SYNTHESIS OF NEODESMOSINE, A CROSSLINKING PYRIDINIUM AMINO ACID OF ELASTIN, VIA A NEGISHI CROSS-COUPLING

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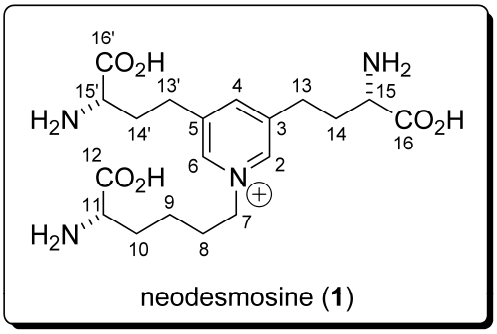
General:

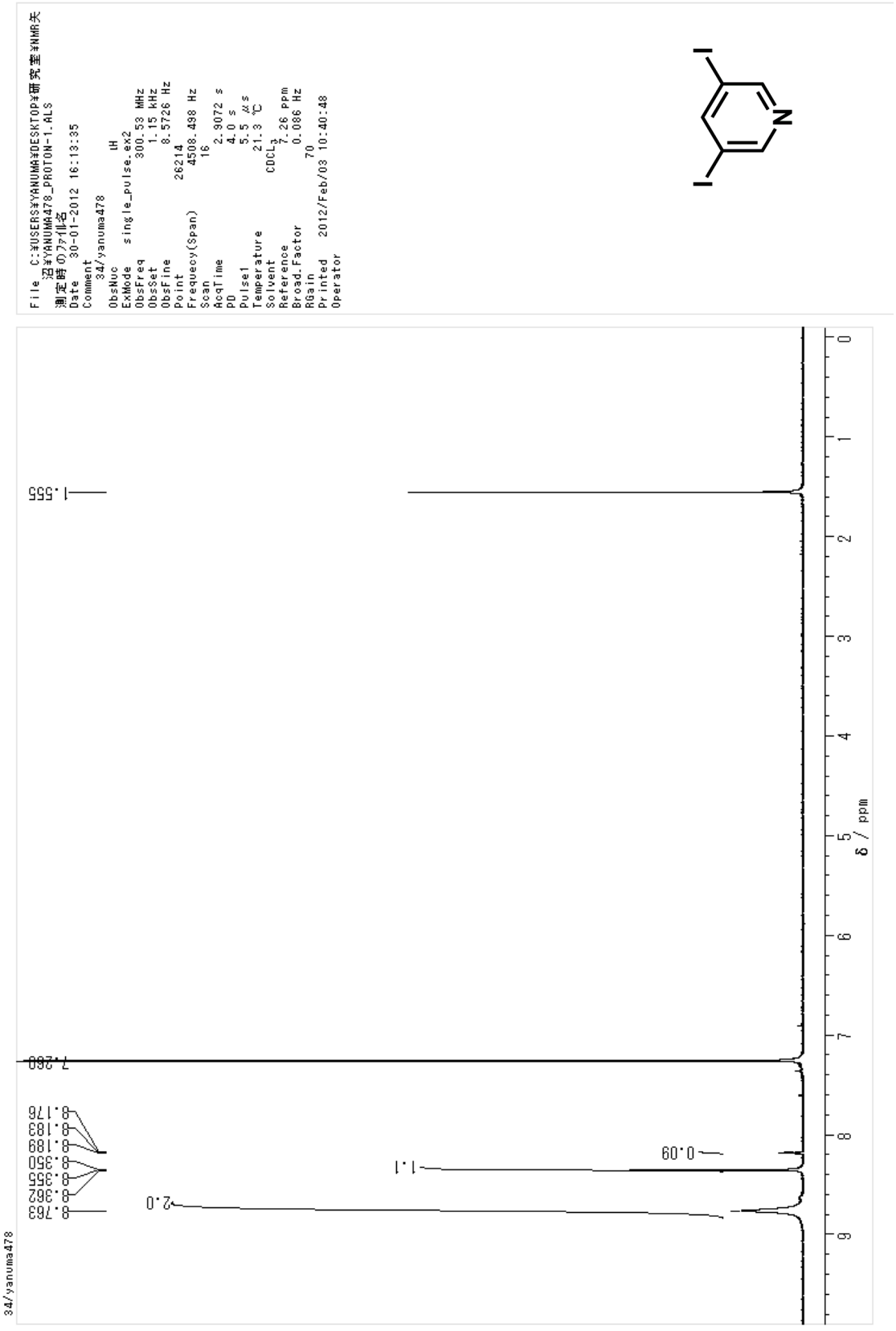
All non-aqueous reactions were conducted under an atmosphere of nitrogen with magnetic stirring using dry solvents unless otherwise indicated. Dimethylformamide (DMF) was dried by distillation and stored over activated molecular sieves. Trimethylsilyl chloride (TMSCl), diisopropylethylamine (iPr$_2$NEt), and nitromethane (MeNO$_2$) were dried by distillation. Dehydrated methanol (MeOH) for the reactions was purchased from Kanto Chemicals (Tokyo, Japan). All reagents were obtained from commercial suppliers and used without further purification unless otherwise stated. Centrifugation was performed by LMS Mini Centrifuge MCF-2360 (6,600 rpm). Analytical thin layer chromatography (TLC) was performed on Silica gel 60 F_{254} plates produced by Merck. Column chromatography was performed with acidic Silica gel 60 (spherical, 40-50 μm) or neutral Silica gel 60N (spherical, 40-50 μm) produced by Kanto Chemicals.

Melting points were measured by an AS one ATM-01 apparatus. Optical rotations were measured on a JASCO P-2200 digital polarimeter at the sodium lamp (λ = 589 nm) D line and are reported as follows: $[\alpha]_D^T$ (c g/100 mL, solvent). UV spectra were recorded on a JASCO V-560 UV/VIS spectrophotometer and are reported in wavelengths (nm). Infrared (IR) spectra were recorded on a JASCO FT-IR 4100 spectrometer and are reported in wavenumbers (cm^{-1}). ^{1}H and ^{13}C NMR spectra were recorded on a JEOL JNM-EXC 300 spectrometer (300 MHz) or on a JEOL JNM-ECA 500 spectrometer (500 MHz). ^{1}H NMR data are reported as follows: chemical shift (δ, ppm), integration, multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants (J) in Hz, assignments. ^{13}C NMR data are reported in terms of chemical shift (δ, ppm). EI-MS spectra were recorded on a Shimadzu GCMS QP-5050 instrument. ESI-MS spectra were recorded on a JEOL JMS-T100LC instrument. Mass spectroscopic data were reported in m/z. JASCO HPLC systems PU-2085, MD-2010, and CO-2060 were used for the purification of neodesmosine **1**.

The carbon numbering on ^{1}H NMR of all compounds is corresponding with **1**.



