Syntheses and Biological Activities of 3′-Azido Disaccharide Analogues of Daunorubicin against Drug-Resistant Leukemia

Guisheng Zhang,[†,#] Lanyan Fang,[‡] Lizhi Zhu,[†] Yanqiang Zhong,[§] Peng George Wang,*[,†] and Duxin Sun*[,‡]

Department of Chemistry and Biochemistry and Division of Pharmaceutics, College of Pharmacy, The Ohio State University, Columbus, Ohio 43210, and School of Chemical and Environmental Sciences, Henan Normal University, Xinxiang, Henan 453007, P. R. China

Received September 15, 2005

Anthracyclines, such as daunorubicin (DNR) and doxorubicin (Dox), are widely used for cancer therapy but are limited by drug resistance and cardiotoxicity. To overcome drug resistance, we synthesized a novel class of disaccharide analogues of DNR against drug-resistant leukemia. In these disaccharide analogues (**1**−**6**) the first (inner) sugar in the carbohydrate chain is a 3-azido-2,3,6-trideoxy-L-*lyxo*-α-hexopyranose; the second (outer) sugars that are linked via α(1→4) to the first sugar are a series of uncommon sugars. Their cytotoxicities were examined in drug-sensitive leukemia cells K562 and doxorubicin-resistant K562/Dox cells by MTS assay. In drug-sensitive cells, compounds **1**−**6** were found to be active against leukemia K562 cells with IC_{50} in the nanomolar range (200−1100 nM), while compounds **2**−**5** with 2,6-dideoxy sugars showed better activity than compounds **1** and **6** with 2,3,6-trideoxy sugars. In doxorubicin-resistant K562/Dox cells, compounds **1**, **3**, and **5** exhibited much better activities (with IC_{50} between 0.29 and 2.0 μM) than DNR (with $IC_{50} > 5$ μM). Compound **3** emerged as the most active compound, showing at least 17-fold higher activity against drug-resistant cells than parent compound DNR. The IC_{50} values of compound **3** in both drug-sensitive and drug-resistant cells are identical, which indicates that compound **3** completely overcomes drug resistance. Structure−activity relationship (SAR) studies showed that the substitution and orientation of the 3-OH group in the second sugar significantly influence its activity against drug-resistant leukemia. These results suggest that sugar modifications of anthracyclines change their activity and overcome drug resistance.

Introduction

The anthracyclines, such as DNR and doxorubicin (Dox) (Figure 1), are among the most potent and clinically used anticancer agents in cancer chemotherapy.[1] Although anthracyclines are widely used in cancer therapy, two major problems limit their clinical application: cardiotoxicity and drug resistance.[2,3]

One of the mechanisms for drug resistance of anthracyclines in cancer cells is mediated by an ABC transporter protein (P-glycoprotein, P-gp, MDR1, ABCB1).[2−5] P-gp is overexpressed in many drug-resistant cancer cells.[2,3,6−11] P-gp actively exports a wide range of drugs from cancer cells, which include anticancer drugs anthracyclines, vinca alkaloids, epipodophyllotoxins, and taxanes; HIV-1 protease inhibitors; immunosuppressants; antibacterial reagents; antifungals; and cardiac glycosides.[12−15] Therefore, P-gp decreases intracellular drug concentration in cancer cells and thus induces drug resistance.

In the search for better therapeutic agents, a wealth of data have been published on the SAR of the aglycone portion and carbohydrate moiety of anthracyclines over the past 30 years. It has been known that the carbohydrate moiety is a critical component for anticancer activities. The orientation of the sugar is critical for DNA binding. α-Glycoside is a required common motif for anthracyclines' anticancer activities, while the β-anomer is even less active than the aglycone itself.[16,17] Chemical and configurational modifications on the sugar moiety of anthracyclines markedly changed their anticancer activity, toxicity, the sequence specificity of DNA break sites, and other cellular process/pathway besides topo II.[18] Indeed, modifications on the sugar structures have led to the second generation of doxorubicin analogues with promising clinical anticancer efficacy, such as the monosaccharides epirubicin and idarubicin.[19−21] However, these new analogues still showed MDR-mediated drug resistance.

Recently, considerable interest in disaccharide anthracyclines has emerged. In the third-generation anthracycline analogues, a novel disaccharide analogue MEN10755[22−24] (MEN, Figure 1) has been found to show a different activity spectrum against doxorubicin-resistant tumors compared to doxorubicin. MEN has the first sugar without the 3′-amino group, substituted for a hydroxyl group, and the second sugar, daunosamine, linked via α(1−4) to the first sugar. In comparison, MAR70 (MAR, Figure 1), a DNR disaccharide analogue, did not show significant antitumor activity compared to the parent drugs.[1] MAR, similar to DNR in the aglycone, does not bear the 14-hydroxyl group. The first sugar in the carbohydrate chain is daunosamine, like in the parent compounds DNR and doxorubicin; the second sugar linked via α(1−4) to the first sugar is 4′-*epi*-2′-deoxyfucose. MEN and MAR have been shown to exhibit different antitumor activities compared with the parent compounds doxorubicin and DNR, respectively. Very interestingly, the crystal structures of MEN and MAR with the same DNA hexamer suggest that the differences in biological activity between MEN and MAR depend on the different DNA binding properties of the sugar moiety.[25] In the MAR complex both sugar rings lie in the minor groove spanning four base pairs. In the complex of MEN and DNA hexamer (CGATCG),[26] two

* To whom correspondence should be addressed. For P.G.W.: phone, (614) 292-9884; fax, (614) 688-3106; e-mail, wang.892@osu.edu. For D.S.: phone, (614) 292-4381; fax, (614) 292-7766; e-mail, sun.176@osu.edu.
[†] Department of Chemistry and Biochemistry, The Ohio State University.
[#] Henan Normal University.
[‡] Division of Pharmaceutics, The Ohio State University.
[§] Present address: Department of Pharmaceutics, College of Pharmacy, The Second Military Medical University, Shanghai 200433, China.

10.1021/jm050916m CCC: $33.50
Published on Web 02/02/2006

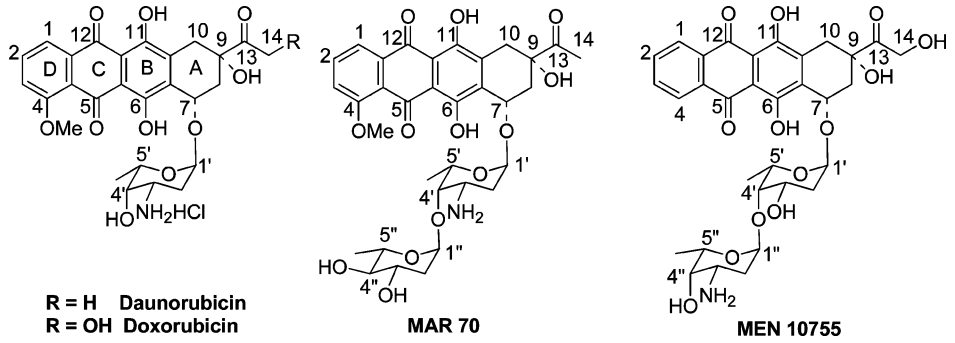


Figure 1. Structures of daunorubicin, doxorubicin, MAR 70, and MEN 10755.

different DNA binding sites were observed. In one binding site, the disaccharide resides in the DNA minor groove; in the other binding site, the second sugar protrudes from the DNA helix and is linked to guanine of another DNA molecular through hydrogen bonds. This peculiar behavior suggests that the second sugar in MEN may interact with other cellular targets such as topoisomerase.[26] In addition, the capability of the second sugar to protrude out of the minor groove is related to the properties of the first sugar. If daunosamine is the first sugar linked to the aglycone, as in the MAR complex, the basic 3′-amino group contributes an electrostatic interaction to the binding free energy. This additional interaction further stabilizes the position of the first sugar, which lies deeply in the minor groove and makes it difficult for the second sugar to protrude out of the minor groove. Disaccharide analogues lacking a basic amino group at the 3′ position in the first sugar, as in the MEN, are more flexible, and the second sugar will not participate in the DNA binding but protrude out of the minor groove to influence external interactions. These interactions could play an important role in the formation of the drug−DNA−topoisomerase complex and may affect the ability of the drug in poisoning both topoisomerases I and II. The interactions with other cellular targets involved in multidrug resistance genes may also be affected.[25]

On the basis of this discovery, a rational design for developing new disaccharide anthracycline drugs has emerged: replacing the 3-amino group of the first sugar with a neutral group and diversifying the second sugar motif with uncommon sugars. The rationale for modifying the sugar moiety in new anthracycline analogues is based on the potential functions of the uncommon sugar structure in anthracyclines and their significant biological effects. Our results and others have demonstrated that sugar structures in many anticancer natural products provide an important function in DNA binding and topoisomerase I/II (topo I/II) inhibition.[27−33] In our previous work, it has been found that the anticancer activities of the disaccharide analogues of anthracyclines correlated with their ability to target topo II mediated genomic DNA damage in vivo. Analogues with various terminal 2,6-dideoxy showed 30- to 60-fold higher anticancer activity than those with 2-deoxy- or 6-deoxy sugar.[32] These results suggested that the second sugar of disaccharide analogues should possess 2,6-dideoxy with α-linkage to the first sugar to exhibit better anticancer activity.

