Aldolase Antibody Activation of Prodrugs of Potent Aldehyde-Containing Cytotoxics for Selective Chemotherapy

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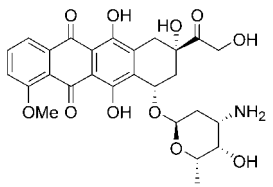
Abstract: Prodrugs of potent aldehyde analogues of the anticancer drug doxorubicin (Dox) were synthesized. These prodrugs were efficiently activated by antibody 93F3 and no drug formation was observed in the absence of 93F3 in either phosphate buffered saline or cell culture media. In the presence of antibody 93F3, these prodrugs were activated and decreased the proliferation of human cancer cells in in vitro proliferation assays.

Keywords: aldolase · antibodies · chemotherapy · doxorubicin · prodrugs

Introduction

To achieve selective chemotherapy with potent chemotherapeutics, new approaches, including antibody-directed enzyme prodrug therapy (ADEPT), are being developed.[1] Catalytic monoclonal antibodies (mAb)[2] were suggested as catalysts for prodrug activation almost a decade ago.[3] In principle, catalytic mAbs are superior to both systemically expressed endogenous human enzymes and externally expressed non-mammalian enzymes. For example, a catalytic mAb can have unique substrates that are not acted on by natural enzymes, thereby increasing the chemical space available for prodrug design. In addition, a humanized catalytic mAb should be less immunogenic than a non-mammalian enzyme. Hence, we have investigated the use of the aldolase mAb 38C2,[4,5] which can be humanized,[6] for the prodrug activation. As described in previous studies, mAb 38C2 effectively catalyzed the activation of the prodrugs of enediyne analogues,[7] camptothecin, etoposide, and doxorubicin (Dox, **1**).[8,9]



1: Doxorubicin (Dox)

As a key constituent of chemotherapeutic regimens, Dox has been used as the first treatment for a variety of cancers.[10] In vitro studies have revealed that it is toxic to most cancer cells in the low micromolar or sub-micromolar range. However, the use of Dox is limited by its systemic toxicity and the ability of cancer cells to develop resistance to it. To have better efficacy and to counter acquired resistance, a number of Dox derivatives have been synthesized that are two to three orders of magnitude more toxic than the parent molecule **1**. These potent derivatives include 2-pyrrolinodoxorubicin (or pyrrolino-Dox, **2**) and 1,3-tetrahydropyridinodoxorubicin (or tetrahydropyridino-Dox, **3**) (Scheme 1).[11] The toxicity of **2** and **3** is believed to originate through the Schiff base intermediates **I** and **II**. In an independent study,[12] it was shown that the products obtained from esterase hydrolysis of diacetoxy derivatives of **4** and **5**, presumably intermediates **I** and **II**, were toxic to the cells with several orders of magnitude higher potency than Dox itself. They

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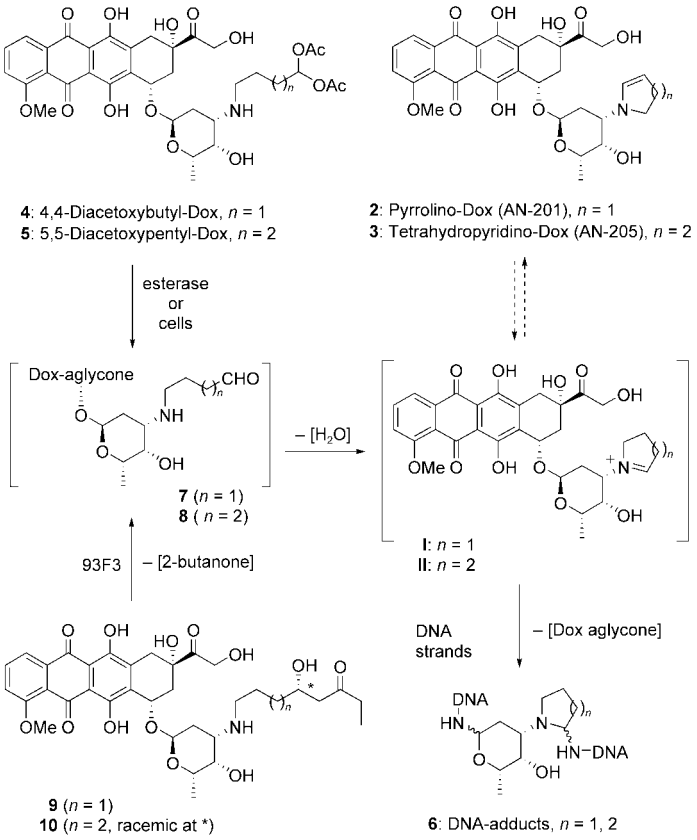


