Dendritic Delivery Systems

Self-Immolative Dendrimers**

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Dendrimers are perfectly cascade-branched, highly defined, synthetic macromolecules, which are characterized by a combination of high number of functional groups and a compact molecular structure.[1] The concept of repetitive growth with branching creates a unique spherical monodisperse dendrimer formation, which is defined by a precise generation number.[2] For example, first-generation dendrimer (G1) will have one branching unit, and the second-generation dendrimer (G2) will have an additional two branching units, etc. The structural precision of dendrimers has motivated numerous studies aimed at developing biological applications,[3–6] such as, the amplification of molecular effects or the creation of high concentrations of drugs,[7] molecular labels, or probe moieties.[8]

Most of the applications of dendrimers rely mainly on the high number of functional groups and not on their unique structures.[9,10] An appropriate dendrimer could be structurally designed to release all of its tail molecules with a single cleavage event at the dendrimer's core. Here we propose the design and synthesis of a novel dendritic structure with a trigger that can initiate the fragmentation of the dendrimer molecule to its building blocks in a self-immolative manner[11,12] with consequent release of the tail-group units.[13]

The design of the first-generation self-immolative dendron (SID) is based on an adaptor molecule that has three functional groups. Two identical functionalities are linked to reporter molecules and the third is attached to a trigger (Figure 1, **I**). Cleavage of the trigger initiates a sequence of self-immolative reactions 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 1, **II**). The head position of the first adaptor unit is linked to a trigger. The G2 dendron can be prepared by this approach, and, similarly, the design can be extended to higher generations of dendrons

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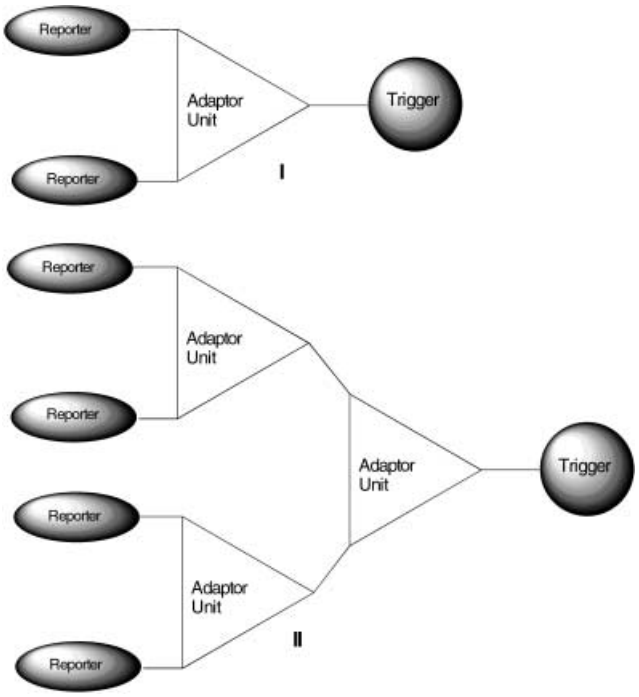
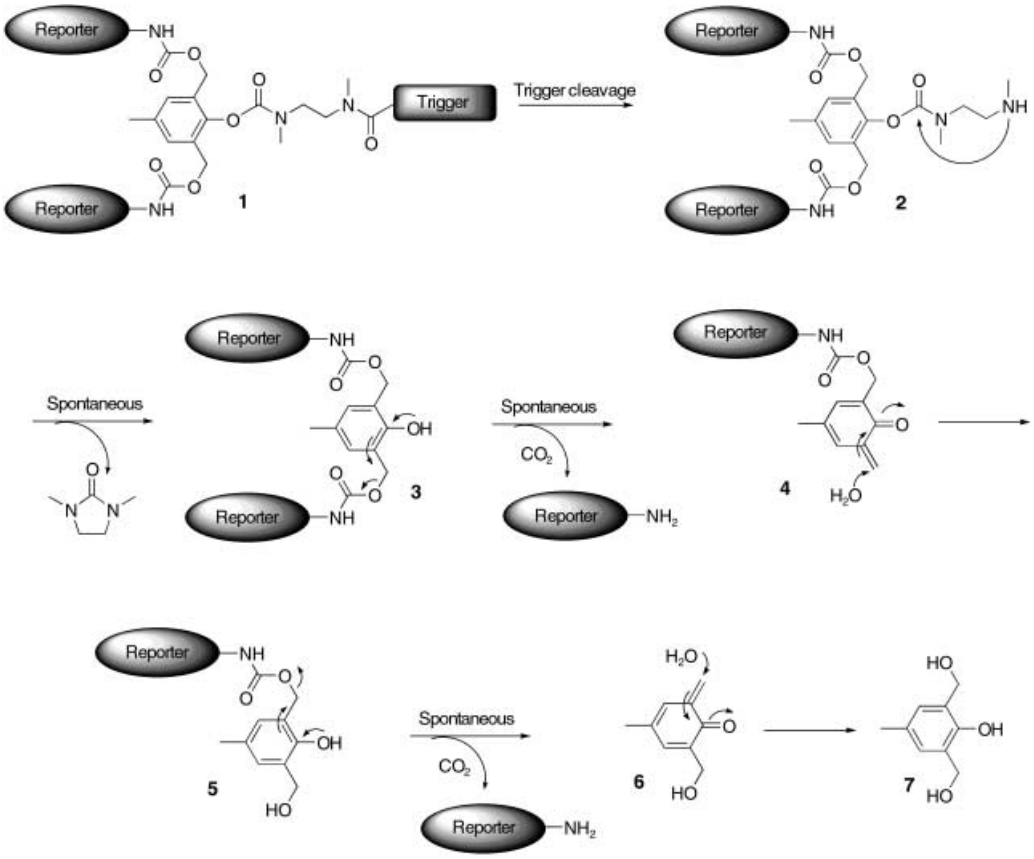


