Pergamon

Bioorganic & Medicinal Chemistry 7 (1999) 1597–1610

BIOORGANIC & MEDICINAL CHEMISTRY

Novel Anthracycline-spacer-β-glucuronide, -β-glucoside, and -β-galactoside Prodrugs for Application in Selective Chemotherapy

Ruben G. G. Leenders,[a,†] Eric W. P. Damen,[a] Edward J. A. Bijsterveld,[a] Hans W. Scheeren,[a,*] Pieter H. J. Houba,[b] Ida H. van der Meulen-Muileman,[b] Epie Boven[b] and Hidde J. Haisma[b]

[a]*Department of Organic Chemistry, NSR-Center for Molecular Structure, Design and Synthesis, University of Nijmegen, Toernooiveld, 6525 ED Nijmegen, The Netherlands*

[b]*Department of Medical Oncology, Academic Hospital Vrije Universiteit, De Boelelaan 1117, 1081 HV Amsterdam, The Netherlands*

Received 7 December 1998; accepted 8 February 1999

Abstract—A series of anthracycline prodrugs containing an immolative spacer was synthesized for application in selective chemotherapy. The prodrugs having the general structure anthracycline-spacer-β-glycoside were designed to be activated by β-glucuronidase or β-galactosidase. Prodrugs with -chloro, -bromo or -*n*-hexyl substituents on the spacer were synthesized as well as prodrugs containing a -β-glucuronyl, -β-glucosyl or -β-galactosyl carbamate specifier. The key step in the synthesis of all prodrugs is the highly β-diastereoselective addition reaction of the anomeric hydroxyl of a glycosyl donor to a spacer isocyanate resulting in the respective β-glycosyl carbamate pro-moieties. The resulting protected pro-moieties were coupled to an anthracycline. Prodrugs were evaluated with respect to activation rate by the appropriate enzyme and additionally, their IC_{50} values were determined. Optimal prodrugs in this study were at least 100- to 200-fold less toxic than their corresponding drug in vitro and were activated to the parent drug in a half-life time of approximately 2 h.

Introduction

Since the discovery of doxorubicin **DOX** (Chart 1) in the 1960s and the assessment of its remarkable activity in the treatment of cancer,[1] more than 2000 analogues of this compound have been synthesized and evaluated for anti-tumor activity. Despite great efforts put into the development of anthracyclines with a higher therapeutic index, no major breakthroughs have been made in this field and doxorubicin remains a frontline cytostatic agent.[2–4] The aggressive nature of cytotoxic chemicals employed in the treatment of cancer, however, raises the need for developing more selective therapies[5,6] to treat this class of disease, especially in the case of anthracyclines where side effects such as myelosuppression and cumulative and irreversible cardiotoxicity occur.[7] A promising approach to increase selectivity is to use a relatively nontoxic prodrug that is selectively activated to the parent cytostatic at the tumor site by the action of an enzyme. When the prodrug-activating enzyme is targeted to the tumor site using a monoclonal antibody (mAb), this is called ADEPT (Antibody Directed Enzyme Prodrug Therapy).[8] In this strategy to activate the prodrug, an enzyme is covalently linked to a mAb and the mAb-enzyme conjugate is administered first. After this conjugate has been situated into the tumor tissue the relatively non-toxic prodrug is administered in the second step. The prodrug is converted to the parent drug by the targeted enzyme. When an endogenous enzyme that is present at an elevated level at the tumor site is activating the prodrug, this is referred to as monotherapy.[9] For example β-glucuronidase is found to be present at highly elevated concentrations in necrotic tumor tissue.[10,11] In this monotherapy only the prodrug is administered. Several approaches toward the synthesis and use of enzyme-activated prodrugs have been described.[12–14] Often, the use of such prodrugs is limited because of a too slow activation rate by the concomitant enzyme,[15] a premature prodrug activation by endogenous enzymes,[16–18] or a too high cytotoxicity of the prodrug.[19]

Our research[20–25] toward the preparation of enzyme-activated prodrugs has focused on using a prodrug in

Key words: Anthracyclines; prodrugs; ADEPT; β-glucuronidase.

* Corresponding author. Tel.: +31-024-365-2331; fax: +31-024-365-2929; e-mail: jsch@sci.kun.nl

† Present address: MercaChem, Toernooiveld 100, 6503 CB Nijmegen, The Netherlands.

0968-0896/99/$ - see front matter
PII: S0968-0896(99)00095-4

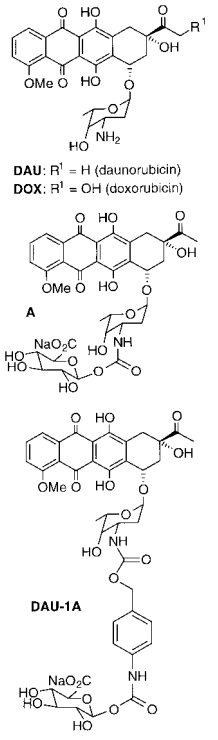
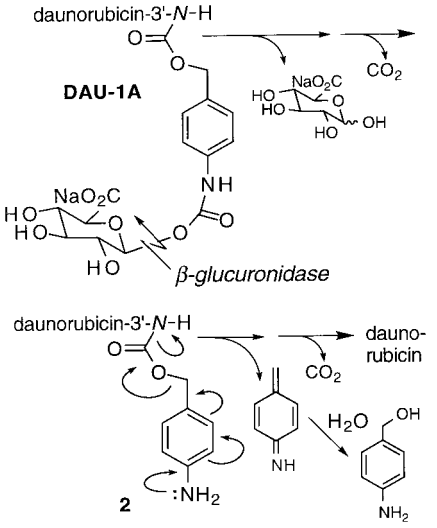


Chart 1.



Scheme 1.

combination with a human enzyme to minimize immuno-response[26] in cases where a mAb-enzyme conjugate is used to activate a prodrug. Prior studies showed that a β-glucuronyl[27–32] carbamate group is useful for prodrug synthesis when attached to a drug via a spacer.[22–24] Prodrug **A** (Chart 1) having a β-glucuronyl carbamate moiety attached without spacer to the 3′-amino group of daunorubicin was activated too slowly by β-glucuronidase to be suitable for use in selective chemotherapy.[21]

The group of prodrugs described in this paper that are designed to be activated analogously to **DAU-1A**[33] are outlined in Scheme 1. Upon enzymatic hydrolysis of the glucuronyl group of the prodrug **DAU-1A** and loss of a molecule of CO_2, the resulting *para*-amino (or *ortho*-amino) benzyloxycarbonyl residue of drug-spacer molecule **2** splits off spontaneously by a 1–6 (or 1–4 in case of **DAU-3A**, Chart 3) electron shift process[34,35] and loss of an additional molecule of CO_2 to yield the free drug **3**.

Here we present the chemical synthesis of **DAU-1A** and the nine derivatives thereof shown in Chart 2, which have the general structure of anthracycline-spacer-specifier. A primary in vitro evaluation of these compounds as prodrugs is reported. A previous paper[24] describes the biological properties of prodrug **DAU-1A**. It was found that **DAU-1A** is activated by β-glucuronidase at an acceptable rate to be useful for selective chemotherapy. The prodrugs described in Chart 2 are designed to study the effect of structural variations in the pro-moiety on enzyme activation, cytotoxicity, and pharmacokinetics. Within this group we prepared: (1) β-glucoside derivative **DAU-1B** and β-galactoside[36] derivative **DAU-1C**, which were designed to be activated by human β-glucosidase and human β-galactosidase, respectively. These prodrugs possess a reduced polarity compared with the more polar glucuronide type prodrugs in order to retard their excretion. (2) For the same reason, prodrug **DAU-2A** was designed to have a lipophylic C_6-tail on the benzylic carbon of the spacer. (3) The *ortho* analogue of **DAU-1A**, prodrug **DAU-3A** (Chart 3). (4) Compounds **DAU-4A–7A** possess a chlorine or bromine atom on the aromatic ring of the spacer. In addition to being electron withdrawing and, hence, facilitating enzymatic hydrolysis of the β-glucuronyl group, bromo- and chloro-aryl groups are known to have a high serum albumin binding potency,[37] which may retard prodrug excretion. Initially prodrugs of daunorubicin were synthesized as this drug is more readily available and easier to handle than doxorubicin. Finally doxorubicin analogue **DOX-1A** was synthesized as doxorubicin is clinically more relevant in the treatment of solid tumor types than daunorubicin. As a primary evaluation of their biological activity the prodrugs have been tested in vitro.

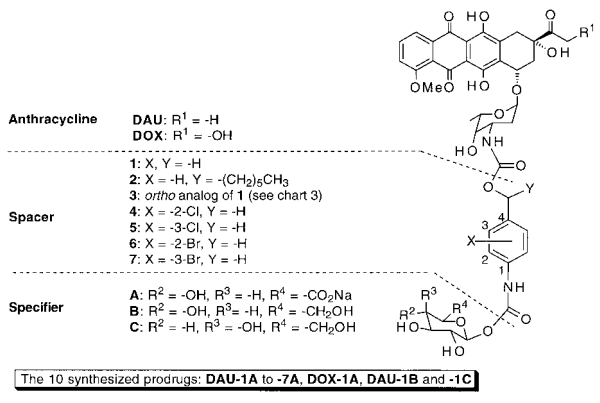


Chart 2.

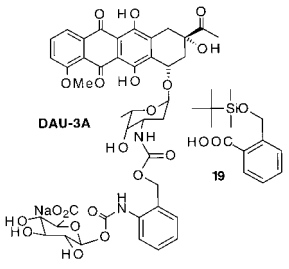


Chart 3.

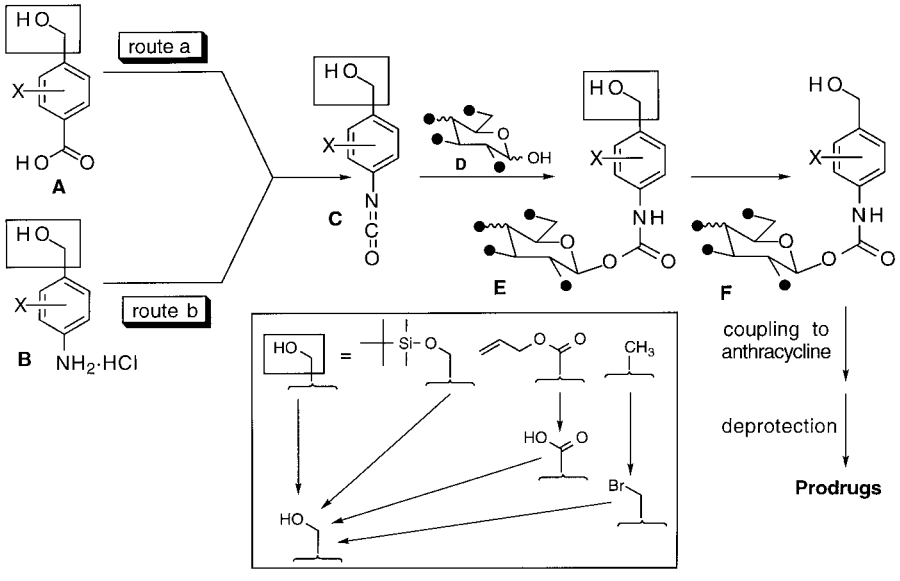
Chemistry

Considering the multiple functionalities present in daunorubicin and doxorubicin, their ease of oxidation and especially the vulnerability of the glycosidic bond connecting the anthracyclinone aglycon and daunosamine sugar, the synthesis of target compounds requires a subtle approach. Preferably, the anthracycline is involved in the synthesis sequence in one of the final reaction steps to prevent side reactions of the anthracycline part.

