Synthesis and Biological Studies of Different Duocarmycin Based Glycosidic Prodrugs for Their Use in the Antibody-Directed Enzyme Prodrug Therapy

Lutz F. Tietze,* Heiko J. Schuster, Birgit Krewer, and Ingrid Schuberth

Institute of Organic and Biomolecular Chemistry Georg-August-University Göttingen, Tammannstrasse 2, D-37077 Göttingen, Germany

Received July 22, 2008

The synthesis and biological evaluation of novel prodrugs for use in the antibody directed enzyme prodrug therapy (ADEPT) of cancer based on the cytotoxic antibiotic duocarmycin SA (**1**) are described. In this approach, we investigated the influence of the sugar moiety of the glycosidic prodrug on the QIC_{50} values as well as on the stability and the water solubility. The best result was found for prodrug **22** containing an α-mannoside moiety with a QIC_{50} value of 4500.

Introduction

One of the major disadvantages of the commonly used chemotherapeutics for the treatment of cancer is their insufficient differentiation between normal and tumor cells. One concept to overcome this problem is the antibody directed enzyme prodrug therapy (ADEPT[a]) introduced by Bagshawe in 1987.[1−5] Over the past decade, we successfully developed novel prodrugs for a use in ADEPT,[6] which are based on the antibiotic duocarmycin SA (**1**).[7] This natural product is an exceptionally 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). One of the best prodrugs developed by us is the glycoside **2** which contains the (1*S*,10*R*)-methyl-*seco*-CBI-skeleton as pharmacophoric unit,[8] a DMAI side chain[9] for binding to the minor groove of DNA and β-D-galactose as protecting group. Compound **2** has a rather low cytotoxicity of $IC_{50} = 3600$ nM but a very high cytotoxicity in the presence of the cleaving enzyme β-galactosidase to give the *seco*-drug **3**. This then reacts very fast in a buffer to the final drug **4** with an IC_{50} value of 0.75 nM by a Winstein cyclization. The difference in toxicities of prodrug **2** and drug **4** results in a QIC_{50} value of 4800 (IC_{50} of prodrug/ IC_{50} of prodrug in the presence of the cleaving enzyme),[10] which together with the high cytotoxicity of **4** makes **2** a promising candidate for a selective treatment of cancer using the ADEPT concept.

Whereas the mode of action of the duocarmycins and CC-1065[11] as well as yatakemycin[12] has been extensively investigated by Boger et al.,[13,14] there is little knowledge about the factors that influence the cytotoxicity of the prodrugs, which is directly correlated to the selectivity of these compounds in the treatment of cancer. Therefore, we have started a research program on the influence of the different components of the prodrugs on the QIC_{50} values. Within these studies, it was found that the detoxifying sugar moiety has a very strong effect on the QIC_{50} value and moreover on the stability of the prodrug toward hydrolysis.[15,16] The results of these investigations are described in the following.

Results and Discussion

The synthesis of the prodrugs containing different sugar components follows a general four-step route as shown in Scheme 2. First, enantiopure phenol (+)-(1*S*,10*R*)-**5**[17] is glycosylated with the corresponding trichloroacetimidates **6**−**11**, then the *N*-*tert*-butyloxycarbonyl group is removed and the formed secondary amine coupled with the DNA-binding unit DMAI·HCl (**12**) in *N*,*N*-dimethylformamide using EDC·HCl. The acetylated prodrugs are then converted into the prodrug under Zemplén deacetylation conditions with sodium methoxide in methanol.

By this way, prodrugs of the disaccharides D-lactose and D-cellobiose as well as of the monosaccharides D-glucose and D-mannose were synthesized; furthermore, L-rhamnose and the glucuronic acid methyl ester were coupled with **5**. First we prepared the corresponding trichloroacetimidates **6**−**11** employing trichloroacetonitrile in the presence of polymer-supported diaza(1,3)bicyclo[5.4.0]undecane (DBU).[18] The glycosidations of **5** with **6**−**11** were carried out in dichloromethane in the presence of molecular sieves (4 Å) and catalytic amounts of $BF_3 \cdot Et_2O$ at −15 °C. The following removal of the *N*-*tert*-butyloxycarbonyl protecting group to allow the introduction of the DNA-binder was accomplished in a one-pot procedure by addition of three more equivalents of $BF_3 \cdot Et_2O$, and the reaction with DMAI·HCl (**12**) was performed at room temperature by using EDC·HCl in *N*,*N*-dimethylformamide for 14−19.5 h to give the acetylated prodrugs **13**−**18** in 54−69% yield over three steps (Table 1). The final deacetylation using the Zemplén procedure then led to the desired prodrugs **19**−**24** in 50−82% yield.

For the purification of the two prodrugs **20** and **21** with a disaccharide moiety, preparative RP-HPLC was used as they show a significant higher polarity than the prodrugs coupled to a monosaccharide. Because of the neighboring participation effect of the acetyl group at C-2,[20,21] the reactions with trichloroacetimidates of the D-glucose, D-cellobiose,[22] and D-lactose[16] led exclusively to the β-glycosides **19**−**21**. On the other hand, the same effect led to the formation of the corresponding α-glycosides **22** and **23** using D-mannose and L-rhamnose, respectively.

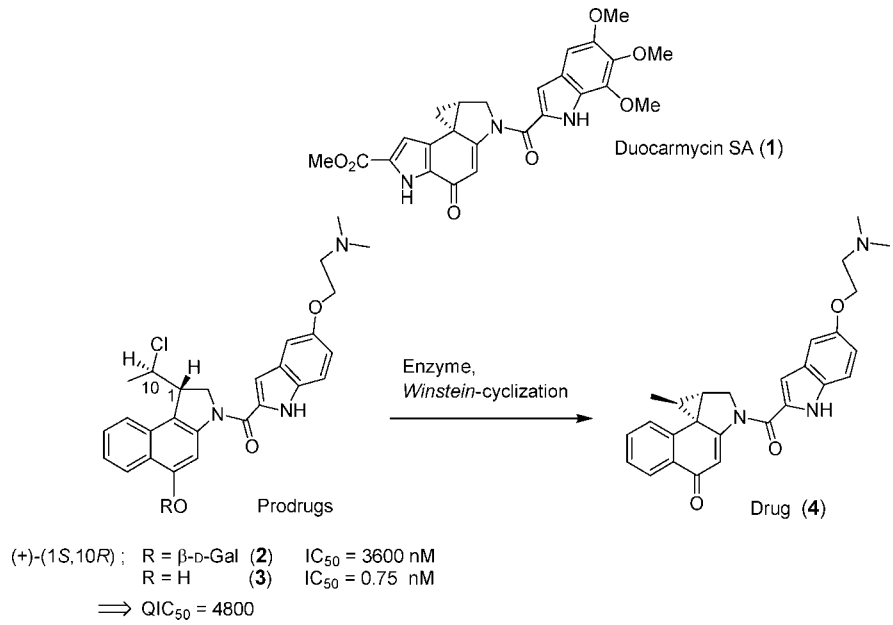
The determination of the cytotoxicity of the prodrugs **19**−**24** was carried out using a HTCFA-assay. The IC_{50} values

* Phone: +49-(0)551-393271. Fax: +49(0)551-399476. E-mail: ltietze@gwdg.de.

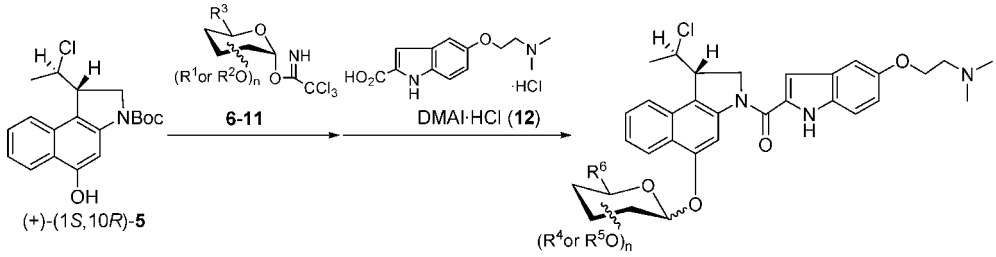
[a] Abbreviations: ADEPT, antibody directed enzyme prodrug therapy; DMAI, *N*,*N*-dimethylamino-ethoxyindole carboxylic acid; HTCFA, human tumor colony forming ability-test; methyl-*seco*-CBI, 1-(10-chloroethyl)-1,2-dihydro-3*H*-benz[*e*]indole.

