Pergamon

Bioorganic & Medicinal Chemistry, Vol. 5, No. 2, pp 405–414, 1997

PII: S0968-0896(96)00249-0
0968-0896/97 $17.00+0.00

Synthesis and Biological Activity of β-Glucuronyl Carbamate-Based Prodrugs of Paclitaxel as Potential Candidates for ADEPT

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Abstract—The syntheses of prodrugs of paclitaxel, which can be used in ADEPT in order to target paclitaxel towards tumor cells, are described. The prodrugs **1** and **2a,b** consist of a spacer molecule connected via a carbamate linkage to a β-glucuronic acid. The spacer molecule is also connected via an ester linkage to the 2′-OH of paclitaxel. Enzyme-catalyzed hydrolysis of the glucuronic acid moiety by human β-glucuronidase results in the liberation of the parent drug paclitaxel via γ or δ lactam formation with half-lives of 45 min and 2 h (**1** and **2b**). The prodrugs **1** and **2b** are two orders of magnitude less cytotoxic than paclitaxel.

Introduction

The lack of selectivity of cytostatic agents for tumor cells is a serious drawback in conventional cancer chemotherapy. The use of monoclonal antibodies (MAb) to target cytotoxic agents towards tumor cells is a promising method to amplify the selectivity of anticancer agents.[1] Using this strategy antibodies, elicited against tumor cells, are utilized to guide a cytotoxic drug to a tumor based on the ability of MAb to recognize and selectively bind to antigens expressed on tumor cells. In ADEPT (antibody-directed enzyme prodrug therapy) enzymes attached to MAbs are used that will, after selective binding to the tumor site, convert a prodrug into a cytotoxic drug at the site of the tumor. Employing this strategy, relatively nontoxic prodrugs can be used having for instance enhanced water solubility. Moreover, the catalytic nature of the enzyme-MAb conjugates allow the activation of many prodrug molecules by one conjugate molecule. The use of ADEPT for targeting cytotoxic drugs has been described for many cytotoxic agents such as methotrexate,[2] etoposide,[3] or doxorubicine.[4]

Paclitaxel,[5] with its unique mechanism of action, is a very attractive candidate for ADEPT, since ADEPT can diminish the cytotoxic effects of paclitaxel on healthy tissues (vide supra). Furthermore, the use of ADEPT allows the administration of more water-soluble paclitaxel prodrugs circumventing problems caused by Cremophor EL oil that is used as a solvent when paclitaxel is administered.[6] Although the synthesis and evaluation of several water-soluble paclitaxel prodrugs have been published,[7] the use of ADEPT in order to target paclitaxel to tumor cells was only recently described by Rodrigues et al.,[8] who used the non-mammalian β-lactamase conjugated to a MAb to activate prodrugs of paclitaxel via ADEPT. In order to circumvent immunogenicity as a result of non-human enzymes,[1d,e,g] we used human β-glucuronidase. This is a lysosomal enzyme, with no activity in blood at neutral pH.[9]

In our study towards paclitaxel prodrugs (**1** and **2a,b**, see Chart 1) as candidates for ADEPT, β-glucuronides

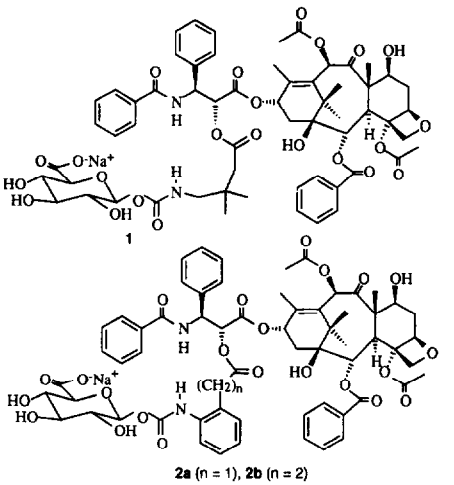

Chart 1. Prodrugs of paclitaxel.

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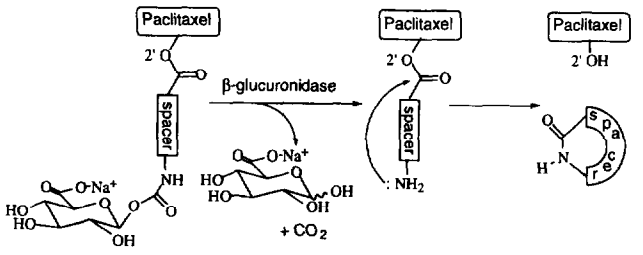


Figure 1. Mechanism for the activation of paclitaxel prodrugs **1** and **2a,b** by human β-glucuronidase.

were coupled to a spacer moiety via a carbamate linkage.[4f] This spacer moiety, esterified to the 2′ hydroxyl function of paclitaxel, should facilitate the β-glucuronidase catalyzed hydrolysis. Upon hydrolysis of the carbohydrate moiety, followed by release of CO_2, the resulting free amine group will attack the ester bond resulting in liberation of paclitaxel (Fig. 1). As the 2′-hydroxyl group is essential for the cytotoxic activity of paclitaxel,[5] functionalization of this hydroxyl moiety will probably result in less toxic prodrugs. Furthermore, the use of polar sugar groups will give rise to better water-soluble paclitaxel prodrugs.

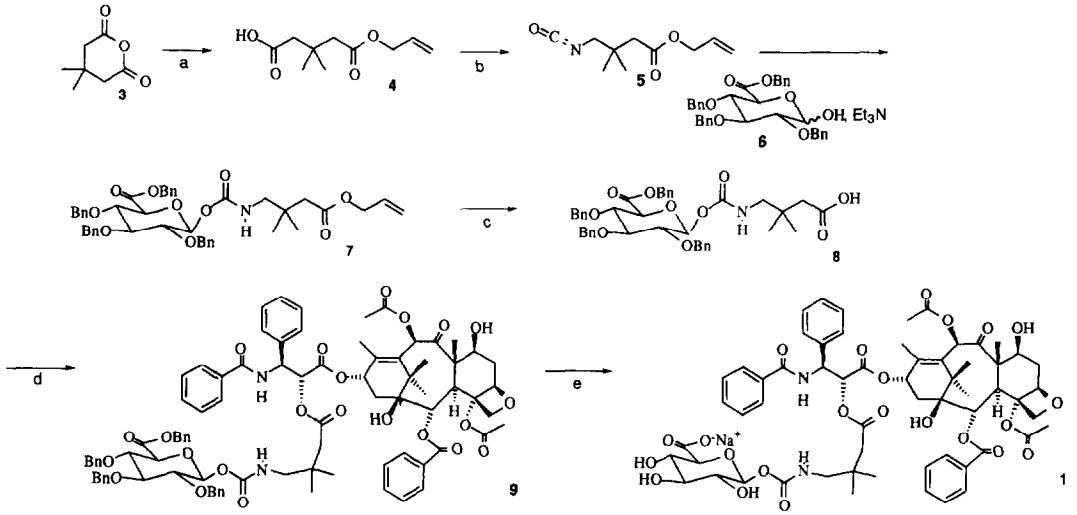
Synthesis

Preparation of the prodrugs **1** (Scheme 1) and **2a,b** (Scheme 2) starts with the ring opening of anhydride **3** or **10** with allyl alcohol[4f] resulting in monoesters **4** or **11a** in a yield of 90 and 85%, respectively, or with the esterification of diacid **12**, with allyl alcohol in the presence of DCC and DMAP, yielding **11b** (91%).

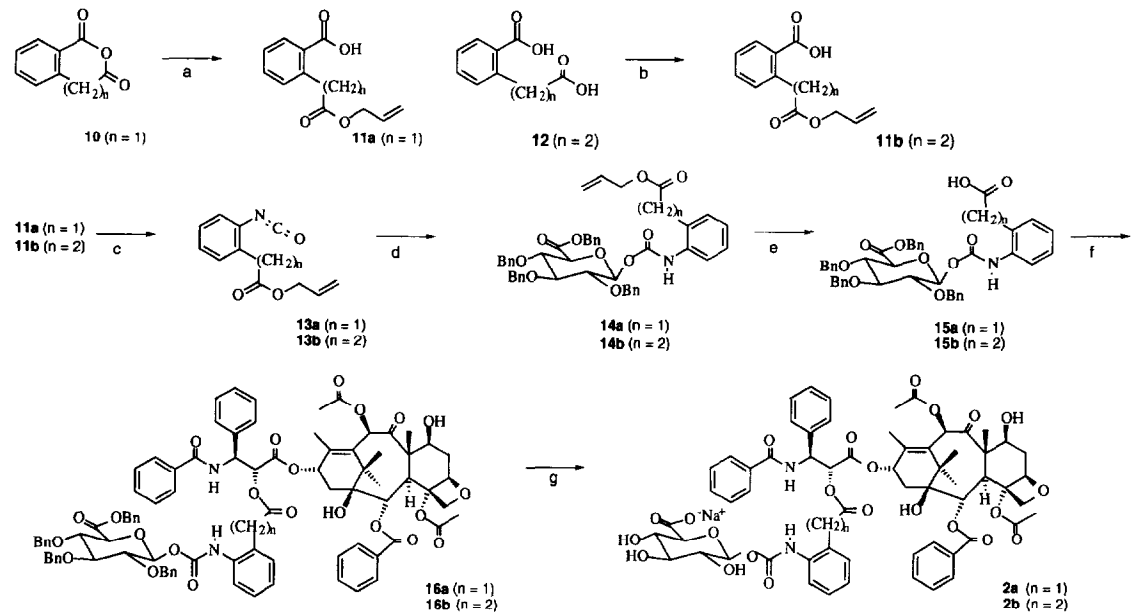
The key step[4f] in the synthesis of prodrugs **1** and **2a,b** is the generation of isocyanates **5** and **13a,b** at which an anomerically unprotected carbohydrate **6**[10] is attached affording sugar carbamates **7** and **14a,b**, respectively. As a result of the desired suicide potential of the spacer, the sugar carbamate moiety cannot be introduced via synthetic steps involving intermediates having a free amino group attached to the spacer moiety because of premature ring closure to the corresponding γ or δ lactam, respectively. For this reason we introduced the sugar carbamate fragment in situ, employing the Curtius rearrangement to generate isocyanates as masked carbamates from carboxylic acids **4** and **11a,b**. In order to synthesize the corresponding acyl azides, essential for the Curtius rearrangement, from carboxylic esters **4** and **11a,b** diphenylphosphoryl azide and triethylamine were added to mono esters **4** and **11a,b**. Subsequent heating afforded, after Curtius rearrangement, isocyanates **5** and **13a,b**. Reaction of the C-1 unprotected sugar derivative **6**[10] with isocyanates **5** and **13a,b** occurred with a high β-selectivity,[4f] resulting in the protected spacer moieties **7** and **14a,b** in a conversion yield of 72, 82, and 76%, respectively. Removal of the allyl protective group resulted in acids **8** and **15a,b** that were subsequently coupled to paclitaxel affording the fully protected paclitaxel prodrugs **9** and **16a,b** employing diisopropylcarbodiimide,[7i] in yields of 79, 100, and 83%, respectively. With dicyclohexylcarbodiimide instead of diisopropylcarbodiimide, we encountered problems during purification by silica gel chromatography caused by dicyclohexylurea. Hydrogenolysis of the benzyl protective groups using hydrogen and palladium on carbon as a catalyst, followed by ion exchange and purification by LH-20 gelfiltration afforded the paclitaxel prodrugs **1** and **2a,b** in yields of 43, 33, and 76%, respectively.

