Organic & Biomolecular Chemistry

Supporting Information

Synthesis and thermal stabilities of oligonucleotides containing 2′-*O*,4′-*C*-methylene bridged nucleic acid with a phenoxazine base

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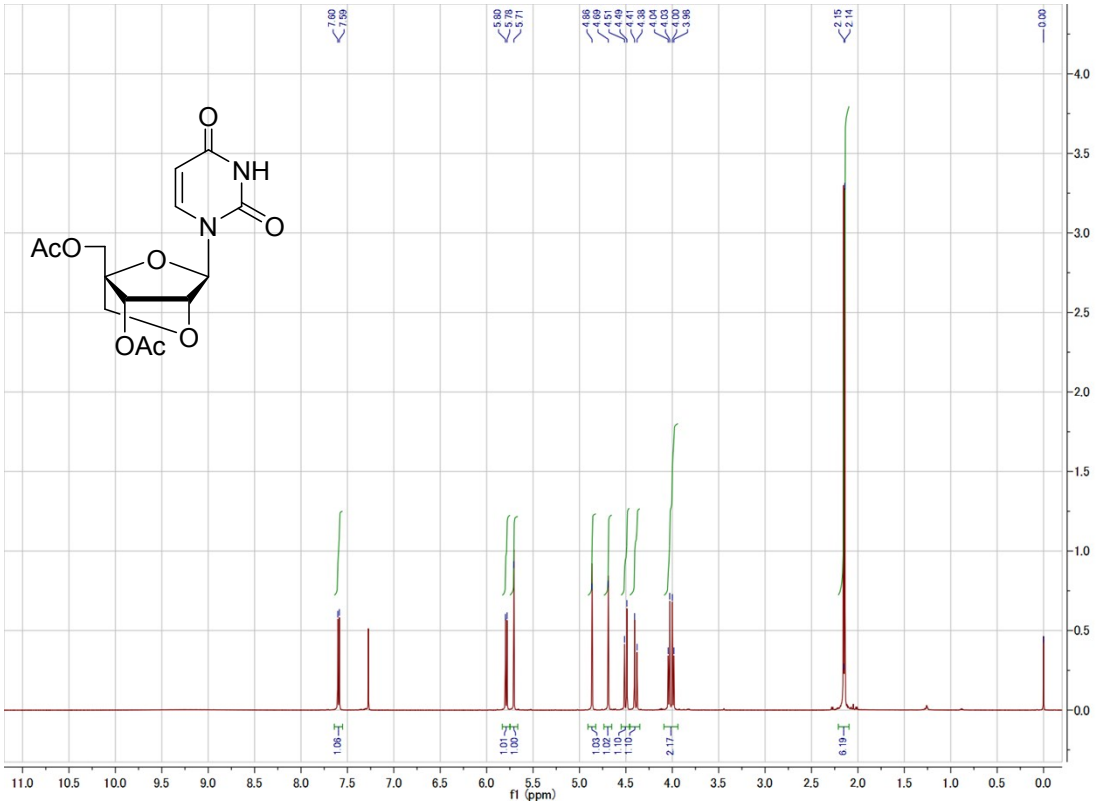
Contents:

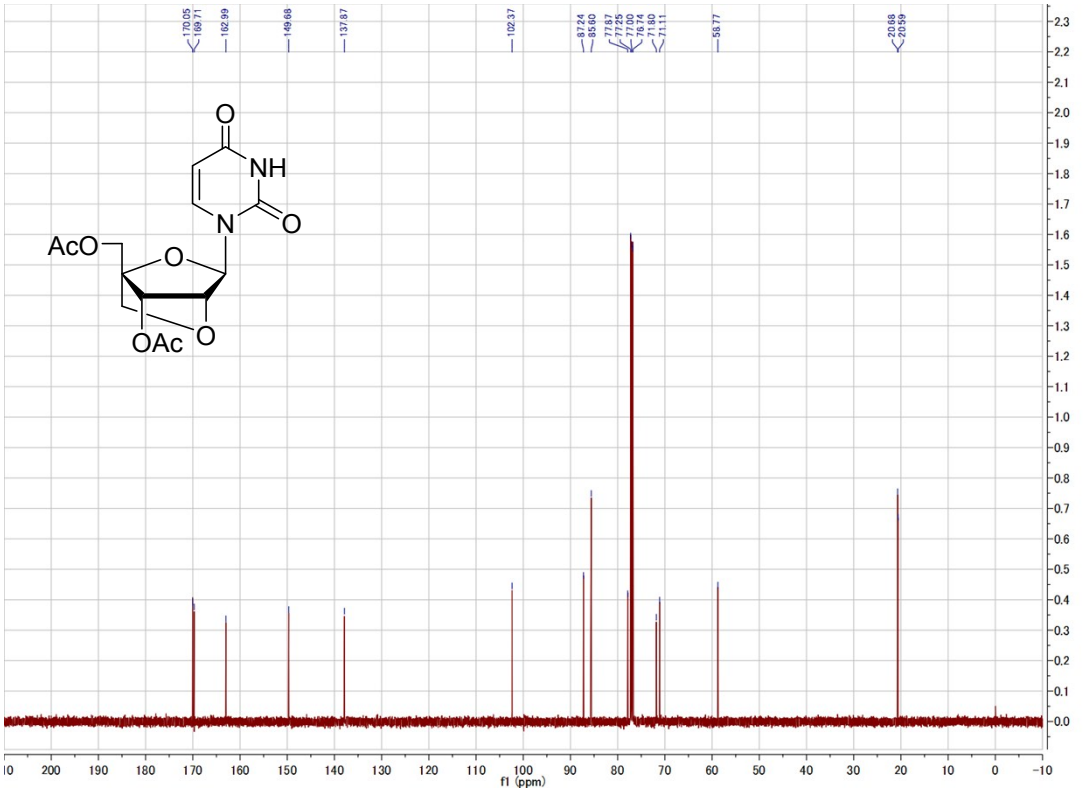
- **Figure S1.** **S2-S7**
  ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR
- **Figure S2.** **S8-S10**
  HPLC and MALDI-TOF-Mass data of oligodeoxynucleotides (ODNs) containing BNAP (**B**)
- **Table S1.** **S11**
  MALDI-TOF Mass Data of ODNs containing 2'-deoxyribonucleoside with phenoxazine (**P**)
- **Figure S3.** **S11**
  Fluorescent spectra of ODNs containing **P** and **B**
- **Figure S4.** **S12**
  UV melting curves for the duplexes
- **Table S2.** **S13**
  Comparison of matched versus mismatched hybridization (**X**-ODN/cRNA)
- **Figure S5.** **S13**
  CD spectra of DNA/DNA and DNA/RNA duplexes (5′-A**X**A-3′)
- **Table S3.** **S14**
  Thermodynamic parameters of duplexes with cDNA and cRNA (5′-A**X**A-3′)
- **Figure S6.** **S14-S16**
  Van't Hoff plots based on T_m results obtained under various concentrations

Figure S1. NMR Spectra (^{1}H, ^{13}C, ^{31}P)

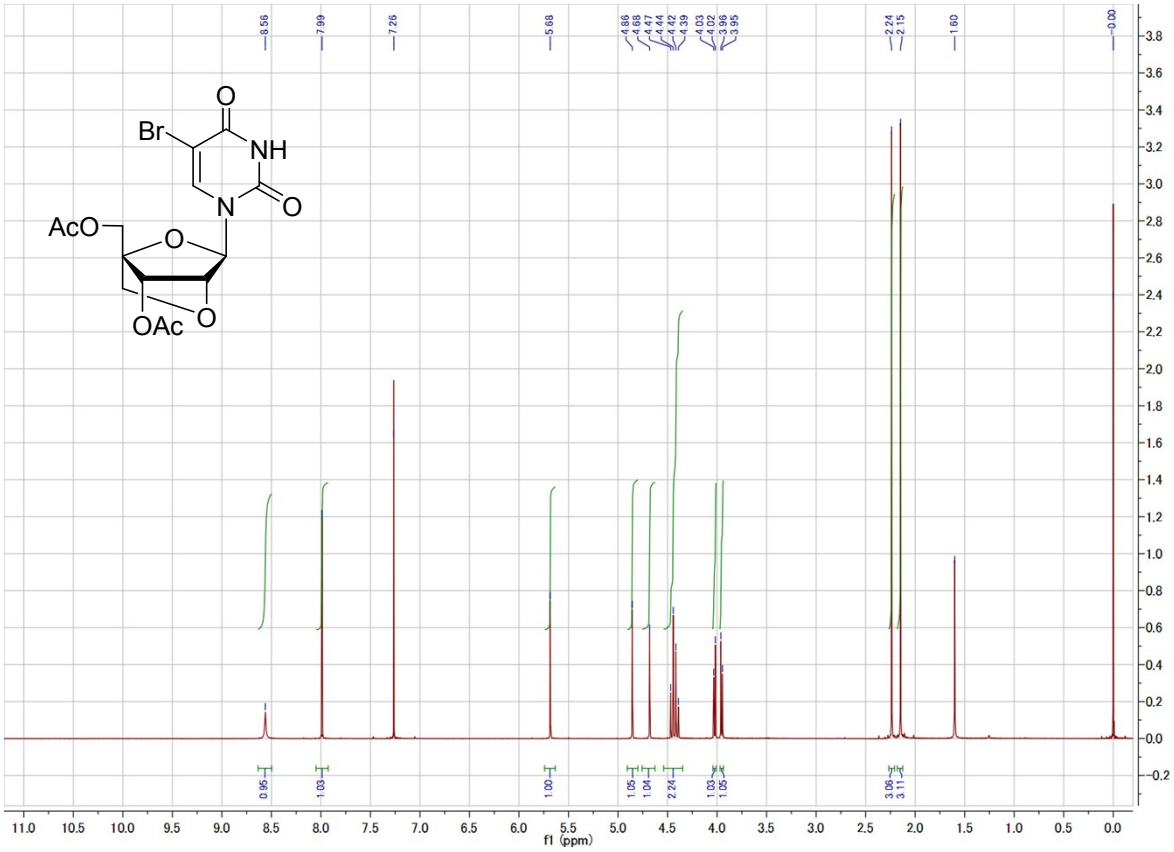
(a) ^{1}H-NMR ($CDCl_3$) of compound **3**



