Prodrugs

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Glycosidic Prodrugs of Highly Potent Bifunctional Duocarmycin Derivatives for Selective Treatment of Cancer**

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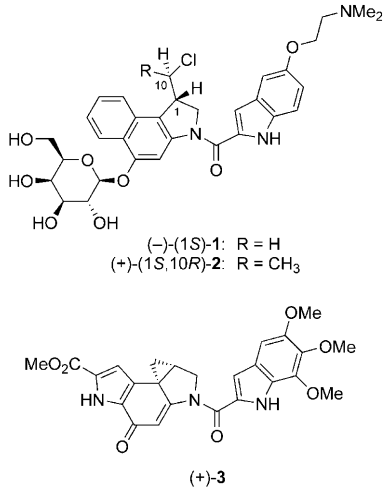
Dedicated to Professor Horst Kunz on the occasion of his 70th birthday

The development of selective chemotherapeutics for treatment of cancer is an important current area of medicinal research. One approach for the reduction of the frequently dose-limiting adverse effects of the currently available cytostatics is the antibody-directed enzyme prodrug therapy (ADEPT),[1,2] in which in a binary approach a conjugate of a tumor-specific antibody and an enzyme in combination with a hardly toxic prodrug is used. By means of the enzyme in the conjugate the prodrug is selectively cleaved in the tumor tissue leading to the formation of a highly cytotoxic compound. The advantages of the ADEPT approach compared to the use of conjugates of antibodies and toxins lies in the far better diffusion of the low-molecular-weight active compound formed in the malignant tumor, the killing also of those cancer cells to which the antibody does not bind, and the enzymatic approach, which gets along with a comparatively low amount of the antibody. One problem of the prodrugs previously developed for ADEPT was that their cytotoxicity was frequently only insignificantly lower than that of the active compounds formed from them (QIC_{50} value; $QIC_{50} = IC_{50}$ of the prodrug/IC_{50} of the prodrug in the presence of the enzyme). Furthermore, the efficacy of the active compound formed was frequently not adequate. We have therefore suggested as minimal requirement a QIC_{50} value of > 1000 and an IC_{50} value of the active compound formed of < 10 nM.[3]

With the compounds (−)-(1*S*)-**1** and (+)-(1*S*,10*R*)-**2** derived from seco analogues of the natural cytostatic duocarmycin SA ((+)-**3**;[4] Scheme 1) we have recently succeeded in developing prodrugs, which with QIC_{50} values of 3500 and 4800, respectively, and IC_{50} values of the active compounds formed of 16 and 750 pM, respectively, best met the requirements of the previously named criteria.[5,6]



Scheme 1. Glycosidic prodrugs (−)-(1*S*)-**1** and (+)-(1*S*,10*R*)-**2**, derived from the natural cytostatic antibiotic duocarmycin SA ((+)-**3**).

Herein we describe new prodrugs and the active compounds derived from them, which with QIC_{50} values of up to almost 1 000 000 and IC_{50} values of the active compounds formed of up to about 100 fM put all previous results well into the shade.[3,5–7] The new compounds contain the same pharmacophore as (−)-(1*S*)-**1** and (+)-(1*S*,10*R*)-**2**, but in this case, however, two of each are coupled through a dicarboxylic acid. The length of the dicarboxylic acid used here has a considerable influence on the biological activity of the prodrugs and the resulting active compounds.[8]

The preparation of the new glycosides (−)-(1′*S*)-**6a–c** ($n =$ 3–5) and (−)-(1′*S*,10′*R*)-**7a–c** ($n = 3$–5) was carried out starting from the enantiomerically pure *N*-Boc-protected seco compounds (−)-(1*S*)-**4**[6,9] and (+)-(1*S*,10*R*)-**5**,[5,10] respectively, by glycosidation using the trichloroacetimidate method,[11] subsequent $BF_3{\cdot}OEt_2$-mediated *tert*-butyloxycarbonyl(Boc) deprotection and coupling of the secondary amines formed with the respective dicarboxylic acid chlorides (Scheme 2). Solvolytic cleavage of the acetyl protecting groups by using the method applied by Zemplén and Pacsu finally afforded the galactosides (−)-(1′*S*)-**6a–c** ($n = 3$–5) and (−)-(1′*S*,10′*R*)-**7a–c** ($n = 3$–5) in yields of 27–55% over three steps.[12] The corresponding secodrugs (−)-(1′*S*)-**8a**,**c** ($n = 3$ and 5) and (+)-(1′*S*,10′*R*)-**9a–c** ($n = 3$–5) were obtained in 65–80% yield from (−)-(1*S*)-**4** and (+)-(1*S*,10*R*)-**5**, respectively by cleavage of the Boc protective

