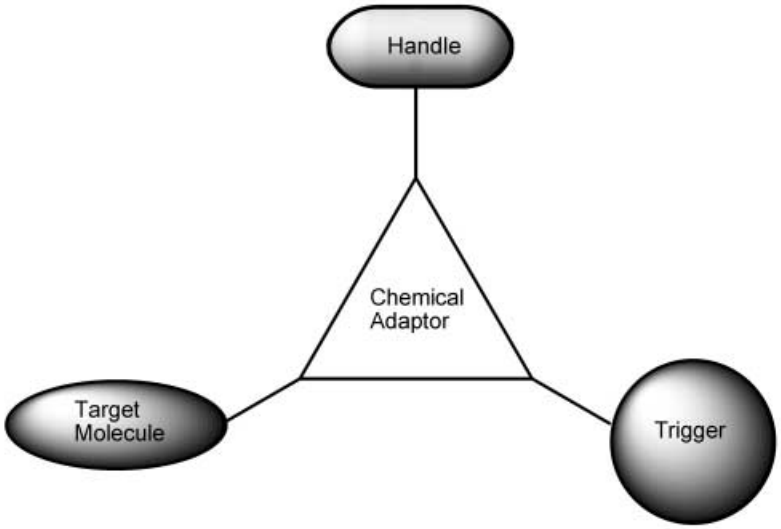
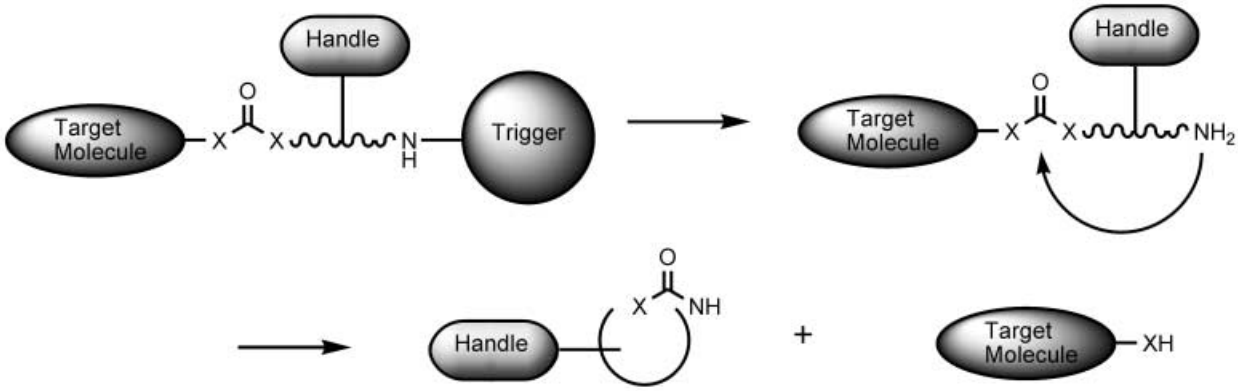
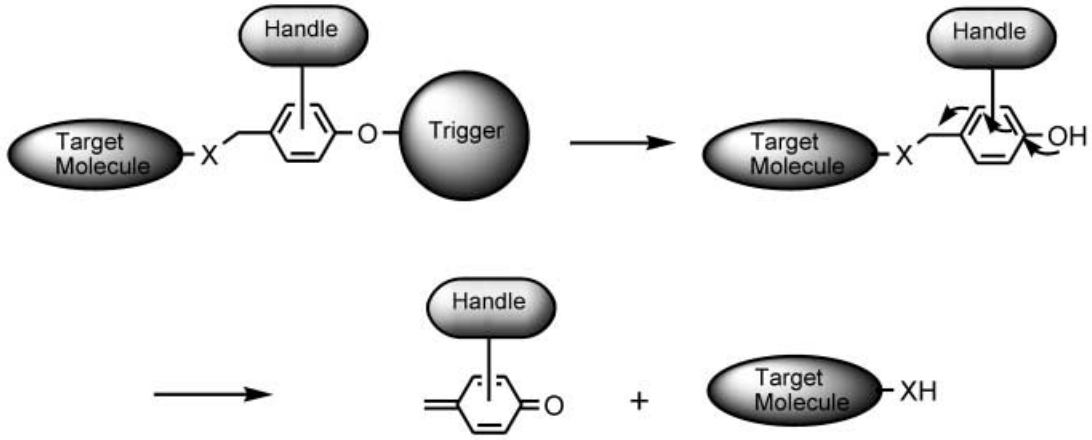
Chemical Adaptor Systems



Cyclization-based chemical adaptor system:



Elimination-based chemical adaptor system:



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Chemical Adaptor Systems

Doron Shabat,* Roey J. Amir, Anna Gopin, Neta Pessah, and Marina Shamis[a]

Abstract: "Chemical adaptor systems" are molecules used to link different functionalities, based on unique reactivity that allows controlled fragmentation. Two different mechanistic reactivities were used to prepare chemical adaptor systems. The first is based on a spontaneous intra-cyclization reaction to form a stable ring molecule. Cleavage of the trigger generates a free nucleophile, for example, an amine group, which undergoes intra-cyclization to release the target molecule from the handle part (e.g., a targeting antibody or a solid support for synthesis). The second applied reactivity is an elimination reaction, which is usually based on a quinone-methide-type rearrangement. Similarly, cleavage of the trigger generates a free phenol functionality, which can undergo a self-elimination reaction through a quinone-methide rearrangement to release the target molecule. The adaptor molecules have been applied in the field of drug delivery to release a drug from a targeting device and in the field of solid-phase synthesis to release a synthetic molecule from the solid support. A chemical adaptor molecule has also been used as a building unit to construct dendrimers with a triggered fragmentation.

