Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
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Supplementary Information

Effect of an *N*-substituent in sulfonamide-bridged nucleic acid (SuNA) on hybridization ability and duplex structure

Yasunori Mitsuoka,[a,b] Hiroshi Aoyama,[a] Akira Kugimiya,[b] Yuko Fujimura,[b] Tsuyoshi Yamamoto,[a] Reiko Waki,[a] Fumito Wada,[a] Saori Tahara,[a] Motoki Sawamura,[a] Mio Noda,[a] Yoshiyuki Hari[a,c] and Satoshi Obika*[,a]

[a] Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan

[b] Discovery Research Laboratory for Innovative Frontier Medicines, Shionogi & Co., Ltd., 3-1-1 Futaba-cho, Toyonaka, Osaka 561-0825, Japan

[c] Faculty of Pharmaceutical Sciences, Tokushima Bunri University, Nishihama, Yamashiro-cho, Tokushima 770-8514, Janan

* Email: obika@phs.osaka-u.ac.jp; Tel: +81 6 6879 8200; Fax: +81 6 6879 8204

Contents

1. X-ray structures of SuNA[NR]-T monomers

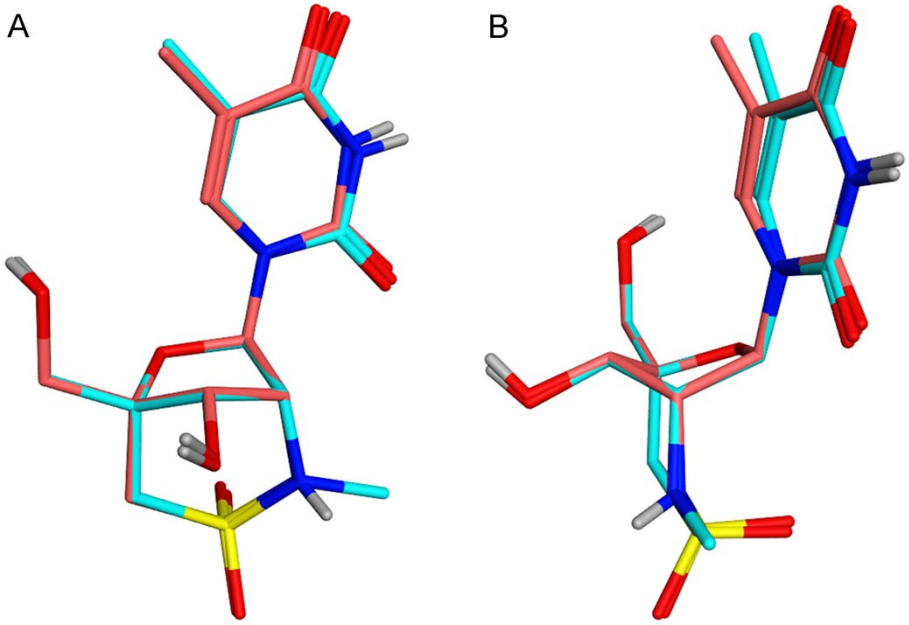


Fig. SI-1. Superposition of SuNA[NMe]-T (**35**) (light blue) and SuNA[NH]-T (**9**) (pink). Carbons are colored in light blue (SuNA[NMe]-T) and pink (SuNA[NH]-T). Oxygen, nitrogen, sulfer and hydrogen atoms are colored in red, blue, yellow, and white, respectively.

2. ESI-TOF mass data of oligonucleotides

Table SI-1. Sequences and ESI-TOF-MS data of oligonucleotides **17–23**

Oligonucleotides		ESI-TOF-MS	
		Calcd. $(M-H)^-$	Found $(M-H)^-$
5'-d(GCGTTt TTTGCT)-3'	(**17**)	3721.58	3722.45
5'-d(GCGTTt Tt TGCT)-3'	(**18**)	3812.56	3813.43
5'-d(GCGt Tt Tt TGCT)-3'	(**19**)	3903.53	3904.43
5'-d(GCGTTt t t TGCT)-3'	(**20**)	3903.53	3904.43
5'-d(GCGTA<u>T</u>ACGC)-3'	(**21**)	3130.53	3130.80
5'-d(GCGTAt ACGC)-3'	(**22**)	3116.51	3116.74
5'-d(TTTTTTTTt T)-3'	(**23**)	3068.47	3069.18

t = SuNA[NH]-T, <u>T</u> = SuNA[NMe]-T

3. Melting profiles

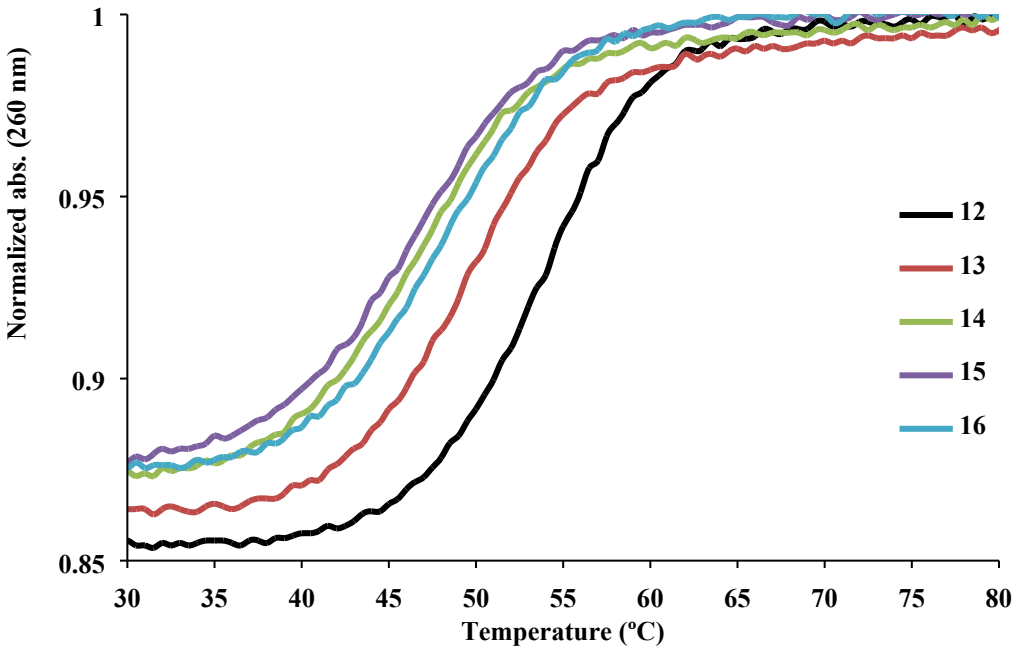


Fig. SI-2. UV melting curves (T_m curves) for the duplexes formed by natural oligonucleotide **12** and SuNA[NMe]–modified oligonucleotides **13**, **14**, **15** and **16** respectively, against the DNA complement (5'-AGCAAAAAACGC-3').

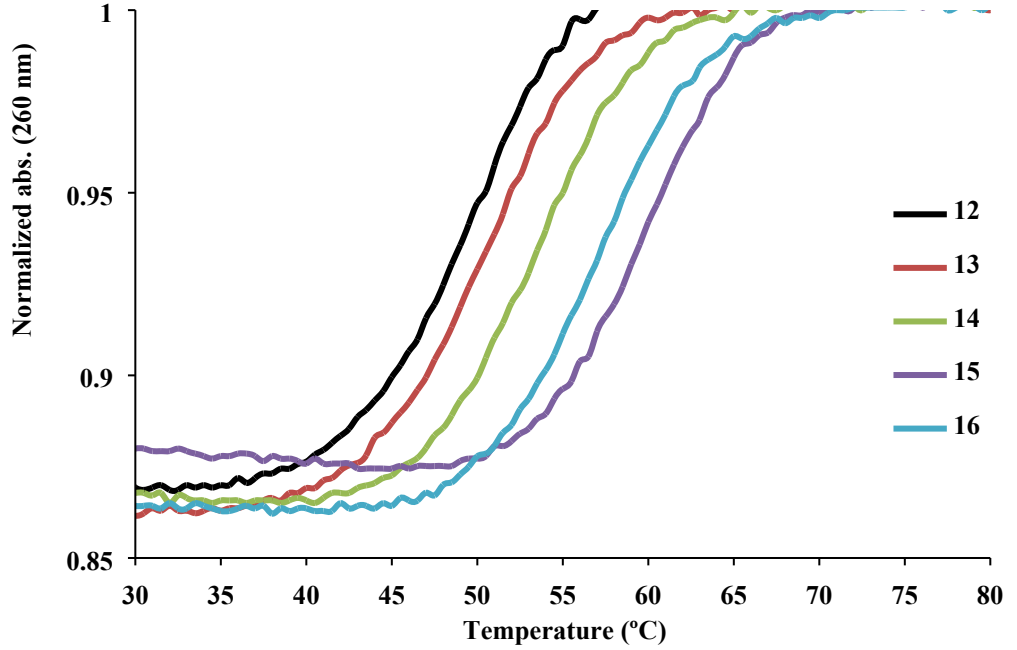


Fig. SI-3. UV melting curves (T_m curves) for the duplexes formed by natural oligonucleotide **12** and SuNA[NMe]–modified oligonucleotides **13**, **14**, **15** and **16** respectively, against the RNA complement (5'-AGCAAAAAACGC-3').

