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Prodrug Mono Therapy: Synthesis and Biological Evaluation of an Etoposide Glucuronide-Prodrug

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Abstract—A glucuronide-based prodrug of etoposide has been synthesized for a Prodrug Mono Therapy strategy. The aim is to selectively liberate the active compound by β-D-glucuronidase already present in necrotic tumours. Outside from these sites, this enzyme is known to be localised inside the lysosomes. The three components of this prodrug are the glucuronic acid (substrate of the enzyme), the spacer (for a faster cleavage), and the active etoposide. In vitro, the prodrug was shown to be less cytotoxic and more water-soluble than etoposide itself. Finally, in the presence of the β-D-glucuronidase, cleavage of the prodrug with complete release of the drug has been observed.

Introduction

Lack of selectivity is a major limitation in cancer chemotherapy. Most drugs are not selective enough to be used in optimal doses without severe drawbacks. A solution is to target the active drugs to the cancer cells. Many trials using for instance antibody-drug have been conducted but without real success[1] until the recently approved mylotarg.[2] Indeed, a number of problems have to be solved such as the stability of the conjugate and its tumour penetration, internalisation and anti-tumour activity.[3]

Prodrugs can be used in order to release the active drug from an inactive precursor. In this particular case, selectivity can be managed by an enzymatic cleavage. The enzyme can be targeted in a first step at the tumour site by a Mab-antigen recognition, like in an Antibody Directed Enzyme Prodrug Therapy (ADEPT) strategy.[4] The second step will be the injection of the prodrug, which results in the liberation of the drug.

A more convenient strategy is the use of an already present enzyme.[5] The prerequisite is to identify an enzyme specifically localized around the tumour cells. Indeed, a lysosomal enzyme, β-glucuronidase, is liberated extracellularly in necrotic tumours and remains active.[6–8] It was unambiguously shown, by enzyme histochemistry, that necrotic areas in human cancers are the site in which lysosomal β-glucuronidase was liberated extracellularly in high local concentration. According to investigations, extracellular β-glucuronidase originates from monocytes and granulocytes concentrated within the necrotic areas. Based on these facts, a new therapeutic procedure called Prodrug Mono Therapy (PMT) was proposed.[6–8] Further investigations showed, in the case of a human perfused lung, that a doxorubicin prodrug (HMR1826) could release the free doxorubicin due to the presence of enzymatically active 'extralysosomal' β-glucuronidase.[9] The level of doxorubicin after lung perfusion with HMR1826 was about 7-fold higher than after perfusion with doxorubicin itself.

All these results led us to consider this strategy as available to increase the delivery of oncostatic drugs in human tumours.[10]

Etoposide **1**, or VP-16, is a semi-synthetic derivative of podophyllotoxin,[11,12] which exerts its antitumour activity by stabilization of the ternary complex involving the drug, DNA and Topoisomerase II.[13–15] Established indications of etoposide are testicular and small-cell lung cancer,[16–18] as well as the use in paediatrics for the treatment of neuroblasma. Cancer leukaemia, and Kaposi's sarcoma represent other clinical indications.

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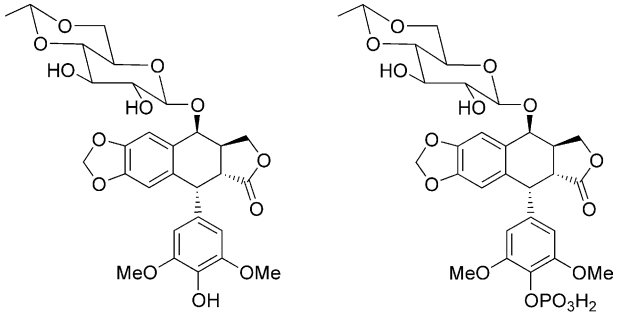
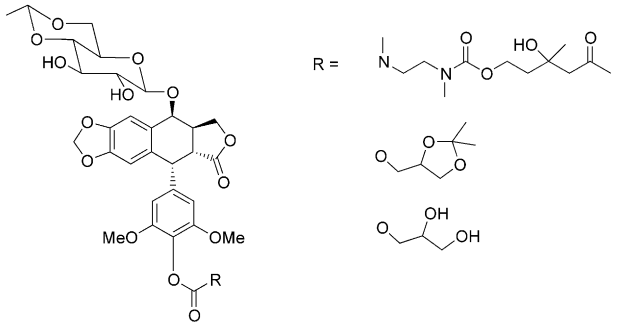
In spite of this widespread clinical use, among limitations is the very poor water-solubility of the drug. Formulation with Tween® 80, polyethylene glycol and ethanol results in acute mortality. To solve this problem, in the 1990′s, the group of Bristol-Myers Squibb initiated a program to discover an appropriate prodrug. This led to the development of etoposide phosphate **2**, BMY-404811.[19–21] Etopophos is rapidly converted to the parent drug in vivo and, therefore, has been introduced in clinics with the same profile as etoposide itself. On this account, although esterification of the phenol function significantly decreases, not only the activity but also the toxicity, both are almost restored by enzymatic cleavage. This indicates that there was no marked gain in selectivity with this type of prodrug.



