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Cleavable Dendrimers**

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Dendrimers are considered as a modern and an elegant class of branched macromolecules.[1] They resemble tree-like molecular architectures since they are built from repetitive monomers with branching point units that are radially connected around a template core. The hydrodynamic volume is dictated by the core, monomer units, branching points, dendrons, generation numbers, and peripheral functions. Dendrimers are discrete macromolecules with a high degree of molecular uniformity and monodispersity. Those features make for an easy characterization and analysis of their tailor-made properties. They are often macromolecules with few defects, whose dense outward (and/or inward) functional groups generate specific properties and functions such as a polyvalent ligand, a molecular receptor, molecular confinement, and a molecular translocator (vector). Multivalency as well as adhesive, amplification, recognition, additive, and cooperative effects have been observed. The expression "dendritic effects" was coined to illustrate those specific chemical behaviors.

Most earlier studies on dendrimers focused on the synthesis, the properties, and the quest for useful applications. However, the first dendrimer disassembly was reported in 1996 and described the enzymatic degradation of chiral polyester dendrimers.[2] In vitro gene delivery by degraded poly(amidoamine) (PAMAM) dendrimers was also mentioned that year.[3] New interest grew from the release of molecular species by covalent fragmentation of dendrimers,[4] which served as a "covalent reservoir" of those species. This approach has led to the concept of "cleavable dendrimers".

The objective of this Highlight is to describe an important new stage in the development of dendrimer chemistry by providing a critical summary of the state of the art on cleavable dendrimers that break down through the dissociation of covalent bonds.

As shown in Figure 1, disassembly of dendrimers can be conceptually divided into three major modes:[5] A) a supramolecular disassembly and liberation of substrate(s) or smaller species;[6] B) a covalent disassembly that could proceed by partial removal of a few functional groups, removal of specific sequences leading to a functional macromolecule, removal of dendrons (or a part of them), removal of a core, or some advanced cleavage of the backbone that leads to full (bio)degradation into simple chemical species, and C) a combined mode in which bond cleavage of a dendritic shell initiates a supramolecular disassembly.[7]

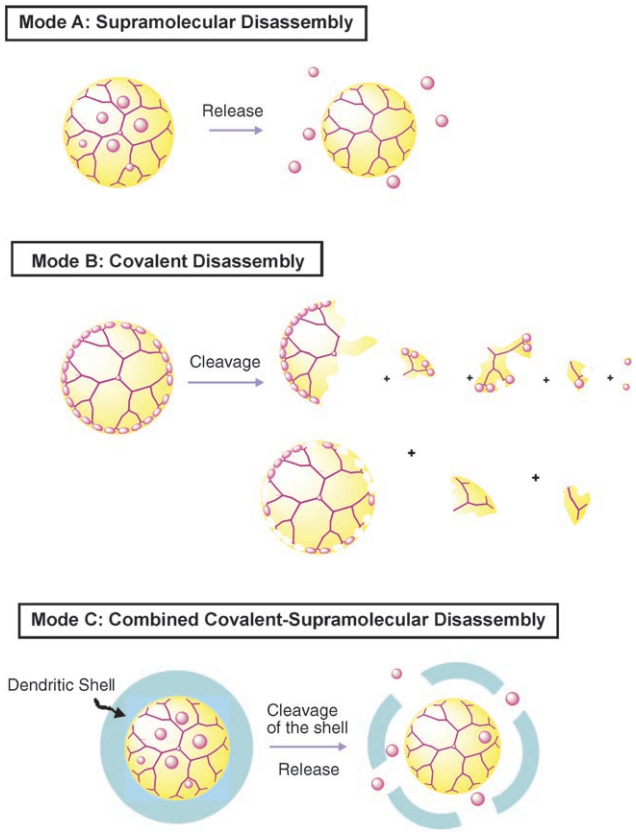

Figure 1. Major modes of dendrimer disassembly (non-exhaustive).

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Table 1: Non-exhaustive list of applications related to cleavable dendrimers and cleaving methods.

Approx. dates of intense activities	Applications	Cleaving methods
1996	first gene transfer with degraded dendrimers and enzymatic cleavage of dendrimers	hydrolytic, pH dependent
2004	glycodendrimers—solid-phase synthesis	hydrolytic, photolytic, metal-catalyzed
2002–2006	degradable biocompatible materials	photolabile, enzymatic, hydrolytic
2003–	tissue repairs, ocular sealant for sutureless eye surgery	biodegradation, hydrolytic
2002–2006	anticancer, chemotherapeutics, drug-release polytherapy, drug solubilization, prodrugs, drug nanocarriers, etc. ...	enzymatic, catalytic antibodies, hydrolytic, metal-mediated, photolabile, chemical trigger
2003	fundamental studies (chemical adaptor units, chemical triggers)	redox, hydrolytic, photolabile, enzymatic, metal-catalyzed, etc.
2000	chemically amplified photoresists	photolabile
2002–2003	monomolecular imprints	hydrolytic
2005	fragrance release	hydrolytic

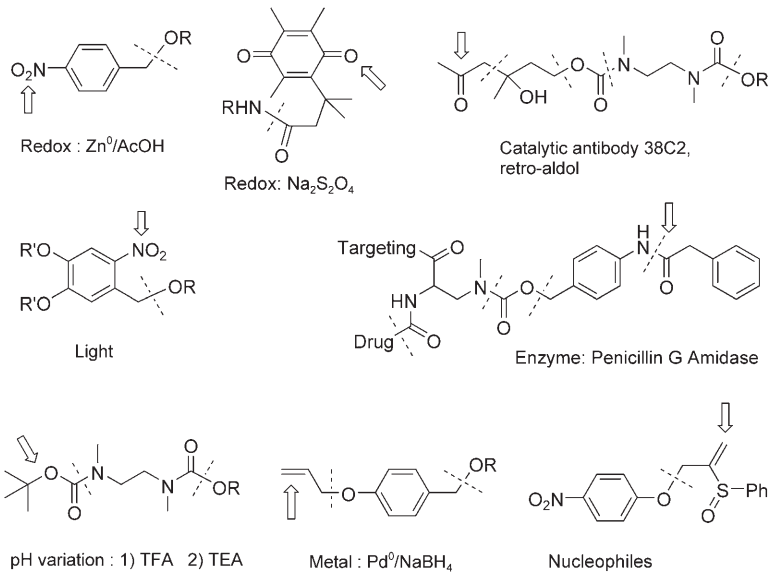
This Highlight will focus on the cleavage of covalent bonds (modes B and C).

