Cancer Chemotherapy: A Paclitaxel Prodrug for ADEPT (Antibody-Directed Enzyme Prodrug Therapy)

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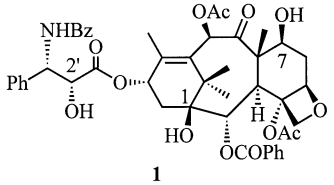
A glucuronide-based prodrug of paclitaxel (taxol®) has been synthesized for use in antibody-directed enzyme prodrug therapy (ADEPT). This three-component prodrug was obtained by coupling a glucuronyl derivative of *N*-methylamino 4-nitrophenol (**10**) to the 2′-hydroxy group of the side-chain of paclitaxel through an aromatic carbamate. Once deprotected, prodrug **2** was shown to be relatively stable in human serum, and to be significantly less cytotoxic (IC_{50} = 65 μM and 90 nM, respectively) than the parent drug. As expected, compound **2** efficiently releases taxol in the presence of β-glucuronidase.

Introduction

Paclitaxel (**1**, Taxol®) was recently introduced in cancer chemotherapy for its remarkable antitumor activity and its unique mechanism of action. This molecule promotes the assembly of stable microtubules from tubulin, and inhibits the disassembly process.[1−2] There are still several limitations for its clinical use, in particular its lack of selectivity and poor solubility in water.



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In order to improve the selectivity of anticancer drugs, the ADEPT (Antibody Directed Enzyme Prodrug Therapy) strategy was proposed several years ago.[3−7] The rationale behind this approach is to achieve selective delivery of a cytotoxic agent from a noncytotoxic prodrug by an enzyme reaction at the tumour cell surface. Thus, an enzyme is first targeted to the tumour cell by antibody-antigen recognition of an enzyme-Mab (monoclonal antibody) conjugate, or a fusion protein. After tumour localisation and systemic conjugate clearance an inactive prodrug is administered. The active compound is finally produced in situ by enzymatic cleavage.

The success of this approach is strongly dependent upon several factors such as the nature of the cytotoxic agent, and the choice of monoclonal antibody and enzyme. Bosslet and co-workers were the first to prepare a fusion protein consisting of a human β-glucuronidase[8−9] and the humanised *N*-terminal domains (or Fab) of the *anti*-CEA (carcinoembryonic antigen) monoclonal antibody BW 431.[10] They subsequently showed by enzyme histochemistry that β-glucuronidase was released in the extracellular medium of necrotic areas.[11−12] Thus, the administration of the glucuronyl prodrug alone in a Prodrug Mono Therapy (PMT) might also be a useful method for tumour-selective therapy.[13]

Over last few years, we have prepared a number of prodrugs which are substrates for human β-glucuronidase. In particular, we synthesized three-component prodrugs[14−16] in which the glucuronyl residue was linked to the drug by means of a self-immolative spacer. In such systems,[17] liberation of the drug occurs by enzymatic cleavage in the first step, followed in a second step by a fast molecular decomposition of the spacer. In the case of bulky aglycons, an increase in the kinetics of enzymatic hydrolysis is expected by this technology. Among the different prodrugs we prepared, one prodrug of doxorubicin was selected for clinical studies under the reference HMR1826,[18−20] and used in the above-mentioned PMT experiment.

Concerning paclitaxel, two classes of prodrugs have already been described. In the first class,[21−23] the main purpose was to increase water solubility by introducing an ionic group into the system which could be removed by a circulating enzyme such as a phosphatase or an esterase. Although this functionalisation increased the solubility of the ensemble, the release of the active compound was still unselective. Furthermore, with the goal of increasing both solubility and selectivity, prodrugs of paclitaxel as potential candidates for ADEPT have also been prepared and reported, including a cephalosporin-taxol prodrug,[24] requiring β-lactamase-mediated activation followed by self-immolation. This approach potentially suffers from two drawbacks: problems of immunogenicity problems, which have

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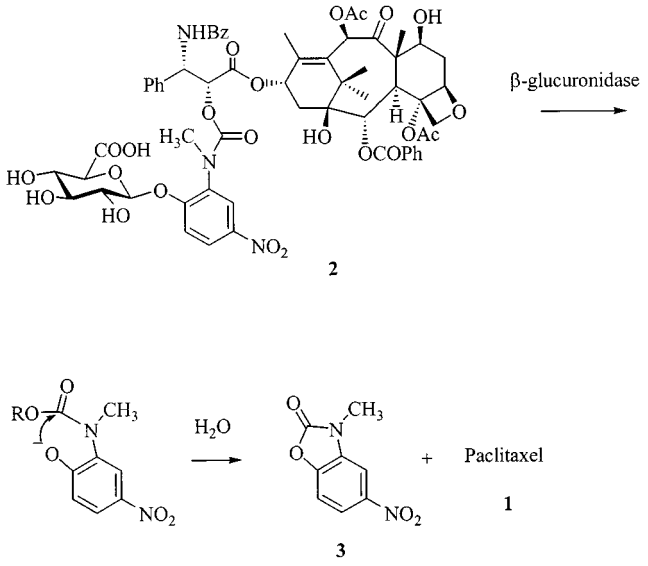
already been encountered with exogenous enzymes,[25] and a rate-limiting step. Indeed, the completion of the self-immolation of the taxol linker required about 16 h, which is inconsistent with the selectivity of release of the active compound.

