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Luminescent heptadentate Tb^{3+} complex with pendant aza-15-crown-5 showing recognition of lactate and salicylate in aqueous solution ☆

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Abstract—The coordinatedly unsaturated neutral complex **TbL1** that possesses two labile metal-bound water molecules provides linear response to lactate in the range of 0–3.0 mM at the simulated extracellular background with the variations of Tb luminescence lifetime as output; the maximal amplification of the luminescence intensity of **TbL1** reaches a factor of 135 upon titration with aromatic antenna salicylate in the physiological pH window.

The selective recognition of anions by artificial receptors plays a significant role in supramolecular chemistry.[1] Despite the importance of bio-active anion recognition in physiological surroundings, a majority of synthetic receptors perform their behaviors only in organic solvents due to the large free hydration energies ΔG_{hyd} of these anions.[2] Recently, luminescence sensors based on well-defined heptadentate Eu^{3+} and Tb^{3+} macrocyclic complexes with two metal-bound water molecules have attracted much attention.[3] The stepwise displacement of water molecules by an anion is signaled by an increase in the luminescence lifetime and the emission intensity due to the removing of diffusing OH (or NH) oscillators, which quench the excited states of Eu and Tb effectively.[4] Moreover, additional advantages of such lanthanide luminescence sensors include in their long-lived excited states (~ms), long emission wavelengths, large stokes shifts, and line-like emission bands in ambient conditions. Previous work showed that the positively charged lanthanide complexes have much higher binding constants to anions than those of neutral or negatively charged complexes in aqueous solution due to the electrostatic attraction.[3a] However, the large osmolality difference between the cationic complex solution and the body fluid causes pain and tissue sloughing, which limit the applications of positively charged lanthanide complexes in vivo.[5] Hence, the neutral complex with high binding constants to the specific anion is the best choice. In this work, we report a neutral complex, **TbL1**,which signals two important bio-active anions lactate and salicylate in aqueous solution.

The synthesis of **TbL1** is depicted in Scheme 1. Treatments of the electrophiles *N*-chloroacetyl-aza-15-crown-5 **1** with 2.5 equiv 1,4,7,10-tetraazacyclododecane (cyclen) gave mono *N*-alkylated product **2** with satisfactory yield.[6] The amide in **2** was reduced to amine in succession. The resulting product **3** reacted with excess *tert*-butyl bromoacetate to afford the corresponding alkylated product **4**. Through the deprotection step, the ligand **L1**[7] was obtained. Treatment of **L1** with $Tb_2(CO_3)_3$ gave the complex **TbL1**.[8] Moreover, to clarify the role of pendant crown ether played in the recognition process, **TbL2**[9] with pendant ethyl amine was prepared. All intermediates and complexes were well characterized using conventional methods. Furthermore, the hydration number q of both **TbL1** and **TbL2** was evaluated as two by measuring their Tb^{3+} luminescence lifetime in H_2O and D_2O, respectively.[10]

Keywords: Recognition; Lactate; Salicylate; Terbium; Luminescence.
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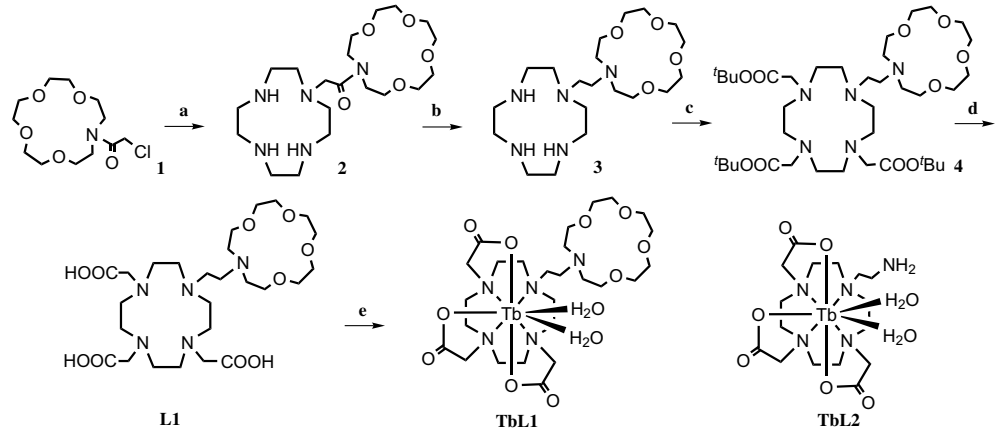

