2,2′-Bis(aminomethyl)-4,4′-bithiazole as a New Cu(II)-Dependent DNA Cleaving Agent

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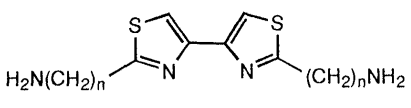
A simple and readily available DNA cleaving agent, 2,2′-bis(aminomethyl)-4,4′-bithiazole at 50 μM concentration showed a significant cleaving activity for plasmid DNA only in the presence of Cu(II) under physiological conditions without any reducing agents.

Key words DNA cleavage; bithiazole; copper complex; DNA; 2,2′-bis(aminomethyl)-4,4′-bithiazole

Recently, one of the authors (H. S.) has reported the synthesis of new DNA cleavers, 2,2′-bis(2-aminoethyl)-4,4′-bithiazole (**1b**)[1a] and *N*,*N*′-bis[2-[4-(3-aminopropylcarbamoyl)-2,4′-bithiazole-2′-yl]ethyl]ethylenediamine (**2**)[1b] and their Co(II)-dependent DNA cleaving activities without any reducing agents. Concerning the functionality of 2,4′-bithiazoles, it is well known that the 2,4′-bithiazole moiety, contained as a part of the C terminus of bleomycin (BLM), plays an important role in the interaction with the double-stranded DNA during the cleavage reaction by BLM.[2] However, it was recently reported that the 2,4′-bithiazole moiety was ineffective for DNA recognition in the DNA cleaving reaction with EDTA-bithiazoles derivatives activated by Fe(III)[3] and 2′-(1,3-diamino-2-propyl)-2,4′-bithiazole derivatives activated by Co(II).[4] On the other hand, 2,2′-disubstituted 4,4′-bithiazole derivatives including 2,2′-diamino-4,4′-bithiazole (**1d**)[5] and a series of macrocycles containing one or more 2,2′-bithiazole rings[6] have been synthesized and their metal binding properties were investigated. Therefore, we were interested in the design and preparation of new bithiazole derivatives, especially those of 4,4′-type with aminoalkyl groups at the 2 and 2′ positions; for example, **1b** is simple and readily available and has a considerable DNA cleaving activity.[1a] Here we report that 2,2′-bis(aminomethyl)-4,4′-bithiazole (**1a**)[7] showed significant DNA cleaving activities in the presence of Cu(II) under physiological conditions. Previously, a number of Cu(II)-activated DNA cleaving agents, such as oligopeptides, phenanthroline, carbazole, and resorsinol derivatives as synthetic compounds, and desferal, podophylotoxin, and epicatechin as natural products, have been reported and their cleaving activities have been examined in the presence or absence of reducing agents.[8] The bithiazole **1a** is the first 4,4′-bithiazole derivative to show DNA cleaving activities only in the presence of Cu(II) without any reducing agents.

As shown in Chart 1, the treatment of (*tert*-butoxycarbonyl)glycinamide (**3a**) with 2,4-bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent) gave (*tert*-butoxycarbonyl)glycinethioamide (**4a**) in 63% yield. Subsequently, **1a** was prepared in 63% yield from **4a** by the condensation of 2 eq of **4a** with 1,4-dibromobutane-2,3-dione to afford the Boc-protected bithiazole (**5a**), followed by acidic deprotection of the amino groups of **5a**. The structure of **1a** was confirmed by the spectral data and elemental analysis, that is, the ^{1}H-NMR (400 MHz, D_2O) spectrum of **1a** shows the signals of methylene protons as a singlet at 4.63 ppm and thiazole ring protons at the 5-position as a singlet at 8.03 ppm, and the IR spectrum shows a characteristic absorption of ammonium groups at around 3300—2400 cm^{-1}. Furthermore, 2,2′-bis(3-aminopropyl)-4,4′-bithiazole (**1c**), a 4,4′-bithiazole structurally related to **1a**, was prepared by the same procedure in 32% overall yield from **3c** as shown in Chart 1.[1a] 2-(Aminomethyl)-4-methylthiazole (**6**)[9] as a monothiazole was obtained by the condensation of **4a** with bromoacetone, followed by deprotection of the Boc group, in 88% overall yield from **4a**. The structures of **1c** and **6** were confirmed by the spectral data and elemental analysis.

