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A new paclitaxel prodrug for use in ADEPT strategy

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Received 3rd June 2003, Accepted 28th July 2003
First published as an Advance Article on the web 29th August 2003

A new paclitaxel prodrug intended for use in Antibody-Directed Prodrug Therapy (ADEPT) or Prodrug Monotherapy (PMT) has been prepared. This prodrug was originally designed to be activated into the drug by human β-glucuronidase. In order to enhance the liberation rate of paclitaxel, an elongated spacer system including a nitro-aromatic derivative and a *N,N′*-methylethylenediamine was incorporated between the sugar moiety and the drug. Indeed, this new prodrug proved to be activated significantly faster than a former paclitaxel prodrug containing a conventional spacer.

Introduction

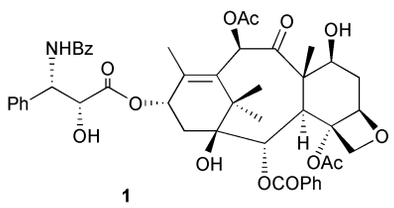
Despite an increasing number of potent anticancer agents, lack of selectivity is still a major drawback in clinical oncology. The use of monoclonal antibodies (MAb) as well as tumor-associated enzymes to efficiently target tumor cells represents a promising way forward.[1–4]

In the Antibody-Directed Enzyme Prodrug Therapy (ADEPT),[5–15] an enzyme linked to a MAb directed at a tumor site catalyses the activation of a prodrug into the corresponding cytotoxic drug. The first step involves the targeting of the desired enzyme to tumor cells by means of antibody–antigen recognition in the form of an enzyme–MAb conjugate or a fusion protein. Then, following tumor localisation and systemic clearance, a non-active prodrug is administered, which, after enzymatic cleavage, will release the active compound only at tumor cells. A higher level of cytotoxic agent in tumor tissue can be achieved *via* this concept, as shown for doxorubicin.[16–17]

The success of a rational approach strongly depends upon several factors. These include the kind of cytotoxic agent, the choice of the MAb and the enzyme. In order to reduce hetero- and immunogenicity, Bosslet and co-workers were the first to build a fusion protein made of human ß-D-glucuronidase combined with a humanised anti-CarcinoEmbryonic Antigen (CEA) MAb BW431.[18–21] Furthermore, following histochemical studies, ß-D-glucuronidase was found in the intercellular medium of necrotic areas.[22–23] This fact allows the potentially useful Prodrug Mono Therapy (PMT) protocol, which is the use of prodrugs alone without any externally added enzyme.[24]

Paclitaxel **1** (Taxol®)[25] has been introduced several years ago in cancer chemotherapy due to its remarkable antitumor activity, especially against ovarian, breast and lung cancers,[26] for which it has been approved by the FDA. It shows a unique mechanism of action by promoting and stabilizing microtubules from tubulin.[26–28] The major drawbacks in the use of paclitaxel are its poor selectivity resulting in a low therapeutic index and side-effects such as myelosuppression, mucositis or peripheral neuropathy.[29–30] Moreover, due to its low water-solubility, it is administered as a solution in Chremophor EL®, a polyoxyethylated castor oil, which may be responsible for hypersensitivity reactions.[29–31] For all these reasons, paclitaxel is an attractive candidate for ADEPT and/or PMT strategies, as a prodrug could solve the problems of toxicity while enhancing both selectivity and solubility.

To date, there are two classes of paclitaxel prodrugs; the first included the addition of ionic groups that could be hydrolysed by circulating enzymes such as esterases or phosphatases.[32–39] They still lack selective release.



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The prodrugs of the second class were designed for enhancing drug selectivity (Fig. 1).

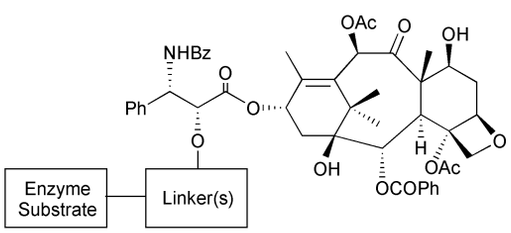
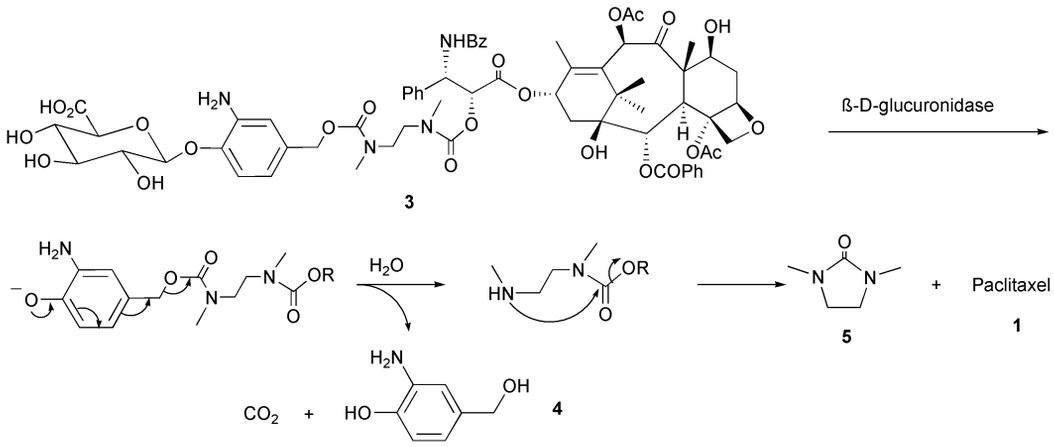


Fig. 1 General structure of prodrugs.

The first ADEPT-designed paclitaxel prodrug was reported by Rodrigues *et al.*[40] It required a ß-lactamase activation followed by self-immolation of an aminobutyryl linker. However, it suffered from a poor release rate, since completion of the self-immolation was reached after 16 h. During this time, the intermediate can escape from the tumor site, which is inconsistent with selective targeting. It also has the potential drawback of immunogenicity already encountered with exogenous enzymes.[41]

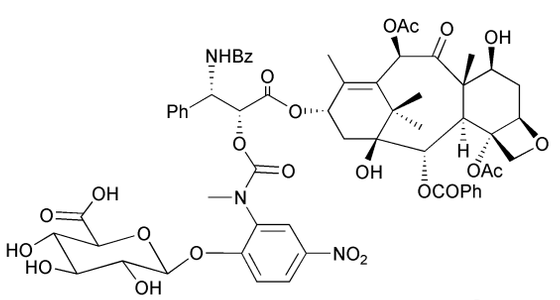
Scheeren and co-workers designed two kinds of paclitaxel prodrugs. The first were devised for ADEPT, including a spacer (aminobutyrate or *p*-aminophenylacetate) connected to ß-glucuronic acid *via* a carbamate function, and linked to the 2′-hydroxy of paclitaxel by an ester bond.[42] Although release of paclitaxel occurred, problems of fast non-specific hydrolysis were encountered in buffer solution (pH = 6.8), and this may be reinforced in plasma, since it is well known that esters of paclitaxel suffer from low chemical stability due to circulating enzymes.[33] They also made prodrugs based on tumor-associated protein plasmin activation (Prodrug MonoTherapy strategy).[43] For this purpose, a tripeptide was linked, *via* an amide bond, to a spacer (*p*-aminobenzyl, ethylenediamine or a combination of both) which in turn was connected to the 2′-hydroxy of paclitaxel by a carbonate or a carbamate function. Recently they designed the first bioreductive-activated prodrugs of paclitaxel with a carbonate bond at the 2′-position.

DOI: 10.1039/b306236h



Scheme 1 Paclitaxel liberation.

For our part, we have previously synthesised the three-component paclitaxel prodrug **2** for ADEPT by linking a glucuronyl derivative of 2-*N*-methylamine-4-nitrophenol to the 2′-hydroxy group through a carbamate linkage.[45] The prodrug displayed a relevant detoxification (about 700-fold) and a high stability in buffer and plasma (half-life of 45.5 ± 4 hours in the latter) with no detected free drug. However, even in the presence of a relative high concentration of enzyme, the enzymatic hydrolysis half-life was still 115 min. On the basis of the already known structure of human ß-D-glucuronidase,[46] molecular modelling calculations indicated that this might be due to steric hindrance.



The design for preparing a glucuronide-containing prodrug of paclitaxel with better enzymatic kinetics led us to choose a linker that would move the glucuronic moiety away from the taxane core. We envisioned using two spacers linked together by a carbamate function such as a *para*-hydroxybenzyl alcohol linked to *N, N′*-dimethyl ethylenediamine; this double spacer has proved efficient with a nitrogen mustard.[47] Interestingly, during the course of our work, a plasmin activated prodrug was published by Scheeren *et al.*[48] incorporating a double spacer. The first spacer was an aniline derivative and the second *N, N′*-dimethyl ethylenediamine derivative linked through carbamate functionalities. This kind of spacer was shown to be effective for the liberation of paclitaxel by plasmin.[48]

The 2′ position of paclitaxel was selected for derivatisation as it is known that its modification leads to a dramatic loss of cytotoxic activity.[32–33] Moreover this position is more reactive than the hindered 7-hydroxy and the tertiary 1-hydroxy.[35] Thus the glucuronide-based paclitaxel prodrug **3**, which was designed for the liberation of the free drug as depicted in Scheme 1, was synthesized and tested *in vitro*. This is the subject of the present report.

Results and discussion

Synthesis

The 3-nitro-4-hydroxybenzaldehyde glucuronide **6**, previously reported during the synthesis of the doxorubicin prodrug HMR 1826,[49] was used as the starting material (Scheme 2). Due to the sensitivity of the paclitaxel skeleton under both acidic or basic conditions,[50–51] the acetyl protecting groups had to be replaced by *tert*-butyldimethylsilyl ethers and the methyl ester by a benzyl ester; both types of protecting groups have been shown to be compatible with our synthetic scheme.[45] The second expected difficulty was the sequential manipulation of the two amine groups of ethylene diamine in order to link one of them to paclitaxel and the other one to the moiety containing the glucuronate.

