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## Daunorubicin Derivatives Obtained from Daunorubicin and Nucleoside Dialdehydes

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## DAUNORUBICIN DERIVATIVES OBTAINED FROM DAUNORUBICIN AND NUCLEOSIDE DIALDEHYDES

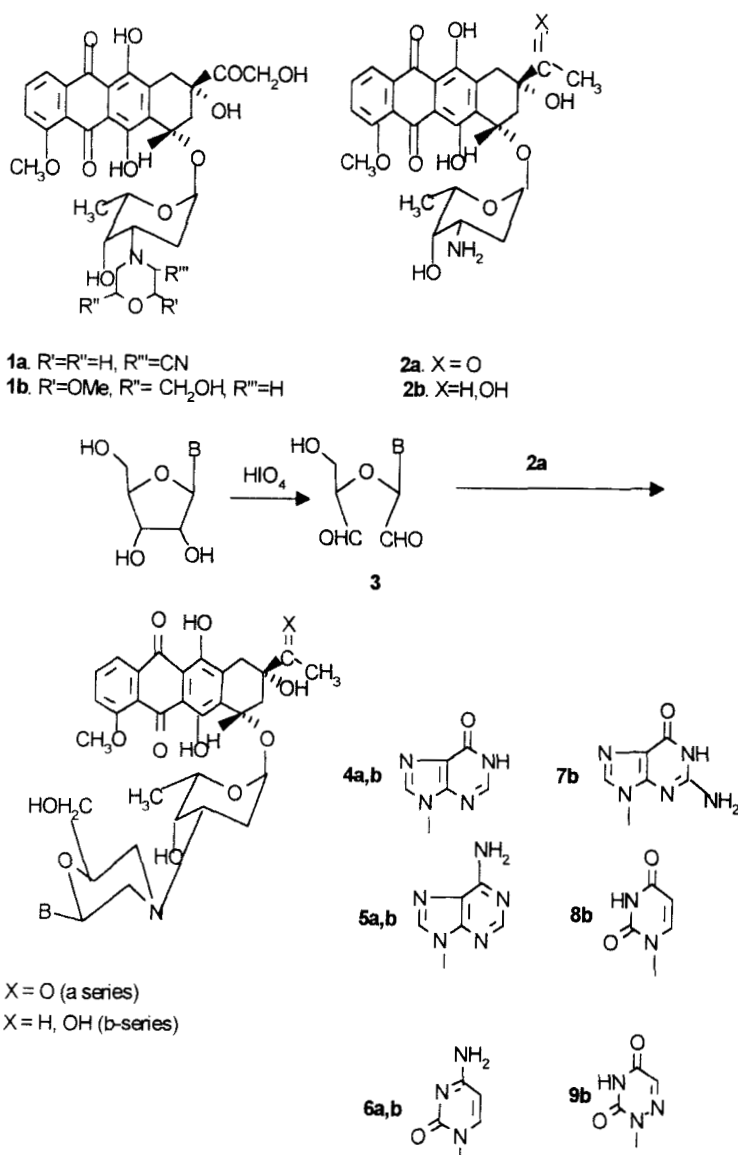
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*Abstract:* Nucleoside dialdehydes were obtained by periodate oxidation of adenosine, cytidine, guanosine, uridine or 6-azauridine in the presence of Dowex (1x8; CH<sub>3</sub>COO<sup>-</sup>). Reductive alkylation of daunorubicin with these dialdehydes in the presence of NaBH<sub>3</sub>CN produced a series of 3'-deamino-3'-(4-morpholino)daunorubicin or 13-(R,S)-dihydrodaunorubicin derivatives, the latter being mixtures of two diastereomers at 13-C atom. The morpholino-daunorubicin derivatives containing nucleic base moieties are less cytotoxic than cyanomorpholino-daunorubicin, morpholino-daunorubicin and even than the parent antibiotic.