The DNA cleaving abilities of **1a** and related compounds (**1b—d** and **6**) in the presence or absence of metals were investigated using supercoiled plasmid pBR322



1a n=1
1b n=2
1c n=3
1d n=0

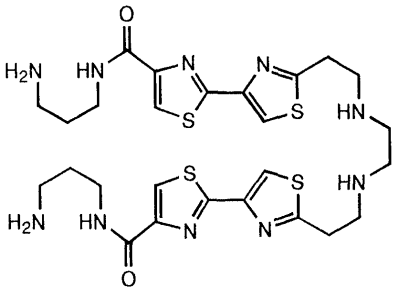


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Fig. 1. Bithiazole Derivatives as DNA Cleaving Agents (**1a, b** and **2**) and Related Compounds (**1c, d**)

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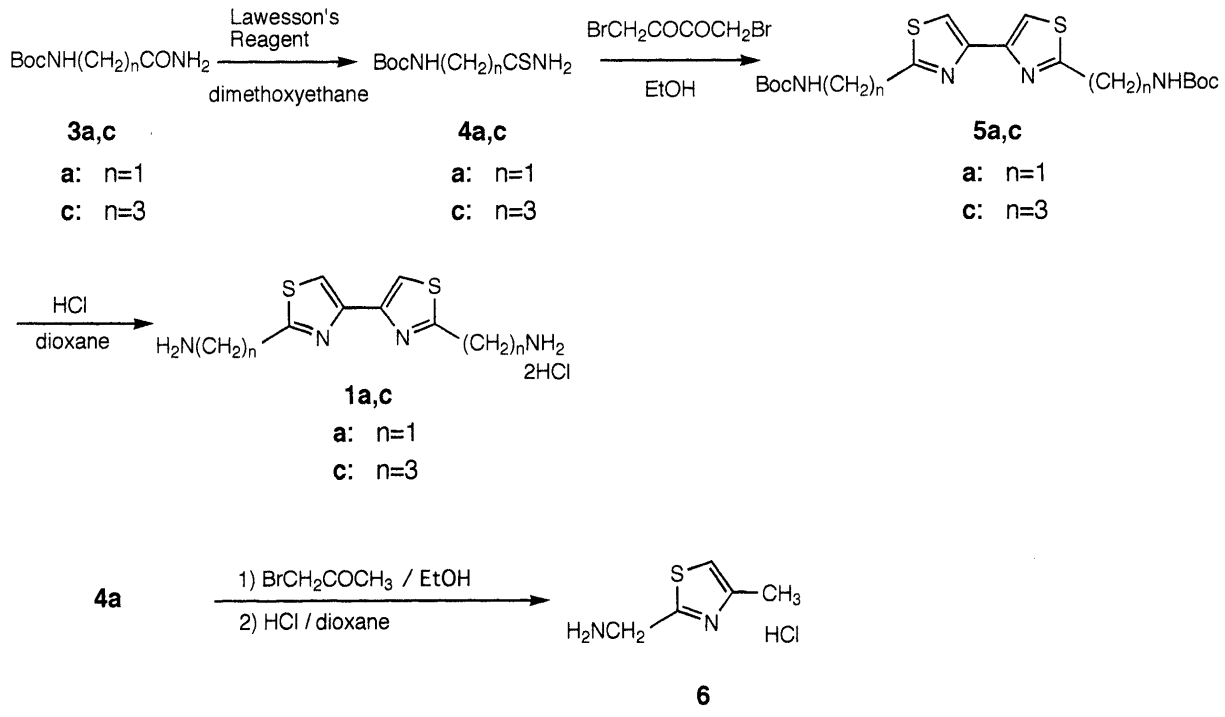

Chart 1

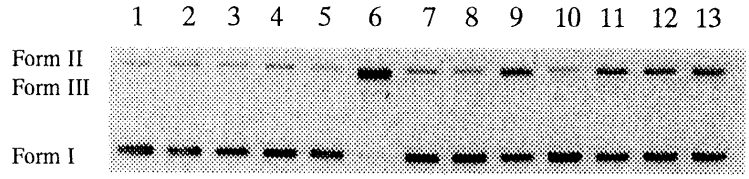

Fig. 2. DNA Cleaving Reactions of Bithiazoles (**1a—d**) and Thiazole (**6**)

