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Phosphorescent Thymidine Triphosphate Sensor Based on a Donor–Acceptor Ensemble System using Intermolecular Energy Transfer

Tae-Hyuk Kwon,[a, b] Hee Jin Kim,[a] and Jong-In Hong*[a]

Abstract: An ensemble sensor system that exhibited selective luminescence enhancement upon binding to thymidine 5′-triphosphate (TTP) in HEPES buffer over other nucleotides was developed. The ensemble system consisted of an energy acceptor (FIrpic–bis(Zn^{2+}–dipicolylamine conjugate, FIrpic = bis[(4,6-difluorophenyl)-pyridinato-*N*,C^{2+}]picolinate) derivative) and an energy donor (mCP–Zn^{2+}–cyclen, mCP = *N*,*N*′-dicarbazolyl-3,5-benzene). Among the nucleotides, the selective recognition and luminescence enhancement for TTP was achieved by the strong binding of the thymine unit to Zn^{2+}–cyclen (cyclen = 1,4,7,10-tetraazacyclododecane) and intermolecular energy transfer between the mCP and FIrpic moieties.

Keywords: donor–acceptor systems · intermolecular energy transfer · luminescence · phosphorescence · sensors

Introduction

Artificial receptor molecules that enable the direct detection of nucleotides have received considerable attention on account of their responsibility for genetic information storage and transfer in cells. Therefore, recognition of a specific nucleotide is challenging.[1a] As an example, the colorimetric adenosine 5′-triphosphate (ATP) receptor[2] and fluorescent quenching guanosine 5′-triphosphate (GTP) receptor[3] have been reported. The Zn^{II} complex of a marcrocyclic tetraamine (Zn^{2+}–cyclen, cyclen = 1,4,7,10-tetraazacyclododecane) is known to be a thymidine receptor because of the strong affinity of Zn^{2+} for the imide part of the thymine base and the two complementary hydrogen bonds between the two NH groups of Zn^{2+}–cyclen and the two carbonyl oxygen atoms of the thymine base.[1a,4] It is known that thymidylate synthase is necessary to produce thymidine triphosphate, an essential building block for DNA replication and cell division.[1b] Because of the central role of thymidylate synthase in growing cells, it has been used as a target in cancer therapy for many years.[1b] We believe that the development of a thymidine 5′-triphosphate (TTP) sensor would enable monitoring of thymidylate synthase. However, with the exception of a TTP sensor, which shows fluorescence quenching through a photoinduced-electron-transfer mechanism,[4b] there is no report on a TTP sensor that shows luminescence enhancement. Furthermore, until recently, fluorescent sensors have been used as analytical tools for examining biological events. However, they might have several problems, such as strong pH dependence, high photobleaching rates, low photostability, small Stokes shifts, and short lifetimes.[5] To overcome these problems, this paper introduces two concepts, energy transfer and phosphorescence (Figure 1). The former will be very useful for enhancing both the signal and signal-to-noise ratio.[6] In addition, transition-metal complexes using phosphorescence can overcome the problems of organic fluorophores.[5] Based on these concepts, this paper proposes a new paradigm for the phosphorescent TTP sensor.

[a] Dr. T.-H. Kwon,[+] H. J. Kim,[+] Prof. Dr. J.-I. Hong
Department of Chemistry, College of Natural Sciences
Seoul National University, Seoul 151-747 (Korea)
Fax: (+82) 2-889-1568
E-mail: jihong@snu.ac.kr

[b] Dr. T.-H. Kwon[+]
Current address: Bio21 Institute, The School of Chemistry
University of Melbourne, Parkville
Victoria, 3010 (Australia)

[+] These authors contributed equally to this work.

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Results and Discussion

Ensemble system: These concepts for a TTP phosphorescent sensor are based on an emission enhancing ensemble system using intermolecular energy transfer, which shows a high sensitivity and selectivity for TTP among the nucleotides and other various anions. This sensor system consisted of an

