JACS
JOURNAL OF THE AMERICAN CHEMICAL SOCIETY
Communication
pubs.acs.org/JACS

Long-Lived Luminogenic Probe for Detection of RNA in a Crude Solution of Living Bacterial Cells

Hisao Saneyoshi,[‡,†] Yoshihiro Ito,*[,‡,⊥] and Hiroshi Abe*[,‡,§,⊥]

[‡]Nano Medical Engineering Laboratory, RIKEN, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan
[§]PRESTO, Japan Science and Technology Agency, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan
[⊥]Emergent Bioengineering Materials Research Team, RIKEN Center for Emergent Matter Science, 2-1 Hirosawa, Wako, Saitama 351-0198, Japan

Supporting Information

ABSTRACT: A pre-type sensitizer for a lanthanide complex on an oligonucleotide was successfully converted to a perfect final structure in a target DNA/RNA-templated reaction, without any chemical reagent or enzyme, under neutral conditions. The final form of the lanthanide−oligonucleotide provided a long-lived luminescence signal, appropriate for time-gated luminescence analysis and signal amplification. Target DNA/RNA-assisted time-gated luminescence analysis is a powerful tool for elimination of autofluorescence and detection of target RNA in living bacterial cells.

Detection and imaging of DNA/RNA species in native cells are gaining importance in biological and biomedical research. However, contamination by autofluorescence disturbs the desired signal. Thus, elimination of severe autofluorescence from biological samples is important for performing sensitive and simple experiments. Generally, the biological environment has several autofluorescent components, such as NADH, FAD, and riboflavin. To reduce the effect of autofluorescence, and thus obtain high signal/background (S/B) ratios, long-lived luminescent compounds may be useful. These can be easily discriminated in terms of large differences in fluorescence lifetime.[1,2]

Time-gated fluorescence measurement using organic fluorescent compounds has been applied to *in vitro* detection of RNA to overcome autofluorescence.[3] However, organic fluorescent compounds provide only a slightly longer fluorescence lifetime than autofluorescence. For example, a pyrene excimer had a 40 ns lifetime, compared with 7 ns of autofluorescence from cellular extracts. In contrast, lanthanide complexes offered a much longer luminescence lifetime (in the order of milliseconds). Lanthanide complexes have been successfully employed in sensing metal ions, monitoring enzymatic activity, and detecting proteins in living cells, time-gated spectroscopy, microscopy, and flow cytometry.[4]

In the field of nucleic acid detection, Oser et al. proposed the use of lanthanide-based probes and applied them to template-mediated formation of lanthanide complexes for the first time.[5] Later, several chemists reported template-mediated lanthanide formation for sensing target nucleic acid sequences.[6] These split-type probes depend on the hybridization affinity and an appropriate distance between the light-harvesting group and chelator with lanthanide ion to obtain luminescence of a similar level as the molecular beacon-type probes recently reported.[7] However, lanthanide-based detection methods have been applied only to the detection of RNA or DNA *in vitro*, and have not been applied to the detection of cellular RNA.

Nucleic acids modified with gold nanoparticles have been used to detect nucleic acids. Sensitive detection at a femtomolar level was achieved *in vitro* through the measurement of light scatter.[8] Fluorogenic gold nanoparticles have been used to detect RNA in living cells.[9] The great benefit of gold nanoparticles is their cell membrane permeability.

Conversely, lanthanide probes with luminogenic properties and long-lived luminescence could provide high S/B ratios, by removing autofluorescence, for the detection of cellular RNA, although these probes require transfection reagents for introduction into cells. To solve the problems associated with previous lanthanide-based oligonucleotide probes, including the inability to amplify signals, we designed a new chemical-reaction-triggered luminogenic DNA probe. A DNA-templated chemical reaction could offer significant advantages, such as high S/B ratio and signal amplification for detection. Various DNA-templated fluorogenic chemical reactions have been reported.[10] However, no chemical-reaction-triggered, lanthanide-based luminogenic probe has been reported. A key issue is how to make a switch for lanthanide-based luminogenic systems. As the switching system, Terai et al.[4e] proposed a photoinduced electron transfer (PeT) system, using aniline attached onto 7-amino-4-methyl-2(1*H*)-quinolinone (carbostyril 124), which was previously used for a labeling study.[11] They demonstrated that the PeT mechanism could be used as an ON-OFF switch, and they successfully applied it to a long-lived protease probe.[4d] For highly sensitive probes for DNA/RNA sensing applications, an ON switch is needed. One possibility, proposed by the same authors, is to use an antenna molecule. The key to this mechanism is the structural change of the antenna molecule,[12a] initiated by Skraup reaction between acrolein and the aniline derivative, to form a quinoline skeleton as an antenna molecule. This absorbs a much longer wavelength than the starting aniline derivative. The antenna molecule is specifically excited to transfer energy to the lanthanide complex. Quite recently, Pershagen et al.[12b] reported

