Supplementary Information

Metal-mediated DNA base pairing of easily prepared 2-oxo-imidazole-4-carboxylate nucleotides

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Contents

1. Experimental procedures

General. All reactions were carried out under nitrogen atmosphere with commercial dehydrated solvents (FUJIFILM Wako Pure Chemical Industries). The reagents for the nucleoside synthesis were purchased from FUJIFILM Wako Pure Chemical Industries and Tokyo Chemical Industry (TCI) and were used without further purification. Nucleoside **3** was prepared according to the reported procedure.[1] Silica gel column chromatography was performed using Merck Silica Gel 60 (230–400 mesh). All NMR spectra were measured on a Bruker AVANCE 500 spectrometer (500 MHz for ^{1}H, 202 MHz for ^{31}P, and 126 MHz for ^{13}C). The spectra were referenced to tetramethylsilane (TMS) in $CDCl_3$ (δ 0 ppm). Electrospray ionisation-time-of-flight (ESI-TOF) mass spectra were recorded on a Waters LCT Premier XE. All the natural DNA strands were purchased from Japan Bio Service Co., Ltd. (Saitama, Japan) at HPLC purification grade. The concentration of DNA strands was determined based on the UV absorbance at 260 nm. The molar extinction coefficients (ε_{260}) of the DNA strands were calculated by the nearest neighbor method. For both **Im$^{\mathbf{OC}}$**- and **Im$^{\mathbf{OA}}$**-containing strands, ε_{260} of the unnatural nucleotides was deemed to be zero. Metal sources were purchased from FUJIFILM Wako Pure Chemical Industries ($CuSO_4 \cdot 5H_2O$ (99.5% purity), $MnCl_2 \cdot 4H_2O$ (99%), $FeCl_2 \cdot 4H_2O$ (99.0–102.0%), $CoCl_2$ (99.0–102.0%), K_2PtCl_4 (98%), $Hg(ClO_4)_2 \cdot 3H_2O$ (99%), $AgNO_3$ (99.8%)), Soekawa Chemical Co. ($NiCl_2 \cdot 6H_2O$ (99%), $ZnSO_4 \cdot 7H_2O$ (99.9%)), and Tokyo Chemical Industry (Na_2PdCl_4 (98%)).

Compound **4**. To a solution containing nucleoside **3** (462 mg, 0.83 mmol) and diisopropylethylamine (545 µL, 3.30 mmol, 4.0 eq) in CH_2Cl_2 (8.3 mL) was added 2-cyanoethyl *N*,*N*-diisopropylchlorophosphoramidite (276 µL, 1.24 mmol, 1.5 eq). After stirred for 0.5 h at room temperature, the reaction mixture was diluted with AcOEt (50 mL) and washed with saturated Na_2CO_3 solutions (50 mL × 2) and brine (50 mL). The organic layer was dried over anhydrous Na_2SO_4 and then evaporated to dryness. The residue was purified by silica gel column chromatography (*n*-hexane:AcOEt = 1:2 containing 1.0% triethylamine) to afford phosphoramidite **4** (488 mg, 0.64 mmol, 78%, a diastereomeric mixture). ^{1}H NMR and ^{31}P NMR spectra indicated the existence of two diastereomers. The ^{13}C NMR spectrum of the diastereomeric mixture was also measured and partially assigned with an HSQC measurement. The phosphoramidite was immediately used for the DNA synthesis.

^{1}H NMR (500 MHz, $CDCl_3$, 300 K): δ 8.13 (br, 1H), 7.47–7.18 (m, 10H), 6.86–6.79 (m, 4H), 6.10–6.04 (m, 1H), 4.65–4.57 (m, 1H), 4.18–4.13 (m, 1H), 3.86–3.53 (m, 13H), 3.36–3.24 (m, 2H), 2.64–2.29 (m, 4H), 1.21–1.02 (m, 12H).

^{31}P NMR (202 MHz, CDCl$_3$, 300 K): δ 148.80, 148.56.

HRMS (ESI-TOF) *m*/*z*: [M + Na]$^+$ calcd for $C_{40}H_{49}N_4O_9PNa$: 783.3135, found: 783.3148.

