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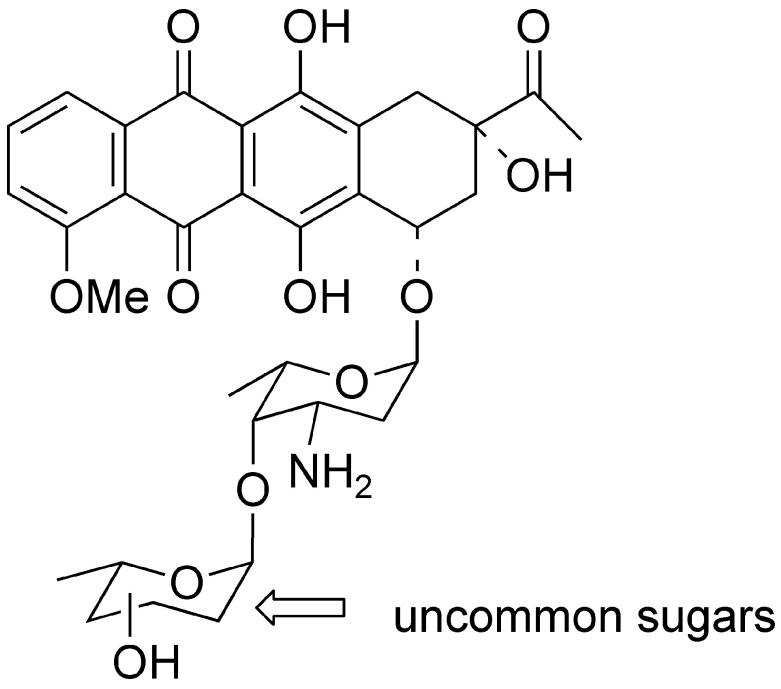

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Syntheses and Biological Activities of Disaccharide Daunorubicins

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Carbohydrate moiety is found in many anticancer nature products. To explore the carbohydrate moiety of daunorubicin in enhancing anticancer efficacy, several daunorubicin derivatives bearing disaccharide (**1**−**8**) have been synthesized. Their cytotoxicities were tested in leukemia K562 and colon cancer SW620 cells. Topoisomerase II (topo II) poisoning was performed with the in vivo complex of topoisomerase bioassay. In both cell lines, compounds with various terminal 2,6-dideoxy sugars (compounds **1**, **3**, **5**, and **8**) showed 30- to 60-fold higher anticancer activity than compounds with 2-deoxy- or 6-deoxy sugar (compounds **6** and **7**). Compounds with an α-linkage between two sugar units (compound **3**) showed 35-fold higher anticancer activity than compounds with a β-linkage (compound **4**). In addition, the anticancer activities of these compounds correlated with their ability to target topo II mediated genomic DNA damage in vivo. Compounds **1** and **3** with 2,6-dideoxy sugars produced more covalent topo−DNA complex than compounds with 2-deoxy sugar (**6**) and 6-deoxy sugar (**7**). Compounds with an α-configuration of terminal 2,6-dideoxy sugar (compounds **1** and **3**) showed higher topo II poisoning than their counterparts with the β-configuration (compounds **2** and **4**). These results indicate that sugar moieties in daunorubicin play a significant role in its anticancer activity and topo II inhibition. The second sugar of disaccharide daunorubicin should possess 2,6-dideoxy with α-linkage to the first sugar to exhibit better anticancer activity.

Introduction

The anthracycline quinone antibiotics daunorubicin (DNR) and doxorubicin (Figure 1) are potent antitumor agents against a variety of solid tumors.[1] In addition to DNA intercalation, topoisomerase II (topo II) is considered as the primary cellular target of anthracyclines.[2,3] A well-accepted mechanism of action is through interaction with DNA−topo II by stabilization of a topo II−DNA−drug ternary complex, which also triggers cell apoptosis. It is noted that DNA binding and interaction is necessary, but not sufficient, for topo II poisoning.[4] In fact, the external (nonintercalating) sugar moieties and the cyclohexane ring A are recognized as crucial moieties for therapeutic efficacy of anthracyclines (Figure 1). The sugar moiety of anthracyclines serves as a minor groove binder of DNA.[5,6] Indeed, modifications on the sugar structures have led to the second generation of doxorubicin analogues as monosaccharides such as epirubicin, valrubicin, and pirarubicin. The third generation of anthracycline as disaccharide (MEN 10755,[7−9] Figure 2) is under clinical trials. MEN 10755 possesses a 2-deoxyfucose linked to the aglycon and daunosamine as the second sugar unit. It exhibits a broader spectrum of antitumor activity than doxorubicin. Structure−activity relationship (SAR) studies

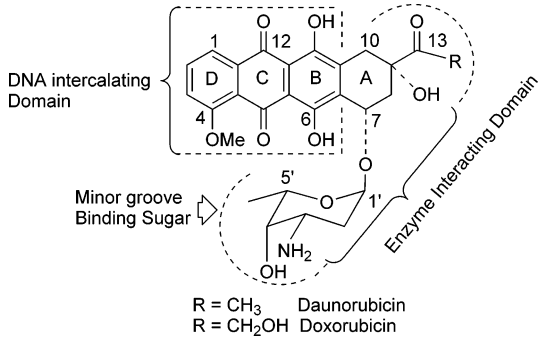

Figure 1.

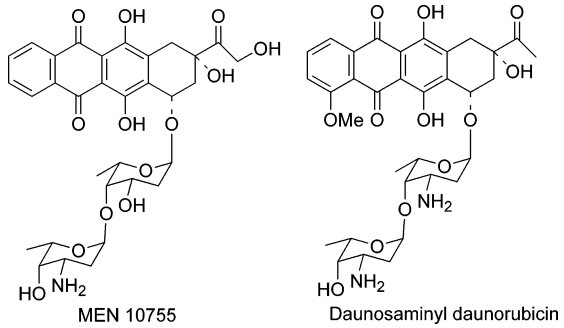

Figure 2.

on MEN 10755 revealed the following. (a) The axial orientation α (1→4) of the second sugar daunosamine is critical for the topo II poisoning ability. Indeed, glycosides with equatorial configuration are ineffective in topo II poisoning.[10,11] (b) The presence of a 4-methoxy group on the aglycone core dramatically decreases its

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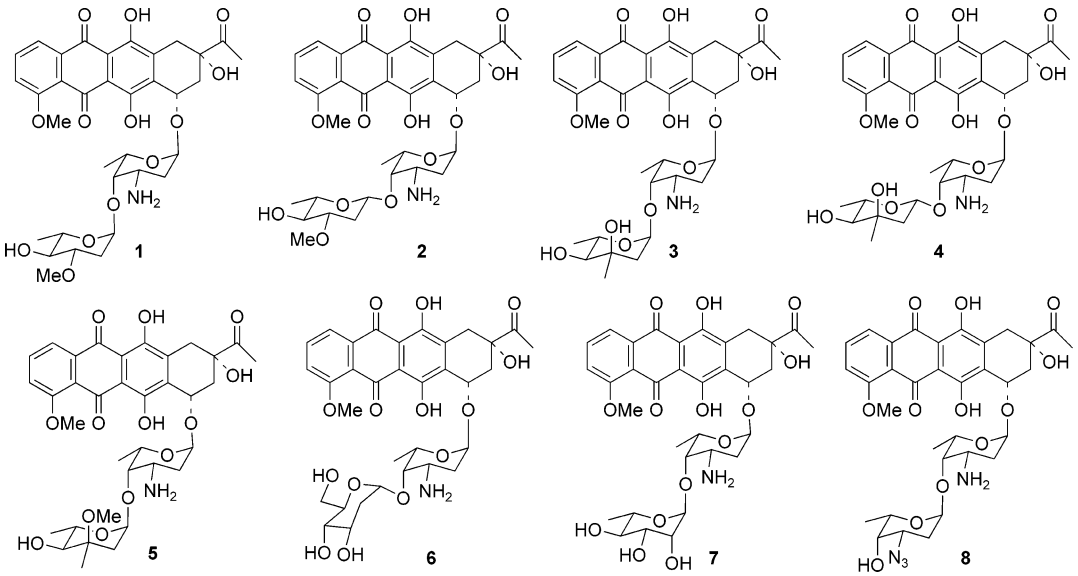


Figure 3. Synthetic compounds.

cytotoxicity because of the reduced topo II poisoning.[10,11] A crystal structure of MEN 10755 and DNA hexamer (CGATCG) complex showed two different DNA binding sites.[12] 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 the guanine of another DNA strand through hydrogen bonds. This peculiar behavior suggested that the second sugar may interact with other cellular target such as topoisomerase.[12]