The second class of prodrugs[26] includes a spacer molecule (aminobutyrate or aminophenyl acetate) connected to β-D-glucuronic acid by a carbamoyl function, and to the 2′-hydroxyl group of the side-chain of paclitaxel by an ester linkage. As expected, liberation of the active paclitaxel occurred in the presence of human β-D-glucuronidase. However, fast nonspecific hydrolysis was observed with one of these prodrugs in buffer solution (pH 6.8). Moreover, a risk of cleavage in plasma (no data given) due to circulating esterases cannot be excluded, since it is well-known that most esters of paclitaxel suffer from low chemical stability.[27] This factor may be a severe drawback to the targeting of the prodrug to tumour cells.

Therefore, at the outset of our project to prepare a glucuronide prodrug of paclitaxel, attention was directed to the use of the carbamate linkage, which is much more stable in the presence of esterases. The 2′ position of paclitaxel was selected for functionalisation as it is known[27–28] that modification of the 2′-hydroxyl group leads to a loss of cytotoxic activity as is required for a prodrug. Moreover, the 2′ position is more chemically reactive relative to the tertiary 1-hydroxyl or the more hindered secondary 7-hydroxyl groups in taxol.[21] This led us to synthesize the taxol glucuronide-based prodrug **2**, which should liberate the free drug at the tumour site by the mechanism depicted in Scheme 1, as previously observed for nitrogen mustard.[14]



Scheme 1

Results and Discussion

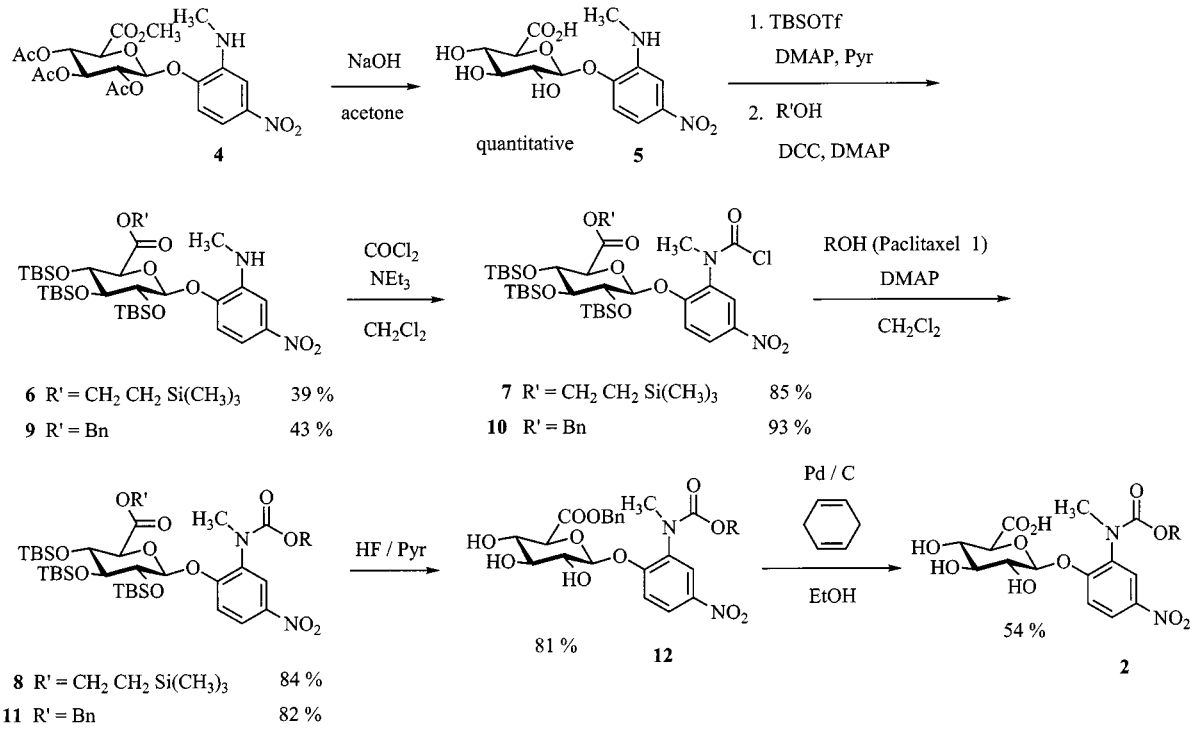
The synthesis of prodrug **2** (Scheme 2) began using a fully protected β-D-glucuronic acid derivative linked to 2-methylamino-4-nitrophenol.[16] However, the fragility of the paclitaxel skeleton in acidic or basic conditions[29–30] did not allow the use of ester protecting groups as in compound **4**.

