Structural Modification Study of Mitoxantrone (DHAQ). Chloro-Substituted Mono- and Bis[(aminoalkyl)amino]anthraquinones

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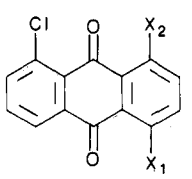
A number of chloro-substituted [(aminoalkyl)amino]anthraquinones were synthesized and evaluated for their antineoplastic and cytotoxic activity. Treatment of 5,8-dichloroquinizarin with substituted amines in pyridine resulted in the replacement of one halogen atom by the amino group to yield mainly 1-chloro-5,8-dihydroxy-4-(substituted amino)anthraquinones. On the other hand, reaction between the dichloroquinizarin and the amines in butanol gave predominately 1,4-dichloro-5-hydroxy-8-(substituted amino)anthraquinones. Other compounds in this series were prepared by displacement of chloro, nitro, or tosyl functions of the appropriate anthraquinone derivatives with various amines by conventional methods. 1,4-Dichloro-5-hydroxy-8-[[2-[(2-hydroxyethyl)amino]ethyl]amino]anthraquinone (**6b**) possesses the highest inhibitory activity against P388 leukemia. Its inhibitory action against B16 melanoma and against the in vitro L1210 screen is also significant. Several other chloro- and hydroxy-substituted aminoanthraquinones (**5a**, **5b**, and **6a**) also showed noticeable activity against P388 in vivo and L1210 in vitro. Structure–activity-relationship examination indicated that the hydroxyl group may contribute to the binding of certain chloroaminoanthraquinones for their biological activity and that the [2-[(2-hydroxyethyl)amino]ethyl]amino side chain seems to be the preferred substituent over other amino side chains.

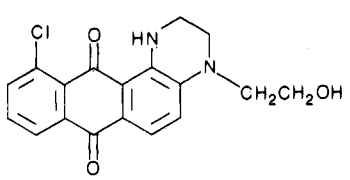
In connection with our continuing efforts on the structural modification study of the potent anticancer agent mitoxantrone (DHAQ),[1,2] some chloro-substituted [(aminoalkyl)amino]anthraquinones were synthesized for acquiring a better understanding of molecular binding characteristics as well as conducting a continued structure–activity–relationship study of compounds of this type. For a direct comparison with DHAQ, compounds chosen to be synthesized are restricted to only those with groups substituted at the α-positions of the anthraquinone ring.

Chemistry

Two monochloro mono(substituted amino)anthraquinones were prepared as follows: 1-Chloro-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]anthrquinone (**1a**), mp 146–148 °C, was prepared by treating 1,5-dichloroanthraquinone[3] with 2-[(2-aminoethyl)amino]ethanol in $CH_3CON(CH_3)_2$. The corresponding 1-chloro-8-(substituted amino)anthraquinone **1b**, mp 144–145 °C, was obtained from 1,8-dichloroanthraquinone[3] and the amine in dioxane. Attempted preparation of **1b** by other investigators, using pyridine as the reaction solvent, resulted in the formation of a tetracyclic compound **2**, mp 216–218 °C.[4]



1a: $X_1 = NH(CH_2)_2NH(CH_2)_2OH$, $X_2 = H$
b: $X_1 = H$, $X_2 = NH(CH_2)_2NH(CH_2)_2OH$



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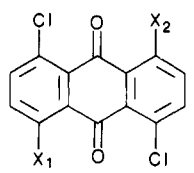
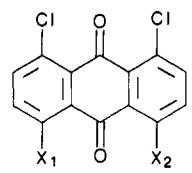
Tosylation of 1,5-dichloro-4,8-dihydroxyanthraquinone,[5] according to the method of Zielske,[6] yielded the ditosyl derivative. One of the two tosyl groups was readily replaced by *N,N*-dimethylethylenediamine in CH_3CN, which, upon hydrolysis, gave 1,5-dichloro-4-[[2-(dimethylamino)ethyl]amino]-8-hydroxyanthraquinone (**3a**). The related (hydroxyalkyl)amino derivative **3b** was prepared in a similar manner. The isomeric 1,8-dichloroanthraquinone compound **4a** was obtained from 1,8-dichloro-4,5-dihydroxyanthraquinone in an analogous fashion.





3a: $X_1 = NH(CH_2)_2N(CH_3)_2$, $X_2 = OH$
b: $X_1 = NH(CH_2)_2NH(CH_2)_2OH$, $X_2 = OH$
c: $X_1 = X_2 = NH(CH_2)_2N(CH_3)_2$

4a: $X_1 = NH(CH_2)_2N(CH_3)_2$, $X_2 = OH$
b: $X_1 = X_2 = NH(CH_2)_2N(CH_3)_2$

The bis(substituted amino)-1,5-dichloroanthraquinone **3c** and its 1,8-dichloro analogue **4b** were prepared by nitration of the appropriate dichloroanthraquinones at 25–35 °C followed by treatment of the resulting dichlorodinitroanthraquinones[7–9] with *N,N*-dimethylethylenediamine in dioxane.

