ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/51207546

Synthesis and Evaluation of the α-D-/α-L-Rhamnosyl and Amicetosyl Digitoxigenin Oligomers as Anti-tumor Agents.

ARTICLE *in* ACS MEDICINAL CHEMISTRY LETTERS · APRIL 2011

Impact Factor: 3.12 · DOI: 10.1021/ml100290d · Source: PubMed

CITATIONS
34

READS
9

3 AUTHORS, INCLUDING:

Hua-Yu Leo Wang
University of Texas Southwestern Medical C…
16 PUBLICATIONS **208** CITATIONS
SEE PROFILE

George A O'Doherty
Northeastern University
208 PUBLICATIONS **3,314** CITATIONS
SEE PROFILE

Available from: Hua-Yu Leo Wang
Retrieved on: 24 January 2016

ACS Medicinal Chemistry Letters

LETTER

pubs.acs.org/acsmedchemlett

Synthesis and Evaluation of the α-D-/α-L-Rhamnosyl and Amicetosyl Digitoxigenin Oligomers as Antitumor Agents

Hua-Yu Leo Wang,[†] Yon Rojanasakul,[*,‡] and George A. O'Doherty[*,†]

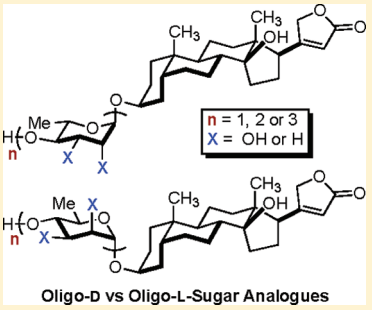
[†]Department of Chemistry and Chemical Biology, Northeastern University, Boston, Massachusetts 02115, United States

[‡]Department of Basic Pharmaceutical Sciences, West Virginia University, Morgantown, West Virginia, 26506, United States

Supporting Information

ABSTRACT: A highly regio- and stereoselective asymmetric synthesis of rhamnosyl- and amicetosyl-digitoxigenin analogues has been established via palladium-catalyzed glycosylation followed by bis-/tris-dihydroxylation or bis-/tris-diimide reduction. The α-L-rhamnose and α-L-amicetose digitoxin monosaccharide analogues displayed stronger apoptosis inducing activity and cytotoxicity against nonsmall cell human lung cancer cells (NCI-H460) than its D-diastereomeric isomers in a sugar-chain length dependent manner.

KEYWORDS: Digitoxin, digitoxigenin trirhamnoside, digitoxigenin triamicetoside, apoptosis, cancer, NCI-H460



Digitoxin **3** (Figure 1) is a well-known cardiac glycoside found in *Digitalis purpurea* that has been used to treat heart congestive failure by the inhibition of plasma membrane Na^+/K^+ ATPase.[1] In this event, the accumulated intracellular Na^+ concentration causes Ca^{2+} influx to enhance myocardial cell contractility. These findings triggered a significant amount of research on the evaluation of the structure activity relationship (SAR) of the carbohydrate moiety in Na^+/K^+ ATPase binding affinity.[2−5] Karlish demonstrated that inhibition of α2- over α1-Na^+/K^+ ATPase isoform could induce cardiac contraction with minimal Ca^{2+} overload and, hence, less cardiotoxicity.[5] Digitoxin has also been recognized for its excellent antitumor activity against a wide range of human cancer cell lines.[6] In fact, a digitalis clinical study on breast tumor patients showed both reduced tumor size and reduced rate of reoccurrence.[7,8]

Digitoxin consists of digitoxigenin (pharmacophore) and the trisaccharide moiety, which is known to be a critical component for both its cardiotoxic and anticancer activity.[9] Although the detailed mechanism of anticancer activity is not fully understood, many apoptosis signals have been found to be affected by digitoxin at subcardiotoxic concentration in plasma.[10] Studies also suggested that an α2-selective cardiac glycoside could result from sugar modification, because the structural differences in these isoforms primarily lie in the extracellular carbohydrate binding loops.[5,11] Thus, sugar modification opens the potential for discovery of new and safer digitoxin analogues with improved anticancer activity. In addition to the study of Na^+/K^+ ATPase in myocardial cells, Thorson and co-workers demonstrated the potential for improving cytotoxicity against a wide range of human cancer cell lines by screening a library of digitoxin *MeNO*-neoglycoside analogues.[12] Although few SAR conclusions could be made, the axial C2′-alcohol in the D-sugar was suggested to be critical for anticancer activity.

