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Duocarmycin-based prodrugs for cancer prodrug monotherapy

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ABSTRACT

The synthesis and biological evaluation of novel prodrugs based on the cytotoxic antibiotic duocarmycin SA (**1**) for a selective treatment of cancer using a prodrug monotherapy (PMT) are described. Transformation of the phenol **8** with the glucuronic acid benzyl ester trichloroacetimidate **9b** followed by reaction with DMAI·HCl (**10**) gives the glucuronide **11b**, which is deprotected to afford the desired prodrug **4a** containing a glucuronic acid moiety. In addition, the prodrug **4b** with a glucuronic methyl ester unit is prepared. The cytotoxicity of the glucuronides is determined using a HTCFA-assay with IC_{50} values of 610 nM for **4a** and 3300 nM for **4b**. In the presence of β-glucuronidase, **4a** expresses an IC_{50} value of 0.9 nM and **4b** of 2.1 nM resulting in QIC_{50} values of about 700 for **4a** and 1600 for **4b**.

1. Introduction

One of the major problems of the commonly used chemotherapeutics in cancer therapy is their insufficient differentiation between normal and cancer cells, which is usually based on the different proliferation rates of cancer and normal cells. However, since several normal cells as intestinal, epithelial and bone marrow cells also show a fast proliferation rate as cancer cells, severe side effects are often encountered. Some strategies to overcome this lack of selectivity make use of non-toxic prodrugs that are selectively activated at the tumour-site by conjugates of monoclonal antibodies and enzymes, which liberate the toxin from the prodrug.

Another approach is based on conjugates of toxins and peptides, which bind to tumour-associated receptors. A third way rests upon enzymes, which are overexpressed in tumours, in combination with an enzymatically cleavable prodrug.

For the first approach, referred to as ADEPT strategy,[1] we have designed novel duocarmycin-based prodrugs as **2** over the last decade.[2] The natural antibiotic duocarmycin SA (**1**)[3] is a highly potent cytostatic compound with an IC_{50} value of about 10 pM against different cancer cell lines and thus one of the strongest anticancer agents known so far (Scheme 1). The newly developed prodrug **2**[4] contains a benzindole skeleton with a chloroethyl group and a dimethylaminoethoxyindole side chain (DMAI),[5] which is necessary for binding to the minor groove of the DNA as well as a galactose moiety for detoxification. After cleaving off the sugar moiety by β-D-galactosidase the in situ formed *seco*-drug **3** undergoes a fast cyclization to give the drug **5** with a spiro-methylcyclopropylcyclohexadienone moiety as pharmacophoric unit.[6] Since the prodrug **2** has an IC_{50} value of 3600 nM in the absence of the cleaving enzyme and of 0.75 nM in its presence it results in a QIC_{50} value of about 5000.[4] Thus, this substance is superior to almost all compounds described so far for the use of ADEPT. Moreover, the in vivo investigations using mice are highly promising.[7]

On the other hand, the ADEPT approach has the disadvantage of being rather costly and must cope with a possible immune response of the patients against the used monoclonal antibody and enzyme.

We therefore also investigated the second approach using the peptide pentagastrin for targeting[8] and the third approach employing glucuronic acid derivatives of the *seco*-drug **3**. Thus, it has been known for quite a long time that the concentration of the enzyme β-glucuronidase is elevated in the extra cellular space of solid necrotic tumours.[9] We as one of the first suggested to use this knowledge for the synthesis of non-toxic prodrugs selectively detoxified by a glucuronic acid moiety.[10] This strategy was later introduced by Bosslet et al.[11] as prodrug monotherapy (PMT). Moreover, this concept is aided by the fact that the activity of β-glucuronidase is very low at neutral pH[12] but elevated at a decreased pH as it is found in the tumour tissue.[13]

So far, several glucuronic acid prodrugs have been synthesized[14,15] but the resulting QIC_{50} values are rather low, and moreover the liberated drugs often show an insufficient cytotoxicity. We have proposed that in prodrug therapy the IC_{50} value of the liberated drug should be <10 nM.[1b,c] Larrick et al. have developed duocarmycin derivatives containing a glucuronic acid moiety

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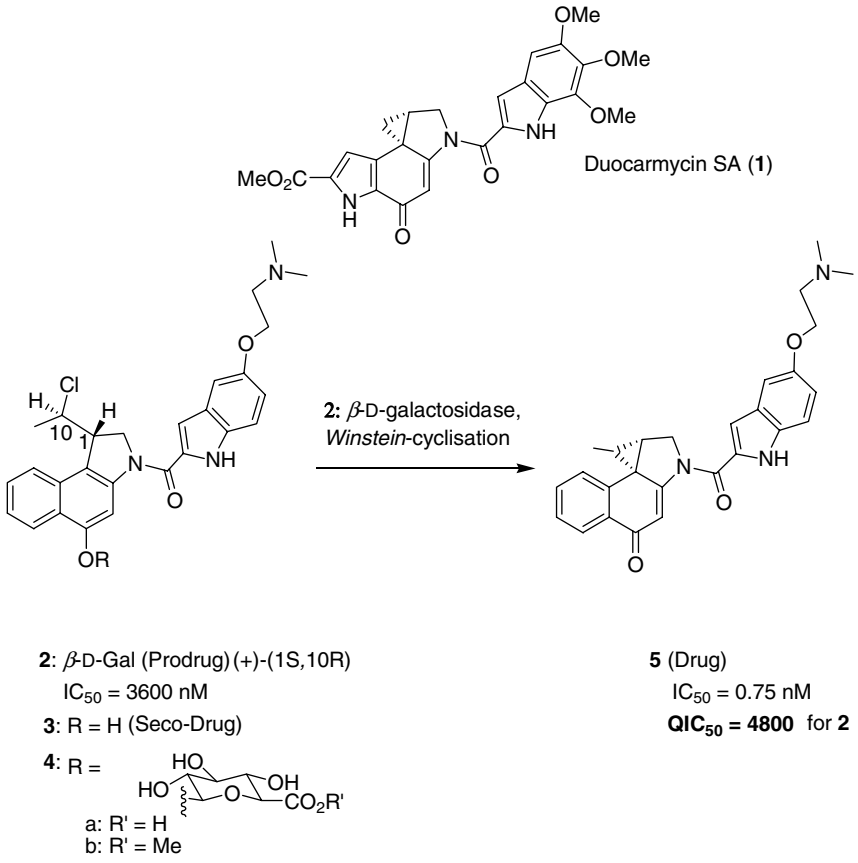