10.1021/jm8009102 CCC: $40.75
Published on Web 12/29/2008

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



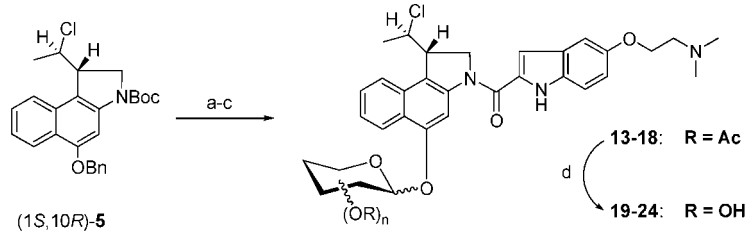
Scheme 2. Synthetic Approach to the Prodrugs

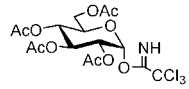
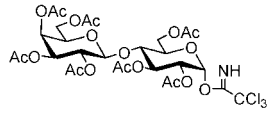
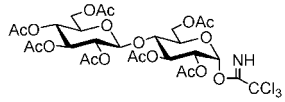
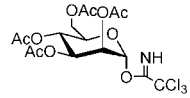
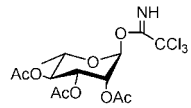
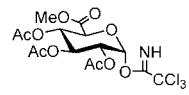


range from 2000 to 3500 nM, respectively (Table 2). Although the rhamnoside **23** has a rather low cytotoxicity, it was not investigated further because α-rhamnosidase is an enzyme laborious to obtain. Purified α-rhamnosidase[23] from naringinase was shown to be used in prodrug delivery systems by Davis et al.,[24] but an adaptation to its use in ADEPT and getting the necessary amounts for in vivo studies is less likely when compared to the other enzymes used. Nonetheless, we prepared this compound to contribute more knowledge on this nonmammalian sugar and its potential use in tumor therapy. In cases of all other prodrugs, the carbohydrate moiety could be cleaved as anticipated using the corresponding enzymes. IC_{50} values between 0.6 and 2.1 nM of the resulting drugs were found that correspond to the different reaction rates of the enzymatic hydrolysis and which correlate to QIC_{50} values between 920 for the cellobioside **21** and 4500 for the mannoside **22**. Although we have employed β-D-glucosidase from different sources, for all prodrugs containing a glucose moiety at the phenolic hydroxyl group, a slower cleavage of the glycosidic bond resulting in lower IC_{50} values was observed when compared with the reaction of the galactoside **2**[25] and the mannoside **22** using β-D-galactosidase and α-D-mannosidase, respectively. The surprising fact that the glucuronic acid methyl ester **24** could be cleaved by the enzyme β-D-glucuronidase can be explained by assuming a foregoing hydrolysis of the ester moiety by ubiquitous esterhydrolases in the cell culture medium.[26] In addition to the HTCFA-assays, the stability of the prodrugs in Ultra-Culture medium at 37 °C was measured using direct HPLC-MS measurements for the determination of the amount of the formed hydrolyzed prodrugs. In no case was a removal of the sugar moiety observed. On the other hand, a replacement of the chloride was found, which to our surprise strongly depends on the type of sugar used for the protection of the phenolic hydroxyl group in **5**. For instance, the galactoside **2** and the mannoside **22** have similar QIC_{50} values, but the mannoside **22** is by far more stable toward hydrolysis and, therefore, more suitable for a possible therapeutic approach. The glucose-bound prodrug **19** and its disaccharide analogues **20** and **21** showed the highest stability; thus, only traces of hydrolyzed products were detected after 24 h. The increased stability of **20** and **21** is furthermore accompanied by a much better water solubility than found for the galactoside **2**.

The QIC_{50} value of the mannoside **22** corresponds very well with the value for the already described galactoside **2**, but **22** has a much better stability. The QIC_{50} values of the other prodrugs are smaller, which can be mostly attributed to the decreased rates of the enzymatic cleavage. However, as already mentioned, they have other favorable properties such as a better stability and water solubility, which makes them also interesting candidates for an ADEPT approach. On the other hand, the higher cytotoxicity of the glucose prodrug **19** compared to **2** and **22** is consistent with earlier observations.[6c] Possible explainations are an active transport through the cell membrane by a glucose-transporter system, e.g., GLUT2 found on human lung epithelial cells,[27] or an activation to give the drug by secreted/endogenous β-glucosidase. The latter fact was not shown so far for A549 cells but can be assumed by knowing the elevated glycolysis of tumor cells.[28] Such a compound might be of interest for the treatment of brain tumors because suitable

Table 1. Synthesis of the Prodrugs **19**−**24** from **5**: (a,b) Trichloroacetimidates **6**−**11**, 0.5 equiv $BF_3 \cdot Et_2O$, CH_2Cl_2, MS (4 Å), −15 to −20 °C, then 3.0 equiv $BF_3 \cdot Et_2O$, RT; (c) DMAI·HCl (**12**), EDC·HCl, DMF, RT, 14−26 h; (d) NaOMe, MeOH, RT, 30 min to 6 h



Prodrug	Trichloroacetimidate		Coupling a-c [%]	Deacetylation d [%]
19		**6 (D-Glc)**	53	82
20		**7 (D-Lac)**	69	75[a]
21		**8 (D-Cel)**	67	50[a]
22		**9 (D-Man)**	65	82
23[b]		**10 (L-Rha)**	54	80
24[c]		**11 (D-MeGlcA)**	59	79

[a] Purification by RP-HPLC (Kromasil 100 C18). [b] *rac*-**5** was used to give a diastereomeric mixture of (1*S*,10*R*)-**23** and (1*R*,10*S*)-**23**. [c] The synthesis has already been described.[19]

Table 2. Measurement of the Cytotoxicity and the Stability of the Prodrugs **2**, **19**−**24**

prodrug	carbohydrate[a]	IC_{50} without enzyme [nM]	IC_{50} with enzyme [nM]	QIC_{50}[c]	stability over 24 h [%][d]	enzyme [UmL^{-1}][f]
19	β-D-glucose	2000	1.9	1050	98	10 and 0.2
20	β-D-lactose	2700	2.1	1290	98	0.2
21	β-D-cellobiose	2400	2.6	920	98	0.2
22	α-D-mannose	2700	0.6	4500	94	0.4
23	α-L-rhamnose[b]	3500	not tested		88	
24	β-D-glucuronic acid methylester	3300	2.1	1600	63[e]	2.1×10^{-3}
2	β-D-galactose	3600	0.8	4800	27	1.0

[a] Carbohydrate moiety on enantiopure (1*S*,10*R*)-methyl-*seco*-CBI-DMAI-pharmacophore. [b] Diastereomeric mixture of (+)-(1*S*,10*R*)- and (−)-(1*R*,10*S*)-**23**. [c] $QIC_{50} = IC_{50}$ of prodrug/IC_{50} of prodrug in presence of the cleaving enzyme. [d] Measured by HPLC-MS (AUC) after 24 h in UltraCulture medium at 37 °C. [e] After 24 h in UltraCulture medium at 37°C a mixture of **24** and the glucuronic acid prodrug was found (1:5). [f] Please read the experimental section on the enzyme sources.

compounds have to pass the blood−brain barrier. As the mode of the cellular uptake of the duocarmycin based drugs and prodrugs is still unknown, we assume that the hydrophilic glycosidic prodrugs except **19** do not penetrate the cell membrane whereas the more lipophilic drugs formed by taking off the sugar moiety can penetrate the cell membrane.

