Communication

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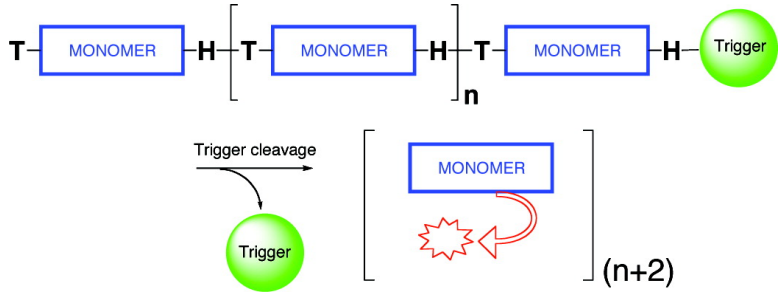

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Self-Immolative Polymers

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The increasing need for effective diagnostic, imaging, and drug delivery systems requires new platforms for the amplification of molecular signals. The polymerase chain reaction (PCR) is a technique widely used in molecular biology to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating millions or more copies of the DNA piece.[1] Recently, chemists have addressed the signal amplification topic with great attention.[2,3] We and others reported the design and synthesis of novel dendritic structures with triggers that initiate the fragmentation of the dendrimer molecule into its building blocks.[4–6] As a consequence of the self-immolative fragmentation, the tail group units are released.[7] A major demand of self-immolative dendrimers is in their multistep synthesis. Furthermore, steric hindrance limits the number of the tail groups that a dendrimer can carry. Here we propose the design and synthesis of novel self-immolative polymeric molecules that can overcome such difficulties. Like self-immolative dendrimers, these self-immolative polymers[8] were designed to sequentially disassemble into their building blocks once the disassembly process is initiated by a triggering event. The polymer is synthesized by polymerization reaction of an appropriate monomer. Capping of the polymer terminal headgroup with a specific protecting group generates a polymeric molecule with a trigger (Figure 1). Selective cleavage of the trigger initiates the polymer sequential fragmentation into its building blocks from the head (**H**) to the tail (**T**).

Our design for a self-immolative polymer is based on compounds such as molecule **1** (Scheme 1). We used a polyurethane backbone with a protecting group on the terminal amine that will act as a trigger. Cleavage of the protecting group will generate amine molecule **2** that will then undergo sequential 1,6-elimination and decarboxylation reactions to form carbon dioxide and azaquinone methide **3**. When the disassembly takes place under aqueous conditions, the highly reactive azaquinone methide will most likely react with a water molecule to form 4-aminobenzylalcohol **4**. The polymeric backbone was synthesized by polymerization[9] (DMF, DBTL as a catalyst, 100 °C) of a blocked isocyanate, in this case, phenyl carbamate **5**, to generate polyurethane **6** (Scheme 2). The terminal isocyanate of the polymeric molecule was then capped with a trigger that has a hydroxyl functional group to form a stable carbamate linkage (polymer **1**). The size of the polymer was controlled through the concentration of the monomer, temperature, and time of the polymerization reaction. For example, when the reaction conditions were 1 M monomer concentration, 110 °C, 15 min, a polymeric chain with an average of about 15−20 monomers was obtained (the average number of the repeating units in the polymer chain was determined by NMR, through the ratio of the benzylic hydrogens at the molecule tail vs those inside the polymeric backbone). The GPC analysis of a representative sample for a polymer such as **1** showed a chromatogram typical of a polymer prepared by condensation reaction with a polydispersity index of roughly 2.

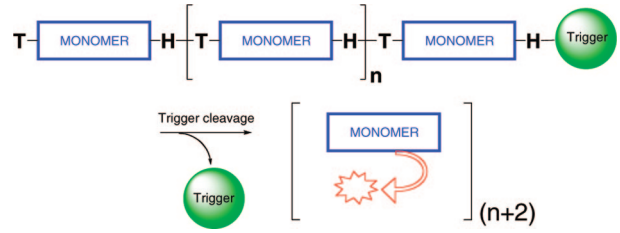
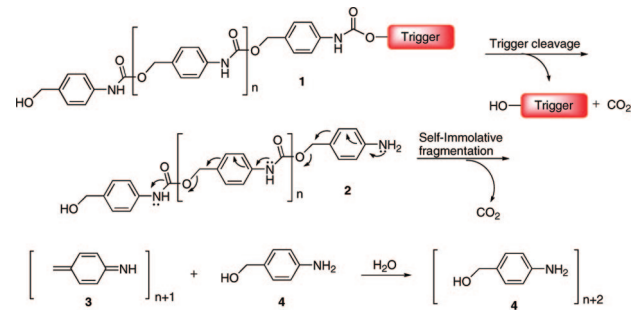


Figure 1. Illustration of the disassembly of a self-immolative polymer.