DNA synthesis. DNA strands containing either **Im^{OA}** or **Im^{OC}** were synthesised on an automated DNA synthesiser (NTS M-4-MX DNA/RNA synthesiser) according to the standard procedure. The DNA synthesis was carried out on a 1-μmol scale in a DMTr-on mode with ultramild deprotection phosphoramidites and reagents (Glen Research). The coupling time for the unnatural nucleosides was extended to 15 min. The oligonucleotides were detached from the solid supports by 2-h incubation with a 0.3 M NaOH (for **Im^{OC}**-containing strands) or a 25% ammonia aqueous solution (for **Im^{OA}**-containing strands) at room temperature. The supernatants were further incubated at 37 °C (for **Im^{OC}**) or 55 °C (for **Im^{OA}**) for 22 h. The obtained products were detritylated and purified using Glen-pak cartridges (Glen research) according to the manufacturer's protocol. Further purification by reverse-phase HPLC (Waters XBridge Oligonucleotide BEH C18 column, buffers: A = MeCN, B = 0.1 M TEAA (pH 7.0) + 2% MeCN, 60 °C) afforded the desired DNA strands, which were identified by ESI-TOF mass spectrometry. The synthesis of the **Im^{C}**-containing strands (**7** and **8**) was reported previously.[2]

Im^{OC}-TXT. 5′-T**Im^{OC}**T-3′. HPLC retention time: 11.4 min (gradient: 2%A (0 min), 4%A (40 min)). ESI MS: *m*/*z* calcd for $[C_{29}H_{38}N_6O_{20}P_2 - H]^-$: 851.15; found: 851.11.

Im^{OA}-TXT. 5′-T**Im^{OA}**T-3′. HPLC retention time: 24.8 min (gradient: 1%A (0 min), 3%A (40 min)). ESI MS: *m*/*z* calcd for $[C_{29}H_{39}N_7O_{19}P_2 - H]^-$: 850.17; found: 850.09.

DNA 1. 5′-CAC ATT A**Im^{OC}**T GTT GTA-3′. HPLC retention time: 13.5 min (gradient: 5%A (0 min), 7%A (40 min)). ESI MS: *m*/*z* calcd for $[C_{147}H_{186}N_{50}O_{92}P_{14} - 4H]^{4-}$: 1138.44; found: 1138.45. $\varepsilon_{260} = 1.40 \times 10^5$ M^{-1} cm^{-1}.

DNA 2. 5′-TAC AAC A**Im^{OC}**T AAT GTG-3′. HPLC retention time: 13.6 min (gradient: 5%A (0 min), 7%A (40 min)). ESI MS: *m*/*z* calcd for $[C_{147}H_{184}N_{56}O_{88}P_{14} - 4H]^{4-}$: 1143.19; found: 1143.09. $\varepsilon_{260} = 1.47 \times 10^5$ M^{-1} cm^{-1}.

DNA 3. 5′-CAC ATT A**Im^{OA}**T GTT GTA-3′. HPLC retention time: 14.4 min (gradient: 5%A (0 min), 7%A (40 min)). ESI MS: *m*/*z* calcd for $[C_{147}H_{187}N_{51}O_{91}P_{14} - 4H]^{4-}$: 1138.19; found: 1138.21. $\varepsilon_{260} = 1.40 \times 10^5$ M^{-1} cm^{-1}.

DNA 4. 5′-TAC AAC A**Im^{OA}**T AAT GTG-3′. HPLC retention time: 14.1 min (gradient: 5%A (0 min), 7%A (40 min)). ESI MS: *m*/*z* calcd for $[C_{147}H_{185}N_{57}O_{87}P_{14} - 4H]^{4-}$: 1142.70; found: 1142.71. $\varepsilon_{260} = 1.47 \times 10^5$ M^{-1} cm^{-1}.

DNA 5. 5′-CAC ATT **Im^{OC}Im^{OC}Im^{OC}** GTT GTA-3′. HPLC retention time: 12.3 min (gradient: 5%A (0 min), 7%A (40 min)). ESI MS: *m*/*z* calcd for $[C_{145}H_{183}N_{47}O_{96}P_{14} - 4H]^{4-}$: 1137.17;

found: 1137.12. ε_{260} = 1.18 × 10^5 M^{-1} cm^{-1}.

DNA 6. 5′-TAC AAC **Im^{OC}Im^{OC}Im^{OC}** AAT GTG-3′. HPLC retention time: 12.2 min (gradient: 5%A (0 min), 7%A (40 min)). ESI MS: *m*/*z* calcd for $[C_{145}H_{181}N_{53}O_{92}P_{14} - 4H]^{4-}$: 1141.68; found: 1141.81. ε_{260} = 1.25 × 10^5 M^{-1} cm^{-1}.

