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Synthesis and Biological Evaluation of Prodrugs Based on the Natural Antibiotic Duocarmycin for Use in ADEPT and PMT**

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Dedicated to Professor Dieter Enders on the occasion of his 65th birthday

Abstract: Chemotherapy of malign tumors is usually associated with serious side effects as common anticancer drugs lack selectivity. An approach to deal with this problem is the antibody-directed enzyme prodrug therapy (ADEPT) and the prodrug monotherapy (PMT). Herein, the synthesis and biological evaluation of new glycosidic prodrugs suitable for both concepts are described. All prodrugs but one are stable in human serum and show QIC_{50} values (IC_{50} of prodrug/IC_{50} of prodrug in the presence of the appropriate glycohydrolase) of up to 6500. This is the best value found so far for compounds interacting with DNA.

Keywords: antitumor agents · cytotoxicity · duocarmycin · glycosides · prodrugs

Introduction

One of the major problems in the chemotherapy of cancer is its so far insufficient selectivity, which is the cause for severe side effects. This has resulted in the development of several new approaches to solve this problem in recent years. One of the most promising concepts is the antibody-directed enzyme prodrug therapy (ADEPT).[1] Herein, selectivity is achieved by monoclonal antibodies that are conjugated to enzymes. After application of such an antibody conjugate a "nontoxic" prodrug is administered, which is converted site-selectively by the enzyme in the conjugate to give a highly cytotoxic compound. This, in principle, would allow the killing of cancer cells without affecting healthy tissue.

Another approach for a more selective treatment of cancer is the prodrug monotherapy (PMT).[2] In contrast to ADEPT, here an antibody is not necessary since the selectivity is based on factors (e.g. enzymes) that are overexpressed in tumor tissue. For instance, it has been known for a long time that the concentration of the enzyme β-D-glucuronidase is elevated in the extracellular space of solid necrotic tumors.[2b,3] As one of the first, we developed "nontoxic" prodrugs bearing a glucuronic acid (GlcA) moiety.[4] A further interesting difference between malignant and normal tissue is the lower pH of cancer cells under hyperglycemic conditions,[5] which has extensively been exploited by us.[4,6]

In recent years, we have developed several highly promising compounds based on the natural antibiotic duocarmycin SA (**1a**) that are suitable for ADEPT and PMT (Scheme 1).[7] Duocarmycin SA (**1a**), found in *Streptomyces* sp., is a highly potent cytostatic compound with an IC_{50} value of about 10 pM against different cancer cell lines.[8] The molecular structure of **1a** shows an indole subunit as DNA minor groove binding component and a spirocyclopropylcyclohexadienone moiety as a pharmacophoric group that causes sequence-selective alkylation of *N*3 of adenine in AT-rich sites of the minor groove of DNA.[9] With pronounced myelotoxicity, duocarmycin SA (**1a**) itself is not applicable in chemotherapy. In our approach we have used derivatives of the seco-analogue seco-CBI[10] (CBI = cyclopropabenzoindole) and methyl-seco-CBI.[11] Thus, we have prepared (+)-(*S*)-**3a** and (+)-(1*S*,10*R*)-**3b** containing a seco-CBI and an anti-methyl-seco-CBI skeleton, respectively, as pharmacophoric units connected to DMAI (5-[2-(*N*,*N*-dimethylamino)ethoxy]-1*H*-indole)[12] as DNA binding moiety (Scheme 1). For a selective treatment of cancer, these seco-

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[**] ADEPT = antibody-directed enzyme prodrug therapy; PMT = prodrug monotherapy.

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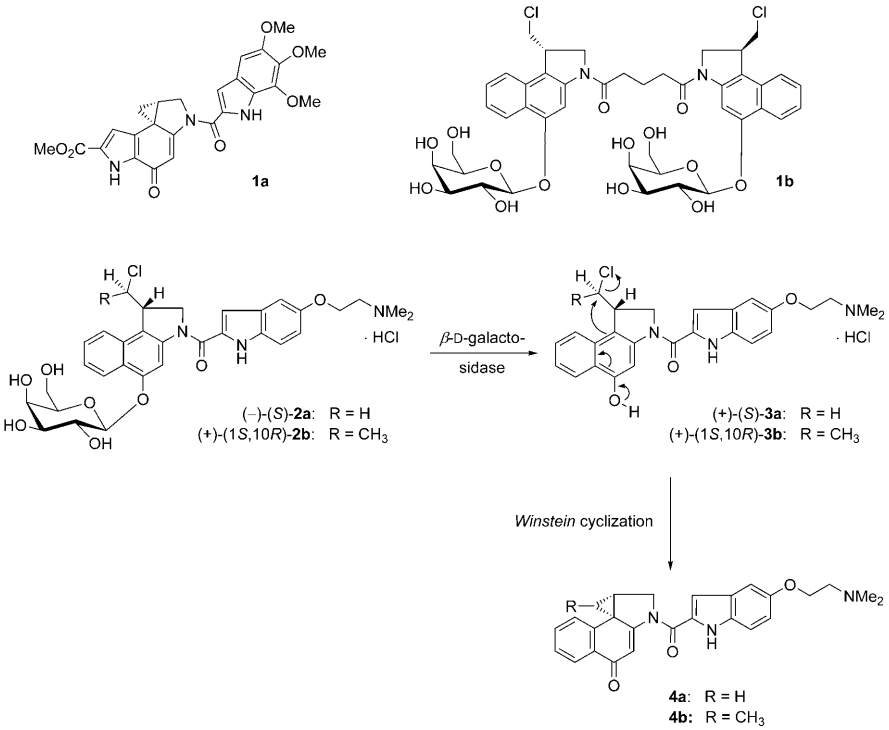


Scheme 1. Duocarmycin SA (**1a**), pseudo-dimeric prodrug **1b**, and prodrugs (−)-(*S*)-2**a** and (+)-(1*S*,10*R*)-**2b** to give the cytotoxic drugs **4a** and **4b** via the seco-drugs (+)-(*S*)-**3a** and (+)-(1*S*,10*R*)-**3b**. Cytotoxic drugs **4a** and **4b** share the spirocyclopropylcyclohexadienone moiety with **1a**.

drugs were transformed into the corresponding glycosides as (−)-(*S*)-**2a** and (+)-(1*S*,10*R*)-**2b** with a highly reduced cytotoxicity by coupling them with different sugar moieties.[7b–e] The so-formed prodrugs can selectively be cleaved again by using the corresponding glycohydrolase or antibody glycohydrolase conjugate to liberate the seco-drugs (+)-(*S*)-**3a** and (+)-(1*S*,10*R*)-**3b**, from which the final cytotoxic drugs **4a** and **4b** are formed in situ by a rapid Winstein cyclization reaction. As proposed by us as a requirement for use in ADEPT, the IC_{50} values of the seco-drugs are below 10 nM with 26 pM for (+)-(*S*)-**3a** and 750 pM for (+)-(1*S*,10*R*)-**3b**.[13]

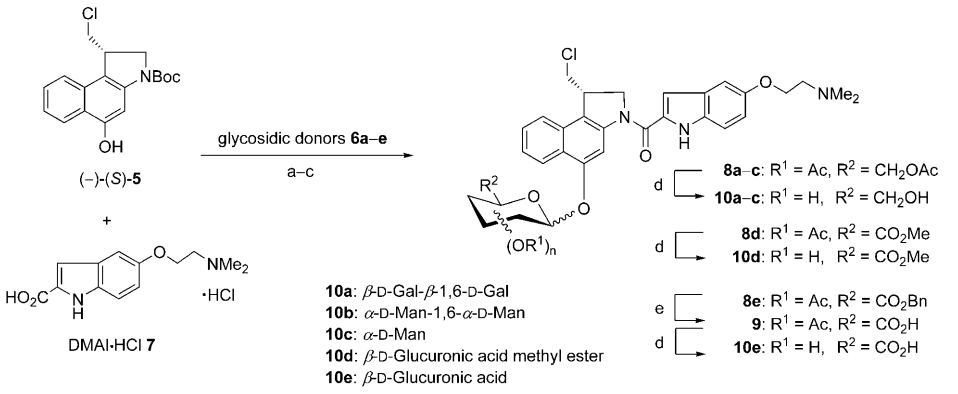
The therapeutic window of these compounds as defined by their QIC_{50} values ($QIC_{50} = IC_{50}$ of prodrug/IC_{50} of prodrug and cleaving enzyme) is very high with QIC_{50} values of up to around 5000 for (+)-(1*S*,10*R*)-**2b**. They well exceed the QIC_{50} value of 1000 that was anticipated by us as a minimum requirement for ADEPT.[13] Quite recently, we were able to improve the QIC_{50} value to almost 1 000 000.[14] However, the new pseudo-dimeric compounds such as **1b** (Scheme 1) with (*S*)-**5** as monomeric unit seem to have a different mode of action. Thus, they do not bind to DNA, which is understandable due to the lack of a DNA binding moiety.

An important objective in designing the prodrugs was the curtailment of their cell-membrane penetration to avoid a dilution effect. Hence, we have also prepared diglycosidic prodrugs by using cellobiose and lactose that are clearly more polar than the monoglycosidic compounds as (+)-(1*S*,10*R*)-**2b** and, therefore, less able to pass through the cell membrane. However, these compounds are not suitable due to an incomplete hydrolysis of the glycosidic bond when using cellulase.[7c] Moreover, the employed anti-methyl-seco-CBI unit showed an insufficient stability in the pharmacokinetic studies.

Here we describe novel disaccharide prodrugs with a mannosyl mannoside (ManMan) and a galactosyl galactoside (GalGal) containing the seco-CBI unit, which is more stable. In addition, we also prepared seco-CBI glucuronides and a seco-CBI mannoside. In the biological evaluation the new compounds very well fulfilled our expectation.

