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Photoactivatable Prodrugs of Highly Potent Duocarmycin Analogues for a Selective Cancer Therapy

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Abstract: A main problem of common cancer chemotherapy is the occurrence of severe side effects caused by insufficient selectivity of the applied drugs. A possible concept to overcome this limitation is light-driven prodrug monotherapy. The synthesis as well as photochemical and biological evaluation of new photoactivatable prodrugs is described. Best results were obtained with prodrug (*S*,*S*)-**7a**. The photochemical labile protecting groups in (*S*,*S*)-**7a** can easily be removed by irradiation with UV-A light in 30 min with a power of only 2 J cm^{-2}. The determination of the in vitro cytotoxicity by using an HTCFA-test reveals a QIC_{50} value of 8200 and the prodrug is more than two million times less cytotoxic than the corresponding seco-drug (−)-(*S*,*S*)-**5** with an IC_{50} value of about 110 fM. The big therapeutic window makes (*S*,*S*)-**7a** very suitable for its use in selective cancer therapy.

Keywords: cancer therapy · cytotoxicity · duocarmycin · photochemistry · prodrugs

Introduction

The development of selective cytostatic drugs for cancer therapy is an important, yet challenging subset in the field of modern medicinal chemistry. Currently available compounds often show severe side effects, which potentially necessitate a reduction of the employed dose, thus leading to a less effective treatment. Therefore, new concepts of therapy were developed, which allow selective killing of cancer cells through a specific targeting of such; by this method, healthy tissue remains almost unaffected. One of the most promising concepts is the ADEPT approach, in which an antibody–enzyme conjugate selectively generates a highly potent drug from a nontoxic prodrug at the tumor site.[1] We have developed the glycosidic prodrugs (−)-(1*S*)-**3** and (−)-(*S*,*S*)-**6** based on the natural antibiotic duocarmycin SA ((+)-**1**) with (−)-(1*S*)-**3** being 6500 times and (−)-(*S*,*S*)-**6** almost a million times less toxic than the corresponding seco-drugs (+)-(1*S*)-**2** and (−)-(*S*,*S*)-**5**, respectively (IC_{50} = 26 pM and 110 fM; Figure 1).[2] These compounds are by now unsurpassed in their activity and selectivity in ADEPT. Furthermore, a new mode of action could be established for the drugs derived from the seco-drugs (−)-(*S*)-**2** and (−)-(*S*,*S*)-**5**.[3] Besides the aforementioned com-

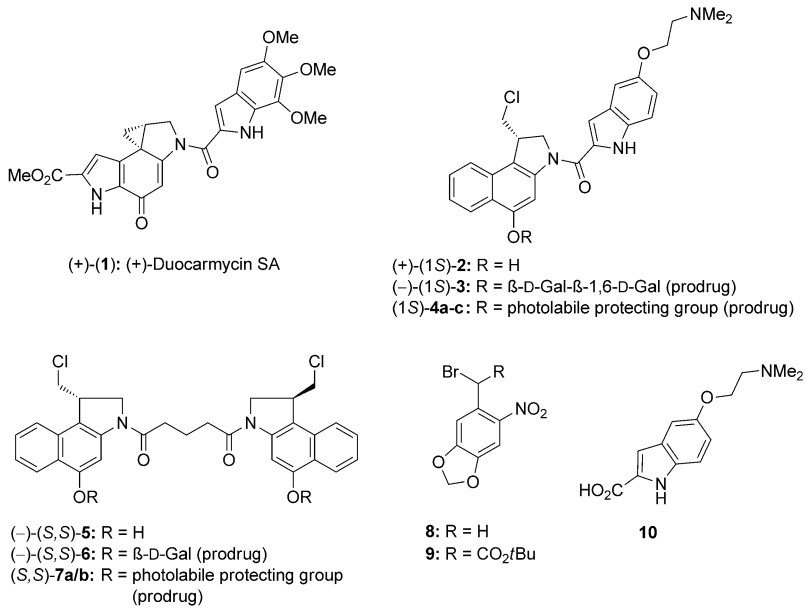

Figure 1. (+)-Duocarmycin SA ((+)-**1**), seco-drugs (+)-(1*S*)-**2** and (−)-(*S*,*S*)-**5**, glycosidic prodrugs (−)-(1*S*)-**3** and (−)-(*S*,*S*)-**6**, photolabile prodrugs (1*S*)-**4a**–**c** and (*S*,*S*)-**7a**/**b** as well as photolabile protecting groups **8** and **9**; DNA binding unit DMAI (5-[2-(*N*,*N*dimethylamino)ethoxy]-1*H*-indole-2-carboxylic acid) **10**.

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pounds of our group for ADEPT, there are several other prodrugs based on duocarmycin that are currently under investigation.[4] However, the use of the antibody–enzyme conjugate and the prodrug in a binary treatment has some drawbacks compared to a monotherapy, which include higher costs and an operatively more complicated application procedure. Herein, we describe the synthesis as well as the photochemical and biological evaluation of several new duocarmycin-based prodrugs (1*S*)-**4a–c** and (*S*,*S*)-**7a/b**, which can be activated by light in a monotherapeutic approach. To the best of our knowledge photolabile prodrugs based on duocarmycins and their analogues have not been described so far.

