Electronic Supplementary Information

Synthesis, duplex-forming ability, enzymatic stability, and *in vitro* antisense potency of oligonucleotides including 2′-*C*,4′-*C*-ethyleneoxy-bridged thymidine derivatives

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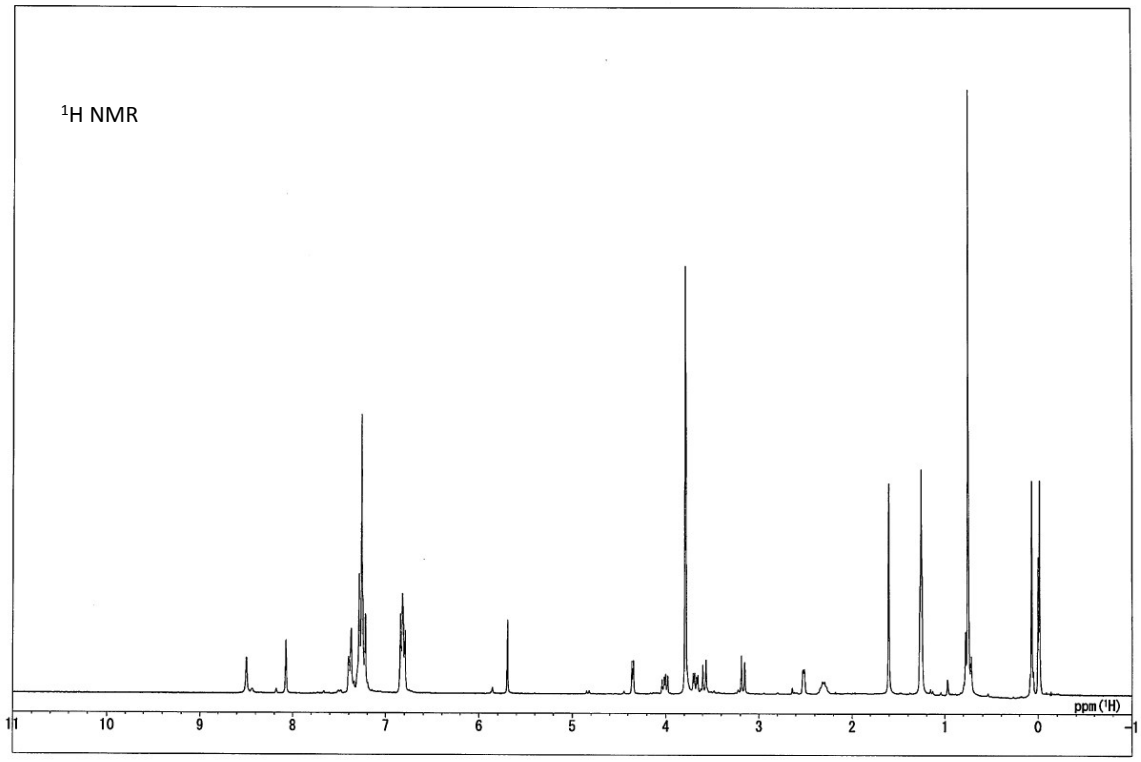

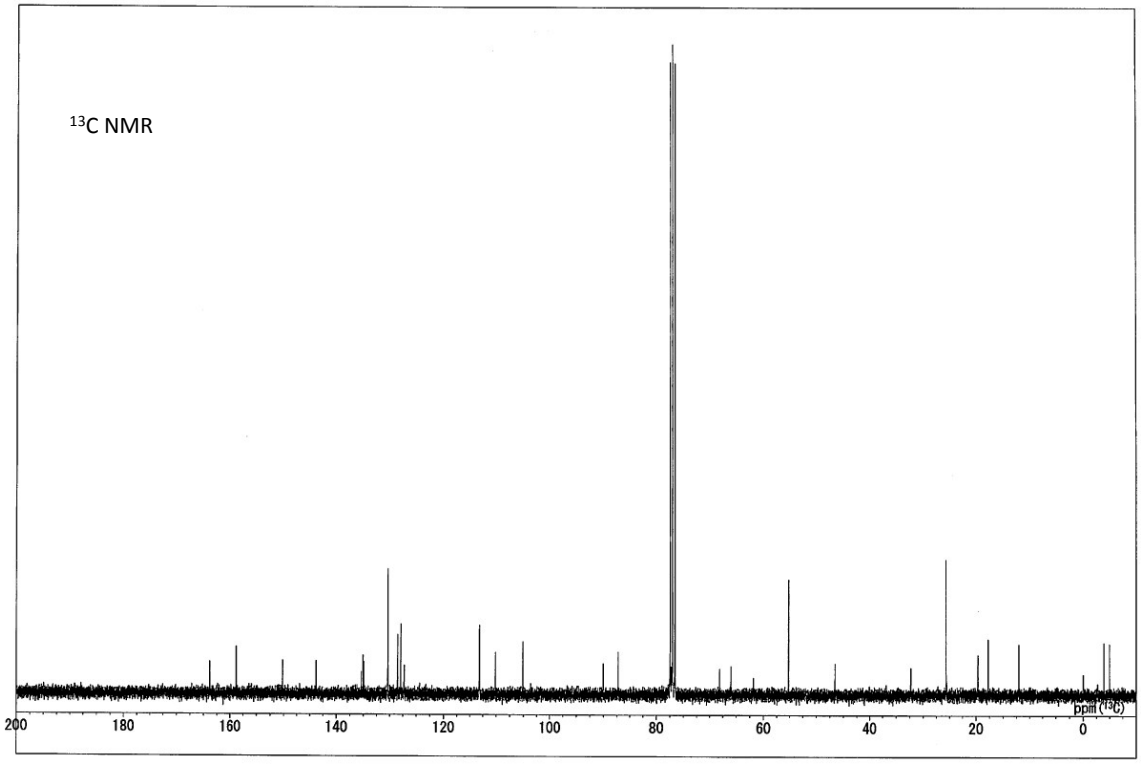

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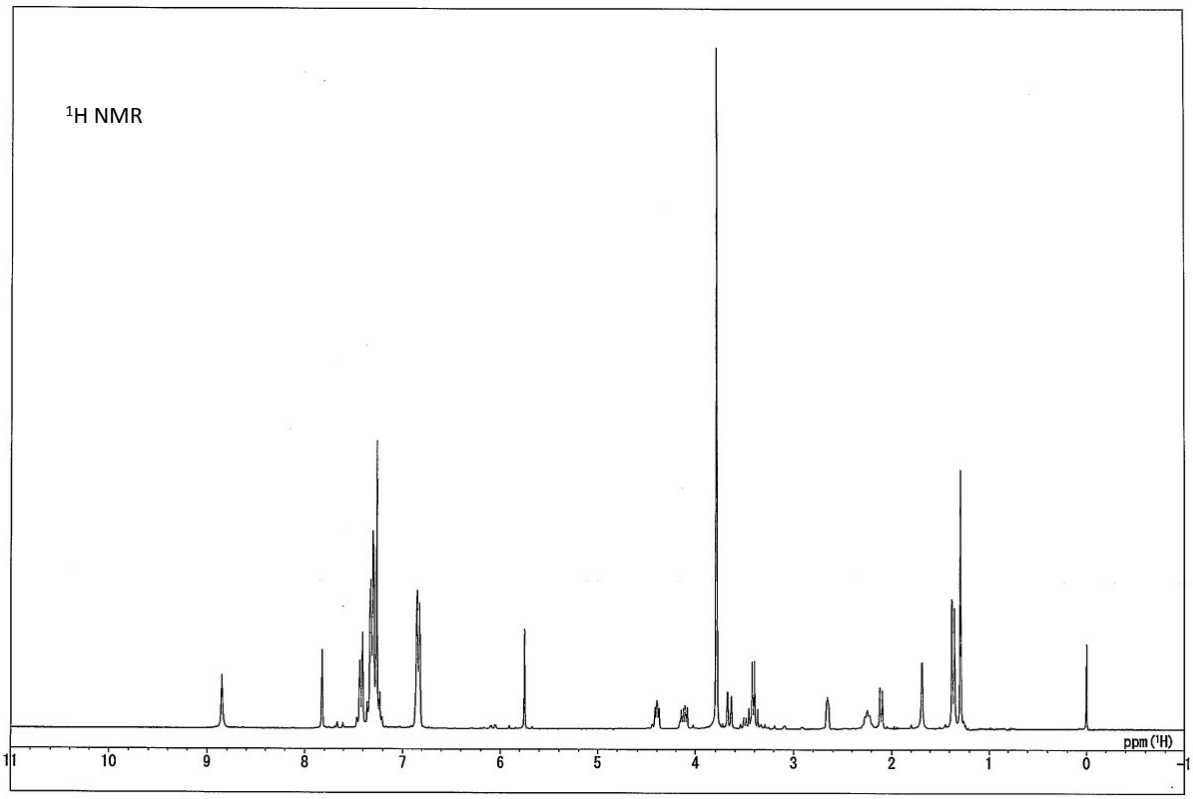

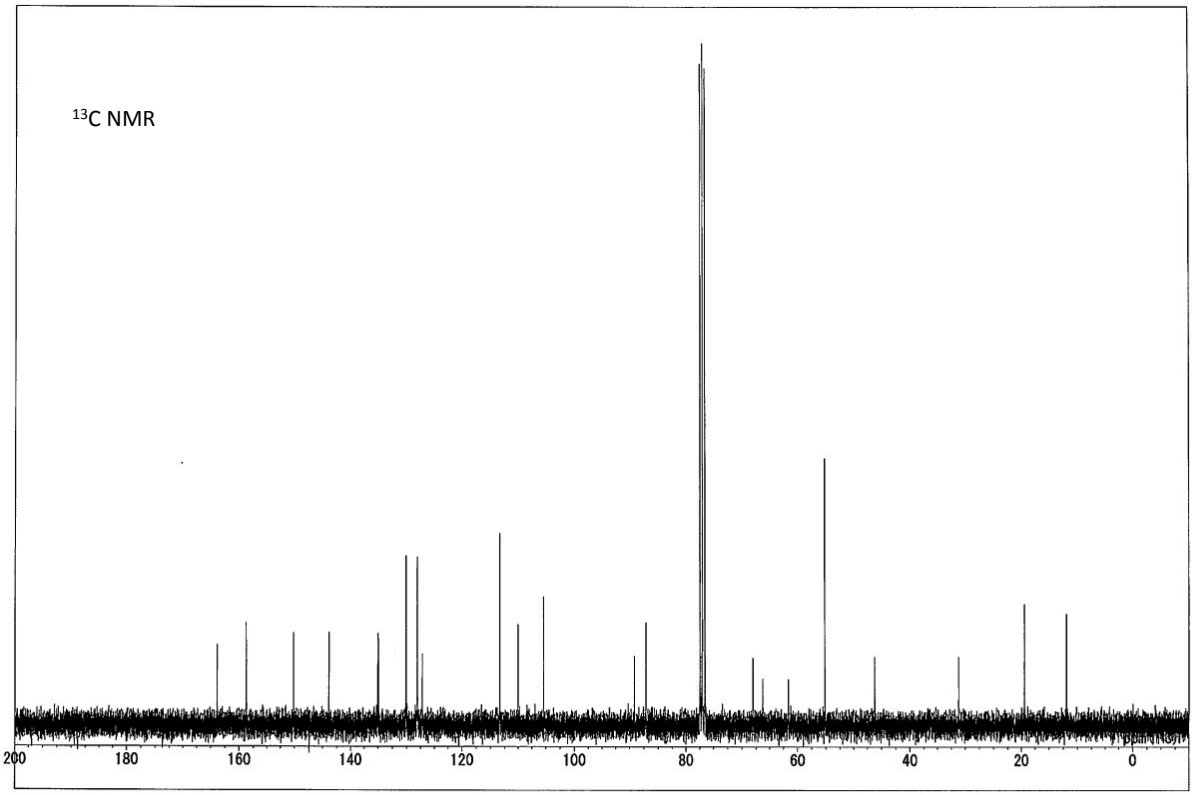

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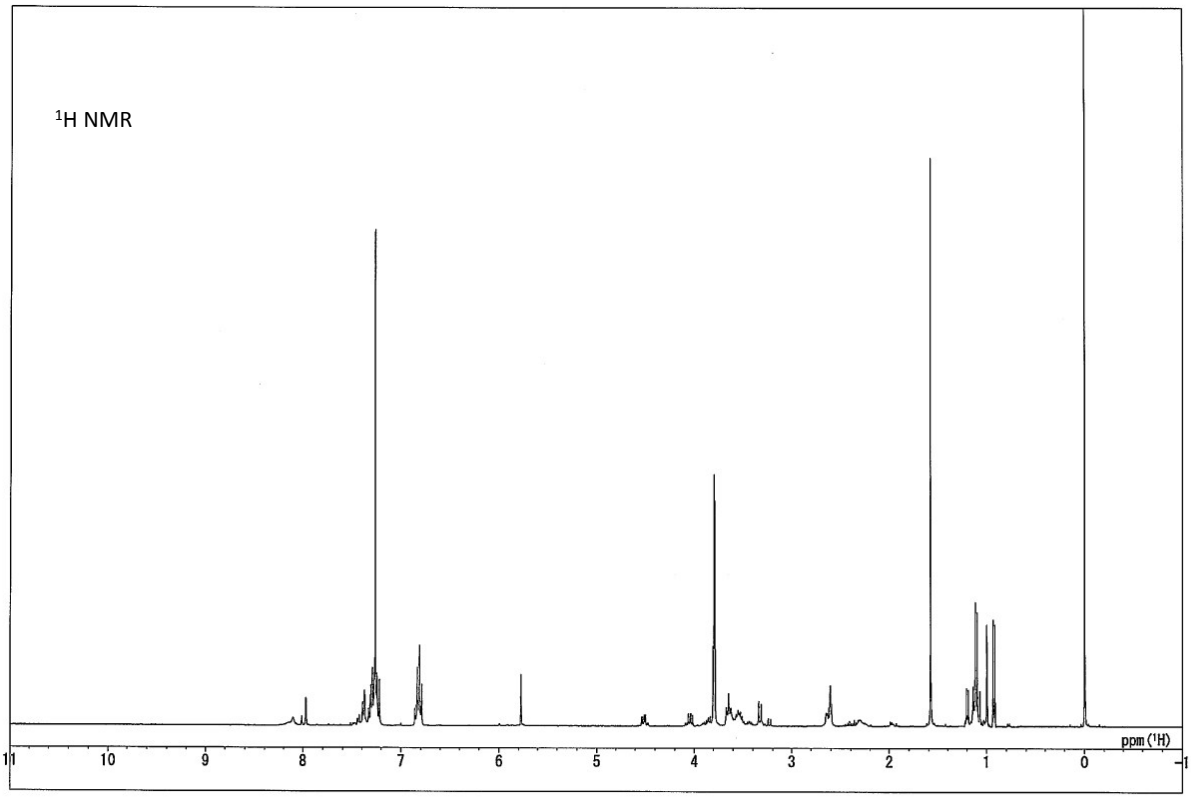

