Supporting Information

Phenanthroline-modified DNA Three-way Junction Structures Stabilized by Interstrand 3:1 Metal Complexation

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1. Experimental methods

General. All reactions were carried out under a nitrogen atmosphere with commercial dehydrated solvents (FUJIFILM Wako Pure Chemical Industries). The reagents were purchased from FUJIFILM Wako Pure Chemical Industries and Tokyo Chemical Industry (TCI), and were used without further purification. 5-Bromomethyl-1,10-phenanthroline[S1] (**1**) and DMTr-protected 2′-amino-2′-deoxyuridine[S2] (**3**) were prepared according to the reported procedures. Silica gel column chromatography was performed using Merck Silica Gel 60 (230–400 mesh) or amine-modified silica gel NH-DM1020 (Fuji Silysia). All NMR spectra were measured on a Bruker AVANCE 500 spectrometer (500 MHz for ^{1}H, 126 MHz for ^{13}C and 202 MHz for ^{31}P). The spectra were referenced to tetramethylsilane (TMS) in $CDCl_3$ (δ 0 ppm) or to the residual solvent signals in CD_3OD (δ 3.31 ppm) and DMSO-d_6 (δ 2.50 ppm). Electrospray ionization-time-of-flight (ESI-TOF) mass spectra were recorded on a Waters LCT Premier XE. The unmodified oligonucleotides and the FAM-labeled strands were purchased from Japan Bio Service Co., Ltd (Saitama, Japan) at HPLC purification grade.

Compound 1. To a solution of 5-bromomethyl-1,10-phenanthroline[S1] (330 mg, 1.21 mmol) in CH_2Cl_2 (45 mL) was added hexamethylenetetramine (156 mg, 1.11 mmol). After stirred at 25 °C for 14 h, the reaction mixture was concentrated under reduced pressure. The residue was washed with CH_2Cl_2 (5 mL) to give hexamethylenetetrammonium salt as beige solid (460 mg). This intermediate was dissolved in ethanol (23 mL) and concentrated aqueous HCl (6 mL) was added. After heated at 80 °C for 20 h, the reaction mixture was concentrated in vacuo. 1 M aqueous NaOH was then added to increase the pH to 10. The product was extracted with CH_2Cl_2 (20 mL × 20). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated under reduced pressure. The residue was purified by alumina column chromatography ($CHCl_3$:CH_3OH = 10:0–9:1) to give the target compound **1** as a pale yellow solid (151 mg, 0.723 mmol, 60%)

^{1}H NMR (500 MHz, CD_3OD, 300 K): δ 9.05 (dd, J = 4.4, 1.6 Hz, 1H), 9.01 (dd, J = 4.4, 1.7 Hz, 1H), 8.56 (dd, J = 8.4, 1.6 Hz, 1H), 8.34 (dd, J = 8.1, 1.7 Hz, 1H), 7.85 (s, 1H), 7.74 (dd, J = 8.4, 4.3 Hz, 1H), 7.70 (dd, J = 8.1, 4.4 Hz, 1H), 4.33 (s, 2H).

^{13}C NMR (126 MHz, CD_3OD, 300 K): δ 150.8, 150,7 146.9, 146.3, 138.0, 137.7, 133.8, 130.0, 128.8, 125.5, 125.0, 124.7, 43.3.

HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{13}H_{12}N_3$ 210.1031, found 210.1004.

Compound 2. To a solution of 5-aminomethyl-1,10-phenanthroline (**1**, 213 mg, 1.02 mmol) in CH_2Cl_2 (5.6 mL) were added succinic anhydride (134 mg, 1.34 mmol, 1.3 eq) and triethylamine (180 μL, 1.20 mmol, 1.2 eq). The solution was stirred at 30 °C for 1 h, and then methanol was added to quench the reaction. The reaction mixture was concentrated under reduced pressure and further coevaporated with ethanol. The residue was washed with CH_2Cl_2 (3 mL) to afford the title compound (**3**) as a beige powder (251 mg, 0.812 mmol, 80%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ 9.11 (dd, J = 4.2, 1.6 Hz, 1H), 9.06 (dd, J = 4.3, 1.7 Hz, 1H), 8.58 (dd, J = 8.3, 1.6 Hz, 1H), 8.55 (t, J = 5.6 Hz, 1H), 8.44 (dd, J = 8.1, 1.7 Hz, 1H), 7.88 (s, 1H), 7.79 (dd, J = 8.3, 4.2 Hz, 1H), 7.76 (dd, J = 8.1, 4.3 Hz, 1H), 4.81 (d, J = 5.6 Hz, 2H), 2.52–2.45 (m, 4H). ^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ 174.4, 171.7, 150.2, 150.0, 146.2, 145.7, 136.3, 134.0, 132.7, 128.3, 127.4, 125.3, 123.9, 123.6, 30.6, 29.7 (One carbon signal may be overlapped with the signals of DMSO). HRMS (ESI-TOF) m/z: $[M + H]^+$ calcd for $C_{17}H_{15}N_3O_3H$ 310.1192, found 310.1163.

