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SYNTHESIS OF NOVEL TARGETED PRO-PRODRUGS OF ANTHRACYCLINES POTENTIALLY ACTIVATED BY A MONOCLONAL ANTIBODY GALACTOSIDASE CONJUGATE (PART 1)

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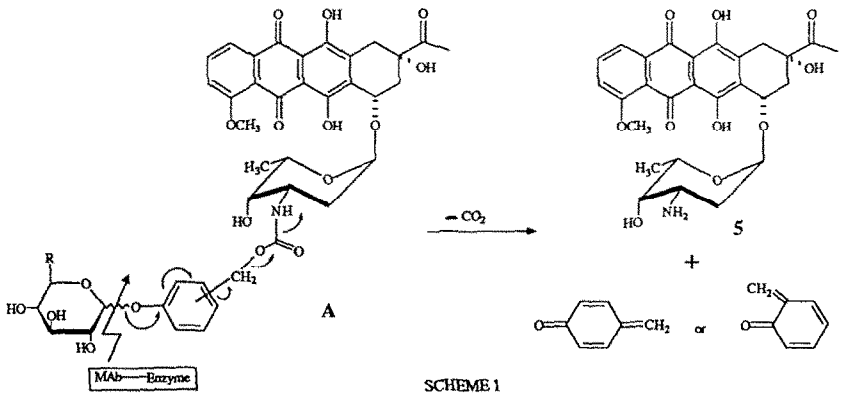
Abstract: *Daunorubicin substituted at N-3' with a benzyloxycarbonyl group (self-immolative spacer) linked to an α-D-galactosyl residue such as 7a and 7b have been prepared as prodrugs. Conversion of 7a and 7b to daunorubicin will be mediated by an immunoconjugate consisting of an α-D-galactosidase enzyme covalently attached to a tumor specific monoclonal antibody.*

Anthracycline antibiotics are widely used in cancer chemotherapy[1] but their clinical efficacy is limited by a severe dose-cumulative cardiotoxicity[2] and by apparition of an acquired resistance[3]. Rational design attempts to avoid these side-effects can be represented either by drug targeting or prodrug synthesis. Combination of both should be the best. In this regard, the combination of prodrug and tumor specific antibody-enzyme conjugates for use as therapeutic agents has been recently reported in the literature[4]. This entails monoclonal antibodies which are directed against a particular tumor and are covalently bonded to a prodrug-cleaving enzyme. Therefore the antibody-enzyme conjugate localizes on the tumor cell surface antigen and the non cytotoxic prodrug is converted into the active cytotoxic species at the tumor cell surface. In order to avoid non-specific liberation of active compound but also the disavantage of an immune response, glycosidases, preferably mammalian such as human α-D-galactosidase[5] or human β-D-glucuronidase[6] which are non immunogenic or of low immunogenicity have been choosen for our studies.

Preliminary experiments having shown that enzymatic hydrolysis was uneffective when galactose or glucuronic acid were directly bonded to the 14-OH of anthracycline through a classical glycosidic linkage, the linkage of these sugars to the primary amino group of daunosamine, the carbohydrate constituent of

daunorubicin and doxorubicin, was considered. However, N-acylation or N-carbamylation of amines to give amide or carbamate prodrugs has only been used to a limited extent due to the relative stability of these derivatives *in vivo*[7]. In contrast o. and p. substituted (OH or NH_2) benzylcarbamate[8] derivatives or (acyloxy)alkyl carbamates[9] can be chemically or enzymatically activated to release the free amino function with concomitant formation of quinone methide and CO_2 or through the intermediate formation of an unstable carbamic acid, respectively.

Based upon these observations, our initial purpose was to prepare potentially enzymatically cleavable prodrugs of daunorubicin **5**, of general formula A with a sugar residue (galactose or glucuronic acid) bonded to daunorubicin through a self-immolative spacer. In this letter we report the initial work which has been done with galactose as sugar residue.