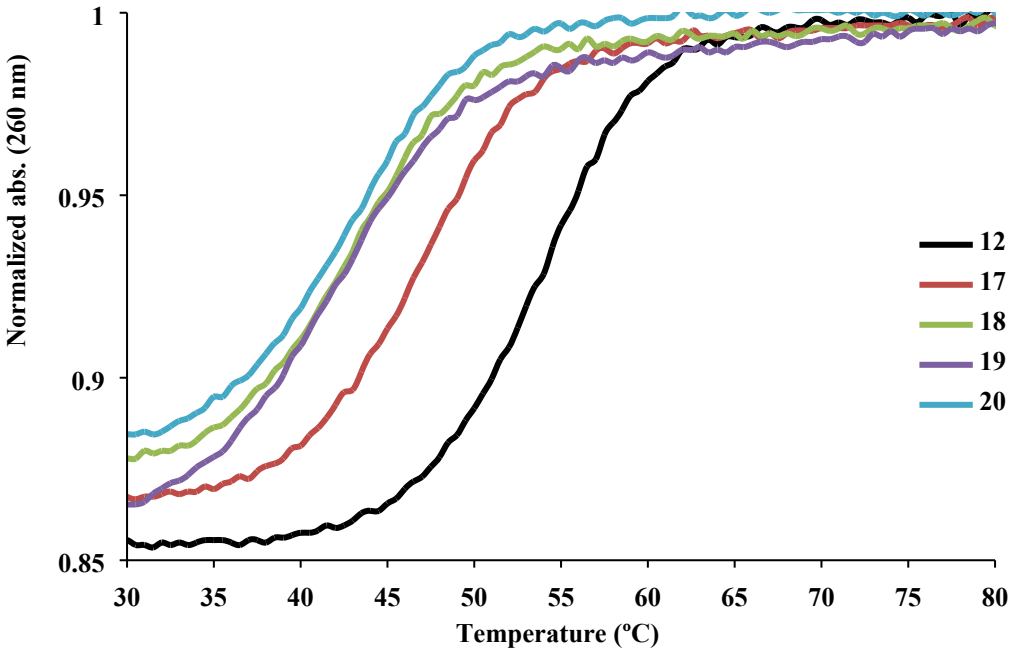


Fig. SI-4. UV melting curves (T_m curves) for the duplexes formed by natural oligonucleotide **12** and SuNA[NH]–modified oligonucleotides **17**, **18**, **19** and **20** respectively, against the DNA complement (5'-AGCAAAAAACGC-3').

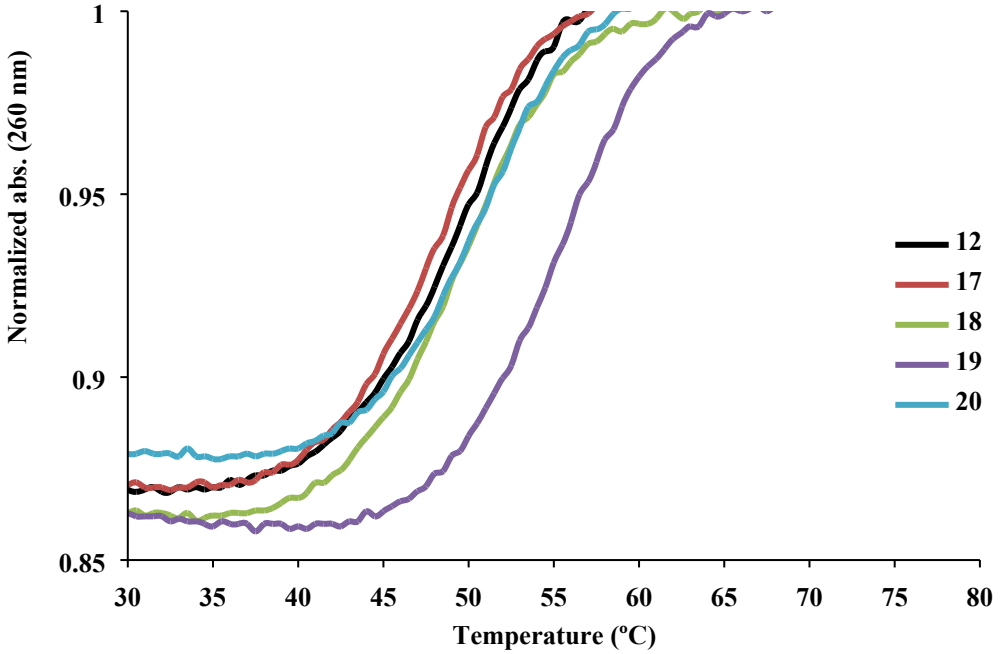


Fig. SI-5. UV melting curves (T_m curves) for the duplexes formed by natural oligonucleotide **12** and SuNA[NH]–modified oligonucleotides **17**, **18**, **19** and **20** respectively, against the RNA complement (5'-AGCAAAAAACGC-3').

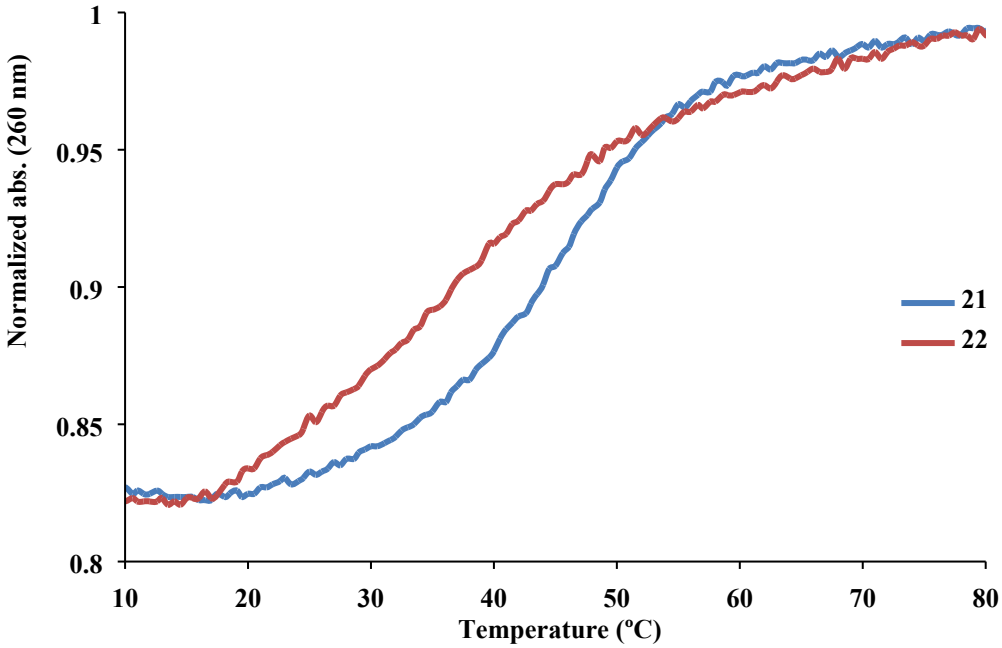


Fig. SI-6. UV melting curves (T_m curves) for the duplexes formed by SuNA[NMe]–modified oligonucleotide **21** and SuNA[NH]–modified oligonucleotide **22**.

4. Nuclease stability of SuNA-modified oligonucleotides

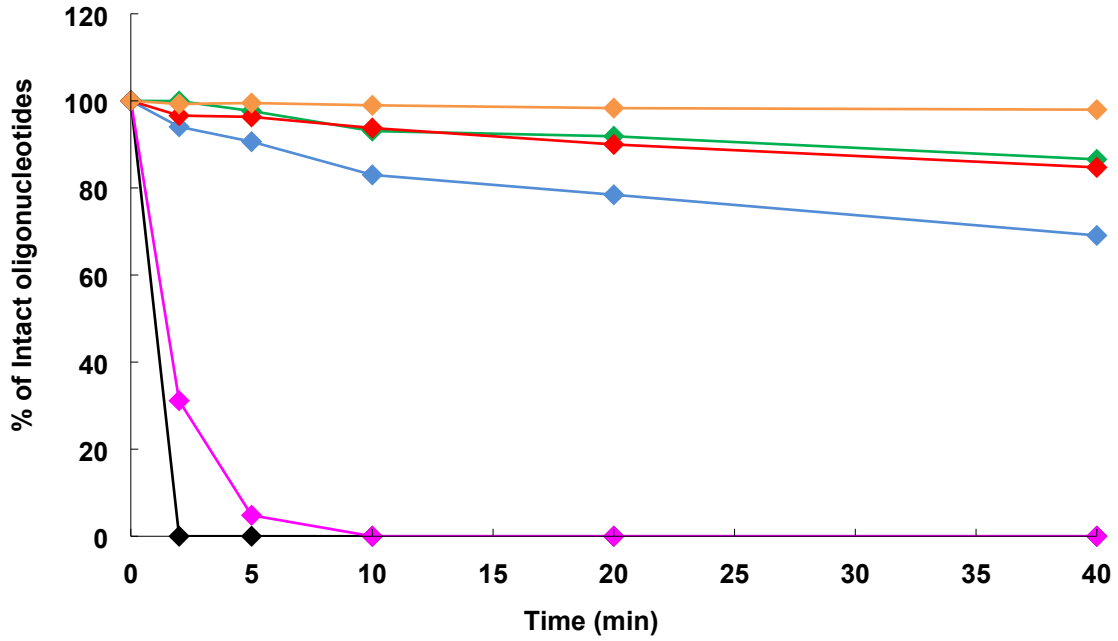


Fig. SI-7. Hydrolysis of oligonucleotides (750 pmol) conducted at 37 °C in buffer (100 µL) containing 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, and phosphodiesterase I (4.0 µg/mL). Sequences: 5'-(TTTTTTTT**T**T)-3', **T** = natural (black, **24**), 2',4'-BNA/LNA (pink, **25**), 2',4'-BNA^{NC}[NMe] [1] (blue, **26**), 2',4'-BNA^{COC} [2] (green, **27**), SuNA[NMe] [3] (red, **28**), SuNA[NH] (orange, **23**).

