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Isomerism in Benzyl-DOTA Derived Bifunctional Chelators: Implications for Molecular Imaging

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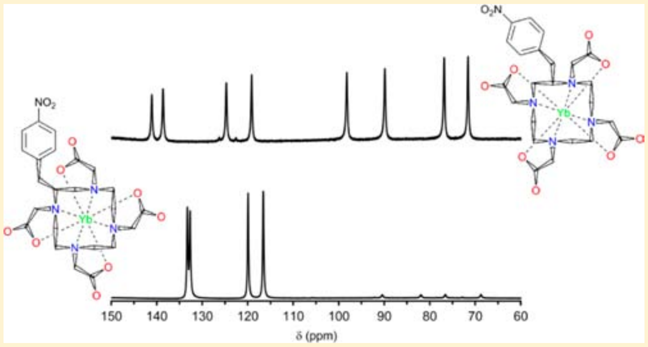
Ⓢ *Supporting Information*



ABSTRACT: The bifunctional chelator IB-DOTA has found use in a range of biomedical applications given its ability to chelate many metal ions, but in particular the lanthanide(III) ions. Gd^{3+} in particular is of interest in the development of new molecular imaging agents for MRI and is highly suitable for chelation by IB-DOTA. Given the long-term instability of the aryl isothiocyanate functional group we have used the more stable nitro derivative (NB-DOTA) to conduct a follow-up study of some of our previous work on the coordination chemistry of chelates of these BFCs. Using a combination of NMR and HPLC to study the Eu^{3+} and Yb^{3+} chelates of NB-DOTA, we have demonstrated that this ligand will produce two discrete regioisomeric chelates at the point at which the metal ion is introduced into the BFC. These regioisomers are defined by the position of the benzylic substituent on the macrocyclic ring: adopting an equatorial position either at the corner or the side of the [3333] ring conformation. These regioisomers are incapable of interconversion and are distinct, separate structures with different SAP/TSAP ratios. The side isomer exhibits an increased population of the TSAP isomer, pointing to more rapid water exchange kinetics in this regioisomer. This has potential ramifications for the use of these two regioisomers of Gd^{3+}-BFC chelates in MRI applications. We have also found that, remarkably, there is little or no freedom of rotation about the first single bond extending from the macrocyclic ring to the benzylic substituent. Since this is the linkage through which the chelate is conjugated to the remainder of the molecular imaging probe, this result implies that there may be reduced local rotation of the Gd^{3+} chelate within a molecular imaging probe. This implies that this type of BFC could exhibit higher relaxivities than other types of BFC.

■ INTRODUCTION

The development of new probes for molecular imaging has been revolutionized by the commercial availability of bifunctional chelators (BFC).[1−3] In principle, BFCs allow almost any molecule, peptide, protein, or particle with properties of interest in vivo to be tagged. By introducing a metal ion with suitable properties for detection with a common imaging modality a molecular imaging probe can be developed. Of the numerous types of BFC commercially available, some are clearly superior to others. Applications, such as molecular imaging, that envisage prolonged in vivo residence lifetimes demand the use of the most robust chelates. The most robust metal chelates are invariably produced through the use of macrocyclic chelators which ensure that significant dechelation of the metal ion cannot occur.[4,5] The nature of the conjugating functional group must also be considered. One common approach is to take a ligating carboxylate and modify it to make a conjugating amide group.[6] This has the disadvantage of altering (possibly negatively) the ligand structure. Isothiocyanates (ITC), by contrast, react selectively with primary amines under mild, aqueous conditions to form a robust thiourea linkage and can be incorporated into a BFC in such a way as to leave the ligand structure essentially unaltered.[6] In light of these considerations IB-DOTA (Chart 1) and its analogues represent one of the more popular, and arguably better, BFC options. Based on the macrocycle cyclen, the DOTA ligand is able to securely chelate a wide range of metal ions, not just the lanthanide (Ln^{3+}) ions, but also other metal ions relevant to molecular imaging such as In^{3+}.