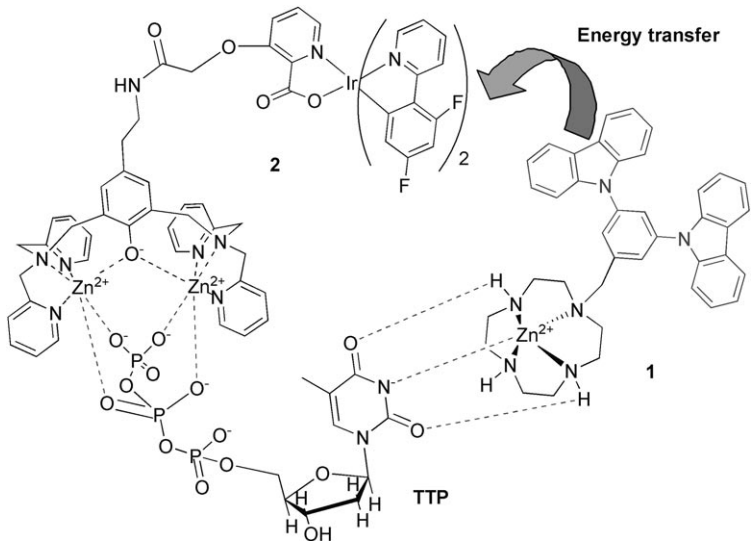


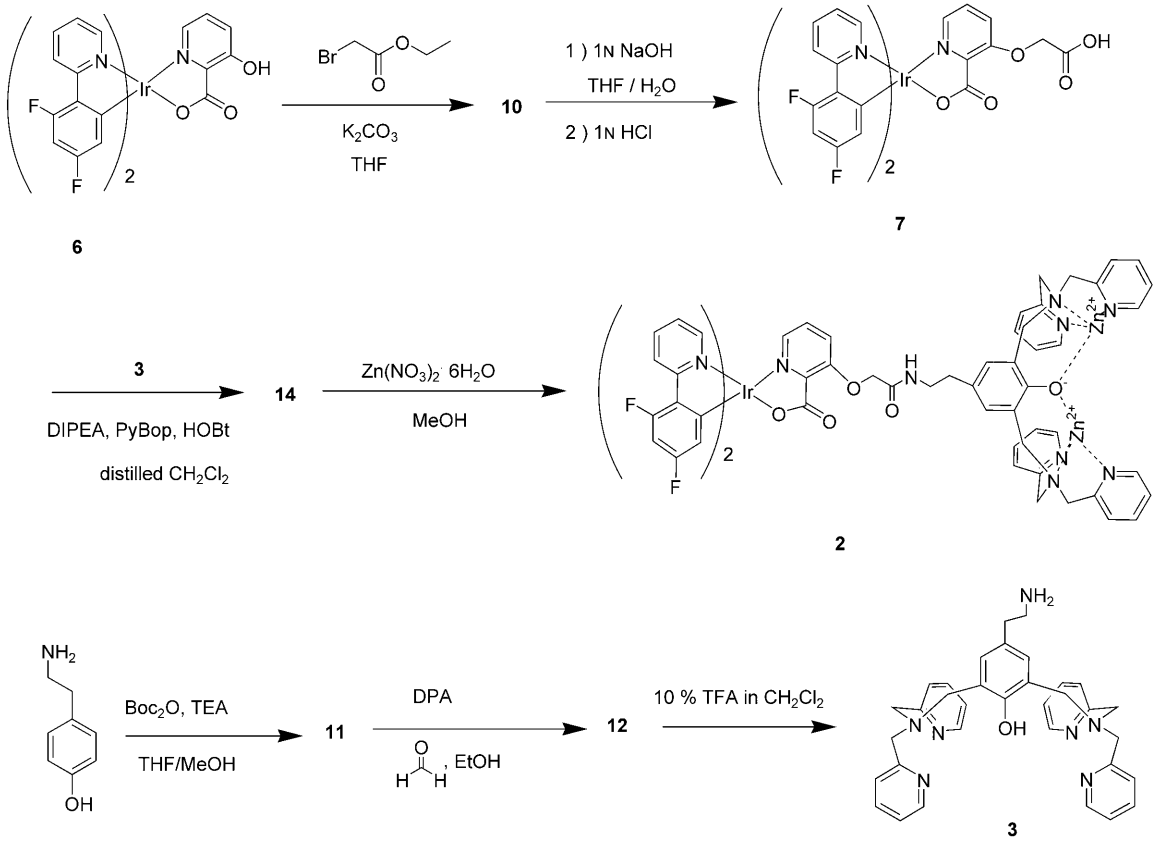
Figure 1. Proposed binding mode between the donor (**1**), TTP, and the acceptor (**2**).

energy acceptor (acceptor (A) = compound **2**, Scheme 2) derived from an iridium(III) complex linked covalently to the Zn^{II}–DPA (DPA = *N,N′*-di(2-picolyl)amine) complex as a binding site for the triphosphate unit of nucleotide guests (ATP, CTP (cytosine 5′-triphosphate), GTP, and TTP), and an energy donor (donor (D) = compound **1**, Scheme 1) derived from the *N,N′*-dicarbazolyl-3,5-benzene (mCP) derivative appended with the Zn^{2+}–cyclen unit (Figure 1). Iridium(III) bis[(4,6-difluorophenyl)-pyridinato-*N,C*$^{2+}$]picolinate (FIrpic), which is a well-known sky-blue dopant material in organic light-emitting diodes, was selected as an energy-acceptor moiety on account of its high quantum efficiency ($\Phi_{PL} = 0.42$). mCP was chosen as an energy-donor unit that demonstrates higher singlet and triplet energies than the acceptor moiety.[7]

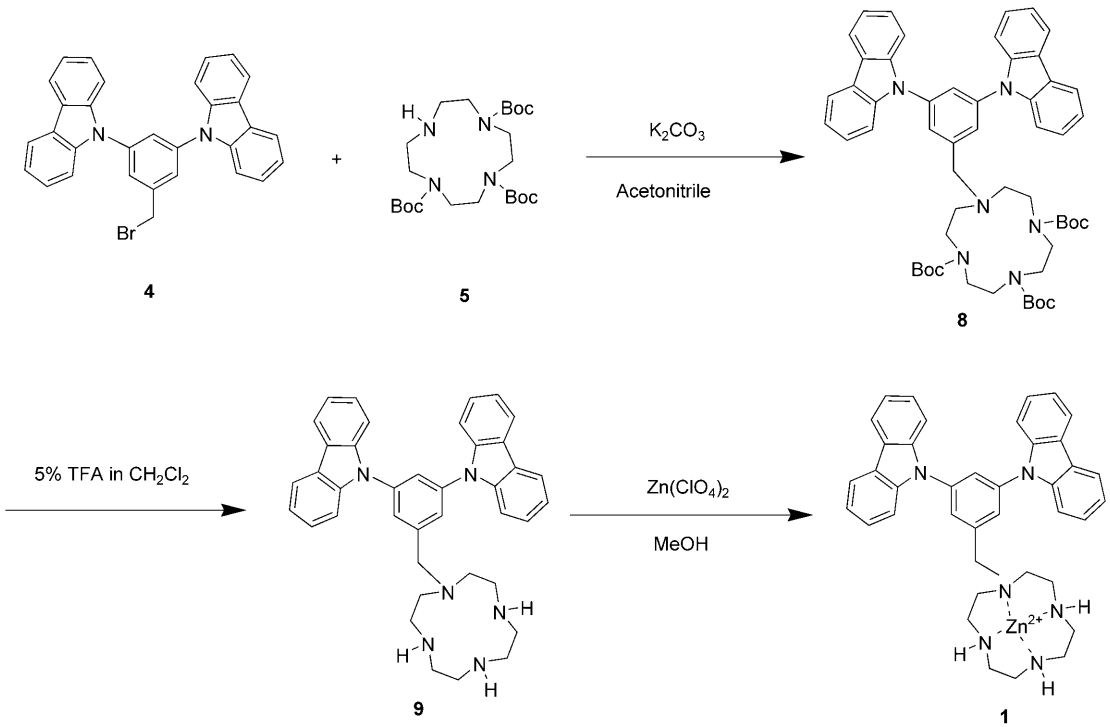
Binding mode: It is expected that both bis(Zn^{2+}–DPA) and Zn^{2+}–cyclen units can be used as multiple bindings for the triphosphate and nucleobase moieties. For example, the bis(Zn^{2+}–DPA) moieties generate an anion-binding site through the formation of a well-known phenoxo-bridged dinuclear metal complex,[8] and two sets of oxygen anions on each phosphorus of a triphosphate moiety bind to the dinuclear zinc complex by binding the two metal ions to give rise to two hexacoordinated Zn^{2+} ions.[8] Zn^{2+}–DPA can also weakly bind with a nucleobase unit of nucleotides, such as imide nitrogen and carbonyl oxygen atoms of the thymine base. Indeed, the Job plot shows 2:1 binding when compound **2** is titrated with TTP in a buffer solution (Figure S1 in the Supporting Information). However, it is believed that the bis(Zn^{2+}–DPA) moiety of **2** binds mainly with the triphosphate unit of TTP. This is because the binding affinity between bis(Zn^{2+}–DPA) and the triphosphate[8] is much stronger than that between bis(Zn^{2+}–DPA) and the imide moiety.