Scheme 1. Duocarmycin SA (**1**) and prodrugs.

which, however, cannot be considered as prodrugs since they easily form the spirocyclopropylcyclohexadiene moiety as the pharmacophoric group under physiological conditions. Hence, the biological investigation gave poor results.[16]

Here, we describe the novel glucuronic acid containing prodrug **4a**, which shows excellent results in HTCFA cell culture experiments using the human bronchial carcinoma cell line A549 (Scheme 1). In addition, we also prepared the corresponding glucuronic acid methyl ester derivative **4b**, which is not cleaved by β-glucuronidase, but could be transformed in situ into the corresponding cleavable acid derivative **4a** by ubiquitous carboxylesterases.[10,17]

2. Results and discussion

A main problem in the formation of the glucuronic acid derivative **4a** was the proper choice of the glucuronic acid donor. First attempts to prepare the corresponding glucoside followed by oxidation of the primary hydroxy group of the sugar moiety using the co-oxidant 2,2,6,6-tetramethylpiperidinyloxyl (TEMPO)[18] and [bis(acetoxy)iodoso]benzene (BAIB), iodosobenzene (PhI=O)/KBr[19] or polymer-supported chlorite (PS–O_2Cl) were not successful.[20]

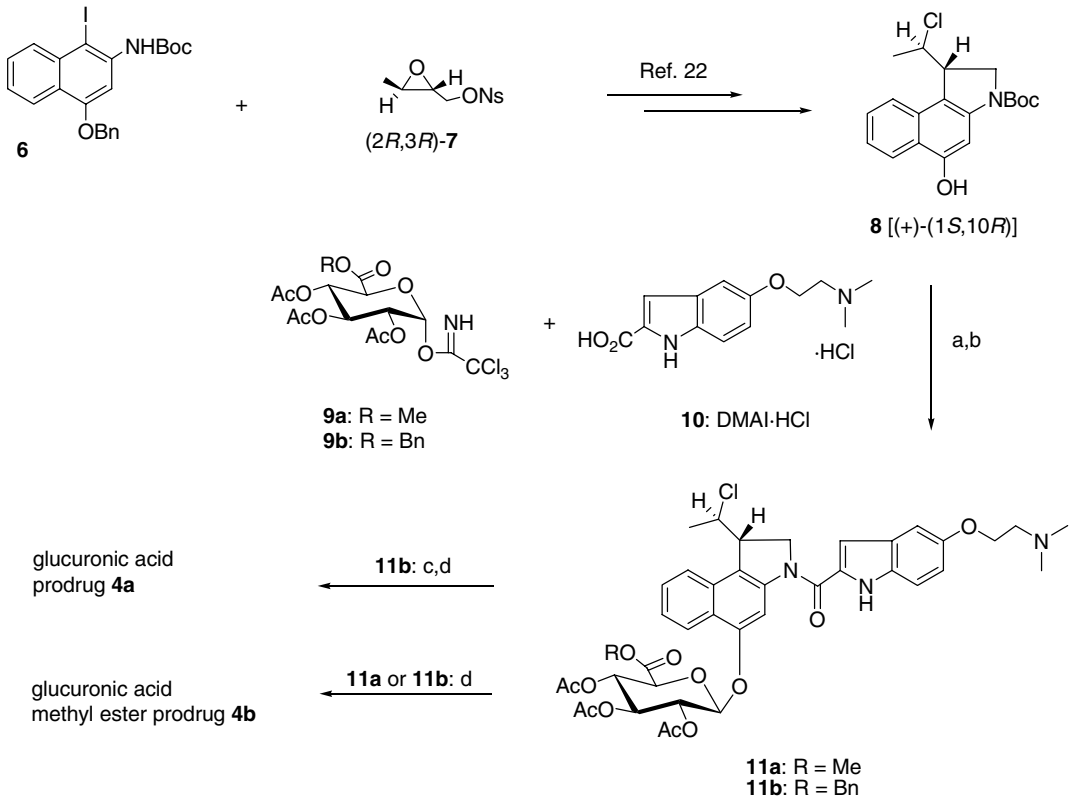
We then employed a route via the glucuronic acid methyl ester **11a**, but hydrolysis of the methyl ester moiety in **11a** could not be performed without severe side reactions due to the lability of the secondary chloride toward basic conditions. On the other hand, the glucuronic acid benzyl ester derivative **11b** could easily be obtained, which allowed the formation of the desired glucuronic acid prodrug **4a** by cleavage of the benzyl ester using a hydrogenolysis under neutral conditions. Furthermore, **11b** could also be employed for the synthesis of the glucuronic acid methyl ester **4b**.

The synthesis of the prodrug **4a** followed the general route shown in Scheme 2. We started from the known phenol (1*S*,10*R*)-**8**, which was obtained in a highly selective way from the known naphthaline derivative **6**[21] and the enantiopure epoxide (2*R*,3*R*)-**7**.[22] Reaction of the phenol (+)-(1*S*,10*R*)-**8** with the glucuronide trichloroacetimidate **9b**[23] in the presence of $BF_3{\cdot}Et_2O$ in dichloromethane and molecular sieves (4 Å) at −10 °C for 3 h gave the corresponding glucuronide which, however, was not isolated, but directly transformed into the desired amide **11b**. For this purpose, the primarily formed glucuronide was treated with additional three equivalents of $BF_3{\cdot}Et_2O$ to remove the *N*-*tert*-butyloxycarbonyl moiety and then reacted with the indole carboxylic acid DMAI·HCl (**10**) in the presence of EDC·HCl in *N*,*N*-dimethylformamide for 24 h to give **11b** in 43% overall yield.

