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Luminescent Tb^{3+} Complex with Pendant Crown Ether Showing Dual-Component Recognition of H^+ and K^+ at Multiple pH Windows

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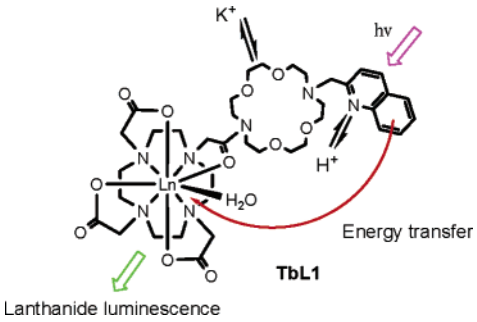
ABSTRACT



A lanthanide luminescent probe that was functionalized with quinoline-alkylated diaza-18-crown-6 showed dual-component recognition of concentrations of H^+ and K^+ at four independent pH ranges, including in the physiological pH window. Moreover, it exhibited pH- and [K^+]-independent long-lived lanthanide luminescent lifetimes in aqueous solution.

The sensitive detection of concentrations of K^+ is essential in many fields, such as analytical, biomedical, and diagnostic science, since the variation of serum potassium concentration is closely related to hypertension, stroke, and cardiovascular disease.[1] Previous work reported that low extracellular K^+ concentration promotes tumor growth,[2] and that the extracellular pH values of tumors (ca. 6.4−6.8) are generally more acidic than those of normal extracellular tissue (ca. 7.4).[3] Due to the importance of investigating the relationship between the diseases and concentrations of K^+ and H^+ in diagnosing these diseases in their early stages, it is crucial to prepare a sensor to monitor the concentration of K^+ and pH values simultaneously in vivo. Recently, considerable successes have been achieved in developing luminescent chemosensors based on well-defined Eu^{3+} and Tb^{3+} macrocyclic complexes, which provide the advantages of high thermodynamic and kinetic stability, as well as unique characteristics such as long-lived excited states (∼ms), long emission wavelengths (500−750 nm), large stokes shifts, and line-like emission bands (10−30 nm band-wide).[4] In this paper, neutral complex **TbL1** was prepared, and it showed

(1) (a) Grimm, R. H., Jr.; Neaton, J. D.; Elmer, P. J.; Svendsen, K. H.; Levin, J.; Segal, M.; Holland, L.; Witte, L. J.; Clearman, D. R.; Kofron, P. *N. Engl. J. Med.* **1990**, *322*, 569−574. (b) Bazzano, L. A.; He, J.; Ogden, L. G.; Loria, C.; Vupputuri, S.; Myers, L.; Whelton, P. K.; Johnston, S. C. *Stroke* **2001**, *32*, 1473−1480. (c) Walsh, C. R.; Larson, M. G.; Leip, E. P.; Vasan, R. S.; Levy, D. *Arch. Intern. Med.* **2002**, *162*, 1007−1012.

(2) (a) Jacobs, M. M. *Nutr. Cancer* **1990**, *14*, 95−101. (b) Jansson, B. *J. Environ. Pathol. Toxicol. Oncol.* **1996**, *15*, 65−73. (c) Negri, E.; La Vecchia, C.; Franceschi, S.; D'Avanzo, B.; Talamini, R.; Parpinel, M.; Ferraroni, M.; Filiberti, R.; Montella, M.; Falcini, F.; Conti, E.; Decarli, A. *Int. J. Cancer* **1996**, *65*, 140−144.

(3) (a) Gillies, R. J.; Lynch, R. M. *Novartis Fdn. Symp.* **2001**, *240*, 7−18. (b) Schornack, P. A.; Gillies, R. J. *Neoplasia* **2003**, *5*, 135−145. (c) Burd, R.; Lavorgna, S. N.; Daskalakis, C.; Wachsberger, P. R.; Wahl, M. L.; Biaglow, J. E.; Stevens, C. W.; Leeper, D. B. *Radiat. Res.* **2003**, *159*, 328−335. (d) Schwickert, G.; Walenta, S.; Sundfor, K.; Rofstad, E. K.; Mueller-Klieser, W. *Cancer Res.* **1995**, *55*, 4757−4759.

