Cyclophanes. VIII.[1] Synthesis and DNA-Cleaving Activities of Novel Heterocyclophanes Containing Two 4,4′-Bithiazole Rings

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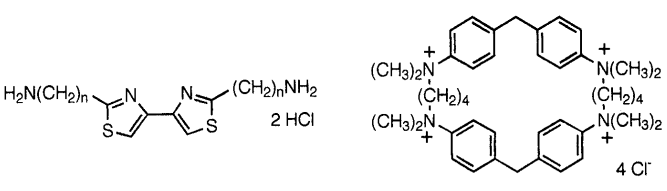
The novel heterocyclophanes, 3,6,21,24-tetraaza[8.8](2,2′)(4,4′-bithiazolophane) (3a) and 3,7,22,26-tetraaza-[9.9](2,2′)(4,4′-bithiazolophane) (3b) were readily synthesized by the cyclization of 1,4-dibromobutane-2,3-dione with *N,N′*-bis(*tert*-butoxycarbonyl)ethylenediamine-*N,N′*-dipropionthioamide and *N,N′*-bis(*tert*-butoxycarbonyl)-trimethylenediamine-*N,N′*-dipropionthioamide, respectively, followed by acidic deprotection. Under physiological conditions, 3a and 3b at 5 μM showed considerable DNA-cleaving activities in the presence of Co(II) without any reducing agent.

Key words cyclophane; 4,4′-bithiazole; DNA cleavage; cobalt complex; heterocyclophane; polyamine

The synthesis and the properties of heterocyclophanes containing five- or six-membered heterocycles have been widely investigated because of their interesting structural properties and ability to bind metals as macrocyclic ligands.[2] Previously, we reported the synthesis of a series of azolophanes, that is, heterocyclophanes containing azole rings such as imidazole and oxazole, and their interesting conformational properties.[1,3] Our synthetic method, that is, the application of azole ring formation to the construction of cyclophane rings, proved very convenient. Recently, we reported the preparation and properties of 2,2′-bis(aminoalkyl)-4,4′-bithiazoles (**1**) as new metal-dependent DNA-cleavers.[4] In particular, 2,2′-bis(2-aminoethyl)-4,4′-bithiazole (**1b**) showed the considerable DNA-cleaving activity in the presence of Co(II).[4b] On the other hand, it has been reported that azoniacyclophane (**2**), having two diphenylmethane moieties linked with two sets of positively charged alkylenediamine bridges, showed considerable electrostatic interaction with DNA.[5] These studies prompted us to prepare novel heterocyclophanes possessing two 4,4′-bithiazole rings bridged with positively charged alkylenediamine moieties.[6] These cyclophanes were expected to have metal-dependent DNA-cleaving ability, because they have both 4,4′-bithiazole moieties as metal-dependent DNA-cleaving domains and positively charged alkylenediamine bridges as electrostatic interaction domains with DNA. Here, we describe the synthesis of 3,6,21,24-tetraaza[8.8]- and 3,7,22,26-tetraaza[9.9](2,2′)(4,4′-bithiazolophanes) (**3a** and **3b**) as novel heterocyclophanes containing two 4,4'-bithiazole rings, as well as the Co(II)-dependent DNA-cleaving activities of these compounds under physiological conditions.

Synthesis and Structures of Bithiazolophanes First, as precursors of the bithiazolophanes (**3**), *N,N′*-bis(*tert*-butoxycarbonyl)alkylenediamine-*N,N′*-dipropionthioamides (**8**) were readily prepared by the following three-step process from the corresponding *N,N′*-bis(2-cyanoethyl)alkylenediamines (**4**).[7] As shown in Chart 1, *N,N′*-bis(*tert*-butoxycarbonyl)ethylenediamine-*N,N′*-dipropionamide (**6a**) or *N,N′*-bis(*tert*-butoxycarbonyl)trimethylenediamine-*N,N′*-dipropionamide (**6b**) was obtained by protection of the amino groups of **4a** or **4b** with di-*tert*-butyl dicarbonate (Boc_2O), followed by hydrolysis of the cyano groups of *N,N′*-bis(*tert*-butoxycarbonyl)-*N,N′*-bis(2-cyanoethyl)ethylenediamine (**5a**) or *N,N′*-bis(*tert*-butoxycarbonyl)-*N,N′*-bis(2-cyanoethyl)trimethylenediamine (**5b**) to amides with alkaline aqueous hydrogen peroxide in 65 or 57% yield from **4a** or **4b**, respectively. *N,N′*-Bis(*tert*-butoxycarbonyl)ethylenediamine-*N,N′*-dipropionthioamide (**8a**) or *N,N′*-bis(*tert*-butoxycarbonyl)trimethylenediamine-*N,N′*-dipropionthioamide (**8b**) was prepared by thiation of the amido carbonyl groups of **6a** or **6b** with 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent) (**7**) in 76 or 81% yield from **6a** or **6b**, respectively. Subsequently, construction of the bithiazolophane ring was carried out by means of Hantzsch's synthesis.[8] The reaction of equimolar amount of **8a** or **8b** with 1,4-dibromobutane-2,3-dione (**9**) at 60 °C for 2 h gave the corresponding *N*-protected bithiazolophane, 3,6,21,24-tetrakis(*tert*-butoxycarbonyl)-3,6,21,24-tetraaza[8.8](2,2′)(4,4′-bithiazolophane) (**10a**) or 3,7,22,26-tetrakis(*tert*-butoxycarbonyl)-3,7,22,26-tetraaza[9.9](2,2′)-(4,4′-bithiazolophane) (**10b**) and subsequent acidic deprotection of the amino groups afforded the bithiazolophanes (**3a** or **3b**) in 22 or 18% overall yield from **8a** or **8b**, respectively.

