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A Fluorescent Probe for Rapid Aqueous Fluoride Detection and Cell Imaging

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Abstract

A stable and highly selective fluorescent probe has been designed and synthesized for the rapid detection of fluoride ions (F^-) in aqueous solution and living cells. The design was based on the high reactivity of F^- toward a silyl group.

As a frequently applied ingredient found in toothpaste, fluoride ion is also used as pharmaceutical agents to treat osteoporosis.[1–3] Besides, fluoride was even added into drinking water to prevent dental caries.[4] On the other hand, in the past few years, it has been proven that consumption of fluoride at an elevated level is the main cause of dental and bone fluorosis.[5] Furthermore, chronic ingestion of low levels of fluoride can cause diseases such as gastric and kidney disorders, urolithiasis and even death.[6, 7] Therefore, fluoride detection in drinking water and living organs has drawn intensive attention. Though the standard Willard-Winter method using an ion-selective electrode and ion chromatography methods are commonly used for F^- analysis,[8–10] they normally require sophisticated procedures and costly instruments. Thus, development of highly selective, sensitive and rapid F^- detection methods has become very important. Along this line, fluorescent probes have been utilized in a wide variety of applications not only because of their high sensitivity but also the ability to conduct analysis in living systems. Fluoride ion is the smallest anion and it has high hydration enthalpy, all of which make aqueous fluoride fluorescent probe design a challenging task. With all available fluorescent fluoride probes,[11–23] most of them can only detect tetrabutylammonium fluoride (TBAF) in organic solvents or require a high level of organic solvents (e.g., DMSO, acetone, and ethanol). In addition, even those that have been used have issues of long reaction time, which is due to the low reactivity between *tert*-butyldiphenylsilyl (TBDPS) and F^-. Thus most of the aqueous fluoride fluorescent probes need tens of minutes or even hours to complete the detection process. The long reaction time is especially a problem if fluoride fluctuation is an issue. To address these issues for biological applications, many groups have made remarkable progress in this field.[24–26] However, the number of fluoride probes suitable for cell-imaging applications is still very limited, mostly because of the stringent requirements a probe has to meet for such applications: (1) high selectivity for F^- in 100% water, (2) high permeability to penetrate cell membrane, and (3) low/no toxicity. To date, there are only four examples.[25–28] Therefore, how to get a fast selective fluorescent fluoride probe suitable for biological system is of our great interests.

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Herein, we describe the design and evaluation of a rapid aqueous fluoride fluorescent probe. One of the commonly used fluoride probe design is based on the chemical affinity of fluoride and a silyl group. *tert*-Butyldimethylsilyl (TBDMS) and TBDPS, firstly reported by Kim and Swager,[29] are frequently chosen as warheads for the fluorophore. Using the same principle, we decided to conjugate a benzothiazole based fluorophore with a silyl group such as TBDMS. Specifically, ethyl 6-hydroxybenzothiazole-2-carboxylate (**1**) was chosen as the fluorophore because it is easy to make, simple to modify, small in size, and biocompatible. We firstly put the TBDMS group on **1** to obtain **BBT** (ethyl 6-((*tert*-butyldimethylsilyl)oxy)benzo[d]thiazole-2-carboxylate **2**). This compound showed good fluorescent turn-on properties upon reaction with TBAF in THF (ESI†). However, **BBT** has poor water solubility, which hinders applications in aqueous solution and in live cell imaging. In order to improve the water solubility, we were interested in appending a hydrophilic group to **1**. Thus, we decided to use sugar as a possible modification moiety as this has been successfully used in similar situations.[27] Firstly, **1** was hydrolyzed to the corresponding carboxylic acid **3** for amidation with glucosamine to afford fluorescent compound **4.** Selective silylation of the phenol hyrdoyl group with TBDMS led to probe **BBTGA** (6-((*tert*-butyldimethylsilyl)oxy)-benzothiazole-2-carboxyl glucosamide **5**).