Biological Activity

The prodrugs **1** and **2a,b** are far more soluble in water than paclitaxel. Concentrations of 10 mM in water and higher were reached, which compare favorably to the solubility of paclitaxel, which is lower than 0.005 μM in water. The chemical stabilities of the prodrugs **1** and



Scheme 1. Synthesis of paclitaxel prodrug **1**. Reagents: (a) allyl alcohol, Et_3N, DMAP; (b) i. diphenylphosphorylazide, Et_3N, rt, 20 h, ii. 65 °C, 2 h; (c) morpholine, $((Ph)_3P)_4Pd$; (d) diisopropylcarbodiimide, DMAP; (e) H_2, 10% Pd/C.

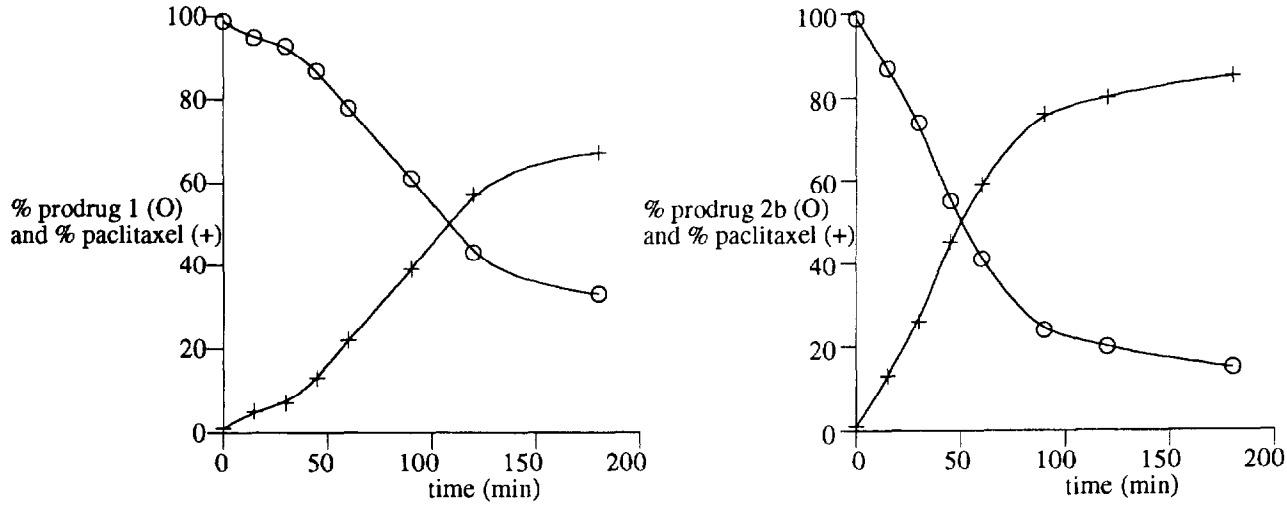


Scheme 2. Synthesis of paclitaxel prodrug **2a,b**. Reagents: (a) allyl alcohol, Et_3N, DMAP; (b) allyl alcohol, dicyclohexylcarbodiimide, DMAP; (c) i. diphenylphosphorylazide, Et_3N, rt ii. destillation, 130 °C, 1 mm Hg; (d) **6**, Et_3N; (e) morpholine, $((Ph)_3P)_4Pd$; (f) diisopropylcarbodiimide, DMAP; (g) H_2, 10% Pd/C.

2b at pH 6.8 at 37 °C were confirmed. Prodrug **2a** hydrolyzed spontaneously towards paclitaxel under these conditions with a half-life of 3.5 h resulting in paclitaxel, as was demonstrated by means of HPLC. No intermediates, having only a spacer molecule attached to paclitaxel, were detected. As a consequence of this fast nonspecific hydrolysis of **2a**, this prodrug was not tested in the β-glucuronidase catalyzed prodrug activation. In combination with the improved water solubility (vide supra) prodrug **2a** may be used as a water soluble prodrug that liberates the parent drug paclitaxel via nonspecific hydrolysis.

The β-glucuronidase-catalyzed prodrug activation of **1** and **2b** was carried out at 100 μM prodrug concentration and 10 μg mL^{-1} human β-glucuronidase[11] at 37 °C by taking an aliquot of the reaction mixture at different time intervals during a time course of 3 h. Analysis of these aliquots by means of HPLC demonstrated an enzyme-catalyzed half-life of prodrugs **1** and **2b** of 2 h and 45 min, respectively (Graph 1). For human β-glucuronidase, preliminary calculations indicate a $K_m > 5000$ μM and a k_{cat} of 2.8 s^{-1} for prodrug **1** and a K_m of 850 μM and a k_{cat} of 5.9 s^{-1} for prodrug **2b**. No intermediates, bearing a spacer moiety attached to paclitaxel, were observed, demonstrating that the intramolecular γ or δ lactam formation is spontaneous, resulting in immediate paclitaxel release after enzyme catalyzed hydrolysis of the β-glucuronic acid moiety.

As the prodrugs **1** and **2b** can be activated, resulting in the release of paclitaxel, by a specific enzyme catalyzed



Graph 1. Enzymatic hydrolysis by human β-glucuronidase of prodrugs **1** and **2b**. For conditions, see text.

mechanism and prodrug **2a** is activated, although in a nonspecific manner (vide supra), we were interested in the cytotoxicity of these prodrugs. The antiproliferative effects of paclitaxel, and the prodrugs **1**, **2a**, and **2b** on OVCAR-3 cells were determined by measuring the cell growth with a protein dye stain in the presence or absence of β-glucuronidase.[12] After 72 h the IC_{50} values were determined and are shown in Table 1. As expected (vide supra), prodrug **2a** is as cytotoxic as paclitaxel both in the presence and in the absence of β-glucuronidase. The prodrugs **1** and **2b** are two orders of magnitude less cytotoxic than the parent drug paclitaxel. Moreover, activation of these prodrugs with β-glucuronidase results in IC_{50} values close to the IC_{50} values of paclitaxel proving that the prodrugs are considerably less cytotoxic than paclitaxel, but upon activation with β-glucuronidase are nearly as active as paclitaxel.

Activation of the prodrugs **1** and **2b** with antibody–enzyme conjugates was studied analogously as described above for the determination of the IC_{50} values. OVCAR-3 cells were pretreated with a conjugate prepared between murine anti-pancarcinoma monoclonal antibody 323/A3 and human β-glucuronidase.[12] The antibody-β-glucuronidase conjugate used retained more than 90% enzyme activity and bound specifically to the OVCAR-3 cells. Prodrug activation was observed, as the IC_{50} values were approximately twofold lower in the presence of conjugate as compared to the prodrug alone. Unfortunately, the amount of enzyme bound to the cells was too low for complete activation of prodrugs **1** and **2b** in the given time (24 h). Human β-glucuronidase or enzyme-immunoconjugate were nontoxic in the concentrations used.

Conclusions

In this paper, we have described novel water-soluble prodrugs of paclitaxel that can release paclitaxel, in the case of **1** and **2b**, via enzyme-catalyzed hydrolysis. The synthesis is based on addition of β-glucuronic acid derivatives to an isocyanate containing spacer molecule. Attachment of this spacer moiety to the 2′-OH of paclitaxel followed by hydrogenolysis, resulted in prodrugs **1** and **2a,b**.

These prodrugs are more water-soluble than paclitaxel and can be activated by β-glucuronidase with a half-life of 2 h or 45 min (**1** and **2b**, respectively). This enzyme-catalyzed hydrolysis was very selective as was demonstrated by the absence of any intermediates, having a spacer moiety attached to paclitaxel. This is in contrast to the recently described cephalosporin-based paclitaxel prodrugs.[8] The IC_{50} value of **2a** is nearly the same as the cytotoxicity of paclitaxel, caused by the fast nonspecific hydrolysis (half-life of 3.5 h). The cytotoxicity of the prodrugs **1** and **2b** is two order of magnitude less than that of paclitaxel, but similar to paclitaxel after enzymatic hydrolysis. Although the enzyme was able to release paclitaxel, treatment of the prodrugs **1** and **2b** with an antibody–β-glucuronidase conjugate did not result in liberation of paclitaxel. This is probably caused by the fact that the concentration of the antibody-β-glucuronidase conjugates was too low. The use of recombinant antibody-β-glucuronidase fusion proteins may be a solution for this problem and are planned for the future. Furthermore, studies are directed to the development of prodrugs with modified spacers that may undergo faster enzyme catalyzed hydrolysis. When for example the same β-glucuronyl carbamate spacer promoieties were coupled to the amino group of daunomycin[4f] hydrolysis by the same enzyme was more than ten times faster, showing that even the used drug has a strong influence on enzyme activity.[13]

Table 1. IC_{50} values of prodrugs **1**, **2a** and **b** in the presence or absence of human β-glucuronidase. For experimental details, see text

(Pro)drug	IC_{50} (nM)	IC_{50} with β-glucuronidase (nM)
1	19±8.8	0.6±0.3
2a	0.23	0.23
2b	27±2.4	1.1±0.6
Paclitaxel	0.2±0.2	

Experimental

General methods

THF was dried by refluxing over $LiAlH_4$ and distilled immediately prior to use. Dichloromethane was distilled from calcium hydride and toluene was distilled from P_2O_5 prior to use.

Unless stated otherwise, materials were obtained from commercial sources and used without further purification. Paclitaxel was a generous gift of PharmaChemie, Haarlem, The Netherlands.

Analytical HPLC was carried out with a LKB 2150 system equipped with a reversed phase (C18) colomn (Lichrospher®, 250 mm × 4 mm, 5 μM) using as eluent a gradient of 10% acetonitrile in water to 90% acetonitrile in water with UV detection at 226 nm.

Gas chromatography was performed on a Hewlett-Packard 5710A GC instrument equipped with a capillary HP crosslinked methyl silicone (25 m × 0.31 mm) column type PAS 017.

Mass spectra were obtained with a double focusing VG 7070E spectrometer. Elemental analysis were determined with a Carbo Erba Ea 1108 instrument.

TLC analysis was performed on Merck precoated silica gel 60 F-254 plates. Spots were visualized with UV light and a 10% H_2SO_4 solution (1 L) containing ammonium molybdate (25 g) and ceric ammonium sulfate (10 g) followed by charring. Column chroma-

tography was carried out on Merck Kieselgel 60H (5–40 μm).

