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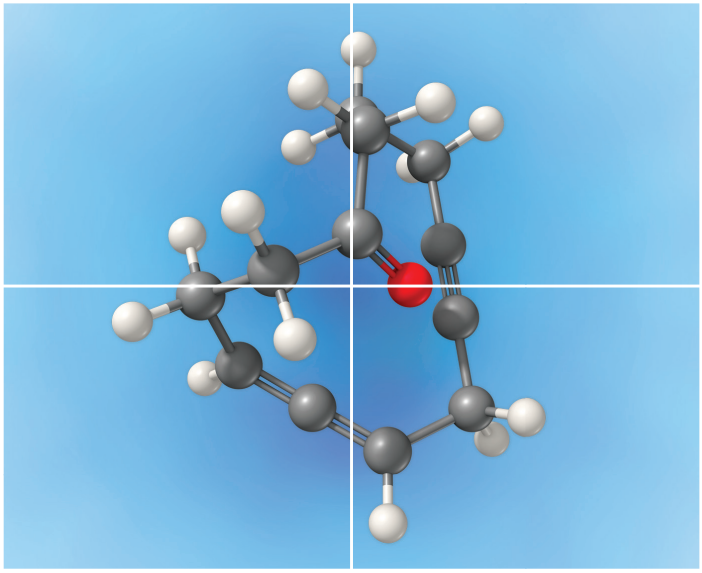

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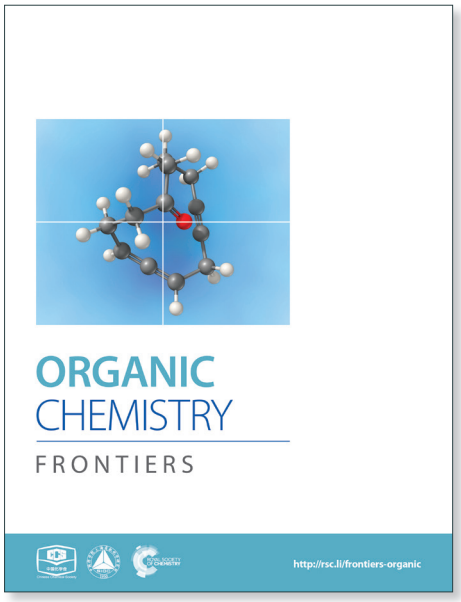

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Chiroptical Sensing of Oligonucleotides with a Cyclic Octapyrrole

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Abstract

A cyclic octapyrrole **8P**, bearing alkyl and phenyl substituents, shows chiroptical responses upon complexations with oligonucleotides in water. It specifically provides a strong CD response with an oligonucleotide that has high thymine content.

Introduction

Dye host molecules, composed of porphyrin and its derivatives that absorb UV and visible light with large absorption coefficients, become chiroptical sensors for asymmetric species.[1–8] In most cases, the host molecule accommodates the asymmetric guest molecule to output circular dichroism (CD) response. Cyclic oligopyrroles and their metal complexes,[9] having helical conformations, show induced CD through noncovalent interactions with the asymmetric guest in the UV–visible region.[10,11] These studies found that their helical conformations, whose enantiomers interconvert dynamically into each other at room temperature, are biased toward one of the enantiomers through chiral molecular recognitions. In those studies, the cyclic hosts recognize chiral structures of the smaller guest molecules through multi point interactions in its inner cavity or metal center.

However, no example has been reported for the chiroptical sensing of larger asymmetric molecules and molecular assemblies with the cyclic oligopyrroles as guest chiroptical sensors. In this study we found that a cyclic octapyrrole **8P**, bearing hydrophobic alkyl and phenyl substituents at *meso* and pyrrole-β positions (Figure 1a), shows the chiroptical response upon complexation with an oligonucleotide in water. **8P** specifically shows a strong CD response with oligonucleotides high in thymine content (Figure 1b).

Results and Discussions

One of the authors previously reported syntheses of **8P**, having a chiral figure-eight loop structure, and its metal complexations.[11] Enantioselective inductions of the helical chirality were achieved by the chiral molecular recognitions of asymmetric compounds such as mandelic acid and 1-(1-phenyl)ethylamine. In this study, we have successfully demonstrated X-ray crystallographic analysis of free-base **8P** (Figure 2). It shows that the cyclic octapyrrole framework adopts the chiral figure-eight loop structure, and is also surrounded by the alkyl and phenyl substituent groups with a molecular diameter of 0.8–1.6 nm. 2,2'-Bipyrrole units with *s-trans* conformations cross at the center of the cavity. The side view shows a rhombic cavity enclosed by four panels of the planar dipyrrin unit.

