Prodrugs

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Antitumor Agents: Development of Highly Potent Glycosidic Duocarmycin Analogues for Selective Cancer Therapy**

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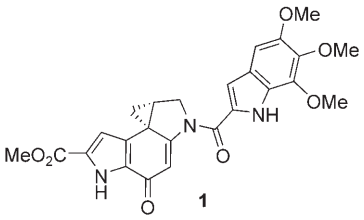
Dedicated to Professor Gerhard Erker on the occasion of his 60th birthday

Effective chemotherapy of malignant tumors is one of the great challenges of modern medicine. Chemotherapy is generally connected with severe side effects as the selectivity of traditional cytostatic agents is mainly based on the difference in the proliferation rate between malignant and normal cells. In comparison with cells particularly from the hematopoietic system, intestinal tract, and hair follicles, this difference is very small. The aim of modern cancer therapy, therefore, should be the targeted removal of malignant cells without impairment of normal cell populations. The approach that we have adopted for selective cancer treatment is "antibody-directed enzyme prodrug therapy" (ADEPT). With this method, nontoxic compounds (prodrugs) are enzymatically transformed into highly cytotoxic agents in cancerous tissue in a targeted manner.[1,2] In this binary approach, selectivity is achieved through the use of monoclonal antibodies that bind to tumor-associated antigens and are covalently bound to the required enzyme. Other approaches for selective cancer therapy depend on the application of immunoconjugates,[3] angiogenesis inhibitors,[4] antitumor vaccines,[5] kinase inhibitors,[6] and conjugates between a neuropeptide and an antitumor agent.[7]

Based on our previous work, which dates back to 1985, we have defined the main criterion for the ADEPT concept to be that the cytotoxic agent derived from the prodrug should achieve an IC_{50}[***] value of < 10 nM. Furthermore, the comparative toxicity ratio between the prodrug and drug, which we define as the QIC_{50} ($QIC_{50} = IC_{50}$(prodrug)/IC_{50}(prodrug+enzyme)),[8] should exceed 1000.

Herein we describe a new prodrug, (+)-**2a**, which we have developed for selective cancer therapy. The compound is based on the cytotoxic duocarmycin antibiotics, which for example, include duocarmycin SA (**1**). Not only does this



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compound fulfill the outlined criteria, but owing to its excellent QIC_{50} value, its good water solubility, and its easy synthesis, it exceeds all prodrugs prepared to date by us[1b,c,9] and others.[1,10]

Duocarmycin SA was isolated from *Streptomyces* DO-113 and has an IC_{50} value of 10 pM in the L1210 tumor cell line.[11] The spirocyclopropylcyclohexadienone moiety, which is necessary for biological activity, can be formed in situ from the corresponding seco compound with a free phenolic hydroxy group.[12] In prodrug (+)-**2a**, the phenolic hydroxy group is protected as a galactoside so that spirocyclization cannot occur. The prodrug can, however, be easily converted to give the required seco compound, (+)-**3**, which further reacts to give the cytotoxic agent (+)-**4** (Scheme 1). The *N*,*N*-dimethylaminoethoxyindole carboxylic acid component[13] not only facilitates intercalation in the minor DNA groove, but also increases water solubility owing to its salification.

The racemic benzyl ether **5**, which was easily obtained by using published methods,[9c,14] was used to synthesize prodrug **2** and seco compound **3**. To synthesize the diastereomerically pure glycosidic prodrugs as well as the enantiomerically pure

