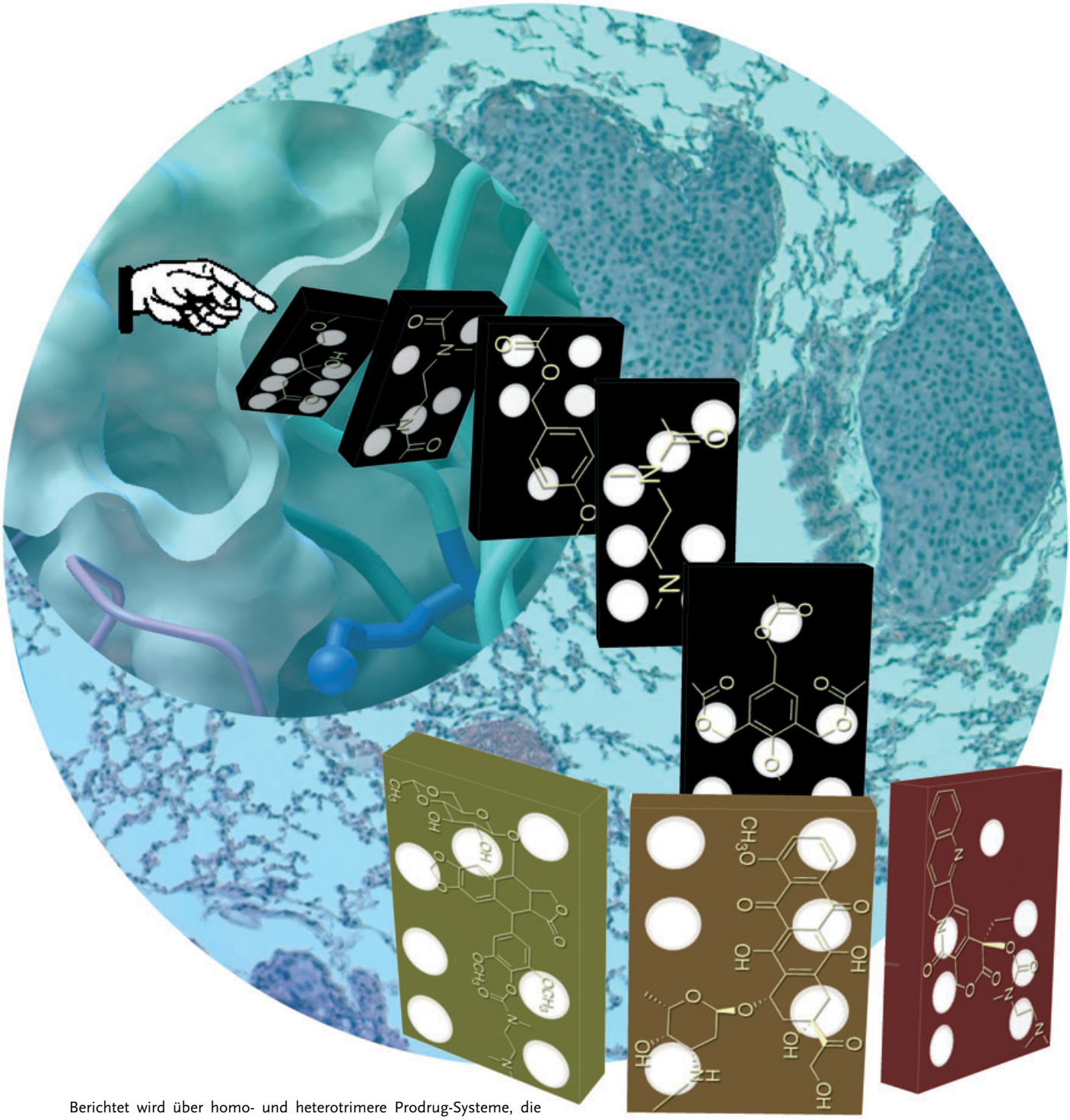
Zuschriften



Berichtet wird über homo- und heterotrimere Prodrug-Systeme, die durch eine einzige enzymkatalysierte Reaktion aktiviert werden und dabei Tumortherapeutika freisetzen. Näheres zum Dominoeffekt bei der Bioaktivierung dieser Prodrug-Plattform finden Sie in der Zuschrift von D. Shabat et al. auf den folgenden Seiten.

