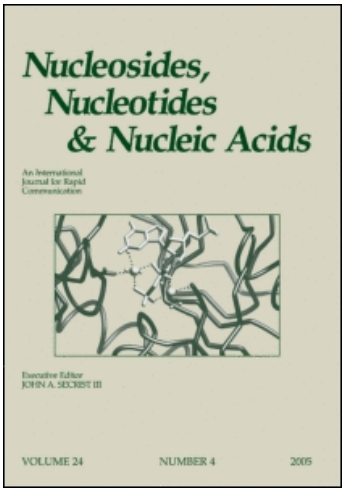

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NOVEL CLASS OF DNA BINDING MOTIFS BASED ON BISTETRAHYDROFURAN AND BISFURAN SKELETON WITH LONG ALKYL CHAINS

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ABSTRACT

Small molecules with DNA-binding affinity within the minor groove have become of great interest. In this paper, new DNA binding molecules; diamino-bistetrahydrofuran (bisTHF) and diamino-bisfuran are reported. The bisTHF ligand with RR configuration at the amino groups and C8 alkyl chains (RR8) stabilized GC-rich duplex. In contrast, bisfuran compounds stabilized AT-rich duplex. The binding affinity of RR8 with 12 mer duplex DNA was determined by isothermal titration calorimetry to be 3.3×10^8 M^{-1}.

DNA-binding molecules with sequence specificity have become of great interest, because of the potential application for the regulation of gene expression at the specific site. Among DNA-binding molecules including triplex-forming oligonucleotides or peptides (TFO or PNA) (1), low molecular weight ligands that bind to DNA within the minor groove have shown rapid development recently (2). Encouraging work has been reported by Dervan's group, in which recognition of full four-codes of DNA sequences within the minor groove has been demonstrated with the use of synthetic pyrrole-imizazole polyamides (3). In this case, stacked- or covalent dimer structures of binding motifs play a significant role for the formation

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specific hydrogen bondings with each of the bases at the bottom of the minor groove. On the other hand, other types of minor groove ligands such as Hoechst 33258 and CC-1065 bind to DNA as a monomer with sequence preference. In the binding of Hoechst 33258 to DNA, hydrophobic interaction has been determined as a major contributor of sequence selectivity (4). In the conceptual mechanism, the first step of minor-groove binding may contain a hydrophobic transfer of the ligand from aqueous solution to the DNA minor groove following arrangement of specific interactions such as hydrogen bondings and van der Waals attraction (5). The shape of the ligand isohelical with the curvature of the groove has been also regarded as an important factor of the binding (6). We have been interested in the development of new ligands based on an aliphatic non-planar structure, because such binders would provide useful information for the design of new ligands. Here we wish to report new high-affinity DNA binding molecules with an emphasis on hydrophobic interactions.

We designed a series of new DNA binders based on a bistetrahydofuran (bisTHF) skeleton bearing cationic groups and long alkyl chains. The idea was originated from the facts that a helix-like conformation was suggested in studies of naturally-occurring bisTHF compounds (7) or in oligoTHF compounds (8). A molecular modeling of the bisTHF molecule also suggested that the dihedral angle between the two tetrahydrofuran rings might be adjustable to fit into the minor groove, and the molecular shape might be isohelical to the groove (Fig. 1).

We first synthesized diamino-bisTHF (**1**(RR8)) with (*R*,*R*)-configuration of the amino groups (9). Interestingly, **1**(RR8) raised the melting temperature of a GC-rich duplex (G16-C16) by 13 degrees but showed no effect on an AT-rich duplex (A16-T16). Since 1(RR8) has neither aromatic systems nor planar structure such as seen in most minor-groove binders, it seemed to be worthy for further investigation. Then, we systematically synthesized several bisTHF compounds (**1**–**4**) having different stereochemistry, length and functionality of the alkyl chains. The bisfuran compounds (**5**) were also synthesized for the comparison with bisTHF ligands (Fig. 2).

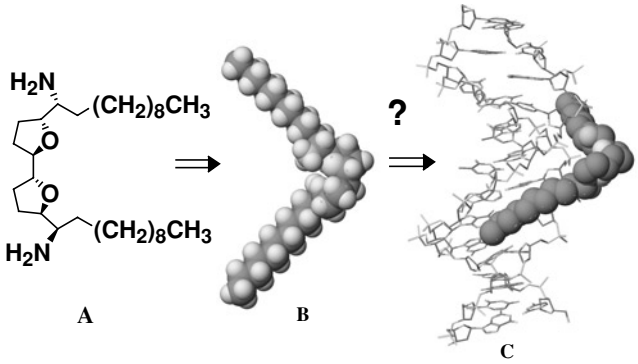

Figure 1.

