SYNTHESIS AND ANTITUMOR ACTIVITY OF NEW ANTHRACYCLINES

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New anthracyclines including 2-deoxy-L-fucose, 2-deoxy-L-rhamnose and 2,6-dideoxy-2-iodo-α-L-mannose as sugar moieties, respectively **8**, **11** and **14**, have been obtained by glycosidation of the 4-demethoxy-9-hydroxymethyl-9-deacetyl daunorubicinone (**1**) with appropriate sugars under Koenigs-Knorr conditions. They were found to display high cytotoxicity on L1210 leukemia, but also an outstanding antileukemic activity in mice in the case of **8** and **14**.

Daunorubicin and doxorubicin are clinically important anthracycline antitumor antibiotics[1]. However, their high activity against various type of human cancer is tempered by undesirable side-effects[2], such as a cumulative cardiotoxicity. The synthesis of analogues of daunorubicin and doxorubicin in which the aglycone or the sugar moiety are structurally modified is of great interest as 4-demethoxydaunorubicin (or idarubicin)[3] is under extensive clinical trials[4,5]. Several 3'-deamino-3'-hydroxydoxorubicin analogues[6,7] have also shown significant activity against a wide range of tumors and may have potential for development as antitumor agents.

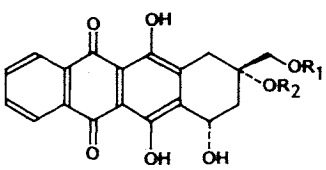
Recently we reported[8,9] the synthesis of a new aglycone, 4-demethoxy-9-hydroxymethyl-9-deacetyl daunorubicinone (**1**), which has also been synthesized simultaneously by the Roche group[10], and later by Japanese authors[11,12]. It has been shown[13] that the corresponding glycosides of this compound with daunosamine or acosamine display significant antitumor activity which led to non cross-resistant novel anthracyclines[14].

In this paper we report on the preparation and preliminary biological data of new deamino anthracyclines including **1** as aglycone moiety.

Chemical Synthesis

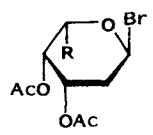
Aglycone **1** was obtained from the α-D-isosaccharino-1,4-lactone, as its *O*-isopropylidene derivative **2**, suitably protected for glycosidation at C-7.

Scheme 1.

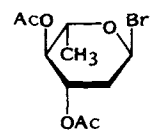


1 $R_1 = R_2 = H$

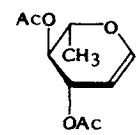
2 $R_1, R_2 =$ 



3a $R = CH_3$
3b $R = C_2H_5$
3c $R = n$-Bu



4



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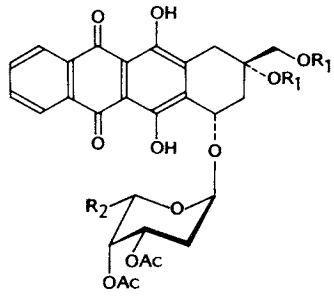
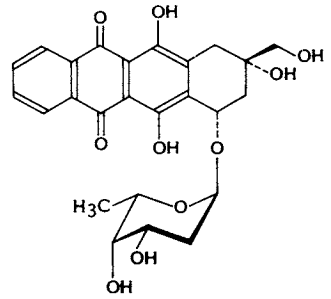
Scheme 2.



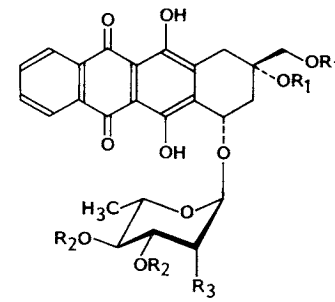
6 $R_1 = \diagup\!\!\diagdown^{CH_3}_{CH_3}$

7 $R_1 = H$

Series **a**: $R_2 = CH_3$ **b**: $R_2 = Et$ **c**: $R_2 = n\text{-}Bu$



8



9 $R_1 = \diagup\!\!\diagdown^{CH_3}_{CH_3}$ $R_2 = Ac$ $R_3 = H$

10 $R_1 = H$ $R_2 = Ac$ $R_3 = H$

11 $R_1 = H$ $R_2 = H$ $R_3 = H$

12 $R_1 = \diagup\!\!\diagdown^{CH_3}_{CH_3}$ $R_2 = Ac$ $R_3 = I$

13 $R_1 = H$ $R_2 = Ac$ $R_3 = I$

14 $R_1 = H$ $R_2 = H$ $R_3 = I$

Glycosidation of **2** with a 2-fold excess of 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranoside bromide[15] (**3a**) was carried out under Koenigs-Knorr conditions (HgO yellow, $HgBr_2$, molecular sieves 4A) giving in a stereoselective manner, the glycoside **6a** in 85% yield based on aglycone used. More lipophilic homologs **3b** and **3c** prepared according to J. P. GESSON *et al.* (unpublished work) were also used as starting materials as lipophilicity and binding to DNA or lipophilicity and cytotoxicity were found to be correlated in a series of anthracyclines[16,17]. This led to glycosides **6b**, **6c** in 90 and 96% yields, respectively. The structure assigned to these glycosides was supported by spectroscopical data (mass[18] and ^1H NMR spectra).