neodesmosine (**1**)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ObsSet 8.5726 Hz
ObsFine 26214
Point 4508.498 Hz
Frequecy(Span) 16
Scan 2.9072 s
AcqTime 4.0 s
PD 5.5 μs
Pulse1 21.3 ℃
Temperature CDCl3
Solvent 7.26 ppm
Reference 0.086 Hz
Broad.Factor 70
RGain
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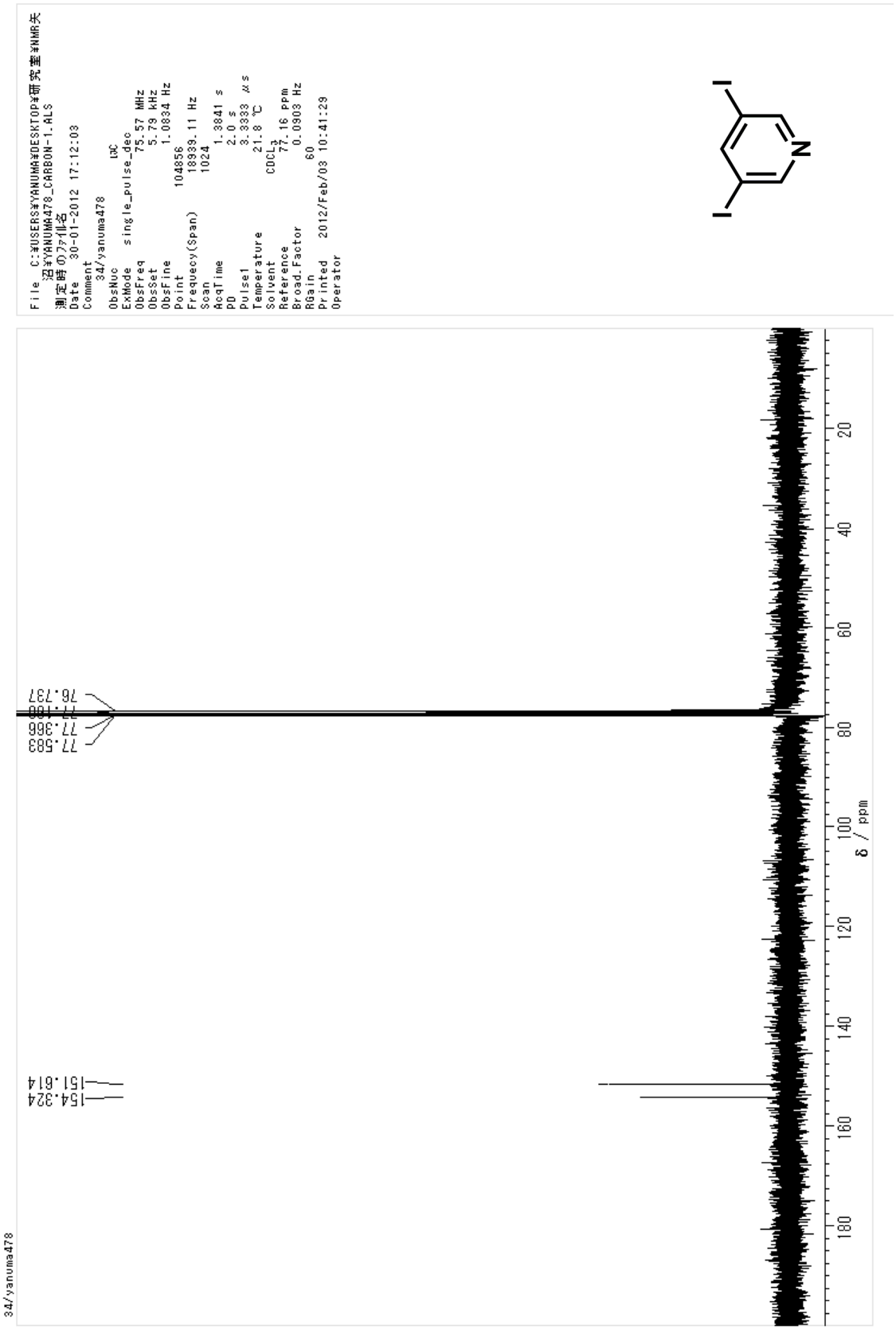
34/yanuma478
8.763
8.362
8.355
8.350
8.189
8.183
8.176
7.269
2.0
1.1
0.09
1.555
9
8
7
6
5
4
3
2
1
0
δ / ppm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77.583
77.386
77.188
76.737
154.324
151.814
δ / ppm
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ObsFine 1.0834 Hz
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Scan 1024
AcqTime 1.3841 s
PD 2.0 s
Pulse1 3.3333 μs
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Solvent CDCl3
Reference 77.16 ppm
Broad.Factor 0.0303 Hz
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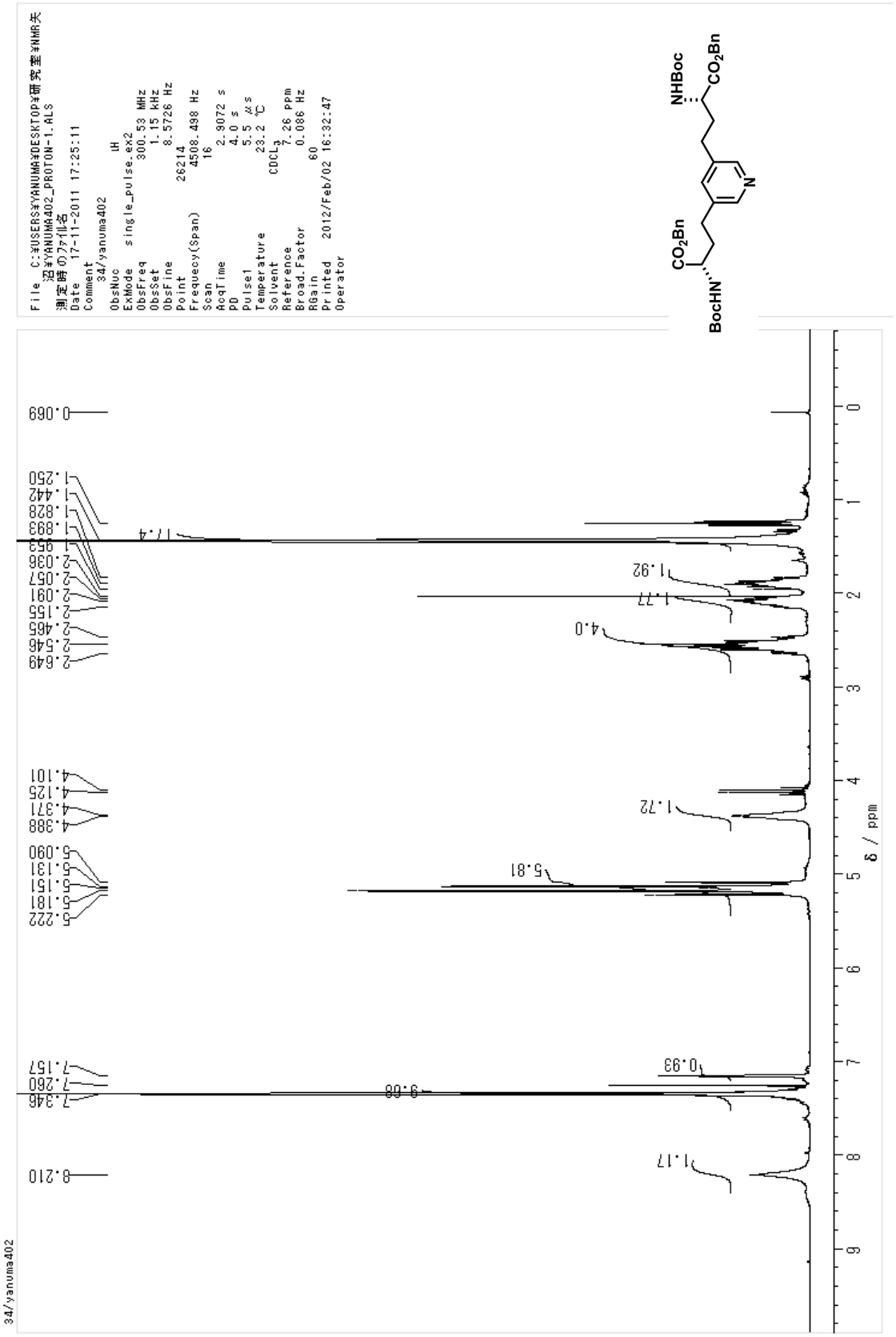
Operator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ObsSet 1.15 kHz
ObsFine 8.5726 Hz
Point 26214
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Scan 16
AcqTime 2.9072 s
PD 4.0 s
Pulse1 5.5 μs
Temperature 23.2 ℃
Solvent CDCl3
Reference 7.26 ppm
Broad.Factor 0.086 Hz
