Organic & Biomolecular Chemistry

Cite this: *Org. Biomol. Chem.*, 2012, **10**, 3595

www.rsc.org/obc

COMMUNICATION

# A small molecule sensor for fluoride based on an autoinductive, colorimetric signal amplification reaction†

**Matthew S. Baker and Scott T. Phillips***

***Received 20th February 2012, Accepted 11th March 2012***
**DOI: 10.1039/c2ob25363a**



**This article describes a small molecule reagent that is capable of detecting fluoride down to 0.12 mM (2.3 ppm) in water. The reagent reveals this level of fluoride through a novel autoinductive signal amplification reaction that produces an unambiguous colorimetric readout.**

Inexpensive and operationally straightforward methods for detecting fluoride in water are needed for measuring the quality of drinking water in resource-limited regions. Herein we describe reagent **1** as a unique sensor for aqueous fluoride (Fig. 1). Reagent **1** functions *via* an autoinductive signal amplification reaction and provides a time-dependent colorimetric readout that reveals the level of fluoride in a sample of water. We also describe an efficient one-step synthesis of **1**, as well as the development and optimization of assay conditions using reagent **1** that enable both semi-quantitative and quantitative measurements of aqueous fluoride down to 2.3 ppm (0.12 mM), which is within the relevant window of the US Environmental Protection Agency's (EPA) recommended fluoride concentration in water (2 ppm)[1] as well as the EPA's mandated upper limit (4 ppm).[1]

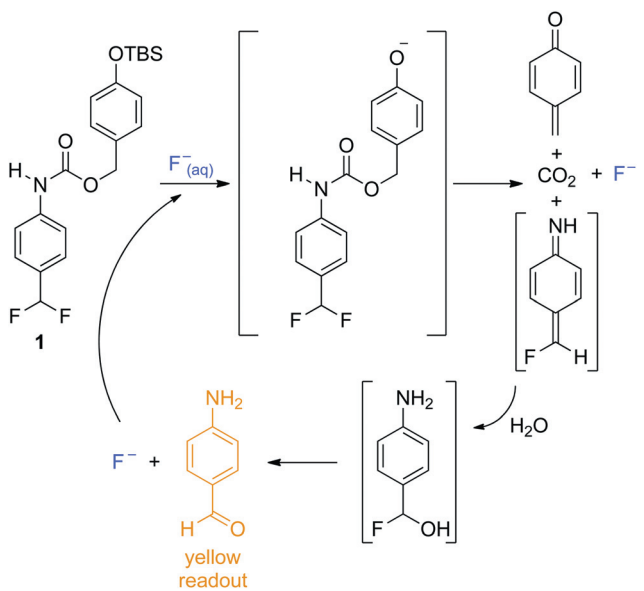



**Fig. 1** Pathway by which reagent **1** detects aqueous fluoride, amplifies 4-aminobenzaldehyde as a colorimetric readout, and releases two more equivalents of fluoride to propagate the autoinductive signal amplification reaction.

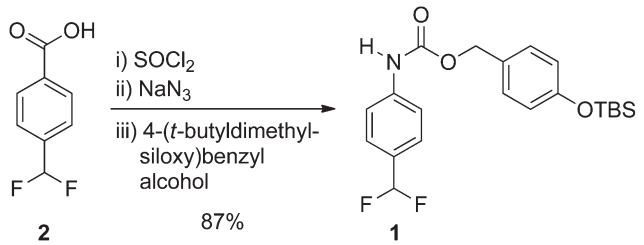
Reagent **1** is unique compared with other reagents for detecting fluoride because of a combination of three features: (i) it detects fluoride in an *aqueous* sample,[2] (ii) it detects fluoride derived from inorganic salts rather than from fluoride bound to a phase-transfer catalyst such as tetrabutylammonium,[3] and (iii) it amplifies signal for the detection event to increase the sensitivity of the assay.[4] These capabilities arise from a designed reaction cascade and network of reactions. Specifically, the reactions are: fluoride-mediated cleavage of the silyl ether group in **1** that induces a cascade elimination reaction (Fig. 1). This cascade reaction releases 4-aminobenzaldehyde (which produces a bright yellow-colored solution) and two new fluoride ions. These two fluoride ions then propagate the signal amplification reaction in a reaction network by reacting with more of **1** to increase the sensitivity of the assay. This approach is complimentary to a recent, independent study conducted by Shabat *et al.*[5] in the area of fluoride detection.