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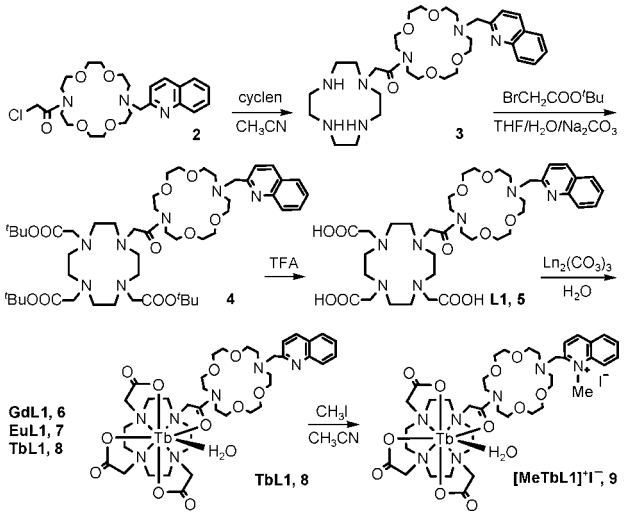
dual-component recognition of H^+ and K^+ in four independent pH windows. Furthermore, an unusually long-lived (~1.8 ms) luminescent lifetime was observed to remain constant in a wide pH range 2.2−12.5, which is very useful in overcoming the disturbances induced by auto-fluorescence and light scattering from the physiological environment.[5] To the best of our knowledge, **TbL1** is the first luminescent probe that can monitor two bioactive cationic species simultaneously in multiple pH windows with variations of characteristic Tb luminescent intensity.

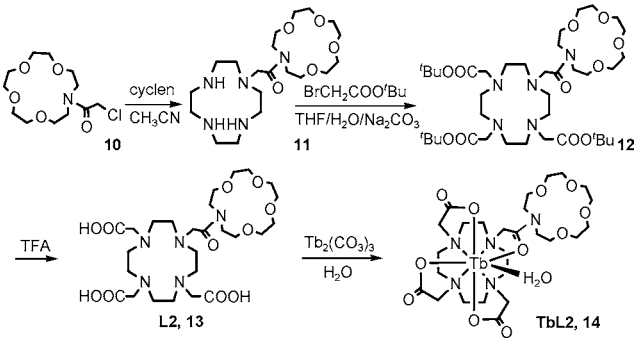
The synthesis of **LnL1** and **[MeTbL1]$^+$I$^-$** is shown in Scheme 1. The reaction of cyclen with halide **2** achieved

Scheme 1. Synthesis of **LnL1** and **MeTbL1**



the mono *N*-alkylated cyclen **3** with a satisfactory yield.[6] Treating **3** with *tert*-butyl bromoacetate afforded **4**. Subsequent deprotection gave **L1** that reacted with $Ln_2(CO_3)_3$ to afford the corresponding complexes **TbL1**, **EuL1**,[7] and **GdL1**. Refluxing **TbL1** with methyl iodide gave **[MeTbL1]$^+$I$^-$** as a precipitate. The preparation of **TbL2** is similar to that of **LnL1** (Scheme 2). The treatment of electrophile **10** with cyclen achieved the mono-*N*-alkylated product **11**. The reaction of **11** with *tert*-butyl bromoacetate gave **12** which resulted in **L2** after the deprotection process

Scheme 2. Synthesis of **TbL2**



in TFA. The targeting **TbL2** was afforded from the reaction between **L2** and $Tb_2(CO_3)_3$.

