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A NEW SPACER GROUP DERIVED FROM ARYLMALONALDEHYDES FOR GLUCURONYLATED PRODRUGS

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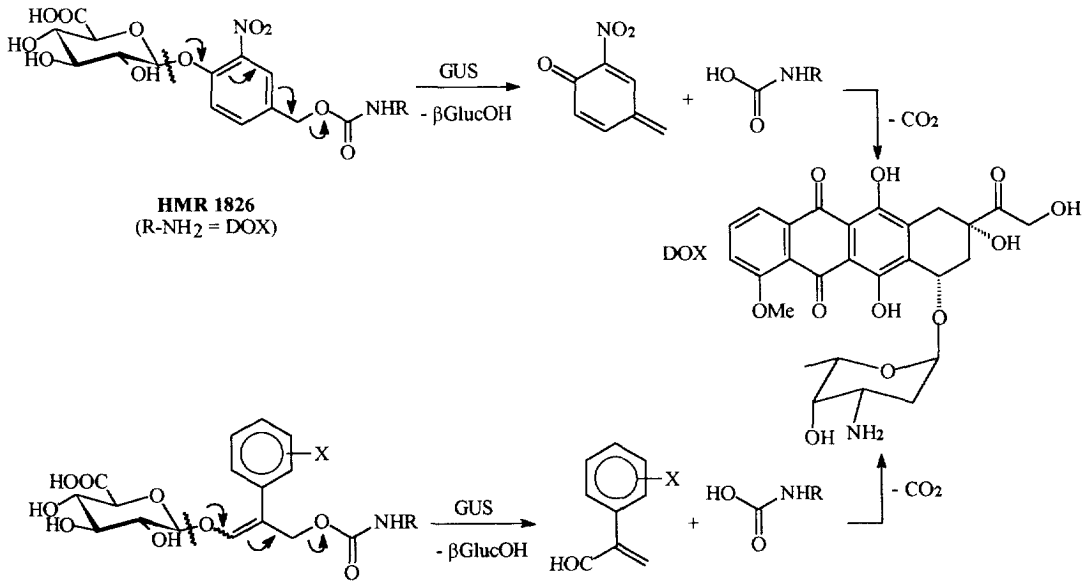
Abstract: A new glucuronylated prodrug of doxorubicin, potentially useful for ADEPT or PMT cancer chemotherapy, has been prepared from 4-methyl phenyl malonaldehyde. The enol ether spacer, linked via a carbamate to the 3'-amino group of doxorubicin is rapidly cleaved after β-glucuronidase (*E coli*) catalyzed hydrolysis at pH 7.2 and 37°C.

Cancer chemotherapy may be improved by targeting potent cytotoxic agents toward cancer cells. In this respect, HMR 1826,[1] a non-toxic prodrug of doxorubicin (DOX) activated by an antibody-enzyme conjuguate (ADEPT strategy)[2] or by β-glucuronidase (GUS) alone (PMT Strategy)[3] has demonstrated superior efficacy in mice and monkeys.[4] The design of such a prodrug relies on the introduction of a spacer group between the active compound (DOX) and the glucuronide moiety.[5,6] This spacer should allow easy recognition by GUS of



Scheme 1

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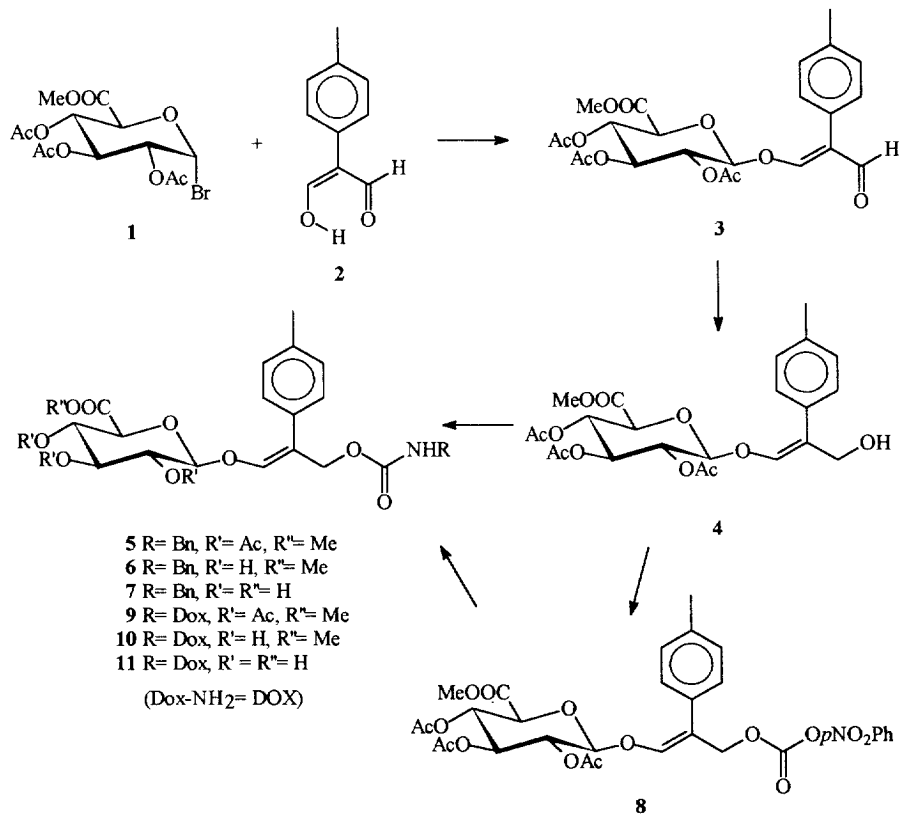
the carbohydrate moiety and, after enzymatic cleavage of the glycosidic bond, should rapidly liberate the free drug under physiological conditions by self-immolative decomposition. The HMR 1826 prodrug is based on the presence of a 4-hydroxymethyl-2-nitro phenol which is connected to the 3'-amino group of DOX by a carbamate. As a consequence of this derivatization, as well as increased hydrophilicity, HMR 1826 is more than 130 times less toxic in animals compared to DOX.[4]