Although the use of IB-DOTA as a BFC for molecular imaging applications was becoming increasingly common 10 years ago, at that time there was precious little data regarding the properties of its chelates to be found in the literature. This omission spurred us to undertake a study of NB-DOTA and its Ln^{3+} chelates; their stability and physicochemical properties.[7] The nitro benzyl (NB) analogue (NB-DOTA) was studied in place of the actual BFC IB-DOTA because, unlike the ITC group, the NO_2 group is stable but unable to appreciably alter

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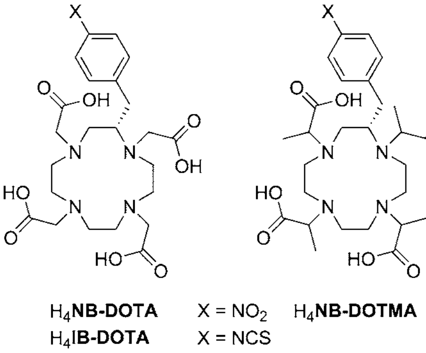
Chart 1. Connectivity of BFC IB-DOTA and Its More Stable Analogue NB-DOTA



the properties of the ligand or its chelates. Our studies[7] showed that NB-DOTA chelates behaved in a broadly similar way to chelates of DOTA. Kinetically and thermodynamically LnNB-DOTA chelates were about as robust as those of DOTA. The kinetics of water exchange between coordination sites on the Gd^{3+} chelate and the bulk solvent were also comparable to GdDOTA; an important consideration if IB-DOTA is used as part of a molecular imaging probe for MRI.

In our previous study[7] we conducted an examination of the coordination chemistry of NB-DOTA chelates and observed that the LnNB-DOTA chelates adopted both of the coordination geometries previously identified for other DOTA-type chelates:[8−10] the square antiprism (SAP) and the twisted square antiprism (TSAP) (Figure 1). However, a second type of isomerism is possible[11−13] in these macrocycle substituted chelates and the related NOTA systems;[14] although this was first reported[13] two years prior to our study of NB-DOTA, its presence in these chelates was not noted at the time. Subsequent work on these NB substituted macrocyclic chelates has found this second isomerism phenomenon to be significant.[11,12,15] In light of these results we began to wonder why it had not been observed for chelates of NB-DOTA. Accordingly we have revisited the question of the coordination chemistry of NB-DOTA, studying the chelates of two Ln^{3+} ions most amenable to NMR: Eu^{3+} and Yb^{3+}.

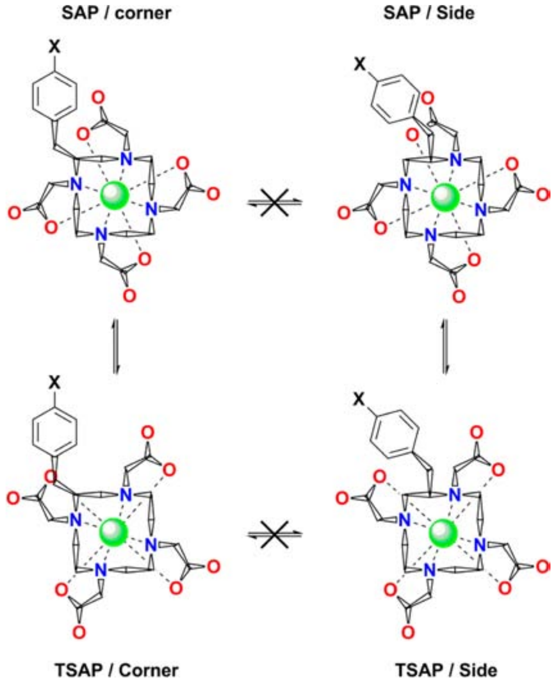


Figure 1. Wedge representations following Dale's conventions[16] of the corner and side isomers of GdNB-DOTA (X = NO_2) or IB-DOTA (X= NCS).

RESULTS AND DISCUSSION

Addition of a benzylic substituent to the cyclen ring of DOTA distinguishes the two carbon atoms of the ethylene bridge on which it is located. In any of the common conformations adopted by cyclen in DOTA chelates (such as the [3333] found in Ln^{3+} chelates)[16,17] one carbon of each ethylene bridge is located at a corner, while the other is on the side. When cyclen is unsubstituted, these two positions are in dynamic interchange.[9,10] However, the benzylic substituent must occupy an equatorial position on the ring to minimize torsional strain, and this has the effect of "freezing out" the interchange process.[7,18] This "freezing out" effect was correctly identified in our previous study.[7] However, by "freezing" the conformation of the ring, the position of the benzylic group is fixed once the chelate has been formed. This differentiation leads to two discrete regioisomeric chelates that are not in exchange with one another. These regioisomers we designate according to Dale's convention:[16] corner and side isomers (Figure 1).