Conclusion

Several novel duocarmycine-based prodrugs for the use in the antibody directed enzyme prodrug therapy (ADEPT) containing different carbohydrates as detoxifying unit have been prepared. The mannoside **22** shows a similar excellent QIC_{50} value of 4500 as the already known galactoside **2**. The other prodrugs have a lower QIC_{50} value ranging from 920 to 1600, which can be attributed to a lower rate of the enzymatic removal of the sugar moiety. Furthermore, we were able to show that the carbohydrate moiety has a strong influence on the prodrug stability. Thus, the amount of the hydrolyzed prodrug **22** by replacement of the chloride for hydroxyl amounts to only 6%

within 24 h, whereas 72% of the hydrolyzed compound employing **2** is found using the same conditions.

Experimental Section

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 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 (CC) 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. IR spectra were determined on a Bruker Vektor 22, UV−vis spectra on a Perkin-Elmer Lambda 2, and mass spectra on a Finnigan MAT 95 for EI-HRMS, and a Bruker Apex IV Fourier transform 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.

The purities and stabilities of the target compounds was checked by HPLC-MS (ESI mass spectrometry with an ion-trap mass spectrometer LCQ (Finnigan)) and found to be between 96 and 99% purity in all cases. The used column was a Phenomenex Synergi Max-RP C12 (150 mm × 2 mm, particle size 4 μm). The HPLC-MS chromatograms can be found in the Supporting Information.

General Procedure for the Glycosylation, Boc-Deprotection, and DNA-Binder coupling (GP 1). A solution of the phenol **5**[17] (1.00 equiv) in dry CH_2Cl_2 (45 mL/mmol) was suspended with freshly activated molecular sieves 4 Å (2.00 g/mmol) and stirred for 30 min at ambient temperature. After addition of the trichloroacetimidate (1.00−1.25 equiv) and cooling to −20 to −18 °C, the promoter $BF_3 \cdot OEt_2$ (0.50 equiv) in dry CH_2Cl_2 (0.5 mL) was added dropwise and the mixture stirred at the given temperature. The end of the reaction was controlled by TLC and subsequently excess $BF_3 \cdot OEt_2$ (3.00 equiv) in CH_2Cl_2 (2.0 mL) was added, and the reaction mixture was warmed to 25 °C and stirred for the given time. After filtration through a small celite pad, the pad was thoroughly washed with CH_2Cl_2, the solvent removed, and the resulting foam dried under high vaccum for 1 h. The formed salt was dissolved in DMF (65 mL/mmol), the stirred solution cooled to 0 °C, and EDC·HCl (3.0 equiv) followed by DMAI-HCl[9] (**12**) (1.5 equiv) added. After stirring at 25 °C for 15−30 h, the mixture was diluted with EtOAc (70 mL/mmol), washed with water (70 mL/mmol) and saturated $NaHCO_3$ solution (25 mL), and the phases were separated and aqueous layer extracted again with EtOAc (4 × 100 mL/mmol). The combined organic layers were washed with saturated NaCl solution (4 × 60 mL/mmol), dried over $MgSO_4$, and the solvent removed in vacuo. The crude material was purified by column chromatography (CC) on silica gel (CH_2Cl_2/MeOH = 10:1) to yield the acetylated prodrugs.

General Procedure for the Zemplén Deacetylation (GP 2). A solution of the acetylated prodrugs (1.0 equiv) in MeOH was treated at 0 °C with a NaOMe solution (100 μL, 30% in MeOH, 0.15−3.0 equiv) in MeOH and stirred at ambient temperature until complete conversion (TLC-control). The mixture was neutralized with HCl (1 m in MeOH from acetylchloride-MeOH) or acetic acid, silica gel (1.5 mg/mg crude material) added, and the solvents removed in vacuo. The crude material was purified by column chromatography (CC) on silica gel (CH_2Cl_2/MeOH = 1:1) and filtered through a membrane filter or purified by RP-HPLC if necessary.

(+)-(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]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside ((+)-13). According to GP 1, the glucose trichloroacetimidate **6** (50.9 mg, 110 μmol, 1.1 equiv) in CH_2Cl_2 (4.5 mL), phenol (+)-(1*S*,10*R*)-**5** (34.6 mg, 100 μmol, 1.0 equiv) and molecular sieves 4 Å (200 mg) were allowed to react under $BF_3 \cdot OEt_2$ (7.1 μL, 50.0 μmol, 0.5 equiv) catalysis at −15 °C for 3.0 h. Additional $BF_3 \cdot OEt_2$ (42.6 μL, 300 μmol, 3.0 equiv), 2.5 h at 25 °C, subsequent reaction with DMAI·HCl (**12**) (42.7 mg, 150 μmol, 1.5 equiv), and EDC·HCl (57.5 mg, 300 μmol, 3.0 equiv) for 18 h gave crude material that was purified by CC to afford the title compound **(+)-13** (42.2 mg, 52.5 μmol, 53%) as colorless solid. R_f = 0.36 (CH_2Cl_2/MeOH = 10:1). ^{1}H NMR (599.8 MHz, DMSO-d_6, 35 °C): μ = 1.64 (d, *J* = 6.6 Hz, 3 H, H_3-11), 2.01, 2.03, 2.05 (3 × s, 12 H, 4 × COCH_3), 2.26 (s, 6 H, NMe_2), 2.68 (t, *J* = 5.8 Hz, 2 H, H_2-2″), 4.07 (m_c, 3 H, H_2-1″, H-6_b‴), 4.25 (m_c, 2 H, H-1, H-5‴), 4.31 (dd, *J* = 11.8, 4.4 Hz, 1 H, H-6_a‴), 4.63 (dd, *J* = 11.0, 1.7 Hz, 1 H, H-2_a), 4.76 (m_c, 1 H, H-2_b), 4.80 (dq, *J* = 12.8, 6.2, 2.4 Hz, 1 H, H-10), 5.10 (t, *J* = 9.6 Hz, 1 H, H-4‴), 5.30 (dd, *J* = 9.7, 8.0 Hz, 1 H, H-2‴), 5.51 (t, *J* = 9.5 Hz, 1 H, H-3‴), 5.64 (d, *J* = 7.4 Hz, 1 H, H-1‴), 6.93 (dd, *J* = 8.9, 2.4 Hz, 1 H, H-6′), 7.17 (s_{br}, 1 H, H-3′), 7.18 (d, *J* = 2.2 Hz, 1 H, H-4′), 7.42 (d, *J* = 8.9 Hz, 1 H, H-7′), 7.47 (t, *J* = 7.6 Hz, 1 H, H-7), 7.59 (t, *J* = 7.6 Hz, 1 H, H-8), 7.98, 8.00 (2 × d, *J* = 8.1 Hz, 2 H, H-6, H-9), 8.22 (s, 1 H, H-4), 11.58 (s, 1 H, NH). HRMS $C_{41}H_{46}ClN_3O_{12}$: calcd 808.28428; found 808.28419 $[M + H]^+$; for further data, see Supporting Information.

(−)-(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-glucopyranoside ((−)-19). Following GP 2, **(+)-13** (165 mg, 204 μmol, 1.0 equiv) in MeOH (30 mL) was treated with NaOMe (5.51 mg, 19.4 μL, 18.6 μmol, 0.5 equiv) and stirred for 3.0 h. Work-up and CC gave the title compound **(−)-19** as slightly ochre-colored solid (106.5 mg, 166 μmol, 82%). R_f = 0.26 (CH_2Cl_2/MeOH = 1:1). ^{1}H NMR (599.8 MHz, DMSO-d_6, 35 °C): μ = 1.65 (d, *J* = 6.5 Hz, 3 H, H_3-11), 2.24 (s, 6 H, NMe_2), 2.66 (t, *J* = 5.7 Hz, 2 H, H_2-2″), 3.30 (m_c, 1 H, H-5‴), 3.34 (t, *J* = 9.0 Hz, 1 H, H-3‴), 3.39 (t, *J* = 9.0 Hz, 1 H, H-4‴), 3.47 (m_c, 1 H, H-2‴), 3.66 (dd, *J* = 11.3, 2.5 Hz, 1 H, H-6_b‴), 3.75 (d, *J* = 11.0 Hz, 1 H, H-6_a‴), 4.07 (t, *J* = 5.8 Hz, 1 H, H_2-1″), 4.26 (d, *J* = 9.0 Hz, 1 H, H-1), 4.37 (s_{br}, 1 H, OH), 4.63 (d, *J* = 11.8 Hz, 1 H, H-2_b), 4.75 (t, *J* = 9.9 Hz, 1 H, H-2_a), 4.83 (m_c, 2 H, H-10), 4.99 (d, *J* = 7.3 Hz, 1 H, H-1‴), 5.15, 5.40 (2 × s_{br}, 3 H, 3 × OH), 6.93 (dd, *J* = 8.8, 2.0 Hz, 1 H, H-6′), 7.18 (m_c, 2 H, H-3′, H-4′), 7.40 (d, *J* = 8.8 Hz, 1 H, H-7′), 7.43, 7.58 (t, *J* = 7.5 Hz, 2 H, H-7, H-8), 7.96, 8.35 (2 × d, *J* = 8.3 Hz, 2 H, H-6, H-9), 8.24 (s, 1 H, H-4), 11.60 (s, 1 H, NH). HRMS $C_{33}H_{38}ClN_3O_8$: calcd 640.24202; found 640.24194 $[M + H]^+$; for further data, see Supporting Information.