5. Crystallization and X-ray structures of DNA duplexes with SuNA[NR] residues

Table SI-2. Selected crystal data and refinement parameters

Oligonucleotide Sequence	5'-d(GCGTA<u>T</u>ACGC)-3' **21**	5'-d(GCGTAtACGC)-3' **22**
Modified nucleotide	<u>T</u> = **SuNA[NMe]**	t = **SuNA[NH]**
Space group	Orthorhombic $P2_12_12_1$	
Unit cell constants [Å] $\alpha=\beta=\gamma=90°$	a=24.42 b=45.12 c=45.45	a=23.68 b=42.90 c=45.20
Resolution [Å]	1.13	0.95
Outer shell [Å]	1.15-1.13	0.97-0.95
Number of unique reflections	19,277	29,154
Completeness (outer shell) [%]	98.6 (98.3)	98.8 (97.1)
R-merge (outer shell) [%]	0.061 (0.378)	0.059 (0.241)
R-work [%]	0.131	0.125
R-free [%]	0.145	0.144
No. of DNA atoms	498	654
No. of waters	131	140
R.m.s.d. bonds [Å]	0.018	0.010
R.m.s.d. angles [°]	2.1	1.6
Avg. B-factor, DNA atoms [Å^2]	10.1	6.4
Avg. B-factor, solvent [Å^2]	28.5	26.1
PDB ID code	5AXF	5AXE

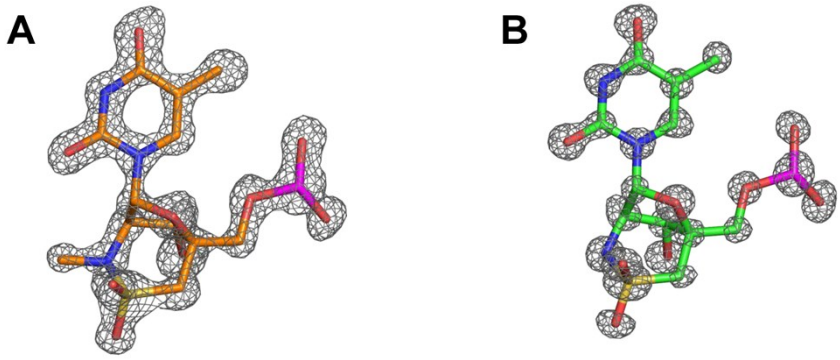


Fig. SI-8. Quality of the final structures. Simulating annealing omit electron density maps around (**A**) SuNA[NMe] and (**B**) SuNA[NH], contoured at 3 σ level (**A**) and 4 σ level (**B**), respectively. Carbons are colored in orange (SuNA[NMe] duplex) and green (SuNA[NH] duplex). Oxygen, nitrogen, sulfur and phosphorus atoms are colored in red, blue, yellow, and pink, respectively. These electron density maps clearly show the structure of SuNA[NMe] and SuNA[NH].

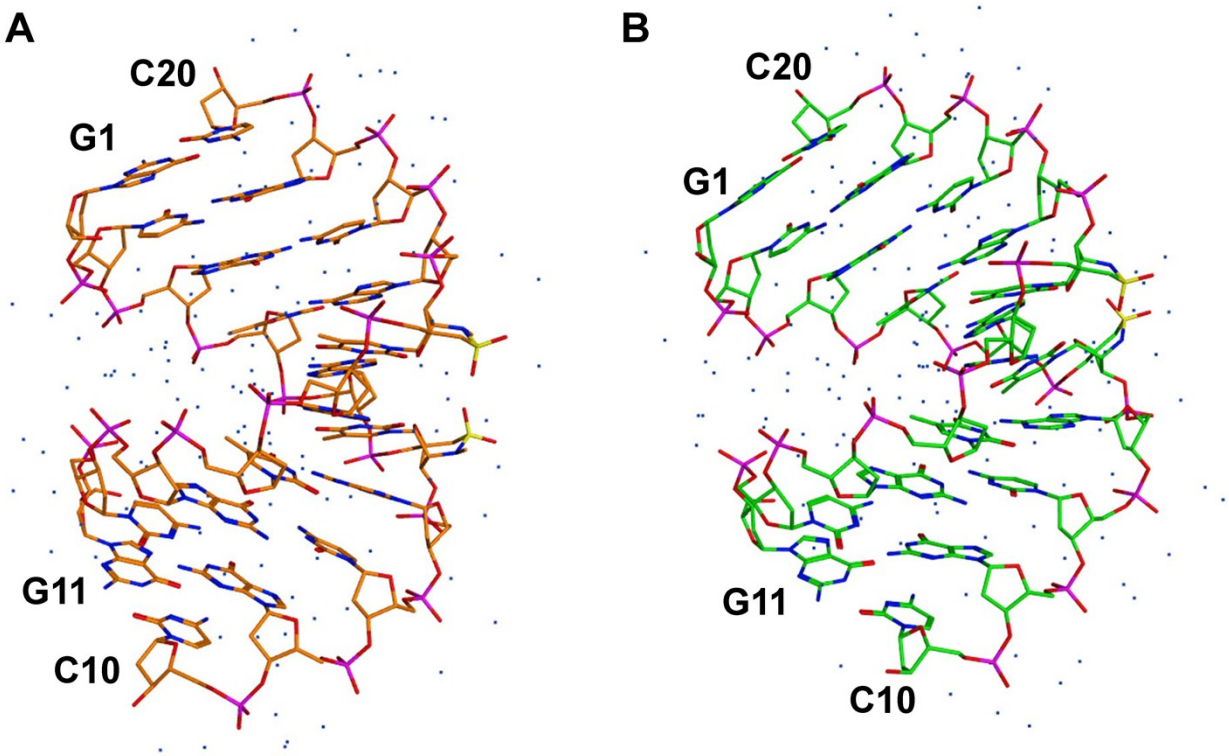
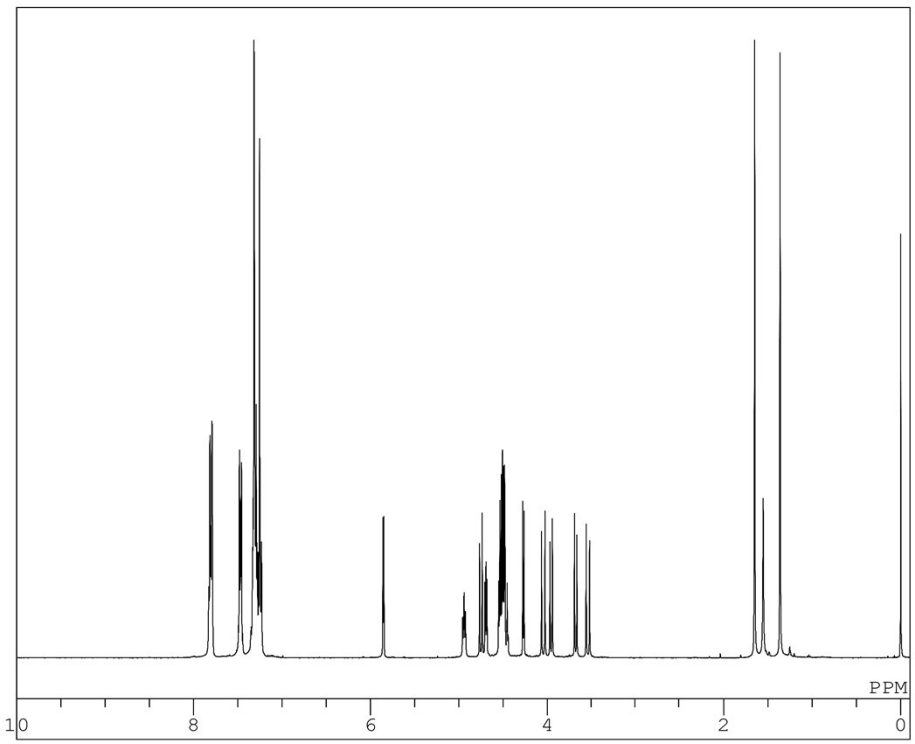


Fig. SI-9. Examples of the overall structures of SuNA[NMe] and SuNA[NH] duplexes. Overall structure of (**A**) the SuNA[NMe] duplex, and (**B**) SuNA[NH] duplex. The view is across the grooves. Carbons are colored in orange (SuNA[NMe] duplex) and green (SuNA[NH] duplex). Oxygen, nitrogen, sulfer and phosphorus atoms are colored in red, blue, yellow, and pink, respectively. Water molecules are cyan spheres.

6. ^{1}H, ^{13}C and ^{31}P spectra for the new compounds

^{1}H & ^{13}C NMR Spectra of compound (**2**)

^{1}H

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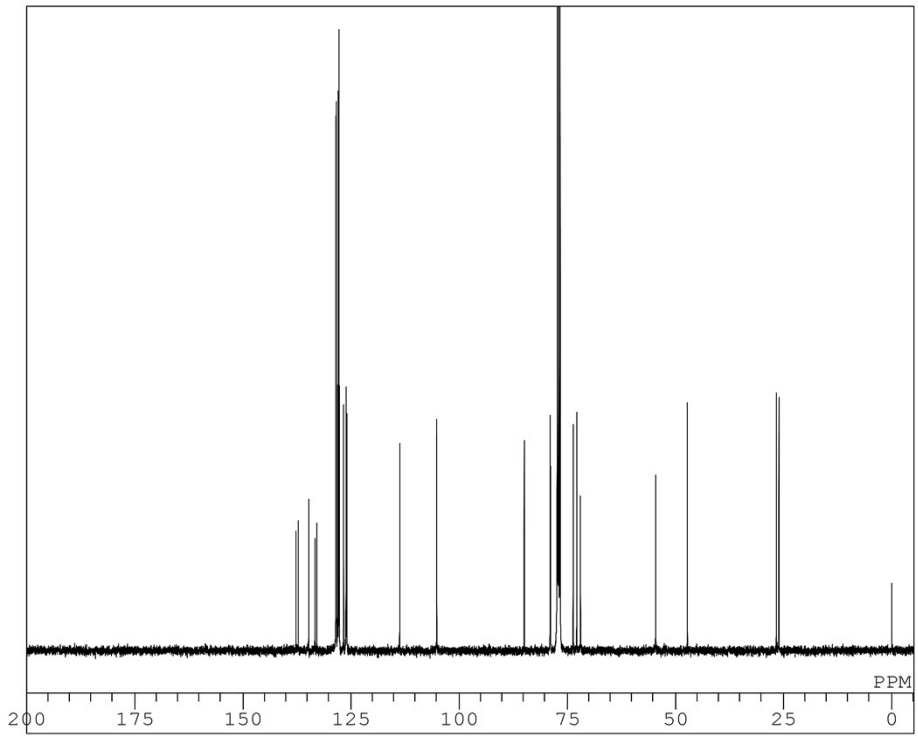
^{13}C

