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Autoinductive Exponential Signal Amplification: A Diagnostic Probe for Direct Detection of Fluoride

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Abstract: A new example of an exponential signal amplification strategy for the direct detection of fluoride is demonstrated. The amplification occurred through reaction of fluoride with a responsive chromogenic probe. The probe activity is based on a unique dendritic chain reaction that generates a fluoride anion, which is the analyte of interest, during the disassembly pathway of the dendritic probe. This autoinductive amplification mechanism may be applied for detection of other analytes by coupling activity of a modified probe with that of the fluoride amplifier.

Keywords: analytes · dendrimers · dyes/pigments · fluoride · reaction mechanisms

Introduction

Signal amplification techniques are often used to boost signals in diagnostic assays for the detection of various analytes and biological markers. One of the most familiar techniques is the enzyme-linked immunosorbent assay (ELISA), a method that uses the catalytic activity of an enzyme–antibody conjugate to amplify a chromogenic signal.[1] In this type of assay, the initial signal is amplified through a linear growth pathway by the catalytic activity of the enzyme. Recently, several groups, including ours, have reported new approaches for signal amplification on the basis of the exponential progress of chemical reactions of small molecules.[2–10] We have introduced a novel modular technique for the exponential amplification of a diagnostic signal: amplification is achieved through a distinctive dendritic chain reaction (DCR),[11] which is generated by the disassembly of self-immolative dendrimers.[12,13] A single activation event initiated by the analyte of interest leads to a chain reaction that results in complete disassembly of all the probe molecules through an exponential progress to ultimately release many copies of dye molecules. By taking advantage of the modular design of the probe, we have applied the DCR technique to the detection of the analytes hydrogen peroxide[14,15] and ubiquitous sulfhydryls.[16] These two examples were based on DCR probes designed to act under aqueous conditions. Here we report a new DCR probe, which undergoes dendritic chain reaction in organic solvent. The probe system exponentially amplifies a diagnostic signal generated by fluoride, the analyte of interest.

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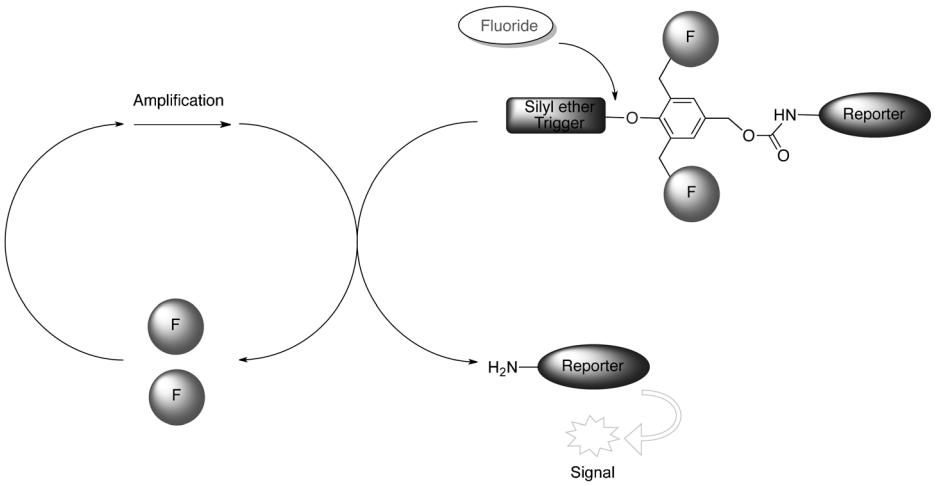
Results and Discussion

Fluoride anions are of interest because fluoride treatment can prevent dental caries and other medical issues such as osteoporosis.[17] An excess amount of fluoride, however, causes fluorosis, a type of fluoride toxicity that is related to an increase in bone density, urolithiasis, or even cancer.[18] A large number of sensors for fluoride anion have been developed to date.[19,20] The United States Environmental Protection Agency (EPA) enforceable standard for drinking water is no more than 4 mg L^{-1} (higher levels may result in osteoflurosis), and ideal levels should be no more than 2 mg L^{-1} to protect against dental fluorosis. Therefore, relevant sensors for fluorides should be able to produce a detectable diagnostic signal this concentration range.

