Self-immolative dendritic probe for direct detection of triacetone triperoxide†

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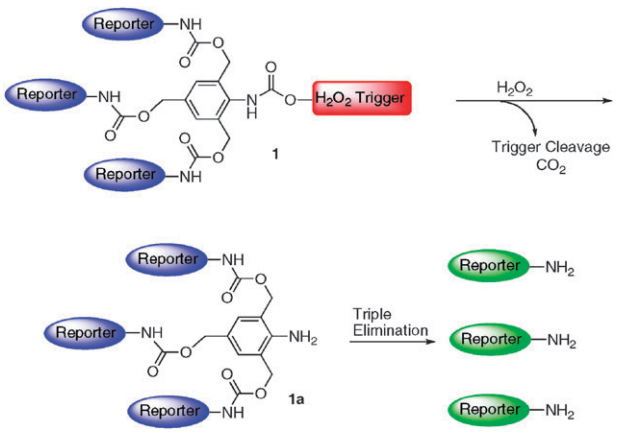
A new self-immolative dendritic probe directly detects triacetone triperoxide through amplification of a single cleavage event initiated by one molecule of hydrogen peroxide into multiple-release of fluorogenic end-groups.

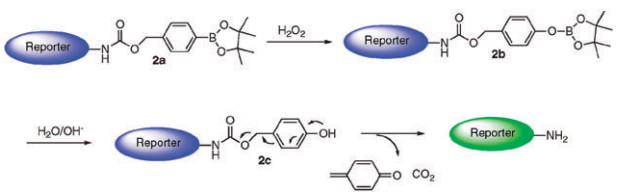
Amplification of molecular signals is a major requirement for the development of sensitive probes in the field of chemical sensing.[1,2] Recently several new approaches have been used to increase the signal-to-noise ratio, improving the sensitivity of detection assays.[3–6] For obvious reasons, particular emphasis has been placed on the development of new techniques for detection of explosives.[7–10] Triacetone triperoxide (TATP) has become an illicit explosive of choice due to its straightforward synthesis using readily available precursor chemicals, acetone and hydrogen peroxide. Treatment of acid with TATP results in the generation of hydrogen peroxide and therefore, a probe for hydrogen peroxide could be used to detect TATP.[11] Here we show that a new self-immolative dendritic-based probe detects TATP at microgram levels.

Self-immolative dendrimers are unique molecules that spontaneously release all of their end-groups following a single activation event.[12–14] The initial activation, or triggering, event induces a cascade of self-eliminations that leads to complete dissociation of the dendrimer into its separate building blocks.[15] Self-immolative dendritic probe **1** is composed of three reporter groups and a trigger that can be activated by hydrogen peroxide (Scheme 1). Exposure of the probe to hydrogen peroxide should result in generation of intermediate **1a** that will subsequently undergo triple elimination to release the three amine-reporters.[16] A single molecule of hydrogen peroxide will generate three molecules of free reporter, which should provide a readable signal.

Aryl borate ester **2a** is known to react with hydrogen peroxide under mild alkaline conditions to generate intermediate **2b**, which can be hydrolyzed to produce phenol **2c** (Scheme 2). The latter undergoes 1,6-elimination and decarboxylation reactions to release the free amine reporter.[17] For that reason, aryl borate ester is used as a protecting group for amine functionalities and the desired amine is obtained upon



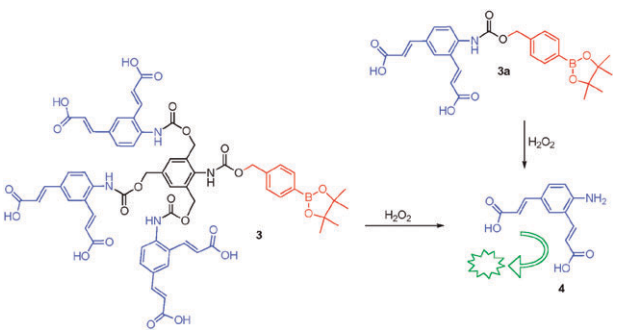
Scheme 1 Activation of dendritic probe **1** by a single molecule of hydrogen peroxide to release three reporter units.



Scheme 2 Deprotection mechanism of aryl borate ester by hydrogen peroxide.

deprotection by hydrogen peroxide. This group should also be suitable for a self-immolative dendrimer trigger.