Compound 4. DMTr-protected 2′-amino-2′-deoxyuridine[S2] (**3**, 229 mg, 0.420 mmol) and phenanthroline **2** (260 mg, 0.841 mmol, 2.0 eq) were dissolved in DMF (4.2 mL). To the mixture, EDC·HCl (201 mg, 1.10 mmol, 2.6 eq) and triethylamine (140 μL, 1.00 mmol, 2.4 eq) were added. After stirred at 30 °C for 2.5 h, phenanthroline **3** (22 mg, 0.07 mmol, 0.2 eq) and EDC·HCl (20 mg, 0.11 mmol, 0.25 eq) were added. After stirred at 30 °C for additional 1.5 h, the reaction was quenched by the addition of methanol. The solvent was removed in vacuo, and the residue was further coevaporated with ethanol. The brown residue was dissolved in CH_2Cl_2 (15 mL) and then washed with a saturated $NaHCO_3$ aqueous solution (15 mL). The product was further extracted with CH_2Cl_2 (15 mL × 4). The combined organic layer was dried over anhydrous $MgSO_4$ and concentrated under reduced pressure. The residue was purified by column chromatography with amine-modified silica gel (AcOEt:CH_3OH = 19:1–17:3). The target compound **4** was obtained as a beige solid (92.1 mg, 0.11 mmol, 26%).

^{1}H NMR (500 MHz, DMSO-d_6, 300 K): δ 11.32 (s, 1H), 9.09 (dd, J = 4.2, 1.5 Hz, 1H), 9.03 (dd, J = 4.2, 1.6 Hz, 1H), 8.58 (dd, J = 8.3, 1.5 Hz, 1H), 8.52 (t, J = 5.6 Hz, 1H), 8.41 (dd, J = 8.1, 1.6 Hz, 1H), 7.98 (d, J = 8.4 Hz, 1H), 7.88 (s, 1H), 7.78 (dd, J = 8.3, 4.2 Hz, 1H), 7.67 (dd, J = 8.1, 4.2 Hz, 1H), 7.64 (d, J = 8.1 Hz, 1H), 7.40 (d, J = 8.1 Hz, 2H), 7.31 (dd, J = 8.1, 7.7 Hz, 2H), 7.26 (dd, J = 9.0, 0.6 Hz, 4H), 7.22 (dd, J = 8.3, 4.2 Hz, 1H), 6.89 (dd, J = 9.0, 0.6 Hz, 4H), 5.88 (d, J = 8.0 Hz, 1H), 5.69 (d, J = 4.7 Hz, 1H), 5.39 (d, J = 8.1 Hz, 1H), 4.79 (m, 2H), 4.64 (m, 1H), 4.16 (m, 1H), 4.01 (m, 1H), 3.72 (s, 6H), 3.25–3.17 (m, 2H), 2.52–2.43 (m, 4H).

^{13}C NMR (126 MHz, DMSO-d_6, 300 K): δ 172.7, 172.1, 163.4, 158.6, 151.2, 150.1, 150.0, 146.1, 145.7, 145.1, 141.1, 136.3, 135.8, 135.6, 134.0, 132.7, 130.3, 128.4, 128.2, 127.4, 127.2, 125.3, 123.9, 123.6, 113.8, 102.3, 86.7, 86.5, 84.9, 70.4, 64.3, 55.5, 54.7, 31.2, 31.0 (One carbon signal may be overlapped with the signals of DMSO). HRMS (ESI-TOF) *m*/*z*: $[M + H]^+$ calcd for $C_{47}H_{44}N_6O_9H$ 837.3248, found 837.3239.