Received: July 2, 2013

   dx.doi.org/10.1021/ja406724k | J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

that an acyclic coumarin precursor was converted into an active antenna, triggered by analytes such as $Pd^{0/2+}$, H_2O_2, F^-, and enzymes. However, to apply this system to our oligonucleotide probe, we ideally needed neutral reaction conditions and no requirement for additional chemicals or enzymes.

Here, we synthesize a new antenna molecule using the Staudinger reaction and apply it to development of a lanthanide-based luminogenic DNA probe. This probe enables sensitive detection of DNA or RNA by time-gated luminescence analysis, as shown in Figure 1. We demonstrate direct detection of RNA in living *Escherichia coli* cells by measuring samples in a cuvette.

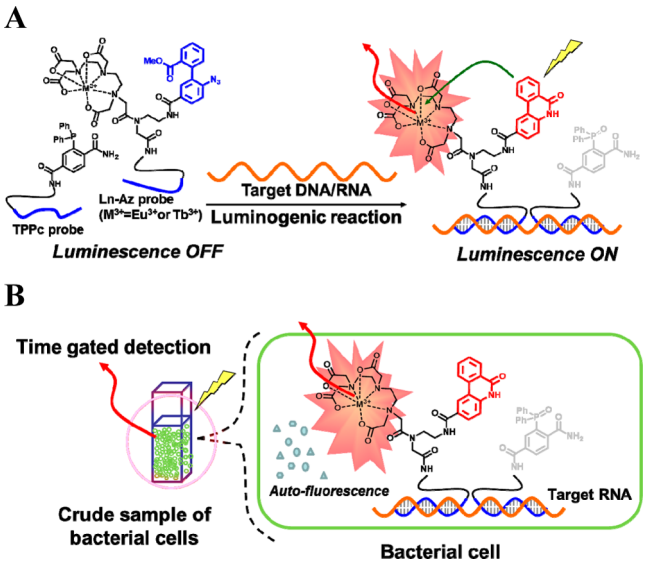


Figure 1. (A) Chemical-reaction-triggered luminogenic reaction. (B) Detection of RNA species in the crude solution of bacterial cells by time-gated luminescence analysis.

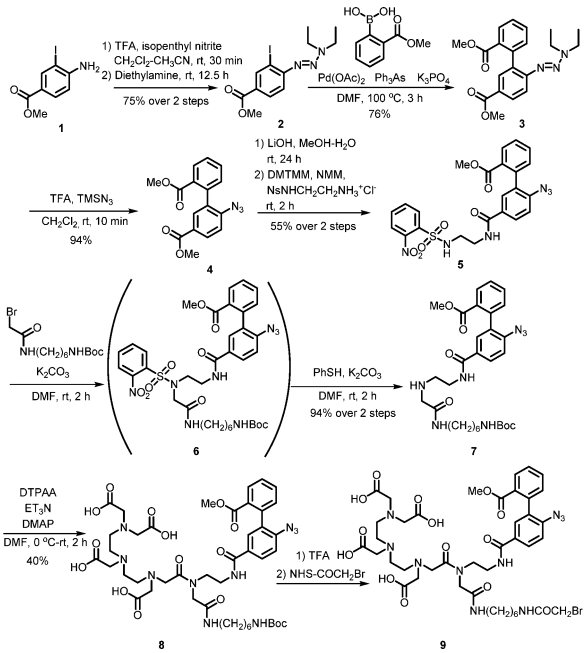
For design of the antenna molecule to be switched by chemical reaction, we separated 2-quinolinone, which is the core structure of carbostyril 124, as shown in Figure S1. When the lactam structure was divided at the amide moiety into ester and amine, no emission was expected. Previous studies have reported the generation of fluorescence by coumarin or quinolinone, starting from *o*-hydroxy- or *o*-amino-cinnamate derivatives via double bond isomerization by photoirradiation.[13] The *Z*-isomer is the critical conformation for the formation of the lactam structure to recover the active antenna molecule structure. However, it is thermally unstable and light sensitive, and rapidly isomerizes to the *E*-isomer, which cannot be converted to a lactam ring under neutral conditions at ambient temperature because the reactive groups are too distant.

