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BIPYRROLE DERIVATIVES AS NEW DNA-MINOR GROOVE BINDERS

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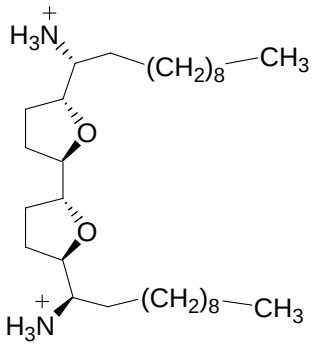
Abstract – Small molecules with capability of binding with DNA or RNA would have broad application as probes in genomic studies, and also as potential candidates for new therapeutic agents. In this study, we synthesized dithioamido-bipyrrole derivatives as new binding molecules to the duplex DNA. It has been revealed from the competitive binding experiments with the known intercalater, ethidum bromide, and the minor groove binder Hoechst 33258 that the dithioamido-bipyrrole derivatives with alkyl chains with medium length bind the duplex DNA in a similar manner with the minor groove binder. As ionic interactions are not included in the binding with the duplex DNA, the new dithioamido-bipyrrole skeleton may provide an interesting motif for DNA binding.

INTRODUCTION

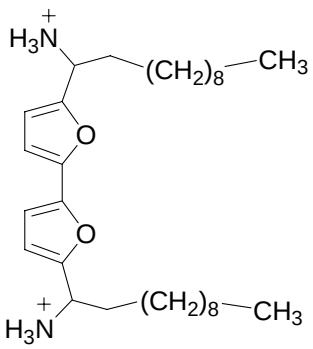
In the current post-genome era, concern has moved to the functional role of the genes. Genome-targeting molecules would have broad application as probes in genomic studies. In particular, small molecules that specifically bind with high affinity to DNA have become of great interest as potential candidates for new therapeutic agents that would provide specific interference in the gene expression. Among the low molecular weight ligands to the duplex DNA, the minor groove binders have shown rapid development recently.[1-3] Encouraging work in this field has been reported by Dervan's group, in which complete recognition of all four gene codes for distinguishing duplex DNA sequences has been achieved with designed pyrrole-imidazole polyamides.[1] In their studies, specific hydrogen bonding between the duplex DNA and the ligands are thought to contribute favorably to the

stability and sequence selectivity. The binding modes of the ligands within the minor groove of the duplex DNA apparently contain hydrophobic and/or van der Waals interactions, that are affected by shape complementarities between the ligand and the minor groove. The development of new binding motifs would be useful for better understanding of the minor groove binding of the duplex DNA, and would become a new candidate for gene-targeting agents.

Previously, we reported a series of new DNA binders based on a bistetrahydrofuran (bisTHF) and bisfuran skeleton bearing cationic groups and long alkyl chains (Figure 1).[4] BisTHF compounds showed binding preference to GC-rich sequences, while the bisfuran ligands bound the AT-rich DNA preferentially. These results have given rise to the question as to how the difference between the tetrahydrofuran and the furan skeleton affects the sequence preferences. Then, we initiated a systematic study for the synthesis of structure variants, such as by altering oxygen atoms to nitrogen or carbon atoms. Here we describe the synthesis and DNA-binding ability of bipyrrole derivatives as the nitrogen variant of the bisfuran ligands. As it was impossible to introduce the amino group at the contiguous position to the pyrrole ring, we attached another functional group for solubility in water. During the search for the suitable functional group, we have found the interesting fact that the thiocarbonyl group can replace the amino group in spite of no positive charge, and that the thiocarbonyl-bearing bipyrrole ligands are competitive to the minor groove binder but not to intercalators or metal cations (Figure 2).

