Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.
This journal is © The Royal Society of Chemistry 2018

Supporting Information

Facile synthesis and fundamental properties of an *N*-methylguanidine-bridged nucleic acid (GuNA[NMe])

Naohiro Horie,[a] Shinji Kumagai,[b] Yutaro Kotobuki,[a] Takao Yamaguchi[a] and Satoshi Obika*[a,c]

[a]Graduate School of Pharmaceutical Sciences, Osaka University, 1-6 Yamadaoka, Suita, Osaka 565-0871, Japan. E-mail: obika@phs.osaka-u.ac.jp

[b]Soyaku. Innovation Reserch Division, Mitsubishi Tanabe Pharma Corporation, 1000 Kamoshida, Aoba-ku, Yokohama 227-0033, Japan.

[c]National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), 7-6-8 Saito-Asagi, Ibaraki, Osaka 567-0085, Japan.

Table of Contents

1. Synthesis of *N*-methylisothiourea derivatives 2b and 2c

1,2-dimethyl-3-(*tert*-butoxycarbonyl)isothiourea (2b) and 1,2-dimethyl-1-(*tert*-butoxycarbonyl)isothiourea (2c)

To a suspension of the 1,2-dimethyl-isothiourea hydroiodide (5.07 g, 21.8 mmol) in CH_2Cl_2 (110 mL), sat. aq. $NaHCO_3$ (110 mL) and di-*tert*-butyl dicarbonate (19 mL, 87.4 mmol) were added at 0 ˚C, and the resulting mixture was stirred for 7 h at room temperature. After completion of the reaction, the product was extracted with CH_2Cl_2. The organic phase was washed with brine, dried (Na_2SO_4), and concentrated under reduced pressure. The product was purified by column chromatography (hexane/AcOEt = 5:1) to afford **2b** (1.69g, 8.27 mmol, 38%) and **2c** (1.92 g, 9.40 mmol, 43%) as an yellow oil. compound **2b**: IR (KBr): 3256, 2977, 2932, 1634, 1583, 1463, 1360, 1270, 1150, 1051 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.48 (9H, s), 2.45 (3H, s), 2.96 (3H, d, J = 3.4 Hz), 9.77 (1H, brs) ppm; ^{13}C NMR ($CDCl_3$) δ: 13.0, 27.7, 29.5, 78.6, 161.7, 174.1 ppm; HRMS (MALDI) calcd. for $C_8H_{16}N_2O_2NaS$ $[M+Na]^+$ 227.0825, found 227.0824; compound **2c**: IR (KBr): 3356, 3292, 2979, 2930, 1715, 1576, 1457, 1423, 1393, 1368, 1344, 1257, 1149, 1044, 1031 cm^{-1}; ^1H NMR ($CDCl_3$) δ: 1.45 (9H, s), 2.23 (3H, s), 3.21 (3H, s), 8.62 (1H, brs) ppm; ^{13}C NMR ($CDCl_3$) δ: 14.8, 27.9, 34.1, 82.5, 153.4, 163.1 ppm; HRMS (MALDI) calcd. for $C_8H_{16}N_2O_2NaS$ $[M+Na]^+$ 227.0825, found 227.0822.

2. ^{1}H NMR, ^{13}C NMR and ^{31}P NMR spectra of new compounds

Figure S1. Compound **2b** (^{1}H NMR, $CDCl_3$, 500 MHz)

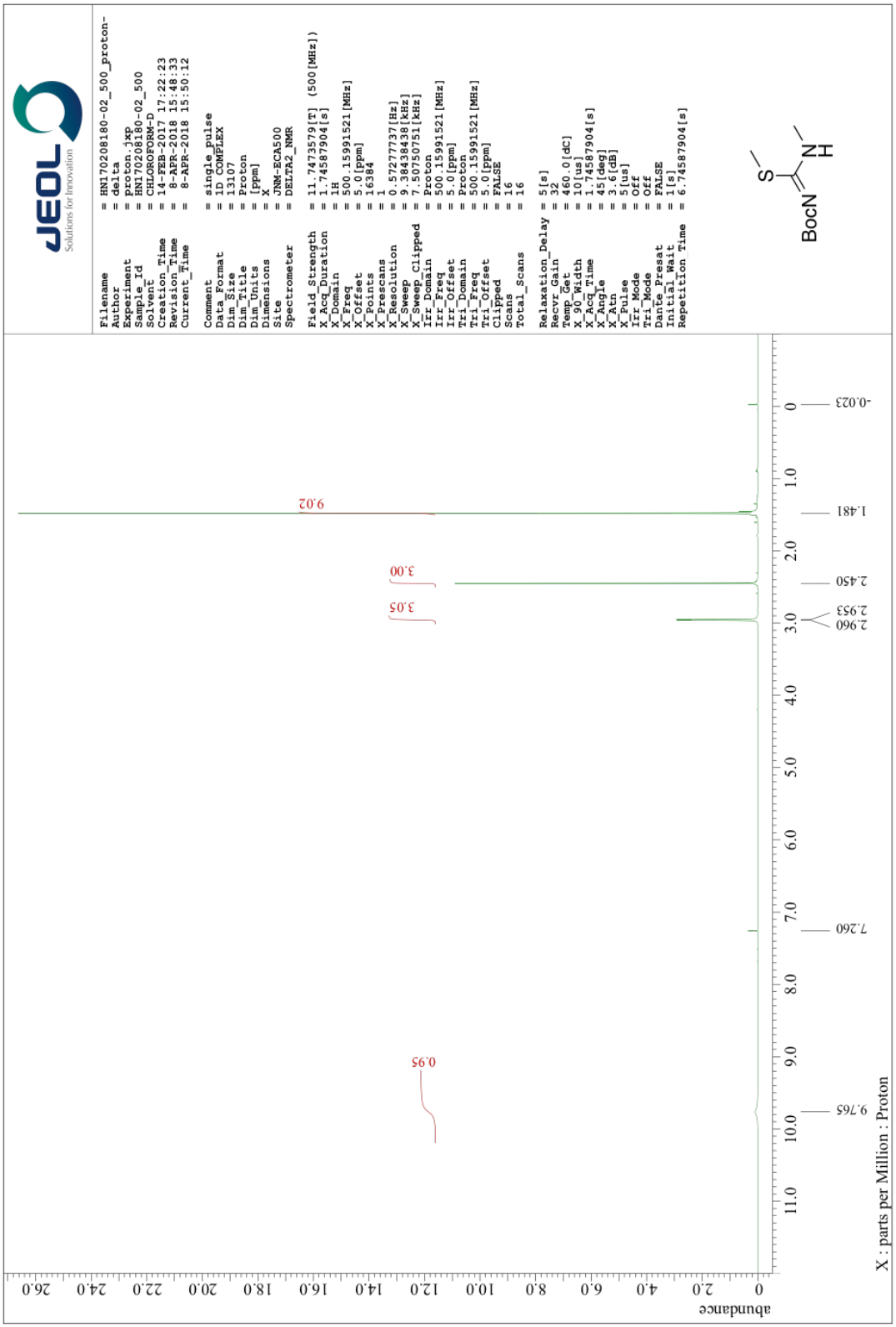


Figure S2. Compound **2b** (^{13}C NMR, $CDCl_3$, 100 MHz)

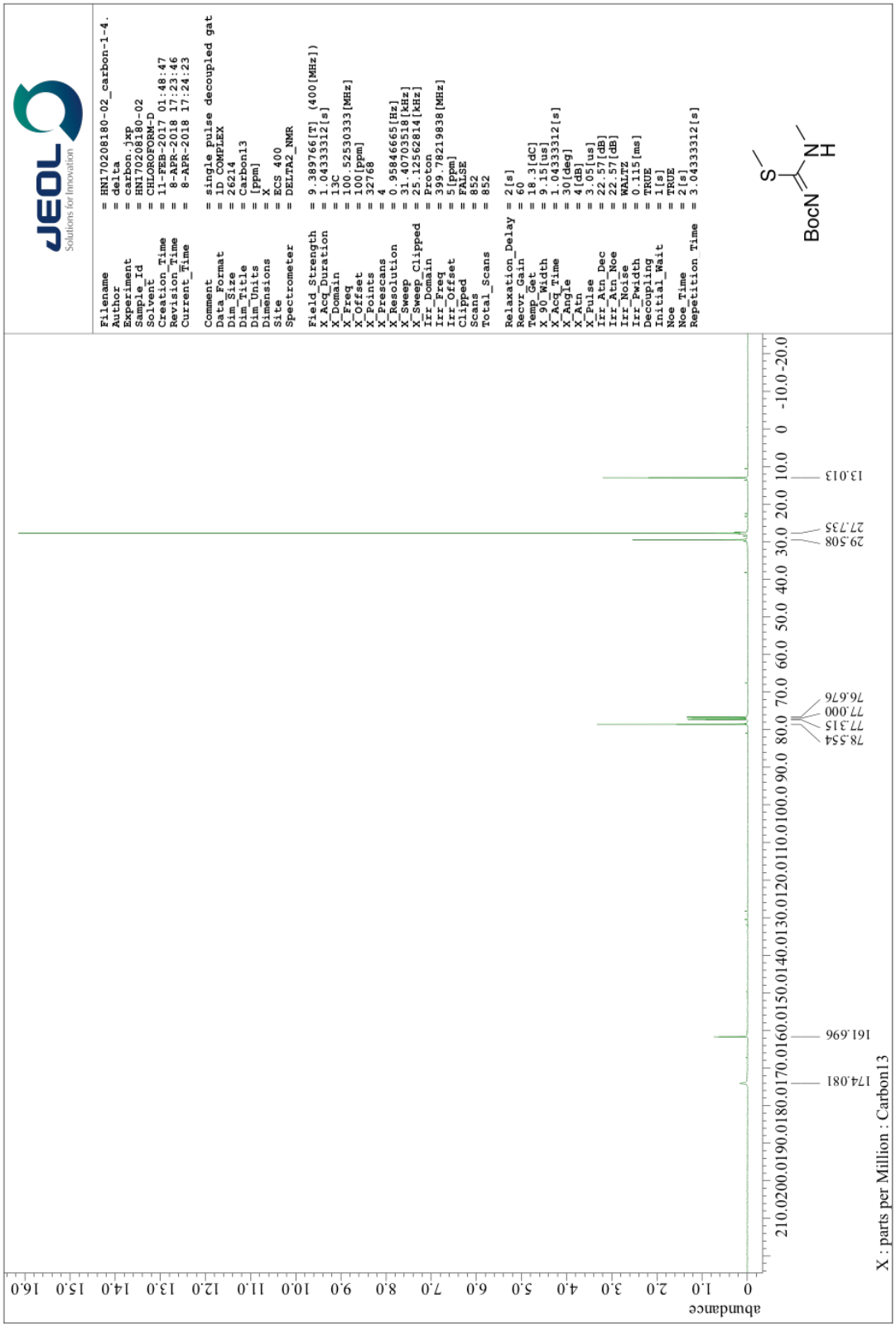


Figure S3. Compound **2c** (^{1}H NMR, $CDCl_3$, 400 MHz)