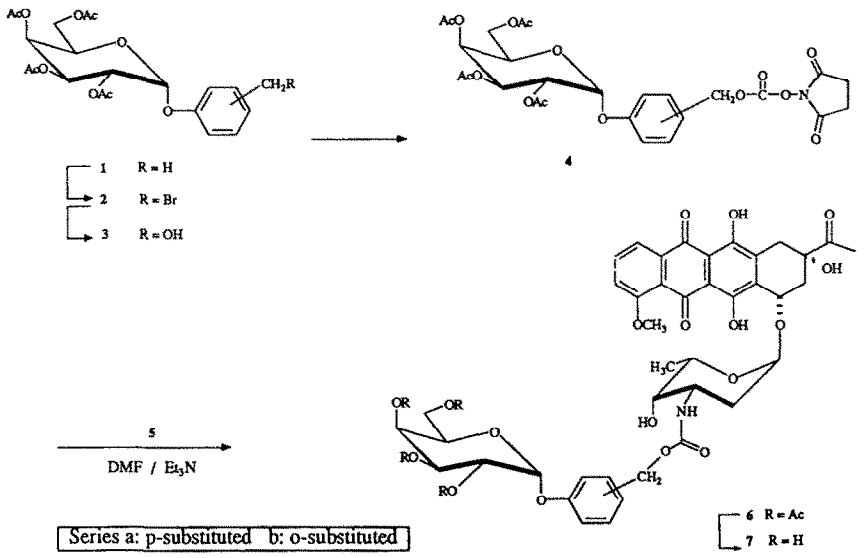
SCHEME 1

The general route used to prepare such compounds involved initial glycosidation of o or p-cresol with peracetyl-D-galactose , benzylic hydroxylation of the resulting o or p-hydroxymethyl phenyl glycosides and activation of the OH with a peptide coupling reagent prior to by condensation with daunorubicin.

As an illustration (scheme 2) , condensation of the penta-*O*-acetyl-D-galactose with p-cresol in the presence of $ZnCl_2$[10] (160°C, 30 min.) stereoselectively afforded the α-glycoside **1a** (m.p. 163-165°C, $[\alpha]_D^{20}$ + 164, 30% yield) which was purified by column chromatography (hexane-EtOAc, 9:1). Benzylic bromination of **1a** was carried out by the use of N-bromosuccinimide in CCl_4, either under irradiation (1000 watts, 15 min.) or in the presence of radical initiator (benzoyl peroxide) , and bromo-derivative **2a** (82% yield) was subsequently converted to alcohol **3a** ($AgNO_3$, acetone-H_2O, 56% yield).[11] Activation of alcohol **3a** with N-hydroxysuccinimidocarbonate led to **4a** (96% yield) which was further condensed with daunorubicin **5** (DMF, Et_3N, r.t.) to give **6a** in 32% yield. Finally N-[4-(α–D-galactopyranosyl)-benzyloxycarbonyl]-daunorubicine **7a** (amorphous solid, $[\alpha]_D^{20}$ + 243) was obtained in 89% yield by transesterification of **6a** with MeONa-MeOH.

Following the same sequence of reactions the corresponding N-[2-(α-D-galactopyranosyl)-benzyloxycarbonyl]-daunorubicin **7b** was prepared from 2-methylphenyl 2,3,4,6-tetra-O-acetyl-α-D galactopyranoside **1b**[12]. Thus bromination of **1b** (NBS,CCl_4) afforded **2b** (80% yield) which was in turn converted ($AgNO_3$) to a mixture of the desired alcohol **3b** (21% yield) and of the corresponding aldehyde (52%). The latter was easily reduced ($NaBH_4$) to **3b** (80%). Therefore, after activation of **3b** using succinimido chloroformate (72%) , condensation of **4b** with daunorubicin **5** (85%) and deprotection of **6b** afforded the desired compound **7b** in 83% yield.

The cytotoxicity of compounds **7a** and **7b** has been investigated against L1210 murine leukemia and, as expected, both exhibit a strongly reduced cytotoxicity (>1 μg/mL) compared to daunorubicin **5** (0.02μg/mL). The stability of these prodrugs in buffer and plasma is now under investigation as well as their cleavability by the enzyme, α–D–galactosidase.