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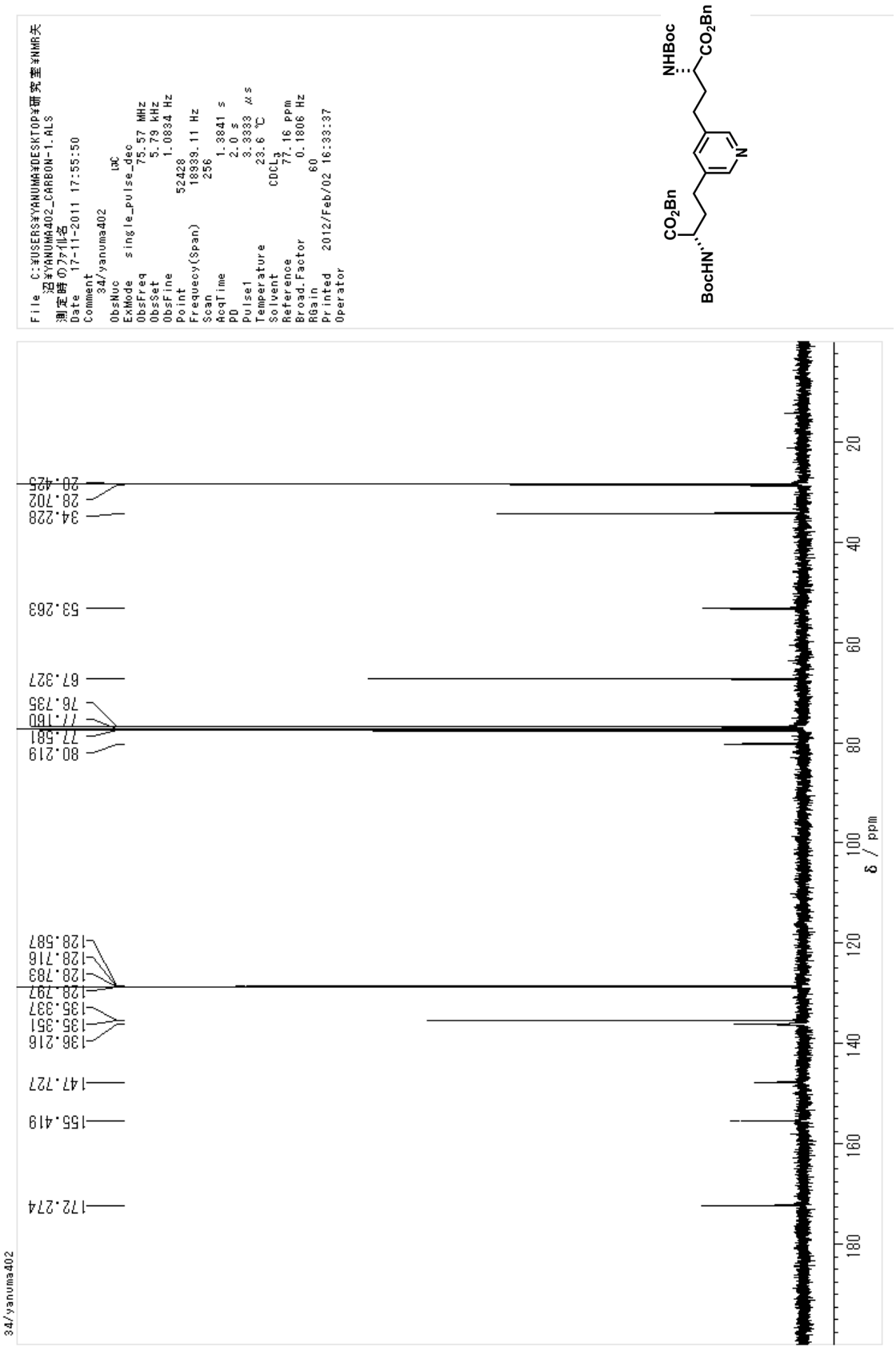
NHBoc
CO2Bn
CO2Bn
BocHN
N
34/yanuma402
δ / ppm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ObsSet 5.79 kHz
ObsFine 1.0834 Hz
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Scan 256
AcqTime 1.3841 s
PD 2.0 s
Pulse1 3.3333 μs
Temperature 23.6 ℃
Solvent CDCl3
Reference 77.16 ppm
Broad.Factor 0.1806 Hz
RGain 60
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Operator
NHBoc
CO2Bn
CO2Bn
BocHN
N
20.425
28.702
34.228
53.263
67.327
76.735
77.160
77.581
80.219
128.587
128.716
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128.797
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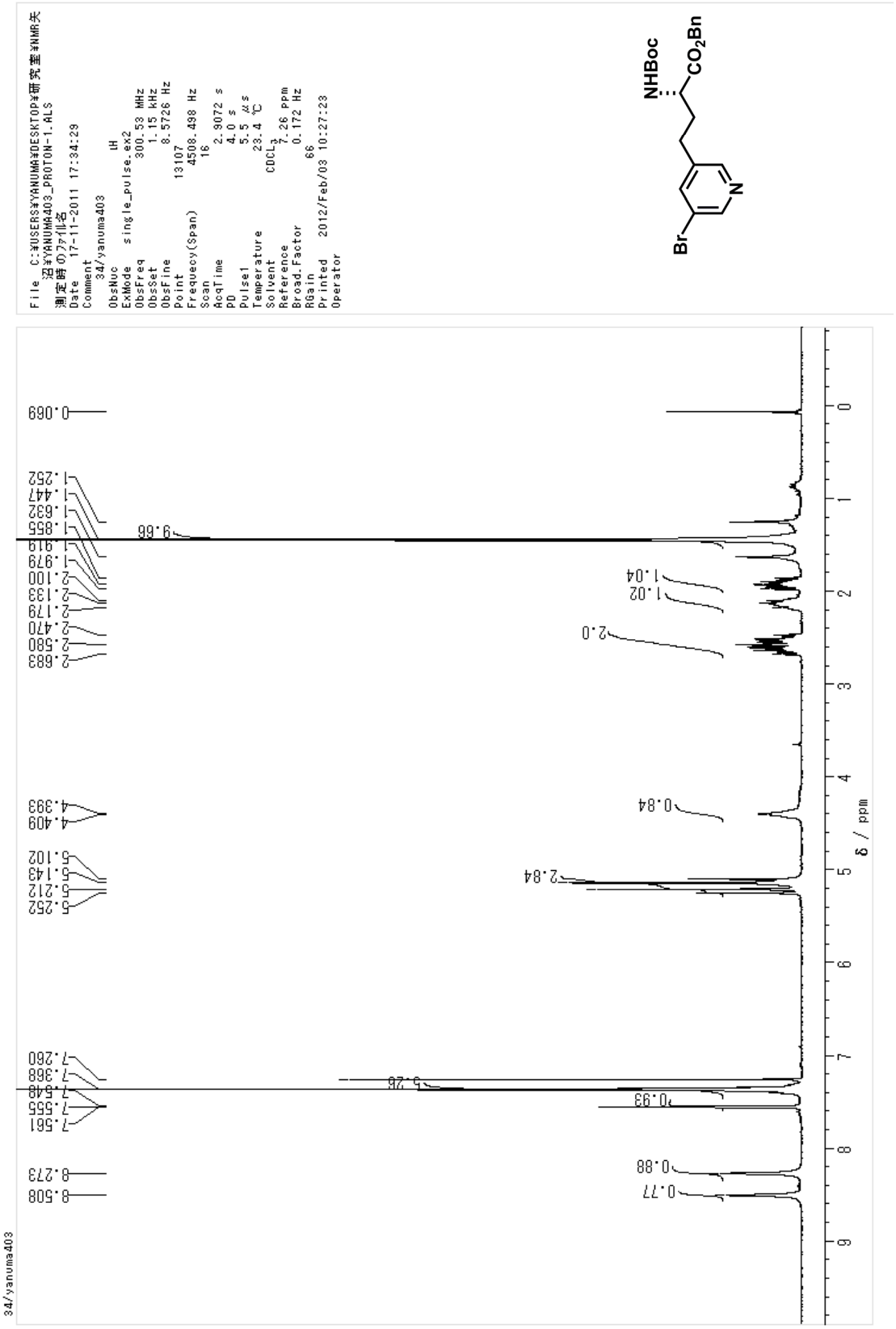
34/yanuma402
δ / ppm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ObsSet 1.15 kHz
ObsFine 8.5726 Hz
Point 13107
Frequency(Span) 4508.498 Hz
Scan 16
AcqTime 2.9072 s
PD 4.0 s
Pulse1 5.5 μs
Temperature 23.4 ℃
Solvent CDCl3
Reference 7.26 ppm
Broad.Factor 0.172 Hz
RGain 66
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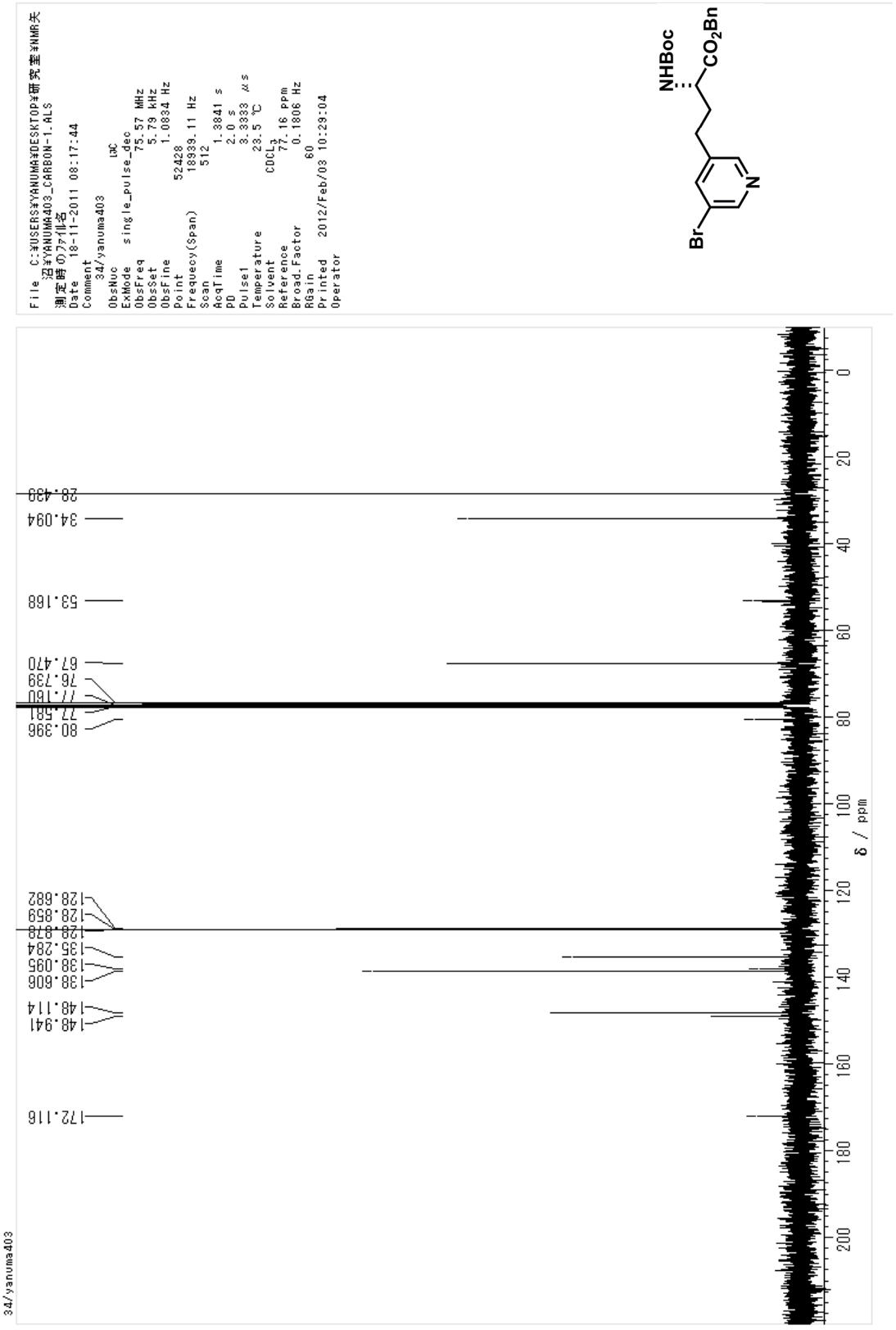
Operator
NHBoc
CO2Bn
Br
N
δ / ppm
34/yanuma403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ObsFreq 75.57 MHz
