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SYNTHESIS OF A NOVEL BIS(2,4'-BITHIAZOLE) DERIVATIVE AS A Co(II)-ACTIVATED DNA CLEAVING AGENT

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A novel DNA cleaving agent, *N,N'*-bis{2-[4-(3-aminopropylcarbamoyl)-2,4'-bithiazol-2'-yl]ethyl}ethylenediamine (**1**), was synthesized. At 25μM concentration, a significant cleaving activity of **1** for plasmid DNA was observed only in the presence of Co(II). Scavengers of active oxygen species could not inhibit the DNA cleavage by **1**.

KEYWORDS DNA; DNA cleavage; bithiazole; thiazole; Co(II) complex; active oxygen

In the site-specific strand scission of DNA by bleomycin, 2,4'-bithiazole moiety with a positive charge center has played a key role in interacting with the DNA double strand.[1] A number of 2,4'-bithiazole derivatives structurally related to C-terminus of BLM were prepared and investigated for interaction with DNA.[2] Hecht and co-workers have reported the photoactivated DNA cleavage by chlorinated 2,4'-bithiazole derivatives at 20nM concentration.[3] Recently, it was reported that 4,4'-bithiazoles with aminoalkyl moieties at 2 and 2' positions of thiazole rings were synthesized and their significant DNA cleaving activities were observed under the physiological conditions.[4] Therefore, of particular interest is the design and synthesis of novel bithiazole derivatives with functional groups such as an amino or an amide group in order to interact with DNA strand. This communication describes synthesis of a novel DNA cleaving agent, *N,N'*-bis{2-[4-(3-aminopropylcarbamoyl)-2,4'-bithiazol-2'-yl]ethyl}ethylenediamine (**1**), having two readily available 2,4'-bithiazole moieties linked with ethylenediamine group and demonstrates Co(II)-activated DNA cleaving activity of **1** under the physiological conditions.

The bis(2,4'-bithiazole) **1** was prepared as shown in Chart 1. The condensation of ethylenediamine-*N,N'*-di(*tert*-butoxycarbonyl)-*N,N'*-di(propionethioamide) (**2**)[5] with two equivalents of ethyl bromopyruvate, following treatment with concentrated ammonium hydroxide, afforded *N,N'*- di(*tert*-butoxycarbonyl)-*N,N'*-bis[2-(4-carbamonylthiazol-2-yl)-

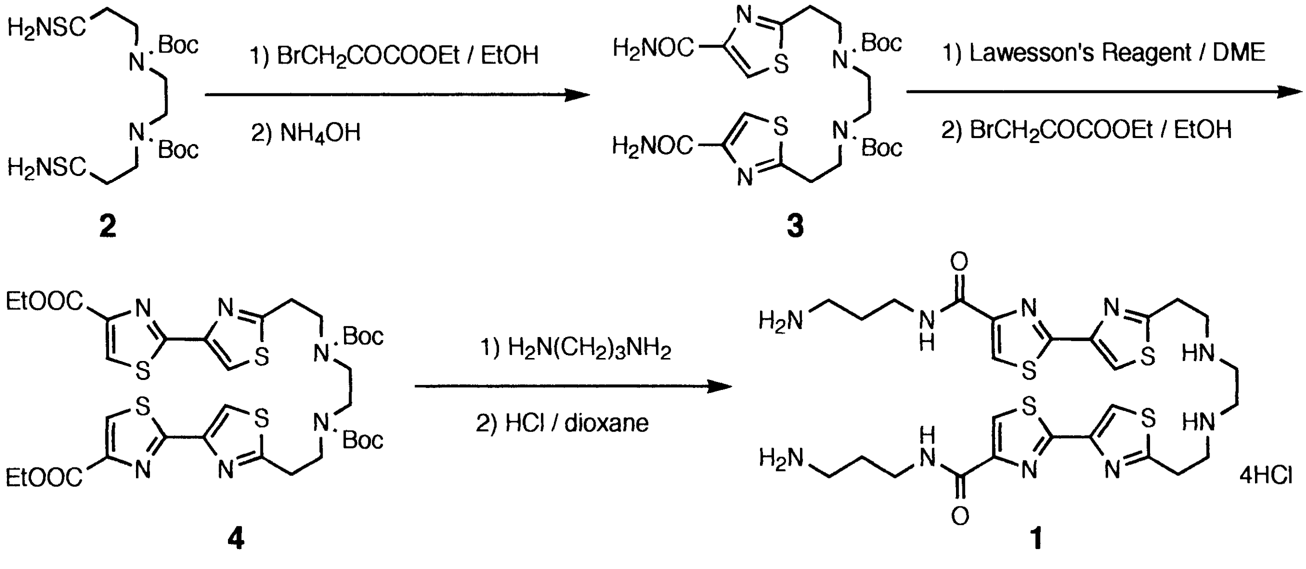
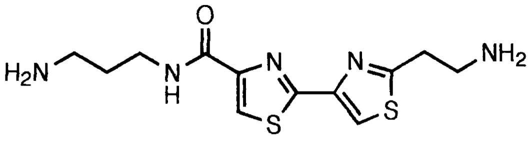


