

Supplementary Data

Proposed Mechanism for the Diterpene Synthases in the Formation of Phomactatriene and Taxadiene: Experimental Results on Biosynthetic Study, Biomimetic Reactions and Examination of the Cyclization Pathway by *ab initio* Calculations

Tetsuo Tokiwano, Taeko Endo, Tae Tsukagoshi, Hitoshi Goto, Eri Fukushi, and Hideaki Oikawa*

Table of Contents:

1. **Table S1.** MMFF94 and *ab Initio* HF/6-31G* Energies of Top Five Conformers Found by CONFLEX Search
-----S2-S3
2. **Figure S1.** Optimized Structures of Top Five Conformers with *ab Initio* HF/6-31G*Calculation
-----S4-S8
3. ¹H and ¹³C NMR spectra for compounds **9a** isolated from *Phoma sp.*
-----S9-S14
4. ¹H and ¹³C NMR spectra for compounds **9b**, **9c**, **13a**, **13b** and **14** obtained from the acid-catalyzed rearrangements of verticillol (**5**)
-----S15-S27

Table S1. MMFF94 and *ab Initio* HF/6-31G* Energies of Top Five Conformers Found by CONFLEX Search**A⁺**

No.	MMFF94	HF/6-31G(d)	
	ΔE^a	E^b	ΔE^a
1	0.00	-776.2916	0.00
2	2.29	-776.2872	2.76
3	2.65	-776.2873	2.66
4	3.40	-776.2862	3.39
5	4.94	-776.2817	6.19

^a kcal/mol. ^b au.**G⁺**

No.	MMFF94	HF/6-31G(d)	
	ΔE^a	E^b	ΔE^a
1	0.00	-776.2906	0.00
2	0.32	-776.2893	0.83
3	1.36	-776.2915	-0.54
4	1.85	-776.2895	0.70
5	1.94	-776.2860	2.93

^a kcal/mol. ^b au.**F⁺**

No.	MMFF94	HF/6-31G(d)	
	ΔE^a	E^b	ΔE^a
1	0.00	-776.2965	0.00
2	3.05	-776.2931	2.12
3	4.07	-776.2891	4.69
4	4.21	-776.2883	5.15
5	4.45	-776.2890	4.74

^a kcal/mol. ^b au.

D⁺

No.	MMFF94	HF/6-31G(d)	
	ΔE^a	E^b	ΔE^a
1	0.00	-776.2877	0.00
2	0.02	-776.2844	2.09
3	0.40	-776.2850	1.70
4	0.84	-776.2813	4.07
5	2.05	-776.2818	3.74

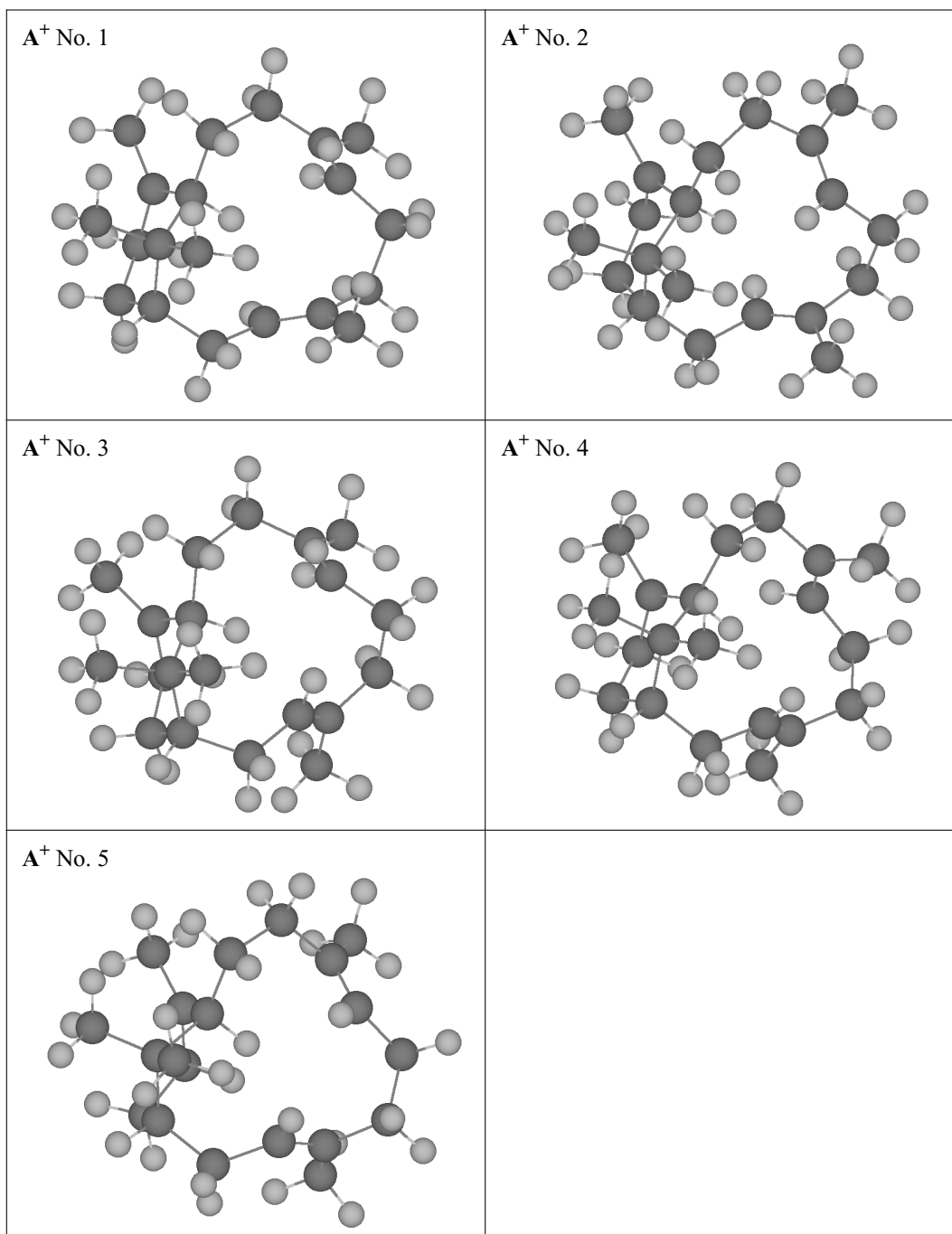
^a kcal/mol. ^b au.

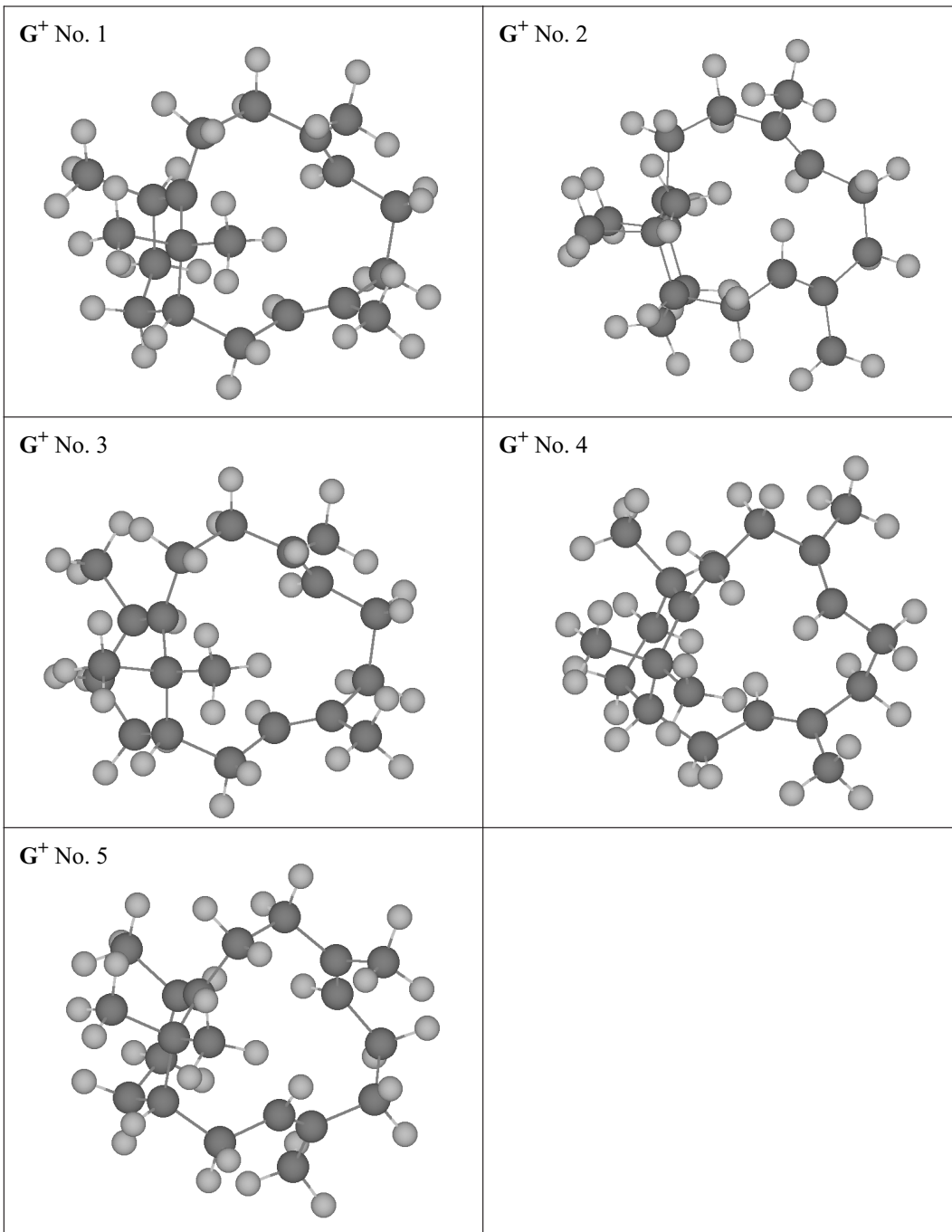
E⁺

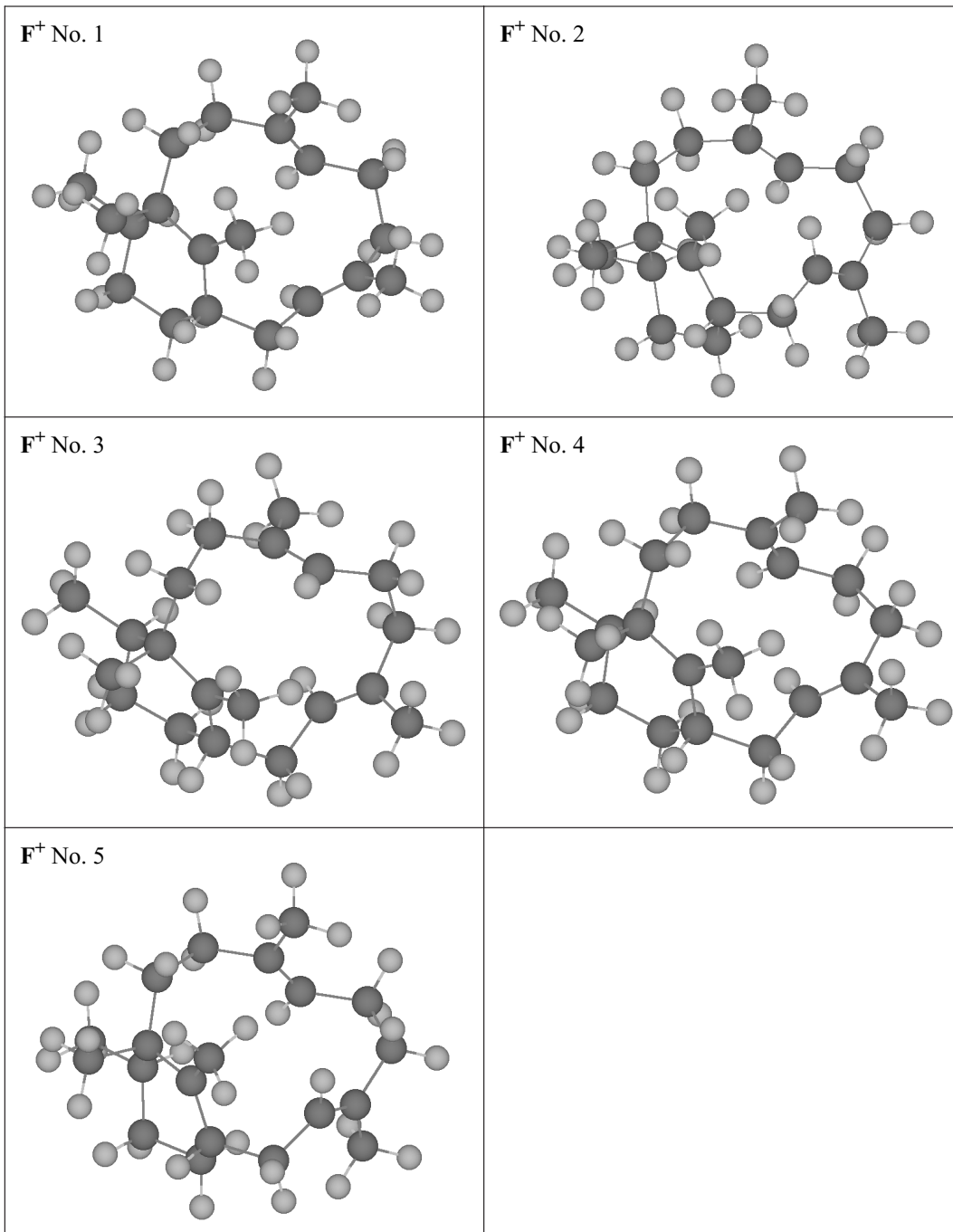
No.	MMFF94	HF/6-31G(d)	
	ΔE^a	E^b	ΔE^a
1	0.00	-776.2856	0.00
2	0.58	-776.2851	0.30
3	0.95	-776.2862	-0.40
4	0.98	-776.2846	0.59
5	1.40	-776.2829	1.67