Scheme 1. Proposed mechanism for the observed potent cytotoxicity of Dox analogues, **2** and **3**, through DNA-adduct formation via intermediates **I** and **II** from **4** and **5** by an esterase-catalyzed reaction or the anticipated activation of the prodrugs **9** and **10**, by using an aldolase mAb 93F3, via aldehydes **7** and **8**.

were also toxic to the Dox-resistant cells. By using **5**, it was also shown that the product caused DNA–DNA crosslinks in HL-60 and HL-60/AMSA cell lines, which suggests that this mechanism contributes to its marked potency.[13] It is believed that the intermediates **I** and **II** undergo nucleophilic attack by a proximate base, such as the 2-amino group of a guanine residue leading to the corresponding DNA adducts (Scheme 1). The hydrolysis of the glycosides in the DNA adducts followed by subsequent reaction with an additional DNA molecule then produce free doxorubicinol aglycone and double-DNA adducts **6**. Therefore, one could imagine that a process, which can selectively generate the markedly potent intermediates, **I** or **II**, from the corresponding less toxic prodrugs will be highly useful for the ADEPT approach. In this communication, we describe the synthesis and in vitro evaluation of the novel prodrugs of aldehyde-based Dox-analogues that can be activated using catalytic aldolase mAb 93F3.[14]

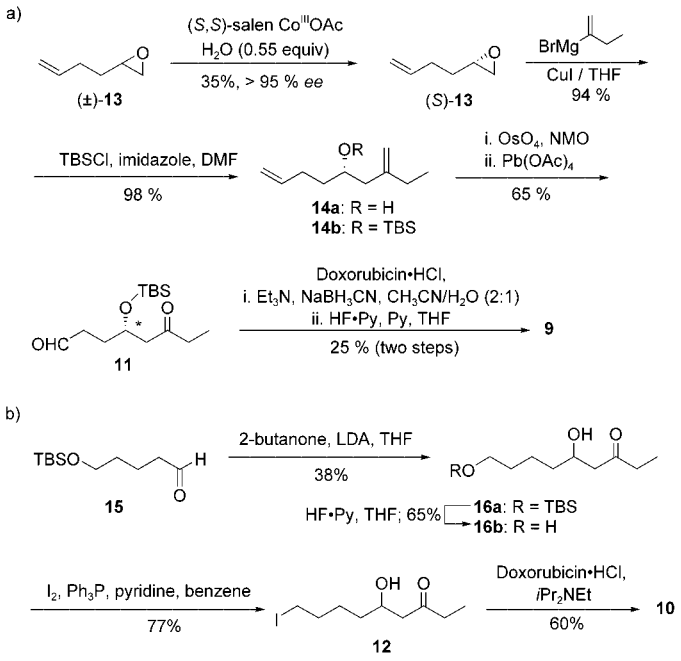
Results and Discussion

Design and synthesis of prodrugs: In the study described above,[12] intermediates **I** and **II** were obtained from the corresponding diacetoxy derivatives **4** and **5**, via the corresponding aldehydes **7** and **8**. We anticipated that aldehydes **7** and **8** could also be obtained by aldolase antibody-catalyzed retro-aldol reactions[15,16] of prodrugs **9** and **10**, respectively, the products of which will then undergo intramolecular cyclization to afford the corresponding carbinolamine derivatives and then dehydrate to produce iminium intermediates **I** and **II** (Scheme 1). Furthermore, unlike compounds **4** and **5**, prodrugs **9** and **10** are expected to be more stable under physiological conditions where esterase activity is abundant. In addition, since substitution of the primary amine functionality of doxorubicin decreases its toxicity in general,[9] prodrugs **9** and **10** are expected to be less toxic in comparison to the parent molecule **1**, as well as to 2-pyrrolino-Dox (**2**, AN-201) and 1,3-tetrahydropyridino-Dox (**3**, AN-205).[11]

Prodrugs **9** and **10** were designed as potential substrates for aldolase mAb 93F3. This aldolase antibody catalyzes retro-aldol reactions of a wide variety of substrates with a very high catalytic rate ($(k_{cat}/K_m)/k_{un} > 10^{13}\,M^{-1}$) and enantioselectivity. We noted that in 93F3-catalyzed reactions,[14,16] a β-substituted β-hydroxyethyl ethyl ketone was retro-aldolized faster than the corresponding β-substituted β-hydroxyethyl methyl ketone. Hence, both prodrugs **9** and **10** were designed to contain a β-hydroxyethyl ethyl ketone as the aldol-triggered linker. Furthermore, only the "*S*" enantiomer (considering the alkyl ketone as second largest group) of an aldol compound was consumed by mAb 93F3 leaving the "*R*" enantiomer intact. Therefore, in order to allow for the highest possible activation using aldolase mAb 93F3, prodrug **9** contained an enantiomerically pure linker with an *S* stereochemistry.

