Synthesis of Water-soluble Chiral DOTA Lanthanide Complexes with Predominantly Twisted Square Antiprism Isomers & CPL Emission

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Supporting Information Placeholder

ABSTRACT: One-step cyclization of a tetraazamacrocycle **5** with 70% yield in a 25-gram scale was performed. Its chiral DOTA derivative has around 93% of TSAP coordination isomer in its Eu(III) and Yb(III) complexes in aqueous solution. **[GdL4]**$^{5-}$ exhibits a very high relaxivity, making it a promising and efficient MRI contrast agent. Very high g_{lum} values of 0.297 (ΔJ = 1) for **[TbL3]**$^{-}$ in DMSO and 0.241 for **[TbL4]**$^{5-}$ in buffer solutions were recorded.

The scaffold of 1,4,7,10-tetraazacyclododecane-N,N',N",N"'-tetraacetic acid (DOTA) is one of the most vastly studied macrocyclic ligands in coordination chemistry due to its favorable chelating effect that form varies of very stable complexes. Numerous kinds of metal-DOTA complexes are known to exhibit excellent stability under physiological conditions hence making it dominant in the field of study for biomedical applications.[1] A notable example is the use of [GdDOTA]$^{-}$ as a clinical contrast agent in magnetic resonance imaging (MRI).[2] DOTA derivatives are also used as chelators of radiometals and trivalent lanthanide ions (Ln) for diagnosis and therapy applications, such as the theranostic pair ^{68}Ga/^{177}Lu-labeled DOTA-TATE, which were approved by U.S. Food and Drug Administration (FDA).[3] Recently, in one of our studies we shown that by introducing chiral groups into the DOTA, this has tremendously enhanced the stability. This has drawn increasing attention to the properties of chiral groups which can be engineered on the carbons of the macrocyclic ring or on the pendant side arms.[5] Our former study with the design of chiral DOTAs where four chiral substituents are artfully situated around the macrocyclic ring, shows that these chelators possess excellent properties that surpass their parent DOTA chelator significantly.[4] Interestingly, these chiral groups also could control the coordination geometry to form only two non-interconvertible isomers. This is important as obtaining pure stereoisomers of chiral luminescent complexes are crucial for circularly polarized luminescence (CPL) applications and ideal for efficient agents as protein tags for NMR studies.[6-8] Regarding T_1-shortening contrast agents for MRI, the conformation of the lanthanide complexes also plays a significant role in the water exchange rate k_{ex} ($k_{ex} = 1/\tau_M$).[9] It has been shown that the k_{ex} in the TSAP (twisted square antiprismatic) configuration is 10 – 100 times faster than SAP (square antiprism), which is useful as T_1-shortening contrast agents.[10] However, the synthetic efficiency of these chiral DOTAs is still very low, and the chiral DOTA complexes with four benzyl groups with predominantly TSAP isomers are not water-soluble, limiting the scope of their bio-applications. Herein, we present our new generation of chiral DOTA complexes with phenyl substituents to improve the water-solubility (Figure 1); the coordination geometry, relaxation behavior, as well as the CPL properties have been studied.

The ligand **L1** and its complexes were synthesized according to our previous report.[4] Based on **L1**, we introduced a hydrophilic amino group on each of the phenyl rings to get **L2**. As shown in Scheme 1, the twelve-membered **2** was synthesized from the aziridine compound **1** (see Scheme S1 in Supporting Information) through a cyclization reaction. We have tried two methods which were optimized in our previous study:[4] Method A uses benzene as solvent and boron trifluoride diethyl etherate as catalyst, reacting at 80 °C for 16 h; Method B uses acetonitrile as solvent and *p*-toluenesulfonic acid monohydrate as catalyst, reacting at ambient temperature for 6 days. Although both conditions could get reasonable yields, the work-up for the first method is more complicated and benzene, a highly toxic solvent, is needed, so we chose the second method in scaling up our reactions. Then the four nitro groups were reduced to amino groups by zinc powder in acetic acid, and subsequently protected by acetic anhydride. The four