In 2009, Park, Hong and co-workers reported that in a 7-hydroxycoumarin-based system the covalent bond character of the Si–O bond in O-TBDMS is much weaker in phosphate buffered saline (PBS) than in pure water. Thus the strong Si–O bond polarization led to fluorescence turn-on in buffer without actual desilylation.[25, 27] Accordingly, we first tested the stability of our probe in PBS. Fortunately, the 6-hydroxybenzothiazole system did not show similar fluorescent auto turn-on problems. The fluorescent intensity increased only 3-fold after 4 hours of incubation in PBS (10 mM, DMSO 0.5%, pH = 7.4) and 5-fold after 10 h (ESI†). On the other hand, addition of 0.1 M NaF led to a fluorescent intensity increase of 30 fold in 10 min (ESI†). With these promising results we then further tested the selectivity of BBTGA for F^- against other anions by adding 0.1 M Cl^-, Br^-, I^-, F^-, $H_2PO_4^-$, N_3^-, NO_2^-, NO_3^-, AcO^-, and SO_4^{2-} (final concentration 50 mM) to its solution in PBS (10 mM, DMSO 0.5%, pH = 7.4), respectively.

Results for the fluorescence selectivity tests are shown in Fig. 1 and 2. It is clear that only F^- ion can trigger significant fluorescence intensity changes.

We then moved forward to the linearity test. The results confirmed that there was an excellent linearity between the fluorescent intensity (λ_{em}: 508 nm) and the F^- concentrations in the range of 0.1 to 1 mM in PBS (10 mM, DMSO 0.5%, pH = 7.4) at 25 °C. Owning to the high reactivity of TBDMS and F^-, each fluorescent spectrum was measured after only 5 min of incubation. This detection time was among the fastest fluorescent fluoride probes.[27] An additional point that we examined was whether the appended sugar could sequester fluoride. Thus we studied the probe's sensitivity toward fluoride in the presence of added *N*-acetylglucosamine and found that the added sugar had no measurable effect on the probe's sensitivity (ESI†). Thus we concluded that the appended sugar only plays a role of improving solubility and has no measurable fluoride sequestration effect.

We explored the application of **BBTGA** in imaging in KB human carcinoma cell lines. Firstly, to evaluate the cytotoxicity of **BBTGA**, we performed standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assays using KB cells at 50 and 100 μM **BBTGA** for 48 h, respectively. The result clearly showed that **BBTGA** was nontoxic to the cultured cells under the experimental conditions (ESI†). We then incubated

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the cells with **BBTGA** (0.1 mM) for 24 h followed by washing with PBS twice. Afterward, the cells were further incubated in MEM medium (ESI[†]) containing 0.1 mM NaF for 2 h and fluorescence measurements were taken. As shown in Fig 4, fluoride treatment led to a significant increase in the fluorescence intensity (Fig. 4c) as compared to the control experiments (Fig. 4a and b).

In conclusion, we have successfully designed, synthesized, and investigated the properties of a new sugar-functionalized fluorescent fluoride probe **BBTGA** based on a desilylation reaction in aqueous medium. This probe has many desired properties such as fast reaction rate, excellent F^- ions selectivity over other anions, and non-cytotoxic to mammalian cells for cellular imaging applications.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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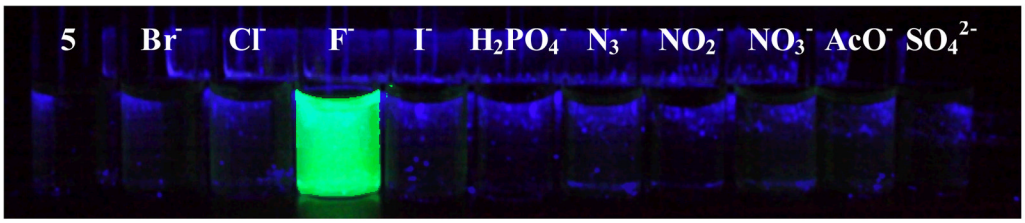


Figure 1.

Direct observation of fluorescence emission changes of **BBTGA** (10 μM) under UV irradiation (λ_{ex}: 365 nm) after reaction for 10 min with various sodium salts (50 mM) in PBS (10 mM, DMSO 0.5%, pH = 7.4) at 25 °C. Left to right: **BBTGA**, Br^-, Cl^-, F^-, I^-, $H_2PO_4^-$, N_3^-, NO_2^-, NO_3^-, AcO^-, SO_4^{2-} (Na salts).