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ or DMSO-d_6 on a Bruker AC100 (100 MHz), a Varian spectrometer (300 MHz) or a Bruker AM 400 (400 MHz) spectrometer. Chemical shifts are given in ppm (δ) relative to TMS as internal standard.

1-Allyl-3,3-dimethylglutaric ester (4). To a soln of 3,3-dimethylglutaric anhydride (2.1 g, 14.7 mmol) in allyl alcohol (10 mL) were added triethylamine (2 mL, 14.7 mmol) and a catalytic amount of dimethylaminopyridine (DMAP). After completion of the reaction (3 h, monitored by GC), the reaction mixture was diluted with EtOAc and washed with an aq soln of 1 N $KHSO_4$ and brine, respectively, followed by drying over anhydrous Na_2SO_4. Evaporation of the solvent afforded **4** as an oil in 90% yield (2.6 g). ^1H NMR (100 MHz, $CDCl_3$): δ 1.14 (s, 6H, Me (both)), 2.47 (s, 4H, CH_2 (both)), 4.60 (dt, 2H, CH_2 (allyl), $J_{vic}=5.6$ Hz, $J_{1,4=CH\alpha}=J_{1,4=CH\beta}=1.2$ Hz), 5.23 (ddt, 1H, $=CH_\alpha$ (allyl), $J_{vic}=10.1$ Hz, $J_{gem}=1.7$ Hz, $J_{1,4}=1.2$ Hz), 5.31 (ddt, 1H, $=CH_\beta$ (allyl), $J_{vic}=17.2$ Hz, $J_{gem}=1.7$ Hz, $J_{1,4}=1.2$ Hz), 5.93 (8 lines, 1H, CH_2-C<u>H</u>$=CH_2$, $J_{CH,CH\beta}=17.2$ Hz, $J_{CH,CH\alpha}=10.1$ Hz, $J_{CH,CH_2}=5.6$ Hz). MS (EI): 200 (M^+).

Allyl 2-(carboxylic acid)phenylacetate (11a). Compound **11a** was prepared as described for the synthesis of **4** using homophthalic anhydride (**10**) (3.25 g, 20 mmol), allyl alcohol (10 mL), triethylamine (3.1 mL, 22 mmol), and a few crystals of DMAP. After completion of the reaction, work-up was carried out as described for **4** (vide supra). Evaporation of the solvent and purification by crystallization from hexanes afforded allyl ester **11a** in 83% (3.65 g), which was pure according to GC. ^1H NMR (100 MHz, $CDCl_3$): δ 3.88 (s, 2H, PhC<u>H</u>$_2$C(O)), 4.40 (ddd, 2H, OCH_2 allyl, $J_{vic}=5.6$ Hz, $J_{CH_2,=CH\alpha}=J_{CH_2,=CH\beta}=1.3$ Hz), 4.95 (ddt, 1H, $=CH_\alpha$, $J_{vic}=17.2$ Hz, $J_{1,4}=J_{gem}=1.3$ Hz), 4.98 (ddt, 1H, $=CH_\alpha$, $J_{vic}=10.2$ Hz, $J_{1,4}=J_{gem}=1.3$ Hz), 5.72 (ddt, 1H, CH_2C<u>H</u>$=CH_2$ allyl, $J_{CH,CH_2}=5.6$ Hz, $J_{CH,CH\alpha}=17.2$ Hz, $J_{CH,CH\beta}=10.2$ Hz), 7.03–7.42 (m, 3H, CH Ph), 7.92 (dd, 1H, C_3H Ph, $J_{vic}=7.60$, $J_{1,4}=1.8$ Hz), 12.0 (s, 1H, C(O)OH). ^{13}C NMR (25.4 MHz, $CDCl_3$): δ 40.8 (<u>C</u>H_2C(O)), 85.6 (OCH_2 allyl), 116.4 ($=CH_2$ allyl), 127.9, 132.0, 132.2, 132.6, and 133.4 (CHPh and CH_2C<u>H</u>$=CH_2$ allyl), 128.7 (C^1 Ph), 138.8 (C^2 Ph), 171.3 and 172.8 (C(O) both). MS (EI): 220 (M^+) and 135 $(M-CO_2allyl)^+$. Elemental analysis: calcd for $C_{12}H_{12}O_4$: C 56.45, H 5.49, measured: C 65.40, H 5.39.

Allyl 2-(carboxylic acid)phenylpropionate (11b). 2-(Carboxylic acid)phenylpropionic acid (**12**) (194 mg, 1 mmol) and allyl alcohol (75 μL, 1.1 mmol) were dissolved in a mixture of dry CH_2Cl_2 (5 mL) and dry DMF (0.5 mL). After cooling of the soln to 0 °C, dicyclohexylcarbodiimide (206 mg, 1 mmol) and a few crystals of dimethylaminopyridine were added, the reaction mixture was stirred at 0 °C for 4 h and another 12 h at room temperature. The reaction mixture was filtrated, the filtrate diluted with CH_2Cl_2, washed with an aq soln of 1 N $KHSO_4$, dried over anhydrous Na_2SO_4 and the solvent was evapd under red. press. Redissolving the obtained ester in hexanes, followed by filtration of traces of dicyclohexylurea and evaporation of the solvent under red. press. afforded **11b** as an oil (91%, 212 mg). ^1H NMR (100 MHz, $CDCl_3$): δ 3.30 (t, 2H, C<u>H</u>$_2$Ph, $J_{vic}=7.7$ Hz), 3.93 (t, 2H, CH_2C(O), $J_{vic}=7.7$ Hz), 5.14 (dt, 2H, OCH_2 allyl, $J_{vic}=5.6$ Hz, $J_{1,4}=1.2$ Hz), 5.77 (10 lines, 1H, $=CH_\alpha$, $J_{vic}=10.1$ H, $J_{gem}=1.2$ Hz, $J_{1,4}=1.2$ Hz), 5.86 (10 lines, 1H, $=CH_\beta$, $J_{vic}=17.1$ Hz, $J_{gem}=1.2$ Hz, $J_{1,4}=1.2$ Hz), 6.46 (12 lines, CH_2C<u>H</u>$=CH_2$, $J_{CH,CH\beta}=17.1$ Hz, $J_{CH,CH\alpha}=10.1$ Hz, $J_{CH,CH_2}=5.5$ Hz), 7.79–8.12 (m, 3H, CHPh), 8.64 (1H. dd, C^3H Ph, $J_{vic}=11.8$ Hz, $J_{1,4}=2.0$ Hz) and 9.34 (s, 1H, C(O)OH). ^{13}C NMR (25.4 MHz, $CDCl_3$): δ 30.1 (PhCH_2), 35.8 (CH_2C(O)), 65.3 (OCH_2 allyl), 118.2 ($=CH_2$ allyl), 126.8 (CH allyl), 128.4 (C1 Ph), 131.6, 132.1, 132.3, and 133.3 (C3, C4, C5, and C6 Ph), 143.5 (C2 Ph), 172.8 and 172.9 (C(O) both). Mass spectrometry (EI): 234 (M^+).

***N*-[Allyl 3,3-dimethylbutanoate]-*O*-[2,3,4,6-tetrabenzyl-β-glucuronyl] carbamate (7)**. To the stirred soln of allyl ester **4** (0.6 g, 3 mmol) in 10 mL of dry CH_2Cl_2 were added triethylamine (0.50 mL, 3.6 mmol) and diphenylphosphorylazide (0.78 mL, 3.6 mmol) and the progress of the reaction was monitored by GC. After completion of the reaction (4 h), the solvent was evapd and the residue was subjected to kugelrohr distillation (130 °C, 1 mm Hg) resulting in isocyanate **5** after Curtius rearrangement (yield: 487 mg, 83%). Isocyanate **5** was immediately dissolved in dry toluene (10 mL), followed by addition of 1-hydroxy-2,3,4,6-tetrabenzyl-β-glucuronic acid (0.91 g, **6**) (1.65 mmol) and a few drops of triethylamine as a catalyst. The reaction mixture was heated to 70 °C for 24 h and then refluxed for another 16 h. The solvent was evaporated under reduced pressure, and the residue was subjected to silica gel column chromatography (silica 60H, eluent 20% EtOAc acetate in hexanes). Compound **7** was obtained as an oil in a conversion yield of 87% (0.81 g). Further elution afforded 1-hydroxy-2,3,4,6-tetrabenzyl β-glucuronic acid (**6**) (0.23 g, 0.41 mmol). ^{13}C NMR (25.4 MHz, $CDCl_3$): δ 25.4 and 25.5 (CH_3 spacer (both)), 34.6 (<u>C</u>$(CH_3)_2$ spacer), 43.8 (<u>C</u>H_2C=O spacer), 50.4 (<u>C</u>H_2NH spacer), 65.1, 67.3, 74.9 and 75.7 (<u>C</u>H_2Ph and OCH_2 allyl), 74.6, 79.3 and 80.5 (C^2, C^3, and C^4 glucuronic acid) 83.7 (C^5 glucuronic acid), 94.8 (C^1 glucuronic acid), 116.6 ($=CH_2$ allyl), 127.6, 128.8, 127.9, 128.2, 128.3, 128.4, and 131.8 (CH Ph, CH_2C<u>H</u>$=CH_2$ allyl), 134.8, 137.5, 137.8, and 138.0 (Cq Ph), 154.3 (NC(O)O), 166.2 and 171.6 (C^6 glucuronic acid and C(O) spacer). ^1H NMR (400 MHz, $CDCl_3$): δ 0.99 and 1.00 (s, 6H, CH_3 spacer (both)), 2.20 (d, 1H, $CH_aC(O)$ spacer, $J_{gem}=13.7$ Hz), 2.26 (d, 1H, $CH_bC(O)$ spacer, $J_{gem}=13.7$ Hz), 3.11 (d, 2H, <u>C</u>H_2NH spacer, $J_{vic}=6.7$ Hz), 3.60 (dd, 1H, CH glucuronic acid, $J_{vic}=J_{vic}=8.2$ Hz), 3.73 (dd, 1H, CH glucuronic acid, $J_{vic}=J_{vic}=8.8$ Hz), 3.82 (dd, 1H, CH glucuronic acid, $J_{vic}=J_{vic}=9.9$ Hz), 4.09 (d, 1H, C^5H glucuronic acid, $J_{vic}=9.9$ Hz), 4.42 and 4.85 (both d, both 1H, C<u>H</u>$_2$Ph

and $C\underline{H}_\beta Ph$, $J_{gem}=10.6$ Hz (both)), 4.56 (d, 2H, $OC\underline{H}_2$ allyl, $J_{vic}=5.8$ Hz), 4.69 and 4.78 (both d, both 1H, $CH_\alpha Ph$ and $C\underline{H}_\beta Ph$, $J_{gem}=11.2$ Hz (both)), 4.74 (s, 2H, $C(O)OC\underline{H}_2Ph$), 5.13 and 5.18 (both d, both 1H, $C\underline{H}_\alpha Ph$ and $C\underline{H}_\beta Ph$, $J_{gem}=12.3$ Hz (both)), 5.22 (t, 1H, NH, $J_{vic}=6.6$ Hz), 5.24 (d, 1H, $=CH_\alpha$, $J_{CH\alpha,CH}=11.1$ Hz), 5.31 (d, 1H, $=CH_\beta$, $J_{CH\beta,CH}=17.0$ Hz), 5.62 (d, 1H, C'H glucuronic acid, $J_{vic}=8.2$ Hz), 5.90 (8 lines, 1H, $CH_2C\underline{H}=CH_2$ allyl, $J_{CH,CH\beta}=17.0$ Hz, $J_{CH,CH\alpha}=11.1$ Hz, $J_{CH,CH_2}=5.9$ Hz), 7.09–7.30 (m, 24H, Ph). FABMS: 752 $(M+H)^+$, 774 $(M+Na)^+$, and 790 $(M+K)^+$.

