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Synthesis of Cardiac Glycoside Analogs by Catalyst-Controlled, Regioselective Glycosylation of Digitoxin

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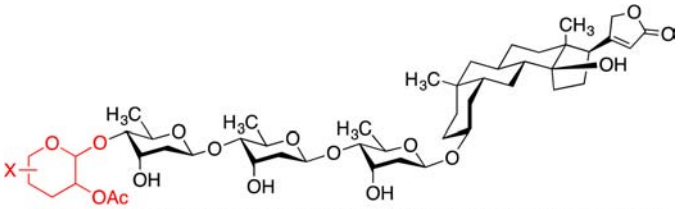
ABSTRACT



Prepared by catalyst-controlled regioselective glycosylation of digitoxin
6 examples, 51–75% yield

The cardiac glycoside natural product digitoxin was selectively glycosylated at one of its five hydroxyl groups using a borinic acid derived catalyst. This method provided access to the glycosylation pattern characteristic of a subclass of natural products from *Digitalis purpurea*. Variation of the glycosyl donor was tolerated, enabling the synthesis of novel cardiac glycoside analogs from readily available materials.

The chemo- or regioselective derivatization of complex natural products is an intriguing chemical challenge and a complementary strategy to total synthesis in terms of the preparation of new analogs. Such analogs can be used to probe structure–activity relationships or mechanisms of action in biological systems, fueled by the importance of natural products and their derivatives as drug candidates.[1] Efficient methods for selective functionalization of natural products have emerged recently, with catalysis (either synthetic or enzymatic) playing a key role. Given that many classes of natural products are hydroxylated, regioselective modification of OH groups has been pursued intensively; synthetic catalyst systems have been developed for selective acylation,[2,3] deoxygenation,[4] and O–H insertion reactions[5] of complex polyol natural products.[6]

Among the possible chemical modifications of polyol natural products, *O*-glycosylation (the covalent attachment of one or more carbohydrate moieties) occupies a prominent position. Numerous bioactive secondary metabolites are glycosylated, including several currently employed therapeutic agents.[7] While elucidation of the roles of the carbohydrate moieties in such compounds is an ongoing task, it is clear that glycosylation contributes significantly and, in certain cases, is essential to biological activity. The regioselective *O*-glycosylation of complex

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natural products has thus generated great interest. Existing solutions to this problem employ enzyme catalysis, either *in vivo* (metabolic engineering) or *in vitro* (chemoenzymatic methods).[8] Progress along this second line has been achieved through the discovery and engineering of enzymes that are tolerant of variation of both the glycosyl donor and acceptor moiety. Among the substrates that have been successfully glycosylated using catalysts of this type are vancomycin, macrolide antibiotics, and flavonoid natural products.[9] On the other hand, the use of synthetic catalysts for regioselective glycosylation of natural products has yet to be demonstrated.

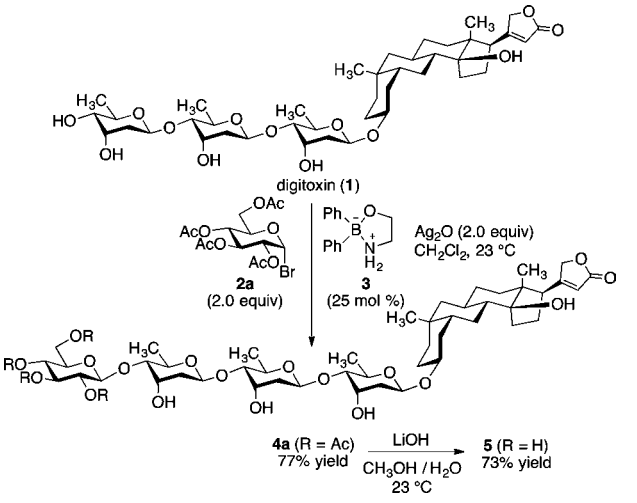
Our laboratory has identified diarylborinic acids as useful catalysts for the regioselective acylation, alkylation, sulfonylation, and glycosylation of minimally protected pyranoside substrates.[10,11] In light of the potential for complementarity with the chemoenzymatic methods mentioned above, we sought to apply this catalyst system to the selective glycosylation of polyol natural products. Here, we describe the organoboron-catalyzed regioselective monoglycosylation of digitoxin, providing access to novel *purpurea*-type cardiac glycosides.