ObsSet 5.79 kHz
ObsFine 1.0834 Hz
Point 52428
Frequecy(Span) 18339.11 Hz
Scan 512
AcqTime 1.3841 s
PD 2.0 s
Pulse1 3.3333 μs
Temperature 23.5 ℃
Solvent CDCl3
Reference 77.16 ppm
Broad.Factor 0.1806 Hz
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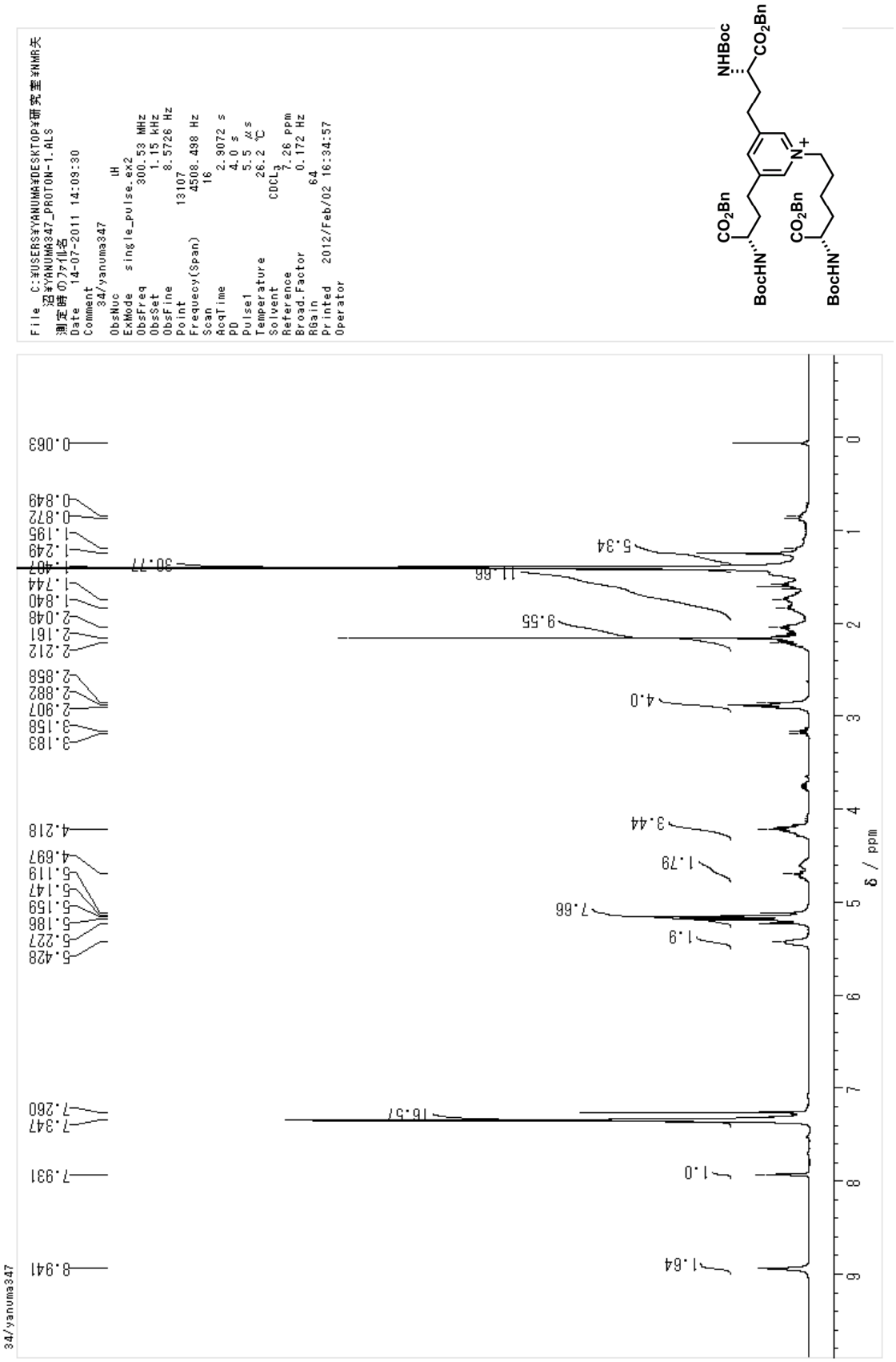
Operator
NHBoc
CO2Bn
Br
N
34/yanuma403
28.439
34.094
53.168
67.470
76.739
77.160
77.581
80.396
128.682
128.859
128.878
135.284
138.095
138.606
148.114
148.941
172.116
δ / ppm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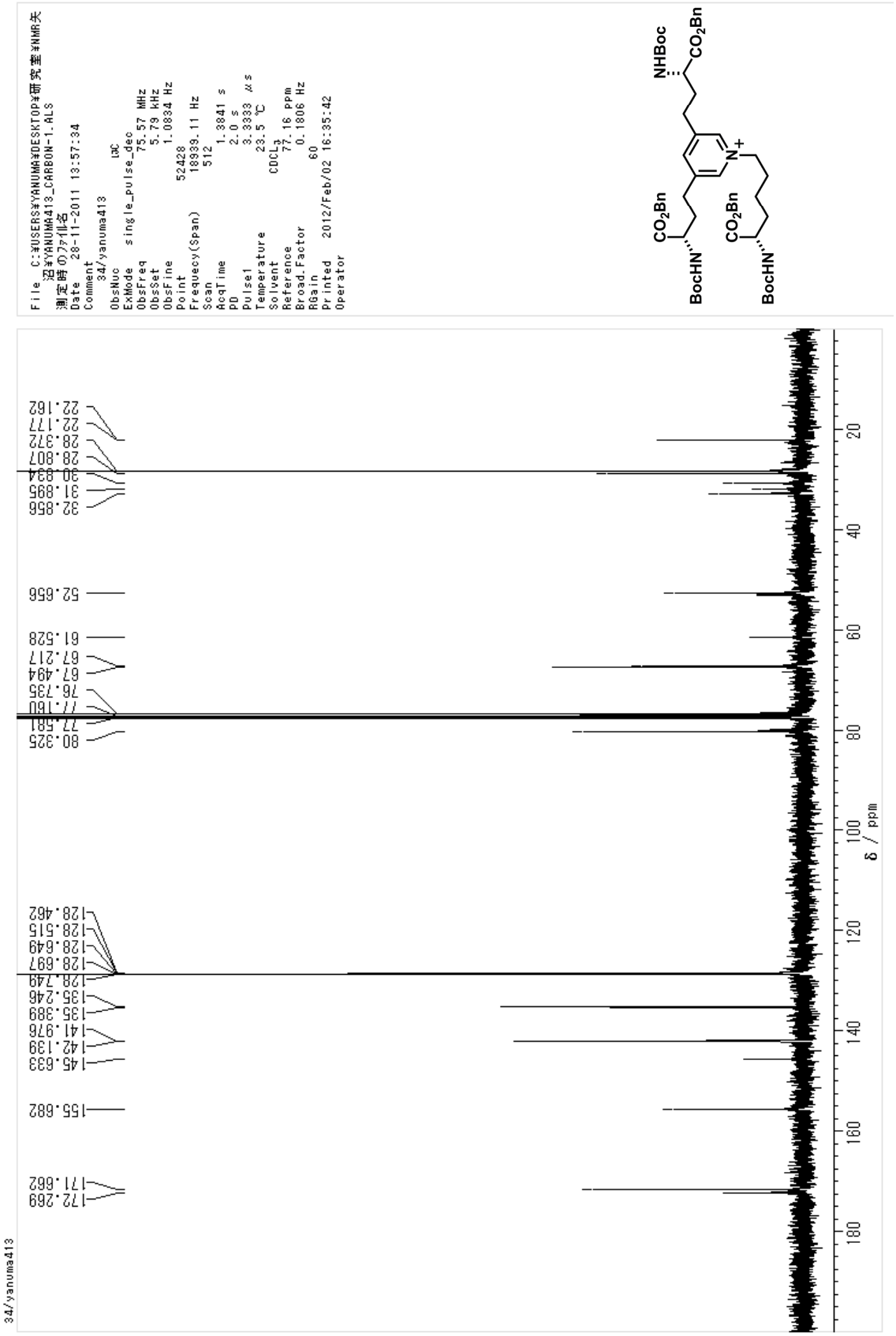
ObsSet 1.15 kHz
ObsFine 8.5726 Hz
Point 13107
Frequecy(Span) 4508.498 Hz
Scan 16
AcqTime 2.9072 s
PD 4.0 s
Pulse1 5.5 μs
Temperature 26.2 ℃
Solvent CDCl3
Reference 7.26 ppm
Broad.Factor 0.172 Hz
RGain 64
Printed 2012/Feb/02 16:34:57
Operator
NHBoc
CO2Bn
CO2Bn
CO2Bn
BocHN
BocHN
N+
34/yanuma347
δ / ppm
0.063
0.849
0.872
1.195
1.249
1.407
1.744
1.840
2.048
2.161
2.212
2.858
2.882
2.907
3.158
3.183
4.218
4.697
5.119
5.147
5.159
5.186
5.227
5.428
7.260
7.347
7.931
8.941
30.77
18.57
11.66
9.55
5.34
4.0
3.44
1.79
7.66
1.9
1.0
1.64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ObsFine 1.0834 Hz
Point 52428
