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Bioorganic & Medicinal Chemistry 13 (2005) 6381–6387
Bioorganic & Medicinal Chemistry

Syntheses and biological activities of daunorubicin analogs with uncommon sugars

Lizhi Zhu,[a] Xianhua Cao,[b] Wenlan Chen,[a] Guisheng Zhang,[a] Duxin Sun[b,*] and Peng George Wang[a,*]

[a]*Department of Chemistry and Biochemistry, The Ohio State University, Columbus, OH 43210, USA*
[b]*Division of Pharmaceutics, College of Pharmacy, The Ohio State University, Columbus, OH 43210, USA*

Received 7 June 2005; accepted 24 June 2005
Available online 1 August 2005

Abstract—To study the effects of the sugar structure on the activity of anthracycline against cancer cells, six daunorubicin analogs containing different uncommon sugars were synthesized. Their cytotoxicities were tested against colon cancer cells by MTS assay. The results showed that the aglycon without sugar moiety has 70–100-fold lower activity against cancer cells than daunorubicin derivatives with various uncommon sugars. It suggests that the sugar structure in daunorubicin plays a critical role in determining its anticancer activity. In the compounds with various sugars, the 4′-OH of the sugar is an important determinant for their activity, while the *axial*-3′-substituent in the sugar interferes with the binding of daunorubicins to DNA. Therefore, 2,6-dideoxy sugars are a better choice for generating biologically active daunorubicin analogs than 6-deoxysugars, 2,3,6-trideoxysugars, or 2,3,4,6-tetradeoxysugars.

1. Introduction

The anthracycline quinone antibiotics daunorubicin and doxorubicin (adriamycin) have been used clinically since the 1970s.[1] So far anthracyclines are among the most effective drugs against a variety of human solid tumors (Fig. 1).[2] The primary cellular target for anthracyclines is DNA topoisomerase II (topo II).[3] The well-accepted mechanism of action is through interaction with DNA topo II by stabilization of the topo II–DNA–drug ternary complex. It is noted that DNA binding and interaction are necessary, but not sufficient, for topo II poisoning.[4] In fact, the external (non-intercalating) moieties involving the sugar structure and the cyclohexane ring A (Fig. 1) are recognized as crucial moieties for therapeutic efficacy of anthracyclines. The sugar moiety of anthracyclines lies in the minor groove of the DNA and contacts base pairs.[5,6] This mechanisms of anthracycline to intercalate DNA causes DNA breakage, produces oxidative stress, and triggers cell apoptosis.[7] The

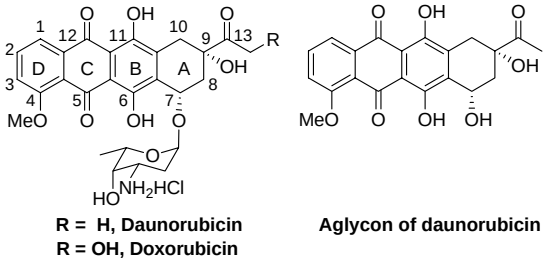

Figure 1. Daunorubicin, doxorubicin, and aglycon of daunorubicin.

major problems associated with anthracycline drugs are cardiotoxicity and drug resistance mediated by the multidrug resistance gene (MDR).[8,9] Many researchers have explored ways to modify the structure of anthracycline to generate various analogs to reduce the side effects and reverse multidrug resistance; however, these efforts only have had limited success.[10,11]

In fact, a wealth of data has been published on the structure–activity relationship (SAR) of DOX's aglycon portion and carbohydrate moiety over the past 30 years. The results show that the carbohydrates moiety is a critical component of anthracycline anticancer activity. For instance, the aglycon itself is nearly inactive when tested

Keywords: Daunorubicin; Glycosylation; Structure–activity relationship (SAR); Anticancer.
* Corresponding authors. Tel.: +1 614 292 9884; fax: +1 614 688 3106 (P.G.W.); tel.: +1 614 292 4381; fax: +1 614 292 7766 (D.S.); e-mail addresses: sun.176@osu.edu; wang.892@osu.edu

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doi:10.1016/j.bmc.2005.06.053

as an anticancer agent. It is well established that the sugar plays a vital role in the binding of anthracycline with the DNA topo II complex and the orientation of sugar is critical for the binding. α-Glycoside is assumed as a common motif for anthracyclines with high anticancer activities, while the β-anomer is even less active than the aglycon itself.[12,13] Chemical and configurational modifications on the sugar moiety, which mainly focus on the 3′- and 4′-positions, markedly determine the difference in anticancer activity, toxicity, the sequence specificity of the DNA break sites, and other cellular process/pathway besides topo II.[14]

Although the chemical modifications of anthracycline so far support the hypothesis that the sugar structure is a very important determinant for its anticancer activity, the diversity of sugar structure in that study is rather limited by the unavailability of the various uncommon sugars. In our research, we have generated an uncommon sugar library, which provides us a powerful tool in the systematic SAR investigation of sugar structures for anticancer activity. We synthesized six daunorubicin analogs, with different uncommon sugars attached, to explore the structure–activity relation of the uncommon sugar moiety of daunorubicin analogs. Their activity against colon cancer cells SW 620 was measured by MTS assay (tetrazolium[3-(4,5-dimethy-thiazol-2-yl)]-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt). Indeed, different uncommon sugars in daunorubicin analogs show distinct cytotoxicity against cancer cells.