In the present study, we propose to synthesize a series of anthracyclines with modified disaccharides to improve the pharmacological efficacy, to overcome MDR-mediated drug resistance, and to obtain more information on the SAR of uncommon sugars as the second sugar moiety of disaccharide anthracyclines. Therefore, we designed six novel disaccharide analogues of DNR (Figure 2) by connecting 2,6-dideoxy sugars to the first sugar to reveal its function in anticancer activity

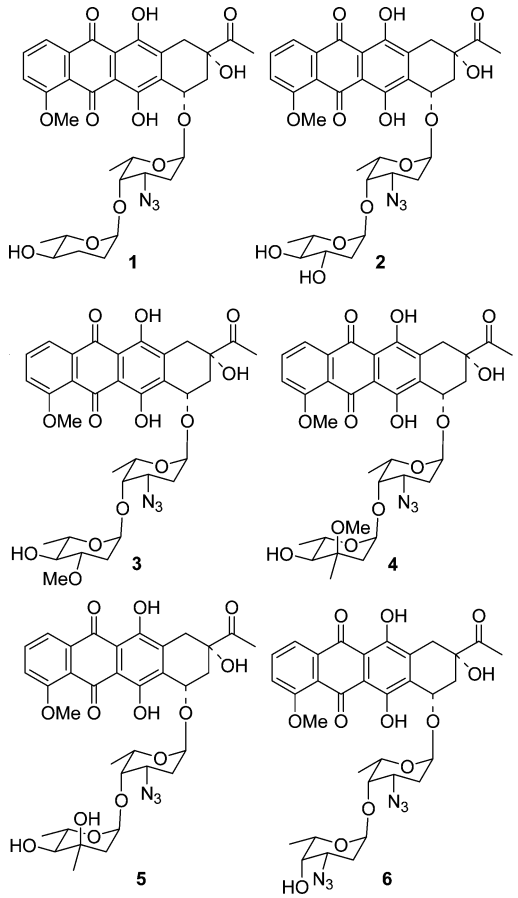
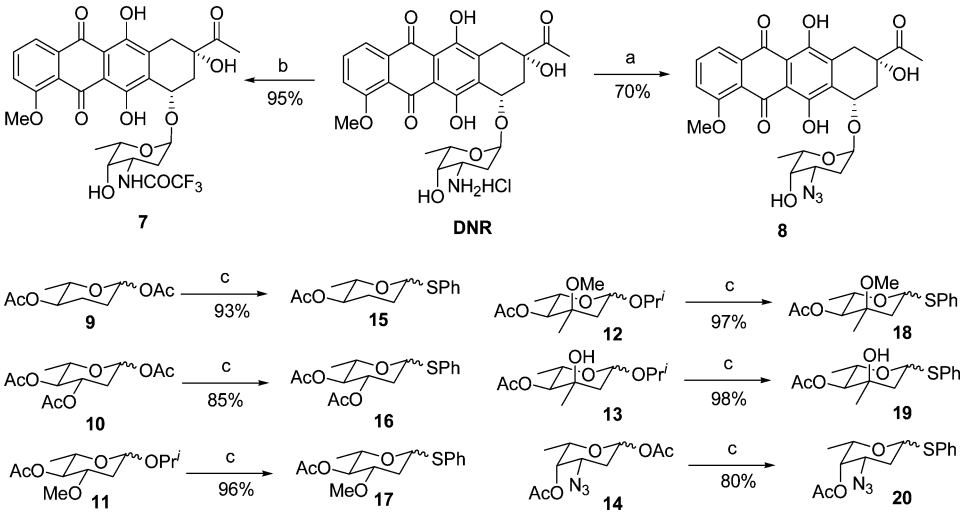


Figure 2. Synthesized compounds.

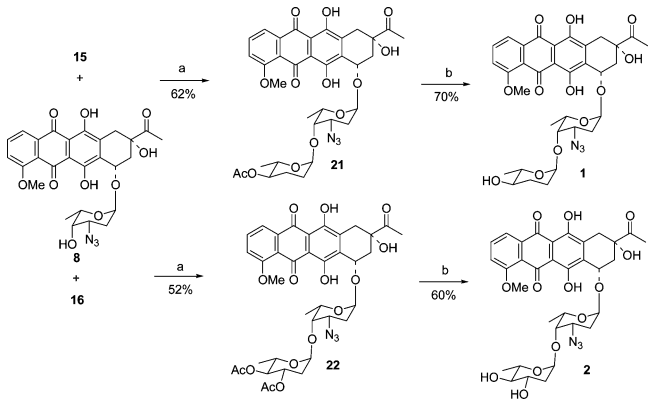
and to overcome drug resistance. In these compounds, the first sugar is an 3-azido-2,3,6-trideoxy-L-*lyxo*-α-hexopyranose; the second sugar linked via α(1→4) to the first sugar is a series of uncommon sugars including four 2,6-dideoxy sugars and two 2,3,6-trideoxysugars. The rationale for introducing the azido group into the first sugar is that the azido group is a neutral group with high electron density, which might avert P-gp recognition to overcome drug resistance. Indeed, our previous research has shown that replacing the amino group on DNR with an azido group can avert the binding of P-gp and overcome multidrug resistance (Fang, L.; Zhang, G.; et al. *J. Med. Chem.*, in press). The cytotoxicities of the synthesized compounds were examined in drug-sensitive leukemia cells K562 and doxorubicin-resistant K562/Dox cells by MTS assay. Indeed, different uncommon sugars in disaccharide analogues of daunorubicin showed distinct cytotoxicity against drug-resistant cancer cells.

Scheme 1[a]



[a] Reagents and conditions: (a) K_2CO_3, $CuSO_4$,TfN_3 solution; (b) $(CF_3CO)_2O$/pyridine, −20 °C, 15 min; (c) PhSH, $BF_3{\cdot}Et_2O/CH_2Cl_2$, 0 °C, 2 h.

Scheme 2[a]



[a] Reagents and conditions: (a) TTBP, $AgPF_6$/ CH_2Cl_2, 0 °C; (b) 0.1 M NaOH/THF, 0 °C.