Keywords: dendrimers · drug delivery · enzymes · prodrugs · self-immolative dendrimers

Introduction

The versatile world of chemistry offers an indefinite number of molecules with a variety of structures and reactivities. Scientists often are required to link a specific chemical or biological activity to a target molecule with a controlled approach. Therefore, several molecules have been designed and synthesized specifically for that purpose. We have named these unique functional molecules "chemical adaptor systems." The adaptor is usually a molecule consisting of at least three functionalities (Figure 1). One of them acts as a handle and is used for direction/anchoring purposes. The second functionality acts as a bio-/chemical switch, and its cleavage triggers a self-immolative spontaneous release of the third functionality, which can be, for example, a drug or a synthetic target molecule. Importantly, unlike conventional self-immolative linkers that connect two functionalities,[1] a chemical adaptor molecule is constructed with at least one additional functionality that adds a new concept to the linking system.

Two different mechanistic reactivities were used by us and others to prepare chemical adaptor systems. The first is based on a spontaneous intra-cyclization reaction to form a stable cyclic molecule (Figure 2A). Cleavage of the trigger generates a free nucleophile, for example, an amine group, which undergoes intra-cyclization to release the target molecule from the handle part (e.g., a targeting antibody or a solid support for synthesis). The second applied reactivity is an elimination reaction, which is usually based on a quinone–methide rearrangement (Figure 2B). Similarly, cleavage of the trigger generates a free phenol functionality, which can undergo a self-elimination reaction through a quinone–methide rearrangement to release the target molecule.

Selective Drug Delivery

A lot of effort has been devoted to the development of new drug delivery systems that mediate drug release selectively at the tumor site.[2] One way to achieve such selectivity is to activate a prodrug, specifically by way of confined enzymatic activity. In this concept, the enzyme either is expressed by the tumor cells, or directed to the tumor by a targeting moiety, such as a monoclonal antibody. The prodrug is converted to an active drug by the local or localized enzyme at the tumor site, thereby minimizing nonspecific toxicity to other tissues.[3]

We recently applied chemical adaptor systems as a platform, which combines a tumor-targeting device, a prodrug,

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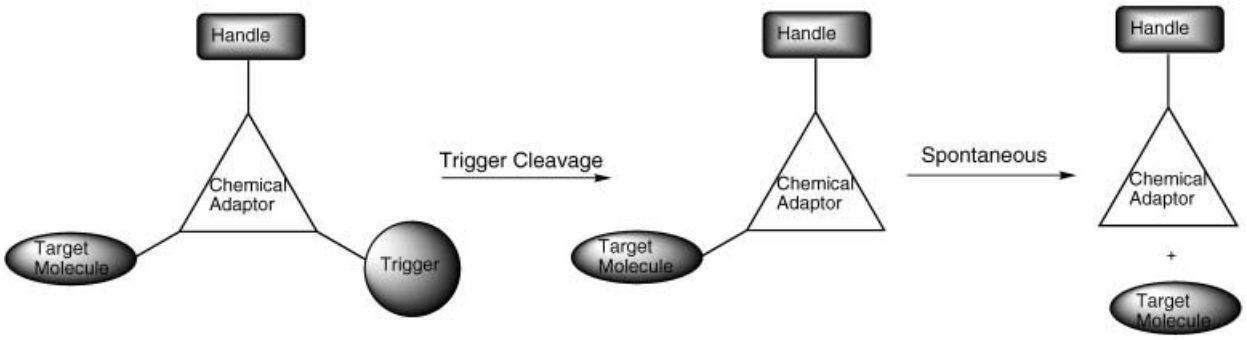


Figure 1. General principle of a chemical adaptor system.