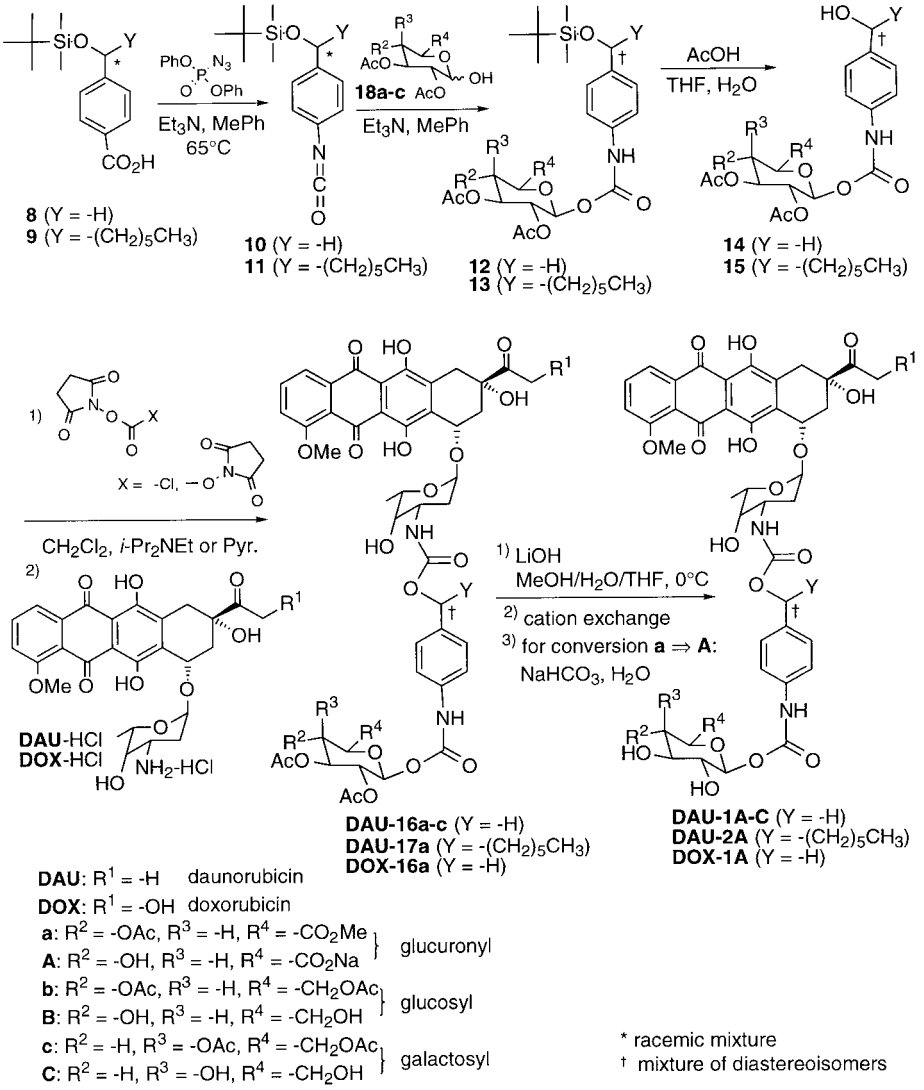
The general strategy is depicted in Scheme 2. The designed prodrugs presented in Chart 2 can be prepared by coupling the benzyl alcohol group of pro-moieties **F** to the anthracycline 3′-amino group. For the preparation of compound **F** via masked intermediates **E**, in routes a and b (Scheme 2), introduction of a β-glycosyl carbamate group to the spacer can be accomplished by the addition reaction of the anomerically unprotected glycosyl donor **D** to spacer isocyanate **C**. This is the key step in the synthesis of all prodrugs and when performed in toluene and triethylamine as the catalyst, leads to the respective spacer-β-glycosyl carbamates **E** in a very high β-diastereoselectivity.[38] Depending on the availability of the starting material for **A** and **B**, the hydroxymethyl precursor was either a protected hydroxymethyl group, an ester, or a methyl group. These units can be converted into a hydroxymethyl group by deprotection, reduction or oxidation, respectively. Following these pathways, pro-moieties **F** can either be prepared from carboxylic acids **A**, aniline-hydrochlorides **B** or commercially available isocyanates **C**.

In case of the synthesis of **DAU-1A** to **1C**, **DOX-1A** and **DAU-2A** and **3A** (Scheme 3) the desired spacer-isocyanates were generated from the respective spacer-carboxylic acids in situ. Employing the Curtius rearrangement using diphenyl phosphoryl azide,[39] spacer-acyl azides were formed from carboxylic acids. Heating of the acyl azides resulted in the formation of the respective spacer-isocyanates **10** and **11**. Following the procedure described earlier by us[38] glycosyl carbamates **12** and **13** were prepared from the respective carboxylic acids **8** and **9** in a one-pot procedure. The protected pro-moieties **12** and **13** were treated with acetic acid to remove the silyl protective group on the benzyl alcohol moiety. The resulting intermediates **14** and **15** were coupled to daunorubicin or doxorubicin making use of di-*N*,*N*′-succinimidyl carbonate[40] or succinimidyl chloroformate leading to the protected prodrugs **DAU-16a–c**, **DAU-17a** and **DOX-16a**. The *ortho* substituted prodrug **DAU-3A** (Chart 3) was synthesized analogously to **DAU-1A** (Scheme 3) starting from the silyl-protected *o*-hydroxymethyl benzoic acid **19** (Chart 3). The starting material for prodrug **DAU-2A** possessing a hexyl substituent on the spacer, compound **9**, was easily obtained by following Scheme 4. Because of the larger steric demand of the additional hexyl group, the coupling of **15** to daunorubicin was accomplished using *N*-succinimidyl chloroformate which is more reactive than di-*N*,*N*′-succinimidyl carbonate.

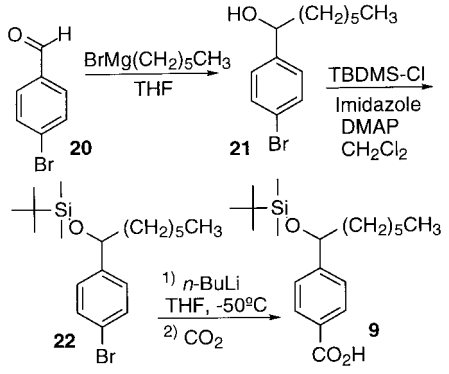
Alternatively, compounds **DAU-4A** to **DAU-7A** (Scheme 5) having a chlorine or bromine atom on the spacer, were synthesized starting from substituted[41] anilines **23**, toluic isocyanates **24** or terephthalic acid derivatives **28**, depending on the availability of the starting material.



Scheme 2.



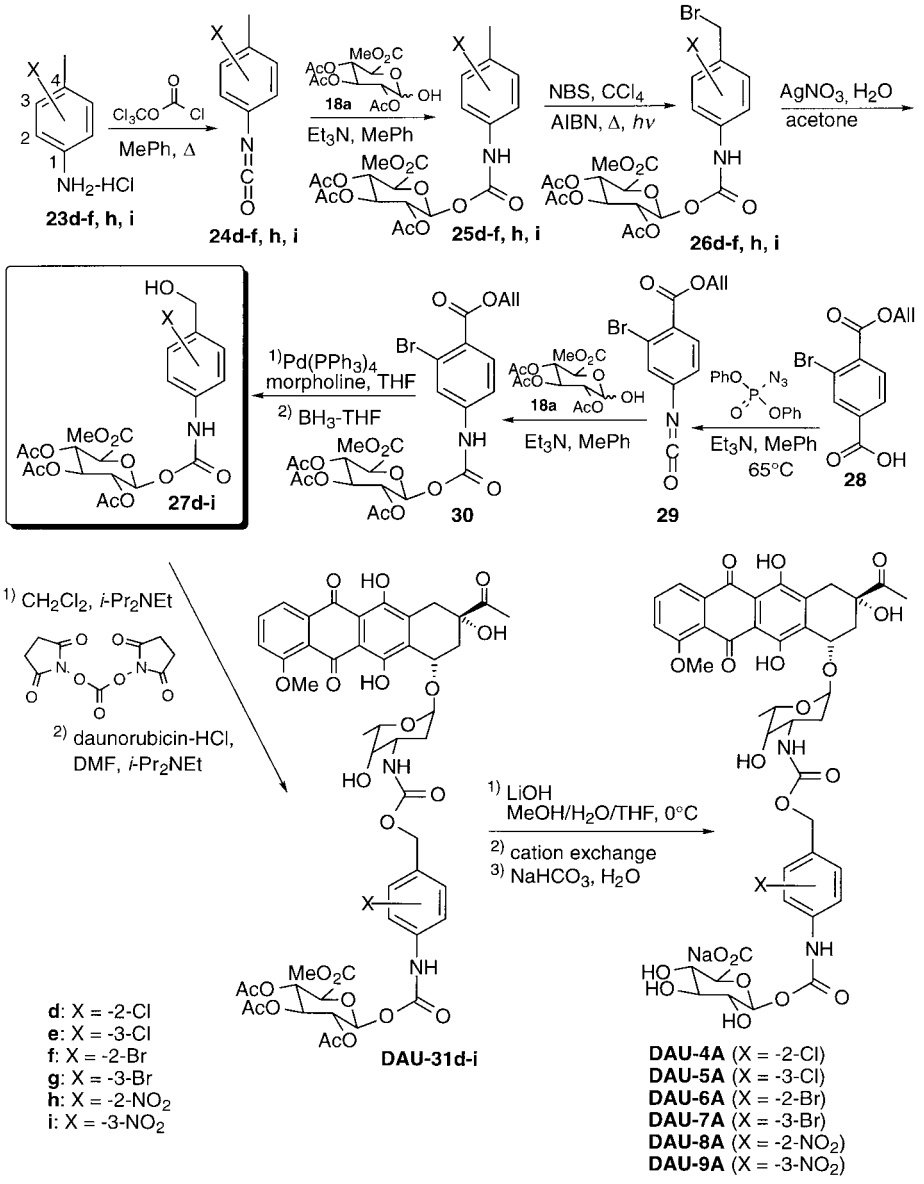
Scheme 3.



Scheme 4.

After the addition of glucuronyl donor **18a** to isocyanates **24d–f**, **h** and **i**, benzylic bromination of the respectively obtained β-glycosyl carbamates **25** and hydrolysis of the resulting bromides **26**, promoieties **27d–f**, **h** and **i** were obtained. Using di-succinimido carbonate, promoieties **27** were coupled to daunorubicin. As isocyanate **24g** was not commercially available, the corresponding promoiety **27g** was synthesized from the bromo terephthalic acid derivative **28** according to the one-pot modified Curtius procedure outlined above for Scheme 3. The monoester **28** was prepared from the corresponding dicarboxylic acid by esterification using DCC and obtained from the mixture of products by selective crystallization. Removal of the allyl protecting group of **30** and reduction of the resulting carboxylic acid to an alcohol group using BH_3-THF resulted in promoiety **27g**.

Deprotection of the carbohydrate promoieties of all protected prodrugs **DAU-16a–c**, **DAU-17a**, **DOX-16a** and **DAU-31d–i** was accomplished using lithium hydroxide in MeOH/H_2O/THF at 0°C. After neutralization of the basic solution with ion exchange resin and conversion of the glucuronide carboxylic acid group to the sodium salt using sodium bicarbonate, the polar prodrugs were purified on a reversed phase C_{18} column. The product fraction was lyophilized to yield the pure prodrugs as red fluffy solids.



Scheme 5.

In the case of prodrug **DOX-1A**, however, during the deprotection of the glucuronyl moiety extensive side product formation took place due to the base lability of doxorubicin.[42] For this deprotection procedure a maximum yield of 37% was attained whereas for the daunorubicin prodrug **DAU-1A** the deprotection was accomplished in 81%. Deprotection of the prodrug precursors **DAU-31d–i** having the electronegative substituent on the 2-position of the aromatic ring of the spacer led to extensive decomposition. In case of both **DAU-4A** and **6A** (X = −2-Cl and -2-Br, respectively), about 35% yield was attained. Deprotection of **DAU-31h** (X = −2-NO_2) did not lead to the desired prodrug **DAU-8A**, but in nearly quantitative yield methyl carbamate **32** (Chart 4) was formed. Deprotection of the prodrug precursors **DAU-31e,g** having the electronegative substituent on the 3-position of the aromatic ring was accomplished in satisfactory yields in case of **DAU-5A** (67%) and **DAU-7A** (50%). Deprotection of **DAU-31i** (X = -3-NO_2) led to extensive

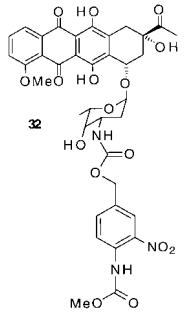


Chart 4.

decomposition and multiple side-product formation and only a small amount of **DAU-9A** was obtained in an impure state. So it seems that electron-withdrawing substituents in the aromatic ring of the spacer which should facilitate hydrolysis of the glucuronide group

also promote base mediated decomposition of the glucuronyl carbamate part. Especially electron-withdrawing substituents in the 2-position of the aromatic ring seem to make the glucuronyl carbamate function more susceptible for nucleophillic attack.[43]

Biological Evaluation of the Prodrugs

From all prodrugs synthesized, in vitro activation rates by the relevant enzyme have been determined. For prodrugs with favorable in vitro activation rates, in vitro antiproliferative effects were determined. The chemical stability of prodrugs **DAU-1A** and **DOX-1A** was greater than 95% over a 24 h incubation period in either PBS/BSA and human serum both at 37°C.

Prodrug activation rate

For the determination of the activation half-lives, the prodrugs were incubated with the concordant enzyme[44] at clinically achievable concentrations (100 μM). All prodrugs of daunorubicin and doxorubicin possessing the β-glucuronyl carbamate promoiety were activated by β-glucuronidase at approximately the same rate. Intermediate drug-spacer molecules were not detected by HPLC. Half-lives between 2 and 3 h were recorded (see Table 1). The glucosyl and galactosyl based prodrugs, however, were activated at a much slower rate than the glucuronyl based prodrugs. Among the derivatives having an electron-withdrawing group on the spacer, only **DAU-5A** (entry 8, Table 1) displayed a slightly higher activation rate than the unsubstituted prodrug **DAU-1A** (entry 1). Because the synthesis of **DAU-1A** is less complicated than that of **DAU-5A**, **DAU-1A** was selected to be further evaluated for use in ADEPT. For the same reason, **DOX-1A** was selected for further evaluation.

Antiproliferative effects

The in vitro antiproliferative effects of prodrugs with a favorable activation rate were determined in OVCAR-3 human ovarian cancer cells and these were compared with the IC_{50} values of the respective parent drugs (see Table 1). The β-glucuronide type prodrugs in entries 1, 2, 6 and 8 proved to be 85 to 200 times less toxic than the parent drug and for that reason seem very apt as prodrugs to be activated by β-glucuronidase.

