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Design, Synthesis and Biological Activity of 7-*O*-(4-*O*-Acetyl-3-iodo-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)daunomycinone and 7-*O*-(3-Chloro-2,3,6-trideoxy-4-*O*-propanoyl-α-L-*lyxo*-hexopyranosyl)daunomycinone

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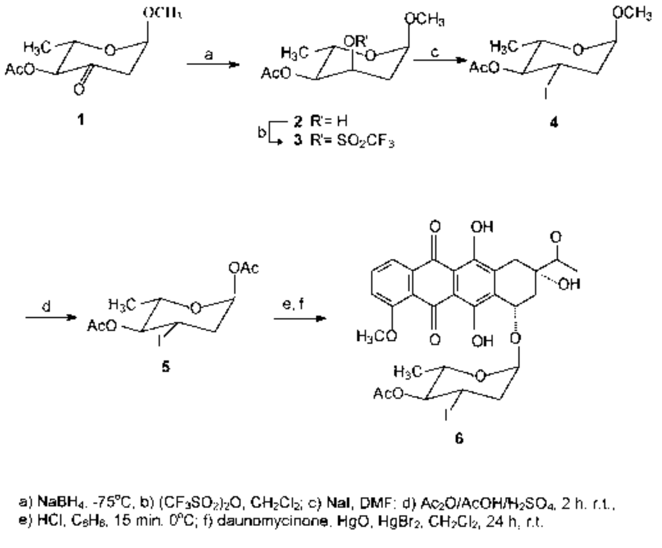
The synthesis and pharmacological evaluation of 7-*O*-(4-*O*-acetyl-3-iodo-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)daunomycinone and 7-*O*-(3-chloro-2,3,6-trideoxy-4-*O*-propanoyl-α-L-*lyxo*-hexopyranosyl)daunomycinone are described. Their cytotoxic activity was evaluated against normal and resistant cell lines. Both compounds exhibited activity against the adriamycin resistant cell line KB-A1. These results support the hypothesis that the increased lipophilicity of the sugar part of anthracyclines is associated with their ability to overcome multidrug resistance (MDR).

Key words anthracycline; cytotoxicity; lipophilicity; multidrug resistance

Over the last two decades the anthracycline antibiotics adriamycin (ADR) and daunomycin have proved to be important cancer chemotherapeutic drugs for the management of hematologic malignancies and a large variety of solid tumors.[1] However their effectiveness is often limited because of the development of cardiotoxicity and tumor recurrence after initially effective systemic chemotherapy, due to multidrug resistance (MDR).[2] This phenomenon is characterized by resistance to several structurally and mechanistically different drugs developed by tumor cells upon treatment with one drug. In spite of intensive investigation the exact mechanism of resistance is not totally understood, but it has been shown that MDR is associated with an overexpression of cell membrane proteins, which enhance efflux of the drug and an overexpression of glutathione transferase, which transforms xenobiotics to glutathione conjugates for excretion.[3] Different approaches to overcome MDR have been tried, among them the modification of the sugar part of anthracyclines, which is known to be a critical determinant of their pharmacological action.[4] Indeed certain anthracycline molecules have been synthesized that have shown *in vitro* to have toxic effects on resistant MDR cells. Earlier reports have shown that 3′-deamino-3′-halo derivatives possessing the *lyxo* configuration, were active against resistant tumor cells,[5,6] suggesting that the increased lipophilicity of the sugar moiety of anthracyclines could be related to their ability to overcome MDR.[7,8]

Having in mind the above mentioned considerations, we have synthesized 7-*O*-(4-*O*-acetyl-3-iodo-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)daunomycinone (**6**) and 7-*O*-(3-chloro-2,3,6-trideoxy-4-*O*-propanoyl-α-L-*lyxo*-hexopyranosyl)daunomycinone (**14**). Compound **6** bears the highly lipophilic iodine substitution at C-3′ and is structurally related to the 3′-deamino-3′-chloro and bromo epidaunomycin analogs, which have shown interesting activity against adriamycin resistant cell lines.[5] On the other hand, compound **14** was prepared in order to clarify the role of stereochemistry at C-4′. The cytotoxicity of the new derivatives on various murine and human cell lines, both sensitive and resistant, have been measured.

The synthesis of the iodo derivative is depicted in Chart 1. We used compound **1**[9] as the starting material, which was reduced with sodium borohydride at −75 °C to give methyl 4-*O*-acetyl-2,6-dideoxy-α-L-*ribo*-hexopyranoside (**2**) as the major product, together with methyl 3-*O*-acetyl-2,6-dideoxy-α-L-*ribo*-hexopyranoside (**7**) in a 16% yield (Fig. 1). When the reduction was carried out at higher temperature we obtained a significant amount of **7**, which reached a yield of nearly 50% at 0 °C. The formation of **7** may proceed through a cyclic intermediate, which is probably due to the nucleophilic attack of the 3-alkoxide anion to the carbonyl of the neighboring acetyl group. As far as the reduction of **1** is concerned, the *syn*-axial attack of the hydride anion is hindered and the axial derivative **2** was obtained, whereas, reduction of the corresponding β-anomer afforded the undesired compound **8** (Fig. 1), which is thermodynamically favored. Treatment of **2** with trifluoromethanesulfonic anhydride provided the highly unstable ester **3**. Attempts to purify this ester resulted in the formation of the 2,3-unsaturated analog,[10,11] consequently, **3** was not isolated, but was immediately sub-



