Cascade-Release Dendrimers

"Cascade-Release Dendrimers" Liberate All End Groups upon a Single Triggering Event in the Dendritic Core**

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Dendrimers are well-defined, branched treelike molecules with multiple end groups.[1,2] Numerous applications of these starlike structures have been explored.[3,4] They can be utilized in areas such as magnetic resonance imaging (MRI), gene therapy,[5] liquid crystals, sensors, and catalysis. The application of dendrimers in drug delivery is currently receiving much scientific attention.[6–9] Most dendrimers reported so far have been constructed as a branched skeleton that incorporates functional molecules either covalently or noncovalently. Biologically active substances that are attached to the termini of dendritic structures[10] can be liberated by chemical or biological methods.[11] Several branched constructs have been reported which contain covalently linked doxorubicin molecules as end groups.[12–14] However, in all the branched structures or dendrimers reported so far, each single drug has to be independently cleaved to be released. Here, we report dendrimers that have been built to completely and rapidly dissociate into separate building blocks upon a single triggering event in the dendritic core. This process induces simultaneous release of all end-group molecules connected to the dendritic termini. We term these multiple-release dendritic systems "cascade-release dendrimers". Such dendrimers collapse into their separate monomeric building blocks after a single (chemical or biological) activation step that triggers a cascade of self-elimination reactions, thereby releasing all the end groups from the periphery of the "exploding" cascade-release dendrimer (see Supporting Information).

Functional dendrimers of this kind may find application in several fields. One particular area is (targeted) drug delivery, where cascade-release dendrimers possess two major advantages over conventional dendrimers: 1) multiple covalently bound drug molecules can be site-specifically released from the targeting moiety by a single cleaving step; and 2) they are selectively as well as completely degraded. Thus, in contrast to conventional dendrimers, they can be easily drained from the body. Clearance of macromolecules from the body may be a limiting factor in current macromolecular drug-delivery systems,[15] as the long-term effects of some synthetic macromolecules in parenteral medicine are not completely known.[16]

To facilitate drug release, linkers are frequently incorporated between the drug molecule and a cleavable carrier, which is termed the "specifier" (Figure 1).

Cascade-release dendrimers are constructed from two or more generations of branched self-elimination linkers, each of which releases multiple leaving groups after a single activation (Figure 2). We present here two such structurally distinct monomeric multiple-release building blocks. Both linkers are based on a general type of single-release linker that is eliminated as a consequence of shifting conjugated electron

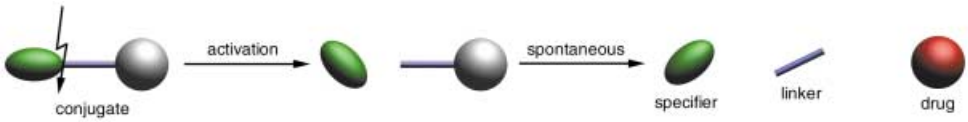

Figure 1. A conjugated drug (gray) is released from an inactive conjugate. Cleavage between the specifier (green) and the linker (blue) induces spontaneous self-elimination of the linker, thus releasing the drug molecule (red) with regained activity.