Duplex melting analysis. All samples were prepared by combining the DNA strands (2.0 μM each) in a 10 mM HEPES buffer (pH 7.0) solution containing 100 mM NaCl or 100 mM $NaNO_3$ (for Hg^{II} and Ag^{I}). After the addition of metal ions, the mixtures were annealed (85 or 60 °C → 4 °C, 1.0 °C/min) prior to the measurements. Absorbance at 260 nm (A_{260}) was recorded on UV-1700, UV-1800 and UV-1900 spectrophotometers (Shimadzu) equipped with a TMSPC-8 temperature controller. The temperature was raised from 4 °C to 60 °C at the rate of 0.2 °C/min. The melting curves were depicted after normalisation as follows:

Normalised $A_{260} = \{A_{260}\ (t\ °C) - A_{260}\ (4\ °C)\}/\ \{A_{260}\ (60\ °C) - A_{260}\ (4\ °C)\} \times 100$.

The melting temperature (T_m) was determined as an inflection point of a melting curve using a T_m analysis software LabSolutions (Shimadzu) with a 17-point adaptive smoothing program. Average T_m values of three independent runs are shown.

CD spectroscopy. The samples were prepared as described above. CD spectra were recorded on a JASCO J-820 spectropolarimeter with 10-time accumulation using a path length of 0.5 cm at 4 °C. The spectra were smoothed using a simple moving average smoothing program.

Mass spectrometry. The metallo-DNA samples were prepared in 20 mM NH_4OAc buffer (pH 7.0) and annealed prior to the measurements (60 °C → 4 °C, 1.0 °C/min). ESI-TOF mass spectra were recorded in a negative mode.

2. Supplementary figures

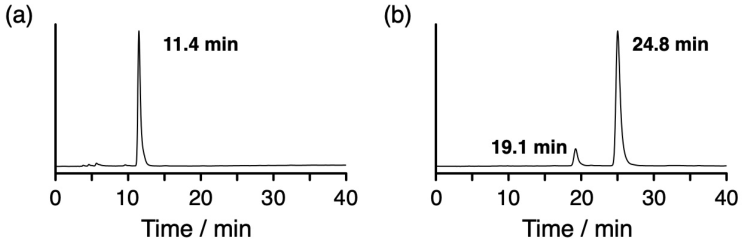


Fig. S1. HPLC analysis of 5′-T**X**T-3′ after deprotection using a (a) NaOH or (b) ammonia solution. The major peak in (b) is the target oligonucleotide 5′-T**Im^{OA}**T-3′ (~ 90% of the whole peak area). Waters XBridge Oligonucleotide BEH C18 column (4.6 × 50 mm), flow rate: 1.0 mL/min, temperature: 60 °C, monitored at 260 nm. Gradient: (a) 0 to 40 min, 2% A to 4% A, (b) 0 to 40 min, 1% A to 3% A (solvent A = MeCN, solvent B = 0.1 M TEAA buffer (pH 7.0) + 2% MeCN).

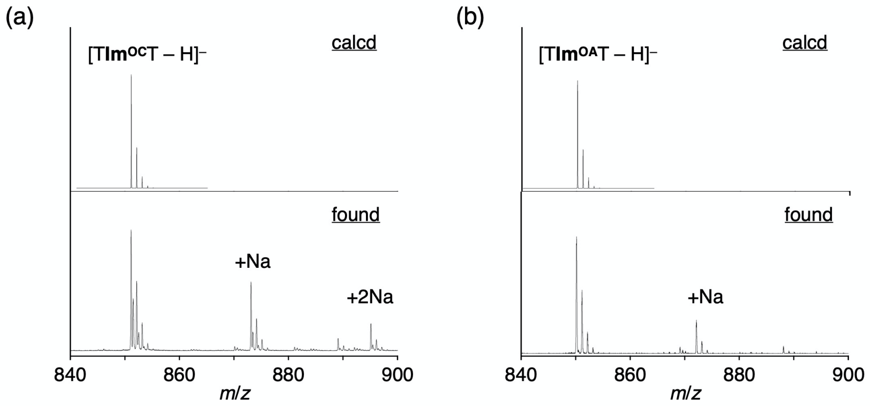


Fig. S2. ESI-TOF mass spectra of DNA strands (a) 5′-T**Im**$^{\mathbf{OC}}$T-3′and (b) 5′-T**Im**$^{\mathbf{OA}}$T-3′. 5′-T**Im**$^{\mathbf{OC}}$T-3′ = $C_{29}H_{38}N_6O_{20}P_2$ (found: 851.11 (z = 1); calcd for $[M–H]^-$: 851.15). 5′-T**Im**$^{\mathbf{OA}}$T-3′ = $C_{29}H_{39}N_7O_{19}P_2$ (found: 850.09 (z = 1); calcd for $[M–H]^-$: 850.17). Negative mode.

