

## Nucleotidylation of Unsaturated Carbasugar in Validamycin Biosynthesis

Jongtae Yang,<sup>1</sup> Hui Xu,<sup>1,2</sup> Yirong Zhang,<sup>2</sup> Linquan Bai,<sup>2</sup> Zixin Deng,<sup>2</sup> Taifo Mahmud<sup>1,\*</sup>

<sup>1</sup>Department of Pharmaceutical Sciences, Oregon State University, Corvallis, OR 97331; <sup>2</sup>Laboratory of Microbial Metabolism and School of Life Sciences & Biotechnology, Shanghai Jiaotong University, Shanghai 200030, China

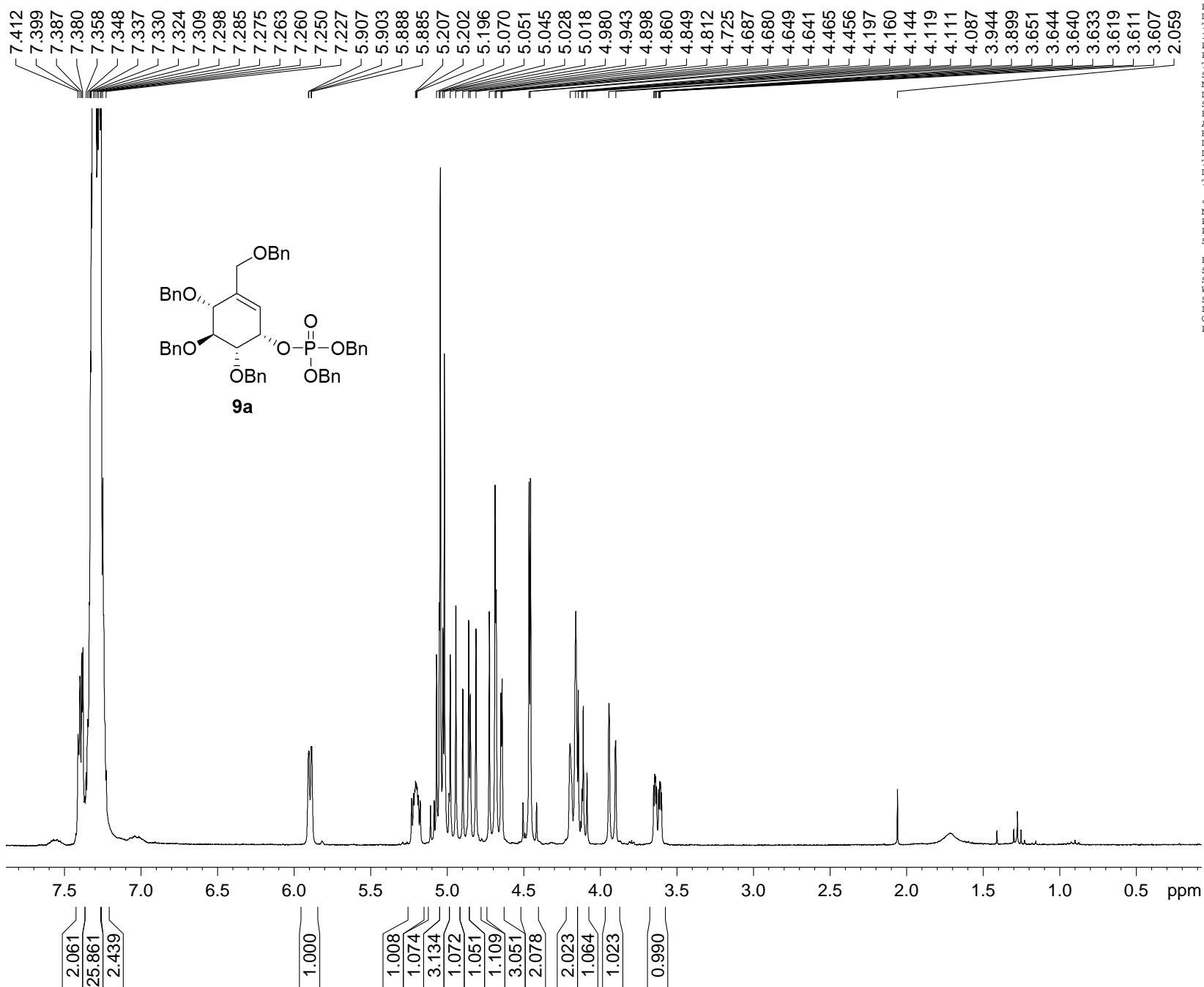
**Table S1. Strains and plasmids used in this study**