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Prodrug Dendrimers

Single-Triggered Trimeric Prodrugs**

*Keren Haba, Mikhail Popkov, Marina Shamis, Richard A. Lerner, Carlos F. Barbas III, and Doron Shabat**

Of the various approaches designed to minimize undesirable properties of drugs while retaining therapeutic activity, the chemical approach of drug derivatization offers the highest flexibility and has been demonstrated to improve drug efficacy.[1] The term "prodrug" indicates that chemical derivatization has been used to alter the physicochemical properties of a drug. The prodrug is converted in vivo by metabolic processes or environmental conditions into the active form. Several anticancer prodrugs have been designed for selective activation in malignant tissues by a specific enzyme secreted within the proximity of the tumor.[2] The amount of drug activated at the targeted tissue is dependent on the rate and concentration of the specific enzyme. To overcome this limitation, our group[3,4] and two others[5,6] have designed dendritic units that are able to release several drug molecules with a single enzymatic cleavage. We have also demonstrated the potential of the dendritic platform with the first bioactivation of dimeric prodrugs.[7] Here we report the advantages of a trimeric prodrug system relative to a conventional monomeric system.

Our prodrug system is based on an AB_3 dendritic unit (Figure 1) in which three drug molecules B are linked to one enzymatic substrate A. The trigger is activated upon enzymatic cleavage and is designed to release the three drug molecules through a mechanism based on self-cyclization and triple quinone methide rearrangement (see Supporting Information for the detailed mechanism). Although a similar conceptual molecule was described previously, it was not examined as a prodrug system.[5]

To evaluate the self-cyclization and triple quinone methide rearrangement, we constructed a model system from three molecules of *p*-nitroaniline to mimic the drug units and a *tert*-butoxycarbonyl (Boc) protecting group to mimic the enzymatic substrate. Cleavage of the Boc group should trigger the release of the *p*-nitroaniline moieties as shown in Figure 2. Initially, we set out to examine whether the release could occur under physiological conditions. The Boc group was removed with anhydrous hydrogen chloride, and the product was then incubated in phosphate buffered saline (PBS) solution at pH 7.4. The progress of the reaction was monitored by HPLC by following the formation of *p*-nitroaniline. Figure 3 shows that free *p*-nitroaniline was rapidly generated after the cleavage of the Boc protecting group. No release was observed when the Boc group remained attached to the platform.

This experiment proved that the triple quinone methide rearrangement could indeed take place under physiological conditions. With this information in hand, we synthesized a trimeric prodrug system in which three molecules of the anticancer drug camptothecin (pro-tCPT) were linked through a retro-aldol, retro-Michael trigger to a substrate for the catalytic antibody 38C2. This antibody catalyzes aldol and retro-aldol reactions by using the enamine mechanism of natural aldolases and has been used for the selective activation of prodrugs.[8–13] Furthermore, we synthesized a

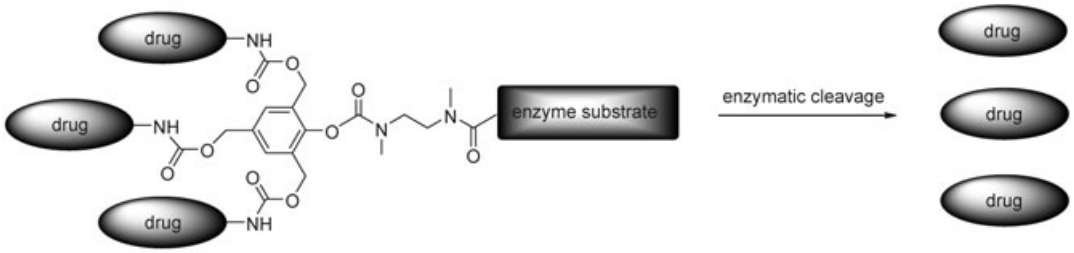

Figure 1. General structure of a single-triggered trimeric prodrug.