To show that the population of the Tb^{3+} ($^5D_4$, 20 500 cm^{-1}) by the pendant chromophore via energy transfer is possible, **L1**'s triplet state was measured as 23696 cm^{-1} for the unprotonated **GdL1** at 77 K (glycerol: 0.05 M borate/HCl buffer = 5:4, pH = 8.8).[8] The absorption spectrum of **TbL1** was highly pH dependent. In alkaline solution (pH 12.5), the absorption bands occurred at 228, 281, and 303 nm. A pH vs absorption titration profile of **TbL1** gave a sigmoidal curve in accordance with simple ion-binding equilibira extending over two pH units. The pK_a of 5.1 (±0.2) was determined, and attributed to the protonation of quinoline nitrogen moiety. Excitation at 313 nm gave pH dependent ligand-based fluorescence. A broad band (λ_{max} = 393 nm) was efficiently enhanced upon acidification, with the pK_a of the excited singlet state of the quinoline as 5.2 (±0.2).

The pH-dependent Tb^{3+} luminescence was evaluated in aqueous solution (I = 0.1 M NMe_4ClO_4, 293 K) (Figure 1).

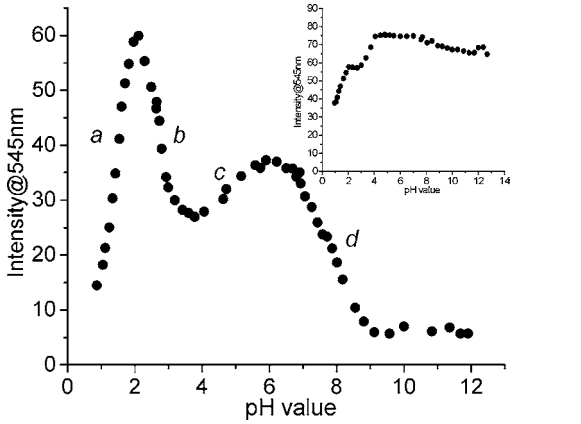


Figure 1. pH-dependent Tb luminescence intensity profile for **TbL1** and **[MeTbL1]$^+$I$^-$** (inset) at 545 nm (10 μM, 0.1 M NMe_4ClO_4, excited at 313 nm).

Only a weak Tb^{3+} emission was observed at 489, 545, 584, and 620 nm for the $^5D_4 \rightarrow {}^7F_J$ (J = 6, 5, 4, 3) transitions after excitation at 313 nm in both strong acidic (pH 0.8−1.0) and alkali (pH 8.8−12.5) solutions, whereas two neighboring

(4) (a) Parker, D.; Dickins, R. S.; Puschmann, H.; Crossland, C.; Howard, J. A. K. *Chem. Rev.* **2002**, *102*, 1977−2010. (b) Parker, D. *Coord. Chem. Rev.* **2000**, *205*, 109−130. (c) Gunnlaugsson, T.; Harte, A. J.; Leonard, J. P.; Nieuwenhuyzen, M. *Supramol. Chem.* **2003**, *15*, 505−519. (d) Li, C.; Wong, W. T. *Tetrahedron Lett.* **2004**, *45*, 6055−6058.

(5) (a) de Silva, A. P.; Gunaratne, H. Q. N.; Rice, T. E. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2116−2118. (b) Licini, M.; Williams, J. A. G. *Chem. Commun.* **1999**, 1943−1944.

(6) Li, C.; Wong, W. T. *Tetrahedron Lett.* **2002**, *43*, 3217−3220.

(7) ^{1}H NMR (500 Hz, D_2O, 273 K) spectra of **EuL1** gave two sets of resonances at 40.5−35.7 ppm and 14.3−10.6 ppm, which can be attributed to the axial protons of the cyclen ring, and indicates that two major diastereoisomers of **LnL1**, "regular (mono-capped) square-antiprismatic isomer (**M**)" and "twisted square-antiprismatic isomer (**m**)" are in a relative ratio, **M**/**m** of 2:1 in aqueous solution.

