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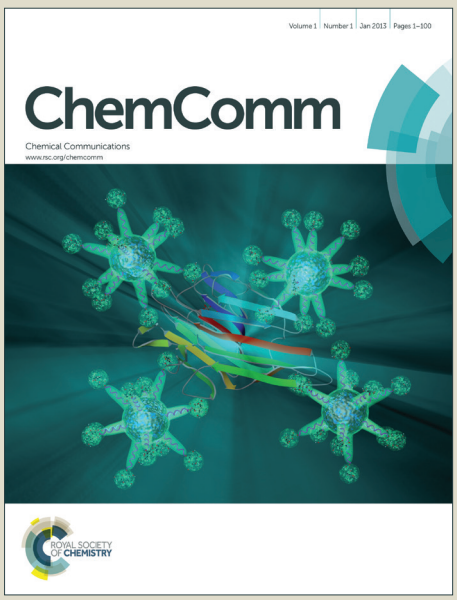

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ARTICLE TYPE

A Bispyrene Derivative as a Selective Fluorescent Probe for RNA†

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A novel bispyrene compound was synthesized to selectively detect RNA through excimer emission "turn-on" in aqueous solution at physiological pH (7.4). The compound was used to successfully image RNA in HeLa cells.

Since their discovery in 1868,[1] ribonucleic acids (RNAs) have attracted great interest due to their critical roles in biological processes. RNA is known to perform active roles in the coding, regulation, and expression of genes, among other functions.[2] Consequently, the development of RNA detection and recognition technology is of great importance. Although there have been many reports regarding fluorescent detection of DNA,[3] G-Quadruplex DNA,[4] and nucleotides[5-6], relatively few examples can be found of RNA detection[7-9].

These probes for RNA detection often require complicated synthetic steps to link the oligonucleotides to fluorophores. In addition, the selectivity for RNA over other nucleic acids is often insufficient. Recently, Chang *et al*.[7] and Kim *et al*.[8] reported the development of RNA-selective probes based on small positively charged molecules. Chang and co-workers reported two small molecules for RNA detection; however, relatively small differences in affinity were observed between RNA and DNA.[7] On the other hand, Kim *et al*. recently reported small cyclophane-containing imidazolium moieties which can selectively detect RNA over DNA in living cells.[8]

As delineated above, selective fluorescent detection of RNA is still a challenging task. Here we report a neutral bispyrene probe with high selectivity and sensitivity for RNA in aqueous solution at physiological pH, 7.4, which can be used for fluorescence imaging of RNA in live cells.

For the synthesis of probe **1**, 1-hydroxypyrene-2-carbaldehyde (compound **2**) was first prepared from 1-bromopyrene in 3 steps following a previously reported procedure.[9] Compound **2** was then reacted with diethylenetriamine to give **1** as a red solid in 80% yield (Scheme 1). Probe **1** was fully characterized by ^{1}H NMR, ^{13}C NMR and HRMS (FAB).

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† This paper is dedicated to the occasion of the 70th Birthday of Seiji Shinkai
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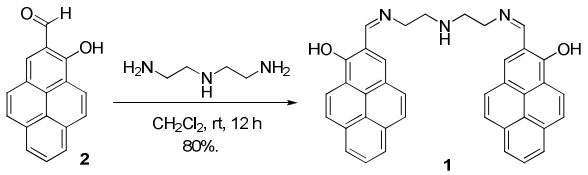
Fluorescence properties were examined in 99.8% Tris-HCl buffer (and 0.2% DMSO) at pH 7.4. The emission spectra are presented in Fig. 1. Upon the addition of 1.75 eq. of ssRNA, ctDNA, UTP, TTP, poly (dA-dT) or poly (dG-dC) to probe **1**,



Scheme 1. Synthesis of fluorescent probe **1**.

no change, including any quenching effect, was observed in the emission of probe **1**. On the other hand, when 1.75 eq. of total RNA was added, a selective fluorescence enhancement was observed at 470 nm, the excimer emission of pyrene fluorophores[10]. Fig. 2 presents the fluorescence titrations of probe **1** with total RNA. As shown in Fig. 2(b), the peak at 470 nm arose from excimer emission of pyrene enhanced by increasing amounts of RNA, which may indicate that the two pyrenes in probe **1** are mostly π-stacked in RNA.

