A Fluorogenic Reaction Based on Heavy-Atom Removal for Ultrasensitive DNA Detection

Deepak K. Prusty and Andreas Herrmann*

Department of Polymer Chemistry, Zernike Institute for Advanced Materials, University of Groningen, Nijenborgh 4, 9747 AG Groningen, The Netherlands

Received June 14, 2010; E-mail: a.herrmann@rug.nl

Abstract: Fluorogenic reactions have recently emerged as a powerful tool for detection, diagnostics, and biosensing applications in a chemical and biological context. However, conventional fluorogenic systems reported to date rely on energy- or photo-induced electron transfer within the probes. Our communication demonstrates a conceptually new approach for generating a strong fluorescence signal through chemical bond formation mediated by a heavy-atom removal process. This method has favorable photophysical properties such as exceptional quantum yield and very low limits of fluorogenic DNA detection.

Fluorogenic reactions are powerful analytical tools that have been employed to detect phenols and nitrosothiols in water[1] and acetylenes and copper in polymers.[2] In a biological context, fluorogenic transformations have been utilized for the labeling of proteins, including virus capsids,[3] bovine serum albumin,[4] and synthetic polypetides,[5] as well as visualizing single biocatalytic events.[6] In particular, DNA-templated fluorogenic reactions[7] have been exploited for nucleic acid detection and monitoring of chemical bond formation in DNA−polymer nanoparticles.[8] These diverse applications are based primarily on two representative classes of fluorogenic reaction. In one strategy, nonfluorescent compounds are reacted in a bi- or trimolecular transformation to yield a fluorescent product.[8,9] The other method relies on the quenching ability of azide functional groups through photoinduced electron transfer. Reaction of the azides can remove this effect and consequently restore the fluorescence.[10]

The transformations described above were suitable for tailored applications. However, all of these systems entailed significant trade-offs between signal-to-noise ratio, detection limit, reaction rate, degree of conversion, reactant stability, and biocompatible photophysical properties. The utility and applicability of fluorogenic reactions would be greatly improved by the development of stable reporter compounds that exhibit longer emission wavelengths and higher fluorescence quantum yields.

To this end, we report here a conceptually new approach for generating strong fluorescence upon chemical bond formation mediated by heavy-atom removal from a profluorescent substrate. Boron dipyrromethane (BODIPY) chromophores were selected to demonstrate this concept and simultaneously address some of the above issues. These materials exhibit high extinction coefficients (ε) and high fluorescence quantum yields (Φ_{fl}), and in addition, their absorption and fluorescence spectra can be easily tuned by simple chemical modification of the pyrrole rings.[11] We describe the synthesis of the profluorescent BODIPY substrate and its conversion into a highly emissive fluorophore via Pd-catalyzed Heck reactions. We then show the power of this approach in a DNA-templated fluorogenic reaction allowing ultrasensitive nucleic acid detection.
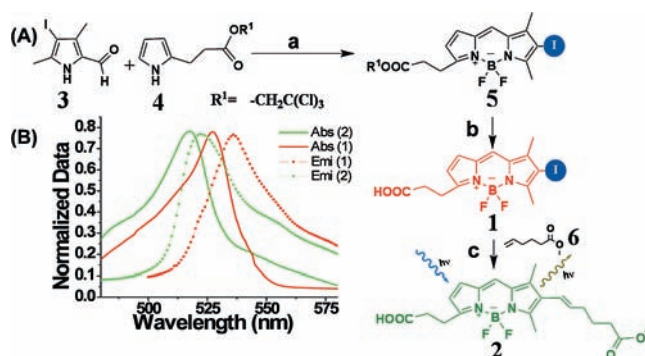

Figure 1. (A) Synthesis and (B) photophysical properties of iodinated BODIPY probe **1** and fluorescent Heck-coupling product **2**. In (A), the reaction conditions are as follows: (a) (i) $POCl_3$, CH_2Cl_2, 0 °C, 6 h; (ii) $BF_3 \cdot OEt_2$, diisopropylethylamine, RT, 12 h; 68% yield for two steps. (b) Acetone, porcine liver esterase, 0.1 M phosphate buffer (pH 8.0), 63% yield. (c) $Pd_2(dba)_3$, $P(t\text{-}Bu)_3$, CY_2NMe, dioxane, RT, 1 h, 53% yield. In (B), separately normalized absorption and emission spectra of **1** ($\lambda_{abs} = 527$, $\lambda_{em} = 536$ nm) and **2** ($\lambda_{abs} = 517$, $\lambda_{em} = 523$ nm) are shown.