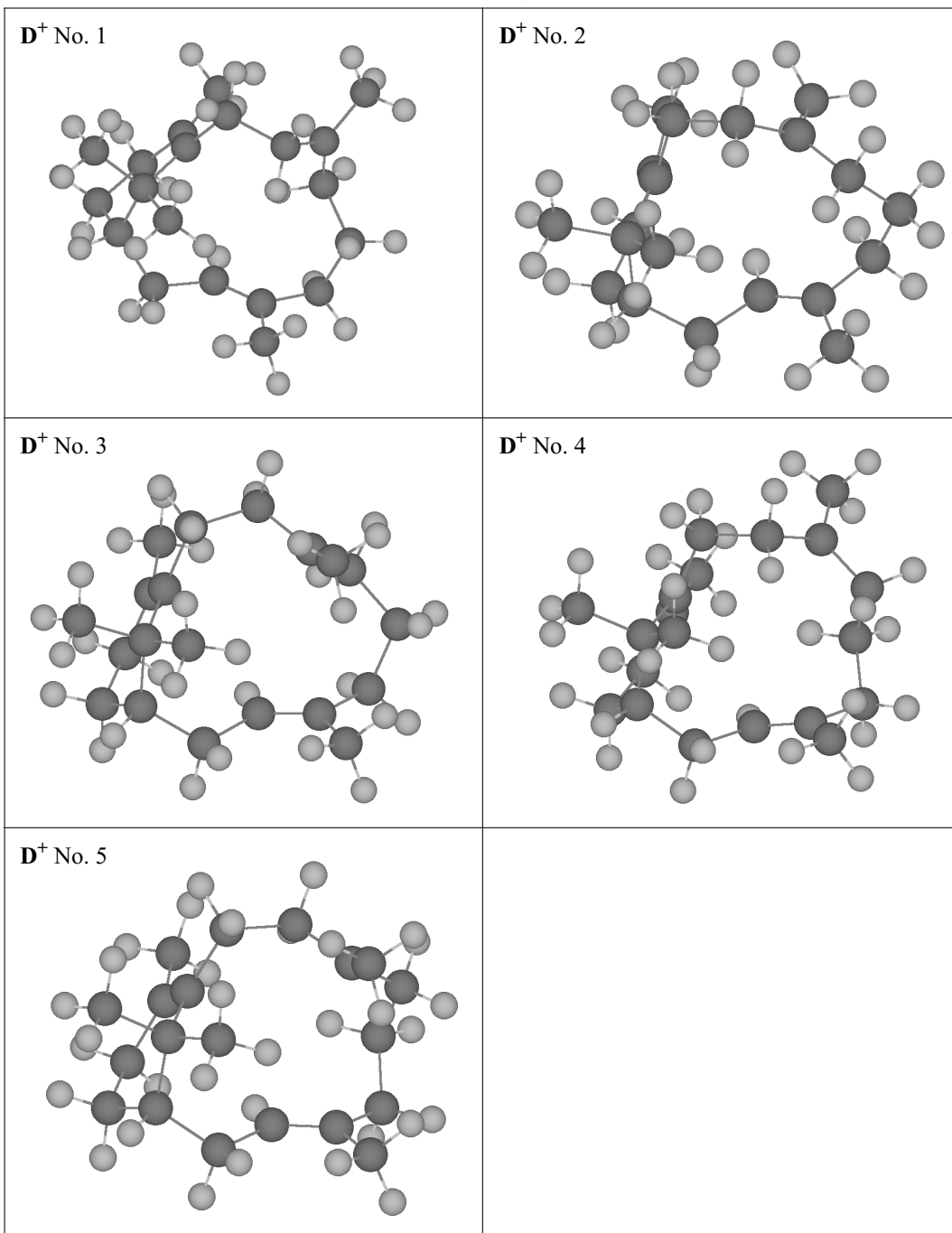
^a kcal/mol. ^b au.

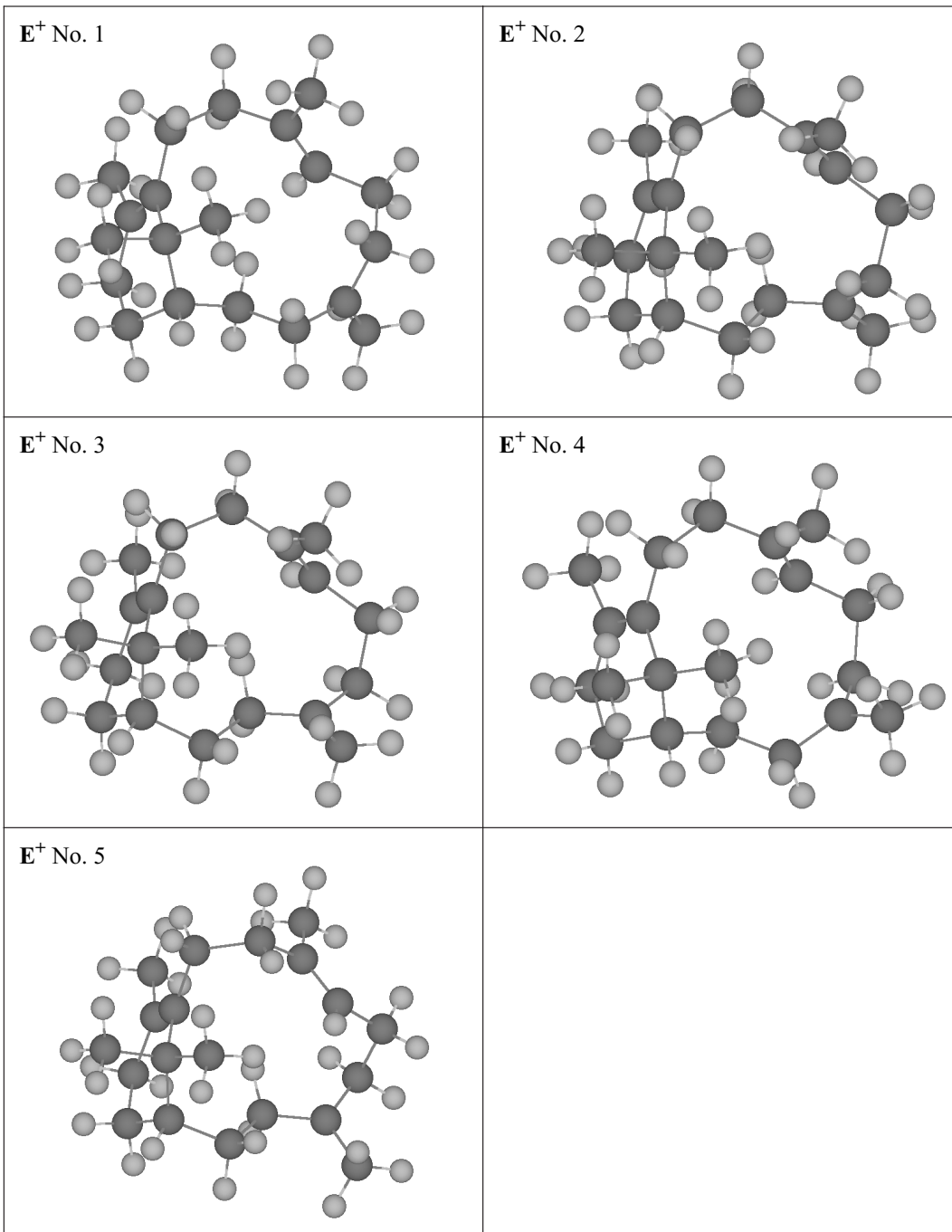
Figure S1. Optimized Structures of Top Five Conformers with *ab Initio* HF/6-31G* Calculation

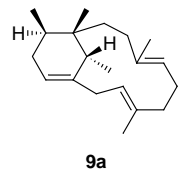
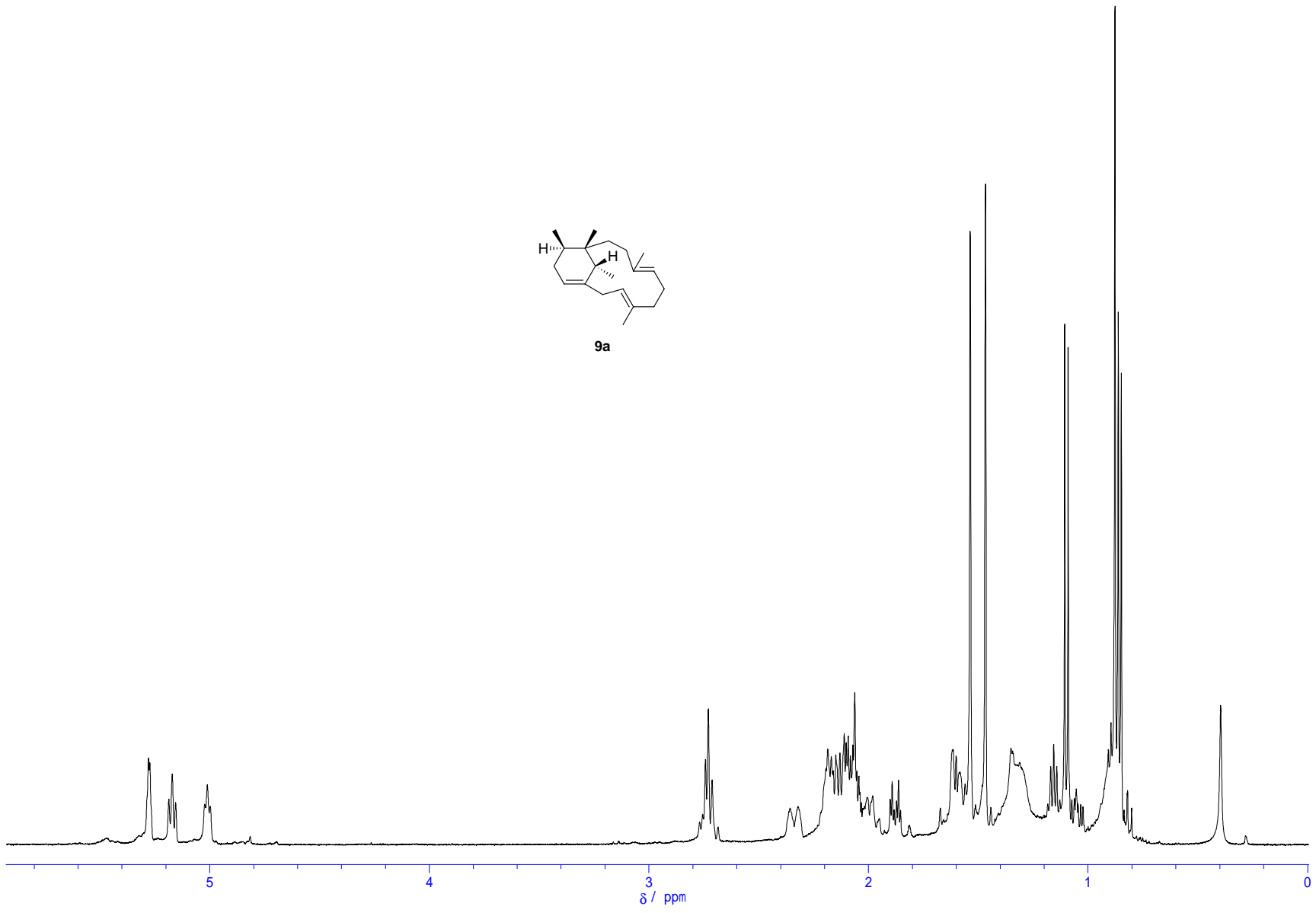