The concentrations of the bithiazoles **1a—d**, the thiazole **6**, and all metals were 50, 100, and 100 μM, respectively. Lane 1, DNA control; lane 2, **1a** alone; lane 3, **1a**+Mn(II); lane 4, **1a**+Co(II); lane 5, **1a**+Ni(II); lane 6, **1a**+Cu(II); lane 7, **1a**+Zn(II); lane 8, **1a**+Cu(II)+EDTA (1 mM); lane 9, **1d**+Cu(II); lane 10, **1b**+Cu(II); lane 11, **1c**+Cu(II); lane 12, **6**+Cu(II); lane 13, Cu(II) alone.

DNA[10] in 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (40 mM, pH 7.0) at 37 °C for 3 h. As shown in Fig. 2, **1a** alone at 50 μM concentration showed no DNA cleaving activity (lane 2), as compared with the DNA control (lane 1). Interestingly, **1a** could efficiently cleave DNA only in the presence of Cu(II) (lane 6), that is, supercoiled DNA (form I) almost completely disappeared and nicked circular DNA (form II) was obtained along with a small amount of linear DNA (form III). In contrast, no DNA cleavage by **1a** was observed in the presence of other metals, such as Mn(II), Co(II), Ni(II), and Zn(II) (lanes 3, 4, 5, and 7, respectively). The addition of a large excess of EDTA (1 mM) to the reaction mixture of **1a** and DNA with Cu(II) efficiently inhibited the DNA cleavage (lane 8). In addition, at 100 μM **1a** in the presence of 100 μM Cu(II), the plasmid DNA was degraded to small pieces, but no DNA cleavage was observed at 10 μM **1a** in the presence of 100 μM Cu(II) (data is not shown). It is clear that the complex formation of **1a** specifically with Cu(II), not other metals, is necessary to the DNA cleavage. Figure 2 also shows the relationship between the structures of the 4,4′-bithiazole derivatives (**1a—d**) and the DNA cleaving activity. Interestingly, except for **1a** (lane 6), relatively little DNA cleaving activity was observed upon incubation of plasmid DNA with bithiazoles (**1b—d**) at 50 μM in the presence of Cu(II). The bithiazoles **1d** having no alkyl chain or **1b** and **1c** possessing ethylene and trimethylene chains, respectively, between the amino group and the 2 position of the thiazole ring, showed little DNA cleaving activities (lanes 9, 10, and 11), the effects being similar to that of Cu(II) alone (lane 13). The application of the thiazole **6** to the DNA cleaving reaction, even at 100 μM concentration, similarly resulted in very little DNA cleaving activity (lane 12). These facts strongly suggested that the specific structure of a 4,4′-bithiazole possessing two 2-aminomethyl groups attached at the 2 and 2′ positions of the bithiazole ring and the presence of Cu(II) are essential for the DNA cleavage. Furthermore, it was interesting that **1a** and **1b**, which differ only slightly in structure, require the co-presence of Cu(II) and Co(II), respectively, in their metal-dependent DNA cleaving reactions.

Since the DNA cleavage of **1a** requires the co-presence of Cu(II), the cleavage reaction may proceed by the oxidative degradation of DNA induced by an active oxygen species. As listed in Table 1, the following experiments were carried out in order to clarify the principal reaction species. Hydroxyl radical scavengers such as ethanol, 2-propanol, dimethyl sulfoxide, and D-mannitol, and a scavenger of singlet oxygen such as sodium azide did not inhibit the cleavage (runs 3—7). Superoxide dismutase (SOD) as an inhibitor of superoxide and catalase as a scavenger of peroxide showed no inhibition of the cleavage (runs 8 and

Table 1. Inhibition of DNA Cleaving Reaction of **1a** in the Presence of Cu(II)

Run No.	1	2	3	4	5	6	7	8	9	10	11
1a/Cu(II)	−	+	+	+	+	+	+	+	+	+	+
Inhibitor	—	—	Ethanol	2-Propanol	DMSO	D-Mannitol	NaN_3	SOD	Catalase	Dark	Anaerobic
Concentration			0.2 M	0.2 M	0.2 M	0.1 M	5 mM	20 mg/ml	20 mg/ml	condition	condition
Form I (%)	93	2	2	3	1	2	2	1	2	2	3
Form III (%)	—	5	7	5	4	4	6	3	2	5	1
Form II (%)	7	93	91	92	95	94	92	96	96	93	96

