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A daunorubicin β-galactoside prodrug for use in conjunction with gene-directed enzyme prodrug therapy

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Abstract

N-[4-(β-D-Galactopyranosyl)-3-nitrobenzyloxycarbonyl]daunomycin was synthesized as a neutral prodrug of daunomycin. Although stable in 0.05 M phosphate buffer, pH 7.4 at 37°C, it was rapidly converted to daunomycin and 3-nitro-4-hydroxybenzyl alcohol under the same conditions in the presence of *E. coli* β-galactosidase.

Keywords: anthracycline; daunomycin; prodrug; β-galactosidase; gene therapy.

Anthracycline antibiotics, particularly doxorubicin and daunorubicin, have been used extensively in the treatment of human malignancies.[1] However, cardiotoxicity and multidrug resistance are significant problems that limit the clinical efficacy of such agents.[2,3] In an effort to design less toxic and more effective antitumor drugs, we have reported various agents[4–11] that can be used in conjunction with antibody-directed enzyme prodrug therapy[12] (ADEPT) and gene-directed enzyme prodrug therapy (GDEPT).[13] In the latter approach, a transduced gene is used to encode an enzyme that can convert a nontoxic prodrug into a cytotoxic product. The two most commonly used prodrug/activating enzyme combinations are: (a) ganciclovir (GCV)/herpes simplex virus thymidine kinase (HSV tk), and (b) 5-fluorocytosine/cytosine deaminase (CD). A limitation of HSV tk and CD as drug-activating enzymes is that they are substrate selective and can only activate prodrugs that closely structurally resemble the natural substrates. The vast majority of clinically useful antitumor agents (e.g. mustards, anthracyclines, antifols, etc.) are not candidates for 'prodrug activation' by these enzymes. In an effort to use such agents in conjunction with ADEPT and GDEPT, we have investigated β-galactosidase[4–7] and β-glucuronidase[8–11] as potential prodrug-activating enzymes. The former is particularly appealing because it can be readily expressed in mammalian cells transduced with the *E. coli* LacZ gene, and has been widely used as a marker enzyme in gene therapy studies.[14] In this communication, we report a

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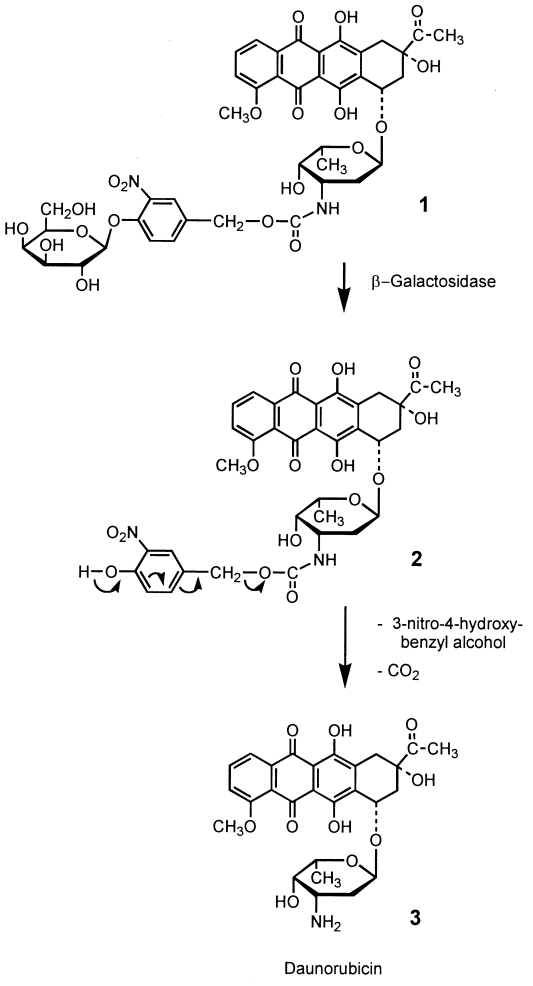
daunomycin prodrug **1** (Scheme 1) that is activated by β-galactosidase. The approach is based on the use of certain *para*-substituted benzyloxycarbonyl groups as bioreversible amine protective groups.[15]

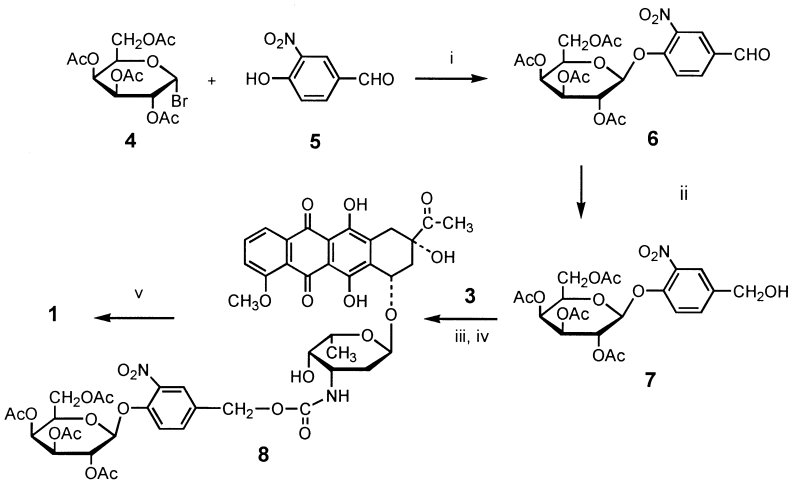


Scheme 1.

