

Supplementary Material

for

Synthesis and evaluation of artificial nucleic acid bearing an oxanorbornane scaffold

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1. Supplementary results

Table S1. T_m values of duplexes formed between oligonucleotides (ON1, ON2, ON4, and ON5) and complementary ssDNA

Sequence	T_m [°C] values at each concentration					
	0.9 μ M	1.48 μ M	2.44 μ M	4.00 μ M	6.52 μ M	13.6 μ M
ON4 5'-d(GCG TTU TTT GCT)-3'	46.5	48.4	49.0	49.9	50.7	52.0
ON1 5'-d(GCG TTX TTT GCT)-3'	40.1	41.2	43.0	45.4	45.6	46.8
ON5 5'-d(GCG UTU TUT GCT)-3'	45.0	45.9	46.9	48.4	49.3	51.9
ON2 5'-d(GCG XTX TXT GCT)-3'	14.1	15.9	17.5	21.0	22.2	24.7

Conditions: 10 mM sodium phosphate buffer (pH 7.2), 100 mM NaCl aq., and 0.90–13.6 μ M each oligonucleotide (six data points). The T_m values reflect the average of at least three measurements. The sequence of the target ssDNA is 5'-d(AGCAAAAACGC)-3'. U = 2'-deoxyuridine, X = OxNorNA-U.

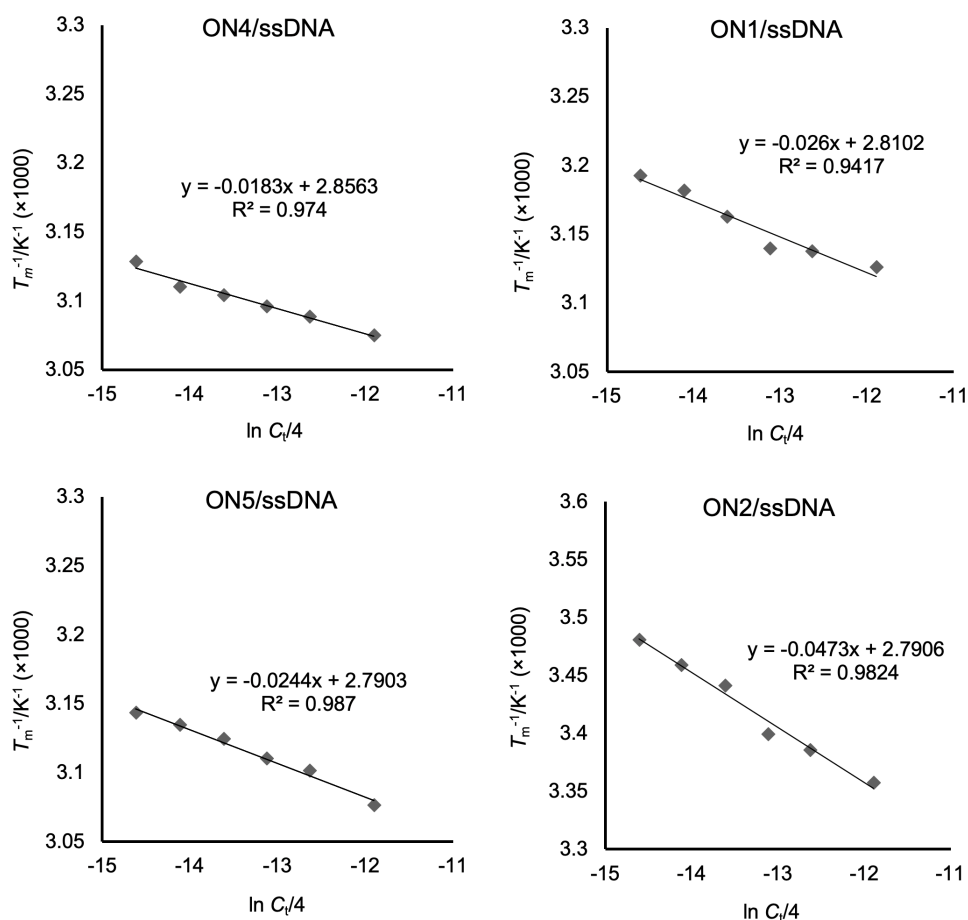


Figure S1. Van't Hoff plots of the duplexes formed between oligonucleotides (ON1, ON2, ON4, and ON5) and complementary ssDNA.

Table S2. T_m values of duplexes formed between oligonucleotides (ON1, ON2, ON4, and ON5) and complementary ssRNA

Sequence		T_m [°C] values at each concentration					
		0.9 μ M	1.48 μ M	2.44 μ M	4.00 μ M	6.52 μ M	13.6 μ M
ON4	5'-d(GCG TTU TTT GCT)-3'	43.3	45.0	46.0	46.5	47.6	49.6
ON1	5'-d(GCG TT <u>X</u> TTT GCT)-3'	38.9	39.3	41.2	42.4	43.1	44.3
ON5	5'-d(GCG UTU TUT GCT)-3'	40.8	42.5	44.4	44.7	46.1	47.1
ON2	5'-d(GCG <u>XTX</u> <u>XTX</u> GCT)-3'	18.9	20.1	21.9	24.7	25.0	26.9

Conditions: 10 mM sodium phosphate buffer (pH 7.2), 100 mM NaCl aq., and 0.90–13.6 μ M each oligonucleotide (six data points). The T_m values reflect the average of at least three measurements. The sequence of the target ssRNA is 5'-r(AGCAAAAACGC)-3'. U = 2'-deoxyuridine, X = OxNorNA-U.

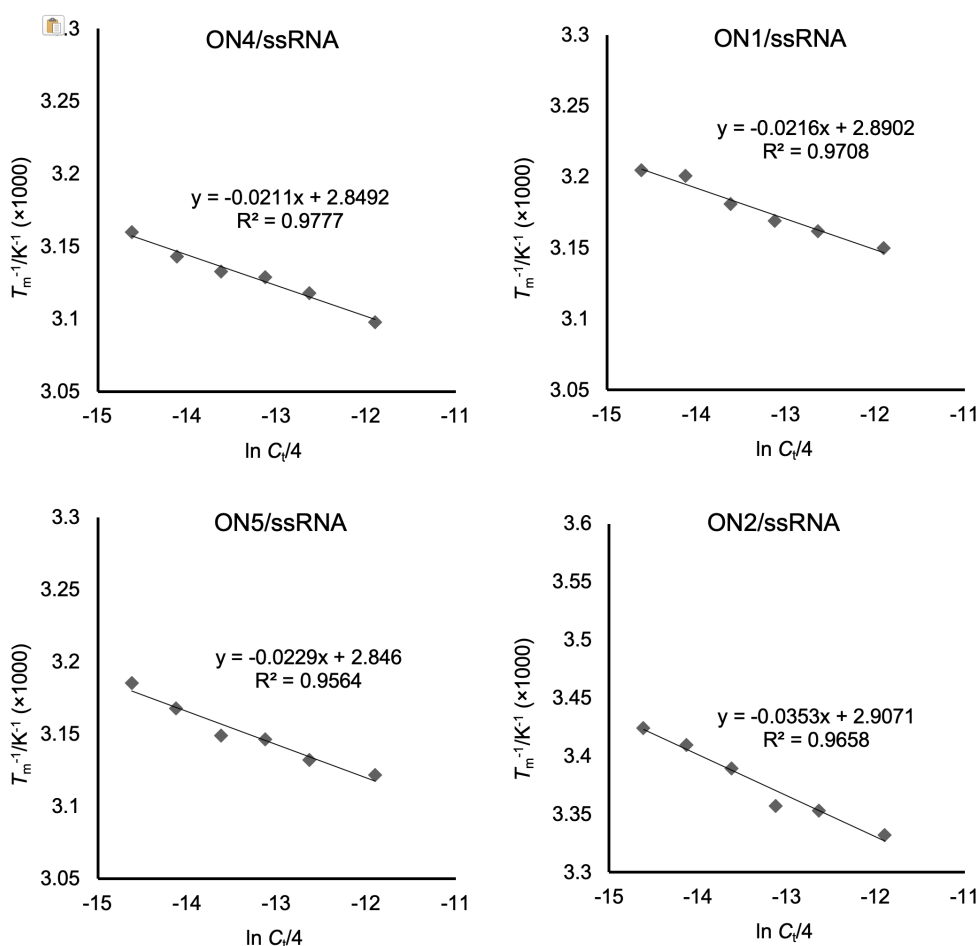
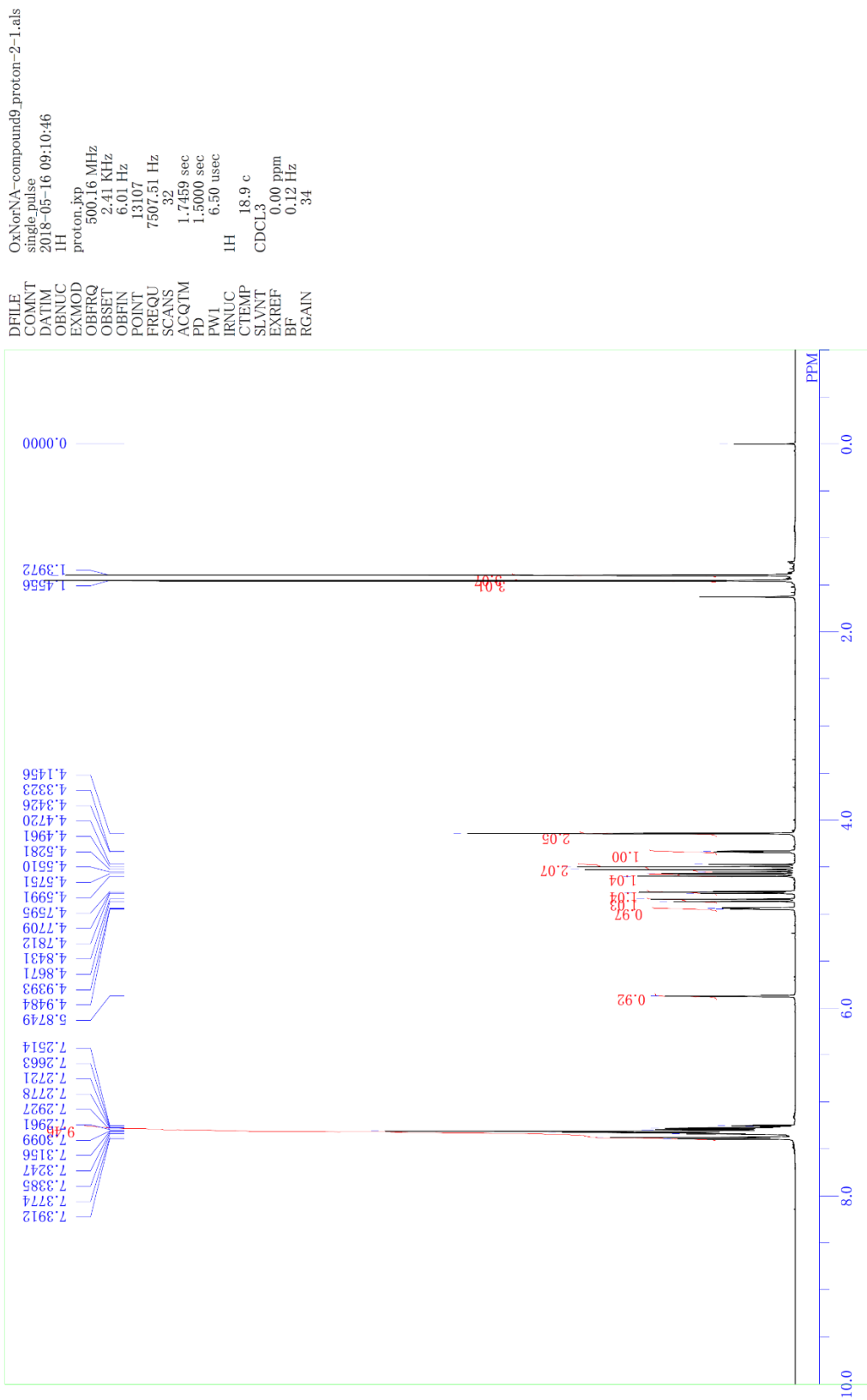


Figure S2. Van't Hoff plots of the duplexes formed between oligonucleotides (ON1, ON2, ON4, and ON5) and complementary ssRNA.

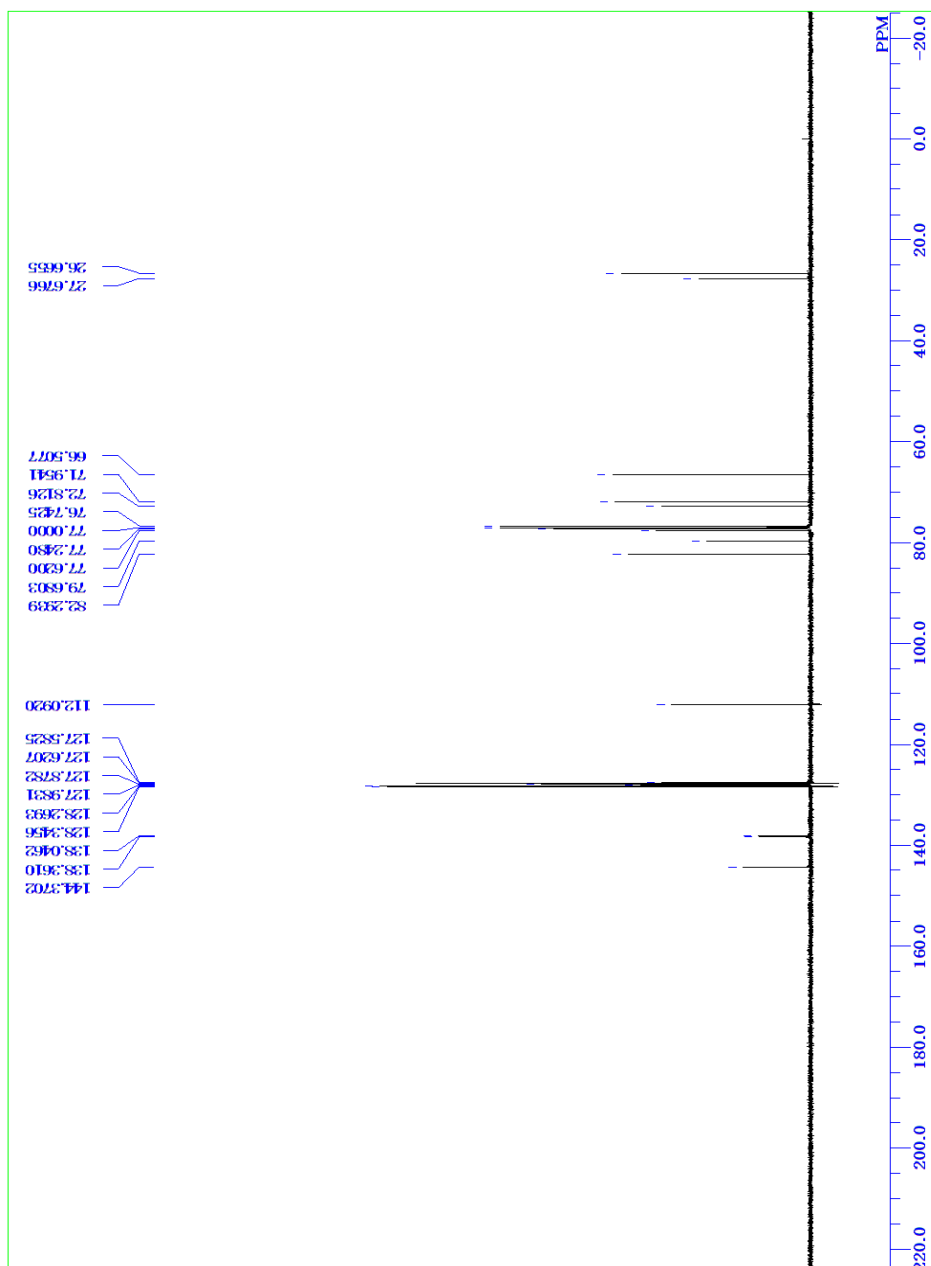
2. Copies of the ^1H , ^{13}C , and ^{31}P NMR spectra of all new compounds
Compound **2** (^1H NMR, CDCl_3 , 500MHz)



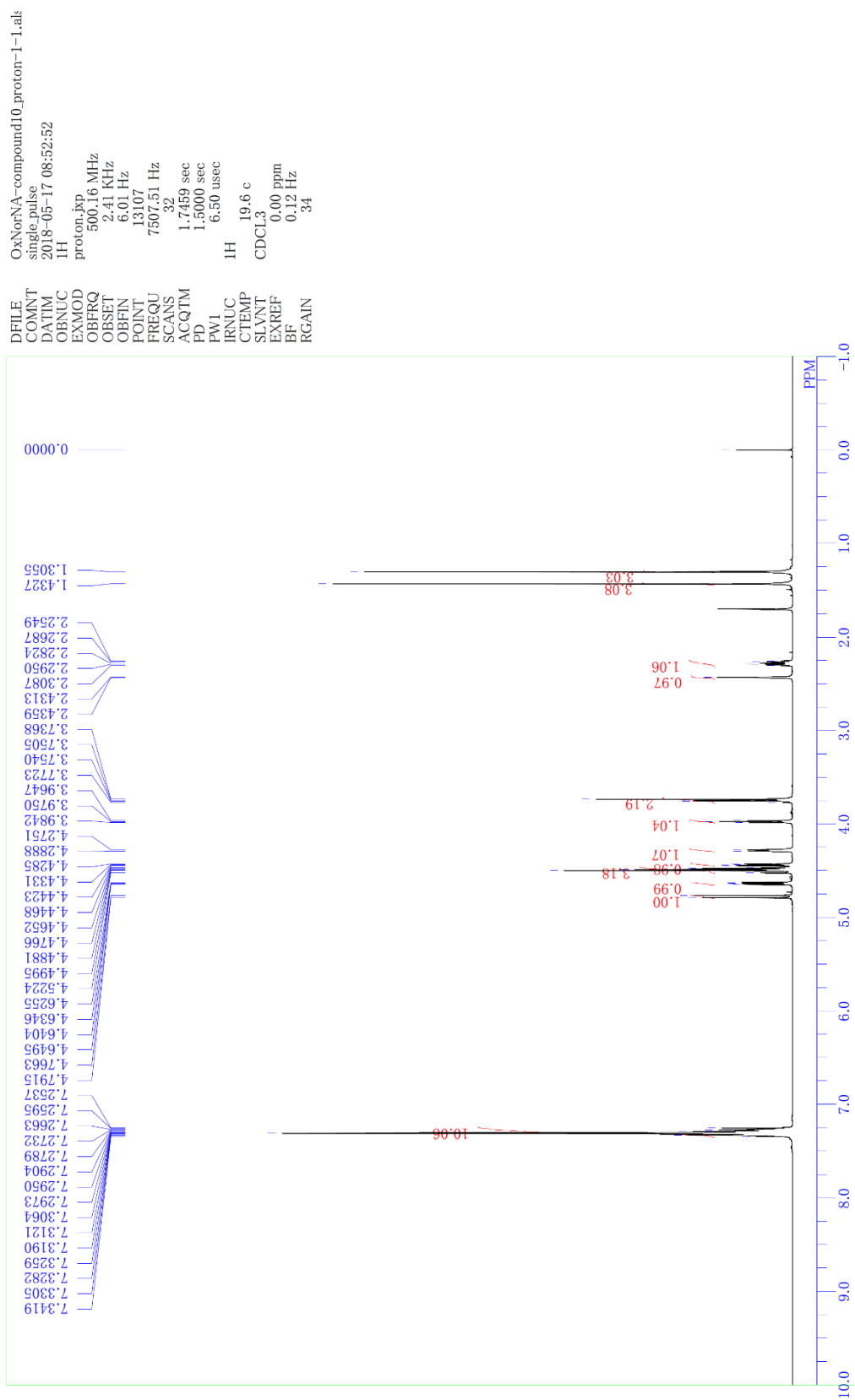
Compound **2** (^{13}C NMR, CDCl_3 , 125MHz)

OxNorNA-compound9_Carbon
 single pulse decoupled gated 1
 2018-05-16 09:15:29
 ^{13}C
 carbon.kp
 125.77 MHz
 7.87 KHz
 4.21 Hz
 26214
 31446.54 Hz
 500
 0.8336 sec
 2.0000 sec
 3.93 usec
 1H 19.6 c
 CDCl_3
 77.00 ppm
 0.12 Hz
 56

DFILE
 COMNT
 DATIM
 OBNUC
 EXMOD
 OBFRO
 OBFRO
 OBFRO
 POINT
 FREQU
 SCANS
 ACQTM
 PD
 PW1
 IRNUC
 CTEMP
 SLVNT
 EXREF
 BF
 RGAIN