Our new DCR probe **1** was constructed of an AB_3 self-immolative dendron[21,22] equipped with silyl ether, the triggering group, which can be cleaved by a fluoride anion (Scheme 1). Removal of this protecting group by fluoride exposes a phenolate, which undergoes rapid disassembly to release a dye reporter and two additional fluorides (Scheme 2). These two anions activate two more molecules of probe **1** to generate an autoinductive disassembly and exponential amplification of the diagnostic signal.

To employ a dendritic chain reaction for detection of fluoride, two major chemical reactivities must be in hand: a "disassembly" chemical pathway that enables the release of a covalently linked fluoride, and a specific protecting group that is cleaved by reaction with a fluoride. These two reactions are illustrated in Scheme 3. The release of a covalently linked fluoride was achieved through the design of molecules like compound **2a**. The synthesis of this model compound and probe **3** was achieved as shown in Scheme 4. 4-



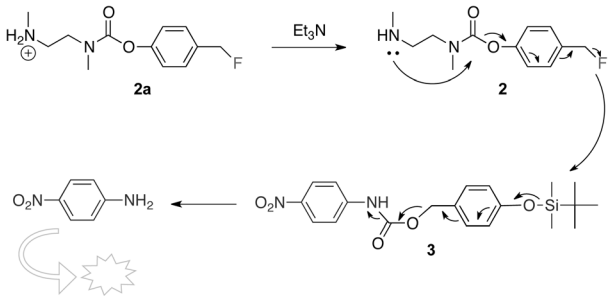
Scheme 1. Autoinductive disassembly of a DCR probe for the detection of fluoride.

Hydroxybenzyl-alcohol was treated with carbamoyl-chloride **2b** to give carbamate **2c**. Fluorination of the latter with (diethylamino)sulfur trifluoride (DAST) gave benzyl fluoride **2d**, which was deprotected under acidic conditions to produce compound **2a** in the form of an ammonium salt. Probe **3** was obtained in quantitative yield by an addition reaction of silyl-protected 4-hydroxybenzyl alcohol to 4-nitrophenyl isocyanate.

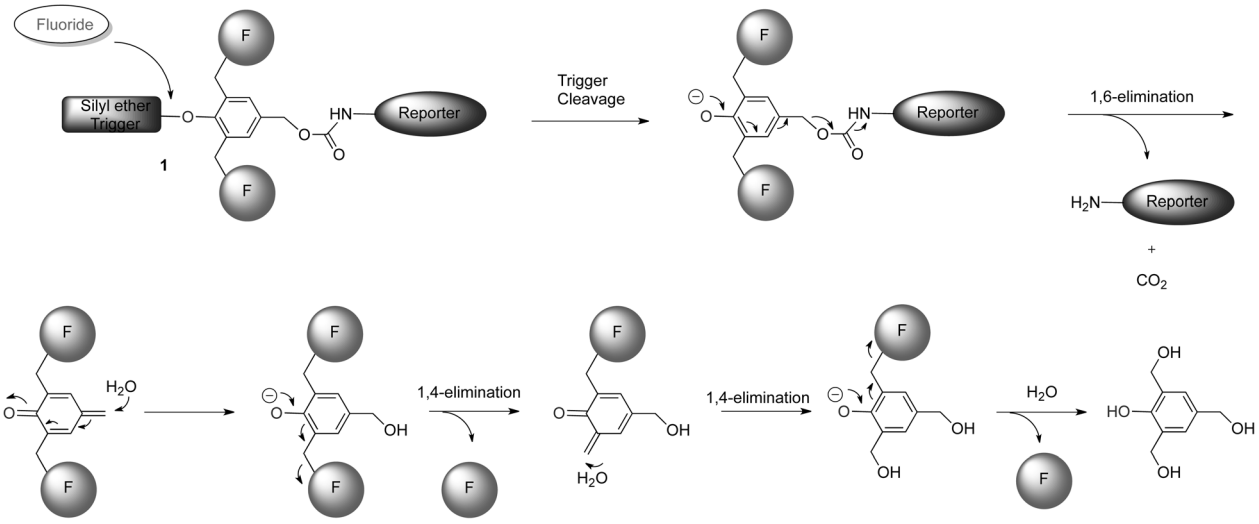
When triethylamine was incubated with ammonium salt **2a**, a rapid cyclization occurred to generate a phenloate that underwent 1,6-elimination to release a fluoride. The free fluoride treated with probe molecule **3** removed the *tert*-butyldimethylsilyl (TBDMS) protecting group, thereby initiating another 1,6-elimination and decarboxylation to release the reporter 4-nitroaniline.

