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First enzymatically activated Taxotere prodrugs designed for ADEPT and PMT

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Abstract—Described here are the syntheses and preliminary biological evaluations of the first two enzymatically activated prodrugs of docetaxel (Taxotere®) reported to date. These prodrugs were designed as potential candidates for selective chemotherapy in ADEPT or PMT. They are constituted of a glucuronic acid moiety, a double spacer and the cytotoxic drug, differing only by the spacer substitution. The prodrugs were stable in a buffer, and the in vitro studies showed good detoxification and hydrolysis kinetics. As docetaxel was efficiently released in both cases, these compounds are very valuable candidates for further biological evaluations.

1. Introduction

Paclitaxel (Taxol®) **1**,[1] a diterpene considered as one of the most promising antitumor drugs of the 1990s, and its semisynthetic analogue docetaxel (Taxotere®) **2**,[2] have become indispensable drugs in clinics (Fig. 1). The main chemical differences between paclitaxel **1** and docetaxel **2** are the substitutions at the 3′-nitrogen on the side chain (BOC instead of benzoyl) and the 10-position of the taxoid core (free alcohol instead of acetate). Although being among the very first examples of a new mechanism of action (promotion and stabilization of microtubules) and showing high potency against solid tumors, they bring about a number of undesirable side effects, and moreover their poor water-solubility hampers their clinical application. Namely, they have to be co-injected with a detergent, which induces reactions such as hypersensitivity.[3,4]

To address these problems, numerous researches on analogues, prodrugs[5] and derivatives of paclitaxel have been carried out; in contrast with docetaxel, very few attempts have been undertaken to overcome these difficulties and improve the drug properties. Previously, some glycosyl derivatives of docetaxel at the 2′, 7 or 10 positions, including a glycolate spacer, were prepared and evaluated;[6–7] some of them displayed better solubilities together with better toxicities than the parent drug. A direct 7-*O*-glucosyl docetaxel analogue was reported to be twice as water-soluble as paclitaxel but with similar activity.[8] Amino acid conjugates linked to the 2′-hydroxyl of a 3′-cyclopropyl docetaxel analogue were also prepared and tested in vivo.[9] Some showed better activity than docetaxel in ILS (Increase of Life Span) on B16 melanoma-bearing mice.

Since our aim was to selectively deliver docetaxel at the tumor site for an increased efficacy of the treatment, we considered converting the active compound into a water-soluble prodrug. The specific delivery will be based on an enzymatic (β-D-glucuronidase) hydrolysis according to an ADEPT[10] (Antibody Directed Enzyme Prodrug Therapy) or a PMT[11] (Prodrug Mono Therapy) strategy. In an ADEPT strategy, the enzyme is first targeted at the tumor site by Mab-antigen recognition. In a second step, the enzyme acts as a catalyst to cleave the injected prodrug locally and release the cytotoxic drug. In a PMT strategy, the already present β-D-glucuronidase found in necrotic tumor areas is used instead.[12]

In the present paper, we report the synthesis of two docetaxel prodrugs **4** and **5** (Fig. 2) in which a glucuronic moiety is linked to a double spacer containing a *para*-hydroxybenzyl alcohol connected to a diamine tether via a carbamate linkage. This system was shown

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to be efficient in releasing drugs in the presence of β-D-glucuronidase.[13,14] For instance a paclitaxel prodrug **3** (Fig. 2) was shown to be efficient.[13] The spacer is linked to the 2′ position of docetaxel **2** via a carbamate. The 2′ position was selected because it is known that its modification leads to a dramatic loss of cytotoxic activity, and the carbamate linkage because it is relatively stable in the presence of esterases. The drug release mechanism from **4** or **5** is depicted on Scheme 1. On the other hand, we decided to use two types of substituents on the aromatic part of the spacer, a nitro or an amino function in the *ortho* position of the phenol involved in the glycosidic linkage. Indeed most of the prodrugs based on a *para*-hydroxybenzyl spacer reported in the ADEPT or PMT literature contained a nitro substituent to decrease the pK_a of the phenol and consequently improve the rate of the self-immolative decomposition of the spacer. However we must remind that for paclitaxel prodrug **3**, we could not access to the corresponding nitro containing spacer prodrug since some intriguing problems related to the reduction of the nitro group occurred during a benzyl hydrogenolysis.[13] Nevertheless, liberation of paclitaxel from the amino containing spacer prodrug was very efficient. Therefore it was of interest to prepare both types in the case of docetaxel and to compare stability, cytotoxicity and kinetics of enzymatic hydrolysis. So, at the outset of our project, we considered the synthesis of prodrugs **4** and **5** as described in the retrosynthetic pathway (Scheme 2). Whereas the preparation of **4** was straightforward and used the same pathway as

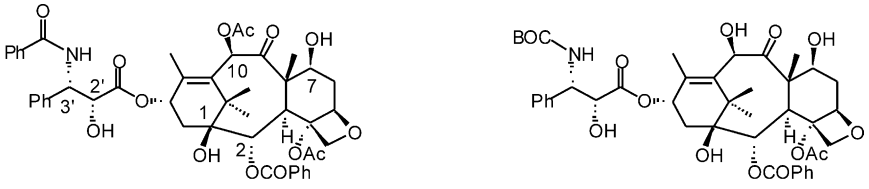


Figure 1. Structures of paclitaxel **1** and docetaxel **2**.

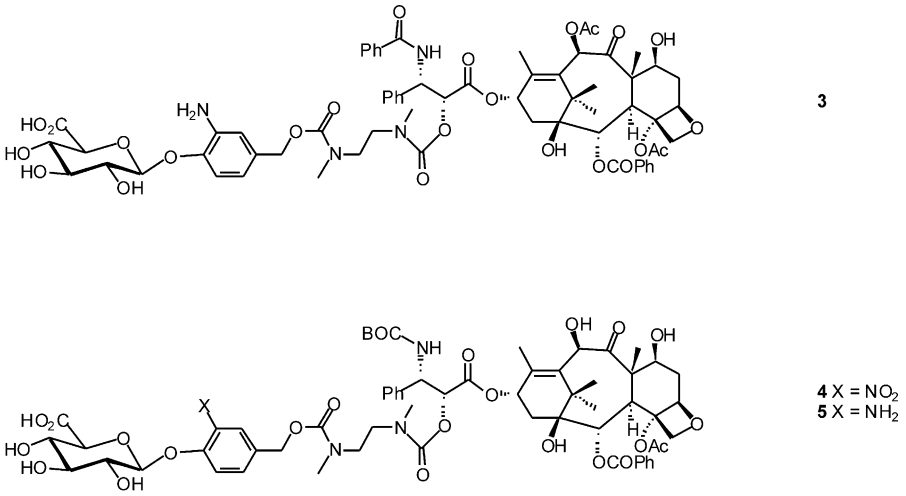
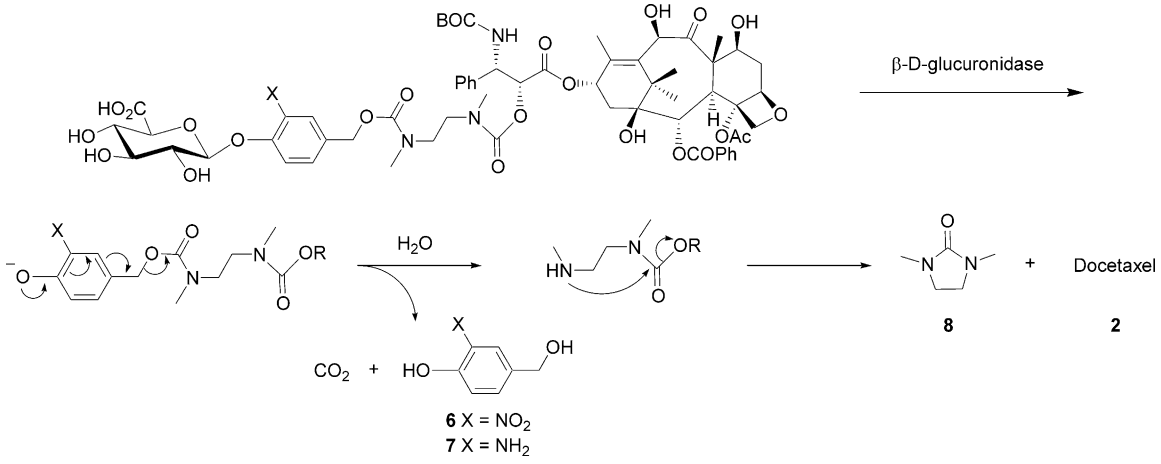