To solve this problem, we conformationally locked the active geometry to add a benzene ring to the double bond moiety for cyclization between ester and amine, as shown in Figure S1. The *o*-amine(de)−*o*-ester pair on the biphenyl skeleton is highly reactive and forms a phenanthridinone ring.[14] To control autocyclization, the amino group was caged as an azide group. After reduction of the azide group with a reducing reagent such as phosphine, we expected to obtain an active antenna structure to excite the lanthanide chelate. The expanded-ring version of 2-quinolinone, phenanthridinone, should have a similar UV absorption wavelength and act as the antenna molecule, stimulating the lanthanide complex. The formation of the new antenna structure was confirmed by UV absorbance following cyclization under reductive conditions (Figure S2). Figure S2 shows that phenyl azide group **4** was reduced by tris-carboxyethylphosphine (TCEP) to a cyclized species, phenanthridinone derivative **4′**. The cyclization reaction ceased after 200 s, as shown in Figure S2.

For the synthesis of the target probe, aniline derivative **1** was chosen as a starting material. The amino group was converted into triazene for protection and pre-activation.[15] The triazene derivative **2** was assembled with *o*-methoxybenzoylboronic acid using Suzuki coupling conditions, to give biaryl compound **3**. Subsequently, the triazene group was rapidly converted into azide derivative **4** by reaction with trimethylsilyl azide ($TMSN_3$) under acidic conditions.

In Scheme 1, methyl benzoate derivative **4** was treated with lithium hydroxide, and then the carboxyl group was

Scheme 1. Synthesis of Pre-antenna-Chelator Complex



immediately coupled with mono-*o*-nitrobenzenesulfonyl (Ns)-protected ethylenediamine hydrochloride, using 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride as a condensation reagent to give **5**. A linker moiety for conjugation with the oligonucleotide probe was attached at the sulfone amide by alkylation, followed by the deprotection of the Ns group with thiophenol.[16] Diethylenetriaminepentaacetic acid (DTPA) was selected as a chelator because of its high stability for lanthanide ions.[17] Diethylenetriaminepentaacetic anhydride was coupled with secondary amine **7** to give compound **8**.

A model luminogenic compound (**8′**) was synthesized from **8** by treatment with $EuCl_3$ in MeOH in the presence of NaOH. UV spectra and time-gated luminescence spectra clearly showed the conversion to a lactam structure (**8″**) and a 1500-fold increase in luminescence, relative to the acyclic form, upon reduction, as shown in Figure 2.

For the synthesis of luminogenic DNA-Az probe (Ln-Az), the Boc group of compound **8** at the alkyl linker moiety was removed under acidic conditions and then coupled with bromoacetyl NHS ester to give the reagent for conjugation. Oligonucleotide-3′-phosphorothioate was synthesized by DNA

dx.doi.org/10.1021/ja406724k | J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

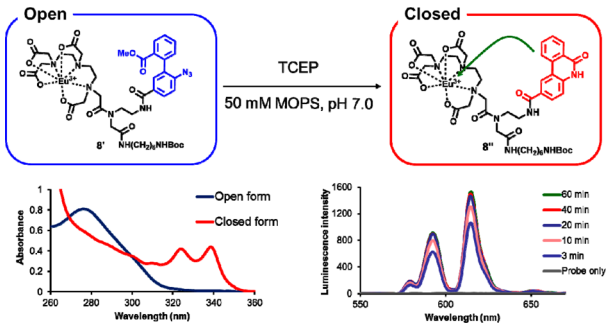


Figure 2. Spectra of luminogenic compound **8′** and luminescent compound **8″**. Left: UV spectra. Right: Time-gated luminescence spectra. UV spectrum conditions: 50 μM probe **8′**, 50 mM MOPS (pH 7.0), 20 mM TCEP. Time-gated luminescence spectra conditions: 1 μM probe, 50 mM MOPS, 2 mM TCEP, λ_{Ex} 340 nm.

synthesizer and coupled with bromoacetylated coupling unit **9**. In addition, Ln-Az probe was treated with TCEP to give the reduced Ln-Az probe (rLn-Az). Each probe was mixed with lanthanide ion (Eu^{3+} and Tb^{3+}) for use in photophysical studies, as shown in Figures 3 and S3 and Table S1.