Etoposide **1** Etopophos **2**

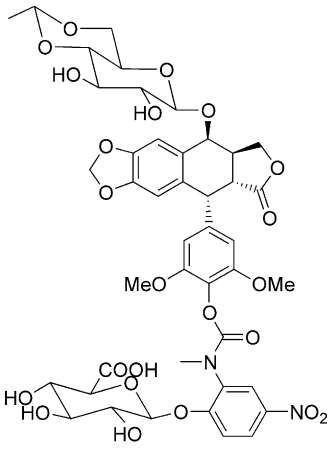
Structures of etoposide and etopophos

For the other two types of prodrugs of etoposide including a carbamate or carbonate spacer at 4′ recently described,[22,23] the release of the drug was induced by a retro-aldol/retro-Michael reaction catalysed by an aldolase antibody 38C2 and by carboxylesterases, respectively.



Structures of etoposide prodrugs

In this paper following our patent,[24] we report the synthesis of a prodrug of etoposide **3**. Our aim was to obtain a water-soluble compound and a selective liberation of the drug **1** at the necrotic tumours due to the presence of active β-glucuronidase within these areas.



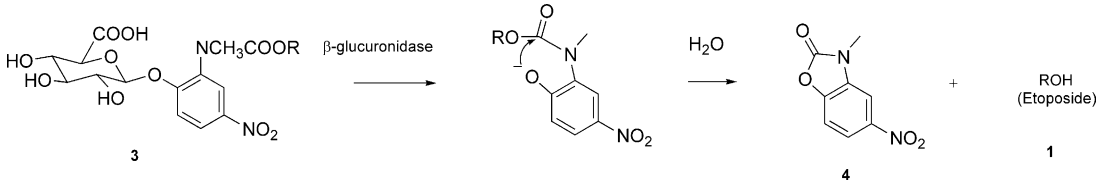
Structure of prodrug **3**

Synthesis

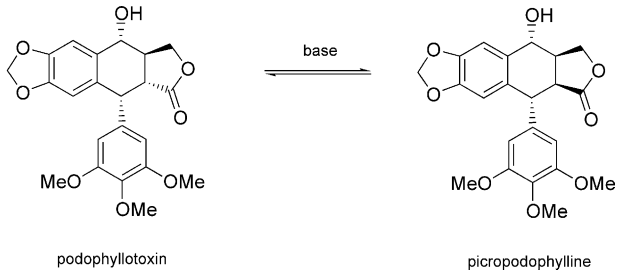
In the prodrug **3**, etoposide was connected to the β-D-glucuronic moiety by a self-immolative spacer. The role of the spacer is to facilitate the enzymatic cleavage of the prodrug as it was shown that, in some cases, direct connection gave a bad enzymatic hydrolysis turnover. We chose a spacer already used in the laboratory for other drugs, with success for the kinetics results.[25–27] The mechanism of drug release was planned as in Scheme 1.

For the synthesis of prodrug **3**, we could not use methyl esters and acetate as protecting groups.[25,26] For the removal of the ester groups from the β-D-glucuronic moiety, the basic conditions are not compatible with the etoposide structure. Even under slightly basic conditions, podophyllotoxin derivatives are known to epimerise and give picropodophyllins which are unfortunately inactive (Scheme 2).[28,29]

The main problems of the synthesis were the protecting groups and the selectivity of fixation between the different hydroxyl groups present in the etoposide structure. Our first choice was silylated groups for both hydroxyl and carboxyl groups of the glucuronic acid. The alcohols were transformed into *tert*-butyldimethylsilyl ethers, and the acid into a trimethylsilylethanol ester.[30,31] The last deprotection step was conducted with fluoride anion. As we could not use the basic TBAF, we tried HF/Pyr or HF/NEt_3. Both reagents permit deprotection of



Scheme 1. Release of etoposide from prodrug **3**.



Scheme 2. Epimerisation of podophyllotoxin.

the alcohols but we did not succeed in removing the silylated ester. For this deprotection, only TBAF was described.[30,31]

Finally, we obtained the prodrug **3** following Scheme 3.