An interesting replacement behavior was noticed with 1,4-dichloro-5,8-dihydroxyanthraquinone.[7] Treatment of the dichlorodihydroxyanthraquinone with aliphatic amines in pyridine under reflux resulted in the replacement of one halogen atom by the amino group to yield mainly 1-chloro-5,8-dihydroxy-4-(substituted amino)anthraquinones **5a–c**. On the other hand, reaction between the dichlorodihydroxyanthraquinone and the amines in refluxing

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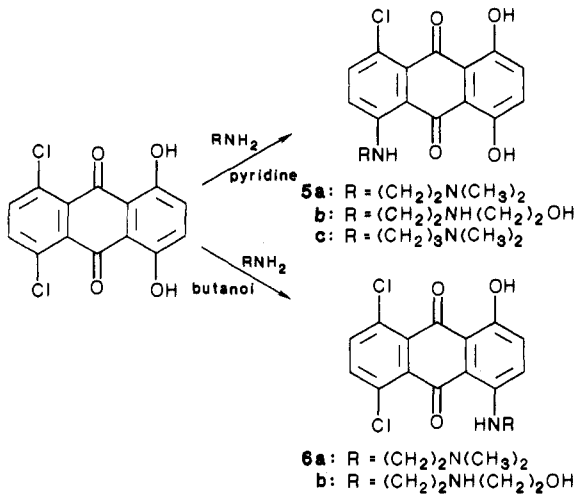
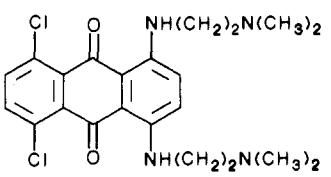


butanol replaced one of the two hydroxy groups by amine with retention of both chloro groups to form predominately 1,4-dichloro-5-hydroxy-8-(substituted amino)anthraquinones **6a,b**. Structures of these products were established by spectroscopic and elemental analyses and confirmed by unequivocal syntheses from the tosyl intermediates. Aromatic amines were reported to replace both chloro groups of the dichlorodihydroxyanthraquinone to form 1,4-dihydroxy-5,8-bis(arylamino)anthraquinones.[10,11]

The dichlorobis(substituted amino)anthraquinone **7** was prepared as follows: The aforementioned dichlorodihydroxyanthraquinone was reduced with Sn and HCl. The resulting leuco derivative was reacted with excess *N,N*-dimethylethylenediamine, followed by oxidizing of the condensed product with O_2. The method is analogous to that for the preparation of DHAQ.[1]



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Biological Activity and Discussion

Available test results are provided in Table I. In general, there is a good correlation between dose potency in in vivo and in vitro activity. Compound **6b** possesses the highest inhibitory activity against P388 leukemia among compounds in this series. Its inhibitory action against B16 melanoma and in the in vitro L1210 screen are likewise outstanding. Other compounds, including **6a**, **5a**, and **5b**, also showed noticeable activity against P388 in vivo and L1210 in vitro. Compounds containing the [2-[(2-hydroxyethyl)amino]ethyl]amino side chain seem to have a slight edge over those containing the [2-(dimethylamino)ethyl]amino side chain. The optimum number of carbon atoms between the two amino nitrogen atoms is two, which is analgous to the structural requirements in the DHAQ series.

Although all the aforementioned four compounds contain a hydroxyl group at the "opposite side" of the anthraquinone ring with regard to the position of the [(substituted amino)alkyl]amino side chain (as is the case of DHAQ and analogous compounds), the presence of the hydroxyl group may be a necessary, but not a sufficient, condition for compounds in the chloro-substituted series since compounds **3a**, **3b**, and **4a**, which also contain the hydroxyl group, are without activity in the present screen tests. Nevertheless, the contribution of a hydroxyl group present at a strategic position of a molecule to the biological activity should not be overlooked, as it has been repeatedly demonstrated by comparison of the activity of DHAQ and that of its unhydroxylated analogue.[1,2,12-15]

Table I. Antineoplastic and Cytotoxic Activities of Chloro-Substituted [(Aminoalkyl)amino]anthraquinones

	P388 in vivo tests[a]			in vitro tests[b]	
no.	treat. sched	T/C	dose/inj, mg/kg	L1210	human colon adenocarcinoma
1a	Q4D × 2	105	200	1.17×10^{-6} M	1.40×10^{-6} M
		100	100		
1b	Q1D × 9	120	160		
		124	80		
		116	40		
3a	Q4D × 2	117	80	1.13×10^{-6} M	
		103	40		
3b	Q4D × 2	111	400		
		96	200		
3c	Q4D × 2	123	400	3.85×10^{-7} M	2.29×10^{-7} M
		128	200		
		115	100		
4a	Q4D × 2	125	400	1.76×10^{-7} M	
		113	200		
4b	Q1D × 5	119	200	1.67×10^{-6} M	
		113	100		
5a	Q1D × 5	169	120	3.62×10^{-8} M	
		137	60		
		121	30		
5b	Q4D × 2	184	160	3.98×10^{-7} M	
		154	80		
		130	40		
		125	20		
		113	10		
5c	Q1D × 5	129	120	2.65×10^{-7} M	
		123	60		
		109	30		
6a	Q1D × 5	176	120	8.69×10^{-8} M	5.22×10^{-7} M
		150	60		
		136	30		
6b	Q1D × 5	219	100[c]	8.66×10^{-8} M	
		180	50		
		203	25		
		146	12.5		
		154	6.25		
7b	Q4D × 2	117	200[d]	1.17×10^{-7} M	1.30×10^{-7} M
		120	100		
		110	50		