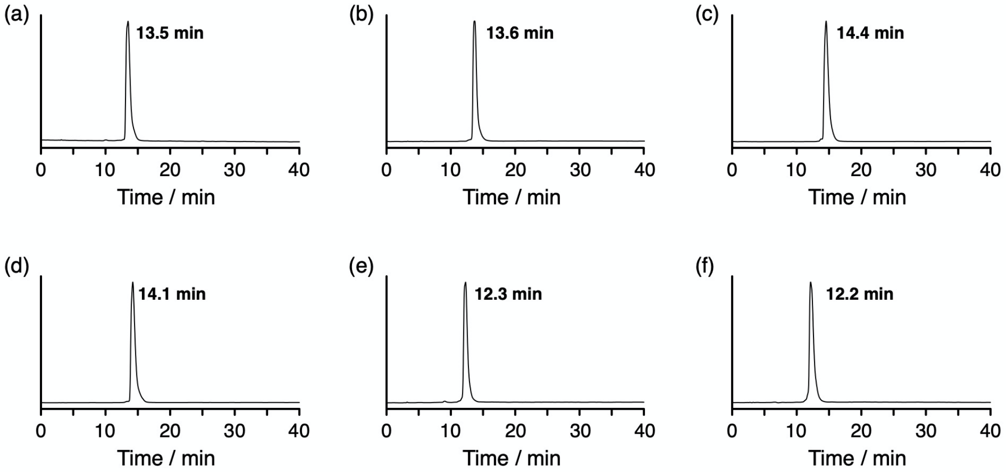


Fig. S3. HPLC analysis of DNA strands containing **Im**$^{\mathbf{OC}}$ or **Im**$^{\mathbf{OA}}$ nucleotides after purification. (a) DNA **1**, (b) DNA **2**, (c) DNA **3**, (d) DNA **4**, (e) DNA **5**, (f) DNA **6**. Waters XBridge Oligonucleotide BEH C18 column (4.6 × 50 mm), flow rate: 1.0 mL/min, temperature: 60 °C, monitored at 260 nm. Gradient: 0 to 40 min, 5% A to 7% A (solvent A = MeCN, solvent B = 0.1M TEAA buffer (pH 7.0) + 2% MeCN).

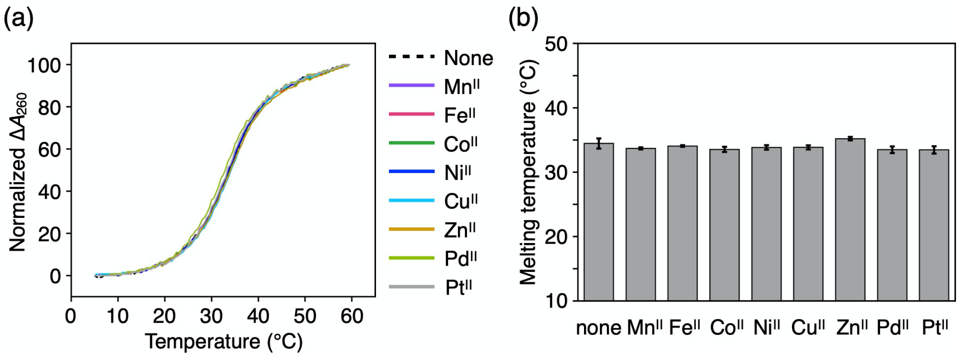


Fig. S4. (a) Melting curves of a DNA duplex containing an **Im**$^{\mathbf{OA}}$–**Im**$^{\mathbf{OA}}$ base pair (**3**·**4**) in the presence of various metal ions. [duplex] = 2.0 μM, [metal ion]/[duplex] = 0 (dashed line) or 1 (solid lines) in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl, 0.2 °C/min. (b) Melting temperatures. $N = 3$. The error bars represent the standard errors.

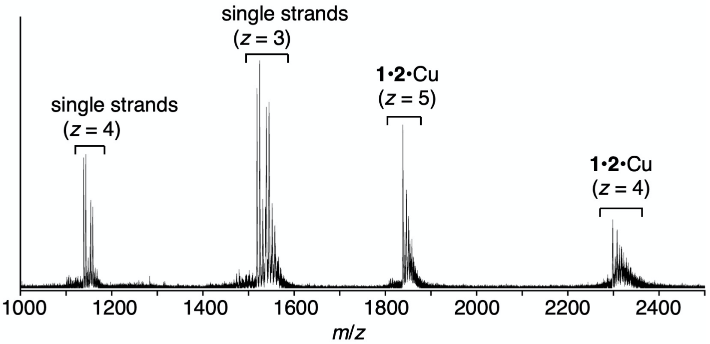


Fig. S5. ESI-TOF mass spectrum of duplex **1**·**2** with 1 equiv of Cu^{II} ions. **1**·**2**·Cu^{II} = $C_{294}H_{368}N_{106}O_{180}P_{28}Cu$ (found: 1838.41 ($z = 5$); calcd for [**1**·**2**·Cu^{II}–5H]$^{5-}$: 1838.47). [duplex] = 100 μM, [Cu^{II}] = 100 μM in 20 mM NH_4OAc (pH 7.0). Negative mode. Signals ascribed to sodium and ammonium adducts were also observed. The magnified spectrum is shown in Fig. 3b.

