

A Genetically Encoded Cyclobutene Probe for Labelling of Live Cells

Electronic Supporting Information, Section 2: NMR spectra

Model studies of inverse electron-demand Diels-Alder (IEDDA)

8-(2,5-Di(pyridin-2-yl)-3,4-diazabicyclo[4.2.0]octa-1,4-dien-7-yl)octanoic acid and

8-(2,5-di(pyridin-2-yl)-3,4-diazabicyclo[4.2.0]octa-2,5-dien-7-yl)octanoic acid (inseparable mixture of tautomers **2a** and **2b**)

¹H p 2

8-(2,5-Di(pyridin-2-yl)-3,4-diazabicyclo[4.2.0]octa-1,3,5-trien-7-yl)octanoic acid (**3**)

¹H 3

¹³C 4

Construction of CbK

((Pent-4-en-1-yloxy)methyl)benzene (**4**).

¹H 5

¹³C 6

cis-3-(3-(benzyloxy)propyl)-2,2-dichlorocyclobutan-1-ol (**5**)

¹H 7

¹³C 8

cis-3-(3-(Benzyloxy)propyl)-2,2-dichlorocyclobutyl methanesulfonate (**6**)

¹H 9

¹³C 10

3-(Cyclobut-2-en-1-yl)propanol (unnumbered; not isolated)

¹H 11

¹³C 12

3-(Cyclobut-2-en-1-yl)propanoic acid (**7**)

¹H 13

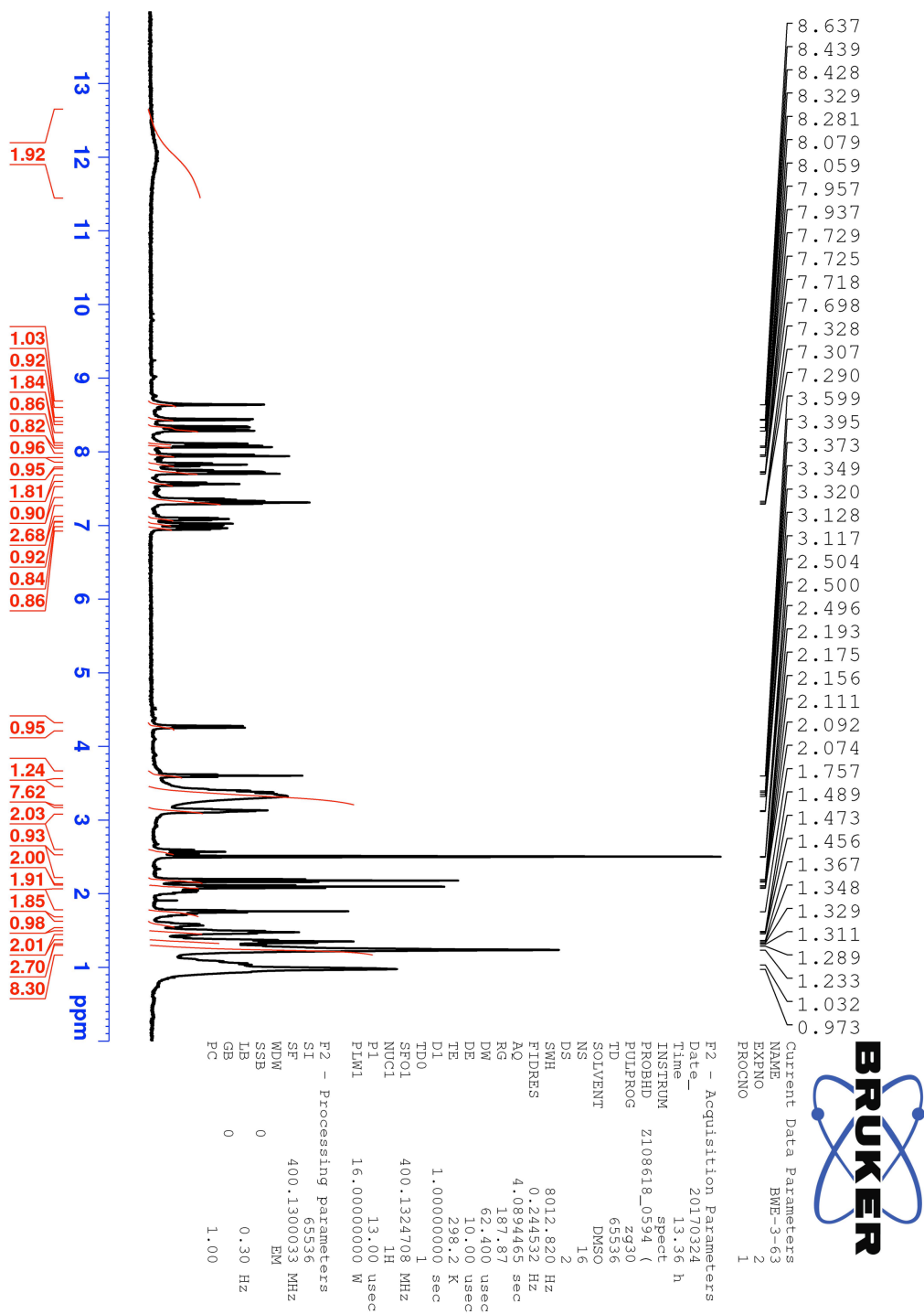
¹³C 14

N6-(3-(Cyclobut-2-en-1-yl)propionyl)-L-lysine (**CbK**).

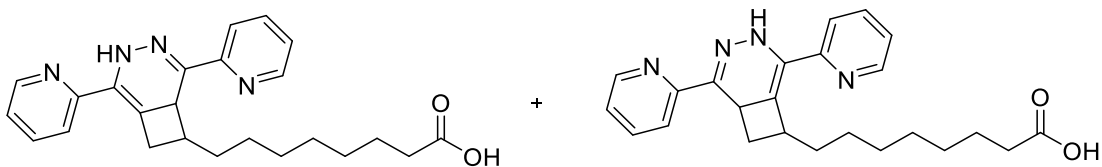
¹H 15

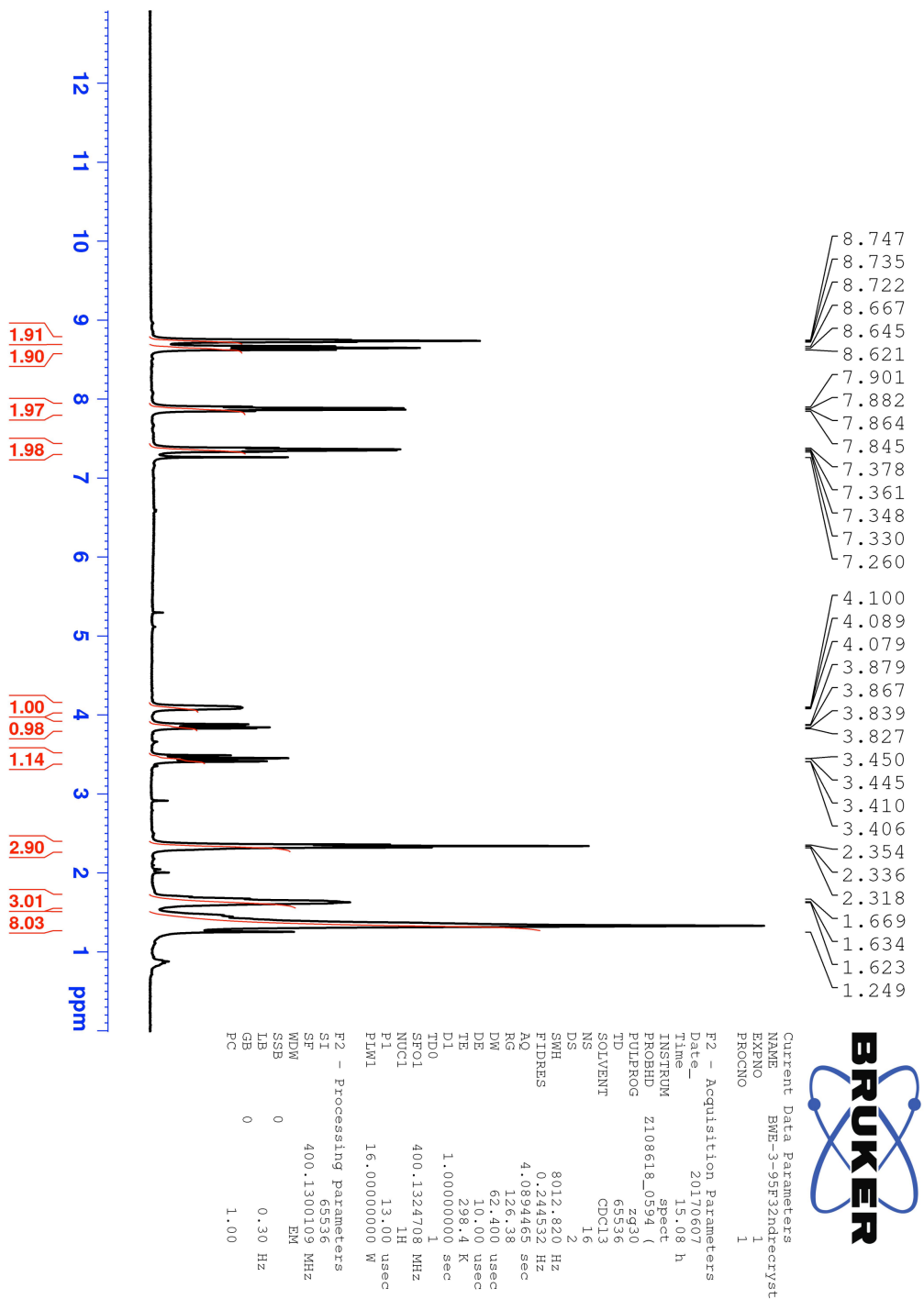
¹³C 16

Model studies of inverse electron-demand Diels-Alder (IEDDA)