*Rationale for developing a fluoride detection reagent.* Studies now indicate that long-term ingestion of water containing more than 4 ppm of fluoride can cause dental and skeletal fluorosis, as well as osteoporosis.[6] Recent estimates suggest that approximately 200 million people from among 25 nations face hazardous levels of naturally occurring fluoride in drinking water.[7] Many of these people live in resource-limited regions where advanced water purification facilities are lacking[8] and where standard analytical equipment[9] (such as ion chromatography, gas chromatography, capillary zone electrophoresis and radioanalysis) are not available or cannot be used to measure the levels of fluoride in drinking water.

As a first step towards mitigating this problem, sources of drinking water that contain excess fluoride must be identified before an appropriate local treatment can be applied. Moreover, once a source of water is treated to remove fluoride, the effectiveness of the treatment must be evaluated. Both of these situations

*Department of Chemistry, The Pennsylvania State University, 104 Chemistry Building, University Park, PA 16802, USA. E-mail: sphillips@psu.edu; Fax: +814 865 5235; Tel: +814 867 2502*

†Electronic supplementary information (ESI) available: Experimental procedures, tables of data, figures providing additional results. See DOI: 10.1039/c2ob25363a




**Scheme 1** Synthesis of detection reagent **1**.

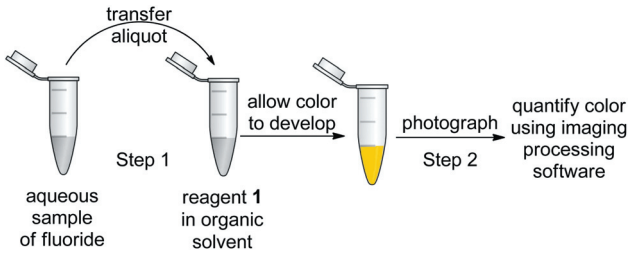



**Fig. 2** Schematic of the assay procedure for detecting fluoride colorimetrically. Step 1 involves diluting a sample that contains aqueous fluoride into a solution of **1**. After a period of time required for the autoinductive signal amplification reaction to proceed, photographs are taken (step 2) and the colorimetric response is measured using image-processing software.

require an inexpensive, yet sensitive and selective sensor for measuring relevant levels of fluoride in water.

The ideal sensor for these environments must be capable of operating under a stringent set of criteria: the sensor must (i) provide an unambiguous result that is easy to interpret by an untrained user; (ii) provide measurements without using instruments or readers; (iii) remain stable for prolonged periods of time (*i.e.*, when transported and stored without refrigeration); (iv) detect fluoride selectively over all other anions present in water; and (v) measure fluoride down to at least 4 ppm in water.[10]

Substantial progress has been made recently towards creating reagents for portable fluoride sensors, some of which satisfy several of the criteria outlined above for the ideal fluoride sensor.[11] These reagents can be classified into four main categories based on their mode of operation: (i) supramolecular recognition,[12] (ii) Lewis acid–base interactions,[13] (iii) hydrogen bonding,[14] and (iv) reaction-based detection.[15] The reagent described in this article falls into the latter category, but unlike most reaction-based sensors that detect fluoride in water our reagent amplifies signal,[4] which provides a method for increasing the sensitivity of the assay.