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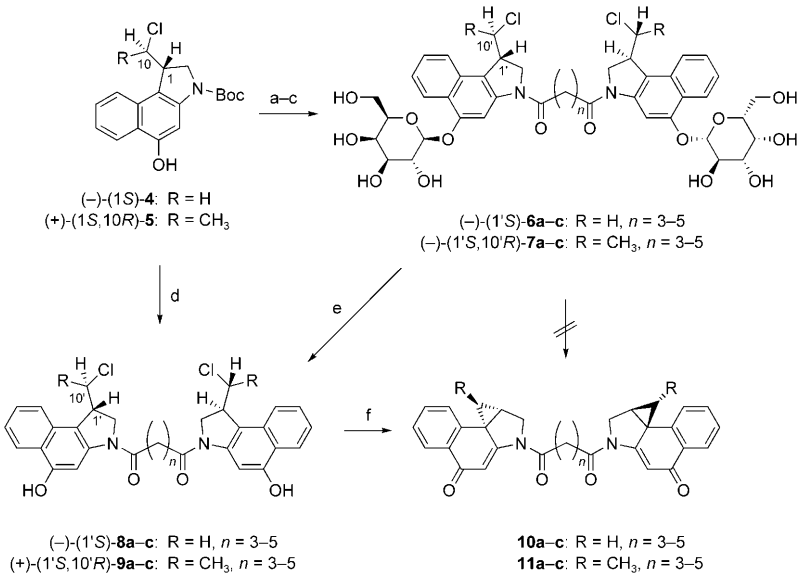
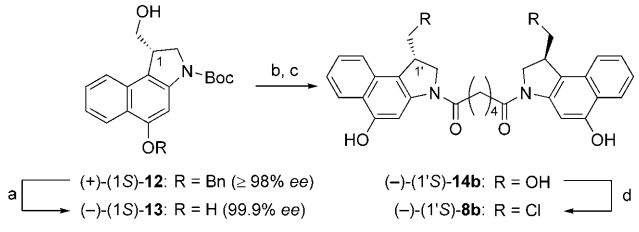


Scheme 2. Synthesis of the prodrugs (a–c) and secodrugs (d; not for (–)-(1′*S*)-**8b**), as well as the enzymatic activation of the prodrugs to the active compounds (e, f): a) α-trichloroacetimidate of D-galactose, $BF_3{\cdot}OEt_2$ (0.5 equiv), CH_2Cl_2, MS (4 Å), −10 °C, 3.5 h, then $BF_3{\cdot}OEt_2$ (3.0 equiv), RT, 5.5 h; b) $ClCO(CH_2)_nCOCl$ (n = 3–5), NEt_3, DMF, RT, 20 h, then preparative HPLC: Kromasil 100 C18 (250×20 mm, 7 μm), A = H_2O, B = MeOH, gradient(A/B) = 30:70→0:100 in 6.5 min; c) NaOMe/MeOH, RT, 2 h, then preparative HPLC: Kromasil 100 C18 (250×20 mm, 7 μm), A = H_2O, B = MeOH, gradient(A/B) = 70:30→0:100 in 15 min; d) 4 N HCl/EtOAc, RT, 3 h, then $ClCO(CH_2)_nCOCl$ (n = 3–5), pyridine, DMF, RT, 20 h, then preparative HPLC: Kromasil 100 C18 (250×20 mm, 7 μm), A = H_2O, B = MeOH, gradient(A/B) = 30:70→0:100 in 6.5 min; e) glycoside cleavage of the prodrugs to the secodrugs by β-D-galactosidase; f) in situ Winstein cyclization of the secodrugs to the active compounds under physiological conditions. DMF = *N*,*N*-dimethylformamide.

group and reaction with the respective dicarboxylic acid chlorides. Surprisingly the preparation of the secodrug (–)-(1′*S*)-**8b** (n = 4) was not successful in this way neither in acceptable yields nor in pure form.