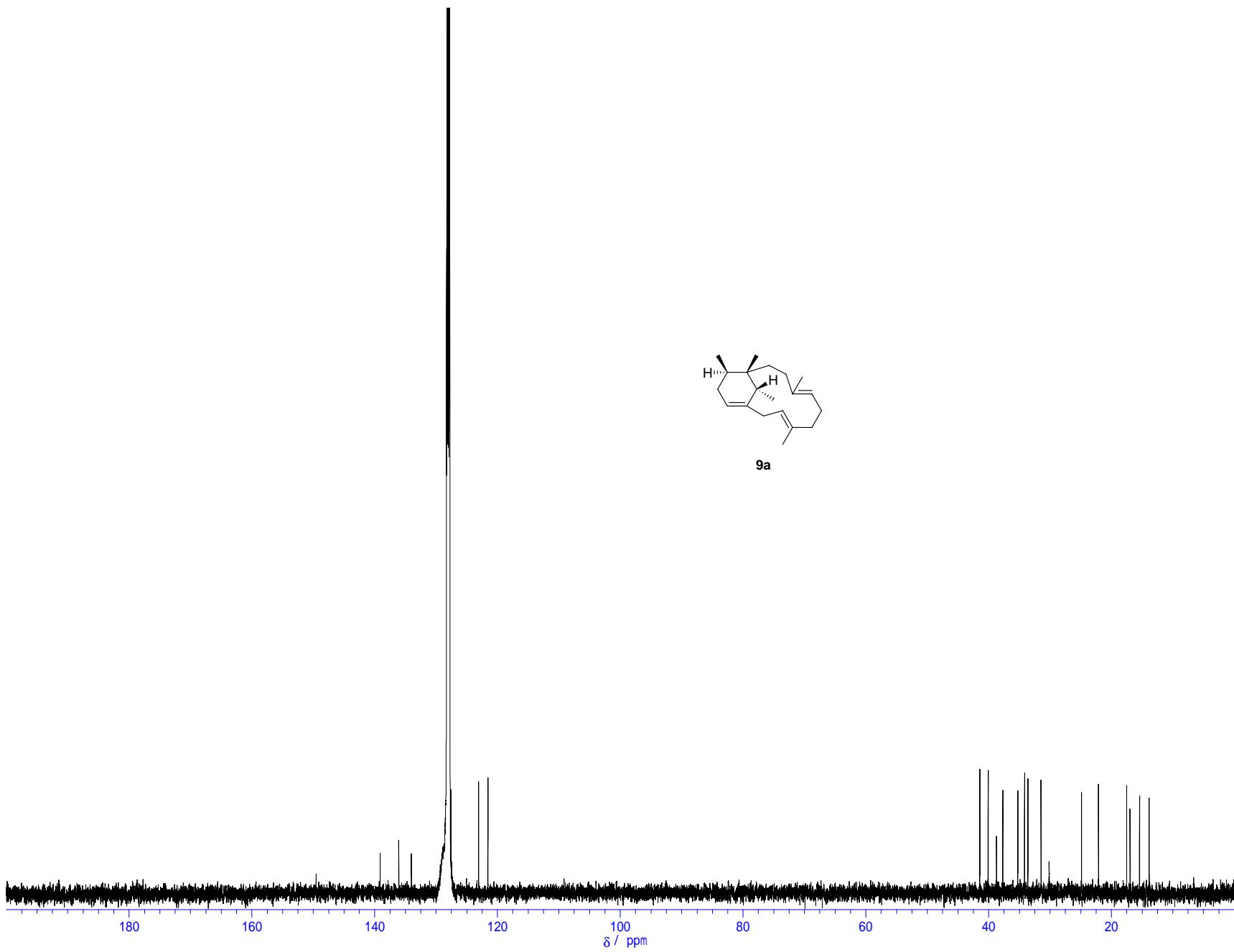




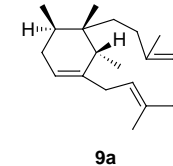
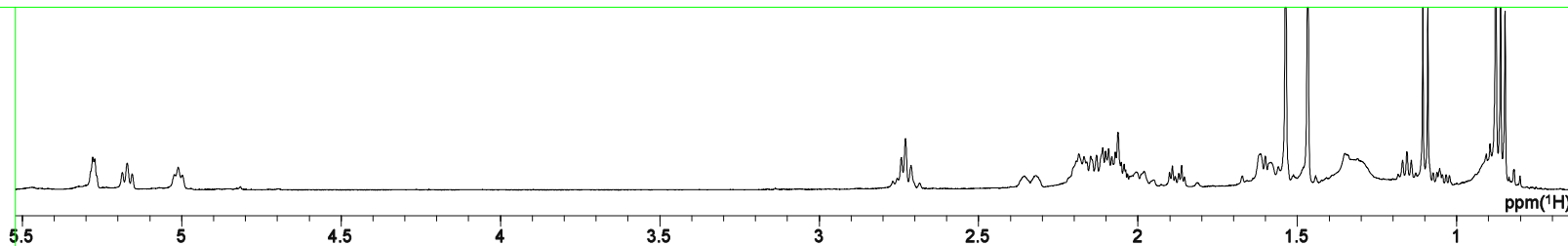




-S10-



Exp 1.2



Comment
File piccpmt/3
COSY
Oikawa PMT 1mg /90uL C6D6
dH(Solv)=7.15ppm, dC(solv)=128.0p
Bruker AMX-500/ GC-MS & NMR Lab.
Grad. Sch. Agric., Hokkaido Univ.
H=piccpmt/1 C=piccpmt/2

\$ F2-Acquisition Parameters

Date Mon Jun 17 16:48:33 2002
PULPROG cosygs
TD 1024
NS 1
DS 2
D[0] 0.000003 sec
D[1] 1.999999 sec
HL1 5 dB
IN[0] 0.000218 sec
P[1] 12.000000 usec
Sf 9.171891 ppm
SWH 4587.155963 Hz
FIDRES 0.223232 Hz
AQ 0.111616 sec
RG 4096
NUCLEUS 1H
SF01 500.131982 MHz
BF1 500.130000 MHz
O1 1982.147592 MHz
SF02 0.000000 MHz
BF2 500.130000 MHz
O2 1982.147592 MHz
LOKNUC 2H
SOLVENT C6D6
PROBHD 2.5 mm DUL 13C-1H-D Z-GR
TE 300 K
GRDPROG 2sine
CNST[21] 10 %
CNST[22] -10 %
D[16] 0.000100 sec
P[16] 1000.000000 usec
L[21] 100

\$ F1-Acquisition Parameters

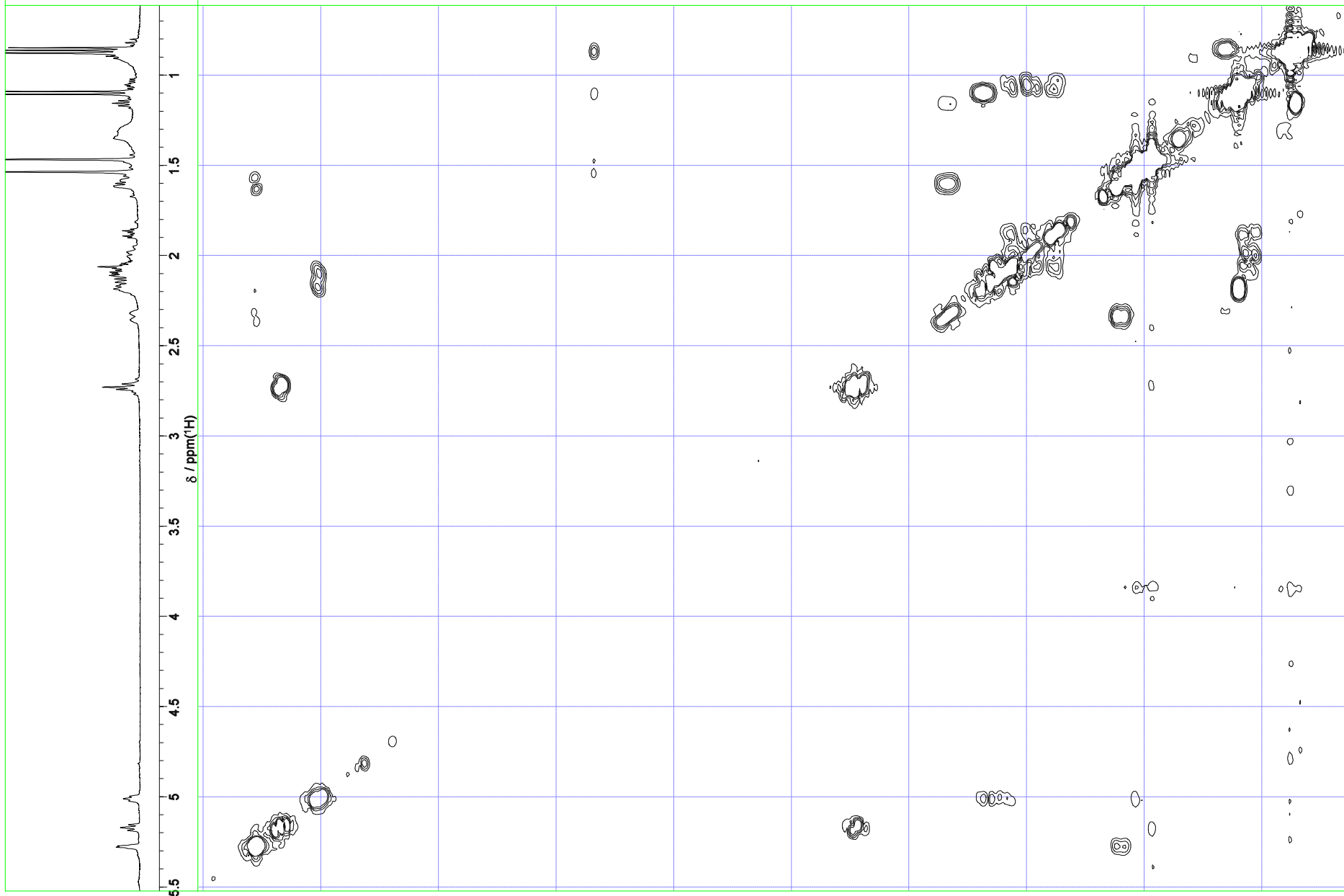
TD 256
ND0 1
SW 9.171891 ppm
SWH 4587.155963 Hz
FIDRES 0.055808 Hz

\$ F2-Processing Parameters

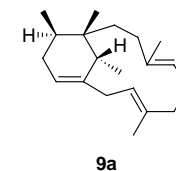
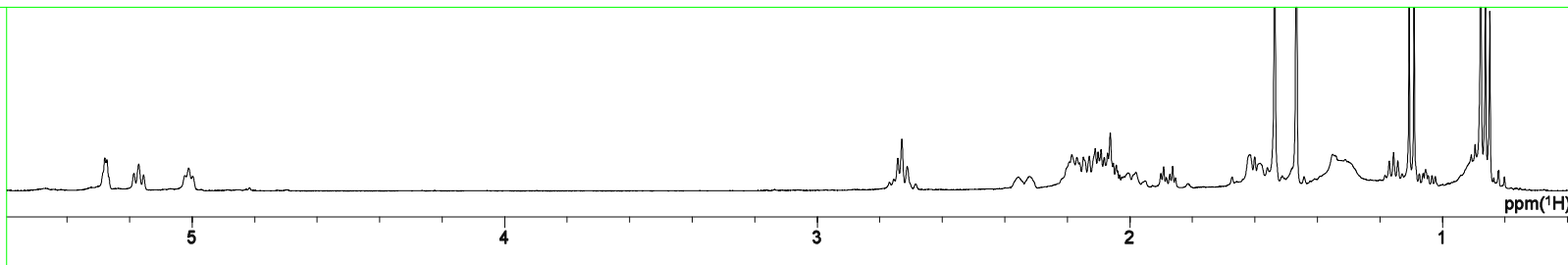
SI 1024
SF 500.130062538213
OFFSET 8.424184
HZpPT 4.479648 Hz
MC2 qf
WDW SINE
SSB 0.000000

\$ F1-Processing Parameters

SI 512
SF 500.130062538213
OFFSET 8.424184
REVERSE yes
HZpPT 8.959297 Hz
MC2 qf
WDW SINE
SSB 0.000000
expt = ca. 9 min



Exp 1.2



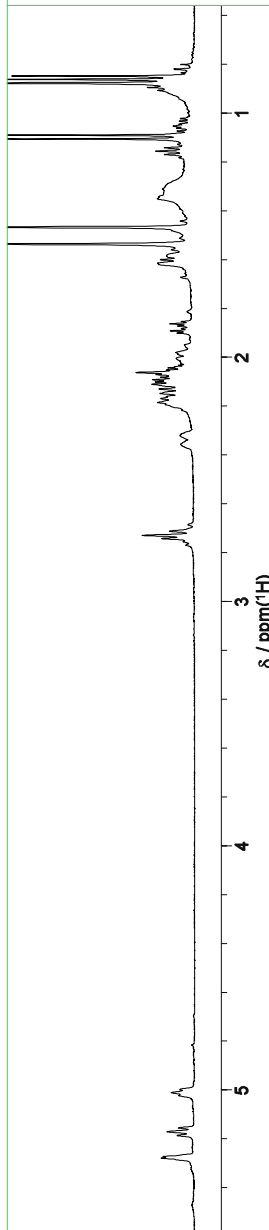
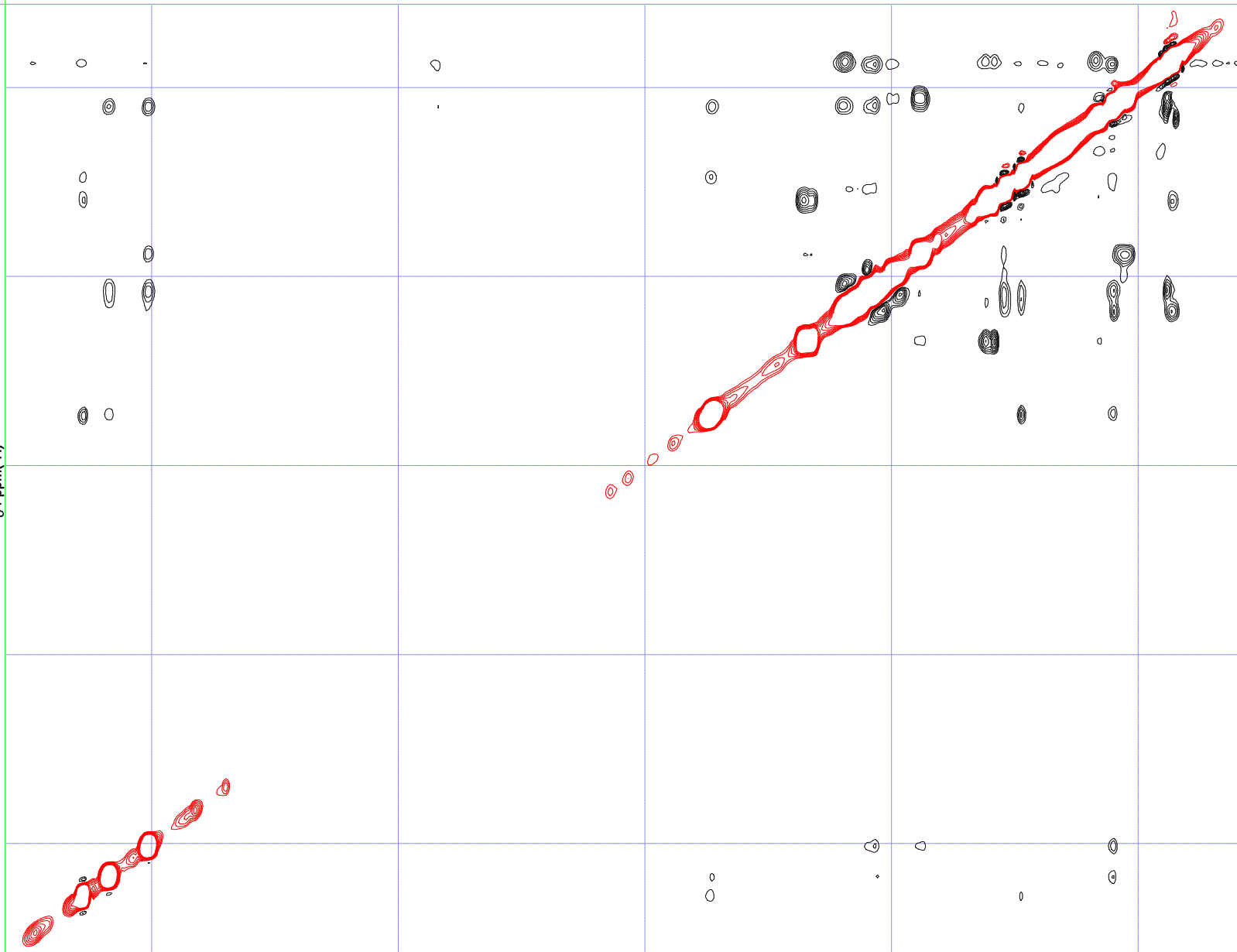
Comment
File piccpmt/4
noesy
Oikawa PMT 1mg /90uL C6D6
dH(Solv)=7.15ppm, dC(solv)=128.0p
Bruker AMX-500/ GC-MS & NMR Lab.
Grad. Sch. Agric., Hokkaido Univ.
H=piccpmt/1 C=piccpmt/2

