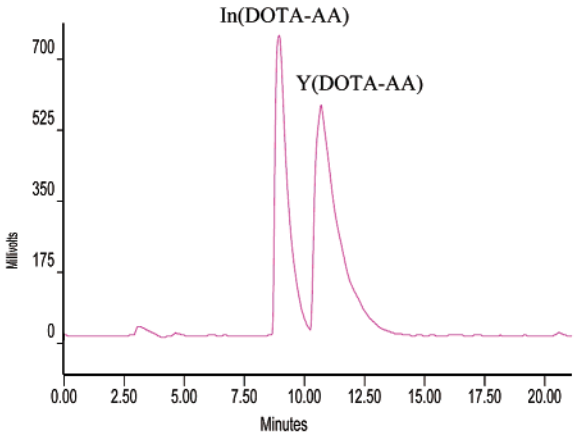

Figure 2. Typical HPLC chromatogram of con-injected In(DOTA-AA) and Y(DOTA-AA).

Results and Discussion

Synthesis of In(DOTA-AA) was straightforward. We prepared In(DOTA-AA) by reacting DOTA-AA with 1 equiv of indium chloride in ammonium acetate buffer (0.5 M, pH ∼ 6). In(DOTA-AA) was isolated from the reaction mixture as pink crystals suitable for X-ray crystallography and has been characterized by elemental analysis, IR, ES-MS, and ^{1}H NMR methods. The IR spectrum of In(DOTA-AA) shows a strong and broad band at 3433 cm^{-1} due to crystallization water molecules, and a strong band at 1625 cm^{-1} due to the coordinated carboxylate and carbonyl groups. Upon coordination, stretching frequencies corresponding to the carboxylic acid ($\nu_{C=O}$ ∼ 1737 cm^{-1}) groups undergo a significant "red-shift" (∼112 cm^{-1}). The ES-MS spectrum of In(DOTA-AA) shows a molecular ion at m/z = 607.2 for $[M + H]^+$ and 605.2 for $[M - H]^+$. The elemental analysis data is completely consistent with the proposed formula and has been confirmed by X-ray crystallography.

For comparison purposes, we also prepared the complex Y(DOTA-AA). HPLC analysis of Y(DOTA-AA) shows >98% purity. ES-MS spectrum of Y(DOTA-AA) shows a molecular ion at m/z = 581.2 for $[M + H]^+$ ($[C_{22}H_{32}YN_6O_7]^+$) and 579.2 for $[M - H]^-$ ($[C_{22}H_{30}YN_6O_7]^-$). A reversed phase HPLC method was used to determine the relative lipophilicity of In(DOTA-AA) and Y(DOTA-AA). The solution containing In(DOTA-AA) and Y(DOTA-AA) was co-injected to avoid chromatographic changes for two consecutive injections. Figure 2 shows the typical HPLC chromatogram of the solution containing In(DOTA-AA) and Y(DOTA-AA). The retention time of In(DOTA-AA) is ∼2.0 min shorter than that of Y(DOTA-AA). This result is consistent with our previous observations that In(DOTA-monoamide) is more hydrophilic than Y(DOTA-monoamide).[29,30]

(32) Otwinowski, Z.; Minor, W. *Methods Enzymol.* **1997**, *276*, 307.

(33) Beurskens, P. T.; Beurskens, G.; deGelder, R.; Garcia-Granda, S.; Gould, R. O.; Israel, R.; Smits, M. M. *The DIRDIF-99 Program System*; Crystallography Laboratory, University of Nijmegen: Nijmegen, The Netherlands, 1999.

(34) Shelderick, G. M. *SHELXL 97. A Program for Crystal Structure Refinement*; University of Göttingen: Göttingen, Germany, 1997.