***N*-[(2-Allylacetate)phenyl]-*O*-[2,3,4,6-tetrabenzyl-β-glucuronyl] carbamate (14a).** To a soln of allylester **11a** (220 mg, 1 mmol) in dry toluene (5 mL) was added triethylamine (0.17 mL, 1.2 mmol) and diphenylphosphorylazide (0.26 mL, 1.2 mmol). Stirring overnight, upon which the corresponding azide was formed, followed by heating of the reaction mixture for 2 h at 65 °C resulted in isocyanate **13a**, which was directly converted without isolation to carbamate **14a** by addition of glucuronic acid derivative **6** (0.28 g, 0.5 mmol) to the reaction mixture containing isocyanate **13a**. After completion of the reaction (2 days), monitored by TLC (eluent EtOAc:hexanes, 1:1, v/v), the reaction mixture was diluted with EtOAc, washed with an aq soln of 1 N $KHSO_4$, an aq soln of satd $NaHCO_3$, brine and water and dried over anhydrous Na_2SO_4. Purification by silica gel chromatography (silica 60H, eluent EtOAc:hexanes, 1:3, v/v) afforded **14a** as a white solid (yield 82%, 315 mg) that was pure according to TLC. ^1H NMR (400 MHz, $CDCl_3$): δ 3.54 and 3.66 (both d, both 1H, $C(O)C\underline{H}_\alpha Ph$ and $C(O)C\underline{H}_\beta Ph$ spacer, $J_{gem}=14.7$ Hz (both)), 3.69 (m, 1H, CH glucuronic acid), 3.77 (dd, 1H, CH glucuronic acid, $J_{vic}=8.8$ Hz), 3.87 (dd, 1H, CH glucuronic acid, $J_{vic}=9.1$ Hz), 4.14 (d, 1H, C^5H glucuronic acid, $J_{vic}=10.0$ Hz), 4.44 and 4.70 (both d, both 1H, $CH_\alpha Ph$ and $CH_\beta Ph$, $J_{gem}=10.6$ Hz (both)), 4.58 (d, 2H, OCH_2 allyl, $J_{vic}=5.9$ Hz), 4.80 (d, 1H, $CH_\alpha Ph$, $J_{gem}=10.6$ Hz), 4.81 (m, 3H, $CH_\beta Ph$ (twice) and NH), 4.88 (d, 1H, $CH_\alpha Ph$, $J_{gem}=11.2$ Hz), 5.14 and 5.18 (both d, both 1H, $CH_\alpha Ph$ and $CH_\beta Ph$, $J_{gem}=12.3$ Hz (both)), 5.23 (d, 1H, $=CH_\alpha$, $J_{CH\alpha,CH}=10.6$ Hz), 5.27 (d, 1H, $=CH_\beta$, $J_{t=CH\beta,CH}=17.2$ Hz), 5.72 (d, 1H, C'H glucuronic acid, $J_{vic}=7.6$ Hz), 5.87 (8 lines, 1H, $CH_2C\underline{H}=CH_2$ allyl, $J_{CH,CH\beta}=17.2$ Hz, $J_{CH,CH\alpha}=10.6$ Hz, $J_{CH,CH_2}=5.9$ Hz), 7.09–8.22 (m, 24H, Ph). ^{13}C NMR (25.4 MHz, $CDCl_3$): δ 38.4 ($\underline{C}H_2C(O)$ spacer), 66.1, 67.4, 74.9 and 75.7 ($\underline{C}H_2Ph$ and CH_2 allyl), 79.3, 80.4 and 83.9 (C^2H, C^3H, C^4H, and C^5H glucuronic acid), 95.1 (C'H glucuronic acid), 118.9 ($=CH_2$ allyl), 125.1, 127.7, 127.8, 128.2, 128.3, 128.4, 128.5, 130.7, and 131.3 ($CH_2\underline{C}H=CH_2$, CH Ph), 134.8, 136.0, 137.5, 137.8, and 138.0 (Cq Ph), 151.8 (C(O) carbamate), 166.2 and 171.8 (C6(O) glucuronic acid and C(O) spacer). FABMS: 794 $(M+Na)^+$. Elemental analysis: measured: C 71.43, H 5.98 and N 1.83, calcd for $C_{46}H_{45}O_{10}N$: C 71.58, H 5.88 and N 1.81.

***N*-[(2-Allylpropionate)phenyl]-*O*-[2,3,4,6-tetrabenzyl-β-glucuronyl] carbamate (14b).** Carbamate **14b** was prepared as described for **14a** using acid **11b** (234 mg, 1 mmol), dry toluene (5 mL), triethylamine (0.17 mL, 1.2 mmol), diphenylphosphorylazide (0.27 mL, 1.2 mmol) and glucuronic acid derivative **6** (240 mg, 0.43 mmol). Work up as described for the synthesis of **14a** (vide supra) resulted in carbamate **14b**, which was further purified by silica gel chromatography (silica 60H, eluent EtOAc:hexanes, 1:4. v/v). Yield 76% (257 mg) that was pure according to TLC. ^1H NMR (400 MHz, $CDCl_3$, T=325 K): δ 2.67 (m, 2H, $PhC\underline{H}_2CH_2C(O)$ spacer), 2.85 (m, 2H, $PhCH_2C\underline{H}_2C(O)$ spacer), 3.69 (dd, 1H, CH glucuronic acid, $J_{vic}=8.2$ Hz), 3.75 (dd, 1H, CH glucuronic acid, $J_{vic}=8.8$ Hz), 3.88 (dd, 1H, CH glucuronic acid, $J_{vic}=9.4$ Hz), 4.14 (d, 1H, C^5H glucuronic acid, $J_{vic}=9.4$ Hz), 4.46 and 4.69 (both d, both 1H, $C\underline{H}_\alpha Ph$ and $C\underline{H}_\beta Ph$, $J_{gem}=10.6$ Hz (both)), 4.48 (d, 2H, OCH_2 allyl, $J_{vic}=5.9$ Hz), 4.76 and 4.83 (both d, both 1H, $C\underline{H}_\alpha Ph$ and $C\underline{H}_\beta Ph$, $J_{gem}=11.7$ Hz), 4.77 and 4.86 (both d, both H, $C\underline{H}_\alpha Ph$ and $C\underline{H}_\beta Ph$, $J_{gem}=11.2$ Hz), 5.13 and 5.17 (both d, both 1H, $C\underline{H}_\alpha Ph$ and $C\underline{H}_\beta Ph$, $J_{gem}=12.0$ Hz), 5.15 (d, 1H, $=CH_\alpha$, $J_{CH_\alpha,CH}=10.6$ Hz), 5.19 (d, 1H, $=CH_\beta$, $J_{CH\beta,CH}=17.0$ Hz), 5.73 (d, 1H, C'H glucuronic acid, $J_{vic}=7.6$ Hz), 5.79 (8 lines, 1H, $CH_2C\underline{H}=CH_2$ allyl, $J_{CH,CH\beta}=17.0$ Hz, $J_{CH,CH\alpha}=10.6$ Hz, $J_{CH,CH_2}=5.9$ Hz), 7.07–7.84 (m, 25H, Ph and NH). ^{13}C NMR (25.4 MHz, $CDCl_3$): δ 25.1 ($PhC\underline{H}_2CH_2C(O)$ spacer), 35.3 ($PhCH_2\underline{C}H_2C(O)$ spacer), 65.8, 67.5 and 75.8 ($\underline{C}H_2Ph$ and $O\underline{C}H_2$ allyl), 79.5, 80.5, and 83.8 (C^2, C^3, C^4, and C^5 glucuronic acid), 95.3 (C' glucuronic acid), 116.9 ($=CH_2$ allyl), 125.3, 127.4, 127.7, 127.9, 128.2, 128.4, 128.5, 128.8, 128.9, 129.7, and 131.7 (CH Ph and $CH_2\underline{C}H=CH_2$ allyl), 132.1, 135.0, 135.2, 137.7, 138.0, and 138.3 (Cq Ph), 152.1 (C(O) carbamate), 166.5 and 174.0 (C6 glucuronic acid and C(O) spacer). Mass spectrometry FABMS: 808 $(M+Na)^+$. Elemental analysis: measured: C 71.14, H 5.97 and N 1.91, calcd for $C_{47}H_{47}O_{10}N$: C 71.83, H 6.03 and N 1.78

***N*-[Paclitaxel-2'-*O*-3,3-dimethyl butanoate]-*O*-[2,3,4,6-tetrabenzyl-β-glucuronyl] carbamate (9).** Allyl ester **7** (0.87 g, 0.12 mmol) was dissolved in THF, followed by addition of morpholine (46 μL, 0.58 mmol). After bubbling of argon gas for 15 min, a few crystals of palladium tetrakistriphenylphosphine were added. When the reaction was complete, as was demonstrated by TLC (eluent EtOAc:hexanes, 1:1, v/v), the mixture was diluted with EtOAc, washed with 1 N $KHSO_4$, dried over anhydrous Na_2SO_4 and the solvent was evapd under red. press. yielding acid **8**, which without further purification was immediately coupled to paclitaxel (vide infra).