[a] Ascites fluid implanted in BDF_1 mice. Route of drug administration and site of tumor inoculation: ip. For the general screening procedure and data interpretation, cf.: Geran, R. I.; Greenberg, N. H.; MacDonald, M. M.; Schumacher, A. M.; Abbott, B. J. *Cancer Chemother. Rep., Part 3* **1972**, *3*, 1. See also: *Screening Data Summary Interpretation and Outline of Current Screen, Instruction Booklet 14*; Drug Evaluation Branch, Division of Cancer Treatment, National Cancer Institute: Bethesda, MD, 1986. [b] Criterion for significant cytotoxic activity is 50% inhibition at a concentration of less than 10^{-6} M; cf.: Leopold, W. R.; Shillis, J. L.; Mertus, A. E.; Nelson, J. M.; Roberts, B. J.; Jackson, R. C. *Cancer Res.* **1984**, *44*, 1928. See also: Leopold, W. R.; Nelson, J. M.; Plowman, J.; Jackson, R. C. *Cancer Res.* **1985**, *45*, 5532. [c] Against B16 melanoma (treatment schedule, Q1D × 9): T/C 326 (160 mg/kg), 270 (100), 271 (80), 252 (50), 231 (40), 199 (25), 224 (20), 172 (12.5), and 166 (6.25). [d] Against B16 melanoma (treatment schedule, Q1D × 9): T/C 133 (50), 141 (25), 143 (12.5), and 117 (6.25).

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A comparison of the test results of compounds in this series with those of DHAQ and related compounds revealed that the aryl chloro-substituted [(aminoalkyl)amino]anthraquinones are, in general, less potent than the corresponding aryl hydroxy-substituted compounds. For example, compound **6b** gave T/C values of 219, 180, 203, 146, and 154 at doses of 100, 50, 25, 12.5, and 6.25 mg/kg, respectively, against P388 leukemia, whereas DHAQ[1a] and T/C values of 280, 277, 299, 280, 200, and 208 at doses of 2, 1, 0.5, 0.25, 0.12, and 0.06 mg/kg, respectively, against the same test system.

Experimental Section

Where analyses are indicated only by symbols of the elements, analytical results obtained for these elements were within ±0.4% of the theoretical values.

1-Chloro-5-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione (1a). A mixture of 11.6 g (40 mmol) of 1,5-dichloroanthraquinone[3] and 17.2 g (160 mmol) of 2-[(2-aminoethyl)amino]ethanol in 60 mL of $CH_3CON(CH_3)_2$ was stirred at room temperature for 1 h. It was then heated at 100 °C for 45 min. The reaction mixture was cooled, and unreacted dichloroanthraquinone was removed by filtration. To the filtrate was added 100 mL of petroleum ether (bp 35–40 °C), and the mixture was cooled overnight. The crude product (8 g, mp 125–128 °C) was collected by filtration [most of the bis(substituted amino)anthraquinone remained in the filtrate]. It was boiled with 2-PrOH, treated with charcoal, and precipitated with petroleum ether to give 2.6 g of dark brown powder, mp 137–140 °C. This was column chromatographed over neutral Al_2O_3 and eluted successively with petroleum ether, Et_2O, and MeOH. Pure **1a** was obtained as rust red powder that melted at 146–148 °C. The yield was 1.2 g (8.3% yield): UV λ_{max} (MeOH) 205 nm (log ϵ 4.41), 248 (4.53), 320 (3.79), 505 (3.87). Anal. ($C_{18}H_{17}ClN_2O_3$) C, H, N.

1-Chloro-8-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione (1b). A mixture of 4.2 g (15 mmol) of 1,8-dichloroanthraquinone,[3] 7.5 g (72 mmol) of 2-[(2-aminoethyl)amino]ethanol, and 90 mL of dioxane was stirred at room temperature for 30 min and then refluxed for 2.5 h. The solvent was removed by distillation in vacuo. The residue was washed successively with 100 mL of H_2O, 10 mL of EtOH, and 100 mL of Et_2O to give, after drying under reduced pressure, 4.7 g of the crude product, mp 130–138 °C. Recrystallization from EtOH gave 3.0 g (58% yield) of **1b**: mp 144–145 °C; UV λ_{max} (MeOH) 213 nm (log ϵ 4.47), 253 (4.96), 331 (3.95), 511 (3.16). Anal. ($C_{18}H_{17}ClN_2O_3$) C, H, N.

