Electronic Supplementary Material (ESI) for Chemical Communications

Guanidine bridged nucleic acid (GuNA): An effect of cationic bridged nucleic acid on DNA binding affinity

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Contents

1. General

All moisture-sensitive reactions were carried out in well-dried glassware under a N_2 atmosphere. Anhydrous dichloromethane, DMF, MeCN, and pyridine were used as purchased. ^{1}H NMR spectra were recorded at 400 MHz, ^{13}C NMR were recorded at 100 MHz, and the ^{31}P spectrum was recorded at 161 MHz. Chemical shift values are expressed in δ values (ppm) relative to tetramethylsilane (TMS) as internal standard and residual solvents for ^{1}H NMR, and $CHCl_3$ (δ = 77.00 ppm) and DMSO (39.50 ppm) for ^{13}C NMR, and 85% H_3PO_4 (δ = 0 ppm) for ^{31}P NMR. Fast atom bombardment mass spectra (FAB-MS) were recorded in positive ion mode. For column chromatography, silica gel PSQ 100B was used. The progress of reaction was monitored by analytical thin layer chromatography (TLC) on pre-coated aluminium sheets (Silica gel 60 F_{254}- sheet-Merck), and the products were visualized by UV light.

2. Synthesis of GuNA monomer and phosphoramidites

1-(2- Amino-3,5-di-*O*-benzyl-4-*C*-*tert*-butyldiphenylsiloxymethyl-*β*-D-ribofuranosyl)thymine (3).

To the solution of **2** (622mg, 0.85 mmol) in methanol (8 mL), nickel chloride (11 mg, 0.085 mmol) was added and placed in an ice-bath. After stirring for few min, sodium borohydride (64 mg, 1.7 mmol) was added slowly and the reaction mixture was stirred for 10 min at room temperature. After completion of the reaction, ice-cold water was added and the product was extracted with ethyl acetate. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The product was purified by column chromatography (0.5% triethylamine in ethyl acetate) to afford **3** (456 mg, 76 %) as a white solid.

$[\alpha]_D^{24}$ -17.3 (c 1.00, $CHCl_3$). IR (KBr): 3175, 3067, 2931, 2857, 1700, 1469, 1267, 1091 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ: 1.04 (9H, s), 1.63 (3H, d, *J* = 1.5 Hz), 3.59, 3.66 (2H, AB, *J* = 10.0 Hz), 3.67 (1H, dd, *J* = 5.5 Hz, 9.0 Hz), 3.79, 3.99 (2H, AB, *J* = 11.0 Hz), 4.06 (1H, d, *J* = 5.5 Hz), 4.55, 4.58 (2H, AB, *J* = 11.0 Hz), 4.67, 4.76 (2H, AB, *J* = 11.0 Hz), 5.81 (1H, d, *J* = 9.0 Hz), 7.19-7.61 (21H, m); ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 12.16, 19.13, 26.94, 58.90, 64.04, 73.61, 73.81, 75.14, 81.48, 87.47, 88.03, 110.93, 127.55, 127.70, 127.72, 127.76, 127.89, 128.01, 128.46, 128.65, 129.75, 129.85, 132.57, 132.87, 135.53, 135.75, 135.82, 137.31, 137.48, 151.03, 136.78. MS (FAB): m/z 706 ($M+H^+$), High-resolution MS (FAB): Calcd. for $C_{41}H_{48}N_3O_6Si$ ($M+H^+$): 706.3307. Found: 706.3326.

1-[3,5-Di-*O*-benzyl-2-(*N*,*N*"-di(*tert*-butoxycarbonyl)guanidinyl)-4-*C*-*tert*-butyldiphenylsiloxymethyl-*β*-D-ribofuranosyl]thymine (4).

To the solution of **3** (50 mg, 0.071 mmol) in dichloromethane (1 mL), *N*,*N*'-di(*tert*-butoxycarbonyl)thiourea (30.4 mg, 0.11 mmol), diisopropylethylamine (9 μL, 0.035 mmol), and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (21 mg, 0.11 mmol) was added and the reaction mixture was stirred at room temperature for 3 h. After completion of the reaction, ice-cold water was added and the product was extracted with dichloromethane. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The product was purified by column chromatography (*n*-hexane : ethyl acetate = 4 : 1) to afford **4** (58 mg, 86 %) as a white solid.

$[\alpha]_D^{24}$ -5.23 (c 1.00, $CHCl_3$). IR (KBr): 3314, 3173, 3022, 2932, 2860, 1720, 1641, 1612, 1554, 1469, 1416, 1367, 1303, 1232,

1156, 1058 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ : 1.04 (9H, s), 1.42 (9H, s), 1.46(9H, s), 1.72 (3H, d, *J* = 1.0 Hz), 3.57, 3.96 (2H, AB, *J* = 10.0 Hz), 3.73, 3.78 (2H, AB, *J* = 11.0 Hz), 4.25 (1H, d, *J* = 8.0 Hz), 4.57, 4.65 (2H, AB, *J* = 11.0 Hz), 4.59, 4.61 (2H, AB, *J* = 9.0 Hz), 4.89 (1H, q, *J* = 8.0 Hz), 5.98 (1H, d, *J* = 8.0 Hz), 7.20-7.69 (22H, m), 8.93 (1H, d, *J* = 8.0 Hz), 11.34 (1H,s) ; ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 12.43, 19.18, 26.95, 27.89, 28.13, 55.76, 63.70, 72.40, 73.65, 75.31, 79.03, 73.30, 83.15, 86.73, 87.58, 110.76, 127.67, 127.74, 127.89, 128.21, 128.65, 128.89, 129.76, 129.84, 132.60, 132.99, 135.60, 135.77, 136.25, 136.43, 137.44, 150.59, 152.52, 156.06, 163.28, 163.61. MS (FAB): m/z 948 ($M+H^+$), High-resolution MS (FAB): Calcd. for $C_{52}H_{66}N_5O_{10}Si$ ($M+H^+$): 948.4573. Found: 948.4567.

1-[3,5-Di-*O*-benzyl-2-(*N*,*N"*-di(*tert*-butoxycarbonyl)guanidinyl)-4-*C*-hydroxymethyl-*β*-D-ribofuranosyl]thymine (5).

To the solution of **4** (106 mg, 0.11 mmol) in THF (1 mL), 1N TBAF in THF (0.14 mL, 0.14 mmol) was added and the reaction mixture was stirred at room temperature for 4.5 h. After completion of the reaction, water was added to the mixture and the product was extracted with ethyl acetate. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The product was purified by flash column chromatography (*n*-hexane : ethyl acetate = 1 : 1) to afford **5** (80 mg, quant.) as a white amorphous solid.

$[\alpha]_D^{23}$ +4.57 (c 1.00, $CHCl_3$). IR (KBr): 3449, 3313, 3172, 2978, 2932, 2872, 1693, 1641, 1611, 1554, 1473, 1414, 1367, 1302, 1155, 1106, 1057 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ : 1.42 (9H, s), 1.50 (9H, s), 1.75 (3H, d, *J* = 1.0 Hz), 3.57, 3.62 (2H, AB, *J* = 10.0 Hz), 3.68, 3.84 (2H, AB, *J* = 11.0 Hz), 4.35 (1H, d, *J* = 7.5 Hz), 4.51, 4.72 (2H, AB, *J* = 11.0 Hz), 4.58, 4.62 (2H, AB, *J* = 11.5 Hz), 4.87 (1H, q, *J* = 7.5 Hz), 6.07 (1H, d, *J* = 7.5 Hz), 7.26-7.52 (11H, m), 7.88 (1H, s), 9.05 (1H, d, *J* = 7.5 Hz), 11.39 (1H,s) ; ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 12.38, 27.96, 28.05, 55.44, 62.89, 71.75, 73.60. 75.25, 79.10, 79.14, 83.29, 87.08, 87.52, 110.92, 127.62, 127.89, 128.46, 128.57, 128.92, 136.16, 136.22, 137.36, 150.77, 152.62, 155.98, 163.11, 163.70. MS (FAB): m/z 710 ($M+H^+$), High-resolution MS (FAB): Calcd. for $C_{36}H_{48}N_5O_{10}$ ($M+H^+$): 710.3396. Found: 710.3418.

[*tert*-Butoxycarbonylimino-[(1*R*.3*R*,4*R*,7*S*)-1-benzyloxymethyl-7-benzyloxy-3-(thymin-1-yl)-(2-oxa-5-aza-bicyclo[2.2.1] heptyl)-methyl]-carbamic acid *tert*-butyl ester (6).

To the solution of **5** (850 mg, 1.20 mmol) in dichloromethane (12 mL), pyridine (0.29 mL, 3.59 mmol) and trifluoromethanesulfonic anhydride (0.3 mL, 1.78 mmol) was added at 0 °C and the reaction mixture was stirred at same temperature for 3 h. After completion of the reaction, ice-cold water was added and the product was extracted with dichloromethane. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The crude product was dissolved in dichloromethane (8 mL) and triethylamine (2 mL) was added. The reaction solution was stirred at room temperature for 27 h. After completion of the reaction, the solution was concentrated and the product was purified by column chromatography (*n*-hexane : ethyl acetate = 1 : 1) to afford **6** (644 mg, 77 %, two steps) as a white solid.

$[\alpha]_D^{24}$ +66.1 (c 1.00, $CHCl_3$). IR (KBr): 3521, 3174, 3067, 2978, 1748, 1694, 1600, 1455, 1266, 1133 cm^{-1},^{1}H-NMR (DMSO) δ : 1.43 (9H, s), 1.48 (9H, s), 1.57 (3H, s), 3.52, 3.54 (1H, m), 3.59, 3.62 (1H, AB, *J* = 12.0 Hz), 3.86, 3.89 12H, AB, *J* = 12.0 Hz), 3.91, 3.94 (1H, AB, *J* = 12.0 Hz), 4.12 (1H, s), 4.59 – 4.65 (3H, m), 4.73, 4.76 (1H, AB, *J* = 12 Hz), 5.52 (1H, br s),

7.31-7.46 (12H, m), 9.63 (1H, s), 11.22 (1H, s); ^{13}C-NMR (100 MHz, DMSO) δ: 11.66, 27.80, 53.05, 65.21, 66.63, 71.19, 72.66, 77.24, 108.28, 127.20, 127.31, 127.37, 127.89, 127.89, 128.01, 134.34, 137.17, 137.70, 149.54, 163.42. High-resolution MS (FAB): Calcd. for $C_{36}H_{46}N_5O_9$ (M+H$^+$): 692.3290. Found: 692.3295.

[*tert*-Butoxycarbonylimino-[(1*R*.3*R*,4*R*,7*S*)-1-(4,4'-dimethoxytrityloxymethyl)-7-hydroxy-3-(thymin-1-yl)-(2-oxa-5-aza-bicyclo[2.2.1]heptyl)-methyl]-carbamic acid *tert*-butyl ester (8).

To the solution of **6** (644 mg, 0.93 mmol) in methanol (10 mL) was added 20% palladium hydroxide in carbon (900 mg) and the reaction vessel was degassed several times with hydrogen. The reaction mixture was stirred at room temperature for 14 h under hydrogen atmosphere. After completion of the reaction, the solution was filtered and concentrated. The crude product **7** (354 mg) was co-evaporated three times with pyridine. To the solution of crude **7** in pyridine (7 mL), 4,4'-dimethoxytritylchloride (469 mg, 1.38 mmol) was added at 0 ºC. The reaction solution was stirred at room temperature for 12 h. After completion of the reaction, ice-cold water was added and the product was extracted with dichloromethane. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The product was purified by column chromatography (*n*-hexane : ethyl acetate = 1 : 1) to afford **8** (442 mg, 54 %, two steps) as a white solid.

$[\alpha]_D^{23}$ -9.80 (c 1.00, $CHCl_3$). IR (KBr): 3216, 2977, 1694,1605, 1509, 1460, 1294, 1252, 1175, 1134 cm^{-1}. ^{1}H-NMR ($CDCl_3$) δ : 1.49 (18H, s), 1.68 (3H, s), 3.46, 3.51 (2H, AB, *J* = 11.0 Hz), 3.57, 3.77 (2H, AB, *J* = 11.0 Hz), 3.80 (3H, s), 4.32 (1H, s), 4.66 (1H, s), 5.57 (1H, s), 6.84 (4H, d, *J* = 8.5 Hz), 7.24-7.36 (7H, m), 7.46 (2H, d, *J* = 8.0 Hz), 7.60 (1H, s), 8.14 (1H, s), 10.13 (1H,s) ; ^{13}C-NMR (100 MHz, $CDCl_3$) δ: 12.56, 28.01, 53.44, 55.20, 59.22, 64.54, 69.46, 86.56, 86.63, 89.01, 110.51, 113.26, 127.04, 128.00, 130.01, 130.04, 134.57, 135.17, 135.34, 144.32, 149.57, 158.62, 163.71. MS (FAB): m/z 814 (M+H$^+$), High-resolution MS (FAB): Calcd. for $C_{36}H_{46}N_5O_9$ (M+H$^+$): 814.3658. Found: 814.3663.

[*tert*-Butoxycarbonylimino-[(1*R*.3*R*,4*R*,7*S*)-7-(2-cyanoethoxy(disopropylamino)phosphinoxy)-1-(4,4'-dimethoxytritylox ymethyl)-3-(thymin-1-yl)-(2-oxa-5-aza-bicyclo[2.2.1]heptyl)-methyl]-carbamic acid *tert*-butyl ester (9).

To the solution of **8** (141 mg, 0.17 mmol) in acetonitrile (2 mL) was added *N,N'*-diisopropylammoniumtetrazolide (39 mg, 0.23 mmol) and 2-cyanoethyl-*N,N,N',N'*-tetraisopropylphosphorodiamidite (73 μL, 0.23 mmol), and the reaction mixture was stirred at toom temperature for 3 h. After completion of the reaction, ice-cold water was added and the product was extracted with dichloromethane. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated. The product was purified by column chromatography (*n*-hexane : ethyl acetate = 3 : 2) to afford **9** (148 mg, 86 %) as a white solid

^{31}P-NMR ($CDCl_3$) δ : 148.14, 148.73, 149.08. MS (FAB): m/z 1014 (M+H$^+$), High-resolution MS (FAB): Calcd. for $C_{52}H_{69}N_7O_{12}P$ (M+H$^+$):1014.4736. Found: 1014.4770.