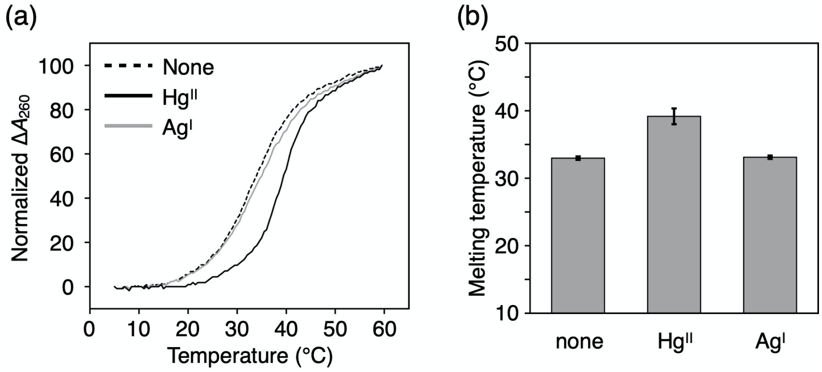


Fig. S6. (a) Melting curves of a DNA duplex containing an **Im^{OA}–Im^{OA}** base pair (**3**·**4**) in the presence of Hg^{II} and Ag^{I} ions. [duplex] = 2.0 μM, [metal ion]/[duplex] = 0 (dashed line) or 1 (solid lines) in 10 mM HEPES buffer (pH 7.0), 100 mM $NaNO_3$, 0.2 °C/min. (b) Melting temperatures. $N = 3$. The error bars represent the standard errors.

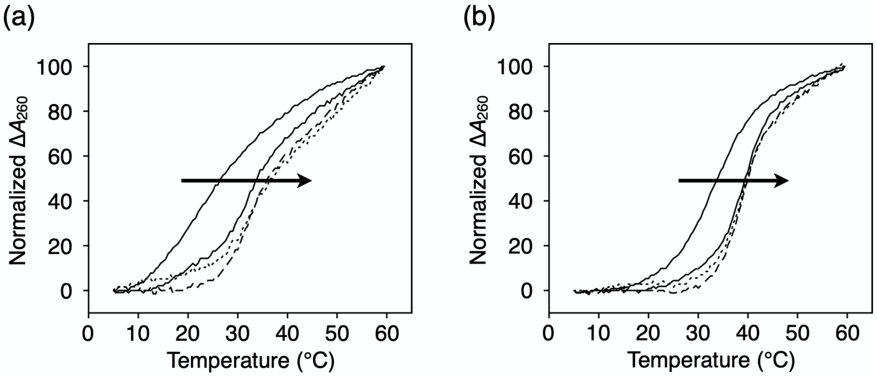


Fig. S7. Melting curves of (a) DNA duplex **1**·**2** containing an **Im^{OC}–Im^{OC}** pair and (b) DNA duplex **3**·**4** containing an **Im^{OA}–Im^{OA}** pair in the presence of different concentrations of Hg^{II} ions. [duplex] = 2.0 μM, [Hg^{II}]/[duplex] = 0, 1 (solid lines), 2 (dashed lines), and 3 (dotted lines) in 10 mM HEPES buffer (pH 7.0), 100 mM $NaNO_3$, 0.2 °C/min.

