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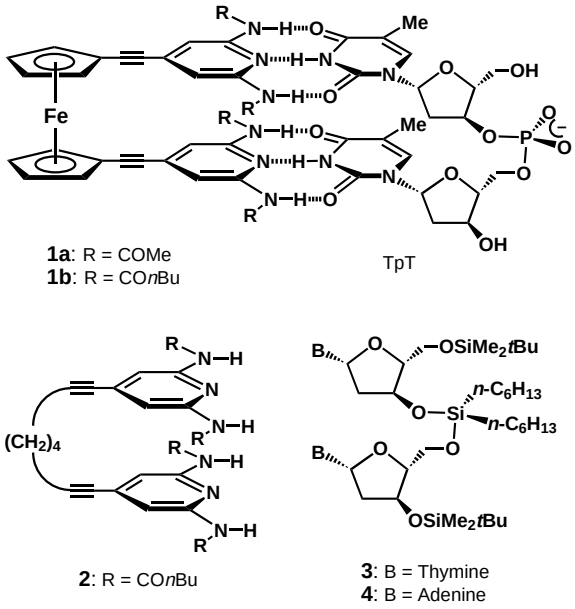
receptors for dinucleotides and demonstrate the highly selective extraction of the dinucleotides into nonpolar solvents by using the receptors.

As a starting point for this project, we chose thymidylyl(3′→5′)thymidine (TpT) as a target dinucleotide because the synthetic recognition site for thymine was already developed and successfully tethered to a ferrocene skeleton.[6] The molecular design of the dinucleotide receptors **1** (Scheme 1) for TpT was based on the inter-ring spacing

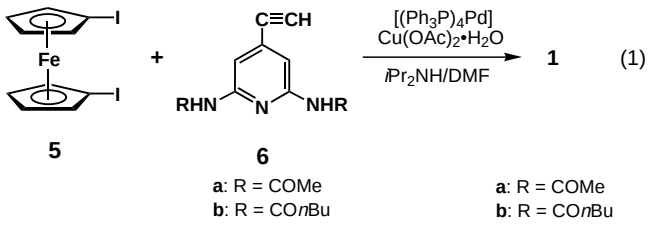


Scheme 1. A multipoint hydrogen-bonded complex between dinucleotide receptor **1** and TpT. Analogues **2**, **3**, and **4** were also investigated.

Specific Binding and Separation of Dinucleotides by Ferrocene-Modified Artificial Receptors

Masahiko Inouye* and Masayoshi Takase

Short oligonucleotides and their derivatives have significant biological activities; for example, they act as antitumor and antiviral agents.[1] Hence, efficient syntheses and simple purification methods of such oligomers on a mg-scale are important.[2, 3] Although many artificial receptors for nucleobase and nucleoside derivatives have been reported,[4] the selective recognition of native oligonucleotides by these receptors remains to be developed.[5] Herein we show the strong binding of designed ferrocene-modified artificial between two cyclopentadienyl (Cp) rings in ferrocene (0.33 nm),[7] which is almost the same as the distance between stacked base pairs in DNA.[8] Thus, when two diamidopyridine moieties (a hydrogen-bonding site for thymine)[9] are connected to the Cp rings of ferrocene through linear ethynediyl spacers, the entropic disadvantages of the receptors during the complexation must be limited to the restricted free rotation of the Cp rings and of the diamidopyridine side chains. This might play a role in the increasing affinities of **1** for TpT. The dinucleotide receptors **1** were synthesized by the Sonogashira reaction between 1,1′-diiodoferrocene (**5**) and 2,6-diamido-4-ethynylpyridines **6** [Eq. (1)].



The recognition abilities of the artificial receptors **1** were first investigated in homogeneous solutions by the use of lipophilic TpT analogue **3** (Scheme 1),[10] to obtain detailed information of the complexation. Receptor **1a** (5.0×10^{-4} M)

