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Expanded Utility of the β-Glucuronide Linker: ADCs That Deliver Phenolic Cytotoxic Agents

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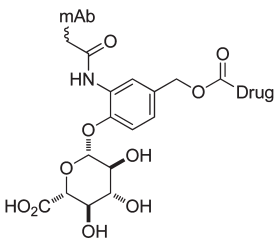
ABSTRACT The β-glucuronide linker has been used for antibody−drug conjugates (ADCs) to deliver amine-containing cytotoxic agents. The linker is stable in circulation, hydrophilic and provides ADCs that are highly active in vitro and in vivo. To extend the utility of the β-glucuronide linker toward phenol-containing drugs, an *N*,*N*′-dimethylethylene diamine self-immolative spacer was incorporated with the linker for release of the potent cytotoxic phenol psymberin A. Exposure of the drug-linker to β-glucuronidase resulted in facile drug release. The corresponding ADCs were active and immunologically selective against CD30-positive L540cy and CD70-positive Caki-1 cell lines.

KEYWORDS β-Glucuronide linker, antibody−drug conjugates, phenolic cytotoxic agents, *N*,*N*′-dimethylethylene diamine, CD30-positive L540cy cell line, CD70-positive Caki-1 cell line



β-Glucuronide Linker

Antibody−drug conjugates (ADCs) consist of three primary components: a monoclonal antibody that targets a tumor antigen with high tumor expression, a cytotoxic agent, and a linker system that releases drug from the antibody upon internalization into cancer cells. When a linker system with high circulation stability and tumor specificity is used, tolerability and efficacy can be significantly augmented.[1−3] In an effort to develop safe and effective ADCs, we have focused on linker systems meeting these criteria. We have reported on ADCs employing the β-glucuronide linker (Figure 1) with several drug classes including auristatins,[4] a highly potent doxorubicin derivative,[5] CBI minor groove binders,[6] and camptothecin analogues.[7] The β-glucuronide linker is readily cleaved by β-glucuronidase, an enzyme present in lysosomes and tumor interstitium.[8,9] The linker is highly stable in circulation and hydrophilic, which allows the use of hydrophobic drugs for ADCs that otherwise would lead to high degrees of aggregation.[6,10]

Potent cytotoxic agents with basic amine residues are attractive molecules for targeted delivery using the β-glucuronide linker because the amine can be used for linker attachment. We were interested in extending the application of the β-glucuronide linker beyond amines to phenol-containing drugs, since the phenol functional group is a common element in many anticancer drugs. For example, the camptothecin derivative SN38 (**1**) and duocarmycins such as **2** possess phenol residues. Another phenol-containing drug, psymberin (aka irciniastatin A, **3**), is a potent anticancer molecule with subnanomolar cytotoxic activity (Table 1).[11] The treatment of cells with **3** leads to apoptosis through an undefined mechanism.

Our goal was to modify the β-glucuronide linker to contain a dimethylethylene diamine (DMED) self-immolative spacer[12] for stable linkage and facile release of **3**. Drug release would involve enzymatic deglucuronidation, 1,6-elimination, decarboxylation, and cyclization of the DMED carbamate to liberate free phenol (Scheme 1). Toward this end, the glucuronide *para*-nitrophenyl (pNP) carbonate **4** was reacted with excess DMED to readily afford the stable coupling partner **5** (Scheme 2). Psymberin (**3**) was activated with pNP carbonate to give the phenolic carbonate **6**. The coupling of **5** and **6** gave a modest yield of **7**. Saponification of the glucuronide acetate and methyl ester groups was accomplished concomitantly with Fmoc removal, and coupling to maleimidocaproyl-*N*-hydroxy succinimide ester provided the final drug-linker **8**.

To confirm the reactivity of **8** toward enzymatic cleavage,[4] the linker was treated with cysteine (Scheme 3) to form the cysteine−succinimide adduct **9** (80 μM), which was then exposed to β-glucuronidase (*Escherichia coli*, 36 μg/mL) at 37 °C. After 10 min, inspection of the reaction mixture by liquid chromatography−mass spectrometry (Figure 2) demonstrated that **9** had been almost completely consumed. The specific activity for enzymatic hydrolysis is 0.21 μmol/min/mg, which is consistent with a previous measurement on an auristatin-based drug-linker.[4] The products were **3** and the residual linker components, specifically the diastereomeric cysteine adduct **10**. The absence of any detectible intermediate carbamate **11** demonstrates that self-immolation of the DMED intermediate **11** to release **3** was rapid.

