

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. Brown²⁾ that 2-substituted anthraquinones were active against leukemia L1210 in mice. Also, 1,2-dihydroxy-anthraquinone 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 ¹H NMR spectrum was identical with the proposed structure.

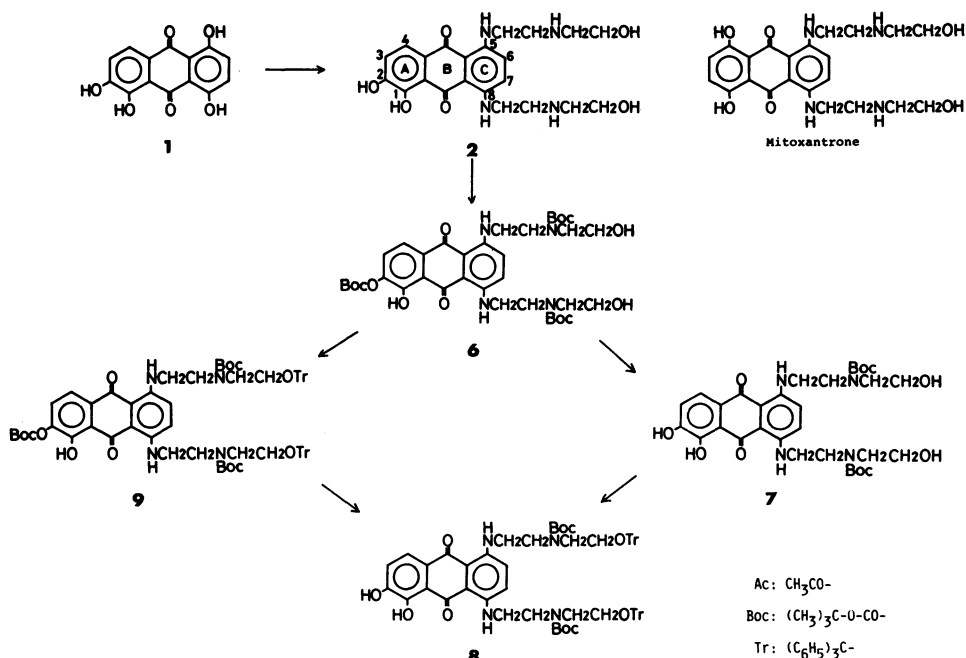
The positional isomer of **3** (**4**), 2-*O*-methyl derivative was prepared by the following process.

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 OF COMPOUNDS **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
4	6.25	71	0/5
	100	Toxic	0/5
	50	50	0/5
	25	25	0/5
	12.5	25	0/5
5	6.25	13	0/5
	100	14	0/5
	50	14	0/5
	25	0	0/5
	12.5	14	0/5
Mitoxantrone	6.25	14	0/5
	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 BDF₁ hybrid mice were inoculated intraperitoneally with 10⁶ 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.

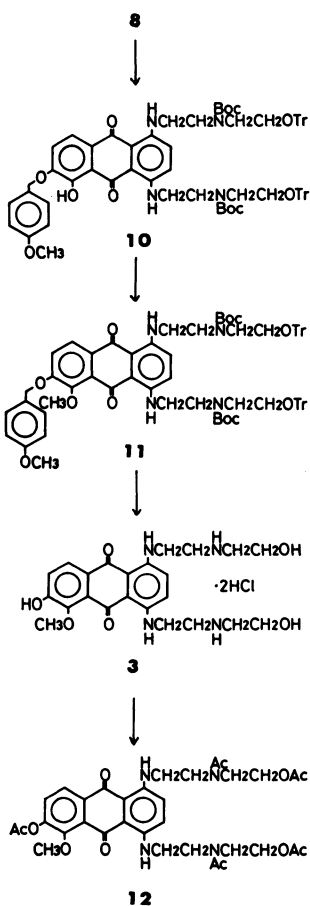
Compound **4** was also obtained by another route as follows. Methylation of **9** with dimethyl sulfate and potassium carbonate afforded a 1-*O*-(*t*-butoxycarbonyl)-

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.

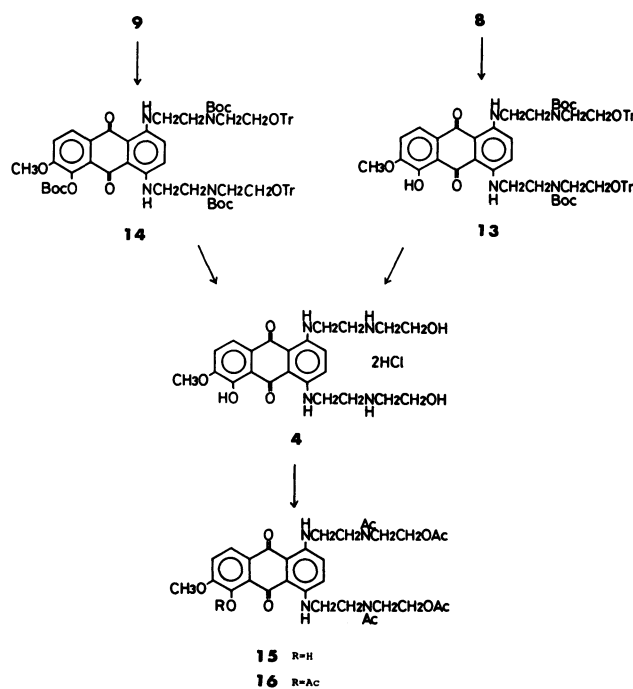
Compound **4** was converted to a tetraacetyl derivative (**15**) and a pentaacetyl derivative (**16**), whose ^1H NMR spectrum was identical with the proposed structure.

