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Single-Triggered AB_6 Self-Immolative Dendritic Amplifiers

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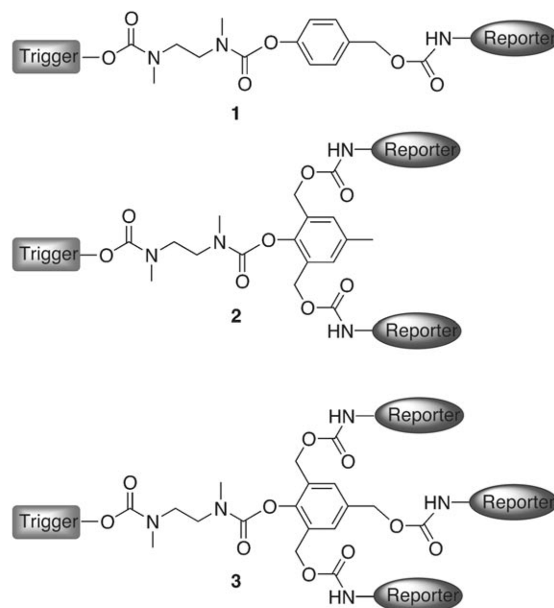
Abstract: Self-immolative dendrimers are a unique class of molecules that are able to disassemble upon undergoing a specific triggering reaction through domino-like fragmentations. We have designed and synthesized a novel AB_6 self-immolative dendritic adaptor that amplifies a single cleavage event into the release of six reporter units. The disassembly mechanism is based on a specifically triggered cleavage event followed by elimination of a cyclic urea derivative and six consecutive quinone methide eliminations. The system was disassembled under both organic and aqueous conditions by either chemical or enzymatic triggering. Various reporter molecules and triggering groups were introduced onto the dendritic adaptor to obtaining sensor molecules with enhanced properties for diagnostics and imaging.

Keywords: dendrimers · fluorescence · molecular amplifiers · prodrugs · self-immolative

Introduction

Amplification of molecular signals is an important task in the fields of diagnostics,[1,2] imaging,[3] and drug delivery.[4] In each of these fields there is a need for the release of active signaling or drug molecules on specific demand. We, and two other groups, have recently reported the design and synthesis of dendrimers that fragment into their building blocks in a self-immolative manner to release the tail-group units after fragmentation has been initiated by a triggering reaction.[5–7] The unique disassembly of these molecules was harnessed for the construction of self-immolative dendritic prodrugs[8] with single or multiple triggering modes of activation.[9–14] The basic building block of a first-generation dendrimer is usually referred to as an AB_n unit, in which A is the head and B is the tail. Single-triggered self-immolative dendritic adaptors AB_1 (**1**), AB_2 (**2**), and AB_3 (**3**) were developed. The release of an active reporter molecule from compound **1** is initiated by cleavage of the trigger, followed by removal of a cyclic dimethylurea derivative, and a 1,6-quinone methide rearrangement. Similarly, dendron **2** undergoes a double 1,4-quinone methide rearrangement to release two reporter units, whereas dendron **3** undergoes a 1,6- and a double 1,4-quinone methide rearrangement to release three reporter units.



Elimination of reporter groups by the quinone methide rearrangement was shown to be an efficient reaction to exploit when designing AB_n self-immolative dendritic adaptors.[15] This elimination takes place when there is a good