Selective hydrolysis of the acetal ring of the aglycone moiety of **6a**, **6b** and **6c** in mild acidic medium

Table 1. Effect on L1210 cells *in vitro* (IC_{50}, μg/ml).

Compounds	Prolifera-tion assay	Stem cell assay		Compounds	Prolifera-tion assay	Stem cell assay	
		Continuous expt	1 hour expt			Continuous expt	1 hour expt
Doxorubicin·HCl	0.022	0.02	0.04	**9**		2.8	>1
6a		>1	>1	**10**		0.021	3.6
7a	0.032	0.021	3.6	**11**		0.025	0.063
8	0.0033	0.025	0.062	**12**		>1	>1
7b	>1	>1	>1	**13**		0.1	0.1
7c	>1	>1	>1	**14**	>1	0.02	0.024

Table 2. ^{1}H NMR chemical shift data ($CDCl_3$, δ) (coupling constants (Hz) in parenthesis).

	6a	7a	8	7b	6c	7c	9	10	11[a]	12	13
Aglycone											
1-H_a	3.27 (d) (19)	3.28 (d) (19)	3.16 (d)	3.18 (d) (18)	3.27 (d) (18)	3.19 (d) (18)	3.24 (d) (18)	3.30 (d) (18)	3.33 (d) (18)	3.28 (d) (18)	3.21 (d) (18)
1-H_b	2.80 (d) (19)	2.62 (d) (19)	2.52 (d)	2.76 (d) (18)	2.88 (d) (18)	2.58 (d) (18)	2.90 (d) (18)	2.66 (d) (18)	3.23 (d) (18)	2.86 (dd) (18,14)	2.59 (d) (19)
3-CH_2	2.35 ~1.86 (m)	2.35 ~1.90 (m)	2.33 ~1.74 (m)	2.10 ~1.80 (m)	2.30 ~2.00 (m)	2.15 ~1.80 (m)	2.38 ~1.76 (m)	2.38 ~1.76 (m)	2.83 ~2.02 (m)	2.49 ~1.79 (m)	2.20 ~1.65 (m)
4-H	5.20 (br s) (W_H~8 Hz)	5.26 (br s) (W_H~5 Hz)	5.26 (br s) (W_H~6 Hz)	5.06	5.08 (br s) (W_H~6 Hz)	5.08 (br s) (W_H~7 Hz)	5.04 (br s) (W_H~8 Hz)	5.28 (br s)	5.39 (br s) (W_H~8 Hz)	5.01 (br s) (W_H~7 Hz)	5.25 (br s) (W_H~8 Hz)
7-H, 10-H	8.28 (m)	8.28 (m)	8.28 (m)	8.25 (m)	8.29 (m)	8.25 (m)	8.35 (m)	8.35 (m)	8.25 (m)	8.28 (m)	8.25 (m)
8-H, 9-H	7.80 (m)	7.80 (m)	7.80 (m)	7.77 (m)	7.81 (m)	7.78 (m)	7.86 (m)	7.92 (m)	7.63 (m)	7.77 (m)	7.75 (m)
13-H_a	3.95 (d) (9)	3.76 (d) (10)	3.70 (d) (10)	3.70 (d) (10)	3.93 (d) (7)	3.70 (d) (7)	3.89 (d) (8)	3.79 (d) (11)	3.96 (s)	3.96 (d) (7)	3.70 (d) (13)
13-H_b	3.85 (d) (9)	3.54 (d) (10)	3.46 (dd)	3.48 (d) (10)	3.87 (d) (7)	3.52 (d) (7)	3.83 (d) (8)	3.56 (d) (11)		3.88 (d) (8)	3.50 (d) (13)
5-OH	13.58 (s)	13.60 (s)	13.60 (s)	13.46 (s)	13.60 (s)	13.50 (s)	13.70 (s)	13.72 (s)		13.55 (s)	13.56 (s)
12-OH	13.30 (s)	13.30 (s)	13.30 (s)	13.20 (s)	13.30 (s)	13.20 (s)	13.44 (s)	13.42 (s)		13.27 (s)	13.30 (s)
Sugar											
1'-H	5.58 (d) (3)	5.56 (d) (3)	5.50 (br s)	5.52 (br s)	5.65 (br s)	5.50 (d) (3)	5.50 (br s)	5.54 (br s)	5.76 (br s) (W_H~8 Hz)	5.75 (s)	5.75 (s)
2'-CH_2	2.35 ~1.86 (m)	2.35 ~1.86 (m)	2.34 ~1.74 (m)	2.06 ~1.30 (m)	2.30 ~2.00 (m)	2.15 ~1.80 (m)	2.38 ~1.76 (m)	2.37 ~1.76 (m)	2.83 ~2.02 (m)	4.51 (d) (4)	4.63 (d) (4.5)
3'-H	5.24 (m)	5.26 (m)	4.30 (m)	5.17 (m)	5.24 (m)	5.25 (m)	5.26 (m)	5.10 (m)	4.52 (m)	4.55 (dd) (9.4)	4.33 (dd) (9.5, 4.5)
4'-H	5.03 (br d)	5.32 (br d)	4.10 (m)	5.17 (m)			4.77 (dd) (10, 10)	4.84 (dd) (10, 10)	3.60 (dd) (10, 9)	5.13 (dd) (9, 9)	5.21 (dd) (9.5, 9.5)
5'-H	4.40 (q) (6.5)	4.40 (q) (6.5)	3.60 (q)	3.96 (m)	4.15 (m)	4.22 (br s) (W_H~7 Hz)	4.20 (qd) (10, 6.5)	4.13 (qd) (10, 6.5)	4.37 (qd) (10, 6.5)	4.28 (qd) (9, 6)	4.13 (qd) (9.5, 6)
6'-CH_3	1.21 (d) (6.5)	1.26 (d) (6.5)	1.35 (d) (6.5)				1.26 (d) (6.5)	1.25 (d) (6.5)	1.67 (d) (6.5)	1.25 (d) (6)	1.32 (d) (6)
OAc	2.15 (s) 1.89 (s)	2.20 (s) 1.98 (s)		2.13 (s) 1.95 (s)	2.15 (s) 1.96 (s)	2.14 (s) 1.93 (s)	2.03 (s) 1.98 (s)			2.02, 2.01 (s)	2.05, 2.03 (s)
CH_3	1.48 (s, 6H)			0.96 (t)	1.52 (s), 1.49 (s), 0.94 (t)	0.92 (t)	1.50 (s, 6H)				
CH_2				2.03 ~1.30 (m)	1.71 ~1.35 (m)	1.76 ~1.10 (m)					