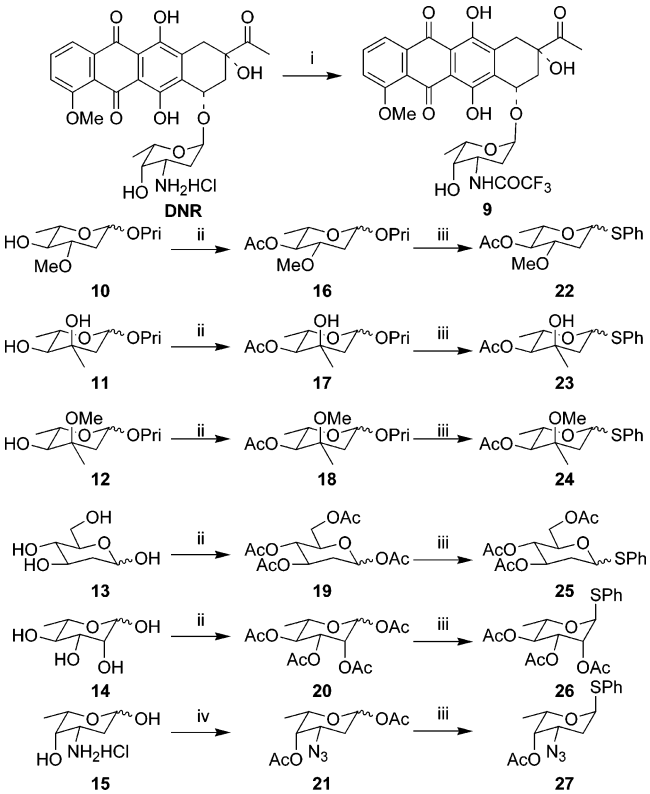
Indeed, natural anthracycline analogues isolated from *Streptomyces* sp. often contain two or more sugars connected by axial (1→4) linkage. The anthracycline disaccharides or oligosaccharides invariably possess amino sugar as the first moiety attached to the aglycone.[1] For instance, daunosaminyl daunorubicin (Figure 2), which was isolated from fermentation, contains a disaccharide with two daunosamines α (1→4). The structures of the terminal sugar and sugar chain length have been shown to significantly influence DNA binding, topoisomerase poisoning, and anticancer efficacy.[13]

Taken together, compelling evidence has shown that extending the monosaccharide to disaccharide with uncommon sugars may have significant impact on topoisomerase inhibition besides DNA binding. Surprisingly, the SAR on anthracyclines has not involved a large variation in the sugar structure. So far, only six uncommon sugars were used in disaccharide anthracycline analogues. We hypothesize that alteration of the second sugar of anthracycline analogues with uncommon sugars may provide more information for rational design to improve the pharmacological efficacy[14] and to reduce side effects.[15−17] Uncommon sugars have received special attention because of the increased recognition of their vital roles in the biological function of many natural products. They add important features to the shape and the stereoelectronic properties of a molecule and often play an essential role in the biological activity of many natural product drugs. Their significance has been studied in cellular adhesion, fertilization, protein folding, neurobiology, xenotransplantation, and target recognition in the immune response.[18−21] However, to date, there have not been systematic studies for SAR of uncommon sugars on the second sugar moiety of anthracycline to depict their role in anticancer activity. In the present study, we designed novel disaccharide daunorubicins with uncommon sugars as terminal sugar moieties to reveal its function in anticancer activity (Figure 3).

Results and Discussion

Chemistry. For disaccharide daunorubicins, the optimal configuration should be an uncommon sugar linked to the 4-position of the first sugar through α (1→4) linkage. In our efforts to gain access to semisynthetic disaccharide daunorubicins, we focused on the glycosylation of 3′-amino protected daunorubicin (**9**) with appropriate glycosyl donors. To make α-glycosylation with 2,6-dideoxy sugars, we anticipate three synthetic challenges: first, the stability of 2,6-dideoxy sugar donors; second, α-selective formation of a glycosidic bond between a ribo-configured sugar (**11**) and an aglycon, and finally the known low reactivity of the axial 4-hydroxyl group in the glycosyl acceptors.[22] Glycosyl sulfide donors are believed to be the best choice for the α-glycosylation of 2,6-dideoxy sugars in this case. Thioglycosides have been introduced as glycosyl donors because they are stable to normal protecting group manipulation encountered in oligosaccharide synthesis.[23−25] Various activating systems can be applied, such as, MeOTf, NBS, BSP/Tf_2O/TTBP,[26] IDCP, NIS/TfOH, and NIS/TMSOTf, etc. For a 2-deoxy sugar, without the stereodirecting ability of 2-substituents, direct and efficient α-selective glycosylations are difficult to realize.[23,27] Strategies to prepare 2-deoxyglycosides selectively in high α- or β-anomeric forms rely heavily on indirect sequences from glycals or latent 2-deoxysugars.[28,29] As such, these strategies require a subsequent reductive step that would be unsuitable for our cases. Recently, Hirama 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.[27] In this paper, we report a concise promoter system $AgPF_6$/TTBP (2,4,6-tri-*tert*-butylpyrimidine) for syntheses of disaccharide daunorubicins. The disaccharide daunorubicins were assembled using

Scheme 1[a]



[a] Reagents and conditions: (i) $(CF_3CO)_2O$/pyridine, −20 °C, 15 min; (ii) Ac_2O/pyridine, room temp, 24 h; (iii) PhSH, $BF_3{\cdot}Et_2O$/CH_2Cl_2, 0 °C, 2 h, (iv) K_2CO_3, $CuSO_4$, TfN_3 solution, then Ac_2O/pyridine.

thiophenyl 2,6-dideoxyglycosides as glycosyl donors. This protocol overcame the low reactivity of the axial 4′-hydroxy group and produced the desired α-glycosidic bond with good yields.

Treatment of DNR with trifluroacetic anhydride in pyridine gave the glycosyl acceptor **9** with a yield of 95%.[30] The acetyl group was used for protecting the hydroxyl functions present in sugar molecule. Acetyl and trifluoroacetyl groups were cleavable under 0.1 M NaOH in THF, which allowed the acid and strong base sensitive aglycon moiety in the anthracycline molecule to be unaffected in the final deprotection. According to the literature procedures, isopropyl glycosides **10**−**12** were obtained through acidic cleavage of the natural products erythromycin, tylosin, and avermectin in isopropyl alcohol instead of water.[31,32] Treatment of **10**−**12** and free sugars **13** and **14** with acetyl anhydride in dry pyridine at room temperature overnight produced the corresponding acetates **16**, **17**, **18**, **19**, and **20** with yields of 90%, 79%, 92%, 87%, and 89%, respectively. It was of interest to note that in the acetylation of **11** 3-OH was intact. However, the acetylation of free mycarose gave 1,3,4-tri-*O*-acetylmycarose in 83% of yield. Therefore, it was suggested that the hindrance of the bulky isopropyl group allowed for 3-OH survival in the acetylation of **11**. The azido sugar **21** could be prepared readily from **15** in an overall yield of 80% according to C.-H. Wong's method.[33] Treatment of **16**−**21** with thiophenol in the presence of boron trifluoride diethyl etherate gave thioglycosides **22**, **23**, **24**, **25**, **26**, and **27** in 90%, 96%, 96%, 85%, 90%, and 80% of yields, respectively (Scheme 1).[34−36]