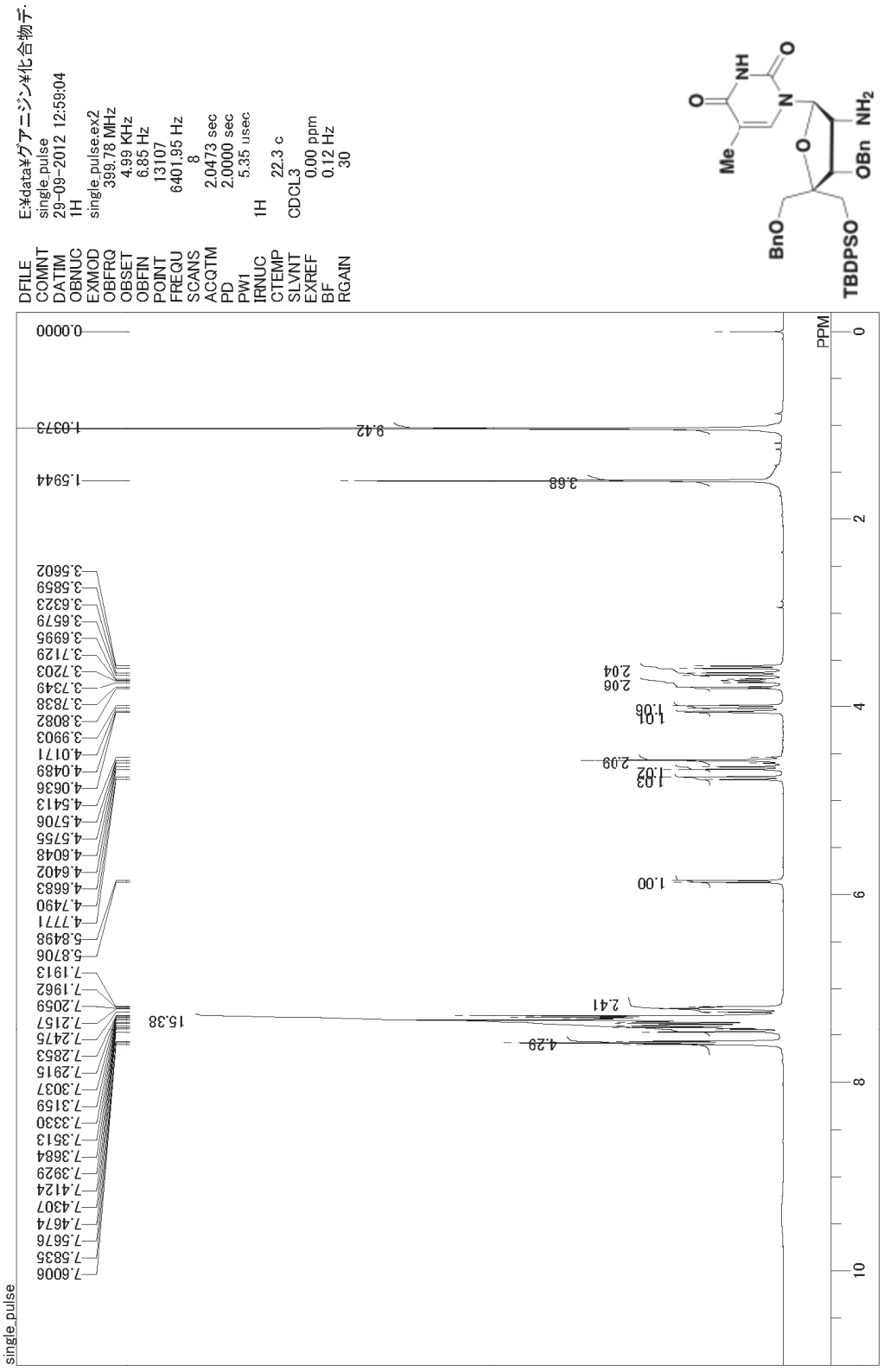

Fig. SI-1: ^{1}H-NMR spectrum of compound **3**

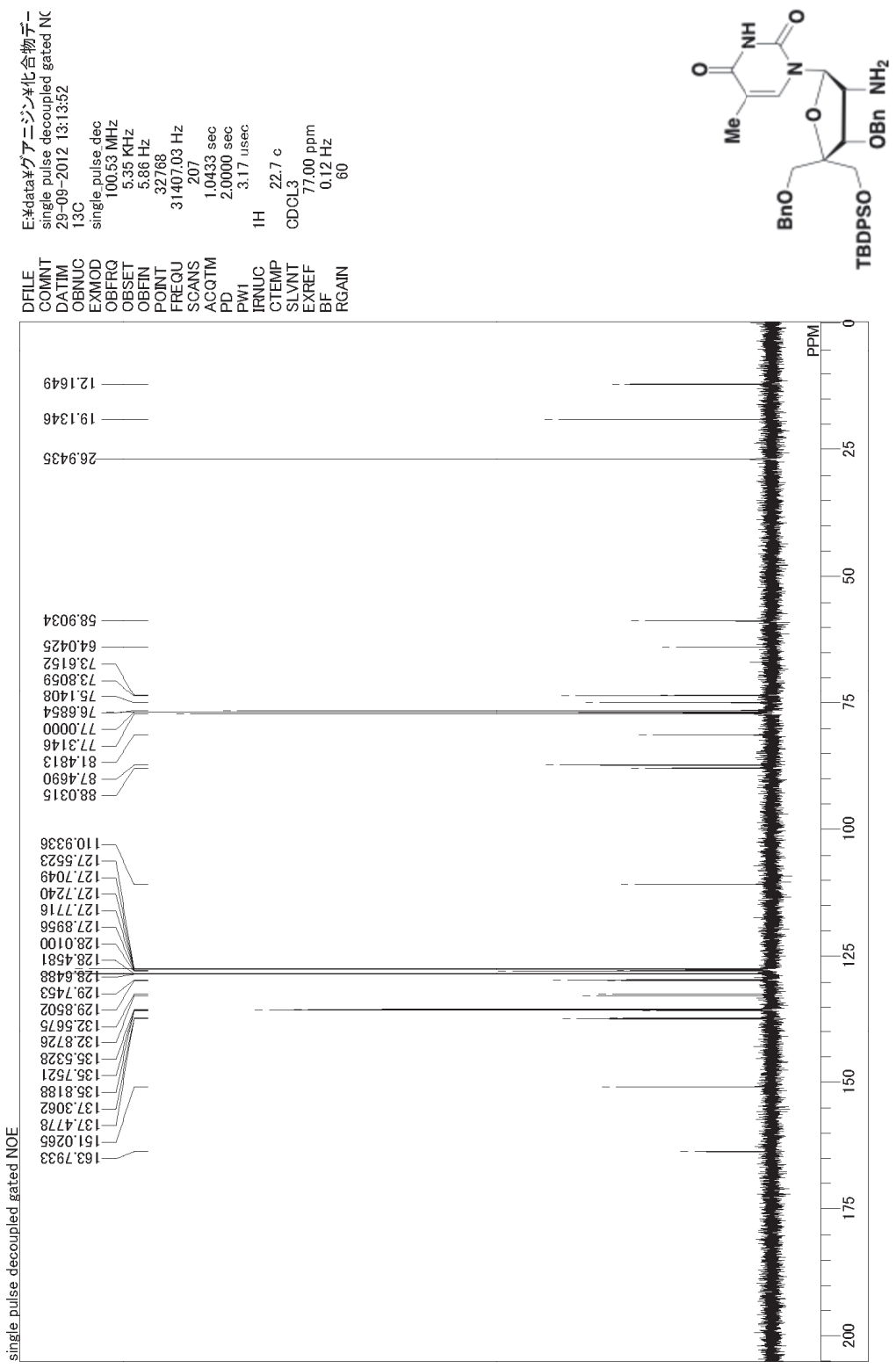

Fig. SI-2: ^{13}C-NMR spectrum of compound **3**

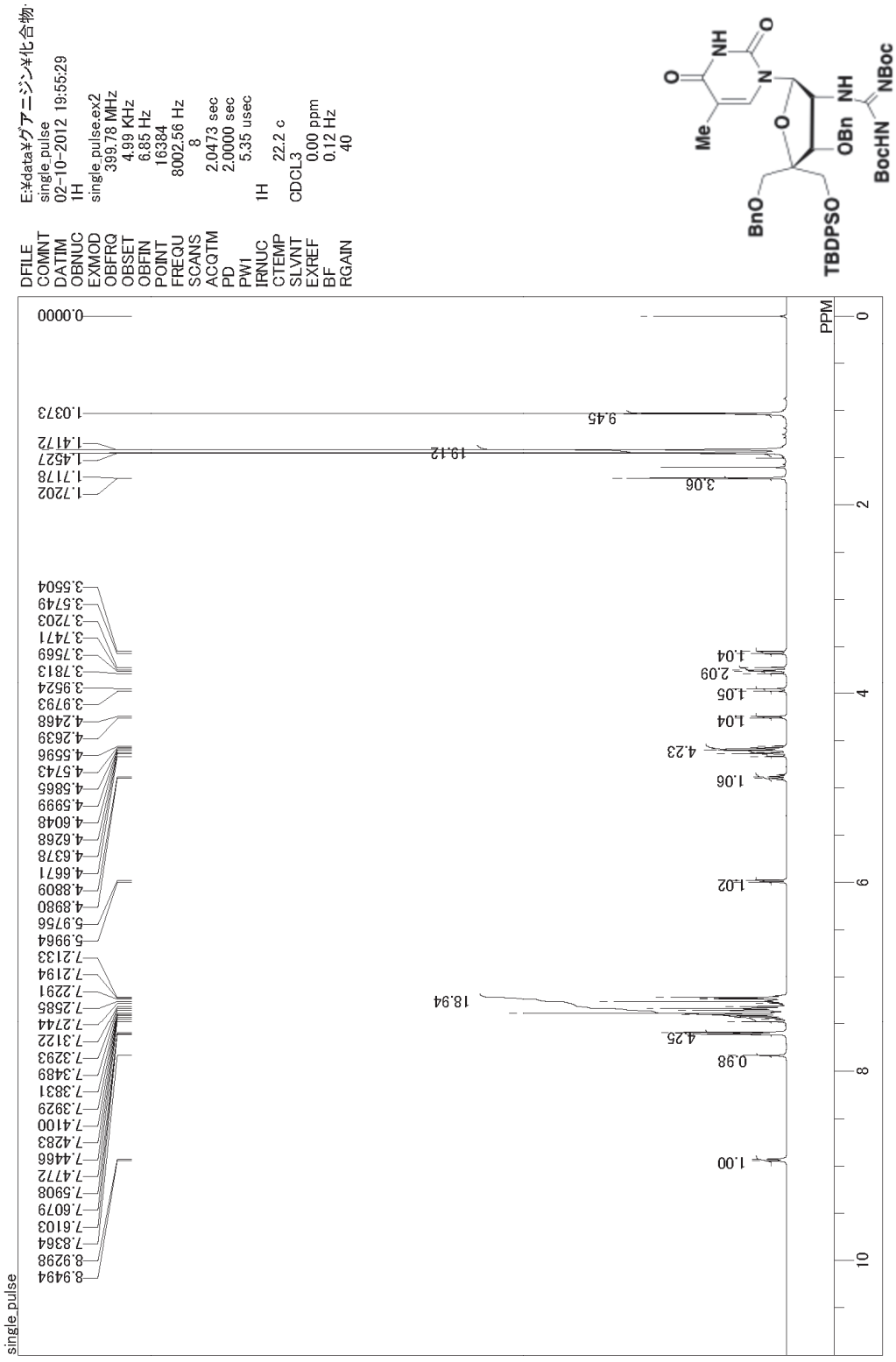

Fig. SI-3: ^{1}H-NMR spectrum of compound **4**

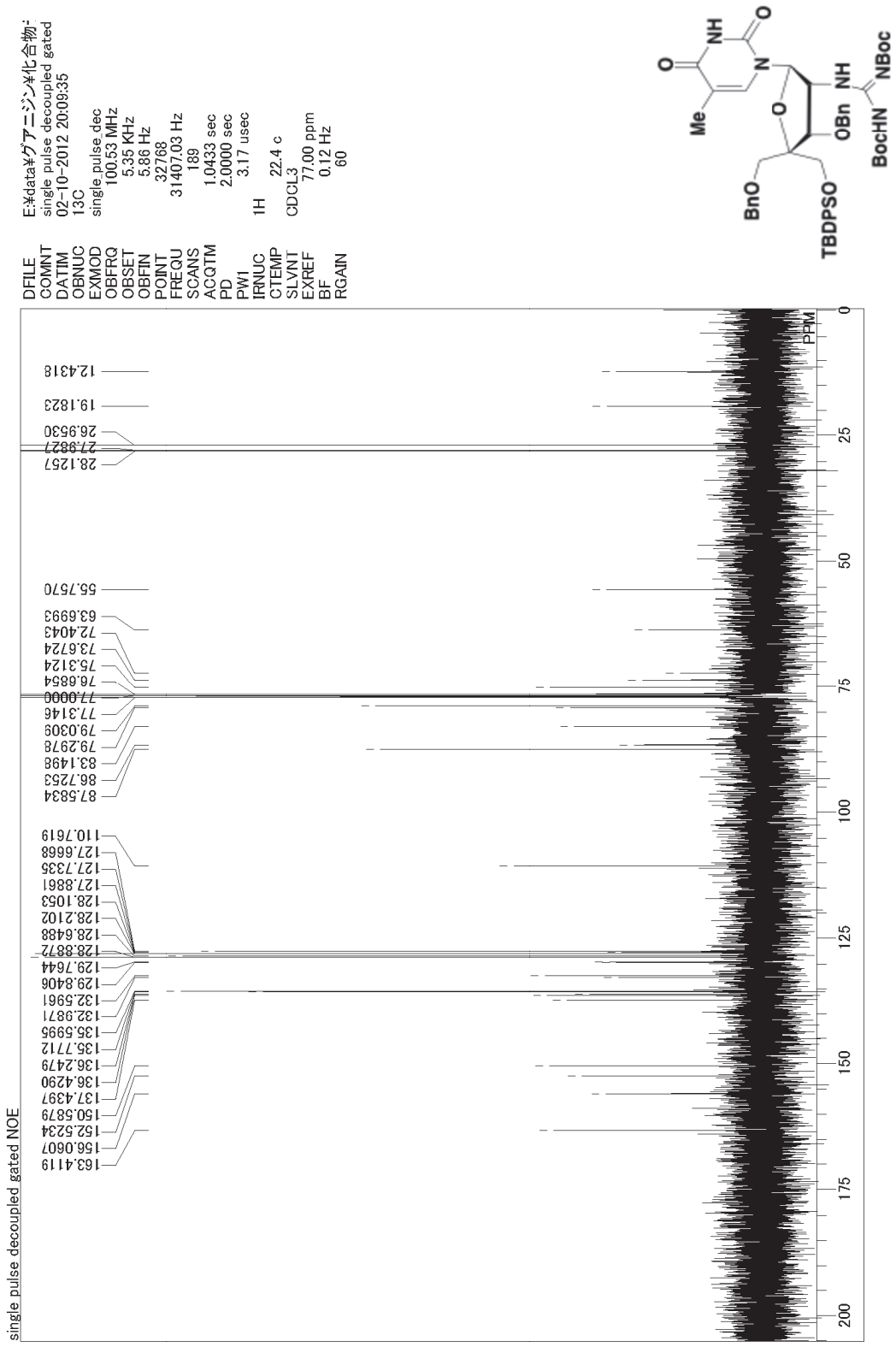

Fig. SI-4: ^{13}C-NMR spectrum of compound **4**

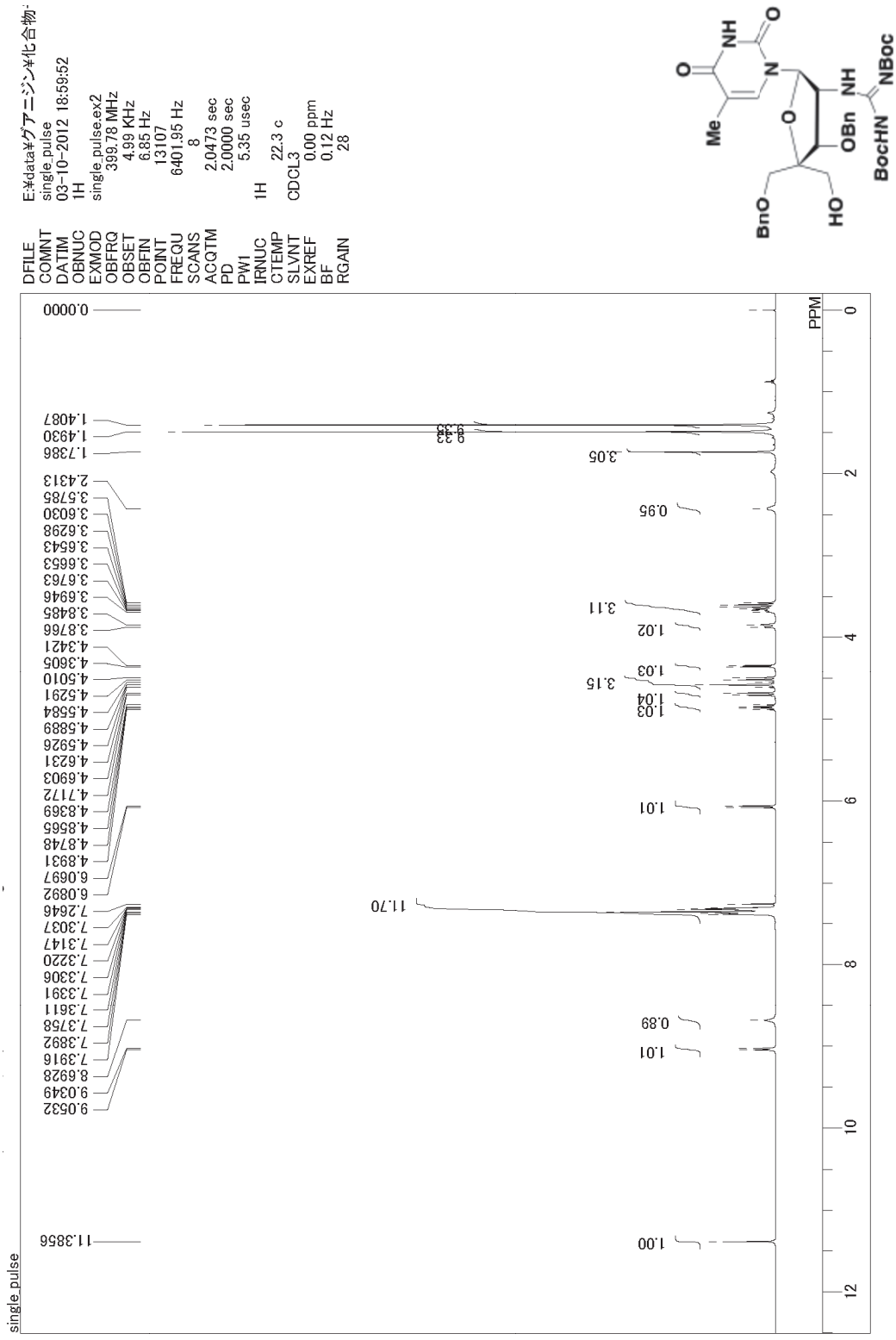

Fig. SI-5: ^{1}H-NMR spectrum of compound **5**

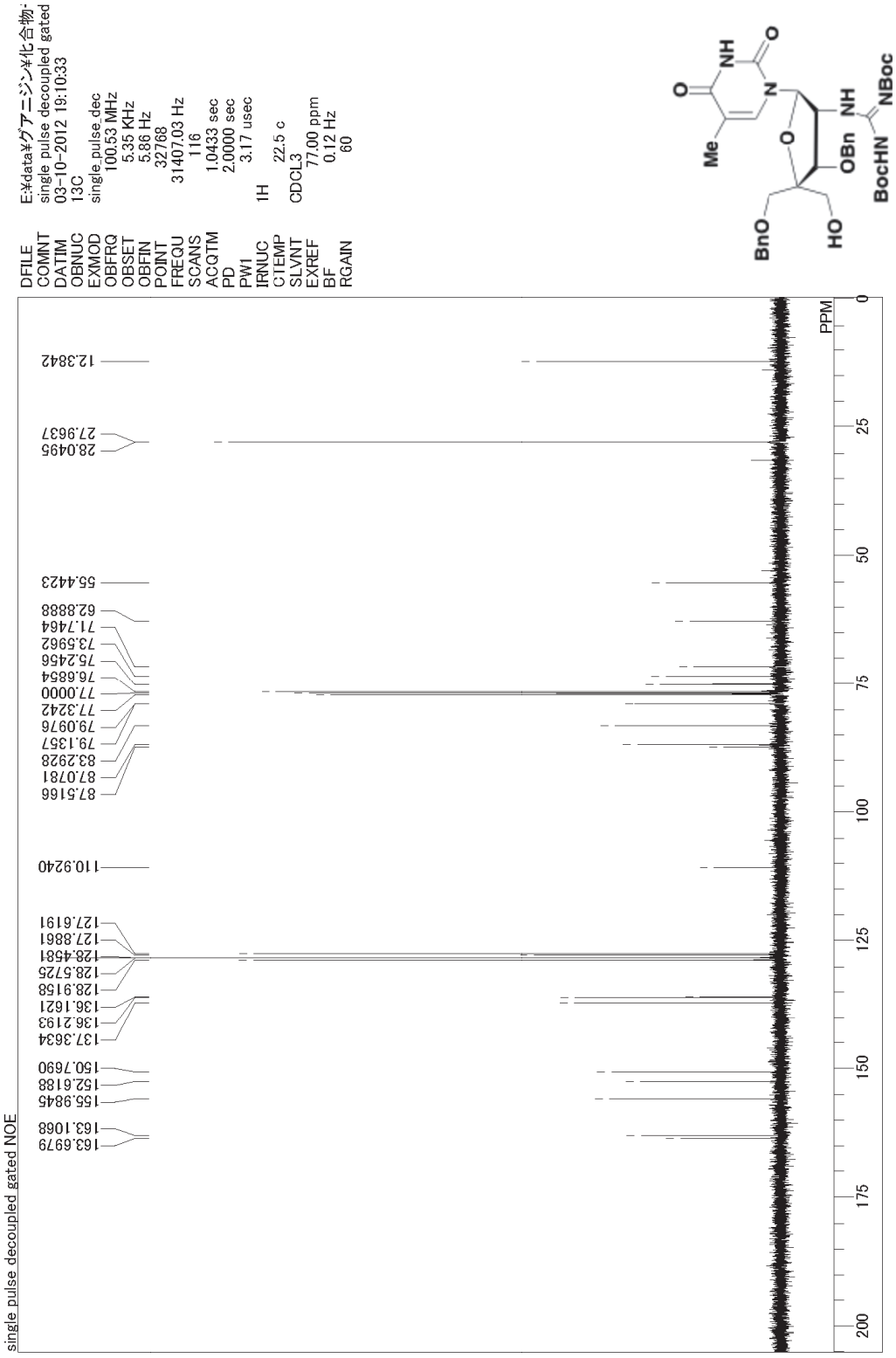

Fig. SI-6: ^{13}C-NMR spectrum of compound **5**

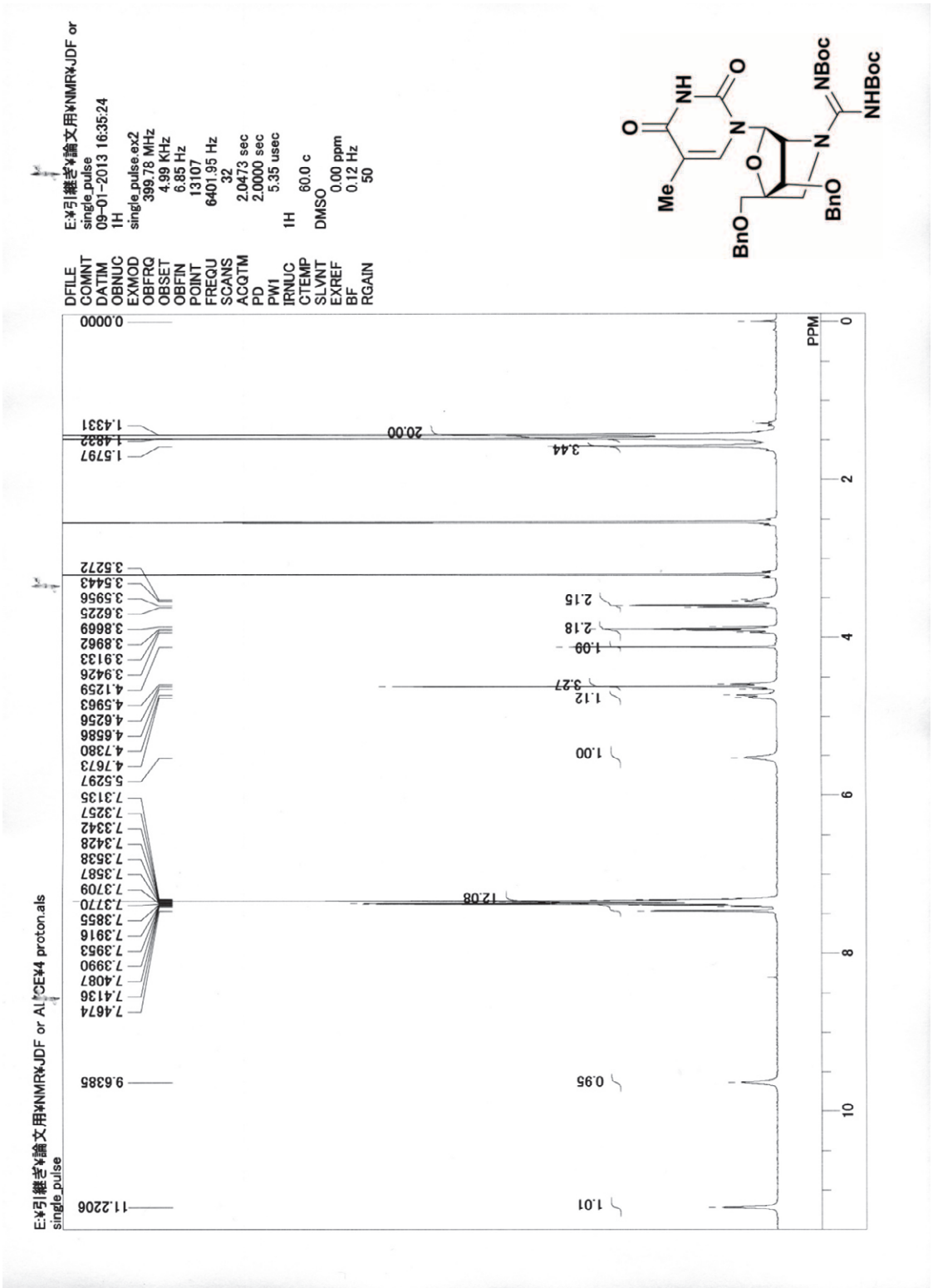

Fig. SI-7: ^{1}H-NMR spectrum of compound **6**

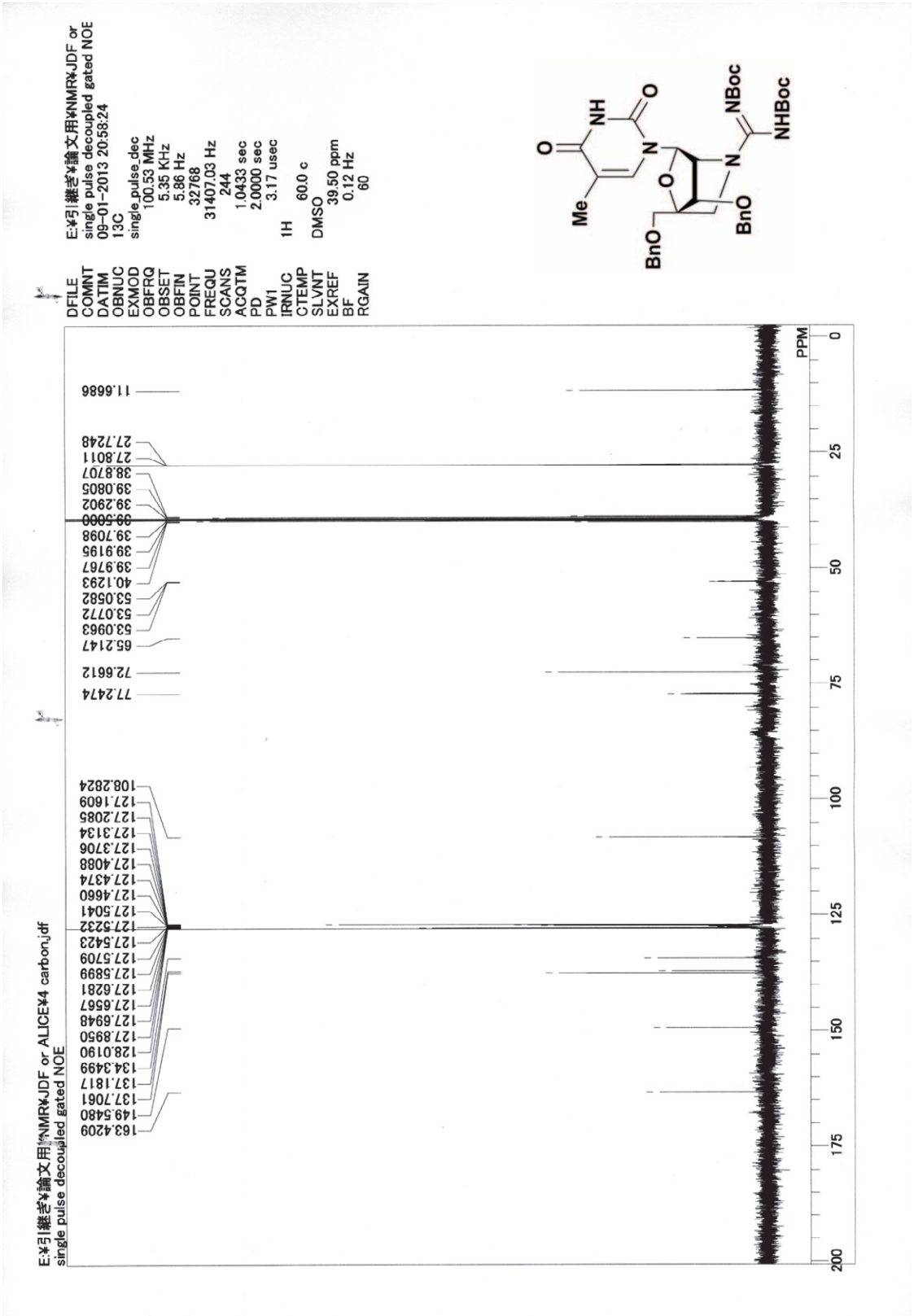

Fig. SI-8: ^{13}C-NMR spectrum of compound **6**

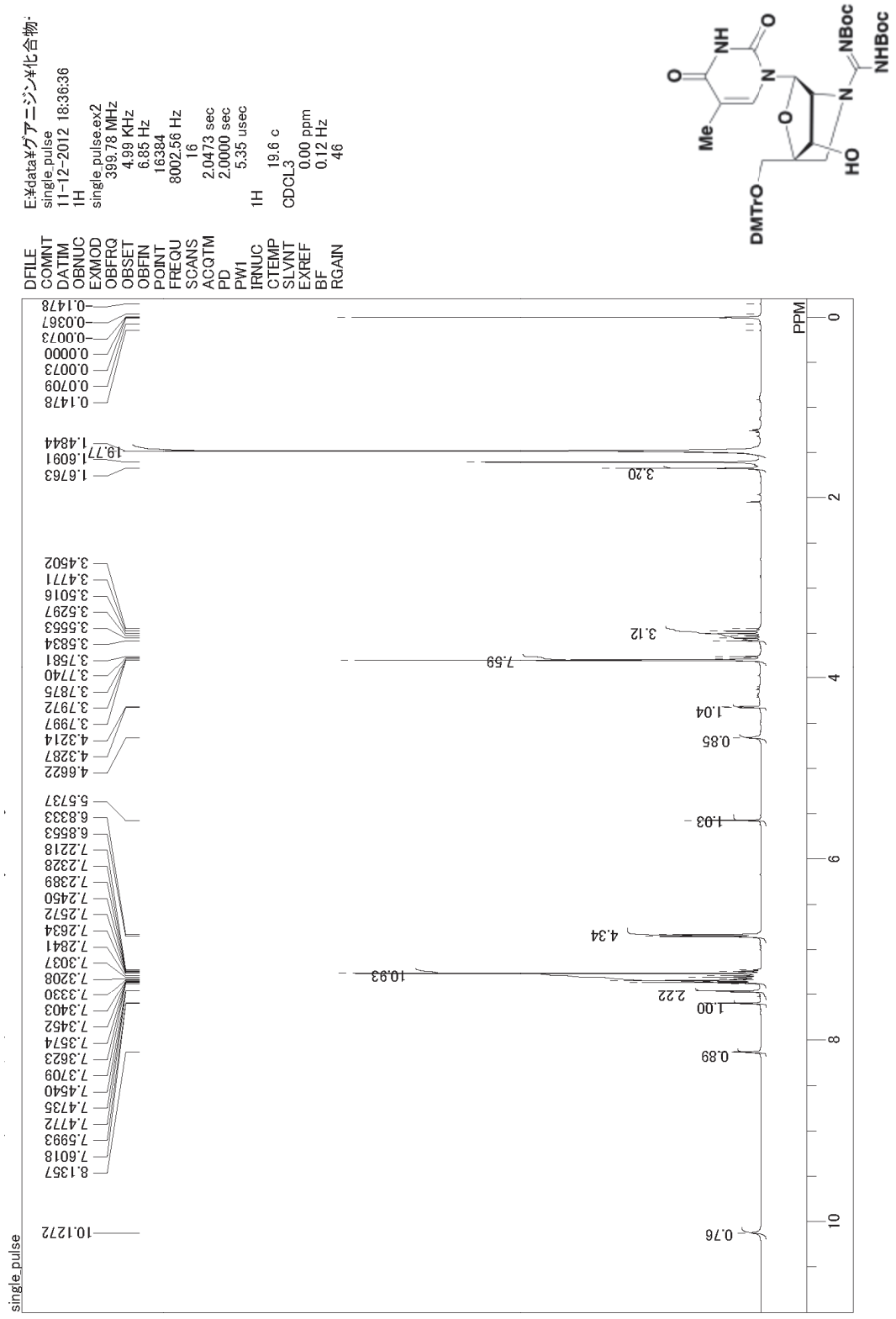

Fig. SI-9: ^{1}H-NMR spectrum of compound **8**

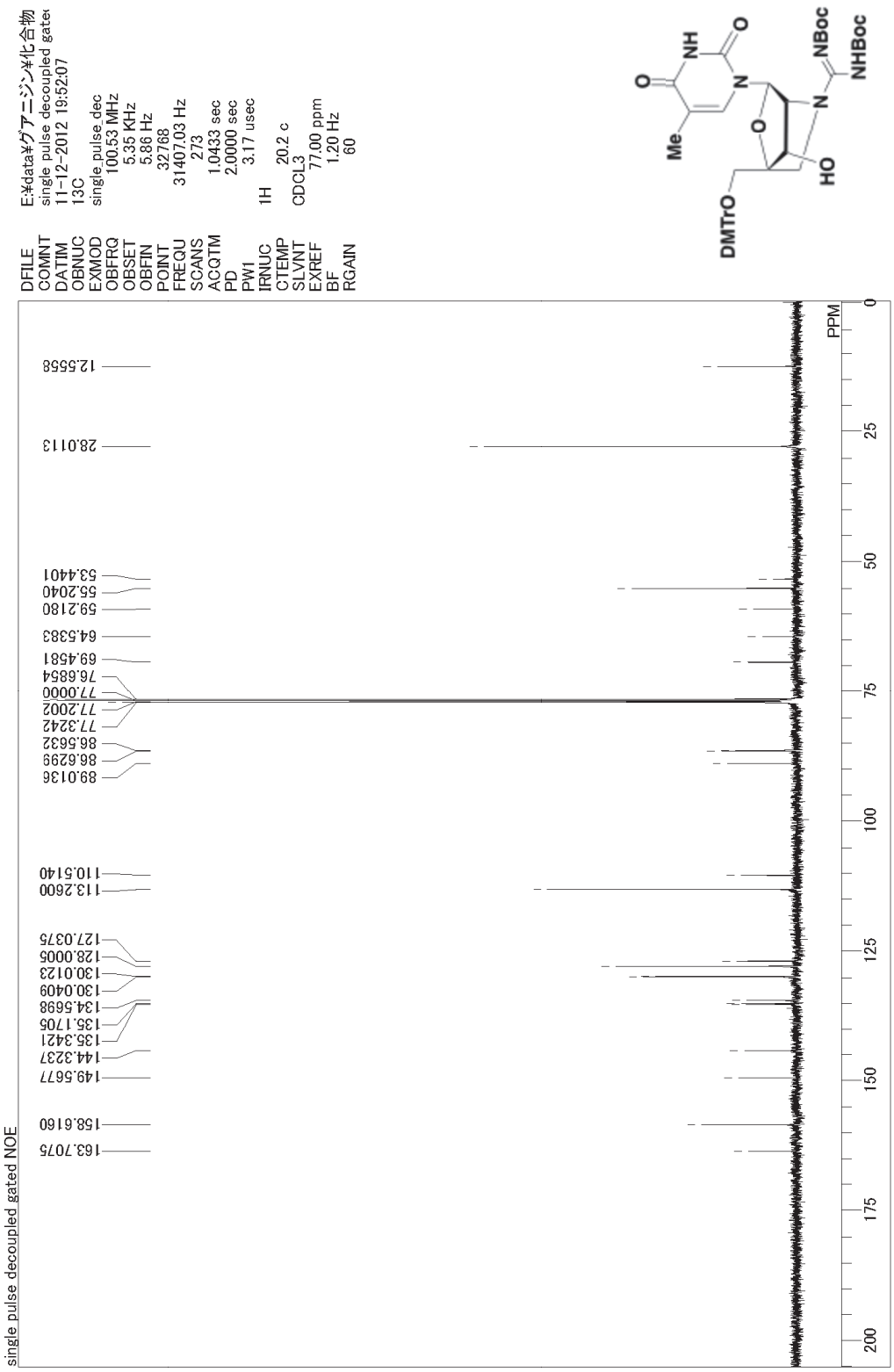

Fig. SI-10: ^{13}C-NMR spectrum of compound **8**

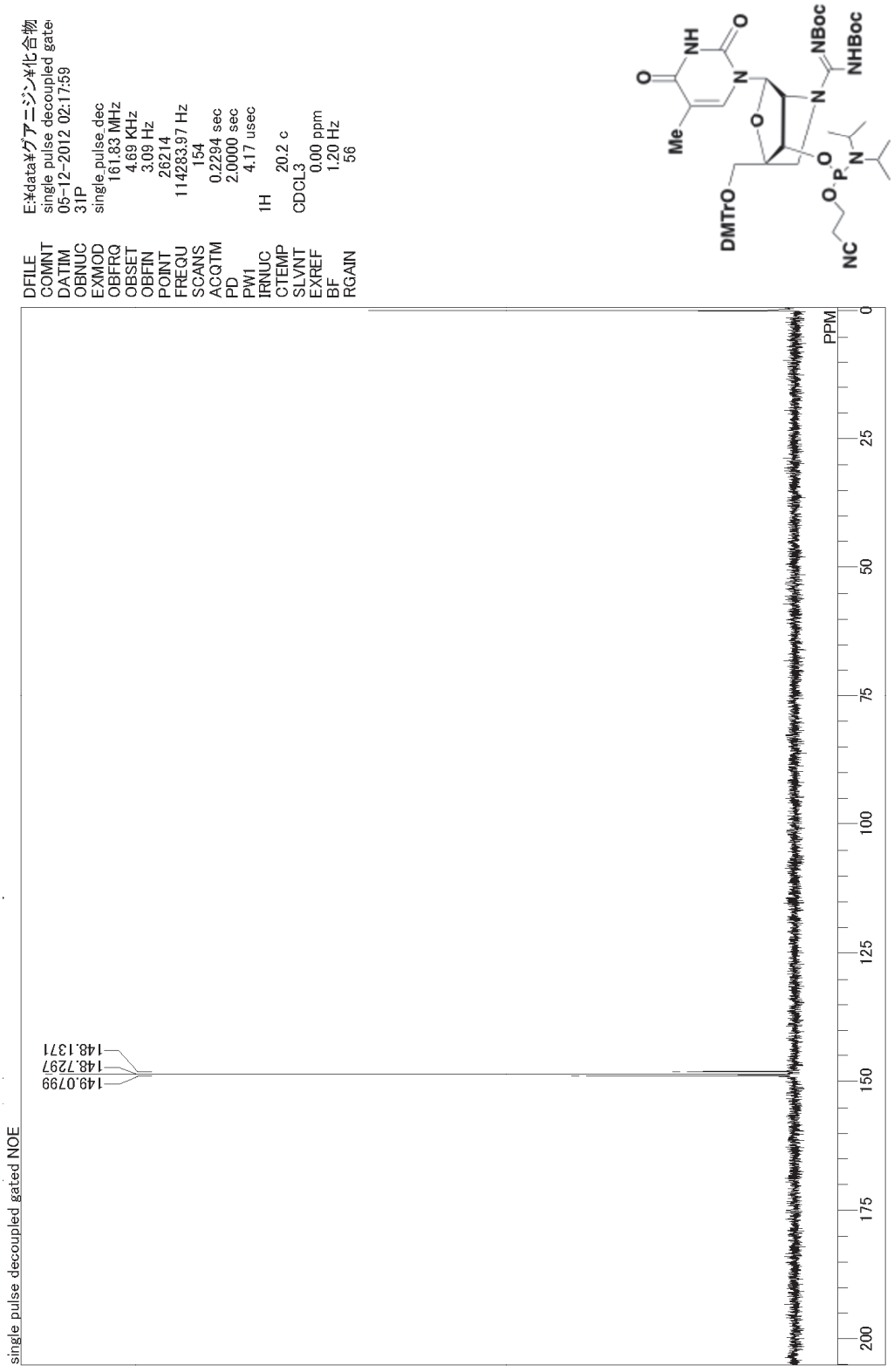

Fig. SI-11: ^{31}P-NMR spectrum of compound **9**

4. Synthesis, purification and characterization of oligonucleotides

Synthesis of 0.2 μmol scale of oligonucleotides **ON-2**, **ON-3**, **ON-4**, **ON-5**, **ON-6**, **ON-14**, **ON-16**, **ON-20**, and **ON-21** modified with GuNA was performed using the nS-8 oligonucleotide synthesizer (GeneDesign, Inc.) according to the standard phosphoroamidite protocol with 0.5 M 1*H*-tetrazole as the activator. The standard synthesis cycle was used for assembly of the reagents and synthesis of the oligonucleotides, except that the coupling time was extended