Organic & Biomolecular Chemistry

Supporting Information

Enhanced Duplex- and Triplex-forming Ability and Enzymatic Resistance of Oligodeoxynucleotides Modified by a Tricyclic Thymine Derivative

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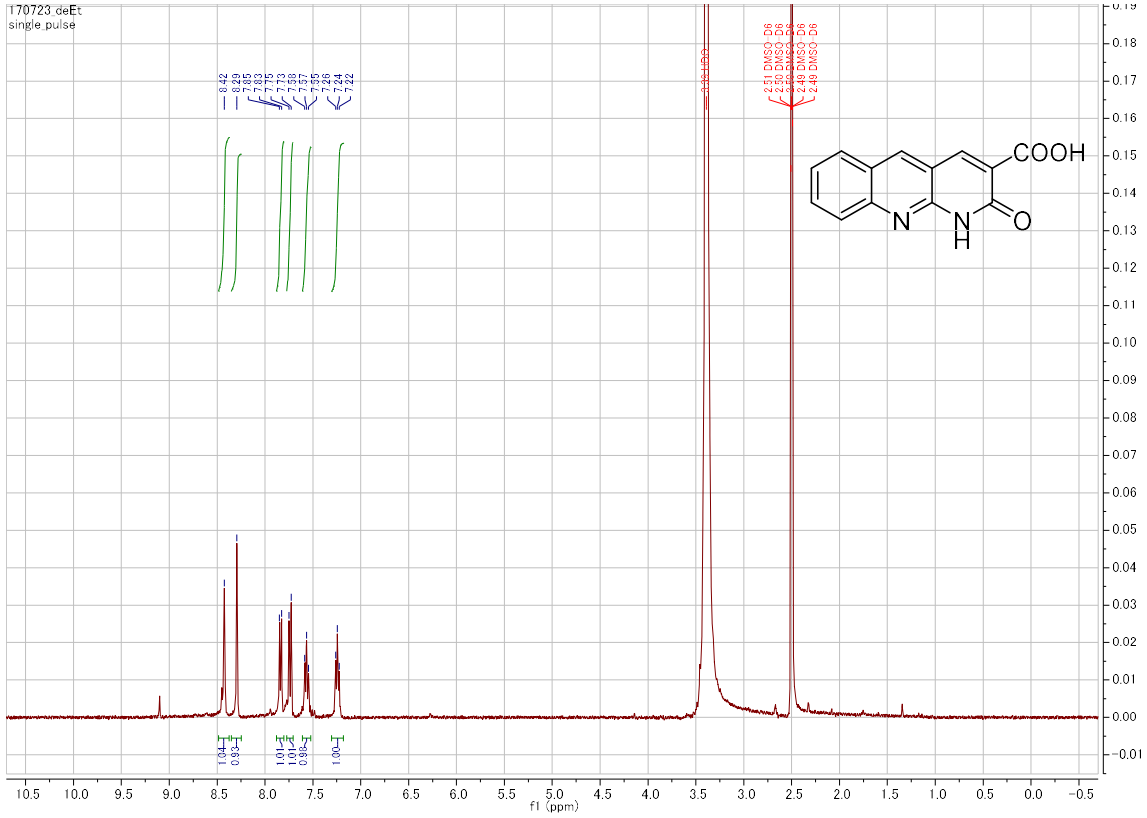
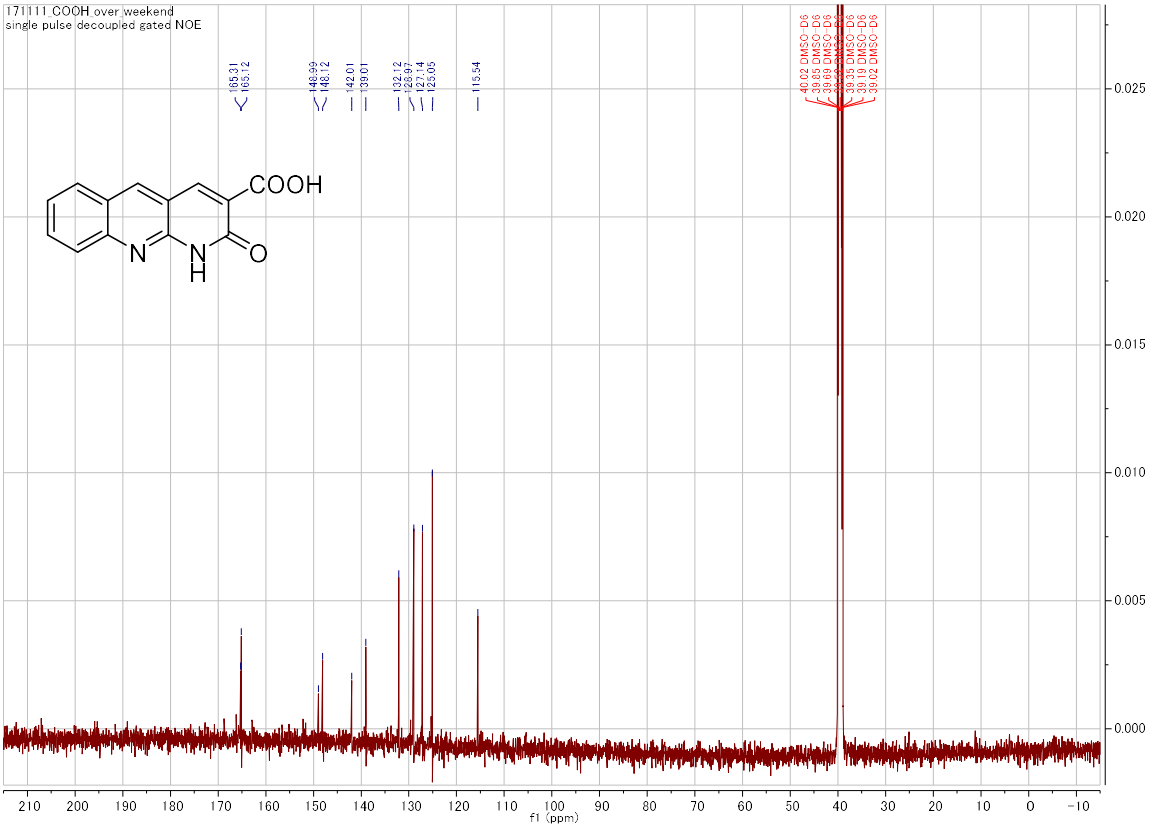
Fig. S1 ^{1}H-NMR, ^{13}C-NMR, ^{31}P-NMR spectra of each compound.

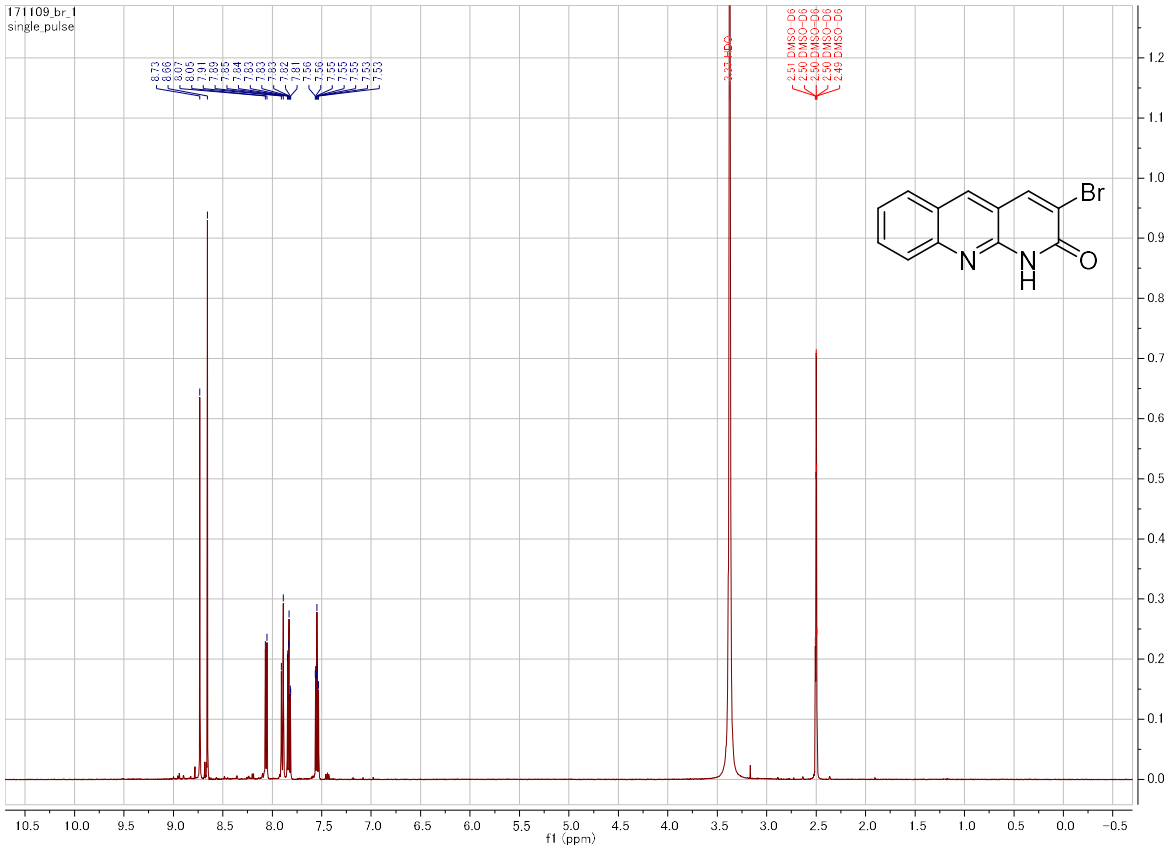
(a) ^{1}H-NMR (DMSO-d_6) of compound **2**



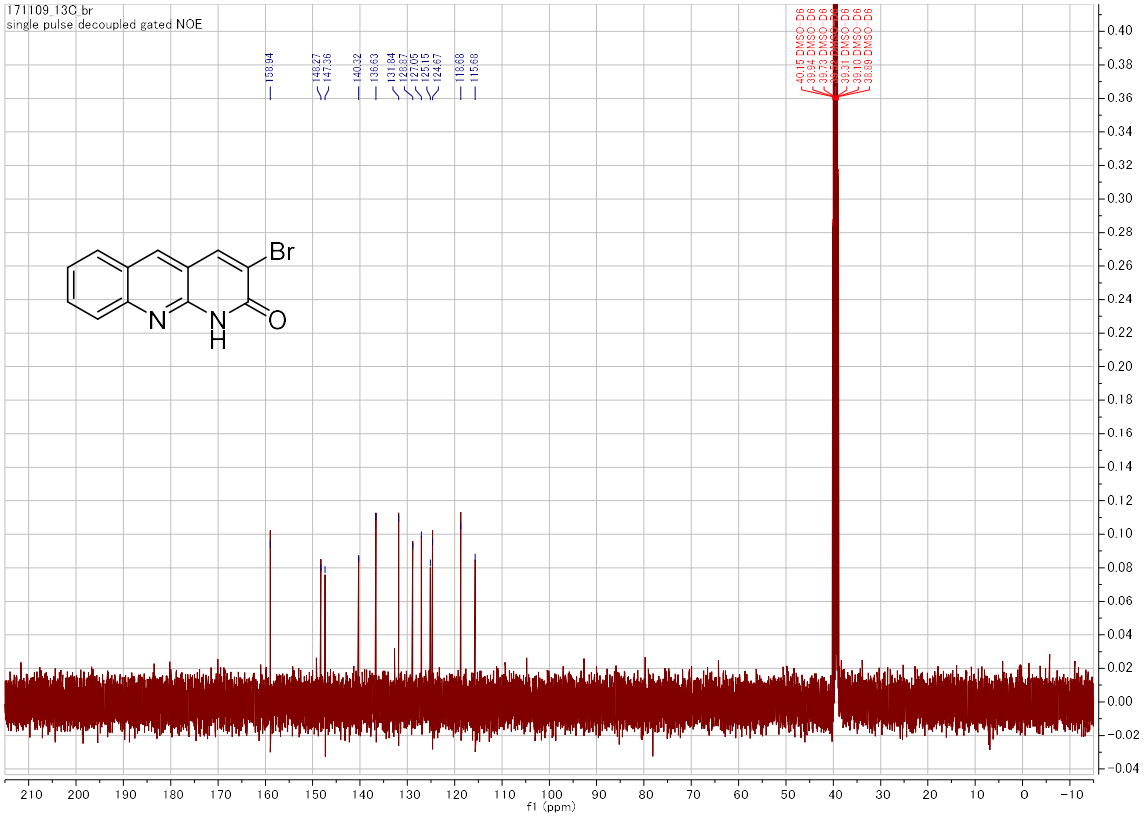
^{13}C-NMR (DMSO-d_6) of compound **2**



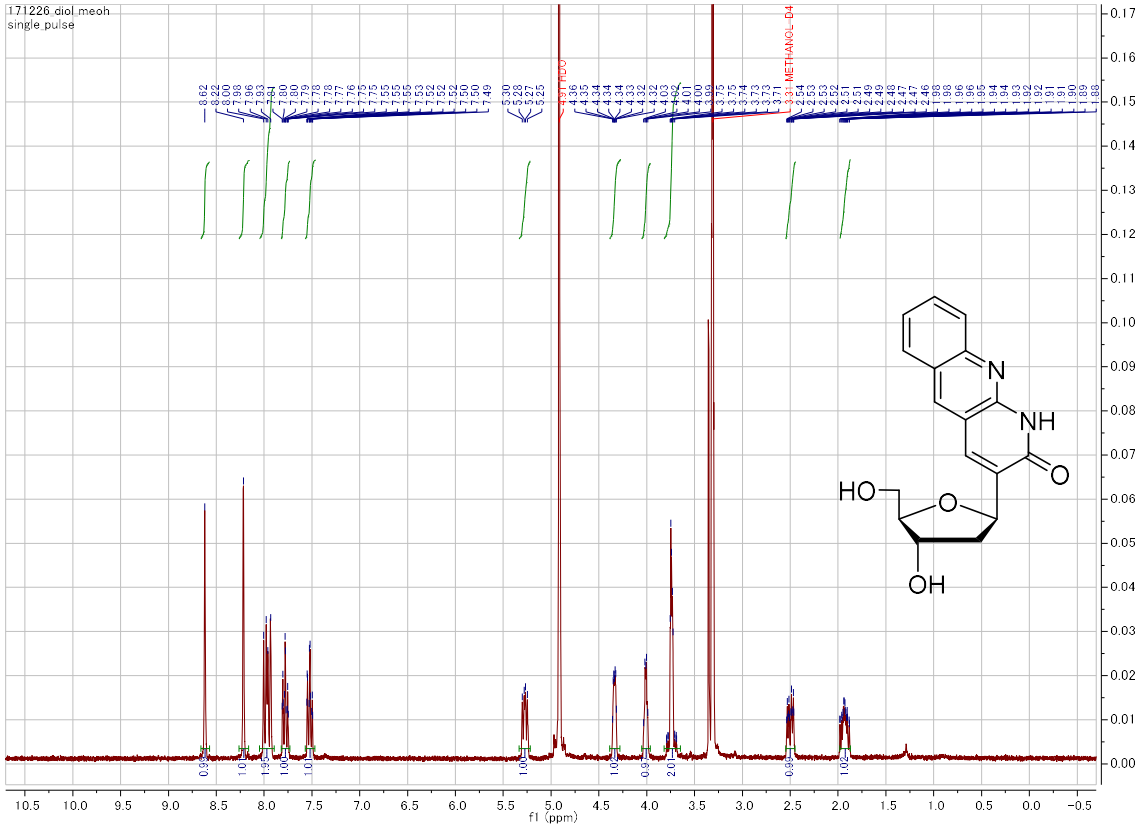
(b) ^{1}H-NMR (DMSO-d_6) of compound **3**