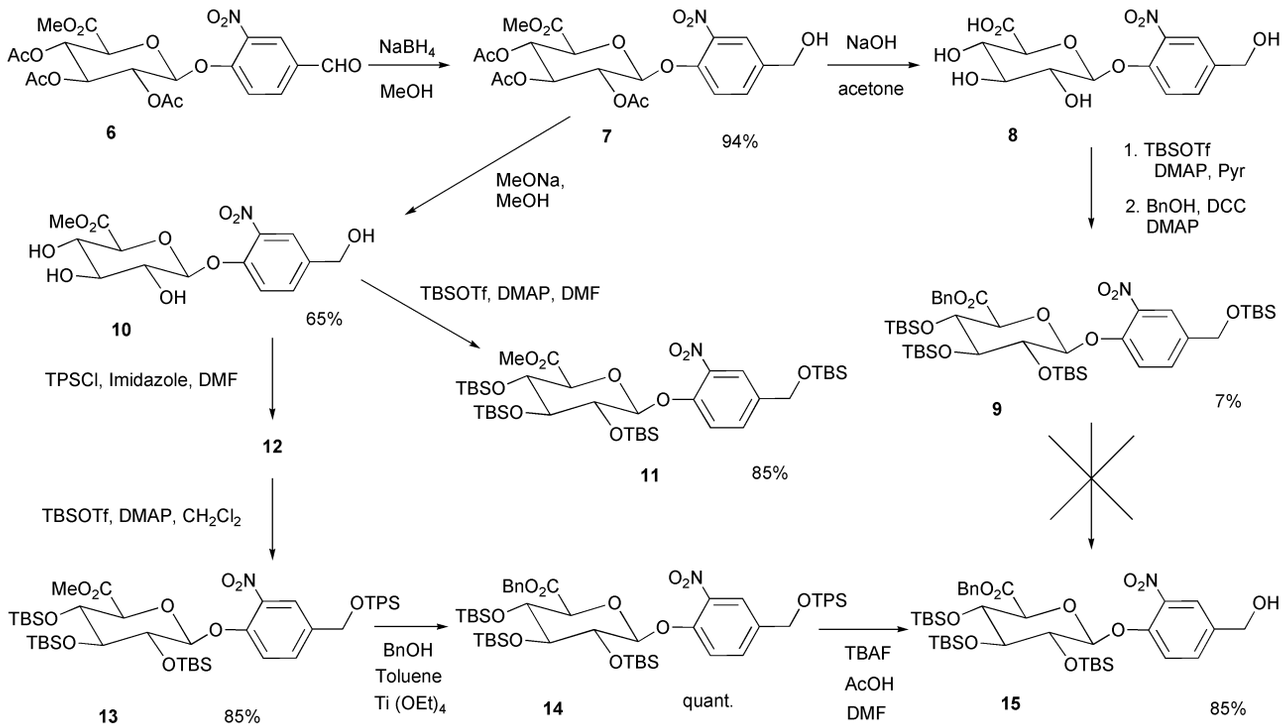
Our approach started with the reduction of aldehyde **6** to alcohol **7** by sodium borohydride. The glucuronate **7** contained three different kinds of hydroxy groups, one primary benzylic alcohol, three secondary alcohols protected by acetate, and one carboxylic acid protected as a methyl ester. Our aim was to obtain a benzyl ester with silylated secondary hydroxy groups and a free primary alcohol. Our first trial was based on the fact that it should be possible to selectively deprotect a primary silylated hydroxy group in the presence of secondary ones.

Treatment of compound **7** with sodium hydroxide in acetone led to the fully deprotected glucuronide **8**. Exhaustive silylation using TBSOTf in pyridine, followed by esterification of the carboxylic function with benzyl alcohol using DCC, gave intermediate **9**. However, the crucial selective desilylation of the benzylic alcohol turned out to be very challenging. Despite many trials (TBAF in THF, HF–pyridine in THF, adjustment of reaction temperature and duration) such selectivity could not be cleanly achieved in the correct yield.

Moreover, the poor yield observed in the preparation of **9**, led us to reconsider our synthetic scheme and use a stepwise process for the deprotection/reprotection steps of the sugar moiety,[52] in order to differentiate the hydroxy on the benzylic position prior to the protection of the sugar hydroxy groups.

Starting from compound **7**, a selective deacetylation giving ester **10** was achieved using NaOMe in dry MeOH. A fully silylated derivative **11** was then obtained by reaction with TBSOTf. The switch from the methyl ester to a benzyl ester was attempted according to a described method by saponification and reesterification,[52] but was unsuccessful on **11** in our hands. As selective desilylation of **9** proved to be also challenging, this approach was consequently abandoned and the introduction of different silyl groups was undertaken.

A selective silylation of the primary benzyl hydroxy could be effected using TPSCl, and then the remaining secondary hydroxy groups were protected using TBSOTf. The switch from the methyl ester to a benzyl ester by saponification and reesterification[52] was unsuccessful on **13** in our hands. A good alternative turned out to be a transesterification procedure using $Ti(OEt)_4$ and benzyl alcohol,[53] which had the advantage of avoiding any free acid handling. So the fully protected compound **14** was obtained from **6** in 44% overall yield.



Scheme 2 Prodrug synthesis (first part).

The next step was the introduction of the diamine tether. Using a reported procedure (1 eq. TBAF–AcOH),[54] selective desilylation of the benzyl hydroxy was cleanly performed to afford **15**. Next this hydroxy was activated as a *p*-nitrophenylchlorocarbonate, and **16** was condensed with the *mono*-protected diamine **17** (Scheme 3).[55] This afforded the benzyl carbamate **18**. It is noteworthy that an improved reaction yield was achieved using a one-pot procedure by avoiding the isolation of the sensitive activated carbonate. Dry 3 M HCl in EtOAc removed the BOC protecting group giving the deprotected amine as a hydrochloride salt **19**. No attempts were made to isolate the free amine, which would obviously cyclise very rapidly on the carbamate function with liberation of the starting free benzylic alcohol. The chlorocarbamoylation of the resulting amine by phosgene cleanly led to the activated carbamoyl chloride **20**, which was ready for the coupling step with paclitaxel. The amine liberated from its salt by triethylamine reacted fast enough with phosgene to avoid the intermolecular cyclisation. Compound **20** proved to be stable enough to be purified by chromatography.

Remarkably, the coupling reaction between compound **20** and paclitaxel **1** was achieved regioselectively in good yield (87%) by using a large excess of DMAP. The three silyl groups of the resulting compound **21** were then easily removed with an excess of the HF–pyridine complex in dry acetonitrile. Finally, reductive cleavage of the benzyl ester needed a large excess of cyclohexadiene with palladium-on-charcoal and gave the prodrug **3**, which was isolated in moderate yield (24%). Moreover, it must be noted that the nitro group was reduced to an amine under these conditions. This was rather surprising compared with the previous behaviour of such a nitro-containing spacer used to obtain prodrug **2**,[45] since this procedure allowed us to keep the nitro group intact.

There is still a precedent with prodrugs including a spacer bearing *ortho* amino groups like NEt_2, $NHCOCH_3$, $NHCOCF_3$ in the literature,[56] but to date, not with NH_2. It remains interesting to know the behaviour of this latter prodrug, and thus, to study its use as prodrug in an ADEPT or PMT strategy.

In summary, prodrug **3** was obtained in 16% overall yield relative to paclitaxel.

Biological results

Our aim was to obtain a prodrug more suitable than the former[45] for the *in vivo* tests, especially concerning the enzymatic cleavage. The spacer was modified and our choice was a cascade of two linkers (Scheme 1), an aromatic part and a derivative of diaminoethylene. Another difference to the former prodrug was the presence of an amino group instead of a nitro group on the aromatic spacer.

Prodrug **3** was tested *in vitro* for detoxification, stability and kinetics of enzymatic cleavage.

A requirement for ADEPT strategy is that a relatively non-cytotoxic prodrug releases a cytotoxic drug. Cytotoxicity measurements were done on L1210 cell lines. IC_{50} of the prodrug (1500 nM) was lower than that of the drug (9.8 nM) by a factor of 153. This value is approximately of the same magnitude as the values found for other stable paclitaxel prodrugs (Table 1).

The cytotoxicity ratio between the prodrug and the free drug depends on the cell line, the stability of the prodrug, and its structure. As a general rule, the more hydrophilic prodrugs are less prone to crossing the cell membranes and reaching their cellular targets. To date, the best detoxification for a paclitaxel prodrug has been reached by plasmin activated prodrugs. These compounds have two amine nitrogens and are used as their bis-chlorhydrate. Usually, it can be considered that a factor of at least 100 is needed for an ADEPT strategy; this is the case for prodrug **3**, even if the value is slightly lower than for prodrug **2** (722). One explanation could be the relative log P (octanol/water partition coefficient) values of paclitaxel and both prodrugs **2** and **3**. They were calculated using the ClogP module from ChemDraw®.

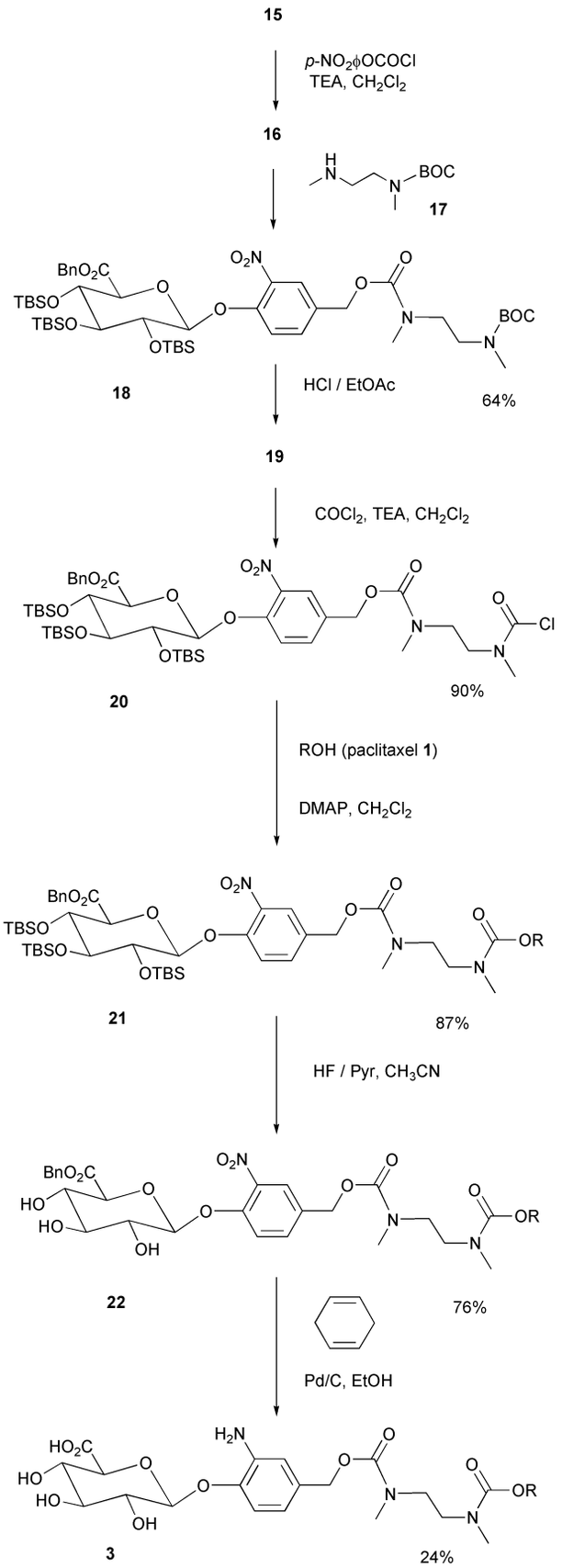
Paclitaxel **1**: 4.7308

Prodrug **2**: 3.5745

Prodrug **3**: 4.062

Prodrug **2** being more hydrophilic than **3** is also the less cytotoxic prodrug.

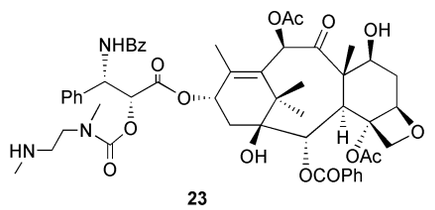
Consistent with these figures is the observation that prodrug **3** is less water-soluble than the former; so, all measurements were conducted with a concentration of 30 μg mL^{-1} (22.9 μmol) instead of 250 μg mL^{-1} for prodrug **2**.