*Design of reagent **1***. We designed reagent **1** with four considerations in mind: (i) the reagent must detect fluoride selectively (the *tert*-butyldimethylsilyl ether group provides this capability[16]); (ii) a colorimetric readout must be generated for each reaction with fluoride to provide an unambiguous readout (generation of 4-aminobenzaldehyde provides this function[16,17]); (iii) two molecules of fluoride must be released per reaction of **1** with fluoride, both to propagate the signal amplification reaction, and to accelerate the rate of the reaction (release of only one molecule of fluoride would propagate, but not accelerate the rate of the reaction); and (iv) the reagent that enables all of these capabilities must be accessible *via* an exceedingly short and inexpensive synthesis that makes the reagent an attractive starting point for use in resource-limited environments.[18]

*Synthesis of reagent **1***. The synthesis of reagent **1** is shown in Scheme 1. Specifically, 4-(difluoromethyl)benzoic acid (**2**) was coupled with *p*-[(*tert*-butyldimethylsilyl)oxy]benzyl alcohol through an efficient Curtius rearrangement[19] to afford **1** in 87% yield. In comparison, our previous synthesis of **1** required three steps,[16] proceeded in only 40% overall yield, and required the use of the expensive and potentially dangerous reagent diethylaminosulfur trifluoride.

*Design of the assay.* Since reagent **1** is hydrophobic, we used a mixed organic–aqueous solution to detect aqueous fluoride, and then optimized the solvent conditions for the assay to enable detection of ~2 ppm fluoride in water. The general assay design is shown in Fig. 2. First, the aqueous sample that contains fluoride is diluted into a solution of **1** in organic solvents. Next, the autoinductive signal amplification reaction depicted in Fig. 1 begins, and a yellow color (from 4-aminobenzaldehyde) forms in the solution. Over time, the quantity of 4-aminobenzaldehyde, due to the autoinductive signal amplification reaction, increases to improve the sensitivity of the assay.

A fixed-time assay using this procedure enables semi-quantitative as well as quantitative measurements of the level of fluoride in the sample. Semi-quantitative assays simply require monitoring the assay solution until an obvious yellow color forms and then comparing the time required to form this color with calibration data relating time (to a yellow color) with known concentrations of fluoride in water. To obtain a quantitative measurement, the assay solution is photographed using a camera-equipped cellular phone, and the intensity of the color in the digital image is measured using image-processing software (this digital analysis process, in theory, could be accomplished off-site by a trained physician through a process referred to as Telemedicine[20]). Once the assay reaches 30% of the maximum colorimetric signal (which is known (*vide infra*) for different concentrations of fluoride), the time required to reach this intensity of signal can be compared with a calibration curve that relates "time to 30% of the maximum colorimetric signal" with "concentration of fluoride in the sample" to obtain the concentration of fluoride in the experimental sample.

*Quantitative detection of aqueous fluoride.* Our first generation assay conditions were as follows: a sample of water (10 μL) that contains fluoride (we used CsF) was transferred to a solution of reagent **1** (0.13 M, 95 μL) in 18 : 1 methanol–pyridine. In this step, the original concentration of fluoride is diluted 10.5×. The resulting diluted solution was agitated by shaking for 5 s and then left undisturbed as the assay developed. The appearance of a visible yellow color marked the end-point of the assay, although in some experiments, we also allowed the reaction to reach completion (*i.e.*, the point at which the yellow color no longer increased in intensity) to obtain full kinetics for the response.

Both the original concentration of fluoride in water (before it was diluted in the assay) and the assay time affect the intensity of the colorimetric response that is obtained with this type of