Figure 2. Structure of prodrugs.

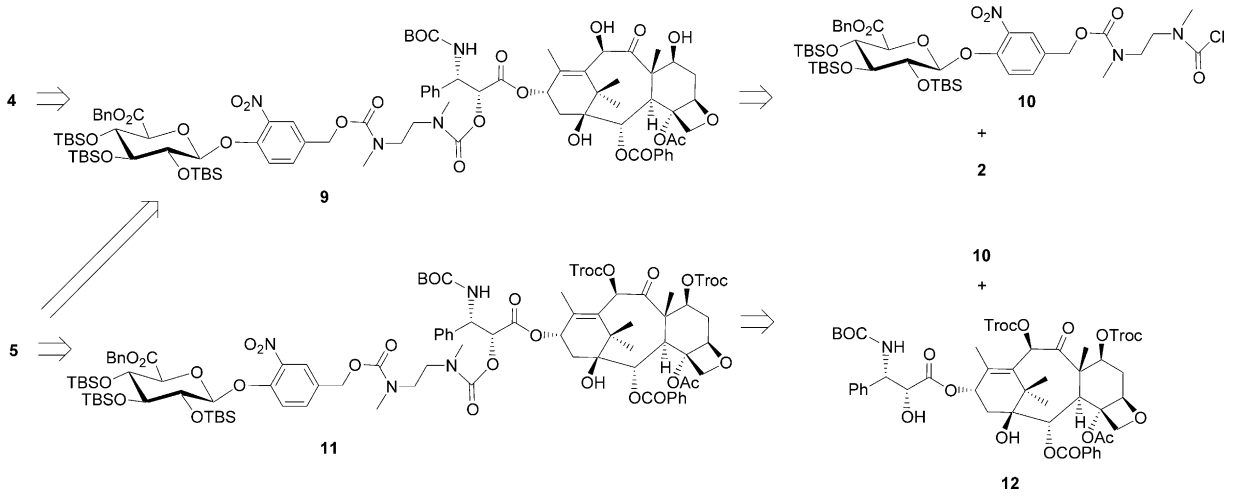


Scheme 1. Release of docetaxel from prodrug.

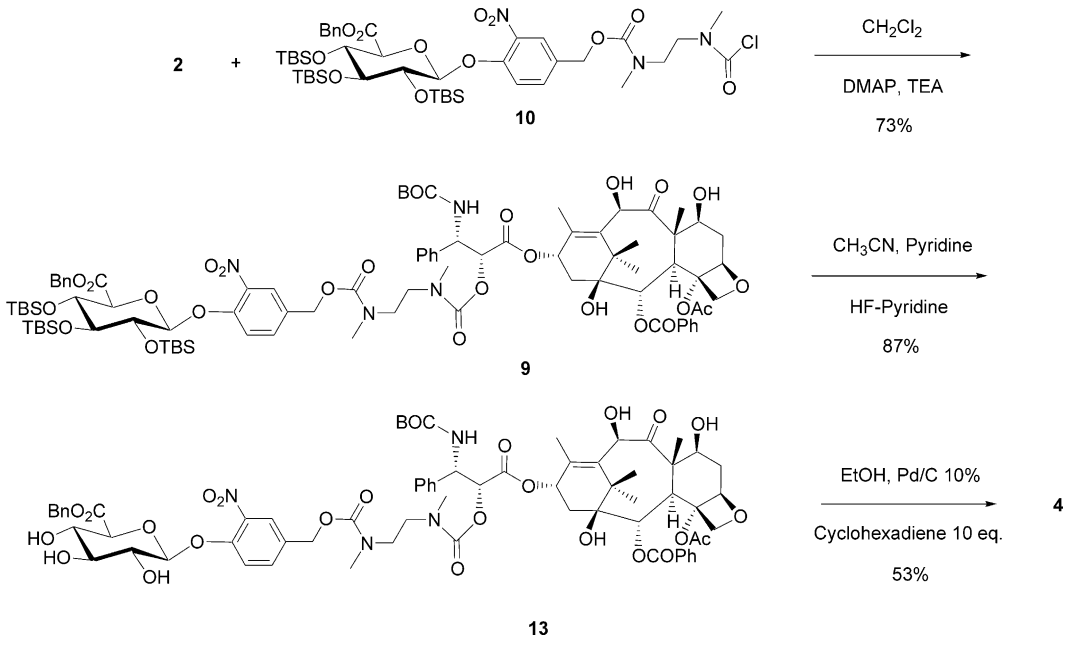
for **3**, we envisioned that **5** could be obtained from the common precursor **9** by means of an hydrogenation, or through troc deprotection conditions (Zn and AcOH) that would also reduce the nitro into an amino group.[15]

2. Chemistry

First of all, docetaxel **2** and its 7,10-ditroc protected derivative **12** were prepared starting from oxazoline and 7,10-ditroc-DAB (10-deacetyl-baccatin III) using published procedures.[16,17] For **4**, a regioselective carbamoylation at the 2′ hydroxyl of **2** with the previously described intermediate **10**[13] cleanly led to the fully protected prodrug **9**; as expected the less reactive hydroxyl groups of docetaxel did not react. Then desilylation of **9** with HF-Pyridine, followed by selective hydrogenolysis of **13** with cyclohexadiene and palladium on charcoal, without reduction of the nitro group on the spacer moiety, yielded **4** (34% from **2**, Scheme 3). This result was quite surprising at first sight, bearing in mind the failure of this reaction using paclitaxel as the drug.[13] Molecular modelling (HyperChem, field MM+) of the compounds may support this fact as we have observed that the two faces of the benzyl ester of prodrug **3** are hindered by π-stacking with the 2-benzoate and interactions with the side chain of paclitaxel on the other side. This steric hindrance in the approach of the benzyl group explains the need for harsher conditions in order to cleave this protecting group by hydrogenolysis, whereas the nitro group of the spacer is very accessible. So we could not succeed in cleaving the benzyl ester without reducing the nitro to an amino group. In comparison, the ester in **13** is relatively free on one face, the other being involved in π-stacking interactions with the phenyl ring of the spacer. This fact could account



Scheme 2. Retrosynthetic scheme of compounds **4** and **5**.



Scheme 3. Synthesis of prodrug **4**.

for its easy removal in our conditions without reduction of the nitro group.