Zn^{2+}–cyclen acts as a monotopic receptor for deoxythymidine (dT) and uridine (U) from all the nucleosides at a physiological pH in aqueous solution through Zn^{2+}–cyclen/imide N^- binding and two complementary hydrogen bonds between the two NH groups of Zn^{2+}–cyclen and the two carbonyl oxygen atoms of the thymine base.[4d] The outstanding Lewis acid properties of a Zn^{2+}–cyclen complex tend to bind with anionic ligands at the vacant fifth coordination site rather than with neutral nitrogen donors.[4b] Therefore, there appears to be a slight interaction between the Zn^{2+}–cyclen complex and the donor sites of nucleobases, such as 2′-deoxyguanosine (dG), 2′-deoxyadenosine (dA), and 2′-deoxycytidine (dC).[4b] However, the Zn^{2+}–cyclen complex can also act as a good monotopic receptor for phosphate moieties in aqueous solution.[4d] Indeed, the Job plot shows 2:1 stoichiometry when compound **1** is titrated with TTP in aqueous solution (Figure S2 in the Supporting Information). Hence, in this ensemble system, there may be a competitive binding mode between the Zn^{2+}–cyclen/triphosphate and bis(Zn^{2+}–DPA)/triphosphate. However, Zn^{2+}–cyclen mainly binds with a nucleobase unit in this ensemble system because the binding constant for bis(Zn^{2+}–DPA)/triphosphate ($\log K \approx 6$–7)[8] is much larger (ca. 3 orders) than that for Zn^{2+}–cyclen/triphosphate ($\log K \approx 3$–4).[4d] To further remove any possibility of Zn^{2+}–cyclen/triphosphate binding, two equivalents of compound **2** were used in the experiment.[9] As a result, it was confirmed that compound **2** binds mainly with the triphosphate unit of TTP, whereas the nucleobase unit of TTP interacts with compound **1** (Figure 1).[9]

Synthesis: Schemes 1 and 2 outline the synthesis of the phosphorescent donor-acceptor ensemble system. Donor **1** was obtained by the three consecutive reactions of alkylation (80% yield), *tert*-butoxycarbonyl (Boc) deprotection (90% yield), and zinc insertion (48% yield; Scheme 1). Acceptor **2** was composed of the iridium complex (**6**) and bis(Zn^{2+}–DPA) derivative (**3**). Compound **3** was synthesized from 4-(2-aminoethyl)phenol, which was protected with Boc_2O and then treated with a solution of DPA and formaldehyde in ethanol. Compound **3** was finally obtained after removing the Boc group with 10% TFA, in an overall yield of 16% (Scheme 2). Compound **6** was coupled with ethyl bromoacetate to give compound **7** in 58% yield after hydrolysis followed by an acid treatment. Amide coupling between compounds **7** and **3** in the presence of *N,N′*-diisopropylethylamine (DIPEA), benzotriazol-1-yl-oxytripyrrolidi-



Scheme 1. Synthesis of Compound 1.



Scheme 2. Synthesis of Compound 2.

nophosphonium hexafluorophosphate (PyBOP), and 1-hydroxybenzotriazole (HOBt), followed by zinc insertion afforded the acceptor **2** of the ensemble system in 19% yield.

Photophysical studies: It was previously reported that the phosphorescent FIrpic acceptor coupled with the dendrimer mCP donor by a nonconjugated bridge showed a dramatic increase in emission intensity compared with that of the acceptor without the donor or just the donor–acceptor blending system when excited at the donor absorption (λ_{ex} = 310 nm).[6] This is because the singlet–singlet (S–S) and triplet–triplet (T–T) energy transfer (ET) can occur efficiently.[6] As shown in Figure 2a, there is a good overlap between the emission spectrum of 5mCP and the absorption spectrum of FIrpic over 350 nm (^{1}MLCT and ^{3}LC region), which ensures S–S ET from mCP to FIrpic. The T–T ET from 5mCP to FIrpic can also occur because the triplet energy of 5mCP is higher than that of FIrpic. Table 1 and Figure 2b shows the