\$ F2-Acquisition Parameters
Date Mon Jun 17 16:59:41 2002
PULPROG noesygs.jp
TD 1024
NS 16
DS 4
D[0] 0.000003 sec
D[1] 2.002048 sec
D[9] 1.000000 sec
HL1 5 dB
IN[0] 0.000109 sec
P[1] 12.000000 usec
V9 2.000000 %
SW 9.171891 ppm
SI 4587.155963 Hz
FIDRES 0.223232 Hz
AQ 0.111616 sec
RG 2048
NUCLEUS 1H
SF01 500.131982 MHz
BF1 500.130000 MHz
O1 1982.147592 MHz
SF02 0.000000 MHz
BF2 500.130000 MHz
O2 1982.147592 MHz
LOKNUC 2H
SOLVENT C6D6
PROBHD 2.5 mm DUL 13C-1H-D Z-GR
TE 300 K
GRDPROG 1sine
CNST[21] 30 %
D[16] 0.000100 sec
P[16] 1000.000000 usec
L[21] 100

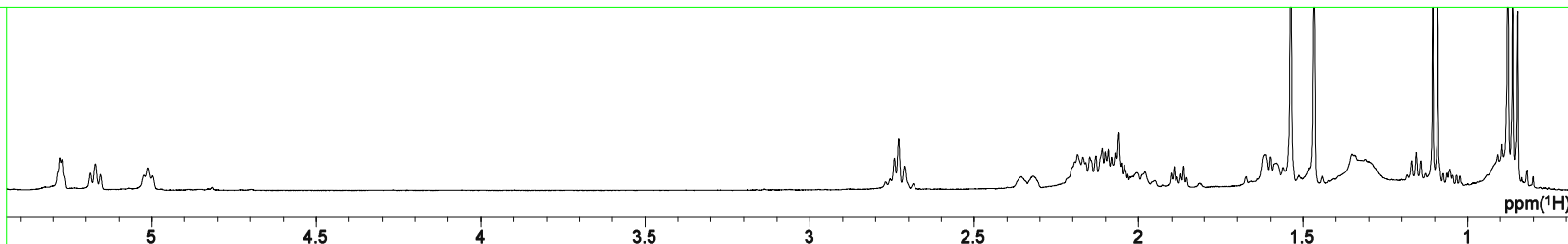
\$ F1-Acquisition Parameters
TD 256
ND0 2
SW 9.171891 ppm
SI 4587.155963 Hz
FIDRES 0.055808 Hz

\$ F2-Processing Parameters
SI 512
SF 500.130062538213
OFFSET 8.424184
HZpPT 8.959297 Hz
MC2 qf
WDW QSINE
SSB 2.000000
PH_mod pk
PHC0 35.422300 degree
PHC1 25.800010 degree

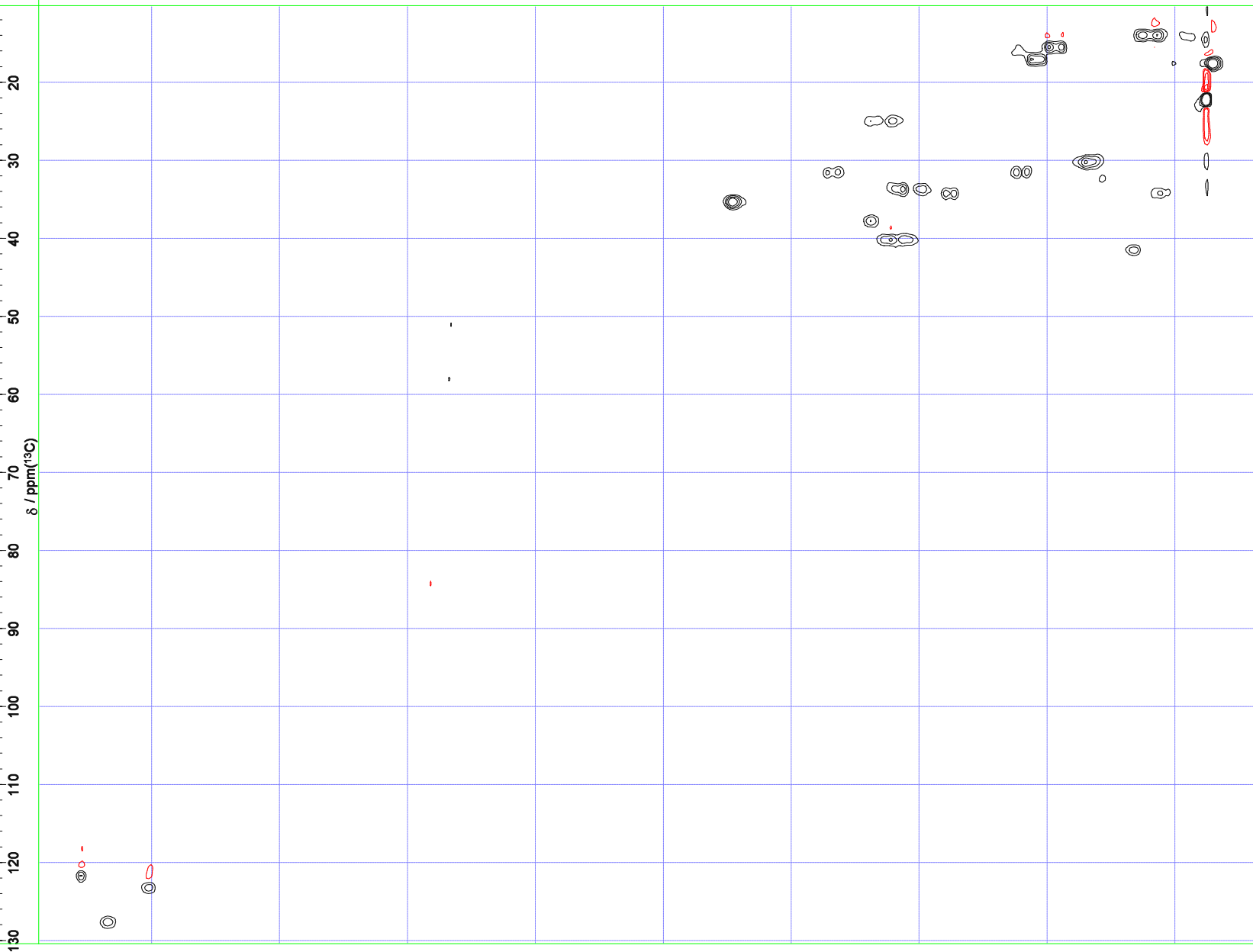
\$ F1-Processing Parameters
SI 512
SF 500.130062538213
OFFSET 8.424184
REVERSE no
HZpPT 8.959297 Hz
MC2 TPP1
WDW QSINE
SSB 2.000000
PH_mod pk
PHC0 15.443750 degree
PHC1 -29.200000 degree
expt = ca. 4 hr



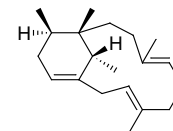
Exp 1.2



Comment
File piccpmt/7
hsqc
Oikawa PMT 1mg /90uL C6D6
dH(Solv)=7.15ppm, dC(sol)=128.0p
Bruker AMX-500/ GC-MS & NMR Lab.
Grad. Sch. Agric., Hokkaido Univ.
Hpiccpmt/1 C=piccpmt/2

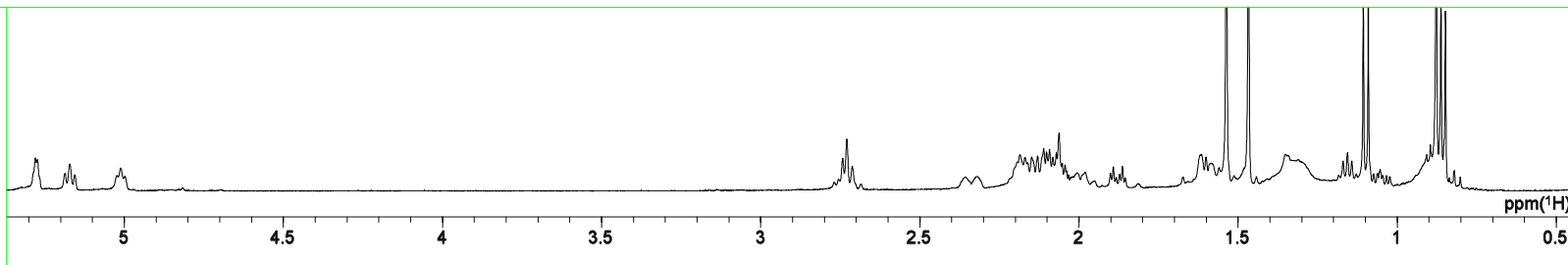


\$ F2-Acquisition Parameters
Date Thu Jun 20 15:38:53 2002
PULPROG invg1stp.m
TD 1024
NS 96
DS 2
D[0] 0.000003 sec
D[1] 2.181247 sec
D[4] 0.001800 sec
DL[0] 19 dB
DL[5] 41 dB
HL1 5 dB
IN[0] 0.000013 sec
P[1] 12.000000 usec
P[3] 5.800000 usec
P[31] 0.000000 usec
SI 9.171891 ppm
SWH 4587.155963 Hz
FIDRES 0.223232 Hz
AQ 0.111616 sec
RG 32768
NUCLEUS 1H
SF01 500.131982 MHz
BF1 500.130000 MHz
O1 1982.147592 MHz
SF02 125.768146 MHz
BF2 125.757739 MHz
O2 10407.375084 MHz
LOKNUC 2H
SOLVENT C6D6
PROBHD 2.5 mm DUL 13C-1H-D Z-GR
TE 300 K
GRDPROG 3sine
CNST[21] 40 %
CNST[22] 15 %
CNST[23] 10 %
D[16] 0.000000 sec
P[16] 1000.000000 usec
L[21] 100
\$ F1-Acquisition Parameters
TD 256
ND0 4
SW 159.022778 ppm
SWH 20000.000000 Hz
FIDRES 0.012800 Hz
\$ F2-Processing Parameters
SI 1024
SF 500.130062538213
OFFSET 8.424184
HZpPT 4.479648 Hz
MC2 cf
WDW QSINE
SSB 2.000000
PH_mod pk
PHC0 -100.325000 degree
PHC1 -25.600000 degree
\$ F1-Processing Parameters
SI 512
SF 125.757762832763
OFFSET 162.0858
REVERSE no
HZpPT 39.062500 Hz
MC2 TPP1
WDW QSINE
SSB 2.000000
PH_mod pk
PHC0 289.530900 degree
PHC1 -253.800000 degree
expt = ca. 16 hr



9a

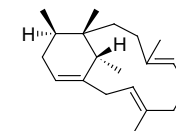
Exp 1.2



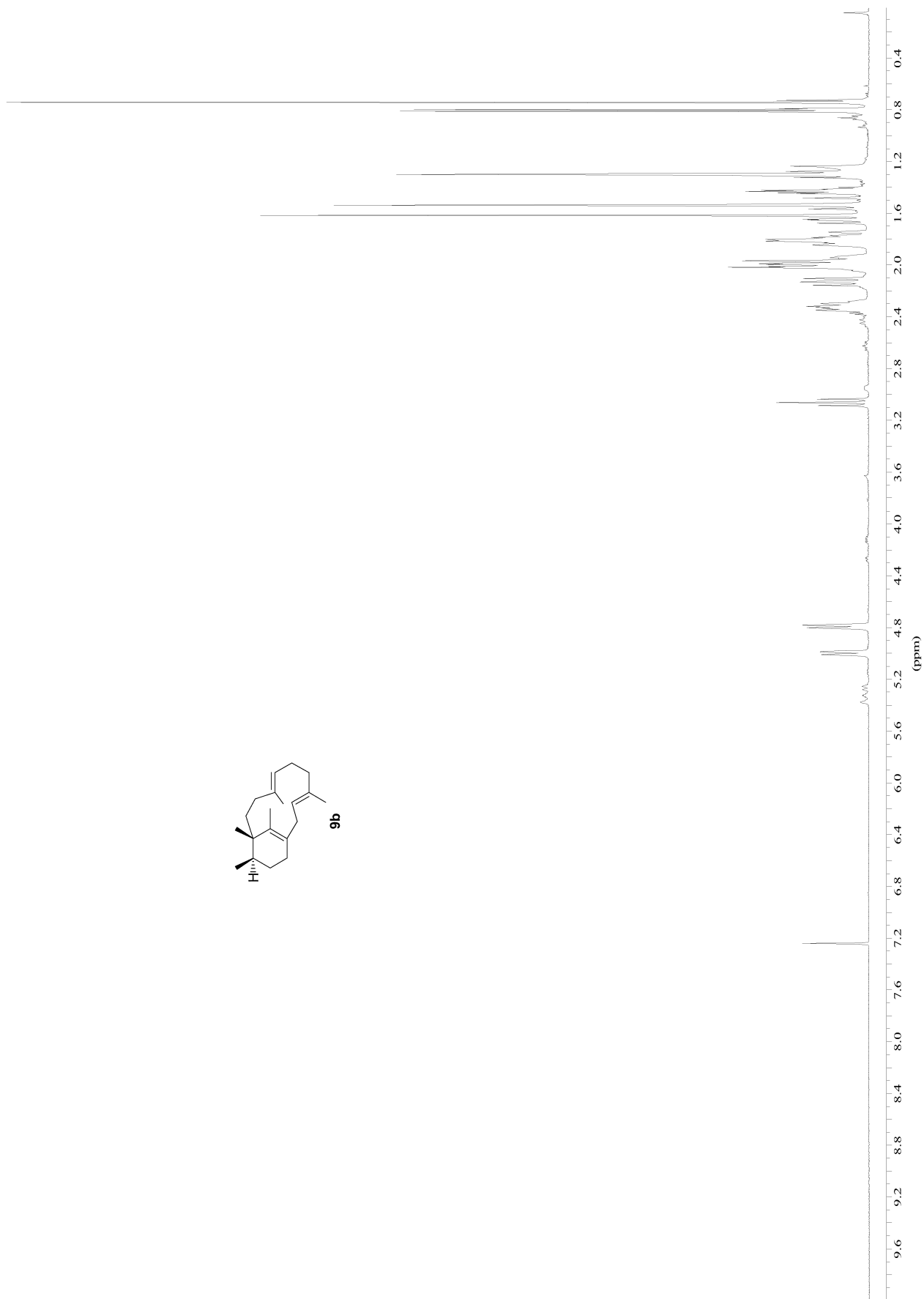
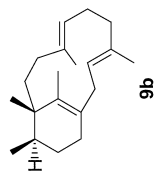
Comment
File piccpmt/6