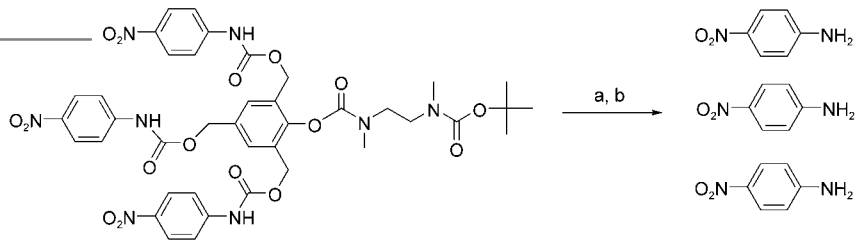

Figure 2. Cleavage of a Boc group triggers the release of *p*-nitroaniline from the trimeric platform. a) HCl; b) PBS, pH 7.4.

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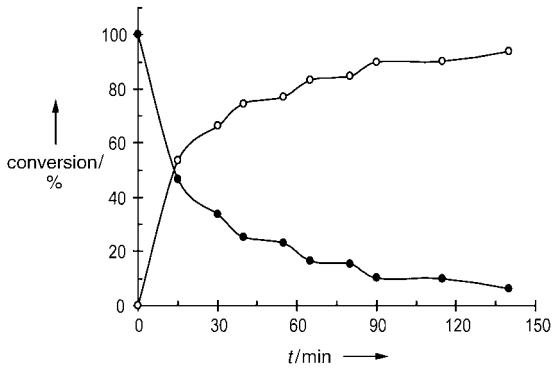

Figure 3. Release (% conversion) of *p*-nitroaniline from the trimeric platform, ○ = *p*-nitroaniline, ● = starting material.

monomeric CPT prodrug (pro-mCPT) with an identical linker (Figure 4 a). Both prodrugs were activated upon incubation with the antibody 38C2, and the release of CPT was confirmed by HPLC analysis (data not shown).

Next, we examined whether the trimeric prodrug system had an advantage over the monomeric system in a cell-growth inhibition assay. We evaluated the ability of the prodrugs to inhibit cell proliferation in the presence of catalytic antibody 38C2 using three different cell lines: the human T-lineage acute lymphoblastic leukemia (ALL) cell line MOLT-3, the human erythroleukemia cell line HEL, and the human acute myeloid leukemia (AML) cell line HL-60. The results are summarized in Table 1 and the data from the MOLT-3 cell

Table 1: IC_{50} values [nM] from cell-growth inhibition assays.

Drug/Prodrug	MOLT-3		HL-60		HEL	
	IC_{50}[a]	IC_{50}[b]	IC_{50}[a]	IC_{50}[b]	IC_{50}[a]	IC_{50}[b]
CPT	2.2	2.0	9.0	7.5	13	11
pro-mCPT	100	15	150	31	400	100
pro-tCTP	80	2.7	100	7.5	200	19

[a] Cells were incubated in medium with drug/prodrug. [b] Cells were incubated in medium with drug/prodrug and 1-μM catalytic antibody 38C2.

line are presented in Figure 4 b. The trimeric prodrug is more potent then the monomeric drug when incubated with the antibody. This behavior is as expected because triple the amount of CPT is released relative to the amount released from an equivalent concentration of the monomeric prodrug.