(+)-(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]-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside ((+)-14). According to GP 1, the lactose trichloroacetimidate **7** (117 mg, 149 μmol, 1.15 equiv) in CH_2Cl_2 (4.0 mL), phenol (+)-(1*S*,10*R*)-**5** (45.0 mg, 130 μmol, 1.0 equiv), and molecular sieves 4 Å (200 mg) were allowed to react under $BF_3 \cdot OEt_2$ (8.2 μL, 65.0 μmol, 0.5 equiv) catalysis at −16 °C for 3.0 h. Additional $BF_3 \cdot OEt_2$ (41.0 μL, 390 μmol, 3.0 equiv), 2.0 h at 25 °C, subsequent reaction with DMAI·HCl (**12**) (59.2 mg, 208 μmol, 1.5 equiv) and EDC·HCl (74.7 mg, 390 μmol, 3.0 equiv) for 15 h gave crude material that was purified by CC to afford the title compound **(+)-14** (98.7 mg, 90.1 μmol, 69%) as colorless solid. R_f = 0.29 (CH_2Cl_2/MeOH = 10:1). ^{1}H NMR (599.7 MHz, DMSO-d_6, 35 °C): μ = 1.64 (d, *J* = 6.6 Hz, 3 H, CH_3-11), 1.91, 2.03, 2.04, 2.06, 2.11 (5 × s, zus. 21 H, 7 × COCH_3), 2.28 (s, 6 H, NMe_2), 2.71 (t, *J* = 5.7, 2 H, H-2″), 4.00 (t, *J* = 9.4 Hz, 1 H, H-4‴), 4.04−4.11 (m, 1 H, H-6_b⁗), 4.09 (t, *J* = 5.8 Hz, 2 H, H-1″), 4.14 (m, 1 H, H-5‴), 4.19−4.27 (m, 3 H, H-1, H-6_a‴, H-6_a⁗), 4.35 (d, *J* = 11.3 Hz, 1 H, H-6_b‴), 4.63 (dd, *J* = 10.9, 1.1 Hz, 1 H, H-2_a), 4.75 (d, *J* = 10.6 Hz, 1 H, H-2_b), 4.79 (m, 1 H, H-10), 4.81 (t, *J* = 8.0 Hz, 1 H, H-1⁗), 4.89 (t, *J* = 10.2, 8.1 Hz, 1 H, H-2⁗), 5.19 (dd, *J* = 10.3, 3.5 Hz, 1 H, H-3⁗), 5.22 (dd, *J* = 9.7, 8.1 Hz, 1 H, H-2‴), 5.26 (d, *J* = 3.4 Hz, 1 H, H-4⁗), 5.40 (t, *J* = 9.4 Hz, 1 H, H-3‴), 5.58 (d, *J* = 7.9 Hz, 1 H, H-1‴), 6.94 (dd, *J* = 8.9, 2.3 Hz, 1 H, H-6′), 7.17 (d, *J* = 1.2 Hz, 1 H, H-4′), 7.18 (d, *J* = 2.0 Hz, 1 H, H-4′), 7.42 (d, *J* = 8.9 Hz, 1 H, H-7′), 7.46 (t, *J* = 7.6 Hz, 1 H, H-7), 7.58 (t, *J* = 7.3 Hz, 1 H, H-8), 7.96

(d, $J = 8.5$ Hz, 1 H, H-9), 7.99 (d, $J = 8.4$ Hz, 1 H, H-6), 8.19 (s_{br}, 1 H, H-4), 11.55 (s_{br}, 1 H, NH). HRMS $C_{53}H_{62}ClN_3O_{20}$: calcd 1096.36880; found 1096.36907 $[M + H]^+$; for further data, see Supporting Information.

(1*S*,10*R*)-1-(10-Chloro-ethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)-ethoxy)indol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[*e*]indol-5-yl]-(D-galactopyranosyl)-(β1→4)-β-D-glucopyranoside (20). Following GP 2, **(+)-14** (67.0 mg, 61.1 μmol, 1.0 equiv) in MeOH (8 mL) was treated with NaOMe (11.6 mg, 41 μL, 214 μmol, 3.5 equiv, in 1 mL MeOH) and stirred for 5.0 h. Work-up with acetic acid and RP-HPLC gave the title compound **20** as colorless solid (36.7 mg, 45.8 μmol, 75%). $R_f = 0.22$ (CH_2Cl_2/MeOH = 1:1.5). ^{1}H NMR (599.7 MHz, DMSO-d_6, 35 °C): $\mu = 1.65$ (d, $J = 6.6$ Hz, 3 H, CH_3-11), 2.27 (s, 6 H, NMe_2), 2.69 (t, $J = 5.8$ Hz, 2 H, H-2″), 3.35 (dd, $J = 9.5$, 3.5 Hz, 1 H, H-3‴″), 3.38 (dd, $J = 9.5$, 7.5 Hz, 1 H, H-2‴″), 3.50 (t, $J = 6.1$ Hz, 1 H, H-5‴″), 3.46−3.64 (m, 5 H, H-2‴, H-3‴, H-5‴″, $H\text{-}6_{a,b}$‴″), 3.66 (d, $J = 2.7$ Hz, 1 H, H-4‴″), 3.82 (m_c, 1 H, $H\text{-}6_{a,b}$‴″), 4.08 (t, $J = 5.9$ Hz 2 H, H-1″), 4.25 (dt, $J = 9.4$, 2.4 Hz, 1 H, H-1), 4.39−4.61 (br, 6 H, 6 × OH), 4.31 (d, $J = 7.5$ Hz, 1 H, H-1‴″), 4.63 (dd, $J = 10.9$, 1.6 Hz, 1 H, $H\text{-}2_a$), 4.76 (d, $J = 9.7$ Hz, 1 H, $H\text{-}2_b$), 4.82 (ddd, $J = 8.9$, 6.4, 1.9 Hz, 1 H, H-10), 5.03 (s_{br}, 1 H, H-1‴), 5.60 (s_{br}, 1 H, OH), 6.92 (dd, $J = 8.9$, 2.3 Hz, 1 H, H-6′), 7.17 (s_{br}, 1 H, H-3′), 7.18 (d, $J = 2.2$ Hz, 1 H, H-4′), 7.40 (d, $J = 8.9$ Hz, 1 H, H-7′), 7.44 (t, $J = 7.6$ Hz, 1 H, H-7), 7.57 (t, $J = 7.6$ Hz, 1 H, H-8), 7.97 (d, $J = 8.4$ Hz, 1 H, H-9), 8.24 (s_{br}, 1 H, H-4), 8.35 (d, $J = 8.5$ Hz, 1 H, H-6), 11.60 (s_{br}, 1 H, NH). HRMS $C_{39}H_{48}ClN_3O_{13}$: calcd 802.29484; found 802.29496 $[M + H]^+$; for further data, see Supporting Information.