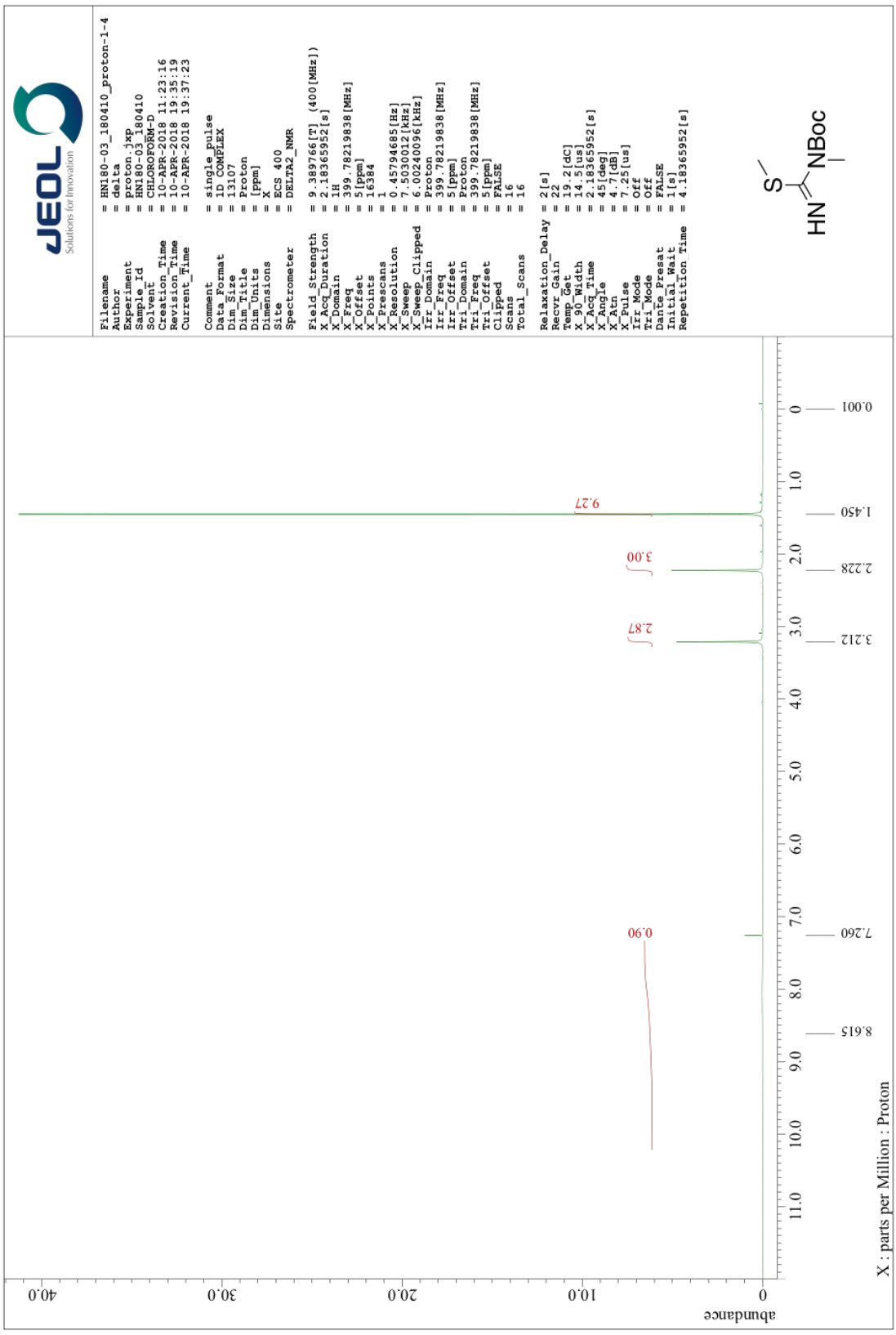


Figure S4. Compound **2c** (^{13}C NMR, $CDCl_3$, 100 MHz)

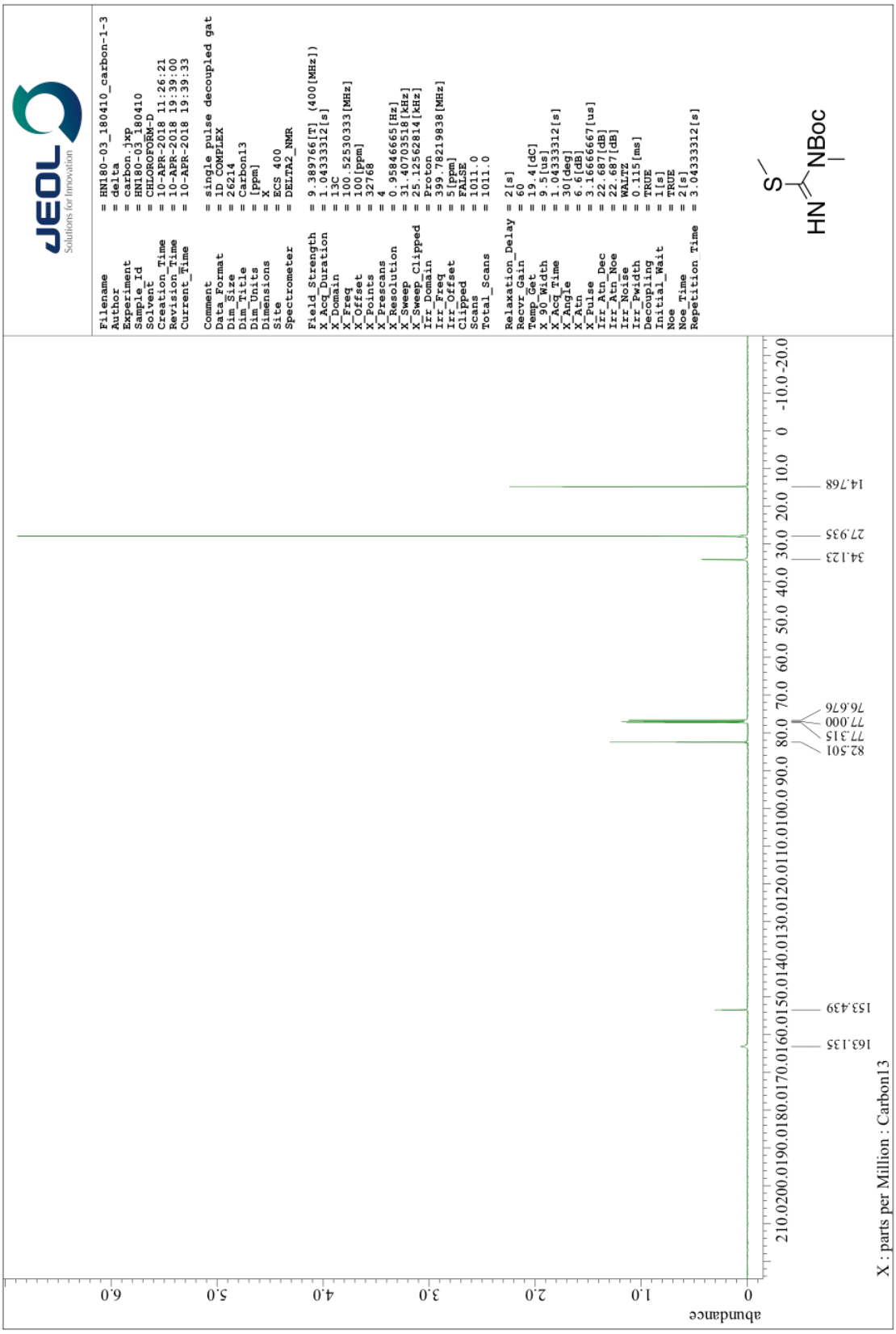


Figure S5. Compound **3b** (^{1}H NMR, $CDCl_3$, 300 MHz)

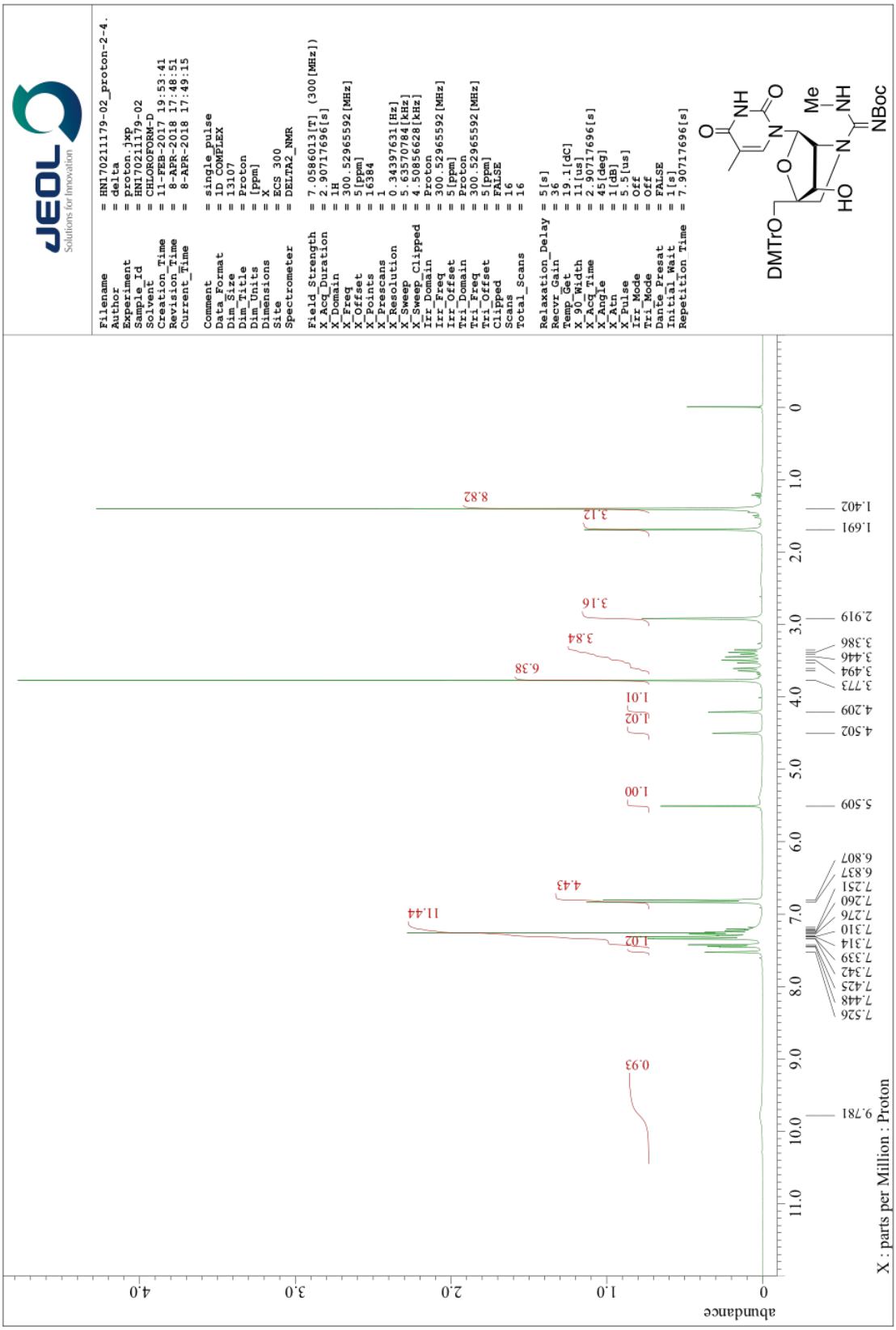


Figure S6. Compound **3b** (^{13}C NMR, $CDCl_3$, 76 MHz)