However, the application of light in cancer therapy is not new, for example, in photodynamic therapy (PDT) in which the tumor cells are destroyed by reactive oxygen species or radicals, which are formed by irradiation of a photosensitizer inside the tumor tissue.[5] Unfortunately, the effect of the therapy is rather unpredictable since the impact of oxidative stress may vary significantly depending on the cell compartment.[6]

Photoactivatable prodrugs based on platinum, sulfur mustards, or antimetabolites have also been described, but so far they exhibit a comparably low therapeutic effectiveness.[7] In contrast, the prodrug (*S*,*S*)-**7a** presented in this publication is more than two million times less cytotoxic than the corresponding seco-drug (−)-(*S*,*S*)-**5** with an IC_{50} value of 110 fM and its activation can be achieved in an effective way with light of a wavelength of $\lambda = 365$ nm.

For PDT there is a wide range of light sources known that can be applied by an endoscope or interstitial.[8] These sources should also be employable for the activation of the prodrug (*S*,*S*)-**7a**.

Results and Discussion

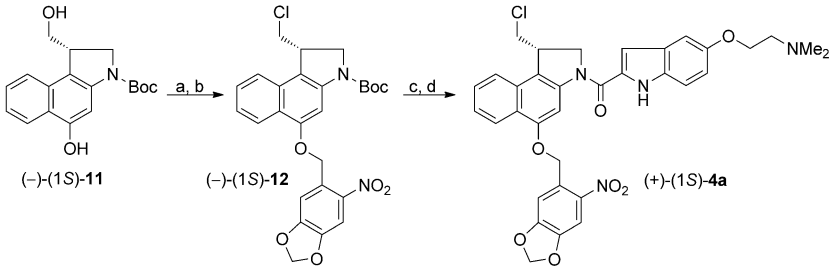
The new photoactivatable prodrugs (1*S*)-**4a–c** and (*S*,*S*)-**7a/b** are based on the seco-analogue (+)-(1*S*)-**2** of duocarmycin with a DNA binding unit and the dimeric duocarmycin analogue (−)-(*S*,*S*)-**5**, respectively. The photolabile protecting groups are bound to the phenolic hydroxyl groups to prevent the formation of the biologically active spiro-cyclopropyl unit. The *ortho*-nitrobenzyl protecting group is by far the most explored photolabile group used in biochemical applications.[9] Disadvantages of this group are the absorption maximum of short wavelength in the UV-B region and the slow cleavage rate. A shift to longer λ_{max} values can be accomplished by introduction of a 3,4-methylenedioxy substitution, which shifts the absorption maximum into the UV-A region. Moreover, introduction of a substituent in the benzylic position allows a strong acceleration of the photolysis (**8** and **9**).[10] To evaluate the influence of the hydrophilicity of the photolabile protecting group on the biological activity of the prodrugs, we employed protecting groups containing either a *tert*-butyl ester (prodrugs **4b** and **7a**) or a carboxylic acid moiety (prodrugs **4c** and **7b**).

For the synthesis of the photolabile prodrugs (1*S*)-**4a–c** and (*S*,*S*)-**7a/b** phenol (−)-(1*S*)-**11** was chosen as a substrate; it is easily accessible in enantiopure form on a large scale.[2a] The following transformations vary slightly for the different prodrugs. For prodrug (+)-(1*S*)-**4a** etherification of (−)-(1*S*)-**11** with **8** was performed, which was followed by an Appel reaction to afford (−)-(1*S*)-**12** by transfer of the hydroxymethyl into a chloromethyl group. This compound was then coupled with the DNA binding unit (5-[2-(*N*,*N*-dimethylamino)ethoxy]-1*H*-indole-2-carboxylic acid) (DMAI, **10**) in a sequence of Boc deprotection and amidation to yield (+)-(1*S*)-**4a** in 39% yield over four steps (Scheme 1).

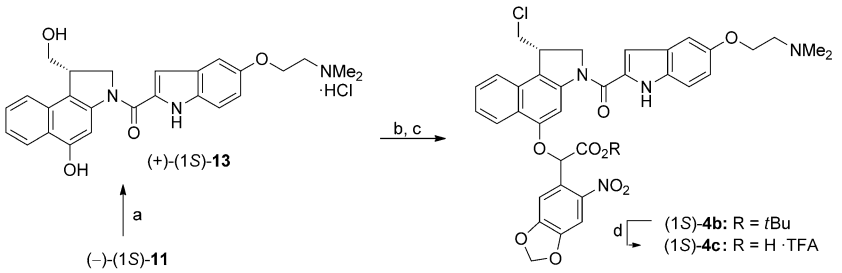
For the synthesis of prodrugs (1*S*)-**4b** and (1*S*)-**4c**, containing an α-substituted *ortho*-nitrobenzyl group, a modified sequence was used. Phenol (−)-(1*S*)-**11** was coupled with the DNA binding unit DMAI (**10**) by using a sequence of Boc deprotection and amidation under EDC activation, thereby affording (+)-(1*S*)-**13**. During the removal of the Boc group in ethyl acetate as the solvent, formation of partially acetylated products was observed; these were finally deacetylated under Zemplén conditions by using NaOMe in MeOH.

