Available online at www.sciencedirect.com
ScienceDirect
Tetrahedron Letters
Tetrahedron Letters 48 (2007) 3159–3162

Anion-induced ring-opening of fluorescein spirolactam: fluorescent *OFF–ON*

Jia-Sheng Wu,[a] Hyun Jung Kim,[a] Min Hee Lee,[a] Jun Hee Yoon,[a] Joung Hae Lee[b] and Jong Seung Kim[a,*]

[a]*Department of Chemistry, Institute of Nanosensor and Biotechnology, Dankook University, Seoul 140-714, Republic of Korea*
[b]*Korea Research Institute of Standards and Science, Taejon 305-600, Republic of Korea*

Received 8 February 2007; revised 8 March 2007; accepted 9 March 2007
Available online 13 March 2007

Abstract—*N*-(2,7-Dichlorofluorescein)lactam-*N*′-phenylthiourea (**L**) was developed as a colorimetric and fluorescent chemosensor for anions such as AcO^-, F^- and $H_2PO_4^-$. The addition of AcO^- anion to the solution of **L** in CH_3CN results in a distinct fluorescence '*ON*' observation as well as color change (from colorless to pink). The H-bonding interaction between AcO^- anion and thiourea moiety of **L** induced the ring-opening of the spirolactam of fluorescein moiety, which gave rise to the dual chromo- and fluorogenic changes.

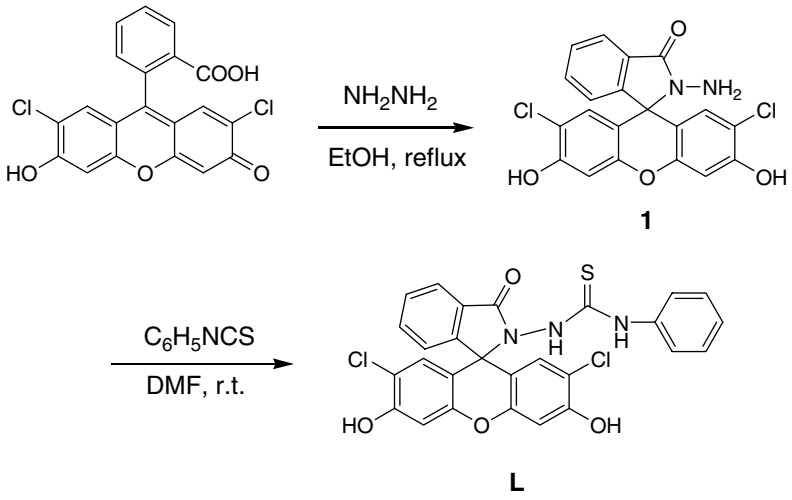
Considerable attention has been focused on the design of fluorescein/rhodamine-based chemosensors through naked-eye observation and/or fluorescence method in recent years because of their particular structural properties.[1] As we know, the fluorescein/rhodamine with spirolactam structure was non-fluorescent, whereas ring-opening of the spirolactam gave rise to a strong fluorescence emission.[2] In fact, this is an ideal mode to construct *OFF–ON* fluorescent switch sensors. Moreover, they have a longer emission wavelength (about 550 nm), which is often preferred to serve as reporting groups for analyte to avoid the influence of the background fluorescence (below 500 nm).[3] Actually, to date, many spirolactam-based chemosensors have been developed for metal cations such as Hg^{2+}, Pb^{2+}, Cu^{2+}, Fe^{2+}, and Zn^{2+}.[2] However, all of these cases are only restricted in cation-induced ring-opening.

On the other hand, there is still a demand to explore anion-induced ring-opening of the spirolactam through anion recognition, though it is a more challenging work compared with the conventional cation recognition because anions such as acetate, fluoride, and phosphate play crucial roles in a range of biological phenomena and are implicated in many disease states.[4,5] However, as we notice, there is no such case till now to illustrate that the ring-opening of spirolactam can also take place by an anionic inducement. With this in mind, we have tried to append a H-bonding donor (thiourea group) at the side of amide moiety of the spirolactam. In deed, the amide-*N*-thiourea has been used to recognize AcO^-, F^-, and dicarboxylate anions when it is appended to other fluorophores.[6] Herein, we applied this structure to the fluorescein matrix and eventually found that anions, in terms of a H-bonding with amide-*N*-thiourea, also could induce ring-opening of the spirolactam in a function of dual chromo- and fluorogenic changes (*OFF–ON*).