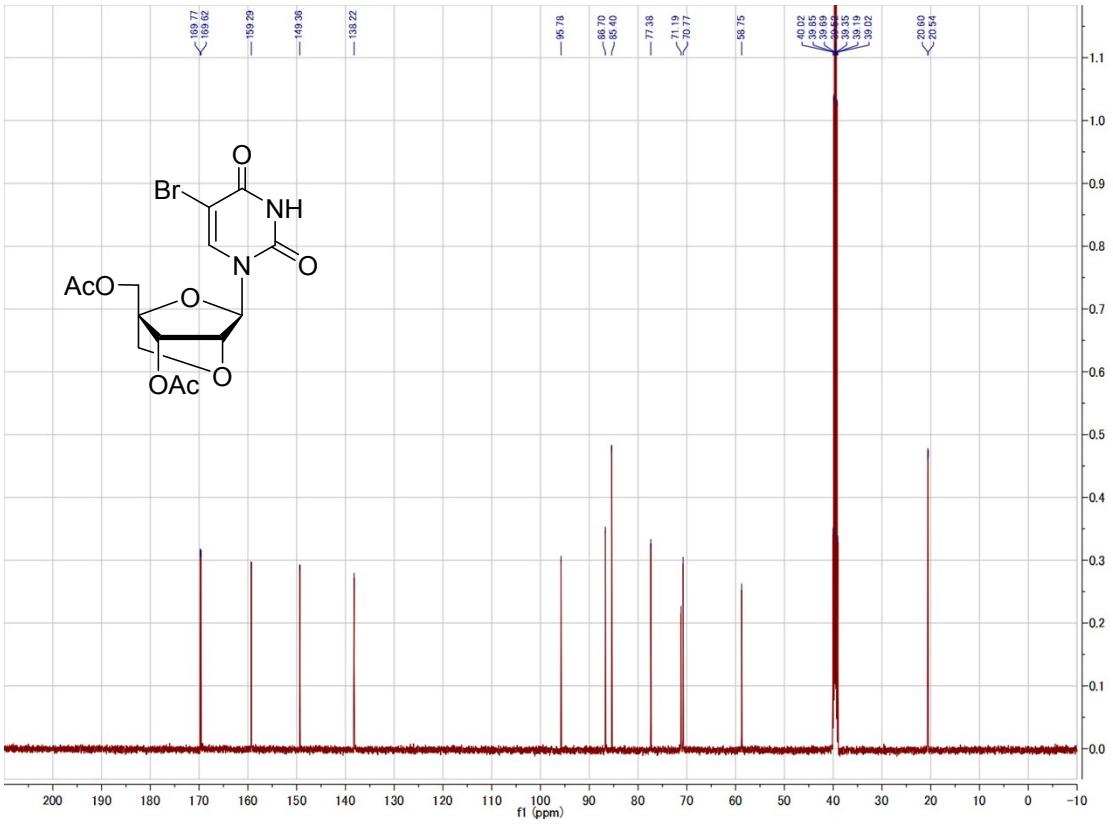
^{13}C-NMR ($CDCl_3$) of compound **3**



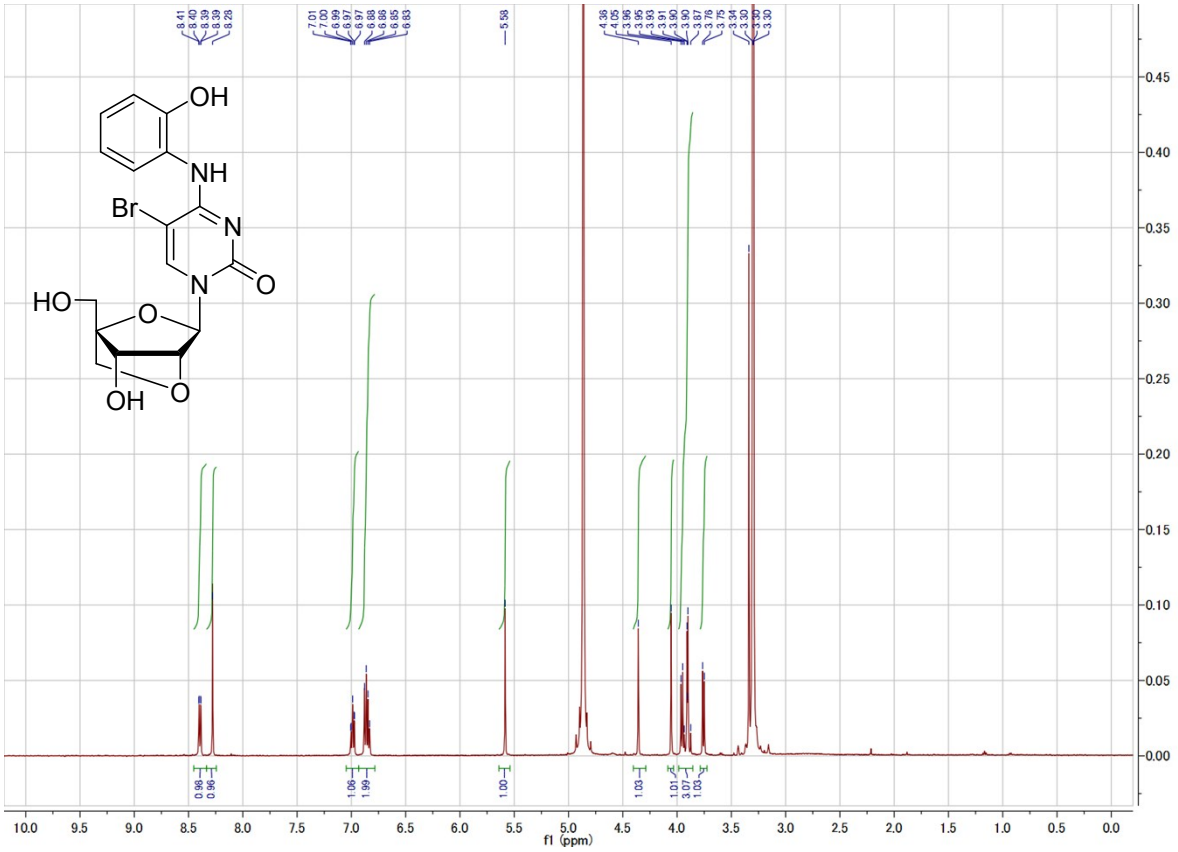
(b) ^{1}H-NMR ($CDCl_3$) of compound **4**