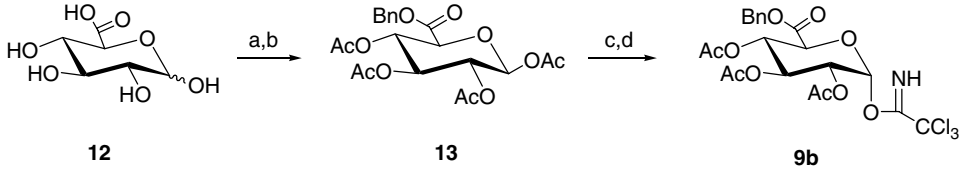
In a similar way the glucuronic acid methyl ester derivative **11a**[24] was prepared in 59% yield using the trichloroacetimidate **9a**. Under *Zemplén* deacetylation conditions using sodium methoxide in methanol,[25] **11a** and **11b** were transformed into the methyl ester prodrug **4b** in 79% and 90% yield, respectively. For the synthesis of the free glucuronic acid prodrug **4a** the benzyl ester **11b** was hydrogenated under a hydrogen atmosphere using palladium on charcoal. The final *Zemplén* deacetylation led to the desired acid prodrug **4a** in 60% yield over two steps, which was purified by chromatography on a reversed phase column (Kromasil 100 C18).

The methyl ester trichloroacetimidate **9a** was prepared using a known procedure starting from D-(+)-glucurono-3,6-lactone in 60% yield over four steps.[26]

The first three steps of the synthesis of the benzyl ester trichloroacetimidate **9b** had first been performed by Vasella et al. in 33% yield starting from the sodium salt of glucuronic acid.[23a] We developed a higher-yielding route to **9b** and its intermediate starting from D-glu-



Scheme 2. Synthesis of the glucuronic acid prodrugs **4a** and **4b**. Reagents and conditions: (a) for **11a**: **9a**, $BF_3{\cdot}Et_2O$, CH_2Cl_2, MS (4 Å), −18 to −10 °C, 3 h, then $BF_3{\cdot}Et_2O$, rt, 3 h; for **11b**: **9b**, $BF_3{\cdot}Et_2O$, CH_2Cl_2, MS (4 Å), −15 °C, 3.5 h, then $BF_3{\cdot}Et_2O$, rt, 4.5 h; (b) **10**, EDC·HCl, DMF, rt, 24 h, **11a**: 59%, **11b**: 43%; (c) Pd/C, H_2, EtOAc/ MeOH (1:10), rt, 12 h, 90%; (d) NaOMe, MeOH, rt, 60 min, then HOAc/MeOH; for **4a**: after RP-HPLC (Kromasil 100 C18), 60% over two steps; for **4b**: from **11a**: 79%, from **11b**: 90%.



Scheme 3. Novel route to the trichloroacetimidate **9b**. Reagents and conditions: (a) Ac_2O, I_2, rt, 2 h, 99%; (b) BnOH, CH_2Cl_2, rt, 16 h, 65%; (c) $NH_2NH_2{\cdot}HOAc$, DMF, rt, 2.5 h, 90% of **14**; (d) CCl_3CN, PS-DBU, CH_2Cl_2, rt, 1 h, 85%.

curonic acid (**12**) itself (Scheme 3). In the first step, a mixed anhydride was formed with simultaneous peracetylation of the hydroxy groups using acetic acid anhydride in the presence of catalytic amounts of iodine. This is then directly converted into the peracetylated benzyl ester **13** by reaction with benzyl alcohol within 16 h in 65% yield over two steps. It follows an anomeric deprotection to give **14** by employing hydrazine acetate in DMF in 90% yield. The transformation into the glucuronic acid donor **9b** containing a trichloroacetimidate moiety was accomplished by using trichloroacetonitrile and polymer-supported DBU[27,28] as base avoiding a purification step of the highly sensitive trichloroacetimidate moiety. This route allows the synthesis of **9b** in 46% over four steps.

The cytotoxicity of the glucuronides was determined using a HTCFA-assay with IC_{50} values of 610 nM for **4a** and 3300 nM for **4b** (Fig. 1). In the presence of β-glucuronidase **4a** expresses an IC_{50} value of 0.9 nM and **4b** a value of 2.1 nM resulting in QIC_{50} values of about 700 for **4a** and 1600 for **4b**. As expected, the IC_{50} value of **4b** in the presence of glucuronidase is lower than the value obtained for **4a**; however, the cytotoxicity is still quite high indicating that the glucuronic acid methyl ester was cleaved in the cell culture medium by a present carboxylesterase. It is important to note that the necessary amount of β-D-glucuronidase for the cleavage of the glucuronide moiety in **4a** and **4b** is rather low and comes up to only four fishman units.

3. Conclusion

We have synthesized the novel glucuronic acid prodrug **4a** as detoxified analogue of the cytotoxic antibiotic duocarmycin SA for a selective treatment of cancer using the prodrug monotherapy with a high QIC_{50} value of 700. Furthermore, we were able to show that also the glucuronic methyl ester **4b** with an even higher QIC_{50} value of 1600 might be also useable since it will be cleaved in situ by ubiquitous carboxylesterases.

4. Experimental

4.1. General

All reactions were performed in flame-dried glassware under an atmosphere of argon. Solvents were dried and purified according to the method defined by Perrin and Armarego. Commercial

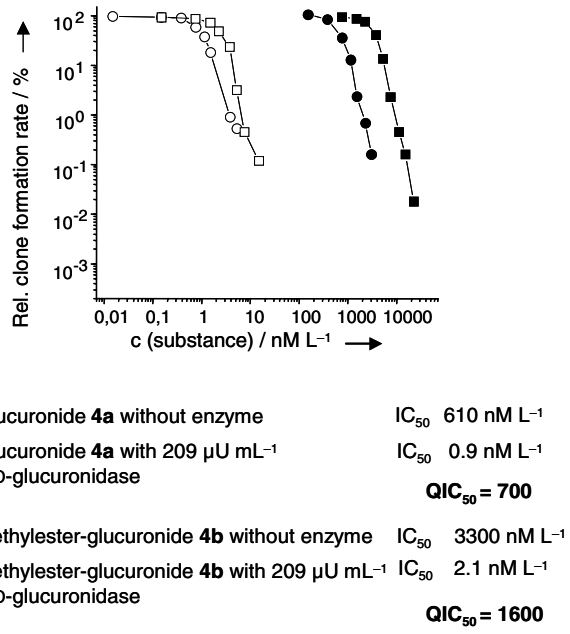


Figure 1. HTCFA assay of the cytotoxicity of **4a** and **4b** against human bronchial carcinoma cells of line A549.