The hydroxyl protecting groups were kept, but the acid was protected as a benzyl ester. The intermediate **5**[26] was deprotected and then reprotected as TBDMS ethers for the hydroxyl groups and as a benzyl ester for the carboxylic acid. The amine function of **7** was activated with phosgene and then coupled to etoposide **1** at the phenolic hydroxyl group. Under the controlled conditions used (overstœchiometric DMAP and one equivalent carbamoyle chloride), the alcohol groups of the etoposide glucose moiety did not react and only the 4′-OH phenol group was coupled. Deprotection of the glucuronide moiety was then achieved to convert **9** into prodrug **3**. The TBDMS groups were removed with HF/Pyridine giving **10** and the benzyl ester with cyclohexadiene over palladium.[32,33] These conditions enabled to keep the aromatic nitro function, contrary to classical hydrogenation conditions which are known to reduce the nitro function very well. The overall yield starting from etoposide was 15%.

Biological Activity

Solubility

In water and under comparable conditions, prodrug **3** is approximately 200-fold more soluble than the corresponding drug. Thus, while the solubility of etoposide is about 0.1 mg/mL, the solubility of prodrug **3** is about 20 mg/mL.

Cytotoxicity

On L1210 cell line, prodrug **3** gave an IC_{50} value of 50.2 μM. After hydrolysis by β-glucuronidase (100 μg/mL), increased cytotoxicity was obtained with an IC_{50} value of 0.93 μM, which was very closely related to the IC_{50} value of the etoposide itself (0.834 μM). This means that the prodrug was detoxified by a factor of about 50.

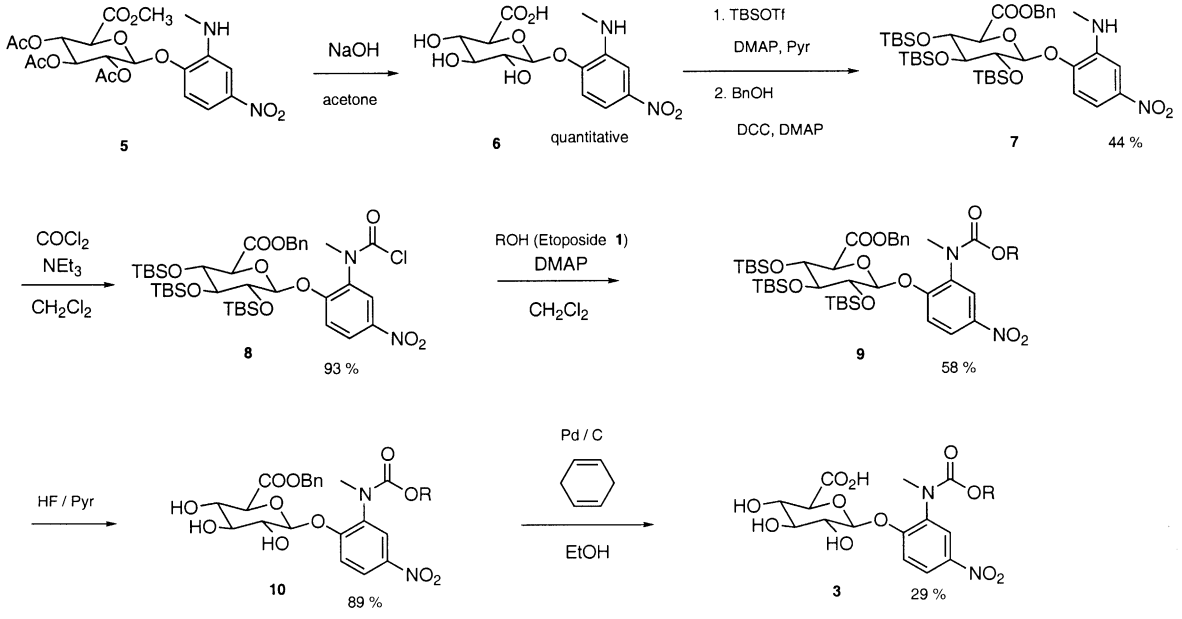
Stability

The stability of **3** was followed by HPLC measurements in buffer solution at pH 7.2 for 24 h. We could not detect any evolution. So, in the absence of β-D-glucuronidase, prodrug **3** is stable in vitro under these conditions.

Kinetics of drug release

Prodrug **3** (500 μg/mL, 0.521 mM) was incubated with *Escherichia coli* β-D-glucuronidase (20 μg/mL, 26.2 units/mL). Aliquot samples were analysed by HPLC at different times (Fig. 1).