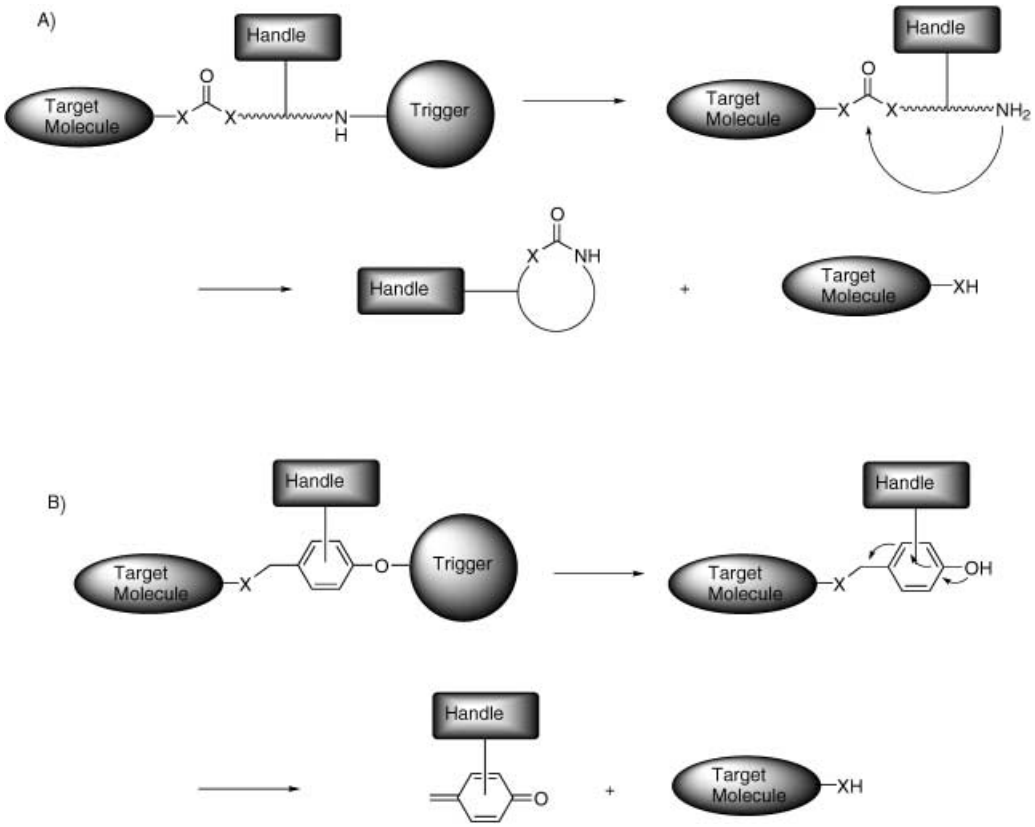


Figure 2. A) Cyclization-based chemical adaptor system. B) Elimination-based chemical adaptor system.

and a prodrug activation trigger.[4] The three functional groups of the adaptor molecule were used as follows. The first functionality (handle) is linked to a targeting moiety, which is responsible for guiding the prodrug to the tumor site; the second (target molecule) is attached to an active drug and, thereby, masks it to yield a prodrug; and the third (trigger) is attached to an enzyme substrate. When the corresponding enzyme cleaves the substrate, it triggers a spontaneous reaction that releases the active drug from the targeting moiety. As a result, prodrug activation will occur preferentially at the tumor site.

The first example applies the quinone–methide rearrangement–elimination reaction, which is described in Figure 2B. The central core of the chemical adaptor (Figure 3) is based on 4-hydroxymandelic acid, which is commercially available and has three functional groups suitable for linkage. Group

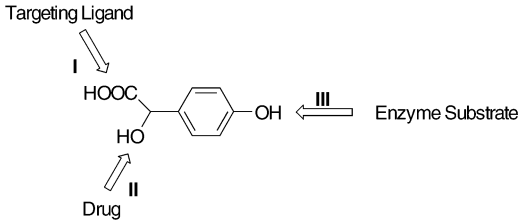


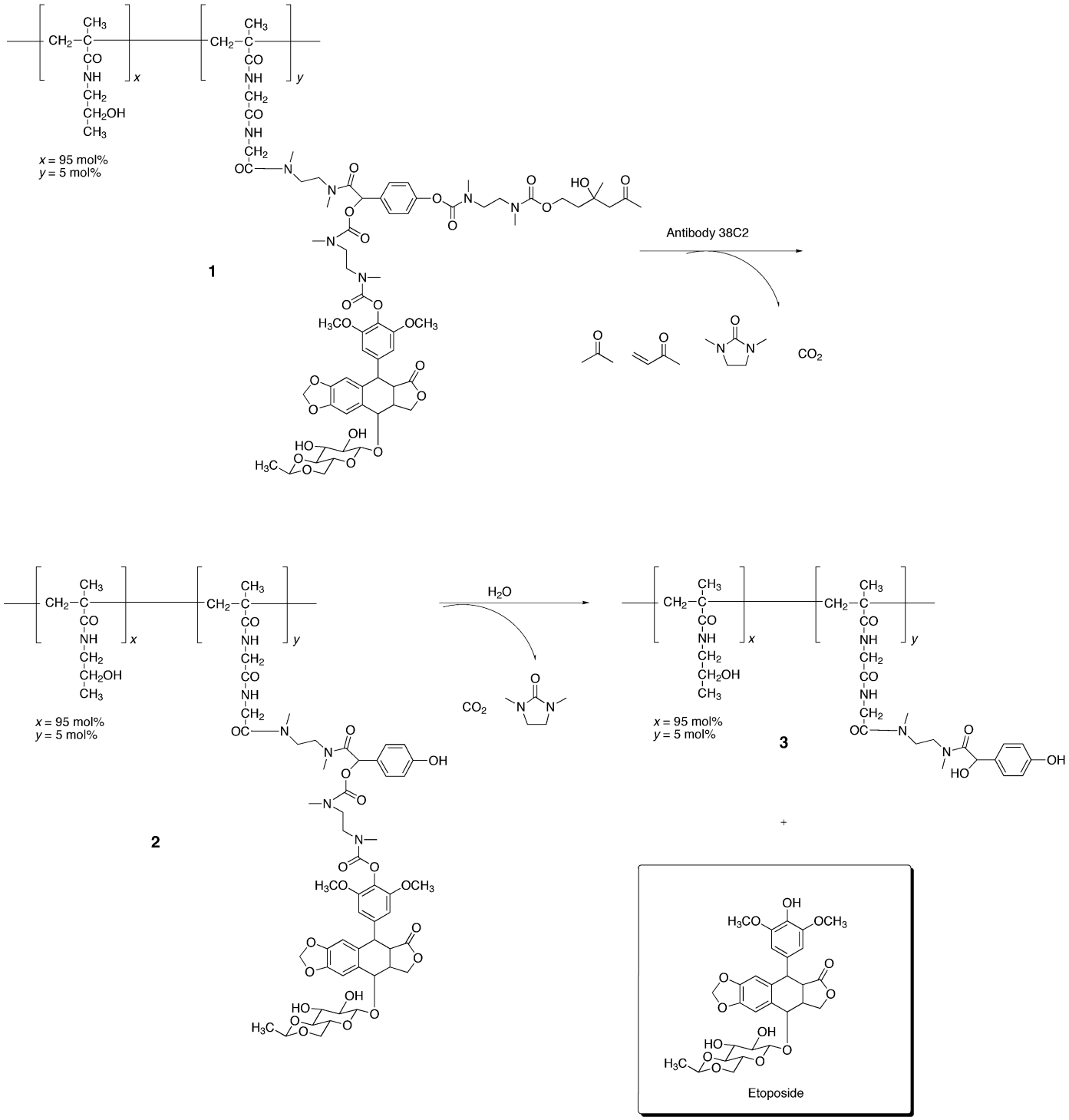
Figure 3. 4-Hydroxymandelic acid as a chemical adaptor molecule.