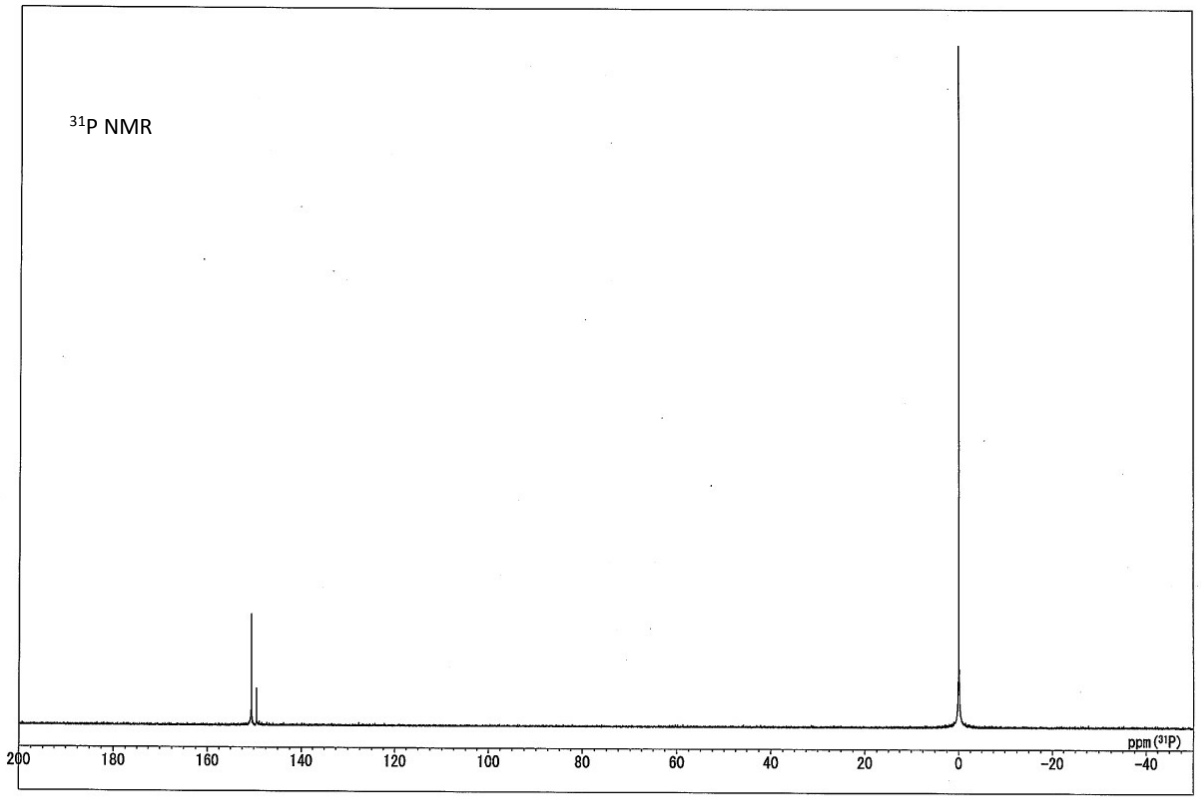

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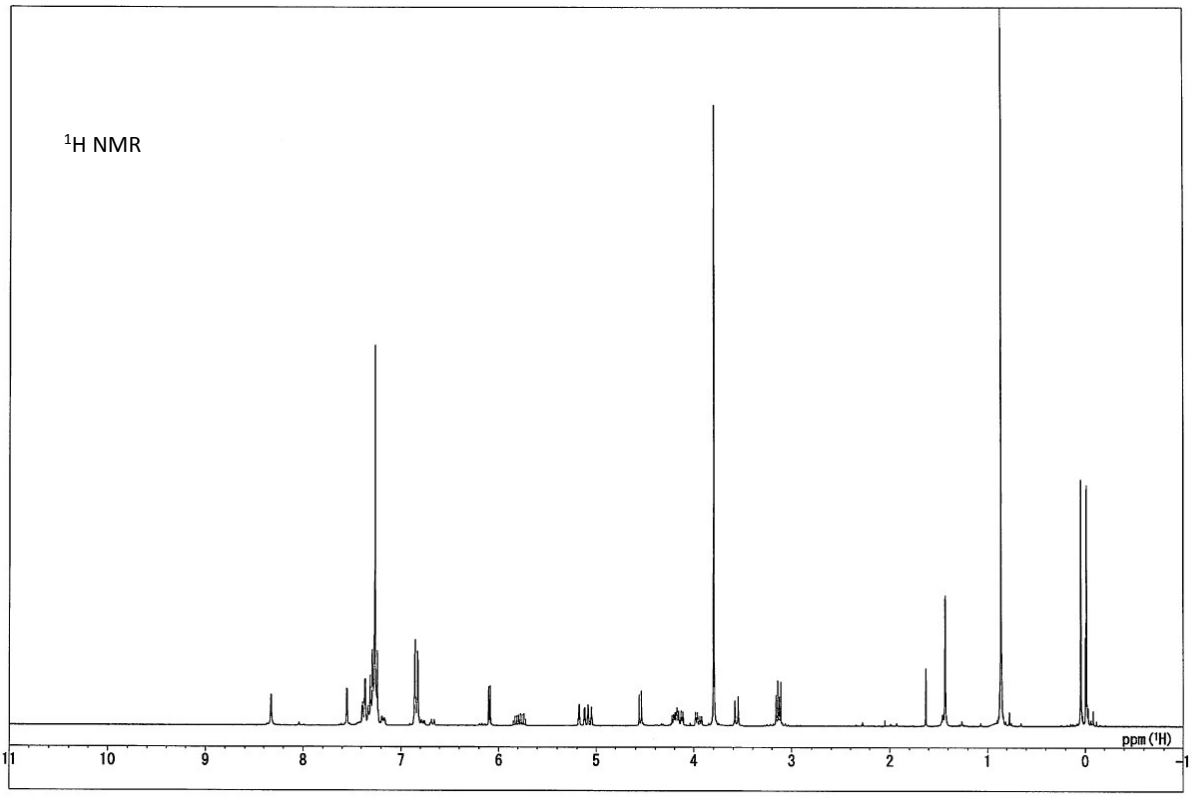

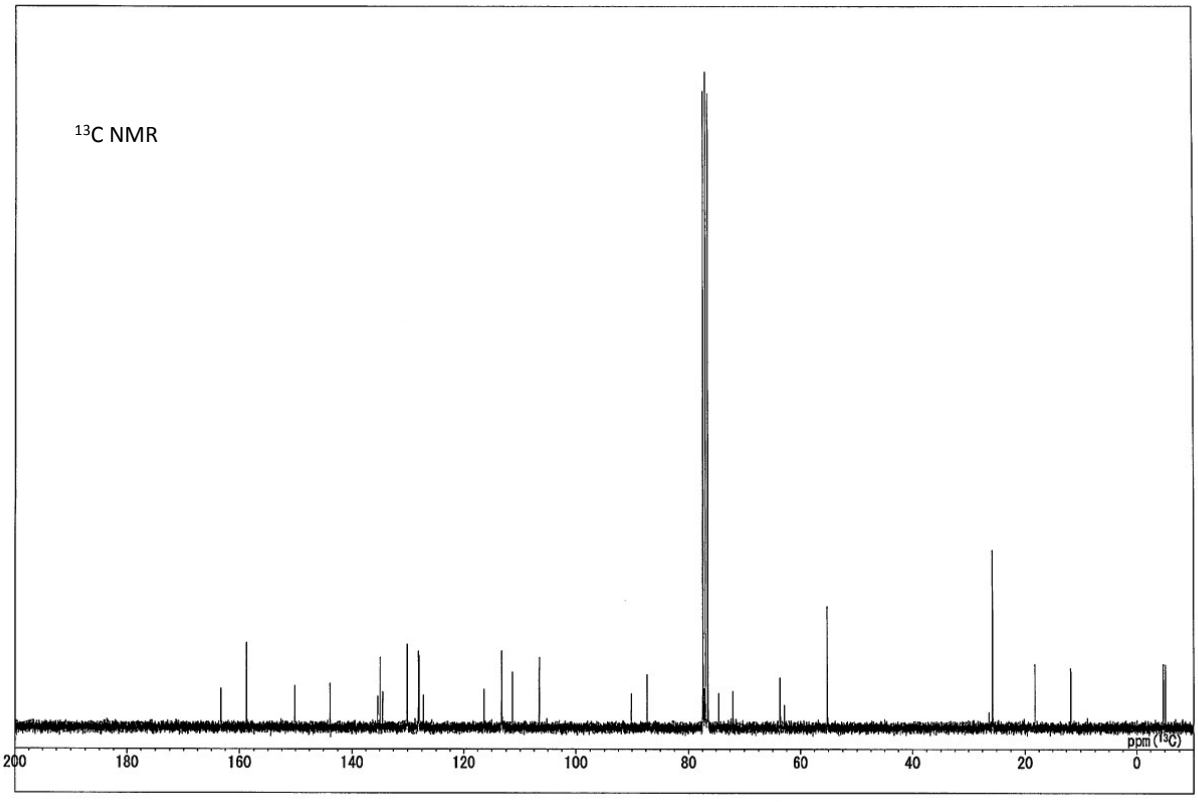

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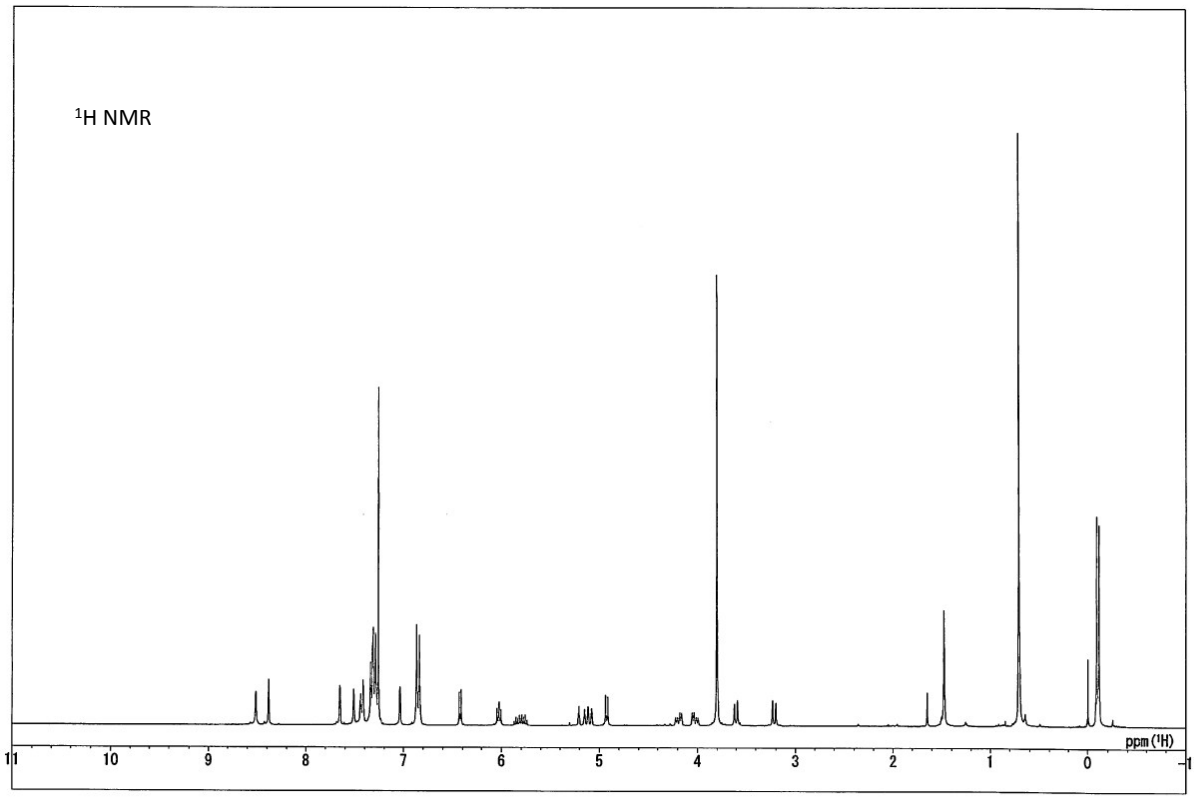

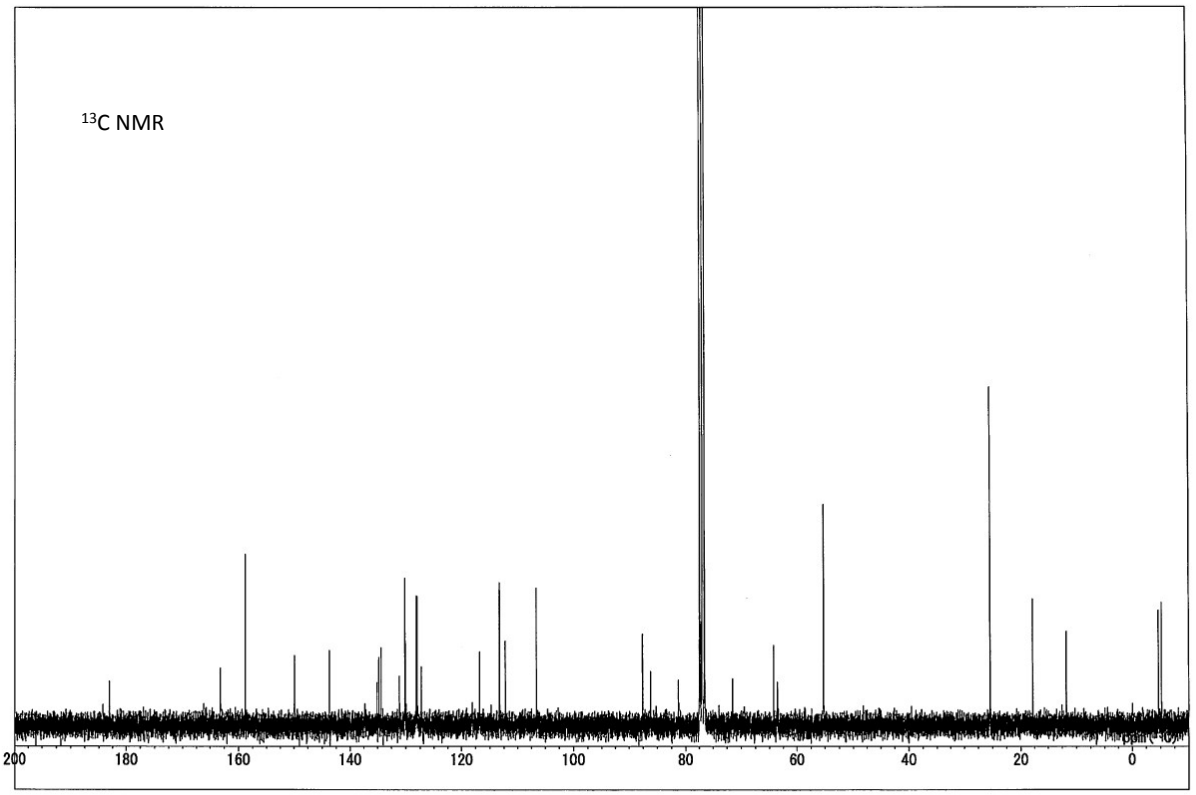

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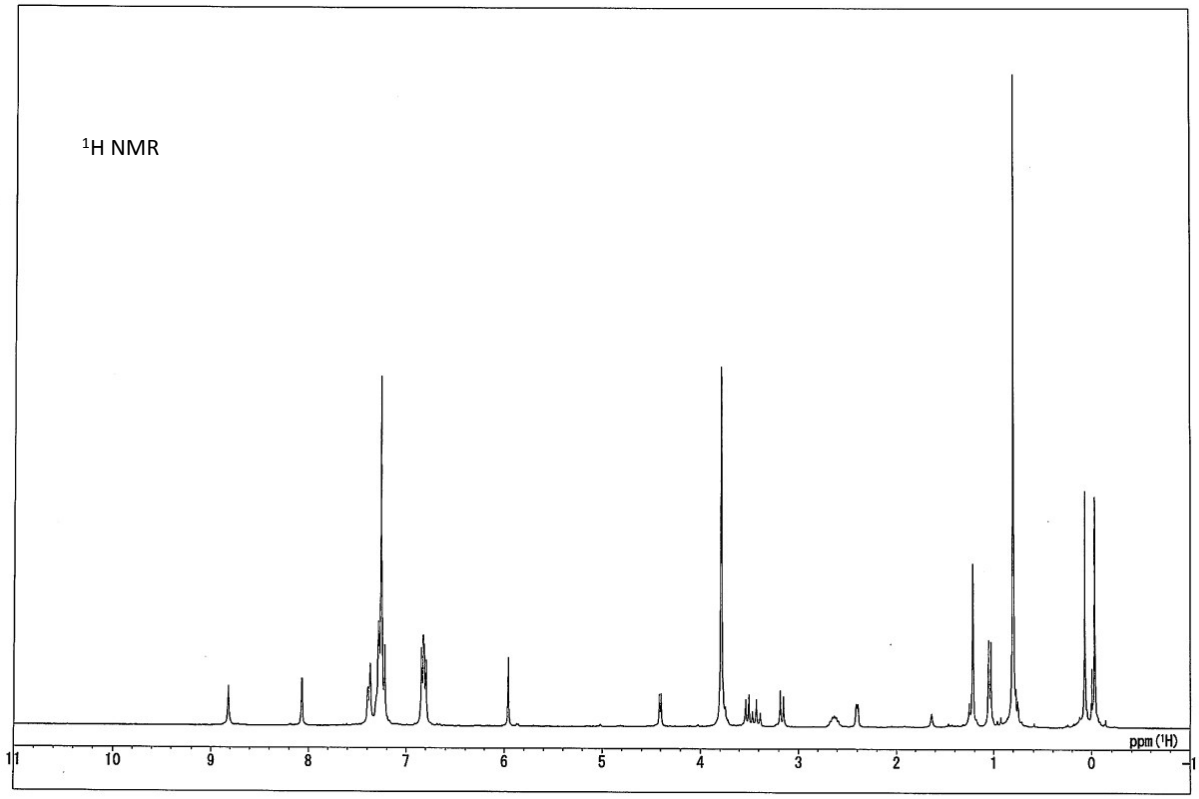