The salt form of amine **2** (ammonium trifluoroacetate **2a**) and probe **3** were incubated together in methanol with or without of triethylamine, and the release of the 4-nitroaniline was monitored by UV/Vis spectroscopy. As expected, the presence of triethylamine with salt **2a** resulted in release of 4-nitroaniline from probe **3**, whereas no release was observed in the absence of the base (Figure 1). This model system clearly demonstrated the proposed disassembly pathway, which resulted in release of a covalently linked fluoride to activate a silyl-ether protecting-group-based probe.

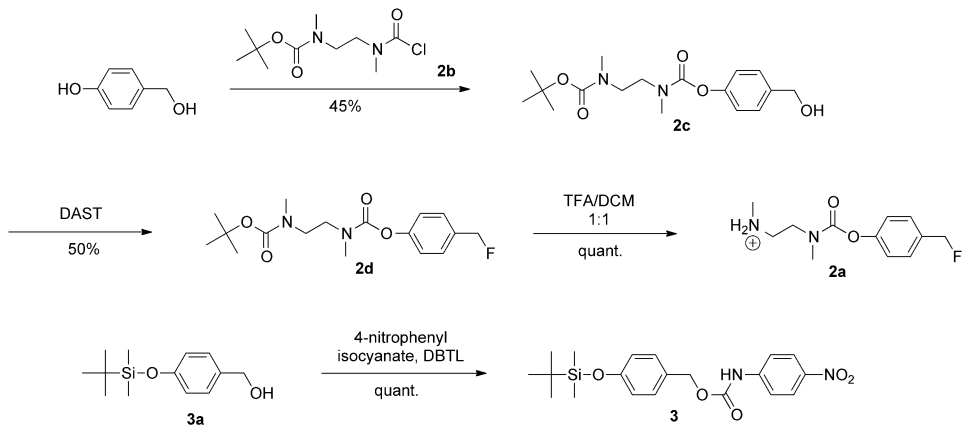
Based on the design presented in Scheme 1, we synthesized DCR probe **1** with a TBDMS protecting group as the trigger and 4-nitroaniline as a reporter (Scheme 5). To achieve the triggered release of fluoride, a fluorine was covalently attached to the benzylic positions of the AB_3 dendron, similar to the synthesis described for compound **2a**. The synthesis of the probe was performed according to Scheme 6. Phenol derivative **1a** was protected with



Scheme 3. Activation of a silyl ether trigger by release of covalently attached fluoride.



Scheme 2. Disassembly pathway of AB_3 self-immolative dendron **1** to release its reporter and the two fluorides.



Scheme 4. Chemical synthesis of compounds **2a** and probe **3**.

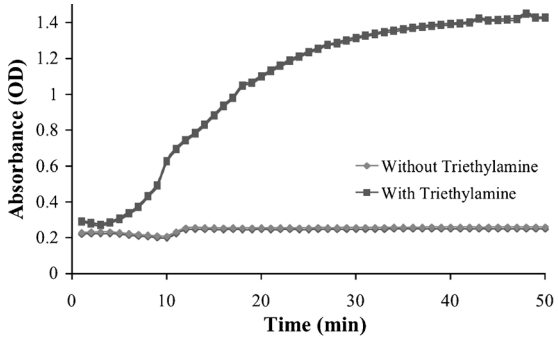
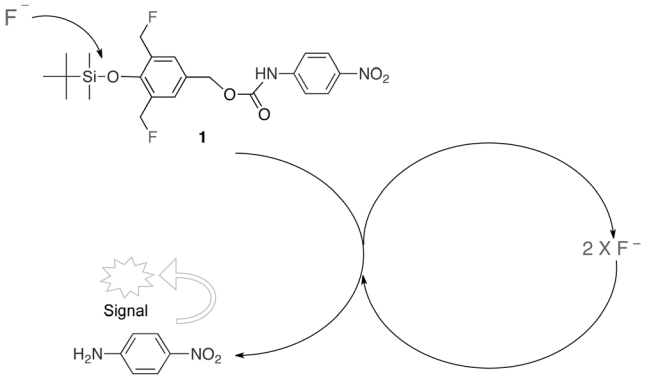


Figure 1. Release of 4-nitroaniline from probe **3** in the presence of the salt-form amine **2a** and triethylamine (squares). No reaction was observed in the absence of triethylamine (triangles).