Cardiac glycosides from *Digitalis purpurea* have been utilized as treatments for heart problems for over 200 years. They are characterized by a steroid core substituted by an unsaturated lactone at C17 and by a variable sugar portion at C3.[12] Digitoxin (**1**, Scheme 1) is a prototypic example, wherein the digitoxigenin core bears a trisaccharide composed of 2,6-dideoxy sugars. A structural feature important to the present study was the presence of a single pyranoside-derived *cis*-vicinal diol grouping, the motif required for efficient organoboron-catalyzed regioselective glycosylation.[10]

The activity of the cardiac glycosides as inhibitors of Na^+/K^+-ATPase results in an increase in intracellular Na^+ and Ca^{2+} in cardiac myocytes, which increases the contractile force of the heart (a positive inotropic effect).[13] While their clinical utility is limited by a small therapeutic window resulting from high toxicity,[14] these drugs have attracted renewed attention as potential therapies for such indications as ischemic stroke, neurodegenerative diseases, and cancer.[15]

To improve the activities of these compounds in disparate disease areas and/or to mitigate their toxic effects, cardiac glycoside analogs have been prepared. Strategies have included (i) modification of the steroid core;[16] (ii) replacement of the natural digitose trisaccharide with monosaccharide moieties;[17] (iii) 'neo-glycorandomization', wherein unprotected sugars were directly conjugated to a digitoxigenin-derived methoxylamine to form *N*-glycosides;[18] and (iv) organocatalytic, selective acylation of the natural products.[3] The reported total syntheses of digitoxin[19] also represent prospective routes to novel analogs. The group of O'Doherty has been active in this regard, employing palladium-catalyzed *de novo* oligosaccharide synthesis to generate derivatives bearing aberrant glycan structures.[20] Structure–activity relationships for

Scheme 1. Preparation of Purpurea Glycoside A (**5**) from Digitoxin (**1**) by Catalyst-Controlled Glycosylation



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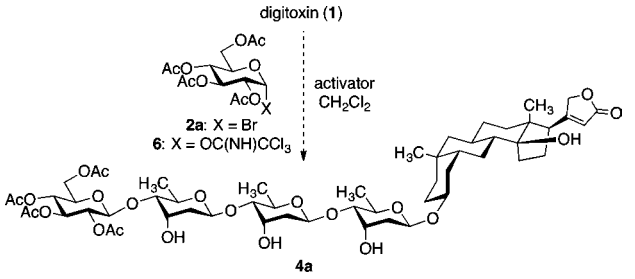
analogs prepared by neoglycorandomization and *de novo* synthesis highlight the significant effects of the configuration and length of the carbohydrate chain on antitumor activity.

Our efforts at catalyst-controlled glycosylation of digitoxin began with coupling of **1** and 2,3,4,6-tetraacetyl-α-D-glucopyranosyl bromide (**2a**: Scheme 1). Little modification of the catalytic conditions developed in our initial study was required: using the borinic ester precatalyst (**3**) and halide abstracting reagent (Ag_2O) identified previously,[10c] a 77% yield of monoglycosylated product **4a** was obtained after variation of the solvent and glycosyl donor stoichiometry (see the Supporting Information). Detailed NMR spectroscopic studies (in particular, $^1H-^{13}C$ HMBC and $^1H-^1H$ COSY correlations) indicated that glycosylation had occurred at the 4″-OH group. This regiochemical outcome was consistent with the pattern of selective activation of the equatorial OH group of *cis*-1,2-diol motifs that has emerged from our previous work on borinic acid activation of pyranoside substrates.[10] The newly formed glycosidic bond was also found to be of a β-configuration, as evidenced by the coupling observed between H1‴ and H2‴ (^3J = 8.0 Hz).

Deacetylation of **4a** was accomplished in 73% yield using lithium hydroxide in methanol/water (Scheme 1).[19c] The 4″-*O*-glucosylated product **5** corresponds to the naturally occurring purpurea glycoside A (desacetyl lanatoside A).[21] To date, the only reported conversions of digitoxin into purpurea glycoside A have made use of whole cell extracts, usually from *Digitalis* species.[22] Given the modest levels of amplification of purpurea glycoside A that have been reported in such studies, the preparative utility of the two-step sequence described here may compare favorably with those of the biotransformation-based protocols.

According to reactivity patterns of secondary OH groups in pyranosides, the 4″-OH group that underwent organoboron-catalyzed glycosylation corresponds to the most sterically accessible position of digitoxin.[23] This assertion is supported by the observations of Kawabata and co-workers, who found that digitoxin was selectively acylated at this position under standard conditions (isobutyric anhydride, DMAP, collidine), with 3″-,4″-bisacylation representing the major byproduct.[3a] We thus carried out control experiments (Table 1) to establish whether substrate-controlled glycosylation of **1** might enable selective access to **4a** without the need for a catalyst.