Compound 5. To a solution of nucleoside **4** (87.7 mg, 0.11 mmol) in CH_2Cl_2 (0.33 mL), diisopropylamine (14.8 μL, 0.11 mmol, 1.0 eq) and tetrazole (7.5 mg, 0.11 mmol, 1.0 eq) were added. A solution of 2-cyanoethyl-*N,N,N′,N′*-tetraisopropylphosphorodiamidite (42 μL, 0.13 mmol, 1.2 eq) in CH_2Cl_2 (0.3 mL) was then added. After stirred at 25 °C for 17 h, the reaction mixture was diluted with AcOEt (10 mL) and washed with a saturated $NaHCO_3$ aqueous solution (10 mL) and brine (10 mL × 2). The organic layer was dried over anhydrous Na_2SO_4 and then evaporated to dryness. The residue was chromatographed on amine-modified silica gel ($CHCl_3$:CH_3OH = 20:0–19:1) to give compound **5** (60 mg, 0.058 mmol, as a diastereomeric mixture) as a colorless solid. The formation of the phosphoramidite derivative was confirmed by ^{31}P NMR and ESI-MS. Although ^{1}H NMR indicated the existence of some impurities, the obtained product was immediately used for DNA synthesis without further purification.
^{1}H NMR (500 MHz, 300 K, $CDCl_3$): δ 9.14 (m, 1H), 9.08 (dd × 2, *J* = 4.3, 1.7 Hz, 1H), 8.41 (dd × 2, *J* = 8.4, 1.6 Hz, 1H), 8.13 (dd × 2, *J* = 8.1, 1.7 Hz, 1H), 7.75–7.74 (s ×2, 1H), 7.72–7.61 (d × 2 , *J* =8.1 Hz, 1H), 7.61–7.58 (m, 1H), 7.49 (dd × 2, *J* = 8.0, 4.3 Hz, 1H), 7.39 (m, 2H), 7.30–7.25 (m, 7H), 7.19–7.15 (m, 2H), 6.84–6.81 (m, 4H), 6.71 (d × 2, *J* = 8.3 Hz, 1H), 6.04 (d × 2, *J* = 8.3 Hz, 1H), 5.39 (d × 2, *J* = 8.1 Hz, 1H), 4.91 (m, 2H), 4.80–4.63 (ddd × 2, *J* = 8.3, 8.3, 6.1 Hz, 1H), 4.67–4.56 (m, 1H), 4.35–4.20 (m, 1H), 3.76 (s × 2, 6H), 3.91–3.57 (m, 6H), 2.75–2.36 (m, 6H), 1.21–1.07 (m, 15H). ^{31}P NMR (202 MHz, 300 K, $CDCl_3$): δ 154.8, 152.6. (diastereomers). ESI-MS (positive) *m*/*z*: $[M + H]^+$ calcd for $C_{56}H_{61}N_8O_{10}PH$ 1037.4326; found 1037.4379.

DNA synthesis. Phen-modified DNA strands (**L1**, **L2**, and **L3**) were synthesized on an NTS M-2-MX DNA/RNA synthesizer at 1 μmol scale (DMTr-on mode) using ultramild deprotection phosphoramidites and reagents (Glen Research). The phosphoramidite derivative of $\mathbf{U_{phen}}$ was dissolved in dry MeCN to prepare a 0.1 M solution for the DNA synthesis. The synthesis was carried out according to the standard protocol except for the extended coupling time (15 min) for $\mathbf{U_{phen}}$ nucleoside. After cleavage from the solid support, the DNA strands were deprotected with 25% aqueous ammonia at room temperature for 2–4 h, and subjected to crude purification and

detritylation on a Poly-Pak II cartridge (Glen Research). After purified by reverse-phase HPLC (Waters XBridge OST C18 column, 0.1 M TEAA (pH 7.0)/MeCN gradient, 60 °C), the DNA strands were characterized by MALDI-TOF mass spectrometry (Brucker Autoflex) using a mixture of 3-hydroxypicolinic acid (3-HPA) and ammonium citrate as a matrix. The DNA strands were quantified based on the UV absorbance at 260 nm. The molar extinction coefficients (ε_{260}) of the phen-modified strands were calculated from the sum of the extinction coefficients of corresponding unmodified DNA strands and that of the phenanthroline ligand, which was approximated to 1.2×10^4 M^{-1} cm^{-1}.

Melting experiments. The samples were prepared by mixing DNA strands (1.0 μM each) in 10 mM MOPS buffer (pH 7.0) containing 100 mM NaCl. After the addition of $NiSO_4 \cdot 7H_2O$ (Soekawa) or EDTA, the samples were annealed by heating to 85 °C and slowly cooling to 5 °C at a rate of 1.0 °C/min. UV melting experiments were performed on UV-1700, UV-1800, and UV-1900 spectrophotometers (Shimadzu) equipped with a TMSPC-8 temperature controller. Absorbance at 260 nm was recorded while the temperature was raised from 5 °C to 85 °C at a rate of 0.2 °C/min. The melting curves were depicted after normalization as follows:

$$\text{Normalized } A_{260} = \{A_{260}(t\ ^\circ\text{C}) - A_{260}(5\ ^\circ\text{C})\}/\{A_{260}(85\ ^\circ\text{C}) - A_{260}(5\ ^\circ\text{C})\} \times 100.$$