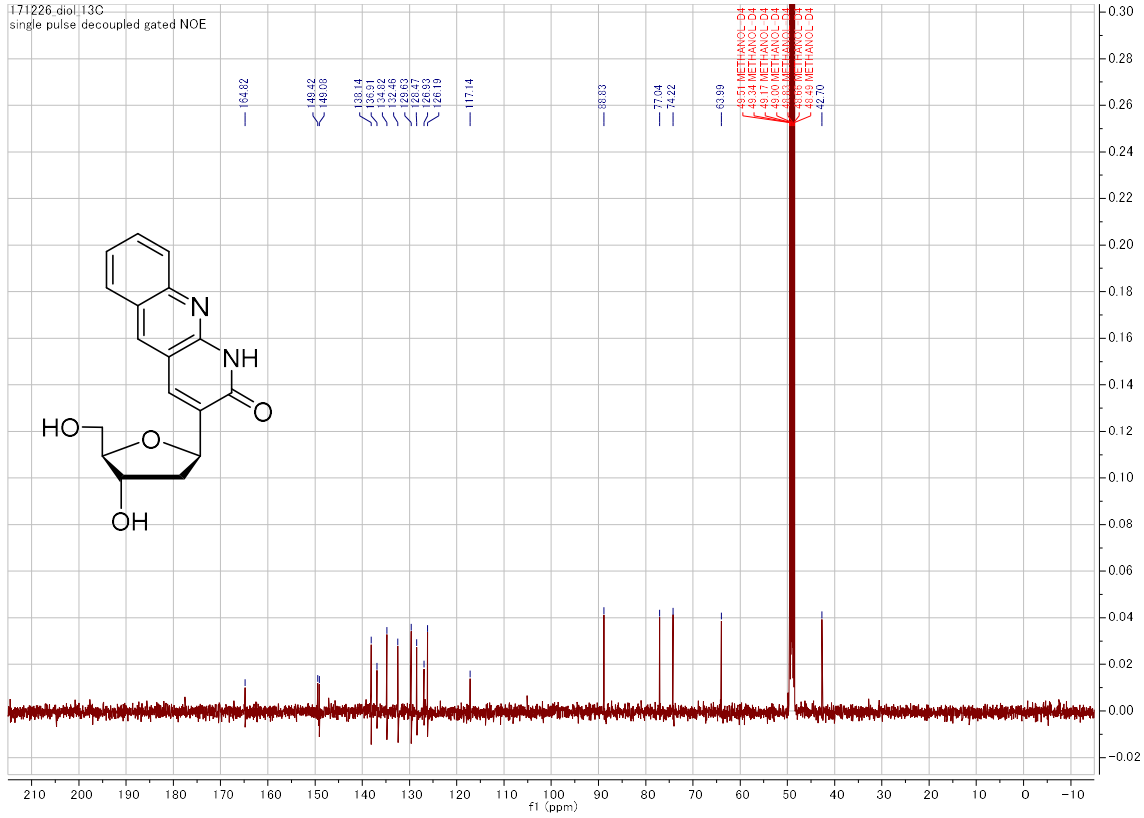
^{13}C-NMR (DMSO-d_6) of compound **3**



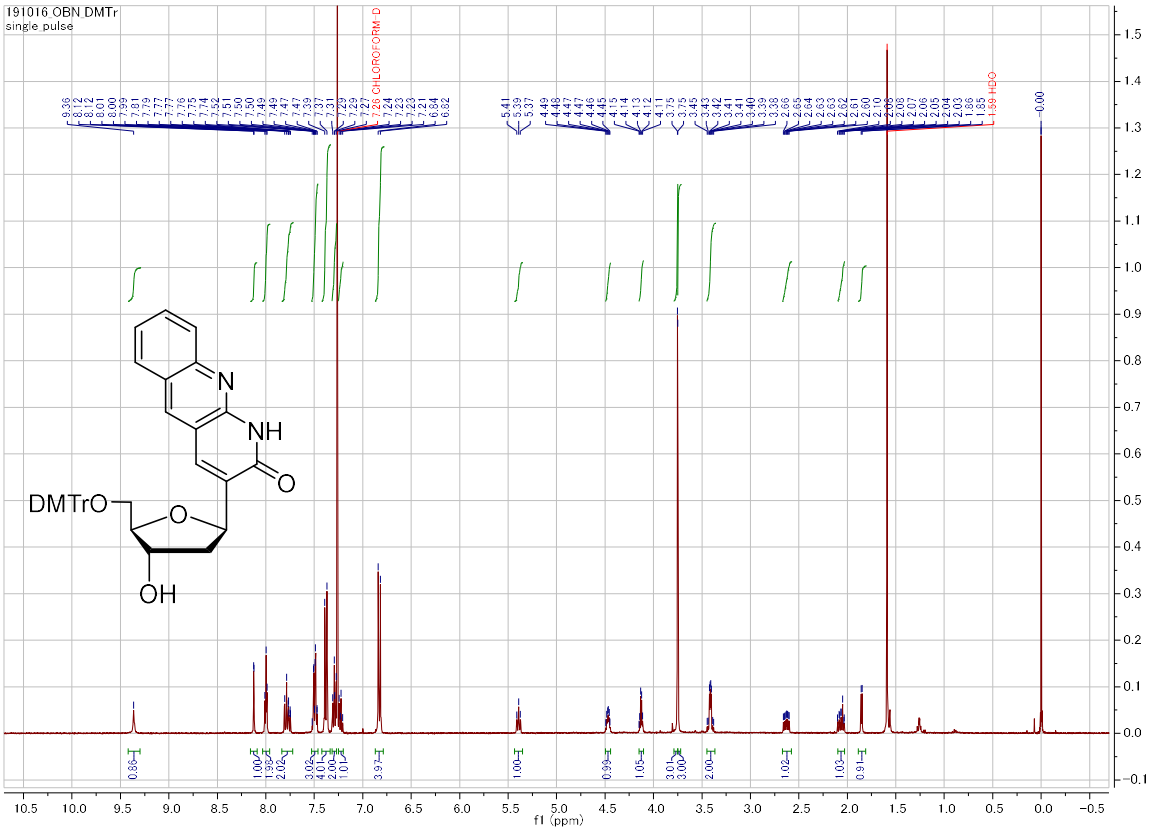
(c) ^{1}H-NMR (CD_3OD) of compound **6**



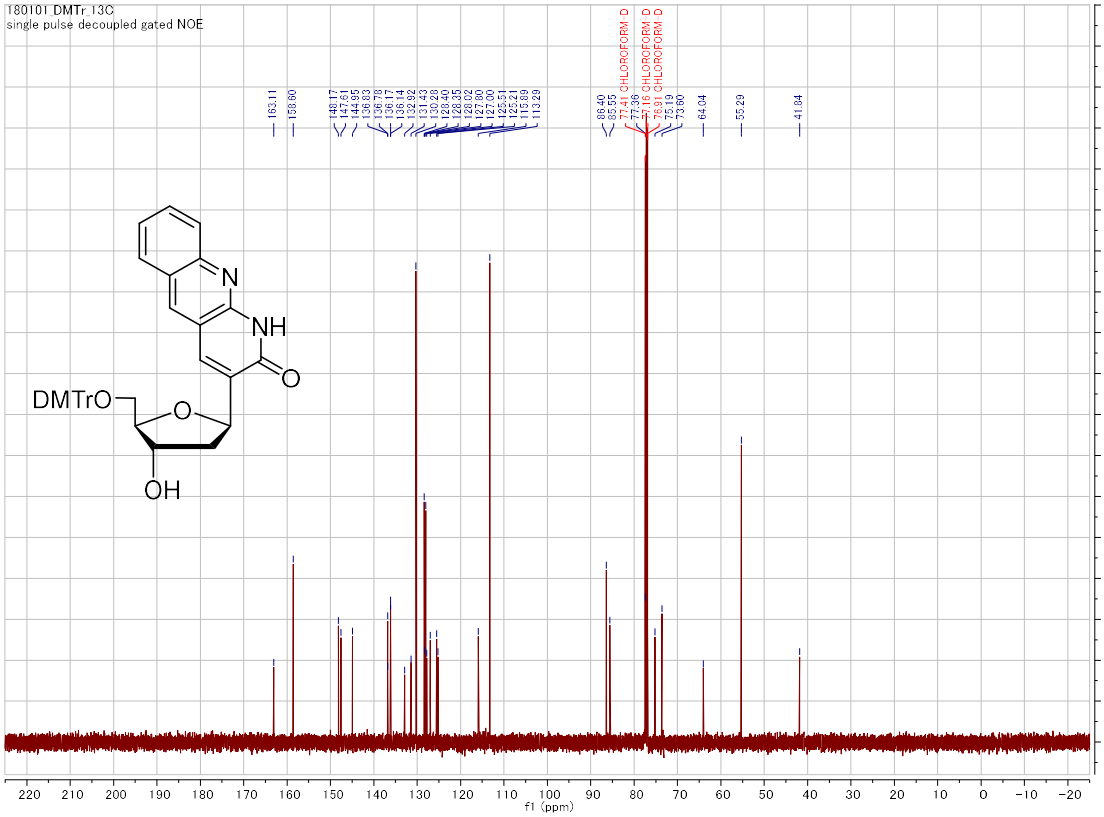
^{13}C-NMR (CD_3OD) compound **6**



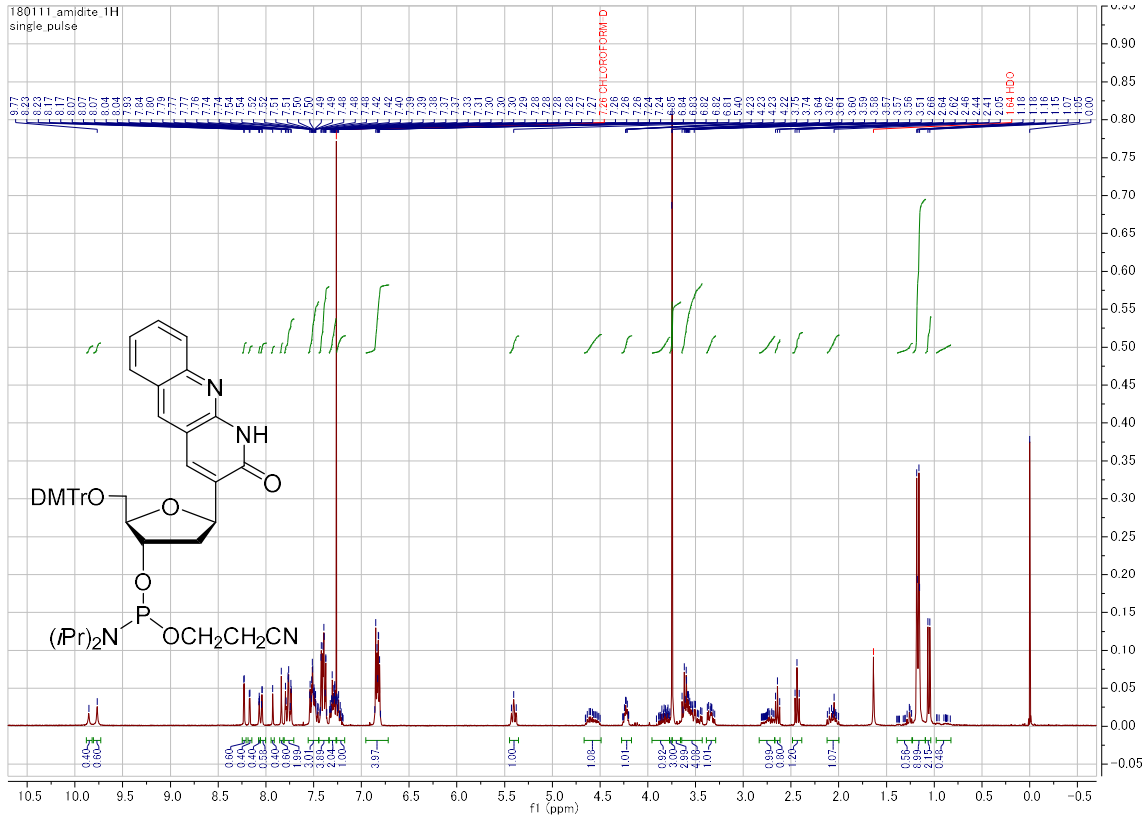
(d) ^{1}H-NMR ($CDCl_3$) compound **7**



^{13}C-NMR ($CDCl_3$) of compound **7**



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



^{31}P-NMR ($CDCl_3$) of compound **8**

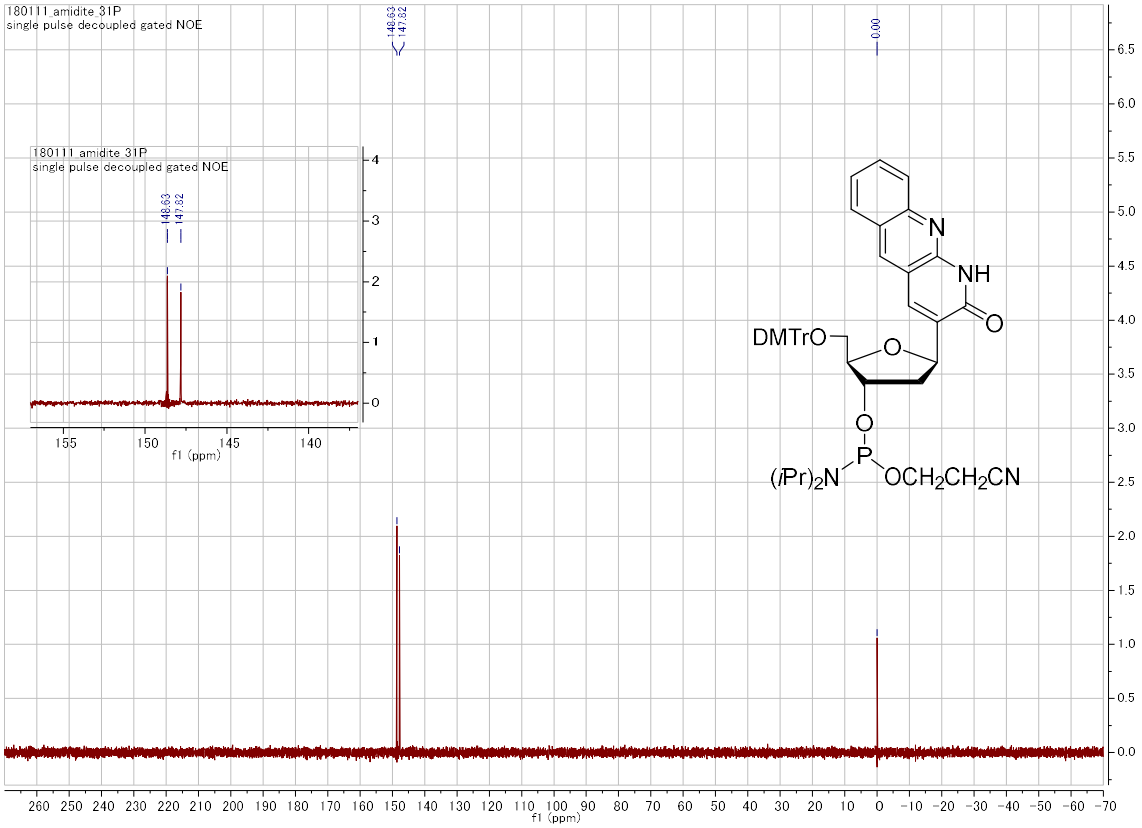


Fig. S2 Absorption spectrum of OBN nucleoside **6**.

OBN nucleoside **6** was dissolved in water containing 0.1% (v/v) of DMSO at 25μM (final concentration). The absorption spectrum was measured by UV-1800 spectrometers (SHIMADZU).

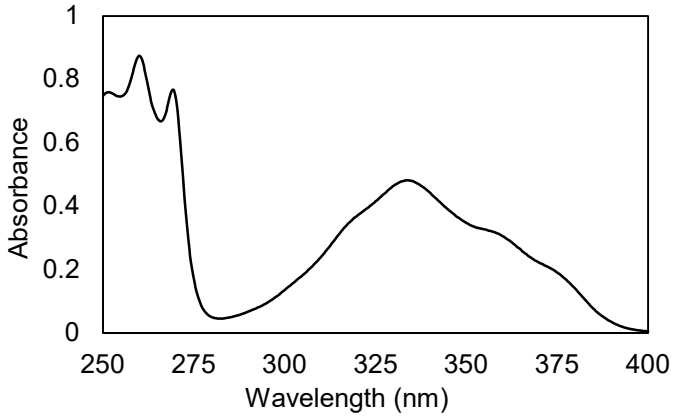


Table S1 Aborption data of OBN nucloiside **6**.

Molar Absorptivity (L/(mol·cm)) was calculated according Lambert-Beer law based on each absorption maximum picked up from Fig S2.

Absorption maximum (nm)	Molar Absorptivity (L/(mol·cm))
260	30,000
269	26,000
334	16,000

Table S2 Sequence and MALDI-TOF-Mass data of OBN-modified ODNs.

Sequence	MALDI-TOF-Mass $[M-H]^-$	
	Calcd.	Found
ODN2: 5′-d(GCGTT**B**TTTGCT)-3′	3702.5	3703.8
ODN3: 5′-d(GCGT**B**T**B**TTGCT)-3′	3772.6	3772.7
ODN4: 5′-d(GCGTT**BB**TTGCT)-3′	3772.6	3772.4
ODN5: 5′-d(GCG**B**T**B**T**B**TGCT)-3′	3842.7	3843.4
ODN6: 5′-d(GCGT**BBB**TTGCT)-3′	3842.7	3843.8
ODN8: 5′-d(GCGTC**B**ATTGCT)-3′	3696.5	3697.3
ODN10: 5′-d(GCGTC**B**CTTGCT)-3′	3672.5	3673.5
ODN12: 5′-d(GCGTA**B**ATTGCT)-3′	3720.5	3722.4
ODN14: 5′-d(GCGTG**B**GTTGCT)-3′	3752.5	3753.2
TFO2: 5′-d(TTTTT<u>C</u>T**B**T<u>C</u>T<u>C</u>T<u>C</u>T)-3′	4566.1	4567.3
TFO3: 5′-d(TTTT**B**<u>C</u>TTT<u>C</u>T<u>C</u>T<u>C</u>T)-3′	4566.1	4565.0
TFO4: 5′-d(TTTTT<u>C</u>**B**TT<u>C</u>T<u>C</u>T<u>C</u>T)-3′	4566.1	4565.6
TFO5: 5′-d(TTTT**B**<u>C</u>**B**TT<u>C</u>T<u>C</u>T<u>C</u>T)-3′	4636.2	4638.8
TFO6: 5′-d(TTTT**B**<u>C</u>TT**B**<u>C</u>T<u>C</u>T<u>C</u>T)-3′	4636.2	4636.6
TFO7: 5′-d(TTTTT<u>C</u>T**BB**<u>C</u>T<u>C</u>T<u>C</u>T)-3′	4636.2	4637.0
TFO8: 5′-d(TTTTT<u>C</u>**BBB**<u>C</u>T<u>C</u>T<u>C</u>T)-3′	4706.3	4706.8
ODN18: 5′-d(TTTTTTTTT**B**)-3′	3049.1	3049.0

B = OBN, <u>C</u> = 2′-deoxy-5-mehtylcytidine

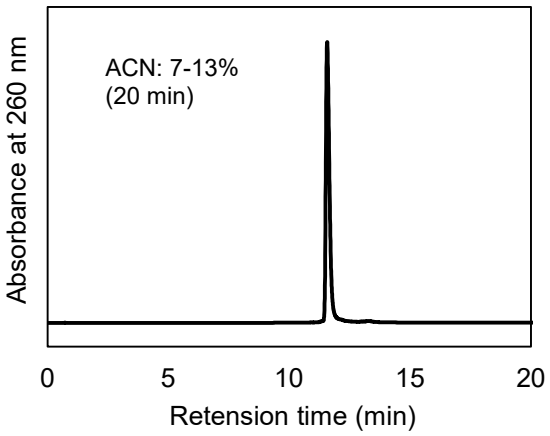
Fig. S3 HPLC chart of oligodeoxynucleotides (ODNs) containing OBN (**B**).