Regioisomerism in LnNB-DOTA Chelates. One of the limitations of our previous study was that no HPLC analysis was performed on either the ligand or chelates studied therein. In this study, to ensure that all chelates produced from the commercial sample of NB-DOTA arose from the same ligand, the sample was purified to a single peak by preparative RP-HPLC. The identity of the ligand was confirmed by mass spectrometry. From this purified sample the Eu^{3+} and Yb^{3+} chelates of NB-DOTA were prepared from the corresponding $LnCl_3$ salt in the same manner as in our previous study:[7] aqueous solution at pH 5.5 and 60 °C. RP-HPLC analysis of the Eu^{3+} and Yb^{3+} NB-DOTA chelates prepared in this way clearly reveals the presence of two primary species in a ratio of 3:1 regardless of metal ion (Figure 2). These two major species were determined to be isomeric by mass spectrometric analysis, confirming that the phenomenon of regioisomerism also arises in NB-DOTA chelates. The two regioisomers (corner and side)

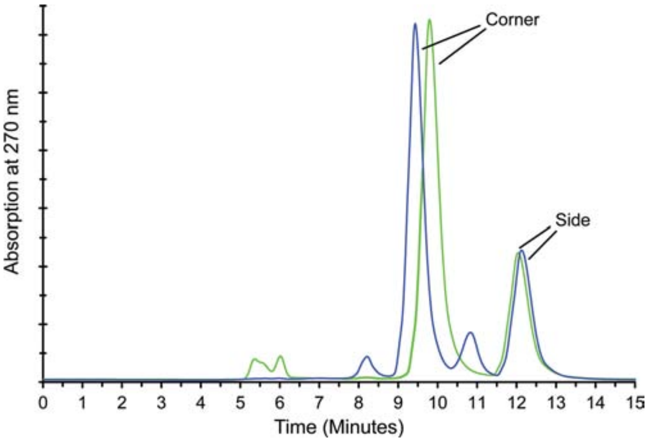


Figure 2. RP-HPLC traces from the purification of the two regioisomers of EuNB-DOTA (blue) and YbNB-DOTA (green). Detection was by absorption monitoring at 270 nm.

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are discriminated at the point of metal approach to the ligand: the direction from which metal and ligand approach one another during recombination determine which regioisomer is produced.[12] This explains how a sample of pure ligand is able to produce two discrete chelates, separable by HPLC. Interestingly the more hydrophilic (corner) isomer always predominates. The RP-HPLC retention time of this regioisomer varies depending upon the size of the metal ion: the more compact Yb^{3+} chelate is more hydrophobic than the more open Eu^{3+} chelate. In contrast, the elution time of the second (side) isomer appears largely unaffected by the size of the metal ion. Separating these two isomeric chelates by preparative RP-HPLC allows them to be properly assigned to the appropriate regioisomer. On the basis of previous observations[11,12] the more hydrophilic major species was preliminarily assigned to the corner isomer and the minor, more hydrophobic one to the side isomer. This assignment was later confirmed by 2D NMR analysis of the EuNB-DOTA chelates (vide supra).

The ^{1}H NMR spectrum of EuNB-DOTA shows a similar distribution of regioisomers (Figure 3). Owing to the lack of symmetry in these chelates most of these spectra are extremely crowded and can only be completely interpreted with the aid of 2D NMR spectroscopy (vide supra). Fortunately the axial protons on the side of the ring (ax^S) are shifted far downfield of all other resonances and can play a diagnostic role in interpreting these spectra; this region of the spectrum of crude EuNB-DOTA is shown in Figure 3. At first glance examination of this region of the spectrum appears to show one SAP isomer and one TSAP isomer. However, on closer inspection some small peaks (labeled †, Figure 3) from what appears to be a second TSAP species may be discerned (these may have been attributed to a ligand impurity in our initial analysis, but that mistake cannot now be made, following HPLC purification of the ligand). There is also one small peak (labeled ‡, Figure 3) visible on the upfield edge of the major SAP peaks. Comparing this spectrum (acquired at higher B_0 field) with that published previously,[7] this small peak appears as just a shoulder on the most upfield major SAP peak.