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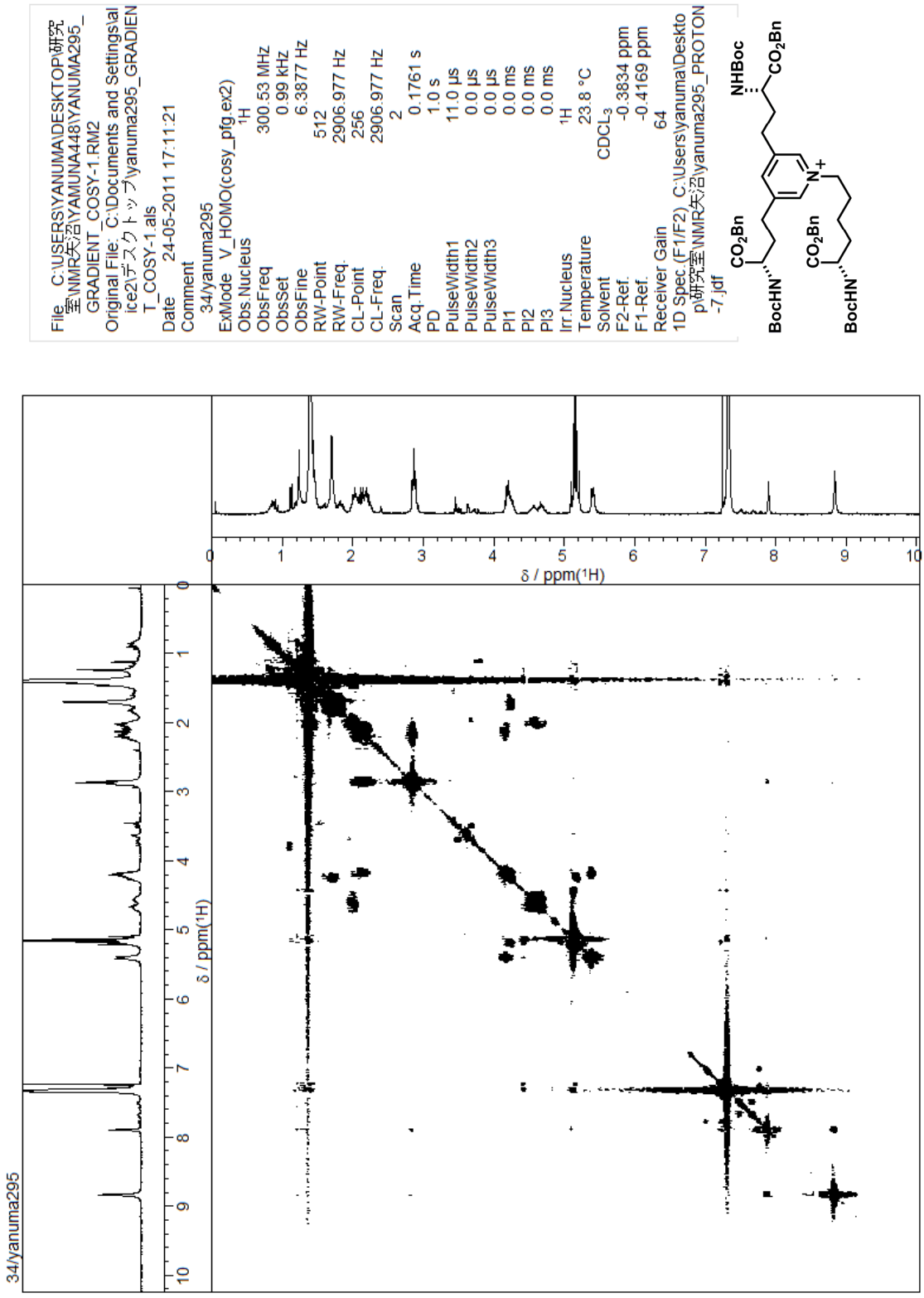
Scan 512
AcqTime 1.3841 s
PD 2.0 s
Pulse1 3.3333 μs
Temperature 23.5 ℃
Solvent CDCL3
Reference 77.16 ppm
Broad.Factor 0.1806 Hz
RGain 60
Printed 2012/Feb/02 16:35:42
Operator
NHBoc
CO2Bn
CO2Bn
BocHN
CO2Bn
BocHN
34/yanuma413
172.269
171.662
155.682
145.633
142.139
141.976
135.389
135.246
128.749
128.697
128.649
128.515
128.462
80.325
77.581
77.160
76.735
67.494
67.217
61.528
52.656
32.856
31.895
30.834
28.807
28.372
22.177
22.162
δ / ppm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ObsSet 0.99 kHz
ObsFine 6.3877 Hz
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RW-Freq. 2906.977 Hz
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CL-Freq. 2906.977 Hz
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PD 1.0 s
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PulseWidth3 0.0 ms
PI1 0.0 ms
PI2 0.0 ms
PI3 0.0 ms
Irr.Nucleus 1H
Temperature 23.8 °C
Solvent CDCl3
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F1-Ref. -0.4169 ppm
Receiver Gain 64
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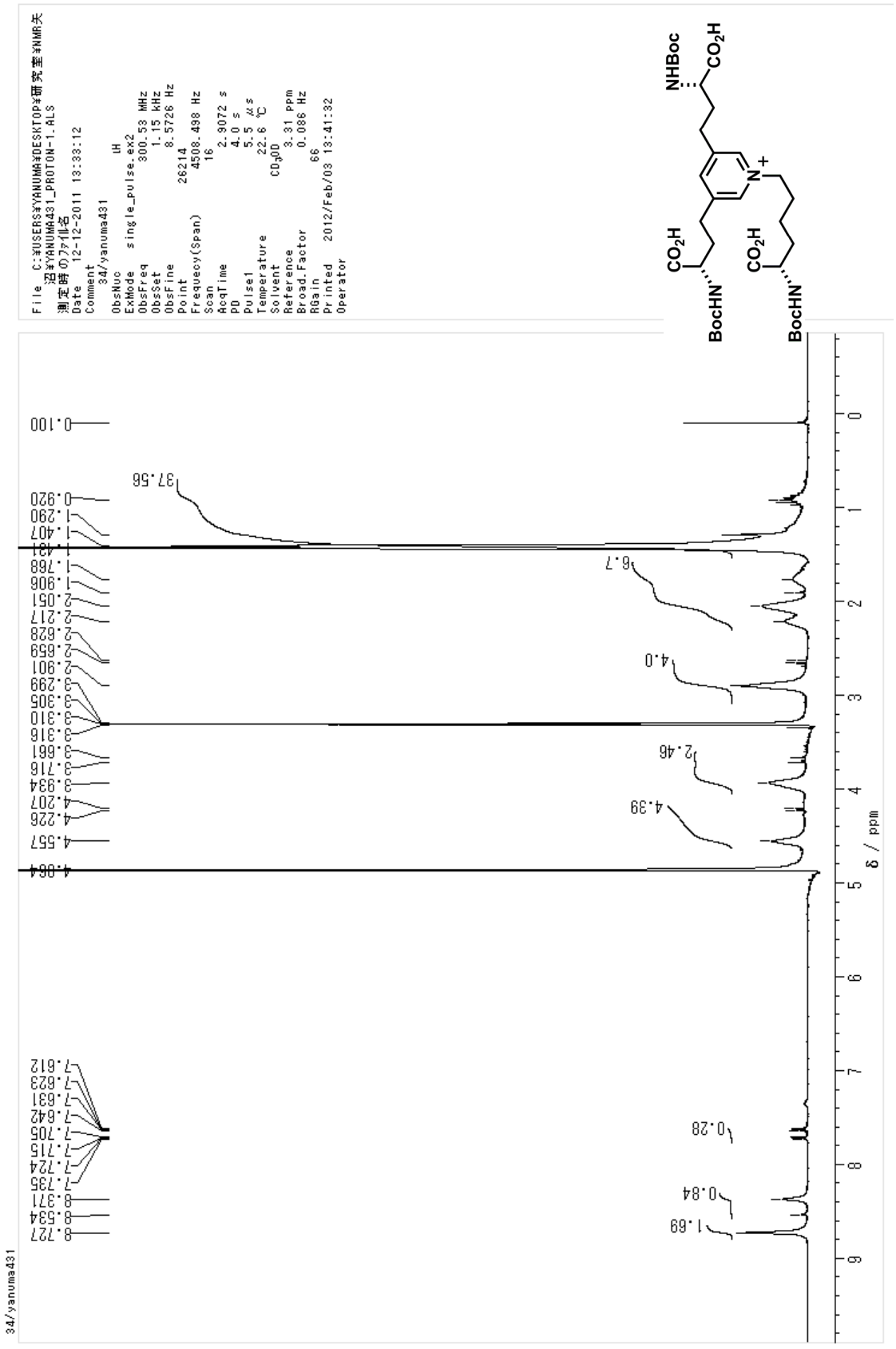
CO2Bn
CO2Bn
CO2Bn
BocHN
BocHN
δ / ppm(1H)
δ / ppm(1H)
34/yanuma295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Comment 34/yanuma431