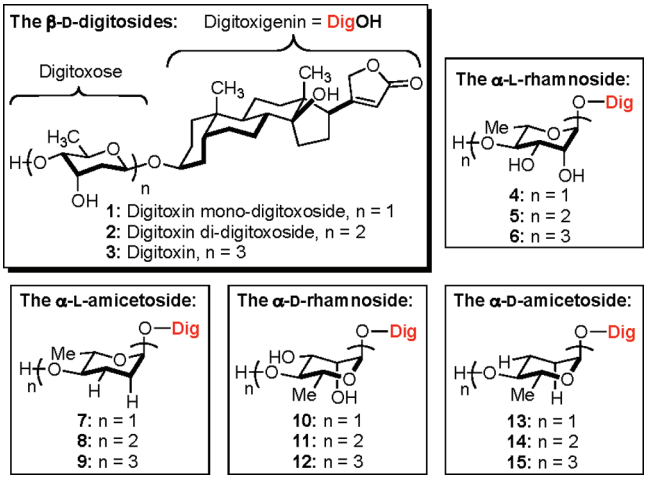


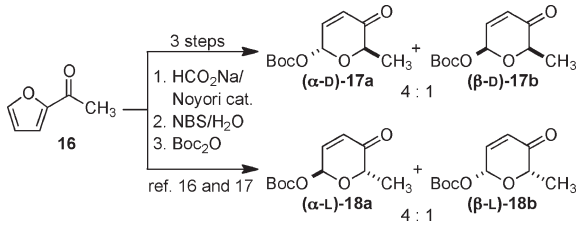
Figure 1. Digitoxin **3** and Related Carbohydrate Analogues.

We later showed that digitoxin *O*-glycosides consistently displayed stronger potency in apoptosis activation than *MeNO*-neoglycosides regardless of sugar-chain length (i.e., mono-**1** is better than di-**2** and trisaccharide **3**; Figure 1).[13] We further investigated the *O*-linked monosaccharides for the optimal stereochemistry and functionality required for initiating apoptosis.[14] Surprisingly, this survey found two L-sugar digitoxin monosaccharides (**4** and **7**) that displayed equal or better growth inhibition (against the NCI panel of 60-cancer cell lines) than the previous

Received: December 6, 2010
Accepted: January 10, 2011
Published: January 24, 2011

ACS Publications © 2011 American Chemical Society

Scheme 1. *De Novo* Approach to the Key Pyranone Building Blocks 17a/b and 18a/b



best, digitoxin monosaccharide **1**. For example, both α-L-rhamnoside (**4**) (IC_{50} 46.7 nM) and α-L-amicetoside (**7**) (IC_{50} 55.7 nM) demonstrated a similar potency to β-D-digitoxoside (**1**) (IC_{50} 74.8 nM) in apoptosis induction against nonsmall cell human lung cancer cell (NCI-H460), which are all ~10 fold more potent than digitoxin (**3**) (IC_{50} 357 nM).[14,15] The effects of C5′ alkyl substituents on cytotoxicity for both the α-L-rhamno- and α-L-amiceto- monosaccharides were also studied (see DOI 10.1021/ml100291n).

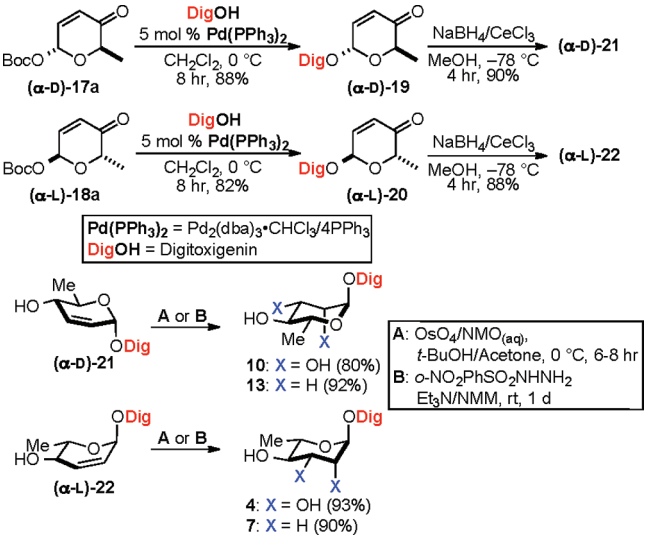
The fact that the structurally different monosaccharide analogues **1**, **4**, and **7** (e.g., sharing only one stereocenter and having different numbers of hydroxyl groups) possess similar activity suggests that the carbohydrate binding site of the target must accommodate several distinct orientations upon binding to its target.[15] To further probe this hypothesis, we decided to test whether di- and trisaccharide analogues of **4** and **7** would have a similarly weakened anticancer activity. Thus, we decided to synthesize and test di- and trisaccharide α-L-rhamnoside (**5** and **6**) and α-L-amicetoside (**8** and **9**) analogues. In addition, all D-rhamnoside (**10**−**12**) and all D-amicetoside (**13**−**15**) analogues could be made and tested as a control group, to account for nonspecific effects such as solubility and membrane transport properties.