Acyclic reference compounds, 2,2′-bis[2-(2-aminoethyl)aminoethyl]-4,4′-bithiazole (**16a**) and 2,2′-bis[2-(3-aminopropyl)aminoethyl]-4,4′-bithiazole (**16b**), were also prepared by the same synthetic procedure as used for **3** (Chart 2) in order to investigate the relationship between the structure of the bithiazolophanes and the DNA-cleaving ability. That is, *N,N′*-bis(*tert*-butoxycarbonyl)-alkylenediamine-*N*-propionthioamides **14** as precursors of **16** were obtained by Boc-protection of *N*-(2-cyano-



1a: n = 1
1b: n = 2

2

Fig. 1. 2,2′-Bis(aminoalkyl)-4,4′-bithiazoles (**1**) and Azoniacyclophane (**2**)

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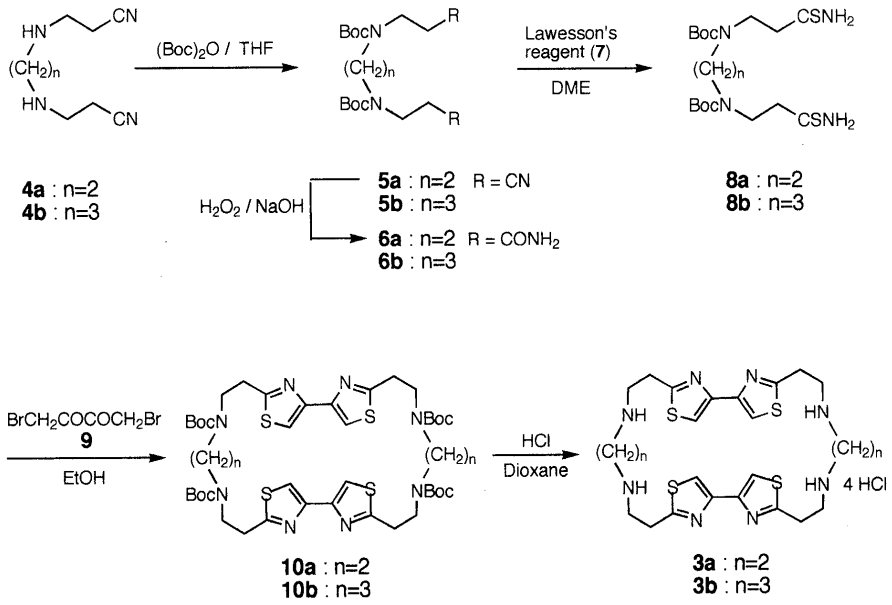

Chart 1

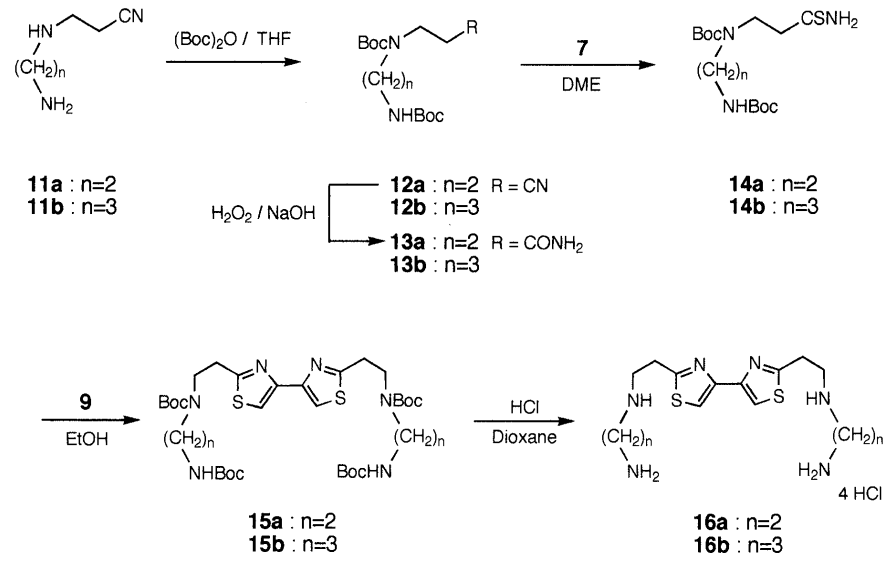

Chart 2

ethyl)alkylenediamines (**11**),[7] hydrolysis of the cyano group of the *N*,*N*′-bis(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)alkylenediamines (**12**) to amide, and thiation of the amido carbonyl group of the *N*,*N*′-bis(*tert*-butoxycarbonyl)alkylenediamine-*N*-propionamides (**13**) in 34 and 36% overall yields from **11a** and **11b**, respectively. Acyclic analogs **16a** and **16b** were synthesized by cyclization of **14a** or **14b** with **9**, followed by acidic deprotection of the amino groups of the 2,2′-bis[*N*,*N*′-bis(*tert*-butoxycarbonyl)-2-(ω-aminoalkyl)aminoethyl]-4,4′-bithiazoles (**15**) in 68 and 70% overall yields from **9**, respectively.