The prodrugs were synthesized starting from Dox (**1**) and (4*S*)-octan-4-(*tert*-butyldimethyl-silyloxy)-6-keto-1-aldehyde (**11**) for prodrug **9** or 1-iodo-7-keto-nonan-5-ol (**12**) for prodrug **10** as shown in Scheme 2. The linkers, **11** and **12**, were prepared starting from racemic 5-hexene-1,2-epoxide, (±)-**13**, and aldehyde **15**,[17] respectively. The enantiomerically pure epoxide (*S*)-**13** was obtained by kinetic resolution of the corresponding racemic epoxide (±)-**13**.[18] Compound (*S*)-**13** was then reacted with 1-buten-2-yl magnesium bromide in the presence of catalytic CuI to afford alcohol **14a** that was then converted to TBS ether **14b**. Both alkene functions were cleaved by dihydroxylation by using OsO_4 and NMO followed by treatment with $Pb(OAc)_4$ to afford the TBS-protected linker **11**. Aldehyde **11** was treated with **1** to give the corresponding imine. The latter was reduced in situ with $NaCNBH_3$; subsequently the TBS group in the product was deprotected by using a solution of HF·Py and pyridine in THF to afford the desired prodrug **9**. For the synthesis of linker **12**, aldehyde **15** underwent aldol reaction with 2-butanone using LDA as a base. The TBS group in **16a** was deprotected using HF·Py to give **16b** and the primary alcohol was then converted to iodide **12**. Finally, alkylation of the amine of Dox (**1**), by using iodide **12** afforded prodrug **10**.

Prodrug activation: Prodrug **9** (500 μM) was incubated with a catalytic amount of 93F3 (34 μM) in PBS buffer at 37 °C, and the activation of prodrug was analyzed by LCMS. Reactions in PBS buffer (pH 7.4) and 10% fetal calf serum in cell cul-



Scheme 2. Synthesis of prodrugs **9** and **10**.

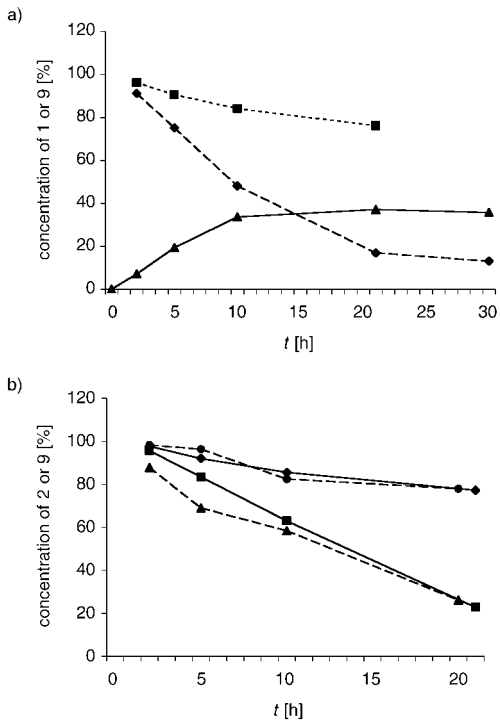


Figure 2. a) Activation of prodrug **9** and production of drug **2** using aldolase mAb 93F3. Shown are the consumption of **9** and production of **2** over time in the 93F3-catalyzed reaction (**9**/cat ◆ and **2**/cat ▲, respectively) and the consumption of **9** in the background reaction (**9**/BKG ■). In these experiments, compound **9** (500 μM) was incubated at 37 °C with 93F3 (34 μM) in PBS buffer or PBS buffer alone. The reaction mixtures were periodically analyzed by using LCMS equipped with column, UV detector (254 nm), and EI-MS. b) Comparison of the stability of prodrug **9** and Dox (**1**) in PBS buffer, and 10 % fetal calf serum in cell culture medium ("S" stands for serum). In these experiments, prodrug **9** (500 μM) or Dox (**1**) (500 μM) were incubated at 37 °C in PBS buffer (pH 7.4) alone (**9**: ◆, **1**: ●) or in 10 % fetal calf serum in cell culture medium (**9**: ■, **1**: ▲), and analyzed by using LCMS as described above.

ture medium (pH 7.4)[19] were used to assess the uncatalyzed reaction. The results are shown in Figures 1 and 2. It is evident from Figure 1 (data collected after 10 h of incubation at 37 °C) that the product, assigned as **2** based on molecular weight, is formed only in the 93F3-catalyzed reactions (A), but not in the background reactions either with the PBS (B) or with the cell culture medium (C). This clearly showed that the activation of the prodrug **9** was 93F3-dependent. The reaction with 93F3 produced about 35 % yield of drug **2** with an approximate rate of 0.009 min^{-1}, before reaching a plateau (see, Figure 2a).