8P is insoluble in water, but its HCl salt (**8P**·**HCl**) is slightly soluble in water. The electronic absorption spectrum of **8P**·**HCl**, whose sample solution was prepared upon 1000-fold dilution of a methanol solution of **8P**·**HCl** with water, showed a broad absorption band in the range of 400–800 nm with λ_{max} at 546 nm, originating from the large π-conjugations over the pyrrole components (Figure 3, black curve). Since ^{1}H NMR spectrum of **8P**·**HCl** in D_2O is notably broadened, **8P**·**HCl** molecules most likely aggregate in the water solution. Dynamic light scattering (DLS) measurement of the sample solution with a concentration of 1.8×10^{-5} M actually showed presence of the aggregates with an average diameter of 42.7 nm (Figure S1).

It is known that **8P** shows chiroptical responses, originating from induction of the

helical structure to one of the enantiomers, upon complexations with optically active acids such as mandelic acid and tartaric acid in CH_2Cl_2.[11] Even in the water solution, **8P·HCl** provided the induced CD spectra in the visible region upon mixing with excess amounts of (*R*)- and (*S*)-mandelic acid (Figure S2). With an expectation that **8P·HCl** also complexes with nucleotide monomers (dAMP, dTMP, dCMP, and dGMP) through electrostatic and/or hydrophobic interactions,[12] we then demonstrated spectroscopic measurements of **8P·HCl** upon mixing with the nucleotide in an acetic acid buffer solution with pH 4.0 at 20 °C. In the electronic absorption spectroscopy, λ_{max} of **8P·HCl** at 546 nm in the lower energy absorption band was blue-shifted to 542–543 nm with increasing absorbance upon addition of 5 equiv. of dAMP, dTMP, dCMP, or dGMP to the solution (Figure 3, black broken curves). These observed spectral changes indicate the complexations of **8P·HCl** with the nucleotide through possible anion–exchanges. However, no notable CD inductions were observed in any of the cases.

In sharp contrast to the cases with nucleotide monomers, when **8P·HCl** was mixed with 0.8 equiv. (10 equiv. for monomer unit) of the homooligonucleotide 12 mer of dA_{12}, dT_{12}, dC_{12}, or dG_{12}, the lowest energy absorption band was significantly red-shifted to λ_{max} = 570–630 nm with a shoulder around 700 nm (Figure 3, red curves and Figure S3). The observed spectral changes indicate that the homooligonucleotides form complexes with **8P·HCl** through essentially different interactions from that with the nucleotide monomers. In these sample solutions, surprisingly, induced CD spectra appeared only in cases with dA_{12} and dT_{12}. The mixture of **8P·HCl** and dA_{12} showed negative CD signals at 787 and 477 nm, and positive ones at 617 and 405 nm. The almost same CD spectral profile was also observed in the mixture of **8P·HCl** and dT_{12} in the same conditions. The electronic transitions along the long axes of the π-conjugated bisdipyrrin chromophores are responsible for the absorption band around 700 nm. CD exciton chirality method for the observed CD signals with negative to positive sign (a negative couplet), corresponding to this absorption band, suggest dominant formation of the *M*,*M* helical conformation.[13] This

characterization is also supported by the calculated CD profile of the metal complex of **8P** reported previously.[11] The homooligonucleotides and their assemblies are known to adopt right-handed helical conformations in crystal structures.[14,15] Since the twist direction of the homooligonucleotides agrees with that for the long molecular axis of (*M*,*M*)-**8P**, the directional hydrophobic interaction between the multi base and substituent groups may bring about the higher stability of the complex.