1H
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ObsFreq 300.53 MHz
ObsSet 1.15 kHz
ObsFine 8.5726 Hz
Point 26214
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Scan 16
AcqTime 2.9072 s
PD 4.0 s
Pulse1 5.5 μs
Temperature 22.6 ℃
Solvent CD3OD
Reference 3.31 ppm
Broad.Factor 0.086 Hz
RGain 66
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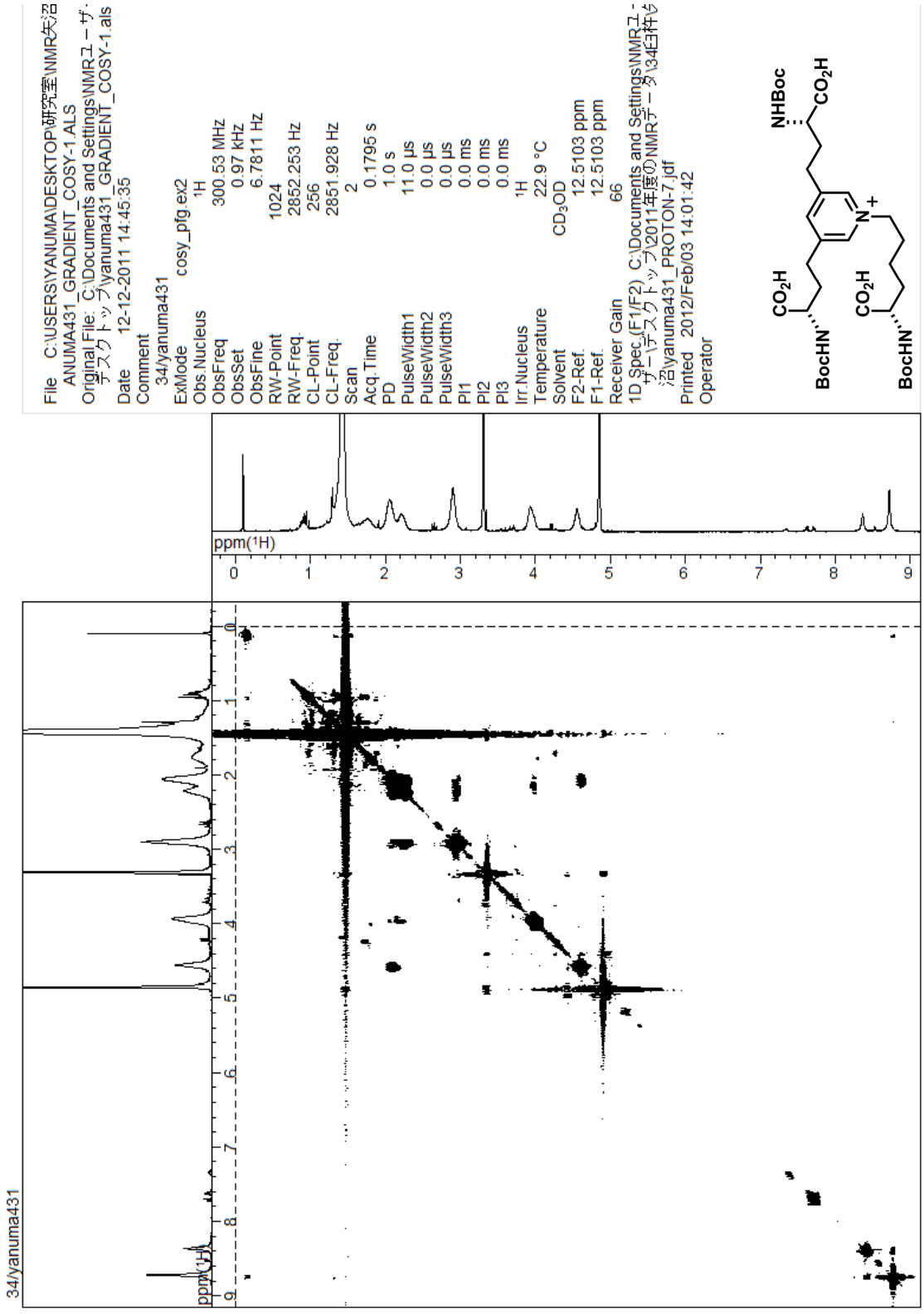
Operator
NHBoc
CO2H
CO2H
BocHN
CO2H
BocHN
N+
34/yanuma431
δ / ppm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PulseWidth3 0.0 µs
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PI2 0.0 ms
PI3 0.0 ms
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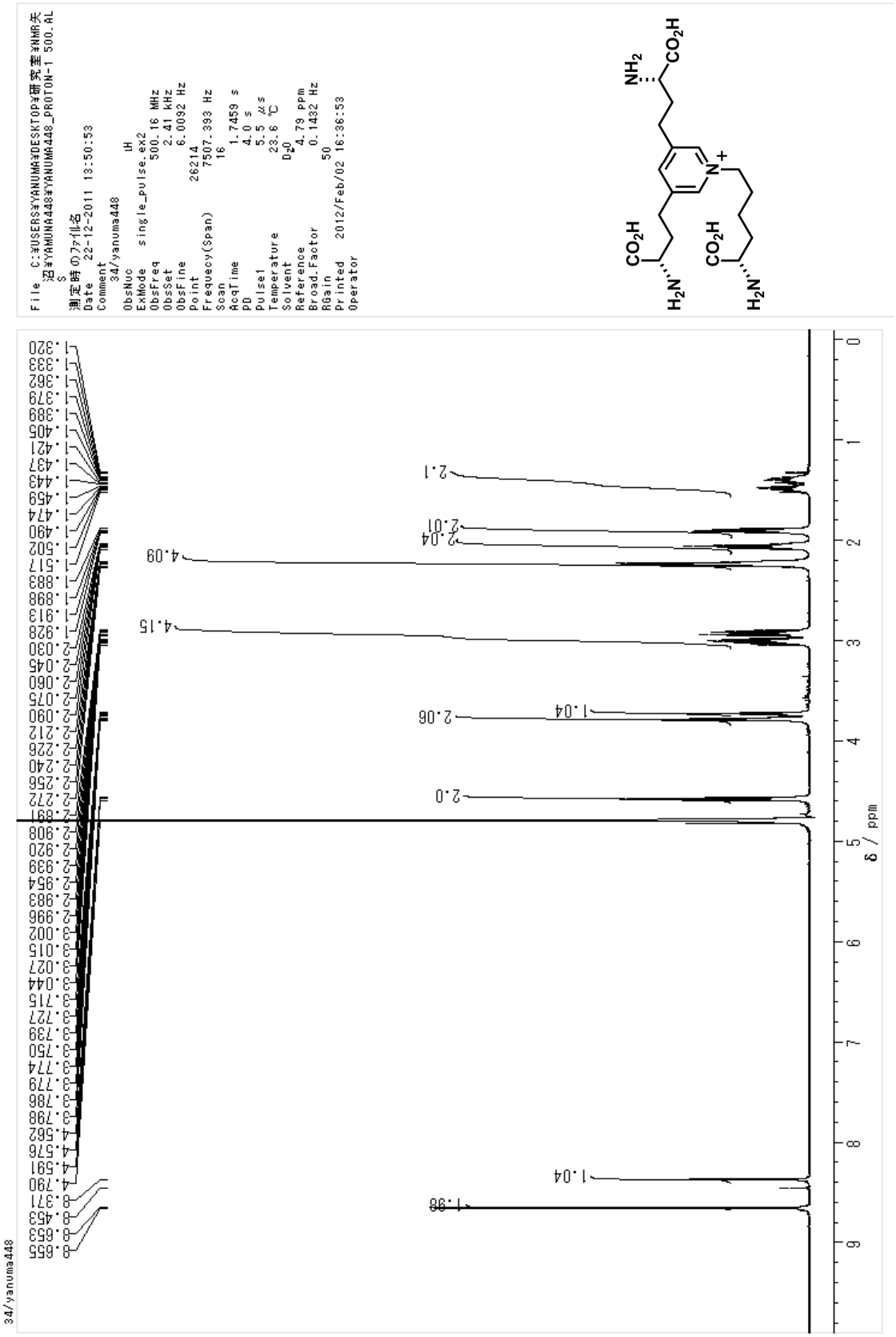
BocHN
CO2H
BocHN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Date 22-12-2011 13:50:53
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ObsFreq 500.16 MHz
ObsSet 2.41 kHz
ObsFine 6.0092 Hz
Point 26214
Frequecy(Span) 7507.393 Hz
Scan 16
AcqTime 1.7459 s
PD 4.0 s
Pulse1 5.5 μs
Temperature 23.6 ℃
Solvent D2O
Reference 4.79 ppm
Broad.Factor 0.1432 Hz
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NH2
CO2H
CO2H
CO2H
H2N
H2N
N+
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2.06