I is a carboxylic acid that is conjugated to a targeting moiety through an amide bond. The drug is linked through the benzyl alcohol group **II** and the enzyme substrate is attached through the phenol group **III** by a carbamate bond.

The system is generic and allows the use of a variety of drugs, targeting devices, and enzymes by introducing the

corresponding substrate as a trigger for drug release in the adaptor molecule. The chemical adaptor system was designed with stable chemical linkages to avoid nonspecific drug release in vivo. Proof of the concept was demonstrated by using etoposide as the drug, an HPMA–copolymer[5,6] as the targeting device, and catalytic antibody 38C2[7,8] as the triggering enzyme (Scheme 1).

We tested whether the etoposide drug can be released from complex **1** by the catalytic activity of antibody 38C2. According to our design, the drug should be spontaneously released after the generation of phenol **2** as illustrated in Scheme 1. We incubated complex **1** with catalytic antibody 38C2 in PBS (pH 7.4) at 37 °C and monitored the appearance of etoposide using an HPLC assay. As we expected, etoposide was released by the catalytic activity of antibody 38C2 to form compound **3** and the free drug. No spontaneous etoposide release was observed in the absence of the antibody. Two additional examples, comprising a penicillin-G-



Scheme 1. Mechanism of etoposide drug release from the HPMA–copolymer, using catalytic antibody 38C2 as the triggering enzyme.

amidase substrate as the enzymatic trigger and camptothecin as the anticancer drug, respectively, were also synthesized and activated.

An additional example of a chemical adaptor system applies the cyclization-based mechanism (described in Figure 2A). The central core of the chemical adaptor (Figure 4) is based on *N*-methyldiaminopropionic acid, which has three functional groups suitable for linkage, similar to those described in the first example. Group **I** is a carboxylic acid that is conjugated to a targeting moiety through an amide bond. The drug is linked through the 2-amino group **II**, and the enzyme substrate is attached through the 3-amino group **III** by a carbamate bond.

