Synthesis of Potential Antineoplastic Anthracenedione Derivatives. II. 1,2-Dihydroxy Derivatives

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In continuation of the preceding paper in this series,[1] four derivatives of 1,2-dihydroxy-5,8-bis[2-(2-hydroxyethylamino)ethylamino]-9,10-anthracenedione: a positional isomer of mitoxantrone have been prepared to establish a relationship between the position of the hydroxyl groups on the A ring and antineoplastic activity against experimental leukemia L1210 in mice. Two compounds were found to be fairly active.

In continuation of the preceding paper in this series,[1] 1,2-dihydroxy-5,8-bis[2-(2-hydroxyethylamino)ethylamino]-9,10-anthracenedione (**2**): a positional isomer of mitoxantrone has been synthesized, together with the 1-, 2- and 1,2-di-*O*-methyl derivatives in order to establish the role of hydroxyl groups in an appearance of antitumor activity. It has been described by J. R. Brown[2] that 2-substituted anthraquinones were active against leukemia L1210 in mice. Also, 1,2-dihydroxyanthraquinone derivatives were found to be cytotoxic and antileukemic compounds.[3]

While, a DNA-intercalating drug, mitoxantrone is a 1,4-dihydroxy analog of **2** and exhibits high antitumor activity against leukemia L1210 in mice.[4-7] The effective binding to DNA is achieved in the C ring and multiple substitutions with hydroxyl groups in the A ring in an anthraquinone skeleton.[2]

From the facts that mitoxantrone (1,4-dihydroxy analog) and 1-hydroxy analog are active,[5] it has been deduced that an existence of a hydroxyl group on the C-1 position is favorable for antitumor activity. In the present study, a role of another hydroxyl group on the C-2 position will be discussed.

Results and Discussion

A treatment of quinalizarin (**1**) with 2-(2-aminoethylamino)ethanol at elevated temperature gave 1,2-dihydroxy-5,8-bis[2-(2-hydroxyethylamino)ethylamino]-9,10-anthracenedione **2** as a crude product.[7-9] The product was converted into the partially protected compound (**6**) in 19% yield. Removal of the protective groups in **6** with methanolic HCl yielded **2** in 79% yield. Overall yield of **2** from quinalizarin was 15%. Mild hydrolysis of **6** in aq acetic acid afforded the partially deprotected compound (**7**) in 34% yield. Tritylation of **7** gave the ditrityl derivative (**8**) in 73% yield. Also, **8** was obtained by an alternative reaction process as follows. Tritylation of **6** afforded the compound (**9**) in 75% yield, which was converted, by heating with potassium carbonate in acetone, to **8** in 94% yield.

To protect one of the hydroxyl groups on the C-2 position selectively, protection of **8** with *p*-methoxybenzyl chloride yielded 2-*O*-(*p*-methoxybenzyl) derivative (**10**) in 87% yield. Methylation of **10** with dimethyl sulfate and potassium carbonate in acetone gave a 1-*O*-methyl derivative (**11**) in 77%, which was further converted into the dihydrochloride (**3**), by treating with methanolic HCl, in 70% yield.

To prove the structure, **3** was converted into a pentaacetyl derivative (**12**), whose ^{1}H NMR spectrum was identical with the proposed structure.

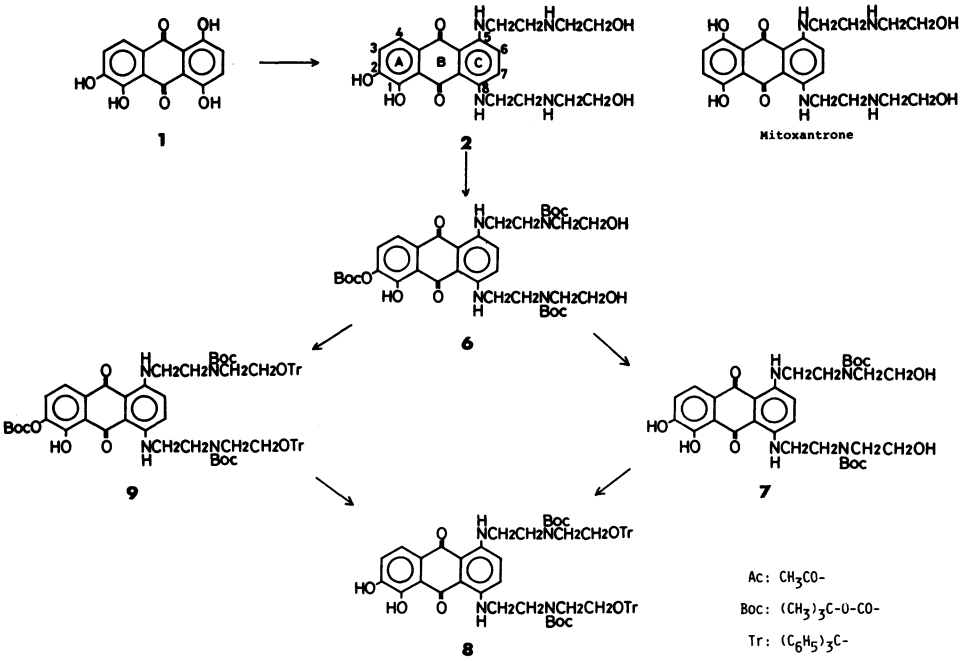
The positional isomer of **3** (**4**), 2-*O*-methyl derivative was prepared by the following process.

