SYNTHESIS AND STRUCTURE-ACTIVITY RELATIONSHIPS OF NEW DIMERIC MITOMYCIN DERIVATIVES; 7-*N*,7′-*N*′-BIS(ω-THIOALKYL)DIMITOMYCINS

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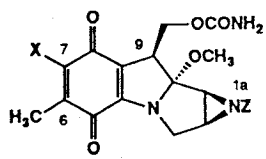
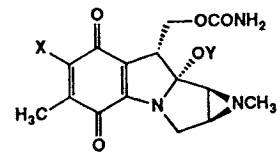
The reaction between mitomycin A (**1**) and cysteamine afforded 7-*N*,7′-*N*′-bis(2-thioethyl)dimitomycin C (**7**), 7-*N*-[2-[(2-aminoethyl)dithio]ethyl]mitomycin C (**8**), and 7-methoxy mitosenes (**10**, **11**). The structures of **7** and **8** were elucidated on the basis of spectroscopy and reactions between **1** and **8**, and **1** and cystamine. The observation of the time course for the reaction revealed the mechanism of the formation of **7** and **8**. The rapid oxidation of cysteamine by the quinone of **1** gave cystamine, which was trapped by **1** to give **8**, and **8** was additionally reacted with **1** to give **7**. Since **7** showed significant antitumor activities, related 7-*N*,7′-*N*′-bis(ω-thioalkyl)dimitomycins were synthesized. They also showed remarkable antitumor activities against HeLa-S_3 *in vitro*, sarcoma 180 (sc-ip), leukemia P388 (ip-ip) *in vivo*. In these evaluations, compound **7** demonstrated unique potency.

Mitomycin C (**3**) is one of the most potent antitumor antibiotics known, especially against solid tumors, *e.g.*, stomach and lung cancers,[1,2] and is widely used in clinical chemotherapy. However it also has strong side effects such as myelosuppression. Numerous approaches have been investigated to reduce its toxicities. Recently great attention has been given to the modification at C-7-N in **3**,[3~5] focusing on changing the physico-chemical characteristics of **3** and instaling other functional groups. By contrast, our research efforts have been first directed toward the naturally occurring mitomycins represented by

Fig. 1.





	X	Z	No.
Mitomycin A	OCH_3	H	**1**
Mitomycin C	NH_2	H	**3**
Mitomycin F	OCH_3	CH_3	**5**

	X	Y	No.
Mitomycin B	OCH_3	H	**2**
Mitomycin D	NH_2	H	**4**
Mitomycin J	OCH_3	CH_3	**6**

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mitomycins A (**1**), B (**2**), and C (**3**). During the course of our study, several new compounds including mitomycins D (**4**), F (**5**), and J (**6**) have been found from the fermentation broth of mitomycins,[6,7] and semi-syntheses of related compounds have been developed.[7~10] On the basis of structure-activity relationships among naturally occurring mitomycins and newly developed related compounds, we have investigated the introduction of novel functional groups at C-7-N[11~14] and C-6 methyl[15~18] in various important mitomycin skeletons.

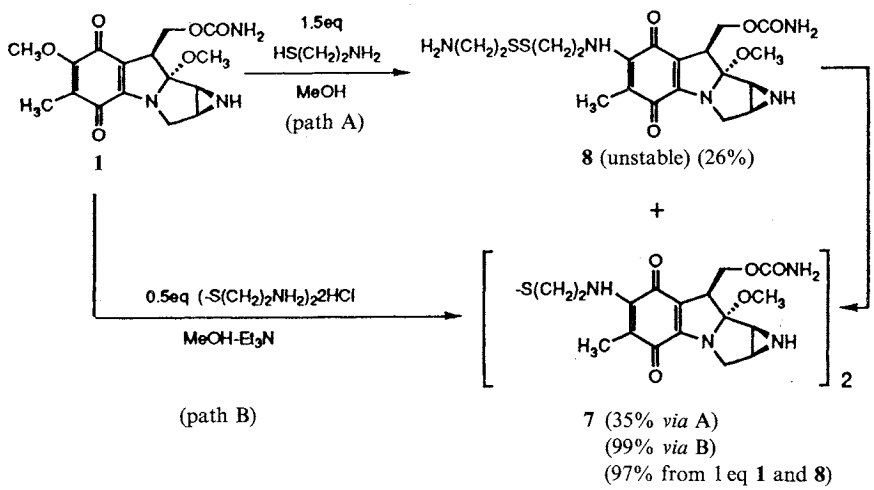
In the early study on the derivatives of mitomycins, COSULICH[19] reported that mitomycin A (**1**) decomposed with a thiol to give yellow compounds. This preceding literature[19] prompted us to start the study of mitomycin derivatives set up for the intramolecular reductive activation by an auxiliary at C-7, *e.g.*, an ω-mercaptoalkylamino substituent. In our first attempts to introduce a 2-mercaptoethylamino group at C-7, we found cysteamine reacted with **1** to afford 7-*N*,7'-*N*'-bis(2-thioethyl)dimitomycin C (**7**) and unstable 7-*N*-[2-[(2-aminoethyl)dithio]ethyl]mitomycin C (**8**) in moderate yields.[20] During our study, REMERS *et al.*[21] reported that a similar reaction gave 7-*N*-(2-mercaptoethyl)mitomycin C (RR-150), which showed remarkable antitumor activities. Recently the structure of RR-150 was revised as its dimer by SENTER *et al.*[22] We independently reexamined the reaction conditions of REMERS[21] and also proved that the structure of RR-150 was that of **7**.[20] These disulfides of **7** and **8** were presumed to be generated *via* oxidation by **1** on the basis of the time course for the reaction and the structures of by-products.