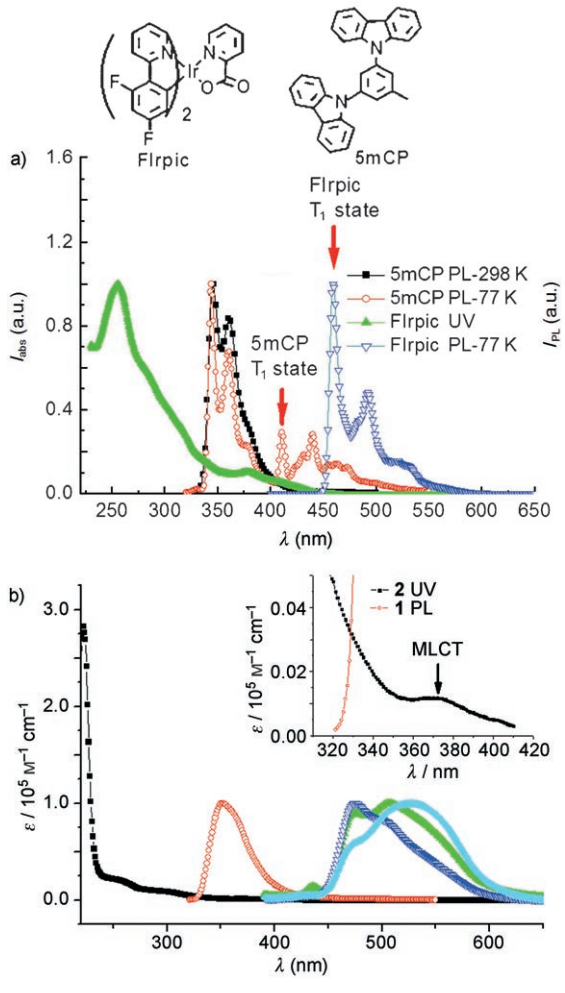


Figure 2. a) UV spectra of FIrpic and PL spectra of 5mCP and FIrpic in 0.02 mM 2-MeTHF at 298 and 77 K. The structures of 5mCP and FIrpic are also shown.[6] b) UV spectra of **2** (■) in 0.01 mM buffer solution, and PL spectra of **1** in 0.01 mM buffer (○), and PL spectra of **2** in 0.01 mM buffer (▲), DMSO (▽), and CH_2Cl_2 (◆), respectively. Inset shows the magnification of the MLCT region of **2**.

Table 1. Photophysical properties of **1** and **2**.

	1	**2**
absorption [nm] ($\varepsilon \times 10^4$ cm^{-1} M^{-1})		
H_2O[a]	221 (29), 289 (0.45) 322 (0.11), 338 (0.11)	222 (28), 253 (21) 300 (0.86), 372 (0.12)
emission [nm]		
H_2O[a]	351, 362 (sh)	475, 507
DMSO[b]	–	475, 500 (sh)
CH_2Cl_2[b]	–	4756, 527

[a] 0.01 mM of **1** or **2** in HEPES buffer solution [b] 0.01 mM solution.

photoluminescence (PL) data of compound **1** and UV and PL data of compound **2** in solution in buffer, CH_2Cl_2, and DMSO. These data are similar to Figure 2a. The luminescence of compound **2** results from both ^{1}MLCT (metal-to-ligand charge transfer, dπ(Ir)→π*(N-O)) and ^{3}LC (ligand centered) in a FIrpic moiety. Photophysical properties of compound **2** are very sensitive to the polarity of the environment due to the MLCT character.[10] Therefore, its emission maximum is found at 475 nm, which is almost identical to that of FIrpic, but emission spectral shape is dependent on the solvent environment (Figure 2b).[11] The absorption spectrum of compound **2** in buffer shows relatively weak MLCT absorption compared with that of FIrpic in 2-methyltetrahydrofuran (2-MeTHF). However, the inset of Figure 2b clearly shows the MLCT region of compound **2**, and the spectral overlap between the absorption spectrum of **2** over 350 nm and the emission spectrum of **1**. We can also assume that **1** and **2** have similar triplet energy values to those of 5mCP and FIrpic, respectively.[12] Therefore, the donor–acceptor ensemble system composed of **1** and **2** is expected to exhibit a good ET from the donor to the acceptor through both the S–S and T–T ET mechanisms. Without TTP, a large amount of donor emission still remained and there is a little acceptor emission intensity upon excitation at the donor (**1**) absorption peak (λ_{ex} = 310 nm) (Figure 4 and Figure S3 in the Supporting Information). This is because the distance between the donor and the acceptor is too far away for efficient intermolecular energy transfer in the absence of TTP. However, the donor emission was reduced dramatically in the 1:1:2 mixture of **1**+TTP+**2** compared with that of **1**+TTP (λ_{ex} = 310 nm), as shown in Figure 3. In addition, the acceptor emission intensity in **1**+TTP+**2** upon excitation at 310 nm increases more than three times compared with that upon excitation at the acceptor absorption (λ_{ex} = 380 nm). This increase is because efficient intermolecular S–S and T–T ET can occur in this ensemble system. Furthermore, it shows little change in luminescence upon the addition of various anions, such as I^-, Cl^-, NO_3^-, CO_3^{2-}, NO_3^{2-}, and ClO_4^-, when excited at 310 nm (Figure 4). This observation means that the 1:1:1 ensemble system does not form in the presence of other anions. Only nucleotides with two strong binding sites can form an ensemble system that enables intermolecular energy transfer.

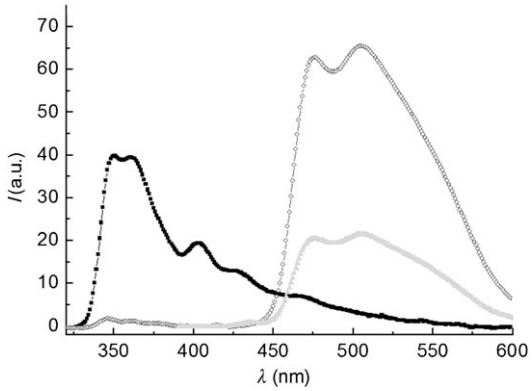


Figure 3. PL spectra of **1** (10 μM) and TTP (10 μM) upon excitation at 310 nm (■) and **1** (10 μM), TTP (10 μM), and **2** (20 μM) upon excitation at 310 nm (○) and 380 nm (▲) upon excitation at 310 nm. respectively, in HEPES buffer at 298 K and pH 7.4.