In 2003/2004, three research groups independently published some "chemical adaptors units"[8] that were designed to release a drug from dendrimers. It definitively launched the field of "cleavable dendrimers" and the disassembly of dendritic backbones.[9] In analogy to polymers, it could be compared to depolymerization. Those chemical adaptors incorporated a specific functional sequence that triggered a cascade cleavage of the dendrimer in a linear or in a geometric way by using an external stimulus (Scheme 1). The latter could be a pH variation,[10] a photochemical reaction,[11] transition metals,[12] enzymes[2,13] (hydrolytic enzymes of esters, amides, and carbamates), catalytic antibodies,[14] a redox reaction,[15] or a thermal process.

As shown in Table 1, recent applications of cleavable dendrimers[4,16–20] are found in drug and gene delivery, pH-responsive devices, smart materials, (bio)degradable materials, release of fragrances and flavors, tissue repairs, supramolecular nanocontainers, diagnostic and imaging, molecular imprints, and photoresists.

Anticancer chemotherapeutics[21] are by far the main representative use of cleavable dendrimers. The concepts of cell targeting, polytherapy, drug solubilization, macromolecular prodrugs, and drug nanocarriers are found in most examples. Dendrimers are now perceived as a promising class of drug scaffolds because they are well defined, monodisperse, readily soluble in solvents, and well characterized. In contrast, it was reported that polydispersity and reproducibility in the preparation of functionalized hyperbranched or linear polymers may lead to irreproducible pharmacokinetic behaviors as a result of the variation in the molecular-weight distribution profile.

Cleavable dendritic backbones are often derived from polyamides, PAMAMs, polyesters, or polyethylene glycols (PEGs). The constitutive units are often PEG as well as succinic or glutamic acids. The main cleavable functionalities are amides, esters, and carbamates. The last two are preferred because of their higher rates of hydrolysis. Until now, only a few therapeutic studies have been achieved, but it is known that molecular size and branching determines whether a cellular uptake mechanism can occur by an endosomal process. Furthermore it is known that enhanced permeation-and-retention (EPR) phenomena in tumors is physically favorable compared to normal cells if the macromolecular size is sufficiently high for long blood circulation times and there is a decreased rate of renal filtration. In other words, there is a physical accumulation of the drug in a tumor because of the less-efficient lymphatic drainage and more permeable endovascular tissues. Biocompatibility must also be ensured by some degradation and renal elimination, which depend on the generation number and degree of branching.[22] Neutral or anionic components in dendrimers are preferred over cationic ones because of



Scheme 1. Chemical adaptor units and the external stimuli involved in a cleavage reaction. The arrows represent the initiating parts of the cascade reactions and the dashed lines indicate the bonds that are cleaved (adapted from Ref. [4]). TFA = trifluoroacetic acid, TEA = triethylamine.

their lower toxicity and avoidance of cell lyses.

Some reports on polyester dendrimers with low cytotoxicity are some of the most advanced studies on the biological evaluation of dendrimers for drug delivery.[16,23,24] Their degradation in vitro, their toxicity, and their in vivo biodistribution in mice were studied using radiolabeling (radioidination), which indicated some accumulation of those macromolecules in the liver and intestines for dendrimers of generation 1, but not in other organs such as heart, lungs, spleen, or stomach. No other generations showed any accumulation. The clearance rate in the blood circulation was faster for smaller polyesters, but a higher macromolecular weight (> 30000–40000 Da) and branching helped for longer plasma circulation times. According to a model of renal filtration through glomerular pores and EPR effects, the accumulation of the dendrimer in tumors is significant in this case. They represent the first systematic studies between the molecular weight/architectures of dendrimers and pharmacokinetics for well-defined macromolecules. An interesting study on the biodistribution and persistence of [^{3}H]PAMAM dendrimers in organ/tumor was achieved. In mice having B16 melanoma or DU145 human prostate cancer, the dendrimers were mainly localized in the lungs, liver, and kidneys, followed by tumors, heart, pancreas, and spleen.[25]

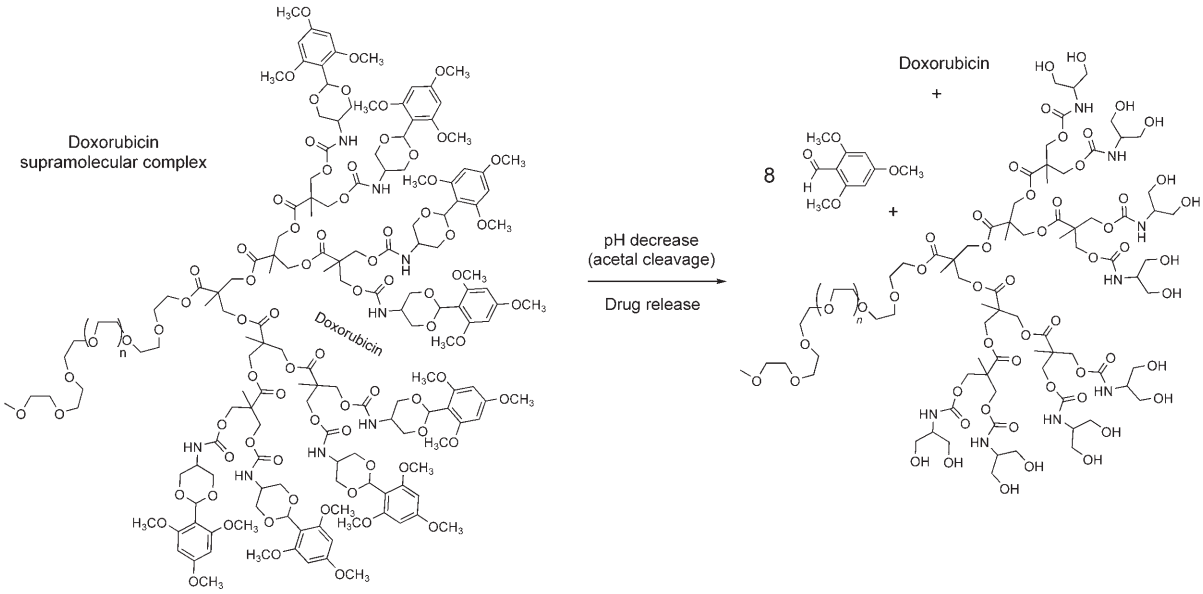
A few representative examples of some anticancer dendritic prodrugs are given below. The drugs involved are: doxorubicin, methotrexate (MTX), camptothecin, etoposide, 5-fluorouracil (5-FU), and paclitaxel (taxol).