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was treated with one equivalent of **3** in $CDCl_3$, and the mixture showed several characteristic changes in the ^1H NMR spectrum: the signals for the NH protons of both **1a** (from $\delta =$ 7.58 to 9.97) and **3** (from $\delta = 7.87$ and 7.89 to 11.40 and 11.60, respectively) had been shifted downfield, thus reflecting the formation of a multipoint hydrogen-bonded complex.[6] The absorption spectra of **1a** at 298 K changed upon the addition of **3** in $CHCl_3$ under the relatively dilute conditions required for assessing the association constant. When **3** was added incrementally to a solution of **1a**, a decrease (at 316, 359, and 456 nm) and an increase (at 380 nm) in the absorbance of **1a** were observed, with several isosbestic points (Figure 1a). From these changes, a 1:1 stoichiometry was confirmed, and the association constant K_a was estimated to be $1.2 \times 10^5\,M^{-1}$ by using an iterative least-squares curve-fitting analysis.[11] Thus, the change in free energy of the complexation ($\Delta G_{298} = -29.0\,kJ\,mol^{-1}$) is, to the best of our knowledge, one of the highest values for artificial receptors for thymine–thymine dinucleotide derivatives.[12]

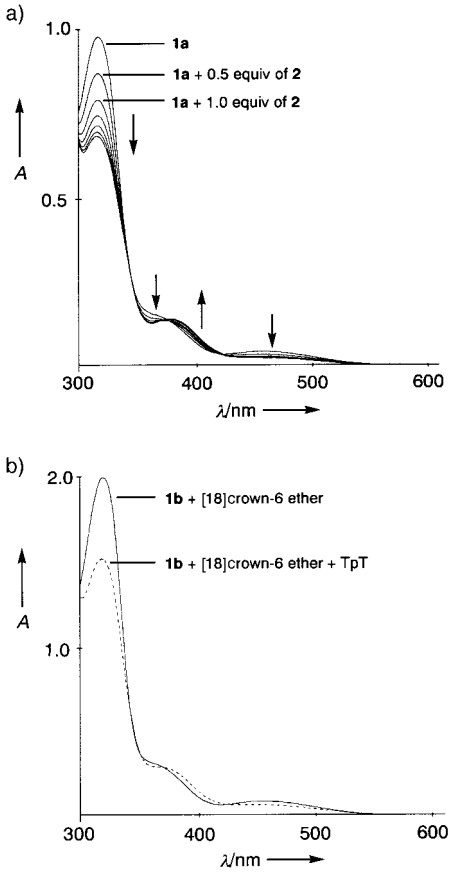

Figure 1. Electronic absorption spectra: a) **1a** (2.5×10^{-5} M) in the presence of **3** (0.5–3.0 equiv). b) **1b** (5.0×10^{-5} M) in the presence of TpT (5.0×10^{-5} M) and [18]crown-6 ether (1.0×10^{-4} M) in $CHCl_3$.

Native dinucleotides are essentially insoluble in $CHCl_3$ and $CDCl_3$. Thus, their extraction into such nonpolar solvents containing the receptors would demonstrate the high recognition efficiency of the receptors. The ammonium salt of native TpT was readily solubilized in $CDCl_3$ by the addition of almost one equivalent of **1b** in the presence of [18]crown-6 ether, and the molar ratio of TpT/**1b** was determined to be approximately 1, as judged from the integration of the ^1H NMR spectrum (Figure 2). A control experiment without

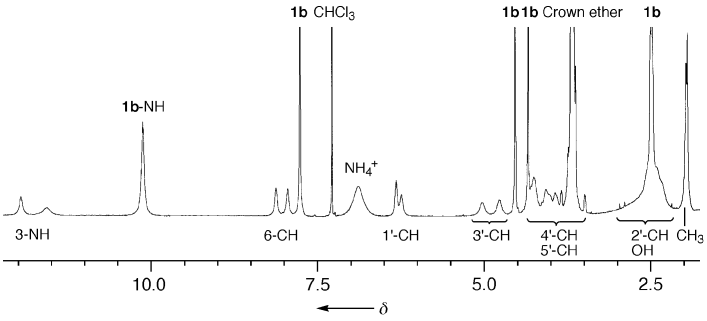

Figure 2. ^1H NMR (400 MHz, $CDCl_3$, 25 °C, $(CH_3)_4Si$) spectrum of the complex between TpT and **1b**. A suspension of TpT (7.7×10^{-3} mmol), **1b** (7.0×10^{-3} mmol), and [18]crown-6 ether (1.4×10^{-2} mmol) in $CDCl_3$ (0.7 mL) was stirred at 25 °C for 1 h. The suspension was filtered, and the filtrate was analyzed directly by ^1H NMR spectroscopy. The signals for receptor **1b** are labeled in the spectrum. Assignments of the TpT signals (depicted below the spectrum) are based on those for **3**. Two sets of signals for the nucleoside residues of TpT cannot be distinguished.