Table 1. Attempted Uncatalyzed Glycosylations of **1**



entry	conditions	yield of **4a**	comments
1	1 equiv of **2a**, Ag_2O, CH_2Cl_2, 23 °C, 20 h	trace	digitoxin recovered
2	2 equiv of **2a**, Ag_2O, CH_2Cl_2, 50 °C, 20 h	none	mixture of orthoesters isolated (41%)
3	1 equiv of **2a**, AgOTf, collidine, CH_2Cl_2, −78 → 23 °C, 20 h	trace	digitoxin recovered
4	1 equiv of **6**, 0.1 equiv of TMSOTf, CH_2Cl_2, 0 °C, 15 min	none	digitoxigenin (aglycon) isolated
5	1 equiv of **6**, 0.1 equiv of TMSOTf, CH_2Cl_2, −78 → 23 °C, 2 h	none	digitoxigenin (aglycon) isolated

Consistent with our earlier kinetic studies,[10c] uncatalyzed glycosylation under the conditions of Scheme 1 was slow and resulted in recovery of unreacted digitoxin (entry 1). Heating the reaction mixture to 50 °C without the catalyst generated a mixture of orthoester regioisomers rather than the glycosylation product **4a** (entry 2). Typical homogeneous activation conditions for the glycosyl bromide, using AgOTf as the halide abstracting reagent,[24] led to very low levels of conversion (entry 3). Under classical Lewis acid activated conditions using trichloroacetimidate donor **6**,[25] the labile β-2,6-dideoxyglycosidic linkages were cleaved, even at low temperature (entries 4 and 5). Because an exhaustive screen of donors and activators was not performed, we cannot rule out the possibility that the regioselective glycosylation of **1** could be effected without a catalyst under some sets of conditions. However, the data suggest that it is not trivial to identify glycosylation conditions that are sufficiently mild to tolerate the labile digitose moiety of **1** while also leading to high levels of regiocontrol.[26,27]

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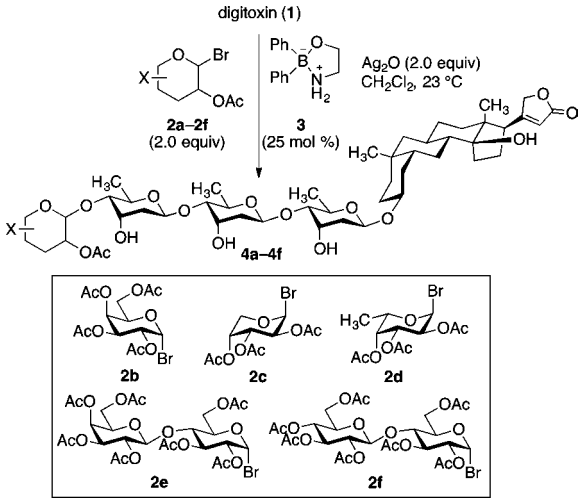
Table 2. Scope of Catalyst-Controlled Glycosylation of Digitoxin



entry	glycosyl donor	yield/%[a]
1	**2a**	77
2	**2b**	63
3	**2c**	63
4	**2d**	51
5	**2e**	64
6	**2f**	74

[a] Isolated yield after purification by chromatography on silica gel.

Having demonstrated the conversion of digitoxin to purpurea glycoside A using borinic acid catalysis, we investigated the preparation of novel analogs by variation of the glycosyl donor (Table 2). Peracetylated glycosyl bromides derived from D-galactose, D-arabinose, L-fucose, lactose, and cellobiose were employed, leading to products **4b**–**4f** having 1,2-*trans*-glycosidic linkages. The levels of regiocontrol for the 4″-*O*-glycosylated isomer were excellent, with the major byproduct of the reactions being unreacted digitoxin rather than glycosylated regioisomers. The exception in this regard was the coupling of galactosyl bromide (entry 2), in which a mixture of the 3″- and 4″-*O*-glycosylated products was observed. The major product was the 4″-*O*-Gal isomer, which could be isolated in 63% yield. The anomalous behavior of the galactosyl donor may reflect a matching/mismatching effect related to this specific donor/acceptor combination. While it is conceivable that the lower level of regiocontrol stems from the increased reactivity of the galactosyl relative to the glucosyl donor,[28] we note that 3″-*O*-glycosylated regioisomers were not evident in reactions of the still less electron-deficient (and thus more reactive) arabinosyl and fucosyl donors.

In conclusion, digitoxin has been regioselectively glycosylated at one of its five OH groups using organoboron catalysis. This work establishes that borinic acid catalysis is useful for the selective glycosylation of natural products and provides the first nonbiocatalytic mode of access to the purpurea glycosylation pattern from digitoxin. Biological assessment of novel analogs prepared by this method is underway. Extension of this strategy to other complex polyols would be of interest. In the case of digitoxin, it is likely that the intrinsic substrate bias was enhanced; cases where substrate bias is overcome using catalysis will be potentially more challenging.

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Supporting Information Available. Experimental procedures and characterization data for all new compounds, including NMR spectra used for structural assignments. The material is available free of charge via the Internet at http://pubs.acs.org.

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The authors declare no competing financial interest.