Results and Discussion

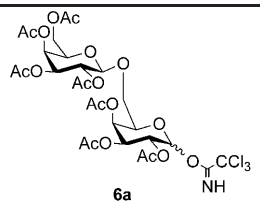
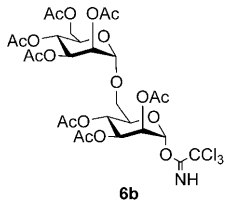
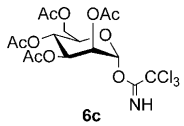
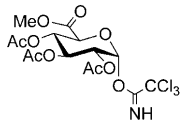
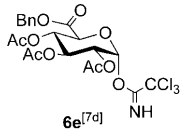
The synthesis of the desired prodrugs **10a–e** was accomplished by following a four- and five-step route, respectively, as depicted in Scheme 2. As starting material, the *tert*-butox-



Scheme 2. Synthesis of the prodrugs **10a–e** (for yields, see Table 1): a) **6a–e** (Table 1), $BF_3{\cdot}OEt_2$, CH_2Cl_2, −10 °C, 3–6.5 h; b) $BF_3{\cdot}OEt_2$, CH_2Cl_2, RT, 5 h; c) **7**, EDC·HCl, DMF, RT, 15–19 h; d) NaOMe, MeOH, RT, 30 min–9 h, prep. RP-HPLC (Kromasil 100 C18, not for **10d**); e) Pd/C, H_2, EtOAc/MeOH 10:1, RT, 6 h.

ycarbonyl Boc-protected phenol (−)-(*S*)-**5**[15] was used. For the transformation into the corresponding prodrugs, (−)-(*S*)-**5** was first glycosylated by using the Schmidt procedure[16] with the trichloroacetimidate donors **6a–e** (Table 1) under BF_3·OEt_2 catalysis in dichloromethane at −10 °C.

Table 1. Glycosidic donors **6a–e** and yields for the synthesis of prodrugs **10a–e** (see Scheme 1).

Product	Glycosidic donor	Coupling of **5**, **6**, and **7** (a–c) [%]	Deacetylation (d) [%]
10a	 **6a**	62	83
10b	 **6b**	62	96
10c	 **6c**	49	90
10d	 **6d**[7d]	39	93
10e	 **6e**[7d]	48	77[a]

[a] Yield for Bn and OAc deprotection (two steps).

The necessary disaccharide donors **6a** (GalGal) and **6b** (ManMan) as well as **6c–e** were prepared according to literature-known procedures with slight variations. Thus, for the formation of the trichloroacetimidates we used trichloroacetonitrile and polymer-supported 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU),[17] which allowed us to improve the yields considerably. Moreover, the obtained products were so clean that a chromatographic purification was unnecessary.

After the glycosylation, the *N*-Boc protecting group was removed in a one-pot protocol by adding three equivalents of BF_3·OEt_2 and keeping the reaction mixture for 5 h at RT. Subsequent amidation of the obtained secondary amine with DMAI (**7**) under EDC·HCl activation in DMF gave the acetylated prodrugs **8a–e** in reasonable yields over three steps (Table 1).

For the synthesis of the glucuronic acid derivative **10e** one more step was required prior to deacetylation. Thus, the benzyl ester **8e** had to be cleaved, which was accomplished by using palladium on charcoal under a H_2 atmosphere in ethyl acetate/methanol to give **9** in 86% yield. The final step in the synthesis of all prodrugs **10** was the solvolysis of the acetyl protecting groups by using the Zemplén procedure with NaOMe in methanol at room temperature. Subsequently, all compounds but **10d** were purified by semipreparative RP-HPLC employing water and methanol or acetonitrile with 0.05% acetic acid to guarantee high purity for the in vitro assays. The methyl glucuronide **10d** was not stable during HPLC under these conditions; thus, partial hydrolysis of the methyl ester **10d** was observed to give the corresponding acid **10e**. Hence, **10d** was further purified by preparative TLC (CH_2Cl_2/MeOH 5:1).

The structures of the molecules and especially the configuration at the glycosidic linkage could be determined by ^{1}H NMR spectroscopy for all compounds but the mannosyl mannoside **10b**. Though, an α-glycosidic bond had to be expected according to the mechanism of the glycosidation, a clear cut evidence of the configuration as α at both anomeric centers was obtained by a 2D-TOCSY-NMR spectroscopic experiment (total correlation spectroscopy) giving the traces as described for α-D-mannoses and α-L-rhamnoses (mixing time: 100 ms).[18] Besides, the $^1J_{C,H}$ coupling constant was measured to be 170 (C-1‴) and 167 Hz (C-1⁗) corresponding to an α-configuration as stated by Pedersen and Jennings.[19]

All new prodrugs were analyzed with regard to their stability in undiluted human serum (Table 2). Over a time span of 24 h, the prodrug-serum mixtures were incubated at 37 °C and substrates and metabolites were examined by HPLC-MS. The stability of the prodrugs **10a–c** and **10e** was very high in the serum showing almost no changes within 24 h. Important to note, the new prodrugs containing the seco-CBI unit have a much better stability in human serum than the corresponding compounds with a seco-methyl-CBI moiety. Only prodrug **10d** showed a change in human serum; however, here the chloromethyl group was not altered as in the case of the seco-methyl-CBI compounds but the ester group of the glucuronic acid methyl ester moiety was cleaved. Obeying a first-order reaction, it is hydrolyzed to give the corresponding GlcA prodrug **10e** with a rate constant of $k = 3.9 \pm 0.2 \times 10^{-5}\ s^{-1}$ and a half-life of $t_{1/2} = 4.9 \pm 0.3$ h (Table 2).

In vitro cytotoxicity: The in vitro cytotoxicities of the novel prodrugs and of the corresponding seco-drug (Table 2) were determined on human bronchial carcinoma cells A549 employing a colony-forming test that reflects the proliferation capacity of single cells (Table 2). These assays also give evidence of the efficiency of drug formation from the prodrug and whether an undesired suicide mechanism inactivating the enzyme takes place. If the IC_{50} value of the prodrug in the presence of the enzyme is similar to that of the corresponding seco-drug, one can assume that the enzymatic

Table 2. In vitro cytotoxicity[a] and stability of the prodrugs (−)-(*S*)-**2a**, **10a–e**, and the seco-drug (+)-(*S*)-**3a**.

Prodrug	Detoxifying unit[b]	IC_{50} without enzyme [nM]	IC_{50} with enzyme [pM]	QIC_{50}[f]	Stability over 24 h [%][g]
(+)-(*S*)-**3a**	–	0.026	–	–	–
(−)-(*S*)-**2a**	β-D-galactose	56.3	16[c]	3500	–
10a	β-D-galactosyl-(1→6)-β-D-galactose	130	20[c]	6500	100
10b	α-D-mannosyl-(1→6)-α-D-mannose	183	1250[d]	150	100
10c	α-D-mannose	42.5	60[d]	700	100
10d	β-D-glucuronic acid methyl ester	57.0	56[e]	1020	98% conversion to **10e**[h]
10e	β-D-glucuronic acid	6.20	13[e]	480	100

[a] Determined by HTCFA test; see the Experimental Section. [b] Carbohydrate moiety on enantiopure (−)-(*S*)-seco-CBI pharmacophor. [c] Enzyme: 4 U mL^{-1} β-D-galactosidase. [d] Enzyme: 0.4 U mL^{-1} α-D-mannosidase. [e] Enzyme: 4 U mL^{-1} β-D-glucuronidase. [f] $QIC_{50} = IC_{50}$ of prodrug/IC_{50} of prodrug in presence of cleaving enzyme. [g] Incubation in human serum at 37 °C; see the Experimental Section. [h] First-order reaction, $[S] = [S]_0 \cdot e^{-kt}$, $k = 3.9 \pm 0.2 \times 10^{-5}$ s^{-1}, $t_{1/2} = 4.9 \pm 0.3$ h, $R^2 = 0.984$.

cleavage is fast and the enzyme is not deactivated by the product formed. For the assay, the cells were exposed to various concentrations of each prodrug **10a–e** in the absence and presence of the corresponding enzyme in serum-free UC medium (UltraCulture medium).

As anticipated, the IC_{50} values of the diglycosidic prodrugs **10a** and **10b** are higher than those found for the monoglycosidic compounds **2a** and **10c** with $IC_{50} = 130$ and $IC_{50} = 183$ nM, respectively, relative to $IC_{50} = 56.3$ and $IC_{50} = 42.5$ nM, respectively. This might be due to a reduced intake of the diglycosidic relative to the monoglycosidic prodrugs due to a higher polarity of the former. The enzymatic cleavage of the GalGal moiety in **10a** seems to be very fast when using β-D-galactosidase from *E. coli* since the IC_{50} value of **10a** in the presence of the enzyme corresponds very well to that of the seco-drug **2a**. This results in a QIC_{50} value of 6500, which is so far the best result for this family of compounds interacting with DNA. In contrast, prodrug **10b** seems to be less suitable since the enzymatic cleavage of the α-D-mannose moieties when using α-D-mannosidase is rather slow resulting in lower cytotoxicity. Here, either the prodrug **10b** is not a good substrate for the enzyme or indeed a suicide mechanism might be operating. A similar effect was found for the monoglycosidic compound **10c** containing a mannose residue.

A highly promising compound for PMT seems to be **10d** bearing a β-D-methyl glucuronide moiety with a QIC_{50} value of 1020. Its primary cytotoxicity is much lower than that of the prodrug **10e** containing a β-D-glucuronide moiety.