(8) (a) Latva, M.; Takalo, H.; Mukkala, V. M.; Matachescu, C.; Rodríguez-Ubis, J. C.; Kankare, J. *J. Luminescence* **1997**, *75*, 149−169. (b) Gunnlaugsson, T.; MacDonaill, D. A.; Parker, D. *J. Am. Chem. Soc.* **2001**, *123*, 12866−12876.

bell-shaped curves with maximal intensity at pH 2.05 (LE ≈ 15) and 5.8−6.2 (LE ≈ 9), consisting of four slopes between pH 1.0−2.1 (a), 2.1−3.4 (b), 3.7−6.0 (c), and 6.2−9.2 (d), were obtained by plotting the intensity of the $^5D_4 \rightarrow {}^7F_5$ transition as a function of the pH values (in the pH range of 1.0−9.5). This intriguing and unique switching profile was fully reversible in the pH range of 1.0−12.5. The pK_a values of 4.8 (±0.2) and 7.6 (±0.2) were determined for slopes (c) and (d), which were assigned to the protonation of heteroaryl nitrogen and tertiary amine, respectively. It is noteworthy that the slope (d) just transpired over the physiological pH range. In terms of slope (b), a meaningful pK_a could not be determined, but it could be related to the protonation of the amide nitrogen. As expected, after the methylation of the nitrogen atom in the chromophore, the lanthanide emission of **[MeTbL1]$^+$I$^-$** showed little variation in the pH range of 3.0−12.5 (Figure 1, inset). The hydration number (q) for **TbL1** was evaluated by measuring the Tb^{3+} luminescent lifetime in H_2O and D_2O. Astonishingly, its lifetimes (τ_{Tb} = 1.7−1.8 and 2.7−2.9 ms in H_2O and D_2O, which gives $q \approx 0.7$) were not only pH independent in the wide pH range 2.2−12.5 but also much longer than those of previously reported Tb^{3+} complexes with similar coordination structures (0.1−1.1 ms in H_2O, pH = 7.4).[9] Upon further acidification (pH 1.0−2.2), both the luminescent intensity and lifetime of **TbL1** decreased significantly and a q value about 1.6 was given, which implies that the coordinated amide oxygen was protonated and that two water molecules were bound to the Tb^{3+} center. To investigate this distinctive luminescent lifetime, **TbL2** with pendant aza-15-crown-5 (Scheme 2) was prepared. Its luminescent lifetime was even longer and totally pH independent (τ_{Tb} = 2.0 and 3.4 ms in H_2O and D_2O respectively, giving q = 0.7) in the wide pH range of 1.0−12.5. The long-lived and pH-independent luminescent lifetime suggests the existence of second sphere H-bonding interactions through which the crown ether shields the metal center from its interactions with water molecules,[10] which offers a design strategy for lanthanide chelates possessing long-lived and steady lanthanide luminescent lifetimes in aqueous solution.

Study of the dual-component sensing behavior of **TbL1** was conducted upon K^+ in a wide pH range of 0.8−12.0 (Figure 2). Tb^{3+} emission was silent and immunized to the added K^+ in the alkali (pH 9.2−12.0) solution. Interestingly, in contrast to the quenching of the emission in the pH range of 1.0−3.4, Tb^{3+} luminescence was enhanced gradually in the pH range of 4.1−9.0 upon addition of K^+ from 0 to 0.5 M. Notably, the Tb-luminescent lifetime remained invariable in the K^+ titration process. According to the three-dimensional model (Figure 2), an exclusive concentration of either K^+ or H^+ can be detected discretionally in four independent pH windows, especially in the physiological pH window (6−8) if the Tb luminescent intensity and the other component's concentration (H^+ or K^+) are known in advance. We assume that the dual-component sensing behavior of **TbL1** is derived from the intramolecular energy transfer efficiency, which is not only determined by the protonation or deprotonation of nitrogen atoms with different pK_a values in