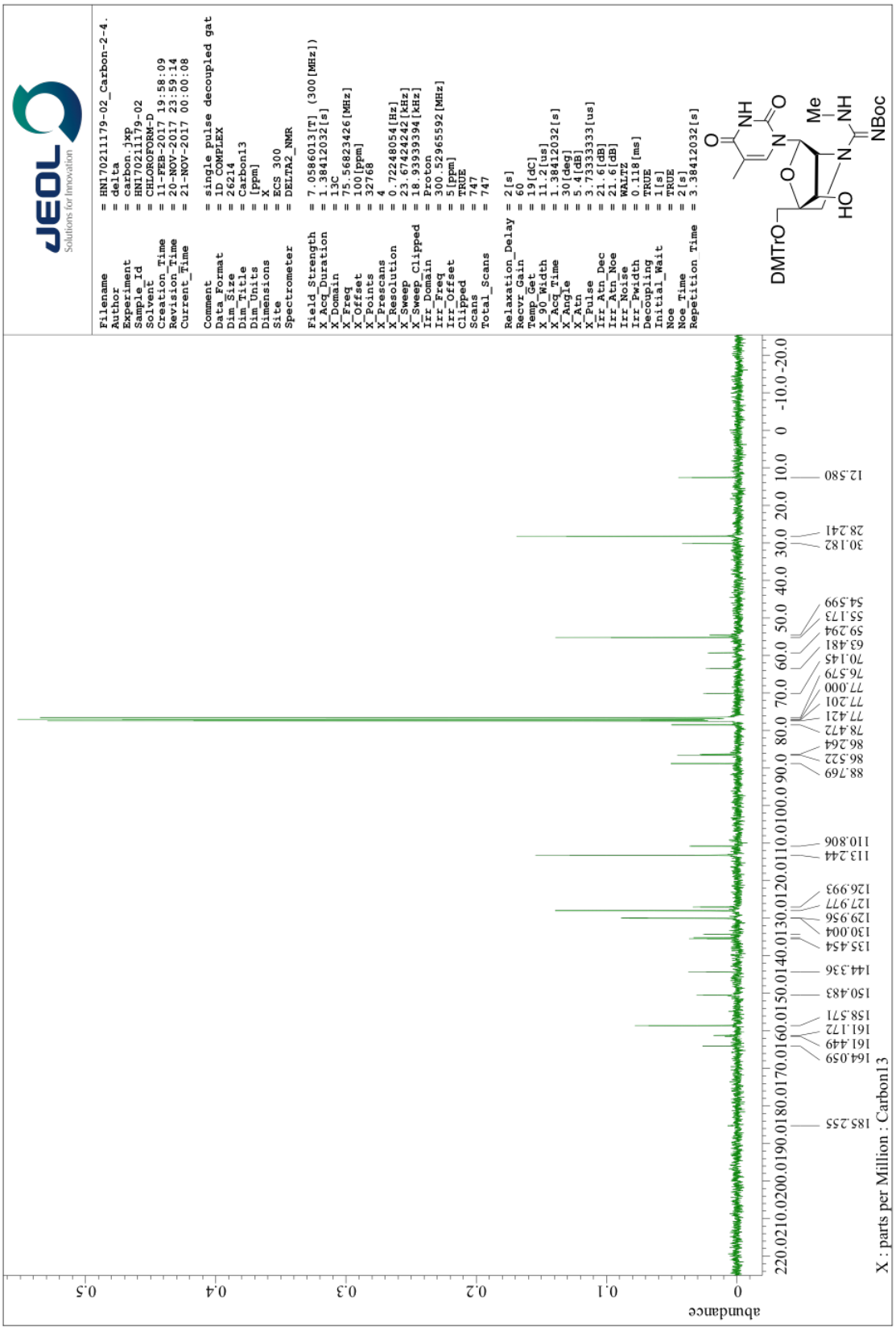


Figure S7. Compound **4** (^{1}H NMR, $CDCl_3$, 300 MHz)

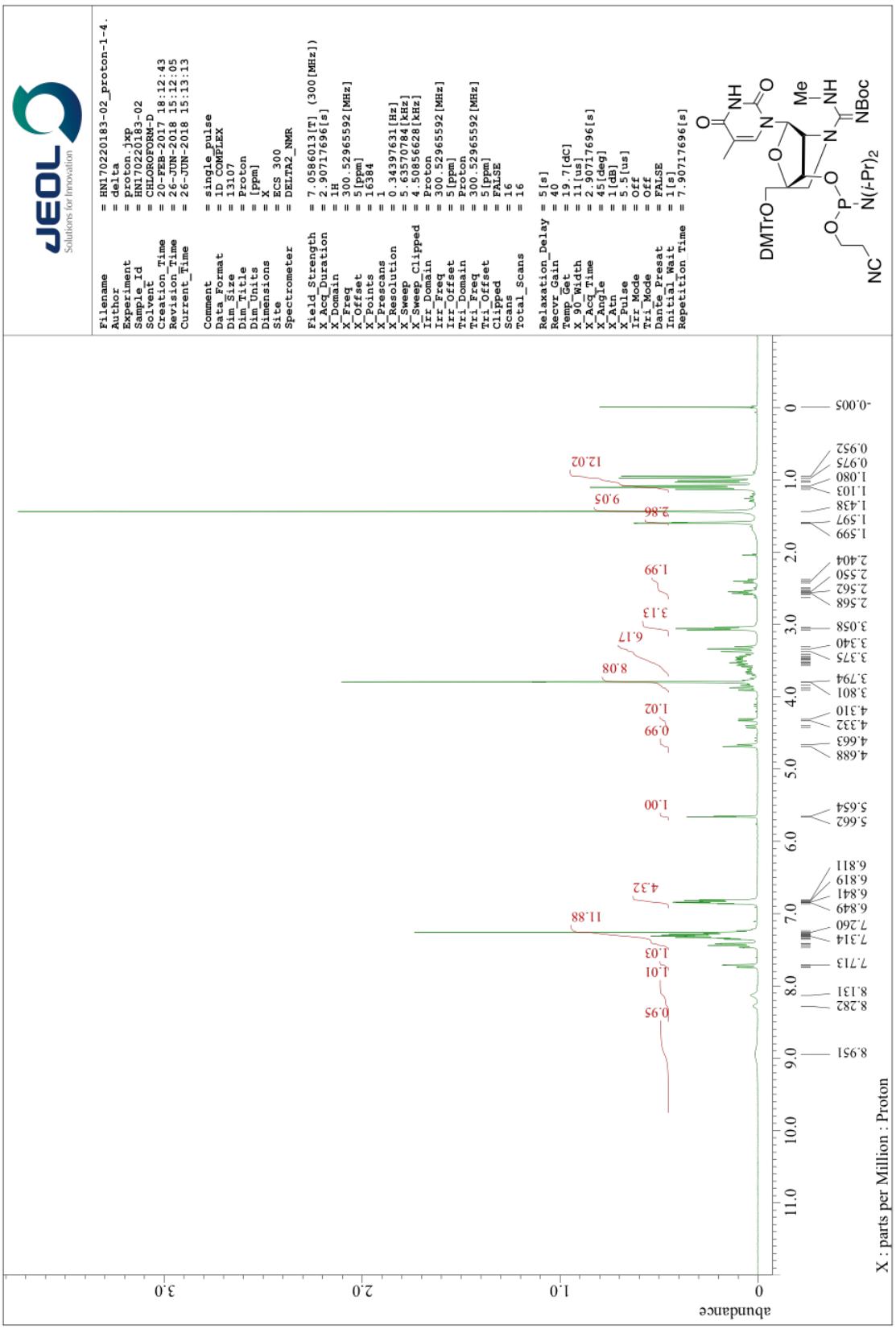


Figure S8. Compound **4** (^{31}P NMR, $CDCl_3$, 202 MHz)

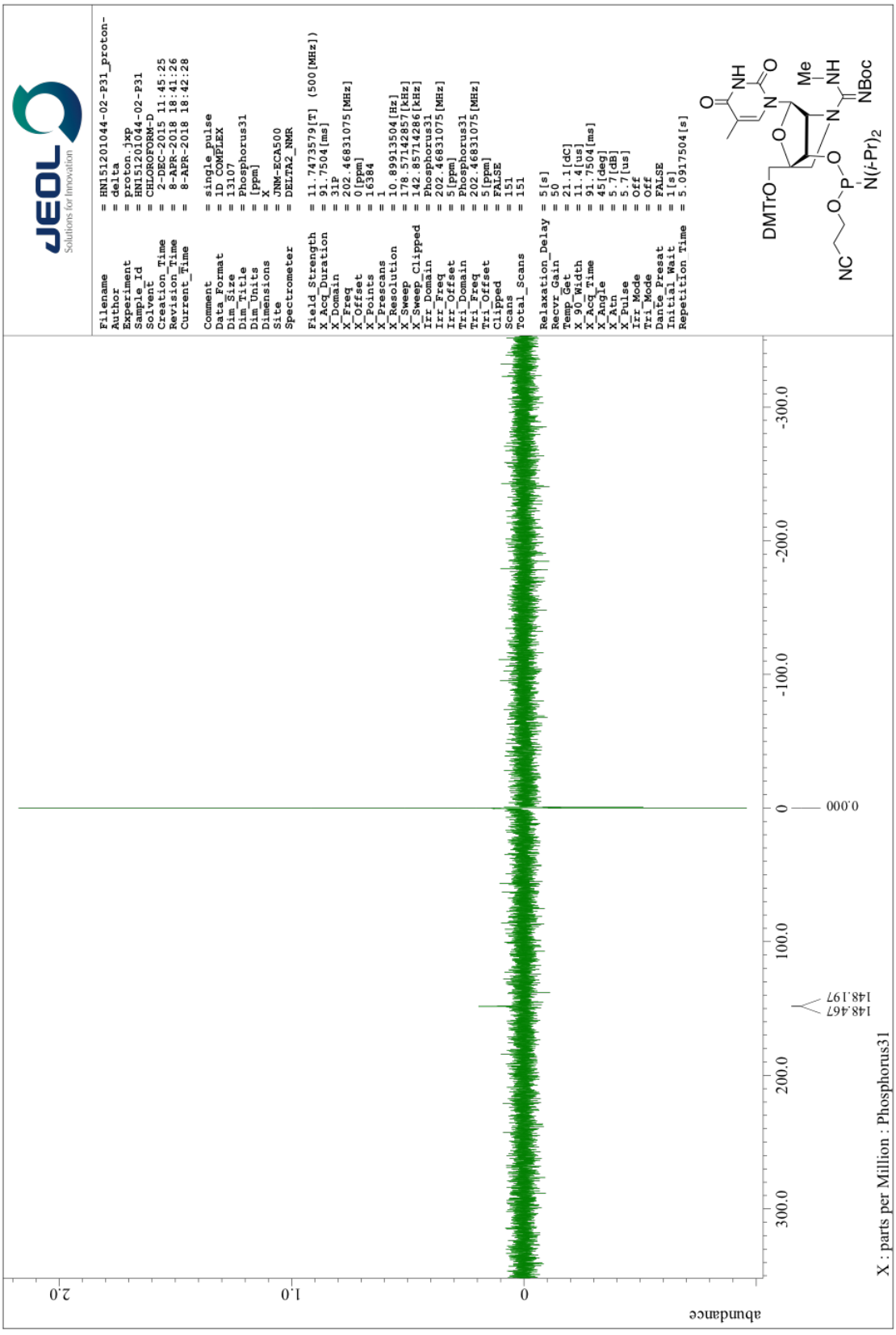


Figure S9. Compound **6** (^{1}H NMR, $CDCl_3$, 500 MHz)

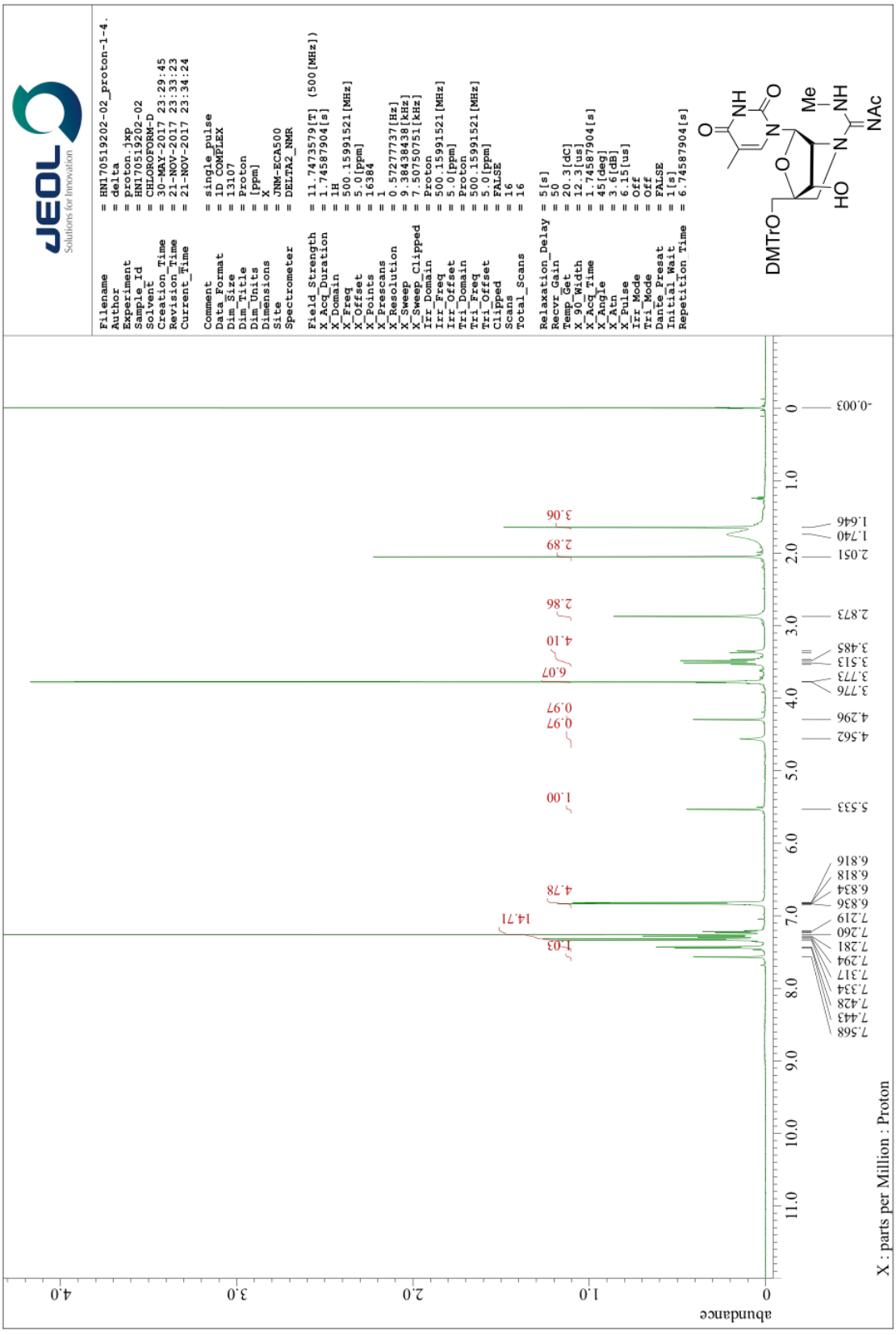


Figure S10. Compound **6** (^{13}C NMR, $CDCl_3$, 126 MHz)