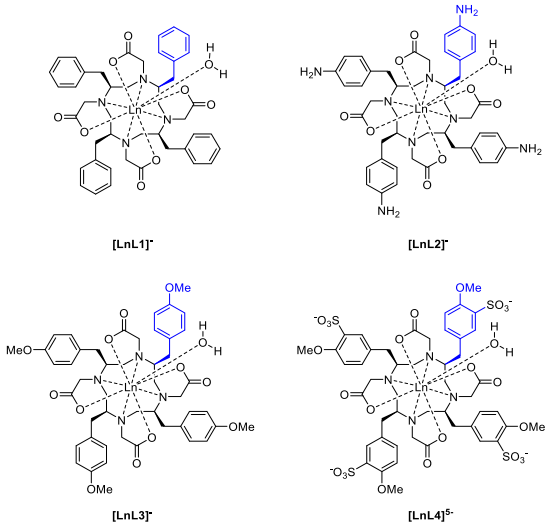
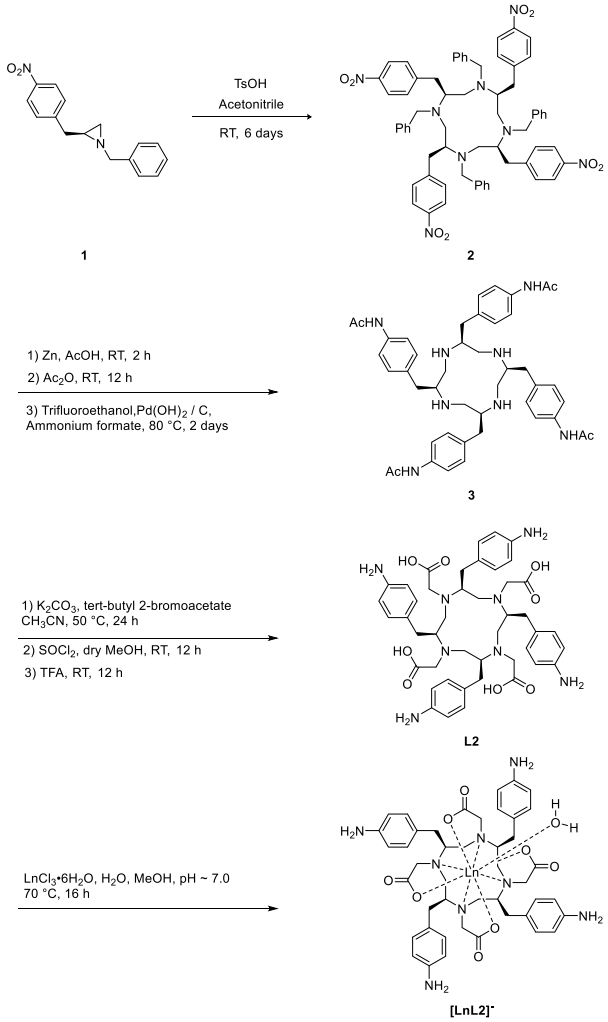


Figure 1. The structures of synthesized lanthanide chiral DOTA complexes ([**LnL1**]$^-$ – [**LnL4**]$^{5-}$). Ln represents Eu(III), Tb(III), Yb(III) and Gd(III). Note for clarity the counterions are not depicted

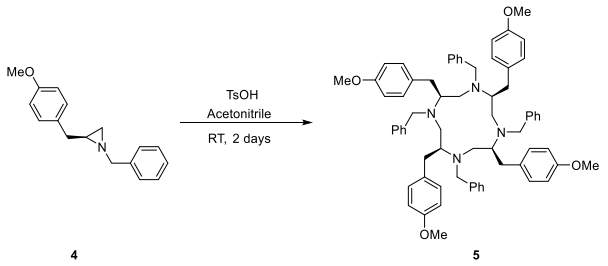
benzyl protecting groups were then deprotected by palladium hydroxide on carbon and ammonium formate, trifluoroethanol was used as solvent as we found this solvent is more efficient than the other alcohol solvents (such as methanol and ethanol), and also because the compound has better solubility. This resulted in the chiral cyclen (1,4,7,10-tetraazacyclododecane) compound **3**. **3** was reacted with tert-butyl 2-bromoacetate in the presence of potassium carbonate to get the fully protected DOTA compound, and acetate groups were then deprotected under an anhydrous condition [11] which gave **L2** after deprotection with TFA. Complexations were performed under neutral conditions. However, despite having a better solubility than [**GdL1**]$^-$, [**GdL2**]$^-$ is not completely water-soluble (< 0.1 mM).