Chromatographic Purification of Crude 20. A solution of 60.0 mg of crude **20** in 3.00 mL $CH_3CN/H_2O = 1:1 + 0.05\%$ HOAc was separated (injection volume 0.50 mL) by semipreparative RP-HPLC (Kromasil 100 C18, 250 mm × 20 mm, particle size: 7 μm, isocratic $CH_3CN/H_2O = 1:3 + 0.05\%$ HOAc, flow: 12 mL·min^{-1}; UV-detector: $\mu = 299$ nm, Jasco module) to provide pure **20** (t_R = 5.6 min).

(+)-(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]-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→4)-2,3,6-tri-*O*-acetyl-β-D-glucopyranoside ((+)-15). As described for the synthesis of **(+)-14** using identical conditions, the cellobiose trichloroacetimdiate **8** afforded the title compound **(+)-15** as colorless foam (95.7 mg, 87.3 μmol, 67%). $R_f = 0.42$ (CH_2Cl_2/MeOH = 10:1). ^{1}H NMR (599.7 MHz, $CDCl_3$): $\mu = 1.63$ (d, $J = 6.4$ Hz, 3 H, CH_3-11), 1.92, 1.98, 2.00, 2.02, 2.03, 2.06 (6 × s, zus. 21 H, 7 × $COCH_3$), 2.27 (s, 6 H, NMe_2), 2.70 (t, $J = 5.5$, 2 H, H-2″), 3.96 (t, $J = 9.4$ Hz, 1 H, H-4‴), 3.97−4.17 (m, 2 H, H-5‴″, $H\text{-}6_b$‴″), 4.08 (t, $J = 5.7$ Hz, 2 H, H-1″), 4.14 (m, 1 H, H-5‴), 4.20 (dd, $J = 11.8$, 6.0 Hz, 1 H, $H\text{-}6_a$‴), 4.26 (m, 1 H, H-1), 4.27 (dd, $J = 12.7$, 4.2 Hz, 1 H, $H\text{-}6_a$‴″), 4.36 (d, $J = 11.6$ Hz, 1 H, $H\text{-}6_b$‴), 4.62 (d, $J = 10.7$ Hz, 1 H, $H\text{-}2_a$), 4.68 (t, $J = 8.8$ Hz, 1 H, H-3‴″), 4.75 (d, $J = 10.6$ Hz, 1 H, $H\text{-}2_b$), 4.79 (m, 1 H, H-10), 4.87 (t, $J = 8.4$ Hz, 1 H, H-1‴″), 4.91 (t, $J = 9.8$ Hz, 1 H, H-4‴″), 5.20 (m, 1 H, H-2‴), 5.27 (t, $J = 9.0$ Hz, 1 H, H-2‴″), 5.39 (t, $J = 9.4$ Hz, 1 H, H-3‴), 5.55 (d, $J = 7.9$ Hz, 1 H, H-1‴), 6.93 (d, $J = 8.6$ Hz, 1 H, H-6′), 7.15, 7.17 (2 × s_{br}, 2 H, H-3′, H-4′), 7.41 (d, $J = 8.9$ Hz, 1 H, H-7′), 7.46 (t, $J = 7.4$ Hz, 1 H, H-7), 7.50 (t, $J = 7.2$ Hz, 1 H, H-8), 7.94 (d, $J = 8.4$ Hz, 1 H, H-9), 7.99 (d, $J = 8.4$ Hz, 1 H, H-6), 8.17 (s_{br}, 1 H, H-4), 11.53 (s_{br}, 1 H, NH).. HRMS $C_{53}H_{62}ClN_3O_{20}$: calcd 1096.36880; found 1096.36914 $[M + H]^+$; for further data, see Supporting Information.

(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]-(D-glucopyranosyl)-(β1→4)-β-D-glucopyranoside (21). Following GP 2, **(+)-15** (65.9 mg, 60.1 μmol, 1.0 equiv) in MeOH (8 mL) was treated with NaOMe (11.4 mg, 40 μL, 210 μmol, 3.5 equiv, in 1 mL MeOH) and stirred for 1.5 h. Work-up with acetic acid and RP-HPLC gave the title compound **21** as colorless cotton-like solid (24.1 mg, 30.1 μmol, 50%). $R_f = 0.25$ (CH_2Cl_2/MeOH = 1:1.5). ^{1}H NMR (599.7 MHz, DMSO-d_6, 35 °C): $\mu = 1.65$ (d, $J = 6.7$ Hz, 3 H, CH_3-11), 2.51 (s_{br}, 6 H, NMe_2), 3.00−3.13 (m, 4 H, H-2″, H-2‴″, H-4‴″), 3.17−3.27 (m, 2 H, H-5‴, H-5‴″), 3.45 (dd, $J = 11.4$, 6.4 Hz, 1 H, $H\text{-}6_a$‴″), 3.48−3.55 (m, 3 H, H-2‴, H-3‴, H-3‴″), 3.62 (t, $J = 8.7$ Hz, 1 H, H-4‴″), 3.73 (dd, $J = 11.3$, 1.5 Hz, 1 H, $H\text{-}6_b$‴″), 3.78 (dd, $J = 10.2$, 1.6 Hz, 1 H, $H\text{-}6_a$‴″), 3.84 (d, $J = 11.3$ Hz, 1 H, $H\text{-}6_b$‴″), 4.20 (t, $J = 5.4$ Hz, 2 H, H-1″), 4.26 (dt, $J = 9.3$, 2.3 Hz, 1 H, H-1), 4.37 (d, $J = 7.9$ Hz, 1 H, H-1‴″), 4.59 (s_{br}, 2 H, 2 × OH), 4.62 (dd, $J = 11.1$, 1.8 Hz, 1 H, $H\text{-}2_a$), 4.75 (d, $J = 10.1$ Hz, 1 H, $H\text{-}2_b$), 4.78 (s_{br}, 1 H, OH), 4.82 (dq, $J = 6.8$, 2.5 Hz, 1 H, H-10), 4.96−5.03 (m_{br}, 4 H, H-1‴, 3 × OH), 5.60 (s_{br}, 1 H, OH), 6.96 (dd, $J = 8.9$, 2.4 Hz, 1 H, H-6′), 7.18 (s_{br}, 1 H, H-3′), 7.21 (d, $J = 2.2$ Hz, 1 H, H-4′), 7.42 (d, $J = 8.8$ Hz, 1 H, H-7′), 7.44 (t, $J = 8.0$ Hz, 1 H, H-7), 7.57 (ddd, $J = 8.0$, 6.9, 1.1 Hz, 1 H, H-8), 7.97 (d, $J = 8.4$ Hz, 1 H, H-9), 8.22 (s_{br}, 1 H, H-4), 8.35 (d, $J = 8.5$ Hz, 1 H, H-6), 11.63 (s_{br}, 1 H, NH). HRMS $C_{39}H_{48}ClN_3O_{13}$: calcd 802.29484; found 802.29482 $[M + H]^+$; for further data, see Supporting Information.

Chromatographic Purification of Crude 21. Identical conditions as described for **20** provided pure **21** (t_R = 4.7 min).