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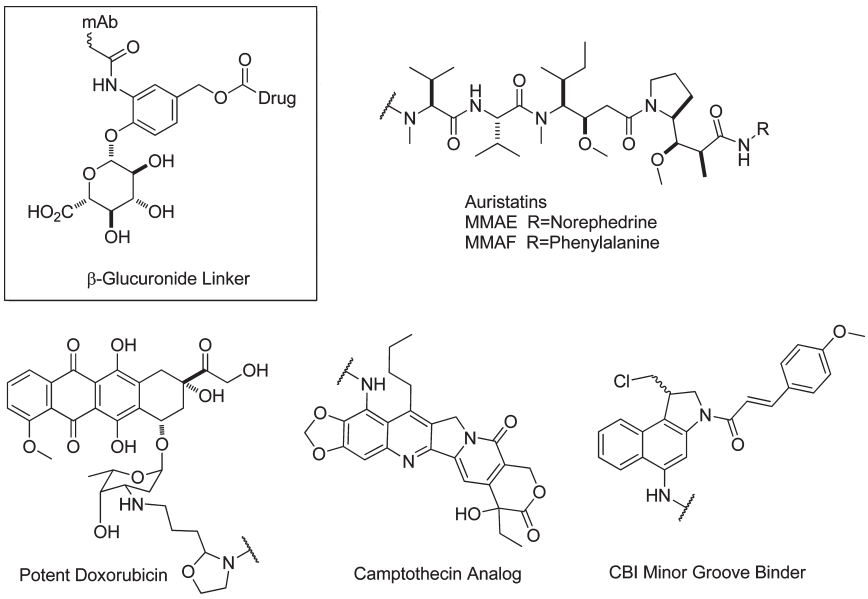

Figure 1. Examples of ADCs with the β-glucuronide linker.

Scheme 1. Release Mechanism from β-Glucuronide Linker

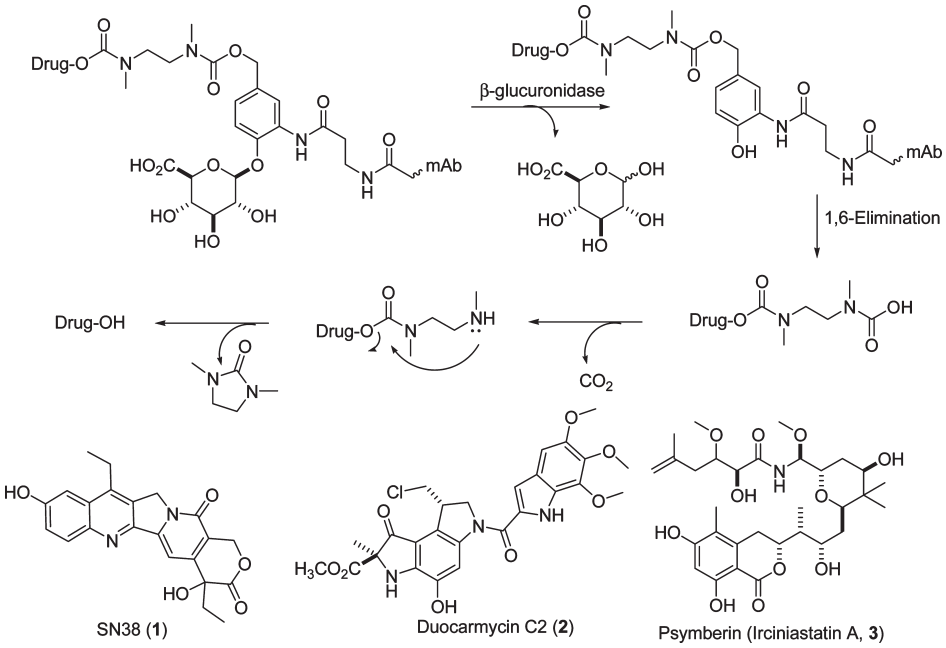

Table 1. IC_{50} Values of Psymberin (**3**) and β-Glucuronide Drug-Linker **8** Conjugates[a]

	Caki-1 (CD70+/CD30−)	L540cy (CD70−/CD30+)
psymberin (**3**)	0.3	0.7
cAC10−**8** (5.4 dr/mAb)	62	0.15
h1F6−**8** (5.4 dr/mAb)	0.8	58.6

[a] Values are listed in nM.