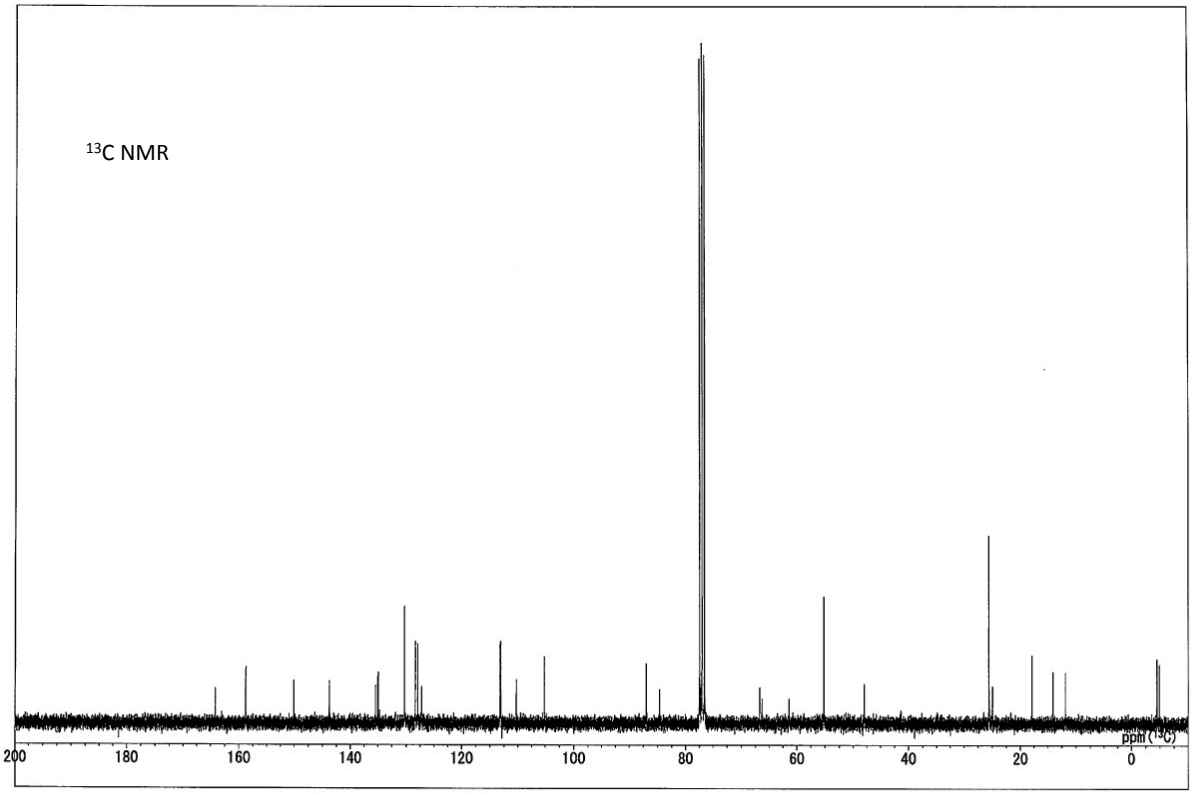

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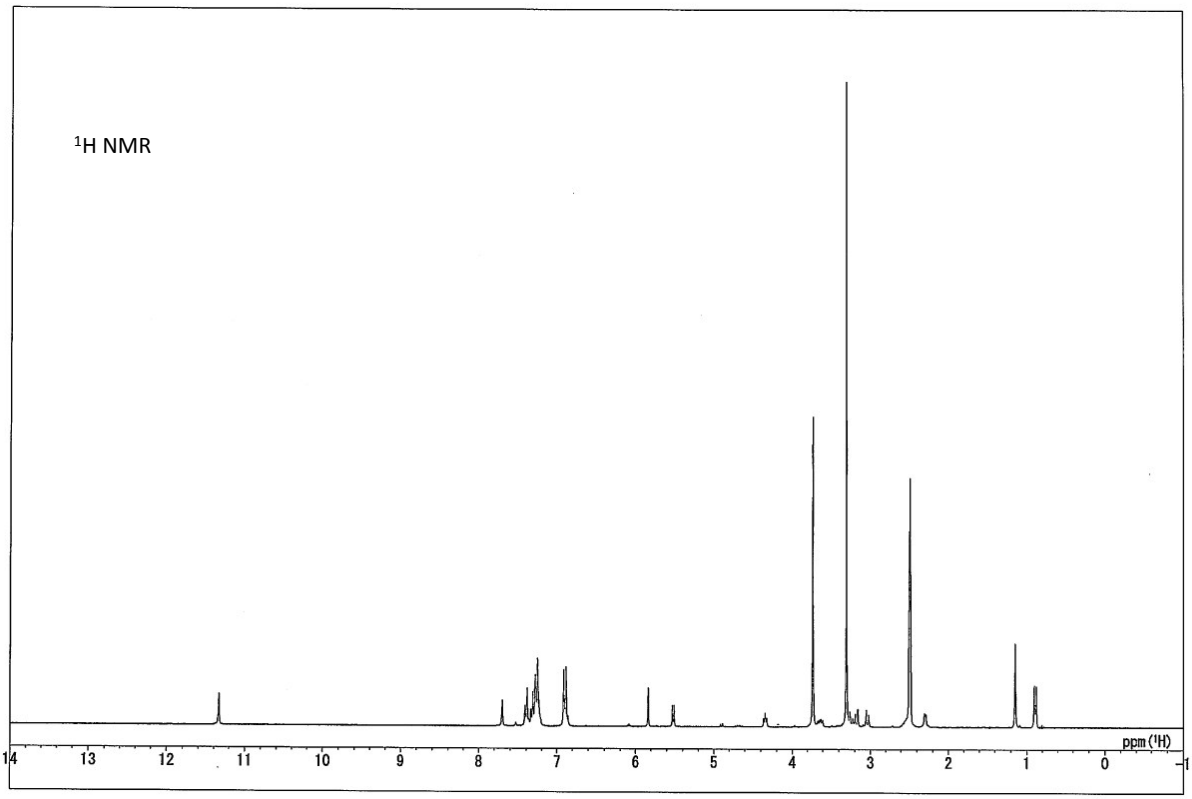

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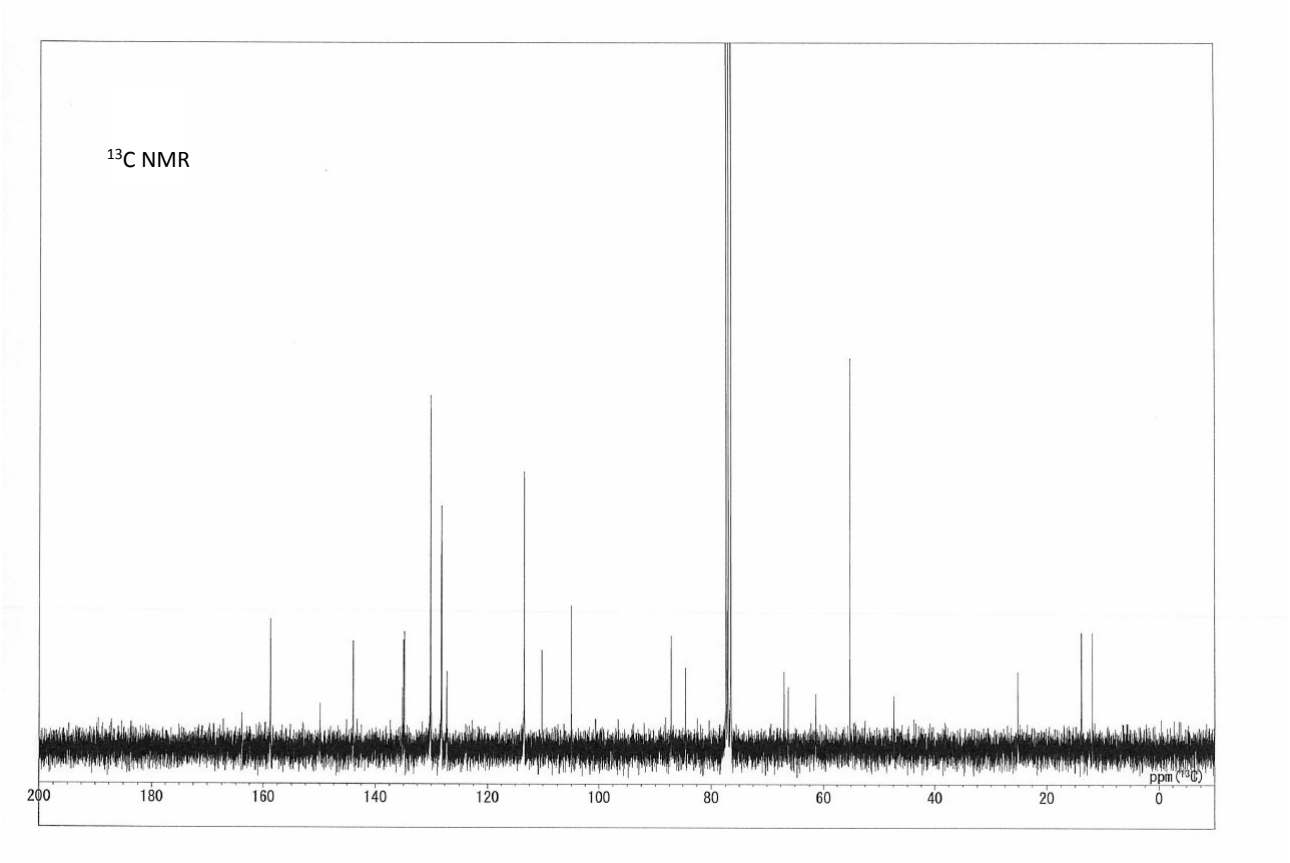

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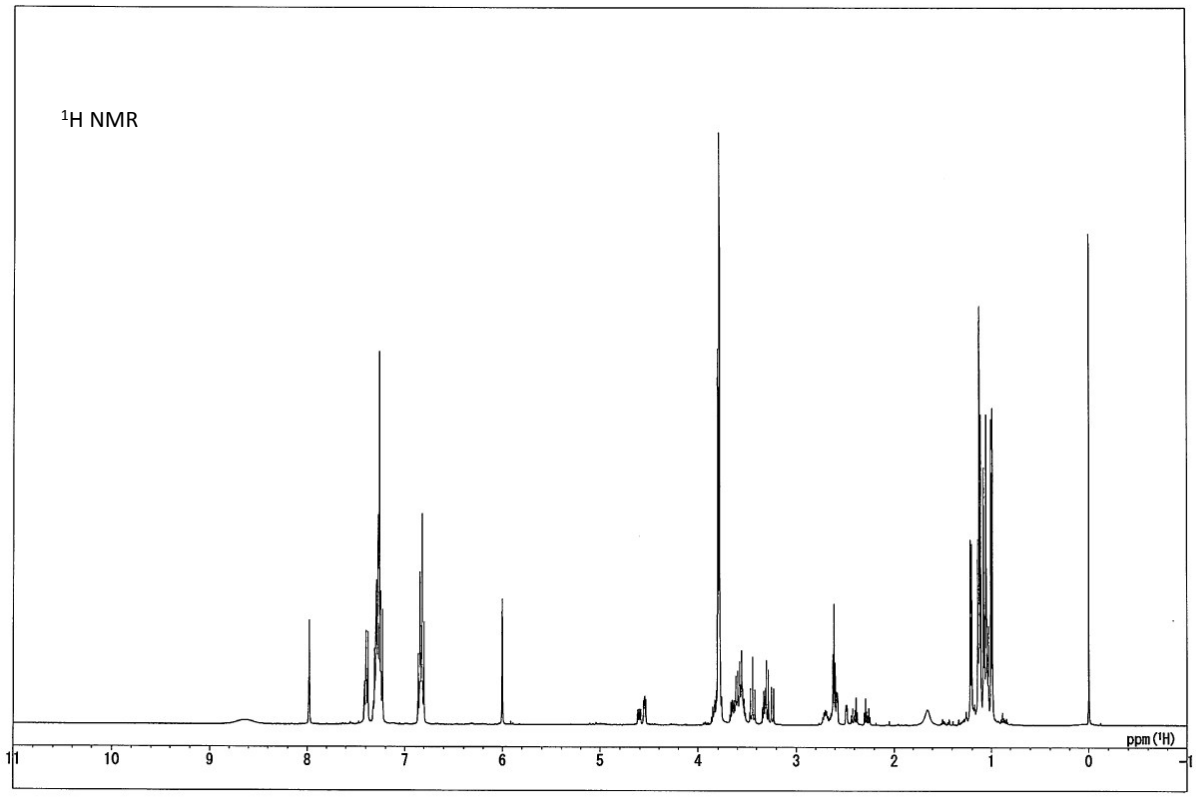

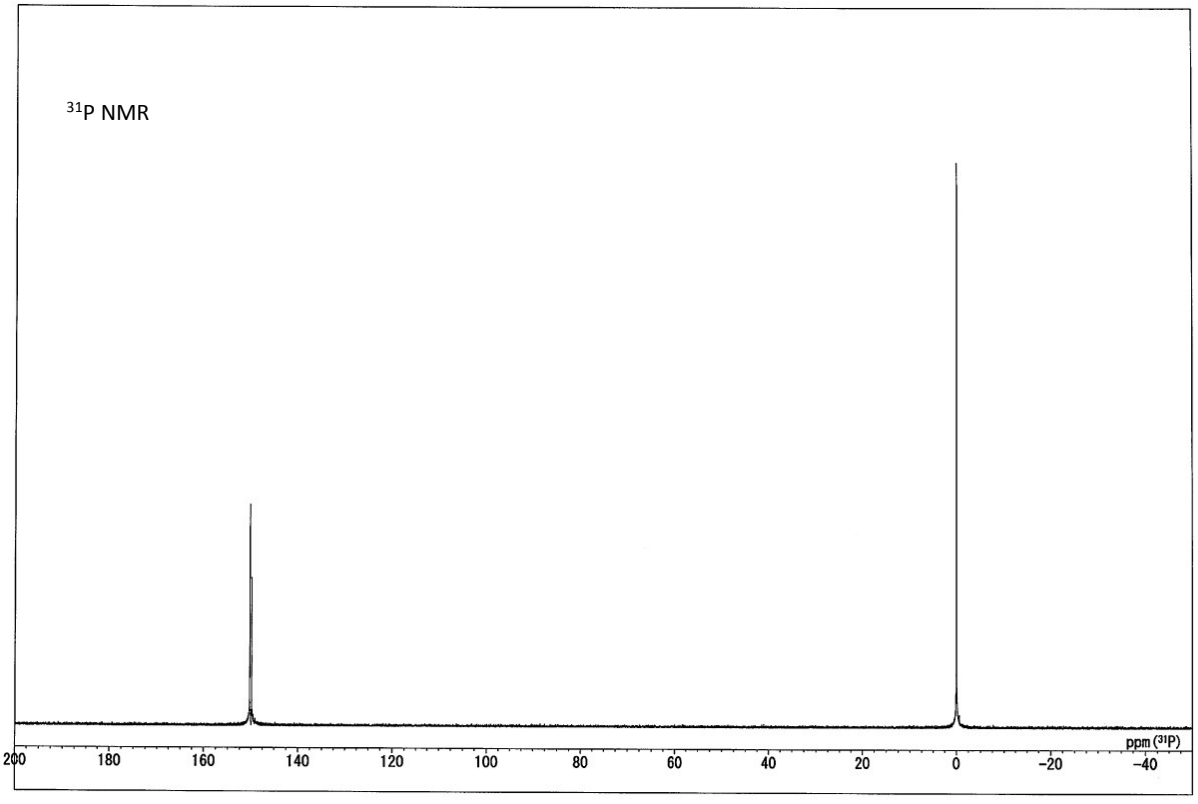

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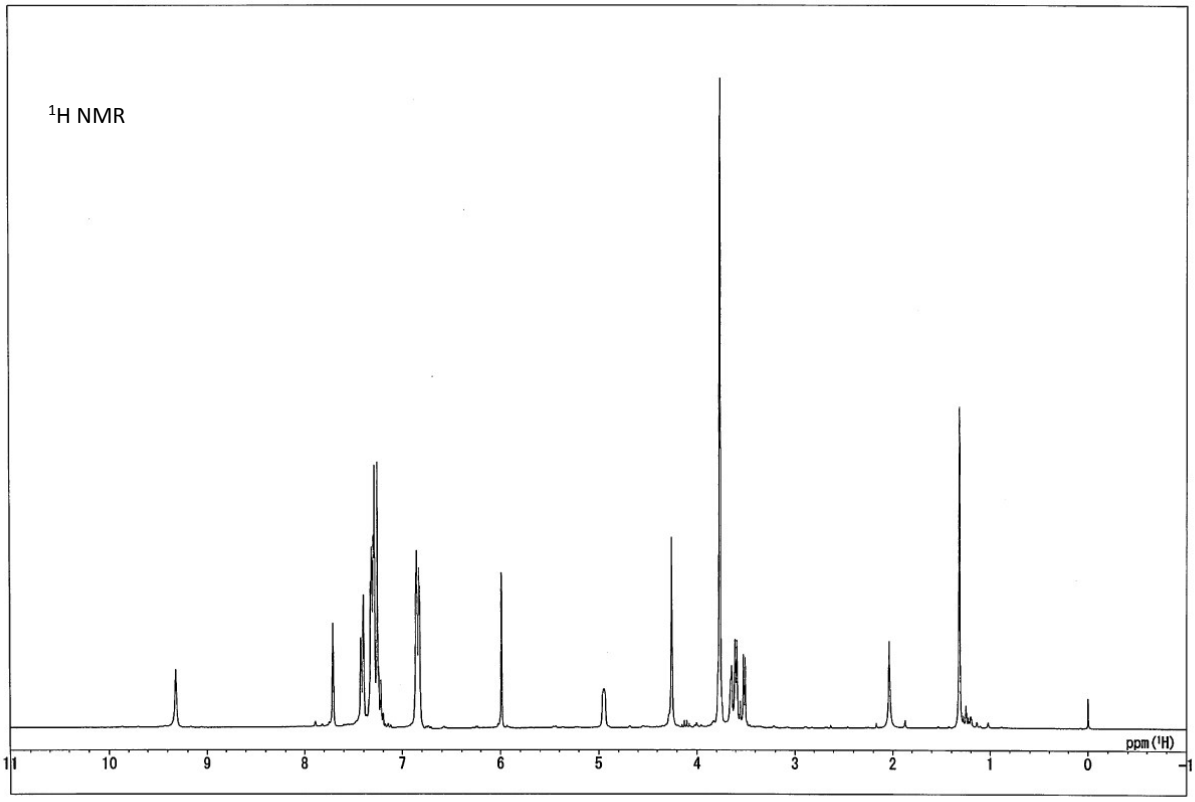