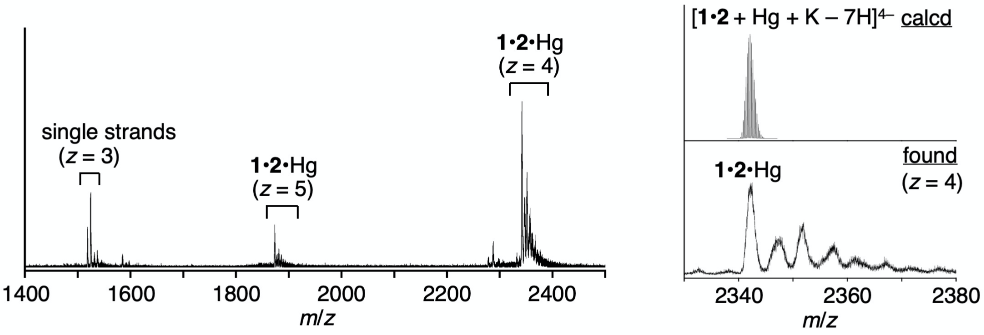


Fig. S8. ESI-TOF mass spectrum of duplex **1**·**2** with 1 equiv of $\mathrm{Hg^{II}}$ ions. **1**·**2**·$\mathrm{Hg^{II}}$ = $\mathrm{C_{294}H_{368}N_{106}O_{180}P_{28}Hg}$ (found: 2342.26 (z = 4); calcd for [**1**·**2**·$\mathrm{Hg^{II}}$+K–5H]$^{4-}$: 2342.11). [duplex] = 100 μM, [$\mathrm{Hg^{II}}$] = 100 μM in 20 mM $\mathrm{NH_4OAc}$ (pH 7.0). Negative mode. Signals ascribed to sodium and ammonium adducts were also observed.

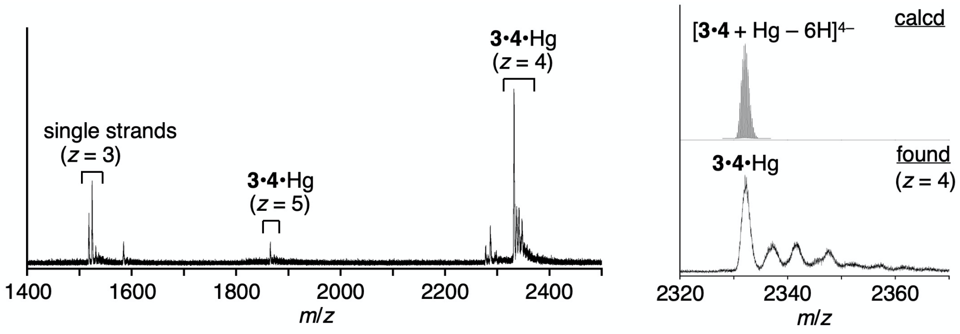


Fig. S9. ESI-TOF mass spectrum of duplex **3**·**4** with 1 equiv of $\mathrm{Hg^{II}}$ ions. **3**·**4**·$\mathrm{Hg^{II}}$ = $\mathrm{C_{294}H_{370}N_{108}O_{178}P_{28}Hg}$ (found: 2332.08 (z = 4); calcd for [**3**·**4**·$\mathrm{Hg^{II}}$–4H]$^{4-}$: 2332.13). [duplex] = 100 μM, [$\mathrm{Hg^{II}}$] = 100 μM in 20 mM $\mathrm{NH_4OAc}$ (pH 7.0). Negative mode. Signals ascribed to sodium and ammonium adducts were also observed.

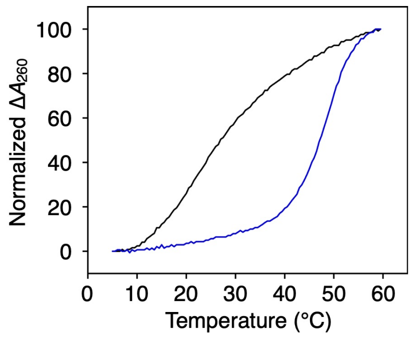


Fig. S10. Melting curves of a DNA duplex containing an **Im$^{\mathbf{OC}}$**–**Im$^{\mathbf{C}}$** base pair (**1**·**8**) in the absence and presence of $\mathrm{Cu^{II}}$ ions. [duplex] = 2.0 μM, [$\mathrm{Cu^{II}}$]/[duplex] = 0 (black line) or 1 (blue line) in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl, 0.2 °C/min.

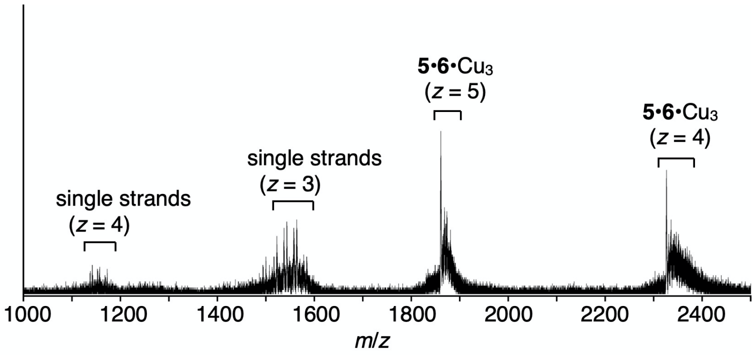


Fig. S11. ESI-TOF mass spectrum of duplex **5**·**6** with 3 equiv of Cu^{II} ions. **5**·**6**·Cu$^{II}_3$ = $C_{290}H_{358}N_{100}O_{188}P_{28}Cu_3$ (found: 1860.92 (z = 5); calcd for [**5**·**6**·Cu$^{II}_3$–5H]$^{5-}$: 1860.84). [duplex] = 100 µM, [Cu^{II}] = 300 µM in 20 mM NH_4OAc (pH 7.0). Negative mode. Signals ascribed to sodium and ammonium adducts were also observed.