The choice of the appropriate protecting groups in **4** was not obvious due to the fragility of the taxane skeleton. With the aim to find one-pot deprotecting conditions suitable with hydroxyl and carboxyl groups, we decided to protect the three hydroxyl groups in the glucuronic moiety as their *tert*-butyl dimethylsilyl ethers and the acid function as a trimethylsilylethyl ester (Scheme 2). Intermediate **4** was thus deprotected with sodium hydroxide in acetone in quantitative yield to give **5** which was subsequently treated with *tert*-butyldimethylsilyl triflate (TBSOTf) and trimethylsilylethanol to give **6** in a moderate yield (39%). Attempts to increase the yield of **6** were unsuccessful for two main reasons: difficulties in purifying the intermediate compound before esterification (even in the presence of a large excess of TBSOTf), and in achieving a full protection of the three hydroxyl groups of the glucuronic moiety.

The subsequent coupling of taxol **1** with the glucuronide-spacer moiety **6** required transformation of the amine function of **6** to the corresponding carbamoyl chloride derivative **7** by treatment with phosgene. A coupling reaction of **1** and **7** was achieved regioselectively in good yield (84%) using stoichiometric quantities of DMAP. Under catalytic DMAP conditions, the carbamoyl chloride intermediate **7** proved to be insufficiently reactive. Deprotection of the silyl groups was first attempted using tetrabutylammonium fluoride (TBAF),[31–32] but under these conditions, simultaneous cleavage of the benzoate ester of paclitaxel occurred. As an alternative, deprotection was attempted using HF/pyridine.[33–34] Unfortunately, although removal of TBS ethers occurred and the taxane skeleton was not modified, the trimethylsilylethyl ester was not cleaved.

In light of these results, attention was turned toward protecting the acid function as a benzyl ester, since this ester can be cleaved under neutral conditions (Scheme 2). Thus, the intermediate **5** was successively converted into **9** through reaction with *tert*-butyldimethylsilyl triflate and benzyl alcohol in the presence of DDC and DMAP. The amine function of **9** was then reacted with phosgene in dichloromethane, and the resulting carbamoyl chloride was condensed with paclitaxel in the presence of a stoichiometric quantity of DMAP. The coupled compound **11** was subsequently deprotected in two steps. First, the three silyl ethers were easily removed with HF/pyridine.[33–34] Then the benzyl ester was reductively cleaved with cyclohexadiene and palladium in ethanol without concomitant reduction of the aromatic nitro function.[35–36] Under these conditions, prodrug **2** was isolated in acceptable yield (54%). It is noteworthy that this prodrug was about 2000-fold more soluble in water than the free drug **1** (10 mM versus 5 μM).

Having achieved the synthesis of **2** in 36% overall yield from paclitaxel, the next step was to determine if prodrug **2** had the required properties for its use in an ADEPT or a PMT strategy. Concerning its stability in a phosphate buffer (pH 7.2) at 37 °C, more than 95% of the initial prodrug was



6 R' = $CH_2CH_2Si(CH_3)_3$ 39 %
9 R' = Bn 43 %
7 R' = $CH_2CH_2Si(CH_3)_3$ 85 %
10 R' = Bn 93 %
8 R' = $CH_2CH_2Si(CH_3)_3$ 84 %
11 R' = Bn 82 %

Scheme 2. Synthesis of the prodrug **2**

recovered after 24 hours, as indicated by HPLC measurement. Control of stability was further carried out in human serum at 37 °C, and the half-life was calculated to be 45.5 ± 4 hours. The main products detected and identified by their UV and mass spectra, were known metabolites,[37−38] such as those resulting from the epimerisation of carbon 7 (β-hydroxy ketone), and from the oxidation of the taxane skeleton. Neither Paclitaxel **1** nor the cyclised spacer **3** was detected.

Whereas the IC_{50} value (Figure 1) for **1** measured for LoVo cells is 90 nM, prodrug **2** is about 700-fold less cytotoxic than paclitaxel with an IC_{50} of 65 μM. The activity of the parent drug is restored by activation by β-glucuronidase, as indicated by measuring the IC_{50} of the prodrug **2** in the presence of β-glucuronidase (100 nM). It should be noted that the liberated cyclised spacer **3** does not interfere with these cytotoxicity measurements as its IC_{50} is 300 μM.

Finally, kinetic measurements of the cleavage of prodrug **2** and the appearance of **1** and **3** were carried out in vitro with *E. coli* β-glucuronidase. This enzyme has substantial sequence homology with the human enzyme[39−40] and could therefore be used as a model for the fusion protein (as shown previously[20]). With a β-glucuronidase concentration of 100 μg/mL, and a prodrug concentration of 250 μg/mL, the enzymatic reaction occurred (Figure 2) with a prodrug half-life of 115 minutes. As the prodrug **2** was consumed, two products appeared, which were identified by HPLC and through comparison with authentic samples as the paclitaxel **1** and the cyclised spacer **3**. No trace of intermediate paclitaxel-spacer was detected during these experiments, meaning that the spacer is cleaved as soon as the glycosidic bond is hydrolysed.