After dissolving acid **8** in CH_2Cl_2 (5 mL), paclitaxel (50 mg, 58 μmol) was added and the mixture was cooled to 0 °C. Then diisopropylcarbodiimide (18 μL, 0.12 mmol) and a few crystals of dimethylaminopyridine were added and the reaction mixture was stirred at 0 °C for 1 h. After the reaction was complete (as was monitored by TLC (eluent CH_2Cl_2:MeOH, 95:5, v/v)) the mixture was diluted with CH_2Cl_2, washed with an aq soln of 1 N $KHSO_4$, satd $NaHCO_3$, water, brine, and dried over anhydrous Na_2SO_4. Evaporation of the solvent under

red. press., followed by purification over silica gel chromatography (silica 60H, eluent EtOAc:hexanes, 1:1, v/v) afforded the fully protected paclitaxel prodrug **9** in a yield of 79% (71.4 mg), which was pure according to TLC (eluent CH_2Cl_2:MeOH, 95:5, v/v) and HPLC. ^{13}C NMR (75.4 MHz, $CDCl_3$): δ 9.6 (C^{19} paclitaxel), 14.7 (C^{18} paclitaxel), 20.8 ($C^{10}OC(O)\underline{C}H_3$ paclitaxel), 22.2 ($C^4OC(O)\underline{C}H_3$ paclitaxel), 22.8 (C^{17} paclitaxel), 25.2 and 26.2 (CH_3 spacer (both)), 26.8 (C^{16} paclitaxel), 34.7 (Cq spacer), 35.5 (C^6 paclitaxel), 35.7 (C^{14} paclitaxel), 42.3 (C^{15} paclitaxel), 43.2 ($\underline{C}H_2C(O)$ spacer), 45.6 (C^3 paclitaxel), 49.4 (CH_2NH spacer), 52.8 ($C^{3'}$ paclitaxel), 58.5 (C^8 paclitaxel), 67.3 ($\underline{C}H_2Ph$), 71.7 (C^7 paclitaxel), 72.1 (C^{13} paclitaxel), 74.7, 75.2, 75.6 79.2, and 80.6 (C^2, C^{10}, and $C^{2'}$ paclitaxel, C^2H, C^3H, and C^4H glucuronic acid), 74.8 and 75.0 ($\underline{C}H_2Ph$), 79.1 (C^{20} and C^1 paclitaxel), 79.2 (C^1 paclitaxel), 81.1 (C^4 paclitaxel), 83.6 (C^5 paclitaxel), 84.5 (C^5H glucuronic acid), 95.0 (C^1H glucuronic acid), 125.5, 126.6, 127.7, 127.7, 127.8, 127.9, 127.9, 128.2, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.9, 130.2, 130.6, and 133.5 (CH Ph), 129.2, 132.7, 134.8, 137.2, 137.6, 138.0, and 138.1 (Cq Ph) 133.9 (C^{11} paclitaxel), 142.8 (C^{12} paclitaxel), 154.5 (C(O) carbamate), 167.0, 167.6, 168.0, 168.2, 170.0, 170.5, and 171.1 ($N^{3'}C(O)$ paclitaxel, $C^2O\underline{C}(O)Ph$ paclitaxel, $C^{1'}$ paclitaxel, $C^4O\underline{C}(O)CH_3$ paclitaxel, $C^{10}O\underline{C}(O)CH_3$ paclitaxel, $C^6(O)$ glucuronic acid, C(O) spacer) and 203.8 (C^9 paclitaxel). ^1H NMR (500 MHz, $CDCl_3$): δ 0.86 (s, 3H, CH_3 spacer), 0.94 (s, 3H, CH_3 spacer), 1.13 (s, 3H, $C^{17}H_3$ paclitaxel), 1.23 (s, 3H, $C^{16}H_3$ paclitaxel), 1.69 (s, 3H, $C^{19}H_3$ paclitaxel), 1.89 (ddd, 1H, C^6H_β paclitaxel, $J_{C6H\beta,C5H}=2.8$ Hz, $J_{C6H\beta,C7H}=10.2$ Hz, $J_{C6H\beta,C6H\alpha}=14.4$ Hz), 1.96 (d, 3H, $C^{18}H_3$ paclitaxel, $J_{1,4}=0.9$ Hz), 2.04 (d, 1H, $CH_\alpha C(O)$ spacer, $J_{gem}=13.0$ Hz), 2.12 (dd, 1H, $C^{14}H_\alpha$ paclitaxel, $J_{gem}=15.4$ Hz, $J_{vic}=9.2$ Hz), 2.23 (s, 3H, $C^{10}OC(O)CH_3$ paclitaxel), 2.28 (d, 1H, $CH_\alpha C(O)$ spacer, $J_{gem}=13.0$ Hz), 2.44 (dd, 1H, $C^{14}H_\alpha$ paclitaxel, $J_{gem}=15.4$ Hz, $J_{vic}=9.2$ Hz), 2.49 (d, 1H, C^7OH paclitaxel, $J_{vic}=4.2$ Hz), 2.57 (ddd, 1H, C^6H_α paclitaxel, $J_{C6H\alpha,C5H}=8.8$ Hz, $J_{C6H\alpha,C7H}=6.6$ Hz, $J_{C6H\alpha,C6H\beta}=14.4$ Hz), 2.58 (s, 3H, $C^4OC(O)CH_3$ paclitaxel), 2.85 (dd, 1H, $C\underline{H}_\beta NH$ spacer, $J_{gem}=14.1$ Hz, $J_{vic}=5.1$ Hz), 3.55 (dd, 1H, C^2H glucuronic acid, $J_{C2H,C1H}=J_{C2H,C3H}=7.5$ Hz), 3.56 (dd, 1H, $C\underline{H}_\alpha NH$ spacer, $J_{gem}=14.1$ Hz, $J_{vic}=8.5$ Hz), 3.58 (dd, 1H, C^3H glucuronic acid, $J_{C3H,C2H}=J_{C3H,C4H}=8.5$ Hz), 3.65 (d, 1H, C^5H glucuronic acid, $J_{C5H,C4H}=9.6$ Hz), 3.78 (dd, 1H, C^4H glucuronic acid, $J_{C4H,C3H}=J_{C4H,C5H}=9.1$ Hz), 3.83 (d, 1H, C^3H paclitaxel, $J_{vic}=7.1$ Hz), 4.22 (d, 1H, $C^{20}H_\beta$ paclitaxel, $J_{gem}=8.4$ Hz), 4.32 (d, 1H, $C^{20}H_\alpha$ paclitaxel, $J_{gem}=8.4$ Hz), 4.39 and 4.66 (both d, both 1H, $C\underline{H}_\alpha Ph$ and $C\underline{H}_\beta Ph$, $J_{gem}=10.9$ Hz (both)), 4.46 (ddd, 1H, C^7H, $J_{C7H,C6H\alpha}=6.6$ Hz, $J_{C7H,C6H\beta}=11.2$ Hz, $J_{C7H,OH}=4.2$ Hz), 4.67 and 4.77 (both d, both 1H, $C\underline{H}_\alpha Ph$ and $C\underline{H}_\beta Ph$, $J_{gem}=11.6$ Hz (both)), 4.71 (dd, 1H, $CH_2N\underline{H}$ spacer, $J_{NH,CH\alpha}=8.5$ Hz, $J_{NH,CH\beta}=5,1$ Hz), 4.77 and 4.82 (both d, both 1H, $C\underline{H}_\alpha Ph$ and $C\underline{H}_\beta Ph$, $J_{gem}=10.9$ Hz (both)), 4.83 and 4.99 (both d, both 1H, $CH_\alpha Ph$ and $CH_\beta Ph$, $J_{gem}=12.1$ Hz (both)), 4.98 (m, 1H, C^5H paclitaxel), 5.38 (d, 1H, C^1H glucuronic acid, $J_{vic}=7.5$ Hz), 5.50 (d, 1H, $C^{2'}H$ paclitaxel, $J_{vic}=3.0$ Hz), 5.86 (d, 1H, C^2H paclitaxel, $J_{vic}=7.2$ Hz), 6.08 (dd, 1H, $C^{3'}H$ paclitaxel, $J_{C3'H,NH}=9.5$ Hz, $J_{C3'H,C2'H}=3.0$ Hz), 6.30 (s, 1H, $C^{10}H$ paclitaxel), 6.31 (dd, 1H, $C^{13}H$ paclitaxel, $J_{C13H,C14H\alpha}=J_{C13H,C14H\beta}=9.6$ Hz), 7.05–8.16 (m, 35H, Ph), 7.99 (d, 1H, NH paclitaxel, $J_{vic}=9.5$ Hz). FABMS: 1547 $(M+H)^+$, 1569 $(M+Na)^+$. Elemental analysis measured C: 67.77, H: 6.49, N: 2.40, calcd for $C_{88}H_{94}N_2O_{23}\cdot H_2O$: C: 67.55, H: 6.18, N: 1.79.

***N*-[Paclitaxel-2′-*O*-(2-amino)phenylacetate]-*O*-[2,3,4,6-tetrabenzyl-β-glucuronyl] carbamate (16a).** Compound **16a** was synthesized as described above for **9** using THF (5 mL), allyl ester **14a** (90 mg, 0.12 mmol), morpholine (50 μL, 0.6 mmol), a few crystals of palladium tetrakistriphenyl-phosphine yielding carboxylic acid **15a** which was not further purified.

Esterfication of paclitaxel (50 mg, 58 μmol) with acid **15a** was carried out as described for **9** (vide supra) using dry CH_2Cl_2 (5 mL), diisopropylcarbodiimide (18 μL, 0.12 mmol), and a few crystals dimethylaminopyridine. Work up was carried out as for **9** followed by purification over silica gel chromatography (silica 60H, eluent EtOAc:hexanes, 1:1, v/v) affording the fully protected paclitaxel prodrug **16a** in quantitative yield (96 mg), which was pure according to TLC (eluent CH_2Cl_2:MeOH, 95:5, v/v) and HPLC. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 9.6 (C^{19} paclitaxel), 14.7 (C^{18} paclitaxel), 20.7 ($C^{10}OC(O)\underline{C}H_3$ paclitaxel), 22.4 ($C^4OC(O)\underline{C}H_3$ paclitaxel), 22.7 (C^{17} paclitaxel), 26.7 (C^{16} paclitaxel), 35.5 (C^6 paclitaxel), 35.6 (C^{14} paclitaxel), 37.9 (CH_2 spacer), 43.1 (C^{15} paclitaxel), 45.5 (C^3 paclitaxel), 52.6 ($C^{3'}$ paclitaxel), 58.4 (C^8 paclitaxel), 67.4 and 74.8, 75.5 and 76.6 ($\underline{C}H_2Ph$), 72.0 (C^7 paclitaxel), 72.1 (C^{13} paclitaxel), 74.8, 75.1, 75.5, and 79.2 (C^2, C^{10}, and $C^{2'}$ paclitaxel and CH glucuronic acid), 79.1 (C^{20} and C^1 paclitaxel), 80.4 (CH glucuronic acid), 82.2 (C^4 paclitaxel), 83.5 (C^5 paclitaxel), 84.4 (C^5H glucuronic acid), 97.6 (C^1H glucuronic acid), 126.5, 127.1, 127.7, 128.3, 128.3, 128.4, 128.5, 128.7, 129.1, 130.2, 131.9, and 133.6 (CH Ph), 129.3, 132.9, 133.3, 134.8, 135.5, 136.6, 137.5, and 137.7 (Cq Ph and C^{11} paclitaxel), 142.3 (C^{12} paclitaxel), 166.9, 168.3, 169.8, and 171.1 ($N^{3'}C(O)$, $C^2O\underline{C}(O)Ph$, $C^{1'}$, $C^4O\underline{C}(O)CH_3$, $C^{10}O\underline{C}(O)CH_3$ paclitaxel, $C^6(O)$ glucuronic acid, C(O) spacer) and 203.7 (C^9 paclitaxel). ^1H NMR (500 Mhz, $CDCl_3$): δ 1.09 (s, 6H, $C^{17}H_3$ and $C^{16}H_3$ paclitaxel), 1.68 (s, 3H, $C^{19}H_3$ paclitaxel), 1.89 (ddd, 1H, C^6H_β, $J_{C6H\beta,C5H}=2.6$ Hz, $J_{C6H\beta,C7H}=11.4$ Hz, $J_{C6H\beta,C6H\alpha}=14.5$ Hz), 1.91 (s, 3H, $C^{18}H_3$ paclitaxel), 2.05 (br s, 1H, $C^{14}H_\beta$ paclitaxel), 2.21 (s, 3H, $C^{10}OC(O)CH_3$ paclitaxel), 2.35 (br s, 1H, $C^{14}H_\alpha$ paclitaxel), 2.47 (br s, 3H, $C^4OC(O)CH_3$ paclitaxel), 2.49 (d, 1H, C^7OH, $J_{vic}=4.0$ Hz), 2.55 (ddd, 1H, C^6H_α, $J_{C6H\alpha,C5H}=9.3$ Hz, $J_{C6H\alpha,C7H}=6.3$ Hz, $J_{C6H\alpha,C6H\beta}=14.5$ Hz), 3.63–3.80 (m, 6H, $OC(O)CH_2Ph$ spacer, C^3 paclitaxel, C^2H C^3H and C^4H glucuronic acid), 4.10 (br s, 1H, C^5H glucuronic acid), 4.20 (d, 1H, $C^{20}H_\beta$ paclitaxel, Jgem = 8.5 Hz), 4.31 (d, 1H, $C^{20}H_\alpha$ paclitaxel, $J_{gem}=8.5$ Hz), 4.42 and 4.68 (both d, both 1H, $CH_\alpha Ph$ and $CH_\beta Ph$, $J_{gem}=10.8$ Hz (both)), 4.44 (m, 1H, C^7H paclitaxel), 4.76 and 4.82 (both d, both 1H, $CH_\alpha Ph$ and $CH_\beta Ph$, $J_{gem}=11.0$ Hz