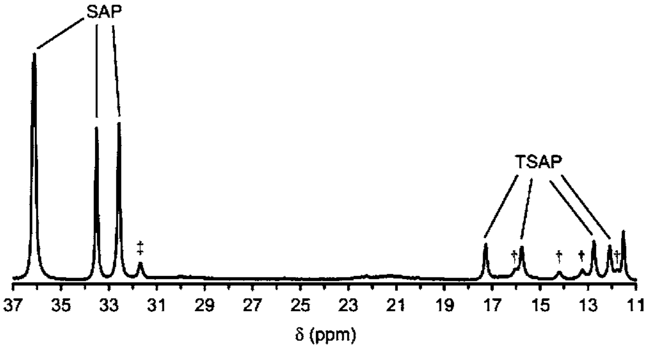

Figure 3. Downfield region of the ^{1}H NMR spectra of crude chelation reaction products of EuNB-DOTA showing the ax^S resonances in D$_2$O (pD 5.5) at 283 K, recorded at 400 MHz.

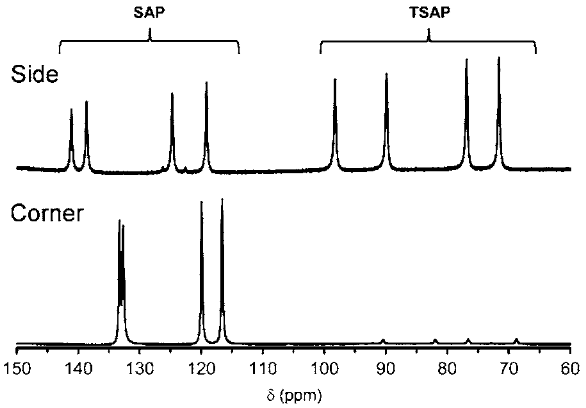

Figure 4. Downfield region of the ^{1}H NMR spectra of each isomer of YbNB-DOTA showing the ax^S resonances in D$_2$O (pD 3.5) at 283 K, recorded at 400 MHz.

After separation of the two regioisomers, ^{1}H NMR spectra were acquired of each isolated regioisomer of YbNB-DOTA, the downfield ax^S resonances for each chelate are shown in Figure 4. For each regioisomer a total of eight ax^S resonances are observed, the shifts of these resonances (and the lack of symmetry in the chelate) are consistent with the presence of one SAP isomer and one TSAP isomer. The configuration at carbon (*S*-) defines these chelates as the $\Lambda(\delta\delta\delta\delta)$ (SAP) and $\Delta(\delta\delta\delta\delta)$ (TSAP) isomers, the $(\lambda\lambda\lambda\lambda)$ ring conformation is rendered inaccessible by the NB substituent.[7,19] What is striking about these spectra is the vastly different SAP/TSAP ratio present in each. The corner isomer exists almost exclusively (~95%) in the SAP coordination geometry. In contrast, the side isomer has an almost equal distribution of the two, favoring the TSAP isomer only marginally (~55%). This may be an indication that the equatorial side location is more sterically demanding then the equatorial corner and the more open TSAP geometry more easily accommodates bulky groups in this position. Almost identical SAP/TSAP distributions are observed for the corner and side isomers of both Eu^{3+} and Yb^{3+} chelates of NB-DOTA (Figures 4 and S1, Supporting Information). This demonstrates that the two most populated species in the spectrum of crude EuNB-DOTA (Figure 3) are not (as previously assigned) the SAP and TSAP isomers of the same chelate. They are in fact the major coordination isomers of the corner and side isomers and these resonances overlap, obscuring those of both minor coordination isomers. Since the rate of water exchange in the TSAP isomer is known to be considerably faster than in the SAP isomer,[18−22] the difference in isomeric distribution between corner and side isomers has potential implications for the use of these chelates as MRI contrast agents.[23] Our previously reported water residence lifetime of GdNB-DOTA (τ_M = 200 ns)[7] represents a weighted average of the lifetimes for all four chelates present in solution, but τ_M for the side isomer (55% TSAP) must be much shorter than this.

