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Synthesis and biological evaluation of *RON*-neoglycosides as tumor cytotoxins

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ABSTRACT

Cardenolides such as digitoxin have been shown to inhibit cancer cell growth, to reduce cancer metastasis, and to induce apoptosis in tumor cells. Among the most potent digitoxin-based cytotoxins identified to date are *MeON*-neoglycosides generated via oxyamine neoglycosylation. Here, we report our studies of oxyamine neoglycosylation aimed at facilitating the elucidation of linkage-diversified digitoxin neoglycoside structure–activity relationships. We identified conditions suitable for the convenient synthesis of digitoxin neoglycosides and found that sugar structure, rather than *RON*-glycosidic linkage, exerts the strongest influence on neoglycoside yield and stereochemistry. We synthesized a library of digitoxin neoglycosides and assessed their cytotoxicity against eight human cancer cell lines. Consistent with previous findings, our data show that the structure of *RON*-neoglycosidic linkages influences both the potency and selectivity of digitoxin neoglycosides.

1. Introduction

Cardiac glycosides have been used for several centuries as drugs to treat congestive heart failure and arrhythmias.[1] However, more recently, cardenolides such as digitoxin (**1**, Fig. 1) have been shown to inhibit cancer cell growth, to reduce cancer metastasis, and to induce apoptosis in tumor cells.[2–10] Thus, the receptor of cardiac glycosides, Na^+/K^+-ATPase,[11] is receiving increasing attention as a novel target for cancer chemotherapy.[12] The primary role of Na^+/K^+-ATPase is to maintain an electrochemical gradient across the plasma membrane of eukaryotes by transporting sodium ions out of cells and potassium ions into cells. The resulting Na^+ gradient plays a role in osmotic regulation and drives secondary transport processes ranging from nutrient intake to Na^+/Ca^{2+} exchange. However, a growing body of evidence suggests that a subset of Na^+/K^+-ATPase, likely localized within plasma membrane caveolae, plays a role in cell signaling instead of ion transport.[13] Inhibition of this population by cardiac glycosides activates the non-receptor tyrosine kinase Src,[14] leading to a number of downstream effects that can influence the development and progression of cancers. These effects include the transactivation of EGRF and activation of the Ras/MAPK signaling cascade,[14b,15] an increase of reactive oxygen species in mitochondria,[16] the regulation of caveolin-1 trafficking,[17] the modulation of the structure of cell–cell tight junctions,[18] and apoptosis.[2–9,10a–c]

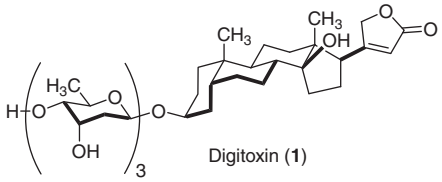

Figure 1. Digitoxin, a cardiac glycoside, is receiving increasing attention for its activity against human cancer cells.

As researchers work to elucidate the complex mechanisms of action associated with the anticancer activities of cardiac glycosides, structure–activity relationship (SAR) studies have identified several structural features of digitoxin derivatives that are critical to Na^+/K^+-ATPase inhibition and cytotoxicity.[9,10,19] The presence of the carbohydrate moiety is critical; cardiac glycosides are invariably better Na^+/K^+-ATPase inhibitors than the corresponding aglycons.[9c,19] The cytotoxicity of both *O*-glycosidic and *MeON*-neoglycosidic analogs of digitoxin is dependent on carbohydrate stereochemistry[10a] and on saccharide chain length,[9a,10b] with monosaccharide derivatives displaying the most potent activities.

Among the most potent digitoxin-based cytotoxins identified to date are L-riboside and L-xyloside *MeON*-neoglycosides[9c] generated via oxyamine neoglycosylation (Fig. 2), a chemoselective glycosylation methodology that employs unprotected, unactivated

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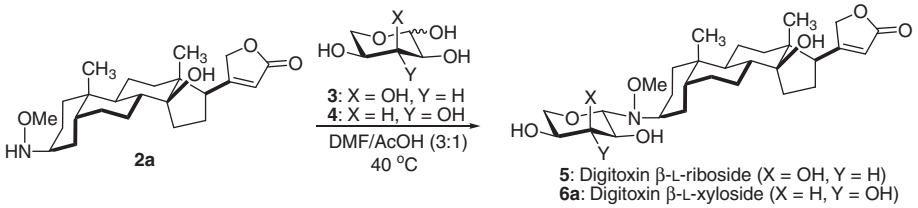


Figure 2. L-Riboside and L-xyloside *MeON*-neoglycosides generated via oxyamine neoglycosylation.

reducing sugars to form the corresponding closed ring neoglycosides in good yields.[9,20–23] The fact that these molecules are active, despite containing non-natural *MeON*-neoglycosidic linkages, led us to realize that glycosidic linkages could represent a third point of diversity to optimize cardiac glycosides, complementing the modification of aglycons and sugars. Although this additional site of diversification has received little attention, we recently showed that oxyamine linkages can be modified to identify structural variants with enhanced tumor selectivities.[9b]

Here, we report our studies of oxyamine neoglycosylation aimed at facilitating the further elucidation of digitoxin neoglycoside structure–activity relationships. First, we explored new oxyamine neoglycosylation reaction conditions, as well as the influence different oxyamine structures have on *RON*-neoglycoside yield, stereochemistry, and stability. Finally, we synthesized and evaluated an expanded library of digitoxin cytotoxins bearing *RON*-neoglycosidic linkages.