As described in Scheme 1, **L** was synthesized from the reaction of 2,7-dichlorofluorescein and hydrazine, followed by the reaction with phenyl isothiocyanate in 61% overall yield.[7,8] The structure of **L** was characterized by ^1H NMR, ^{13}C NMR, mass spectroscopy and elemental analysis. **L** was selected for anion sensing mainly based on the following considerations: (i) in the case of Jiang's example,[6a] amide-*N*-thiourea moiety was attached to *N*,*N*-dimethylaminophenyl moiety to produce a ratiometric fluorescence signal, herein, fluorescein spirolactam was used to give a fluorescent and colorimetric *OFF–ON* signal; (ii) the introduction of thiourea makes ring-opening of the spirolactam possible when the strong H-bonding acceptors are introduced. In our present work, the addition of AcO^- or F^- resulted

* Corresponding author. Tel.: +82 2 799 1351; fax: +82 2 797 3277; e-mail: jongskim@dankook.ac.kr

0040-4039/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetlet.2007.03.060



Scheme 1. Synthetic route of *N*-(2,7-dichlorofluorescein)lactam-*N*′-phenylthiourea (**L**).

in distinct dual chromo- and fluorescent changes of **L** in CH_3CN, $H_2PO_4^-$ only induced a smaller spectral change, whereas Cl^-, Br^-, HSO_4^-, and I^- did not lead to any spectral changes.

Figure 1 shows spectral variations of **L** in CH_3CN upon the addition of AcO^- anion (countercations of all anions are tetrabutylammonium). From UV–vis titration curves (Fig. 1a), we found two distinctly new absorption bands centered at 530 and 323 nm upon gradual addition of acetate anion, whilst a small shoulder peak at 484 nm was also observed. The new peak at 530 nm was increased with the concentration of AcO^- and kept silent when more than 20 equiv of AcO^- anion was added. In addition, an obvious color change from colorless to pink was also observed with naked eyes. Job's plot analysis (inset of Fig. 1a) indicates the formation of 1:1 complex between **L** and AcO^- in CH_3CN. On the basis of 1:1 stoichiometry, the binding constant (K_{ass}) was calculated to be $2.7 \times 10^5\ M^{-1}$ in ENZFITTER program based on UV–vis titration data.[9]

The above process was also conducted by fluorescence titration experiment using 10 μM of **L** in CH_3CN (Fig. 1b). Upon the addition of AcO^- anion, a new emissive band at 550 nm appeared and increased with the concentration of AcO^- anion. This observation is also obviously due to a ring-opening of the fluorescein spirolactam upon the addition of acetate anion, which is in a good consistency with the results of UV–vis spectra. Thus, this is such a case of dual spectral changes applying for fluorescent and colorimetric switch sensor (*OFF–ON*).

From the spectral results above and possible structural change of **L** upon the addition of acetate anion, the new absorption band at 530 nm is attributed to ring-opening of the fluorescein spirolactam, whereas the peak at 323 nm arises from deprotonated phenoxide of fluorescein matrix, which can be deduced from the absorption spectra of **1** (only lacking thiourea unit compared with **L**). From Figure 2, the addition of AcO^- anion to **1** only leads to a new band appeared at 323 nm, but no peak at 530 nm, implying that the band at 323 nm corresponds to deprotonated phenoxide and has nothing to do with the introduction of thiourea moiety and ring-opening of spirolactam. In addition, fluorescence spectra of **1** were also kept silent upon addition of