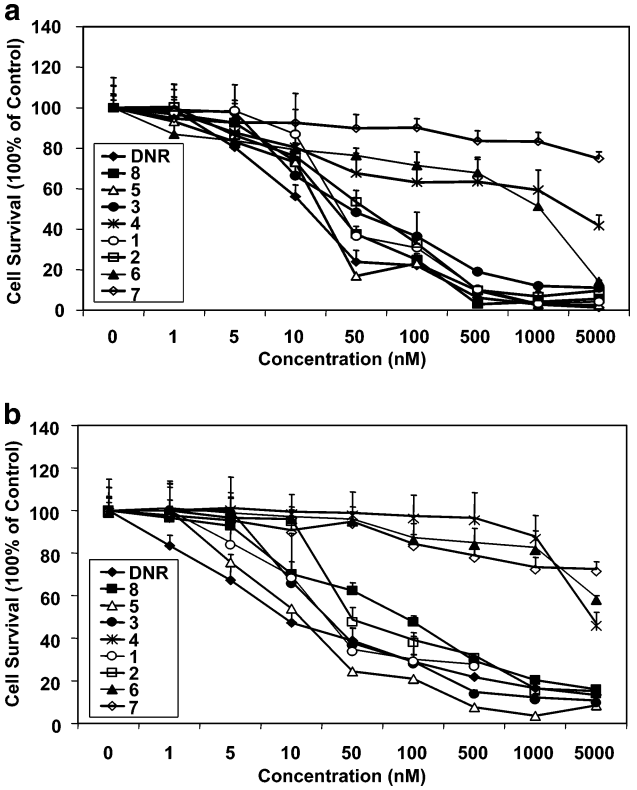
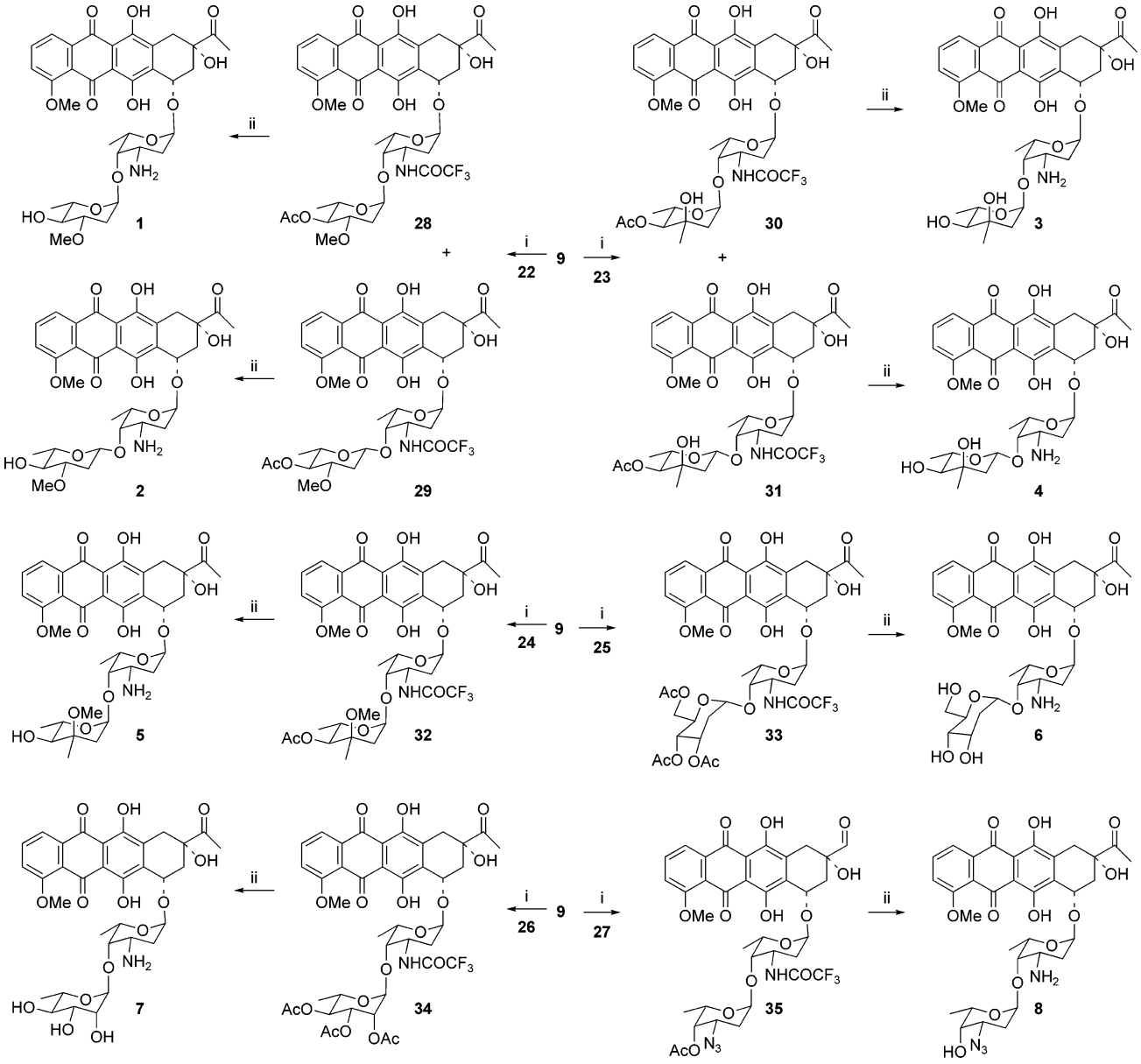


Figure 4. Cytotoxicity of compounds **1**−**8** on leukemia K562 cells (a) and colon cancer SW620 cells (b). The 2000−10000 cells were incubated with 0.005−5 μM tested compounds for 72 h. MTS/PMS solution was added, and cells were incubated for another 1−4 h. Then the absorbance was recorded at 490 nM. The survival rate was expressed as a percentage of control group.

Condensation of the glycosyl acceptor **9** with donors **24**−**27** in the presence of TTBP and $AgPF_6$ exclusively formed the α-products **32**, **33**, **34**, and **35** with yields of 81%, 40%, 28%, and 82%, respectively. Under the same reaction conditions, however, the reactions of **9** with **22** and **23** gave anomeric mixtures ($\alpha{:}\beta$ = 2:1) in yields of 83% and 60%, respectively (Scheme 2). Mild deprotection of **28**−**35** with 0.1 M NaOH in THF afforded the final products **1**, **2**, **3**, **4**, **5**, **6**, **7**, and **8** in good yields (88%, 81%, 76%, 75%, 85%, 62%, 67%, and 66%, respectively).

Biology. Cytotoxicity. The cytotoxicity of these compounds was examined against leukemia cell line K562 cells (Figure 4a) and colon cancer cell line SW620 cells (Figure 4b) with MTS assay as described.[37−39] 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 the percentage of cell control group without treatment. In both cell lines, compounds **1**, **3**, **5**, and **8** with various terminal 2,6-dideoxy sugars showed very potent cytotoxicity with IC_{50} of 20−60 nM. Compounds **6** (with 2-deoxy terminal sugar) and **7** (with 6-deoxy terminal sugar), when compared to other compounds, showed much worse activity (30- to 60-fold less) than compounds **1**, **3**, and **5** (with 2,6-dideoxy terminal sugar). This suggests that the presence of 2-OH or 6-OH groups in a terminal sugar (compounds **6** and **7**) decreases cytotoxicity, while compound **3** with 3-OH and compound **5** with 3-OMe in the terminal 2,6-dideoxy-

Scheme 2[a]



[a] Reagents and conditions: (i) TTBP, $AgPF_6/CH_2Cl_2$, 0 °C, 2 h; (ii) 0.1 M NaOH/THF, 0 °C, 6 h.

Table 1. Anticancer Activity (IC_{50}) of Synthesized Compounds in Two Cancer Cell Lines

	compounds								
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	DNR
IC_{50} in K562 (nM)	39.5	45.8	44.3	1531.6	21.0	1378.3	>4000	31.3	15.6
IC_{50} in SW620 (nM)	18.2	38.9	21.7	>1000	11.4	>1000	>1000	60.3	8.6

sugar showed very similar activity. Thus, 2,6-dideoxy sugars are necessary for cytotoxicity, while modification of other sites does not affect its activity significantly. Very interestingly, compound **3** with an α-configuration of the terminal 2,6-dideoxy sugar moiety showed 35-fold higher cytotoxicity than compound **4** with the *β*-configuration. The IC_{50} values of all these compounds against these two cell lines are summarized in Table 1. It was demonstrated that these compounds have similar IC_{50} range and comparable efficacy in K562 and SW620 cells. Altogether, these data suggest that modification on the terminal sugar of daunorubicin can significantly change its cytotoxicity against cancer cells. The second sugar of disaccharide daunorubicin should possess a 2,6-dideoxy withan α-linkage to the first sugar to exhibit better activity.

Inhibition of Topoisomerase II. Compounds **1**−**8** were also tested for their ability to mediate topo II cleavage (or covalent DNA) complexes in vivo using the ICT (in vivo complex of topoisomerase) bioassay in HeLa cells. Formation of the covalent topo−DNA complex is essential for the cytotoxic action of the topoisomerase poisons. The unique nature of topoisomerase−DNA interaction (formation of the covalent intermediate) can be exploited to quantify topoisomerase-mediated DNA damage in the intact cell. In the presence of topo II poisons, the topo-mediated DNA breaks are stabilized and a covalent topo−DNA complex can be trapped upon

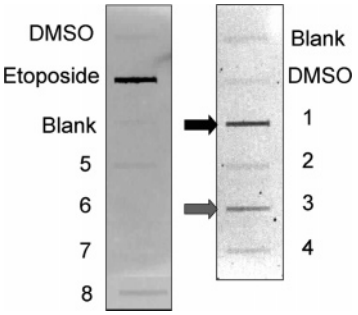


Figure 5. Inhibition of topoisomerase II by compounds **1**−**8**. Exponentially growing HeLa cells were incubated with 50 μM etoposide or tested compounds for 30 min at 37 °C. DNA and denatured proteins were fractionated by CsCl gradient centrifugation. The bottom fraction was collected. Each fraction (50 μL) was slotted onto a Hybond ECL membrane, and the blot was probed with anti-topo-II antibody. The immunoblot signals were visualized by chemiluminescence.

addition of protein denaturants. DNA is then purified (without proteases) by CsCl gradient centrifugation, which completely dissociates noncovalent protein−DNA interactions and allows quantitative separation and recovery of DNA. An antibody is then used to quantify topo II levels in the DNA peak fraction from the gradient as shown in Figure 5. The anticancer activities of these compounds correlated with their ability to target topo II mediated genomic DNA damage in vivo (Figure 5). Clearly, compound **1** produced more covalent topo−DNA complex than compounds **2**−**8**. Among these compounds, compounds **1** and **3** with an α-configuration of the terminal 2,6-dideoxy sugar showed high topo II targetability than their counterparts with β-configuration (compounds **2** and **4**). By comparison of compounds **3** and **5**, 3-OH in the terminal sugar seems to play a more important role than 3-OMe in poisoning topo II. However, compound **1** with only an equatorial 3-OMe showed higher topo II poison than compounds **3** and **5** with both axial 3-OMe (or OH) and equatorial 3-*C*-Me groups. Compounds **6** and **7** with 6-deoxy or 2-deoxy terminal sugar did not show any topo II inhibition. These results indicate that sugar moieties in daunorubincin play a significant role in topo II inhibition, in which 2,6-dideoxy sugars with an α-linkage are required for maintaining anthracycline cytotoxicity against cancer cells.