Table 1. Enzyme activation half-lives and antiproliferative effects

Entry	Compound	Activation $t_{1/2}$ (min)	IC_{50} (μM)[a]
1	**DAU-1A**	135[b]	10
2	**DOX-1A**	170[b]	10
3	**DAU-1B**	300[c]	1.5
4	**DAU-1C**	400[c]	2.5
5	**DAU-2A**	950[b]	n.d.
6	**DAU-3A**	125[b]	11
7	**DAU-4A**	150[b]	n.d.
8	**DAU-5A**	90[b]	8.5
9	**DAU-6A**	150[b]	n.d.
10	**DAU-7A**	170[b]	n.d.
11	Daunorubicin	—	0.1
12	Doxorubicin	—	0.05

[a] Inhibition concentration [(pro) drug concentrations that gave 50% growth inhibition in OVCAR-3 cells when compared with control cell growth].
[b] 100 μM Prodrug, 0.03 U/mL human β-glucuronidase, pH 6.8, 37°C.
[c] 100 μM Prodrug., 0.3 U/mL bovine liver β-galactosidase,[44] pH 6.8, 37°C.

Conclusions

A flexible convergent synthesis has been developed for the anthracycline prodrugs collected in Chart 2. In this synthesis (generally outlined in Scheme 2) specifier, spacer and drug can be varied independently.

Enzymatic activation rates determined for all prodrugs showed that (1) the β-glucuronide based prodrugs seem better substrates than the β-glucoside and β-galactoside prodrugs, (2) prodrugs containing a 1,6-elimination spacer showed faster hydrolysis than prodrugs with a 1,4-elimination spacer, (3) chlorine or bromine substituents at the aromatic ring of the spacer do not have a profound effect on the enzymatic activation rate. A small accelerating effect was found on the enzymatic activation rate for prodrug **DAU-5A** having a 3-chlorine substituted spacer when compared with **DAU-1A**.

Unfortunately it was not possible to prepare prodrugs with stronger electron-withdrawing substituents, for example, a nitro-substituted spacer (**DAU-8A** and **-9A**) for which a higher enzymatic activation rate than **DAU-1A** are expected. Because of extensive decomposition during the final deprotection step these prodrugs were not obtained.

DAU-1A and **DOX-1A** have shown promising results in monotherapy experiments using mice bearing human tumor xenografts. These results are published separately.[25] Of these, prodrug **DOX-1A** has been selected for further evaluation in a phase I trial in cancer patients.

Experimental

Biological evaluation

Prodrug stability. The plasma stability of **DAU-1A** and **DOX-1A** was determined as described before[24] in a 0.1% (w/v) BSA/PBS solution of pH 7.4 as well as in human serum, both over a 24-h period at 37°C. 'Half-life' in this context refers to release of daunorubicin or doxorubicin from the tested prodrug.

Prodrug activation half-lives. Half-lives of enzyme hydrolysis were determined by incubating 100 μM prodrug at pH 6.8[45] with 0.03 U/mL of human β-glucuronidase[46] or 0.3 U/mL bovine liver β-galactosidase[44] (purchased from Sigma, G1875) at 37°C. Samples were prepared and analyzed on reversed phase SiO_2–C_{18} HPLC as described.[24]

Antiproliferative effects. The antiproliferative effects of daunorubicin, doxorubicin and of all 10 prodrugs on OVCAR-3 cells were determined by measuring cell growth with a protein dye stain.[15] Cells were harvested

with 0.25% trypsin and 0.2% EDTA in PBS to obtain a single cell suspension and seeded in 96-wells tissue culture plates (50,000 cells/mL 100 μL/well, in supplemented DMEM, three wells per concentration). After 24 h drug or prodrug was added (100 μL/well in supplemented DMEM) at different concentrations with a range of three or more logs and the cells were allowed to proliferate for 72 h. Cells were fixed with 25% trichloroacetic acid for 1 h at 4°C and washed with water. After staining the cells with 0.4% sulforhodamine B in 1% (v/v) acetic acid for 15 min at room temperature, they were washed with 1% acetic acid and air-dried. The bound dye was solubilized with 10 mM unbuffered Tris and the absorbance was read at 492 nm. The absorbance was linear with cell concentrations from 1000 to 200,000 cells/well. Separate wells were fixed 24 h after seeding to subtract background staining. The antiproliferative effects were determined and expressed as IC_{50} values which are the (pro)drug concentrations that gave 50% growth inhibition when compared with control cell growth.

Chemistry

General. Daunorubicin and doxorubicin hydrochlorides were a generous gift of Pharmachemie bv (Haarlem, The Netherlands). Chromatotron model 7924-T Harrison Research (Palo Alto, CA, USA) equipped with plates (thickness 2 mm, diameter 8.5 cm) made from Merck silicagel 60 PF_{254} which contains gypsum (art. 7749) was used when circular chromatography is indicated. When cation exchange material was indicated, amberlite resin IR-120 (Na) BDH (Poole, Dorset, UK) was converted to the H^+-form using 1 N HCl prior to use. Reversed phase chromatography was performed with a liquid chromatography pump LC-410 (Kontron) using a pre-packed column (24 cm, diameter 11 mm) containing octadecylsilane (4063 μm) Merck (Darmstadt, Germany). Prior to use the RP-C_{18} column was equilibrated with demineralized water. ^{1}H NMR spectra at 400 MHz were obtained on a Bruker AM-400. Chemical shifts are expressed in ppm downfield from internal standard Me_4Si. Et_3N, *i*-Pr_2NEt, CH_2Cl_2, pyridine and CCl_4 were dried by distillation over CaH_2, PhMe was dried by distillation over sodium and THF by distillation over $LiAlH_4$. In all cases demineralized H_2O was used. Allyl alcohol was dried by distillation over Mg (with a catalytic amount of I_2 added).

Synthesis of *N*-[4-(daunorubicin-*N*-carbonyl oxymethyl)-phenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-1A)

***N*-[4-(*t*-Butyldimethylsilyloxymethyl)phenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (12a).** 954 mg (3.59 mmol) of **8**[47] was stirred with 853 μL (1.1 equiv) of $(PhO)_2P(O)N_3$ and 549 μL (1.1 equiv) of Et_3N in 10 mL of PhMe under an argon atmosphere. After 12 h the reaction mixture was stirred at 80°C for 3 h. The mixture was cooled to ambient temperature and 719 mg (0.6 equiv) of methyl 2,3,4-tri-*O*-acetyl glucuronic acid **18a** was added. The reaction was worked-up as previously described[38] to yield 1.227 g of **12a**, 95% from **18a**. Physical data are according to the literature.[38]

***N*-[4-(Hydroxymethyl)phenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (14a).** Compound **12a** (1.227 g, 2.055 mmol) was stirred in 120 mL of THF/H_2O/AcOH (1/1/1). The course of the deprotection reaction was followed by TLC (SiO_2, Et_2O). After 3 h, no starting material was detected, the reaction mixture was diluted with 200 mL of H_2O and the THF was removed by evaporation. The aqueous layer was washed five times with 100 mL portions of CH_2Cl_2, the organic extracts were combined and washed with aqueous saturated $NaHCO_3$ until gas evolution ceased. After that the organic layer was dried with brine and Na_2SO_4 successively and evaporated. The resulting foam was sonicated in *i*-Pr_2O and filtered to give 911 mg of **14a**, mp 173°C, 92%. **1**H NMR (100 MHz, $CDCl_3$) δ 1.96 (9H,s), 3.64 (3H,s), 4.13 (1H, d, $J=9.3$ Hz), 4.61 (2H, s), 5.00–5.35 (3H, m), 5.71 (1H, d, $J=7.5$ Hz), 6.92 (1H, s), 7.19–7.28 (4H, m).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl)phenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl β-glucuronyl) carbamate (DAU-16a).** Compound **14a** (600 mg, 1.448 mmol) was stirred with 369 mg (1.1 equiv) of di-*N*-*N*′-succinimidyl carbonate and 550 μL (2.5 equiv) of *i*-Pr_2NEt in 25mL of CH_2Cl_2. After 1.5 h, no starting material was detected on TLC (SiO_2, Et_2O) and a solution of 790 mg (1.1 equiv) of **DAU**-HCl and 550 μL (2.5 equiv) of *i*-Pr_2NEt in 10 mL of DMF were added. The course of the reaction was monitored by TLC (SiO_2, CH_2Cl_2/EtOH, 10/1). After all of the active ester starting material had disappeared, the reaction mixture was diluted with 600 mL of CH_2Cl_2 and washed with 100 mL portions of aqueous 0.5 N $KHSO_4$ (3×), H_2O, aqueous saturated $NaHCO_3$ (2×), H_2O (2×), and with brine. The organic layer was dried over Na_2SO_4 and evaporated. The resulting red residue was purified twice by means of circular chromatography using a chromatotron supplied with a 2 mm silica plate and CH_2Cl_2/EtOH (10/1 and 30/1, respectively). After evaporation of the eluent, the resulting red product was sonicated in *i*-Pr_2O and filtrated to yield 1.076 g of **DAU-16a**, 84%, mp 163–164°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.28 (3H, d, $J=6.6$ Hz), 1.78 (1H, dt, $J=12.9$ Hz, $J=3.8$ Hz), 1.87 (1H, dd, $J=13.3$, Hz $J=5.8$ Hz), 2.05 (9H, s), 2.09 (1H, dd, $J=15.2$, Hz $J=3.7$ Hz), 2.30 (1H, d, $J=14.9$ Hz), 2.41 (3H, s), 2.88 (1H, d, $J=18.8$ Hz), 3.20 (1H, d, $J=18.8$ Hz), 3.67 (1H, s), 3.72 (3H, s), 3.88 (1H, m), 4.05 (3H, s), 4.22 (1H, d, $J=9.8$ Hz), 4.15–4.25 (1H, m), 4.48 (1H, s), 4.89 (1H, d, $J=12.2$ Hz), 4.95 (1H, d, $J=12.2$ Hz), 5.15–5.30 (4H, m), 5.38 (1H, t, $J=9.3$ Hz), 5.47 (1H, d, $J=3.1$ Hz), 5.77 (1H, d, $J=8.0$ Hz), 7.16 (1H, s), 7.18 (2H, d, $J=8.0$ Hz), 7.24 (2H, d, $J=8.0$ Hz) 7.37 (1H, d, $J=8.4$ Hz), 7.76 (1H, t, $J=8.0$ Hz), 8.00 (1H, d, $J=7.7$ Hz), 13.22 (1H, s), 13.94 (1H, s).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl)phenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-1A).** To 1.255 g (1.193 mmol) of **DAU-16a** was added 57.3 mL (6 equiv) of a 0.10 N LiOH solution in MeOH/H_2O/THF (2.5/1.0/0.5), the resulting deep blue solution was stirred at 0°C under an argon atmosphere. Progress of the deprotection was monitored on reversed-phase TLC (SiO_2–C_{18} MeCN/H_2O, 1/1). After 2 h of deprotection, the

reaction mixture was diluted with 150 mL of H_2O and neutralized by adding ca. 10 g of amberlite cation exchange material (H^+ form), 10 mL of THF was added to homogenize the suspension. The amberlite was removed by filtration and ca. 150 mg of $NaHCO_3$ were added. The MeOH and THF suspended in the water layer were removed by evaporation and the red aqueous product solution was transferred to a reversed phase column packed with RP-C_{18} material and eluted with 300 mL of H_2O to remove the excess of $NaHCO_3$. The column was successively washed with MeCN/H_2O (1/4) to elute the product and the MeCN was removed by evaporation. Freeze drying of the aqueous product solution gave 892 mg of 81%, mp 175°C (dec.). Anal. calcd for $C_{42}H_{43}N_2O_{20}Na.4H_2O$: C, 50.91; H, 5.19; N, 2.83. Found: C, 50.73; H, 4.96; N, 2.94. MS (FAB$^+$) m/z 942 ([M + 1 + Na]$^+$), 941 ([M + Na]$^+$), 920 ([M + 1 + H]$^+$), 919 ([M + H]+). ^{1}H NMR (400 MHz, $(CD_3)_2SO$)) δ 1.11 (3H, d, J = 6.6 Hz), 1.47 (1H, dd, J = 12.1 Hz J = 3.4 Hz), 1.82 (1H, dt, J = 11.5 Hz J = 3.4 Hz), 2.07 (1H, dd, J = 14.1 Hz J = 6.1 Hz), 2.19 (1H, dd, J = 14.1 Hz, J = 3.1 Hz), 2.27 (3H, s), 3.11 (1H, d, J = 18.5 Hz), 3.13 (1H, d, J = 18.5 Hz), 3.20–3.65 (4H, m), 3.71 (1H, m), 3.95 (3H, s), 4.16 (1H, q, J = 6.6 Hz), 4.70 (1H, d, J = 5.1 Hz), 4.76–5.32 (4H, m), 4.84 (1H, d, J = 12.8 Hz), 4.88 (1H, d, J = 12.8 Hz), 4.90 (1H, t, J = 5.1 Hz), 5.20 (1H, d, J = 3.0 Hz), 5.28 (1H, d, J = 8.2 Hz), 5.52 (1H, s), 6.84 (1H, d, J = 8.0 Hz), 7.23 (2H, d, J = 8.4 Hz), 7.43 (2H, d, J = 8.4 Hz), 7.60 (1H, dd, J = 6.8 Hz J = 3.0 Hz), 7.80–7.90 (2H, m), 9.90 (1H, s) 13.25 (1H, s), 13.99 (1H, s).