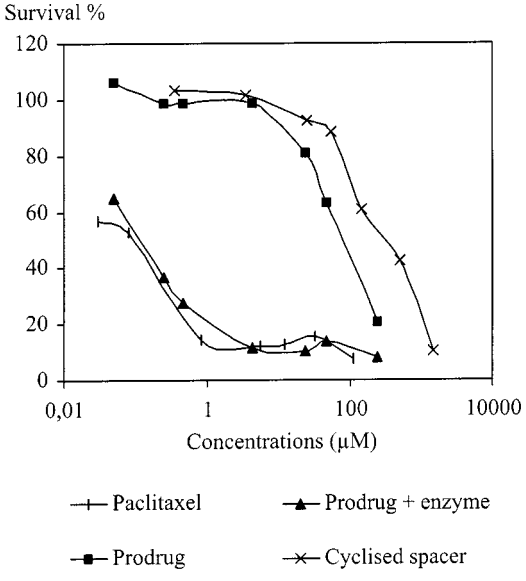


Figure 1. Cytotoxicity measurements

Although the enzyme was able to release paclitaxel, the level of enzyme required for a fast release is most probably difficult to reach under in vivo conditions, especially in PMT conditions. However, the turnover of the enzyme and the stability of the prodrug should permit a slower cleavage. On the basis of the already known structure of β-glucuronidase,[20] molecular modelling calculations were initiated which indicated that this relatively slow hydrolysis may be due to problems of steric hindrance.

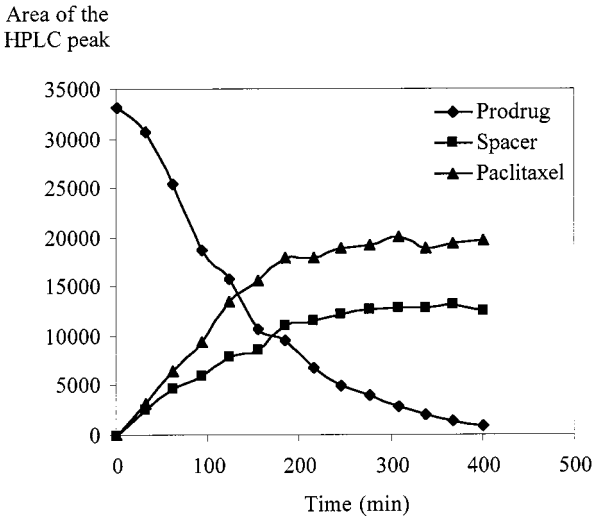


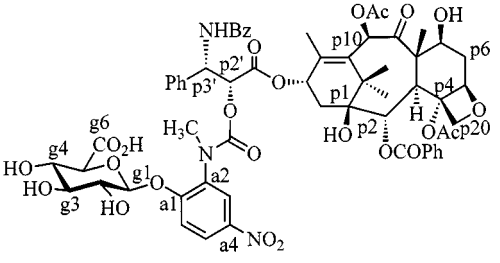
Figure 2. Enzymatic hydrolysis of the prodrug **2**

Conclusion

A water-soluble prodrug of paclitaxel designed for an ADEPT Strategy was obtained. The synthetic problems were solved and the compound gave good preliminary biological in vitro results. The prodrug had a good stability in human serum and showed adequate detoxification. The active paclitaxel was liberated from the prodrug by action of β-D-glucuronidase. The only limitation is the relatively large concentration of enzyme needed for a fast release. Elaboration of slightly different spacers to overcome this limitation is ongoing.

Experimental Section

General: Melting points were measured on a Reichert apparatus or on a Koffler Bench and are uncorrected. Optical rotations were obtained on a Perkin−Elmer 241 polarimeter. Infrared spectra were measured on a Perkin−Elmer 1710 FT/IR spectrometer. ^{1}H NMR (300 MHz) spectra were recorded on a Bruker AC 300 spectrometer (chemical shifts δ in ppm and *J* in Hz). For the descriptions, the atoms were labelled as following: a for the spacer aromatic ring, g for the glucuronic moiety and p for the paclitaxel. Some publications[41−42] were helpful for the NMR assignments of paclitaxel.



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Chemical ionization mass spectra (CI-MS or FAB) were recorded on a NERMAG R10−10C spectrometer. Electrospray ionization spectra were acquired using a quadrupole instrument with an *m/z* range of 2000. Column chromatography was carried out on Merck silica Kieselgel 60 (230−400 Mesh). Analytical HPLC was carried out using a Gilson HPLC system with UV detection at 226 nm. The separation was performed on a reversed phase column (Spherisorb C18, 250 × 4.6 mm) using isocratic conditions (1 mL/min) of 40% 0.02 M phosphate buffer (pH 3) and 60% acetonitrile.

Stability of Compounds in a Buffer Solution: A solution of 250 μL of prodrug in 0.02 M phosphate buffer (pH 7.2) was incubated at 37 °C. Aliquots were taken at various times and analysed by HPLC after dilution with eluent.

Stability of Compounds in Serum: After incubation of the prodrug at 37 °C in human serum ($c_0 = 10^{-4}$ M, 3% DMSO), aliquots (80 μL) were analysed according to the "on-line cleaning HPLC/UV/MS" methodology.[43] The parent prodrug and its metabolites were separated by HPLC (Alliance, WATERS), then quantified by UV detection (PPA 996, WATERS); this allowed pharmacokinetic studies. Information on the possible structures of some metabolites was obtained from their UV spectra and from coupling with an ESI mass spectrometer (SSQ-7000, THERMOQUEST).