Thus for (–)-(1′*S*)-**8b** an alternative synthetic route was developed starting from (+)-(1*S*)-**12**,[6,13] a precursor of (–)-(1*S*)-**4** (Scheme 3). First cleavage of the benzyl group was carried out in a transfer hydrogenation that led to the formation of the diol (–)-(1*S*)-**13**,[13] which after cleavage of the Boc protective group was coupled with adipoyl chloride. In this way a mixture of (–)-(1′*S*)-**14b** and differently acylated



Scheme 3. Alternative synthetic route to (–)-(1′*S*)-**8b**: a) Pd/C/NH_4HCO_2, THF, RT, 4.5 h, then crystallization from *n*-hexane/EtOAc, 61%; b) 4 N HCl/EtOAc, RT, 100 min, then $ClCO(CH_2)_4COCl$, pyridine, DMF, RT, 19 h; c) NaOMe/MeOH, DMF, RT, 4 h, 65% over two steps; d) PPh_3 resin, DMF/CCl_4 (10:1), RT, 7.5 h, then crystallization from DMF/CH_2Cl_2, 19%. Bn = Benzyl.

products was obtained which, however, could be transformed completely into the desired diamide (–)-(1′*S*)-**14b** by solvolysis in the presence of catalytic amounts of NaOMe in MeOH. In the last step the desired chloride (–)-(1′*S*)-**8b** was formed from (–)-(1′*S*)-**14b** in an Appel reaction. Overall, (–)-(1′*S*)-**8b** was obtained in pure form in 8% yield over four steps from (+)-(1*S*)-**12** by subsequent crystallization (*N*,*N*-dimethylformamide/dichloromethane).

The determination of the in vitro cytotoxicity of the new compounds was carried out in a clonogenicity assay based on the HTCFA (Human Tumor Colony Forming Ability) test on human bronchial carcinoma cells of the line A549, which reflects the proliferation ability of individual cells. For the prodrugs (–)-(1′*S*)-**6a–c** and (–)-(1′*S*,10′*R*)-**7a–c** the investigations (Table 1) gave practically identical cytotoxicities in the presence of β-D-galactosidase as were obtained for the corresponding secodrugs (–)-(1′*S*)-**8a–c** and (+)-(1′*S*,10′*R*)-**9a–c**. The reversible detoxification thus established forms the basic requirement for an application within the framework of the ADEPT concept. In agreement with earlier results the secodrugs that contained the pharmacophoric *anti*-methyl-seco-CBI unit ((+)-(1′*S*,10′*R*)-**9a–c**) showed a less pronounced cytotoxicity than the analogous compounds with a seco-CBI unit ((–)-(1′*S*)-**8a–c**).[5,6] In addition the cytotoxicity of the secodrugs within these two homologous series each fell within the sequence $n = 3 > 5 > 4$. Highlighted is the prodrug (–)-(1′*S*)-**6a** (n = 3), which has a QIC_{50} value of 970000 and in the presence of β-D-galactosidase a cytotoxic activity in the femtomolar range (IC_{50} = 0.15 pM).

Under physiological conditions the secodrugs are transformed in situ into the corresponding active compounds **10a–c** and **11a–c** (Scheme 2) with a spirocyclopropyl group as in (+)-**3**. By using the secodrugs generated from (–)-(1*S*)-**1** und (+)-(1*S*,10*R*)-**2** and related compounds we had been able to show by mass spectrometry on oligonucleotides and CD spectroscopy on living cells that the resulting active compounds are embedded very rapidly into the minor grooves of the DNA and subsequently alkylate more slowly an adenine residue sequence specifically.[14] The cytotoxic activity of the active compounds released from (–)-(1*S*)-**1** and (+)-(1*S*,10*R*)-**2** is thus presumably attributable to a stabilization of the double strand of the DNA, whereby the alkylation causes a fixation of the molecules in the minor grooves. In contrast the formation of DNA intra- or DNA interstrand cross-links (ICLs) without incorporation into the minor grooves of the DNA could be held responsible for the comparably high cytotoxicity of the new secodrugs (–)-(1′*S*)-**8a–c** and (+)-(1′*S*,10′*R*)-**9a–c** and of other comparable bifunctional active compounds and hybrid structures,[8,15–17,18] since these substances have no DNA-binding unit for a

Table 1: Total yields and results of the HTCFA assay for the investigation of the cytotoxicity of the new prodrugs and secodrugs against human bronchial carcinoma cells (A549).[a]