Scheme 5. DCR-based probe for detection of fluorides.

3 equiv of TBSCl to give tri(silyl ether) **1b**. Reduction of the ester functional group with diisobutylaluminum hydride (DIBAL-H) afforded benzyl alcohol **1c**, which was treated with 4-nitrophenyl isocyanate to give carbamate **1d**. Selective deprotection of the benzylic silyl ethers by using *p*-toluene sulfonic acid (*p*-TsOH) gave diol **1e**, which then was fluorinated by DAST to produce probe **1**.

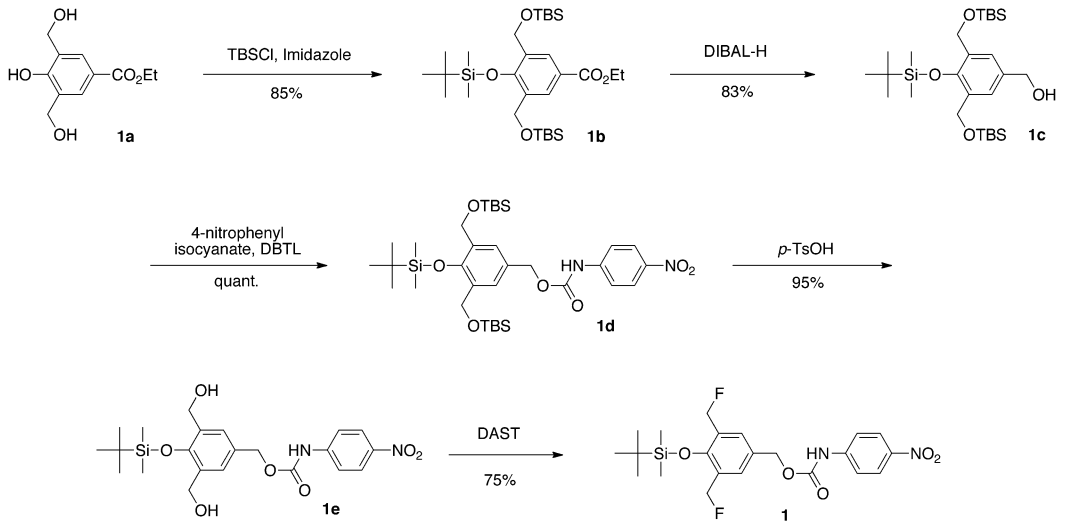
The DCR amplification cycle obtained by reaction of fluoride with probe **1** is illustrated in Scheme 5. Cleavage of the TBDMS trigger by a fluoride anion generates the unprotected phenol, which is further disassembled through triple quinone methide elimination to release 4-nitroaniline and two fluoride anions. The two fluorides can then activate two additional AB_3 dendrons. The rate of the disassembly should increase exponentially until all of the 4-nitroaniline molecules have been released. The signal can be detected by monitoring the yellow color of the released 4-nitroaniline.

To test whether an exponential amplification was indeed produced by the DCR probe, probe **1** was incubated in acetonitrile/dimethylsulfoxide (1:1) with 5% H_2O and various amounts of tetrabutyl-ammoniumfluoride (TBAF). The release of 4-nitroaniline was monitored by visible spectroscopy (Figure 2).

When 1.0 equiv of TBAF was used, the probe disassembled immediately (data not shown) to give a signal indicative of the complete release of the 4-nitroaniline. As expected, probe **1** reached complete disassembly after a longer period of time when less fluoride was used. Complete reaction occurred with as little as 0.004 equiv of fluoride (2 μM). The sigmoidal plots observed for various equivalents of TBAF are characteristic of an exponential progress of disassembly. The background signal due to some spontaneous hydrolysis of probe **1** in the absence of fluoride was also amplified. Nevertheless, the background amplification commenced only after about 10 h. Since the ideal and enforceable standards for fluoride in drinking water are between 1–4 $mg\,mL^{-1}$, the designed colorimetric probe is sufficiently sensitive for fluoride anion detection.

