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A cascade reaction based fluorescent probe for rapid and selective fluoride ion detection†

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A cascade reaction-based colorimetric and fluorescent probe for selective fluoride ion detection is reported. The probe displays a fast response ($t_{1/2}$ = 2.41 min) and 550-fold fluorescence enhancement during sensing of fluoride ions. Application of the probe in live cell imaging is demonstrated.

The development of artificial molecular systems for sensing anions has received great research interest as anions play a pivotal role in a wide range of biological, metabolic processes and participate in various enzymatic reactions.[1,2] Fluoride, the smallest anion with high charge density, contributes towards diverse health and environmental issues.[3,4] The presence of fluoride in low levels has proven to be critical for the prevention of dental and skeletal fluorosis, enamel demineralization while wearing orthodontic appliances and during osteoporosis treatment. The optimal level of the ions in the human body is routinely maintained from toothpaste, drinking water, foods and other fluoride supplements. However, excess intake of fluoride can result in metabolic disorders, skeletal disease, mottled teeth, inhibition of neuro-transmitter biosynthesis in fetuses, nephrolithiasis. For these reasons, the controlled consumption of fluoride in the human body is a serious concern throughout the world.[5,6] Therefore, it is of great necessity to develop techniques for selective detection of fluoride ions. Fluorescent probes offer promising approach owing to its simplicity, high selectivity and sensitivity.

A large number of fluorescent probes have been developed for detection of F^- ions. Synthetic chemosensors recognize the ion through either hydrogen bonding interactions (involving functional groups such as phenol, urea, thiourea, amide, pyrrolic and indolic N–Hs, *etc.*)[7,8] or Lewis acid/base coordination[9,10] or anion-π interactions.[11–13] Chemodosimeters on the other hand, are known to undergo irreversible chemical reactions with the ion and provide better selectivity. Generally, the high affinity of F^- ions towards either boron or silicon has been extensively used for the development of fluoride selective probes. Facile cleavage of either the C–Si[14–18] or the O–Si[19–23] bond by the F^- ion has been the key to designing the reaction sites in these probes. Based on these strategies various chemodosimeters for fluoride sensing were reported. These deprotection strategies were further maneuvered to develop new probes *via* the cascade reaction process. Kim and coworkers have reported a probe consisting of the 4-(hydroxymethyl)phenol linker between the fluorophore and the silyl group. Fluoride mediated reaction resulted in the release of the fluorophore along with the formation of 4-methylenecyclohexa-2,5-dien-1-one as the by-product.[24] Alternately, desilylation followed by cyclization forming either coumarin or iminocoumarin was reported for efficient F^- ion detection.[25–27] However, most of these probes suffered from a common drawback of longer response times and poor off–on response.

Cascade reactions offer advantages such as atom economy as well as economies of time and waste generation. As such, cascade reactions can be considered to fall under the category of "green chemistry".[28] Herein, we report the design, synthesis, photophysical characterization of a new cascade reaction based highly selective and sensitive colorimetric and fluorescent off–on probe **1** for F^- ions (Scheme 1). The non-fluorescent species **1** upon treatment with fluoride was expected to undergo a cleavage of the Si–O bond to release *tert*-butyldimethylsilyl fluoride **2**. A subsequent cyclization was proposed to form phthalide **3** with the release of carboxyfluorescein-based fluorophore **4**. The cascade reaction is expected to be fast as the process is entropically favorable.

The synthesis of probe **1** was carried out in two steps (Scheme S1, ESI†). First, **3** was converted to free acid **5** in one-pot three-step strategy *via* opening of the lactone using methanolic KOH followed by silyl protection of both benzylic alcohol and –COOH groups. Subsequent selective deprotection of silyl ester provided acid **5** with an overall 32% yield. Acid **5** was then coupled with the carboxyfluorescein derivative **6** under EDC·HCl and DMAP/DIPEA conditions to form **1** in 58% yield.