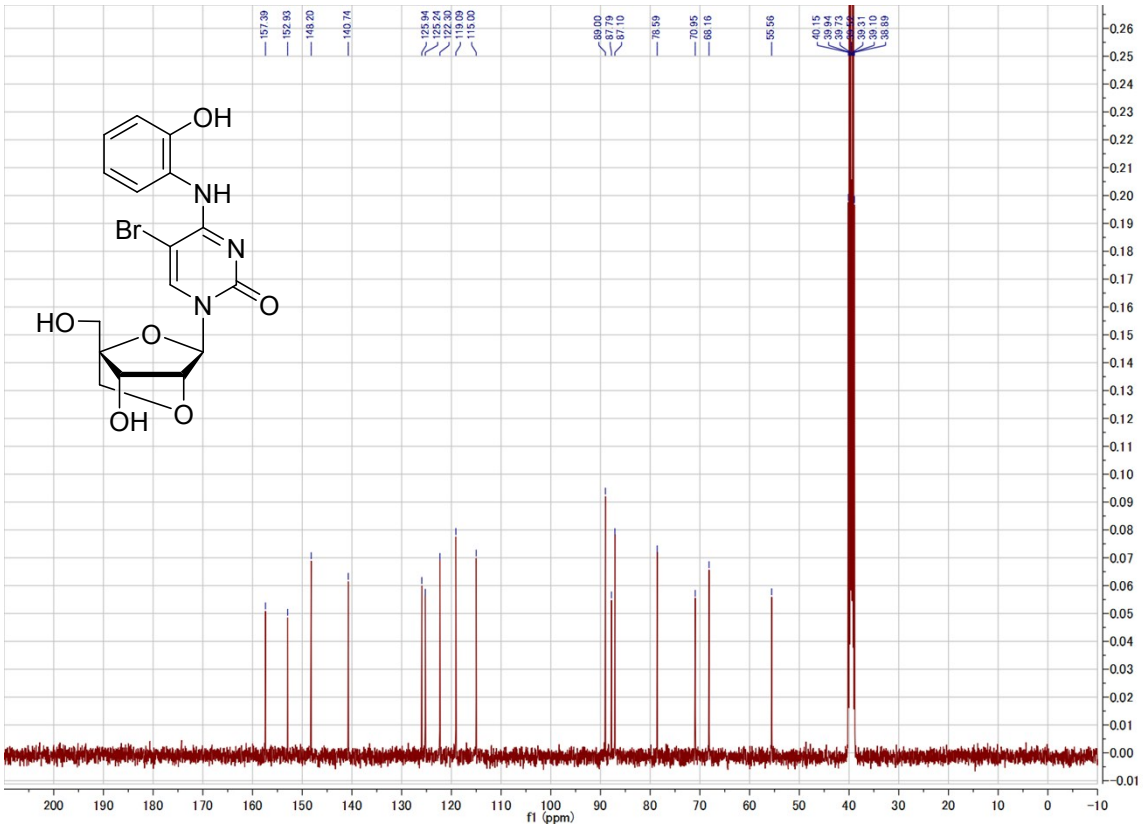
^{13}C-NMR (DMSO-d_6) of compound **4**



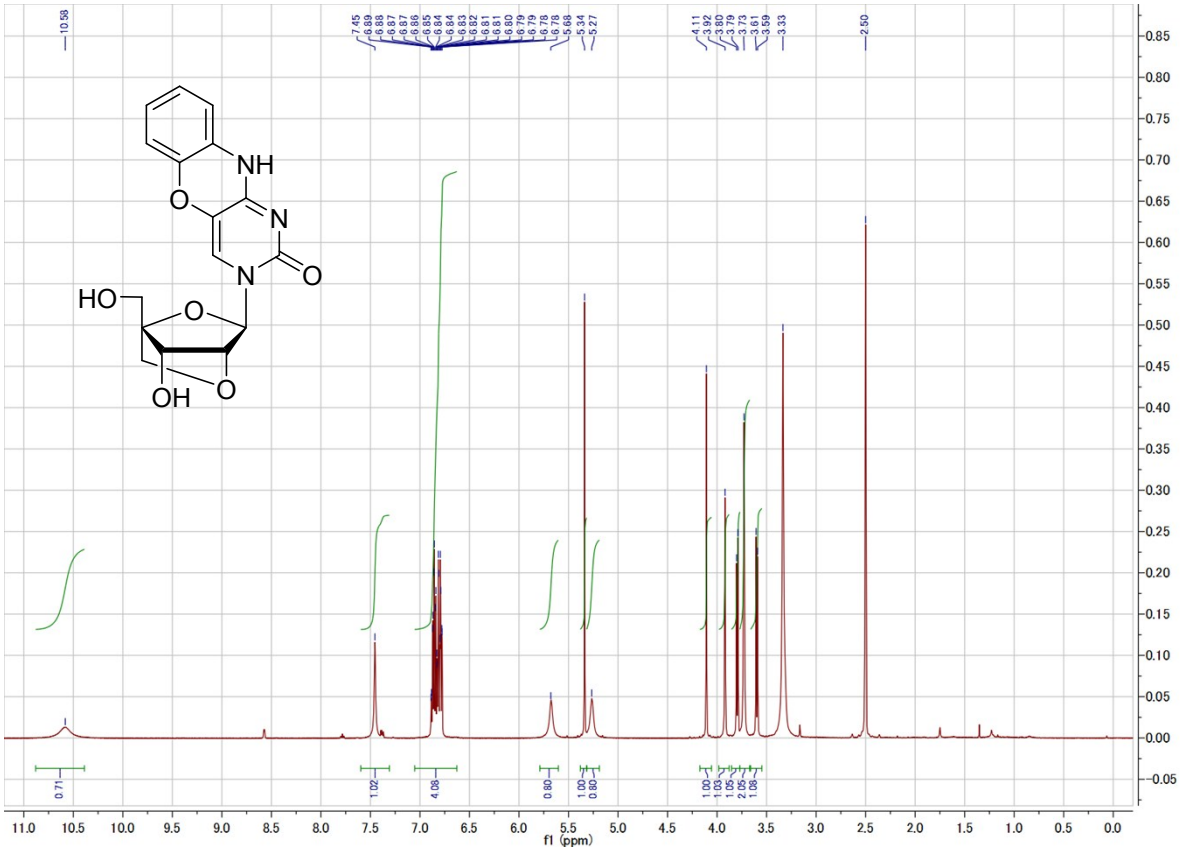
(c) ^{1}H-NMR (CD_3OD) of compound **6**