As the extension of this study, we synthesized various symmetrical disulfides with several mitomycin skeletons and spacers to investigate potent symmetrical mitomycin disulfides. We found that several disulfide mitomycins including **7** showed excellent activities against rodent tumors. Here we describe the syntheses of symmetrical mitomycin disulfides and their structure-activity relationships.

Chemistry

The yellow product (λ_{max}^{MeOH} 244, 321, 470 nm) gained by COSULICH *et al.*[19] in the reaction between **1** and cysteamine was assumed to have a mitosene skeleton. We reexamined the similar reaction to confirm our hypothesis that an intramolecular quinone reduction by an introduced 7-*N*-mercaptoethylamino auxiliary afforded a so-called activated mitomycin[23~25] and the following ring opening of the aziridine gave a mitosene. However, the reaction of **1** with cysteamine (freshly purified by sublimation, 1.5 equivalents,

Scheme 1.

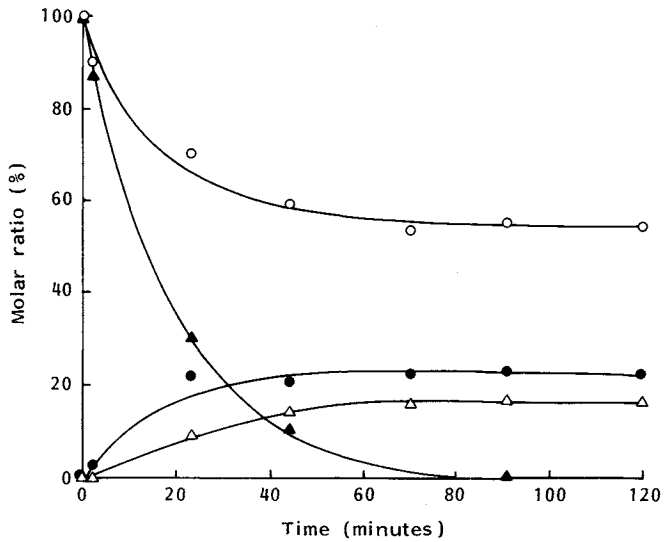


in MeOH, room temperature (rt) under a nitrogen atmosphere) resulted in two bluish purple products, *i.e.*, **7** and **8**, accompanied by a few yellowish orange by-products (described later). Although **8** was unstable and converted, in part, to **7** and red purplish unstable compounds (structures unknown) on silica gel TLC within several tens of minutes, **7** and **8** were purified on silica gel flash chromatography for structural elucidation ($CHCl_3$ - MeOH = 85 : 15, then 6 : 4, v/v).

In the EI-MS of **7**, the molecular ion peak could not be observed. By contrast, the SI-MS showed the ion peak *m/z* 789 $(M+3)^+$ (described later). The ^1H NMR showed the presence of a thioethylamino moiety at C-7, however there was no thiol proton, suggesting a dimeric structure. The inspection of the ^{13}C NMR in pyridine-d_5 also supported the symmetrical dimer, *i.e.*, δ 43.8 (t) and 38.6 (t) were assigned to 7-*N*α and 7-*N*β carbons of **7** respectively on the basis of comparison between ^{13}C NMR of cysteamine hydrochloride (δ 41.8 and 21.2) and cystamine dihydrochloride (δ 37.8 and 33.9) in DMSO-d_6. The ^1H NMR (pyridine-d_5) of **8** showed a complicated multiplet (8 H) assigned to two sets of ethylene of the cystamino moiety. For corroboration of these structures, freshly purified **8** was treated with 1 equivalent of **1** to afford **7** in a very good yield (97%). The amination reaction of **1** by a half equivalent of cystamine also gave a high yield of **7**. Thus the structures of **7** and **8** were unambiguously determined by spectroscopy and their modes of formation.

While we had been studying this reaction, REMERS *et al.*[21] reported that RR-150 with a mercaptoethylamino moiety at C-7 was obtained in the reaction of **1** and cysteamine hydrochloride. In order to reexamine their experiment and to investigate the mechanism of this reaction, the time course for the reaction between **1** and cysteamine hydrochloride (2 equivalents, in the presence of 8 equivalents of triethylamine, in methanol, rt, under a nitrogen atmosphere) was observed by HPLC equipped with a photo-diode array detector.

Fig. 2. Time course for the reaction of mitomycin A with cysteamine.
○ Total mitomycins, ▲ mitomycin A (**1**), △ **7**, ● **8**.



$$\mathbf{1} \xrightarrow[Et_3N/MeOH]{\text{2 eq } HS(CH_2)_2NH_2 \cdot \text{HCl salt}} \mathbf{7}+\mathbf{8}$$

Scheme 2.

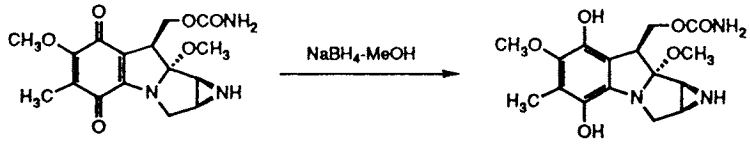


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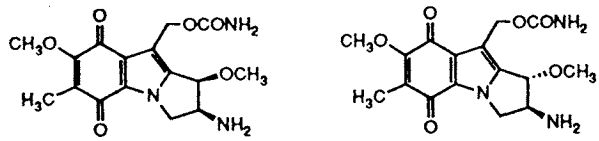
Fig. 3.