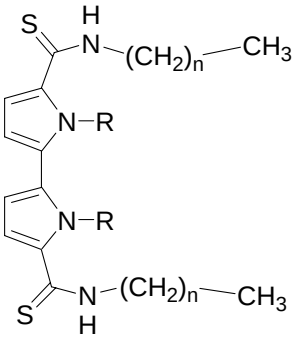


Diamino-bisTHF



Diamino-bisfuran

Figure 1



	R=	n=
1a	H	5
1b	H	8
2a	CH_3	0
2b	CH_3	5
2c	CH_3	8

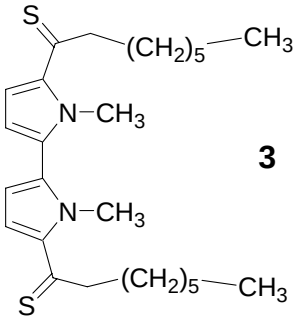


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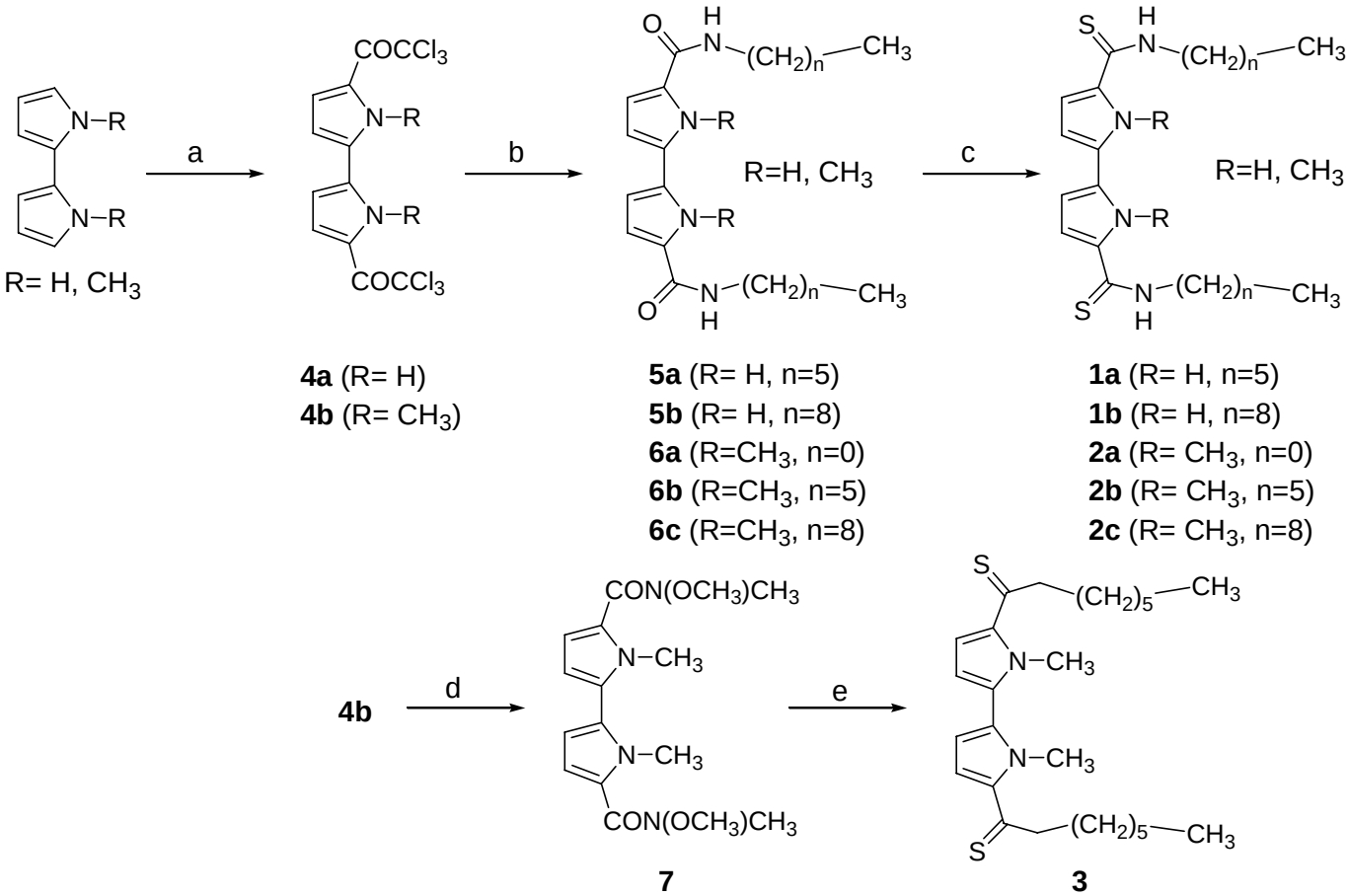
Figure 2

SYNTHESIS

The bipyrrole derivatives (**1, 2** and **3**) were synthesized starting from bipyrrole[5] and *N*-methylbipyrrole[6] (Scheme 1). Trichloroacetyl intermediates (**4a,b**) were obtained by using trichloroacetyl chloride,[7] which were treated with primary amines with an alkyl chain of various lengths to afford amides (**5** and **6**). Dithioamide-bipyrroles (**1** and **2**) were obtained by using Lawesson's reagent. For the synthesis of dithioketo-bipyrrole, trichloroacetyl intermediate (**4b**) was transformed to Weinreb amide (**7**) through the corresponding dicarboxylate. Alkylation with Grignard reagents afforded diketo-bipyrrole, followed by treatment with Lawesson's reagent to furnish dithioketo-bipyrrole (**3**).

Scheme 1



4a (R= H)
4b (R= CH_3)

5a (R= H, n=5)
5b (R= H, n=8)
6a (R=CH_3, n=0)
6b (R=CH_3, n=5)
6c (R=CH_3, n=8)

1a (R= H, n=5)
1b (R= H, n=8)
2a (R= CH_3, n=0)
2b (R= CH_3, n=5)
2c (R= CH_3, n=8)

7 **3**

a) Trichloroacetyl chloride, THF; b) $CH_3(CH_2)_nNH_2$, THF; c) Lawesson's reagent, THF; d) (1) CH_3CH_2ONa, CH_3CH_2OH; (2) 2 M aqueous NaOH; (3) $SOCl_2$, Pyridine, CH_2Cl_2; (4) $NH(OCH_3)CH_3$•HCl, pyridine; e) (1) $CH_3(CH_2)_5MgBr$, THF, rt; (2) Lawesson's reagent, THF.