Alternatively, the ditroc derivative **12** was used instead of **9** to achieve the synthesis of prodrug **5** (Scheme 4). Coupling of **12** with **10** led to **14** (70% yield), which was first deprotected with Zn/AcOH with simultaneous reduction of the nitro group to an amine giving **15**. The next desilylation step proved to be very challenging and compound **16** was obtained in a rather modest yield (20%). Indeed, since the HF/Pyridine reagent was not strong enough to deprotect all the silyl groups, we instead used the $Et_3N/3HF$ reagent, which had been efficient to remove silyl groups from nucleosides and nucleotides.[18] In fact, the best way to afford **5** appeared to be the hydrogenation of compound **13** which quantitatively led to the deprotected amino prodrug (Scheme 4). So, the troc protecting groups were removed prior to coupling with the carbamoyle chloride **10**, and then hydrogenolysis with H_2 and Pd/C cleaved the benzyl ester with concomitant reduction of the nitro group to yield **5** quantitatively.

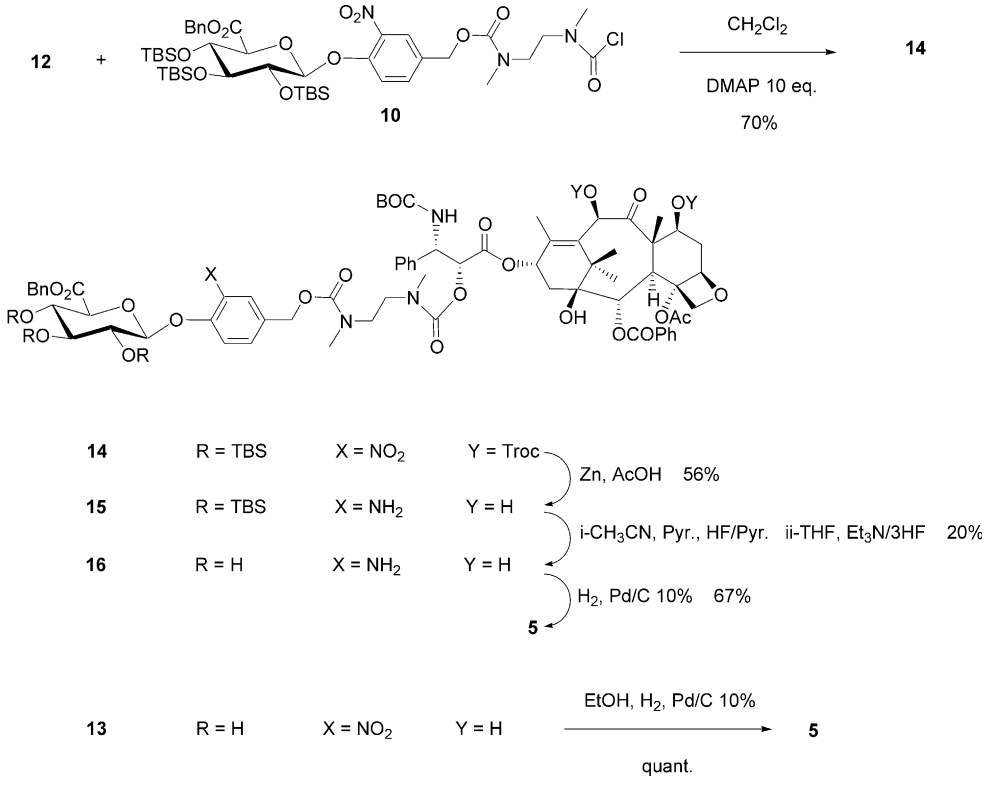
For HPLC comparison, spacers **6** and **7** were prepared as described in Scheme 5.

3. Biological results

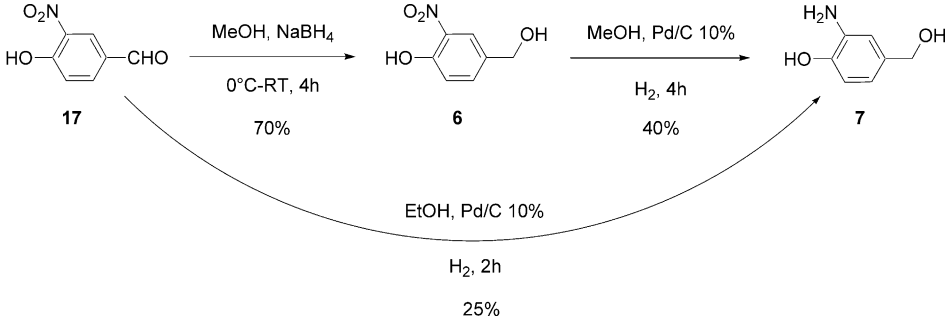
The key issues of these studies were to quantify the differences between the two docetaxel prodrugs (with a nitro- or an amino-substituted spacer) as well as between the previously obtained paclitaxel prodrug and the new docetaxel ones containing the same kind of spacer.

3.1. Stability

First of all, stabilities of the prodrugs **4** and **5** were measured by HPLC (UV detection) in a phosphate



Scheme 4. Synthesis of prodrug **5**.



Scheme 5. Synthesis of spacers **6** and **7**.

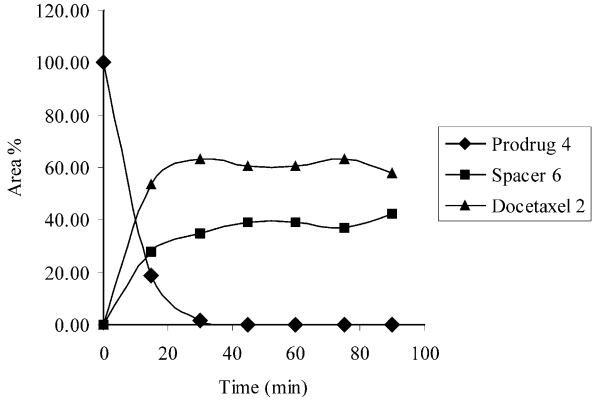


Figure 3. Enzymatic cleavage of prodrug **4**.

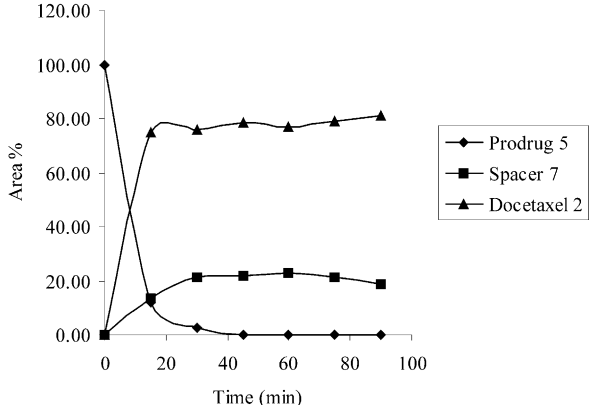


Figure 4. Enzymatic cleavage of prodrug **5**.

buffer at 37 °C (pH 7.2). Both prodrugs proved to be stable during a 24 h-run, and no docetaxel was liberated from the prodrugs during this time.

3.2. Cytotoxicity

Cytotoxities of the prodrug were also measured. For an ADEPT or a PMT strategy, the prodrug must be less cytotoxic than the corresponding drug.

