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New Taxol® (paclitaxel) prodrugs designed for ADEPT and PMT strategies in cancer chemotherapy

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Abstract—Two new glucuronide paclitaxel prodrugs have been synthesized. Linked to the 2′-OH of the drug by a carbonate function, they include a self-immolative spacer bearing an arylnitro or arylamino group between the drug and the glucuronic acid residue. Both prodrugs were well detoxified and easily cleaved in the presence of β-D-glucuronidase with fast removal of the spacer, releasing paclitaxel. The arylamino spacer-containing prodrug, more stable than the corresponding nitro analogue, was selected for further studies.

1. Introduction

Despite recent progress in cancer therapy, a major challenge remains to increase antitumour selectivity of cytotoxic drugs that are still currently used. Indeed, the lack of selectivity of such drugs is a serious drawback typically associated with severe side-effects, insufficient drug concentrations that would completely eradicate the tumour and, for most of them, emergence of drug resistance after prolonged treatment. Among different ways to overcome these problems, one is to use non-cytotoxic prodrugs designed to locally deliver the antitumour agent by specific activation.[1–3] According to this strategy called 'tumour activated prodrug' (TAP),[4–6] some prodrugs have reached the preclinical or clinical trial steps.[7] This tumour-specific activation may occur by hypoxic environment of solid tumours, by localisation to tumour antigen, by tumour-associated proteases (PSA, PSMA) or by enzyme selectively present in the tumour. In the case of tumour-associated enzyme activation, the active enzyme may be present in the tumour, like in PMT[8,9] strategies, or targeted to the tumour site, as in ADEPT, GDEPT, or VDEPT approaches.[10–13,6]

Paclitaxel (Taxol®) and Docetaxel (Taxotere®) are potent anticancer agents that are currently used for the treatment of patients with ovarian, breast, non-small cell lung (NSCL) and prostate cancers. They possess a unique mechanism of action by binding to tubulin and promoting stable and non-functional microtubule formation[14,15] which finally leads to disrupted mitosis and cell death. Recent findings have shown that paclitaxel initiates apoptosis through multiple mechanisms.[16,17] However, the clinical usefulness of these drugs is particularly hampered by their poor solubility, along with the aforementioned general problems, that is, lack of selectivity and development of multidrug resistance in clinic.[18,19] Therefore, it remains of interest to improve their use, at least to gain in solubility and selectivity, by preparing water-soluble prodrugs selectively activable in tumour areas. Since endogenous extracellular β-D-glucuronidase[20–23] is highly present in necrotic tumours, glucuronide prodrugs fulfil these two requirements and can be used in prodrug monotherapy (PMT). Moreover, in the case of non-necrotic areas of tumours where the enzyme concentration is low, following the antibody directed enzyme prodrug therapy (ADEPT) strategy, introduction of exogenous β-D-glucuronidase via a fusion protein consisting of an anti-CEA antibody linked to a β-D-glucuronidase has been already assessed.[24] Therefore, some time ago, we undertook and reported the synthesis of paclitaxel prodrugs designed to be activated by β-glucuronidase such as prodrugs **1**[25] and **2**[26] consisting of a β-glucuronide

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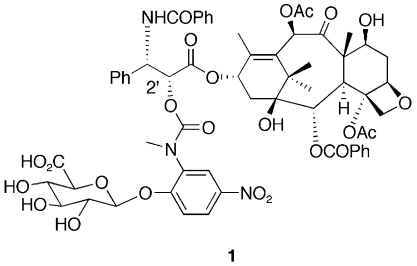
moiety, a self-immolative spacer and paclitaxel. In the case of **1**, relevant detoxification of the drug was observed[25] but along with an inefficient enzyme activation of the prodrug, which was attributed to a steric hindrance of the glycosidic linkage, as ascertained by molecular modelling. With still a suitable detoxification, an improved enzymatic cleavage was obtained[26] by using an elongated spacer as in **2**, but the synthesis of such a prodrug became laborious for two main reasons: removal, at the last step, of the benzyl ester as the protecting group of the acidic function of the glucuronic acid gave moderate yields (54% and 24%, respectively) but also led to partial reduction of the nitro function.[26]

2. Chemistry

The priority was then to investigate the possibility of replacing the benzyl ester of the glucuronic acid moiety by an allyl ester. As, to our knowledge, this protecting group had never been used in the case of paclitaxel glucuronides, the challenge was to find adequate conditions to remove it in the presence of the very sensitive taxane skeleton. Simultaneously, we expected to either keep or reduce the nitro group in view of better understanding the role of the nitro or amino-containing spacer with respect to the prodrug stability and to the enzymatic cleavage Figure 1.

At the same time, we envisioned improving the kinetics of cleavage by attaching the spacer to the 2′-OH of paclitaxel by a carbonate instead of a carbamate. The stability of a carbonate function in position 2′ of the paclitaxel depends on its structural environment. In the literature, some 2′-paclitaxel carbonates have been reported to be hydrolysed in serum,[27] whereas others were stable enough to be used in TAP strategies. This is the case of several prodrugs to be activated by β-D-glucuronidase, plasmine or bioreduction in order to release the free paclitaxel such as those described by Scheeren et al.[28–30]



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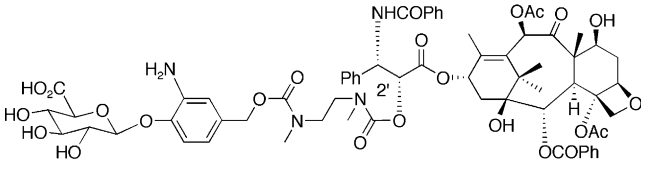

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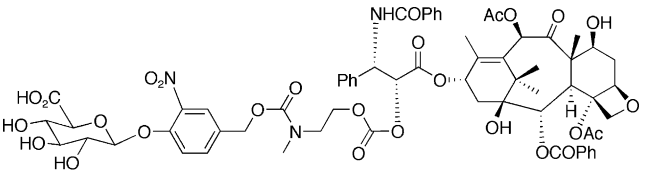
Figure 1. Structures of prodrugs **1** and **2**.



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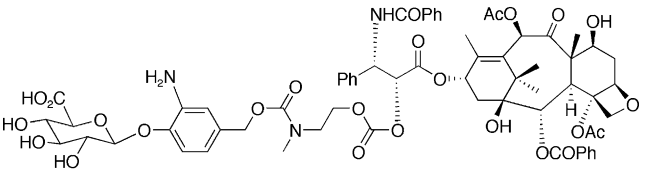

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Figure 2. Structures of prodrugs **3** and **4**.

The two planned molecules were **3** and **4** (Fig. 2).