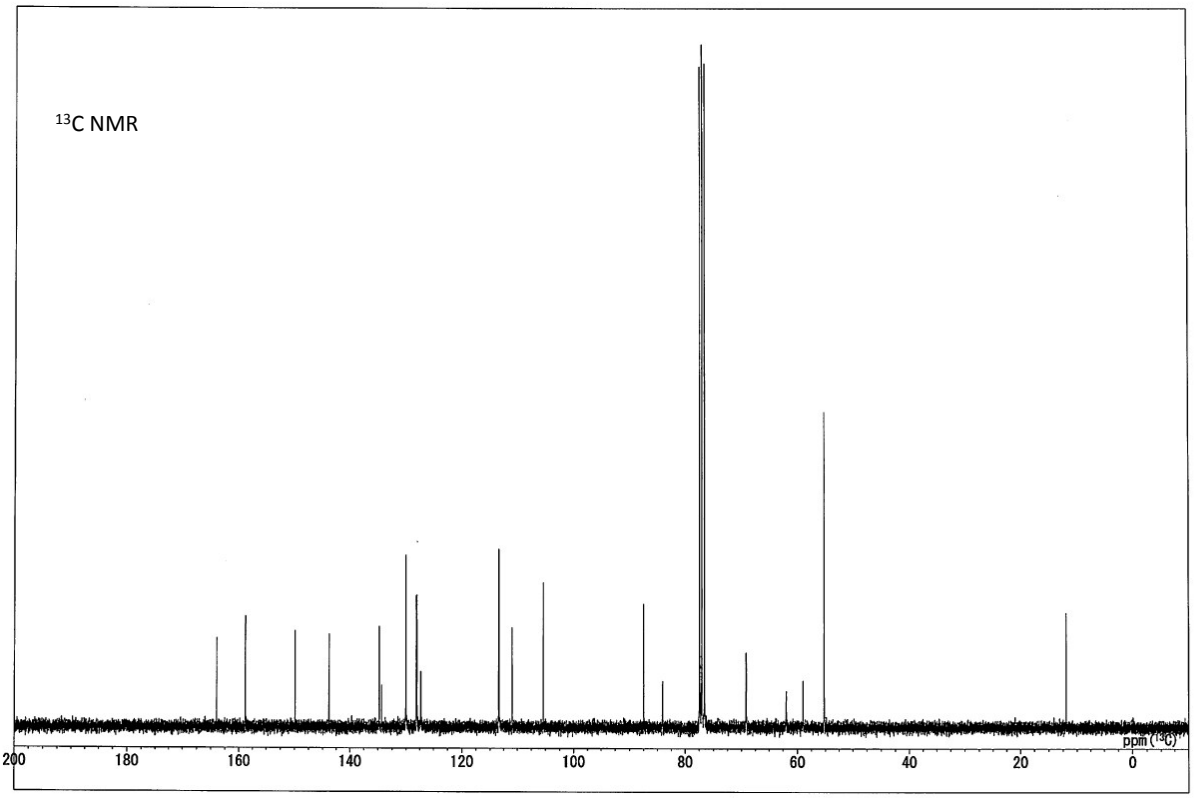

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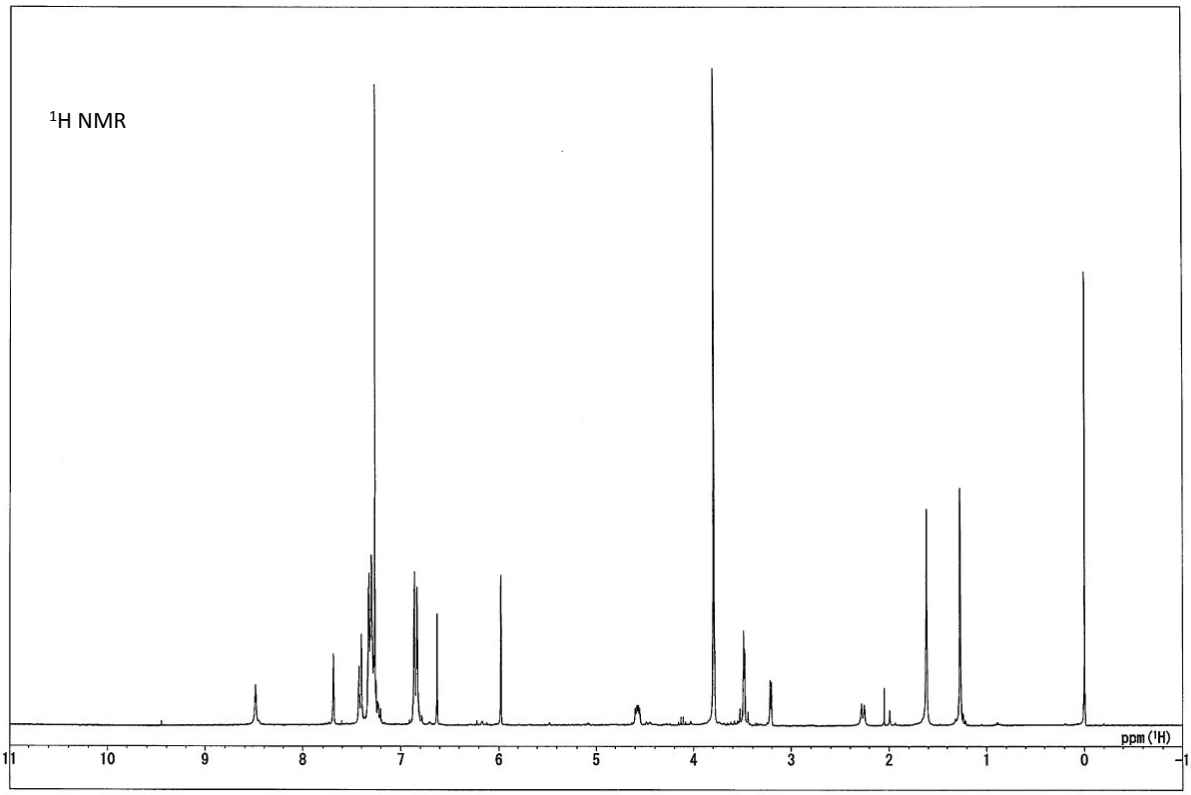

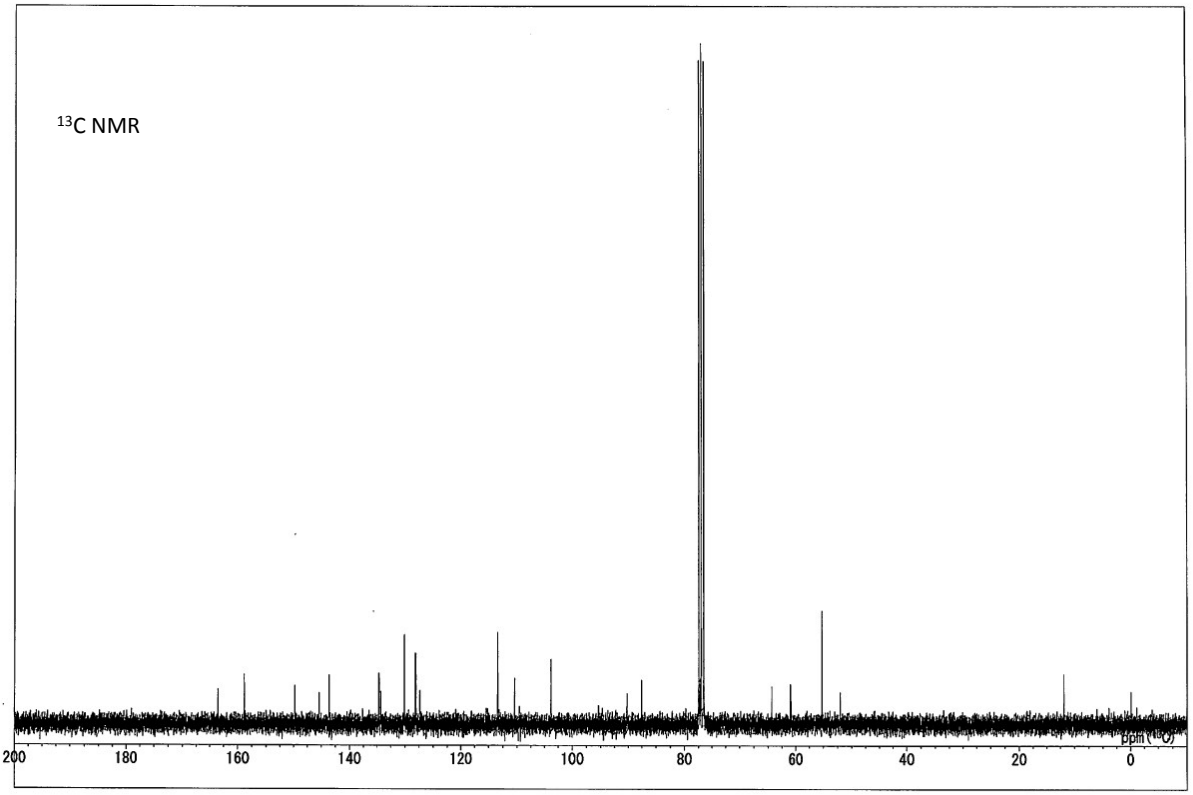

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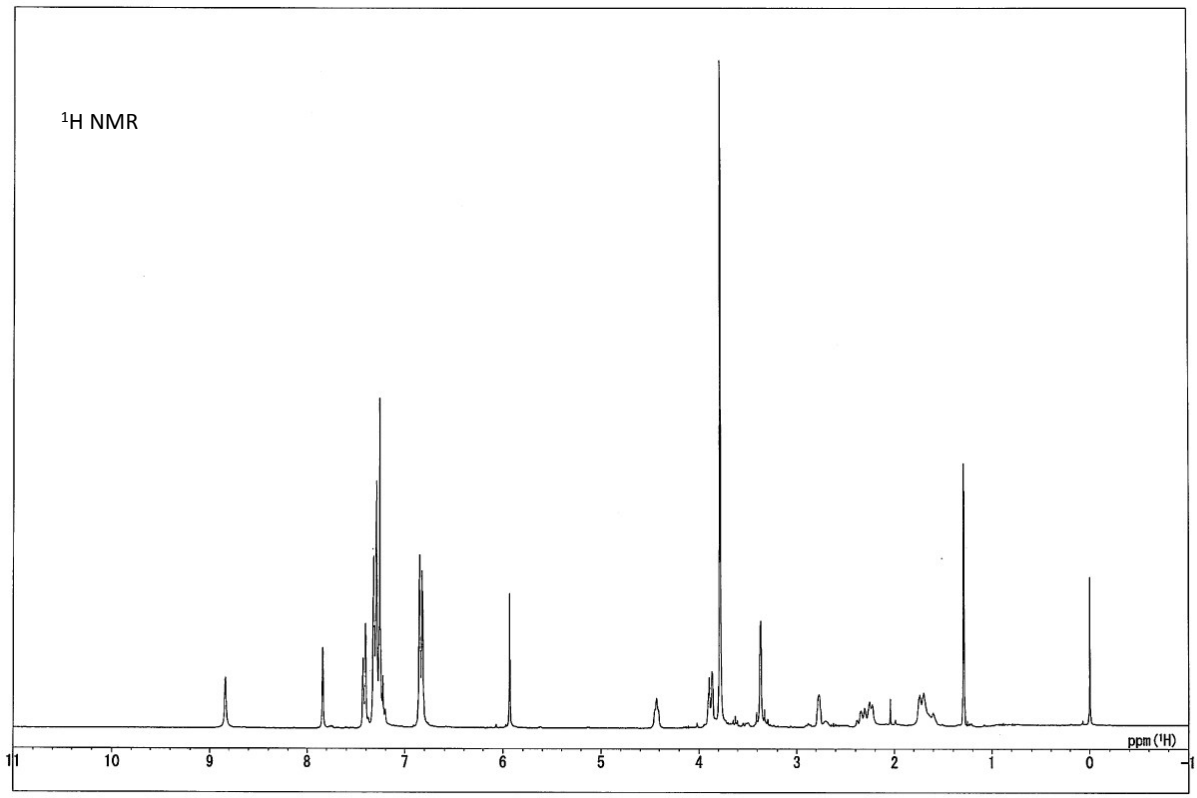

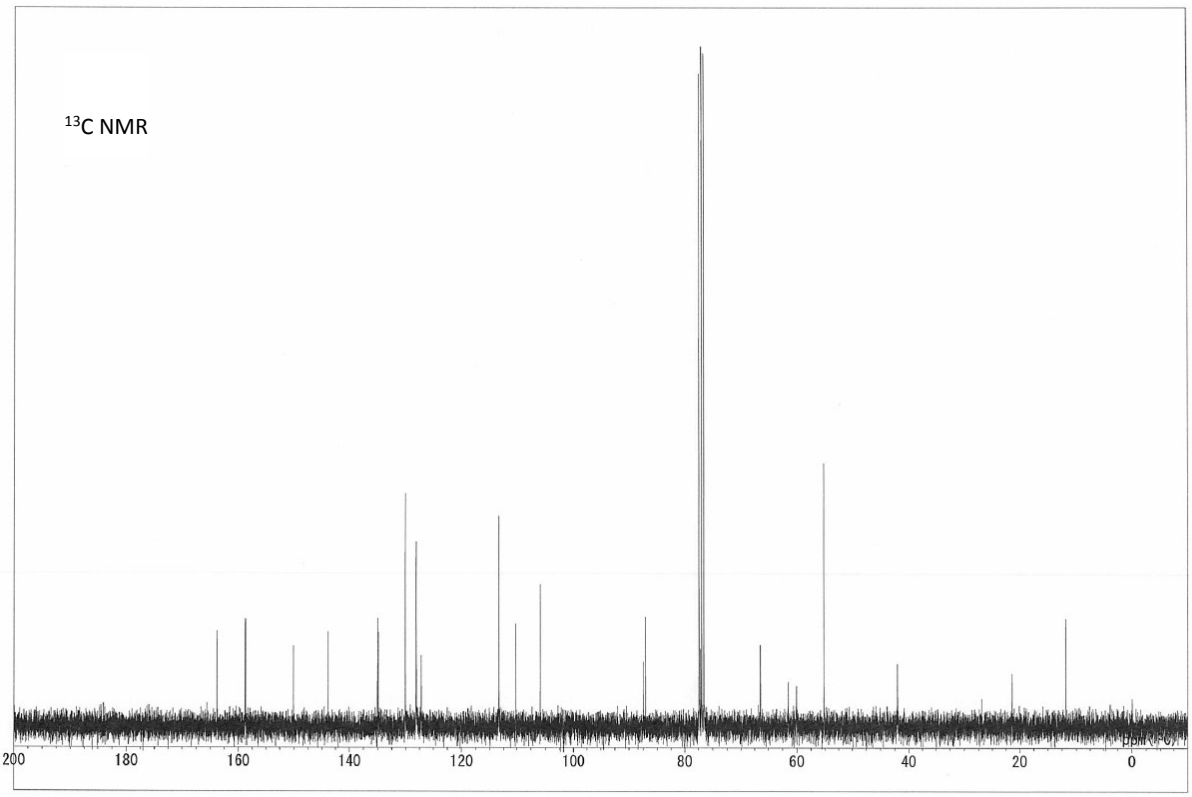

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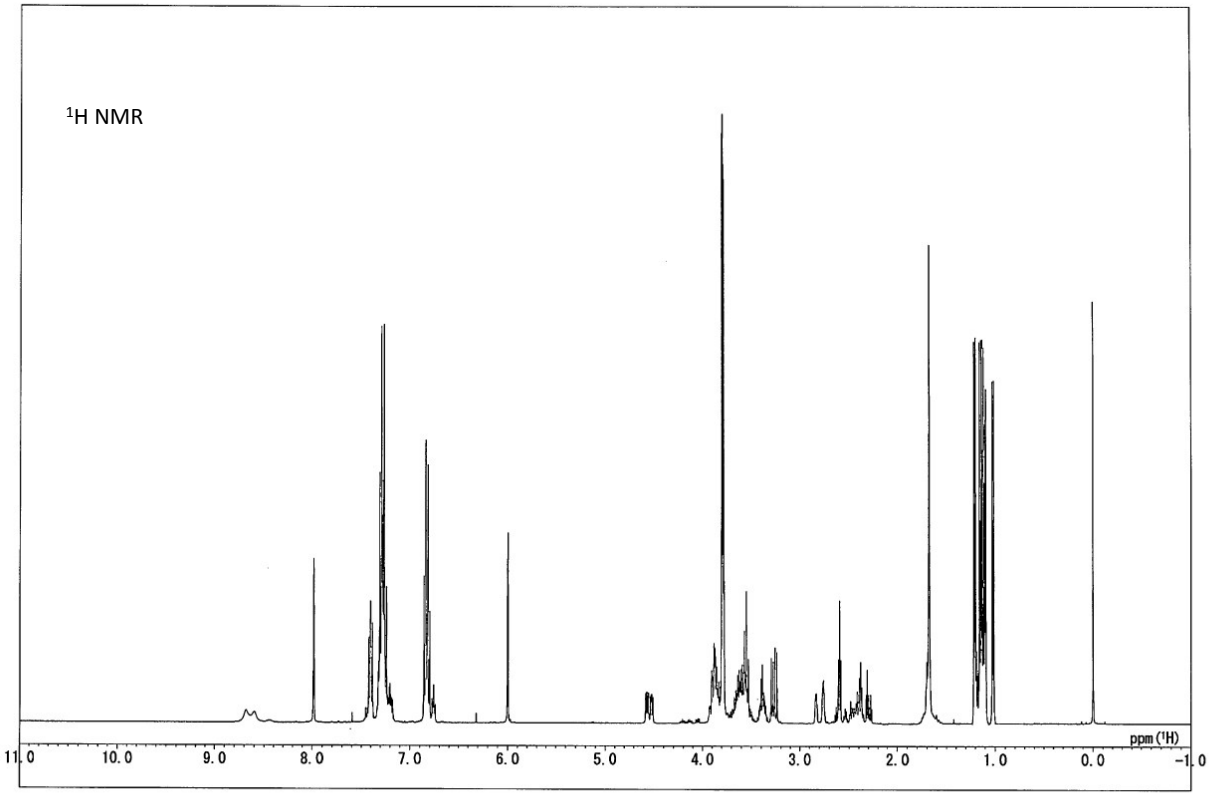