Bisiodinated BODIPY derivatives have been shown to exhibit the heavy-atom quenching effect.[12] It is well-known that the introduction of iodine into a chromophore favors triplet formation through intersystem crossing due to spin−orbit coupling and thereby depopulates the emissive singlet state. For this study, we synthesized a monoiodinated BODIPY derivative with a carboxylic acid functional group, 4,4-difluoro-5,7-dimethyl-6-iodo-4-bora-3a,4a-diaza-*s*-indacene-3-propionic acid (**1**), that allows straightforward chemical attachment to biological analytes. We prepared profluorescent substrate **1** in two steps from pyrrole derivatives with aldehyde and propionic ester functional groups as starting materials (**3** and **4**, Figure 1A) with an overall yield of 68%.[13,14] The two starting materials were condensed in the presence of boron trifluoride to yield BODIPY trichloroethyl ester **5**. This was subsequently converted into the carboxylic acid **1** by enzymatic hydrolysis employing porcine liver esterase.

To test the suitability of **1** as a substrate for fluorogenic reactions, it was subjected to a Heck reaction with the methyl ester of 5-hexenoic acid (**6**), which produced highly fluorescent **2** in 53% yield. The contrast between the photophysical properties of substrate **1** and product **2** is significant. The absorption and emission maxima of **2** are blue-shifted relative to those for **1** by 12 and 13 nm, respectively. Moreover, the fluorescence quantum yield of fluorophore **2** ($\Phi_{fl2} = 0.75$) is 25 times greater than that of the precursor **1** ($\Phi_{fl1} = 0.03$), as determined in chloroform against the reference dye cresyl violet in methanol. Notably, the Heck reaction product **2** exhibits a relatively long emission wavelength ($\lambda_{em} = 523$ nm) and, to the best of our knowledge, the highest fluorescence quantum yield (75%) reported to date for fluorogenic systems.[15]

As a proof of concept, this fluorogenic conversion was evaluated for nucleic acid detection in an aqueous environment along the lines

of DNA-templated Heck reactions reported by Liu and co-workers.[16] Compounds **1** and **6** were coupled to amino-modified oligonucleotides (ODNs) via the corresponding *N*-hydroxysuccinimidyl esters [Schemes S3 and S4 in the Supporting Information (SI)]. The sequences and measured molecular masses are presented in Table S2 and Figure S3. These ODN conjugates were used in DNA-templated Heck reactions in the presence of a water-soluble Pd catalyst with two DNA template architectures, linear (L) and tritemplate (T) (Figure 2A, left and right, respectively). These templates gave conversion yields of 42 and 60%, respectively, as determined by densitometric gel analysis (Figure 2B, the lowest mobility bands in lanes 1) and HPLC (Figure S4). The isolated reaction products were confirmed by MALDI-TOF mass spectrometry (Figures S5 and S6). In contrast, no product formation was observed in poly(acrylamide) gel electrophoresis (PAGE) for either template architecture in the absence of catalyst or with single-base mismatches. Further analysis of the fluorescence spectra of T-type conversions revealed excellent mismatch sensitivity, with a 20-fold increase in fluorescence intensity for fully matched versus single-base-mismatched templates. Indeed, the fluorescence intensities for single-base mismatches were comparable to those of negative controls, indicating that virtually no reaction occurs without a fully matched template (Figure 2C).
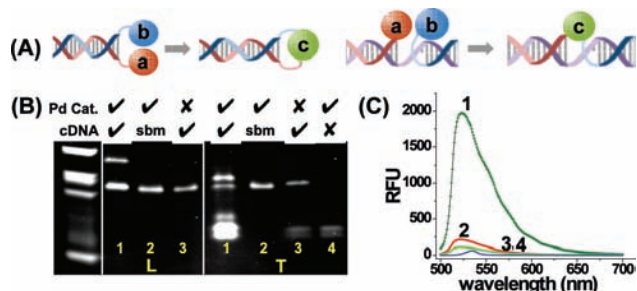

Figure 2. (A) Schematic representation of (left) linear template (L) and (right) tritemplate (T) architectures employed for fluorogenic DNA-templated reaction. Spheres marked **a** and **b** represent iodo-BODIPY reporter **1** and Heck reagent **6**, respectively, and **c** represents fluorescent product **2**, as shown in Figure 1A. (B) PAGE results of L- and T-type reactions and controls. Single-base mismatches are labeled "sbm". (C) Fluorescence spectra ($\lambda_{ex} = 515$ nm) of T-type reactions. Numbers in (B) and (C) denote experimental conditions: fully complementary DNAs (cDNAs) with catalyst (lanes 1), single-base-mismatched DNAs with catalyst (lanes 2), cDNAs without catalyst (lanes 3), and catalyst without template (lane 4).

