Chiral Recognition

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Detection and Chiral Recognition of α-Hydroxyl Acid through ^{1}H and CEST NMR Spectroscopy Using a Ytterbium Macrocyclic Complex

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Abstract: *Chiral α-hydroxyl acids are of great importance in chemical synthesis. Current methods for recognizing their chirality by ^{1}H NMR are limited by their small chemical shift differences and intrinsic solubility problem in organic solvents. Herein, we developed three YbDO3A(ala)$_3$ derivatives to recognize four different commercially available chiral α-hydroxyl acids in aqueous solution through ^{1}H NMR and chemical exchange saturation transfer (CEST) spectroscopy. The shift difference between chiral α-hydroxyl acid observed by proton and CEST NMR ranged from 15–40 and 20–40 ppm, respectively. Our work demonstrates for first time, that even one chiral center on the side-arm chain of cyclen could set the stage for rotation of the other two non-chiral side chains into a preferred position. This is ascribed to the lower energy state of the structure. The results show that chiral YbDO3A-like complexes can be used to discriminate chiral α-hydroxyl acids with a distinct signal difference.*

The sensitivity of chiral compound recognition is a central theme in chiral sensing and detecting field.[1–3] In principle, among all the chiral-recognition analytical techniques, NMR spectroscopy is often used to discriminate enantiomers due to its simplicity and convenience.[4–7] As one of the most important chiral agents, α-hydroxyl acids are frequently used in natural product synthesis and are also involved in asymmetrical synthetic steps.[8–13] The present methods to recognize α-hydroxyl acids are mostly through fluorescence, proton NMR spectroscopy and circular dichroism using a chiral solvent agent and all of them are performed in organic solvents.[1,14] Until now, there is no report for α-hydroxyl acid sensing in aqueous solution. As a result, asymmetrical reactions that take place in H_2O will not be determined directly. Moreover, the sensitivity of ^{1}H NMR spectroscopy to discriminate those compounds is always less than 1 ppm, which makes it difficult to distinguish the isomers in a complicated system. Thus, the realization of detection of chiral molecules with significant signal differences in aqueous solution is an attractive goal.

Chemical exchange saturation transfer (CEST) is an imaging technique that has been used for tumor diagnosis in MRI.[15,16] Basically, any component with an exchangeable labile proton can generate a decreased water signal with proper saturation power and delay to show the CEST effect. Samuel et al. have used glucose as an internal CEST agent for imaging the metabolic stage of tumor tissue using labile protons present in glucose and several other small metabolic molecules have been studied extensively over the last 15 years.[17] Due to the lanthanide induced shift (LIS) effect, metal complexes containing a bound water molecule that coordinates to lanthanide ions would also be considered as CEST agents with the CEST signal further downfield-shifted than that of a normal agent.[18] Normally, a macrocyclic lanthanide complex has four different diastereoisomers in solution, which are caused by side-arm rotation and ring-flip effect. This is associated with a δδδδ or λλλλ conformation of ethyenediamine moieties in the tetraazamacrocyclic and the Λ or Δ raised from the orientation of the coordinating arm. Sherry and co-workers showed that YbDO3A(Gly)$_3$ could bind with chiral lactate to form two diastereomers, Λ(δδδδ) and Δ(λλλλ), which has been demonstrated to be present in solution by ^{1}H NMR spectroscopy and corresponding DFT calculations. However, a Yb complex containing one δ-position chiral center, such as YbDO3A(Ala)$_3$, exhibits high enantioselectivity by ^{1}H NMR spectroscopy and CEST upon binding with chiral lactic acid. In that case, only isomers with the structure of Λ(δδδδ) were detected by NMR spectroscopy. Several other similar chiral Yb complexes present the same behavior in terms of CEST recognition ability.[19–23] Although this is successful for lactic acid, the Yb complex bearing one or two chiral centers have not been studied and the recognition ability of those complexes toward α-hydroxyl acid is unclear.

