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Chiral Bis(1,10-phenanthroline) with Tröger's Base Skeleton. Synthesis and Interaction with DNA

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Chiral bis(1,10-phenanthroline) with Tröger's base skeleton (**1**) was synthesized from 5-amino-1,10-phenanthroline with formaldehyde and HCl. Racemic **1** could interact with DNA from CD spectroscopy and could cleave DNA when it was complexed with copper(I).

Since the discovery of left-handed DNA, much attention has been focused on the chiral nature of DNA. DNA is an optically active naturally occurring macromolecule like protein and polysaccharide. However, there have been only a few studies concerning the chiral recognition with DNA.[1] Wilson et al. prepared racemic intercalators containing anthracene or anthraquinone, but the enantiomeric recognition of them in binding with DNA could not be directly observed.[2] Recently, Barton and coworkers have successfully demonstrated that the enantiomers of tris(1,10-phenanthroline) complexes of transition metals could discriminate in binding between right- and left-handed DNA.[3] This may be the first excellent example of the chiral recognition of racemic compounds by DNA.

Here, we report the synthesis of new chiral bis(1,10-phenanthroline) (**1**) with Tröger's base skeleton in order to examine the possibility of the chiral recognition with DNA. The chemistry of Tröger's base analogues has been extensively studied by Wilcox et al.[4] and dibenzodiazocine unit has been incorporated into macrocyclic and nonmacrocyclic synthetic receptors.[4,5] However, most of the analogues were derived from substituted aniline derivatives. The analogues derived from (hetero)-cyclic aromatic amines were scarcely known to our knowledge.

Compound **1** possesses a few outstanding characteristics; 1) **1** is a relatively rigid chiral molecule in which two phenanthroline rings would be held in a position well defined, since the structures of Tröger's base and its para-substituted analogues are determined by X-ray analyses and

the dihedral angles between the planes of two aromatic rings are estimated to range from 89° to 104°[6]; 2) compound **1** will form polychelation complexes with transition metals such as copper, ruthenium, and cobalt; 3) especially, a complex of copper(I) will be expected to cleave DNA, because bis(1,10-phenanthroline)copper(I) discovered by Sigman et al.[7] is well known as the first artificial chemical nuclease.[8]

Compound **1** was easily prepared by the same procedures of Wagner[9] and Wilcox[4] from 5-aminophenanthroline, which was prepared by reducing 5-nitrophenanthroline in ethanol with stannous chloride and HCl.[10] 5-Aminophenanthroline was treated with formaldehyde and HCl in ethanol at 60 °C for 48 h to provide racemic Tröger's base analogue **1** in 22% yield.[11] Figure 1 shows the ^{1}H NMR spectrum of **1** in CD_2Cl_2. All signals were fully assigned by ^{1}H-^{1}H-COSY, -NOESY, and differential NOE experiments. The assignments of the endo and exo protons of cyclic diazocine unit of **1** were carried out by irradiation of the bridging methylene (Hc). Only the intensity of the lower field signal was enhanced, and therefore, the lower field doublet was assigned to the exo and the higher field doublet endo. This assignment agreed well with that done by Wilcox et al. for Tröger's base analogue.[12]

Since compound **1** absorbs in the same UV region as DNA (λ_{max} 280 nm (ε 3.81 x 10^4) in phosphate buffer, pH 7.8), circular dichroism rather than UV absorption spectroscopy was used to detect the interaction with DNA. CD spectrum was dramatically changed when **1** was titrated into a solution of DNA and a new strong positive peak appeared at around 280 nm (Fig. 2)