Total cellular RNA consists of ssRNA, tRNA, rRNA and microRNA. As shown in Fig. 1, excimer peak enhancement was not observed for ssRNA or tRNA, unlike with total RNA. Thus, one can propose that rRNA, accounting for ~80% of

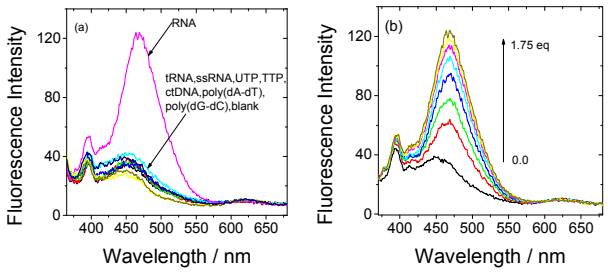


Fig 1. (a) Fluorescence changes of **1** (20 μM) upon the addition of RNA, tRNA, ssRNA, ctDNA, UTP, TTP, poly (dA-dT), poly (dG-dC) (1.75 equiv. each) in 0.05 M Tris-HCl (pH 7.4). (b) Fluorescence titrations of **1** (20 μM) with total RNA (0.0, 0.25, 0.5, 0.75, 1.0, 1.25, 1.5, 1.75 equiv.) (λ_{ex} = 348 nm).

total RNA, induces the excimer peak enhancement. Fig. 2 presents the ^{1}H NMR spectra of probe **1** with total RNA. The aromatic protons of the pyrene moieties were assigned based on TOCSY and ROESY 2D NMR experiments as shown in Fig. S4. After adding 1 equiv. of total RNA, aliphatic protons

at 3.79 and 3.04 showed small downfield shifts to 3.83 and 3.10, respectively, (Fig. 2). In addition, the H10 resonances of pyrene ring (doublet, 8.29 and 8.32) slightly shifted upfield to 8.29 and 8.32 ppm (Fig. 2). However, the other aromatic protons showed no significant chemical shift changes (Fig. 2). These results can be attributed to the possible hydrogen bonding interaction between the OH group and phosphate backbone of RNA. When the unique conformation of rRNA induces π–π stacking interaction between two pyrenes in probe **1** through intermolecular hydrogen bonding interaction, the probe **1** exhibits fluorescence emission.

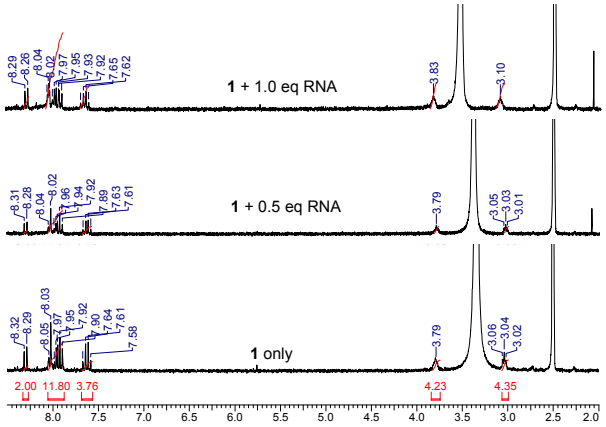


Fig 2. ^{1}H NMR titration of **1** (1 mM) in DMSO-d_6 with 0.0, 0.5, 1.0 equiv. RNA (from bottom to up). RNA was dissolved in D_2O.

To test the RNA imaging properties of probe **1**, HeLa cells (human epithelial adenocarcinoma) were incubated with probe **1** (20 μM in 0.2% DMSO) in the culture media at 37 ºC for 1 h. In the absence of probe **1**, the cells exhibited no fluorescence, but the cells incubated with probe **1** exhibited strong fluorescence (Fig. 3). To determine whether probe **1** binds to DNA or RNA, the cells were fixed and treated with