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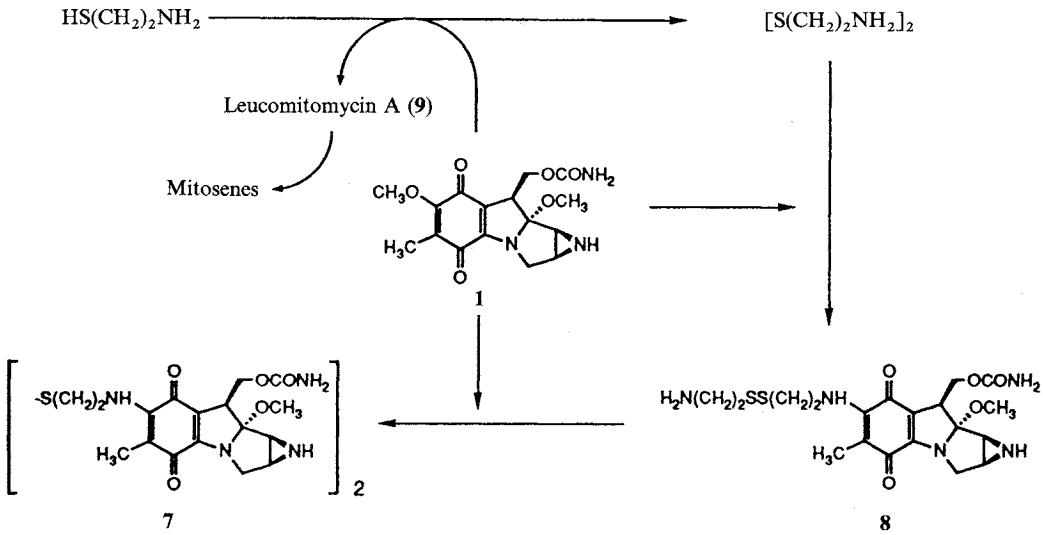
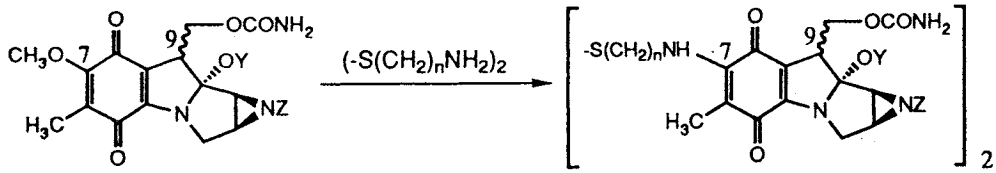
Scheme 3. Possible mechanism leading to the formation of **7** and **8**.



After 2 hours, when the reaction was completed, the total molar number of the consumed mitomycins were nearly equal to the sum of those of **7** and **8**, suggesting that the oxidation reaction to introduce the cystamine from cysteamine was linked with the quinone reduction of **1**. The observed UV spectrum and retention time for the unstable dihydromitomycin coincided with those of leucomitomycin A (**9**)[26] generated from **1** with sodium borohydride. This quinone reduction was rapid, when 4 equivalents of sublimed cysteamine were used. The characteristic deep reddish purple color of **1** changed to yellow within few minutes. This indicated the formation of **9**. Moreover the structures of the isolated mitosenes reinforced this proposal for the initiation mechanism. That is, the by-products structures (**10**, **11**), determined preliminarily by ^{1}H NMR, showed the presence of a 7-methoxy group. Thus it was concluded that the rapid oxidation of cysteamine by the quinone of **1** caused the formation of cystamine, which was then trapped by the remaining **1** to give **8**, and then **8** subsequently reacted with **1** to give **7**.

We then synthesized other 7-*N*,7′-*N*′-bis(ω-thioalkyl)dimitomycins to evaluate their antitumor activi-

Table 1. Reaction of 7-methoxymitomycins with bis(ω-thioalkyl)diamine.



1, **2**, **5**, or **6**

No.	n	9	Y	Z	Yield (%)	SI-MS (*m/z*)
7	2	β	CH_3	H	99	789 (M+3)
12	2	β	CH_3	CH_3	97	—
13	2	α	H	CH_3	93	728 ((M+2)−61)
14	2	α	CH_3	CH_3	96	—
15	3	β	CH_3	H	58	818 (M+4)
16	3	α	H	CH_3	59	819 (M+5)
17	4	β	CH_3	H	97	845 (M+3)
18	6	β	CH_3	H	95	901 (M+3)
19	8	β	CH_3	H	88	959 (M+5)
20	12	β	CH_3	H	66	1,071 (M+5)

—: Not done.

ties. The mitomycin skeleton of **7** was converted to other mitomycins and the spacer ethylene between the dithio moiety and 7-amino group of **7** was changed from propylene to dodecamethylene. Among naturally occurring mitomycins[6,7] with 7-methoxy group, **2**, **5**, and **6** were selected, since they were readily available potent mitomycin skeletons. The reaction of these 7-methoxy mitomycins with cystamine gave **12**, **13**, and **14** in good yields. The reaction of **1** and bis(ω-thioalkyl)diamines[27,28] also gave desired dimeric mitomycins (**15**, **16**, **17**, **18**, **19**, **20**) in good to moderate yields. SI-MS of these compounds showed *m/z* from $(M+1)^+$ to $(M+5)^+$, indicating that the hydrogenation of quinone occurred in the ionization process of the series of these compounds.[29),†,††] The presence of the ion peak $(M+4)^+$ and $(M+5)^+$ could be explained by the existence of 2 hydroquinones in an ionized molecule.