HPLC conditions: Detection: UV 260 nm, flow rate: 1.0 mL/min, mobile Phase (A): 0.1 M TEAA (pH 7.0), (B): acetonitrile (ACN), column: waters XBridge™ OST C18 2.5μm (4.6×50 mm), temperature: 50 ˚C.

(**B**: OBN, C: 2′-deoxy5-methylcytidine)

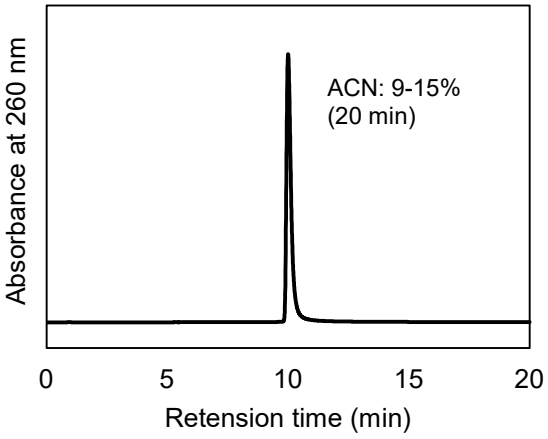
(a) 5'-d(GCGTT**B**TTTGCT)-3' (ODN**2**)

[B conc. ACN: 7-13% (20 min)]



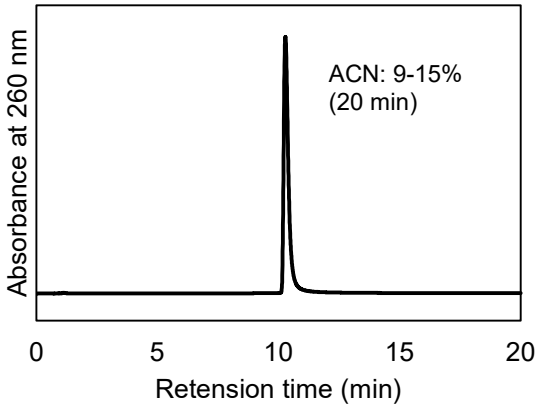
(b) 5'-d(GCGT**B**T**B**TTGCT)-3' (ODN**3**)

[B conc. ACN: 9-15% (20 min)]



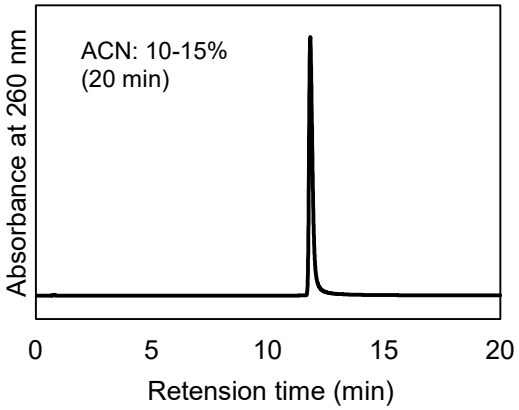
(c) 5'-d(GCGTT**BB**TTGCT)-3' (ODN**4**)

[B conc. ACN: 9-15% (20 min)]



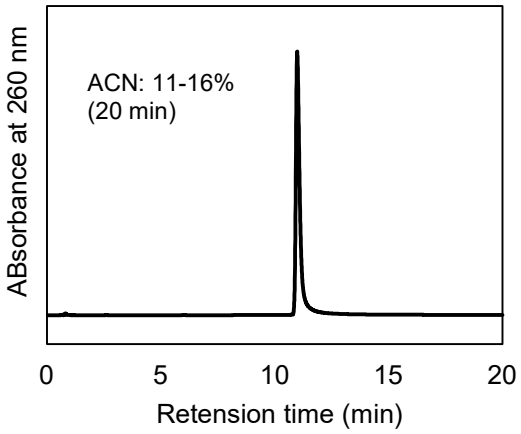
(d) 5'-d(GCG**B**T**B**T**B**TGCT)-3' (ODN**5**)

[B conc. ACN: 10-15% (20 min)]



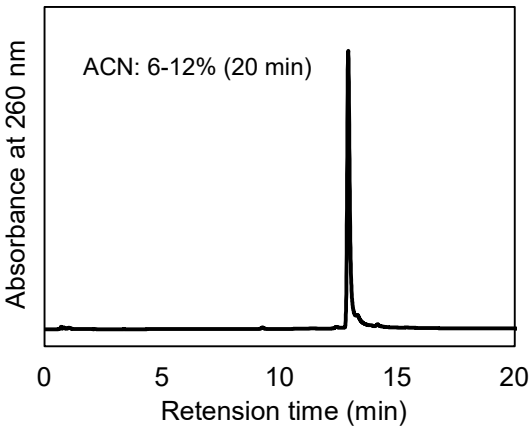
(e) 5'-d(GCGT**BBB**TTGCT)-3'

[B conc. ACN: 11-16% (20 min)]



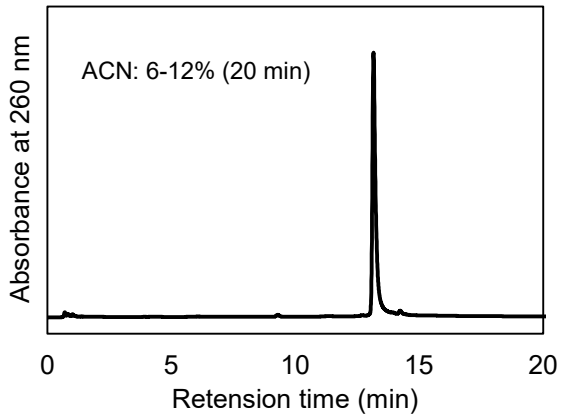
(f) 5'-d(GCGTC**B**AGTTGCT)-3' (ODN**8**)

[B conc. ACN: 6-12% (20 min)]



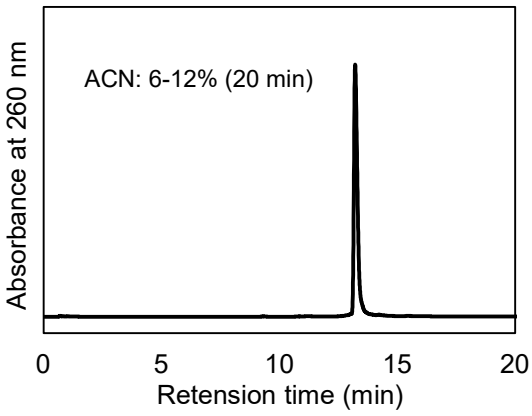
(g) 5'-d(GCGTC**B**CTTGCT)-3' (ODN**10**)

[B conc. ACN: 6-12% (20 min)]



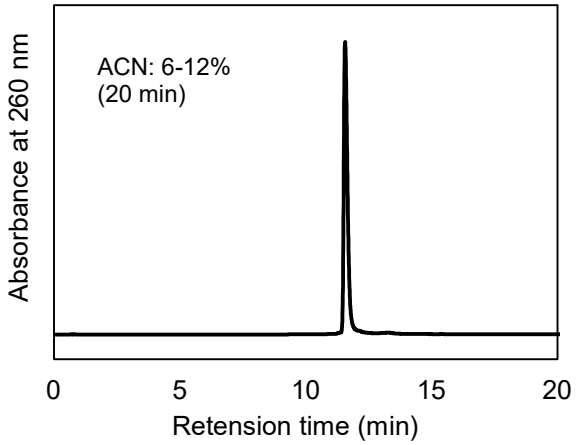
(h) 5'-d(GCGA**B**ATGCT)-3' (ODN**12**)

[B conc. ACN: 6-12% (20 min)]



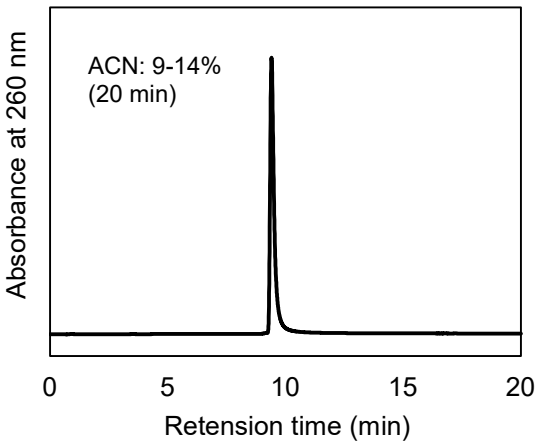
(i) 5'-d(GCGTG**B**GTTGCT)-3' (ODN**14**)

[B conc. ACN: 6-12% (20 min)]

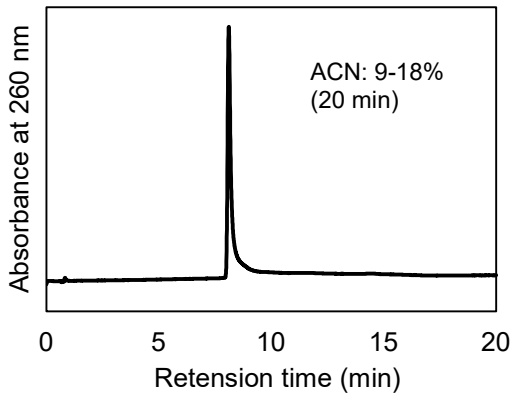


(j) 5'-d(TTTTTC̲T**B**TC̲TC̲TC̲T)-3' (TFO**2**)

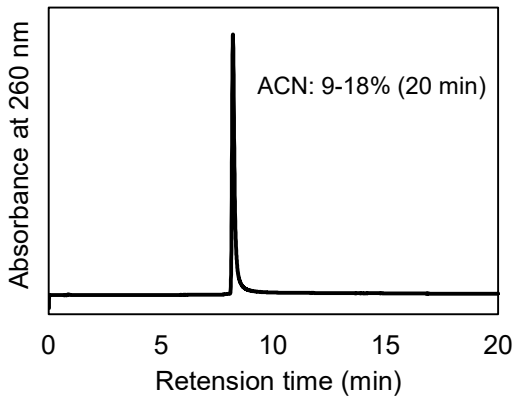
[B conc. ACN: 9-14% (20 min)]



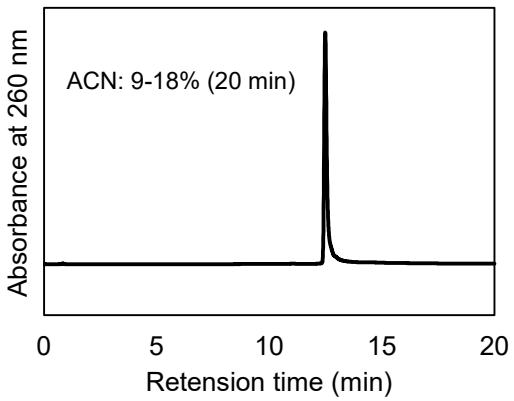
(k) 5'-d(TTTT**B**C̲TTTC̲TC̲TC̲T)-3' (TFO**3**)
[B conc. ACN: 9-18% (20 min)]



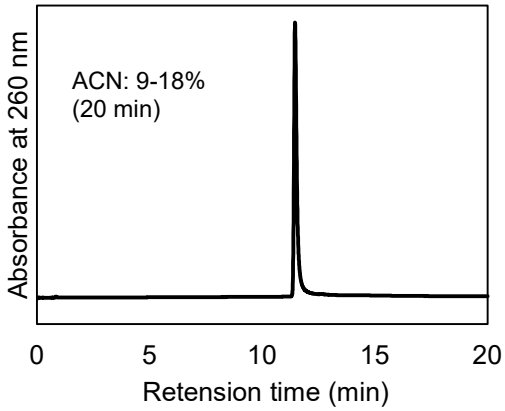
(l) 5'-d(TTTTTC̲**B**TTC̲TC̲TC̲T)-3' (TFO**4**)
[B conc. ACN: 9-18% (20 min)]



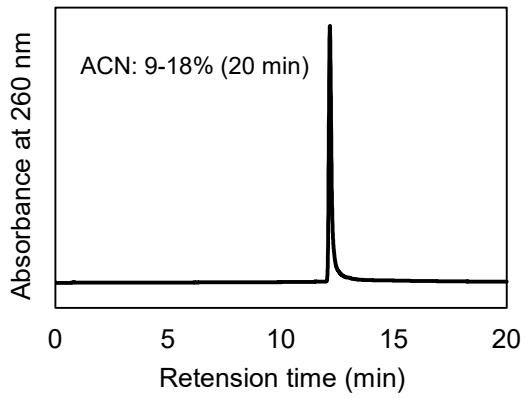
(m) 5'-d(TTTT**B**C̲**B**TTC̲TC̲TC̲T)-3' (TFO**5**)
[B conc. ACN: 9-18% (20 min)]



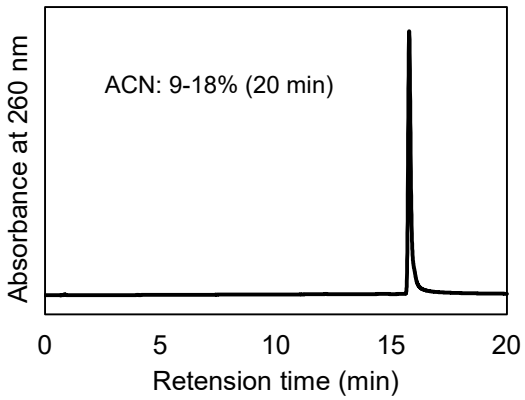
(n) 5'-d(TTTT**B**C̲TT**B**C̲TC̲TC̲T)-3' (TFO**6**)
[B conc. ACN: 9-18% (20 min)]



(o) 5'-d(TTTTTC̲**BB**TC̲TC̲TC̲T)-3' (TFO**7**)
[B conc. ACN: 9-18% (20 min)]



(p) 5'-d(TTTTTC̲**BBB**C̲TC̲TC̲T)-3' (TFO**8**)
[B conc. ACN: 9-18% (20 min)]



(q) 5'-d(TTTTTTTTT**B**)-3' (ODN**18**)

[B conc. ACN: 9-18% (20 min)]

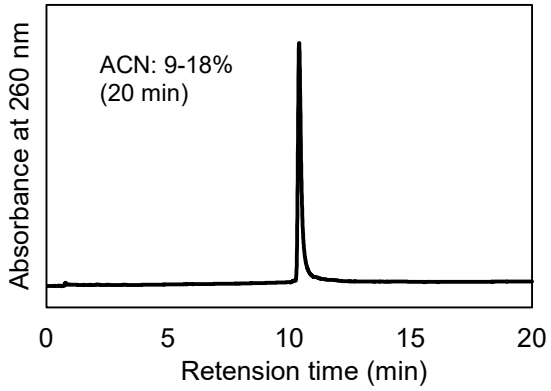
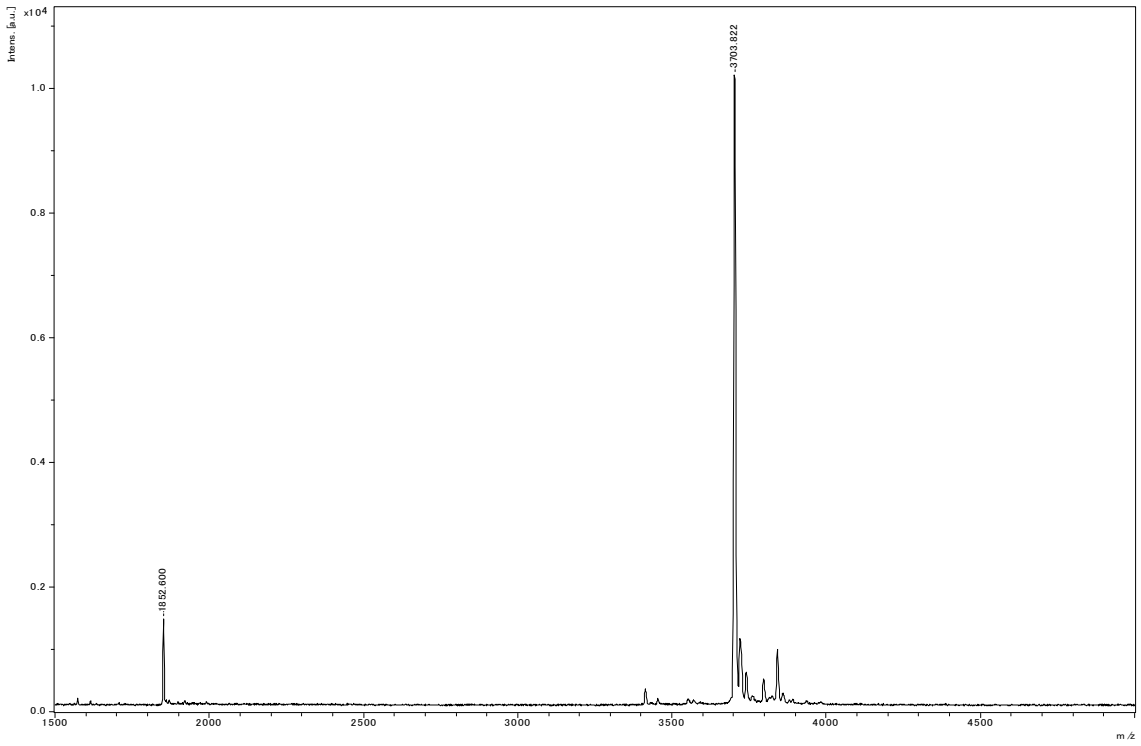


Fig. S4 MALDI-TOF mass spectrometry data obtained for ODNs containing OBN (**B**).