to 20 minutes. The synthesis was carried out in trityl on mode and was treated with concentrated ammonium hydroxide at room temperature for 1 h to cleave the synthesized oligonucleotides from the solid support. The oligonucleotides were initially purified by Waters Sep-Pak Plus C_{18} Environmental Cartridge. The Boc-protecting group of the synthesized oligonucleotides were deprotected by 75% trifluoroacetic acid (TFA) and purified by Nap-10 column. The oligonucleotides were further purified by reverse-phase HPLC with Waters XBridge^{TM} C_{18} (4.6 X 50 mm analytical and 10 mm × 50 mm preparative) columns with a linear gradient of MeCN (5-10% over 30 min) in 0.1 M triethylammonium acetate (pH 7.0). The oligonucleotides were analyzed for purity by HPLC and characterized by MALDI-TOF mass spectroscopy.

Table SI-1. Sequences of oligonucleotides used in this study and their MALDI-TOF-mass. The GuNA-T modifications are indicated by bold **X**.

Entry	Oligonucleotide sequence	MALDI-TOF-MASS	
		Calcd. $[M - H]^-$	Found $[M - H]^-$
1	5'-d(TTTT**X**TTTTT)-3' (**ON-2**)	3048.03	3048.26
2	5'-d(TTTT**X**T**X**TTT)-3' (**ON-3**)	3117.09	3117.06
3	5'-d(TT**X**T**X**T**X**TTT)-3' (**ON-4**)	3186.16	3187.39
4	5'-d(TTT**XXX**TTT)-3' (**ON-5**)	3186.16	3185.63
5	5'-d(**X**T**X**T**X**T**X**T**X**T)-3' (**ON-6**)	3324.29	3323.75
6	5'-d(TTTTTTTT**X**T)-3' (**ON-14**)	3048.03	3048.69
7	5'-d(TTTTTTTTT**X**)-3' (**ON-16**)	3048.03	3048.03
8	5'-d(TTTT**X**TTTCT)-3' (**ON-20**)	3033.02	3032.87
9	5'-d(TT**X**T**X**T**X**TCT)-3' (**ON-21**)	3171.15	3171.47