Plot of the CD spectral intensity change of **8P**·**HCl** at 400 nm by varying concentrations of the added dA_{12} shows that the spectral intensity increases until it reaches a plateau at 1:1 ratio, with no further effect at higher concentration of dA_{12} (Figure 4a, black circles). This result indicates 1:1 complexation of **8P**·**HCl** and dA_{12}. However, the observed profile with dT_{12} shows stepwise increases of the CD intensity to show the first and the second plateaus around 1:1 and 1:2 ratios, respectively (Figure 4a, red circles). The observed CD intensity with 1:2 mixture is approximately three times larger than that observed in the 1:1 mixture. The absorption spectrum of the 1:2 mixture shows a sharp absorption band with increasing absorbance at λ_{max} = 617 nm, and is also markedly different from that observed in the 1:1 mixture (Figure 3b, blue and red curve, respectively). These characteristic spectral changes indicate stepwise formations of the 1:1 and 1:2 complexes of **8P**·**HCl** and dT_{12}.

To clarify the molecular interactions between **8P**·**HCl** and dT_{12}, we then performed ^{1}H NMR spectroscopy of their mixture solution. When **8P**·**HCl** was mixed with 2 equiv. of dT_{12} in D_2O, no notable shifts of signals corresponding to the deoxyribose components were observed in dT_{12}, but downfield shifts ($\Delta\delta$ = ~0.03 ppm) of the signals were observed in the thymine nucleobase component (Figure 5). The host molecules, when complexed with porphyrin derivatives having a large aromatic macrocyclic ring, generally show upfield shifts of the peaks owing to the ring current effects of the porphyrin. The observed downfield shifts of the peaks of dT_{12}, when complexed with **8P**·**HCl** may, thus, originate from the non-conjugated macrocyclic structure of **8P** carrying HCl that generally brings

about the downfield shifts of the interacting molecules. The observed characteristic peak shifts indicate that dT_{12} envelops **8P·HCl** in water solutions through hydrophobic interactions (Figure 6). Here, the thymine component, having a hydrophobic methyl group without an amino group, in dT_{12} may allow its directed supramolecular complexations with **8P·HCl**, having hydrophobic substituent groups, to cause the helical induction. As a support of this hypothesis, the stronger induced CD appeared upon mixing of **8P·HCl** with a heterooligonucleotide dT_nA_{12-n} having higher thymine content (Figure 4b). The CD intensity nonlinearly increases with increasing the hydrophobic thymine component in the oligonucleotide 12 mer. Further, the CD intensity shows length dependence of the oligonucleotide. The CD intensity increases as the number of the nucleotide components of dT_n increases, and a plot of the intensity with respect to the number of thymine components (n) shows a sigmoidal profile (Figure 4c). The CD response appears over dT_4, and it increases with increasing numbers of oligonucleotide units, and reaches a plateau around dT_{12}. The result can be readily understand by a CPK model study, showing that dT_{12} can accommodate **8P·HCl** in its pseudo hydrophobic cavity to form a size-favorable complex as observed in foldamers (Figure S4).[6] At higher concentrations of dT_{12}, it can be expected that **8P·HCl** is more efficiently isolated from water by two molecules of dT_{12} through the 1:2 complexation (Figure 6b).

Conclusion

In this study we found that a cyclic octapyrrole **8P**, having a figure-eight loop structure, is capable of sensing chiroptically the molecular chirality of oligonucleotides, and gives a strong CD response to oligonucleotides high in thymine content. With the advantage of **8P** at sensing molecular chirality by its basic inner cavity or hydrophobic outer substituents, the chiroptical sensing of natural biomolecules, such as DNA, and proteins will lead to fascinating future projects.