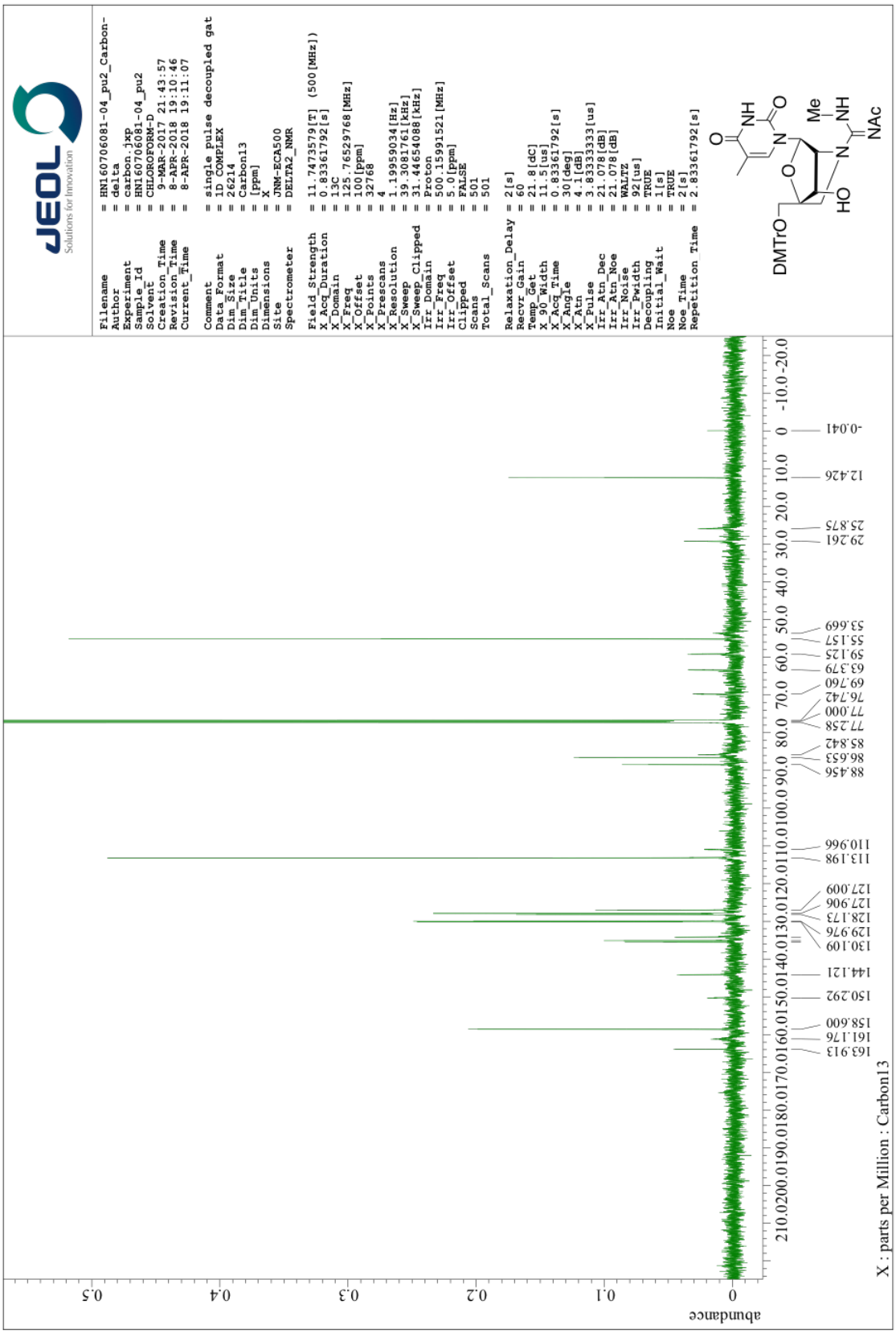


Figure S11. Compound 7 (^{1}H NMR, $CDCl_3$, 400 MHz)

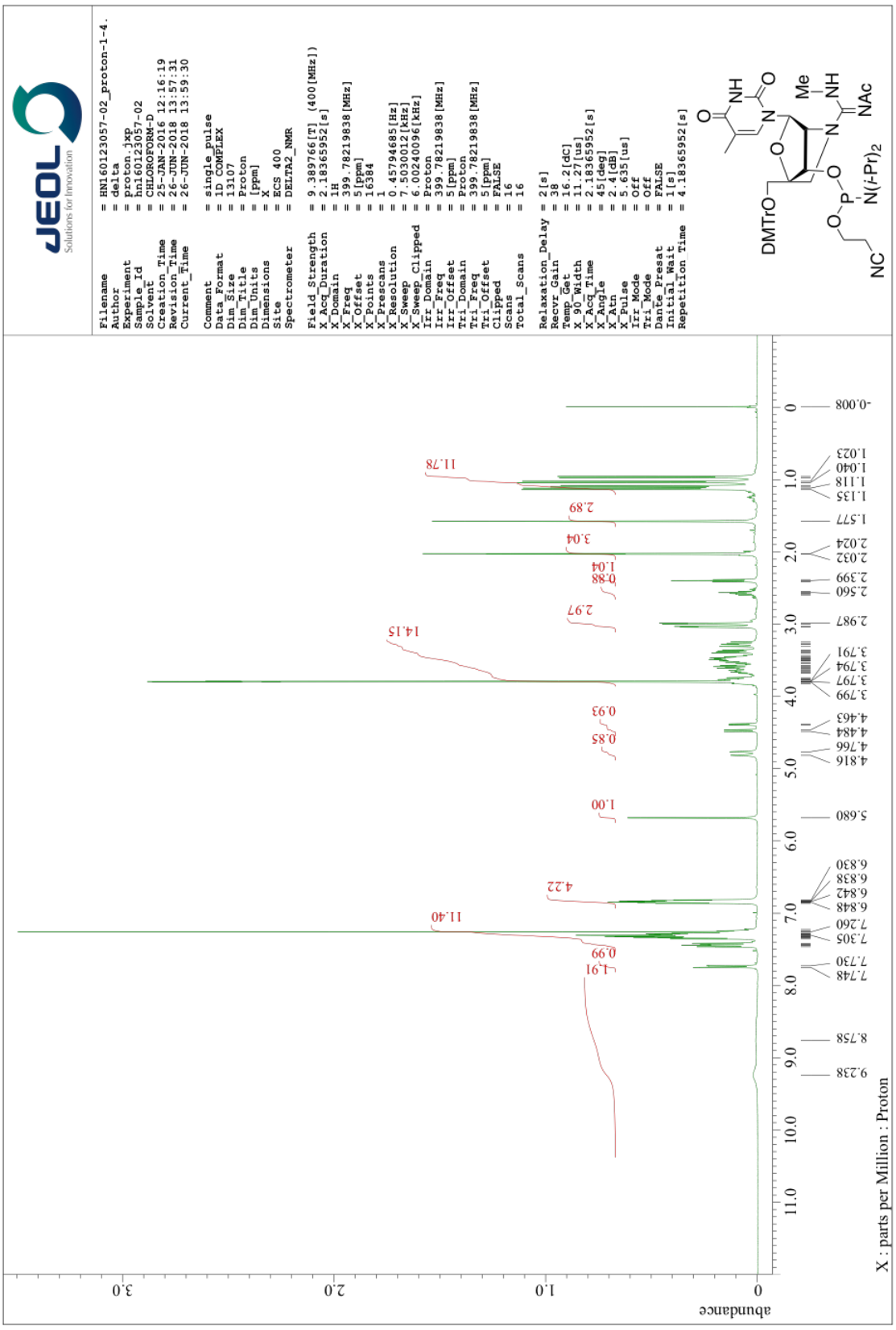
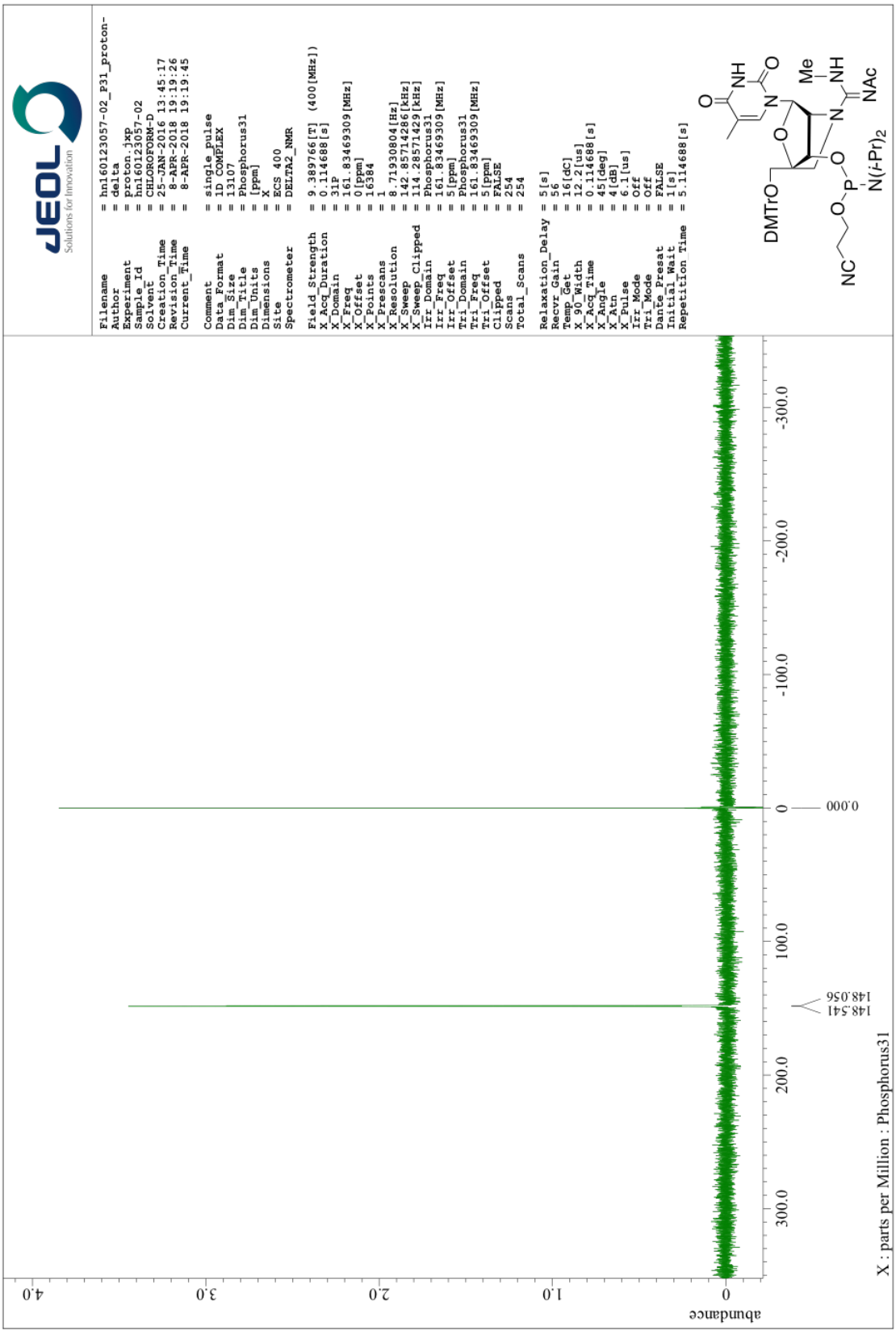