Examination of the curves indicated that the prodrug was rapidly hydrolysed, the only products detected being the etoposide **1** and the cyclised spacer **4**. No intermediate containing the spacer still attached to the etoposide was seen. This is consistent with a fast enzymatic cleavage (half-life of **3** is <25 min) and a fast cleavage of the spacer.



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

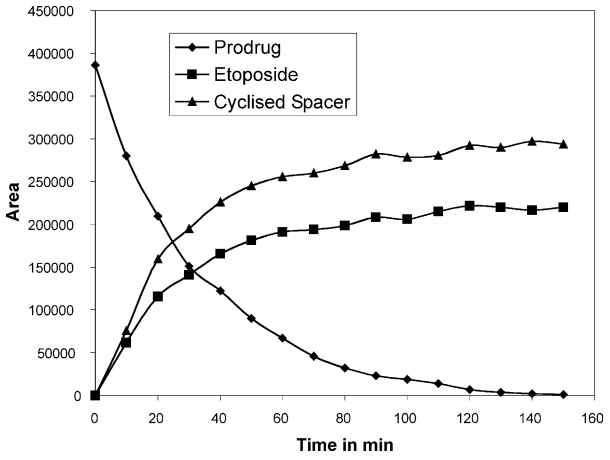


Figure 1. Enzymatic cleavage of prodrug **3**.

It is also assumed that the liberated compound is indeed etoposide and not picroetoposide, which was synthesised following a described procedure.[34] HPLC examination by comparison of the retention times was in agreement with the fact that, during all the synthesis, the *trans*-fused lactone was not epimerised into *cis*-fused lactone.

As concentration of the enzyme in necrotic area is dependent on the nature and localisation of the tumour, it is difficult to predict whether the concentration needed for the cleavage of etoposide prodrug will be reached in vivo. Moreover, it must be taken into consideration that human β-D-glucuronidase is less efficient than *E. coli* β-D-glucuronidase at physiological pH.[35] Nevertheless, it has been established that doxorubicin was liberated efficiently from the prodrug HMR1826 in a human perfused lung.[9]

Conclusions

The prodrug **3** as described has all the in vitro requirements for use in a PMT strategy in cancer chemotherapy. Less cytotoxic than the free drug, this prodrug is stable at pH 7.2 and releases etoposide by the action of β-D-glucuronidase. In vivo experiments are planned to evaluate more precisely the enzymatic cleavage inside necrotic tumours.

Experimental

Melting points (mp) were taken on a Koffler Bench and are uncorrected. Optical rotations were obtained on a Perkin-Elmer 241 polarimeter (589 nm). Specific rotations ($[\alpha]_D$) are reported in deg/dm, and the concentration (c) is given in g/100 mL in the specific solvent. Infrared spectra were recorded on a Perkin-Elmer 1600 FTIR spectrometer (ν in cm^{-1}). ^1H 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, positive ion mode) or FAB (positive ion mode) 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. Chromatographies were conducted over silica gel [Merck 60 (230–400 mesh)].

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 g/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.

For the cell cycle analysis, L1210 cells (5×10^5 cells/mL) 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 RNAse and 50 μg/mL propidium iodide for 30 min at 20 °C. For each sample, 10,000 cells were analysed on a XLMCL flow cytometer (Beckman Coulter, France).

HPLC conditions

Good separation in a short delay was obtained with a reversed-phase phenyl analytical column (Spherisorb 250×4.6) using isocratic conditions (1 mL/min) of 60% phosphate buffer (0.02 M, pH 3) and 40% acetonitrile with UV detection at 254 nm. Using these conditions, the retention times of **1**, **3**, **4** were respectively 4.9, 3.4, and 5.8 min.

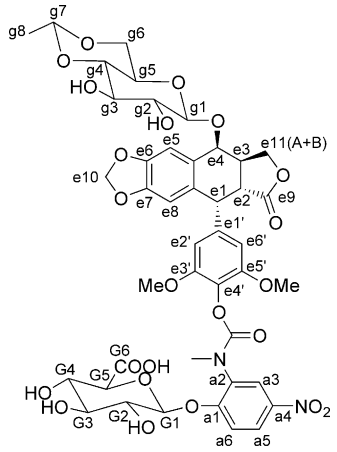
Stability of compounds in a buffer solution

A solution of 500 μL of prodrug in 0.02 M phosphate buffer (pH 7.2) was incubated for various times at 37 °C. Aliquots (100 μL) were taken at various times and analysed by HPLC after dilution with eluent (300 μL).