In the trimeric system, one cleavage by the antibody releases three times the amount of CPT as a cleavage in the monomeric prodrug system. We selected one cell line (MOLT-3 leukemia) for further studies with fixed concentrations of the prodrug and varying concentrations of antibody 38C2. To have equal amounts of CPT, the concentration of the monomeric prodrug was three times that of the trimeric prodrug (36 nM and 12 nM for the monomeric and trimeric prodrugs, respectively). The results are shown in Figure 5. The trimeric prodrug inhibited cell growth up to three times more effectively than the monomeric prodrug in the concentration range of 15–150 nM of the antibody. In other words, the

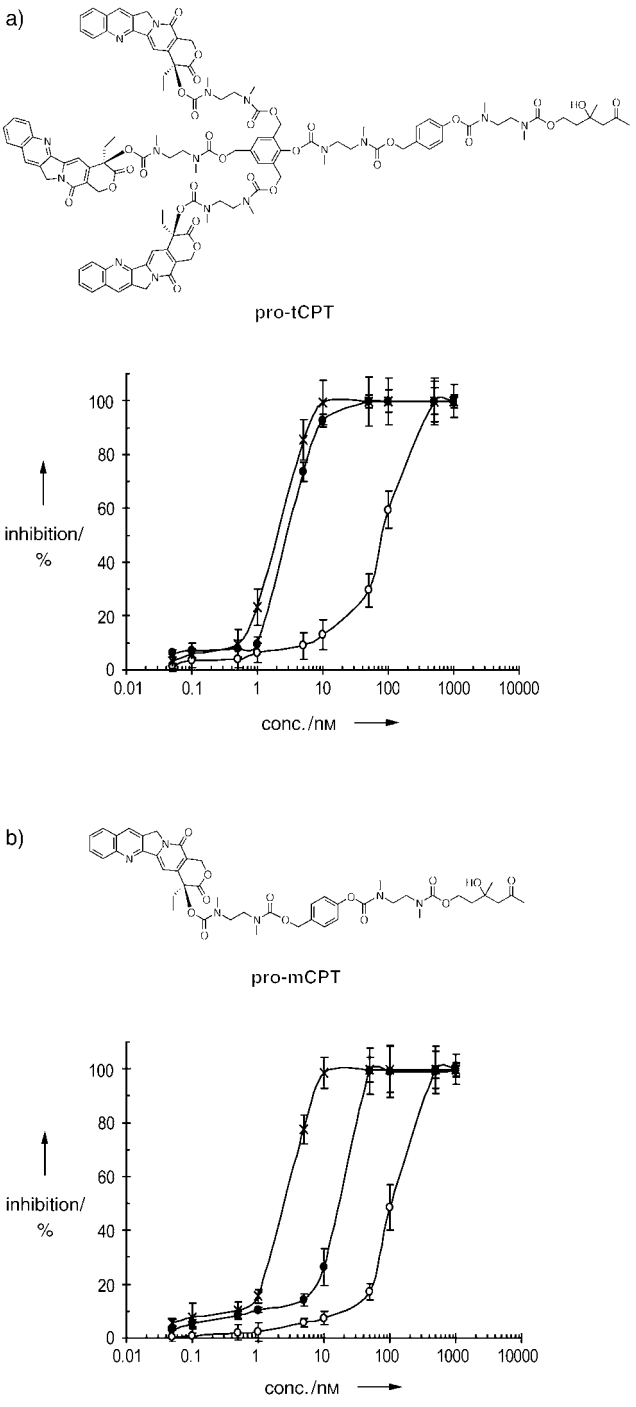

Figure 4. Molecular structures of the single-triggered CPT trimeric prodrug (pro-tCPT) and the classic CPT monomeric prodrug (pro-mCPT), which both have identical triggers, and the growth inhibition assays of human MOLT-3 leukemia cell line with the prodrugs in the presence and absence of catalytic antibody 38C2. a) pro-tCPT: ○ = pro-tCPT, ○ = pro-tCPT + 38C2, × = CPT; b) pro-mCPT: ○ = pro-mCPT, ● = pro-mCPT + 38C2, × = CPT.