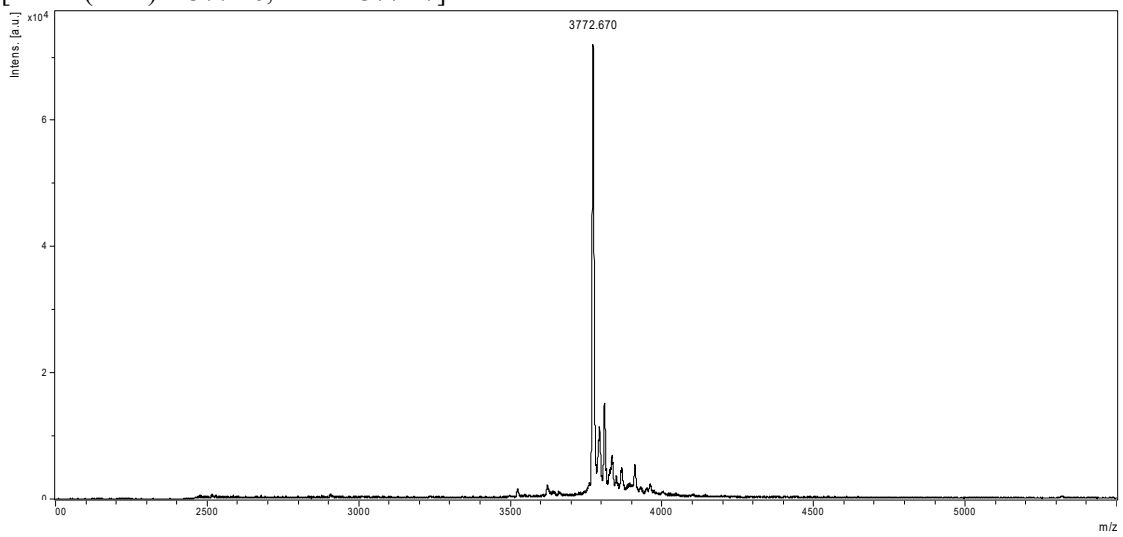
(a) ODN**2**: 5'-d(GCGTT**B**TTTGCT)-3' (**B**: OBN)

[calcd. (M-H)$^-$: 3702.5, found: 3703.8]



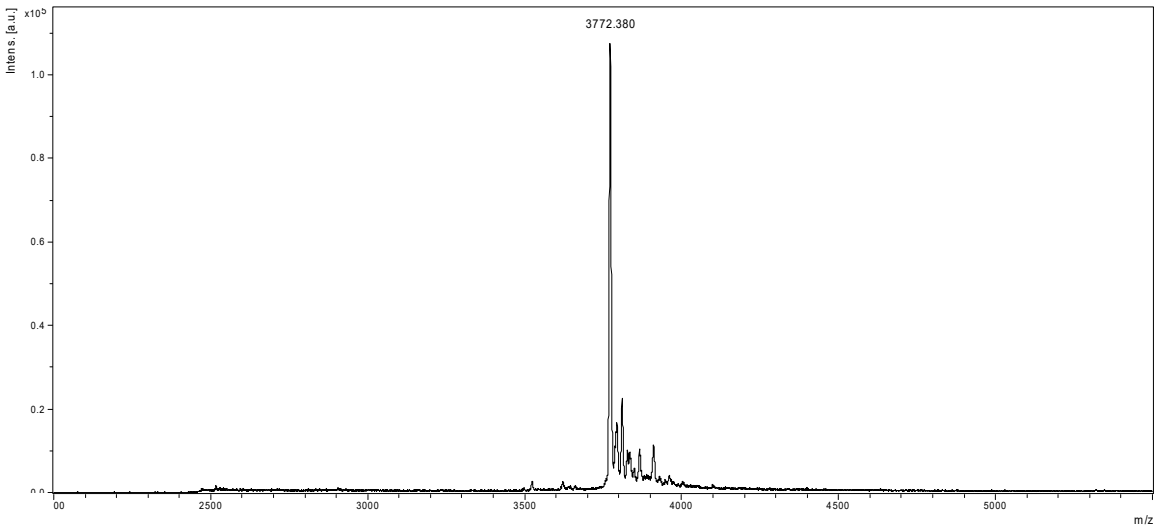
(b) ODN3: 5'-d(GCGT**B**T**B**TTGCT)-3'　　(**B**: OBN)

[calcd. (M-H)$^-$: 3772.6, found: 3772.7]



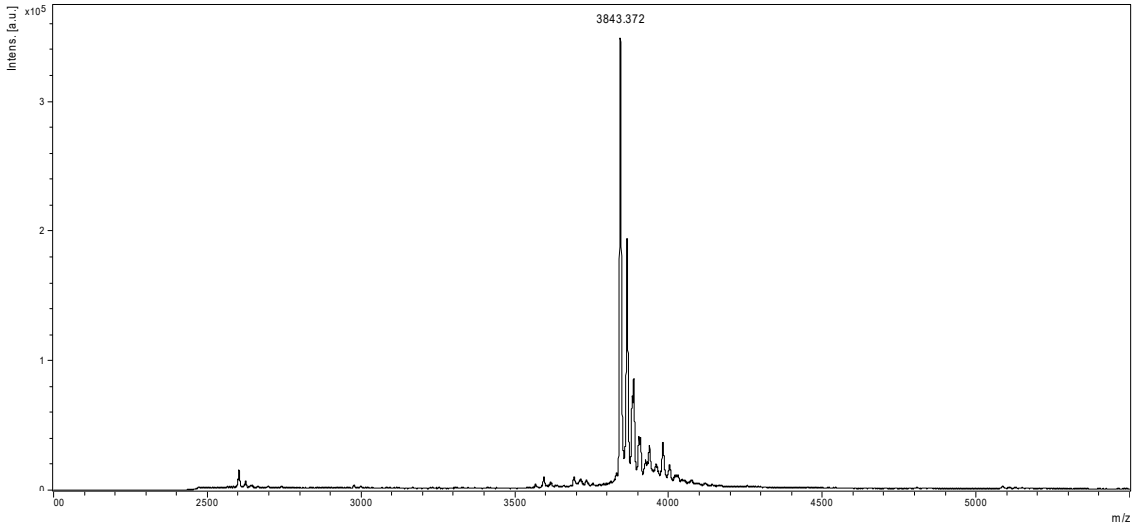
(c) ODN**4**: 5'-d(GCGTT**BB**TTGCT)-3'　　(**B**: OBN)

[calcd. (M-H)$^-$: 3772.6, found: 3772.4]



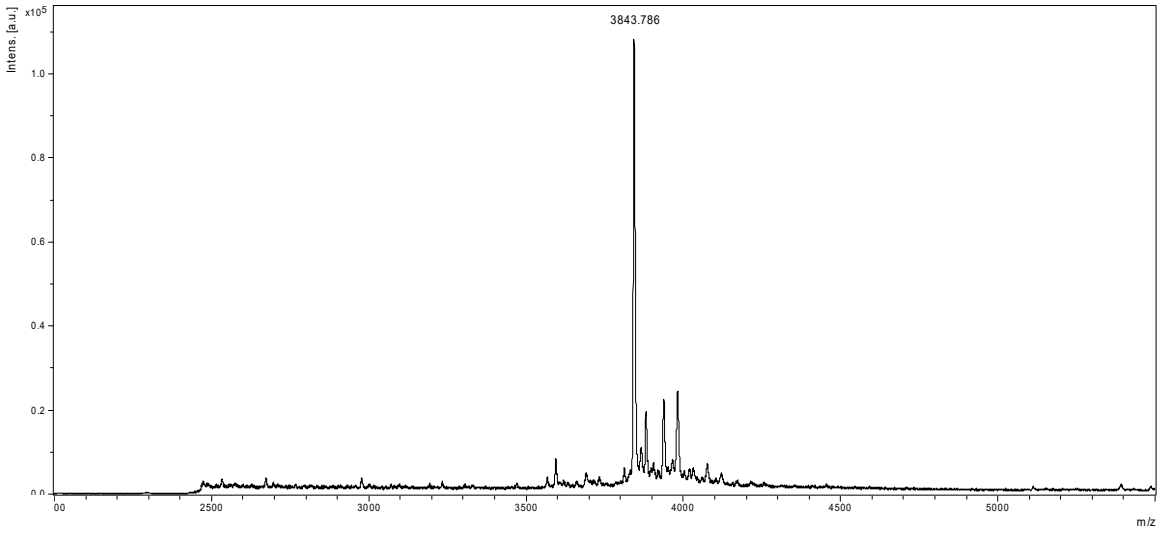
(d) ODN**5**: 5'-d(GCG**B**T**B**T**B**TGCT)-3'　(**B**: OBN)

[calcd. (M-H)$^-$: 3842.7, found: 3843.4]



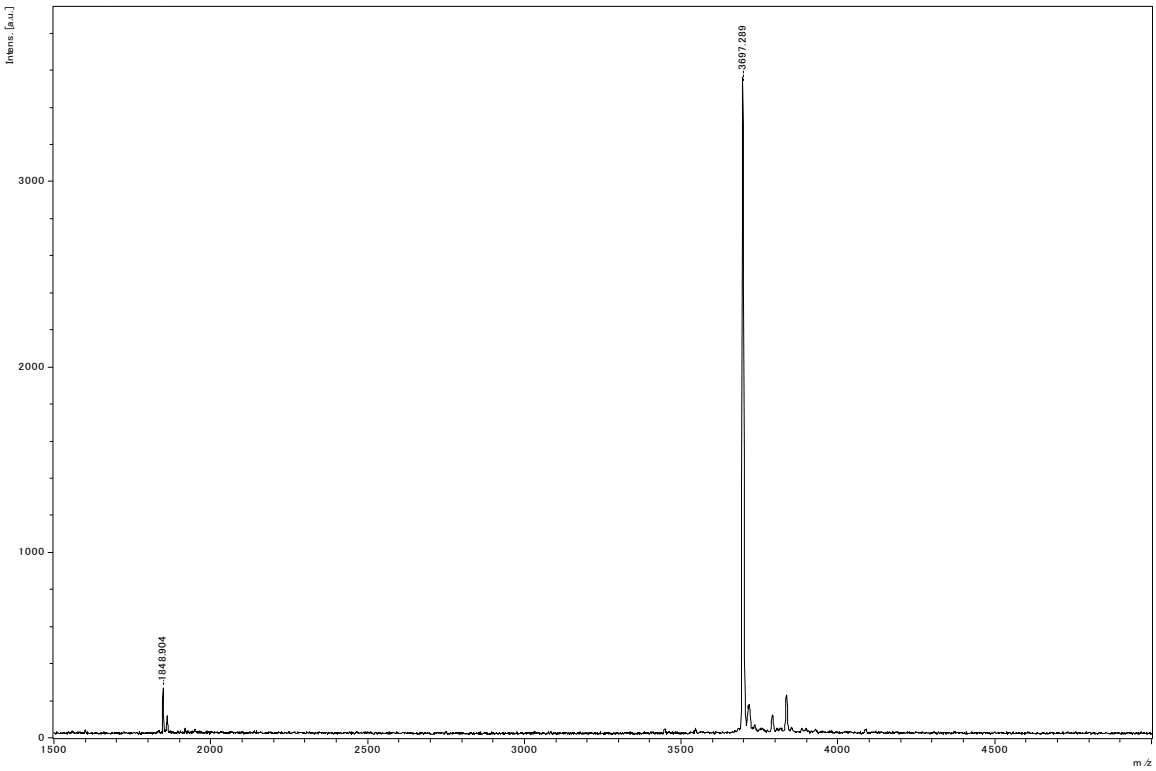
(e) ODN**6**: 5'-d(GCGT**BBB**TTGCT)-3'　(**B**: OBN)

[calcd. (M-H)$^-$: 3842.7, found: 3843.8]



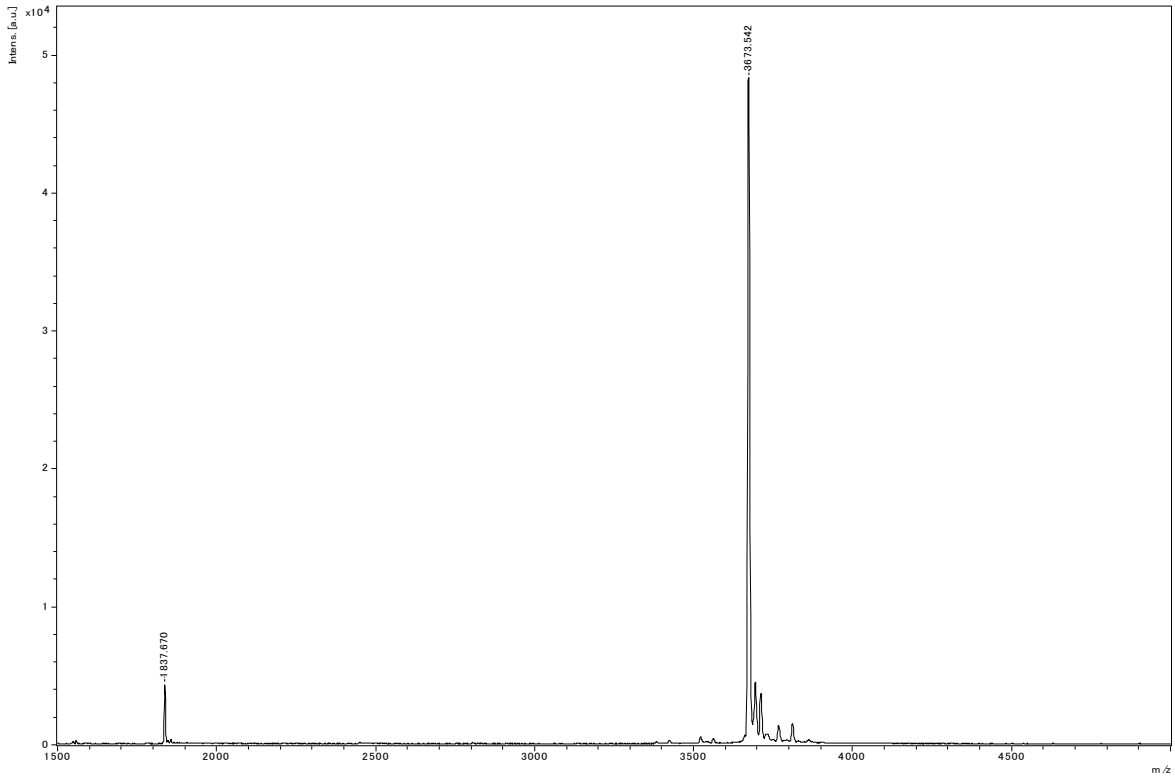
(f) ODN**8**: 5'-d(GCGTC**B**ATTGCT)-3'　　(**B**: OBN)

[calcd. (M-H)$^-$: 3696.5, found: 3697.3]

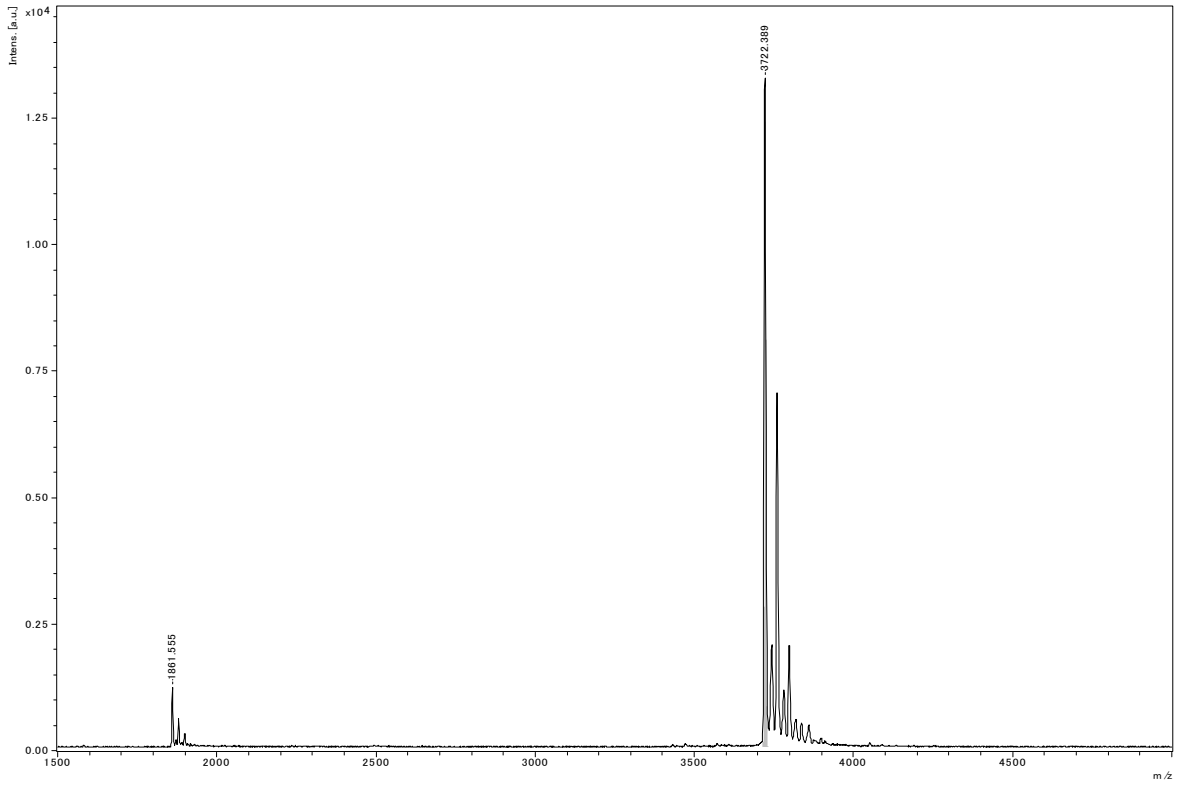


(g) ODN**10**: 5'-d(GCGTC**B**CTTGCT)-3'　　(**B**: OBN)