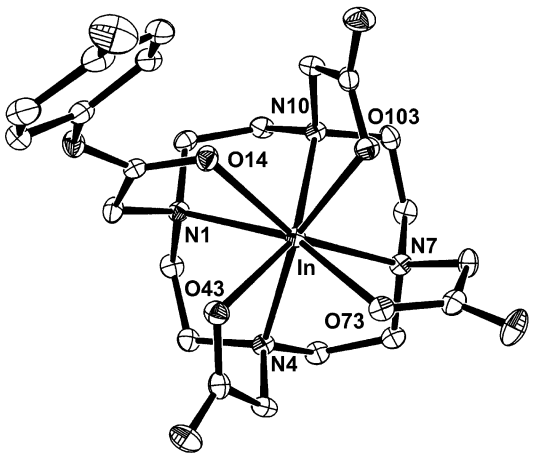

Figure 3. ORTEP drawing of In(DOTA-AA) (ellipoids are at 50% probability). Crystallization water and hydrogen atoms are omitted for the sake of clarity.

An ORTEP view of the structure of In(DOTA-AA) is shown in Figure 3. The selected crystallographic data are listed in Table 1. There are four In(DOTA-AA) molecules in each unit cell, along with four crystallization water molecules surrounding each In(DOTA-AA) molecule. DOTA-AA acts as an octadentate ligand in bonding to the In^{3+} with four amine-nitrogen, one carbonyl-oxygen, and three carboxylate-oxygen atoms. In^{3+} is deeply buried inside the N_4O_4 coordination cavity of the DOTA-AA chelator at a distance of ~1.3 Å above the four-nitrogen plane and ~1.2 Å below the four-oxygen plane. The four-nitrogen square plane is almost parallel with the four-oxygen plane. The twist angle between these two planes is ~28°, resulting in a coordination geometry between prismatic (theoretical twist angle of 0°) and antiprismatic (theoretical twist angle of 45°). The relative orientation of the four-oxygen plane is different from that found in Y(DOTA-D-Phe-NH_2).[31] The coordination geometry is best defined as "inverted square antiprism" arrangement (traditionally termed as m isomer) to differentiate it from the usual square antiprismatic geometry (traditionally termed as M isomer) found in Y(DOTA-D-Phe-NH_2) and many other lanthanide complexes of DOTA and its derivatives.[35−41]

Table 2 lists selected In−N and In−O bond distances in the coordination sphere. The average In−N bond length is 2.430(2) Å, which is very close to that of Y−N bonds (2.418(6) Å) in Y(DOTA-D-Phe-NH_2) even though the ionic radius of Y^{3+} (1.02 Å for eight-coordinated Y^{3+}) is about 0.1 Å longer than that of In^{3+} (0.92 Å for eight-coordinated In^{3+}).[42] The average bond distance between In^{3+} and three carboxylate-oxygen atoms is 2.254(2) Å. The In−O (carbonyl) bond length (2.3143(19) Å) is only slightly longer than those of In−O (carboxylate) bonds (varying from 2.2185(19) to 2.2746(19) Å). The In−O bond distances are almost identical to the Y−O bond distances (2.241(6)−2.282(6) Å for Y−O (carboxylate) and 2.318(7) Å for Y−O (carbonyl)) found in Y(DOTA-D-Phe-NH_2).[31] This suggests that In^{3+} might not fit perfectly in the cavity of the DOTA-AA. Both In−N and In−O bond distances in the complex In(DOTA-AA) are about 0.075 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) found in In(DO3A).[43] These In−O and In−N bond differences in In(DOTA-AA) and In(DO3A) may be caused by the changes in the coordination number.