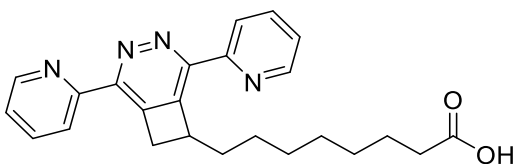


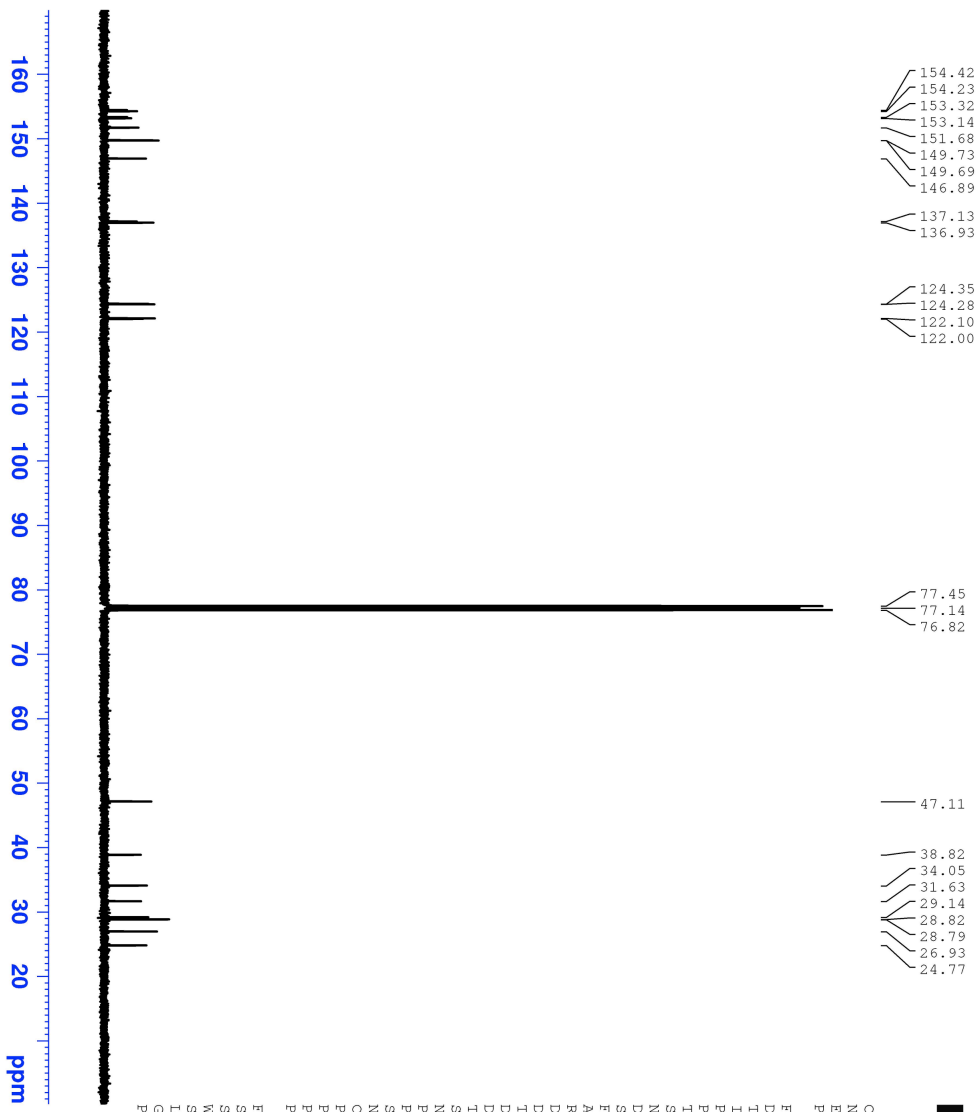
8-(2,5-Di(pyridin-2-yl)-3,4-diazabicyclo[4.2.0]octa-1,4-dien-7-yl)octanoic acid and -2,5-dien-7-yl tautomer. (2a, 2b)





8-(2,5-Di(pyridin-2-yl)-3,4-diazabicyclo[4.2.0]octa-1,3,5-trien-7-yl)octanoic acid (one-pot cycloaddition/aromatization). ¹H spectrum





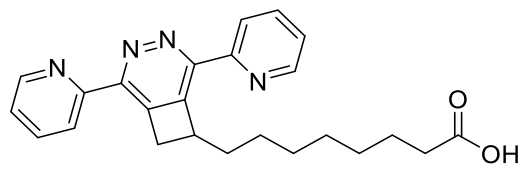
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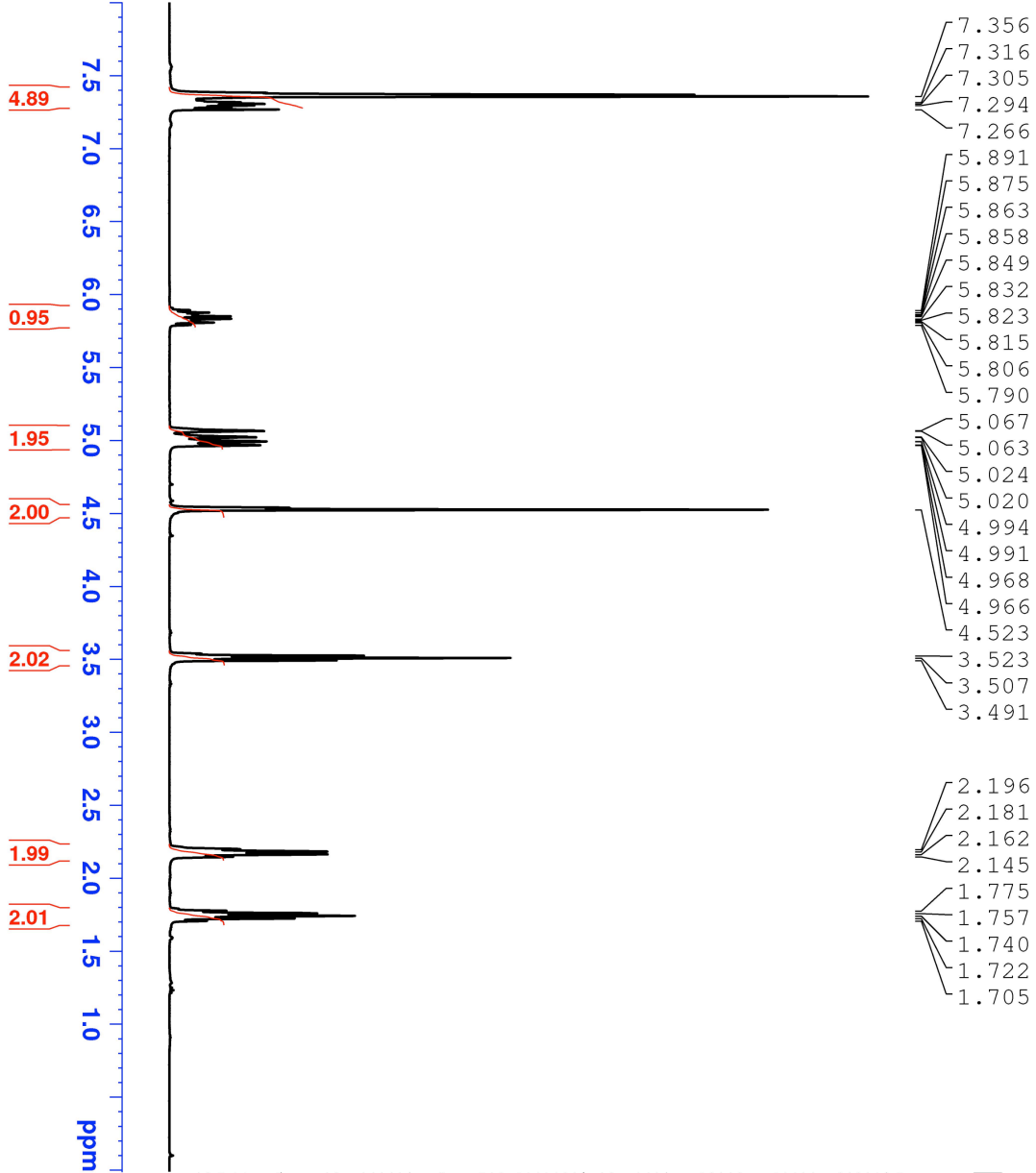
F2 - Acquisition Parameters
 Date_ 20170608
 Time 8.57 h
 INSTRUM spect
 PROBHD Z108618_0594 (zgrg30)
 PULPROG zgpg30
 TD 65536
 SOLVENT CDCl3
 NS 503
 DS 4
 SWH 24038.461 Hz
 FIDRES 0.733596 Hz
 AQ 1.3631488 sec
 RG 210.59
 DW 20.800 usec
 DE 10.00 usec
 TE 298.1 K
 D1 10.00000000 sec
 D11 0.03000000 sec
 TD0 1
 SF01 100.628233 MHz
 NUC1 13C
 P1 9.00 usec
 P1M1 58.00000000 W
 SF02 400.1316005 MHz
 NUC2 1H
 P2 16.00000000 W
 PLW2 0.2844001 W
 PLWI2 0.14307000 W
 PLWI3 0.14307000 W

