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β-Glucuronyl Carbamate Based Pro-moieties Designed for Prodrugs in ADEPT

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Abstract: A number of pro-moieties **8a** - **e** designed for prodrug preparation have been synthesized (chart 2). The pro-moieties, containing a glucuronyl carbamate group linked to a spacer possessing a terminal carboxylic acid group, have been synthesized from isocyanates **6** and anomerically unprotected glucuronic acids **10** (chart 2). The requisite isocyanates had to be prepared using the Curtius rearrangement. Glucuronyl carbamates proved to be excellent substrates for human β-glucuronidase. The pro-moieties **8a** - **e** can be coupled to hydroxy- or amino group containing drugs. The resulting prodrugs are designed to be activated by β-glucuronidase (chart 1) and to be used in ADEPT. Application is demonstrated with the synthesis of daunomycin prodrugs **12a** - **e** (chart 3).

The lack of selectivity of cytostatic agents for tumor cells is a serious drawback in conventional cancer chemotherapy. This major problem dictates research in the field of selective chemotherapy. In this context the selectivity of monoclonal antibodies (mAb) for their target tumor cells can be used to selectively deliver cytotoxicity to tumor tissue. In the ADEPT[1] (Antibody Directed Enzyme Prodrug Therapy) approach, specific enzymes are delivered to tumor sites by using mAb's. These enzymes are selected to convert a prodrug, which is administered in a second step after localization of the mAb-enzyme conjugate, to the parent cytotoxic drug[2]. We focused our attention on the β-glucuronidase (GUS) / β-glucuronide prodrug[3] couple. This system possesses the advantage that GUS is a human lysosomal enzyme present only at minor concentrations in blood[4] and that β-glucuronides are highly polar compounds which have a poor cell-membrane permeability.

In our approach towards glucuronide based prodrugs, a β-glucuronide is attached to a spacer molecule *via* a carbamate linkage. A spacer was used in order to couple the β-glucuronide to a hydroxy- or amino group containing drug as well as to facilitate GUS hydrolysis[5].

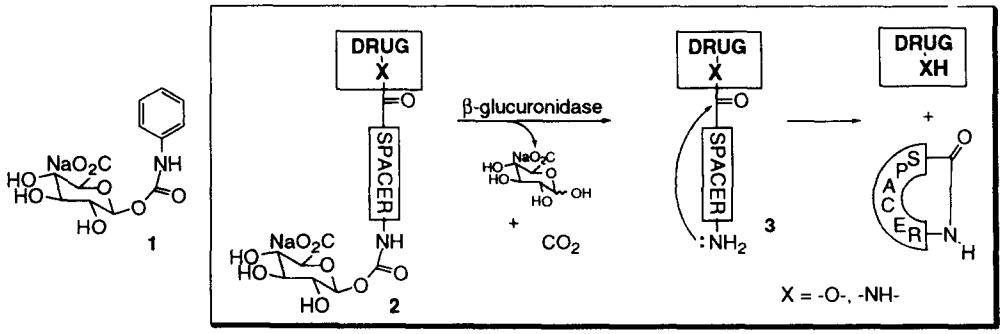


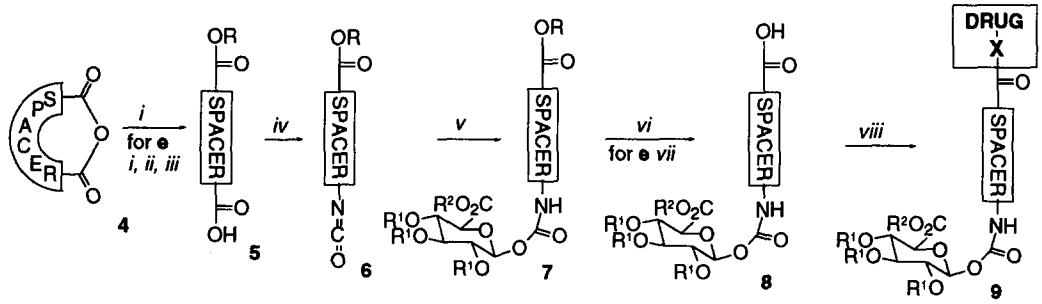
CHART 1

In a pilot study, *N*-phenyl β-glucuronyl carbamate **1** was synthesized and proved to be hydrolyzed by GUS at a rate comparable to that of *p*-nitrophenyl glucuronide[6], demonstrating that β-glucuronyl carbamates are

excellent substrates for GUS. By using these glucuronyl carbamate pro-moieties in prodrugs with general structure **2**, after GUS hydrolysis, a drug-spacer molecule **3** is generated having an γ-amino group which is apt to attack the carbonyl of the -C(O)-X-drug function and thereby liberating the drug-XH (chart 1).

In literature, amides comparable to our spacers are described in which a γ- or δ- nucleophilic group (hydroxy[7]- or amino[8]) is generated after a triggering step, producing lactones and lactams respectively after cyclization, with expulsion of an amine. To date, no pro-moieties comparable to those presented in this paper have been described in which spacer cyclization is triggered by enzymatic hydrolysis, effecting in drug liberation.

The different spacers **a** - **e** were chosen to study their influence on GUS hydrolysis rates and rates of lactam formation. To test the cyclization ability of the spacers, the mono ester mono carboxylic acids **5b** and **5c** were transformed to isocyanates to which benzyl alcohol was added. The obtained benzyl carbamates were subjected to hydrogenolytic conditions after which the γ-lactams were instantaneously formed as indicated by ^{1}H-NMR experiments. The synthesis of pro-moieties **8a** - **e** is presented in chart 2.