reagents were used without further purification. Thin-layer chromatography (TLC) was carried out on precoated Alugram SIL G/UV254 (0.25 mm) plates from Macherey-Nagel & Co. Column chromatography was carried out on silica gel 60 from Merck with particle size 0.063–0.200 mm for normal pressure and 0.020–0.063 mm for flash chromatography (P = pentane). IR spectra were determined on a Bruker Vektor 22, UV–vis spectra on a Perkin-Elmer Lambda 2, and mass spectra on a Varian MAT 311A, Varian MAT 731 for EI-HRMS, and a Bioapex fourier transformation ion cyclotron resonance mass spectrometer for ESI-HRMS.

^{1}H NMR spectra were recorded either on a Varian UNITY-300 MHz, Varian Inova 500 MHz, or Varian Inova 600 MHz. ^{13}C NMR spectra were recorded at 75, 125, or 150 MHz. Spectra were taken at room temperature except otherwise stated in deuterated solvents as indicated using the solvent peak as internal standard.

4.2. Benzyl (1,2,3,4-tetra-*O*-acetyl-β-D-glucopyran)uronate (13)

To a stirred suspension of D-glucuronic acid (7.50 g, 38.6 mmol, 1.00 equiv) in acetic anhydride (113 mL, 122 g, 1.20 mol) was slowly added at room temperature iodine (530 mg, 1.93 mmol, 0.05 equiv) and stirring was continued for 2 h. The solvent was removed in vacuo, the residue taken up in toluene followed by distillation in vacuo (3× 200 mL) to remove the iodine and traces of acetic acid to give the analytically pure mixed anhydride as slightly yellow solid (15.5 g, 38.4 mmol, 99%). Then, a solution of 10.4 g (25.8 mmol, 1.00 equiv) of the mixed anhydride in CH_2Cl_2 (70 mL) and benzyl alcohol (5.01 g, 4.80 mL, 46.7 mmol, 1.80 equiv) was stirred for 16 h at 25 °C. The solution was diluted with EtOAc (250 mL), washed with 1 M $Na_2S_2O_3$ solution (100 mL), 1 M HCl solution (100 mL), satd $NaHCO_3$ solution (100 mL), and brine (100 mL). The organic phase was dried ($MgSO_4$), the solvent removed in vacuo and the residue purified by column chromatography on silica gel (pentane/EtOAc = 2:1) to give **13** as a colorless solid (6.76 g, 14.9 mmol, 65%) which can be recrystallised from EtOH. R_f = 0.53 (pentane/EtOAc = 1:1); δ_H (300 MHz, $CDCl_3$) = 1.76, 1.96, 2.01, 2.09 (4× s, 12H, 4× COC*H*$_3$), 4.19 (dd, *J* = 9.5, 4.5 Hz, 1H, 5-H), 5.08 (d, *J* = 12.0 Hz, 1H, OC*H*$_2$Ph), 5.11 (dd, *J* = 9.0, 7.7 Hz, 1H, 2-H), 5.13 (d, *J* = 12.0 Hz, 1H, OC*H*$_2$Ph), 5.19–5.29 (m, 2H, 3-H, 4-H), 5.74 (d, *J* = 7.7 Hz, 1H, 1-H), 7.29–7.38 (m, 5H, Ph-H); δ_C (75.5 MHz, $CDCl_3$) = 20.21, 20.51, 20.74 (4× *C*OCH$_3$), 67.98 (O*C*H$_2$Ph), 68.80 (C-4), 70.11 (C-3), 71.87 (C-2), 72.94 (C-5), 91.28 (C-1), 128.6, 128.7, 128.8 (Ph-C$_o$, Ph-C$_m$, Ph-C$_p$), 134.5 (Ph-C$_i$), 166.3, (C-6), 168.8, 169.2, 169.3, 169.9 (4× *C*OCH$_3$); $C_{21}H_{24}O_{11}$ (452.41).

4.3. Benzyl (2,3,4-tri-*O*-acetyl-α/β-D-glucopyran)uronate (14)

To a stirred solution of **13** (1.94 g, 4.29 mmol, 1.00 equiv) in DMF (20 mL) was added hydrazine acetate (503 mg, 5.47 mmol, 1.28 equiv) and stirring was continued for 2.5 h at 25 °C. After dilution with EtOAc (150 mL) the solution was washed with ice-water (60 mL). The water phase was extracted with EtOAc (2× 25 mL), and the combined organic layers were washed successively with satd $NaHCO_3$ solution (2× 70 mL), ice-water (70 mL), 1 N HCl solution (70 mL), and brine (70 mL). After drying (Na_2SO_4), removal of the solvent in vacuo, and column chromatography on silica gel (pentane/EtOAc = 1.5:1) **14** was obtained as a colorless solid (1.58 g, 3.85 mmol, 90%) at a α/β ratio of 6:1; R_f = 0.35 (pentane/EtOAc = 1:1); α-anomer: δ_H (300 MHz, $CDCl_3$): 1.71, 1.96, 2.03 (3× s, 9H, 3× COC*H*$_3$), 4.27 (s$_{br}$, 1H, OH), 4.62 (d, *J* = 10.2 Hz, 1H, 5-H), 4.84 (dd, *J* = 10.3, 3.5 Hz, 1H, 2-H), 5.05 (d, *J* = 12.0 Hz, 1H, OC*H*$_2$Ph), 5.10 (t, *J* = 10.0 Hz, 3-H), 5.19 (d, *J* = 11.9 Hz, 1H, OC*H*$_2$Ph), 5.55 (d, *J* = 4.0 Hz, 1H, 1-H), 5.56 (t, *J* = 9.8 Hz, 1H, 4-H), 7.28–7.43 (m, 5H, Ph-H); δ_C (75.5 MHz, $CDCl_3$): 20.27, 20.62, 20.65 (3× COCH$_3$), 67.91 (O*C*H$_2$Ph), 67.99 (C-2), 69.16, 69.39 (C-3, C-4), 70.62 (C-5), 90.19 (C-1), 128.6, 128.7, 128.7 (Ph-C$_o$, Ph-C$_m$, Ph-C$_p$), 134.5 (Ph-C$_i$), 168.0 (C-6), 169.6, 170.0, 170.1 (3× *C*OCH$_3$); $C_{19}H_{22}O_{10}$ (410.37).