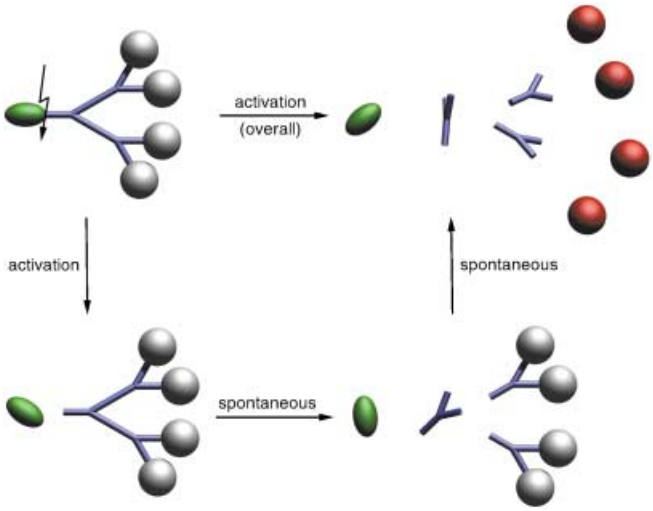

Figure 2. A single activation of a second generation cascade-release dendrimer triggers a cascade of self-eliminations and induces release of all end groups. Covalently bound end groups are depicted in gray, branched self-elimination linkers in blue, and the specifier in green. The released end groups are depicted in red.

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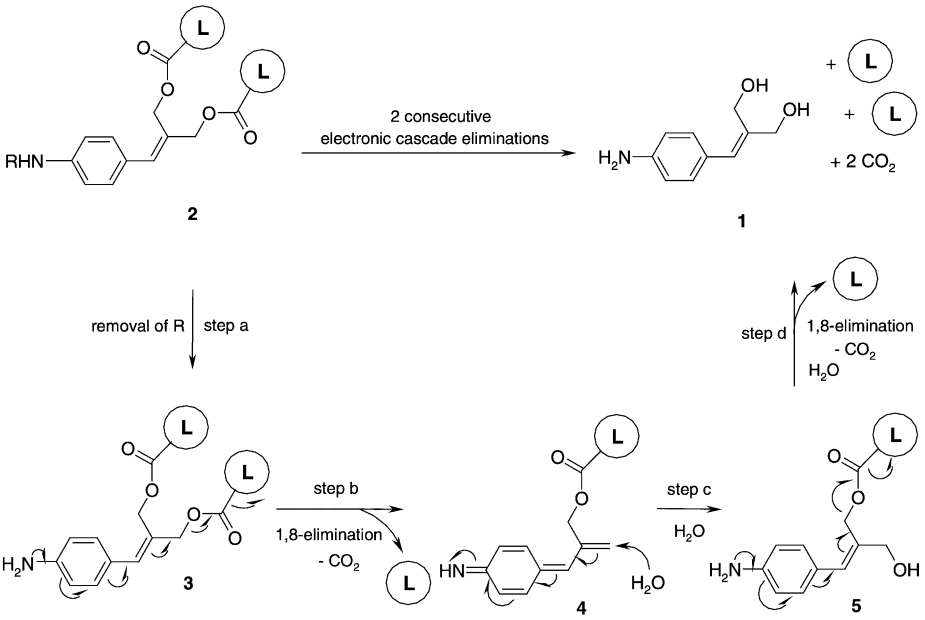
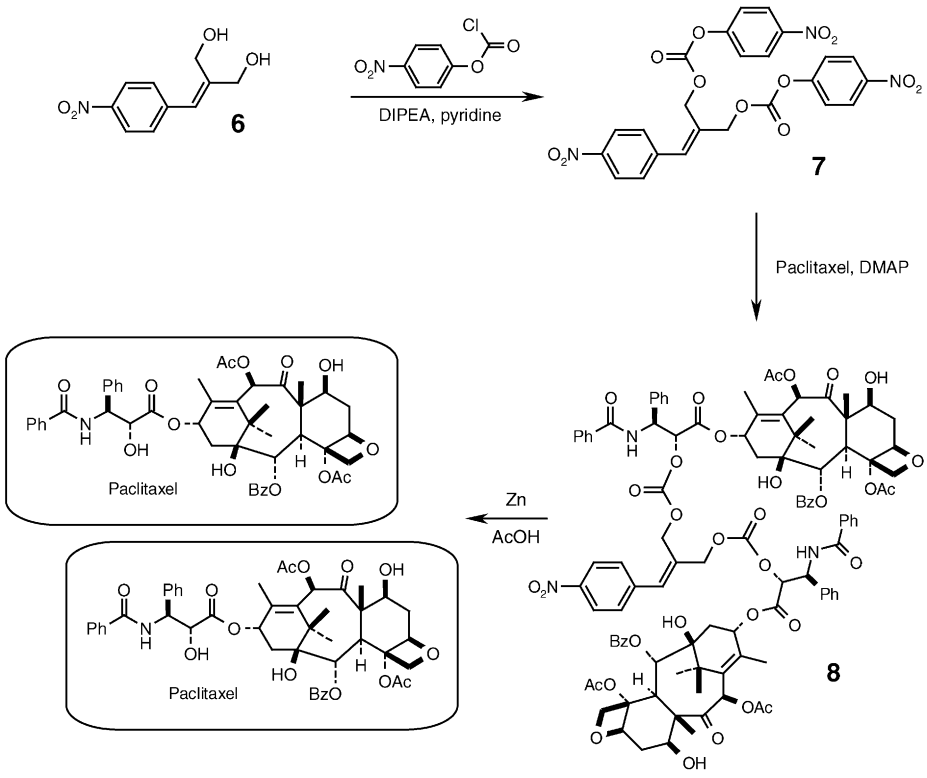
pairs, which leads to expulsion of the leaving group (for example, a drug). The most prominent example of this self-elimination linker type is the highly versatile and well-known 4-aminobenzyl alcohol linker.[17] We recently reported that the elongation of this conventional 1,6-elimination linker results in enhanced rates of drug release.[18] It was shown that the elongated 4-aminocinnamyl alcohol linker released paclitaxel (taxol) following 1,8-elimination.[19] Herein, proof of principle is reported for both the release of two leaving groups from a branched double-release linker, which can be obtained by introduction of branching into the 4-aminocinnamyl alcohol linker, and for the release of three leaving groups from a branched triple-release linker, which is based on the 1,6-elimination linker. Furthermore, we demonstrate that a cascade-release dendrimer which contains two generations of double-release linkers completely falls apart into its free monomers and releases all end groups upon a single activating event.