5. UV melting experiments and melting profiles

The UV melting experiments were carried out on SHIMADZU UV-1650 spectrometer equipped with a T_m analysis accessory. Equimolecular amounts of the target RNA or DNA strand and oligonucleotide were dissolved in 20 mM cacodylate buffer at pH 6.8 containing 200 mM NaCl to give final strand concentration of 4 μM. The samples were annealed by heating at 95 °C followed by slow cooling to room temperature. The melting profile was recorded at 260 nm from 0 to 90 °C at a scan rate of 0.5 °C /min. The T_m was calculated as the temperature of the half-dissociation of the formed duplexes, determined by the first derivative of the melting curve.

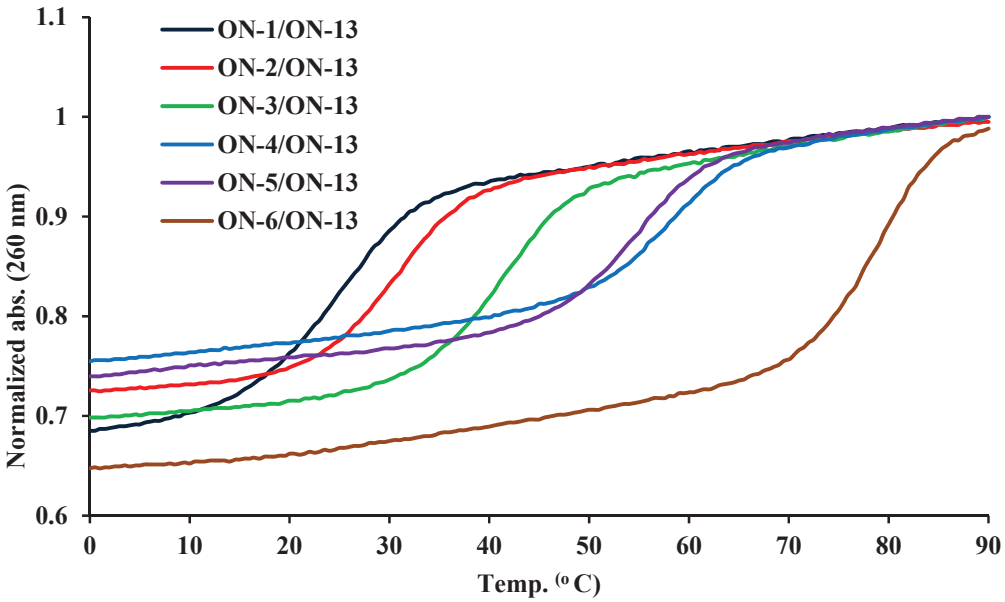

Fig SI-12. UV melting curves (T_m curves) for the duplexes formed by natural oligonucleotide **ON-1** and GuNA–modified oligonucleotides **ON-2**, **ON-3**, **ON-4**, **ON-5**, and **ON-6** respectively, against the complementary DNA **ON-13**.

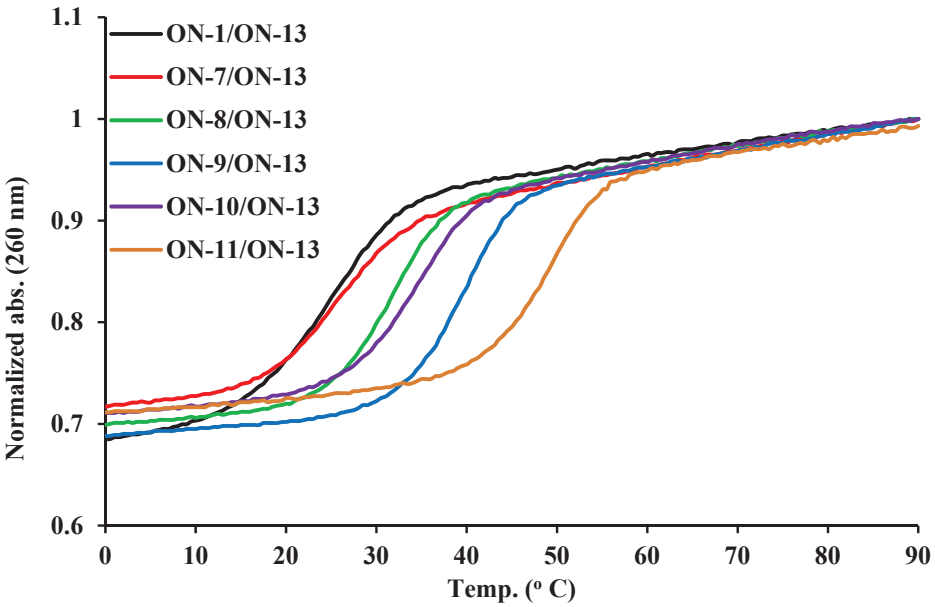

Fig SI-13. UV melting curves (T_m curves) for the duplexes formed by natural oligonucleotide **ON-1** and 2',4'-BNA/LNA–modified oligonucleotides **ON-7**, **ON-8**, **ON-9**, **ON-10**, and **ON-11** respectively, against the complementary DNA **ON-13**.

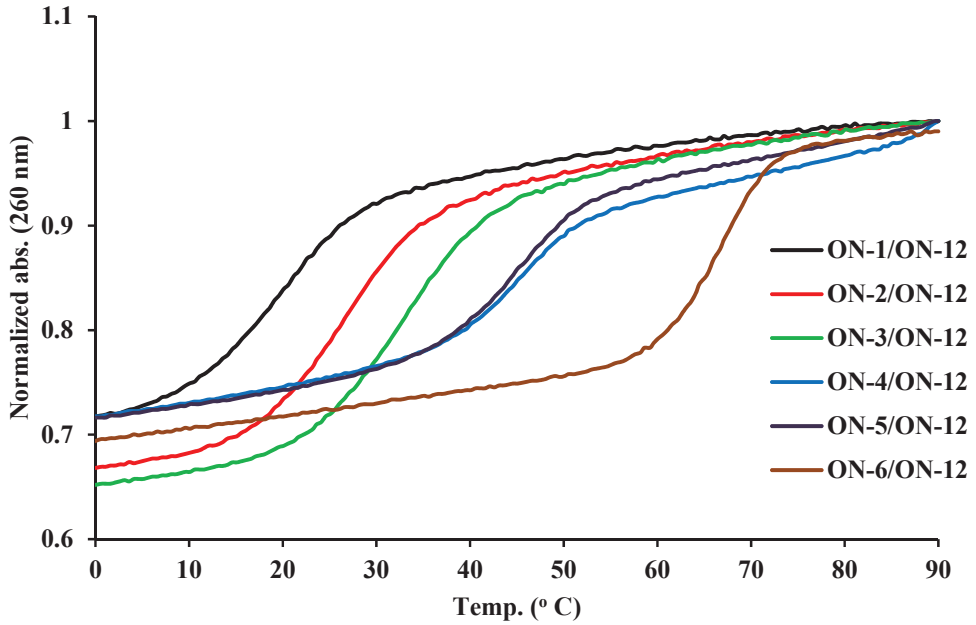

Fig. SI-14. UV melting curves (T_m curves) for the duplexes formed by natural oligonucleotide **ON-1** and GuNA–modified oligonucleotides **ON-2**, **ON-3**, **ON-4**, **ON-5**, and **ON-6** respectively, against the complementary RNA **ON-12**.