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

Hydrolysis was investigated by incubating a solution of 500 mg/mL (0.521 mM) of prodrug **3** and 20 μg/mL (26.2 units/mL) of *E. coli* β-D-glucuronidase in 0.02 M phosphate buffer (pH 7.2) at 37 °C. Aliquots (100 μL) were taken at various times and analysed by HPLC after dilution with 300 μL of eluent.

For the NMR description, the following numeration was chosen: 'a' for aromatic, 'e' for Etoposide, 'g' for glucose, 'G' for glucuronic acid.



NMR numeration of prodrug **3**

For the NMR attribution, some publications were very helpful.[36–39]

2-Methylamino-4-nitrophenyl-β-D-glucopyranosiduronic acid (6). To a solution of **5**[26] (2 g, 4.13 mmol) in 50 mL acetone at 0 °C, a 1 N NaOH aqueous solution (50 mL) was added dropwise. After stirring for 5 min at 0 °C, the mixture was neutralized with 1 N HCl at pH 4, evaporated and purified by column chromatography (CH_3CN/H_2O: 80/20). The solid was heated in boiling methanol and filtered to eliminate silica. After evaporation, a bright orange solid (100%) was obtained. $C_{13}H_{16}N_2O_9$; mp 172 °C; $[\alpha]_D$ −53 (*c* 0.96 in MeOH); ν_{max}/cm^{-1} (KBr) 3400 (O–H), 1588 (aromatics), 1530, 1343 (NO_2); δ_H (DMSO) 7.46 (1H, dd, $J_m = 3$, $J_o = 9$, a5), 7.19 (1H, d, $J_m = 3$, a6), 7.12 (1H, d, $J_o = 9$, a3), 6.04 (1H, q, $J = 5$, N-H), 5.68 (1H, br, s, OH), 5.13 (1H, br, s, OH), 4.85 (1H, d, $J = 7$, G1), 3.57–3.17 (4H, G2, G3, G4, G5), 2.80 (3H, d, $J = 5$, N–CH_3); δ_C (DMSO) 172.4 (G6), 149.8 (a1), 143.0 (a2), 140.5 (a4), 113.3 (a5), 111.6 (a6), 102.3 (a3), 101.4 (G1), 75.6, 74.1, 73.0, 72.1 (G2, G3, G4, G5), 29.4 (NMe); *m/z* (ES^-) 343 $[M-H]^-$.

2-Methylamino-4-nitrophenyl-2,3,4-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranosiduronic acid. DMAP (0.1 g) was added to a solution of **6** (1.87 g, 5.43 mmol) in 20 mL pyridine. The mixture was cooled to 0 °C and TBS triflate (12 mL, 52.3 mmol) were added dropwise. After 48 h at room temperature, the mixture was evaporated and the residue was taken in toluene (200 mL). The insoluble pyridinium triflate was filtered, and the filtrate evaporated. The product, obtained as a yellow resin (3.68 g), was used crude in the next step. Any attempt of purification by chromatography resulted in loss of the compound.

Benzyl [2-methylamino-4-nitrophenyl-2,3,4-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranosid]uronate (7). DMAP (0.3 g, 2.45 mmol) was added to the preceding crude compound (3.64 g, 5.31 mmol) in 20 mL CH_2Cl_2. After cooling to 0 °C, benzyl alcohol (0.5 mL, 4.9 mmol) and DCC (1.095 g, 5.31 mmol) were successively added. After 12 h at room temperature, the mixture was evaporated and taken in cyclohexane (250 mL). The insoluble urea was filtered. The filtrate was evaporated and purified by two successive chromatographies, the first with CH_2Cl_2, and the second with CH_2Cl_2/cyclohexane: 5/1. Compound **7** was obtained as a yellow resin (1.83 g, 44% from **6**). $C_{38}H_{64}NO_9Si_3$; $[\alpha]_D$ −2.3 (*c* 0.98 in $CHCl_3$); ν_{max}/cm^{-1}($CDCl_3$) 1762 (C=O ester), 1623 (aromatics), 1530, 1343 (NO_2); δ_H ($CDCl_3$) 7.53 (1H, dd, $J_o = 9$, $J_m = 3$, a5), 7.35 (1H, d, $J_m = 3$, a3), 7.33–7.26 (5H, Ph), 6.83 (1H, d, $J_o = 9$, a6), 5.62 (1H, d, $J = 6$, G1), 5.11 (s, 2H, CH_2Ph), 4.59 (1H, q, $J = 5$, NH), 4.52 (1H, G3), 4.38 (1H, G4), 4.02 (1H, t, $J = 6$, G2), 3.87 (1H, d, $J = 3.5$, G5), 2.86 (3H, d, $J = 5$, N–CH_3), 0.91 (18H, Si–C–CH_3), 0.86 (9H, Si–C–CH_3), 0.15 (3H, Si–CH_3), 0.14 (3H, Si–CH_3), 0.12 (6H, Si–CH_3), 0.08 (3H, Si–CH_3), −0.01 (3H, Si–CH_3); δ_C ($CDCl_3$) 168.4 (G6), 148.8 (a1), 143.7 (a2), 140.5 (a4), 135.1 (Ph quaternary), 128.5–128.4–128.3 (Ph tertiary), 112.5 (a5), 112.0 (a6), 104.1 (a3), 98.9 (G1), 78.9 (G3), 77.2 (G5), 75.7 (G2), 72.1 (G4), 67.0 ($\underline{C}H_2Ph$), 29.4 (NMe), 25.7 (Si–C–$\underline{C}H_3$), 18.0–17.9 (Si–$\underline{C}$–CH_3)−4.5–4.6–4.7–5 (Si–$\underline{C}H_3$); *m/z* (CI) 777 $[M+H]^+$.