Antitumor Activity

In Vitro Evaluation

The new symmetrical disulfide mitomycins were initially evaluated for antitumor activity against HeLa-S_3 cell culture.[31] All of them showed *in vitro* activity; especially all derivatives with the ethylene spacer exhibited excellent activity and **7**, **12**, and **14** were apparently superior to **3**. However elongation of the spacer resulted in less activity *in vitro*.

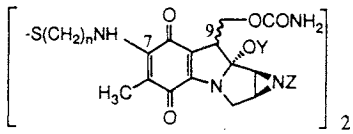
In Vivo Evaluation

In order to estimate the viability of a candidate for clinical study, many *in vivo* tests have been evaluated[32] with emphsis on efficacy and toxicity. As the first system, we employed sarcoma 180 (sc-ip) as a solid tumor and P388 (ip-ip) as a leukemia. In the evaluation of sarcoma 180, CI (a range of effective dosage), WBC_{4000}/ED_{50} (a level of myelosuppression compared to **3**), and ED_{50} (an amount of effective dosage) were regarded as significant. While in the evaluation of P388, ILS_{max} (an increase of life span) was the dominant factor.

† FAB-MS spectrometry of quinones commonly gives large M+2 and M+3 peaks.

†† On the basis of our results, SANO *et al.* studied SI-MS spectrometry of quinones.[30]

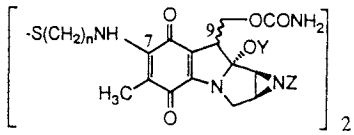
Table 2. Growth inhibition effect in HeLa-S_3 cell culture.



(μg/ml)

No.	n	9	Y	Z	1 hour	72 hours
7	2	β	CH_3	H	0.012	0.0051
12	2	β	CH_3	CH_3	0.0076	0.0026
13	2	α	H	CH_3	0.26	0.021
14	2	α	CH_3	CH_3	0.044	0.0036
15	3	β	CH_3	H	4.8	0.90
17	4	β	CH_3	H	8.3	1.1
18	6	β	CH_3	H	1.3	1.4
19	8	β	CH_3	H	25	1.9
20	12	β	CH_3	H	3.8	0.36
3		β	CH_3	H	0.21	0.027

Table 3. Antitumor activity of mitomycin derivatives with symmetrical disulfide.



No.	n	9	Y	Z	LD_{50}	Sarcoma 180 (sc-ip)				P388 (ip-ip)
						ED_{50}	Cl[a]	WBC_{4000}	$(WBC_{4000}/ED_{50})^a$	ILS_{max}[a]
7	2	β	CH_3	H	18.8	6.3	1.56	16.6	2.96	2.50
12	2	β	CH_3	CH_3	33.8	15.7	1.02	21.0	2.23	-
13	2	α	H	CH_3	>60	20.1	>1.42	44.8	3.82	1.38
14	2	α	CH_3	CH_3	50	28.6	1.10	24.3	1.05	>1.07
15	3	β	CH_3	H	90	25.4	1.56	99.7	5.61	0.79
17	4	β	CH_3	H	200	32.6	2.26	118.5	5.14	>1.04
18	6	β	CH_3	H	>200	34.6	>3.41	42.3	0.92	0.69
19	8	β	CH_3	H	37.5	10.1	1.94	18.1	2.80	0.56
20	12	β	CH_3	H	>200	—	—	>200	—	-
3		β	CH_3	H	8.4	3.2~4.9	1	2.6~6.3	1	1

LD_{50}, ED_{50}, WBC_{4000}: mg/kg, Cl: LD_{50}/ED_{50}, [a]: ratio to mitomycin C, —: not determined, -: not done.

All new symmetrical disulfide mitomycins besides **20** showed remarkable activity against sarcoma 180 (sc-ip). The compounds **7**, **13**, and **15** demonstrated fairly wide ranges of effective dosage and **17**, **18**, and **19** were the derivatives with the widest Cl that had ever been observed. The myelosuppression of all compounds besides **14** and **18** appeared to be reduced compared to **3**. The compound **7** showed a ED_{50} close to that of **3** and compound **19** possessed high potency in spite of its relatively weak cytotoxicity. This modification from C-7-amino to C-7-(ω-thioalkyl)amino including C-7-(2-thioethyl)amino group was entirely successful in **4** because the ED_{50} of **13** was 7.5 times as strong as that of **4** (data not shown). Compound **7** demonstrated the highest activity against P388 and compounds **13**, **14**, and **17** showed almost the same activity as **3**.

The ED_{50} values against sarcoma 180 (sc-ip) were not directly proportional to the *in vitro* IC_{50} values, suggesting the *in vivo* activation or quenching of the intact molecules. By contrast, the tendency of ILS_{max} against P388 (ip-ip) in this series of compounds basically met with that of the values of IC_{50}, implying that the ip-ip system is closer to the *in vitro* system than the sc-ip system.

The dominant compound in the metabolic pathway *in vivo* in this series of compounds is not yet known. Moreover, the essential active species upon reacting the DNA target is obscure. So far as we are concerned, neither 7-*N*-(2-mercaptoethyl)mitomycins nor 7-*N*-(2-mercaptoethyl)mitosenes were detected under the normal reaction conditions between 7-methoxy mitomycins and cysteamine. The studies of the metabolism and the molecular mechanism of action of this series of mitomycins are currently under way.