Figure 1. Graphical representation of a first generation (G1) self-immolative dendron with a trigger and two tail units (**I**). Graphical representation of a second generation (G2) self-immolative dendron with a trigger and four tail units (**II**).

and dendrimers. The cleavage of the trigger will initiate self-immolative chain reactions that will consequently fragment the dendrimer and release all of the tail molecules.

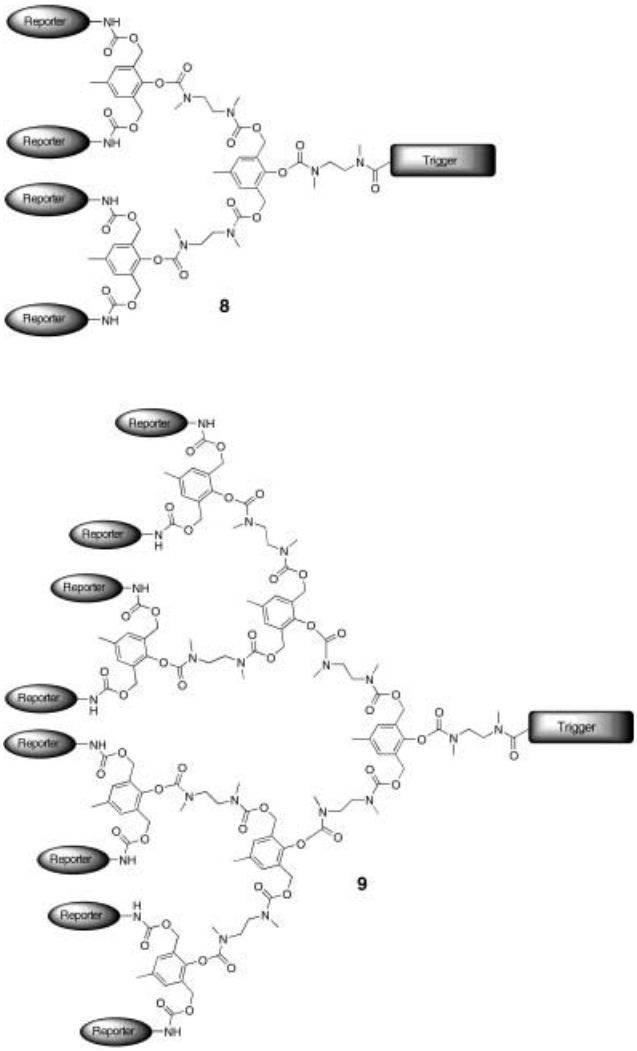
The adaptor unit of our dendrimer is based on 2,6-bis(hydroxymethyl)-*p*-cresol (**7**), a commercially available compound, which has three functional groups (Scheme 1). The two hydroxybenzyl groups are attached through a carbamate linkage to reporter molecules and the phenol functionality is linked to a trigger through a short spacer (*N*,*N*′-dimethylethylenediamine) to form **1**. Cleavage of the trigger initiates a sequence of self-immolative reactions of the amine intermediate **2**, starting with spontaneous cyclization to form an *N*,*N*′-dimethylurea derivative. The generated phenol **3** undergoes a 1,4-quinone methide rearrangement, followed by spontaneous decarboxylation to liberate one of the reporter molecules. The quinone methide species **4** is rapidly trapped by a water molecule (from the reaction solvent) to form a phenol **5**, which again undergoes a 1,4-quinone methide rearrangement to liberate the second reporter molecule. The generated quinone methide species **6** is then trapped by another water molecule to form **7**. As far as we know, this is the first reported example of a double 1,4-quinone methide rearrangement for this type of aromatic system.