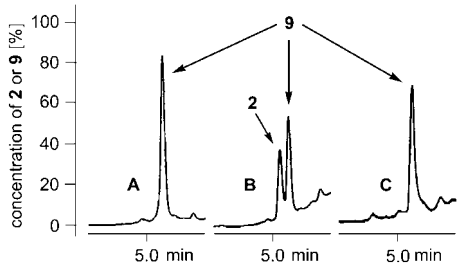


Figure 1. Comparison of the chromatograms (B) from antibody 93F3-catalyzed activation of prodrug **9** to produce drug **2**, and background reactions in A) PBS buffer (pH 7.4) and C) 10 % fetal calf serum in cell culture medium. In these experiments, compound **9** (500 μM) was incubated at 37 °C with 93F3 (34 μM) in given buffer for 10 h and the reaction mixtures were analyzed by using LCMS equipped with column, UV detector (254 nM), and EI-MS. The unconsumed prodrug **9** or the produced drug **2** is shown as percent on *y* axis. Retention times for **2** and **9** are 5.15 min and 5.30 min, respectively, as shown on the x axis of the chromatograms A)–C).

As shown in Figure 2a and b, the concentration of prodrug **9** also decreased in the absence of mAb 93F3, especially in the cell culture medium. Since the formation of **2** was not observed in the absence of mAb 93F3, it is evident that prodrug **9** degrades by a pathway similar to that of the parental drug Dox, **1**, which is known in the literature to degrade rapidly in plasma and blood.[20] In various cell culture media, the half-life of Dox has been estimated to be only 3 h.[21] We compared the stability of Dox and prodrug **9** in PBS and the cell culture media at 37 °C (Figure 2b). The decomposition of Dox and prodrug **9** are comparable, which suggests that the formation of intermediate **I** and **II** from the activation of prodrugs **9** and **10** will be achieved only by mAb 93F3, and thus they may not be formed in vivo by endogenous enzyme-mediated reactions.

In vitro evaluations: Biological activities of prodrugs **9** and **10** were evaluated, in vitro, by tumor cell proliferation assays using human Kaposi's sarcoma (SLK) and breast cancer (MDA-MB-435) cell lines. The experiments with prodrugs **9** and **10** both in the presence and absence of antibody 93F3 were carried out as reported earlier,[9] and the results are shown in Figures 3 and 4. As shown in Figure 3, proliferation of human breast cancer MDA-MB-435 and human Kaposi's sarcoma cells was inhibited by 50 % in the presence of prodrug **10** (2 μM) and mAb 93F3 (1 μM). Doxorubicin also

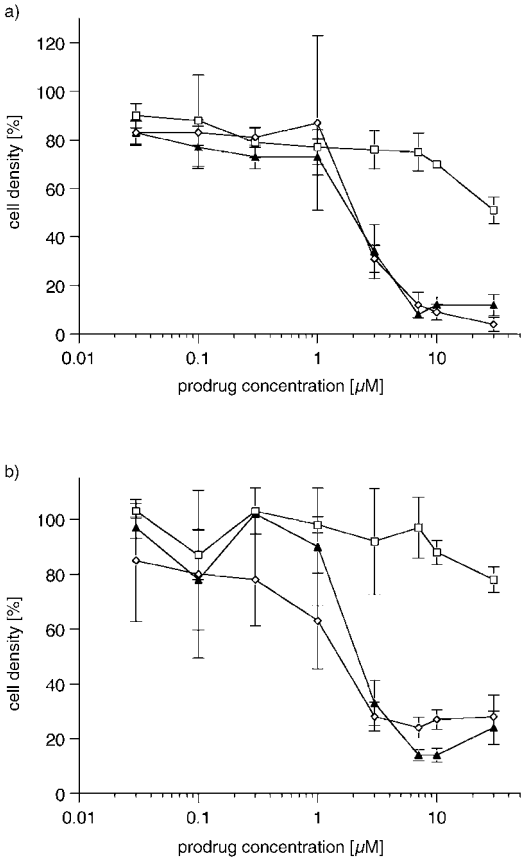


Figure 3. Growth inhibition of a) human breast cancer cell line (MDA-MB-435) and b) human Kaposi's sarcoma (SLK) cell line by prodrug **10** in the presence and absence of 1 μM mAb 93F3. The growth inhibition in the presence of Dox (**1**) is shown for comparison. **1**: ◇, **10**: □, **10**+93F3: ▲.

showed toxicity identical to the mixture of prodrug **10** (2 μM) and mAb 93F3 (1 μM). In contrast, 2 μM prodrug **10** in the absence of mAb 93F3 was nontoxic.