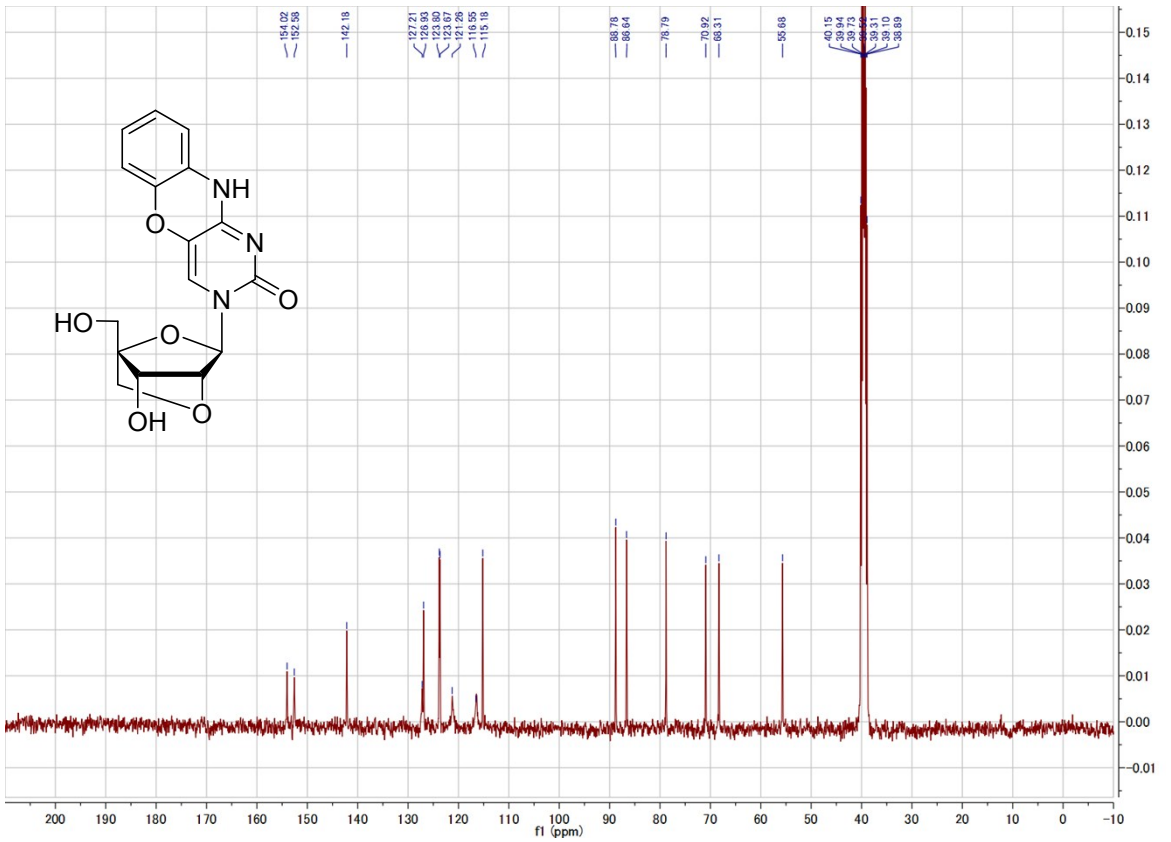
^{13}C-NMR (DMSO-d_6) compound **6**



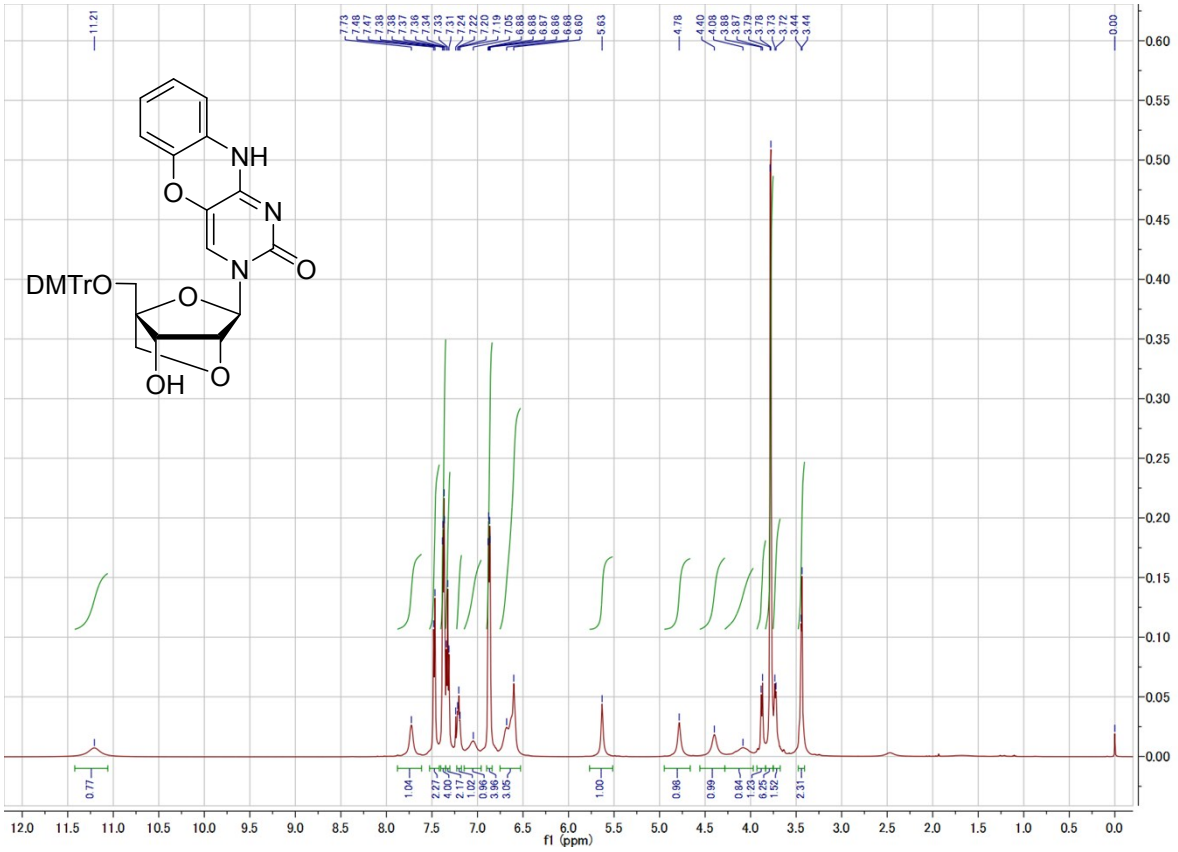
(d) ^{1}H-NMR (DMSO-d_6) compound **7**



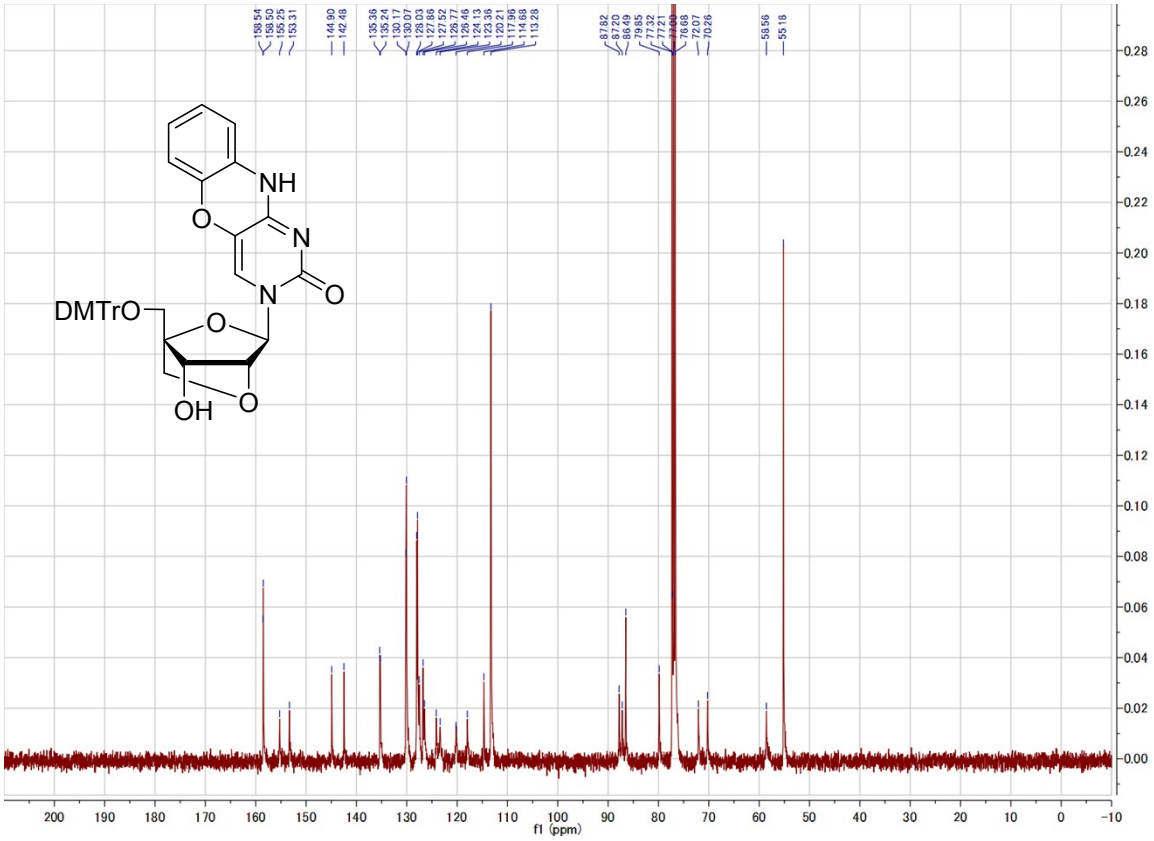
^{13}C-NMR (DMSO-d_6) of compound **7**



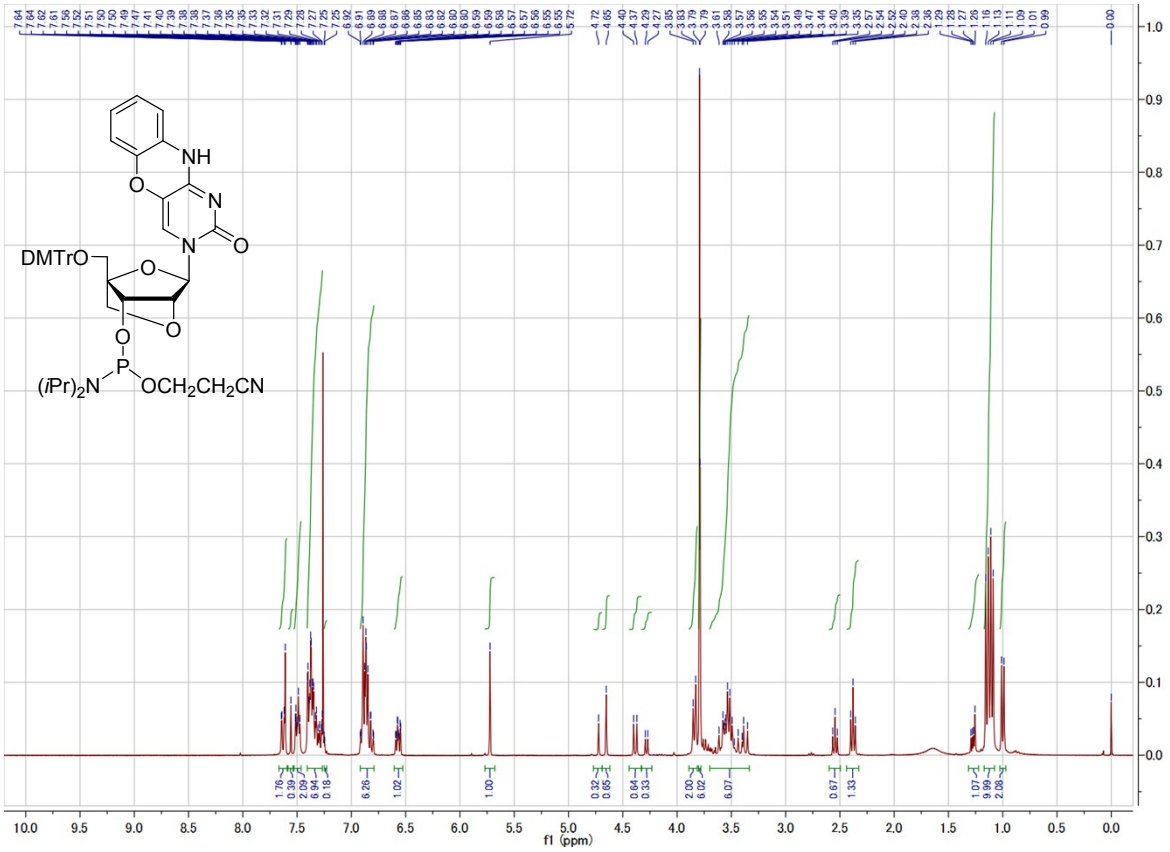
(e) ^{1}H-NMR ($CDCl_3$) of compound **8**



^{13}C-NMR ($CDCl_3$) of compound **8**



(f) ^{1}H-NMR ($CDCl_3$) of compound **9**



^{31}P-NMR ($CDCl_3$) of compound **9**

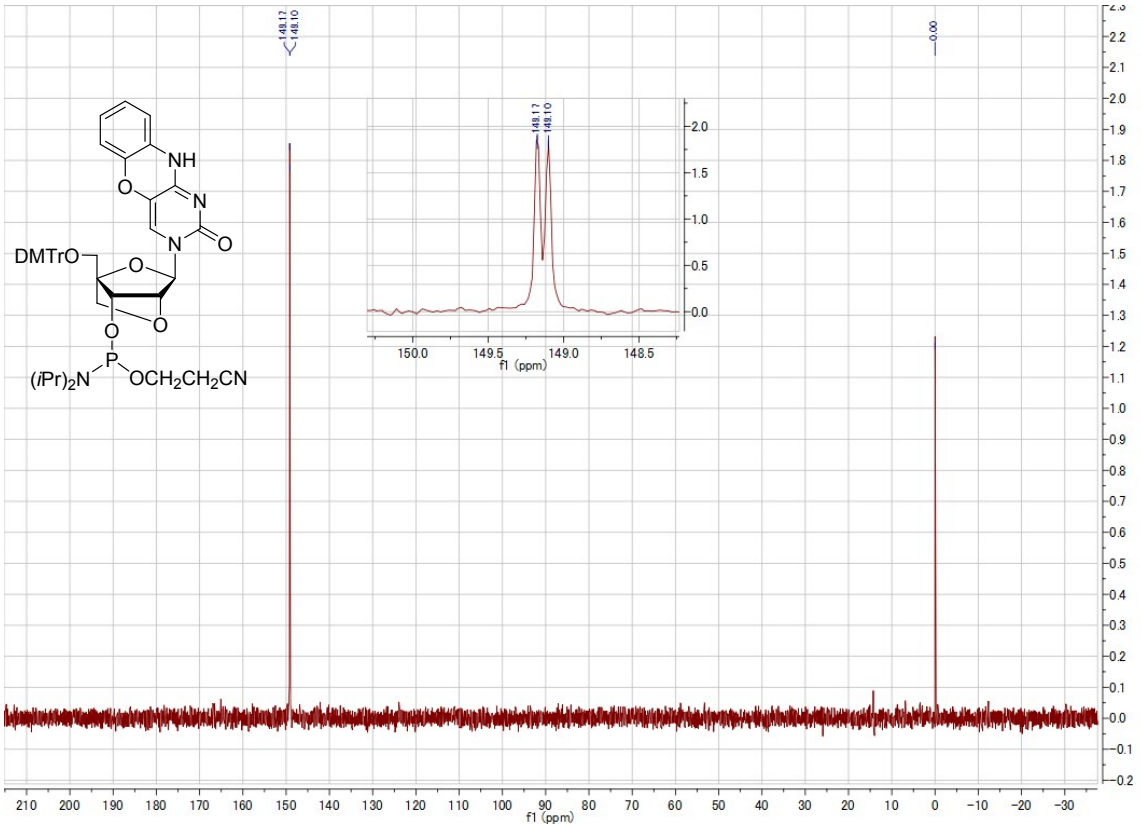
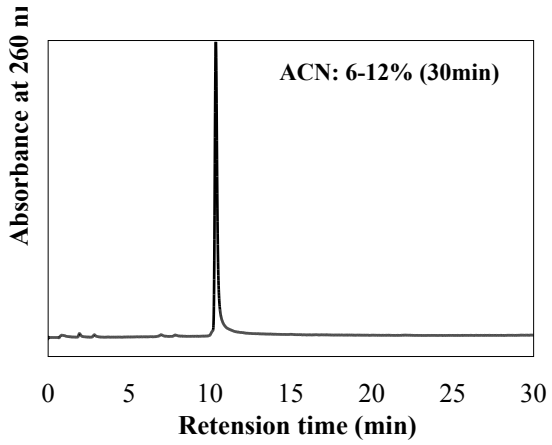