Scheme 1. Reagents and conditions: (a) 2.5 equiv cyclen, K_2CO_3/CH_3CN, 50–60 °C, 12 h, 86%; (b) BH_3·THF/THF, reflux, 12 h, 81%; (c) 3.3 equiv *tert*-butyl bromoacetate, Na_2CO_3/THF–H_2O, rt, 4 h, 84%; (d) TFA/CH_2Cl_2, rt, 2 h, 96%; (e) $Tb_2(CO_3)_3$, H_2O, 80 °C, 10 h, 92%.

Monitoring the concentration of lactate in vivo plays an important role in the observation, diagnosis, and treatment of diseases such as ischemia, stroke, and epilepsy.[11] To investigate the selective recognition of the Tb^{3+} complexes to bio-active anions, a series of titration experiments were carried out in buffered aqueous solutions (pH = 7.4). As shown in Figure 1, the luminescence lifetime of **TbL1** increased by 48% from 1.45 ms to its maximum of 2.15 ms upon the addition of 3.0 equiv of lactate. Concomitantly, its q value decreased from 2 to 0. In the titration with HCO_3^-, the lifetime of **TbL1** only increased by about 19% to 1.73 ms even after 14.0 equiv of anion was added. Similar behaviors were observed upon the addition of $H_2PO_4^-$ and AcO^-, and the lifetime of **TbL1** achieved 12% and 6% enhancements, respectively, in the presence of 14 equiv of anion. Meanwhile, the addition of Cl^- did not lead to any significant changes of luminescence lifetime or the q value. In the case of **TbL2**, it showed no obvious selectivity to these anions. The changing patterns of luminescence lifetime upon addition of lactate, HCO_3^-, and $H_2PO_4^-$ are quite similar, and 20%, 14% and 11% enhancements of luminescence lifetime were recorded in the presence of 14 equiv of anion.

Changes in the Tb luminescence lifetime of **TbL1** and **TbL2** were further investigated upon the addition of lactate in a stimulated extracellular ionic background (0.1 M NaCl, 30 mM HCO_3^-, 0.9 mM $H_2PO_4^-$, 0.13 mM citrate, pH 7.4, 0.05 M Tris/HCl). As illustrated in Figure 2, in comparison to the lifetime of **TbL2** increasing by about 9% in the presence of 4 mM lactate, the lifetime of **TbL1** increased linearly by 35% from 1.72 to 2.32 ms as the concentration of lactate increased from 0 to 3 mM. It is noteworthy that the concentration of lactate in physiological fluids is in the range of 0.35–2.3 mM,[12] which means **TbL1** can be used as a potential probe for monitoring the concentration of lactate in vivo. To clarify the selective recognition of **TbL1** to lactate, binding constants K_a of **TbL1** and **TbL2** for