The remarkable activities and relatively weak myelosuppression of this series of compounds have prompted us to synthesize other series of compounds with the same essential $S(CH_2)_nNH$ at C-7 in mitomycins.[32~34]†

Experimental

Melting points were recorded on a Yanagimoto melting point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were recorded on a Bruker AM 400, AM 500, and a JEOL FX 100 spectrometers. MS spectra were recorded on a Hitachi M-80B spectrometer. IR spectra were recorded on a JASCO IR-810 spectrometer. TLC was carried out on E. Merck Reagents 60-F_{254} plates. The purity of mitomycin derivatives except **8** was confirmed by TLC and elemental analysis within an error of 0.4%.

Preparation of 7-*N*,7′-*N*′-Bis(2-thioethyl)dimitomycin C (**7**) and 7-*N*-[2-[(2-Aminoethyl)dithio]ethyl]-mitomycin C (**8**) (reaction of mitomycin A (**1**) with cysteamine)

Cysteamine (33 mg) was purified by sublimation and dissolved without delay in methanol (3 ml), to which was added mitomycin A (**1**, 100 mg). The reaction mixture was stirred at room temperature for 25 minutes. The solvent was removed *in vacuo*. As quickly as possible after drying, the residue was purified by column chromatography on silica gel with chloroform - methanol (85 : 15, then 6 : 4, v/v). The first blue fractions were collected and concentrated under reduced pressure to give blackish purple solids, which resulted in bluish gray powders of **7** (39 mg, 35%) from *n*-hexane - acetone. The second blue fractions were collected and concentrated *in vacuo* to give a blackish purple paste of **8** (35 mg, 26%), which was used directly for spectral analysis and reaction with **1** as quickly as possible. **7**: mp 142~145°C; SI-MS *m/z* 789 $(M+3)^+$; ^1H NMR (100 MHz, pyridine-d_5) δ 2.14 (3H, s), 2.75 (1H, dd), 3.00 (2H, t), 3.13 (1H, d), 3.22 (3H, s), 3.59 (1H, dd), 3.95 (2H, q), 3.99 (1H, dd), 4.52 (1H, d), 5.02 (1H, t), 5.36 (1H, dd), 7.27 (1H, t), 7.58 (2H, br); ^{13}C NMR (25 MHz, pyridine-d_5) δ 10.0 (C-6-CH_3), 32.7 (C-2), 36.6 (C-1), 38.6 (C-7-*N*-Cβ), 43.8 (7-*N*-Cα), 44.3 (C-9), 49.7 (C-9a-OCH_3), 50.5 (C-3), 62.4 (C-10), 104.5 (C-6), 106.8 (C-9a), 110.8 (C-8a), 147.1 (C-7), 155.7 (C-4a), 158.0 (OCON), 176.7 (C-8), 179.1 (C-5); IR (KBr) 3290, 2920, 1714, 1632, 1554, 1507, 1448, 1325, 1217, 1062, 752 cm^{-1}. **8**: ^1H NMR (400 MHz, pyridine-d_5) δ 2.12 (3H, s), 2.76 (1H, dd, J=4.3, 1.8), 3.00 (2H, t, J=6.8), 3.15 (1H, d, J=4.3), 3.23 (3H, s), 3.28 (2H, br t, J=6.8), 3.51 (2H, br t, J=6.2), 3.61 (1H, dd, J=12.7, 1.8), 3.94 (2H, t, J=7.1), 3.99 (1H, dd, J=11.1, 4.2), 4.52 (1H, d, J=12.7), 5.03 (1H, br t, J=10.7), 5.37 (1H, dd, J=10.4, 4.2), 7.29 (1H, br t, J=6.7), 7.66 (2H, br); IR (KBr) 3280, 2920, 1712, 1632, 1552, 1508, 1449, 1330, 1061, 752 cm^{-1}.

Preparation of 7-*N*,7′-*N*′-Bis(2-thioethyl)dimitomycin C (**7**) (reaction of mitomycin A (**1**) with cystamine dihydrochloride)

Cystamine dihydrochloride (129 mg) was dissolved in methanol (24 ml), to which was added triethylamine (1.2 ml) and mitomycin A (**1**, 400 mg). The reaction mixture was stirred at room temperature for 10 hours. To this was added water and the resulting mixture was extracted with chloroform. The

† The development and structure-activity relationships of mitomycin derivatives with unsymmetrical disulfide will be described elsewhere.

extracted solution was washed with brine and dried over anhydrous sodium sulfate. The drying agent was removed by filtration and the chloroform by evaporation. The residue was purified by column chromatography on silica gel with chloroform - methanol (85 : 15, v/v) to afford **7** (black paste, 533 mg, 99%).

Preparation of 7-*N*,7′-*N*′-Bis(2-thioethyl)dimitomycin C (**7**) (reaction of mitomycin A (**1**) with 7-*N*-[2-[(2-aminoethyl)dithio]ethyl]mitomycin C (**8**))

7-*N*-[2-[(2-aminoethyl)dithio]ethyl]mitomycin C (**8**, 10 mg) was dissolved in methanol (0.2 ml), to which was added mitomycin A (**1**, 7.4 mg). The reaction mixture was stirred at room temperature for 10 hours. The solvent was removed by evaporation. The residue was purified by column chromatography on silica gel with chloroform - methanol (85 : 15, v/v) to afford **7** (16.3 mg, 97%).