F2 - Processing parameters
 SI 32768
 SF 100.6127578 MHz
 WDW EM
 SSB 0 Hz
 LB 0
 GB 0
 PC 1.40



8-(2,5-Di(pyridin-2-yl)-3,4-diazabicyclo[4.2.0]octa-1,3,5-trien-7-yl)octanoic acid (one-pot cycloaddition/aromatization). ¹H spectrum





Current Data Parameters
 NAME BDE-7-66-pdt
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20160218
 Time 9.23

INSTRUM spect
 PROBHD 5 mm PABBO BB/
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 8
 DS 2

SWH 8012.820 Hz
 FIDRES 0.122266 Hz
 AQ 4.0894465 sec
 RG 62.76
 DW 62.400 usec
 DE 10.00 usec
 TE 298.1 K
 D1 1.00000000 sec
 TDO 1

==== CHANNEL f1 =====
 SF01 400.1324710 MHz
 NUCL1 1H
 P1 12.00 usec
 PLW1 16.00000000 W

F2 - Processing parameters
 SI 65536
 SF 400.1300072 MHz
 WDM EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

((Pent-4-en-1-yloxy)methyl)benzene (1). ¹H spectrum.



Current Data Parameters
NAME BDE-7-66-Carbon
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160218
Time 9.33

INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 107
DS 4

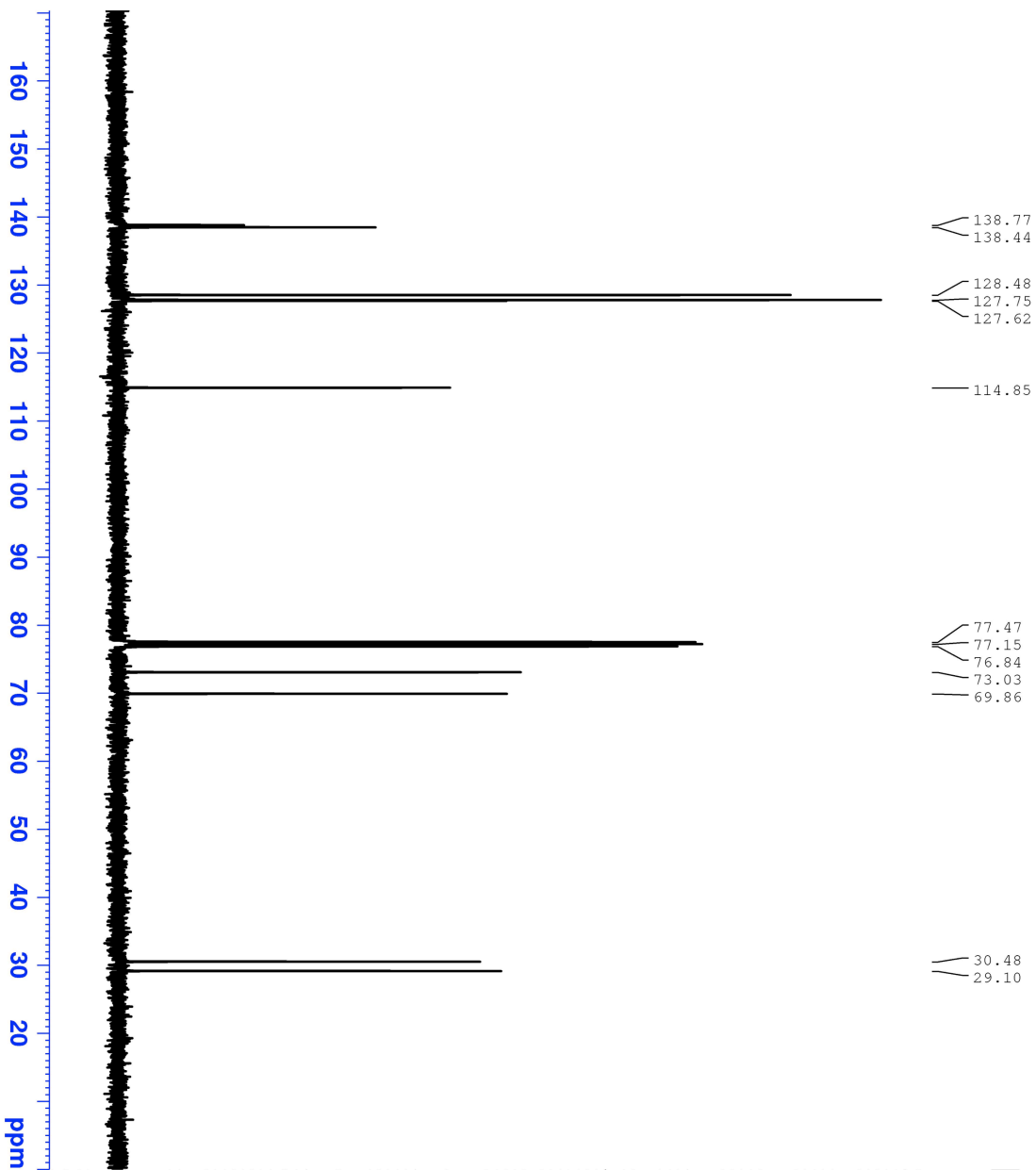
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FIDRES 0.366798 Hz
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RG 210.59
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DE 10.00 usec
TE 298.2 K
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D11 0.03000000 sec
TD0 1

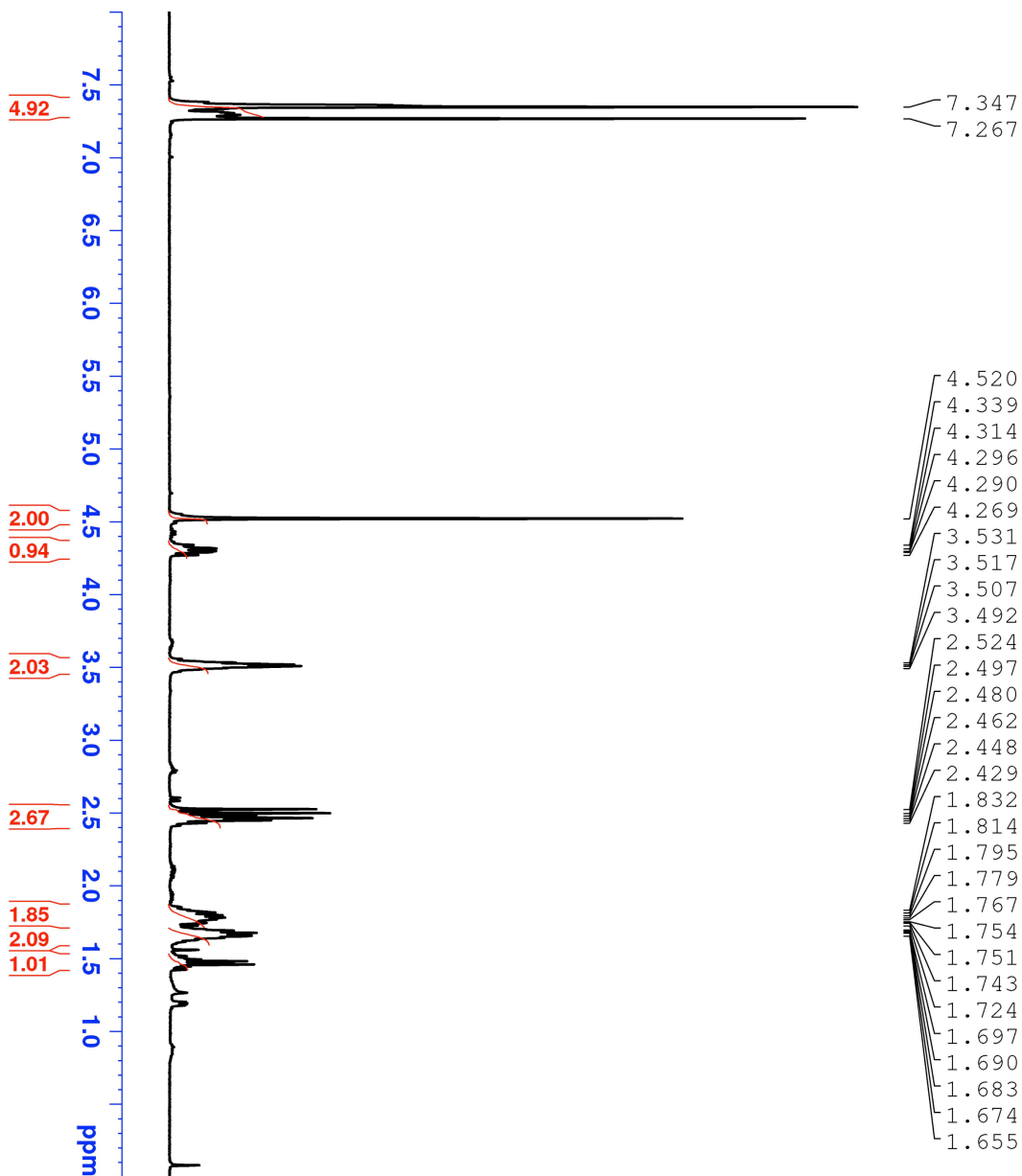
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SFO1 100.6228293 MHz
NUC1 13C
P1 9.00 usec
PLW1 58.00000000 W