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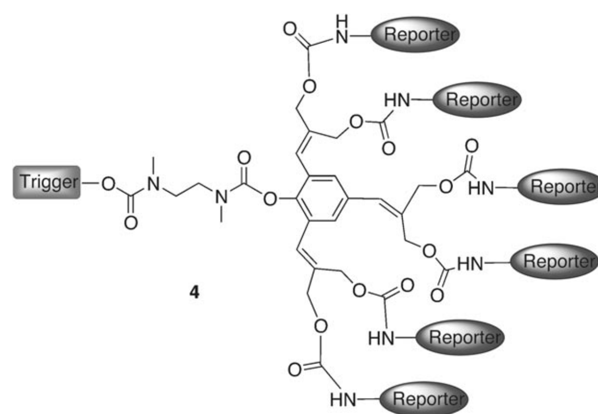
leaving group *para* or *ortho* to the phenolic oxygen. Therefore, the maximum number of reporter units linked to one benzene ring is limited to three because there are only two *ortho* and one *para* positions available. Herein we report the design, synthesis, and disassembly of a novel AB_6 self-immolative dendritic molecule in which the number of substituents that are conjugated to the phenolic oxygen has been doubled. In this AB_6 dendron, a single cleavage event releases six reporter units.

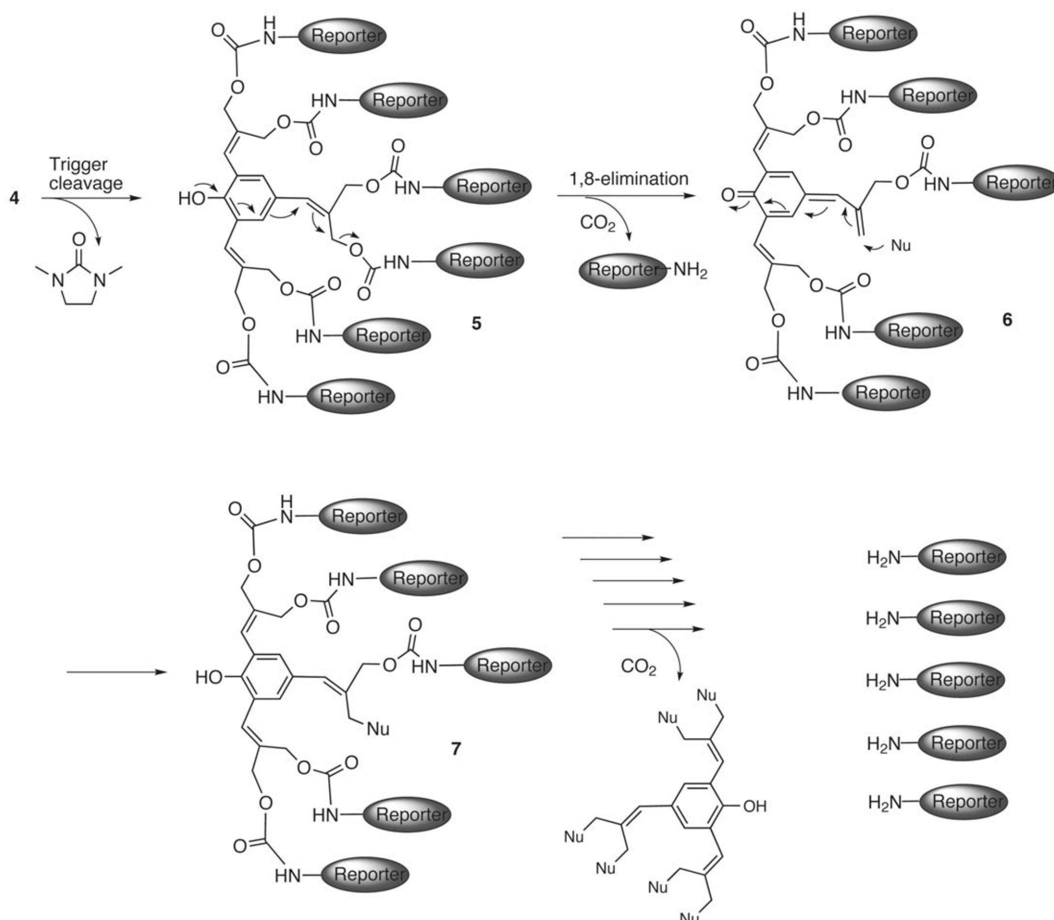


Results and Discussion

To generate an AB_6 self-immolative dendritic adaptor from a benzene ring, we designed dendron **4**. The number of substituents that were conjugated to the phenolic oxygen was doubled through a short split extension. This molecular design allows a single cleavage reaction to be amplified to release six active reporter units.