Scheme 3 Prodrug synthesis (second part).

Stability of prodrug **3** was measured by HPLC (UV detection) in a phosphate buffer at 37 °C (pH 7.2). The prodrug proved to be stable during a 24-hour run, and no paclitaxel was liberated from the prodrug during this time.

Enzymatic cleavage was studied using *E. coli* β-D-glucuronidase at 37 °C, at pH 7.2 in 0.02 M phosphate buffer. Starting from a peak corresponding to the prodrug **3**, three peaks appeared successively. The last one was identified by HPLC comparison with the free paclitaxel. The first one corresponded to the spacer **4**. The second was an intermediate of the reaction, since it first appeared then disappeared progressively. For identification of this instable compound, we performed LC/MS experiments. We could obtain the mass spectra of this compound and characterise it as being the paclitaxel still linked to the ethylenediamino derivative **23**.



23

The half-life of intermediate **23** was measured by using an excess of enzyme (50 μg mL^{-1}, 240 units mL^{-1}) in order to cleave the glucuronic moiety almost instantaneously. The curve corresponding to **23** gave a half-life of 13 minutes (Fig. 2). Scheeren *et al* observed the same intermediate starting from a different prodrug and measured a $T_{1/2\ cycl}$ of 47 minutes.[48] The difference could be explained by the concentration of starting prodrug and the nature of buffer and enzyme in the solution.

Cleavage of prodrug **3** was followed by using an enzymatic concentration of 2.5 μg mL^{-1} (12 units mL^{-1}) (Fig. 3).

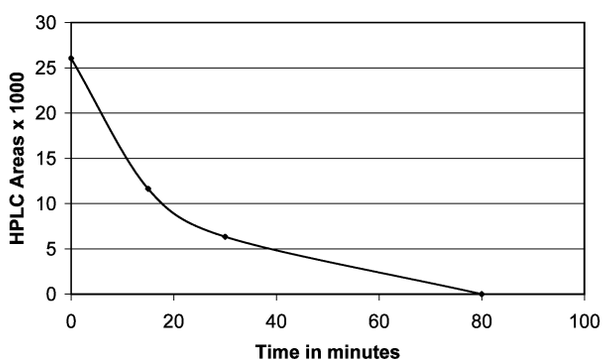


Fig. 2 Disappearance of intermediate **23**. Prodrug **3** concentration: 30 μg mL^{-1} (22.9 μmol) Enzyme concentration: 240 units mL^{-1}.

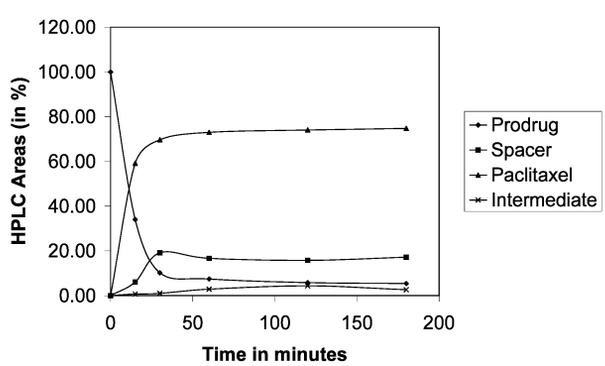


Fig. 3 Enzymatic cleavage of prodrug **3**. Prodrug **3** concentration: 30 μg mL^{-1} (22.9 μmol) Enzyme concentration: 12 units mL^{-1}.

Two facts were very different from the data of the former prodrug **2**.[45] The cleavage required a much lower β-D-glucuronidase concentration, but an intermediate species was observed.

For prodrug **3** (30 μg mL^{-1}), the half-life of the prodrug was 10 minutes for an enzyme concentration of 12 units mL^{-1}. For comparison, we measured the kinetics of cleavage of prodrug **2** with the same concentration of prodrug. In the latter case, an

Table 1 Comparison of cytotoxicities for paclitaxel prodrugs

References	Activation enzyme	Cytotoxicity ratio[a]	Cell lines	ClogP (from ChemDraw®)
40	β-lactamase	10	SK-BR-3	2.9236
42	β-D-glucuronidase	95	OVCAR-3	3.3781
		1.1		2.7830
		135		3.2735
43	Plasmin	1100–20000 (average 8000)	MCF-7	4.9453–7.1763 (calculations on neutral species, not on the chlorhydrates)
			EVSA-T	
			WIDR	
			IGROV	
			M19	
			A498	
			H226	
44	Bioreduction	1–462	H226	4.8784–7.5460
			MCF7	
			EVSA-+T	
			WIDR	
			IGROV	
			M19	
			A498	
48	Plasmin	1.6–213	MCF-7	6.7667–7.0700 (calculations on neutral species, not on the chlorhydrates)
			EVSA-T	
			WIDR	
			IGROV	
			M19	
			A498	
			H226	
45 (compound **2**)	β-D-glucuronidase	722	LoVo	3.5745

[a] Measured as IC_{50}(prodrug)/IC_{50}(Free paclitaxel).

enzyme concentration of 240 units mL^{-1} was needed to obtain a half-life of 5.5 hours. At this enzyme concentration, cleavage of prodrug **3** was almost instantaneous. The difference can be explained by steric interactions. In prodrug **2**, the glucuronate and the paclitaxel are in *ortho* positions on the aromatic spacer, giving a bad steric interaction for the approach of the enzyme towards the glycosidic bond. In prodrug **3**, the glucuronate and paclitaxel are in *para* positions, so the glycosidic bond is much more accessible and the kinetics are much faster.

These results showed also that good kinetic results can be observed with an amino aromatic spacer instead of a nitro aromatic one. It was known that spacers with substituted amino groups like NEt_2, $NHCOCH_3$, $NHCOCF_3$ were compatible,[56] but to our knowledge it is the first time that it has also been proved for a NH_2 spacer with a prodrug suitable for an ADEPT strategy.

Conclusion

In conclusion, we have prepared a new paclitaxel prodrug, which contains two self-immolative spacers. Such a prodrug is endowed with acceptable reduced cytotoxic effect and activated by β-glucuronidase to release paclitaxel under conditions compatible with *in vivo* experiments. It is also noteworthy that for this class of glucuronic acid-containing prodrugs this work represents the first example of a spacer bearing an amino group in the *ortho* position of the phenol involved in the glycosidic linkage.

Experimental

General

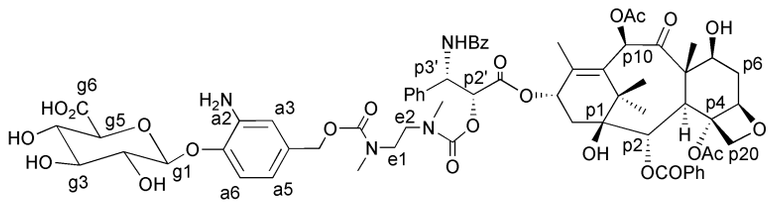
Melting points (mp) were measured using an Electrothermal digital melting point apparatus and are uncorrected. ^{1}H-NMR (300 MHz) and ^{13}C-NMR (75 MHz) spectra were recorded on Bruker AC 300 spectrometer – chemical shifts δ in ppm and J in Hz. Chemical ionisation CI-MS (NH^3) or FAB mass spectra, were recorded on a Nermag R 10-10C spectrometer. Electrospray ionisation mass spectra (ESI-MS) were acquired on an API3000 LC-MS-MS spectrometer, and HR-MS were recorded on a LCTOF (Micromass) Spectrometer. Chromatography was conducted over silica gel (Merck 60 (230–400 Mesh)).

For the NMR description, the following numeration was chosen: "a" for aromatic "g" for glucuronic acid, "e" for ethylenediamine, and "p" for paclitaxel (see Fig. 4).

If there was any ambiguity in 1D-NMR experiments, ^{1}H and ^{13}C assignments were determined by 2-D-NMR experiments (COSY, HMQC).

Methyl {[2-nitro-4-(hydroxymethyl)phenyl]-2,3,4-tri-*O*-acetyl-β-D-glucopyranosid}uronate 7[49]**.** Aldehyde **6** (1.45 g, 3 mmol) in $CHCl_3$ (20 mL) and *i*PrOH (5 mL) was degassed under argon. After addition of 2.25 g of silica, the medium was cooled to 0 °C and $NaBH_4$ (230 mg, 12 eq.) was added portionwise. After 1 h at 0 °C, the solution was allowed to reach room temperature. The solution was filtered on Celite 545 and the pad washed with CH_2Cl_2 (20 mL). The filtrate was concentrated under vacuum, and the compound was recrystallized from ethanol, affording 1.19 g (82%) of the reduction compound as a microcrystalline powder; mp 175 °C (from EtOH); (Found: C, 49.75; H, 4.81; N, 2.96. $C_{20}H_{23}NO_{13}$ requires C, 49.49; H: 4.78; N, 2.89%); δ_H (DMSO-d_6): 7.80 (1H, d, J 1.5 Hz, a3), 7.62 (1H, dd, J 8.3 and 1.3 Hz, a5), 7.38 (1H, d, J 8.7 Hz, a6), 5.71 (1H, d, J 7.8 Hz, g1), 5.45 (2H, m, OH, g3), 5.11 (2H, m, g2, g4), 4.72 (1H, d, J 9.9 Hz, g5), 4.51 (2H, s, CH_2OH); 3.64 (3H, s, CH_3O); 2.01 (9H, s, $CH_3C(O)$); δ_C (DMSO-d_6): 169.5, 169.3 and 168.8 (MeC(O)), 167.0 (CO_2Me), 146.9, 140.2 and 138.6 ($Cq_{aromatics}$), 132.0 (a5), 122.3 (a3), 117.7 (a6), 98.1 (g1), 71.0 (g5), 70.8 (g3), 70.0 (g2), 68.7 (g4), 61.3 (CH_2OH), 52.7 (CH_3O), 20.6 ($CH_3C(O)$); m/z [CI + NH_3] 503 $(M + NH_4)^+$.