2. Results and discussion

2.1. Chemistry

Previous research has indicated that the linkage between the sugar moiety and the aglycon is very important. Only the α-linked daunorubicin monosaccharide analogs are biologically active. Therefore, the uncommon sugar has to be introduced stereoselectively. Glycosyl sulfide donors are believed to be the best choice for the α-glycosylation of 2,6-dideoxy sugars in this case. Thiolglycosides have been introduced as glycosyl donors because they are stable to normal protecting group manipulation encountered in oligosaccharide syntheses.[15–17] Various activating systems can be applied, such as, MeOTf, NBS, BSP/Tf_2O/TTBP (2,4,6-tri-*tert*-butylpyrimidine),[18] IDCP, NIS/TfOH, and NIS/TMSOTf. For 2-deoxy sugars, without the stereodirecting ability of 2-substituents, strategies to prepare 2-deoxyglycosides selectively in high α- or β-anomeric forms rely heavily on indirect sequences from glycols or latent 2-deoxysugars.[19,20] In addition, direct and efficient α-selective glycosylations are difficult to realize.[15,21] Recently, Hirama and co-workers reported a direct and efficient α-selective glycosylation protocol for the kedarcidin sugar and L-mycarose using $AgPF_6$ as a remarkable activator of 2-deoxythioglycosides.[21]

In this paper, we report a concise promoter system $AgPF_6$/TTBP for syntheses of α-linked monosaccharide daunorubicin analogs. The monosaccharide daunorubicins were synthesized using thiophenyl glycoside of 2,6-dideoxysugar donors in the presence of $AgPF_6$ and TTBP. This protocol produced the desired α-glycosidic bond with good yields. Taking advantage of our uncommon sugar library, we synthesized six daunorubicin analogs (Fig. 2) using this glycosylation method. The biological activities of these compounds have been tested with colon cancer cell line SW620.

The syntheses of daunorubicin analogs started with the preparation of the aglycon. The aglycon (**DNR-A**) was readily obtained from the hydrolysis of daunorubicin hydrochloride with dilute HCl at 90 °C for 1 h. **DNR-A** was obtained after filtration as a red powder (Scheme 1) and was used directly for further glycosylations. The corresponding sugar donors were prepared from their corresponding precursors (compounds **1–6**). 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 aglycon moiety in the anthracycline not to be affected in the final deprotection procedures. As shown in Scheme 1, after treatment with phenylthiol in the presence of $BF_3{\cdot}Et_2O$ at 0 °C for 4 h, the desired sugar donors (compounds **7–12**) were obtained in excellent yields (>94%). The thiolglycosides were obtained as a mixture of α- and β-isomers. Since both the isomers are able to be used for the glycosylation to produce the desired α-linked daunorubicin derivatives, separation of them is not necessary. Previous research[22] found that the tertiary hydroxyl group would not affect further glycosylation, thus compound **2** was directly converted to the sugar donor **8**. With the aglycon and the sugar donors in hand, the glycosylation was performed subsequently.

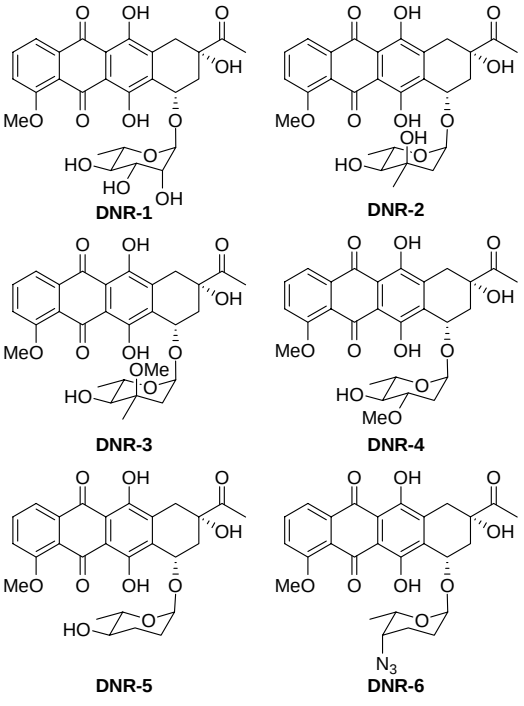
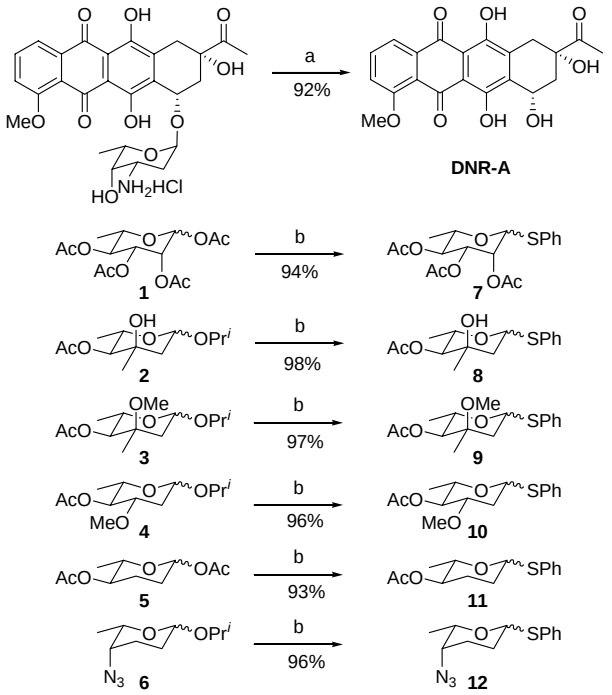


Figure 2. Synthetic daunorubicin derivatives.



Scheme 1. Reagents and conditions: (a) 0.2 M HCl, 90 °C, 1 h; (b) PhSH, $BF_3{\cdot}Et_2O/CH_2Cl_2$, 0 °C, 4 h.