To rapidly assess this structurally diverse set of analogues, we employed our *de novo* asymmetric synthesis of carbohydrates to systematically prepare both stereo- and oligo-isomers of rhamnosyl and amicetosyl digitoxin derivatives. Herein, we report the synthesis and biological activity of digitoxin α-D-/α-L-rhamnose and amicetose mono-, di-, and trisaccharide analogues (**4**−**15**, Figure 1) against human lung cancer NCI-H460 cell line.

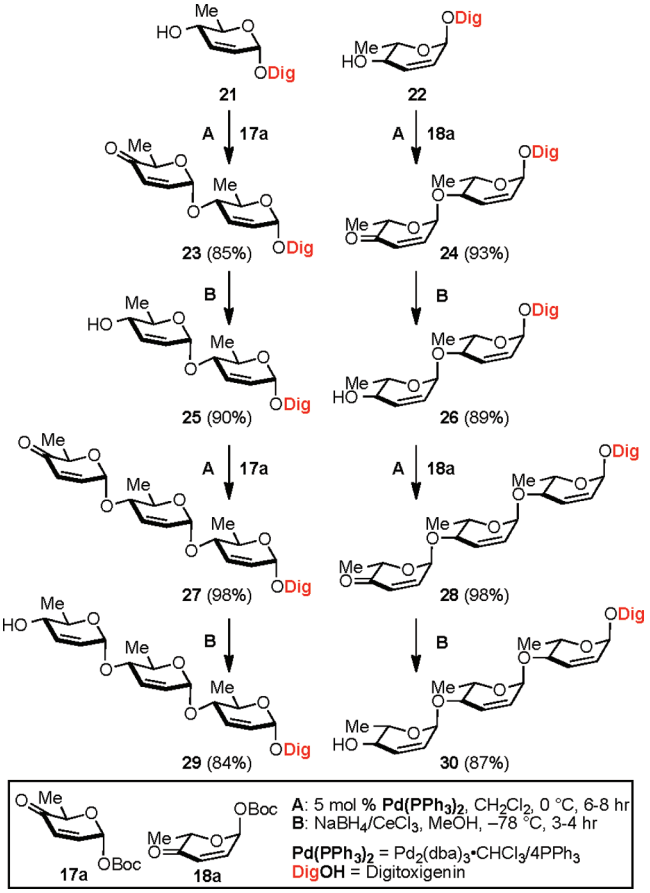
Retrosynthetically, we imagined that the desired target molecules could be obtained by coupling with the digitoxigenin (**DigOH**) and the α-D/L-Boc pyranones (D-**17a** and L-**18a**, Scheme 1). Previously, we have described a *de novo* approach where all the α/β-D/L-Boc pyranones could be prepared in three steps from acetylfuran **16**.[16,17] Briefly, the absolute D- and L-sugar stereochemistry was installed by Noyori asymmetric reduction.[18] The pyranyl ring was prepared by Achmatowicz oxidative rearrangement (NBS/H_2O), with subsequent Boc-protection under various conditions to give **17a**/**b** and **18a**/**b** in good overall yield (60−70%).[19]

With the desired α-D/α-L-sugar building blocks **17a** and **18a** in hand, we next employed our palladium-catalyzed glycosylation to couple them with digitoxigenin to afford the corresponding digitoxin pyranones **19** and **20**, with complete stereocontrol at the anomeric center (Scheme 2). The desired C4′-hydroxyl group was stereoselectively installed via Luche reduction to give allylic alcohols **21** and **22**, respectively. The resulting C2′−C3′ olefin was readily oxidized or reduced in order to provide rhamnosyl **10** and **4** or amicetosyl **13** and **7** digitoxin analogues under Upjohn dihydroxylation or diimide reduction conditions.[20,21]

Scheme 2. Stereodivergent Approach to D- and L-Digitoxin Monosaccharides



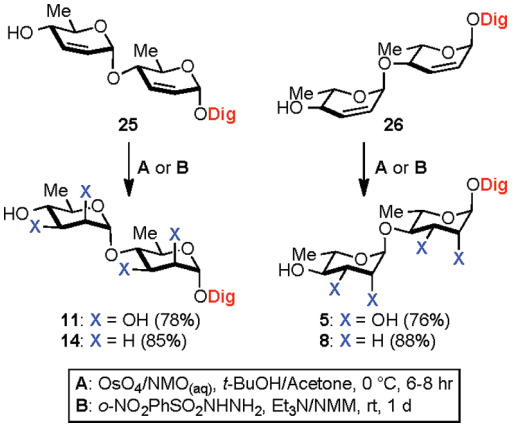
Scheme 3. Stereodivergent Approach to D- and L-Digitoxin Bis- and Tris-pyran Oligomers