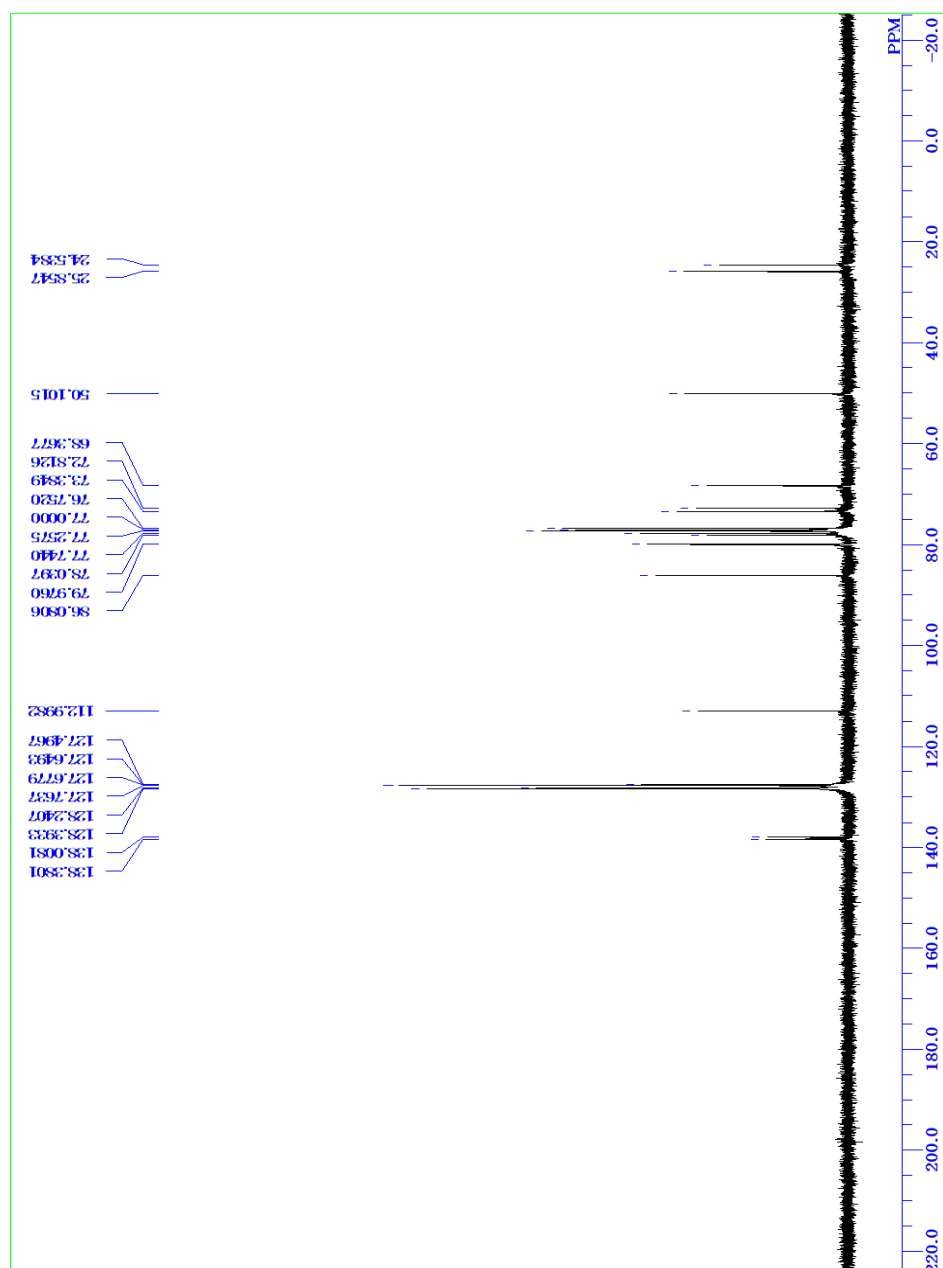
Compound **3** (^1H NMR, CDCl_3 , 500MHz)



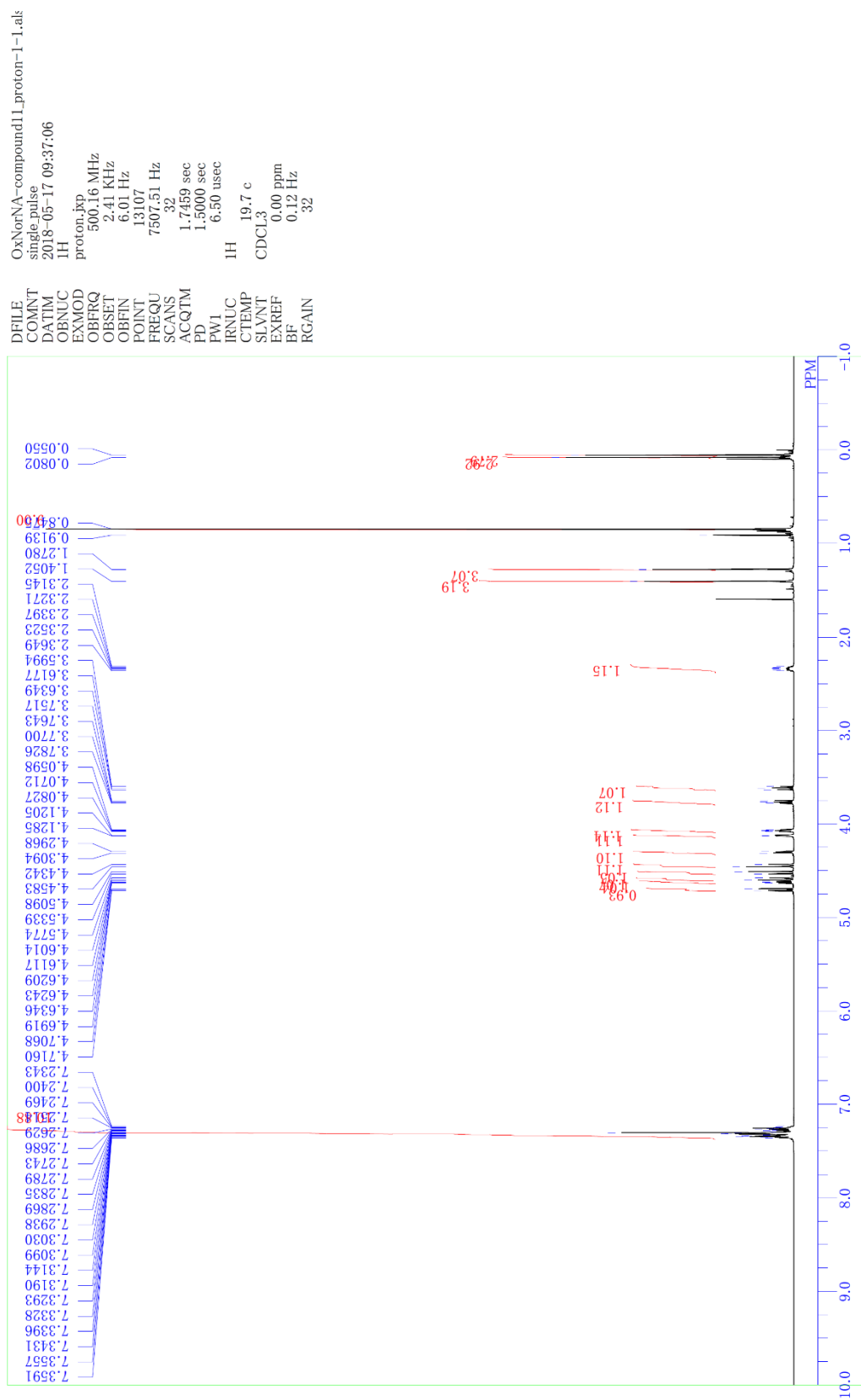
Compound **3** (^{13}C NMR, CDCl_3 , 125MHz)

OxNorNA-compound10_Carbo
single pulse decoupled gated N
2018-05-16 10:23:21
13C
carbon.kp
125.77 MHz
7.87 KHz
4.21 Hz
26214
31446.54 Hz
500
0.8336 sec
2.0000 sec
3.93 usec
IH 19.8 c
CDCL3
77.00 ppm
0.12 Hz
60

DFILE
COMINT
DATIM
OBNUC
EXMOD
OBFRO
OBSET
OBFIN
POINT
FREQU
SCANS
ACQTM
PD
PWL
IRNLC
CTEMP
SLVNT
EXREF
BF
RGAIN



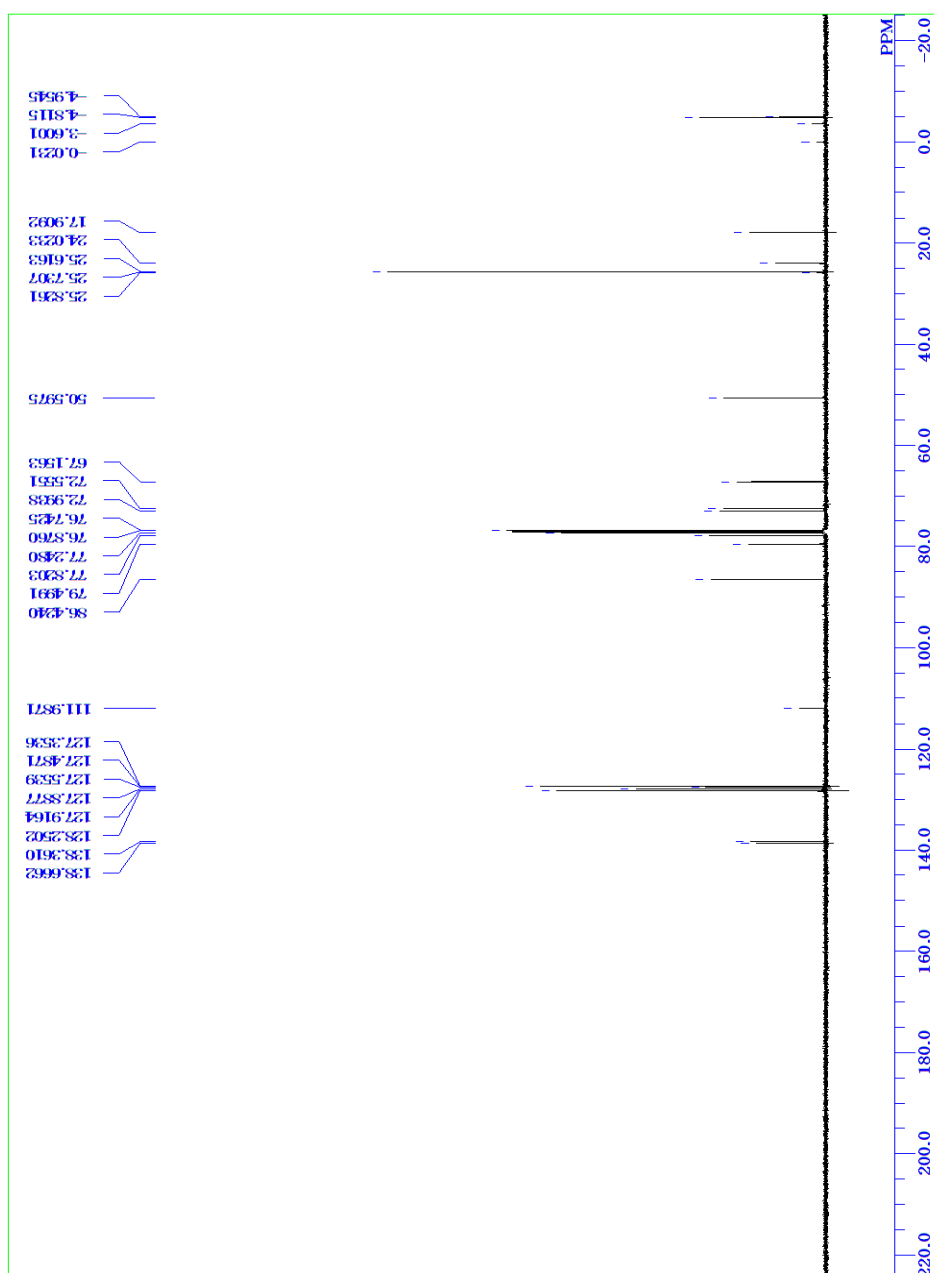
Compound **4** (^1H NMR, CDCl_3 , 500MHz)



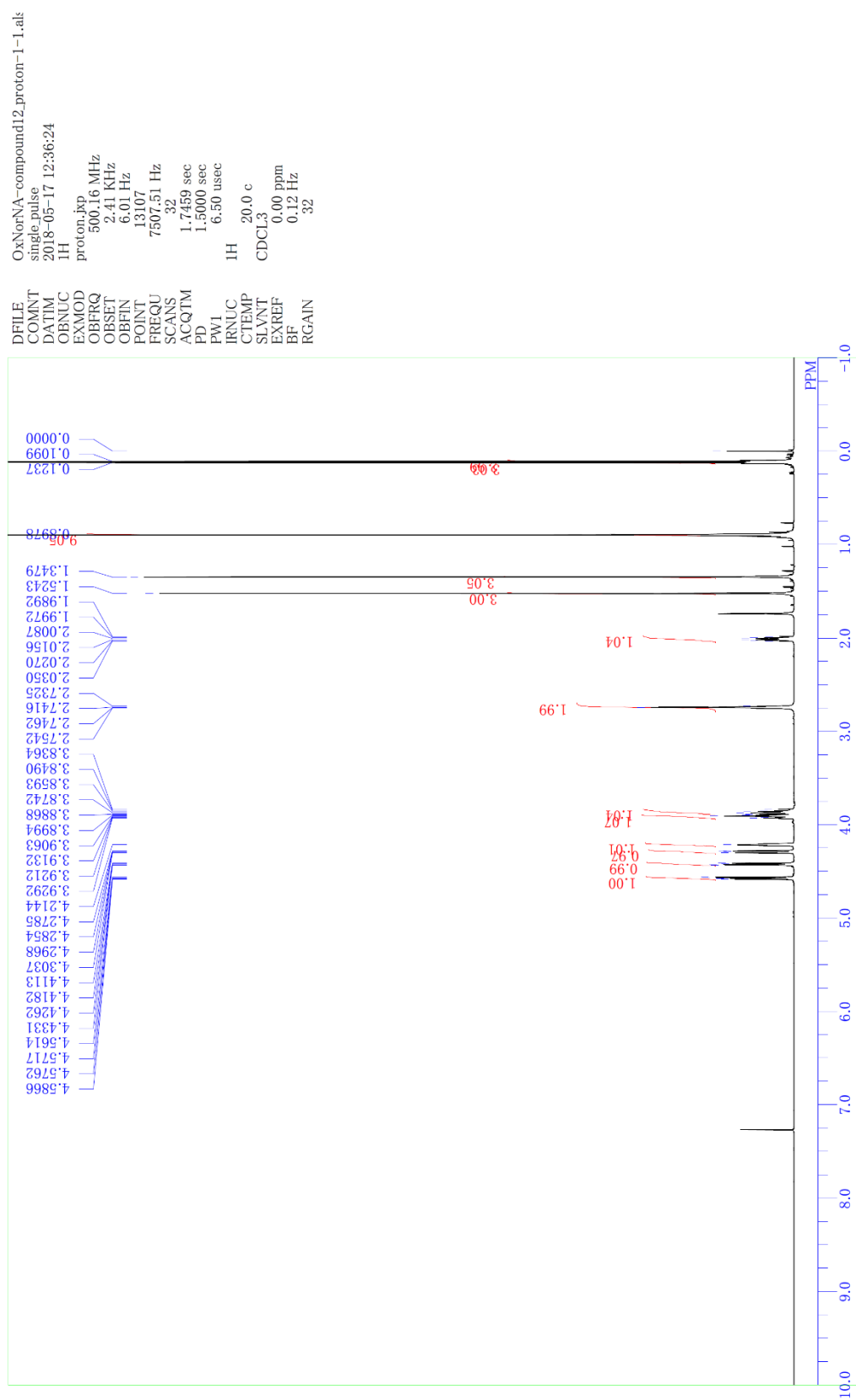
Compound **4** (^{13}C NMR, CDCl_3 , 125MHz)

OxNorNA-compound11_Carbo
single pulse decoupled gated
2018-05-17 09:41:32
 ^{13}C
carbon.jpg
125.77 MHz
7.87 KHz
4.21 Hz
26214
31446.54 Hz
500
0.8336 sec
2.0000 sec
3.93 usec
1H
20.4 c
 CDCl_3
77.00 ppm
0.12 Hz
54

DFILE
COMNT
DATIM
OBNUC
EXMOD
OBFRO
OBFRO
OBFRO
POINT
FREQU
SCANS
ACQTM
PD
FWI
IRNUC
CTEMP
SLVNT
EXREF
BF
RGAIN



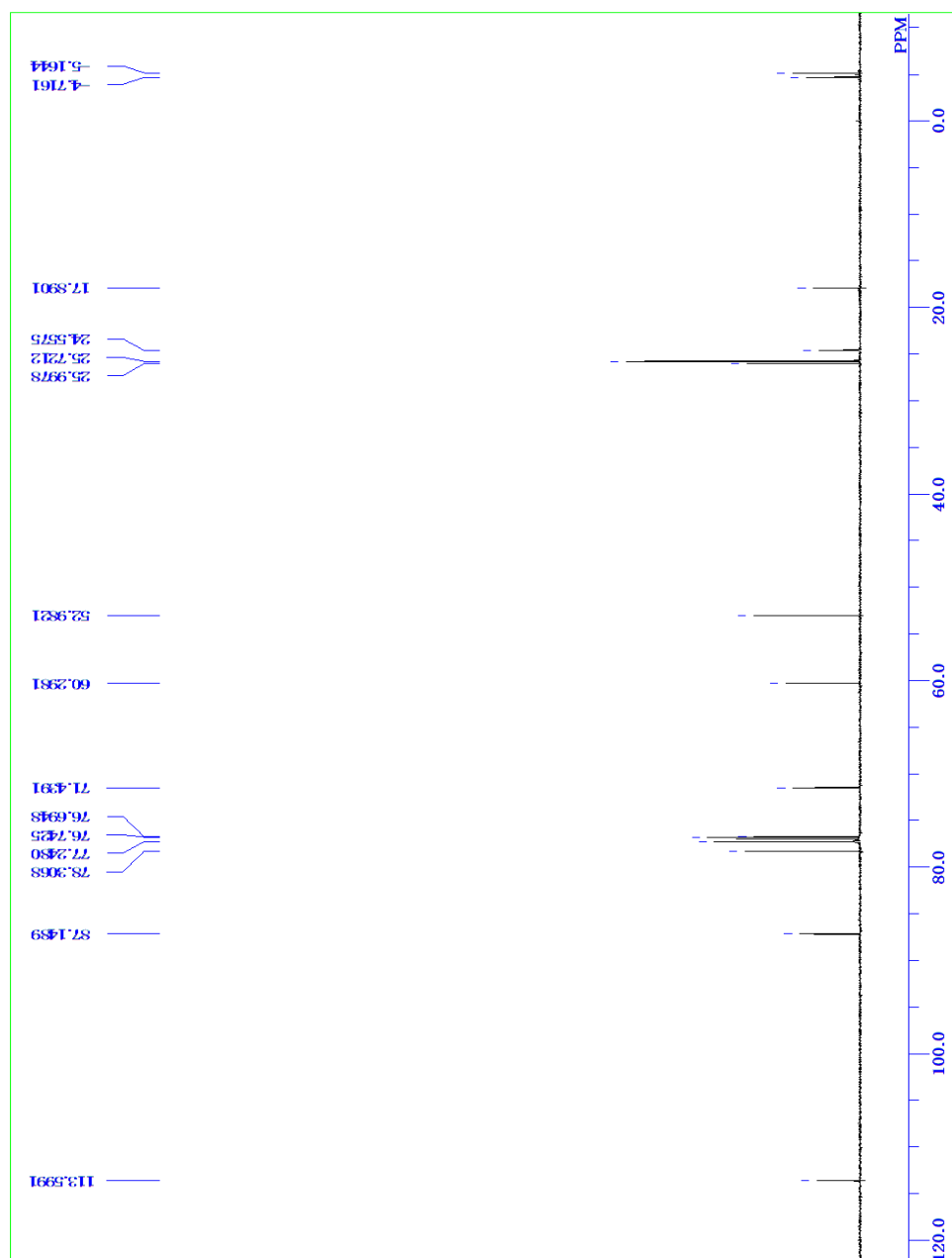
Compound 5 (^1H NMR, CDCl_3 , 500MHz)



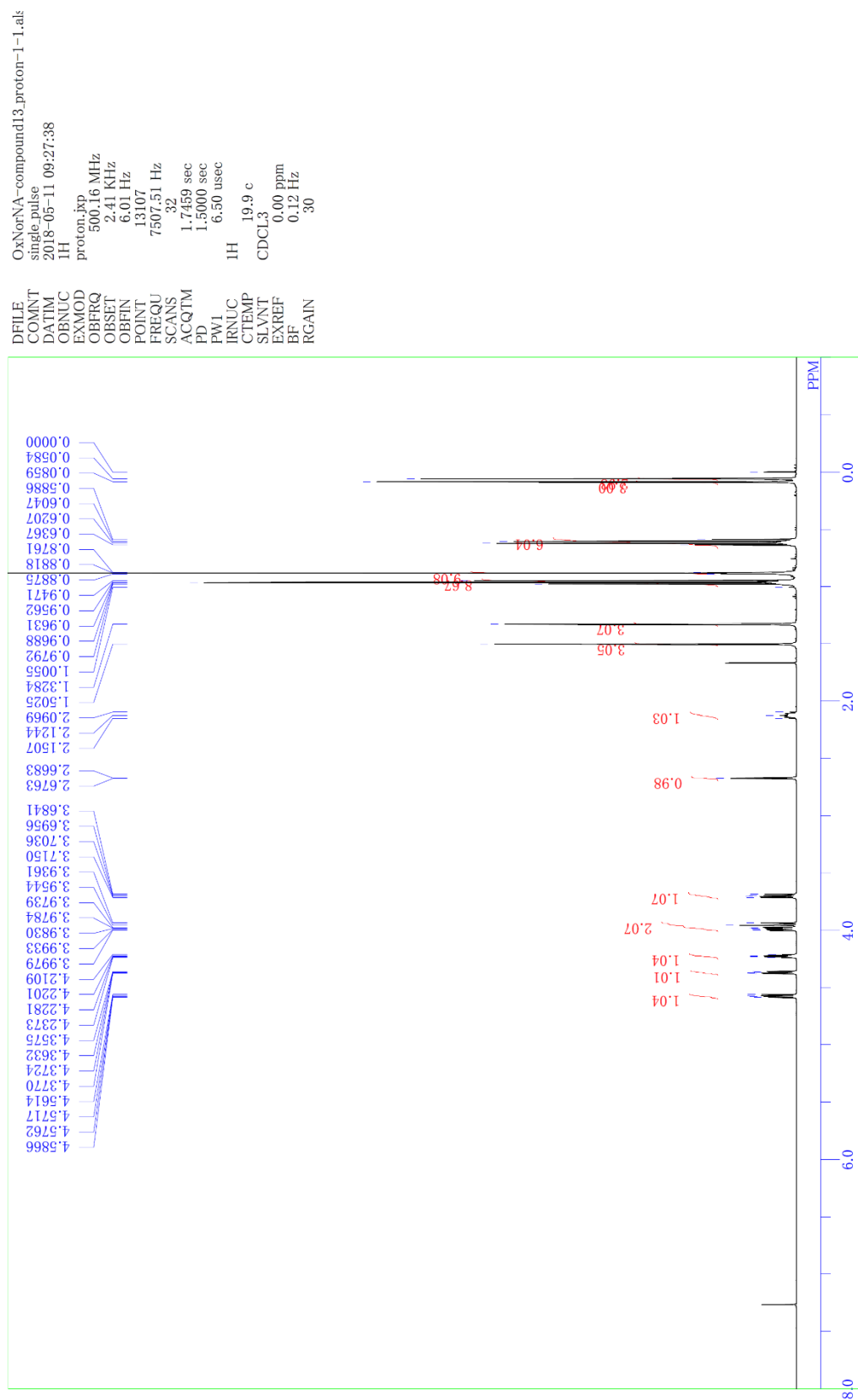
Compound 5 (^{13}C NMR, CDCl_3 , 125MHz)