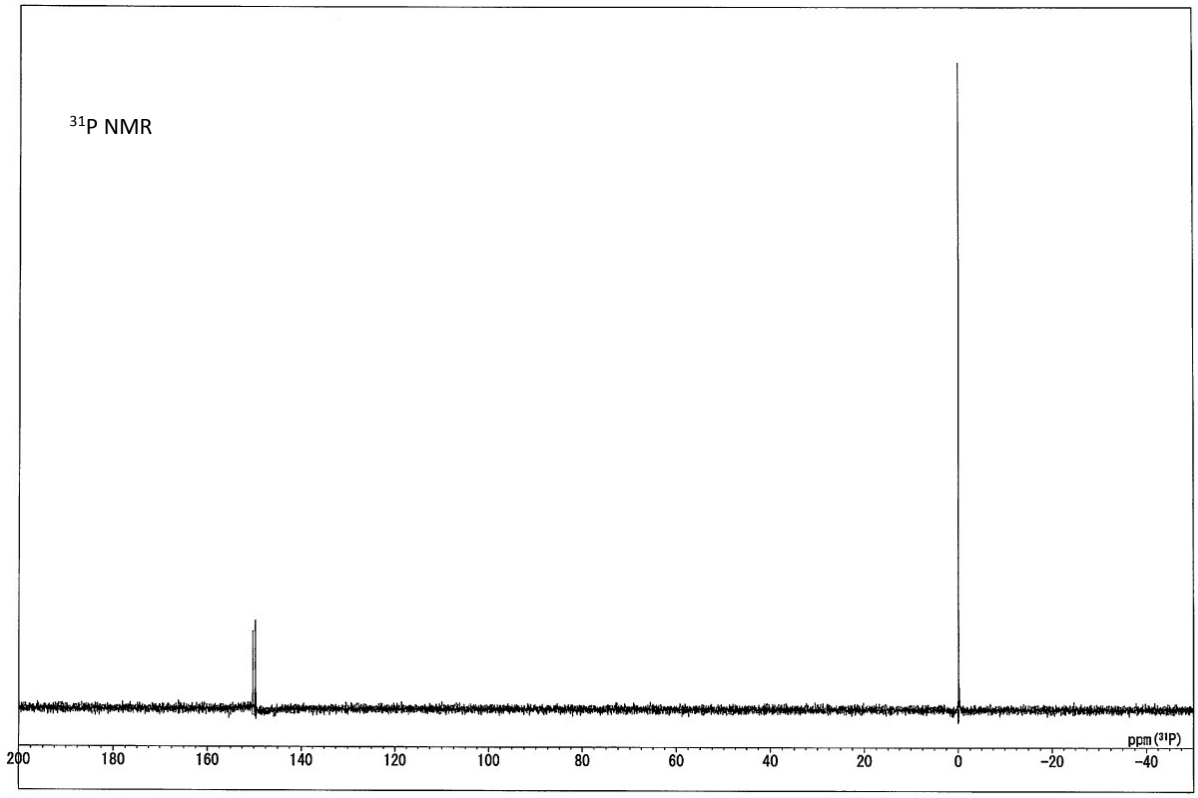

2. H-H COSY and NOESY spectra of compounds 3 and 9

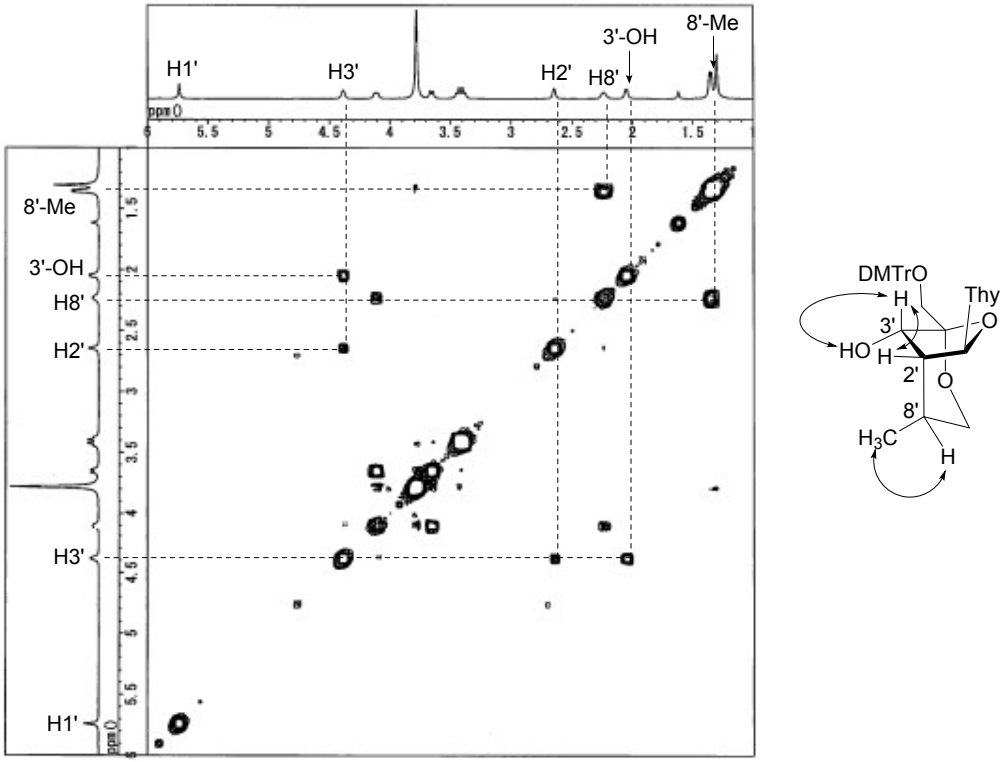

Fig. S1. H-H COSY spectrum of compound **3** in $CDCl_3$.

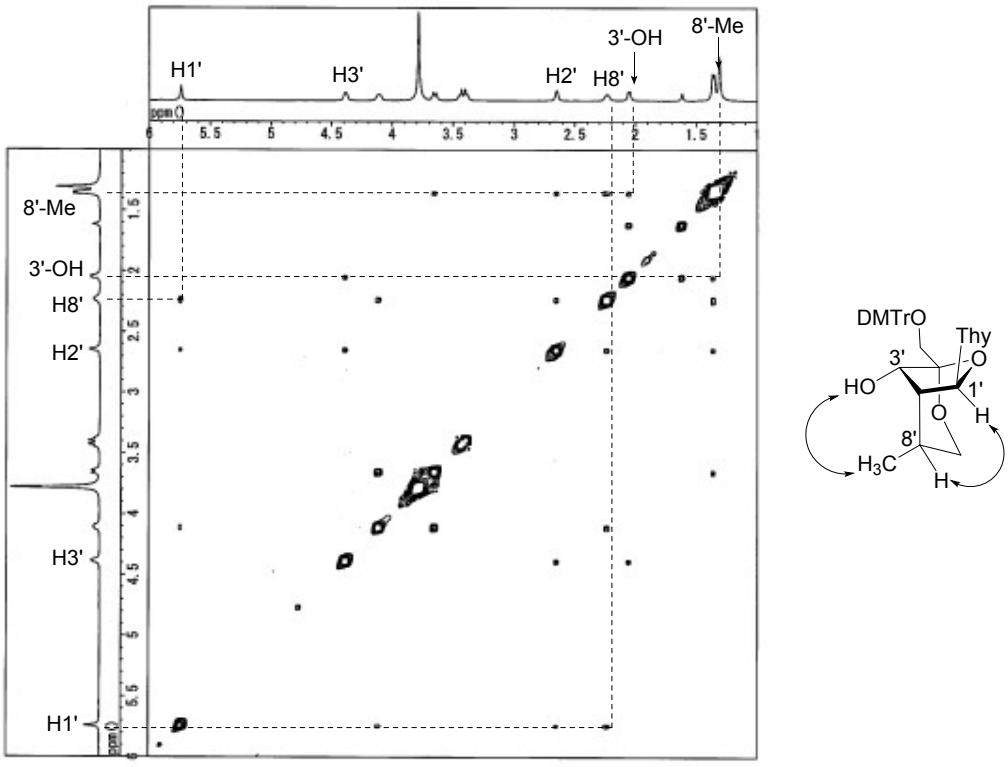

Fig. S2. NOESY spectrum of compound **3** in $CDCl_3$.

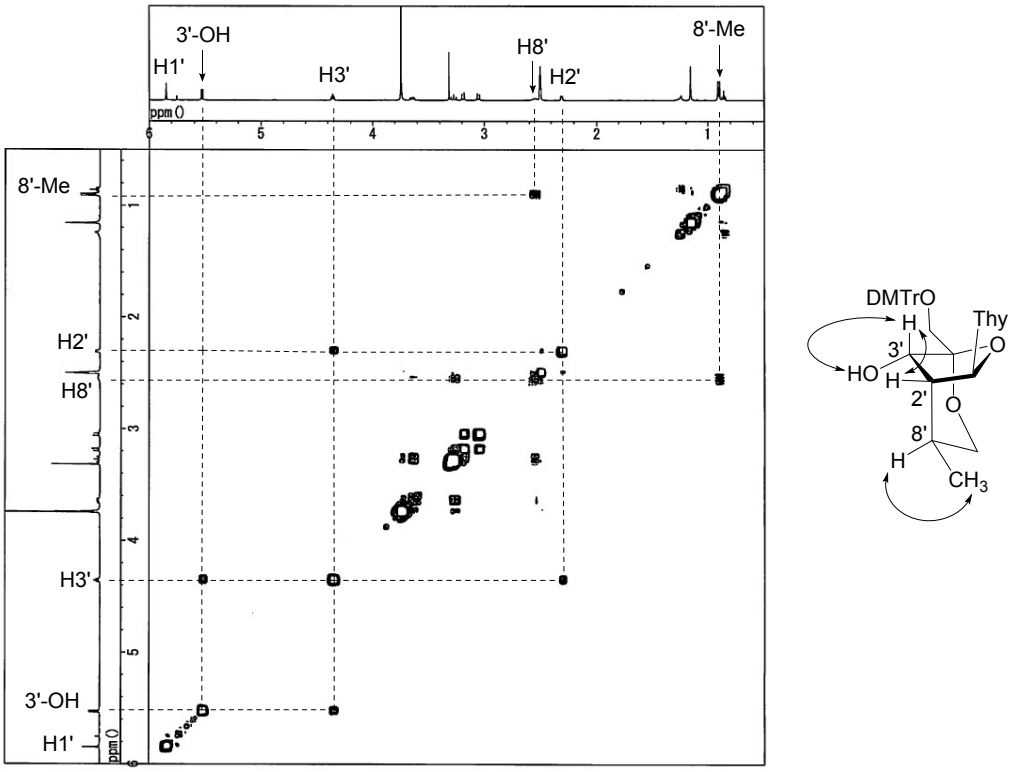

Fig. S3. H-H COSY spectrum of compound **9** in DMSO-d_6.

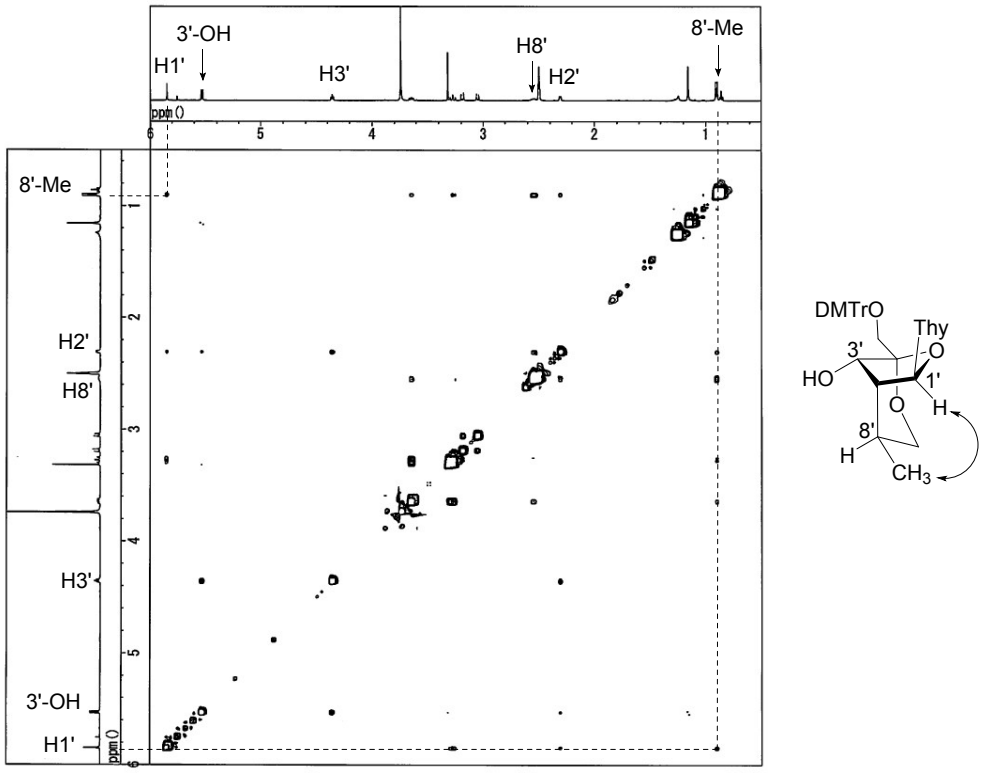

Fig. S4. NOESY spectrum of compound **9** in DMSO-d_6.

3. MALDI-TOF mass data of new oligonucleotides

Table S1. Sequences and MALDI-TOF mass spectra data of new oligonucleotides

Oligonucleotides (5′-3′)	Calcd. $[M-H]^-$	Found $[M-H]^-$
d(T^{m}CTT^{m}CTT**T**TT^{m}CT^{m}CT)	4233.86	4235.21
d(T^{m}CTT^{m}CT**TTT**T^{m}CT^{m}CT)	4317.93	4317.13
d(T^{m}CTT^{m}C**T**T**T**T**T**^{m}CT^{m}CT)	4317.93	4317.49
d(T^{m}CT**<u>T</u>**^{m}C**T**T**T**T**T**^{m}C**<u>T</u>**^{m}CT)	4402.60	4403.26
d(T^{m}CTT^{m}CTT*T*TT^{m}CT^{m}CT)	4247.86	4247.59
d(T^{m}CTT^{m}CT*TTT*T^{m}CT^{m}CT)	4359.98	4360.84
d(T^{m}CTT^{m}C*T*T*T*T^{m}CT^{m}CT)	4359.98	4359.95
d(T^{m}CT*T*^{m}C*T*T*T*T^{m}C*T*^{m}CT)	4472.11	4471.77
d(T^{m}CTT^{m}CTT<u>*T*</u>TT^{m}CT^{m}CT)	4247.86	4247.39
d(T^{m}CTT^{m}CT<u>*TTT*</u>T^{m}CT^{m}CT)	4359.98	4359.86
d(T^{m}CTT^{m}C<u>*T*</u>T<u>*T*</u>T<u>*T*</u>^{m}CT^{m}CT)	4359.98	4359.52
d(T^{m}CT<u>*T*</u>^{m}C<u>*T*</u>T<u>*T*</u>T<u>*T*</u>^{m}C<u>*T*</u>^{m}CT)	4472.11	4472.42
TTTTTTTTT**T**T	3021.21	3021.68
TTTTTTTT*T*T	3035.02	3034.29
TTTTTTTT<u>*T*</u>T	3035.02	3034.21
d(**TT**CAGCATTGG**T**A**TT**C) (**ASO1**)	5071.38	5071.56
d(*TT*CAGCATTGG*T*A*TT*C) (**ASO2**)	5141.52	5141.32
d(<u>*TT*</u>CAGCATTGG<u>*T*</u>A<u>*TT*</u>C) (**ASO3**)	5141.52	5140.11
d(**tt**CAGCATTGG**t**A**tt**C) (**ASO4**)	5131.44	5131.34
d(*tt*CAGCATTGG*t*A*tt*C) (**ASO5**)	5201.57	5202.01
d(<u>tt</u>CAGCATTGG<u>t</u>A<u>tt</u>C) (**ASO6**)	5201.57	5202.01

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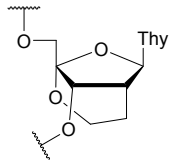

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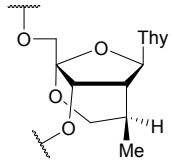

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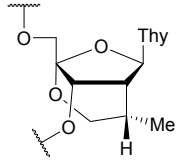