Figure S2. HPLC charts and MALDI-TOF Mass spectra of ODNs containing BNAP (**B**)

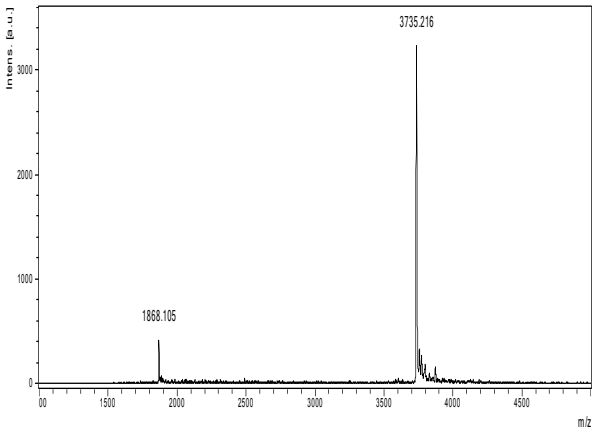
HPLC conditions: Detection: UV 260 nm, Flow rate: 1.0 mL/min, Mobile Phase (A): 0.1 M TEAA (pH 7.0), (B): acetonitrile, Column: Waters XBridge™ OST C18 2.5µm (4.6×50 mm), Temperature: 50°C.

(a) 5'-d(GCGTT**B**TTTGCT)-3'

HPLC

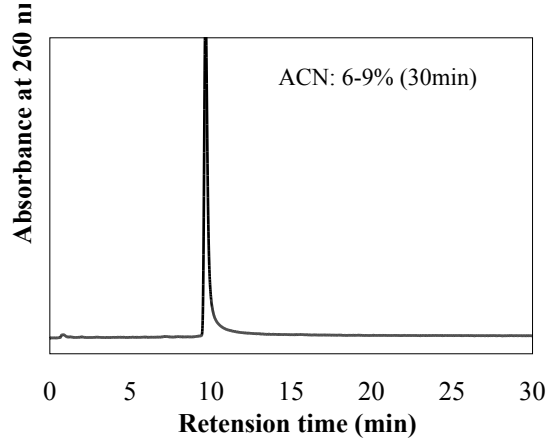


MALDI-TOF-Mass

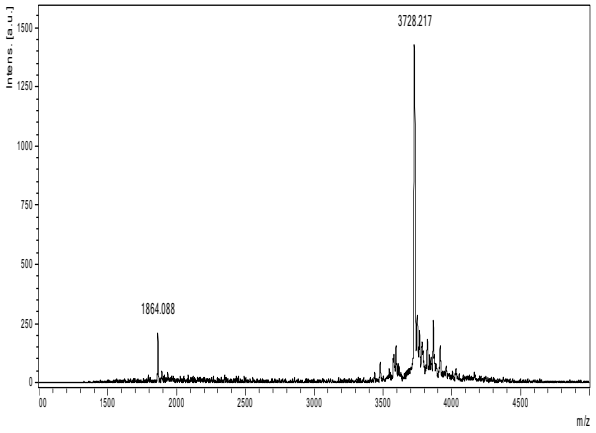


(b) 5'-d(GCGTC**B**ATTGCT)-3'

HPLC

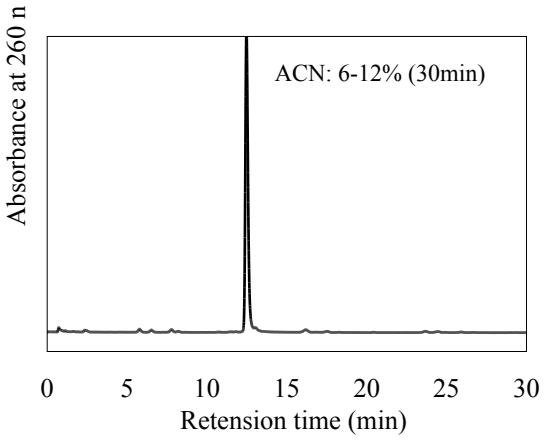


MALDI-TOF-Mass

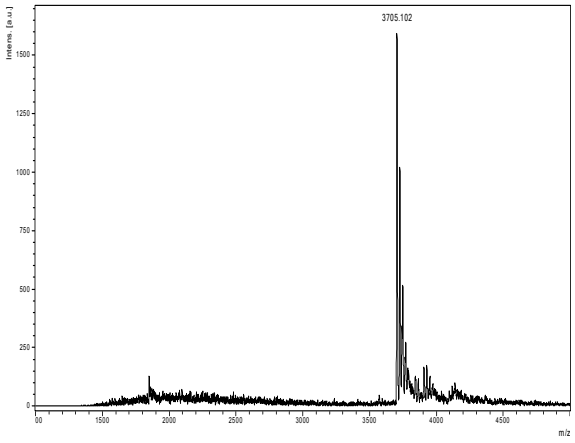


(c) 5'-d(GCGTC**B**CTTGCT)-3'

HPLC

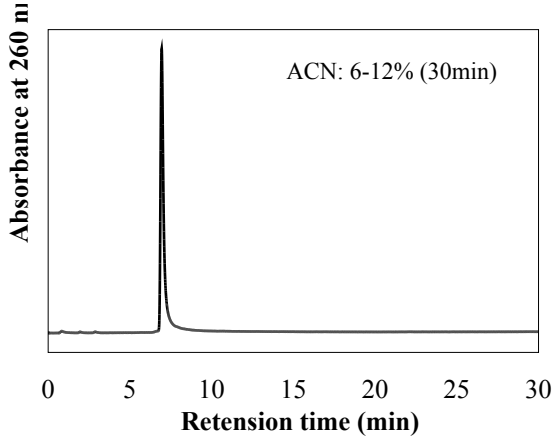


MALDI-TOF-Mass

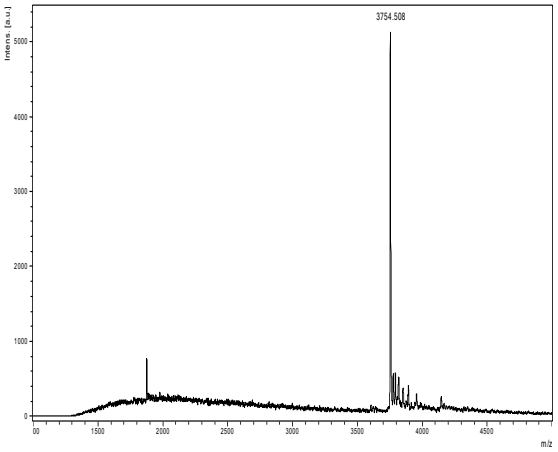


(d) 5'-d(GCGTA**B**ATTGCT)-3'

HPLC

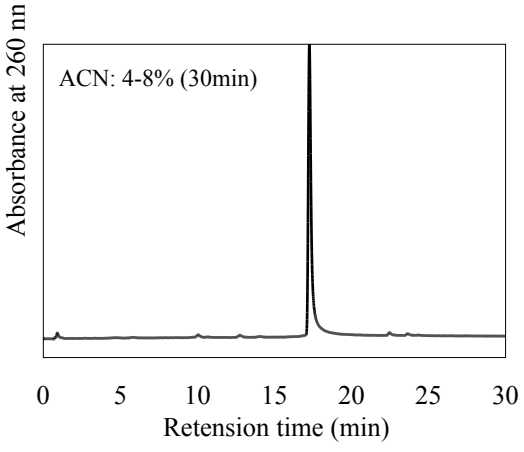


MALDI-TOF-Mass

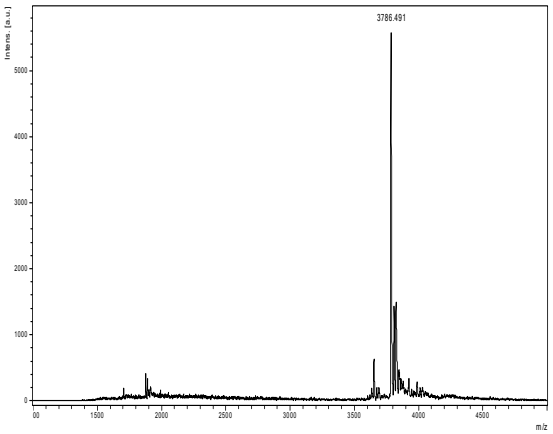


(e) 5'-d(GCGTG**B**GTTGCT)-3'