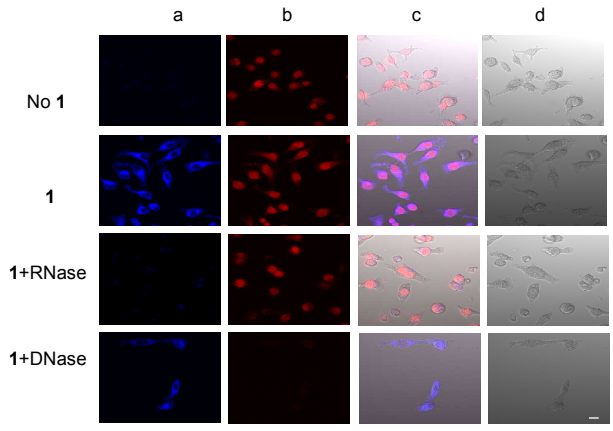


Fig 3. Fluorescence images of probe **1** in HeLa cell. Cells were fixed with 4 % paraformaldehyde and treated with RNase or DNase in the 40 mM Tris-HCl, pH 7.5, 5 mM $MgCl_2$ buffer for 30 min at 37 ºC. And the cells were incubated in the absence or presence of 20 μM JEJ-1 for 1 h at 37 ºC. DRAQ5 was used as DNA stain. (a) **1** only (b) DRAQ5 (c) merge (d) DIC. Scale bars : 10 μm.

DNase or RNase. When the cells were treated with RNase, no fluorescence was observed; however, cells treated with DNase

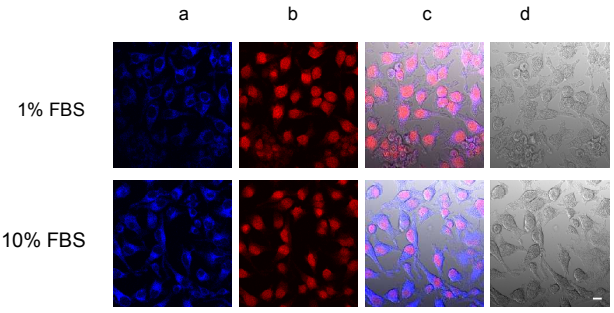


Fig 4. Fluorescence intensities of probe **1** according to different serum concentrations. The HeLa cells were cultured in 1 % fetal bovine serum, RPMI 1640 media for 24 h and then exchanged 10 % fetal bovine serum, RPMI 1640 media for 8 h. After 1 h incubation with **1** and 10 min incubation with DRAQ5, the cell images were captured using a confocal microscopy. (a) **1** only (b) DRAQ5 (c) merge (d) DIC. Scale bars: 10 μm.

exhibited fluorescence, as shown in Fig. 3. DRAQ5 stain was used as a DNA staining agent.

Serum (FBS, fetal bovine serum) in the culture media is essential to cellular growth and RNA synthesis.[11] Cells were cultured with 1% FBS in RPMI-1640 media for 24 h, and then a subset thereof were cultured with 10% FBS in RPMI-1640 media for an additional 8 h. After probe **1** was administered to the live cells for 1 h, the cells were examined by confocal microscopy. While the cells cultured only with 1% FBS displayed relatively weak fluorescence, the cells cultured with 10% FBS displayed strong fluorescence (Fig. 4). In addition, these data confirm that the live cell membrane is permeable to probe **1**.

Actinomycin D is an antineoplastic antibiotic that inhibits cell proliferation. This compound inhibits the proliferation of cells nonspecifically by forming a stable complex with double-stranded DNA (via deoxyguanosine residues), thus inhibiting DNA-primed RNA synthesis.[12] Actinomycin D (2 μg/ml) was administered to cells for 4 h, and the fluorescence intensity of probe **1** in these cells was measured. The fluorescence intensity was reduced in cells receiving actinomycin D treatment, supporting the finding that probe **1** binds to RNA specifically (Fig. 5).

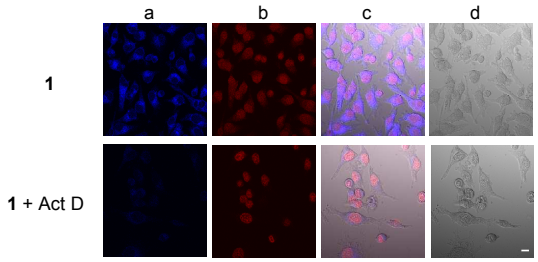


Fig 5. Fluorescence changes of probe **1** in the cell after the treatment of actinomycin D. Actinomycin D treated to the cell 2 μg/ml for 4 h and washed with DPBS and incubated with 20 μM **1** for 1 h. (a) **1** only (b) DRAQ5 (c) merge (d) DIC. Scale bars : 10 μm.