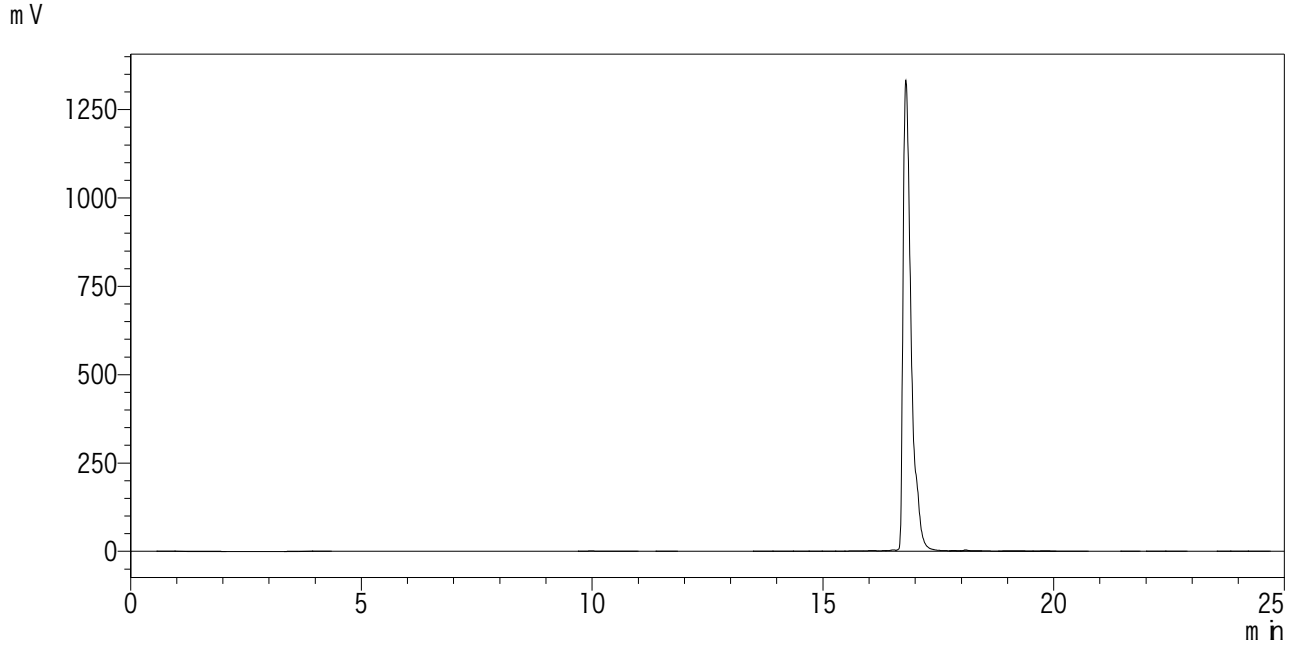
Figure S12. Compound **7** (^{31}P NMR, $CDCl_3$, 162 MHz)