Conclusion

Five new glycosidic prodrugs based on duocarmycin SA that differ in the detoxifying sugar moiety have been prepared for application in ADEPT and PMT. All compounds except **10d** are stable in human serum, which is essential for application in vivo. The ester **10d** showing a good QIC_{50} value of 1020 can act as a "double-prodrug" as it is hydrolyzed to give the corresponding glucuronic acid prodrug **10e** in serum and during cell culture experiments, which then can be activated by the enzyme *β*-D-glucuronidase. With a $QIC_{50} = 6500$ the GalGal prodrug **10a** shows the best selectivity of all prodrugs prepared so far based on compounds interacting with DNA; thus, **10a** is very suitable for in vivo studies.

Experimental Section

General: All reactions were carried out under argon in flame-dried glassware. Solvents were distilled prior to use by common laboratory methods or used from commercial sources in p.a. grade and stored over molecular sieves. All reagents purchased from commercial sources were used without further purification. TLC was performed on silica gel 60 F_{254} plates from Merck and silica gel 60 (0.032–0.063 mm, Merck) was used for column chromatography. Vanillin in methanolic sulfuric acid was used as the staining reagent for TLC. Preparative HPLC for purification of the target compounds was performed on a Jasco system, equipped with two solvent pumps PU-2087 PLUS, UV detector UV-2075 PLUS, and a semi-preparative RP column Kromasil 100 C18 (250×20 mm, 7 μm, Jasco). UV spectra were taken with a Perkin–Elmer Lambda 2 spectrometer. IR spectra were recorded as KBr pellets with a Bruker IFS 25 spectrometer. Optical rotations were measured on a Perkin–Elmer 241 polarimeter in the solvent indicated. ^{1}H and ^{13}C NMR spectra were recorded with Mercury-300, Unity-300, Inova-500, Inova-600 (Varian) spectrometers. Spectra were taken at room temperature or 35 °C in deuterated solvents as indicated by using the solvent peak as an internal standard. For ^{13}C numbering see the Supporting Information. Mass spectra (ESI) were obtained from an ion-trap mass spectrometer LCQ (Finnigan) and a TOF spectrometer micrOTOF (Bruker). HRMS was performed with 7 T FTICR-MS APEX IV equipment (Bruker) and micrOTOF (Bruker). The purities and stabilities of the prodrugs were analyzed by HPLC-MS by using ESI mass spectrometry with the ion-trap mass spectrometer LCQ (Finnigan). The HPLC system comprises of solvent pump Rheos 400, degasser ERC-3415α (Flux Instruments), autosampler 851 (Jasco), and a diode array detector (Thermo). The column was a Synergi Max-RP C12 (150×2 mm, 4 μm, phenomenex). Mobile phases were water (A) and methanol (B) (hypergrade for LC-MS, Merck) with 0.05% formic acid (Roth) each. The flow was 300 μL min^{-1} with the following gradient: 0–15 min: 70A/30B→0A/100B, 15–22 min: 0A/100B, 22–23 min: 0A/100B→70A/30B, 23–29 min: 70A/30B. Pooled human serum was obtained from the Klinikum Göttingen, dept. of transfusion medicine, and used without dilution.

General procedure A for the synthesis of the trichloroacetimidate donors: Polymer-supported DBU[17] (1.26 mmol g^{-1}, 0.50 equiv) and trichloroacetonitrile (4.0–10.0 equiv) were added to a solution of the anomeric deprotected sugar (1.0 equiv) in dry CH_2Cl_2 (8–13 mL mmol^{-1}). After stirring for 1–3.5 h at room temperature, the brown suspension was filtered over Celite to separate the polymer and the Celite was washed with CH_2Cl_2. The solvent was removed at room temperature in vacuo to

give the clean trichloroacetimidate as a slightly yellow syrup. Yields: **6a**: 97%, **6b**: 95%, **6c**: 78%, **6d**: 82%, **6e**: 85%.

General procedure B for the glycosylation, Boc-deprotection, and DNA-binder coupling: A mixture of the phenol (−)-(*S*)-**5** (1.00 equiv), trichloroacetimidate **6** (1.18–1.45 equiv), and freshly activated molecular sieves 4 Å (1.80–2.40 g mmol^{-1}) in dry CH_2Cl_2 (33–46 mL mmol^{-1}) was stirred for 30 min at RT. After cooling to the indicated temperature, the pre-cooled promoter BF_3·OEt_2 (0.5 equiv) in dry CH_2Cl_2 (10.5 mL mmol^{-1} BF_3·OEt_2) was added dropwise and the mixture stirred at −10 °C for the given time. To remove the Boc-protecting group, an excess of BF_3·OEt_2 (3.0 equiv) in CH_2Cl_2 (1.5 mL mmol^{-1} BF_3·OEt_2) was added, the mixture warmed to room temperature, and stirred for 5 h. The reaction mixture was transferred into a second flask by transfer cannula to separate the molecular sieves, which were then thoroughly washed with CH_2Cl_2. The solvent was removed under reduced pressure and the resulting foam dried under high vacuum for 1 h. The formed salt was dissolved in DMF (45 mL mmol^{-1}), the stirred solution cooled to 0 °C, and EDC·HCl (3.0 equiv) followed by DMAI·HCl (**7**) (1.5 equiv) were added. After stirring at RT for 15–19 h, the mixture was diluted with EtOAc (70 mL mmol^{-1}), water (70 mL mmol^{-1}), and saturated $NaHCO_3$ solution (55 mL mmol^{-1}), the phases separated, and the water layer extracted again with EtOAc (4×110 mL mmol^{-1}). The combined organic layers were washed with brine (2×90 mL mmol^{-1}), dried over $MgSO_4$, and the solvent removed under reduced pressure. The crude material was purified by column chromatography on silica gel (CH_2Cl_2/MeOH 10:1) to yield the acetylated prodrugs **8**.

General procedure C for the Zemplén deacetylation: At 0 °C a solution of the acetylated prodrug (1.0 equiv) in MeOH was treated with a NaOMe solution (25 or 30% in MeOH, 0.5–3.0 equiv) and the mixture stirred at RT until complete conversion was observed (TLC control). The mixture was neutralized with acidic ion-exchange resin Amberlite IR120 or 1 M methanolic acetic acid, respectively. After evaporation of the solvent, the crude material was purified by RP-HPLC unless otherwise stated.

Compound 8a: According to general procedure B, a stirred mixture of the trichloroacetimidate **6a** (200 mg, 256 μmol, 1.22 equiv), phenol (−)-(1*S*)-**5** (70 mg, 210 μmol, 1.0 equiv), and molecular sieves 4 Å (450 mg) in CH_2Cl_2 (8 mL) were treated with BF_3·OEt_2 (13.3 μL, 105 μmol, in CH_2Cl_2, 0.5 equiv) at −16 °C and stirring was continued at −10 °C for 3.5 h. Additional BF_3·OEt_2 (79.7 μL, 629 μmol, in 0.9 mL CH_2Cl_2, 3.0 equiv) was added and the mixture kept at RT for 5 h. Evaporation and subsequent reaction with DMAI·HCl (**7**) (89.6 mg, 315 μmol, 1.5 equiv) and EDC·HCl (121 mg, 629 μmol, 3.0 equiv) in DMF (10 mL) for 16 h yielded crude material that was purified by column chromatography to afford the peracetylated prodrug **8a** (140 mg, 130 μmol, 62%) as a pale-yellow solid. R_f=0.39 (CH_2Cl_2/MeOH 10:1); α=+4.3° (*c*=0.21 in MeOH); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=1.88 1.89, 1.90, 1.96 (4×s, 4×3H; 4×C(O)CH$_3$), 2.06 (s, 6H; 2×C(O)CH$_3$), 2.17 (s, 3H; C(O)CH$_3$), 2.26 (s, 6H; NMe$_2$), 2.68 (t, *J*=5.8 Hz, 2H; 2″-H), 3.72 (m$_c$, 1H; 6‴-H$_a$), 3.78 (m$_c$, 1H; 6‴-H$_b$), 3.94 (dd, *J*=11.2, 6.9 Hz, 1H; 10-H$_a$), 3.98–4.06 (m, 3H; 6⁗-H$_a$, 6⁗-H$_b$), 4.08 (t, *J*=5.9 Hz, 2H; 10-H$_b$, 1‴-H), 4.14 (t, *J*=6.7 Hz, 1H; 5⁗-H), 4.33 (m$_c$, 1H; 1-H), 4.38 (m$_c$, 1H; 5‴-H), 4.60 (dd, *J*=10.9, 2.0 Hz, 1H; 2-H$_a$), 4.71 (d, *J*=8.0 Hz, 1H; 1⁗-H), 4.83 (t, *J*=10.0 Hz, 1H; 2-H$_b$), 4.87 (dd, *J*=10.3, 8.0 Hz, 1H; 2⁗-H), 5.02 (dd, *J*=10.4, 3.6 Hz, 1H; 3⁗-H), 5.21 (d, *J*=3.4 Hz, 1H; 4⁗-H), 5.34 (d, *J*=2.0 Hz, 1H; 4‴-H), 5.36–5.43 (m, 2H; 2‴-H, 3‴-H), 5.56 (d, *J*=6.6 Hz, 1H; 1‴-H), 6.93 (dd, *J*=8.9, 2.4 Hz, 1H; 6′-H), 7.11 (d, *J*=1.7 Hz, 1H; 3′-H), 7.18 (d, *J*=2.3 Hz, 1H; 4′-H), 7.39 (d, *J*=8.9 Hz, 1H; 7′-H), 7.48 (ddd, *J*=8.1, 6.8, 0.8 Hz, 1H; 7-H), 7.60 (ddd, *J*=8.1, 6.9, 1.1 Hz, 1H; 8-H), 7.96 (d, *J*=8.4 Hz, 1H; 9-H), 8.01 (d, *J*=8.5 Hz, 1H; 6-H), 8.24 (brs, 1H; 4-H), 11.57 ppm (d, *J*=1.6 Hz, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=20.2 (4 signals; 4×C(O)*C*H$_3$), 20.3 (2×C(O)*C*H$_3$), 20.5 (C(O)*C*H$_3$), 41.1 (C-1), 45.4 (NMe$_2$), 47.4 (C-10), 54.9 (C-2), 57.7 (C-2″), 61.0 (C-6⁗), 64.8 (C-6‴), 66.1 (C-1″), 66.6 (C-4‴), 67.0 (C-4⁗), 68.3 (C-2⁗), 68.6 (C-2‴), 69.8 (C-5⁗), 70.0 (C-3‴), 70.2 (C-3⁗), 70.5 (C-5‴), 99.0 (C-1⁗), 99.6 (C-1‴), 103.1 (C-4′), 103.4 (C-4), 105.2 (C-3′), 113.0 (C-7′), 115.7 (C-6′), 119.4 (C-9b), 122.0 (C-6), 122.7 (C-5a), 122.9 (C-9), 124.3 (C-7), 127.3 (C-3a′), 127.5 (C-8), 129.4 (C-9a), 130.6 (C-2′), 131.5 (C-7a′), 141.7 (C-3a), 152.7 (C-5), 152.8 (C-5′), 160.2 (C=O), 168.7, 169.1, 169.2 (2 signals), 169.3, 169.5 ppm (2 signals; 7×*C*(O)CH$_3$); IR (KBr): $\tilde{\nu}$=2942, 1753, 1627, 1519, 1462, 1370, 1221, 1068, 760 cm^{-1}; UV (MeOH): λ_{max} (lg ε)=202.5 (4.7014), 298.5 (4.4572), 332.5 nm (4.443); HRMS (ESI): *m*/*z*: calcd for $C_{52}H_{60}ClN_3O_{20}$: 1082.3532; found: 1082.3537 [*M*+H]$^+$.