9). In the dark and under anaerobic conditions, the cleaving activities of **1a** were not reduced (runs 10 and 11) as compared with that of the control (lane 2 in Fig. 2). These results imply that photocleavage and oxidative degradation by active oxygen species are not involved in the DNA cleaving reactions of **1a**. The mechanism may therefore be a hydrolytic mechanism, though this is speculative at the present stage. Further investigations are in progress.

In conclusion, a new DNA cleaver, 2,2′-bis(aminomethyl)-4,4′-bithiazole (**1a**) could be readily prepared. The Cu(II) complex of **1a** would efficiently cleave DNA without light, molecular oxygen, or any reducing agent under physiological conditions. This DNA cleavage did not proceed *via* a photoactivated process or an oxidative pathway.

Experimental

All melting points were taken on a Yanagimoto micro melting point determination apparatus and are uncorrected. IR spectra were recorded on a Hitachi model 270-30 infrared spectrophotometer. ^{1}H-NMR spectra were taken at 400 MHz with a Bruker AM-400 spectrometer and at 60 MHz with a Hitachi R-600 spectrometer using tetramethylsilane (TMS) in $CDCl_3$ and 4,4-dimethyl-4-silapentanesulfonic acid sodium salt (DSS) in D_2O as internal references.

(*tert*-Butoxycarbonyl)glycinethioamide (4a) 2,4-Bis(4-methoxyphenyl)-1,3-dithia-2,4-diphosphetane-2,4-disulfide (Lawesson's reagent) (10.1 g, 25 mmol) was added at once to a stirred suspension of (*tert*-butoxycarbonyl)glycinamide (**3a**) (8.7 g, 50 mmol) in 1,2-dimetoxyethane (100 ml) at room temperature. The mixture was refluxed for 2 h with stirring, and the organic solvent was removed under reduced pressure. A mixture of EtOAc (250 ml) and an NaOH aqueous solution (2 mol/l, 50 ml) was poured onto the residue and the organic layer was separated, washed with an NaOH aqueous solution (2 mol/l, 30 ml) and brine (30 ml), and dried over anhydrous $MgSO_4$. The solvent was evaporated off, and the residue was purified by silica gel flash column chromatography with a 1 : 1 mixture of acetone and hexane to give 6.0 g (63%) of **4a**, mp 128—129 °C (ether). IR (KBr): 3448, 3312, 1684, 1622, 1530, 1162, 626 cm^{-1}. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.47 (9H, s, Boc-H), 4.18 (2H, d, J=6.0 Hz, $-CH_2-$), 5.41 (1H, br s, BocN<u>H</u>–). *Anal.* Calcd for $C_7H_{14}N_2O_2S$: C, 44.19; H, 7.42; N, 14.72. Found: C, 44.29; H, 7.25; N, 14.84.

2,2′-Bis[(*tert*-butoxycarbonyl)aminomethyl]-4,4′-bithiazole (5a) 1,4-Dibromobutane-2,3-dione (2.43 g, 10 mmol) was added at once to a stirred solution of (*tert*-butoxycarbonyl)glycinethioamide (**4a**) (4.1 g, 20 mmol) in dry ethanol (100 ml) at room temperature. The mixture was gently heated to 50 °C for 2 h with stirring. Excess triethylamine (4.0 g, 40 mmol) was added to the mixture, and the organic solvent was removed under reduced pressure. 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 dried over anhydrous $MgSO_4$. The solvent was evaporated off, and the residue was purified by silica gel flash column chromatography with a 1 : 1 mixture of acetone and hexane to give 2.84 g (64%) of **5a**, mp 206—208 °C (ethanol). IR (KBr): 3364, 1694, 1524 cm^{-1}. ^{1}H-NMR (400 MHz, $CDCl_3$) δ: 1.48 (18H, s, Boc-H), 4.65 (4H, d, J=6.0 Hz, $-CH_2-$), 5.31 (2H, br s, BocN<u>H</u>–), 7.66 (2H, s, thiazole 5-H). *Anal.* Calcd for $C_{18}H_{26}N_4O_4S_2$: C, 50.69; H, 6.14; N, 113.13. Found: C, 50.57; H, 6.02; N, 13.14.