Benzyl {[2-nitro-4-(*O*-*tert*-butyldimethylsilylmethyl)phenyl]-2,3,4-tri-(*O*-*tert*-butyldimethylsilyl)-ß-D-glucopyranosid}uronate 9. Benzyl alcohol **7** (1.6 g, 3.3 mmol) was dissolved in 40 mL of



3

Fig. 4 NMR attributions for prodrug **3**.

acetone at 0 °C. A molar solution (25 mL) of aqueous sodium hydroxide was added dropwise and stirring was continued for 5 min. The medium was then neutralized with 1 M HCl, evaporated and the compound was purified on a silica gel column (eluent CH_3CN–H_2O, 7 : 3). The recuperated compound was dissolved in distilled pyridine (15 mL) in the presence of DMAP (5.6 mg). After cooling to 0 °C, 15 mL of TBSOTf were added dropwise to the medium under argon, and the reaction was left for 18 h at room temperature. The reaction mixture was then concentrated, the residue taken up in toluene (120 mL) and the insoluble pyridinium triflate salt filtered. The filtrate was washed with 20 mL of water, decanted and dried over $MgSO_4$. Finally the organic layer was concentrated under reduced pressure. The residue was then solubilized in anhydrous CH_2Cl_2 (10 mL) under argon, in the presence of 200 mg of DMAP. The mixture was cooled to 0 °C, benzyl alcohol (0.4 mL, 1 eq.) was added, then DCC (770 mg, 1 eq.) as a solution in 3 mL of anhydrous CH_2Cl_2, and stirring was maintained overnight. The solution was concentrated, taken up with cyclohexane, the insoluble urea was filtered, and the compound was purified twice by chromatography (CH_2Cl_2–CyH, 5 : 1 then Tol–MeOH, 1 : 0⟶97.5 : 2.5), finally leading to 230 mg (7%) of a yellow oil. δ_H ($CDCl_3$): 7.73 (1H, d, *J* 2.1 Hz, a3), 7.38 (1H, dd, *J* 8.7 and 2.1 Hz, a5), 7.30 (5H, m, Bn), 7.08 (1H, d, *J* 8.7 Hz, a6), 5.52 (1H, d, *J* 5.7 Hz, g1), 5.11 (2H, s, Bn), 4.65 (2H, s, CH_2OSi), 4.44 (1H, d, *J* 1.8 Hz, g3), 4.37 (1H, m, g4), 4.03 (1H, d, *J* 5.6 Hz, g2), 3.82 (1H, d, *J* 3.7 Hz, g5), 0.90, 0.87, 0.83 and 0.80 (9H, s, $(CH_3)_3C$), 0.14 (3H, s, CH_3Si), 0.11 (3H, s, CH_3Si), 0.03 (3H, s, CH_3Si), −0.02 (3H, s, CH_3Si), 0.07 (6H, s, CH_3Si); δ_C ($CDCl_3$): 168.6 (CO_2), 149.1 and 140.2 ($Cq_{aromatics}$), 135.3 (2 $Cq_{aromatics}C$), 131.4 (a5), 128.6, 128.4 and 128.4 ($CH_{aromatics}$), 123.1 (a3), 116.9 (a6), 99.7 (g1), 78.8 (g3), 77.0 (g5), 75.2 (g2), 72.3 (g4), 67.0 (CH_2Ph), 63.6 (CH_2OTBS), 26.0, 25.9 and 25.8 ($(CH_3)_3C$), 18.5, 18.1 and 18.0 (Me_3CSi), −4.4, −4.5, −4.6, −4.7, −4.9, −5.0 and −5.2 (CH_3Si); *m*/*z* [CI + NH_3] 909 $(M + NH_4)^+$.

Methyl [2-nitro-4-(hydroxymethyl)phenyl-β-D-glucopyranosid]uronate 10. To a solution of the triacetylated benzyl alcohol **7** (2 g, 4.12 mmol) in 40 mL of methanol cooled to −15 °C, 84 mg (0.5 eq.) of NaOMe were added. The mixture was allowed to reach room temperature overnight. The medium was then neutralized with Dowex 50X8–200 resin and the compound was purified by chromatography on a silica gel column (eluent CH_2Cl_2–MeOH, 9 : 1). After recrystallization from ethanol, the deacetylated compound was obtained as a white powdery solid (958 mg, 65%); mp 175 °C; (Found: C, 47.05; H, 4.81; N, 3.94. $C_{20}H_{23}NO_{13}$ requires C, 46.80; H: 4.77; N, 3.90%); δ_H (CD_3OD): 7.80 (1H, d, *J* 2.0 Hz, a3), 7.56 (1H, dd, *J* 8.7 and 2.1 Hz, a5), 7.36 (1H, d, *J* 8.7 Hz, a6), 5.16 (1H, d, *J* 7.2 Hz, g1), 4.61 (2H, s, CH_2OH), 4.08 (1H, d, *J* 9.7 Hz, g5), 3.76 (3H, s, CH_3O), 3.64 (1H, t, *J* 9.4 Hz, g4), 3.56–3.46 (2H, m, g2, g3); δ_C (CD_3OD): 171.1 (CO_2), 150.3, 142.7 and 138.5 ($Cq_{aromatics}$), 133.5, 124.6 and 119.3 ($CH_{aromatics}$), 103.0 (g1), 77.5 (g2 or g3), 77.2 (g5), 74.8 (g2 or g3), 73.1 (g4), 64.0 (CH_2OH), 58.7 (CH_3O); *m*/*z* [CI + NH_3] 377 $(M + NH_4)^+$.

Methyl {[2-nitro-4-(*O*-*tert*-butyldimethylsilylmethyl)phenyl]-2,3,4-tri-(*O*-*tert*-butyldimethylsilyl)-ß-D-glucopyranosid}uronate 11. To a solution of the deacetylated compound **10** (470 mg, 1.3 mmol) in 10 mL DMF were added 875 mg (5.8 mmol) of TBSCl, 792 mg (11.6 mmol) of imidazole and 79 mg (0.65 mmol) of DMAP. The solution was heated at 80 °C for 48 h, and allowed to return to room temperature. 5 mL of anhydrous pyridine and 5 g (18.9 mmol) of TBSOTf were added dropwise to the solution. After stirring at room temperature for one night and evaporation, the residue was taken up in toluene and the insoluble pyridinium triflate was removed by filtration. After washing with water, brine, and drying over $MgSO_4$, the compound was purified on silica gel (eluent CH_2Cl_2). An oil was obtained, which crystallized; after recrystallization from ethanol, 900 mg (85%) of the methyl ester were collected as white crystals; mp 90–91 °C (from EtOH) (Found; C, 55.62; H, 9.01; N, 1.74; Si, 13.85. $C_{38}H_{73}NO_{10}Si_4$ requires C, 55.91; H, 9.01; N, 1.72; Si, 13.76); δ_H ($CDCl_3$): 7.79 (1H, d, *J* 2.1 Hz, a3), 7.47 (1H, dd, *J* 8.7 and 2.1 Hz, a5), 7.12 (1H, d, *J* 8.7 Hz, a6), 5.58 (1H, d, *J* 5.9 Hz, g1), 4.70 (2H, s, CH_2O), 4.51 (1H, d, *J* 1.4 Hz, g3), 4.39 (1H, d, *J* 6.0 Hz, g4), 4.04 (1H, d, *J* 5.9 Hz, g2), 3.84 (1H, d, *J* 3.8 Hz, g5), 3.72 (3H, s, CH_3O), 0.94 (18H, s, $(CH_3)_3CSi$), 0.87 and 0.83 (9H, s, $(CH_3)_3CSi$), 0.17–0.09 (18H, m, CH_3Si), 0.07 and 0.00 (3H, s, CH_3Si); δ_C ($CDCl_3$): 169.6 (CO_2), 149.1, 140.0 and 135.3 ($Cq_{aromatics}$), 131.5 (a5), 123.2 (a3), 116.4 (a6), 99.4 (g1), 79.0 (g3), 77.0 (g2), 75.4 (g5), 72.5 (g4), 63.6 (CH_2O), 52.5 (CH_3O), 26.0, 25.9 (2C) and 25.8 ($(CH_3)_3C$), 18.5, 18.1 (2C) and 18.0 (Me_3CSi), −4.4, −4.5, −4.6, −4.6, −4.7–5.0, −5.1 and −5.2 (2C) (CH_3Si); *m*/*z* [CI + NH_3] 833 $(M + NH_4)^+$.

Methyl [2-nitro-4-(*O*-*tert*-butyldiphenylsilylmethyl)phenyl-ß-D-glucopyranosid]uronate 12. To a solution of 400 mg (1.11 mmol) of methyl [2-nitro-4-(hydroxymethyl)phenyl-β-D-glucopyranosid]uronate **10** and 173 mg (2.3 eq.) of imidazole in 10 mL of anhydrous DMF, 0.49 mL (1.7 eq.) of TBDPSCl were added dropwise; after 3 h, an extra quantity of 0.1 mL was added. After stirring for 5 h at room temperature, 30 mL of water were added to the medium, which was then extracted with 4 × 20 mL of EtOAc. After drying over $MgSO_4$ and concentration under high vacuum, the compound was purified by chromatography on a silica gel column (eluent CH_2Cl_2–MeOH, 97.5 : 2.5), giving 568 mg (85%) of the monosilylated compound as a white foam; mp 60–61 °C; δ_H ($CDCl_3$): 7.68 (1H, d, *J* 1.6 Hz, a3), 7.61 (4H, dd, *J* 7.5 and 1.4 Hz, H_o $SiPh_2$), 7.42 (1H, dd, *J* 8.8 and 1.7 Hz, a5), 7.35 (6H, m, $H_{m,\,p}$ $SiPh_2$), 7.26 (1H, d, *J* 8.7 Hz, a6), 5.17 (1H, s, OH), 5.07 (1H, d, *J* 6.8 Hz, g1), 4.83 (1H, s, OH), 4.67 (1H, s, OH), 4.64 (2H, s, CH_2O), 4.12 (1H, d, *J* 9.5 Hz, g5), 3.89 (1H, m, H_4), 3.79 (2H, m, g2, g3), 3.76 (3H, s, CH_3O), 1.05 (9H, s, $(CH_3)_3CSi$); δ_C ($CDCl_3$): 168.9 (CO_2), 148.9, 140.1 and 136.1 ($Cq_{aromatics}$), 135.1 ($C_{o,m}$ $SiPh_2$), 132.8 ($Cq_{aromatics}$ $SiPh_2$), 131.7 (a5), 129.9 (C_p $SiPh_2$), 127.8 ($C_{o,m}$ $SiPh_2$), 122.7 (a3), 118.1 (a6), 101.9 (g1), 75.2 (g2 or g3), 75.0 (g5), 72.7 (g2 or g3), 71.0 (g4), 64.0 (CH_2), 52.7 (CH_3O), 26.7 ($(CH_3)_3C$), 19.2 (Me_3CSi); *m*/*z* [CI + NH_3] 615 ($M + NH_4^+$) (Found: $M + NH_4^+$, 615.2369, $C_{30}H_{39}O_{10}N_2Si$ requires 615.2374); *m*/*z* [FAB + NaI]: 620 $(M + Na)^+$.