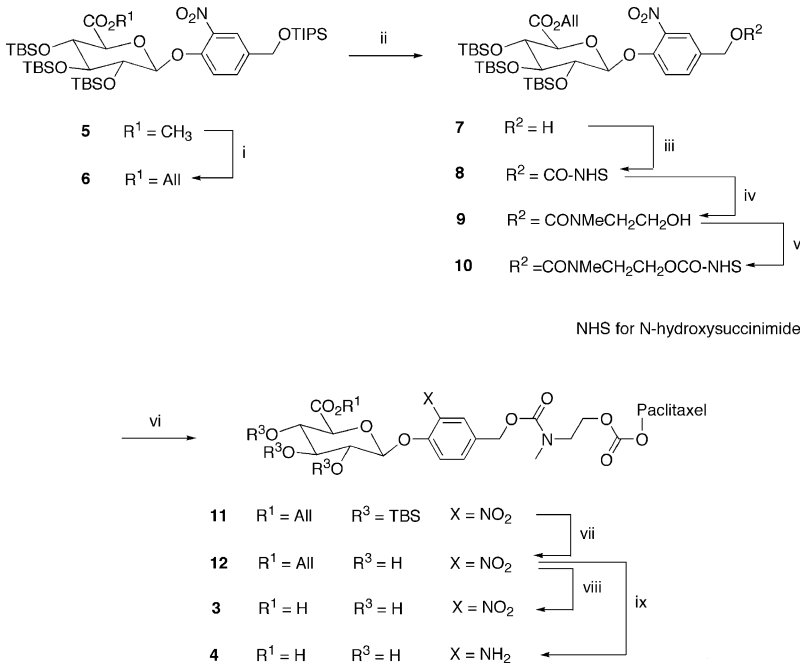
Prodrug **3** was synthesized according to Scheme 1 starting from the intermediate **5** already described by us.[26]

The first step consisted in replacing the methyl ester group by an allyl ester. In order to avoid successive saponification and reesterification, we tried a one-step transesterification reaction in the presence of an excess of allyl alcohol. A catalyst compatible with the silyl ethers was found within the titanium IV derivatives. Whereas, by using the commercial titanium tetra-isopropoxide, a mixture of isopropyl and allyl ester was obtained, in situ preparation of titanium tetra-allylate and subsequent addition of the methyl ester gave exclusively the expected ester **6**.

Selective deprotection of the TIPS group from the primary benzylic alcohol in the presence of the secondary TBS ethers was achieved by using TBAF/AcOH in DMF according to the literature.[31]

The benzylic alcohol of **7** was then activated with DSC as a *N*-hydroxy succinimide ether in the presence of a base. The obtained carbonate **8**, too unstable to be purified, was directly engaged in the next step by addition of the required amino-alcohol to the crude reaction mixture. Carbamate **9**, obtained in good yield (96%), was subsequently activated again with DSC as a *N*-hydroxy succinimide carbonate. Carbonate **10** could be purified in 79% yield. Next step was the coupling with paclitaxel which worked fine (yield: 93%) by using stoichiometric DMAP in dichloromethane. Under theses conditions, only the more reactive C-2′ alcohol of the side chain of paclitaxel reacted. Deprotection of the TBS groups was conducted with HF/Pyr complex.

The last step involved removal of the allyl ester group. Our first trials with classical reagents such as $Pd(PPh_3)_4$/pyrrolidine[32] or $Pd(PPh_3)_4$/morpholine[27] resulted in the C-2′ carbonate cleavage of the paclitaxel, probably due to the nucleophilicity of these heterocyclic bases. However, the allyl ester was successfully removed



Scheme 1. Synthesis of prodrugs. Reagents: (i) $Ti(OAll)_4$/AllOH (69%); (ii) TBAF/AcOH/DMF (88%); (iii) DSC/$^iPr_2NEt$; (iv) $MeNHCH_2CH_2OH$ (96% two steps); (v) DSC/$^iPr_2NEt$/DMAP (79%); (vi) paclitaxel/DMAP/NEt_3 (93%); (vii) HF/Pyr (75%); (viii) $Pd(PPh_3)_4/NEt_3/CH_3CO_2H$ (80%); (xi) $Pd(PPh_{34}/NEt_3/HCO_2H$ (81%).

using the described reagent $Pd(PPh_3)_4$/formic acid/triethylamine.[33,34] During this reaction, the nitro group of the spacer was reduced to an amino group leading to **4** in good yield (81%). In order to improve the yield of the reaction, we tried to use the same reactant in acetic acid instead of formic acid. Interestingly, by using $Pd(PPh_3)_4$/acetic acid/triethylamine, deprotection of the allyl ester occurred without reduction of the nitro group, allowing **3** directly from **12** in 80% yield. To our knowledge, it is the first time that such selectivity was observed for this kind of reaction by replacing formic acid by acetic acid in the reagent. An explanation could be that formates act as reducing agents in the presence of the palladium catalyst.

3. Biological results

3.1. Stability

Stability of prodrugs **3** and **4** was measured by HPLC in a phosphate buffer solution at pH 7.2 or in a 10% FCS (foetal calf serum) in a phosphate buffer solution. Prodrug **4** was stable over 24 h, with no visible cleavage of the carbonate bond in position 2′ of the paclitaxel. Prodrug **3**, with its nitro group on the spacer, was less stable in the same solutions. Monitoring HPLC analysis with UV detection showed a 57% decrease of the latter prodrug peak in a phosphate buffer solution and a 76% decrease in the serum solution over 24 h. Since the nitro group causes the molecule to be more sensitive, it appeared preferable to use an amino group on the spacer, like in prodrug **4**, for further studies.

3.2. Cytotoxicity

The cytotoxicities of prodrugs **3** and **4** in the presence or in the absence of β-D-glucuronidase were measured on HT-29 (colon cancer) cell lines and compared to the cytotoxicity of the free drug.

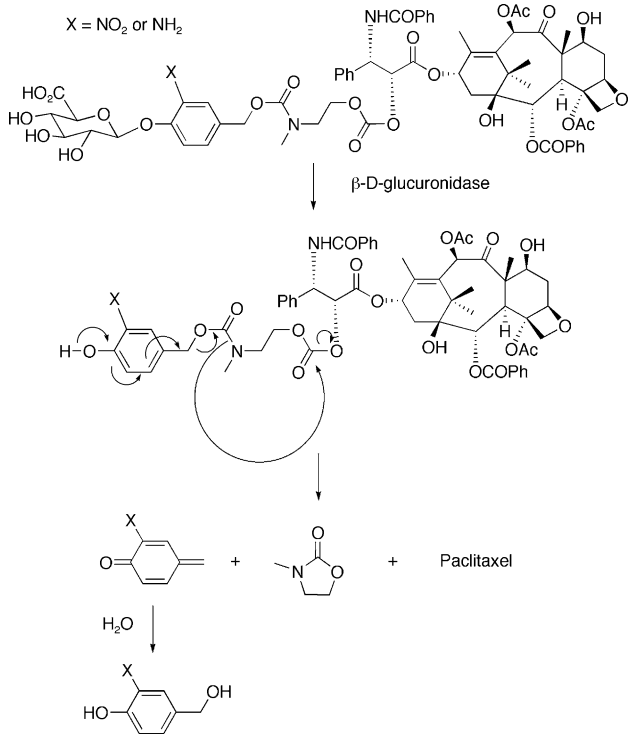
The IC_{50} of prodrugs **3**, **4** and free paclitaxel were, respectively, 28.9, 11.3 and 0.16 nM. In the presence of β-D-glucuronidase, the cytotoxicities of prodrugs **3** and **4** were restored to that of the free paclitaxel. The ratios of cytotoxicities between the prodrug and the free drug were, respectively, 180 and 70 for **3** and **4**. These values are compatible with ADEPT or PMT strategies where a relatively non-cytotoxic prodrug releases a cytotoxic compound.