Interconversion of Regioisomers of LnNB-DOTA. Studies on rigid and tetraamide NB-cyclen analogues have shown unambiguously that it is not possible to exchange corner and side isomers through conformational changes.[11,12] In the case of LnNB-DOTA chelates this is evidenced by the fact that each regioisomer can be isolated and is stable in solution (at close to neutral pH) for several weeks. Under these conditions no conversion of either isomer to the other was observed by ^{1}H NMR. For the rigid analogue YbNB-DOTMA it was observed that by heating a sample of pure corner isomer under strongly acidic conditions (pD 1, 363 K) it was possible to obtain a sample containing a mixture of both regioisomers.[12] Conversion of the corner isomer of the Yb^{3+} chelate back to an equilibrium distribution of both isomers is slow; the half-life for this process is about 3.25 weeks at pH 1.[12] In contrast, when the same experiment is attempted with YbNB-DOTA, equilibrium is reached much more quickly: within 1 week! The only mechanism that can convert one regioisomer to the other is dechelation of the metal ion, followed by recombination. Since the regiochemistry of the recombination product is independent of the regiochemistry of the initial chelate, an equilibrium distribution of corner and side isomers can be reached. The speed with which an equilibrium distribution of regioisomers of YbNB-DOTA is reached raises the question: just how inert to loss of metal ion is NB-DOTA relative to the much less labile NB-DOTMA analogues?

Assignment of the Regioisomers of LnNB-DOTA. Figure 5 shows the ^{1}H−^{1}H COSY spectrum of the major regioisomer of EuNB-DOTA; this spectrum allows the complete assignment of the major species observed in the spectrum of crude EuNB-DOTA (Figure 2). The assignment of the major SAP isomer is consistent with that previously published.[11] It clearly shows that one of the corner equatorial protons (eq^C) is missing and this position must therefore be occupied by the NB group. This is also visible in the absence of the vicinal cross-coupling peak between the $ax^S \leftrightarrow eq^C$ protons on this ethylene bridge. The presence of coupling cross-peaks between both benzylic (*Bn* and *Bn*′) protons and the axial corner (ax^C) proton of the same ethylene bridge further supports assignment of this species to the corner isomer. The SAP/TSAP ratio is comparable to that observed for the Yb^{3+} chelate (SAP ≈ 95%, Figure 4). This suggests that the ionic radius of the metal ion has comparatively little effect on this ratio; although as in the case of DOTA itself, this observation may be coincidental.[7,8] The preponderance of SAP isomer in this case makes it comparatively easy to assign the SAP spectrum in Figure 5. Assignment of the minor TSAP spectrum is possible only by reference to the EXSY spectrum (Supporting Information Figure S3).

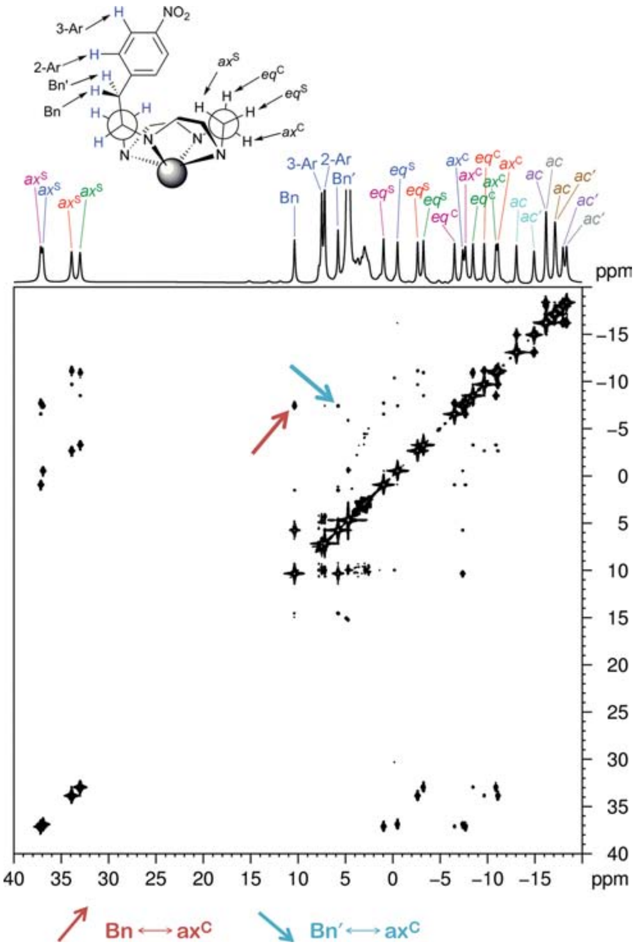

Figure 5. ^{1}H−^{1}H COSY NMR spectrum (283 K, 400 MHz) of the more hydrophilic corner isomer of EuNB-DOTA.