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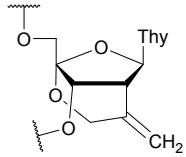

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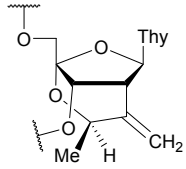

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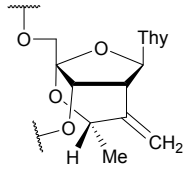
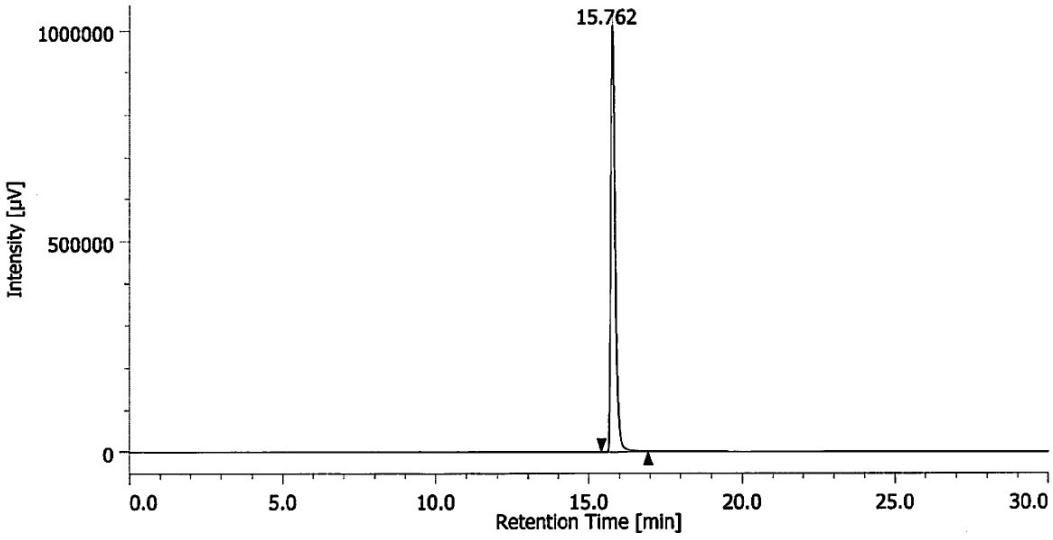


4. HPLC charts of new oligonucleotides

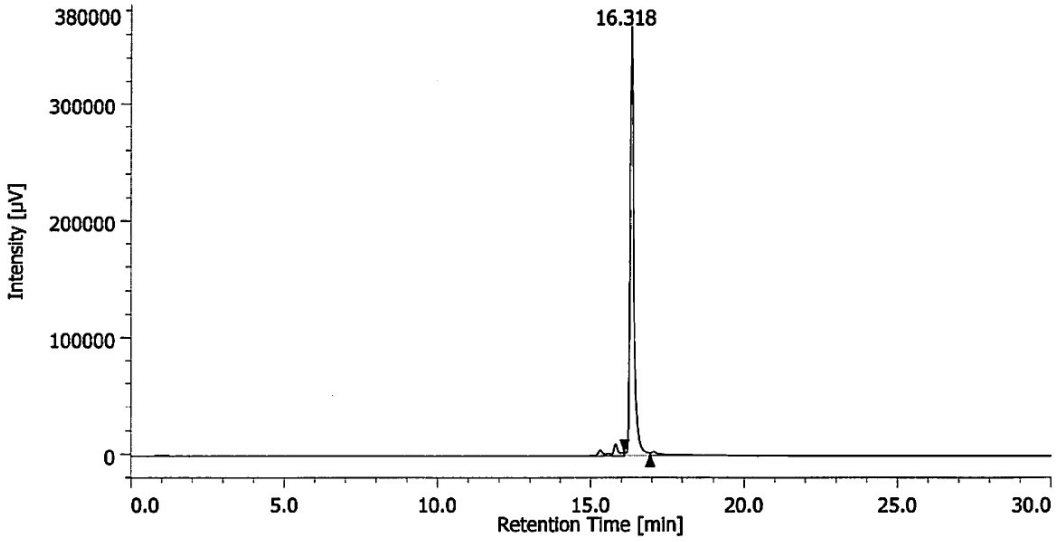
5′-d(T^{m}CTT^{m}CTT**T**TT^{m}CT^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



5′-d(T^{m}CTT^{m}CT**TTT**T^{m}CT^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



5′-d(T^{m}CTT^{m}C**T**T**T**T**T**^{m}CT^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
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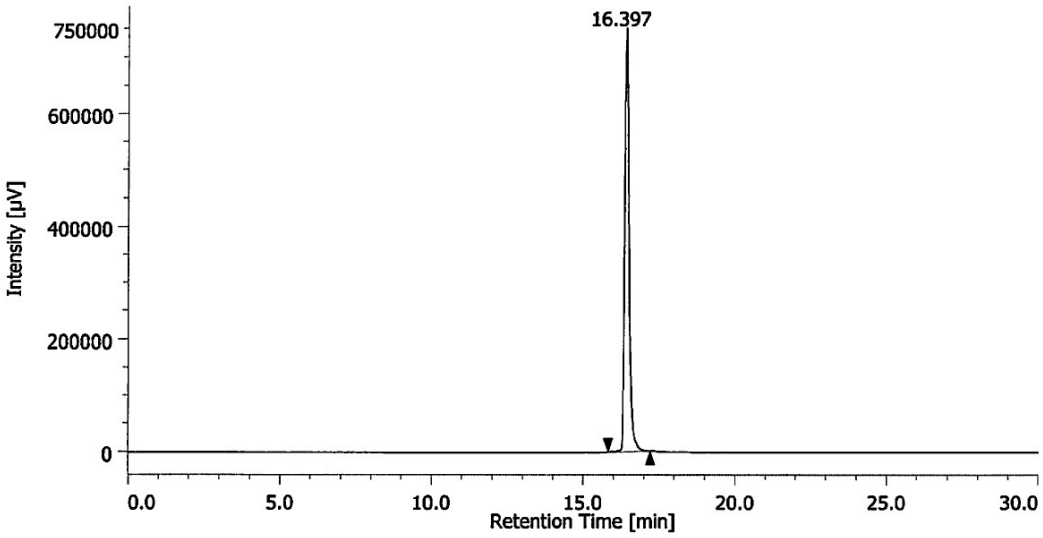
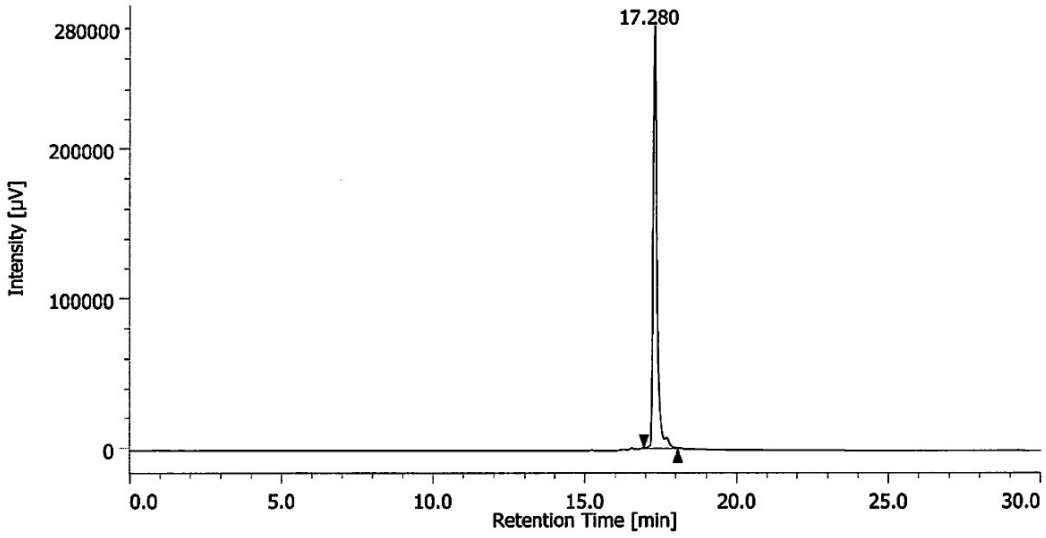


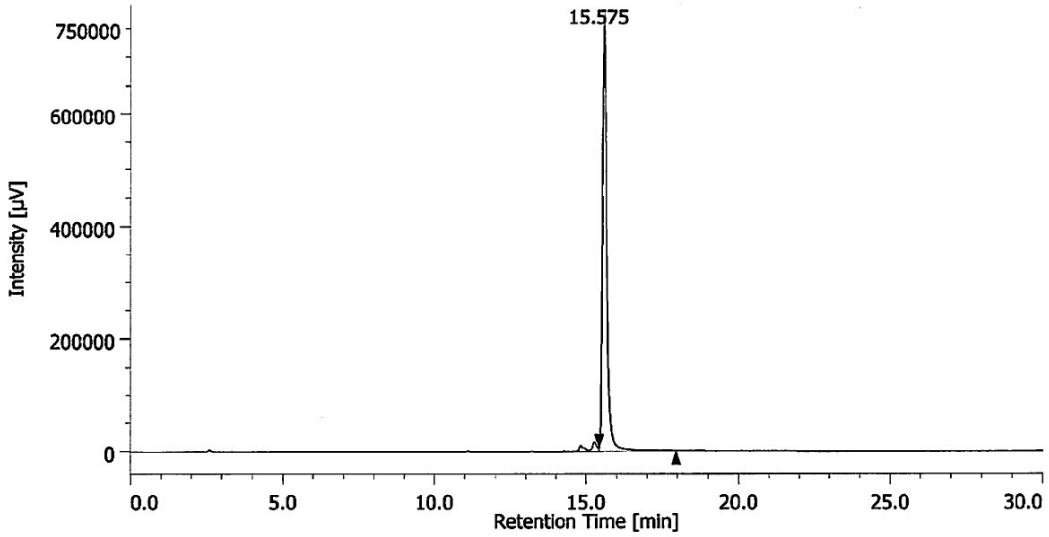
5′-d(T^{m}CT**T**^{m}C**T**T**T**T**T**^{m}C**T**^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



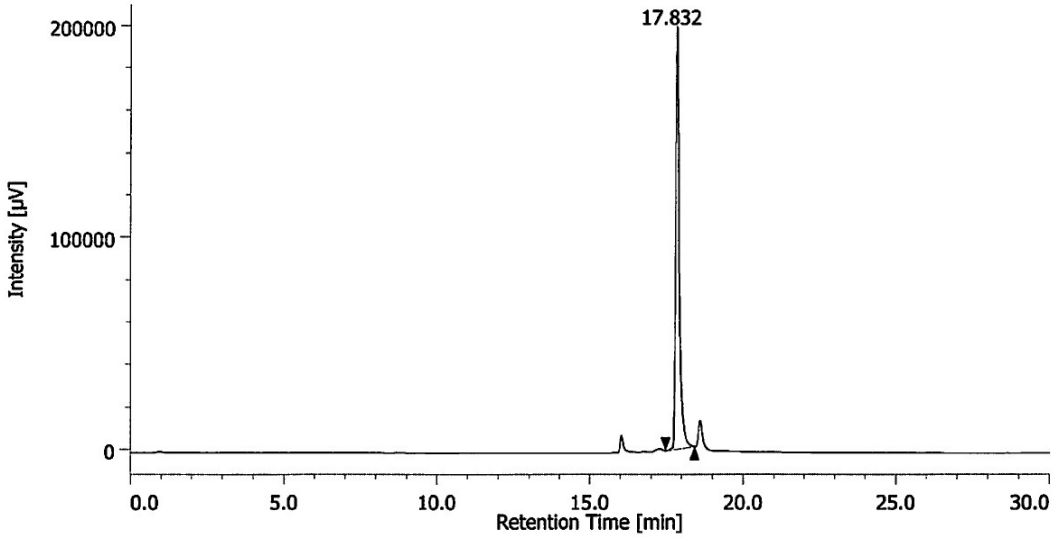
5′-d(T^{m}CTT^{m}CTT*T*TT^{m}CT^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



5′-d(T^{m}CTT^{m}CT*TTT*T^{m}CT^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



5′-d(T^{m}CTT^{m}C*T*T*T*T^{m}CT^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
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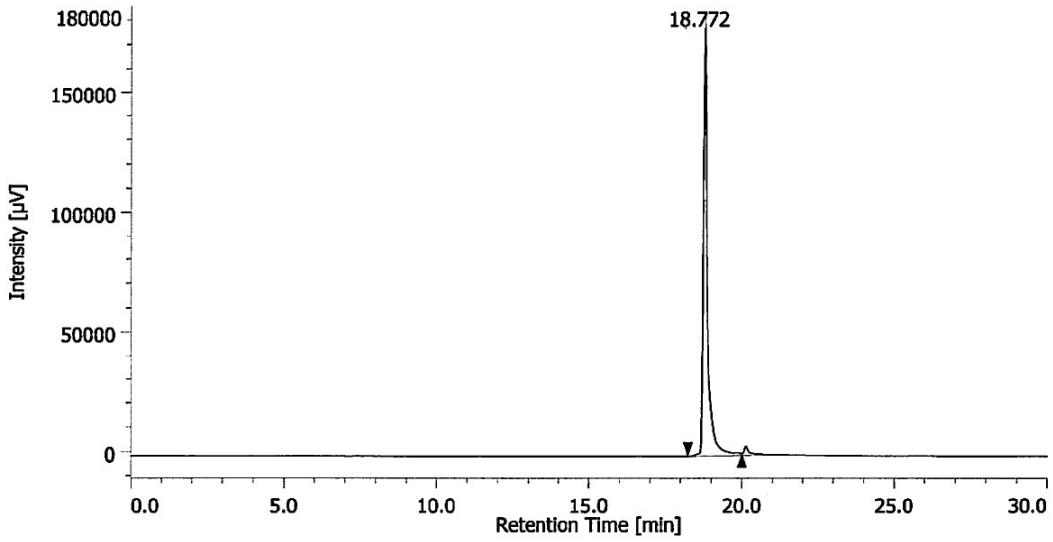

5′-d(T^{m}CT*T*^{m}C*T*T*T*T^{m}C*T*^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
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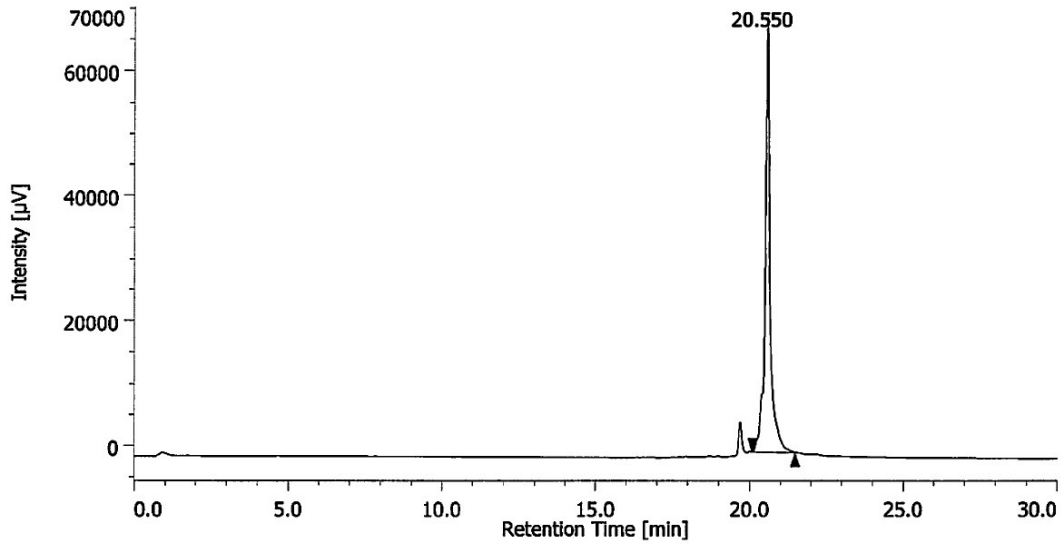
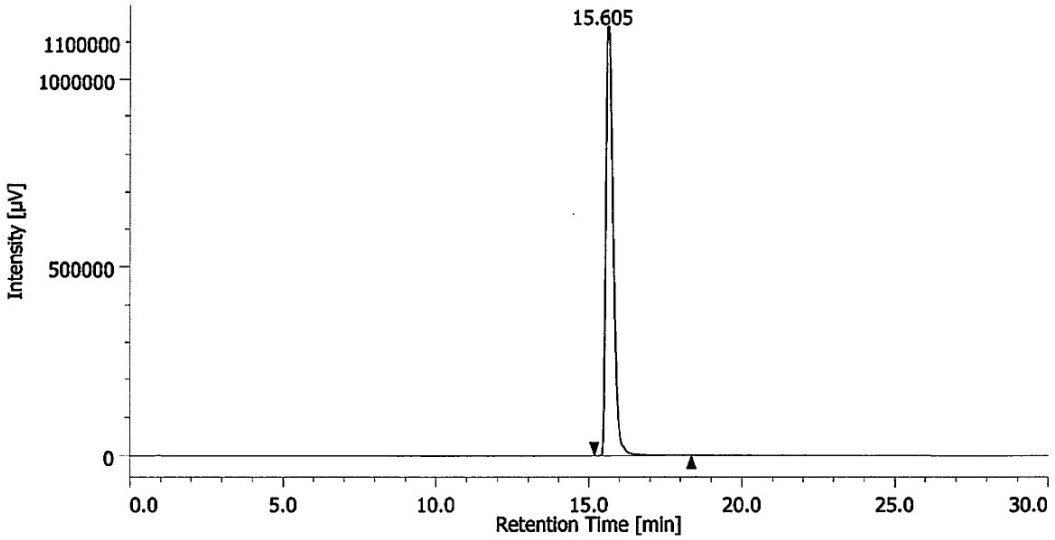