1.04
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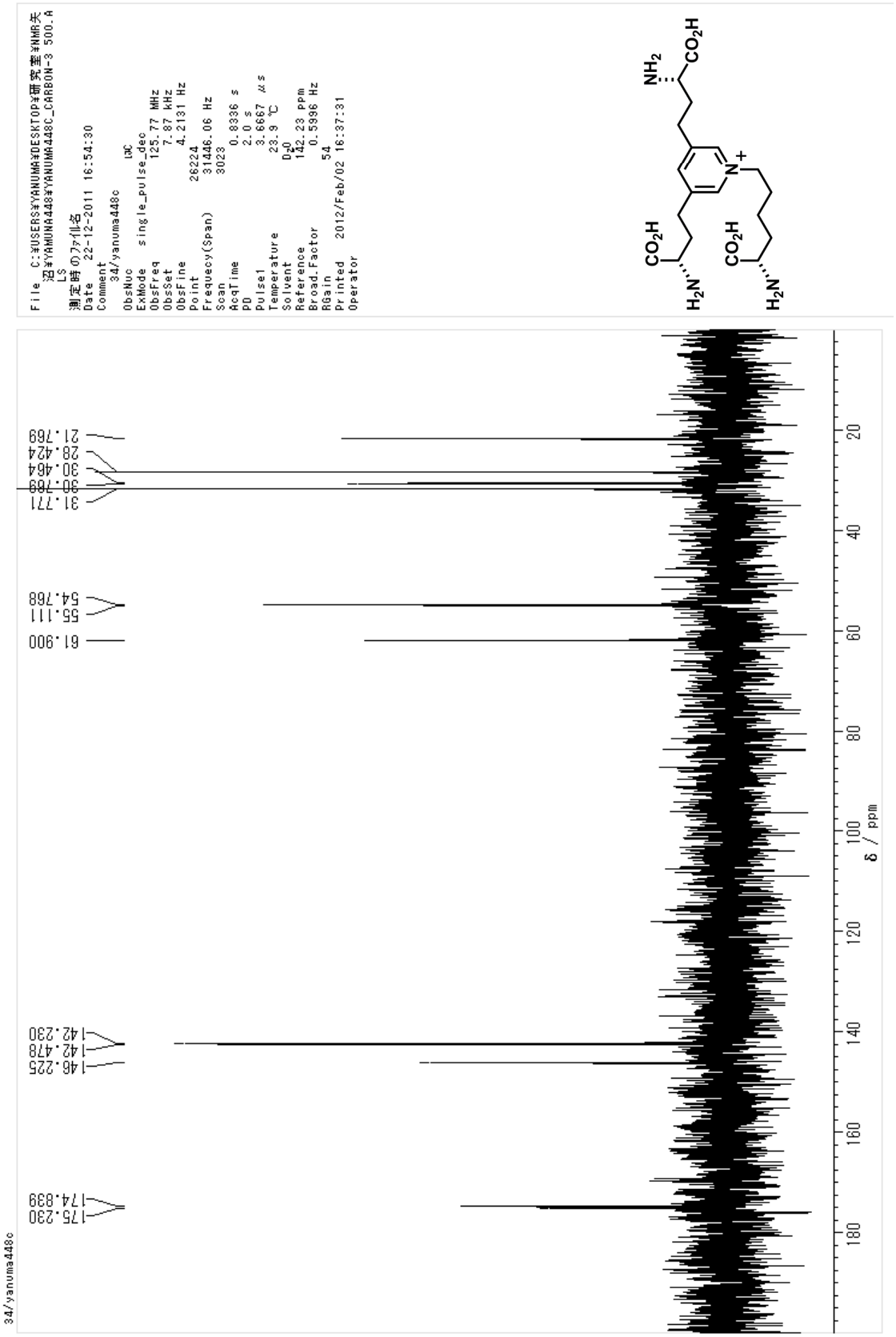
1.98
δ / ppm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ObsSet 7.87 kHz
ObsFine 4.2131 Hz
Point 26224
Frequecy(Span) 31446.06 Hz
Scan 3023
AcqTime 0.8336 s
PD 2.0 s
Pulse1 3.6667 μs
Temperature 23.9 ℃
Solvent D2O
Reference 142.23 ppm
Broad.Factor 0.5996 Hz
Again 54
Printed 2012/Feb/02 16:37:31
Operator
NH2
CO2H
CO2H
H2N
CO2H
H2N
N+
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30.464
30.769
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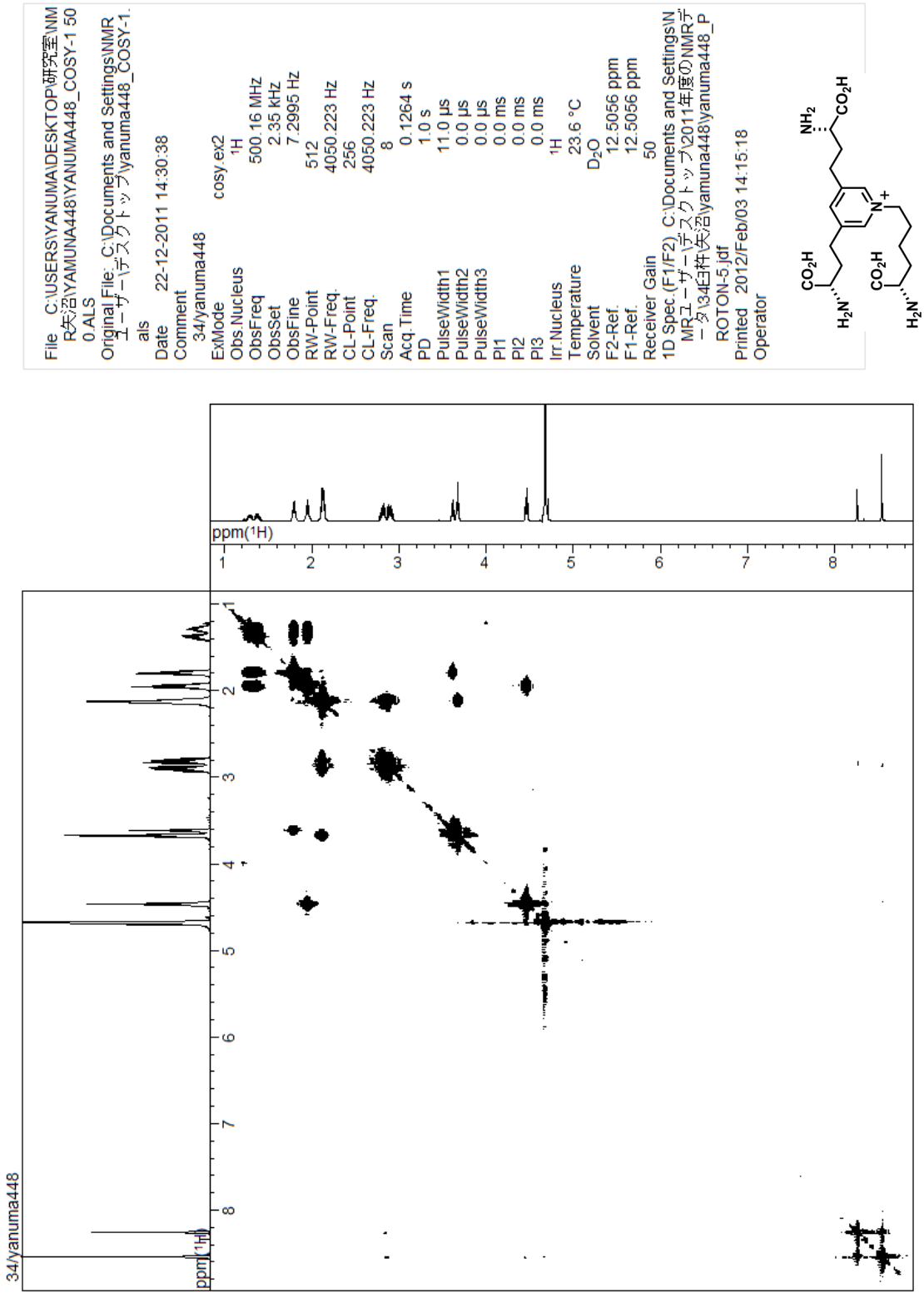
34/yanuma448c
δ / ppm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ObsSet 2.35 kHz
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RW-Freq. 4050.223 Hz
CL-Point 256
CL-Freq. 4050.223 Hz
Scan 8
Acq.Time 0.1264 s
PD 1.0 s
PulseWidth1 11.0 µs
PulseWidth2 0.0 µs
PulseWidth3 0.0 µs
Pl1 0.0 ms
Pl2 0.0 ms
Pl3 0.0 ms
Irr.Nucleus 1H
Temperature 23.6 °C
Solvent D2O
F2-Ref. 12.5056 ppm
F1-Ref. 12.5056 ppm
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34/yanuma448
ppm(1H)
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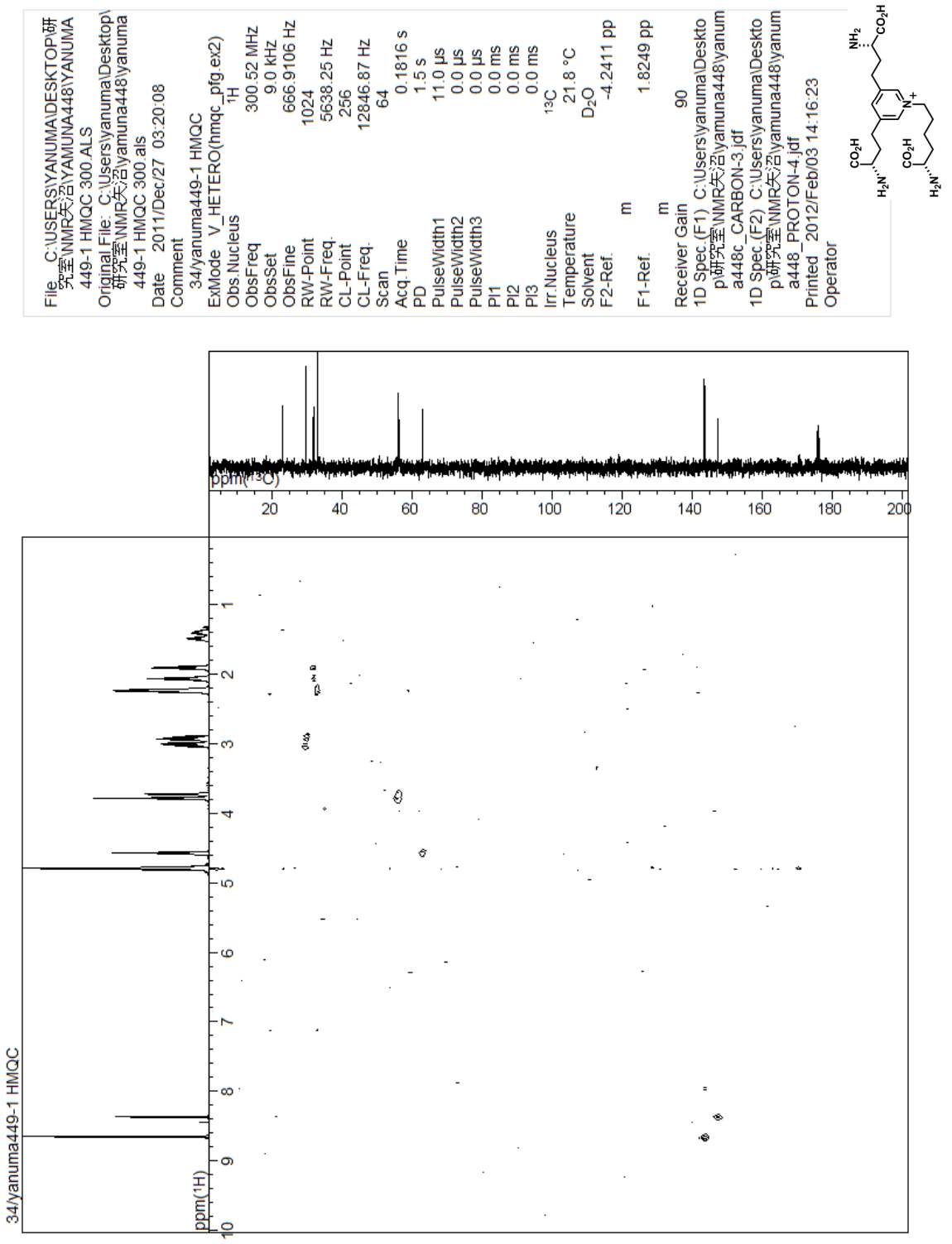
CO2H
H2N
H2N