Methyl {[2-nitro-4-(*O*-*tert*-butyldiphenylsilylmethyl)phenyl]-2,3,4-tri-(*O*-*tert*-butyldimethylsilyl)-ß-D-glucopyranosid}uronate 13. Methyl [2-nitro-4-(*O*-*tert*-butyldiphenylsilylmethyl)phenyl-ß-D-glucopyranosid] uronate **12** (568 mg, 0.95 mmol), pyridine (6.25 mL) and DMAP (187.5 mg, 1.53 mmol) were dissolved in 50 mL of freshly distilled CH_2Cl_2, placed under argon and cooled in an ice-bath. Then, TBSOTf (5.5 mL, 23.9 mmol) was cautiously added dropwise. The reaction medium was allowed to reach room temperature overnight. After evaporation, the residue was taken up in toluene, the insoluble salt was filtered, and the organic layer was washed with brine and dried over $MgSO_4$. After chromatography (eluent CH_2Cl_2), 775 mg (84%) of a yellowish oil were obtained. δ_H ($CDCl_3$): 7.77 (1H, d, *J* 2.1 Hz, a3), 7.69 (4H, m, H_o $SiPh_2$), 7.48 (1H, dd, *J* 8.8 and 2.0 Hz, a5), 7.42 (6H, m, $H_{m,\ p}$ $SiPh_2$), 7.15 (1H, d, *J* 8.7 Hz, a6), 5.61 (1H, d, *J* 5.8 Hz, g1), 4.73 (2H, s, CH_2O), 4.53 (1H, d, *J* 1.6 Hz, g3), 4.43 (1H, dd, *J* 3.6 and 1.6 Hz, g4), 4.10 (1H, d, *J* 5.8 Hz, g2), 3.88 (1H, d, *J* 3.7 Hz, g5), 3.74 (3H, s, CH_3O), 1.11 (9H, s, $(CH_3)_3CSiPh_2$), 0.96, 0.90 and 0.86 (9H, s, $(CH_3)_3CSiMe_2$), 0.19 (6H, s, CH_3Si), 0.17, 0.16, 0.10 and 0.04 (3H, s, CH_3Si); δ_C ($CDCl_3$): 169.6 (CO_2), 149.1, 140.1 and 134.9 ($Cq_{aromatics}$), 135.6 ($C_{o,m}$ $SiPh_2$), 133.1 ($Cq_{aromatics}$ $SiPh_2$), 131.5 (a5), 130.0 (C_p $SiPh_2$), 127.9 ($C_{o,m}$ $SiPh_2$), 123.3 (a3), 116.5 (a6), 99.5 (g1), 78.9 (g3), 77.0 (g5), 75.4 (g2), 72.5 (g4), 64.3 (CH_2), 52.4 (CH_3O), 26.9 ($(CH_3)_3CSiPh_2$), 25.9 and 25.8 ($CH_3)_3CSiMe_2$), 19.3 (Me_3CSiPh_2), 18.1, 18.0 and 18.0 ($(CH_3)_3CSiMe_2$), −4.4, −4.5, −4.6, −4.7, −4.9 and −5.0 (CH_3Si); *m/z* [CI + NH_3] 957 $(M + NH_4)^+$ (Found: M + NH_4^+, 957.4977, $C_{40}H_{81}O_{10}N_2Si_4$ requires 957.4968).

Benzyl {[2-nitro-4-(*O*-*tert*-butyldiphenylsilylmethyl)phenyl]-2,3,4-tri-(*O*-*tert*-butyldimethylsilyl)-ß-D-glucopyranosid}uronate 14. Methyl {[2-nitro-4-(*O*-*tert*-butyldiphenylsilylmethyl)-phenyl]-2,3,4-tri-(*O*-*tert*-butyldimethylsilyl)-ß-D-glucopyrano-sid} **13** uronate (775 mg, 0.827 mmol), 2 mL (19.2 mmol) of BnOH, 0.2 mL (0.8 mmol) of $Ti(OEt)_4$ (*pract*. 85%) and 5 g of 4 Å molecular sieves were reacted at reflux for 48 h in 30 mL of anhydrous toluene. The mixture was then diluted with 50 mL of water and extracted with Et_2O (5 × 30 mL), then successively washed with water and brine, dried over $MgSO_4$ and concentrated under high vacuum. After chromatography (eluent CH_2Cl_2), 602 mg (72%) of a colourless oil were obtained. δ_H ($CDCl_3$): 7.71 (1H, d, *J* 2.3 Hz, a3), 7.66 (5H, m, H_o $SiPh_2$ and a5), 7.42 (6H, m, $H_{m,\ p}$ $SiPh_2$), 7.31 (5H, m, Bn), 7.13 (1H, d, *J* 8.7 Hz, a6), 5.56 (1H, d, *J* 5.6 Hz, g1), 5.15 (2H, s, Bn), 4.69 (2H, s, CH_2O), 4.52 (1H, d, *J* 1.8 Hz, g3), 4.42 (1H, m, g4), 4.07 (1H, d, *J* 5.5 Hz, g2), 3.86 (1H, d, *J* 3.6 Hz, g5), 1.10 (9H, s, $(CH_3)_3CSiPh_2$), 0.91, 0.88 and 0.85 (9H, s, $(CH_3)_3CSiMe_2$), 0.17, 0.12, 0.07 and 0.03 (3H, s, CH_3Si), 0.13 (6H, s, CH_3Si); δ_C ($CDCl_3$): 168.6 (CO_2), 149.1, 140.2, 135.4 and 134.9 ($Cq_{aromatics}$), 135.6 ($C_{o,m}$ $SiPh_2$), 133.1 ($Cq_{aromatics}$ $SiPh_2$), 131.4 (a5), 130.0 (C_p $SiPh_2$), 128.6 and 128.4 ($C_{o,\ m}$ Bn), 128.4 (C_p Bn), 127.9 ($C_{o,m}$ $SiPh_2$), 123.2 (a3), 117.0 (a6), 99.7 (g1), 78.8 (g3), 77.0 (g5), 75.2 (g2), 72.4 (g4), 67.1 (CH_2 Bn), 64.3 (CH_2OSi), 26.9 ($(CH_3)_3CSiPh_2$), 25.9 and 25.8 ($(CH_3)_3CSiMe_2$), 19.4 (Me_3CSiPh_2), 18.1, 18.1 and 18.0 ($(CH_3)_3CSiMe_2$), −4.4, −4.5, −4.6, −4.7, −4.9 and −4.9 (CH_3Si); *m/z* [CI + NH_3] 1033 $(M + NH_4)^+$ (Found: M + NH_4^+,1033.5288, $C_{54}H_{85}O_{10}N_2Si_4$ requires 1033.5281).

Benzyl [(2-nitro-4-hydroxymethylphenyl)-2,3,4-tri-(*O*-*tert*-butyldimethylsilyl)-ß-D-glucopyranosid]uronate 15. Compound **14** (276 mg, 0.298 mmol) and acetic acid (17 µL, 1 eq.) were dissolved in 15 mL of anhydrous DMF and placed under argon. Then, 298 µL (1 eq.) of TBAF (1 M in THF) were added dropwise. The reaction was followed by TLC (it lasted notably longer when larger quantities were used). The reaction was then quenched with 15 g of ice and the medium was extracted with ether (6 × 20 mL). After washing with a saturated aqueous $NaHCO_3$ solution then with water, drying ($MgSO_4$) and concentration, the compound was purified on a silica gel column (eluent CH_2Cl_2–MeOH, 98 : 2), giving 198 mg (85%) of the benzyl alcohol as a white solid; mp 111–112 °C; δ_H ($CDCl_3$): 7.77 (1H, d, *J* 2.0 Hz, a3), 7.44 (1H, dd, *J* 8.7 and 2.1 Hz, a5), 7.31 (5H, m, Bn), 7.12 (1H, d, *J* 8.7 Hz, a6), 5.56 (1H, d, *J* 5.7 Hz, g1), 5.14 (2H, s, Bn), 4.66 (2H, s, CH_2OH), 4.52 (1H, d, *J* 1.5 Hz, g3), 4.41 (1H, m, g4), 4.05 (1H, d, *J* 5.6 Hz, g2), 3.85 (1H, d, *J* 3.6 Hz, g5), 2.03 (1H, se, OH), 0.91, 0.88 and 0.83 (9H, s, $(CH_3)_3CSiMe_2$), 0.16, 0.11, 0.06 and 0.00 (3H, s, CH_3Si), 0.12 (6H, s, CH_3Si); δ_C ($CDCl_3$): 168.6 (CO_2), 149.4, 140.1, 135.3 and 134.8 ($Cq_{aromatics}$), 132.3 (a5), 128.6 and 128.4 ($C_{o,\ m}$ Bn), 128.4 (C_p Bn), 123.8 (a3), 116.9 (a6), 99.6 (g1), 78.9 (g3), 76.8 (g5), 75.2 (g2), 72.2 (g4), 67.1 (CH_2, Bn), 63.7 (CH_2OH), 25.9 (2C) and 25.8 ($(CH_3)_3CSiMe_2$), 18.1 and 18.0 ($(CH_3)_3CSiMe_2$), −4.4, −4.5, −4.6, −4.7, −5.0 and −5.0 (CH_3Si); *m/z* [CI + NH_3] 795 $(M + NH_4)^+$ (Found: M + NH_4^+, 795.4096, $C_{38}H_{67}O_{10}N_2Si_3$ requires 795.4104).

Benzyl [(2-nitro-4-(4-nitrophenoxycarbonyloxymethyl)-phenoxy)-2,3-*tert*-butyldimethylsilyl)-ß-D-glucopyranosid]-uronate 16. Compound **15** (145 mg, 0.187 mmol) with one drop of anhydrous pyridine was dissolved in 5 mL of anhydrous CH_2Cl_2 and placed under argon. Then, 85 mg (2.3 eq.) of 4-nitrophenyl chloroformate were poured onto the mixture in one portion. After leaving overnight at room temperature, water was added and the organic layer was carefully washed with a saturated $NaHCO_3$ solution, and with H_2O, then dried ($MgSO_4$). After chromatography on silica gel (eluent CH_2Cl_2), 95 mg (54%) of the expected activated carbonate were obtained as a yellow oil. δ_H ($CDCl_3$): 8.27 (2H, d, *J* 9.2 Hz, $H_{ar/o\text{-}NO2}$), 7.90 (1H, d, *J* 2.0 Hz, a3), 7.53 (1H, dd, *J* 8.7 and 2.1 Hz, a5), 7.38 (2H, d, *J* 9.2 Hz, $H_{ar/m\text{-}NO2}$), 7.31 (5H, m, Bn), 7.19 (1H, d, *J* 8.7 Hz, a6), 5.60 (1H, d, *J* 5.6 Hz, g1), 5.23 (2H, s, Bn), 5.13 (2H, s, CH_2O), 4.52 (1H, d, *J* 1.2 Hz, g3), 4.41 (1H, m, g4), 4.06 (1H, d, *J* 5.5 Hz, g2), 3.86 (1H, d, *J* 3.6 Hz, g5), 0.90, 0.88 and 0.83 (s, 9H, $(CH_3)_3CSiMe_2$), 0.16, 0.11, 0.06 and 0.01 (3H, s, CH_3Si), 0.12 (6H, s, CH_3Si); δ_C ($CDCl_3$): 168.4 (CO_2), 155.4, 152.4, 150.6, 145.5, 140.1 and 135.1 ($Cq_{aromatics}$ and CO_3), 134.3 (a5), 128.5 and 128.4 ($C_{o,\ m,\ p}$ Bn), 127.8 ($Cq_{aromatics}$), 126.1 (a3), 125.4 ($C_{ar/o\text{-}NO2}$), 121.8 ($H_{ar/m\text{-}NO2}$), 115.7 (a6), 99.5 (g1), 78.9 (g3), 76.6 (g5), 75.1 (g2), 72.1 (g4), 69.2 (CH_2 Bn), 67.1 (CH_2O), 25.8 (2C) and 25.8 ($(CH_3)_3CSiMe_2$), 18.0 (2C) and 18.0 ($(CH_3)_3$-$CSiMe_2$), −4.5, −4.6, −4.6, −4.8 and −5.0 (2C) (CH_3Si); *m/z* [CI + NH_3] 960 $(M + NH_4)^+$.