| Strain/plasmid                 | Relevant genotype/comments  | Source/ref. |
|--------------------------------|---|-------------|
| <b><i>Escherichia coli</i></b> |   |             |
| DH10B                          | <i>F</i> <i>mcrA</i> $\Delta$ ( <i>mrr-hsdRMS-mcrBC</i> ) $\phi$ 80 <i>lacZ</i><br><i><math>\Delta</math>M15 <math>\Delta</math>lacX74 recA1 endA1 araD139 <math>\Delta</math>(ara, leu)7697 galU galK <math>\lambda</math> rspL nupG</i> | GibcoBRL    |
| ET12567(pUZ8002)               | <i>dam dcm hsdS</i> , pUZ8002   | 1           |
| BW25113                        | K12 derivative: <i><math>\Delta</math>araBAD, <math>\Delta</math>rhaBAD</i>   | 2           |
| <b><i>S. hygroscopicus</i></b> |   |             |
| 5008                           | wild-type producer of validamycin   | 3           |
| ZYR-2                          | 5008 derivative generated by replacement of a 936-bp DNA fragment internal to <i>valB</i> with the 1.40-kb <i>oriT-aac(3)IV</i> cassette  | This work   |
| ZYR-2/pJTU918                  | ZYR-2 complemented with pJTU918 harboring full-length <i>valB</i>   | This work   |
| <b>Plasmids</b>                |   |             |
| pHZ1358                        | <i>tsr, bla, oriT, ori</i> (pIJ101)   | 4           |

|                |   |            |
|----------------|---|------------|
| pHZ2229        | pBluescript II SK(+) with the 6.0 kb BamHI fragment from cosmid 20E1  | 3          |
| pIJ790         | $\lambda$ -RED ( <i>gam</i> , <i>bet</i> , <i>exo</i> ), <i>cat</i> , <i>araC</i> , <i>rep101</i> <sup>ts</sup> | 2          |
| pIJ773         | <i>aac(3)IV</i> , <i>oriT</i>   | 2          |
| pRSET-B        | P <sub>T7</sub> RBS 6×His Xpress <sup>TM</sup> Epitope EK, <i>bla</i>   | Invitrogen |
| pMD18 T-vector | pUC18 derivative  | TaKaRa     |
| pPM927         | <i>tsr</i> , <i>oriT</i> , <i>int</i> , <i>attP</i>   | 5          |
| pJTU968        | pRSET-B derivative <i>bla</i> , <i>PermE</i> *  | 6          |
| pJTU700        | 5.80-kb BamHI fragment from pHZ2229 cloned in BamHI-digested pHZ1358  | This work  |
| pJTU702        | pJTU700 recombinant with 1384-bp <i>oriT</i> - <i>aac(3)IV</i> cassette through Redirect Technology             | This work  |
| pJTU707        | 1.10-kb <i>valB</i> fragment cloned in pRSET-B  | This work  |
| pJTU915        | 1.10-kb NdeI/EcoRI fragment from pJTU707 cloned in pJTU968  | This work  |
| pJTU918        | pPM927 carrying <i>PermE</i> * and <i>valB</i>  | This work  |

---

**Figures S1 – S24: NMR Spectra**



Current Data Parameters  
NAME JTY-1-37-04  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20070507  
Time 12.30  
INSTRUM DRX300  
PROBHD 5 mm BBO BB/B  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 9  
DS 4  
SWH 4496.403 Hz  
FIDRES 0.068610 Hz  
AQ 7.2876530 sec  
RG 287.4  
DW 111.200 usec  
DE 158.64 usec  
TE 296.4 K  
D1 1.6000002 sec  
TDO 1

==== CHANNEL f1 =====  
NUC1 1H  
P1 11.00 usec  
PL1 0.00 dB  
SFO1 300.1319508 MHz

F2 - Processing parameters  
SI 32768  
SF 300.1300063 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

Figure S1. <sup>1</sup>H NMR spectrum of compound **9a**

Current Data Parameters  
NAME JTY-1-37-04c  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20070507  
Time 13.20  
INSTRUM DRX300  
PROBHD 5 mm BBO BB/BB  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 1360  
DS 8  
SWH 22727.273 Hz  
FIDRES 0.346791 Hz  
AQ 1.4418420 sec  
RG 3251  
DW 22.000 use  
DE 6.00 use  
TE 296.7 K  
D1 0.3000001 sec  
d11 0.03000000 sec  
DELTA 0.2000002 sec  
TDO 1

===== CHANNEL f1 =====  
NUC1 13C  
P1 8.95 use  
PL1 0.00 dB  
SFO1 75.4783145 MHz

===== CHANNEL f2 =====  
CPDPRG2 waltz16  
NUC2 1H  
PCPD2 85.00 use  
PL2 0.00 dB  
PL12 19.00 dB  
PL13 19.00 dB  
SFO2 300.1312005 MHz

F2 - Processing parameters  
SI 32768  
SF 75.4677404 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40