A few systems combined the supramolecular encapsulation of a drug and its release after cleavage of some of the covalent bonds of the dendritic shell (Figure 1, mode C).[7] Micelles made from such dendrimers enabled the delivery of doxorubicin (Scheme 2).[10c] This system comprised a PEG polymer coupled to a dendritic wedge having cleavable ester and carbamate groups as well as a shell of ketal/acetal functional groups. The drug could be delivered after hydrolysis of the ketal/acetal functions under mild acidic conditions, similar to the pH present in endosomes, with the hope of obtaining better delivery to the cancer cells. Other studies made use of polyglycerol or poly(ethyleneimine) (PEI) dendrimers functionalized with ketals or imines that could also be hydrolyzed after reactions of the dendritic shell.[7a] Of the covalent prodrug systems, metothrexate conjugates made from either PAMAM–MTX dendrimers or benzyl ether–MTX dendrimers were reported as a statistical mixture.[26]

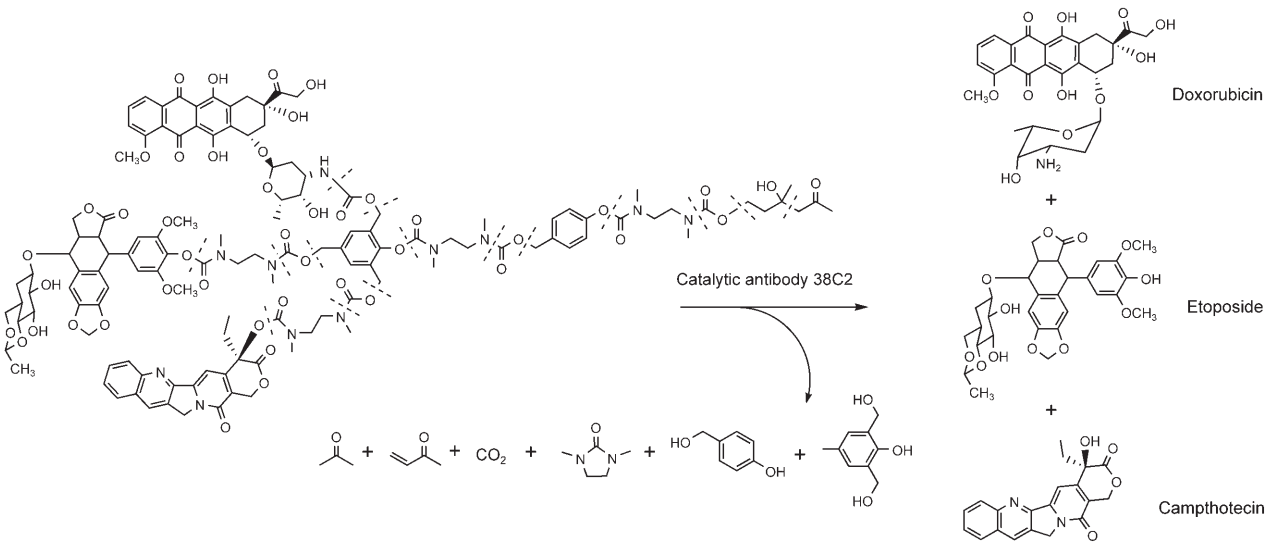
The concept of cancer tritherapy[14] was reported in an elegant study in which camptothecin, doxorubicin, and etoposide were attached to a single chemical adaptator unit (Scheme 3). By triggering the degredation process with catalytic antibody 38C2, it was possible to release three different drugs from the same scaffold in a cascade reaction starting from a single chemical event (a retro-aldol reaction). The heterodendritic trimeric prodrug was more potent than individual monomeric drugs when incubated with the antibody. This study was an extension of a similar bioactivation method in which self-immolative heterodendritic produgs were used in bitherapy.[14]

De Groot et al. introduced "cascade-release dendrimers" with an amazing self-eliminating chemical adaptator unit that liberated paclitaxel (taxol; Scheme 4).[8c,13i] The reduction of a nitro to an amino group served as a redox trigger to release paclitaxel molecules (taxol). Majoros et al. reported another study on PAMAM dendrimers conjugated with taxol, fluorescein isothiocyanate (for imaging), and folic acid (for targeting). The dendrimer bioactivities were tested in vitro against some cancer cells.[13b]