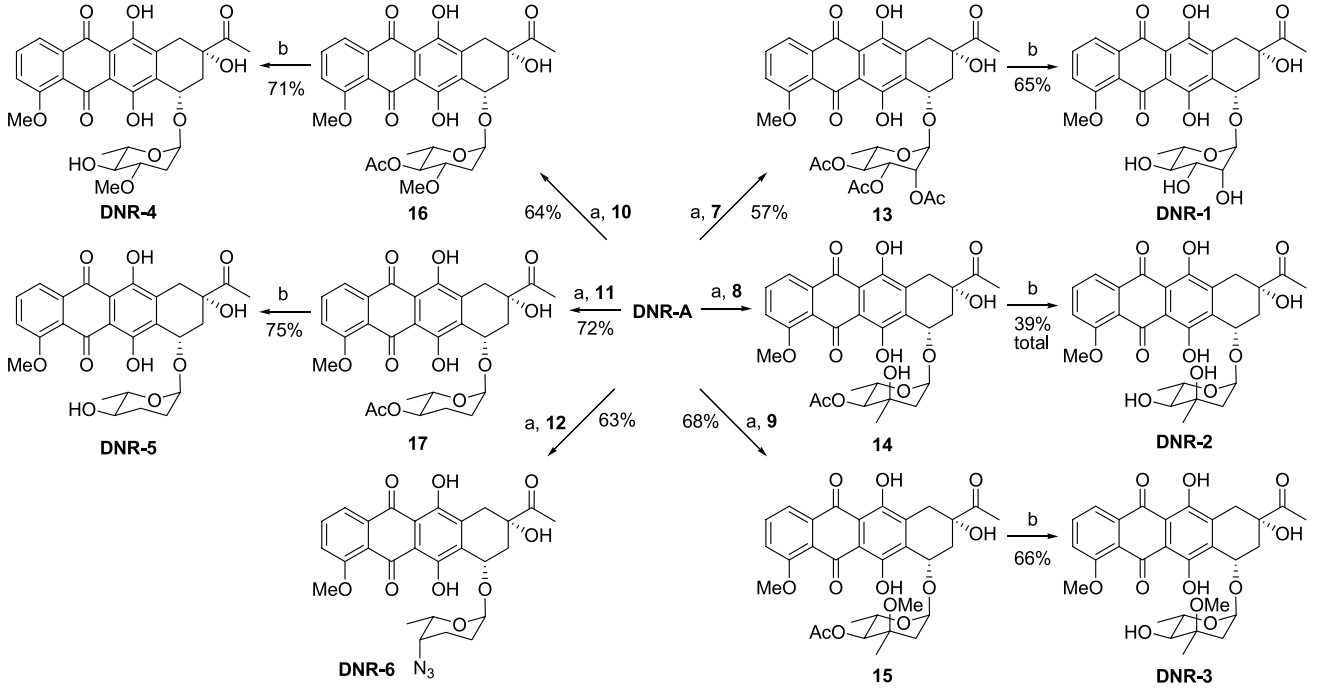
The mixture of aglycon (**DNR-A**) and sugar donors **7**–**12**, in the presence of TTBP and 4 Å molecular sieves, was treated with $AgPF_6$ at 0 °C for 4 h to give the glycosylated products **13**–**17** and **DNR-6** in around 60% yields (Scheme 2). The ^1H NMR data indicated that the desired α-linkage was formed predominantly (α:β > 9:1). However, glycosylation of **DNR-A** with sugar donor **10** produced a mixture of both isomers (α:β = 3:1). In addition, compound **14** was unable to be separated from the unreacted aglycon (same R_f value using 100:1 CH_2Cl_2/MeOH as eluent). Finally, the pure **DNR-2** was obtained after the deprotection step in 39% overall yield for two steps. Compounds **13**–**17** were treated with 0.1 M NaOH for 6 h, and the acetyl groups were successfully removed to allow the isolation of the desired **DNR-1**–**DNR-5** in 65–75% yields.

2.2. Cytotoxicity

The cytotoxicity of these compounds was examined in colon cancer cell line SW620 cells (Fig. 3) with MTS assay. Five thousand cells were incubated with 0.001–10 μM DNR and its derivatives for 72 h. Then 20 μL MTS/PMS assay solution was added to each well and the absorbance was recorded. The cell survival was calculated as a percentage of the cell control group (without treatment with drug). The IC_{50} of all these compounds against colon cancer cell line SW620 are summarized in Table 1. The IC_{50} values were calculated by WinNonlin 4.1 (Pharsight) from the dose–response curves of percentage of cell growth with the model: $E = E_{max} - (E_{max} - E_0) \times [C/(C + EC_{50})]$.

The results showed that the aglycon, **DNR-A**, exhibited 70–100-fold lower cytotoxicity than daunorubicin derivatives with various uncommon sugars. This suggests that the sugar structure in daunorubicin plays a critical role in determining its anticancer activity. Indeed, this finding has also been confirmed by other reports.[20,21]

Compound **DNR-4** with a 3′-OMe terminal 2,6-dideoxysugar showed very potent cytotoxicity with IC_{50} of 104 nM (Fig. 3A). Importantly, compared to compounds **DNR-2** and **DNR-3** (with an *axial*-3′-OMe or



Scheme 2. Reagents and conditions: (a) TTBP, $AgPF_6/CH_2Cl_2$, 4 Å MS, 0 °C, 4 h; (b) 0.1 M NaOH/THF, 0 °C, 6 h.