HMC
Oikawa PMT 1mg /90uL C6D6
dH(Solv)=7.15ppm, dC(solV)=128.0p
Bruker AMX-500/ GC-MS & NMR Lab.
Grad. Sch. Agric., Hokkaido Univ.
H=piccpmt/1 C=piccpmt/2

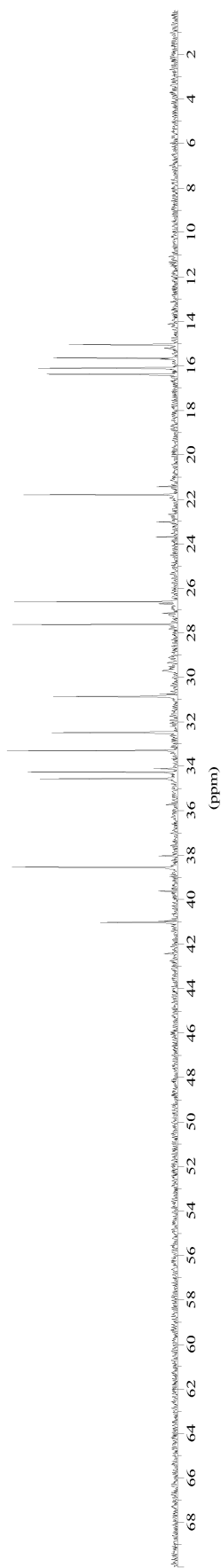
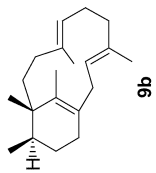
\$ F2-Acquisition Parameters
Date Tue Jun 18 02:02:20 2002
PULPROG inv4gsplrnd.jp
TD 1024
NS 192
DS 2
D[0] 0.000003 sec
D[1] 2.000000 sec
D[2] 0.003450 sec
D[6] 0.070000 sec
DL[0] 19 dB
HL1 5 dB
IN[0] 0.000025 sec
P[1] 12.000000 usec
P[3] 5.800000 usec
SI 9.171691 ppm
SWH 4587.155963 Hz
FIDRES 0.223232 Hz
AQ 0.111616 sec
RG 32768
NUCLEUS 1H
SF01 500.131982 MHz
BF1 500.130000 MHz
O1 1982.147592 MHz
SF02 125.768146 MHz
BF2 125.757739 MHz
O2 10407.375084 MHz
LOKNUC 2H
SOLVENT C6D6
PROBHD 2.5 mm DUL 13C-1H-D Z-GR
TE 300 K
GRDPROG 3sine
CNST[21] 10 %
CNST[22] 10 %
CNST[23] 5 %
D[16] 0.000000 sec
P[16] 1000.000000 usec
L[21] 100
\$ F1-Acquisition Parameters
TD 256
ND0 2
SW 159.022778 ppm
SWH 20000.000000 Hz
FIDRES 0.012800 Hz
\$ F2-Processing Parameters
SI 1024
SF 500.130062538213
OFFSET 8.424184
HZpPT 4.479648 Hz
MC2 qf
WDW GM
GB 0.300000
LB -10.000000
\$ F1-Processing Parameters
SI 512
SF 125.757762832763
OFFSET 162.0858
REVERSE no
HZpPT 39.062500 Hz
MC2 qf
WDW QSINE
SSB 6.000000
expt = ca. 29 hr



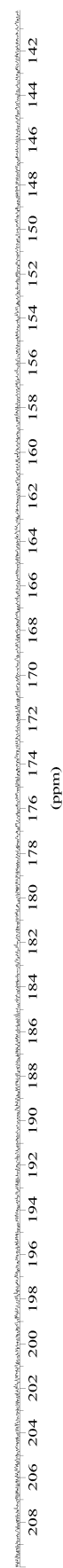
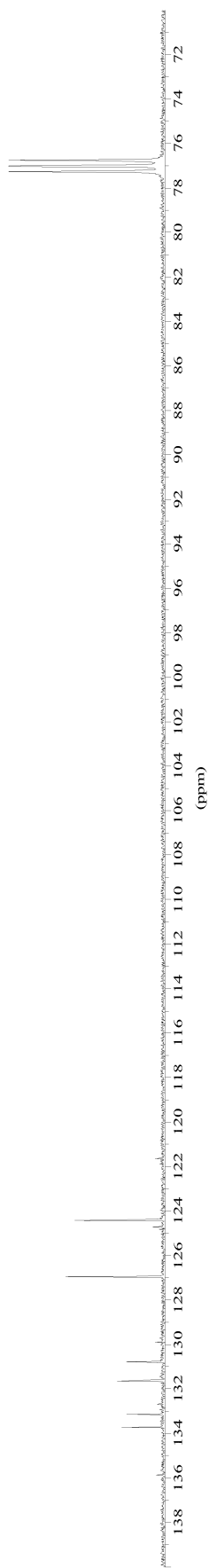
9a



-S15-

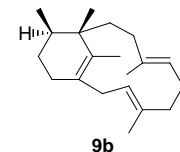
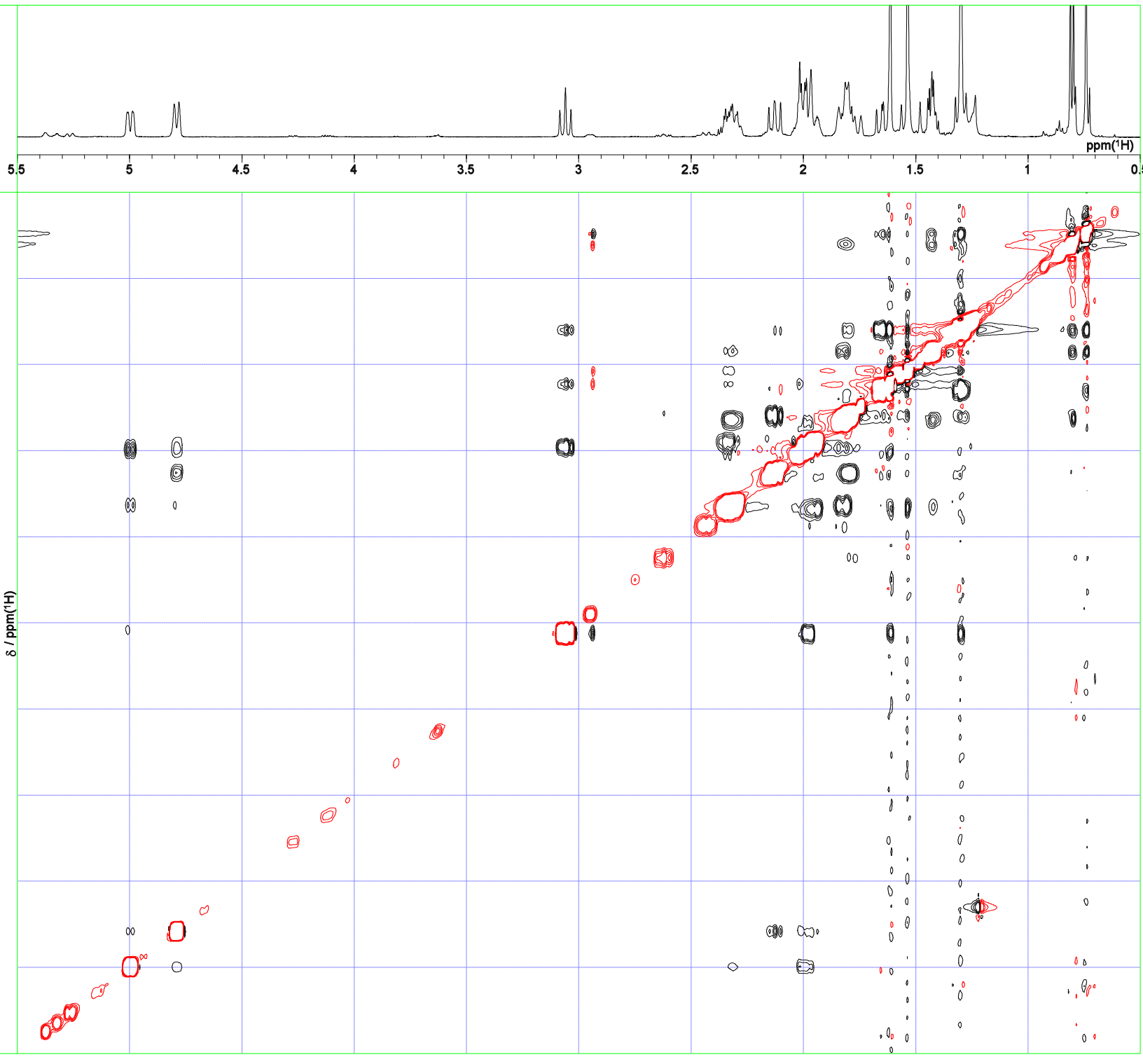