Scheme 1. Design and Disassembly Mechanism of Self-Immolative Polymer



Scheme 2. General Synthesis of Self-Immolative Polymer with a Trigger

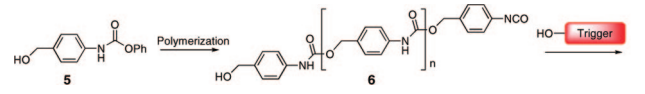
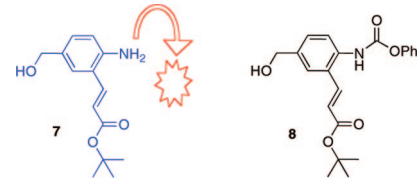


Chart 1. Chemical Structure of Fluorescent Amine **7** and Monomer **8**



In order to obtain a clear visual signal for the polymer disassembly, we designed and synthesized amine **7** (with an *ortho*-acrylate substituent) as the building block (Chart 1). This aromatic amine has a strong fluorescence emission at a wavelength of 510 nm. The fluorescence was significantly reduced when the amine was masked as a carbamate as in compound **8** (see Supporting Information). Therefore, the trigger cleavage of a polymer prepared from monomer **8** should initiate

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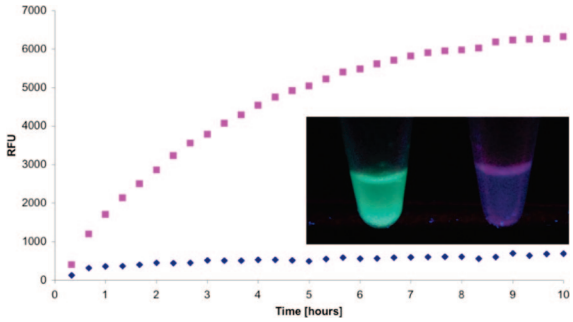
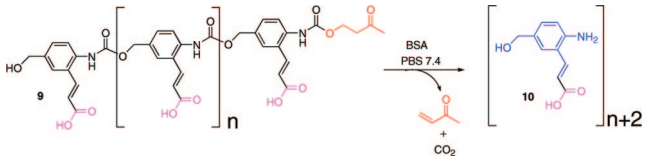
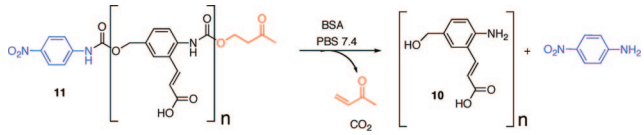


Figure 2. Emitted fluorescence ($\lambda_{ex} = 270$ nm, $\lambda_{em} = 510$ nm) of **9** [500 μM] in the presence (■) and in the absence (♦) of BSA (1.0 mg/mL) in PBS as a function of time. The left eppendorf shows the fluorescence generated from polymer **9** with BSA versus the background reaction on the right eppendorf (without BSA).

Scheme 3. BSA Catalyzed the Disassembly of Self-Immolative Polymer **9** ($n = 15$) to Release Fluorescent Building Blocks



Scheme 4. BSA Catalyzed the Disassembly of Self-Immolative Polymer **11** to Release the Tail Reporter 4-Nitroaniline



the polyurethane disassembly to release building blocks with detectable fluorescence signal. Furthermore, removal of the *t*-butyl protecting groups will generate a water-soluble polymer with ionized carboxyl groups.

Polymer **9** was synthesized from monomer **8**, capped with 4-hydroxy-2-butanone as a trigger, and deprotected with TFA. The trigger was designed for removal through a β-elimination reaction catalyzed by the protein BSA (Scheme 3).[10] The polymer was incubated in phosphate buffered saline, pH 7.4 (PBS), with or without BSA, and the release of amine **10** was monitored by spectrophotometry (Figure 2). The fluorescence emission was gradually increased over 10 h when BSA was present in the polymer solution; in the absence of BSA, almost no fluorescence increase was observed. The rate-determining step of the polymer disassembly was the β-elimination of the trigger catalyzed by BSA.[10] These results support the disassembly pathway of polymer **9** suggested above.

In order to demonstrate that the disassembly of the polymeric molecule occurred all the way from the polymer head through a domino-like fragmentation toward the tail, we synthesized self-immolative polymer **11** with the 4-hydroxy-2-butanone trigger and a 4-nitroaniline reporter at the tail (Scheme 4). The polymer was incubated in PBS, pH 7.4, with BSA, and the release of 4-nitroaniline was monitored by RP-HPLC. The reporter, 4-nitroaniline, was gradually released to the solution in the presence of BSA over 10 h (see Supporting Information). This observation confirms the polymer sequential fragmentation presented in Figure 1.

Self-immolative polymer **9** amplified a single cleavage reaction into multiple release of fluorogenic molecules. The synthesis of such polymers is achieved by three simple synthetic steps—monomer polymerization, trigger insertion, and protecting group removal. Since the building blocks of the polymer are disassembled into fluorogenic molecules, a large number of reporters can be effortlessly incorporated in a self-immolative polymer. We demonstrated the potential of a self-immolative polymeric probe to detect biocatalytic activity of BSA. The system could be applied to prepare highly sensitive molecular sensors with large signal-to-noise ratios. These sensors should be useful for the detection of a wide range of biological and chemical activities through insertion of the appropriate trigger at the polymer head.

In summary, we have introduced a novel kind of "smart" polymer[11–13] (polymers that respond to external stimuli) that undergoes head-to-tail disassembly through a sequential fragmentation once the head trigger is activated. Insertion of additional hydroxymethyl substituents at the ortho-position of each monomer will generate self-immolative comb-polymers with side-releasable groups that could be used as a drug delivery system.[7,14,15] Current efforts of our laboratory are focused on combining the self-immolative dendritic molecules with the polymers to construct self-immolative dendronized polymers.[16]

Acknowledgment. Financial support has been provided by the Israel Science Foundation (ISF) and the Binational Science Foundation (BSF). Dedicated to Professor Ehud Keinan on the occasion of his 60th birthday.

Supporting Information Available: Full experimental details, characterization data of all new compounds (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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