Methylation of **8** with dimethyl sulfate and potassium carbonate gave a 2-*O*-methyl derivative (**13**) exclusively in 93% yield. Deprotection of **13** in methanolic HCl

Table 1. Antitumor activity ofcompounds **2**, **3**, **4** and **5** against mouse leukemia L1210[a]

Compound	Dose (mg/kg)	ILS[b] (%)	60-day survival
2	50	Toxic	0/5
	37.5	>160	2/5
	25	>757	5/5
	12.5	>100	1/5
	6.25	114	0/5
3	200	88	0/5
	100	114	0/5
	50	114	0/5
	25	71	0/5
	12.5	71	0/5
	6.25	71	0/5
4	100	Toxic	0/5
	50	50	0/5
	25	25	0/5
	12.5	25	0/5
	6.25	13	0/5
5	100	14	0/5
	50	14	0/5
	25	0	0/5
	12.5	14	0/5
	6.25	14	0/5
Mitoxantrone	25	Toxic	0/5
	12.5	>157	1/5
	6.25	329	0/5
	3.12	>757	3/5
	1.5	>129	1/5

a) Male BDF_1 hybrid mice were inoculated intraperitoneally with 10^6 cells of lymphoid leukemia L1210. Compounds were dissolved in distilled water and administered intraperitoneally at a volume of 0.1 ml/animal once at 24 h after the tumor implantation. b) Percentage increase in life span of treated animals compared with control tumor bearers [100(T/C-1)].



Scheme 1.

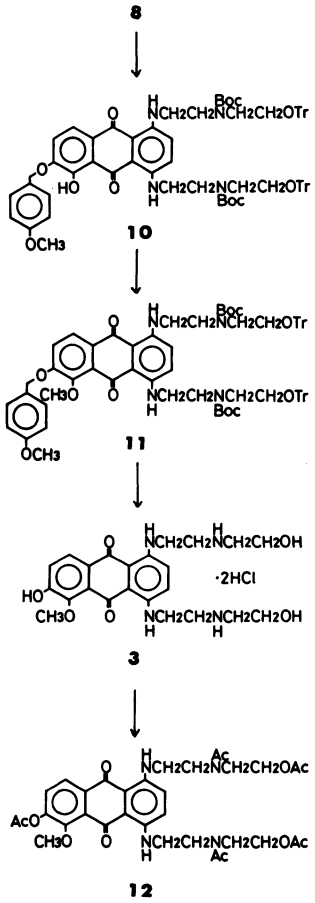
gave **4** in 56% yield.

Compound **4** was also obtained by another route as follows. Methylation of **9** with dimethyl sulfate and potassium carbonate afforded a 1-*O*-(*t*-butoxycarbonyl)-2-*O*-methyl derivative (**14**) in 64% yield. The reaction involved a migration of the *t*-butoxycarbonyl (Boc) group from 2-*O* to 1-*O*. Deprotection of **14** in methanolic HCl gave **4** in 87% yield.

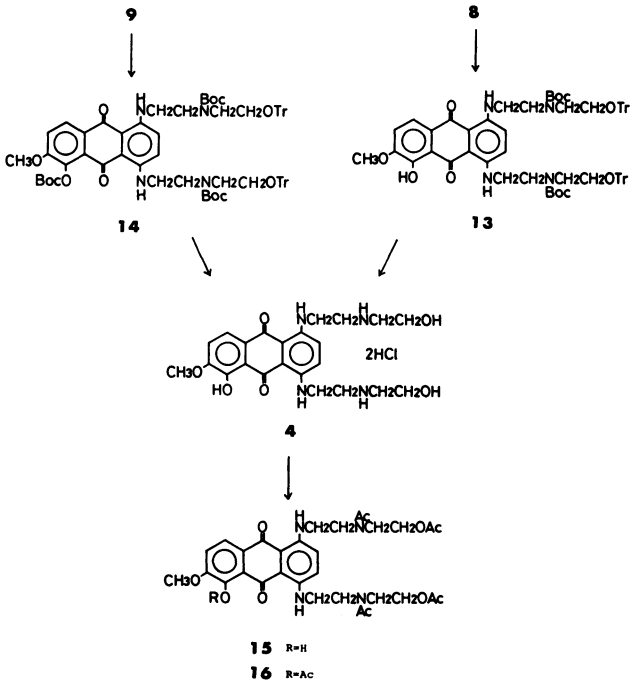
Compound **4** was converted to a tetraacetyl derivative (**15**) and a pentaacetyl derivative (**16**), whose ^{1}H NMR spectrum were identical with the proposed structure.