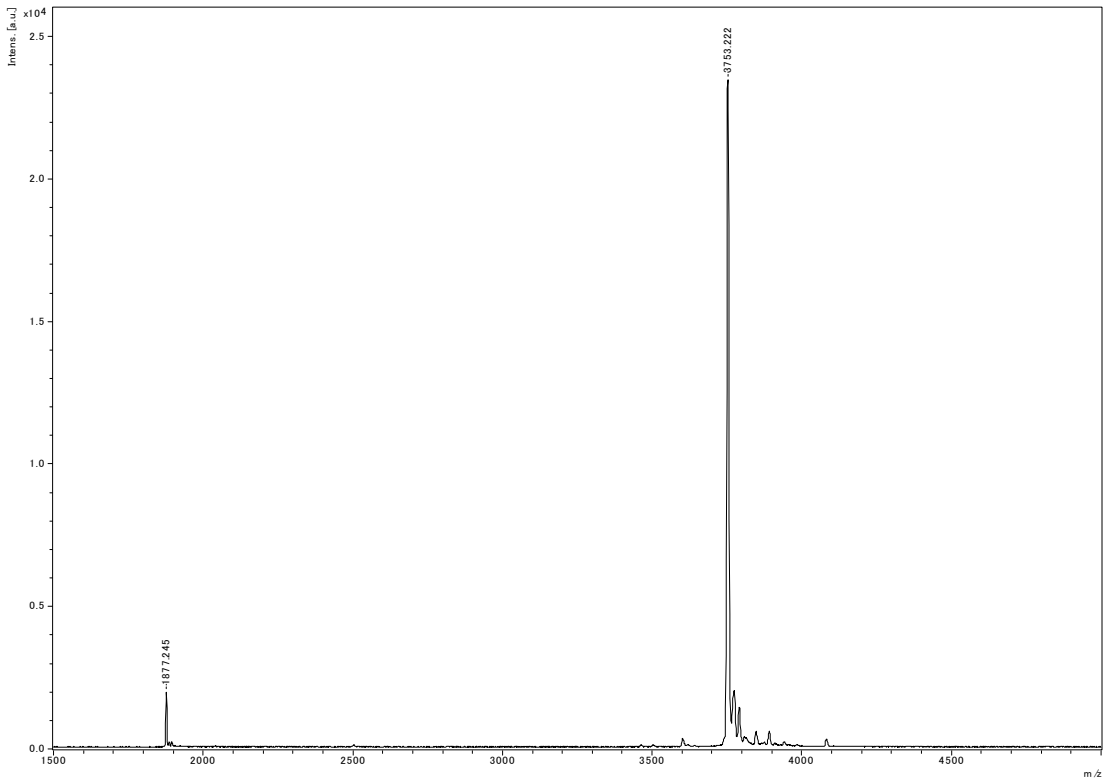
[calcd. (M-H)$^-$: 3672.5, found: 3673.5]



(h) ODN**12**: 5'-d(GCGTA**B**ATTGCT)-3'　(**B**: OBN)
[calcd. (M-H)$^-$: 3720.5, found: 3722.4]

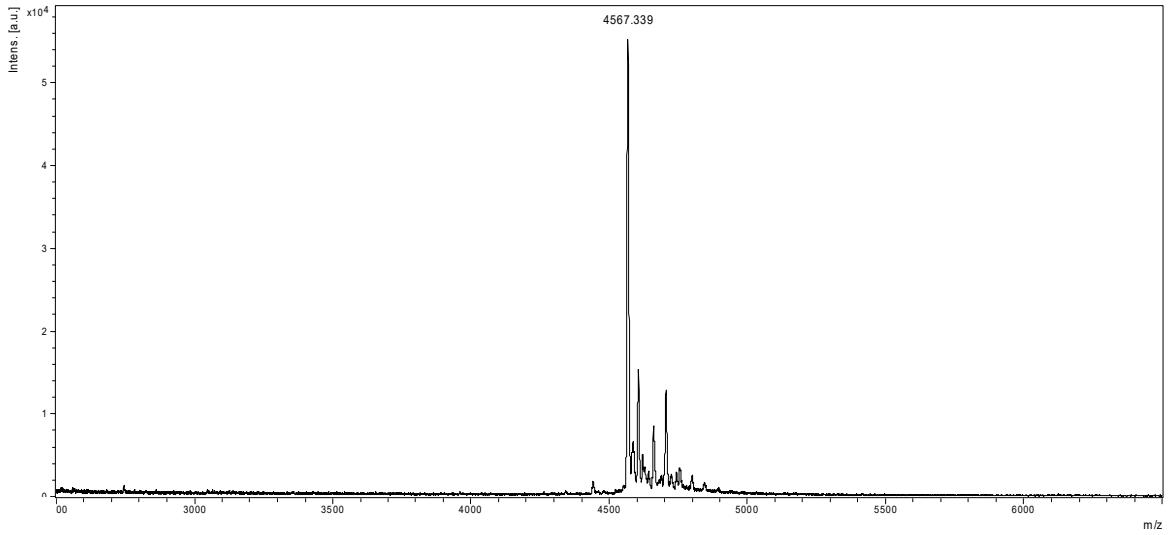


(i) ODN**14**: 5'-d(GCGTG**B**GTTGCT)-3'　(**B**: OBN)
[calcd. (M-H)$^-$: 3752.5, found: 3753.2]



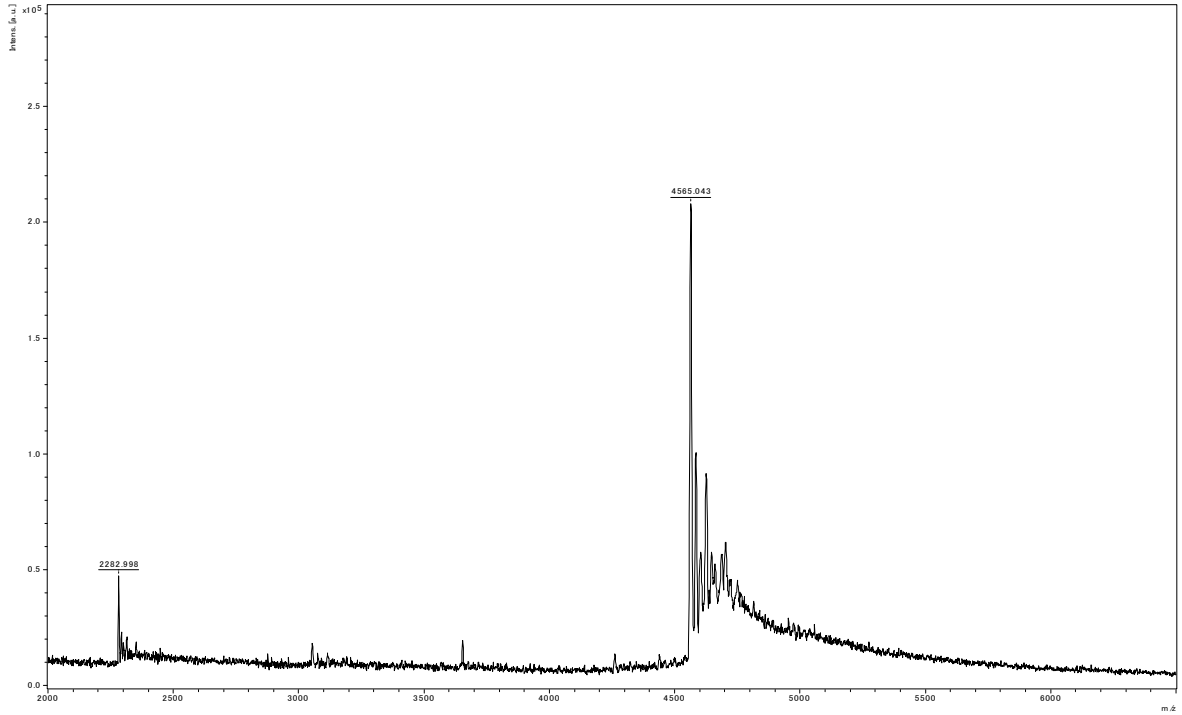
(j) TFO**2**: 5'-d(TTTTTC̲T**B**TC̲TC̲TC̲T)-3'　(**B**: OBN, C̲: 2′-deoxy5-methylcytidine)

[calcd. $(M-H)^-$: 4566.1, found: 4567.3]



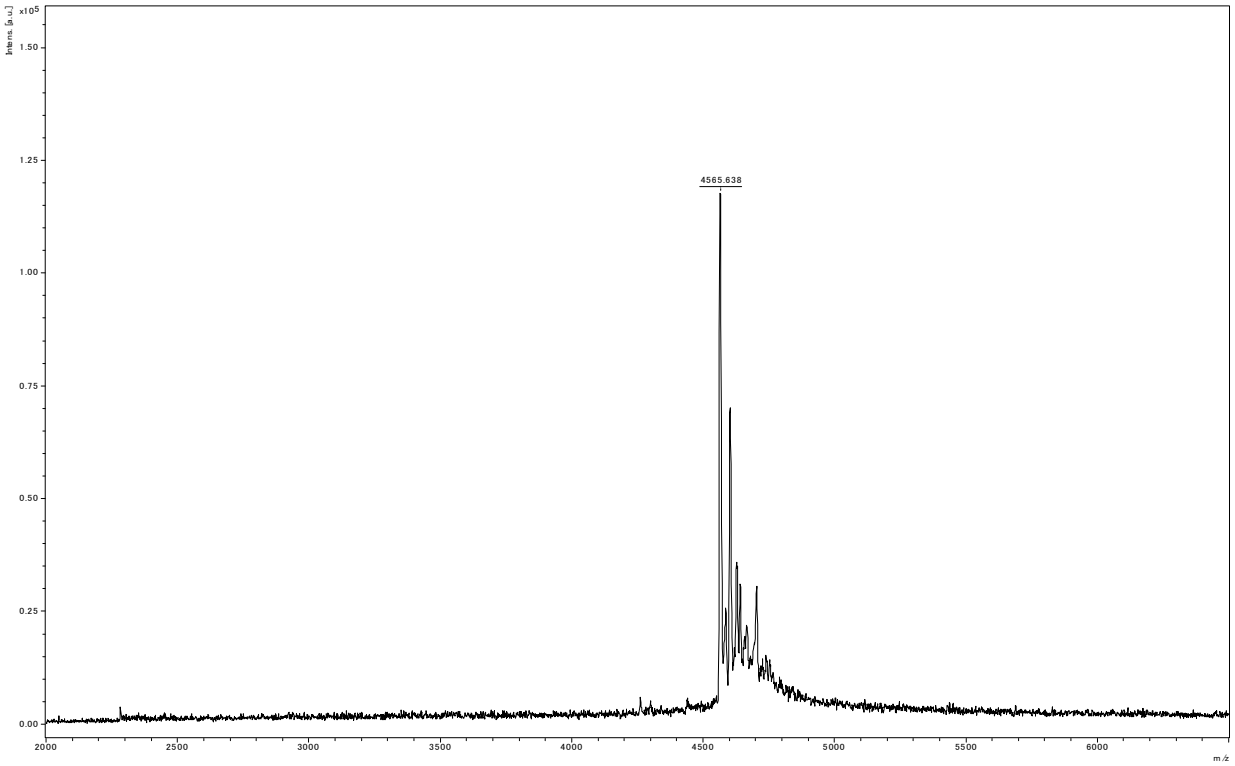
(k) TFO**3**: 5'-d(TTTT**B**C̲TTTC̲TC̲TC̲T)-3' (**B**: OBN, C̲: 2′-deoxy5-methylcytidine)

[calcd. $(M-H)^-$: 4566.1, found: 4565.0]



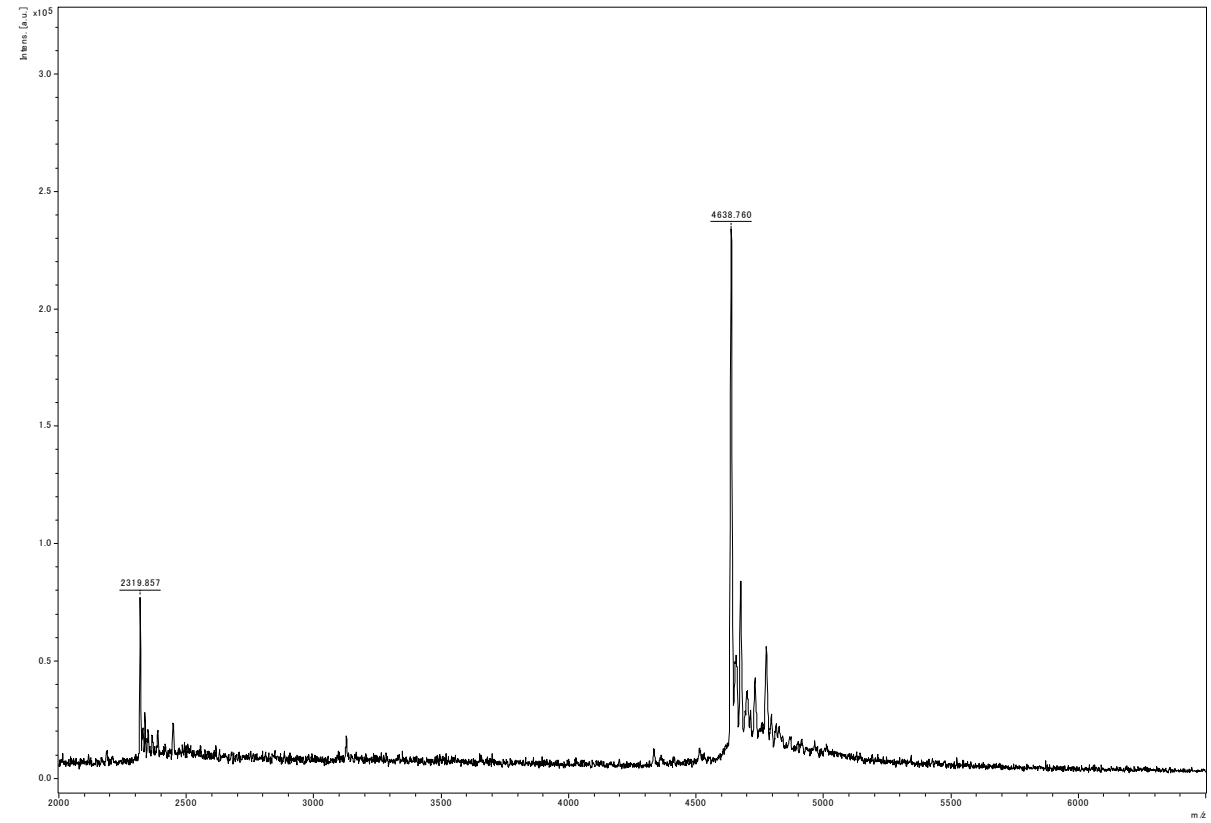
(l) TFO**4**: 5'-d(TTTTTC**B**TTCTCTCT)-3'(**B**: OBN, C: 2′-deoxy-5-methylcytidine)

[calcd. (M-H)$^-$: 4566.1, found: 4565.6]