The structures of the newly obtained compounds (**3**, **5**, **6**, **8**, **10**, and **12**—**16**) were confirmed by the spectral data and elemental analysis. In particular, the ^{1}H-NMR spectrum of **10b** exhibited broad signals due to the bridge methylene protons, but a sharp singlet due to the thiazole ring protons. Moreover, no sharp singlet appeared in the spectrum of **10a** having a smaller cyclophane ring. Conformational change of the bridge methylene groups of **10** would be severely restricted because of the presence of the four bulky Boc groups at the N atoms. These trends were also observed for the acyclic reference compounds (**15**). In contrast, the ^{1}H-NMR spectra of **3** showed sharp signals due to the thiazole ring protons and bridge methylene protons. The signals of the protons at the 5-position of the thiazole rings of **3a** and **3b** appeared as sharp singlets at δ 7.83 and δ 7.84, respectively, which were similar to those of the corresponding protons of **16a**, **16b**, and **1b** (δ 7.90, δ 7.88, and δ 7.87,[4b] respectively). Furthermore, the UV spectra of **3a** and **3b** showed very small shifts at the absorption maximum as compared with those of the acyclic reference compounds **16a**, **16b**, and **1b** [UV λ_{max} nm (log ε): 251 (3.19)]. Therefore, it was considered that the bithiazolophane rings of **3** have almost no restriction of conformational change, and the transannular ring current effect and the locations of the bithiazole moieties of **3** should be essentially the same as those of **16** and **1b**. A consideration of space-filling molecular models (CPK models) of **3** supported these flexible structures and allowed us to estimate the diameter

Table 1. Inhibition of DNA-Cleaving Reactions of **3a** and **3b** in the Presence of Co(II)

Run No.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
Compound	—	**3a**	**3a**	**3b**	**3a**	**3b**	**3a**	**3b**	**3a**	**3b**	**3a**	**3b**	**3a**	**3b**	**3a**	**3b**	**3a**	**3b**
Inhibitor	—	—	EDTA		Ethanol		2-Propanol		DMSO		D-Mannitol		DABCO[a]		SOD		Catalase	
Concentration			1 mM		0.2 M		0.2 M		0.2 M		0.1 M		5 mM		20 mg/ml		20 mg/ml	
Form I (%)	93	1	93	92	2	3	3	2	4	3	3	2	1	3	2	3	3	4
Form II (%)	7	99	7	8	98	97	97	98	96	97	97	98	99	97	98	97	97	96

a) 1,4-Diazabicyclo[2.2.2]octane.

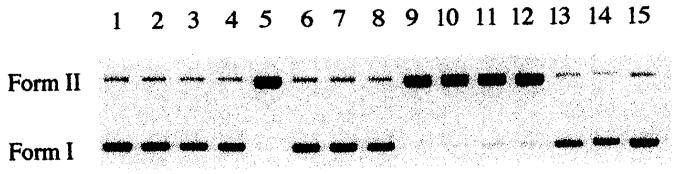

Fig. 2. Cleavage of Supercoiled Plasmid pBR322 DNA by Bithiazolophanes (**3a** and **3b**) and Acyclic Reference Compounds (**1b**, **16a**, and **16b**)

The concentration of metals was 50 μM. Lane 1, DNA control; lane 2, **3a** (10 μM) alone; lane 3, **3b** (10 μM) alone; lanes 4—8, **3a** (10 μM) + Mn(II), Co(II), Ni(II), Cu(II), and Zn(II), respectively; lane 9, **3a** (5 μM) + Co(II); lane 10, **3b** (10 μM) + Co(II): lane 11, **3b** (5 μM) + Co(II), lane 12, **1b** (10 μM) + Co(II); lane 13, **16a** (100 μM) + Co(II); lane 14, **16b** (100 μM) + Co(II); lane 15, Co(II) alone.

of the intramolecular cavities of **3a** and **3b** as 0.56 and 0.70 nm, respectively. It would be of interest to investigate **3** as an inclusion host, since the amino groups make **3**, which possesses a hydrophobic intramolecular cavity, highly soluble in water.

DNA-Cleaving Reactions of Bithiazolophanes and Related Compounds In order to investigate the DNA-cleaving abilities of the bithiazolophanes (**3a** and **3b**), DNA-cleaving reaction using supercoiled plasmid pBR322 DNA[9] was carried out in 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (40 mM, pH 7.0) at 37 °C for 30 min, as shown in Fig. 2. No DNA-cleaving activity was observed at 10 μM **3a** or **3b** alone (lanes 2 and 3), or at 50 μM Co(II) alone (lane 15), as is evident from a comparison with the DNA control (lane 1). Only in the presence of Co(II), **3a** or **3b** at 10 μM (lanes 5 and 10) or bithiazole (**1b**) at 10 μM (lane 12) show significant DNA cleavage, that is, nicking of the DNA strand to convert supercoiled DNA (form I) into nicked circular DNA (form II). Even at 5 μM **3a** or **3b**, giving a similar concentration of the bithiazole residue to that in the case of **1b** at 10 μM, considerable DNA-cleaving activity was observed (lanes 9 and 11). However, DNA cleavage could not be observed in the presence of other metals such as Mn(II), Ni(II), Cu(II), and Zn(II) under the same conditions (lanes 4 and 6—8). In the presence of excess EDTA (1 mM) the DNA scission by **3a** or **3b** was completely inhibited (Runs 3 and 4 in Table 1). These facts suggest that complex formation of **3a** and **3b** with Co(II) plays an important role in these cleavage reactions. On the other hand, **16a** and **16b** could not cleave DNA at 100 μM concentration under the same conditions (lanes 13 and 14).[10] Since **16a** and **16b** have flexible acyclic chain structures possessing positively charged amino groups, like those of polyamines such as spermidine and spermine, which are DNA minor groove binders,[11] the interactions of **16a** and **16b** with DNA may be stronger than those of the bithiazolophanes (**3a** and **3b**). In order to estimate the strength of the interaction between DNA and bithiazolophanes (**3**) or bithiazoles (**16** and **1b**), the C_{50} values of **3**, **16**, and **1b** were measured, *i.e.*, the concentrations giving a 50% reduction in the fluorescence intensity of ethidium bromide intercalated with DNA.[12] Based on these C_{50} values, acyclic reference compounds (**16a** and **16b**) having low C_{50} values (16 and 7.0 μM, respectively) interacted more efficiently with DNA than did the bithiazolophanes (**3a** and **3b**) (82 and 72 μM, respectively) or **1b** (105 μM). In contrast, the interactions of **3a** and **3b** with DNA were quite similar to that of **1b** as judged from the similar C_{50} values. Since the DNA-cleaving activities of **3a** and **3b** are similar to that of **1b** and both bithiazole rings with two aminoethyl groups in **3a** and **3b** are spatially separated from each other, **3a** and **3b** should be able to form similar complexes with Co(II) to that of **1b** with Co(II). These considerations suggest that the structure of the complex of **16** with Co(II) may be different from that of **3** with Co(II), and the presence of Co(II) is essential for the DNA cleavage of **3**.

