ORGANIC LETTERS

2003
Vol. 5, No. 9
1419−1422

Fluorescence Sensing of Ammonium and Organoammonium Ions with Tripodal Oxazoline Receptors

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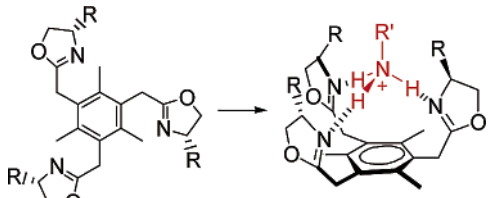
Received January 9, 2003

ABSTRACT



A new class of fluorescence sensors for ammonium and organoammonium ions has been disclosed. One of the sensors, an alaninol-derived tripodal oxazoline (1a) shows significant fluorescence enhancement upon binding NH_4^+ but little response toward K^+, Na^+, and Mg^{2+} ions. Owing to its chiral environment, a phenylglycinol-derived tripodal oxazoline (1b) shows chiral discrimination in fluorescence upon binding enantiomeric guests.

The selective recognition and sensing of ammonium and organoammonium ions have received considerable research interest in light of the potential applications in medical, biochemical, and environmental areas.[1] In the course of our study on the molecular recognition of biologically active amines through their ammonium salts using oxazoline-based receptors,[2] we found that the receptor system can be developed into a new class of fluorescence sensors for ammonium and organoammonium ions. A variety of fluorescence sensors have been reported for various analytes including organoammonium ions, for which a photoinduced electron transfer (PET) mechanism has been widely used as the fluorescence signaling process.[3,4] Recently, the conformational restriction of fluorophores has also been utilized as a useful way of fluorescence signaling.[5] Herein, we wish to report the fluorescence sensing of ammonium and organo-

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(1) Bühlmann, P.; Pretsch, E.; Bakker, E. *Chem. Rev.* **1998**, *98*, 1593.

(2) (a) Ahn, K. H.; Kim, S.-G.; Jung, J.; Kim, K.-H.; Kim, J.; Chin, J.; Kim, K. *Chem. Lett.* **2000**, 170. (b) Kim, S.-G.; Ahn, K. H. *Chem. Eur. J.* **2000**, *6*, 3399. (c) Kim, S.-G.; Kim, K.-H.; Jung, J.; Shin, S. K.; Ahn, K. H. *J. Am. Chem. Soc.* **2002**, *124*, 591.

(3) (a) *Fluorosensors for Ion and Molecular Recognition*; Czarnik, A. W., Ed.; American Chemical Society: Washington, DC, 1994; Vol. 538. (b) de Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E. *Chem. Rev.* **1997**, *97*, 1515. (c) Valeur, B.; Leray, I. *Coord. Chem. Rev.* **2000**, *205*, 3. Prodi, L.; Bolletta, F.; Montalti, M.; Zaccheroni, N. *Coord. Chem. Rev.* **2000**, *205*, 59. (d) Fabbrizzi, L.; Licchelli, M.; Rabaioli, G.; Taglietti, A. *Coord. Chem. Rev.* **2000**, *205*, 85.

(4) For the fluorescence sensing of organoammonium ions based on the PET process, see: (a) de Silva, A. P. *Angew. Chem., Int. Ed. Engl.* **1990**, *29*, 1173. (b) Ballardini, R.; Balzani, V.; Credi, A.; Gandolfi, M. T.; Kotzyba-Hibert, F.; Lehn, J.-M.; Prodi, L. *J. Am. Chem. Soc.* **1994**, *116*, 5741. The fluorescence sensing of chiral organoamines through a quenching mode, not organoammonium ions, based on binaphthol derivatives, see: (d) Pugh, V. J.; Hu, Q.-S.; Pu, L. *Angew. Chem., Int. Ed.* **2000**, *39*, 3638. (e) Beer, C.; Rurack, K.; Daub, J. *Chem. Commun.* **2001**, 1138. (f) Reetz, M. T.; Sostmann, S. *Tetrahedron* **2001**, *57*, 2515. Lin, J.; Zhang, H.-C.; Pu, L. *Org. Lett.* **2002**, *4*, 3297.

10.1021/ol034041m CCC: $25.00
Published on Web 04/10/2003

ammonium ions using the tripodal oxazoline receptors. Our receptor system contains a benzene fluorophore, which is a rare case.