Compound 10a: According to the general procedure C, a solution of the acetylated prodrug **8a** (131 mg, 121 μmol, 1.0 equiv) in MeOH (18 mL) was treated with NaOMe (67.1 μL, 362 μmol, 30% solution in MeOH, 3.0 equiv) for 1 h at room temperature. Workup and purification by RP-HPLC with water (A), MeOH (B)+0.05% acetic acid as the eluent (gradient: 0–15 min: 70A/30B→0A/100B, 15–20 min: 0A/100B, 20–21 min: 0A/100B→70A/30B, 21–26 min: 70A/30B, flow: 18 mL min^{-1}; λ=254 nm; injection volume: 0.8 mL; t_R=7.0 min) gave **10a** as a slightly ocher solid (78.9 mg, 100 μmol, 83%). R_f=0.15 (MeOH/H$_2$O 5:1, 0.5% HOAc); α=−14.1° (*c*=0.21 in DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=2.25 (s, 6H; NMe$_2$), 2.66 (t, *J*=5.9 Hz, 2H; 2″-H$_2$), 3.29 (dd, *J*=9.5, 3.4 Hz, 1H; 3⁗-H), 3.36–3.41 (m, 2H; 2⁗-H, 5⁗-H), 3.48 (dd, *J*=9.6, 2.8 Hz, 1H; 3‴-H), 3.54 (m$_c$, *J*=10.9, 6.2 Hz, 2H; 6⁗-H$_a$, 6⁗-H$_b$), 3.62 (d, *J*=3.3 Hz, 1H; 4⁗-H), 3.65 (dd, *J*=10.2, 5.6 Hz, 1H; 6‴-H$_a$), 3.79 (m$_c$, 2H; 2‴-H, 5‴-H), 3.84 (d, *J*=2.8 Hz, 1H; 4‴-H), 3.91 (dd, *J*=11.2, 7.4 Hz, 1H; 10-H$_a$), 3.98 (dd, *J*=10.2, 6.6 Hz, 1H; 6‴-H$_b$), 4.04–4.09 (m, 3H; 10-H$_b$, 1″-H$_2$), 4.17 (d, *J*=7.6 Hz, 1H; 1⁗-H), 4.30 (m$_c$, 1H; 1-H), 4.60 (dd, *J*=10.8, 1.7 Hz, 1H; 2-H$_a$), 4.83 (t, *J*=10.0 Hz, 1H; 2-H$_b$), 4.92 (d, *J*=7.3 Hz, 1H; 1‴-H), 5.32 (brs, 1H; OH), 6.93 (dd, *J*=8.9, 2.4 Hz, 1H; 6′-H), 7.13 (d, *J*=1.6 Hz, 1H; 3′-H), 7.18 (d, *J*=2.3 Hz, 1H; 4′-H), 7.44 (m$_c$, 2H; 7-H, 7′-H), 7.58 (t, *J*=7.6 Hz, 1H; 8-H), 7.93 (d, *J*=8.4 Hz, 1H; 9-H), 8.28 (brs, 1H; 4-H), 8.36 (d, *J*=8.5 Hz, 1H; 6-H), 11.46 ppm (d, *J*=1.2 Hz, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=41.2 (C-1), 45.5 (NMe$_2$), 47.4 (C-10), 54.9 (C-2), 57.7 (C-2″), 60.5 (C-6⁗), 66.2 (C-1″), 67.2 (C-6‴), 67.9 (C-4⁗), 68.1 (C-4‴), 70.3 (C-2‴), 70.4 (C-2⁗), 72.8 (C-3‴), 73.1 (C-3⁗), 73.5 (C-5‴), 75.1 (C-5⁗), 102.1 (C-4), 102.3 (C-1‴), 103.0 (C-4′), 103.4 (C-1⁗), 105.4 (C-3′), 113.1 (C-7′), 115.8 (C-6′), 118.1 (C-9b), 122.6 (C-9), 122.9 (C-5a), 123.2 (C-6), 123.7 (C-7), 127.3 (2 signals; C-8, C-3a′), 129.3 (C-9a), 130.5 (C-2′), 131.5 (C-7a′), 141.8 (C-3a), 152.8 (C-5′), 153.6 (C-5), 160.2 ppm (C=O); IR (KBr): $\tilde{\nu}$=3386, 1589, 1516, 1462, 1413, 1267, 1075, 761 cm^{-1}; UV (MeOH): λ_{max} (lg ε)=206.5 (4.629), 300.0 (4.4603), 336.5 nm (4.4265); HRMS (ESI): *m*/*z*: calcd for $C_{38}H_{46}ClN_3O_{13}$: 810.2611; found: 810.2629 [*M*+Na]$^+$.

Compound 8b: According to general procedure B, a stirred mixture of the trichloroacetimidate **6b** (220 mg, 281 μmol, 1.25 equiv), phenol (−)-(1*S*)-**5** (75 mg, 225 μmol, 1.0 equiv), and molecular sieves 4 Å (500 mg) in CH_2Cl_2 (8 mL) was treated with BF_3·OEt_2 (14.3 μL, 113 μmol, in 1.2 mL CH_2Cl_2, 0.5 equiv) at −16 °C and stirring was continued at −10 °C for 3.5 h. Additional BF_3·OEt_2 (85.5 μL, 675 μmol, in 1.0 mL CH_2Cl_2, 3.0 equiv) was added and the mixture kept at RT for 5 h. Evaporation and subsequent reaction with DMAI·HCl (**7**) (96.0 mg, 338 μmol, 1.5 equiv) and EDC·HCl (129 mg, 675 μmol, 3.0 equiv) in DMF (10 mL) for 17.5 h gave crude material that was purified by column chromatography to afford the peracetylated prodrug **8b** (150 mg, 139 μmol, 62%) as a pale-yellow solid. R_f=0.32 (CH_2Cl_2/MeOH 10:1); α=+74.0° (*c*=0.2 in MeOH); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=1.89, 1.92, 1.95, 2.05, 2.07 (2 signals), 2.19 (7×s, 7×3H; 7×C(O)CH$_3$), 2.25 (s, 6H; NMe$_2$), 2.67 (t, *J*=5.8 Hz, 2H; 2″-H$_2$), 3.55 (dd, *J*=11.5, 2.6 Hz, 1H; 6‴-H$_a$), 3.71 (dd, *J*=11.5, 3.6 Hz, 1H; 6‴-H$_b$), 3.83 (ddd, *J*=9.6, 5.4, 2.5 Hz, 1H; 5⁗-H), 3.91–3.96 (m, 2H; 10-H$_a$, 6⁗-H$_a$), 4.07 (m$_c$, 5H; 10-H$_b$, 1″-H$_2$, 5‴-H, 6⁗-H$_b$), 4.33 (m$_c$, 1H; 1-H), 4.60 (dd, *J*=10.8, 1.9 Hz, 1H; 2-H$_a$), 4.83 (t, *J*=10.0 Hz, 1H; 2-H$_b$), 4.86 (d, *J*=1.3 Hz, 1H; 1⁗-H), 5.04 (t, *J*=10.0 Hz, 1H; 4⁗-H), 5.10 (dd, *J*=10.2, 3.4 Hz, 1H; 3⁗-H), 5.15 (dd, *J*=3.4, 1.6 Hz, 1H; 2⁗-H), 5.46 (t, *J*=10.1 Hz, 1H; 4‴-H), 5.52 (dd, *J*=3.2, 2.0 Hz, 1H; 2‴-H), 5.61 (dd, *J*=10.2, 3.3 Hz, 1H; 3‴-H), 5.91 (s, 1H; 1‴-H), 6.92 (dd, *J*=8.9, 2.4 Hz, 1H; 6′-H), 7.08 (d, *J*=1.8 Hz, 1H; 3′-H), 7.17 (d, *J*=2.3 Hz, 1H; 4′-H), 7.39 (d, *J*=8.9 Hz, 1H; 7′-H), 7.54 (ddd, *J*=8.1, 7.0, 0.8 Hz, 1H; 7-H), 7.63 (ddd, *J*=8.1, 7.0, 1.0 Hz, 1H; 8-H), 7.99 (d, *J*=8.4 Hz, 1H; 9-H), 8.16 (d, *J*=8.4 Hz, 1H; 6-H), 8.25 (brs, 1H; 4-H), 11.50 ppm (d, *J*=1.6 Hz, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=20.2, 20.3 (2 signals; 3×C(O)*C*H$_3$), 20.4 (2 signals; 3×C(O)*C*H$_3$), 20.5 (C(O)*C*H$_3$), 41.1 (C-1), 45.4 (NMe$_2$), 47.4 (C-10), 54.9 (C-2), 57.7 (C-2″), 61.7 (C-6⁗), 65.0 (C-4‴), 65.1 (C-4⁗), 65.4 (C-6‴),