Table 2. DNA-Cleaving Reactions of **3a** and **3b** in the Presence of Co(II) in the Dark and under Anaerobic Conditions

Run No.	1	2	3	4
Compound	**3a**	**3a**	**3b**	**3b**
Co(II)	+	+	+	+
Condition	Dark	Anaerobic	Dark	Anaerobic
Form I (%)	2	2	1	3
Form II (%)	98	98	99	97

It was reported that the DNA cleavage of **1b** in the presence of Co(II) did not proceed through oxidative degradation.[4b] In order to investigate the mechanism of the DNA-cleaving reactions of **3a** and **3b**, some inhibition experiments were conducted. As listed in Table 1, hydroxyl radical and singlet oxygen scavengers (runs 5—14), superoxide dismutase (SOD) (runs 15 and 16), or catalase (runs 17 and 18) did not inhibit the cleavage. Since the cleaving reaction did not require any reducing agent, it was considered that active oxygen species could not be induced by a redox reaction on cobalt ion. Moreover, in the dark or under anaerobic conditions, the DNA-cleaving activities of **3a** and **3b** were not reduced, as shown in Table 2. Based on these results, the DNA cleavages by **3a** and **3b**, as well as **1b**, appear not to proceed through either photoactivated or oxidative DNA degradation. Further investigations on the mechanism of the DNA-cleaving reaction are in progress in our laboratory.

In conclusion, it was demonstrated that our synthetic method for azolophanes could be successfully applied for the preparation of novel bithiazolophanes, the first

reported heterocyclophanes possessing two 4,4′-bithiazole rings. At 5 μM under physiological conditions, the bithiazolophanes (**3a** and **3b**) containing two 2,2′-bis(2-aminoethyl)-4,4′-bithiazole moieties as a DNA-cleaving domain showed considerable DNA-cleaving abilities in the presence of Co(II) without any reducing agent.

Experimental

All melting points were taken on a Yanagimoto micro melting point determination apparatus and are uncorrected. IR and UV spectra in H_2O were recorded on a Hitachi 270-30 infrared spectrophotometer and a Hitachi U-3000 spectrophotometer, respectively. ^{1}H-NMR spectra were taken at 400 MHz with a Bruker DPX-400 spectrometer using tetramethylsilane in $CDCl_3$ and 4,4-dimethyl-4-silapentanesulfonic acid sodium salt in D_2O as internal references. Fluorescence spectra were recorded on a Hitachi F-3000 spectrophotometer and C_{50} values were measured by the same procedure as described in ref. 12.

***N,N′*-Bis(*tert*-butoxycarbonyl)-*N,N′*-bis(2-cyanoethyl)ethylenediamine (5a) and *N,N′*-Bis(*tert*-butoxycarbonyl)-*N,N′*-bis(2-cyanoethyl)-trimethylenediamine (5b)** General Procedure: Di-*tert*-butyl dicarbonate (21.8 g, 100 mmol) was added portionwise to a solution of an *N,N′*-bis(2-cyanoethyl)alkylenediamine (**4**, 50 mmol)[7] in a mixture of tetrahydrofuran (THF, 300 ml) and an aqueous sodium hydroxide (NaOH) solution (2 mol/l, 60 ml), and the mixture was stirred for 2 h at room temperature. The organic solvent was removed under reduced pressure and ethyl acetate (EtOAc, 100 ml) was poured into the residual aqueous solution. The organic layer was separated, washed twice with 10% aqueous citric acid solution (20 ml) and brine (20 ml), and then dried over anhydrous $MgSO_4$. The solvent was removed *in vacuo* to yield a colorless oil, which was purified by silica gel flash column chromatography. Elution of the column with hexane–EtOAc (1 : 1) gave a crude product.

5a: Yield: 17.9 g (97%), colorless prisms (ether), mp 120—121 °C. IR (KBr): 2980, 2248, 1694 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (18H, s, Boc-H), 2.52—2.68 (4H, m, –C<u>H</u>$_2$CN), 3.40—3.55 [8H, m, –C<u>H</u>$_2$N-(Boc)C<u>H</u>$_2$C<u>H</u>$_2$N(Boc)C<u>H</u>$_2$–]. *Anal.* Calcd for $C_{18}H_{30}N_4O_4$: C, 59.00; H, 8.25; N, 15.29. Found: C, 59.14; H, 8.39; N, 15.45.