The IC_{50} measured for L1210 cell lines were:

Prodrug **4**: 4.86 μM	Docetaxel **2**:	Spacer **6**: 75.3 μM
Prodrug **5**: 2.69 μM	14.4 nM	Spacer **7**: 45.8 μM

It is noteworthy that the spacers are much less cytotoxic than the drug and therefore cannot interfere. The ratios of IC_{50} between prodrug and drug are 337 for **4** and 187 for **5**, which correspond to a good detoxification. These values are compatible with an ADEPT or a PMT strategy.

3.3. Kinetics of drug release

The in vitro kinetics studies are very important because they first validate the approach if the prodrug is cleaved and then are used to select the best prodrugs for in vivo experiments. Prodrugs **4** and **5** were incubated with *Escherichia coli* β-D-glucuronidase at 37 °C and the solutions were analysed by HPLC (UV detection). The enzymatic hydrolyses of both prodrugs were very similar, showing the appearance of two peaks characterized as docetaxel and spacer **6** for **4** and spacer **7** for **5** by comparison with the synthesized samples (Figs 3 and 4). The peak corresponding to the prodrug decreased until its complete disappearance and both spacer and docetaxel reached a plateau. The observed half-lives of prodrug disappearance (Fig. 5) were approximatically 10 min for both prodrugs, which was almost the same as the one previously reported **3**[13] for the same concentration of prodrug (29.6 μmol), and of enzyme (12 units/mL).

The absence of major differences between the kinetics for **4** and **5** could be explained by the fact that the immolative step involving two different substituted phenols is not rate-determining.

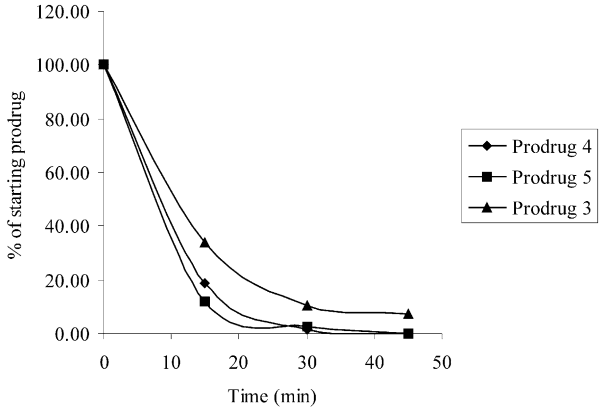
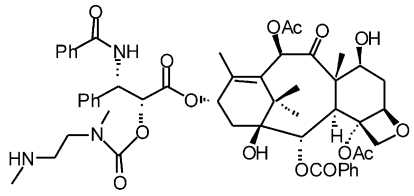


Figure 5. Comparison of the disappearance of the three prodrugs.



18

Figure 6. Structure of intermediate **18**.

The striking difference between **3** and **4** or **5** was that, for the two latter examples, no intermediate compound such as docetaxel linked to the diamine tether could be detected, whereas for **3**, compound **18** (Fig. 6) was detected and characterized by LC-MS.[13] This again stressed the divergent reactivities between paclitaxel and docetaxel already observed in the hydrogenolysis step (see above). It seems that this ethylenediamine-docetaxel intermediate cyclised too fast to be detected and only docetaxel was seen.

4. Conclusion

We have reported the synthesis of the first docetaxel prodrugs to date aimed at enzyme activation in ADEPT or PMT strategies. These prodrugs differ only by the substitution of the aromatic ring. Indeed, a nitro group

on the aromatic ring of the spacer could be preserved during the hydrogenolysis step, a reaction that could not have been achieved during deprotection of the already reported paclitaxel prodrug **3**.[13] Therefore nitro- and amino-spacer containing prodrugs of docetaxel were obtained and compared. Their in vivo evaluation showed a relevant detoxification and good kinetics of enzymatic hydrolysis.

The double self-immolative spacer used was found to be as effective with docetaxel as with paclitaxel, with only a slight difference for the spacer release between docetaxel prodrugs containing the nitro and the amino spacer. These are encouraging results in the field and will be further investigated.

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 ppm and *J* in Hz. 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 with 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. The chromatographies were conducted over silica gel [Merck 60 (230–400 Mesh)].

For the NMR descriptions, the numbers correspond to the baccatin core, the ′ to the taxoid side chain and the ″ to the glucuronic- spacer moiety.

5.1. In vitro cytotoxicity

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

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

5.2. 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 53% phosphate buffer (0.02 M, pH 3) and 47% acetonitrile with UV detection at 226 nm (extracted from PDA 3-D spectra).

5.3. Stability of compounds in a buffer solution

A solution of 29.6 μmol of prodrug in 0.02 M phosphate buffer (pH 7.2) was incubated at 37 °C. Aliquots (50 μL) were taken at various times and analysed by HPLC after dilution with eluent (150 μL).

5.4. Enzymatic cleavage by *E. coli* β-D-glucuronidase

A solution of 29.6 μmol of prodrug in 0.02 M phosphate buffer (pH 7.2) was incubated at 37 °C in the presence of 12 units/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).