### INTRODUCTION

Anthracycline antibiotics (daunorubicin, doxorubicin and some others) are well known clinically used antitumor drugs. Second generation drug design in these series is directed to compounds active against tumors resistant to the clinically used antibiotics and devoid of negative side effects such as myelosuppression and cardiotoxicity. Highly cytotoxic compounds were found among 3'-deamino-3'-(4-morpholino)daunorubicin derivatives of anthracycline antibiotics, e.g. 3'-deamino-3'-(3-cyano-4-morpholino)-doxorubicin (**1a**) or 3'-deamino-3'-(2-methoxy-6-hydroxymethyl-4-morpholino)-doxorubicin (**1b**)<sup>1</sup>. Isomers of the latter compound were synthesized by reductive alkylation of doxorubicin with dialdehydes obtained by periodate oxidation of methyl  $\alpha$ - or  $\beta$ -D-glucopyranosides. Earlier starting from daunorubicin (**2a**) or its 13-dihydroderivative (**2b**) and an inosine dialdehyde, we have obtained 3'-deamino-3'-(2-hypoxantyl-6-hydroxymethyl-4-morpholino)dauno-



rubicin (**4a**) or corresponding 13-dihydroderivative (**4b**) containing 1'C-O-4'C-5'C moiety of inosine backbone, which were ten times less cytotoxic than the parent antibiotics<sup>2</sup>. As anthracyclines interact with DNA, RNA and DNA-dependent enzymes<sup>3,4</sup>, the biological activity of the conjugates of anthracyclines with the nucleoside dialdehydes might be dependent on the nature of the nucleoside aglycones. Here we report the synthesis and some biological properties of novel hybrids of the antitumor antibiotic daunorubicin with adenosine, guanosine, cytidine, uridine, and 6-azauridine nucleoside dialdehydes.

## RESULTS AND DISCUSSION

Nucleoside dialdehydes **3**, containing adenine, cytidine, guanine, uracil and 6-azauracil residues were obtained from the corresponding nucleosides using a modified method of the periodate cleavage by  $\text{HIO}_4$  in the presence of anion exchange resin Dowex 1x8 ( $\text{CH}_3\text{COO}^-$ ) which permits one to obtain pure nucleoside dialdehydes in practically quantitative yields<sup>5</sup>. Attempts to use for the condensation with daunorubicin nucleoside dialdehydes obtained from nucleosides with the use of  $\text{NaIO}_4$  led to complex mixtures of reaction products which were difficult to separate. The interaction of **2a** with adenosine dialdehyde and  $\text{NaBH}_3\text{CN}$  (molar ratio 1:20:2.5) (method *A*) produced 3'-deamino-3'-[(2R)-(adenin-9-yl)-(6S)-hydroxymethyl-4-morpholino]daunorubicin (**5a**) in 28% yield and 13-(R,S)-dihydro-derivative (**5b**) in 18% yield, as separated by column chromatography. When **2a**, **3** ( $\text{B} = \text{Ade}$ ) and  $\text{NaBH}_3\text{CN}$  were taken in 1:5:5 a ratio (method *B*), the yield of **5b** increased to 41% but **5a** was not formed. By the same method *A*, 3'-deamino-3'-[(2R)-(cytosin-1-yl)-(6S)-hydroxymethyl-4-morpholino]-daunorubicin (**6a**) and the corresponding 13-(R,S)-derivative **6b** were obtained from **2a** and cytidine dialdehyde **3** ( $\text{B} = \text{Cyt}$ ). Morpholino derivatives **7b**, **8b** and **9b** were synthesized from dialdehydes of guanosine, uridine, and 6-azauridine (**3**, when  $\text{B} = \text{Gua}$ , *Ura* or 6-*aza-Ura*), and **2a** using method *B* (Table 1). When larger amounts of corresponding dialdehydes and lower amounts of  $\text{NaBH}_3\text{CN}$  were used (method *A*), attempts to isolate compounds of the **a** series for **7**, **8** and **9** were not successful.