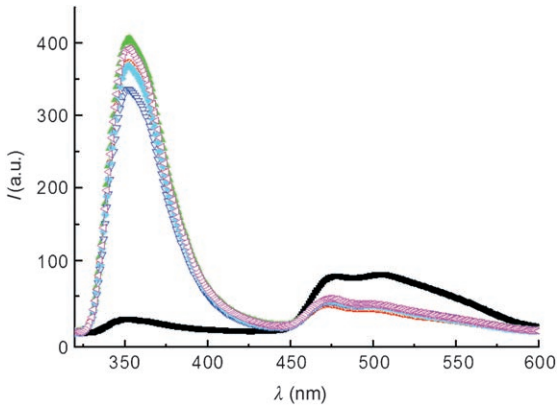


Figure 4. Photoluminescence spectra of **1** (10 μM) and various anions (TTP (■), I^-(○), Cl^- (▲), ClO_4^- (▽), CO_3^{2-} (◆), NO_3^- (◁)) (each 10 μM) and **2** (10 μM) in HEPES buffer at 298 K and pH 7.4, upon excitation at 310 nm.

Energy transfer efficiency and TTP selectivity: Table 2 shows the ET efficiency obtained using the steady-state PL method. The ET efficiency was determined from the extent of luminescence quenching of the donor in the presence of the acceptor.[6] This was measured from the relative ratio between the integrated area of **1**+NTP (I_D) and that of the residual donor peak in **1**+NTP+**2** (I_{D-A}) at 298 K. A decrease in donor emission intensity of the **1**+TTP+**2** system results from intermolecular ET. The ET efficiency from the donor to the acceptor at 298 K was calculated to be 91, 74, 76, and 79% for TTP, ATP, GTP, and CTP, respectively (Table 2; also see the Supporting Information). ET efficiency appears to increase according to the binding strength between the Zn^{2+}–cyclen unit and nucleobase. Therefore, TTP shows the highest ET efficiency of all nucleotides due to Zn^{2+}–cyclen/imide N^- binding and two complementary hydrogen bonds between the two NH groups of Zn^{2+}–cyclen and the two carbonyl O atoms of the thymine base.

Table 2. Energy transfer efficiency.

	Φ_{ET}[a] [%]
TTP	91
ATP	74
GTP	76
CTP	79

[a] The ET efficiency was measured from the relative ratio between the integrated area of **1**+NTP (I_D) and that of the residual donor peak in **1**+NTP+**2** (I_{D-A}). Energy transfer efficiency (%) = (**1**−I_{D-A}/I_D) × 100.

Due to the highest ET efficiency of TTP among the nucleotides examined, the residual donor emission was greatly decreased and the emission intensity of the acceptor in **1**+TTP+**2** was enhanced slightly compared with those of the other nucleotide ensemble systems when excited at the donor absorption (λ_{ex} = 310 nm). In contrast, there was no selectivity when it was excited at the acceptor absorption (λ_{ex} = 380 nm; see the Supporting Information for more details). The acceptor (**2**) emission intensity (I_A) in the TTP ensemble system increases only 1.2 times compared with the other ensemble systems, as shown in the inset of Figure 5a. However, if the ET efficiency is considered, the current ensemble system shows good selectivity for TTP from all nucleotides. Figure 5b shows that TTP can be recognized selectively when the acceptor (**2**) emission intensity (I_A) is divided by the residual donor (**1**) emission intensity (I_D). The

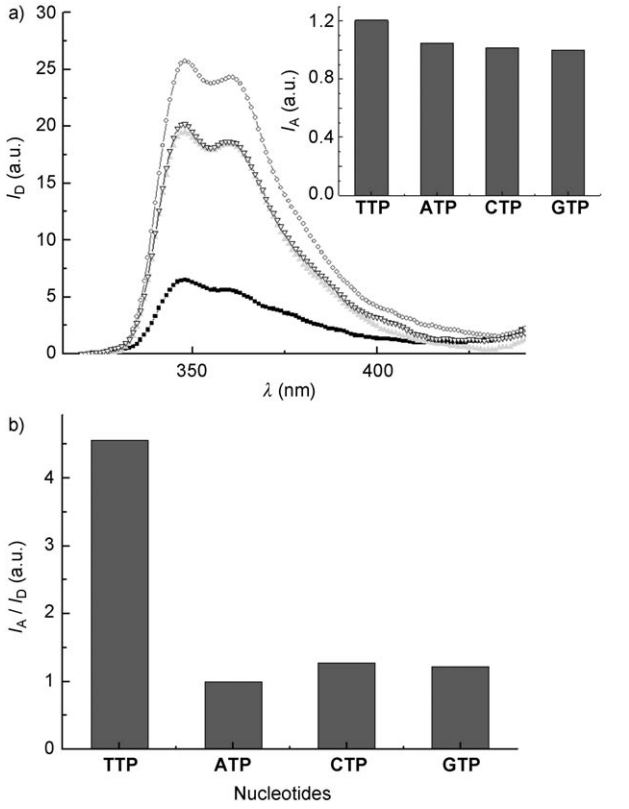


Figure 5. PL spectra of the ensemble system excited at 310 nm and TTP selectivity. a) Donor emission intensity (I_D) of compound **1** (10 μM), TTP (10 μM), and **2** (20 μM) ensemble system, excited at 310 nm. The inset shows the relative acceptor emission intensity at 476 nm (I_A). b) The relative ratios when the acceptor emission intensity (I_A) at 476 nm is divided by the residual donor emission (I_D) at 348 nm.

ratio of I_A/I_D in the TTP ensemble system shows more than four times the enhancement compared with that of other nucleotide ensemble systems. This ensemble system also displayed a relatively small energy transfer effect for ADP, AMP, and $H_2PO_4^{2-}$ (Figure S6 in the Supporting Information). Therefore, TTP selectivity was achieved.