The disassembly mechanism of dendron **4** is illustrated in Scheme 1. Cleavage of the trigger initiates the cyclization of a dimethylurea derivative to release phenol **5**. The latter can undergo 1,8-elimination followed by decarboxylation to release one reporter unit and generate quinone methide **6**. In the next step, a nucleophile (most likely a solvent molecule) reacts with the highly electrophilic quinone methide to generate phenol **7**. Similarly, we hypothesize that another 1,8-elimination and four 1,6-eliminations occur to lead to the release of all six reporter units.

Next, we synthesized AB_6 dendron **8** that contains six molecules of aminomethylpyrene as the reporter units and the *t*-butyloxycarbonyl (Boc) protecting group as the trigger



Scheme 1. Disassembly mechanism of AB_6 self-immolative dendron **4**.

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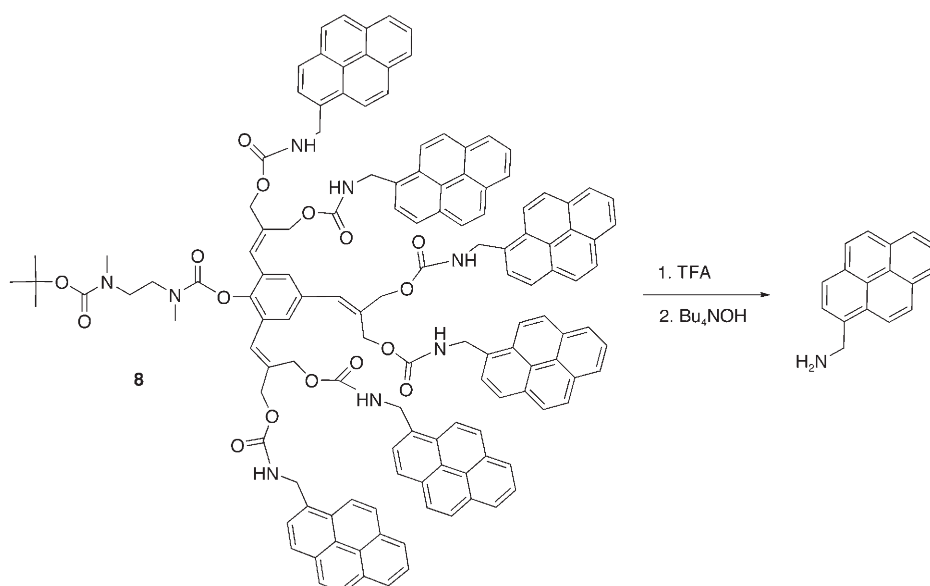
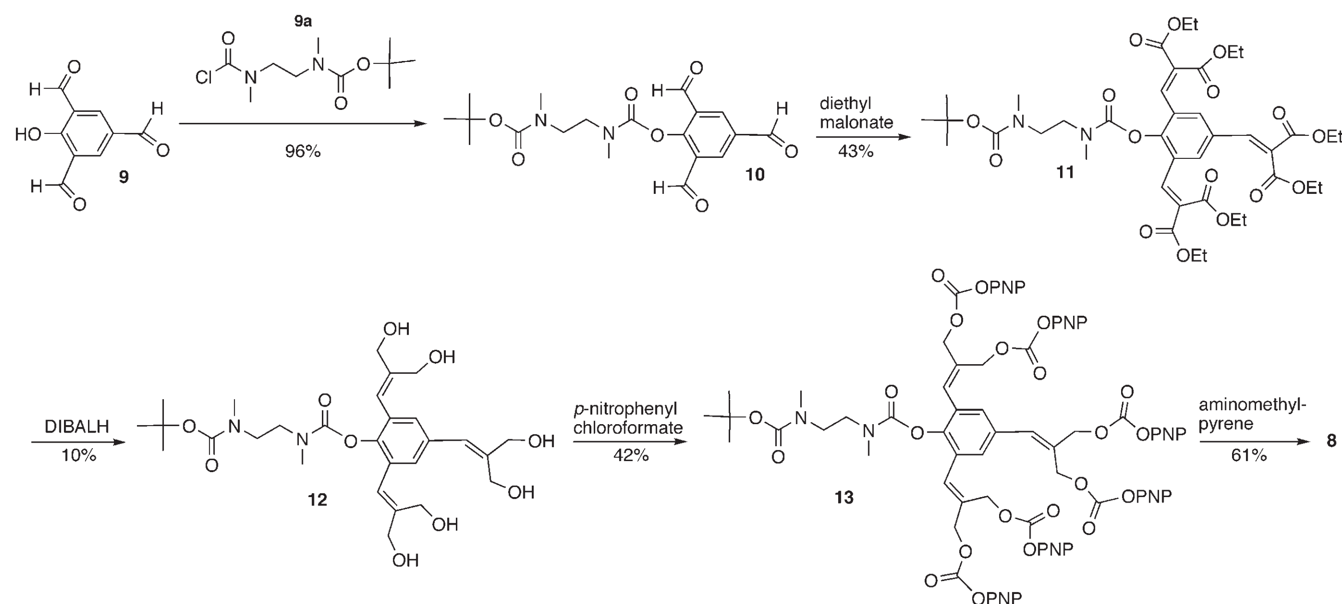