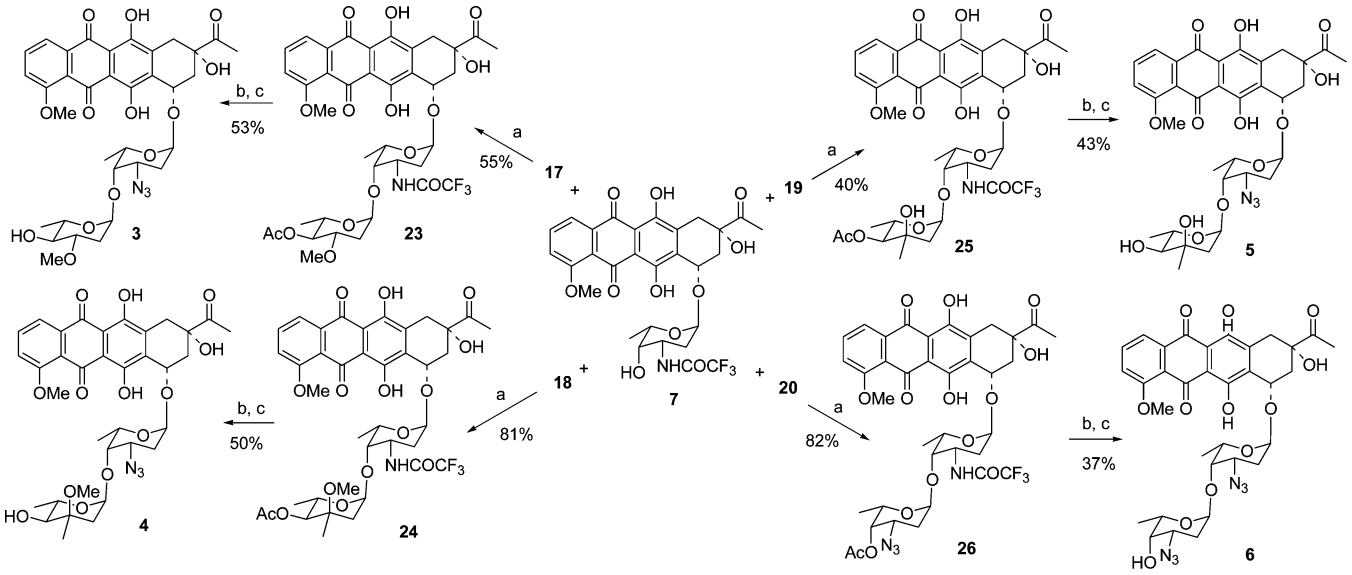
Results and Discussion

Chemistry. Our results and others have demonstrated that the optimal configuration of disaccharide analogues of anthracyclines should be an uncommon sugar linked to the 4 position of the first sugar through an α(1→4) linkage.[22−25,32] Indeed, natural anthracycline analogues often contain two or more sugars connected by *axial* (1→4) linkage. Therefore, for the synthesis of 3′-azido disaccharide analogues of daunorubicin, the second uncommon sugars have to be introduced stereoselectively. In our previous work, we developed a concise promoter system $AgPF_6$/TTBP (2,4,6-*tert*-butylpyrimidine) of 2-deoxythioglycosides for syntheses of the disaccharide anthracyclines.[32] This protocol produced the desired α-glycosidic bond with good yields.

In this work, two strategies were investigated to produce the 3′-azido disaccharide analogues of DNR. Both of them are based on glycosylation using our catalyst system ($AgPF_6$/TTBP). One strategy was to transform the amino group of DNR to an azido group through Wong's method[34] to produce 3′-azidodeaminodaunorubicin (**8**) as a glycosyl acceptor for the glycosylation with the second 2,6-dideoxy sugars (Scheme 2). The second one was to apply 3′-*N*-trifluoroacetyldaunorubicin (**7**) as the glycosyl acceptor for the glycosylation reaction. The amino group was converted to an azido group after glycosylation and deprotection (Scheme 3). Both strategies were proven to be effective for the synthesis of these types of disaccharide anthracyclines.

The synthetic routes of glycosyl acceptors (**7** and **8**) and glycosyl donors are outlined in Scheme 1. The glycosyl acceptor **8** was readily prepared by treatment of DNR with TfN_3 solution[34] in 70% yield. Treatment of DNR with trifluoroacetic anhydride in pyridine at −20 °C for 15 min gave the glycosyl acceptor **7** in 95% yield.[32] The corresponding glycosyl donors were prepared from their corresponding precursors (compounds **9**−**14**). The acetyl group was used for protecting the hydroxyl groups present in the sugar molecule because they were cleavable under 0.1 M NaOH in THF, which allowed the acid and strong base sensitive aglycone moiety in the anthracycline molecule not to be affected in the final deprotection manipulations. As shown in Scheme 3, after treatment with phenylthiol in the presence of $BF_3{\cdot}Et_2O$ at 0 °C for 2 h, the desired sugar donors (compounds **15**−**20**) were obtained in excellent yields. The thiolglycosides were obtained as a mixture of α- and *β*-isomers. Since both isomers are able to be used for the glycosylation to produce the desired α-linked daunorubicin derivatives, separation of them was unnecessary. Previous research[32] has found that the tertiary hydroxyl group would not affect further glycosylation; thus, compound **13** can be directly converted to the sugar donor **19**. With the glycosyl acceptors and donors in hand, the glycosylation was subsequently performed. The mixture of glycosyl acceptors (**7** and **8**) and donors **15**−**20** in the presence of TTBP and 4 Å molecular sieves was treated with $AgPF_6$ at 0 °C for 2−4 h to give the products **21**−**26** in good yields (Schemes 2 and 3). Glycosylation of **8** with glycosyl donors **15** and **16** gave exclusively the α-products **21** and **22** in yields of 62% and 52%, respectively. The ^{1}H NMR data indicated that the desired α-linkage was formed predominantly $\alpha/\beta > 5{:}1$. Mild deprotection of the ester groups with 0.1 M NaOH in THF afforded the final products **1** and **2** in yields of 70%, and 60%, respectively. Condensation of **7** with glycosyl donors **18** and **20** exclusively formed the α-products **24** and **26** with yields of 81% and 82%, respectively. However, condensation of **7** with the glycosyl donors **17** and **19** gave the anomeric mixtures ($\alpha/\beta = 2{:}1$) with yields of 83% and 60%, respectively. After glycosylation, deprotection of the desired disaccharide intermediates **21**−**26** with 0.1 M NaOH in THF followed by treatment with TfN_3 solution in CH_2Cl_2

Scheme 3[a]



[a] Reagents and conditions: (a) TTBP, $AgPF_6$/ CH_2Cl_2, 0 °C; (b) 0.1 M NaOH/THF, 0 °C; (c) K_2CO_3, $CuSO_4$,TfN_3 solution.

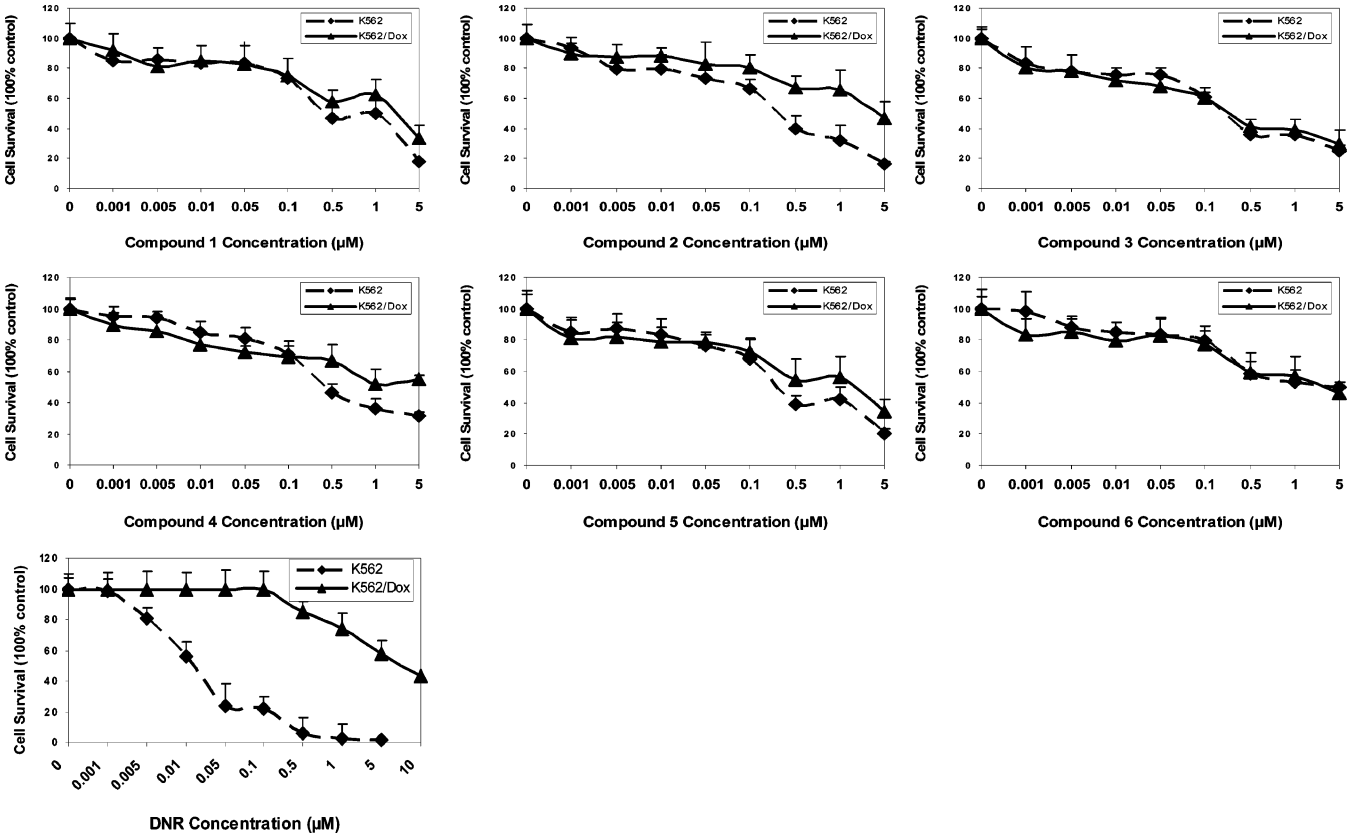


Figure 3. Cytotoxicity of synthesized compounds on K562 and K562/Dox cells.

afforded the final compounds **3**, **4**, **5**, and **6** with overall yields of 53%, 50%, 43%, and 37%, respectively.