==== CHANNEL f2 =====
SFO2 400.1316005 MHz
NUC2 1H
PCPD2 waitz16
PLW2 90.00 usec
PLM2 16.00000000 W
PLM12 0.28444001 W
PLM13 0.23040000 W

F2 - Processing parameters
SI 32768
SF 100.6127578 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



((Pent-4-en-1-yloxy)methyl)benzene (1). ¹³C spectrum.



7.347
7.267

4.520
4.339
4.314
4.296
4.290
4.269
3.531
3.517
3.507
3.492
2.524
2.497
2.480
2.462
2.448
2.429
1.832
1.814
1.795
1.779
1.767
1.754
1.751
1.743
1.724
1.697
1.690
1.683
1.674
1.655



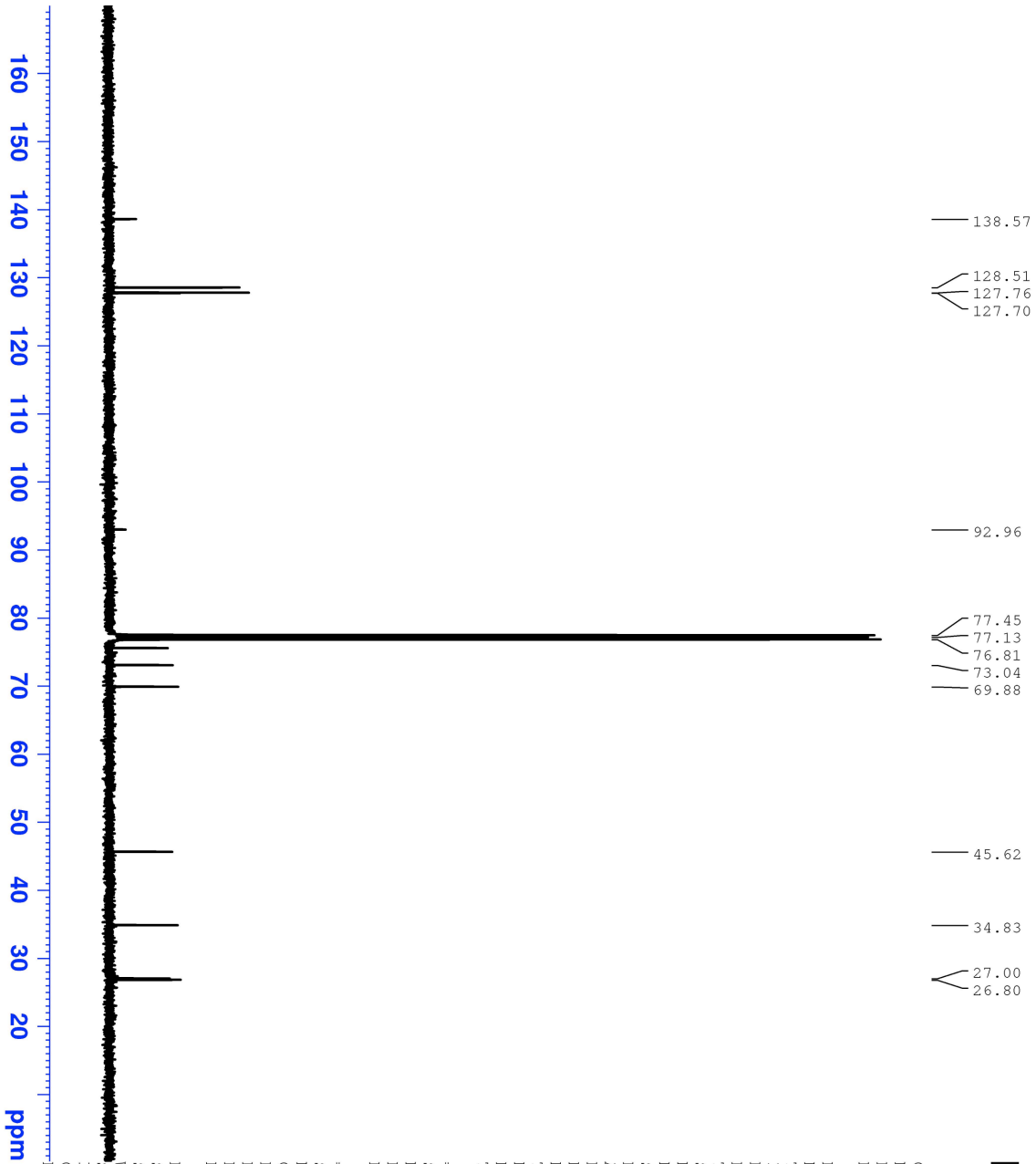
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EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
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Time 14.51
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zg30
ID 65536
SOLVENT CDCl3
NS 10
DS 2
SWH 8012.820 Hz
FIDRES 0.122266 Hz
AQ 4.0894465 sec
RG 210.59
DW 62.400 usec
DE 10.00 usec
TE 298.2 K
D1 1.00000000 sec
TDO 1

==== CHANNEL f1 =====
SFO1 400.1324710 MHz
NUC1 1H
PI 12.00 usec
PLW1 16.00000000 W

F2 - Processing parameters
SI 65536
SF 400.1300070 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

cis-3-(3-(Benzyloxy)propyl)-2,2-dichlorocyclobutan-1-ol (2). ¹H spectrum.



Current Data Parameters
 NAME BDE-7-77-Carbon
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20160222
 Time 15.13

INSTRUM spect
 PROBHD 5 mm PABBO BB/
 PULPROG zgpg30
 TD 65536
 SOLVENT CDCl3
 NS 296
 DS 4