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IRNUC
CTEMP            31.2 c
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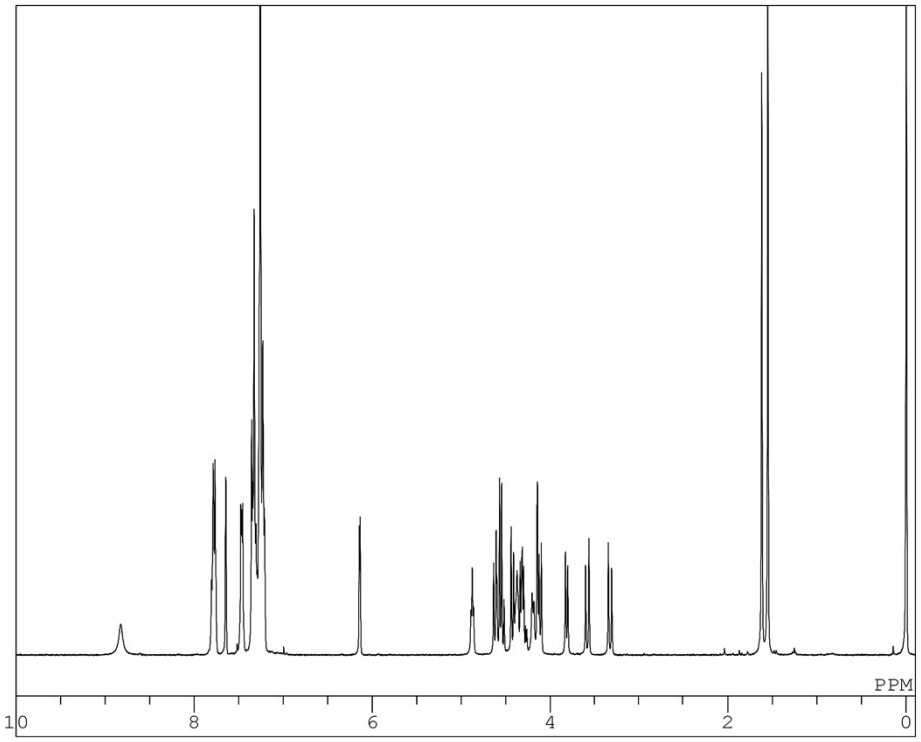
```

^{1}H & ^{13}C NMR Spectra of compound (**7**)

^{1}H

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RGAIN             512
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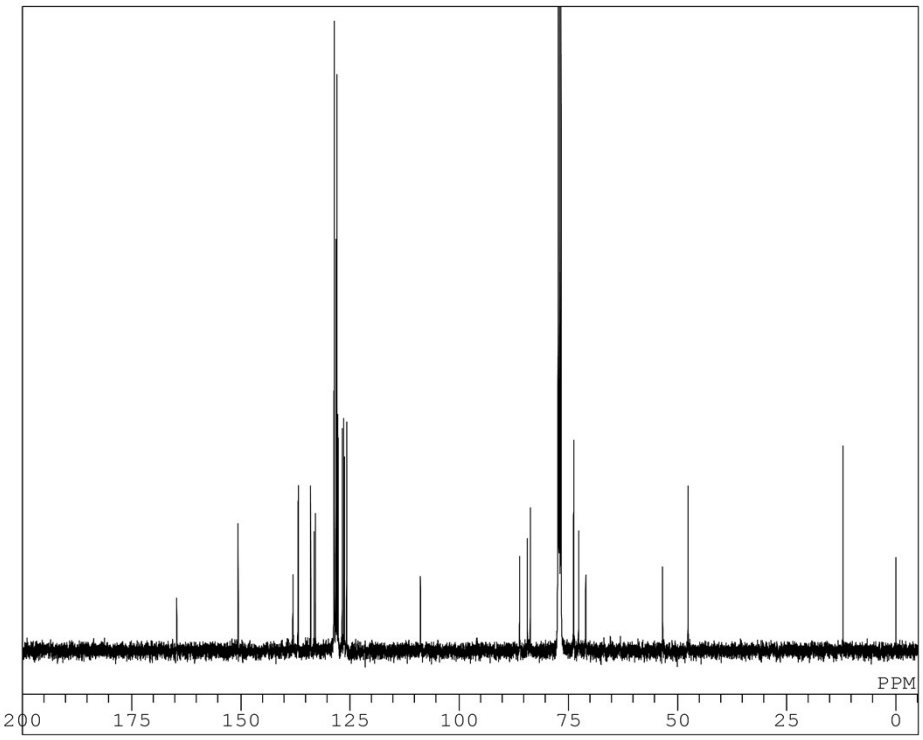
^{13}C

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PD             2.0000 sec
PW1             10.00 usec
IRNUC
CTEMP            30.8 c
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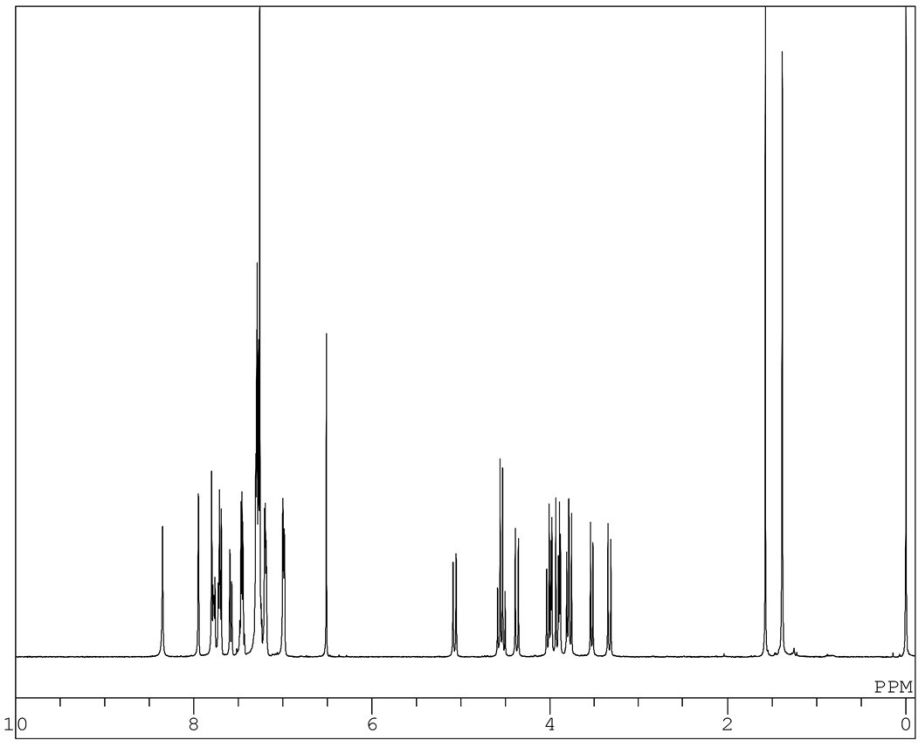
RGAIN             202
```

^{1}H & ^{13}C NMR Spectra of compound (**8**)

^{1}H

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PD            1.0000 sec
PW1            10.00 usec
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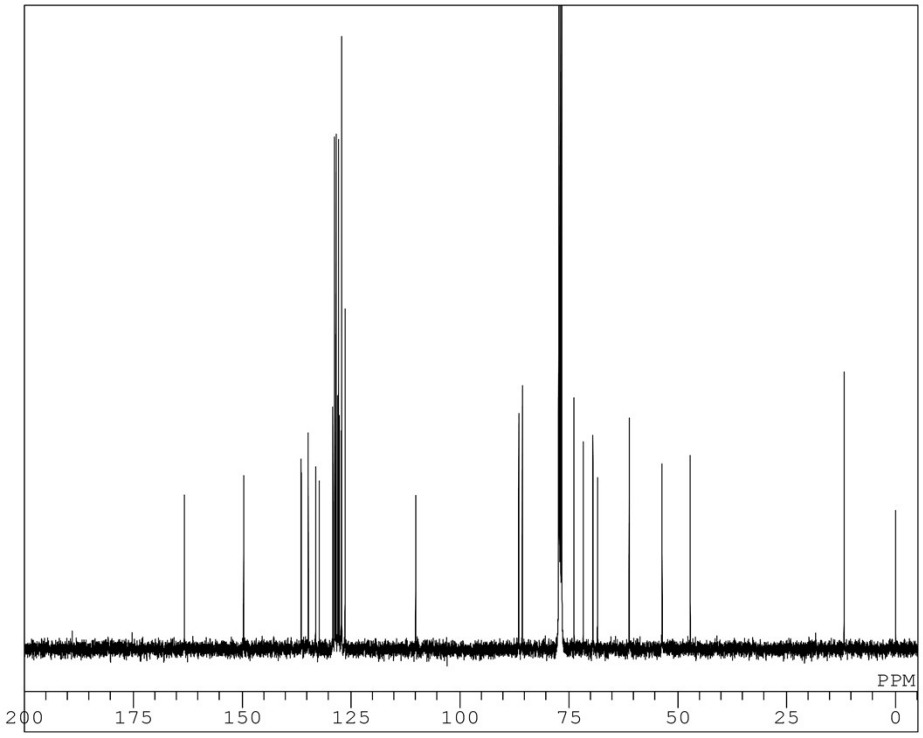
CTEMP           26.7 c
SLVNT  CDCl3
EXREF          16.43 ppm
BF              0.30 Hz
RGAIN             81
```

^{13}C

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FREQU      24038.46 Hz
SCANS          9000
ACQTM         1.3632 sec
PD            2.0000 sec
PW1            10.00 usec
IRNUC
CTEMP           31.4 c
SLVNT  CDCl3
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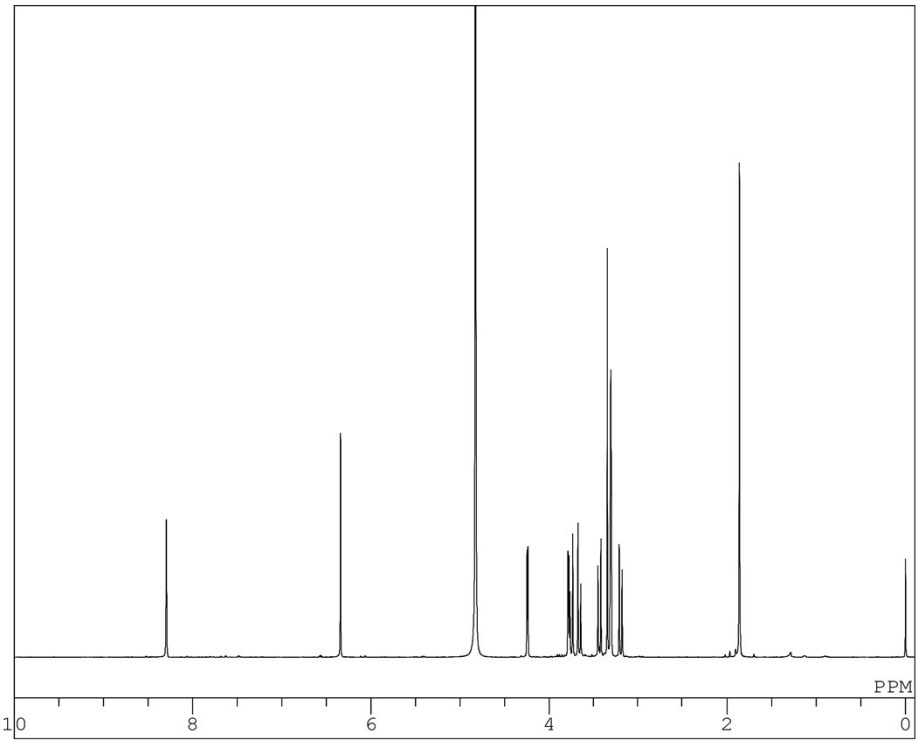
BF              1.00 Hz
RGAIN            202
```