The Observation of Time Course for the Reaction of Mitomycin A (**1**) with Cysteamine Hydrochloride

The HPLC system was composed of a detector; Shimadzu SPD-M1A (photo-diode array detector), a column; Chemcosorb ODS-H (5 μm) 4.0 i.d. × 150 mm, a solvent system; 0.1 M phosphate buffer (pH 6.0) - acetonitrile (7 : 3, v/v), and a flow rate; 1 ml/minute at ambient temperature. Cysteamine hydrochloride (10.1 mg) was dissolved in dry methanol (1.2 ml) with triethylamine (50 μl) under a nitrogen atmosphere. Mitomycin A (**1**, 15.3 mg) was added to the solution and stirred for 2 hours at room temperature. In the course of reaction, the reaction mixture was injected to HPLC, and products were analyzed quantitatively by comparison with authentic samples. UV spectra of each peaks were detected by a photo-diode array detector.

Preparation of Leukomitomycin A (**9**)

Mitomycin A (21.1 mg) was dissolved in methanol (1 ml) and cooled to −78°C under a nitrogen atmosphere. Sodium borohydride (8.8 mg) was added with stirring, and the reaction mixture was analyzed with the HPLC system (described above).

Preparation of Mitosenes (**10**, **11**)

Cysteamine hydrochloride (55.5 mg) was dissolved in methanol (7 ml) with triethylamine (270 μl) under a nitrogen atmosphere. Mitomycin A (**1**, 88.4 mg) was added to the solution and stirred for 2 hours at room temperature. After removal of the solvent *in vacuo*, the residue was purified by preparative thin-layer chromatography with chloroform - methanol (85 : 15, v/v) to give yellowish orange **10** (11.4 mg, 12%) and **11** (8.6 mg, 10%) accompanying by **8** (9.6 mg, 8%) and **7** (34.5 mg, 36%). **10**: SI-MS *m/z* 350 $(M+1)^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 1.91 (3H, s, 6-CH_3), 3.51 (3H, s, 1β-OCH_3), 4.16 (1H, dd, J=12.3, 7.8, 3β-H), 4.44 (1H, td, J=7.8, 5.6, 2α-H), 4.71 (1H, dd, J=12.3, 7.8, 3α-H), 4.99 (1H, d, J=5.6, 1α-H), 5.25 (1H, d, J=13.3, 10-Ha), 5.30 (1H, d, J=13.3, 10-Hb); ^{13}C NMR (125 MHz, CD_3OD) δ 8.6 (q), 41.9 (t), 55.7 (d), 57.8 (q), 59.2 (t), 61.7 (q), 74.1 (d), 117.5 (s), 124.8 (s), 128.7 (s), 129.4 (s), 140.0 (s), 158.8 (s), 159.4 (s), 179.8 (s), 180.3 (s). **11**: SI-MS *m/z* 350 $(M+1)^+$; ^{1}H NMR (500 MHz, CD_3OD) δ 1.91 (3H, s, 6-CH_3), 3.49 (3H, s, 1α-OCH_3), 4.34 (1H, dd, J=13.9, 1.5, 3β-H), 4.38 (1H, br d, J=5.9, 2α-H), 4.61 (1H, dd, J=13.9, 5.9, 3α-H), 4.94 (1H, br s, 1β-H), 5.24 (1H, d, J=13.3, 10-Ha), 5.29 (1H, d, J=13.3, 10-Hb); ^{13}C NMR (125 MHz, CD_3OD) δ 8.6 (q), 51.4 (t), 57.7 (q), 59.1 (t), 59.9 (d), 61.7 (q), 79.8 (d), 117.7 (s), 125.7 (s), 128.3 (s), 129.4 (s), 139.9 (s), 158.8 (s), 159.5 (s), 179.8 (s), 180.4 (s).

Preparation of 7-*N*,7′-*N*′-Bis(2-thioethyl)diporfiromycin (**12**)

The compound **12** (black powder) was prepared from mitomycin F (**5**) and cystamine dihydrochloride by a similar procedure used in the preparation of **7** from **1** and cystamine dihydrochloride. The yield was 97%. **12**: mp 67.5~70°C; ^{1}H NMR (100 MHz, pyridine-d_5) δ 2.15 (3H, s), 2.15 (1H, dd), 2.25 (3H, s), 2.53 (1H, d), 3.00 (2H, t), 3.20 (3H, s), 3.51 (1H, d), 3.95 (3H, m), 4.45 (1H, d), 4.77 (1H, t), 5.30 (1H, dd), 7.25 (1H, t), 7.58 (2H, br s); IR (KBr) 3280, 2920, 1721, 1633, 1568, 1510, 1447, 1327, 1060 cm^{-1}.

Preparation of 7-*N*,7′-*N*′-Bis(2-thioethyl)dimitomycin D (**13**)

The compound **13** (green prisms) was prepared from mitomycin B (**2**) and cystamine dihydrochloride by a similar procedure used in the preparation of **7** from **1** and cystamine dihydrochloride. The yield was 93%. **13**: mp >220°C (dec); ^{1}H NMR (100 MHz, pyridine-d_5) δ 2.10 (3H, s), 2.13 (3H, s), 2.22 (1H, d), 2.47

(1H, d), 2.91 (2H, t), 3.66 (1H, d), 3.87 (2H, q), 4.23 (1H, dd), 4.44 (1H, d), 5.21 (1H, t), 5.47 (1H, dd), 7.26 (1H, t), 7.46 (2H, br s); IR (KBr) 3420, 3250, 1703, 1543, 1503, 1453, 1342 cm^{-1}.