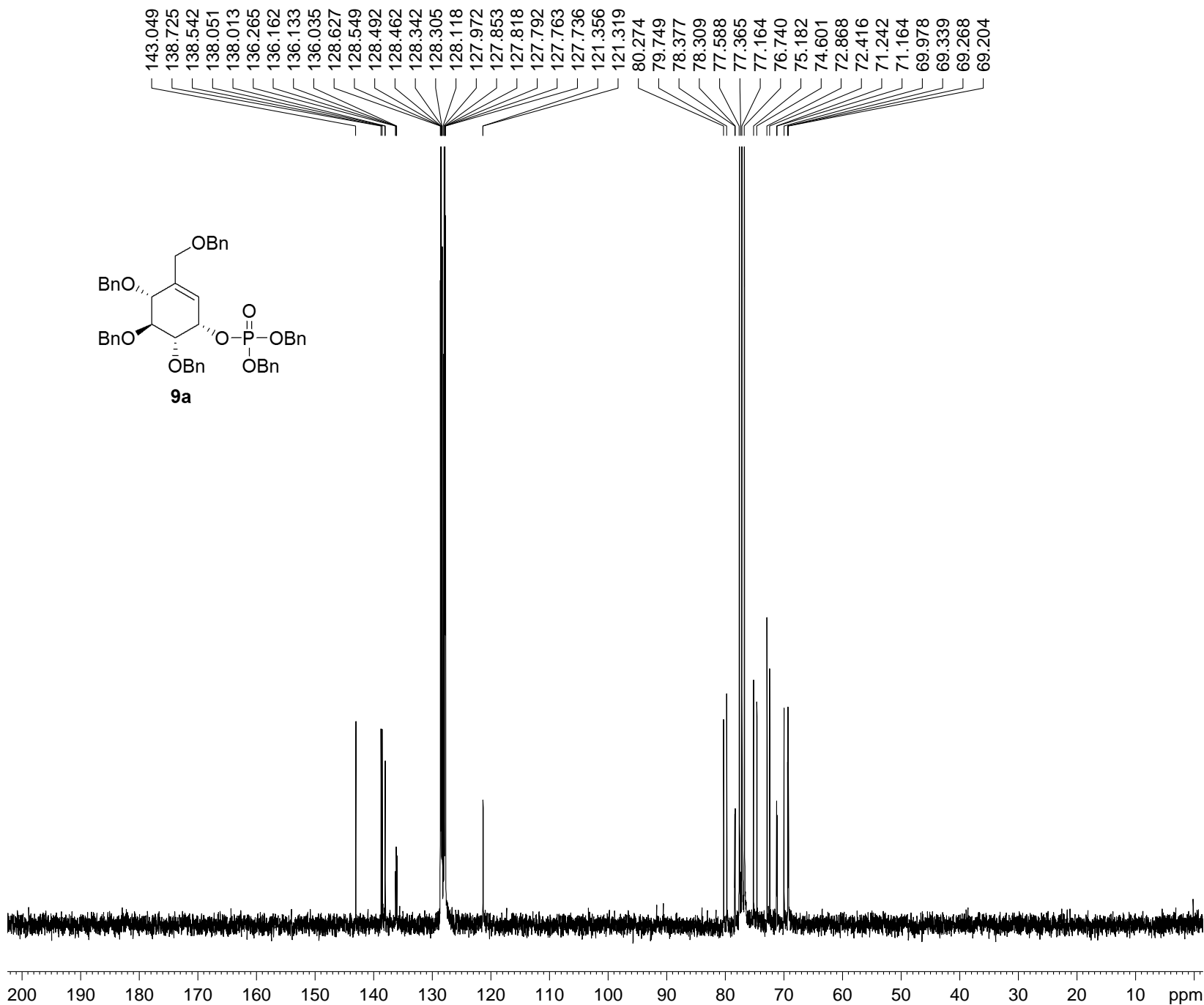
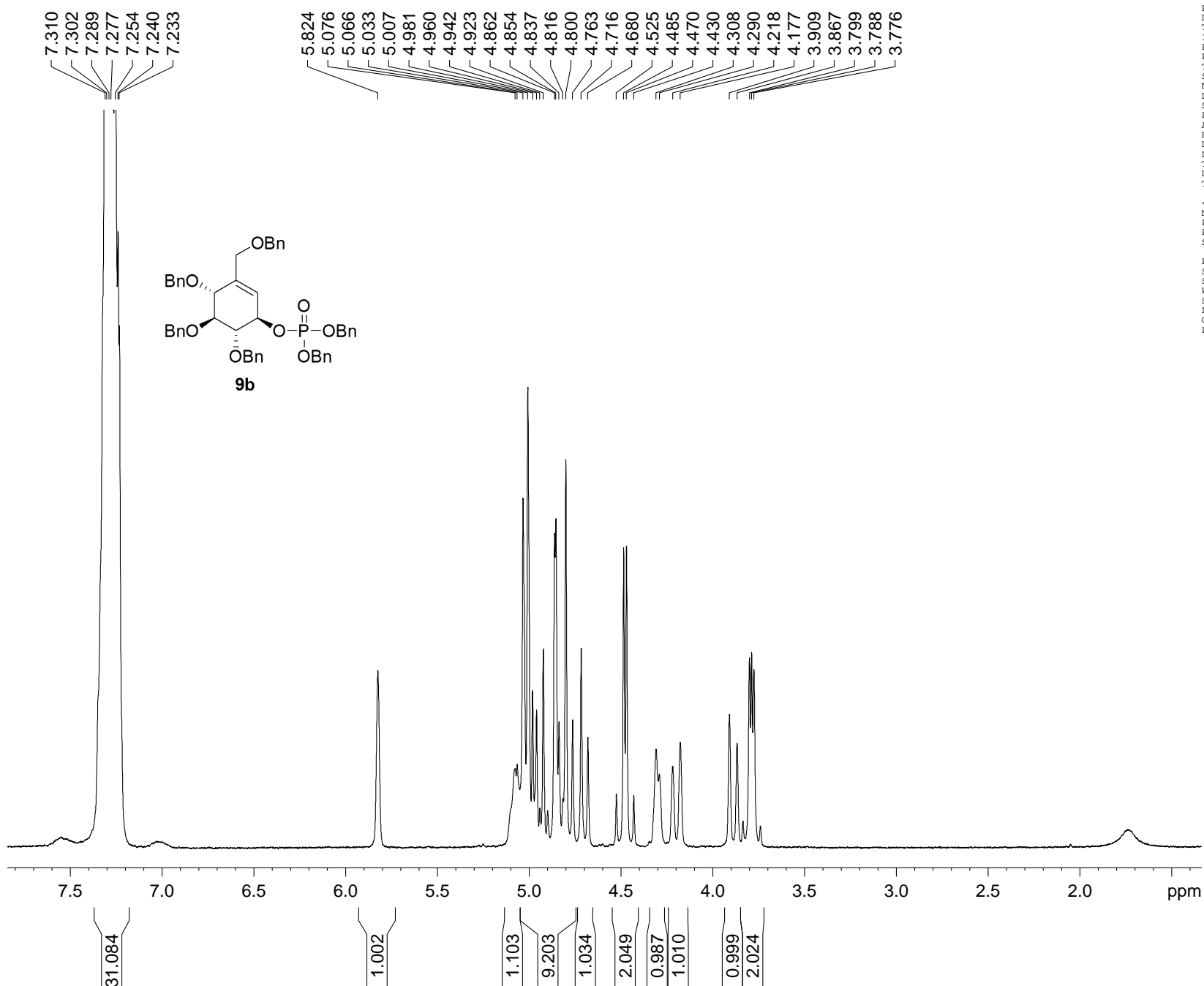