OxNorNA-compound12 Carbo
 single pulse decoupled gated 1
 2018-05-17 12:40:55
 ^{13}C
 carbon, bp
 125.77 MHz
 7.87 KHz
 4.21 Hz
 26214
 31446.54 Hz
 500
 0.8336 sec
 2.0000 sec
 3.93 usec
 1H
 20.6 c
 CDCl_3
 77.00 ppm
 0.12 Hz
 5#

DFILE
 COMNT
 DATIM
 OBNUC
 EXMOD
 OBFRQ
 OBSET
 OBFIN
 POINT
 FREQU
 SCANS
 ACQTM
 PD
 PW1
 IRNUC
 CTEMP
 SLVNT
 EXREF
 BF
 RGAIN



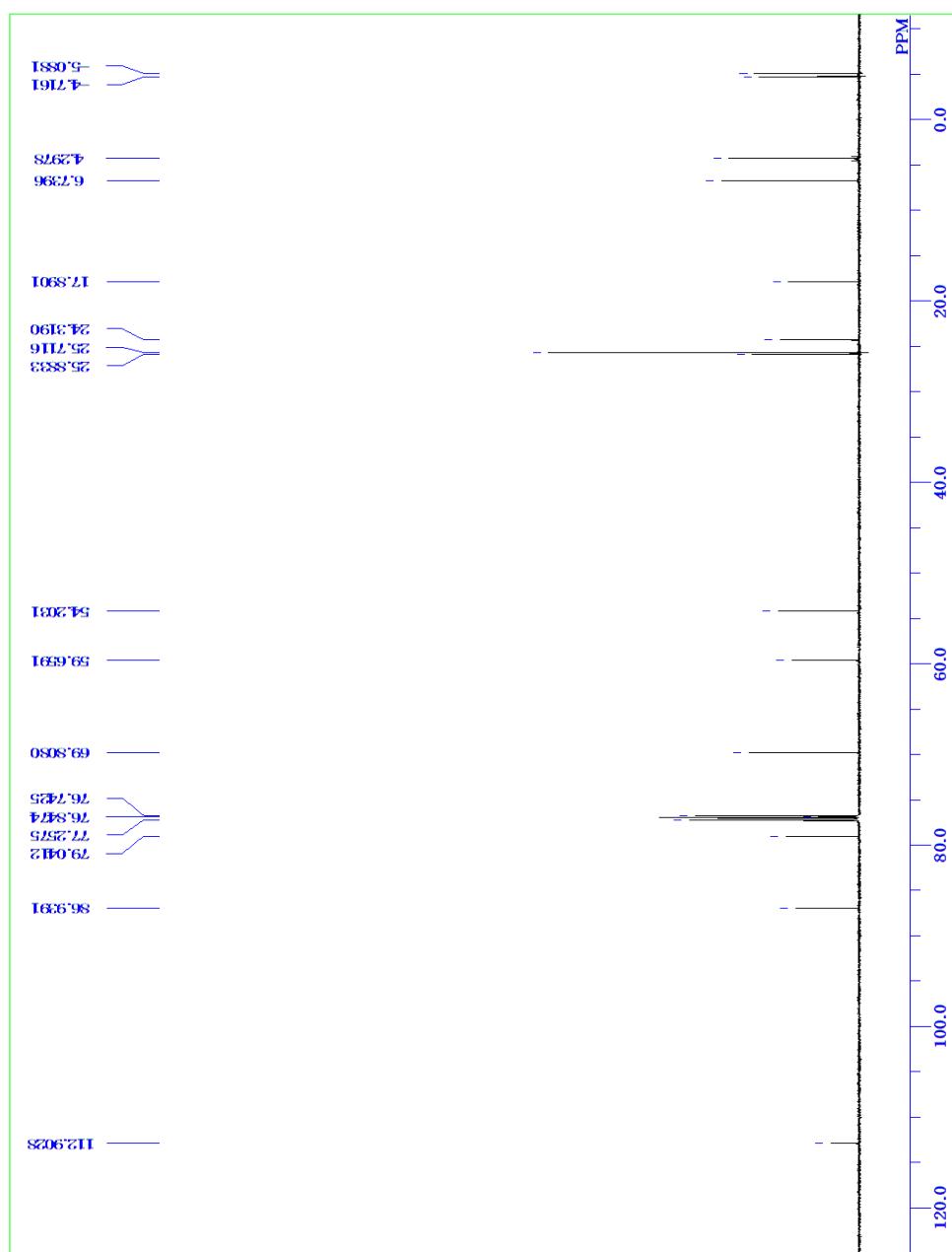
Compound 6 (^1H NMR, CDCl_3 , 500MHz)



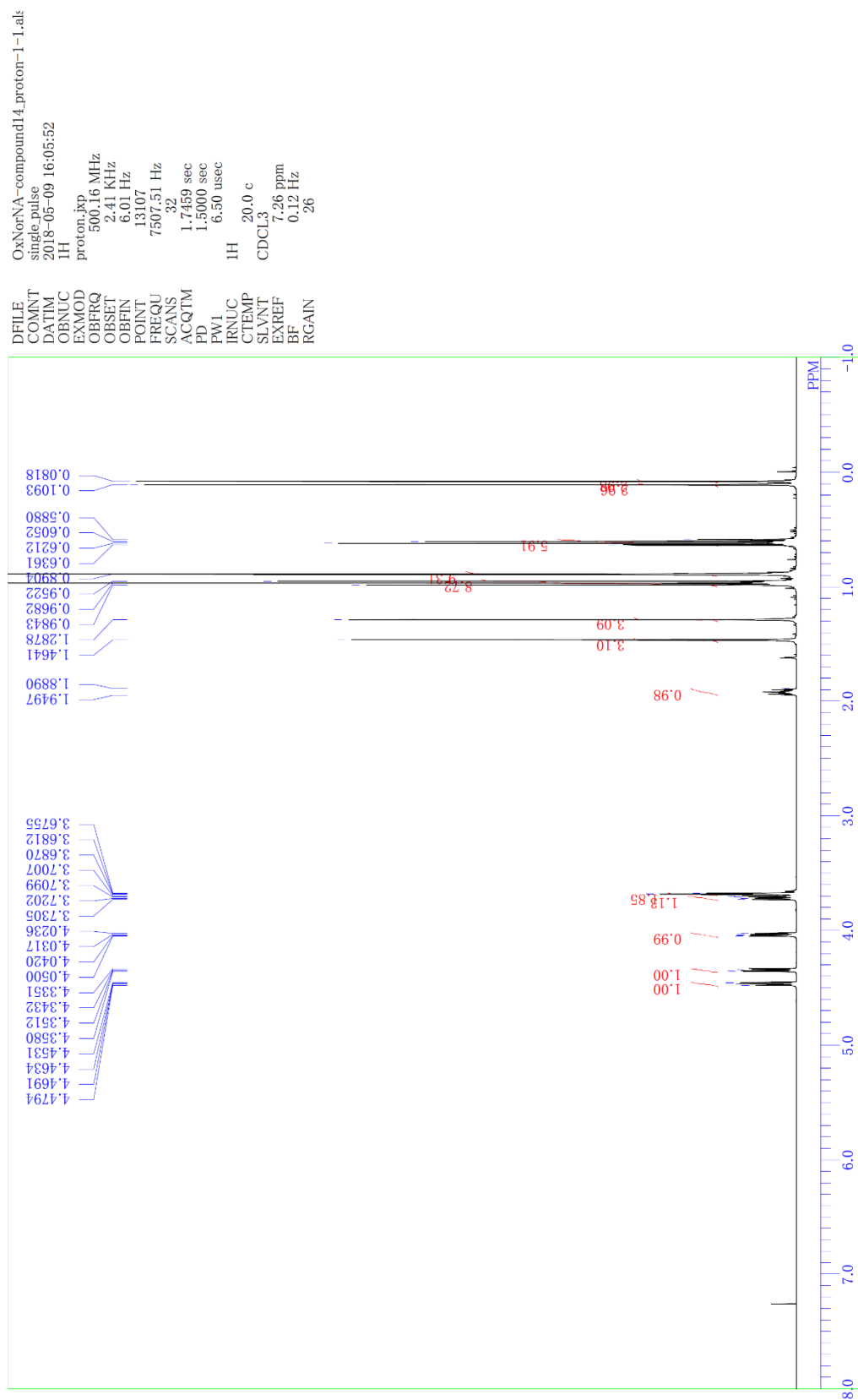
Compound **6** (^{13}C NMR, CDCl_3 , 125MHz)

OxNorNA-compound13_Carbo
single pulse decoupled gated ^{13}C
2018-05-11 09:32:03
 ^{13}C
carbon.kp
125.77 MHz
7.87 KHz
4.21 Hz
26214
31446.54 Hz
500
0.8336 sec
2.0000 sec
PD
3.93 usec
1H
20.8 c
 CDCl_3
77.00 ppm
0.12 Hz
60

DFILE
COMNT
DATIM
OBNUC
EXMOD
OBFRQ
OBSET
OBFIN
POINT
FREQU
SCANS
ACQTM
PD
PW1
IRNUC
CTEMP
SLVNT
EXREF
BF
RGAIN



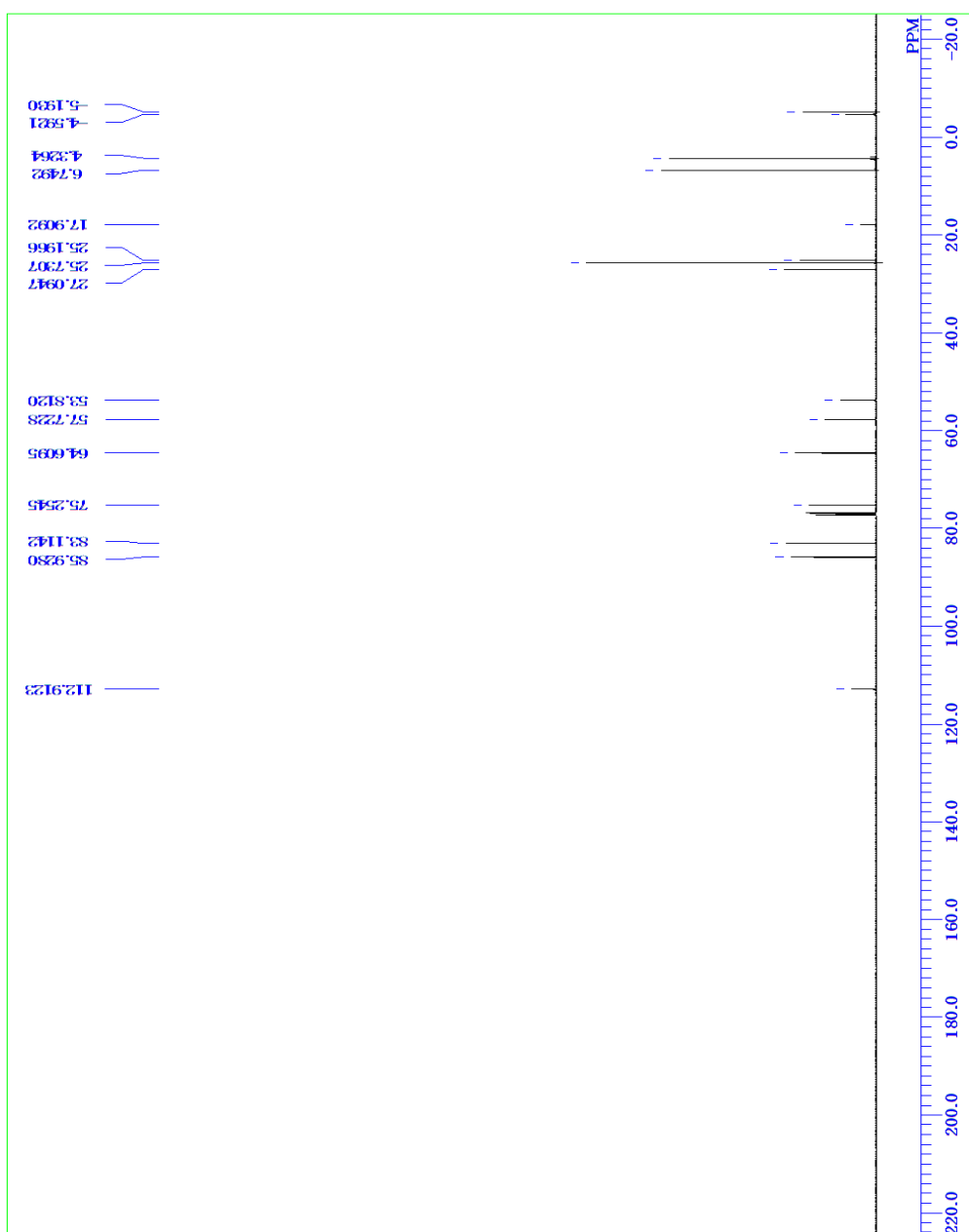
Compound 7 (^1H NMR, CDCl_3 , 500MHz)



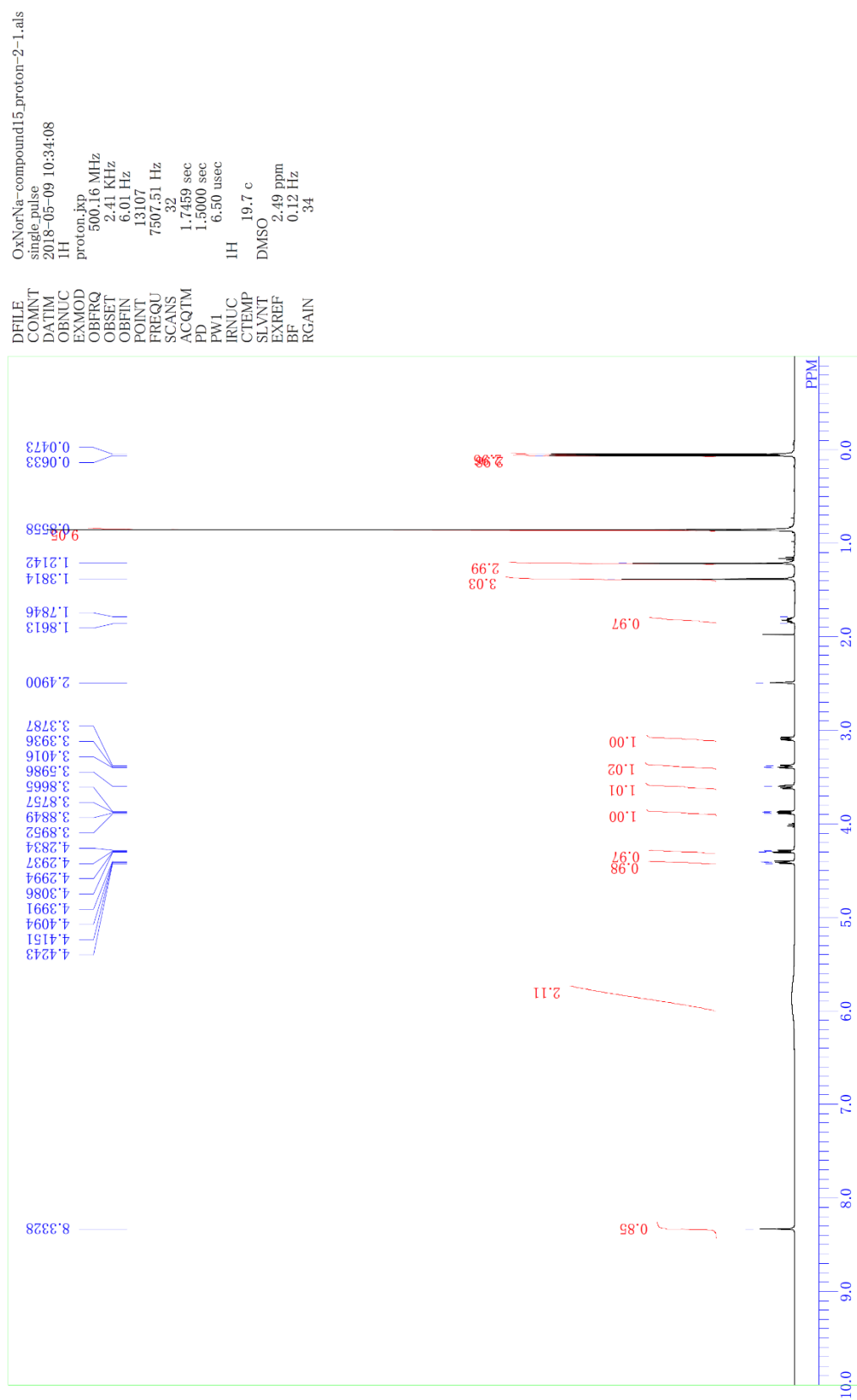
Compound 7 (^{13}C NMR, CDCl_3 , 125MHz)

OxNorNA-compound14_Carbo
single pulse decoupled gated N
2018-05-09 16:10:24
13C
carbon.jpg
125.77 MHz
7.87 KHz
4.21 Hz
26214
31446.54 Hz
600
0.8336 sec
2.0000 sec
3.93 usec
1H
20.5 c
CDCl₃
77.00 ppm
0.12 Hz
58

DFILE
COMNT
DATIM
OBNUC
EXMOD
OBPRQ
OBSET
OBFIN
POINT
FREQU
SCANS
ACQTM
PD
PW1
IRNUC
CTEMP
SLVNT
EXREF
BF
RGAIN

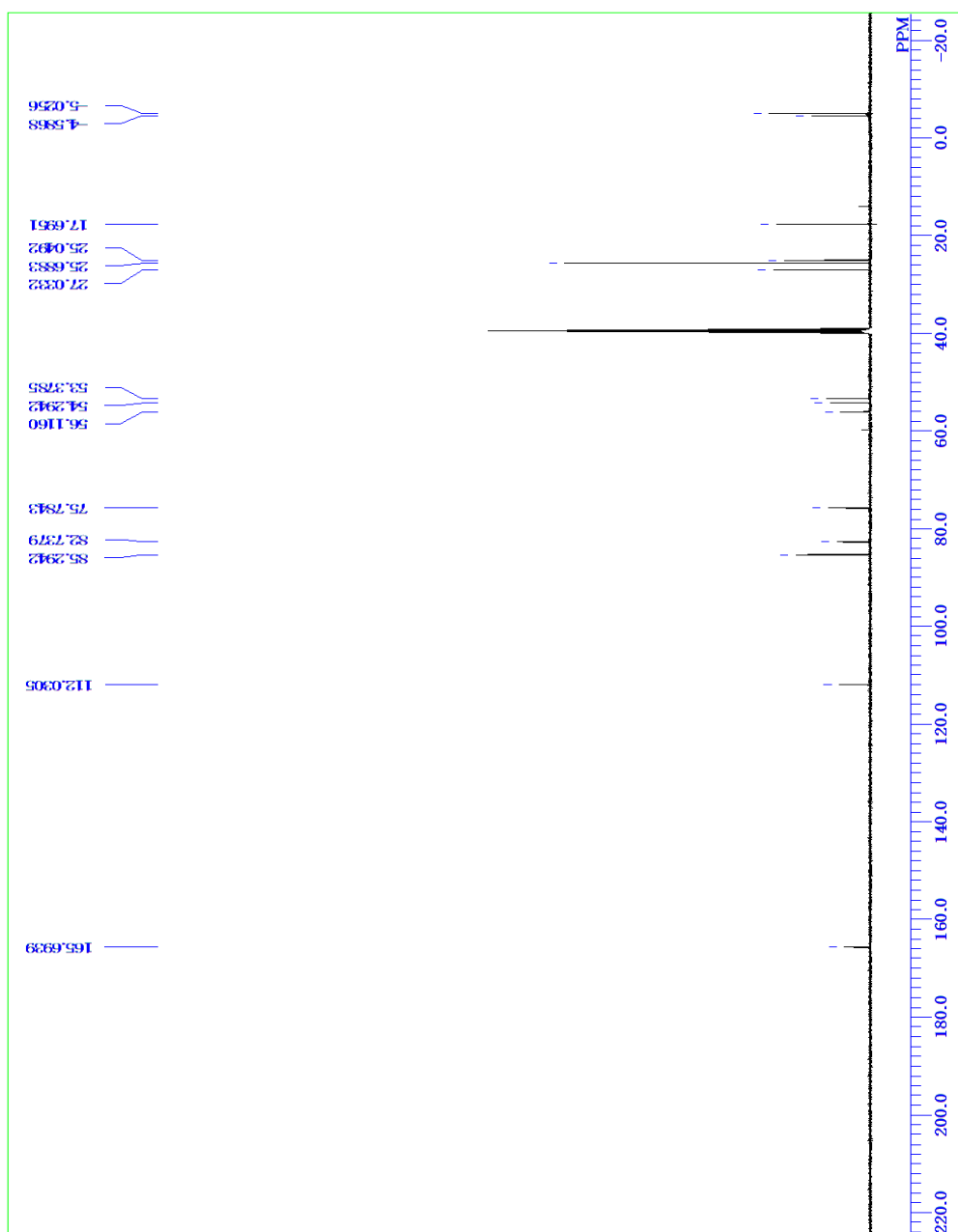


Compound 8 (^1H NMR, $\text{DMSO-}d_6$, 500MHz)