Biology. Cytotoxicity. The anticancer activities of these compounds were examined in leukemia cells K562 and doxorubicin-resistant K562/Dox cells by MTS assay as described.[35−37] The drug-resistant K562/Dox cell line was induced by doxorubicin treatment. The cell line was cultured in 0.1 μM doxorubicin 1 week per month, followed by 10 days of culture without doxrubicin before experiment. This is to maintain similar high levels of P-gp expression in each experiment. In drug-resistant K562/Dox cells, MDR1 mRNA was induced by 600-fold higher than drug-sensitive K562 cells as measured by real-time PCR (data not shown). The 2000−10000 cells were incubated with 0.001−5 μM DNR and its derivatives for 72 h. Then 20 μL of MTS/PMS assay solution was added to each well and the absorbance was recorded. The cell survival was calculated as a percentage of cell control group without treatment. The anticancer activities are summarized in Figure 3 and Table 1. The IC_{50} values were calculated by WinNonlin 4.1 (Pharsight) from the dose-response curves of percentage

Table 1. Cytotoxicity (IC_{50}) and Drug Resistance Index (DRI) of Disaccharide Daunorubicins and DNR

	compounds							
	1	**2**	**3**	**4**	**5**	**6**	**8**	DNR
IC_{50} in K562 (μM)	0.787	0.287	0.278	0.225	0.448	1.132	0.075	0.015
IC_{50} in K562/Dox (μM)	1.926	5.437	0.289	12.070	1.628	6.388	1	>5
DRI	2.45	18.94	1.04	53.64	3.63	5.64	13.3	>333

of cell growth with the model

$$E = E_{max} - (E_{max} - E_0)\frac{C}{C + EC_{50}}$$

In drug-sensitive cells, compounds **1**−**6** were found to be active against leukemia K562 cells with IC_{50} values in the nanomolar range (200−1100 nM); yet their IC_{50} values are higher than that of parent compound DNR (15 nM). Compounds **2**−**5** with 2,6-dideoxy sugars showed 3- to 4-fold better activity than compounds **1** and **6** with 2,3,6-trideoxy sugars. These results combined with our previous results[32] indicate that 2,6-dideoxy sugars are the best choice to modify the sugar chain of disaccharide anthracyclines compared with 2-deoxy-, 6-deoxy-, and 2,3,6-trideoxy sugars.

In doxorubicin-resistant K562/Dox cells, compounds **1**, **3**, and **5** exhibit much better activities (with $IC_{50} = 0.29-2.0\ \mu M$) than DNR (with $IC_{50} > 5\ \mu M$). The drug resistance index (DRI, ratio of IC_{50} in drug-resistant cells to IC_{50} in drug-sensitive cells) is a good indicator of the drug's ability to overcome resistance. The smaller DRI indicates better capacity to overcome drug resistance. As summarized in Table 1, DRI values of compounds **1**−**6** were 6.2- to 320-fold lower than that of DNR with a value of 333. In comparison, monosaccharide daunorubicin with a 3-azido sugar (compound **8**) also showed strong cytotoxicity against drug-resistant K562 with a DRI of 13.3 (Table 1). Among the synthesized compounds, compound **3** emerged as the most active compound against drug-resistant cells with at least 17-fold higher activity than DNR. It completely overcomes the drug resistance in this leukemia cell line (DRI = 1.04, 320-fold lower than that of parent compound DNR). It is worthy to be further evaluated as a new drug candidate.

Compound **2** with a 3″-equatorial hydroxyl group and compound **3** with a 3″-equatorial methoxyl group possess similar sugar moieties and showed similar activity in drug-sensitive cells. However, compound **3** exhibited 18-fold higher activity against doxorubicin-resistant cells than compound **2**. The substitution of hydrogen atom in the equatorial 3-OH group of the second sugar with methyl group resulted in a significant increase of activity against doxorubicin-resistant cells. In contrast, compound **5** with a 3″-axial hydroxyl group exhibited 14-fold higher activity against drug-resistant K562 than compound **4** with a 3″-axial methoxyl group. These data suggest that the substitution and orientation of the 3-OH group in the second sugar may significantly influence its binding to P-gp and activity against drug-resistant cells. Further SAR studies are required to clarify this finding.

Conclusion

In summary, we have developed two effective approaches to synthesize these novel disaccharide analogues from the parent DNR using $AgPF_6$/TTBP as a mild activating system for glycosylation with 2,6-dideoxythioglycosides. A series of the novel disaccharide analogues have been synthesized and evaluated for their cytotoxicity against drug-resistant leukemia. Compound **3** emerged as the most active compound, which completely overcomes drug resistance with at least 17-fold higher activity against drug-resistant cells than the parent compound (DNR). SAR studies showed that the substitution and orientation of the 3-OH group in the second sugar significantly influence its activity against drug-resistant cancer cells. These data indicate that chemical modification of the sugar structures of anthracyclines not only changes the anticancer activity but also overcomes drug resistance.

Experimental Section

Chemistry. All solvents were dried with a solvent-purification system from Innovative Technology, Inc. All reagents were used as received from commercial sources. Analytical TLC was carried out on E. Merck silica gel 60 F254 aluminum-backed plates. Preparative TLC was carried out on EMD Chemicals, Inc. silica gel 60 F254 plates (20 cm × 20 cm, 1 mm). The 230−400 mesh size of the same absorbent was utilized for all chromatographic purifications. ^1H and ^{13}C NMR spectra are at the indicated field strengths. The high-resolution mass spectra were recorded at The Ohio State University Campus Chemical Instrumentation Center.

3′-Azidodaunorubicin (8). Daunorubicin hydrochloride (5.24 g, 9.3 mmol) was dissolved in water (30 mL) and treated with potassium carbonate (1.92 g, 13.9 mmol) and $CuSO_4 \cdot 5H_2O$ (14 mg, 88 μmol). MeOH (60 mL) was added to the sugar solution, and the TfN_3 solution was made using 2 equiv of Tf_2O.[34] Then, adequate MeOH was added to homogeneity. The mixture was stirred overnight. The mixture was diluted with H_2O (100 mL) and extracted with CH_2Cl_2. The combined extractions were dried over anhydrous Na_2SO_4. After removal of the solvent, the residue was purified through a silica gel column using MeOH/CH_2Cl_2 (1:50). Product **8** was obtained as a red solid (4 g, 70%): HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{27}H_{27}N_3O_{10}Na^+$ 576.1589, found 576.1612. ^1H NMR (500 MHz, $CDCl_3$) δ 13.95 (1H, s), 13.19 (1H, s), 7.98 (1H, d), 7.74 (1H, t), 7.36 (1H, d), 5.54 (1H, d, $J = 3.6$ Hz, H-1′), 5.23 (1H, d, $J = 1.9$ Hz, H-7), 4.37 (1H, s), 4.10 (1H, m), 4.05 (3H, s), 3.69 (1H, br), 3.60 (1H, m), 3.15 (1H, d), 2.87 (1H, d), 2.38 (3H, s), 2.28 (1H, m), 2.09 (2H, m), 1.91 (1H, m), 1.30 (3H, d). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.5, 186.9, 186.7, 161.1, 156.3, 155.7, 135.7, 135.5, 134.2, 133.9, 120.8, 119.8, 118.5, 111.5, 111.3, 100.6, 76.7, 70.1, 69.5, 67.1, 56.8, 56.7, 34.9, 33.3, 28.5, 24.7, 16.8.