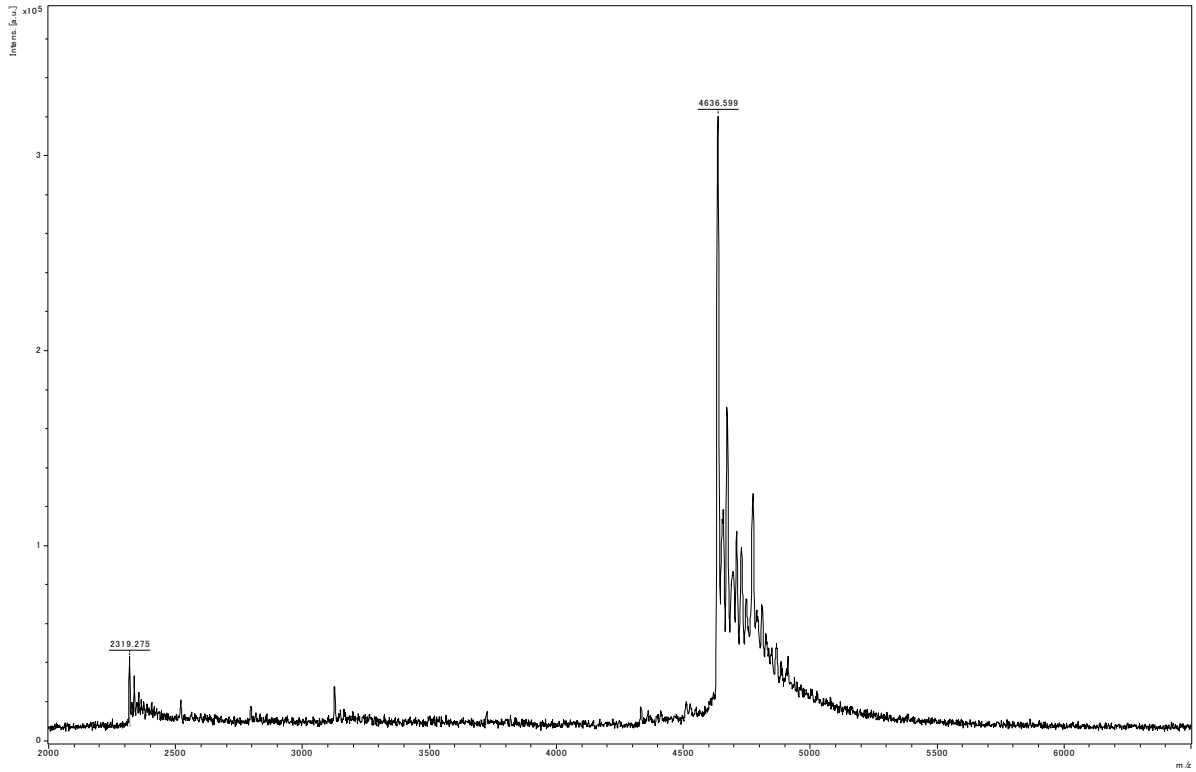
(m) TFO**5**: 5'-d(TTTT**B**C**B**TTCTCTCT)-3'　(**B**: OBN, C: 2′-deoxy-5-methylcytidine)

[calcd. (M-H)$^-$: 4636.2, found: 4638.8]



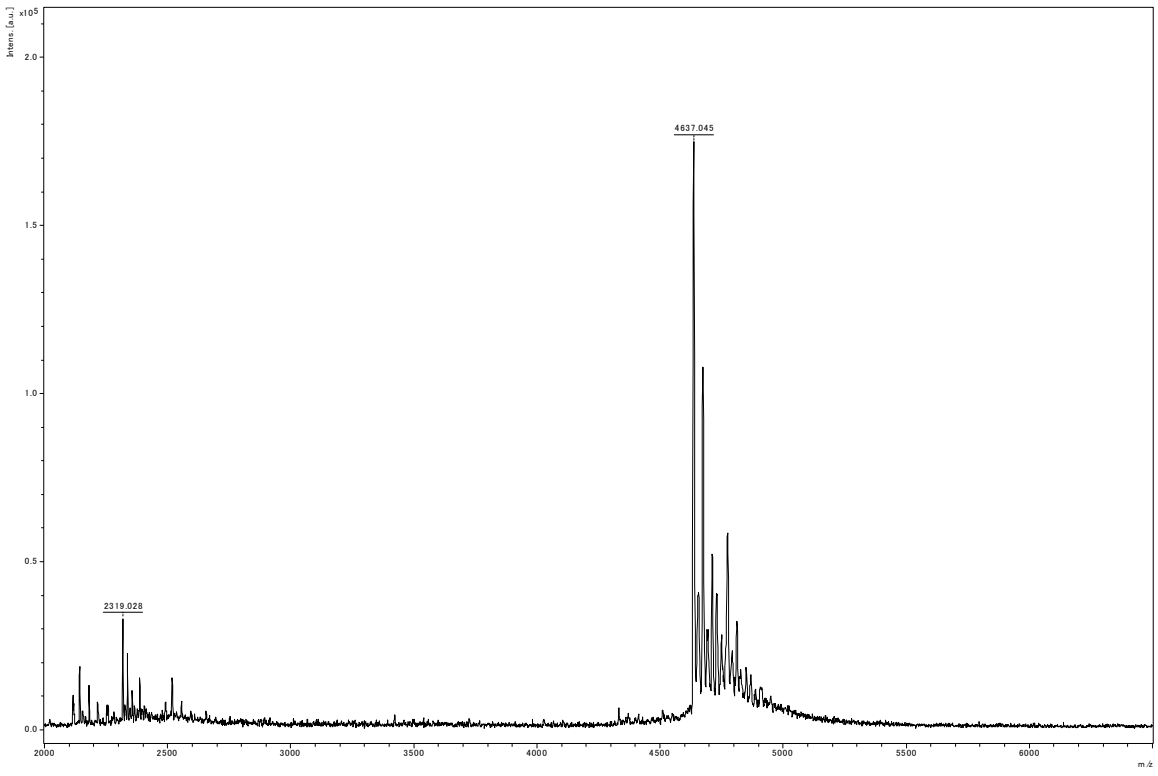
(n) TFO**6**: 5'-d(TTTT**B**C̲TT**B**C̲TC̲TC̲T)-3' (**B**: OBN, C̲: 2′-deoxy-5-methylcytidine)

[calcd. (M-H)$^-$: 4636.2, found: 4636.6]



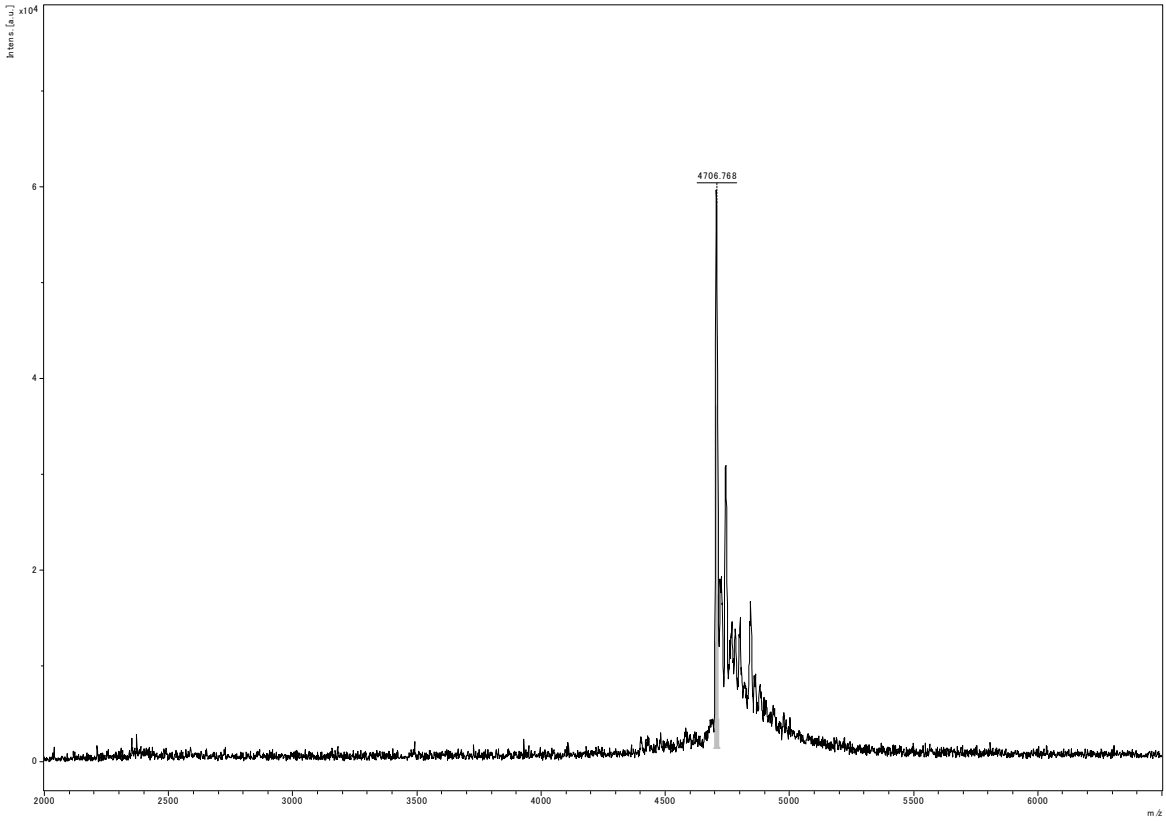
(o) TFO**7**: 5'-d(TTTTTC̲**BB**TC̲TC̲TC̲T)-3' (**B**: OBN, C̲: 2′-deoxy-5-methylcytidine)

[calcd. (M-H)$^-$: 4636.2, found: 4637.0]



(p) TFO**8**: 5'-d(TTTTTC**BBB**CTCTCT)-3'　(**B**: OBN, C: 2′-deoxy-5-methylcytidine)

[calcd. (M-H)$^-$: 4706.3, found: 4706.8]



(q) ODN**18**: 5'-d(TTTTTTTTT**B**)-3' (**B**: OBN)

[calcd. (M-H)$^-$: 3049.1, found: 3049.0]

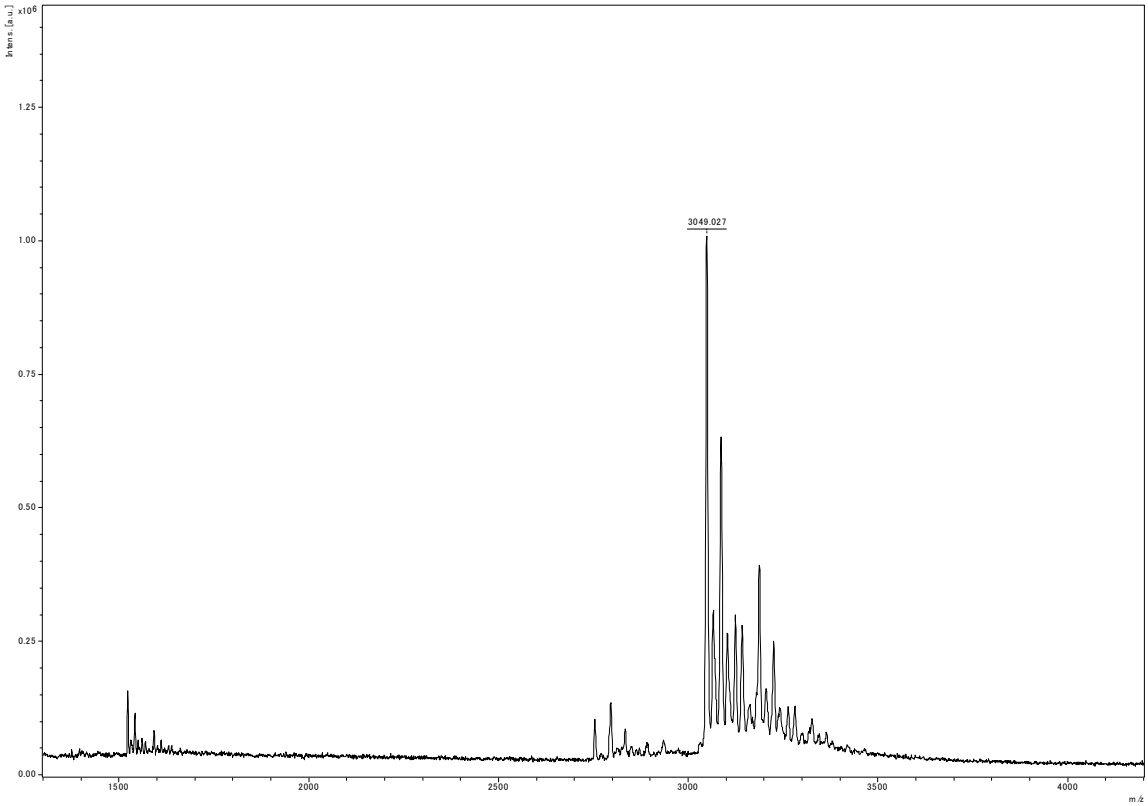
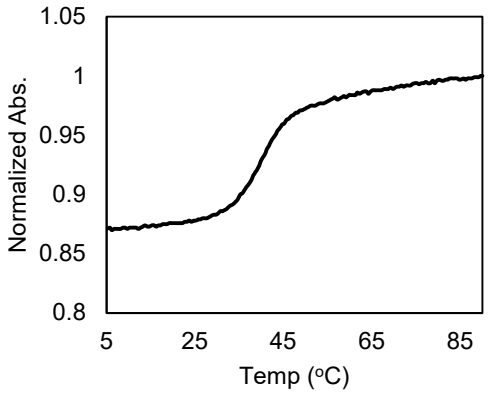


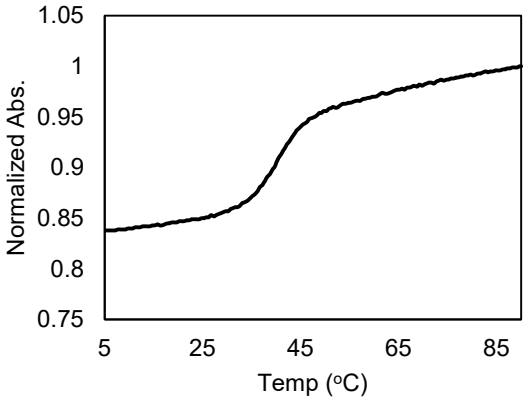
Fig. S5 UV melting curves for the duplexes containing OBN (**B**).

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.
Sequence: 5′-d(GCG**N**TGCT)-3′/3′-CGCA**Q**A**Q**AACGA-5′
(**N**: A, C, T, G or **B** (=OBN), **Q**: Corresponding matching base (A, C, T (or U), or G)).

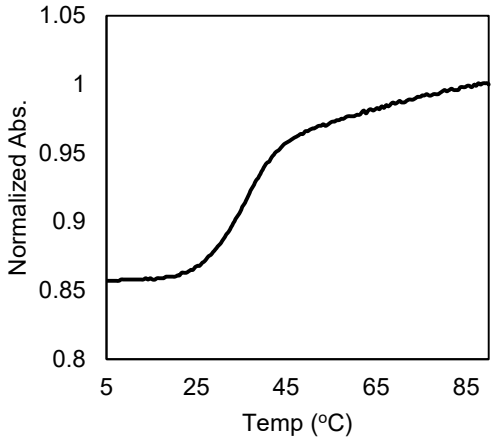
(a) 5′-d(GCGTT**B**TTTGCT)-3′ (ODN**2**) /cDNA**1**



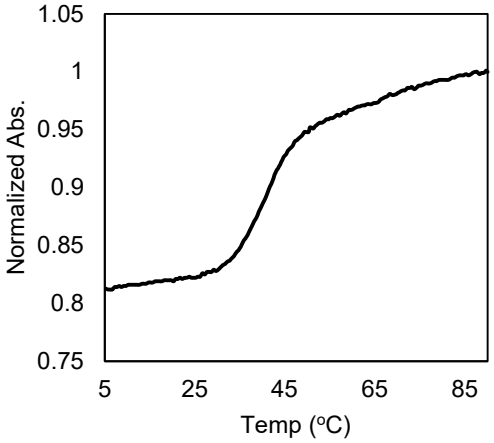
(b) 5′-d(GCGTT**B**TTTGCT)-3′ (ODN**2**) /cRNA**1**



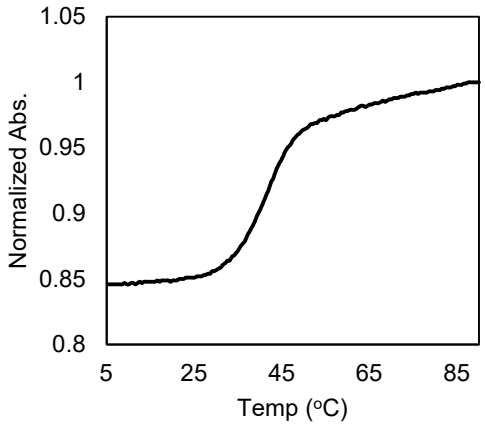
(c) 5′-d(GCGT**B**T**B**TTGCT)-3′ (ODN**3**) /cDNA**1**



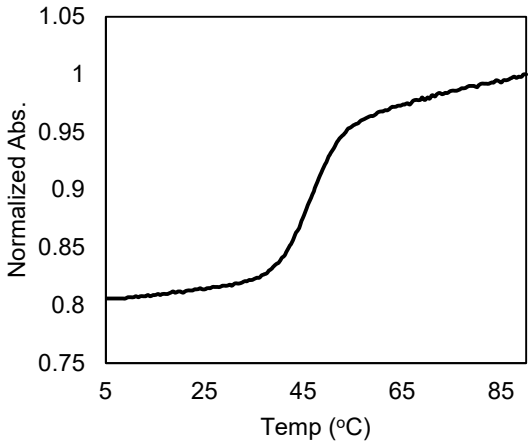
(d) 5′-d(GCGT**B**T**B**TTGCT)-3′ (ODN**3**) /cRNA**1**



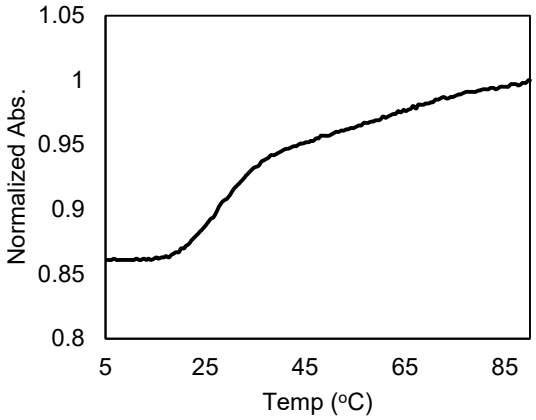
(e) 5′-d(GCGTT**BB**TTGCT)-3′ (ODN**4**) /cDNA**1**