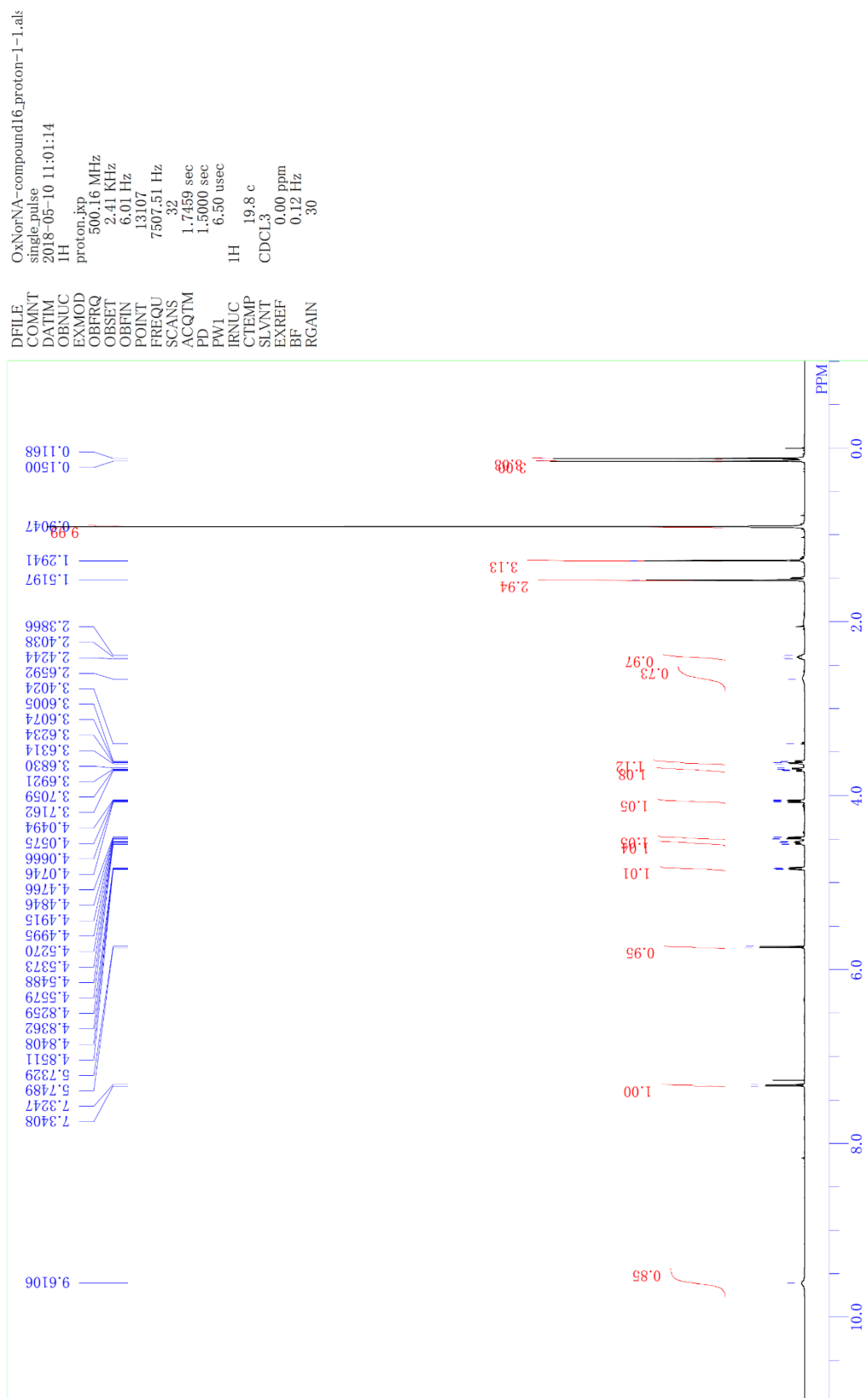


Compound **8** (^{13}C NMR, $\text{DMSO-}d_6$, 125MHz)

DFILE OxNorNa-compound15_Carboi
 COMNT single pulse decoupled gated N
 DATIM 2018-05-09 10:38:37
 OBNUC ^{13}C
 EXMOD carbon.jpg
 OBFREQ 125.77 MHz
 OBSSET 7.87 KHz
 OBFIN 4.21 Hz
 POINT 26214
 FREQU 31446.54 Hz
 SCANS 500
 ACQTM 0.8336 sec
 PD 2.0000 sec
 PW1 3.93 usec
 IRNUC ^1H
 CTEMP 19.8 c
 SLVNT DMSO
 EXREF 39.50 ppm
 BF 0.12 Hz
 RGAIN 58

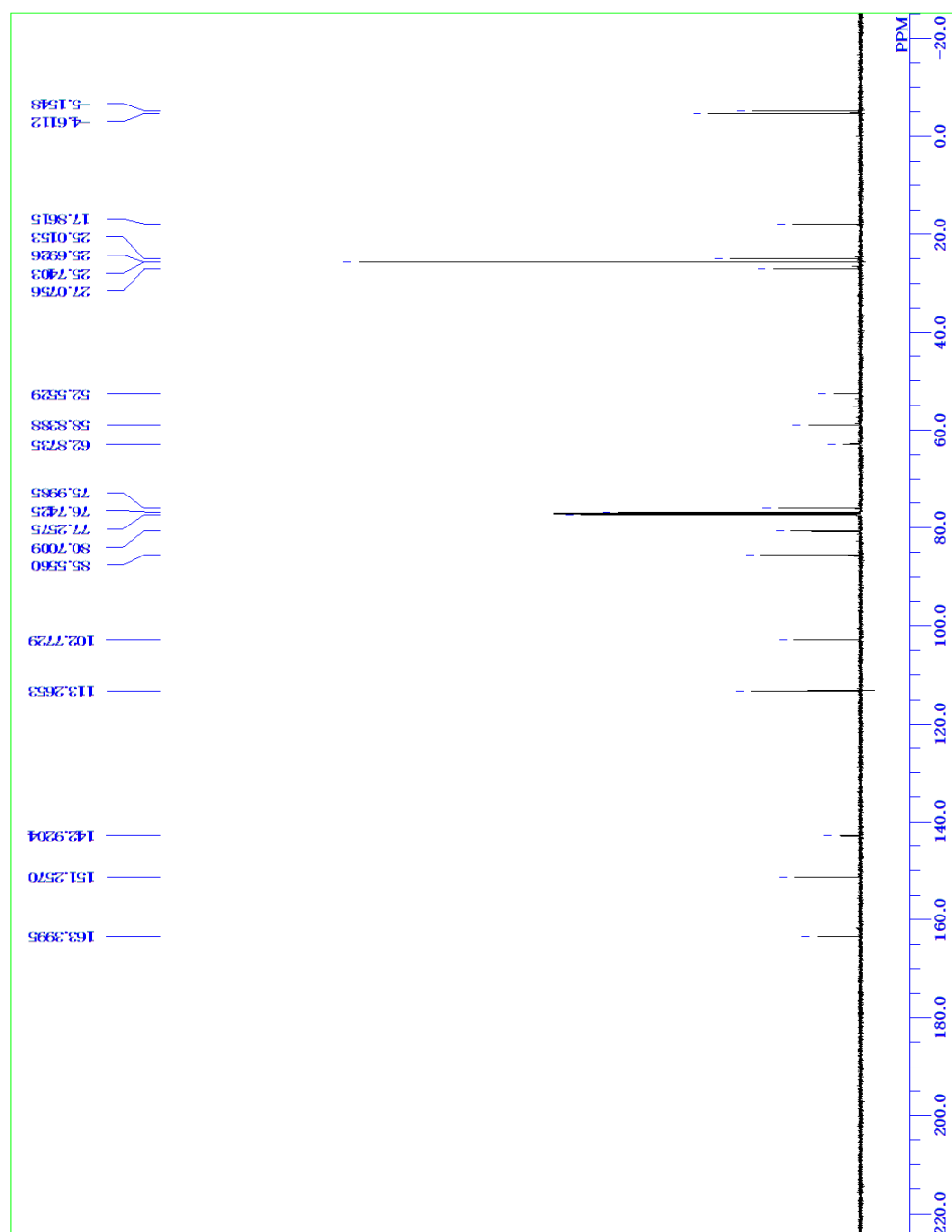


Compound **9** (^1H NMR, CDCl_3 , 500MHz)

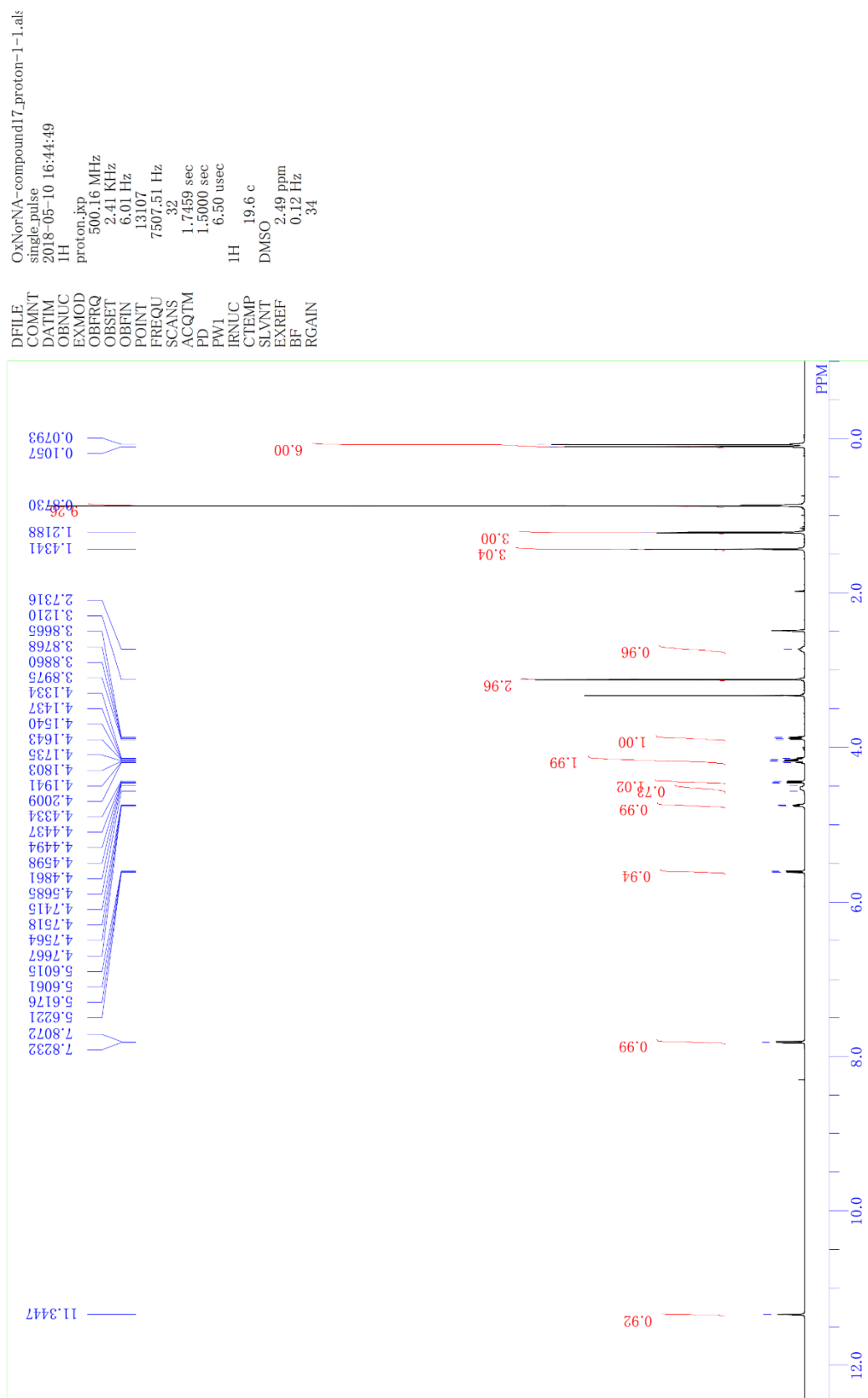


Compound **9** (^{13}C NMR, CDCl_3 , 125MHz)

DFILE OxNorNA-compound16_Carbo
 COMNT single pulse decoupled gated 1
 DATIM 2018-05-10 11:05:36
 OBNUC ^{13}C
 EXMOD carbon-jxp
 OBFREQ 125.77 MHz
 OBSFET 7.87 KHz
 OBFIN 4.21 Hz
 POINT 26214
 FREQU 31446.54 Hz
 SCANS 500
 ACQTM 0.8336 sec
 PD 2.0000 sec
 PW1 3.93 usec
 IRNUC ^1H
 CTEMP 20.6 c
 SLVNT CDCl_3
 EXREF 77.00 ppm
 BF 0.12 Hz
 RGAIN 60



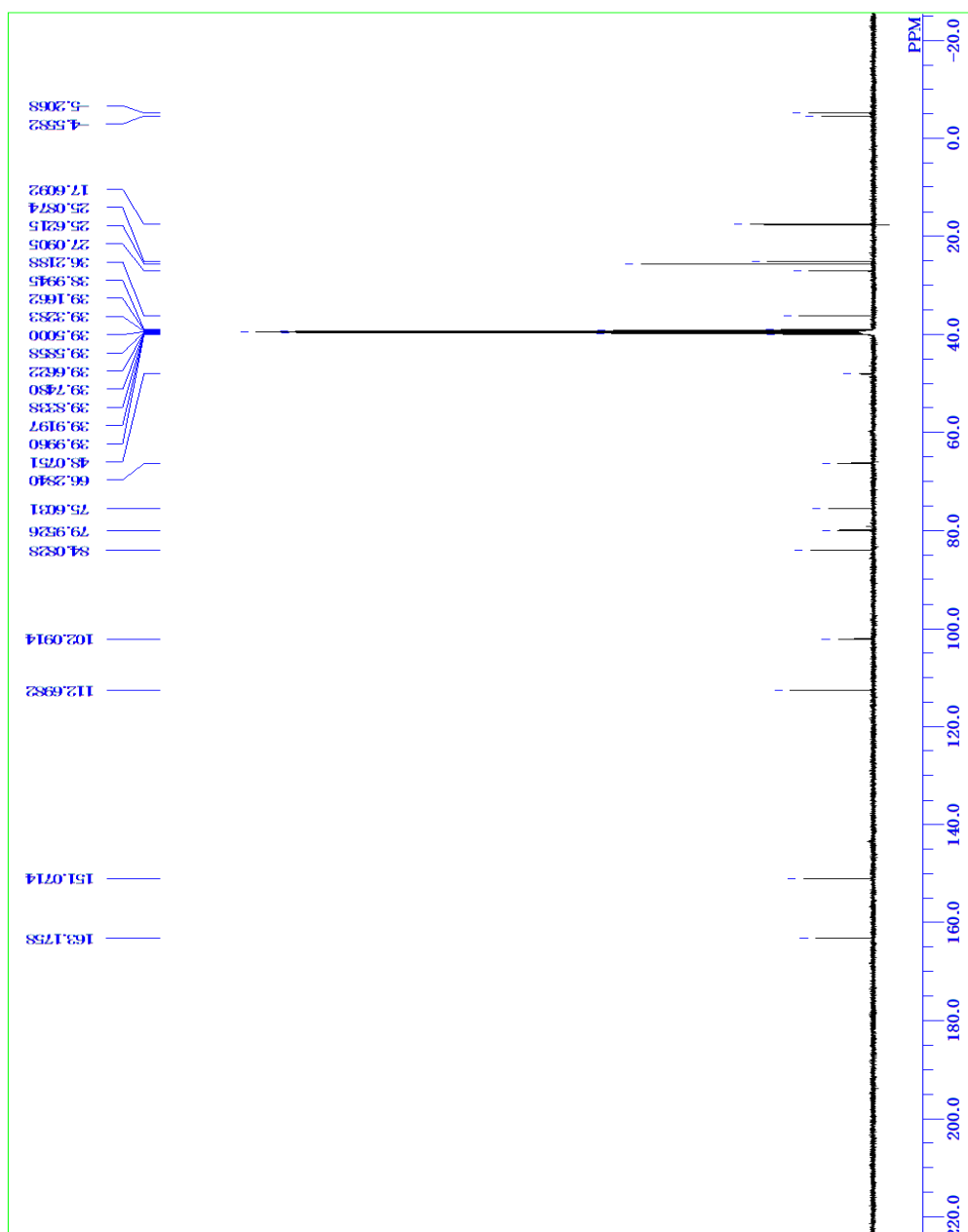
Compound **10** (^1H NMR, $\text{DMSO}-d_6$, 500MHz)



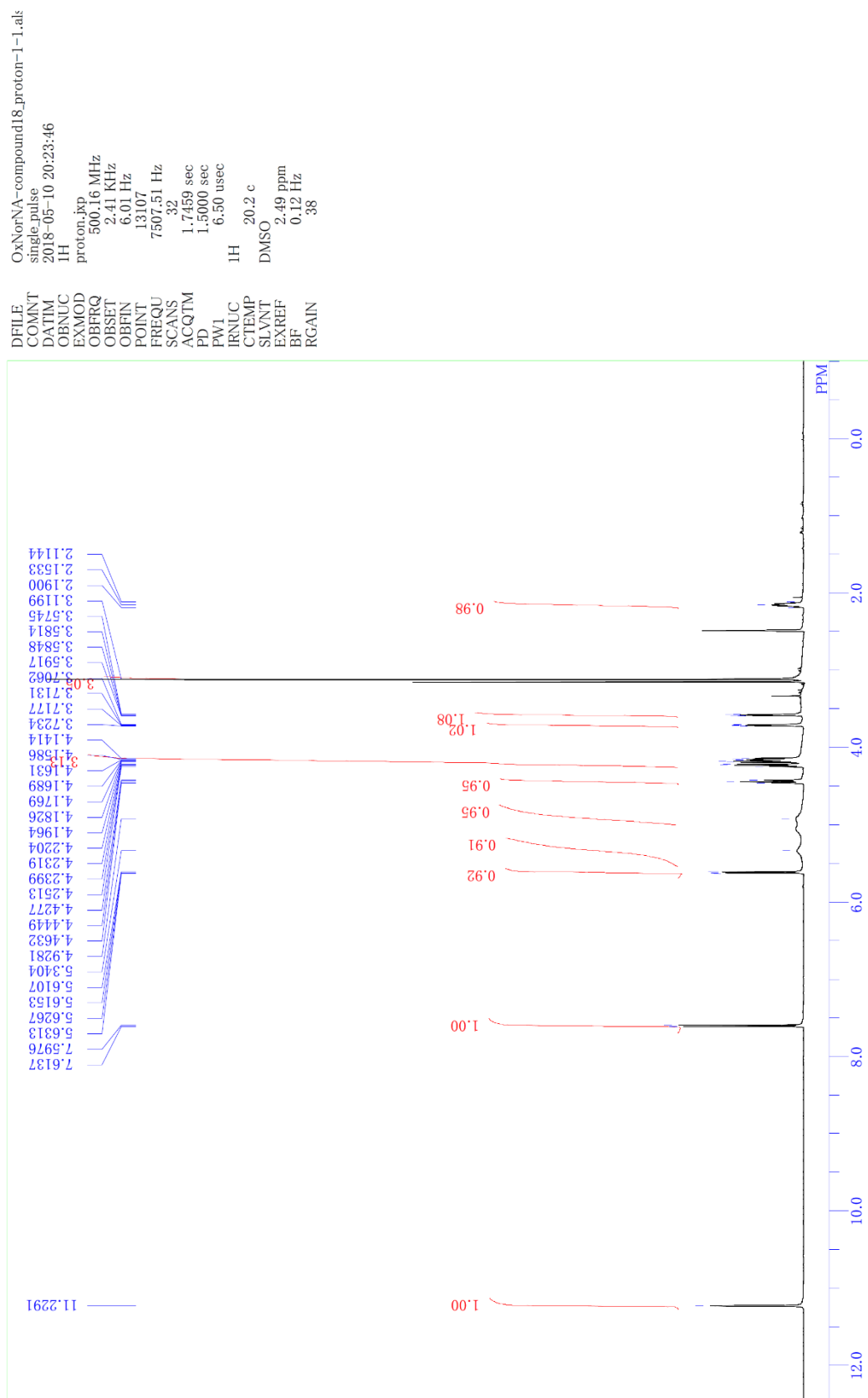
Compound **10** (^{13}C NMR, $\text{DMSO}-d_6$, 125MHz)