Experimental Section

Materials: Most reagents and solvents were used as received from commercial sources without further purification. (*R*)- and (*S*)-Mandelic Acid (> 99%) were purchased from Aldrich. 2´-Deoxyadenosine-, 2´-deoxycitidine-, and 2´-deoxyguanosine-5´-monophosphate disodiumsalts (dAMP, dCMP, and dGMP, respectively) were purchased from MP Biomedicals. 2´-Deoxythymidine-5´-monophosphate disodiumsalt (dTMP) was purchased from Nacalai Tesque. Oligonucleotides were purchased from Hokkaido System Science Co., Ltd. **8P** was synthesized according to the method described in the literature.[11] Recrystallization from benzene/acetonitrile gave crystals for X-ray crystallographic analysis. Crystal data: $C_{86}H_{92}N_8 \cdot C_6H_6$, M_r=1315.79, orthorhombic, space group *Pnn2*, a = 15.865(4), b = 16.430(4), c = 14.961(4) Å, a = 90, b = 90, g = 90°, V = 3899.7(17) Å^3, Z = 2, ρ_{calcd} = 1.120 Mgm^{-3}, $\mu(Mo_{K\alpha})$ = 0.065 mm^{-1}, T = 173(2) K, crystal size: 0.40 × 0.20 × 0.15 mm. A total of 18780 unique reflections were collected ($1.78 < 2\theta < 25.50$) by using graphite-monochromated $Mo_{K\alpha}$ radiation. The structure was solved by the direct method by using the SHELX97 package.[16] The positions of all non-hydrogen atoms were refined anisotropically (533 parameters). All hydrogen atoms were placed at ideal positions and included in the refinement. R_1 = 0.0433, wR_2 = 0.1132 for 6562 reflections with $I > 2.00\sigma(I)$; R_1 = 0.0511, wR_2 = 0.1077 for all data; GOF (on F^2) = 1.034. CCDC 1027733 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Preparation of 8P·HCl salt: A CH_2Cl_2 solution of **8P** was stirred upon mixing with a small amount of conc. HCl(aq). The sample solution was evaporated to dryness under reduced pressure to leave **8P**·*n***HCl** (*n* = 1–4) as dark purple powdery solid.

Measurements: CD spectra were recorded on a JASCO J-820 spectropolarimeter equipped

with a JASCO PTC-423L temperature/stirring controller. Electronic absorption spectra were recorded using a JASCO type V-670 UV/VIS/NIR spectrometer equipped with a JASCO type ETC-717 temperature/stirring controller. Prior to spectral measurements, sample water solutions ([**8P**·HCl] = 1.8×10^{-5} M) were prepared by dilution of a methanol solution of **8P**·HCl ([**8P**·HCl] = 1.8×10^{-2} M) with a water solution containing oligonucleotide, and allowed to stand in the dark at 10 °C for 6 h under stirring. ^{1}H NMR spectra were recorded on a Bruker AVANCE 500 spectrometer (500 MHz). Chemical shifts (δ in ppm) were reported with respect to trimethylsilyl propanoic acid (TMSP) as the internal standard. Dynamic light scattering (DLS) measurements were performed using an Otsuka model ELS-Z2 instrument. X-ray diffraction data were collected on a Bruker APEX II Ultra CCD diffractometer using Mo$K\alpha$ radiation (λ = 0.71073 Å) at 298 K.

Acknowledgement

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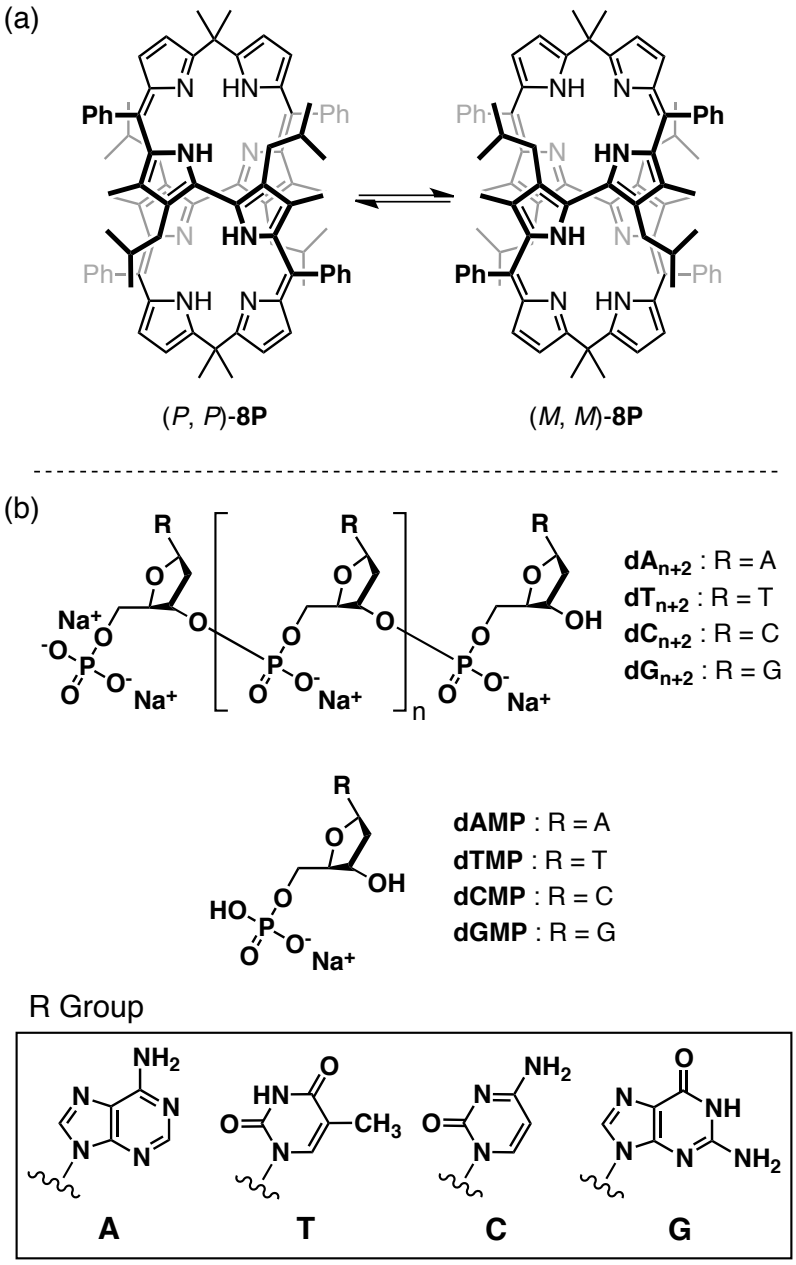