2,2′-Bis(aminomethyl)-4,4′-bithiazole Dihydrochloride (1a) A solution of HCl in dioxane (7.2 mol/l, 10 ml) was added dropwise to a stirred solution of **5a** (2.13 g, 5 mmol) in dry dioxane (50 ml) at room temperature. The mixture was stirred overnight at room temperature to give a precipitate, which was collected by filtration. The off-white powder was recrystallized from 10% aqueous ethanol to give **1a** as colorless prisms (1.46 g, 98%), mp >300 °C (ethanol : H_2O=4 : 1). IR (KBr): 3300—2400 (br), 1568, 1482, 772 cm^{-1}. ^{1}H-NMR (400 MHz, D_2O) δ: 4.63 (4H, s, $-CH_2-$), 8.03 (2H, s, thiazole 5-H). *Anal.* Calcd for $C_8H_{12}Cl_2N_4S_2$: C, 32.11; H, 4.04; N, 18.72. Found: C, 32.14; H, 3.92; N, 18.67.

4-[(*tert*-Butoxycarbonyl)amino]butanethioamide (4c) Lawesson's reagent (10.1 g, 25 mmol) was added at once to a stirred suspension of 4-[(*tert*-butoxycarbonyl)amino]butanamide (**3c**) (10.1 g, 50 mmol) in 1,2-dimethoxyethane (100 ml) at room temperature. The mixture was refluxed for 2 h with stirring, and the organic solvent was removed under reduced pressure. A mixture of EtOAc (250 ml) and an NaOH aqueous solution (2 mol/l, 50 ml) was poured onto the residue and the organic layer was separated, washed with an NaOH aqueous solution (2 mol/l, 30 ml) and brine (30 ml), and dried over anhydrous $MgSO_4$. The solvent was evaporated off, and the residue was purified by silica gel flash column chromatography with a 1 : 1 mixture of acetone and hexane to give 5.32 g (48%) of **4c**, mp 94—95 °C (ether). IR (KBr): 3296, 3148, 1692, 1662, 1538, 1164, 736 cm^{-1}. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.51 (9H, s, Boc-H), 1.70—2.50 (6H, m, $-CH_2-$), 5.41 (1H, br s, BocN<u>H</u>–). *Anal.* Calcd. for $C_9H_{18}N_2O_2S$: C, 49.51; H, 8.31; N, 12.83. Found: C, 49.41; H, 8.61; N, 12.82.

2,2′-Bis[3-(*tert*-butoxycarbonyl)aminopropyl]-4,4′-bithiazole (5c) 1,4-Dibromobutane-2,3-dione (2.43 g, 10 mmol) was added at once to a stirred solution of **4c** (4.36 g, 20 mmol) in dry ethanol (100 ml) at room temperature. The mixture was gently heated to 50 °C for 2 h with stirring. Excess triethylamine (4.0 g, 40 mmol) was added to the mixture, and the organic solvent was removed under reduced pressure. 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 dried over anhydrous $MgSO_4$. The solvent was evaporated off, and the residue was purified by silica gel flash column chromatography with a 1 : 1 mixture of acetone and hexane to give 3.4 g (71%) of **5c**, mp 125—126 °C (EtOAc). IR (KBr): 3376, 2980, 1686, 1526, 1170 cm^{-1}. ^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.47 (18H, s, Boc-H), 2.06 (4H, quintet, J=7.0 Hz, $-CH_2C\underline{H}_2CH_2-$), 2.80—3.50 (8H, m, $-C\underline{H}_2CH_2C\underline{H}_2-$), 5.05 (2H, br s, BocN<u>H</u>–), 7.63 (2H, s, thiazole 5-H). *Anal.* Calcd for $C_{22}H_{34}N_4O_4S_2$: C, 54.75; H, 7.10; N, 11.61. Found: C, 54.56; H, 7.11; N, 11.63.