Reaction of (+)-(1*S*)-**13** with the benzylic bromide **9** under basic conditions led to a selective etherification of the phenolic hydroxyl group and the following Appel reaction afforded prodrug (1*S*)-**4b** in 20% yield over five steps (Scheme 2).

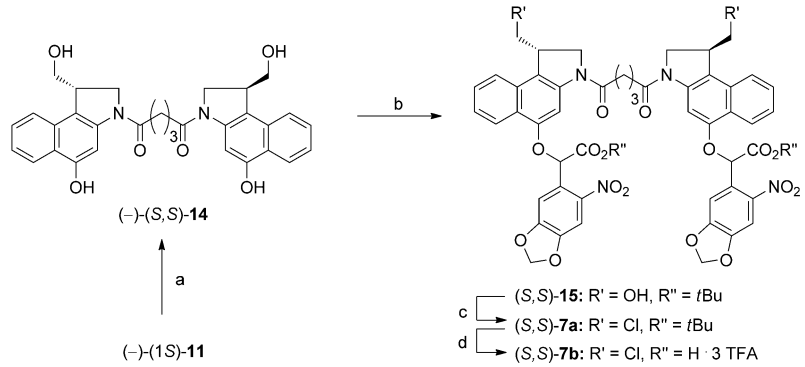
Treatment with TFA converted (1*S*)-**4b** into prodrug (1*S*)-**4c** containing a carboxylic acid moiety. The prodrugs (1*S*)-**4b** and (1*S*)-**4c** were obtained as a mixture of two enantiomerically pure diastereomers, since **9** was used as a racemic mixture. However, this shouldn't have any effect on the biological activity, since the removal of the photosensitive pro-



Scheme 1. Synthesis of photoactivatable prodrug (+)-(1*S*)-**4a**: a) K_2CO_3, DMF, RT, 20 min, then **8**, DMF, RT, 7.5 h, 67%; b) PPh_3, CCl_4, CH_3CN/CH_2Cl_2, RT, 4.5 h, 91%; c) 1) 4 M HCl/EtOAc, RT, 3 h; 2) DMAI·HCl (**10**·HCl), EDC·HCl, DMF, RT, 43.5 h, 64%; d) preparative HPLC: Kromasil 100 C18 (250×20 mm, 7 μm), A = H_2O, B = CH_3CN, gradient: A/B 80:20→10:90 in 40 min. Boc = *tert*-butoxycarbonyl; EDC = *N*′-(3-dimethylaminopropyl)-*N*-ethylcarbodiimide.



Scheme 2. Synthesis of the photoactivatable prodrugs (1*S*)-**4b** and (1*S*)-**4c**: a) 1) 4 M HCl/EtOAc, RT, 2 h; 2) DMAI·HCl (**10**·HCl), EDC·HCl, DMF, 0 °C→RT, 16 h; 3) NaOMe, MeOH/DMF, RT, 3 h, 61 %; b) **9**, K_2CO_3, DMF, RT, 24 h, 51 %; c) PPh_3, CCl_4, DMF, RT, 15 h, 64 %; d) TFA, RT, 1 h, 95 %. TFA = trifluoroacetic acid.



Scheme 3. Synthesis of prodrugs (*S*,*S*)-**7a** and (*S*,*S*)-**7b**: a) 1) 4 M HCl/EtOAc, RT, 2 h; 2) glutaryl dichloride, pyridine, DMF, 0 °C→RT, 18.5 h; 3) NaOMe, MeOH/DMF, RT, 5 h, 76 %; b) **9**, K_2CO_3, DMF, RT, 16 h, 74 %; c) 1) MsCl, NEt_3, CH_2Cl_2, 0 °C→RT 45 min; 2) LiCl, DMF, 75 °C, 5.5 h; 3) HPLC: Kromasil 100 C18 (250× 20 mm, 7 μm), A = H_2O, B = MeOH, gradient: A/B 30:70→0:100 in 25 min, 82 %; d) TFA, RT, 30 min, 90 %.

tecting group would be almost identical for both diastereomers and the seco-drug is formed as a pure enantiomer.

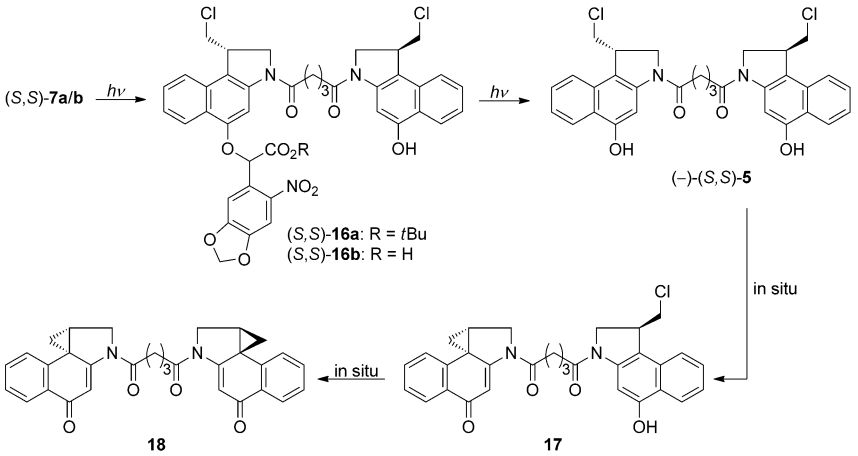
For the synthesis of the bifunctional prodrugs (*S*,*S*)-**7a** and (*S*,*S*)-**7b**, we applied an approach similar to the synthesis of (1*S*)-**4b** and (1*S*)-**4c** (Scheme 3). After removal of the *N*-Boc protecting group in (−)-(1*S*)-**11**, the resulting dihydrobenzoindole was coupled with glutaryl dichloride and treated with NaOMe in MeOH to give diphenol (−)-(*S*,*S*)-**14**. Selective etherification of the phenolic hydroxyl groups of (−)-(*S*,*S*)-**14** with benzylic bromide **9** led to (*S*,*S*)-**15** in 56 % yield over four steps. A conversion of this compound into prodrug (*S*,*S*)-**7a** by an Appel reaction did not give the desired product but led to decomposition of the starting material. Therefore, the prodrug (*S*,*S*)-**7a** was synthesized in a two-step procedure by mesylation and S_N2 reaction with LiCl in 82 % yield after HPLC purification.