5b: Yield: 18.0 g (95%), colorless oil. IR (neat): 2976, 2252, 1694 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (18H, s, Boc-H), 1.81 (2H, quintet, J = 7.3 Hz, –CH$_2$C<u>H</u>$_2$CH$_2$–), 2.54—2.72 (4H, m, –C<u>H</u>$_2$CN), 3.29 (4H, t, J = 7.3 Hz, –C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$–), 3.48 (4H, t, J = 6.5 Hz, –C<u>H</u>$_2$CH$_2$CN).

***N,N′*-Bis(*tert*-butoxycarbonyl)ethylenediamine-*N,N′*-dipropionamide (6a) and *N,N′*-Bis(*tert*-butoxycarbonyl)trimethylenediamine-*N,N′*-dipropionamide (6b)** General Procedure: A hydrogen peroxide (H_2O_2) solution (30%, 20 ml) was added dropwise to a stirred solution of **5** (25 mmol) and NaOH (100 mmol) in a mixture of water (50 ml) and ethanol (100 ml) for 30 min, keeping the temperature below 5 °C. The mixture was stirred overnight at room temperature, then excess H_2O_2 was decomposed by the addition of a 20% sodium bisulfite solution. The insoluble material was removed by filtration, the organic solvent in the filtrate was evaporated *in vacuo*, and the resulting aqueous solution was extracted 5 times with EtOAc (20 ml). The combined organic layer was washed with brine (20 ml), and then dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by silica gel flash column chromatography with $CHCl_3$–methanol (9 : 1) to give a viscous oil. This oil was dissolved in ether and the resulting solution was allowed to stand overnight at −20 °C to afford a colorless crystalline mass.

6a: Yield: 6.7 g (67%), colorless prisms ($CHCl_3$: methanol = 9 : 1), mp 212—214 °C. IR (KBr): 3392, 3196, 2976, 2940, 1676, 1630 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.59 (18H, s, Boc-H), 2.45—2.57 (4H, m, –C<u>H</u>$_2$CONH$_2$), 3.32—3.56 [8H, m, –C<u>H</u>$_2$N(Boc)C<u>H</u>$_2$C<u>H</u>$_2$N(Boc)-C<u>H</u>$_2$–]. *Anal.* Calcd for $C_{18}H_{34}N_4O_6$: C, 53.71; H, 8.51; N, 13.92. Found: C, 53.58; H, 8.72; N, 13.85.

6b: Yield: 6.2 g (60%), colorless prisms (ethanol), mp 125—127 °C. IR (KBr): 3388, 3210, 2974, 2932, 1704, 1659 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.45 (18H, s, Boc-H), 1.70—1.80 (2H, m, –CH$_2$C<u>H</u>$_2$CH$_2$–), 2.51 (4H, br s, –C<u>H</u>$_2$CONH$_2$), 3.26 (4H, br s, –C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$–), 3.48 (4H, t, J = 6.4 Hz, –C<u>H</u>$_2$CH$_2$CONH$_2$). *Anal.* Calcd for $C_{19}H_{36}N_4O_6$: C, 54.79; H, 8.70; N, 13.45. Found: C, 54.66; H, 8.56; N, 13.40.

***N,N′*-Bis(*tert*-butoxycarbonyl)ethylenediamine-*N,N′*-dipropionthioamide (8a) and *N,N′*-Bis(*tert*-butoxycarbonyl)trimethylenediamine-*N,N′*-dipropionthioamide (8b)** General Procedure: Lawesson's reagent (**7**) (2.2 g, 5.5 mmol) was added in one portion to a stirred suspension of **6** (10 mmol) in 1,2-dimethoxyethane (DME, 100 ml) at room temperature. The mixture was heated to 60 °C for 2 h with stirring, and the organic solvent was removed under reduced pressure. A mixture of EtOAc (100 ml) and aqueous NaOH solution (2 mol/l, 20 ml) was poured onto the residue and the organic layer was separated, washed with aqueous NaOH solution (2 mol/l, 20 ml) and brine (30 ml), and then dried over anhydrous $MgSO_4$. The solvent was evaporated *in vacuo*, and the residue was purified by silica gel flash column chromatography with acetone–hexane (1 : 1) to give a crude product.

8a: Yield: 3.3 g (76%), colorless prisms (ethanol), mp 200—201 °C. IR (KBr): 3344, 3188, 2980, 1678, 1650 cm^{-1}. ^{1}H-NMR ($CDCl_3$: CD_3OD = 1 : 1) δ: 1.49 (18H, s, Boc-H), 2.76—2.90 (4H, m, –C<u>H</u>$_2$CSNH$_2$), 3.36—3.43 [4H, br s, –N(Boc)C<u>H</u>$_2$C<u>H</u>$_2$N(Boc)–], 3.60 (4H, m, –C<u>H</u>$_2$CH$_2$CSNH$_2$). *Anal.* Calcd for $C_{18}H_{34}N_4O_4S_2$: C, 49.74; H, 7.89; N, 12.89. Found: C, 49.48; H, 7.76; N, 12.73.

8b: Yield: 3.6 g (81%), colorless prisms (ether), mp 144—145 °C. IR (KBr): 3360, 3200, 2980, 2936, 1676 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (18H, s, Boc-H), 1.77 (2H, quintet, J = 7.2 Hz, –CH$_2$C<u>H</u>$_2$CH$_2$–), 2.99 (4H, br s, –C<u>H</u>$_2$CSNH$_2$), 3.16—3.26 (4H, m, –C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$–), 3.53—3.63 (4H, m, –C<u>H</u>$_2$CH$_2$CSNH$_2$). *Anal.* Calcd for $C_{19}H_{36}N_4O_4S_2$: C, 50.87; H, 8.08; N, 12.49. Found: C, 51.07; H, 8.11; N, 12.26.