1,5-Dichloro-4,8-bis(tosyloxy)-9,10-anthracenedione. To 12.4 g (40 mmol) of 1,5-dichloro-4,8-dihydroxyanthraquinone in 400 mL of CH_2Cl_2 was added 22.4 mL (160 mmol) of Et_3N followed by 19.1 g (100 mmol) of p-$CH_3C_6H_4SO_2Cl$. The reaction mixture was stirred at room temperature for 24 h. It was transferred to a separatory funnel and washed with H_2O (3 × 150 mL). The organic layer was separated and dried over anhydrous Na_2SO_4. After the solvent was removed, there was obtained 23 g of solid, mp 175–178 °C. Recrystallization from a mixture of $CHCl_3$ and EtOH gave 20.2 g (81.5% yield) of product: mp 185–187 °C; UV λ_{max} (MeOH) 230 nm (log ϵ 4.59), 345 (3.70). This compound was used for following reactions without further characterization.

1,5-Dichloro-4-[[2-(dimethylamino)ethyl]amino]-8-(tosyloxy)-9,10-anthracenedione. To a stirring mixture of 6.2 g (10 mmol) of the ditosylanthraquinone in 250 mL of CH_3CN was added 2.0 g (22 mol) of *N,N*-dimethylethylenediamine. The mixture was refluxed for 20 h and then was evaporated to dryness under reduced pressure. The residue was dissolved in 100 mL of $CHCl_3$ and washed with H_2O (2 × 50 mL). The $CHCl_3$ extract was dried (Na_2SO_4) to give, after removal of solvent, 3.2 g (61% yield) of the product, mp 180–183 °C. The product was purified by recrystallization from EtOH: mp 195–197 °C; UV λ_{max} (MeOH) 230 nm (log ϵ 4.60), 250 (4.64), 335 (3.77), 510 (3.90); NMR ($CDCl_3$) δ 2.3 (s, 6 H, CH_3), 2.4 (s, 3 H, CH_3), 2.6 (t, 2 H, CH_2), 3.4 (q, 2 H, CH_2), 6.8–8.0 (m, 8 H, Ar H), 9.2 (br s, 1 H, NH).

1,5-Dichloro-4-[[2-(dimethylamino)ethyl]amino]-8-hydroxy-9,10-anthracenedione (3a). To a solution of 4.5 g (8 mmol) of the preceding tosyl intermediate in 150 mL of EtOH was added 150 mL of 5% NaOH. The mixture was heated at 75–80 °C for 2.5 h and filtered while hot. The filtrate was acidified with 5 N HCl to pH 1 and then made basic with NH_4OH. The solid thus formed was collected by filtration, washed with H_2O, and dried in vacuo to yield 3.7 g of crude **3a**, mp 126–128 °C. It was recrystallized from EtOH to give 2.7 g (85% yield) of pure **3a**: mp 129–131 °C dec; UV λ_{max} (MeOH) 233 nm (log ϵ 4.62), 325 (370), 410 (3.58), 525 (3.92); NMR ($CDCl_3$) δ 2.4 (s, 6 H, CH_3) 2.7 (t, 2 H, CH_2), 3.4 (q, 2 H, CH_2), 7.0–7.8 (m, 4 H, Ar H), 10.1 (br s, 1 H, NH); mass spectrum 379 (M^+), 380 ($M^+ + 1$). Anal. ($C_{18}H_{16}Cl_2N_2O_3$) C, H, N.

1,5-Dichloro-4-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-8-(tosyloxy)-9,10-anthracenedione. A mixture of 3.08 g (5 mmol) of the aforementioned ditosylanthraquinone, 100 mL of CH_3CN, and 1.04 g (10 mmol) of 2-[(2-aminoethyl)amino]-ethanol was refluxed for 5 h and evaporated to dryness. The residue was purified by chromatography through a SiO_2 column to give 1.96 g (71% yield) of the product, mp 102–105 °C. This intermediate was used for the preparation of **3b** without further purification.

1,5-Dichloro-4-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-8-hydroxy-9,10-anthracenedione (3b). To a solution of 0.9 g (1.6 mmol) of the preceding tosyl compound in 25 mL of EtOH was added 25 mL of 7% NaOH. The mixture was refluxed for 4 h and filtered while hot. The filtrate was acidified to pH 1 with 2 N HCl and then made basic with NH_4OH. The solid thus formed was collected by filtration, washed with H_2O, and dried in vacuo to give 0.48 g of crude **3b**. It was purified by chromatography over SiO_2 to give 0.32 g (49% yield) of **3b**; mp 116–119 °C dec; UV λ_{max} (MeOH) 235 nm (log ϵ 4.58), 330 (3.60), 415 (3.51), 525 (3.85); mass spectrum 395 (M^+), 397 ($M^+ + 2$). Anal. ($C_{18}H_{16}Cl_2N_2O_4$) C, H, N.