N+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ObsSet 9.0 kHz
ObsFine 666.9106 Hz
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RW-Freq. 5638.25 Hz
CL-Point 256
CL-Freq. 12846.87 Hz
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PulseWidth2 0.0 µs
PulseWidth3 0.0 µs
Pl1 0.0 ms
Pl2 0.0 ms
Pl3 0.0 ms
Irr.Nucleus 13C
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Solvent D2O
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F1-Ref. 1.8249 pp m
Receiver Gain 90
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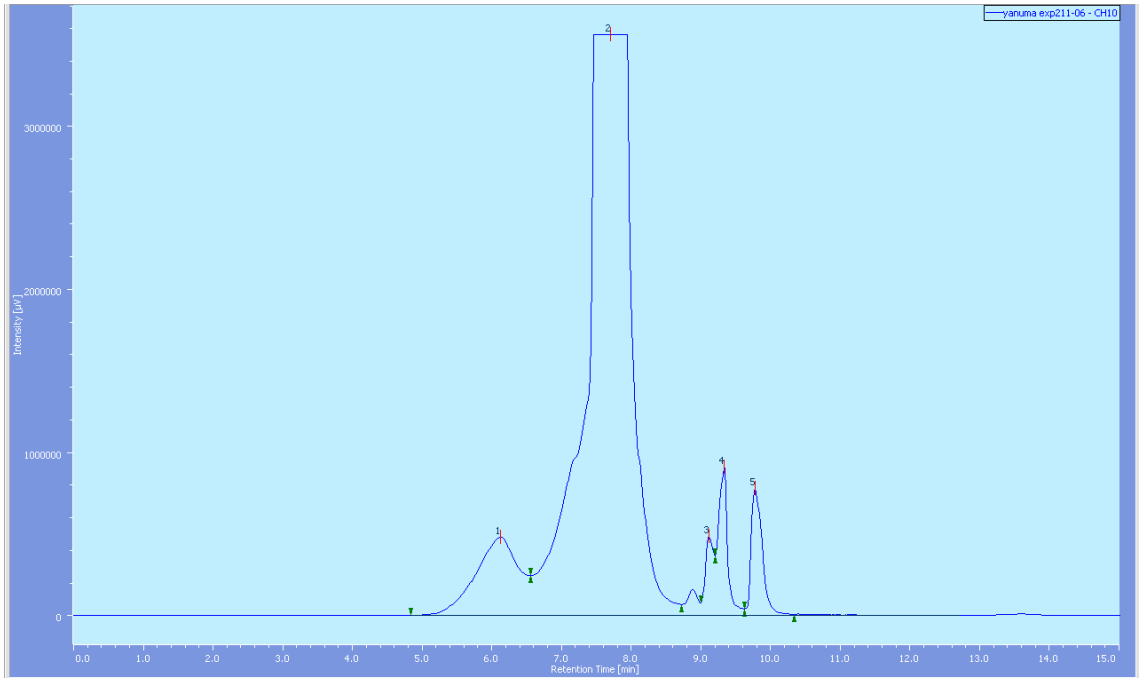
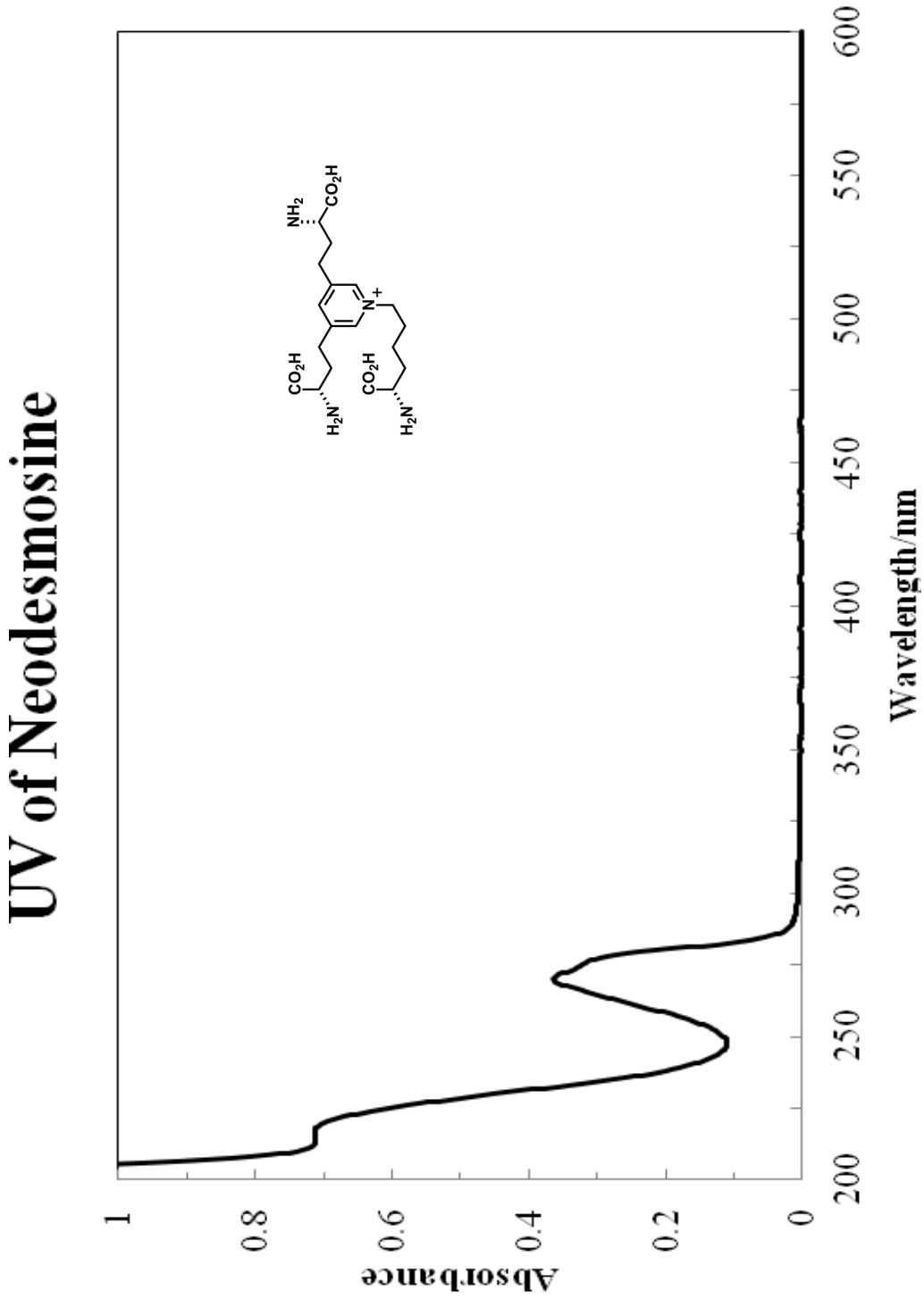
1D Spec.(F2) C:\Users\yanuma\Desktop\研究室\NMR実習\yamuna448\yanuma448c_CARBON-3.jdf
Printed 2012/Feb/03 14:16:23
Operator
34/yanuma449-1 HMQC
ppm(1H)
ppm(13C)
NH2
CO2H
H2N
N+

HPLC chromatogram of neodesmosine.



Column:	COSMOSIL 5C_{18}-AR-II (10×250, Manf.No. K52111)
Solvents:	MeOH/H_2O = 1/9
Flow rate:	1.5 mL/min
Temperature:	40 °C
Injection volume:	200 μL
Injection concentration:	52.0 mmol/L
Detection:	270 nm (λ_{max} of neodesmosine)
Peak of neodesmosine:	peak 2 (retention time: 6.5-8.7 min)


UV of Neodesmosine
Absorbance
Wavelength/nm
0
0.2
0.4
0.6
0.8
1
200
250
300
350
400
450
500
550
600
NH2
CO2H
CO2H
CO2H
H2N
H2N
N+