Scheme 2. Molecular structure of a self-immolative AB_6 dendron with aminomethylpyrene reporter units and a Boc protecting group as a trigger.

(Scheme 2). The dendron was designed to release its six tail units upon removal of the protecting group.

The synthesis of **8** was performed by using the synthetic strategy shown in Scheme 3. Trialdehyde **9** was prepared as previously described.[16] Acylation of the phenol group of **9** by using the chlorocarbamoyl of *N,N'*-dimethylethylenediamine (**9a**) generated trialdehyde **10**. The latter was condensed with three equivalents of diethyl malonate to give hexaethyl ester **11**, which was reduced with diisobutyl aluminumhydride to afford hexaol **12**. Acylation of **12** with excess *p*-nitrophenyl (PNP) chloroformate generated hexacarbonate **13**, which was further treated with six equivalents of aminomethylpyrene to afford dendron **8**.

To evaluate the disassembling activity of dendron **8**, the Boc trigger was removed by using trifluoroacetic acid (TFA) to generate the corresponding ammonium salt; this was incubated in 1:1 MeOH/DMSO in a 2% aqueous solution of tetrabutylammonium hydroxide. As a control reaction, dendron **8**, which contained an intact trigger unit was incubated under identical conditions. The release of free aminomethylpyrene was monitored by reverse-phase HPLC (RP-HPLC). Figure 1 shows that the pyrene tail units were completely released within 6 h. Importantly, no release was observed in the control solution. In the HPLC chromatogram, the signal that corresponds to aminomethylpyrene was directly observed from the disassembly of the ammonium salt of dendron **8** without the formation of any other intermediates. This observation suggests that the rate-determining step of the disassembly process is the cyclization of the amine to release the dimethylurea derivative and phenol **5** (Scheme 1). The latter has a short lifetime and the six elimination reactions, which occur to release aminomethylpyrene that is observed are probably fast.



Scheme 3. Chemical synthesis of AB_6 self-immolative dendron **8**.

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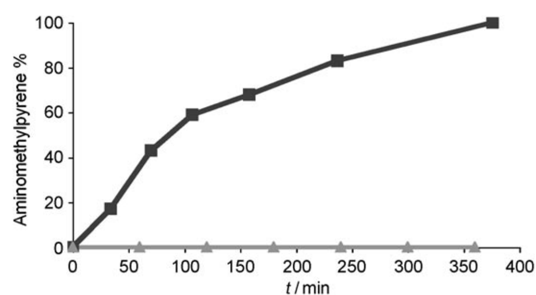


Figure 1. Release of aminomethylpyrene from dendron **8** upon removal of the Boc-protecting group trigger (■; 1:1 MeOH/DMSO mixture that contains 2 % aqueous solution of Bu_4NOH). Control reaction under similar conditions with dendron **8** with the Boc trigger intact (▲).