Treatment of prodrug (*S*,*S*)-**7a** with TFA gave prodrug (*S*,*S*)-**7b** with two carboxyl acid groups, which contained three equivalents of TFA that could not be removed even after extended evacuation.

Kinetic studies on the removal of the photolabile protecting groups in the prodrugs (1*S*)-**4a–c** and (*S*,*S*)-**7a/b** were performed in PBS buffer with 1 and 10 % DMSO, respectively, by using an UV-transilluminator (Biostep, model UST-20L-8K, 365 nm, 1.1 mW cm^{-2}). The products of the reactions and their relative ratios were determined by LC-MS analysis.

After removal of the *ortho*-nitrobenzyl protecting groups, the resulting seco-drugs (+)-(1*S*)-**2** and (−)-(*S*,*S*)-**5** cyclize in situ to give the corresponding drugs. In the case of the bifunctional prodrugs (*S*,*S*)-**7a/b**, a two-step mechanism was observed as expected (Scheme 4). First, the mono-seco-drugs (*S*,*S*)-**16a/b** containing one protecting group and then the seco-drug (−)-(*S*,*S*)-**5** were generated followed by the formation of the mono-cyclo-seco-drug **17** with one and the drug **18** with two spiro-cyclopropyl groups, respectively. The cyclization products of the mono-seco-drugs (*S*,*S*)-**16a/b** were not observed.

Prodrug (*S*,*S*)-**7b** containing two carboxyl groups showed the best results in the irradiation experiments (Figure 2).



Scheme 4. Photochemical activation of dimeric seco-drugs (*S*,*S*)-**7a/b** (observed sequence).

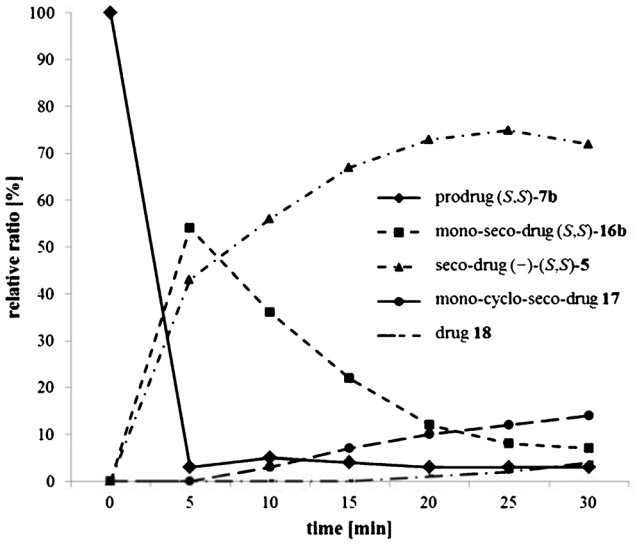


Figure 2. Photochemical cleavage of prodrug (*S*,*S*)-**7b**. ◆: prodrug (*S*,*S*)-**7b**; ■: mono-seco-drug (*S*,*S*)-**16b**; ▲: seco-drug (−)-(*S*,*S*)-**5**; ●: mono-cyclo-seco-drug **17**; -----: drug **18**.

The photochemical activation is very fast and the prodrug is almost completely converted into the mono-seco-drug (*S*,*S*)-**16b** and the seco-drug (−)-(*S*,*S*)-**5** after 5 min of irradiaton. The quantum yield was determined as 0.45 (calculated from the LC-MS chromatograms, see the Supporting Information). After 30 min, the seco-drug (−)-(*S*,*S*)-**5** and its cyclized analogues **17** and **18** were detected almost exclusively.

The prodrug (*S*,*S*)-**7a** containing two *tert*-butyl ester moieties instead of the carboxylic acid groups as in (*S*,*S*)-**7b** showed still a good photochemical transformation though it was slightly slower and gave more side products than (*S*,*S*)-**7b** (as seen by LC-MS); even so, after 30 min of irradiation the mono-cyclo-seco-drug **17** was the predominant product.