OxNorNA-compound17_Carbo
single pulse decoupled gated N
2018-05-10 16:49:25
 ^{13}C
carbon.jpg
125.77 MHz
7.87 kHz
4.21 Hz
26214
31446.54 Hz
500
0.8336 sec
2.0000 sec
3.93 usec
1H
DMSO
20.2 c
39.50 ppm
0.12 Hz
56

DFILE
COMNT
DATIM
OBNUC
EXMOD
OBPRQ
OBSET
OBFIN
POINT
FREQU
SCANS
ACQTM
PD
PW1
IRNUC
CTEMP
SLVNT
EXREF
BF
RGAIN



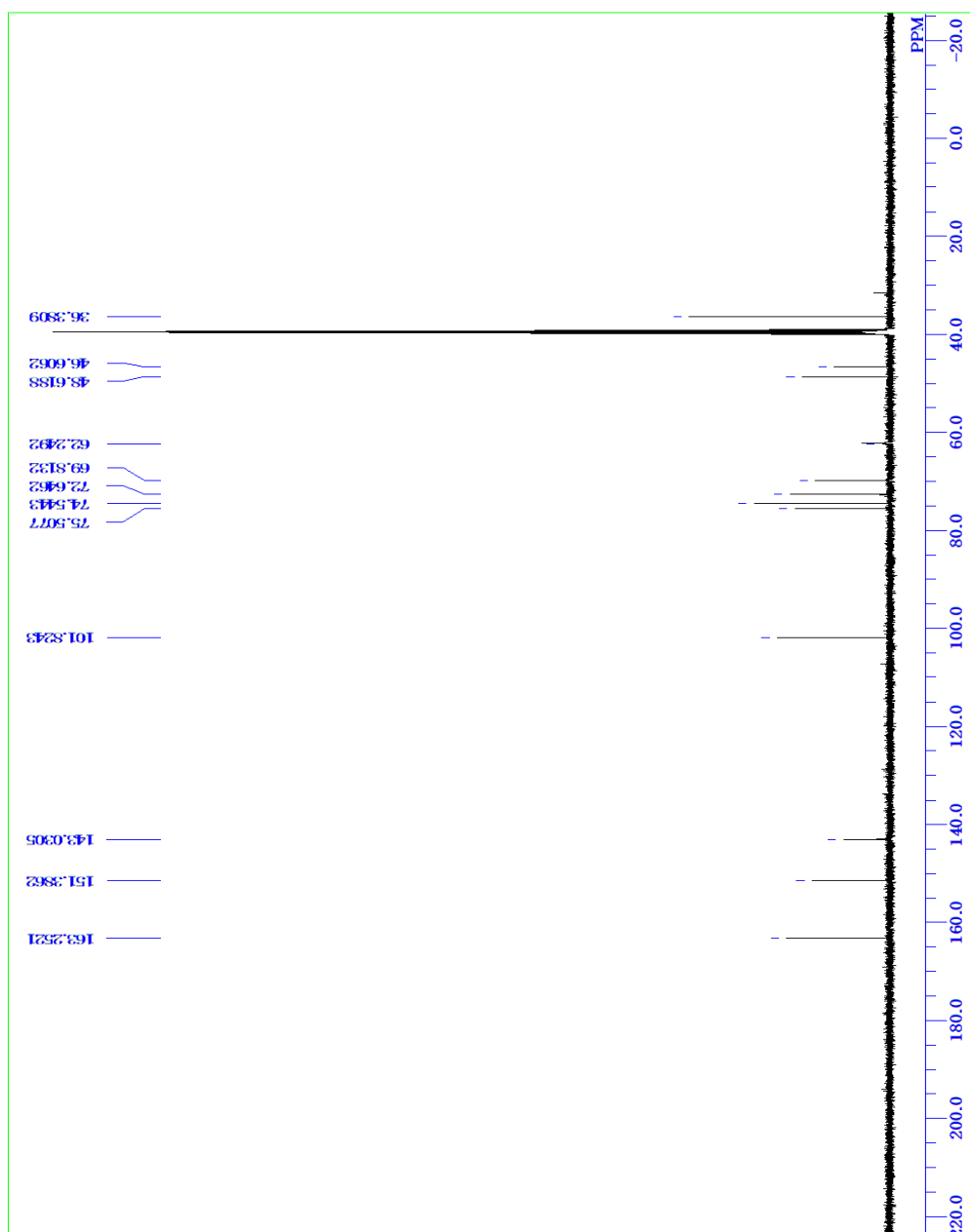
Compound **11** (^1H NMR, $\text{DMSO-}d_6$, 500MHz)



Compound **11** (^{13}C NMR, $\text{DMSO}-d_6$, 125MHz)

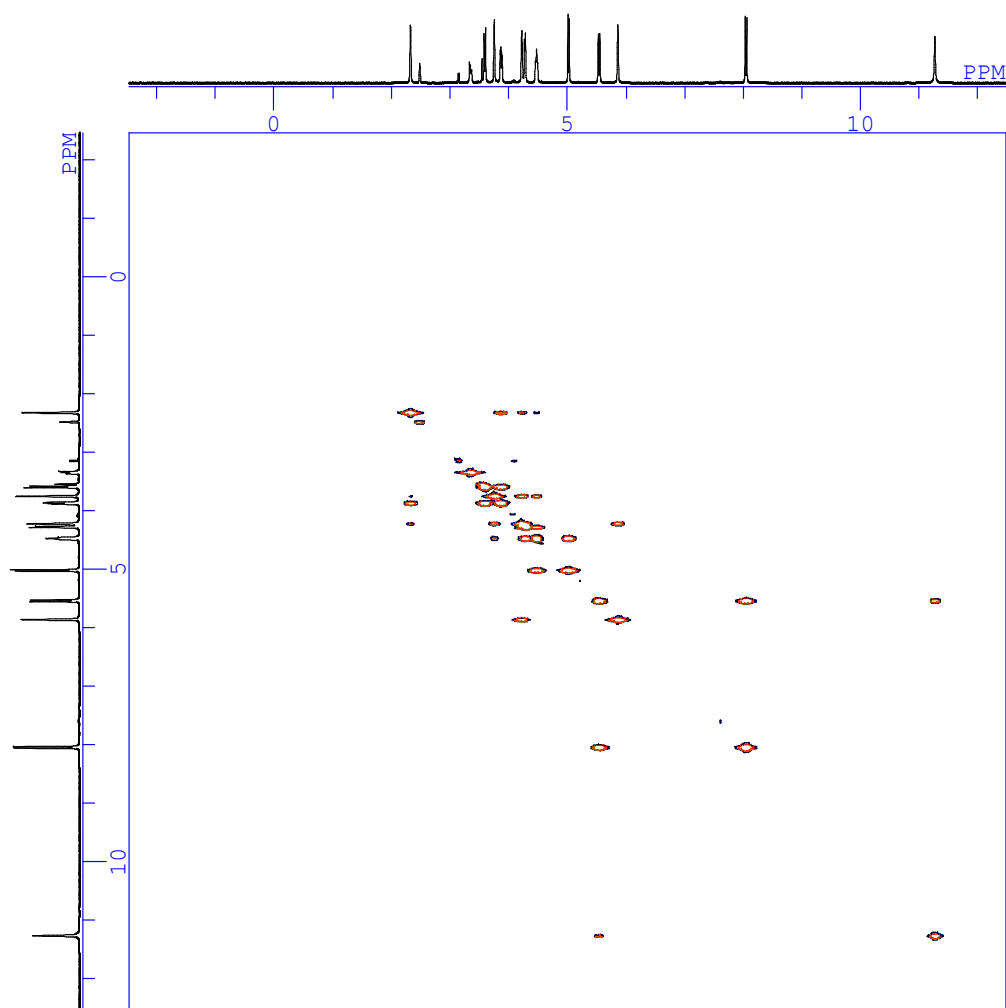
OxNorNA-compound18 Carbo
single pulse decoupled gated N
2018-05-10 20:28:24
13C
carbon.jpg
125.77 MHz
7.87 KHz
4.21 Hz
26214
31446.54 Hz
500
0.8336 sec
2.0000 sec
3.93 usec
1H
20.3 c
DMSO
39.50 ppm
0.12 Hz
58

DFILE
COMNT
DATIM
OBNUC
EXMOD
OBFRQ
OBSET
OBFIN
POINT
FREQU
SCANS
ACQTM
PD
PW1
IRNUC
CTEMP
SLVNT
EXREF
BF
RGAIN

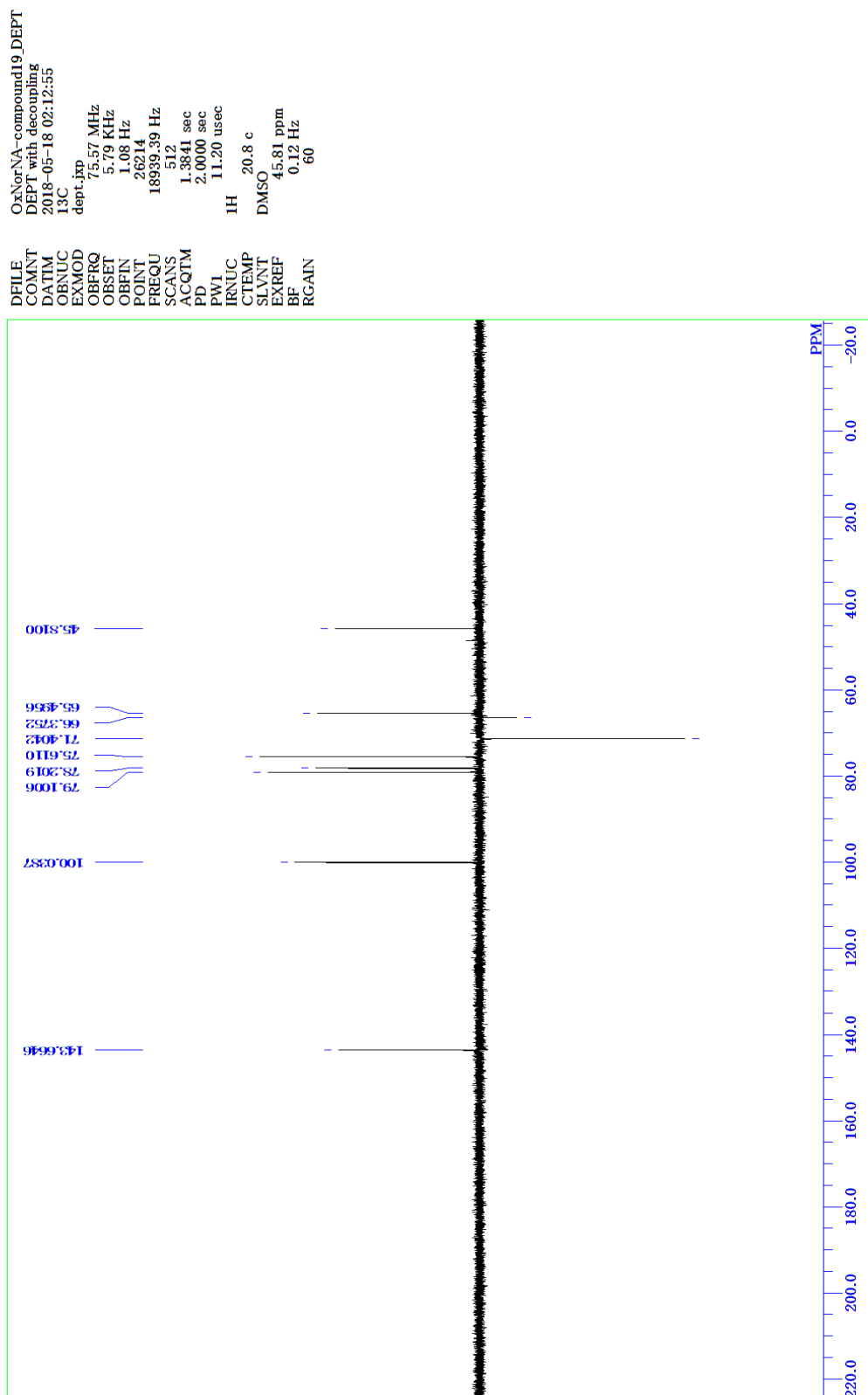


Compound **11** (^1H - ^1H COSY NMR, $\text{DMSO-}d_6$, 300MHz)

DATIM 29-05-2018 21:22:22
 DFILE OxNorNA-Compound19_csy
 OBNUC ^1H
 EXMOD cosy.jxp
 OFR 300.53 MHz
 OBSET 1.15 KHz
 OBFIN 8.57 Hz
 POINT 1024
 FREQU 4508.57 Hz
 SCANS 4
 ACQTM 0.2271 sec
 PD 1.5000 sec
 PW1 11.00 usec
 IRN
 CTEMP 20.9 c
 SLVNT DMSO
 EXREF 2.49 ppm
 BF 4.20 Hz
 RGAIN 44

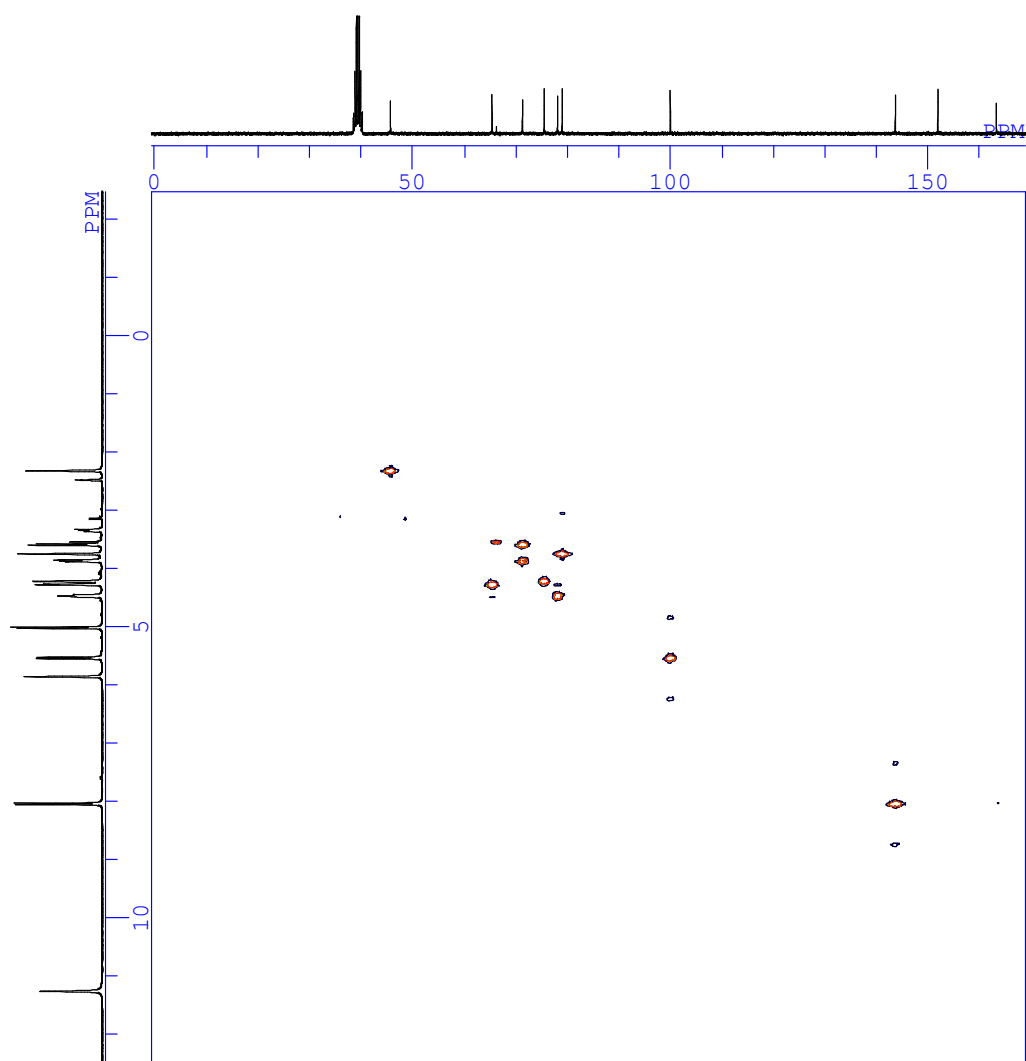


Compound **11** (DEPT NMR, DMSO-*d*₆, 76MHz)



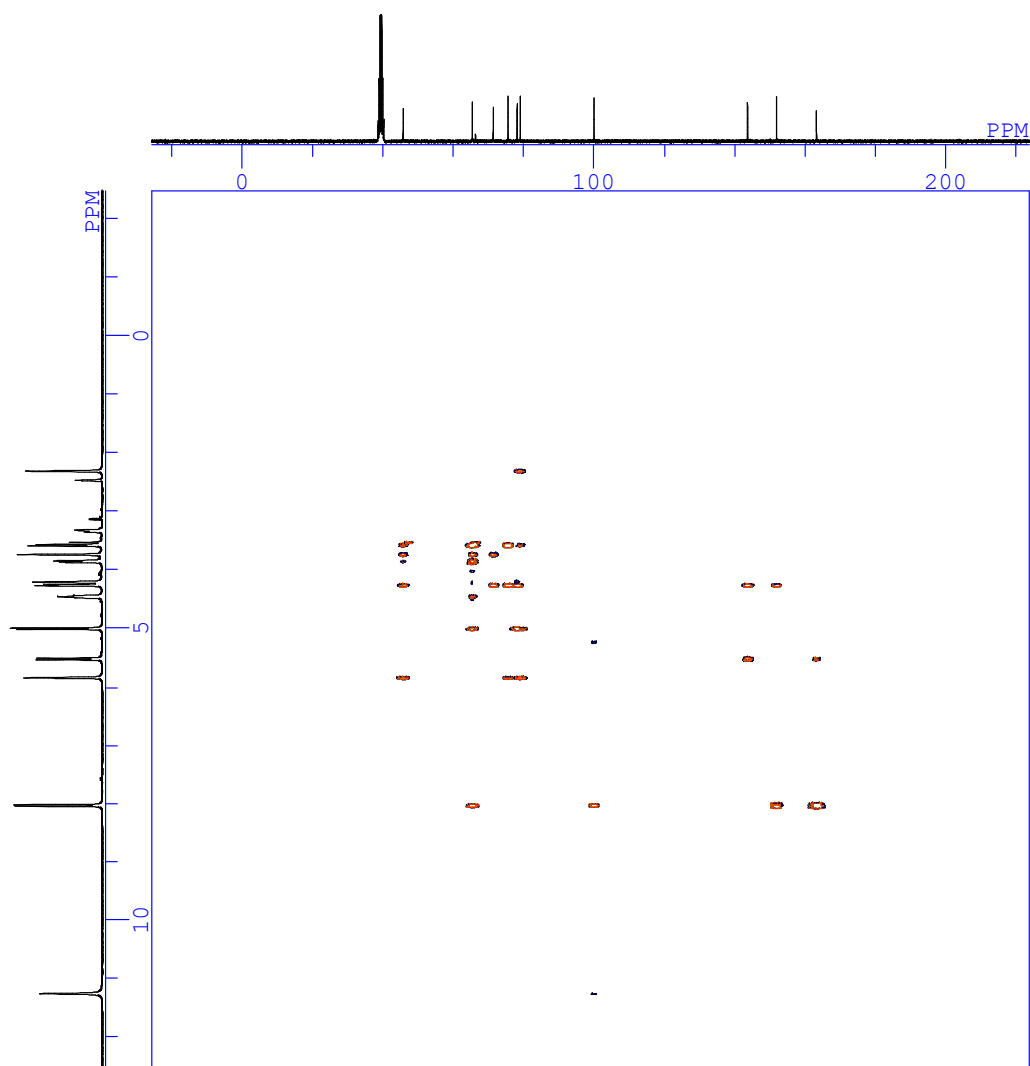
Compound **11** (HMQC NMR, DMSO-*d*₆, 300MHz)

DATIM 18-05-2018 03:14:34
 DFILE OxNorNA-compound19_HMQC
 OBNUC 1H
 EXMOD hmqc.jxp
 OFR 300.53 MHz
 OBSET 1.15 KHz
 OBFIN 8.57 Hz
 POINT 819
 FREQU 4510.60 Hz
 SCANS 4
 ACQTM 0.1816 sec
 PD 1.5000 sec
 PW1 11.00 usec
 IRN
 CTEMP 20.6 c
 SLVNT DMSO
 EXREF 2.49 ppm
 BF 4.20 Hz
 RGAIN 84