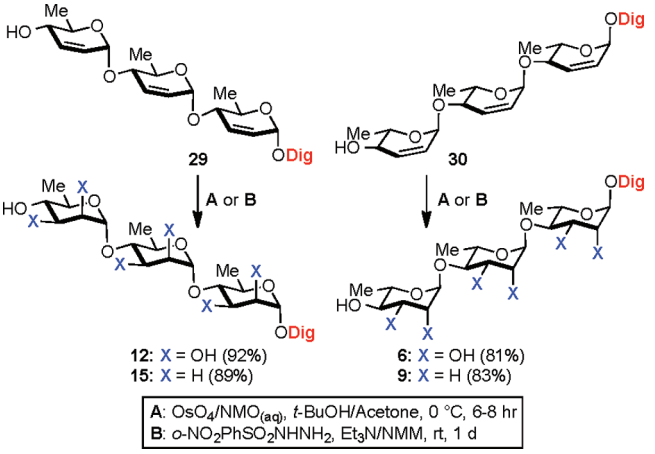
To rapidly construct the α-linked 1,4-oligosaccharide digitoxin (Scheme 3), we utilized the versatile digitoxin C4′-allylic

dx.doi.org/10.1021/ml100290d |ACS Med. Chem. Lett. 2011, 2, 264–269

Scheme 4. Post-glycosylation Installation of Bis-*rhamno*-/*amiceto*-functionality



Scheme 5. Post-glycosylation Installation of Tris-*rhamno*-/*amiceto*-functionality



alcohol intermediates **21** and **22** as the desired glycosyl receptors to couple with α-D/L-Boc-pyranones **17a** and **18a** via palladium mediated glycosylation.[22,23] The resulting digitoxin bis-enones **23** and **24** were reduced by $NaBH_4$ to give exclusively C4′-equatorial alcohols **25** and **26**. By simply repeating these two steps of glycosylation and reduction, the corresponding digitoxin tris-pyranyl alcohols **29** and **30** were prepared in both excellent yield and diastereoselectivity.

The remaining stereocenters of C2′/C3′-hydroxyl groups were rapidly installed via bis-dihydroxylation to give the desired *rhamno*-stereochemistry in **11** and **5** (Scheme 4). Alternatively, α-D-/L-amicetose digitoxin disaccharides were prepared by bis-diimide reduction to give **14** and **8** in relatively high yield. Finally, the syntheses of the rhamnosyl **12** and **6** and amicetosyl **15** and **9** derivatives of digitoxin trisaccharide were completed by repeating these two versatile dihydroxylation and diimide reduction transformations (Scheme 5). The key to this success relied on the highly stereoselective bis-/tris-dihydroxylation and diimide reduction to install four/six stereocenters in one transformation without any reliance on protecting groups. And these complex digitoxin trirhamnoside/amicetoside analogues were concisely prepared in a total of seven linear steps, with 41%−46% overall yield from the sugar building blocks.

We next evaluated the anticancer activity of the diastereo-/oligo-isomers of the rhamnosyl and amicetosyl digitoxigenin