Prodrugs (1*S*)-**4a–c**, with a CBI–DMAI backbone showed unsatisfactory reactivity under photochemical excitation. The removal of the protecting groups proceeded slowly and several side products in higher concentrations were formed.

The determination of the in vitro cytotoxicity of the prodrugs (1*S*)-**4a–c** and (*S*,*S*)-**7a/b** was performed by using an HTCFA-derived (human tumor colony forming ability) test on human bronchial carcinoma cells A549 (Table 1). This test allows the determination of the proliferative ability of single cells and, therefore, also shows sub-lethal effects of the investigated toxins. For each test run a PBS-buffered solution containing the prodrug was irradiated externally with UV-A light for 30 min with a power of 2 J cm^{-2} (Biostep, model UST-20L-8K, 365 nm, 1.1 mW cm^{-2}) and then added to the cell culture. These conditions are very mild relative to those used for PDT or other photoactivatable prodrugs. Thus, for PDT, an irradiation power of up to 100 J cm^{-2} of visible light is used.[8a] In the case of other photoactivatable prodrugs irradiation power of the applied UV light is up to 25 J cm^{-2}.[7a,b,f] Usually the application of the toxins to the cells is performed in UltraCULTURE™ medium. Irradiation of the cells was not possible in this medium due to the presence of phenol red and a phenol red free UltraCULTURE™ medium was not commercially available. We therefore switched to the above mentioned external liberation of the drug from the prodrug. A control in PBS buffer showed only a little effect of the UV light on the cells' proliferative behavior.

Table 1. In vitro cytotoxicity of prodrugs (1*S*)-**4a–c** and (*S*,*S*)-**7a/b** and seco-drugs (+)-(1*S*)-**2** and (−)-(*S*,*S*)-**5**, cell line A 549.

Compound	IC_{50} without UV irradiation [nM]	IC_{50} after UV irradiation [nM]	QIC_{50}[a]
(+)-(1*S*)-**2**	0.026	–	–
(+)-(1*S*)-**4a**	4.5	5.5	–
(1*S*)-**4b**	11	11	–
(1*S*)-**4c**	0.03	5	–
(−)-(*S*,*S*)-**5**	1.1×10^{-4}	–	–
(*S*,*S*)-**7a**	230–245	0.03–0.08	3000–8200
(*S*,*S*)-**7b**	<0.001	0.03	–

[a] $QIC_{50}=(IC_{50}$ without irradiation)/(IC_{50} after irradiation).

Prodrug (*S*,*S*)-**7a** carrying two *tert*-butyl ester groups displayed very good results, with an IC_{50} value of 230–245 nM without irradiation. This corresponds to a difference in toxicity relative to the seco-drug (−)-(*S*,*S*)-**5** of more than two million, which is so far the highest difference obtained for any prodrug. After 30 min of irradiation of the prodrug (*S*,*S*)-**7a** followed by application to the cell culture, an IC_{50} value of 30–80 pM was found. This demonstrates a rapid conversion of prodrug (*S*,*S*)-**7a** into the seco-drug (−)-(*S*,*S*)-**5** and a high QIC_{50} value of 3000–8200 was calculated (for definition of QIC_{50} see the caption of Table 1). The results of the biological evaluation of the structurally related prodrug (*S*,*S*)-**7b**, containing two carboxyl acid moieties, are rather astounding. In this case, an unexpectedly low IC_{50} of <1 pM was found without irradiation. A LC-MS-based investigation of the stability of (*S*,*S*)-**7b** as a PBS-buffered solution showed no cleavage in the absence of light; furthermore, a decarboxylation was not detected during synthesis or workup. Upon irradiation, prodrug (*S*,*S*)-**7b** gave the seco-drug (−)-(*S*,*S*)-**5** with a decrease of biological activity. Thus, the introduction of an *ortho*-nitrobenzyl moiety carrying a carboxylic acid group enhances the overall cytotoxicity of the molecule. This is the first time that such an effect was observed for this class of protecting groups.[11] At the moment we cannot give a conclusive explanation for this phenomenon. A possible mechanism might involve a fast active transport of the prodrug to the active site, in which it could operate either as an intact compound or as the seco-drug (−)-(*S*,*S*)-**5**. The biological profile of prodrugs (1*S*)-**4a–c** in the presence or absence of light precludes their use as therapeutic agents. Interestingly, the carboxylic acid derived prodrug (1*S*)-**4c** showed also a higher cytotoxicity without irradiation as described for (*S*,*S*)-**7b**.

Conclusion

In conclusion, with prodrug (*S*,*S*)-**7a** we have successfully developed a photoactivatable prodrug whose cytotoxicity could be dramatically reduced by a factor of 2×10^6 in comparison to the parent seco-drug (−)-(*S*,*S*)-**5** ($IC_{50}=110$ fM). Compound (−)-(*S*,*S*)-**5** can be formed from (*S*,*S*)-**7a** by exposure to UV-A light under relatively mild conditions with an irradiation-power of only 2 J cm^{-2}. With a QIC_{50} of up to 8200 it is well suited for a potential use in a selective cancer therapy. In contrast, the introduction of an *ortho*-nitrobenzyl moiety carrying a carboxylic acid group enhances the overall cytotoxicity. Such an effect has not been described previously for this class of protecting groups.[11]