Figure S2. <sup>13</sup>C NMR spectrum of compound 9a



Current Data Parameters  
NAME JTY-1-41-02  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20100209  
Time 16.19  
INSTRUM DRX300  
PROBHD 5 mm BBO BB-1H  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 6  
DS 4  
SWH 4496.403 Hz  
FIDRES 0.068610 Hz  
AQ 7.2876530 sec  
RG 322.5  
DW 111.200 usec  
DE 158.64 usec  
TE 303.0 K  
D1 1.6000002 sec  
TDO 1

==== CHANNEL f1 =====  
NUC1 1H  
P1 11.00 usec  
PL1 0.00 dB  
SFO1 300.1319508 MHz

F2 - Processing parameters  
SI 32768  
SF 300.1300061 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

Figure S3. <sup>1</sup>H NMR spectrum of compound **9b**

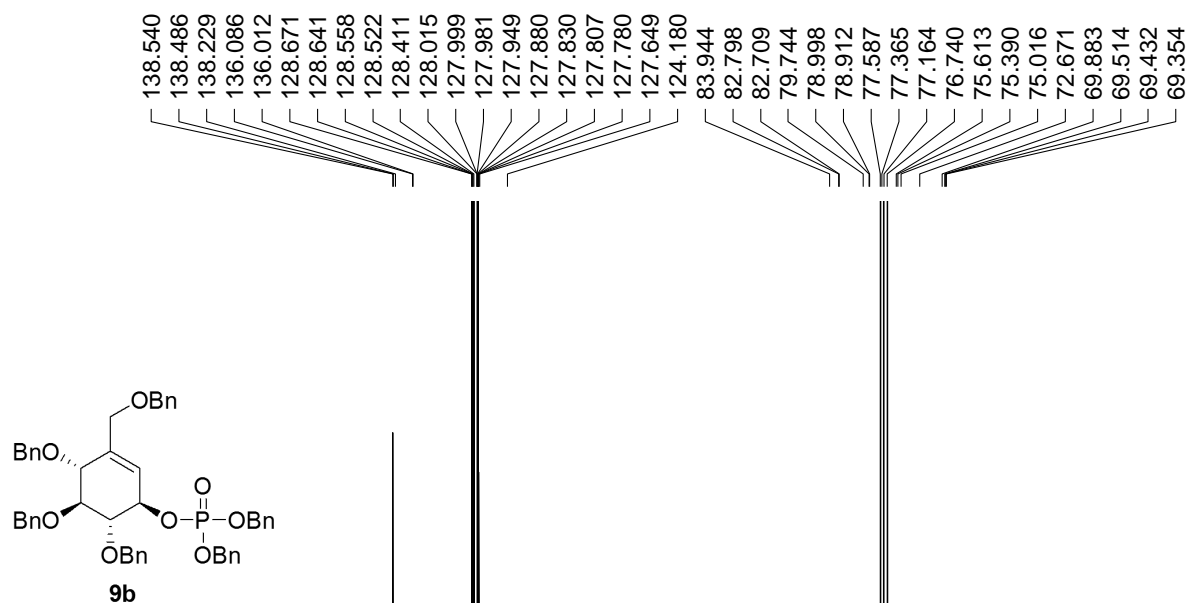
```
Current Data Parameters
NAME      JTY-1-41-02c
EXPNO     2
PROCNO    1

F2 - Acquisition Parameters
Date_     20100209
Time      18.23
INSTRUM   DRX300
PROBHD    5 mm BBO BB-1H
PULPROG   zgpg30
TD        65536
SOLVENT   CDCl3
NS         4000
DS         8
SWH       22727.273 Hz
FIDRES    0.346791 Hz
AQ         1.4418420 sec
RG         32768
DW         22.000 usec