Figure 1. (a) Dynamic inversions between left- and right-handed helical enantiomers of **8P**. (b) Nucleotides and oligonucleotides used in this study.

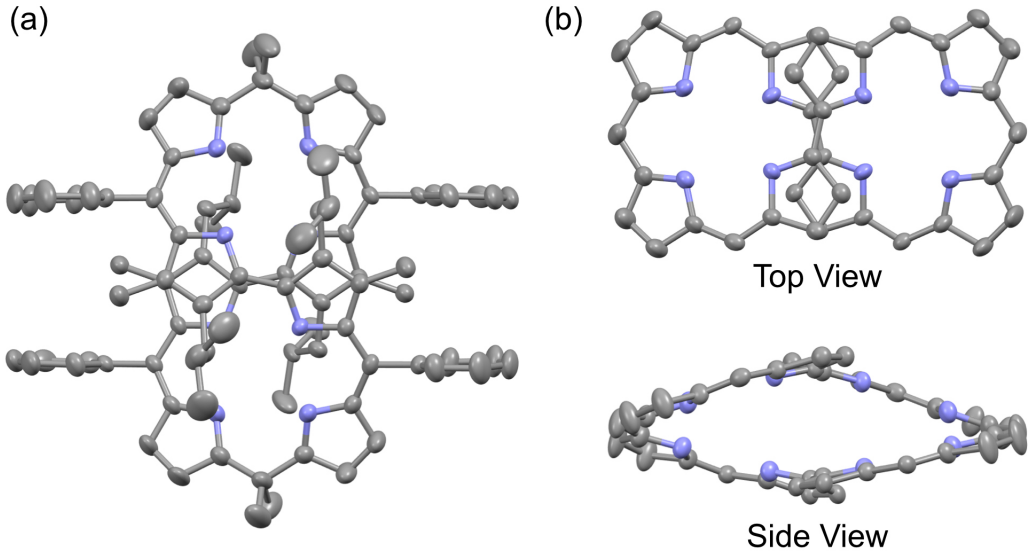

Figure 2. X-ray crystal structure of **8P**; (a) whole view, where hydrogen atoms are omitted for clarity, (b) top and side views, where hydrogen atoms and all substituent groups are omitted for clarity.