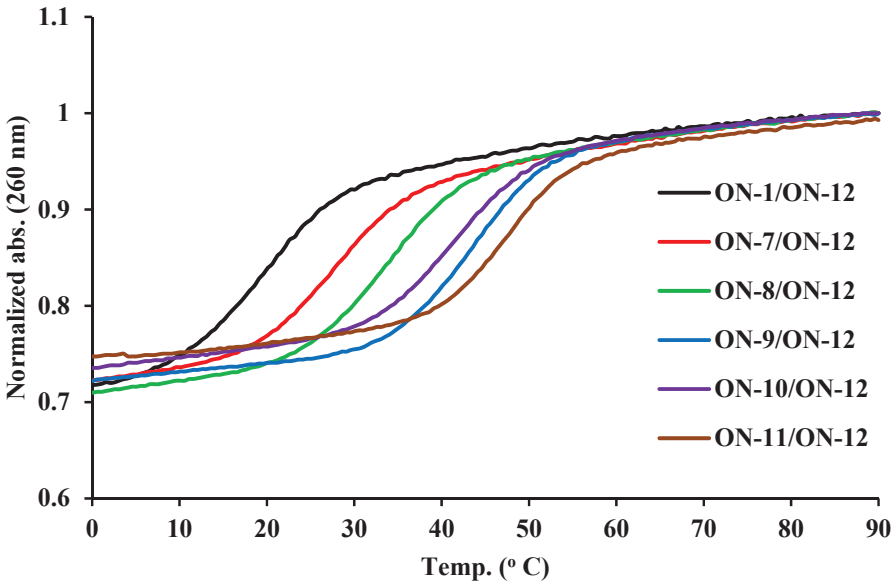

Fig.SI-15. UV melting curves (T_m curves) for the duplexes formed by natural oligonucleotide **ON-1** and 2',4'-BNA/LNA–modified oligonucleotides **ON-7**, **ON-8**, **ON-9**, **ON-10**, and **ON-11** respectively, against the complementary RNA **ON-12**

Table SI-2. T_m (°C) values of duplex formed by GuNA and 2',4'-BNA/LNA-modified oligonucleotides with complementary ssDNA

Oligonucleotides	**T_m(ΔT_m / mod.) (°C)**
	DNA complement
5'- TTTTTTTTCT-3' (**ON-19**)	29
5'-TTTT**X**TTTCT-3' (**ON-20**)	32 (+6.0)
5'-TT**X**T**X**T**X**TCT-3' (**ON-21**)	50 (+7.0)
5'-TTTT**Y**TTTCT-3 (**ON-22**)	29 (0.0)
5'-TT**Y**T**Y**T**Y**TCT-3' (**ON-23**)	35 (+2.0)

Conditions : 20 mM Cacodylate buffer (pH 6.8), 200 mM KCl, 4 μM each oligonucleotide, 0.5°C/min, 260 nm. Target strand: 3'-d(AAAAAAAAGA)-5' (**ON-24**), **X** = GuNA-T, **Y** = 2',4'-BNA/ LNA-T

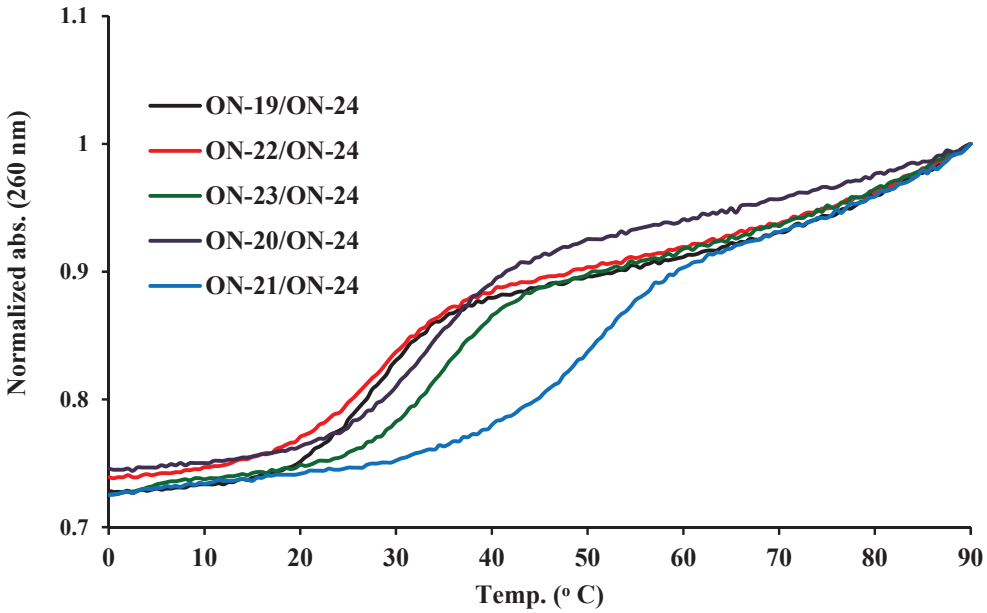

Fig. SI-16. UV melting curves (*Tm* curves) for the duplexes formed by natural oligonucleotide **ON-19** and 2',4'-BNA/LNA–modified oligonucleotides **ON-22** and **ON-23**, and GuNA-modified oligonucleotides **ON-20**, and **ON-21**, respectively, against the complementary DNA **ON-24**

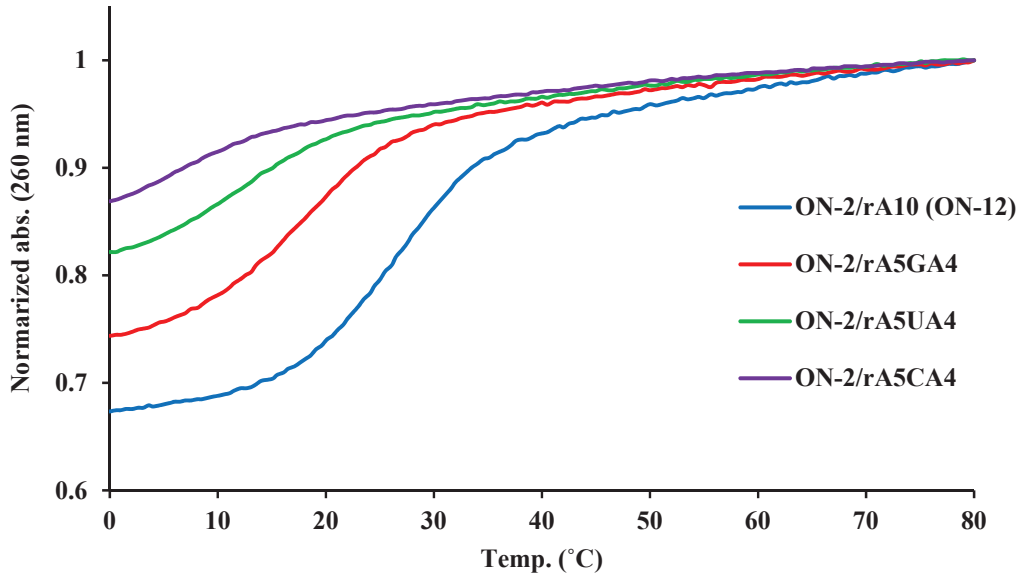

Fig. SI-17. Mismatch discrimination studies. UV melting curves (T_m curves) for the duplexes formed by GuNA-modified oligonucleotide (**ON-2**) against match RNA target (**ON-12**) and the mismatched RNAs; target RNAs = 5'-r(AAAAA**X**AAAA)-3'; **X** = A (**ON-12**), **X**= G (r**A5GA4**), **X** = U (r**A5UA4**), **X** = C (**rA5CA4**).

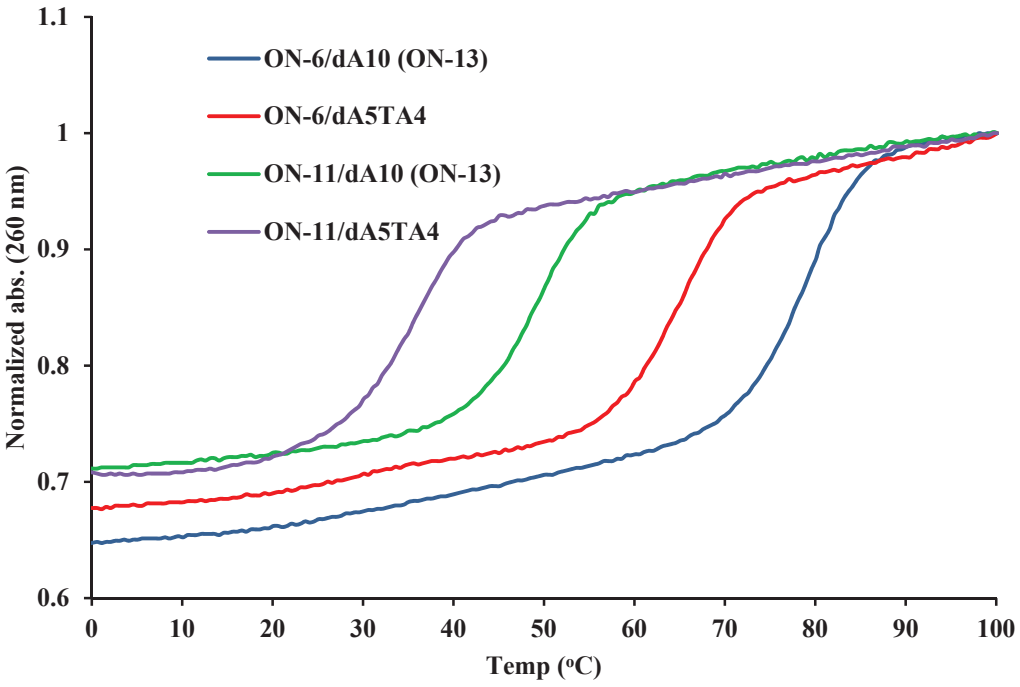

Fig. SI-18. Mismatch discrimination studies. Comparative UV melting curves (T_m curves) for the duplexes formed by GuNA-modified oligonucleotide (**ON-6**) and 2',4'-BNA/LNA-modified oligonucleotide (**ON-11**) against match DNA target (**ON-13**) and the mismatched DNAs; target DNAs = 5'-d(AAAAA**X**AAAA)-3'; **X** = A (**ON-13**), **X**= T (d**A5TA4**).

6. Nuclease resistance study

The sample solutions were prepared by dissolving 0.75 μmol of oligonucleotides in 50 mM Tris-HCl buffer (pH 8.0) containing 10 mM $MgCl_2$. In each sample solutions, 0.08 or 0.25 μg CAVP was added and the cleavage reaction was carried out at 37 °C. A portion of each reaction mixture was removed at timed intervals and heated to 90 °C for 5 min to deactivate the nuclease. Aliquots of the timed samples were analyzed by RP-HPLC to evaluate the amount of intact oligonucleotides remaining. The percentage of intact oligonucleotides in each sample was calculated and plotted against the digestion time to obtain a degradation curve with time.

When the experiment was carried out with the oligonucleotide sequence 5'-d(TTTTTTTT**X**T)-3', GuNA-modified oligonucleotide (**ON-14**) showed appearance of new peak at longer elution time on a HPLC profile. The intensity of the new peak increased with respect to time and no other peaks were observed, indicating no further digestion of the oligonucleotide. The MALDI-Tof mass of the degraded oligonucleotide confirmed that the natural T at 3'-terminal was digested and remaining sequence with GuNA at 3'-terminal was highly resistant to nuclease. In our next experiment, we used modified

oligonucleotides with modifications at 3'-terminal [5'-d(TTTTTTTTT**X**)-3']. As expected, GuNA-modified oligonucleotide (**ON-16**) showed high resistance towards the exonuclease which was comparable to that of S-oligo (**ON-18**). Note that we used more concentrated 3'-exonuclease (0.25 μg) in this experiment than in the previous one (0.08 μg). It was noted that nuclease resistance of GuNA was 2 times greater than that of 2',4'-BNA/LNA when modified at (n-1) position of 3'-terminal, while it was 15 times greater when modified at the 3'-terminal.

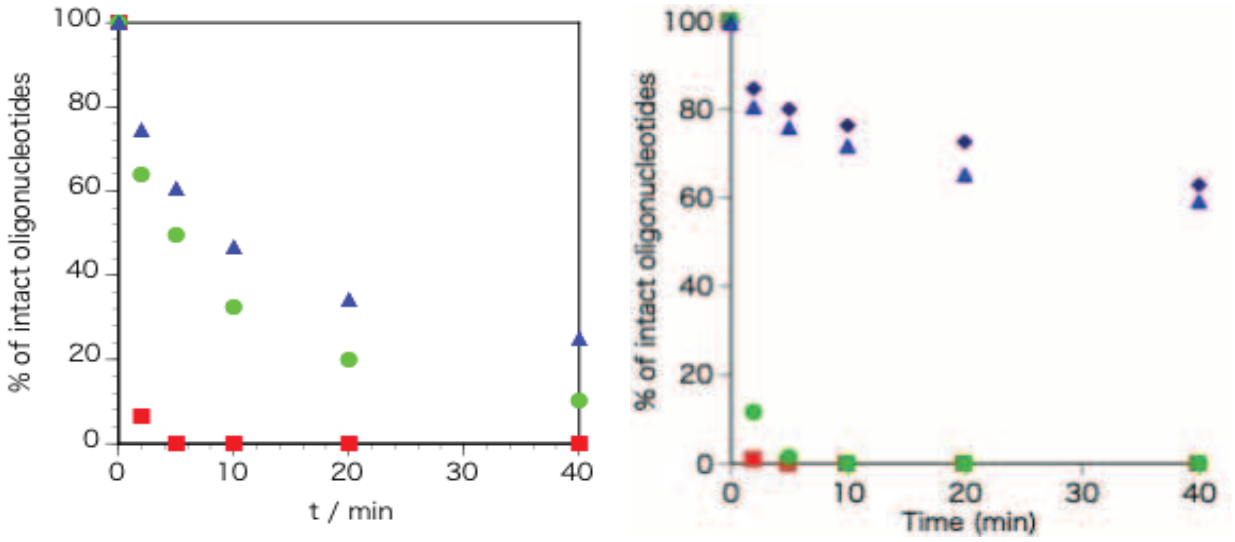

Fig. SI-19. (A) Nuclease resistance of 5'-d(TTTTTTTT**X**T)-3' against CAVP. **X** = GuNA-T (blue closed triangle) (**ON-14)**; natural DNA-T (red closed square) (**ON-1**); 2',4'-BNA/LNA-T (green closed circle) (**ON-15)**; (**B**) Nuclease resistance of 5'-d(TTTTTTTTT**X**)-3' against CAVP. **X** = S-oligo (purple closed diamond) (**ON-18**); GuNA (blue closed triangle) (**ON-16)**; 2',4'-BNA/LNA-T (green closed circle) (**ON-17)**; Experiments were performed at 37 ^{o}C in 100 μL of buffer containing 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, CAVP [0.08 μg for (**A**) and 0.25 μg for (**B**)] , and 7.5 μM of oligonucleotide.