***N,N′*-Dimethyl-*N*-benzyl-[4-(2,3,4-tri-*O*-*tert*-butyldimethyl-silyl-ß-D-glucopyranosyl)uronate-3-nitrobenzyloxycarbonyl]-*N′*-(*tert*-butoxycarbonyl) ethylenediamine 18.** Method 1: to 81 mg (0.085 mmol) of the activated carbonate **16** and 170 mg (11 eq.) of the mono-BOC amine **17**[55] in solution in 5 mL of dry CH_2Cl_2 were added 3 drops of pyridine. The mixture was stirred for 5 h at room temperature, diluted with CH_2Cl_2 (20 mL) and water (10 mL), then extracted with 3 × 30 mL of a saturated $NaHCO_3$ solution and dried (Na_2SO_4). After purification by chromatography (eluent CH_2Cl_2–MeOH, 98.5 : 1.5), 38 mg (45%) of the diamino compound were obtained.

Method 2: benzyl alcohol **15** (600 mg, 0.771 mmol) and 4 drops of Et_3N were dissolved in 30 mL of anhydrous CH_2Cl_2 and placed under argon. Then, 250 mg (1.265 mmol) of 4-nitrophenyl chloroformate in solution in anhydrous CH_2Cl (5 mL) were added dropwise. After stirring for 2.5 h at room temperature, 1.5 g (8.25 mmol) of the amine **17** was added to the medium and stirring was continued overnight. The same treatment as in method 2 enabled us to obtain 488 mg (64%) of the desired coupling compound as a pale yellow syrup.

δ_H ($CDCl_3$): 7.81 (1H, s, a3), 7.44 (1H, m, a5), 7.31 (5H, m, Bn), 7.14 (1H, d, *J* 8.7 Hz, a6), 5.57 (1H, d, *J* 5.7 Hz, H_1), 5.13 (2H, s, Bn), 5.06 (2H, s, CH_2O), 4.51 (1H, s, g3), 4.40 (1H, m, g4), 4.05 (1H, d, *J* 5.5 Hz, g2), 3.85 (1H, d, *J* 3.2 Hz, g5), 3.38 (4H, m, e1, e2), 2.95 (3H, s, CH_3N), 2.87–2.80 (3H, m, CH_3N),

1.43 (9H, s, $(CH_3)_3CO$), 0.90, 0.87 and 0.83 (9H, s, $(CH_3)_3CSiMe_2$), 0.16, 0.06 and 0.01 (3H, s, CH_3Si), 0.11 (9H, s, CH_3Si); δ_C ($CDCl_3$): 168.5 (CO_2), 155.8 and 149.9 (NCO_2), 140.1, 135.3 (2C) and 130.5 ($Cq_{aromatics}$), 133.5 (a5), 128.6 and 128.4 ($C_{o,\ m,\ p}$ Bn), 125.1 (a3), 117.0 (a6), 99.4 (g1), 79.7 (Me_3CO), 78.8 (g3), 76.8 (g5), 75.2 (g2), 72.2 (g4), 67.1 (CH_2, Bn), 65.7 and 65.4 (CH_2O), 46.8 (m, 2C, e1, e2), 35.5, 35.0 and 34.6 (2C, CH_3N), 28.5 ($(CH_3)_3CO$), 25.9 and 25.8 (2C) ($(CH_3)_3CSiMe_2$), 18.1 (2C) and 18.0 ($(CH_3)_3CSiMe_2$), −4.4, −4.5, −4.6, −4.7, −4.9 and −5.0 (CH_3Si); *m/z* [CI + NH_3] 1009 $(M + NH_4)^+$.

***N,N′*-Dimethyl-*N*-benzyl-[4-(2,3,4-tri-*O*-*tert*-butyldimethylsilyl-ß-D-glucopyranosyl)uronate-3-nitrobenzyloxycarbonyl]-ethylenediamine hydrochlorate 19.** BOC derivative **18** (270 mg, 0.27 mmol) was suspended in a freshly prepared 3 M HCl solution in EtOAc (20 mL) previously cooled to 0 °C. Stirring was maintained at this temperature until complete BOC disappearance (TLC monitoring). The reaction medium was then concentrated and the crude salt obtained was used directly for the next step. δ_H (CD_3OD): 7.91 (1H, s, a3), 7.64 (1H, m, a5), 7.36 (5H, m, Bn), 7.27 (1H, d, *J* 8.2 Hz, a6), 5.65 (1H, d, *J* 6.0 Hz, g1), 5.16 (4H, s, Bn and CH_2O), 4.71 (1H, s, H_3), 4.46 (1H, d, *J* 3.5 Hz, g4), 4.01 (1H, d, *J* 6.0 Hz, g2), 3.91 (1H, d, *J* 3.5 Hz, g5), 3.63 (2H, m, CH_2N), 3.21 (2H, m, CH_2N), 3.00 (3H, s, CH_3N), 2.72 (3H, s, CH_3N), 0.94, 0.90 and 0.85 (9H, s, $(CH_3)_3CSiMe_2$), 0.16, 0.15, 0.08 and 0.00 (3H, s, CH_3Si), 0.14 (6H, s, CH_3Si); δ_C (CD_3OD): 170.0 (CO_2), 150.5 (NCO_2), 141.5, 136.6 (2C) and 129.6 ($Cq_{aromatics}$), 132.2 (a5), 129.5 and 129.4 ($C_{o,\ m,\ p}$ Bn), 129.2 (a3), 117.4 (a6), 100.4 (g1), 80.3 (g3), 78.2 (g5), 77.1 (g2), 73.5 (g4), 68.3 (CH_2, Bn), 67.1 (CH_2O), 49.4 (m, 2C, CH_2N), 34.0 (2C, CH_3N), 26.4, 26.3 and 26.2 ($(CH_3)_3CSiMe_2$), 18.9 ($(CH_3)_3CSiMe_2$), −4.2, −4.2, −4.5, −4.6, −4.6 and −4.8 (CH_3Si); *m/z* [FAB +] 892 $(M - HCl + H)^+$.

***N,N′*-Dimethyl-*N*-benzyl-[4-(2,3,4-tri-*O*-*tert*-butyldimethylsilyl-ß-D-glucopyranosyl)uronate-3-nitrobenzyloxycarbonyl]-ethylenediamine carbamoyl chloride 20.** Salt **19** (250 mg, 0.027 mmol) and 0.1 mL (0.2 mmol) of phosgene in solution in 20% toluene were suspended in 50 mL of anhydrous CH_2Cl_2, placed under an argon atmosphere and cooled to 0 °C. Then, 3 drops of Et_3N were added and the temperature was maintained at 0 °C for 2.5 h. The reaction mixture was then concentrated and purified on a silica gel column (eluent EtOAc); 230 mg (90%) of carbamoyl chloride were obtained as a pale yellow oil. δ_H ($CDCl_3$): 7.82 (1H, s, a3), 7.47 (1H, m, a5), 7.31 (5H, m, Bn), 7.15 (1H, d, *J* 8.6 Hz, a6), 5.58 (1H, d, *J* 5.5 Hz, g1), 5.13 (2H, s, Bn), 5.07 (2H, s, CH_2O), 4.51 (1H, s, g3), 4.40 (1H, m, g4), 4.05 (1H, d, *J* 6.0 Hz, g2), 3.85 (1H, d, *J* 3.6 Hz, g5), 3.75–3.35 (4H, m, e1, e2), 3.13–2.96 (6H, m, CH_3N), 0.90, 0.87 and 0.83 (9H, s, $(CH_3)_3CSiMe_2$), 0.16, 0.12, 0.11, 0.07, 0.06 and 0.01 (3H, s, CH_3Si); δ_C ($CDCl_3$): 168.1 (CO_2), 156.0, 155.8, 155.4 and 155.3 (NCO_2), 149.9, 149.7, 149.5 and 149.5 (NCOCl), 148.5, 139.8 and 135.0 ($Cq_{aromatics}$), 133.9, 133.4 and 133.2 (a5), 130.4, 130.3, 130.1 and 130.0 ($Cq_{aromatics}$), 130.0 ($C_{o,m}$ Bn), 128.3 ($C_{o,m}$ and Bn), 125.3, 125.2, 124.8 and 124.8 (a3), 116.6 (a6), 99.1 (g1), 78.6 (g3), 76.6 (g5), 75.0 (g2), 71.9 (g4), 66.3 (CH_2, Bn), 65.7, 65.5, 65.4 and 65.3 (CH_2O), 50.4, 50.1, 48.9 and 48.2 (e1, e2), 46.8, 46.4, 46.0 and 45.7 (CH_2N), 38.9, 38.6, 37.0 and 36.8 (CH_3N), 35.2, 35.0, 34.9 and 34.4 (CH_3N), 25.6 (2C) and 25.5 ($(CH_3)_3CSiMe_2$), 17.8 (2C) and 17.7 ($(CH_3)_3CSiMe_2$), −4.7, −4.8, −4.9, −5.0, −5.2 and −5.3 (CH_3Si); *m/z* [CI + NH_3] 971 (100%), 973 (61.4) $(M + NH_4)^+$ (Found: $M + NH_4^+$, 971.4451 (100%), 973.4450 (63.9), $C_{44}H_{76}O_{12}N_4ClSi_3$ requires 971.4456, 973.4427).