Enzymatic Cleavage by *E. coli* β-D-Glucuronidase: Hydrolysis was investigated by incubating a solution of 250 μg/mL of prodrug 2 and 100 μg/mL of *E. coli* β-glucuronidase in 0.02 M phosphate buffer (pH 7.2) at 37 °C. Aliquots (100 μL) were taken at various times and analysed by HPLC after dilution with 300 μL of eluent.

In vitro Cytotoxicity: Cytotoxicity was tested against LoVo (human colon cancer cell line) cells using the methylene blue assay. The concentration of prodrug or drug inducing 50% of inhibition (IC_{50}) was calculated from the dose-response curve.

2-Methylamino-4-nitrophenyl-β-D-glucopyranosiduronic Acid (5): To a solution of **4**[16] (2 g, 4.13 mmol) in 50 mL acetone at 0 °C was added dropwise a 1 N NaOH aqueous solution (50 mL). After stirring for 5 min. at 0 °C, the mixture was acidified with 1 N HCl to pH 4, the solvents evaporated and the remaining mixture purified by column chromatography (MeCN/H_2O, 80:20). The solid was heated in a small volume of boiling methanol and filtered to eliminate most of the silica. After evaporation, a bright orange solid (100%) was obtained. − M.p. 172 °C; $[\alpha]_D^{20} = -53$ (*c* = 0.96 in MeOH). − IR (KBr): $\tilde{\nu}$ = 3400 (O−H), 1588 (aromatics), 1530, 1343 (NO_2) cm^{-1}. − ^{1}H NMR (DMSO): δ = 7.46 (dd, J_m = 3, J_o = 9, 1 H, a5), 7.19 (d, J_m = 3, 1 H, a3), 7.12 (d, J_o = 9, 1 H, a6), 6.04 (q, *J* = 5, 1 H, N−H), 5.68 (br. s, 1 H, OH), 5.13 (br. s, 1 H, OH), 4.85 (d, *J* = 7, 1 H, g1), 3.57−3.17 (4 H, g2, g3, g4, g5), 2.8 (d, *J* = 5, 3 H, N−CH_3). − MS (ES^-): *m/z* = 343 [M − H]$^-$.

2-(Trimethylsilyl)ethyl [2-Methylamino-4-nitrophenyl-2,3,4-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranoside]uronate (6): DMAP (3 mg) was added to a solution of **5** (910 mg, 2.64 mmol) in 9 mL pyridine. The mixture was cooled to 0 °C and TBS triflate (7 mL, 30.50 mmol) was added dropwise. After 48 h at room temperature, the solvents were evaporated and toluene (100 mL) was added to the residue. The insoluble pyridinium triflate was removed by filtration, and the filtrate was washed with water, dried over magnesium sulfate and the solvents evaporated. The product was obtained as a yellow resin (1.5 g) and used without purification in the next step. Any attempt at purification by chromatography resulted in loss of the compound. A solution of DMAP (24 mg, 0.20 mmol) in 3 mL CH_2Cl_2 was added to the crude product (457 mg, 0.67 mmol). After cooling to 0 °C, 2-trimethylsilylethanol (0.21 mL, 1.33 mmol) and a 1 M DCC solution in CH_2Cl_2 (1.34 mL, 1.34 mmol) were successively added. After stirring for 12 hours at room temperature, the

solvents were evaporated and cyclohexane (50 mL) added. The insoluble urea was removed by filtration. The solvents were again removed and the mixture purified by chromatography (toluene). Compound **6** was obtained as a yellow resin (247 mg, 39%). − $[\alpha]_D^{20}$ = 15 (c = 0.9 in $CHCl_3$). − IR ($CDCl_3$): $\tilde{\nu}$ = 1755 (C=O ester), 1588 (aromatics), 1530, 1343 (NO_2) cm^{-1}. − ^1H NMR ($CDCl_3$): δ = 7.58 (dd, J_o = 9, J_m = 3, 1 H, a5), 7.38 (d, J_m = 3, 1 H, a3), 6.83 (d, J_o = 9, 1 H, a6), 5.64 (d, J = 6, 1 H, g1), 4.59 (q, J = 5, 1 H, NH), 4.45 (1 H, g3), 4.38 (1 H, g4), 4.17 (m, 2 H, −$COOCH_2$-), 4.01 (t, J = 6, 1 H, g2), 3.87 (d, J = 3.5, 1 H, g5), 2.91 (d, J = 5, 3 H, N−CH_3), 0.97 (m, 2 H, Si−CH_2), 0.92 (9 H, Si−C−CH_3), 0.90 (9 H, Si−C−CH_3), 0.86 (9 H, Si−C−CH_3), 0.20−0.04 (27 H, Si−CH_3). − MS (CI): m/z = 787 $[M + H]^+$.