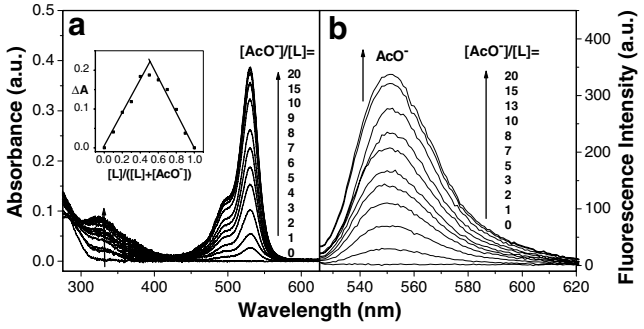


Figure 1. (a) UV–vis and (b) fluorescence titration spectra of **L** (10 μM) in CH_3CN upon the addition of increasing concentrations of AcO^- anion (tetrabytylammonium salt, 0–20 equiv) with an excitation wavelength of 520 nm (Inset: Job's plot of **L** vs AcO^-).

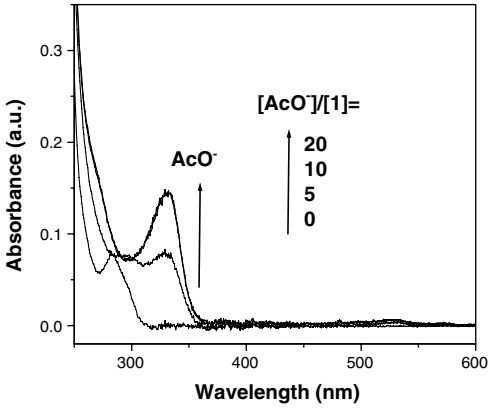


Figure 2. UV–vis titration curves of **1** (10 μM) in CH_3CN upon the addition of different concentrations of AcO^- (tetrabytylammonium salt, 0–20 equiv).

20 equiv of AcO^- anion. Therefore, it is noteworthy that the ring-opening of the spirolactam of **L** to give fluorescence changes is mainly due to the H-bonding between the acetate anion and thiourea group rather than deprotonation of the phenolic OH of the fluorescein matrix, as depicted in Scheme 2.

Addition of F^- anion into the solution of **L** in CH_3CN also resulted in the similar spectral variation as AcO^- did, whereas $H_2PO_4^-$ only led to a smaller spectral change compared with AcO^- and F^- because of the weaker H-bonding acceptor ability. Within 1:1 stoichiometry, the binding constants (K_{ass}) of **L**–F^- and **L**–$H_2PO_4^-$ were calculated to be $3.2 \times 10^5\ M^{-1}$ and $4.6 \times 10^4\ M^{-1}$, respectively.[9] However, introducing other anions such as Cl^-, Br^-, HSO_4^- and I^- did not lead to any obvious spectral changes of **L** even if 100 equiv of such anions were added, and their binding constants were too small to be calculated. The color and fluorescence changes of **L** upon the addition of various anions are shown in Figure 3.

The direct evidence of the ring-opening of spirolactam comes from ^{13}C NMR data. For free **L**, ^{13}C chemical shift of *quaternary-C* was 67.5 ppm, whereas in the presence of 10 equiv of fluoride anion, it was shifted to the aryl region, indicating that the ring-opening of spirolactam took place when a strong H-bonding acceptor was added.