66.1 (C-1″), 67.8 (C-5‴), 68.3 (C-2‴), 68.5 (C-3‴), 68.6 (C-2‴), 68.7 (C-3‴), 69.8 (C-5‴), 95.4 (C-1‴), 96.8 (C-1‴), 101.7 (C-4), 103.1 (C-4′), 105.2 (C-3′), 112.9 (C-7′), 115.7 (C-6′), 119.0 (C-9b), 121.8 (C-6), 122.3 (C-5a), 123.1 (C-9), 124.4 (C-7), 127.3 (C-3a′), 127.6 (C-8), 129.5 (C-9a), 130.6 (C-2′), 131.4 (C-7a′), 141.8 (C-3a), 151.0 (C-5), 152.8 (C-5′), 160.0 (C=O), 169.1 (2 signals; 3×*C*(O)CH$_3$), 169.3, 169.4, 169.6 ppm (2 signals; 4×*C*(O)CH$_3$); IR (KBr): $\tilde{\nu}$ = 2945, 1752, 1627, 1519, 1462, 1371, 1225, 1085, 1048, 759 cm^{-1}; UV (MeOH): λ_{max} (lg ε) = 205.5 (4.6778), 298.5 (4.5086), 335.0 nm (4.4884); HRMS (ESI): *m*/*z*: calcd for $C_{52}H_{60}ClN_3O_{20}$: 1082.3531; found: 1082.3557 [*M*+H]$^+$.

Compound 10b: By following general procedure C, a solution of the acetylated prodrug **8b** (133 mg, 123 μmol, 1.0 equiv) in MeOH (10 mL) was treated with NaOMe (68.5 μL, 370 μmol, 30% solution in MeOH, 3.0 equiv) and the mixture stirred for 2 h at RT. Workup and purification by RP-HPLC with water (A), MeOH (B)+0.05% acetic acid as the eluent (gradient: 0–15 min: 70A/30B→0A/100B, 15–20 min: 0A/100B, 20–21 min: 0A/100B→70A/30B, 21–26 min: 70A/30B, flow: 18 mL min^{-1}; λ = 254 nm; injection volume: 0.8 mL; t_R = 8.0 min) gave **10b** as a slightly ocher solid (93.2 mg, 118 μmol, 96%). R_f = 0.15 (MeOH/H$_2$O 5:1, 0.5% HOAc); α = +72.6° (*c* = 0.17 in DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 2.24 (s, 6H; NMe$_2$), 2.66 (t, *J* = 5.8 Hz, 2H; 2″-H$_2$), 3.35–3.40 (m, 2H; 4‴-H, 5‴-H), 3.43 (dd, *J* = 11.4, 5.1 Hz, 1H; 6‴-H$_a$), 3.46 (d, *J* = 10.9 Hz, 2H; 6‴-H$_a$, 3‴-H), 3.51 (dd, *J* = 3.1, 1.5 Hz, 1H; 2‴-H), 3.58 (m$_c$, 2H; 5‴-H, 6‴-H$_b$), 3.70 (t, *J* = 9.5 Hz, 1H; 4‴-H), 3.74 (dd, *J* = 11.2, 4.9 Hz, 1H; 6‴-H$_b$), 3.89–3.94 (m, 2H; 10-H$_a$, 3‴-H), 4.03–4.09 (m, 4H; 10-H$_b$, 1″-H$_2$, 2‴-H), 4.30 (m$_c$, 1H; 1-H), 4.51 (d, *J* = 1.2 Hz, 1H; 1‴-H), 4.58 (dd, *J* = 10.8, 2.0 Hz, 1H; 2-H$_a$), 4.85 (t, *J* = 9.9 Hz, 1H; 2-H$_b$), 5.62 (s, 1H; 1‴-H), 6.92 (dd, *J* = 8.9, 2.4 Hz, 1H; 6′-H), 7.10 (s, 1H; 3′-H), 7.17 (d, *J* = 2.2 Hz, 1H; 4′-H), 7.41 (d, *J* = 8.9 Hz, 1H; 7′-H), 7.47 (t, *J* = 7.7 Hz, 1H; 7-H), 7.58 (t, *J* = 7.5 Hz, 1H; 8-H), 7.93 (d, *J* = 8.3 Hz, 1H; 9-H), 8.13 (d, *J* = 8.4 Hz, 1H; 6-H), 8.24 (s, 1H; 4-H), 11.61 ppm (brs, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 41.1 (C-1), 45.5 (NMe$_2$), 47.5 (C-10), 54.9 (C-2), 57.8 (C-2″), 61.0 (C-6‴), 65.0 (C-6‴), 66.1 (C-4‴), 66.2 (C-1″), 66.9 (C-4‴), 70.0 (C-2‴), 70.1 (C-2‴), 70.8 (C-3‴), 71.1 (C-3‴), 73.2 (C-5‴), 73.4 (C-5‴), 98.9 (C-1‴), 99.3 (C-1‴), 101.6 (C-4), 103.1 (C-4′), 105.2 (C-3′), 113.0 (C-7′), 115.6 (C-6′), 117.9 (C-9b), 122.3 (C-6), 122.7 (C-5a), 122.9 (C-9), 123.9 (C-7), 127.3 (2 signals) (C-8, C-3a′), 129.5 (C-9a), 130.8 (C-2a′), 131.5 (C-7a′), 141.9 (C-3a), 151.8 (C-5), 152.8 (C-5′), 160.1 ppm (C=O); IR (KBr): $\tilde{\nu}$ = 3383, 1580, 1516, 1462, 1413, 1065, 972, 760 cm^{-1}; UV (MeOH): λ_{max} (lg ε) = 202.0 (5.3727), 203.0 (4.7069), 300.0 (4.4287), 335.5 nm (4.4016); HRMS (ESI): *m*/*z*: calcd for $C_{38}H_{46}ClN_3O_{13}$: 788.2792; found: 788.2788 [*M*+H]$^+$.

Compound 8c: According to general procedure B, a stirred solution of the mannose trichloroacetimidate **6c** (386 mg, 782 μmol, 1.45 equiv), phenol (−)-(1*S*)-**5** (180 mg, 539 μmol, 1.0 equiv), and molecular sieves 4 Å (1.30 g) in CH_2Cl_2 (25 mL) was treated with BF_3·OEt_2 (34.0 μL, 270 μmol, in 2.8 mL CH_2Cl_2, 0.5 equiv) at −20 °C and the mixture stirred at −10 °C for 6.5 h. Additional BF_3·OEt_2 (205 μL, 1.62 mmol, in 2.4 mL CH_2Cl_2, 3.0 equiv) was added and the mixture kept at RT for 5 h. Evaporation and subsequent reaction with DMAI·HCl (**7**) (230 mg, 809 μmol, 1.5 equiv) and EDC·HCl (310 mg, 1.62 mmol, 3.0 equiv) in DMF (25 mL) for 16 h after purification gave prodrug **8c** (209 mg, 263 μmol, 49%) as a pale-yellow solid. R_f = 0.38 (CH_2Cl_2/MeOH 10:1); α = +67.4° (*c* = 0.5 in MeOH); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 1.87 (s, 3H; C(O)CH$_3$), 2.04 (2 signals, 2×s, 2×3H; 2×C(O)CH$_3$), 2.19 (s, 3H; C(O)CH$_3$), 2.24 (s, 6H; NMe$_2$), 2.66 (t, *J* = 5.8 Hz, 2H; 2″-H$_2$), 3.95 (dd, *J* = 11.3, 6.6 Hz, 1H; 10-H$_a$), 3.96 (dd, *J* = 12.4, 2.6 Hz, 1H; 6‴-H$_a$), 4.04–4.08 (m, 3H; 10-H$_b$, 1″-H$_2$), 4.10 (dd, *J* = 5.8, 2.6 Hz, 1H; 5‴-H), 4.21 (dd, *J* = 12.3, 5.7 Hz, 1H; 6‴-H$_b$), 4.34 (m$_c$, 1H; 1-H), 4.60 (dd, *J* = 10.8, 2.1 Hz, 1H; 2-H$_a$), 4.82 (t, *J* = 10.0 Hz, 1H; 2-H$_b$), 5.26 (t, *J* = 10.0 Hz, 1H; 4‴-H), 5.55 (dd, *J* = 3.5, 1.8 Hz, 1H; 2‴-H), 5.57 (dd, *J* = 10.0, 3.5 Hz, 1H; 3‴-H), 5.88 (s, 1H; 1‴-H), 6.93 (dd, *J* = 8.9, 2.4 Hz, 1H; 6′-H), 7.10 (d, *J* = 1.9 Hz, 1H; 3′-H), 7.17 (d, *J* = 2.3 Hz, 1H; 4′-H), 7.40 (d, *J* = 8.9 Hz, 1H; 7′-H), 7.54 (t, *J* = 7.6 Hz, 1H; 7-H), 7.63 (t, *J* = 7.7 Hz, 1H; 8-H), 7.99 (d, *J* = 8.4 Hz, 1H; 9-H), 8.15 (d, *J* = 8.4 Hz, 1H; 6-H), 8.29 (brs, 1H; 4-H), 11.55 ppm (d, *J* = 1.5 Hz, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 20.1, 20.3, 20.4, 20.5 (4 x C(O)*C*H$_3$), 41.1 (C-1), 45.5 (NMe$_2$), 47.5 (C-10), 54.9 (C-2), 57.8 (C-2″), 61.7 (C-6‴), 65.2 (C-4‴), 66.3 (C-1″), 68.6 (2 signals; C-2‴, C-3‴), 69.0 (C-5‴), 95.7 (C-1‴), 101.8 (C-4), 103.2 (C-4′), 105.3 (C-3′), 113.1 (C-7′), 115.9 (C-6′), 119.0 (C-9b), 121.9 (C-6), 122.3 (C-5a), 123.1 (C-9), 124.5 (C-7), 127.4 (C-3a′), 127.7 (C-8), 129.6 (C-9a), 130.7 (C-2′), 131.6 (C-7a′), 141.9 (C-3a), 151.2 (C-5), 153.0 (C-5′), 160.1 (C=O), 169.4, 169.6, 169.7, 169.8 ppm (4×*C*(O)CH$_3$); IR (KBr): $\tilde{\nu}$ = 2952, 1752, 1627, 1518, 1462, 1397, 1228, 1050, 760 cm^{-1}; UV (MeOH): λ_{max} (lg ε) = 204.5 (4.6945), 299.0 (4.5186), 335.0 nm (4.5033); HRMS (ESI): *m*/*z*: calcd for $C_{40}H_{44}ClN_3O_{12}$: 794.2686; found: 794.2676 [*M*+H]$^+$.