DE         6.00 usec
TE         303.0 K
D1         0.30000001 sec
d11        0.03000000 sec
DELTA     0.20000002 sec
TD0        1

===== CHANNEL f1 =====
NUC1       13C
P1         8.95 usec
PL1        0.00 dB
SFO1       75.4783145 MHz

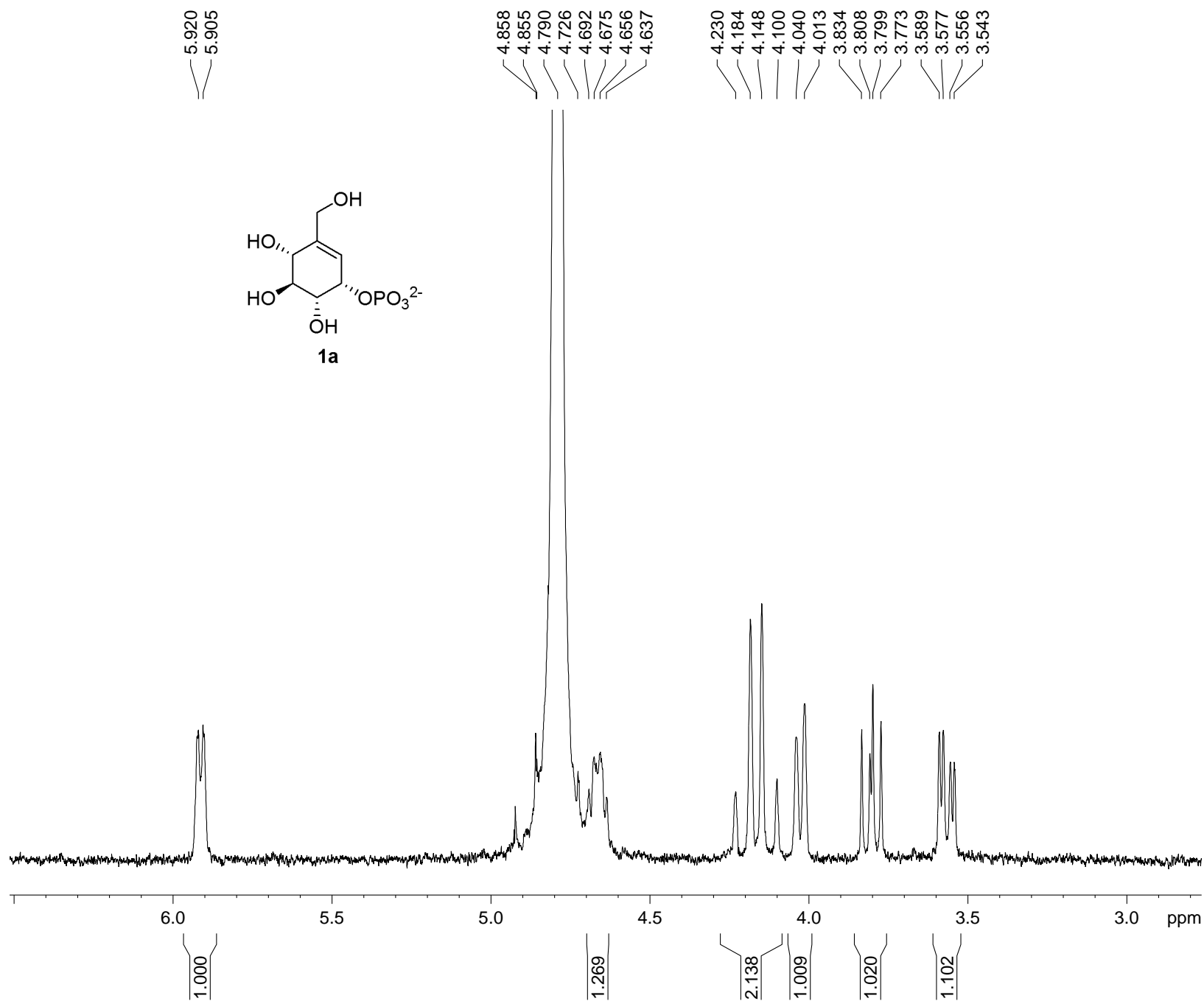
===== CHANNEL f2 =====
CPDPRG2    waltz16
NUC2        1H
PCPD2      85.00 usec
PL2         0.00 dB
PL12        19.00 dB
PL13        19.00 dB
SFO2       300.1312005 MHz

F2 - Processing parameters
SI         32768
SF         75.4677364 MHz
WDW        EM
SSB         0
LB         1.00 Hz
GB          0
PC         1.40
```



200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

Figure S4. <sup>13</sup>C NMR spectrum of compound 9b



Current Data Parameters  
NAME JTY-1-V-1P  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20091109  
Time 17.59  
INSTRUM DXX300  
PROBHD 5 mm BBO BB-1H  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 17  
DS 2  
SWH 4496.403 Hz  
FIDRES 0.068610 Hz  
AQ 7.2876530 sec  
RG 1024  
DW 111.200 usec  
DE 158.64 usec  
TE 293.7 K  
D1 1.79999995 sec  
TDO 1