The structural assignment of the morpholino derivatives was supported by the  $^1\text{H}$  NMR data and FAB mass spectra (TABLE 1). In  $^1\text{H}$  NMR spectra, all signals of the daunomycin residue and corresponding nucleoside aglycons were present, as were signals of the substituted morpholine moiety. Similar to the product of condensation of **2a** with inosine dialdehyde (**3**,  $\text{B} = \text{Hyp}$ ), the reduction of 13-CO-group by  $\text{NaBH}_3\text{CN}$  occurs nonspecifically to afford a mixture of two diastereomers epimeric at C-13. This was supported by the  $^1\text{H}$  NMR spectra of the compounds of the **b** series, in which two sets for signals for 14- $\text{CH}_3$ ; 4- $\text{OCH}_3$ ; 5'- $\text{CH}_3$ ; H-1; H-2 and H-3 of the dihydrodaunorubicin moiety, and protons of the corresponding nucleic bases are present. The morpholine ring in compound **5a** has a chair conformation  $^0\text{C}_N$ , where the 2''-H proton ( $\delta$  5,80 ppm,  $J_{2'',3''\text{ax}} = 10.0$  Hz) and 6''-H proton ( $\delta$  2,15 ppm,  $J_{6'',5''\text{ax}} = 11.0$  Hz) are in axial orientation and the nucleoside aglycone and  $\text{CH}_2\text{OH}$  group are in equatorial positions. As the starting

nucleosides have 1'(R), 4'(S) configuration, the same configuration can be assigned to the 2'' and 6'' asymmetric atoms of the morpholine derivatives.

In the  $^1\text{H}$  NMR spectra of all the compounds (4 - 9) the signals of solvents were absent what corresponded to less than 5 % of a solvent content; in visible spectra  $\varepsilon$  values of the compounds (4 - 9) (TABLE 1) at 484 nm versus daunorubicin correspond to  $\sim 80$  % of the  $\varepsilon$  value of daunorubicin what can be connected with decrease of absorbancy due to stacking or self-association effects<sup>6,7</sup> as well as with the admixtures of water or other invisible compounds. It can be concluded that the content of admixtures in the compounds (4-9) is below 20 %.

The FAB mass spectra of compounds **5a**, **6a**, and **7b-9b** all contain ions  $(\text{M}+\text{H})^+$  corresponding to the expected molecular weights (Table.1). In the mass spectra of these compounds, the ions 2 m/z or 3 m/z higher than the expected molecular ions are present  $[(\text{M}+2\text{H})^+ \text{ and } (\text{M}+3\text{H})^+]$ ; which is typical for mass spectra of quinone compounds including anthracycline antibiotics<sup>2,8</sup>.

The minimal inhibitory concentrations ( $\text{IC}_{50}$ ) of the morpholino derivatives 4-9 required to inhibit by 50 % proliferation of murine leukemia L1210 and human T-lymphoblast (Molt 4/C8 and CEM/0) cells were compared with the  $\text{IC}_{50}$  of daunorubicin **2a**. The  $\text{IC}_{50}$  values are presented in TABLE 2.

All the morpholine compounds obtained are 1-2 orders of magnitude less cytotoxic than the starting antibiotic **2a**. The morpholine derivatives of the **b** series (cf. **5a** and **5b**, **6a** and **6b**) are several times less toxic than the morpholine derivatives of **a** series, in agreement with the lower cytotoxicity of 13-dihydrodaunorubicin (**2b**) versus with daunorubicin (**2a**)<sup>9</sup>. The uridine and cytidine derivatives **8b** and **6a** are the most cytotoxic of all nucleic base-containing morpholine derivatives. Earlier it was shown that uridine dialdehyde is the most cytotoxic of all natural nucleoside dialdehydes and is more cytotoxic than 6-azauridine dialdehyde or 5-fluorouridine dialdehyde<sup>10</sup>, a comparison of toxic effects of conjugates of uridine dialdehyde and 5-fluorouridine dialdehyde with human serum albumin (HSA) revealed that uridine dialdehyde-HSA conjugates (containing a uracilyl-morpholine moiety) are several times more toxic than 5-fluorouridine dialdehyde-HSA conjugates<sup>11</sup>. High toxicity seems to be a common feature of different uracilyl morpholino derivatives. All the compounds investigated did not inhibit HIV-1 and HIV-2 proliferation at subtoxic concentrations.