General Procedure for Preparation of the Glycosyl Donors (15−20). To a stirred solution of acetated sugar **12** (0.2 g, 0.77 mmol) in CH_2Cl_2 (5.0 mL) at 0 °C were added benzenethiol (0.1 mL, 0.98 mmol) and $BF_3 \cdot Et_2O$ (0.11 mL, 0.87 mmol). The reaction mixture was allowed to warm slowly to room temperature. After the mixture was stirred for 4 h, aqueous NaOH (0.1 M, 10 mL) was added to quench the reaction. Then the reaction mixture was washed with water. The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by flash column chromatography, giving compound **18** as a syrup (0.23 g, 97% yield).

General Procedure for the Preparation of the Protected Disaccharide Analogues of Daunorubicin (21−26). A mixture of sugar donor (4.8 mmol), aglycone (7.2 mmol), TTBP (17.3 mmol), and molecular sieves (4 Å, <5 μm, freshly activated) in CH_2Cl_2 (50 mL) was stirred at room temperature for 2 h under N_2, then cooled to 0 °C (ice bath). Powdered $AgPF_6$ (17.3 mmol) was added and stirred for 3 h. Subsequently pyridine (23 mL) was added, and the mixture was stirred for a further 0.5 h. Filtration (through a Celite pad), concentration, and chromatographic purification provided the products.

7-[4-*O*-(4-*O*-Acetyl-2,3,6-trideoxy-α-L-*erythro*-hexopyranosyl)-3-azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (21). Pure **21** was obtained as a red solid with a yield of 62% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:150). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{35}H_{39}N_3O_{13}Na^+$ 732.2375, found 732.2378. ^1H NMR (500 MHz, $CDCl_3$) δ 13.84 (1H, s), 13.07 (1H, s), 7.90 (1H, d, $J = 7.7$ Hz), 7.70 (1H, t, $J = 8.1$ Hz), 7.31 (1H, d, $J = 8.5$ Hz), 5.49 (1H, d, $J = 3.0$ Hz, H-1′), 5.17 (1H, s, H-7), 4.82 (1H, s, H-1″), 4.39 (2H, m), 4.16 (1H, m), 4.04 (1H, m), 4.02 (3H, s), 3.71 (2H, m), 3.01 (1H, d, $J = 18.6$ Hz), 2.78 (1H, d, $J = 18.6$ Hz), 2.36 (3H, s), 2.23 (1H, m), 2.09−1.78 (11H, m), 1.22 (3H, d, $J = 6.5$ Hz), 0.1.11 (3H, d, $J = 6.2$ Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.5, 186.8, 186.4, 170.4, 160.9, 156.3, 155.6, 135.7, 135.3, 134.2, 133.9, 120.7, 119.7, 118.4, 111.3, 111.2, 100.6, 98.2, 76.7, 75.1, 73.5, 69.9, 67.9, 67.6, 56.7, 56.6, 34.9, 33.2, 29.6, 29.1, 24.7, 24.1, 21.2, 17.9, 17.5.

7-[4-*O*-(3,4-*O*-Diacetyl-2,6-dideoxy-α-L-*arabino*-hexopyranosyl)-3-azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (22). The pure **22** was obtained as a red solid with a yield of 52% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:150). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{37}H_{41}N_3O_{15}Na^+$ 790.2430, found 790.2423. ^1H NMR (500 MHz, $CDCl_3$) δ 13.94 (1H, s), 13.23 (1H, s), 8.00 (1H, d, $J = 7.7$ Hz), 7.75 (1H, t, $J = 8.1$ Hz), 7.37 (1H, d, $J = 8.5$ Hz), 5.52 (1H, s, H-1′), 5.25 (1H, m), 5.24 (1H, s, H-7), 4.95 (1H, d, $J = 3.3$ Hz, H-1″), 4.69 (1H, t, $J = 9.6$ Hz), 4.40 (1H, br), 4.21 (1H, m), 4.04 (5H, m), 3.79 (1H, d, $J = 12.6$ Hz), 3.68 (1H, s), 3.20, (1H, d, $J = 18.7$ Hz), 2.92 (1H, d, $J = 18.4$ Hz), 2.38 (3H, s), 2.37 (1H, m), 2.26 (1H, m), 2.12−1.95 (8H, m), 1.85 (2H, m), 1.24 (3H, d, $J = 6.0$ Hz), 1.15 (3H, d, $J = 6.2$ Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.5, 187.0, 186.8, 170.3, 170.2, 161.1, 156.4, 155.8, 135.7, 135.5, 134.2, 133.9, 120.9, 119.8, 118.5, 111.5, 111.4, 100.6.

7-[4-*O*-(4-*O*-Acetyl-2,6-dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-2,3,6-trideoxy-3-*N*-trifluoroacetyl-α-L-*lyx*o-hexopyranosyl]-daunorubicinone (23). The pure **23** was obtained as a red solid with a yield of 55% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:100). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{38}H_{42}F_3NO_{15}Na^+$ 832.2398, found 832.2387. ^1H NMR (500 MHz, $CDCl_3$) δ 13.89 (1H, s), 13.15 (1H, s), 8.11 (1H, d), 7.94 (1H, d), 7.71 (1H, t), 7.34 (1H, d), 5.51 (1H, d, $J = 3.3$ Hz, H-1′), 5.23 (1H, d, $J = 1.8$ Hz), 4.89 (1H, br), 4.71 (1H, m), 4.35 (1H, br), 4.21 (2H, m), 4.20 (3H, s), 3.95 (1H, m), 3.65 (1H, m), 3.53 (1H, br), 3.36 (3H, s), 3.11 (1H, d), 2.85 (1H, d), 2.37 (3H, s), 2.25 (2H, m), 2.09 (4H, m), 1.83 (3H, m,), 1.27 (3H, d), 1.20 (3H, d). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.9, 186.9, 186.6, 170.1, 160.9, 156.7, 156.3, 155.7, 135.6, 135.4, 134.3, 133.9, 120.8, 119.8, 118.4, 116.9, 111.5, 111.3, 100.4, 99.3, 80.1, 76.7, 75.4, 73.3, 69.9, 69.4, 67.4, 57.1, 56.6, 45.5, 35.1, 34.1, 33.4, 30.6, 24.1, 21.0, 17.2, 17.0.

7-[4-*O*-(4-*O*-Acetyl-2,6-dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)-2,3,6-trideoxy-3-*N*-trifluoroacetyl-α-L-*lyxo*-hexopyranosyl]daunorubicinone (24). The pure **24** was obtained as a red solid with a yield of 81% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:100). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{39}H_{44}F_3NO_{15}Na^+$ 846.2555, found 846.2545. ^1H NMR (500 MHz, $CDCl_3$) δ 13.90 (1H, s), 13.20 (1H, s), 7.98 (1H, d), 7.73 (2H, m), 7.34 (1H, d), 5.48 (1H, d, $J = 3.5$ Hz, H-1′), 5.24 (1H, d, $J = 1.6$ Hz, H-7), 4.92 (1H, d, $J = 4.3$ Hz, H-1″), 4.68 (1H, d), 4.38 (1H, s), 4.26 (2H, m), 4.18 (1H, m), 4.04 (3H, s), 3.61 (1H, br), 3.30 (3H, s), 3.17 (1H, d), 2.85 (1H, d), 2.46 (1H, m), 2.37 (3H, s), 2.25 (1H, m), 2.12 (4H, m), 1.83 (2H, m), 1.65 (1H, m), 1.29 (3H, d), 1.14 (3H, s), 1.08 (3H, d). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.9, 187.0, 186.6, 170.6, 161.0, 156.7, 156.4, 155.8, 135.6, 135.5, 134.3, 133.9, 120.9, 119.8, 118.4, 116.9, 111.5, 111.3, 100.5, 98.2, 77.7, 76.9, 76.7, 72.9, 69.9, 67.8, 63.9, 56.7, 49.8, 45.6, 35.6, 35.1, 33.4, 30.7, 24.8, 21.1, 20.8, 17.8, 17.1.