Synthesis of *N*-[4-(doxorubicin-*N*-carbonyl oxymethyl) phenyl] *O*-β-glucuronyl carbamate sodium salt (DOX-1A)

***N*-[4-(Doxorubicin-*N*-carbonyl oxymethyl)phenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (DOX-16a).** Analogously to the preparation of **DAU-16a** from 570 mg (1.18 mmol) of **14a**. Compound **DOX**-16a was isolated in 69% yield (850 mg), mp 165–167°C. **1**H NMR (400 MHz, $CDCl_3$) δ 1.28 (3H, d, J = 6.5 Hz), 1.75–1.90 (3H, m), 2.05 (9H, s), 2.14 (1H, dd, J = 14.7 Hz, J = 3.9 Hz), 2.32 (1H, d, J = 14.7 Hz), 2.97 (1H, d, J = 18.9 Hz), 3.06 (1H, s, 14-OH), 3.24 (1H, d, J = 18.9 Hz), 3.66 (1H, m), 3.72 (3H, s), 3.86 (1H, m), 4.06 (3H, s), 4.13 (1H, q, J = 6.5 Hz), 4.22 (1H, d, J = 9.7 Hz), 4.55 (1H, s), 4.75 (2H, s), 4.93 (1H, d, J = 12.4 Hz), 4.96 (1H, d, J = 12.4 Hz), 5.15–5.30 (4H, m), 5.36 (1H, t, J = 9.3 Hz), 5.48 (1H, d, J = 3.0 Hz), 5.77 (1H, d, J = 8.0 Hz), 7.20 (2H, d, J = 7.0 Hz), 7.20–7.35 (3H, m), 7.38 (1H, d, J = 8.5 Hz), 7.78 (1H, t, J = 8.0 Hz), 8.01 (1H, d, J = 7.8 Hz), 13.19 (1H, s), 13.93 (1H, s).

***N*-[4-(Doxorubicin-*N*-carbonyl oxymethyl)phenyl] *O*-β-glucuronyl carbamate sodium salt (DOX-1A).** Analogously to the preparation of **DAU-1A** from 850 mg (0.808 mmol) of **DOX-16a**. Prodrug **DOX-1A** was isolated in 37% yield (280 mg), mp 191°C (dec.). Anal. calcd for $C_{42}H_{43}N_2O_{21}Na.4H_2O$: C, 50.10; H, 5.11; N, 2.78. Found: C, 50.31; H, 4.81; N, 3.00. MS (FAB+) m/z 958 ([M + 1 + Na]$^+$), 957 ([M + Na]$^+$). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 1.12 (3H, d, J = 6.3 Hz), 1.47 (1H, d, J = 12.3 Hz), 1.83 (1H, dt, J = 12.9 Hz J = 3.5 Hz), 2.12 (1H, dd, J = 14.2 Hz J = 5.6 Hz), 2.20 (1H, d, J = 11.9 Hz), 2.95 (1H, d, J = 18.8 Hz), 3.01 (1H, d, J = 18.8 Hz), 3.05–3.60 (4H, m), 3.71 (1H, m), 3.99 (3H, s), 4.15 (1H, q, J = 6.3 Hz), 4.57 (2H, s), 4.62–4.71 (1H, m), 4.68 (1H, d, J = 4.9 Hz), 4.80–5.30 (4H, m), 4.88 (2H, s), 4.94 (1H, t, J = 4.2 Hz), 5.21 (1H, d, J = 2.7 Hz), 5.33 (1H, d, J = 8.0 Hz), 5.46 (1H, s), 6.83 (1H, d, J = 8.0 Hz), 7.24 (2H, d, J = 8.1 Hz), 7.43 (2H, d, J = 8.1 Hz), 7.65 (1H, t, J = 4.8 Hz), 7.85–7.95 (2H, m), 9.91 (1H, s) 13.28 (1H, s), 14.03 (1H, s).

Synthesis of *N*-[4-(daunorubicin-*N*-carbonyl oxymethyl) phenyl] *O*-β-glucosyl carbamate (DAU-1B)

***N*-[4-(*t*-Butyldimethylsilyloxymethyl)phenyl] *O*-(2,3,4,6-tetra-*O*-acetyl-β-glucosyl) carbamate (12b).** Analogously to the preparation of **12a** from 500 mg (1.88 mmol) of **8** and 339 mg (0.5 equiv) of **18b**. Compound **12b** was isolated in 77% yield (calculated from **18b**) (452 mg). Physical data are according to literature.[38]

***N*-[4-(Hydroxymethyl)phenyl] *O*-(2,3,4,6-tetra-*O*-acetyl-β-glucosyl) carbamate (14b).** Analogously to the preparation of **14a** from 240 mg (0.394 mmol) of **12b**. Compound **14b** was isolated in 68% yield (132 mg) as an oil. ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.91, 1.92, 1.94 and 1.96 (4s, 12H), 3.70–3.85 (1H, m), 3.98 (1H, dd, J = 12.4 Hz, J = 2.0 Hz), 4.22 (1H, dd, J = 12.4 Hz J = 4.3 Hz), 4.54 (2H, s), 4.90–5.20 (3H, m), 5.62 (1H, d, J = 7.8 Hz), 6.93 (1H, s), 7.15–7.25 (4H, m).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl)phenyl] *O*-(2,3,4,6-tetra-*O*-acetyl-β-glucosyl) carbamate (DAU-16b).** Analogously to the preparation of **DAU-16a** from 80 mg (0.161 mmol) of **14b**. Compound **DAU-16b** was isolated in 58% yield (97 mg), mp 145–148°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (3H, d, J = 6.5 Hz), 1.70–1.95 (3H, m), 2.02, 2.04, 2.06 and 2.07 (4s, 12H), 2.11 (1H, dd, J = 14.8 Hz J = 3.9 Hz), 2.31 (1H, d, J = 14.8 Hz), 2.41 (3H, s), 2.91 (1H, d, J = 18.7 Hz), 3.22 (1H, d, J = 18.7 Hz), 3.65 (1H, m), 3.85–3.95 (2H, m), 4.07 (3H, s), 4.12 (1H, d, J = 11.7 Hz), 4.21 (1H, q, J = 6.5 Hz), 4.31 (1H, dd, J = 11.7 Hz J = 4.6 Hz), 4.48 (1H, s), 4.92 (1H, d, J = 12.2 Hz), 4.97 (1H, d, J = 12.2 Hz), 5.10–5.35 (5H, m), 5.48 (1H, d, J = 3.3 Hz), 5.75 (1H, d, J = 8.1 Hz), 7.10 (1H, s), 7.22 (2H, d, J = 7.9 Hz), 7.31 (2H, d, J = 7.9 Hz), 7.38 (1H, d, J = 8.6 Hz), 7.78 (1H, t, J = 8.0 Hz), 8.02 (1H, d, J = 7.5 Hz), 13.26 (1H, s), 13.96 (1H, s).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl)phenyl] *O*-β-glucosyl carbamate (DAU-1B).** Analogously to the preparation **DAU-1A** (with the following modifications: After the amberlite was filtered off, no $NaHCO_3$ was added. Prior to RP-18 column chromatography, the crude product suspension was homogenized by adding ca. 10% (v/v) MeCN) from 12.5 mg (11.9 μmol) of **DAU-16b**. Prodrug **DAU-1B** was isolated in 79% (8.7 mg), mp 182–184°C. Anal. calcd for $C_{42}H_{46}N_2O_{19}.2.5H_2O$: C, 54.37; H, 5.54; N, 3.02. Found: C, 54.45; H, 5.24; N, 2.88. MS (FAB+) m/z 906 ([M + 1 + Na]$^+$), 905 ([M + Na]$^+$). ^{1}H NMR (400 MHz, $(CD_3)_2$ SO) δ −0.06 (s, 4H), 1.13 (3H, d, J = 6.5 Hz), 1.47 (1H, d, J = 12.8 Hz), 1.83 (1H, dt, J = 12.8 Hz J = 3.5 Hz),

2.08 (1H, dd, $J=14.4$ Hz, $J=5.4$ Hz), 2.20 (1H, d, $J=14.4$ Hz), 2.27 (3H, s), 2.93 (2H, s), 3.00–3.80 (10H, m), 3.97 (3H, s), 4.17 (1H, q, $J=6.5$ Hz), 4.87 (2H, s), 4.92 (1H, t, $J=4.4$ Hz), 5.21 (1H, d, $J=2.6$ Hz), 5.35 (1H, d, $J=8.2$ Hz), 6.83 (1H, d, $J=8.0$ Hz), 7.24 (2H, d, $J=8.3$ Hz), 7.42 (2H, d, $J=8.3$ Hz), 7.62 (1H, dd, $J=6.2$ Hz $J=3.5$ Hz), 7.85–7.90 (2H, m), 9.88 (1H, s) 13.26 (1H, s), 14.00 (1H, s).

Synthesis of *N*-[4-(daunorubicin-*N*-carbonyl oxymethyl) phenyl] *O*-β-galactosyl carbamate (DAU-1C)

***N*-[4-(*t*-Butyldimethylsilyloxymethyl)phenyl] *O*-(2,3,4,6-tetra-*O*-acetyl-β-galactosyl) carbamate (12c).** Analogously to the preparation of **12a** from 400 mg (1.50 mmol) of **8**. Compound **12c** was isolated in 57% yield (261 mg) (calculated from **18c**). Physical data are according to literature.[38]

***N*-[4-(Hydroxymethyl)phenyl] *O*-(2,3,4,6-tetra-*O*-acetyl-β-galactosyl) carbamate (14c).** Analogously to the preparation of **14a** from 225 mg (0.368 mmol) of **12c**. Compound **14c** was isolated in quantitative yield (183 mg) as an oil. **1**H NMR (100 MHz, $CDCl_3$) δ 1.92, 1.95, 1.99 and 2.04 (4s, 12H), 3.90–4.20 (3H, m), 4.55 (2H, s), 5.05 (1H, dd, $J=10.1$ Hz, $J=3.3$ Hz), 5.27 (1H, t, $J=7.9$ Hz), 5.37 (1H, d, $J=2.9$ Hz), 5.64 (1H, d, $J=7.8$ Hz), 7.20 (2H, d, $J=10.1$ Hz), 7.30 (2H, d, $J=10.1$ Hz), 7.65 (1H, s).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl)phenyl] *O*-(2,3,4,6-tetra-*O*-acetyl-β-galactosyl) carbamate (DAU-16c).** Analogously to the preparation of **DAU-16A** from 179 mg (0.36 mmol) of **14c**. Compound **DAU-16c** was isolated in 45% yield (171 mg), mp 170–173°C. **1**H NMR (400 MHz, $CDCl_3$) δ 1.29 (3H, d, $J=6.5$ Hz), 1.77 (1H, s), 1.78 (1H, dt, $J=13.1$ Hz, $J=4.0$ Hz), 1.88 (1H, dd, $J=13.2$ Hz, $J=5.0$ Hz), 2.00, 2.03, 2.05 and 2.17 (4s, 12H), 4.11 (1H, dd, $J=14.8$ Hz, $J=4.0$ Hz), 2.31 (1H, d, $J=14.9$ Hz), 2.42 (3H, s), 2.91 (1H, d, $J=18.8$ Hz), 3.22 (1H, d, $J=18.8$ Hz), 3.67 (1H, d, $J=5.9$ Hz), 3.91 (1H, bs), 4.07 (3H, s), 4.05–4.25 (4H, m), 4.49 (1H, s), 4.92 (1H, d, $J=12.3$ Hz), 4.98 (1H, d, $J=12.3$ Hz), 5.12 (1H, dd, $J=10.4$ Hz $J=3.1$ Hz), 5.19 (1H, d, $J=8.32$ Hz), 5.25 (1H, s), 5.35 (1H, t, $J=9.4$ Hz), 5.4 (1H, d, $J=3.1$ Hz), 5.49 (1H, d, $J=3.4$ Hz), 5.71 (1H, d, $J=8.3$ Hz), 7.14 (1H, s), 7.22 (2H, d, $J=7.9$ Hz), 7.31 (2H, d, $J=7.9$ Hz), 7.38 (1H, d, $J=8.5$ Hz), 7.78 (1H, t, $J=8.1$ Hz), 8.02 (1H, d, $J=7.7$ Hz), 13.25 (1H, s), 13.96 (1H, s).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl)phenyl] *O*-β-galactosyl carbamate (DAU-1C).** Analogously to the preparation of **DAU-1B** from 62 mg (59.1 μmol) of **DAU-16c**. Prodrug **DAU-1C** was isolated in 77% yield (40 mg), mp 206–210°C. Anal. calcd for $C_{42}H_{46}N_2O_{19}.2H_2O$: C, 54.90; H, 5.48; N, 3.05. Found: C, 54.90; H, 5.44; N, 3.10. MS (FAB+) *m/z* 906 ($[M+1+Na]^+$), 905 ($[M+Na]^+$). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 1.11 (3H, d, $J=6.4$ Hz), 1.46 (1H, dd, $J=12.8$ Hz $J=3.8$ Hz), 1.82 (1H, dt, $J=12.8$ Hz $J=3.5$ Hz), 2.08 (1H, dd, $J=14.1$ Hz, $J=5.7$ Hz), 2.19 (1H, d, $J=14.7$ Hz), 2.25 (3H, s), 2.93 (2H, t, $J=19.8$ Hz), 3.30–3.75 (8H, m), 3.97 (3H, s), 4.16 (1H, q, $J=6.6$ Hz), 4.50 (1H, d, $J=3.9$ Hz), 4.63 (1H, d, $J=5.0$ Hz), 5.68 (1H, d, $J=5.5$ Hz), 4.86 (2H, s), 4.92 (1H, t, $J=4.3$ Hz), 5.04 (1H, d, $J=4.6$ Hz), 5.20 (1H, d, $J=2.6$ Hz), 5.30 (1H, d, $J=8.0$ Hz), 5.51 (1H, s), 6.82 (1H, d, $J=8.0$ Hz), 7.23 (2H, d, $J=8.4$ Hz), 7.421 (2H, d, $J=8.3$ Hz), 7.62 (1H, t, $J=7.6$ Hz), 7.85–7.90 (2H, m), 9.83 (1H, s) 13.24 (1H, s), 14.00 (1H, s).