(both)), 4.67–4.78 (m, 2H, C$\underline{H}_2$Ph), 4.96 (d, 1H, C^{5}H paclitaxel $J_{C5H,C6H\alpha}$=9.4 Hz), 5.12 (m, 2H, C$\underline{H}_2$Ph), 5.43 (br s, 1H, C$^{2'}$H paclitaxel), 5.66 (m, 2H, C^{2}H paclitaxel and C^{1}H glucuronic acid), 5.92 (br s, 1H, C$^{3'}$H paclitaxel), 6.23 (m, 1H, C^{13}H paclitaxel), 6.26 (s, 1H, C^{10}H paclitaxel), 6.62 (br s, 1H, NH paclitaxel) 7.09–8.16 (m, 35H, Ph paclitaxel). FABMS: 1589 $(M+Na)^+$. Elemental analysis measured C: 68.93, H: 5.88, N: 2.04, calcd for $C_{90}H_{90}N_2O_{23}\cdot H_2O$: 68.17, H: 5.85, N: 1.77.

***N*-[Paclitaxel-2′-*O*-(2-amino)phenylpropionate]-*O*-[2,3,4,6-tetrabenzyl-β-glucuronyl] carbamate (16b).** The synthesis of **16b** was carried out as described for **9** using allyl ester **14b** (94 mg, 0.12 mmol), morpholine (50 μL, 0.6 mmol), and a few crystals of palladium tetrakistriphenylphosphine. The obtained acid **15b**, without further purification, was directly esterfied with paclitaxel using dry CH_2Cl_2 (5 mL), paclitaxel (50 mg, 58 μmol), diisopropylcarbodiimide (18 μL, 0.12 mmol), and a few crystals of dimethylaminopyridine. Work up, as described for **9**, followed by purification by chromatography (silica gel 60H, eluent EtOAc:hexanes, 45:55, v/v) afforded **15b** which was pure according to TLC and HPLC. Yield 83% (75.7 mg). ^{13}C NMR (75.5 MHz, $CDCl_3$) 9.6 (C^{19} paclitaxel), 14.6 (C^{18} paclitaxel), 20.7 (C^{10}OC(O)$\underline{C}H_3$ paclitaxel), 22.0 (C^{4}OC(O)$\underline{C}H_3$ paclitaxel), 22.6 (C^{17} paclitaxel), 25.0 (Ph$\underline{C}H_2CH_2C$(O) spacer), 26.8 (C^{16} paclitaxel), 29.6 (PhC$H_2\underline{C}H_2$C(O) spacer), 34.1 (C^6 paclitaxel), 35.6 (C^{14} paclitaxel), 43.1 (C^{15} paclitaxel), 45.6 (C^3 paclitaxel), 52.8 (C$^{3'}$ paclitaxel), 58.5 (C^8 paclitaxel), 67.5, 74.9, 75.4, and 76.4 ($\underline{C}H_2$Ph), 71.9 (C^7 paclitaxel), 72.0 (C^{13} paclitaxel), 74.2, 74.8, 75.1, 75.5, and 80.3 (C^2, C^{10}, and C$^{2'}$ paclitaxel, C^{2}H, C^{3}H, and C^{4}H glucuronic acid), 79.1 (C^{20} and C^1 paclitaxel), 81.0 (C^4 paclitaxel), 83.7 (C^5 paclitaxel), 84.4 (C^{5}H glucuronic acid), 95.1 (C^{1}H glucuronic acid), 126.5, 127.0, 127.4, 127.7, 127.8, 127.9, 128.2, 128.3, 128.4, 128.5, 128.6, 128.7, 128.8, 129.0, 130.2, and 131.9 (CH Ph), 132.9, 133.6, 134.6, 134.8, 136.7, 137.6, 137.9, and 138.8 (Cq Ph), 133.6 (C^{11} paclitaxel), 142.4 (C^{12} paclitaxel), 151.4 (C(O) carbamate), 167.0, 167.1, 167.9, 168.4, 169.8, 171.0, and 172.7 (N$^{3'}$C(O), C^{2}O$\underline{C}$(O)Ph, C$^{1'}$(O), C^{4}O$\underline{C}$(O)CH$_3$, C^{10}O$\underline{C}$(O)CH$_3$ paclitaxel, C^6(O) glucuronic acid, and C(O) spacer) and 203.7 (C^9 paclitaxel). ^{1}H NMR (500 MHz, $CDCl_3$): δ 1.13 (s, 3H, C^{17}H$_3$ paclitaxel), 1.21 (s, 3H, C^{16}H$_3$ paclitaxel), 1.68 (s, 3H, C^{19}H$_3$ paclitaxel), 1.87 (ddd, 1H, C^{6}H$_\beta$, $J_{C6H\beta,C5H}$=2.4 Hz, $J_{C6H\beta,C7H}$=9.1 Hz, $J_{C6H\beta,C6H\alpha}$=14.0 Hz), 1.87 (d, 3H, C^{18}H$_3$ paclitaxel, $J_{1,4}$=1.2 Hz), 2.12 (dd, 1H, C^{14}H$_\beta$ paclitaxel, J_{gem}=15.4 Hz, J_{vic}=8.9 Hz), 2.20 (s, 3H, C^{10}OC(O)CH$_3$ paclitaxel), 2.33 (dd, 1H, C^{14}H$_\alpha$ paclitaxel, J_{gem}=15.4 Hz, J_{vic}=8.9 Hz), 2.39 (s, 3H, C^{4}OC(O)CH$_3$ paclitaxel), 2.55 (d, 1H, C^{7}OH, J_{vic}=4.2 Hz), 2.56 (ddd, 1H, C^{6}H$_\alpha$, $J_{C6H\alpha,C5H}$=9.7 Hz, $J_{C6H\alpha,C7H}$=6.7 Hz, $J_{C6H\alpha,C6H\beta}$=14.0 Hz), 2.81 (m, 4H, PhC$\underline{H}_2$C$\underline{H}_2$C(O) spacer), 3.66 (m, 1H, C^{2}H glucuronic acid), 3.75 (dd, 1H, C^{3}H glucuronic acid, $J_{C3H,C2H}$=$J_{C3H,C4H}$=8.5 Hz), 3.79 (d, 1H, C^3 paclitaxel, J_{vic}=7.1 Hz), 3.84 (dd, 1H, C^{4}H glucuronic acid, $J_{C4H,C3H}$=$J_{C4H,C5H}$=8.5 Hz), 4.10 (d, 1H, C^{5}H glucuronic acid, $J_{C5H,C4H}$=8.5 Hz), 4.19 (d, 1H, C^{20}H$_\beta$ paclitaxel, J_{gem}=8.4 Hz), 4.31 (d, 1H, C^{20}H$_\alpha$ paclitaxel, J_{gem}=8.4 Hz), 4.39 and 4.66 (both d, both 1H, CH$_\alpha$Ph and CH$_\beta$Ph, J_{gem}=10.9 Hz (both)), 4.44 (m, 1H, C^{7}H paclitaxel), 4.43 and 4.69 (both d, both 1H, CH$_\alpha$Ph and CH$_\beta$Ph, J_{gem}=10.9 Hz (both)), 4.78–4.86 (m, 4H, C$\underline{H}_2$Ph), 4.96 (dd, 1H, C^{5}H paclitaxel $J_{C5H,C6H\alpha}$=9.6 Hz, $J_{C5H,C6H\beta}$=2.0 Hz), 5.12 (m, 2H, C$\underline{H}_2$Ph), 5.52 (d, 1H, C$^{2'}$H paclitaxel, J_{vic}=3.7 Hz), 5.67 (d, 1H, C^{2}H paclitaxel, J_{vic}=7.1 Hz), 5.69 (d, 1H, C^{1}H glucuronic acid, J_{vic}=8.0 Hz), 5.94 (br s, 1H, C$^{3'}$H paclitaxel), 6.21 (dd, 1H, C^{13}H paclitaxel, $J_{C13H,C14H\alpha}$=$J_{C13H,C14H\beta}$=8.9 Hz), 6.28 (s, 1H, C^{10}H paclitaxel), 7.09–8.14 (m, 35H, Ph and NH paclitaxel). FABMS: 1603 $(M+Na)^+$. Elemental analysis measured C: 68.12, H: 6.27, N: 2.84, calcd for $C_{92}H_{92}N_2O_{23}\cdot H_2O$: 68.32, H: 5.92, N: 1.75.