7-[4-*O*-(4-*O*-Acetyl-2,6-dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-2,3,6-trideoxy-3-*N*-trifluoroacetyl-α-L-*lyxo*-hexopyranosyl]daunorubicinone (25). The pure **25** was obtained as a red solid with a yield of 40% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:75). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{38}H_{42}F_3NO_{15}Na^+$ 832.2398, found 832.2391. ^1H NMR (500 MHz, $CDCl_3$) δ 13.87 (1H, s), 13.83 (1H, s), 7.99 (1H, d, $NHCOCF_3$), 7.87 (1H, d), 7.66 (1H, t), 7.30 (1H, d), 5.44 (1H, d, $J = 3.3$ Hz, H-1′), 5.14 (1H, s), 4.97 (1H, d, $J = 3.5$ Hz, H-1″), 4.65 (1H, d), 4.19 (4H, m), 3.99 (3H, s), 3.61(1H, s), 3.09 (1H, d), 2.80 (1H, d), 2.35 (3H, s), 2.10, 1.91, (9H, m), 1.27 (3H, d), 1.45 (6H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.7, 186.7, 186.5, 170.4, 160.9, 156.7, 156.3, 155.6, 135.6, 135.3, 134.1, 133.8, 120.7, 119.7, 118.4, 116.9, 111.5, 111.2, 100.4, 99.9, 79.7, 76.6, 70.2, 69.4, 67.2, 64.6, 56.6, 45.5, 41.28, 35.1, 33.3, 30.7, 26.0, 24.8, 20.7, 17.5, 17.1.

7-[4-*O*-(4-*O*-Acetyl-3-azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-2,3,6-trideoxy-3-*N*-trifluoroacetyl-α-L-*lyxo*-hexopyranosyl]-daunorubicinone (26). The pure **26** was obtained as a red solid with a yield of 82% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:75). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{37}H_{39}F_3N_4O_{14}Na^+$ 843.2307, found 843.2331. ^1H NMR (500 MHz, $CDCl_3$) δ 13.94 (1H, s), 13.21 (1H, s), 8.60 (1H, d), 7.99 (1H, d), 7.73 (1H, m), 7.33 (1H, m), 5.50 (1H, d, $J = 3.4$ Hz, H-1′), 5.24 (1H, br, H-7), 5.21 (1H, br), 4.99 (1H, br, H-1″), 4.20 (3H, m), 4.04 (3H, s), 3.92 (1H, m), 3.58 (1H, br), 3.18 (1H, d), 2.90 (1H, d), 2.38 (3H, s), 2.17 (1H, m), 2.17 (3H, s). 2.13 (2H, m), 2.02 (1H, m), 1.86 (1H, m), 1.77 (1H, m), 1.27 (3H, d), 1.14 (3H, d). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.8, 187.1, 186.7, 170.3, 161.0, 156.3, 155.8, 150.4, 135.7, 135.5, 134.3, 133.8, 124.3, 120.9, 119.8, 118.4, 111.5, 111.4, 100.4, 100.2, 80.9, 76.6, 69.9, 69.7, 67.4, 67.3, 56.7, 53.9, 45.6, 35.1, 33.5, 30.8, 30.1, 24.8, 20.7, 17.3, 16.4.

General Procedure for the Preparation of Disaccharide Analogues of Daunorubicin (1 and 2). A solution of protected disaccharide daunorubicin (100 mg) in THF (5 mL) was cooled in an ice bath to 0 °C, and then 0.1 M NaOH aqueous solution (70 mL), which was cooled in advance in an ice bath, was added. After being stirred for 6−8 h, the reaction mixture was neutralized with 0.1 M citric acid and extracted with $CHCl_3$. The extractions were washed with saturated aqueous $NaHCO_3$ solution. Concentration and chromatographic purification provided the products.

7-[4-*O*-(2,3,6-Trideoxy-α-L-*erythro*-hexopyranosyl)-3-azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (1). Pure **1** was obtained as a red solid with a yield of 70% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:75). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{33}H_{37}N_3O_{12}Na^+$ 690.2269, found 690.2267. ^1H NMR (500 MHz, $CDCl_3$) δ 13.92 (1H, s), 13.17 (1H, s), 7.96 (1H, d, $J = 7.6$ Hz), 7.74 (1H, t, $J = 8.0$ Hz), 7.35 (1H, d, $J = 8.5$ Hz), 5.53 (1H, s, H-1′), 5.21 (1H, s, H-7), 4.83 (1H, s, H-1″), 4.83 (1H, br), 4.43 (4H, m), 3.95 (1H, m), 3.72 (2H, m), 3.24 (1H, m), 3.13 (1H, d, $J = 18.8$ Hz), 2.87 (1H, d, $J = 18.8$ Hz), 2.37 (3H, s), 2.25 (1H, m), 2.08 (3H, m), 1.83 (6H, m), 1.24 (6H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.6, 186.9, 186.6, 161.0, 156.3, 155.7, 135.7, 135.4, 134.2, 133.9, 120.8, 119.8, 118.4, 111.4, 111.3, 100.6, 98.1, 76.7, 74.9, 72.1, 70.0, 69.8, 68.0, 56.7, 56.6, 34.9, 33.3, 29.7, 29.6, 29.5, 27.6, 24.7, 17.9, 17.5.

7-[4-*O*-(2,6-Dideoxy-α-L-*arabino*-hexopyranosyl)-3-azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (2). Pure **2** was obtained as a red solid with a yield of 60% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:40). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{33}H_{37}N_3O_{12}Na^+$ 706.2218, found 706.2231. ^1H NMR (500 MHz, $CDCl_3$) δ 13.93 (1H, s), 13.16 (1H, s), 7.96 (1H, d, $J = 7.3$ Hz), 7.73 (1H, t, $J = 7.9$ Hz), 7.35 (1H, d, $J = 8.4$ Hz), 5.51 (1H, s, H-1′), 5.21 (1H, s, H-7), 4.93 (1H, d, $J = 2.4$ Hz, H-1″), 4.04 (3H, s), 3.95 (4H, m), 3.70 (3H, m), 3.13 (2H, m), 2.87 (1H, d, $J = 18.4$ Hz), 2.38 (3H, s), 2.07 (3H, m), 1.83 (1H, m), 1.71 (2H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.6, 186.9, 186.6, 161.0, 156.3, 155.7, 135.7, 135.4, 134.2, 133.9, 120.8, 119.8, 118.4, 111.5, 111.3, 100.6, 99.4, 78.0, 75.4, 69.8, 69.0, 68.7, 67.8, 56.7, 56.5, 37.7, 34.9, 33.3, 29.7, 29.6, 24.7, 17.7, 17.5.

General Procedure for the Preparation of Disaccharide Analogues of Daunorubicin (3−6). A solution of protected disaccharide analogues (150 mg) in THF (8 mL) was cooled in an ice bath to 0 °C, and then 0.1 M NaOH aqueous solution (90 mL), which was cooled in advance in an ice bath, was added. After being stirred for 6−8 h, the reaction mixture was neutralized with 0.1 M citric acid and extracted with $CHCl_3$. The extractions were washed with saturated aqueous $NaHCO_3$ solution. Removal of the solvent

provided the crude deprotected products, which were used in the next reaction without further purification.

The crude compound (100 mg) was mixed in water (0.4 mL) and treated with potassium carbonate (24 mg) and $CuSO_4 \cdot 5H_2O$ (0.2 mg). MeOH (1.8 mL) and the TfN_3 solution was made using 2 equiv of Tf_2O.[34] Then, adequate MeOH was added to homogeneity. The mixture was stirred overnight. The mixture was diluted with H_2O (10 mL) and extracted with CH_2Cl_2. The combined extractions were dried over anhydrous Na_2SO_4. After removal of solvent, the residue was purified on a column of silica gel to afford the final product.

7-[4-*O*-(2,6-Dideoxy-3-*O*-methyl-α-L-*arabino*-hexopyranosyl)-3-azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (3). Pure **3** was obtained as a red solid with a yield of 53% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:75). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{34}H_{39}N_3O_{13}Na^+$ 720.2375, found 720.2382. ^1H NMR (500 MHz, $CDCl_3$) δ 13.91 (1H, s), 13.13 (1H, s), 7.95 (1H, d, $J = 7.5$ Hz), 7.73 (1H, t, $J = 8.2$ Hz), 7.34 (1H, d, $J = 8.4$ Hz), 5.51 (1H, d, $J = 3.4$ Hz, H-1′), 5.21 (1H, d, $J = 1.9$ Hz, H-7), 4.93 (1H, d, $J = 3.5$ Hz, H-1″), 4.40 (1H, br), 4.06 (2H, m), 4.04 (3H, s), 3.70 (2H, m), 3.50 (1H, m), 3.40 (3H, s), 3.12 (2H, m), 2.83 (1H, d, $J = 18.7$ Hz), 2.40 (1H, m), 2.39 (3H, s), 2.24 (1H, m), 2.08 (2H, m), 1.83 (1H, m), 1.52 (1H, m), 1.27 (6H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.6, 186.9, 186.6, 161.0, 156.3, 155.7, 135.7, 135.4, 134.2, 133.9, 120.8, 119.8, 118.4, 111.4, 111.3, 100.6, 99.3, 78.1, 76.7, 75.2, 69.8, 68.5, 67.9, 56.7, 56.6, 56.5, 34.9, 34.0, 33.3, 29.5, 24.7, 17.8, 17.5.