5.4.1. 2′-*O*-[*N*,*N*′-Dimethyl-*N*′-benzyl-[4(−2,3,4-tri-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl uronate-3-nitrobenzyloxycarbonyl]ethylenediamin]docetaxel (9). 200 mg (0.248 mmol) of docetaxel **2** and a large excess of DMAP (300 mg, 2.45 mmol) were dissolved in 20 mL of freshly distilled CH_2Cl_2 and placed under argon atmosphere. Then 284 mg (1.1 equiv) of the carbamoyl chloride **10**[13] were added, followed by dropwise addition of 0.2 mL of TEA. After 6 h, the mixture was diluted with 20 mL of CH_2Cl_2, washed with water and brine, and the compound was purified by column chromatography (eluent CH_2Cl_2–MeOH 97.5/2.5→95/5) to give the coupling product **9** 267 mg (73%) as a gum. ^{1}H NMR ($CDCl_3$) δ 8.15 (m, 2H, H_o Bz$_2$), 7.85 (m, 1H, H_a), 7.48 (m, 4H, H_m Bz, H_p Bz, H_b), 7.30 (m, 10H, H_{aro}), 7.13 (d, *J* = 8.7 Hz, 1H, H_c), 6.99 (d, *J* = 9.5 Hz, 1H, NH), 6.32 (m, 1H, H_{13}), 5.65 (m, 1H, $H_{3'}$), 5.58 (d, *J* = 5.6 Hz, 1H, $H_{1''}$), 5.48–5.24 (m, 3H, $H_{2,\ 3,\ 10}$), 5.13 (m, 2H, CH_2 Bn), 5.09 (m, 2H, CH_2O), 4.98 (m, 1H, H_5), 4.50 (m, 1H, $H_{3''}$), 4.40 (s, 1H, $H_{4''}$), 4.29–4.06 (m, 3H, H_7, H_{20}), 4.17 (d, *J* = 3.6 Hz, 1H, $H_{2''}$), 4.00 (m, 1H, $H_{2'}$), 3.89 (d, *J* = 2.2 Hz, 1H, $H_{5''}$), 3.62–3.02 (m, 4H, CH_2N), 2.92–2.81 (m, 6H, CH_3N), 2.52 (m, 4H, $H_{6\alpha}$, C$\underline{H}_3$ Ac$_4$), 2.43–2.15 (m, 2H, H_{14}), 1.96 (s, 3H, H_{18}), 1.84 (m, 1H, $H_{6\beta}$), 1.73 (m, 3H, H_{19}), 1.28 (m, 9H, Me BOC), 1.21 (s, 3H, H_{16}), 1.10 (s, 3H, H_{17}), 0.89, 0.87, 0.83 (s, 9H) (C$\underline{H}_3$)$_3$CSiMe$_2$), 0.16, 0.11, 0.11, 010, 0.05, 0.01 (s, 3H) (CH_3Si); ^{13}C NMR ($CDCl_3$) δ 211.5 (C_9), 169.8, 168.9, 168.3, 167.0 (Me$\underline{C}$(O)C$_4$, ϕ$\underline{C}O_2$, $C_{1'}$, $\underline{C}O_2$Bn), 155.9, 154.9, 149.9 (N$\underline{C}O_2$), 140.1, 139.3, 138.0, 135.4, 135.1, 129.3 (Cq_{aro}, C_{11}, C_{12}); 133.9, 133.5, 130.2, 128.7, 128.5, 128.3, 127.7, 126.3 (CH_{aro}), 125.5 (C_a), 116.8 (C_c), 99.3 ($C_{1''}$), 84.4 (C_5), 81.0 (C_4), 79.5 (Me$_3\underline{C}$), 78.9 (C_1), 78.8 ($C_{3''}$), 76.6 (C_{20}), 77.8, 75.8, 75.5 ($C_{2/10}$, $C_{2''}$ and $C_{5''}$), 75.2 ($C_{3'}$), 74.5 ($C_{2/10}$), 72.2 ($C_{4''}$), 71.7 (C_{13}), 71.5 (C_7), 67.0 (CH_2 Bn), 65.7 (CH_2O), 57.5 (C_8), 54.2 (C_3), 50.6, 47.4, 47.0, 46.2 (CH_2N), 46.5 ($C_{2'}$), 43.1 (C_{15}), 36.8 (C_6), 35.6, 35.5 (CH_3N), 28.2, 28.2 (Me BOC), 26.3 (C_{16}), 25.8 (2C), 25.7 ((C$\underline{H}_3$)$_3$CSiMe$_2$), 22.7 (C$\underline{H}_3$ Ac$_4$), 22.6 (C_{14}), 21.0 (C_{17}), 18.0 (2C), 17.9 ((CH$_3$)$_3\underline{C}$SiMe$_2$), 14.3 (C_{18}), 10.0 (C_{19}), −4.5, −4.6, −4.7, −4.8, −5.0, −5.1 (CH_3Si); *m*/*z* (FAB$^+$ MB + NaI) 1747.8 M + Na$^+$, 1748.8 M + Na + H$^+$; HRMS: ($C_{87}H_{124}O_{26}N_4Si_3$Na) *m*/*z* calcd: 1747.7709, found: 1747.7657.

5.4.2. 2′-*O*-[*N*,*N*′-Dimethyl-*N*-benzyl-4-(β-D-glucopyranosiduronate)-3-nitrobenzyloxycarbonylethylenediamin]-docetaxel (13). 267 mg (0.155 mmol) of the silylated

compound **9** were dissolved in 10 mL of anhydrous CH_3CN with 0.5 mL of anhydrous pyridine. The reaction mixture was placed under argon and cooled to 0 °C in an ice bath. 1.5 mL of 70% HF–pyridine complex were then added dropwise, and the mixture allowed to reach rt overnight. The solution was cooled again and quenched with 50 mL of satd $NaHCO_3$. After extraction with 2×50 mL of EtOAc and drying over Na_2SO_4, the residue was purified on a silica gel column (eluent CH_2Cl_2–MeOH 95/5→9/1) to afford 185 mg (87%) of the desilylated compound **13** as a sticky foam, together with 10 mg of the starting material and 50 mg of a monosilylated compound. ^1H NMR ($CDCl_3$) δ 8.07 (s, 2H, H_o Bz_2), 7.79 (m, 1H, H_a), 7.56 (m, 1H, H_p Bz), 7.48 (m, 3H, H_m Bz, and H_b), 7.32 (m, 11H, H_{aro}), 6.63 (m, 1H, NH), 6.19 (m, 1H, H_{13}), 5.60 (s, 1H, $H_{3'}$), 5.50–4.94 (m, 5H, $H_{2,\ 3,\ 5,\ 10,\ 1''}$), 5.17 (m, 4H, CH_2 Bn, CH_2O), 4.46 (s, 1H, H_7), 4.26 (s, 1H, $H_{3''}$), 4.14 (s, 1H, $H_{4''}$), 4.26 and 4.14 (s, 1H, H_{20}), 3.87 (m, 2H, $H_{2',\ 5''}$), 3.76 (m, 4H, $H_{2''}$, OH $_{2',\ 3'',\ 4''}$), 3.33–3.00 (m, 4H, CH_2N), 2.91–2.76 (m, 6H, CH_3N), 2.67 (m, 1H, $H_{6\alpha}$), 2.60–2.20 (m, 2H, H_{14}), 2.44 (s 3H, C$\underline{H}_3$ Ac_4), 1.88 (s, 3H, H_{18}), 1.86 (m, 1H, $H_{6\beta}$), 1.28 (s, 9H, Me BOC), 1.14 (s, 3H, H_{16}), 1.05 (s, 3H, H_{17}); ^{13}C NMR ($CDCl_3$) δ 211.5 (C_9), 170.1, 169.0, 168.4, 167.0 (Me$\underline{C}$(O)C_4, ϕ$\underline{C}O_2$, $C_{1'}$, $\underline{C}O_2$Bn), 155.7, 155.2, 149.8 (NCO_2), 140.2, 139.4, 137.8, 135.4, 135.1, 131.9, 129.4 (Cq_{aro}, C_{11}, C_{12}), 133.6, 130.2, 128.8, 128.6, 128.4, 128.1, 126.5 (CH_{aro}), 124.9 (C_a), 118.4 (C_c), 101.7 ($C_{1''}$), 84.4 (C_5), 81.0 (C_1), 80.3 ($Me_3\underline{C}$), 78.9 (C_4), 76.5 (C_{20}), 75.2, 75.0, 74.5, 72.7, 71.7, 70.8 ($C_{2,7,10,13,2'',3'',4'',5''}$), 67.4 ($CH_2$ Bn), 65.7 (CH_2O), 57.5 (C_8), 54.5 (C_3), 46.5 ($C_{2'}$), 47.7, 46.3 (CH_2N), 43.1 (C_{15}), 36.6 (C_6), 35.4 (CH_3N), 34.5 (C_{14}), 28.3, 28.2 (Me BOC), 26.4 (C_{16}), 22.6 ($\underline{C}H_3Ac_4$), 21.0 (C_{17}), 14.3 (C_{18}), 10.1 (C_{19}); *m/z* (FAB^+ MB + NaI) 1405.8 M + Na^+; HRMS: ($C_{69}H_{82}O_{26}N_4Na$) *m/z* calcd: 1405.5115, found: 1405.5145.