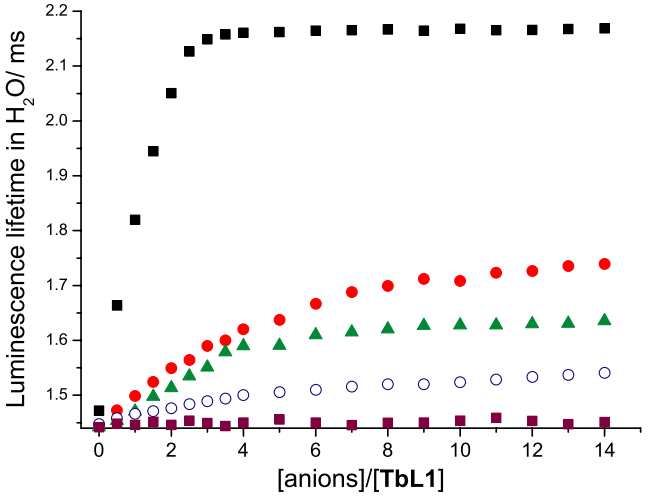

Figure 1. Plots of the luminescence lifetime of **TbL1** (λ_{em} = 545 nm, λ_{ex} = 228 nm) in H_2O as a function of the ratio [anions]/[**TbL1**]; black square, lactate; red circles, HCO_3^-; green triangles, $H_2PO_4^-$; open circle, AcO^-; purple square, Cl^- (1.0 mM complex, pH 7.4, 0.1 M Tris/HCl, 295 K).

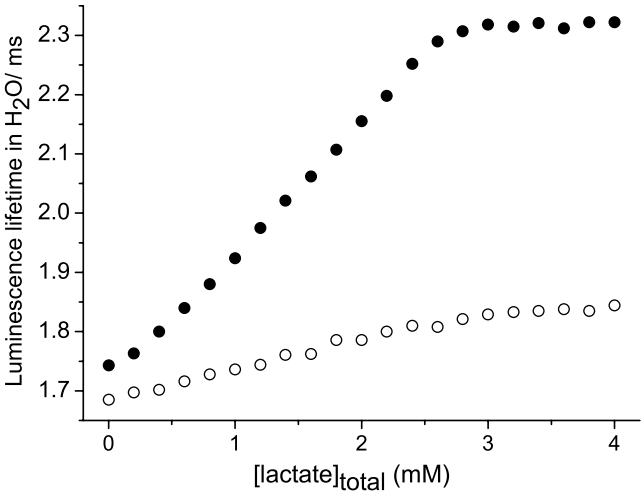

Figure 2. Plots of the luminescence lifetime of **TbL1** (filled circle) and **TbL2** (open circle) as a function of added lactate (1.0 mM complex, pH 7.4, 0.05 M Tris/HCl, in a simulated extracellular background: 100 mM NaCl, 30 mM HCO_3^-, 0.9 mM $H_2PO_4^-$, 0.13 mM citrate).

Table 1. Binding constants[a] ($\log K_a \pm 0.2$) for 1:1 complex formation and the hydration numbers, q^{Tb} (±15%)[b] (295 K, 0.05 M Tris/HCl buffer, pH 7.4)

Anion	Log K_a (q^{Tb})	
	TbL1	**TbL2**
Lactate	4.1 (0)	3.1 (0.4)
HCO_3^-	2.6 (1.0)	2.6 (1.1)
AcO^-	2.0 (1.2)	1.9 (1.3)
$H_2PO_4^-$	3.3 (0.8)	3.0 (1.0)
Cl^-	n.d.[c] (1.8)	n.d.[c] (1.8)
Benzoate (**1**)	2.9 (0.7)	2.7 (0.7)
Salicylate (**2**)	3.9 (0)	2.9 (0.4)

[a] Values of K_a were the mean of three determinations in the presence of 1×10^{-3} M Tb^{3+} complexes.
[b] Values of q^{Tb} were measured in the presence of 10 equiv of anion.
[c] Not detected.

anionic species were determined by plotting the emission values against the concentration of anions, and the results are summarized in Table 1. The binding constant of **TbL1** for lactate is not only larger than those for HCO_3^-, $H_2PO_4^-$, AcO^-, and Cl^-, but also larger than that of **TbL2** for lactate, indicating that the pendant crown ether promotes the binding specificity to lactate in aqueous solution.