We have shown that benzene-based tripodal oxazolines **1a** (Me-BTO) and **1b** (Ph-BTO) are strong and selective receptors toward ammonium and organoammonium ions, respectively.[2,6] During the studies, we observed that our receptors exhibited fluorescence when monitored by silica gel TLC. This observation led us to investigate their fluorescence behavior in detail. We surmised that a tripodal oxazoline complex of the H_3O^+ ion in the silica gel was responsible for the fluorescence. The oxazolines **1** (Figure 1) and ammonium or organoammonium ions are also expected to form similar complexes that exhibit fluorescence, which are disclosed in our study.

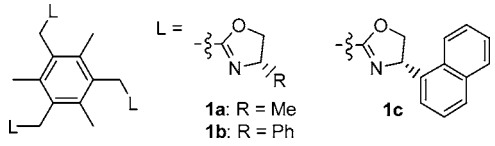

Figure 1. Tripodal oxaoline receptors.

We have studied the basic fluorescence behavior of the alaninol-derived oxazoline **1a**, a strong ammonium ion binder. **1a** showed two absorption bands with λ_{max} at 210 and 230, respectively, and one weak band with λ_{max} at 272 nm in CH_3CN (Figure S1). The emission spectrum of **1a**, recorded at each of the three absorption maxima, exhibited significant fluorescence only for the case of the excitation at 272 nm and with sample concentrations of millimolar ranges. The high concentration required is due to a low absorptivity of the benzene chromophore.[7]

We titrated **1a** with varying concentrations of NH_4^+ in CH_3CN at 295 K.

The fluorescence intensity increased gradually and reached a plateau when an equimolar amount of NH_4^+ was added, and little change was observed after that point (Figure 2). This saturation behavior indicates that a strong 1:1 host−guest complex forms, which results in enhanced fluorescence.

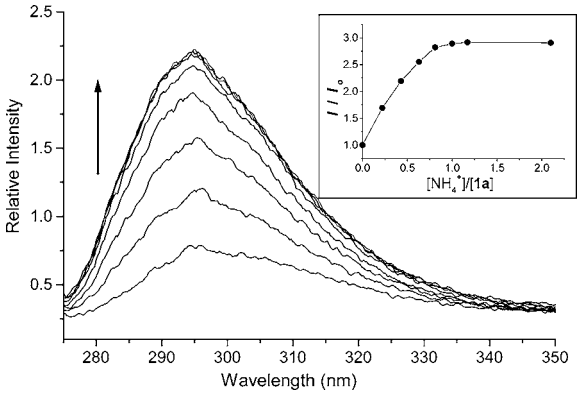

Figure 2. Fluorescence emission changes of **1a** (1.0 mM) upon addition of NH_4^+ (as ClO_4-salt; 0.0, 0.22, 0.43, 0.63, 0.81, 1.00, 1.17, and 2.10 equiv from the bottom) in CH_3CN following 272-nm excitation. Inset: Changes of the relative maximum intensity of fluorescence with respect to the $[NH_4^+]/[\mathbf{1a}]$ ratio.

At the saturation point, the increase in the fluorescence intensity amounted to 2.75 times that of the receptor only. Such enhancement was not observed in polar solvents such as methanol and water.[8]

We measured the quantum yield for the fluorescence enhancement by using quinine sulfate as a reference compound.[9] The quantum yield increased as $[NH_4^+]$ did, and reached a plateau when an equimolar amount of the guest was added (Figure 3). Thus the quantum yield of **1a** increased

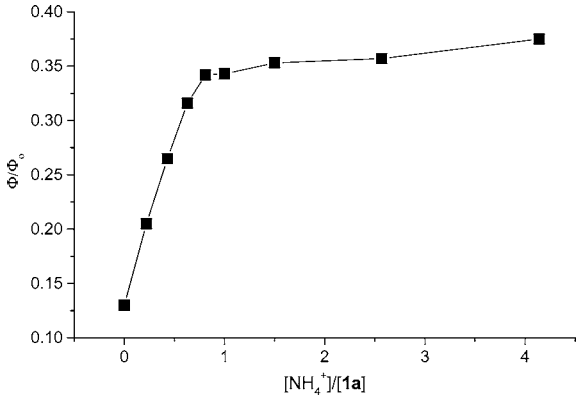

Figure 3. Changes of the quantum yield of Me-BTO **1a** upon addition of NH_4^+.