Preparation of 7-*N*,7′-*N*′-Bis(2-thioethyl)dimitomycin E (**14**)

The compound **14** (dark bluish purple powder) was prepared from mitomycin J (**6**) and cystamine dihydrochloride by a similar procedure used in the preparation of **7** from **1** and cystamine dihydrochloride. The yield was 96%. **14**: mp 132.5~134°C; ^1H NMR (100 MHz, $CDCl_3$) δ 2.01 (3H, s), 2.22 (1H, d), 2.31 (3H, s), 2.37 (1H, dd), 2.85 (2H, t), 3.30 (3H, s), 3.57 (1H, d), 3.86 (2H, q), 4.02 (1H, d), 4.45 (1H, dd), 4.74 (2H, br s), 4.80 (1H, dd), 6.47 (1H, t); IR (KBr) 3300, 2930, 1716, 1553, 1510, 1445, 1333, 1050 cm^{-1}.

Preparation of 7-*N*,7′-*N*′-Bis(3-thiopropyl)dimitomycin C (**15**)

The compound **15** (dark bluish purple powder, from chloroform - methanol - *n*-hexane) was prepared from mitomycin A (**1**) and homocystamine dihydrochloride by a similar procedure used in the preparation of **7** from **1** and cystamine dihydrochloride. The yield was 58%. **15**: mp 122~135°C (the accurate mp was not observed due to dark color); SI-MS *m/z* 816 $(M+2)^+$, 818 $(M+4)^+$; ^1H NMR (100 MHz, pyridine-d_5) δ 2.00 (3H, quint, J=7.1), 2.14 (3H, s), approx 2.7 (1H, br), 2.80 (2H, t, J=7.1), 3.14 (1H, br s), 3.22 (3H, s), 3.61 (1H, br d, J=12.7), 3.66 (2H, q, J=6.7), 4.00 (1H, dd, J=11.0, 4.4), 4.54 (1H, d, J=12.7), 5.04 (1H, t, J=10.6), 5.38 (1H, dd, J=10.3, 4.4), 7.08 (1H, t, J=7.0), 7.63 (2H, br s).; IR (KBr) 3300, 2940, 1716, 1631, 1550, 1509, 1445, 1328, 1059 cm^{-1}.

Preparation of 7-*N*,7′-*N*′-Bis(3-thiopropyl)dimitomycin D (**16**)

The compound **16** (dark bluish green powder, from chloroform - methanol - *n*-hexane) was prepared from mitomycin B (**2**) and homocystamine dihydrochloride by a similar procedure used in the preparation of **7** from **1** and cystamine dihydrochloride. The yield was 59%. **16**: mp 153~157°C; SI-MS *m/z* 816 $(M+2)^+$, 817 $(M+3)^+$, 819 $(M+5)^+$; ^1H NMR (100 MHz, pyridine-d_5) δ 1.93 (3H, quint, J=7.1), 2.10 (3H, s), 2.13 (3H, s), 2.23 (1H, dd, J=4.9, 1.7), 2.47 (1H, d, J=4.9), 2.74 (2H, t, J=7.1), 3.59 (2H, q, J=7.1), 3.67 (1H, dd, J=12.9, 1.7), 4.22 (1H, dd, J=9.8, 3.7), 4.45 (1H, d, J=12.9), 5.20 (1H, t, J=10.1), 5.47 (1H, dd, J=10.5, 3.7), 7.07 (1H, t, J=6.6), 7.47 (2H, br s), 8.32 (1H, br s); IR (KBr) 3300, 2955, 1710, 1631, 1547, 1510, 1450, 1331, 1054 cm^{-1}.

Preparation of 7-*N*,7′-*N*′-Bis(4-thiobutyl)dimitomycin C (**17**)

Mitomycin A (**1**, 175 mg) was dissolved in methanol (4 ml), to which was added triethylamine (104.5 μl) and 4,4′-dithiodibutylamine dihydrochloride (70.3 mg). The reaction mixture was stirred at room temperature for 5.5 hours and kept at 5°C in a refrigerator overnight. The reaction mixture was extracted with ethyl acetate (150 ml) and washed with aqueous 5% sodium bicarbonate solution (50 ml) and dried over anhydrous sodium sulfate, followed by removal of the solvent by evaporation. The residue was purified by column chromatography on silica gel with chloroform - methanol (93 : 7, v/v). The blue fractions were collected and concentrated under reduced pressure. To the concentrated solution was added *n*-hexane and the residue was dried under reduced pressure to give dark bluish purple powder (205 mg, 97%). **17**: mp 126~134°C (the accurate mp was not observed due to dark color); SI-MS *m/z* 843 $(M+1)^+$, 844 $(M+2)^+$, 845 $(M+3)^+$; ^1H NMR (100 MHz, pyridine-d_5) δ 1.72 (4H, m), 2.14 (3H, s), 2.76 (2H, t, J=6.6), approx 2.8 (1H, br), 3.15 (1H, br s), 3.23 (3H, s), 3.53 (2H, q, J=6.4), 3.61 (1H, br d, J=12.7), 4.00 (1H, dd, J=11.0, 4.4), 4.56 (1H, d, J=12.7), 5.03 (1H, t, J=10.6), 5.38 (1H, dd, J=10.5, 4.4), 7.02 (1H, t, J=6.4), 7.62 (2H, br s); IR (KBr) 3310, 2950, 1720, 1636, 1553, 1510, 1448, 1329, 1059 cm^{-1}.