1b revealed that no extraction occurred, which shows that the crown ether only plays a role in the capture of the ammonium countercation. On the other hand, **2** (a flexible analogue of **1**; Scheme 1) and lipophilic dApdA analogue **4** (an antisense strategy) showed much lower extractabilities than **1b** (TpT/**2** $\leq$ 0.3 and TpT/**4** $\leq$ 0.2) under identical experimental conditions. The flexible linkages of **2** and **4** may result in lower extractabilities than that of **1** because of the expected entropic loss during the complexation (see above). In the ^1H NMR spectrum of the extracted solution, the signal for the NH proton of **1b** was shifted downfield from $\delta = 7.58$ to 10.12, and the amide NH protons of the native TpT gave rise to signals at $\delta = 11.58$ and 11.91, which are almost the same chemical shifts as those of the amide NH protons of the bound lipophilic TpT analogue **3** (see above). The absorption spectrum of **1b** showed similar changes to those observed for **1a** and **3** after the extraction (Figure 1b). Furthermore, the complex of achiral **1b** and chiral TpT showed a characteristic induced circular dichroism (ICD) in the visible region of the spectrum of **1b** ($\Delta\varepsilon_{326} = 3.46$; $\Delta\varepsilon_{384} = -0.61$; $\Delta\varepsilon_{430} = 0.23$; $\Delta\varepsilon_{510} = 0.61$). The ICD, which reflects the twisted orientation of the two hydrogen-bonding sites of **1b**, suggests that the Cp rings of the ferrocene modulator can rotate to give optimal hydrogen bonding to the two nucleobase residues of the dinucleotide. The NMR, UV, and CD spectroscopic data obtained from the extraction experiments are extremely important and show that the hydrogen-bonding interaction of the receptors may be a major driving force even in the recognition of native TpT (Scheme 1).

Attempts were made to extract the other native dinucleotides into $CDCl_3$ containing **1b** to assess the specificity of the hydrogen-bonding interaction of **1**. We could not detect any traces of dApdA, dCpdG, TpdA, and even pT under identical experimental conditions. The specific affinity of **1b** to TpT was further demonstrated by the selective extraction of TpT from a mixture of various nucleotides, including TpT. A

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mixture of eight dinucleotides and four mononucleotides was added to a solution of **1b** in $CHCl_3$. The suspension was filtered, and the filtrate was re-extracted with water. HPLC analysis revealed that only TpT existed in the aqueous phase (Figure 3), whereas **1b** remained unchanged in the $CHCl_3$ phase and could be reused without any loss of extraction ability. Excellent selectivity was also observed between