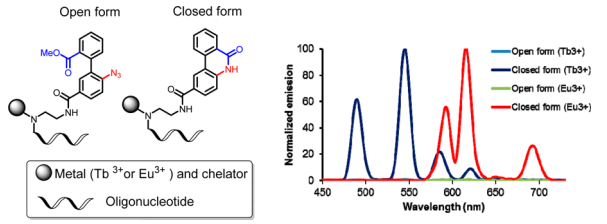


Figure 3. Structure of Ln-Az (open form) and rLn-Az probe (closed form) and their spectral properties.

The spectra of the new Ln-Az probe (open form) show that the open form did not emit fluorescence at 340 nm excitation. However, the rLn-Az probe (closed form) effectively emitted luminescence in the 490−700 nm range, depending on the central metals in the chelator (Figure 3). Maximum emission of the Eu^{3+} and Tb^{3+} probes was observed at 615 and 545 nm, respectively. The lifetimes of luminescence of these probes were 0.6 and 1.8 ms, respectively, which are sufficiently long to eliminate background fluorescence, as shown in Figure S5 and Table S1.[18] The lanthanide complex was relatively photostable under consecutive irradiation, compared with an organic fluorescent dye, pyrene (Figure S4).

Next, to know whether Ln-Az probe can discriminate a single base difference, detection of the *ras* gene was performed *in vitro* using time-gated spectroscopy. In the presence of target sequence, activation of Ln-Az (Eu^{3+}) probe by reduction of the triphenylphosphine carboxamide (TPPc)−oligonucleotide probe immediately generated a long-lived luminescence signal (Figure 4). On the other hand, experiments with mismatch-containing target or without target showed no luminescence. The maximum S/B ratio after 10 min was 400. The most intriguing property, compared with previous lanthanide-based oligonucleotide probes, was the ability to amplify signal during the detection process (Figure S6). We confirmed that the probe could amplify signal by at least 10-fold when a 100-fold excess of probe versus target DNA was used, as described in Figure S7.

Finally, the direct detection of RNA species in crude solution of living *E. coli* cells was performed. Ln-Az (Tb^{3+}) and TPPc

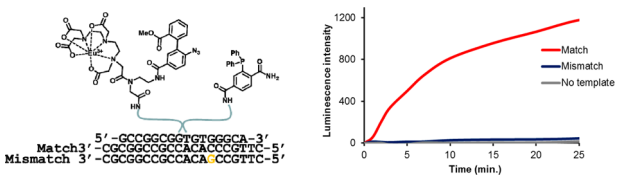


Figure 4. Time course of luminogenic reaction with full-match DNA, mismatch DNA, and without template. Conditions: 50 mM MOPS (pH 7.0), 100 mM NaCl, 10 μg/mL BSA, λ_{Ex} 340 nm, delay time 0.05 ms, gate time 3 ms, slit width, 10 nm. Reaction was monitored at λ_{Em} 615 nm. Ln-Az probe (Eu^{3+}): 5′-GCCGGCGG-Ln-Az (Eu^{3+})-3′, 250 nM. TPPc probe: 5′-TPPc-TGTGGGCA-3′, 500 nM. Match-DNA: 3′-CGCGGCCGCCACACCCGTTC-5′, 250 nM. Mismatch-DNA: 3′-CGCGGCCGCCACAGCCGTTC-5′, 250 nM.

probes were designed to target 23S rRNA. Probes (Match pair or Scramble pair) and cells were mixed in buffer in a cuvette and directly analyzed by fluorescence spectrometry. The autofluorescence from *E. coli* and the luminescent signal overlapped in the 400−600 nm range and could not be distinguished without time-gated monitoring, as shown in Figure 5A. However, using a wait time of 0.1 ms,