Preparation of 7-*N*,7′-*N*′-Bis(6-thiohexyl)dimitomycin C (**18**)

The compound **18** (dark bluish purple powder, from chloroform - methanol - *n*-hexane) was prepared from mitomycin A (**1**) and 6,6′-dithiodihexylamine dihydrochloride by a similar procedure used in the preparation of **7** from **1** and cystamine dihydrochloride. The yield was 95%. **18**: mp 106~113°C (the accurate mp was not observed due to dark color); SI-MS *m/z* 900 $(M+2)^+$, 901 $(M+3)^+$; ^1H NMR (100 MHz, pyridine-d_5) δ approx 1.2~1.8 (8H, m), 2.15 (3H, s), 2.76 (2H, t, J=6.6), approx 2.8 (1H, br), 3.15 (1H, d, J=4.4), 3.23 (3H, s), 3.48 (2H, q, J=6.6), 3.62 (1H, dd, J=12.7, 1.7), 4.00 (1H, dd, J=11.0, 4.4), 4.57 (1H, d, J=12.7), 5.04 (1H, t, J=10.6), 5.40 (1H, dd, J=10.3, 4.4), 6.96 (1H, t, J=6.6), 7.61 (2H, br s);

IR (KBr) 3290, 2910, 1713, 1628, 1548, 1504, 1441, 1320, 1052 cm^{-1}.

Preparation of 7-*N*,7'-*N*'-Bis(8-thiooctyl)dimitomycin C (**19**)

Mitomycin A (**1**, 175 mg) was dissolved in methanol - chloroform (2 : 1, v/v, 15 ml), to which was added triethylamine (104.5 μl) and 8,8'-dithiodioctylamine dihydrochloride (98.3 mg). After stirring at room temperature for 7.5 hours, triethylamine (69.7 μl) and 8,8'-dithiodioctylamine dihydrochloride (9.8 mg) was added to the reaction mixture and stirring was kept overnight. The reaction mixture was diluted and extracted with ethyl acetate (150 ml) and washed with aqueous 5% sodium bicarbonate solution (50 ml) and dried over anhydrous sodium sulfate, followed by removal of the solvent by evaporation. The residue was purified by column chromatography on silica gel with chloroform - methanol (92 : 8, v/v). The blue fractions were collected and concentrated under reduced pressure. To the concentrated solution was added *n*-hexane and the residue was dried under reduced pressure to give dark bluish purple powder (211 mg, 88%). **19**: mp. The hue was changed in the range of 93~98°C and 102~108°C, but the mp was not observed; SI-MS *m/z* 959 $(M+5)^+$; ^1H NMR (100 MHz, pyridine-d_5) δ approx 1.2~1.8 (12H, m), 2.16 (3H, s), 2.79 (2H, t, J=6.8), approx 2.8 (1H, br), 3.11 (1H, br d, J=4.9), 3.23 (3H, s), 3.48 (2H, q, J=6.4), 3.60 (1H, br d, J=12.7), 4.01 (1H, dd, J=11.0, 4.4), 4.58 (1H, d, J=12.7), 5.07 (1H, t, J=10.8), 5.41 (1H, dd, J=10.3, 4.4), 6.97 (1H, t, J=6.4), 7.62 (2H, br s); IR (KBr) 3300, 2930, 1719, 1639, 1558, 1518, 1450, 1330, 1060 cm^{-1}.

Preparation of 7-*N*,7'-*N*'-Bis(12-thiododecyl)dimitomycin C (**20**)

Mitomycin A (**1**, 150.8 mg) was dissolved in methanol (10 ml), to which was added triethylamine (301 μl) and crude 12,12'-dithiodidodecylamine dihydrobromide (200 mg, including approx 72 mg of phthalic acid). After stirring at room temperature for 40 minutes, chloroform (5 ml) was added to the reaction mixture and stirring was kept overnight. The reaction mixture was diluted and extracted with ethyl acetate (150 ml) and washed with aqueous 5% sodium bicarbonate solution (50 ml) and brine and dried over anhydrous sodium sulfate, followed by removal of the solvent by evaporation. The residue was purified by column chromatography on silica gel with chloroform - methanol (93 : 7, v/v). The blue fractions were collected and concentrated under reduced pressure. To the concentrated solution was added *n*-hexane and the residue was dried under reduced pressure to give dark bluish purple powder (165 mg, 66%). **20**: mp. The hue was changed in the range of 88~102°C and 147°C, but the mp was not observed; SI-MS *m/z* 1,071 $(M+5)^+$; ^1H NMR (100 MHz, pyridine-d_5) δ approx 1.2~1.9 (20H, m), 2.17 (3H, s), 2.81 (2H, t, J=6.6), approx 2.8 (1H, br s), 3.11 (1H, br d, J=4.4), 3.23 (3H, s), 3.50 (2H, q, J=6.6), 3.61 (1H, br d, J=12.9), 4.01 (1H, dd, J=11.0, 4.4), 4.58 (1H, d, J=12.9), 5.07 (1H, t, J=10.8), 5.41 (1H, dd, J=10.5, 4.4), 6.98 (1H, t, J=6.1), 7.63 (2H, br s); IR (KBr) 3310, 2930, 1720, 1640, 1560, 1514, 1452, 1328, 1060 cm^{-1}.

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