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**).

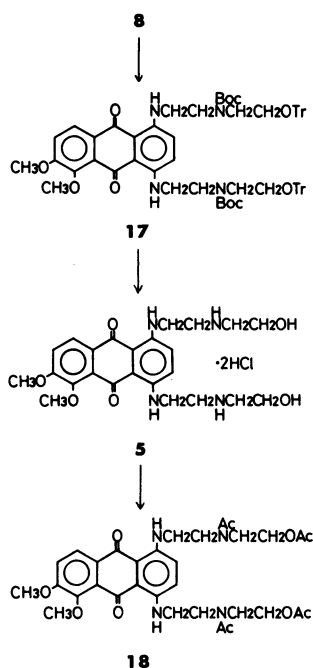
Acetylation of **5** gave a tetraacetyl derivative (**18**).



Scheme 2.



Scheme 3.



Scheme 4.

When ^1H NMR spectra of **12**, **16**, and **18** were compared each other, characteristic chemical shifts of acetoxy groups on C-1 and 2 provided a good evidence for the structures. The chemical shift of the acetoxy group on C-2 was attributable to δ 2.37 and that of the acetoxy 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.¹¹

1, 2-Dihydroxy-5, 8-bis[2-(2-hydroxyethylamino)ethylamino]-9, 10-anthracenedione 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 $214\text{--}219^\circ\text{C}$; UV (H_2O) 245 (log ϵ , 4.56), 591 (4.28), 635 nm (4.27); IR (KBr) 1615 cm^{-1} (quinone). ^1H NMR (D_2O): $\delta=3.33\text{--}3.93$ (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 $\text{C}_{22}\text{H}_{30}\text{N}_4\text{Cl}_2\text{O}_6\cdot\text{H}_2\text{O}$: 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, 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 $154\text{--}155^\circ\text{C}$; UV (CHCl_3) 260 (log ϵ , 4.41), 381 (3.68), 606 (4.25), 656 nm (4.36). ^1H NMR: $\delta=1.46$ (18H, s, NBoc $\times 2$), 1.61 (9H, s, OBoc), 2.93—3.61 (12H, m, $\text{NHCH}_2\text{CH}_2\text{N}(\text{Boc})\text{CH}_2\text{CH}_2\text{OH}$), 3.64—3.90 (4H, m, $\text{CH}_2\text{CH}_2\text{OH}$), 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 $\text{C}_{37}\text{H}_{52}\text{N}_4\text{O}_{12}$: C, 59.66; H, 7.04; N, 7.52%.

5, 8-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 $164\text{--}166^\circ\text{C}$; UV (CHCl_3) 260 (log ϵ , 4.36) (shoulder), 274 (4.26) (shoulder), 565 (3.87) (shoulder), 602 (4.19), 650 nm (4.27). ^1H NMR: $\delta=1.47$ (18H, s, NBoc $\times 2$), 3.44 (12H, bs, $\text{NHCH}_2\text{CH}_2\text{N}(\text{Boc})\text{CH}_2\text{CH}_2\text{OH}$), 3.76 (4H, bs, $\text{CH}_2\text{CH}_2\text{OH}$), 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 $\text{C}_{32}\text{H}_{44}\text{N}_4\text{O}_{10}$: 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 $140\text{--}143^\circ\text{C}$; UV (CHCl_3) 261 (log ϵ , 4.56) (shoulder), 560 (4.02), (shoulder), 602 (4.36), 652 nm (4.46). ^1H NMR: $\delta=1.40$ (18H, bs, NBoc $\times 2$), 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_6H_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 $\text{C}_{70}\text{H}_{78}\text{N}_4$

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). 1H NMR: δ =1.35 (18H, bs, NBoc $\times$ 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_6H_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-hexane 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). 1H NMR: δ =1.17–1.63 (18H, m, NBoc $\times$ 2), 3.01–3.66 (16H, m, CH_2 of side chain), 3.76 (3H, s, $CH_2C_6H_4OCH_3$), 5.17 (2H, s, $CH_2C_6H_4OCH_3$), 6.86 (2H, d, J =9.0 Hz, aromatic protons of MBn¹³), 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 (20 ml), dimethyl sulfate (0.5 ml) and potassium carbonate (794 mg) were added, heated under reflux. After 6 h, the mixture was filtered and 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 **11**, mp 101–106 °C; UV ($CHCl_3$) 273 (log ϵ , 4.56), 597 (4.23), 645 nm (4.26). 1H NMR: δ =1.17–1.59 (18H, m, NBoc $\times$ 2), 3.02–3.64 (16H, m, CH_2 of side chain), 3.76 (3H, s, $CH_2C_6H_4OCH_3$), 3.95 (3H, s, 1- OCH_3), 5.15 (2H, s, $CH_2C_6H_4OCH_3$), 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 solu-