Next, we studied the effect of prodrug **9** in the presence and absence of mAb 93F3 using human Kaposi's sarcoma cells (SLK) and compared the results using prodrug **10**. The IC_{50} of prodrug **9** was found to be 0.06 μM (Figure 4) in the presence of mAb 93F3, whereas the IC_{50} of prodrug **10** was only 2 μM (Figure 3) under the same conditions. These results are consistent with the higher toxicity of **2** in comparison to **3**. It is noteworthy that drug **2** and prodrug **9** generate intermediate **I**, whereas drug **3** and prodrug **10** produce intermediate **II**. In both cases, the prodrugs were less toxic in the absence than in the presence of mAb 93F3. Prodrugs **9** and **10** alone were 20–30 times less toxic than in the presence of mAb 93F3, which is comparable to prodrug-to-drug toxicity ratios of other doxorubicin prodrugs.[9]

In conclusion, we have synthesized novel prodrugs, **9** and **10**, of two Dox analogues, pyrrolino-Dox and tetrahydropyridino-Dox and evaluated their toxicities in cell culture assays. These prodrugs, unlike many other prodrugs, contained short retro-aldol activated linker. Unlike other prodrugs designed to work together with aldolase antibodies,[7,9,22] coupling of other reactions together with the retro-aldol reaction is not required for drug activation. Moreover,

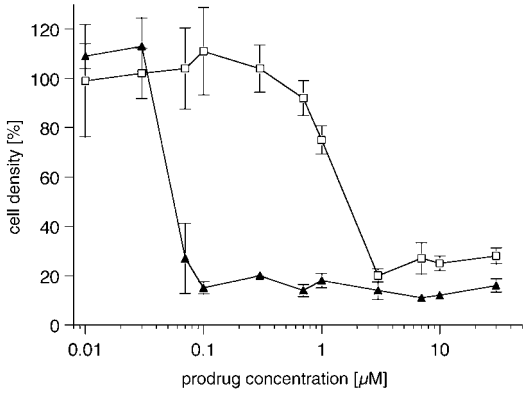


Figure 4. Growth inhibition of human Kaposi's sarcoma (SLK) cell line by prodrug **9** in the presence and absence of 1 μM mAb 93F3. **9**: □, **9**+93F3: ▲.

the linkers of prodrugs **9** and **10** were selectively activated using the proficient aldolase mAb 93F3. Prodrugs **9** and **10** effectively inhibited the proliferation of cancer cells upon activation with mAb 93F3 as observed by in vitro evaluation by using human breast cancer (MDA-MB-435) and Kaposi's sarcoma (SLK) cell lines. These characteristics suggest that prodrugs **9** and **10** are promising candidates for in vivo study.

Experimental Section

General methods: TLC was performed on glass sheets precoated with silica gel (Merck, Kieselgel 60, F254, Art. 5715). Column chromatographic separations were performed on silica gel (Merck, Kieselgel 60, 230–400 mesh, Art. No. 9385) under pressure. All commercially available reagents were used without further purification. Solvents were either used as purchased or distilled by using common practices where appropriate. All reactions were carried out under dry argon.

Compound (*S*)-13: A mixture of (±)-**13** (1.96 g, 20 mmol), (*S*,*S*)-salen $Co^{III}OAc$ catalyst (136 mg, 0.2 mmol), and H_2O (198 μL, 11 mmol) was stirred at room temperature for 24 h. The product was purified by distillation. ^1H NMR (500 MHz, $CDCl_3$): δ = 5.84 (m, 1H), 5.06 (m, 1H), 5.01 (m, 1H), 2.94 (m, 1H), 2.76 (dd, J=5.1, 4.1 Hz, 1H), 2.49 (dd, J=5.1, 2.9 Hz, 1H), 2.26–2.21 (m, 2H), 1.70–1.60 ppm (m, 2H).

Compound 14a: A solution of 1-buten-2-yl magnesium bromide (12.76 mL, 0.75 M in THF), prepared from 2-bromo-1-butene (2.02 g, 15 mmol) and Mg (0.4 g, 16.5 mmol) in dry THF (17 mL), was added to a stirred suspension of CuI (155 mg, 0.8 mmol) in anhydrous THF (5 mL) under argon at −10 °C. Then the yellow suspension was further cooled to −20 °C, a solution of epoxide **13** (313 mg, 3.19 mmol) in dry THF (5 mL) was added dropwise. The resulting mixture was stirred from −20 to 0 °C for 2 h, it was worked up by using aqueous solution of NH_4Cl and Et_2O, the organic layer was washed with NH_4OH and brine, dried over $MgSO_4$. After filtration, the solvents were removed under vacuum, and the residue was purified over silica gel by using EtOAc/hexanes to afford pure **14a** (460 mg, 94%). ^1H NMR (500 MHz, $CDCl_3$): δ = 5.89–5.81 (m, 1H), 5.05 (m, 1H), 4.97 (m, 1H), 4.90 (s, 1H), 4.83 (s, 1H), 3.76–3.71 (m, 1H), 2.28–1.98 (m, 6H), 1.60–1.55 (m, 2H), 1.05 ppm (t, J=7.7 Hz, 3H); $[\alpha]_D$ = −10.9 (c = 1.0, $CHCl_3$).