^{1}H & ^{13}C NMR Spectra of compound (**9**)

^{1}H

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RGAIN             81
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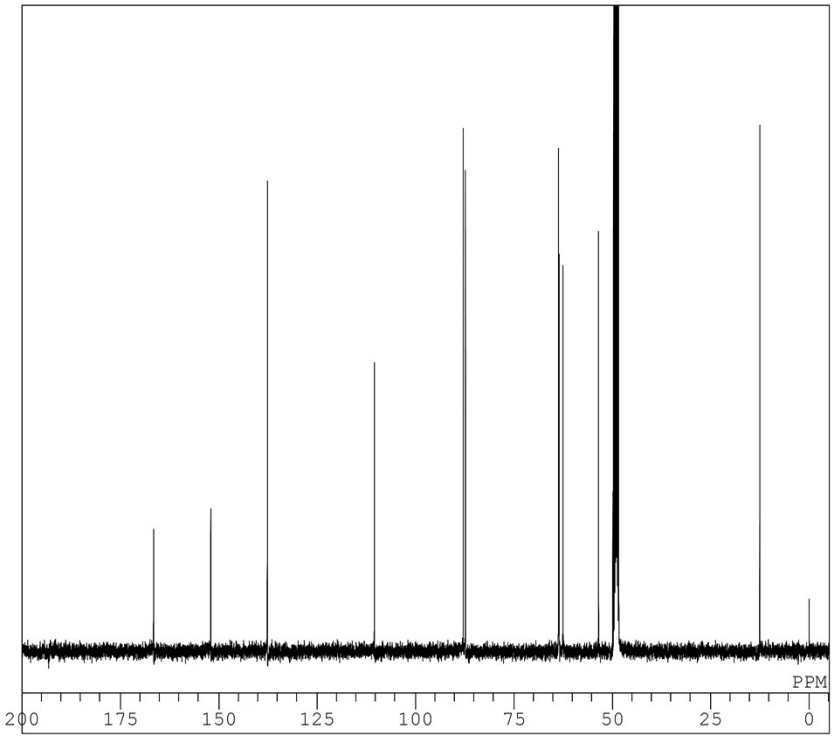
^{13}C

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OBFIN          9.33 Hz
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FREQU      24038.46 Hz
SCANS          9000
ACQTM        1.3632 sec
PD           2.0000 sec
PW1           10.00 usec
IRNUC
CTEMP           32.1 c
SLVNT  MeOD
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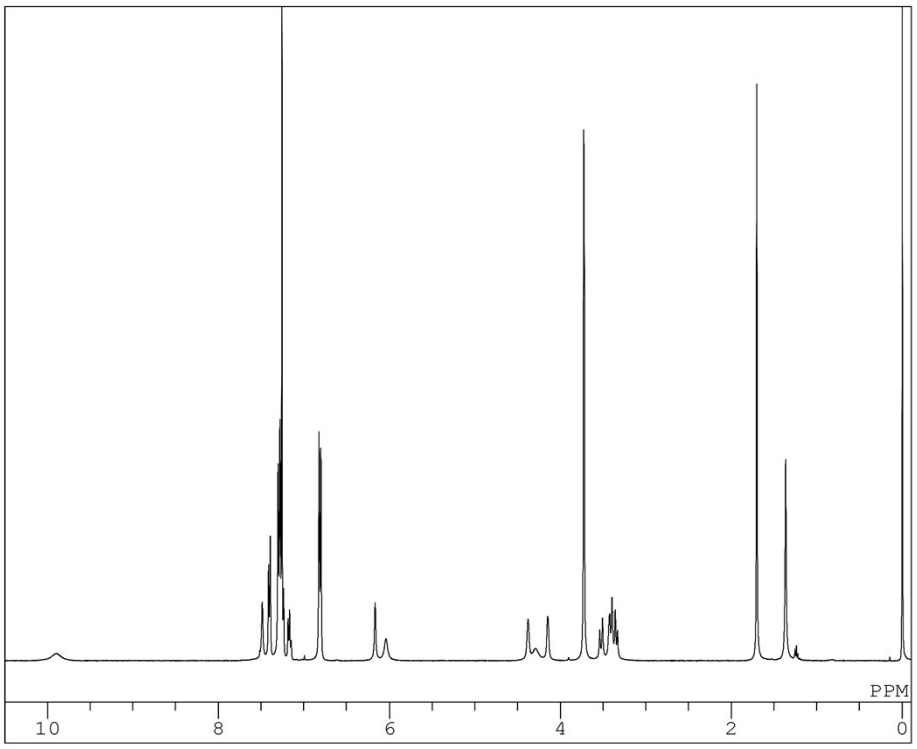
RGAIN            202
```

^{1}H & ^{13}C NMR Spectra of compound (**10**)

^{1}H

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OBFIN          0.97 Hz
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FREQU       8223.68 Hz
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PD            1.0000 sec
PW1            10.00 usec
IRNUC
CTEMP           28.0 c
SLVNT  CDCl3
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RGAIN             89
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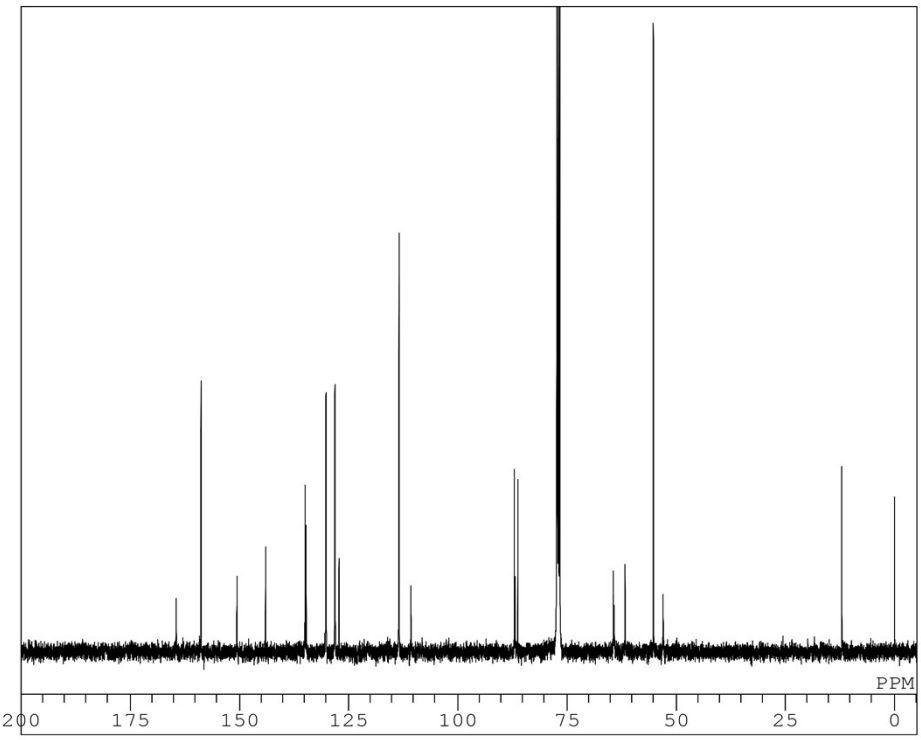
^{13}C

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PD            2.0000 sec
PW1            10.00 usec
IRNUC
CTEMP           32.5 c
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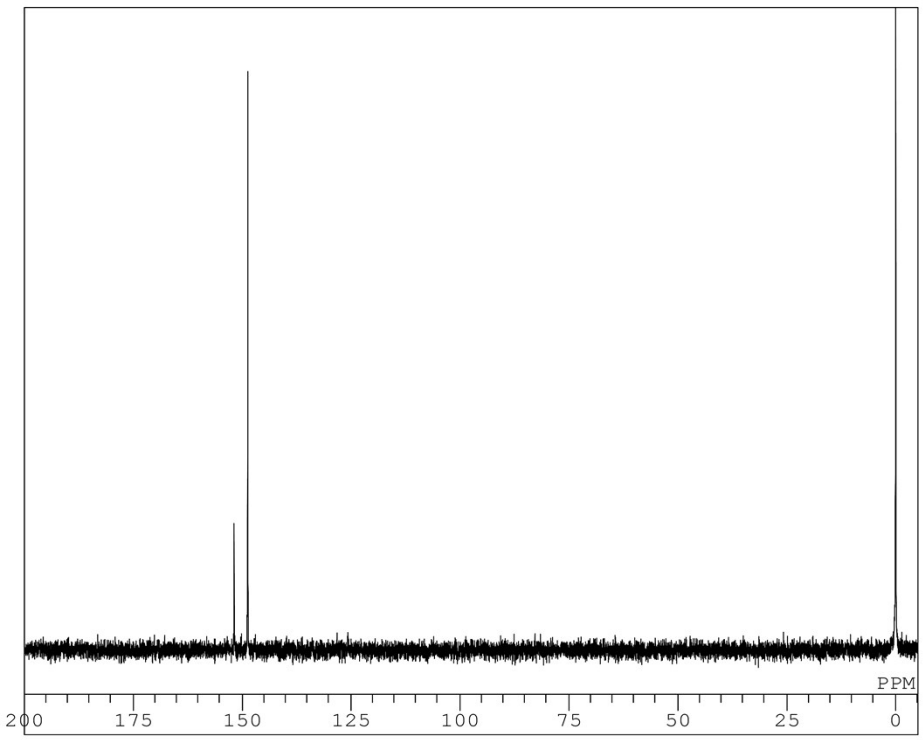
RGAIN            202
```