[a] In pyridine-d_5.

led to **7a**, **7b** and **7c**. Fully deprotected anthracycline **8**[19], was obtained in the subsequent step by treatment of **7a** with 0.25 N aqueous NaOH at 0°C and purification by flash chromatography.

The corresponding isomeric *arabino* glycosides were prepared from 3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*arabino*-hexopyranosyl bromide[15] (**4**) as the glycoside donor. The condensation of **2** with **4** under Koenigs-Knorr conditions afforded **9** in 86% yield. The same two-step deprotection sequence afforded successively **10** and **11**.

On the other hand, glycosidation of **2** with 3,4-di-*O*-acetyl-2,6-dideoxy-L-*arabino*-hex-1-enitol (**5**) (or di-*O*-acetyl-L-rhamnal) in the presence of *N*-iodosuccinimide[20] gave the 2'-iodo anthracycline of L-*manno* configuration **12** and, after successive deprotection of the aglycone and of the sugar moieties, compounds **13** and **14**.

Biological Activity

Cytotoxicity on L1210 cells of the new anthracyclines was compared[21] to that of doxorubicin and the results are reported in Table 1.

With the exception of the acetal derivatives **6a**, **9** and **12** and the 6-*C*-methyl and 6-*C*-propyl analogues of **7a**, *i.e.* **7b** and **7c**, the new glycosides were found to be highly cytotoxic, sometimes like the parent antibiotic. Comparisons of **7a** and **8**, or **10** and **11** or **13** and **14** shows that the cytotoxicity was almost identical before and after cleavage of the protecting groups on the sugar moieties if the continuous exposure was used for incubation in a stem cell assay. On the contrary, after 1 hour exposure, the compounds were only active after cleavage of the protecting groups. This may indicate that the presence of the protecting groups will decrease the membrane transport or membrane permeability of **7a** and **10**. A slow enzymatic deacetylation might also explain the low activity of **7a** and **10**.