3.3. Kinetics of drug release

The kinetics of transformation of the prodrugs **3** and **4** into the active drug were measured by HPLC and compared to that of the previously obtained prodrug **2**. The planned enzymatic cleavage reaction was as depicted in Scheme 2.

Escherichia coli β-D-glucuronidase was used as the activating enzyme. For both prodrugs **3** and **4**, cleavage of the prodrug was observed with simultaneous appearance of Taxol® and cyclized spacer (Fig. 3).

In our comparison, we used the same concentrations of prodrugs **2**, **3** and **4** (190 μM) and of β-D-glucuronidase (4.3 U/mL) and then measured the disappearance of the



Scheme 2. Hydrolysis of prodrugs **3** and **4**.

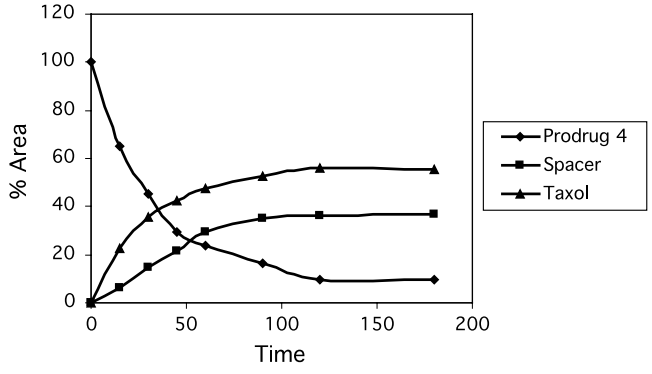


Figure 3. Cleavage of prodrug **4** by β-D-glucuronidase.

starting prodrug (Fig. 4). The results clearly showed the faster cleavage of prodrugs **3** and **4** (Fig. 4). So, the prodrugs **3** and **4** bearing a carbonate function exhibited faster enzymatic cleavage than the prodrug **2** with a carbamate function.

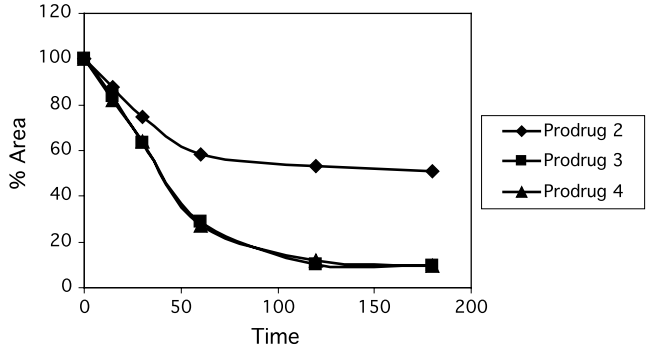


Figure 4. Comparison of cleavage between prodrugs **2**, **3** and **4**.

Between prodrugs **3** and **4**, which differ only by a nitro or an amino group on the spacer, the kinetics were almost identical.

This similarity between amino and nitro spacers was previously observed with docetaxel prodrugs.[35] An explanation could be that the glycosidic enzymatic cleavage is rate-determining and that the following step, namely self-immolation of both spacers, is fast (Scheme 2).

4. Conclusion

In the light of these experiments it appears that, from the two new prodrugs obtained, prodrug **4** can be selected for further in vivo tests for its better stability and liberation kinetics of the active compound in the presence of the activating enzyme.

The spacer of prodrug **4** could also be used in the synthesis of other prodrugs, such as prodrugs of antitumour compounds possessing a free hydroxy group, like etoposide or camptothecin derivatives like SN-38.

During this synthesis, we have shown a difference in reactivity between two reagents ($Pd(PPh_3)_4$/formic acid/triethylamine and $Pd(PPh_3)_4$/acetic acid/triethylamine) for the removal of an allyl ester-protecting group. This can be applied to other syntheses in which the starting material contains a sensitive and reducible group.

5. Experimental

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 a Bruker AC 300 spectrometer—chemical shifts δ in parts per million and J in hertz. Chemical ionisation (CI-MS; NH_3, positive ion mode) or FAB (positive ion mode, either MB ('magic bullet'), or NBA (3-nitrobenzyl alcohol) as matrix) mass spectra were recorded on a Nermag R 10-10C spectrometer. Electrospray ionisation mass spectra (ESI-MS) were acquired using a quadripole instrument with a mass of charge (m/z) range of 2000. The Nermag R 10-10 mass spectrometer used was equipped with an analytical atmospheric pressure electrospray source. Chromatographies were conducted over silica gel (Merck 60 (230–400 Mesh)).

For the NMR descriptions, the numbers are as in Figure 5.

5.1. Allyl {[2-nitro-4-(*O*-*tert*-butyldiphenylsilylmethyl)phenyl]-2,3,4-tri-(*O*-*tert*-butyldimethylsilyl)-β-D-glucopyranosid}uronate (6)

To a solution of Ti-isopropoxide (0.094 mL, 0.32 mmol) in toluene (10 mL) under argon, allyl alcohol (0.058 mL,

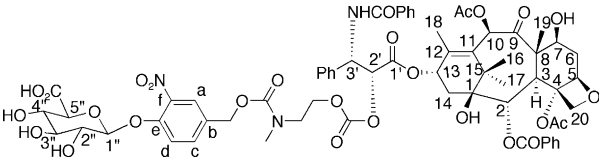


Figure 5. NMR attributions.