Compound 10c: By following general procedure C, a solution of the acetylated prodrug **8c** (170 mg, 214 μmol, 1.0 equiv) in MeOH (9 mL) was treated with NaOMe (40 μL, 214 μmol, 30% solution in MeOH, 1.0 equiv) for 30 min at RT. Workup and purification by RP-HPLC with water (A), MeOH (B)+0.05% acetic acid as the eluent (gradient: 0–15 min: 70A/30B→0A/100B, 15–20 min: 0A/100B, 20–21 min: 0A/100B→70A/30B, 21–26 min: 70A/30B, flow: 18 mL min^{-1}; λ = 254 nm; injection volume: 1 mL; t_R = 8.4 min) gave **10c** as a slightly ocher solid (120 mg, 192 μmol, 90%). R_f = 0.20 (MeOH/H$_2$O 5:1, 0.5% HOAc); α = +68.6° (*c* = 0.45 in DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 2.25 (s, 6H; NMe$_2$), 2.66 (t, *J* = 5.9 Hz, 2H; 2″-H$_2$), 3.43 (m$_c$, *J* = 9.4, 5.0, 2.2 Hz, 1H; 5‴-H), 3.50 (dd, *J* = 11.8, 5.1 Hz, 1H; 6‴-H$_a$), 3.57 (dd, *J* = 11.8, 2.1 Hz, 1H; 6‴-H$_b$), 3.64 (t, *J* = 9.5 Hz, 1H; 4‴-H), 3.89–3.94 (m, 2H, 10-H$_a$; 3‴-H), 4.03–4.09 (m, 4H; 10-H$_b$, 1″-H$_2$, 2‴-H), 4.29 (m$_c$, 1H; 1-H), 4.59 (dd, *J* = 10.8, 1.9 Hz, 1H; 2-H$_a$), 4.84 (t, *J* = 10.0 Hz, 1H; 2-H$_b$), 5.68 (s, 1H; 1‴-H), 6.93 (dd, *J* = 8.9, 2.4 Hz, 1H; 6′-H), 7.11 (d, *J* = 1.7 Hz, 1H; 3′-H), 7.18 (d, *J* = 2.2 Hz, 1H; 4′-H), 7.40 (d, *J* = 8.9 Hz, 1H; 7′-H), 7.47 (t, *J* = 7.6 Hz, 1H; 7-H), 7.58 (t, *J* = 7.6 Hz, 1H; 8-H), 7.93 (d, *J* = 8.4 Hz, 1H; 9-H), 8.14 (d, *J* = 8.4 Hz, 1H; 6-H), 8.23 (brs, 1H; 4-H), 11.58 ppm (brs, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 41.1 (C-1), 45.5 (NMe$_2$), 47.5 (C-10), 55.1 (C-2), 57.8 (C-2″), 60.7 (C-6‴), 66.2 (C-1″), 66.5 (C-4‴), 70.1 (C-2‴), 71.0 (C-3‴), 75.4 (C-5‴), 98.5 (C-1‴), 101.0 (C-4), 103.2 (C-4′), 105.3 (C-3′), 113.1 (C-7′), 115.9 (C-6′), 117.8 (C-9b), 122.4 (C-6), 122.7 (C-5a), 123.0 (C-9), 124.0 (C-7), 127.4 (C-3a′), 127.5 (C-8), 129.6 (C-9a), 130.8 (C-2′), 131.6 (C-7a′), 142.1 (C-3a), 151.9 (C-5), 153.0 (C-5′), 160.2 ppm (C=O); IR (KBr): $\tilde{\nu}$ = 3385, 1611, 1514, 1461, 1415, 1264, 1181, 1065, 973, 755 cm^{-1}; UV (MeOH): λ_{max} (lg ε) = 204.5 (4.6462), 300.0 (4.4595), 336.5 nm (4.4273); HRMS (ESI): *m*/*z*: calcd for $C_{32}H_{36}ClN_3O_8$: 626.2264; found: 626.2263 [*M*+H]$^+$.

Compound 8d: According to general procedure B, a stirred mixture of the trichloroacetimidate **6d** (170 mg, 355 μmol, 1.18 equiv), phenol (−)-(1*S*)-**5** (100 mg, 300 μmol, 1.0 equiv), and molecular sieves 4 Å (550 mg) in CH_2Cl_2 (10 mL) was treated with BF_3·OEt_2 (19.0 μL, 150 μmol, in 1.6 mL CH_2Cl_2, 0.5 equiv) at −18 °C and stirring was continued at −10 °C for 4 h. Additional BF_3·OEt_2 (114 μL, 900 μmol, in 1.3 mL CH_2Cl_2, 3.0 equiv) was added and the mixture kept at RT for 5 h. Evaporation and subsequent reaction with DMAI·HCl (**7**) (128 mg, 150 μmol, 1.5 equiv) and EDC·HCl (172 mg, 900 μmol, 3.0 equiv) in DMF (14 mL) for 19 h after purification yielded **8d** (89.5 mg, 155 μmol, 39%) as a slightly ocher solid. R_f = 0.35 (CH_2Cl_2/MeOH 10:1); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 2.02, 2.03 (2 signals; 3×s, 3×3H; 3×C(O)CH$_3$), 2.25 (s, 6H; NMe$_2$), 2.67 (t, *J* = 5.8 Hz, 2H; 2″-H$_2$), 3.67 (s, 3H; OCH$_3$), 3.93 (dd, *J* = 11.2, 7.0 Hz, 1H; 10-H$_a$), 4.03–4.10 (m, 3H; 10-H$_b$, 1″-H$_2$), 4.31 (m$_c$, 1H; 1-H), 4.60 (dd, *J* = 10.8, 2.0 Hz, 1H; 2-H$_a$), 4.74 (d, *J* = 9.8 Hz, 1H; 5‴-H), 4.83 (t, *J* = 10.0 Hz, 1H; 2-H$_b$), 5.15 (t, *J* = 9.6 Hz, 1H; 4‴-H), 5.34 (dd, *J* = 9.7, 7.8 Hz, 1H; 2‴-H), 5.60 (t, *J* = 9.6 Hz, 1H; 3‴-H), 5.81 (d, *J* = 7.8 Hz, 1H; 1‴-H), 6.93 (dd, *J* = 8.9, 2.4 Hz, 1H; 6′-H), 7.11 (d, *J* = 1.7 Hz, 1H; 3′-H), 7.18 (d, *J* = 2.3 Hz, 1H; 4′-H), 7.41 (d, *J* = 8.9 Hz, 1H; 7′-H), 7.48 (ddd, *J* = 8.1, 6.9, 0.9 Hz, 1H; 7-H), 7.60 (ddd, *J* = 8.1, 7.0, 1.1 Hz, 1H; 8-H), 7.96 (d, *J* = 8.4 Hz, 1H; 9-H), 7.99 (d, *J* = 8.5 Hz, 1H; 6-H), 8.23 (s, 1H; 4-H), 11.61 ppm (d, *J* = 1.5 Hz, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 20.2, 20.3 (2 signals; 3×C(O)*C*H$_3$), 41.1 (C-1), 45.5 (NMe$_2$), 47.4 (C-10), 52.5 (OCH$_3$), 55.0 (C-2), 57.7 (C-2″), 66.2 (C-1″), 69.0 (C-4‴), 70.6 (2 signals; C-2‴, C-3‴), 71.0 (C-5‴), 98.2 (C-1‴), 102.4 (C-4), 103.1 (C-4′), 105.2 (C-3′), 113.0 (C-7′), 115.8 (C-6′), 119.4 (C-9b), 121.8 (C-6), 122.4 (C-5a), 122.9 (C-9), 124.3 (C-7), 127.3 (C-3a′), 127.6 (C-8), 129.3 (C-9a), 130.5 (C-2′), 131.5 (C-7a′), 141.7 (C-3a), 152.3 (C-5), 152.8 (C-5′), 160.0 (C(O)N), 166.8 (C-6‴), 169.0 (2 signals), 169.1 ppm (3×*C*(O)CH$_3$); IR (KBr): $\tilde{\nu}$ = 1759, 1626, 1517, 1462, 1413, 1218, 1040, 757 cm^{-1}; UV (MeOH): λ_{max} (lg ε) = 204.0 (4.1497), 298.5 (3.932), 335.0 nm (3.9196); HRMS (ESI): *m*/*z*: calcd for $C_{39}H_{42}ClN_3O_{12}$: 780.2530; found: 780.2525 [*M*+H]$^+$.