The potential cytotoxic effects of probe **1** were also examined in HeLa cells (Fig. S6). Cells were incubated with each concentration of probe **1** for 4 h and 24 h. Cell death was not detected after 4 h of incubation, but 83% cell death occurred after 24 h incubation with 50 μM probe **1**. This result can be attributed to the binding of RNA by probe **1** causing

inhibition of RNA translation or degradation.

In conclusion, we have shown that a relatively simple bispyrene derivative, probe **1,** can serve as a fluorescent probe for selectively detecting RNA within living cells. This probe is suited for biological conditions and is designed to specifically target RNA out of many other biological molecules. Cell imaging of RNA was also successfully performed in HeLa cells. The molecular interaction of probe **1** with specific RNA sequences will be explored in the future.

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Notes and references

1. R. Dahm, *Developmental Biology*, 2005, **278**, 274-288.
2. (a) E. Szathmáry, *Trends Genet.*, 1999, **15**, 223-229; (b) G. North, *Nature*, 1987, **328**, 18.
3. (a) H. N. Kim, J. Lim, H. N. Lee, J.-W. Ryu, M. J. Kim, J. Lee, D.-U. Lee, Y. Kim, S.-J. Kim, K. D. Lee, H.-S. Lee and J. Yoon, *Org. Lett.*, 2011, **13**, 1314; (b) A. Okamoto, A. Kanatani and I. Saito, *J. Am. Chem. Soc.*, 2004, **126**, 4820; (c) A. Okamoto, Y. Ochi and I. Saito, *Chem. Commun.*, 2005, 1128; (d) M. Kitamura, A. Nimura, K. Yamana and T. Shimidzu, *Nucleic Acids Res. Symp. Ser.*, 1991, **25**, 67; F. D. Lews, Y. Zhang and R. L. Letsinger, *J. Am. Chem. Soc.*, 1997, **119**, 5451.
4. (a) Y. J. Seo, I. J. Lee, J. W. Yi and B. H. Kim. *Chem Commun.*, 2007, 2817; (b) H. N. Kim, E.-H. Lee, Z. Xu, H.-E. Kim, H.-S. Lee, J.-H. Lee and J. Yoon, *Biomaterials*, 2012, **33**, 2282; (c) C. Allain, D. Monchaud and M. P. Teulade-Fichou, *J. Am. Chem. Soc.*, 2006, **128**, 11890; (d) P. Yang, A. De Cian, M. P. Teulade-Fichou, J. L. Mergny and D. Monchaud, *Angew. Chem. Int. Ed.*, 2009, **48**, 2188.
5. (a) Y. Zhou, Z. Xu, J. Yoon, *Chem. Soc. Rev*. **2011**, *40*, 2222; (b) T. Sakamoto, A. Ojida and I. Hamachi, *Chem. Commun*., 2009, **45**, 141.
6. (a) C. Li, M. Numata, M. Tekeuchi and S. Shinkai, *Angew. Chem. Int. Ed.*, 2005, **44**, 6371; (b) J. Y. Kwon, N. J. Singh, H. Kim, S. K. Kim, K. S. Kim and J. Yoon, *J. Am. Chem. Soc.*, 2004, **126**, 8892; (c) P. J. Santangelo, A. W. Lifland, P. Curt, Y. Sasaki, G. J. Bassell, M. E. Lindquist and J. E. Crowe Jr, *Nature Methods*, 2009, **6**, 347; (d) S. Ikeda, T. Kubota, H. Yanagisawa, M. Yuki and A. Okamoto, *Nuceic Acids Symposium Series*, 2009, **53**, 155; (e) N. A O'Connor, N. Stevens, D. Samaroo, M. R. Solomon, A. A. Martí, J. Dyer, H. Vishwasrao, D. L. Akins, E. R. Kandel and N. J. Turro, *Chem. Commun.