To extend the synthesis to higher generations of dendrons, two identical units of G1 dendrons can be attached to the hydroxybenzyl functionalities of the first unit to give a



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

G2 dendron **8** (Scheme 2). The attachment is performed through a double carbamate linkage using *N*,*N*-dimethylethylenediamine as a spacer. The G3 dendrimer can similarly be obtained by linking two G2 units to the hydroxybenzyl groups of the first unit to give **9**. The carbamate linkage between the units is highly stable until the trigger is cleaved and the self-immolative reaction sequence is initiated.



Scheme 2. General chemical structure of G2 and G3 self-immolative dendrons.

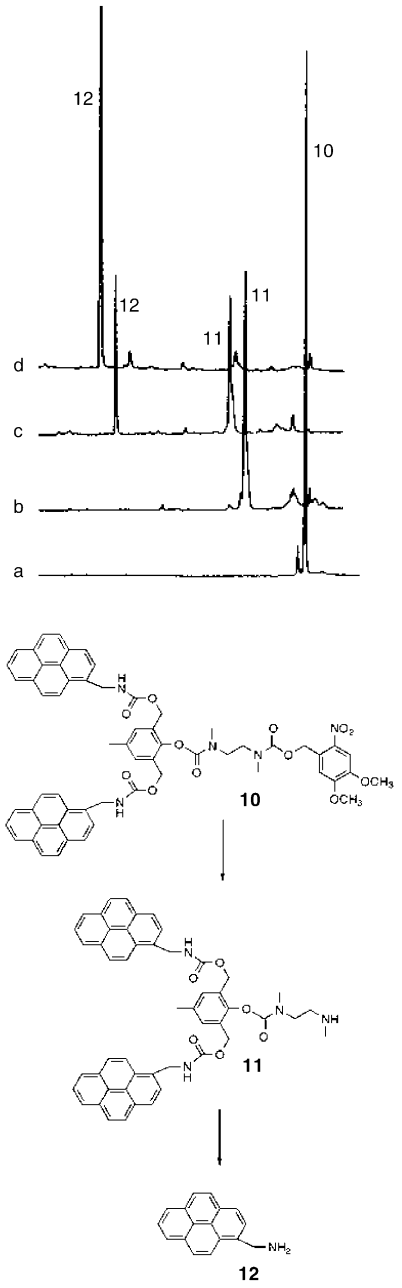


Figure 2. HPLC chromatograms showing the self-immolative dendron activation of G1 to release aminomethylpyrene (50 μM **10** in methanol with 10% triethylamine): a) before radiation; b) after radiation, $t=0$; c) $t=4$ h; d) $t=11$ h.