tion 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). 1H NMR (D_2O): δ =3.33–3.65 (8H, m, $NHCH_2CH_2NHCH_2CH_2OH$), 3.68–3.95 (7H, m, $NHCH_2CH_2NH$ and 1- OCH_3), 3.95–4.20 (4H, m, CH_2CH_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 (quantitative yield) of **12** as an amorphous solid. 1H NMR: δ =2.05, (6H, s, Ac $\times$ 2), 2.15 (6H, s, Ac $\times$ 2), 2.37 (3H, s, 2-OAc) 3.09–3.78 (12H, m, $NHCH_2CH_2N(Ac)CH_2CH_2OAc$) 3.92 (3H, s, 1- OCH_3), 4.18 (4H, t, J =6.0 Hz, CH_2CH_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-anthracenedione (**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). 1H NMR: δ =1.35 (18H, bs, NBoc $\times$ 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_6H_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). 1H NMR: δ =1.37 (18H, bs, NBoc $\times$ 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_6H_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). 1H 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 $C_{29}H_{30}N_4O_6 \cdot 2HCl \cdot 2H_2O$: 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. 1H NMR: δ =2.04 (6H, s, $Ac \times 2$), 2.14 (6H, s, $Ac \times 2$), 3.57 (12H, bs, $NHCH_2CH_2N(Ac)CH_2CH_2OAc$), 3.96 (3H, s, $2-OCH_3$), 4.17 (4H, t, J =6.0 Hz, CH_2CH_2OAc), 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). 1H NMR: δ =2.02 (6H, s, $Ac \times 2$), 2.13 (6H, s, $Ac \times 2$), 2.46 (3H, s, 1-OAc), 3.24–3.80 (12H, m, $NHCH_2CH_2N(Ac)CH_2CH_2OAc$), 3.92 (3H, s, $2-OCH_3$), 4.16 (4H, t, J =6.0 Hz, CH_2CH_2OAc), 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). 1H NMR: δ =1.22 (18H, bs, $NBoc \times 2$), 3.01–3.72 (16H, m, CH_2 of side chain), 3.94 (6H, s, $OCH_3 \times 2$), 6.89–7.50 (33H, m, H-3,6,7 and $C(C_6H_5)_3$), 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} . 1H 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). 1H NMR: δ =2.04 (6H, s, $Ac \times 2$), 2.13 (6H, s, $Ac \times 2$), 3.40–3.78 (12H, m, $NHCH_2CH_2N(Ac)CH_2CH_2OAc$), 3.95 (6H, s, $OCH_3 \times 2$), 4.17 (4H, t, J =6.0 Hz, CH_2CH_2OAc), 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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References

- 1) K. Nakamura, T. Asai, T. Hara, N. Minorikawa, and T. Suami, *Bull. Chem. Soc. Jpn.*, submitted.
- 2) S. Bennett, D. Sharples, and J. R. Brown, *J. Med. Chem.*, **25**, 369 (1982).
- 3) P. Chang, K.-H. Lee, T. Shingu, T. Hirayama, and I. H. Hall, *J. Natural Products*, **45**, 206 (1982).
- 4) D. D. Von Hoff, E. Pollard, J. Kuhn, E. Murray, and C. A. Coltman, Jr., *Cancer Res.*, **40**, 1516 (1980).
- 5) R. K. Jhonson, R. K. Y. Zee-Cheng, W. W. Lee, F. M. Acton, D. W. Henry, and C. C. Cheng, *Cancer Treat. Res.*, **63**, 425 (1979).
- 6) R. E. Wallace, K. C. Murdock, R. B. Angier, and F. E. Durr, *Cancer Res.*, **39**, 1570 (1979).
- 7) R. K. Y. Zee-Cheng and C. C. Cheng, *J. Med. Chem.*, **21**, 291 (1978).
- 8) R. K. Y. Zee-Cheng, E. G. Podrebarac, C. S. Menon, and C. C. Cheng, *J. Med. Chem.*, **22**, 501 (1979).
- 9) M. Kikuchi, T. Yamagishi, and M. Hida, *Chem. Lett.*, **1981**, 1391.
- 10) F. Arcamone, G. Cassinelli, F. DiMatteo, S. Forenza, M. C. Ripamonti, G. Rivola, A. Vigevari, J. Clardy, and T. Ripamonti, G. Rivola, A. Vigevari, J. Clardy, and T. McCabe, *J. Am. Chem. Soc.*, **102**, 1462 (1980).
- 11) The antitumor activity was determined by Dr. Iwao Umezawa, Kitasato Institute, Shirogane, Minato-ku, Tokyo by the established protocol.¹²⁾
- 12) J. L. Hartwell, *Cancer Chemother. Rep.*, Part 1, 25 (1962).
- 13) MBn: *p*-Methoxybenzyl.