*, 2009, 2640; (f) A. W. gifland, C. Zurla and P. J. Santangelo, *Bioconjugate Chem.*, **2010,** *21,* 483; (g) S.Sando and E.T. Kool, *J. Am. Chem. Soc.*, 2002, **124**, 9686; (h) R. Kierzek, , Y. Li, D. H. Turner, and P. C. Bevilacqua, *J. Am. Chem. Soc.*, 1993, **115**, 4985; (i) Z. Xu, N. R. Song, J. H. Moon, J. Y. Lee and J. Yoon, *Org. Biomol. Chem.*, 2011, **9**, 8340; (j) S. K. Silverman, and T. R. Cech, *Biochemistry*, 1999, **38**, 14224; (k) K. Yamana, R. Iwase, S. Furutani, H. Tsuchida, H. Zako, T. Yamaoka, and A. Murakami, *Nucleic Acids Res.*, 1999, **27**, 2387; (l) A. Mahara, R. Iwase, T. Sakamoto, K. Yamana, T. Yamaoka and A. Murakami, *Angew. Chem. Int. Ed.*, 2002, **41**, 3648; (m) H. S. Jeong, S. Kang, J. Y. Lee and B. H. Kim, *Org. Biomol. Chem.*, 2009, **7**, 921; (n) H. Jeon, S. Lee, Y. Li, S. Park and J. Yoon, *J. Mater. Chem.*, 2012, **22**, 3795; (o) G. V. Zyryanov, M. A. Palacios and P. Anzenbacher, Jr. *Angew. Chem. Int. Ed.,* 2007, **46**, 7849; (p) Y. Kurishita, T. Kohira, A. Ojida and I. Hamachi, *J. Am. Chem. Soc.,* 2010, **132**, 13290; (q) X. Chen, M. J. Jou and J. Yoon, *Org. Lett.*, 2009, **11**, 2181; (r) H.-W. Rhee, H.-Y. Choi, K. Han and J.-I. Hong, *J. Am. Chem. Soc.,* 2007, **129**, 4524; (s) T. Noguchi, A. Dawn, D. Yoshihara, Y. Tsuchiya, T. Yamamoto and S. Shinkai, *Macromol. Rapid Commun.*, 2013, **34**, 779.
7. (a) Q. Li, Y. K . Kim, J. Namm, A. Kulkarni, G. Rosania, Y. H. Ahn, Y. T. Chang, *Chem. Biol.*, 2006, **13**, 615; (b) S. Feng, Kim, Y. K.; S. Yang, Y. T. Chang, *Chem. Commun.*, 2010, **46**, 436.
8. B. Shirinfar, N. Ahmed, Y. S. Park, G.-S. Cho, I. S. Youn, J.-K. Han, H. G. Nam and K. S. Kim, *J. Am. Chem. Soc.*, 2013, **135**, 90.
9. (a) Y. Zhou, W. Fang, Y. Kim, S. Kim and J. Yoon, *Org. Lett.*, 2009, **11**, 4442; (b) P. Demerseman, J. Einhorn, J. F. Gourvest, R. Royer, *J. Heterocyclic Chem.*, 1985, **22**, 39.
10. (a) J. Wu, Y. Zou, C. Li, W. Sicking, I. Piantanida, T. Yi and C. Schmuck, *J. Am. Chem. Soc.*, 2012, **134**, 1958; (b) Z. Xu, N. J. Singh, J. Lim, J. Pan, H. N. Kim, S. Park, K. S. Kim and J. Yoon, *J. Am. Chem. Soc.*, 2009, **131**, 15528; (c) H. S. Jung, M. Park, D. Y. Han, E. Kim, C. Lee, S. Ham and J. S. Kim, *Org. Lett.*, 2009, **11**, 3378.
11. A. Zetterberg and O. Skold, *Experimental Cell Research*, 1969, **57**, 114; (b) C. M. Smith and J. A. Steitz, *Mole. and Cell. Biol*., 1998, **18**, 6897.

A Bispyrene Derivative as a Selective Fluorescent Probe for RNA

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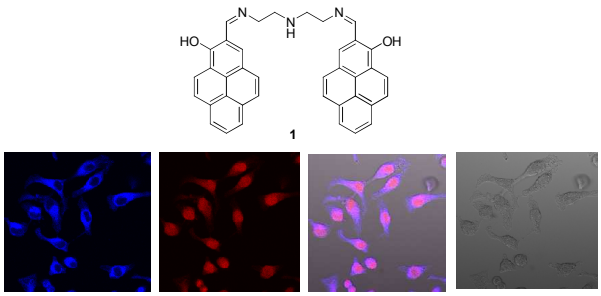

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A novel bispyrene compound was synthesized to selectively detect RNA through excimer emission “turn-on” at physiological pH.