As the active form of Aspirin®, salicylate performs various pharmacological actions in clinical use, such as anti-inflammation, anti-thrombopoiesis, and potential anti-tumor activities.[13] The recognition of **TbL1** and **TbL2** for the aromatic antenna benzoate and salicylate was conducted in pH 7.4 Tris/HCl buffer. These two aromatic anions not only possess triplet states that are high enough to sensitize Tb^{3+} $^5D_4$ ($E = 20500\,cm^{-1}$),[14] but they can also form ternary complexes with the heptadentate Tb^{3+} ion. As expected, **TbL1** was emissive silence in the absence of the chromophores when excited at 270–350 nm (Fig. 3). During the titration process, **TbL1** became emissive upon addition of salicylate while

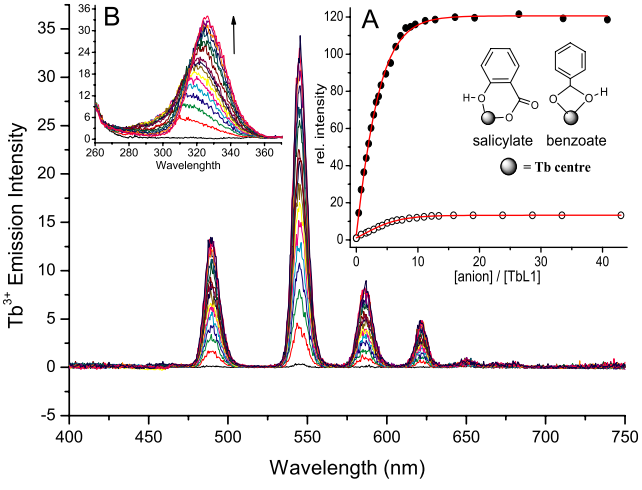

Figure 3. Emission trace of **TbL1** upon addition of salicylate in water ($\lambda_{em} = 545$ nm, $\lambda_{ex} = 324$ nm). Inset A: Tb^{3+} emission at 545 nm as functions of [salicylate]/[**TbL1**] 'filled circles' and [benzoate]/[**TbL1**] 'open circles'; the lines present the fit to a 1:1 binding mode (0.1 mM complex, pH 7.4, 0.05 M Tris/HCl). Inset B: plots of the excitation trace of **TbL1** upon the addition of salicylate.

excited at 324 nm. A maximal luminescence enhancement factor of ca. 135 was recorded for the band at 545 nm as the $^5D_4 \rightarrow {}^7F_5$ transition. Moreover, the binding constant $\log K_a$ was estimated as 3.9 (±0.2) M^{-1}. **TbL1** emission upon benzoate addition was also measured. The maximal amplification factor ($\lambda_{em} = 545$ nm) of ca. 12 was detected, and the binding constant was about 2.9 (±0.2) M^{-1}. During the binding process with salicylate, the two liable coordination sites of the Tb^{3+} center can be taken up by the two oxygen atoms of the carboxylate, to form a four-membered ring chelate, or via one oxygen atom from carboxylate and the other from phenolic oxygen to form a six-membered ring chelate (Fig. 3, inset A). However, in the case of benzoate, only a four-membered ring chelate can be achieved through the binding of two oxygen atoms in carboxylate with a Tb center. For **TbL2**, the excited triplet state can also be sensitized efficiently by benzoate and salicylate, and the maximal intensity enhancement factors of 14 and 21 were measured, respectively. Moreover, the binding constants between **TbL2** and these two aromatic anions were calculated as 2.7 and 2.9 M^{-1}.

The results of the titration experiments with bio-active anions suggest that **TbL1** with pendant aza-15-crown-5 prefers to bind with anion, which can form a larger membered ring chelate with lanthanide center. For example, more efficient population of the Tb^{3+} $^5D_4$ excited state and larger binding constants can be achieved through the six- or five-membered ring chelates between the Tb^{3+} center and salicylate or lactate. This specificity determined by binding mode indicates the existence of sterical interaction between the crown ether and the specific anion in the axial binding sites of Tb center,[15] and further investigations into these features are currently in progress.

In this work, **TbL1** with pendant aza-15-crown-5 shows recognition of two important bio-active anions: lactate and salicylate in physiological surroundings with the variation of luminescence lifetime and intensity, respectively. Moreover, as far as we know, this is the first time that the recognition of an anion in aqueous solution with the variation of long-lived lanthanide luminescence lifetime has been observed.