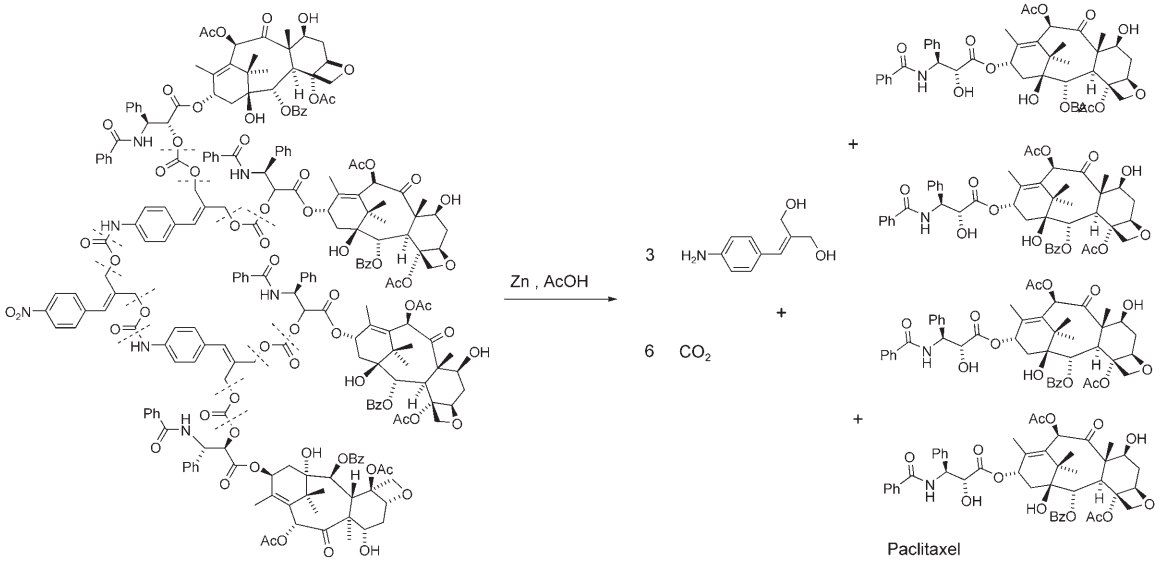
A dendritic doxorubicin prodrug was synthesized as a conjugate from a three-arm PEG core and polyester dendrons (Scheme 5).[27] An acid-labile hydrazone linkage with doxorubicin was demonstrated. This system had a stable polymeric backbone, low polydispersity, water solubility, and negligible toxicity. Biological evaluations were achieved



Scheme 2. A doxorubicin polyester dendrimer complex with a cleavable acetal shell for liberating the anticancer drug (based on Ref. [10c]).



Scheme 3. A heterodendritic dendrimer prodrug that demonstrates tritherapy by the simultaneous delivery of three anticancer drugs by using the catalytic antibody 38C2 as the trigger (based on Ref. [14a]).



Scheme 4. Cascade-release dendrimer liberating four paclitaxel (taxol) leaving groups upon reduction (based on Ref. [8c]). Bz = benzoyl.

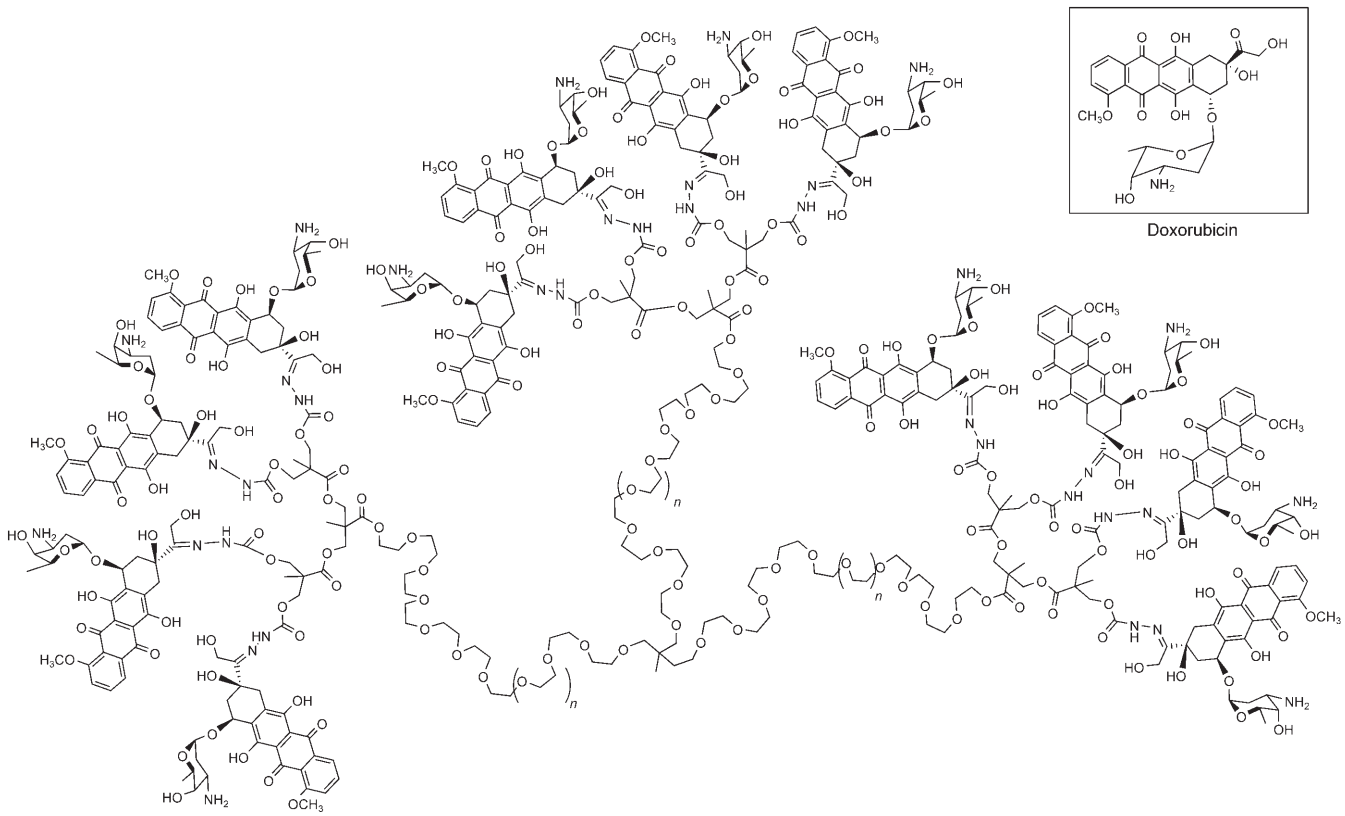
in vitro and in vivo (with mice), and cell viability, biodistribution, and the use of confocal microscopy were discussed.