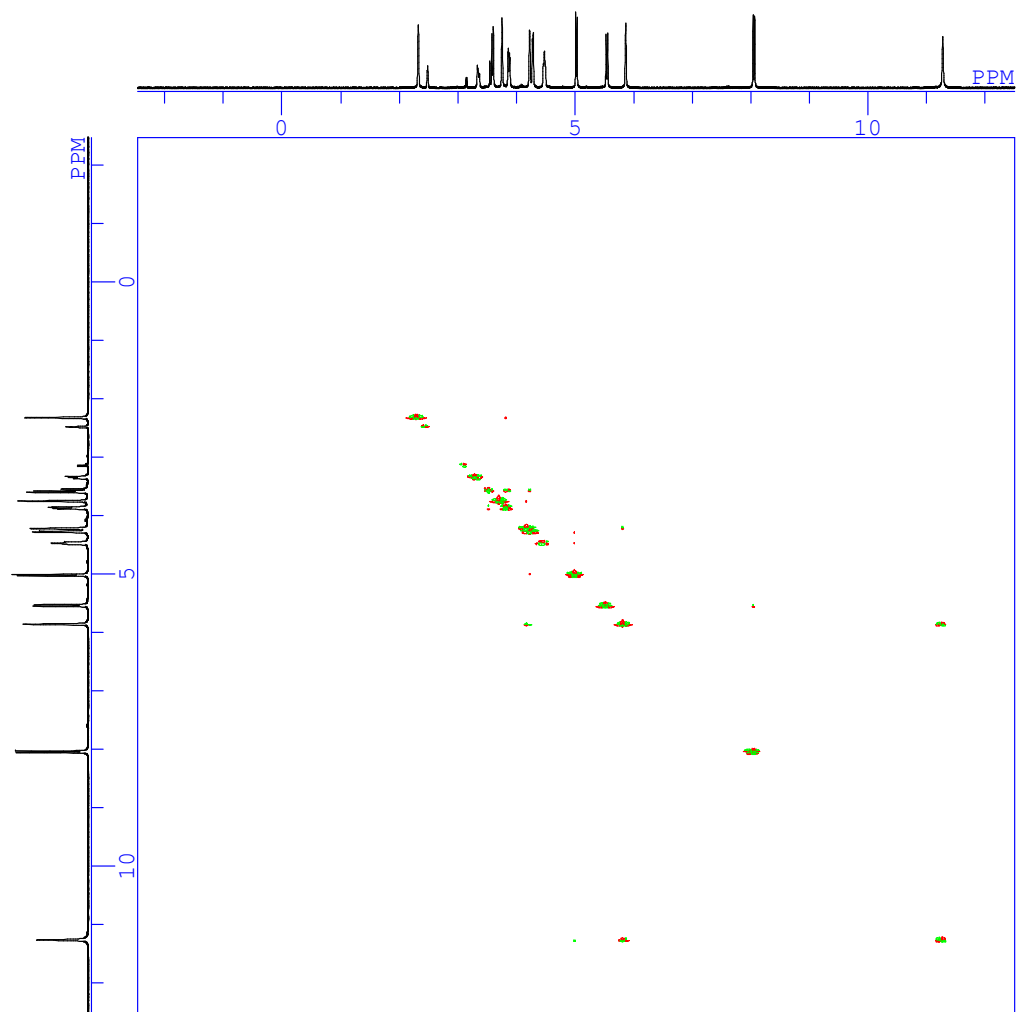
Compound **11** (HMBC NMR, DMSO-*d*₆, 300MHz)

DATIM 18-05-2018 03:45:45
 DFILE OxNorNA-compound19_HMBC
 OBNUC 1H
 EXMOD hmbc.jxp
 OFR 300.53 MHz
 OBSET 1.15 KHz
 OBFIN 8.57 Hz
 POINT 1638
 FREQU 4510.60 Hz
 SCANS 8
 ACQTM 0.3632 sec
 PD 1.5000 sec
 PW1 11.00 usec
 IRN
 CTEMP 20.4 C
 SLVNT DMSO
 EXREF 2.49 ppm
 BF 4.20 Hz
 RGAIN 84

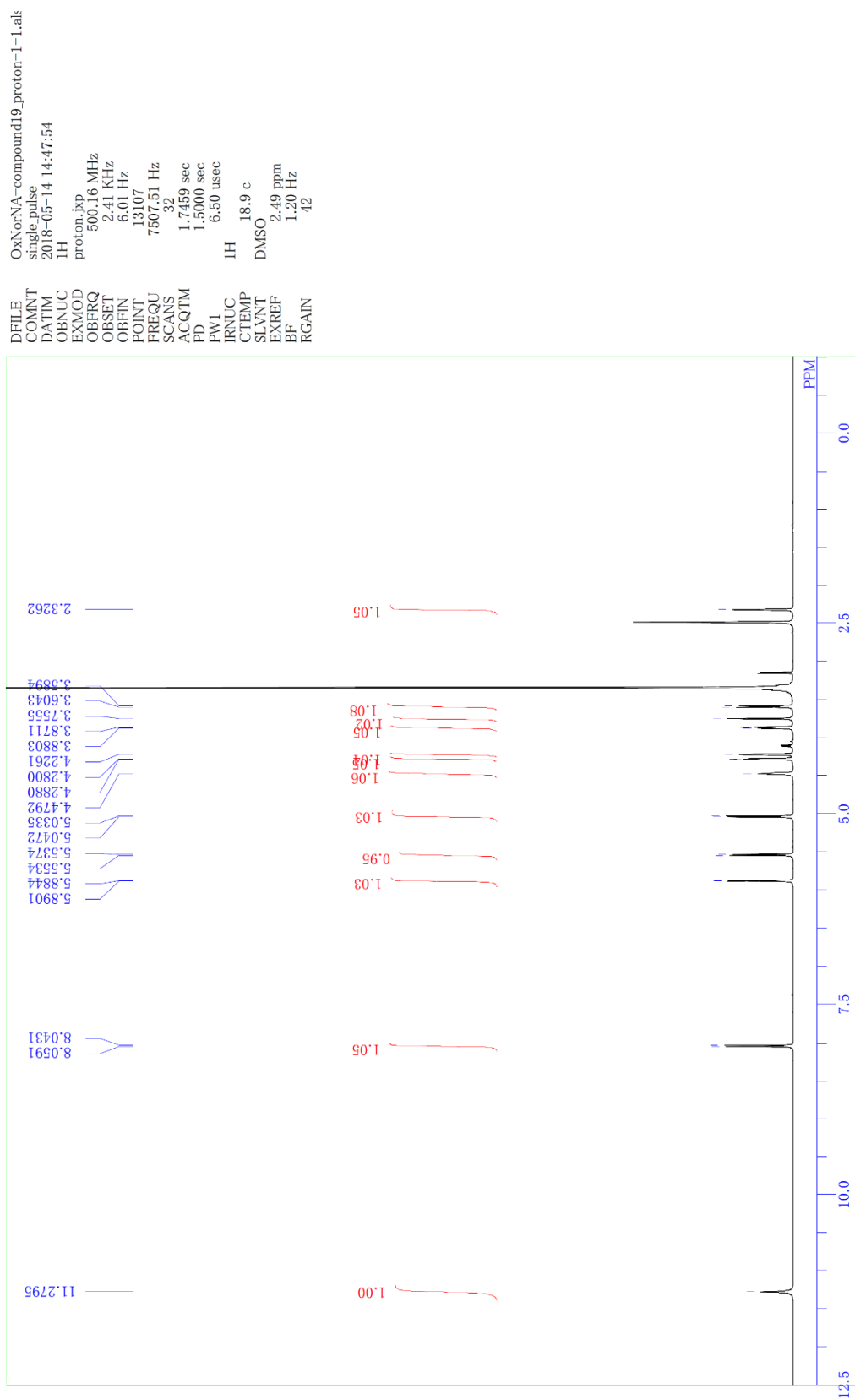


Compound **11** (NOESY NMR, DMSO-*d*₆, 300MHz)

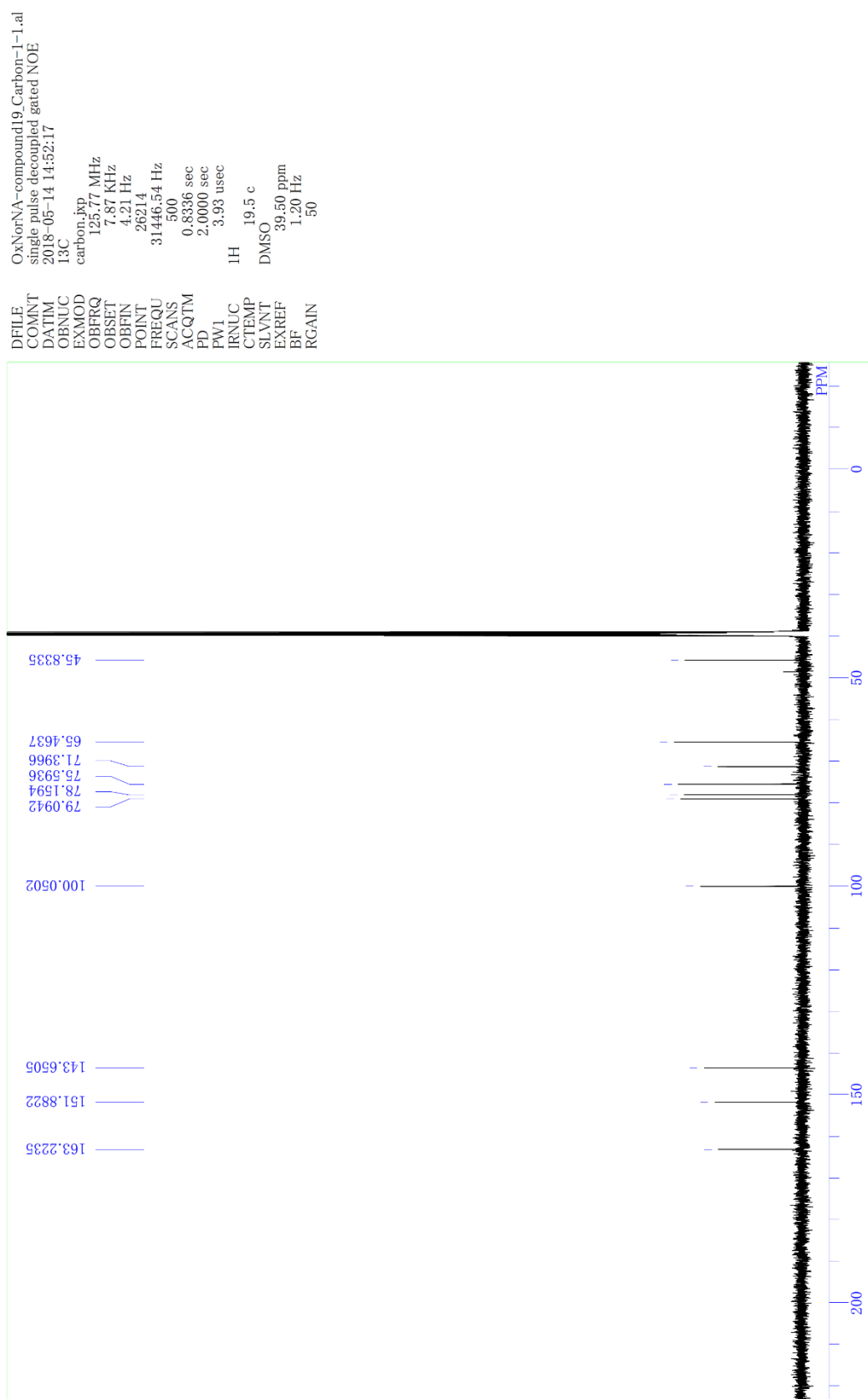
DATIM 18-05-2018 04:52:42
 DFILE OxNorNA-compound19_NOESY
 OBNUC 1H
 EXMOD noesy.jxp
 OFR 300.53 MHz
 OBSET 1.15 KHz
 OBFIN 8.57 Hz
 POINT 819
 FREQU 4510.60 Hz
 SCANS 8
 ACQTM 0.1816 sec
 PD 1.5000 sec
 PW1 11.00 usec
 IRN
 CTEMP 20.5 c
 SLVNT DMSO
 EXREF 2.49 ppm
 BF 4.20 Hz
 PGAIN 46



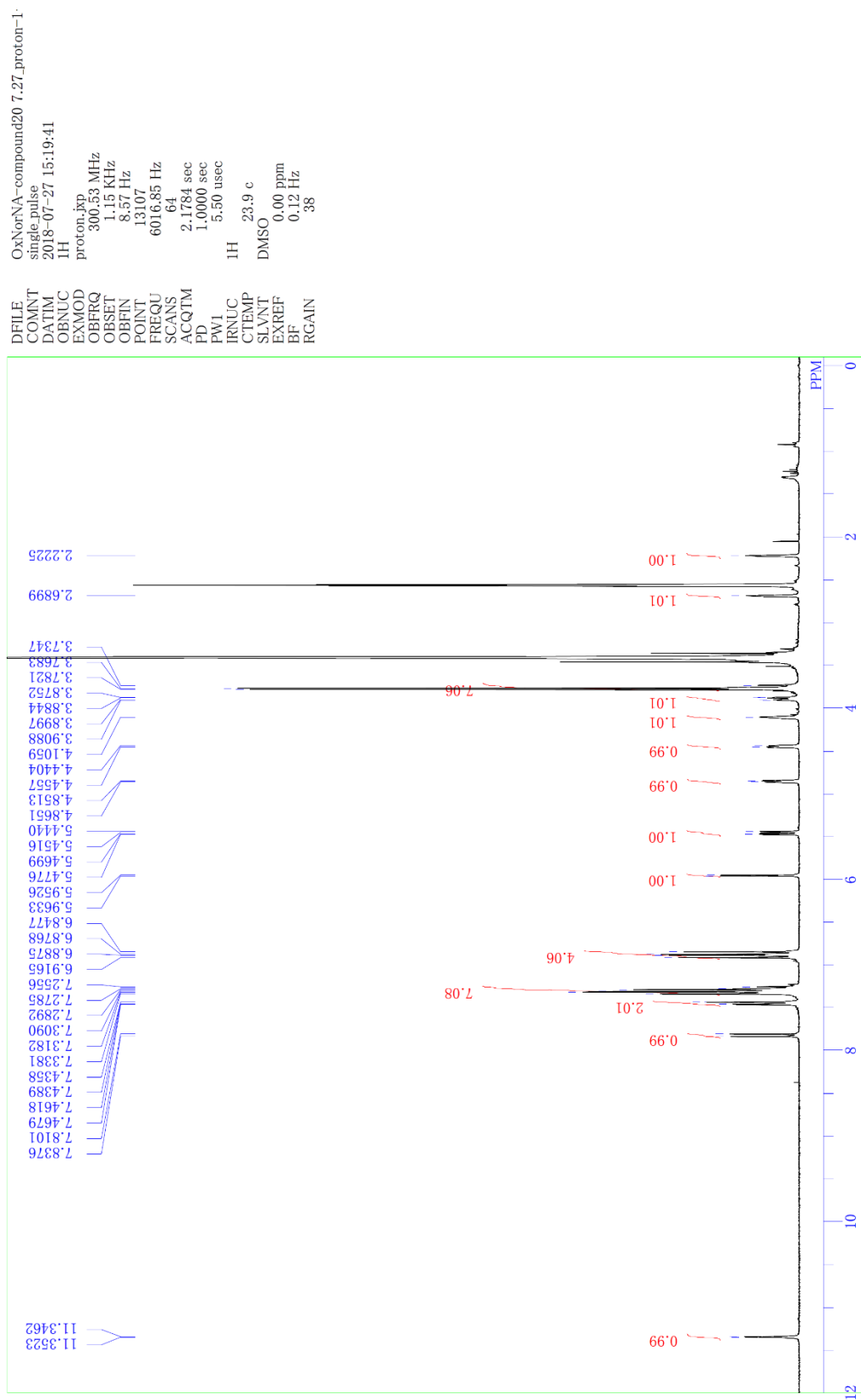
Compound **12** (^1H NMR, $\text{DMSO-}d_6$, 500MHz)



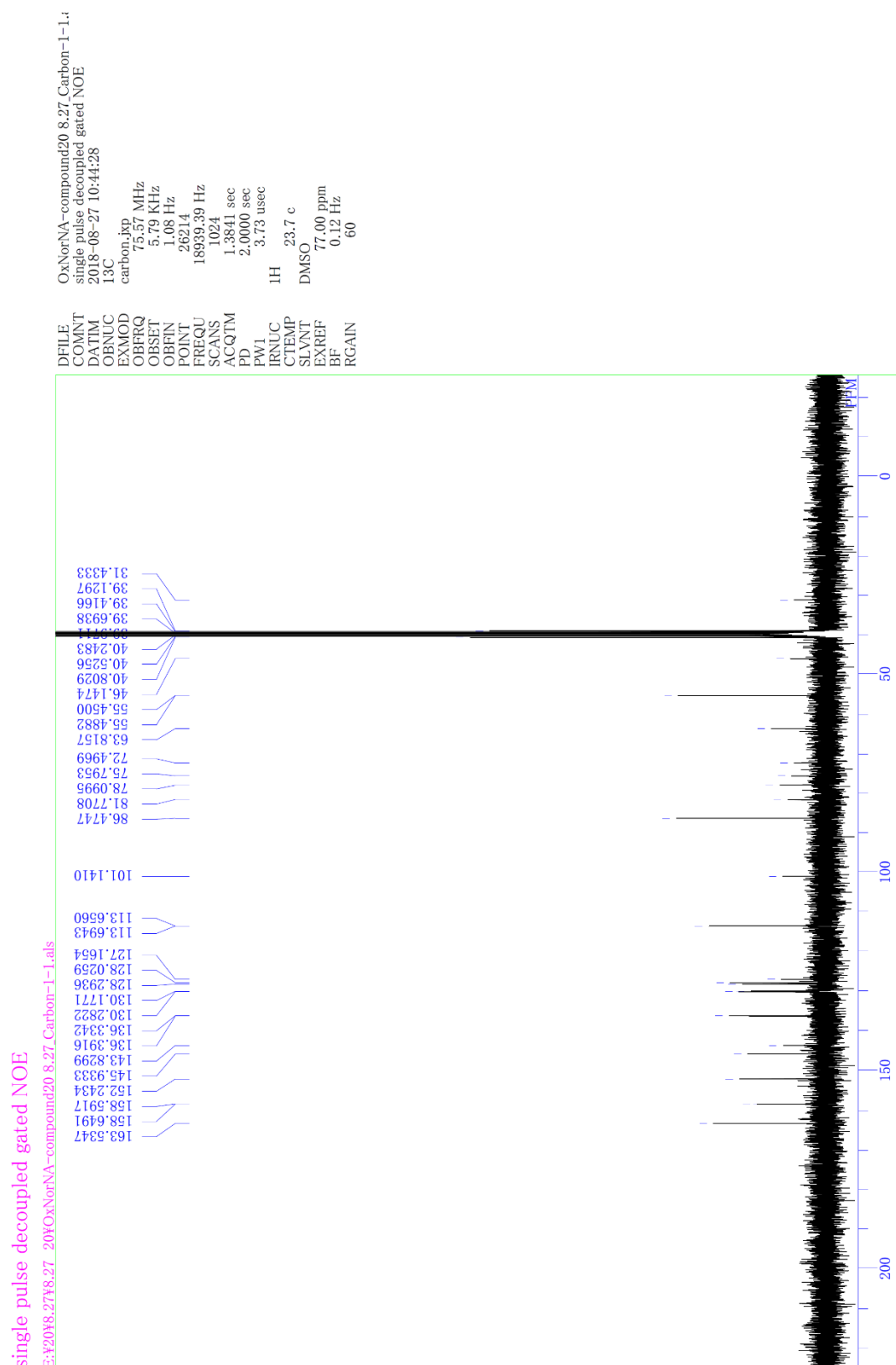
Compound **12** (^{13}C NMR, $\text{DMSO}-d_6$, 125MHz)



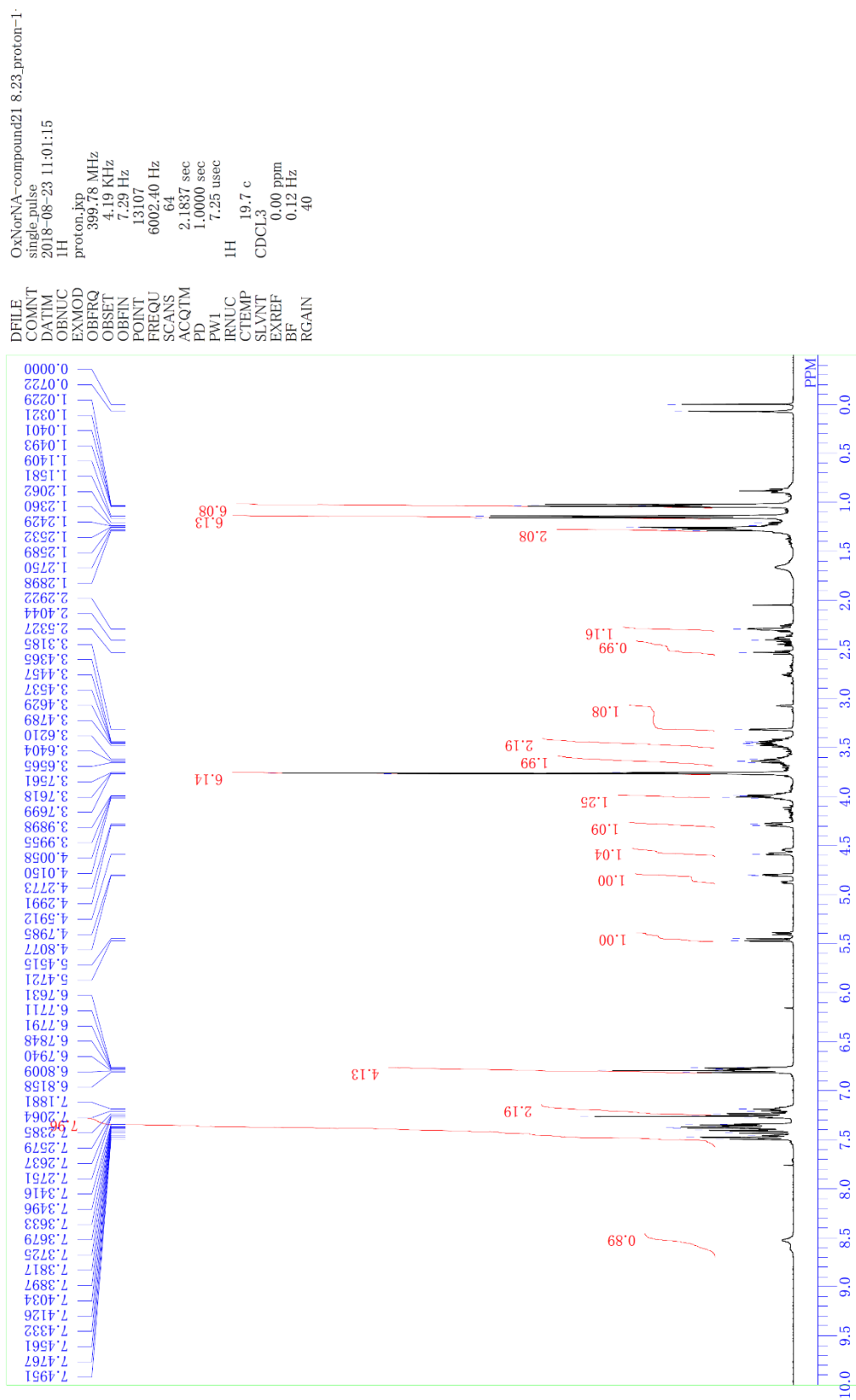
Compound **13** (^1H NMR, $\text{DMSO-}d_6$, 300MHz)