TABLE 1. Physicochemical data for compounds **5** - **9**

Comp	Mol. formula (mol. weight)	FAB-MS*	<sup>1</sup> H-NMR spectra (in CD <sub>3</sub> OD): signals of nucleobase moiety	TLC R <sub>f</sub> (system)	UV-visible spectra (in 0.05 N aq. HCl) λ <sub>max</sub> nm, (lg ε)	Yield (method)
<b>5a**</b>	C <sub>37</sub> H <sub>40</sub> N <sub>6</sub> O <sub>12</sub> (760)	<u>761 (M+H)<sup>+</sup></u> <u>762 (M+2H)<sup>+</sup></u> <u>763 (M+H)<sup>+</sup></u>	8.20 s (1H, 2-H ade); 7.95 (1H, 8-H ade)	0.59 (A) 0.84 (B)	235 (4.51) 253 (4.43) 484 (3.96)	28% (A)
<b>5b</b>	C <sub>37</sub> H <sub>42</sub> N <sub>6</sub> O <sub>12</sub> (762)	<u>763 (M+H)<sup>+</sup></u> <u>764 (M+2H)<sup>+</sup></u>	8.45 (s) and 8.44 (s) (2-H ade); 8.30 (s) and 8.29 (s) (8-H ade)	0.51 (A) 0.77 (B)	235 (4.52) 253 (4.43) 484 (3.97)	18% (A) 41% (B)
<b>6a</b>	C <sub>36</sub> H <sub>40</sub> N <sub>4</sub> O <sub>13</sub> (736)	<u>737 (M+H)<sup>+</sup></u> <u>738 (M+2H)<sup>+</sup></u> <u>739 (M+3H)<sup>+</sup></u>	7.72 (J <sub>6,5</sub> 7.4) (1H, 6-H cyt); 5.86 (1H, d, 5-H cyt)	0.29 (A) 0.54 (B)	234.5 (4.50) 255 (4.47) 485 (3.99)	18% (A)
<b>6b</b>	C <sub>36</sub> H <sub>42</sub> N <sub>4</sub> O <sub>13</sub> (738)	<u>739 (M+H)<sup>+</sup></u> <u>740 (M+2H)<sup>+</sup></u> <u>741 (M+3H)<sup>+</sup></u>	7.72 (J <sub>6,5</sub> 7.4) (1H, 6-H cyt); 6.08 (1H, d, 5-H cyt)	0.25 (A) 0.50 (B)	234.5 (4.49) 255 (4.47) 485 (3.98)	16% (A)
<b>7b***</b>	C <sub>37</sub> H <sub>42</sub> N <sub>6</sub> O <sub>13</sub> (778)	<u>779 (M+H)<sup>+</sup></u> <u>780 (M+2H)<sup>+</sup></u> <u>781 (M+3H)<sup>+</sup></u>	7.74 (1H, s, 8-H gua)	0.27 (A) 0.52 (B)	232 (4.45) 255 (4.37) 481 (3.95)	72% (B)
<b>8b</b>	C <sub>36</sub> H <sub>41</sub> N <sub>3</sub> O <sub>14</sub> (739)	<u>740 (M+H)<sup>+</sup></u> <u>741 (M+2H)<sup>+</sup></u>	7.69 (J <sub>6,5</sub> 8.1) (1H, d, 6-H ura); 5.62 (1H, d, 5-H ura)	0.31 (A) 0.56 (B)	234 (4.48) 251 (4.46) 484 (3.95)	37% (B)
<b>9b</b>	C <sub>35</sub> H <sub>40</sub> N <sub>4</sub> O <sub>14</sub> (740)	<u>741 (M+H)<sup>+</sup></u> <u>742 (M+2H)<sup>+</sup></u>	7.37 (1H, s, 5-H azaurac)	0.33 (A) 0.58 (B)	233.5 (4.21) 249 (4.44) 485 (3.98)	30% (B)