Chart 1

ethyl]ethylenediamine (**3**) in 87% yield. *N,N'*-Di(*tert*-butoxycarbonyl)-*N,N'*-bis[2-(4-ethoxycarbonyl-2,4'-bithiazol-2'-yl)ethyl]ethylenediamine (**4**) was obtained by the thiation of **3** with Lawesson's reagent, followed by the condensation with two equivalents of ethyl bromopyruvate in 55% yield. Subsequently, **4** was treated with 1,3-diaminopropane, followed by the acidic deprotection of amino groups to give **1** in 32% total yield from **2**. The structure of **1** was confirmed by the spectral data and analysis.[6]

The DNA cleaving ability of **1** in the presence or absence of a metal was investigated using supercoiled plasmid pBR322 DNA[7] by the incubation in Tris-HCl buffer (40mM, pH8.0) at 37°C for 1.5h. As shown in Figure 1, **1** or Co(II) alone shows no DNA cleavage (lanes 2 and 3) as compared with DNA control (lane 1). However, only in the presence of Co(II) could **1** efficiently cleave DNA at 25μM concentration (lane 4);[8] that is, supercoiled DNA (form I) completely disappeared and nicked circular DNA (form II) was produced along with a small amont of linear DNA (form III). In contrast, no DNA cleavage by **1** was observed in the presence of other metals, such as Ni(II), Cu(II), and Zn(II) (lanes 5, 6, and 7, respectively). The addition of excess EDTA (1mM) efficiently inhibited the DNA cleavage by **1** in the presence of Co(II) (lane 8). It is clear that the complex formation of **1** with Co(II) is necessary to the present DNA cleavage. Furthermore, in order to investigate the relationship between the structure of 2,4'- bithiazole derivatives and the cleaving activity, *N*-(3-aminopropyl)-2'-(2-aminoethyl)-2,4'-bithiazole-4-carboxamide (**5**)[2a] as a 2,4'-bithiazole and*N*-(3-aminopropyl)-2-(2-aminoethyl)thiazole-4-carboxamide (**6**)[9] as a thiazole were examined for the same DNA scission reaction. In the presence of Co(II), the bithiazole **5** slightly cleaved DNA and the thiazole **6** showed no DNA cleavage at 50μM and 100μM concentrations, respectively, as shown in Figure 1 (lanes 9 and 10). These facts strongly indicate that the specific structure of bis(2,4'-bithiazole) linked with ethylenediamine group, with 3-aminopropylcarbamoyl groups at 4 position of each bithiazole ring and the presence of Co(II) are essential for the DNA cleavage.



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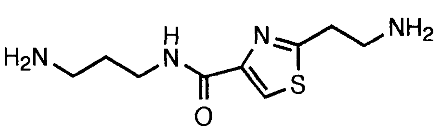

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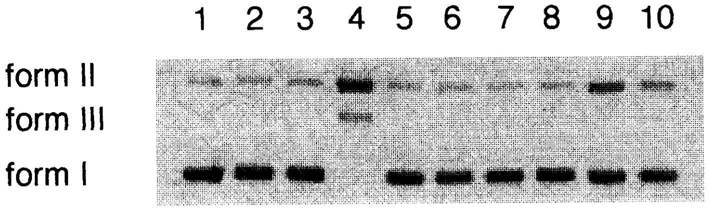

Fig. 1. Cleavage of Supercoiled Plasmid pBR322 DNA(form I) by **1**
Reaction solution contained 0.1μg of supercoiled plasmid pBR322 DNA in 40mM Tris-HCl (pH 8.0) buffer. All cleavage reactions were run at 37°C for 1.5h , and the electrophoresis was carried out at 50V (1.8h) on a 1.2% agarose gel. The gel patterns were developed by soaking the gels in ethidium bromide buffer solution (1mg/1ml). The concentrations of **1** and all metal(II) chlorides are 25μM and 100μM, respectively.[8] lane 1, DNA control; lane 2, **1** alone; lane 3, Co(II) alone; lane 4, **1**+Co(II); lane 5, **1**+Ni(II); lane 6, **1**+Cu(II); lane 7, **1**+Zn(II); lane 8, **1**+Co(II)+ EDTA (1 mM), lane 9, **5**(50μM)+Co(II); lane 10, **6**(100μM)+Co(II).