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

PAGE analysis. Samples were prepared by mixing DNA strands (1.0 μM each) in 10 mM MOPS buffer (pH 7.0) containing 100 mM NaCl. After the addition of $NiSO_4 \cdot 7H_2O$ (Soekawa) or EDTA, the samples were annealed by heating to 85 °C and slowly cooling to 5 °C at a rate of 1.0 °C/min.. Native polyacrylamide gel electrophoresis (PAGE) was carried out on 18% (19:1) gel using TAMg buffer (40 mM Tris, 76 mM $MgCl_2$, 14 mM acetic acid, pH 8.0) in a cool incubator (4 °C). After combined with a loading buffer (not containing urea or EDTA), the samples were applied on the gel. The gels were visualized by Gel Doc EZ Imager and analyzed using Image Lab software (Bio-Rad). Each product on the gel was quantified by comparing the band intensity of the FAM-labeled DNA strands. The average values of at least three independent experiments are shown.

2. Supporting figures and tables

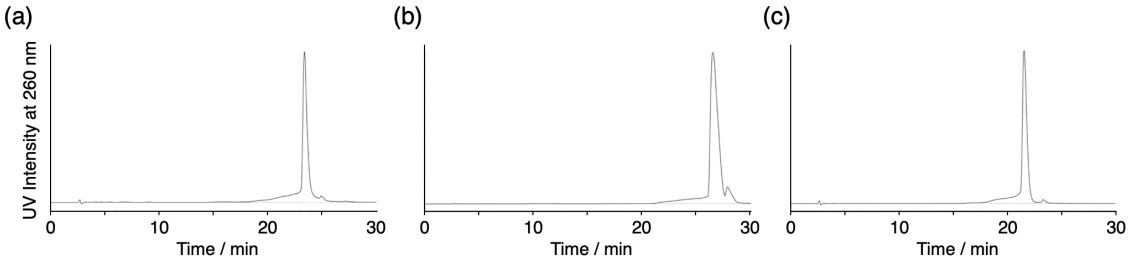


Figure S1. HPLC analysis of the phen-modified DNA strands. (a) **L1**, (b) **L2**, (c) **L3** after HPLC purification. Waters XBridge OST C18 column (10 × 50 mm), flow rate: 1.0 mL/min, temperature: 60 °C, monitored at 260 nm. Gradient: 0 to 30 min, 4% A to 9.5% A (solvent A = CH_3CN, solvent B = 0.1 M TEAA buffer (pH 7.0) + 2% CH_3CN).

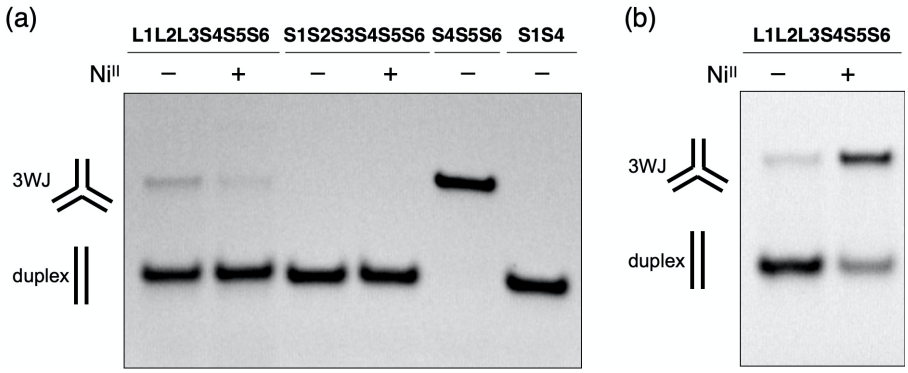


Figure S2. Native PAGE analysis of mixtures of DNA strands in the absence and presence of Ni^{II} ions. (a) The mixture of phen-modified strands (**L1**, **L2**, and **L3**) and their complementary strands (**S4**, **S5**, and **S6**). The results are also shown for the mixture of unmodified strands (**S1**, **S2**, **S3**, **S4**, **S5**, and **S6**). 3WJ **S4S5S6** and duplex **S1S4** were used as markers. (b) The mixture of bpy-modified strands (**L1**, **L2**, and **L3**) and their complementary strands (**S4**, **S5**, and **S6**).[S3] Strand **S4** was labeled with FAM. [DNA strands] = 1.0 μM each, [EDTA] = 10 μM or [Ni^{II}] = 1.0 μM in 10 mM MOPS buffer (pH 7.0), 100 mM NaCl. The bands were detected by FAM fluorescence.