3. Supplementary tables

Table S1. Melting temperatures of duplexes **1**·**2** and **3**·**4** in the presence of 1 equiv of transition metal ions.[a]

Metals	**1·2**		**3·4**	
	T_m/ °C	ΔT_m/ °C[b]	T_m/ °C	ΔT_m/ °C[b]
None	23.0 ± 0.8	–	34.5 ± 0.8	–
Mn^{II}	21.4 ± 0.3	–1.6	33.7 ± 0.2	–0.8
Fe^{II}	21.4 ± 0.1	–1.6	34.1 ± 0.1	–0.4
Co^{II}	23.3 ± 0.9	+0.3	33.5 ± 0.4	–0.9
Ni^{II}	24.0 ± 1.0	+1.0	33.8 ± 0.3	–0.6
Cu^{II}	43.3 ± 0.6	+20.3	33.9 ± 0.3	–0.6
Zn^{II}	20.8 ± 0.7	–2.2	35.2 ± 0.3	+0.7
Pd^{II}	23.3 ± 0.7	+0.3	33.5 ± 0.5	–1.0
Pt^{II}	22.8 ± 0.5	–0.2	33.5 ± 0.6	–1.0

[a] [duplex] = 2 µM, [metal ion] = 2 µM in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl. N = 3. The standard errors are also shown. [b] ΔT_m represents the temperature difference from the T_m value of the metal-free duplex.

Table S2. Melting temperatures of duplexes **1**·**2** and **3**·**4** in the presence 1 equiv of Hg^{II} and Ag^{I} ions.[a]

Metals	**1·2**		**3·4**	
	T_m/ °C	ΔT_m/ °C[b]	T_m/ °C	ΔT_m/ °C[b]
None	20.6 ± 0.5	–	33.0 ± 0.2	–
Cu^{II}	42.1 ± 0.8	+21.5	–	–
Ag^{I}	21.4 ± 1.3	+0.9	33.1 ± 0.2	+0.1
Hg^{II}	31.9 ± 0.9	+11.4	39.2 ± 1.2	+6.2

[a] [duplex] = 2 µM, [metal ion] = 2 µM in 10 mM HEPES buffer (pH 7.0), 100 mM $NaNO_3$. N = 3. The standard errors are also shown. [b] ΔT_m represents the temperature difference from the T_m value of the metal-free duplex.

Table S3. Melting temperatures of duplexes **1·2** and **1·2N** in the absence and presence of 1 equiv of Cu^{II} ions.[a]

Duplexes	None	1 equiv of Cu^{II}	
	T_m / °C	T_m / °C	ΔT_m / °C[b]
1·2	23.0 ± 0.8	43.3 ± 0.6	+20.3
1·2A	38.6 ± 0.3	38.4 ± 0.4	–0.2
1·2T	27.3 ± 0.5	28.3 ± 0.2	+1.0
1·2C	22.1 ± 0.4	25.1 ± 1.2	+2.9
1·2G	33.0 ± 0.6	35.0 ± 0.2	+2.0

[a] [duplex] = 2 μM, [metal ion] = 2 μM in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl. $N = 3$. The standard errors are also shown. [b] ΔT_m represents the temperature difference from the T_m value of the metal-free duplex.

Table S4. Melting temperatures of duplexes **1·2**, **1·8**, and **7·8** in the absence and presence of 1 equiv of Cu^{II} ions.[a]