By the process of elimination the minor, more hydrophobic species must be the side isomer. This assignment was confirmed by ^{1}H−^{1}H COSY and EXSY spectroscopy (Supporting Information Figures S4 and S5). In this case the SAP/TSAP ratio is very close; the TSAP isomer predominates, but only slightly (∼55%) (Figure 4). This makes it very difficult to distinguish which peaks and cross-peaks arise from which coordination isomer. A full assignment of these spectra is only possible by a simultaneous analysis of both COSY and EXSY spectra (Supporting Information Figures S4, S5, and S6). However, the assignment as the side isomer is comparatively easy for both SAP and TSAP isomers. In each case a cross-peak corresponding to the geminal couple between the ax^S and eq^S protons is absent on one ethylene bridge. This indicates that one eq^S position is occupied by the NB substituent. Further confirmation of this assignment is provided by observation of the coupling between the ax^S proton and one benzylic proton in both the SAP and TSAP isomers (Supporting Information Figure S4). We have previously noted that the presence of an NB substituent causes distortion of the ethylene bridge on which it is located.[11,24] This distortion alters the torsion angle between vicinal axial and equatorial protons, and on the basis of the Karplus relationship,[25] distortion is therefore apparent from differences in the magnitude of vicinal couplings in the COSY data. It is more commonly found that this torsion angle is increased by substitution,[11,24] although it can decrease.[24] The complexity of the COSY spectrum of the side isomer of EuNB-DOTA renders a careful analysis of this phenomenon almost impossible. In the COSY spectrum of the corner isomer the ax^S−eq^C cross-peak is absent, again frustrating attempts to probe this distortion. Although there is no reason to suppose that a similar distortion of the substituted ethylene bridge does not occur in this case, there is no definitive evidence for it or indication of which direction it is distorted.

Exchange Spectroscopy of LnNB-DOTA Chelates. The conclusion of our previous exchange spectroscopy study of a crude sample of EuNB-DOTA was correct.[7] The ring inversion motion of LnDOTA-type chelates is "frozen out" by substitution of the macrocycle. This is supported by the data acquired in this work: the EXSY spectra of both corner and side isomers of EuNB-DOTA (Supporting Information Figures S3 and S5) show cross-peaks corresponding to $ax^S{}_{SAP}$−$ax^S{}_{TSAP}$ exchange. This is consistent with interconversion through pendant arm rotation; the positions of protons on the macrocyclic ring are unaffected by this exchange process. Cross-peaks corresponding to ring inversion ($ax^S{}_{SAP}$−$eq^C{}_{TSAP}$ or $ax^S{}_{TSAP}$−$eq^C{}_{SAP}$) are absent in both EXSY spectra, indicating that interconversion does not occur through ring inversion.

One further aspect of the NMR exchange data deserves attention. In all NB-DOTA-type chelates studied to date, the two benzylic protons have different chemical shifts and hence must be experiencing quite different chemical environments.[11,12,18,24,26,27] The shift patterns differ between the corner and side isomers. In the corner isomer, one benzylic proton appears to be largely unaffected by the proximity of a paramagnetic ion; the other is substantially shifted downfield.

In the side isomer, both benzylic protons experience hyperfine shifts; one downfield, but the other one significantly upfield. These shift patterns are evident in the data for EuNB-DOTA presented in Figure 6. The shifts of these benzylic protons can be interpreted on the basis of dipolar contributions alone, i.e., the spatial location of the benzylic protons relative to the paramagnetic ion. The shift patterns are consistent with the different orientations of the NB determined for the corner and side isomers in EuNB-DOTMA.[11,15] Given the shift differences between the two benzylic protons it is clear that rotation about the macrocycle-benzyl bond is not fast on the NMR time-scale. This strongly suggests that for chelates of NB-DOTA the orientation of the substituent of the macrocyclic ring is relatively inflexible and different in each regioisomer of the chelate.

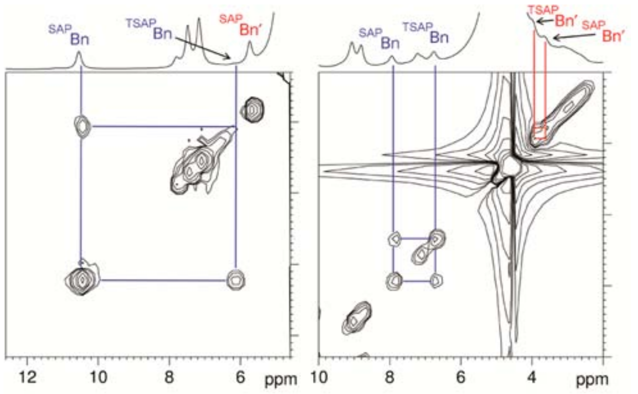

Figure 6. ^{1}H EXSY spectra of the corner (left) and side (right) isomers of EuNB-DOTA, focusing on the exchange of the benzylic protons. Spectra were recorded in D_2O, at 400 MHz and 283 K.