a) $NaBH_4$, -75°C, b) $(CF_3SO_2)_2O$, CH_2Cl_2; c) NaI, DMF; d) $Ac_2O/AcOH/H_2SO_4$, 2 h, r.t., e) HCl, C_6H_6, 15 min, 0°C; f) daunomycinone, HgO, $HgBr_2$, CH_2Cl_2, 24 h, r.t.

Chart 1

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jected to reaction with sodium iodide in dry dimethylformamide (DMF), to afford methyl 4-*O*-acetyl-3-iodo-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside (**4**). Compound **4** was subsequently converted by acetolysis to an $\alpha:\beta$ mixture of the corresponding 1-*O*-acetyl derivatives, containing the α anomer (**5**) as the predominant component (90% yield). The reaction of **5** with dry hydrogen chloride in benzene gave the corresponding glycosyl chloride, which was used immediately and in excess for coupling with daunorubicinone to provide a 40% yield of **6**, contaminated with a small amount of the β-L anomer.

The synthetic procedure for the preparation of compound **14** is shown in Chart 2. We used as the starting material the anomeric mixture of methyl 4-*O*-acetyl-3-chloro-2,3,6-trideoxy-L-*arabino*-hexopyranoside (**9**), which has been prepared according to known procedures, starting from 3,4-di-*O*-acetyl-L-rhamnal.[12] Transesterification with sodium methoxide in methanol provided hexopyranoside **10**, which was treated with trifluoromethanesulfonic anhydride in dichloroethane in the presence of 4-dimethylaminopyridine (DMAP) to give a mixture of both anomers of **11** in very good yield. Treatment of the anomeric mixture of **11** with cesium propionate in anhydrous DMF afforded both anomers of methyl 4-*O*-propanoyl-3-chloro-2,3,6-trideoxy-L-*lyxo*-hexopyranoside (**12**). The anomeric mixture of **12** was subsequently converted by acetolysis to a mixture of both anomers of the 1-*O*-acetyl derivative **13** and then, to the corresponding glycosyl chloride. Coupling of this chloride with daunorubicinone was carried out in dichloromethane solution, under modified Köenings-Knorr conditions (yellow mercury II oxide and mercury II bromide), to give only the α anomer of compound **14** in 45% yield, after purification through column chromatography. For analytical purposes, both anomers of compounds **10**—**13** were separated by column chromatography and fully characterised.

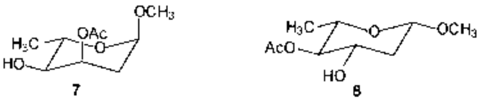
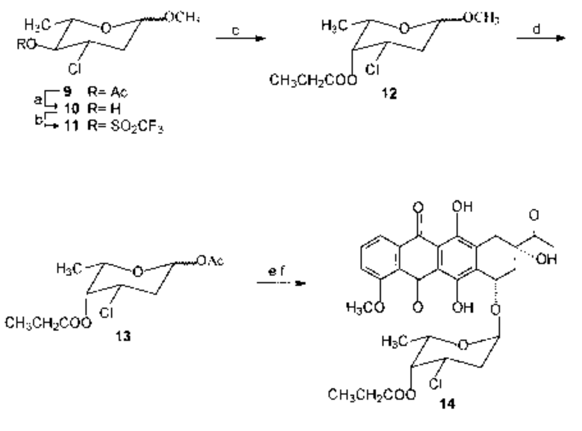


Fig. 1



a) MeONa. MeOH; b) $(CF_3SO_2)_2O$ $ClCH_2CH_2Cl$. DMAP. c) $CH_3CH_2COO^-$ Cs^+, DMF: d) $Ac_2O/AcOH/H_2SO_4$, 2 h r.t., e) HCl. C_6H_6. 15 min. 0°C; f) daunomycinone, HgO, $HgBr_2$. CH_2Cl_2, 24 h, r.t.

Chart 2

The newly synthesized compounds were screened for their cytotoxicity against two leukemia cell lines (P388 leukemia, L1210 leukemia) and a panel of solid tumors: the non-small cell lung cancers A-549 and NCI-H460, the colon cancer HT-29, the ovarian cancer (OVCAR-3), the B16 melanoma, the human epidermoid carcinoma cell line KB-3-1 and its 600-fold resistant to ADR subline KB-A1. The results are summarized in Table 1. Both derivatives have reduced potency compared to ADR, when tested on the above mentioned panel of cell lines, but exhibited a very interesting activity towards the resistant cell line (KB-A1). The effect on the cellular cycle was also examined and was found to be that expected for a DNA intercalating agent. Interestingly, as presented in Table 2, both compounds induced a massive accumulation of HT-29 cells in the G2+M phase of the cell cycle. These findings support the hypothesis that the enhanced lipophilicity of the sugar part of anthracyclines may favor the potency against resistant cell lines. On the other hand the configuration at C4′ seems to be less important in the case of the resistant cell line, since no great biological difference between the two compounds was observed.