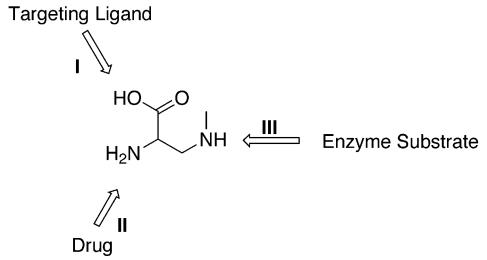
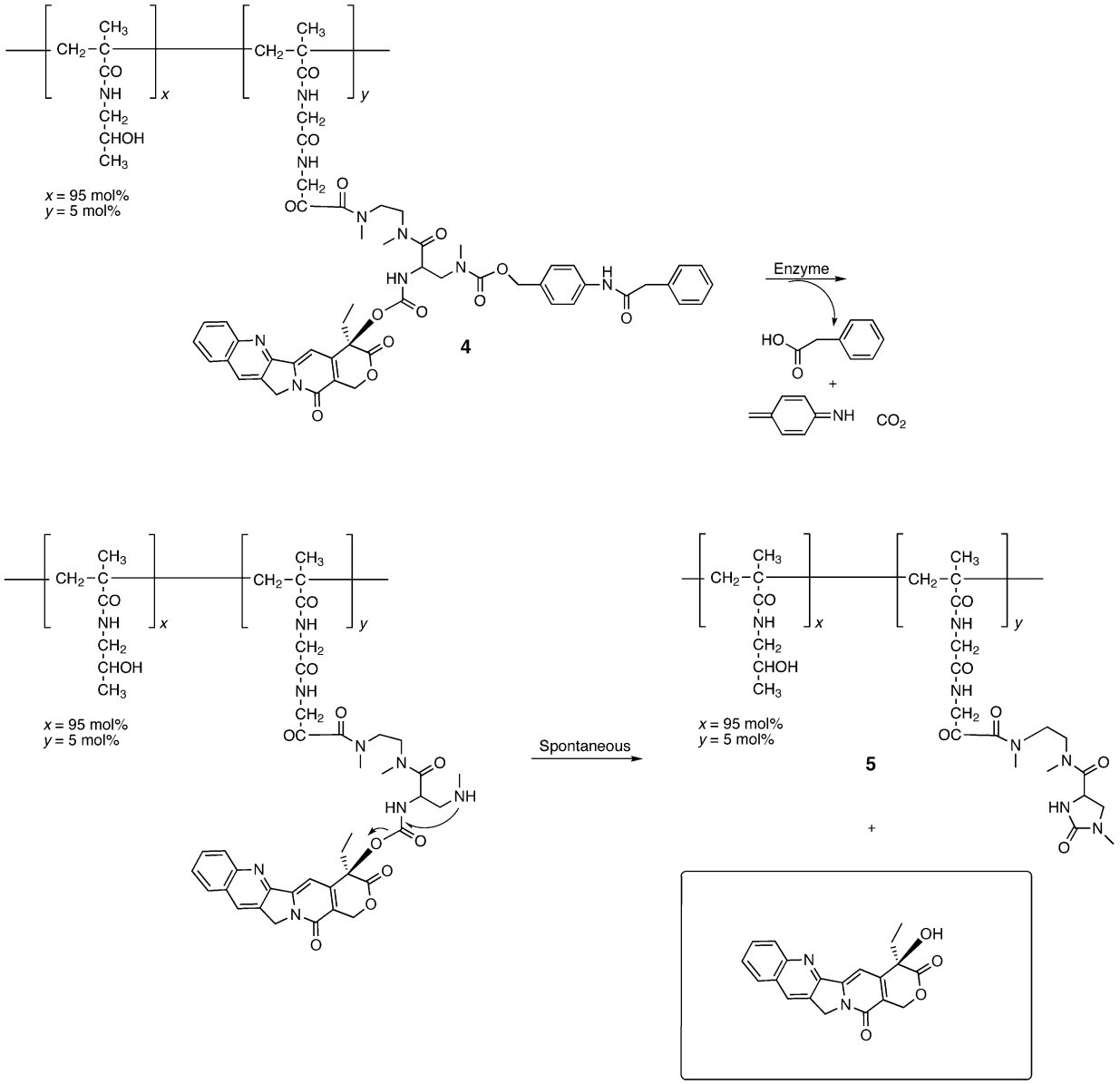


Figure 4. 2-Amino-3-methylamino-propionic-acid, the central core of the cyclization-based chemical adaptor unit.

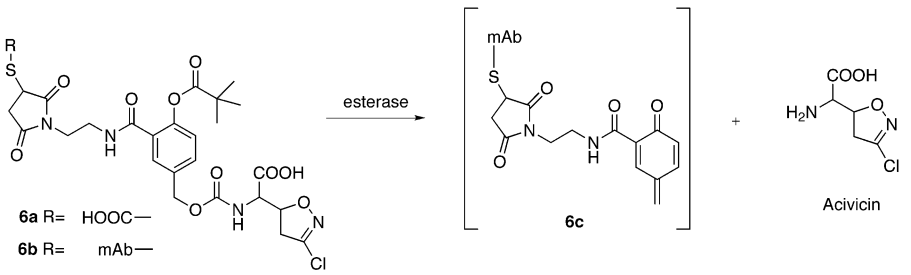
Similarly to the previous example, we designed a pilot system for which we chose *Escherichia coli* penicillin-G-amidase as the triggering enzyme. The water-soluble synthetic copolymer *N*-(2-hydroxypropyl)methacrylamide (HPMA) was chosen as a targeting device and camptothecin as the anticancer drug. We incubated complex **4** with PGA in PBS (pH 7.4) at 37 °C and monitored the appearance of free CPT, using an HPLC assay Scheme 2. Camptothecin was indeed released by the catalytic activity of PGA to form compound **5** and the free drug. No spontaneous CPT release



Scheme 2. Mechanism of camptothecin drug release from the HPMA-copolymer, using penicillin-G-amidase as the triggering enzyme.

was observed in the absence of the enzyme (Data will be reported shortly).

A similar adaptor drug delivery system was also reported by Monneret et. al.[9] They used a carboxy derivative of 4-hydroxybenzyl alcohol to link the anticancer drug acivicin with an esterase substrate and a maleimide group, readily available for linkage with a targeting antibody (Scheme 3). Incubation of pig liver esterase with compound **6a** was followed by the release of free acivicin through an elimination reaction generating quinone-methide **6c**. However, the adaptor molecule has not been tested when it is conjugated with a targeting antibody (complex **6b**).



Scheme 3. Elimination-based chemical adaptor system that is activated by an esterase.

Organic Synthesis on Solid Support

Interestingly, very similar molecules have been used by Waldmann et al. for a completely different application. They used an enzyme-labile linker for the release of a target synthetic molecule from a solid support.[10,11] One system utilized an elimination-based chemical adaptor with an ester trigger, which can be cleaved by a lipase (Figure 5 A). The incubation of the enzyme with complex **7**, generated intermediate **8** which spontaneously released the target molecule. An additional example utilizing a cyclization-based chemical adaptor, was elegantly demonstrated (Figure 5 B). System **9** was prepared with phenylacetamide as a trigger, which can be cleaved with penicillin-G-acylase to generate amine **10**. The later undergoes spontaneous intra-cyclization to release the target molecule. In both examples, the researchers used the advantages of solid support for multistep synthesis for the target molecule and then utilized the chemical adaptor system to release the synthesized mol-