Since there is no time-averaging of the two benzylic proton signals, this raises the question of whether the chelate is able to rotate about this bond at all, even though it is only a single bond. Examining the EXSY spectra of both regioisomers of EuNB-DOTA (Figure 6) reveals cross-peaks corresponding to SAP/TSAP exchange for each benzylic proton, but the position of each benzylic proton (*Bn* or *Bn′*) is always conserved. There is no evidence that the two benzylic positions in the same isomer can exchange, i.e., no rotation. Cognizant of the fact that the absence of an EXSY cross-peak is not evidence of the absence of exchange we examined the shifts of the two benzylic positions of the SAP-corner isomer of EuNB-DOTA as a function of temperature (Figure 7). The two resonances exhibit very different behaviors. The resonance designated "*Bn*" broadens rapidly and shifts to higher field as the temperature rises from 278 to 328 K. This appears to be the result of SAP/TSAP exchange between the two benzylic positions; the ^{TSAP}Bn resonance can be identified in Figure 6 at about 6.2 ppm at 278 K. At 328 K these two resonances appear to be close to coalescence. In contrast the "*Bn′*" resonance moves a barely perceptible amount across this temperature range. The line-width also hardly increases. This resonance appears to exchange only with the $^{TSAP}Bn'$ resonance (which has an almost identical shift) but is not close to coalescence with the ^{SAP}Bn resonance. These observations also indicate that rotation about the macrocycle–benzylic bond is very slow, even at 328 K, and on the basis of the shift difference the rate constant is even less than 1×10^{-3} s^{-1}. This has implications for the incorporation of Gd^{3+} chelates into molecular imaging agents using this type of BFC. The maximum rate of rotation about this bond (the first linkage between chelate and targeting group) is more than 5 orders of magnitude slower than would be necessary to have a modulating effect on relaxivity.[28,29] There appears to be a high degree of rigidity in the orientation of and rotation about the macrocycle–benzyl linker between this type of BFC derived Gd^{3+}-based MRI contrast agent and the remainder of the conjugate.

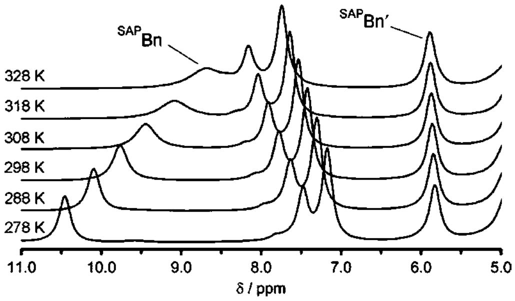

Figure 7. Benzylic and aromatic region of the ^{1}H NMR spectra of the corner isomer of EuNB-DOTA, recorded at 400 MHz in D_2O at temperatures from 278 to 328 K in 10 K increments.

■ CONCLUSIONS

Like its more rigid analogues, NB-DOTA does produce two regioisomeric chelates during introduction of the metal ion into the ligand. Since the conventional use of BFCs involves tagging biomolecules of interest with the BFC prior to introduction of the metal ion, this observation has implications for the use of IB-DOTA as a BFC. It is clear now that this more conventional approach will produce a distribution of products derived from the corner and side isomers of the BFC. These two regioisomers have different physicochemical properties, and from the point of view of developing molecular imaging probes for MRI, these differences seem likely to render one regioisomer more favorable than the other.[23]

There remain several questions that should be addressed. For instance, is it possible to favor the production of one isomer over the other? From this limited data set it appears that the size of the Ln^{3+} ion has relatively little influence over the regioisomeric distribution. However, previous studies have shown that conditions in the chelation reaction, such as temperature, the counterion, and pH, can influence the amount of each isomer produced.[12,14] Second, it is important to know whether both chelates are equally stable and robust to dissociation. We have previously calculated that the side isomer is higher in energy than the corner isomer.[12] In light of the relatively rapid rate of re-equilibration through metal release and recombination, properly characterizing the rates of metal ion release of each regioisomer is critical. Additionally, the question of whether the corner or side isomer represents the more effective component of a molecular imaging probe for MRI is worthy of greater attention. Conventional wisdom has always suggested that the chelate with the faster water exchange will tend to afford the more effective contrast agent. However, recent results in our laboratory on structurally related systems have suggested that optimal water exchange is actually significantly slower than previously appreciated.[23,30] On this basis it may be that the corner isomer (the more slowly exchanging isomer) functions more effectively as a component in an MRI probe.