\*The most intensive peak is underlined. \*\* NMR spectrum was recorded in CDCl<sub>3</sub> - CD<sub>3</sub>OD (95:5). \*\*\* NMR spectrum was recorded in d<sub>6</sub>-DMSO.

**TABLE 2.** Inhibitory effects of daunorubicin derivatives on the proliferation of murine leukemia L1210 and human T-lymphoblast (Molt4/C8 and CEM/0) cells

Compound	IC <sub>50</sub> *( $\mu$ M)		
	L1210	Molt4/C8	CEM/0
<b>2a</b>	0.52		
<b>4a</b>	5.12		
<b>5a</b>	2.9 $\pm$ 1.2	0.94 $\pm$ 0.54	1.4 $\pm$ 0.01
<b>5b</b>	8.1 $\pm$ 3.7	4.9 $\pm$ 3.8	8.7 $\pm$ 1.3
<b>6a</b>	2.7 $\pm$ 0.88	2.9 $\pm$ 1.6	4.9 $\pm$ 1.7
<b>6b</b>	27 $\pm$ 7.8	27 $\pm$ 7.1	49 $\pm$ 9.4
<b>7b</b>	60 $\pm$ 5.2	46 $\pm$ 0.9	$\geq$ 100
<b>8b</b>	3.0 $\pm$ 0.26	1.9 $\pm$ 0.19	6.2 $\pm$ 1.3
<b>9b</b>	22 $\pm$ 9.7	18 $\pm$ 13	22 $\pm$ 13

\* Concentration required to inhibit cell proliferation by 50 %.

## EXPERIMENTAL

*General methods.* Daunorubicin (**2a**) was produced at the Pilot Plant of the Institute of New Antibiotics, Russian Academy of Medical Sciences. All NMR data were obtained on a Varian VXR-400 instrument operated at 400 MHz; chemical shifts are given in ppm relative to the signals of the solvents used (CD<sub>3</sub>OD,  $\delta$  3.32 ppm; CDCl<sub>3</sub> 7.21 ppm). FAB-Ms were obtained on a MAT-90 spectrometer (Finigan, USA) using glycerol matrix and 8 keV Xe-atoms to bombard the sample. UV and visible spectra were recorded using Hitachi U2000 Spectrophotometer (Japan) at concentrations below  $1 \times 10^{-4}$ . Thin-layer chromatography (TLC) was performed on the plates coated with silica gel 60 F<sub>254</sub> (Merck) in a chloroform-benzene-methanol 10:1:3 (A) or chloroform - methanol-water 13:6:1 (B) mixture. All evaporations were carried out under reduced pressure at or below 40°C. For column chromatography, silica gel 60 (Merck) (230 -400 mesh ASTM) was used.

**Nucleoside dialdehydes.** To a stirred solution of metaperiodic acid dihydrate (HIO<sub>4</sub>·2H<sub>2</sub>O) (0.88 mmol) in water (5 ml) in dark at room temperature was added Dowex resin (1x8,

$\text{CH}_3\text{COO}^-$ ) to pH 2.4 followed by a nucleoside (0.75 mmol). After 6 h of stirring, the resin was separated and rinsed with water (3 x 3 ml). The combined solutions of nucleoside dialdehydes were evaporated in vacuum with n-butanol addition to give the solid residues of nucleoside dialdehydes, which were used without purification. Solid dialdehydes were obtained in almost quantitative yields.