Conclusion

In summary, we have developed an effective method to prepare disaccharide daunorubicins from DNR using $AgPF_6$/TTBP as a mild activating system for simple 2,6-dideoxythioglycosides. The biological activities of these compounds in two cancer cell lines show that compounds **1**, **3**, **5**, and **8** with various terminal 2,6-dideoxy sugars display 30- to 60-fold better cytotoxicity than compounds **6** and **7**, which contain either a 2- or 6-deoxy sugar. Meanwhile, structural modification on other sites in the second sugar moiety (such as the 3-position, −OH or −OMe) does not affect the activity significantly. This suggests that 2,6-dideoxy sugars are required for anticancer activity of daunorubicin and its derivatives. In addition, the results show that a compound bearing an α-configuration of the terminal 2,6-dideoxy sugar moiety is 35- to 50-fold more potent than compounds with the β-configuration. The anticancer activity of these compounds correlated with their ability to target topo II mediated genomic DNA damage in vivo. Compounds with the α-configuration of the terminal 2,6-dideoxy sugar showed higher topo II targetability than their counterparts with the β-configuration, while compounds with 6-deoxy or 2-deoxy terminal sugar do not show any topo II inhibition. These results indicate that the structures and conformations of sugar moieties are important for their anticancer activity and topo II targeting. The second sugar 2,6-dideoxy with α-linkage to the first sugar of daunorubicin disaccharide is preferred to exhibit anticancer activity.

Experimental Section

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

***N*-(Trifluoroacetyl)daunorubicin (9).** Daunorubicin hydrochloride (16.0 g, 28.4 mmol) was stirred in dry pyridine (16 mL) at −20 °C for 0.5 h. Trifluoroacetic anhydride (28 mL) in anhydrous ether (200 mL) was added dropwise over a 1.5 h period. After 0.5 h, water (250 mL) was added and stirring was continued for 0.5 h. The reaction mixture was extracted with ethyl acetate, and the extracts were washed with water. After the mixture was dried over Na_2SO_4, the solvent was filtered and concentrated and the residue was heated at reflux with MeOH (50 mL) for 0.5 h. MeOH was removed on the rotary evaporator, and the residue was precipitated from chloroform−pentane to afford **9** as a deep-red solid (16.8 g, 95%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{29}H_{28}F_3NO_{11}Na^+$ 646.1507, found 646.1493. ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.98 (1H, s, OH-6), 13.25 (1H, s, OH-11), 8.02 (1H, d, J = 7.8 Hz, H-1), 7.68 (1H, t, J = 8.1 Hz, H-2), 7.37 (1H, d, J = 8.2 Hz, H-3), 6.63 (1H, d, J = 8.5 Hz, $NHCOCF_3$), 5.50 (1H, d, J = 3.9 Hz, H-1′), 5.25 (1H, dd, J = 1.9, 4.0 Hz, H-7), 4.24 (2H, m, H-3′, H-5′), 4.06 (3H, s, OMe-4), 3.65 (1H, d, J = 2.2 Hz, H-4′), 3.25 (1H, dd, J = 1.9, 18.8 Hz, H-10), 2.92 (1H, d, J = 18.8 Hz, H-10), 2.39 (3H, s, H-14), 2.29 (1H, m, Ha-8), 2.14 (1H, m, Hb-8), 1.94 (1H, m, Ha-2′), 1.80 (1H, m, Hb-2′), 1.28 (3H, d, J = 6.6 Hz, H-6′).

General Procedure A for the Preparation of Isopropyl Glycosides (16−20). A mixture of substrate (12.3 mmol), acetyl anhydride (30 mL), and dry pyridine (36 mL) was stirred overnight at room temperature. Then methanol (10 mL) was added and the mixture was stirred for 0.5 h. The reaction mixture was diluted with EtOAc (200 mL) and washed with ice−water, saturated aqueous $NaHCO_3$ solution, and brine. Concentration at the rotary evaporator and chromatography on silica gel gave the protected glycosides.

Isopropyl 4-*O*-Acetyl-L-oleandroside (16). Compound **16** was prepared from glycoside **10** according to general procedure A with 90% yield (purified on a column of silica gel using EtOAc/hexanes 1:9). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{12}H_{22}O_5Na^+$ 269.1359, found 269.1359. ^{1}H NMR (250 MHz, $CDCl_3$), β-anomer, δ 4.59 (1H, m), 4.49 (1H, d, J = 8.2 Hz, H-1), 3.93 (1H, m), 3.29 (5H, m), 2.25 (1H, m), 2.06 (3H, s), 1.59 (1H, m), 1.15 (9H, m); α-anomer, δ 4.59 (1H, m), 4.49 (1H, d, J = 8.2 Hz, H-1), 3.93 (1H, m), 3.29 (5H, m), 2.25 (1H, m), 2.06 (3H, s), 1.59 (1H, m), 1.15 (9H, m).

Isopropyl 4-*O*-Acetyl-L-mycaroside (17). Compound **17** was prepared from glycoside **11** according to general procedure A with 79% yield (purified on a column of silica gel using EtOAc/hexane 1:4). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{12}H_{22}O_5Na^+$ 269.1359, found 269.1362. ^{1}H NMR (250 MHz, $CDCl_3$), β-anomer, δ 4.89 (1H, dd, J = 2.2 Hz, 9.6 Hz, H-1), 4.60 (1H,

d, J = 9.7 Hz, H-4), 3.96 (1H, quint, J = 6.2 Hz), 3.81 (1H, m), 2.11 (3H, s, OAc), 1.92 (1H, m), 1.76 (1H, br, OH), 1.21 (3H, d, J = 6.2 Hz, H-6), 1.14 (9H, m).

Isopropyl 4-*O*-Acetyl-L-cladinoside (18). Compound **18** was prepared from glycoside **12** according to general procedure A with 92% yield (purified on a column of silica gel using EtOAc/hexanes 1:9). HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{13}H_{24}O_5Na^+$ 283.1516, found 283.1529. ^{1}H NMR (250 MHz, $CDCl_3$) δ 4.73 (2H, m), 3.93 (2H, m), 3.25 (3H, s, OMe-3), 2.12 (4H, m), 1.46 (1H, m), 1.59 (12H, m).

2-Deoxy-1,3,4,6-tetra-*O*-acetyl-D-glucopyranose (19). Compound **19** was prepared from 2-deoxyglucopyranose **13** according to general procedure A with 87% yield (purified on a column of silica gel using EtOAc/hexanes 1:4).

1,2,3,4-Tetra-*O*-acetyl-L-rhamnopyranose (20). Compound **20** was prepared from rhamnopyranose **14** according to general procedure A with 89% yield (purified on a column of silica gel using EtOAc/hexane 1:4).

3-Azido-1,4-di-*O*-acetyl-2,3,6-trideoxy-L-*lyxo*-hexopyranose (21). Daunosamine hydrochloride **15** (184.0 mg, 1.0 mmol) was dissolved in water (3 mL) and treated with potassium carbonate (207.0 mg, 1.5 mmol) and $CuSO_4{\cdot}5H_2O$ (1.5 mg, 0.01 mmol). To the sugar solution was added MeOH (6 mL) and the TfN_3 solution made using 2 equiv of Tf_2O.[33] Then more MeOH was added to obtain homogeneity. The reaction mixture was allowed to be stirred at room temperature for 18 h, and the solvent was removed. The residue was acetylated using Ac_2O (3 mL) and pyridine (5 mL) with catalytic DMAP. It was worked up by removal of solvent and extraction with water from EtOAc. Column chromatography of the organic phase on silica gel using EtOAc/hexanes (1:4) afforded the product **21** as a yellow oil (205 mg, 80%).

General Procedure B for the Preparation of Thiophenyl Glycosides (22−27). A solution of substrate (6.2 mmol) and thiophenol (678 mg, 6.2 mmol) in toluene (10 mL) was cooled to 0 °C (ice bath), and then boron trifluoride ethyl etherate (678 μL) was added. The mixture was warmed to room temperature and aged for 2 h. After the mixture was cooled to 0 °C by an ice bath, aqueous NaOH (5%, 15 mL) was added. The organic phase was separated, and the aqueous phase was extracted with toluene (50 mL). The combined organic phases were washed with saturated NaCl and concentrated under vacuum to give the crude products.

Thiophenyl 4-*O*-Acetyl-L-oleandroside (22). Compound **22** was prepared from glycoside **16** according to general procedure B (purified on a column of silica gel, EtOAc/benzene 1:19) as a pale-yellow solid (90%, α:β = 1.7:1). HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{15}H_{20}O_4SNa^+$ 319.0974, found 319.0970. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (m, Ar), 7.31 (m, Ar), 5.65 (d, J = 5.6 Hz, H-1$_\alpha$), 4.71 (m), 4.29 (m), 3.65 (m), 3.41 (m), 3.39 (s, H−OMe$_\alpha$), 3.37 (s, H−OMe$_\beta$), 2.48(m), 2.14 (s, H−OAc$_\alpha$), 2.11 (s, H−OAc$_\beta$), 2.07 (m), 1.26 (d, J = 6.2 Hz, H-6$_\beta$), 1.20 (d, J = 6.3 Hz, H-6$_\alpha$).