from $\Phi_o = 0.13$ to $\Phi = 0.34$ at the equivalent point, then slightly increased after that point ($\Phi = 0.37$ at the point of $[NH_4^+]/[\mathbf{1a}] = 4.1$). The increase in the quantum yield from the receptor only to the 1:1 complex corresponds to the

(5) For chemosensors that utilize the binding-induced conformational restriction of fluorophores, see: (a) Sandanayake, K.; Nakashima, H.; Shinkai, S. *Chem. Commun.* **1994**, 1621. (b) Takeuchi, M.; Mizuno, T.; Shinmori, H.; Nakashima, M.; Shinkai, S. *Tetrahedron*, **1996**, *52*, 1195. (c) Black, C. B.; Andrioletti, B.; Try, A. C.; Ruiperez, C.; Sessler, J. L. *J. Am. Chem. Soc.* **1999**, *121*, 10438. (d) McFarland, S. A.; Finney, N. S. *J. Am. Chem. Soc.* **2001**, *123*, 1260. (e) McFarland, S. A.; Finney, N. S. *J. Am. Chem. Soc.* **2002**, *124*, 1178.

(6) For selected examples of molecular recognition with other C_3 symmetric, benzene-based tripodal receptors, see: (a) Metzger, A.; Lynch, V. M.; Anslyn, E. V. *Angew. Chem., Int. Ed. Engl.* **1997**, *36*, 862. (b) Niikura, K.; Metzger, A.; Anslyn, E. V. *J. Am. Chem. Soc.* **1998**, *120*, 8533. (c) Sato, K.; Arai, S.; Yamagishi, T. *Tetrahedron Lett.* **1999**, *40*, 5219. (d) Chin, J.; Walsdorff, C.; Stranix, B.; Oh, J.; Chung, H. J.; Park, S.-M.; Kim, K. *Angew. Chem., Int Ed.* **1999**, *38*, 2756. (e) Lavigne, J. L.; Anslyn, E. V. *Angew. Chem., Int. Ed.* **1999**, *38*, 3666. (f) Kim, H.-J.; Kim, Y.-H.; Hong, J.-I. *Tetrahedron Lett.* **2001**, *42*, 5049.

(7) This problem may be solved, for example, by introducing conjugation moieties such as arylvinyl groups to the benzene ring instead of the 2,4,6-trimethyl groups, which is underway.

(8) For practical purposes, prior extraction of NH_4^+ out of water with **1a** or analogues[2a,c] followed by fluorescence analysis may be used.

(9) Lakowicz, J. R. *Principles of Fluorescence Spectroscopy*, 2nd ed.; Kluwer Academic: New York, 1999.

fluorescence intensity changes in Figure 2, supporting evidence that the fluorescence enhancement is due to the formation of a 1:1 inclusion complex.

Next, to evaluate the possibility of photoinduced electron transfer (PET) from the oxazoline nitrogen to the benzene fluorophore as a fluorescence mechanism, we synthesized naphthyl-substituted oxazoline **1c**.[10]

This experiment was also designed to examine whether a similar PET would operate from the naphthyl-oxazoline moiety; in this case, more intense fluorescence was anticipated. The new oxazoline was synthesized according to the established procedure[2b] starting with (*S*)-2-amino-2-(naphthalene-1-yl)ethanol.[11] The fluorescence change of the binding process was measured under similar conditions as described above. In this case, however, the fluorescence from the naphthalene ring was predominant, which appeared as broad peaks in the range of 320−340 nm when excited at an absorption maximum of 282 nm (Figure S2). Above all, little changes in the fluorescence resulted upon increasing [NH_4^+] (Figure S3). The small fluorescence change suggests that the naphthyl-substituted oxazoline does not constitute a PET system, in contrast to the well-known (aminomethyl)-aryl fluorophores in the literature (Figure 4).[3] Moreover, the

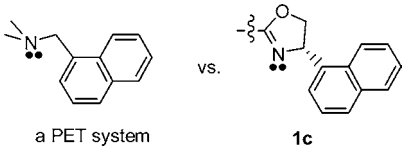

Figure 4.

titration of oxazoline **1a** with CF_3CO_2H resulted in quenching rather than fluorescence enhancement.[12] These results raise a question regarding the possibility of a PET process for the fluorescence enhancement in the case of oxazoline **1a** or **1b** upon guest binding.