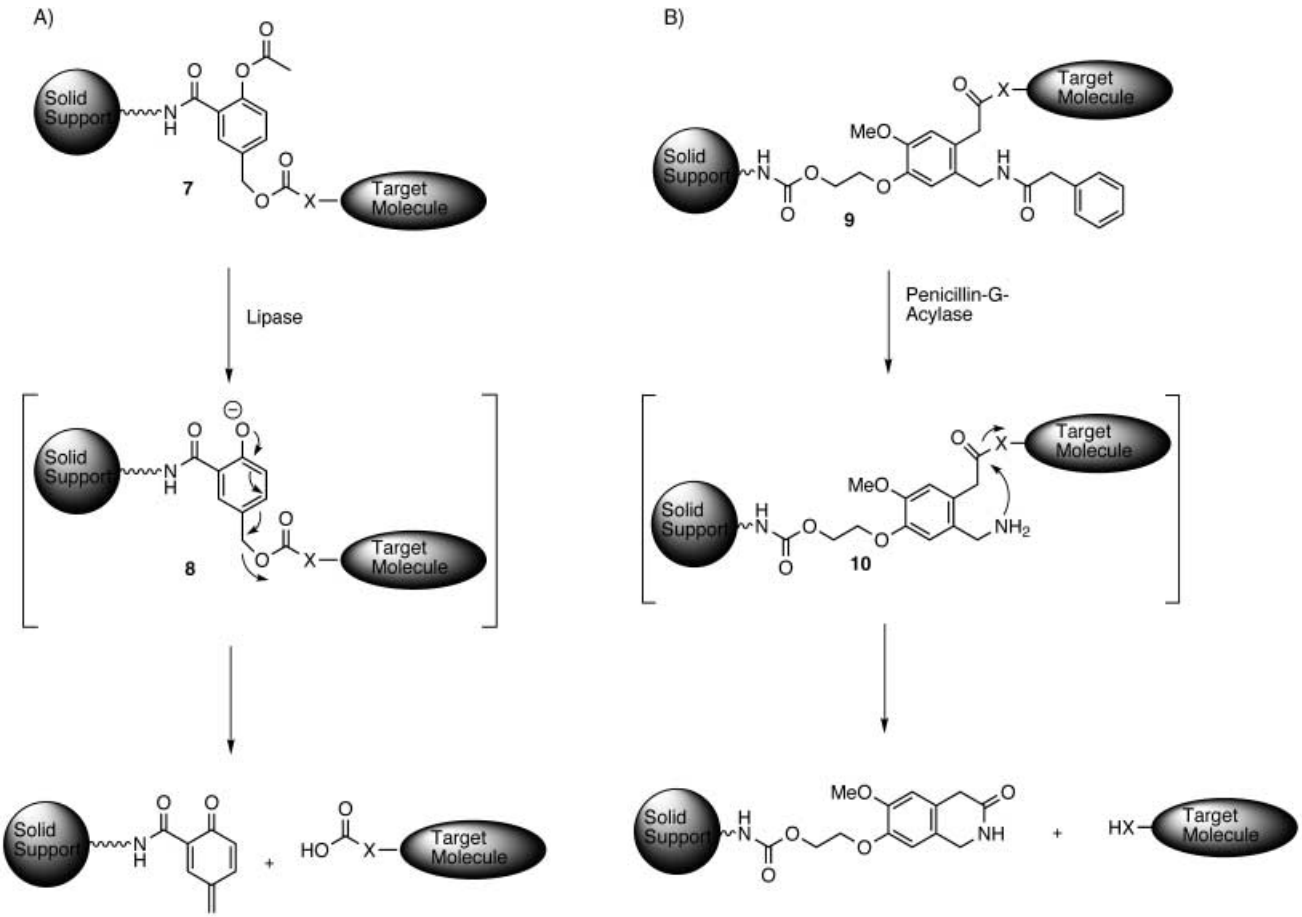


Figure 5. A) Elimination-based chemical adaptor system activated by a lipase. B) Cyclization-based chemical adaptor system activated by penicillin-G-acylase.

ecule from the solid support with the described enzymatic activity.

Self-Immolative Dendrimers

Very recently, we[12,13] and others[14,15] introduced a new class of dendritic molecules that were termed self-immolative dendrimers (SIDs). These structurally unique dendrimers can release all of their tail units, through a self-immolative chain fragmentation, which is initiated by a single cleavage at the dendrimer's core. The first-generation design of a self-immolative dendron is based on a chemical adaptor molecule that has three functional groups. Two identical functionalities are linked to reporter molecules, and the third is attached to a trigger (Figure 6, **I**). The cleavage of the trigger initiates a self-immolative reaction sequence that leads to a spontaneous release of the two reporter molecules. The adaptor molecule can be linked to two additional identical units, which are each attached to two reporter molecules (Figure 6, **II**). The head position of the first adaptor unit is linked to a trigger. In this approach, the G2-dendron can be prepared, and, similarly, the design can be extended to higher generations of dendrons and dendrimers. The cleavage of the trigger will initiate self-immolative chain re-