The selectivity of the DCR probe was tested by the addition of other common anions. As shown in Figure 3, only fluoride anions elicited a strong visual signal after 10 h from probe **1**, whereas iodide, bromide, chloride, and acetate caused no signal significantly above background under similar conditions.

The principles of the DCR system are based on autoinductive amplification generated by the release of trigger molecules. Once these molecules are free, they acquire the activity of the analyte of interest. Thus the free reagents have than the ability to trigger the disassembly of additional probes and thereby initiate new diagnostic cycles. The DCR approach offers considerable advantages over diagnostic probes that are based on a stoichiometric reaction between the analyte and the probe. In the DCR technique, the stoichiometric reaction of the analyte and the probe is exponentially amplified to generate a strong diagnostic signal. The DCR-based assay presented here can be extended to amplify diagnostic signals for the detection of other analytes by simply coupling it with another probe activity. During the preparation of this manuscript, a closely related example for signal amplification based on fluoride, was reported by Phil-



Scheme 6. Chemical synthesis of probe **1**.

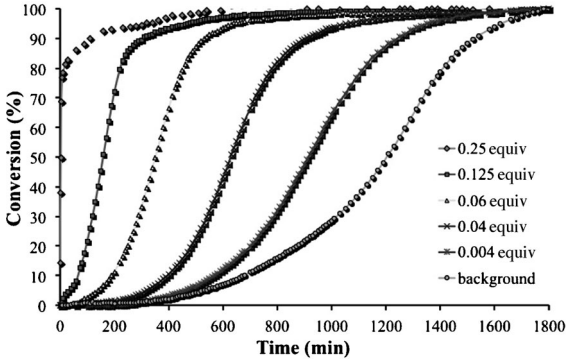


Figure 2. Release of 4-nitroaniline from probe **1** (500 μM) in acetonitrile/dimethylsulfoxide (1:1) upon addition of the indicated equivalents of TBAF. The reaction progress was monitored at a wavelength of 400 nm for the indicated time period.

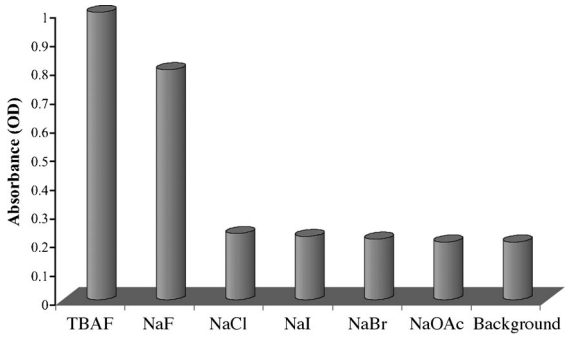


Figure 3. Absorbance of free 4-nitroaniline [400 nm] released from probe **1** (500 μM) upon addition of various anions (0.1 equiv) in acetonitrile/dimethylsulfoxide (1:1); 10 h incubation time, 25 °C.

lips' group.[23] That report elegantly demonstrates how a similar probe can amplify a diagnostic signal for the detection of threshold levels of palladium(II) when coupled with an additional component. We have previously demonstrated how DCR amplification can also be achieved by a two-component dendritic chain reaction (2CDCR).[15] This approach is based on disassembly mechanisms of a dendritic amplifier moiety and a chromogenic probe. Both are equipped with identical triggering systems designed for activation by a specific analyte. The amplifier component releases reagent units with the same chemical reactivity of the analyte, thereby initiating amplification cycles, and the probe component generates a chromogenic signal. The synthesis of this two-component amplification system is rather simple compared to the synthesis of a DCR probe that includes both the analyte and the chromogenic probe. In addition, the two-component system provides additional flexibility. Once the amplifier component is in hand, the diagnostic assay can be performed in combination with different types of probe molecules. Furthermore, the produced diagnostic signal could then be tuned by adjusting the ratio of the amplifier and the chromogenic probe. The 2CDCR approach could also be applied in the current example, in which the amplifier component releases three equivalents of fluoride and the second component equipped with the same silyl trigger generates the chromogenic molecule.