To address those challenges, we have designed chemosensors with one, two, and three chiral centers at the δ position on the side-arm rings of the YbDO3A(Gly)$_3$ complexes. We hypothesize that the α-hydroxyl acid molecule binding will induce the formation of single isomeric com-

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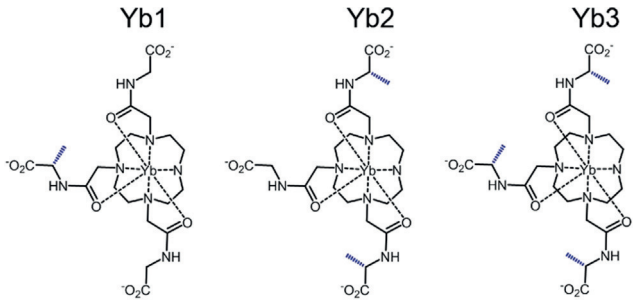
plexes thereby changing the chemical shift of the −OH proton coordinated with the lanthanide ion. Critical to the success of this method is the fact that the radius of the Yb ion is very small, allowing the α-hydroxyl acid to induce a tiny difference on its structure, thereby changing the chemical shifts on both −CH and −OH protons noticeably. The synthetic procedures are either given in Supporting Information or described previously.[19]

Herein, we examine the ^{1}H and CEST NMR properties of Yb complexes of ligands (Scheme 1) with one equivalent of



Scheme 1. Chemical structures discussed in this study.

commercially available α-hydroxyl acid (Table 1). The ligands were designed to have three glycinate side-arm chains bearing one, two, and three chiral centers on the δ-position to evaluate the impact of multiple chiral centers on the structural behavior upon binding with the guest molecules.

The ^{1}H NMR spectra of the all three complexes binding with different guest molecules are recorded under the same conditions (Figure 1). The highly shifted ^{1}H NMR signals observed for Yb^{3+} complexes are largely pseudo-contact in origin and produced large lanthanide induced shifts with negligible contact contributions. This makes the Yb ion the preferable lanthanide for structural elucidations of metal-complexes by NMR spectroscopy. Significant sharp peaks of single H_4 protons were detected across all proton spectra in

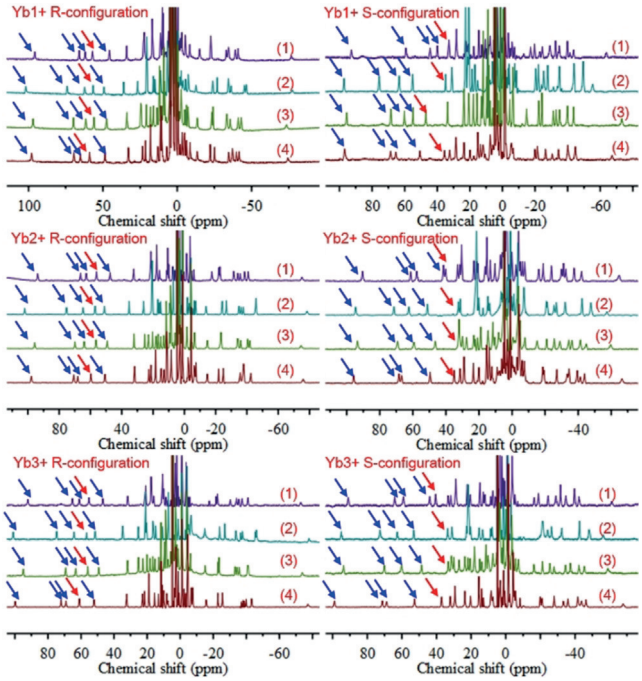
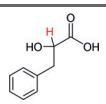
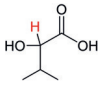
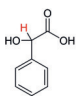


Figure 1. ^{1}H NMR spectra of Yb_x complexes containing 1:1 *R*-α-hydroxyl acids or 1:1 *S*-α-hydroxyl acids. All spectra were recorded at 25 °C in D_2O at 400 MHz. pD = 7.4. Blue arrows point to the H_4 proton resonances. Red arrows represent the −CH proton resonance. 1 mg of analyte was used for the experiments using a 500 MHz NMR spectrometer (the number of scans is 128).

the readily recognized Yb complex square antiprism (SAP) conformation, while the free complexes exhibit a blurry baseline (Figure S9). This further demonstrates that the interconversion of isomers in the free Yb macrocyclic complex is fast. It becomes much slower when α-hydroxyl acids are bound. One may conclude that the unusually sharp peaks present here (Figure 1) originate from the higher ligand field effect, which demonstrates the strong affinity of guest molecules towards di-aquo Yb macrocyclic complexes. The R/S molecule binds with complexes differently as the spectra present different chemical shifts of the cyclen H_4 and α-hydroxyl acid CH proton (Table 1). In all samples, resonance between the single alpha-hydroxyl acid and −CH group was observed. This further demonstrates that the host–guest complexes exist as a single isomer in solution. Considering the difference between the structure of Δ-(λλλλ) and Λ(δδδδ), the former isomer has a tendency to maintain the −COOH group in a pseudo-axial position while the latter leaves the $-CH_3$ group in the pseudo-axial position. The ^{1}H NMR results indicate that the

Table 1: Chemical shifts (in ppm) of the α-hydroxyl acid-OH and -CH proton resonances for each of the *R* or *S* isomer Yb_x complexes, CEST chemical shift at 298 K with $B_1 = 600$ Hz.