The fully deprotected new anthracyclines **8** and **14** were also tested against L1210 leukemia in mice in comparison with doxorubicin·HCl. The results reported in Table 3 indicate that the 3'-deamino-3'-hydroxy-L-*lyxo* analogue, **8** displays good activity which is somewhat inferior to the antitumor effectivity of doxorubicin. On the other hand, the 2'-iodo analogue **14** was highly active in the L1210 mouse tumor system and the effect was comparable to the effect of doxorubicin.

Cytotoxicity of **8** was also investigated against the human erythro leukemia K 562 cells (ID_{50} at 0.3 μM against 0.02 μM for doxorubicin). As previously reported with doxorubicin[22], anthracycline **8** induces the appearance of a differentiated K 562 phenotype. These findings suggest that **8** could belong to the group of chemotherapeutic agents which exert a therapeutic effect through a combination of cell growth inhibition and cell maturation[23].

Table 3. Activity of daunorubicin analogues against L1210 leukemia in mice.

Compounds	Dose (mg/kg)	Schedule	T/C (%)	LTS	Toxic deaths
Doxorubicin·HCl	2.1	3 ip/ip Q4D	141	—	—
	2.8		248	2/6	2/6
	3.75		350	4/6	1/6
8	0.88		116	—	—
	1.18		116	—	—
	1.58		142	—	1/6
14	1.3	3 ip/ip Q4D	235	2/6	—
	1.7		323	4/6	—
	2.2		341	4/6	2/6

From these different results, it appears that anthracycline **14** is worthy of further investigations.

Experimental

MP's (Kofler hot stage microscope) are uncorrected. IR spectra were recorded on a Perkin-Elmer Model 257 spectrophotometer, calibrated against polystyrene film, and were expressed in cm^{-1}. Specific rotations were measured on a Perkin-Elmer model 241 at 589 nm. ^1H NMR spectra at 270 MHz were obtained on a Bruker HX 270 in $CDCl_3$ except when signaled. MS (DCI - NH_3) were recorded on a Nermag R 1010C. Silica gel for chromatography and flash chromatography were Merck Silica gel No. 7736 and 9385, respectively. Microanalyses were performed by the "Laboratoire de Microanalyse du C.N.R.S.", Gif-sur-Yvette and Lyon.

(2*S*,4*S*)-4-*O*-(3',4'-Di-*O*-acetyl-2',6'-dideoxy-α-L-*lyxo*-hexopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-2,13-*O*-isopropylidene-6,11-naphtacene Dione (**6a**)

To a solution of bromo-sugar **3a** (prepared from di-*O*-acetyl-L-fucal[15] (840 mg, 4 mmol)) in dry dichloromethane was added molecular sieves 4A (7 g). After 5 minutes, a solution of aglycone **2** (792 mg, 2 mmol) in dichloromethane (100 ml), yellow HgO (5 g, 23 mmol) and $HgBr_2$ (1.3 g, 3.6 mmol) were successively added. The mixture was stirred for 18 hours at room temperature and filtered. The filtrate was concentrated under reduced pressure and the crude residue was dissolved in THF and then precipitated by addition of equal amount of MeOH to give **6a** (1 g, 81%), pure enough for the next step of the synthesis. A sample was recrystallized for analysis from hexane - acetone: MP 199~200°C; $[\alpha]_D^{20}$ +105° (*c* 0.04, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1740 (CO), 1625, 1590 (H-bonded quinone); Rf 0.23 (hexane - EtOAc, 4:1) or 0.45 (toluene - acetone, 9:1).

Anal Calcd for $C_{32}H_{34}O_{12}$ (610.59): C 62.94, H 5.61.
Found: C 62.80, H 5.63.

(2*S*,4*S*)-4-*O*-(3',4'-Di-*O*-acetyl-2',6'-dideoxy-α-L-*lyxo*-hexopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-6,11-naphtacene Dione (**7a**)

A solution of **6a** (610 mg, 1 mmol) in a mixture of MeOH (500 ml), 2 N aqueous solution of HCl (25 ml) and THF (10 ml) was stirred at room temperature for 24 hours. After neutralization by dropwise addition of aqueous 5 N NaOH, the reaction mixture was extracted with dichloromethane. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated. Flash chromatography of the residue (hexane - acetone, 2:1) gave 400 mg (70%) of **7a** which crystallized from hexane - acetone (1:1): MP 207~209°C; $[\alpha]_D^{20}$ +91° (*c* 0.05, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1730 (CO), 1625, 1590 (H-bonded quinone); MS (DCI - NH_3) *m/z* 588 ($M+NH_4^+$), 356, 155.