HPLC

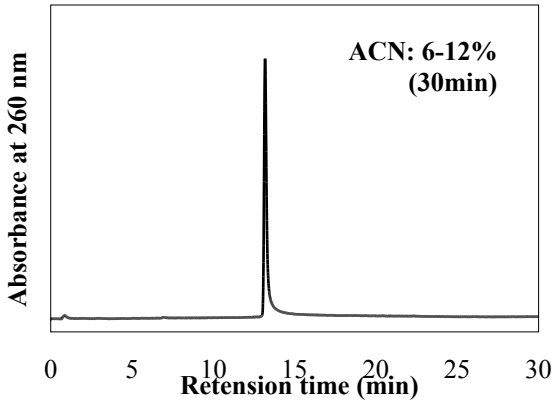


MALDI-TOF-Mass

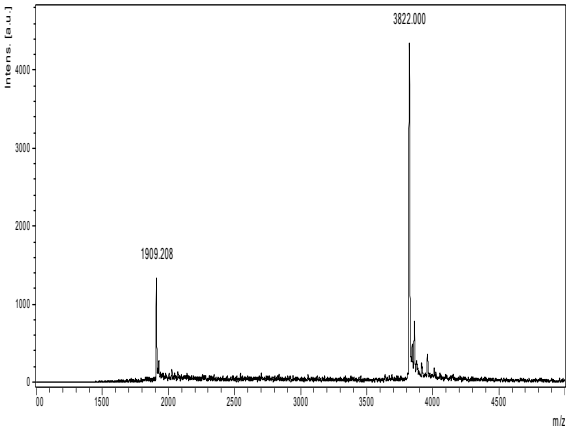


(f) 5'-d(GCGT**B**C**B**GTTGCT)-3'

HPLC

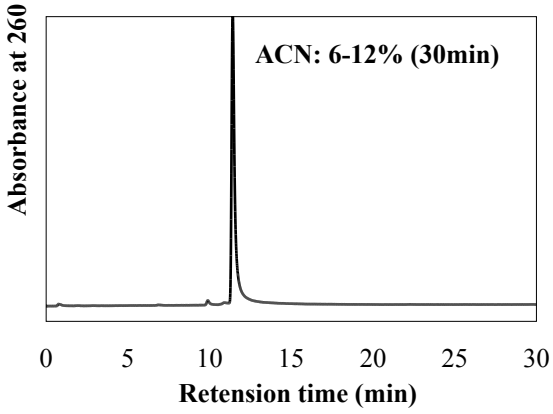


MALDI-TOF-Mass

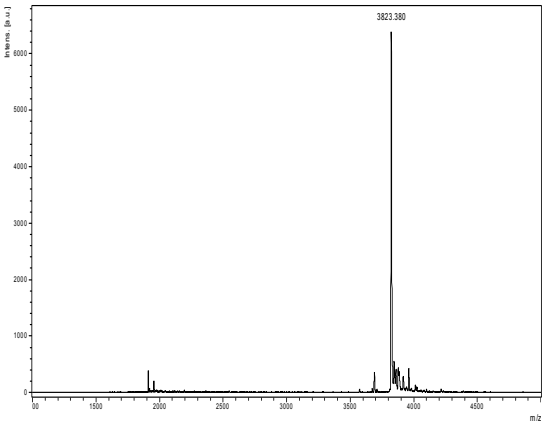


(g) 5'-d(GCGTC**BB**TTGCT)-3'

HPLC

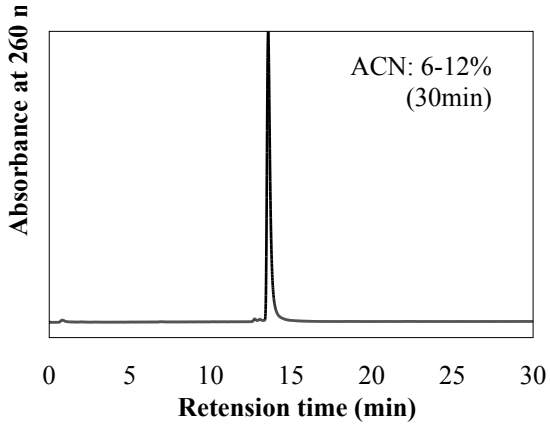


MALDI-TOF-Mass



(h) 5'-d(GCGT**BBB**TTGCT)-3'

HPLC



MALDI-TOF-Mass

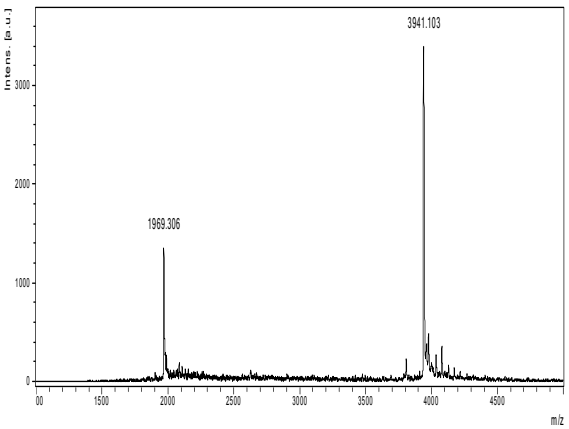
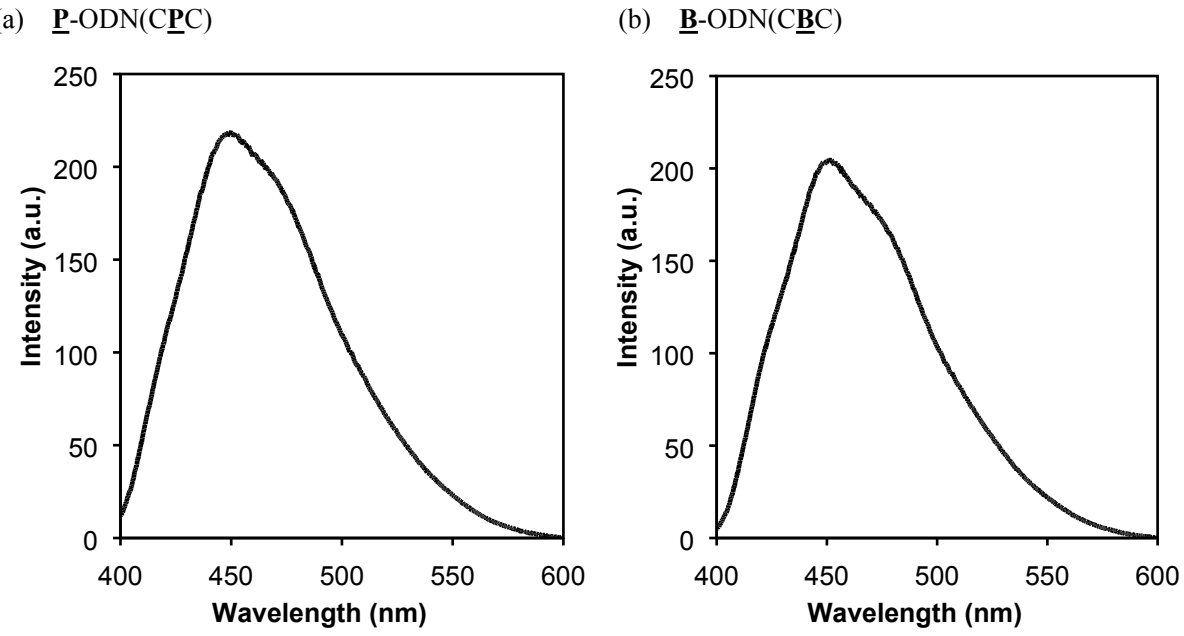


Table S1. MALDI-TOF mass data of ODNs containing 2'-deoxyribonucleoside with phenoxazine (**P**)

Sequences	Calcd. $(M-H)^-$	Found
5'-d(GCGTT**P**TTTGCT)-3'	3707.5	3706.9
5'-d(GCGTC**P**ATTGCT)-3'	3701.5	3702.1
5'-d(GCGTC**P**CTTGCT)-3'	3677.4	3676.9
5'-d(GCGTA**P**ATTGCT)-3'	3725.5	3725.2
5'-d(GCGTG**P**GTTGCT)-3'	3757.5	3756.9
5'-d(GCGT**P**C**P**TTGCT)-3'	3767.5	3767.8
5'-d(GCGTC**PP**TTGCT)-3'	3767.5	3767.6
5'-d(GCGT**PPP**TTGCT)-3'	3857.6	3858.2

ODNs containing 2',4'-BNA/LNA with 5-methylcytosine (**L**) were purchased from *GeneDesign* Inc., Japan.

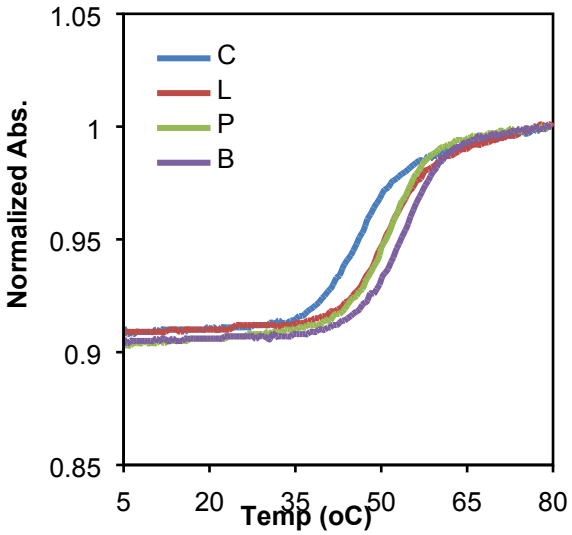
Figure S3. Fluorescent spectra of ODNs containing **P** and **B**



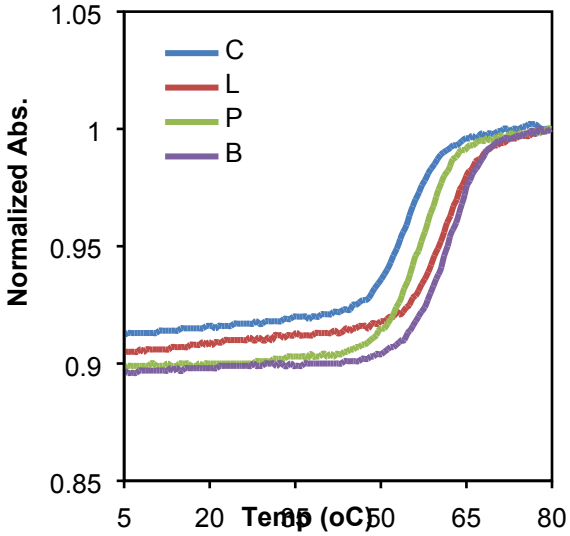
Conditions: 2 mM sodium phosphate buffer (pH 7.2), 20 mM NaCl, each strand 2 μM. Ex.: 365 nm, rt.
Sequence: 5′-d(GCGT**CXC**TTGCT)-3′ (**X**: **P** or **B**).