Compound 10d: By following general procedure C, a solution of **8d** (30 mg, 38.5 μmol, 1.0 equiv) in MeOH (12 mL) was treated with NaOMe (4.4 μL, 19.2 μmol, 25% solution in MeOH, 0.5 equiv) and stirred for 9 h at room temperature. After workup and purification by column chromatography (CH_2Cl_2/MeOH 1:1) compound **10d** (23.5 mg, 35.9 μmol, 93%, 99% purity (LC-MS)) was obtained as a slightly ocher solid. R_f=0.23 (CH_2Cl_2/MeOH 1:1); α=−24.8° (c=0.20 in DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=2.31 (s, 6H; NMe_2), 2.75 (m$_c$, 2H; 2″-H$_2$), 3.40 (t, J=9.0 Hz, 1H; 3‴-H), 3.46–3.55 (m, 2H; 2‴-H, 4‴-H), 3.68 (s, 3H; OCH_3), 3.93 (dd, J=11.1, 7.3 Hz, 1H; 10-H$_a$), 3.97 (d, J=9.6 Hz, 1H; 5‴-H), 4.06 (dd, J=11.1, 2.9 Hz, 1H; 10-H$_b$), 4.10 (t, J=5.7 Hz, 2H; 1″-H$_2$), 4.31 (m$_c$, 1H; 1-H), 4.59 (d, J=10.8 Hz, 1H; 2-H$_a$), 4.82 (t, J=10.1 Hz, 1H; 2-H$_b$), 5.12 (d, J=7.7 Hz, 1H; 1‴-H), 5.28, 5.40, 5.61 (3×brs, 3×1H; 3×OH), 6.93 (dd, J=8.9, 2.2 Hz, 1H; 6′-H), 7.10 (s, 1H; 3′-H), 7.18 (d, J=1.7 Hz, 1H; 4′-H), 7.41 (d, J=8.9 Hz, 1H; 7′-H), 7.45 (t, J=7.6 Hz, 1H; 7-H), 7.59 (t, J=7.6 Hz, 1H; 8-H), 7.93 (d, J=8.3 Hz, 1H; 9-H), 8.16 (brs, 1H; 4-H), 8.33 (d, J=8.5 Hz, 1H; 6-H), 11.62 ppm (s, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=41.2 (C-1), 45.2 (NMe_2), 47.4 (C-10), 51.9 (OCH_3), 54.9 (C-2), 57.5 (C-2″), 65.8 (C-1″), 71.4 (C-4‴), 73.0 (C-2‴), 75.3 (C-3‴), 75.5 (C-5‴), 101.3 (C-1‴), 102.0 (C-4), 103.2 (C-4′), 105.2 (C-3′), 113.1 (C-7′), 115.7 (C-6′), 118.4 (C-9b), 122.7 (C-9), 122.8 (C-5a), 123.1 (C-6), 123.8 (C-7), 127.3 (C-3a′), 127.5 (C-8), 129.4 (C-9a), 130.7 (C-2′), 131.6 (C-7a′), 141.8 (C-3a), 152.7 (C-5′), 153.0 (C-5), 160.0 (C(O)N), 168.8 ppm (C-6‴); IR (KBr): $\tilde{\nu}$= 3386, 1745, 1625, 1516, 1462, 1414, 1267, 1178, 1065, 759 cm^{-1}; UV (MeOH): λ_{max} (lg ε)=271.0 (5.0708), 334.0 nm (4.3873); HRMS (ESI): m/z: calcd for $C_{33}H_{36}ClN_3O_9$: 654.2213; found: 654.2212 $[M+H]^+$.

Compound 8e: According to general procedure B, a stirred mixture of the trichloroacetimidate **6e** (312 mg, 562 μmol, 1.25 equiv), phenol (−)-(1*S*)-**5** (150 mg, 449 μmol, 1.0 equiv), and molecular sieves 4 Å (820 mg) in CH_2Cl_2 (20 mL) was treated with BF_3·OEt_2 (28.0 μL, 225 μmol, in 2.4 mL CH_2Cl_2, 0.5 equiv) at −18 °C and stirring was continued at −10 °C for 3 h. Additional BF_3·OEt_2 (171 μL, 1.35 mmol, in 2.0 mL CH_2Cl_2, 3.0 equiv) was added and the mixture kept at RT for 5 h. Evaporation and subsequent reaction with DMAI·HCl (**7**) (192 mg, 674 μmol, 1.5 equiv) and EDC·HCl (259 mg, 1.35 mmol, 3.0 equiv) in DMF (20 mL) for 15 h yielded the peracetylated prodrug **8e** (185 mg, 216 μmol, 48%) as slightly a ocher solid after purification. R_f=0.38 (CH_2Cl_2/MeOH 10:1); α=−4.7° (c=0.22 in $CHCl_3$); ^{1}H NMR (600 MHz, $CDCl_3$): δ= 1.85, 2.04, 2.05 (3×s, 3×3H; 3×C(O)CH_3), 2.39 (s, 6H; NMe_2), 2.81 (t, J=5.6 Hz, 2H; 2″-H$_2$), 3.40 (t, J=11.0 Hz, 1H; 10-H$_a$), 3.95 (dd, J=11.4, 3.1 Hz, 1H; 10-H$_b$), 4.13 (t, J=5.7 Hz, 2H; 1″-H$_2$), 4.15–4.19 (m, 1H; 1-H), 4.35 (d, J=8.8 Hz, 1H; 5‴-H), 4.66 (t, J=9.6 Hz, 1H; 2-H$_a$), 4.78 (dd, J=10.6, 1.7 Hz, 1H; 2-H$_b$), 5.10 (d, J=2.1 Hz, 2H; OCH_2Ph), 5.37 (t, J=9.2 Hz, 1H; 3‴-H), 5.43 (t, J=9.4 Hz, 1H; 4‴-H), 5.43 (d, J= 7.3 Hz, 1H; 1‴-H), 5.51 (dd, J=8.9, 7.7 Hz, 1H; 2‴-H), 7.01 (d, J= 1.5 Hz, 1H; 3′-H), 7.02 (dd, J=9.0, 2.3 Hz, 1H; 6′-H), 7.13 (d, J=2.0 Hz, 1H; 4′-H), 7.23–7.27 (m, 3H; 3×Ph-H), 7.27–7.32 (m, 3H; 7′-H, 2×Ph-H), 7.40 (t, J=7.6 Hz, 1H; 7-H), 7.54 (t, J=7.5 Hz, 1H; 8-H), 7.69 (d, J=8.3 Hz, 1H; 9-H), 8.13 (d, J=8.5 Hz, 1H; 6-H), 8.35 (brs, 1H; 4-H), 9.36 ppm (brs, 1H; NH); ^{13}C NMR (125 MHz, $CDCl_3$): δ=20.4, 20.7 (2 signals; 3×C(O)CH_3), 43.2 (C-1), 45.9 (NMe_2), 46.1 (C-10), 54.9 (C-2), 58.4 (C-2″), 66.5 (C-1″), 67.7 (OCH_2Ph), 69.2 (C-4‴), 70.8 (C-2‴), 71.9 (C-3‴), 72.7 (C-5‴), 99.1 (C-1‴), 102.2 (C-4), 103.5 (C-4′), 105.9 (C-3′), 112.6 (C-7′), 117.3 (C-6′), 118.7 (C-9b), 122.0 (C-9), 123.3 (C-6), 123.5 (C-5a), 124.6 (C-7), 127.9 (C-8), 128.2 (C-3a′), 128.3, 128.4 (2 signals; Ph-C$_o$, Ph-C$_m$, Ph-C$_p$), 129.6 (C-9a), 130.4 (C-2′), 131.2 (C-7a′), 134.7 (Ph-Ci), 141.7 (C-3a), 153.4 (C-5), 153.7 (C-5′), 160.3 (C(O)N), 166.1 (C-6‴), 169.2, 169.3, 169.8 ppm (3×C(O)CH_3); IR (KBr): $\tilde{\nu}$=1759, 1626, 1516, 1461, 1396, 1216, 1039, 755 cm^{-1}; UV (MeOH): λ_{max} (lg ε)=299.0 (3.8214), 334.0 nm (3.796); HRMS (ESI): m/z: calcd for $C_{45}H_{46}ClN_3O_{12}$: 856.2843; found: 856.2866 $[M+H]^+$.