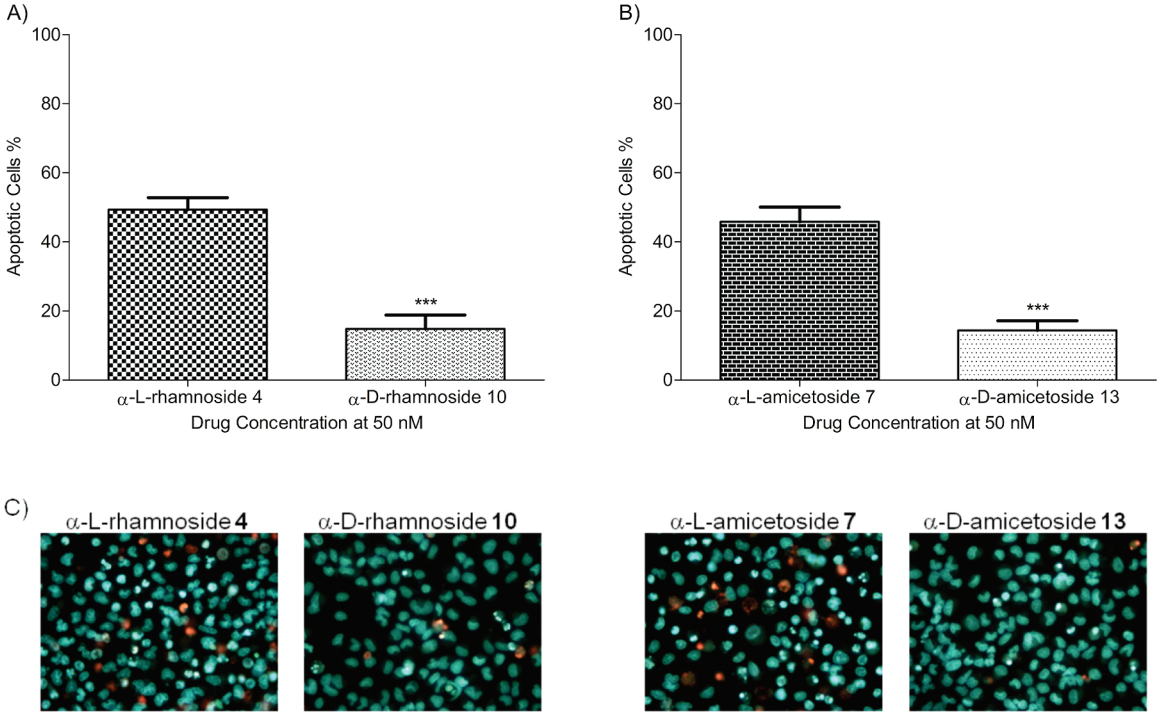


Figure 2. Apoptotic cell death as effect of stereochemistry. (A and B) Apoptotic cell death (%) was compared for each α-L-/D-pair of digitoxin rhamnoside and amicetoside at 50 nM concentration (Student *t* test; ***, $P < 0.001$). (C) Hoechst stained apoptotic cell appears blue and propidium iodide stained necrotic cell appears red at 50 nM drug concentration.