Further investigation, concerning the effect of various modifications at the sugar part of anthracyclines in the biological activity, is currently in progress in our laboratories.

Experimental

General Remarks Optical rotations were measured with a Perkin-Elmer 341 polarimeter. ^{13}C-NMR spectra were recorded on a Bruker AC200 spectrometer and ^1H-NMR spectra on a Bruker DRX400 instrument in deuterated solvents and were referenced to tetramethylsilane (TMS) (δ scale). Mass spectra were recorded with a Nermag R 10-10C spectrometer using ES-MS or CID-MS (reagent gas, NH_3) techniques. Column chromatography was performed on silica gel [Merck, 0.04—0.06 mm (flash chromatography)]. Analytical thin layer chromatography (TLC) was carried out on precoated (0.25 mm) Merck silica gel F-254 plates.

Methyl 4-*O*-Acetyl-2,6-dideoxy-α-L-*ribo*-hexopyranoside (2) To a solution of **1** (0.13 g, 0.64 mmol) in methanol (8 ml) at −75 °C, was added sodium borohydride (0.05 g, 1.32 mmol). The reaction mixture was stirred at

Table 2. Cell Cycle Effect of **6** and **14** on HT-29 Cell Line

	% of cells in the indicated phase		
	G1	S	G2+M
Control	53	25	22
6 (2.5 μM)	16	13	71
14 (10 μM)	15	9	76

Table 1. Inhibition of the Proliferation IC_{50} (μM)

Cell line	P388	L1210	B16	HT-29	NCI-H460	A549	OVCAR-3	KB-3-1	KB-A1
ADR	0.019	0.024	0.007	0.064	0.027	0.039	0.024	0.021	12.6
6	0.63	>100	0.28	2.15	0.66	0.65	1.73	0.70	1.78
14	0.64	2.58	0.45	1.44	0.50	0.42	1.53	1.70	0.75

−75 °C for 1 h and then allowed to warm up at room temperature. The mixture was then neutralized by the addition of Amberlite IR-120, the insoluble material was filtered off and washed with methanol. The solvent was evaporated to dryness and the residue was purified by column chromatography (silica gel), using a mixture of CH_2Cl_2/MeOH 99/1 as the eluent, to provide **2** (0.08 g, 61%), together with **7** (0.02 g, 16%).

Compound **2**: Syrup, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.20 (3H, d, *J*=6.2 Hz, H-6), 1.95 (1H, ~dt, *J*=14.5, 3.7 Hz, H-2ax), 2.10 (3H, s, CH_3COO-4), 2.11 (1H, dd, *J*=14.5, 3.3 Hz, H-2eq), 3.37 (3H, s, CH_3O), 4.15-4.05 (2H, m, H-5/H-3), 4.51 (1H, dd, *J*=9.9, 2.9 Hz, H-4), 4.77 (1H, d, *J*=3.7 Hz, H-1). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 17.5 (C-6), 21.1 ($\underline{C}H_3COO$-4), 35.2 (C-2), 55.2 (CH_3O), 61.2 (C-3), 65.5 (C-5), 74.9 (C-4), 98.5 (C-1), 170.0 ($CH_3\underline{C}OO$-4). CID-MS *m/z*: 222 ($M+NH_4^+$). $[\alpha]_D^{20}$ −79.1° (*c*=0.27, $CHCl_3$).

Methyl 3-*O*-acetyl-2,6-dideoxy-α-L-*ribo*-hexopyranoside **7**: Syrup, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.29 (3H, d, *J*=6.2 Hz, H-6), 1.92 (1H, ~dt, *J*=15.3, 4.1, 3.7 Hz, H-2ax), 2.09 (3H, s, CH_3COO-3), 2.21 (1H, ddd, *J*=15.3, 3.3, 1.2 Hz, H-2eq), 3.31 (3H, s, CH_3O), 3.39 (1H, dd, *J*=9.9, 3.3 Hz, H-4), 3.94 (1H, m, H-5), 4.63 (1H, dd, *J*=4.1, 1.2 Hz, H-1), 5.05 (1H, ~q, *J*=3.7, 3.3, 3.3 Hz, H-3). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 17.4 (C-6), 21.0 ($\underline{C}H_3COO$-3), 33.0 (C-2), 55.1 (CH_3O), 64.0 (C-5), 69.4 (C-3), 71.5 (C-4), 96.8 (C-1), 169.1 ($CH_3\underline{C}OO$-4). CID-MS *m/z*: 222 ($M+NH_4^+$). $[\alpha]_D^{20}$ −121.5° (*c*= 0.20, $CHCl_3$).