Compound 14b: TBSCl (590 mg, 3.9 mmol) was added to a solution of **14a** (460 mg, 3.0 mmol) and imidazole (408 mg, 6.0 mmol) in dry DMF (1 mL) at 0 C. After the mixture was stirred at this temperature for 12 h, it was worked up by using water and Et_2O. The combined organic layer was washed with brine, dried over $MgSO_4$, and filtered. The solvents were removed under vacuum, and the residue was purified over silica gel

by using EtOAc/hexane 1:50 to afford pure **14b** (790 mg, 98%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.87–5.74 (m, 1H), 5.03–4.91 (m, 2H), 4.76 (s, 1H), 4.72 (s, 1H), 3.80 (m, 1H), 2.27–1.99 (m, 6H), 1.63–1.42 (m, 2H), 1.03 (t, J=7.2 Hz, 3H), 0.90 (s, 9H), 0.06 (s, 3H), 0.04 ppm (s, 3H); MS: m/z: 269 $[M+Na]^+$; $[\alpha]_D$=−11.1 (c = 1.0, $CHCl_3$).

Compound 11: OsO_4 (0.2 M in toluene, 0.1 mL, 0.02 mmol) and NMO (50% w/w in H_2O, 0.23 mL, 1.1 mmol) were added to a solution of **14b** (100 mg, 0.37 mmol) in acetone/water (3:1, 4 mL) at room temperature. After the mixture was stirred for 12 h at this temperature, excess oxidants were destroyed by using 10% solution of $Na_2S_2O_3$ and then the resulting mixture was extracted with EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4. The insoluble materials were filtered out and the solvents were removed under vacuum. The residue was passed over a short bed of silica gel (EtOAc), and the product (tetrol) was taken to next step without further purification.

Lead tetraacetate (576 mg, 1.3 mmol) was added in portions to a solution of the above-described tetrol in CH_2Cl_2 (5 mL) at 0°C. After the mixture was stirred for 2 h, it was worked up using aqueous $Na_2S_2O_5$ and EtOAc. The combined organic layer was dried over anhydrous Na_2SO_4. The insoluble materials were filtered out and the solvents were removed under vacuum. The residue was purified over silica gel (hexane/EtOAc 4:1), to afford pure aldehyde **11** (60 mg, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 9.75 (s, 1H), 4.24 (dt, J=11.1, 6.0 Hz, 1H), 2.61 (dd, J=15.6, 6.3 Hz, 1H), 2.50 (dd, J=7.5, 1.8 Hz, 1H), 2.45 (m, 1H), 2.43 (dd, J=15.6, 5.7 Hz, 1H), 1.92–1.68 (m, 2H), 1.02 (t, J=7.5 Hz, 3H), 0.86 (s, 9H), 0.06 (s, 3H), 0.01 ppm (s, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 209.4, 201.7, 67.8, 49.5, 39.6, 37.6, 29.6, 25.9, 18.0, 7.6 ppm; MS: m/z: 241 $[M+H]^+$, 263 $[M+Na]^+$; $[\alpha]_D$=17.8 (c = 0.7, $CHCl_3$).

Prodrug 9: A solution of $NaBH_3CN$ (1 M in THF) (24 μL, 0.67 equiv) was added to a stirred solution of Dox-hydrochloride (20 mg, 0.035 mmol) and aldehyde **11** (27.7 mg, 0.102 mmol) in CH_3CN/H_2O (2:1, 5 mL). The mixture was stirred at room temperature in the dark for 2 h. The reaction mixture was diluted with water and extracted repeatedly (10×10 mL) with a mixture of $CHCl_3$/MeOH 5:1. The combined organic layer was dried over Na_2SO_4. After filtration, the solvents were removed under vacuum, and the residue was purified by using PTLC (CH_2Cl_2/MeOH 5:1) to afford the TBS ether protected **9** (5.8 mg, 22%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.01 (d, J=7.3 Hz, 1H), 7.78 (t, J=8.1 Hz, 1H), 7.39 (d, J=8.1 Hz, 1H), 5.54 (brs, 1H), 5.30 (brs, 1H), 4.77 (s, 2H), 4.13 (m, 1H), 4.08 (s, 3H), 4.01 (q, J=6.7 Hz, 1H), 3.79 (brs, 1H), 3.49 (s, 1H), 3.26 (d, J=18.7 Hz, 1H), 3.00 (d, J=18.7 Hz, 1H), 2.99 (m, 1H), 2.70 (m, 2H), 2.56 (dd, J=15.4, 7.0 Hz, 1H), 2.44–2.36 (m, 4H), 2.16 (m, 1H), 1.95 (m, 1H), 1.77 (m, 1H), 1.59 (m, 2H), 1.46 (m, 2H), 1.35 (d, J=6.6 Hz, 3H), 0.98 (t, J=7.3 Hz, 3H), 0.78 (s, 9H), −0.01 (s, 3H), −0.04 ppm (s, 3H); MS: m/z: 800 $[M+H]^+$, 798 $[M-H]^-$, 834 $[M+Cl]^-$.