Chemical structure of α-hydroxyl acid		Yb1 (CEST)		Yb2 (CEST)		Yb3 (CEST)		Yb1 (−CH)		Yb2 (−CH)		Yb3 (−CH)	
		R	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*	*R*	*S*
1:		163	128	159	116	164	126	57.1	32.9	56.1	40.5	55.3	40.4
2:		183	151	184	147	169	144	55.4	34.9	57.0	32.5	56.7	33.4
3:		179	144	165	136	187	149	54.9	32.9	56.5	32.2	56.0	31.4
4:		167	147	165	138	171	142	59.0	35.7	59.7	35.2	61.0	32.0

[a] ^{1}H NMR spectra were collected in D_2O. [b] CEST experiment was performed with 50 mM complex plus 50 mM guest molecule. Pre-saturation pulse of 4 s with $B_1 = 600$ Hz was applied for each sample.

pseudo-equatorial position prefers the smaller chemical group and, in this occasion, the $-CH_3$ group is larger (tetrahedron form) than the −COOH group (planar form) as shown in Figure S10. Consequently, arm rotation would place the bulkier group in a pseudo-axial position, which results in an unfavorable higher energy state as discussed in previous work.[19] Because of the orientation of the pendant arms, only two of the four stereoisomers are accessible. They are the SAP and twisted square antiprism (TSAP), respectively. Thus, the single isomer that we observe from the ^{1}H NMR spectra indicates that all the guest-Yb complexes belong to Λ(δδδδ) isomer (SAP).

The chemical shifts of the four alpha-hydroxyl acid protons differ in the range of 15–40 ppm across their *R*- and *S*- configurations. When the *R*-configuration guest molecule binds with Yb complexes, all the −CH protons have been downshifted to the range of 54.9–61.0 ppm. While those protons appear in a similar position to the H_4 resonance region in the *R*-configuration. This is supported by our previous observations and it indicates that the geometrical positions of the −CH protons in *R*-α-hydroxyl acids Yb_x complexes and *S*-α-hydroxyl acids Yb_x complexes are different as the magnetic axes governing the Yb-induced pseudo-contact shifts.[19] To our best knowledge, this is the first report of ^{1}H NMR chiral recognition with highly distinguishable chemical shift difference. The observation of discrete signals at precise chemical shifts that are not concentration-dependent indicates the formation of static complexes on the NMR time scale. As a consequence, for a given solvent like D_2O, each enantiomer can be correlated to an NMR signal with a precise chemical shift. Figure 1 illustrates the ability of $Yb_{1\text{-}3}$ to resolve all the enantiomers. One noteworthy feature is the one chiral center in Yb_1, which can rotate two non-chiral pendant arms into a preferable, lower energy structural state.

We next investigate the CEST behavior of these guest Yb complexes systems. As shown in Figure 2, the chemical shifts of the exchanging guest −OH protons in the Yb_x complexes are larger compared to the shifts observed in EuDO3A.[24] For one chiral complex, mandelic acid as an example, two sharp CEST peaks at 142 and 171 ppm were detected for *R* and *S* configurations, which might attribute to the Λ(δδδδ) isomer in different structures. This is consistent with enantiomeric discrimination of *R* and *S* guest molecules by the Yb_x complexes. The *R*-isomers have larger chemical shifts than *S* isomers in all occasions. The amplitude of the CEST exchange proton peaks for the *R* and *S* molecule in each complex varied little as the pH of the solution slightly changed between 7.0 and 7.6. From the results described above, this can be attributed to slightly different positions of the −OH protons with respect to the orientation of the magnetic axes. The signal provides the complex-distinguishing information and shows that the Yb complexes have the ability to identify the α-hydroxyl acids by CEST.