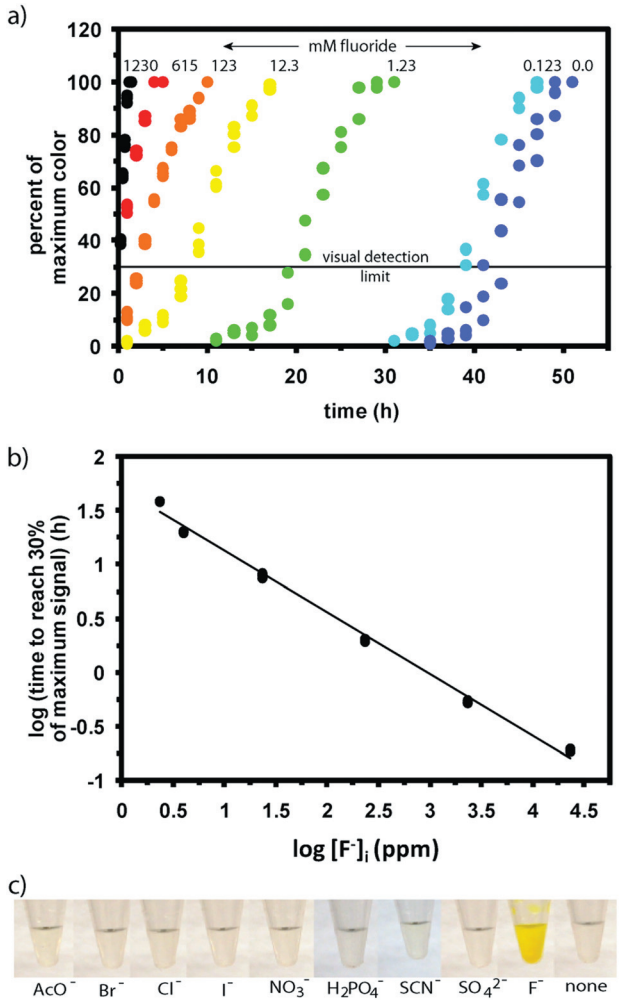



**Fig. 3** Assay results using reagent **1** to detect aqueous fluoride according to the procedure outlined in Fig. 2. (a) Quantity of color produced when **1** reacts with various initial quantities of fluoride *via* the autoinductive cycle shown in Fig. 1. The solution turns a bright, visible yellow color when 30% of the maximum possible color is produced (*i.e.*, when 30% of **1** is consumed). The experiments were performed in triplicate and all data are plotted on the graph. (b) A log–log calibration curve for quantitatively measuring the level of fluoride in a sample. Overlapping triplicate measurements are plotted on the graph. (c) Effect of anions on the colorimetric response of reagent **1**. The photographs were taken 3 h after **1** (0.13 M in 18 : 1 MeOH–pyr) was exposed to 0.5 equiv of various anions (10 μL from 0.62 M solutions in nanopure water).

assay (Fig. 3a). Fig. 3a reveals four key features about this first generation version of the assay: (i) reagent **1** is consumed completely even when substoichiometric quantities of fluoride are present in the assay; (ii) the sigmoidal response profile is characteristic of an autoinductive signal amplification reaction, as expected;[16] (iii) an intense yellow color is visible after only 30% of reagent **1** is consumed (see the solid indicator line); and (iv) background signal in the absence of applied fluoride becomes an issue only after long assay times (>33 h).

For quantitative assays, the calibration curve relating time (to a visible color) with concentration of fluoride in water is shown in Fig. 3b. To quantitatively measure the concentration of fluoride in a sample, one must simply run the assay until the

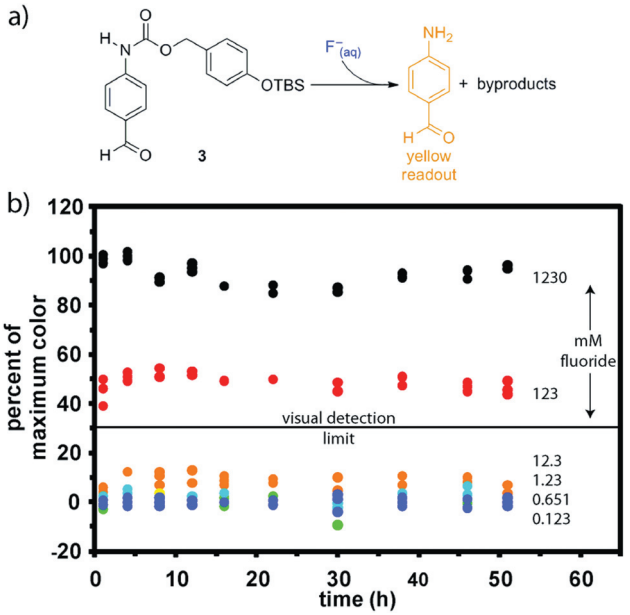



**Fig. 4** A control reagent (compound **3**) for determining the sensitivity for detecting fluoride without the contribution of an autoinductive signal amplification reaction. The assay conditions were identical to those used in Fig. 3. (a) Reaction of reagent **3** with fluoride to produce 4-aminobenzaldehyde. (b) Reagent **3** provides a non-amplified response to fluoride.

intensity that corresponds to 30% completion is reached (the first sign of a distinctly yellow color), and then compare the measurement of time with the calibration curve.