A solution of HF·Py (0.01 mL) in THF/pyridine (4:1, 1 mL) was added to a solution of the product described above in THF (0.5 mL) at 0°C. The mixture was stirred at this temperature for 12 h, and then neutralized using $NaHCO_3$ solution and extracted 5× with CH_2Cl_2/MeOH 5:1. Solvents were removed under vacuum and the residue was purified by using PTLC (CH_2Cl_2/MeOH 5:1) to afford pure **9** (1.4 mg, 52%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.04 (dx, J=7.7 Hz d,), 7.81 (t, J=7.7 Hz, 1H), 7.41 (d, J=8.5 Hz, 1H), 5.56 (d, 1H), 5.28 (m, 1H), 4.76 (s, 2H), 4.10 (s, 3H), 4.04 (q, J=6.6 Hz, 1H), 3.87 (s, 1H), 3.46 (s, 1H), 3.43 (m, 1H), 3.28 (dd, J=19.0 Hz, 1H), 3.10 (m, 1H), 3.04 (d, J=19.0 Hz, 1H), 2.84 (m, 1H), 2.76 (m, 1H), 2.68 (dd, J=17.6, 9.2 Hz, 1H), 2.51 (dd, J=17.2, 3.3 Hz, 1H), 2.46 (m, 2H), 2.36 (dt, J=14.7 Hz, 1H), 2.17 (dd, 1H), 1.95–1.60 (m, 4H), 1.55 (m, 2H), 1.30 (d, J=7.4 Hz, 3H), 1.04 ppm (t, J=7.4 Hz, 3H); MS: m/z: 686 $[M+H]^+$, 708 $[M+Na]^+$, 684 $[M-H]^-$, 720 $[M+Cl]^-$.

Compound 16a: Butanone (4.5 mL, 50.8 mmol) in THF (5 mL) was dropwise added to a solution of LDA (1.9 M in THF, 26.7 mL, 50.8 mmol) in dry THF (30 mL) at −100°C. After the mixture was stirred for 15 min, aldehyde **15** (10.0 g, 46.2 mmol) in THF (2 mL) was added and stirred for 3 h at −78°C. The mixture was quenched using aqueous solution of NH_4Cl and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified over silica gel (hexane/EtOAc 5:1) to afford **16a** (5.06 g, 38%). ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.12 (m, 1H), 3.69 (t, J=6.3 Hz, 2H), 3.15 (d, J=2.6 Hz, 1H), 2.68 (dd, J=2.6, 17.6 Hz, 1H), 2.58 (dd, J=9.2, 17.6 Hz, 1H), 2.53 (q, J=7.3 Hz, 2H), 1.65–1.42 (m, 6H), 1.14 (t, J=7.3 Hz, 3H), 0.97 (s, 9H), 0.12 ppm (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 212.2, 67.4, 62.8, 48.5, 36.5, 36.1, 32.4, 25.7, 21.6, 7.3, −5.5 ppm; MALDI-FTMS: m/z: calcd for $C_{15}H_{32}O_3SiNa$: 311.2013; found 311.2003 $[M+Na]^+$.

Compound 16b: HF·Py (2 mL) was added to a solution of **16a** (770 mg, 2.67 mmol) in THF (10 mL) at −20°C. The mixture was stirred at this temperature for 2 h, and then neutralized using $NaHCO_3$ solution and extracted with EtOAc. The combined organic layer was washed with brine, dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The residue was purified over silica gel (hexane/EtOAc 3:1) to afford **16b** (300 mg, 65%). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 4.05 (m, 1H), 3.65 (t, J=6.3 Hz, 2H), 3.2 (br, 1H), 2.61 (dd, J=2.9, 17.6 Hz, 1H), 2.51 (dd, J=9.1, 17.6 Hz, 1H), 2.45 (q, J=7.3 Hz, 2H), 1.80–1.37 (m, 7H), 1.06 ppm (t, J=7.3 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ = 212.4, 67.3, 61.9, 48.8, 36.6, 36.0, 32.0, 21.4, 7.3 ppm.