Experimental Section

General: All reactions were performed in flame-dried flasks under an argon atmosphere. Solvents were dried according to common laboratory techniques and freshly distilled prior to use. All reagents purchased from commercial sources were used directly without further purification. TLC analysis was performed on precoated silica gel SIL G/UV254 plates from Merck and Silica gel 60 (0.040–0.063 mm) from Merck was used for column chromatography. Vanillin in methanolic sulphuric acid (0.5 g vanillin, 3 mL conc. H_2SO_4, 85 mL methanol and 10 mL acetic acid) was used as the staining agent for TLC analysis. Preparative separations were performed on a HPLC system from Jasco equipped with two PU-2087 PLUS solvent pumps and a UV-2075 PLUS detector. As the stationary phase a Kromasil 100 C18 (250×20 mm, particle size 7 μm) column in combination with a Kromasil 100 C18 (50×20 mm, 5 μm) guard column (both from Jasco) was used. UV spectra were recorded by using a Lambda 2 spectrometer from Perkin–Elmer or a V-630 spectrometer from Jasco. IR spectra were recorded on a Bruker Vector 22 spectrometer (KBr discs). An FTIR-4100 instrument from Jasco was used as well. ^{1}H and ^{13}C NMR spectra were recorded on a Mercury 300, Unity 300 or Inova 600 from Varian. Chemical shifts are reported in δ (ppm). Residual peaks of the deuterated solvents indicated were used as internal standards. For HPLC-MS the ESI mass spectrometry with the ion-trap mass spectrometer LCQ (Finnigan) was used. The HPLC system comprises of solvent pump Rheos 400, degasser ERC-3415a (Flux Instruments), autosampler 851 (Jasco), and a diode array detector (Thermo). The column was a Synergi Max-RP C12 (150 2 mm, 4 mm, phenomenex).

Compound (*S*,*S*)-15: A solution of diphenol (−)-(*S*,*S*)-**14** (220 mg, 418 μmol, 1.00 equiv), K_2CO_3 (173 mg, 1.25 mmol, 3.00 equiv), and protective group **11** (331 mg, 919 μmol, 2.20 equiv) in abs. DMF (30 mL) was stirred for 16 h at room temperature. The solution was poured into brine (100 mL) and extracted with CH_2Cl_2 (4×150 mL). The combined organic layers were washed with brine (3×150 mL), dried over $MgSO_4$, and the solvent was removed under reduced pressure. The product (335 mg, 308 μmol, 74 %) was obtained by column chromatography on silica (CH_2Cl_2/MeOH 75:1→25:1) as a yellowish solid. $R_f=0.38$ (CH_2Cl_2/MeOH 25:1); UV (CH_3CN): λ_{max} (lg ε) = 194 (4.859), 256 (4.996), 264 (4.983), 318 nm (4.514); IR (ATR): $\tilde{\nu}$ = 1738, 1653, 1626, 1579, 1525, 1505, 1466, 1394, 1369, 1333, 1259, 1148, 1133, 1095, 1030, 928, 844, 757 cm^{-1}; ^{1}H NMR (600 MHz, [D$_6$]DMSO; signals of diastereomers are assigned if possible): δ = 1.37, 1.38 (2 s, 18 H; C(*CH*$_3$)$_3$), 1.88–1.97 (m, 2 H; 3-H$_2$), 2.51–2.71 (m$_c$, 4 H; 2-H$_2$, 4-H$_2$), 3.37–3.45 (m, 1 H; 10′-H$_a$), 3.71–3.88 (m, 2 H; 10′-H$_b$, 1′-H), 4.22 (m, 2 H; 2′-H$_a$, 2′-H$_b$), 4.88–4.97 (m, 2 H; CH$_2$*OH*), 6.23–6.31 (m, 2 H; O*CH*$_2$O), 6.42 (s, 1 H; O*CH*CO$_2$*t*Bu), 7.30, 7.33 (2 s, 1 H; 2″-H), 7.39–7.45 (m, 1 H; 7′-H), 7.55 (t, J = 7.6 Hz, 1 H; 7′-H), 7.76 (s, 1 H; 5″-H), 7.84 (d, J = 8.3 Hz, 1 H; 9′-H), 8.10, 8.12 (2 s, 1 H; 4′-H), 8.17 ppm (d, J = 8.4 Hz, 1 H; 6′-H); ^{13}C NMR (125 MHz, [D$_6$]DMSO; signals of diastereomers are assigned if possible): δ = 19.08 (C-3), 27.29, 27.30 (C(*CH*$_3$)$_3$), 34.32, 34.40 (C-2, C-4), 41.71, 41.74 (C-1′), 52.07 (C-2'), 63.05 (C-10'), 75.35, 75.53, 75.55 (*CH*CO$_2$*t*Bu), 82.64 (*C*-(CH$_3$)$_3$), 99.35, 99.52 (C-4′), 103.78 (O*CH*$_2$O), 105.68 (C-5″), 106.85 (C-2″), 118.54, 118.63, 121.95, 122.01 (C-5a′, C-9b′), 122.35, 122.38 (C-6′), 123.21 (C-9′), 123.60, 123.62 (C-7′), 127.00, 127.11, 127.22 (C-8′, C-1″), 130.03 (C-9a′), 141.07, 141.11, 141.89, 141.91 (C-6″, C-3a′), 147.99, 148.03 (C-4″), 151.97 (C-3″), 152.44, 152.47 (C-5′), 166.45, 166.48 (C=O), 170.92 ppm (CON); HRMS (ESI): m/z: calcd for $C_{57}H_{56}N_4O_{18}$: 1107.3482; found: 1107.3481 [M+Na]$^+$.