As a result, this phosphorescent ensemble system can be used for the selective recognition of TTP due to its intermolecular ET efficiency.

Conclusion

A phosphorescent TTP sensor was developed through a donor–acceptor ensemble system using intermolecular ET. Out of all nucleotides, TTP shows the best ET efficiency (>90%) due to the strong binding between Zn^{2+}–cyclen and the nucleobase. This system can be used for the selective sensing of TTP from the nucleotides on account of the intermolecular ET.

Experimental Section

Reagents: Buffer A solution contained 10 mM HEPES adjusted to pH 7.4 with NaOH. To make compounds **1** and **2** soluble in aqueous solvent, a cosolvent system (H_2O/DMSO = 24:1) was used. The tetrasodium salt of TTP and the disodium salt of GTP were purchased from Fluka. The disodium salt of ATP was purchased from Sigma. The disodium salt of CTP was purchased from Aldrich. DIPEA, HOBt, and PyBOP were purchased from Aldrich. Analytical thin-layer chromatography was performed on Kieselgel 60F-254 plates obtained from Merck. Column chromatography was carried out on Merck silica gel 60 (70–230 mesh). All solvents and reagents were commercially available and used without further purification unless otherwise noted.

Instruments: ^{1}H and ^{13}C NMR spectra were recorded using an Advance 300 MHz Bruker spectrometer in $CDCl_3$ and [D$_6$]DMSO. UV/Vis spectra were recorded on a Beckman DU 650 spectrophotometer. Mass spectra were obtained using a MALDI-TOF mass spectrometer from Bruker or a gas chromatography–mass spectrometer from JEOL. Fluorescence spectra were recorded on a Jasco FP-7500 spectrophotometer.

Compound (4): This compound was prepared according to the literature procedure.[6]

Compound (5): This compound was prepared according to the literature procedure.[13]

Compound 8: (10-(3,5-Bis-carbazole-9-yl-benzyl)-1,4,7-tris(*tert*-butyloxycarbonyl)-1,4,7,10-tetraazacyclododecane): A mixture of 5mCP-Br (**4**) (100 mg, 0.188 mmol), 3Boc–cyclen (**5**, 170 mg, 0.360 mmol), K_2CO_3 (62.4 mg, 0.451 mmol) was heated at reflux in acetonitrile for 24 h. After cooling to room temperature, the solvent was removed in vacuo and dissolved in ethyl acetate. The organic phase was washed with water, washed with brine, and dried over Na_2SO_4. The solvent was evaporated to give the crude product, which was purified by column chromatography on silica gel using ethyl acetate/hexane (1:3, v/v) as the eluent to provide the desired product as a white powder (132 mg, 78.5%). ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 8.23 (d, J(H,H) = 7.7 Hz, 4H), 7.81 (s, 3H), 7.61 (d, J(H,H) = 8.2 Hz, 4H), 7.50 (t, J(H,H) = 7.5 Hz, 4H), 7.31 (t, J(H,H) = 7.4 Hz, 4H), 4.06 (s, 2H), 3.62 (br, 4H), 3.46 (br, 8H), 2.86 (br, 4H), 1.47 (s, 9H), 1.28 ppm (s, 18H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 156.3, 155.9, 141.3, 140.7, 139.2, 128.0, 126.3, 124.7, 123.6, 120.4, 120.3, 109.6, 79.7, 79.5, 56.8, 56.0, 55.2, 50.3, 47.8, 28.7, 28.4 ppm; MALDI-TOF: m/z calcd for $C_{54}H_{66}N_6O_6$ $[M+2H]^+$: 894.504; found: 894.417.

Compound 9: Trifluoroacetic acid (0.26 mL) was slowly added to a solution of compound **8** (118 mg, 0.132 mmol) in CH_2Cl_2 (5 mL). After being stirred at room temperature for 24 h, the solvent was removed in vacuo and dissolved in CH_2Cl_2. The organic phase was washed with water and dried over Na_2SO_4. The reaction mixture was concentrated under reduced pressure. The resulting crude powder was crystallized from CH_2Cl_2/hexane to provide the desired product as a white powder (70 mg, 89.4%). ^{1}H NMR (300 MHz, [D$_3$]acetonitrile): δ = 8.22 (d, J(H,H) = 7.7 Hz, 4H), 7.78 (s, 3H), 7.64 (d, J(H,H) = 8.2 Hz, 4H), 7.50 (t, J(H,H) = 8.2 Hz, 4H), 7.34 (t, J(H,H) = 7.4 Hz, 4H), 4.10 (s, 2H), 3.20 (br, 4H) 3.02 (m, 8H), 2.88 ppm (br, 4H); ^{13}C NMR (125 MHz, [D$_6$]acetone): δ = 140.9, 140.8, 139.4, 127.7, 126.3, 124.9, 123.4, 120.2, 120.2, 110.1, 56.5, 48.6, 45.0, 42.7, 42.6 ppm; HRMS (FAB): m/z calcd for $C_{39}H_{41}N_6$ $[M+H]^+$: 593.3393; found: 593.3395.