0.85 mmol) was added. The reaction mixture was refluxed and isopropanol was removed. Then, after 1 h, fresh allyl alcohol (0.85 mmol) was added and the same procedure was repeated three times. Tetrasilyl compound **5** in 10 mL toluene was added to the reaction vessel and reflux was maintained for four additional hours. Water was added to the reaction mixture, and the solution was filtered through Celite and extracted with diethyl ether (6× 20 mL). After drying over $MgSO_4$ and evaporation, the crude residue was purified by chromatography (toluene). A pale yellow liquid (0.07 g, 69.0%) was isolated. On multigram scale, the yield was less reproducible ranging from 55% to 70%; $R_f = 0.674$ (cyclohexane/EtOAc 7:3); $[\alpha]_D^{20}$ −12.0 (*c* 1.13, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.74 (d, 1H, $J = 1.9$ Hz, Ha), 7.68–7.64 (m, 4H, $H_o\phi_2Si$), 7.42 (m, 7H, $H_{m/p}\phi_2Si$, Hc), 7.18 (d, 1H, $J = 8.1$ Hz, Hd), 5.93–5.80 (m, 1H, $CH_2{=}CH{-}CH_2$), 5.58 (d, 1H, $J = 5.6$ Hz, $H_{1''}$), 5.33–5.19 (dt, 2H, $J = 10.4$ Hz and $J' = 1.2$ Hz, $CH_2{=}CH$), 4.70 (s, 2H, CH_2O_{arom}), 4.61 (d, 2H, $J = 5.6$ Hz, $CH{=}CH_2O$), 4.51 (d, 1H, $J = 1.6$ Hz, $H_{3''}$), 4.41 (m, 1H, $H_{4''}$), 4.06 (d, 1H, $J = 5.5$ Hz, $H_{2''}$), 3.85 (d, 1H, $J = 3.4$ Hz, $H_{5''}$), 1.10 (s, 9H, $(CH_3)_3CSi\phi_2$), 0.93. 0.88 and 0.87 (s, 27H, $(CH_3)_3CSi$), 0.16, 0.14, 0.13, 0.07 and 0.02 (s, 18H, $(CH_3)_2Si$); ^{13}C NMR ($CDCl_3$) δ 170.3 (CO_2 allyl), 150.8 (Ce), 141.9 (Cf), 137.3 ($CH_{o/m}\phi_2Si$), 136.6 (Cb), 134.8 (Cc), 133.3 and 133.2 (Cqaromϕ_2Si, $CH_2{=}CH$), 131.7 ($CH_p\phi_2Si$), 129.6 ($CH_{o/m}\phi_2Si$), 124.9 (Ca), 120.7 ($CH_2{=}CH$), 118.5 (Cd), 101.3 ($C_{1''}$), 80.5 ($C_{3''}$), 79.2 ($C_{5''}$), 77.0 ($C_{2''}$), 74.1 ($C_{4''}$), 67.8 (CH–CH_2O), 66.0 (CH_{2arom}), 28.6 ($(CH_3)_3CSi\phi_2$), 27.6 and 27.5 ($(CH_3)_3CSi$), 21.0 ($(CH_3)_3CSi\phi_2$), 19.8 and 19.7 ($(CH_3)_3CSi$), −2.6, −2.7, −2.8, −2.9, −3.1 and −3.2 ($(CH_3)_2Si$); *m/z* [CI+NH_3] 983.5 $(M+NH_4)^+$.

5.2. Allyl {(2-nitro-4-hydroxymethylphenyl)-2,3,4-tri-(*O*-*tert*-butyldimethylsilyl)-β-D-glucopyranosid}uronate (7)

To a solution of allyltetrasilyl **6** (0.1 g, 0.1 mmol) in DMF (5.4 mL) and acetic acid (6.2 μL, 0.1 mmol) under argon, TBAF (1 M in THF, 0.103 mL, 0.15 mmol) was added and stirred at room temperature for 2 h. The solution was extracted with ether (6× 20 mL). The ethereal layer was washed with saturated $NaHCO_3$ (3× 10 mL), then with water. The organic layer was dried over $MgSO_4$ and evaporated. The crude residue was purified by chromatography (MeOH/CH_2Cl_2 2:8). A white solid (66 mg, 88.0%) was isolated; $R_f = 0.261$ (cyclohexane/EtOAc 7:3); mp 136 °C; $[\alpha]_D^{20}$ −19.2 (*c* 1.65, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.81 (d, 1H, $J = 1.9$ Hz, Ha), 7.52 (dd, 1H, $J = 2.0$ Hz and $J = 8.6$ Hz, Hc), 7.16 (d, 1H, $J = 8.6$ Hz, Hd), 5.92–5.79 (m, 1H, $CH_2{=}CH{-}CH_2$), 5.58 (d, 1H, $J = 5.7$ Hz, $H_{1''}$), 5.32–5.19 (dt, 2H, $J = 10.4$ Hz and $J' = 1.2$ Hz, $CH_2{=}CH$), 4.68 (s, 2H, CH_2O_{arom}), 4.60 (d, 2H, $J = 5.8$ Hz, $CH{=}CH_2O$), 4.51 (d, 1H, $J = 1.3$ Hz, $H_{3''}$), 4.40 (d, 1H, $J = 3.2$ Hz, $H_{4''}$), 4.04 (d, 1H, $J = 5.7$ Hz, $H_{2''}$), 3.85 (d, 1H, $J = 3.4$ Hz, $H_{5''}$), 0.92, 0.87 and 0.82 (s, 27H, $(CH_3)_3CSi$), 0.15, 0.13, 0.12, 0.06 and 0.00 (s, 18H, $(CH_3)_2Si$); ^{13}C NMR ($CDCl_3$) δ 170.0 (CO_2 allyl), 149.8 (Ce), 141.5 (Cf), 137.3 (Cb), 132.9 and 132.8 (Cc, $CH_2{=}CH$), 124.7 (Ca), 119.2 ($CH_2{=}CH$), 117.2 (Cd), 100.3 ($C_{1''}$), 80.1 ($C_{3''}$), 78.4 ($C_{5''}$), 77.2 ($C_{2''}$), 73.5 ($C_{4''}$), 67.1 (CH–CH_2O), 63.5 (CH_{2arom}), 26.4. 26.3 and 26.1 ($(CH_3)_3CSi$), 18.8 (2C) ($(CH_3)_3CSi$), −4.2, −4.3, −4.4, −4.6 and −4.7 ($(CH_3)_2Si$); *m/z* [CI+NH_3] 745.5 $(M+NH_4)^+$.