Acknowledgements

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7. Selected data for 1-(2-ethyl-aza-15-crown-5)-4,7,10-tris(acetic acid)-1,4,7,10-tetraazacyclododecane **L1**: **L1** was obtained as a colorless oil (171.5 mg, yield: 96%). ^{1}H NMR (400 MHz, D_2O, pD = 7.6): δ 4.12–3.96 (4H, br), 3.74–3.62 (4H, br), 3.60–3.42 (14H, m), 3.41–3.12 (14H, m), 3.10–2.79 (10H, br). ^{13}C NMR (100 MHz, D_2O): 171.7 (C), 169.9 (C), 169.7 (C), 69.4 ($2\times CH_2$), 68.9 ($2\times CH_2$), 68.4 ($2\times CH_2$), 63.5 ($2\times CH_2$), 54.9 ($2\times CH_2$), 54.8 ($3CH_2$), 52.9 (CH_2), 51.9 (CH_2), 51.3 (CH_2), 50.7 ($3\times CH_2$), 48.6 ($3\times CH_2$), 46.4 (CH_2); ESI-MS m/z 592.4 $(M+H)^+$; HRFAB-MS m/z 592.3560 $(M+H)^+$ [Calcd for $C_{26}H_{50}N_5O_{10}$ $(M+H)^+$, 592.3558].
8. Selected data for **TbL1**: **TbL1** was obtained as a white solid that was very hygroscopic and stored in a desiccator under inert gas (58.5 mg, yield: 92%). ESI-MS m/z 748.3 $(M+H)^+$; HRFAB-MS m/z 748.2545 $(M+H)^+$ [Calcd for $C_{26}H_{47}N_5O_{10}Tb$ $(M+H)^+$, 748.2576]; Anal. Calcd for $C_{26}H_{46}N_5O_{10}Tb\cdot 3H_2O$: C, 38.96; H, 6.54; N, 8.74. Found: C, 38.78; H, 6.86; N, 8.61.
9. Selected data for 1-(2-ethylamine)-4,7,10-tris(acetic acid)-1,4,7,10-tetraazacyclotetradecane **L2**: **L2** was isolated as a colorless oil (107.7 mg, yield: 92%). ^{1}H NMR (400 MHz, D_2O): δ 4.05–3.96 (2H, t, $J = 7.1$ Hz), 3.78–3.66 (4H, m), 3.56–3.40 (2H, s), 3.32–2.70 (18H, m); ^{13}C NMR (100 MHz, D_2O): 171.9 (C), 169.7 ($2\times$C), 55.7 ($2\times CH_2$), 53.8 (CH_2), 51.2 ($2\times CH_2$), 50.8 ($2\times CH_2$), 50.0 ($2\times CH_2$), 48.8 ($2\times CH_2$), 39.1 (CH_2), 37.3 (CH_2); ESI-MS m/z 390.2 $(M+1)^+$; HRFAB-MS m/z 390.2357 $(M+1)^+$ [Calcd for $C_{16}H_{32}N_5O_6$ $(M+1)^+$, 390.2353]. Selected data for **TbL2**: **TbL2** was obtained as a white solid, and stored in a desiccator under inert gas (65.5 mg, yield: 90%). ESI-MS m/z 546.1 $(M+H)^+$; HRFAB-MS m/z 546.1389 $(M+H)^+$ [Calcd for $C_{16}H_{29}N_5O_6Tb$ $(M+H)^+$, 546.1371]; Anal. Calcd for $C_{16}H_{28}N_5O_6Tb\cdot 2H_2O$: C, 33.05; H, 5.55; N, 12.05. Found: C, 33.31; H, 5.36; N, 12.32.
10. The hydration number q is calculated by using the Horrocks equation: $q^{Tb} = 5(1/\tau_{H2O} - 1/\tau_{D2O} - 0.06)$, where τ_{H2O} and τ_{D2O} are the luminescence lifetime of Tb^{3+} complex in H_2O and D_2O, respectively. The uncertainty of the q value is ±10%.
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