5-Fluorouracil (5-FU) is a classic anticancer drug for the treatment of colon cancer. PAMAM dendrimers were used as a molecular scaffold for making a 5-FU conjugate prodrug.[10d]

A recent model for diagnostic and imaging was based on a biodegradable drug carrier from the coupling of PAMAM or PEI dendritic cores, poly(L-glutamic acid) as the arms, folic acid moieties for targeting cancer cells, and a near-infrared absorbing indocyanine dye.[13c] The resulting conjugate polymers could be degraded by the endosomal enzyme cathepsin B, and bound selectively to tumor cells expressing folate receptors. Fluorescence microscopy was used for imaging. Radioiodination and fluorescence confocal microscopy also demonstrated that the dendritic structure incorporating doxorubicin was located in cancer cells.[10c]

Besides anticancer therapy, the anti-inflammatory naproxen drug was covalently bound to a PAMAM core to make a polyester or a polyamide dendritic prodrug that could be cleaved by esterases in plasma or by a variation in the pH value. This study confirmed that the amide linkage was too unreactive for a slow release of the drugs, although the ester functions were slowly cleaved after many hours.[10a]

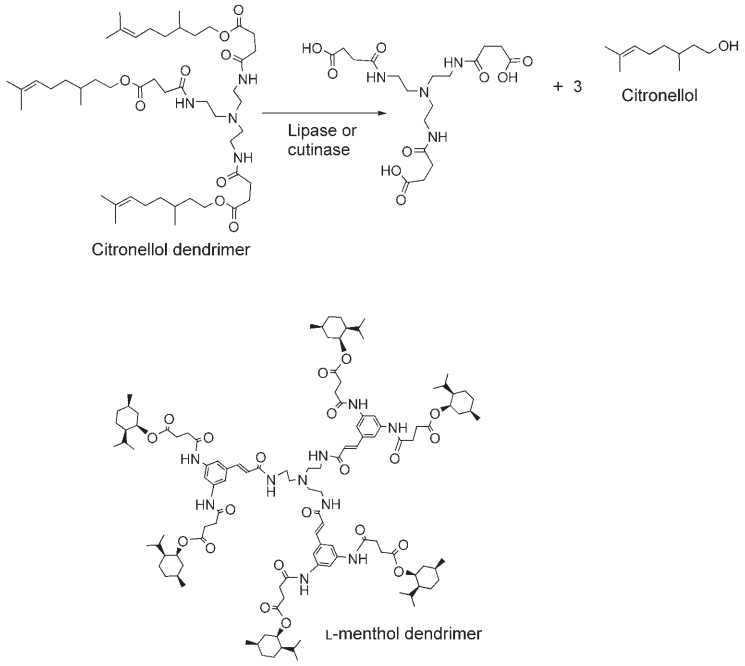
Biodegradable materials are important for biomedical applications, for example, in the repair of cartilage tissue.[28] However, the high water content, biocompatibility, and rate of degradation of the matrix material must be



Scheme 5. A cleavable polyester–PEG dendrimer as a macromolecular doxorubicin prodrug.[27]

tuned to the synthesis of the extracellular matrix. The role of photo-cross-linkable biodendrimer-based hydrogel scaffolds was promoted as a means to use methacrylated dendrimers for increasing the cross-linking and to avoid swelling in the solvent, which could be detrimental to the adjustment of precise geometric entities. Multiple branching of a PEG_{3400} core to polyester wedges produced a dendrimer that underwent considerable degradation before the cross-linked network broke down, thus providing materials with a better mechanical strength. In a similar application, Carnahan and Grinstaff used methacrylated biodendrimer wedges and PEG; this system assisted in the repair of corneal tissue by acting as an ocular sealant for sutureless eye surgery.[29,30]

Branched, cleavable polyamides have also been used for the release of the fragrances citronellol and L-menthol (Scheme 6). The combination of amide functions incorporated in a dendritic backbone and covalent attachment of the organoleptic compounds through ester functional groups enable several enzymes to be used. In these specific cases, a lipase (*Candida cylindracea*) and a cutinase (*Fusarium solani pisii*) were chosen. Those investigations revealed a partial enzymatic cleavage of the ester functionalities, which became more difficult as the branching, the rigidity, or the bulkiness of the polyamide–fragrance conjugates increased.[31]



Scheme 6. Fragrance-release dendrimers liberating citronellol or L-menthol after ester hydrolysis by either a lipase or cutinase.[31]

Photoresist technology is used to fabricate electronic circuitry and DRAM (dynamic random access memories). The first example of chemically amplified lithography resist materials based on dendrimers was created by using dendrimers with thermally labile end groups (*tert*-butoxycarbonyl, *t*Boc). Lithography with conventional e-beam irradiation produced lines in the 50–100 nm range. It is believed that the dendritic shape relative to the long polymeric chains is responsible for the increase in the resolution.[32]

Redox-driven shaving dendrimers used a redox stimuli (with $Na_2S_2O_4$) for cleaving peripheral substituted quinone end groups (Scheme 7).[15a] Reduction of the latter induced an intramolecular nucleophilic cyclization which led to the cleavage products.

A new concept called monomolecular imprinting, in which metathesis polymerization of homoallyloxy-terminated dendrimers was used for interdendron cross-linking and formation of a dendritic matrix incorporating a dendritic core (a porphyrin or trimesitoic acid) is shown in Figure 2.[33] The core can eventually be extracted after cleaving the ester functionalities to leave a molecular imprint of the core (a porphyrin or trimesoic acid). The beneficial features of this approach for the field of molecular imprinting rely on a dendritic scaffold that undergoes a controlled reversible metathesis polymerization that self-adjusts with the template because of the equilibrium and produces less defects in the imprints compared to classic molecular imprinting. The quantitative removal of the template is noteworthy in the field.