Compound 1: Compound **9** (40 mg, 0.0612 mmol) in MeOH (1 mL) and $Zn(ClO_4)_2 \cdot 6H_2O$ in MeOH (1 mL) were combined and heated at reflux for 1 h under nitrogen. Most of the solvent was removed in vacuo, and the precipitated product was collected by filtration and dried in vacuo to yield the product as a white powder (22 mg, 48.4%). M.p. 200°C; ^{1}H NMR (300 MHz, CD_3CN): δ = 8.24 (d, J(H,H) = 7.7 Hz, 4H), 8.01 (d, J(H,H) = 15.9 Hz, 1H), 7.75 (d, J(H,H) = 11.5 Hz, 2H), 7.62 (t, J(H,H) = 8.2 Hz, 4H), 7.56–7.50 (m, 4H), 7.36 (t, J(H,H) = 7.4 Hz, 4H), 4.21 (s, 1H), 4.10 (s, 1H), 3.22–2.74 ppm (m, 16H); ^{13}C NMR (125 MHz, CD_3CN): δ = 140.7, 139.3, 138.5, 128.8, 128.0, 126.4, 125.4, 123.4, 120.5, 109.9, 55.9, 49.2, 47.6, 45.0, 44.5, 43.7, 42.5, 42.3, 41.7 ppm; HRMS (FAB): m/z calcd for $C_{39}H_{40}N_6ZnClO_4$ $[M]^+$: 755.2091; found: 755.2072.

Compound 6: This compound was prepared according to the literature procedure.[6]

Compound 10: A mixture of **6** (100 mg, 0.141 mmol), bromoacetic acid ethyl ester (94 mg, 0.563 mmol), and K_2CO_3 (29 mg, 0.210 mmol) was heated at reflux in THF for 24 h. After cooling to room temperature, the solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2. The organic phase was washed with water, washed with brine, and dried over Na_2SO_4. The solvent was evaporated to give the crude product, which was purified by column chromatography on silica gel with CH_2Cl_2/methanol (100:1, v/v) as the eluent to provide the desired product as a yellow powder (75 mg, 67%). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 8.59 (d, J(H,H) = 5.3 Hz, 1H), 8.31–8.23 (m, 2H), 8.11–8.01 (m, 2H), 7.74 (d, J(H,H) = 8.6 Hz, 1H), 7.67 (d, J(H,H) = 5.5 Hz, 1H), 7.56–7.49 (m, 2H), 7.37–7.33 (m, 2H), 6.87–6.76 (m, 2H), 5.68 (dd, J(H,H) = 8.6, 2.2 Hz, 1H), 5.45 (dd, J(H,H) = 8.6, 2.2 Hz, 1H), 4.99 (s, 2H), 4.15 (q, J(H,H) = 7.1 Hz, 2H), 1.18 ppm (t, J(H,H) = 7.1 Hz, 3H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 170.2, 168.6, 163.5, 158.3, 153.9, 149.3, 148.5, 142.1, 139.8, 138.9, 130.4, 128.3, 126.4, 124.4, 124.0, 123.1, 114.1, 98.0, 66.2, 61.3, 14.4 ppm; MALDI-TOF: m/z calcd for $C_{32}H_{23}F_4IrN_3O_5$ $[M+H]^+$: 798.120; found: 798.448.

Compound 7: A 1N aqueous solution of NaOH (1 mL) was added to a solution of **10** (75 mg, 0.0941 mmol) in THF/H_2O (1 mL/1 mL). After stirring for 2 h at room temperature, the reaction mixture was acidified with 1N HCl. The solvent was removed in vacuo and dissolved in CH_2Cl_2. The organic phase was washed with water and dried over Na_2SO_4. The solvent was evaporated to give the desired product as a yellow powder (63 mg, 87%). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 13.3 (br, 1H), 8.59 (d, J-(H,H) = 5.3 Hz, 1H), 8.30–8.22 (m, 2H), 8.09–8.00 (m, 2H), 7.78 (d, J-(H,H) = 8.6 Hz, 1H), 7.67 (d, J(H,H) = 5.4 Hz, 1H), 7.58–7.49 (m, 2H), 7.39–7.33 (m, 2H), 6.86–6.73 (m, 2H), 5.68 (dd, J(H,H) = 8.6, 2.1 Hz, 1H), 5.46 (dd, J(H,H) = 8.6, 2.2 Hz, 1H), 4.92 ppm (s, 2H); ^{13}C NMR (125 MHz, [D$_6$]DMSO): δ = 170.7, 170.0, 164.0, 158.5, 153.5, 149.4, 148.5, 142.2, 139.9, 138.7, 130.6, 128.3, 126.8, 124.5, 124.1, 123.4, 114.1, 98.0, 66.7 ppm; MALDI-TOF: calcd for $C_{30}H_{19}F_4IrN_3O_5$ $[M+H]^+$: 770.089; found: 770.530.

Compound 14: A mixture of compound **7** (32 mg, 0.042 mmol), DIPEA (11 mg, 0.085 mmol), PyBOP (21 mg, 0.041 mmol), and HOBt (3 mg, 0.040 mmol) was stirred in distilled CH_2Cl_2 (4 mL) for 1 h , followed by slowly adding compound **3** in CH_2Cl_2 (1 mL). After stirring at room temperature for 7 h, the mixture was diluted with CH_2Cl_2, and washed with water and brine. The organic phase was dried over Na_2SO_4 and evaporated to dryness. The residue was purified by column chromatography on