The double-release self-elimination AB_2-type monomer 2-(4-aminobenzylidene)propane-1,3-diol (**1**) and the proposed mechanism of the release of two leaving groups upon activation are depicted in Scheme 1. The multiple-release system is stable as long as the amine function in **2** is capped by a protecting group. Unmasking (step a) the protected amine **2** triggers two 1,8-elimination reactions from amine **3**, in which two molecules of CO_2 and two leaving groups L are liberated. The intermediate non-aromatic species **4** that is formed after the first self-elimination (step b) is trapped by a nucleophile, such as water, in step c to regenerate an aromatic species **5** that can undergo the second self-elimination (step d). Quenching of the intermediate with water (for example, under physiological conditions) will generate aminodiol building block **1**.



Scheme 1. Structure of double-release linker **1** and the proposed mechanism of elimination of both leaving groups L upon activation of **2**.

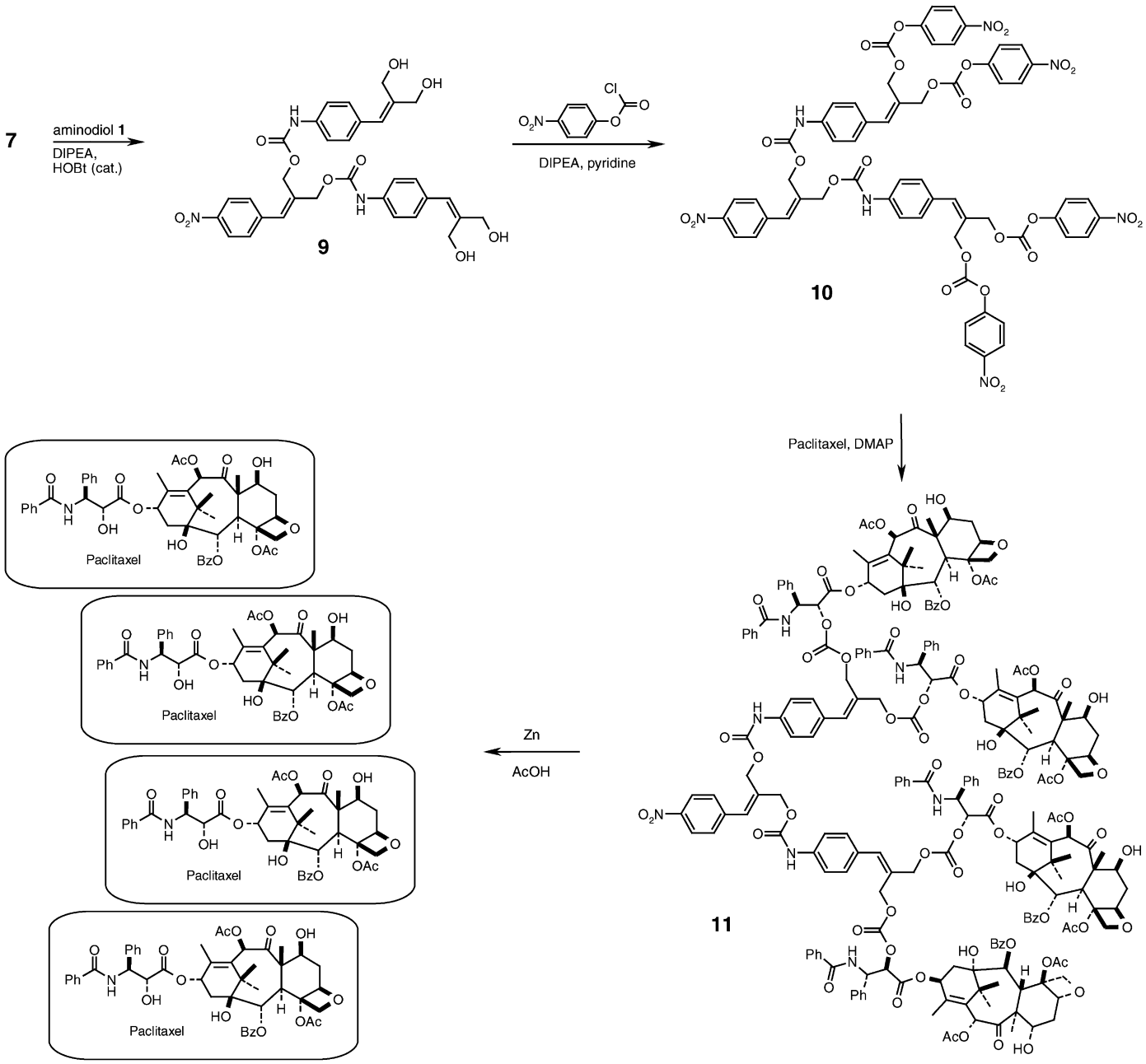
Proof of concept for complete release of both end groups from the double-release linker was firmly established in a branched system in which nitrodiol **6**[20] formed the core (Scheme 2). The nitro function can be considered as a masked amine. Chemical reduction of the nitro group to the amine should trigger the cascade of self-eliminations. Nitrodiol **6** was activated using 4-nitrophenyl chloroformate to give the corresponding bis(4-nitrophenyl carbonate) **7**, which was subsequently coupled with two equivalents of paclitaxel to yield dendron **8**. To demonstrate the generality of the concept, the chemotherapeutic drug paclitaxel was chosen as a model compound because of its complex structure and bulkiness. At 0°C paclitaxel reacted specifically with the activated carbonates through its most reactive 2′-hydroxy function. The nitro function was then



Scheme 2. Synthesis of branched construct **8** and elimination of two paclitaxel (taxol) molecules upon a single triggering reaction (reduction of the nitro function). DIPEA = *N*,*N*,-diisopropylethylamine, DMAP = 4-dimethylaminopyridine, Bz = benzoyl.