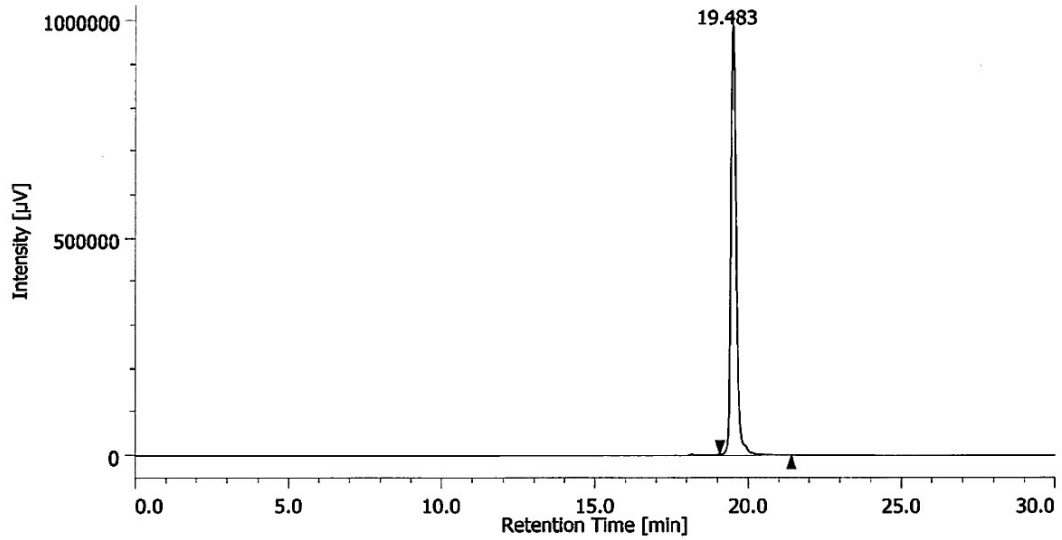
5′-d(T^{m}CTT^{m}CTT*T*TT^{m}CT^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



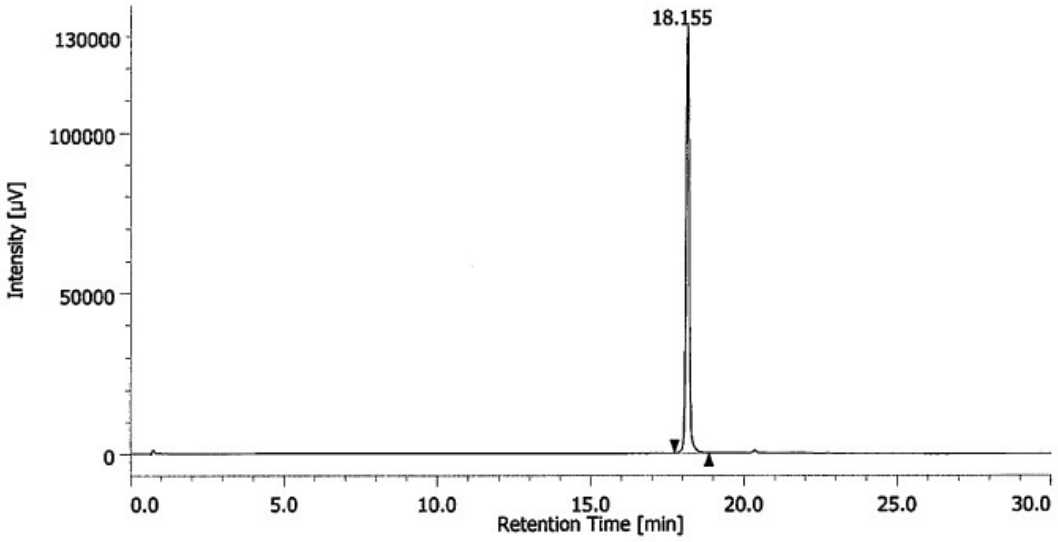
5′-d(T^{m}CTT^{m}CT*TTT*T^{m}CT^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



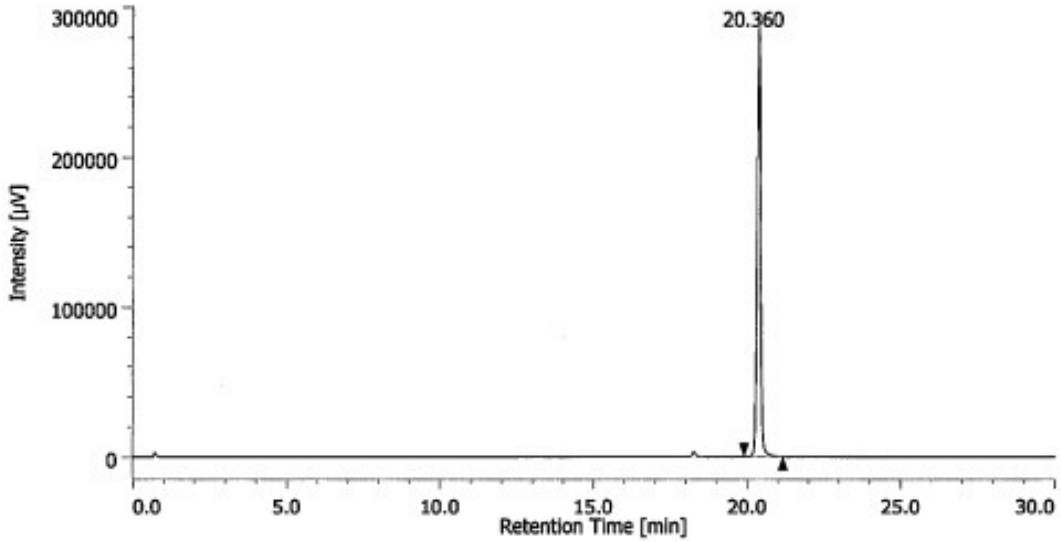
5′-d(T^{m}CTT^{m}C*T*T*T*T^{m}CT^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



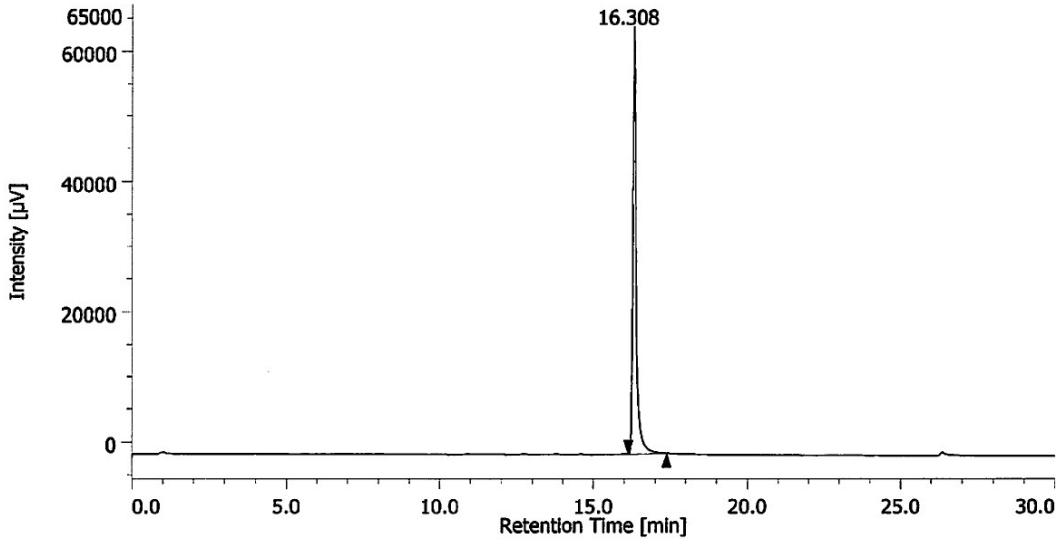
5′-d(T^{m}CT*T*^{m}C*T*T*T*T^{m}C*T*^{m}CT)-3′

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



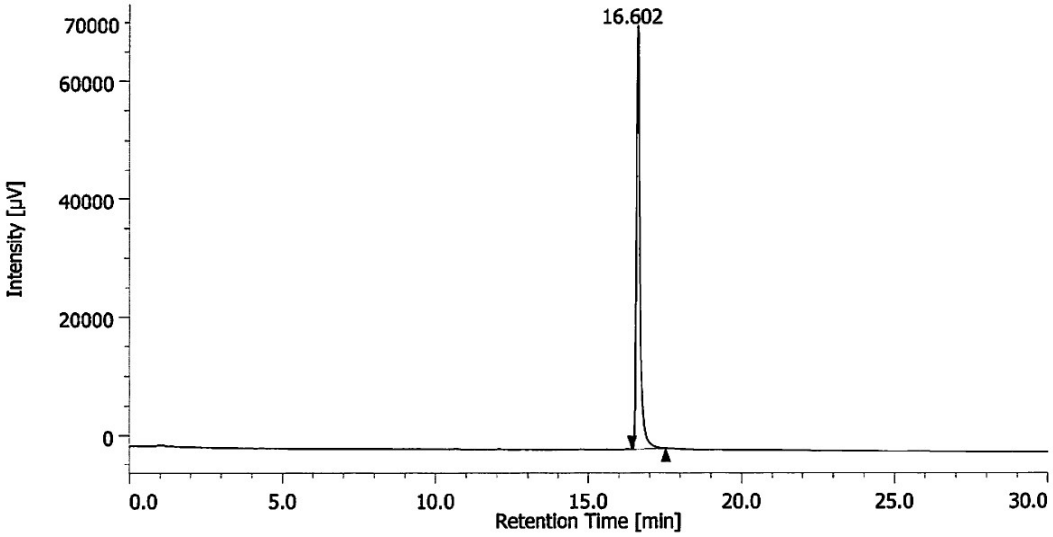
5′-TTTTTTTT**T**T-3′

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



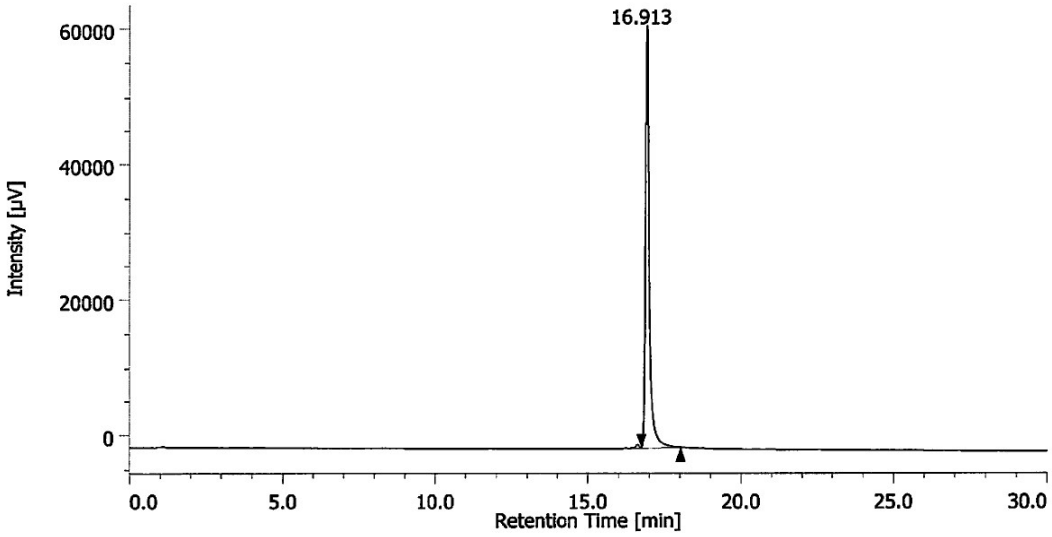
5′-TTTTTTTT*T*T-3′

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



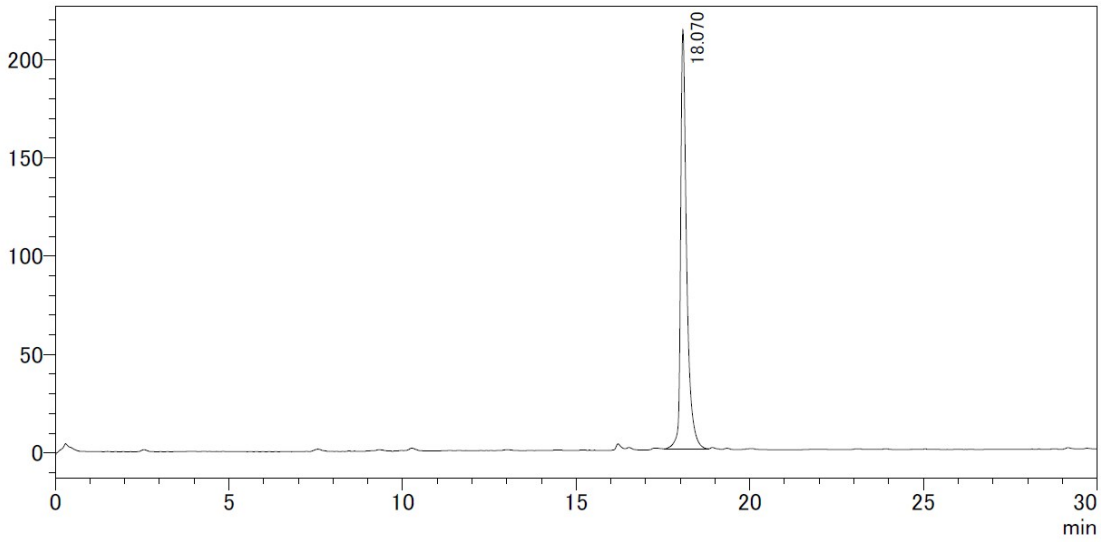
5′-TTTTTTTT*T*T-3′

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 40˚C.



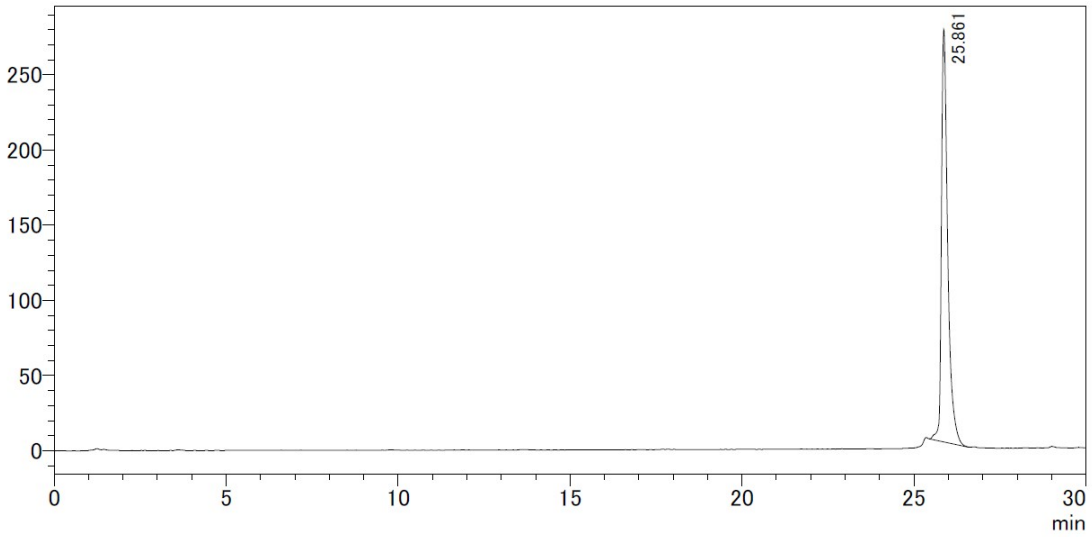
5′-d(**TT**CAGCATTGG**T**A**TT**C)-3′ (**ASO1**)

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 10-25% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 50˚C.



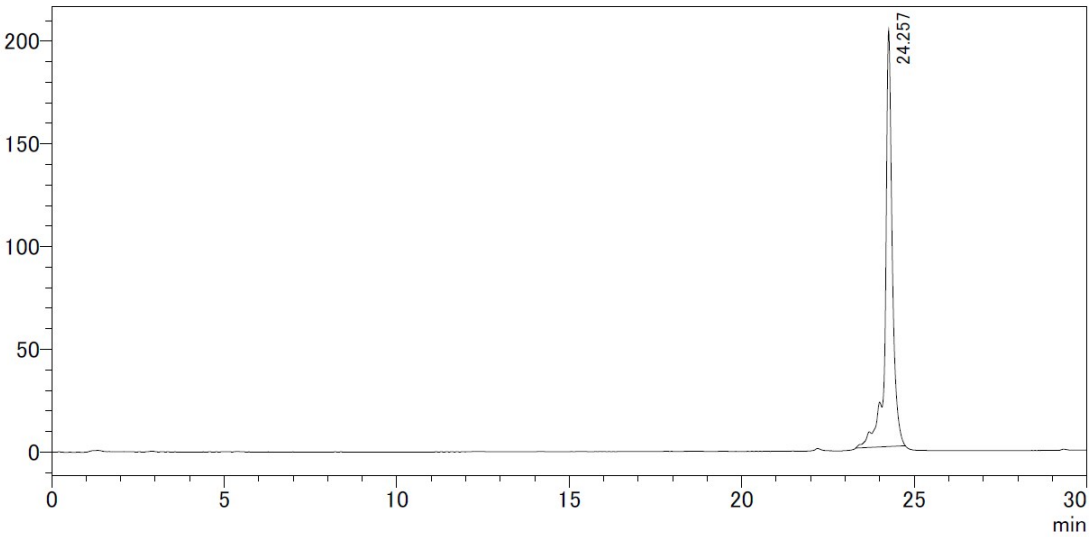
5′-d(*TT*CAGCATTGG*TA**TT***C)-3′ (**ASO2**)

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 5-20% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 50˚C.