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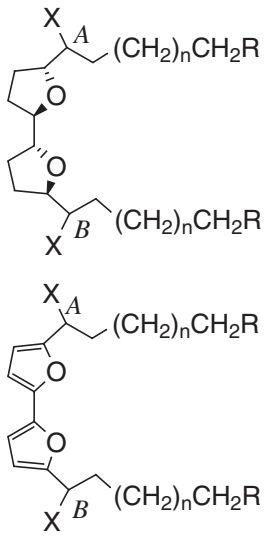

	(*A*, *B*)	n	X	R
1(RR8)	(*R*, *R*)	8	NH_2	H
1(RR7)	(*R*, *R*)	7	NH_2	H
1(RR6)	(*R*, *R*)	6	NH_2	H
1(RR5)	(*R*, *R*)	5	NH_2	H
1(RR4)	(*R*, *R*)	4	NH_2	H
1(RR0)	(*R*, *R*)	0	NH_2	H
1(RS8)	(*R*, *S*)	8	NH_2	H
1(SS8)	(*S*, *S*)	8	NH_2	H
2	(*R*, *R*)	8	OH	NH_2
3	(*R*, *R*)	8	NH_2	OTHP
4	(*R*, *R*)	8	NH_2	OH

5(meso)	meso	8	NH_2	H
5(racemi)	racemi	8	NH_2	H

Figure 2.

The diamino-bisTHFs (**1**–**4**) were synthesized from respective diepoxy intermediates (**6**) which were derived from diethyl isopropylidene D-tartrate (Scheme 1) (10). Each epoxydes (**6**) was alkylated using Grignard reagents with catalytic amount of CuBr to afford the secondary alcohols (**7**–**8**). Mitsunobu reaction of these alcohols with HN_3 gave diazido compounds involving the stereochemical inversion at both hydroxyl groups. Finally, diamino-bisTHFs (**1**, **3**, **4**) were obtained by reduction of the azido group with lithium aluminum hydride (**LAH**). The ligand (**2**) was synthesized in a similar manner. For the synthesis of diamino-bisfurans, 5-bromo-2-furoic acid amyl ester was first coupled with Cu powder in DMF under reflux to afford bisfuran building block **9**. Diester **9** was transformed into a hydroxyalkyl intermediate **10**. Hydroxyl groups of **10** were displaced by phthalimide by Mitsunobu reaction to afford corresponding phthalimide derivatives as mixture of stereoisomers. The isomers of dl- and meso form were separated by fractional recrystallization, and the configuration of theless soluble derivative was determined by X-ray crystallographic analysis to be meso. Finally, bothof meso and racemic compounds were refluxed with hydrazine to furnish **5**. The diamino-bidTHFs and diamino-bisfurans were used as hydrochlorides in the following binding experiments.

Interaction between the duplex DNA and the ligand was first evaluated by measuring effect on the melting temperature (Tm) of the 16bp duplex (G16-C16 and A16-T16) (Table 1). Tm value of G16-C16 duplex was raised with **1**(RR8) by 13 degrees, whereas that of A16-T16 duplex was not affected, indicating that **1**(RR8) stabilized G16-C16 selectively. Stereochemistry of the amino groups influenced the stabilizing effect; order of the Tm values of G16-C16 duplex decreased in the order of **1**(RR8) > **1**(RS8) > **1**(SS8), suggesting that the stereochemistry