Thiophenyl 4-*O*-Acetyl-L-mycaroside (23). Compound **23** was prepared from glycoside **17** according to general procedure B (purified on a column of silica gel, EtOAc/benzene 1:11) as a pale-yellow solid (96%, α:β = 1:3). HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{15}H_{20}O_4SNa^+$ 319.0974, found 319.0976. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (m, Ar), 7.26 (m, Ar), 5.48 (dd, J = 3.0 Hz, J = 4.6 Hz, H-1$_\alpha$), 5.15 (dd, J = 2.2 Hz, J = 11.9 Hz, H-1$_\beta$), 4.65 (m), 3.87 (m), 2.36 (s), 2.13 (m), 1.82 (m).

Thiophenyl 4-*O*-Acetyl-L-cladinoside (24). Compound **24** was prepared from glycoside **18** according to general procedure B to give **24** (purified on a column of silica gel, EtOAc/benzene 1:19) as a pale-yellow solid (96%, α:β = 1.8:1). HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{15}H_{20}O_4SNa^+$ 283.1516, found 283.1529. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.48 (m, Ar), 7.30 (m, Ar), 5.65 (d, J = 6.3 Hz, H-1$_\alpha$), 5.04 (dd, J = 1.8 Hz, J = 11.8 Hz, H-1$_\beta$), 4.63 (m), 4.01 (m), 3.34 (s, H−OMe$_\alpha$), 3.24 (s, H−OMe$_\beta$), 2.48-(d, J = 15.0 Hz), 2.26 (dd, J = 1.8 Hz, J = 14.3 Hz), 2.14 (s, H−OAc$_\alpha$), 2.11 (s, H−OAc$_\beta$), 2.03 (m), 1.66 (m), 1.14 (m).

Thiophenyl 2-Deoxy-3,4,6-tri-*O*-acetyl-D-glucopyranoside (25). Compound **25** was prepared from glycoside **19** according to general procedure B (purified on a column of silica gel, EtOAc/hexane 1:4) as a colorless oil (85%, α:β = 1.5:1). HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{18}H_{22}O_7SNa^+$ 405.0978, found 405.0970. ^{1}H NMR (500 MHz, $CDCl_3$), α-anomer, δ 7.45 (2H, m, Ar), 7.29 (3H, m, Ar), 5.47 (1H, d, J = 5.5 Hz, H-1), 5.28 (1H, m, H-3), 4.99 (1H, m, H-4), 4.51 (1H, m, H-5), 4.30 (1H, dd, J = 5.3 Hz, J = 12.3 Hz, Ha-6), 4.01 (1H, dd, J = 2.2 Hz, J = 12.3 Hz, Hb-6), 2.43 (1H. m, H-2), 2.23 (1H, m, H-2), 2.05 (3H, s, OAc), 2.01 (3H, s, OAc), 2.00 (3H, s, OAc); β -anomer, 7.49 (2H, m, Ar), 7.29 (3H, m, Ar), 4.98 (1H, m, H-3), 4.96 (1H, t, J = 9.6 Hz, H-4), 4.78 (1H, dd, J = 1.9 Hz, 11.9 Hz, H-1), 4.22 (1H, m, Ha-6), 4.13 (1H, m, Hb-6), 3.62 (1H, m, H-5), 2.45 (1H. m, H-2), 1.84 (1H, m, H-2), 2.06 (3H, s, OAc), 2.02 (3H, s, OAc), 1.99 (3H, s, OAc).

Thiophenyl 2,3,4-Tri-*O*-acetyl-L-rhamnopyranoside (26). Compound **26** was prepared from glycoside **20** according to general procedure B (purified on a column of silica gel, EtOAc/hexane 1:4) as a pale-yellow solid (90%, α-anomer only). HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{18}H_{22}O_7SNa^+$ 405.0978, found 405.0972. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (2H, m, Ar), 7.29 (3H, m, Ar), 5.47 (1H, dd, J = 1.6 Hz, J = 3.3 Hz, H-2), 5.38 (1H, d, J = 1.4 Hz, H-1), 5.27 (1H, dd, J = 3.4 Hz, J = 10.1 Hz, H-3), 5.12 (1H, t, J = 9.8 Hz, H-4), 4.34 (1H, m, H-5), 2.12 (3H, s, OAc), 2.05 (3H, s, OAc), 1.98 (3H, s, OAc), 1.22 (3H, d, J = 6.3 Hz, H-6).

Thiophenyl 4-*O*-Acetyl-3-azido-2,3,6-trideoxy-L-*lyxo*-hexopyranoside (27). Compound **27** was prepared from glycoside **21** according to general procedure B (purified on a column of silica gel, 5% Et_2O in hexane) as a pale-yellow oil (80%, α-anomer). HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{14}H_{17}N_3O_3SNa^+$ 330.0883, found 330.0885. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.44 (2H, m, Ar), 7.29 (3H, m, Ar), 5.71 (1H, d, J = 5.6 Hz, H-1), 5.19 (1H, d, J = 2.4 Hz, H-4), 4.45 (1H, q, J = 6.5 Hz, H-5), 3.18 (1H, m, H-3), 2.44, 2.12 (2H, m, H-2), 2.16 (3H, s, OAc), 1.12 (3H, d, H-6).

General Procedure C for the Preparation of the Protected Disaccharide Daunorubicins (28−35). A mixture of 2,6-dideoxythioglycoside (4.8 mmol), compound **9** (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 the mixture was 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-α-L-oleandrosyl)-2,3,6-trideoxy-3-trifluoroacetoamido-α-L-*lyxo*-hexopyranosyl]daunorubicinone (28) and 7-[4-*O*-(4-*O*-Acetyl-*β*-L-oleandrosyl)-2,3,6-trideoxy-3-trifluoroacetoamido-α-L-*lyxo*-hexopyranosyl]daunorubicinone (29). The glycoside **22** was reacted according to general procedure C to give α-anomer **28** and β-anomer **29** (separated on a column of silica gel, MeOH/CH_2Cl_2 1:100) as red solids.

28. Yield, 55%. HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{38}H_{42}F_3NO_{15}Na^+$ 832.2398, found 832.2387. ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.89 (1H, s, HO-6), 13.15 (1H, s, HO-11), 8.11 (1H, d, J = 8.3 Hz, NHCOCF$_3$), 7.94 (1H, d, J = 7.7 Hz, H-1), 7.71 (1H, t, J = 8.2 Hz, H-2), 7.34 (1H, d, J = 8.1 Hz, H-3), 5.51 (1H, d, J = 3.3 Hz, H-1′), 5.23 (1H, d, J = 1.8 Hz, H-7), 4.89 (1H, br, H-1″), 4.71 (1H, m, H-4″), 4.35 (1H, br, OH-9), 4.21 (2H, m, H-3′, H-5′), 4.20 (3H, s, OMe-4), 3.95 (1H, m, H-5″), 3.65 (1H, m, H-3″), 3.53 (1H, br, H-4′), 3.36 (3H, s, OMe-3″), 3.11 (1H, d, J = 18.7 Hz, Ha-10), 2.85 (1H, d, J = 18.7 Hz, Hb-10), 2.37 (3H, s, H-14), 2.25 (2H, m, Ha-2″, Ha-8), 2.09 (4H, m, OAc-4″, Hb-8), 1.83 (3H, m, Hb-2″, H-2′), 1.27 (3H, d, J = 6.4 Hz, H-6′), 1.20 (3H, d, J = 6.7 Hz, H-6″). ^{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.

29. Yield, 27%. HRMS (M + Na)$^+$ (ESI$^+$) calcd for $C_{38}H_{42}F_3NO_{15}Na^+$ 832.2398, found 832.2381. ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.87 (1H, s, HO-6), 13.14 (1H, s, HO-11), 7.94 (1H, d, J = 7.6 Hz, H-1), 7.72 (1H, t, J = 8.4 Hz, H-2), 7.33 (1H, d,

J = 8.5 Hz, H-3), 6.89 (1H, d, J = 7.6 Hz, $NHCOCF_3$), 5.48 (1H, d, J = 3.3 Hz, H-1′), 5.19 (1H, d, J = 1.9 Hz, H-7), 4.58 (2H, m, H-1″, H-4″), 4.31 (1H, s, OH-9), 4.17 (2H, m, H-3′, H-5′), 4.02 (3H, s, OMe-4), 3.89 (1H, br, H-4′), 3.35 (5H, m, H-5″, H-3″, OMe-3″), 3.11 (1H, d, J = 18.7 Hz, Ha-10), 2.85 (1H, d, J = 18.7 Hz, Hb-10), 2.37 (3H, s, H-14), 2.27 (2H, m, Ha-2″, Ha-8), 2.09 (4H, m, OAc-4″, Hb-8), 1.89 (2H, m, H-2′), 1.65 (1H, m, H-2″), 1.27 (3H, d, J = 6.6 Hz, H-6′), 1.15 (3H, d, J = 6.2 Hz, H-6″). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.9, 186.9, 186.5, 170.1, 160.9, 156.3, 156.2, 155.7, 135.5, 135.4, 134.3, 133.9, 120.8, 119.7, 118.4, 116.8, 111.4, 111.2, 100.2, 99.5, 77.9, 76.6, 75.0, 73.2, 70.8, 70.0, 66.8, 56.8, 56.6, 46.1, 35.5, 35.0, 33.3, 29.8, 24.8, 20.8, 17.5, 17.3.