5.4.3. 2′-*O*-[*N*,*N*′-Dimethyl-*N*-4-(β-D-glucopyranosiduronate)-3-nitrobenzyloxycarbonylethylenediamin]docetaxel 4. 64 mg (46 μmol) of **13** in solution in 4 mL of EtOH in the presence of 10% Pd/C (60 mg) was heated at 40–50 °C, then 50 μL of cyclohexa 1,4-diene (10 equiv) were added to the mixture. After 2 h, 50 μL of diene were added again and the reaction was continued overnight at rt. A third equivalent of diene was added and, after an additional 24 h stirring, the mixture was filtered on Celite 545. The crude product was then purified by chromatography on silica gel (eluent CH_3CN–H_2O 9/1) to give 43 mg (53%) of the solid nitro prodrug **4**. Mp 160 °C (dec.) ^1H NMR ($CDCl_3$) δ 8.12 (d, J = 6.4 Hz, 2H, H_o Bz), 7.83 (m, 1H, H_a), 7.63–7.39 (m, 9H, $H_{p,\ m}$ Bz, H_{aro}, H_b), 7.24 (1H, H_c), 6.09 (m, 1H, H_{13}), 5.63 (d, J = 6.4 Hz, 1H, $H_{3'}$), 5.46 (br s, 1H, NH), 5.29 (s, 2H, $H_{3,\ 10}$), 5.16–5.00 (m, 5H, $H_{2,\ 5,\ 1''}$, CH_2O), 4.19 (s, 3H, H_7, H_{20}), 3.82 (m, 2H, $H_{2',\ 5''}$), 3.75–3.50 (m, 8H, $H_{2'',\ 3'',\ 4''}$, CH_2N), 3.02–2.78 (m, 6H, CH_3N), 2.41 (m, 4H, $H_{6\alpha}$, C$\underline{H}_3$ Ac_4), 2.60–2.20 (m, 2H, H_{14}), 1.93 (s, 3H, H_{18}), 1.82 (m, 1H, $H_{6\beta}$), 1.68 (s, 3H, H_{19}), 1.38 (s, 9H, Me BOC), 1.14 (s, 3H, H_{16}), 1.12 (s, 3H, H_{17}); ^{13}C NMR ($CDCl_3$) δ 211.4 (C_9), 171.5, 171.0, 167.7 (Me$\underline{C}$(O)C_4, ϕ$\underline{C}O_2$, $C_{1'}$), 157.9, 157.1, 151.2 (NCO_2), 141.7, 140.0, 139.8, 139.0, 137.7, 130.0 (Cq_{aro}, C_{11}, C_{12}), 134.7 (C_b), 132.7, 131.4, 131.2, 129.8, 129.6, 128.2 (CH_{aro}), 123.1 (C_a), 117.5 (C_c), 102.3 ($C_{1''}$), 82.2, 80.8, 79.2 ($C_{1,\ 4}$, $Me_3\underline{C}$), 77.5, 77.2, 76.6, 76.5, 75.6, 74.5, 73.4, 73.1 ($C_{2,7,10,20,3',2'',3'',4'',5''}$), 66.9 ($CH_2O$), 58.8 ($C_8$), 56.4 ($C_3$), 49.6, 49.3, 49.0 ($CH_2N$), 47.7 ($C_{2'}$), 44.4 ($C_{15}$), 37.5 ($C_6$), 36.6 ($CH_3N$), 28.8 (Me BOC), 28.0 ($C_{16}$), 23.3 ($\underline{C}H_3Ac_4$), 21.9 ($C_{17}$), 20.5 ($C_{14}$), 14.8 ($C_{18}$), 10.5 ($C_{19}$); *m/z* ($ESI^-$) 1291.5 M−$H^-$; HRMS: ($C_{62}H_{76}O_{26}N_4$) *m/z* calcd: 1291.4670, found: 1291.4608.

5.4.4. 2′-*O*-[*N*,*N*′-Dimethyl-*N*′-benzyl-[4(−2,3,4-tri-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl uronate-3-nitrobenzyloxycarbonyl]ethylenediamin]-7,10-ditroc-docetaxel (14). To 100 mg (97 μmol) of ditroc-docetaxel **12** and 174 mg of DMAP (1.42 mmol) in 15 mL of CH_2Cl_2 were added 131 mg (1.3 eq.) of the carbamoyl chloride **10**[13] dissolved into 1.5 mL of CH_2Cl_2. After 2 h, the mixture was diluted with CH_2Cl_2, washed with water and brine, and dried over Na_2SO_4. After chromatography (eluent CH_2Cl_2–MeOH 98/2), 187 mg (93%) of the coupling product **14** were isolated as a gum. ^1H NMR ($CDCl_3$) δ 8.11 (m, 2H, H_o Bz_2), 7.86 (m, 1H, H_a), 7.60 (m, 1H, H_p Bz), 7.52 (m, 3H, H_m Bz, H_b), 7.31 (m, 10H, H_{aro}), 7.13 (d, J = 8.7 Hz, 1H_c), 7.04 (d, J = 10.6 Hz, 1H, NH), 6.34 (m, 1H, H_{13}), 6.26 (s, 1H, H_{10}), 5.70 (s, 1H, H_2), 5.58 (m, 2H, $H_{5,\ 1''}$), 5.36 (m, 2H, CH_2O), 5.23 (m, 1H, $H_{3'}$), 5.13 (s, 2H, CH_2 Bn), 5.00 (m, 2H, $H_{7,\ 2'}$), 4.89 (m, 1H), 4.76 (m, 2H, CH_2CCl_3), 4.59 (m, 1H) (CH_2CCl_3), 4.50 (s, 1H, $H_{3''}$), 4.40 (s, 1H, $H_{4''}$), 4.33 and 4.18 (m, 1H, H_{20}), 4.05 (d, J = 5.6 Hz, 1H, $H_{2''}$), 3.94 (m, 1H, H_3), 3.84 (d, J = 3.6 Hz, 1H, $H_{5''}$), 3.75–3.00 (m, 4H, CH_2N), 2.88 (m, 6H, CH_3N), 2.67 (m, 1H, $H_{6\alpha}$), 2.62 (s, 3H, C$\underline{H}_3Ac_4$), 2.43 (m, 2H, H_{14}), 2.09 (s, 3H, H_{18}), 2.05 (m, 1H, $H_{6\beta}$), 1.85 (s, 3H, H_{19}), 1.34 (s, 3H, H_{16}), 1.27 (s, 9H, Me BOC), 1.18 (s, 3H, H_{17}), 0.90 (m, 9H), 0.87, 0.83 (s, 9H) (($C\underline{H}_3)_3$CSi), 0.16, 0.05, 0.00 (s, 3H), 0.11 (s, 9H) (CH_3Si); ^{13}C NMR ($CDCl_3$) δ 201.1 (C_9), 170.1, 169.1, 168.4, 167.0 (Me$\underline{C}$(O)C_4, ϕ$\underline{C}O_2$, $C_{1'}$, $\underline{C}O_2$Bn), 156.0, 154.8, 153.3, 153.1, 150.0 (NCO_2), 143.8, 140.2, 138.0, 135.2, 129.1 (Cq_{aro}, C_{11}, C_{12}), 133.9 (C_b), 131.4, 130.3, 128.8, 128.6, 128.4, 127.8, 126.4, 125.6 (CH_{aro}), 125.1 (C_a), 116.9 (C_c), 99.4 ($C_{1''}$), 94.2 (CCl_3), 83.8 (C_5), 80.7 (C_4), 80.3 ($Me_3\underline{C}$), 79.6 (C_{10}), 79.3 ($C_{3''/4''}$), 75.8 (CH_2O), 75.2 ($C_{3'}$), 74.4 (C_2), 72.2 ($C_{3''/4''}$), 71.4 (C_4), 67.1 (CH_2 Bn), 65.8 ($C_{2'}$), 76.8 ($\underline{C}H_2CCl_3$), 76.5 ($C_{5''}$), 75.2 (C_{20}), 74.4 ($C_{2''}$), 56.1 (C_3), 50.9 (C_8), 46.9 (m, CH_2N), 43.2 (C_{15}), 35.6 (m, CH_3N), 33.3 (C_6), 28.3, 28.2 (Me BOC), 26.3 (C_{16}), 25.9 (2C), 25.8 (($\underline{C}H_3)_3$CSi), 22.7 ($\underline{C}H_3$ Ac_4), 22.6 (C_{14}), 21.3 (C_{17}), 18.0 (2C), 17.9 (($CH_3)_3\underline{C}$Si), 14.7 (C_{18}), 10.8 (C_{19}), −4.5, −4.6, −4.6, −4.7−4.9, −5.0 (CH_3Si); *m/z* (FAB^+ NBA + NaI) calcd: 2097.8 M ($^{35}Cl_5{}^{37}Cl$) + Na^+ (88%), 2098.8 M ($^{35}Cl_5{}^{37}Cl$) + Na + H^+ (88%), 2099.8 M ($^{35}Cl_4{}^{37}Cl_2$) + Na^+ (100%); HRMS: ($C_{139}H_{126}O_{30}N_4{}^{35}Cl_6Si_3Na$) *m/z* calcd: 2095.5794, found: 2095.5823 (32%), ($C_{139}H_{126}O_{30}N_4{}^{35}Cl_5{}^{37}ClSi_3Na$) *m/z* calcd: 2097.5764, found: 2097.5806 (77%), ($C_{139}H_{126}O_{30}N_4{}^{35}Cl_4{}^{37}Cl_2Si_3Na$) *m/z* calcd: 2099.5837, found: 2099.5734 (100%), ($C_{139}H_{126}O_{30}N_4{}^{35}Cl_3{}^{37}Cl_3Si_3Na$) *m/z* calcd: 2101.5702; found: 2101.5823 (67%).