COMPETITIVE DISPLACEMENT ASSAY

The binding affinity of these ligands with the duplex DNA was estimated by a competitive displacement assay using ethidium bromide (ETBr)[8] and Hoechst 33258.[9] The emission intensity of fluorescent ligands (ETBr or Hoechst 33258) is increased by binding with duplex DNA, and is diminished by another

competitive DNA-binding ligand. The binding modes of ethidium bromide with DNA contain intercalation as the major attractive force together with strong electrostatic interaction. It has been also reported that inhibition of emission of ethidium bromide does not reflect the binding mode of the competitive ligands. On the other hand, Hoechst 33258 binds within the minor groove of dsDNA with AT sequence selectivity,[10] and competitive minor groove binders with AT-selectivity inhibit Hoechst 33258-DNA binding most effectively. Concentrations of the ligand to inhibit 50% of fluorescence intensity are expressed as IC_{50} values, and correspond to the relative binding affinity of the ligands.

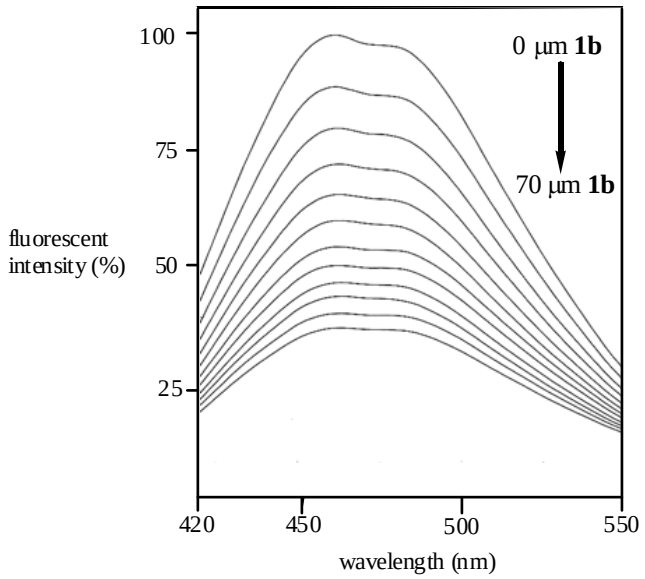


Figure 3. Change of Fluoresecnt Spectra in Displacement Assay of Hoechst 33258-CA12 with **1b**. Emission spectra was obtained with λ_{ex}= 355 nm. See footnote of Table 1 for CA12.

In this study, self-complementary 12 bp DNA duplexes CT12 and CA12 were used for measurement of binding affinity. CA12 includes an AATT region as the ligand binding site, while CT12 has a CGCG region at the middle of the duplex. Displacement assay was done at 20 ^{o}C using a solution of the duplex DNA (CT12 or CA12, 1 μM) in 0.01 M SHE buffer (9.4 mM NaCl, 2 mM HEPES, and 10 μM EDTA, pH 7.0).

Dithioamide-bipyrroles (**1** and **2**) and dithioketo-bipyrrole (**3**) did not show significant inhibition of ETBr-DNA binding. In contrast, they displayed clear displacement to the binding of Hoechst 33258, indicating minor-groove binding of these new ligands (**1**, **2** and **3**) (Figure 3). The IC_{50} values thus obtained are summarized in Table 1. There is no difference in IC_{50} values between the dithioamide and dithioketo ligands (**2b** *vs* **3**), and also between *N*-methylpyrrole and pyrrol ligands **(1** *vs* **2**), suggesting

that hydrogen bonds are not included in their binding to the minor groove of DNA. It seems that the length of alkyl chains reaches optimal at the number of 5 (**1a** and **2b**).

Table 1. IC_{50} Values in the Displacement Assay with ETBr and Hoechst 33258.

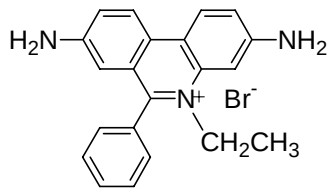
compound	*N*-R (R=)	$(CH_2)_n$ (n=)	IC_{50} (μM) ETBr CT12	ETBr CA12	Hoechst 33258 CA12
1a	H	5	non-competitive		35
1b	H	8	non-competitive		56
2a	Me	0	non-competitive		62
2b	Me	5	non-competitive		36
2c	Me	8	non-competitive		41
3	Me	6	non-competitive		37
Diamino-Bisfuran (meso)			17	4	23
Distamycin			19	>7 [a)]	0.7

1.0 μM each of DNA and a fluorescent ligand were used in 0.01 M SHE buffer at pH 7.0, 20 °C. Fluorescent spectra were obteined by excitation at 546 nm with ethidium bromide or at 355 nm with Hoechst 33258. a) partial displacement.