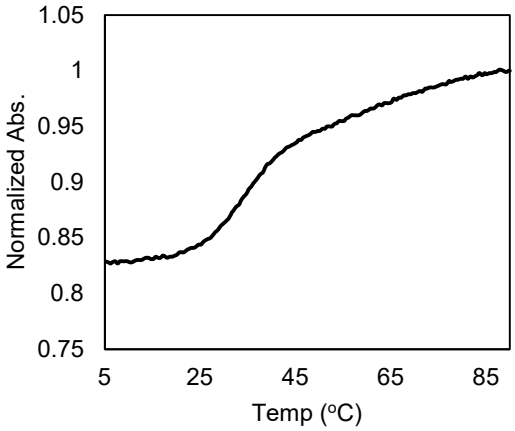
(f) 5′-d(GCGTT**BB**TTGCT)-3′ (ODN**4**) /cRNA**1**



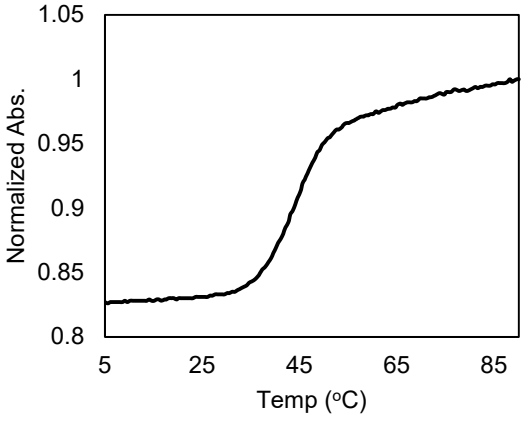
(g) 5′-d(GCG**B**T**B**T**B**TGCT)-3′ (ODN**5**) /cDNA1



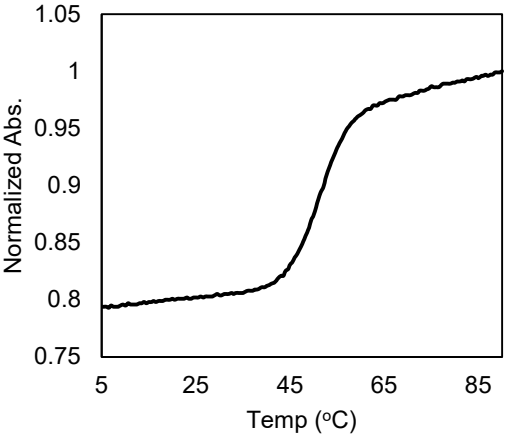
(h) 5′-d(GCG**B**T**B**T**B**TGCT)-3′ (ODN**5**) /cRNA1



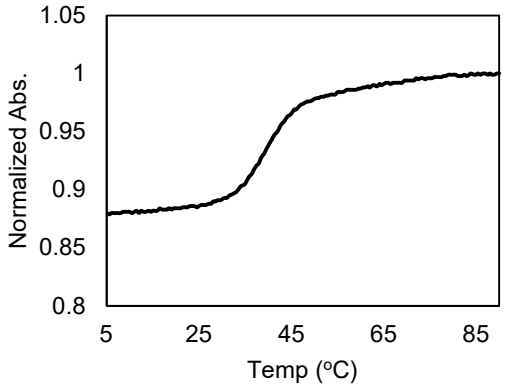
(i) 5′-d(GCGT**BBB**TTGCT)-3′ (ODN**8**) /cDNA1



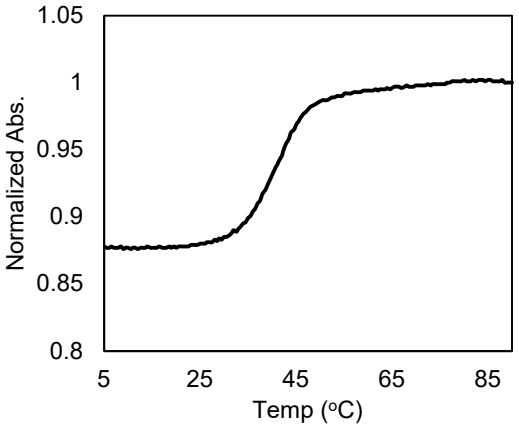
(j) 5′-d(GCGT**BBB**TTGCT)-3′ (ODN**8**) /cRNA1



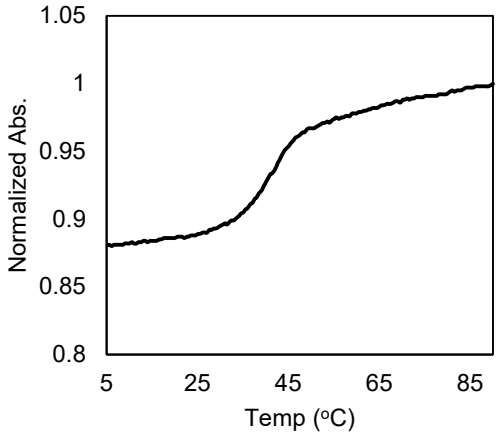
(k) 5′-d(GCGTC**B**ATTGCT)-3′ (ODN**8**) /cDNA2



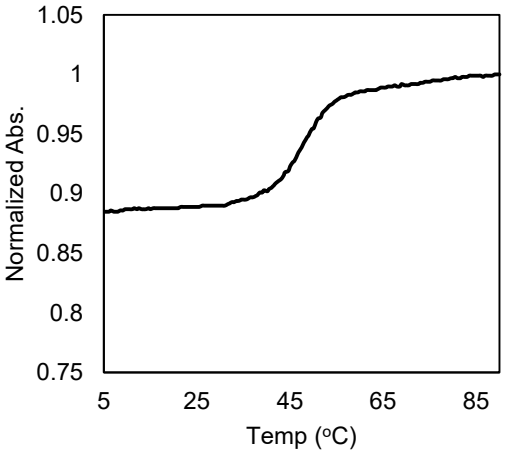
(l) 5′-d(GCGTC**B**ATTGCT)-3′ (ODN**8**) /cRNA2



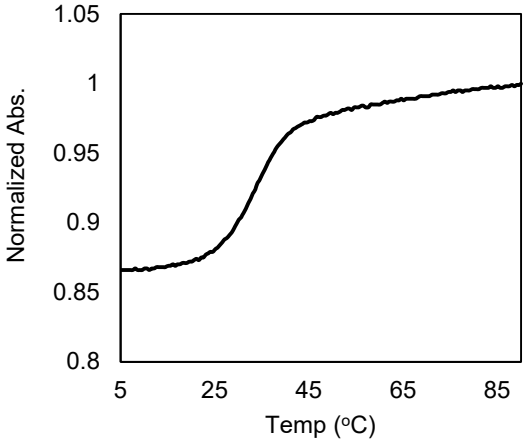
(m) 5′-d(GCGTC**B**CTTGCT)-3′ (ODN**10**) /cDNA3



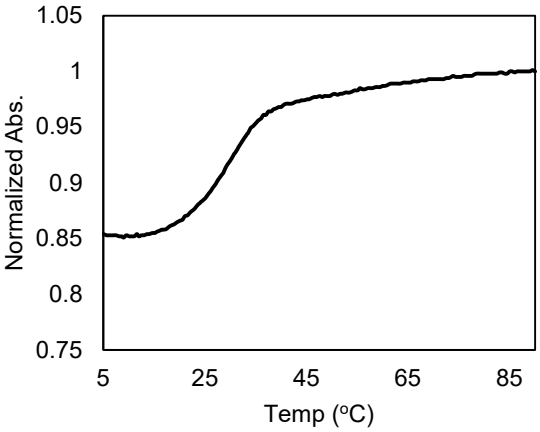
(n) 5′-d(GCGTC**B**CTTGCT)-3′ (ODN**10**) /cRNA3



(o) 5′-d(GCGTA**B**ATTGCT)-3′ (ODN**12**) /cDNA4



(p) 5′-d(GCGTA**B**ATTGCT)-3′ (ODN**12**) /cRNA4



(q) 5′-d(GCGTG**B**GTTGCT)-3′ (ODN**14**) /cDNA5 (r) d(GCGTG**B**GTTGCT)-3′ (ODN**14**) /cRNA5

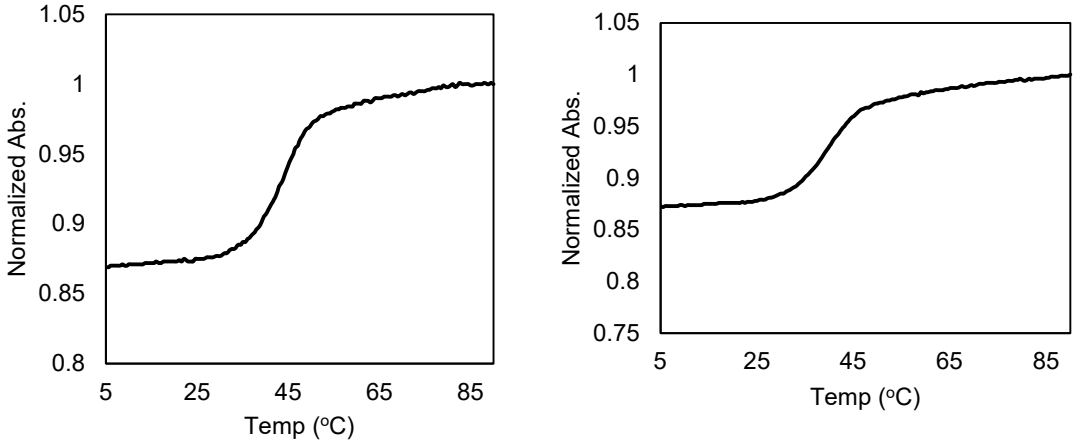
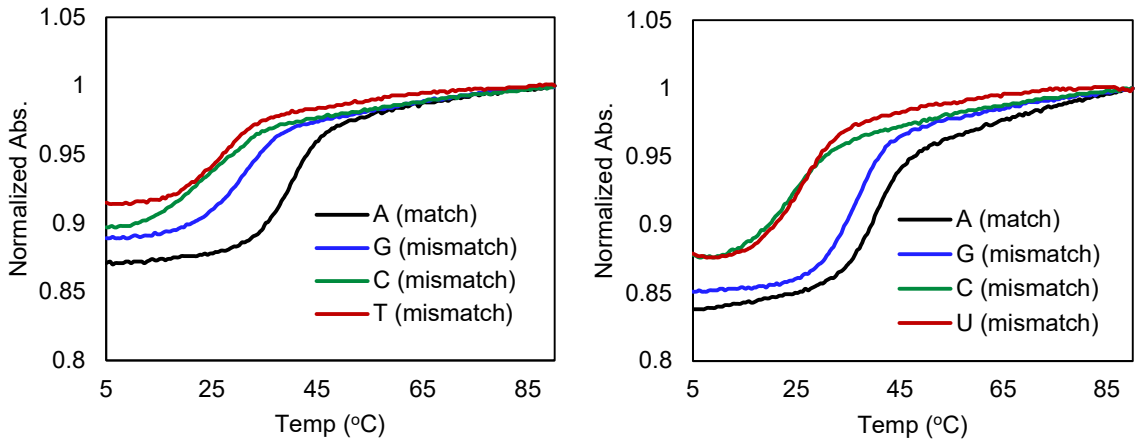


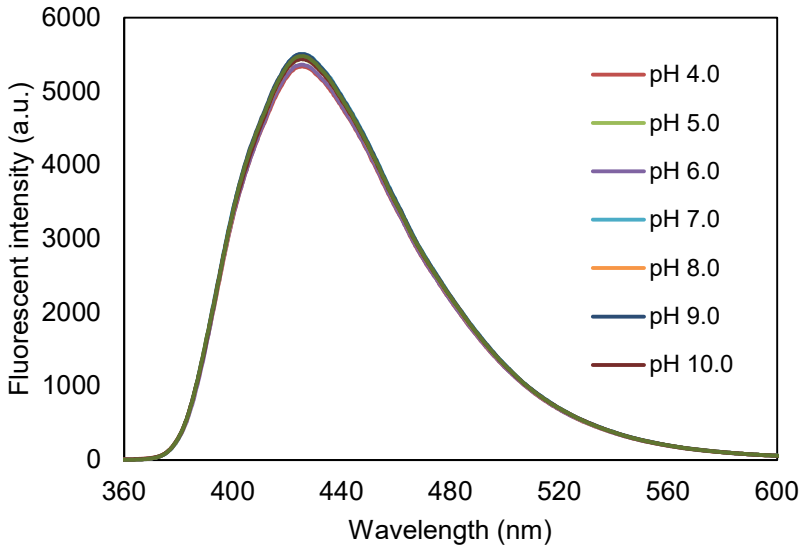
Fig. S6 UV melting curves for the duplexes contaning OBN (**B**) with matched and mismatched base pairs.

(a) 5′-d(GCGTT**B**TTTGCT)-3′ (ODN**2**) /ssDNA (b) 5′-d(GCGTT**B**TTTGCT)-3′ (ODN**2**) /ssRNA



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.
Sequence: 5′-d(GCGTT**B**TTTGCT)-3′ (ODN**2**) /3′-(CGCA**Y**AAACGA-5′) (**Y**: A, G, C, and T (or U)).
B: OBN.

Fig. S7 Fluorescent spectrum of OBN-modified ODN**2** (5′-d(GCGTT**B**TTTGCT)-3′).



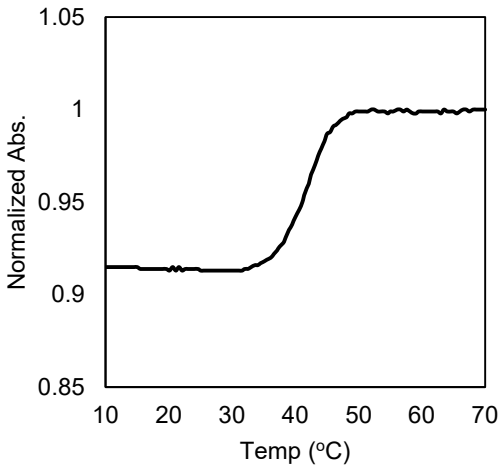
Fluorescent spectrums of ODN**2** (4μM) were meassured in the 10 mM of citric acid buffer (pH 4.0 and 5.0), 10 mM of phosphate buffer (6.0, 7.0, and 8.0), NaOH-glysine buffer (pH 9.0 and 10.0) at 10 °C. Excited at 335 nm.

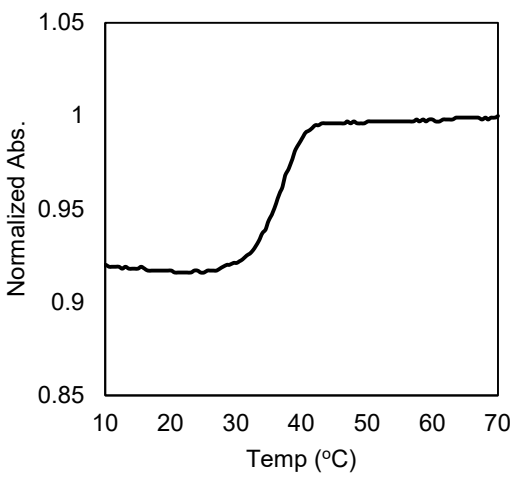
Fig. S8 UV melting curves for the triplexes containinig TFO**2**-**8**.