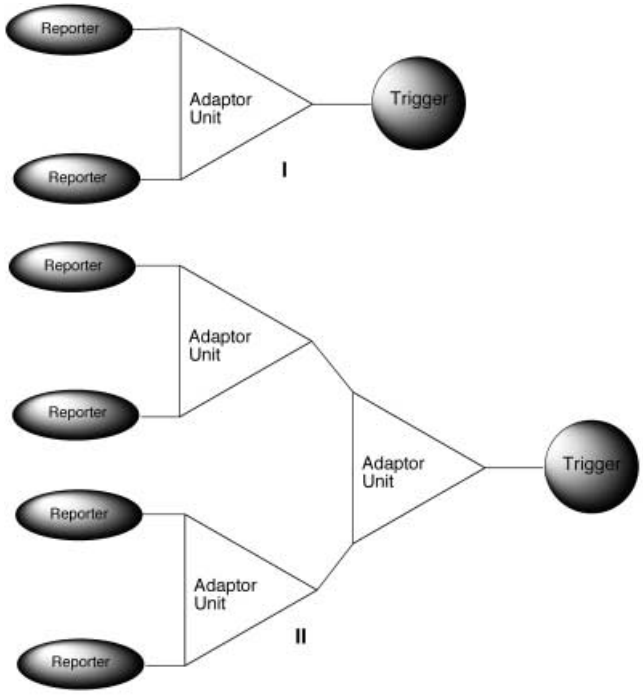


Figure 6. **I**) Graphical structure of a first-generation (G1) self-immolative dendron with a trigger and two tail units. **II**) Graphical structure of a second-generation (G2) self-immolative dendron with a trigger and four tail units

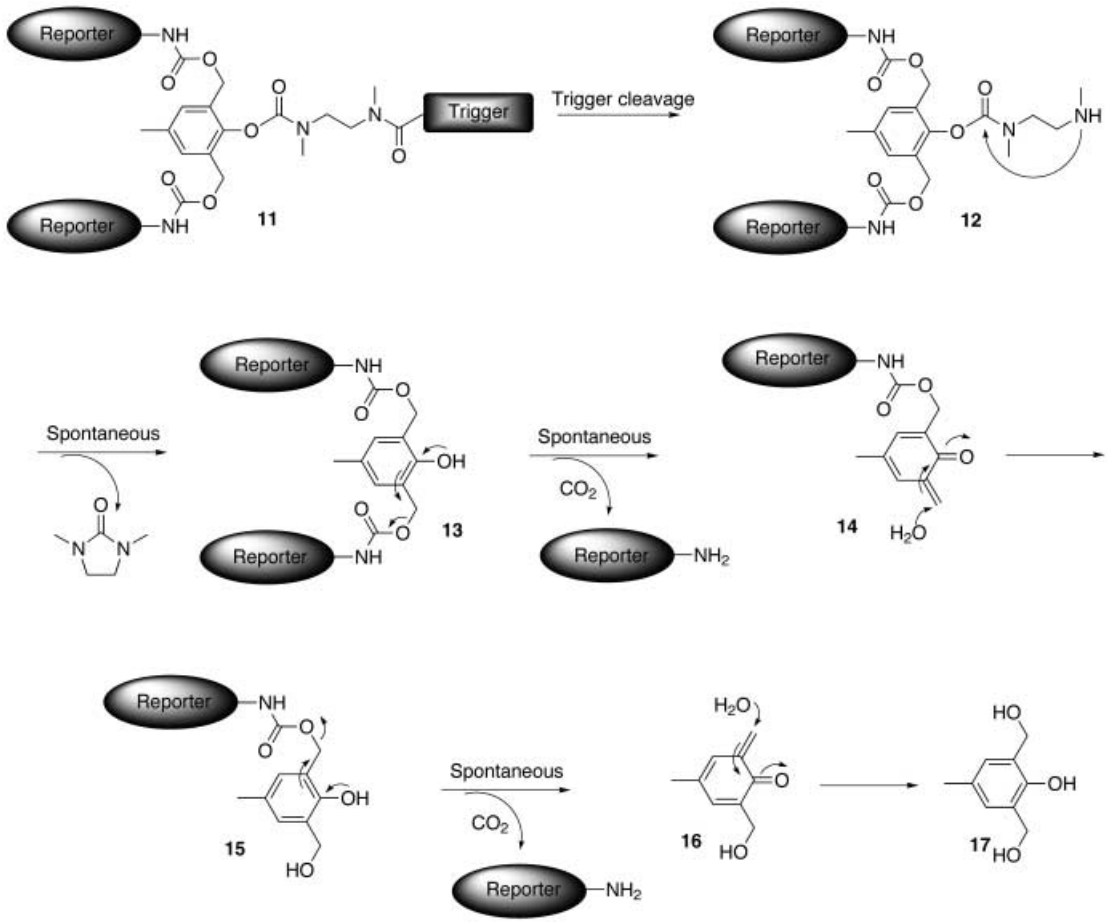


Figure 7. Schematic representation of the G1-self-immolative dendron activation through a spontaneous chain reaction, based on cyclization and 1,4-quinone-methide rearrangement.