Anal Calcd for $C_{29}H_{30}O_{12}$ (570.56): C 61.05, H 5.30.
Found: C 60.92, H 5.35.

(2*S*,4*S*)-4-*O*-(2',6'-Dideoxy-α-L-*lyxo*-hexopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-6,11-naphtacene Dione (**8**)

Compound **7a** (600 mg, 1.05 mmol) was dissolved in dioxane (50 ml) and MeOH (10 ml) and the solution was stirred for 3 hours at 0°C with 0.25 N aqueous solution of NaOH (2.5 ml). The mixture was made neutral by dropwise addition of 1 N aqueous solution of HCl until the blue reaction mixture became red. The solution was washed with brine (30 ml), extracted with EtOAc, dried over sodium sulfate, and evaporated. Purification was achieved by flash chromatography (CH_2Cl_2 - MeOH, 9:1) giving **8** (Rf 0.46, 306 mg, 60%) as a crystalline compound: MP 224~227°C; $[\alpha]_D^{20}$ +94° (*c* 0.04, MeOH); IR ν_{max} ($CHCl_3$) cm^{-1} 3450 (OH), 1625, 1590 (H-bonded quinone); MS (DCI - NH_3) *m/z* 504 ($M+NH_4^+$), 374, 356 (aglycone), 340, 321, 220, 205, 180, 148.

Anal Calcd for $C_{25}H_{26}O_{10}$ (486.48): C 61.72, H 5.38, O 32.89.
Found: C 61.85, H 5.29, O 32.73.

(2*S*,4*S*)-4-*O*-(3′,4′-Di-*O*-acetyl-2′,6′-dideoxy-6′-*C*-methyl-α-L-fucopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-6,11-naphtacene Dione (**7b**)

Molecular sieve (1 g) was added to a solution of the bromo-sugar **3b** (prepared as for **3a**[15]) from 200 mg of the corresponding glycal[16]) in dry dichloromethane (100 ml). After 5 minutes, a solution of aglycone **2** (180 mg, 0.45 mmol), yellow HgO (1 g, 4.6 mmol) and $HgBr_2$ (300 mg, 0.8 mmol) were successively added. The mixture was stirred at room temperature for 15 hours. After filtration and evaporation of the filtrate under reduced pressure, the residue was dissolved in MeOH (50 ml), H_2O (2 ml), THF (1 ml) in the presence of conc HCl (0.4 ml). After stirring at room temperature for 15 hours, a solution of brine was added (20 ml) and the mixture was extracted with EtOAc. Usual work-up afforded 131 mg of **6b** (50%) as a crystalline compound: MP 76~78°C; $[\alpha]_D^{20}$ −22° (*c* 0.04, THF); IR ν_{max} ($CHCl_3$) cm^{-1} 1730 (CO), 1625, 1590 (H-bonded quinone); Rf 0.32 (toluene-acetone, 5:1); MS (DCI-NH_3) *m/z* 602 ($M+NH_4^+$), 549, 526, 356 (aglycone), 305, 169.

Anal Calcd for $C_{30}H_{32}O_{12}$ (584.58): C 61.64, H 5.52.
Found: C 61.50, H 5.64.

(2*S*,4*S*)-4-*O*-(3′,4′-Di-*O*-acetyl-2′,6′-dideoxy-6′-*C*-propyl-α-L-fucopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-2,13-*O*-isopropylidene-6,11-naphtacene Dione (**6c**)

Molecular sieve (1 g) was added to a solution of the bromo-sugar **3c** (prepared as for **3a**[15]) from 150 mg of the corresponding glycal[16]) in dry dichloromethane (100 ml). After 5 minutes, a solution of aglycone **2** (115 mg, 0.29 mmol), yellow HgO (725 mg, 3.3 mmol) and $HgBr_2$ (188 mg, 0.52 mmol) were added successively. The mixture was stirred for 18 hours at room temperature and treated as previously indicated for preparation of **6a**. This afforded after flash chromatography (toluene-acetone, 95:5) and crystallization from MeOH, 183 mg of **6c** (96%): MP 85~90°C (dec); $[\alpha]_D^{20}$ +85° (*c* 0.06, THF); IR ν_{max} ($CHCl_3$) cm^{-1} 1735 (CO), 1620, 1585 (H-bonded quinone); Rf 0.40 (toluene-acetone, 20:1); MS (DCI-NH_3) *m/z* 670 ($M+NH_4^+$), 396, 381, 292, 274, 197.

Anal Calcd for $C_{35}H_{40}O_{12}$ (652.69): C 64.40, H 6.17.
Found: C 64.65, H 6.25.