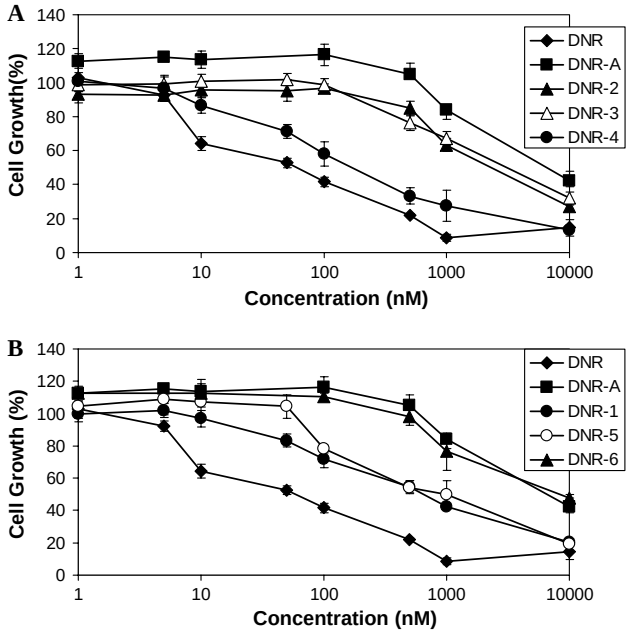


Figure 3. Cytotoxicity of compounds **DNR**, **DNR-A**, **DNR-2–4** (A) and compounds **DNR**, **DNR-A**, **DNR-1**, **DNR-5–6** (B) against colon cancer SW620 cells. Five thousand cells were incubated with 0.001–10 μM tested compounds for 72 h. MTS/PMS solution was added and further incubated with cells for 2.5–3 h. Then the absorbance was recorded at 490 nM. The survival rate was expressed as percentage of control group.

axial-3′-OH group), **DNR-4** (with an *equatorial*-3′-OMe group) showed 10–20-fold higher anticancer activity. This suggested that the *axial*-3′-substituent in the sugar (such as in compounds **DNR-2** and **DNR-3**) may interfere daunorubicin binding to DNA.

Compounds **DNR-1** and **DNR-5** with an *equatorial*-4′-OH showed similar activity, while **DNR-6** (substituted 4′-OH with an *axial*-4′-N_3 in the sugar moiety), which is similar to the aglycon **DNR-A**, lost its cytotoxicity (Fig. 3B). This suggests that the 4′-OH in the sugar may also be important for sugar containing anthracycline as an anticancer agent. Further SAR studies are required to clarify this finding.

From the published results,[4] it is found that the α-face of the sugar is lying toward the DNA base pairs when binding to DNA. Therefore, increasing the size of the α-face will decrease the binding ability of the sugar moiety to DNA, thus decreasing cytotoxicity decreases. The IC_{50} values of **DNR-1**, **DNR-4**, **DNR-5**, and **DNR-6** showed that compound **DNR-4** had the best activity followed by **DNR-1**, **DNR-5**, and **DNR-6**. This tendency suggests that the 2,6-dideoxy sugars are better choices for making biologically active daunorubicin analogs than 6-deoxysugars, 2,3,6-trideoxysugars, or 2,3,4,6-tetradeoxysugars. These data also indicate that the sugar moiety is not just simply lying in the minor groove of the DNA, the functional group of sugar rather interact with the base pairs for the biological activity of daunorubicin analogs.

3. Conclusions

In summary, six daunorubicin analogs with different uncommon sugars were synthesized. Their biological activities were tested against colon cancer cell line SW620 cells. The results showed that the aglycon of anthracycline without sugar moiety decreased its activity by 70–100-fold. This suggests that the sugar moiety in these analogs plays a crucial role in determining the cytotoxicities of these compounds. In addition, different modifications in sugar structures of anthracylcine indicate that the *axial*-3′-substituent in sugar (such as in compounds **DNR-2** and **DNR-3**) interferes with the binding of daunorubicin to DNA, while the 4′-OH of sugar is a critical determinant for the activity of daunorubicin. Therefore, increasing the hindrance of the α-face of the sugar leads to decreased cytotoxicity against cancer cells. The 2,6-dideoxy sugars are the best choice to modify daunorubicin compared with 6-deoxysugar, 2,3,6-trideoxysugars, and 2,3,4,6-tetradeoxysugars.

4. Experimental

4.1. Chemistry

4.1.1. General information. All the solvents were dried with solvent-purification system from Innovative Technology, Inc. All the reagents were purchased from commercial sources and used without further purification. Analytical TLC was carried out on silica gel 60 F_{254} aluminum-backed plates (E. Merck). The preparation TLC was carried out on silica gel 60 F_{254} plates (EMD Chemicals, Inc. 20 × 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 were recorded at the indicated field strengths with the indicated solvent. The high-resolution mass spectra were obtained at The Ohio State University Campus Chemical Instrumentation Center.

4.1.2. General procedure for preparation of the sugar donor. To a stirred solution of acetated sugar **3** (0.2 g, 0.77 mmol) in CH_2Cl_2 (5.0 mL) at 0 °C, phenylthiol (0.1 mL, 0.98 mmol) and $BF_3{\cdot}Et_2O$ (0.11 mL, 0.87 mmol) were added. The reaction mixture was allowed to warm slowly to room temperature. After stirring for 4 h, the reaction mixture was quenched with aqueous NaOH (0.1 N, 10 mL). 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

Table 1. Cytotoxicity (IC_{50}) of synthesized compounds against colon cancer SW620 cells

Compounds	DNR-1	DNR-2	DNR-3	DNR-4	DNR-5	DNR-6	DNR-A	DNR
IC_{50} in SW620 (nM)	264.6	>1000	>1000	104	350	>1000	>2000	33.4

column chromatography (50:1 petroleum ether–EtOAc as eluent) and provided donor **9** as syrup (0.23 g, 97% yield).