Synthesis of *N*-[4-(daunorubicin-*N*-carbonyl-1-oxyheptyl)-phenyl]*O*-β-glucuronyl carbamate sodium salt (DAU-2A)

4-(1-Hydroxyheptyl) bromobenzene (21 (Scheme 4)). 0.78 g (0.9 equiv) of Mg turnings, a crystal of I_2 and 30 mL of THF were brought into a three-necked flask connected with a dropping funnel and a reflux condenser under an argon atmosphere. From 6.26 g (=1 equiv) of *n*-hexyl bromide in 10 mL of THF in the dropping funnel, approximately one third was added to the reaction vessel. The Grignard reagent was agitated and the rest of the *n*-hexyl bromide solution was added. When the magnesium had almost disappeared, the reaction mixture was cooled to −10°C and 7.0 g (1.0 equiv) of 4-bromobenzaldehyde **20** in 10 mL of THF were added slowly. Stirring was continued for 10 min at −10°C and the reaction mixture was quenched on 20 g of ice, 10 mL of 15% H_2SO_4 and the reaction mixture was extracted with 350 mL portions of Et_2O. The organic layer was washed with brine and dried over Na_2SO_4. Purification by column chromatography (SiO_2-*n*-hexane) yielded 2.9 g, 34%, of **21** as an oil. ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.86 (3H, t, $J=5.6$ Hz), 1.10–1.70 (10H, m), 2.35 (1H, bs), 4.58 (1H, t, $J=6.3$ Hz), 7.17 (2H, d, $J=8.3$ Hz), 7.45 (2H, d, $J=8.3$ Hz).

4-(1-*t*-Butyldimethylsilyloxyheptyl) bromobenzene (22). 655 mg (2.42 mmol) of **21**, 247 mg (1.5 equiv) of imidazole, 1.46 g (4.0 equiv) of TBDMS-Cl and a catalytic amount of DMAP were dissolved in 20 mL of CH_2Cl_2 and stirred for 3 days under an argon atmosphere. After that, the reaction mixture was diluted with 200 mL of Et_2O, washed with 100 mL portions of, respectively, aqueous 0.5 N $NaHSO_4$, aqueous saturated $NaHCO_3$ and brine. The organic layer was dried over $NaSO_4$ and evaporated. The product was purified by means of column chromatography (SiO_2, *n*-hexane) to give 602 mg of **22**, 65%, as an oil. ^{1}H NMR (100 MHz, $CDCl_3$) δ −0.14 (3H, s), 0.02 (3H, s), 0.67 (9H, s), 0.70–1.75 (13H, m), 4.58 (1H, t, Alk1-H, $J=5.8$ Hz), 7.15 (2H, d, $J=8.5$ Hz), 7.42 (2H, d, $J=8.4$ Hz).

4-(1-*t*-Butyldimethylsilyloxyheptyl) benzoic acid (9). To 1.1 mL (1.6 equiv) of a 1.6 N *n*-BuLi solution and 1 mL of THF in a 3-necked flask connected with a dropping funnel under an argon atmosphere, 437 mg (=1.0 equiv) of **22** dissolved in 10 mL of THF were added slowly at −78°C. The reaction mixture was stirred at −50°C for 15 min and carefully added to a few grams of solid CO_2 in 10 mL of THF under an argon atmosphere. The reaction mixture was quenched with ice and 20 mL of aqueous 0.5 N $KHSO_4$ were added. The product was extracted with three 100 mL portions of Et_2O and the combined organic layer was washed with brine and dried over Na_2SO_4. The product was purified by means

of column chromatography (SiO_2, CH_2Cl_2/EtOH, 10/1) to yield 179 mg, 45%, of **9** as an oil. ^{1}H NMR (100 MHz, $CDCl_3$) δ −0.14 (3H, s), 0.03 (3H, s), 0.88 (9H, s), 0.70–1.70 (13H, m), 4.69 (1H, t, J = 5.5 Hz), 7.37 (2H, d, J = 8.1 Hz), 8.04 (2H, d, J = 8.1 Hz).

***N*-[4-(1-*t*-Butyldimethylsilyloxyheptyl)phenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (13a).** Analogously to the preparation of **12a** from 189 mg (0.539 mmol) of **9** and 135 mg (0.75 equiv) of **18a**. After column chromatography (SiO_2, Et_2O/*n*-hexane, 1/1) compound **13a** was isolated in 73% (201 mg) (calculated from **18a**) as an oil. Due to the racemic starting material, a diastereomeric mixture of **13a** must be formed, conversely, this was not observed in the proton NMR spectrum. ^{1}H NMR (100 MHz, $CDCl_3$) δ −0.23 (3H, s), 0.07 (3H, s, 0.59 (9H, s), 0.52–1.63 (13H, m), 1.98 (bs, 9H), 3.66 (3H, s), 4.15 (1H, d, J = 9.4 Hz), 4.51 (1H, t, J = 6.3 Hz), 5.10–5.45 (3H, m,), 5.73 (1H, d, J = 7.6 Hz), 6.95 (1H, bs), 7.13 (2H, d, J = 8.7 Hz), 7.03 (2H, d, J = 8.7).

***N*-[4-(1-Hydroxyheptyl)phenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (15a).** Analogously to the preparation of **14a** from 226 mg (0.331 mmol) of **13a**. Compound **15a** was isolated in 92% yield (173 mg) as an oil. ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.80–1.90 (13H, m), 2.05 (9H, s), 3.73 (3H, s), 4.19 (1H, d, J = 8.9 Hz), 4.63 (1H, t, J = 6.8 Hz), 5.15–5.30 (3H, m), 5.76 (1H, d, J = 7.4 Hz), 7.12 (1H, bs), 7.22 (4H, bs).

***N*-[4-(Daunorubicin-*N*-carbonyl-(1-oxyheptyl))phenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (DAU-17a).** 173 mg (0.305 mmol) of **15a**, 109 mg (2.0 equiv) of *N*-succinimidyl chloroformate and 74 μL (3.0 equiv) of pyridine in 10 mL of CH_2Cl_2 were stirred for 2 h at room temperature under an argon atmosphere. A solution of 431 mg (2.5 equiv) of **DAU**-HCl, 333 μL of *i*-Pr_2NEt (6.0 equiv) in 10 mL of DMF was added and stirring was continued for 18 h. The product was purified as described for **DAU-16a** to give 122 mg, 36% of **DAU-17a**, mp 71°C. Because of chirality of the spacer -ArC*H(alkyl)(O)-, a diastereomeric mixture was formed in a ratio of 1/1, determined by dividing the integrals of the clear singlets of the respective ArN*H*-signals found at 6.77 and 6.86 ppm. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.80–1.90 (18H, m), 2.00–2.05 (9H, m), 2.09 (1H, dd, J = 15.2 Hz J = 3.7 Hz), 2.30 (1H, d, J = 14.9 Hz), 2.40 (3H, bs), 2.88 (1H, d, J = 18.8 Hz), 3.20 (1H, d, J = 18.8 Hz), 3.65–3.90 (5H, m), 4.06 and 4.09 (3H, s), 4.22 (1H, bd, J = 9.8 Hz), 4.15–4.25 (1H, m), 4.48 (1H, s), 4.90 (7H, m), 5.79 (1H, bd, J = 8.0 Hz), 6.77 and 6.86 (1H, s), 7.15–7.25 (4H, m), 7.38 (1H, bd, J = 9.4 Hz), 7.77 (bt, 1H, J = 9.0 Hz), 8.02 (1H, bd, J = 7.5 Hz), 13.26 and 13.28 (1H, s), 13.95 and 13.98 (1H, s).

***N*-[4-(Daunorubicin-*N*-carbonyl-(1-oxyheptyl))phenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-2A).** Analogously to the preparation of **DAU-1A** (except that the product was eluted from the reversed-phase column with CH3CN/H_2O, 1/2) from 60 mg (0.054 mmol) of **DAU-17a**. Prodrug **DAU-2A** was isolated in 39% yield (21 mg), mp 193°C (dec.). Anal. calcd for $C_{48}H_{55}N_2O_{20}Na.5H_2O$: C, 52.75; H, 5.99; N, 2.56. Found: C, 52.73; H, 5.69; N, 2.70. MS (FAB$^+$) m/z 1026 ([M + 1 + Na]$^+$), 1025 ([M + Na]$^+$), 1004 ([M + 1 + H]$^+$), 1003 ([M + H]$^+$). Because of chirality of the spacer −ArC*H (alkyl)(O)-, a diastereomeric mixture was formed in a ratio of 1/0.2, determined by dividing the integrals of the clear singlets of the respective ArN*H*- signals found at 9.78 and 9.84 ppm in the proton-NMR spectrum. ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 0.76 and 0.81 (2H, t, J = 6.8 Hz resp. 6.7 Hz), 1.05–1.25 (13H, m), 1.39 and 1.46 (1H, dd, J = 12.3 Hz, J = 4.1 Hz resp. 12.3 and 4.0 Hz), 1.79 (1H, dt, J = 12.9 Hz, J = 3.9 Hz), 2.08 (1H, m), 2.15 (1H, d, J = 13.4 Hz), 2.23 (3H, s), 2.90 (1H, d, J = 18.4 Hz), 2.97 (1H, d, J = 18.4 Hz), 3.00–3.45 (4H, m), 3.65 (1H, m), 3.97 and 3.98 (s), 4.12 (1H, q, J = 6.2 Hz), 4.66 and 4.68 (1H, d, J = 5.4 Hz resp. J = 5.5 Hz), 4.91 (1H, t, J = 4.3 Hz), 5.00 (1H, t, J = 4.7 Hz), 5.18 and 4.21 (1H, d, J = 1.6 Hz resp J = 2.4 Hz), 5.10–5.15 (1H, m), 5.25 (1H, d, J = 8.0 Hz), 5.50 (1H, s), 6.72 (1H, d, J = 7.8 Hz), 7.16 and 7.20 (2H, d, J = 8.5 Hz resp. J = 8.5 Hz), 7.37 and 7.41 (2H, d, J = 8.2 Hz resp. J = 8.3 Hz), 7.63 (1H, m), 7.85–7.95 (2H, m), 9.78 and 9.84 (1H, s), 13.19 (1H, s), 14.01 (1H, s).

Synthesis of *N*-[2-(daunorubicin-*N*-carbonyl oxymethyl)-phenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-3A)

***N*-[2-(*t*-Butyldimethylsilyloxymethyl)phenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (*ortho*-12a).** Analogously to the preparation of **12a** from 400 mg (1.50 mmol) of 19[48] (Chart 3). Compound ***ortho*-12a** was isolated in 69% yield (310 mg) (calculated from **18a**), mp 92–94°C. ^{1}H NMR (100 MHz, $CDCl_3$) δ 0.00 (3H, s, $Si\mathit{Me}_a\mathrm{Me}_b$-), 0.05 (3H, s), 0.83 (9H, s), 1.96 (9H, s), 3.66 (3H, s), 4.16 (1H, d, J = 9.4 Hz), 4.60 (1H, d, J = 18.1 Hz), 4.72 (1H, d, J = 18.1 Hz), 5.01–5.32 (3H, m), 5.76 (1H, d, J = 7.8 Hz), 6.90–7.35 (3H, m), 7.90 (1H, d, J = 8.0 Hz), 8.61 (1H, s).

***N*-[2-(Hydroxymethyl)phenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (*ortho*-14a).** Analogously to the preparation of **14a**, from 310 mg (0.52 mmol) of *ortho*-12a. Compound ***ortho*-14a** was isolated in 68% yield (171 mg), mp 132°C. **1**H NMR (100 MHz, $CDCl_3$) δ 1.96 (9H, s), 3.64 (3H, s), 4.11 (1H, d, J = 9.4 Hz), 4.59 (2H, s), 5.09–5.21 (3H, m), 5.74 (1H, d, J = 7.5 Hz), 6.95–7.30 (3H, m), 7.70 (1H, d, J = 7.7 Hz), 8.15 (1H, s).