2-(Trimethylsilyl)ethyl [2-(*N*-chloroformyl-*N*-methylamino)-4-nitrophenyl-2,3,4-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranoside]-uronate (7): To a solution of **6** (350 mg, 0.445 mmol) in 20 mL CH_2Cl_2 at 0 °C was added a solution of phosgene (0.685 mL, 1.330 mmol) in toluene. Triethylamine (0.184 mL, 1.330 mmol) was then added dropwise. After 30 min. at 0 °C, the reaction was quenched with 10 mL water. The organic phase was separated and washed with 10 mL brine, dried over magnesium sulfate and evaporated. The residue was purified by chromatography (EtOAc/cyclohexane, 1:9) to obtain a colourless viscous oil (322 mg, 85%). − $[\alpha]_D^{20}$ = 12 (c = 0.89 in $CHCl_3$). − IR ($CDCl_3$): $\tilde{\nu}$ = 1753 (C=O esters), 1733 (C=O carbamoyl chloride), 1595 (aromatics), 1528, 1349 (NO_2) cm^{-1}. − ^1H NMR ($CDCl_3$): δ = 8.29 (dd, J_o = 9, J_m = 3, 1 H, a5), 8.18 (d, J_m = 3, 1 H, a3), 7.22 (d, J_o = 9, 1 H, a6), 5.72 (d, J = 5.5, 1 H, g1), 4.39 (1 H, g3), 4.37 (1 H, g4), 4.12 (m, 2 H, −$COOCH_2$-), 3.95 (d, J = 6, 1 H, g2), 3.85 (d, J = 3, 1 H, g5), 3.50 (s, 3 H, N−CH_3), 0.97 (m, 2 H, Si−CH_2), 0.96−0.85 (27 H, Si−C−CH_3), 0.17−0.04 (27 H, Si−CH_3). − MS (CI): m/z = 866 $[M + NH_4]^+$.

2-(Trimethylsilyl)ethyl {4-Nitrophenyl-2-[(paclitaxel-2′-*O*-carbonyl)methylamino]-2,3,4-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranoside}uronate (8): To a solution of chloride **7** (480 mg, 0.565 mmol) and paclitaxel (418 mg, 0.513 mmol) in 50 mL CH_2Cl_2 were added DMF (0.1 mL) and DMAP (128 mg, 1.050 mmol). Triethylamine (0.139 mL, 1 mmol) was then added dropwise. After stirring for 15 h at room temperature, the mixture was diluted with 20 mL CH_2Cl_2, washed three times with 20 mL water, dried over magnesium sulfate and evaporated. The residue was purified by chromatography (CH_2Cl_2/MeOH, 97:3) to obtain a white solid (720 mg, 84%). − M.p. 155 °C. − $[\alpha]_D^{20}$ = −57 (c = 1 in $CHCl_3$). − IR ($CDCl_3$): $\tilde{\nu}$ = 3645−3452 (OH, NH), 1730 (C=O ester), 1676 (C=O carbamate), 1594 (aromatics), 1528, 1348 (NO_2) cm^{-1}. − ^1H NMR ($CDCl_3$): δ = 8.16 (d, J = 8, 2 H, p2-Bz), 7.8−7.2 (16 H, aromatics a + p), 7.05 (t, J = 10, 1 H, p3′-NHBz), 6.29 (s, 1 H, p10), 6.19 (t, J = 8, 1 H, p13), 5.75 (1 H, p3′), 5.67 (1 H, p2), 5.66 (1 H, g1), 5.35 (1 H, p2′), 5.00 (d, J = 9, 1 H, p5), 4.48 (1 H, g3), 4.45 (m, 1 H, p7), 4.34 (AB, J = 8, 1 H, p20α), 4.27 (1 H, g4), 4.20 (AB, J = 8, 1 H, p20β), 4.10 (m, 2 H, $COOCH_2$), 3.95 (dd, J = 5, 1 H, g2), 3.84 (d, J = 4, 1 H, g5), 3.82 (d, J = 7, 1 H, p3), 3.23 (s, 3 H, N−CH_3), 2.52 (m, 1 H, p6α), 2.45 (s, 3 H, p4-OAc), 2.33 (m, 2 H, p14), 2.23 (s, 3 H, p10-OAc), 1.95 (m, 1 H, p6β), 1.68 (s, 3 H, p18), 1.63 (s, 3 H, p19), 1.21 (s, 3 H, p16), 1.13 (s, 3 H, p17), 0.98−0.75 (29 H, Si−C−CH_3 + Si−CH_2), 0.18−0.04 (27 H, Si−CH_3). − MS (FAB + NaCl): m/z = 1689 $[M + Na]^+$.