We therefore designed and synthesized monomeric probe **3a** and trimeric probe **3** (Scheme 3). Aryl borate ester is used as a trigger designed for activation by hydrogen peroxide and



Scheme 3 Activation of probes **3** and **3a** by hydrogen peroxide to release reporter **4**.

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amine **4** is used as a fluorescent reporter. While exposure of probe **3a** to one molecule of hydrogen peroxide will result in the release of one reporter, three reporters should be released from probe **3**. Amine **4** (for synthesis, see the ESI†) was selected as a reporter unit since it is water-soluble and has strong fluorescence in its free amine form.

The fluorescence spectra of probes **3** and **3a** and reporter **4** are presented in Fig. 1. Unmasking the carbamate derivative of amine **4** to produce free aromatic amine functional group (of probes **3** and **3a**) results in a red shift of the maximum emission wavelength from 450 nm to 510 nm. Therefore, the release of free reporter from probes **3** and **3a** can be conveniently monitored by a fluorescence reader at a wavelength higher than 510 nm.

Initially, we evaluated the disassembly of probes **3** and **3a** by RP-HPLC assay. The probes were incubated with hydrogen peroxide in aqueous $NaHCO_3$ (pH = 8.3) and the release of reporter **4** was monitored. The data shown in Fig. 2 confirmed the release of the reporter units from both probes. The total amount of reporter **4** released from probe **3** was about three times greater than that released from probe **3a**. The disassembly of probe **3a** was rapid, whereas that of probe **3** took approximately 90 min since it required several steps.

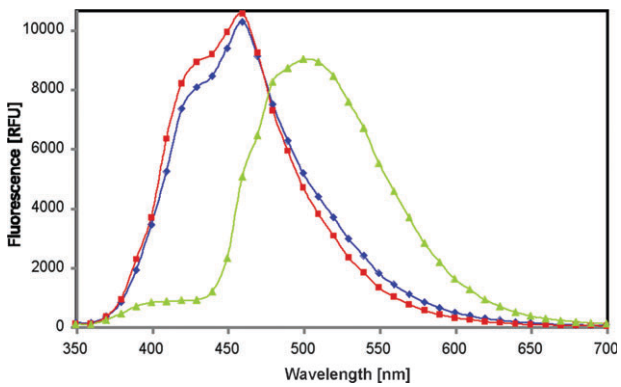

Fig. 1 Fluorescence spectra (λ_{ex} = 270 nm) of compound **4** (-■-) [500 μM], compound **3a** (-♦-) [500 μM] and compound **3** (-▲-) [500 μM] in $NaHCO_3$ pH = 8.3.

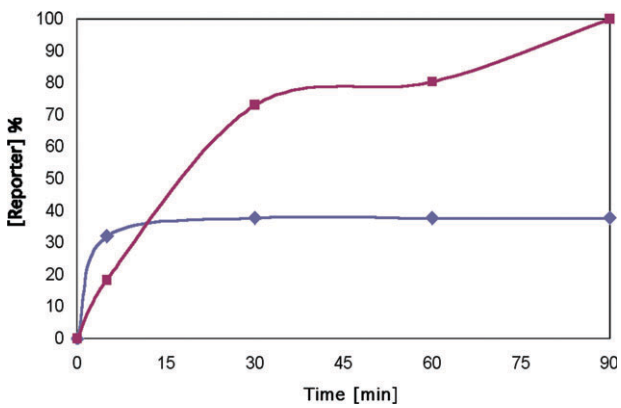

Fig. 2 Release of reporter **4** from probes **3** (-■-) [500 μM] and **3a** (-♦-) [500 μM] upon incubation with H_2O_2 [500 μM] in aqueous $NaHCO_3$, pH = 8.3.

Next, we measured the emitted fluorescence generated upon exposure of the probes to hydrogen peroxide. In order to evaluate the amplification due to the self-immolative dendritic platform, we used three equivalents of probe **3a** and one equivalent of probe **3**. The probes were incubated with various amounts of hydrogen peroxide for 90 min in aqueous $NaHCO_3$, then the emitted fluorescence was recorded at wavelength of 560 nm (Fig. 3). As expected, the fluorescence generated by dendritic probe **3** upon reaction with hydrogen peroxide was approximately three-fold higher than the fluorescence generated of probe **3a**. Hydrogen peroxide was detected without any difficulty at a concentration of 1 μM. The intensity of the background fluorescence emitted by the probes in the absence of hydrogen peroxide under these conditions was less than 100 RFU and did not change during the 90 min incubation time.