Finally, the 1,2-di-*O*-methyl derivative (**5**) was prepared by the analogous methylation for a prolonged period and successive deprotection in methanolic HCl from **8** *via* compound (**17**).

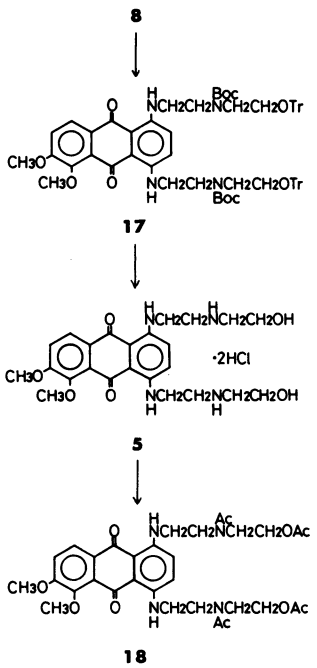
Acetylation of **5** gave a tetraacetyl derivative (**18**).



Scheme 2.



Scheme 3.



Scheme 4.

When ^{1}H NMR spectra of **12**, **16**, and **18** were compared each other, characteristic chemical shifts of acetoxyl groups on C-1 and 2 provided a good evidence for the structures. The chemical shift of the acetoxyl group on C-2 was attributable to δ 2.37 and that of the acetoxyl group on C-1 was δ 2.46, since the latter was deshielded to the down field by the quinone carbonyl group on C-9.[10]

Antitumor activities of **2**, **3**, **4**, and **5** were determined against experimental leukemia L1210 in mice.[11] Compound **2** was highly active, producing ILS value (>757%) and curing all mice by day 60. While mitoxantrone produced the same ILS value (>757%) but was curing 3 animals in 5 mice by day 60. Therefore, **2** is some what superior to mitoxantrone in number of curing animals by day 60. In the case of **5** where the two hydroxyl groups on C-1 and 2 were methylated, the compound did not show any activity. When the hydroxyl group on C-1 was methylated, **3** retained considerable activity producing 114% ILS value. While, the hydroxyl group on C-2 was methylated, **4** was fairly toxic. Considering from these facts, an existence of the free hydroxyl group on C-2 enhances antitumor activity, together with a presence of the hydroxyl group on C-1.

Experimental

General Methods. The same methods were used as described in the preceding paper.[1]

1,2-Dihydroxy-5,8-bis[2-(2-hydroxyethylamino)ethylamino]-9,10-anhracenedione Dihydrochloride (2). *(a) From quinalizarin (1)*: To a suspension of quinalizarin (**1**, 3.00 g) in 1-butanol (30 ml), a solution of 2-(2-aminoethylamino)ethanol (6.0 g) in 1-butanol (20 ml) was added under N_2 and stirred at 150 °C for 8 h. To the mixture, hexane was added and stirred overnight. The supernatant liquid was removed by decantation and the residue was washed with hot ethanol to give 4.56 g of **2**.

(b) From **6**: Compound **6** (200 mg) was dissolved in methanolic HCl (8 ml) under ice cooling. After 7 h, the precipitates were collected by filtration and recrystallized from 5 : 5 : 2 (v/v) ethanol–methanol–water to give 109 mg (79%) of **2**, mp 214—219 °C; UV (H_2O) 245 (log ε, 4.56), 591 (4.28), 635 nm (4.27); IR (KBr) 1615 cm^{-1} (quinone). ^{1}H NMR (D_2O): δ=3.33—3.93 (12H, m, CH_2 of side chain), 4.00—4.27 (4H, m, CH_2 of side chain), 6.63 (1H, d, J=9.0 Hz, aromatic proton), 6.85—7.33 (3H, m, aromatic protons).

Found: C, 49.72; H, 5.77; N, 10.16; Cl, 12.93%. Calcd for $C_{22}H_{30}N_4Cl_2O_6\cdot H_2O$: C, 49.35; H, 6.02; N, 10.46; Cl, 13.24%.