Table 2. Selected Bond Distances (Å) for In(DOTA-AA)·$4H_2O$

atom 1	atom 2	distance
In(5a)	O(43)	2.2185(19)
In(5a)	O(73)	2.269(2)
In(5a)	O(103)	2.2746(19)
In5(a)	O(14)	2.3143(19)
In(5a)	N(7)	2.372(2)
In(5a)	N(10)	2.413(2)
In(5a)	N(4)	2.417(2)
In(5a)	N(1)	2.518(2)

In^{3+} and Y^{3+} are trivalent cations. The difference between In^{3+} and Y^{3+} is their size. As a result, In^{3+} and Y^{3+} complexes of DTPA and DOTA derivatives often show different coordination chemistry with respect to their coordination number and solution properties of their complexes. For example, Y^{3+} has an ionic radius of 1.02 Å, which fits perfectly into the cavity of DOTA derivatives. It is not surprising that Y^{3+} complexes of DOTA derivatives are able to maintain their rigid eight-coordinated structure in solution.[30] In^{3+} has an ionic radius of 0.92 Å, which is much smaller than that of Y^{3+}. The coordination number for In^{3+} is typically 6 or 7.[44−48] As a matter of fact, the complex In(DOTA-AA) described in this report represents a rare example of eight-coordinated In^{3+} complexes.[49−51]

On the basis of the solid state structure, it is quite clear that the DOTA-monoamide chelator forms In^{3+} and Y^{3+} complexes with different coordination geometries. The difference between In(DOTA-monoamide) and Y(DOTA-mono-

(35) Chang, C. A.; Francesconi, L. C.; Malley, M. F.; Kumar, K.; Cougoutas, J. Z.; Tweedle, M. F. *Inorg. Chem.* **1993**, *32*, 3501.
(36) Kumar, K.; Chang, C. A.; Francesconi, L. C.; Dischino, D. D.; Malley, M. F.; Cougoutas, J. Z.; Tweedle, M. F. *Inorg. Chem.* **1994**, *33*, 3567.
(37) Aime, S.; Botta, M.; Ermondi, G.; Terreno, E.; Anelli, P. L.; Dedeli, F.; Uggeri, F. *Inorg. Chem.* **1996**, *35*, 2726.
(38) Aime, S.; Batsanow, A. S.; Botta, M.; Dickins, R. S.; Faulkner, S.; Foster, E. C.; Howard, J. A. k.; Moloney, J. M.; Norman, T. J.; Parker, D.; Royle, L.; Williams, J. A. G. *J. Chem. Soc., Dalton Trans.* **1997**, 3623.
(39) Aime, S.; Barge, A.; Benetollo, F.; Bombieri, G.; Botta, M.; Uggeri, F. *Inorg. Chem.* **1997**, *36*, 4287.
(40) Morrow, J. R.; Amin, S.; Lake, C. H.; Churchill, M. R. *Inorg. Chem.* **1993**, *32*, 4566.
(41) Aime, S.; Barge, A.; Botta, M.; Fasano, M.; Ayala, J. D.; Bombieri, G. *Inorg. Chim. Acta* **1996**, *246*, 423.
(42) Shannon, R. D. *Acta Crystallogr.* **1976**, *A32*, 751.
(43) Riesen, A.; Kaden, T. A.; Ritter, W.; Mäcke, H. R. *J. Chem. Soc., Chem. Commun.* **1989**, 460.
(44) Liu, S.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1992**, *31*, 5400.
(45) Liu, S.; Wong, E.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1993**, *32*, 4268.
(46) Liu, S.; Wong, E.; Karunaratne, V.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1993**, *32*, 1756.
(47) Wong, E.; Caravan, P.; Liu, S.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1996**, *35*, 715.
(48) Wong, E.; Liu, S.; Lügger, T.; Hahn, F. E.; Orvig, C. *Inorg. Chem.* **1995**, *34*, 93.
(49) Mäcke, H. R.; Riesen, A.; Ritter, W. *J. Nucl. Med.* **1989**, *30*, 1235.
(50) Malyrick M. A.; Ilyhin, A. B.; Petrosyans, S. P. *Main Group Met. Chem.* **1994**, *17*, 707.
(51) Maeke, H.; Scherer, G.; Heppeler, A.; Henig, M. *Eur. J. Nucl. Med.* **2001**, *28*, 967 (Abstract OS-27).