reduced under mild conditions (Zn, acetic acid; Scheme 2). Analysis by thin-layer chromatography indicated complete disappearance of the starting material and formation of unconjugated paclitaxel within 30 minutes. ^{1}H NMR spectroscopic studies unambiguously confirmed the complete release of both paclitaxel molecules (see Supporting Information). Although the NMR spectra of large paclitaxel derivatives are generally complicated, the 2′-H signal of **8** can be identified as a clearly distinguishable signal at $\delta = 5.46$ ppm, which is indicative of a 2′-hydroxy group functionalized with an alkyloxycarbonyl group. This signal completely vanished following the multiple-release cascade, thus indicating that no conjugated paclitaxel was present, whereas the characteristic paclitaxel 2′-H signal at $\delta = 4.74$ ppm was clearly present. Mass spectrometric studies showed that compound **8**, its reduced congener, or any of the intermediates similar to the ones in Scheme 1 were no longer present, and clearly showed a major peak corresponding to free paclitaxel. No degradation or formation of side products had taken place (TLC, NMR), thus showing the validity of the concept. The kinetics for electronic cascade self-eliminations are generally fast.[17] Paclitaxel was immediately formed, which left the triggering reaction as the rate-limiting step for complete dissociation.

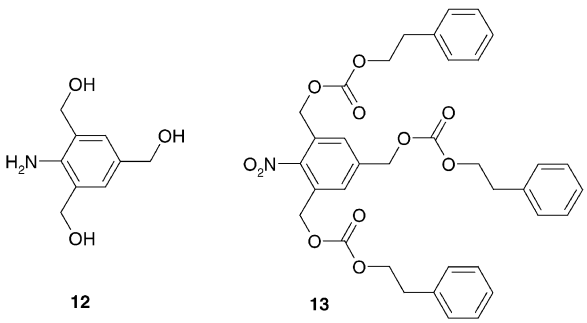
With the double-release linker in hand, we now focused on the construction of a dendritic structure to unequivocally establish that multiple release occurred from a cascade-release dendrimer. A divergent route, starting with the dendrimer core (Scheme 3), was used to synthesize cascade-



Scheme 3. Synthesis of second-generation cascade-release dendrimer **11** and release of four paclitaxel leaving groups upon reduction. HOBt = 1-hydroxy-1*H*-benzotriazole.

release dendrimer **11**. Two generations of double-release linkers were connected through carbamate linkages by coupling two molecules of aminodiol **1** to the bis(4-nitrophenyl carbonate) **7** to yield **9**. The four terminal alcohol groups were then activated to give tetracarbonate **10**, which was coupled to four equivalents of paclitaxel to yield the second-generation cascade-release dendrimer **11** loaded with four paclitaxel molecules at the dendritic termini. To prove the validity of the dendritic cascade-release concept, second generation dendrimer **11** was subjected to reduction using Zn and acetic acid (Scheme 3). Chromatographic analysis once again indicated rapid and complete disappearance of the starting compound and formation of paclitaxel. ^{1}H NMR spectroscopic analysis demonstrated complete formation of liberated paclitaxel and no degradation products. No conjugated paclitaxel was present, thus demonstrating that **11** behaves as a cascade-release dendrimer that releases all end groups according to the sequence depicted in Figure 2.

We have also designed and synthesized a second multiple-release linker, (2-amino-3,5-di(hydroxymethyl)phenyl)methanol (**12**, Scheme 4). This triple-release linker is an aniline



Scheme 4. Structures of triple-release linker **12** and its functionalized nitroaromatic derivative **13**.

derivative with a hydroxymethyl group at the 2-, 4-, and 6-positions, and it combines one 1,6- with two 1,4-elimination systems in a single linker.[17] We synthesized a nitrotriol derivative of this AB_3-type monomeric building block[21] (**13**) containing three phenethyl alcohol molecules as leaving groups connected by carbonate linkages. All the starting material was, again, completely converted into free phenethyl alcohol (MS, NMR) upon reduction of the nitro function, thus validating aminotriol **12** as a triple-release monomeric linker.