Although the four amino groups could be used to conjugate with even higher hydrophilic compounds to improve the water solubility, the ratio of TSAP/SAP in the complexes of [**EuL2**]$^-$ and [**YbL2**]$^-$ decreased drastically compared to the complexes of **L1** (*vide infra*); this phenomenon was also observed in similar systems with amino groups,[4] so we changed our design to the compound with methoxyl groups on the *para*-position of the phenyl rings. These electron donor groups make it possible to perform sulfonylation reactions at their nearby positions, and sulfonylation is one of the best ways to improve a compound's water solubility,[12] which is shown in ascending order [**GdL1**]$^-$ ~ [**GdL3**]$^-$ < [**GdL2**]$^-$ < [**GdL4**]$^{5-}$ (see Supporting Information). To

Scheme 1. Synthesis of [LnL2]$^-$. Ln represents Eu(III), Yb(III) and Gd(III).



Scheme 2. Synthesis of compound 5.



test the effect of the methoxy groups on the para-position of the phenyl groups on ratios of TSAP/SAP, **L3** and its complexes were synthesized as shown in Scheme S3. It should be noted that the chiral cyclen compound **5** was published as a total synthesis.[13] Similar to our **L1** and its complexes, the single crystal structure showed that the four methoxybenzyl groups on the macrocyclic ring were located on one side of the ring, and the other four benzyl groups on the nitrogen positions are located on the other side of the ring.[13] To our interest, the cyclization reaction of this compound gave nearly quantitative conversion and (as shown in Scheme 2), as monitored by TLC, the reaction was almost finished after stirring at ambient temperature for 2 days with no obvious byproduct observed. The work-up was also very simple: 2% $NaHCO_3$ was poured into the reaction mixture to quench the reaction and after stirring

for 20 minutes, a simple filtration was performed and the resulting white solid was dried to give the product in a yield of 70% (filtrate unrecycled). The high yield was maintained after scaling up with four batches of 12.5 grams and one batch of 25 grams of **4**. This demonstrated the feasibility of large-scale production of this compound, and this is the first time a chiral cyclen compound is obtained in such a high yield and with a simple synthesis.

The sulfonation reaction was also unexpectedly smooth (as shown in Scheme 3). **5** was dissolved in dry dichloromethane and chlorosulfonic acid was dropped into the reaction mixture at 0 – 10 °C. After stirring for 12 hours at ambient temperature - the reaction was monitored by mass spectrometry, only the signal of the product and its hydrolyzed products were detected. After successful reaction of the first two steps, the latter steps were performed similarly to the synthesis of **L1** and its complexes.[4]

The TSAP/SAP isomers ratios of the synthesized Eu(III) and Yb(III) complexes were determined by ^{1}H NMR. As the two sets of protons in the two geometric isomers could be easily identified on the ^{1}H NMR spectrum.[10] As shown in Figure 2, the TSAP/SAP

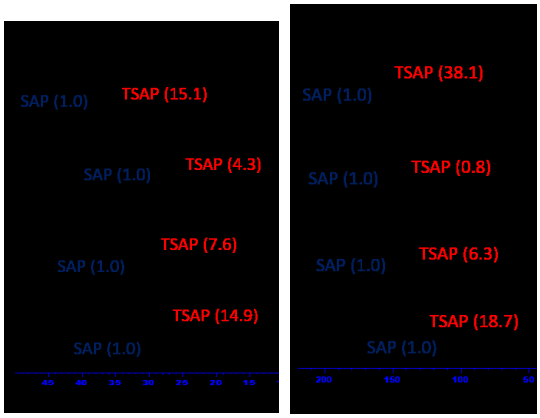
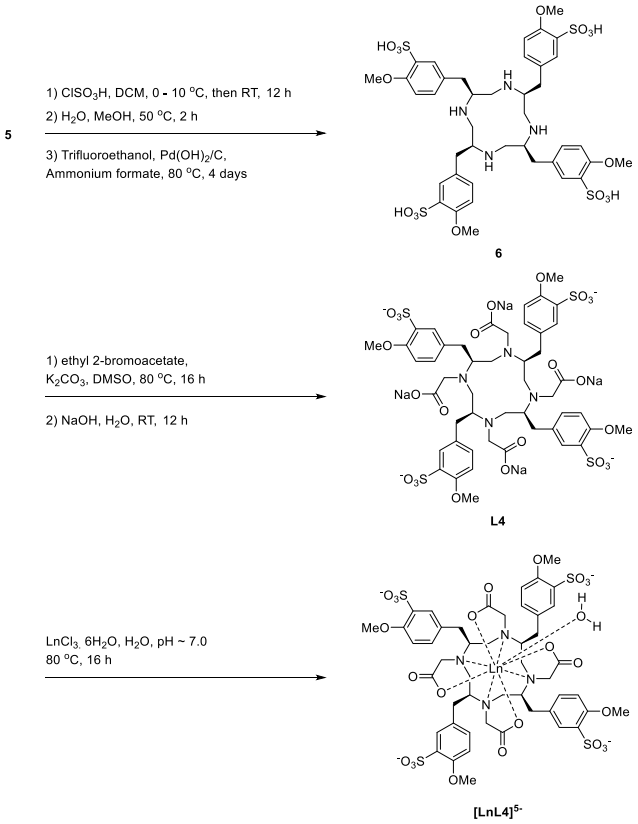