1,5-Bis[[2-(dimethylamino)ethyl]amino]-4,8-dichloro-9,10-anthracenedione (3c). A mixture of 5.4 g (14.7 mmol) of 1,5-dichloro-4,8-dinitroanthraquinone[7-9] in 30 mL of dioxane was heated to 50 °C with stirring. To the resulting solution was added dropwise (20 min) 8 g (90 mmol) of *N,N*-dimethylethylenediamine in 20 mL of dioxane. The resulting mixture was heated at 50–60 °C with sitrring for 2 h. Solvent was removed under reduced pressure. To the residue was added 100 mL of H_2O. After the mixture was stirred for 30 min at room temperature, the solid was collected by filtration and washed well with H_2O and then with Et_2O. The solid product was recrystallized from a small amount of MeOH to yield a crude product, mp 186–188 °C. It was further purified by column chromatography over SiO_2 and eluted seccessively with benzene and 5:1 benzene–acetone to give 2.4 g (36% yield) of **3c**: mp 189–191 °C; UV λ_{max} (MeOH) 206 nm (log ϵ 4.39), 237 (4.48), 318 (3.72), 524 (3.95); mass spectrum 450 (M^+). Anal. ($C_{22}H_{26}Cl_2N_4O_2 \cdot 0.25H_2O$) C, H, N.

1,8-Dichloro-4,5-bis(tosyloxy)-9,10-anthracenedione. To a solution of 12.4 g (40 mmol) of 1,8-dichloro-4,5-dihydroxyanthraquinone[5] in 400 mL of CH_2Cl_2 was added 22.4 mL of Et_3N followed by 19.1 g (100 mmol) of p-$CH_3C_6H_4SO_2Cl$. The mixture was refluxed for 12 h and filtered. The filtrate was washed with H_2O (3 × 5 mL) in a separatory funnel and dried (Na_2SO_4). The solvent was removed under reduced pressure, and the residue was triturated with a small amount of Et_2O. The resulting solid was collected by filtration, washed with a small amount of CH_2Cl_2, and dried to yield 18.5 g of a crude product, mp 198–201 °C. It was recrystallized from $CHCl_3$ to give 16.1 g (65% yield) of the ditosyl compound: mp 207–209 °C; UV λ_{max} (MeOH) 220 nm (log ϵ 4.57), 250 (4.44), 350 (3.50). This compound was used for the preparation of **4a** without further characterization.

1,8-Dichloro-4-[[2-(dimethylamino)ethyl]amino]-5-hydroxy-9,10-anthracenedione (4a). A mixture of 10.8 g (17.5 mmol) of the preceding ditosylanthraquinone, 3.0 g (35 mmol) of *N,N*-dimethylethylenediamine, and 250 mL of CH_3CN was refluxed for 7 h. After removal of the solvent, the residue was dissolved in 150 mL of EtOH. To the solution was added 150 mL of 7% NaOH. The mixture was refluxed for 4.5 h and filtered while hot. The filtrate was acidified with 5 N HCl and then made basic with NH_4OH. The solid thus formed was collected by

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filtration, washed with H_2O, and dried. Purification through column chromatography over SiO_2 with $CHCl_3$–EtOH (95:5) as eluent followed by recrystallization from EtOH gave 3.2 g (39.4% yield) of **4a**: mp 151–153 °C; UV λ_{max} (MeOH) 207 nm (log ϵ 4.39), 235 (5.63), 326 (3.58), 525 (4.06). Anal. ($C_{18}H_{16}Cl_2N_2O_3 \cdot 0.25H_2O$) C, H, N.

1,8-Bis[[2-(dimethylamino)ethyl]amino]-4,5-dichloro-9,10-anthracenedione (4b). To a stirred solution of 2.6 g (7.1 mmol) of 1,8-dichloro-4,5-dinitroanthraquinone in 20 mL of dioxane at 100 °C was added dropwise 6.2 g (270 mmol) of *N,N*-dimethylethylenediamine in 8 mL of dioxane. The mixture was refluxed for 2 h (TLC showed the absence of starting material). Excess solvent as removed under reduced pressure. To the residue was added 50 mL of H_2O. After the mixture was stirred for 30 min at room temperature, the solid was collected by filtration and washed with H_2O, cold EtOH, and then Et_2O. It was recrystallized initially from MeOH and then from 2-PrOH, to give 0.7 g (21% yield) of **4b**: mp 185–187 °C; UV λ_{max} (MeOH) 204 nm (log ϵ 4.37), 236 (4.50), 318 (3.92), 544 (4.04); mass spectrum 450 (M^+). Anal. ($C_{22}H_{26}Cl_2N_4O_2 \cdot H_2O$) C, H, N.

1-Chloro-5,8-dihydroxy-4-[[2-(dimethylamino)ethyl]amino]-9,10-anthracenedione (5a). To a stirred solution of 12.4 g (40 mmol) of 1,4-dichloro-5,8-dihydroxyanthraquinone[7] in 150 mL of pyridine was added 8.8 g (100 mmol) of *N,N*-dimethylethylenediamine. The mixture was refluxed with continuous stirring for 90 min. Excess solvent was removed by evaporation under reduced pressure. The residue was triturated with petroleum ether to yield 15 g of a crude product. It was chromatographed over a SiO_2 column and eluted successively with $CHCl_3$, 95:5 $CHCl_3$–EtOH, and 9:1 $CHCl_3$–EtOH to give, after recrystallization from EtOH, 8.2 g (57% yield) of **5a** as a purple solid: mp 168–170 °C; UV λ_{max} (MeOH) 240 nm (log ϵ 4.65), 290 (4.00), 550 (4.13), 572 (4.12); NMR ($CDCl_3$) δ 2.3 (s, 6 H, NCH_3), 2.7 (t, 2 H, CH_2), 3.3 (t, 2 H, CH_2), 6.8–7.5 (m, 4 H, Ar H), 10.0 (br s, 1 H, NH), 12.9 (br s, 2 H, OH). Anal. ($C_{18}H_{17}ClN_2O_4$) C, H, N.