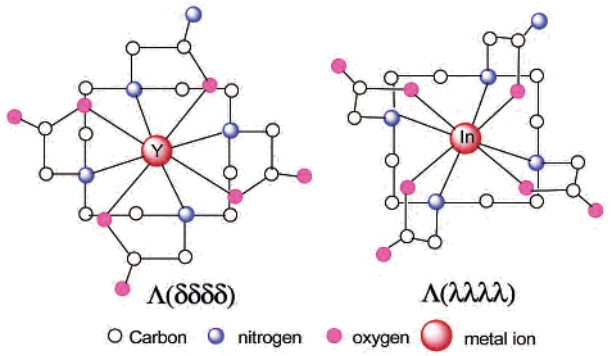

Figure 4. Relative orientations of the acetamide/acetate arms and ethylenic bridges in complexes Y(DOTA-Phe-NH_2) and In(DOTA-AA).

amide) is the conformation of ethylenic bridges and the relative orientation of the acetamide and three acetate arms (Figure 4). This slight structural difference might be able to explain the lipophilicity difference between In(DOTA-BA) and Y(DOTA-BA),[30] but it hardly explains the fact that In(DOTA-BA) is fluxional at room temperature while Y(DOTA-BA) only becomes fluxional at elevated temperatures (>45 °C).[30] From this point of view, the differences in lipophilicity and solution behaviors between In(DOTA-AA) and Y(DOTA-AA) are most likely caused by their different solution structures.

Figure 5 shows the aliphatic region of the ^{1}H NMR spectra of In(DOTA-AA) in H_2O/D_2O (90:10 = v:v) at 25 °C (top) and 65 °C (bottom). At room temperature, all resonance signals in the aliphatic region are broad. The broad singlet at 3.67 ppm is tentatively assigned to the two methylene hydrogens of the acetamide group and the broad singlet at 3.30 ppm to methylene hydrogens of the three acetate arms. The ratio of the integrated intensity for the two broad peaks at 3.67 and 3.30 ppm is 1:3. The broad singlet at 3.10 ppm and the multiplet at 2.73 ppm are from methylene hydrogens of the macrocyclic framework.

The observation of two broad singlets at 3.67 and 3.30 ppm at room temperature is significant and suggests that the coordinated DOTA-AA has become partially fluxional due to dissociation of the carbonyl-oxygen and rotation of the