Conclusion

In summary, we have demonstrated a new DCR probe for detection of fluoride that exponentially amplifies a visual diagnostic signal. The probe is based on a dendritic chain reaction that generates a fluoride anion, the analyte of interest, during the disassembly pathway of the probe molecule. Aqueous fluoride solutions can be directly analyzed by diluting them into organic solvent mixture of acetonitrile and dimethylsulfoxide. The sigmoidal curves observed upon reaction of the DCR probe with substoichiometric quantities of fluoride provide a clear indication that the autoinductive amplification mechanism occurs through the designed disassembly pathway.

Experimental Section

General methods: All reactions requiring anhydrous conditions were performed under an argon or N_2 atmosphere. All reactions were carried out at room temperature unless stated otherwise. Chemicals and solvents were either analytical reagent grade or purified by standard techniques. Thin-layer chromatography (TLC) was carried out on silica gel plates Merck 60 F254. The compounds were visualized by irradiation with UV light. Flash chromatography (FC) was carried out using silica gel Merck 60 (particle size 0.040–0.063 mm); the eluent is given in parentheses. ^{1}H NMR spectra were measured using a Bruker Avance instrument operated at 400 MHz as indicated. ^{13}C NMR spectra were measured using a Bruker Avance instrument operated at 100 MHz as indicated. ^{19}F NMR spectra were measured using a Bruker Avance instrument operated at 188 MHz as indicated. The chemical shifts (δ) are expressed relative to TMS (0 ppm), and coupling constants (J) are in Hz. The spectra were recorded in $CDCl_3$ as solvent at room temperature unless stated otherwise. All general reagents, including salts and solvents, were purchased from Sigma–Aldrich.

Compound 2c: Commercially available 4-hydroxybenzyl alcohol (200 mg, 1.61 mmol) was dissolved in pyridine (2 mL), and compound **2b** (483 mg, 1.93 mmol) and catalytic amount of DMAP (5 mg) were added. The reaction was stirred in room temperature overnight and was monitored by TLC (EtOAc/hexane 50:50). Upon completion, the reaction was diluted with EtOAc and washed twice with saturated NH_4Cl solution. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexane 50:50) to give compound **2c** (250 mg, 45%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.25 (d, J=6.4 Hz, 2H), 7.01 (d, J=6.4 Hz, 2H), 4.53 (s, 2H), 3.51–3.42 (m, 4H), 3.07–2.85 (m, 6H), 1.44 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=156.30, 155.51, 151.12, 139.18, 128.48, 122.44, 80.69, 64.93, 47.45, 35.91, 35.43, 29.11 ppm; MS (ESI+): m/z calcd for $C_{17}H_{26}N_2O_5$: 338.1; found: 361.2 $[M+Na]^+$.

Compound 2d: Compound **2c** (122 mg, 0.36 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled to −78°C. DAST (71 μL, 0.54 mmol) was added and the reaction was stirred for 35 min and monitored by TLC (EtOAc/hexane 30:70). Upon completion, the reaction was quenched with water (2 mL), diluted with EtOAc, and washed with brine. The organic layer was dried over magnesium sulfate, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexane 20:80) to give compound **2d** (61 mg, 50%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.35 (d, J=8.4 Hz, 2H), 7.10 (d, J=8.4 Hz, 2H), 5.31 (d, J=47.8 Hz, 2H), 3.55–3.46 (m, 4H), 3.09–2.87 (m, 6H), 1.43 ppm (s, 9H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=155.47, 152.47, 133.87, 129.41, 122.80, 85.59, 83.94, 47.67, 35.97, 35.28, 28.40 ppm; ^{19}F NMR (188 MHz, $CDCl_3$): −205.95 ppm (t, J=47.8 Hz); MS (ESI+): m/z calcd for $C_{17}H_{25}FN_2O_4$: 340.1; found: 363.2 $[M+Na]^+$.

Compound 2a: Compound **2a** was obtained in quantitative yield as a white solid after compound **2d** was dissolved in a mixture of CH_2Cl_2/trifluoroacetic acid (TFA) (1:1), stirred for 5 min, and the solvents were removed under reduced pressure.