Compound (*S*,*S*)-7a: A solution of alcohol (*S*,*S*)-**15** (300 mg, 277 μmol, 1.00 equiv) in CH_2Cl_2 (30 mL) was cooled to 0 °C and treated with triethylamine (5.75 mL, 41.5 mmol, 150 equiv) and methanesulfonyl chloride (430 μL, 5.53 mmol, 20.0 equiv) and stirred for 45 min at room temperature. The solution was poured into sat. $NaHCO_3$ (75 mL) and extracted with CH_2Cl_2 (3×75 mL). The combined organic layers were dried over $MgSO_4$ and the solvent was removed under reduced pressure. The residue was dried under high vacuum together with LiCl (410 mg, 9.68 mmol, 20.0 equiv) for 1 h. Abs. DMF (30 mL) was added and the solution was stirred for 5.5 h at 75 °C. The reaction was monitored by HPLC analysis. After cooling to room temperature, the solution was poured into brine (90 mL) and extracted with CH_2Cl_2 (3×75 mL). Combined organic layers were dried over $MgSO_4$, the solvent was removed, and the residue was taken up in CH_2Cl_2 and filtered over a hydrophobic syringe filter. Purification on preparative HPLC gave the product (254 mg, 226 μmol, 82 %) as a colorless solid. HPLC (analytical) t_R = 12.32 min (Kromasil 100 C-18 250×4.6 mm, 5 μm) on a gradient: A = MeOH, B = water; 0–6 min: 70A/30B→100A/0B, 6–9.5 min: 100A/0B, 9.5–10 min: 100A/0B→70A/30B, 10–15 min: 70A/30B, flow = 0.8 mL min^{-1}; HPLC (preparative) t_R = 26.18 min (Kromasil 100 C-18 250×4.6 mm, 5 μm) on a gradient: A = MeOH, B = water; 0–25 min: 70A/30B→100A/0B, 25–30 min: 100A/0B, 30–31 min: 100A/0B→70A/30B, 31–40 min: 70A/30B, flow = 18 mL min^{-1}; UV (CH_3CN): λ_{max} (lg ε) = 196 (4.920), 255 (4.828), 263 (4.820), 305 (4.260), 317 nm (4.326); IR (ATR): $\tilde{\nu}$ = 2954, 2920, 2851, 1736, 1661, 1628, 1579, 1525, 1505, 1483, 1465, 1395, 1368, 1332, 1310, 1258, 1147, 1133, 1097, 1081, 1032, 965, 929, 844, 818, 755, 718 cm^{-1}; ^{1}H NMR (600 MHz, [D$_6$]DMSO; signals of diastereomers are assigned if possible): δ = 1.37 (s, 18 H; C(*CH*$_3$)$_3$), 1.88–1.98 (m, 2 H; 3-H$_2$), 2.50–2.73 (m$_c$, 4 H; 2-H$_2$, 4-H$_2$), 3.79–3.92 (m, 1 H; 10′-H$_a$), 3.96–4.04 (m, 1 H; 10′-H$_b$), 4.14–4.27 (m, 2 H; 1′-H, 2′-H$_a$), 4.30–4.40 (m, 1 H; 2′-H$_b$), 6.28 (t, J = 9.2 Hz, 2 H; O*CH*$_2$O), 6.45, 6.46 (2 s, 1 H; O*CH*CO$_2$*t*Bu), 7.31, 7.35 (2 s, 1 H; 2″-H), 7.43–7.47 (m, 1 H; 7′-H), 7.56–7.60 (m, 1 H; 8′-H), 7.76, 7.77 (2 s, 1 H; 5″-H), 7.88 (d, J = 8.3 Hz, 1 H; 9′-H), 8.12, 8.14 (2 s, 1 H, 4′-H), 8.20 ppm (d, J = 8.4 Hz, 1 H; 6′-H); ^{13}C NMR (125 MHz, [D$_6$]DMSO; signals of diastereomers are assigned if possible): δ = 19.07 (C-3), 27.32 (C-(*CH*$_3$)$_3$), 34.36 (C-2, C-4), 40.60, 40.71 (C-1′), 47.53, 47.62 (C-10′), 52.63 (C-2′), 75.32, 75.48 (O*CH*CO$_2$*t*Bu), 82.71 (*C*(CH$_3$)$_3$), 99.15, 99.32 (C-4′), 103.80 (O*CH*$_2$O), 105.68, 105.70 (C-5″), 106.88, 106.92 (C-2″), 116.83, 116.92 (C-5a′), 122.00, 122.08 (C-6′), 122.53, 122.59 (C-9b′), 122.76 (C-9′), 123.85 (C-7′), 126.85, 126.99 (C-1″), 127.72 (C-8′), 129.74 (C-9a′), 141.64 (C-3a′), 141.93, 141.95 (C-6″), 148.04, 148.07 (C-4″), 151.98 (C-3″), 153.06, 153.14 (C-5′), 166.40, 166.43 (CO$_2$*t*Bu), 170.87, 170.90 ppm (CON); HRMS (ESI): m/z calcd for $C_{57}H_{54}Cl_2N_4O_{16}$: 1143.2804; found: 1143.2811 [M+Na]$^+$.