The selectivity of the assay is equally as important as the sensitivity, and, as expected for an activity-based detection mechanism,[21] reagent **1** is highly selective for detecting fluoride over many other anions that might be expected in an aqueous sample (Fig. 3c).

Likewise, the stability of the reagent is crucial if **1** (or, more likely, future derivatives of **1**) is ever to be used in resource-limited environments. While we have not performed an exhaustive stability study at this early stage in development, we have found that, as a solid, reagent **1** shows no signs of decomposition when stored open to the air at 37 °C for four weeks.

*Effect of the signal amplification reaction on the sensitivity of the assay.* To test the importance of the signal amplification reaction on the sensitivity of the assay, we prepared reagent **3** (Fig. 4a). Upon reaction with fluoride, reagent **3** releases 4-aminobenzaldehyde (which also is released by reagent **1**), but does not release two new equivalents of fluoride, and, hence, does not amplify signal (Fig. 4b). The graph in Fig. 4b was obtained using a procedure analogous to that described in Fig. 2.

Comparison of Fig. 4b and 3a shows that **3** provides only a linear response to fluoride (as expected), whereas **1** provides an amplified response. More importantly, reagent **3** is at least 1000× less sensitive than **1** in detecting fluoride (refer to the visual detection limits in Fig. 3a and 4b), and is capable of detecting only 123 mM (2300 ppm) fluoride in water, which is well outside the EPA-recommended range.

*Optimizing the assay.* Given that the autoinductive signal amplification reaction is necessary for achieving the desired sensitivity, we further optimized the assay to enable detection of

even 2 ppm fluoride in water and to increase the rate of the signal amplification reaction to decrease the assay time. Our assay strategy (Fig. 2) remained nearly the same, but we reasoned that increasing the polarity of the assay medium would stabilize the presumed azaquinone methide transition state (Fig. 1) that releases the first equivalent of fluoride; as a consequence, the rate of the signal amplification reaction should increase.[22] In addition, we recognized that we could increase the relative proportion (v/v) of the aqueous sample in the assay solution if different solvents were used. By increasing the volume of the aqueous sample relative to the organic solvent, we would decrease the extent to which the fluoride is diluted in Step 1 (Fig. 2) and further increase the rate of the detection event.

To achieve these goals we conducted two sets of solvent screens. First, preliminary experiments showed that pyridine accelerates the amplification reaction. More specifically, ~1% pyridine (relative to the total volume of solvent) is sufficient to maintain this accelerated rate (Scheme S1†); higher concentrations of pyridine offered no additional benefit, and, in fact, concentrations above 24% slowed the signal amplification reaction. Second, we screened polar protic solvents (data not shown) to identify conditions that maintain a high solvent polarity while still effectively solvating reagent **1**, even after addition of the aqueous sample in Step 1 (Fig. 2).

The solvent ratio of 10 : 4 : 1 iPrOH–aqueous sample–pyridine proved most effective for increasing the rate of the signal amplification reaction when 2300 ppm (0.122 M) fluoride was present in the aqueous sample (the assay used 0.16 M of **1** and was conducted at 23 °C). With this solvent combination, the aqueous sample now makes up 27% of the total assay volume, which means that the fluoride in the aqueous sample is diluted only 3.8× in step 1 (for comparison, the first-generation solvent conditions resulted in 10.5× dilution). The consequence of these changes is that an assay for 2300 ppm (0.122 M) fluoride now is ~3.1× faster than when the original solvent conditions are used.

Likewise, this acceleration in reaction rate has the added benefit of increasing the sensitivity of the assay – now even 2.3 ppm (0.12 mM) fluoride can be distinguished easily from the background reaction (Fig. 5a). This increase in reaction rate and sensitivity, however, does not impact the selectivity of the assay: as shown in Fig. 5c, fluoride remains the only anion tested that provides an observable signal.