2-(t-Butoxycarbonyloxy)-5,8-bis[2-(N-t-butoxycarbonyl-2-hydroxyethylamino)ethylamino]-1-hydroxy-9,10-anthracenedione (6). To a stirred solution of crude **2** (2.00 g) in 1 M NaOH solution (50 ml) and dioxane (25 ml) (1 M=1 mol dm^{-3}), di-*t*-butyl dicarbonate (4.91 g) was added. After 16 h, 1 M HCl solution (50 ml) was added under ice cooling, and the mixture was extracted with ethyl acetate. The Organic layer was washed with water, dried over anhydrous Na_2SO_4 and concentrated. The residue was purified on a column using 1 : 3 (v/v) acetone–toluene. Fractions homogeneous (R_f 0.47) on TLC in 1 : 1 (v/v) acetone–toluene were collected and concentrated. The residue was recrystallized from benzene–hexane to give 651 mg (19%) of **6**, mp 154—155 °C; UV ($CHCl_3$) 260 (log ε, 4.41), 381 (3.68), 606 (4.25), 656 nm (4.36). ^{1}H NMR: δ= 1.46 (18H, s, NBoc×2), 1.61 (9H, s, OBoc), 2.93—3.61 (12H, m, NHC$\underline{H_2}$C$\underline{H_2}$N(Boc)C$\underline{H_2}$$CH_2$OH), 3.64—3.90 (4H, m, CH_2C$\underline{H_2}$OH), 6.73—6.89 (2H, m, H-6 and 7), 7.29 (1H, d, J=9.0 Hz, H-3), 7.58 (1H, d, J=9.0 Hz, H-4), 10.12 (1H, bs, aromatic NH), 10.77 (1H, bs, aromatic NH), 13.73 (1H, s, 1-OH).

Found: C, 59.43; H, 6.89; N, 7.25%. Calcd for $C_{37}H_{52}N_4O_{12}$: C, 59.66; H, 7.04; N, 7.52%.

5,5-Bis[2-(N-t-butoxycarbonyl-2-hydroxyethylamino)ethylamino]-1,2-dihydroxy-9,10-anthracenedione (7). Compound **6** (400 mg) was dissolved in 80% aqueous acetic acid (10 ml) and stirred at 100 °C for 30 min. The mixture was concentrated and the residue was purified on a column using 1 : 7 (v/v) ethanol–toluene. Fractions homogeneous (R_f 0.43) on TLC in 1 : 5 (v/v) ethanol–toluene were collected and concentrated. The residue was recrystallized from benzene–hexane to give 118 mg (34%) of **7**, mp 164—166 °C; UV ($CHCl_3$) 260 (log ε, 4.36) (shoulder), 274 (4.26) (shoulder), 565 (3.87) (shoulder), 602 (4.19), 650 nm (4.27). ^{1}H NMR: δ=1.47 (18H, s, NBoc×2), 3.44 (12H, bs, NHC$\underline{H_2}$C$\underline{H_2}$N(Boc)C$\underline{H_2}$$CH_2$OH), 3.76 (4H, bs, CH_2C$\underline{H_2}$OH), 6.54—7.54 (5H, m, aromatic protons and 2-OH), 9.81 (1H, bs, aromatic NH), 10.51 (1H, bs, aromatic NH), 13.48 (1H, s, 1-OH).

Found: C, 59.82; H, 6.89; N, 8.41%. Calcd for $C_{32}H_{44}N_4O_{10}$: C, 59.61; H, 6.88; N, 8.69%.

5,8-Bis[2-[N-(t-butoxycarbonyl)-2-(trityloxy)ethylamino]ethylamino]-1,2-dihydroxy-9,10-anthracenedione (8). *(a) From* **7**: To a stirred solution of **7** (80 mg) in pyridine (5 ml), trityl chloride (206 mg) was added. After 17 h at 60 °C, the mixture was poured into ice cold water. Precipitates were collected by filtration and purified on a column using 1 : 10 (v/v) ethyl acetate–toluene. Fractions homogeneous (R_f 0.36) on TLC in 1 : 5 (v/v) ethyl acetate–toluene were collected and concentrated. The residue was recrystallized from ethanol to give 101 mg (73%) of **8**, mp 140—143 °C; UV ($CHCl_3$) 261 (log ε, 4.56) (shoulder), 560 (4.02), (shoulder), 602 (4.36), 652 nm (4.46). ^{1}H NMR: δ=1.40 (18H, bs, NBoc×2), 2.96—4.75 (16H, m, CH_2 of side chain), 6.63 (1H, bs, 2-OH), 6.83—7.54 (33H, m, H-3,6,7 and C($C_6\underline{H_5}$)$_3$), 10.10 (1H, bs, aromatic NH), 10.76 (1H, bs, aromatic NH), 13.65 (1H, bs, 1-OH).