Benzyl [2-(*N*-chloroformyl-*N*-methylamino)-4-nitrophenyl-2,3,4-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranosid]uronate (8). To a solution of **7** (350 mg, 0.45 mmol) in 20 mL CH_2Cl_2 at 0 °C, a solution of phosgene (700 μL, 1.35 mmol) in toluene was added. Then triethylamine (1.13 mL, 8.16 mmol) was added dropwise. After 30 min at 0 °C, the reaction was quenched with 10 mL water. The organic phase was separated and washed with 10 mL brine, dried over magnesium sulphate and evaporated. The residue was purified by chromatography (EtOAc/cyclohexane: 1/13) to afford a colourless viscous oil (352 mg, 93%). $C_{39}H_{63}N_2O_{10}ClSi_3$; $[\alpha]_D$ −5 (*c* 1 in $CHCl_3$); ν_{max}/cm^{-1} ($CDCl_3$), 1734 (C=O carbamoyle chloride), 1594 (aromatics), 1528, 1349 (NO_2); δ_H ($CDCl_3$) 8.24 (1H, dd, $J_o = 9$, $J_m = 3$, a5), 8.14 (1H, d, $J_m = 3$, a3), 7.34–7.28 (5H, Ph), 7.22 (1H, d, $J_o = 9$, a6), 5.73 (1H, d, $J = 5.5$, G1), 5.07–5.05 (2H, 2 s, CH_2Ph), 4.43 (1H, G3), 4.40 (1H, G4), 3.96 (1H, G2), 3.88 (1H, d, $J = 3$, G5), 3.25 (3H, s, N–CH_3), 0.95–0.86 (27H, Si–C–CH_3), 0.08 (12H, Si–CH_3), 0.07 (3H, Si–CH_3), 0.02 (3H, Si–CH_3); δ_C ($CDCl_3$) 168.0 (G6), 157.5 (NCOCl), 148.6 (a1), 142.1 (a2), 134.7 (a4), 133.1 (Ph quaternary), 128.3–128.1–125.7 (Ph tertiary), 125.5 (a5), 115.9 (a6), 99.3 (a3), 99.2 (G1), 78.6 (G3), 76.4 (G5), 75.5 (G2), 71.6 (G4), 66.9 ($\underline{C}H_2Ph$), 45.5–44.2 (NMe), 25.6–25.5 (Si–C–$\underline{C}H_3$), 17.7–13.5–12.6 (Si–$\underline{C}$–CH_3) −4.6–4.8–4.9–5.2 (Si–$\underline{C}H_3$); *m/z* (CI) 856 $[M+NH_4]^+$.

Benzyl [4-nitrophenyl-2-[(etoposide-4′-*O*-carbonyl)methylamino]-2,3,4-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-glucopyranosid]uronate (9). DMAP (124.5 mg, 1.035 mmol) was added to a solution of **8** (0.51 g, 0.609 mmol) and etoposide (287 mg, 0.487 mmol) in CH_2Cl_2 (107 mL). Triethylamine (0.14 mL, 1.035 mmol) was added dropwise, and the mixture was stirred for 16 h at room temperature. After evaporation, the residue was purified by chromatography (CH_2Cl_2/CH_3CN: 8/2). A white solid (0.39 g, 57%) was isolated. $C_{68}H_{94}N_2O_{23}Si_3$; mp 171 °C; $[\alpha]_D$ +1.3 (*c* 0.99 in $CHCl_3$); ν_{max}/cm^{-1} ($CDCl_3$) 1770 (CO ester), 1729 (CO carbamate), 1601 (aromatics), 1525, 1348 (NO_2); δ_H ($CDCl_3$) 8.67 (d, $J_m = 2$, 1H, a3), 8.10 (dd, $J_o = 9$, $J_m = 2$, 1H, a5), 7.30 (5H, Ph 7.04 (d,