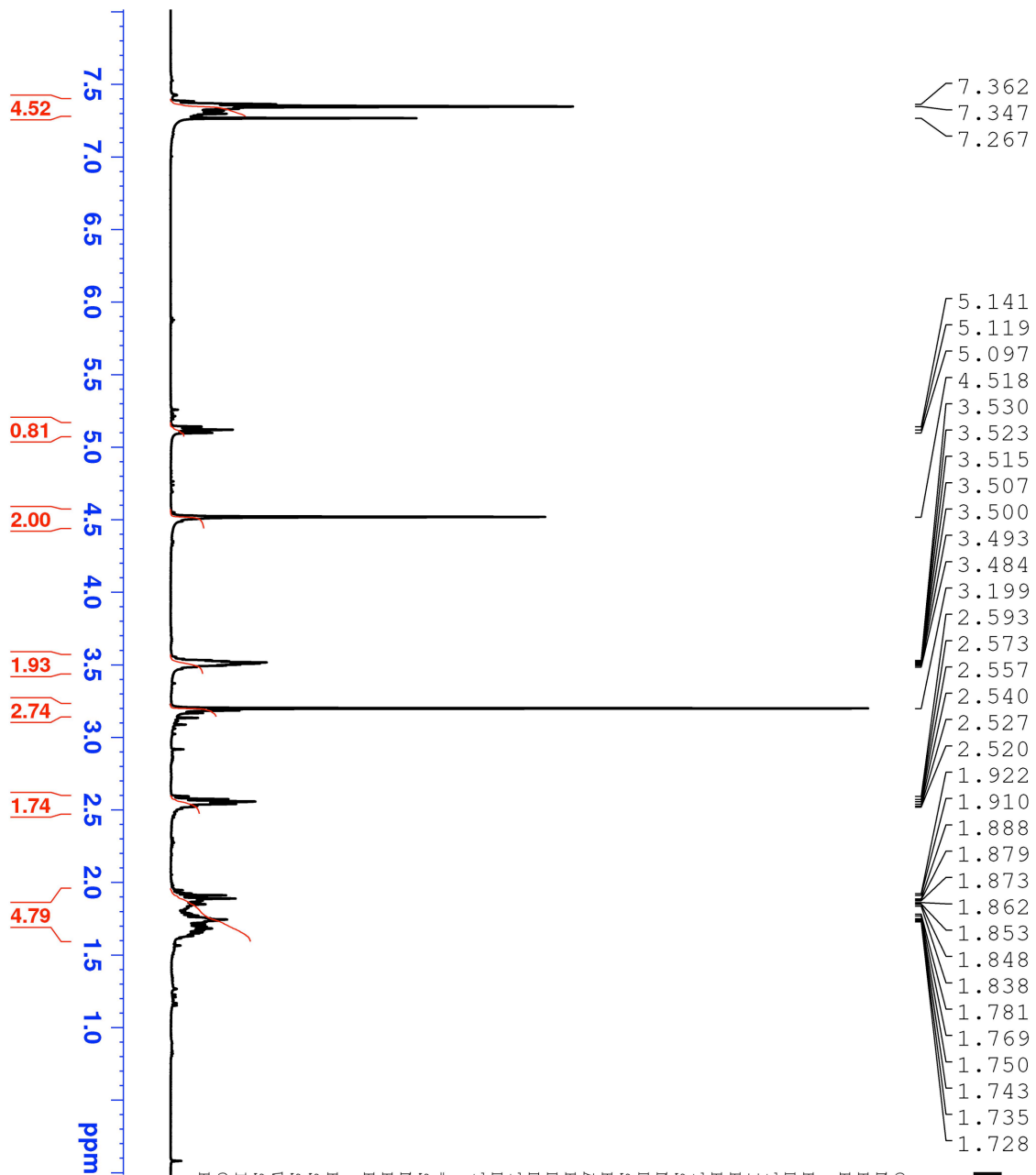
SWH 24038.461 Hz
 FIDRES 0.366798 Hz
 AQ 1.3631488 sec
 RG 210.59
 DW 20.800 usec
 DE 10.00 usec
 TE 298.2 K
 D1 2.00000000 sec
 D11 0.03000000 sec
 TD0 1

==== CHANNEL f1 =====
 SFO1 100.6228293 MHz
 NUC1 13C
 P1 9.00 usec
 PLW1 58.00000000 W

==== CHANNEL f2 =====
 SFO2 400.1316005 MHz
 NUC2 1H
 CPDPRG12 waltz16
 PCPD2 90.00 usec
 PLM2 16.00000000 W
 PLM12 0.28444001 W
 PLM13 0.23040000 W

F2 - Processing parameters
 SI 32768
 SF 100.6127578 MHz
 WDW EM
 SSB 0
 GB 0
 PC 1.40

cis-3-(3-(benzyloxy)propyl)-2,2-dichlorocyclobutan-1-ol (2). ¹³C spectrum.



Current Data Parameters
 NAME BDE-7-78
 EXPNO 1
 PROCNO 1

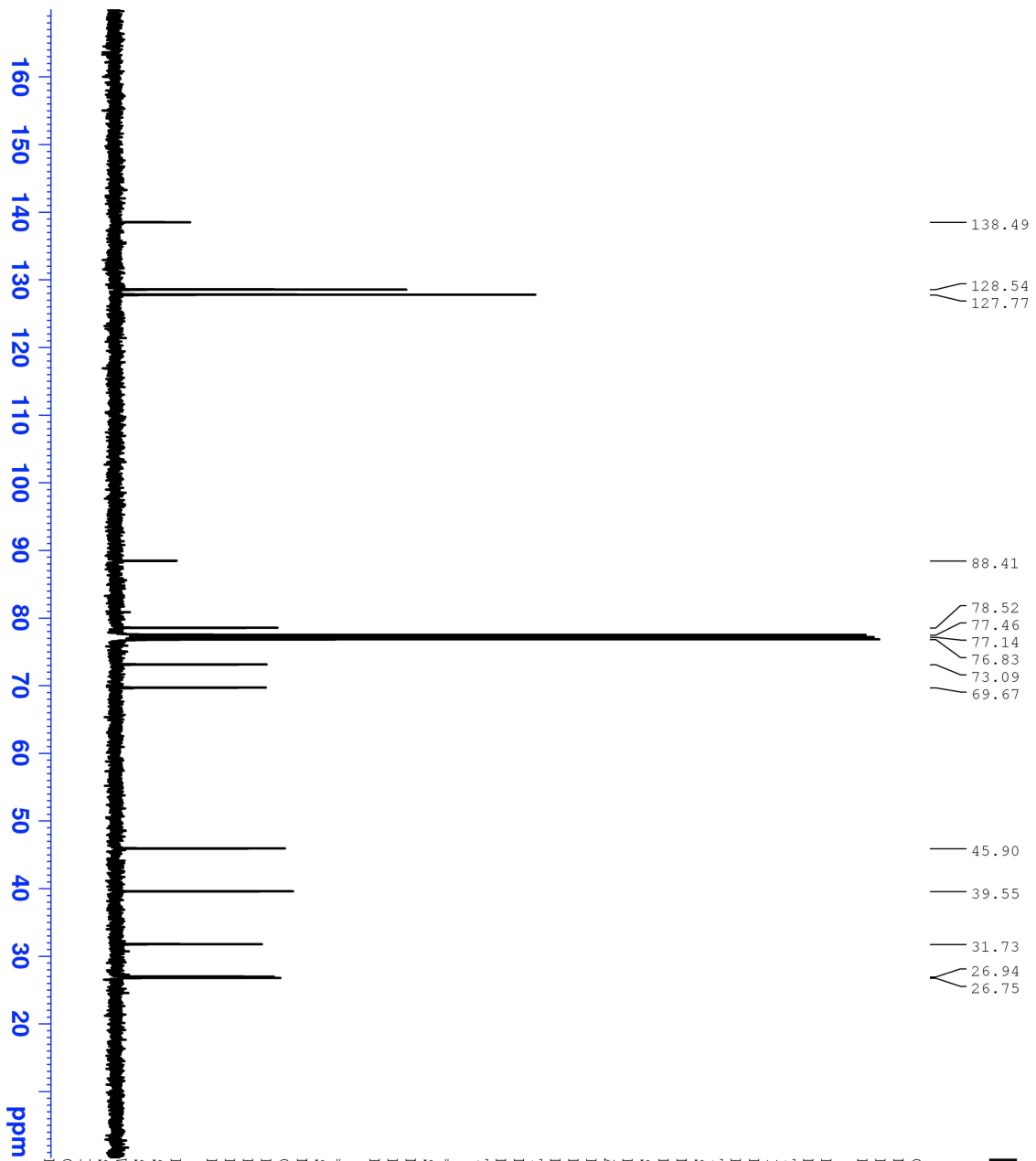
F2 - Acquisition Parameters

Date_ 20160229
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 INSTRUM spect
 PROBHD 5 mm PABBO BB/
 PULPROG zg30
 TD 65536
 SOLVENT CDCl3
 NS 16
 DS 2
 SWH 8012.820 Hz
 FIDRES 0.122266 Hz
 AQ 4.0894465 sec
 RG 103.57
 DW 62.400 usec
 DE 10.00 usec
 TE 298.1 K
 D1 1.00000000 sec
 TDO 1

===== CHANNEL f1 =====
 SFO1 400.1324710 MHz
 NUC1 1H
 P1 12.00 usec
 P1M1 16.00000000 W

F2 - Processing parameters
 SI 65536
 SF 400.1300072 MHz
 WDW EM
 SSB 0
 LB 0.30 Hz
 GB 0
 PC 1.00

cis-3-(3-(Benzyloxy)propyl)-2,2-dichlorocyclobutyl methanesulfonate (3). ¹H spectrum.