4.1.3. General procedure for glycosylation. The mixture of donor **9** (0.17 g, 0.55 mmol), aglycon (0.29 g, 0.75 mmol), 4 Å MS, and TTBP (0.55 g, 2.2 mmol) in CH_2Cl_2 (5.0 mL) was stirred (avoid light) at room temperature for 1.5 h. Then the mixture was cooled to 0 °C and $AgPF_6$ (0.43 g, 1.7 mmol) was added. After stirring for 6 h at 0 °C, pyridine (2 mL) was added, and the reaction mixture was stirred for a further 0.5 h at 0 °C. The mixture was filtered through Celite, and the residue was thoroughly washed with CH_2Cl_2. The crude product was purified by flash column chromatography (100:1 dichloromethane–methanol as eluent) to give compound **15** (220 mg, 68% yield) as a red powder.

4.1.4. General procedure for deprotection. To a solution of compound **15** (180 mg, 0.3 mmol) in THF (5 mL) at 0 °C, aqueous NaOH (0.1 M, 70 mL) was added. After stirring at 0 °C for 6 h, the reaction mixture was neutralized with citric acid (10 wt %). The aqueous layer was extracted with CH_2Cl_2, and the combined organic layers were washed with saturated $NaHCO_3$ and water. After drying with $MgSO_4$ and concentrating under vacuum, the crude product was purified by flash column chromatography (80:1 dichloromethane–methanol) to give **DNR-3** (110 mg, 66% yield) as a red powder.

4.2. Biology

4.2.1. Cell culture. Colon cancer cell line SW620 was cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% non-essential 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.

4.2.2. Cytotoxicity of synthesized compounds against cancer cells by MTS assay. Colon cancer SW620 cells (5000) 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 incubation, tetrazolium[3-(4,5-dimethy-thiazol-2-yl)]-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2*H*-tetrazolium, inner salt (MTS, 2 mg/mL), and phenazine methosulfate (PMS, 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 carbohydrate–drug conjugates for cytotoxicity were calculated by WinNonlin 4.1 (Pharsight) from the dose–response curves of percentage of cell growth versus control (no compound added).

4.3. Spectroscopic data

4.3.1. Phenylthio-4-azido-2,3,4,6-tetradeoxy-α-L-*erythro*-hexopyranoside (12). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.45 (d, J = 7.7 Hz, 2H), 7.29 (t, J = 7.3 Hz, 2H), 7.22 (t, J = 7.4 Hz, 1H), 5.62 (d, J = 5.4 Hz, 1H), 4.49 (m, 1H), 3.53 (s, 1H), 2.37 (m, 1H), 2.14 (tt, J = 14.1, 3.8 Hz, 1H), 2.04 (dd, J = 14.3, 3.4 Hz, 1H), 1.82 (dt, J = 14.2, 3.2 Hz, 1H), 1.23 (d, J = 6.5 Hz, 3H); ^{13}C NMR (125.69 MHz, $CDCl_3$) δ 135.2, 130.9 (2C), 128.9 (2C), 126.9, 84.5, 66.4, 60.0, 25.3, 24.6, 17.8. The parent ion of this compound was not observed by HRMS, due to the decomposition of this compound under the analysis conditions.

4.3.2. 7-(2,3,4-*O*-Triacetyl-α-rhamnosyl)daunorubicinone (13). ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.86 (s, 1H), 13.14 (s, 1H), 7.93 (d, J = 7.7 Hz, 1H), 7.72 (t, J = 8.2 Hz, 1H), 7.34 (d, J = 8.5 Hz, 1H), 5.33 (d, J = 1.5 Hz, 1H), 5.31 (m, 1H), 5.26 (d, J = 2.1 Hz, 1H), 5.10 (s, 1H), 5.08 (s, 1H), 4.07 (m, 1H), 4.03 (s, 3H), 3.19 (dd, J = 18.5, 1.0 Hz, 1H), 2.85 (d, J = 18.7 Hz, 1H), 2.41 (s, 3H), 2.32 (d, J = 15.0 Hz, 1H), 2.16 (dd, J = 14.8, 4.3 Hz, 1H), 2.15 (s, 3H), 2.04 (m, 1H), 2.02 (s, 3H), 1.91 (s, 3H), 1.27 (d, J = 6.3 Hz, 3H); ^{13}C NMR (125.69 MHz, $CDCl_3$) δ 211.6, 186.7, 186.6, 170.0, 169.7, 169.6, 161.0, 156.1, 155.4, 137.3, 135.3, 134.3, 132.9, 120.7, 119.7, 118.5, 111.5 (2C), 100.5, 76.4, 70.8, 70.4, 69.4, 69.1, 67.7, 56.6, 35.1, 33.3, 24.8, 20.9, 20.8, 20.6, 17.4; HRMS (ESI) *m*/*z* calcd for $C_{33}H_{34}O_{15}Na$ (M+Na$^+$) 693.1790, found 693.1768.

4.3.3. 7-(4-*O*-Acetyl-α-cladinosyl)daunorubicinone (15). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.83 (s, 1H), 13.11 (s, 1H), 7.92 (d, J = 7.6 Hz, 1H), 7.71 (t, J = 8.1 Hz, 1H), 7.33 (d, J = 8.5 Hz, 1H), 5.35 (d, J = 4.5 Hz, 1H), 5.27 (s, 1H), 5.15 (m, 1H), 4.73 (d, J = 10.0 Hz, 1H), 4.43 (m, 1H), 4.04 (s, 3H), 3.09 (d, J = 20.0 Hz, 1H), 3.09 (s, 3H), 2.78 (d, J = 19.0 Hz, 1H), 2.41 (s, 3H), 2.31 (d, J = 14.5 Hz, 1H), 2.19 (d, J = 15.1 Hz, 1H), 2.13 (s, 3H), 2.03 (m, 1H), 1.63 (m, 1H), 1.17 (d, J = 6.3 Hz, 3H), 1.06 (s, 3H); ^{13}C NMR (125.69 MHz, $CDCl_3$) δ 213.0, 186.9, 186.3, 170.6, 160.9, 156.3, 155.9, 135.5, 135.4, 134.8, 134.6, 120.8, 119.6, 118.2, 111.1, 110.9, 99.7, 78.4, 76.4, 73.3, 69.5, 63.3, 56.6, 50.0, 37.1, 34.8, 33.3, 24.9, 20.9, 20.7, 17.2; HRMS (ESI) *m*/*z* calcd for $C_{31}H_{34}O_{12}Na$ 621.1942 (M+Na$^+$), found 621.1913.