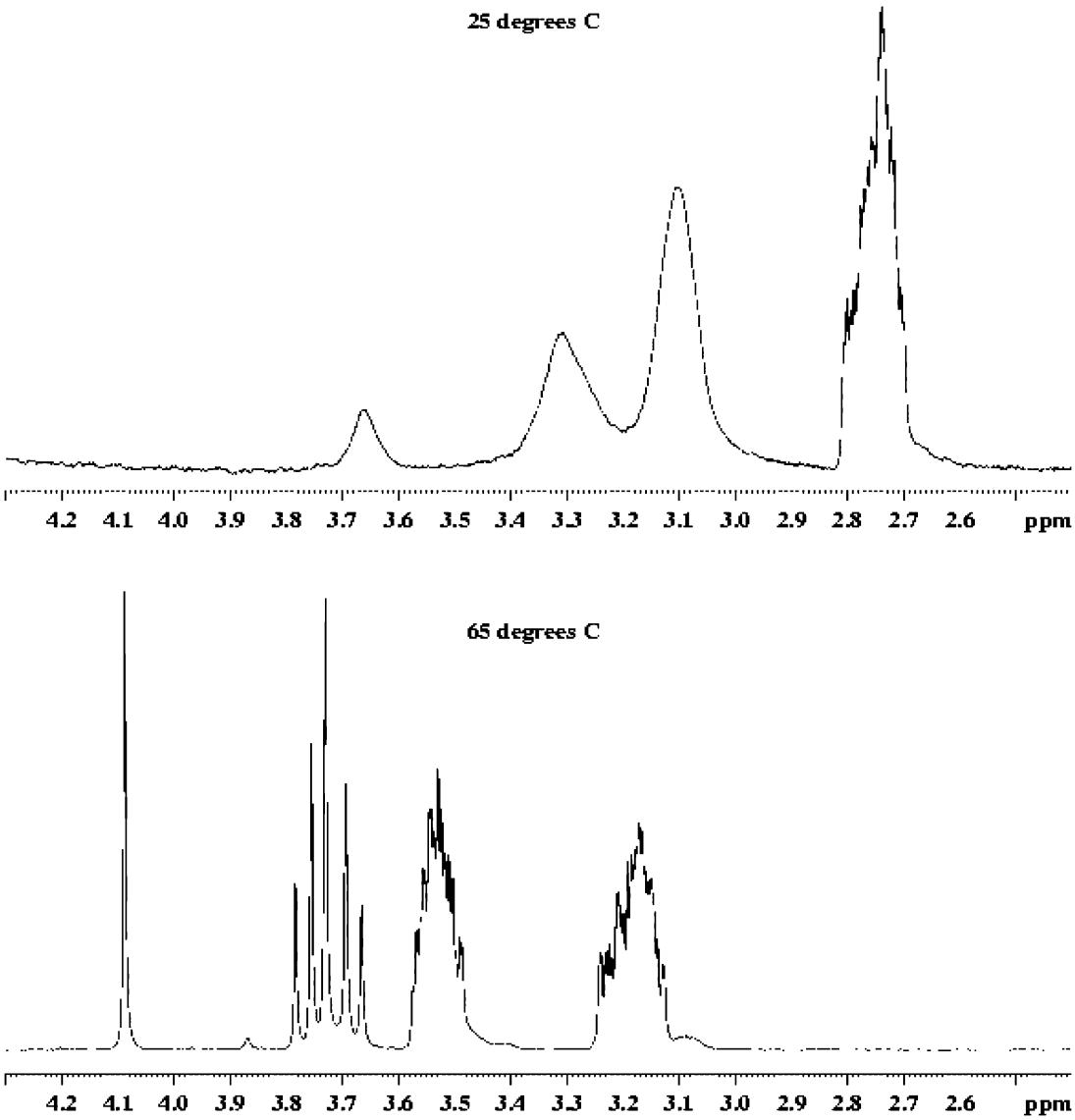

Figure 5. Aliphatic region of the ^{1}H NMR (600 MHz) spectrum of In(DOTA-AA) (in H_2O/D_2O = 90:10) at 25 °C (top) and 65 °C (bottom).