Figure 2. ^{1}H NMR spectra (25 °C, 400 MHz, pD 7.0) of **[EuL1]**$^-$ - **[EuL4]**$^{5-}$ (a) and **[YbL1]**$^-$ - **[YbL4]**$^{5-}$ (b) showing the variation in the TSAP/SAP isomer ratios. (**[LnL4]**$^{5-}$ in D_2O, the others are in mixture of d^6-DMSO-D_2O (~1:1) because of solubility issues).

Scheme 3. Synthesis of [LnL4]$^{5-}$. Ln represents Eu(III), Tb(III), Yb(III) and Gd(III).



isomers ratio in **[EuL1]**$^-$ is 15.1, this ratio decreased to 4.3 in **[EuL2]**$^-$, this is because there are four amino groups on the *para*-position of the phenyl groups, we have found the hydrogen bond of the amino groups could affect the coordination geometry of the lanthanide complexes.[4] While the ratio increased to 7.6 for **[EuL3]**$^-$, to our expected, the ratio went back to 14.9 for the complex of **[EuL4]**$^{5-}$. The TSAP/SAP ratios change tendency of 38.1 – 0.8 – 6.3 – 18.7 in the system of Yb(III) complexes also very similar to the Eu(III) complexes. As the ionic radius of Gd(III) is between Eu(III) and Yb(III), we could expect the abundance of TSAP isomer in **[GdL4]**$^{5-}$ would be around 95%. Such a high abundance of TSAP geometry in Gd(III) complex is ideally for MRI contrast agents.

Gd(III) complexes are mostly used as T_1-shortening MRI contrast agents, and their efficiencies are commonly evaluated in terms of longitudinal relaxivity (r_1), which is the enhancement of the water proton relaxation rate (T_1^{-1}) in solutions containing 1 mM of the paramagnetic solute.[14] The relaxivity of **[GdL4]**$^{5-}$ was compared against the commercial available [GdDOTA]$^-$ (Dotarem) as shown in Figure S1. The relaxivity of **[GdL4]**$^{5-}$ is 6.8 mM^{-1}s^{-1}, which is higher than two-times of [GdDOTA]$^-$ (3.2 mM^{-1}s^{-1}) under the same conditions (1.5 T, 37 °C). This means **[GdL4]**$^{5-}$ is more efficient than [GdDOTA]$^-$ as MRI contrast agent.

Luminescent chiral lanthanide complexes are also capable of giving off circularly polarized luminescence (CPL). Lanthanide cations with a spherical nature, tend to avoid the problem of anisotropy, and if judiciously designed, can afford high luminescence dissymmetry factor (g_{lum}), which is defined as $g_{lum} = 2(I_L - I_R)/(I_L + I_R)$) ($I_L$ and I_R are the emission intensity of left- and right-handed circularly polarized light, respectively).[15] Typical g_{lum} values of organic compounds are in the 10^{-4} – 10^{-3} range, while g_{lum} for chiral lanthanide complexes

can be as high as over 0.1.[15] As mentioned above, the complexes of **[LnL3]**$^{-}$ and **[LnL4]**$^{5-}$

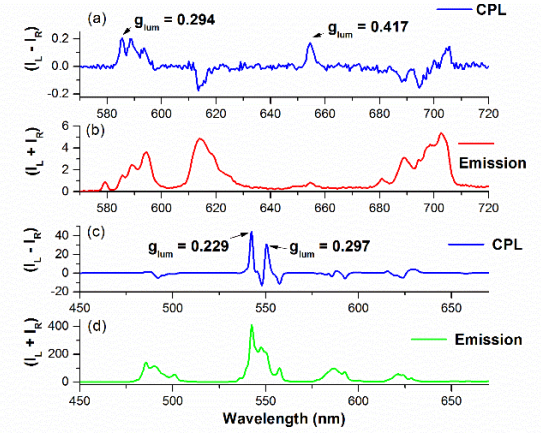


Figure 3. Total emission and CPL spectra of **[EuL3]**$^{-}$ and **[TbL3]**$^{-}$ in DMSO, λ_{ex} = 285 nm. (a) CPL spectrum of **[EuL3]**$^{-}$; (b) Total emission spectrum of **[EuL3]**$^{-}$; (c) CPL spectrum of **[TbL3]**$^{-}$; (b) Total emission spectrum of **[TbL3]**$^{-}$.