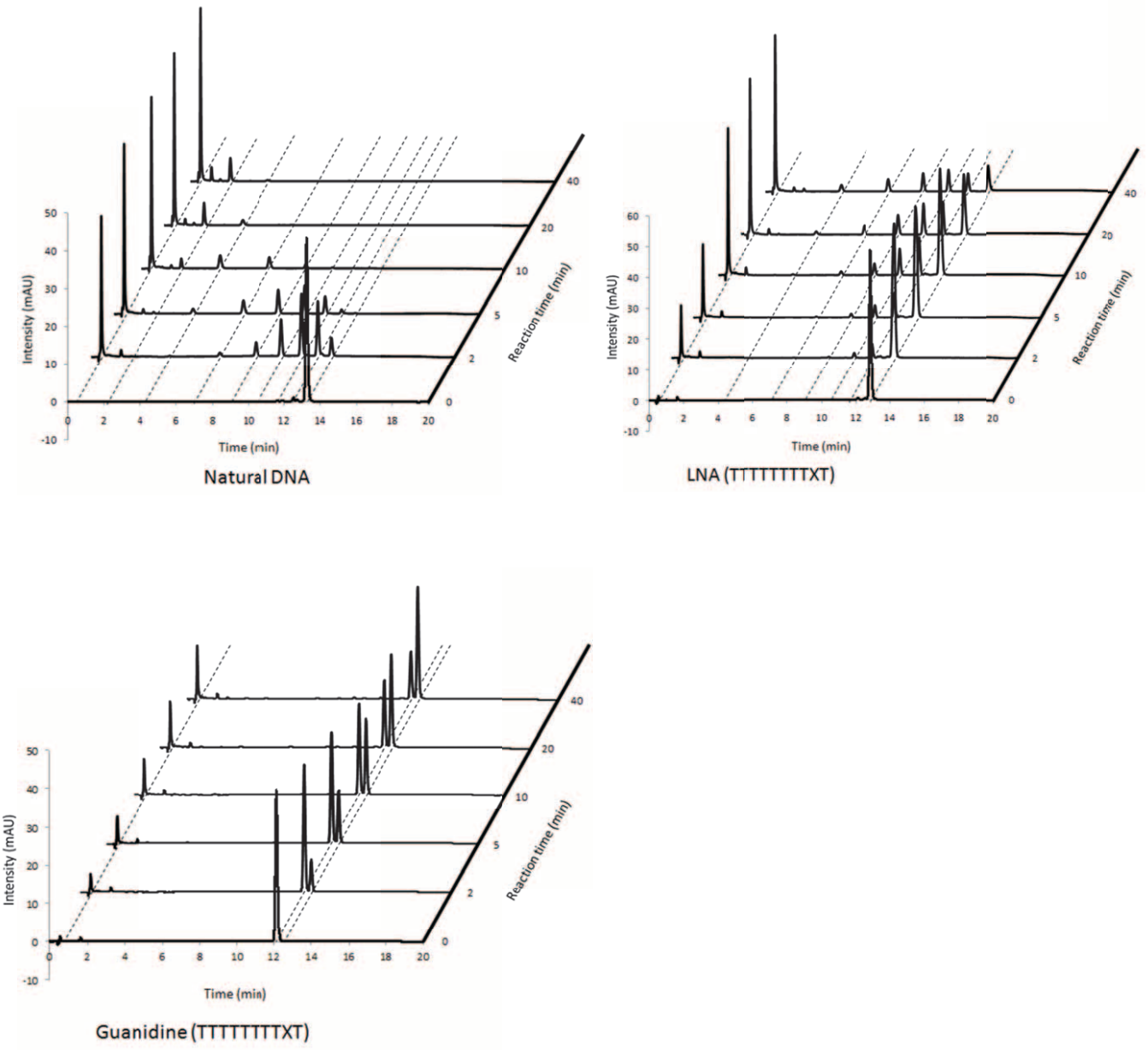

Fig. SI-20. HPLC profiles of the enzymatic degradation of Natural DNA (**ON-1**), 2',4'-BNA/LNA-modified (**ON-15),** and GuNA modified (**ON-14)** oligonucleotides with respect to time. Experiments were performed at 37 ^{o}C in 100 µL of buffer containing 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, CAVP (0.08 µg) and 7.5 µM of oligonucleotide.

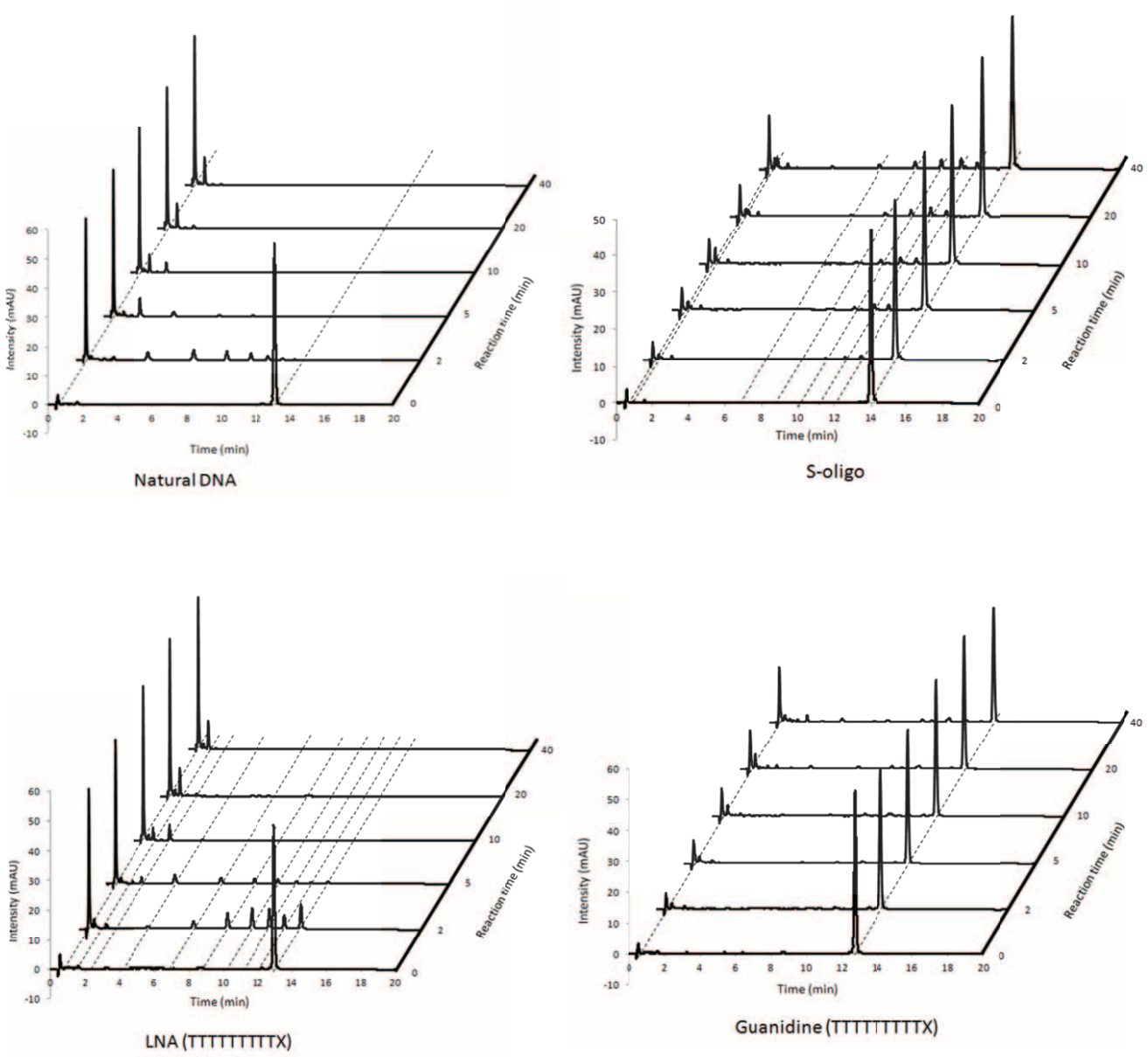

Fig. SI-21. HPLC profiles of the enzymatic degradation of Natural DNA (**ON-1**), S-oligo (**ON-18**), 2',4'-BNA/LNA-modified (**ON-17),** and GuNA modified (**ON-16)** oligonucleotides with respect to time. Experiments were performed at 37 ^{o}C in 100 µL of buffer containing 50 mM Tris-HCl (pH 8.0), 10 mM $MgCl_2$, CAVP (0.25 µg) and 7.5 µM of oligonucleotide.

7. CD spectroscopic analysis of the helical structure of GuNA-modified oligonucleotide duplexes.

Circular Dichorism (CD) spectra of the duplexes formed by GuNA with complementary RNA was found to be comparable to that of 2',4'-BNA/LNA, showing that GuNA-RNA heteroduplex adopts a conformation that closely resembles A-form geometry of RNA-RNA duplex (Fig. SI-21). In case of GuNA-DNA heteroduplex, the CD spectra of the duplex containing one to three GuNA modifications showed similarities with that of natural DNA-DNA duplex. Whereas, the GuNA-DNA heteroduplex containing five GuNA modifications showed characteristically different spectral pattern, with a prominent band at 260nm, than that of 2',4'-BNA/LNA – DNA and natural DNA – DNA duplex (Fig. SI-20).

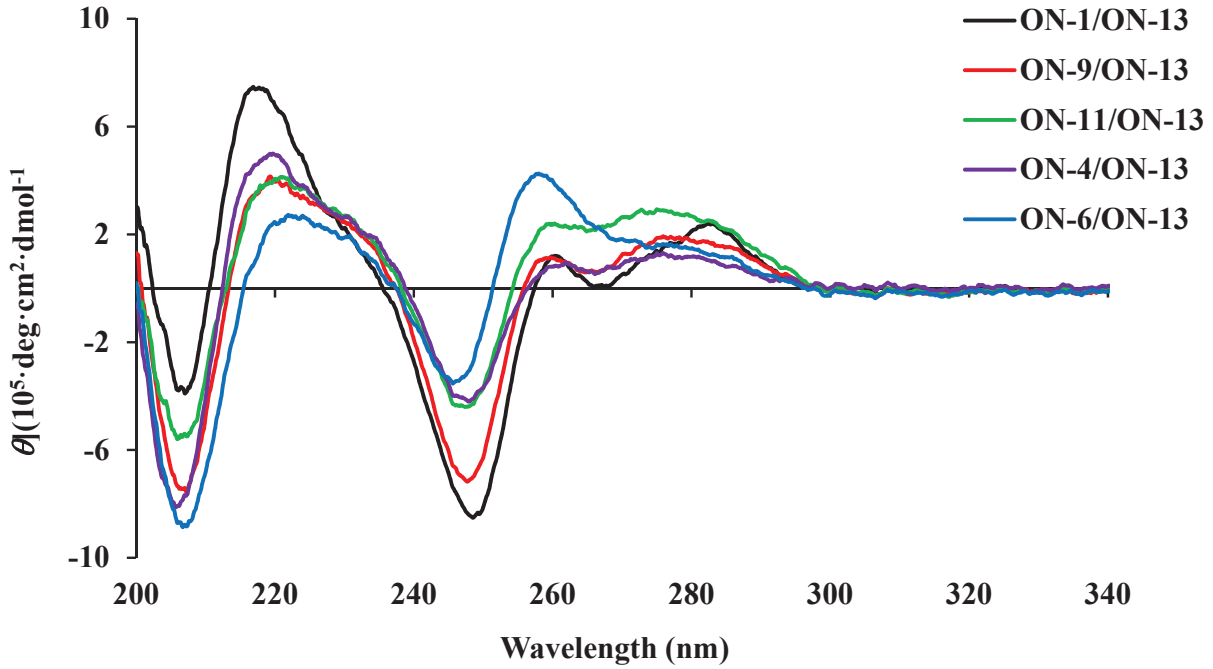

Fig SI-22. CD spectra for the duplex involving oligonucleotides **ON-1**, **ON-4**, **ON-6**, **ON-9**, and **ON-11**, and their complementary DNA, 5'-d(AAAAAAAAAA)-3' (**ON-13**). Condition: 20 mM Cacodylate buffer (pH 6.8), 200 mM KCl, 4 μM each oligonucleotide. Cell path length = 0.1 cm. Temperature = 4 ^{o}C.

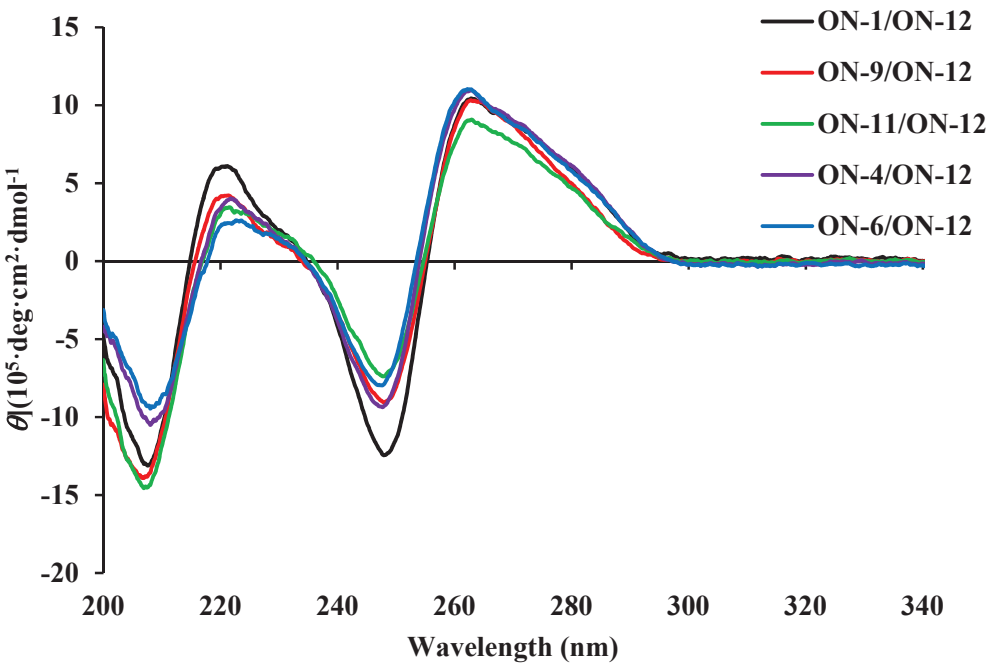

Fig. SI-23. CD spectra for the duplex involving oligonucleotides **ON-1**, **ON-4**, **ON-6**, **ON-9**, and **ON-11**, and their complementary RNA, 5'-r(AAAAAAAAAA)-3' (**ON-12**). Condition: 20 mM Cacodylate buffer (pH 6.8), 200 mM KCl, 4 μM each oligonucleotide. Cell path length = 0.1 cm. Temperature = 4 °C.