To prove that the conditions used for the reduction of the nitro function in the synthesized compounds did not directly effect cleavage of the paclitaxel carbonate or phenethyl carbonate linkages, we treated reference compounds dibenzyl carbonate and 2′-*O*-(cinnamyloxycarbonyl)paclitaxel with Zn/acetic acid. Both compounds remained fully intact under these conditions (as observed by ^{1}H NMR spectroscopy), thus indicating that the nitro-containing multiple-release compounds are only degraded on reduction of the nitro group.

Since the reported cascade-release dendrimers may be useful for drug delivery, we have evaluated both multiple-release monomers aminodiol **1** and aminotriol **12**, which are expected to be regenerated under physiological conditions upon dendritic disintegration, for their cytotoxicity in a panel of seven well-characterized human tumor cell lines. Neither compound displayed any toxicity. Details concerning the experimental procedures and in vitro cytotoxicity are enclosed as Supporting Information.

Cascade-release dendrimers, as well as the multiple-release monomers, may show utility in fields such as (targeted) drug delivery, biodegradable materials, controlled release, and diagnostics. Much room is left for the variation of individual components for the design of a cascade-release dendrimer with desired properties. The characteristics of the activation can be modified by the choice of specifier. An appropriate specifier can, for example, be a specific peptide substrate for a disease-associated enzyme, or it may contain an additional targeting moiety, such as, for example, an antibody. Two or more different parent compounds can be attached as end groups. Linear self-elimination linkers can be incorporated between the specifier and the first branched linker to improve the efficiency of drug release.[18] Linear self-elimination linkers can be incorporated between generations of branched monomers to increase the outer sphere surface and the number of end groups that can be accommodated.

Cascade-release dendrimers can be used for drug delivery, particularly for tumor-targeted drug delivery,[22,23] where they may potentially induce a substantial therapeutic advantage over single-release unbranched conjugates.[24–26] The cascade-release dendrimer amplifies the effect of one tumor-specific activating reaction, by triggering a "cytotoxic explosion". Cascade-release dendrimers of sufficient size will also passively target by the enhanced permeability and retention (EPR) effect,[27] and, as for conventional dendrimers, they can be manufactured as monodisperse and homogeneous compounds. The monomers presented here are appropriate for tumor targeting, because self-elimination of the double- and triple-release monomers proceeds with a short half-life,[17,18] and because the monomers can be connected through carbamate linkages that are generally stable under physiological conditions.[28] We have reported chemical proof of the concept of cascade-release dendrimers. Their potential in tumor targeting should be exploited, for example, by choosing a specifier that is a substrate for tumor-associated or tumor-targeted enzymes and a highly toxic anticancer agent for attachment at the dendritic termini.

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[1] D. A. Tomalia, A. M. Naylor, W. A. Goddard III, *Angew. Chem.* **1990**, *102*, 119–157; *Angew. Chem. Int. Ed. Engl.* **1990**, *29*, 138–175.
[2] G. R. Newkome, A. Nyak, R. K. Behera, C. N. Moorefield, G. R. Baker, *J. Org. Chem.* **1992**, *57*, 358–362.
[3] M. Fischer, F. Vögtle, *Angew. Chem.* **1999**, *111*, 934–956; *Angew. Chem. Int. Ed.* **1999**, *38*, 884–905.
[4] A. W. Bosman, H. M. Janssen, E. W. Meijer, *Chem. Rev.* **1999**, *99*, 1665–1688.