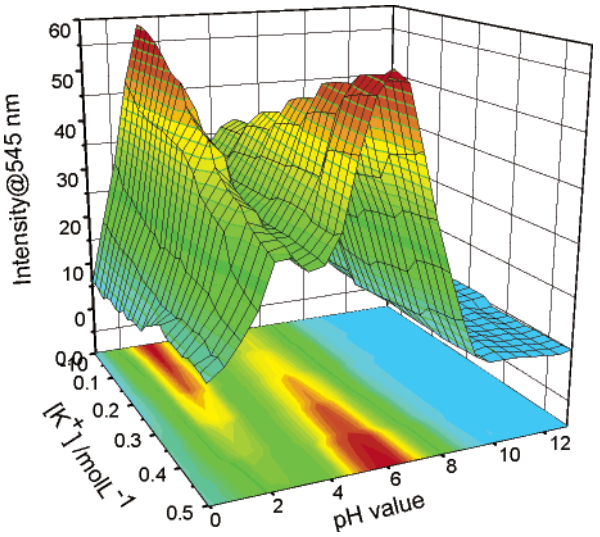


Figure 2. 3D matrix showing Tb luminescence behavior as a function of pH and [K^+] in aqueous solution (10 μM **TbL1**, 0.1 M NMe_4ClO_4, excited at 313 nm).

specific pH windows,[9c,11] but also by the variation of the crown ether ring's conformation and rigidity during the complexation process, which changes the oxidation potential of the receptor.[9c,11]

The selectivity of **TbL1** for K^+ over other bioactive cations is particularly important because these cations in body fluids can bind with crown ether competitively and, hence, interfere with the response of potassium ion. The luminescence profiles of **TbL1** in the presence of selected cations are shown in buffered solutions with pH values of 6.3 and 2.2, respectively (Figure 3). On the basis of the experimental

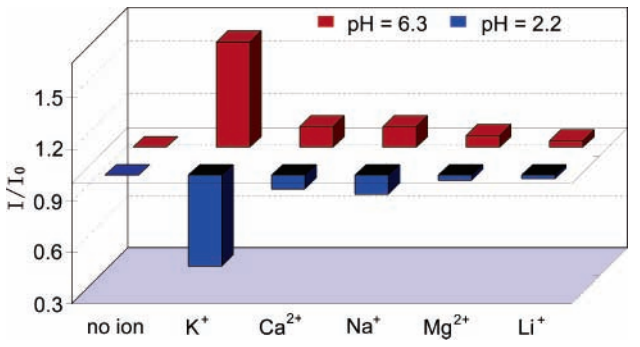


Figure 3. Lanthanide luminescence intensity of **TbL1** (1×10^{-5} M) upon selected bioactive cations at pH 6.3 and 2.2, respectively (excited at 313 nm).

results, similar cation dependent Tb luminescent changing patterns were monitored upon addition of K^+, Na^+, Li^+, Ca^{2+}, and Mg^{2+}. During the titration processes, the luminescence intensity was enhanced at pH 6.3, while it quenched simultaneously at pH 2.2. Notably, the enhancements or

quenching induced by K^+ were much more significant than that induced by other cations added.[12] It seems that **TbL1** can perform satisfactory selectivity toward K^+ in the physiological pH range of around 6.0−7.4.

As indicated in Table 1, compared to the luminescent silence of **TbL2**, which is short of chromophore, the highest value of quantum yield of **TbL1** (0.037−0.038) appeared at pH 2.0 with the absence of K^+ and at pH 5.9 with the presence of 0.5 M K^+. This value is five times greater than the values measured in strong acidic (pH < 0.8) and alkali (pH > 9.0) solutions. This fact provides further confirmation that the intramolecular energy transfer efficiency of **TbL1** can be determined by pH and [K^+] corporately.