***N*-[Paclitaxel-2′-*O*-3,3-dimethyl butanoate]-*O*-[β-glucuronyl] carbamate sodium salt (1).** Protected prodrug **9** (49.4 mg, 32 μmol) was dissolved in MeOH (20 mL), transferred to an autoclave, filled with nitrogen and a catalytic amount of Pd on C (10%) was added. Subsequently, the reaction mixture was treated with hydrogen gas (50 atm) and stirred for 24 h. After completion of the reaction, as was monitored by HPLC, the mixture was centrifuged at 5000 rpm for 5 min, the supernatants was decanted followed by evapn of the solvent under red. press. Dissolving the residue in *tert*-butanol:H_2O (1:1, v/v, 20 mL), addition of Dowex-Na, in order to prepare the sodium salt, lyophilizing the solvent followed by purification using LH-20 gelfiltration (eluent: acetonitrile:H_2O, 85:15, v/v) afforded compound **1** in a yield of 43% (16.8 mg) that was pure according to HPLC. ^{1}H NMR (400 MHz, DMSO-d_6, T=305 K): δ 0.92 (s, 3H, CH$_3$ spacer), 0.97 (s, 3H, CH$_3$ spacer), 1.06 (s, 3H, C^{17}H$_3$ paclitaxel), 1.09 (s, 3H, C^{16}H$_3$ paclitaxel), 1.56 (s, 3H, C^{19}H$_3$ paclitaxel), 1.57 (m, 1H, C^{6}H$_\beta$ paclitaxel), 1.69 (m, 2H, CH$_\beta$C(O) spacer and C^{14}H$_\beta$ paclitaxel), 1.84 (s, 3H, C^{18}H$_3$ paclitaxel), 1.89 (dd, 1H, C^{14}H$_\alpha$ paclitaxel, J_{gem}=14.9 Hz, J_{vic}=9.1 Hz), 2.16 (s, 3H, C^{10}OC(O)CH$_3$ paclitaxel), 2.35 (m, 5H, CH$_\alpha$C(O) spacer, C^{4}OC(O)CH$_3$ paclitaxel and C^{6}H$_\alpha$ paclitaxel), 2.96 (dd, 1H, C$\underline{H}_\beta$NH spacer, J_{gem}=13.5 Hz, J_{vic}=5.9 Hz), 3.03 (dd, 1H, C$\underline{H}_\alpha$NH spacer, J_{gem}=13.5 Hz, J_{vic}=6.5 Hz), 3.18 (m, 1H, C^{2}H glucuronic acid), 3.66 (d, 1H, C^{3}H paclitaxel, J_{vic}=7.2 Hz), 4.06 (d, 1H, C^{20}H$_\beta$ paclitaxel, J_{gem}=8.2 Hz), 4.10 (d, 1H, C^{20}H$_\alpha$ paclitaxel, J_{gem}=8.2 Hz), 4.19 (ddd, 1H, C^{7}H, $J_{C7H,C6H\alpha}$=6.9 Hz, $J_{C7H,C6H\beta}$=10.4 Hz, $J_{C7H,OH}$=6.5 Hz), 4.64 (s, 1H, C^{1}OH paclitaxel), 4.93 (d, 1H, C^{7}O$\underline{H}$, paclitaxel), 4.97 (d, 1H, C^{5}H paclitaxel, $J_{C5H,C6H\alpha}$=10.6), 5.12 (br s, 1H, C^{2}O$\underline{H}$ glucuronic acid), 5.16 (d, 1H, C^{5}H glucuronic acid, $J_{C5H,C4H}$=5.3 Hz), 5.30 (d, 1H, C^{1}H glucuronic acid, J_{vic}=7.6 Hz), 5.44 (d, 1H, C$^{2'}$H paclitaxel, J_{vic}=8.8 Hz), 5.47 (d, 1H, C^{2}H paclitaxel, J_{vic}=7.0 Hz), 5.62 (dd, 1H, C$^{3'}$H paclitaxel, $J_{C3'H,NH}$=$J_{C3'H,C2'H}$=8.2 Hz), 5.87 (dd, 1H, C^{13}H paclitaxel, $J_{C13H,C14H\alpha}$=$J_{C13H,C14H\beta}$=9.1 Hz), 6.36 (s, 1H, C^{10}H paclitaxel), 7.16–7.98 (m, 35H, Ph), 9.17 (d, 1H, NH paclitaxel, J_{vic}=8.2 Hz). FABMS: 1209 $(M+H)^+$, 1231 $(M+Na)^+$.

***N*-[Paclitaxel-2′-*O*-(2-amino)phenylacetate]-*O*-[β-glucuronyl] carbamate sodium salt (2a).** Hydrogenolysis of

16a was carried out as described for the preparation of **1** using compound **16a** (58.7 mg, 37 μmol), methanol (20 mL), a catalytic amount of Pd on C (10%) and H_2 gas (50 atm). After 1 day the hydrogenolysis was complete as was shown by HPLC. Work up and purification analogously to **1** afforded prodrug **2a** in a yield of 33% (15.2 mg) that was pure according to HPLC. ^{1}H NMR (400 MHz, DMSO-d_6, T=298 K): δ 0.99 (s, 3H, $C^{17}H_3$ paclitaxel), 1.01 (s, 3H, $C^{16}H_3$ paclitaxel), 1.47 (m, 4H, $C^{19}H_3$ paclitaxel and $C^{14}H_\beta$ paclitaxel), 1.60 (m, 1H, C^6H_β paclitaxel), 1.75 (s, 3H, $C^{18}H_3$ paclitaxel), 1.81 (dd, 1H, $C^{14}H_\alpha$ paclitaxel, J_{gem}=15.2 Hz, J_{vic}=9.7 Hz), 2.09 (s, 3H, $C^{10}OC(O)CH_3$ paclitaxel), 2.23 (s, 3H, $C^4OC(O)CH_3$ paclitaxel) 2.30 (m, 1H, C^6H_α paclitaxel), 3.56 (d, 1H, C^3H paclitaxel, J_{vic}=8.2 Hz), 3.79 (d, 1H, $C^{20}H_\beta$ paclitaxel, J_{gem}=16.3 Hz), 3.86 (d, 1H, $C^{20}H_\alpha$ paclitaxel, J_{gem}=16.3 Hz), 3.99 (s, 2H, CH_2 spacer), 4.09 (m, 1H, C^7H), 4.60 (s, 1H, C^1OH paclitaxel), 4.89 (d, 1H, C^5H paclitaxel, J_{gem}=10.3), 4.92 (m, 1H, C^7OH paclitaxel), 5.12 (br s, 1H, C^2OH, paclitaxel), 5.22 (d, 1H, C^5H glucuronic acid, J_{vic}=5.2 Hz), 5.33 (d, 1H, $C^{2'}$H paclitaxel, J_{vic}=8.2 Hz), 5.40 (d, 2H, C^2H paclitaxel and C^1H glucuronic acid, J_{vic}=8.7 Hz), 5.54 (dd, 1H, $C^{3'}$H paclitaxel, $J_{C3'H,NH}$=$J_{C3'H,C2'H}$=8.2 Hz), 5.82 (m, 1H, C^{13}H paclitaxel), 6.27 (s, 1H, C^{10}H paclitaxel), 6.91–7.98 (m, 19H, Ph). FABMS: 1229 $(M+H)^+$ and 1251 $(M+Na)^+$.

***N*-[Paclitaxel-2′-*O*-(2-amino)phenylpropionate]-*O*-[β-glucuronyl] carbamate sodium salt (2b)**. Compound **16b** (39.3 mg, 25 μmol) was hydrogenolyzed using methanol (20 mL), a catalytic amount of Pd on C (10%) and H_2O gas (50 atm). After completion of the reaction, work up and purification were carried out as described for **1** resulting in prodrug **2b** in a yield of 76% (23.6 mg) that was pure according to HPLC analysis. ^{1}H NMR (400 MHz, DMSO-d_6, T=298 K): δ 0.99 (s, 3H, $C^{17}H_3$ paclitaxel), 1.01 (s, 3H, $C^{16}H_3$ paclitaxel), 1.46 (m, 1H, $C^{14}H_\beta$ paclitaxel), 1.48 (s, 3H, $C^{19}H_3$ paclitaxel), 1.61 (m, 1H, C^6H_β paclitaxel), 1.78 (m, 1H, $C^{14}H_\alpha$ paclitaxel), 1.80 (s, 3H, $C^{18}H_3$ paclitaxel), 2.10 (s, 3H, $C^{10}OC(O)CH_3$ paclitaxel), 2.22 (s, 3H, $C^4OC(O)CH_3$ paclitaxel) 2.31 (m, 1H, C^6H_α paclitaxel), 2.65–2.95 (m, 4H, CH_2 spacer, both), 3.57 (d, 1H, C^3H paclitaxel, J_{vic}=7.0 Hz), 3.99 (br s, 2H, $C^{20}H_\beta$ and $C^{20}H_\alpha$ paclitaxel), 4.11 (m, 1H, C^7H), 4.59 (s, 1H, C^1OH paclitaxel), 4.90 (d, 1H, C^5H, paclitaxel, J =10.0 Hz), 4.93 (m, 1H, C^7OH paclitaxel), 5.09 (br s, 1H, OH glucuronic acid), 5.21 (d, 1H, C^1H glucuronic acid, J_{vic}=4.7 Hz), 5.33 (d, 1H, $C^{2'}$H paclitaxel, J_{vic}=7.6 Hz), 5.40 (m, 3H, C^2H paclitaxel, C^5H glucuronic acid and OH), 5.47 (dd, 1H, $C^{3'}$H paclitaxel, $J_{C3'H,NH}$= $J_{C3'H,C2'H}$=8.7 Hz), 5.79 (dd, 1H, C^{13}H paclitaxel, $J_{C13H,C14H\alpha}$=$J_{C13H,C14H\beta}$=8.9 Hz), 6.29 (s, 1H, C^{10}H paclitaxel), 7.04–7.98 (m, 19H, Ph), 9.59 (br s, 1H, NH, paclitaxel). FABMS: 1243 $(M+H)^+$ and 1265 $(M+Na)^+$.

Biological activity

Enzyme-catalyzed activation of prodrug 1. To 140 μL PBS buffer (pH 6.8) were added 20 μL of a 1 mM soln of prodrug **1** in PBS buffer (pH 6.8) and 20 μL of 1% bovine serum albumin also in PBS buffer (pH 6.8). After incubation of the mixture for 10 min at 37 °C, 20 μL of a soln of 0.1 mg mL^{-1} human β-glucuronidase[11] or PBS buffer (control) was added. Directly after the addition of the enzyme to the reaction mixture an aliquot of 10 μL was taken and diluted with 90 μL cold acetonitrile (−20 °C) in order to stop the enzyme reaction. The reaction mixture was incubated for 3 h at 37 °C and samples were taken at 15, 30, 45, 60, 90, 120, and 180 min. All samples were quenched by dilution with cold acetonitrile (−20 °C). The samples from both experiments were analysed by HPLC. The ratio of prodrug **1** to paclitaxel, as shown in Table 1, is determined by intergration the peak areas of the appropriate signals. In the experiment without human β-glucuronidase, no paclitaxel was found within the time course of the experiment demonstrating that the hydrolysis found was caused by the enzyme. All experiments were carried out in duplicate.