4.3.4. 7-(4-*O*-Acetyl-α-oleaudrosyl)daunorubicinone (16). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.92 (s, 1H), 13.16 (s, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.74 (t, J = 8.3 Hz, 1H), 7.35 (d, J = 8.2 Hz, 1H), 5.49 (d, J = 3.1 Hz, 1H), 5.20 (d, J = 2.2 Hz, 1H), 4.67 (t, J = 9.3 Hz, 1H), 4.52 (s, 1H), 4.04 (s, 3H), 4.93 (m, 1H), 3.40 (m, 1H), 3.24 (s, 3H), 3.15 (dd, J = 18.8, 1.8 Hz, 1H), 2.84 (d, J = 18.7 Hz, 1H), 2.40 (s, 3H), 2.31 (d, J = 14.8 Hz, 1H), 2.25 (m, 1H), 2.09 (m, 1H), 2.07 (s, 3H), 1.66 (m, 1H), 1.19 (d, J = 6.5 Hz, 3H); ^{13}C NMR (125.69 MHz, $CDCl_3$) δ 211.7, 186.9, 186.6, 170.2, 161.0, 156.4, 155.7, 135.7, 135.4, 134.4, 134.0, 120.8, 119.8, 118.4, 111.4, 111.3, 101.0, 76.7, 75.8, 75.5, 70.2, 67.2, 56.9, 56.7, 35.0, 34.7, 33.3, 24.8, 21.1, 17.5; HRMS (ESI) *m*/*z* calcd for $C_{30}H_{32}O_{12}Na$ 607.1786 (M+Na$^+$), found 607.1799.

4.3.5. 7-(4-*O*-Acetyl-α-amicetosyl)daunorubicinone (17). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.97 (s, 1H), 13.29 (s, 1H), 8.02 (d, J = 7.7 Hz, 1H), 7.76 (t, J = 8.1 Hz, 1H),

7.37 (d, J = 8.5 Hz, 1H), 5.39 (m, 1H), 5.32 (m, 1H), 4.81 (s, 1H), 4.52 (m, 1H), 4.07 (s, 3H), 3.96 (m, 1H), 3.24 (dd, J = 18.9, 1.9 Hz, 1H), 2.97 (d, J = 18.8 Hz, 1H), 2.43 (s, 3H), 2.38 (m, 1H), 2.10 (dd, J = 14.8, 4.1 Hz, 1H), 2.04 (s, 3H), 1.90 (m, 1H), 1.82 (m, 2H), 1.59 (m, 1H), 1.21 (d, J = 6.3 Hz, 3H); ^{13}C NMR (125.69 MHz, $CDCl_3$) δ 212.0, 187.2, 186.8, 170.3, 161.1, 156.5, 156.0, 135.7, 135.6, 134.6, 134.3, 121.1, 119.8, 118.4, 111.5, 111.4, 99.8, 76.9, 73.2, 69.5, 68.0, 56.7, 35.0, 33.5, 29.7, 28.9, 24.8, 21.2, 17.8; HRMS (ESI) *m/z* calcd for $C_{29}H_{30}O_{11}Na$ 577.1680 ($M+Na^+$), found 577.1688.

4.3.6. 7-(α-Rhamnosyl)daunorubicinone (DNR-1). ^{1}H NMR (500 MHz, DMSO-d_6) δ 14.03 (s, 1H), 13.24 (s, 1H), 7.90 (m, 2H), 7.64 (m, 1H), 5.45 (s, 1H), 5.02 (s, 1H), 4.95 (s, 1H), 4.70 (dd, J = 13.4, 5.1 Hz, 2H), 4.41 (d, J = 5.6 Hz, 1H), 3.98 (s, 3H), 3.77 (m, 1H), 3.58 (s, 1H), 3.33 (m, 1H), 3.22 (m, 1H), 2.99 (d, J = 17.7 Hz, 1H), 2.87 (d, J = 17.9 Hz, 1H), 2.55 (s, 1H), 2.24 (s, 3H), 2.16 (m, 1H), 1.18 (d, J = 6.2 Hz, 3H); ^{13}C NMR (125.69 MHz, DMSO-d_6) δ 212.0, 187.0, 186.9, 161.3, 156.6, 150.1, 136.7, 136.6, 136.0, 135.2, 135.1, 120.5, 120.2, 119.5, 111.3, 111.2, 104.2, 75.8, 72.3, 71.1, 71.0, 69.6, 57.1, 37.1, 32.1, 24.4, 18.2; HRMS (ESI) *m/z* calcd for $C_{27}H_{28}O_{12}Na$ 567.1743 ($M+Na^+$), found 567.1465.

