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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 derivatives, **L4**, has around 93% of TSAP coordination isomer in its Eu(III) and Yb(III) complexes in aqueous solution. **[GdL4]$^{5-}$** exhibits a high relaxivity, making it a promising and efficient MRI contrast agent. High g_{lum} values of 0.285 ($\Delta J = 1$) for **[TbL3]$^{-}$** and 0.241 ($\Delta J = 1$) 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. Hence numerous kinds of metal-DOTA complexes are known to exhibit excellent stability under physiological conditions, hence dominating 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 showed that by introducing chiral groups into DOTA, the stability of the complexes was tremendously enhanced.[4] 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] Interestingly, our chiral DOTAs also could control the coordination geometry such that only two non-interconvertible isomers can be formed. This is important as obtaining pure stereoisomers of chiral luminescent complexes is crucial for circularly polarized luminescence (CPL) applications and ideal for use 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, this limits the scope of their bio-applications. Herein, we present a new generation of chiral DOTA complexes (Figure 1) with phenyl substituents aiming to improve their water-solubility; their coordination geometry, relaxation behavior, as well as the CPL properties were 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** through a cyclization reaction (see Scheme S1). We have two methods which were optimized in our previous study:[4] Method A uses benzene as solvent and boron trifluoride diethyl etherate as catalyst, and the reaction takes place at 80 °C for 16 h; method B uses acetonitrile as solvent and *p*-toluenesulfonic acid monohydrate as catalyst, and the reaction takes place 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. The four nitro groups were then reduced to amino groups by zinc powder in acetic acid, and subsequently protected by acetic anhydride. The four benzyl protecting groups were then deprotected by palladium hydroxide on carbon and ammonium formate with trifluoroethanol used as solvent as we found this solvent is more efficient than the other alcohol solvents (such as methanol and ethanol), and the compound has better solubility in it.

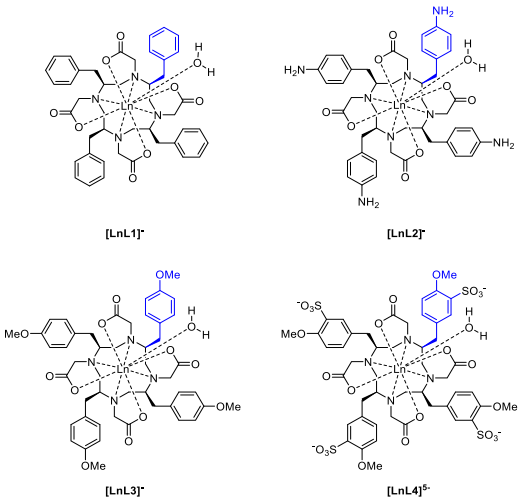
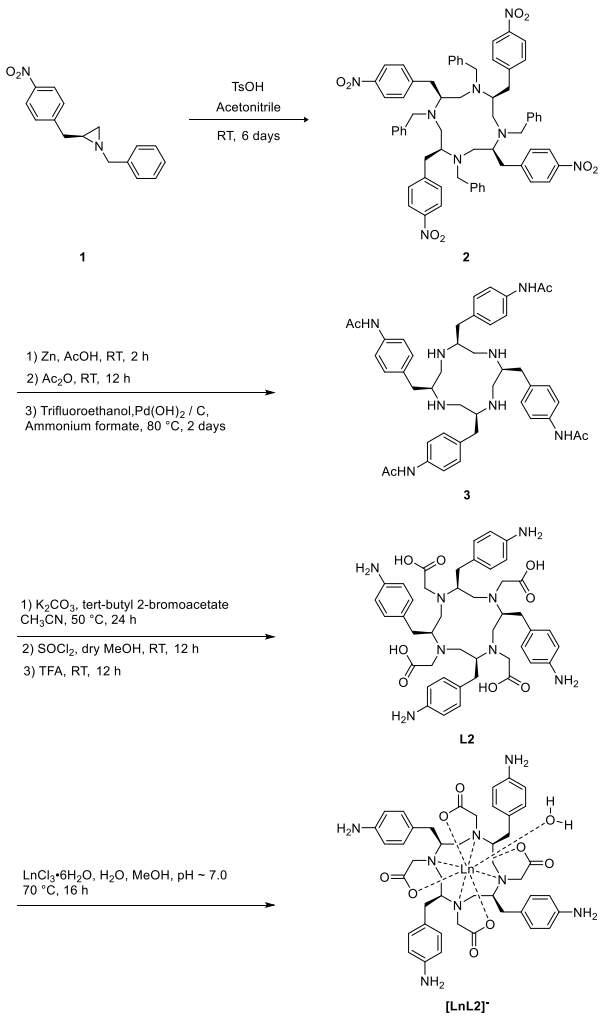