Compound (*S*,*S*)-7b: Ester (*S*,*S*)-**7a** (36.3 mg, 32.6 μmol) was dissolved in TFA (2.5 mL) and stirred for 30 min at room temperature. The solvent was removed under reduced pressure and the residue was dried under high vacuum. The product (39.9 mg, 29.5 μmol, 90 %) was obtained as a light-yellow solid. HPLC (analytical) t_R = 16.56, 17.00, 17.36 min (Kromasil 100 C-18 250×4.6 mm, 5 μm) on a gradient: A = MeOH, B = water + 0.03 % HCl; 0–15 min: 50A/50B→100A/0B, 15–25 min: 100A/0B, 25–27 min: 100A/0B→50A/50B, 27–35 min: 50A/50B, flow = 0.8 mL min^{-1}; UV (CH_3CN): λ_{max} (lg ε) = 195 (4.879), 255 (5.004), 263 (4.986), 306 (4.431), 317 nm (4.500); IR (ATR): $\tilde{\nu}$ = 1724, 1652, 1627, 1579, 1523, 1505, 1467, 1396, 1332, 1259, 1131, 1023, 982, 927, 756 cm^{-1}; ^{1}H NMR (600 MHz, [D$_6$]DMSO; signals of diastereomers are assigned if possible): δ = 1.87–1.96 (dd, J = 14.3, 7.1 Hz, 2 H; 3-H$_2$), 2.51–2.71 (m$_c$, 4 H; 2-H$_2$, 4-H$_2$), 3.80–3.91 (m, 1 H; 10′-H$_a$), 4.01 (dd, J = 14.2, 6.4 Hz, 1 H; 10′-H$_b$), 4.15–4.26 (m, 2 H; 1′-H, 2′-H$_a$), 4.30–4.40 (m, 1 H; 2′-H$_b$), 6.20–6.30 (m, 2 H; OCH$_2$O), 6.55, 6.56 (2 s, 1 H; O*CH*CO$_2$H), 7.29, 7.33 (2 s, 1 H; 2″-H), 7.44 (t, J = 7.6 Hz, 1 H; 7′-H), 7.58 (t, J = 7.6 Hz, 1 H; 8′-H), 7.74, 7.75 (2 s,

1H, 5″-H), 7.87 (d, $J=8.4$ Hz, 1H; 9′-H), 8.09, 8.11 (2s, 1H; 4′-H), 8.22 ppm (d, $J=8.5$ Hz, 1H; 6′-H); ^{13}C NMR (125 MHz, [D$_6$]DMSO; signals of diastereomers are assigned if possible): $\delta=18.98$ (C-3), 34.37 (C-2, C-4), 40.59, 40.69 (C-1′), 47.46, 47.59 (C-10′), 52.61 (C-2′), 74.46, 74.52 (O*CH*CO$_2$H), 99.74, 99.78 (C-4′), 103.73 (OCH$_2$O), 105.62, 105.63 (C-5″), 107.03, 107.12 (C-2″), 116.60, 116.63 (5a, 9a, or 9b), 121.98 (C-6′), 122.58, 122.64 (C-9′), 122.70 (5a′, 9a′, or 9b′), 123.78 (5a′, 9a′, or 9b′), 127.31, 127.33 (C-1″), 127.67 (5a′, 9a′, or 9b′), 129.72 (5a′, 9a′, or 9b′), 141.63, 141.98 (C-6″), 148.00, 148.02 (C-4″), 151.95 (C-3″), 153.01, 153.08 (C-5′), 168.74, 168.78 (CO$_2$H), 170.89, 170.90 ppm (CON); HRMS (ESI): *m/z*: calcd for $C_{55}H_{41}Cl_2F_9N_4O_{22}$: 1053.1372; found: 1053.1368 [M−TFA−H+2Na]$^+$.

Photochemical investigations: The prodrugs (+)-(1*S*)-**4a**, (1*S*)-**4b**, (1*S*)-**4c**, (*S*,*S*)-**7a**, or (*S*,*S*)-**7b** were dissolved in DMSO and the solution was diluted with PBS-buffer to give a total volume of 4 mL with 1% DMSO in the case of (1*S*)-**4c**, (*S*,*S*)-**7a**, and (*S*,*S*)-**7b** or 10% DMSO for (+)-(1*S*)-**4a** or (1*S*)-**4b**, respectively. The concentrations of the solutions were 0.009–0.03 mM. The solution was placed in a $\varnothing=5$ cm petri dish and the 0 min sample was taken with an Eppendorf pipett. Then irradiation with the transilluminator (Biostep, model UST-20L-8E, 365 nm, ca. 1100 μW cm^{-2}) was started and samples were taken after 5, 10, 15, 20, 25, and 30 min. Each sample was an amount of 100 μL. After the 30 min sample was taken, all samples were injected into the LC-MS and spectra were recorded.

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