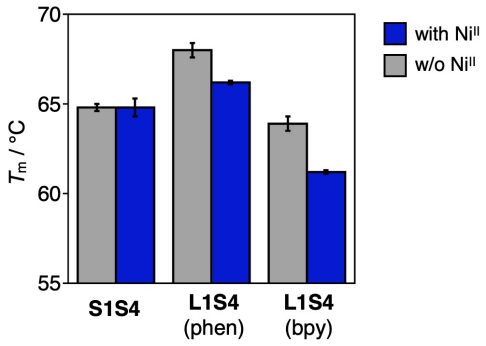


Figure S3. Melting temperatures (T_m) of the unmodified DNA duplex (**S1S4**), phen-modified duplex (**L1S4**), and bpy-modified duplex[S3] in the absence and presence of Ni^{II} ions. [DNA duplex] = 1.0 μM, [EDTA] = 10 μM or [Ni^{II}] = 1.0 μM in 10 mM MOPS buffer (pH 7.0), 100 mM NaCl. Error bars indicate the standard errors.

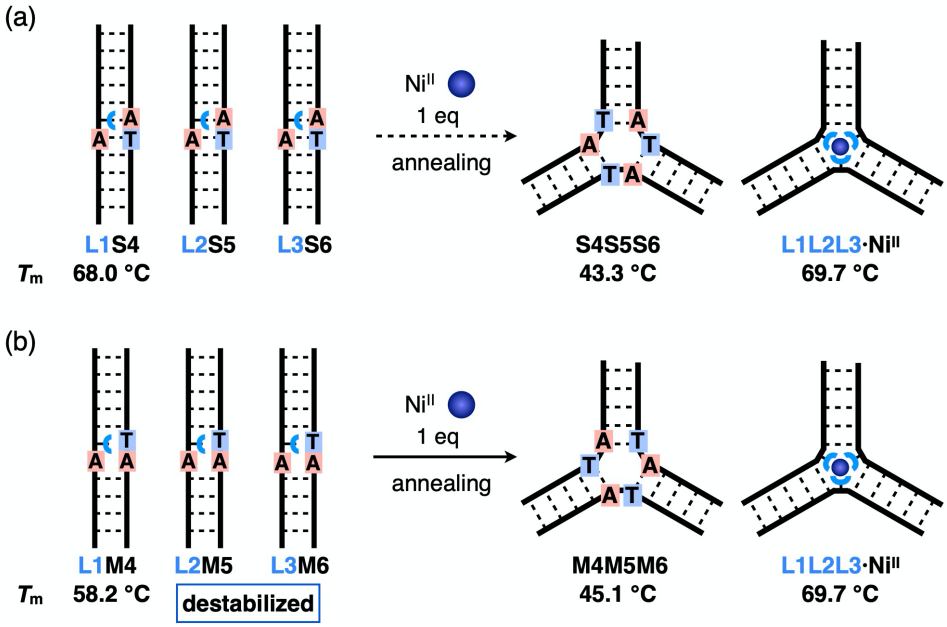


Figure S4. Schematic representation of the Ni^{II}-mediated formation of the 3WJs. (a) With fully matched counter strands (**S4**, **S5**, and **S6**). (b) With mutated counter strands (**M4**, **M5**, and **M6**). Melting temperatures are also shown. The sequences are shown in Table 1.