Drug-linker **8** was conjugated to the monoclonal antibodies cAC10 (anti-CD30 mAb) and h1F6 (anti-CD70 mAb) via reduced interchain disulfides. An average loading of 5.4 drugs/mAb was achieved, and the conjugates were >95% monomeric. Exposure to the CD30-expressing cell line L540cy (Hodgkin's lymphoma) to cAC10−**8** over a 96 h period gave an IC_{50} value of 0.15 nM (Table 1). The cell killing was immunologically selective as exposure to the CD30-negative cell line Caki-1 (renal cell carcinoma) gave an IC_{50}

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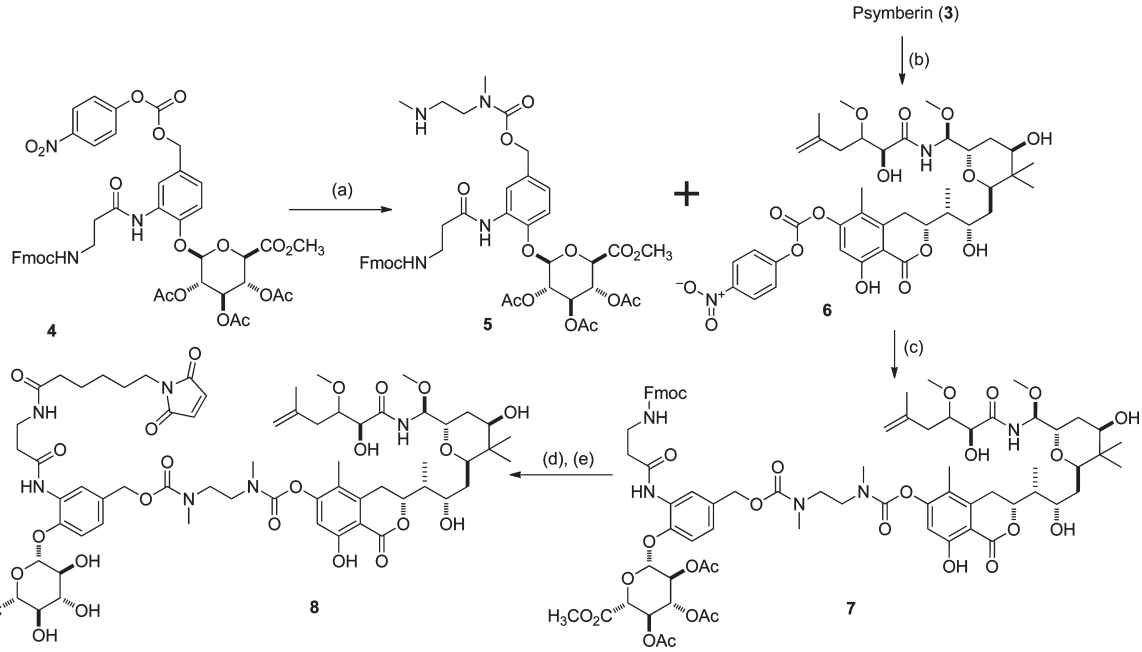
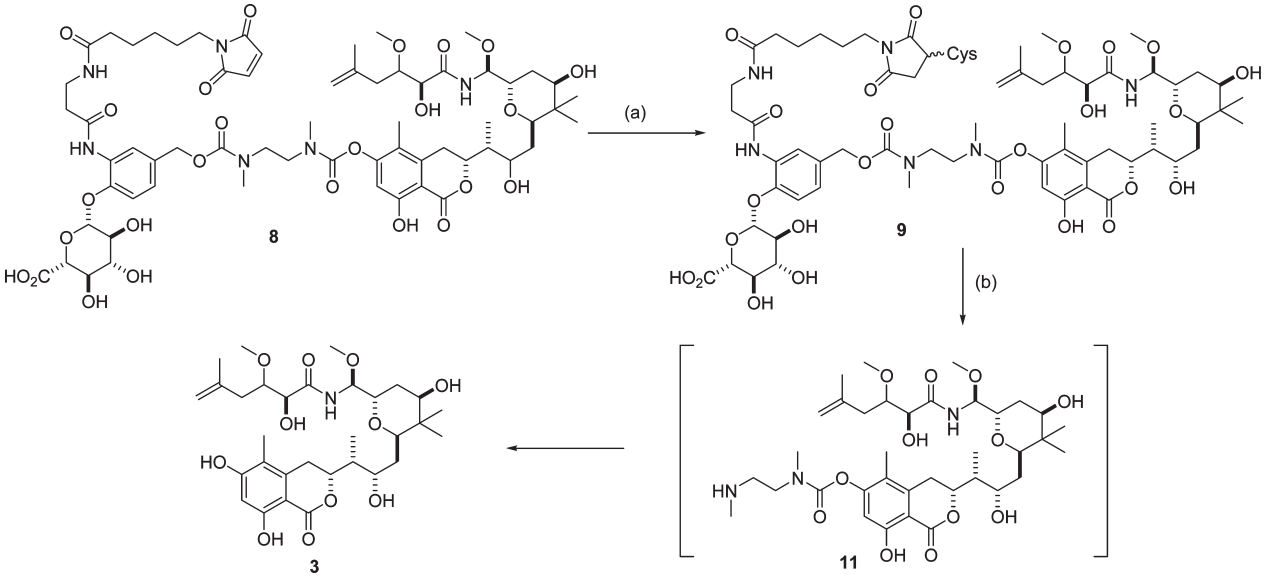
Scheme 2[a]