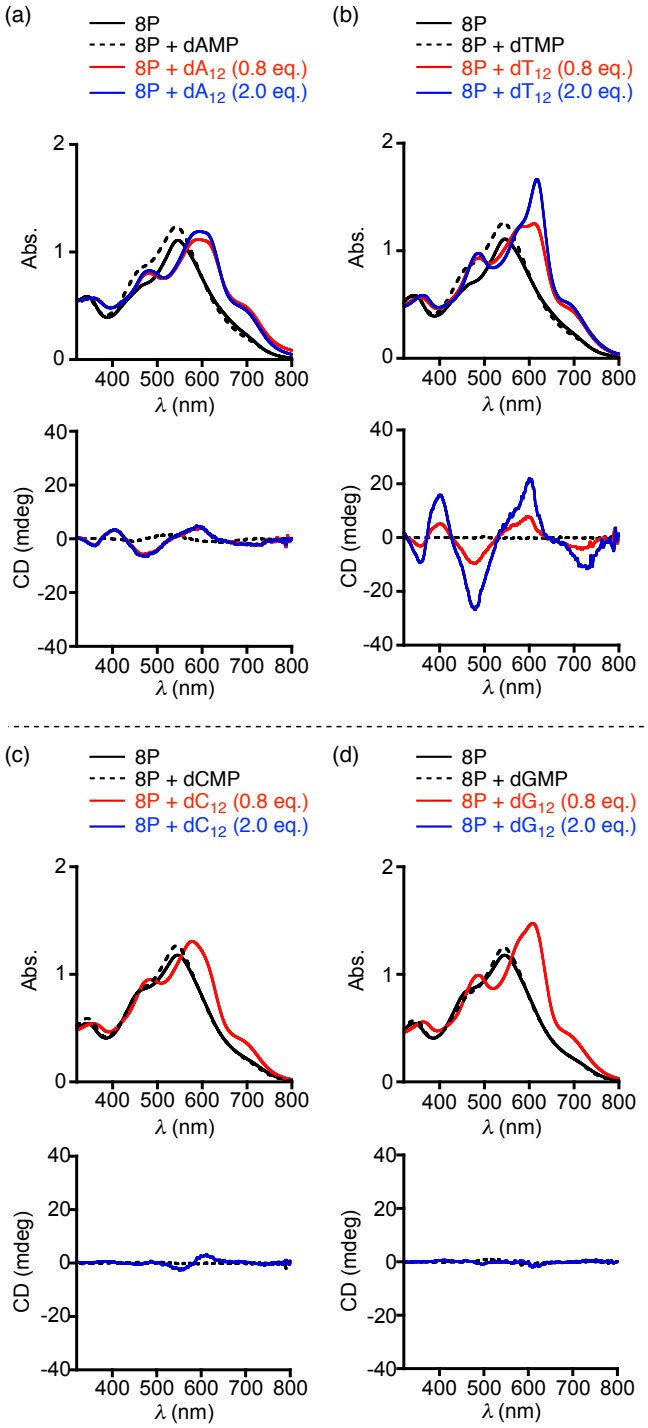

Figure 3. Absorption and CD spectra of **8P·HCl** in the presence and absence of nucleotide (dAMP, dTMP, dCMP, or dGMP) or oligonucleotide (dA_{12}, dT_{12}, dC_{12}, or dG_{12}) at 10 °C in a 10 mM acetate buffer solution at pH 4.0. [**8P·HCl**] = 1.8×10^{-5} M, [nucleotide] = 9.0×10^{-5} M, [oligonucleotide] = 1.4×10^{-5} or 3.6×10^{-5} M.