Compound **13** (^{13}C NMR, $\text{DMSO}-d_6$, 75MHz)

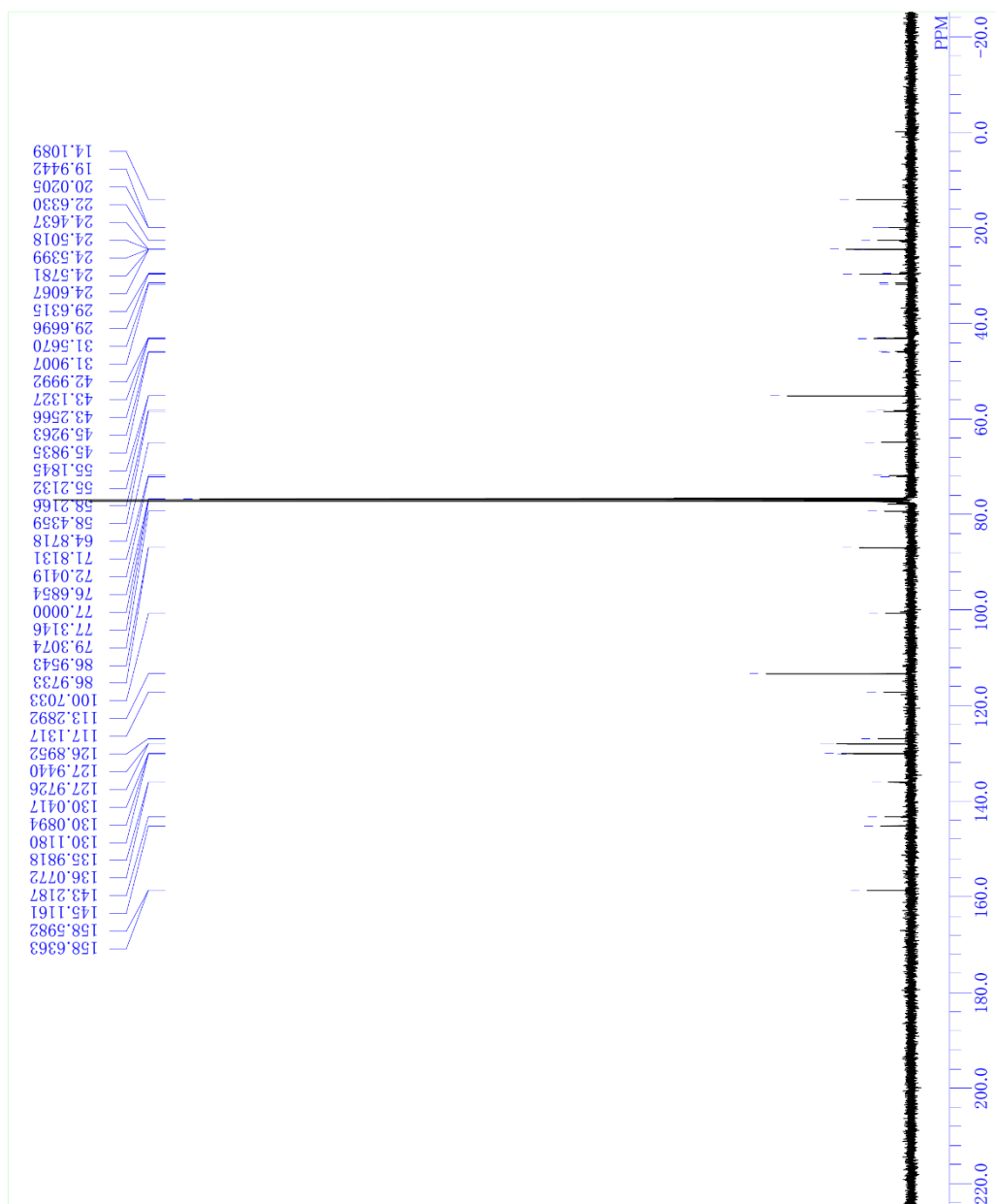


Compound **14** (^1H NMR, CDCl_3 , 400MHz)



Compound **14** (^{13}C NMR, CDCl_3 , 100MHz)

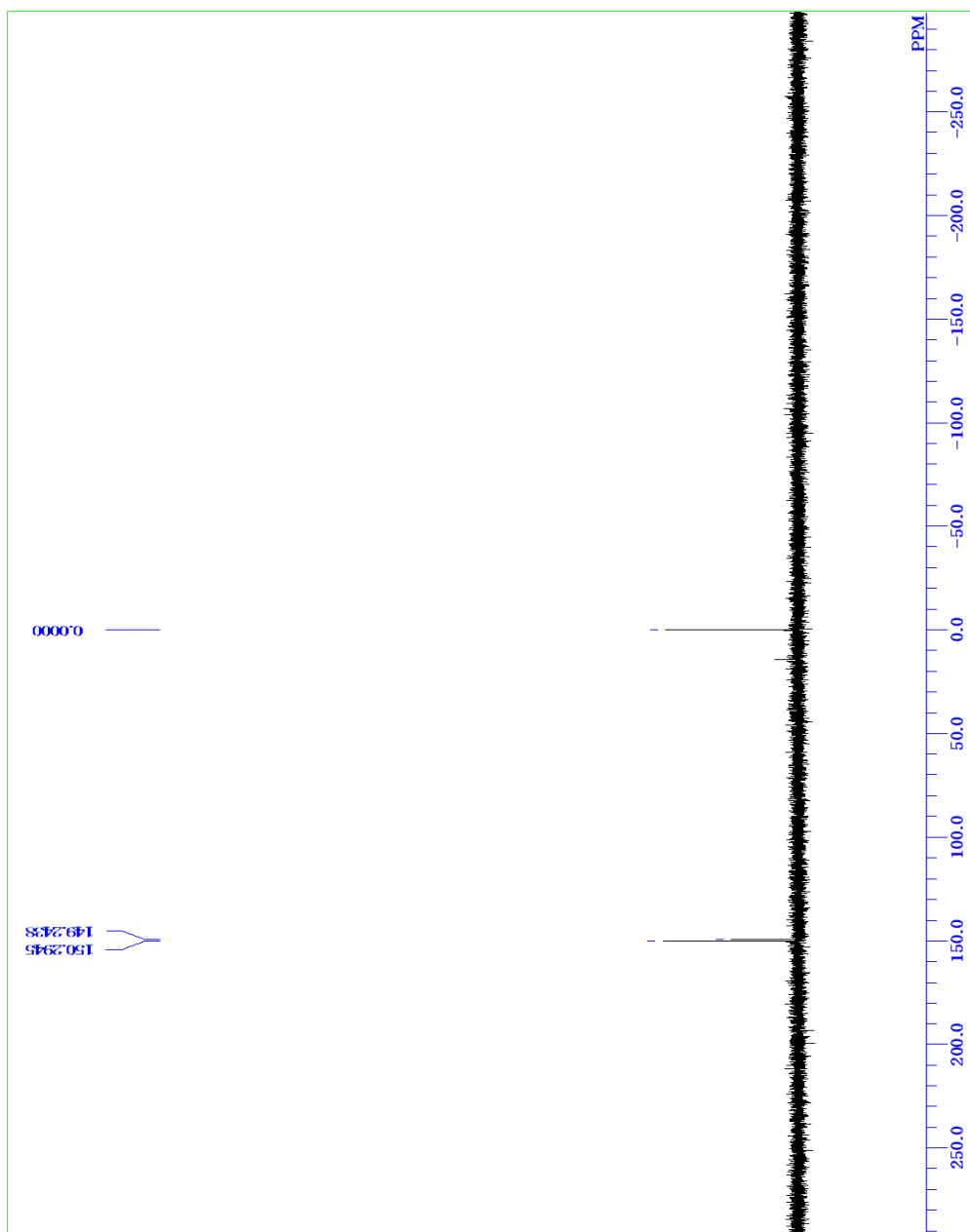
DFILE OxNorVA-compound21 8,23 carbon-1
 COMNT single pulse decoupled gated NOE
 DATIM 2018-09-20 10:13:33
 OBNUC ^{13}C
 EXMOD carbon.jpg
 OBSFQ 100.53 MHz
 OBSF 5.35 KHz
 OBSF 5.86 Hz
 POINT 26214
 FREQU 25125.63 Hz
 SCANS 1025
 ACQTM 1.0433 sec
 PD 2.0000 sec
 PW1 3.17 usec
 IRNUC ^1H
 CTEMP 19.9 c
 SLVNT CDCl_3
 EXREF 77.00 ppm
 BF 0.12 Hz
 RGAIN 60



Compound **14** (^{31}P NMR, CDCl_3 , 162MHz)

OxNorNA-compound21 8.23 s
 single pulse decoupled gated N
 2018-08-23 10:46:52
 31P
 single_pulse_dec
 161.83 MHz
 4.69 KHz
 3.09 Hz
 26214
 114285.71 Hz
 10
 0.2294 sec
 2.0000 sec
 4.97 usec
 1H
 19.2 c
 CDCL3
 0.00 ppm
 0.12 Hz
 56

DFILE
 COMINT
 DATIM
 OBNUC
 EXMOD
 OBFRQ
 OBSET
 OBFIN
 POINT
 FREQU
 SCANS
 ACQTM
 PD
 PW1
 IRNUC
 CTEMP
 SLVNT
 EXREF
 BF
 RGAIN



3. Copies of the HPLC and MALDI-TOF MS charts of the synthesized oligonucleotides

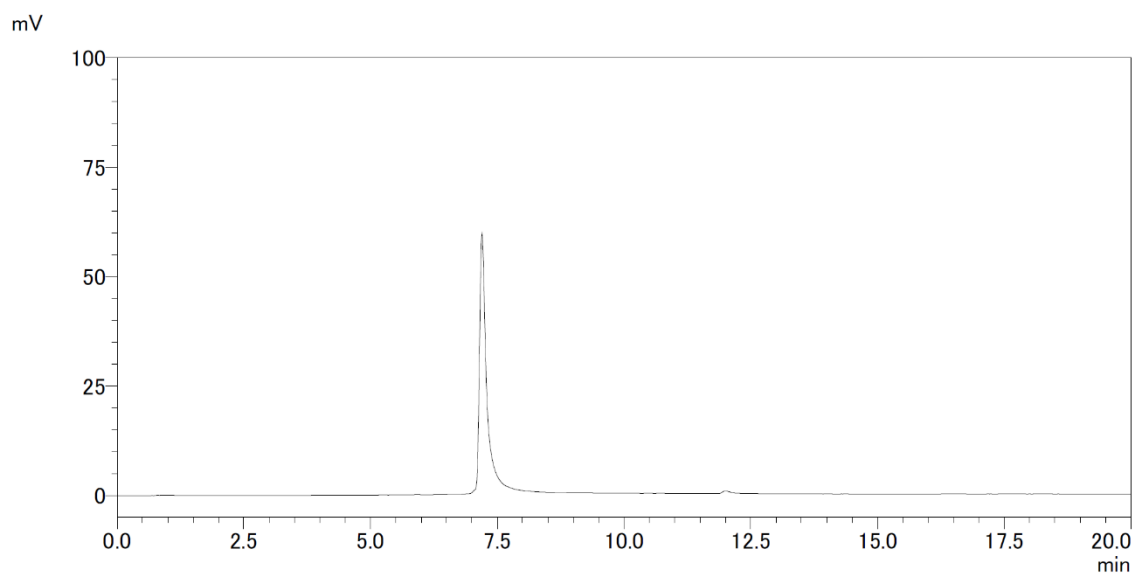
Synthetic protocol for natural oligonucleotides (ON4 and ON5)

The natural DNA oligonucleotides (**ON4** and **ON5**) were synthesized on a 0.2 μmol scale by standard phosphoramidite chemistry (DMTr-off mode) utilizing an nS-8 Oligonucleotide Synthesizer (GeneDesign) and 2'-deoxyuridine phosphoramidite (**Table S3**). The resulting oligonucleotides were detached from the solid support by standard ammonia treatment (28% NH_4OH , rt, 4h), deprotected (28% NH_4OH , 55 $^{\circ}\text{C}$, overnight), and purified quickly with NAPTM-10 SephadexTM G-25 DNA Grade columns and by reverse-phase HPLC.

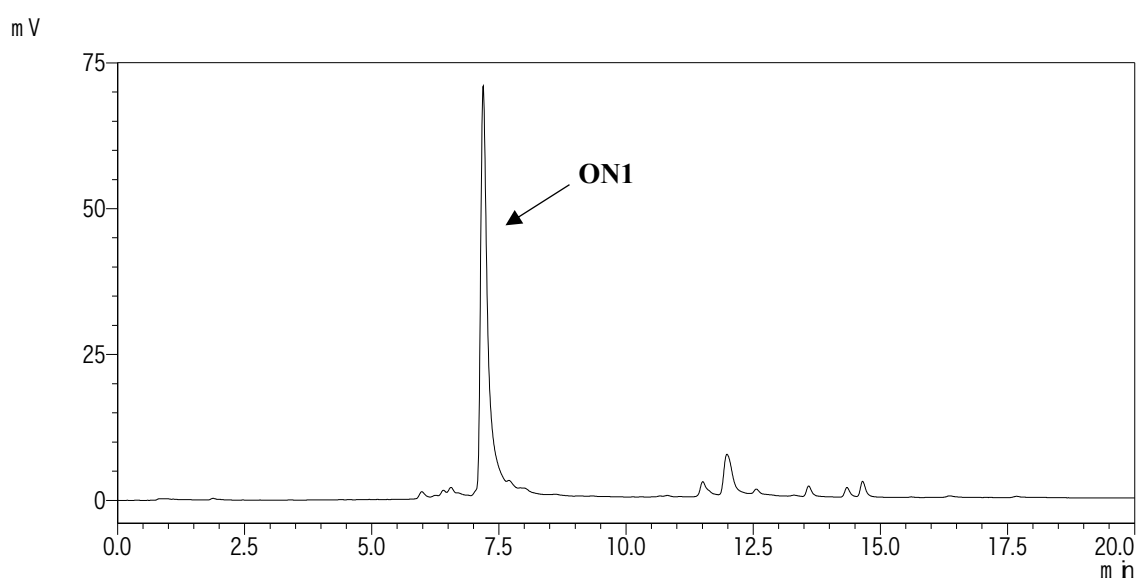
Table S3. Isolated yields of natural oligonucleotides, together with MALDI-TOF MS data

	Sequence ^a	Yield (%)	[M – H] [–]	
			Calcd	Found
ON4	5'-d(GCG TTU TTT GCT)-3'	74	3618.4	3617.9
ON5	5'-d(GCG UTU TUT GCT)-3'	66	3590.3	3589.9

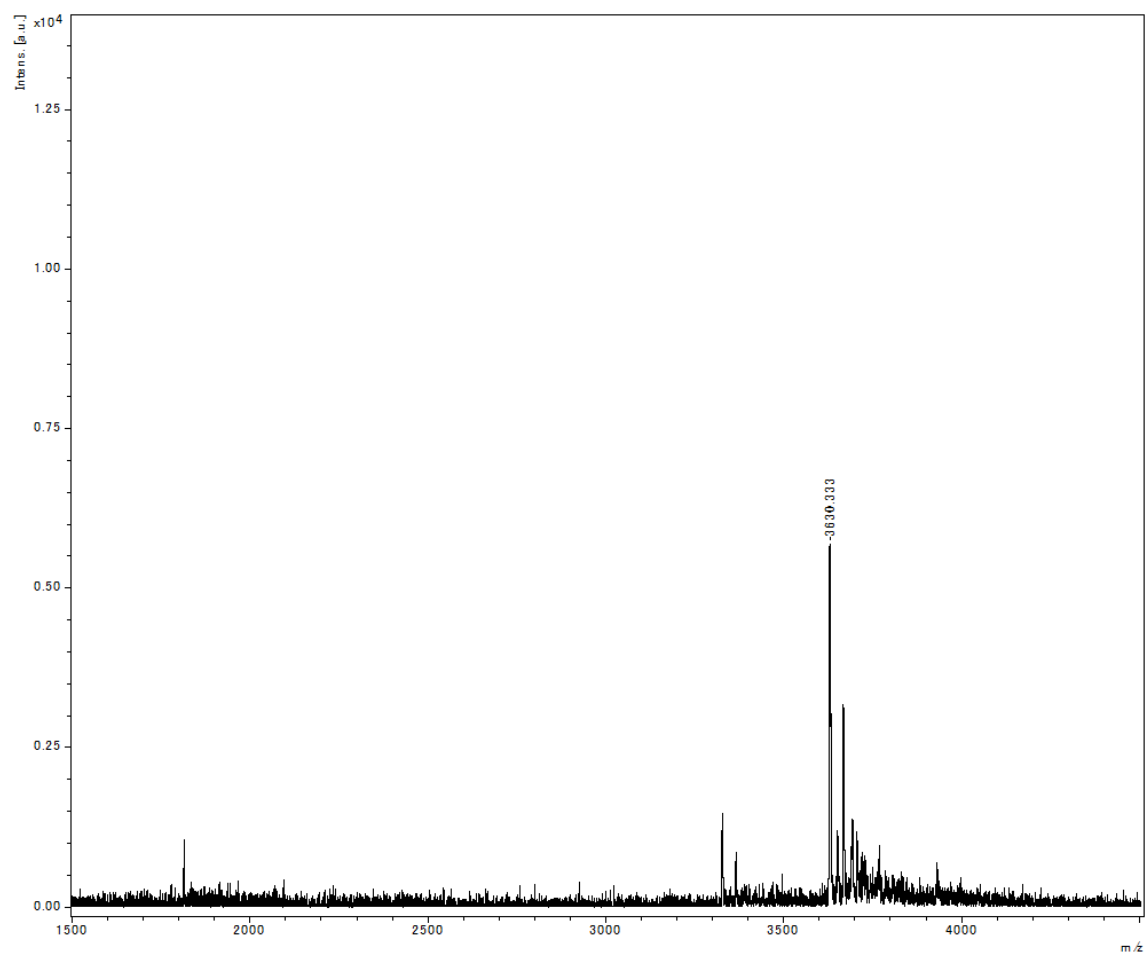
HPLC (purified **ON1**) [eluent A: 0.1 M TEAA, eluent B: MeCN, gradient: 6–15% of eluent B]



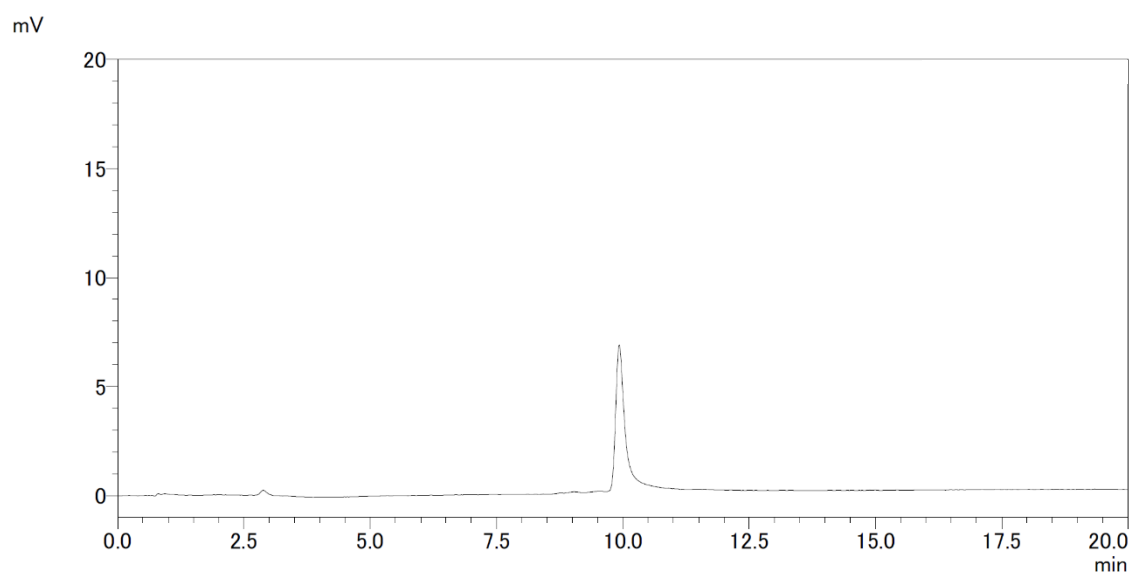
HPLC (crude **ON1** after Sep-Pak) [eluent A: 0.1 M TEAA, eluent B: MeCN, gradient: 6–15% of eluent B]