To prove our concept we initially synthesized a G1 SID **10** with aminomethylpyrene as the tail unit and a photolabile trigger (see the Supporting Information). The dendrimer was dissolved in methanol, the solution was irradiated with UV light ($\lambda=360$ nm) to cleave the trigger, and 10% triethylamine was added to initiate the self-immolative reactions (the triethylamine is needed to generate the mildly basic media which is required for the quinone methide rearrangement). The release of aminomethylpyrene (**12**) was monitored by HPLC and the results are shown in Figure 2. Cleavage of the photolabile trigger generated amine **11**, which gradually degraded to the tail units through the previously explained self-immolative process. The release of aminomethylpyrene was completed after 11 h. Since no intermediates other than amine **11** were observed we concluded that the rate-limiting step of the self-immolative sequence is the cyclization of amine **11** to form an *N*,*N*′-dimethylurea derivative and a phenol which rapidly rearranges to release the tail units. We have characterized amine **11** by HRMS and by comparison with the HPLC spectrum of a reference compound.

Next, we synthesized a G2 SID **13** with a similar tail unit and a photolabile trigger. We repeated the previous experi-

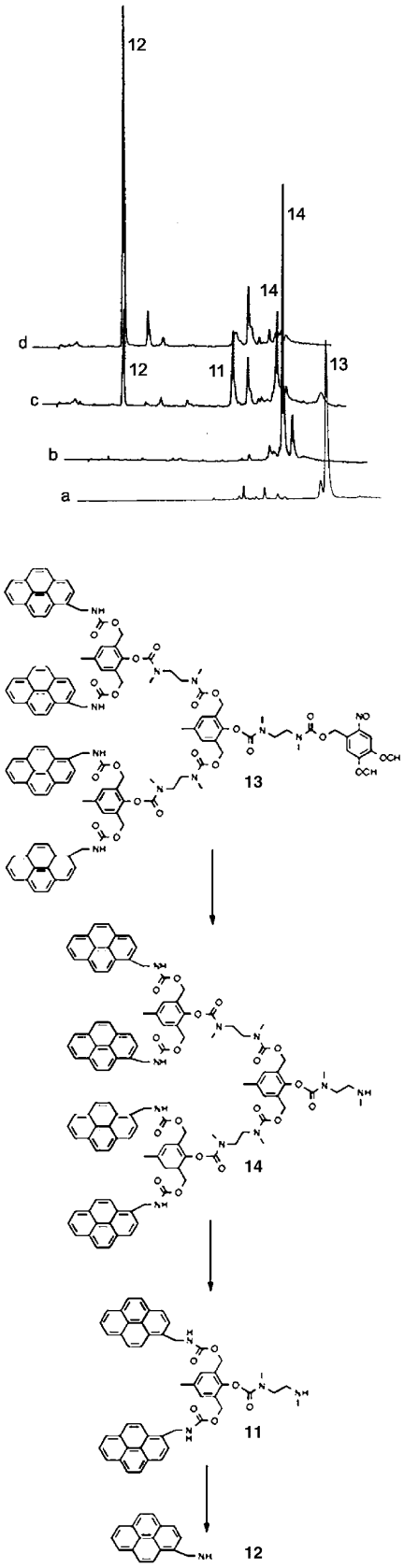


Figure 3. HPLC chromatograms showing the self-immolative dendron activation of G2 to release aminomethylpyrene (50 μM **13** in methanol with 10% triethylamine): a) before radiation; b) after radiation, $t=0$; c) $t=6$ h; d) $t=20$ h.