Figure 1. The structures of synthesized lanthanide chiral DOTA complexes (**[LnL1]$^{-}$** – **[LnL4]$^{5-}$**). Ln represents Eu(III), Tb(III), Yb(III) and Gd(III). Note for clarity the counterions are not depicted

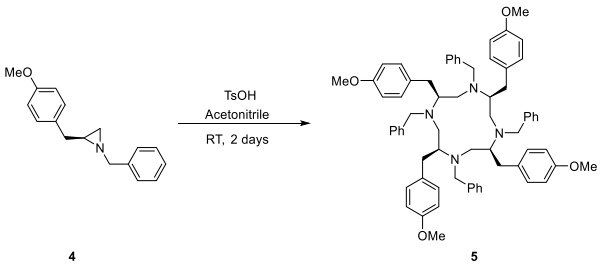
This resulted in the chiral cyclen (1,4,7,10-tetraazacyclododecane) compound **3**. **3** was reacted with tert-butyl 2-bromoacetate in the presence of potassium carbonate to get the fully protected DOTA compound, and acetate groups were then deprotected under an anhydrous condition [11] which gave **L2** after deprotection by TFA. Complexations were performed under neutral conditions. 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 methoxyl groups on the *para*-position of the phenyl rings. These electron donating groups make it possible to perform sulfonylation reactions at their nearby positions, as sulfonylation is one of the best ways to improve a compound's water solubility.[12] After sulfonylation, the complexes exhibit the following solubility in ascending order **[GdL1]**$^-$ ~ **[GdL3]**$^-$ < **[GdL2]**$^-$ < **[GdL4]**$^{5-}$ (see Supporting Information). To test the effect of methoxy groups on the *para*-position of the phenyl groups on TSAP/SAP ratio, **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 **L1** and its complexes, the single crystal structure of compound 5 showed that the four methoxybenzyl groups on the macrocyclic ring were located on one side of the ring, and the other four

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



Scheme 2. Synthesis of compound 5.



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 (Scheme 2) and, 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 with 70% yield (filtrate unrecycled). The high yield was maintained after scaling up to 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 reactions of the first two steps, the latter steps were performed similarly to the synthesis of **L1** and its complexes.[4]

The TSAP/SAP isomer ratios of the synthesized Eu(III) and Yb(III) complexes were determined by ^{1}H NMR as the two sets of protons of two geometric isomers could be easily identified on the ^{1}H NMR spectra.[10] As shown in Figure 2, the TSAP/SAP isomer ratio in **[EuL1]**$^-$ is 15.1. This ratio decreased to 4.3 in **[EuL2]**$^-$ because there are four amino groups on the *para*-position of the phenyl groups. We have found that the hydrogen bonds on the amino groups could affect the coordination geometry of the lanthanide complexes.[4] While the ratio increased to 7.6 for **[EuL3]**$^-$, to our expectation, the ratio went back to 14.9 for **[EuL4]**$^{5-}$. The TSAP/SAP ratios range from 38.1 – 0.8 – 6.3 – 18.7 in Yb(III) complexes, the