5.3. *N*-Methyl-*N*-allyl-[4-(2,3,4-tri-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)uronate-3-nitrobenzyloxycarbonyl]-2-amino-ethanol (9)

To a solution of silyl compound **7** (0.05 g; 0.069 mmol) in dry CH_2Cl_2 (2.0 mL) under argon, *N*,*N*-disuccimidyl carbonate (0.026 g, 0.1 mmol) and $(^iPr)_2NEt$ (0.03 mL, 0.17 mmol) were added. The reaction mixture was stirred for 2 h at room temperature, and *N*-methyl-ethanol (0.006 mL, 0.084 mmol) was added. The reaction mixture was then stirred at room temperature for 1 h. After evaporation, the residue was purified by chromatography (CH_2Cl_2/MeOH: 99:1 then 95:5). A pale yellow liquid (54.7 mg, 96.0%) was isolated; $R_f = 0.264$ (CH_2Cl_2/MeOH 9.75:0.25); $[\alpha]_D^{20}$ −13.8 (*c* 1.84, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, 1H, $J = 2.1$ Hz, Ha), 7.53 (dd, 1H, $J = 2.1$ Hz and $J = 8.6$ Hz, Hc), 7.17 (d, 1H, $J = 8.6$ Hz, Hd), 5.92–5.78 (m, 1H, $CH_2{=}CH{-}CH_2$), 5.59 (d, 1H, $J = 5.6$ Hz, $H_{1''}$), 5.32–5.19 (dt, 2H, $J = 10.3$ Hz and $J' = 1.4$ Hz, $CH_2{=}CH$), 5.09 (s, 2H, CH_2O_{arom}), 4.60 (d, 2H, $J = 5.8$ Hz, $CH{=}CH_2O$), 4.49 (d, 1H, $J = 1.4$ Hz, $H_{3''}$), 4.40 (s, 1H, $H_{4''}$), 4.05 (d, 1H, $J = 5.6$ Hz, $H_{2''}$), 3.85 (d, 1H, $J = 3.5$ Hz, $H_{5''}$), 3.79 (s, 2H, CH_2OH), 3.46 (s, 2H, CH_2N), 2.99 (s, 3H, NCH_3), 0.95, 0.92, 0.86, 0.84 and 0.83 (s, 27H, $(CH_3)_3CSi$), 0.18–0.01 (s, 18H, $(CH_3)_2Si$); ^{13}C NMR ($CDCl_3$) δ 168.5 (CO_2 allyl), 157.3 ($OCONCH_3$), 150.0 (Ce), 140.2 (Cf), 133.8 (Cb), 131.7 (Cc), 130.8 and 130.6 ($CH_2{=}CH$), 125.4 (Ca), 119.1 ($CH_2{=}CH$), 117.2 (Cd), 99.4 ($C_{1''}$), 78.9 ($C_{3''}$), 76.9 ($C_{5''}$), 75.4 ($C_{2''}$), 72.4 ($C_{4''}$), 68.2 (CH–CH_2O), 65.8 (CH_{2arom}), 61.2 and 60.7 (CH_2OH), 52.1 and 51.1 (CH_2N), 35.9 and 35.5 (NCH_3), 26.8, 26.3 and 24.9 ($(CH_3)_3CSi$), 18.3, 18.2 and 18.1 ($(CH_3)_3CSi$), −4.4 (2C) and −4.7 ($(CH_3)_2Si$); *m/z* [CI+NH_3] 846.2 $(M+NH_4)^+$.

5.4. 1-{*N*-Methyl-*N*-allyl-[4-(2,3,4-tri-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)uronate-3-nitrobenzyloxycarbonyl]-2-amino-ethoxycarbonyl}-pyrrolidine-2,5-dione (10)

To a solution of silyl compound **9** (311 mg, 0.375 mmol) in dry dichloromethane, *N*,*N*-disuccimidylcarbonate (480 mg, 2.25 mmol, three times 2 equiv every 2 h, added by small portions), DMAP (91.6 mg, 0.75 mmol, 2 equiv) and $(^iPr)_2NEt$ (163.3 μL, 0.973 mmol, 2.5 equiv) were added. The reaction mixture was stirred

overnight at room temperature. After evaporation, the residue was purified by chromatography on a silica gel column (CH_2Cl_2/EtOAc 1:1) affording 288 mg (79%) of compound **10** as an oil; $R_f = 0.465$ (cyclohexane/EtOAc 1:1); $[\alpha]_D^{20}$ −14.7 (*c* 1.02, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 7.84 (d, 1H, *J* = 1.6 Hz, Ha), 7.53 (d, 1H, *J* = 8.1 Hz, Hc), 7.17 (d, 1H, *J* = 8.1 Hz, Hd), 5.83 (m, 1H, CH_2=C*H*–CH_2), 5.58 (d, 1H, *J* = 5.3 Hz, $H_{1''}$), 5.22 (m, 2H, CH_2=CH), 5.12 (d, 2H, *J* = 4.5 Hz, CH_2O_{arom}), 4.58 (d, 2H, *J* = 5.6 Hz, CH=CH_2O), 4.49 (s, 2H, $H_{3''}$ and $H_{4''}$), 4.39 (s, 2H, CH_2OCO), 4.03 (d, 1H, *J* = 5.3 Hz, $H_{2''}$), 3.84 (d, 1H, *J* = 3.3 Hz, $H_{5''}$), 3.61 (m, 2H, CH_2N), 3.01 (s, 3H, NCH_3), 2.83 (s, 4H, CH_2–CH_2), 0.92, 0.86 and 0.82 (s, 27H, $(CH_3)_3CSi$), 0.15, 0.13, 0.11, 0.05 and −0.00 (s, 18H, $(CH_3)_2Si$); ^{13}C NMR ($CDCl_3$) δ 168.6, 168.5 and 168.3 (CO esters, CO anhydride), 155.9 and 155.6 (OCO and O*C*ONCH$_3$), 151.5 (Ce), 149.7 (Cf), 140.0 (Cb), 133.8 (Cc), 131.4 (CH_2=*C*H), 125.5 (Ca), 118.9 (*C*H_2=CH), 116.6 (Cd), 99.2 ($C_{1''}$), 78.7 ($C_{3''}$), 76.7 ($C_{5''}$), 75.1 ($C_{2''}$), 72.1 (*C*H_2OCO), 69.1 ($C_{4''}$), 66.0, 65.8 and 65.6 (CH_{2arom} and CH–CH_2O), 48.3 (CH_2N), 35.9 (NCH_3), 25.8, 25.7 and 25.4 ((*C*H_3)$_3$CSi) and (CH_2–CH_{2anhyd}), 17.9 (3C) ((CH$_3$)$_3$*C*Si), −4.5, −4.6, −4.7, −5.0 and −5.1 ((*C*H_3)$_2$Si); *m*/*z* [CI+NH_3] 987.5 $(M+NH_4)^+$.