(2*S*,4*S*)-4-*O*-(3′,4′-Di-*O*-acetyl-2′,6′-dideoxy-6′-*C*-propyl-α-L-fucopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-6,11-naphtacene Dione (**7c**)

A solution of **6c** (50 mg, 0.07 mmol) in MeOH (50 ml), H_2O (2 ml), THF (1 ml) and conc HCl (0.4 ml) was stirred at room temperature overnight. The same work-up as indicated for **7a** followed by flash chromatography (toluene-acetone, 95:5) gave **7c** (Rf 0.35, 12 mg, 28%) as an amorph solid: MP 192°C; $[\alpha]_D^{20}$ +122° (*c* 0.04, dioxane); MS (DCI-NH_3) *m/z* 630 ($M+NH_4^+$), 613 ($M+H^+$), 374, 356, 339, 292.

(2*S*,4*S*)-4-*O*-(3′,4′-Di-*O*-acetyl-2′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-2,13-*O*-isopropylidene-6,11-naphtacene Dione (**9**)

A solution of **2** (150 mg, 0.38 mmol) in anhydrous dichloromethane was treated with the bromo-sugar **4** (prepared from 190 mg of di-*O*-acetyl-L-rhamnal **5** as for **3a**)[15] under the conditions previously used for the preparation of **6a**. This gave a crude mixture (250 mg) which was chromatographed on silica gel (hexane-EtOAc, 4:1) and **9** was isolated (Rf 0.23; 200 mg, 86%) as a crystalline compound. After recrystallization from EtOAc: MP 235~236°C; $[\alpha]_D^{20}$ +148° (*c* 0.04, $CHCl_3$); MS (DCI-NH_3) *m/z* 628 ($M+NH_4^+$), 484, 464, 250, 232, 215, 190, 175, 155 (base peak).

Anal Calcd for $C_{32}H_{34}O_{12}$ (610.62): C 62.95, H 5.61.
Found: C 63.08, H 5.80.

(2*S*,4*S*)-4-*O*-(3′,4′-Di-*O*-acetyl-2′,6′-dideoxy-α-L-*arabino*-hexopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-6,11-naphtacene Dione (**10**)

A solution of **9** (200 mg) in methanol (50 ml) was stirred overnight in the presence of 1 N HCl (10 ml). After neutralization with saturated aqueous solution of $NaHCO_3$ and addition of brine (20 ml), extraction with EtOAc afforded 200 mg of crude material. Chromatography on silica gel (hexane-EtOAc, 2:1) led to 65 mg of crystalline **10** (34%) and 60 mg (30%) of recovered starting material **9**. After recrystallization of **10** from hexane-acetone: MP 216~217°C; $[\alpha]_D^{20}$ +110° (*c* 0.04, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 3550

(OH), 1740, 1625, 1590 (H-bonded quinone); Rf 0.20 (toluene - acetone, 8 : 2) and 0.67 (hexane - acetone, 1 : 1).

Anal Calcd for $C_{29}H_{30}O_{12}$ (570.55): C 61.04, H 5.30.
Found: C 61.22, H 5.17.

(2*S*,4*S*)-4-*O*-(2′,6′-Dideoxy-α-L-*arabino*-hexopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-6,11-naphtacene Dione (**11**)

A solution of **10** (60 mg) in dichloromethane (20 ml) and methanol (20 ml) was stirred for 3 hours at 0°C in the presence of 1 N aqueous NaOH (0.7 ml). After neutralization with dropwise addition of pure AcOH and concentration under reduced pressure, addition of brine (~10 ml) to the residue was followed by extraction with a mixture of EtOAc and THF (1 : 1). Crystallization of the residue from methanol gave 30 mg of **11**: MP 217~218°C; $[\alpha]_D^{20}$ +125° (*c* 0.056, MeOH); MS (DCI - NH_3) *m/z* 504 ($M+NH_4^+$), 487 ($M+H^+$), 376, 148 (base peak).

Anal Calcd for $C_{25}H_{26}O_{10}$ (486.48): C 61.72, H 5.39.
Found: C 61.87, H 5.23.