Found: C, 74.24; H, 6.47; N, 4.71%. Calcd for $C_{70}H_{72}$-

N_4O_{10}: C, 74.44; H, 6.43; N, 4.96%.

(b) From ***9***: To a stirred solution of **9** (636 mg) in acetone (15 ml), potassium carbonate (358 mg) was added and heated under reflux. After 7 h, the mixture was filtered and filtrate was concentrated. The residue was dissolved in ethyl acetate, washed with water, dried over anhydrous Na_2SO_4 and concentrated to give 501 mg (94%) of **8**.

*2-(t-Butoxycarbonyloxy)-5,8-bis[2-[N-(t-butoxycarbonyl)-2-(trityloxy) ethylamino] ethylamino]-1-hydroxy-9, 10-anthracenedione (****9****).* Compound **6** (100 mg) was tritylated analogously as described in the preparation of **8**. Fractions homogenous (R_f 0.22) on TLC in 1 : 3 (v/v) ethyl acetate–hexane were collected and concentrated. The residue was recrystallized from ethanol–methanol to give 114 mg (75%) of **9**, mp 190—215 °C; UV (EtOH) 210 (log ε, 4.93), 368 (3.77), 564 (3.96), 603 (4.33), 652 nm (4.45). ^{1}H NMR: δ=1.35 (18H, bs, NBoc×2), 1.53 (9H, s, OBoc), 3.00—3.77 (16H, m, CH_2 of side chain), 6.93—7.60 (33H, m, H-3,6,7 and $C(C_6\underline{H}_5)_3$), 7.80 (1H, d, J=9.0 Hz, H-4), 10.50 (1H, bs, aromatic NH), 11.05 (1H, bs, aromatic NH), 13.85 (1H, bs, 1-OH).

Found: C, 73.38; H, 6.65; N, 4.33%. Calcd for $C_{75}H_{80}N_4O_{12}$: C, 73.27; H, 6.56; N, 4.56%.

*5,8-Bis[2-[N-(t-butoxycarbonyl)-2-(trityloxy)ethylamino]ethylamino]-1-hydroxy-2-(p-methoxybenzyloxy)-9,10-anthracenedione (****10****).* To a stirred solution of **8** (300 mg) in dichloromethane (5 ml) and DMF (5 ml), *p*-methoxybenzyl chloride (0.5 ml) and potassium carbonate (184 mg) were added. After 4 h, the mixture was filtered and filtrate was washed with water, dried over anhydrous Na_2SO_4, and concentrated. The residue was washed with hexane and purified on a column using 1 : 3 (v/v) ethyl acetate–hexane. Fractions homogeneous (R_f 0.40) on TLC in 1 : 2 (v/v) ethyl acetate–hexaen were collected and concentrated. The residue was crystallized from ethanol to give 289 mg (87%) of **10**, mp 103—106 °C; UV ($CHCl_3$) 260 (log ε, 4.51) (shoulder), 280 (4.34) (shoulder), 568 (3.95) (shoulder), 602 (4.28), 654 nm (4.38). ^{1}H NMR: δ=1.17—1.63 (18H, m, NBoc×2), 3.01—3.66 (16H, m, CH_2 of side chain), 3.76 (3H, s, $CH_2C_6H_4OC\underline{H}_3$), 5.17 (2H, s, $C\underline{H}_2C_6H_4OCH_3$), 6.86 (2H, d, J=9.0 Hz, aromatic protons of MBn[13]), 6.99—7.51 (35H, m, aromatic protons), 7.73 (1H, d, J=9.0 Hz, H-4), 10.30 (1H, bs, aromatic NH), 10.83 (1H, bs, aromatic NH), 13.62 (1H, bs, 1-OH).

Found: C, 75.13; H, 6.51; N, 4.41%. Calcd for $C_{78}H_{81}N_4O_{11}$: C, 74.92; H, 6.53; N, 4.48%.