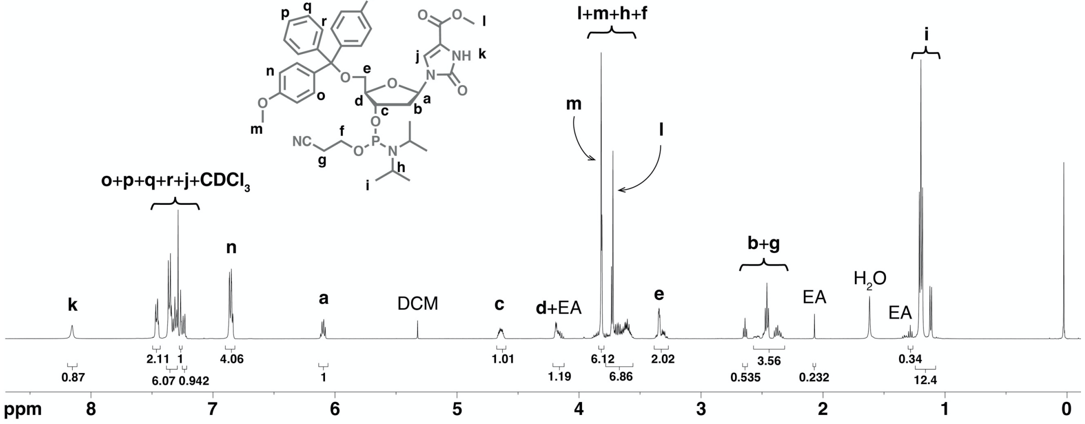
Duplexes	None	1 equiv of Cu^{II}	
	T_m / °C	T_m / °C	ΔT_m / °C[b]
1·2	23.0 ± 0.8	43.3 ± 0.6	+20.3
1·8	23.3 ± 0.5	49.4 ± 0.2	+26.1
7·8[c]	22.5 ± 1.1	57.7 ± 0.8	+35.2

[a] [duplex] = 2 μM, [metal ion] = 2 μM in 10 mM HEPES buffer (pH 7.0), 100 mM NaCl. $N = 3$. The standard errors are also shown. [b] ΔT_m represents the temperature difference from the T_m value r of the metal-free duplex. [c] Reproduced from the previous study.[2]

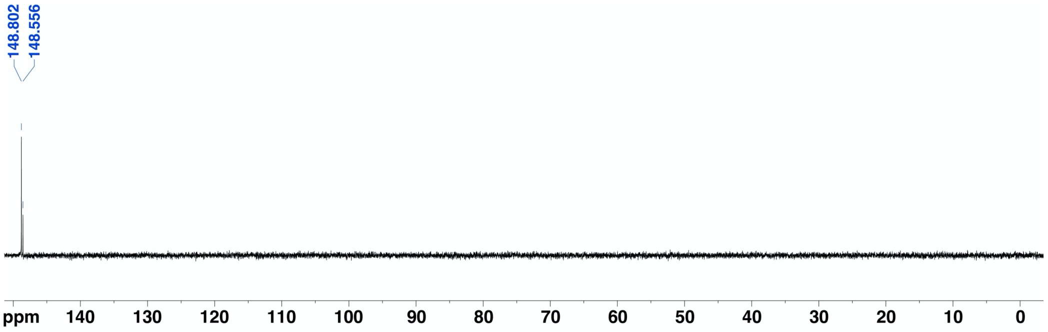
4. NMR spectra

Compound 4

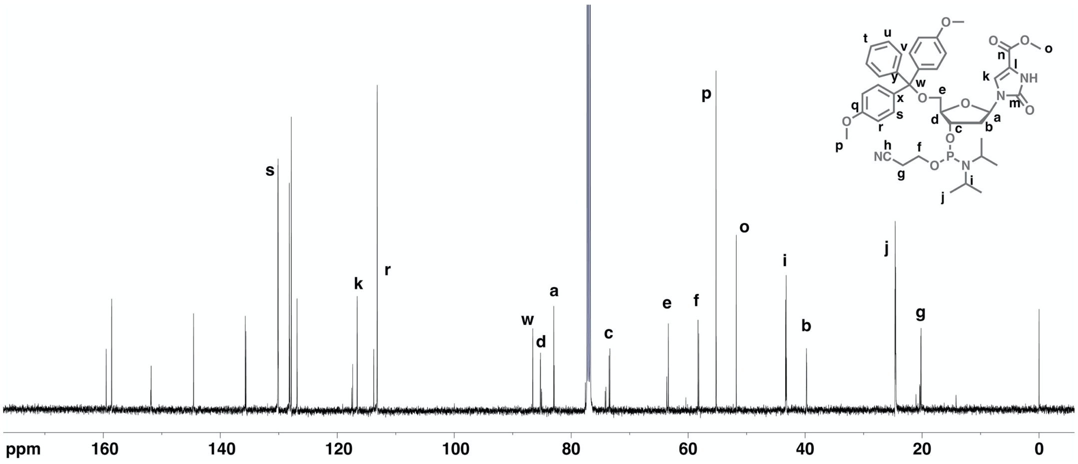
^1H NMR (500 MHz, 300K, $CDCl_3$).



^{31}P NMR (202 MHz, 300K, $CDCl_3$).



^{13}C NMR (126 MHz, 300K, $CDCl_3$).



5. References

[1] C. Cadena-Amaro and S. Pochet, *Tetrahedron*, 2005, **61**, 5081.

[2] Y. Takezawa, L. Hu, T. Nakama and M. Shionoya, *Angew. Chem., Int. Ed.*, 2020, **59**, 21488.