-S16-

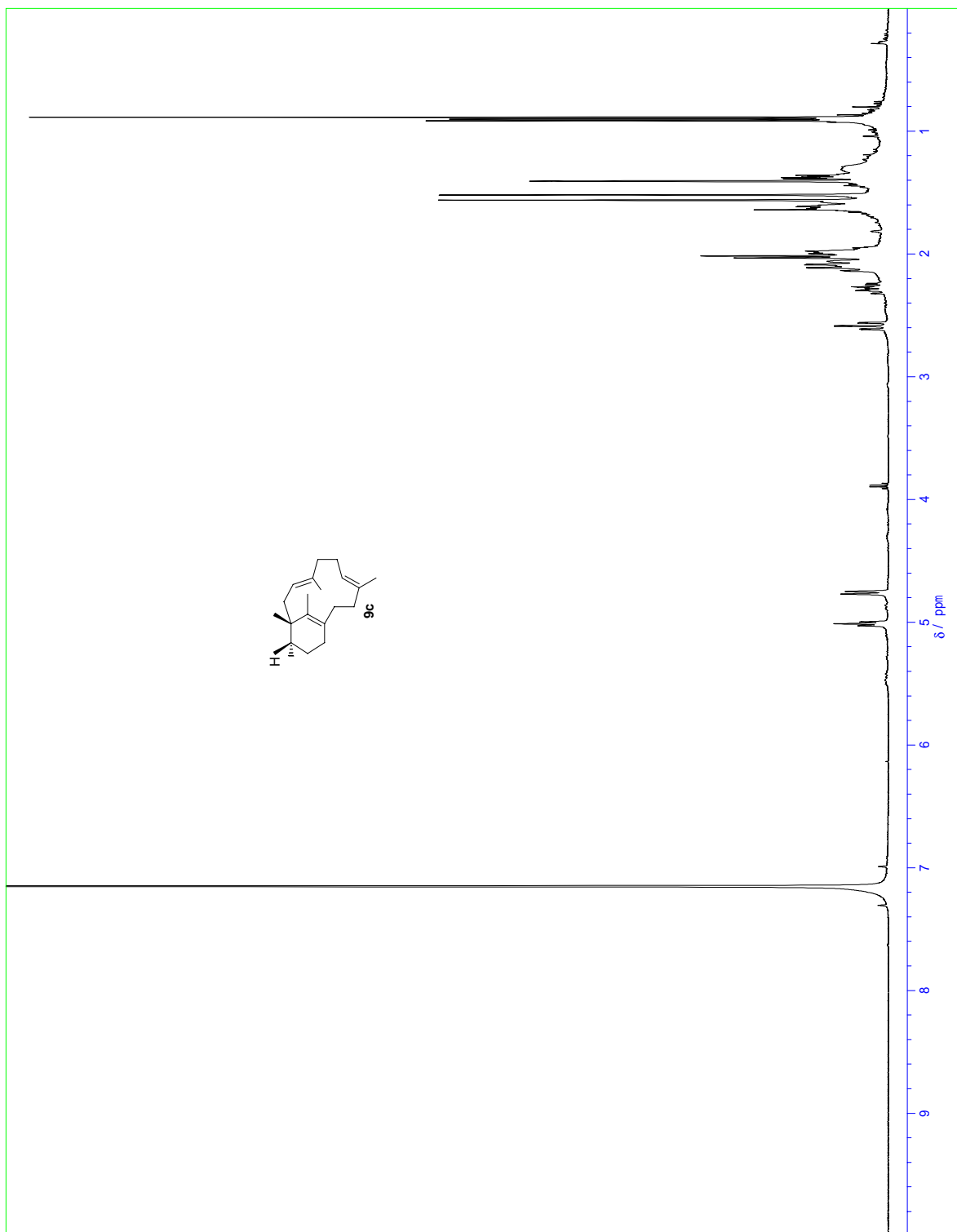


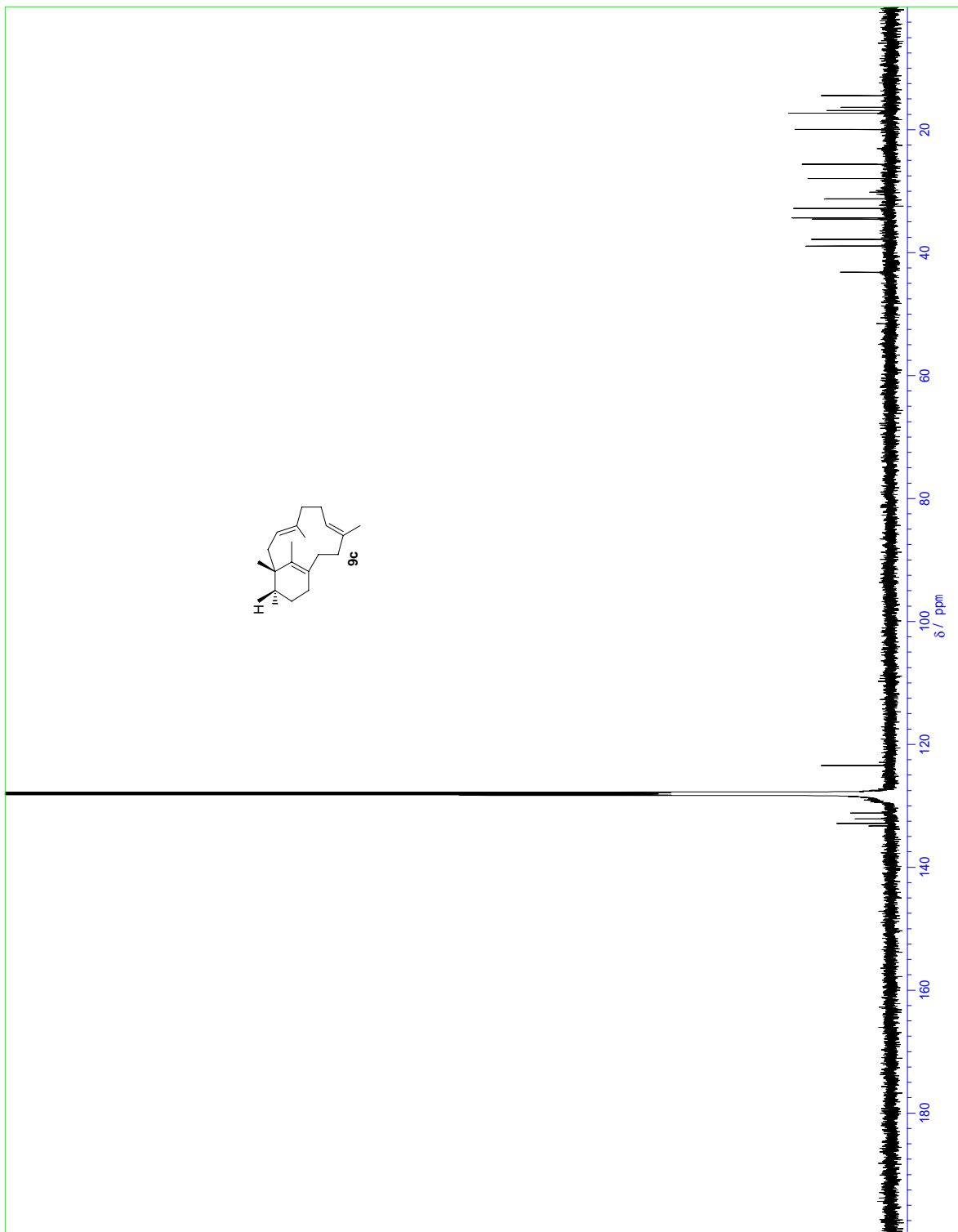
Comment
 File piccpmt/104
 noesy
 Oikawa verticillol-(BF3)-(-78)-f
 r3 ? mg /60uL CDCl3
 dH(Solv)=7.24ppm, dC(solv)=77.0p
 pm
 Bruker AMX-500/ GC-MS & NMR Lab.
 Grad. Sch. Agric., Hokkaido Univ
 H=piccpmt/101 C=piccpmt/102

\$ F2-Acquisition Parameters
 Date Sat Nov 15 15:07:52 200
 3
 PULPROG noesygs.jp
 TD 1024
 NS 32
 DS 4
 D[0] 0.000003 sec
 D[1] 1.943680 sec
 D[9] 1.000000 sec
 HL1 5 dB
 INFO 0.000166 sec
 P[1] 12.000000 usec
 V9 2.000000 %
 SW 6.022513 ppm
 SWH 3012.048193 Hz
 FIDRES 0.339968 Hz
 AQ 0.169984 sec
 RG 1024
 NUCLEUS 1H
 SF01 500.131493 MHz
 BF1 500.130000 MHz
 O1 1492.510573 MHz
 SF02 0.000000 MHz
 BF2 500.130000 MHz
 O2 1492.510573 MHz
 LOKNUC 2H
 SOLVENT CDCl3
 PROBHD 2.5 mm DUL 13C-1H-D Z-G
 RD Z5541/008
 TE 300 K
 GRDPROG lsine
 CNST[21] 30 %
 D[16] 0.000100 sec
 P[16] 1000.000000 usec
 L[21] 100
 \$ F1-Acquisition Parameters
 TD 256
 NDO 2
 SW 6.022513 ppm
 SWH 3012.048193 Hz
 FIDRES 0.084992 Hz
 \$ F2-Processing Parameters
 SI 512
 SF 500.130023435046
 OFFSET 5.948652
 HZpPT 5.882910 Hz
 MC2 of
 WDW QSINE
 SSB 2.000000
 PH_mod pk
 PHC0 -11.780910 degree
 PHC1 104.200000 degree
 \$ F1-Processing Parameters
 SI 512
 SF 500.130023435046
 OFFSET 5.948652
 REVERSE no
 HZpPT 5.882910 Hz
 MC2 TPP1
 WDW QSINE
 SSB 2.000000
 PH_mod pk
 PHC0 11.223440 degree
 PHC1 -23.200000 degree
 expt = ca. 7 hr

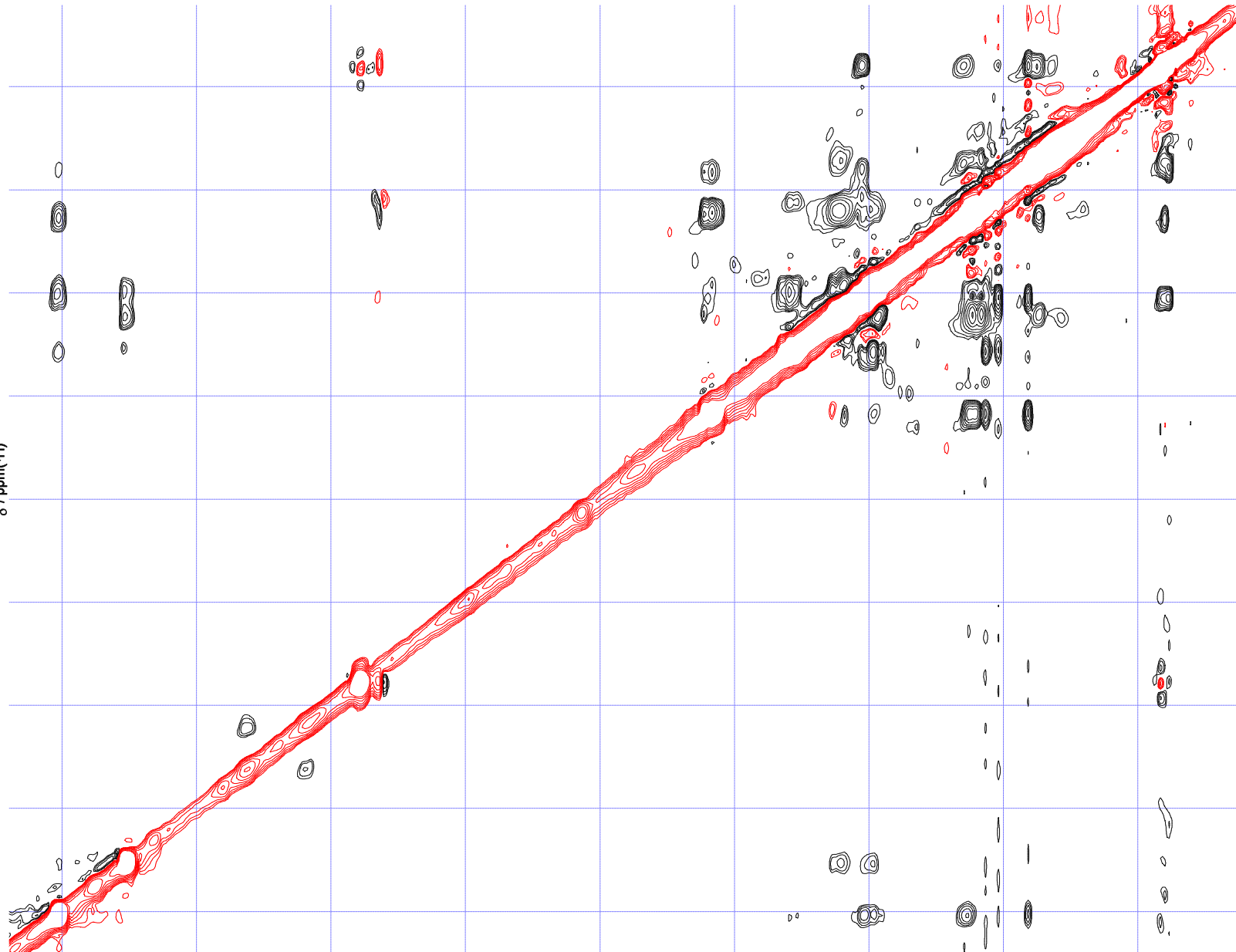
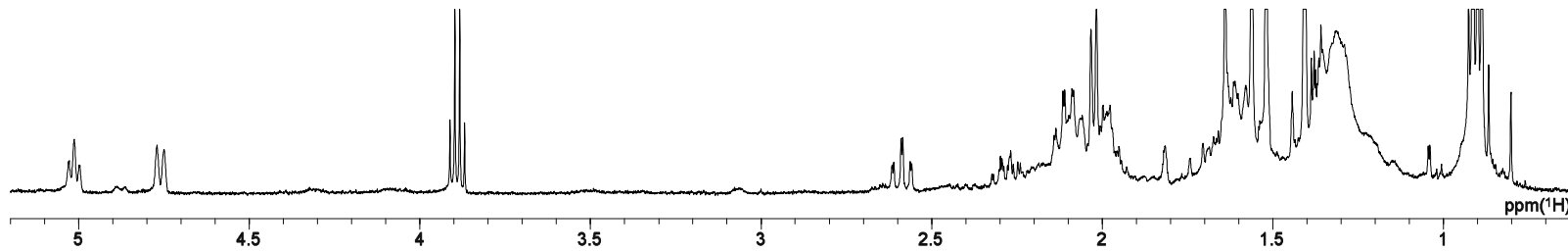


-S17-



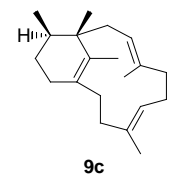


Exp 1.5

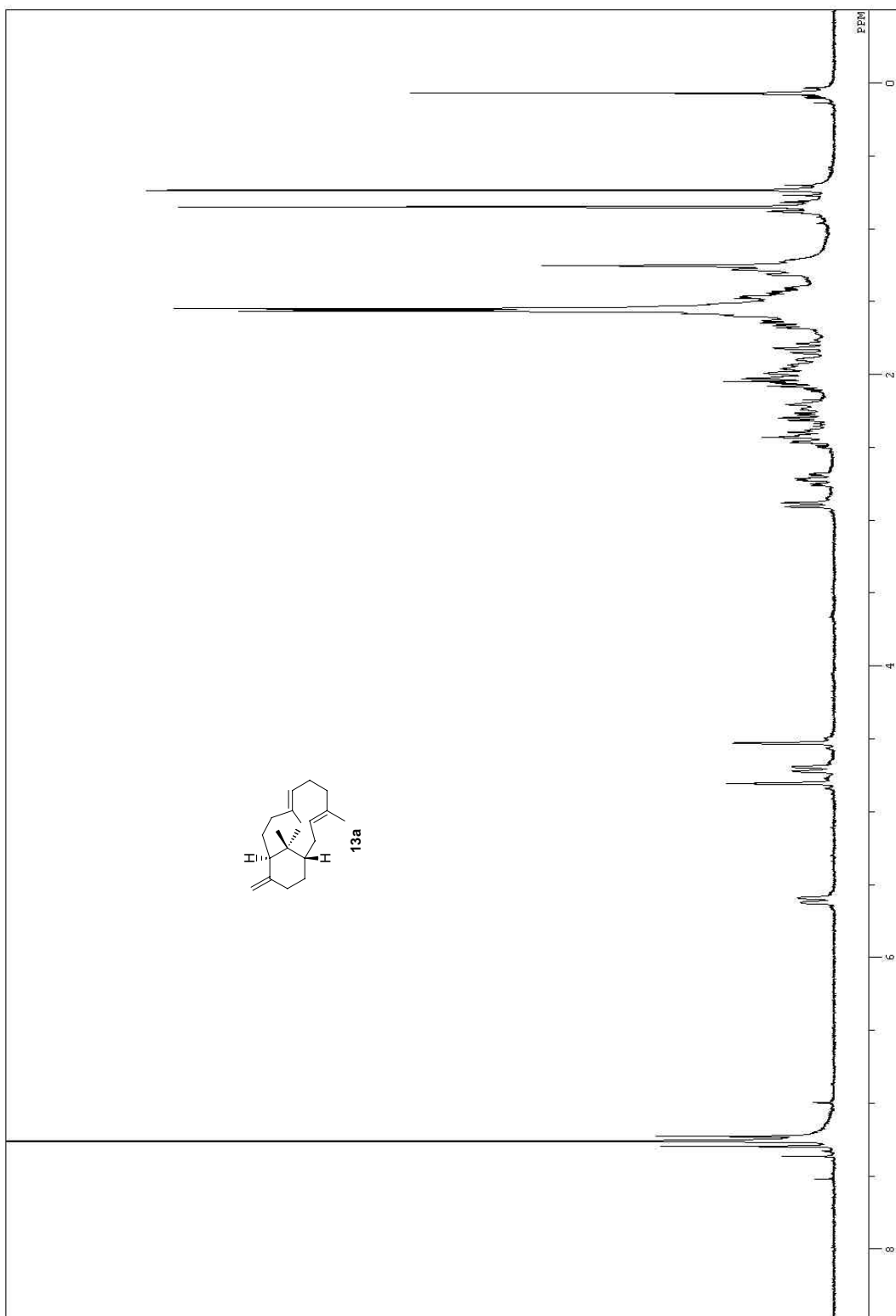


Comment
File piccpmt/206
noesy
Oikawa No.2 (verticillol-27.5-L
(OTf)3-10min-m/z=121,135) 0.3mg
/90uL C6D6
dh(sol)=7.15ppm, dC(sol)=128.
ppm
Bruker AMX-500/ GC-MS & NMR Lab
Grad. Sch. Agric., Hokkaido Uni
H=piccpmt/201 C=piccpmt/202