Methyl 4-*O*-Acetyl-2,6-dideoxy-α-L-*arabino*-hexopyranoside (8) Reduction of methyl 4-*O*-acetyl-2,6-dideoxy-β-L-*erythro*-hexopyranosid-3-ulose (0.03 g, 0.15 mmol), under the conditions described for compound **2**, and the subsequent chromatographic purification of the product using a mixture of CH_2Cl_2/MeOH 99/1 as the mobile phase, afforded the title compound (0.022 g, 73%) as a syrup: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.22 (3H, d, *J*=6.4 Hz, H-6), 1.63 (1H, dt, *J*=12.7, 12.7, 9.3 Hz, H-2ax), 2.11 (3H, s, $\underline{C}H_3COO$-4), 2.26 (1H, ddd, *J*=12.7, 5.4, 1.9 Hz, H-2eq), 3.40 (1H, m, H-5), 3.47 (3H, s, CH_3O), 3.71 (1H, m, H-3), 4.38 (1H, dd, *J*=9.3, 1.9 Hz, H-1), 4.45 (1H, t, *J*=9.3 Hz, H-4). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 17.9 (C-6), 21.3 ($\underline{C}H_3COO$-4), 39.6 (C-2), 56.4 (CH_3O), 70.0 (C-5), 70.32 (C-3), 78.9 (C-4), 100.8 (C-1), 169.8 ($CH_3\underline{C}OO$-4). CID-MS *m/z*: 222 ($M+NH_4^+$). $[\alpha]_D^{20}$ 0.0° (*c*=0.18, 0.55, $CHCl_3$).

Methyl 4-*O*-Acetyl-3-iodo-2,3,6-trideoxy-α-L-*arabino*-hexopyranoside (4) A solution of methyl 4-*O*-acetyl-2,6-dideoxy-α-L-*ribo*-hexopyranoside (**2**, 0.06 g, 0.29 mmol) in dry dichloromethane (1.5 ml), was added dropwise to a stirred solution of pyridine (0.062 ml) and trifluoromethanesulfonic anhydride (0.12 ml, 0.71 mmol) in dry dichloromethane (3 ml) at −10 °C. Stirring was continued for 1 h at −10 °C, and then ice-water was added, the mixture was extracted with dichloromethane, the organic layer was dried (Na_2SO_4), and vacuum evaporated to provide methyl 4-*O*-acetyl-2,3,6-trideoxy-3-*O*-trifluoromethanesulfonyl-α-L-*ribo*-hexopyranoside (**3**). This compound, without further purification, was dissolved in dry DMF (3 ml), sodium iodide (0.12 g, 0.80 mmol) was added and the mixture was stirred at room temperature for 1 h. The solvent was then removed *in vacuo* and the residue was purified by column chromatography (silica gel) using a mixture of CH_2Cl_2/MeOH 99.5/0.5 as the eluent, to provide pure **4** (0.04 g, 44%) as a syrup: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.16 (3H, d, *J*=6.4 Hz, H-6), 2.11 (3H, s, CH_3COO-4), 2.41 (1H, dt, *J*=13.2, 13.2, 3.4 Hz, H-2ax), 2.55 (1H, dd, *J*=13.2, 4.6 Hz, H-2eq), 3.30 (3H, s, CH_3O), 3.77 (1H, m, H-5), 4.35 (1H, m, H-3), 4.47 (1H, d, *J*=3.4 Hz, H-1), 4.88 (1H, ~t, *J*=10.3, 9.8 Hz, H-4). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 18.2 (C-6), 21.1 ($\underline{C}H_3COO$-4), 22.9 (C-3), 43.0 (C-2), 54.6 (CH_3O), 67.4 (C-5), 77.3 (C-4), 98.4 (C-1), 169.7 ($CH_3\underline{C}OO$-4). CID-MS *m/z*: 332 ($M+NH_4^+$). $[\alpha]_D^{20}$ −44.6° (*c*=0.12, $CHCl_3$).

1,4-Di-*O*-acetyl-3-iodo-2,3,6-trideoxy-α-L-*arabino*-hexopyranose (5) A solution of 0.5% of sulfuric acid in acetic anhydride (0.025 ml), was added dropwise under stirring to a mixture of **4** (0.040 g, 0.127 mmol), acetic anhydride (0.25 ml) and acetic acid (0.25 ml). Stirring was continued at room temperature for 1 h and then water was added, the mixture was extracted with diethyl ether, the organic layer was dried (Na_2SO_4) and the solvent was evaporated to dryness to give a mixture of both anomers of **5** (0.039 g, 0.114 mmol, 90%). In this mixture, the α-anomer was the predominant one and the β-anomer was only detected by TLC. Data of the α-anomer: syrup, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.17 (3H, d, *J*=6.3 Hz, H-6), 2.12, 2.09 (each s of 3H, CH_3COO-4, -1), 2.63—2.50 (2H, m, H-2ax/H-2eq), 3.87 (1H, m, H-5), 4.32 (1H, m, H-3), 4.92 (1H, ~t, *J*=10.1, 9.4 Hz, H-4), 5.89 (1H, dd, *J*=3.3, 1.3 Hz, H-1). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 18.2 (C-6), 20.8 (C-3), 21.0 ($\underline{C}H_3COO$-1, -4), 41.7 (C-2), 70.0 (C-5), 76.5 (C-4), 91.5 (C-1), 169.0 ($CH_3\underline{C}OO$-1), 169.5 ($CH_3\underline{C}OO$-4). CID-MS *m/z*: 360 ($M+NH_4^+$). $[\alpha]_D^{20}$ −37.7° (*c*= 0.18, $CHCl_3$).