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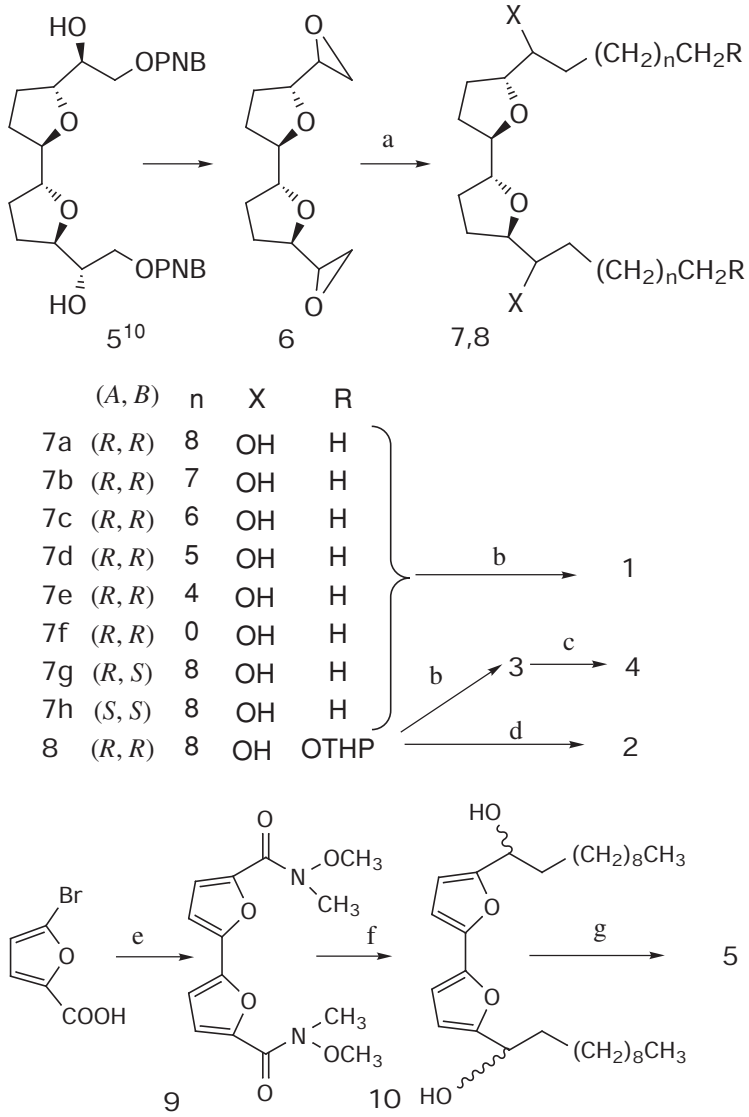

[a] a) $RCH_2(CH_2)_nMgBr$, CuBr, THF, b) (1) HN_3, Ph_3P, DEAD, THF, (2) $LiAlH_4$, THF, c) 1M HCl, THF.d) (1) TsOH, H_2O/EtOH/THF, (2) phthalimide, Ph_3P, DEAD, THF, (3) hydrazine, EtOH, e) 1) p-TsOH•EH_2O, PeOH, 2) Cu powder, DMF, 3) 1N $NaOH_{aq}$, MeOH, 4) $SOCl_2$,Pyridine, CH_2Cl_2, 5) *N,O*-Dimethylhydroxylamine, pyridine, CH_2Cl_2, f)1) $CH_3(CH_2)_9MgBr$, CH_2Cl_2, 2) $NaBH_4$, CH_2Cl_2-EtOH, g) 1) phthalimide, Ph_3P, DEAD, THF, (2) hydrazine, EtOH.

Scheme 1.

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Table 1. Melting Temperature (T_m) Changes by Diamino-bisTHFs and Diamino-bisfurans

Compound	DNA sequence[a)]	ΔT_m(°C)
1(RR8)[b]	G16-C16	13
	A16-T16	0
1(RS8)[b]	G16-C16	7
	A16-T16	0
1(SS8)[b]	G16-C16	0
	A16-T16	0
5(meso)[c]	G16-C16	0
	A16-T16	4
5(racemi)[c]	G16-C16	0
	A16-T16	9

a) 1μM DNA in the buffer containing 100 mM KCL, 5 mM Na_2HPO_4 at pH 5.0 for G16-C16 or at pH 7.0 for A16-T16, b) 20 μM **1** was used, c) 50 μM **5** was used.

```
G16-C16      5' GGGGGAGGGGGCGGGA 3'
(Tm=62°C)    3' CCCCCTCCCCCGCCCT 5'

A16-T16      5' AAAGAAAAAGAAAAAC 3'
(Tm=40°C)    3' TTTCTTTTTCTTTTTG 5'
```

of the amino group has a key role in DNA stabilization. In contrast, diamino-bisfuran **5**(meso) and **5** (racemic) raised Tm of A16-T16 duplex selectively by 4 and 9 degrees, respectively. The stereochemistry of the amino groups of bisfuran also affected DNA stabilization. It should be noted that bisTHF and bisfuran skeletons apparently play a key role in sequence preference of the ligands.

We next evaluated binding affinity of the ligands by a competitive binding assay using fluorescent ethidium bromide (ETBr). Emission intensity of ETBr is increased by binding with duplex DNA, and is quenched in the presence of a competitive binder. Concentration of the ligand to inhibit 50% of fluorescence intensity of ETBr (C_{50}) corresponds to a relative binding affinity of the ligand (Table 2).