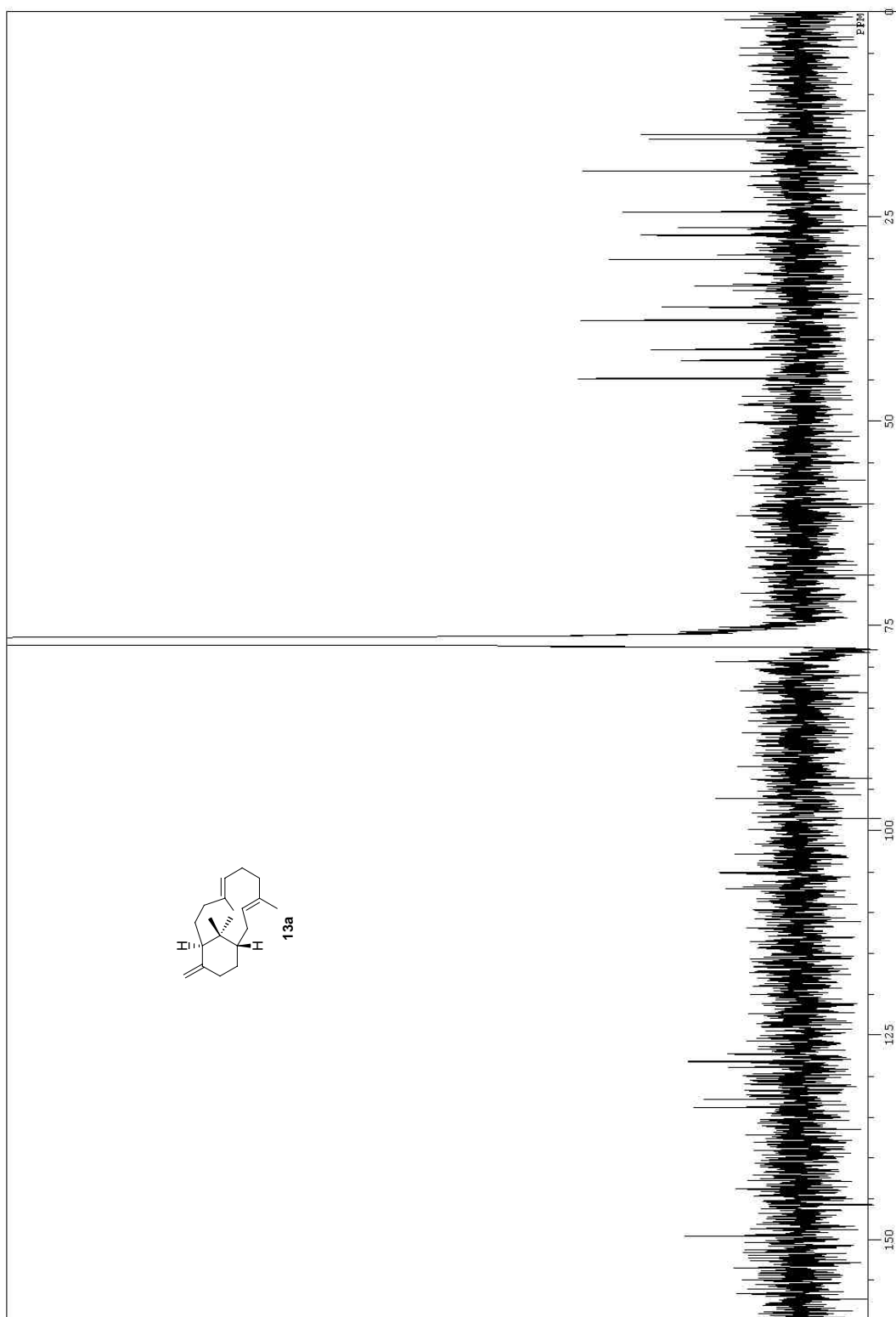
\$ F2-Acquisition Parameters
Date Wed Feb 11 10:36:27 20
4
PULPROG noesy.jp
TD 1024
NS 256
DS 4
D[0] 0.000003 sec
D[1] 1.750000 sec
D[9] 1.000000 sec
HL1 5 dB
IN[0] 0.000123 sec
P[1] 12.000000 usec
v9 2.000000 %
SW 8.127936 ppm
SWH 4065.040650 Hz
FIDRES 0.251904 Hz
AQ 0.125952 sec
RG 2048
NUCLEUS 1H
SF01 500.131973 MHz
BF1 500.130000 MHz
O1 1972.771511 MHz
SF02 0.000000 MHz
BF2 500.130000 MHz
O2 1972.771511 MHz
LOKNUC 2H
SOLVENT C6D6
PROBHD 2.5 mm DUL 13C-1H-D Z-
RD Z5541/008
TE 300 K
\$ F1-Acquisition Parameters
TD 128
ND0 2
SW 8.127936 ppm
SWH 4065.040650 Hz
FIDRES 0.031488 Hz
\$ F2-Processing Parameters
SI 512
SF 500.130062049003
OFFSET 7.884435
HZpPT 7.939531 Hz
MC2 qf
VDW QSINE
SSB 2.000000
PH_mod pk
PHC0 23.159380 degree
PHC1 47.200000 degree
\$ F1-Processing Parameters
SI 512
SF 500.130062049003
OFFSET 7.884435
REVERSE no
HZpPT 7.939531 Hz
MC2 TPP1
VDW QSINE
SSB 2.000000
PH_mod pk
PHC0 11.223440 degree
PHC1 -23.200000 degree
ME_mod LPfr
NCOEF 16
LPBIN 256
expt = ca. 27 hr