7-*O*-(4-*O*-Acetyl-3-iodo-2,3,6-trideoxy-α-L-*arabino*-hexopyranosyl)-daunomycinone (6) Dry hydrogen chloride was purged for 15 min into a solution of **5** (0.04 g, 0.12 mmol) in dry benzene (5 ml) at 0 °C. The solvent was then vacuum evaporated, and the last traces of HCl were azeotropically removed with toluene. The residue was dissolved in dry dichloromethane and yellow mercury II oxide (0.20 g, 0.92 mmol), mercury II bromide (0.10 g, 0.28 mmol), a solution of daunomycinone (0.02 g, 0.05 mmol) in dry dichloromethane (5 ml) were added and the mixture was stirred at room temperature for 4 h. The insoluble material was then filtered off, washed with dichloromethane and the filtrate was washed with a saturated $NaHCO_3$ solution. The organic layer was dried (Na_2SO_4), the solvent was evaporated to dryness and the residue was purified by column chromatography using a mixture of toluene/EtOAc 95/5 as the eluent. Compound **6** (0.014 g, 40%) was obtained as a syrup, in a ratio α/β=95/5. Data of the α-anomer: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.25 (3H, d, *J*=6.2 Hz, CH_3-5′), 2.11 (1H, d, *J*=14.9 Hz, H-8b), 2.12 (3H, s, CH_3CO-4′), 2.30 (1H, ~d, *J*=14.9 Hz, H-8a), 2.41 (3H, s, CH_3CO-9), 2.45 (1H, dt, *J*=12.9, 12.9, 3.7 Hz, H-2′ax), 2.59 (1H, dd, *J*=12.9, 4.6 Hz, H-2′eq), 2.93 (1H, d, *J*=18.7 Hz, H-10b), 3.25 (1H, dd, *J*=18.7, 1.6 Hz, H-10a), 3.98 (1H, m, H-5′), 4.06 (3H, s, CH_3O), 4.17 (1H, m, H-3′), 4.40 (1H, s, OH-9), 4.91 (1H, dd, *J*=10.4, 9.5 Hz, H-4′), 5.25 (2H, m, H-7, H-1′), 7.38 (1H, d, *J*=8.3 Hz, H-3), 7.79 (1H, t, *J*=8.3 Hz, H-2), 8.05 (1H, d, *J*=8.3 Hz, H-1), 13.29 (1H, s, OH-11), 14.00 (1H, s, OH-6). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 18.1 (C-6′), 21.9 ($\underline{C}H_3COO$-4′), 24.7 (C-3′), 26.7 ($\underline{C}H_3CO$-9), 33.3 (C-10), 35.0 (C-8), 43.0 (C-2′), 56.7 (CH_3O), 68.9 (C-5′), 70.1 (C-7), 77.6 (C-4′), 101.0 (C-1′), 118.4 (C-3), 119.8 (C-1), 135.8 (C-2), 169.9 ($CH_3\underline{C}OO$-4). ES-MS *m/z*: 703 (M+Na). $[\alpha]_D^{20}$ +108.9° (*c*=0.06, $CHCl_3$).

Methyl 3-Chloro-2,3,6-trideoxy-L-*arabino*-hexopyranoside (10) Sodium methoxide (0.43 g, 7.96 mmol) was added to an ice-cold solution of **9** (1.0 g, 4.49 mmol) in dry methanol (20 ml). The mixture was stirred at 4 °C for 4 h and was then neutralized by the addition of Amberlite IRC-50. The insoluble material was filtered off, washed with methanol, the solvent was evaporated to dryness and the residue was purified by column chromatography (silica gel), using a mixture of cyclohexane/EtOAc 95/5 as the eluent, to provide both anomers of **10** (0.77 g, 95%) in a ratio α/β=4/1.

Compound **10α**: Syrup, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.29 (3H, d, *J*=6.3 Hz, H-6), 2.01 (1H, dt, *J*=13.2, 13.2, 3.1 Hz, H-2ax), 2.35 (1H, ddd, *J*=13.2, 4.6, 1.0 Hz, H-2eq), 2.85 (1H, OH), 3.25 (1H, t, *J*=10.0 Hz, H-4), 3.30 (3H, s, CH_3O), 3.68 (1H, m, H-5), 4.17 (1H, m, H-3), 4.68 (1H, dd, *J*=3.4, 1.0 Hz, H-1). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 18.0 (C-6), 39.7 (C-2), 54.5 (CH_3O), 60.2 (C-3), 68.1 (C-5), 77.6 (C-4), 97.7 (C-1). CID-MS *m/z*: 198 ($M+NH_4^+$). $[\alpha]_D^{20}$ −150.8° (*c*=0.24, $CHCl_3$).