SCHEME 2

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13 *Compound 2a*: $C_{21}H_{25}O_{10}Br$ = 517. M.p. 187°C; $(\alpha)_D$ + 26°; ^1H NMR : δ 7.33 (d, 2H, J = 8 Hz), 7.02 (d, 2H, J = 8 Hz), 5.78 (d, 1H, J = 4 Hz), 5.60 (dd, 1H, J = 10, J' = 4 Hz), 5.55 (dd. 1H, J = 4, J' = 1 Hz), 5.29 (dd, 1H, J = 10, J' = 4 Hz), 4.49 (s, 2H), 4.32 (ddd, 1H, J = 7, J' = 6, J" = 1 Hz), 4.12 (dd, 1H, J = 12, J' = 6 Hz), 4.06 (dd, 1H, J = 12, J' = 7 Hz), 2.17, 2.07, 2.03, 1.94 (4s, 4 x 3H). *Compound 3a*: $C_{21}H_{26}O_{11}$ = 454. M.p. 153°C; $(\alpha)_D$ + 144°; ^1H NMR: δ 7.30 (d, 2H, J = 8 Hz), 7.04 (d, 2H, J = 8 Hz), 5.60 (d, 1H, J = 4 Hz), 5.54 (dd, 1H, J = 10, J' = 4 Hz), 5.51 (dd, 1H, J= 4, J' = 1 Hz), 5.28 (dd, 1H, J = 10, J ' = 4 Hz), 4.64 (s, 2H), 4.35 (dd, 1H, J = 7, J' = 6, J" = 1 Hz), 4.09 (dd, 1H, J = 12, J' = 6 Hz), 4.07 (dd, 1H, J = 12, J' = 7 Hz), 2.17, 2.08, 2.03, 1.96 (4s, 4 x 3H); MS: m/z 472 $(M + NH_4)^+$, 331. *Compound 4a*: $C_{26}H_{29}NO_{15}$ = 595. Foam; $(\alpha)_D$ + 142°; ^1H NMR: δ 7.33 (d, 2H, J = 8 Hz), 7.07 (d, 2H, J = 8 Hz), 5.76 (d, 1H, J = 4 Hz), 5.55 (dd, 1H , J = 10, J' = 4 Hz), 5.51 (dd, 1H, J = 4, J' = 1 Hz), 5.29 (dd, 1H, J = 10, J' = 4 Hz), 5.26 (s, 2H), 4.30 (ddd, 1H, J = 7, J' = 6, J" = 1 Hz), 4.11 (dd, 1H, J = 12, J' = 6 Hz), 4.07 (dd, 1H, J = 12, J' = 7 Hz), 2.83 (s, 4H), 2.16, 2.07, 2.03, 1.90 (4s, 4 x 3H); MS : m/z 613 $(M + NH_4)^+$, 437, 348. *Compound 6a*: $C_{49}H_{53}NO_{22}$ = 1007. Foam ; $(\alpha)_D$ + 278°; ^1H NMR (DMSO-d_6) δ 7.88 (m, 2H), 7.64 (m, 1H), 7.26 (d, 2H, J = 8 Hz), 7.03 (d, 2H, J = 8 Hz), 6.84 (d, 1H, J = 8 Hz), 5.74 (d, 1H, J = 4 Hz), 5.53 (s, 1H), 5.37 (m, 2H), 5.17 (m, 1H), 5.10 (dd, 1H, J = 10, J' = 4 Hz), 4.88 (m, 3H), 4.70 (d, 1H, J = 6 Hz), 4.30 (ddd, 1H, J = 7, J '= 6, J" = 1 Hz), 4.13 (dd, 1H, J = 7; J' = 4 Hz), 4.00-3.93 (m, 5H), 3.66 (m, 1H, J = 12, J'= 8, J" = 4 Hz), 3.31 (m, 1H), 2.91 (s, 2H), 2.20, 2.08, 1.96, 1.91, 1.76 (5s, 5 x 3H), 2.20-1.40 (m, 4H), 1.08 (d, 3H, J = 7 Hz); MS: m/z 1025 $(M + NH_4)^+$, 376. *Prodrug* **7a**: $C_{41}H_{45}NO_{18}$ = 839.Foam $(\alpha)_D$ + 243° (c 0.01). *Compound 3b*: $C_{21}H_{26}O_{11}$ = 454. M.p. 96°C; ^1H NMR : δ7.40-7.00 (m, 4H), 5.72 (d, 1H, J = 4 Hz), 5.60-5.30 (m, 3H), 4.89 (d, 1H, J = 12.5 Hz), 4.57 (d, 1H, J = 12.5 Hz), 4.43 (t, 1H, J = J'= 6 Hz), 4.15 (m, 2H), 2.19 (s, 3H), 2.19, 2.09, 2.03, 1.98 (4s, 4 x 3H). *Compound 4b*: $C_{26}H_{29}NO_{15}$ = 595. ^1H NMR : δ 7.50-7.20 (m, 3H), 7.10 (t, 1H, J= J'= 8 Hz), 5.90-5.40 (m, 5H), 5.33 (dd, 1H, J = 12 , J'= 4 Hz), 5.16 ((d, 1H, J = 12.5 Hz), 4.57 (d, 1H, J = 12 Hz), 4.43 (t, 1H, J = J'= 6 Hz), 4.15 (m, 2H), 2.87 (sl, 4H), 2.17 , 2.06, 1.99, 1.98 (4s, 4 x 3H). *Compound 6b*: $C_{49}H_{53}N0_{22}$ = 1007. M.p. 130°C; $(\alpha)_D$ + 216°; ^1H NMR: δ 13.98 (s, 1H, OH), 13.28 (s, 1H, OH), 8.03 (d, 1H, J = 8 Hz), 7.78 (t, 1H, J = J'= 8 Hz), 7.50-7.00 (m, 4H), 5.85 (d, 1H, J = 4 Hz), 5.70-5.20 (m, 7H), 5.10 (ABq, 2H, J = 9 Hz), 4.08 (s, 3H), 4.20-3.70 (m, 6H), 3.66 (s, 1H), 3.23 (d, 1H, J = 18 Hz), 2.94 (d, 1H, J = 18 Hz), 2.41, 2.12, 2.08, 2.00, 1.87 (5s, 5 x 3H). *Prodrug* **7b** : $C_{41}H_{45}N0_{18}$. m.p. 130°C.

For *series a*: ^1H NMR at 270 MHz and for series b ^1H NMR at 200 MHz in $CDCl_3$ except for compound **6a**; (α)D in $CHCl_3$ except for **7a** in MeOH ; MS (DCI/NH_3).