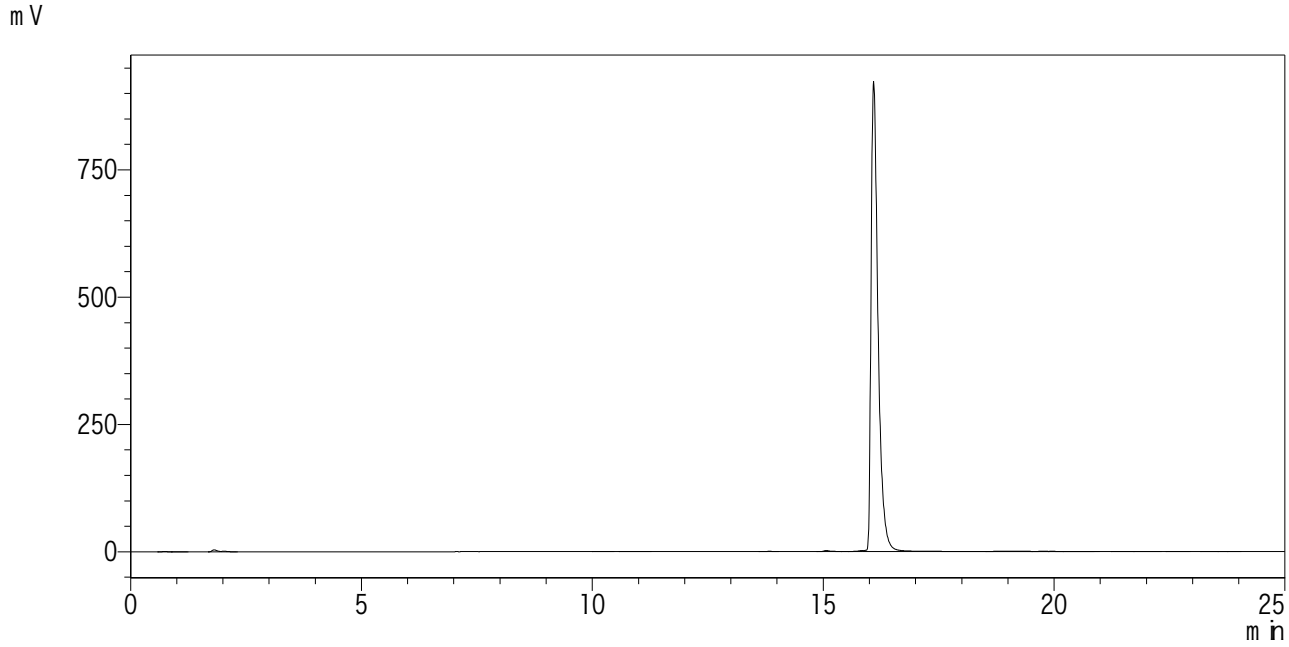
3. Characterisation of oligonucleotides

Figure S13. HPLC charts of all new oligonucleotides

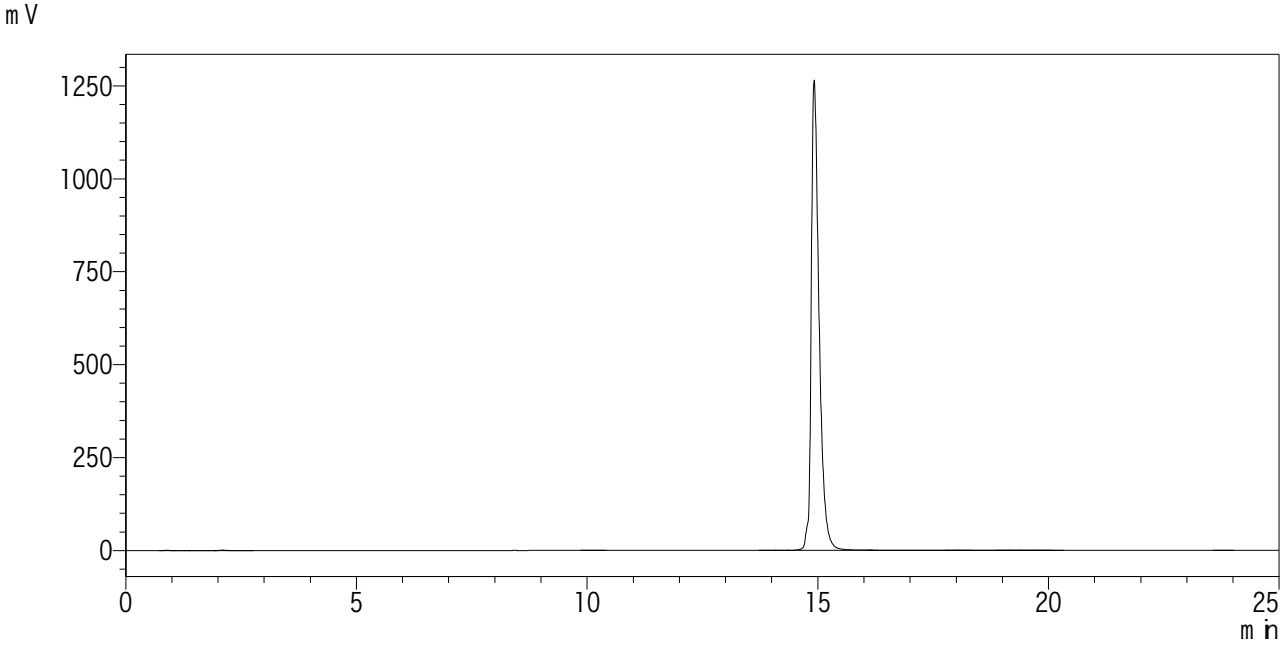
ON1



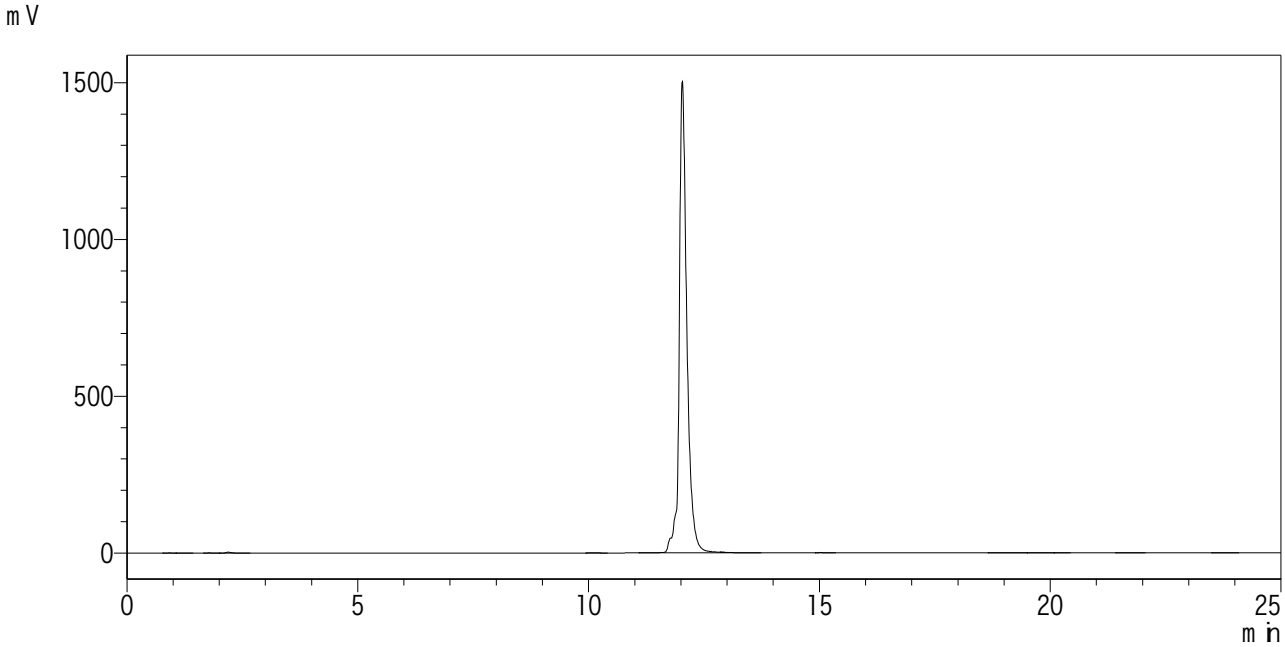
ON2



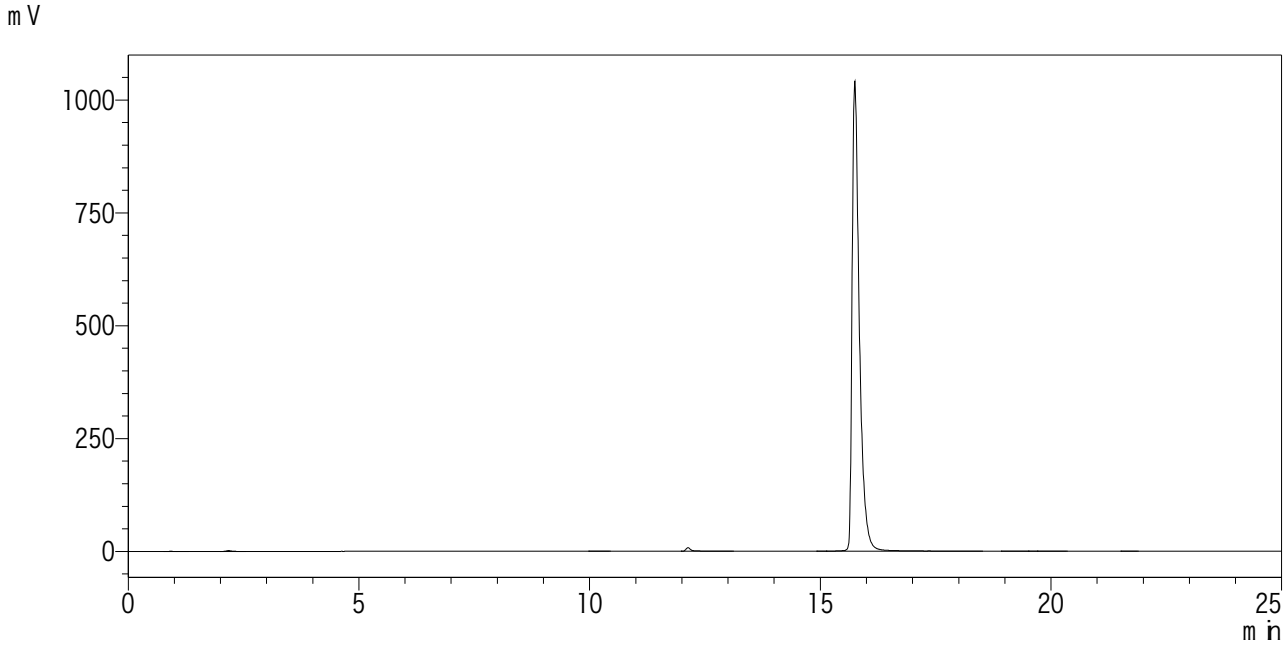
ON3


mV
1250
1000
750
500
250
0
0
5
10
15
20
25
min

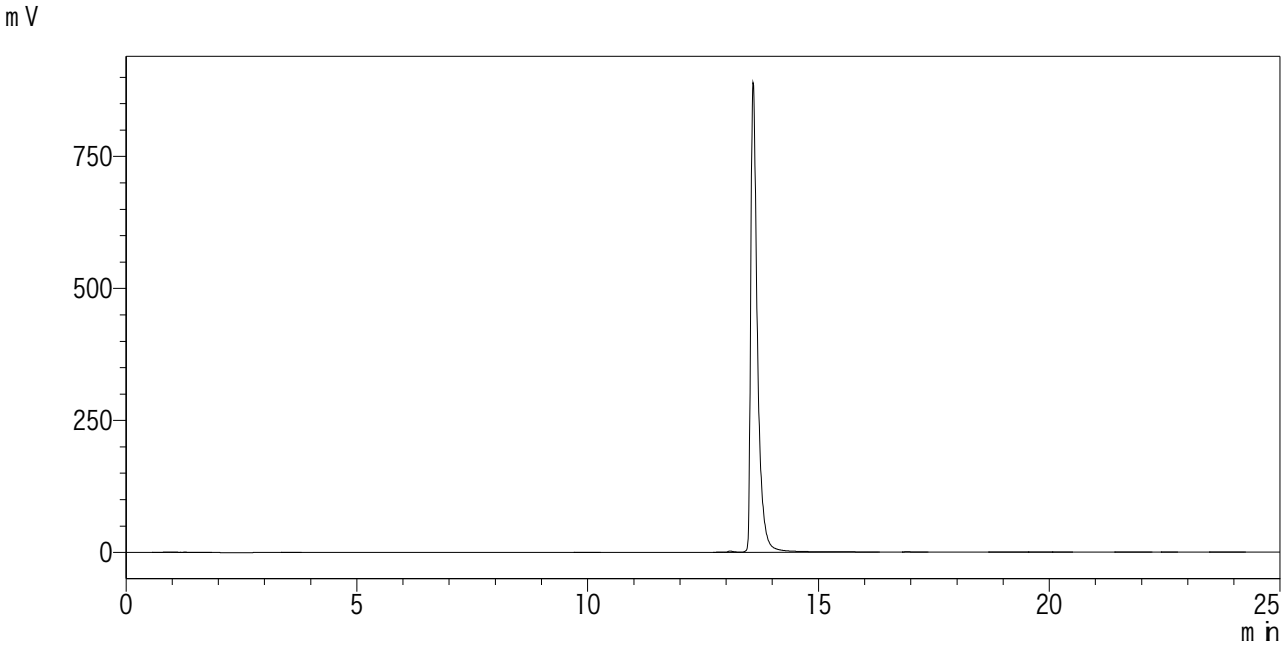
ON4


mV
1500
1000
500
0
0
5
10
15
20
25
min

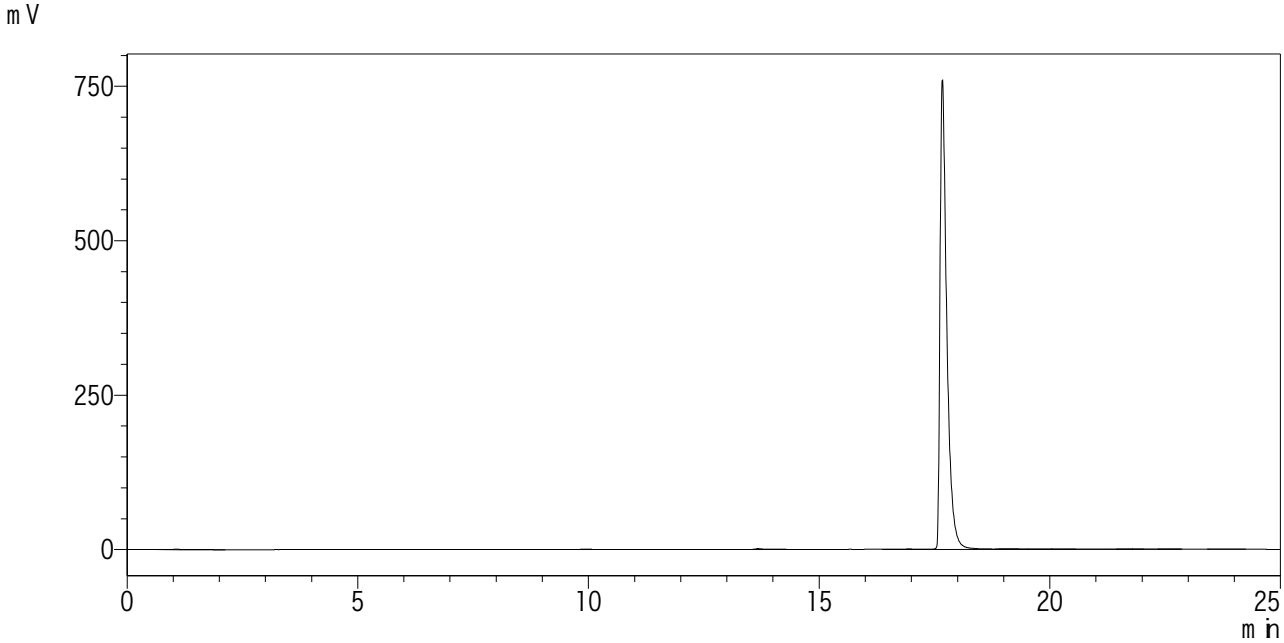
ON5


mV
1000
750
500
250
0
0
5
10
15
20
25
min

ON6


mV
750
500
250
0
0
5
10
15
20
25
min

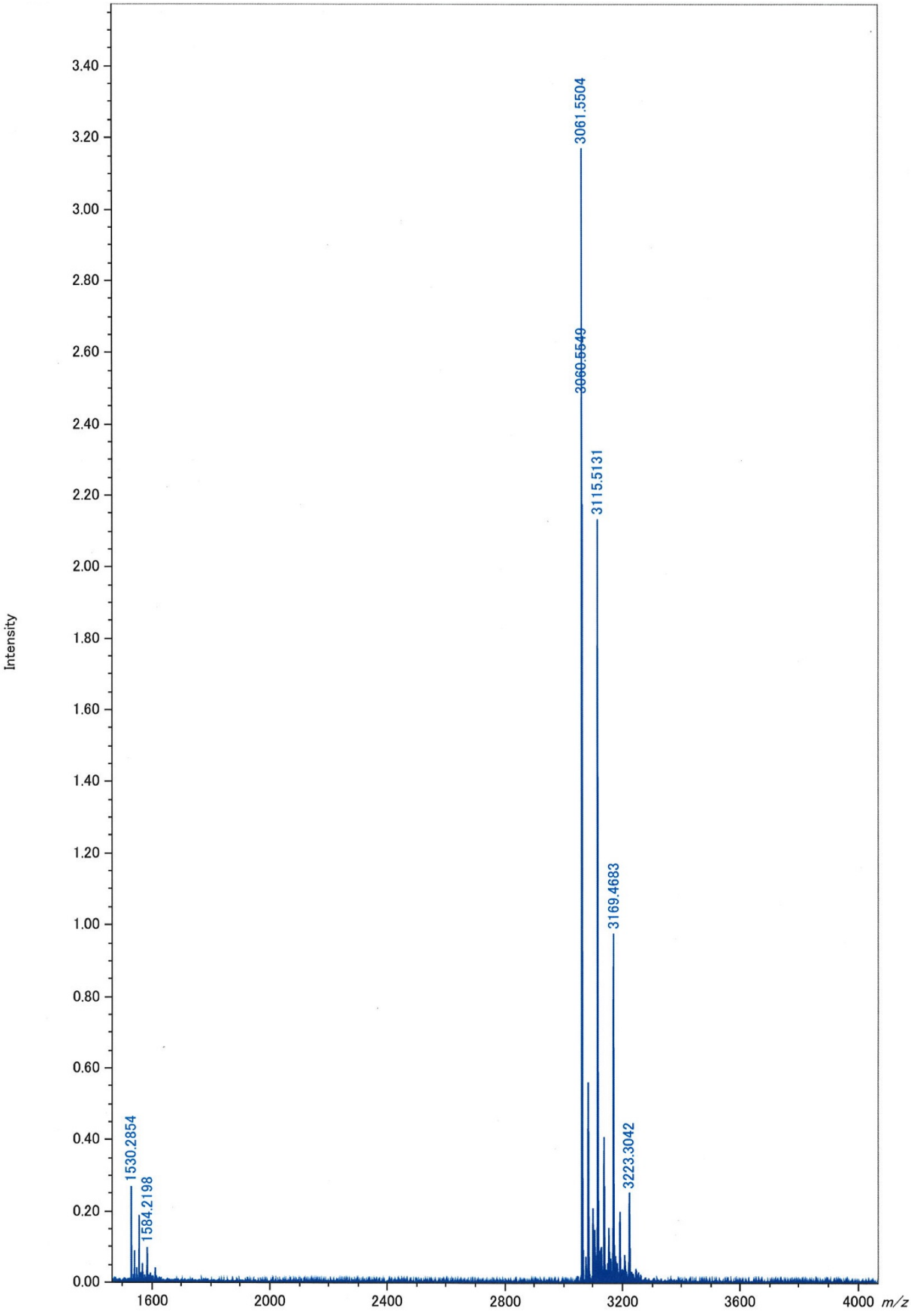