Current Data Parameters
 NAME BDE-7-78-Carbon
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters

Date_ 20160229
 Time 10.31
 INSTRUM spect
 PROBHD 5 mm PABBO BB/
 PULPROG zgpg30
 TD 65536
 SOLVENT CDCl3
 NS 205
 DS 4
 SWH 24038.461 Hz
 FIDRES 0.366798 Hz
 AQ 1.3631488 sec
 RG 210.59
 DW 20.800 usec
 DE 10.00 usec
 TE 298.2 K
 D1 2.00000000 sec
 D11 0.03000000 sec
 TD0 1

==== CHANNEL f1 =====

SFO1 100.6228293 MHz
 NUC1 13C
 P1 9.00 usec
 PLW1 58.00000000 W

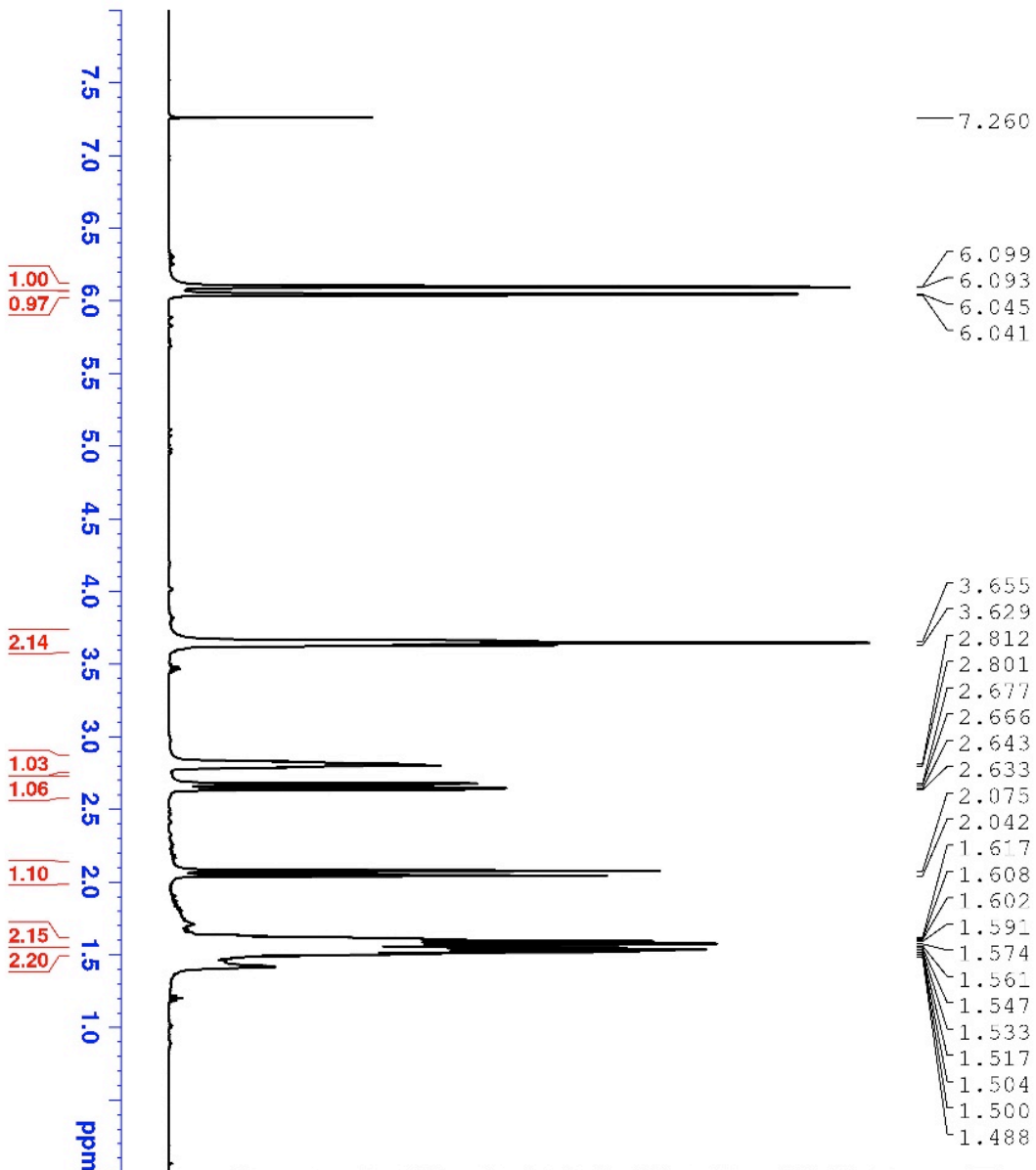
==== CHANNEL f2 =====

SFO2 400.1316005 MHz
 NUC2 1H
 CPDPRG12 waltz16
 PCPD2 90.00 usec
 PLM2 16.00000000 W
 PLW2 0.28444001 W
 PLW13 0.23040000 W

F2 - Processing parameters

SI 32768
 SF 100.6127578 MHz
 WDW EM
 SSB 0
 GB 0
 PC 1.40

cis-3-(3-(Benzyloxy)propyl)-2,2-dichlorocyclobutyl methanesulfonate (3). ¹³C spectrum.



Current Data Parameters
 NAME: BMR 4 59D
 EXPNO: 7
 ZPROGNO: 1

F2 - Acquisition Parameters
 Date_ 20170819
 Time 21:15:16
 INSTRUM spect
 PROBU 2108618_0394 (1
 PULPROG zgpg30
 TD 65536
 SOLVENT CCO13
 NS 16
 DS 2
 SWH 8012.920 Hz
 FIDRES 0.244832 Hz
 AQ 4.0894465 sec
 RG 82.47
 DK 62.400 usec
 DE 10.00 usec
 JE 298.0 K
 DL 1.000000000 sec
 DQ 1
 SECT 400.1324798 MHz
 NUC1 1H
 P1 13.00 usec
 P1M 16.000000000 W
 P1M1

F2 Processing parameters
 S 65536
 SF 400.1300092 MHz
 KDW 64
 DR 0
 CR 0
 PC 1.00



3-(Cyclobut-2-en-1-yl)propanol. ¹H spectrum of partially purified reaction aliquot.



Current Data Parameters
 NAME BME-4-59D1
 EXPNO 10
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20170819
 Time 21.33 h
 INSTRUM spect
 PROBHD z108618_0594 /
 PULPROG zgpg30
 TD 65536
 SOLVENT CDCl3
 NS 288
 DS 4

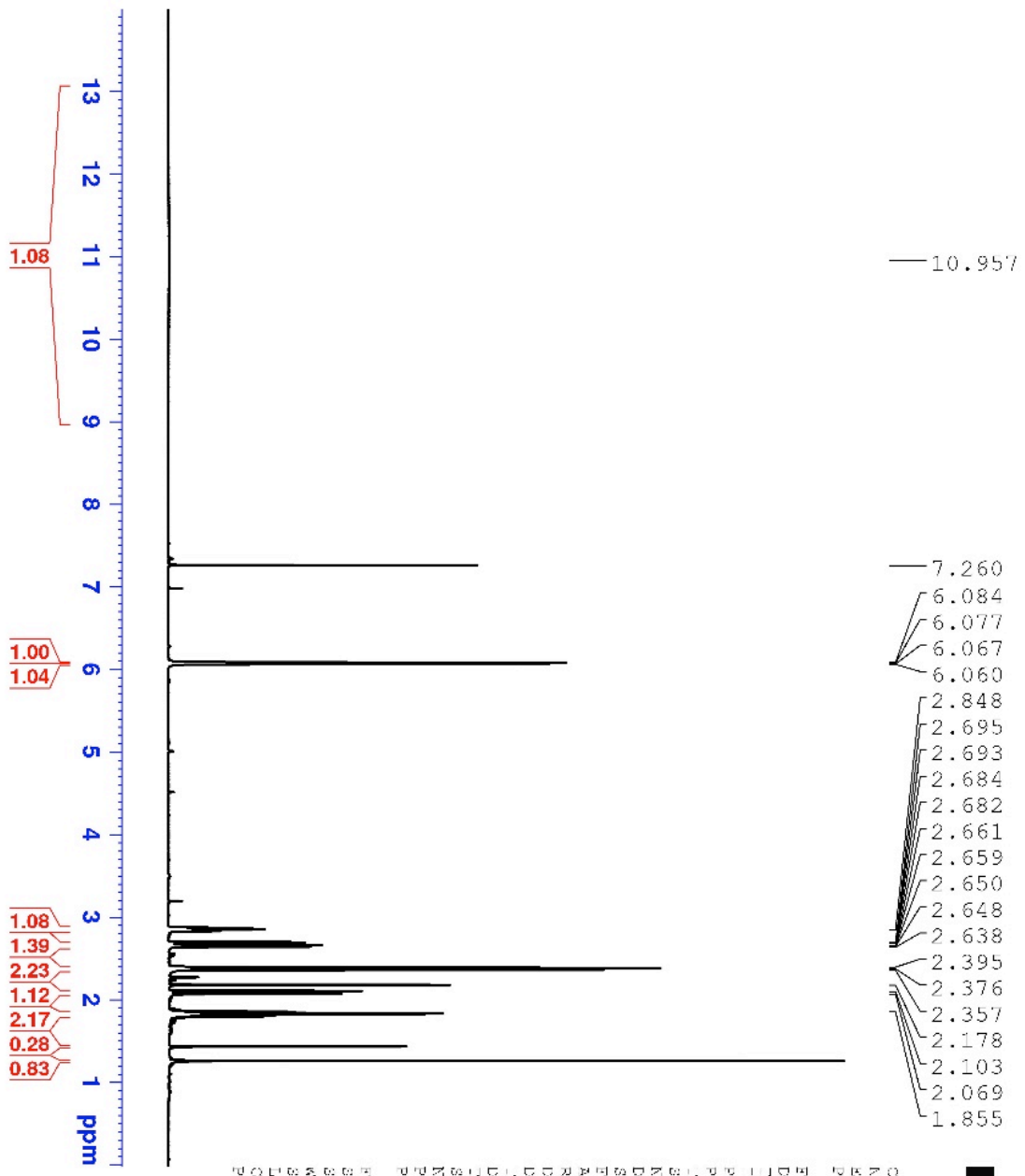
SWH 24038.461 Hz
 FIDRES 0.733596 Hz
 AQ 1.3631488 sec
 RG 210.59
 DW 20.800 usec
 DE 10.00 usec
 TE 298.0 K