Benzyl [2-Methylamino-4-nitrophenyl-2,3,4-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranoside]uronate (9): Compound **9** was obtained following the same procedure as described for **6**. Thus, treatment of **5** (1.87 g, 5.43 mmol) afforded **9** as a yellow resin (1.83 g, 44% from **5**) after chromatography with CH_2Cl_2 and then with CH_2Cl_2/cyclohexane (5:1). − $[\alpha]_D^{20}$ = −2.3 (c = 0.98 in $CHCl_3$). − IR ($CDCl_3$): $\tilde{\nu}$ = 1762 (C=O ester), 1623 (aromatics), 1530, 1343 (NO_2) cm^{-1}. − ^1H NMR ($CDCl_3$): δ = 7.53 (dd, J_o = 9, J_m = 3, 1 H, a5), 7.35 (d, J_m = 3, 1 H, a3), 7.33−7.26 (5 H, Bn), 6.83 (d, J_o = 9, 1 H, a6), 5.62 (d, J = 6, 1 H, g1), 5.11 (s, 2 H, −$COOCH_2$-), 4.59 (q, J = 5, 1 H, NH), 4.50 (d, J = 6, 1 H, g3), 4.38 (d, J = 4, 1 H, g4), 4.02 (d, J = 6, 1 H, g2), 3.87 (d, J = 4, 1 H, g5), 2.86 (d, J = 5, 3 H, N−CH_3), 0.91 (18 H, Si−C−CH_3), 0.86 (9 H, Si−C−CH_3), 0.16−0.01 (18 H, Si−CH_3). − MS (CI): m/z = 777 $[M + H]^+$.

Benzyl [2-(*N*-chloroformyl-*N*-methylamino)-4-nitrophenyl-2,3,4-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranoside]uronate (10): To a solution of **9** (350 mg, 0.45 mmol) in 20 mL CH_2Cl_2 at 0 °C, was added a solution of phosgene (0.700 mL, 1.35 mmol) in toluene. Triethylamine (1.13 mL, 8.16 mmol) was then added dropwise. After 30 min. at 0 °C, the reaction was quenched with 10 mL water. The organic phase was separated and washed with 10 mL brine, dried over magnesium sulfate and evaporated. The residue was purified by chromatography (EtOAc/cyclohexane, 1:13) to obtain a colourless viscous oil (352 mg, 93%). − $[\alpha]_D^{20}$ = −5 (c = 1 in $CHCl_3$). − IR ($CDCl_3$): $\tilde{\nu}$ = 1734 (C=O carbamoyl chloride), 1594 (aromatics), 1528, 1349 (NO_2) cm^{-1}. − ^1H NMR ($CDCl_3$): δ = 8.24 (dd, J_o = 9, J_m = 3, 1 H, a5), 8.14 (d, J_m = 3, 1 H, a3),7.35−7.25 (5 H, Bn), 7.22 (d, J_o = 9, 1 H, a6), 5.73 (d, J = 5.5, 1 H, g1), 5.07 (AB, J = 12, 1 H, $COOCH_2$), 5.05 (AB, J = 12, 1 H, $COOCH_2$), 4.43 (1 H, g3), 4.40 (1 H, g4), 3.96 (d, J = 5, 1 H, g2), 3.88 (d, J = 3, 1 H, g5), 3.25 (3 H, s, N−CH_3), 0.95−0.86 (27 H, Si−C−CH_3), 0.08 (12 H, Si−CH_3), 0.07 (3 H, Si−CH_3), 0.02 (3 H, Si−CH_3). − MS (CI): m/z = 856 $[M + NH_4]^+$.

Benzyl {4-Nitrophenyl-2-[(paclitaxel-2′-*O*-carbonyl)methylamino]-2,3,4-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranoside}uronate (11): Chloride **10** (338 mg, 0.403 mmol) was condensed with paclitaxel (313 mg, 0.367 mmol) under the same conditions as described for **8**. After chromatography (CH_2Cl_2/MeOH, 98:2), compound **11** was obtained as a white solid (498 mg, 82%). − $[\alpha]_D^{20}$ = −59 (c = 1 in $CHCl_3$). − IR ($CDCl_3$): $\tilde{\nu}$ = 3647−3452 (OH, NH), 1724 (C=O ester), 1667 (C=O carbamate), 1593 (aromatics), 1526, 1349 (NO_2) cm^{-1}. − ^1H NMR ($CDCl_3$): δ = 8.16 (d, J = 8, 2 H, p2-Bz), 7.78−7.15 (21 H, aromatics a + p + Bn), 7.07 (t, J = 10, 1 H, p3′-NHBz), 6.29 (s, 1 H, p10), 6.23 (t, J = 9, 1 H, p13), 5.75 (1 H, p3′), 5.68 (d, J = 7, 1 H, p2), 5.63 (d, J = 5.5, 1 H, g1), 5.37 (1 H, p2′), 5.13 (d, J = 9, 1 H, p5), 5.02 (AB, J = 15, 1 H, $COOCH_2$), 4.98 (AB, J = 15, 1 H, $COOCH_2$), 4.45 (m, 1 H, p7), 4.39 (1 H, g3), 4.35 (AB, J = 8, 1 H, p20α), 4.34 (1 H, g4), 4.21 (AB, J = 8, 1 H, p20β), 3.94 (d, J = 5, 1 H, g2), 3.86 (d, J = 4, 1 H, g5), 3.82 (d, J = 7, 1 H, p3), 3.17 (s, 3 H, N−CH_3), 2.48 (m, 1 H, p6α), 2.47 (s, 3 H, p4-OAc), 2.34 (m, 2 H, p14), 2.21 (s, 3 H, p10-OAc), 1.98 (m, 1 H, p6β), 1.70 (s, 3 H, p18), 1.66 (s, 3 H, p19), 1.22 (s, 3 H, p16), 1.15 (s, 3 H, p17), 0.96−0.73 (27 H, Si−C−CH_3), 0.16−0.04 (27 H, Si−CH_3). − MS (FAB + NH_3): m/z = 1674 $[M + NH_4]^+$.