ON7



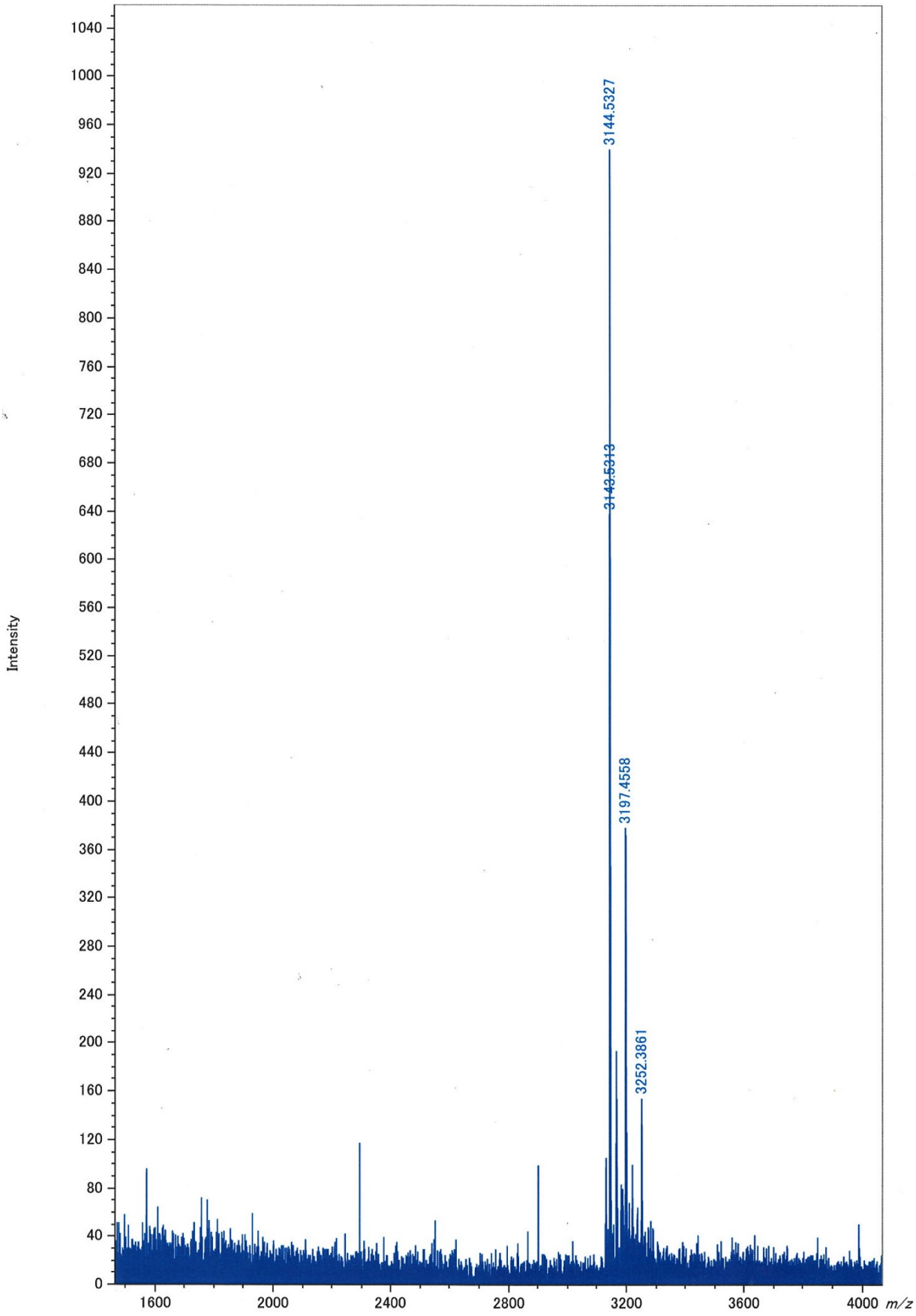
HPLC condition: reversed-phase HPLC (Waters XBridge™ C18 column) with a linear gradient of acetonitrile (2.5 to 15% over 25 min) in 0.1 M triethylammonium acetate buffer (pH 7.0).

Figure S14. MALDI-TOF MS charts of all new oligonucleotides

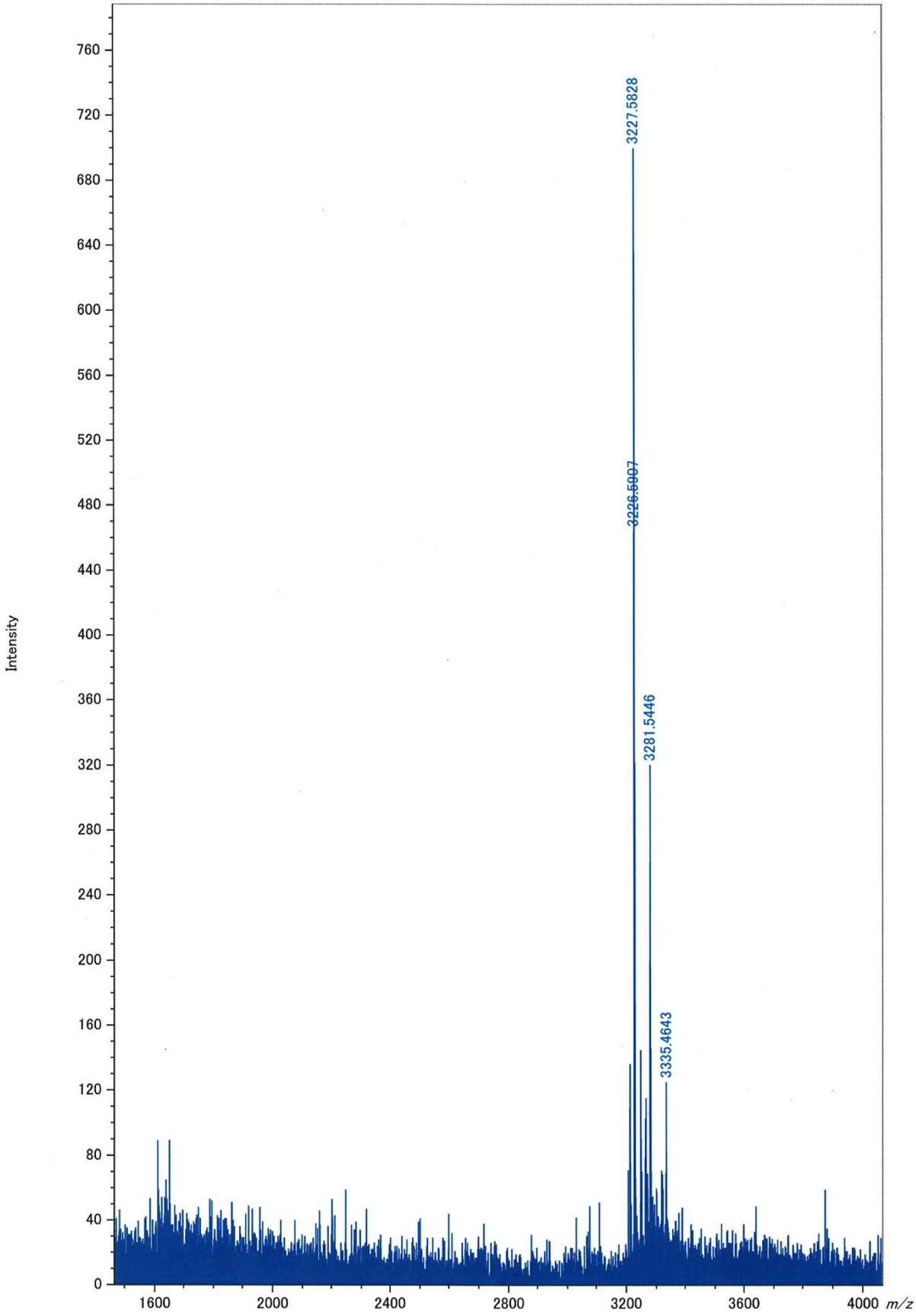
ON1



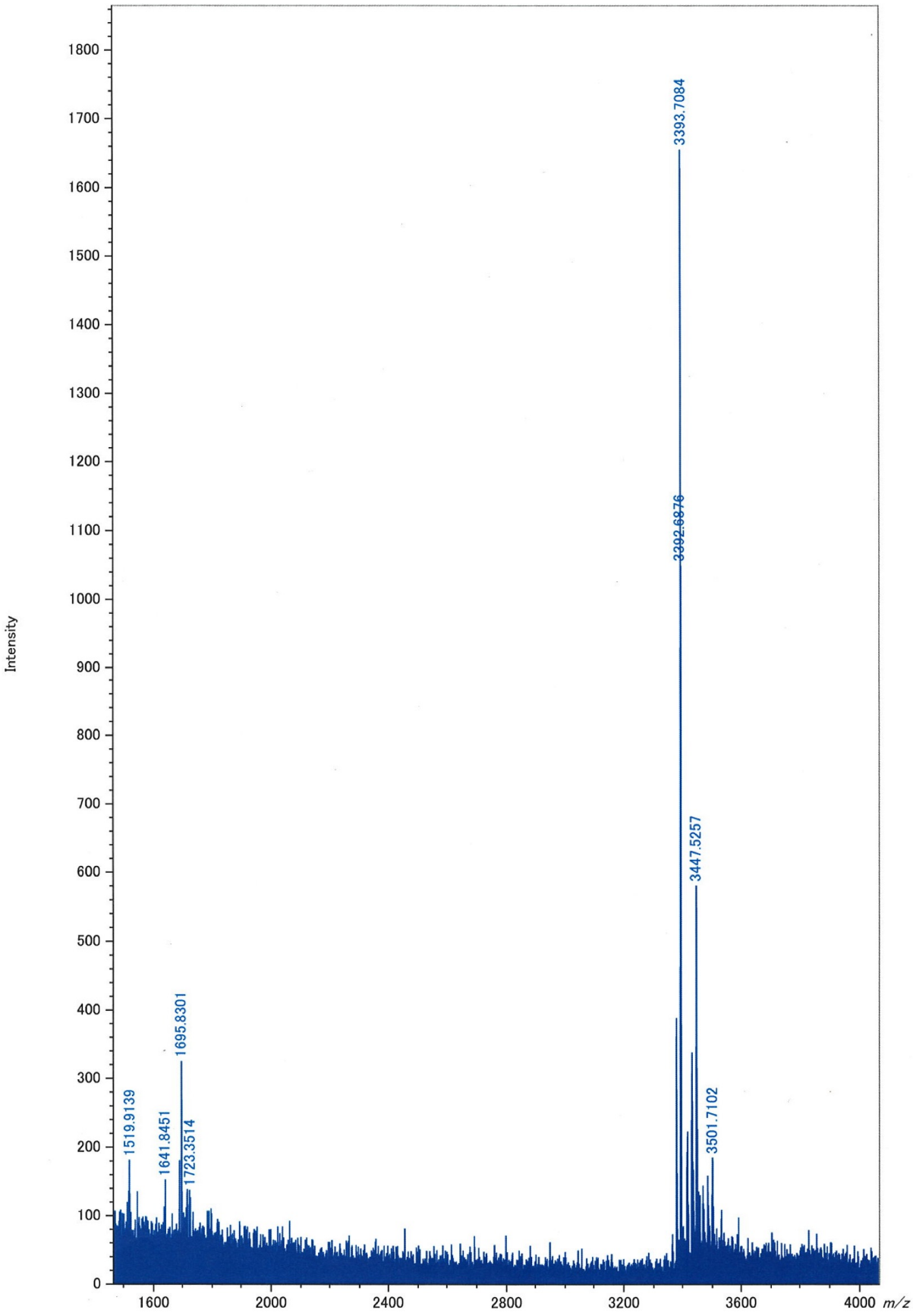
ON2


Intensity
1040
1000
960
920
880
840
800
760
720
680
640
600
560
520
480
440
400
360
320
280
240
200
160
120
80
40
0
3144.5327
3143.5313
3197.4558
3252.3861
1600
2000
2400
2800
3200
3600
4000
m/z