A new spacer group is now proposed: key features are a conjugated enol ether and a carbamate group (Scheme 1). It is anticipated that such a structure will allow fast enzymatic hydrolysis of the glucuronide and that the intermediate enol will rapidly eliminate a carbamic acid (or eventually other leaving groups) leading then to a free amine after decarboxylation. This new spacer could also be modified by the introduction of different substituents in the aryl moiety and thus a modulation of prodrug pharmacokinetics should be possible from readily available aryl malonaldehydes.[7]

This strategy has been first implemented with 4-methylphenyl malonaldehyde **2** (which exists mainly as the *Z* enol tautomer)[7] which was converted to glycoside **3**[8] (54 %) with 1 eq of bromide **1** in presence of Ag_2O (1.5 eq) in CH_3CN (rt, 6 h). Aldehyde **3** was then reduced to **4** (44 %, $NaBH_4$, MeOH/THF 1/1). At this stage, the β configuration of the anomeric center was confirmed by the presence of a doublet (*J* 7.4 Hz, δ 4.83 ppm)



5 R= Bn, R'= Ac, R"= Me
6 R= Bn, R'= H, R"= Me
7 R= Bn, R'= R"= H
9 R= Dox, R'= Ac, R"= Me
10 R= Dox, R'= H, R"= Me
11 R= Dox, R' = R"= H

(Dox-NH_2= DOX)

Scheme 2

in ^{1}H NMR. Furthermore NOEDIFF experiments allow the determination of the double bond configuration as *E* based on a 10% nOe between the vinylic proton and the allylic methylene protons. This result is in agreement with previous observations by Lubineau[9] and Stoodley[10] for related glycosidations of 1,3-enols with glucose derivatives.

Then, in order to test the deprotection steps and stability of the targeted prodrugs, alcohol **4** was treated with benzyl isocyanate, in the presence of CuCl, to give **5** (54 %, 88 % based on starting material consumption). Deprotection with cat. MeONa in MeOH afforded **6** (62%) which was converted to **7** (94 %) using $Ba(OH)_2.8\ H_2O$ in MeOH.[1] No decomposition of **6** was detected after 20 h in 0.02 M phosphate buffer at pH 7.2 and 37°C.

The preparation of the DOX prodrug **11** was then carried out from **4**. Indeed, activation of the latter with 4-nitrophenyl chloroformate in pyridine afforded the mixed carbonate in only 32 % yield. This material proved to be rather unstable under mild conditions and thus one-pot activation (1 eq *p*NO_2PhOCOCl, pyr) and coupling with DOX (0.9 eq, 2.5 eq Et_3N) was carried out to give **9**, mp 149°C, $[\alpha]_D$ + 99 (c 0.03, $CHCl_3$) in 12 % yield (60 % based on recovered DOX). Transesterification, as above, gave methyl ester **10** as a red solid, mp 148°C (35 %). However, hydrolysis of **10** with $Ba(OH)_2$ resulted in the formation of an undetermined side product along with **11**. The latter could not be separated by chromatography.

Enzymatic hydrolysis of methyl ester and glucuronate was then carried out in one-pot from **10** (0.05 mg/mL) with PLE (*E Coli*, Sigma ref E3019, 6 U/mL) in 0.02 M phosphate buffer at pH 7.2 and 37°C.[11] Conversion of **10** to **11** was observed by HPLC[12] ($t_{½}$ 60 min) together with formation of *c.a.* 10 % of DOX resulting from direct carbamate hydrolysis (Figure 1). After complete disappearance of **10**, addition of GUS (*E Coli*, Sigma ref G 7896, 40 U/mL) resulted in a clean conversion to DOX ($t_{½}$ 60 min).

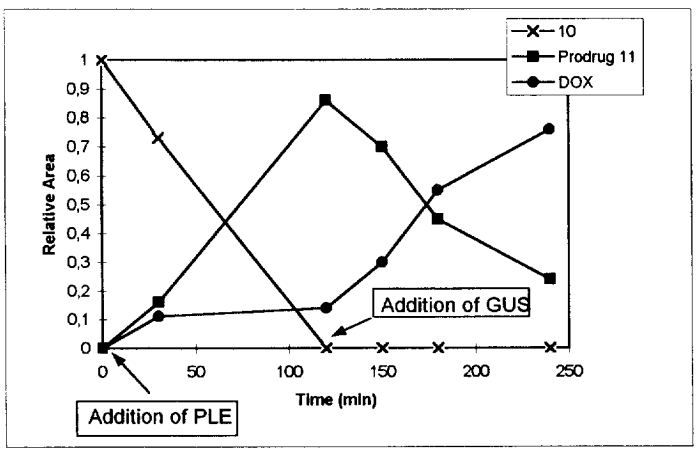


Figure 1

In conclusion, a new prodrug of DOX has been prepared in few steps from 4-methyl phenyl malonaldehyde. However, the instability of the intermediate activating group (carbonate) results in a low yield in the coupling reaction with DOX compared to direct synthesis of the carbamate model compound by reaction with benzyl isocyanate. Nevertheless, the clean conversion of **11** to DOX shows that this new spacer group may be of interest and further experiments are underway to improve the preparation of such prodrugs.

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