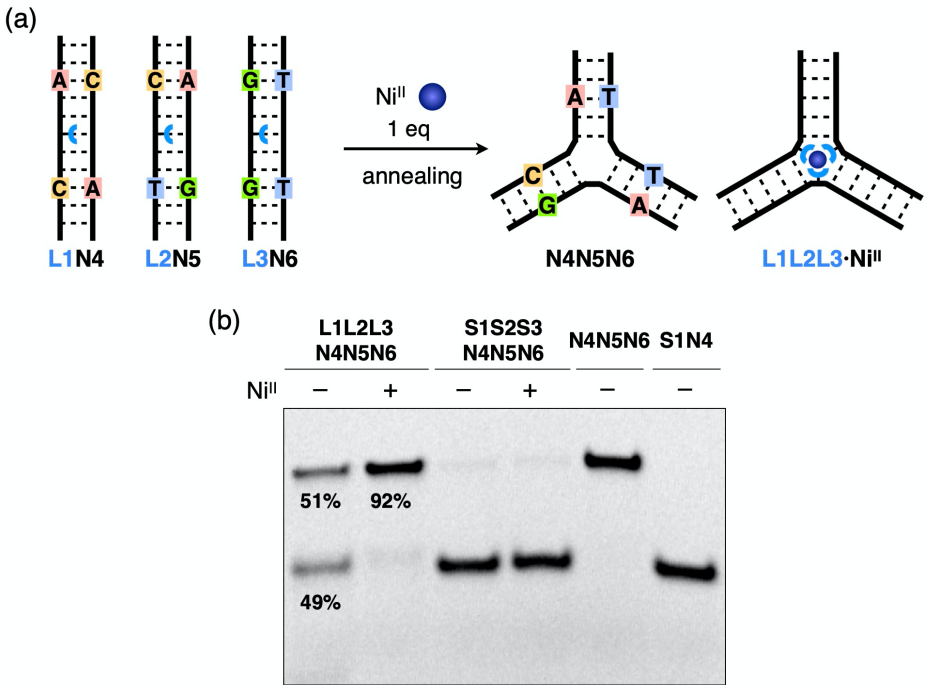


Figure S5. Ni^{II}-mediated 3WJ formation with another mutated counter strands (**N4**, **N5**, and **N6**). (a) Scheme. (b) Native PAGE analysis. Strand **N4** was labeled with FAM. [DNA strands] = 1.0 μM each, [EDTA] = 10 μM or [Ni^{II}] = 1.0 μM in 10 mM MOPS buffer (pH 7.0), 100 mM NaCl. The bands were detected by FAM fluorescence. **N4**: 5′-CTG CGA ATG TAC GCT CCT TC-3′, **N5**: 5′-GAA GGA GCG TAG CAT GGA AC-3′, **N6**: 5′-GTT CCA TGC TAC ATT CGC AG-3′.

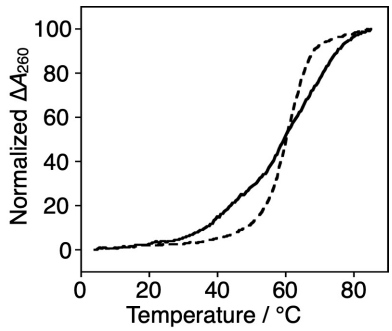


Figure S6. Melting curves of a mixture of six strands **L1**, **L2**, **L3**, **M4**, **M5**, and **M6** in the absence (broken line) and presence of Ni^{II} ions (solid line). [DNA strand] = 1.0 μM each, [EDTA] = 10 μM or [Ni^{II}] = 1.0 μM in 10 mM MOPS buffer (pH 7.0), 100 mM NaCl, 0.2 °C/min.

Table S1. Characterization of phen-modified DNA strands

DNA strands	Composition	Calc.	Obs.
L1	$[C_{211}H_{254}N_{88}O_{115}P_{19}]^-$	6448.2	6447.8
L2	$[C_{208}H_{259}N_{67}O_{125}P_{19}]^-$	6283.1	6286.1
L3	$[C_{212}H_{256}N_{84}O_{120}P_{19}]^-$	6486.2	6486.6

Table S2. Melting temperatures of DNA 3WJs, duplexes, and a mixture of six strands (**L1**, **L2**, **L3**, **M4**, **M5**, and **M6**) in the absence and presence of one equiv. of Ni^{II} ions[a]

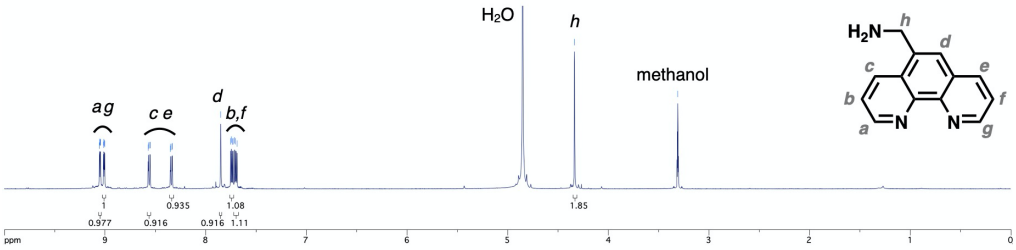
	Metal-free[b]	1 eq of Ni^{II}	
	T_m / °C	T_m / °C	ΔT_m / °C[c]
Three-way junctions			
L1L2L3	52.8 ± 2.2	69.7 ± 1.5	+16.9
S1S2S3[d]	42.1 ± 0.3	42.6 ± 0.5	+0.5
S4S5S6[d]	43.3 ± 0.5	43.0 ± 0.4	–0.3
M4M5M6[d]	45.1 ± 0.8	44.9 ± 0.7	–0.2
Duplexes			
L1S4	68.0 ± 0.4	66.2 ± 0.1	–1.8
L1M4	58.2 ± 1.0	58.0 ± 1.2	–0.2
Mixture of 6 strands			
L1, **L2**, **L3**, **M4**, **M5**, and **M6**	59.6 ± 0.9	69.9 ± 0.3	+10.3
		41.0 ± 1.1	–18.6

[a] Standard errors are also shown. [b] In the presence of 10 equiv. of EDTA. [c] ΔT_m represents the difference in the T_m values relative to that of the metal-free DNAs. [d] From Ref. S4.