**3'-Deamino-3'-[(2R)-(adenin-9-yl)-(6S)-hydroxymethyl-4-morpholino]daunorubicin (5a) and 13-(R,S)-dihydro-3'-deamino-3'-[(3R)-(adenin-9-yl)-(6S)-hydroxymethyl-morpholin-4-yl]daunorubicin (5b).** *Method A.* Adenosine dialdehyde (1400 mg, 5.28 mmol) and  $\text{NaBH}_3\text{CN}$  (40 mg, 0.65 mmol) were added to a stirred solution of daunorubicin

hydrochloride **2a** (140 mg, 0.25 mmol) in water (30 ml) and acetonitrile (30 ml). The reaction mixture was stirred for 3 h at 20° C, and daunomycinone formed as a by-product was extracted with chloroform. Afterwards, a sodium bicarbonate solution (5%, 30 ml) was added to adjust pH to 8 and the crude products were extracted with a chloroform-methanol (15:1) mixture. After evaporation in a vacuum, the reaction products were purified by column chromatography (50 x 2 cm, system B). The fractions containing **5a** or **5b** yielded **5a** (28%) and **5b** (18%).  $^1\text{H}$  NMR spectrum of **5a** in  $\text{CDCl}_3+\text{CD}_3\text{OD}$  (95:5 v/v),  $\delta$  ppm, (J, Hz): 8.21 (1H, s, 2-ade); 7.99 (1H, d,  $J_{1,2}$  7.6, 1-H); 7.95 (1H, s, 8-ade); 7.75 (1H, t,  $J_{2,1}$  7.6,  $J_{2,3}$  8.3, 2-H); 7.36 (1H, d,  $J_{3,2}$  8.3, 3-H); 5.80 (1H, dd,  $J_{2'',3''\text{ax}}$  10.0,  $J_{2'',3''\text{eq}}$  2.3, 2''- $\text{H}_{\text{ax}}$ ); 5.52 (1H, m, 1'-H); 5.22 (1H, d,  $J_{7,8}$  4.0, 7-H); 4.04 (3H, s,  $0\text{CH}_3$ ); 4.01 (1H, q,  $J_{5',4'}$  6.6, 5' $\text{-H}_{\text{ax}}$ ); 3.91 (1H, m, 6''- $\text{H}_{\text{ax}}$ ); 3.66 (1H, br. s, 4'-H); 3.59 (2H, m, 7''-2H); 3.31 (1H, d,  $J_{\text{gem}}$  10.0, 3''- $\text{H}_{\text{eq}}$ ); 3.16 (1H, d,  $J_{\text{gem}}$  18.5, 10- $\text{H}_{\text{eq}}$ ); 2.92 (1H, d,  $J_{\text{gem}}$  18.5, 10- $\text{H}_{\text{ax}}$ ); 2.88 (1H, d,  $J_{\text{gem}}$  11.0, 5''- $\text{H}_{\text{eq}}$ ); 2.41 (1H, t,  $J_{3'',2''}$  10.0,  $J_{\text{gem}}$  10.0, 3''- $\text{H}_{\text{ax}}$ ); 2.50 (1H, m, 3'-H); 2.34 (3H, s, 14- $\text{CH}_3$ ); 2.29 (1H, d,  $J_{\text{gem}}$  14.7, 8- $\text{H}_{\text{eq}}$ ); 2.15 (1H, t,  $J_{5'',6''}$  11.0,  $J_{\text{gem}}$  11.0, 5''- $\text{H}_{\text{ax}}$ ); 2.05 (1H, dd,  $J_{\text{gem}}$  14.7,  $J_{8,7}$  4.0, 8- $\text{H}_{\text{ax}}$ ); 1.90 (1H, ddd,  $J_{\text{gem}}$  12.0,  $J_{2',3'}$  4.0, 2'- $\text{H}_{\text{ax}}$ ); 1.81 (1H, dd,  $J_{\text{gem}}$  12.0,  $J_{2',3'}$  4.0, 2'- $\text{H}_{\text{eq}}$ ); 1.28 (3H, d,  $J_{6',5'}$  6.6, 6'- $\text{CH}_3$ ).