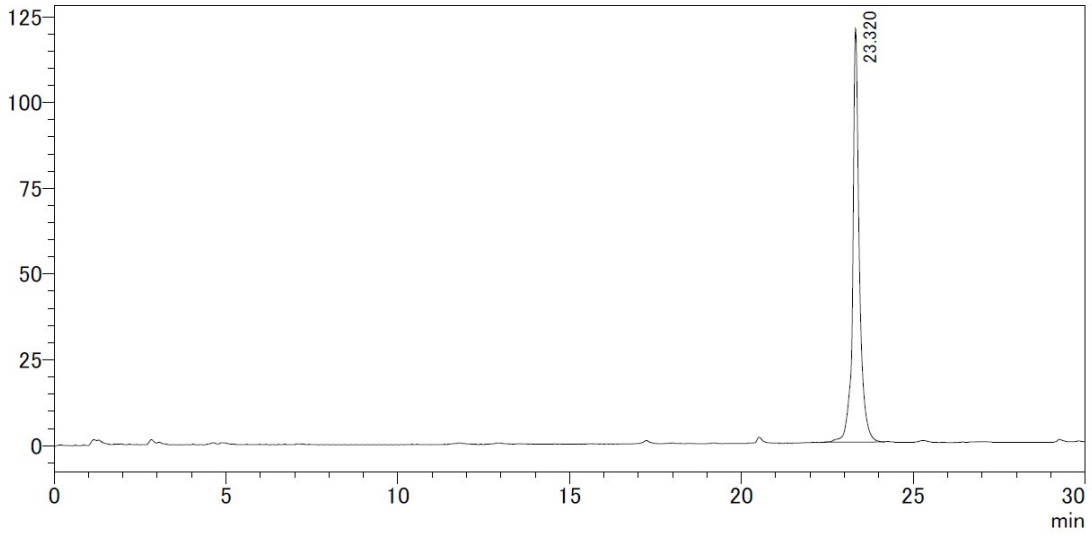
5′-d(*TT*CAGCATTGG*TA**TT***C)-3′ (**ASO3**)

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 10-25% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 50˚C.



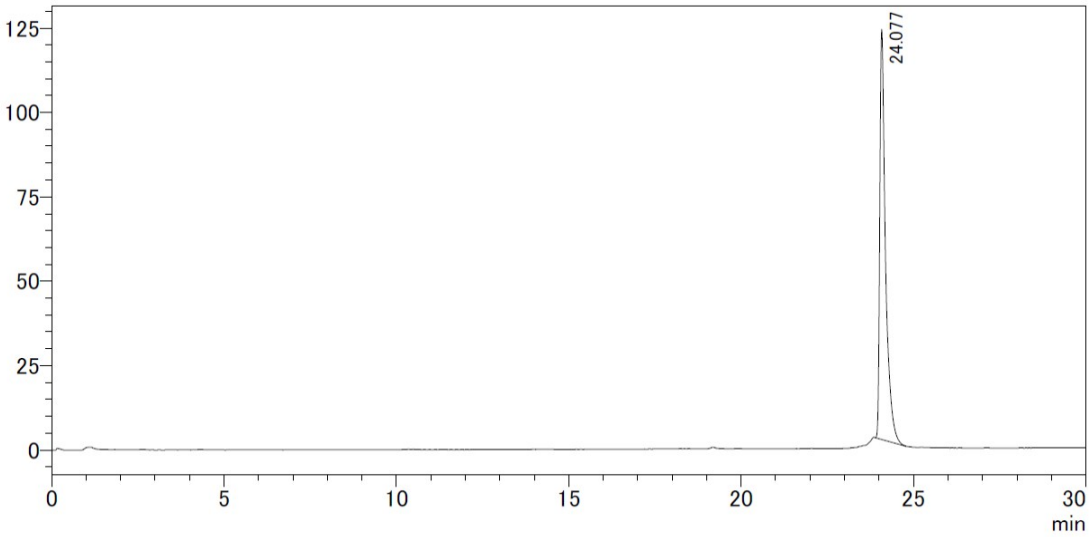
5′-d(**tt**CAGCATTGG**t**A**tt**C)-3′ (**ASO4**)

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 10-25% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 50˚C.



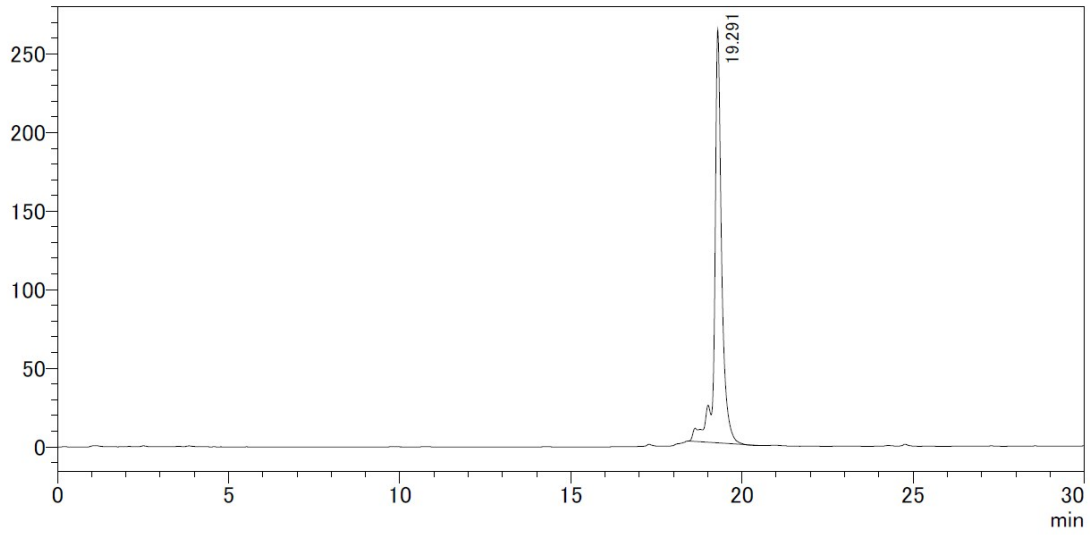
5′-d(*tt*CAGCATTGG*t*A*tt*C)-3′ (**ASO5**)

Column : Waters XBridge® MS C_{18} 2.5 µm, 4.6 × 50 mm.
Gradient : 10-25% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 50˚C.



5′-d(ttCAGCATTGGtAttC)-3′ (**ASO6**)

Column : Waters XBridge® MS C_{18} 2.5 μm, 4.6 × 50 mm.
Gradient : 15-30% MeCN in triethylammonium acetate (0.1 M, pH 7.0) buffer for 30 min.
Flow rate : 1.0 mL/min.
Column temp. : 50˚C.



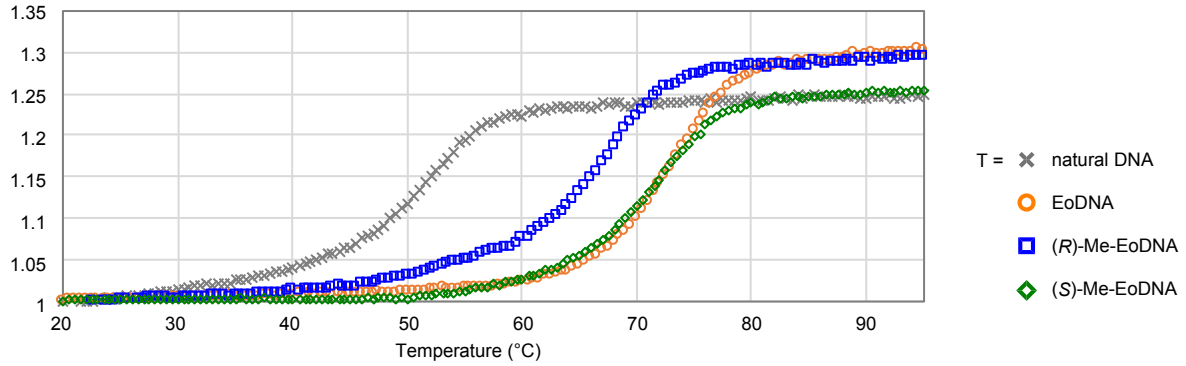
5. Representative UV-melting data

Table S2. T_m values of duplexes formed by oligonucleotides including EoDNAs with complementary ssRNA and ssDNA[a]

Oligonucleotides (5′-3′)	T_m (°C) (ΔT_m/mod.)	
	ssRNA	ssDNA
d(T^{m}CTT^{m}CTTTTT^{m}CT^{m}CT)	51	50
d(T^{m}CTT^{m}CTT**T**TT^{m}CT^{m}CT)	54 (+3.0)	47 (−3.0)
d(T^{m}CTT^{m}CT**TTT**T^{m}CT^{m}CT)	61 (+3.3)	50 (±0.0)
d(T^{m}CTT^{m}C**T**T**T**T**T**^{m}CT^{m}CT)	63 (+4.0)	49 (−0.3)
d(T^{m}CT**T**^{m}C**T**T**T**T**T**^{m}C**T**^{m}CT)	72 (+4.2)	52 (+0.4)
d(T^{m}CTT^{m}CTT*T*TT^{m}CT^{m}CT)	53 (+2.0)	45 (−5.0)
d(T^{m}CTT^{m}CT*TTT*T^{m}CT^{m}CT)	55 (+1.7)	45 (−1.7)
d(T^{m}CTT^{m}C*T*T*T*T^{m}CT^{m}CT)	60 (+3.0)	44 (−2.0)
d(T^{m}CT*T*^{m}C*T*T*T*T^{m}C*T*^{m}CT)	67 (+3.2)	47 (−0.6)
d(T^{m}CTT^{m}CTT$\underline{T}$TT^{m}CT^{m}CT)	54 (+3.0)	46 (−4.0)
d(T^{m}CTT^{m}CT$\underline{TTT}$T^{m}CT^{m}CT)	60 (+3.0)	48 (−0.7)
d(T^{m}CTT^{m}C$\underline{T}$T$\underline{T}$T$\underline{T}$^{m}CT^{m}CT)	62 (+3.7)	46 (−1.3)
d(T^{m}CT$\underline{T}$^{m}C$\underline{T}$T$\underline{T}$T$\underline{T}$^{m}C$\underline{T}$^{m}CT)	71 (+4.6)	50 (±0.0)

[a] Conditions: 10 mM sodium cacodylate buffer (pH 7.2), 140 mM KCl, and 4 μM of each oligonucleotide. ^{m}C = 2′-deoxy-5-methylcytidine, **T** = EoDNA, *T* = (*R*)-Me-EoDNA, $\underline{T}$ = (*S*)-Me-EoDNA. The sequences of target ssRNA and ssDNA are 5′-r(AGAGAAAAAGAAGA)-3′ and 5′-d(AGAGAAAAAGAAGA)-3′, respectively. ΔT_m/mod.: the change in T_m value (ΔT_m) per modification compared to the unmodified natural strand.

(A) With ssRNA



(B) With ssDNA

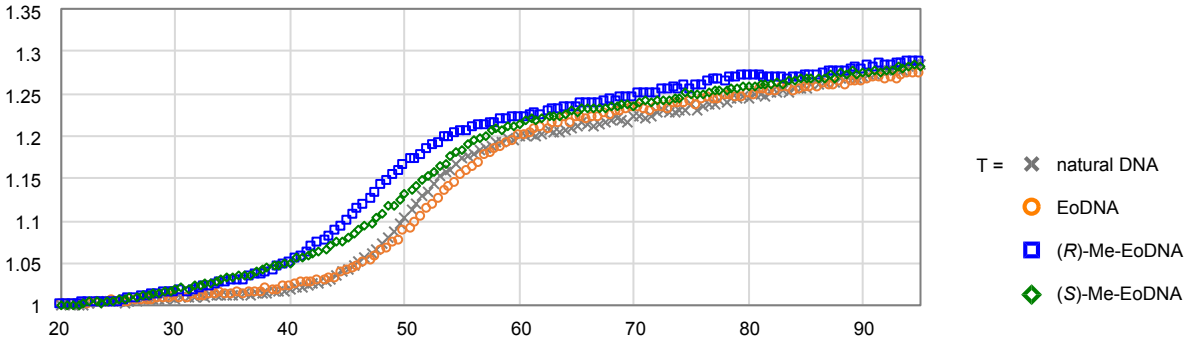


Fig. S5. UV-melting data of duplex formed by oligonucleotides [5′-d(T^{m}CTT^{m}CTTTTT^{m}CT^{m}CT)-3′] with ssRNA (A) and with ssDNA (B).

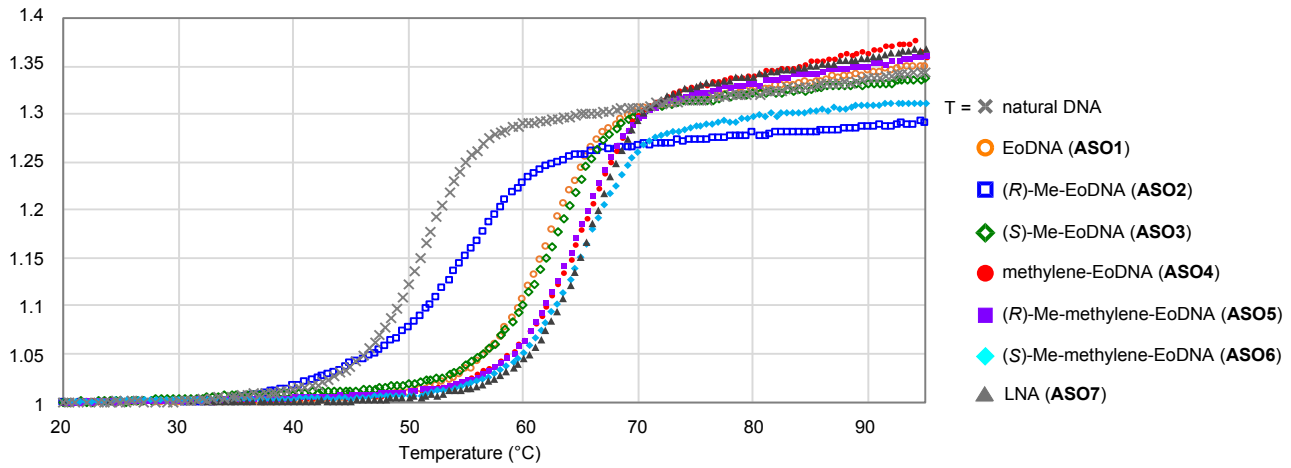


Fig. S6. UV-melting data of duplex formed by ASOs with ssRNA shown in Table 1.

6. Conformational analysis of (*R*)-Me-EoDNA and (*S*)-Me-EoDNA

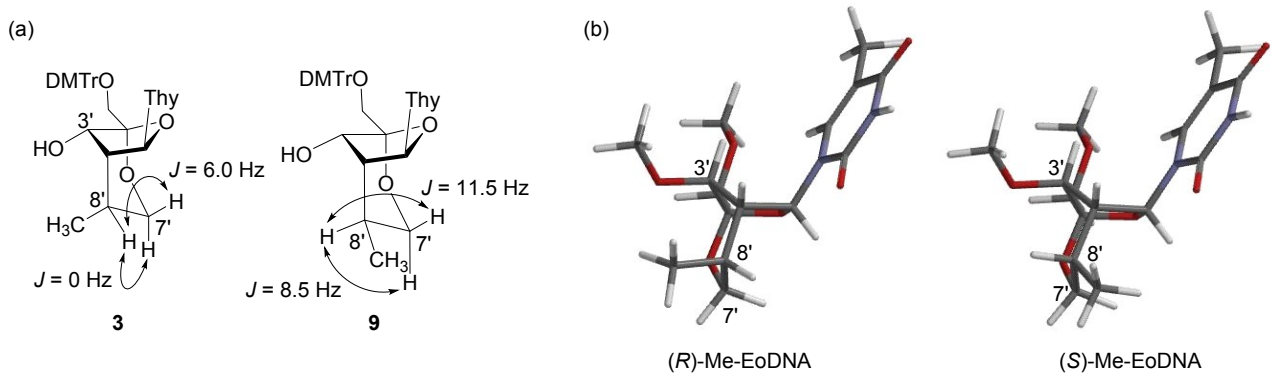


Fig. S7. (a) Coupling constants between H7′ and H8′ in ^{1}H NMR spectra of (*R*)-Me-EoDNA **3** and (*S*)-Me-EoDNA **9**. (b) Optimized structures of 3′-*O*,5′-*O*-bismethyl analogues of (*R*)-Me-EoDNA and (*S*)-Me-EoDNA (HF/6-31G*).