dx.doi.org/10.1021/ml100290d |*ACS Med. Chem. Lett.* 2011, 2, 264–269

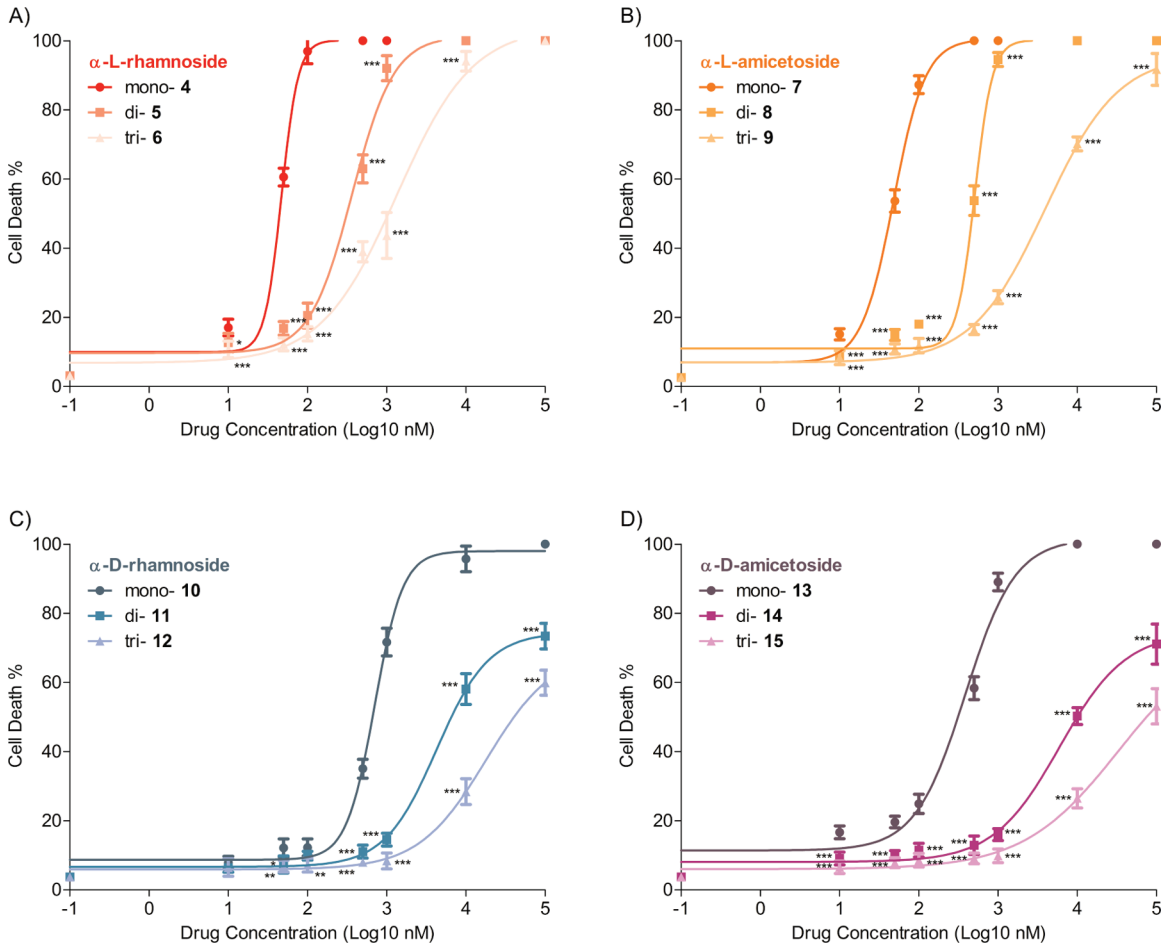


Figure 3. Cytotoxicity as a function of drug concentration in the comparison of sugar stereochemistry and chain length. The dose response curve of total cell death (apoptosis/necrosis) mediated by digitoxin analogues in 12 h treatment at increasing concentrations (10 nM to 100 μM). All data were analyzed by two-way ANOVA ($N = 6$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).

Table 1. Cytotoxicity of Digitoxin Analogues on NCI-H460 Epithelial Human Lung Cancer Cells

compd	IC_{50} (nM) ± S.E.[a]	compd	IC_{50} (nM) ± S.E.[a]
α-L-rhamnoside		α-D-rhamnoside	
mono-**4**	47 ± 1	mono-**10**	706 ± 1
di-**5**	365 ± 1	di-**11**	4271 ± 1
tri-**6**	1347 ± 1	tri-**12**	18032 ± 1
α-L-amicetoside		α-D-amicetoside	
mono-**7**	48 ± 1	mono-**13**	387 ± 1
di-**8**	510 ± 1	di-**14**	5992 ± 1
tri-**9**	3963 ± 1	tri-**15**	33098 ± 2

[a] All values represent the standard error of the mean value of three independent experiments with duplicate determinations.

analogues with both α-L-rhamnose (**4**) and α-L-amicetose (**7**) as a control against NCI-H460 human lung cancer cells. To identify the occurrence of apoptosis, we used Hoechst 33342 nuclear stain and propidium iodide to differentiate cells that undergo apoptosis or necrosis.[24] Comparing the effects of D- vs L-stereochemistry on apoptosis induction, both D-rhamnose (**10**) (13.6%) and D-amicetose (**13**) (15.3%) exhibited a significantly reduced apoptosis activity as compared to L-rhamnose (**4**) (48.6%) and L-amicetose (**7**) (45.7%, Figure 2A and B). Thus, the change in the D-/L-stereochemistry of the sugar has a greater effect on the degree of apoptosis activation than substitution at the C-2/C-3 position of the sugar (i.e., L-rhamnose (**4**) is significantly more active than D-rhamnose (**10**) (~20 fold), whereas L-rhamnose (**4**) and L-amicetose (**7**) are equally active ($P > 0.05$)). Regardless of sugar substitution and stereochemistry, the major mode of cell death is apoptosis (>85%). As shown in Figure 2C, NCI-H460 cells underwent apoptosis, with condensed and fragmented nuclei seen in blue Hoechst nuclear stain, whereas cells appeared completely ruptured in red propidium iodide stain, indicative of necrosis. It is worth noting that the ratio of apoptosis and necrosis become difficult to estimate at high dose concentration (>500 nM), due to the high cell mortality rate.

To further study the anticancer activity as the effect of sugar chain length, the cytotoxicity assay was conducted in a 12 h exposure of drugs at increasing concentrations (10 nM to 100 μM). Our result clearly demonstrated that both L-rhamnose (**4**) and L-amicetose (**7**) induced cell death (apoptosis and necrosis) in both concentration dependent and sugar-chain length dependent manners (Figure 3A and B). Significantly, both L-rhamnose (**4**) (IC_{50} 47 nM) and L-amicetose (**7**) (IC_{50} 48 nM) showed at least ~10-fold stronger potency than the corresponding di- (**5** and **8**) and trisaccharide analogues (**6** and **9**, Table 1). We found

dx.doi.org/10.1021/ml100290d |ACS Med. Chem. Lett. 2011, 2, 264–269

for these rhamnosyl and amicetosyl glycosides that the α-L-sugar stereochemistry is essential for the potency (cf., D-analogues **10** and **13**, Table 1). It is worth noting that α-L-amicetose (**7**) has previously shown greater growth inhibitory effect against an NCI-panel of 60 human cancer cell lines than α-D-amicetose (**13**) (see Supporting Information). Consistent with our previous hypothesis, all the D-rhamnose (**10**−**12**) and D-amicetose (**13**−**15**) showed a dramatically reduced cytotoxic activity with increasing sugar chain length (Table 1).