4.3.7. 7-(α-Mycarosyl)daunorubicinone (DNR-2). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.87 (s, 1H), 13.18 (s, 1H), 7.95 (d, J = 7.6 Hz, 1H), 7.71 (t, J = 8.1 Hz, 1H), 7.32 (d, J = 8.5 Hz, 1H), 5.40 (d, J = 3.5 Hz, 1H), 5.15 (s, 1H), 4.50 (s, 1H), 4.03 (s, 3H), 3.93 (m, 1H), 3.70 (s, 1H), 3.01 (m, 3H), 2.37 (s, 3H), 2.36 (s, 1H), 2.29 (d, J = 14.5 Hz, 1H), 2.09 (dd, J = 14.6, 4.1 Hz, 1H), 1.99 (d, J = 14.6 Hz, 1H), 1.75 (dd, J = 14.6, 4.1 Hz, 1H), 1.32 (d, J = 6.2 Hz, 3H), 1.17 (s, 3H); ^{13}C NMR (125.69 MHz, $CDCl_3$) δ 211.2, 186.9, 186.5, 161.0, 156.4, 155.6, 135.6, 135.5, 134.9, 134.0, 120.9, 119.8, 118.4, 111.4, 111.1, 99.8, 76.7, 76.0, 69.6, 68.0, 66.4, 56.7, 41.2, 35.1, 32.8, 26.1, 24.2, 17.9; HRMS (ESI) *m/z* calcd for $C_{28}H_{30}O_{11}Na$ 565.1680 ($M+Na^+$), found 565.1664.

4.3.8. 7-(α-Cladinosyl)daunorubicinone (DNR-3). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.84 (s, 1H), 13.14 (s, 1H), 7.93 (d, J = 7.6 Hz, 1H), 7.71 (t, J = 8.1 Hz, 1H), 7.34 (d, J = 8.3 Hz, 1H), 5.28 (d, J = 4.3 Hz, 1H), 5.20 (s, 1H), 5.12 (s, 1H), 4.05 (m, 1H), 4.04 (s, 3H), 3.05 (m, 2H), 3.00 (s, 3H), 2.81 (d, J = 18.8 Hz, 1H), 2.39 (s, 3H), 2.32 (m, 1H), 2.25 (d, J = 11.1 Hz, 1H), 2.19 (d, J = 15.4 Hz, 1H), 1.99 (dd, J = 14.7, 3.8 Hz, 1H), 1.50 (dd, J = 15.4, 4.6 Hz, 1H), 1.30 (d, J = 6.3 Hz, 3H), 1.16 (s, 3H); ^{13}C NMR (125.69 MHz, $CDCl_3$) δ 212.7, 186.9, 186.4, 160.9, 156.3, 156.0, 135.6, 135.5, 134.9, 134.7, 120.9, 119.7, 118.3, 111.1, 111.0, 99.8, 77.8, 76.6, 73.2, 69.8, 65.9, 56.6, 49.3, 35.4, 34.8, 33.2, 24.8, 21.4, 17.9; HRMS (ESI) *m/z* calcd for $C_{29}H_{32}O_{11}Na$ 579.1839 ($M+Na^+$), found 579.1856.

4.3.9. 7-(α-Oleaudrosyl)daunorubicinone (DNR-4). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.92 (s, 1H), 13.15 (s, 1H), 7.96 (d, J = 7.7 Hz, 1H), 7.73 (t, J = 8.0 Hz, 1H), 7.35 (d, J = 8.5 Hz, 1H), 5.50 (d, J = 3.7 Hz, 1H), 5.27 (s, 1H), 5.20 (m, 1H), 4.54 (s, 1H), 4.05 (s, 3H), 3.85 (m, 1H), 3.27 (m, 1H), 3.26 (s, 3H), 3.18 (t, J = 9.1 Hz, 1H), 3.13 (dd, J = 18.8, 1.9 Hz, 1H), 2.80 (d, J = 18.7 Hz, 1H), 2.39 (s, 3H), 2.32 (d, J = 14.8 Hz, 1H), 2.25 (dd, J = 13.1, 4.5 Hz, 1H), 2.08 (dd, J = 14.8, 4.2 Hz, 1H), 1.51 (m, 1H), 1.33 (d, J = 6.2 Hz, 3H); ^{13}C NMR (125.69 MHz, $CDCl_3$) δ 212.1, 186.9, 186.5, 160.9, 156.4, 155.8, 135.6, 135.4, 134.6, 134.1, 120.8, 119.7, 118.4, 111.3, 111.2, 101.3, 78.0, 75.8, 70.1, 68.9, 56.6, 56.4, 35.0, 33.9, 33.3, 24.8 (2C), 17.7; HRMS (ESI) *m/z* calcd for $C_{28}H_{30}O_{11}Na$ 565.1680 ($M+Na^+$), found 565.1706.

4.3.10. 7-(α-Amicetosyl)daunorubicinone (DNR-5). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.95 (s, 1H), 13.24 (s, 1H), 8.00 (d, J = 7.7 Hz, 1H), 7.76 (t, J = 8.1 Hz, 1H), 7.37 (d, J = 8.4 Hz, 1H), 5.38 (d, J = 3.0 Hz, 1H), 5.30 (m, 1H), 4.85 (s, 1H), 4.07 (s, 3H), 3.77 (m, 1H), 3.32 (br, 1H), 3.20 (dd, J = 18.9, 1.9 Hz, 1H), 2.92 (d, J = 18.8 Hz, 1H), 2.42 (s, 3 H), 2.38 (dt, J = 14.5, 2.1 Hz, 1H), 2.09 (dd, J = 14.8, 4.1 Hz, 1H), 1.82 (m, 3H), 1.55 (m, 2H), 1.32 (d, J = 6.2 Hz, 3H); ^{13}C NMR (125.69 MHz, $CDCl_3$) δ 212.3, 187.0, 186.6, 161.0, 156.5, 155.9, 135.6, 135.5, 134.7, 134.4, 120.9, 119.8, 118.3, 111.4, 111.2, 99.7, 76.9, 71.8, 70.9, 69.3, 56.6, 34.9, 33.5, 29.4, 27.6, 24.9, 17.8; HRMS (ESI) *m/z* calcd for $C_{27}H_{28}O_{10}Na$ 535.1575 ($M+Na^+$), found 535.1566.