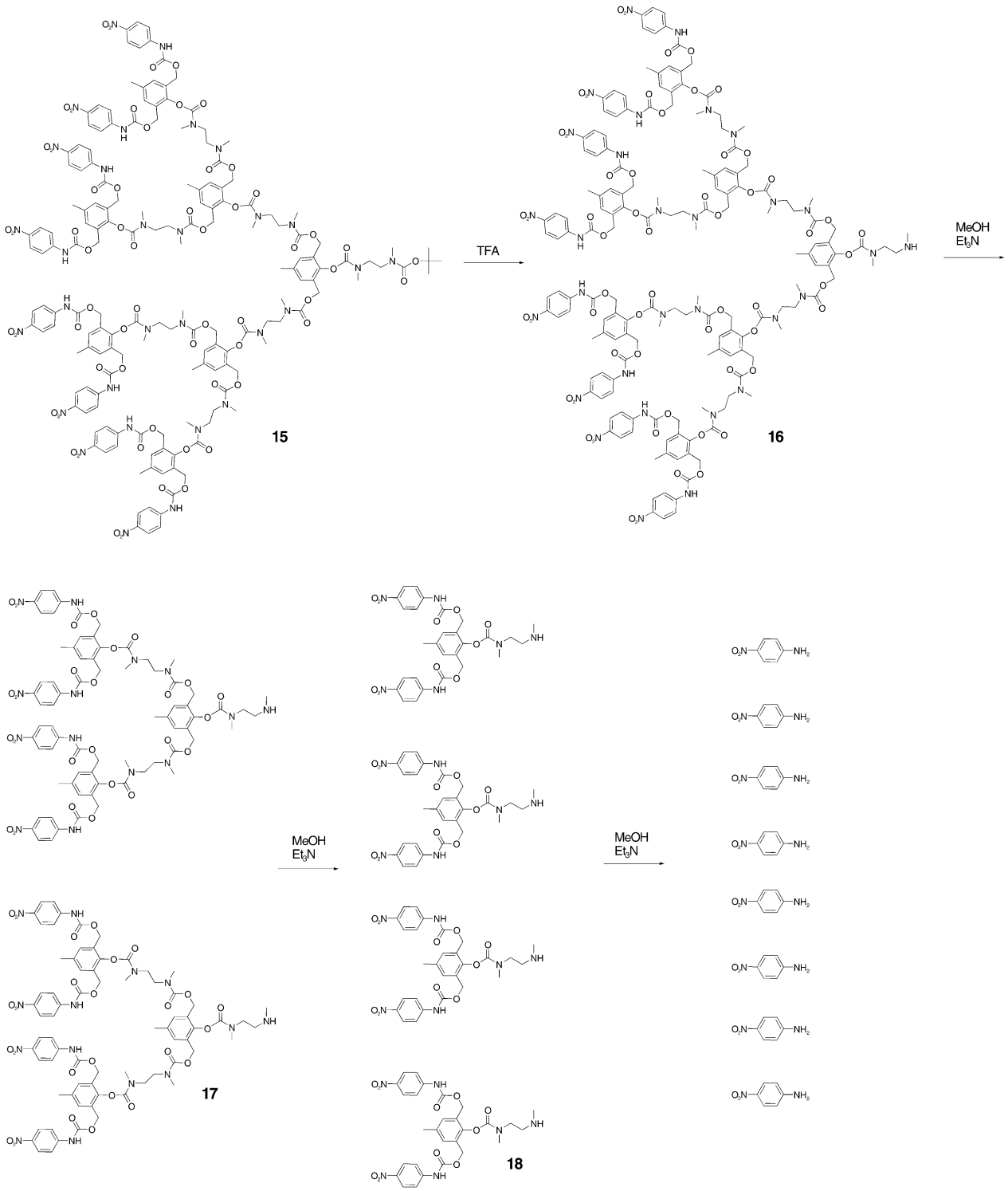
ment and monitored the release of the tail molecules. It was clearly shown (Figure 3) that upon cleavage of the trigger, to form amine **14**, the self-immolative release of aminomethylpyrene **12** is initiated and completed after 21 h.

Our attempts to synthesize a G3 SID with an aminomethylpyrene as the tail unit have not succeeded so far. A possible obstacle could be that eight molecules of pyrene do not fit into the limited space of the dendrimer's partial sphere because of possible steric problems. To test this hypothesis, we tried to synthesize a G3 SID with 4-nitroaniline instead of pyrene. 4-Nitroaniline is a significantly smaller molecule than pyrene and has a yellow color that is easy to observe when it is in a free state. To our delight, we successfully accomplished the synthesis, thus possibly confirming our previous hypothesis. The G3 SID **15** was synthesized with a trigger group (*tert*-butoxycarbonyl, Boc) that can be chemically removed by trifluoroacetic acid to form amine **16**. The deprotected dendrimer **16** was dissolved in methanol containing 10% triethylamine and the release of 4-nitroaniline was monitored by HPLC and UV analysis. The expected pattern of the self-immolative process was observed (Scheme 3). The intermediates **17** and **18** were gradually generated and then disappeared to finally release eight molecules of 4-nitroaniline. The dendrimers were found to be highly stable in control experiments as long as the trigger was not removed, and no decomposition was observed for at least 72 h.