Compound 9: A mixture of the benzyl-protected acid **8e** (167 mg, 195 μmol, 1.0 equiv) and palladium on charcoal (10%, 125 mg, 117 μmol, 0.6 equiv of Pd) in MeOH/EtOAc (66.0 mL, 1:10) was stirred under a H_2 atmosphere (1 atm) at RT for 6 h. Filtration over Celite, washing with MeOH, and evaporation of the solvent yielded the crude product, which was then purified by column chromatography on silica gel (CH_2Cl_2/MeOH 3:1). After membrane filtration, the debenzylated compound **9** (128 mg, 167 μmol, 86%) was obtained as a slightly yellow solid. R_f=0.33 (CH_2Cl_2/MeOH 3:1); α=+34.7° (c=0.20 in MeOH); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=1.92, 1.98 (2 signals; 3×s, 3×3H; 3×C(O)CH_3), 2.27 (s, 6H; NMe_2), 2.68 (t, J=5.8 Hz, 2H; 2″-H$_2$), 3.91 (dt, J=19.4, 9.7 Hz, 1H; 10-H$_a$), 4.03–4.12 (m, 4H; 10-H$_b$, 1″-H$_2$, 5‴-H), 4.31 (m$_c$, 1H; 1-H), 4.60 (dd, J=10.8, 2.0 Hz, 1H; 2-H$_a$), 4.82 (t, J=10.0 Hz, 1H; 2-H$_b$), 5.14 (t, J=9.7 Hz, 1H; 4‴-H), 5.26 (dd, J=9.8, 7.9 Hz, 1H; 2‴-H), 5.41 (t, J=9.6 Hz, 1H; 3‴-H), 5.60 (d, J=7.8 Hz, 1H; 1‴-H), 6.91 (dd, J=8.9, 2.4 Hz, 1H; 6′-H), 7.11 (d, J=1.7 Hz, 1H; 3′-H), 7.17 (d, J= 2.3 Hz, 1H; 4′-H), 7.40 (d, J=8.9 Hz, 1H; 7′-H), 7.45 (ddd, J=8.1, 6.9, 1.0 Hz, 1H; 7-H), 7.58 (ddd, J=8.2, 6.9, 1.1 Hz, 1H; 8-H), 7.95 (d, J= 8.4 Hz, 1H; 9-H), 8.01 (d, J=8.4 Hz, 1H; 6-H), 8.23 (brs, 1H; 4-H), 11.80 ppm (brs, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=20.3, 20.4, 20.6 (3×C(O)CH_3), 41.1 (C-1), 45.4 (NMe_2), 47.4 (C-10), 54.9 (C-2), 57.7 (C-2″), 66.1 (C-1″), 70.4 (C-4‴), 71.1 (C-2‴), 72.2 (C-3‴), 74.5 (C-5‴), 98.2 (C-1‴), 102.1 (C-4), 103.1 (C-4′), 105.3 (C-3′), 113.0 (C-7′), 115.8 (C-6′), 118.8 (C-9b), 122.0 (C-6), 122.6 (C-5a), 122.8 (C-9), 124.1 (C-7), 127.3 (C-3a′), 127.5 (C-8), 129.3 (C-9a), 130.6 (C-2′), 131.6 (C-7a′), 141.8 (C-3a), 152.6 (C-5), 152.7 (C-5′), 160.0 (C(O)N), 168.3 (C-6‴), 168.6, 169.1, 169.2 ppm (3×C(O)CH_3); IR (KBr): $\tilde{\nu}$=2945, 1756, 1623, 1516, 1463, 1415, 1229, 1038, 761 cm^{-1}; UV (MeOH): λ_{max} (lg ε)=206.0 (4.6307), 298.5 (4.425), 334.0 nm (4.4237); HRMS (ESI): m/z: calcd for $C_{38}H_{40}ClN_3O_{12}$: 764.2228; found: 764.2226 $[M-H]^-$.

Compound 10e: By following general procedure C, a solution of the acetylated prodrug **9** (50 mg, 65.3 μmol, 1.0 equiv) in MeOH (10 mL) was treated with NaOMe (37.3 μL, 163 μmol, 25% solution in MeOH, 2.5 equiv) and stirred for 3 h at room temperature. After workup and purification by RP-HPLC with water (A), CH_3CN (B)+0.05% acetic acid as the eluent (gradient: 0–10 min: 80A/20B→60A/40B, 10–15 min: 60A/40B, 15–16 min: 60A/40B→80A/20B, 16–22 min: 80A/20B, flow: 12 mL min^{-1}; λ=299 nm; injection volume: 0.8 mL; t_R=11.8 min) the prodrug **10e** was obtained as a colorless solid (37.4 mg, 58.4 μmol, 89%). R_f=0.12 (CH_2Cl_2/MeOH 1:5); α=−23.2° (c=0.2 in DMSO); ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ=2.37 (s, 6H; NMe_2), 2.82 (s, 2H; 2″-H$_2$), 3.29 (m$_c$, 1H; 5‴-H), 3.35 (t, J=8.9 Hz, 1H; 3‴-H), 3.40 (t, J=9.1 Hz, 1H; 4‴-H), 3.48 (m$_c$, 1H; 2‴-H), 3.68 (brs, 1H; OH), 3.92 (dd, J=11.2, 7.5 Hz, 1H; 10-H$_a$), 4.06 (dd, J=11.2, 3.2 Hz, 1H; 10-H$_b$), 4.11 (m$_c$, J= 10.1, 4.9 Hz, 2H; 1″-H$_2$), 4.31 (m$_c$, 1H; 1-H), 4.59 (dd, J=8.8, 1.8 Hz, 1H; 2-H$_a$), 4.80 (t, J=10.0 Hz, 1H; 2-H$_b$), 5.05 (d, J=7.0 Hz, 1H; 1‴-H), 5.16, 5.49 (2×brs, 2×1H; 2×OH), 6.92 (dd, J=8.9, 2.4 Hz, 1H; 6′-H), 7.09 (s, 1H; 3′-H), 7.18 (d, J=2.2 Hz, 1H; 4′-H), 7.39 (d, J=8.9 Hz, 1H; 7′-H), 7.44 (ddd, J=8.1, 6.8, 1.0 Hz, 1H; 7-H), 7.58 (ddd, J=8.1, 6.8, 1.1 Hz, 1H; 8-H), 7.93 (d, J=8.4 Hz, 1H; 9-H), 8.17 (brs, 1H; 4-H), 8.33 (d, J=8.5 Hz, 1H; 6-H), 11.70 ppm (s, 1H; NH); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ=41.2 (C-1), 45.0 (NMe_2), 47.4 (C-10), 54.8 (C-2), 57.3 (C-2″), 65.5 (C-1″), 71.6 (C-4‴), 73.0 (C-2‴), 75.2 (C-5‴), 75.9 (C-3‴), 100.9 (C-1‴), 101.5 (C-4), 103.2 (C-4′), 105.1 (C-3′), 113.0 (C-7′), 115.7 (C-6′), 118.0 (C-9b), 122.6 (C-9), 122.8 (C-5a), 123.1 (C-6), 123.7 (C-7), 127.3 (C-3a′), 127.4 (C-8), 129.4 (C-9a), 130.7 (C-2′), 131.6 (C-7a′), 141.8 (C-3a), 152.6 (C-5′), 153.2 (C-5), 160.0 (C(O)N), 170.1 ppm (C-6‴); IR (KBr): $\tilde{\nu}$=3386, 1615, 1516, 1462, 1399, 1290, 1233, 1179, 1063, 759 cm^{-1}; UV (MeOH): λ_{max} (lg ε)=209.0 (4.6106), 239.0 (4.4127), 299.5 (4.3174), 329.5 nm (4.3425); HRMS (ESI): m/z: calcd for $C_{32}H_{34}ClN_3O_9$: 638.1911; found: 638.1915 $[M-H]^-$.

Serum stability studies: Preincubated human serum (495 μL, 37 °C) was treated with the appropriate prodrug (**10a–e**, 5.00 μL, 240 nmol, 48.0 mM stock solution in DMSO) to give a 480 μM reaction mixture at a final DMSO concentration of 1%. The solution was mixed for 5 s by using a vortex mixer and a sample of 40 μL (19.2 nmol prodrug) was taken immediately. Protein precipitation was initiated by adding an acetonitrile working solution of the standard **11** (100 μL, 4.80 nmol, 48 μM solution in CH_3CN, for structure see the Supporting Information) and mixing for 15 s. After centrifugation at 14000 U min^{-1}, 4 °C, 5 min) the supernatant was analyzed by HPLC-MS (see General Information). The remaining reaction mixture was incubated for 24 h at 37 °C. Further samples were taken at t=0.5, 1.0, 1.5, 2.0, 3.0, 4.0, 5.0, 6.0, 24.0 h and workup as well as HPLC-MS were done as described. Concerning interpretation, for all investigated times the area under the curve (AUC) of the corresponding ion currents of substrate and metabolite(s) was determined. The integrals

were normalized by referencing to the AUC of the standard **11**. The relative AUCs were plotted against time and the rate constant determined by using OriginPro 8G and Dynafit 3.[20]

In vitro cytotoxicity assays: Adherent cells of line A549 (human bronchial carcinoma cells) were sown in triplicate in 6 multiwell plates at concentrations of 10^2, 10^3, and 10^4 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, Lonza). Incubation with compounds **10a–e** was then performed in UltraCulture medium at 6–8 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 DMSO concentration 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 9–10 days. The medium was removed and the clones were dried, stained with Löffler's methylene blue (Merck, Darmstadt, Germany) and 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 0.4 U mL^{-1} α-D-mannosidase (EC 3.2.1.24, 1 U = 1 μmol min^{-1}, Sigma), 4.0 U mL^{-1} β-D-galactosidase (EC 3.2.1.23, 1 U = 1 μmol min^{-1}, Sigma), 4 U mL^{-1} β-D-glucuronidase (EC 3.2.1.31, 1 U = 0.0523 nmol min^{-1}, Sigma) to the cells during incubation with the substances.

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