2′-*O*-{*N,N′*-Dimethyl-*N*-benzyl-[4-(2,3,4-tri-*O*-*tert*-butyldimethylsilyl-ß-D-glucopyranosyl)uronate-3-nitrobenzyloxycarbonyl] ethylenediamine}paclitaxel 21. Carbamoyl chloride **20** (80 mg, 0.083 mmol) and 116 mg (*ca.* 10 eq.) of DMAP were dissolved in 10 mL of freshly distilled CH_2Cl_2 over P_2O_5. Under argon, 32 mg (0.037 mmol) of paclitaxel, then, dropwise, 0.2 mL of Et_3N were added. After 6 h, the solution was diluted with CH_2Cl_2 (20 mL), washed with water and brine, and dried over Na_2SO_4. Purification on a thin silica gel column (60, 0.015–0.040 mm, Merck) (eluent CH_2Cl_2–MeOH, 98 : 2), yielded 57 mg (87%) of the coupling compound as a white foam; mp 183 °C; δ_H ($CDCl_3$): 8.53 (1H, d, *J* 9.7 Hz, p3′-NH), 8.15 (2H, d, *J* 7.2 Hz, p2-Bz (*o*)), 7.78 (3H, m, p2-Bz (*m*) and a3), 7.65–7.15 (17H, m, $H_{aromatics}$), 7.10 (1H, m, a6), 6.30 (2H, m, p10, p13), 6.12 (1H, m, p3′), 5.66 (1H, m, p1), 5.57 (1H, d, *J* 6.3 Hz, g1), 5.48 (1H, d, *J* 2.7 Hz, p2′), 5.13 (2H, m, CH_2, Bn), 4.98 (1H, m, p5), 4.64 (2H, ABq, *J* 12.6 Hz, CH_2O), 4.50 (1H, m, g3), 4.47 (1H, m, p7), 4.40 (1H, s, g4), 4.17 (2H, ABq, *J* 8.6 Hz, p20), 4.05 (1H, d, *J* 5.4 Hz, g2), 3.84 (2H, m, p3, g5), 3.65–3.02 (4H, m, e1, e2), 2.95–2.89 (6H, m, CH_3N), 2.58 (s, p4-OAc), 2.52 (1H, m, p6α), 2.42 (2H, d, *J* 2.3 Hz, p14), 2.21 (3H, s, p10-OAc), 2.09 (3H, m, 3H, p18), 2.00 (1H, m, p6β), 1.68 (3H, s, p19), 1.22 (3H, s, p16), 1.11 (3H, s, p17), 0.90, 0.87 and 0.83 (9H, s, $(CH_3)_3CSiMe_2$), 0.16, 0.06, and 0.00 (3H, s, CH_3Si), 0.12 (9H, s, CH_3Si); δ_C ($CDCl_3$): 204.1 (p9), 171.4, 170.1, 169.0, 168.4, 168.3 and 167.1 (MeC (O)C_4, Me*C*(O)C_{10}, Ph*C*O_2, p3′-Ph*C*(O), p1′ and *C*O_2Bn), 155.9 and 149.9 (NCO_2), 154.9, 143.3, 140.1 (p12), 137.6, 135.2, 134.6, 132.5, 130.0 and 129.3 (7 $Cq_{aromatics}$ and p11), 133.6, 133.3, 131.4, 130.3, 129.0, 128.8 (2C), 128.6, 128.4, 128.1, 127.9, 127.1, 126.7 and 125.0 (14 $CH_{aromatics}$), 116.8 (a6), 99.4 (g1), 84.5 (p5), 81.0 (p4), 79.2 (p1); 78.9 (g3), 76.7, 75.7 (2C) and 75.2 (2C) (p2, p10, p2′, g2, g5), 72.2 (p7, g4), 71.4 (p13), 76.5 (p20), 67.1 (CH_2, Bn), 65.5 (CH_2O), 58.5 (p8), 52.8 (p3′), 47.4 and 46.1 (e1, e2), 45.6 (p3), 43.2 (p15), 35.7 (CH_3N), 35.6 (p6), 26.8 (p16), 25.9 (2C) and 25.8 ($(CH_3)_3CSiMe_2$), 22.8 (p14 and p4-OAc), 22.4 (p17), 20.9 (p10-OAc), 18.0 (2C) and 18.0 ($(CH_3)_3CSiMe_2$), 14.9 (p18), 9.7 (p19), −4.5, −4.6, −4.6, −4.8, −5.0 and −5.0 (CH_3Si); *m/z* [FAB + NaI] 1794 $(M + Na)^+$ (Found: $M + Na^+$, 1793.7540, $C_{91}H_{122}O_{26}N_4Si_3Na$ requires 1793.7553).

2′-*O*-[*N,N′*-Dimethyl-*N*-benzyl-4-(ß-D-glucopyranosiduronate)-3-nitrobenzyloxycarbonylethylenediamine]paclitaxel 22. To 150 mg (0.085 mmol) of the protected prodrug **21** and 0.3 mL of anhydrous pyridine dissolved in anhydrous CH_3CN (5 mL) under argon was dropwise added, at 0 °C, 0.8 mL of a 70% HF–pyridine complex. After 3 h at 0 °C, the mixture was allowed to reach room temperature and stirring was continued overnight. Hydrolysis at 0 °C was performed with saturated $NaHCO_3$ (125 mL), then the medium was extracted with EtOAc (3 × 50 mL), dried over Na_2SO_4, concentrated and chromatographed on silica gel (eluent CH_2Cl_2–MeOH, 95 : 5), affording 90 mg (76%) of the desilylated compound as a white foam; mp 154 °C; δ_H ($CDCl_3$): 8.43 (1H, d, *J* 9.5 Hz, p3′-NH), 8.13 (2H, p2-Bz (*o*)), 7.78 (3H, m, p2-Bz (*m*) and a3), 7.65–7.24 (18H, m, $H_{aromatics}$), 6.28 (2H, m, p10 and p13), 6.12 (1H, dd, *J* 9.3 and 3.0 Hz, p3′), 5.66 (1H, m, p2), 5.46 (1H, d, *J* 2.9 Hz, p2′), 5.23 (2H, CH_2, Bn), 4.94 (1H, m, p5), 4.88 (1H, d, *J* 7.2 Hz, g1), 4.64 (2H, ABq, *J* 13 Hz, CH_2O), 4.50 (1H, m, g3), 4.47 (1H, m, p7), 4.10 (2H, ABq, *J* 8.4 Hz, p20), 3.95 (1H, g5), 3.88 (1H, m, g4), 3.81 (1H, m, p3), 3.80–3.05 (8H, m, g3, e1, e2, OH(g2, g3, g4)), 2.93–2.88 (6H, m, CH_3N), 2.55 (3H, s, p4-OAc), 2.45 (1H, m, p6α), 2.39 (2H, m, p14), 2.21 (3H, s, p10-OAc), 1.98 (3H, s, p18), 1.88 (1H, m, p6β), 1.67 (3H, s, p19), 1.18 (3H, s, p16), 1.11 (3H, s, p17); δ_C ($CDCl_3$): 204.0 (p9), 171.4, 170.1, 169.0, 168.4, 168.2 and 167.1 (p4-OAc, P10-OAc, Ph*C*O_2, Ph*C*(O)N_3, p1′ and *C*O_2Bn), 155.9 and 149.9 (NCO_2), 154.9, 143.2, 140.3 (p12), 137.5, 134.9, 134.5, 132.6 132.1 and 129.3 (7 $Cq_{aromatics}$ and p11), 133.7, 131.5, 130.4, 128.8 (2C), 128.7 (2C), 128.7, 128.3, 128.1, 128.0, 127.1, 126.7 and 124.7 (14 $CH_{aromatics}$), 119.1 (a6), 102.7 (g1), 84.5 (p5), 81.0 (p4), 79.3 (p1), 75.7 (2C), 75.2, 75.0 and 74.8 (p2, p10, p2′, g2 or g3, g5), 76.8 (p20), 72.8, 72.1, 71.4 and 70.9 (p7, p13, g2 or g3, g4), 67.7 (CH_2 Bn), 65.4 (CH_2O), 58.5 (p8), 53.0 (p3′), 47.4 and 46.1 (e1,

e2), 45.7 (p3), 43.2 (p15), 35.7 (CH_3N), 35.6 (p6), 26.8 (p16), 22.8 (p17), 22.6 (p14), 22.3 (p4-OAc), 20.9 (p10-OAc), 15.0 (p18), 9.7 (p19); *m/z* [FAB + NaI] 1451 $(M + Na)^+$. (Found: $M + Na^+$, 1451.4979, $C_{73}H_{80}O_{26}N_4Na$ requires 1451.4959).

2′-*O*-[*N*,*N*′-Dimethyl-*N*-4-(ß-D-glucopyranosiduronate)-3-aminobenzyloxycarbonylethylenediamine]paclitaxel 3. To a suspension of 90 mg (0.063 mmol) benzyl ester **22** and 10% Pd/C (100 mg) in 10 mL abs. ethanol, 0.3 mL (50 eq.) cyclohexa-1,4-diene were added and the mixture heated at 45 °C for 24 hours. After filtration over Celite 545, the product was purified by chromatography on silica gel (eluent CH_3CN–H_2O, 9 : 1). Lyophilisation afforded 20 mg (24%) of prodrug **3** as a white foam; mp 190 °C; δ_H (CD_3OD): 9.12 (1H, m, p3′-NH), 8.13 (2H, p2-Bz (*o*)), 7.81 (2H, m, p2-Bz (*m*)), 7.67–7.28 (11H, m, $H_{aromatics}$), 7.05 (1H, m, a5), 6.67 (1H, m, a3), 6.50 (1H, m, a6), 6.46 (1H, m, p10), 6.15 (1H, m, p13), 6.05 (1H, m, p3′), 5.64 (1H, m, p2), 5.40 (1H, m, p2′), 4.99 (1H, m, p5), 4.76 (1H, m, g1), 4.76 (2H, m, CH_2O), 4.35 (1H, m, p7), 4.19 (4H, m, p20, CH_2O), 3.83 (2H, m, p3, g5), 3.60–3.34 (7H, m, g2, g3, g4, e1, e2), 2.99–2.81 (6H, m, CH_3N), 2.49–2.39 (4H, s, p4-OAc, p6α), 2.19 (2H, m, p14), 2.16 (3H, s, p10-OAc), 1.97 (3H, s, p18), 1.80 (1H, m, p6β), 1.66 (3H, s, p19), 1.12 (6H, s, p16, p17); δ_C (CD_3OD): 205.3 (p9), 171.6, 171.3, 171.0, 170.9, 167.7 (p4-OAc, p10-OAc, Ph*C*O_2, Ph*C*(O)N_3, p1′), 158.0 and 146.6 (NCO_2), 156.7, 142.5, 138.3 ($Cq_{aromatics}$), 135.7, 134.8, 133.8, 131.4, 131.1, 130.5, 130.0, 129.5, 129.1, 128.7, 128.4, 128.1, and 124.4 ($CH_{aromatics}$), 118.6 (a5, a6), 117.4 (a3), 104.6 (g1), 85.9 (p5), 82.2 (p4), 78.0, 77.4, 76.9 (2C) and 76.3 (2C) (p2, p10, p20, p2′, g3, g5), 74.6, 73.3, 72.7 and 72.2 (p7, p13, g2, g4), 69.7 (CH_2O), 59.2 (p8), 54.8 (p3′), 47.5 (p3), 47.1 and 46.9 (e1, e2), 44.5 (p15), 37.5 (p6), 37.1–35.2 (CH_3N), 27.2 (p16), 23.3, 22.5, 22.4 (p17, p14, p4-OAc), 20.8 (p10-OAc), 14.7 (p18), 10.5 (p19); *m/z* [ES+] 1309 $(M + H)^+$. (Found: $M + H^+$, 1309.4926, $C_{66}H_{77}O_{24}N_4$ requires 1309.4928).

2-Amino-4-hydroxymethylphenol 4. A solution of 4-hydroxy-3-nitrobenzaldehyde (141 mg, 0.833 mmol) in methanol (20 mL) was hydrogenated for 4 hours in the presence of 10% Pd/C (96 mg). After filtration over Celite 545 and chromatography (eluent CH_2Cl_2–MeOH, 95 : 5), 50 mg of a brown powder were isolated; mp 142–144 °C; δ_H (CD_3OD): 6.75 (1H, d, *J* 1.7 Hz, a3), 6.66 (1H, d, *J* 8.0 Hz, a6), 6.58 (1H, dd, *J* 8.0 Hz and *J* 1.7 Hz, a5), 4.42 (2H, s, CH_2); δ_C (CD_3OD): 145.6 ($C_{quaternary}$–O), 135.6 and 133.8 ($C_{quaternary}$), 119.2 (a5), 116.5 (a3), 115.1 (a6), 61.3 (CH_2); *m/z* [CI + NH_3] 140 $(M + NH4)^+$ (Found: $M + H^+$, 140.0710, $C_7H_{10}O_2N$ requires 140.0712).

Cytotoxicity measurements

Cytotoxicity was tested against L1210 (mouse leukemic cell line) cells using the microculture tetrazolium assay (MTA).