Combinatorial solid-phase synthesis of dendritic glycoclusters was described as a promising approach for studying cluster effects in glycobiology and in carbohydrate–protein interactions. A dendritic wedge containing peripheral galactoside units is presented in Scheme 8.[34] By using photochemistry,

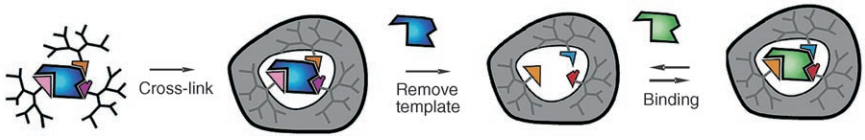
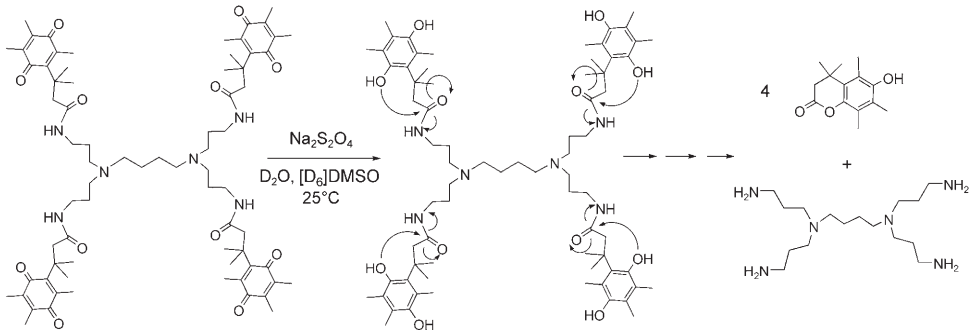
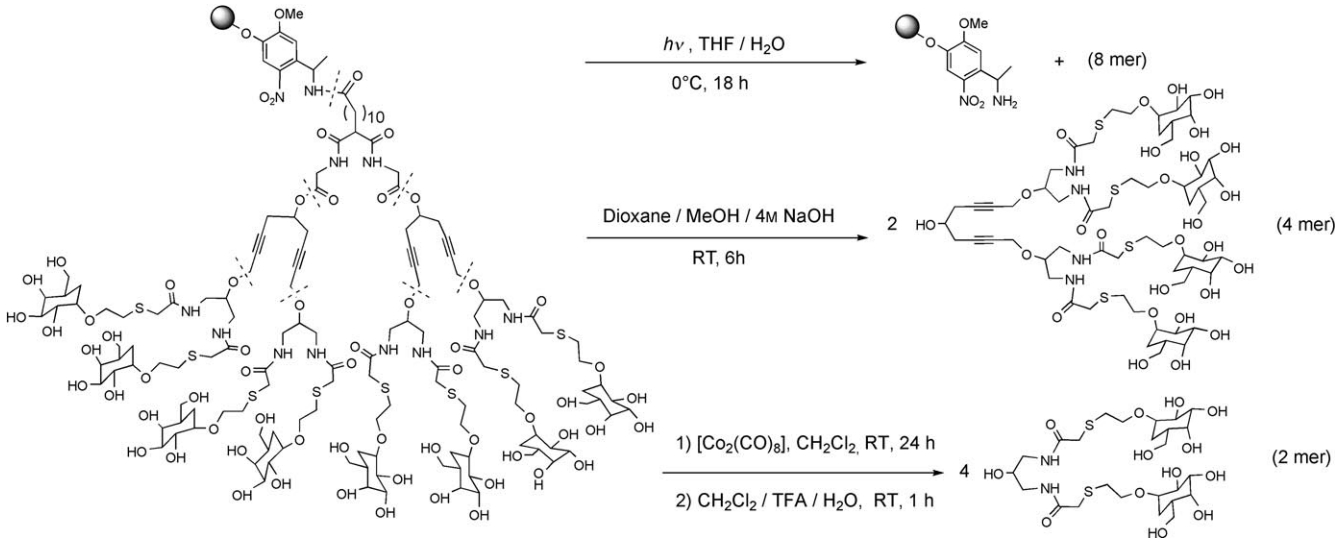


Figure 2. General concept of monomolecular imprinting inside dendrimers (modified and reproduced with the kind permission from Ref. [33]).



Scheme 7. Redox-shaving dendrimers based on the reduction of quinone derivatives as chemical adaptator units (based on Ref. [15a]).



Scheme 8. Cleavage of a dendritic carbohydrate cluster with three types of orthogonally cleavable groups formed by solid-phase synthesis. The shaded circle represents the solid support.[34]

it was possible to release the 8-mer glycodendrimer from the resin by dissociation of a covalent bond. A basic hydrolysis of the esters produced the 4-mer compounds. The propargyloxy group could be cleaved by using $[Co_2(CO)_8]$ to generate the 2-mer products. In short, orthogonal methods for the selective cleavage of the resin-bound glycodendrimer were demonstrated.

A new stage in the development of dendrimer chemistry was set by creating cleavable dendrimers with well-defined monodisperse structures. These features make them more attractive than dendritic polymers with high polydispersity. Some "chemical adaptor units" for dendrimer disassembly through cascade reactions that are triggered by several external stimuli, such as light, redox reactions, enzymes, and pH variation, were recently reported. Such results could be compared to "smart materials" already used in multiple applications, especially in the field of anticancer prodrugs and drug delivery. Recent biological evaluations are extremely encouraging in regard to some new modes of biodistribution, cell targeting, controlled release of drugs, and polytherapy, while taking advantage of the EPR effects. Dendrimers can be considered as covalent nanocarriers, where the properties of the molecular scaffold could be tailor-made. They have brought new insights into tissue repairs, diagnostics and imaging, molecular imprints, and photoresists. Cleavable dendrimers should bring a new dimension in the fields of nano-oncology,[16] nanomedicine,[35] and (bio)nanotechnology, as well as supramolecular and materials chemistry.

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[1] a) "Dendrimers and Dendritic Polymers": *Prog. Polym. Sci.* (Eds.: D. A. Tomalia, J. M. Fréchet), Elsevier, Amsterdam, **2005**; b) A. M. Caminade, J. P. Majoral, *Acc. Chem. Res.* **2004**, *37*, 341 – 348; c) "Dendrimers V: Functional and Hyperbranched Building Blocks, Photophysical Properties, Applications in Materials and Life Sciences": *Top. Curr. Chem.* (Eds.: C. A. Schalley, F. Vögtle), Springer, Berlin, **2003**.

[2] D. Seebach, G. F. Herrmann, U. D. Lengweiler, B. M. Bachmann, W. Amrein, *Angew. Chem.* **1996**, *108*, 2969 – 2972; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2795 – 2796.