exist as predominantly TSAP isomers, making them promise for CPL studies. Tb(III) and Eu(III) complexes were selected for synthesis due to their favorable characteristic and sensitive luminescent profile and long-lived lifetimes in the visible region.[16] Figure 3 shows the total emission and CPL spectra of **[EuL3]**$^{-}$ and **[TbL3]**$^{-}$ in DMSO. It is well-known that the magnetic-dipole allowed f-f transition gives higher CPL intensity. The g_{lum} value of the magnetic-dipole allowed transition of **[EuL3]**$^{-}$ ($^5D_0 \rightarrow {}^7F_1$) was 0.294 (585 nm); this is one of the highest for Eu(III) complexes with macrocyclic chelators. The CPL intensity of Tb complex is much higher than the Eu(III) complex and high g_{lum} values were measured at 542 nm (g_{lum} = 0.229) and 551 nm (g_{lum} = 0.297), both of these two peaks corresponding to the magnetic-dipole allowed $^5D_4 \rightarrow {}^7F_5$ transition, the value of 0.297 is amongst the highest g_{lum} values recorded thus far for Tb(III) complexes.[17] Figure 4 shows the total emission and CPL spectra of **[TbL4]**$^{5-}$ in HEPES buffer (0.1 M, pH 7.4) under the excitation of 280 nm. Comparing the CPL spectrum of **[TbL3]**$^{-}$ in DMSO, the shape of CPL spectrum of **[TbL4]**$^{5-}$ in the aqueous solution is with a certain degree of deviation. However, the very high g_{lum} values were maintained in this complex. The highest g_{lum} was observed at the highest CPL intensity (0.241 at $^5D_4 \rightarrow {}^7F_5$, 542.5 nm). It needs to be noted that CPL of Tb(III) complexes are even more promising than the Eu(III) complexes because while the $^5D_0 \rightarrow {}^7F_1$ transition of Eu(III) complexes often has the highest g_{lum} values, this transition always has very low emission and CPL intensity.

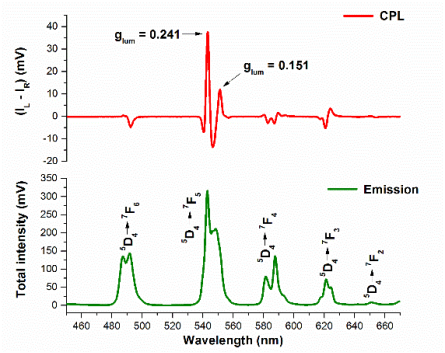


Figure 4. Total emission (down) and CPL (upper) spectra of **[TbL4]**$^{5-}$ in 0.1 M of Hepes buffer, pH 7.4, λ_{ex} = 285 nm.

In conclusion, we have described an efficient strategy for the synthesis of water-soluble lanthanide chiral DOTA complexes with very high twisted square antiprismatic (TSAP) coordination geometry. The key intermediate of chiral cyclen **5** with four 4-methoxybenzyl groups was synthesized with high yields in the scale of dozens of grams, and this compound could be easily functionalized to create variations of DOTA chelates. Complexes **[LnL4]**$^{5-}$ have very good water-solubility and exist up to 95% of the TSAP isomer. **[GdL4]**$^{5-}$ shows very high relaxivity at 1.5 T, 37 °C, while g_{lum} values of **[TbL3]**$^{-}$ and **[TbL4]**$^{5-}$ are amongst the highest g_{lum} values of chiral Tb(III) complexes, making them promising for a diverse range of applications such as MRI contrast agents or for CPL applications. Further biological studies and applications of these compounds are currently in progress in our laboratory.

ASSOCIATED CONTENT

Supporting Information

This material is available free of charge via the Internet at http://pubs.acs.org.
Experimental procedures, full characterization of products, and NMR spectra (PDF)

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Author Contributions

G.-L.L. conceived and supervised the project. All authors have given approval to the final version of the manuscript.

Notes

Any additional relevant notes should be placed here.

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