With these results in hand, we evaluated the sensitivity of our dendritic probe as a sensor for direct detection of TATP. As in the previous experiment, three equivalents of probe **3a** and one equivalent of dendritic probe **3** were used. The probes were exposed to various amounts of TATP (without any pretreatment) for 120 min and the emitted fluorescence was recorded. As shown in Fig. 4, the fluorescence generated from dendritic probe **3** was about three-fold higher than the fluorescence generated from probe **3a**. Remarkably, TATP samples could be straightforwardly detected on a microgram scale.

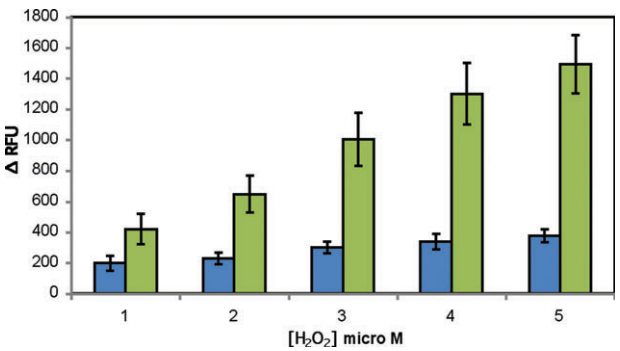

Fig. 3 Fluorescence response (λ_{ex} 270 nm, λ_{em} 560 nm) of probes **3a** (blue) [150 μM] and **3** (green) [150 μM] in aqueous $NaHCO_3$ pH = 8.3, upon incubation with various concentrations of hydrogen peroxide.

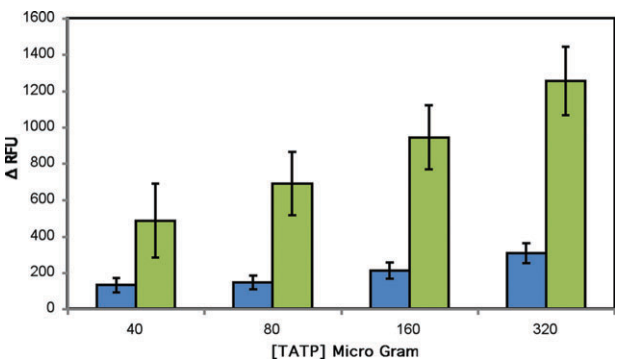

Fig. 4 Fluorescence response (λ_{ex} 270 nm, λ_{em} 560 nm) of probes **3a** (blue) [150 μM] and **3** (green) [150 μM] in aqueous $NaHCO_3$ pH = 8.3, upon incubation with various concentrations of TATP.

The amplification effect of the dendritic platform is due to the multiple-release of the dendrimer end-groups after a single cleavage event. In contrast, with classic chromogenic probes, one cleavage event generates a single free reporter molecule. The fluorescence reporter **4** has certain features essential for an efficient detection system: It emits strong fluorescence when the amine functional group is free, while almost no fluorescence was detected from the reporter when the amine was masked in the form of a carbamate. In addition, reporter **4** has two ionized carboxyl acid functional groups that result in significant water solubility, allowing this dendritic system to act under aqueous conditions. Most of the chromogenic assays developed for detection of TATP require pretreatment of the sample with acid; this treatment results in decomposition of the acetonide peroxide to hydrogen peroxide, which then reacts with the probe system to generate a visual signal.[11] Our dendritic probe proved sufficiently sensitive for direct detection of TATP without acid pretreatment. The sensitivity of our dendritic probe could be increased further by constructing a higher-generation dendrimer with the same trigger. This would increase the signal to noise ratio and would allow detection of an even lower concentration of TATP. While the two major matrices for TATP detection are gas and solid samples, our approach is most suitable for detection of solid traces. However, it should be possible to collect vapor samples and after a simple concentration protocol to apply our probe for the trace detection.

In summary, we have developed a new molecular probe for detection of TATP. The probe is based on a self-immolative dendritic platform that can amplify a single cleavage event generated by hydrogen peroxide into a multiple-release of fluorogenic end-groups. The signal intensity produced by the dendritic probe is about three times higher than that of the non-dendritic one and background noise is comparable. The dendritic probe was used to detect TATP in micrograms amounts in aqueous buffer without any pretreatment.

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