5.5. 2′-*O*-{*N*-Methyl-*N*-allyl-[4-(2,3,4-tri-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl)uronate-3-nitrobenzyloxycarbonyl]-2-amino-ethyl}-oxycarbonyl-paclitaxel (11)

Carbonate **10** (200 mg, 0.206 mmol) and 50.3 mg (0.412 mmol, 2 equiv) of DMAP were dissolved in 20 mL of freshly distilled dichloromethane. Under argon, 123 mg (0.144 mmol) of Taxol and then 50 μL (0.412 mmol, 2 equiv) of triethylamine were added. After 24 h, the solution was diluted with CH_2Cl_2 (25 mL), washed with water, brine, dried over $MgSO_4$ and concentrated. After purification on a silica gel column (CH_2Cl_2/EtOAc 1:1), 230 mg (93%) of the coupling compound was isolated as a white solid; $R_f = 0.340$ (CH_2Cl_2/MeOH 97:3); mp 128–129 °C; $[\alpha]_D^{20}$ −38.4° (*c* 0.85, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, 2H, *J* = 7.3 Hz, H_oBz_2), 7.75 (m, 3H, H_mBz and Ha), 7.59 (d, 1H, *J* = 7.2 Hz, Hc), 7.53–7.25 (m, 11H, H_{arom}), 7.14 (m, 1H, Hd), 6.29 (s, 2H, H_{10} and H_{13}), 6.01 (m, 1H, $H_{3'}$), 5.82 (m, 1H, CH_2=C*H*–CH_2), 5.69 (d, 1H, *J* = 7.02 Hz, H_2), 5.59 (d, 1H, *J* = 5.6 Hz, $H_{1''}$), 5.43 (d, 1H, *J* = 1.9 Hz, $H_{2'}$), 5.25 (m, 2H, CH_2=CH), 4.99 (d, 3H, *J* = 9.1 Hz, H5 and CH_2O_{arom}), 4.59 (d, 2H, *J* = 5.5 Hz, CH=CH_2O), 4.49 (s, 1H, $H_{3''}$), 4.40 (s, 1H, $H_{4''}$), 4.32 (d, 2H, *J* = 8.2 Hz, CH_2OCO), 4.21 (m, 1H, H_7), 4.12 (2H, ABq, *J* = 7.1 Hz, H_{20}), 4.04 (d, 1H, *J* = 5.3 Hz, $H_{2''}$), 3.85 (m, 2H, H_3 and $H_{5''}$), 3.51 (m, 2H, CH_2N), 2.91 (s, 3H, NCH_3), 2.55 (m, 1H, $H_{6\alpha}$), 2.46 (s, 3H, CH_3Ac$_4$), 2.40 (m, 1H, H_{14}), 2.22 (s, 3H, CH_3Ac$_{10}$), 2.20 (m, 1H, H_{14}), 1.93 (s, 3H, H_{18}), 1.84 (m, 1H, $H_{6\beta}$), 1.67 (s, 3H, H_{19}), 1.24 (s, 3H, H_{16}), 1.13 (s, 3H, H_{17}), 0.92, 0.86 and 0.83 (s, 27H, $(CH_3)_3CSi$), 0.15, 0.13, 0.11, 0.06, 0.03 and −0.00 (s, 18H, $(CH_3)_2Si$); ^{13}C NMR ($CDCl_3$) δ 203.7 (C_9), 171.1, 169.8, 168.2, 167.8, 167.2 and 166.8 (Me*C*O(C_4), Me*C*O(C_{10}), ϕ*C*O$_2$., ϕ*C*ON$_{3'}$, $C_{1'}$, *C*O$_2$ allyl), 155.9, 155.8 and 144.9 (OCO and O*C*ONCH$_3$), 149.7 (Cf), 142.5 (C_{12}), 140.1, 137.1, 133.5, 132.8, 131.9 and 129.2 (Cq_{arom} and C_{11}), 131.4, 130.2, 129.0, 128.7, 128.6, 128.4 and 127.1 (CH_{arom}, Cc, CH_2=*C*H), 126.6 (Ca), 118.8 (*C*H_2=CH), 116.8 (Cd), 99.3 ($C_{1''}$), 84.4 (C_5), 81.0 (C_4), 78.9 (C_1), 78.7 ($C_{3''}$), 76.6, 76.4 and 75.5 (C_{10}, $C_{2'}$, $C_{2''}$, C_7, $C_{4''}$ and $C_{5''}$), 75.1 (2C) (C_{20}, C_2), 72.1 (C_{13}), 66.5 (*C*H_2O-CO), 65.9 (CH–*C*H_2O), 65.6 (CH_{2arom}), 58.4 (C_8), 52.7 ($C_{3'}$), 47.3 and 46.2 (CH_2N), 45.5 (C_3), 43.1 (C_{15}), 35.6 (2C) (NCH_3 and C_6), 26.7 (C_{16}), 25.8 and 25.7 ((*C*H_3)$_3$CSi), 22.6 (C_{14}), 22.1 (*C*H_3Ac$_4$), 25.8 and 25.7 ((CH$_3$)$_3$*C*Si), 20.9 (C_{17}), 20.8 (*C*H_3Ac$_{10}$), 17.9 ((CH$_3$)$_3$CSi), 14.7 (C_{18}), 9.6 (C_{19}), −4.5, −4.6, −4.7, −5.0 and −7.0 ((*C*H_3)$_2$Si); *m*/*z* [FAB+NaI] 1730.2 $(M+Na)^+$, 1731.4 $(M+Na+1)^+$ (Found $M+Na^+$, 1730.7131, $C_{86}H_{117}N_3O_{27}Si_3Na$ requires 1730.7080).

5.6. 2′-*O*-{[*N*-Methyl-*N*-allyl-4-(β-D-glucopyranosiduronate)-3-nitrobenzyloxycarbonyl]-2- amino-ethyl}-oxycarbonyl-paclitaxel (12)