Compound	R	*n*	Yield [%][b]	β-D-Galactosidase[c]	IC_{50} [pM]	QIC_{50}
(−)-(1′*S*)-**6a**	H	3	51	−	1.46×10^5	9.7×10^5
(−)-(1′*S*)-**6a**	H	3		+	0.15	
(−)-(1′*S*)-**8a**	H	3	76	−	0.11	
(−)-(1′*S*)-**6b**	H	4	44	−	1.29×10^5	2.2×10^4
(−)-(1′*S*)-**6b**	H	4		+	5.8	
(−)-(1′*S*)-**8b**	H	4	< 6 (8[d])	−	9.0	
(−)-(1′*S*)-**6c**	H	5	55	−	1.80×10^5	1.4×10^5
(−)-(1′*S*)-**6c**	H	5		+	1.3	
(−)-(1′*S*)-**8c**	H	5	65	−	1.0	
(−)-(1′*S*,10′*R*)-**7a**	CH_3	3	30	−	3.26×10^5	2.2×10^3
(−)-(1′*S*,10′*R*)-**7a**	CH_3	3		+	1.5×10^2	
(+)-(1′*S*,10′*R*)-**9a**	CH_3	3	80	−	1.6×10^2	
(−)-(1′*S*,10′*R*)-**7b**	CH_3	4	27	−	5.3×10^6	1.9×10^3
(−)-(1′*S*,10′*R*)-**7b**	CH_3	4		+	2.86×10^3	
(+)-(1′*S*,10′*R*)-**9b**	CH_3	4	76	−	3.14×10^3	
(−)-(1′*S*,10′*R*)-**7c**	CH_3	5	35	−	3.83×10^7	1.5×10^5
(−)-(1′*S*,10′*R*)-**7c**	CH_3	5		+	2.6×10^2	
(+)-(1′*S*,10′*R*)-**9c**	CH_3	5	79	−	1.7×10^2	

[a] The incubation of the cells (A549) with the respective compound was carried out for 24 h at 37 °C; after subsequent cultivation for 12 days the relative clone formation rate was determined compared relative to untreated controls. The experiments were carried out as replicates (at least three) of duplicates. β-D-Galactosidase: *Escherichia coli*, 4 U mL^{-1}. [b] Each total yield based on (−)-(1*S*)-**4** or (+)-(1*S*,10*R*)-**5**. [c] Addition of β-D-galactosidase. [d] Total yield based on (+)-(1*S*)-**12**.

noncovalent interaction with the DNA as have duocarmycin SA ((+)-**3**) and the cytotoxic active substances generated from (−)-(1*S*)-**1** and (+)-(1*S*,10*R*)-**2**.

To estimate the reactivity of the new bifunctional secodrugs towards double-stranded DNA and to investigate the structure–activity relationships, the compounds (−)-(1′*S*)-**8a** ($n=3$), (−)-(1′*S*)-**8c** ($n=5$), and (+)-(1′*S*,10′*R*)-**9a–c** ($n=3$–5) were incubated with different double-strand oligodeoxynucleotides in aqueous solution (1% DMSO) for 24 h at 37 °C and then analyzed with high-resolution electrospray ionization Fourier transform ion cyclotron resonance mass spectrometry (ESI-FTICR-MS).[14] The ESI-FTICR-MS experiments showed no significant formation of ICLs with any of the compounds investigated. An analysis by circular dichroism gave no indication of a characteristic interaction of the active compounds with the oligodeoxynucleotides.[14b] It is therefore unlikely that alkylation and cross-linking of double-stranded DNA is responsible for the high cytotoxicity of these new compounds.

In conclusion, the glycosidic prodrugs (−)-(1′*S*)-**6a–c** ($n=3$–5) and (−)-(1′*S*,10′*R*)-**7a–c** ($n=3$–5), which are constructed from seco analogues of the pharmacophoric units of duocarmycin SA ((+)-**3**) coupled with one another through dicarboxylic acids, are excellently suited for use within the scope of the ADEPT concept. Worthy of note is (−)-(1′*S*)-**6a** ($n=3$), which with a QIC_{50} value of almost 1 000 000 and an IC_{50} value of the underlying active compound of 0.11–0.15 pM exceeds by far all previously known compounds. In addition, the biological activity of the compounds is most probably not attributable to DNA intra- or DNA interstrand cross-linking. The cause of the high cytotoxicity is presumably another, as yet unknown mechanism.

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