[3] M. X. Tang, C. T. Redemann, F. C. Szoka, Jr., *Bioconjugate Chem.* **1996**, *7*, 703 – 714.

[4] General discussion: a) D. Shabat, *J. Polym. Sci. Part A* **2006**, *44*, 1569 – 1578; b) D. V. McGrath, *Mol. Pharm.* **2005**, *2*, 253 – 263; c) C. C. Lee, J. A. MacKay, J. M. J. Fréchet, F. C. Szoka, *Nat. Biotechnol.* **2005**, *23*, 1517 – 1526.

[5] Supramolecular versus covalent mode: A. K. Patri, J. F. Kukowska-Latallo, J. R. Baker, Jr., *Adv. Drug Delivery Rev.* **2005**, *57*, 2203 – 2214.

[6] a) A. D'Emanuele, D. Attwood, *Adv. Drug Delivery Rev.* **2005**, *57*, 2147 – 2162; b) D. K. Smith, *Chem. Commun.* **2006**, 34 – 44.

[7] a) R. Haag, F. Kratz, *Angew. Chem.* **2006**, *118*, 1218 – 1237; *Angew. Chem. Int. Ed.* **2006**, *45*, 1198 – 1215; b) see Ref. [10c]; c) R. Haag, *Angew. Chem.* **2004**, *116*, 280 – 284; *Angew. Chem. Int. Ed.* **2004**, *43*, 278 – 282; d) M. Krämer, J.-F. Stumbé, H. Türk, S. Krause, A. Komp, L. Delineau, S. Prokhorova, H. Kautz, R. Haag, *Angew. Chem.* **2002**, *114*, 4426 – 4431; *Angew. Chem. Int. Ed.* **2002**, *41*, 4252 – 4256; e) J. F. G. A. Jansen, E. W. Meijer, E. M. M. de Brabander-van den Berg, *J. Am. Chem. Soc.* **1995**, *117*, 4417 – 4418; f) J. F. G. A. Jansen, E. M. M. de Brabander-van den Berg, E. W. Meijer, *Science* **1994**, *266*, 1226 – 1229.

[8] a) D. Shabat, R. J. Amir, A. Gopin, N. Pessah, M. Shamis, *Chem. Eur. J.* **2004**, *10*, 2626 – 2634; b) A. Gopin, C. Rader, D. Shabat, *Bioorg. Med. Chem.* **2004**, *12*, 1853 – 1858; c) F. M. H. de Groot, C. Albrecht, R. Koekkoek, P. H. Beusker, H. W. Scheeren, *Angew. Chem.* **2003**, *115*, 4628 – 4632; *Angew. Chem. Int. Ed.* **2003**, *42*, 4490 – 4494; d) S. Li, M. L. Szalai, R. M. Kevwitch, D. V. McGrath, *J. Am. Chem. Soc.* **2003**, *125*, 10516 – 10517; e) R. J. Amir, N. Pessah, M. Shamis, D. Shabat, *Angew. Chem.* **2003**, *115*, 4632 – 4637; *Angew. Chem. Int. Ed.* **2003**, *42*, 4494 – 4499; f) R. Madec-Lougerstay, J.-C. Florent, C. Monneret, *J. Chem. Soc. Perkin Trans. 1* **1999**, 1369 – 1375.

[9] E. W. Meijer, M. H. P. van Genderen, *Nature* **2003**, *426*, 128 – 129.

[10] Among the many references: a) M. Najlah, S. Freeman, D. Attwood, A. D'Emanuele, *Int. J. Pharm.* **2006**, *308*, 175 – 182; b) X. Li, Y. Su, Q. Chen, Y. Lin, Y. Tong, Y. Li, *Biomacromolecules* **2005**, *6*, 3181 – 3188; c) E. R. Gillies, J. M. J. Fréchet, *Bioconjugate Chem.* **2005**, *16*, 361 – 368; d) R. X. Zhuo, B. Du, Z. R. Lu, *J. Controlled Release* **1999**, *57*, 249 – 257.

[11] a) N. Nishiyama, A. Iriyama, W.-D. Jang, K. Miyata, K. Itaka, Y. Inoue, H. Takahashi, Y. Yanagi, Y. Tamaki, H. Koyama, K. Kataoka, *Nat. Mater.* **2005**, *4*, 934 – 941; b) J. R. R. Majjigapu, A. N. Kurchan, R. Kottani, T. P. Gustafson, A. G. Kutateladze, *J. Am. Chem. Soc.* **2005**, *127*, 12458 – 12459; c) I. Grabchev, R. Betcheva, V. Bojinov, D. Staneva, *Eur. Polym. J.* **2004**, *40*, 1249 – 1254; d) M. L. Szalai, D. V. McGrath, *Tetrahedron* **2004**, *60*, 7261 – 7266; e) I. Grabchev, V. Bojinov, J.-M. Chovelon, *Polymer* **2003**, *44*, 4421 – 4428; f) R. M. Kevwitch, D. V. McGrath, *Synthesis* **2002**, 1171 – 1175; g) M. Smet, L.-X. Liao, W. Dehaen, D. V. McGrath, *Org. Lett.* **2000**, *2*, 511 – 513.

[12] a) M. L. Szalai, R. M. Kevwitch, D. V. McGrath, *J. Am. Chem. Soc.* **2003**, *125*, 15688 – 15689; b) see Ref. [8d].