7-[4-*O*-(4-*O*-Acetyl-α-L-mycarosyl)-2,3,6-trideoxy-3-trifluoroacetoamido-α-L-*lyxo*-hexopyranosyl]daunorubicinone (30) and 7-[4-*O*-(4-*O*-acetyl-*β*-L-mycarosyl)-2,3,6-trideoxy-3-trifluoroacetoamido-α-L-*lyxo*-hexopyranosyl]daunorubicinone (31). The glycoside **23** was reacted according to general procedure C to give α-anomer **30** and *β*-anomer **31** (separated on a column of silica gel, MeOH/CH_2Cl_2 1:75) as red solids.

30. Yield, 40%. HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{38}H_{42}F_3NO_{15}Na^+$ 832.2398, found 832.2438. ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.87 (1H, s, HO-6), 13.83 (1H, s, HO-11), 7.99 (1H, d, J = 8.8 Hz, $NHCOCF_3$), 7.87 (1H, d, J = 7.7 Hz, H-1), 7.66 (1H, t, J = 8.0 Hz, H-2), 7.30 (1H, d, J = 7.6 Hz, H-3), 5.44 (1H, d, J = 3.3 Hz, H-1′), 5.14 (1H, s, H-7), 4.97 (1H, br, J = 3.5 Hz, H-1″), 4.65 (1H, d, J = 9.9 Hz, H-4″), 4.19 (4H, m, H-3′, H-5′, H-5″, H-4′), 3.99 (3H, s, OMe-4), 3.61 (1H, s, OH-9), 3.09 (1H, d, J = 18.9 Hz, Ha-10), 2.80 (1H, d, J = 18.9 Hz, Hb-10), 2.35 (3H, s, H-14), 2.10, 1.91, (9H, m, H-8, H-2′, H-2″, OAc), 1.27 (3H, d, J = 6.2 Hz, H-6′), 1.45 (6H, m, CH_3-3″, H-6″). ^{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.

31. Yield, 20%. HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{38}H_{42}F_3NO_{15}Na^+$ 832.2398, found 832.2368. ^{1}H NMR (500 MHz, $CDCl_3$) δ 14.06 (1H, s, HO-6), 13.34 (1H, s, HO-11), 8.01 (1H, d, J = 7.6 Hz, H-1), 7.77 (1H, t, J = 8.1 Hz, H-2), 7.35 (1H, d, J = 8.3 Hz, H-3), 6.48 (1H, d, J = 8.5 Hz, $NHCOCF_3$), 5.48 (1H, br, H-1′), 4.97 (1H, d, J = 5.5 Hz, H-1″), 4.88 (1H, br, H-1″), 4.75 (1H, m, H-3′), 4.55 (1H, m, H-5′), 4.38 (1H, d, J = 10.0 Hz, H-4″), 4.07 (3H, s, OMe-4), 3.67 (3H, m, Ha-10, H-5″, H-4′), 2.96 (1H, d, J = 18.9 Hz, Hb-10), 2.55 (1H, m, Ha-8), 2.31 (3H, s, H-14), 2.1−1.8 (8H, m, H-2″, Hb-8, H-2′, OAc), 1.29 (3H, d, J = 6.6 Hz, H-6′), 1.05 (3H, s, CH_3-3″), 0.46 (3H, d, J = 6.2 Hz, H-6″). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.9, 186.9, 186.5, 170.1, 160.9, 156.3, 156.2, 155.7, 135.5, 135.4, 134.3, 133.9, 120.8, 119.7, 118.4, 116.8, 111.4, 111.2, 100.2, 99.5, 77.9, 76.6, 75.0, 73.2, 70.8, 70.0, 66.8, 56.8, 56.6, 46.1, 35.5, 35.0, 33.3, 29.8, 24.8, 20.8, 17.5, 17.3.

7-[4-*O*-(4-*O*-Acetyl-α-L-cladinosyl)-2,3,6-trideoxy-3-trifluoroacetoamido-α-L-*lyxo*-hexopyranosyl]daunorubicinone (32). Compound **32** was prepared from glycoside **24** according to general procedure C (purified on a column of silica gel, MeOH/CH_2Cl_2 1:100) as a red solid (81%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{39}H_{44}F_3NO_{15}Na^+$ 846.2555, found 846.2545. ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.90 (1H, s, HO-6), 13.20 (1H, s, HO-11), 7.98 (1H, d, J = 7.7 Hz, H-1), 7.73 (2H, m, H-2, $NHCOCF_3$), 7.34 (1H, d, J = 8.5 Hz, H-3), 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, J = 9.7 Hz, H-4″), 4.38 (1H, s, OH-9), 4.26 (2H, m, H-3′, H-5″), 4.18 (1H, m, H-5′), 4.04 (3H, s, OMe-4), 3.61 (1H, br, H-4′), 3.30 (3H, s, OMe-3″), 3.17 (1H, d, J = 18.8 Hz, Ha-10), 2.85 (1H, d, J = 18.8 Hz, Hb-10), 2.46 (1H, m, Ha-2″), 2.37 (3H, s, H-14), 2.25 (1H, m, Ha-8), 2.12 (4H, m, OAc-4″, Hb-8), 1.83 (2H, m, H-2′), 1.65 (1H, m, Hb-2″), 1.29 (3H, d, J = 6.6 Hz, H-6′), 1.14 (3H, s, CH_3-3″), 1.08 (3H, d, J = 6.3 Hz, H-6″). ^{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*-(2-Deoxy-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl)-2,3,6-trideoxy-3-trifluoroacetoamido-α-L-*lyxo*-hexopyranosyl]daunorubicinone (33). Compound **33** was prepared from glycoside **25** according to general procedure C (purified on a column of silica gel, MeOH/CH_2Cl_2 1:75) as a red solid (40%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{41}H_{44}F_3NO_{18}Na^+$ 918.2403, found 918.2387. ^{1}H NMR (125 MHz, $CDCl_3$) δ 13.95 (1H, s, HO-6), 13.18 (1H, s, HO-11), 7.97 (1H, d, J = 7.6 Hz, H-1), 7.74 (1H, m, H-2), 7.34 (1H, d, J = 8.5 Hz, H-3), 6.69 (1H, d, J = 7.1 Hz, $NHCOCF_3$), 5.56 (1H, d, J = 3.5 Hz, H-1′), 5.28 (1H, m, H-3″), 5.18 (1H, d, J = 2.2 Hz, H-7), 5.04 (1H, t, J = 9.7 Hz, H-4″), 4.88 (1H, d, J = 1.6 Hz, H-1″), 4.28−4.06 (7H, m, H-3′, H-3″, H-5′, H-5″, H-6″, OH-9), 4.03 (3H, s, MeO-4), 3.84 (1H, br, H-4′), 3.14 (1H, d, J = 18.7 Hz, Ha-10), 2.85 (1H, d, J = 18.7 Hz, Hb-10), 2.38 (3H, s, H-14), 2.28 (2H, m, Ha-2″, Ha-8), 2.06 (2H, m, Ha-2′, Hb-8), 2.05, 2.03, 2.02 (9H, 3s, OAc), 1.83 (2H, m, Hb-2′, Hb-2″), 1.31 (3H, d, J = 6.6 Hz, H-6′). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.8, 187.0, 186.6, 170.7, 170.7, 169.7, 161.0, 156.4, 156.3, 155.7, 135.7, 135.5, 134.4, 133.8, 120.8, 119.8, 118.4, 116.7, 111.5, 111.3, 100.4, 100.3, 78.6, 76.5, 70.4, 69.3, 69.1, 68.8, 66.4, 62.4, 56.6, 46.9, 35.4, 35.1, 33.3, 29.6, 24.8, 20.9, 20.7, 20.6, 17.3.

7-[4-*O*-(2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-2,3,6-trideoxy-3-trifluoroacetoamido-α-L-*lyxo*-hexopyranosyl]-daunorubicinone (34). Compound **34** was prepared from glycoside **26** according to general procedure C (purified on a column of silica gel, MeOH/CH_2Cl_2 1:75) as a red solid (28%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{41}H_{44}F_3NO_{18}Na^+$ 918.2403, found 918.2411. ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.95 (1H, s, HO-6), 13.24 (1H, s, HO-11), 8.02 (1H, d, J = 7.7 Hz, H-1), 7.76 (1H, t, J = 8.3 Hz, H-2), 7.52 (1H, d, J = 8.7 Hz, $NHCOCF_3$), 7.37 (1H, d, J = 8.5 Hz, H-3), 5.53 (1H, d, J = 3.2 Hz, H-1′), 5.36 (1H, m, H-3″), 5.29 (1H, br, H-2″), 5.25 (1H, br, H-7), 5.10 (1H, t, H-4″), 4.78 (1H, d, J = 2.3 Hz, H-1″), 4.31 (1H, s, OH-9), 4.25 (1H, m, H-3′), 4.23 (1H, m, H-5′), 4.05 (3H, s, MeO-4), 4.04 (1H, m, H-5″), 3.64 (1H, br, H-4′), 3.20 (1H, d, J = 18.8 Hz, Ha-10), 2.91 (1H, d, J = 18.8 Hz, Hb-10), 2.38 (3H, s, H-14), 2.27 (1H, m, Ha-8), 2.16 (1H, m, Hb-8), 2.14, 2.07, 2.01 (9H, 3s, OAc), 1.87 (2H, m, H-2′), 1.33 (3H, d, J = 6.5 Hz, H-6′), 1.24 (3H, d, J = 6.3 Hz, H-6″). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.8, 187.1, 186.7, 170.1, 169.9, 169.8, 161.1, 156.4, 156.3, 155.8, 135.7, 135.6, 134.4, 133.8, 121.0, 119.8, 118.4, 116.7, 111.6, 111.4, 100.3, 100.2, 80.9, 76.7, 70.5, 70.2, 69.5, 68.6, 68.5, 67.2, 56.7, 45.5, 35.1, 33.4, 30.6, 24.8, 20.9, 20.8, 20.7, 17.2, 17.1.