5.4.5. 2′-*O*-[*N*,*N*′-Dimethyl-*N*′-benzyl-[4(−2,3,4-tri-*O*-*tert*-butyldimethylsilyl-β-D-glucopyranosyl uronate-3-aminobenzyloxycarbonyl]ethylenediamin]docetaxel (15). Compound **14** (187 mg, 0.09 mmol) was dissolved in 20 mL

of EtOAc under vigorous stirring in the presence of a large excess of zinc (ca. 250 mg) and 2 mL of AcOH. After heating at 45 °C for 4 h, the reaction medium was filtered, then quenched with water and extracted with 2×30 mL of EtOAc. The organic layers were washed with water and brine, and dried over Na_2SO_4. Flash chromatography (eluent:EtOAc) gave 85 mg (56%) of the amino compound **15** as an oil. ^{1}H NMR ($CDCl_3$) δ 8.11 (m, 2H, H_o Bz_2), 7.52 (m, 1H, H_p Bz_2), 7.48 (m, 2H, H_m Bz), 7.31 (m, 11H, H_{aro}, NH), 6.82–6.70 (m, 3H, $H_{a,\ b,\ c}$), 6.30 (m, 2H, H_{13}), 5.67 (m, 2H, $H_{2,\ 10}$), 5.47 (m, 1H, $H_{1''}$), 5.24 (m, 1H, $H_{3'}$), 5.12 (s, 2H, CH_2 Bn), 4.98 (m, 1H, H_5), 4.80 (m, 2H, CH_2O), 4.44 (m, 1H, $H_{3''}$), 4.29–4.13 (m, 5H, $H_{7,\ 20,\ 2',\ 4''}$), 3.96 (m, 2H, $H_{3,\ 2''}$), 3.84 (m, 1H, $H_{5''}$), 3.60–3.00 (m, 4H, CH_2N), 2.88–2.80 (m, 6H, CH_3N), 2.58 (m, 1H, $H_{6\alpha}$), 2.54 (s, 3H, C$\underline{H}_3$Ac$_4$), 2.44 (m, 2H, H_{14}), 1.97 (s, 3H, H_{18}), 1.85 (m, 1H, $H_{6\beta}$), 1.74 (s, 3H, H_{19}), 1.31 (s, 3H, H_{16}), 1.25 (s, 9H, Me BOC), 1.11 (s, 3H, H_{17}), 0.88 (s, 18H), 0.82 (s, 9H) (($C\underline{H}_3)_3$CSi), 0.15, 0.11 (s, 3H), 0.09 and 0.05 (s, 6H) (CH_3Si); ^{13}C NMR ($CDCl_3$) δ 211.6 (C_9), 171.2, 169.9, 168.8, 167.1 (Me$\underline{C}$(O)C_4, ϕ$\underline{C}O_2$, $C_{1'}$ and $\underline{C}O_2$Bn), 156.4, 155.9, 155.2, 154.9 (NCO_2), 144.5, 139.4, 138.5, 138.2, 135.3, 131.5, 129.4 (Cq_{aro}, $C_{11,}$ C_{12}), 133.5, 130.2, 128.7, 128.5, 128.3, 128.3, 127.6, 126.4 (CH_{aro}), 118.4, 116.0, 115.9 ($C_{a,\ b,\ c}$), 100.3 ($C_{1''}$), 84.4 (C_5), 81.0 (C_4), 80.0 (C_1), 79.2 ($C_{3''}$), 76.6 (C_{20}), 76.5, 75.4 ($C_{2'',\ 4''}$), 76.1 (C_{10}), 74.9 (C_2), 74.5 ($C_{3'}$), 72.5 ($C_{7,\ 2'}$), 71.7 (C_{13}), 67.9 (CH_2Bn), 67.4 ($C_{5''}$), 66.9 (CH_2O), 57.6 (C_8), 47.7, 46.4 (CH_2N), 43.2 (C_{15}), 46.5 (C_3), 43.1 (C_{15}), 35.5 (CH_3N), 29.7 (C_6), 28.2 (Me BOC), 25.9 (2C), 25.8 (($C\underline{H}_3)_3$CSi), 25.6 (C_{14}), 22.7 ($\underline{C}H_3$ Ac_4), 21.0 (C_{17}), 18.0, 18.0, 17.9 (($CH_3)_3\underline{C}$Si), 14.3 (C_{18}), 11.5 (C_{19}), −4.1, −4.6, −4.6, −4.7, −4.8, −5.0 (CH_3Si); *m*/*z* (FAB$^+$ NBA + NaI) 1718.5 M + Na + H$^+$, 1717.5 M + Na$^+$.

5.4.6. 2′-*O*-[*N*,*N*′-Dimethyl-*N*-benzyl-4-(β-D-glucopyranosiduronate)-3-aminobenzyloxycarbonylethylenediamin]-docetaxel (16). To 110 mg (0.80 mmol) of the amine **15** dissolved in 5 mL of anhydrous CH_3CN and 0.5 mL of pyridine, cooled to 0 °C, 1 mL of the HF–Pyridine complex was added dropwise. The mixture was allowed to reach rt and stirring was pursued for 48 h. The reaction was not completed, as compounds of intermediary polarity which showed partial desilylation, were isolated and characterized by mass spectrography. The mixture of compounds was taken up, dissolved in 5 mL of THF dried over 4 Å molecular sieves, then 0.1 mL (1.5 equiv) of NEt_3/3HF were added. After 4 h, NEt_3 was added and the medium was concentrated and purified by chromatography (eluent CH_2Cl_2–MeOH 95/5→9/1) affording 22 mg (20%) of the desilylated compound **16** as an oil. As the problems encountered during the reaction and the purification of the final product did not allow full characterization of **16**, only the spectroscopic mass analysis is indicated; *m*/*z* (FAB$^+$ MB) 1353.9 M + H$^+$.