==== CHANNEL f1 =====  
NUC1 1H  
P1 11.00 usec  
PL1 0.00 dB  
SF01 300.1319508 MHz

F2 - Processing parameters  
SI 32768  
SF 300.1299715 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

Figure S5. <sup>1</sup>H NMR spectrum of compound 1a

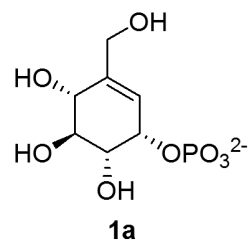
Current Data Parameters  
NAME JTY-1-V-1P-03c  
EXPNO 1  
PROCNO 2

F2 - Acquisition Parameters  
Date\_ 20091111  
Time 9.16  
INSTRUM DRX300  
PROBHD 5 mm BBO BB-1H  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 23769  
DS 8  
SWH 22727.273 Hz  
FIDRES 0.346791 Hz  
AQ 1.4418420 sec  
RG 1625.5  
DW 22.000 usec  
DE 6.00 usec  
TE 293.0 K  
D1 0.3000001 sec  
d11 0.0300000 sec  
DELTA 0.2000002 sec  
TD0 1

===== CHANNEL f1 =====  
NUC1 13C  
P1 8.95 usec  
PL1 0.00 dB  
SFO1 75.4783145 MHz

===== CHANNEL f2 =====  
CPDPRG2 waltz16  
NUC2 1H  
PCPD2 85.00 usec  
PL2 0.00 dB  
PL12 19.00 dB  
PL13 19.00 dB  
SFO2 300.1312005 MHz

F2 - Processing parameters  
SI 32768  
SF 75.4675366 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.20



144.693

124.619  
124.586

75.825  
74.841  
73.388  
73.334  
72.230  
72.165  
64.024

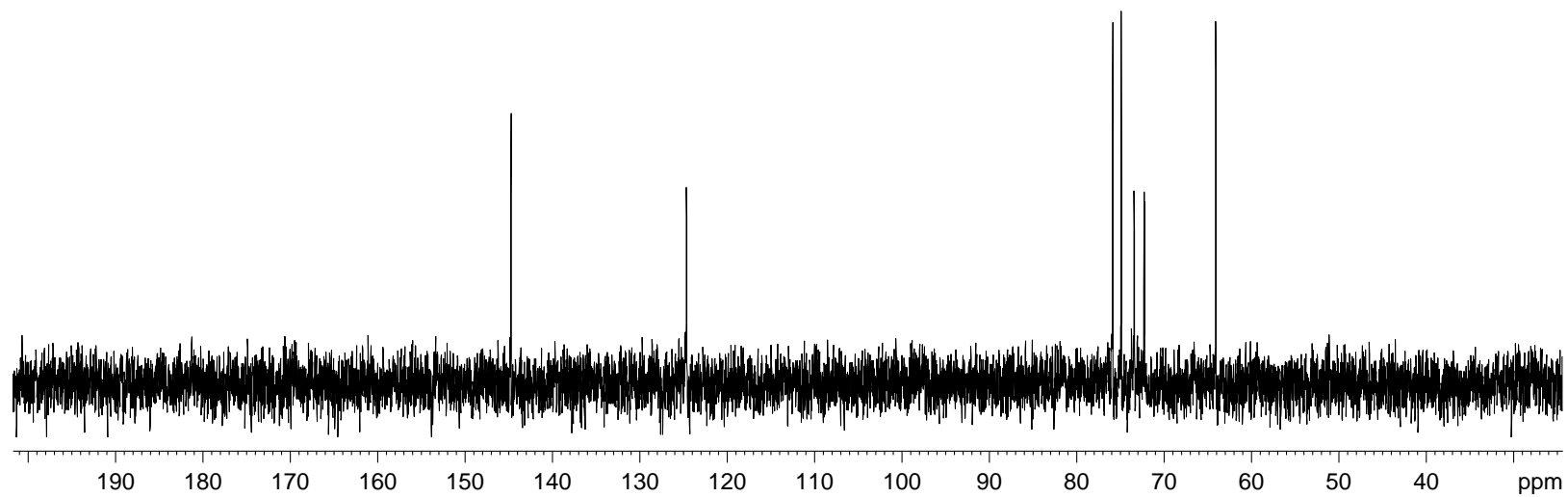
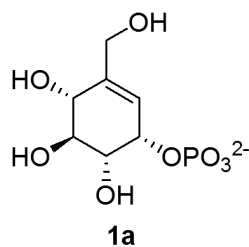
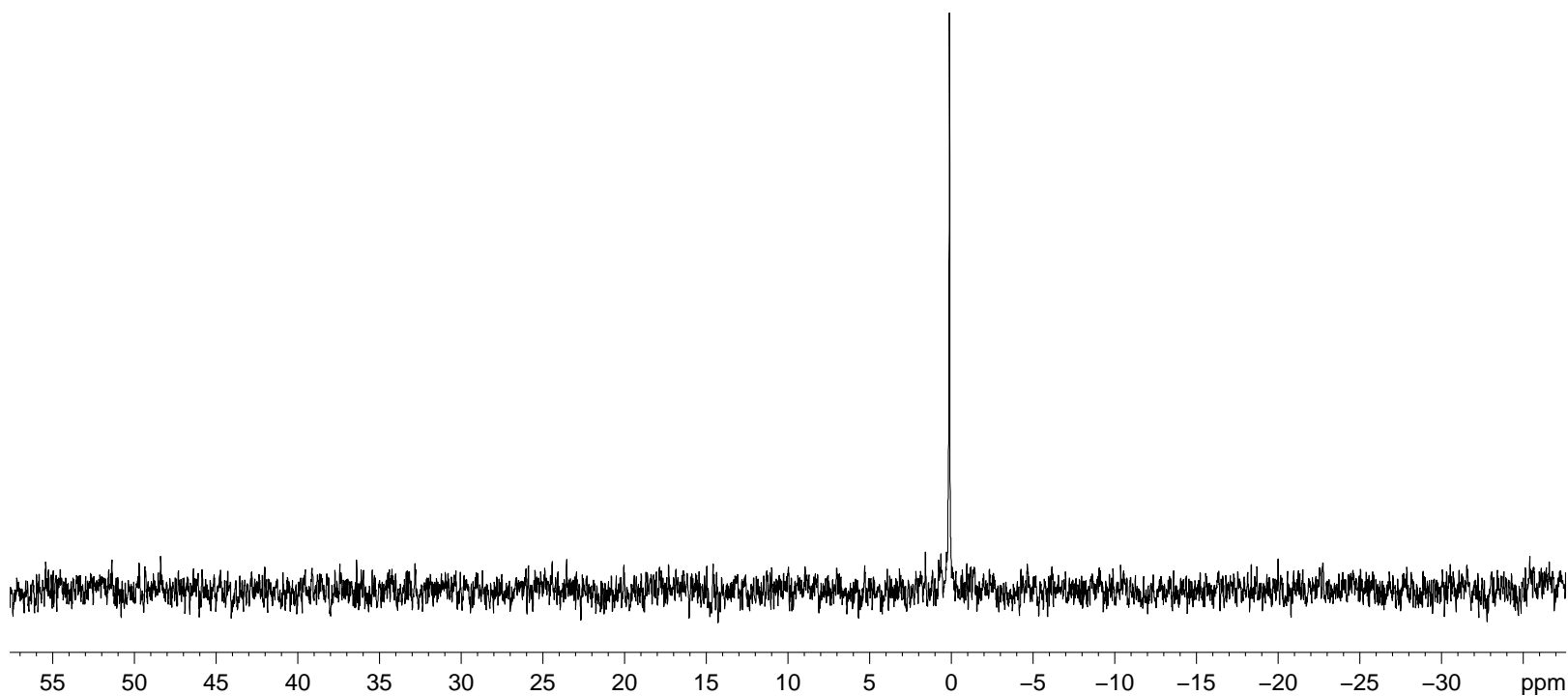