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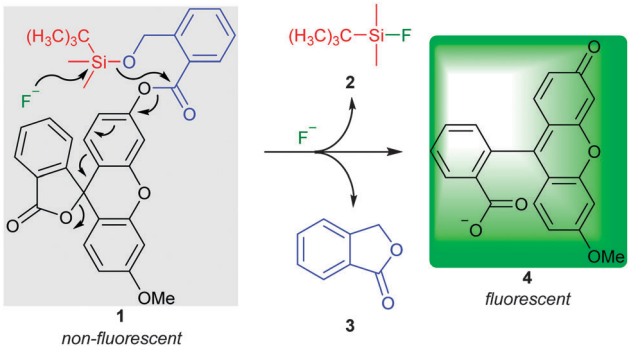
† Electronic supplementary information (ESI) available: Experimental procedures, supplemental data, and the ^{1}H-, ^{13}C-NMR spectrum. See DOI: 10.1039/c4cc01665c



Scheme 1 Structure and fluoride ion sensing mechanism of probe **1**.

The photophysical properties of probe **1** were investigated in DMSO. Probe **1** did not show any characteristic UV-visible absorption band between 300 and 700 nm and was non-fluorescent. Upon addition of tetrabutylammonium fluoride, TBAF (300 equivalent) to the solution of probe **1** (10 μM), a fluorescence band centered at λ_{em} = 523 nm (λ_{ex} = 460 nm) was observed (Fig. 1A). Pseudo first order reaction kinetics was observed with the rate constant k = 0.28 min^{-1} and $t_{1/2}$ = 2.41 min (Fig. 1A) and the sensing process was completed in ~7 min. As probe **1** was not capable of F^- detection under aqueous conditions, to overcome this limitation we prepared the stock of F^- in water and the assay was carried out in DMSO solvent.[29,30] The sensitivity of **1** towards F^- was comparable irrespective of the medium of the F^- source (water or THF). When the response of **1** (10 μM) towards increasing concentrations of TBAF (0–350 equivalents) was monitored, a stepwise increase in the fluorescence was observed upto 300 equivalents of the salt (Fig. 1B). When fluorescence intensity measured at λ = 523 nm was plotted against the concentration, a linear increase was observed within 0–200 equivalents of F^- ions (Fig. S3, ESI†). Sensing of F^- ions by **1** was independent of pH when analyzed over the range 3–11 (Fig. S5, ESI†).

The detection limit of probe **1** toward F^- was evaluated to be 1.03 μM (19.6 ppb) which was well below 4 ppm, the allowed concentration level of F^- in drinking water specified by the USEPA[25] (Fig. S4, ESI†). The low detection limit of **1** shows its potential for

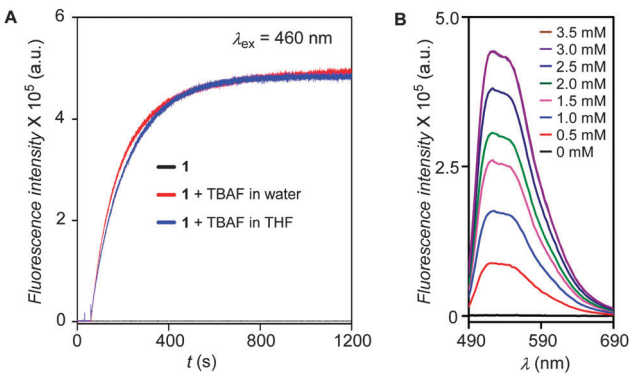


Fig. 1 Fluorescence kinetics of **1** (10 μM) in presence of TBAF (3 mM in either THF or H_2O) in DMSO at λ = 523 nm (A). Fluorescence spectra of **1** with increasing concentration of TBAF (0–3.5 mM) in DMSO (B).