(+)-(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]-2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranoside ((+)-16). According to GP 1, mannose trichloroacetimidate **9** (77.6 mg, 168 μmol, 1.1 equiv) in CH_2Cl_2 (7.0 mL), phenol (+)-(1*S*,10*R*)-**5** (52.8 mg, 152 μmol, 1.0 equiv), and molecular sieves 4 Å (380 mg) were allowed to react under $BF_3 \cdot OEt_2$ (9.6 μL, 76.0 μmol, 0.5 equiv) catalysis at −20 °C for 100 min. Additional $BF_3 \cdot OEt_2$ (57.8 μL, 456 μmol, 3.0 equiv), 2.0 h at 25 °C, subsequent reaction with DMAI·HCl (**12**) (65.0 mg, 228 μmol, 1.5 equiv) and EDC·HCl (87.3 mg, 456 μmol, 3.0 equiv) for 15 h gave crude material that was purified by CC to afford the title compound **(+)-16** (80.0 mg, 99.0 μmol, 65%) as colorless solid. $R_f = 0.43$ (CH_2Cl_2/MeOH = 9:1). ^{1}H NMR (599.8 MHz, DMSO-d_6, 35 °C): $\mu = 1.65$ (d, $J = 6.7$ Hz, 3 H, H_3-11), 1.87, 2.04, 2.19 (3 × s, zus. 12 H, 4 × $COCH_3$), 2.24 (s, 6 H, NMe_2), 2.64 (t, $J = 5.9$ Hz, 2 H, H-2″), 3.96 (dd, $J = 12.2$, 2.4 Hz, 1 H, $H\text{-}6_b$‴), 4.07 (t, $J = 5.9$ Hz, 1 H, H-1″), 4.09 (ddd, $J = 10.0$, 5.6, 2.3 Hz, 1 H, H-5‴), 4.21 (dd, $J = 12.3$, 5.7 Hz, 1 H, $H\text{-}6_a$‴), 4.28 (td, $J = 9.3$, 2.3 Hz, 1 H, H-1), 4.64 (dd, $J = 11.0$, 2.3 Hz, 1 H, $H\text{-}2_a$), 4.77(dd, $J = 10.6$, 9.9 Hz, 1 H, $H\text{-}2_b$), 4.81 (ddd, $J = 13.4$, 6.2, 2.4 Hz, 1 H, H-10), 5.27 (t, $J = 10.1$ Hz, 1 H, H-4‴), 5.55 (dd, $J = 3.5$, 1.7 Hz, 1 H, H-2‴″), 5.58 (dd, $J = 10.0$, 3.5 Hz, 1 H, H-3‴), 5.90 (d, $J = 1.1$ Hz, 1 H, H-1‴), 6.93 (dd, $J = 8.9$, 2.4 Hz, 1 H, H-6′), 7.18 (2 × d, $J = 2.2$ Hz, 2 H, H-3′, H-4′), 7.40 (d, $J = 8.9$ Hz, 1 H, H-7′), 7.54 (ddd, $J = 8.2$, 6.8, 1.1 Hz, 1 H, H-7), 7.62 (ddd, $J = 8.3$, 6.9, 1.2 Hz, 1 H, H-8), 8.03 (d, $J = 8.4$ Hz, 1 H, H-9), 8.16 (d, $J = 8.5$ Hz, 1 H, H-6), 8.30 (s, 1 H, H-4), 11.56 (s, 1 H, NH). HRMS $C_{41}H_{46}ClN_3O_{12}$: calcd 808.28456; found 808.28428 $[M + H]^+$; for further data, see Supporting Information.

(+)-(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]-α-D-mannopyranoside ((+)-22). Following GP 2, **(+)-16** (28.1 mg, 34.8 μmol, 1.0 equiv) in MeOH (30 mL) was treated with NaOMe (0.94 mg, 3.3 μL, 17.4 μmol, 0.5 equiv) and stirred for 30 min. Work-up and CC gave the title compound **(+)-22** as colorless solid (18.3 mg, 28.6 μmol, 82%), which can be crystallized from a minimum amount methanol/ *n*-hexane. $R_f = 0.45$ (CH_2Cl_2/MeOH = 1:1). ^{1}H NMR (599.8 MHz, DMSO-d_6, 35 °C): $\mu = 1.65$ (d, $J = 6.7$ Hz, 3 H, H-11), 2.24 (s, 6 H, NMe_2), 2.65 (t, $J = 5.8$ Hz, 2 H, H-2″), 3.43 (ddd, $J = 9.2$, 4.4, 2.3 Hz, 1 H, H-5‴), 3.51 (dd, $J = 11.8$, 4.4 Hz, 1 H, $H\text{-}6_a$‴), 3.56 (dd, $J = 11.8$, 2.2 Hz, 1 H, $H\text{-}6_b$‴), 3.66 (t, $J = 9.5$ Hz, 1 H, H-4‴), 3.94 (dd, $J = 9.3$, 3.0 Hz, 1 H, H-3‴), 4.07 (m_c, 3 H, H-1″, H-2‴), 4.23 (dt, $J = 9.2$, 2.2 Hz, 1 H, H-1), 4.38 (s_{br}, 1 H, OH), 4.62 (dd, $J = 11.0$, 2.0 Hz, 1 H, $H\text{-}2_a$), 4.78 (m_c, 2 H, H-10, $H\text{-}2_b$), 4.88, 4.95, 5.20 (3 × s_{br}, 3 H, 3 × OH), 5.69 (s_{br}, 1 H, H-1‴), 6.93 (dd, $J = 8.8$, 2.2 Hz, 1 H, H-6′), 7.18 (m_c, 2 H, H-3′, H-4′), 7.40 (d, $J = 8.9$ Hz, 1 H, H-7′), 7.46, 7.58 (t, $J = 8.0$ Hz, 2 H, H-7, H-8), 7.97, 8.15 (2 × d, $J = 8.1$ Hz, 2 H, H-6, H-9), 8.22 (s, 1 H, H-4), 11.60 (s, 1 H, NH). HRMS $C_{33}H_{38}ClN_3O_8$: calcd 640.24202; found 640.24211 $[M + H]^+$; for further data, see Supporting Information.

(1*SR*,10*RS*)-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-α-l-rhamnopyranosides (17). According to GP 1, the rhamnose trichloroacetimidate **10** (66.9 mg, 154 μmol, 1.05 equiv) in CH_2Cl_2 (7.0 mL), phenol (±)-(1*RS*,10*SR*)-**5** (50.7 mg, 146 μmol, 1.0 equiv), and molecular sieves 4 Å (350 mg) were allowed to react under $BF_3 \cdot OEt_2$ (9.3 μL, 73.2 μmol, 0.5 equiv) catalysis at −20 °C for 105 min. Additional $BF_3 \cdot OEt_2$ (55.6 μL, 439 μmol, 3.0 equiv), 1.5 h at 25 °C, workup, subsequent reaction with DMAI·HCl (**12**) (62.3 mg, 219 μmol, 1.5 equiv) and EDC·HCl (83.8 mg, 438 μmol, 3.0 equiv) for 19.5 h gave crude material that was purified by CC to afford the title compound **17** (60.0 mg, 81.0 μmol, 54%) as colorless solids. $R_f = 0.45$ (CH_2Cl_2/MeOH = 10:1). ^{1}H NMR (300.1 MHz, DMSO-d_6, 35 °C): μ = 1.16, 1.20 (2 × d, J = 6.2 Hz, 3 H, H_3-6), 1.66 (d, J = 6.6 Hz, 3 H, H_3-11), 2.04, 2.07, 2.08, 2.17, 2.19 (5 × s, zus. 9 H, 3 × COCH_3), 2.26 (s, 6 H, NMe_2), 2.67 (t, J = 5.8 Hz, 2 H, H_2-2″), 4.00 (dq, J = 9.8, 6.3 Hz, 1 H, H-5‴), 4.07 (t, J = 5.7 Hz, 2 H, H_2-1″), 4.28 (dd, J = 6.0, 2.5 Hz, 1 H, H-1), 4.65 (d, J = 10.9 Hz, 1 H, H-2_a), 4.75 (d, J = 10.0 Hz, 1 H, H-2_b), 4.68.4.78 (m, 1 H, H-10), 5.09, 5.10 (2 × t, J = 9.9 Hz, 1 H, H-4‴), 5.51−5.58 (m, 2 H, H-2‴, H-3‴), 5.78, 5.83 (2 × s_{br}, 1 H, H-1‴), 6.92, 6.95 (2 × s_{br}, 1 H, H-6′), 7.06−7.28 (s_{br}, 2 H, H-3′, H-4′), 7.41 (d, J = 8.9 Hz, 1 H, H-7′), 7.53 (t, J = 7.6 Hz, 1 H, H-7), 7.61 (t, J = 7.6 Hz, 1 H, H-8), 8.01 (d, J = 8.3 Hz, 1 H, H-9), 8.17 (d, J = 8.3 Hz, 1 H, H-6), 8.32, 8.36 (2 × s_{br}, 1 H, H-4), 11.60, 11.64 (2 × s_{br}, 1 H, NH); $C_{33}H_{38}ClN_3O_8$: calcd 750.23; for further data, see Supporting Information.