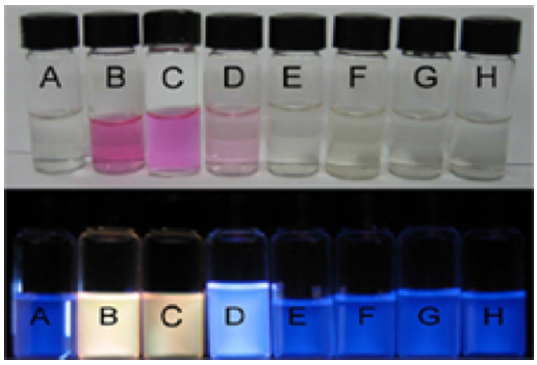
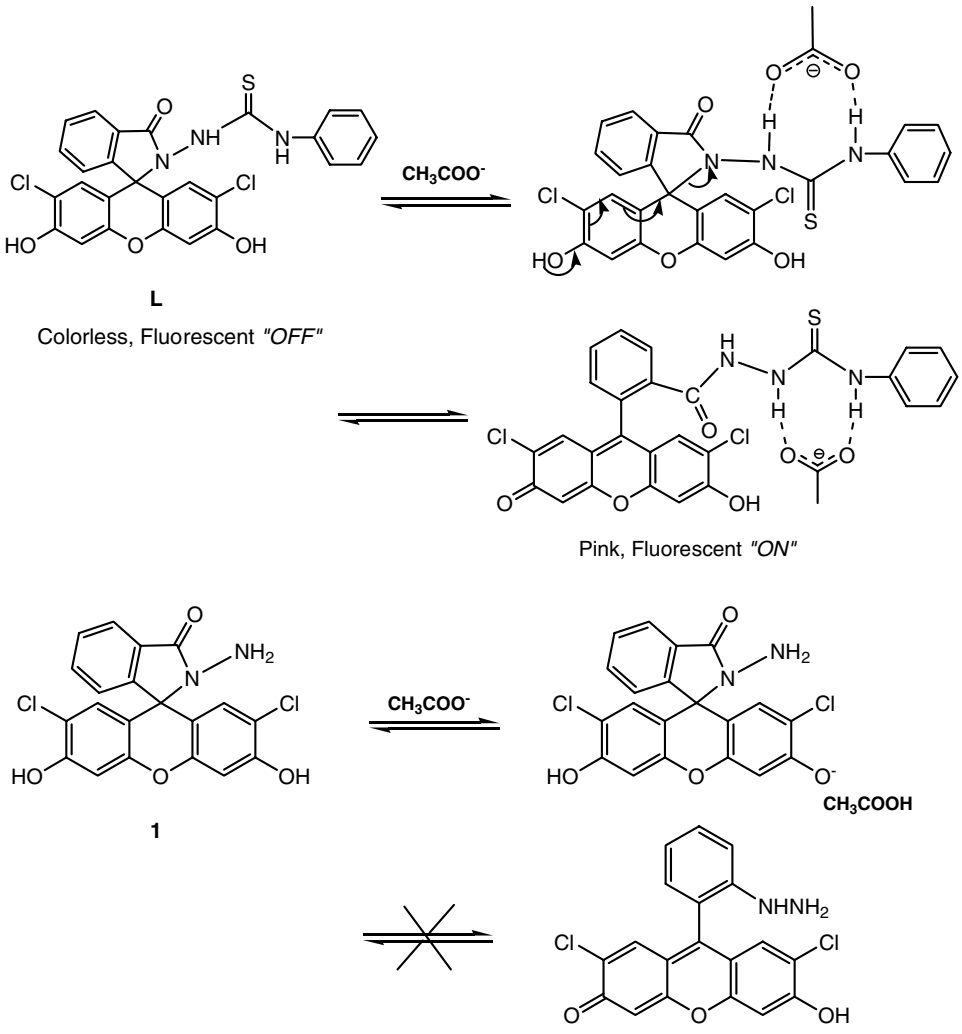


Figure 3. Color changes (first row) and fluorescence changes (second row) of **L** (10 μM) in the presence of 20 equiv of different anions: (A) **L** only, (B) AcO^-, (C) F^-, (D) $H_2PO_4^-$, (E) HSO_4^-, (F) Cl^-, (G) Br^-, (H) I^-.

L shows a high selectivity for the strong H-bonding acceptors, such as AcO^-, F^-, and $H_2PO_4^-$ among other



Scheme 2. Proposed ring-opening mechanism of **L** induced by AcO^- anion.

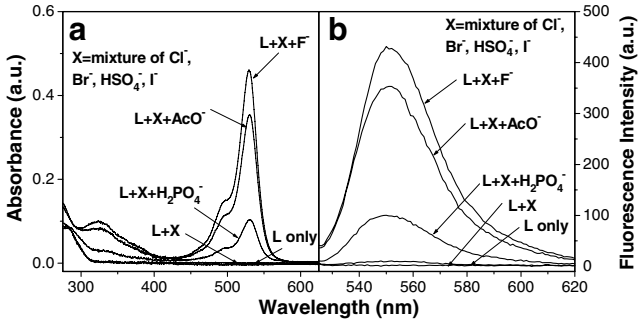


Figure 4. (a) UV–vis spectra and (b) fluorescence spectra of **L** (10 μM) + mixed anions including Cl^-, Br^-, HSO_4^-, I^- (100 μM) in CH_3CN upon addition of F^-, AcO^-, $H_2PO_4^-$ (20 equiv, respectively) with an excitation wavelength of 520 nm.