Compound 12: Iodine (349 mg, 1.37 mmol) was added in portion to a solution of **16b** (171 mg, 0.98 mmol), PPh_3 (386 mg, 1.47 mmol) and pyridine (0.24 mL, 2.94 mmol) in benzene (20 mL) at room temperature. The mixture was stirred under reflux for 30 min. After cooling to room temperature, the mixture was filtered by Celite and washed with EtOAc. The filtrate was concentrated and the residue was purified over silica gel (hexane/EtOAc 20:1) to afford iodide **12** (215 mg, 77%); ^{1}H NMR (500 MHz, $CDCl_3$): δ = 4.02 (m, 1H), 3.18 (t, J=7.0 Hz, 2H), 3.15 (br, 1H), 2.60 (dd, J=2.6 Hz, 17.6 Hz, 1H), 2.50 (dd, J=9.2 Hz, 17.6 Hz, 1H), 2.45 (q, J=7.3 Hz, 2H), 1.88–1.77 (m, 2H), 1.60–1.35 (m, 4H), 1.05 ppm (t, J=7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 212.7, 67.2, 48.4, 36.7, 35.1, 33.2, 26.4, 7.5, 6.8 ppm.

Prodrug 10: Iodide **12** (147 mg, 0.517 mmol) was added to a solution of Dox-hydrochloride (10.0 mg, 0.0172 mmol) in DMF (0.2 mL) followed by iPr$_2$NEt (6.0 mL, 0.034 mmol), and the mixture was stirred overnight at room temperature for 16 h. The mixture was purified over silica gel (CH_3Cl/MeOH 10:1) to afford **10** (7.2 mg, 60%). ^{1}H NMR (600 MHz, CD_3OD): δ = 7.94 (d, J=7.8 Hz, 1H), 7.83 (t, J=7.8 Hz, 1H), 7.57 (d, J=7.8 Hz, 1H), 5.47 (s, 1H), 5.10 (s, 1H), 4.74 (d, J=18 Hz, 1H), 4.70 (d, J=18 Hz, 1H), 4.60 (brs, 1H), 4.27 (q, J=6.6 Hz, 1H), 4.04 (s, 3H), 3.96 (m, 1H), 3.80 (s, 1H), 4.49 (m, 1H), 3.10 (d, J=19 Hz, 1H), 2.97 (d, J=19 Hz, 1H), 2.96–2.70 (m, 3H), 2.56–2.40 (m, 4H), 2.35 (d, J=15 Hz, 1H), 2.20–1.95 (m, 3H), 1,70–1.30 (m, 6H), 1.30 (d, J=6 Hz, 3H), 0.97 ppm (t, J=7.3 Hz, 3H); MALDI-FTMS: m/z: calcd for $C_{36}H_{45}NO_{13}Na$: 722.2783; found 722.2810 $[M+Na]^+$.

In vitro cell growth assay: Briefly, human breast cancer cells (MDA-MB-435) and Kaposi's sarcoma cells (SLK) were plated at a density of 5×10^3 cells per well in 96-well tissue culture plates and maintained in culture. After 24 h, the media was gently removed from the 96-well plates and all wells were washed 2× with media, without disturbing the cells. Prodrugs were added immediately after washing. For the antibody experiments, prodrug and 93F3 IgG were mixed just before adding the activated prodrug solution to the cells. The final concentration of antibody in all solutions was 1 μM. Each concentration of prodrug added in triplicate. After prodrug addition, the cells were maintained at 37°C in 5% CO_2 for 1 h. After incubation, 20 μL of the Promega Substrate MTS (2H-tetrazolium, 5-[3-carboxymethoxy)phenyl]-3-(4,5-dimethyl-2-thiazolyl)-2-(4-sulophenyl)-inner salt (9Cl)) were directly added to every well of the plate, which already contained 100 μL of the prodrug (+antibody) sample. The MTS substrate is quickly converted to a red formazan product in the presence of lactate dehydrogenase, which is released from living cells. After 1 and 2 h, the absorbance at 490 nm was recorded in an ELISA plate reader to quantify the number of surviving cells. Six wells without cells had been kept blank throughout the entire experiment except for the addition of the MTS substrate, and the A_{490} values from these wells was averaged and subtracted from every other well. Three wells containing untreated cells and three wells containing cells with only antibody added were averaged, and the A_{490} values were set as 100% cell survival for comparison with addition of prodrug and addition of prodrug+antibody, respectively. The standard deviation for each triplicate experiment was also calculated after correction of background and is reported in Figures 3 and 4.

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