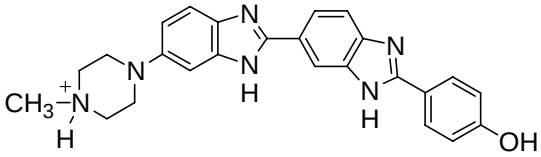
CT12 : CGTACGCGTACG
GCATGCGCATGC

CA12 : CGCGAATTCGCG
GCGCTTAAGCGC

Ethidium Bromide (ETBr)



Hoechst 33258



As electrostatic interaction plays a role in minor-groove binding, ligands must have positive charges in the molecules for strong competition to ETBr. The diamino-bisfuran has two ammonium groups, and displayed higher inhibitory activity to ETBr than to Hoechst 33258 binding. It is interesting, although the new ligands (**1a-e** and **2**) have no cationic group in the molecule, that they exhibit displacement activity to Hoechst 33258-DNA binding with potent affinity comparable to that of diamino-bisfuran. These facts have suggested that the binding force of the ligands (**1** and **2**) does not include electrostatic interaction as a major factor. In order to confirm this hypothesis, we then investigated the effect of Na^+ concentration on the IC_{50} values in Hoechst-displacement assay with the use of **2b** and distamycin for comparison. As clearly shown in Table 2, the strong inhibitory effect of distamycin was diminished to a great extent. On the other hand, the IC_{50} value of **2b** was shown not to be dependent on Na^+ concentration. The ratio ($\log(IC_{50}[Na^+]_{8mM}/IC_{50})$) was plotted against Na^+ concentration in order to compare the IC_{50} value obtained at each Na^+ concentration to that obtained at 8 mM Na^+ (Figure 4),

indicating clearly that the binding of **2b** to DNA is hardly affected by Na^+ concentration. Thus, it is confirmed that electrostatic interactions are not included as the major binding force of the new ligands.

Table 2. Na^+-Dependent Displacement of Hoechst 33258.

Na^+ (mM)	IC_{50} (μM)	
	Distamycin	**2b**
8	0.7	36
15	8.7	-
25	9.3	-
50	10.3	38
100	11.9	40

Displacement assay was carried out similary as decribed in the footnote of Table 1 except for NaCl concentrations.

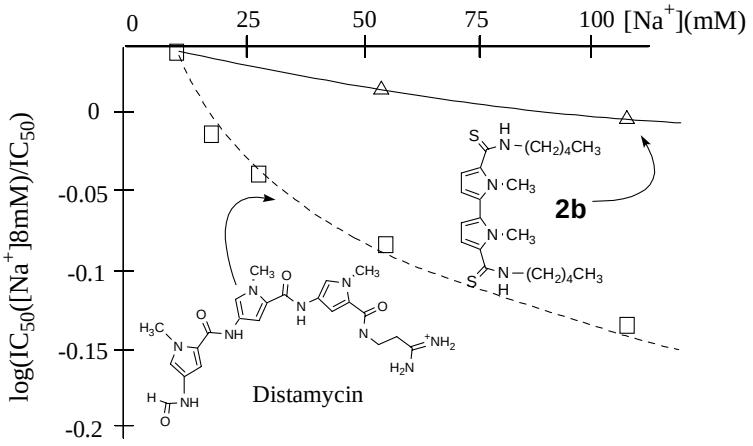


Figure 4. Comparison of Decrease of IC_{50} Values Depending on NaCl Concentrations.

CONCLUSION

In conclusion, we have developed new DNA binding molecules with dithioamide-bipyrroles as a binding motif. These ligands showed DNA binding affinity in a competitive manner to Hoecst 33258 that is a potent minor-goove binder with selectivity to the AT-rich region. The characteristic point of the new ligands is that electrostatic interactions are not included as the major binding force, and the binding affinity is hardly affected by metal cations. As metal cations are present in high concentration under physiological conditions, this feature may be beneficial for the design of new minor groove binders for use *in vivo.*

EXPERIMENTAL

General

^{1}H-NMR (270, 400, or 500 MHz) spectra were recorded on a JEOL GX-270, Varian UNITY-400, INOVA-500, or INOVA-600 spectrometer. ^{13}C-NMR (100 MHz) spectra were recorded on a Varian UNITY-400. IR spectra were obtained using a SHIMADZU FTIR-8400 spectrophotometer. HRMS analyses were recorded on Applied Biosystems Mariner System 5299 spectrometer using bradykinin, neurotensin and angiotensin as an internal standard.