In summary, we have prepared and evaluated the anticancer activity of the diastereo- and oligo-isomers of rhamnosyl and amicetosyl digitoxigenin. The syntheses of digitoxin di-/trirhamnoside and di-/triamicetoside analogues were successfully achieved in a linear and highly stereoselective fashion from a commercially available acetylfuran. Based on the SAR study in the comparison of our previously found potent digitoxin α-L-rhamnose (**4**) and α-L-amicetose (**7**), we identified a significant change of cytotoxicity by altering the diastereomeric relationship; L-sugar stereochemistry is suggested to exhibit a distinct binding orientation to its target. In addition, we demonstrated that the important structural motif for inducing anticancer activity can be greatly optimized by shortening the carbohydrate chain of digitoxin analogues. Most importantly, this work illustrates the use of palladium-catalyzed glycosylation and *de novo* asymmetric synthesis to install an array of rare sugars, which are not readily accessible via enzymatic synthesis, in digitoxin for the development of a potential cardiac glycoside anticancer drug.

■ ASSOCIATED CONTENT

Supporting Information. Assay protocols, statistical analysis data, synthetic procedures, characterization data, and NMR spectra. This information is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: yrojan@hsc.wvu.edu; g.odoherty@neu.edu.

Author Contributions

All the experimental work was performed by H.-Y. L.W. The experimental design, data analysis and manuscript preparation was performed by all the authors.

■ ACKNOWLEDGMENT

We thank Anand Krishnan Iyer (Hampton University), Todd A. Stueckle, and Yongju Lu (West Virginia University) for their advice on cell culturing and cytotoxicity assays. We also thank Jonathan Boyd (West Virginia University) for his help with data analysis. We are grateful to the NIH (GM090259 and GM088839) and NSF (CHE-0749451) for the support of our research.

■ REFERENCES

(1) Greeff, K. *Cardiac Glycosides, Part 1: Experimental Pharmacology. Handbook of Experimental Pharmacology*; Springer-Verlag: Berlin, New York, 1981; Vol. 56.

(2) Yoda, A. Structure−Activity Relationships of Cardiotonic Steroids for the Inhibition of Sodium- and Potassium-Dependent Adenosine Triphosphatase. *Mol. Pharmacol.* **1973**, *9*, 51–60.

(3) Brown, L.; Erdmann, E.; Thomas, R. Digitalis structure−activity relationship analyses: Conclusions from indirect binding studies with cardiac ($Na^+ + K^+$)-ATPase. *Biochem. Pharmacol.* **1983**, *32*, 2767–2774.

(4) Rathore, H.; From, A. H. L.; Ahmed, K.; Fullerton, D. S. Cardiac Glycosides. 7. Sugar Stereochemistry and Cardiac Glycoside Activity. *J. Med. Chem.* **1986**, *29*, 1945–1952.

(5) Katz, A.; Lifshitz, Y.; Bab-Dinitz, E.; Kapri-Pardes, E.; Goldshleger, R.; Tal, D. M.; Karlish, S. J. D. Selectivity of Digitalis Glycosides for Isoforms of Human Na,K-ATPase. *J. Biol. Chem.* **2010**, *285*, 19582–19592.

(6) Newman, R. A.; Yang, P.; Pawlus, A. D.; Block, K. I. Cardiac Glycosides as Novel Cancer Therapeutic Agents. *Mol. Interventions* **2008**, *8*, 36–49.

(7) Stenkvist, B.; Bengtsson, E.; Eriksson, O.; Holmquist, J.; Nordin, B.; Westman-Naeser, S. Cardiac glycosides and breast cancer. *Lancet* **1979**, *1*, 563.

(8) Stenkvist, B.; Bengtsson, E.; Dahlqvist, B.; Eriksson, O.; Jarkrans, T.; Nordin, B. N. Cardiac glycosides and breast cancer, revisited. *N. Engl. J. Med.* **1982**, *306*, 484.

(9) Lopez-Lazaro, M.; Pastor, N.; Azrak, S. S.; Ayuso, M. J.; Austin, C. A.; Cortes, F. Digitoxin Inhibits the Growth of Cancer Cell Lines at Concentrations Commonly Found in Cardiac Patients. *J. Nat. Prod.* **2005**, *68*, 1642–1645.