When we measured the absorption spectra of **1a**-NH_4^+ complexes varying [NH_4^+], we observed spectra very similar to that of **1a** only (Figure S4). Thus the possibility of charge-transfer complex formation between **1a** and NH_4^+ also can be excluded based on this result.

From these results, the fluorescence enhancement shown in Figure 2 may be explained by the conformational restriction of the receptor **1a** upon binding NH_4^+, which results in reduced nonradiative decay.[13] This is relevant to the recent examples of fluorescence enhancement through the binding-induced conformational restriction of fluorophores.[5] Our result suggests that the fluorescence enhancement from conformational restriction may be extended to the whole receptor molecule as well as to the fluorophore itself. Extension of this approach to receptors with different fluorophores other than benzene may provide useful fluorescence sensors.

Next, we evaluated the fluorescence behavior of **1a** toward metal cations such as K^+, Na^+, and Mg^{2+} under the same experimental conditions. As shown in Figure 5, **1a** showed

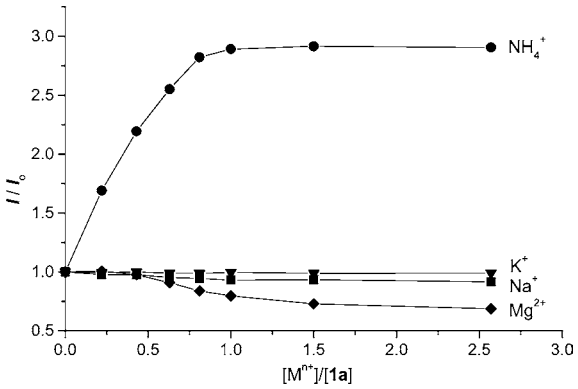

Figure 5. Changes of the relative maximum intensity of fluorescence with respect to the [M^{n+}]/[**1a**] ratio, wherein M^{n+} denotes the metal ions (perchlorate salts).

little response toward K^+ and Na^+, and slight quenching in the case of Mg^{2+}. Also, the titration of **1a** with NH_4^+ in the presence of excess K^+ (10 molar equiv) resulted in little fluorescence increase compared to the case without K^+ (Figure S5). Thus the fluorescence titration of NH_4^+ can be performed in the presence of an excess amount of K^+. The excellent selectivity is notable considering that K^+ is similar to NH_4^+ in terms of the charge and size. The little fluorescence changes toward the metal cations can be ascribed to a markedly lower binding affinity of **1a** toward the metal cations compared to NH_4^+,[14] thus producing less tightly bound inclusion complexes.

The interesting fluorescence behavior of **1a** was further extended to Ph-BTO **1b**, which was shown to be an efficient and unique receptor for the selective recognition of linear and chiral organoammonium ions.[2b,c] Ph-BTO **1b** also showed similar fluorescence enhancement upon binding NH_4^+ (Figure S6). A similar fluorescence enhancement was observed when we changed the analyte from NH_4^+ to $PhCH_2CH_2NH_3^+$ (as perchlorate salt), a basic structure of neurotransmitter catecholamines (Figure S7). In this case, the

(10) The oxazoline **1c** was synthesized following the similar route described in ref 2b: $[\alpha]_D^{18}$ +192.9 (*c* 0.52, $CHCl_3$); mp 128.2−130.0 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.87−7.36 (m, 7H), 5.88 (t, J = 9.4 Hz, 1H), 4.85 (dd, J = 10.2, 8.4 Hz, 1H), 4.00−3.95 (m, 3H), 2.63 (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.0, 139.4, 136.6, 134.4, 131.5, 131.2, 129.6, 128.3, 126.8, 126.3, 126.2, 123.8, 123.4, 75.1, 66.9, 31.0, 18.1; HRMS (FAB) calcd for $C_{51}H_{45}N_3O_3$ [M + H]$^+$ 748.3539, found 748.3527.

(11) Takacs, J. M.; Jaber, M. R.; Vellekoop, A. S. *J. Org. Chem.* **1998**, *63*, 2742.

(12) The fluorescence of **1a** was almost quenched when 3 molar equiv of CF_3CO_2H was added. The result suggests that the protonation of the oxazoline nitrogen perturbs the fluorescence of **1a**. A further study is necessary to address the quenching mechanism.