Compound 3: Compound **3a**[24] (203 mg, 0.85 mmol) was dissolved in dry THF (5 mL). 4-Nitrophenyl isocyanate (168 mg, 1.02 mmol) was added, followed by a catalytic amount of dibutyltin dilaurate (DBTL). The reaction mixture was heated to 50°C, stirred for 1 h under an Ar atmosphere, and monitored by TLC (EtOAc/hexane 30:70). After completion, the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexane 20:80) to give compound **3** (342 mg, 100%) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.21 (d, J=9.2 Hz, 2H), 7.53 (d, J=9.2 Hz, 2H), 7.28 (d, J=8.6 Hz, 2H), 7.05 (brs, 1H), 6.84 (d, J=8.6 Hz, 2H), 5.15 (s, 2H), 0.98 (s, 9H), 0.20 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=156.07, 152.75, 143.87, 142.86, 130.15, 127.91, 125.08, 120.18, 117.68, 67.50, 25.61, 25.45, 18.09, −4.61 ppm; MS (ESI+): m/z calcd for $C_{20}H_{26}N_2O_5Si$: 402.1; found: 425.1 $[M+Na]^+$.

Compound 1b: Compound **1a**[21] (500 mg, 2.25 mmol) was dissolved in DMF (2 mL) and cooled to 0°C. Imidazole (766 mg, 11.25 mmol) and TBSCl (1.69 gr, 11.25 mmol) were added. The reaction was allowed to warm to room temperature and was stirred for an additional 2 h. The reaction was monitored by TLC (EtOAc/hexane 5:95). Upon completion, the reaction was diluted with diethyl ether and washed with saturated NH_4Cl solution followed by brine. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOH/hexane 5:95) to give compound **1b** (1.07 g, 85%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.06 (s, 2H), 4.67 (s, 4H), 4.31 (q, J=7.2 Hz, 2H), 1.33 (t, J=7.2 Hz, 3H), 0.98 (s, 9H), 0.93 (s, 18H), 0.16 (s, 6H), 0.07 ppm (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=170.01, 156.19, 132.63, 128.57, 124.26, 61.19, 60.03, 26.72, 26.64, 19.57, 19.11, 14.98, −2.53, −4.54 ppm; MS (CI+): m/z calcd for $C_{29}H_{56}O_5Si_3$: 568.3; found: 569.4 $[M+H]^+$.

Compound 1c: Compound **1b** (983 mg, 1.72 mmol) was dissolved in dry THF (10 mL) under an Ar atmosphere and cooled to −78°C. DIBAL-H (1M in toluene, 6.91 mL, 6.91 mmol) was added dropwise. The reaction was stirred under these conditions for 30 min and was monitored by TLC (EtOAc/hexane 10:90). Upon completion, the reaction was quenched with saturated NH_4Cl solution (5 mL) and diluted with diethyl ether. Celite was added, and the reaction mixture was stirred at room temp for 30 min. After filtration, the organic layer was dried over magnesium sulfate, and the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexane 10:90) to give compound **1c** (750 mg, 83%) as a colorless oil. ^{1}H NMR (400 MHz, $CDCl_3$): δ=7.37 (s, 2H), 4.71 (s, 4H), 4.63 (d, J=5.8 Hz, 2H), 1.02 (s, 9H), 0.94 (s, 18H), 0.18 (s, 6H), 0.15 ppm (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=165.87, 132.58, 128.56, 125.79, 66.49, 61.33, 26.71, 26.66, 19.57, 19.12, −2.61, −4.52 ppm; MS (CI+): m/z calcd for $C_{27}H_{54}O_4Si_3$: 526.3; found: 526.2 $[M]^+$.