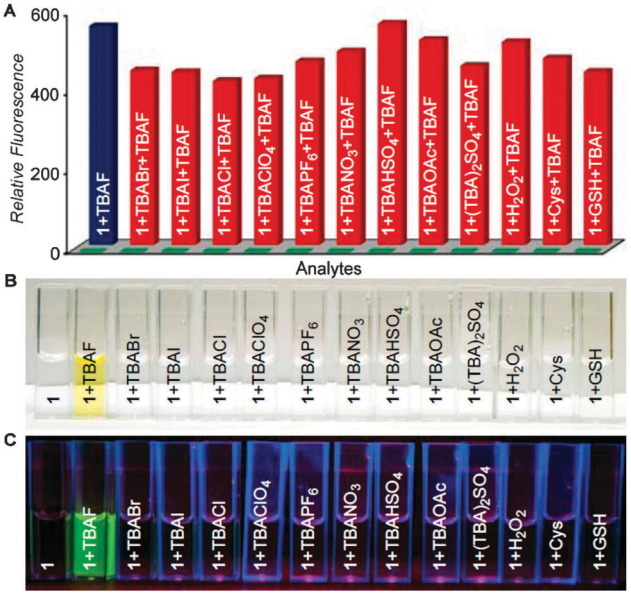


Fig. 2 Relative fluorescence of **1** (10 μM) in presence of either TBAF (3 mM) or various analytes (front row) and subsequent addition of TBAF (back row) to the same sample (A). Colour change for **1** in presence of various analytes under visible light (B). Emission colour change for **1** in presence of various analytes (C). Cuvettes were photographed under the hand-held UV-lamp with λ_{ex} = 365 nm.

application in the detection of F^- in biological and environmental science as a higher F^- concentration is toxic (0.1 mM for drinking water standard and >3 mM in biological systems).[31] The detection limit and response time for probe **1** were either comparable or better than reported probes (Table S1, ESI†).

To evaluate the selectivity of probe **1** towards F^- ion over other possibly competitive analytes, the fluorescence measurements at λ_{em} = 523 nm (λ_{ex} = 460 nm) were carried out in the presence of various interfering ions (Br^-, I^-, Cl^-, ClO_4^-, PF_6^-, NO_3^-, HSO_4^-, OAc^- and SO_4^{2-}) and analytes (H_2O_2, Cys and GSH). When probe **1** (10 μM) in DMSO was treated with 300 equivalents of TBAF, a 550-fold enhancement in the fluorescence intensity was observed within 7 minutes of stirring at room temperature (Fig. 2A, blue bar) but other analytes (3 mM) did not show any change in emission intensity (Fig. 2A, front row). The probe **1** showed fluorescence intensity enhancement only after addition of TBAF into these solutions, confirming the selectivity of the probe towards F^- ions, even in the presence of competitive analytes (Fig. 2A, back row). The resulting solution also provided a quantum yield Φ = 0.06 (standard: fluorescein, Φ = 0.85 in 0.1 N NaOH). The response of **1** towards F^- ions under ambient light was associated with the colour change from colourless to yellow and inertness towards other analytes was supported by no change in color (Fig. 2B). The fluorescence turn-on response upon sensing of F^- ions was also confirmed by the appearance of green fluorescence of the reaction mixture when cuvette images were taken under a hand-held UV-lamp (Fig. 2C). These results confirmed that probe **1** is highly selective towards fluoride.

The proposed mechanism of F^- ion sensing by probe **1** was confirmed by ^{1}H-NMR titration studies (Fig. 3). Upon addition of