2,2′-Bis(3-aminopropyl)-4,4′-bithiazole Dihydrochloride (1c) A solution of HCl in dioxane (7.2 mol/l, 10 ml) was added dropwise to a stirred solution of **5c** (1.25 g, 2.5 mmol) in dry dioxane (20 ml) at room temperature. The mixture was stirred overnight at room temperature to give a precipitate, which was collected by filtration and recrystallized from 10% aqueous ethanol to give **1c** (0.84 g, 95%) as colorless prisms, mp 295—298 °C (dec.). IR (KBr): 3300—2500 (br), 1600, 1504, 1488, 794 cm^{-1}. ^{1}H-NMR (400 MHz, D_2O) δ: 2.36 (4H, quintet, J=7.5 Hz, $-CH_2C\underline{H}_2CH_2-$), 3.00—3.60 (8H, m, $-C\underline{H}_2CH_2C\underline{H}_2-$), 8.07 (2H, s, thiazole 5-H). *Anal.* Calcd for $C_{12}H_{20}Cl_2N_4S_2\cdot H_2O$: C, 38.60; H, 5.94; N, 15.01. Found: C, 38.45; H, 5.96; N, 14.99.

2-Aminomethyl-4-methylthiazole Dihydrochloride (6) Bromoacetone (0.82 g, 6 mmol) was added at once to a stirred solution of **4a** (1.31 g, 6 mmol) in dry ethanol (30 ml) at room temperature. The mixture was stirred for 2 h at room temperature. The organic solvent was removed under reduced pressure, and a mixture of EtOAc (100 ml) and H_2O (20 ml) was poured onto the residue. The organic layer was separated, washed with brine (20 ml), and dried over anhydrous $MgSO_4$. The solvent was evaporated off, and the residue was purified by silica gel flash column chromatography with a 2 : 3 mixture of EtOAc and hexane to give 1.4 g (91%) of 2-(*tert*-butoxycarbonyl)aminomethyl-4-methylthiazole, mp 59—60 °C (hexane). IR (KBr): 3380, 3084, 2988, 1688, 1520, 1168 cm^{-1}.

^{1}H-NMR (60 MHz, $CDCl_3$) δ: 1.49 (9H, s, Boc-H), 2.45 (3H, s, $-CH_3$), 4.59 (2H, d, $J=6$ Hz, $-CH_2-$), 5.20 (1H, br s, BocNH–), 6.82 (1H, s, thiazole 5-H). *Anal.* Calcd. for $C_{10}H_{16}N_2O_2S$: C, 52.61; H, 7.06; N, 12.27. Found: C, 52.53; H, 7.02; N, 12.26.

The obtained thiazole (0.45 g, 2 mmol) was dissolved in dry dioxane (10 ml) and a solution of HCl in dioxane (7.2 mol/l, 5 ml) was added dropwise at room temperature. The mixture was stirred overnight at room temperature to give a precipitate, which was collected by filtration. The off-white powder was recrystallized from 10% aqueous ethanol to give **6** (0.39 g, 97%) as colorless prisms, mp 180—182 °C. IR (KBr): 3300—2300 (br), 1600, 1454 cm^{-1}. ^{1}H-NMR (400 MHz, D_2O) δ: 2.55 (3H, s, $-CH_3$), 4.09 (2H, s, $-CH_2-$), 7.52 (1H, s, thiazole 5-H). *Anal.* Calcd. for $C_5H_8N_2S\cdot 2HCl$: C, 29.86; H, 5.01; N, 13.93. Found: C, 29.90; H, 4.89; N, 13.92.

DNA Cleaving Reactions of 4,4′-Bithiazoles (1a—d) and Thiazoles (6) Plasmid pBR322 DNA (a supercoiled DNA) was purchased from Nippon Gene Co., Ltd. or Cosmo Bio Co., Ltd. Each reaction solution contained 0.1 mg of supercoiled plasmid pBR322 DNA in 40 mM MOPS (pH 7.0) buffer. All cleavage reactions were run for 3 h 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 buffer (pH 8.1) containing 2 mM EDTA. The gel patterns were developed by soaking the gels in ethidium bromide buffer solution (1 mg/1 ml) and photographed with an instant camera.

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10) Commercially available pBR322 plasmid DNA contains supercoiled DNA (form I) together with a small amount of nicked circular DNA (form II) as an impurity.