Figure S6. <sup>13</sup>C NMR spectrum of compound 1a





0.10



```
Current Data Parameters
NAME      JTY-1-40-03
EXPNO     1
PROCNO    1

F2 - Acquisition Parameters
Date_     20070510
Time      13.45
INSTRUM   DPX300
PROBHD    5 mm QNP 1H/1
PULPROG   zgpg30
TD        16384
SOLVENT   D2O
NS        109
DS        2
SWH       11574.074 Hz
FIDRES    0.706425 Hz
AQ        0.7078388 sec
RG        20642.5
DW        43.200 usec
DE        6.00 usec
TE        298.2 K
D1        2.00000000 sec
d11       0.03000000 sec
DELTA     1.89999998 sec
TDO       1

===== CHANNEL f1 =====
NUC1      31P
P1        7.65 usec
PL1       -3.00 dB
SFO1      121.4960659 MHz

===== CHANNEL f2 =====
CPDPRG2   waltz16
NUC2      1H
PCPD2     80.00 usec
PL2       -3.00 dB
PL12      17.55 dB
PL13      17.55 dB
SFO2      300.1312005 MHz

F2 - Processing parameters
SI        32768
SF        121.4948510 MHz
WDW       EM
SSB       0
LB        3.00 Hz
GB        0
PC        1.40
```

Figure S7. 31P NMR spectrum of compound 1a

```
Current Data Parameters
NAME      JTY-1-98-16
EXPNO    1
PROCNO   2

F2 - Acquisition Parameters
Date_    20080131
Time     16.26
INSTRUM  DRX300
PROBHD   5 mm BBO BB-1H
PULPROG  zg30
TD        65536
SOLVENT  D2O
NS        21
DS        2
SWH       4496.403 Hz
FIDRES    0.068610 Hz
AQ        7.2876530 sec
RG        1824.6
DW        111.200 usec
DE        158.64 usec
TE        294.1 K
D1        1.60000002 sec
TD0       1

===== CHANNEL f1 =====
NUC1      1H
P1        11.00 usec
PL1       0.00 dB
SFO1      300.1319508 MHz

F2 - Processing parameters
SI        32768
SF        300.1299716 MHz
WDW       EM
SSB       0
LB        0.30 Hz
GB        0
PC        1.00
```