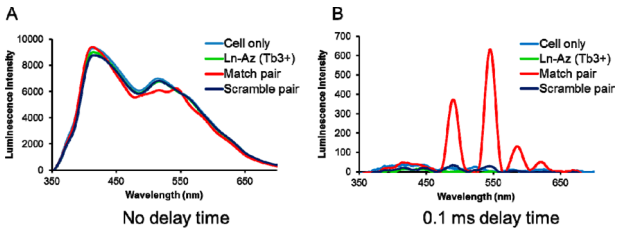


Figure 5. Detection of 23S rRNA in *E. coli* cells by direct measurement of cuvette sample: (A) no delay time and (B) 0.1 ms delay time. Conditions: (*E. coli*, JM109) 1OD, 50 mM MOPS (pH 7.0), 1 M NaCl, 0.05% SDS, λ_{Ex} 340 nm, delay time 0−0.1 ms, gate time 6 ms, slit width 10 nm. Ln-Az probe (Tb^{3+}): 5′-CTGGCGGTCTGGGTT-Ln-Az (Tb^{3+})-3′, 100 nM. TPPc probe with matched sequence: 5′-TPPc-GTTTCCCTCTTCACG-3′, 200 nM. TPPc with scramble sequence: 5′-TPPc-GCCTTCTCCCGAAGT-3′, 200 nM.

autofluorescence was reduced to negligible levels (Figure 5B), and the desired signal was observed with a high S/B ratio. Further, we tested detection of DNA sequences in solutions containing a high concentration of bovine serum albumin (BSA) or 10% fetal bovine serum (Figure S8A−D). In both solutions, no signal was detected in no-delay mode, but a distinct signal was observed in time-gated mode. Ln-Az probe was stable in these solutions since no significant increase of signal was observed without target DNA during the time course measurement (Figure S8E,F). Thus, we confirmed that time-gated luminescence analysis using these probes could sensitively detect RNA signals in biological samples. Although other fluorescence-based methods can detect RNA in bacterial cells by fluorescence microscopy,[10l,p] the direct detection of RNA in a crude solution of bacterial cells by fluorescence spectrometry has never been reported.

In this paper, we first designed a new phenanthridinone derivative (**8″** in Figure 2 or **4′** in Figure S2) that functioned as an antenna molecule. This means that the excited phenanthridinone prefers to transition from a singlet state to a triplet state. Based on this result, we designed a chemically switched antenna molecule (**9**) that was derivatized from phenanthridi-

dx.doi.org/10.1021/ja406724k | J. Am. Chem. Soc. XXXX, XXX, XXX−XXX

none. This reduction-triggered activation of an antenna molecule for a lanthanide sensor is a new system.

In conclusion, we designed and synthesized lanthanide-based luminogenic DNA probes, with two colors originating from the different metals in the chelator. The luminogenic DNA probes were successfully activated by chemical reaction only in the presence of target DNA or RNA and produced long-lived luminescence (0.6−1.8 ms), long enough to eliminate autofluorescence. It also amplified signal by multiple template reactions on the target. These luminogenic DNA probes represent a promising new tool for imaging using time-gated analysis. We are now expanding this concept for drug screening in multi-well plates and cell separation.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Experimental details and NMR spectra of all new compounds, Figures S1−S8, Scheme S1, and Table S1. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Authors

y-ito@riken.jp

h-abe@pharm.hokudai.ac.jp

Present Address

[†]H.S.: Department of Material and Life Chemistry, Faculty of Engineering, Kanagawa University, 3-27-1 Rokkakubashi, Kanagawa-ku, Yokohama 221-8686, Japan

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

H.A. was financially supported by the Ministry of Education, Culture, Sports, Science, and Technology (MEXT), Precursory Research for Embryonic Science and Technology (PREST), and the New Energy and Industrial Technology Development Organization (NEDO). H.S. financially supported by a Grant-in-Aid for Young Scientists (B). We are grateful for the support received from the Brain Science Institute (BSI) Research Resource Center for mass spectrum analysis.

■ REFERENCES

(1) (a) Selvin, P. R. *Annu. Rev. Biophys. Biomol. Struct.* **2002**, *31*, 275. (b) Bünzli, J. C. *Chem. Rev.* **2010**, *110*, 2729. (c) Binnemans, K. *Chem. Rev.* **2009**, *109*, 4283.

(2) Berezin, M. Y.; Achilefu, S. *Chem. Rev.* **2010**, *110*, 2641.

(3) Martí, A. A.; Li, X.; Jockusch, S.; Li, Z.; Raveendra, B.; Kalachikov, S.; Russo, J. J.; Morozova, I.; Puthanveettil, S. V.; Ju, J.; Turro, N. J. *Nucleic Acids Res.* **2006**, *34*, 3161.