REAGENTS AND CONDITIONS

i: AllOH, DMAP (R = -All)
ii: isobutene, H_2SO_4, CH_2Cl_2 (R = -*t*-Bu)
iii: $Pd(PPh_3)_4$, morpholine, THF (R = -*t*-Bu)
iv: $(PhO)_2P(O)N_3$, Et_3N, PhMe, 65 °C
v: **10** Et_3N, PhMe, 65°C
vi: $Pd(PPh_3)_4$, morpholine, THF (R = -All)
vii: CF_3COOH, CH_2Cl_2 (R = -*t*-Bu)
viii: coupling to DRUG-XH

R^1 = -Ac, R^2 = -Me or R^1 = R^2 = -Bn

X = -O-, -NH-

SPACER	a	b	c	d	e
R	-All	-All	-All	-All	-*t*-Bu
c.y. (%) **5** ⇒ **7**	74	82	81	86	68

CHART 2

As a result of the desired lactamization potential, the glucuronyl carbamate moiety can not be introduced *via* synthetic steps involving intermediates having a free γ–amino group because of premature ring closure to the corresponding γ-lactams. For this reason we introduced the glucuronyl carbamate fragment *in situ*, employing the Curtius rearrangement to generate isocyanates as masked carbamates from carboxylic acids using diphenylphosphoryl azide[9]. The 2,3,4,6-protected anomerically unprotected glucuronic acid **10**[10], was added to the isocyanates **6** to yield carbamates **7** in a high β-selective (> 95%[11]) reaction. In this way the γ-amino group is introduced *via* a masked functionality in a one pot procedure starting from carboxylic acids **5**. Carboxylic acids **5** are prepared by opening of the cyclic anhydrides **4** with allyl alcohol. In case of the asymmetric anhydride **4c**,

the ring opening resulted in a 7/2 mixture of the γ-dimethyl **5c** and α-dimethyl carboxylic acid esters respectively. This mixture could not be separated and was used further as such. Later on in the synthesis, when pro-moiety **8c** was coupled to daunomycin **11** (chart 3) the major isomer **12c** could be separated.Homophthalic anhydride **4d** reacted with allyl alcohol yielding exclusively the phenyl acetic acid ester derivative **5d** (R = -All). *t*-Butyl ester formation of **5d** with isobutene and removal of the allyl group resulted in **5e** (R = -*t*-Bu) which finally gave pro-moiety **8e**.

As a first drug to be coupled to these novel pro-moieties, we selected daunomycin which belongs to the anthracycline anti tumor agents, one of the most widely used class of cytostatics[12]. The model prodrugs **13a** - **e** were conveniently prepared from pro-moieties **8a** - **e** and daunomycin **11**[13]. It is well known from structure activity studies[14] that the 3'-amino function of the amino sugar requires to be unsubstituted for optimal activity, indicating that prodrugs should have a pro-moiety preferably attached to the 3'-amino group in order to obtain inactive prodrugs

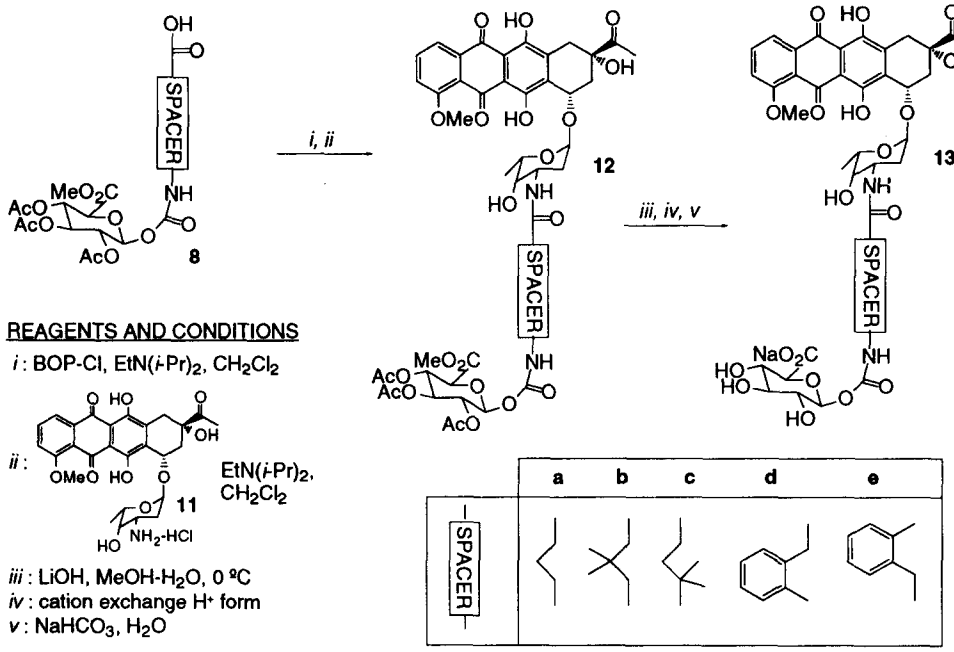


CHART 3

The synthesis of prodrugs **13** (chart 3) was effected by activating the carboxylic acid function of the pro-moieties **8** with BOP-Cl[15] and consecutive coupling of the resulting active ester to daunomycin **11**. The deprotection of the coupled products **12** was readily accomplished in a yield > 95% using a solution of LiOH in a H_2O - methanol mixture. After neutralization of the reaction mixture, the glucuronide was transformed to its sodium salt using $NaHCO_3$ affording prodrugs **13a** - **e**. The spacer based glucuronyl carbamate prodrugs **13a** - **e** are currently being tested. *in vitro*, including cytotoxicity assays and GUS hydrolysis rate determinations.

The fast cyclization of the spacers after generation of the γ-amino group, together with their convenient preparation, suggest that the pro-moieties described in the present paper can be of great value for the synthesis of prodrugs which can be applied in ADEPT.

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