Compound **10β**: Syrup, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.36 (3H, d, *J*=6.2 Hz, H-6), 1.93 (1H, dt, *J*=12.7, 12.7, 9.3 Hz, H-2ax), 2.40 (1H, ddd, *J*=12.7, 5.2, 1.9 Hz, H-2eq), 3.22 (1H, t, *J*=9.8 Hz, H-4), 3.36 (1H, m, H-5), 3.48 (3H, s, CH_3O), 3.88 (1H, m, H-3), 4.36 (1H, dd, *J*=9.3, 1.9 Hz, H-1). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 18.1 (C-6), 40.8 (C-2), 56.5 (CH_3O), 61.0 (C-3), 72.8 (C-5), 77.0 (C-4), 100.4 (C-1). CID-MS *m/z*: 198 ($M+NH_4^+$). $[\alpha]_D^{20}$ +42.6° (*c*= 0.26, $CHCl_3$).

Methyl 3-Chloro-2,3,6-trideoxy-4-*O*-trifluoromethanesulfonyl-L-*arabino*-hexopyranoside (11) To an ice-cold solution of **10** (0.60 g, 3.32 mmol) and DMAP (1.0 g, 8.2 mmol) in dry 1,2-dichloroethane (40 ml) was added dropwise, under Ar, trifluoromethanesulfonic anhydride (0.62 ml, 3.67 mmol). The mixture was stirred for 2 h and then poured into ice-water, extracted with dichloromethane, dried (Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography (silica gel flash) using a mixture of cyclohexane/EtOAc (95/5) as the eluent to give both anomers of **11** (0.62 g, 60%) in a ratio α/β=4/1.

Compound **11α**: Syrup, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.35 (3H, d, *J*=6.2 Hz, H-6), 2.07 (1H, dt, *J*=13.1, 13.1, 3.1 Hz, H-2ax), 2.43 (1H, ddd, *J*=13.1, 5.0, 1.0 Hz, H-2eq), 3.32 (3H, s, CH_3O), 3.91 (1H, m, H-5), 4.33 (1H, m, H-3), 4.67 (1H, dd, *J*=3.4, 1.0 Hz, H-1), 4.51 (1H, t, *J*=9.7 Hz, H-4). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 17.8 (C-6), 40.4 (C-2), 53.6 (C-3) , 54.9 (CH_3O), 65.8 (C-5), 90.3 (C-4), 97.1 (C-1). CID-MS *m/z*: 330 ($M+NH_4^+$). $[\alpha]_D^{20}$ −70.3° (*c*=0.78, $CHCl_3$).

Compound **11β**: Syrup, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.35 (3H, d, *J*=6.2 Hz, H-6), 1.92 (1H, dt, *J*=13.0, 13.0, 9.3 Hz, H-2ax), 2.42 (1H, ddd, *J*=13.0, 5.0, 2.0 Hz, H-2eq) , 3.40 (3H, s, CH_3O), 3.52 (1H, m, H-5), 4.00 (1H, m, H-3), 4.32 (1H, dd, *J*=9.3, 2.0 Hz, H-1) , 4.43 (1H, t, *J*=9.8 Hz, H-4). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 17.9 (C-6), 41.3 (C-2) , 54.8 (C-3), 56.8 (CH_3O), 70.9 (C-5), 89.6 (C-4), 100.3 (C-1). CID-MS *m/z*: 330 ($M+NH_4^+$). $[\alpha]_D^{20}$ +21.6° (*c*= 0.08, $CHCl_3$).

Methyl 3-Chloro-2,3,6-trideoxy-4-*O*-propanoyl-L-*lyxo*-hexopyranoside (12) Cesium propionate (1.0 g, 4.8 mmol) was added to a solution of **11** (0.60 g, 1.92 mmol) in dry DMF (20 ml) and the mixture was heated at 60 °C for 1.5 h. The solvent was then vacuum evaporated and the residue was purified by column chromatography (silica gel, flash) using a mixture of cyclohexane/EtOAc 95/5 as the eluent to provide both anomers of **12** (0.30 g, 66 %) in a ratio $\alpha/\beta=4/1$.

Compound **12*α***: Syrup, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.11 (3H, d, $J=6.4$ Hz, H-6), 1.18 (3H, t, $J=7.3$ Hz, $C\underline{H}_3CH_2CO$), 2.02 (1H, dd, $J=12.7$, 5.4 Hz H-2eq), 2.18 (1H, dt, $J=12.7$, 12.7, 3.4 Hz, H-2ax), 2.45 (2H, q, $J=7.3$ Hz, $CH_3C\underline{H}_2CO$), 3.31 (3H, s, CH_3O), 3.99 (1H, dq, $J=6.4$, 1.4 Hz, H-5), 4.41 (1H, m, H-3), 4.75 (1H, d, $J=3.4$ Hz, H-1), 5.16 (1H, dd, $J=3.4$, 1.4 Hz, H-4). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 9.4 ($\underline{C}H_3CH_2CO$), 17.0 (C-6), 27.5 ($CH_3\underline{C}H_2CO$), 34.9 (C-2), 53.5 (C-3), 54.9 (CH_3O), 65.6 (C-5), 71.0 (C-4), 98.4 (C-1), 173.5 ($CH_3CH_2\underline{C}O$–). CID-MS *m/z*: 254 ($M+NH_4^+$). $[\alpha]_D^{20}$ −67.7° ($c=0.23$, $CHCl_3$).