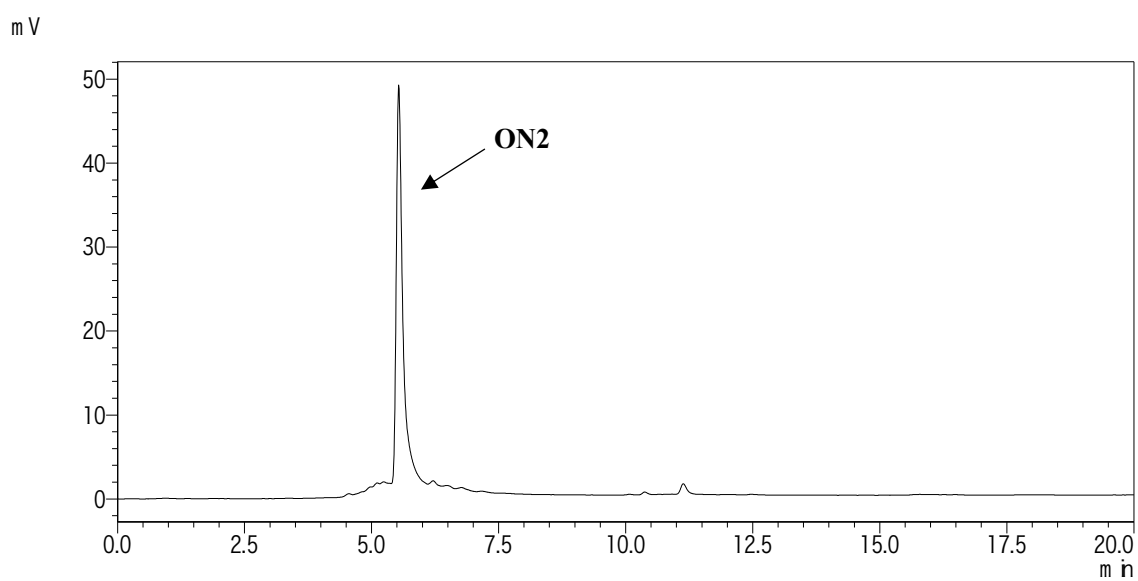
MALDI-TOF MS (purified **ON1**)



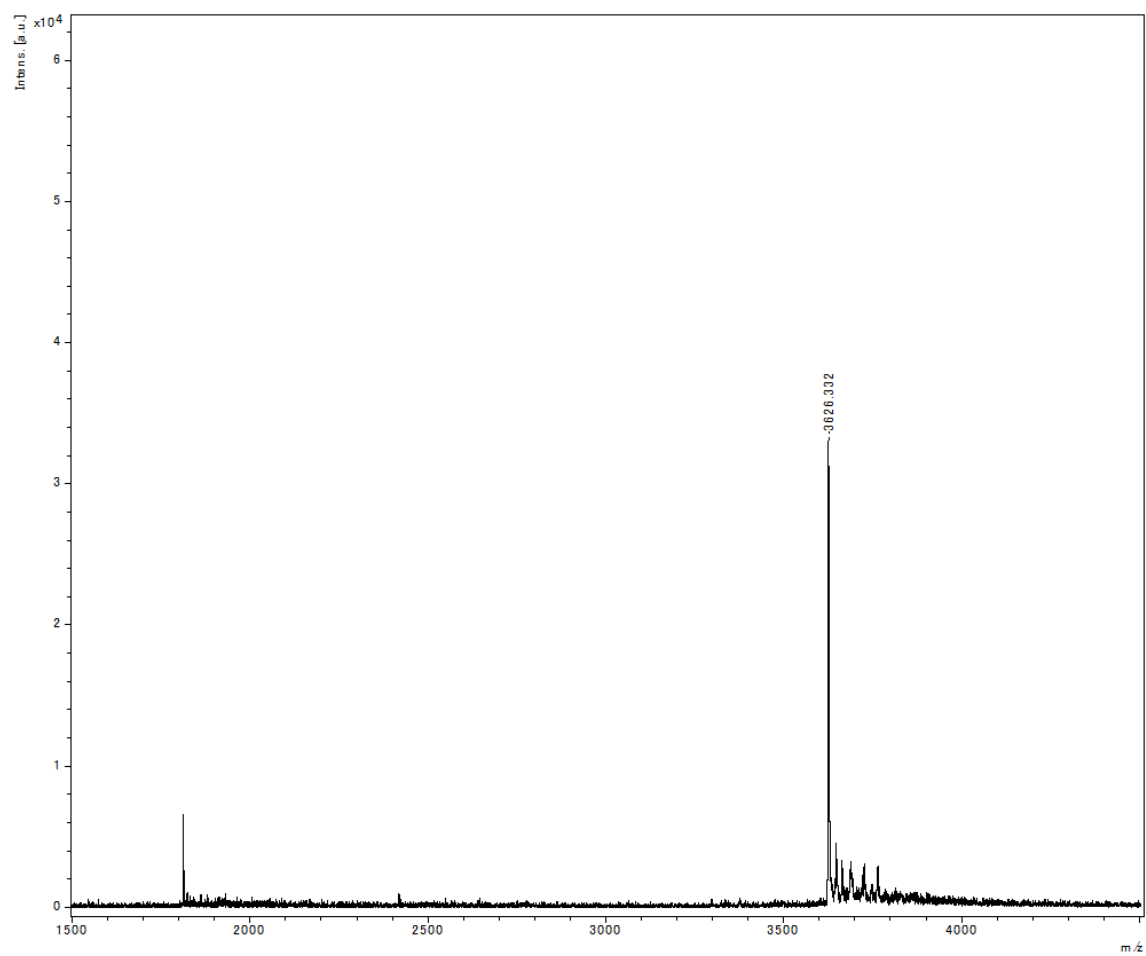
HPLC (purified **ON2**) [eluent A: 0.1 M TEAA, eluent B: MeCN, gradient: 5–9% of eluent B]



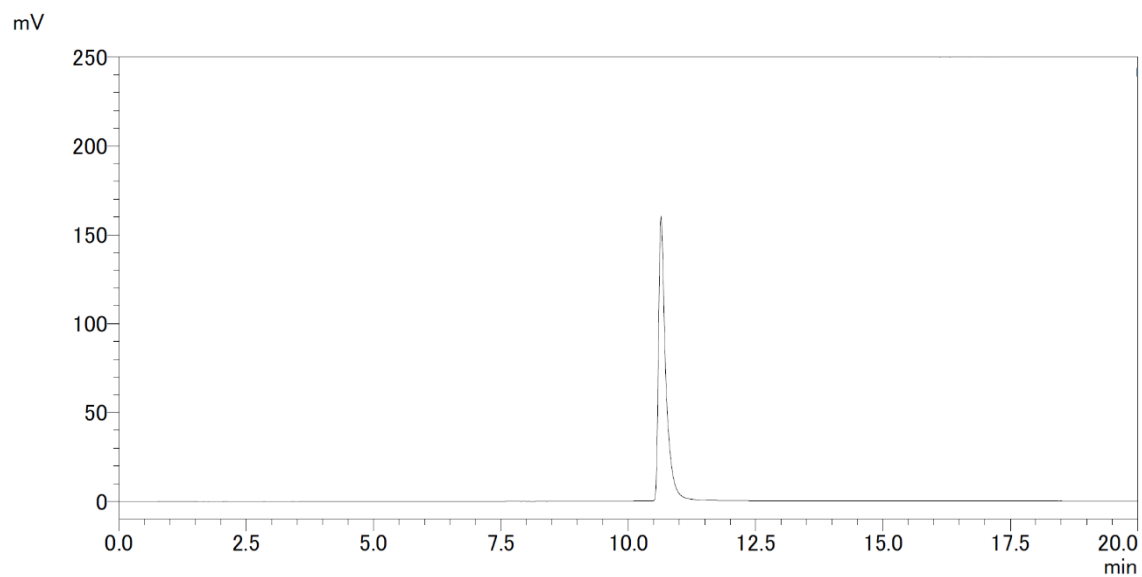
HPLC (crude **ON2** after Sep-Pak) [eluent A: 0.1 M TEAA, eluent B: MeCN, gradient: 6–15% of eluent B]



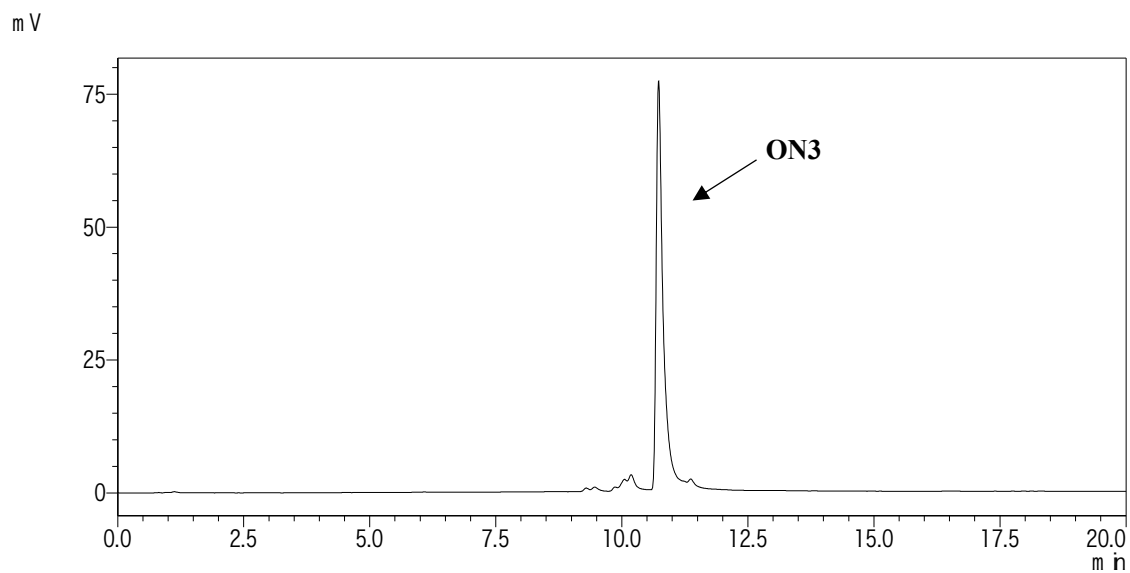
MALDI-TOF MS (purified **ON2**)



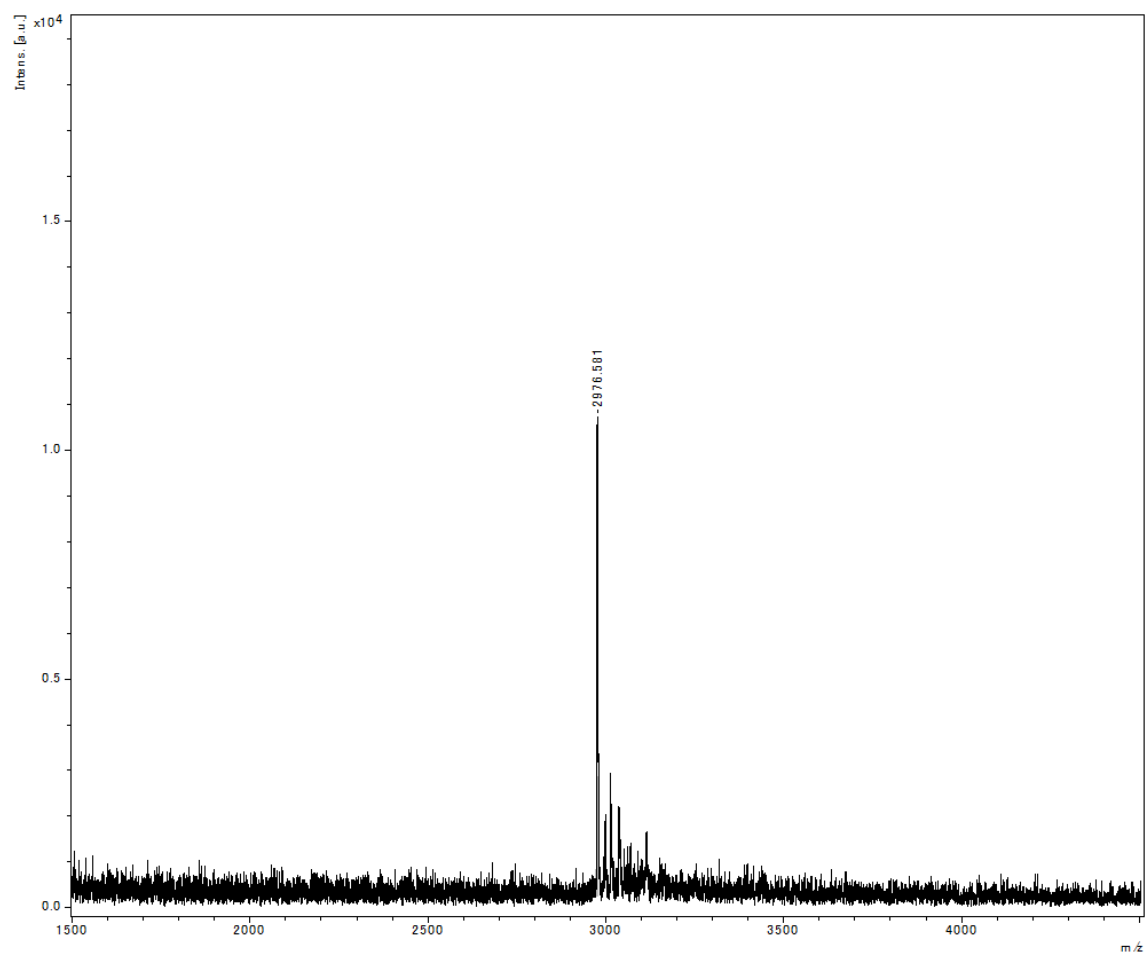
HPLC (purified **ON3**) [eluent A: 0.1 M TEAA, eluent B: MeCN, gradient: 6–15% of eluent B]



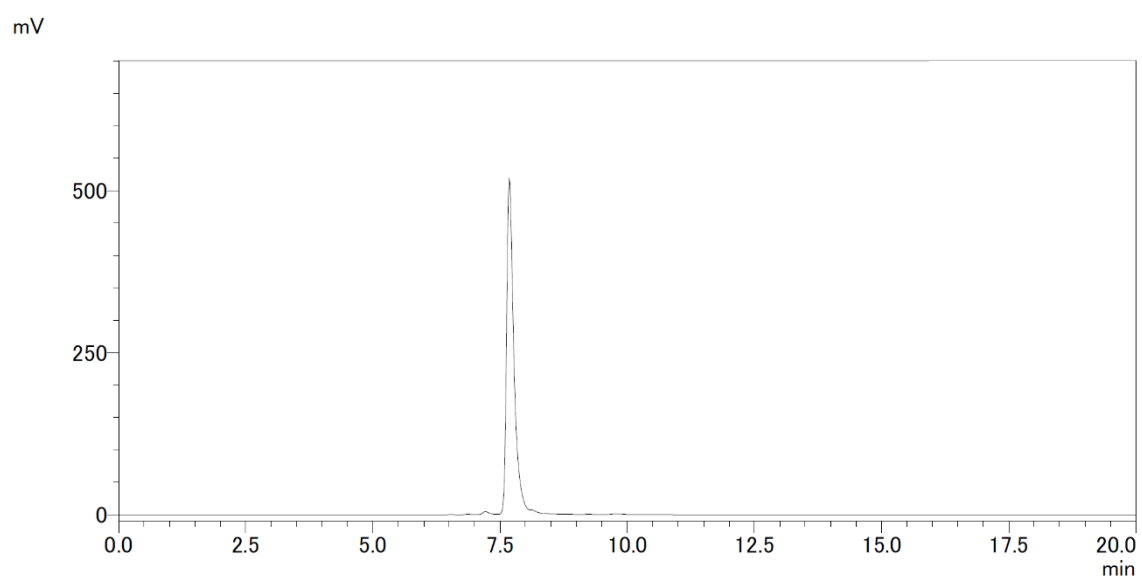
HPLC (crude **ON3** after Sep-Pak) [eluent A: 0.1 M TEAA, eluent B: MeCN, gradient: 6–15% of eluent B]



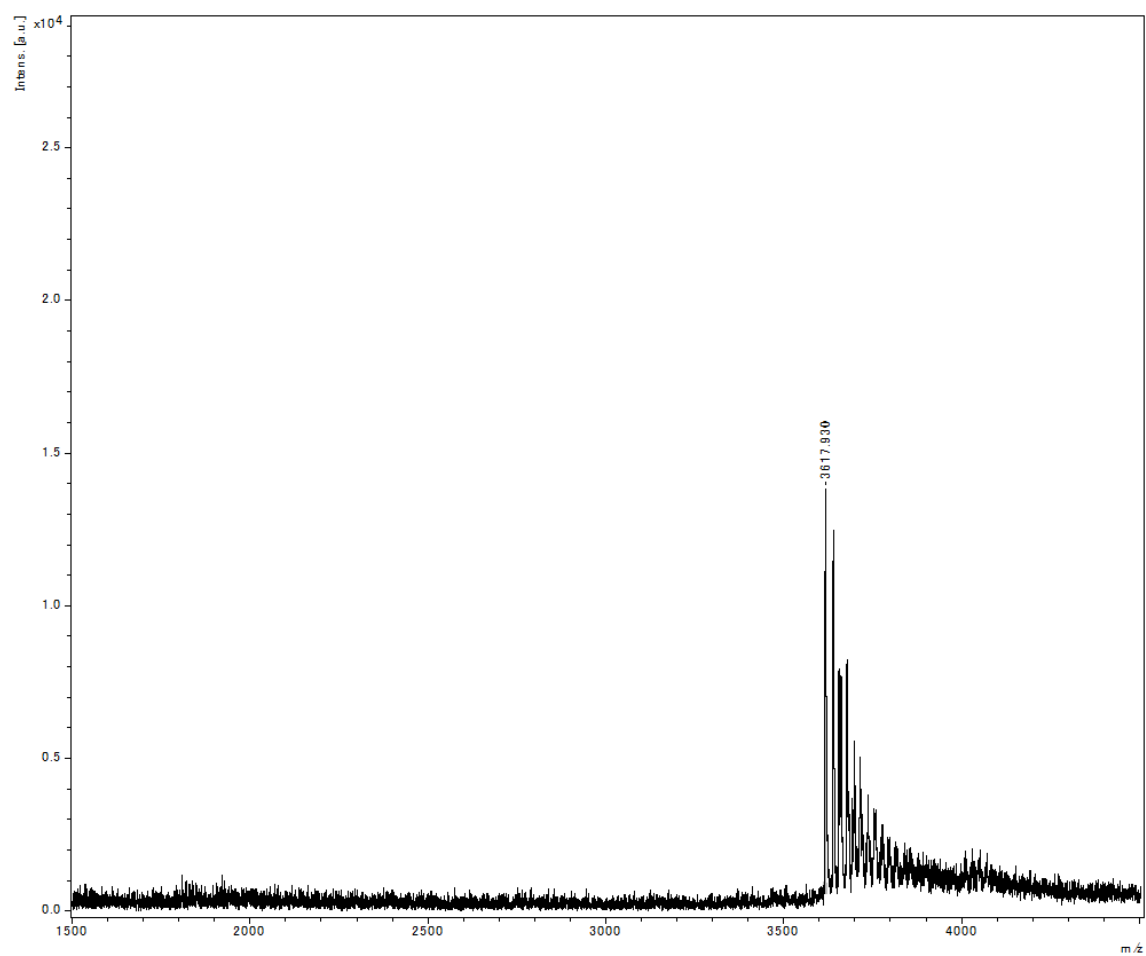
MALDI-TOF MS (purified **ON3**)



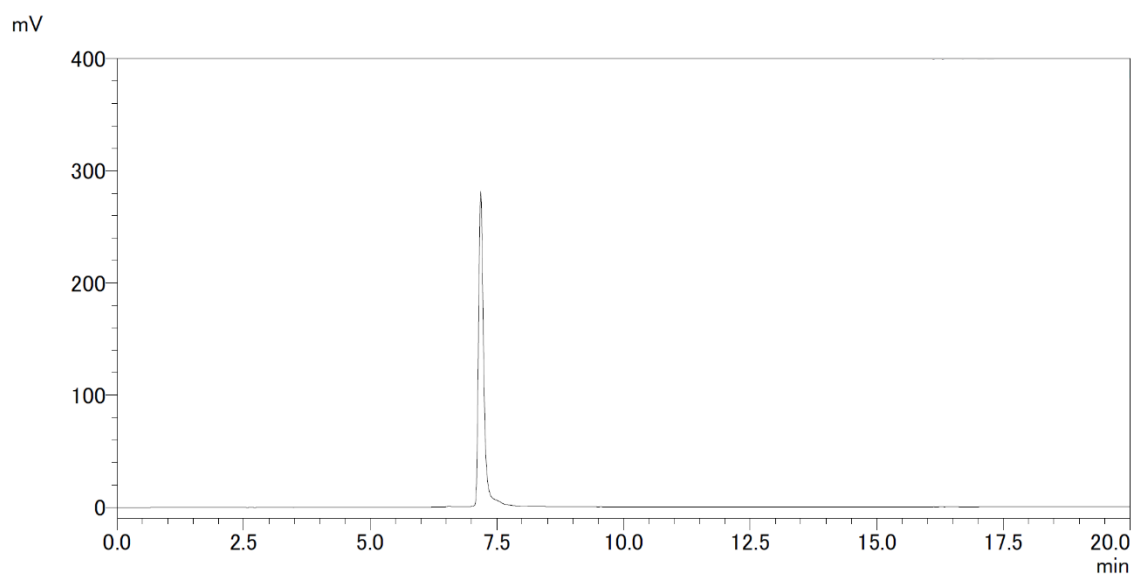
HPLC (purified **ON4**) [eluent A: 0.1 M TEAA, eluent B: MeCN, gradient: 6–15% of eluent B]



MALDI-TOF MS (purified **ON4**)



HPLC (purified **ON5**) [eluent A: 0.1 M TEAA, eluent B: MeCN, gradient: 6–15% of eluent B]



MALDI-TOF MS (purified **ON5**)