*5,8-Bis[2-[N-(t-butoxycarbonyl)-2-(trityloxy) ethylamino] ethylamino]-1-methoxy-2-(p-methoxybenzyloxy)-9,10-anthracenedione (****11****).* To a stirred solution of **10** (1.43 g) in acetone (20ml), dimethyl sulfate (0.5 ml) and potassium carbonate (794 mg) were added, heated under reflux. After 6 h, the mixture was filtered snd the filtrate was diluted with ethyl acetate. The solution was washed with water, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified on a column using 15 : 1 (v/v) benzene–ethyl acetate. Fractions homogeneous (R_f 0.58) on TLC in 5 : 1 (v/v) benzene–ethyl acetate were collected and concentrated. The residue was recrystallized from ethanol to give 1.11 g (77%) of **10**, mp 101—106 °C; UV ($CHCl_3$) 273 (log ε, 4.56), 597 (4.23), 645 nm (4.26). ^{1}H NMR: δ=1.17—1.59 (18H, m, NBoc×2), 3.02—3.64 (16H, m, CH_2 of side chain), 3.76 (3H, s, $CH_2C_6H_4OC\underline{H}_3$), 3.95 (3H, s, 1-$OCH_3$), 5.15 (2H, s, $C\underline{H}_2C_6H_4OCH_3$), 6.85 (2H, d, J= 9.0 Hz, aromatic protons of MBn), 6.98—7.51 (35H, m, aromatic protons), 8.08 (1H, d, J=9.0 Hz, H-4), 10.23 (2H, bs, aromatic NH).

Found: C, 74.91; H, 6.61; N, 4.44%. Calcd for $C_{79}H_{83}N_4O_{11}$: C, 75.04; H, 6.62; N, 4.43%.

*2-Hydroxy-5,8-bis[2-(2-hydroxyethylamino) ethylamino]-1-methoxy-9,10-anthracenedione dihydrochloride (****3****).* To a stirred solution of **11** (1.11 g) in dichloromethane (5 ml), methanolic HCl (20 ml) was added under ice cooling. The precipitates were collected by filtration and recrystallized from 95% aqueous ethanol to give 342 mg (70%) of **3**, mp 209—211 °C; UV (H_2O) 217 (log ε, 4.50), 239 (4.50), 271 (4.50), 586 (4.20), 629 nm (4.20); IR (KBr) 1640 cm^{-1} (quinone). ^{1}H NMR (D_2O): δ=3.33—3.65 (8H, m, $NHCH_2C\underline{H}_2NHC\underline{H}_2CH_2OH$), 3.68—3.95 (7H, m, $NHC\underline{H}_2CH_2NH$ and 1-OCH_3), 3.95—4.20 (4H, m, $CH_2C\underline{H}_2OH$), 7.00—7.28 (3H, m, H-3,6 and 7), 7.55 (1H, d, J=9.0 Hz, H-4).

Found: C, 49.56; H, 5.83; N, 9.70; Cl, 13.05%. Calcd for $C_{23}H_{30}N_4O_6\cdot 2HCl\cdot 1.5\,H_2O$: C, 49.47; H, 6.31; N, 10.03; Cl, 12.69%.

*2-Acetoxy-5,8-bis[2-(N-acetyl-2-acetoxyethylamino) ethylamino]-1-methoxy-9,10-anthracenedione (****12****).* Compound **3** (15 mg) was acetylated with acetic anhydride (0.5 ml) in pyridine (1 ml) under ice cooling. The mixture was extracted with ethyl acetate and washed with H_2O, dried over Na_2SO_4. The solution was concentrated to give 22 mg (quantative yield) of **12** as an amorphous solid. ^{1}H NMR: δ=2.05, (6H, s, Ac×2), 2.15 (6H, s, Ac×2), 2.37 (3H, s, 2-OAc) 3.09—3.78 (12H, m, $NHC\underline{H}_2C\underline{H}_2N(Ac)C\underline{H}_2CH_2OAc$) 3.92 (3H, s, 1-$OCH_3$), 4.18 (4H, t, J=6.0 Hz, $CH_2C\underline{H}_2OAc$), 7.22—7.48 (3H, m, H-3,6 and 7), 8.15 (1H, d, J=9.0 Hz, H-4), 10.46 (2H, bs, aromatic NH).

*5,8-Bis[2-[N-(t-butoxycarbonyl)-2-(trityloxy) ethylamino] ethylamino]-1-hydroxy-2-methoxy-9,10-anthracedione (****13****).* Compound **8** (70 mg) was methylated analogously as described in the preparation of **11**. The crude product was purified on a column using 1 : 10 (v/v) ethyl acetate–toluene. Fractions homogeneous (R_f 0.43) on TLC in 1 : 5 (v/v) ethyl acetate–toluene were collected and concentrated. The residue was recrystallized from ethanol to give 66 mg (93%) of **13** as amorphous solids, mp 110—114 °C; UV ($CHCl_3$) 262 (log ε, 4.52) (shoulder), 278 (4.38), 564 (3.99) (shoulder), 520 (4.33), 652 nm (4.43). ^{1}H NMR: δ=1.35 (18H, bs, NBoc×2), 2.97—3.63 (16H, m, CH_2 of side chain), 3.82 (3H, s, 2-OCH_3), 6.80—7.67 (33H, m, H-3,6,7 and $C(C_6\underline{H}_5)_3$), 7.78 (1H, d, J=9.0 Hz, H-4), 10.30 (1H, bs, aromatic NH), 10.87 (1H, bs, aromatic NH), 13.79 (1H, bs, 1-OH).