concentration of antibody needed to inhibit the cell growth by 50% with pro-tCPT is about three times less than that needed with pro-mCPT. Note that the cytotoxicity of the platform degradation products in cell-growth inhibition was previously evaluated. The degradation products were found to have

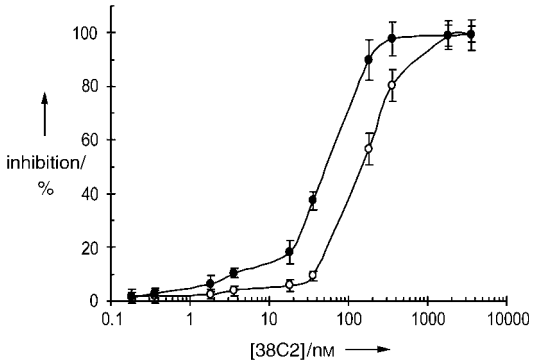

Figure 5. Growth inhibition assay of the human MOLT-3 leukemia cell line at a fixed concentration of the prodrug and upon variation of the concentration of the catalytic antibody 38C2. Cells were incubated for 72 h. ○ = 36-nM pro-mCPT, ● = 12-nM pro-tCPT.

negligible or no toxicity at all within the concentration range for the drug in the cell assay.[5]

It is also possible to incorporate three different drug molecules on the same prodrug platform. This would effectively allow triple-drug therapy in a single molecule. We prepared a heterotrimeric system with the anticancer drugs CPT, doxorubicin, and etoposide by using the retro-aldol, retro-Michael trigger activated by antibody 38C2 (Figure 6). Upon single-activation cleavage by the catalytic antibody, it was anticipated that this prodrug system should almost simultaneously release three different chemotherapeutic drugs at the same location. HPLC analysis confirmed the release of the drugs in the presence of antibody 38C2.

The heterotrimeric prodrug system was also evaluated in a cell-growth inhibition assay (Figure 7). The prodrug was incubated with MOLT-3 leukemia cells in the presence and in the absence of catalytic antibody 38C2. The inhibition of cell growth by the heterotrimeric prodrug was increased approximately 15-fold upon activation by antibody 38C2. We

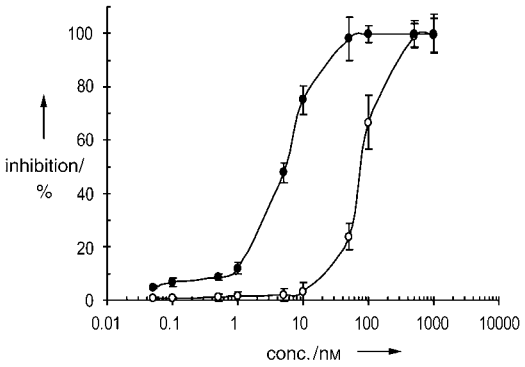

Figure 7. Growth inhibition assay of the human MOLT-3 leukemia cell line. Cells were incubated for 72 h. a) ● = heterotrimeric prodrug (pro-HT) + 1-μM catalytic antibody 38C2; b) ○ = pro-HT.