UV melting profiles were measured in 7 mM sodium phosphate buffer (pH 7.0 or pH 6.0) containing 140 mM KCl and 10 mM $MgCl_2$ at a scan rate of 0.5°C/min at 260 nm. The concentration of oligonucleotide used was 1.5 μM for each strand. The error in T_m values was ±0.5°C. (**B**: OBN, C: 2′-deoxy-5-methylcytidine)

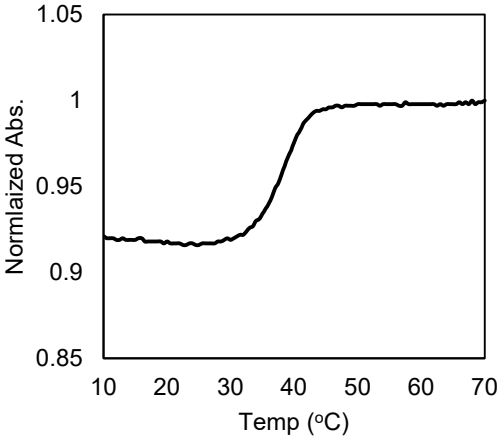
(I) Under pH 7.0 contidions

(a) 5′-d(TTTTTCT**B**TCTCTCT)-3' (TFO**2**) /dsDNA (b) 5′-d(TTTT**B**CTTTCTCTCT)-3' (TFO**3**) /dsDNA

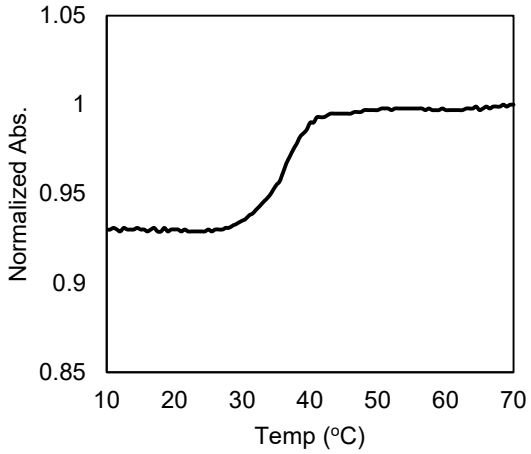




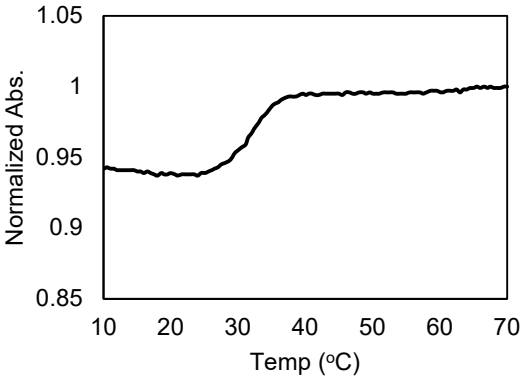
(c) 5′-d(TTTTTC**B**TTCTCTCT)-3' (TFO**4**) /dsDNA



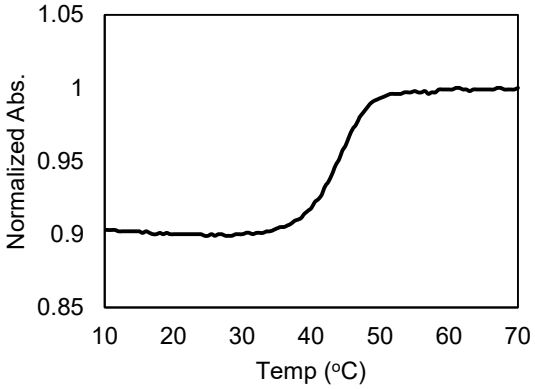
(d) 5′-d(TTTT**B**C**B**TTCTCTCT)-3' (TFO**5**) /dsDNA



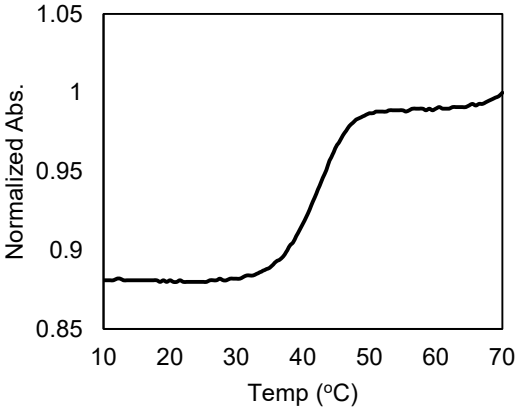
(e) 5′-d(TTTT**B**CTT**B**CTCTCT)-3' (TFO**6**) /dsDNA



(f) 5′-d(TTTTTC**BB**TCTCTCT)-3' (TFO**7**) /dsDNA

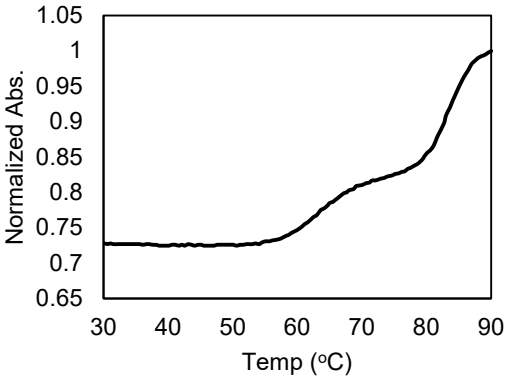


(g) 5'-d(TTTTTC**BBB**CTCTCT)-3' (TFO**8**) /dsDNA

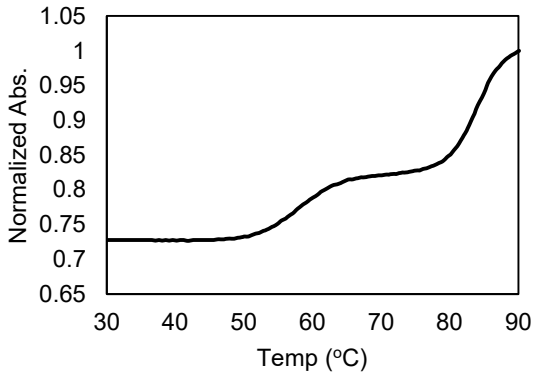


(II) Under pH 6.0 conditions

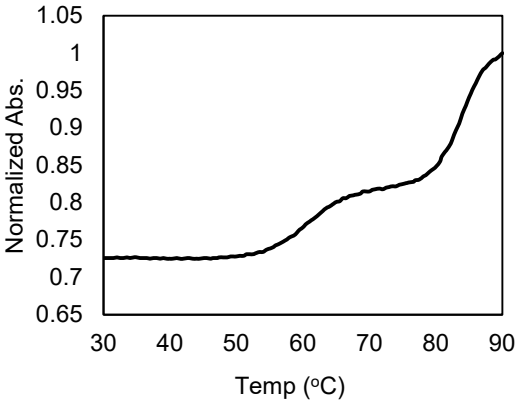
(a) 5′-d(TTTTTC<u>T</u>**B**T<u>C</u>T<u>C</u>T<u>C</u>T)-3' (TFO**2**) /dsDNA



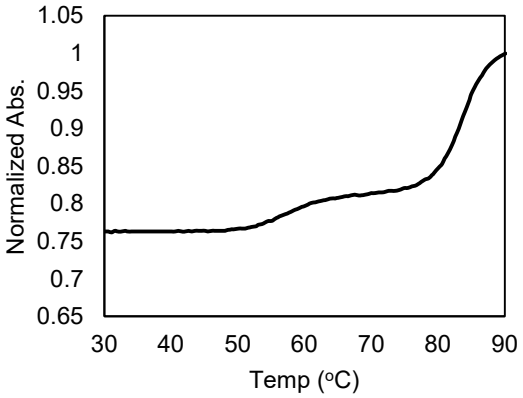
(b) 5′-d(TTTT**B**<u>C</u>TTT<u>C</u>T<u>C</u>T<u>C</u>T)-3' (TFO**3**) /dsDNA



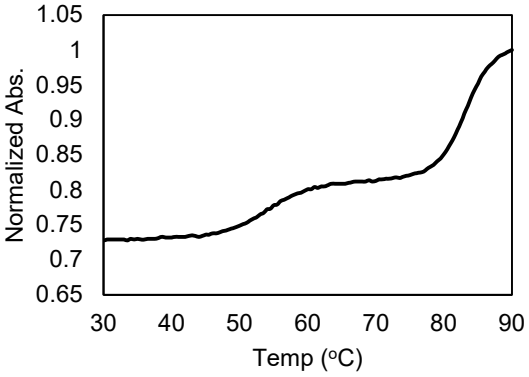
(c) 5′-d(TTTTTC**B**TT<u>C</u>T<u>C</u>T<u>C</u>T)-3' (TFO**4**) /dsDNA



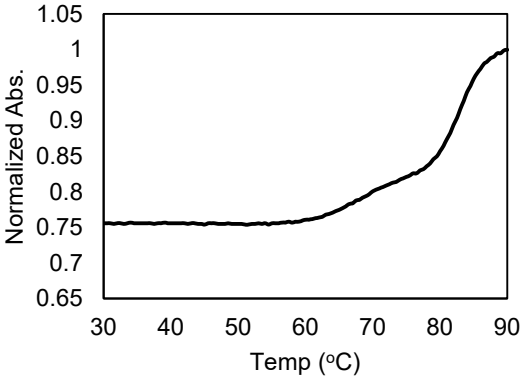
(d) 5′-d(TTTT**B**<u>C</u>**B**TT<u>C</u>T<u>C</u>T<u>C</u>T)-3' (TFO**5**) /dsDNA



(e) 5′-d(TTTT**B**<u>C</u>TT**B**<u>C</u>T<u>C</u>T<u>C</u>T)-3' (TFO**6**) /dsDNA



(f) 5′-d(TTTTTC**BB**T<u>C</u>T<u>C</u>T<u>C</u>T)-3' (TFO**7**) /dsDNA



(g) 5'-d(TTTTTC**BBB**CTCTCT)-3' (TFO**8**) /dsDNA

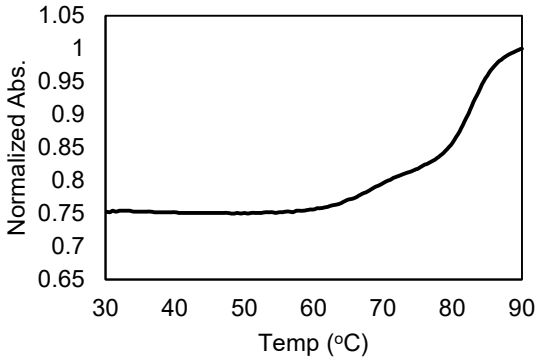
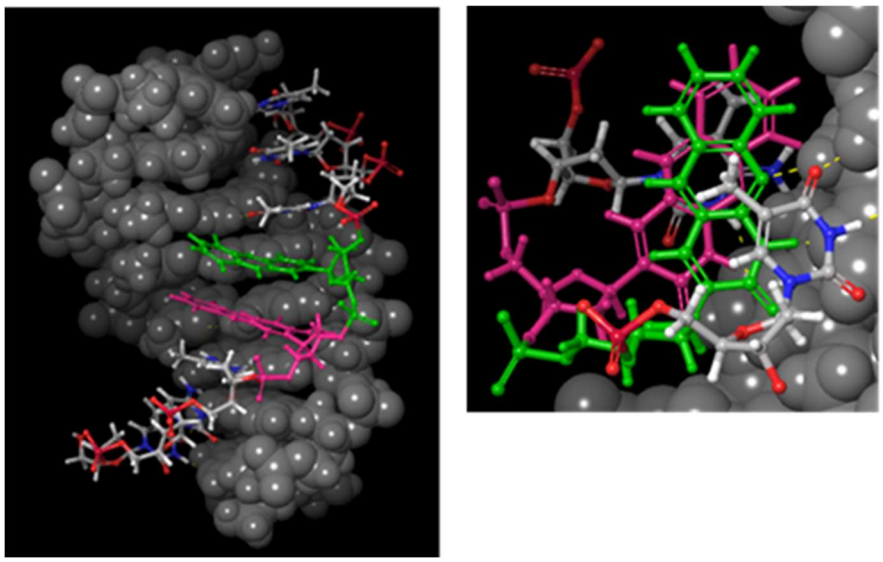


Fig. S9 Snapshots of molecular dynamics (MD) caluculation of OBN-modified ODN/dsDNA.



MD calculation was conducted by MacroModel software [Schrödinger, LLC]; OPLS3 force field (in water at 300 K for 2.0 ns). The initial structure was constructed with 1D3X [PDB ID: 1D3X[1]]. The sequence of TFO is 5′-d(TCC**B**$_1$**B**$_2$TTT)-3′. The sequences of target dsDNA are 5′-d(AGGAAAAA)-3′ and 3’-d(TCCTTTTT)-5′. Purple: **B**$_1$ (= OBN) at 5′-adjacent of another OBN (= **B**$_2$). Green: **B**$_2$ (= OBN) at 5′-adjacent of thymidine. C: 2′-deoxy-5-methylcytidine. Gray dsDNA is shown as space-filling model.

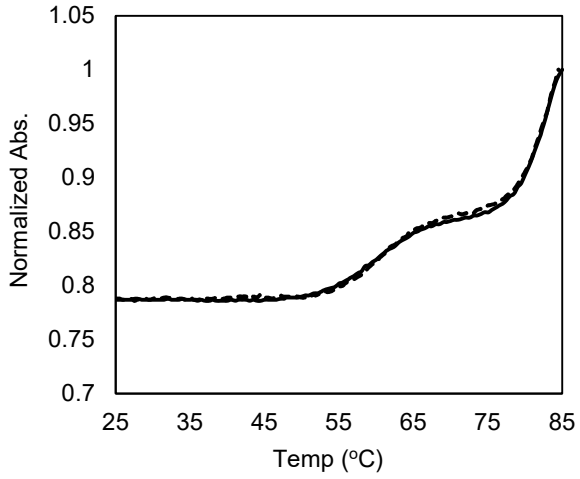
Fig. S10 Hysteresis of sigmoidal curve in TFO/dsDNA

UV melting profiles were measured in 7 mM sodium phosphate buffer (pH 6.0) containing 140 mM KCl and 10 mM $MgCl_2$ at a scan rate of 0.2°C/min or 0.5°C/min at 260 nm. The concentration of oligonucleotide used was 1.5 μM for each strand. The error in T_m values was ±0.5°C. Plain; sigmoidal curve with cooling, dashed; sigmoidal curve with heating. (**B**: OBN, C: 2′-deoxy-5-methylcytidine)

(I) Ramp rate: 0.2°C/min

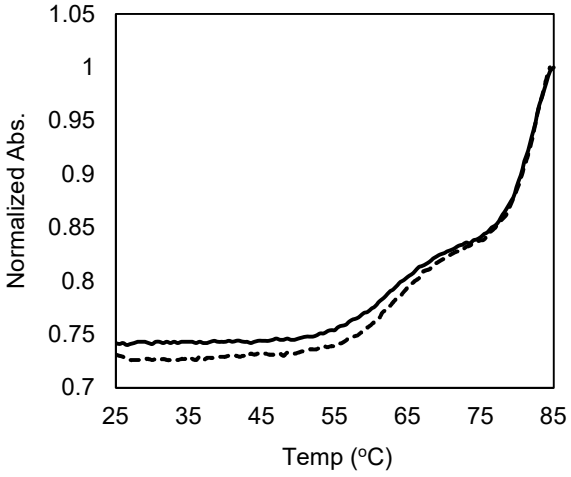
(a) 5'-d(TTTTTCTTTCTCTCT)-3' (TFO**1**) /dsDNA

T_m: 61 °C (with heating), 60 °C (with cooling)



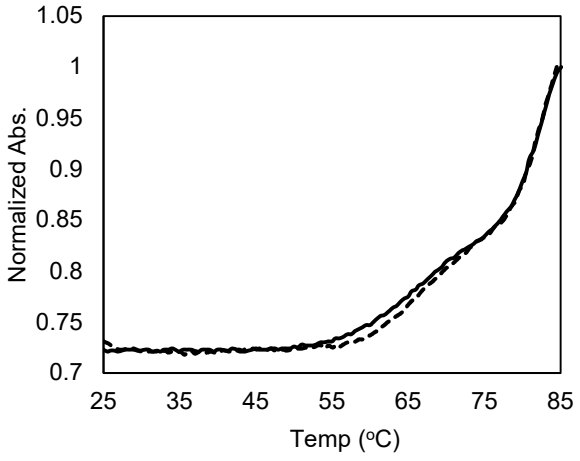
(b) 5'-d(TTTTTCT**B**TCTCTCT)-3' (TFO**2**) /dsDNA

T_m: 63°C (with heating), 63°C (with cooling)



(c) 5'-d(TTTTTC**BB**TCTCTCT)-3' (TFO**7**) /dsDNA

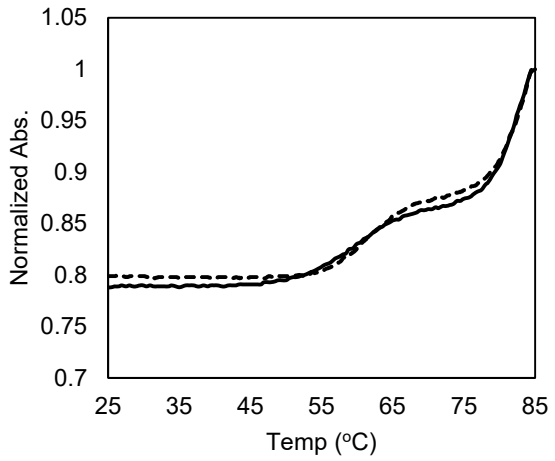
T_m: 67 °C (with heating), 67 °C (with cooling)



(II) Ramp rate: 0.5°C/min

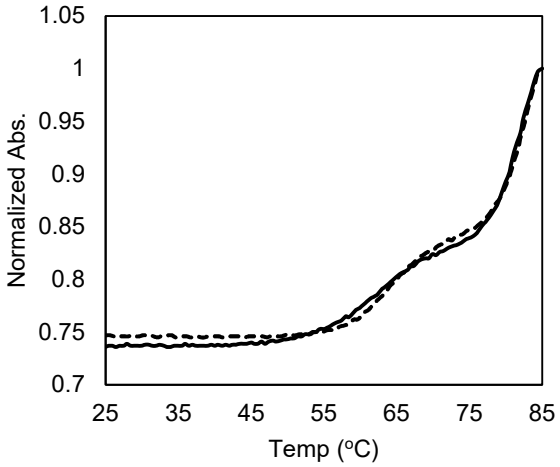
(a) 5'-d(TTTTTC̲TTTC̲TC̲TC̲T)-3' (TFO**1**) /dsDNA

T_m: 62 °C (with heating), 61 °C (with cooling)



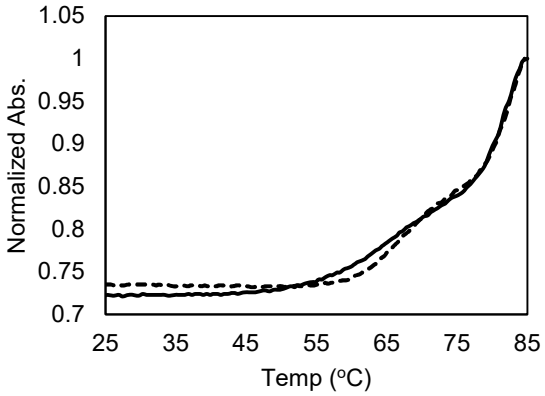
(b) 5'-d(TTTTTC̲T**B**TC̲TC̲TC̲T)-3' (TFO**2**) /dsDNA

T_m: 64 °C (with heating), 63 °C (with cooling)



(C) 5'-d(TTTTTC̲**BB**TC̲TC̲TC̲T)-3' (TFO**7**) /dsDNA

T_m: 67 °C (with heating), 64 °C (with cooling)



Reference

1) M. Tarkçy, A. K. Phipps, P. Schultze, J. Feigon, *Biochemistry*, 1998, **37**, 5810-5819.