^{31}P NMR Spectra of compound (**11**)

^{31}**P**

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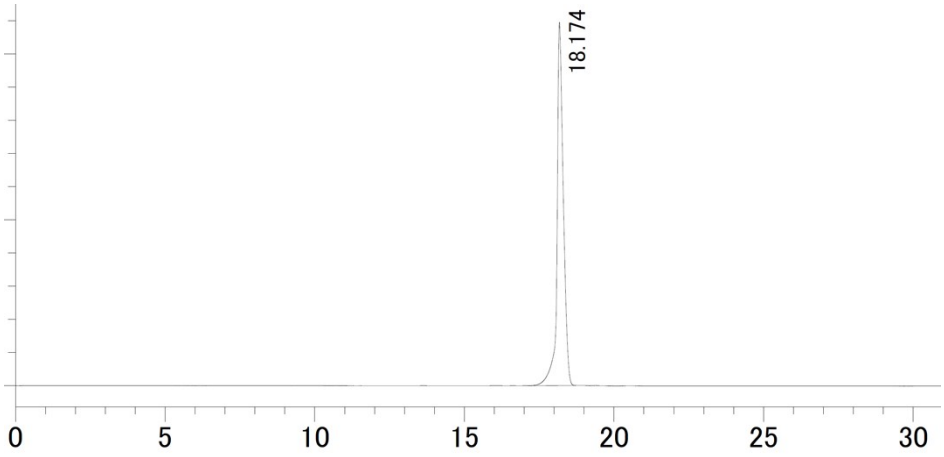
ACQTM        0.4020 sec
PD           2.0000 sec
PW1            8.00 usec
IRNUC
CTEMP          28.6 c
SLVNT  CDCl3
EXREF          0.00 ppm
BF             1.00 Hz
RGAIN           202
```

7. HPLC and ESI-TOF-Mass spectra for the new oligonucleotides

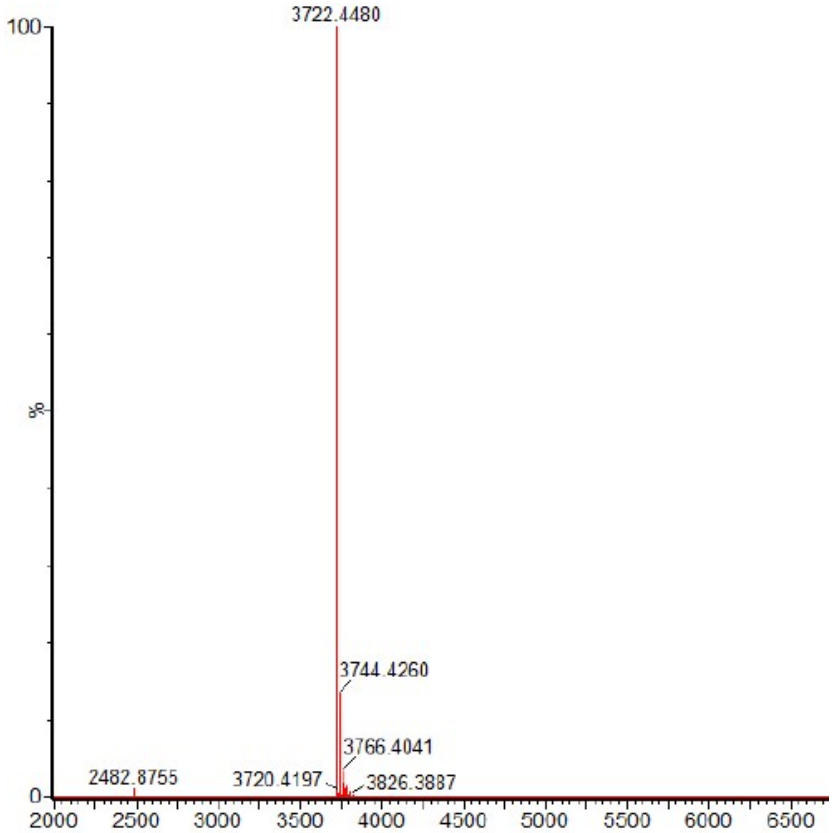
Oligonucleotide **17**

HPLC

A : 10mM TEAA
B : 10mM TEAA / MeCN = 1 / 1
Column : YMC Hydrosphere C18 Column (5.0 μm, 4.6 x 100 mm)
Gradient : B 12-20% (30 min)
Flow rate : 1.0 mL/min
Column Temp. : 50°C



ESI-TOF-MS



Oligonucleotide **18**

HPLC

A : 10mM TEAA
B : 10mM TEAA / MeCN = 1 / 1
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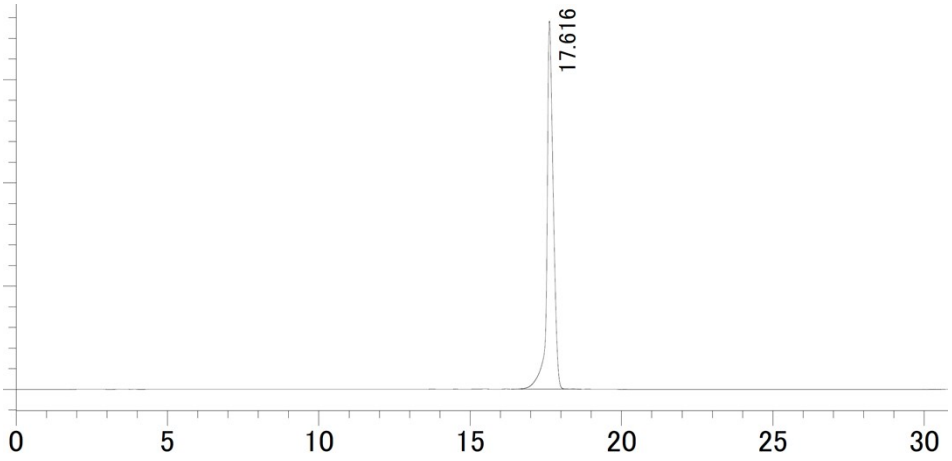
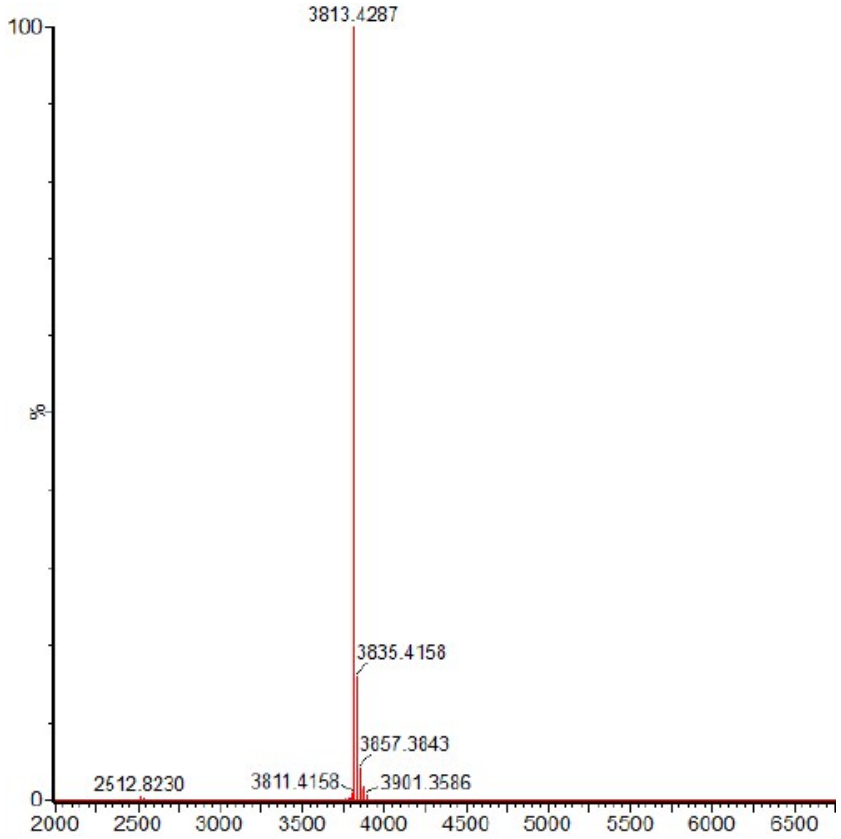
Gradient : B 12-20% (30 min)
Flow rate : 1.0 mL/min
Column Temp. : 50°C



ESI-TOF-MS



Oligonucleotide **19**