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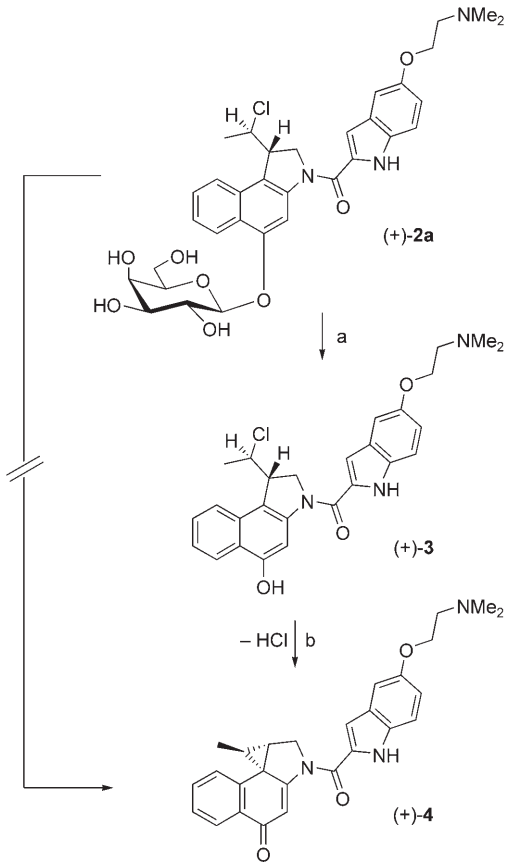
[***] IC_{50}: drug concentration at which 50% of cell growth is inhibited.

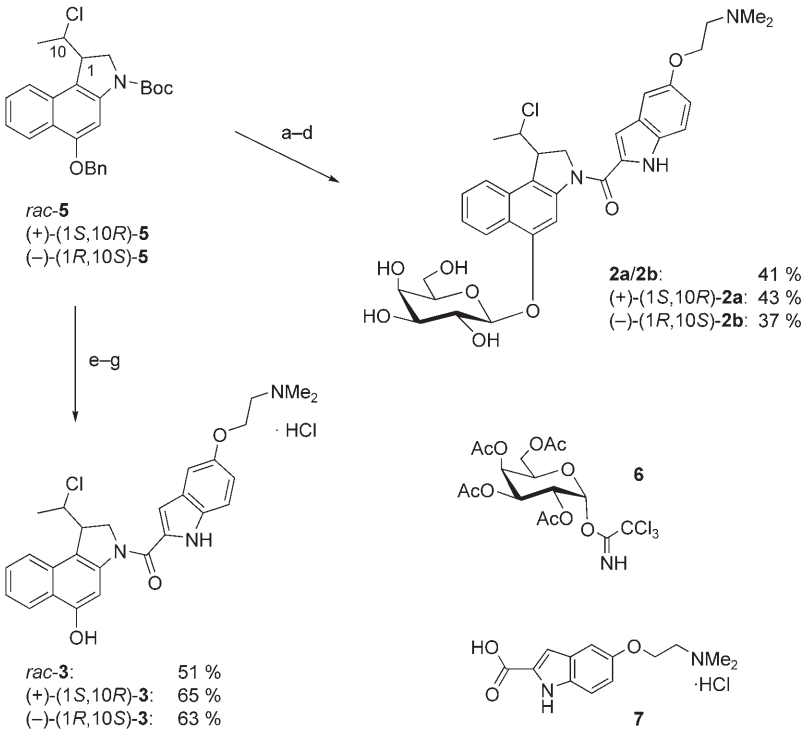


Scheme 1. Enzymatic toxification of the glycosidic prodrug (+)-**2a**: a) Transformation into the seco compound (+)-**3** and b) Winstein cyclization in situ to give the drug (+)-**4**, which is an analogue of the antibiotic (+)-duocarmycin SA (**1**).

toxins, it was necessary to maintain **5** in an enantiomerically pure form. Owing to the sensitivity of the free amine of **5**, it was not possible to use classical resolution methods, such as formation of diastereomeric salts or derivatives with enantiomerically pure reagents. However, efficient preparative HPLC separation was carried out by using a column with a chiral stationary phase (Chiralpak IA).[15] The absolute configuration of (+)-**5** was determined after derivatization with 3,5-dibromobenzoic acid by using X-ray crystal structure analysis.[16]