(10) Haux, J.; Solheim, O.; Isaksen, T.; Angelsen, A. Digitoxin, in non-toxic concentrations, inhibits proliferation and induces cell death in prostate cancer cell lines. *Z. Onkol.* **2000**, *32*, 11–16.

(11) Wansapura, A. N.; Lasko, V.; Xie, Z.; Fedorova, O. V.; Bagrov, A. Y.; Lingrel, J. B.; Lorenz, J. N. Marinobufagenin enhances cardiac contractility in mice with ouabain-sensitive α1 Na^+-K^+-ATPase. *Am. J. Physiol. Heart Circ. Physiol.* **2009**, *296*, 1833–1839.

(12) Langenhan, L. M.; Peters, N. R.; Guzei, I. A.; Hoffmann, M.; Thorson, J. S. Enhancing the anticancer properties of cardiac glycosides by neoglycorandomization. *Proc. Natl. Acad. Sci. U.S.A.* **2005**, *102*, 12305–12310.

(13) Iyer, A. K. V.; Zhou, M.; Azad, N.; Elbaz, H.; Wang, L.; Rogalsky, D. K.; Rojanasakul, Y.; O'Doherty, G. A.; Langenhan, J. M. Direct Comparison of the Anticancer Activities of Digitoxin MeON-Neoglycosides and O-Glycosides: Oligosaccharide Chain Length-Dependent Induction of Caspase-9-Mediated Apoptosis. *ACS Med. Chem. Lett.* **2010**, *1*, 326–330.

(14) Wang, H.-Y. L.; Xin, W.; Zhou, M.; Stueckle, T. A.; Rojanasakul, Y.; O'Doherty, G. A. Stereochemical Survey of Digitoxin Monosaccharides. *ACS Med. Chem. Lett.* **2011**, *2* (1), pp 73–78.

(15) It is worth noting that other D- and L-sugar monosaccharides were significantly less active than monosaccharides **1**, **4**, and **7**; see ref 13.

(16) For the synthesis of α-/β-D-Boc pyranones, see: Zhou, M.; O'Doherty, G. A. De Novo Approach to 2-Deoxy-β-glycosides: Asymmetric Syntheses of Digoxose and Digitoxin. *J. Org. Chem.* **2007**, *72*, 2485–2493.

(17) For the synthesis of α-/β-L-Boc pyranones, see: Guo, H.; O'Doherty, G. A. De Novo Asymmetric Synthesis of Anthrax Tetrasaccharide and Related Tetrasaccharide. *J. Org. Chem.* **2008**, *73*, 5211–5220.

(18) Fujii, A.; Hashiguchi, S.; Uematsu, N.; Ikariya, T.; Noyori, R. Ruthenium (II)-Catalyzed Asymmetric Transfer Hydrogenation of Ketones Using a Formic Acid-Triethylamine Mixture. *J. Am. Chem. Soc.* **1996**, *118*, 2521–2522.

(19) Achmatowicz, O.; Bukowski, P.; Szechner, B.; Zwierzchowska, Z.; Zamojski, A. Synthesis of methyl 2,3-dideoxy-DL-alk-2-enopyranosides from furan compounds: A general approach to the total synthesis of monosaccharides. *Tetrahedron* **1971**, *27*, 1973–1996.

(20) VanRheenen, V.; Kelly, R. C.; Cha, D. Y. An improved catalytic OsO_4 oxidation of olefins to cis-1,2-glycols using tertiary amine oxides as the oxidant. *Tetrahedron Lett.* **1976**, *17*, 1973–1976.

(21) Haukaas, M. H.; O'Doherty, G. A. Enantioselective Synthesis of 2-Deoxy- and 2,3-Dideoxyhexoses. *Org. Lett.* **2002**, *4*, 1771–1774.

(22) Babu, R. S.; Zhou, M.; O'Doherty, G. A. De Novo Synthesis of Oligosaccharides Using a Palladium-Catalyzed Glycosylation Reaction. *J. Am. Chem. Soc.* **2004**, *126*, 3428–3429.

(23) Zhou, M.; O'Doherty, G. A. The *De Novo* Synthesis of Oligosaccharides: Application to the Medicinal Chemistry SAR-Study of Digitoxin. *Curr. Top. Med. Chem.* **2008**, *8*, 114–125.
(24) Chanvorachote, P.; Nimmannit, U.; Stehlik, C.; Wang, L.; Jiang, B.-H.; Ongpipatanakul, B.; Rojanasakul, Y. Nitric Oxide Regulates Cell Sensitivity to Cisplatin-Induced Apoptosis through *S*-Nitrosylation and Inhibition of Bcl-2 Ubiquitination. *Cancer Res.* **2006**, *66*, 6353–6360.