7-[4-*O*-(4-*O*-Acetyl-3-azido-α-L-*lyxo*-hexopyranosyl)-2,3,6-trideoxy-3-trifluoroacetoamido-α-L-*lyxo*-hexopyranosyl]-daunorubicinone (35). Compound **35** was prepared from glycoside **27** according to general procedure C (purified on a column of silica gel, MeOH/CH_2Cl_2 1:150) as a red solid (82%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{37}H_{39}F_3N_4O_{14}Na^+$ 843.2307, found 843.2331. ^{1}H NMR (500 MHz, $CDCl_3$) δ 13.94 (1H, s, HO-6), 13.21 (1H, s, HO-11), 8.60 (1H, d, J = 4.3 Hz, $NHCOCF_3$), 7.99 (1H, d, J = 7.9 Hz, H-1), 7.73 (1H, m, H-2), 7.33 (1H, m, H-3), 5.50 (1H, d, J = 3.4 Hz, H-1′), 5.24 (1H, br, H-7), 5.21 (1H, br, H-4″), 4.99 (1H, br, H-1″), 4.20 (3H, m, H-3′, H-5′, H-5″), 4.04 (3H, s, MeO-4), 3.92 (1H, m, H-3″), 3.58 (1H, br, H-4′), 3.18 (1H, d, J = 18.8 Hz, Ha-10), 2.90 (1H, d, J = 18.8 Hz, Hb-10), 2.38 (3H, s, H-14), 2.17 (1H, m, Ha-8), 2.17 (3H, s, OAc), 2.13(2H, m, Ha-2″, Hb-8), 2.02 (1H, m, Hb-2″), 1.86 (1H, m, Ha-2′), 1.77 (1H, m, Hb-2′), 1.27 (3H, d, J = 6.5 Hz, H-6′), 1.14 (3H, d, J = 6.6 Hz, H-6″). ^{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 D for the Preparation of the Deprotected Disaccharide Daunorubicins (1−8). 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 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-[3-Amino-4-*O*-(α-L-oleandrosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (1). Compound **1** was prepared from protected glycoside **28** according to the general procedure (purified on a column of silica gel, MeOH/CH_2Cl_2 1:25 to 1:15) as a red solids (88%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{34}H_{41}NO_{13}Na^+$ 694.2470, found 694.2483. ^1H NMR (500 MHz, $CDCl_3$) δ 7.94 (1H, d, J = 7.6 Hz, H-1), 7.71 (1H, t, J = 8.0 Hz, H-2), 7.32 (1H, d, J = 8.5 Hz, H-3), 5.45 (1H, d, J = 2.9 Hz, H-1′), 5.20 (1H, br, H-7), 4.92 (1H, d, J = 2.5 Hz, H-1″), 4.05 (4H, m, H-5′, OMe-4), 3.85 (1H, m, H-5″), 3.53 (2H, m, H-3′, H-4′), 3.39 (3H, s, OMe-3″), 3.12 (2H, m, H-4″, Ha-10), 2.95 (1H, m, H-3″), 2.83 (1H, d, J = 10.8 Hz, Hb-10), 2.37 (4H, m, Ha-8, H-14), 2.26 (1H, m, Ha-2″), 2.07 (1H, m, Hb-8), 1.74 (2H, m, H-2′), 1.53 (1H, m, Hb-2″), 1.23 (6H, m, H-6′, H-6″). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.9, 186.8, 186.5, 160.9, 156.4, 155.7, 135.6, 135.4, 134.3, 134.2, 120.8, 119.7, 118.4, 111.3, 111.2, 101.1, 100.3, 81.9, 77.9, 75.9, 69.4, 68.8, 68.2, 56.6, 56.5, 46.7, 34.8, 34.3, 33.4, 29.7, 25.1, 24.7, 17.8, 17.6.

7-[3-Amino-4-*O*-(β-L-oleandrosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (2). Compound **2** was prepared from protected glycoside **29** according to general procedure D (purified on a column of silica gel, MeOH/CH_2Cl_2 1:10) as a red solids (81%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{34}H_{41}NO_{13}Na^+$ 694.2470, found 694.2473. ^1H NMR (500 MHz, $CDCl_3$) δ 7.94 (1H, d, J = 7.7 Hz, H-1), 7.73 (1H, t, J = 8.1 Hz, H-2), 7.32 (1H, d, J = 8.3 Hz, H-3), 5.47 (1H, d, J = 3.1 Hz, H-1′), 5.22 (1H, br, H-7), 4.64 (1H, d, J = 8.6 Hz, H-1″), 4.06 (4H, m, H-5′, OMe-4), 3.69 (1H, br, H-4′), 3.78 (H, m, OMe-3″, H-4″), 3.17 (4H, m, H-3″, Ha-10, H-3′, H-5″), 2.91 (1H, d, J = 18.8 Hz, Hb-10), 2.39 (5H, m, Ha-8, H-14, Ha-2″), 2.04 (1H, m, Hb-8), 1.74 (2H, m, H-2′), 1.51 (1H, m, Hb-2″), 1.30 (6H, m, H-6′, H-6″). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.9, 186.9, 186.6, 161.0, 156.4, 155.8, 135.6, 135.4, 134.5, 134.4, 120.9, 119.7, 118.4, 111.3, 111.2, 100.9, 100.6, 80.7, 77.2, 75.5, 71.5, 69.5, 67.8, 56.6, 56.2, 47.0, 35.1, 34.8, 33.3, 29.7, 24.9, 24.7, 17.8, 17.5.

7-[3-Amino-4-*O*-(α-L-mycarosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (3). Compound **3** was prepared from protected glycoside **30** according to general procedure D (purified on a column of silica gel, MeOH/CH_2Cl_2 1:25−1:10) as a deep red solid (76%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{34}H_{41}NO_{13}Na^+$ 694.2470, found 694.2454. ^1H NMR (500 MHz, $CDCl_3$) δ 7.89 (1H, d, J = 7.6 Hz, H-1), 7.71 (1H, t, J = 8.0 Hz, H-2), 7.30 (1H, d, J = 8.4 Hz, H-3), 5.41 (1H, d, J = 3.0 Hz, H-1′), 5.13 (1H, br, H-7), 4.95 (1H, J = 2.6 Hz, H-1″), 4.07 (1H, m, H-5′), 3.98 (3H, s, OMe-4), 3.87 (1H, m, H-5″), 3.53 (1H, br, H-4′), 3.07 (2H, m, Ha-10, H-3′), 2.94 (1H, d, J = 79.6 Hz, H-4″), 2.79 (1H, d, J = 18.7 Hz, Hb-10), 2.34 (3H, s, H-14), 2.15 (2H, m, Ha-2″, Ha-8), 2.01 (1H, m, Hb-8), 1.81 (1H, m, Hb-2″), 1.68 (2H, m, H-2′), 1.24 (9H, m, H-6′, H-6″, CH_3-3″). ^{13}C NMR (125 MHz, $CDCl_3$) δ 211.7, 186.6, 186.4, 160.9, 156.3, 155.6, 135.6, 135.3, 134.2, 134.1, 120.7, 119.6, 118.4, 111.2, 111.1, 100.9, 99.8, 81.6, 76.7, 76.5, 69.7, 69.5, 67.8, 66.7, 56.6, 46.5, 41.0, 34.8, 34.3, 33.2, 25.4, 24.7, 17.9, 17.7

7-[3-Amino-4-*O*-(β-L-mycarosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (4). Compound **4** was prepared from protected glycoside **31** according to general procedure D (purified on a column of silica gel, MeOH/CH_2Cl_2 1:10−1:6) as a deep red solids (75%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{34}H_{41}NO_{13}Na^+$ 694.2470, found 694.2462. ^1H NMR (500 MHz, $CDCl_3$) δ 7.99 (1H, d, J = 7.7 Hz, H-1), 7.77 (1H, t, J = 8.1 Hz, H-2), 7.37 (1H, d, J = 8.3 Hz, H-3), 5.51 (1H, d, J = 3.9 Hz, H-1′), 4.98 (1H, d, J = 7.3 Hz, H-1″), 4.85 (1H, J = 3.5 Hz, H-7), 4.05 (4H, m, H-5′, OMe-4), 3.76 (1H, d, J = 2.5 Hz, J = 18.8 Hz, Ha-10), 3.55 (1H, m, H-3′), 3.45 (1H, br, H-4′), 2.94 (1H, m, H-5″), 2.75 (1H, d, J = 18.8 Hz, Hb-10), 2.69 (1H, d, J = 9.7 Hz, H-4″), 2.43 (1H, m, Ha-8), 2.29 (3H, s, H-14), 2.03 (1H, m, Ha-2″), 1.91 (1H, m, Hb-8), 1.74 (3H, m, Hb-2″, H-2′), 1.32 (3H, d, J = 6.6 Hz, H-6′), 1.10 (3H, s, CH_3-3″), 0.72 (3H, d, J = 6.1 Hz, H-6″). ^{13}C NMR (125 MHz, $CDCl_3$) δ 208.3, 187.1, 186.6, 161.1, 157.3, 155.4, 135.6, 135.5, 135.0, 134.9, 121.1, 119.8, 118.5, 111.1, 111.0, 101.8, 95.5, 82.4, 76.3, 71.0, 69.9, 69.1, 66.9, 66.8, 56.7, 41.9, 37.9, 34.3, 29.5, 27.3, 25.7, 24.3, 17.5, 17.1.