HPLC

A : 10mM TEAA
B : 10mM TEAA / MeCN = 1 / 1
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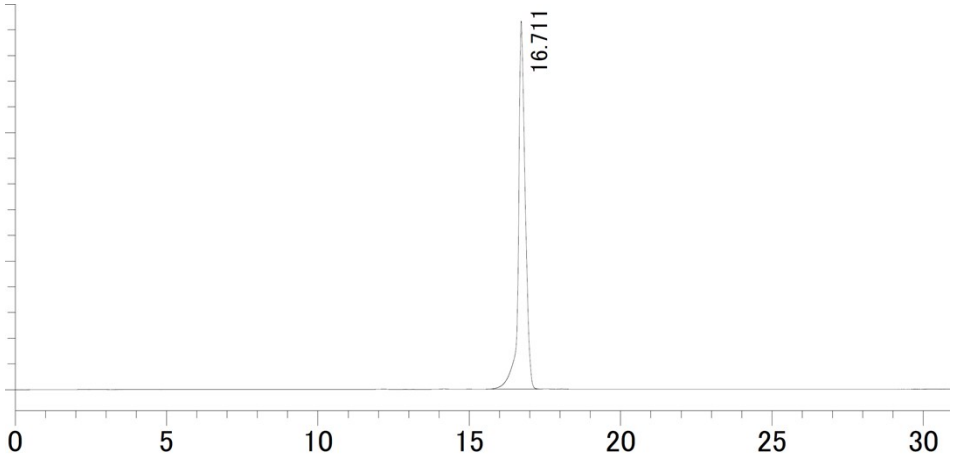
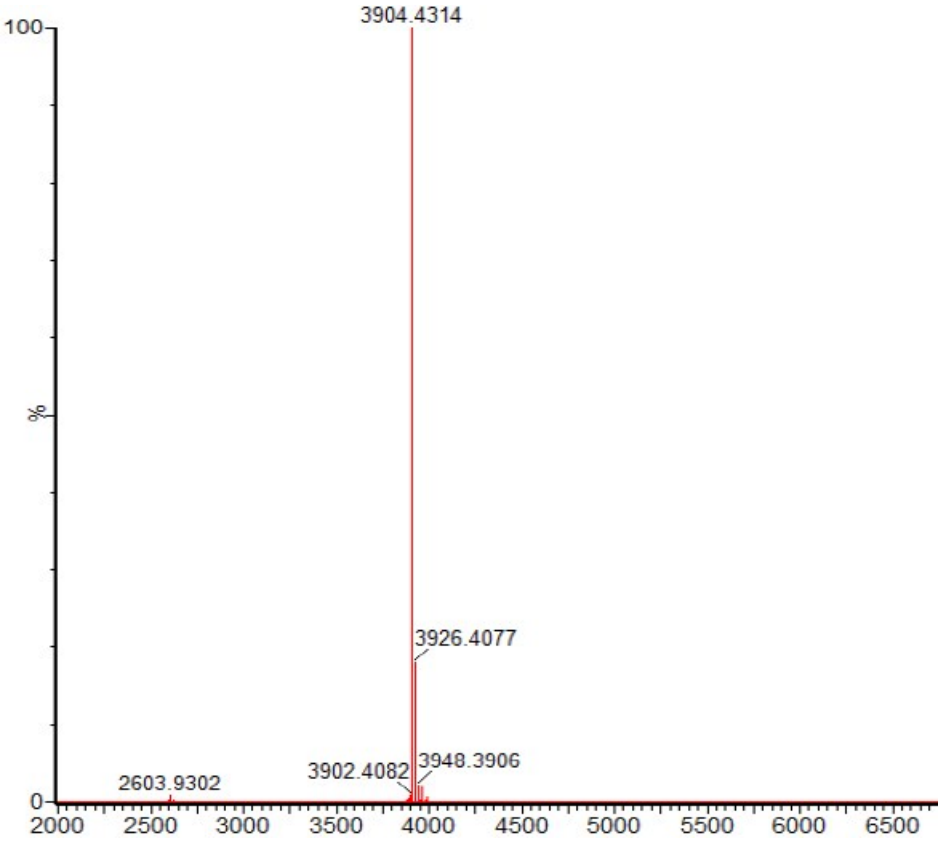
Gradient : B 12-20% (30 min)
Flow rate : 1.0 mL/min
Column Temp. : 50°C



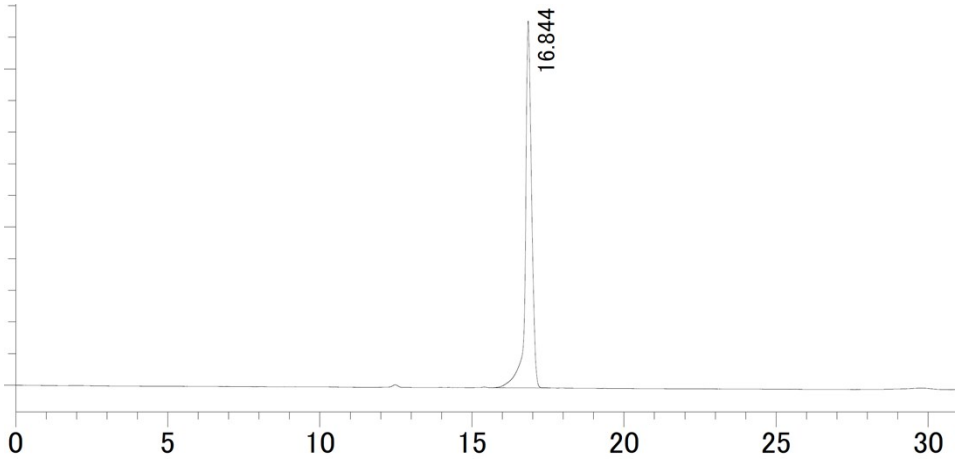
ESI-TOF-MS



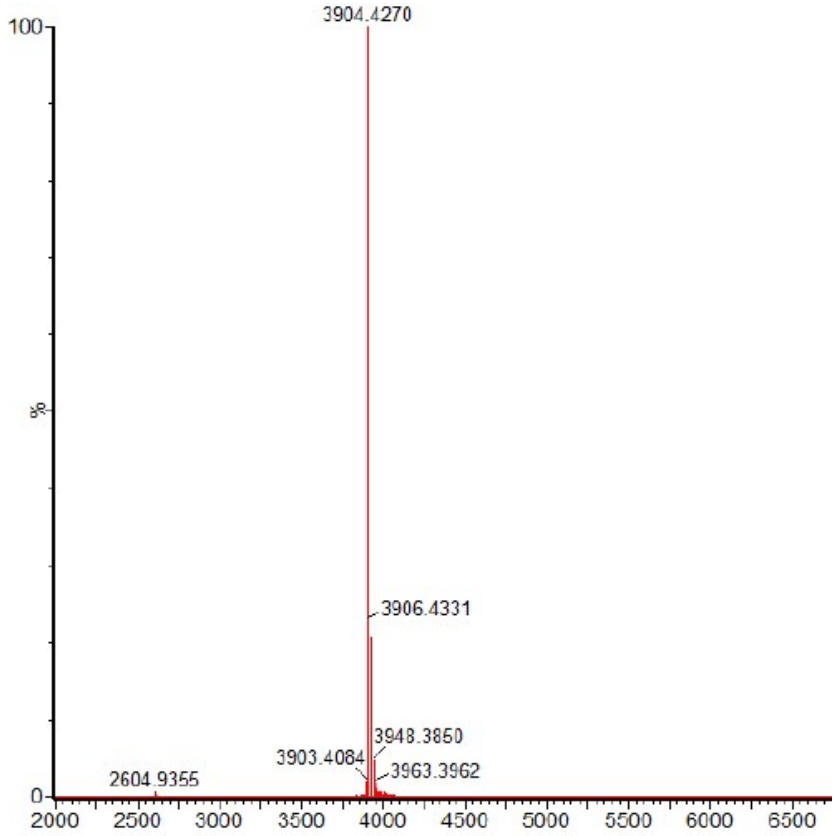
Oligonucleotide **20**

HPLC

A : 10mM TEAA
B : 10mM TEAA / MeCN = 1 / 1
Column : YMC Hydrosphere C18 Column (5.0 μm, 4.6 x 100 mm)
Gradient : B 12-20% (30 min)
Flow rate : 1.0 mL/min
Column Temp. : 50°C



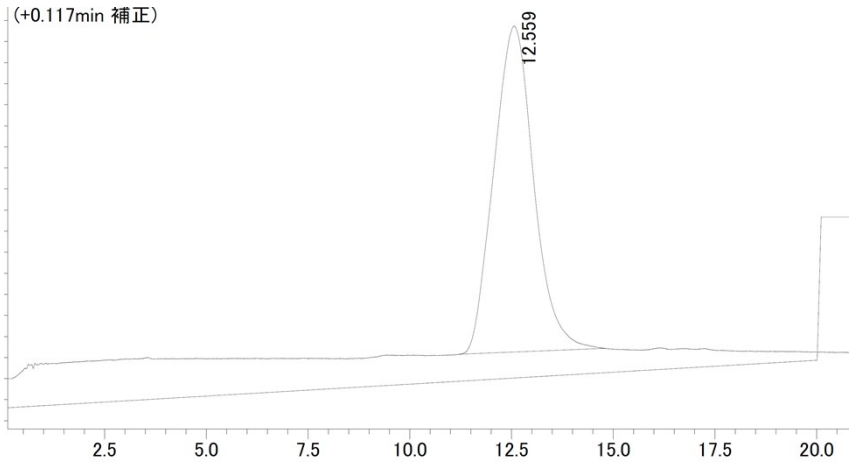
ESI-TOF-MS



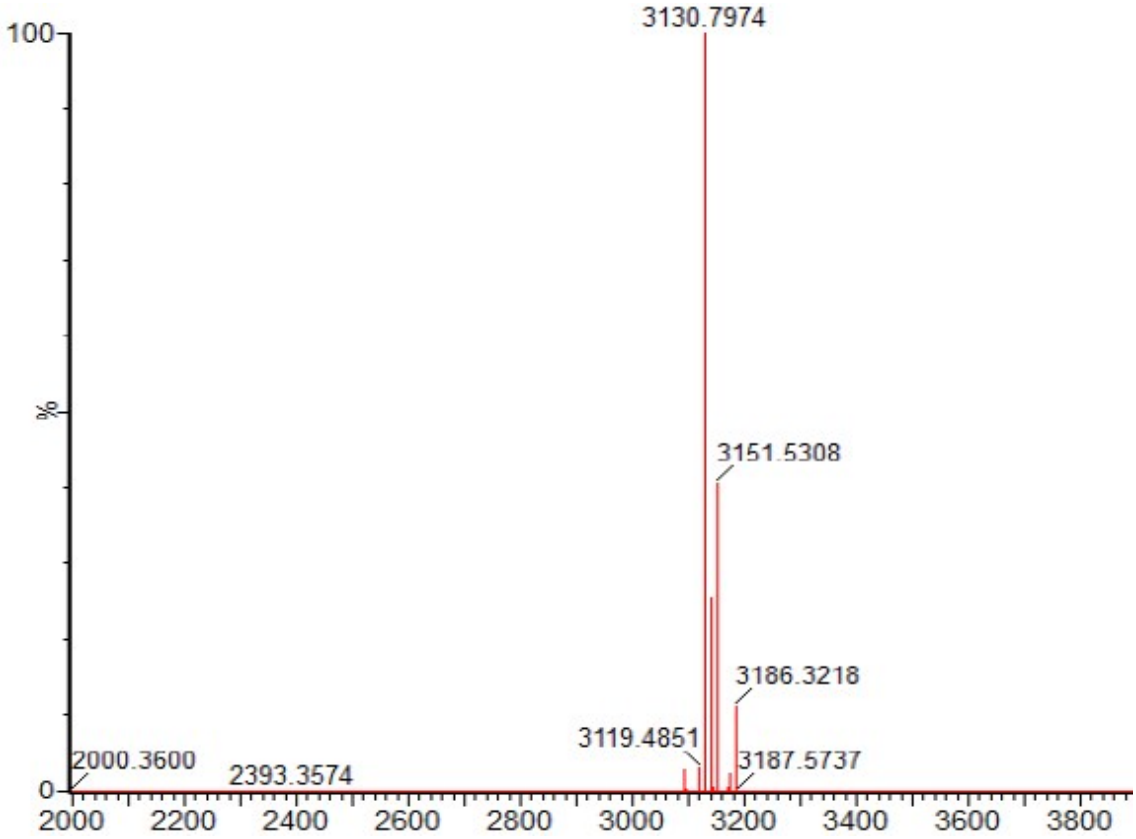
Oligonucleotide **21**

HPLC

A : 200 mM HFIP (Hexafluoroisopropanol) / 8 mM TEA aqueous solution
B : MeOH
Column : XBridge™ C18 Column (5.0 μm, 4.6 x 50 mm)
Gradient : B 10-20% (20 min)
Flow rate : 1.0 mL/min
Column Temp. : rt



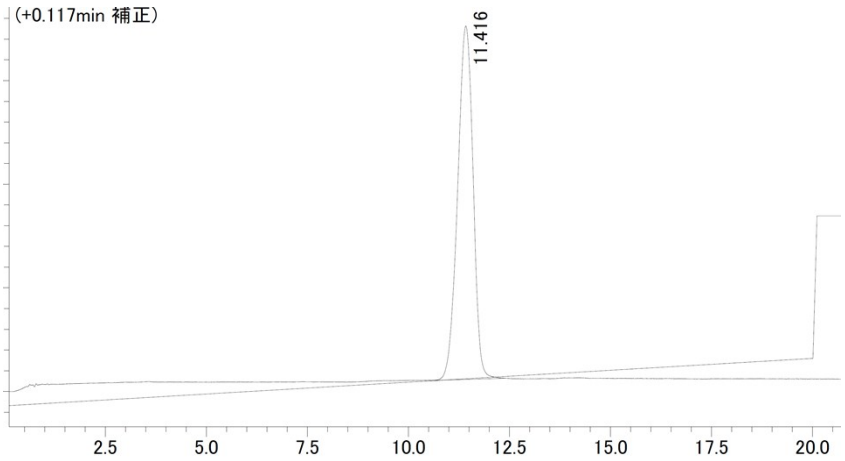
ESI-TOF-MS



Oligonucleotide **22**