***N*-[2-(Daunorubicin-*N*-carbonyl oxymethyl)phenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (*ortho*-DAU-16a).** 100 mg (0.213 mmol) of ***ortho*-14a** was stirred with 43 mg (1.1 equiv) of succinimidyl chloroformate and 21 μL (1.05 equiv) of pyridine in 10 mL of CH_2Cl_2 and coupled to 144 mg (1.2 equiv) of **DAU**-HCl using 73 μL (2 equiv) of *i*-Pr_2NEt and 10 mL of DMF (analogously to **DAU-16a**). 62% (133 mg) of ***ortho*-DAU-16a** was obtained, mp 151–153°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (3H, d, J = 6.5 Hz), 1.80–1.85 (3H, m) 2.00, 2.04 and 2.05 (3s, 9H), 2.16 (1H, dd, J = 14.8 Hz, J = 4.1 Hz), 2.33 (1H, d, J = 15.2 Hz), 2.43 (3H, s), 2.95 (1H, d, J = 18.9 Hz), 3.23 (1H, d, J = 18.9 Hz), 3.67 (1H, d, J = 6.7 Hz), 3.72 (3H, s), 3.90 (1H, m), 4.07 (3H, s), 4.20–4.25 (2H, m), 4.46 (1H, s), 4.96 (1H, d, J = 12.6 Hz), 5.07 (1H, d, J = 12.6 Hz), 5.16 (1H, t,

$J = 8.0$ Hz), 5.25–5.35 (4H, m), 5.50 (1H, m), 5.80 (1H, d, $J = 7.5$), 7.08 (1H, t, $J = 7.4$ Hz), 7.26 (1H, d, $J = 8.8$ Hz), 7.32 (1H, t, $J = 7.7$ Hz), 7.39 (1H, d, $J = 8.5$ Hz), 7.78 (1H, t, $J = 8.1$ Hz), 7.84 (1H, m), 8.03 (1H, d, $J = 7.7$ Hz), 8.20 (1H, s), 13.28 (1H, s), 13.97 (1H, s)

***N*-[2-(Daunorubicin-*N*-carbonyl oxymethyl)phenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-3A).** Analogously to the preparation of **DAU-1A** from 20 mg (19 μmol) of ***ortho*-DAU-16a**. Prodrug **DAU-3A** was isolated in 69% yield (12.2 mg), mp 171°C (dec). Anal. calcd for $C_{42}H_{43}N_2O_{20}Na.3.5H_2O$: C, 51.38; H, 5.13; N, 2.85. Found: C, 51.36; H, 4.86; N, 2.69. MS (FAB$^+$) m/z 942 ([M + 1 + Na]$^+$), 941 ([M + Na]$^+$), 920 ([M + 1 + H]$^+$), 919 ([M + H]$^+$). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 1.12 (3H, d, $J = 6.4$ Hz), 1.46 (1H, dd, $J = 12.6$ Hz), 1.85 (1H, dt, $J = 12.6$ Hz, $J = 3.5$ Hz), 2.11 (1H, dd, $J = 14.4$ Hz, $J = 5.8$ Hz), 2.20 (1H, d, $J = 14.4$ Hz), 2.26 (3H, s), 2.93 (1H, d, $J = 18.2$ Hz), 2.99 (1H, d, $J = 18.2$ Hz), 3.15–3.65 (4H, m), 3.72 (1H, m), 3.99 (3H, s), 4.17 (1H, q, $J = 6.4$ Hz), 4.70–4.90 (2H, m), 4.94 (1H, t, $J = 4.4$ Hz), 4.98 (2H, s), 5.20–5.25 (2H, m), 5.22 (1H, d, $J = 3.1$ Hz), 5.32 (1H, d, $J = 5.5$ Hz), 5.34 (1H, d, $J = 8.0$ Hz), 5.55 (1H, s), 7.03 (1H, d, $J = 8.0$ Hz), 7.15 (1H, t, $J = 7.3$), 7.27 (1H, t, $J = 7.7$), 7.33 (1H, d, $J = 7.6$ Hz), 7.40 (1H, d, $J = 7.8$ Hz), 7.66 (1H, dd, $J = 5.9$ Hz $J = 3.9$ Hz), 7.88–7.94 (2H, m), 9.25 (1H, s) 13.29 (1H, s), 14.04 (1H, s).

N-[4-(Daunorubicin-*N*-carbonyl oxymethyl) 2-chlorophenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-4A)

***N*-(4-Methyl 2-chlorophenyl) *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (25d).** 4-Methyl-2-chlorophenyl isocyanate **24d** was prepared from 500 mg of 4-methyl-2-chloroaniline-HCl **23d** by refluxing it for 2 h with 339 μL (1.0 equiv) of diphosgene in 20 mL of PhMe under an argon atmosphere. The reaction mixture was allowed to cool to ambient temperature and 1.5 mL (ca. 4 equiv) of Et_3N were added to combine with the liberated HCl. 469 mg (0.5 equiv) of **18a** were added. After 0.5 h, **18a** had disappeared and the reaction mixture was taken to dryness (100% β-isomer by ^{1}H NMR of the crude reaction mixture) dissolved in Et_2O and washed with 5% aqueous $KHSO_4$, saturated aqueous $NaHCO_3$, brine, dried over Na_2SO_4 and evaporated. The crude product was purified by column chromatography (SiO_2, Et_2O/*n*-hexane, 1/1, to elute the apolar symmetrical urethane and Et_2O to elute the product) and the product fraction was crystallized from *i*-Pr_2O to give 645 mg, 92%, of **25d** as white crystals, mp 142–143°C. ^{1}H NMR (100 MHz, $CDCl_3$) δ 2.05 (9H, s), 2.29 (3H, s), 3.74 (3H, s), 4.26 (1H, d, $J = 9.5$ Hz), 5.10–5.55 (3H, m), 5.86 (1H, d, $J = 7.4$ Hz), 7.07 (1H, d, $J = 8.3$ Hz), 7.10–7.30 (2H, m,), 7.92 (1H, d, $J = 8.3$ Hz).

***N*-[4-(Bromomethyl) 2-chlorophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (26d).** Compound **25d** (325 mg, 0.65 mmol) was dissolved in 10 mL of CCl_4 and 127 mg (1.1 equiv) of NBS and a catalytic amount of AIBN was added. The solution was radiated for 1 h using a 250 W lamp and the temperature raised to reflux. After cooling, the reaction mixture was filtered, evaporated and dried under reduced pressure (0.1 mm Hg) to give the crude **26d** which was used in the next step without further purification. ^{1}H NMR (100 MHz, $CDCl_3$) δ 2.05 (9H, s), 3.75 (3H, s), 4.25 (1H, d, $J = 9.2$ Hz), 4.42 (2H, s), 5.10–5.45 (3H, m), 5.85 (1H, d, $J = 7.6$ Hz), 7.10–7.50 (3H, m), 8.09 (1H, d, $J = 8.4$ Hz).

***N*-[4-(Hydroxymethyl) 2-chlorophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (27d).** The crude **26d** was dissolved in 10 mL of acetone and 10 mL (3 equiv) of an aqueous 0.2 N $AgNO_3$ solution were added. After 2 days, the mixture was filtered and evaporated. The resulting oil was redissolved in CH_2Cl_2 and washed with saturated aqueous $NaHCO_3$ (2×), with brine and dried over Na_2SO_4. After the CH_2Cl_2 had been evaporated, **27d** was crystallized from Et_2O to give 175 mg, 52%, from **25d**, as white crystals. ^{1}H NMR (100 MHz, $CDCl_3$) δ 1.98 (9H, s), 3.67 (3H, s), 4.16 (1H, d, $J = 9.3$ Hz), 4.56 (2H, s), 5.00–5.40 (3H, m), 5.75 (1H, d, $J = 7.5$ Hz), 7.17 (1H, d, $J = 8.4$ Hz), 7.31 (2H, bs), 7.37 (1H, d, $J = 8.4$ Hz).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl) 2-chlorophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (DAU-31d).** Analogously to the preparation of **DAU-16a** from 45 mg (0.87 mmol) of **27d**. Compound **DAU-31d** was isolated in 28% yield (26 mg), mp 150–155°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.29 (3H, d, $J = 6.6$ Hz), 1.74 (1H, dt, $J = 13.1$ Hz, $J = 4.1$ Hz), 1.90 (1H, dd, $J = 13.1$ Hz $J = 4.8$ Hz), 2.05 (9H, s), 2.12 (1H, dd, $J = 14.9$ Hz, $J = 4.1$ Hz), 2.32 (1H, d, $J = 14.8$ Hz), 2.41 (3H, s), 2.95 (1H, d, $J = 18.8$ Hz), 3.25 (1H, d, $J = 18.8$ Hz), 3.65 (1H, s), 3.73 (3H, s), 3.87 (1H, m), 4.08 (3H, s), 4.21 (1H, d, $J = 9.5$ Hz), 4.20–4.25 (1H, m), 4.44 (1H, s), 4.95 (1H, d), 5.10–5.30 (4H, m), 5.35 (1H, t, $J = 8.9$ Hz), 5.49 (1H, d, $J = 3.7$ Hz), 5.81 (1H, d, $J = 7.9$ Hz), 7.21 (1H, d, $J = 8.3$ Hz), 7.26 and 7.34 (2s, 2H), 7.40 (1H, d, $J = 8.4$ Hz), 7.79 (1H, t, $J = 8.1$ Hz), 8.00–8.10 (2H, m), 13.29 (1H, s), 13.99 (1H, s).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl) 2-chlorophenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-4A).** Analogously to the preparation of **DAU-1A** from 26 mg (0.024 mmol) of **DAU-31d**. Prodrug **DAU-4A** was isolated in 37% (8.5 mg), mp 175–179°C. Anal. calcd for $C_{42}H_{42}N_2O_{20}ClNa.5H_2O$: C, 48.38; H, 5.02; N, 2.69. Found: C, 48.22; H, 4.70; N, 3.08. MS (FAB$^+$) m/z 975 ([M + Na]$^+$), 955 ([M + 2 + H]$^+$), 954 ([M + 1 + H]$^+$), 953 ([M + H]$^+$). ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 1.12 (3H, d, $J = 6.4$ Hz), 1.48 (1H, d, $J = 12.6$), 1.85 (1H, t, $J = 13.4$ Hz), 2.05 (2H, m), 2.25 (3H, s), 2.90 (6H, m), 3.73 (1H, m), 3.98 (3H, s), 4.16 (1H, m), 4.73 (1H, d, $J = 5.1$ Hz), 4.85 (2H, m), 5.00 (1H, d, $J = 5.2$ Hz), 5.23 (1H, bs), 5.27 (1H, d, $J = 7.9$ Hz), 5.53 (1H, s), 7.27 (1H, d, $J = 7.3$ Hz), 7.44 (1H, s,), 7.57 (1H, d, $J = 8.12$ Hz), 7.64 (1H, m), 7.85–8.00 (2H, m), 9.22 (1H, s).

Synthesis of *N*-[4-(daunorubicin-*N*-carbonyl oxymethyl) 3-chlorophenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-5A)

***N*-(4-Methyl 3-chlorophenyl) *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (25e).** 200 mg (1.19 mmol) of

commercially available 4-methyl-3-chlorophenyl isocyanate **24e** was dissolved in 10 mL of PhMe under an argon atmosphere. 199 mg (0.5 equiv) of **18a** and one drop of Et_3N were added. The course of the reaction was followed by means of TLC (SiO_2, Et_2O). After **18a** had disappeared, the reaction mixture was taken to dryness (α/β of the crude reaction mixture was 1/10 by ^1H NMR) and sonicated in Et_2O. The solid material was removed and the solution was concentrated and refrigerated overnight. 434 mg, 81%, of **25e** was obtained as white crystals, mp 155°C. The α-isomer stayed in solution. ^1H NMR (100 MHz, $CDCl_3$) δ 1.99 (9H, s), 2.24 (3H, s, 3.66 (3H, s), 4.16 (1H, d, $J=9.4$ Hz), 5.00–5.45 (3H, m), 5.71 (1H, d, $J=7.6$ Hz), 6.90–7.15 (3H, m), 7.39 (1H, s).

***N*-[4-(Bromomethyl) 3-chlorophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl β-glucuronyl) carbamate (26e).** Analogously to the preparation of **26d** from 332 mg (0.66 mmol) of **25e**. The crude **26e** which was obtained was used in the next step without further purification. ^1H NMR (100 MHz, $CDCl_3$) δ 1.99 (9H, s), 3.66 (3H, s), 4.20 (1H, d, $J=8.9$ Hz), 4.47 (2H, s), 5.05–5.45 (3H, m), 5.71 (1H, d, $J=7.5$ Hz), 6.90–7.50 (3H, m), 7.63 (1H, s).