4.3.11. 7-(4-Azido-2,3,4,6-tetradeoxy-α-L-*erythro*-hexopyranosyl) daunorubicinone (DNR-6). ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.94 (s, 1H), 13.26 (s, 1H), 8.01 (d, J = 7.7 Hz, 1H), 7.77 (t, J = 8.2 Hz, 1H), 7.38 (d, J = 8.4 Hz, 1H), 5.45 (m, 1H), 5.29 (d, J = 5.3 Hz, 1H), 4.75 (s, 1H), 4.19 (m, 1H), 4.08 (s, 3H), 3.52 (m, 1H), 3.21 (d, J = 19.3 Hz, 1H), 2.94 (d, J = 18.8 Hz, 1H), 2.40 (s, 3H), 2.33 (d, J = 14.5 Hz, 1H), 2.10 (dd, J = 14.8, 3.9 Hz, 1H), 1.98–1.88 (m, 3H), 1.60–1.55 (m, 1H), 1.27 (d, J = 6.5 Hz, 3H); ^{13}C NMR (125.69 MHz, $CDCl_3$) δ 211.7, 187.1, 186.7, 161.1, 156.4, 155.9, 135.7, 135.6, 134.4, 134.3, 121.0, 119.8, 118.4, 111.5, 111.3, 100.4, 76.9, 69.6, 66.5, 59.7, 56.7, 34.9, 33.5, 24.7, 23.9, 22.9, 17.9; HRMS (ESI) *m/z* calcd for $C_{27}H_{27}N_3O_9Na$ 560.1640 ($M+Na^+$), found 560.1651.

Acknowledgments

This work was supported by NSF (CH-0316806) and a fund from Michigan Life Science Corridor Fund (1632) to P. G. Wang. This work was partially supported by ACS grant IRG-98-278-04, Research Starter Grant from PhRMA Foundation, and NIP from AACP to D. Sun. The authors thank The Ohio State University for providing research resources, analysis instruments, and funding support.

References and notes

1. Weiss, R. B.; Sarosy, G.; Clagett-Carr, K.; Russo, M. *Cancer Chemother. Pharmacol.* **1986**, *18*, 185–197.
2. Arcamone, F. In *Doxorubicin, Anticancer antibiotics In Medicinal Chemistry Series*; Academic Press: New York, 1981; 17, pp 1–369.

3. Wang, J. C. *Ann. Rev. Biochem.* **1996**, *65*, 635–692.
4. Zunino, F.; Capranico, G. *Anti-Cancer Drug Des.* **1990**, *5*, 307–317.
5. Capranico, G.; Palumbo, M.; Tinelli, S.; Mabilia, M.; Pozzan, A.; Zunino, F. *J. Mol. Biol.* **1994**, *235*, 1218–1230.
6. Capranico, G.; Butelli, E.; Zunino, F. *Cancer Res.* **1995**, *55*, 312–317.
7. Aligiannis, N.; Pouli, N.; Marakos, P.; Skaltsounis, A. L.; Florent, J. C.; Perchellet, E. M.; Sperfslage, B. J.; McIlvain, C. J.; Perchellet, J. P. *J. Antibiot.* **2002**, *55*, 181–190.
8. Gottesman, M. M. *Cancer Res.* **1993**, *53*, 747–754.
9. Kaye, S. B. *Br. J. Cancer* **1988**, *58*, 691–694.
10. Garnier-Suillerot, A.; Marbeuf-Gueye, C.; Salerno, M.; Loetchutinat, C.; Fokt, I.; Krawczyk, M.; Kowalczyk, T.; Priebe, W. *Curr. Med. Chem.* **2001**, *8*, 51–64.
11. Lehne, G. *Cur. Drug Targets* **2000**, *1*, 85–99.
12. Roche, C. J.; Berkowitz, D.; Sulikowski, G. A.; Danishefsky, S. J.; Crothers, D. M. *Biochemistry* **1994**, *33*, 936–942.
13. Israel, M.; Murray, R. J. *J. Med. Chem.* **1982**, *25*, 24–28.
14. Capranico, G.; Supino, R.; Binaschi, M.; Capolongo, L.; Grandi, M.; Suarato, A.; Zunino, F. *Mol. Pharmacol.* **1994**, *45*, 908–915.
15. Nicolaou, K. C.; Seitz, S. P.; Papahatjis, D. P. *J. Am. Chem. Soc.* **1983**, *105*, 2430–2434.
16. Veeneman, G. H.; Van Leeuwen, S. H.; Van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334.
17. Veeneman, G. H.; Van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 275–278.
18. Crich, D.; Smith, M. *J. Am. Chem. Soc.* **2001**, *123*, 9015–9020.
19. Marzabadi, C. H.; Franck, R. W. *Tetrahedron* **2000**, *56*, 8385–8417.
20. Toshima, K.; Tatsuta, K. *Chem. Rev.* **1993**, *93*, 1503–1531.
21. Lear, M. J.; Yoshimura, F.; Hirama, M. *Angew. Chem., Int. Ed.* **2001**, *40*, 946–949.
22. Zhang, G; Fang, L.; Zhu, L.; Aimiuwu, J. E.; Shen, J.; Cheng, H.; Muller, M. T.; Lee, G. E.; Sun, D.; Wang, P. G. *J. Med. Chem.* **2005**, *48*, in press.