common anions, which can be illustrated from Figure 4 as well. The absorption and fluorescence spectra of **L** are not markedly changed upon the addition of mixed anions including Cl^-, Br^-, HSO_4^-, I^- (100 μM, respectively). To this solution (**L** + mixed anions), addition of F^-, AcO^- or $H_2PO_4^-$ leads to distinct fluorescence and color changes. Thus, anion-induced ring-opening of **L** is according to the following sequence: $AcO^- \sim F^- > H_2PO_4^- \gg HSO_4^- > Cl^- \sim Br^- \sim I^-$. This is to say, the occurrence of recognition event here is based on the H-bonding interaction between **L** and anions.

In summary, we herein demonstrate a fluorescein-based dual chromo- and fluorogenic chemosensor for anions mainly due to a H-bonding interaction. To the best of our knowledge, this is the first case to explore anion-induced ring-opening of the spirolactam of fluorescein/rhodamine derivatives. Investigations along these lines for development of more sophisticated systems for recognition of anions, especially in buffered aqueous solution, are still in progress.

Acknowledgements

This work was supported by the SRC program (R11-2005-008-02001-0(2006)), Basic Science Research of KOSEF (R01-2006-000-10001-0) and BK21.

References and notes

1. (a) Bencic-Nagale, S.; Sternfeld, T.; Walt, D. R.. *J. Am. Chem. Soc.* **2006**, *128*, 5041; (b) Mottram, L. F.; Boonyarattanakalin, S.; Kovel, R. E.; Peterson, B. R. *Org. Lett.* **2006**, *8*, 581; (c) Goldsmith, C. R.; Jaworski, J.; Sheng, M.; Lippard, S. J. *J. Am. Chem. Soc.* **2006**, *128*, 418; (d) Komatsu, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2005**, *127*, 10197; (e) Takakusa, H.; Kikuchi, K.; Urano, Y.; Sakamoto, S.; Yamaguchi, K.; Nagano, T. *J. Am. Chem. Soc.* **2002**, *124*, 1653; (f) Nolan, E. M.; Lippard, S. J. *Inorg. Chem.* **2004**, *43*, 8310; (g) Sarpara, G. H.; Hu, S. J.; Palmer, D. A.; French, M. T.; Evans, M.; Miller, J. N. *Anal. Commun.* **1999**, *36*, 19.
2. (a) Zheng, H.; Qian, Z.-H.; Xu, L.; Yuan, F.-F.; Lan, L.-D.; Xu, J.-G. *Org. Lett.* **2006**, *8*, 859; (b) Wu, J.-S.; Hwang, I.-C.; Kim, K. S.; Kim, J. S. *Org. Lett.* **2007**, *9*, 907; (c) Xiang, Y.; Tong, A.; Jin, P.; Ju, Y. *Org. Lett.* **2006**, *8*, 2863; (d) Yang, Y.-K.; Yook, K.-J.; Tae, J. *J. Am. Chem. Soc.* **2005**, *127*, 16760; (e) Xiang, Y.; Tong, A. *Org. Lett.* **2006**, *8*, 1549; (f) Kwon, J. Y.; Jang, Y. J.; Lee, Y. J.; Kim, K. M.; Seo, M. S.; Nam, W.; Yoon, J. *J. Am. Chem. Soc.* **2005**, *127*, 10107; (g) Dujols, V.; Ford, F.; Czarnik, A. W. *J. Am. Chem. Soc.* **1997**, *119*, 7386.
3. (a) Grynkiewicz, G.; Poenie, M.; Tsien, R. Y. *J. Biol. Chem.* **1985**, *260*, 3440; (b) Minta, A.; Tsien, R. Y. *J. Biol. Chem.* **1989**, *264*, 19449; (c) Kimura, E.; Koike, T. *Chem. Soc. Rev.* **1998**, *27*, 179.