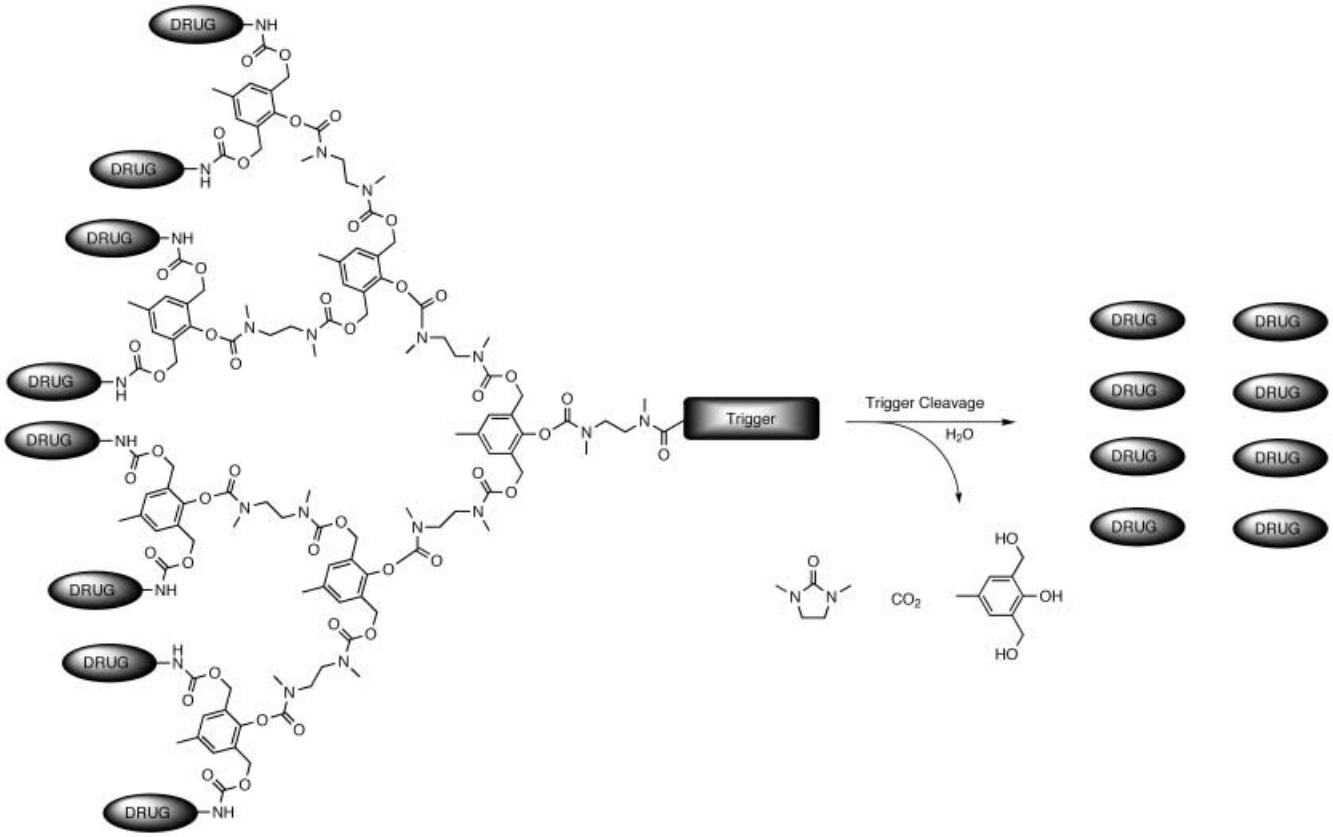


Figure 8. Chemical structure of a G3 self-immolative dendron with eight drug molecules, which are released by a single triggering event.

actions that consequently will fragment the dendrimer and release all of the tail molecules.

Our dendrimer's chemical adaptor unit is based on 2,6-bis(hydroxymethyl)-*p*-cresol **17**, a commercially available compound, which has three functional groups (Figure 7). The two hydroxybenzyl groups are attached, through a carbamate linkage, to reporter molecules, and the phenol functionality is linked to a trigger through a short-spacer *N*,*N*′-dimethylethylenediamine (compound **11**). The cleavage of the trigger initiates a self-immolative reaction sequence of amine intermediate **12**, starting with spontaneous cyclization to form an *N*,*N*′-dimethylurea derivative. The generated phenol **13** undergoes a 1,4-quinone–methide rearrangement, followed by spontaneous decarboxylation to liberate one of the reporter molecules. The quinone–methide species **14** is trapped rapidly by a water molecule (from the reaction solvent) to form a phenol (compound **15**), which again undergoes a 1,4-quinone–methide rearrangement to liberate the second reporter molecule. The generated quinone–methide species **16** is trapped again by a water molecule to form 2,6-bis(hydroxymethyl)-*p*-cresol **17**.

We synthesized first-to-third generation of SIDs with chemical and enzymatic triggers. Cleavage of these triggers indeed resulted in a domino breakdown of the dendritic molecule and the release of the reporter units. The double elimination-based adaptor molecule (2,6-bis(hydroxymethyl)-*p*-cresol) is the key structural unit of the SID and is responsible for this elegant fragmentation. Replacement of the reporter units with drug molecules potentially can apply SIDs as new drug delivery systems (Figure 8).

Conclusion

Chemical adaptor molecules offer unique possibilities for linking different functionalities and reactivities. Two categories of reactions have been used in the chemistry of adaptor systems. One is based on elimination reactions, and the other on intra-cyclization reactions. The adaptor molecules were applied in the field of drug delivery to release a drug from a targeting device and in the field of solid-phase synthesis to release a synthetic molecule from the solid support. An adaptor molecule was also used as a building unit to construct dendrimers with a triggered fragmentation. These molecules have begun to play a role only recently, although their chemical reactivities are not new. However, we expect that their potential to provide solutions for a variety of applications will promote their wide use.

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