[5] R. F. Service, *Science* **1995**, *267*, 458 – 459.
[6] M. Liu, J. M. J. Fréchet, *Pharm. Sci. Technol. Today* **1999**, *2*, 393 – 401.
[7] N. Malik, R. Wiwattanapatapee, R. Klopsch, K. Lorenz, H. Frey, J. W. Weener, E. W. Meijer, W. Paulus, R. Duncan, *J. Controlled Release* **2000**, *65*, 133 – 148.
[8] O. L. Padilla De Jesús, H. R. Ihre, L. Gagne, J. M. J. Fréchet, F. C. Szoka, Jr., *Bioconjugate Chem.* **2002**, *13*, 453 – 461.
[9] A. K. Patri, I. J. Majoros, J. R. Baker, Jr., *Curr. Opin. Chem. Biol.* **2002**, *6*, 466 – 471, and references therein.
[10] M. J. Cloninger, *Curr. Opin. Chem. Biol.* **2002**, *6*, 742 – 748.
[11] D. Seebach, G. F. Herrmann, U. D. Lengweiler, B. M. Bachmann, W. Amrein, *Angew. Chem.* **1996**, *108*, 2969 – 2971; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2795 – 2797.
[12] H. D. King, D. Yurgaitis, D. Willner, R. A. Firestone, M. B. Yang, S. J. Lasch, K. E. Hellstrom, P. A. Trail, *Bioconjugate Chem.* **1999**, *10*, 279 – 288.
[13] D. Wang, P. Kopeckova, T. Minko, V. Nanayakkara, J. Kopecek, *Biomacromolecules* **2000**, *1*, 313 – 319.
[14] M. Kovář, J. Strohalm, T. Etrych, K. Ulbrich, B. Říhová, *Bioconjugate Chem.* **2002**, *13*, 206 – 215.
[15] L. W. Seymour, *Crit. Rev. Ther. Drug Carrier Syst.* **1992**, *9*, 135 – 187.
[16] A. C. Hunter, S. M. Moghimi, *Drug Discovery Today* **2002**, *7*, 998 – 1001.
[17] P. L. Carl, P. K. Chakravarty, J. A. Katzenellenbogen, *J. Med. Chem.* **1981**, *24*, 479 – 480.
[18] 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.
[19] E. W. P. Damen, T. J. Nevalainen, T. J. M. Van den Bergh, F. M. H. de Groot, H. W. Scheeren, *Bioorg. Med. Chem.* **2002**, *10*, 71 – 77.
[20] P. Vanelle, J. Maldonado, M. P. Crozet, K. Senouki, F. Delmas, M. Gasquet, P. Timon-David, *Eur. J. Med. Chem.* **1991**, *26*, 709 – 714.
[21] F. Piozzi, *Gazz. Chim. Ital.* **1953**, *83*, 673 – 676.
[22] G. M. Dubowchik, M. A. Walker, *Pharmacol. Ther.* **1999**, *83*, 67 – 123.
[23] F. M. H. de Groot, E. W. P. Damen, H. W. Scheeren, *Curr. Med. Chem.* **2001**, *8*, 1093 – 1122.
[24] D. DeFeo-Jones, V. M. Garsky, B. K. Wong, D. M. Feng, T. Bolyar, K. Haskell, D. M. Kiefer, K. Leander, E. McAvoy, P. Lumma, J. Wai, E. T. Senderak, S. L. Motzel, K. Keenan, M. van Zwieten, J. H. Lin, R. Freidinger, J. Huff, A. Oliff, R. E. Jones, *Nat. Med.* **2000**, *6*, 1248 – 1252.
[25] P. H. J. Houba, E. Boven, I. H. van der Meulen-Muileman, R. G. G. Leenders, J. W. Scheeren, H. M. Pinedo, H. J. Haisma, *Int. J. Cancer* **2001**, *91*, 550 – 554.
[26] F. M. H. de Groot, L. W. A. van Berkom, H. W. Scheeren, *J. Med. Chem.* **1999**, *42*, 5277 – 5283.
[27] H. Maeda, Y. Matsumura, *Crit. Rev. Ther. Drug Carrier Syst.* **1989**, *6*, 193 – 210.
[28] F. M. H. de Groot, L. W. A. van Berkom, H. W. Scheeren, *J. Med. Chem.* **2000**, *43*, 3093 – 3102.