***N*-[4-(Hydroxymethyl) 3-chlorophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (27e).** Analogously to the preparation of **27d** from the crude **26e** obtained as described above. After workup the crude product was sonicated in *i*-Pr_2O and filtered to give **27e** in 76% yield (259 mg) (calculated from **25e**). ^1H NMR (100 MHz, $CDCl_3$) δ 1.99 (9H, s), 3.66 (3H, s), 4.16 (1H, d, $J=9.3$ Hz), 4.63 (2H, s), 5.00–5.45 (3H, m), 5.69 (1H, d, $J=7.5$ Hz), 7.10–745 (3H, m), 7.55 (1H, s).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl) 3-chlorophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (DAU-31e).** Analogously to the preparation of **DAU-16a** from 100 mg (0.193 mmol) of **27e**. Compound **DAU-31e** was obtained in 76% yield (82 mg), mp 154–158°C. ^1H NMR (400 MHz, $CDCl_3$) δ 1.29 (3H, d, $J=6.5$ Hz), 1.78 (1H, dt, $J=13.0$ Hz $J=3.9$ Hz), 1.85–1.90 (2H, m), 2.05 (9H, s), 2.05–2.15 (1H, m), 2.28 (1H, d, $J=14.9$ Hz), 2.42 (3H, s), 2.87 (1H, d, $J=19.0$ Hz), 3.19 (1H, d, $J=18.8$ Hz), 3.68 (1H, bs), 3.74 (3H, s), 3.87 (1H, bs), 4.05 (3H, s), 4.15–4.20 (1H, m), 4.24 (1H, d, $J=9.8$ Hz), 4.46 (1H, s), 4.97 (1H, d, $J=12.7$ Hz), 5.01 (1H, d, $J=12.7$ Hz), 5.12–5.40 (4H, m), 5.48 (1H, d, $J=2.4$ Hz), 5.73 (1H, d, $J=8.0$ Hz), 7.00–7.50 (5H, m), 7.76 (1H, t, $J=8.1$ Hz), 8.00 (1H, d, $J=8.2$ Hz), 13.24 (1H, s), 13.95 (1H, s).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl) 3-chlorophenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-5A).** Analogously to the preparation of **DAU-1A** from 45 mg (42 μmol) of **DAU-31e**. Prodrug **DAU-5A** was obtained in 67%, (27 mg), mp 205°C (dec.). Anal. calcd for $C_{42}H_{42}N_2O_{20}ClNa.6H_2O$: C, 47.53; H, 5.13; N, 2.64. Found: C, 47.25; H, 4.75; N, 2.64. MS (FAB$^+$) *m*/*z* 977 ($[M+2+Na]^+$), 976 ($[M+1+Na]^+$), 975 ($[M+Na]^+$), 955 ($[M+2+H]^+$), 954 ($[M+1+H]^+$), 953 ($[M+H]^+$). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 1.11 (3H, d, $J=6.4$ Hz), 1.46 (1H, dd, $J=12.0$ Hz, $J=3.6$ Hz), 1.81 (1H, dd, $J=12.7$ Hz, $J=3.2$ Hz), 2.08 (1H, dd, $J=13.9$ Hz $J=5.2$ Hz), 2.17 (1H, d, $J=13.6$ Hz), 2.24 (3H, s), 3.00 (6H, m 4′-H), 3.70 (1H, m), 3.96 (3H, s), 4.13 (1H, q, $J=6.5$ Hz), 4.69 (1H, d, $J=3.9$ Hz), 4.94 (2H, s), 5.07 (1H, bs), 5.21 (1H, s), 5.28 (1H, d, $J=8.1$ Hz), 5.51 (1H, s), 6.95 (1H, d, $J=7.9$ Hz), 7.23 (1H, d, $J=8.4$ Hz), 7.35–7.40 (2H, m), 7.55–7.60 (2H, m), 7.80–7.95 (2H, m), 10.14 (1H, s).

Synthesis of *N*-[4-(daunorubicin-*N*-carbonyl oxymethyl) 2-bromophenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-6A)

***N*-[4-(Methyl) 2-bromophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (25f).** Analogously to the preparation of **25d** from 263 mg (1.20 mmol) of **23f**. The crude product was purified by means of column chromatography (SiO_2, Et_2O/*n*-hexane, 1/1) to give 310 mg (95% from **18a**) of **25f**, mp 139°C. ^1H NMR (100 MHz, CDCl3) δ 2.05 (9H, s), 2.30 (3H, s), 3.75 (3H, s), 4.22 (1H, d, $J=9.1$ Hz), 5.10–5.45 (3H, m), 5.83 (1H, d, $J=7.8$ Hz), 7.15–7.45 (2H, m), 7.35 (1H, d, $J=2.0$ Hz) 7.93 (1H, d, $J=8.3$ Hz).

***N*-[4-(Bromomethyl) 2-bromophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (26f).** Analogously to the preparation of **26d** from 250 mg (0.458 mmol) of **25f**. The crude bromide **26f** which was obtained was used without further purification in the next step. ^1H NMR (100 MHz, $CDCl_3$) δ 2.05 (9H, s), 3.75 (3H, s), 4.23 (1H, d, $J=9.1$ Hz), 4.42 (3H, s), 5.10–5.45 (3H, m), 5.83 (1H, d, $J=7.8$ Hz), 7.35 (1H, dd, $J=8.4$ Hz $J=2.0$ Hz), 7.40 (1H, s), 7.59 (1H, d, $J=2.0$ Hz), 8.09 (1H, d, $J=8.4$ Hz).

***N*-[4-(Hydroxymethyl) 2-bromophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (27f).** Analogously to the preparation of **27d** from 0.458 mmol of crude **26f** described above. Compound **27f** was obtained in 80% (206 mg) (calculated from **25f**) as an oil. ^1H NMR (100 MHz, $CDCl_3$) δ 2.04 (9H, s), 3.75 (3H, s), 4.22 (1H, d, $J=9.2$ Hz), 4.65 (3H, s), 5.20–5.39 (3H, m), 5.83 (1H, d, $J=7.5$ Hz), 7.20–7.40 (2H, m), 7.57 (1H, d, $J=1.8$ Hz), 8.07 (1H, d, $J=8.1$ Hz).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl) 2-bromophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (DAU-31f).** Analogously to the preparation of **DAU-16a** from 178 mg (0.317 mmol) of **27f**. Compound **DAU-31f** was obtained in 14% yield (51 mg), mp 149°C. ^1H NMR (400 MHz, $CDCl_3$) δ 1.22 (3H, d, $J=6.4$ Hz), 1.58 (1H, s), 1.70 (1H, dt, $J=13.2$ Hz, $J=4.2$ Hz), 1.82 (1H, dd, $J=13.3$ Hz, $J=5.1$ Hz), 1.95 (9H, s), 2.04 (1H, dd, $J=14.9$ Hz, $J=4.1$ Hz), 2.24 (1H, d, $J=14.4$ Hz), 2.34 (3H, s), 2.83 (1H, d, $J=18.8$ Hz), 3.16 (1H, d, $J=18.8$ Hz), 3.59 (1H, m), 3.68 (3H, s), 3.81 (1H, m), 4.01 (3H, s), 4.15 (1H, d, $J=9.6$ Hz), 4.10–4.20 (1H, m), 4.38 (1H, s), 4.86 (1H, d, $J=12.5$ Hz), 4.89 (1H, d, $J=12.5$ Hz), 5.05–5.25 (4H, m), 5.28 (1H, t, $J=9.3$ Hz), 5.42 (1H, d, $J=3.7$ Hz), 5.74 (1H, d, $J=8.0$ Hz), 7.15–7.25 (2H, m), 7.32 (1H, d, $J=8.6$ Hz), 7.41 (1H, s,), 7.71 (1H, t, $J=8.0$ Hz), 7.96 (1H, d, $J=7.6$ Hz), 7.90–8.00 (1H, m), 13.19 (1H, s), 13.90 (1H, s).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl) 2-bromophenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-6A).** Analogously to the preparation of **DAU-1A** from 37 mg (33 μmol) of **DAU-31f**. Prodrug **DAU-6A** was obtained in 34% yield (11.2 mg), mp 213°C (dec). Anal. calcd for $C_{42}H_{42}N_2O_{20}BrNa.3H_2O$: C, 47.96; H, 4.60; N, 2.66. Found: C, 47.75; H, 4.59; N, 3.08. MS (FAB^+) *m/z* 1021 ($[M+2+Na]^+$), 1019 ($[M+Na]^+$), 999 ($[M+2+H]^+$), 997 ($[M+H]^+$). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 1.13 (3H, d, *J* = 6.5 Hz), 1.48 (1H, dd, *J* = 12.4 Hz, *J* = 5.3 Hz), 1.83 (1H, dt, *J* = 13.3 Hz *J* = 3.5 Hz), 2.10–2.20 (2H, m), 2.26 (3H, s), 2.94 (1H, d, *J* = 18.5 Hz), 3.10–3.65 (5H, m 4′-H), 3.73 (1H, m), 3.99 (3H, s), 4.18 (1H, q, *J* = 6.3 Hz), 4.72 (1H, d, *J* = 5.6 Hz), 4.75–5.20 (2H, m), 4.93 (2H, s), 5.22 (1H, d, *J* = 3.5 Hz), 5.27 (1H, d, *J* = 8.1 Hz), 5.55 (1H, s), 6.98 (1H, d, *J* = 8.0 Hz), 7.31 (1H, d, *J* = 8.4 Hz), 7.52 (1H, d, *J* = 8.2 Hz), 7.61 (1H, s,), 7.67 (1H, t, *J* = 7.8 Hz), 7.90–8.00 (2H, m). 8.31 (1H, s).

Synthesis of *N*-[4-(daunorubicin-*N*-carbonyl oxymethyl) 3-bromophenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-7A)

3-Bromo terephthalic acid allyl ester (28). Bromoterephthalic acid (3.0 g, 12 mmol) was dissolved in 150 mL of THF under an argon atmosphere and 1.25 mL (1.5 equiv) of AllOH and a catalytic amount of DMAP was added. After cooling the mixture to 0°C, 2.2 g (1.0 equiv) of DCC were added and the reaction mixture was stirred at 0°C for 2 h and overnight at room temperature. The reaction mixture was filtrated and evaporated, the product was purified by means of column chromatography (SiO_2, CH_2Cl_2/EtOH, 10/1) and crystallized from *n*-hexane to yield 893 mg, 26%, of **28** as white needles, mp 117°C. ^1H NMR (100 MHz, $CDCl_3$) δ 4.88 (2H, d, *J* = 5.6 Hz), 5.30–5.55 (2H, m), 5.90–6.25 (1H, m), 7.79 (1H, d, *J* = 8.0 Hz), 8.10 (1H, dd, *J* = 8.0 Hz, *J* = 1.5 Hz), 8.40 (1H, d, *J* = 1.5 Hz).

***N*-[4-(Carboxylic acid allyl ester) 3-bromophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (30).** 341 mg (1.20 mmol) of **28**, 284 μL (1.1 equiv) of $(PhO)_2P(O)N_3$ and 183 μL (1.1 equiv) of Et_3N were stirred overnight at room temperature under an argon atmosphere in 15 mL of PhMe. Subsequently, the reaction mixture was stirred at 85°C for 1 h, cooled to ambient temperature and 197 mg (0.5 equiv) of anomerically unprotected glucuronic acid **18a** were added and the mixture was treated as for **12a**. The crude product was purified by means of column chromatography (SiO_2, Et_2O/*n*-hexane, 3/1) to give 294 mg, 80%, of **30** as a white powder, mp 61°C. ^1H NMR (100 MHz, $CDCl_3$) δ 2.05 (9H, s), 3.72 (3H, s), 4.24 (1H, d, *J* = 9.5 Hz), 4.79 (2H, d, *J* = 5.6 Hz), 5.10–5.50 (5H, m), 5.77 (1H, d, *J* = 7.6 Hz), 5.70–6.25 (1H, m), 7.34 (1H, dd, *J* = 8.6 Hz, *J* = 2.2 Hz), 7.58 (1H, s), 7.74 (1H, d, *J* = 2.2 Hz), 7.83 (1H, d, *J* = 8.6 Hz)

***N*-[4-(Carboxylic acid) 3-bromophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate.** 263 mg (0.427 mmol) of **30**, 186 μL (5 equiv) of morpholine in 20 mL of THF were stirred for 2 h at room temperature while bubbling argon through the solution. After that, a catalytic amount of $Pd(PPh_3)_4$ was added. After 15 min **30** had disappeared on TLC (SiO_2, Et_2O) and the reaction mixture was diluted with 100 mL of Et_2O and washed with 100 mL portions of aqueous 0.5 N $KHSO_4$ (3×) and with brine. The organic layer was dried over Na_2SO_4 and evaporated to obtain 246 mg of the carboxylic acid in a quantitative yield, mp 110°C. This was used without further purification in the next step. ^1H NMR (100 MHz, $CDCl_3$) δ 2.04 (9H, s), 3.73 (3H, s), 4.23 (1H, d, *J* = 9.5 Hz), 5.10–5.40 (3H, m), 5.77 (1H, d, *J* = 7.6 Hz), 7.36 (1H, dd, *J* = 8.6 Hz *J* = 2.2 Hz), 7.56 (1H, s), 7.79 (1H, d, *J* = 2.2 Hz) 7.96 (1H, d, *J* = 8.6 Hz).