5,5'-Bistrichloroacetyl-2,2'-bipyrrole (4a). Trichloroacetyl chloride (0.25 mL, 2.27 mmol) was added to a solution of bipyrrole (60 mg, 0.45 mmol) in ether (5 mL) under argon at rt, and the mixture was stirred at 40 ^{o}C. The reaction mixture was diluted with 10% Na_2CO_3, extracted with ether and the

organic layer was washed with brine, dried (Na_2SO_4) and concentrate *in vacuo* to give a green solid **(4a)**. It could be used in the next reaction without further purification. IR (nujol) 3300, 1612 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.41 (2H, d, *J*= 4.29 Hz), 6.84 (2H, d, *J*= 4.28 Hz); FABMS m/z 423.9 $(M+H)^+$.

5,5'-Bistrichloroacetyl-2,2'-bi(*N*-methyl)pyrrole (4b). Trichloroacetyl chloride (0.14 mL, 1.25 mmol) was added to a solution of bi(*N*-methyl)pyrrole (40 mg, 0.25 mmol) in THF (2 mL) under argon at rt, and the mixture was stirred at 40 ^oC. The reaction mixture was diluted with saturated $NaHCO_3$, extracted with EtOAc and the organic layer was washed with H_2O, brine, dried (Na_2SO_4) and concentrate *in vacuo* to give a green solid. Column chromatography (silica gel, hexane/ether, 5:1) afforded **5b** (69 mg, 65%) as a yellow solid: IR (neat) 1670 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.61 (2H, d, *J*= 4.29 Hz), 6.39 (2H, d, *J*= 4.62 Hz), 3.84 (6H, s); FABMS m/z 451 $(M)^+$.

A general procedure for transformation of trichloroacety group to carboxamido group.

A primary amine (four equivalents to **4**) was added to a solution of **4** in dry THF under argon at rt. After 24 h, the mixture was concentrated *in vacuo*, and triturated by ether to give the product.

5,5'-Bis(*N*-hexylcarboxamido)-2,2'-bipyrrole (5a). **5a** was obtained from **4a** (127 mg, 0.3 mmol) as a green solid (90.7 mg, 78 %): mp 243-244 ^oC; IR (KBr) 1604, 1545 cm^{-1}; ^{1}H-NMR (270 MHz, DMSO-d_6) δ 11.66 (2H, br s), 7.94 (2H, br t, *J*= 5.77 Hz), 6.75 (2H, dd, *J*= 2.31, 3.63 Hz), 6.43 (2H, dd, *J*= 2.50, 3.46 Hz), 3.36 (4H, m), 1.48-1.45 (4H, m), 1.28-1.26 (12H, m), 0.88-0.86 (6H, m); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 160.2, 127.4, 126.2, 110.7, 106.0, 38.4, 31.0, 29.3, 26.1, 22.1, 13.9; FABMS m/z 387.2 $(M+H)^+$; HRMS (APCI) calcd for $C_{22}H_{35}N_4O_2$ $(M+H)^+$ 387.2755, found 387.2764.

5,5'-Bis(*N*-nonylcarboxamido)-2,2'-bipyrrole (5b). **5b** was obtained from **4a** (200 mg, 0.473 mmol) as a yellowish-green solid (176 mg, 79 %): mp 222 ^oC; IR(KBr) 1604, 1545 cm^{-1}; ^{1}H-NMR (270 MHz, DMSO-d_6) δ 11.66 (2H, br s), 7.93 (2H, br t, *J*= 5.77 Hz), 6.75 (2H, dd, *J*= 1.65, 3.63 Hz), 6.43 (2H, dd, *J*= 1.97, 3.63 Hz), 3.19 (4H, q, *J*= 6.77 Hz), 1.48 (4H, m), 1.24 (24H, m), 0.86-0.82 (6H,m); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 160.2, 127.5, 126.2, 110.6, 106.0, 38.4, 31.2, 29.4, 28.9, 28.8, 28.6, 26.4, 22.0, 13.9; FABMS m/z 471.4 $(M+H)^+$; HRMS (ESI) calcd for $C_{28}H_{47}N_4O_2$ $(M+H)^+$ 471.3694, found 471.3683.

5,5'-Bis(*N*-methylcarboxamido)-2,2'-bi(*N*-methyl)pyrrole (6a). **6a** was obtained from **4b** (3 g, 6.65 mmol) as a pale yellow powder (1.68 g, 85%). mp 244-246 ^oC; IR (KBr) 1634, 1547 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 6.56 (2H, d, *J*= 3.96 Hz), 6.16 (2H, d, *J*= 3.95 Hz), 5.96 (2H, br s), 3.73 (6H, s), 2.86 (6H, d, *J*= 4.94 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 161.7, 128.3, 127.3, 111.3, 110.2, 33.4, 25.5; FABMS m/z 274.2 $(M)^+$; HRMS (APCI) calcd for $C_{14}H_{18}N_4O_2$ $(M+H)^+$ 275.1503, found 275.1507.