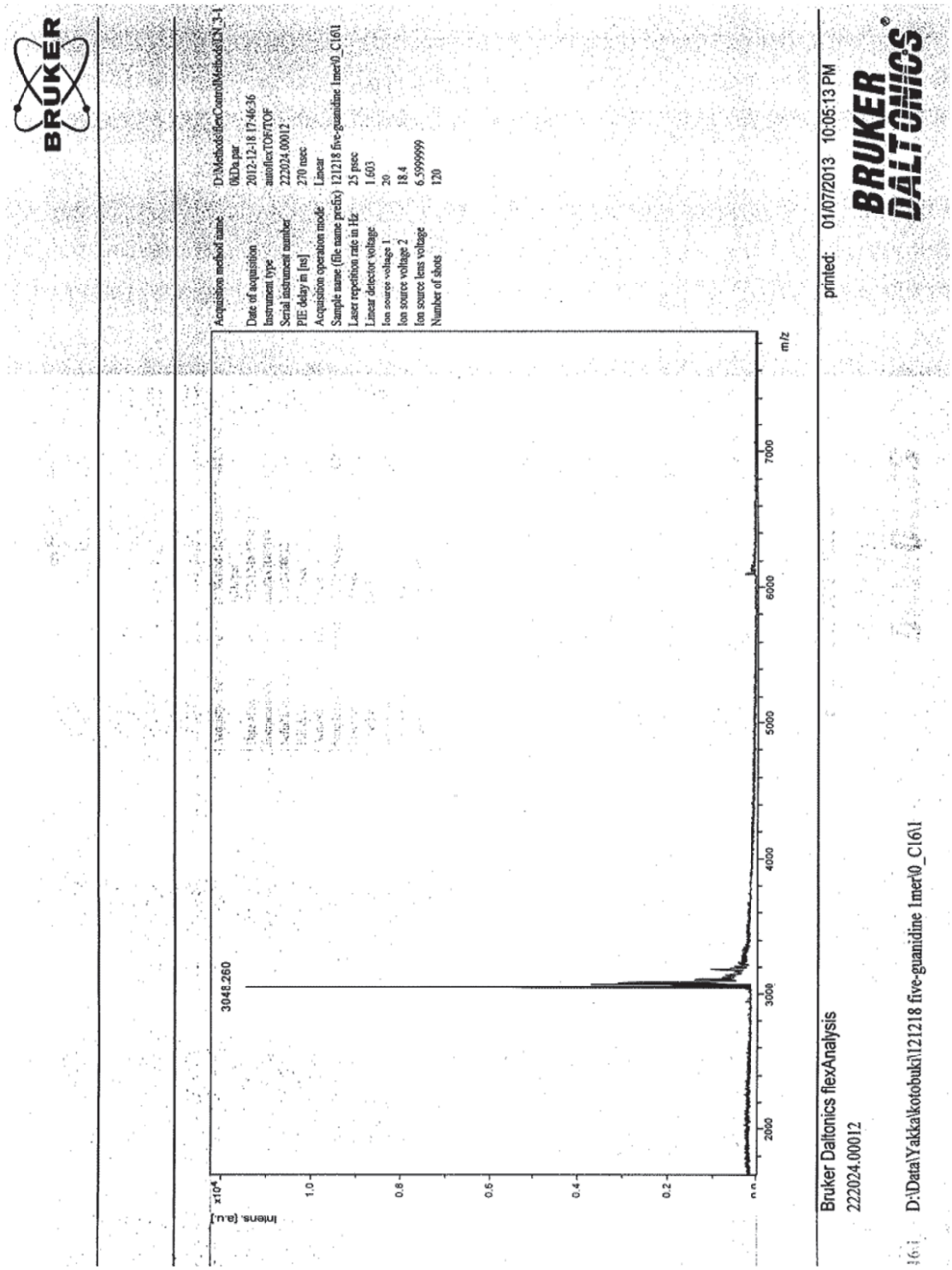

Fig. SI-24: MALDI-TOF-Mass of 5'-d(TTTT**X**TTTTT)-3' (**ON-2**)

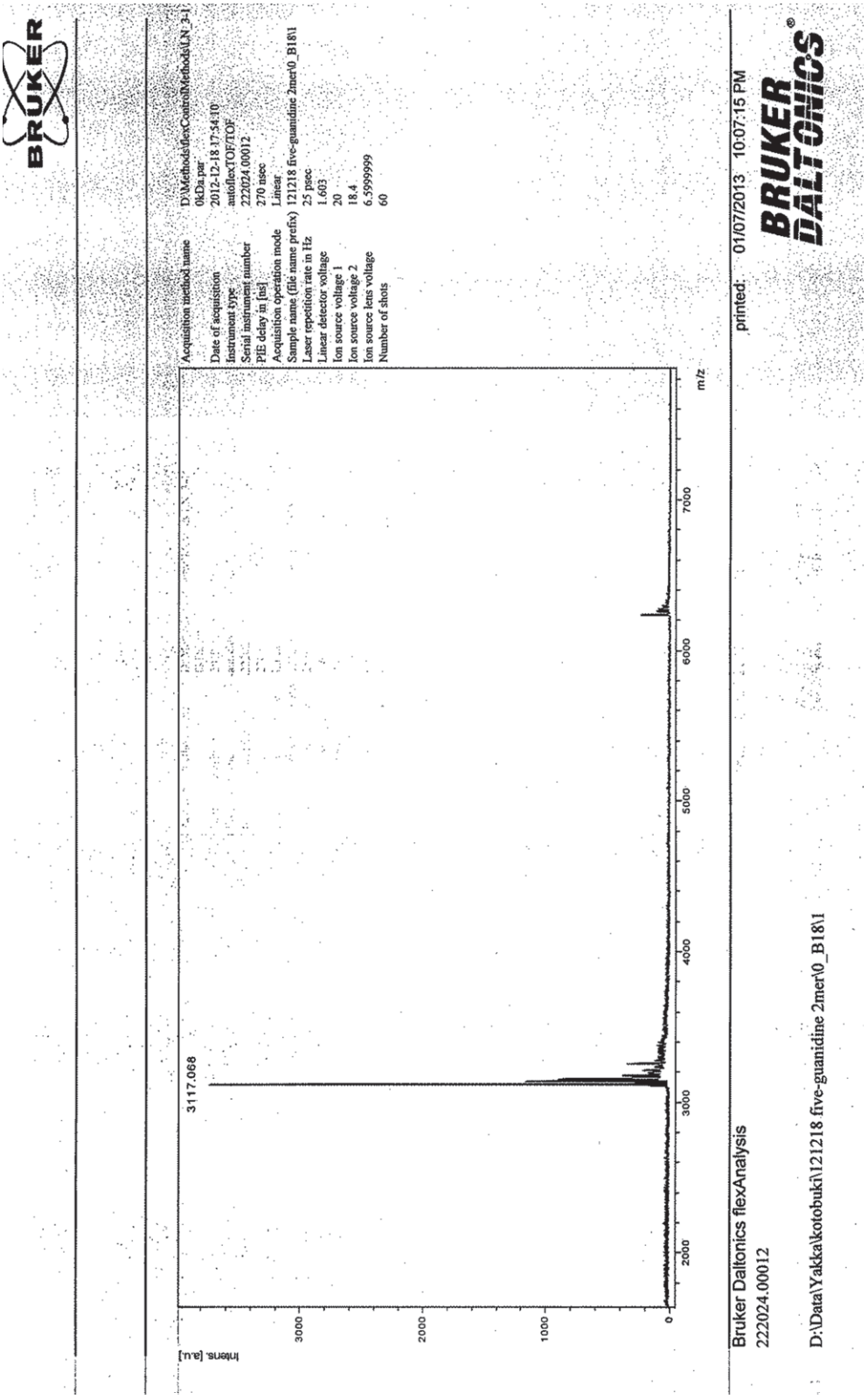

Fig. SI-25. MALDI-TOF mass of 5'-d(TTTT**X**T**X**TTT)-3' (**ON-3**)

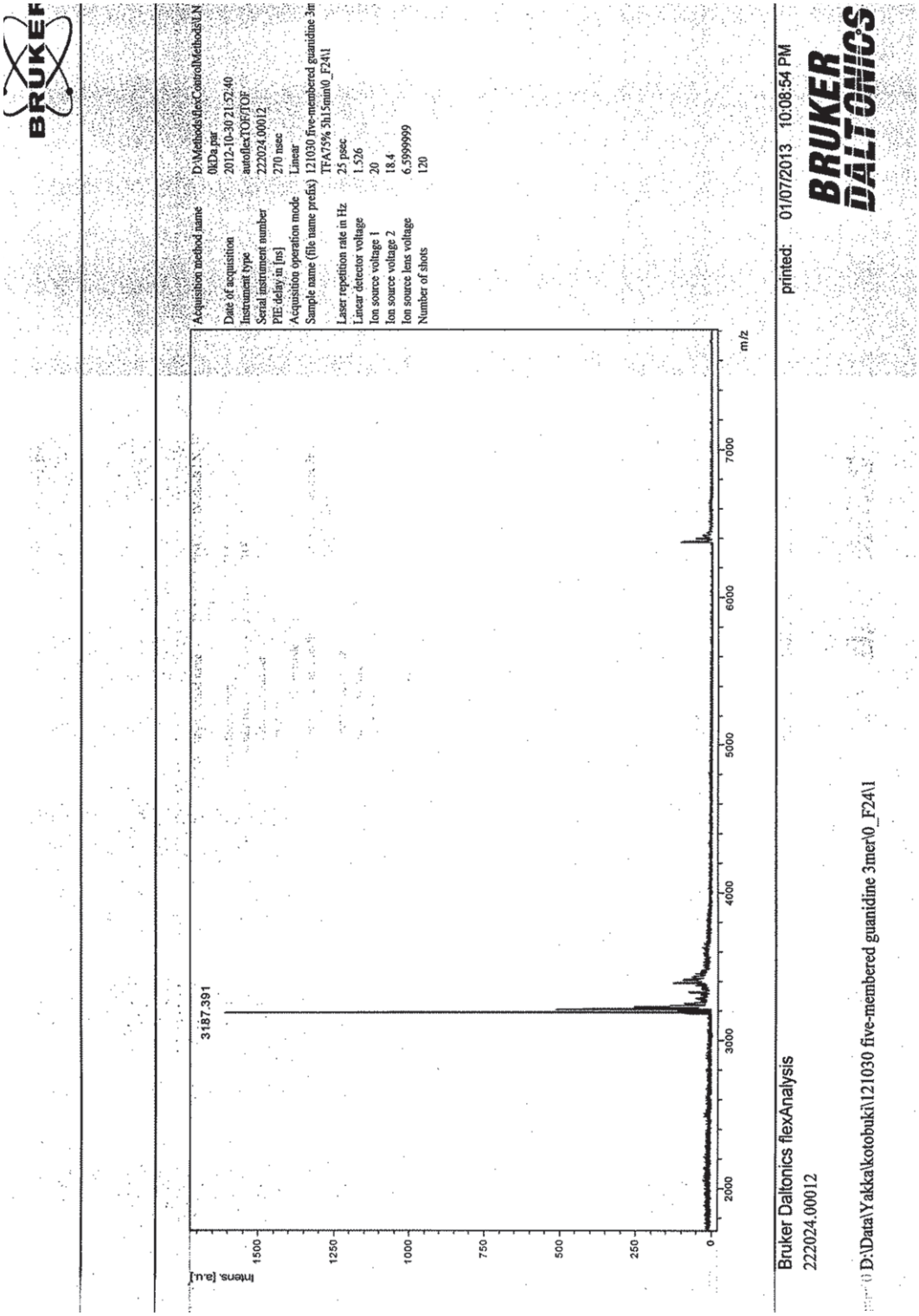

Fig. SI-26. MALDI-TOF mass of 5'-d(TT**X**T**X**T**X**TTT)-3' (**ON-4**)

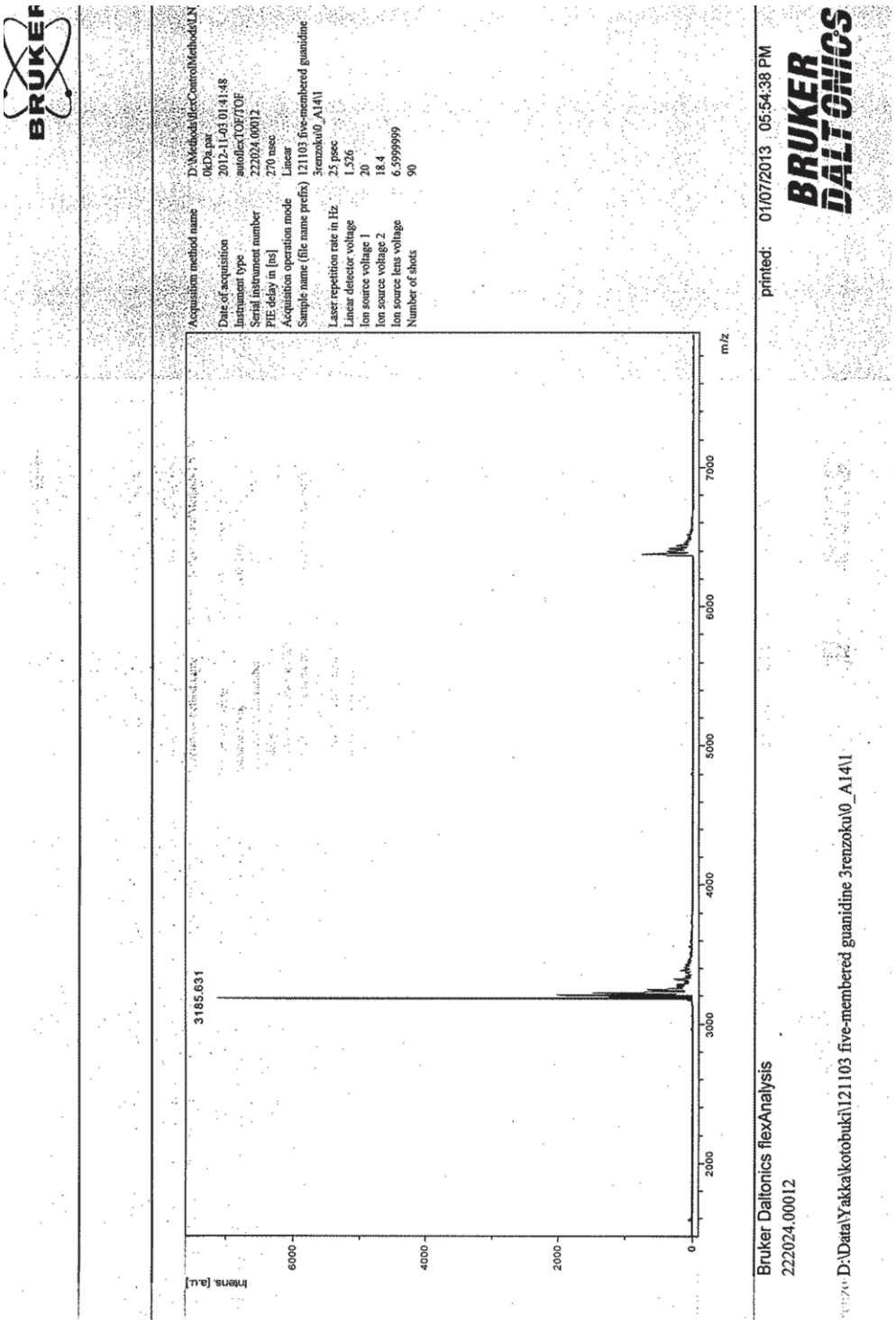

Fig. SI-27. MALDI-TOF mass of 5'-d(TTT**XXX**TTT)-3' (**ON-5**)

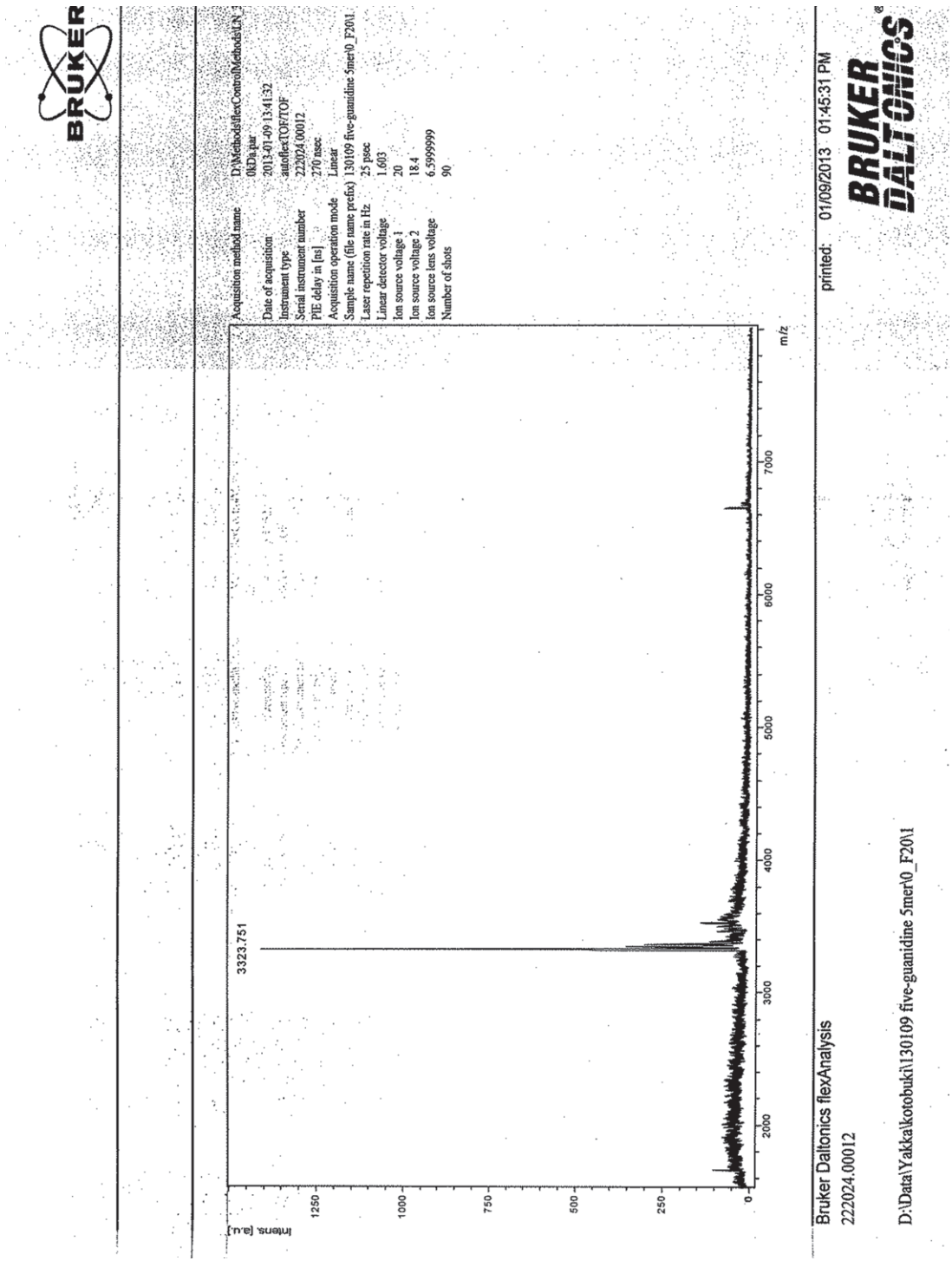

Fig. SI-28. MALDI-TOF mass of 5'-d(**X**T**X**T**X**T**X**T)-3' (**ON-6**)