Additional support for this disassembly mechanism was obtained by monitoring the release of the pyrene tail units by fluorescence spectroscopy. The confined nature of the pyrene units in the dendritic molecule results in the formation of excimers. Excimer fluorescence generates a broad band at a wavelength of 470 nm in the emission spectrum of dendron **8** (Figure 2). Upon the release of the pyrene units from the dendritic platform, the band at $\lambda = 470$ nm disappeared from the spectrum and the band at $\lambda = 408$ nm, which corresponds to free aminomethylpyrene, increased. Similar to the results obtained from the HPLC assay, no decrease in the excimer band was observed for the control reaction that contained dendron **8** with an intact Boc trigger unit (data not shown). Similar photophysical behavior was observed in the fluorescence spectrum of pyrene-functionalized poly(propylene imine) dendrimers.[17,18]

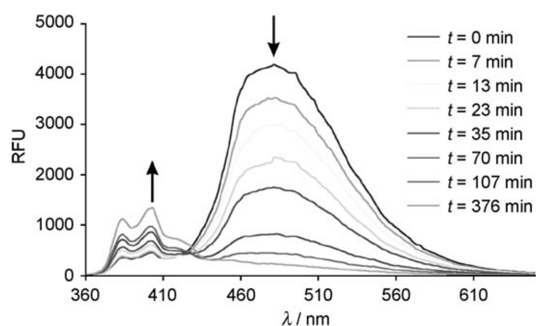
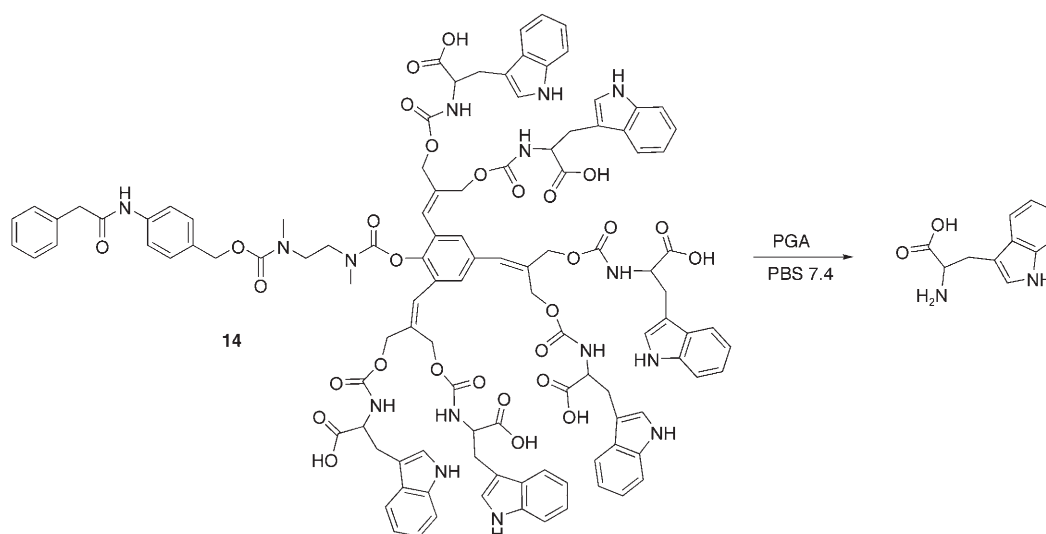


Figure 2. Emission fluorescence spectra ($\lambda_{ex} = 340$ nm) of dendron **8** [50 μM] upon removal of the Boc trigger upon incubation in a 1:1 MeOH/DMSO mixture that contains 2 % aqueous solution of Bu_4NOH.