3,6,21,24-Tetrakis(*tert*-butoxycarbonyl)-3,6,21,24-tetraaza[8.8](2,2′)-(4,4′-bithiazolophane) (10a) and 3,7,22,26-Tetrakis(*tert*-butoxycarbonyl)-3,7,22,26-tetraaza[9.9](2,2′)(4,4′-bithiazolophane) (10b) General Procedure: 1,4-Dibromobutane-2,3-dione (**9**) (1.2 g, 5 mmol) was added in one portion to a stirred solution of **8** (5 mmol) in dry ethanol (100 ml) at room temperature. The mixture was gently heated to 60 °C for 2 h with stirring. The addition of excess triethylamine (2.0 g, 20 mmol) to the mixture, and the organic solvent was evaporated *in vacuo*. A mixture of EtOAc (100 ml) and H_2O (20 ml) was poured onto the residue and the organic layer was separated, washed with brine (20 ml), and then dried over anhydrous $MgSO_4$. The solvent was evaporated off and the residue was purified by silica gel flash column chromatography with acetone–hexane (1 : 1) to give a crude product.

10a: Yield: 0.58 g (24%), colorless prisms (methanol), mp 204—205 °C. IR (KBr): 3116, 2972, 1704, 1160 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.32—1.52 (36H, m, Boc-H), 2.98—3.80 (24H, m, –CH$_2$–), 7.40—7.65 (4H, m, thiazole 5-H). *Anal.* Calcd for $C_{44}H_{64}N_8O_8S_4 \cdot CH_3OH$: C, 54.41; H, 6.90; N, 11.28. Found: C, 54.56; H, 6.83; N, 11.25.

10b: Yield: 0.47 g (19%) , colorless prisms (ether : hexane = 1 : 1), mp 136—138 °C. IR (KBr): 3108, 2976, 1682, 1144 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.38—1.52 (36H, m, Boc-H), 2.36 (4H, br s, –CH$_2$C<u>H</u>$_2$CH$_2$–), 3.07 (8H, br s, –C<u>H</u>$_2$-thiazole), 3.20—3.40 (8H, m, –C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$–), 3.85—3.93 (4H, m, –C<u>H</u>$_2$CH$_2$–thiazole), 7.31 (4H, s, thiazole 5-H). *Anal.* Calcd for $C_{46}H_{68}N_8O_8S_4$: C, 55.84; H, 6.92; N, 11.33. Found: C, 55.70; H, 6.88; N, 11.14.

3,6,21,24-Tetraaza[8.8](2,2′)(4,4′-bithiazolophane) (3a) and 3,7,22,26-Tetraaza[9.9](2,2′)(4,4′-bithiazolophane) (3b) General Procedure: A solution of hydrogen chloride in dioxane (5.2 mol/l, 2 ml) was added dropwise to a solution of **10** (0.2 mmol) in dioxane (5 ml) at room temperature. The mixture was stirred overnight at room temperature, and the precipitates were collected by suction to give a crude product.

3a: Yield: 0.13 g (92%), colorless prisms (ethanol : H_2O = 5 : 1), mp > 300 °C. IR (KBr): 2400—3150, 1630, 1424, 1122 cm^{-1}. ^{1}H-NMR (D_2O) δ: 3.59 (8H, t, J = 6.1 Hz, –C<u>H</u>$_2$-thiazole), 3.67 (8H, s, –NHC<u>H</u>$_2$C<u>H</u>$_2$NH–), 3.71 (8H, t, J = 6.1 Hz, –C<u>H</u>$_2$CH$_2$–thiazole), 7.83 (4H, s, thiazole 5-H). UV λ_{max} nm (log ε): 249 (3.38). C_{50} (μM): 82. *Anal.* Calcd for $C_{24}H_{32}N_8S_4 \cdot 4HCl$: C, 40.79; H, 5.14; N, 15.86. Found: C, 40.60; H, 5.04; N, 15.97.

3b: Yield: 0.14 g (95%), colorless prisms (ethanol : H_2O = 5 : 1), mp > 300 °C. IR (KBr): 2400—3150, 1606, 1424, 1116 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.34 (4H, quintet, J = 6.5 Hz, –CH$_2$C<u>H</u>$_2$CH$_2$–), 3.49—3.54 (8H, m, –C<u>H</u>$_2$–thiazole), 3.58 (8H, t, J = 6.5 Hz, –C<u>H</u>$_2$CH$_2$C<u>H</u>$_2$–), 3.63—3.67 (8H, m, –C<u>H</u>$_2$CH$_2$–thiazole), 7.84 (4H, s, thiazole 5-H). UV λ_{max} nm (log ε): 250 (3.36). C_{50} (μM): 72. *Anal.* Calcd for $C_{26}H_{36}N_8S_4 \cdot 4HCl$: C,42.51; H, 5.48; N, 15.25. Found: C, 42.38; H, 5.35; N, 15.21.

***N,N′*-Bis(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)ethylenediamine (12a) and *N,N′*-Bis(*tert*-butoxycarbonyl)-*N*-(2-cyanoethyl)trimethylenediamine (12b)** According to the general procedure described above for the preparation of **5**, the treatment of *N*-(2-cyanoethyl)alkylenediamines (**11**, 50 mmol)[7] with Boc_2O (21.8 g, 100 mmol) in a mixture of THF (200 ml) and aqueous NaOH solution (2 mol/l, 50 ml) gave a crude product, which was purified by silica gel flash column chromatography with

EtOAc–hexane (1 : 1).