1-Chloro-5,8-dihydroxy-4-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione (5b). This compound was prepared in a manner similar to that for **5a** from 9.3 g (30 mmol) of 1,4-dichloro-5,8-dihydroxyanthraquinone,[7] 6.3 g (60 mmol) of 2-[(aminoethyl)amino]ethanol, and 150 mL of pyridine. After column chromatography ($CHCl_3$, 95:5 $CHCl_3$–EtOH, 8:2 $CHCl_3$–EtOH, and 7:3 $CHCl_3$–EtOH successively) and crystallization from EtOH, 3.1 g (28% yield) of **5b** was obtained as purple crystals: (softened at 185 °C) 204–206 °C dec; UV λ_{max} (MeOH) 235 nm (log ϵ 4.52), 285 (3.89), 545 (3.96), 565 (3.95); mass spectrum 376 (M^+), 377 ($M^+ + 1$). Anal. ($C_{18}H_{17}ClN_2O_5 \cdot 1.25H_2O$) C, H, N.

1-Chloro-5,8-dihydroxy-4-[[3-(dimethylamino)propyl]amino]-9,10-anthracenedione (5c). This compound was prepared in a manner similar to those for **5a** and **5b** from 4.5 g (14.5 mmol) of 1,4-dichloro-5,8-dihydroxyanthraquinone,[7] 3.4 g (33 mmol) of *N,N*-dimethylpropylenediamine, and 100 mL of pyridine to give, after column chromatography and recrystallization from a mixture of $CHCl_3$ and petroleum ether, 3.3 g (60% yield) of a purple solid: mp 134–136 °C dec; UV λ_{max} (MeOH) 240 nm (log ϵ 4.70), 290 (4.04), 550 (4.16), 575 (4.14); NMR ($CDCl_3$) δ 1.9 (q, 2 H, CH_2), 2.3 (s, 6 H, NCH_3), 2.4 (q, 2 H, CH_2), 3.3 (q, 2 H, CH_2), 6.7–7.4 (m, 4 H, Ar H), 9.8 (br s, 1 H, NH), 12.8 (br s, 2 H, OH); mass spectrum 374 (M^+). Anal. ($C_{19}H_{19}ClN_2O_4$) C, H, N.

The hydrochloride salt of **5c** was also prepared: mp 266–268 °C; UV λ_{max} (MeOH) 204 nm (log ϵ 4.27), 234 (4.63), 285 (3.99), 540 (4.07), 570 (4.20); mass spectrum 374 (M^+). Anal. ($C_{19}H_{19}ClN_2O_4 \cdot HCl \cdot 0.5H_2O$) C, H, N.

1,4-Dichloro-5-[[2-(dimethylamio)ethyl]amino]-8-hydroxy-9,10-anthracenedione (6a). To a stirred mixture of 9.3 g (30 mmol) of 1,4-dichloro-5,8-dihydroxyanthraquinone[7] in 160 mL of BuOH was added a solution of 5.5 g (40 mmol) of *N,N*-dimethylethylenediamine in 40 mL of BuOH. The mixture was heated at 135 °C for 5 h and then cooled. The resulting dark purple solid was collected by filtration, washed with petroleum ether (3 × 20 mL), and dried to give 6 g (51% yield) of crude **6a**, mp 125–130 °C. It was purified through a SiO_2 column with $CHCl_3$ as the eluting solvent. The resulting evaporated solid was recrystallized from a small amount of EtOH to give 0.9 g (8% yield) of pure **6a**: mp 146–148 °C; UV λ_{max} (MeOH) 215 nm (log ϵ 4.31), 238 (4.33), 290 (4.13), 345 (3.65), 540 (3.91), 570 (3.85). Anal. ($C_{18}H_{16}Cl_2N_2O_3 \cdot 0.5H_2O$) C, H, N.

1,4-Dichloro-5-hydroxy-8-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-9,10-anthracenedione (6b). To a mixture of 3.1 g (10 mmol) of 1,4-dichloro-5,8-dihydroxyanthraquinone[7] in 60 mL of BuOH was added, with stirring, a solution of 1.5 g (15 mmol) of 2-[(aminoethyl)amino]ethanol in 20 mL of BuOH. The mixture was refluxed for 5 h with continuous stirring. It was cooled, and the resulting dark purple crystals were collected by filtration. The solid was washed with petroleum ether (3 × 20 mL) and dried to give 4.0 g (76% yield) of crude product, mp 85–100 °C. Recrystallization from a mixture of 500 mL of EtOH and 500 mL of petroleum ether (bp 60–68 °C) gave 750 mg (18.5% yield) of purified **6b**: mp 178–180 °C; UV λ_{max} (MeOH) 206 nm (log ϵ 4.27), 239 (4.42), 258 (4.31), 540 (3.91), 570 (3.97); mass spectrum 395 (M^+), 397 (isotope Cl). Anal. ($C_{18}H_{16}Cl_2N_2O_4 \cdot 0.5H_2O$) C, H, N.