Despite the production of two regioisomeric chelates during introduction of the metal ion into macrocycle substituted BFCs these ligands may offer one particular advantage in the development of new MRI contrast media. There is distinctly less freedom of rotation about the linkage between the DOTA chelate and the substituent of the macrocycle than inspection of the structure might suggest. In fact, rotation of the chelate about the macrocycle−benzylic bond appears to be so slow that it is unable to modulate (or limit) the relaxivity of a contrast agent derived from this type of BFC.

■ EXPERIMENTAL PROCEDURES

General Remarks. All solvents and reagents were purchased from commercial sources and used as received. NB-DOTA was purchased from Macrocyclics. The BFC was purified by preparative RP-HPLC on a Waters 2545 quaternary gradient module equipped with a Phenomenex Luna C18(2) 250 × 50 mm column at a flow rate of 50 mL min^{-1}. Detection was by absorption, monitored at 270 nm on a Waters 2489 UV/visible detector. For all solvent systems H_2O contained 0.037% HCl. The ligand was purified by eluting with 80% H_2O/20% MeCN for 3 min followed by a linear gradient over 10 min to 77% H_2O/23% MeCN. The BFC was obtained as a colorless solid (HPLC R_T = 9.3 min). One- and two-dimensional ^1H NMR spectra were recorded on a Bruker Avance IIa spectrometer operating at 400.13 MHz using a 5 mm broadband probe. The temperature was controlled using the installed variable temperature controller model 2416 with BCU-05 chiller. COSY corner and side spectra were acquired using 4096 × 1024 points and 32 (corner) and 64 (side) transients per FID. EXSY spectra were acquired using 4096 × 512 points and 128 transients per FID; the mixing time was 25 ms. The conversion of the corner isomer of YbNB-DOTA to an equilibrium distribution of regioisomers was followed at 600.13 MHz on a Bruker Avance III spectrometer. The sample was sealed in a 5 mm NMR tube at pD 1 and heated to 363 K. The sample was cooled to ambient temperature prior to acquisition of NMR data. The temperature was controlled at 298 K with the installed variable temperature controller model 2416 with BCU-05 chiller. Mass spectra were recorded on an electrospray (30000) LTQ-Orbitrap mass spectrometer (Thermo Scientific LTQ Orbitrap XL).

The Eu^{3+} and Yb^{3+} chelates of NB-DOTA were prepared from the purified ligand and respective trichlorides using the procedure described previously.[7] The resulting chelates were purified by preparative RP-HPLC eluting with 78% H_2O/22% MeCN for 3 min followed by a linear gradient over 10 min to 70% H_2O/30% MeCN.

EuNB-DOTA. Corner: HPLC R_T = 9.43 min; m/z (ESI-): 688.11 (100%, $[M]^-$). Side: HPLC R_T = 12.13 min; m/z (ESI-): 688.11 (100%, $[M]^-$). Appropriate isotope patterns were observed in each case.

YbNB-DOTA. Corner: HPLC R_T = 9.80 min; m/z (ESI-): 710.13 (100%, $[M]^-$). Side: HPLC R_T = 12.05 min; m/z (ESI-): 710.13 (100%, $[M]^-$). Appropriate isotope patterns were observed in each case.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional 1-D NMR spectra; ^1H EXSY spectra of both the corner and side isomers of EuNB-DOTA; $^1H-^1H$ COSY spectrum of the side isomer of EuNB-DOTA; and assignment of the spectra of both SAP and TSAP isomers of the side isomer of EuNB-DOTA. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

DOTA, 1,4,7,10-Tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-1,4,7,10-tetraacetate; NB-DOTA, *S*-2-(*p*-Nitrobenzyl)-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-1,4,7,10-tetraacetate; IB-DOTA, *S*-2-(*p*-Isothiocyanatobenzyl)-1,4,7,10-tetraazacyclododecane-*N*,*N*′,*N*″,*N*‴-1,4,7,10-tetraacetate

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