Since the presence of Co(II) is required for this DNA scission by **1**, the cleavage reaction may be considered to proceed by the oxidative degradation of DNA induced by active oxygen species. However, hydroxyl radical scavengers such as ethanol, 2-propanol, dimethyl sulfoxide, and *D*-mannitol did not inhibit the cleavage of **1**. A scavenger of singlet oxygen, 1,4-diazabicyclo[2.2.2]octane (DABCO), also induced no inhibition of the cleavage. Furthermore, in the additions of large excess of superoxide dismutase (SOD) or catalase to the mixture of **1** and DNA in the presence of Co(II), a similar DNA cleavage to that in a control reaction or a little inhibition were, respectively, observed. These results indicate that this DNA cleavage is little produced by **1** in the presence of Co(II) by active oxygen species. On the other hand, it was considered that active oxygen species could not be induced by redox reaction involving the reduction of Co(II) to Co(I) species because this DNA cleavage reaction did not require any reducing agents. Under dark conditions, the cleavage reaction was slightly inhibited, compared with the result of a control reaction exposed to daylight. It was suggested that the cleavage reaction must be operated at least in part through photocleavage reaction. Further investigations on the mechanism of the present DNA cleavage by **1** including hydrolytic mechanism and the structure activity relationship in the family of bis(2,4'-bithiazole) are in progress in my laboratory.

REFERENCES AND NOTES

1) H. Kasai, H. Naganawa, T. Takita, H. Umezawa, *J. Antibiot.*, **31,** 1316 (1978).

2) a) J.-P. Hénichart, J.-L. Bernier, N. Helbecque, R. Houssin, *Nucleic Acids Res.*, **13**, 6703 (1985); b) L. M. Fisher, R. Kuroda, T. T. Sakai, *Biochemistry*, **24**, 3199 (1985); c) J. Kross, D. Henner, W. A. Haseltine, L. Rodriguez, M. D. Levin, S. M. Hecht, *Biochemistry*, **21**, 3711 (1982); d) S. Y. Lin, A. P. Grollman, *Biochemistry*, **20**, 7589 (1981); e) T. T. Sakai, J. M. Riordan, T. E. Booth, J. D. Glickson, *J. Med. Chem.*, **24**, 279 (1981).

3) J. C. Quada Jr., M. J. Levy, S. M. Hecht, *J. Am. Chem. Soc.*,**115,** 12171(1993).

4) H. Sasaki, *Tetrahedron Lett.*, **35**, in press; H. Sasaki, *Chem. Pharm. Bull.*, in preparation.

5) As a precursor of **1**, compound **2** was prepared from ethylenediamine by four steps, that is, cyanoethylation, Boc protection of amino group, hydrolysis of cyano groups to amides, and the thiation of amido carbonyl groups by Lawesson's reagent in 45% total yield from ethylenediamine.

6) Hygroscopic needles from MeOH:H_2O(3:1). mp 249-250°C. ^{1}H-NMR (400MHz, D_2O, DSS) δ: 2.10(4H, m, $-CH_2C\underline{H}_2CH_2-$), 3.18(4H, t, J=7.7Hz, $H_2NC\underline{H}_2CH_2CH_2-$), 3.57(4H, t, J=6.8Hz, $-CH_2C\underline{H}_2NHCO-$), 3.63(4H, t, J=6.3Hz, $-HNCH_2C\underline{H}_2$-thiazole), 3.75(4H, s, $-HNC\underline{H}_2C\underline{H}_2NH-$), 3.78(4H, t, J=6.3Hz, $-HNC\underline{H}_2CH_2$-thiazole), 8.12(2H, s, thiazole 5'-H), 8.15(2H, s, thiazole 5-H). IR(KBr); 2900-3600(broad), 1650 cm^{-1}. *Anal.* Calcd for $C_{10}H_{14}N_4S_2\cdot 4HCl\cdot H_2O$: C, 38.42; H, 5.21; N, 17.37. Found: C, 38.58; H, 5.56; N, 17.12.

7) Commercially available pBR322 plasmid DNA contains a small amount of nicked circular DNA (form II) as an impurity.

8) When the effect of **1** and $CoCl_2$ concentrations for the cleavage was tested, significant cleavages were observed at equimolar concentrations of 50μM or above and at 25μM of **1** with over 50μM of $CoCl_2$. The cleavage efficiency is dependent on both concentrations, the optimal concentrations being 25μM and 100μM for **1** and $CoCl_2$, respectively, under the present conditions.

9) Thiazole **6** was prepared by the reaction of ethyl 2-[2-(*tert*-butoxycarbonyl)aminoethyl]thiazole-4-carboxylate with 1,3-diaminopropane, followed by deprotection of amino group in 85% yield.

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