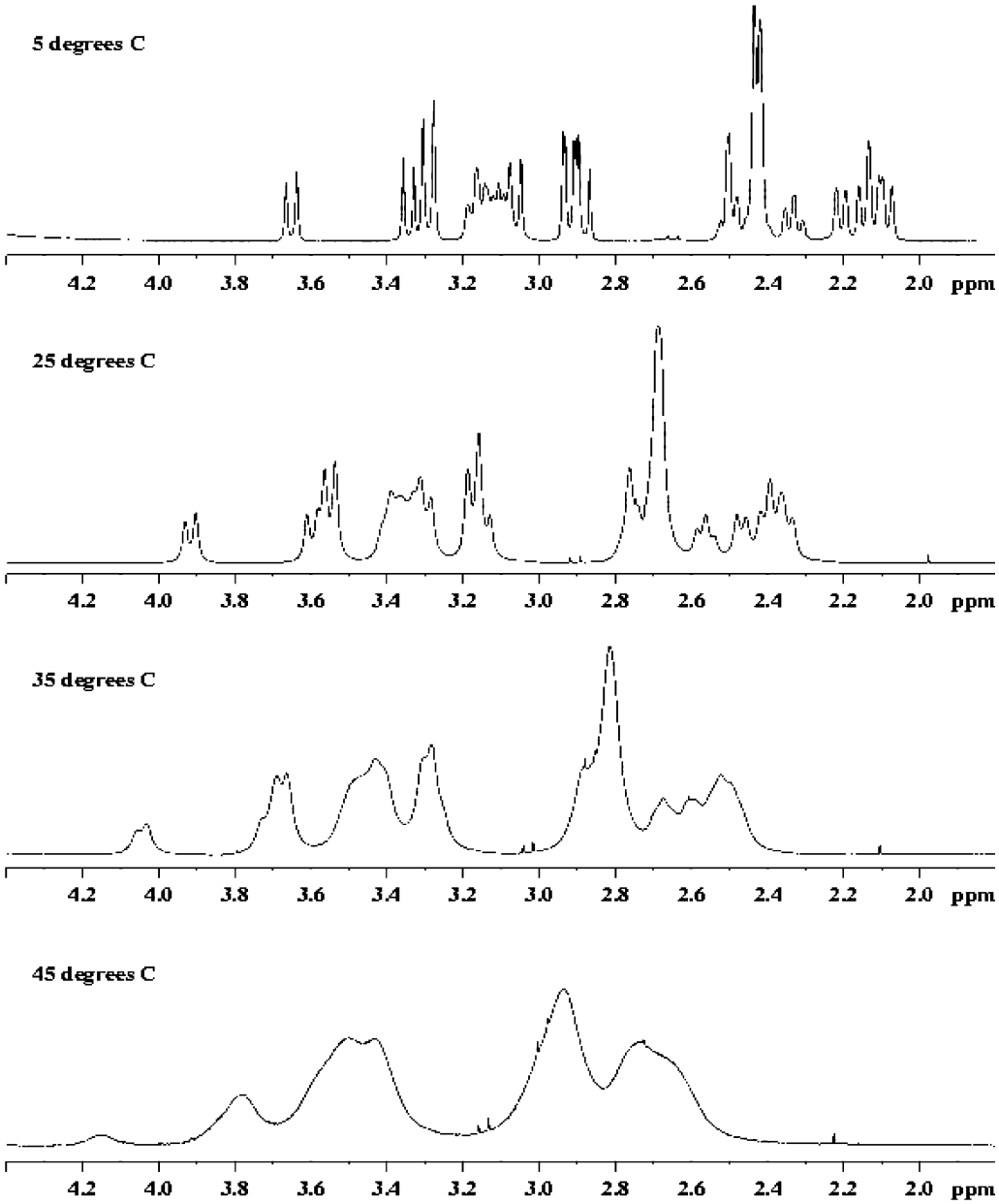

Figure 6. Variable temperature ^{1}H NMR (600 MHz) spectrum of Y(DOTA-AA) (in H_2O/D_2O = 90:10) in the aliphatic region.

three acetate arms. As the temperature increases, the coordinated DOTA-AA becomes more fluxional. As a result, all resonance signals in the aliphatic region become sharper. The coalescence point for the complex In(DOTA-AA) is ~35 °C, at which temperature the signal is at its broadest and splitting cannot yet be observed. At 65 °C, the resonance signal from the two methylene hydrogens of the acetamide group appears as a singlet at 4.08 ppm. The two methylene hydrogens of the acetate arm opposite to the acetamide group show a singlet at 3.74 ppm while methylene hydrogens from the two remaining acetate arms appear as an AB quartet pattern at 3.72 ppm. If the In^{3+} in In(DOTA-AA) were still eight-coordinated in solution, there would have been two AB quartets for methylene hydrogens of the acetamide group and those of the opposite acetate arm as observed in ^{1}H NMR spectra of Y(DOTA-AA) (Figure 6).

Dissociation of the carbonyl-oxygen in solution (Figure 7) may contribute to the high hydrophilicity of In(DOTA-AA) and at the same time makes it easier for the three acetate arms to rotate. The rapid rotation of three acetate arms and inversion of ethylenic groups makes In(DOTA-AA) more symmetrical. However, all three carboxylate-oxygen donors remain firmly bonded to the In^{3+} as evidenced by the presence of the AB quartet pattern at 3.71 ppm in the NMR spectrum of In(DOTA-AA) at 65 °C. If any of these three carboxylate-oxygen donors were dissociated, there would have been three singlets: one from methylene hydrogens of the acetamide, one from methylene hydrogens of the opposite acetate arm, and one from the two remaining acetate arms.