In this experiment, self-complementary 12bp duplexes CT12 and CA12 were used, in which a GCGC or an AATT regions are included in the middle of CT12 or CA12, respectively. High affinity of **1**(RR8) to both duplexes was confirmed with affinity as high as distamycin. Importance of the stereochemistry of **1** has been again observed in this experiment, that is, the affinity decreased in the order of **1**(RR8) > **1**(RS8) > **1**(SS8). The sequence preference of the bisTHF ligands was not clear. The high affinity of **5**(meso) as well as sequence preference to CA12 was shown in this assay. Either the ligand **2** or the ligand **4** did not show significant affinity. Protection of the hydroxyl group of **4** with tetrahydropyranyl group (**3**) recovered binding affinity, indicating that hydrophobicity of the alkyl chain terminal

Table 2. Comparison of the DNA Binding Affinity[a)]

	C_{50} (μM)	
Compound	CT12 ($K_{ETBr=2.4\times10^6}$)	CA12 ($K_{ETBr=7.6\times10^6}$)
1(RR8)	7	5.5
1(RS8)	19	13
1(SS8)	30	30
1(RR7)	34	23
1(RR6)	135	125
1(RR5)	760	880
2	300	330
3	32	27
4	490	540
5(meso)	17	4
1(racemi)	>100	>100
distamycin	19	>15

a) 1.5 μM DNA and 1.5 μM ETBr were used in the buffer containing 9.4 mM NaCl, 2.0 mM HEPES, 10 mM EDTA, pH 7.0.

```
CT12: 5' CGTAGCGCTACG 3'  CA12: 5' CGCGAATTCGCG 3'
         GCATCGCGATGC                GCGCTTAAGCGC
```

is also important for binding. These results have suggested that the hydrophobic nature of the termini of alkyl chains is also important for binding affinity. It is quite interesting that the linear correlation was between C_{50} values and the length of alkyl chains of bisTHF ligand **1**. These results clearly suggest that hydrophobic interaction is the major contributor in the binding of bisTHF ligands.

A similar displacement assay was done with Hoechst33258, a strong minor-groove binder with high affinity of about 3×10^8 to CA12 (4). In this assay, 5(meso) displaced Hoechst33258 almost completely with C_{50} value of 23 μM, indicating that the bisfuran ligand shares the binding site of CA12 with Hoechst33258, probably at the AATT region, with relatively low affinity. Interestingly, addition of the bisTHF ligand **1**(RR8) to the complex between Hoechst33258 and CA12 changed the fluorescence spectra, indicating second binding between **1**(RR8) and Hoechst33258• CA12 complex. As **1**(RR8) has been suggested to be GC-selective, **1**(RR8) might bind the terminal GCGC region of CA12. A similar displacement assay with CT12 and Hoechst33258 could not be done because of weak complexation between these two. The binding affinity of **1**(RR8) with CT12 was determined by isothermal titration calorimetric (ITC) experiments to be $K_a = 3.36 \times 10^8\ M^{-1}$.

In conclusion, we have developed new DNA-binding molecules with unusual binding motifs, diamino-bisTHF and diaminobisfuran. These molecules were expected to take a stable conformation with a crescent shape that seemed to be suitable to fit to the minor groove. As a result, the binding affinity of **1**(RR8) has been determined to be as high as a binding constant of $10^8\ M^{-1}$. It has been also clearly shown

that both the stereochemistry of the amino groups and the alkyl chain length play major roles for the DNA binding. The importance of the hydrophobic interaction in DNA binding has been now clearly confirmed in this study. In the measurement of melting temperature, diaminobisTHF **1**(RR8) showed sequence preference to GC-rich duplex, and diaminobisfuran **5** indicated preference to the AT-rich duplex. Most minor-groove binders have preference to AT-tract, therefore, GC-preference of the new ligand **1**(RR8) will provide useful information for binding with GC-rich sequence. A further investigation is now ongoing with footprinting analysis to determine sequence selectivity in detail.

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9. Abbreviation in parentheses following compound number indicates the stereochemistry of the amino groups and the alkyl chain length. For example, **1**(RR8) means that the compound **1** has R, R configuration at the A and B position and a nonyl group ($(CH_2)_8CH_3$) for alkyl chains. All of the bisTHF compounds used in this study have (R,R,R,R) configuration as shown in Figure. 2.
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