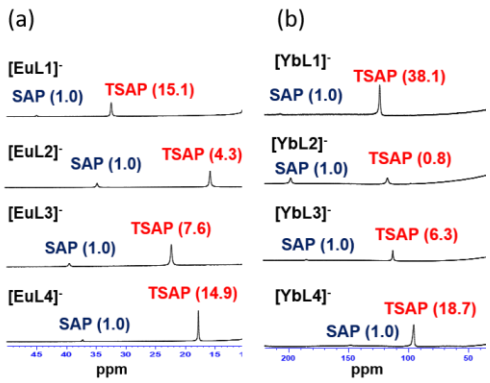
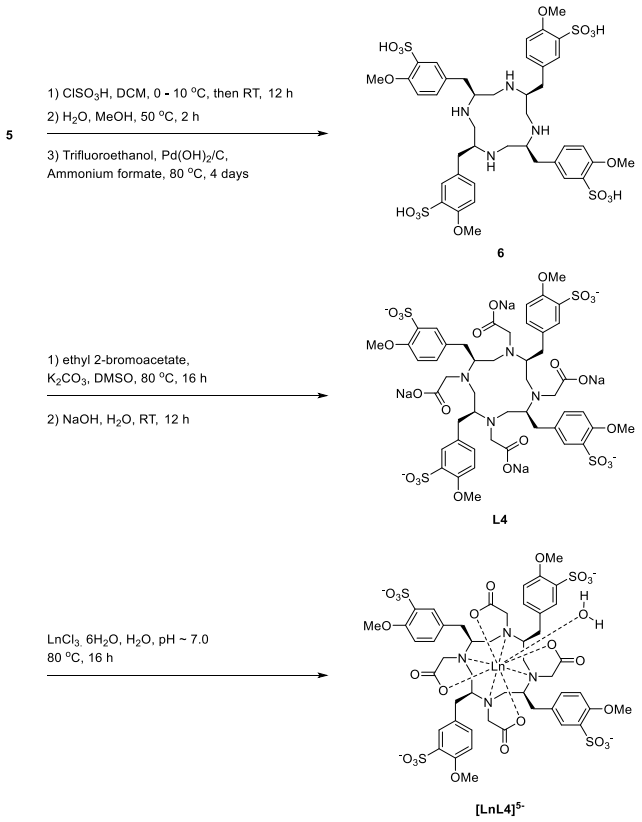


Figure 2. ^{1}H NMR spectra (25 °C, 400 MHz, pD 7.0) of **[EuL1]**$^-$ - **[EuL4]**$^{5-}$ (a) and **[YbL1]**$^-$ **- [YbL4]**$^{5-}$ (b) showing the variation in the TSAP/SAP isomer ratios. (**[LnL4]**$^{5-}$ in D_2O, the others are in mixture of d^6-DMSO-D_2O (~1:1) because of solubility issues).

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



trend is 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 93%. Such a high abundance of TSAP geometry in Gd(III) complex is ideal as 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) (Figure S1). The relaxivity of **[GdL4]$^{5-}$** is 6.8 mM^{-1}s^{-1}, 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 an MRI contrast agent.

Luminescent chiral lanthanide complexes are capable of emitting circularly polarized luminescence (CPL), especially from the magnetic-dipole allowed f-f transitions which give higher intensity.[6] Spherical lanthanide cations can 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). Typical g_{lum} values of organic compounds are in the $10^{-4} - 10^{-3}$ range, while those of chiral lanthanide complexes can be as high as over 0.1.[15] As mentioned above, the complexes of **[LnL3]$^-$** and **[LnL4]$^{5-}$** exist as predominantly TSAP isomers, making them promising for CPL studies. Pure TSAP isomers of **[EuL3]$^-$** and **[TbL3]$^-$** were obtained by reversed phase semi-preparative HPLC. Samples for photophysical measurements were prepared in 0.1 M HEPES with 5% DMSO due to solubility issues. CPL signals from **[EuL3]$^-$** could not be detected due to its very weak emission (Figure S13), a result of poor energy transfer due to the large energy gap between the

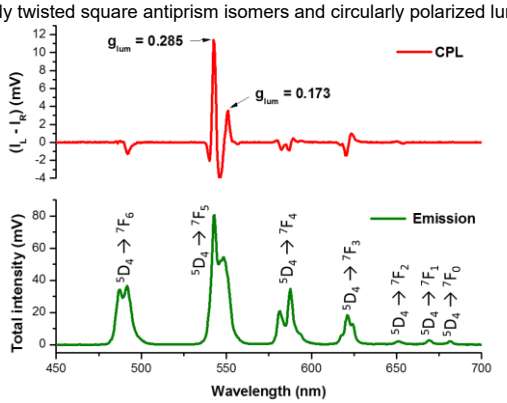


Figure 3. Total emission (lower) and CPL (upper) spectra of TSAP isomer of **[TbL3]$^-$** in 0.1 M of HEPES buffer (with ~5% of DMSO), pH 7.4, abs = 0.3, λ_{ex} = 280 nm.