Figure S4. UV melting curves for the duplexes

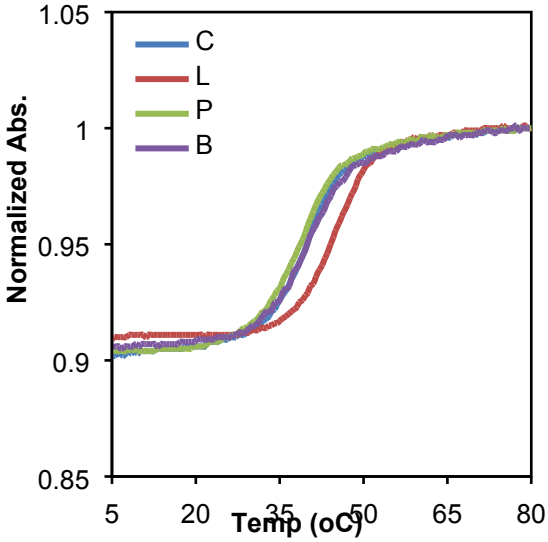
(a) **X**-ODN(C**X**C)/cDNA



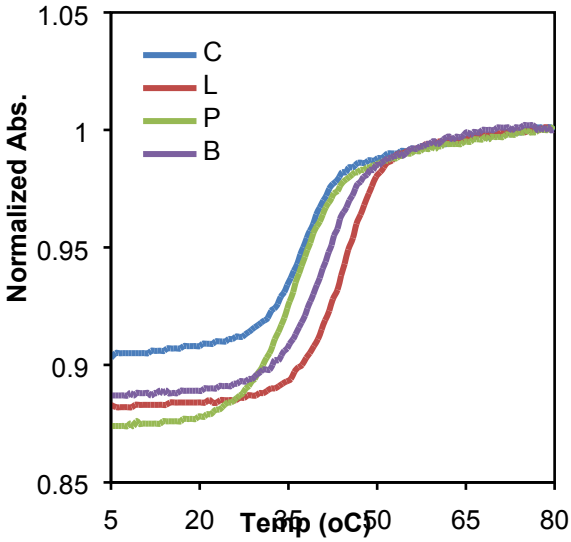
(b) **X**-ODN(C**X**C)/cRNA



(c) **X**-ODN(A**X**A)/cDNA



(d) **X**-ODN(A**X**A)/cRNA



UV melting profiles were measured in 2 mM sodium phosphate buffer (pH 7.2) containing 20 mM NaCl at a scan rate of 0.5°C/min at 260 nm. The concentration of oligonucleotide used was 2 μM for each strand. The error in T_m values was ±0.5°C. **C**: 2′-deoxycytidine, **L**: 2′,4′-BNA/LNA with 5-methylcytosine, **P**: 2′-deoxyribonucleoside with phenoxazine, **B**: BNAP.
Sequence: 5′-d(GCGT**NNN**TTGCT)-3′/3′-CGC**QGQ**AACGA-5′ (**X**: **C**, **L**, **P** or **B**) (**Q**: Corresponding matching base).

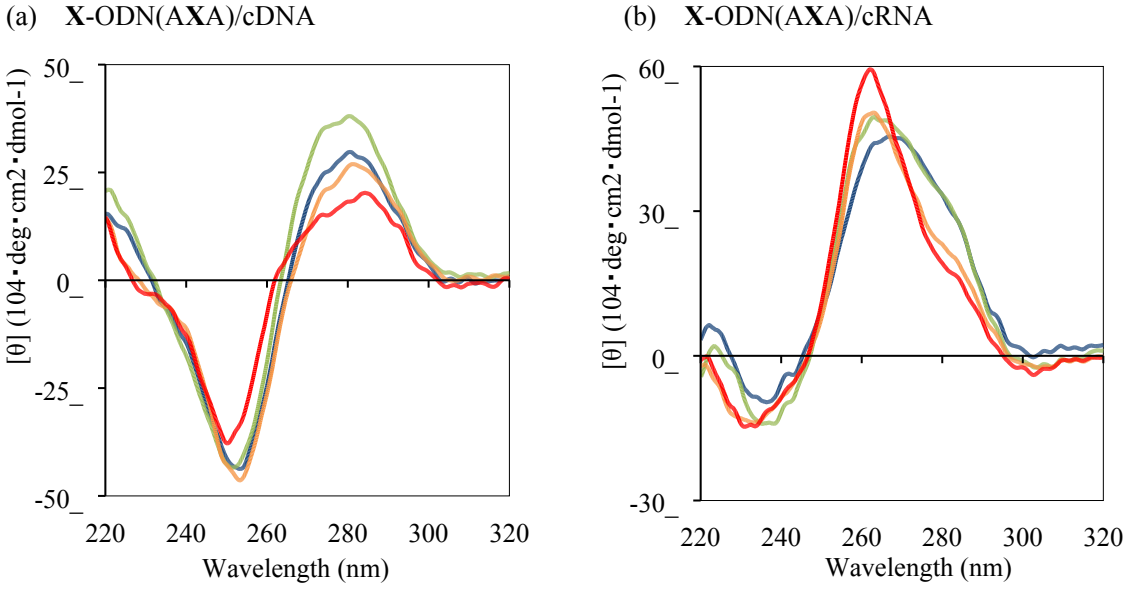
Table S2. Comparison of matched versus mismatched hybridization (**X**-ODN/cRNA)

5′-d(GCGTC**X**CTTGCT)-3′
3′-r(CGCAG**Y**GAACGA)-5′

X =	T_m (ΔT_m: T_m[mismatch]-T_m[match]) (°C)			
Y =	G	A	U	C
C	54	39 (−15)	31 (−23)	32 (−22)
L	61	45 (−16)	40 (−21)	38 (−23)
P	56	43 (−13)	39 (−17)	35 (−21)
B	62	48 (−14)	44 (−18)	39 (−23)

Values in parentheses are the difference between the T_m when the ODN was bound with the guanine-containing target and the T_m when the ODN was bound with a mismatched base. The buffer conditions were the same as given in Table 2. **C**: 2′-deoxycytidine, **L**: 2′,4′-BNA/LNA with 5-methylcytosine, **P**: 2′-deoxyribonucleoside with phenoxazine, **B**: BNAP.

Figure S5. CD spectra of DNA/DNA (panel a) and DNA/RNA (panel b) duplexes (5′-A**X**A-3′)



Conditions: 2 mM sodium phosphate buffer (pH 7.2), 20 mM NaCl, each strand 2 μM. **C**: 2′-deoxycytidine (blue), **L**: 2′,4′-BNA/LNA with 5-methylcytosine (green), **P**: 2′-deoxyribonucleoside with phenoxazine (orange), **B**: BNAP (red).
Sequence: 5′-d(GCGT**AXA**TTGCT)-3′/3′-CGC**TCT**AACGA-5′ (**X**: **C**, **L**, **P** or **B**).

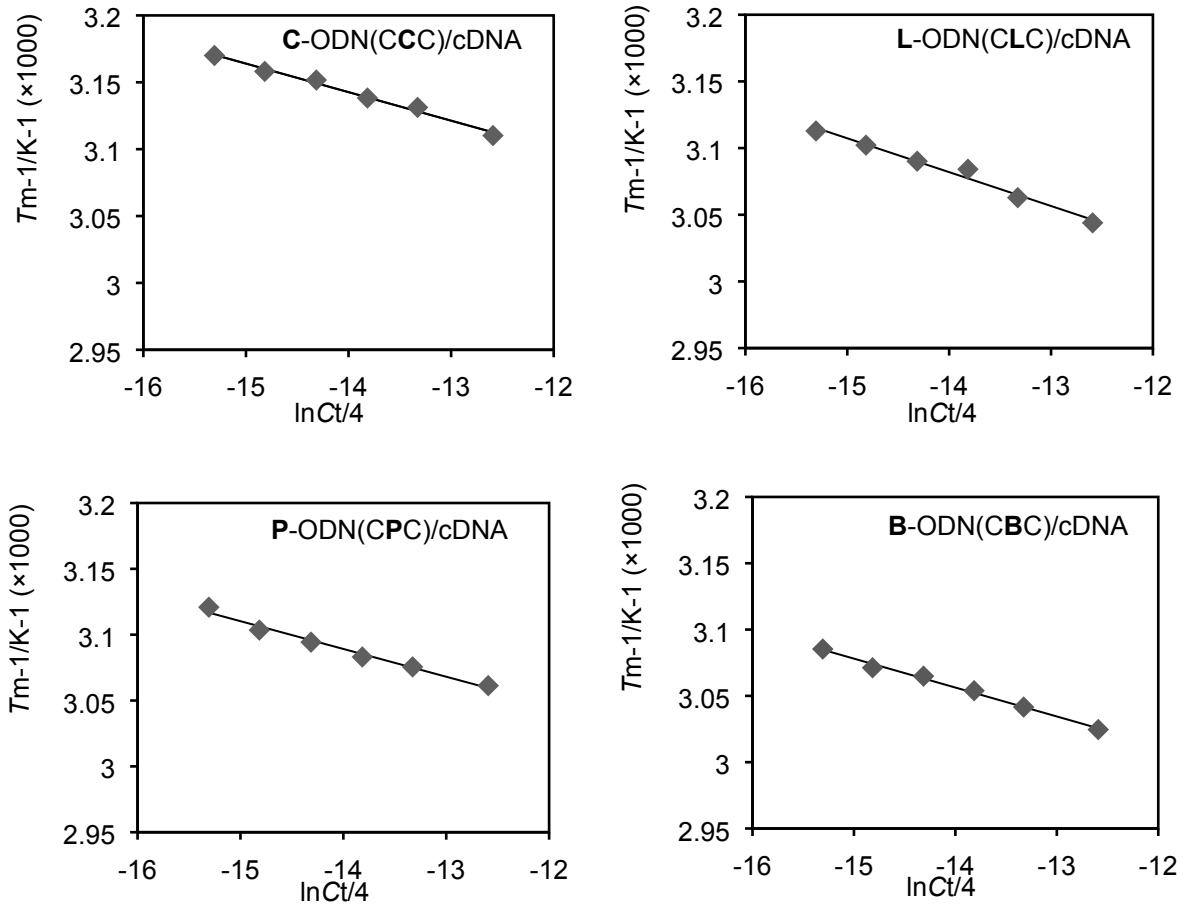
Table S3. Thermodynamic parameters of duplexes with cDNA and cRNA (5′-A**X**A-3′)