We also wanted to evaluate the disassembly of our dendritic system under physiological conditions. Thus, we synthesized self-immolative AB_6 dendron **14** that contained water-soluble tryptophan tail units and a phenylacetamide head as the trigger unit (Scheme 4) to evaluate disassembly in aqueous conditions. The phenylacetamide is selectively cleaved by the bacterial enzyme penicillin G amidase (PGA).[19] The trigger was designed to disassemble through an azaquinone methide rearrangement and cyclic dimethylurea elimination to release a phenol intermediate that will undergo six quinone methide elimination reactions to release the tryptophan tail units.

The synthesis of dendron **14** was performed by using the synthetic strategy shown in Scheme 5. Hexacarbonate **13** was reacted with excess tryptophan to give dendron **15**. The Boc protecting group of **13** was removed by using TFA to generate an ammonium salt, which was further treated with linker **16** (prepared as reported in ref. [12]) to afford dendron **14**.

Dendron **14** was incubated in phosphate buffer saline (PBS, pH 7.4) in the presence of and in the absence of PGA. The progress of the disassembly process was monitored by RP-HPLC and the results are presented in Figure 3. Tryptophan was gradually released from dendron **14** upon incubation with PGA. The release of tryptophan was completed within 48 h in the presence of PGA, whereas no release was observed for the control reaction in which PGA was absent. Although disassembly of this dendron oc-



Scheme 4. Molecular structure of a self-immolative AB_6 dendron with tryptophan tail units and phenylacetamide group as a trigger.

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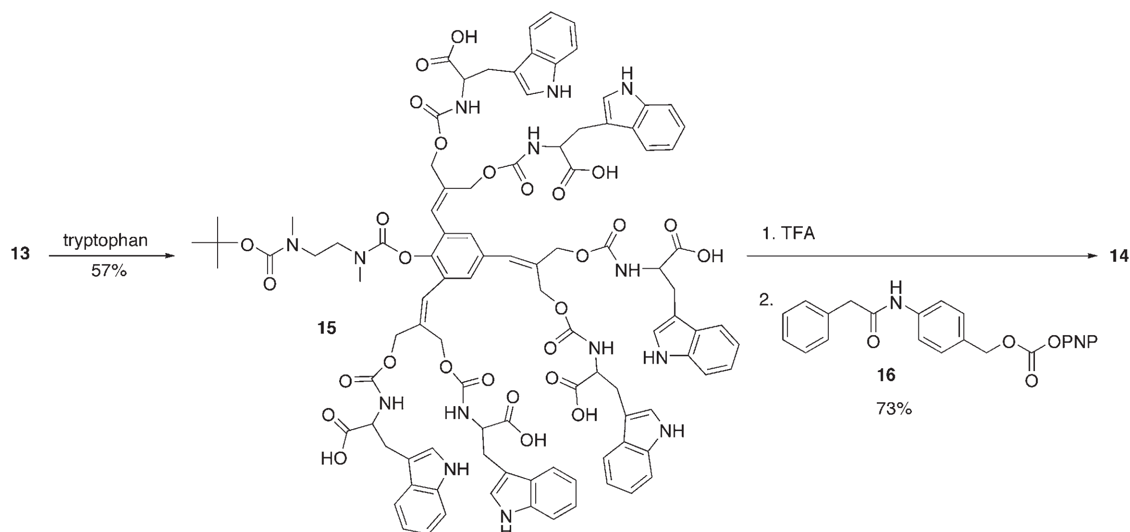

Scheme 5. Chemical synthesis of self-immolative AB_6 dendron **14**.