12a: Yield: 14.4 g (92%), colorless prisms (ether), mp 69.5—70 °C. IR (KBr): 2976, 2248, 1690 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.44 and 1.48 (18H, each s, Boc-H), 2.50—2.70 (2H, m, $-CH_2CN$), 3.20—3.35 (2H, m, $BocNHCH_2-$), 3.40 (2H, t, J=6.2 Hz, $BocNHCH_2CH_2-$), 3.50 (2H, t, J=6.7 Hz, $-CH_2CH_2CN$), 4.65—5.15 (1H, m, BocNH-). *Anal.* Calcd for $C_{15}H_{27}N_3O_4$: C, 57.49; H, 8.68; N, 13.41. Found: C, 57.22; H, 8.80; N, 13.40.

12b: Yield: 14.7 g (90%), colorless oil. IR (neat): 2980, 2252, 1694 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.44 and 1.48 (18H, each s, Boc-H), 1.64—1.76 (2H, m, $-CH_2CH_2CH_2-$), 2.54—2.70 (2H, m, $-CH_2CN$), 3.06—3.18 (2H, m, $BocNHCH_2-$), 3.34 [2H, t, J=6.7 Hz, $-CH_2CH_2CH_2N(Boc)-$], 3.46 (2H, t, J=6.7 Hz, $-CH_2CH_2CN$).

***N,N'*-Bis(*tert*-butoxycarbonyl)ethylenediamine-*N*-propionamide (13a) and *N,N'*-Bis(*tert*-butoxycarbonyl)trimethylenediamine-*N*-propionamide (13b)** According to the general procedure described above for the preparation of **6**, **12** (30 mmol) was treated with a mixture of aqueous NaOH solution (2 mol/l, 30 ml) and ethanol (100 ml) in the presence of H_2O_2 (30% aqueous solution, 50 ml) to afford a crude product, which was purified by silica gel flash column chromatography with EtOAc–hexane (3 : 1).

13a: Yield: 6.0 g (60%), colorless microcrystals (ether), mp 85—86 °C. IR (KBr): 3364, 3200, 2976, 1674, 1638 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.43 and 1.47 (18H, each s, Boc-H), 1.82 (2H, br s, $-CONH_2$), 2.51 (2H, br s, $-CH_2CONH_2$), 3.20—3.40 (4H, m, $BocNHCH_2CH_2-$), 3.53 (2H, t, J=6.8 Hz, $-CH_2CH_2CONH_2$), 4.65—5.15 (1H, m, BocNH-). *Anal.* Calcd for $C_{15}H_{29}N_3O_5 \cdot 1/4H_2O$: C, 53.63; H, 8.85; N, 12.51. Found: C, 53.59; H, 8.77; N, 12.30.

13b: Yield: 6.4 g (62%), colorless microcrystals (ether), mp 125—127 °C. IR (KBr): 3360, 3196, 2980, 1696, 1674 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.44 and 1.47 (18H, each s, Boc-H), 1.66 (2H, quintet, J=6.7 Hz, $-CH_2CH_2CH_2-$), 2.45—2.58 (2H, m, $-CH_2CONH_2$), 3.08—3.12 (2H, m, $BocNHCH_2-$), 3.24—3.33 [2H, m, $-CH_2CH_2CH_2N(Boc)-$], 3.49 (2H, t, J=6.6 Hz, $-CH_2CH_2CONH_2$). *Anal.* Calcd for $C_{16}H_{31}N_3O_5$: C, 55.63; H, 9.05; N, 12.16. Found: C, 55.52; H, 8.95; N, 12.13.

***N,N'*-Bis(*tert*-butoxycarbonyl)ethylenediamine-*N*-propionthioamide (14a) and *N,N'*-Bis(*tert*-butoxycarbonyl)trimethylenediamine-*N*-propionthioamide (14b)** According to the general procedure described above for the preparation of **8**, the treatment of **13** (10 mmol) with Lawesson's reagent (4.0 g, 10 mmol) in DME (100 ml) for 2 h at 60 °C gave a crude product, which was purified by silica gel flash column chromatography with EtOAc–hexane (2 : 1).

14a: Yield: 2.1 g (61%), colorless microcrystals (ether : hexane = 5 : 1), mp 108—108.5 °C. IR (KBr): 3388, 3176, 2980, 1678, 1652 cm^{-1}. ^{1}H-NMR ($CDCl_3$: CD_3OD = 1 : 1) δ: 1.43 and 1.47 (18H, each s, Boc-H), 2.80—3.05 (2H, m, $-CH_2CSNH_2$), 3.20—3.40 [4H, m, $BocNHCH_2CH_2-$], 3.55—3.65 (2H, m, $-CH_2CH_2CSNH_2$), 4.65—5.15 (1H, m, BocNH-). *Anal.* Calcd for $C_{15}H_{29}N_3O_4S$: C, 51.85; H, 8.41; N, 12.09. Found: C, 52.13; H, 8.71; N, 12.19.