Found: C, 74.75; H, 6.71; N, 4.90%. Calcd for $C_{71}H_{74}N_4O_{10}$: C, 74.58; H, 6.52; N, 4.90%.

*1-(t-Butoxycarbonyloxy)-5,8-bis[2-[N-(t-butoxycarbonyl)-2-(trityloxy)ethylamino]ethylamino]-2-methoxy-9, 10-anthracenedione (****14****).* Compound **9** (400 mg) was methylated analogously as described in the preparation of **11**. The crude product was purified on a column using 1 : 4 (v/v) ethyl acetate–hexane. Fractions homogeneous (R_f 0.16) on TLC in 1 : 3 (v/v) ethyl acetate–hexane were collected and concentrated. The residue was recrystallized from ethanol to give 257 mg (64%) of **14**, mp 108—111 °C; UV ($CHCl_3$) 272 (log ε, 4.63), 599 (4.32), 646 nm (4.37). ^{1}H NMR: δ=1.37 (18H, bs, NBoc×2), 1.55 (9H, s, OBoc), 2.90—3.67 (16H, m, CH_2 of side chain), 3.83 (3H, s, 2-OCH_3), 6.90—7.67 (33H, m, H-3,6,7 and $C(C_6\underline{H}_5)_3$), 8.32 (1H, d, J=9.0 Hz, H-4), 10.62 (2H, bs, aromatic NH).

Found: C, 73.13; H, 6.69; N, 4.26%. Calcd for $C_{76}H_{82}N_4O_{12}$: C, 73.41; H, 6.65; N, 4.51%.

*1-Hydroxy-5,8-bis[2-(2-hydroxyethylamino)ethylamino]-2-methoxy-9,10-anthracenedione dihydrochloride (****4****).* *(a) From* ***13***: Compound **13** (150 mg) was treated with methanolic HCl (10 ml) under ice cooling. After 4 h, precipitates were collected and recrystallized from methanol to give 39 mg (56%) of **4**, mp 199—201 °C; UV (H_2O) 227 (log ε, 4.24) (shoulder), 243 (4.45), 260 (4.38), 589 (4.15), 635 nm (4.17); IR (KBr) 1640 cm^{-1} (quinone). ^{1}H NMR (D_2O): δ=6.73—7.00 (4H, m, H-3,4,6 and 7).

Found: C, 48.77; H, 5.85; N, 9.54; Cl, 12.50%. Calcd for $C_{23}H_{30}N_4O_6 \cdot 2HCl \cdot 2H_2O$: C, 48.68; H, 6.39; N, 9.87; Cl, 12.49%.

(b) From ***14***: Compound **14** (150 mg) was treated analogously as described above in (a) to give 56 mg (87%) of **4**,.

*5,8-Bis[2-(N-acetyl-2-acetoxyethylamino) ethylamino]-1-hydroxy-2-methoxy-9,10-anthracenedione (**15**).* Compound **4** (30 mg) was acetylated with acetic anhydride (0.5 ml) in pyridine (0.5 ml) under ice cooling. The solution was diluted with ethyl acetate and washed with water, dried over anhydrous Na_2SO_4. The solution was concentrated and the residue was purified on a column using 1 : 6 (v/v) ethanol–toluene were collected and concentrated to give 23 mg (66%) of **15** as syrup. ^{1}H NMR: δ=2.04 (6H, s, Ac×2), 2.14 (6H, s, Ac×2), 3.57 (12H, bs, NHC$\underline{H}_2$C$\underline{H}_2$N(Ac)C$\underline{H}_2$CH$_2$OAc), 3.96 (3H, s, 2-OCH$_3$), 4.17 (4H, t, J=6.0 Hz, CH$_2$C$\underline{H}_2$OAc), 6.87—7.51 (3H, m, H-3,6, and 7), 7.70 (1H, d, J=9.0 Hz, H-4), 10.19 (1H, bs, aromatic NH), 10.72 (1H, bs, aromatic NH), 13.42—13.78 (1H, m, 1-OH).