5,5'-Bis(*N*-hexylcarboxamido)-2,2'-bi(*N*-methyl)pyrrole (6b). **6b** was obtained from **4b** (50 mg, 0.12 mmol) as a white powder (38 mg, 78%). mp 169-171 ^oC; IR (KBr) 1620 cm^{-1}; ^{1}H-NMR (270 MHz,

$CDCl_3$) δ 6.55 (2H, d, *J*= 3.96 Hz), 6.16 (2H, d, *J*= 3.96 Hz), 5.92-5.87 (2H, m), 3.72 (6H, s), 3.38 (4H, dt, *J*= 7.26, 5.94 Hz), 1.40-1.25 (16H, m), 0.89 (6H, t, *J*= 6.60 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 161.1, 128.3, 127.3, 111.4, 110.1, 38.4, 33.4, 31.0, 29.2, 26.1, 22.0, 13.9; HRMS (ESI) calcd for $C_{24}H_{38}N_4O_2$ $(M+H)^+$ 415.3068, found 415.3045.

5,5'-Bis(*N*-nonylcarboxamido)-2,2'-bi(*N*-methyl)pyrrole (6c). **6c** was obtained from **4b** (400 mg, 0.887 mmol) as a white powder (282 mg, 64%). mp 154-156 ^{o}C; IR (KBr) 3310, 1630, 1539 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 6.55 (2H, d, *J*= 3.96 Hz), 6.16 (2H, d, *J*=3.96 Hz), 5.90-5.88 (2H, m), 3.72 (6H, s), 3.38 (4H, dt, *J*= 6.60, 6.27 Hz), 1.49-1.27 (28H, m), 0.88 (6H, t, *J*= 6.60 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 161.1, 128.3, 127.4, 111.4, 110.1, 38.4, 33.4, 31.2, 29.3, 28.9, 28.7, 28.6, 26.4, 22.0, 13.9; HRMS (ESI) calcd for $C_{30}H_{50}N_4O_2$ $(M+H)^+$ 499.4007, found 499.3969.

General Procedure for the Formation of Dithioamido Derivative.

Lawesson's reagent (4 eq.) was added to a solution of diamido-bipyrrole in dry THF under argon at rt, and the mixture was stirred at 70 ^{o}C for 15 h. The mixture was concentrated *in vacuo*. Column chromatography (silica gel, $CHCl_3$) afforded the dithioamido-bypyrrile.

5,5'-Bis(*N*-hexylthiocarboxamido)-2,2'-bipyrrole (1a). **1a** was obtained from **5a** (20.8 mg, 0.054 mmol) as a brown solid (22.7 mg, quantitative). IR ($CHCl_3$) 1697 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 9.79 (2H, br s), 7.24 (2H, m), 6.57 (2H, dd, *J*= 2.31, 3.96 Hz), 6.43 (2H, dd, *J*= 2.97, 3.96 Hz), 3.85-3.78 (4H, m), 1.76-1.68 (4H, m), 1.45-1.31 (12H, m), 0.93-0.88 (6H, m); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 183.6, 132.3, 129.3, 109.4, 108.2, 44.5, 31.0, 27.7, 26.2, 22.0, 13.9; FABMS m/z 419.2 $(M+H)^+$; HRMS (ESI) calcd for $C_{22}H_{35}N_4S_2$ $(M+H)^+$ 419.2298, found 419.2343.

5,5'-Bis(*N*-nonylthiocarboxamido)-2,2'-bipyrrole (1b). **1b** was obtained from **5b** (66 mg, 0.140 mmol) as a yellow solid (51.9 mg, 73%). mp 192-193 ^{o}C; IR (KBr) 3400 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 9.78 (2H, br s), 7.24 (2H, m), 6.57 (2H, dd, *J*= 2.31, 3.96 Hz), 6.43 (2H, dd, *J*= 2.97, 3.96 Hz), 3.85-3.77 (4H, m), 1.76-1.62 (4H, m), 1.52-1.19 (24H, m), 0.91-0.82 (6H, m); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 183.6, 132.4, 129.3, 109.3, 108.2, 44.5, 31.2, 28.9, 28.7, 28.6, 27.7, 26.4, 22.0, 13.9; HRMS (ESI) calcd for $C_{28}H_{47}N_4S_2$ $(M+H)^+$ 503.3237, found 503.3227.

5,5'-Bis(*N*-methylthiocarboxamido)-2,2'-bi(*N*-methylpyrrole) (2a). **2a** was obtained from **6a** (1 g, 3.6 mmol) as a pale yellow powder (890 mg, 81%). mp 230 ^{o}C; IR (KBr) 1508, 1038 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.52 (2H, br s), 6.47 (2H, d, *J*= 3.96 Hz), 6.18 (2H, d, *J*= 3.96 Hz), 3.80 (6H, s), 3.30 (6H, d, *J*= 4.62 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 187.2, 135.4, 129.3, 110.4, 110.1, 34.1, 32.1; FABMS m/z 306.2 $(M)^+$; HRMS (APCI) calcd for $C_{14}H_{18}N_4O_2$ $(M+H)^+$ 307.1046, found 307.1072.