[a] Reagents and conditions: (a) DMED (10 equiv), CH_2Cl_2, 61%. (b) pNP carbonate, DIPEA, THF, 49%. (c) Compound **5** and **6**, CH_2Cl_2, 42%. (d) LiOH (9 equiv), CH_3OH, THF, H_2O. (e) MC−OSu, DMF, DIPEA, 62% two steps.

Scheme 3[a]



[a] Reagents and conditions: (a) Cysteine. (b) β-Glucuronidase (*E. coli*), 30 min.

value of 62 nM. Conversely, the h1F6−**8** ADC showed good activity on the CD70-expressing line Caki-1 but significantly reduced activity against the CD30-negative line L540cy. Unconjugated **3** was highly potent on these cell lines. The selectivity observed over the 96 h exposure assay suggests a high degree of stability, which is consistent with previous findings with the β-glucuronide linker.[7]

ADCs continue to demonstrate their value in the clinic,[3,13−16] and as a consequence, investigations into new chemotypes and linkers for ADCs will continue. The favorable properties

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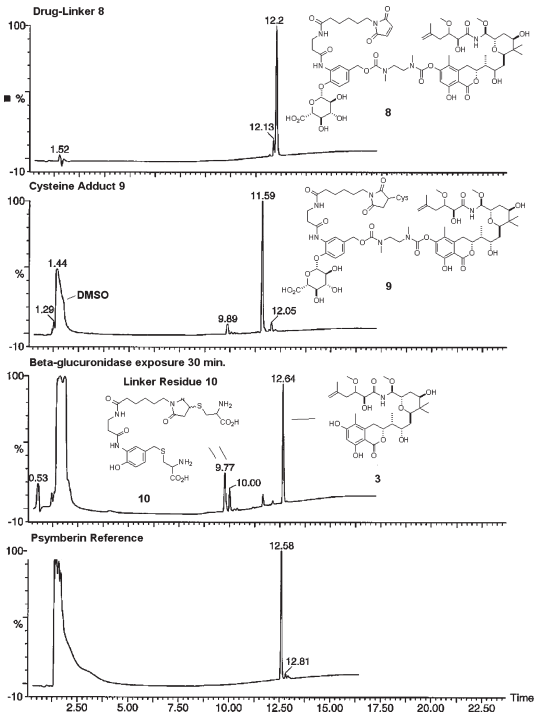

Figure 2. HPLC analysis of β-glucuronidase cleavage of psymberin drug linker **9**. Peaks were observed using a diode array detector.

of the β-glucuronide linker, including its hydrophilicity, stability in circulation, tumor selectivity, and efficient drug release inside tumor cells elevate this linker as an appealing component for new ADC development and chemotype screening. The expansion of its utility for the release of a phenol-containing cytotoxic such as psymberin (**3**) has been demonstrated in this communication and will be an avenue of continued investigation in our laboratory.

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