HPLC

A : 200 mM HFIP (Hexafluoroisopropanol) / 8 mM TEA aqueous solution
B : MeOH
Column : XBridge™ C18 Column (5.0 μm, 4.6 x 50 mm)
Gradient : B 10-20% (20 min)
Flow rate : 1.0 mL/min
Column Temp. : rt



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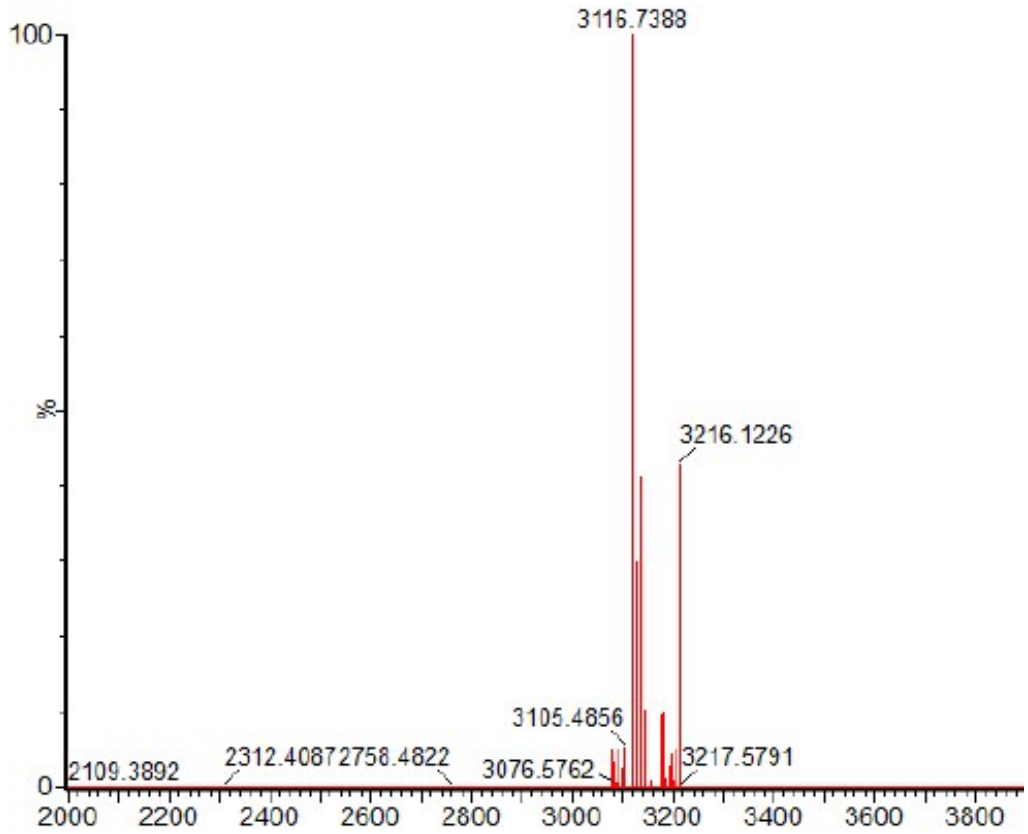
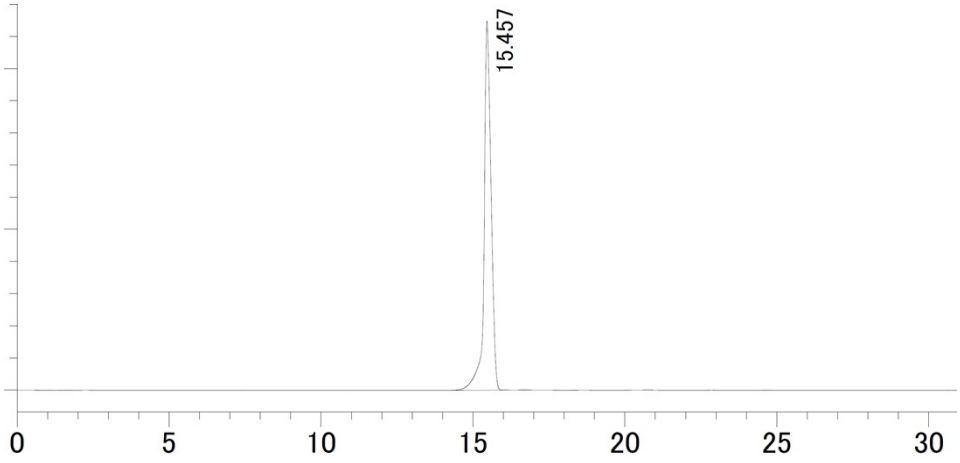


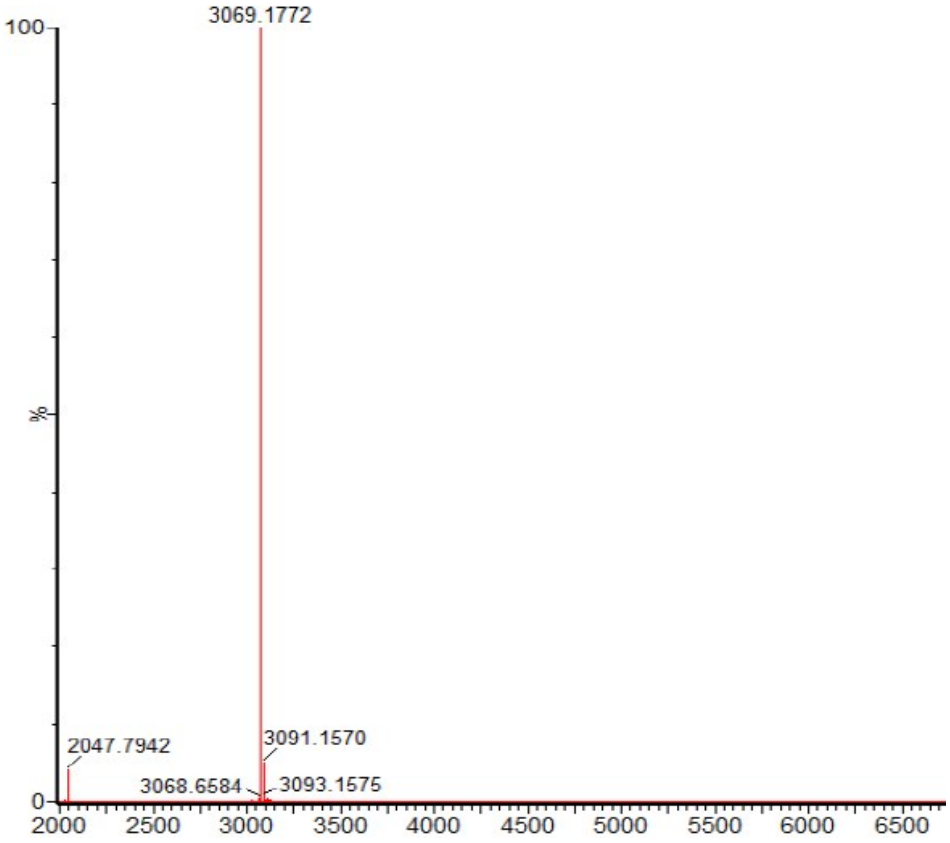
Oligonucleotide **23**

HPLC

A : 10mM TEAA
B : 10mM TEAA / MeCN = 1 / 1
Column : YMC Hydrosphere C18 Column (5.0 μm, 4.6 x 100 mm)
Gradient : B 16-24% (30 min)
Flow rate : 1.0 mL/min
Column Temp. : 50°C



ESI-TOF-MS



8. References

1) K. Miyashita, S. M. A. Rahman, S. Seki, S. Obika and T. Imanishi, *Chem. Commun.*, 2007, 3765.
2) Y. Hari, S. Obika, R. Ohnishi, K. Eguchi, T. Osaki, H. Ohishi and T. Imanishi, *Bioorg. Med. Chem.*, 2006, **14**, 1029.
3) Y. Mitsuoka, Y. Fujimura, R. Waki, A. Kugimiya, T. Yamamoto, Y. Hari and S. Obika, *Org. Lett.*, 2014, **16**, 5640.