[13] Among the many references: a) H. Yang, S. T. Lopina, *J. Biomed. Mater. Res. Part A* **2006**, *76*, 398 – 407; b) I. J. Majoros, A. Myc, T. Thomas, C. B. Mehta, J. R. Baker, Jr., *Biomacromolecules* **2006**, *7*, 572 – 579; c) P. Kolhe, J. Khandare, O. Pillai, S. Kannan, M. Lieh-Lai, R. M. Kannan, *Biomaterials* **2006**, *27*, 660 – 669; d) J. Khandare, P. Kolhe, O. Pillai, S. Kannan, M. Lieh-Lai, R. M. Kannan, *Bioconjugate Chem.* **2005**, *16*, 330 – 337; e) R. J. Amir, D. Shabat, *Chem. Commun.* **2004**, 1614 – 1615; f) W. Tansey, S. Ke, X.-Y. Cao, M. J. Pasuelo, S. Wallace, C. Li, *J. Controlled Release* **2004**, *94*, 39 – 51; g) M. Ternon, M. Bradley, *Chem. Commun.* **2003**, 2402 – 2403; h) D. A. Sarracino, C. Richert, *Bioorg. Med. Chem. Lett.* **2001**, *11*, 1733 – 1736; i) F. M. H. de Groot, W. J. Loos, R. Koekkoek, L. W. A. van Berkom, G. F. Busscher, A. E. Seelen, C. Albrecht, P. de Bruijn, H. W. Scheeren, *J. Org. Chem.* **2001**, *66*, 8815 – 8830.

[14] Tritherapy: a) K. Haba, M. Popkov, M. Shamis, R. A. Lerner, C. F. Barbas III, D. Shabat, *Angew. Chem.* **2005**, *117*, 726 – 730; *Angew. Chem. Int. Ed.* **2005**, *44*, 716 – 720; bitherapy: b) M. Shamis, H. N. Lode, D. Shabat, *J. Am. Chem. Soc.* **2004**, *126*, 1726 – 1731; others: c) N. Pessah, M. Reznik, M. Shamis, F. Yantiri, H. Xin, K. Bowdish, N. Shomron, G. Ast, D. Shabat, *Bioorg. Med. Chem.* **2004**, *12*, 1859 – 1866; d) A. Cordüva, K. D. Janda, *J. Am. Chem. Soc.* **2001**, *123*, 8248 – 8259.

[15] a) W. Ong, R. L. McCarley, *Chem. Commun.* **2005**, 4699 – 4701; b) M. L. Szalai, D. V. McGrath, *Polym. Prepr.* **2004**, *45*, 110 – 111; c) see Ref. [8c].

[16] N. G. Portney, M. Ozkan, *Anal. Bioanal. Chem.* **2006**, *384*, 620 – 630.

[17] S. Svenson, D. A. Tomalia, *Adv. Drug Delivery Rev.* **2005**, *57*, 2106 – 2129.

[18] U. Boas, P. M. H. Heegaard, *Chem. Soc. Rev.* **2004**, *33*, 43 – 63.
[19] a) E. R. Gillies, J. M. J. Fréchet, *Drug Discovery Today* **2005**, *10*, 35 – 43; b) F. Aulenta, W. Hayes, S. Rannard, *Eur. Polym. J.* **2003**, *39*, 1741 – 1771; c) M. J. Cloninger, *Curr. Opin. Chem. Biol.* **2002**, *6*, 742 – 748.
[20] K. Ulbrich, V. Šubr, *Adv. Drug Delivery Rev.* **2004**, *56*, 1023 – 1050.
[21] M. Liu, J. M. J. Fréchet, *Pharm. Sci. Technol. Today* **1999**, *2*, 393 – 401.
[22] R. Duncan, L. Izzo, *Adv. Drug Delivery Rev.* **2005**, *57*, 2215 – 2237.
[23] E. R. Gillies, E. Dy, J. M. J. Fréchet, F. C. Szoka, Jr., *Mol. Pharm.* **2005**, *2*, 129 – 138.
[24] H. R. Ihre, O. L. Padilla De Jesús, F. C. Szoka, Jr., J. M. J. Fréchet, *Bioconjugate Chem.* **2002**, *13*, 443 – 452.
[25] S. S. Nigavekar, L. Y. Sung, M. Llanes, A. El-Jawahri, T. S. Lawrence, C. W. Becker, L. Balogh, M. K. Khan, *Pharm. Res.* **2004**, *21*, 476 – 483.
[26] a) S. Gurdag, J. Khandare, S. Stapels, L. H. Matherly, R. M. Kannan, *Bioconjugate Chem.* **2006**, *17*, 275 – 283; b) I. J. Majoros, T. P. Thomas, C. B. Mehta, J. R. Baker, Jr., *J. Med. Chem.* **2005**, *48*, 5892 – 5899; c) K. Kono, M. Liu, J. M. J. Fréchet, *Bioconjugate Chem.* **1999**, *10*, 1115 – 1121.
[27] O. L. Padilla De Jesús, P. L. Gagne, J. M. J. Fréchet, F. C. Szoka, Jr., *Bioconjugate Chem.* **2002**, *13*, 453 – 461.
[28] S. H. M. Söntjiens, D. L. Nettles, M. A. Carnahan, L. A. Setton, M. W. Grinstaff, *Biomacromolecules* **2006**, *7*, 310—316..
[29] M. A. Carnahan, M. W. Grinstaff, *J. Am. Chem. Soc.* **2001**, *123*, 2905 – 2906.
[30] "Dendrimers: an Innovative and Enhanced Ocular Drug Delivery System": J. M. Loutsch, D. Ong, J. M. Hill in *Ophthalmic Drug Delivery System* (Ed.: A. K. Mitra), Marcel Dekker, New York, **2003**, chap. 15, pp. 467 – 492.
[31] F. Aulenta, M. G. B. Drew, A. Foster, W. Hayes, S. Rannard, D. W. Thornthwaite, T. G. A. Youngs, *Molecules* **2005**, *10*, 81 – 97.
[32] D. C. Thully, A. R. Trimble, J. M. J. Fréchet, *Adv. Mater.* **2000**, *12*, 1118 – 1122.
[33] a) S. C. Zimmerman, I. Zharov, M. S. Wendland, N. A. Rakow, K. S. Suslick, *J. Am. Chem. Soc.* **2003**, *125*, 13504 – 13518; b) S. C. Zimmerman, M. S. Wendland, N. A. Rakow, I. Zhaov, K. S. Suslick, *Nature* **2002**, *418*, 399 – 403.
[34] T. Amaya, H. Tanaka, T. Takahashi, *Synlett* **2004**, 503 – 507.
[35] O. M. Koo, I. Rubinstein, H. Onyuksel, *Nanomedecine* **2005**, *1*, 193 – 212.