14b: Yield: 2.3 g (65%), colorless microcrystals (acetone : hexane = 5 : 1), mp 118—119 °C. IR (KBr): 3372, 3156, 2984, 1696, 1648 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.43 and 1.47 (18H, each s, Boc-H), 1.69 (2H, quintet, J=6.8 Hz, $-CH_2CH_2CH_2-$), 2.98 (2H, br s, $-CH_2CSNH_2$), 2.97—3.14 (2H, m, $BocNHCH_2-$), 3.23—3.32 [2H, m, $-CH_2CH_2CH_2N(Boc)-$], 3.56 (2H, t, J=6.8 Hz, $-CH_2CH_2CSNH_2$). *Anal.* Calcd for $C_{16}H_{31}N_3O_4S$: C, 53.16; H, 8.64; N, 11.62. Found: C, 53.01; H, 8.45; N, 11.47.

2,2'-Bis[*N,N'*-bis(*tert*-butoxycarbonyl)-2-(2-aminoethyl)aminoethyl]-4,4'-bithiazole (15a) and 2,2'-Bis[*N,N'*-bis(*tert*-butoxycarbonyl)-2-(3-aminopropyl)aminoethyl]-4,4'-bithiazole (15b) According to the general procedure described above for the preparation of **10**, the treatment of **14** (5 mmol) with **9** (1.2 g, 5 mmol) in dry ethanol (100 ml) for 2 h at 50 °C gave a crude product, which was purified by silica gel flash column chromatography with EtOAc–hexane (1 : 1).

15a: Yield: 1.4 g (73%), colorless microcrystals (acetone), mp 204—205 °C. IR (KBr): 3340, 3140, 2980, 1710, 1680, 1168 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (36H, s, Boc-H), 3.28 (12H, br s, $BocNHCH_2CH_2-$), 3.65 (4H, br s, $-CH_2-$thiazole), 5.00—5.40 (1H, m, BocNH-), 7.65 (2H, s, thiazole 5-H). *Anal.* Calcd for $C_{34}H_{56}N_6O_8S_2$: C, 55.11; H, 7.62; N, 11.34. Found: C, 55.21; H, 7.74; N, 11.16.

15b: Yield: 1.5 g (78%), colorless microcrystals (ether), mp 132—133 °C. IR (KBr): 3380, 3096, 2976, 1712, 1686, 1174 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.43 and 1.47 (36H, each s, Boc-H), 1.65 (2H, quintet, J=6.4 Hz, $-CH_2CH_2CH_2-$), 3.04—3.13 (2H, m, $BocNHCH_2-$), 3.18—3.33 [4H, m, $-CH_2N(Boc)CH_2-$], 3.58—3.62 (2H, m, $-CH_2-$thiazole), 7.63 (2H, s, thiazole 5-H). *Anal.* Calcd for $C_{36}H_{60}N_6O_8S_4$: C, 56.23; H, 7.86; N, 10.93. Found: C, 55.99; H, 7.84; N, 10.67.

2,2'-Bis[2-(2-aminoethyl)aminoethyl]-4,4'-bithiazole (16a) and 2,2'-Bis[2-(3-aminopropyl)aminoethyl]-4,4'-bithiazole (16b) According to the general procedure described above for the preparation of **10**, a solution of HCl in dioxane (5.2 mol/l, 10 ml) was added to a solution of **15** (2 mmol) in dioxane (50 ml) at room temperature and the mixture was stirred overnight to give a crude product.

16a: Yield: 0.87 g (93%), colorless plates (methanol : H_2O = 4 : 1), mp > 300 °C. IR (KBr): 3150—2400, 1600, 1496, 1192 cm^{-1}. ^{1}H-NMR (D_2O) δ: 3.45—3.60 (8H, m, $-NHCH_2CH_2NH-$), 3.56—3.72 (8H, m, $-CH_2CH_2-$thiazole), 7.90 (2H, s, thiazole 5-H). UV λ_{max} nm (log ε): 251 (3.14). C_{50} (μM): 16. *Anal.* Calcd for $C_{14}H_{24}N_6S_2 \cdot 4HCl$: C, 34.72; H, 5.41; N, 17.35. Found: C, 34.60; H, 5.67; N, 17.22.

16b: Yield: 0.93 g (90%), colorless plates (methanol : H_2O = 4 : 1), mp > 300 °C. IR (KBr): 3150—2400, 1600, 1496, 1180 cm^{-1}. ^{1}H-NMR (D_2O) δ: 2.17 (4H, quintet, J=6.5 Hz, $-CH_2CH_2CH_2-$), 3.10—3.35 (8H, m, $-CH_2CH_2CH_2-$), 3.50—3.70 (8H, m, $-CH_2CH_2-$thiazole), 7.88 (2H, s, thiazole 5-H). UV λ_{max} nm (log ε): 250 (3.16). C_{50} (μM): 7.0. *Anal.* Calcd for $C_{16}H_{28}N_6S_2 \cdot 4HCl$: C, 37.36; H, 6.27; N, 16.34. Found: C, 37.25; H, 6.15; N, 16.26.

DNA-Cleaving Reactions of Bithiazolophanes (3a and 3b) and Acyclic Reference Compounds (1b, 16a, and 16b) Plasmid pBR322 DNA purchased from Nippon Gene Co., Ltd., or Cosmo Bio Co., Ltd., was used as a supercoiled DNA. Each reaction solution contained 0.1 mg of supercoiled plasmid pBR322 DNA in 40 mM MOPS buffer (pH 7.0). All cleavage reactions were run for 30 min at 37 °C, and the electrophoresis was carried out at 50 V (1.8 h) on a 1.2% agarose gel in 40 mM Tris–acetate containing 2 mM EDTA (TAE) buffer (pH 8.1). The gel patterns were developed by soaking the gels in ethidium bromide buffer solution (1 mg/1 ml of TAE buffer) and photographed with an instant camera.

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