$J_m = 2$, 1H, a6), 6.84 (s, 1H, e5), 6.55 (s, 1H, e8), 6.38–6.22 (2H, e2′, e6′), 6.03 (d, $J = 1$, 1H, e10A), 6.02 (s, $J = 1$, 1H, e10B), 5.77 (d, $J = 6$, 1H, G1), 5.16 (AB, $J = 12$, 1H, CH_2(A)Ph), 5.09 (AB, $J = 12$, 1H, CH_2(B)Ph), 4.92 (d, $J = 3$, 1H, g4), 4.77 (q, $J = 5$, 1H, g7), 4.68 (d, $J = 8$, 1H, g1), 4.59 (d, $J = 5.5$, 1H, e1), 4.52 (br, 1H, G3), 4.41 (br, 2H, e11A, G4), 4.22–4.15 (2H, g6equ, e11B), 4.05 (d, $J = 6$, 1H, G2), 3.91 (d, $J = 3.5$, 1H, G5), 3.82–3.68 (7H, g3, OCH_3), 3.59 (t, $J = 10$, 1H, g6ax), 3.44 (t, $J = 8$, 1H, g2), 3.36 (1H, g5), 3.27 (5H, e2, e4,N–CH_3), 2.86 (m, 1H, e3), 1.39 (d, $J = 5$, 3H, g8), 0.96 (9H, Si–C–CH_3), 0.91 (9H, Si–C–CH_3), 0.89 (9H, Si–C–CH_3), 0.21– (−0.02) (18H, Si–CH_3); δ_C ($CDCl_3$) 174.9 (e9), 168.4 (G6), 156.2, 153.5, 153.2, 152.1, 148.9, 147.4, 142.0, 137.6, 135.2, 132.7, 132.5 (C quaternary, carbamate) 128.6, 128.5, 128.3 (Ph), 126.6 (a3), 123.6 (a5), 114.3 (a6), 110.9 (e8), 109.1 (e5), 106.8 (e2′, e6′), 102.1 (e10), 101.7 (g1), 99.9 (g7), 98.1 (G1), 79.8 (g5), 79.0 (G3), 77.0 (G5), 76.8 (G2), 74.6 (g2), 73.9 (g4), 73.1 (g3), 72.4 (G4), 68.1 (g6), 67.9 (e11), 67.0 (CH_2Ph), 66.5 (e4), 56.0 (O-CH_3), 44.0 (e1), 41.3 (e2, e3), 37.5, 37.0 (N-CH_3), 26.6, 25.9, 25.8, 25.7 (Si–C–$\underline{C}H_3$), 20.3 (g8), 18.1, 18.0, 17.9 (Si–$\underline{C}$–CH_3,), −4.2, −4.$\overline{5}$, −4.6, −4.7, −4.9, −5.7 (Si–CH_3); m/z (FAB$^+$) 1413 $[M + Na]^+$.