5,5'-Bis(*N*-hexylthiocarboxamido)-2,2'-bi(*N*-methylpyrrole) (2b). **2b** was obtained from **6b** (500 mg, 1.2 mmol) as a green powder (420 mg, 78 %). mp 95 ^{o}C; IR (KBr) 3400-3200, 2100, 1620 cm^{-1};

^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.39 (2H, br s), 6.45 (2H, d, *J*= 3.96 Hz), 6.18 (2H, d, *J*= 3.96 Hz), 3.79 (6H, s), 1.76-1.68 (6H, m), 1.46-1.34 (22H, m), 0.91 (6H, t, *J*= 6.93 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 186.5, 135.7, 129.1, 110.6, 110.0, 44.7, 34.0, 30.9, 27.2, 26.1, 22.0, 13.8; HRMS (ESI) calcd for $C_{24}H_{39}N_4S_2$ $(M+H)^+$ 447.2611, found 447.2587.

5,5'-Bis(*N*-nonylthiocarboxamido) -2,2'-bi(*N*-methylpyrrole) (2c). **2c** was obtained from **6c** (100 mg, 0.24 mmol) as a colorless solid. mp 95-99 ^{o}C; IR (KBr) 3400-3200, 2100, 1625 cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.37-7.36 (2H, m), 6.44 (2H, d, *J*= 3.96 Hz), 6.18 (2H, d, *J*= 3.96 Hz), 3.79 (6H, s), 3.78-3.72 (4H, m), 1.75-1.66 (4H, m), 1.48-1.23 (24H, m), 0.89 (6H, t, *J*= 6.59 Hz); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 187.7, 136.2, 130.9, 110.8, 109.2, 45.4, 34.5, 31.8, 29.4, 29.2, 29.1, 28.3, 27.0, 22.6, 14.0; HRMS (ESI) calcd for $C_{30}H_{50}N_4S_2$ $(M+H)^+$ 531.3550, found 531.3508.

5,5'-Bis(ethoxycarbonyl)-2,2'-bi(*N*-methyl)pyrrole. **5b** (500 mg) was added to a stirred solution of 0.32 M EtONa in dry ethanol (30 mL) under Ar, at rt, and the mixture was stirred for 1.5 h. The mixture was concentrated *in vacuo* to afford a brown oil, which was diluted with EtOAc, and washed with 10% aq HCl, H_2O and brine. The organic layer was dried (Na_2SO_4) and concentrated *in vacuo* to give a brown oil. Column chromatography (silica gel, hexane/ether, 4:1) afforded the desired compound (244 mg, 72%) as a white solid: mp 42 ^{o}C. IR ($CHCl_3$) 1697cm^{-1}; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 7.02 (2H, d, *J*= 3.96 Hz), 6.21 (2H, d, *J*= 3.96 Hz), 4.31 (4H, q, *J*= 7.04 Hz), 3.74 (6H, s), 1.37 (6H, t, *J*= 7.01 Hz); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 160.3, 130.4, 123.5, 116.7, 111.3, 59.6, 33.7, 14.2; HRMS (APCI) calcd for $C_{16}H_{20}N_2O_4$ $(M+H)^+$ 305.1496, found 305.1511.

5,5'-Dicarboxy-2,2'-bi(*N*-methyl)pyrrole. A 2 M aqueous solution of NaOH (0.8 mL) was added to a solution of the above oil (72.2 mg, 0.237 mmol) in MeOH, and the mixture was stirred at 55 ^{o}C for 3 h. The mixture was acidified with aqueous 10% HCl (5 mL) and diluted with H_2O, and extracted with EtOAc. The organic layer was dried (Na_2SO_4), and concentrated *in vacuo* to give the desired compound as a white solid (57.7 mg, 98%). mp >300 ^{o}C; IR (KBr) 3300-2700, 1676 cm^{-1}; ^{1}H-NMR (270 MHz, CD_3OD) δ 7.01 (2H, d, *J*= 3.96 Hz), 6.23 (2H, d, *J*= 3.96 Hz), 3.70 (6H, s); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 161.9, 130.3, 124.1, 116.7, 111.1, 33.6; Anal. Calcd for $C_{12}H_{12}N_2O_4$: C, 58.04; H, 4.87; N, 11.29. Found: C, 57.88; H, 4.91; N, 11.04.