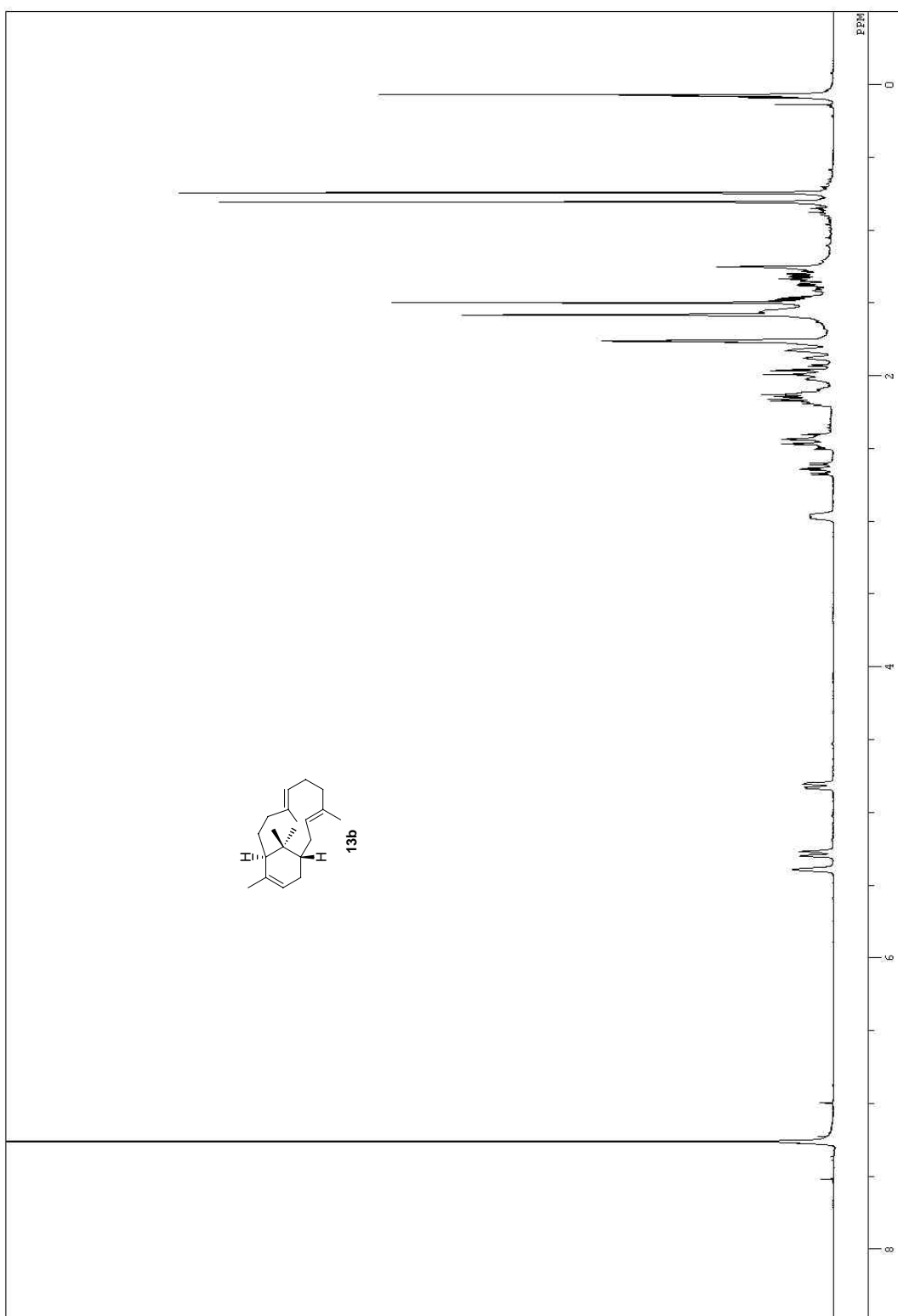


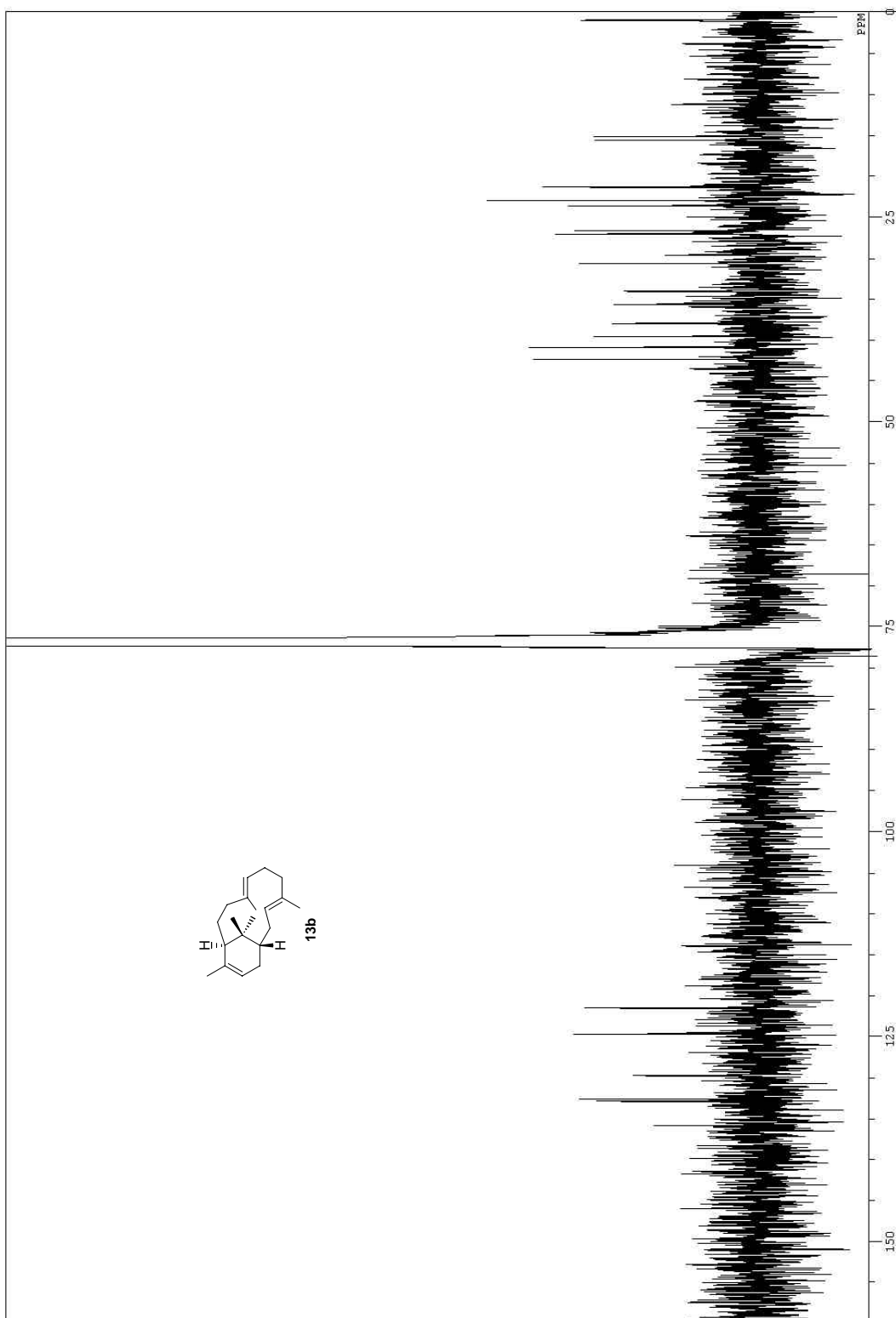
-S20-

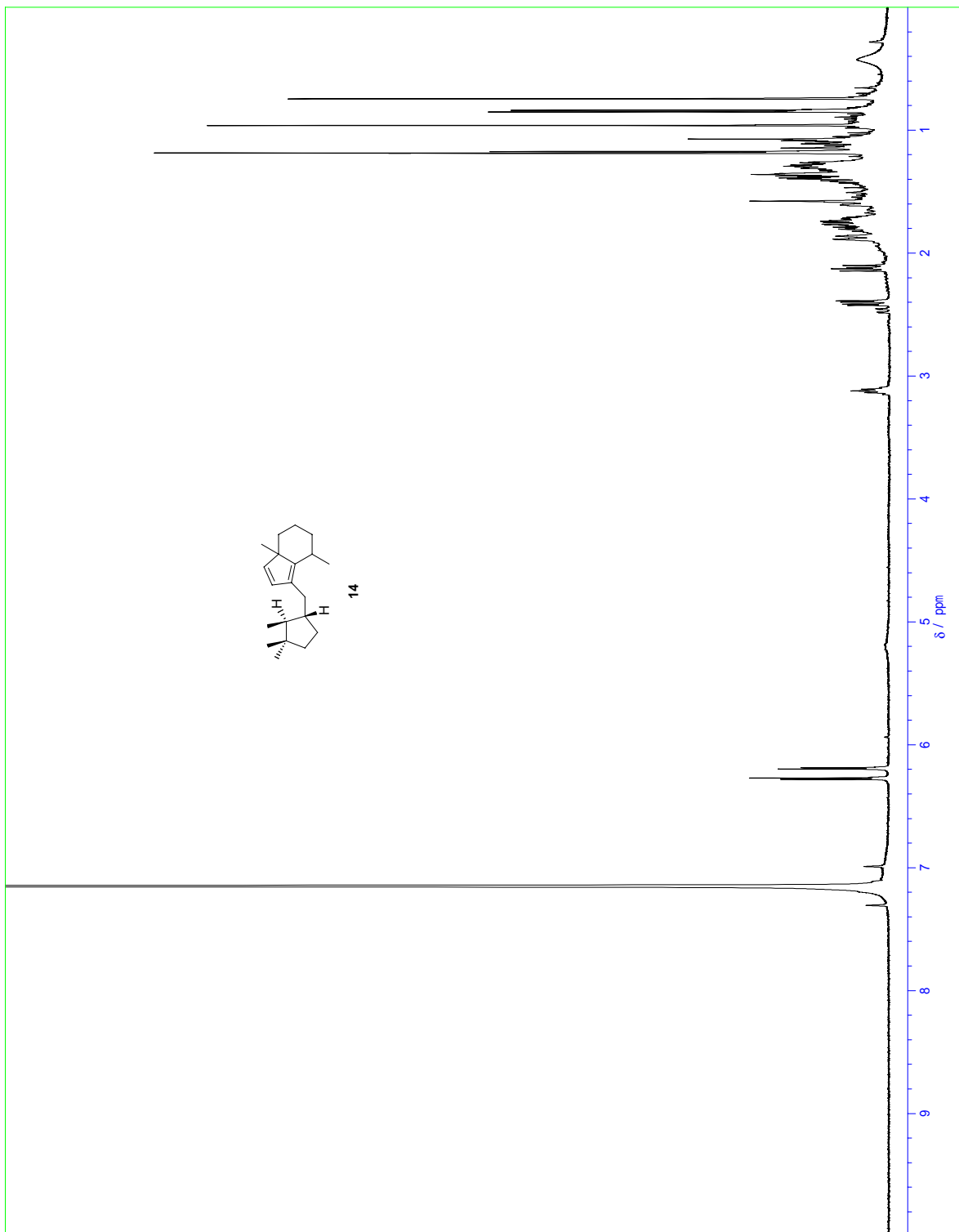


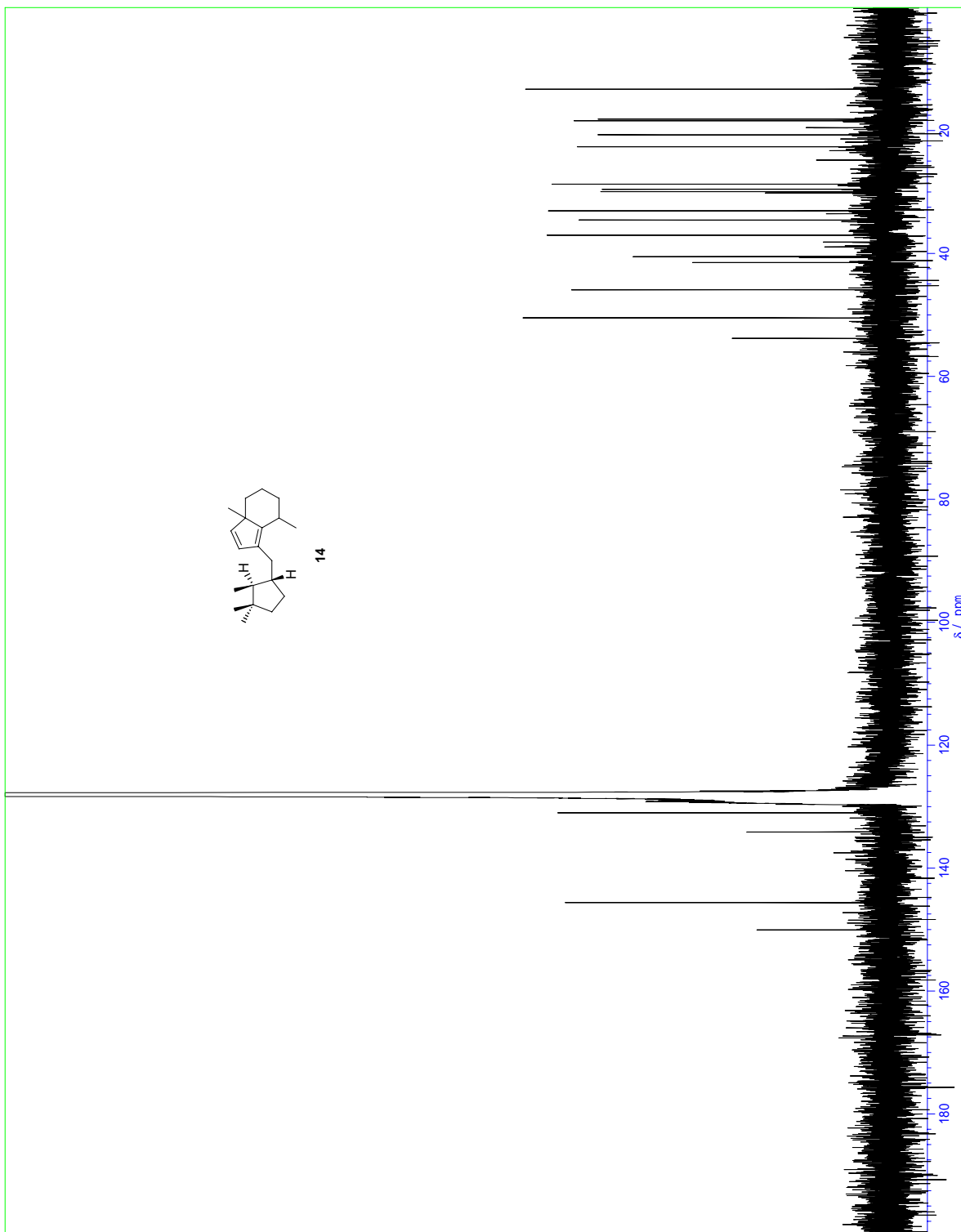
-S21-



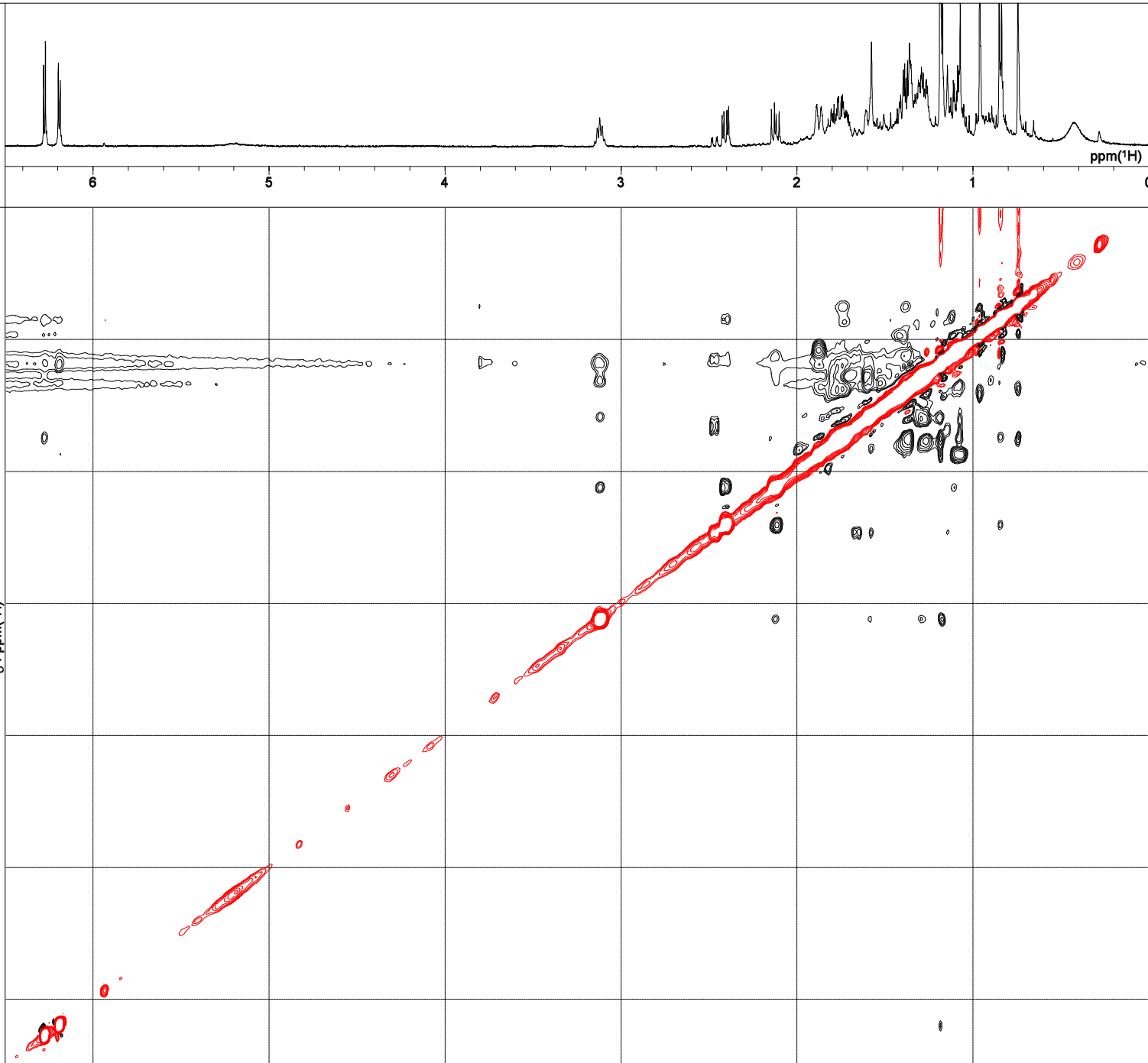








Exp 1.5



Comment
File piccpmt/506
noesy
Oikawa Ver2-7 TiCl4-95 mg /90u
L C6D6
dH(Solv)=7.15ppm, dC(solv)=128.
Oppm
Bruker AMX-500/ GC-MS & NMR Lab
Grad. Sch. Agric., Hokkaido Uni
v.
H=piccpmt/501 C=piccpmt/502

\$ F2-Acquisition Parameters
Date Mon Aug 30 18:28:05 20
04
PULPROG noesy.jp
TD 1024
NS 256
DS 4
D[0] 0.000003 sec
D[1] 1.750000 sec
D[9] 1.000000 sec
HL1 5 dB
IN[0] 0.000123 sec
P[1] 12.000000 usec
V9 2.000000 %
SW 8.127936 ppm
SWH 4065.040650 Hz
FIDRES 0.251904 Hz
AQ 0.125952 sec
RG 2048
NUCLEUS 1H

\$ F1-Acquisition Parameters
TD 128
NDO 2
SW 8.127936 ppm
SWH 4065.040650 Hz
FIDRES 0.031488 Hz

\$ F2-Processing Parameters
SI 512
SF 500.130062049003
OFFSET 7.884435
HZpPT 7.939531 Hz
MC2 qf
WDW QSINE
SSB 2.000000
PH_mod pk
PHC0 45.822670 degree
PHC1 55.400020 degree

\$ F1-Processing Parameters
SI 512
SF 500.130062049003
OFFSET 7.884435
REVERSE no
HZpPT 7.939531 Hz
MC2 TPPI
WDW QSINE
SSB 2.000000
PH_mod pk
PHC0 11.223440 degree
PHC1 -23.200000 degree
ME_mod LPfr
NCOEF 16
LPBIN 256
expt = ca. 27 hr