X =	Target DNA						Target RNA					
	ΔH	ΔS	$\Delta G_{(37°C)}$	$\Delta\Delta H$	$\Delta\Delta S$	$\Delta\Delta G_{(37°C)}$	ΔH	ΔS	$\Delta G_{(37°C)}$	$\Delta\Delta H$	$\Delta\Delta S$	$\Delta\Delta G_{(37°C)}$
A**C**A	−82.8	−237	−9.4	–	–	–	−92.4	−270	−8.8	–	–	–
A**L**A	−92.0	−262	−10.9	−9.2	−25	−1.5	−86.8	−246	−10.5	+5.6	+24	−1.7
A**P**A	−75.0	−213	−8.9	+7.8	+24	+0.5	−82.8	−240	−8.2	+9.6	+30	+0.6
A**B**A	−76.4	−216	−9.3	+6.4	+21	+0.1	−81.8	−233	−9.4	+10.6	+37	−0.6

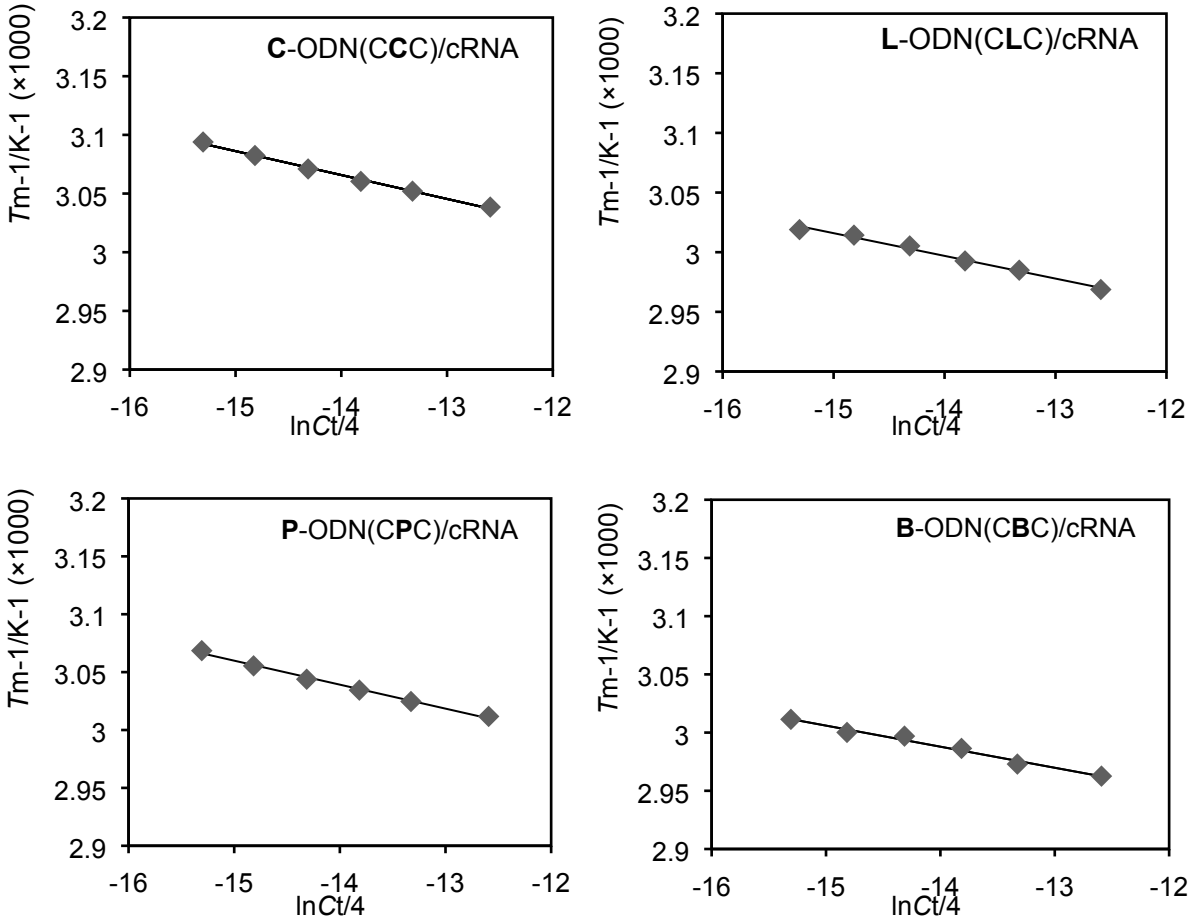
C: 2′-deoxycytidine, **L**: 2′,4′-BNA/LNA with 5-methylcytosine,
P: 2′-deoxyribonucleoside with phenoxazine, **B**: BNAP.
Sequence: 5′-d(GCGT**AXA**TTGCT)-3′/3′-CGC**TCT**AACGA-5′ (**X**: **C**, **L**, **P** or **B**).
The units of ΔH, ΔS and ΔG are kcal/mol, cal/mol・K and kcal/mol, respectively. $\Delta\Delta H$, $\Delta\Delta S$ and $\Delta\Delta G$ indicate the difference of the corresponding ΔH, ΔS and ΔG value from that of **C**-ODN/cDNA (or cRNA) duplex.

Figure S6. Van't Hoff plots based on T_m results obtained under various concentrations

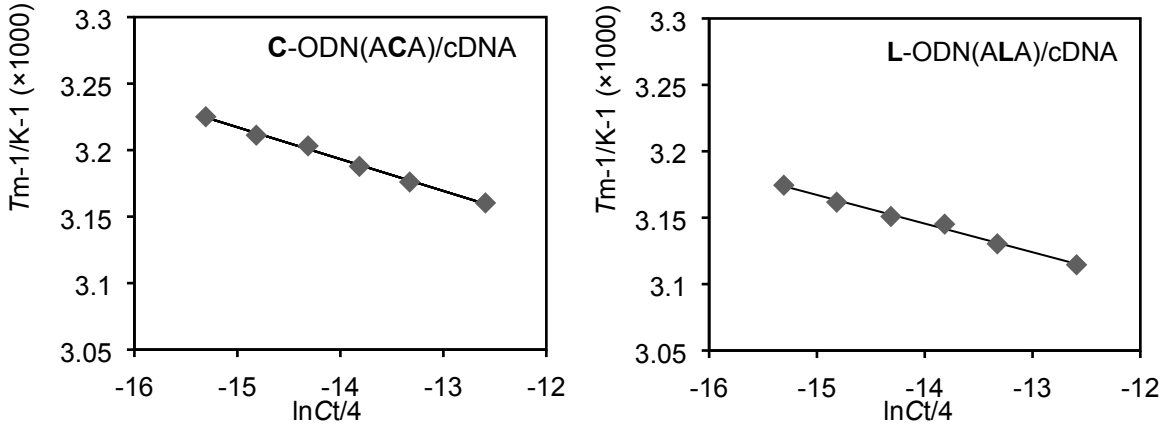
(a) **X**-ODN(C**X**C)/cDNA (**X**: **C**, **L**, **P** or **B**)

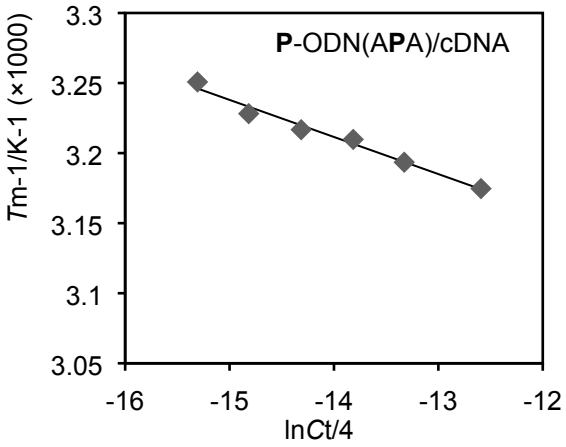


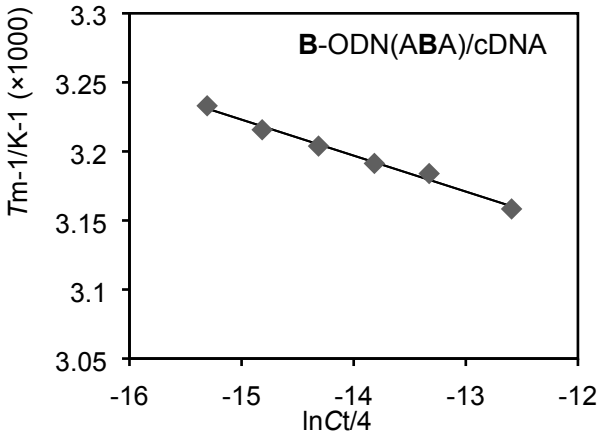
(b) **X**-ODN(C**X**C)/cRNA (**X**: **C**, **L**, **P** or **B**)



(c) **X**-ODN(A**X**A)/cDNA (**X**: **C**, **L**, **P** or **B**)







(d) **X**-ODN(A**X**A)/cRNA (**X**: **C**, **L**, **P** or **B**)