(1*SR*,10*RS*)-1-(10-Chloroethyl)-3-[(5-(2-(*N*,*N*-dimethylamino)-ethoxy)indol-2-yl)carbonyl]-1,2-dihydro-3*H*-benz[*e*]indol-5-yl]-α-l-rhamnopyranosides (23). Following GP 2, (1*SR*,10*RS*)-**17** (30 mg, 40 μmol, 1.0 equiv) in MeOH (30 mL) was treated with NaOMe (1.1 mg, 3.8 μL, 20 μmol, 0.94 mg, 0.5 equiv) and stirred for 30 min. Work-up and CC gave the title compound **23** as slightly yellow solids (20 mg, 32 μmol, 80%). $R_f = 0.42$ (CH_2Cl_2/MeOH = 1:1). ^{1}H NMR (599.7 MHz, DMSO-d_6, 35 °C): μ = 1.12, 1.19 (2 × d, J = 6.2 Hz, 3 H, H_3-6), 1.65, 1.66 (2 × d, J = 6.5 Hz, 3 H, H_3-11), 2.24 (s, 6 H, NMe_2), 2.66 (t, J = 5.8 Hz, 2 H, H-2″), 3.33−3.41 (m, 1 H, H-4‴), 3.54, 3.65 (2 × dq, J = 9.7, 6.3 Hz, 1 H, H-5‴), 3.86, 3.88 (2 × dd, J = 9.2, 3.3 Hz, 1 H, H-3‴), 4.02−4.10 (m_c, 3 H, H-1″, H-2‴), 4.25 (dd, J = 9.3, 1.9 Hz, 1 H, H-1), 4.63 (2 × d, J = 10.8, 2.2 Hz, 1 H, H-2_a), 4.76−4.85 (m, 3 H, H-2_b, H-10, OH), 4.91, 5.15 (2 × s_{br}, 2 H, 2 × OH), 5.50, 5.59 (2 × s_{br}, 1 H, H-1‴), 6.92 (2 × dd, J = 8.9, 1.9 Hz, 1 H, H-6′), 7.17 (s_{br}, 2 H, H-3′, H-4′), 7.29, 7.40 (2 × d, J = 8.9 Hz, 1 H, H-7′), 7.46 (t, J = 7.6 Hz, 1 H, H-7), 7.58 (t, J = 7.5 Hz, 1 H, H-8), 7.98 (d, J = 8.3 Hz, 1 H, H-9), 8.13 (d, J = 8.4 Hz, 1 H, H-6), 8.25, 8.32 (2 × s, 1 H, H-4), 11.59, 11.62 (2 × s, 1 H, NH). HRMS $C_{33}H_{38}N_3O_7Cl$: calcd 624.24710; found 624.24707 $[M + H]^+$; for further data, see Supporting Information.

In Vitro Cytotoxicity Assays. Adherent cells of line A549 were sown in triplicate in 6 multiwell plates at concentrations of 10^2, 10^3, 10^4, and 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 Lonza). Incubation with compounds **19**−**24** 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 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 9−10 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 10 U mL^{-1} β-D-glucosidase (EC 3.2.1.21, from almonds G 0395 (Sigma)), 0.17 U mL^{-1} cellulase (EC 3.2.1.4, from *Trichoderma viride* C 1794 (Sigma), 209 μU mL^{-1} β-glucuronidase (E.C. 3.2.1.31, from *Escherichia coli* G 7646 (Sigma), or 0.4 U mL^{-1} α-mannosidase (E.C. 3.2.1.24, from *Canavalia ensiformis* M 7257 (Sigma) to the cells during incubation with the substances.

Acknowledgment. This research was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. H.J.S. thanks the Friedrich-Ebert-Stiftung and B.K. the Deutsche-Telekom-Stiftung for a Ph.D. scholarship.

Supporting Information Available: Complete set of all analytical data of compounds **7**, **8**, **13**−**23**, as well as cellobiose and lactose heptaacetate. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) (a) First publication on the ADEPT concept: Bagshawe, K. D. Antibody directed enzymes revive anti-cancer prodrugs concept. *Br. J. Cancer* **1987**, *56*, 531–532. (b) Review: Bagshawe, K. D. *Expert Rev. Anticancer Ther.* **2006**, *6*, 1421–1431.

(2) Review: (a) Denny, W. A. Tumor-activated Prodrugs—A New Approach to Cancer Therapy. *Cancer Invest.* **2004**, *22*, 604–619.

(3) Review: (a) Tietze, L. F.; Feuerstein, T. Enzyme and Proton-Activated Prodrugs for a Selective Cancer Therapy. *Curr. Pharm. Des.* **2003**, *9*, 2155–2175.

(4) Review: (a) Tietze, L. F.; Feuerstein, T. Highly Selective Compounds for the Antibody-Directed Enzyme Prodrug Therapy of Cancer. *Aust. J. Chem.* **2003**, *56*, 841–854.

(5) Review: (a) Senter, P. D. Activation of Prodrugs by Antibody−Enzyme Conjugates: A New Approach to Cancer Therapy. *FASEB J.* **1990**, *4*, 188–193.

(6) (a) Tietze, L. F.; Feuerstein, T.; Fecher, A.; Haunert, F.; Panknin, O.; Borchers, U.; Schuberth, I.; Alves, F. Proof of Principle in the Selective Treatment of Cancer by Antibody Directed Enzyme Prodrug Therapy. The Development of a Highly Potent Prodrug. *Angew. Chem.* **2002**, *114*, 785; *Angew. Chem., Int. Ed.* **2002**, *41*, 759−761. (b) Tietze, L. F.; Lieb, M.; Herzig, T.; Haunert, F.; Schuberth, I. A Strategy for Tumor-Selective Chemotherapy by Enzymatic Liberation of *seco*-Duocarmycin SA-Derivatives from Nontoxic Prodrugs. *Bioorg. Med. Chem.* **2001**, *9*, 1929–1939. (c) Tietze, L. F.; Hannemann, R.; Buhr, W.; Lögers, M.; Menningen, P.; Lieb, M.; Starck, D.; Grote, T.; Döring, A.; Schuberth, I. Prodrugs of the Cytostatic CC-1065 that Can Be Activated in a Tumor-Selective Manner. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2674–2677.

(7) (a) Ichimura, M.; Ogawa, T.; Takahashi, K.; Kobayashi, E.; Kawamoto, I.; Yasuzawa, T.; Takahashi, I.; Nakano, H. Duocarmycin SA, a new antitumor antibiotic from *Streptomyces* sp. *J. Antibiot.* **1990**, *43*, 1037–1038. (b) Yasuzawa, T.; Saitoh, Y.; Ichimura, M.; Takahashi, I.; Sano, H. Structure of duocarmycin SA, a potent antitumor antibiotic. *J. Antibiot.* **1991**, *44*, 445–447.

(8) Tietze, L. F.; Herzig, T.; Fecher, A.; Haunert, F.; Schuberth, I. Highly Selective Glycosylated Prodrugs of Cytostatic CC-1065 Analogs for Antibody Directed Enzyme Tumor Therapy. *ChemBioChem* **2001**, *2*, 758–765.

(9) Milbank, J. B. J.; Tercel, M.; Atwell, G. J.; Wilson, W. R.; Hogg, A.; Denny, W. A. Synthesis of 1-substituted 3-(chloromethyl)-6-aminoindoline (6-amino-seco-CI) DNA minor groove alkylating agents and structure−activity relationships for their cytotoxicity. *J. Med. Chem.* **1999**, *42*, 649–658.

(10) Tietze, L. F.; Major, F.; Schuberth, I.; Spiegl, D. A.; Krewer, B.; Maksimenka, K.; Bringmann, G.; Magull, J. Selective Treatment of Cancer: Synthesis, Biological Evaluation and Structural Elucidation of Novel Analogues of the Antibiotic CC-1065 and the Duocarmycins. *Chem.—Eur. J.* **2007**, *13*, 4396.