To 115 mg (0.067 mmol) of the coupling compound **11**, dissolved in 1.5 mL of anhydrous pyridine at 0 °C, was added, dropwise, at 0 °C, 1 mL of a 70% HF–pyridine complex. After 2 h at 0 °C, the mixture was allowed to reach room temperature and stirring was continued for 26 h. The reaction mixture was cooled to 0 °C and 15 mL of saturated $NaHCO_3$ was added. The medium was then extracted with EtOAc (4× 10 mL), washed with water, dried over $MgSO_4$, concentrated and chromatographed on a silica gel (CH_3CN/toluene 1:1 then 8:2). A white solid (69 mg, 75%) was isolated; $R_f = 0.245$ (CH_2Cl_2/MeOH 95:5); mp 116 °C; $[\alpha]_D^{20}$ −20.2 (*c* 1.0, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 8.15 (d, 2H, *J* = 7.4 Hz, H_oBz_2), 7.72 (m, 3H, H_mBz and Ha), 7.53–7.13 (m, 13H, H_{arom}), 6.31 (m, 2H, H_{10} and H_{13}), 6.03 (d, 1H, *J* = 1.9 Hz, $H_{3'}$), 5.91 (m, 1H, CH_2=C*H*–CH_2), 5.69 (d, 1H, *J* = 6.8 Hz, H_2), 5.47 (d, 1H, *J* = 1.9 Hz, $H_{2'}$), 5.31 (m, 2H, CH_2=CH), 4.98 (m, 4H, H_5, CH_2O_{arom} and $H_{1''}$), 4.70 (d, 2H, *J* = 5.2 Hz, CH=CH_2O), 4.50 (m, 1H, $H_{3''}$), 4.32–4.21 (m, 4H, $H_{4''}$, CH_2OCO and H_7), 4.18–3.61 (m, 7H, H_{20}, H_3, $H_{5''}$, $H_{2''}$, 2OH″), 3.51 (m, 2H, CH_2N), 3.13 (m, 1H, OH′), 2.91 (d, 3H, *J* = 3.4 Hz, NCH_3), 2.56 (m, 1H, $H_{6\alpha}$), 2.48 (s, 3H, CH_3Ac$_4$), 2.35 (s, 3H, CH_3Ac$_{10}$), 1.99 (s, 2H, H_{14}), 1.91 (s, 3H, H_{18}), 1.68 (s, 3H, H_{19}), 1.44 (m, 1H, $H_{6\beta}$), 1.21 (s, 3H, H_{16}), 1.13 (s, 3H, H_{17}); ^{13}C NMR ($CDCl_3$) δ 203.8 (C_9), 171.2, 170.0, 168.0, 167.9, 167.2 and 166.9 (Me*C*O(C_4), Me*C*O(C_{10}), ϕ*C*O$_2$, ϕ*C*ON$_{3'}$, $C_{1'}$, *C*O$_2$ allyl), 155.8, 155.7 and 154.0 (OCO and O*C*ONCH$_3$), 149.7 (Cf), 142.4 (C_{12}), 140.3, 136.6, 133.6, 132.8, 132.0 and 129.2 (Cq_{arom} and C_{11}), 131.1, 130.2, 129.0, 128.7, 128.6, 128.5, 128.2, 127.2, 127.0, 126.6, 125.2, 124.7 and 124.5 (CH_{arom}, Ca, Cc, CH_2=*C*H), 119.0 (*C*H_2=CH), 118.7 (Cd), 102.4 ($C_{1''}$), 84.4 (C_5), 81.0 (C_4), 79.0 (C_1), 75.6, 75.0 and 74.8 (C_{10}, C_2, $C_{2'}$, $C_{2''}$, C_7 and $C_{4''}$), 72.8, 72.0 (2C) and 70.9 (C_{13}, C_{20}, $C_{3''}$ and $C_{5''}$), 66.3 and 65.4 (*C*H_2OCO, CH–*C*H_2O, CH_{2arom}), 58.3 (C_8), 52.7 ($C_{3'}$), 47.5 and 46.3 (CH_2N), 45.7 (C_3), 43.1 (C_{15}), 35.8 and 35.5 (NCH_3), 33.8 (C_6), 26.7 (C_{16}), 24.3 (C_{17}), 22.6 (C_{14}), 22.1 (*C*H_3Ac$_4$), 20.8 (*C*H_3Ac$_{10}$), 14.7 (C_{18}), 9.7 (C_{19}); *m*/*z* [FAB+NaI] 1388.6 $(M+Na)^+$ (Found $M+Na^+$ 1388.4526, $C_{68}H_{75}N_3O_{27}Na$ requires 1388.4486).

5.7. 2′-*O*-{[*N*-Methyl-*N*-4-(β-D-glucopyranosiduronate)-3-nitrobenzyloxycarbonyl]-2-amino-ethyl}-oxycarbonyl-paclitaxel (3)

Allyl ester **12** (30 mg, 0.022 mmol) was dissolved in 1 mL of THF, and then was added a mixture of Et_3N (9.2 μL, 0.066 mmol)–AcOH (0.88 μL, 0.044 mmol) in 23 μL THF. After bubbling of argon gas for 10 min, a few crystals of palladium tetrakistriphenylphosphine were added. After 30 min under stirring at room temperature (no starting material was detected on TLC), the volatiles were removed in vacuo, and the residue was purified on silica gel (CH_3CN/H_2O 95:5). Lyophilisation afforded 23.2 mg (80%) of prodrug **3** as a white solid; $R_f = 0.197$ (CH_3CN/H_2O 9:1); mp 180 °C; $[\alpha]_D^{20}$ −56 (*c* 0.9, MeOH); ^1H NMR (300 MHz, MeOD) δ 9.12 (m, 1H, $NH_{3'}$), 8.13 (d, 2H, $J = 6.9$ Hz, $HoBz_2$), 7.77–7.24 (m, 16H, H_{arom}, Ha, Hc, Hd), 6.45 (s, 1H, H_{10}), 6.08 (m, 1H, H_{13}), 5.83 (m, 1H, $H_{3'}$), 5.65 (d, 1H, $J = 6.8$ Hz, H_2), 5.45 (m, 1H, $H_{2'}$), 5.15 (d, 1H, $J = 5.7$ Hz, H_5), 4.98 (s, 1H, $H_{1''}$), 4.36 (m, 3H, CH_2O_{arom} and H_7), 4.18 (s, 2H, H_{20}), 3.99 (d, 1H, $J = 8.9$ Hz, $H_{5''}$), 3.82 (d, 1H, $J = 6.8$ Hz, H_3), 3.72 (m, 2H, CH_2OCO), 3.61–3.39 (m, 5H, CH_2N, $H_{2''}$, $H_{3''}$,$H_{4''}$), 2.90 (s, 3H, NCH_3), 2.47–2.40 (m, 4H, $H_{6\alpha}$ and CH_3Ac_4), 2.25 (m, 2H, H_{14}), 2.02 (s, 3H, CH_3Ac_{10}), 1.94 (m, 3H, H_{18}), 1.86 (m, 1H, $H_{6\beta}$), 1.64 (s, 3H, H_{19}), 1.13 (s, 6H, H_{16} and H_{17}); ^{13}C NMR (MeOD) δ 203.7 (C_9), 170.2, 169.9, 168.8 (2C) and 166.2 (Me*C*O(C_4), Me*C*O(C_{10}), $\phi C O_2$, $\phi C ON_{3'}$, $C_{1'}$, CO_2), 156.4 (2C), 153.9 and 149.7 (OCO and O*C*ONCH$_3$), 149.6 (Cf), 140.8 (2C), 136.6 (Cq_{arom}), 133.9, 133.5, 133.2, 132.3, 131.7, 131.5, 131.0, 129.9, 129.8, 128.7, 128.4, 128.3, 128.1 and 127.1 (CH_{arom}), 124.1 and 117.4 (Cd, Cc, Ca), 100.9 ($C_{1''}$), 84.4 (C_5), 80.8 (C_4), 77.5 (C_1), 77.1, 76.2, 76.0 and 75.3 (C_{20}, C_{10}, C_2, $C_{2'}$, $C_{3''}$, $C_{5''}$), 74.8, 73.0 (2C), 71.8 and 70.8 (C_{13}, C_7, $C_{2''}$ and $C_{4''}$), 65.4 and 65.4 (*C*H_2OCO, CH_{2arom}), 57.7 (C_8), 53.7 ($C_{3'}$), 47.0 and 46.7 (C_3, CH_2N), 43.1 (C_{15}), 36.0 (C_6), 35.0 (NCH_3), 25.5 and 25.0 (C_{16}), 21.8 (2C) and 20.9 (C_{17}, C_{14}), (*C*H_3Ac$_4$), 19.4 (*C*H_3Ac$_{10}$), 13.5 (C_{18}), 9.0 (C_{19}); *m/z* [ESI$^-$] 1324.24 $(M+H)^+$ (Found $M+Na^+$, 1348.4121, $C_{65}H_{71}N_3O_{27}Na$ requires 1348.4173).