The galactosides **2** were prepared from **5** after removal of the benzyl protecting group with palladium on activated carbon and ammonium formate as the hydrogen source.[17] Glycosylation was carried out with **6** by using the trichloroacetimidate method developed by Schmidt.[18] Subsequently, $BF_3{\cdot}OEt_2$ was employed for *N*-*tert*-butoxycarbonyl (*N*-Boc) deprotection and the obtained secondary free amines were coupled with the indolecarboxylic acid hydrochloride **7**.[13] Finally, base-catalyzed solvolysis of the acetyl groups resulted in the desired galactosides **2** in very good final yields of 37–43% in four steps (Scheme 2). The free toxins **3**, which were required as control compounds for the in vitro experiments, were obtained from **5** by *N*-Boc deprotection, followed by coupling with **7** and final debenzylation to give **3** in yields of 51–65%. Direct glycosylation of **3** with **6** was not successful.

The cytotoxic effects of the newly synthesized compounds were tested on human tumor cells by using a colony-forming assay carried out in triplicate (Table 1). This test reflects the proliferation capacity of single cells and is based on the HTCFA assay (human tumor colony forming ability test)[9d]. In experiments using human bronchial carcinoma cell line A549, a QIC_{50} of 6600 was achieved with the mixture of diastereomeric prodrugs **2a**/**2b**, and for the diastereomerically pure prodrug (+)-(1*S*,10*R*)-**2a**, a QIC_{50} value of 4800 was observed. The cytotoxicity of seco compound (+)-(1*S*,10*R*)-**3** was determined as $IC_{50} = 0.75$ nM. It was not possible to assign the QIC_{50} value of prodrug (−)-(1*R*,10*S*)-**2b** owing to its low cytotoxicity. Thus, a higher quantity of this prodrug had to be used, which then led to solubility problems. Interestingly, the underlying seco compound (−)-(1*R*,10*S*)-**3**, with an IC_{50} value of 560 nM, showed an almost 1000-fold lower cytotoxicity than its enantiomer, (+)-(1*S*,10*R*)-**3**. The results of the cytotoxicity assays are in good agreement with MS data obtained from alkylation of duplex DNA with (+)-(1*S*,10*R*)-**2a**, (+)-(1*S*,10*R*)-**3**, and (−)-(1*R*,10*S*)-**3**.[19] As a further important finding from the cytotoxicity assays in vitro, it should be emphasized that after treatment of prodrug (+)-(1*S*,10*R*)-**2a** with β-D-galactosidase, the observed cytotoxicity was identical to that determined for seco compound (+)-(1*S*,10*R*)-**3**.



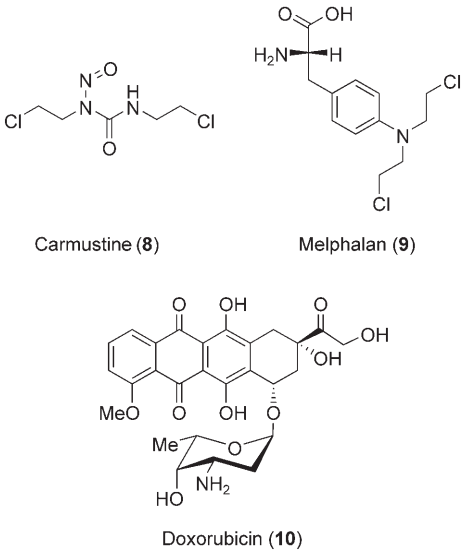
Scheme 2. Synthesis of prodrugs **2** and seco compounds **3**: a) $Pd/C/NH_4HCO_2$, THF, 40°C, 15 min; b) **6**, $BF_3{\cdot}OEt_2$, CH_2Cl_2, molecular sieves (4 Å), −10°C, 3.5 h, then $BF_3{\cdot}OEt_2$, RT, 5 h; c) **7**, EDC·HCl, DMF, RT, 20 h; d) NaOMe/MeOH, RT, 2 h; e) 4 M HCl/EtOAc, RT, 2 h; f) **7**, EDC·HCl, DMF, RT, 19 h; g) 4 M HCl/EtOAc, RT, 2 h, followed by $Pd/C/NH_4HCO_2$, THF, 40°C, 2 h. Bn = Benzyl, DMF = *N*,*N*-dimethylformamide ,EDC = 3-(3-dimethylaminopropyl)-1-ethylcarbodiimide.