4. Tomich, J. M.; Wallace, D.; Henderson, K.; Mitchell, K. E.; Radke, G.; Brandt, R.; Ambler, C. A.; Scott, A. J.; Grantham, J.; Sullivan, L.; Iwamoto, T. *Biophys. J.* **1998**, *74*, 256.
5. (a) Kirk, K. L. *Biochemistry of the Halogens and Inorganic Halides*; Plenum Press: New York, 1991; p 58; (b) Kleerekoper, M. *Endocrinol. Metab. Clin. North Am.* **1998**, *27*, 441.
6. (a) Wu, F.-Y.; Li, Z.; Wen, Z.-C.; Zhou, N.; Zhao, Y.-F.; Jiang, Y.-B. *Org. Lett.* **2002**, *4*, 3203; (b) Gunnlaugsson, T.; Kruger, P. E.; Jensen, P.; Tierney, J.; Ali, H. D. P.; Hussey, G. M. *J. Org. Chem.* **2005**, *70*, 10875.
7. *Synthesis of 2,7-dichlorofluorescein hydrazide* (**1**): 2,7-Dichlorofluorescein (1.0 mmol, 401 mg) was dissolved in 20 mL of absolute ethanol, followed by the addition of hydrazine monohydrate (5.7 mmol, 0.3 mL). The mixture was refluxed for 12 h till the fluorescence of the solution was disappeared. The reaction mixture was dissolved in ethyl acetate (100 mL), then washed with water several times. The organic phase was collected, dried over anhydrous Na_2SO_4, filtered, and evaporated of the solvent. The crude product was recrystallized from methanol to give **1** (335 mg, 81.2% yield). $R_f = 0.57$ (silica, CH_2Cl_2/methanol = 10/1, v/v); ^{1}H NMR (DMSO-d_6): δ 7.78 (d, 1H, ArH), 7.52 (t, 2H, ArH), 7.04 (d, 1H, ArH, $J = 2.4$ Hz), 6.79 (s, 2H, ArH), 6.43 (s, 2H, ArH), 4.63 (s, 2H, NH_2). ^{13}C NMR (DMSO-d_6) δ: 170.9, 165.5, 153.9, 150.7, 150.5, 133.0, 129.2, 127.4, 123.5, 122.7, 115.6, 111.0, 103.8, 64.2 ppm.
8. *Synthesis of N-(2,7-dichlorofluorescein)lactam-N′-phenylthiourea* (**L**): A portion of **1** (207 mg, 0.5 mmol) and phenyl isothiocyanate (0.19 mL, 1.0 mmol) were combined and dissolved in freshly distilled DMF (3 mL). The reaction was stirred at room temperature overnight. The reaction was added CH_2Cl_2 (100 mL) and washed with water several times, dried and evaporated of the solvent. The crude product was further purified by silica-gel column chromatography (elution: CH_2Cl_2/methanol = 10/1, v/v, R_f = 0.63) to give **L** (206 mg, 75.1%). ^{1}H NMR (CD_3OD) δ 8.06–7.97 (t, 2H), 7.81–7.73 (m, 2H), 7.31–7.05 (m, 7H), 6.79 (s, 2H). ^{13}C NMR (CD_3OD) δ: 183.8, 168.0, 164.8, 156.2, 153.6, 149.9, 139.6, 135.7, 131.2, 131.0, 129.3, 126.9, 126.6, 125.6, 124.9, 118.1, 111.4, 104.9, 67.5 ppm. FAB–MS ($M+H^+$): Calcd 550.0. Found: 550.0. Anal. Calcd for $C_{27}H_{21}Cl_2N_3O_6S$: C, 55.30; H, 3.61. Found: C, 55.06; H, 3.44.
9. (a) Association constants were obtained using the computer program ENZFITTER, available from Elsevier-BIOSOFT, 68 Hills Road, Cambridge CB2 1LA, United Kingdom; (b) Conners, K. A. *Binding Constants, The Measurement of Molecular Complex Stability*; Wiley: New York, 1987.