*1-Acetoxy-5,8-bis[2-(N-acetyl-2-acetoxyethylamino) ethylamino]-2-methoxy-9,10-anthracenedione (**16**).* Compound **4** (30 mg) was acetylated analogously as described in the preparation of **15**. Fractions homogeneous (R_f 0.30) on TLC in 1 : 3 (v/v) ethanol–toluene were collected and concentrated to give 13 mg (35%) of **16** as amorphous solids, UV ($CHCl_3$) 274 (log ε, 4.48), 594 (4.14), 640 nm (4.17). ^{1}H NMR: δ=2.02 (6H, s, Ac×2), 2.13 (6H, s, Ac×2), 2.46 (3H, s, 1-OAc), 3.24—3.80 (12H, m, NHC$\underline{H}_2$C$\underline{H}_2$N(Ac)C$\underline{H}_2$CH$_2$OAc), 3.92 (3H, s, 2-OCH$_3$), 4.16 (4H, t, J=6.0 Hz, CH$_2$C$\underline{H}_2$OAc), 6.98—7.67 (3H, m, H-3,6 and 7), 8.25 (1H, d, J=9.0 Hz, H-4), 10.56 (2H, bs, aromatic NH).

Found: C, 59.25; H, 6.01; N, 8.09%. Calcd for $C_{33}H_{40}N_4O_{11}$: C, 59.32; H, 6.03; N, 8.39%.

*5,8-Bis[2-[N-(t-butoxycarbonyl)-2-(trityloxy) ethylamino]ethylamino]-1,2-dimethoxy-9,10-anthracenedione (**17**).* Compound **8** (110 mg) was methylated analogously as described in the preparation of **11**. The product was purified on a column using 1 : 8 (v/v) ethyl acetate–toluene. Fractions homogeneous (R_f 0.56) on TLC in 1 : 3 (v/v) ethyl acetate–toluene were collected and concentrated. The residue was recrystallized from hexane to give 37 mg (32%) of **17**, mp 85—90 °C; UV (EtOH) 212 (log ε, 4.83), 271 (4.49), 593 (4.18), 640 nm (4.21). ^{1}H NMR: δ=1.22 (18H, bs, NBoc×2), 3.01—3.72 (16H, m, CH$_2$ of side chain), 3.94 (6H, s, OCH$_3$×2), 6.89—7.50 (33H, m, H-3,6,7 and C(C$_6\underline{H}_5$)$_3$), 8.13 (1H, d, J=9.0 Hz, H-4), 10.19 (2H, bs, aromatic NH).

Found: C, 74.18; H, 6.91; N, 4.85%. Calcd for $C_{72}H_{76}N_4O_{10}$: C, 74.72; H, 6.62; N, 4.84%.

*5,8-Bis[2-(2-hydroxyethylamino) ethylamino]-1,2-dimethoxy-9,10-anthracenedione dihydrochloride (**5**).* Compound **17** (100 mg) was dissolved in methanolic HCl (4 ml), after 2 h, precipitates were collected by filtration and recrystallized from ethanol to give 35 mg (81%) of **5**, mp 203—205 °C; UV (H_2O) 221 (log ε, 4.39), 239 (4.41), 272 (6.41), 584 (4.12), 627 nm (4.12); IR (KBr) 1635 cm^{-1}. ^{1}H NMR (D_2O): δ=7.10 (2H, bs, H-6 and 7), 7.32 (1H, d, J=9.0 Hz, H-3), 7.72 (1H, d, J=9.0 Hz, H-4).

Found: C, 51.29; H, 6.03; N, 9.61; Cl, 11.77%. Calcd for $C_{24}H_{32}N_4O_6 \cdot 2HCl \cdot H_2O$: C, 51.16; H, 6.44; N, 9.94; Cl, 12.58%.

*5,8-Bis[2-(N-acetyl-2-acetoxyethylamino)ethylamino]-1,2-dimethoxy-9,10-anthracenedione (**18**).* Compound **5** (20 mg) was acetylated analogously as described in the preparation of **15**. Fractions homogeneous (R_f 0.33) on TLC in 1 : 3 (v/v) ethanol–toluene were collected and concentrated to give 14 mg (50%) of **18** as amorphous solids; UV (EtOH) 221 (log ε, 4.08), 239 (4.06), 273 (4.08), 590 (3.78), 635 nm (3.79). ^{1}H NMR: δ=2.04 (6H, s, Ac×2), 2.13 (6H, s, Ac×2), 3.40—3.78 (12H, m, NHC$\underline{H}_2$C$\underline{H}_2$N(Ac)C$\underline{H}_2$CH$_2$OAc), 3.95 (6H, s, OCH$_3$×2), 4.17 (4H, t, J=6.0 Hz, CH$_2$C$\underline{H}_2$OAc), 7.00—7.60 (3H, m, H-3,6 and 7), 8.12 (1H, d, J=9.0 Hz, H-4), 10.12 (2H, bs, aromatic NH).

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