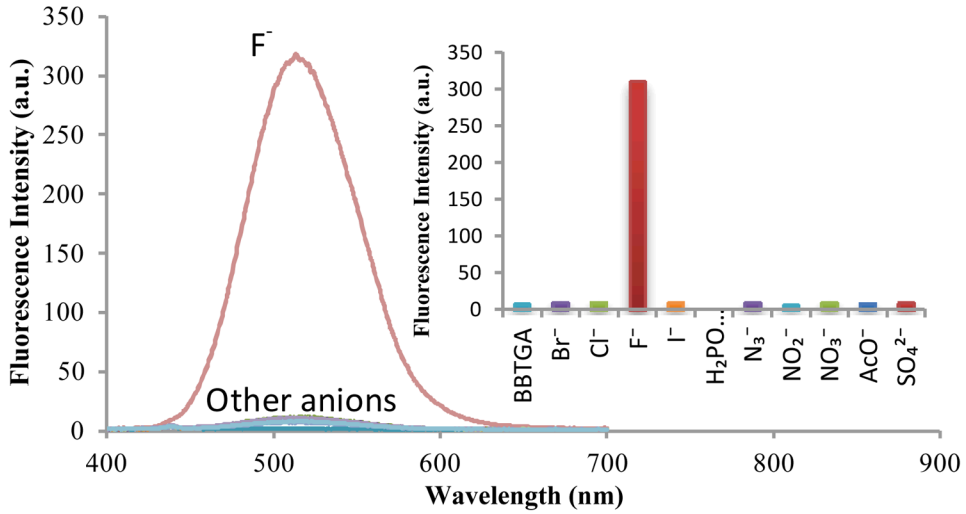


Figure 2.

Anion selectivity of **BBTGA** in PBS (DMSO 0.5%, pH 7.4). A single analyte including 100 mM anions (Br^-, Cl^-, F^-, I^-, $H_2PO_4^-$, N_3^-, NO_2^-, NO_3^-, AcO^-, and SO_4^{2-}) was added to **BBTGA** (10 μM) in PBS (10 mM, DMSO 0.5%, pH = 7.4) at 25 °C. Then the solution was incubated for 10 min. (λ_{ex}: 380 nm, λ_{em}: 508 nm)

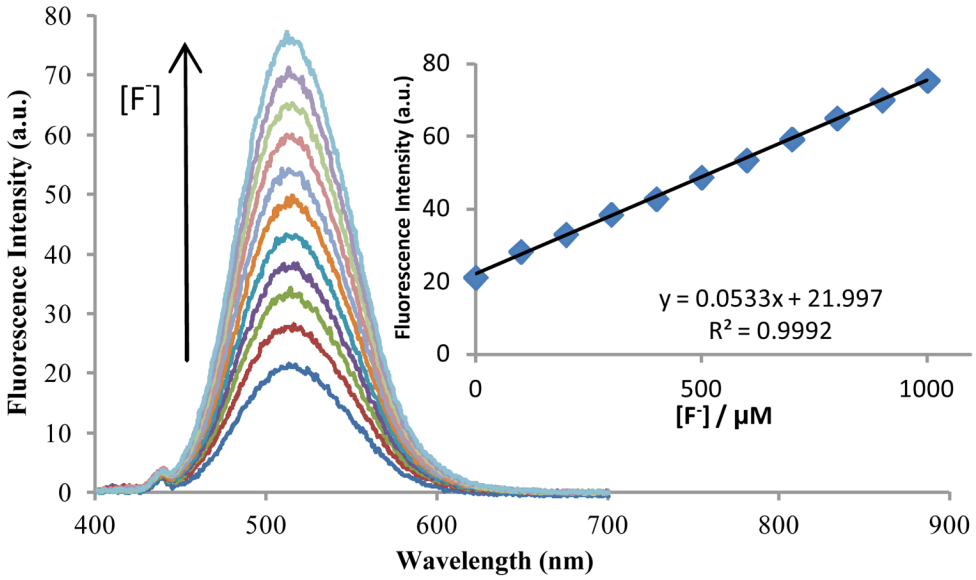


Figure 3.

Fluorescence intensity of **BBTGA** in the presence of F^- at various concentrations in PBS (DMSO 0.5%, pH 7.4 F^- final concentration: 0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1 mM). Each spectrum was obtained after F^- addition at at 25 °C for 5 min (λ_{ex}: 380 nm, λ_{em}: 508 nm).