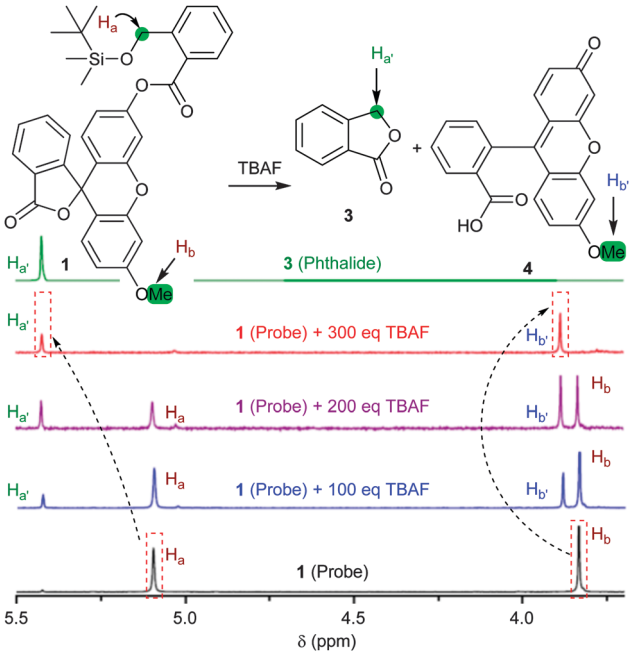


Fig. 3 ^{1}H-NMR spectral changes for probe **1** in DMSO-d_6 with increasing concentration of TBAF (0–300 equivalent).

TBAF (0–300 equivalent) to **1** (4.3 mg in 0.7 mL DMSO-d_6) for 7 min, the characteristic singlet at δ = 5.10 ppm (H_a protons) disappeared gradually and a new singlet at δ = 5.43 ppm, corresponding to $H_{a'}$ protons of phthalide **3** appeared. Similarly, another singlet at δ = 3.83 ppm (H_b protons) for the –OMe group of probe **1** disappeared and a new singlet at δ = 3.89 ppm ($H_{b'}$ protons) corresponding to the –OMe group of compound **4** appeared. Formation of **4** was further confirmed by the appearance of *m*/*z* = 347 in the mass spectrometry (MALDI) data (Fig. S6, ESI†).

To demonstrate the practical application of the probe **1**, a live cell imaging experiment was carried with the human cervical cancer cell line (HeLa cells). No significant fluorescence was observed when HeLa cells were incubated with only **1** (10 μM in 1 : 100 DMSO–DNEM v/v, pH = 7.4) at 37 °C for 2 h (Fig. 4B). When the same cells were again incubated with 20 mM NaF (1 : 100 H_2O–DMEM, pH = 7.4) at 37 °C for 30 min, a strong fluorescence was observed inside the cell (Fig. 4E). These data indicate that **1** can be applied for the selective detection of intracellular F^- ions.

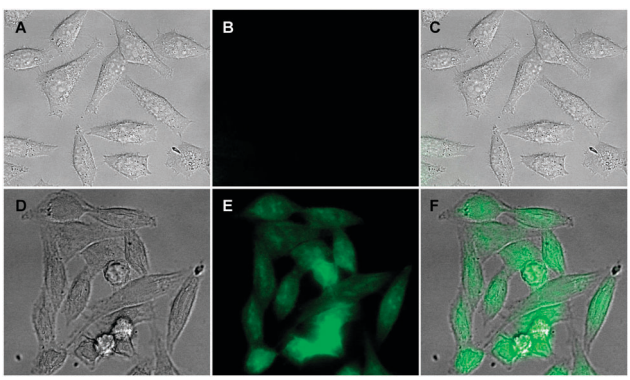


Fig. 4 Live cell image of HeLa cell: brightfield (A), fluorescence (B), and overlay (C), image of HeLa cell incubated with probe **1** (10 μM) for 2 h. (D–F) are the respective brightfield, fluorescence and overlay image of HeLa cell fist incubated with probe **1** (10 μM) for 2 h followed by incubation with NaF (20 mM) for 30 min.

In conclusion, we have successfully designed, synthesized and investigated the selective F^- ion sensing properties of probe **1**. The non-fluorescent probe undergoes a cascade reaction in the presence of the ion to release the lactone phthalide and a carboxyfluorescein derivative as the strong fluorescent species. The fast response of the probe towards the F^- provided $t_{1/2}$ = 2.41 min and 550-fold fluorescence enhancement with a detection limit of 19.6 ppb. Most importantly, the F^- ion detection ability of the probe in living cells was demonstrated using confocal microscopy.

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