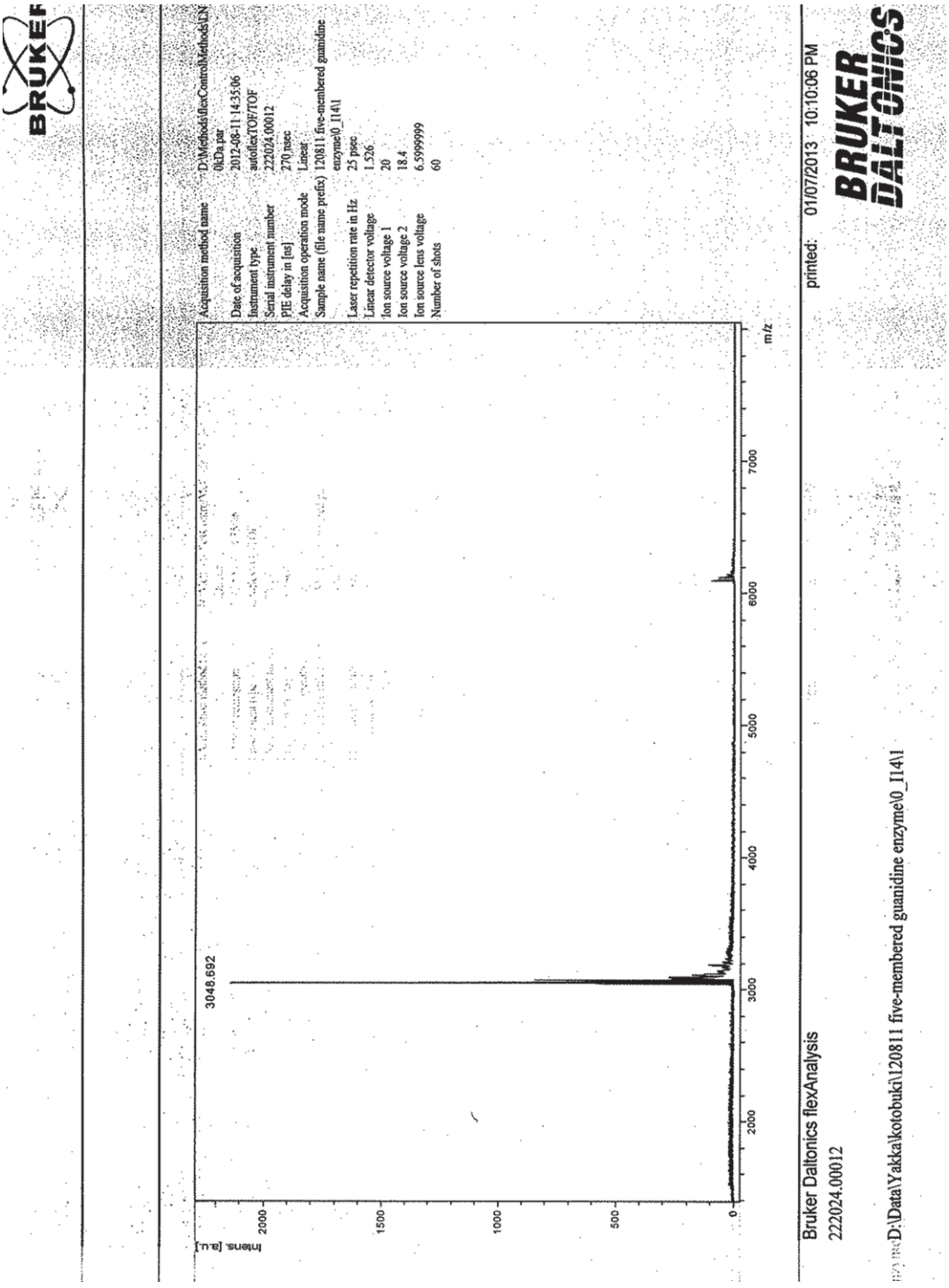

Fig. SI-29. MALDI-TOF mass of 5'-d(TTTTTTTT**X**T)-3' (**ON-14**)

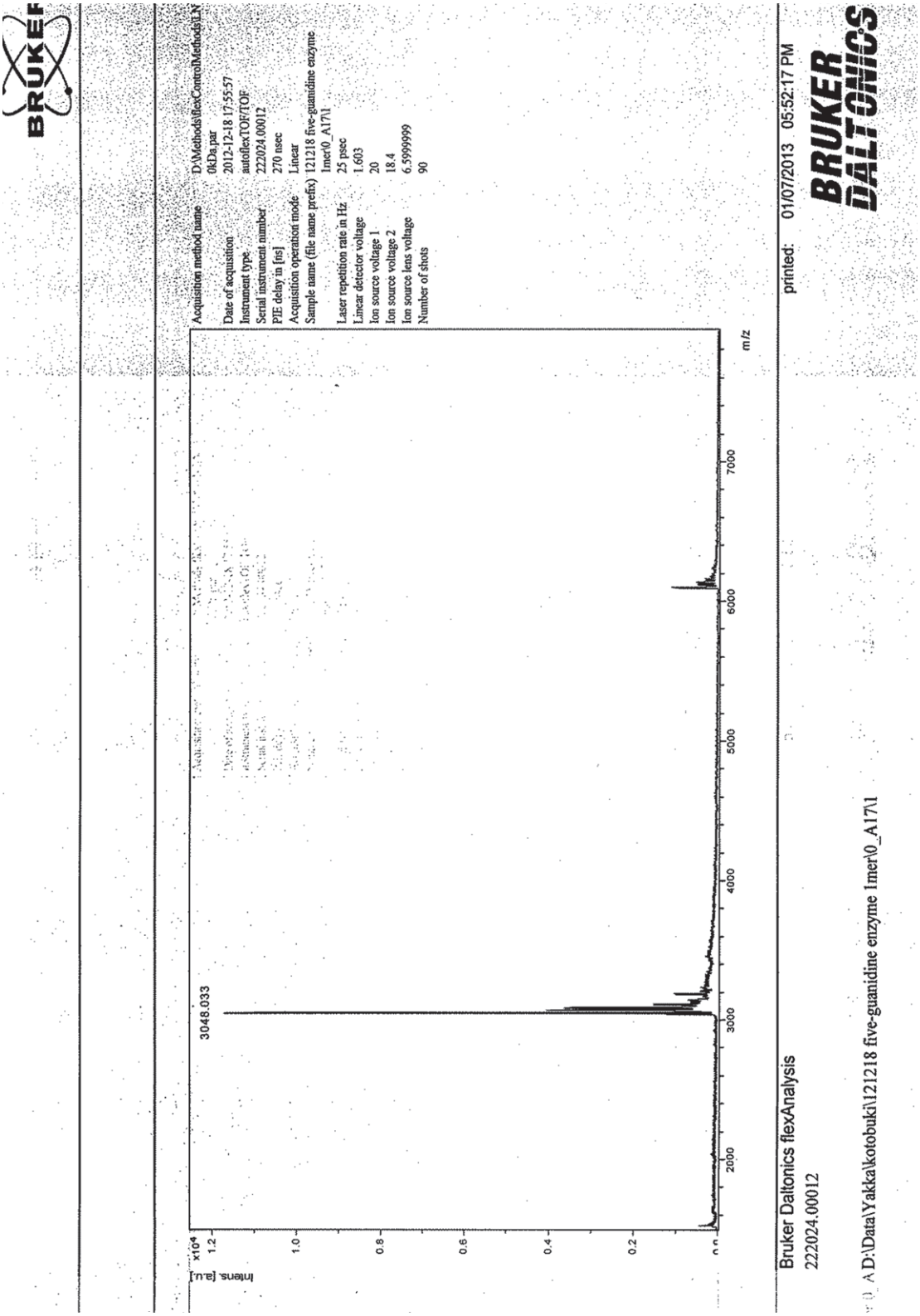

Fig. SI-30. MALDI-TOF mass of 5'-d(TTTTTTTTT**X**)-3' (**ON-16**)

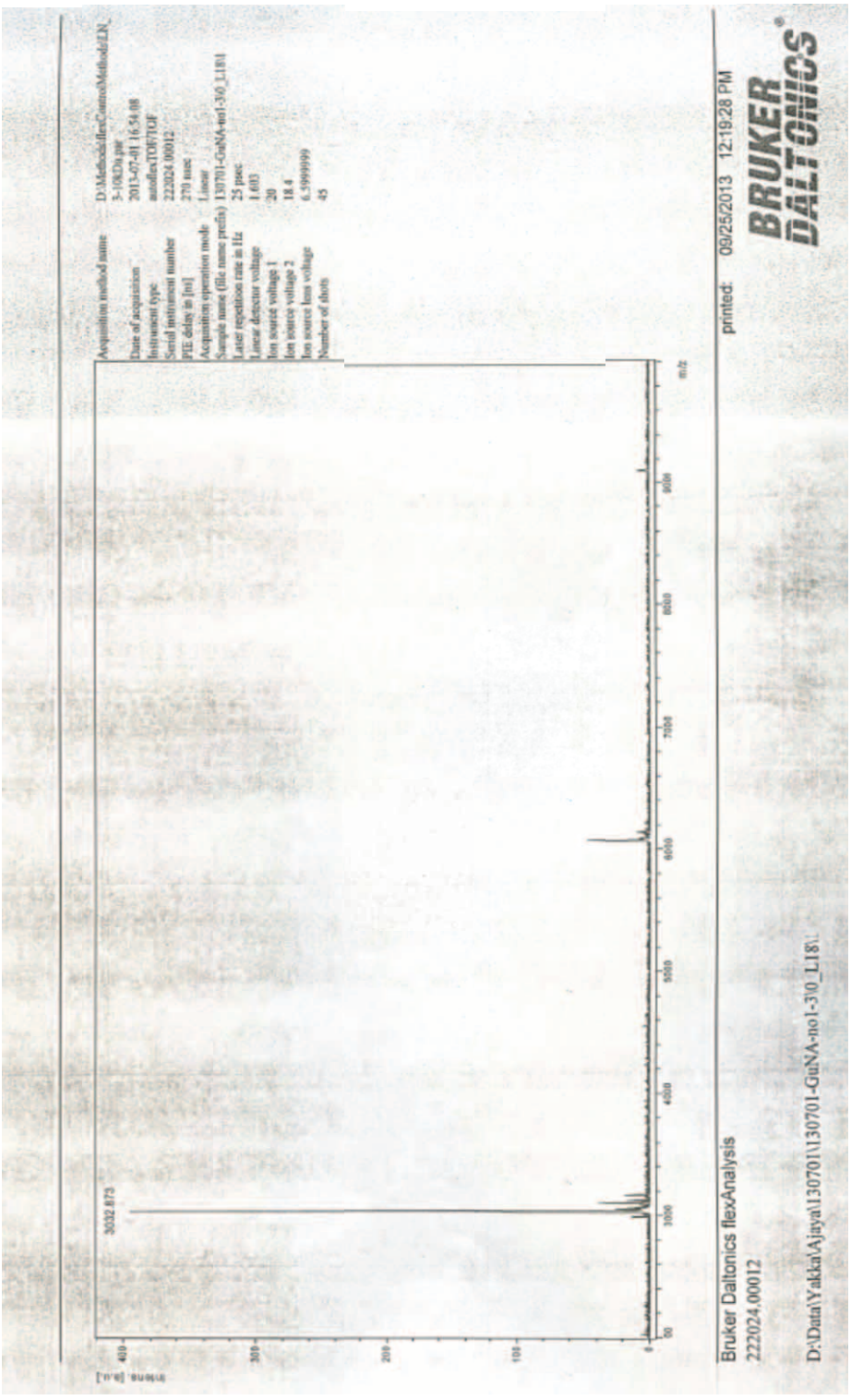

Fig. SI-31. MALDI-TOF mass of 5'-d(TTTT**X**TTTCT)-3' (**ON-20**)

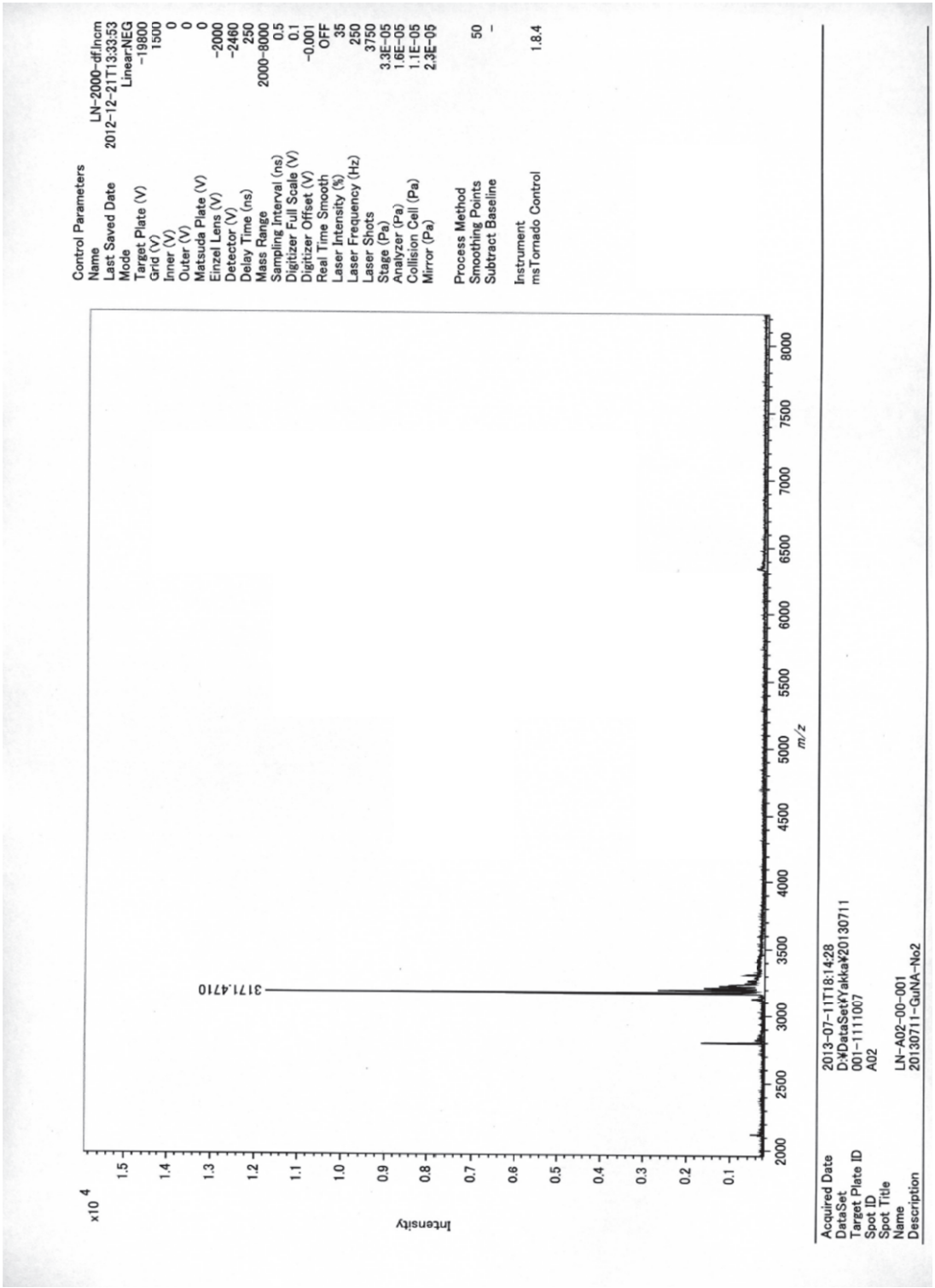

Fig. SI-32. MALDI-TOF mass of 5'-d(TT**X**T**X**T**X**TCT)-3' (**ON-21**)