The structures of compounds **6a** and **6b** were confirmed by unequivocal syntheses from the tosyl intermediates. Following is a description of the synthesis of **6b** via the ditosyl intermediate.

To a mixture of 12.4 g (40 mmol) of 1,4-dichloro-5,8-dihydroxyanthraquinone[7] and 19.1 g (100 mmol) of *p*-$CH_3C_6H_4SO_2Cl$ in 400 mL of CH_2Cl_2 was added dropwise, in 15 min, 29 mL (200 mL) of Et_3N. The mixture was stirred at room temperature for 20 h and then decomposed with 400 mL of H_2O. The organic layer was separated, washed with H_2O (2 × 400 mL), and dried (Na_2SO_4). The solvent was removed by evaporation under reduced pressure to give 24 g of 1,4-dichloro-5,8-ditosyl-9,10-anthracenedione, mp 178–180 °C. Recrystallization from a mixture of $CHCl_3$ and petroleum ether gave the ditosyl compound as white crystals: mp 181–183 °C; UV λ_{max} (MeOH), 204 nm (log ϵ 4.49), 220 (4.61), 345 (3.62).

To a solution of 6.2 g (10 mmol) of the ditosyl compound in 130 mL of CH_2Cl_2 was added dropwise, with stirring, 2.6 g (25 mmol) of 2-[(2-aminoethyl)amino]ethanol. The mixture was stirred at room temperature for 30 min and then heated under reflux for 10 h. The reaction mixture was cooled and the organic layer separated, dried (Na_2SO_4), and evaporated under reduced pressure. The residual solid was added to a solution of 10.8 g of KOH in 150 mL of EtOH and 30 mL of H_2O and heated at 80 °C for 2 h. The resulting mixture was acidified with 10% HCl and then neutralized with NH_4OH to give, after cooling, 8.8 g of solid. Column chromatography of this material over SiO_2 with $CHCl_3$–MeOH (9:1) as eluent gave a product, mp 178–180 °C (mixed mp 178–180 °C). Its IR, UV absorption, and TLC characteristics are identical with those of the product obtained by the aforementioned BuOH method.

1,4-Bis[[2-(dimethylamino)ethyl]amino]-5,8-dichloro-9,10-anthracenedione (7). A mixture of 8 g (25 mmol) of 1,4-dichloroleucoquinizarin (prepared by heating 12.4 g of 1,4-dichloro-5,8-dihydroxyanthraquinone in 23.5 g of Sn, 70 mL of HCl, and 400 mL of AcOH at 90–95 °C for 22 h, collecting the insoluble solid, washing it with H_2O, and drying it in vacuo, λ_{max} (MeOH) 430 and 448 nm) and 18 g (200 mmol) of *N,N*-dimethylethylenediamine was heated with stirring at 55 °C for 2 h. The reaction was cooled, and to it was added 20 mL of 2-PrOH. It was stirred overnight under O_2. To the resulting mixture was added 100 mL of petroleum ether. After the mixture was stirred for 1 h, the solid was collected by filtration, washed with petroleum ether (3 × 30 mL), and dried in vacuo to give 10.3 g of the crude product. This was dissolved in 200 mL of hot MeOH saturated with HCl and 200 mL of Et_2O. The mixture was stirred for 10 min. The solid was collected by filtration, washed with Et_2O, and dried to give 12 g of the HCl salt of the crude product, mp 249–251 °C. Recrystallization from BuOH gave 4.5 g (33.5% yield) of **7**: mp 260–262 °C; UV λ_{max} (MeOH) 218 nm (log ϵ 4.46), 242 (4.50), 328 (3.67), 610 (4.04), 649 (3.99). Anal. ($C_{22}H_{26}Cl_2N_4O_2 \cdot 2HCl \cdot 0.25H_2O$) C, H, N.