From a practical viewpoint, to determine whether a sample has more or less than 2 ppm fluoride (the EPA recommended limit[1]), the assay solution must simply be checked to see if it turns a visible yellow color by 9 h (Fig. 5b); if it does not, then it likely contains less than 2 ppm fluoride. If it turns a visible yellow color before 9 h, then it likely contains more than 2 ppm fluoride.

In conclusion, while the reagent described in this Communication is comparable in sensitivity to other state-of-the-art reagents that detect fluoride in water, the design of the reagent offers two key advances in the area of fluoride detection: (i) it provides a unique and necessary signal amplification reaction for detecting relevant levels of fluoride; and (ii) it can be used to measure the level of fluoride in a sample of water, both in semi-quantitative threshold-type assays, and in quantitative assays. Moreover, because it is accessible in only one synthetic step, reagent **1** provides a useful starting point for designing the type of ideal detection reagent that is needed for detecting aqueous fluoride in resource-limited regions.

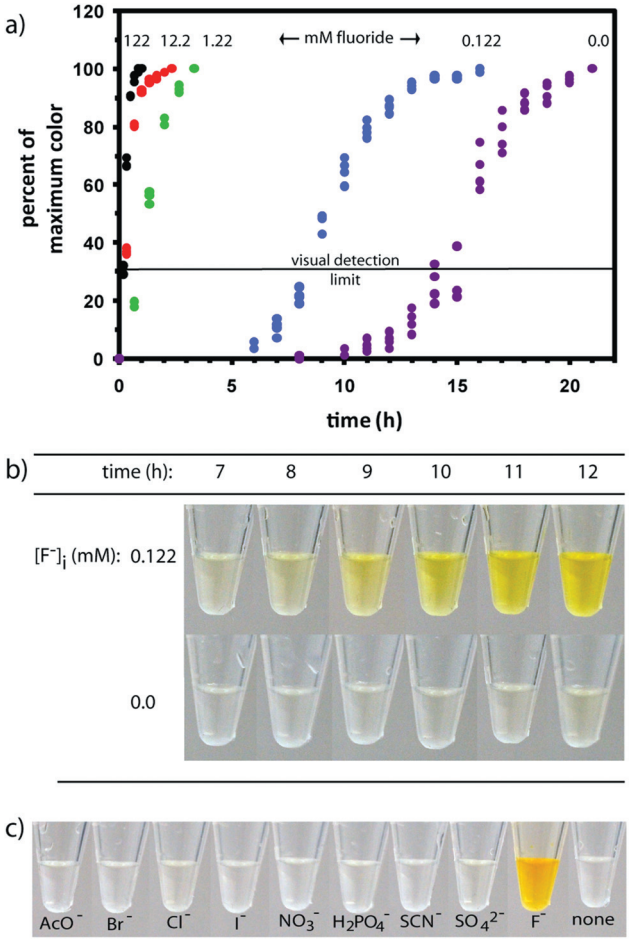



**Fig. 5** Optimized conditions for increasing the rate and sensitivity of the assay for detecting fluoride. (a) Response of **1** to various quantities of fluoride in an aqueous sample. The optimized assay conditions involved transferring 20 μL (step 1, Fig. 2) of an aqueous sample into 55 μL (10 : 1 iPrOH–pyridine) containing 0.22 M **1** and then allowing the color to develop (step 2, Fig. 2) at 23 °C. The assays were repeated in triplicate and all data are shown on the graph. (b) Photographs showing that even 2.3 ppm (0.12 mM) aqueous fluoride can be detected easily over the background reaction. (c) Photographs showing that the selectivity for fluoride has not been affected by the optimized assay conditions. The photographs were taken 2 h after **1** was exposed to 0.2 equiv of each anion under the assay conditions described in (a).

## Acknowledgements

We acknowledge financial support from the Arnold and Mabel Beckman Foundation, The Camille and Henry Dreyfus Foundation, Mr Louis Martarano, and The Pennsylvania State University. We thank Dr Landy K. Blasdel for assistance in preparing the manuscript.