(4) (a) Hanaoka, K.; Kikuchi, K.; Kojima, H.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2004**, *126*, 12470. (b) Thibon, A.; Pierre, V. C. *J. Am. Chem. Soc.* **2008**, *131*, 434. (c) Halim, M.; Tremblay, M. S.; Jockusch, S.; Turro, N. J.; Sames, D. *J. Am. Chem. Soc.* **2007**, *129*, 7704. (d) Mizukami, S.; Tonai, K.; Kaneko, M.; Kikuchi, K. *J. Am. Chem. Soc.* **2008**, *130*, 14376. (e) Terai, T.; Kikuchi, K.; Iwasawa, S.; Kawabe, T.; Hirata, Y.; Urano, Y.; Nagano, T. *J. Am. Chem. Soc.* **2006**, *128*, 6938. (f) New, E. J.; Parker, D.; Smith, D. G.; Walton, J. W. *Curr. Opin. Chem. Biol.* **2010**, *14*, 238. (g) Rajapakse, H. E.; Reddy, D. R.; Mohandessi, S.; Butlin, N. G.; Miller, L. W. *Angew. Chem., Int. Ed.* **2009**, *48*, 4990. (h) Rajapaksea, H. E.; Gahlauta, N.; Mohandessia, S.; Yub, D.; Turnerb, J. R.; Millera, L. W. *Proc. Natl. Acad. Sci. U.S.A.* **2010**, 13582. (i) Pazos, E.; Torrecilla, D.; López, M. V.; Castedo, L.; Mascaren, J. L.; Vidal, A.; Vázquez, M. E. *J. Am. Chem. Soc.* **2008**, *130*, 9652. (j) Jin, D.; Connally; Piper, R. J. *Cytometry* **2007**, *71A*, 783.

(5) Oser, A.; Valet, G. *Angew. Chem., Int. Ed. Engl.* **1990**, *10*, 1167.

(6) (a) Wang, G.; Yuan, J.; Matsumoto, K.; Hu, Z. *Anal. Biochem.* **2001**, *299*, 169. (b) Kitamura, Y.; Ihara, T.; Tsujimura, Y.; Tazaki, M.; Jyo, A. *Chem. Lett.* **2005**, 1606. (c) Karhunen, U.; Jaakkola, L.; Wang, Q.; Lamminmä ki, U.; Soukka, T. *Anal. Chem.* **2010**, *82*, 751.

(7) (a) Krasnoperov, L. N.; Marras, S. A. E.; Kozlov, M.; Wirpsza, L.; Mustaev, A. *Bioconjugate Chem.* **2010**, *21*, 319. (b) Li, J.; Zhou, W.; Ouyang, X.; Yu, H.; Yang, R.; Tan, W.; Yuan, J. *Anal. Chem.* **2011**, *83*, 1356.

(8) (a) Cao, Y. C.; Jin, R.; Mirkin, C. A. *Science* **2002**, *297*, 1536. (b) Alhasan, A. H.; Kim, D. Y.; Daniel, W. L.; Watson, E.; Meeks, J. J.; Thaxton, C. S.; Mirkin, C. A. *Anal. Chem.* **2012**, *84*, 4153.

(9) (a) Seferos, D. S.; Giljohann, D. A.; Hill, H. D.; Prigodich, A. E.; Mirkin, C. A. *J. Am. Chem. Soc.* **2007**, *129*, 15477. (b) Prigodich, A. E.; Randeria, P. S.; Briley, W. E.; Kim, N. J.; Daniel, W. L.; Giljohann, D. A.; Mirkin, C. A. *Anal. Chem.* **2012**, *84*, 2062.