D1 2.00000000 sec
 D11 0.03000000 sec
 TDO 1
 SFO1 100.62828293 MHz
 NUC1 13C
 P1 9.00 usec
 PL1 58.00000000 W
 SFO2 400.1316005 MHz

NUC2 1H
 CPDPRG12 waltz16
 PCPD2 90.00 usec
 PLW2 16.00000000 W
 PLM12 0.28444001 W
 PLM13 0.14307000 W

F2 - Processing parameters
 SI 32768
 SF 100.6127578 MHz
 WDM EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

3-(Cyclobut-2-en-1-yl)propanol; ¹³C spectrum of partially purified reaction aliquot.



Current Data Parameters
 NAME BMR 4 G2
 EXPNO 6
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20170820
 Time 1:29 h

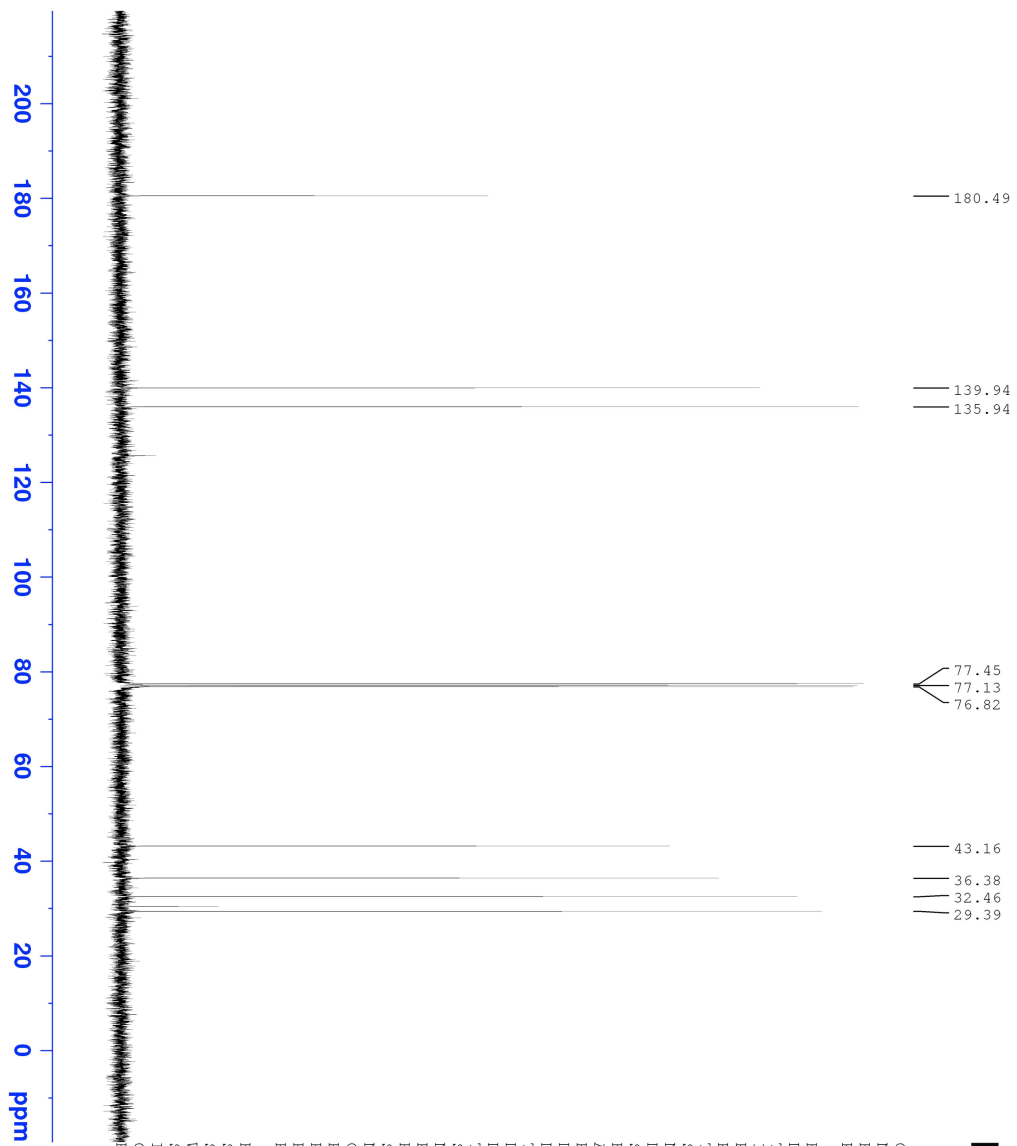
INSTRUM spect
 PROBHD 5mm 1H
 PULPROG zgpg30
 ID 65536
 SOLVENT CCl4
 NS 16
 DS 2

SM 8012.820 Hz
 FIDRES 0.244532 Hz
 AQ 4.0894455 sec
 RG 187.87
 DK 62.400 usec
 DE 10.00 usec
 DR 298.2 K
 DL 1.00000000 sec

TD0 1
 SF01 400.1324708 MHz
 NUCL 1H
 P1 13.00 usec
 PL1 16.00000000 W

F2 Processing parameters
 SI 65536
 SR 400.1300000 MHz
 MDW EM
 SSB 0
 LB 0.30 Hz
 CB 0
 PC 1.00

3-(Cyclobut-2-en-1-yl)propanoic acid (4). ¹H spectrum



Current Data Parameters
 NAME BME-4-62
 EXPNO 3
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20170821
 Time 15.10 h
 INSTRUM spect
 PROBHD z108618_0594 (zggp30
 PULPROG zgpg30
 TD 65536
 SOLVENT CDCl3
 NS 43
 DS 4
 SWH 24038.461 Hz
 FIDRES 0.733596 Hz
 AQ 1.3631488 sec
 RG 210.59
 DM 20.800 usec
 DE 10.00 usec
 TE 298.0 K
 D1 2.00000000 sec
 D11 0.03000000 sec
 TDO 1
 SFO1 100.62828293 MHz
 NUC1 13C
 P1 9.00 usec
 PL1 58.00000000 W
 SFO2 400.1316005 MHz
 NUC2 1H
 CPDPRG[2] waltz16
 PCPD2 90.00 usec
 PLM2 15.00000000 W
 PLM12 0.28444001 W
 PLM13 0.14307000 W

F2 - Processing parameters
 SI 32768
 SF 100.6127578 MHz
 WDM EM
 SSB 0
 IB 1.00 Hz
 GB 0
 PC 1.40



3-(Cyclobut-2-en-1-yl)propanoic acid (4). ¹³C spectrum.