Benzyl {4-Nitrophenyl-2-[(paclitaxel-2′-*O*-carbonyl)methylamino]-β-D-glucopyranoside}uronate (12): To a solution of **11** (472 mg, 0.285 mmol) in 4.7 mL pyridine at 0 °C, was added dropwise a 70% solution of HF in pyridine (4 mL, 1.42 mmol). After stirring at 0 °C for 3 h and for 15 h at room temperature, the solvents were evaporated. The residue was dissolved in CH_2Cl_2, filtered in order to remove the insoluble pyridinium fluoride and the solvents evaporated again. Purification by chromatography (CH_2Cl_2/MeOH, 95:5) gave **12** as a white solid (304 mg, 81%). − M.p. 182 °C. − $[\alpha]_D^{20}$ = −99 (c = 1 in $CHCl_3$). − IR ($CDCl_3$): $\tilde{\nu}$ = 3452−3352 (OH, NH), 1724 (C=O ester), 1594 (aromatics), 1526, 1349 (NO_2) cm^{-1}.

− ^{1}H NMR ($CDCl_3$): δ = 8.08 (d, *J* = 8, 2 H, p2-Bz), 7.78−7.15 (21 H, aromatics a + p + Bn), 7.08 (t, *J* = 10, 1 H, p3′-NHBz), 6.39 (s, 1 H, p10), 6.23 (t, *J* = 9, 1 H, p13), 5.71 (1 H, p3′), 5.65 (d, *J* = 8, 1 H, p2), 5.18 (s, 2 H, $COOCH_2$), 5.11 (d, *J* = 9, 1 H, p5), 4.93 (1 H, p2′), 4.87 (d, *J* = 7, 1 H, g1), 4.46 (m, 1 H, p7), 4.26 (AB, *J* = 8, 1 H, p20α), 4.15 (AB, *J* = 8, 1 H, p20β), 3.96 (1 H, g3), 3.84 (1 H, g4), 3.75 (d, *J* = 6, 1 H, p3), 3.62 (d, *J* = 4, 1 H, g5), 3.50 (1 H, g2), 3.13 (s, 3 H, N−CH_3), 2.50 (m, 1 H, p6α), 2.36 (s, 3 H, p4-OAc), 2.33 (2 H, p14), 2.18 (s, 3 H, p10-OAc), 1.97 (m, 1 H, p6β), 1.70 (s, 3 H, p18), 1.65 (s, 3 H, p19), 1.18 (s, 3 H, p16), 1.12 (s, 3 H, p17). − MS (FAB + NaCl): *m*/*z* = 1336 [M + Na]$^+$.

4-Nitrophenyl-2-[(paclitaxel-2′-*O*-carbonyl)methylamino]-β-D-glucopyranosiduronic Acid (2): Pd/C (10%, 184 mg) was added to a solution of **12** (184 mg, 0.14 mmol) in 0.553 mL ethanol. After dropwise addition of 1,4-cyclohexadiene (0.141 mL, 1.40 mmol), the mixture was heated at 40 °C for 4 h. The mixture was filtered over celite and evaporated. The product was purified by chromatography (MeCN/water, 90:10) giving a white solid (93 mg, 54%). − M.p. 189 °C. − $[\alpha]_D^{20}$ = −79 (*c* = 0.5 in MeOH). − IR (KBr): $\tilde{\nu}$ = 3600−3429 (OH, NH), 1718 (C=O ester), 1631 (C=O carbamate), 1526, 1349 (NO_2) cm^{-1}. − ^{1}H NMR (CD_3OD): δ = 8.09 (d, *J* = 8, 2 H, p2-Bz), 7.72−7.37 (16 H, aromatics a + p), 7.12 (t, *J* = 10, 1 H, p3′-NHBz), 6.45 (s, 1 H, p10), 5.98 (t, *J* = 9, 1 H, p13), 5.60 (d, *J* = 7, 1 H, p2), 5.48 (1 H, p3′), 5.14 (1 H, p2′), 5.12 (d, *J* = 9, 1 H, p5), 4.98 (d, *J* = 7, 1 H, g1), 4.33 (m, 1 H, p7), 4.16 (s, 2 H, p20), 3.76 (d, *J* = 7, 1 H, p3), 3.80−3.50 (4 H, g2 + g3 + g4 + g5), 3.21 (s, 3 H, N−CH_3), 2.48 (m, 1 H, p6α), 2.35 (2 H, p14), 2.31 (s, 3 H, p4-OAc), 2.17 (s, 3 H, p10-OAc), 1.92 (s, 3 H, p18), 1.79 (m, 1 H, p6β), 1.63 (s, 3 H, p19), 1.12 (s, 6 H, p16 + p17). − MS (ES$^-$;NH_4OH/MeCN 2%): *m*/*z* = 1222 [M − H]$^-$.

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