We performed further investigations of the kinetics of fluorogenic heavy-atom displacement in the T-type architecture by fluorescence spectroscopy. The time courses of the fluorescence signals for the T-type conversion and controls are shown in Figure 3A. In the presence of fully complementary ODNs and catalyst, 90% of the fluorescence maximum was reached after only 10 min, and saturation was achieved within 20 min (Figure 3A, curve 1). The use of a single-base-mismatched template slowed the reaction dramatically, reducing the initial intensity growth 10-fold (Figure 3A, curve 2).

Because of the promising selectivity and favorable reaction kinetics of this DNA-templated fluorogenic conversion in the T architecture, the limit of detection was subsequently determined using a standard spectrophotometer. The fluorescence intensity after reaction completion was measured for concentrations of all ODNs ranging from 1 nM to 1 pM (Figure 3b). The limit of detection was calculated using a reported method[17] (see section 6 in the SI). The detection limit of our system was found to be an exceptional 10 pM, which, again to the best of our knowledge, is 1 order of magnitude lower than that reported for any other DNA-templated fluorogenic reaction.[18,19]
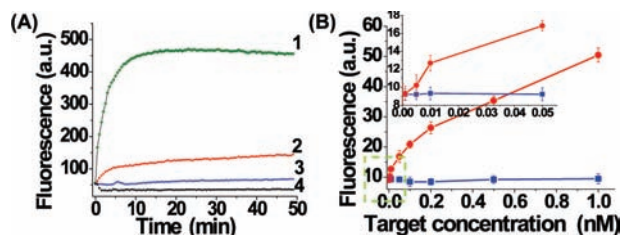
Figure 3. Reaction kinetics and limit of detection for T-type architecture. (A) Evolution of fluorescence intensity over time: cDNAs with catalyst (curve 1), single-base-mismatched DNAs with catalyst (curve 2), cDNAs without catalyst (curve 3), and catalyst without template (curve 4). See section 5 in the SI for reaction conditions. (B) Fluorescence intensities for a range of template concentrations (●) and without template (■). Inset: magnification of the 1−50 pM range. Fluorescence was monitored at 523 nm ($\lambda_{ex} = 515$ nm).

In conclusion, we have demonstrated the generation of a potent fluorophore by the Pd-catalyzed displacement of iodine from a BODIPY core and the powerful application of this reaction in ultrasensitive DNA detection. The favorable photophysical properties of the dye can be applied to a broader range of analytes because of the carboxylic acid functionality, which allows straightforward conjugation to diverse biomolecules. The use of iodine as a quencher species affords still greater flexibility. Though we selected the Heck reaction as a proof of concept, there are several reactions that could be used for the iodine displacement, including enzymatic and named reactions such as Sonogashira−Hagihara and Suzuki−Miyaura couplings. As such, we believe this novel concept of fluorescent probes based on heavy-atom release through chemical bond formation has the potential to greatly improve the power and scope of fluorogenic reactions for diagnostics and biosensing.

Supporting Information Available: Synthetic and experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) Guo, H. M.; Minakawa, M.; Tanaka, F. *J. Org. Chem.* **2008**, *73*, 3964. (b) Pan, J.; Downing, J. A.; McHale, J. L.; Xian, M. *Mol. BioSyst.* **2009**, *5*, 918.

(2) (a) O'Reilly, R. K.; Joralemon, M. J.; Hawker, C. J.; Wooley, K. L. *Chem.−Eur. J.* **2006**, *12*, 6776. (b) Ievins, A. D.; Wang, X. F.; Moughton, A. O.; Skey, J.; O'Reilly, R. K. *Macromolecules* **2008**, *41*, 2998.