4.4. Benzyl (2,3,4-tri-*O*-acetyl-α-D-glucopyran)uronate trichloroacetimidate (9b)

To a solution of **14** (1.00 g, 2.44 mmol, 1.00 equiv) in CH_2Cl_2 (35.0 mL) was added polymer-supported DBU (0.50 mmol/g, 2.44 g, 1.22 mmol 0.50 equiv) and trichloroacetonitrile (1.76 g, 1.22 mL, 12.1 mmol, 5.00 equiv). After stirring at 25 °C for 60 min the suspension was filtered over Celite, and the solvent removed in vacuo. The residue was taken up in toluene and the toluene distilled off in vacuo (3× 15 mL) to give the clean trichloroacetimidate **9b** as slightly yellow syrup (1.15 g, 2.07 mmol, 85%), which was used for the next step without further purification; R_f = 0.49 (pentane/EtOAc = 3:1); δ_H (300.0 MHz, $CDCl_3$): 1.79, 2.02, 2.04, (3× s, 9H, 3× COC*H*$_3$), 4.54 (d, *J* = 10.5 Hz, 1H, 5-H), 5.15 (s, 2H, OC*H*$_2$Ph), 5.16 (dd, *J* = 9.8, 4.1 Hz, 1H, 2-H), 5.27 (t, *J* = 9.8 Hz, 1H, 4-H), 5.62 (t, *J* = 9.8 Hz, 1H, 3-H), 6.66 (d, *J* = 3.9 Hz, 1H, 1-H), 7.37 (s, 5H, Ph-H), 8.75 (s, 1H, NH); δ_C (75.8 MHz, $CDCl_3$): 20.23, 20.39, 20.63 (3× COCH$_3$), 68.07 (*C*H$_2$Ph), 68.94 (C-2), 69.00, 69.38 (C-3, C-4), 70.49 (C-5), 91.76 (*C*Cl$_3$), 92.58 (C-1), 128.6, 128.7, 128.8 (Ph-C$_o$, Ph-C$_m$, Ph-C$_p$), 134.5 (Ph-C$_i$), 160.5 (C=NH),166.6, 169.3, 169.6, 169.7 (4× *C*OCH$_3$); $C_{21}H_{22}Cl_3NO_{10}$ (554.76).

4.5. (+)-Benzyl {(1*S*,10 *R*)-1-(10-chloroethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)ethoxy)indol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[e]indol-5-yl]}-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronate (11b)

A suspension of the trichloroacetimidate **9b** (646 mg, 1.16 mmol, 1.25 equiv), phenol (+)-(1*S*,10*R*)-**8** (325 mg, 938 µmol, 1.00 equiv) and molecular sieves 4 Å (1.75 g) in CH_2Cl_2 (42.0 mL) was stirred at 25 °C for 30 min. The mixture was cooled to −18 °C and $BF_3{\cdot}OEt_2$ (59.4 µL, 468 µmol, 0.50 equiv) in CH_2Cl_2 (1.0 mL) was added dropwise, then, the mixture was allowed to warm to −10 °C. After stirring for 3 h at this temperature additional $BF_3{\cdot}OEt_2$ (200 µL, 1.58 mmol, 2.00 equiv) in CH_2Cl_2 (7.0 mL)

was added, the suspension warmed to 25 °C, stirred for 3 h and the reaction quenched by filtration over a Celite pad. The solvent was removed in vacuo and the resulting salt dried under high vacuum for 1 h to give a foam which was dissolved in DMF (60.0 mL). To the obtained solution was added at 0 °C DMAI·HCl (**10**) (400 mg, 1.41 mmol, 1.50 equiv) and EDC·HCl (539 mg, 2.81 mmol, 3.00 equiv) and stirring was continued at 25 °C for 24 h. Then, EtOAc (70 mL) was added and the mixture washed with ice-water (70 mL) and satd $NaHCO_3$ solution (25 mL). The layers were separated and the water phase reextracted with EtOAc (4× 100 mL). The combined organic layers were washed with brine (4× 60 mL), dried over $MgSO_4$ and the solvent removed in vacuo. Column chromatography on silica gel (CH_2Cl_2/MeOH = 10:1) yielded the acetylated prodrug **11b** as colorless solid (352 mg, 40.4 μmol, 43%); R_f = 0.44 (CH_2Cl_2/MeOH = 10:1); $[\alpha]_D^{20}$ +12.1° (*c* 0.87, MeOH); δ_H (600 MHz, DMSO-d_6, 35 °C): 1.65 (d, *J* = 6.7 Hz, 3H, 11-H_3), 1.83, 2.01, 2.03 (3× s, 9H, 3× COCH_3), 2.28 (s, 6H, N(CH_3)$_2$), 2.70 (t, *J* = 5.8 Hz, 2H, H_2-2″), 4.09 (t, *J* = 5.8 Hz, 2H, 1″-H_2), 4.28 (dt, *J* = 9.4, 2.3 Hz, 1H, 1-H), 4.64 (dd, *J* = 10.0, 2.0 Hz, 1H, 2_a-H), 4.78 (m_c, 2H, 2_b-H, 5‴-H), 4.81 (dq, *J* = 6.6, 2.3 Hz, 1H, 10-H), 5.13, 5.16 (2× d, *J* = 12.3 Hz, 2H, OCH_2Ph), 5.20 (t, *J* = 9.6 Hz, 1H, 4‴-H), 5.34 (dd, *J* = 9.6, 7.7 Hz, 1H, 2‴-H), 5.57 (t, *J* = 9.6 Hz, 1H, 3‴-H), 5.79 (d, *J* = 7.7 Hz, 1H, 1‴-H), 6.93 (dd, *J* = 8.9, 2.3 Hz, 1H, 6′-H), 7.19 (s_{br}, 2H, 3′-H, 4′-H), 7.26–7.38 (m_c, 5H, Ph-H), 7.41 (d, *J* = 8.9 Hz, 1H, 7′-H), 7.47, 7.59 (2× t, *J* = 7.7 Hz, 2H, 7-H, 8-H), 8.00 (d, *J* = 8.9 Hz, 2H, 6-H, 9-H), 8.25 (s_{br}, 1H, 4-H), 11.61 (s, 1H, NH); δ_C (150.8 MHz, DMSO-d_6, 35 °C): 19.99, 20.20, 20.28 (3× COCH_3), 23.31 (11-CH_3), 45.34 (N(CH_3)$_2$), 45.93 (C-1), 52.06 (C-2), 57.65 (C-2″), 61.18 (C-10), 66.08 (C-1″), 66.96 (OCH_2Ph), 69.08 (C-4‴), 70.70 (C-2‴), 70.96 (C-3‴), 71.28 (C-5‴), 98.71 (C-1‴), 103.0 (C-4), 103.3 (C-4′), 105.5 (C-3′), 113.2 (C-7′), 115.9 (C-6′), 120.6 (C-5a), 122.1 (C-6), 122.7 (C-9b), 123.2 (C-9), 124.4 (C-7), 127.5 (C-8, C-3a′), 128.2, 128.3, 128.4 (5× Ph-C_o, Ph-C_m, Ph-C_p), 129.5, 130.7, 131.8 (C-2′, C-7a′, C-9a), 134.9 (Ph-C_i), 141.8 (C-3a), 152.4, 152.9 (C-5, C-5′), 160.2 (NC═O), 166.4 (C-6‴), 169.0, 169.2, 169.4 (3× *C*OCH$_3$); IR (KBr): $\tilde{\nu}$ = 3418 cm^{-1}, 2937, 1759, 1626, 1517, 1461, 1412, 1216, 1038, 758; MS (ESI): *m*/*z*: calcd for $C_{46}H_{49}ClN_3O_{12}$ [M+H]$^+$: 870.3 (100) [M+H]$^+$, 1740.8 (12) [2M+H]$^+$; 868.4 (100) [M−H]$^-$, 1739.1 (72) [2M−H]$^-$; HRMS (ESI): *m*/*z*: calcd for $C_{46}H_{49}ClN_3O_{12}$ [M+H]$^+$: 870.29993; found: 870.30003.