5.8. 2′-*O*-{[*N*-Methyl-*N*-4-(β-D-glucopyranosiduronate)-3-aminobenzyloxycarbonyl]-2-amino-ethyl}-oxycarbonyl-paclitaxel (4)

Allyl ester **12** (40 mg, 0.029 mmol) was dissolved in 1.5 mL THF, followed by addition of Et_3N (12.3 μL, 0.087 mmol)–HCOOH (0.88 μL, 0.058 mmol) mixture in 25 μL THF. After bubbling of argon gas for 10 min, a few crystals of palladium tetrakistriphenylphosphine were added. After 30 min under stirring at room temperature (no starting material was detected on TLC), the volatiles were removed in vacuo, and the residue was purified on silica gel (eluent CH_3CN/H_2O 95:5). Lyophilisation afforded 31 mg (81%) of prodrug **4** as a white solid; $R_f = 0.157$ (CH_3CN/H_2O 9:1); mp 178–179 °C; $[\alpha]_D^{20}$ −65 (*c* 1.25, MeOH); ^1H NMR (300 MHz, MeOD) δ 9.20 (m, 1H, $NH_{3'}$), 8.12 (d, 2H, $J = 5.9$ Hz, H_oBz_2), 7.97–7.25 (m, 16H, H_{arom}), 6.45 (s, 1H, H_{10}), 6.08 (m, 1H, H_{13}), 5.85 (m, 1H, $H_{3'}$), 5.64 (d, 1H, $J = 5.1$ Hz, H_2), 5.42–5.16 (m, 2H, $H_{2'}$ and H_5), 4.97 (s, 1H, $H_{1''}$), 4.68 (m, 3H, CH_2O_{arom} and H_7), 4.32–4.23 (m, 5H, H_{20}, CH_2OCO and $H_{5''}$), 3.80 (m, 1H, H_3), 3.53 (m, 5H, CH_2N, $H_{2''}$, $H_{3''}$, $H_{4''}$), 2.89 (s, 3H, NCH_3), 2.45 (m, 4H, $H_{6\alpha}$ and CH_3Ac_4), 2.14 (m, 2H, H_{14}), 1.93–1.80 (m, 7H, CH_3Ac_{10}, H_{18} and $H_{6\beta}$), 1.64 (s, 3H, H_{19}), 1.12 (s, 6H, H_{16} et H_{17}); RMN ^{13}C (MeOD) δ 203.7 (C_9), 170.2, 169.9, 168.8 and 166.2 (2C) (Me*C*O(C_4), Me*C*O(C_{10}), $\phi C O_2$, $\phi C ON_{3'}$, $C_{1'}$, CO_2), 156.4 (2C), 153.9 and 149.4 (OCO and O*C*ONCH$_3$), 149.4 (Cf), 140.8, 140.3 and 136.6 (Cq_{arom}), 133.9, 133.5, 133.2, 131.5, 131.2, 129.9, 129.8, 128.7, 128.4, 127.1, 126.6 and 124.1 (CH_{arom}), 117.7 and 117.5 (Cd, Cc, Ca), 101.0 ($C_{1''}$), 84.4 (C_5), 80.8 (C_4), 77.5 (C_1), 77.2, 76.0 (2C) and 75.3 (C_{20}, C_{10}, C_2, $C_{2'}$, $C_{3''}$, $C_{5''}$), 74.8, 71.8 and 70.8 (C_{13}, C_7, $C_{2''}$ and $C_{4''}$), 68.6, 65.8 and 65.4 (*C*H_2OCO, CH_{2arom}), 57.7 (C_8), 53.7 ($C_{3'}$), 47.0 and 46.7 (C_3, CH_2N), 43.1 (C_{15}), 36.1 (C_6), 35.0 (NCH_3), 25.5 (C_{16}), 22.8, 21.8 and 20.9 (C_{17}, C_{14}), (*C*H_3Ac$_4$), 19.4 (*C*H_3Ac$_{10}$), 13.6 (C_{18}), 9.0 (C_{19}); *m/z* [MALDI] 1296.54 $(M+H)^+$ (Found $M+Na^+$, 1318.4459, $C_{65}H_{73}N_3O_{25}Na$ requires 1318.4431).

5.9. In vitro cytotoxicity

HT 29 (human colorectal adenocarcinoma) cells were grown in RPMI 1640 (+)(L)-glutamine medium supplemented with 10% (v/v) foetal calf serum, 100 UI/mL penicillin, 100 μg/mL streptomycin and 1.5 μg/mL fungizone and kept under 5% CO_2 at 37 °C.

Ninety-six-well plates were seeded with 800 HT 29 cells per well in 200 μL medium.

Twenty-four hours later, chemicals dissolved in DMSO were added for 72 h at a final concentration ranging between 0.5 nM and 10 μM in a fixed volume of DMSO (1% final concentration). Controls received an equal volume of DMSO. The number of viable cells was measured at 490 nm with the MTS reagent (Promega, Madison, WI) and IC_{50} was calculated as the concentration of compound eliciting a 50% inhibition of cell proliferation.

5.10. HPLC conditions

Analysis was carried out on a reverse-phase column (Lichrospher RP18e, 125 × 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).

5.11. Stability of compounds in a buffer solution

A solution of 190 μM of prodrug in 0.02 M phosphate buffer (pH 7.2) or in 10% FCS (fetal calf serum) in phosphate buffer was incubated at 37 °C. Aliquots (50 μL) were taken at various times and analysed by HPLC after dilution with eluent (150 μL).

5.12. Enzymatic cleavage by *Escherichia coli* β-D-glucuronidase

A solution of 190 μM of prodrug in 0.02 M phosphate buffer (pH 7.2) was incubated at 37 °C in the presence

of 4.3 U/mL of β-D-glucuronidase (*E. coli*). Aliquots (50 μL) were taken at various times and analysed by HPLC after dilution with eluent (150 μL).

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2006.03.002.

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