Benzyl [4-nitrophenyl-2-[(etoposide-4′-*O*-carbonyl)methylamino]-β-D-glucopyranosid]uronate (10). To a solution of **9** (223.2 mg, 0.16 mmol) in pyridine (2.65 mL) at 0 °C, HF/pyridine (2.65 mL, 70%) was added dropwise. The mixture was stirred for 4 h at 0 °C, then 10 h at room temperature. After evaporation, the residue was taken in 200 mL CH_2Cl_2, and washed with water; the aqueous phase was extracted with CH_2Cl_2. The organic phases were dried over magnesium sulphate, and the compound was purified by chromatography (CH_3CN). The obtained product **10** was a beige solid (150 mg, 89%). $C_{50}H_{52}N_2O_{23}$; mp 170 °C; $[\alpha]_D$ −9.2 (*c* 1.1 in $CHCl_3$); ν_{max}/cm^{-1}($CDCl_3$) 3406 (O–H), 1752 (CO ester), 1713 (CO carbamate), 1602 (aromatics), 1525, 1346 (NO_2); δ_H ($CDCl_3$) 8.20 (br, 2H, a3, a5), 7.39 (br, 5H, Ph), 7.12 (d, $J = 9$, 1H, a6), 6.95 + 5.62 (2H, e2′, e6′), 6.65 (s, 1H, e5), 6.57 (s, 1H, e8), 6.07 (s, 2H, e10), 5.34 (AB, $J = 12$, 1H, CH_2(A)Ph), 5.27 (AB, $J = 12$, 1H, CH_2(B)Ph), 5.13 (d, $J = 6$, 1H, G1), 5.03 (d, $J = 2$, 1H, G5), 4.70 (2H, g7, e1), 4.46 (2H, e11), 4.38 (1H, g1), 4.23 (dd, $J = 10$, $J = 4$, 1H, g4), 4.15 (d, $J = 10$, 1H, G4), 3.91 (br, 1H, G3), 3.68 (G2), 3.66–3.52 (4H, e2, e4, g3, g6), 3.51 (s, 9H, OCH_3, NCH_3), 3.42 (1H, g2), 3.33 (1H, g5), 2.99 (1H, e3), 1.40 (d, $J = 5$, 1H, g8); δ_C ($CDCl_3$) 176.4 (e9), 167.5 (G6), 156.0, 152.9, 150.8, 148.0, 146.0, 141.5, 137.4, 134.0, 131.7, 131.3, 126.4, 125.5 (C quaternary, carbamate), 127.8, 127.7, 127.4 (Ph tertiary), 123.3, 122.1 (a3, a5), 113.5 (a6), 110.5 (e8), 109.0 (e5), 107.8 (e2′, e6′), 100.8 (e10, G1), 98.8 (g7), 96.4 (g1), 78.8 (g4), 73.9 (G4, g2), 72.9 (G2), 72.5 (e4), 71.2 (G3), 69.7 (G5), 67.4 (g3, g6), 67.0–66.7 (e11, CH_2Ph), 65.0 (g5), 55.3 (O-CH3), 43.2 (e1), 38.8–38.1 (e2, e3), 36.6 (N–CH_3), 19.4 (g8); m/z (FAB$^+$) 1071 $[M + Na]^+$.

[4 - Nitrophenyl - 2 - [(etoposide - 4′ - *O* - carbonyl)methylamino]-β-D-glucopyranosid]uronic acid (3). Palladium over charcoal (137 mg, 10%) and 1.4 cyclohexadiene (0.54 mL, 5.7 mmol) were added to a solution of **10** (63.6 mg, 0.06 mmol) in ethanol (1.8 mL). The mixture was stirred at 45 °C for 15 h. After filtration over Celite and evaporation, the crude product was purified by chromatography (CH_3CN/H_2O: 90/10). The compound **3** was isolated as a beige powder (17 mg, 29%). $C_{43}H_{46}N_2O_{23}$; mp 186 °C; $[\alpha]_D$ +6.5 (*c* 0.85 in MeOH); ν_{max}/cm^{-1} (KBr) 3426 (O–H), 1770 (CO ester), 1717 (CO carbamate), 1603 (aromatics), 1505, 1378 (NO_2); δ_H (DMSO) 8.40 (1H, a3), 8.17 (d, $J = 9$, 1H, a5), 7.46 (d, $J = 9$, 1H, a6), 7.02 (s, 1H, e5), 6.55 (s, 1H, e8), 6.28 (2H, e2′, e6′), 6.02 (s, 2H, e10), 5.29 (2H, OH), 5.18 (1H, G1), 4.95 (1H, OH), 4.72 (q, $J = 5$, 1H, g7), 4.58 (2H, g1, e1), 4.27 (1H, e11A), 4.08 (1H, e11B), 3.66 (s, 6H, OCH_3), 3.62–3.09 (14H, NCH_3, e4, g2, g3, g4, g5, g6, G2, G3, G4, G5), 3.07 (1H, e2), 2.91 (1H, e3), 1.24 (d, $J = 5$, 3H, g8); δ_C (DMSO) 175.2 (e9), 172.2 (G6), 158.2, 153.2, 151.8, 148.45, 147.0, 141.6, 139.1, 132.7, 129.6, 128.0 (C quaternary, carbamate), 126.0 (a3), 124.6 (a5), 116.9 (a6), 110.6 (e5), 110.4 (e8), 108.0 (e2′, e6′), 102.2 (g1), 102.0 (e10), 101.9 (G1), 99.9 (g7), 80.8 (g4), 75.0 (e2), 74.4–74.0 (G4, g2), 73.4 (G2), 72.5 (G3), 68.4 (e11), 68.0 (G5), 66.4 (g3, g5, g6, e4), 56.5 (OCH_3), 43.9 (e1), 41.0 (e3), 37.8 (N–CH_3), 21.0 (g8); m/z (ES$^+$) 981 $[M + Na]^+$, 997 $[M + K]^+$.

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