chromophore's excited states and Eu(III) excited states. Alternatively, the emitting state of Tb(III) matches well with the chromophore's excited state, as for efficient energy transfer, the ideal energy gap is ~4000 ± 500cm $^{-1}$. (Figure S16). Figure 3 shows the total emission and CPL spectra of the TSAP isomer of **[TbL3]$^-$**. The g_{lum} values of the magnetic-dipole allowed transitions of **[TbL3]$^-$** ($^5D_4 \rightarrow {}^7F_5$, $\Delta J = 1$) were 0.285 (542.5 nm) and 0.173 (551 nm). **[TbL4]$^{5-}$**, with ~93% TSAP isomer, has g_{lum} values of 0.241 (542.5 nm) and 0.151 (551 nm. Nevertheless, the spectral characteristics of the emission and CPL spectra of **[TbL3]$^-$** and **[TbL4]$^{5-}$** are nearly identical (Figure 4). To the best of our knowledge, the g_{lum} values, 0.285 and 0.241, are amongst the highest for Tb(III) complexes.[16] Conversely, although higher g_{lum} values were reported for chiral Eu(III) complexes,[15,17,18] most of them are formed from multi-component ligands and their solubility and stability in aqueous solution are very poor; moreover, the pure magnetic-dipole nature of the $^5D_0 \rightarrow {}^7F_1$ transition of Eu(III) renders much weaker emission intensity. As a result, our Tb(III) complexes are impressively balanced for practical CPL applications.

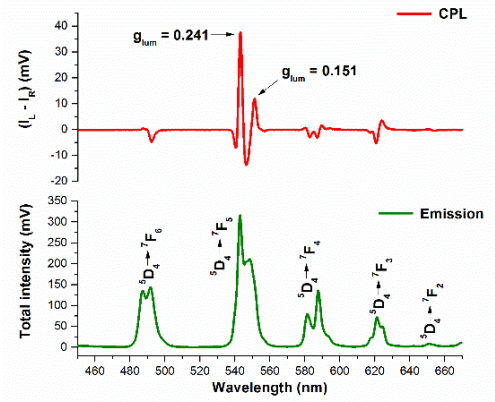


Figure 4. Total emission (down) and CPL (upper) spectra of **[TbL4]$^{5-}$** in 0.1 M of HEPES buffer, pH 7.4, abs = 0.3, λ_{ex} = 280 nm.

In conclusion, we have described an efficient strategy for the synthesis of water-soluble lanthanide chiral DOTA complexes with very high ratio of 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 as up to 93% of

the TSAP isomer. **[GdL4]$^{5-}$** shows very high relaxivity at 1.5 T, 37 ^{o}C, while g_{lum} values of **[TbL3]$^{-}$** and **[TbL4]$^{5-}$** are amongst the highest in chiral Tb(III) complexes, making them promising for a diverse range of applications such as MRI, sensing 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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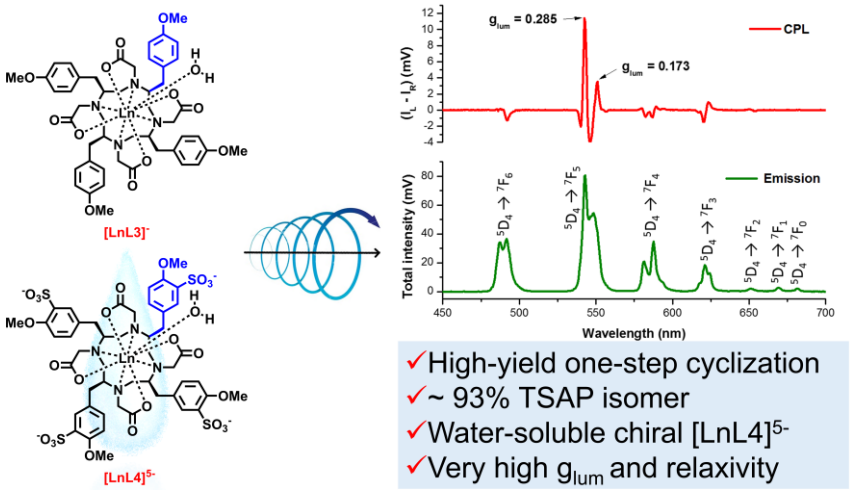

Exceptionally high yield (70 %) of a one-step cyclization of a chiral DOTA was achieved in a 25-g scale consisting of 93% TSAP co-ordination isomer in its lanthanide complex in aqueous solution; the [GdL4] compounds exhibits high relaxivity of 6.7 mM-1s-1, and the Tb analogues have very high glum value of 0.241, 0.285 atΔ J =1