Enzyme-catalyzed activation of prodrug 2b. For the determination of the enzyme catalyzed hydrolysis rate of prodrug **2b**, 20 μL of a 1 mM soln of prodrug **2b** was used following the procedure as described for **1**. Results are shown in Graph 1.

In vitro cytotoxicity assay. The antiproliferative effects of paclitaxel, and the prodrugs **1**, **2a** and **b** on OVCAR-3 cells were determined by measuring the cell growth with a protein dye stain.[12] Cells were seeded in 96-well tissue culture plates (5000 cells/well) and 24 h later (pro)drugs **1**, **2a** or **b** or paclitaxel were added with or without excess human β-glucuronidase. After 72 h the cells were fixed with 25% trichloroacetic acid, stained with 0.4% sulforhodamine B, washed with 1% acetic acid and air-dried. The bound dye was then solubilized with 10 mM Tris and the absorbance read at 492 nm. The antiproliferative effects were expressed as IC_{50} values, which are the (pro)drug concentrations that gave 50% growth inhibition when compared to control cell growth (Table 1).

The effect of a conjugate on the antiproliferative effects of paclitaxel, and the prodrugs **1**, **2a** and **b** was measured by pretreating OVCAR-3 cells with antibody-enzyme conjugate at 10 μg mL^{-1}, followed by measuring the cell growth with a protein dye stain.[12] The conjugate was prepared using SATA- (Pierce, Oud Beierland, The Netherlands) treated human β-glucuronidase and SMCC- (Pierce, Oud Beierland, The Netherlands) treated anti-pancarcinoma monoclonal-antibody 323/A3.[14] The conjugate was purified on a Superose S300 (Pharmacia) column. Enzyme activity of the conjugate was more than 90% when compared to human β-glucuronidase. Cells, pretreated with conjugate or PBS, were seeded in 96-well tissue culture plates (200000 cells/well) and prodrugs **1**, **2a** or **b** or paclitaxel were added. After incubation for 24 h, 200 μL culture medium was added. After 72 h the cells were fixed and the IC_{50} values were determined (vide supra).

Acknowledgement

We are grateful for financial support for this work from Parmachemie B.V., Haarlem, the Netherlands.

References and Notes

1. For review see: (a) Koppel, G. A. *Bioconjugate Chem.* **1990**, *1*, 13. (b) Bundgaard, H. *Drugs of the Future* **1991**, *16*, 443. (c) Jungheim L. N.; Shepherd, T. A. *Chem. Rev.* **1994**, *94*, 1553. (d) Bagshawe K. D. *J. Contr. Release* **1994**, *28*, 187. (e) Bagshawe K. D. *Clin. Pharmacokinet.* **1994**, *27*, 368. (f) Wallace P. M.; Senter P. D. *Find. Exp. Clin. Pharmacol.* **1994**, *16*, 505. (g) Bagshawe K. D. *Drug Devel. Res.* **1995**, *34*, 220. (h) Georg, G. I.; Boge, T. C.; Cheruvallath, Z. S.; Clowers, J. S.; Harriman, G. C. B.; Hepperle, M.; Park, H. In *Taxol® Science and Applications*; Suffness, M., Ed.; CRC: Boca Raton, 1995; pp 317–375. (i) Vyas, D. M. In *The Chemistry and Pharmacology of Taxol® and its Derivatives*; Farina, V., Ed.; Elsevier: Amsterdam, 1995, pp 103–130.

2. Vitols, K. S.; Haenseler, E.; Montejano, Y.; Bear, T.; Huennekens, F. M. *Pteridines* **1992**, *3*, 125. Kuefner, U.; Lohrmann, U.; Montejano, Y. D.; Vitols, K. S.; Huennekens, F. M. *Biochemistry* **1989**, *28*, 2288. Kuefner, U.; Esswein, A.; Lohrmann, U.; Montejano, Y.; Vitols, K. S.; Huennekens, F. M. *Biochemistry* **1990**, *29*, 10540. Haenseler, E.; Esswein, A.; Vitols, K. S.; Montejano, Y.; Mueller, B. A.; Reisfeld, R. A.; Huennekens, F. M. *Biochemistry* **1992**, *31*, 891.

3. Senter, P. D.; Saulnier, M. G.; Schreiber, G. J.; Hirschberg, D. L.; Brown, J. P.; Hellström, I.; Hellström, K. E. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 4842. Haisma, H. J.; Pinedo, H. M.; Boven, E.; van Muijen, M.; de Vries, R. *Cancer Immunol. Immunother.* **1992**, *34*, 343.

4. (a) Jungheim, L. N.; Shepherd, T. A.; Kling, J. K. *Heterocycles* **1993**, *35*, 339. (b) Haisma, H. J.; Boven, E.; van Muijen, M.; de Jong, J.; van der Vijgh, W. J. F.; Pinedo, H. M. *Br. J. Cancer* **1992**, *66*, 474. (c) Jaquesy, J.-C.; Gesson, J.-P.; Monneret, C.; Renoux, B.; Florent, J.-C.; Koch, M.; Tillequin, F.; Sedlacek, H. H.; Kolar, C.; Gaudel, G. *Demande de Brevet Européen* **1992**, EP 0 511 917 A1. (d) Bosslet, K.; Czech, J.; Hoffmann, D. *Cancer Res.* **1994**, *54*, 2151. (e) Gesson, J.-P.; Jaquesy, J.-C.; Mondon, M.; Petit, Renoux, B.; Andrianomenjanahary, S.; Dufat-Trinh Van, H.; Koch, M.; Michel, S.; Tillequin, F.; Florent, J.-C.; Monneret, C.; Bosslet, K.; Czech, J.; Hoffmann, D. *Anti-Cancer Drug Design* **1994**, *9*, 409. (f) Leenders, R. G. G.; Gerrits, K. A. A.; Ruijtenbeek, R.; Scheeren, H. W.; Haisma, H. J.; Boven, E. *Tetrahedron Lett.* **1995**, *36*, 1701. (g) Vrudhula, V. M.; Svensson, H. P.; Senter, P. D. *J. Med. Chem.* **1995**, *38*, 1380.

5. For recent reviews, see: Guénard, D.; Guéritte-Voegelein, F.; Potier, P. *Acc. Chem. Res.* **1993**, *26*, 160. Heinstein, P. F.; Chang, C.-J. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1994**, *45*, 663. Nicolaou, K. C.; Dai, W.-M.; Guy, R. K. *Angew. Chem.* **1994**, *106*, 38. Spencer, C. M.; Faulds, D. *Drugs* **1994**, *48*, 794. Chen, S.H.; Farina, V. *The Chemistry and Pharmacology of Taxol® and its Derivatives*; Farina, V., Ed.; Elsevier: Amsterdam, 1995; pp 165–255.

6. Sharma, A.; Straubinger, R. M. *Pharm. Res.* **1994**, *6*, 889. Liebmann, J.; Cook, J. A.; Lipschultz, C.; Teague, D.; Fisher, J.; Mitchell, J. B. *Cancer Chemother. Pharmacol.* **1994**, *33*, 331.

7. (a) Deutsch, H. M.; Glinski, J. A.; Hernandez, M.; Haugwitz, R. D.; Narayanan, V. L.; Suffness, M.; Zalkow, L. H. *J. Med. Chem.* **1989**, *32*, 788. (b) Haugwitz R. D.; Suffness, M.; Narayanan, V.; Deutsch, H. M. *Int. Patent* **1989**, WO 89/08453. (c) Mathew, A. E.; Mejillano, M. R.; Nath, J. P.; Himes, R. H.; Stella, V. *J. Med. Chem.* **1992**, *35*, 145. (d) Nicolaou, K. C.; Riemer, C.; Kerr, M. A.; Rideout, D.; Wrasidlo, W. *Nature (Lond.)* **1993**, *364*, 464. (e) Vyas, D. M.; Wong, H.; Crosswell, A. R.; Casazza, A. M.; Knipe, J. O.; Mamber, S. W.; Doyle, T. W. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1357. (f) Ueda, Y.; Mikkilineni, A. B.; Knipe, J. O.; Rose, W.; Casazza, A. M.; Vyas, D. M. *Bioorg. Med. Chem. Lett.* **1993**, *3*, 1761. (g) Nicolaou, K. C.; Guy, R. K.; Nicolaou Pitsinos, E.; Wrasidlo, W. *Angew. Chem.* **1994**, *106*, 1672. (h) Ueda, Y. Wong, H.; Matiskella, J. D.; Mikkilineni, A. B.; Farina, V.; Fairchild, G.; Rose, W.; Mamber, S. W.; Long, B. H.; Kerns, E. H.; Casazza, A. M.; Vyas, D. M. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 1861. (i) Greenwald, R. B.; Pendri, A.; Bolikal, D.; Gilbert, C. W. *Bioorg. Med. Chem. Lett.* **1994**, *4*, 2465. (j) Greenwald, R. B.; Pendri, A.; Bolikal, D. *J. Org. Chem.* **1995**, *60*, 331. (k) Ueda, Y.; Matiskella, J. D.; Mikkilineni, A. B.; Farina, V.; Knipe, J. O.; Rose, W.; Casazza, A. M.; Vyas, D. M. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 247. (l) Mamber, S. W.; Mikkilineni, A. B.; Pack, E. J.; Rosser, M. P.; Wong, H.; Ueda, Y.; Forenze, S. *J. Pharm. Exp. Ther.* 1995, *274*, 877.

8. Rodrigues, M. L.; Carter, P.; Wirth, C.; Mullins, S.; Lee, A.; Blackburn, B. K. *Chem. Biol.* **1995**, *2*, 223.

9. Sedlacek, H. H.; Seeman, G.; Hoffman, D.; Czech, J.; Lorenz, P.; Kolar, C.; Bosslet, K. *Antibodies as Carriers of Cytotoxicity*; Karger: Basel, 1992.

10. van Boeckel, C. A. A.; Delbrissine, L. P. C.; Kaspersen, F. M. *Recl. Trav. Chim. Pays-Bas* 1985, *104*, 259. Keglevic, D.; Ljevakovic, D. *Carbohydr. Res.* **1978**, *64*, 319.

11. Haisma, H. J.; van Muijen, M.; Scheffer, G.; Scheper, R. J.; Pinedo, H. M.; Boven, E. A. *Hybridoma* **1995**, *14*, 377.

12. Haisma, H. J.; van Muijen, M.; Pinedo, H. M.; Boven, E. A. *Cell Biophys.* **1994**, *24/25*, 18.

13. Houba, P. H. J.; Leenders, R. G. G.; Boven, E.; Scheeren, J. W.; Pinedo, H. M.; Haisma, H. J. *Biochem. Pharm.* **1996**, *52*, 455.

14. Edwards, D. P.; Grzyb, K. T.; Dressler, L. G.; Mansel, R. E.; Zara, D. T.; Sledge, G. W. *Cancer Res.* **1986**, *46*, 1306.

(*Received in U.S.A.* 20 *March* 1996; *accepted* 29 *September* 1996)