Compound **12*β***: Syrup, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.18 (3H, t, $J=7.5$ Hz, $C\underline{H}_3CH_2CO$), 1.20 (3H, d, $J=6.4$ Hz, H-6), 2.02 (1H, dt, $J=12.7$, 12.7, 9.1 Hz, H-2ax), 2.15 (1H, ddd, $J=12.7$, 5.3, 2.6 Hz, H-2eq), 2.46 (2H, q, $J=7.5$ Hz, $CH_3C\underline{H}_2CO$), 3.49 (3H, s, CH_3O), 3.64 (1H, dq, $J=6.4$, 1.1 Hz, H-5), 4.07 (1H, m, H-3), 4.36 (1H, dd, $J=9.1$, 2.6 Hz, H-1), 5.10 (1H, dd, $J=3.3$, 1.1 Hz, H-4). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 9.4 ($\underline{C}H_3CH_2CO$), 16.9 (C-6), 27.4 ($CH_3\underline{C}H_2CO$), 36.4 (C-2), 55.2 (C-3), 56.7 (CH_3O), 69.8 (C-4), 70.6 (C-5), 101.2 (C-1), 173.3 ($CH_3CH_2\underline{C}O$–). CID-MS *m/z*: 254 ($M+NH_4^+$). $[\alpha]_D^{20}$ +9.9° ($c=0.29$, $CHCl_3$).

1-*O*-Acetyl-3-chloro-2,3,6-trideoxy-4-*O*-propanoyl-L-*lyxo*-hexopyranose (13) A mixture of **12** (0.10 g, 0.42 mmol), acetic acid (0.6 ml) and acetic anhydride (0.6 ml), was treated under vigorous stirring with a 5% solution of sulfuric acid in acetic anhydride (0.06 ml) and the resulting solution was stirred at room temperature for 1.5 h. Water was then added and the mixture was extracted with ether. The organic phase was washed with a saturated $NaHCO_3$ solution and water, dried (Na_2SO_4) and evaporated to dryness. The residue was purified by column chromatography (silica gel, flash) using a mixture of cyclohexane/EtOAc 95/5 as the eluent to give both anomers of **13** (0.075 g, 67%) in a ratio 9/1.

Compound **13*α***: Syrup, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.12 (3H, d, $J=6.2$ Hz, H-6), 1.19 (3H, t, $J=7.5$ Hz, $C\underline{H}_3CH_2CO$), 2.05 (1H, dd, $J=13.2$, 5.0 Hz H-2eq), 2.08 (3H, s, CH_3COO), 2.32 (1H, dt, $J=13.2$, 13.2, 3.3 Hz, H-2ax), 2.45 (2H, q, $J=7.5$ Hz, $CH_3C\underline{H}_2CO$), 4.11 (1H, dq, $J=6.2$, 1.6 Hz, H-5), 4.42 (1H, m, H-3), 5.21 (1H, dd, $J=3.4$, 1.6 Hz, H-4), 6.18 (1H, d, $J=3.3$ Hz, H-1). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 9.3 ($\underline{C}H_3CH_2CO$), 17.0 (C-6), 21.1 ($\underline{C}H_3COO$), 27.4 ($CH_3\underline{C}H_2CO$), 33.7 (C-2), 52.7 (C-3), 68.3 (C-5), 70.4 (C-4), 91.7 (C-1), 169.2 ($CH_3\underline{C}OO$-1), 173.8 ($CH_3CH_2\underline{C}O$–). CID-MS *m/z*: 282 ($M+NH_4^+$). $[\alpha]_D^{20}$ −45.0° ($c=0.20$, $CHCl_3$).

Compound **13*β***: Syrup, ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.19 (3H, d, $J=6.4$ Hz, H-6), 1.19 (3H, t, $J=7.7$ Hz, $C\underline{H}_3CH_2CO$), 2.11 (3H, s, CH_3COO), 2.20—2.12 (2H, m, H-2eq/ H-2ax), 2.47 (2H, q, $J=7.7$ Hz, $C\underline{H}_3C\underline{H}_2CO$), 3.78 (1H, dq, $J=6.4$, 1.1 Hz, H-5), 4.13 (1H, m, H-3), 5.14 (1H, dd, $J=2.9$, 1.1 Hz, H-4), 5.68 (1H, dd, 7.3, 4.8 Hz, H-1). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 9.3 ($\underline{C}H_3CH_2CO$), 16.8 (C-6), 21.0 ($\underline{C}H_3COO$), 27.4 ($CH_3\underline{C}H_2CO$), 35.1 (C-2), 54.5 (C-3), 69.4 (C-4), 71.8 (C-5), 92.0 (C-1), 169.0 ($CH_3\underline{C}OO$-1), 173.5 ($CH_3CH_2\underline{C}O$–). CID-MS *m/z*: 282 ($M+NH_4^+$). $[\alpha]_D^{20}$ −49.9° ($c=0.22$, $CHCl_3$).