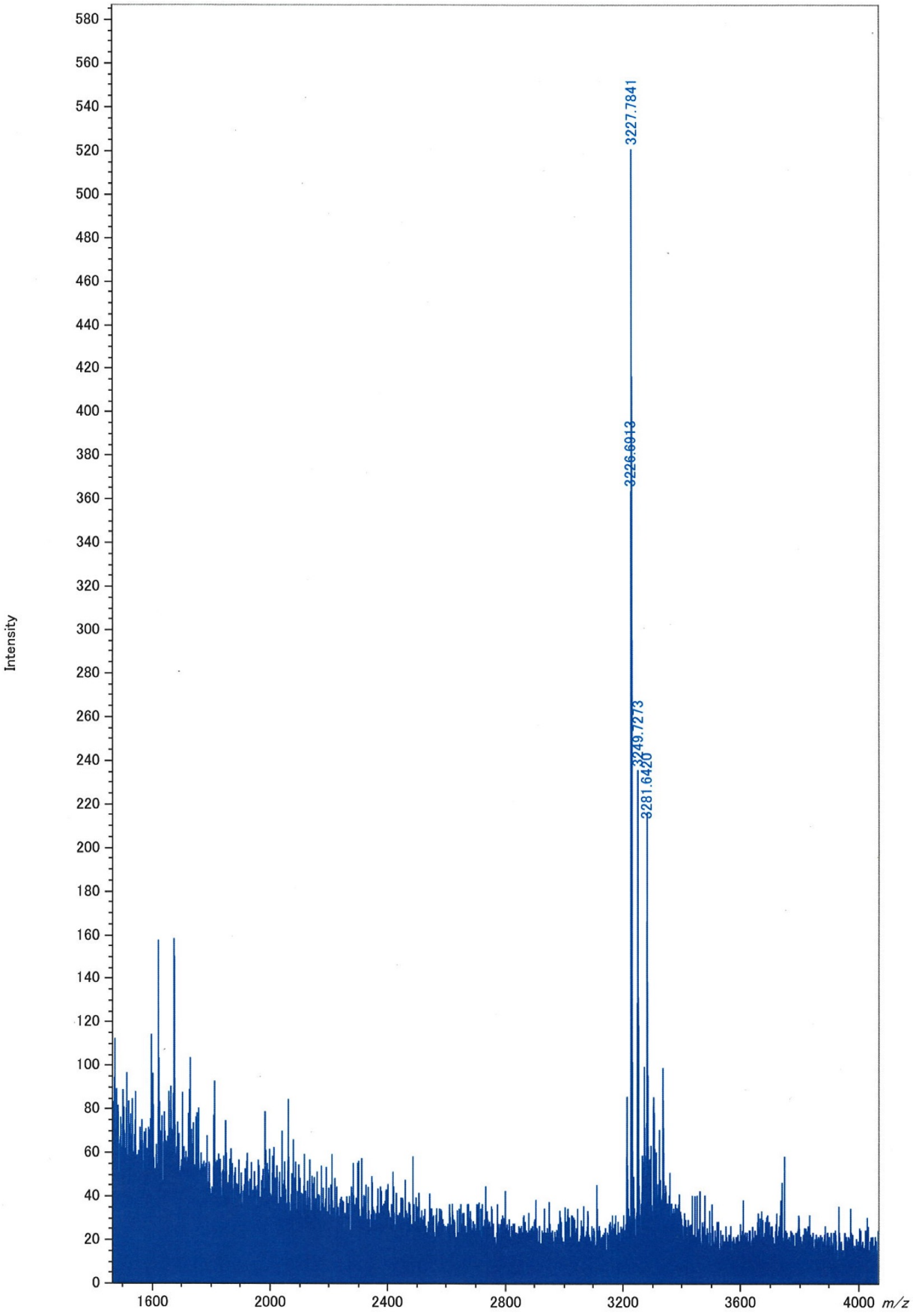
ON3


Intensity
760
720
680
640
600
560
520
480
440
400
360
320
280
240
200
160
120
80
40
0
1600
2000
2400
2800
3200
3600
4000
m/z
3227.5828
3226.5907
3281.5446
3335.4643

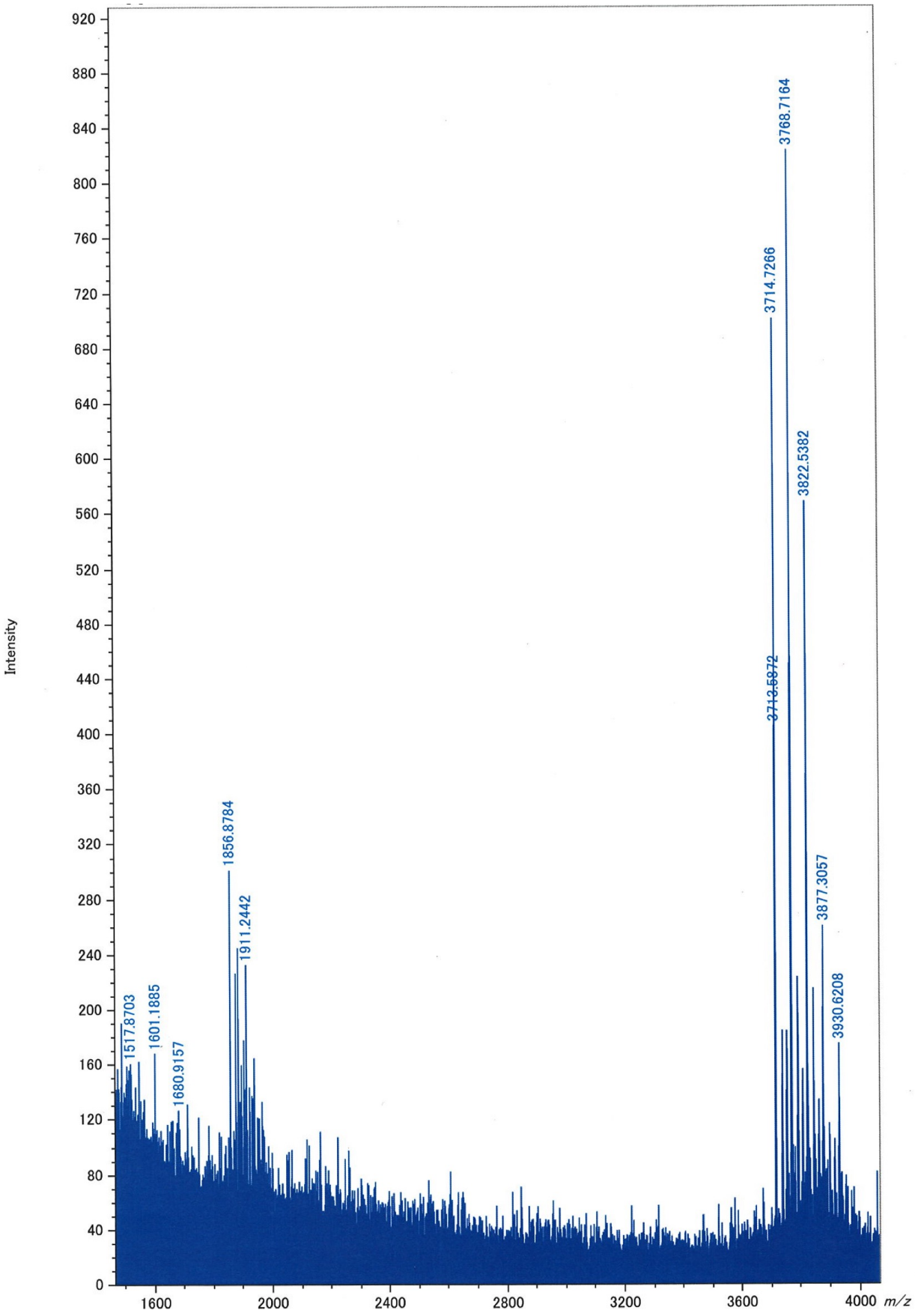
ON4


Intensity
1800
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
600
500
400
300
200
100
0
1519.9139
1641.8451
1695.8301
1723.3514
3392.6876
3393.7084
3447.5257
3501.7102
1600
2000
2400
2800
3200
3600
4000
m/z

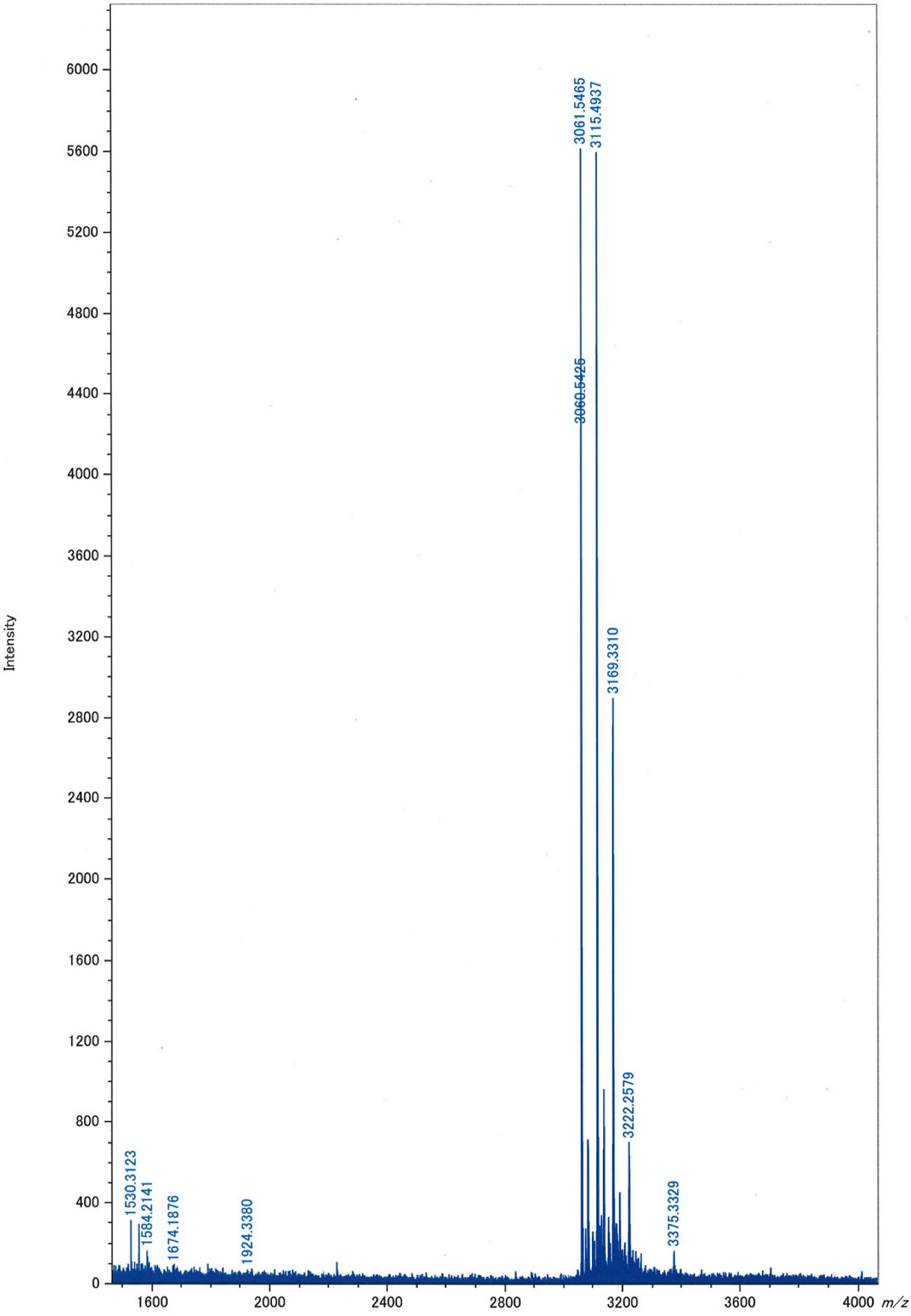
ON5



ON6


Intensity
920
880
840
800
760
720
680
640
600
560
520
480
440
400
360
320
280
240
200
160
120
80
40
0
1517.8703
1601.1885
1680.9157
1856.8784
1911.2442
3714.7266
3768.7164
3822.5382
3877.3057
3930.6208
1600
2000
2400
2800
3200
3600
4000
m/z

ON7


Intensity
6000
5600
5200
4800
4400
4000
3600
3200
2800
2400
2000
1600
1200
800
400
0
1530.3123
1584.2141
1674.1876
1924.3380
3060.5425
3061.5465
3115.4937
3169.3310
3222.2579
3375.3329
1600
2000
2400
2800
3200
3600
4000
m/z