(2*S*,4*S*)-4-*O*-(3′,4′-Di-*O*-acetyl-2′,6′-dideoxy-2′-iodo-α-L-mannopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-2,13-*O*-isopropylidene-6,11-naphtacene Dione (**12**)

To a cooled (0°C) solution of **2** (1.1 g, 2.7 mmol) and di-*O*-acetyl-L-rhamnal **5** (3 g, 13 mmol) in acetonitrile (250 ml) was added a solution of *N*-iodosuccinimide (3 g, 13 mmol) in acetonitrile (50 ml). The reaction mixture was stirred at 30°C overnight and then extracted with dichloromethane (300 ml) with washings with 10% aqueous solution of sodium thiosulfate and H_2O. Evaporation of the solvent under reduced pressure gave a residue which was dissolved in methanol (~10~15 ml). Addition of H_2O and crushed ice gave a precipitate (1.4 g, 70%) which contained exclusively glycoside **12** without any traces of sugar residue. Purity of **12** was controlled by TLC (hexane - acetone, 2 : 1). A sample was recrystallized from methanol: MP 155~160°C; $[\alpha]_D^{20}$ +29° (*c* 0.15, $CHCl_3$); IR ν_{max} ($CHCl_3$) cm^{-1} 1740 (CO), 1625, 1590 (H-bonded quinone); MS (DCI - NH_3) *m/z* 754 ($M+NH_4^+$, base peak), 737 ($M+H^+$), 414, 396 (aglycone), 358, 341.

Anal Calcd for $C_{32}H_{33}O_{12}I$ (736.51): C 52.19, H 4.52, I 17.23.
Found: C 52.40, H 4.47, I 17.35.

(2*S*,4*S*)-4-*O*-(3′,4′-Di-*O*-acetyl-2′,6′-dideoxy-2′-iodo-α-L-mannopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-6,11-naphtacene Dione (**13**)

10 ml of aqueous 1 N HCl were added to a solution of **12** (1.2 g, 1.6 mmol) in a mixture of methanol - THF (600 ml, 30 : 1). After stirring at room temperature overnight, the reaction mixture was extracted with dichloromethane (500 ml) and washed with brine until neutrality. Concentration under reduced pressure afforded **13** (800 mg, 70%) as a syrup, pure enough for the next step. A sample was recrystallized from methanol: MP 234~235°C; $[\alpha]_D^{20}$ +141° (*c* 0.06, $CHCl_3$); IR *cf.* **12**; Rf 0.55 (hexane - acetone, 1 : 1), 0.18 (hexane - EtOAc, 1 : 1 or hexane - acetone, 2 : 1); MS (DCI - NH_3) *m/z* 714 ($M+NH_4^+$), 713, 695.

Anal Calcd for $C_{29}H_{29}O_{12}I$ (696.45): C 50.01, H 4.19, I 18.22.
Found: C 50.12, H 4.25, I 18.33.

(2*S*,4*S*)-4-*O*-(2′,6′-Dideoxy-2′-iodo-α-L-mannopyranosyl)-1,2,3,4-tetrahydro-2,4,5,12-tetrahydroxy-2-hydroxymethyl-6,11-naphtacene Dione (**14**)

A solution of **13** (800 mg, 1.14 mmol) in a mixture of dioxane (60 ml) and methanol (10 ml) was stirred for 3 hours at 0°C in the presence of 0.25 N NaOH (0.5 ml). The same treatment as previously used for the preparation of **11** afforded a crude residue (700 mg). Crystallization from acetone gave 500 mg of pure compound: MP 180°C; $[\alpha]_D^{20}$ +32° (*c* 0.038, MeOH); Rf 0.25 (hexane - acetone, 1 : 1), 0.30 (CH_2Cl_2 - MeOH, 9 : 1).

Anal Calcd for $C_{25}H_{25}O_{10}I$ (612.37): C 49.03, H 4.11, I 20.72.
Found: C 49.12, H 4.15, I 20.89.