L1210 cells were cultivated in RPMI 1640 medium (Gibco) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 units mL^{-1} penicillin, 100 μg mL^{-1} streptomycin, and 10 mM HEPES buffer (pH = 7.4). Cells were exposed to increasing concentrations of drug (nine serial dilutions in triplicate) for 48 h. Results are expressed as IC_{50}: the concentration which reduced the optical density of the treated cells by 50% with respect to the optical density of untreated controls.

For the cell cycle analysis, L1210 cells (5×10^5 cells mL^{-1}) were incubated for 21 h with various concentrations of drugs. Cells were then fixed by 70% ethanol (v/v), washed, and incubated in PBS containing 100 μg mL^{-1} RNAse and 50 μg mL^{-1} propidium iodide for 30 min at 20 °C. For each sample, 10,000 cells were analysed on a XLMCL flow cytometer (Beckman Coulter, France).

Stability measurements

A solution of 30 μg mL^{-1} of prodrug in 0.02 M phosphate buffer (pH 7.2) was incubated at 37 °C. Aliquots (100 μL) were taken at various times and analyzed by HPLC after dilution with eluent (300 μL).

HPLC conditions: analysis was carried out on a reverse-phase column (Lichrospher RP18e, 250 × 4 mm, 5 μm) using isocratic conditions (1 mL/min) of 50% phosphate buffer (0.02 M, pH 3) and 50% acetonitrile with UV detection at 226 nm (extracted from PDA 3D spectra).

Kinetic measurements

A solution of 30 μg mL^{-1} of prodrug in 0.02 M phosphate buffer (pH 7.2) was incubated at 37 °C in the presence of 12 units mL^{-1} of β-D-glucuronidase (*E. coli*). Aliquots (100 μL) were taken at various times and analyzed by HPLC after dilution with eluent (300 μL).

LC/MS measurements

LC/MS spectra were recorded on an API3000 LC-MS-MS spectrometer with electrospray ionisation. The HPLC separation was carried out on a reverse-phase column (Nucleosil C18, 150 × 2.1 mm, 3 μm) using isocratic conditions (0.2 mL min^{-1}) of 50% ammonium formate buffer (5 mM, pH 3.5) and 50% acetonitrile.

Acknowledgements

We are grateful to the Association pour la Recherche sur le cancer (ARC) for Grant Number 5544 and a partial fellowship to E. B. Cytotoxic measurements were performed at the Laboratoires Servier. We thank Jean-Marc Clavel for effecting the LC/MS measurements.

References

1 M. von Mehren and L. Weiner, *Curr. Opin. Oncol.*, 1996, **8**, 493–498.
2 A. Scott and S. Welt, *Curr. Opin. Oncol.*, 1997, **9**, 717–722.
3 P. Carter, *Nat. Rev. Cancer*, 2001, **1**, 118–129.
4 L. Sorbera and X. Rabasseda, *Drugs Future*, 1998, **23**, 1078–1082.
5 K. Bagshawe, *Br. J. Cancer*, 1987, **56**, 531–532.
6 K. Bagshawe, *Drug Dev. Res.*, 1995, **34**, 220–230.
7 R. Melton, R. Knox and T. Connors, *Drugs Future*, 1996, **21**, 167–181.
8 I. Niculescu-Duvaz and C. Springer, *Curr. Med. Chem.*, 1995, **2**, 687–706.
9 W. Denny and W. Wilson, *J. Pharm. Pharmacol.*, 1998, **50**, 387–394.
10 P. Senter and K. Hellstrom, *Bioconjugate Chem.*, 1993, **4**, 3–9.
11 K. Bagshawe, *Clinical Pharmacokinet.*, 1994, **27**, 368–376.
12 K. Bagshawe, *Cell Biophys.*, 1994, **24**, 2583–2591.
13 K. Bagshawe, *Biochem. Soc. Trans.*, 1990, **18**, 750–752.
14 F. Huennekens, *Trends Biotechnol.*, 1994, **12**, 234–239.
15 M. Deonarain and A. Epenetos, *Br. J. Cancer*, 1994, **70**, 786–794.
16 K. Bosslet, J. Czech, G. Seemann, C. Monneret and D. Hoffmann, *Cell Biophys.*, 1994, **24–25**, 51–63.
17 J.-C. Jacquesy, J.-P. Gesson, C. Monneret, M. Mondon, B. Renoux, J.-C. Florent, M. Koch, F. Tillequin, H. Sedlacek, M. Gerken, C. Kolar and G. Gaudel, Patent WO 92/19639, 1992.
18 K. Bosslet, J. Czech and D. Hoffmann, *Cancer Res.*, 1994, **54**, 2151–2159.
19 K. Bosslet, J. Czech, H. Lorenz, M. Sedlacek, M. Schuermann and G. Seemann, *Br. J. Chem.*, 1992, **65**, 234–238.
20 C. Monneret, J.-C. Florent, J.-C. Gesson, J.-C. Jacquesy, F. Tillequin and M. Koch, *ACS Symp. Ser.*, 1995, **574**, 78–99.
21 K. Bosslet, J. Czech, P. Lorenz, H. Sedlacek, M. Schuermann and G. Seemann, *Br. J. Cancer*, 1992, **65**, 234–238.
22 K. Bosslet, R. Straub, M. Gerken, H. Petrul, J. Czech, J.-C. Florent, M. Koch, F. Tillequin, F. Schmidt, C. Monneret and J.-P. Gesson, ***4***. Salzburger Symposium zur Lebensqualität chrinisch Kranker, 1996, Georg Thieme Verlag, Stuttgart-New York, pp. 123–130.
23 K. Bosslet, R. Straub, M. Blumrich, J. Czech, J. M. Gerken, B. Sperker, H. Kroemer, J.-P. Gesson, M. Koch and C. Monneret, *Cancer Res.*, 1998, **58**, 1195–1201.
24 T. Murdter, B. Sperker, K. Kivisto, M. McClellan, P. Fritz, G. Friedel, A. Linder, K. Bosslet, H. Toomes, R. Dierkesmann and H. Kroemer, *Cancer Res.*, 1997, **57**, 2440–2445.
25 M. Wani, H. Taylor, M. Wall, P. Coggon and A. McPhail, *J. Am. Chem. Soc.*, 1971, **93**, 2325–2337.

26 K. Nicolaou, W.-M. Dai and R. Guy, *Angew. Chem., Int. Ed. Engl.*, 1994, **33**, 15–44.
27 D. Guénard, F. Guéritte-Voegelein and P. Potier, *Acc. Chem. Res.*, 1993, **26**, 160–167.
28 D. Kingston, *Trends Biotechnol.*, 1994, **12**, 222–227.
29 W. Slichenmyer and D. Von Hoff, *Anti-Cancer Drugs*, 1991, **2**, 519–530.
30 B. Chabner, *Principles & Practice of Oncology Updates*, 1991, **5**, 1–10.
31 J. Szebeni, F. Muggia and C. Alving, *J. Natl. Cancer Inst.*, 1998, **90**, 300–306.
32 A. Whal, F. Guéritte-Voegelein, D. Guénard, M. Le Goff and P. Potier, *Tetrahedron*, 1992, **48**, 6965–6974.
33 M. Hepperle and G. Georg, *Drugs Future*, 1994, **19**, 573–584.
34 R. Greenwald, A. Pendri and D. Bolikal, *J. Org. Chem.*, 1995, **60**, 331–336.
35 A. Mathew, M. Mejillano, J. Nath, R. Himes and V. Stella, *J. Med. Chem.*, 1992, **35**, 145–151.
36 H. Deutsch, J. Glinski, M. Hernandez, R. Haugwitz, V. Narayanan, M. Suffness and L. Zalkow, *J. Med. Chem.*, 1989, **32**, 788–792.
37 A. Pendri, C. Conover and R. Greenwald, *Anti-Cancer Drug Des.*, 1998, **13**, 387–395.
38 N. Harada, K. Ozaki, T. Yamaguchi, H. Arakawa, A. Ando, K. Oda, N. Nakanishi, M. Ohashi, T. Hashiyama and K. Tsujihara, *Heterocycles*, 1997, **46**, 241–258.
39 Z. Zhao, D. Kingston and A. Crosswell, *J. Nat. Prod.*, 1991, **54**, 1607–1611.
40 M. Rodrigues, P. Carter, C. Wirth, S. Mullins, A. Lee and B. Blackburn, *Chem. Biol.*, 1995, **2**, 223–227.
41 K. Bagshawe and R. Begent, *Adv. Drug Delivery Rev.*, 1996, **22**, 365–367.
42 D. de Bont, R. Leenders, H. Haisma, I. van der Meulen-Muileman and H. Scheeren, *Bioorg. Med. Chem.*, 1997, **5**, 405–414.
43 F. de Groot, L. van Berkom and H. Scheeren, *J. Med. Chem.*, 2000, **43**, 3093–3102.
44 E. Damen, T. Nevalainen, T. vandenBergh, F. de Groot and H. Scheeren, *Bioorg. Med. Chem*, 2002, **10**, 71–77.
45 F. Schmidt, I. Ungureanu, I. R. Duval, A. Pompon and C. Monneret, *Eur. J. Org. Chem.*, 2001, 2129–2134.
46 S. Jain, W. Drendel, Z.-W. Chen, F. Matthews, W. Sly and J. Grubb, *Nat. Struct. Biol.*, 1996, **3**, 375–381.
47 R. Lougerstay-Madec, J.-C. Florent, C. Monneret, F. Nemati and M.-F. Poupon, *Anti-Cancer Drug Des.*, 1998, **13**, 995–1007.
48 F. de Groot, W. Loos, R. Koekoek, L. van Berkom, L. Busscher, A. Seelen, C. Albrecht and P. de Bruijn, *J. Org. Chem.*, 2001, **66**, 8815–8830.
49 J.-C. Florent, X. Dong, G. Gaudel, S. Mitaku, C. Monneret, J.-P. Gesson, J.-C. Jacquesy, M. Mondon, B. Renoux, S. Andrianomenjanahary, S. Michel, M. Koch, F. Tillequin, M. Gerken, J. Czech, R. Straub and K. Bosslet, *J. Med. Chem.*, 1998, **41**, 3572–3581.
50 S. Chen, S. Huang, J. Wei and V. Farina, *Tetrahedron.*, 1993, **49**, 2805–2828.
51 G. Samaranayake, N. Magri, C. Jitrangsri and D. Kingston, *J. Org. Chem.*, 1991, **56**, 5114–5119.
52 M. Tanaka, M. Okita and I. Yamatsu, *Carbohydr. Res.*, 1993, **241**, 81–88.
53 N. Mignet, C. Chaix, B. Rayner and J.-L. Imbach, *Carbohydr. Res.*, 1997, **303**(1), 17–24.
54 S. Higashibayashi, K. Shinko, T. Ishizu, K. Hashimoto, H. Shirahama and M. Nakata, *Synlett*, 2000, **9**, 1306–1308.
55 W. Saari, J. Schwering, P. Lyle, S. Smith and E. Engelhardt, *J. Med. Chem.*, 1990, **33**(1), 97–101.
56 S. Desbène, H. Dufat-Trinh, S. Michel, M. Koch, F. Tillequin, G. Fournier, N. Farjaudon and C. Monneret, *Anti-Cancer Drug Des.*, 1998, **13**, 955–968.