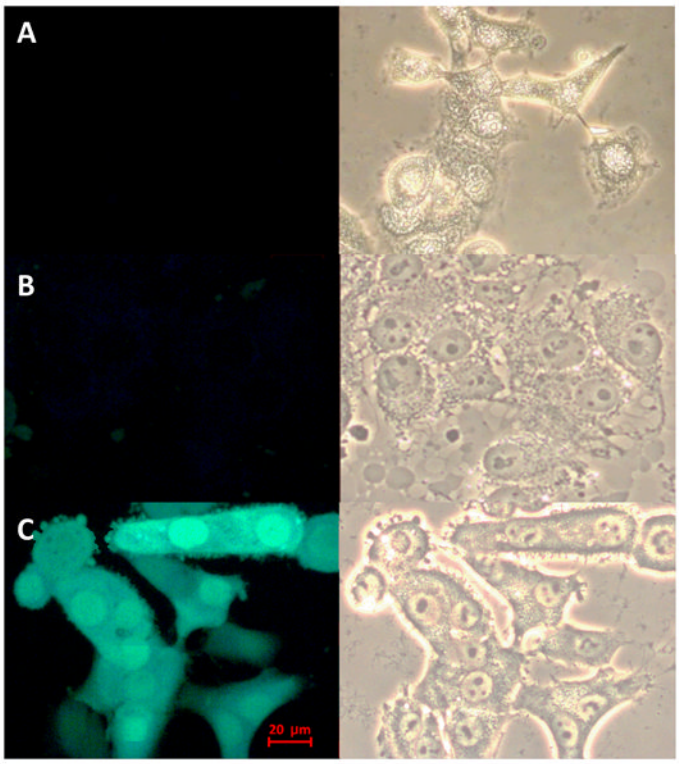
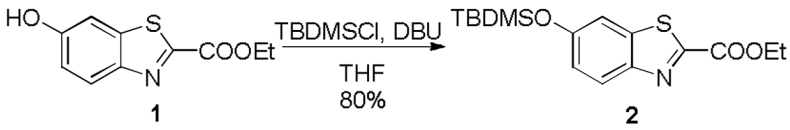
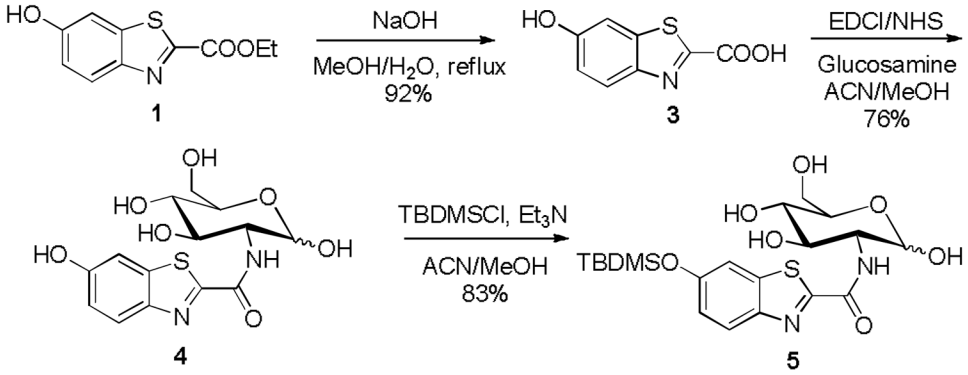


Figure 4.

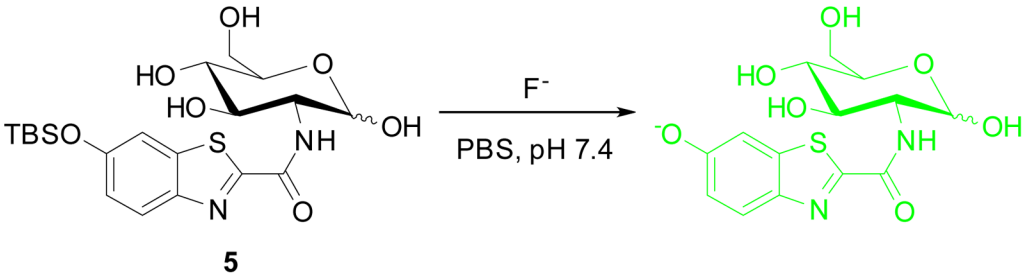
Fluorescence and bright field images of KB human carcinoma cells: (A) KB cells incubated for 24 h without BBTGA and subsequently 2 h with 0.1 mM of NaF at 37 °C as blank, (B) KB cells incubated for 24 h with **BBTGA** and subsequently 2 h without NaF at 37 °C as negative control, (C) KB cells incubated for 24 h with **BBTGA** and subsequently 2 h with 0.1 mM of NaF at 37 °C. The scale bar represents 20 μm.



Scheme 1.
Synthetic route of **2** from compound **1**



Scheme 2.
Synthetic route of **BBTGA 5** from compound **1**



Scheme 3.
Sensing reaction of **BBTGA** for the detection of F^-