4.6. (−)-{(1*S*,10*R*)-1-(10-Chloroethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)ethoxy)indol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[e]indol-5-yl]}-β-D-glucopyranuronate (4a)

A suspension of **11b** (150 mg, 172 μmol, 1.00 equiv) and palladium on charcoal (10 %, 104 mg, 98.0 μmol, 0.57 equiv of Pd) in MeOH/EtOAc (66.0 mL, 1:10) was stirred under a H_2-atmosphere for 12 h at normal pressure and 25 °C. Filtration over Celite, washing with MeOH and evaporation of the solvent yielded an analytically pure colorless solid (121 mg, 155 μmol, 90%; R_f = 0.79, CH_2Cl_2/MeOH = 3:1). The crude salt (121 mg) was dissolved in MeOH (40.0 mL) and a NaOMe solution (30% in MeOH, 70.0 μL, 366 μmol, 2.50 equiv) added dropwise. After stirring at 25 °C for 2 h the solution was neutralized by addition of HOAc. Removal of the solvent under high vacuum gave the crude prodrug, which was purified by reversed phase column chromatography to give **4a** as a colorless solid (61.0 mg, 93.0 μmol, 60%). R_f = 0.12 (CH_2Cl_2/MeOH = 1:1.5); $[\alpha]_D^{20}$ −5.6° (*c* 0.6, DMSO); δ_H (600 MHz, DMSO-d_6, 35 °C): 1.65 (d, *J* = 6.6 Hz, 3H, H_3-11), 2.39 (s, 6H, N(CH_3)$_2$), 2.83–2.88 (m_c, 2H, H_2-2″), 3.36 (d, *J* = 9.1 Hz, 1H, H-5‴), 3.37–3.43 (m_c, 1H, H-3‴), 3.48 (t, *J* = 8.1 Hz, 1H, H-2‴), 3.63–3.70 (m_c, 1H, H-4‴), 4.00 (s_{br}, 1H, OH), 4.12 (t, *J* = 5.7 Hz, 2H, H_2-1″), 4.25 (dt, *J* = 9.5, 2.4 Hz, 1H, H-1), 4.62 (m_c, 1H, H-2_a), 4.73 (t, *J* = 9.7 Hz, 1H, H-2_b), 4.81 (dq, *J* = 6.4, 2.4 Hz, 1H, H-10), 5.34 (m_c, 1H, H-1‴), 5.47 (s_{br}, 2H, 2× OH), 6.92 (dd, *J* = 8.9, 2.2 Hz, 1H, H-6′), 7.15 (s_{br}, 1H, H-3′) 7.18 (d, *J* = 1.3 Hz, 1H, H-4′), 7.39 (d, *J* = 8.9 Hz, 1H, H-7′), 7.44, 7.57 (2× m_c, 2H, H-7, H-8), 7.97 (d, *J* = 8.4 Hz, 1H, H-9), 8.17 (s_{br}, 1H, H-4), 8.34 (d, *J* = 8.4 Hz, 1H, H-6), 11.70 (s, 1H, NH); δ_C (125.7 MHz, DMSO-d_6, 35 °C): 23.34 (11-CH_3), 44.76 (N(*C*H$_3$)$_2$), 45.89 (C-1), 51.93 (C-2), 57.12 (C-2″), 61.21 (C-10), 65.35 (C-1″), 71.70 (C-3‴), 73.05 (C-2‴), 75.24 (C-4‴), 76.03 (C-5‴), 101.2 (C-1‴), 101.6 (C-4), 103.4 (C-4′), 105.4 (C-3′), 113.1 (C-7′), 115.8 (C-6′), 119.1 (C-5a), 122.9, 123.3, 123.7 (C-6, C-7, C-9, C-9b), 127.3, 127.4 (C-8, C-3a′), 129.5, 130.9, 131.7 (C-2′, C-7a′, C-9a), 141.9 (C-3a), 152.7, 153.2 (C-5, C-5′), 160.0 (NC═O), 171.9 (C-6‴); UV/vis (MeOH): λ_{max} (lg ε): 209.5 nm (1.6010), 240.5 (1.4051), 299.5 (1.2945), 330.0 (1.3307); IR (KBr): $\tilde{\nu}$ = 3406 cm^{-1}, 1615, 1592, 1516, 1465, 1415, 1290, 1267, 1234, 1179, 1061, 762; MS (ESI): *m*/*z*: calcd for $C_{33}H_{37}ClN_3O_9$ [M+H]$^+$: 654.2 (100) [M+H]$^+$; HRMS (ESI): *m*/*z*: calcd for $C_{33}H_{37}ClN_3O_9$ [M+H]$^+$: 654.22128; found: 654.22095.