In summary, we have demonstrated a new detecting and sensing scheme based on Yb-chiral complexes for the detection and differentiation of α-hydroxyl acids in aqueous solution. The downfield shift of ^{1}H NMR and CEST signals upon binding with Yb complexes is self-consistent. This supports our model that chiral guest molecules induce Yb-chiral complexes into a single conformation that allows for *R*- and *S*-α hydroxyl acid detection and differentiation. This method is simple and robust for the differentiation of chiral α-

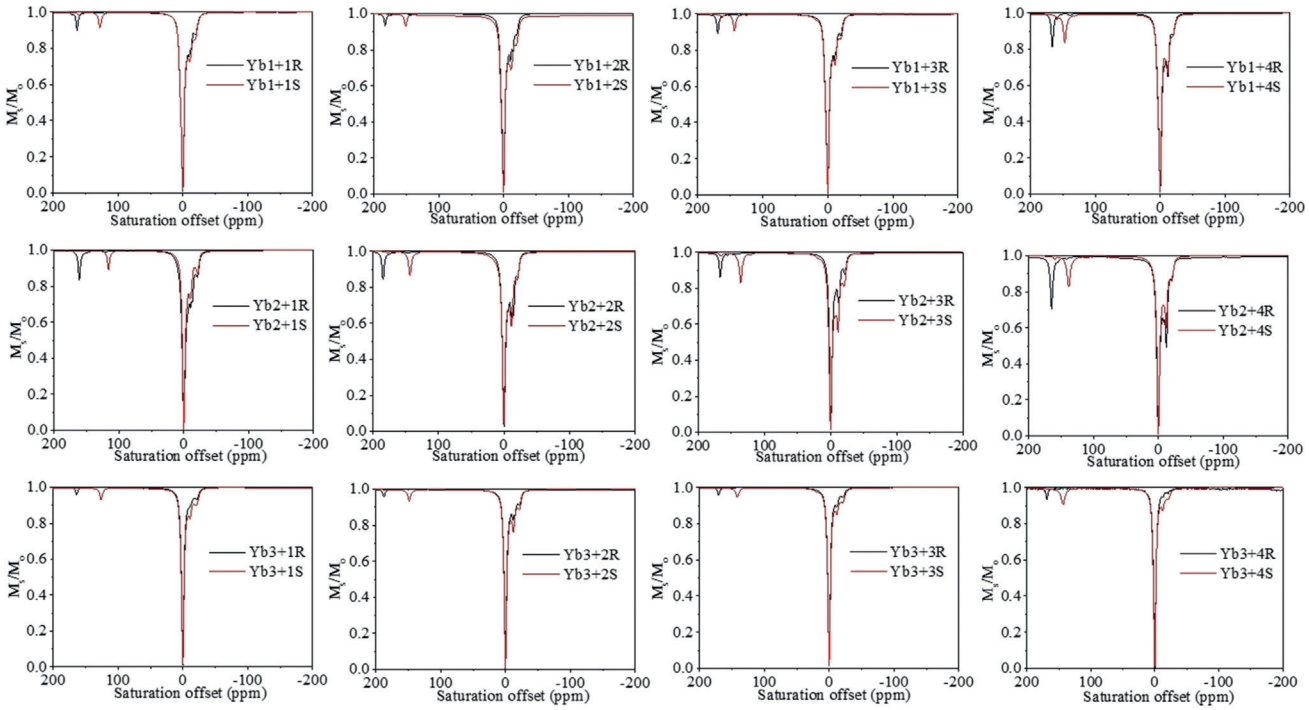


Figure 2. CEST spectra of Yb_x-complexes with either *S* or *R* α-hydroxyl acid in H_2O; 50 mM complex with 50 mM guest molecule. Pre-saturation pulse of 3 s with B_1 of 600 Hz was applied at 298 K using a 400 MHz NMR spectrometer. The pH of the samples was adjusted to the range of 7.0–7.6 prior to experiment.

hydroxyl acids that are not easily resolved with chiral HPLC. The key to the success of this approach is to bind enantiomers with an equal amount of a chiral Yb complex. This is helpful to produce a stronger ligand field effect, inducing sharp peaks on the cyclen H_4 resonance position, while the CEST can detect the non-equivalent amount of chiral guest molecules with a much greater chemical shift difference. The larger chemical shift difference between chiral compounds observed through the ^{1}H NMR and CEST method make it attractive for the detection of other chiral compounds such as amino acids or chiral amines when the ligands are appropriately designed. We believe this strategy will establish a powerful platform for broadly sensing and detection of chiral compounds relevant to the chiral synthesis of biological molecules.

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Conflict of interest

The authors declare no conflict of interest.

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