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Registry No. **1a**, 109217-80-3; **1b**, 109217-81-4; **3a**, 109217-82-5; **3b**, 109217-83-6; **3c**, 109217-84-7; **4a**, 109217-85-8; **4b**, 109217-86-9; **5a**, 109217-87-0; **5b**, 109217-88-1; **5c**, 109217-89-2; **5c**·HCl, 109217-90-5; **6a**, 109217-91-6; **6b**, 109217-92-7; **7**·2HCl, 109217-93-8; mitoxantrone, 65271-80-9; 1,5-dichloroanthraquinone, 82-46-2; 2-[(2-aminoethyl)amino]ethanol, 111-41-1; 1,8-dichloroanthraquinone, 82-43-9; 1,5-dichloro-4,8-dihydroxyanthraquinone, 6837-97-4; 1,5-dichloro-4,8-bis(tosyloxy)-9,10-anthracenedione, 109217-79-0; *N,N*-dimethylethylenediamine, 108-00-9; 1,5-dichloro-4-[[2-(dimethylamino)ethyl]amino]-8-(tosyloxy)-9,10-anthracenedione, 109241-78-3; 1,5-dichloro-4-[[2-[(2-hydroxyethyl)amino]ethyl]amino]-8-(tosyloxy)-9,10-anthracenedione, 109241-79-4; 1,5-dichloro-4,8-dinitroanthraquinone, 6305-89-1; 1,8-dichloro-4,5-dihydroxyanthraquinone, 66227-51-8; 1,8-dichloro-4,5-bis(tosyloxy)-9,10-anthracenedione, 109241-80-7; 1,8-dichloro-4,5-dinitroanthraquinone, 6305-90-4; 1,4-dichloro-5,8-dihydroxyanthraquinone, 2832-30-6; *N,N*-dimethylpropylenediamine, 109-55-7; 1,4-dichloro-5,8-bis(tosyloxy)-9,10-anthracenedione, 91441-77-9; 1,4-dichloroleucoquinizarin, 98809-43-9.

Improved Synthesis and Antitumor Activity of 1-Deazaadenosine

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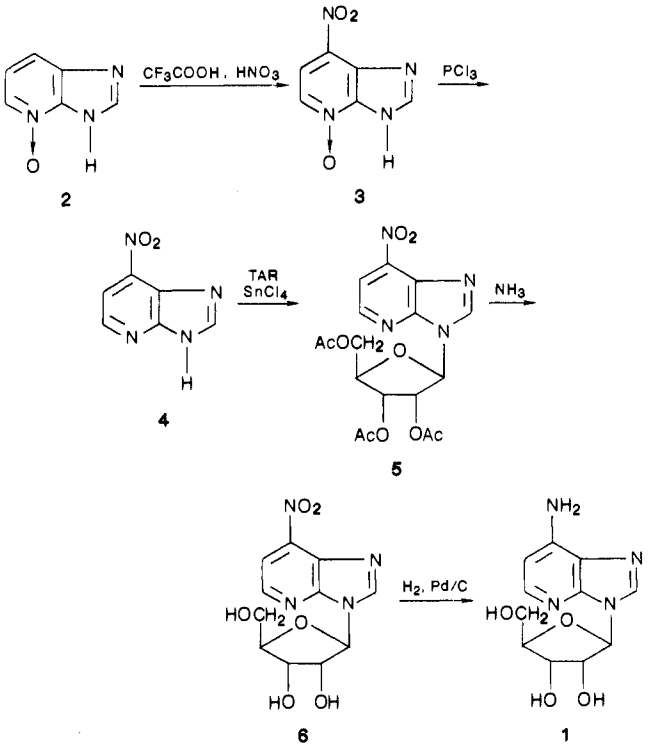
A more convenient synthetic route to 1-deazaadenosine (1) by reduction of the new nucleoside 7-nitro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (**6**) is reported. Compound **6** was obtained by reaction of 7-nitroimidazo[4,5-*b*]pyridine with 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose in the presence of stannic chloride followed by treatment with methanolic ammonia. 1-Deazaadenosine (1) showed good activity in vitro as inhibitor of HeLa, KB, P388, and L1210 leukemia cell line growth, with ID_{50} values ranging from 0.34 μM (KB) to 1.8 μM (P388). The nitro derivative **6** demonstrated moderate activity against the same cell lines.

The adenosine analogue 1-deazaadenosine (1) was found to have a wide spectrum of biological properties. Recently our studies stressed its activity as an inhibitor of blood platelet aggregation[1] and of adenosine deaminase[2] and as an agonist of the adenosine receptors.[3] Furthermore, Japanese authors have reported, without providing experimental data, that 1 shows potent antileukemic activity.[4] Several syntheses of 1 have been reported[5] and the most convenient one appears to be that described by Itoh and co-workers, who have obtained in six steps the title compound in 25% overall yield, starting from imidazo[4,5-*b*]pyridine 4-oxide (**2**).[5d] Recently we reported an improved synthesis of 7-nitroimidazo[4,5-*b*]pyridine 4-oxide (**3**) accomplished by nitration of **2** with a nitric acid–trifluoroacetic acid mixture.[1] The possibility that such a compound might be an useful intermediate in the synthesis of 1-deazaadenosine prompted us to design a new synthetic route to 1.

Chemistry

The synthesis of 1-deazaadenosine (1) was carried out according to Scheme I. Reaction of compound **3** with phosphorus trichloride in acetonitrile gave the deoxygenated derivative **4**, which was condensed with an equimolar amount of 1,2,3,5-tetra-*O*-acetyl-β-D-ribofuranose (TAR) in acetonitrile in the presence of a catalytic amount of stannic chloride to provide 7-nitro-3-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (**5**) in 82% yield. The 7-amino-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (1-deazaadenosine, 1) was easily obtained from the deblocked nucleoside **6** in 93% yield by hydrogenation

Scheme I



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