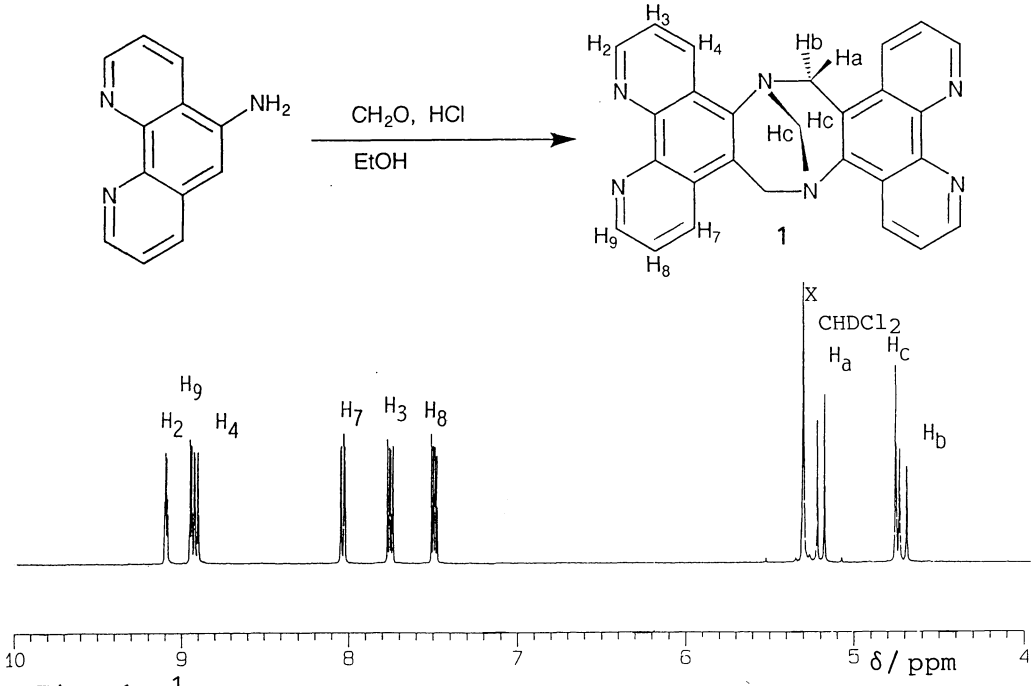

Fig. 1. ^{1}H NMR spectrum of 1 in CD_2Cl_2 at 27°C (400 MHz).

Interestingly, almost no change was observed in the circular dichroism upon addition of 1,10-phenanthroline to the DNA solution as previously reported.[13] These results indicate that phenanthroline alone would not interact with DNA, but the two phenanthrolines of **1** will be favorably located for interacting with DNA so that **1** could bind to DNA. In this study, we used racemic **1** because of difficulty of optical resolution.[14] Further studies will be done by using optically pure isomers towards the chiral recognition by DNA.

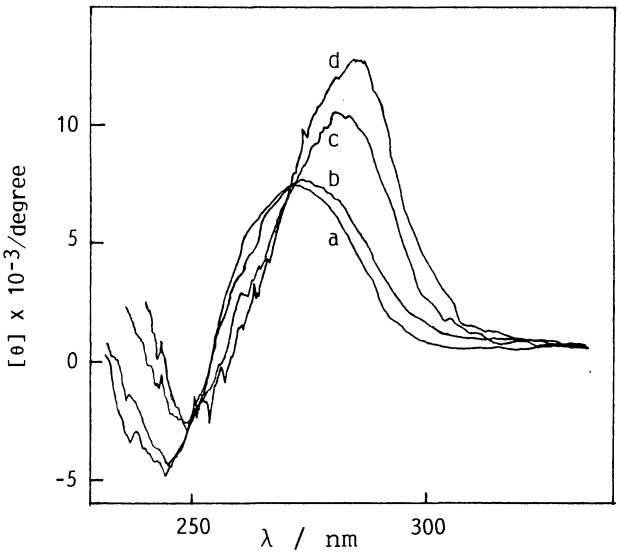

Fig. 2. CD spectra of calf thymus DNA and compound **1** in 1 x 10^{-2} mol dm^{-3} Tris, 1 x 10^{-3} mol dm^{-3} EDTA with 5 x 10^{-2} mol dm^{-3} NaCl (pH 7.8) at 25 °C. Molar ratios of **1** to DNA are 0 (a), 0.10 (b), 0.26 (c), and 0.52 (d), respectively.

The chemical nuclease activity of **1** and copper (I) complex was preliminarily investigated according to Sigman's method.[7),15] **1**-Cu(I) caused strand scission as determined by the almost complete conversion of the covalently closed circular pUC18 plasmid to open circular DNA. The detailed mechanism of the cleavage and the binding properties of **1**-Cu(I) to DNA will be performed in the near future.

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11) Compound **1**: mp > 250 °C; ^{1}H NMR(400 MHz, $CDCl_3$); 4.72 (2H, d, J=16.9 Hz, H_b), 4.76 (2H, s, H_c), 5,22 (2H, d, J=16.9 Hz, H_a), 7.08 (2H, dd, J=4.4, 8.4 Hz, H_8), 7.53 (2H, dd, J=4.4, 8.4 Hz, H_3), 8.05 (2H, dd, J=1.8, 8.4, H_7), 8.90 (2H, dd, J=1.8, 8.4 Hz, H_4), 9.05 (2H, dd, J=1.8, 4.4 Hz, H_9), 9.20 (2H, dd, J=1.8, 4.4 Hz, H_2); ^{13}C NMR (100 MHz, $CDCl_3$); 53.1, 67.4, 119.8, 123.1, 123.2, 125.7, 126.8, 129.4, 131.2, 141.1, 144.5, 146.5, 149.2, 150.1. Anal. Found: C, 74.60; H, 4.39; N, 19.20%. Calcd for $C_{27}H_{18}N_6 \cdot 1/2H_2O$: C, 74.46; H, 4.40; N, 19.30%.
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15) 10 μg/ml of pUC18 DNA (20 μl) was incubated with the addition of 10 μM (1 M = 1 mol dm^{-3}) **1** and/or 1 μM Cu^{2+} and/or 7 mM 2-mercaptopropionic acid and each reaction mixture was applied to a agarose gel (0.7%) by electrophoresis using ethidium bromide stain. The cleavage of the DNA took place only when all the components are present.

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