*Method B.* To a solution of metaperiodic acid dihydrate ( $\text{HJO}_4 \cdot 2\text{H}_2\text{O}$ ) (200 mg, 0.88 mmol) in water (5 ml) was added Dowex resin (1x8,  $\text{CH}_3\text{COO}^-$ ) followed by adenosine (200 mg, 0.75 mmol). The mixture was stirred for 2 h in the dark at room temperature: then, the resin was filtered off and rinsed with water (3 x 3 ml). The combined solutions containing the adenosine dialdehyde were adjusted to pH 5 with  $\text{NaHCO}_3$  solution: then,  $\text{NaBH}_3\text{CN}$  (50 mg, 0.79 mmol) was added. After 15 min of stirring at room temperature, a solution of daunorubicin hydrochloride (85 mg, 0.15 mmol) in water (2 ml) was added. The mixture was stirred for 3 h at room temperature and monitored by TLC. The compounds formed



were extracted with a chloroform-methanol 15:1 mixture (3 x 25 ml). The extracts were evaporated under vacuum and dissolved in a chloroform-methanol 10:1 mixture. Then, **5b** was isolated by column chromatography upon elution with a chloroform-methanol (10:1) mixture. Evaporation yielded 49 mg (42%) of red solid **5b**.

**3'-Deamino-3'-[(2R)-(cytosin-1-yl)-(6S)-hydroxymethylmorpholin-4-yl]daunorubicin (6a)** and **13-(R,S)-dihydro-3'-deamino-3'-[(2R)-(cytosin-1-yl)-(6S)-hydroxymethylmorpholin-4-yl]daunorubicin (6b)** were obtained by method *A* starting from daunorubicin hydrochloride **2a** (140 mg, 0.30 mmol) and cytidine dialdehyde (1250 mg, 5.20 mmol) in yields 18% (**6a**) and 16% (**6b**).

**13-(R,S)-Dihydro-3'-deamino-3'-[(2R)-(guanine-9-yl)-(6S)-hydroxymethylmorpholin-4-yl]daunorubicin (7b)** was obtained by method *B* starting from daunorubicin hydrochloride **2a** (85 mg, 0.15 mmol) and guanosine dialdehyde (200 mg, 0.75 mmol) in the yield 72%.

**13-(R,S)-Dihydro-3'-deamino-3'-[(2R)-(uracil-1-yl)-(6S)-hydroxymethylmorpholin-4-yl]daunorubicin (8b)** was obtained by method *B* starting from daunorubicin hydrochloride **2a** (85 mg, 0.15 mmol) and uridine dialdehyde (184 mg, 0.75 mmol) in the yield 37%.

**13-(R,S)-Dihydro-3'-deamino-3'-[(2R)-(6-azauracil-1-yl)-(6S)-hydroxymethylmorpholin-4-yl]daunorubicin (9b)** was obtained by method *B* starting from daunorubicin hydrochloride **2a** (85 mg, 0.15 mmol) and 6-azauridine dialdehyde (183 mg, 0.75 mmol). The extraction was performed with *n*-butanol (instead of a chloroform-methanol mixture); the extracts were evaporated under vacuum and purified by column chromatography as described for **5b**, to yield **9b** in the 30% yield.

**Cytotoxicity Assays.** All cytotoxicity assays were performed in 96-well microliter plates (Falcon, Grenoble, France). To each well were added  $5 - 7.5 \times 10^4$  cells and a given amount of test compound. The cells were allowed to proliferate for 48 h (L1210), or 72 h (Molt 4/C8 or CEM) at 37°C in a humidified CO<sub>2</sub>-controlled atmosphere. At the end of the incubation period, the cells were counted in a Coulter Counter (Coulter Electronics LTD, United Kingdom). The IC<sub>50</sub> (50 % inhibiting concentrations) were defined as the concentrations of compound that reduced number of viable cells by 50 %.

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