5,5'-Bis(*N*-Methoxy-*N*-methylcarboxamido)-bi(*N*-methyl)pyrrole (7). Dry pyridine (90 μL, 1.03 mmol) and $SOCl_2$ (75 μL, 1.11 mmol) were added slowly to a suspension of (48.1 mg, 0.193 mmol) in dry CH_2Cl_2 (4.2 mL) under argon at rt. After 45 h, the mixture was concentrate *in vacuo* to give the corresponding acid chloride as a yellow solid. A solution of *N,O*-dimethylhydroxylamine hydrochloride (55 mg, 0.563 mmol) in dry pyridine (78 μL, 0.964 mmol) was added to a solution of this oil in dry CH_2Cl_2 (3 mL) at -20 ^{o}C, and the mixture was stirred for 1 h. The mixture was washed with brine, and

the organic layer was dried (Na_2SO_4), concentrated *in vacuo* to give a brown solid. Column chromatography (silica gel, $CHCl_3/CH_3OH$, 99:1) afforded **7** (58.8 mg, 91%) as a pale yellow solid. mp 83-84 ^oC; IR (KBr) 1618 cm^{-1}; ^1H-NMR (270 MHz, $CDCl_3$) δ 6.95 (2H, d, *J*= 3.96 Hz), 6.22 (2H, d, *J*= 4.29 Hz), 3.72 (6H, s), 3.69 (6H, s), 3.35 (6H, s); ^{13}C-NMR (400 MHz, DMSO-d_6): δ 161.8, 128.8, 125.0, 114.3, 110.7, 33.9, 33.4; HRMS(APCI) calcd for $C_{16}H_{23}N_4O_5$ $(M+H)^+$ 335.1714, found 335.1733.

5,5'-Diheptanoyl-2,2'-bi(*N*-methyl)pyrrole. A solution of *n*-hexylmagnesium bromide (0.25 M, THF, 4 mL) was added to a solution of **7** (53 mg, 0.16 mmol) in dry THF (0.5 mL) at 0 ^oC. After 1 h, the reaction mixture was quenched with saturated NH_4Cl, and the solution was extracted with $CHCl_3$. The organic layers were washed with H_2O and brine, dried (Na_2SO_4) and concentrated *in vacuo* to give a pale yellow oil. Column chromatography (silica gel, $CHCl_3$) afforded the desired compound (53.2 mg, 81%) as colorless needles. IR (KBr) 2854, 1651 cm^{-1}; ^1H-NMR (270 MHz, $CDCl_3$) δ 7.03 (2H, d, *J*= 4.29 Hz), 6.23 (2H, d, *J*= 4.29 Hz), 3.76 (6H, s), 2.80 (2H, d, *J*= 7.42 Hz), 1.75-1.69 (4H, m), 1.34-1.28 (16H, m), 0.88 (6H, m); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 192.0, 132.3, 131.9, 118.2, 111.6, 39.5, 34.9, 31.7, 29.4, 29.2, 25.3, 22.6, 14.1; FABMS m/z 413.4 $(M+H)^+$; HRMS (ESI) calcd for $C_{26}H_{40}N_2O_2$ $(M+H)^+$ 413.3163, found 413.3126.

5,5'-Bis(heptanethionyl)-2,2'-bi(*N*-methyl)pyrrole (3). Lawesson's reagent (163 mg, 0.404 mmol) was added to a solution of the above oil (33.3 mg, 0.081 mmol) in dry THF (2 mL) under argon at rt, and the mixture was stirred at 70 ^oC for 2 h. The mixture was concentrated *in vacuo* to afford a red solid. Column chromatography (silica gel, Hexane/$CHCl_3$, 1:1) afforded the **3** (32.9 mg, 73%) as a yellowish-red solid. IR (KBr) 2854 cm^{-1}; ^1H-NMR (270 MHz, $CDCl_3$) δ 7.17 (2H, d, *J*=4.29 Hz), 6.35 (2H, d, *J*=4.29 Hz), 3.89 (6H, s), 3.30-3.25 (4H, m), 1.88-1.82 (4H, m), 1.38-1.29 (16H,m), 0.90-0.85 (6H, m); ^{13}C-NMR (400 MHz, $CDCl_3$): δ 224.8, 143.5, 136.6, 116.2, 112.3, 51.7, 37.5, 32.2, 31.7, 29.4, 29.1, 22.6, 14.1; HRMS (ESI) calcd for $C_{26}H_{40}N_2S_2$ $(M+H)^+$ 445.2706, found 445.2747.

Binding Experiments

Fluorescent spectra were recorded on a Jasco FP-750 fluorescence spectrophotometer at 20^oC using a solution in 0.01M SHE buffer (9.4 mM NaCl, 2 mM HEPES, and 10 μM EDTA, pH 7.0). DNA was dissolved in the measurement buffer and was annealed from 80 to 10^oC during 70 min. Stock solutions of ethidium bromide and Hoechst33258 were prepared immediately before use. Emission spectra of ethidium bromide were recorded between 550 and 650 nm at excitation wavelength of 546 nm, and changes of intensity at 595 nm were analyzed. Emission spectra of Hoechst33258 were recorded between 400 and 600 nm at excitation wavelength of 355 nm, and changes of intensity at 485 nm were analyzed.

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