(13) Nijegorodov, N. I.; Downey, W. S. *J. Phys. Chem.* **1994**, *98*, 5639 and references therein.

(14) The association constants of **1a** toward NH_4^+ and K^+ were 2.5 × 10^7 and 5.7 × 10^4 M^{-1}, respectively, determined for the picrate salts by the extraction-UV titration method (ref 2a).

maximum enhancement reached 1.72 times when 2.3 molar equiv of the analyte was added. This result indicates that **1b** and its derivatives can be used as new fluorescent sensors toward related organoammonium ions of biological importance.

Since Ph-BTO **1b** provides a chiral environment, we also examined enantioselective fluorescence sensing toward both enantiomeric salts of 1-phenylethylamine (1-PEA). The fluorescence enhancement was larger in the case of the (*R*)-salt, which is expected from its larger association constant compared to the (*S*)-salt[2c] (Figure 6). At a fixed concentration

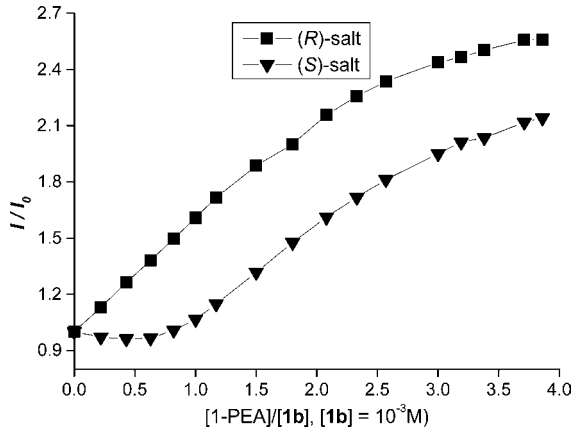

Figure 6. Fluorescence intensity changes of **1b** upon addition of (*R*)- and (*S*)-salts of 1-PEA.

of the perchlorate salt of 1-PEA (1.0 mM in CH_3CN), various known mixtures of the enantiomers were subjected to fluorescence measurements. As shown in Figure 7, a polynominal plot of the relative maximum intensity of the fluorescence versus the (*R*)-isomer percentage shows that the present system can be applied for real-time monitoring of the enantiomeric purity of a racemic 1-PEA sample.

In summary, we have presented a novel example of fluorescence sensing of ammonium and organoammonium ions with oxazoline receptors. The alaninol-derived oxazoline

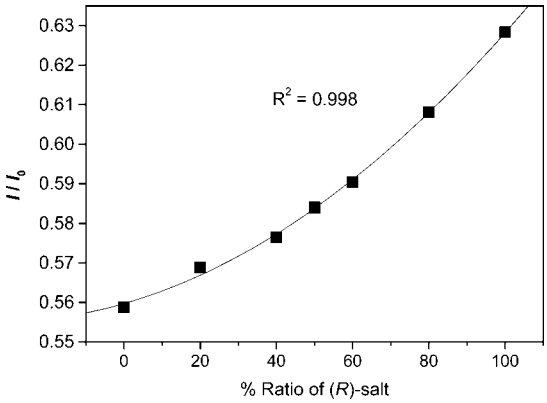

Figure 7. A plot of fluorescence maximum intensity vs the percent enantiomeric ratio of racemic 1-PEA samples (1.0 mM, CH_3CN).

receptor **1a** shows significant fluorescence enhancement upon binding NH_4^+, whereas it shows little enhancement upon binding metal cations such as K^+, Na^+, and Mg^{2+}. The phenylglycinol-derived oxazoline **1b** is shown to be a promising fluorescence sensing system toward organoammonium ions, including a chiral one. A further study to develop tripodal oxazoline analogues that operate at longer excitation wavelengths is in progress.

Acknowledgment. This work was supported by the Korea Science & Engineering Foundation (Basic Research Program, Grant No. R02-2002-000-00103-0) and the Center for Integrated Molecular Systems. K.H.A. thanks Professors S.-M. Park, T. H. Joo (POSTECH, Korea), and J. P. Bolender (University of San Diego, USA) for helpful discussions.

Supporting Information Available: Figures S1−S7 giving UV absorption spectra of **1a** and **1c**, fluorescence emission changes of **1c**, **1a**, and **1b** under different conditions, and changes of UV absorption spectra of **1a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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