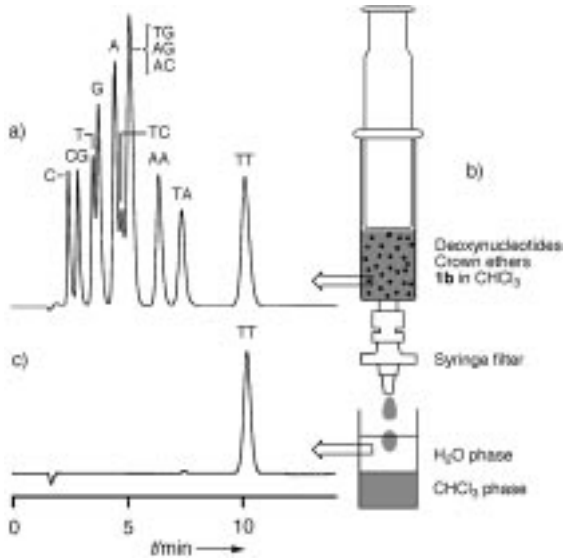

Figure 3. HPLC analyses (column: ODS; eluent: nBu$_4$NBr (5 mM) + K_2HPO_4 (20 mM) in CH_3CN buffer (6% v/v); flow rate: 1.0 mL min^{-1}; temperature: ambient; detection: UV 254 nm). The suspension of a mixture of eight dideoxynucleotides (ammonium salts: 1.1×10^{-3} mmol), four monodeoxynucleotides (sodium salts of 5′-monophosphate: 2.2×10^{-3} mmol), [18]crown-6 ether (for the capture of the ammonium cation; 2.0×10^{-3} mmol), [15]crown-5 ether (for the capture of the sodium cation; 4.0×10^{-3} mmol), and **1b** (1.0×10^{-3} mmol) in $CHCl_3$ (0.2 mL) was stirred at 25 °C for 1 h. a) A small quantity of the suspension was added to H_2O, and the H_2O solution was analyzed by HPLC. The rest of the suspension was filtered, and the filtrate was added to H_2O (0.1 mL). The mixed phases were stirred at 25 °C for 10 min, and b) the H_2O phase was separated by centrifugation. c) The solution in H_2O was analyzed by HPLC, and by ^{1}H NMR spectroscopy.

oligomers of thymidine: only negligible if any extractions of pT (monomer), TpTpT (trimer), or TpTpTpT (tetramer) were observed. These extraction experiments clearly showed that the artificial receptors can perfectly distinguish one particular dinucleotide from other mono- and higher oligomeric nucleotides. Furthermore, the extracted TpT, after evaporation of the water, was found to be very pure by ^{1}H NMR spectroscopic analysis. HPLC purification of nucleotides usually uses various buffer solutions as eluents, so the removal of the buffer agents of inorganic and organic salts is essential to obtain pure nucleotides. The results clearly illustrated the practicability of our receptors: pure dinucleotides can be obtained from mixtures simply by mixing with the receptors, followed by filtration and reextraction.

We also found that the ferrocene linkage was remarkably effective as a modulator of two hydrogen-bonding sites for dinucleotide receptors. There are many synthetic recognition motifs for other nucleobases; these motifs have different recognition strengths and properties, and can easily be connected to ferrocene and other modulator molecules. Thus, the present system demonstrates a new general strategy for the development of practical receptors, each with a unique recognition ability for various di- and oligonucleotides.

Experimental Section

A solution of **5** (162 mg, 0.37 mmol),[13] **6a** (400 mg, 1.84 mmol),[6] [$Pd(Ph_3P)_4$] (35 mg, 30 μmol), and $Cu(OAc)_2 \cdot H_2O$ (6.0 mg, 30 μmol) in iPr$_2$NH (4.0 mL) and *N*,*N*-dimethylformamide (4.0 mL) was stirred at 100 °C for 2 h. After removal of the solvent, the residue was poured into water and extracted with $CHCl_3$. The $CHCl_3$ extract was evaporated and purified by chromatography (silica gel; eluent, CH_2Cl_2/CH_3OH 20:1) to give **1a** (172 mg, 0.28 mmol, 76%). M.p. 170–171 °C; IR (KBr): $\tilde{\nu} = 3169$, 2212, 1684 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, $(CH_3)_4Si$): $\delta = 7.84$ (br s, 4H), 7.74 (br s, 4H), 4.58 (s, 4H), 4.39 (s, 4H), 2.23 (s, 12H); ^{13}C NMR (100 MHz, [D$_6$]DMSO, 25 °C): $\delta = 169.22$, 150.29, 133.66, 109.64, 90.51, 85.65, 72.73, 71.39, 65.66, 24.10; FAB-MS (3-nitrobenzyl alcohol matrix): m/z (%): 617 (100) [MH$^+$]. The reaction of **5** (109 mg, 0.25 mmol) and **6b** (300 mg, 1.00 mmol)[6] in a manner similar to that described for **1a**, led to the formation of **1b** (94 mg, 0.12 mmol, 48%). M.p. 94–95 °C; IR (KBr): $\tilde{\nu} = 3286$, 2214, 1676 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C, $(CH_3)_4Si$) $\delta = 7.93$ (br s, 4H), 7.61 (br s, 4H), 4.59 (t, $J = 1.5$ Hz, 4H), 4.37 (t, $J = 1.5$ Hz, 4H), 2.39 (t, $J = 7.4$ Hz, 8H), 1.75–1.68 (m, 8H), 1.46–1.37 (m, 8H), 0.96 (t, $J = 7.4$ Hz, 12H); ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): $\delta = 171.52$, 149.39, 136.45, 111.14, 92.87, 84.96, 73.31, 72.32, 65.06, 37.49, 27.37, 22.31, 13.80; FAB-MS (3-nitrobenzyl alcohol matrix): m/z (%): 785 (100) [MH$^+$].

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