Compound 1d: Compound **1c** (228 mg, 0.43 mmol) was dissolved in dry THF (5 mL). 4-Nitrophenyl isocyanate (85 mg, 0.52 mmol) was added, followed by catalytic amount of DBTL. The reaction mixture was heated to 50°C, stirred for 45 min under an Ar atmosphere, and monitored by TLC (EtOAc/hexane 10:90). After completion, the solvent was removed under reduced pressure. The crude product was purified by column chromatography on silica gel (EtOAc/hexane 10:90) to give compound **1d** (298 mg, 100%) as a yellowish solid. ^{1}H NMR (400 MHz, $CDCl_3$): δ=8.19 (d, J=9.2 Hz, 2H), 7.55 (d, J=9.2 Hz, 2H), 7.43 (s, 2H), 6.96 (brs, 1H), 5.22 (s, 2H), 4.72 (s, 4H), 1.04 (s, 9H), 0.94 (s, 18H), 0.19 (s, 6H), 0.10 ppm (s, 12H); ^{13}C NMR (100 MHz, $CDCl_3$): δ=153.54, 149.09, 144.77, 143.64, 132.81, 129.21, 127.03, 125.88, 118.39, 68.75, 61.25, 26.72, 19.50, 19.15, −2.56, −4.55 ppm; MS (ESI+): m/z calcd for $C_{34}H_{58}N_2O_7Si_3$: 690.3; found: 713.6 $[M+Na]^+$.

Compound 1e: Compound **1d** (250 mg, 0.36 mmol) was dissolved in MeOH (2 mL). A catalytic amount of p-TsOH was added to the suspension. The reaction mixture was stirred at room temperature for 20 min and monitored by TLC (EtOAc/hexane 40:60). Upon completion, the reaction was diluted with EtOAc and washed with saturated $NaHCO_3$ solution followed by brine. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by using column chromatography on silica gel (EtOAc/hexane 30:70) to give compound **1e** (157 mg, 95%) as a yellow solid. ^{1}H NMR (400 MHz, MeOD): δ=8.10 (d, J=9.0 Hz, 2H), 7.51 (d, J=9.0 Hz, 2H), 7.30 (s, 2H), 5.07 (s, 2H), 4.57 (s, 2H), 0.94 (s, 9H), 0.10 ppm (s, 6H); ^{13}C NMR (100 MHz, MeOD): δ=153.55, 150.32, 145.44, 143.29, 132.84, 130.01, 128.20, 125.75, 118.38, 67.71, 60.66, 26.57, 19.33, −2.97 ppm; MS (ESI+): m/z calcd for $C_{22}H_{30}N_2O_7Si$: 462.1; found: 485.2 $[M+Na]^+$.

Compound 1: Compound **1e** (150 mg, 0.32 mmol) was dissolved in CH_2Cl_2 (5 mL) and cooled to −78°C. DAST (206 mg, 1.3 mmol) was added and the reaction was stirred for 5 min and monitored by TLC (EtOAc/hexane 20:80). Upon completion the reaction was quenched with 2 mL water, diluted with EtOAc and washed with brine. The organic layer was dried over magnesium sulfate and the solvent was removed under reduced pressure. The crude product was purified by column chro-

matography on silica gel (EtOAc/hexane 10:90) to give compound **1** (112 mg, 75%) as a yellow powder. ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 8.03$ (d, $J = 9.2$ Hz, 2H), 7.54 (d, $J = 9.2$ Hz, 2H), 7.47 (s, 2H), 7.00 (br s, 1H), 5.54 (s, 2H), 5.27 (d, $J = 47.6$ Hz, 4H), 1.04 (s, 9H), 0.09 ppm (s, 6H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 153.62$, 151.68, 145.39, 143.31, 131.33, 130.34, 126.17, 118.41, 117.37, 81.28, 79.64, 66.94, 26.43, 19.27, −2.96 ppm; ^{19}F NMR (200 MHz, $CDCl_3$): −209.74 ppm (t, $J = 47.6$ Hz); MS (ESI+): m/z calcd for $C_{22}H_{28}F_2N_2O_5Si$: 466.1; found: 489.1 $[M+Na]^+$.

Assay conditions for detection of fluoride by spectrophotometer: A stock solution of compound **1** was prepared in DMSO, and a stock solution of TBAF was prepared in THF. Stock solutions of all other reagents were prepared in H_2O. In a typical assay, compound **1** (500 μM) was incubated in MeCN/DMSO (1:1). Various amounts of TBAF or a different reagent were added, and the release of 4-nitroaniline was monitored using a 96-well microplate reader ($\lambda = 400$ nm).

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