(11) (a) Hanka, L. J.; Dietz, A.; Gerpheide, S. A.; Kuentzel, S. L.; Martin, D. G. A new antitumor antibiotic. Production, in vitro biological activity, microbiological assays, and taxonomy of the producing microorganisms. *J. Antibiot.* **1978**, *31*, 1211–1217. (b) Martin, D. G.; Biles, C.; Gerpheide, S. A.; Hanka, L. J.; Krueger, W. C.; McGovren, J. P.; Mizsak, S. A.; Neil, G. L.; Stewart, J. C.; Visser, J. CC-1065 (NSC-218223), a potent new antitumor agent, improved production and isolation, characterization, and antitumor activity. *J. Antibiot.* **1981**, *34*, 1119–1125.

(12) (a) Igarashi, Y.; Futamata, K.; Fujita, T.; Sekine, A.; Senda, H.; Naoki, H.; Furumai, T. Yatakemycin, a Novel Antifungal Antibiotic Produced by Streptomyces sp. TP-A0356. *J. Antibiot.* **2003**, *56*, 107–113. (b) Tichenor, M. S.; Kastrinsky, D. B.; Boger, D. L. Total Synthesis,

Structure Revision, and Absolute Configuration of (+)-Yatakemycin. *J. Am. Chem. Soc.* **2004**, *126*, 8396–8398.

(13) (a) Boger, D. L. Jr.; Searcey, M.; Jin, Q. Critical role of the linking amide in CC-1065 and the duocarmycins: implications on the source of DNA alkylation catalysis. *J. Am. Chem. Soc.* **1998**, *120*, 11554–11557. (b) Boger, D. L.; Garbaccio, R. M.; Jin, Q. Synthesis and Evaluation of CC-1065 and Duocarmycin Analogues Incorporating the Iso-CI and Iso-CBI Alkylation Subunits: Impact of Relocation of the C-4 Carbonyl. *J. Org. Chem.* **1997**, *62*, 8875–8891. (c) Boger, D. L.; Johnson, D. S.; Yun, W.; Tarby, C. M. Molecular basis for sequence selective DNA alkylation by (+)- and ent-(−)-CC-1065 and related agents: alkylation site models that accommodate the offset AT-rich adenine N3 alkylation selectivity. *Bioorg. Med. Chem.* **1994**, *2*, 115–135.

(14) (a) Boger, D. L. The duocarymcins: synthetic and mechanistic studies. *Acc. Chem. Res.* **1995**, *28*, 20–29. (b) Boger, D. L.; Johnson, D. S. CC-1065 and the duocarmycins: unraveling the keys to a new class of naturally derived DNA alkylating agents. *Proc Natl. Acad. Sci. U.S.A.* **1995**, *92*, 3642–3649.

(15) Dobhal, M. P.; Li, G.; Gryshuk, A.; Graham, A.; Bhatanager, A. K.; Khaja, S. D.; Joshi, Y. C.; Sharma, M. C.; Oseroff, A.; Pandey, R. K. Structural Modifications of Plumieride Isolated from *Plumeria bicolor* and the Effect of These Modifications on in Vitro Anticancer Activity. *J. Org. Chem.* **2004**, *69*, 6165–6172.

(16) Cheng, H.; Cao, X.; Xian, M.; Fang, L.; Cai, T. B.; Ji, J. J.; Tunac, J. B.; Sun, D.; Wang, P. G. Synthesis and Enzyme-Specific Activation of Carbohydrate−Geldanamycin Conjugates with Potent Anticancer Activity. *J. Med. Chem.* **2005**, *48*, 645–652.

(17) Tietze, L. F.; Schuster, H. J.; Hampel, S.; Rühl, S.; Pfoh, R. Enantio- and Diastereoselective Synthesis of Duocarmycine-Based Prodrugs for a Selective Treatment of Cancer by Epoxide Opening. *Chem.—Eur. J.* **2008**, *14*, 895–901.

(18) Chiara, J. L.; Encinas, L.; Díaz, B. A. Study of Polymer-Supported Bases for the Solution Phase Synthesis of Glycosyl Trichloroacetimidates. *Tetrahedron Lett.* **2005**, *46*, 2445–2448.

(19) Tietze, L. F.; Schuster, H. J.; Schmuck, K.; Schuberth, I.; Alves, F. Duocarymcin-based prodrugs for cancer prodrug monotherapy. *Bioorg. Med. Chem.* **2008**, *16*, 6312–6318.

(20) (a) Schmidt, R. R. New methods for the synthesis of glycosides and oligosaccharides. Are there alternatives to the Koenigs-Knorr method? *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 212–235. (b) Schmidt, R. R. Synthesis of Glycosides In *Comprehensive Organic Chemistry*, 1st ed.; Trost, B. M., Fleming, I., Eds.; Pergamon Press: Elmsford, NY, 1991; pp 33−61.

(21) Zhang, Z.; Ollmann, I. R.; Ye, X.-S.; Wischnat, R.; Baasov, T.; Wong, C.-H. Programmable One-Pot Oligosaccharide Synthesis. *J. Am. Chem. Soc.* **1999**, *121*, 734–753.

(22) Urban, F. J.; Moore, B. S.; Breitenbach, P. Synthesis of Tigogenyl β-D-Cellobioside Heptaacetate and Glycosidic Tetraacetate via Schmidt's Trichloroacetimidate Method; Some New Obeservations. *Tetrahedron Lett.* **1990**, *31*, 4421–4424.

(23) (a) Young, N. M.; Johnston, R. A. Z.; Richards, J. C. Purification of the α-l-rhamnosidase of *Penicillium decumbens* and characterisation of two glycopeptide components. *Carbohydr. Res.* **1989**, *191*, 53–62. (b) Tarentino, A. L.; Trimble, R. B.; Maley, F. Endo-beta-*N*-acetylglucosaminidase from *Streptomyces plicatus*. *Methods Enzymol.* **1978**, *50*, 574–580.

(24) Robinson, M. A.; Charlton, S. T.; Garnier, P.; Wang, X.-t.; Davis, S. S.; Perkins, A. C.; Frier, M.; Duncan, R.; Savage, T. J.; Wyatt, D. A.; Watson, S. A.; Davis, B. D. LEAPT: Lectin-directed enzyme-activated prodrug therapy. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 14527–14532.

(25) Tietze, L. F.; Major, F.; Schuberth, I. Antitumor Agents: Development of Highly Potent Glycosidic Duocarmycin Analogues for Selective Cancer Therapy. *Angew. Chem.* **2006**, *118*, 6724–6727; *Angew. Chem., Int. Ed.* **2006**, *45*, 6574−6577.

(26) (a) Papot, S.; Combaud, D.; Gesson, J. P. A new spacer group derived from arylmalonaldehydes for glucuronylated prodrugs. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2545–2548. (b) Tietze, L. F.; Seele, R.; Leiting, B.; Krach, T. Stereoselective synthesis of (1-alkoxyalkyl) α- and β-D-glucopyranosiduronates (acetal-glucopyranosiduronates): a new approach to specific cytostatics for the treatment of cancer. *Carbohydr. Res.* **1988**, *108*, 253–262. (c) de Graaf, M.; Nevalainen, T. J.; Scheeren, H. W.; Pinedo, H. M.; Haisma, H. J.; Boven, E. A methylester of the glucuronide prodrug DOX-GA3 for improvement of tumor-selective chemotherapy. *Biochem. Pharmacol.* **2004**, *68*, 2273–2281.

(27) Kalsi, K. K.; Baker, E. H.; Medina, R. A.; Rice, S.; Wood, D. M.; Ratoff, J. C.; Philips, B. J.; Baines, D. L. Apical and basolateral localisation of GLUT2 transporters in human lung epithelial cells. *Pflugers Arch.—Eur. J. Physiol.* **2008**, *456*, 991–1003.

(28) (a) Gillies, R. J.; Robey, I.; Gatenby, R. A. Causes and consequences of increased glucose metabolism of cancers. *J. Nucl. Med.* **2008**, *49* (Suppl. 2), 24S–42S. (b) Greulich, K. O.; Altenberg, B. Genes of glycolysis are ubiquitously overexpressed in 24 cancer classes. *Genomics* **2004**, *84*, 1014–1020.

JM8009102