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References

1) ARCAMONE, F.: Doxorubicin anticancer antibiotics. *In* Medicinal Chemistry. Ser. 17. pp. 194~258, Academic Press, 1981
2) LEFRAK, E. A.; J. PITHA, S. ROSENHEIM, R. M. O'BRYAN, M. A. BURGESS & J. A. GOTTLIEB: Adriamycin (NSC-123127) cardiomyopathy. Cancer Chemother. Rep. 6: 203~214, 1975
3) ARCAMONE, F.; L. BERNARDI, P. GIARDINO, B. PATELLI, A. DIMARCO, A. M. CASAZZA, G. PRATESI & P. REGGIANI: Synthesis and antitumor activity of 4-demethoxydaunorubicin, 4-demethoxy-7,9-diepidaunorubicin, and their β-anomers. Cancer Treat. Rep. 60: 829~834, 1976
4) WEISS, R.; G. SAROSY, K. CLAGETT-CARR, M. RUSSO & B. LEYLAND-JONES: Anthracycline analogs: The past, present, and future. Cancer Chemother. Pharmacol. 18: 185~197, 1986
5) YOUNG, C. W. & V. RAYMOND: Clinical assessment of the structure-activity relationships of anthracyclines and related synthetic substances. Cancer Treat. Rep. 70: 51~63, 1986
6) HORTON, D.; W. PRIEBE & W. R. TURNER: Synthesis and antitumor activity of 7-*O*-(3,4-di-*O*-acetyl-2,6-dideoxy-α-L-*lyxo*-hexopyranosyl)adriamycinone. Carbohydr. Res. 94: 11~25, 1981
7) HORTON, D.; W. PRIEBE & O. VARELA: Synthesis and antitumor activity of 3'-deamino-3'-hydroxydoxorubicin. A facile procedure for the preparation of doxorubicin analogs. J. Antibiotics 37: 853~858, 1984
8) BENNANI, F.; J.-C. FLORENT, M. KOCH & C. MONNERET: An efficient synthesis of optically active 4-demethoxy anthracyclinones. Tetrahedron 40: 4669~4676, 1984
9) GENOT, A.; J. C. FLORENT & C. MONNERET: Anthracyclinones; 3. Chiral pool synthesis of anthracyclines *via* tetraline intermediates. J. Org. Chem. 52: 1057~1063, 1987
10) BROADHURST, M. J.; C. H. HASSALL & G. J. THOMAS: Total synthesis of some new 4-demethoxyanthracyclinones. Tetrahedron 40: 4649~4656, 1984
11) SUZUKI, M.; T. MATSUMOTO, M. OHSAKI, Y. KIMURA & S. TERASHIMA: Novel syntheses of optically active 4-demethoxyanthracyclinones carrying a hydroxymethyl or a carbamoyloxymethyl group at the C_9-position. Chem. Letters 1986: 1739~1742, 1986
12) IZAWA, T.; Z.-G. WANG, Y. NISHIMURA, S. KONDO & H. UMEZAWA: A novel enantiocontrolled synthesis of (+)-9-deacetyl-9-(hydroxymethyl)-4-demethoxydaunomycinone. Chem. Letters 1987: 1655~1656, 1987
13) TWENTYMAN, P. R.; N. E. FOX, K. A. WRIGHT, M. J. BROADHURST, J. A. MARTIN & N. M. BLEEHEN: The *in vitro* effects and cross resistance patterns of some novel anthracyclines. Br. J. Cancer 53: 585~594, 1986
14) SCOTT, C. A.; D. WESMACOTT, M. J. BROADHURST, G. J. THOMAS & M. J. HALL: 9-Alkyl anthracyclines absence of cross resistance to adriamycin in human and murine cell cultures. Br. J. Cancer 53: 595~600, 1986
15) EL KHADEM, H. S.; D. L. SWARTZ, J. K. NELSON & S. UMEZAWA: Synthesis of 2,6-dideoxy-α-L-*lyxo* and 2,6-dideoxy-α-L-*arabino*-hexopyranosyl halides. Carbohydr. Res. 58: 230~234, 1977
16) YEN, S.-F.; W. D. WILSON, S. W. PEARCE & E. J. GABBAY: A new series of reductive amination derivatives of daunorubicin: Syntheses, partition coefficients, and DNA binding. J. Pharm. Sci. 73: 1575~1579, 1984
17) FACHETTI, I. & A. VIGEVANI: Lipophilic character and biological activity of anthracyclines. QSAR Drug Design and Toxicology 1987: 138~140, 1987
18) MONNERET, C. & A. SELLIER: Desorption chemical ionization mass spectrometry of anthracyclines and trisaccharides related to aclacinomycin A and marcellomycin. Biomed. Envr. Mass Spectrom. 13: 319~326, 1986
19) ANNE, A.; F. BENNANI, J.-C. FLORENT, J. MOIROUX & C. MONNERET: New anthracyclines synthesis: Electrochemical reduction, rate of glycoside elimination. Tetrahedron Lett. 26: 2641~2644, 1985
20) THIEM, J.; H. KARL & J. SCHWENTNER: Synthese α-verknupfter 2'-deoxy-2'-iododisaccharide. Synthesis 1978: 696~698, 1978
21) KRAEMER, H. P. & H. H. SEDLACEK: A modified screening system to select new cytostatic drugs. Behring Institute Mitteilungen 74: 301~328, 1984
22) JEANNESSON, P.; L. GINOT, M. MANFAIT & J. C. JARDILLIER: Induction of hemoglobin synthesis in human leukemic K 562 cells by adriamycin. Anticancer Res. 4: 47~52, 1984
23) REISS, M.; GAMBA-VITALO & A. C. SARTORELLI: Induction of tumor cell differentiation as a therapeutic approach; preclinical models for hematopoietic and solid neoplasms. Cancer Treat. Rep. 70: 201~208, 1986