7-[4-*O*-(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-*ribo*-hexopyranosyl)-3-azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (4). Pure **4** was obtained as a red solid with a yield of 50% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:100). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{35}H_{41}N_3O_{13}Na^+$ 734.2531, found 734.2515. ^1H NMR (500 MHz, $CDCl_3$) δ 13.87 (1H, s), 13.08 (1H, s), 7.93 (1H, d, $J = 7.2$ Hz), 7.71 (1H, t, $J = 7.9$ Hz), 7.33 (1H, d, $J = 8.1$ Hz), 5.47 (1H, s, H-1′), 5.18 (1H, s, H-7), 4.75 (1H, d, $J = 3.9$ Hz, H-1″), 4.40 (1H, br), 4.21 (1H, m), 4.02 (3H, s), 3.74 (1H, m), 3.63 (1H, s), 3.30 (3H, s), 3.10 (1H, d, $J = 18.6$ Hz), 2.98 (1H, d, $J = 9.6$ Hz), 2.42 (1H, d, $J = 18.6$ Hz), 2.40 (1H, m), 2.36 (3H, s), 2.25 (1H, m), 2.01 (3H, m), 1.81 (1H, m), 1.55 (1H, m), 1.22 (9H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.6, 186.8, 186.5, 160.9, 156.3, 155.6, 135.7, 135.3, 134.2, 133.9, 120.7, 119.8, 118.4, 111.3, 111.2, 100.7, 98.2, 78.1, 76.7, 75.5, 72.7, 69.8, 67.9, 56.7, 56.6, 49.3, 35.4, 34.8, 33.2, 29.8, 24.7, 21.5, 17.9, 17.5.

7-[4-*O*-(2,6-Dideoxy-3-*C*-methyl-α-L-*ribo*-hexopyranosyl)-3-azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (5). Pure **5** was obtained as a red solid with a yield of 43% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:75). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{34}H_{39}N_3O_{13}Na^+$ 720.2375, found 720.2390. ^1H NMR (500 MHz, $CDCl_3$) δ 13.90 (1H, d, $J = 6.4$ Hz), 13.16 (1H, d, $J = 9.8$ Hz), 7.97 (1H, d, $J = 6.8$ Hz), 7.72 (1H, t, $J = 8.1$ Hz), 7.35 (1H, d, $J = 8.3$ Hz), 5.47 (1H, s, H-1′), 5.19 (1H, s, H-7), 4.99 (1H, d, $J = 3.4$ Hz, H-1″), 4.30 (1H, br), 4.07 (2H, m), 4.04 (3H, s), 3.84 (1H, m), 3.75 (1H, s), 3.15 (1H, m), 2.96 (1H, d, $J = 9.8$ Hz), 2.81 (1H, m), 2.37 (3H, s), 2.18 (2H, m), 2.11 (1H, m), 1.88 (1H, m), 1.82 (2H, m), 1.30 (1H, d, $J = 6.4$ Hz), 1.23 (6H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.4, 186.8, 186.5, 161.0, 156.3, 155.6, 135.7, 135.4, 134.1, 133.8, 120.2, 119.8, 118.5, 111.3, 111.2, 100.4, 99.2, 76.6, 76.5, 75.1, 70.0, 69.7, 67.4, 66.6, 56.7, 56.5, 40.8, 34.9, 33.2, 30.0, 25.6, 24.7, 18.1, 17.6.

7-[4-*O*-(3-Azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-3-azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (6). Pure **6** was obtained as a red solid with a yield of 37% (separated on a column of silica gel, MeOH/CH_2Cl_2 1:100). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{33}H_{36}N_6O_{12}Na^+$ 731.2283, found 731.2266. ^1H NMR (500 MHz, $CDCl_3$) δ 13.87 (1H, s), 13.11 (1H, s), 7.94 (1H, d, $J = 7.7$ Hz), 7.72 (1H, t, $J = 8.2$ Hz), 7.34 (1H, d, $J = 8.5$ Hz), 5.50 (1H, d, $J = 3.3$ Hz, H-1′), 5.18 (1H, s, H-7), 5.01 (1H, d, $J = 2.9$ Hz, H-1″), 4.36 (1H, br), 4.29 (1H, q), 4.06 (1H, m), 4.03(3H, s), 3.76 (4H, m), 3.12 (1H, d, $J = 18.7$ Hz), 2.82 (1H, d, $J = 18.7$ Hz), 2.36 (3H, s), 2.26 (1H, $J = 14.8$ Hz), 2.06 (5H, m), 1.84 (1H, m), 1.23 (6H, m). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.5, 186.8, 186.5, 161.0, 156.3, 155.6, 135.7, 135.4, 134.2, 133.9, 120.7, 119.8, 118.5, 111.4, 111.2, 100.5, 98.6, 76.7, 75.2, 69.9, 69.8, 67.7, 66.9, 56.8, 56.6, 34.9, 33.2, 29.6, 28.5, 24.7, 17.5, 16.8.

Biology. Cell Culture. Drug-sensitive cells K562 and drug-resistant cells K562/Dox were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acid, and penicillin (100 units/mL)/streptomycin (100 μg/mL) in a humidified atmosphere of 5% CO_2 and 95% air at 37 °C. The culture mediums were changed every 2−3 days. Before each experiment, K562/Dox cells were stimulated with 0.1 μM doxorubicin for 1 week per week, and then cultured for 10 days without doxorubicin stimulation. It was ensured that the P-gp expression level was similar in every experiment.

Cytotoxicity of Daunorubicin Analogues by MTS Assay. Drug-sensitive K562 and drug-resistant K562/Dox cells (2000−10000) were seeded in 96-well plates in RPMI-1640 and incubated for 24 h. The exponentially growing cancer cells were incubated with various concentrations of compounds for 72 h at 37 °C (5% CO_2, 95% humidity). After 72 h of incubation, tetrazolium-[3-(4,5-dimethythiazol-2-yl)]-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS, final concentration, 2 mg/mL) and phenazine methosulfate (PMS, final concentration of 25 μM) were mixed and added directly to the cells. After incubation for 3 h at 37 °C, the absorbance of formazan (the metabolite of MTS by viable cells) was measured at 490 nm. The IC_{50} values of the compounds for cytotoxicity were calculated by WinNonlin software from the dose-response curves.

Acknowledgment. This work was supported by NSF (Grant CH-0316806) and a fund from Michigan Life Science Corridor Fund (Grant 1632) to P. G. Wang. This work was partially supported by a research grant from Ohio Cancer Research Associates to D. Sun. D. Sun thanks The Ohio State University for providing research resources, analysis instruments, and funding support.

Supporting Information Available: ^1H and ^{13}C NMR and HRMS spectra for **1**−**6**, **21**, and **22**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Arcamone, F. *Doxorubicin, Anticancer Antibiotics; Medicinal Chemistry Series*; Academic Press: New York, 1981; Vol. 17, pp 1−369.
(2) Gottesman, M. M. How cancer cells evade chemotherapy: sixteenth Richard and Hinda Rosenthal Foundation Award Lecture. *Cancer Res.* **1993**, *53*, 747−754.
(3) Kaye, S. B. The multidrug resistance phenotype. *Br. J. Cancer* **1988**, *58*, 691−694.
(4) Hirose, M.; Hosoi, E.; Hamano, S.; Jalili, A. Multidrug resistance in hematological malignancy. *J. Med. Invest.* **2003**, *50*, 126−135.
(5) di Bartolomeo, S.; Spinedi, A. Differential chemosensitizing effect of two glucosylceramide synthase inhibitors in hepatoma cells. *Biochem. Biophys. Res. Commun.* **2001**, *288*, 269−274.
(6) Lee, V. H. Membrane transporters. *Eur. J. Pharm. Sci.* **2000**, *11* (Suppl. 2), S41−S50.
(7) Hosoya, K. I.; Kim, K. J.; Lee, V. H. Age-dependent expression of P-glycoprotein gp170 in Caco-2 cell monolayers. *Pharm. Res.* **1996**, *13*, 885−890.
(8) Efferth, T. The human ATP-binding cassette transporter genes: from the bench to the bedside. *Curr. Mol. Med.* **2001**, *1*, 45−65.
(9) Lepage, P.; Gros, P. Structural and functional aspects of P-glycoproteins and related transport proteins. *Curr. Opin. Nephrol. Hypertens.* **1993**, *2*, 735−743.
(10) Germann, U. A. Molecular analysis of the multidrug transporter. *Cytotechnology* **1993**, *12*, 33−62.
(11) Arias, I. M.; Gatmaitan, Z.; Mazzanti, R.; Shu, H.; Kumamoto, Y. Structure and function of P-glycoprotein in the normal liver and intestine. *Princess Takamatsu Symp.* **1990**, *21*, 229−239.
(12) Yu, D. K. The contribution of P-glycoprotein to pharmacokinetic drug−drug interactions. *J. Clin. Pharmacol.* **1999**, *39*, 1203−1211.