4.7. Chromatographic purification of crude (4a)

A solution of 30.0 mg of crude **4a** in 4.00 mL CH_3CN/H_2O = 1:3 + 0.05% HOAc was separated (injection volume 0.80 mL) by semipreparative RP-HPLC (Kromasil 100 C18, 250 × 20 mm, particle size: 7 μm, gradient from 20% to 25% CH_3CN in H_2O + 0.05% HOAc within 5 min, then CH_3CN/H_2O = 1:3 + 0.05% HOAc, flow: 12 mL min^{-1}; UV-detector: λ = 299 nm, *Jasco*-module) to provide pure **4a** (t_R = 15.8 min).

4.8. (+)-Methyl {(1*S*,10*R*)-1-(10-chloroethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)ethoxy)indol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[e]indol-5-yl]}-2,3,4-tri-*O*-acetyl-β-D-glucopyranuronate (11a)

A suspension of the trichloroacetimidate **9a** (109 mg, 227 μmol, 1.05 equiv), phenol (+)-(1*S*,10*R*)-**8** (75.0 mg, 216 μmol, 1.00 equiv) and molecular sieves 4 Å (450 mg) in CH_2Cl_2 (10.0 mL) was stirred at 25 °C for 30 min. The mixture was cooled to −20 °C and BF_3·OEt_2 (13.7 μL, 108 μmol, 0.50 equiv) in CH_2Cl_2 (150 μL) was added dropwise. After stirring for 3.5 h, additional BF_3·OEt_2 (82.2 μL, 648 μmol, 3.00 equiv) in CH_2Cl_2 (2.00 mL) was added, the suspension warmed to 25 °C, and stirring was continued for 4.5 h. The reaction mixture was filtered over a Celite pad, the filtrate evaporated in vacuo and the resulting salt dried under high vacuum for 1 h to give a foam which was dissolved in DMF (60.0 mL). To the obtained solution were added at 0 °C DMAI·HCl (**10**) (92.3 mg, 324 μmol, 1.50 equiv) and EDC·HCl (124 mg, 648 μmol, 3.00 equiv) and the mixture was stirred at 25 °C for 24 h. Work-up and purification was performed as described for **11b** to give the acetylated prodrug **11a** as colorless solid (109 mg, 146 μmol, 68%). R_f = 0.34 (CH_2Cl_2/MeOH = 10:1); $[\alpha]_D^{20}$ +1.6° (*c* 0.1, MeOH); δ_H (300 MHz, DMSO-d_6, 35 °C): 1.64 (d, *J* = 6.7 Hz, 3H, 11-H_3), 2.01, 2.02 (3× s, 9H, 3× COCH_3), 2.37 (s, 6H, N(CH_3)$_2$), 2.84 (t, *J* = 5.7 Hz, 2H, 2″-H_2), 3.67 (s, 3H, OCH_3), 4.14 (t, *J* = 5.7 Hz, 2H, 1″-H_2), 4.26 (dd, *J* = 8.9, 2.4 Hz, 1H, 1-H), 4.63 (dd, *J* = 11.4, 1.5 Hz, 1H, 2_a-H), 4.71–4.83 (m, 3H, 2_b-H, 5‴-H, 10-H), 5.15 (t, *J* = 9.7 Hz, 1H, 4‴-H), 5.32 (dd, *J* = 9.7, 7.8 Hz, 1H, 2‴-H), 5.60 (t, *J* = 9.6 Hz, 1H, 3‴-H), 5.80 (d, *J* = 7.8 Hz, 1H, 1‴-H), 6.94 (dd, *J* = 9.0, 2.3 Hz, 1H, 6′-H), 7.18, 7.18 (2× s_{br}, 2H, 3′-H, 4′-H), 7.41 (d, *J* = 9.0 Hz, 1H, 7′-H), 7.47 (t, *J* = 7.4 Hz, 1H, 7-H), 7.60 (t, *J* = 7.6 Hz, 1H, 8-H), 7.99, 8.01 (2× d, *J* = 8.1 Hz, 2H, 6-H, 9-H), 8.21 (s, 1H, 4-H), 11.63 (s, 1H, NH); δ_C (125.7 MHz, DMSO-d_6, 35 °C): 20.14, 20.24, 20.29 (3× COCH_3), 23.32 (11-CH_3), 45.02 (N(CH_3)$_2$), 45.88 (C-1), 52.11 (C-2), 52.53 (OCH_3), 57.36 (C-2″), 61.24 (C-10), 65.66 (C-1″), 69.06 (C-4‴), 70.70 (C-2‴, C-3‴), 71.09 (C-5‴), 98.40 (C-1‴), 102.6 (C-4), 103.4 (C-4′), 105.5 (C-3′), 113.2 (C-7′), 115.9 (C-6′), 120.5 (C-5a), 122.0 (C-6), 122.6 (C-9b), 123.3 (C-9), 124.4 (C-7), 127.4, 127.6 (C-8, C-3a′), 129.5, 130.7, 131.4 (C-2′, C-7a′, C-9a), 141.7 (C-3a), 152.4, 152.8 (C-5, C-5′), 160.1

(NC═O), 167.0 (C-6‴), 169.2, 169.3, 169.4 (3× $COCH_3$); UV/vis (MeOH): λ_{max} (lg ε): 205.0 nm (1.5788), 299.0 (1.3633), 334.0 (1.3523); IR (KBr): $\tilde{\nu} = 2928\ cm^{-1}$, 2759, 1626, 1516, 1462, 1414, 1217, 1054, 764; $C_{36}H_{44}ClN_3O_{12}$ (745.85).