The dendrimers' platform is constructed of building blocks which, upon triggering, have the capability to fragment through a process of self-immolative chain reactions, based on cyclization (*N*,*N*′-dimethylethylenediamine) and elimination (1,4-quinone methide rearrangement) reactions. To establish additional support for the suggested release mechanism, we carried out first-order kinetic calculations and found an excellent correlation with the experimental results. The calculated first-order rate constant of $2.2\times10^{-3}\,\mathrm{min}^{-1}$ was observed for the G1, G2, and G3 SIDs (see Supporting Information).

The structural unit of the SIDs, 2,6-bis(hydroxymethyl)-*p*-cresol, can be viewed as an amplifier of a chemical signal. The input of the process is the cleavage of a single chemical bond, which is amplified to an output of two signals in the form of a double cleavage. The linking of an additional two structural units to the output of the first, will consequently amplify one signal into four, etc. We also determined that the self-immolative fragmentation can readily take place in aqueous medium. Therefore, SIDs can potentially be used to amplify chemical or biological signals, depending on the applied trigger and reporter units. For example, the trigger could be an enzyme substrate and the reporter could be a drug. This type of SID will release all of its tail drug molecules by a single catalytic cleavage of the enzyme substrate.

In conclusion, we have designed and synthesized a new class of dendritic molecules that we have termed self-immolative dendrimers. This structurally unique dendrimer can release all of its tail units through a self-immolative chain fragmentation, which is initiated by a single cleavage at the dendrimer's core. SIDs may be applied as a general platform for prodrugs or sensor molecules for enzymatic activity. We



Scheme 3. G3 self-immolative dendron triggered with trifluoroacetic acid (TFA) to release the 4-nitroaniline tail units.

have recently accomplished the bioactivation of dendritic prodrugs with catalytic antibodies.[14]

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[1] D. A. Tomalia, J. M. J. Frechet, *J. Polym. Sci. Part A* **2002**, *40*, 2719.
[2] D. A. Tomalia, A. M. Naylor, W. A. Goddard III, *Angew. Chem.* **1990**, *102*, 119; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 138.
[3] M. W. Grinstaff, *Chem. Eur. J.* **2002**, *8*, 2839.
[4] D. Seebach, G. F. Herrmann, U. D. Lengweiler, B. M. Bachmann, W. Amrein, *Angew. Chem.* **1996**, *108*, 2969; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2795.

[5] Y. Kim, S. C. Zimmerman, *Curr. Opin. Chem. Biol.* **1998**, *2*, 733.
[6] O. L. Padilla De Jesus, H. R. Ihre, L. Gagne, J. M. J. Frechet, F. C. Szoka, Jr., *Bioconjugate Chem.* **2002**, *13*, 453.
[7] A. K. Patri, I. J. Majoros, J. R. Baker, *Curr. Opin. Chem. Biol.* **2002**, *6*, 466.
[8] S.-E. Stiriba, H. Frey, R. Haag, *Angew. Chem.* **2002**, *114*, 1385; *Angew. Chem. Int. Ed.* **2002**, *41*, 1329.
[9] C. Kojima, K. Kono, K. Maruyama, T. Takagishi, *Bioconjugate Chem.* **2000**, *11*, 910.
[10] H. R. Ihre, O. L. Padilla De Jesus, F. C. Szoka, Jr., J. M. J. Frechet, *Bioconjugate Chem.* **2002**, *13*, 443.
[11] R. Madec-Lougerstay, J.-C. Florent, C. Monneret, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1369.
[12] R. Lougerstay-Madec, J.-C. Florent, C. Monneret, F. Nemati, M. F. Poupon, *Anti-Cancer Drug Des.* **1998**, *13*, 995.
[13] D. Shabat, , U.S. provisional patent AN 60/406,958, **2002**
[14] M. Shamis, D. Shabat, unpublished results.