(13) Yu, D. S.; Chang, S. Y.; Ma, C. P. The correlation of membranous glycoprotein-gp-170, cytoplasmic glutathione and glucose-6-phosphate dehydrogenase levels with multidrug resistance in transitional cell carcinoma cell lines of the urinary tract. *J. Urol.* **1997**, *157*, 727−731.
(14) Yu, D. S.; Chang, S. Y.; Ma, C. P. The expression of mdr-1-related gp-170 and its correlation with anthracycline resistance in renal cell carcinoma cell lines and multidrug-resistant sublines. *Br. J. Urol.* **1998**, *82*, 544−7.
(15) Chiou, W. L.; Chung, S. M.; Wu, T. C.; Ma, C. A comprehensive account on the role of efflux transporters in the gastrointestinal absorption of 13 commonly used substrate drugs in humans. *Int. J. Clin. Pharmacol. Ther.* **2001**, *39*, 93−101.
(16) Israel, M.; Murray, R. J. Adriamycin analogs. 1. Preparation and antitumor evaluation of 7-*O*-(b-D-glucosaminyl)daunomycinone and 7-*O*-(b-D-glucosaminyl)adriamycinone and their *N*-trifluoroacetyl derivatives. *J. Med. Chem.* **1982**, *25*, 24−28.
(17) Roche, C. J.; Berkowitz, D.; Sulikowski, G. A.; Danishefsky, S. J.; Crothers, D. M. Binding affinity and site selectivity of daunomycin analogs. *Biochemistry* **1994**, *33* (4), 936−942.
(18) Capranico, G.; Supino, R.; Binaschi, M.; Capolongo, L.; Grandi, M.; Suarato, A.; Zunino, F. Influence of structural modifications at the 3′ and 4′ positions of doxorubicin on the drug ability to trap topoisomerase II and to overcome multidrug resistance. *Mol. Pharmacol.* **1994**, *45*, 908−915.
(19) Arlin, Z.; Case, D. C., Jr.; Moore, J.; Wiernik, P.; Feldman, E.; Saletan, S.; Desai, P.; Sia, L.; Cartwright, K., Randomized multicenter trial of cytosine arabinoside with mitoxantrone or daunorubicin in previously untreated adult patients with acute nonlymphocytic leukemia (ANLL). Lederle Cooperative Group. *Leukemia* **1990**, *4*, 177−183.
(20) Berman, E.; Heller, G.; Santorsa, J.; McKenzie, S.; Gee, T.; Kempin, S.; Gulati, S.; Andreeff, M.; Kolitz, J.; Gabrilove, J.; et al., Results of a randomized trial comparing idarubicin and cytosine arabinoside with daunorubicin and cytosine arabinoside in adult patients with newly diagnosed acute myelogenous leukemia. *Blood* **1991**, *77*, 1666−1674.
(21) Wiernik, P. H.; Banks, P. L.; Case, D. C., Jr.; Arlin, Z. A.; Periman, P. O.; Todd, M. B.; Ritch, P. S.; Enck, R. E.; Weitberg, A. B. Cytarabine plus idarubicin or daunorubicin as induction and consolidation therapy for previously untreated adult patients with acute myeloid leukemia. *Blood* **1992**, *79*, 313−319.
(22) Arcamone, F.; Animati, F.; Capranico, G.; Lombardi, P.; Pratesi, G.; Manzini, S.; Supino, R.; Zunino, F. New developments in antitumor anthracyclines. *Pharmacol. Ther.* **1997**, *76*, 117−124.
(23) Arcamone, F. M. From the pigments of the actinomycetes to third generation antitumor anthracyclines. *Biochimie* **1998**, *80*, 201−206.
(24) Binaschi, M.; Bigioni, M.; Cipollone, A.; Rossi, C.; Goso, C.; Maggi, C. A.; Capranico, G.; Animati, F. Anthracyclines: selected new developments. *Curr. Med. Chem.: Anti-Cancer Agents* **2001**, *1*, 113−130.
(25) Temperini, C.; Cirilli, M.; Aschi, M.; Ughetto, G. Role of the amino sugar in the DNA binding of disaccharide anthracyclines: crystal structure of the complex MAR70/d(CGATCG). *Bioorg. Med. Chem.* **2005**, *13*, 1673−1679.
(26) Temperini, C.; Messori, L.; Orioli, P.; Di Bugno, C.; Animati, F.; Ughetto, G., The crystal structure of the complex between a disaccharide anthracycline and the DNA hexamer d(CGATCG) reveals two different binding sites involving two DNA duplexes. *Nucleic Acids Res.* **2003**, *31*, 1464−1469.
(27) Arimondo, P. B.; Helene, C. Design of new anti-cancer agents based on topoisomerase poisons targeted to specific DNA sequences. *Cur. Med. Chem.: Anti-Cancer Agents* **2001**, *1*, 219−235.
(28) Bailly, C.; Colson, P.; Houssier, C.; Rodrigues-Pereira, E.; Prudhomme, M.; Waring, M. J. Recognition of specific sequences in DNA by a topoisomerase I inhibitor derived from the antitumor drug rebeccamycin. *Mol. Pharmacol.* **1998**, *53*, 77−87.
(29) Bailly, C.; Qu, X.; Graves, D. E.; Prudhomme, M.; Chaires, J. B. Calories from carbohydrates: energetic contribution of the carbohydrate moiety of rebeccamycin to DNA binding and the effect of its orientation on topoisomerase I inhibition. *Chem. Biol.* **1999**, *6*, 277−286.
(30) Gewirtz, D. A. A critical evaluation of the mechanisms of action proposed for the antitumor effects of the anthracycline antibiotics adriamycin and daunorubicin. *Biochem. Pharmacol.* **1999**, *57*, 727−741.
(31) Wang, J. C., DNA topoisomerases. *Annu. Rev. Biochem.* **1996**, *65*, 635−692.
(32) Zhang, G.; Fang, L.; Zhu, L.; Aimiuwu, J. E.; Shen, J.; H., C.; Muller, M. T.; Lee, G. E.; Sun, D.; Wang, P. G. Syntheses and biological activities of disaccharide daunorubicins. *J. Med. Chem.* **2005**, *48*, 5269−5278.
(33) Zhang, G.; Shen, J.; Cheng, H.; Zhu, L.; Fang, L.; Luo, S.; Muller, M. T.; Lee, G. E.; Wei, L.; Du, Y.; Sun, D.; Wang, P. G. Syntheses and biological activities of rebeccamycin analogues with uncommon sugars. *J. Med. Chem.* **2005**, *48*, 2600−2611.
(34) Alper, P. B.; Hung, S.-C.; Wong, C.-H. Metal catalyzed diazo transfer for the synthesis of azides from amines. *Tetrahedron Lett.* **1996**, *37*, 6029−6032.
(35) Qian, J.; Zhou, C. H.; Qian, Z.; Nan, F. J.; Ye, Q. Z. Development of a K562 cell-based assay for screening anticancer agents. *Acta Pharmacol. Sin.* **2001**, *22*, 821−826.
(36) Chen, Z.; Eggert, U. S.; Dong, S. D.; Shaw, S. J.; Sun, B.; LaTour, J. V.; Kahne, D. Structural requirements for VanA activity of vancomycin analogues. *Tetrahedron Lett.* **2002**, *58*, 6585−6594.
(37) Wang, M. J.; Liu, Y. X.; Li, X. L.; Shi, J.; Liu, S. L.; Zheng, D. X. Chemotherapeutic drugs enhanced rsTRAIL tumoricidal activity. *Zhongguo Yixue Kexueyuan Xuebao* **2004**, *26*, 524−528.

JM050916M