## Notes and references

1 EPA National Primary Drinking Water Standards 2009, see http://water.epa.gov/drink/contaminants/ for more information.

2 The vast majority of fluoride sensors are designed to detect fluoride in organic solvents where issues related to solvent interactions with fluoride are minimized. Several recent fluoride sensors now are capable of detecting fluoride in water. These sensors are highlighted in ref. 12, 13, 15.
3 Tetrabutylammonium fluoride (TBAF) contains substantial quantities of hydroxide.[23] This hydroxide likely cleaves silyl ethers and contributes to the overall activity-based detection event when TBAF is used as the source of fluoride. In other words, the use of TBAF may not accurately reflect the ability of a fluoride sensor to detect fluoride that is dissolved in water.
4 Only two other fluoride sensors have been reported that amplify signal for the detection event. They are ref. 15*a*, which detects fluoride in organic solvent, and ref. 5, which is complimentary to the sensor described in this Communication.
5 R. Perry-Feigenbaum, E. Sella and D. Shabat, *Chem.–Eur. J.*, 2011, **17**, 12123.
6 (*a*) S. Jagtapt, M. K. Yenkie, N. Labhsetwart and S. Rayalu, *Chem. Rev.*, 2012, DOI: 10.1021/cr2002855; (*b*) M. Mohapatra, S. Anand, B. K. Mishra, D. E. Giles and P. Singh, *J. Environ. Manage.*, 2009, **91**, 67.
7 S. Ayoob and A. K. Gupta, *Crit. Rev. Environ. Sci. Technol.*, 2006, **36**, 433.
8 Certain resource-limited regions in India provide a case-in-point. It is estimated that approximately 60 million people in India are ingesting water that contains levels of fluoride that are above the recommended limit, and that of these 60 million, 6 million now have crippling osteoporosis as a result.D. R. Ready, *Neurol. India*, 2009, **57**, 7.
9 C.-Q. Zhu, J.-L. Chen, H. Zheng, Y.-Q. Wu and J.-G. Xu, *Anal. Chim. Acta*, 2005, **539**, 311.
10 Since the deleterious health effects associated with fluoride are the consequence of long-term ingestion of water that contains high levels of fluoride, a sensor for use in resource-limited environments does not necessarily need to provide rapid results for an assay.
11 (*a*) Z.-H. Lin, Y.-G. Zhao, C.-Y. Duan, B.-G. Zhang and Z.-P. Bai, *Dalton Trans.*, 2006, 3678; Z.-H. Lin, S.-J. Ou, C.-Y. Duan, B.-G. Zhang and Z.-P. Bai, *Chem. Commun.*, 2006, 624(*b*) R. Hu, J. Feng, D. Hu, S. Wang, S. Li, Y. Li and G. Yang, *Angew. Chem., Int. Ed.*, 2010, **49**, 4915.
12 (*a*) M. Takeuchi, T. Shioya and T. M. Swager, *Angew. Chem., Int. Ed.*, 2001, **40**, 3372; (*b*) S. Guha and S. Saha, *J. Am. Chem. Soc.*, 2010, **132**, 17674; (*c*) S. Watanabe, H. Seguchi, K. Yoshida, K. Kifune, T. Tadaki and H. Shiozaki, *Tetrahedron Lett.*, 2005, **46**, 8827.