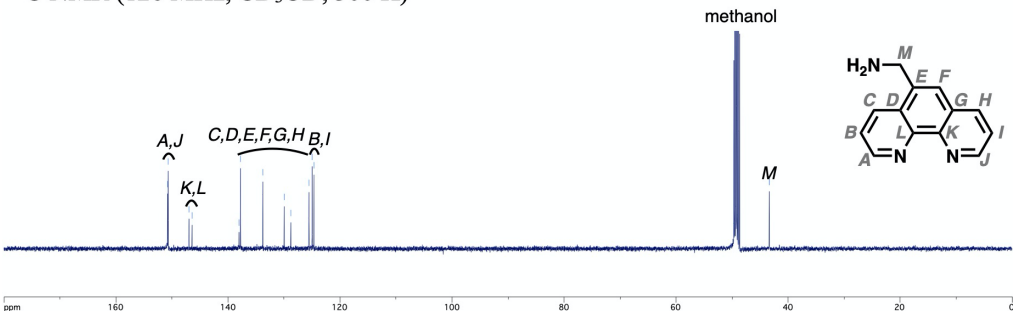
3. NMR spectra

Compound 1

^{1}H NMR (500 MHz, CD_3OD, 300 K)

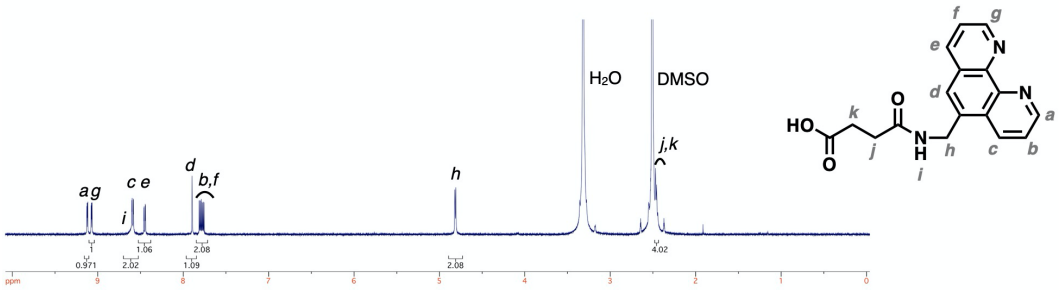


^{13}C NMR (126 MHz, CD_3OD, 300 K)

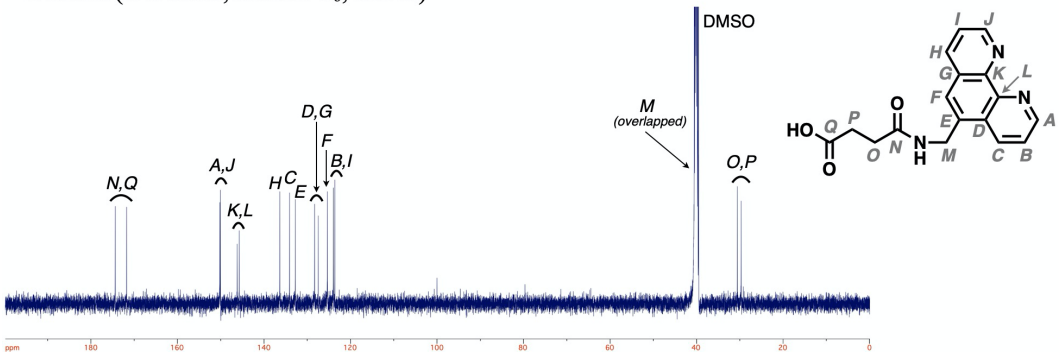


Compound 2

^{1}H NMR (500 MHz, DMSO-d_6, 300 K)

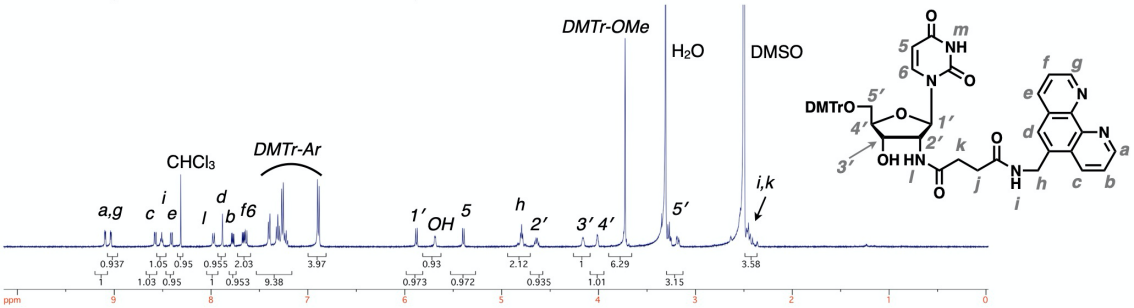


^{13}C NMR (126 MHz, DMSO-d_6, 300 K)