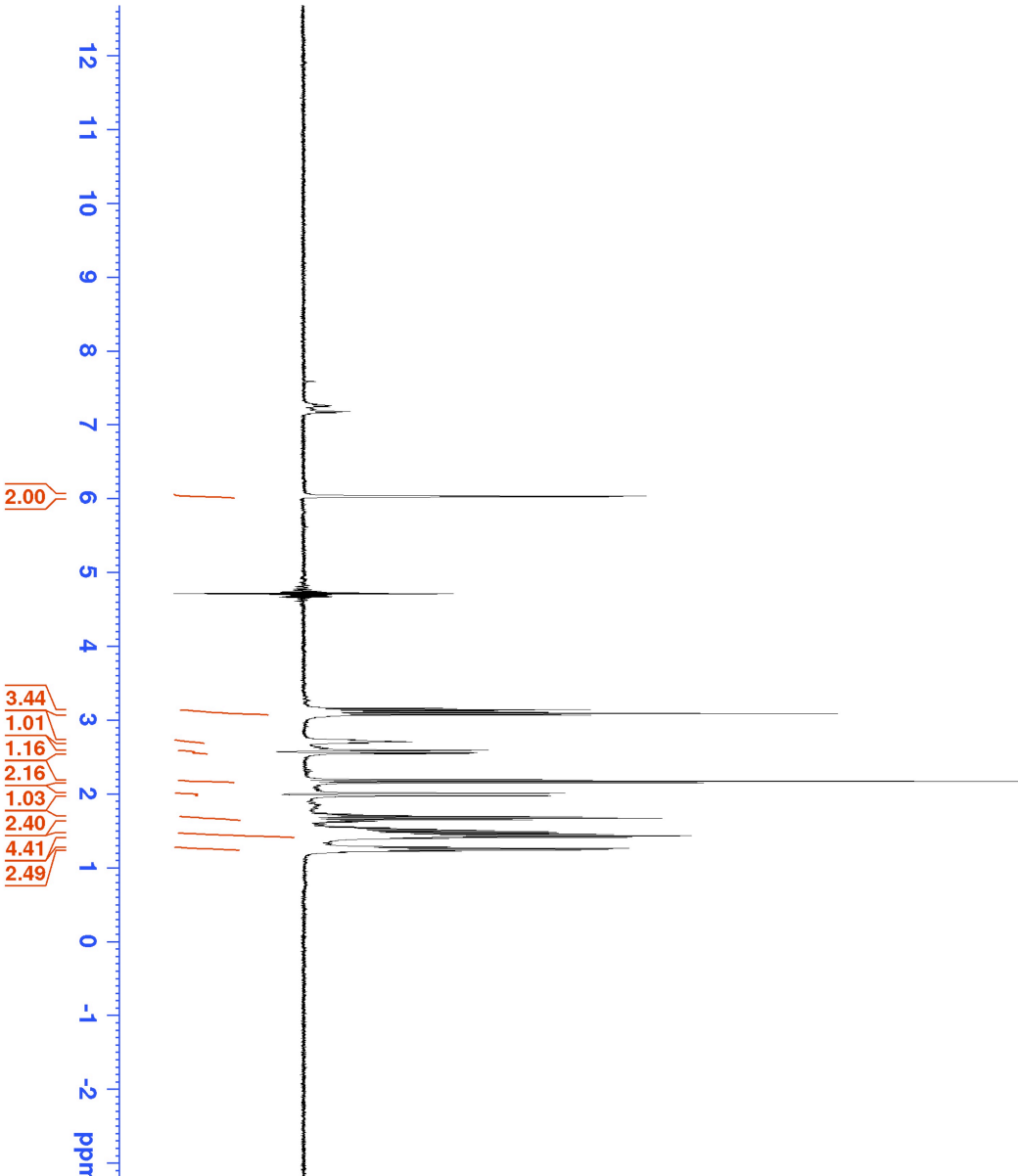
Current Data Parameters
 NAME Klin-CbK-H-D2O-NaOH
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20161123
 Time 15.46
 INSTRUM spect
 PROBHD 5 mm PARBO BB/
 PULPROG zgpg30
 ID 32768
 SOLVENT D2O
 NS 4
 DS 4
 SWH 6393.862 Hz
 FIDRES 0.195125 Hz
 AQ 2.5624576 sec
 RG 210.59
 DW 78.200 usec
 DE 10.00 usec
 TE 298.1 K
 D1 1.00000000 sec
 D12 0.00020000 sec
 D16 0.00020000 sec
 TDO 1

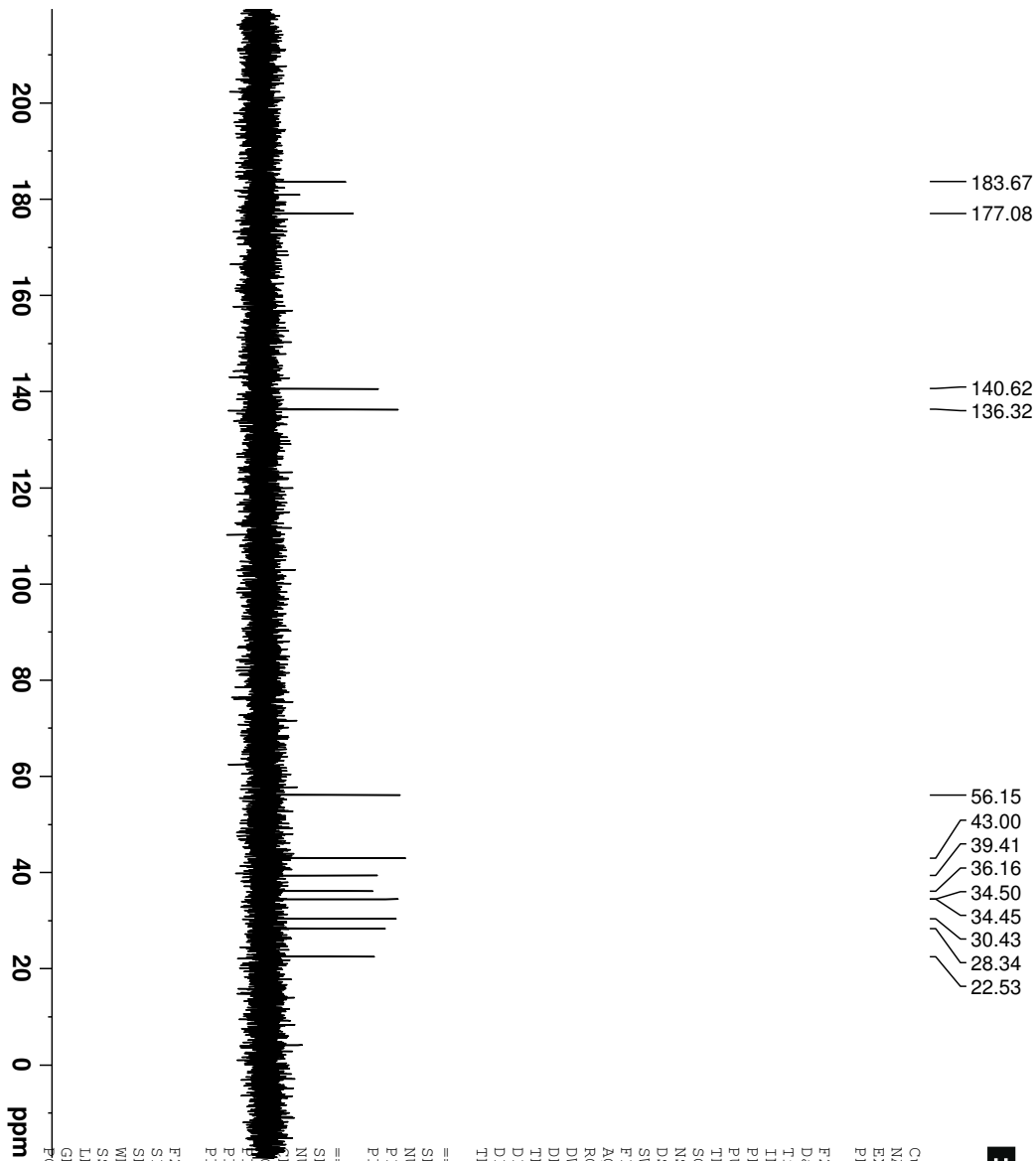
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 SFO1 400.1318776 MHz
 NUC1 1H
 P1 13.00 usec
 P2 26.00 usec
 P12 2000.00 usec
 PLW0 0 W
 PLW1 16.00000000 W
 SPVAM[1] Squa100.1000
 SPOAL1 0.500
 SPOFSL 0 Hz
 SEW1 0.00270400 W

==== GRADIENT CHANNEL =====
 GENVAM[1] SMSQ10.100
 GENVAM[2] SMSQ10.100
 GPZ1 31.00 %
 GPZ2 11.00 %
 Pl6 1000.00 usec

F2 - Processing parameters
 SI 32768
 SE 400.1300000 MHz
 WDW EM
 SSB 0
 GB 0
 PC 1.00



***N*₆-(3-(Cyclobut-2-en-1-yl)propionyl)-L-lysine (CbK). ¹H spectrum**



Current Data Parameters
 NAME K11u-CbK-C--D2O--NaOH
 EXPNO 1
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20161123
 Time 16.24

INSTRUM spect
 PROBHD 5 mm PABBO BB/
 PULPROG zgpg30
 TD 65536
 SOLVENT D2O
 NS 589
 DS 4

SWH 24038.461 Hz
 FIDRES 0.366798 Hz
 AQ 1.3631488 sec
 RG 210.59
 DW 20.800 usec
 DE 10.00 usec
 TE 298.2 K
 D1 2.00000000 sec
 D11 0.03000000 sec
 TD0 1

==== CHANNEL f1 =====
 SFO1 100.6228293 MHz
 NUC1 13C
 P1 9.00 usec
 PLW1 58.00000000 W

==== CHANNEL f2 =====
 SFO2 400.1316005 MHz
 NUC2 1H
 P2 16.00000000 W
 PLW2 0.33383000 W
 PLW12 0.27039999 W
 PLW13 0.27039999 W

F2 - Processing parameters
 SI 32768
 SF 100.6127578 MHz
 WDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40

N_6 -(3-(Cyclobut-2-en-1-yl)propionyl)-L-lysine (CbK). ^{13}C spectrum