A similar strategy has been reported with other drug-activating enzymes including β-galactosidase[16] and β-glucuronidase.[17] Conference reports of this work have appeared.[4,5]

Prodrug **1** was prepared as shown in Scheme 2.

Experimental: A solution of 4-hydroxy-3-nitrobenzaldehyde, **5** (1.26 g, 7.55 mmol) in CH_3CN (15 ml) was added, with stirring, to a solution of tetra-*O*-acetyl-α-D-galactopyranosyl bromide, **4**[18] (3.1 g, 7.55 mmol) in CH_3CN (50 ml) containing freshly-prepared, dry Ag_2O (8.0 g, 32.33 mmol). After 4 h at room temperature, the mixture was filtered to remove Ag salts, and the solvent was evaporated. The crude product was purified by column chromatography on silica (200–400 mesh, Merck Inc.) using CH_2Cl_2:MeOH (95:5) as mobile phase to give the β-anomer **6** as a white solid (2.6 g, 69%), mp 191–192°C. A solution of **6** (2.49 g, 5.0 mmol) in a mixture of dry $CHCl_3$ (30 ml) and dry $(CH_3)_2CHOH$ (7 ml) was stirred with $NaBH_4$ (0.4 g, 10.4 mmol) for 2 h



Scheme 2. Reagents: (i) Ag_2O–CH_3CN; (ii) $NaBH_4$, $CHCl_3$–*i*PrOH; (iii) DSC–Et_3N, CH_3CN; (iv) DMF–Et_3N; (v) MeOH–MeONa

at 0–5°C. After work up, the alcohol **7** was obtained as a white solid (1.8 g, 73%), mp 195–196°C. A solution of **7** (0.25 g, 0.5 mmol) in anhydrous CH_3CN (5 ml) was treated with a solution of disuccinimidyl carbonate (0.25 g, 1.0 mmol) in the same solvent (5 ml) and the mixture was stirred for 90 min. The solution was evaporated to dryness and the residue was taken up in DMF (1 ml), and a solution of daunorubicin hydrochloride, **3** (0.15 g, 0.3 mmol) in DMF (2 ml) was added followed by one drop of Et_3N. After 25 min at room temperature, the DMF was evaporated and the residue was chromatographed on silica (200–400 mesh, Merck Inc.) using $CHCl_3$:MeOH (95:5) as mobile phase to give **8** (0.2 g, 38% with respect to **7**). Compound **8** (0.03 g, 0.028 mmol) was deacylated with MeONa (2 mg) in MeOH (2.5 ml) at 0–5°C for 2.5 h to give **1**[19] as a red solid (0.012 g, 47%).

*Stability and Cytotoxicity Studies of **1***. Compound **1** was completely stable when incubated at a concentration of 10^{-6} M in 0.05 M phosphate buffer, pH 7.4, at 37°C for up to six days. Under similar conditions in the presence of 2 units of *E. coli* β-galactosidase per μmole of prodrug, **1** was degraded with a half-life of 8.4 min (Fig. 1). HPLC analysis[20] of the reaction mixture showed progressive disappearance of the peak attributable to **1** (retention time, 5.1 min), and the

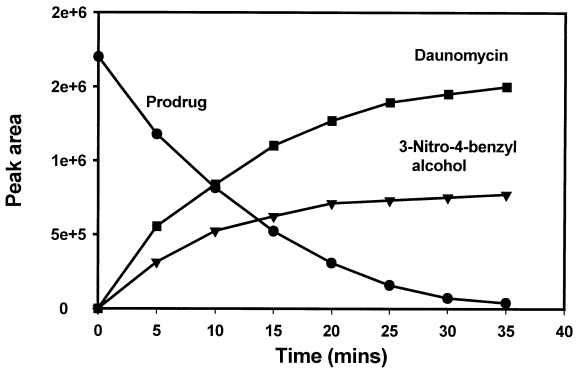


Figure 1. Degradation of daunomycin prodrug, **1** (10^{-6} M) in 0.05 M phosphate buffer, pH 7.4, in the presence of *E. coli* β-galactosidase (2 units per μmole of **1**)

appearance of two new, progressively increasing, peaks with retention times identical to those of authentic samples of 3-nitro-4-hydroxybenzyl alcohol and daunorubicin, (2.5 min and 3.1 min, respectively.)

Evidence that the reaction was enzyme-mediated was obtained from the observation that the degradation half-lives of **1** increased to 25 min and 41 min, respectively, when the concentration of β-galactosidase in the incubation mixture was reduced by 50 and 75%. When incubated in culture with LacZ-transduced (i.e. β-galactosidase-expressing) human prostate, breast, or leukemic cancer cells, **1** showed a 100 to 300-fold enhancement in cytotoxicity compared to mock-transduced control cells. These findings demonstrate that **1** is a good substrate for β-galactosidase and suggests that the compound may be therapeutically useful in an ADEPT or GDEPT setting. Antitumor evaluation studies of **1** are in progress.

Acknowledgements

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19. High resolution FAB-MS *m/e* 907.2380 $(M+Na)^+$.
20. HPLC conditions: A C-18 reverse-phase column (Phenomenex, 150 mm×3.90 mm) was used. The mobile phase was 0.05 M NH_4OAc buffer (pH 4.7)–CH_3CN (3:2) at a flow rate of 1.0 ml per min. Prodrug **1** and the enzyme reaction products were monitored by a variable wavelength UV detector set at 254 nm with 0.01 AUFS sensitivity (Waters 484 model) and quantitated electronically as a function of time using a NEC Pinwriter P6200 integrator. The half-lives were determined by non-linear regression analysis. β-Galactosidase was used as received from Sigma Company (catalog number G-2513).