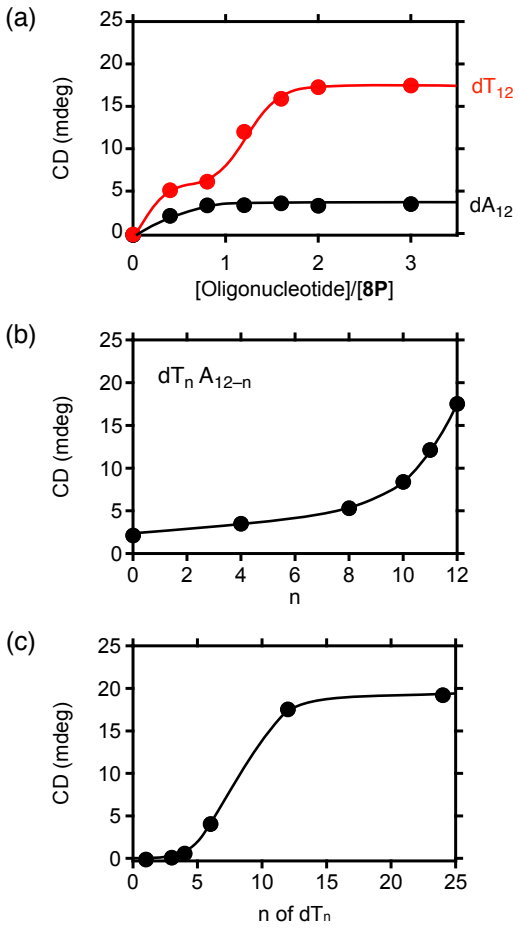

Figure 4. Plots of observed CD intensities of mixtures of **8P·HCl** (1.8×10^{-5} M) and oligonucleotide at 10 °C by varying (a) concentration of dA_{12} and dT_{12}, (b) ratio of adenine (A) and thymine (T) in the oligonucleotide 12 mer (2 equiv.), and (c) number of nucleotide units in dT_n (n = 1, 3, 4, 6, 12, and 24) with the concentration of nucleotide unit at 43.0×10^{-5} M (2 equiv. in dT_{12}). Base sequences of the oligonucleotide in (b): AATAATAATAAT, TTATTATTATTA, ATTTTTTTTTTA, and TTTTTTATTTTT for n = 4, 8, 10, and 11, respectively.

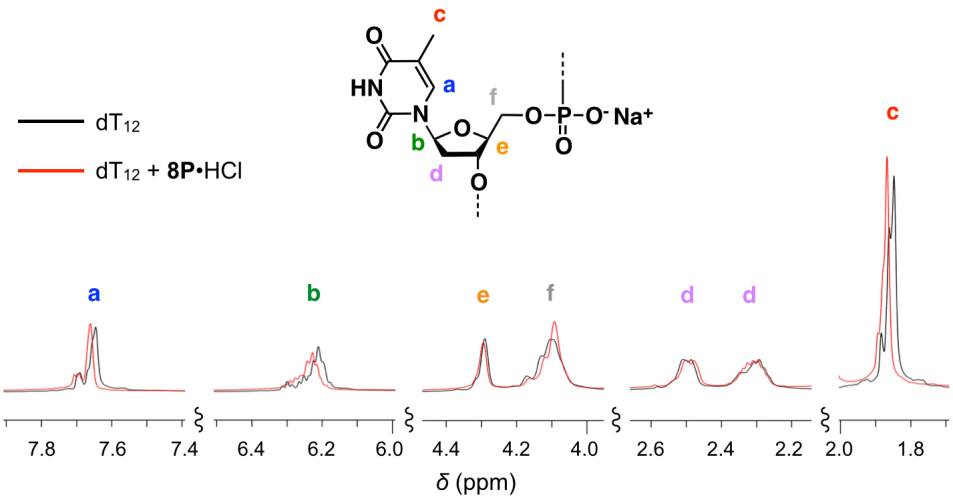

Figure 5. Changes in ^{1}H NMR spectrum of dT_{12} upon mixing with 0.5 equiv. amount of **8P**·HCl at 10 °C in D_2O containing 10 mM CD_3CO_2D. [**8P**·HCl] = 1.5×10^{-3} M, [dT_{12}] = 3.0×10^{-3} M.

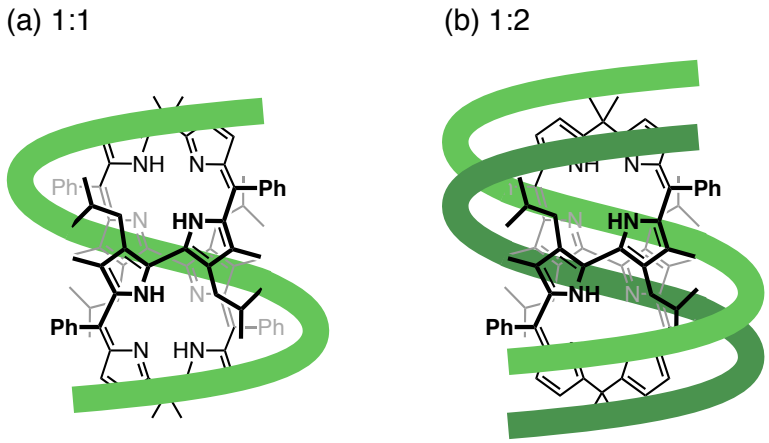

Figure 6. Possible associations of **8P** and dT_{12} in (a) 1:1 and (b) 1:2 complexations.

TOC

A cyclic octapyrrole **8P**, bearing alkyl and phenyl substituents, shows chiroptical responses upon complexations with oligonucleotides in water. It specifically provides a strong CD response with an oligonucleotide that has high thymine content.