13 (*a*) C. R. Wade, A. E. J. Broomsgrove, S. Aldridge and F. P. Gabbaï, *Chem. Rev.*, 2010, **110**, 3958; (*b*) S. Yamaguchi, S. Akiyama and K. Tamao, *J. Am. Chem. Soc.*, 2001, **123**, 11372; (*c*) Q. Zhao, F. Li, S. Liu, M. Yu, Z. Liu, T. Yi and C. Huang, *Inorg. Chem.*, 2008, **47**, 9256; (*e*) I. H. A. Badr and M. E. Meyerhoff, *J. Am. Chem. Soc.*, 2005, **127**, 5318; (*e*) M. J. Bayer, S. S. Jalisatgi, B. Smart, A. Herzog, C. B. Knobler and M. F. Hawthorne, *Angew. Chem., Int. Ed.*, 2004, **43**, 1854; (*f*) A. Oehlke, A. A. Auer, I. Jahre, B. Walfort, T. Rueffer, P. Zoufala, H. Lang and S. Spange, *J. Org. Chem.*, 2007, **72**, 4328; (*g*) Y. Kim and F. P. Gabbaï, *J. Am. Chem. Soc.*, 2009, **131**, 3363; (*h*) C. R. Wade, I.-S. Ke and F. P. Gabbaï, *Angew. Chem., Int. Ed.*, 2012, **51**, 478.
14 (*a*) Y. Li, C. Lifeng and H. Tian, *J. Org. Chem.*, 2006, **71**, 8279; (*b*) D. A. Jose, D. K. Kumar, B. Ganguly and A. Das, *Org. Lett.*, 2004, **6**, 3445; (*c*) Y. Qu, J. Hua and H. Tian, *Org. Lett.*, 2010, **12**, 3320; (*d*) Y. Shiraishi, H. Maehara, T. Sugii, D. Wang and T. Hirai, *Tetrahedron Lett.*, 2009, **50**, 4293; (*e*) C. B. Black, B. Andrioletti, A. C. Try, C. Ruiperez and J. L. Sessler, *J. Am. Chem. Soc.*, 1999, **121**, 10438; (*f*) H. Miyaji, W. Sato and J. L. Sessler, *Angew. Chem., Int. Ed.*, 2000, **39**, 1777.
15 (*a*) T.-H. Kim and T. M. Swager, *Angew. Chem., Int. Ed.*, 2003, **42**, 4803; (*b*) S. Y. Kim and J.-I. Hong, *Org. Lett.*, 2007, **9**, 3109; (*c*) C.-Q. Zhu, J.-L. Chen, H. Zheng, Y.-Q. Wu and J.-G. Xu, *Anal. Chim. Acta*, 2005, **539**, 311; (*d*) J. F. Zhang, C. S. Lim, S. Bhuniya, B. R. Cho and J. S. Kim, *Org. Lett.*, 2011, **13**, 1190; (*e*) O. A. Bozdemir, F. Sozmen, O. Buyukcakir, R. Guliyev, Y. Cakmak and E. U. Akkaya, *Org. Lett.*, 2010, **12**, 1400.
16 M. S. Baker and S. T. Phillips, *J. Am. Chem. Soc.*, 2011, **133**, 5170.
17 S. A. Nuñez, K. Yeung, N. S. Fox and S. T. Phillips, *J. Org. Chem.*, 2011, **76**, 10099.
18 We previously described reagent **1** as a general signal amplification reagent that could be paired with a second detection reagent that released fluoride upon reaction with a specific analyte (we demonstrated the detection of Pd(0)).[16] In this article, we now describe several advances that arise as a result of a year-long study on the mechanism and kinetics of the reaction of **1** with fluoride. These advances include (i) the development of a convenient assay procedure for detecting aqueous fluoride, (ii) the development of optimized assay conditions that enable **1** to detect relevant levels of fluoride in water, and (iii) the development of an exceedingly efficient synthesis route that provides **1** in high yield in a *single* synthetic step.
19 M. B. Smith and J. March, *March's Advanced Organic Chemistry*, Wiley, Hoboken, New Jersey, 6th edn, 2007, p. 1609.
20 A. Martinez, S. T. Phillips, E. Carrilho, S. W. Thomas III, H. Sindi and G. M. Whitesides, *Anal. Chem.*, 2008, **80**, 3699.
21 D.-G. Cho and J. L. Sessler, *Chem. Soc. Rev.*, 2009, **38**, 1647.
22 K. M. Schmid and S. T. Phillips, unpublished work.
23 (*a*) D. P. Cox, J. Terpinski and W. Lawrynowicz, *J. Org. Chem.*, 1984, **49**, 3216; (*b*) A. S. Pilcher, H. L. Ammon and P. DeShong, *J. Am. Chem. Soc.*, 1995, **117**, 5166.