silica gel with CH_2Cl_2/methanol (20:1, v/v) as the eluent to provide the desired product as a yellow powder (10 mg, 18.6%). ^{1}H NMR (300 MHz, [D$_6$]DMSO): δ = 9.04 (br, 1H), 8.54–8.50 (m, 5H), 8.25 (d, J(H,H) = 8.3 Hz, 2H), 8.06–8.00 (m, 3H), 7.81 (d, J(H,H) = 8.6 Hz, 1H), 7.74–7.67 (m, 6H), 7.61–7.57 (m, 1H), 7.44 (t, J(H,H) = 6.7 Hz, 1H), 7.38–7.27 (m, 8H), 7.11 (s, 2H), 6.90–6.76 (m, 2H), 5.66 (dd, J(H,H) = 8.7, 2.2 Hz, 1H), 5.46 (dd, J(H,H) = 8.6, 2.1 Hz, 1H), 4.65 (s, 2H), 4.01 (br, 12H), 3.02 (br, 2H), 2.66 ppm (t, J(H,H) = 7.1 Hz, 2H); ^{13}C NMR (125 MHz, [D$_6$]acetone): δ = 172.1, 167.1, 164.4, 163.9, 157.9, 155.6, 155.0, 153.9, 149.3, 149.0, 148.3, 142.5, 139.2, 138.5, 137.2, 131.8, 130.6, 129.8, 128.4, 125.7, 123.7, 123.5, 123.2, 122.9, 121.4, 114.3, 97.4, 68.3, 58.1, 55.1, 46.1, 34.2; HRMS (FAB): m/z calcd for $C_{64}H_{54}F_4IrN_{10}O_5$ $[M+H]^+$: 1311.3844; found: 1311.3820.

Compound 2: An aqueous solution of $Zn(NO_3)_2{\cdot}6H_2O$ (7 mg, 0.024 mmol) was added dropwise to a solution of **14** (15 mg, 0.011 mmol) in methanol (1.5 mL), and the mixture was stirred for 30 min at room temperature. The solvent was evaporated to give the desired product as a yellow powder (19 mg, 100%). M.p. 233 °C; MALDI-TOF: m/z calcd for $C_{64}H_{52}F_4IrN_{10}O_5{\cdot}2Zn{\cdot}2NO_3^-$ $[M]^+$: 1561.2027; found: 1561.2048.

Compound 11: This compound was prepared according to the literature procedure.[11]

Compound 12: DPA (4.17 g, 20.9 mmol) was added slowly to a solution of 37% aqueous formaldehyde (1.86 g, 22.9 mmol) and ethanol (5 mL). The reaction mixture was heated at reflux for 12 h, and compound **11** (2.36 g, 9.95 mmol) in ethanol (20 mL) was added. After being heated at reflux for 5 d, the reaction mixture was cooled down to room temperature. The solvent was removed in vacuo and the residue was dissolved in ethyl acetate. The organic phase was washed with water and dried over Na_2SO_4. The solvent was evaporated to give the crude product, which was purified by column chromatography on silica gel with CH_2Cl_2/methanol (200:1, v/v) as the eluent to provide the desired product as a brown sticky liquid (1.72 g, 26.2%). ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 10.95 (s, 1H), 8.52 (d, J(H,H) = 4.7 Hz, 4H), 7.72 (t, J(H,H) = 7.6 Hz, 4H), 7.58 (d, J(H,H) = 7.8 Hz, 4H), 7.22 (t, J(H,H) = 6.1 Hz, 4H), 7.11 (s, 2H), 6.02 (s, 1H), 3.86 (s, 8H), 3.74 (s, 4H), 3.27 (q, J(H,H) = 6.7 Hz, 2H), 2.69 (t, J(H,H) = 7.3 Hz, 2H), 1.37 ppm (s, 9H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 159.0, 155.9, 154.3, 148.6, 136.3, 129.5, 128.6, 123.8, 122.8, 121.8, 78.3, 59.6, 54.7, 42.0, 35.2, 28.3; MALDI-TOF: m/z calcd for $C_{39}H_{46}N_7O_3$ $[M+H]^+$: 660.366; found: 660.588.

Compound 3: Trifluoroacetic acid (2.2 mL) was added slowly to a solution of **12** (467 mg, 0.71 mmol) in CH_2Cl_2 (20 mL). After being stirred at room temperature for 12 h, the solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2. The organic phase was washed with water and neutralized with an aqueous solution of $NaHCO_3$, and dried over Na_2SO_4. The solvent was evaporated to give the desired product as a brown sticky liquid (326 mg, 82.3%). ^{1}H NMR (300 MHz, [D$_6$]acetone): δ = 8.52 (d, J(H,H) = 4.4 Hz, 4H), 7.71 (t, J(H,H) = 7.6 Hz, 4H), 7.58 (d, J(H,H) = 7.8 Hz, 4H), 7.22 (t, J(H,H) = 6.0 Hz, 4H), 7.12 (s, 2H), 3.86 (s, 8H), 3.79 (s, 4H), 3.41 (t, J(H,H) = 7.4 Hz, 2H), 2.76 ppm (t, J(H,H) = 7.4 Hz, 2H); ^{13}C NMR (125 MHz, $CDCl_3$): δ = 158.6, 154.6, 148.6, 136.6, 129.7, 126.8, 123.9, 123.1, 122.3, 59.9, 54.5, 41.2, 33.3; MALDI-TOF: m/z calcd for $C_{34}H_{38}N_7O$ $[M+H]^+$: 560.314; found: 560.500.

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[9] In this system, two equivalents of compound **2** were used because an excess of compound **2** can prevent any possible binding between the Zn^{2+}–cyclen moiety of compound **1** and the phosphate moiety of TTP. Although two equivalents of compound **2** and one equivalent of TTP can form 2:1 binding, the addition of one equivalent of compound **1** to this solution would cause weak binding between the Zn^{2+}–DPA unit of compound **2** and the imide unit of TTP to be easily broken and then replaced with stronger binding between the Zn^{2+}–cyclen unit of compound **1** and the imide part of TTP. In addition, the residual compound **2** has little influence on the emission intensity when excited at the donor absorption because of the long distance between the donor and the acceptor, as shown in the Supporting Information. Therefore, we can expect that the ensemble system shows 1:1:1 binding mode, as shown in Figure 1.

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[12] We assume that the mCP moiety of **1** and the FIrpic moiety of **2** are located within the effective Dexter energy-transfer distance (<20 Å) for a good wavefunction overlap between mCP and FIrpic upon 1:1:1 complexation, as shown in Figure 1.[6]

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