(3) Bruckman, M. A.; Kaur, G.; Lee, L. A.; Xie, F.; Sepullvecla, J.; Breitenkamp, R.; Zhang, X.; Joralemon, M.; Russell, T. P.; Emrick, T.; Wang, Q. *ChemBioChem* **2008**, *9*, 519.

(4) Dirks, A. J.; Cornelissen, J. J. L. M.; Nolte, R. J. M. *Bioconjugate Chem.* **2009**, *20*, 1129.

(5) (a) Beatty, K. E.; Liu, J. C.; Xie, F.; Dieterich, D. C.; Schuman, E. M.; Wang, Q.; Tirrell, D. A. *Angew Chem., Int. Ed.* **2006**, *45*, 7364. (b) Beatty, K. E.; Xie, F.; Wang, Q.; Tirrell, D. A. *J. Am. Chem. Soc.* **2005**, *127*, 14150.

(6) (a) Velonia, K.; Flomenbom, O.; Loos, D.; Masuo, S.; Cotlet, M.; Engelborghs, Y.; Hofkens, J.; Rowan, A. E.; Klafter, J.; Nolte, R. J. M.; De Schryver, F. C. *Angew. Chem., Int. Ed.* **2005**, *44*, 560. (b) Roeffaers, M. B. J.; De Cremer, G.; Uji-i, H.; Muls, B.; Sels, B. F.; Jacobs, P. A.; De Schryver, F. C.; De Vos, D. E.; Hofkens, J. *Proc. Natl. Acad. Sci. U.S.A.* **2007**, *104*, 12603.

(7) (a) Silverman, A. P.; Kool, E. T. *Chem. Rev.* **2006**, *106*, 3775. (b) Franzini, R. M.; Kool, E. T. *Org. Lett.* **2008**, *10*, 2935. (c) Shibata, A.; Abe, H.; Ito, M.; Kondo, Y.; Shimizu, S.; Aikawa, K.; Ito, Y. *Chem. Commun.* **2009**, 6586.

(8) Alemdaroglu, F. E.; Ding, K.; Berger, R.; Herrmann, A. *Angew. Chem., Int. Ed.* **2006**, *45*, 4206.

(9) Huang, Y.; Coull, J. M. *J. Am. Chem. Soc.* **2008**, *130*, 3238.

(10) Le Droumaguet, C.; Wang, C.; Wang, Q. *Chem. Soc. Rev.* **2010**, *39*, 1233.

(11) Ulrich, G.; Ziessel, R.; Harriman, A. *Angew. Chem., Int. Ed.* **2008**, *47*, 1184.

(12) Yogo, T.; Urano, Y.; Ishitsuka, Y.; Maniwa, F.; Nagano, T. *J. Am. Chem. Soc.* **2005**, *127*, 12162.

(13) Wan, C. W.; Burghart, A.; Chen, J.; Bergstrom, F.; Johansson, L. B. A.; Wolford, M. F.; Kim, T. G.; Topp, M. R.; Hochstrasser, R. M.; Burgess, K. *Chem.—Eur. J.* **2003**, *9*, 4430.

(14) Malan, S. F.; van Marle, A.; Menge, W. M.; Zuliana, V.; Hoffman, M.; Timmerman, H.; Leurs, R. *Bioorg. Med. Chem.* **2004**, *12*, 6495.

(15) (a) Sivakumar, K.; Xie, F.; Cash, B. M.; Long, S.; Barnhill, H. N.; Wang, Q. *Org. Lett.* **2004**, *6*, 4603. (b) Xie, F.; Sivakumar, K.; Zeng, Q. B.; Bruckman, M. A.; Hodges, B.; Wang, Q. *Tetrahedron* **2008**, *64*, 2906. (c)

Furukawa, K.; Abe, H.; Hibino, K.; Sako, Y.; Tsuneda, S.; Ito, Y. *Bioconjugate Chem.* **2009**, *20*, 1026.

(16) Gartner, Z. J.; Kanan, M. W.; Liu, D. R. *Angew. Chem., Int. Ed.* **2002**, *41*, 1796.

(17) Baeumner, A. J.; Pretz, J.; Fang, S. *Anal. Chem.* **2004**, *76*, 888.

(18) Pianowski, Z. L.; Winssinger, N. *Chem. Commun.* **2007**, 3820.

(19) Grossmann, T. N.; Seitz, O. *J. Am. Chem. Soc.* **2006**, *128*, 15596.

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