This confirms the principle of reversible detoxification, the stability of the prodrugs under test conditions, and the nondisruptive effects of the enzyme activity.

Table 1: Results of the HTCFA assay for the in vitro analysis of the cytotoxicity of **2** and **3** against human bronchial carcinoma cells of line A549.[a]

Compound	β-D-gal.[b]	IC_{50} [nM]	QIC_{50}
2a/**2b**	−	7.9×10^{3}	6600
2a/**2b**	+	1.2	
(+)-(1*S*,10*R*)-**2a**	−	3.6×10^{3}	4800
(+)-(1*S*,10*R*)-**2a**	+	0.75	
(−)-(1*R*,10*S*)-**2b**	−	$>7.8\times10^{4}$	>140
(−)-(1*R*,10*S*)-**2b**	+	5.5×10^{2}	
rac-**3**	−	1.5	
(+)-(1*S*,10*R*)-**3**	−	0.75	
(−)-(1*R*,10*S*)-**3**	−	5.6×10^{2}	

[a] Cells were incubated with the corresponding compounds for 24 h at 37 °C; after 12 days incubation, the clone formation rates were determined in comparison with untreated control cells (β-D-galactosidase: *Escherichia coli*, 4 U mL^{-1}). [b] Presence or absence of β-D-galactosidase.



Scheme 3. The antitumor agents carmustine (**8**), melphalan (**9**), and doxorubicin (**10**).

To compare the compounds **2** and **3** that we have developed with other cytotoxic drugs, HTCFA assays were performed on the A549 cell line with carmustine (**8**), melphalan (**9**) and doxorubicin (**10**), which are currently used as anticancer agents (Scheme 3 and Figure 1). The results confirm that in the presence of the enzyme β-D-galactosidase, the new prodrug (+)-(1*S*,10*R*)-**2a** has a higher biological activity than compounds **8**–**10**. Consequently, the new compound has considerable benefits for use within the framework of the ADEPT concept.

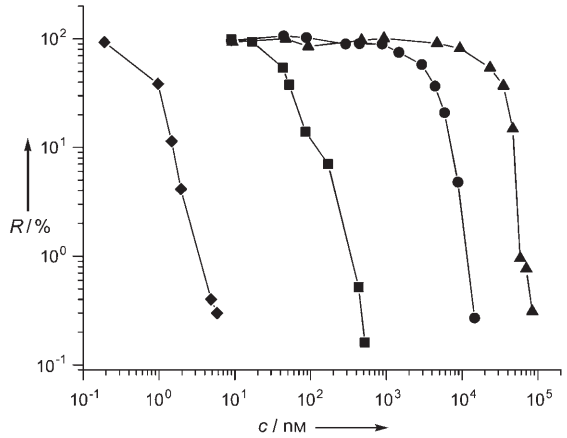


Figure 1. Comparison of the cytotoxicities in vitro of various antitumor agents against human bronchial carcinoma cells of line A549. (◆) (+)-(1*S*,10*R*)-**2a** in the presence of β-D-galactosidase (4 U mL^{-1}): IC_{50} = 0.75 nM; (▲) carmustine (**8**): $IC_{50}=2.6\times10^{4}$ nM; (●) melphalan (**9**): $IC_{50}=3.4\times10^{3}$ nM; (■) doxorubicin (**10**): IC_{50} = 45 nM. *R* = relative clone-formation rate.

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[15] Preparative HPLC separation: Chiralpak IA column (250 × 20 mm, particle size: 5 μm), mobile phase: *n*-heptane/dichloromethane (4:1), flow rate: 18 mL min^{-1}, $\alpha = 2.05$, (+)-**5**: 99.9 % *ee*, (−)-**5**: 99.9 % *ee*.

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