4.9. (−)-Methyl {(1*S*,10*R*)-1-(10-chloroethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)ethoxy)indol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[*e*]indol-5-yl]}-β-D-glucopyranuronate (4b)

To a stirred solution of **11a** (25.0 mg, 33.4 μmol, 1.00 equiv) in MeOH (10.0 mL) were added dropwise NaOMe in MeOH (30% in MeOH, 0.90 mg, 3.20 μL, 16.8 μmol, 0.50 equiv in 1.00 mL MeOH) and stirring was continued at 25 °C for 2 h. Column chromatography on silica gel (CH_2Cl_2/ MeOH = 1:1) and filtration through a membrane filter gave **4b** as a colorless solid (17.6 mg, 26.4 μmol, 79%); R_f = 0.29 (MeOH/CH_2Cl_2 = 1:1); $[\alpha]_D^{20}$ −8.2° (*c* 0.6, MeOH); δ_H (600 MHz, DMSO-d_6, 35 °C): 1.65 (d, *J* = 6.6 Hz, 3H, 11-H_3), 2.25 (s, 6H, N(CH_3)$_2$), 2.67 (t, *J* = 5.7 Hz, 2H, 2″-H_2), 3.38 (t, *J* = 9.0 Hz, 1H, 3‴-H), 3.47–3.57 (m, 1H, 2‴-H, 4‴-H), 3.68 (s, 3H, OCH_3), 3.97 (d, *J* = 9.3 Hz, 1H, 5‴-H), 4.08 (t, *J* = 5.9 Hz, 2H, 1″-H_2), 4.26 (dd, *J* = 9.4, 2.1 Hz, 1H, 1-H), 4.62 (dd, *J* = 10.9, 2.2 Hz, 1H, 2_b-H), 4.75 (t, *J* = 10.3 Hz, 1H, 2_a-H), 4.81 (dq, *J* = 6.5, 2.3 Hz, 1H, 10-H), 5.11 (d, *J* = 7.5 Hz, 1H, 1‴H), 5.38, 5.62 (2× s_{br}, 3H, 3× OH), 6.92 (dd, *J* = 8.8, 2.4 Hz, 1H, 6′-H), 7.17, 7.18 (2× d, *J* = 2.2 Hz, 2H, 3′-H, 4′-H), 7.40 (d, *J* = 8.9 Hz, 1H, 7′-H), 7.45, 7.58 (2× t, *J* = 7.5 Hz, 2H, 7-H, 8-H), 7.97 (d, *J* = 8.3 Hz, 1H, 9-H), 8.16 (s, 1H, 4-H), 8.33 (d, *J* = 8.4 Hz, 1H, 6-H), 11.59 (s, 1H, NH); δ_C (125.7 MHz, DMSO-d_6, 35 °C): 23.36 (11-CH_3), 45.49 (N(CH_3)$_2$), 45.93 (C-1), 51.92 (OCH_3), 52.02 (C-2), 57.77 (C-2″), 61.31 (C-10), 65.66 (C-1″), 69.06 (C-4‴), 70.70 (C-2‴, C-3‴), 71.09 (C-5‴), 101.5 (C-4), 102.1 (C-1‴), 103.3 (C-4′), 105.5 (C-3′), 113.2 (C-7′), 115.9 (C-6′), 120.5 (C-5a), 123.0 (C-9, C-9b), 123.3 (C-6), 123.9 (C-7), 127.4, 127.5 (C-8, C-3a′), 129.5, 130.8, 131.7 (C-2′, C-7a′, C-9a), 141.9 (C-3a), 153.0, 153.1 (C-5, C-5′), 160.1 (NC═O), 169.0 (C-6‴); HRMS (ESI): *m*/*z*: calcd for $C_{34}H_{39}ClN_3O_9$ $[M+H]^+$: 668.23748; found: 668.23791.

4.10. Cell culture

Human bronchial carcinoma cells of line A549 (ATCC CCL 185) were kindly provided by the Institut für Zellbiologie, Universität Essen, and were maintained as exponentially growing cultures at 37 °C and 7.5% CO_2 in air in Dulbecco's modified Eagle's medium (DMEM) (Biochrom, Berlin, Germany) supplemented with 10% fetal calf serum (heat-inactivated for 30 min at 56 °C, Gibco–BRL, Karlsruhe, Germany), 44 mM $NaHCO_3$ (Biochrom, Berlin, Germany) and 4 mM L-glutamine (Gibco–BRL, Karlsruhe, Germany).

4.11. In vitro cytotoxicity assays

Adherent cells of line A549 were sown in triplicate in six-multiwell plates at concentrations of 10^2–10^5 cells per cavity. Culture medium was sucked off after 24 h and cells were washed in the incubation medium Ultraculture (UC, serum-free special medium, purchased from BioWhittaker Europe, Verviers, Belgium). Incubation with compounds **4a** and **4b** was then performed in Ultraculture medium at various concentrations for 24 h. All substances were used as freshly prepared solutions in DMSO (Merck, Darmstadt, Germany) diluted with incubation medium to a final concentration of DMSO of 1% in the wells. After 24 h of exposure the test substance was removed and the cells were washed with fresh medium. Cultivation was done at 37 °C and 7.5% CO_2 in air for 12 days. The medium was removed and the clones were dried and stained with Löffler's methylene blue (Merck, Darmstadt, Germany). They were then counted macroscopically.

The IC_{50} values are based on the relative clone forming rate, which was determined according to the following formula: relative clone forming rate [%] = 100 × (number of clones counted after exposure)/(number of clones counted in the control).

Liberation of the drugs from their glycosidic prodrugs was achieved by addition of 4.0 U mL^{-1} β-D-galactosidase (EC 3.2.1.23, Grade X, purchased from Sigma Germany, Deisenhofen, Germany) to the cells during incubation with the substances.

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