(10) (a) Ma, Z.; Taylor, J.-S. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 11159. (b) Ma, Z.; Taylor, J.-S. *Bioorg. Med. Chem.* **2001**, *9*, 2501. (c) Ma, Z.; Taylor, J.-S. *Bioconjugate Chem.* **2003**, *14*, 679. (d) Sando, S.; Sasaki, T.; Kanatani, K.; Aoyama, Y. *J. Am. Chem. Soc.* **2003**, *125*, 15720. (e) Cai, J.; Li, X.; Yue, X.; Taylor, J. S. *J. Am. Chem. Soc.* **2004**, *126*, 16324. (f) Cai, J.; Li, X.; Taylor, J. S. *Org. Lett.* **2005**, *7*, 751. (g) Dose, C.; Ficht, S.; Seitz, O. *Angew. Chem., Int. Ed.* **2006**, *45*, 5369. (h) Grossmann, T. N.; Seitz, O. *J. Am. Chem. Soc.* **2006**, *128*, 15596. (i) Pianowski, Z. L.; Winssinger, N. *Chem. Commun.* **2007**, 3820. (j) Dose, C.; Seitz, O. *Bioorg. Med. Chem.* **2008**, *16*, 65. (k) Franzini, R. M.; Kool, E. T. *ChemBioChem* **2008**, *9*, 2981. (l) Abe, H.; Wang, J.; Furukawa, K.; Oki, K.; Uda, M.; Tsuneda, S.; Ito, Y. *Bioconjugate Chem.* **2008**, *19*, 1219. (m) Franzini, R. M.; Kool, E. T. *Org. Lett.* **2008**, *10*, 2935. (n) Furukawa, K.; Abe, H.; Hibino, K.; Sako, Y.; Tsuneda, S.; Ito, Y. *Bioconjugate Chem.* **2009**, *20*, 1026. (o) Pianowski, Z.; Gorska, K.; Oswald, L.; Merten, C. A.; Winssinger, N. *J. Am. Chem. Soc.* **2009**, *131*, 6492. (p) Franzini, R. M.; Kool, E. T. *J. Am. Chem. Soc.* **2009**, *131*, 16021. (q) Shibata, A.; Abe, H.; Ito, M.; Kondo, Y.; Shimizu, S.; Aikawa, K.; Ito, Y. *Chem. Commun.* **2009**, 6586. (r) Li, H.; Franzini, R. M.; Bruner, C.; Kool, E. T. *ChemBioChem* **2010**, *11*, 2132. (s) Prusty, D. K.; Herrmann, A. *J. Am. Chem. Soc.* **2010**, *132*, 12197. (t) Arian, D.; Kovbasyuk, L.; Mokhir, A. *Inorg. Chem.* **2011**, *50*, 12010. (u) Röthlingshöfer, M.; Gorska, K.; Winssinger, N. *J. Am. Chem. Soc.* **2011**, *133*, 18110. (v) Röthlingshöfer, M.; Gorska, K.; Winssinger, N. *Org. Lett.* **2011**, *14*, 482. (w) Prusty, D. K.; Kwak, M.; Wildeman, J.; Herrmann, A. *Angew. Chem., Int. Ed.* **2012**, *51*, 11894.

(11) (a) Selvin, P. R.; Hearst, J. E. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 10024. (b) Li, M.; Selvin, P. R. *J. Am. Chem. Soc.* **1995**, *130*, 8132. (c) Chen, J. Y.; Selvin, P. R. *J. Am. Chem. Soc.* **2000**, *122*, 657. (d) Ge, O. H.; Selvin, P. R. *Bioconjugate Chem* **2004**, *15*, 1088.

(12) (a) Togashi, M.; Urano, Y.; Kojima, H.; Terai, T.; Hanaoka, K.; Igarashi, K.; Hirata, Y.; Nagano, T. *Org. Lett.* **2010**, *12*, 1704. (b) Pershagen, E.; Nordholm, J.; Borbas, K. E. *J. Am. Chem. Soc.* **2012**, *134*, 9832.

(13) (a) Hershfield, R.; Schmir, G. L. *J. Am. Chem. Soc.* **1973**, *95*, 7359. (b) Gagey, N.; Neveu, P.; Benbrahim, C.; Goetz, B.; Aujard, I.; Baudin, J.-B.; Jullien, L. *J. Am. Chem. Soc.* **2007**, *129*, 9986. (c) Horaguchi, T.; Hosokawa, N.; Tanemura, K.; Suzuki, T. *J. Heterocycl. Chem.* **2002**, *39*, 61.

(14) (a) Siddiqui, M. A.; Snieckus, V. *Tetrahedron Lett.* **1988**, *29*, 5463. (b) Leenders, R. G. G.; Scheeren, H. W. *Tetrahedron Lett.* **2000**, *41*, 9173.

(15) (a) Knepper, K.; Vanderheiden, S.; Bräse, S. *Eur. J. Org. Chem.* **2006**, 1886. (b) Avemaria, F.; Zimmermann, V.; Bräse, S. *Synlett* **2004**, *7*, 1163.

(16) Fukuyama, T.; Jow, C.-K.; Cheung, M. *Tetrahedron Lett.* **1995**, *36*, 6373.

(17) Martell, A. E.; Smith, R. M. *Critical stability constants*; Plenum Press: New York, 1989.

(18) (a) 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. (b) Lakowicz, J. R. *Principle of fluorescence spectroscopy*; Springer: Berlin, 2006.