***N*-[4-(Hydroxymethyl) 3-bromophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (27g).** 153 mg (0.265 mmol) of the latter carboxylic acid were stirred in 15 mL of THF at room temperature under an argon atmosphere. 1.33 mL (5.0 equiv) of a 1 M BH3-THF solution were added slowly over a period of 20 min. The reaction mixture was stirred overnight at room temperature. When the acid had almost disappeared on TLC (SiO_2, Et_2O), the reaction mixture was quenched with 15 mL of H_2O. When gas evolution ceased, the reaction mixture was diluted with 200 mL of EtOAc and washed with 100 mL portions of saturated aqueous $NaHCO_3$ (3×) and with brine. The organic layer was dried over Na_2SO_4 and evaporated to yield 140 mg, 94%, of the pure **27g** as an oil, ^1H NMR (100 MHz, $CDCl_3$) δ 2.00 (9H, s), 3.67 (3H, s), 4.14 (1H, d, *J* = 9.6 Hz), 4.63 (2H, s), 5.00–5.30 (3H, m), 5.69 (1H, d, *J* = 7.6 Hz), 7.15–7.40 (3H, m), 7.63 (1H, d, *J* = 2.2 Hz).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl) 3-bromophenyl] *O*-(methyl 2,3,4-tri-*O*-acetyl-β-glucuronyl) carbamate (DAU-31g).** Analogously to the preparation of **DAU-16a** from 50 mg (89 μmol) of **27g**. Compound **DAU-31g** was obtained in 56% (56 mg), mp 147°C. ^1H NMR (400 MHz, $CDCl_3$) δ 1.13 (3H, d, *J* = 6.5 Hz), 1.62 (1H, s), 1.81 (1H, dt, *J* = 13.2 Hz, *J* = 3.3 Hz), 1.89 (1H, dd, *J* = 13.2 Hz, *J* = 5.0 Hz), 2.06 (9H, s), 2.09 (1H, dd, *J* = 14.7 Hz, *J* = 3.8 Hz), 2.30 (1H, d, *J* = 14.7 Hz), 2.42 (3H, s), 2.88 (1H, d, *J* = 18.8 Hz), 3.20 (1H, d, *J* = 18.7 Hz), 3.69 (1H, m), 3.72 (3H, s), 3.90 (1H, m), 4.04 (3H, s), 4.20–4.25 (1H, m), 4.50 (1H, s), 4.95 (1H, d, *J* = 12.8 Hz), 5.00 (1H, d, *J* = 12.8 Hz), 5.15–5.30 (4H, m), 5.40 (1H, t, *J* = 9.4 Hz), 5.47 (1H, d, *J* = 2.7 Hz), 5.75 (1H, d, *J* = 8.0 Hz), 7.08 and 7.16 (2H, 2d, *J* = 8.2 Hz), 7.36 (1H, d, *J* = 8.5 Hz), 7.54 and 7.60 (2H, 2s), 7.76 (1H, t, *J* = 8.0 Hz), 7.99 (1H, d, *J* = 7.8 Hz), 13.22 (1H, s), 13.94 (1H, s).

***N*-[4-(Daunorubicin-*N*-carbonyl oxymethyl) 3-bromophenyl] *O*-β-glucuronyl carbamate sodium salt (DAU-7A).** Analogously to the preparation of **DAU-1A** from 41.5 mg (37.2 μmol) of **DAU-31g**. Prodrug **DAU-7A** was obtained in 50% yield (18.7 mg), mp 181°C. Anal. calcd for $C_{42}H_{42}N_2O_{20}BrNa.5H_2O$: C, 46.38; H, 4.82; N, 2.58. Found: C, 46.37; H, 4.49; N, 2.68. MS (FAB^+) *m/z* 1021 ($[M+2+Na]^+$), 1019 ([M + Na]+), 999 ([M + 2 + H]+), 997 ([M + H]+). ^1H NMR (400 MHz, $(CD_3)_2SO$) δ 1.11 (3H, d, *J* = 6.4 Hz), 1.47 (1H, dd, *J* = 11.6 Hz, *J* = 3.1 Hz), 1.82 (1H, dt, *J* = 12.4 Hz, *J* = 3.3 Hz), 2.08 (1H, dd, *J* = 13.4 Hz, *J* = 5.0 Hz), 2.19 (1H, dd, *J* = 13.4 Hz, *J* = 2.5 Hz), 2.25 (3H, s), 3.05–3.65 (7H, m),

3.97 (3H, s), 4.16 (1H, q, $J=6.6$ Hz), 4.70 (1H, d, $J=5.5$ Hz), 4.85–4.95 (1H, m), 4.90 (1H, d, $J=13.5$ Hz), 4.93 (1H, d, $J=13.5$ Hz), 5.20–5.25 (2H, m), 5.28 (1H, d, $J=8.0$ Hz), 5.54 (1H, s), 6.96 (1H, d, $J=7.9$ Hz), 7.36 and 7.44 (2H, 2d $J=8.4$ Hz), 7.63 (1H, t, $J=4.5$ Hz), 7.77 (1H, s), 7.85–7.90 (2H, m), 10.15 (1H, s), 13.23 (1H, s), 14.01 (1H, s).

Acknowledgements

We would like to thank Pharmachemie BV Haarlem, The Netherlands and the Dutch Cancer Foundation, Nederlandse Kankerbestrijding Koningin Wilhelmina Fonds, for their financial support.

References and Notes

1. Arcamone, F. *Doxorubicin Anticancer Antibiotics*; Academic: New York, 1981.
2. Weiss, R. B. *Seminars in Oncology* **1992**, 670–686.
3. Lown, J. W. *Chem. Soc. Rev.* **1993**, 165.
4. Priebe, W., Ed. *Anthracycline Antibiotics*. ACS Symposium Series 574. ACS: Washington, DC, 1995.
5. For recent reviews see: Eisenbrand, G.; Lauck-Birkel, S.; Tang, W.C. *Synthesis* **1996**, 1246: and see ref. 6.
6. Sinhababu, A. K.; Thakker, D. R. *Adv. Drug Del. Rev.* **1996**, *19*, 241.
7. Arcamone, F. *Doxorubicin Antibiotics*. Medicinal Chemistry, a Series of Monographs, Vol. 17; De Stevens, G., Ed.; Academic Press: New York, 1982.
8. For a recent review see: Niculescu-Duvaz, I.; Springer, C. J. *Curr. Med. Chem.* **1995**, *2*, 687.
9. Bosslet, K.; Czech, J.; Hoffmann, D. *Tumor Targeting* **1995**, *1*, 45.
10. This has been known for more than 50 years, see: Fishman, W. H.; Anlyan, A. J. *J. Biol. Chem.* **1947**, *169*, 449.
11. For a review on the role of β-glucuronidase in drug targeting see: Sperker, B.; Backman, J. T.; Kroemer, H. K. *Clin. Pharmacokinet.* **1997**, *33*, 18.
12. For recent reviews see: Jungheim, L. N.; Shephard, T. A. *Chem. Rev.* **1994**, *94*, 1553; and see refs 13 and 14.
13. Sinhababu, A. K.; Thakker, D. R. *Adv. Drug Del.* **1996**, *19*, 241.
14. Eisenbrand, G., Lauck-Birkel, S., Tang, W. C. *Synthesis* **1996**, *1246*.
15. Haisma, H. J.; Boven, E.; v Muijen, M.; de Jong, J.; van der Vijgh, W. J. F.; Pinedo, H. M. *Br. J. Cancer* **1992**, *66*, 474.
16. Senter, P. D.; Schreiber, G. J.; Hirschberg, D. L.; Ashe, S. A.; Hellström, K. E.; Hellström, I. *Cancer Res.* **1989**, *49*, 5789.
17. Senter, P. D.; Saulnier, M. G.; Schreiber, G. J.; Hirschberg, D. L.; Brown, J. P.; Hellström, I.; Hellström, K. E. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 4842.
18. Wallace, P. M.; Senter, P. D. *Bioconj. Chem.* **1991**, *2*, 349.
19. Jungheim, L. N.; Shepard, T. A.; Meyer, D. L. *J. Org. Chem.* **1992**, *57*, 2334.
20. Leenders, R. G. G.; Gerrits, K. A. A.; Ruijtenbeek, R.; Scheeren, J. W.; Haisma, H. J.; Boven, E. *Tetrahedron Lett.* **1995**, *36*, 1701.
21. Leenders, R. G. G.; Scheeren, H. W.; Houba, P. H. J.; Boven, E.; Haisma, H. J. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 2975.
22. Leenders, R. G. G.; Damen, E. W. P.; Scheeren, J. W.; Haisma, H. J.; Houba, P. H. J.; de Vos, D. *European Patent*, 95201747, 1995.
23. De Bont, H. B. A.; Leenders, R. G. G.; Scheeren, J. W.; Haisma, H. J.; De Vos, D. European Patent, 95203671, 1995.
24. Houba, P. H. J.; Leenders, R. G. G.; Boven, E.; Scheeren, J. W.; Pinedo, H. M.; Haisma, H. J. *Biochem. Pharmacol.* **1996**, *52*, 455.
25. Houba, P. H. J.; Boven, E.; Erkelens, C. A. M.; Leenders, R. G. G.; Scheeren, J. W.; Pinedo, H. M.; Haisma, H. J. *Br. J. Cancer* **1998**, *78*, 1600.
26. Sharma, S. K.; Bagshawe, K. D.; Melton, R. G.; Sherwood, R. F. *Cell. Biophys.* **1992**, *21*, 109.
27. β-Glucuronyl carbamates as prodrugs have not been reported so far by others whereas several approaches toward the use of β-glucuronides as prodrugs are described in literature. For example, see refs 28–32.
28. Bosslet, K.; Czech, J.; Hoffmann, D. *Cancer Res.* **1994**, *54*, 2151.
29. Jacquesy, J.-C.; Gesson, J.-P.; Monneret, C.; Mondon, M.; Renoux, B.; Florent, J.-C.; Koch, M.; Tillequin, F.; Sedlacek, H. H.; Gerken, M.; Kolar, C.; Gaudel, G. Patent, WO 92/19639, 1992.
30. Wang, S. M.; Chern, J. W.; Yeh, M. Y.; Ng, J. C.; Tung, E.; Roffler, S. R. *Cancer Res.* **1992**, *52*, 4484.
31. Haisma, H. J.; van Muijen, M.; Pinedo, H. M.; Boven, E. *Cell Biophysics* **1994**, *24–25*, 185.
32. Schmidt, F.; Florent, J.-C.; Monneret, C.; Straub, R.; Czech, J.; Gerken, M.; Bosslet, K. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 1071.
33. In other publications this prodrug is referred to as "daunorubicin-GA3" or "DNR-GA3" see refs 24,25.
34. For the activation pathway of the prodrugs described in this paper see: Wakselman, M. *New J. Chem.* **1983**, *7*, 439, and see ref 35.
35. Carl, P. L.; Chakravarty, P. K.; Katzenellenbogen, J. A. J. *Med. Chem.* **1981**, *24*, 479.
36. An α-galacosyl carbamate derivative has already been described in literature: Azoulay, M.; Florent, J.-C.; Monneret, C.; Gesson, J. P.; Jacquesy, J.-C.; Tillequin, F.; Koch, M.; Bosslet, K.; Czech, J.; Hoffman, D. *Anti-Cancer Drug Design* **1995**, *10*, 441.
37. Yamashita, S.; Suda, Y.; Masada, M.; Madai, T.; Sumi, M. *Chem. Pharm. Bull.* **1989**, *37*, 2861.
38. Leenders, R. G. G.; Ruytenbeek, R.; Damen, E. W. P.; Scheeren, J. W. *Synthesis* **1996**, *1309*.
39. Shioiri, T.; Ninomiya, K.; Yamada, S.-I. *J. Am. Chem. Soc.* **1972**, *94*, 6203.
40. Ghosh, A. K.; Duong, T. T.; McKee, S. P.; Thompson, W. J. *Tetrahedron Lett* **1992**, *33*, 2781.
41. For convenience the aromatic ring of the spacer is numbered starting on the lowest carbon.
42. Tong, G. L.; Wu, H. Y.; Smith, T. H.; Henry, D. W. *J. Med. Chem.* **1979**, *22*, 912.
43. This may also explain the lower yields in the coupling reaction to the amino-group of daunorubicin of pro-moieties **27d**, **f** and **h** having electron withdrawing substituents in the 2-position, (see Experimental). Side products may be formed by nucleophillic attack of the daunorubicin-3′-NH_2 on the carbamate carbonyl of **27d**, **f**, and **h**.
44. Bovine liver β-galactosidase was used to determine the hydrolysis rates of both **DAU-1B** and **DAU-1C**. This enzyme hydrolyses both β-galactosidase and β-glucosidase substrates.
45. pH 6.8 was used for activation rate measurements, reflecting the tumor interstitial pH; see: Martin, G. R.; Jain, R. K. *Cancer Res.* **1994**, *54*, 5670.
46. Human β-glucuronidase was produced by a mouse cell line transfected with human β-glucuronidase gene and secreted in the culture medium from which it was isolated. See: Houba, P. H. J.; Boven, E.; Haisma, H. J. *Bioconjugate Chem.* **1996**, *7*, 606.
47. Kita, Y.; Akai, S.; Ajimura, N.; Yoshigi, M.; Tsugoshi, T.; Yasuda, H.; Tamura, Y. *J. Org. Chem.* **1986**, *51*, 4150.
48. Compound **19** was prepared from phthalide by ring opening according to Cain, B. F. *J. Org. Chem.* **1976**, *41*, 2029, followed by silylation according to ref 47.