7-*O*-(3-Chloro-2,3,6-trideoxy-4-*O*-Propanoyl-*α*-L-*lyxo*-hexopyranosyl)daunomycinone (14) This compound was prepared by a procedure analogous to the one followed for **6**. Purification was carried out by column chromatography (silica gel, flash) using a mixture of CH_2Cl_2/MeOH (99.5/0.5) as the eluent, to afford **14** (0.024 g, 45%) as a syrup: ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.17 (3H, d, $J=6.2$ Hz, H-6′), 1.20 (3H, t, $J=7.5$ Hz, $C\underline{H}_3CH_2CO$), 2.30—2.00 (4H, m, H-2′ax / H-2′eq / H-8a / H-8b), 2.39 (3H, s, CH_3CO-9), 2.48 (2H, q, $J=7.5$ Hz, $CH_3C\underline{H}_2CO$), 2.95 (1H, d, $J=18.5$ Hz, H-10b), 3.23 (1H, dd, $J=18.5$, 1.5 Hz, H-10a), 4.08 (3H, s, CH_3O), 4.29—4.18 (2H, m, H-5′/ H-3′), 5.21 (1H, br s, H-4′), 5.27 (1H, br s, H-7), 5.52 (1H, d, $J=3.7$ Hz, H-1′), 7.39 (1H, d, $J=7.9$ Hz, H-3), 7.78 (1H, t, $J=7.9$ Hz, H-2), 8.03 (1H, d, $J=7.9$ Hz, H-1), 13.28 (1H, s, OH-11), 14.00 (1H, s, OH-6). ^{13}C-NMR (50 MHz, $CDCl_3$) δ: 9.4 ($\underline{C}H_3CH_2CO$), 17.0 (C-6′), 21.0, 24.7 (C-14), 27.5 ($CH_3\underline{C}H_2CO$), 33.4 (C-10), 34.7 (C-2′), 35.0 (C-8), 53.1 (C-3′), 56.7 (CH_3O), 67.0 (C-5′), 69.9 (C-7), 70.7 (C-4′), 100.9 (C-1′), 118.4 (C-3), 119.8 (C-1), 135.8 (C-2), 173.6 ($CH_3CH_2\underline{C}O$–). ES-MS *m/z*: 625 (M+Na). $[\alpha]_D^{20}=+153.15$ ($c=0.03$, $CHCl_3$).

Cytotoxicity The *in vitro* cytotoxic activity of compounds **6** and **14** was evaluated using the murine tumors P388 leukemia and L1210 leukemia, which were provided by Mario Negri Institute (Milan, Italy). B16 melanoma was obtained from the DCT Tumor Depository (NCI, Frederic, MD). The human tumor cell lines HT-29 and A-549 were obtained from the American Tissue Culture Collection (Bethesda, MD); the cell line NCI-H460 was provided by the DCT Tumor Depository. The human epidermoid carcinoma cell line KB-3-1 and its 340-fold resistant to ADR subline KB-A1 were kindly provided by Dr. M. Gottesman (Bethesda, MD). All cells were maintained in RPMI1640 medium (except the KB-3-1 and KB-A1 cells cultivated in (DMEM) supplemented with 10% fetal calf serum, 2 mM L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 10 mM Hepes, pH 7.4. The cytotoxicity was measured by the Microculture Tetrazolium Assay as previously described.[13] Briefly, adherent cells were trypsinized and seeded in 96 well-microplates at densities previously determined to maintain control cells in exponential phase of growth for the duration of experiment and to obtain a linear relationship between the optical density and the number of viable cells.[14] Cells were then incubated for about 4 doubling times, in the presence of graded concentrations of drugs (9 concentrations in triplicate). At the end of this period, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, 0.5 mg/ml) was added and cells were incubated for 4 h at 37 °C. The plates were centrifuged ($1600\times \boldsymbol{g}$) for 5 min and culture medium supernatant was removed from wells by slow aspiration and replaced with 100 μl of dimethylsulfoxide. The plates were shaken and read at 540 nm with a MCC Multiscan spectrophotometer (Flow Laboratories). Results are expressed as IC_{50}, the drug concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells.

Cell Cycle Analysis Concerning the cell cycle analysis, HT-29 cells (2.5×10^5/ml) were incubated for two doubling times with various concentrations of cytotoxic drugs. Cells were then fixed by 70% ethanol, washed twice with phosphate-buffered saline (PBS) and incubated for 30 min in PBS containing 100 μg/ml RNAse, 25 μg/ml propidium iodide (PI). For each sample, 10000 cells were analyzed on an Epics XL Coulter flow cytometer. Results are expressed as the percentage of cells in G1, S and G2+M phases of the cell cycle.

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