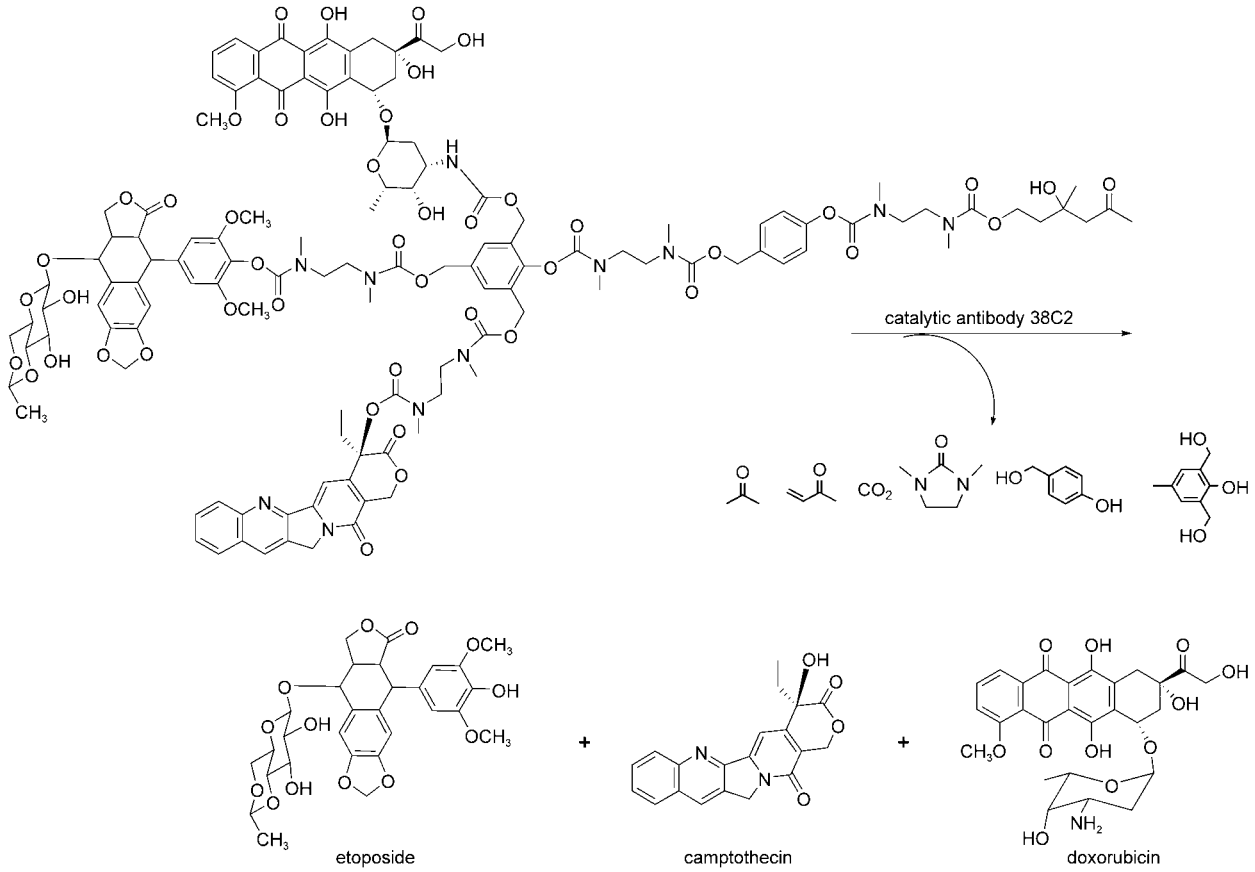

Figure 6. Single-triggered heterotrimeric prodrug system with the anticancer drugs CPT, doxorubicin, and etoposide, and a retro-aldol, retro-Michael substrate.

previously reported that a heterodimeric prodrug system was more effective in the inhibition of cell growth than two monomeric prodrugs.[7] Single-triggered heterotrimeric prodrugs may offer additional synergy in chemotherapy.

Dendritic prodrugs that are activated through a single catalytic reaction by a specific enzyme could offer significant advantages in the inhibition of tumor growth, especially if the targeted or secreted enzyme exists at relatively low levels in the malignant tissue. We have prepared the first example of a single-triggered trimeric prodrug system with the anticancer drug CPT and activated by catalytic antibody 38C2. We anticipate that single-triggered dendritic prodrugs will be exploited to further improve selective chemotherapeutic approaches in cancer therapy.

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Keywords: aldol reaction · catalytic antibodies · dendrimers · drug delivery · prodrugs

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