2. Results and discussion

2.1. Oxyamine neoglycosylation optimization

Many conditions for oxyamine neoglycosylation have been published;[9,20–23] most take place in either aqueous acidic buffers or organic solvents containing acetic acid. Previous efforts to generate digitoxin analogs via oxyamine neoglycosylation successfully employed DMF/AcOH (3:1),[9c,20,21] but we have found that the difficulties associated with evaporating DMF from crude reaction mixtures significantly diminish our ability to generate neoglycoside libraries efficiently. Thus, we set out to determine the compatibility of lower boiling solvent mixtures with oxyamine neoglycosylation. We conducted this study using a simple, achiral secondary oxyamine (**7a**) that we generated from inexpensive starting materials (Fig. 3).

Methoxyamine **7a** was treated with D-glucose (1.1 equiv) for two days in 10 different solvent mixtures that contained either a molar equivalent of AcOH or AcOH as a cosolvent; percent conversions were estimated using LC–MS (Table 1). We were gratified to find that all conditions we examined provided good to excellent percent conversions to neoglucoside **8a**. Although conditions employing AcOH as a cosolvent generally provided the highest yields, it was simpler to evaporate solvents from reaction mixtures containing only a single molar equivalent of AcOH. Because digitoxin oxyamines **2** were sparingly soluble in alcoholic solvents, somewhat soluble in MeOH/$CHCl_3$ (4:1), and readily soluble in MeOH/$CHCl_3$ (9:1), we chose the latter conditions for our subsequent studies.

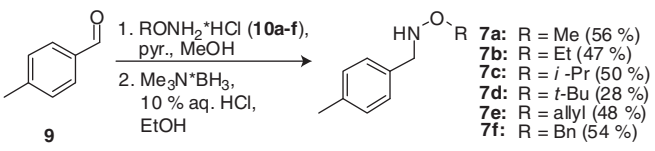
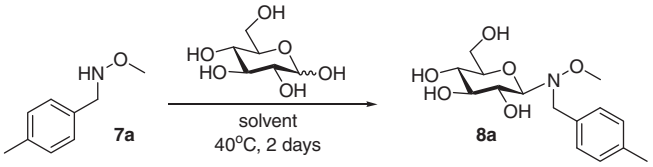


Figure 3. Synthesis of model aglycons.

Table 1
Effect of reaction conditions on percent conversion of neoglycosylation



Entry	Solvent	Conversion[a] (%)
1	DMF/AcOH (3:1)	94
2	Pyridine/AcOH (3:1)	97
3	Acetone/AcOH (3:1)	93
4	THF/AcOH (3:1)	95
5	AcOH	94
6	MeOH[b]	87
7	EtOH[b]	84
8	*i*PrOH[b]	81
9	MeOH/$CHCl_3$ (9:1)[b]	87
10	MeOH/$CHCl_3$ (4:1)[b]	92
11	MeOH/$CHCl_3$ (1:1)[b]	72

[a] As estimated by LC–MS.
[b] With 1 eq AcOH.

2.2. *RON*-neoglycoside yield and stereochemistry

Because of the expense associated with generating digitoxin oxyamines, we decided to employ model aglycons to study systematically the influence of oxyamine structure on *RON*-neoglycoside yield, stereochemistry, and stability. Model secondary oxyamine aglycons **7a**–**f** were synthesized from *p*-tolualdehyde (**9**) by a simple reductive oxyamination strategy as shown in Figure 3. These oxyamines were reacted with D-glucose, D-galactose, D-mannose, *N*-acetylglucosamine, and *N*-acetylgalactosamine under the optimized conditions discussed above to form *RON*-neoglycosides **8a**–**y** (Table 2).

For a given sugar, oxyamine structure appeared not to significantly influence yield until a steric threshold was reached; nearly all of the tested oxyamine neoglycosylations proceeded with good to excellent yields, the chief exception being those involving sterically bulky oxyamine **7d** (R = *t*-Bu). Interestingly, among the glycosylated *O*-*tert*-butylhydroxylamines, the mannoside provided a significantly higher yield (46%) than the other glycosides (17–19%). It is possible that the axial O-2 group of the mannoside does not interact as strongly with the *tert*-butyl group as does the equatorial O-2 of the other derivatives. In accord with previously published work,[20] isomer ratios varied dramatically as a function of the sugar, with glucose derivatives affording β-pyranose products, galactose derivatives affording β-pyranose/β-furanose mixtures, and D-mannose affording β-pyranose/α-pyranose/α-furanose mixtures. Attempts to resolve neoglycoside product mixtures are known to fail due to rapid equilibration between product isomers.[9c]