Table 1. Photophysical Properties of **TbL1** and **TbL2** at Selected pH Values

pH	$\Phi_{\mathbf{TbL1}}$[a] (QE)[b]	$\Phi_{\mathbf{TbL1}-K}$[c]	$\Phi_{\mathbf{TbL2}}$	log K_a[d]
0.9	0.008 (1.14)	0.007	<0.001	*e*
2.0	0.037 (5.29)	0.0015	<0.001	*e*
3.8	0.016 (2.29)	0.016	<0.001	*e*
5.9	0.024 (3.43)	0.038	<0.001	*e*
7.4	0.018 (2.57)	0.022	<0.001	1.63
9.6	0.007	0.007	<0.001	1.84
MeOH	0.023	0.038	<0.001	2.14

[a] Luminescence quantum yields obtained by comparison with quinine sulfate in 0.1 N H_2SO_4, λ_{ex} = 313 nm. [b] pH-induced quantum yield enhancements (e.g., QE = $\Phi_{\text{specific pH value}}/\Phi_{\text{pH9.6}}$). [c] In the presence of 0.5 M K^+. [d] The binding constants of the **TbL1**−K^+ interaction were determined according to $\log K_a = \log[(I_F - I_{F\min})/(I_{F\max} - I_F)] - \log[K^+]$. [e] Spectroscopic change is too small to permit measurement.

In summary, we have described a novel Tb^{3+} luminescent complex that shows dual-component recognition of H^+ and K^+ at four independent pH ranges, especially in the physiological pH window, with long-lived luminescent lifetime that remains constant as a function of pH and [K^+] in aqueous solution. Furthermore, by carefully choosing appropriate chromophores, ionophores, and proton receptors in the modular ligand system, it is possible to develop a dual or even multiple component Ln^{3+} luminescent sensor for signaling selected cations in a specific pH window with higher sensitivity and selectivity.

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Supporting Information Available: Experimental procedures, full characterization for compounds **1**−**14**, pH-dependent UV−vis, fluorescence, lanthanide luminescence, and *q* values; lanthanide luminescence as a function of selected cations at specific pH values. This material is available free of charge via the Internet at http://pubs.acs.org.

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(9) (a) Bruce, J. I.; Dickins, R. S.; Govenlock, L. J.; Gunnlaugsson, T.; Lopinski, S.; Lowe, M. P.; Parker, D.; Peacock, R. D.; Perry, J. J. B.; Aime, S.; Botta, M. *J. Am. Chem. Soc.* **2000**, *122*, 9674−9684. (b) Beeby, A.; Clarkson, I. M.; Dickins, R. S.; Faulkner, S.; Parker, D.; Royle, L.; de Sousa, A. S.; Williams, J. A. G.; Woods, M. *J. Chem. Soc., Perkin Trans. 2* **1999**, 493−503. (c) Parker, D.; Senanayake, P. K.; Williams, J. A. G. *J. Chem. Soc., Perkin Trans. 2* **1998**, 2129−2139.

(10) (a) Aime, S.; Botta, M.; Fasano, M.; Terreno, E. *Chem. Soc. Rev.* **1998**, *27*, 19−29. (b) Botta, M. *Eur. J. Inorg. Chem.* **2000**, 399−407. (c) Li, C.; Wong, W. T. *Chem. Commun.* **2002**, 2034−2035.

(11) (a) Gunnlaugsson, T.; Leonard, J. P.; Sénéchal, K.; Harte, A. J. *J. Am. Chem. Soc.* **2003**, *125*, 12062−12063. (b) Gunnlaugsson, T.; Leonard, J. P. *Chem. Commun.* **2003**, 2424−2425. (c) de Silva, A. P.; Gunaratne, H. Q. N.; Rice, T. E.; Steward, S. *Chem. Commun.* **1997**, 1891−1892.

(12) The luminescence intensity profiles were conducted in the presence of 0.5 M K^+, Na^+, Li^+, 0.12 M Ca^{2+}, and 0.11 M Mg^{2+} respectively.