7-[3-Amino-4-*O*-(α-L-cladinosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (5). Compound **5** was prepared from the protected glycoside **32** according to general procedure D (purified on a column of silica gel, MeOH/CH_2Cl_2 1:30−1:15) as a deep-red solid (85%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{35}H_{43}NO_{13}Na^+$ 708.2626, found 708.2601. ^1H NMR (500 MHz, $CDCl_3$) δ 7.95 (1H, d, J = 7.8 Hz, H-1), 7.71 (1H, t, J = 8.2 Hz, H-2), 7.32 (1H, d, J = 8.1 Hz, H-3), 5.43 (1H, d, J = 3.1 Hz, H-1′), 5.21 (1H, br, H-7), 4.79 (1H, d, J = 4.3 Hz, H-1″), 4.01 (5H, m, H-5′, H-5″, OMe-4), 3.48 (1H, br, H-4′), 3.29 (3H, s, OMe-3″), 3.14 (1H, d, J = 18.7 Hz, Ha-10), 2.98 (2H, m, H-3′, H-4″), 2.84 (1H, d, J = 18.7 Hz, Hb-10), 2.43 (1H, m, Ha-2″), 2.36 (3H, s, H-14), 2.25 (1H, m, Ha-8), 2.05 (1H, m, Hb-8), 1.73 (2H, m, H-2′), 1.55 (1H, m, Hb-2″), 1.21 (9H, m, H-6′, CH_3-3″, H-6″). ^{13}C NMR (125 MHz, $CDCl_3$) δ 212.0, 186.7, 186.5, 160.9, 156.4, 155.8, 135.6, 135.5, 134.4, 134.4, 120.9, 119.7, 118.4, 111.3, 111.2, 101.3, 98.1, 79.9, 77.9, 76.8, 72.8, 69.4, 68.3, 65.7, 56.6, 49.5, 46.6, 35.5, 34.8, 34.3, 33.4, 24.8, 21.5, 17.9, 17.8.

7-[3-Amino-4-*O*-(2-deoxy-l-α-D-glucopyranosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (6). Compound **6** was prepared from glycoside **33** according to the general procedure (purified on a column of silica gel, MeOH/CH_2Cl_2 1:15) as a red solid (62%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{33}H_{39}NO_{14}Na^+$ 696.2263, found 696.2245. ^1H NMR (500 MHz, MeOH-d_4) δ 7.55 (2H, br, H-1, H-2), 7.25 (1H, br, H-3), 5.36 (1H, br, H-1′), 4.91 (1H, d, J = 3.2 Hz, H-7), 4.84 (1H, br, H-1″), 4.34 (1H, m, H-5′), 4.24 (1H, m, H-3′), 3.95 (1H, br, H-4′), 3.93 (1H, m, H-5″), 3.84 (3H, s, MeO-4), 3.81 (2H, m, H-3″, Ha-6″), 3.74 (1H, m, Hb-6″), 3.28 (1H, t, H-4″), 2.85 (1H, m, Ha-10), 2.73 (1H, m, Hb-10), 2.36 (3H, s, H-14), 2.30 (1H, m, Ha-8), 2.23 (2H, m, Ha-2″, Hb-8), 2.05 (1H, m, Hb-2″), 1.80 (1H, m, Ha-2′), 1.60 (1H, m, Hb-2′), 1.32 (3H, d, J = 6.5 Hz, H-6′). ^{13}C NMR (125 MHz, MeOH-d_4) δ 212.3, 186.5, 186.3, 160.7, 157.4, 155.8, 135.5, 134.4, 134.3, 134.1, 118.9, 118.5, 117.1, 110.6, 110.4, 100.4, 99.6, 75.8, 75.0, 73.5, 71.8, 70.2, 68.3, 66.7, 61.3, 55.5, 48.3, 37.4, 35.5, 31.9, 28.7, 23.3, 17.0.

7-[3-Amino-4-*O*-(α-L-rhamnopyranosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (7). Compound **16** was prepared from glycoside **34** according to general procedure D (purified on a column of silica gel, MeOH/CH_2Cl_2 1:6) as a red solid (67%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{33}H_{39}NO_{14}Na^+$ 696.2263, found 696.2265. ^1H NMR (500 MHz, MeOH-d_4) δ 7.68 (2H, m, H-1, H-2), 7.39 (1H, d, J = 7.8 Hz, H-3), 5.37 (1H, d, J = 2.0 Hz, H-1′), 4.94 (1H, br, H-7), 4.82 (1H, br, H-1″), 4.28 (1H, m, H-5′), 4.23 (1H, m, H-2″), 3.93 (3H, s, MeO-4), 3.79 (2H, m, H-3″, H-5″), 3.67 (1H, br, H-4′), 3.48 (1H, t, J = 9.1 Hz, H-4″), 3.19 (1H, m, H-3′), 2.91 (1H, d, J = 18.2 Hz, Ha-10), 2.77 (1H, d, J = 18.2 Hz, Hb-10), 2.28 (1H, m, Ha-8), 2.06 (1H, m, Hb-8), 1.83 (2H, m, H-2′), 1.32 (6H, m, H-6′, H-6″). ^{13}C NMR (125 MHz, MeOH-d_4) δ 212.3, 186.7, 186.5, 161.1, 156.0, 154.9, 135.7, 135.1, 134.6, 134.3, 120.3, 119.1, 118.9, 111.1, 110.8, 103.0, 100.5, 80.8, 76.0, 72.2, 70.9, 70.7, 70.2.3, 70.0, 67.5, 55.7, 48.4, 46.5, 35.5, 32.3, 31.9, 16.6, 16.3.

7-[3-Amino-4-*O*-(3-azido-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl)-2,3,6-trideoxy-α-L-*lyxo*-hexopyranosyl]daunorubicinone (8). Compound **8** was prepared from glycoside **35** according to general procedure D (purified on a column of silica gel, MeOH/CH_2Cl_2 1:35) as a red solid (66%). HRMS $(M + Na)^+$ (ESI^+) calcd for $C_{33}H_{38}N_4O_{12}Na^+$ 705.2378, found 705.2375. ^1H NMR (500 MHz, MeOH-d_4) δ 7.65 (2H, m, H-1, H-2), 7.37 (1H, m, H-3), 5.34 (1H, br, H-1′), 5.04 (1H, d, J = 2.4 Hz, H-7), 4.93 (1H, br, H-1″), 4.24 (1H, m, H-5′), 4.08 (1H, m, H-5″), 3.91 (3H, s, MeO-4), 3.76 (2H, m, H-3′, H-4′), 3.62 (1H, br, H-4″), 3.16 (1H, m, H-3″), 2.87 (1H, d, J = 18.3 Hz, Ha-10), 2.76 (1H, d, J = 18.3 Hz, Hb-10), 2.33, 2.20, 2.00, 1.80 (6H, m,

H-8, H-2′, H-2″), 1.28 (3H, d, J = 6.4 Hz, H-6′), 1.21 (3H, d, J = 6.2 Hz, H-6″). ^{13}C NMR (125 MHz, MeOH-d_4) δ 212.3, 186.1, 185.9, 160.9, 155.9, 154.7, 135.6, 134.6, 134.4, 134.2, 119.8, 118.9, 118.7, 110.7, 110.5, 101.0, 99.9, 81.0, 77.5, 76.0, 72.0, 69.8, 69.5, 67.7, 55.7, 55.6, 46.4, 32.8, 31.9, 29.5, 28.4, 16.5, 15.9.

Cell Culture. Cell lines K562 and SW620 were cultured in RPMI 1640 supplemented with 10% fetal bovine serum (FBS), 1% nonessential amino acid, penicillin (100 units/mL), and 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.

Cytotoxicity of Synthesized Compounds (Daunorubicin (DNR), 1−8) (MTS Assay). Leukemia K562 cells and colon cancer SW620 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, 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 software from the dose−response curves of the percentage of cell growth vs control (no compound added).

Inhibition of Topoisomerase II with ICT Bioassay. ICT bioassay was performed according to previously described procedures.[40−42] Briefly, HeLa cells were incubated with or without 50 μM positive control etoposide or tested compounds for 30 min during exponential growth at 37 °C. Following detergent lyses with sarkosyl, DNA and denatured proteins were fractionated by CsCl gradient centrifugation. The fraction was collected from the bottom. The DNA and covalently bonded proteins are associated with fractions, while free proteins and debris are at the top of the gradient. Each fraction (50 μL) was slotted onto a Hybond ECL membrane (Amersham Biosciences), and the blot was probed with anti-topo-I antibody. The immunoblot signals were visualized by chemiluminescence (Amersham Biosciences).

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