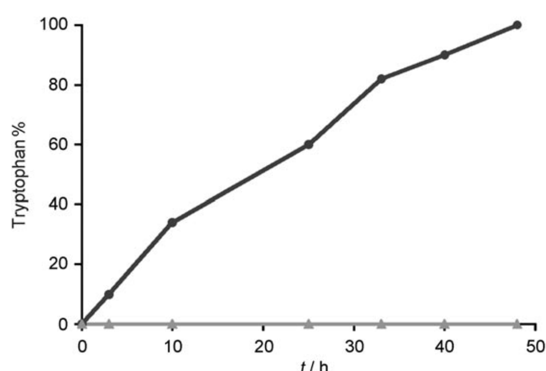

Figure 3. Release of tryptophan from dendron **14** upon incubation with PGA. Conditions: dendron **14**, [50 μM]; PGA, [0.1 mg mL^{-1}], (●). Control reaction under similar conditions without PGA (▲).

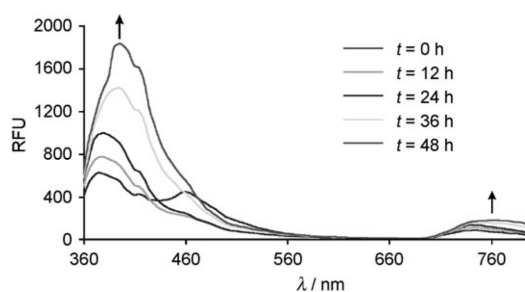

Figure 4. Emission fluorescence spectra ($\lambda_{ex}=260$ nm) of dendron **14** [12 μM] upon incubation in PBS, 37 °C with PGA [0.1 mg mL^{-1}].

curred more slowly under physiological conditions than disassembly of dendron **8** in the MeOH/DMSO environment (Figure 1), PGA cleaved the phenylacetamide from dendron **14** and the resulting amine intermediate was disassembled to release a total of six molecules of tryptophan.

As tryptophan is also a fluorescent molecule, we monitored the disassembly process through spectroscopic measurements. It was found that within the AB_6 dendron (**14**), the tryptophan fluorescence was significantly quenched owing to its confined nature, which is caused by the dendritic skeleton. Upon disassembly, we observed a gradual increase in two bands at $\lambda = 400$ and 760 nm that correspond to free tryptophan molecules (Figure 4).

The mechanism of double 1,8-elimination by azaquinone methide rearrangement in a prodrug system was reported by Firestone.[20] He reported the synthesis of an AB_2 dendron that was linked with two molecules of the anticancer drug doxorubicin. The drug was released through enzymatic activation by using cathepsin B followed by double 1,8-elimination. We have extended that approach into a single-triggered AB_6 dendritic system by including the two *ortho* and the *para* substituents as dendritic arms. A single AB_6 dendritic subunit is able to give a sixfold amplification of a single triggering event, whereas previous AB_2 and AB_3 self-immolative dendritic subunits multiplied a triggering event only two- or threefold, respectively. Thus, AB_6-based self-immolative dendrimers achieve higher degrees of signal amplification at lower dendrimer generations. As higher generation dendrimers are typically more difficult to synthesize, the AB_6-based dendrimer may be a more efficient dendritic amplification subunit.

The quinone methide intermediates generated during the disassembly process are highly reactive electrophiles and rapidly react with any available nucleophiles (methanol or tetrabutylammonium hydroxide in organic solvents). We could not isolate any significant amount of material that derived from the core molecule, probably owing to the generation of a mixture of compounds by the addition of different nucleophiles to the quinone methide.

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Conclusions

In summary, we have developed a simple synthesis for a new AB_6 self-immolative dendritic adaptor. This molecule acts as an amplifier for a cleavage reaction. Thus, a single cleavage event in the dendron focal point is translated into the release of six tail units. This is the first report of a molecule that is capable of six consecutive elimination reactions through a quinone methide rearrangement mechanism. The disassembly process was performed in organic solvents by using chemical activation and under aqueous conditions by enzyme activation. Various reporter molecules and triggering groups could be introduced into the dendritic adaptor and thereby create sensors with enhanced properties that are suitable for use in diagnostics and imaging. Incorporation of drug molecules as the tail units and by using a specific enzymatic substrate as the trigger should generate a single-triggered prodrug system that would be selectively activated at the target site.

Acknowledgements

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