Figure 6 shows the VT ^{1}H NMR spectra of Y(DOTA-AA) in a mixture of H_2O/D_2O (90:10 = v/v). At 5 °C, resonance signals from methylene hydrogens of the acetamide

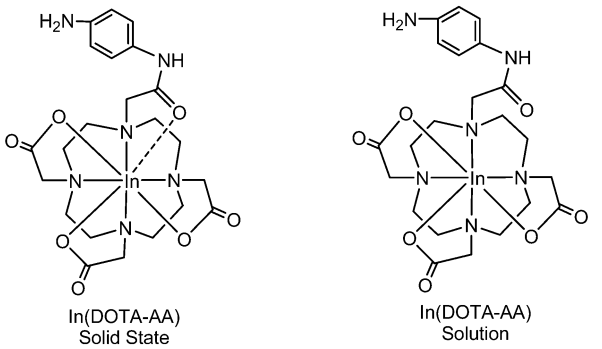

Figure 7. Structures of In(DOTA-AA) in the solid state (left) and solution (right).

and three acetate arms appear as three well-separated AB quartets with the integrated intensity of 1:2:1 while resonance signals from methylene hydrogens of the macrocyclic backbone appear as several overlapped multiplets in the region between 2.0 and 3.7 ppm. At room temperature, the splitting pattern of all resonance signals due to methylene hydrogens of the acetamide and three acetate arms remains relatively unchanged despite a significant signal shift downfield. The observation of three AB quartet patterns at room temperature is significant and suggests that the coordinated DOTA-AA in Y(DOTA-AA) is rigid. There is neither a dissociation of the carbonyl-oxygen nor rapid rotation of three acetate arms, at least at the NMR time scale. As the temperature increases above 45 °C, all resonance signals in the aliphatic region start to collapse. Unfortunately, we were unable to observe the coalescence point for Y(DOTA-AA) even at temperatures >85 °C. On the basis of the VT NMR data, it is clear that the coordinated DOTA-AA in Y(DOTA-AA) is more rigid than that in In(DOTA-AA).

Conclusions

In conclusion, a new model compound, In(DOTA-AA), for ^{111}In-labeled DOTA-BM conjugates has been prepared and characterized by spectroscopic methods and X-ray crystallography. In the solid state, all eight donor atoms (N_4O_4) are bonded to the In^{3+} and form a rare example of eight-coordinated In^{3+} complexes with a twisted square antiprismatic geometry. The ^{1}H NMR data showed that In(DOTA-AA) is fluxional in solution at room temperature most likely due to the dissociation of the acetamide-oxygen from In^{3+}. Although In^{3+} and Y^{3+} are eight-coordinated in solid state structures of their DOTA-monoamide complexes, differences in their solution structures are most likely responsible for their different lipophilicity and solution behaviors. It should be noted that the radiometal chelate is only one part of the ^{111}In- and ^{90}Y-labeled DOTA-BM conjugate. The ^{111}In and ^{90}Y chelates may have different solution structures, which causes the difference in lipophilicity between the ^{111}In- and ^{90}Y-labeled DOTA-BM conjugate. Ultimately, it will be the bioequivalence that determines if the ^{111}In-labeled DOTA-BM conjugate can be used to accurately predict the radiation dosimetry of the corresponding ^{90}Y analogue.

Acknowledgment is made to Mr. M. John Pietryka at Bristol Myers Squibb (BMS) Medical Imaging for his help with variable temperature ^{1}H NMR studies on In(DOTA-AA) and Y(DOTA-AA).

Supporting Information Available: X-ray crystallographic file in CIF format for the reported structure. This material is available free of charge via the Internet at http://pubs.acs.org.

IC0349914