5.4.7. 2′-*O*-[*N*,*N*′-Dimethyl-*N*-4-(β-D-glucopyranosiduronate)-3-aminobenzyloxycarbonylethylenediamin]docetaxel (5). Method 1: 18 mg (13 μmol) of the benzyl compound **16** were dissolved in 5 mL of pure ethanol in the presence of 60 mg of Pd/C 10%, then stirred for 24 h under hydrogen atmosphere (1 bar). After filtration on Celite 545 and concentration of the filtrate, the compound was purified by chromatography (eluent CH_3CN–H_2O 9/1) to give 11 mg (67%) of the prodrug **5**.

Method 2: 35 mg (25 μmol) of the nitrobenzyl **13** were suspended in 5 mL of pure EtOH with 10% Pd/C (60 mg), then stirred for a further 4 h under hydrogen atmosphere (1 bar). After filtration on Celite 545 and concentration of the filtrate, the compound was purified by chromatography (eluent CH_3CN–H_2O 9/1), giving the solid prodrug **5** in quantitative yield (32 mg).

The obtained quantities of **5** were gathered together and lyophilised. Mp 202 °C (dec.) ^{1}H NMR ($CDCl_3$) δ 8.12 (m, 2H, H_o Bz_2), 7.64 (m, 1H, H_p Bz_2), 7.57 (m, 2H, H_m Bz), 7.38 (m, 4H, H_{aro}), 7.10 (m, 1H, H_{aro}), 6.99–6.64 (m, 3H, $H_{a,\ b,\ c}$), 6.09 (m, 2H, H_{13}), 5.64 (m, 1H, H_2), 5.44 (m, 2H, $H_{10,\ 3'}$), 5.12 (m, 1H, H_5), 5.01 (m, 2H, CH_2O), 4.65 (m, 1H, $H_{1''}$), 4.19 (m, 3H, $H_{7,\ 20}$), 3.88 (m, 2H, H_3), 3.72 (m, 1H, $H_{2'}$), 3.50 (m, 4H, $H_{2'',\ 3'',\ 4'',\ 5''}$), 3.39–3.01 (m, 4H, CH_2N), 2.86 (m, 6H, CH_3N), 2.44 (m, 1H, $H_{6\alpha}$), 2.40 (s, 3H, C$\underline{H}_3$ Ac_4), 2.18 (m, 1H, $H_{14\alpha}$), 2.05 (m, 1H, $H_{14\beta}$), 1.93 (s, 3H, H_{18}), 1.86 (m, 1H, $H_{6\beta}$), 1.68 (s, 3H, H_{19}), 1.39 (s, 9H, Me BOC), 1.15 (s, 3H, H_{16}), 1.12 (s, 3H, H_{17}); ^{13}C NMR ($CDCl_3$) δ 211.3 (C_9), 171.5, 170.3, 167.8 (Me$\underline{C}$(O)C_4, ϕ$\underline{C}O_2$, $C_{1'}$), 157.8 (m, NCO_2), 146.8, 145.0, 139.9, 139.6, 137.7, 135.0 (Cq_{aro}, C_{11}, C_{12}); 134.5, 131.4, 131.2, 129.9, 129.7, 129.3, 128.3, 127.9 (CH_{aro}), 118.8, 118.2, 113.9 ($C_{a,\ b,\ c}$), 104.6 ($C_{1''}$), 86.0 (C_5), 82.2 (C_4), 80.8 (C_1), 79.2 (C_{20}), 77.6, 77.5, 74.7, 73.4 ($C_{2'',\ 3'',\ 4'',\ 5''}$), 76.5 ($C_2$), 75.6 ($C_{10}$), 73.0 ($C_{13}$), 72.6 ($C_7$), 68. 9 ($C_{2'}$), 68.4 ($CH_2O$), 58.8 ($C_8$), 56.4 ($C_{3'}$), 47.9 ($C_3$), 44.5 ($C_{15}$), 46.5 ($C_3$), 43.1 ($C_{15}$), 39.7 (m, CH_2N), 37.5 (C_6), 35.3 (CH_3N), 28.7 (Me BOC), 23.3 ($\underline{C}H_3$ Ac_4), 22.1 (C_{14}), 21.7 (C_{17}), 14.8 (C_{18}), 10.5 (C_{19}); HRMS (ESI$^+$): ($C_{139}H_{126}O_{30}N_4$) *m*/*z* calcd: 1263.5096; found: 1263.5084.

5.4.8. 2-Nitro-4-hydroxymethylphenol (6). A solution of 4-hydroxy-3-nitro-benzaldehyde (200 mg, 1.19 mmol) in methanol (20 mL) was cooled to 0 °C; then $NaBH_4$ (100 mg) was added. After 4 h at rt, the mixture was quenched with 50 mL water and the solvent was evaporated. After extraction with 3×50 mL EtOAc and drying over Na_2SO_4, the residue was purified by chromatography (eluent CH_2Cl_2–MeOH 97/3), 141 mg (70%) 6 were isolated as yellow needles. Mp 142–144 °C ^{1}H NMR ($CDCl_3$) δ 10.56 (sl, 1H, OH), 8.11 (d, 1H, J = 2.2 Hz, H_3), 7.60 (dd, 1H, J = 8.6 and 2.2 Hz, H_5), 7.16 (1H, d, J = 8.6 Hz, H_6), 4.69 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$): 154.5(C_q–O), 136.3 (C_5), 135.6 and 133.2 (C_q), 123.0 (C_3), 120.2 (C_6), 63.6 (CH_2); *m*/*z* [CI + NH_3] 170 [M + H]$^+$; HRMS ($C_7H_{10}NO_2$) *m*/*z* calcd: 170.0453, found: 170.0459.

5.4.9. 2-Amino-4-hydroxymethylphenol (7). Method 1: A solution of 4-hydroxy-3-nitro-benzaldehyde **17** (205 mg, 1.23 mmol) in methanol (20 mL) was hydrogenated during 2 h in the presence of 10% Pd/C (206 mg). After filtration over Celite 545 and chromatography (eluent CH_2Cl_2–MeOH/95-5), 60 mg (25%) 7 were isolated as a brown powder.

Method 2: A solution of nitrophenol **6** (141 mg, 0.833 mmol) in methanol (20 mL) was hydrogenated during 4 h in the presence of 10% Pd/C (96 mg). After filtration over Celite 545 and chromatography (eluent CH_2Cl_2–MeOH/95-5), 47 mg (40%) **7** were isolated.

Mp 142–144 °C ^{1}H NMR (CD_3OD) δ 6.75 (d, 1H, $J = 1.7$ Hz, H_3), 6.66 (dd, 1H, $J = 8.0$ Hz, H_6), 6.58 (d, 1H, J 8.0 Hz and $J = 1.7$ Hz, H_s), 4.42 (s, 2H, CH_2); ^{13}C NMR (CD_3OD) δ 145.6 ($C_{quaternary}$-O), 135.6 and 133.8 ($C_{quaternary}$), 119.2 (C_5), 116.5 (C_3), 115.1 (C_6), 61.3 (CH_2); m/z [CI + NH_3] 140 $[M+H]^+$; HRMS ($C_7H_{10}O_2N$) m/z calcd: 140.0712, found: 140.0710.

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