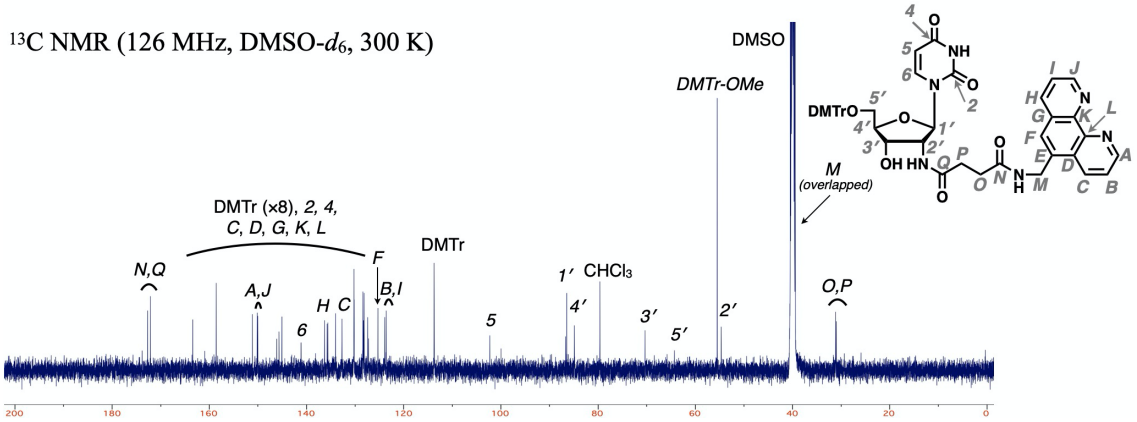


Compound 4

^{1}H NMR (500 MHz, DMSO-d_6, 300 K)

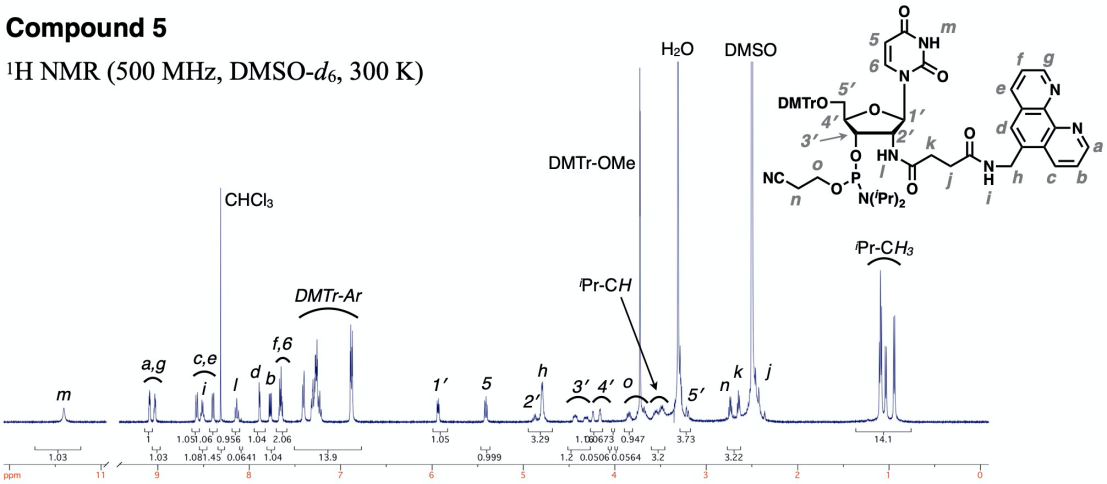


^{13}C NMR (126 MHz, DMSO-d_6, 300 K)

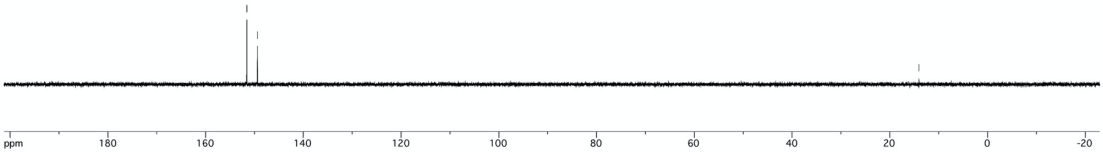


Compound 5

^{1}H NMR (500 MHz, DMSO-d_6, 300 K)



^{31}P NMR (202 MHz, DMSO-d_6, 300 K)



4. References

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