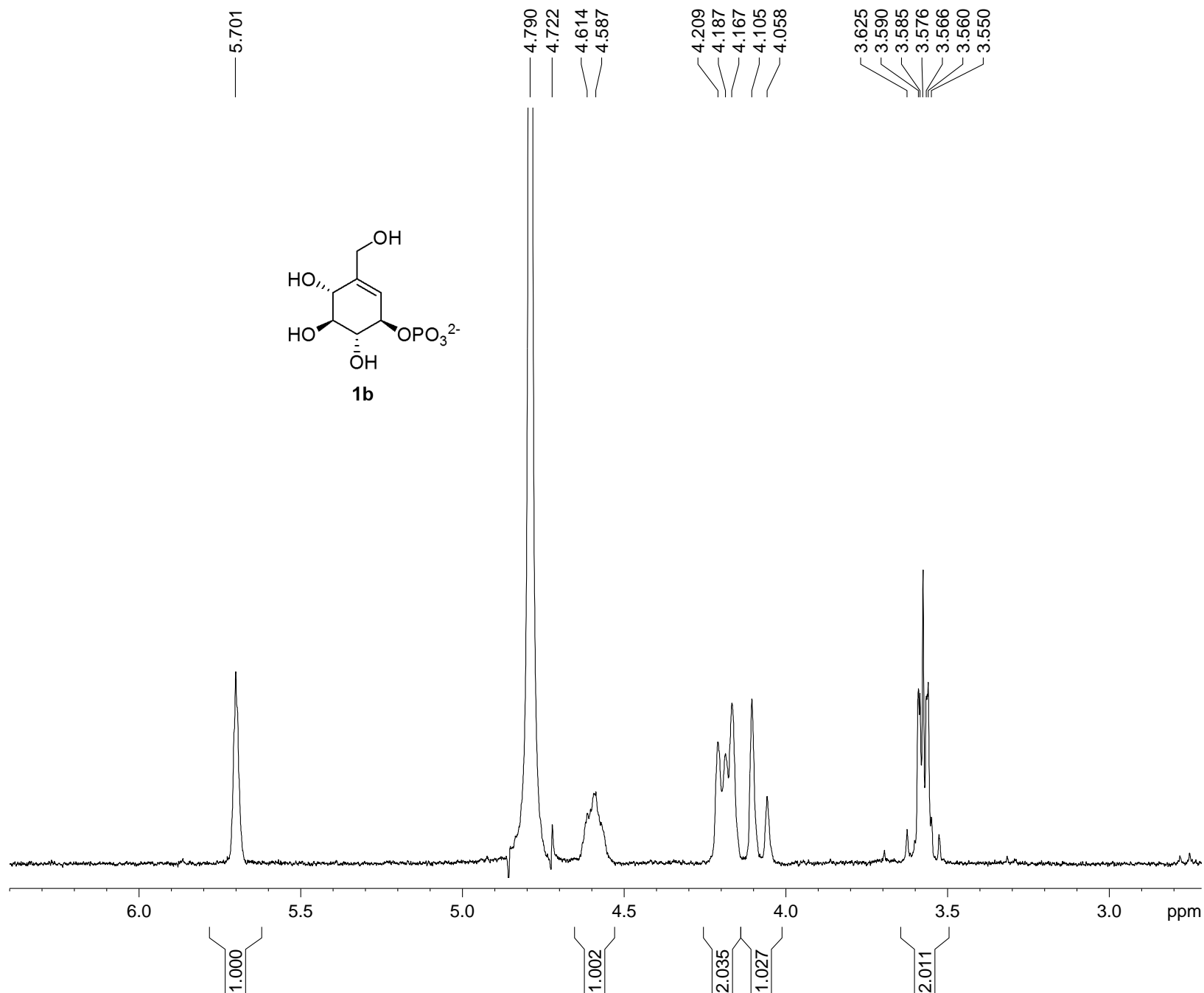
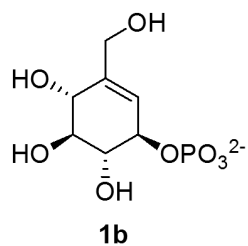


Figure S8. <sup>1</sup>H NMR spectrum of compound **1b**



```
Current Data Parameters
NAME      JTY-1-98-16c
EXPNO     1
PROCNO    2

F2 - Acquisition Parameters
Date_     20080202
Time      7.55
INSTRUM   DRX300
PROBHD    5 mm BBO BB-1H
PULPROG   zgpg30
TD         65536
SOLVENT   CDCl3
NS         33744
DS         8
SWH        22727.273 Hz
FIDRES     0.346791 Hz
AQ         1.4418420 sec
RG         4096
DW         22.000 usec
DE         6.00 usec
TE         292.8 K
D1         0.30000001 sec
d11       0.03000000 sec
DELTA     0.20000002 sec
TD0       1

===== CHANNEL f1 =====
NUC1      13C
P1        8.95 usec
PL1       0.00 dB
SF01     75.4783145 MHz

===== CHANNEL f2 =====
CPDPRG2   waltz16
NUC2      1H
PCPD2     85.00 usec
PL2       0.00 dB
PL12      19.00 dB
PL13      19.00 dB
SF02     300.1312005 MHz

F2 - Processing parameters
SI        32768
SF        75.4675345 MHz
WDW       EM
SSB       0
LB        1.00 Hz
GB        0
PC        1.20
```

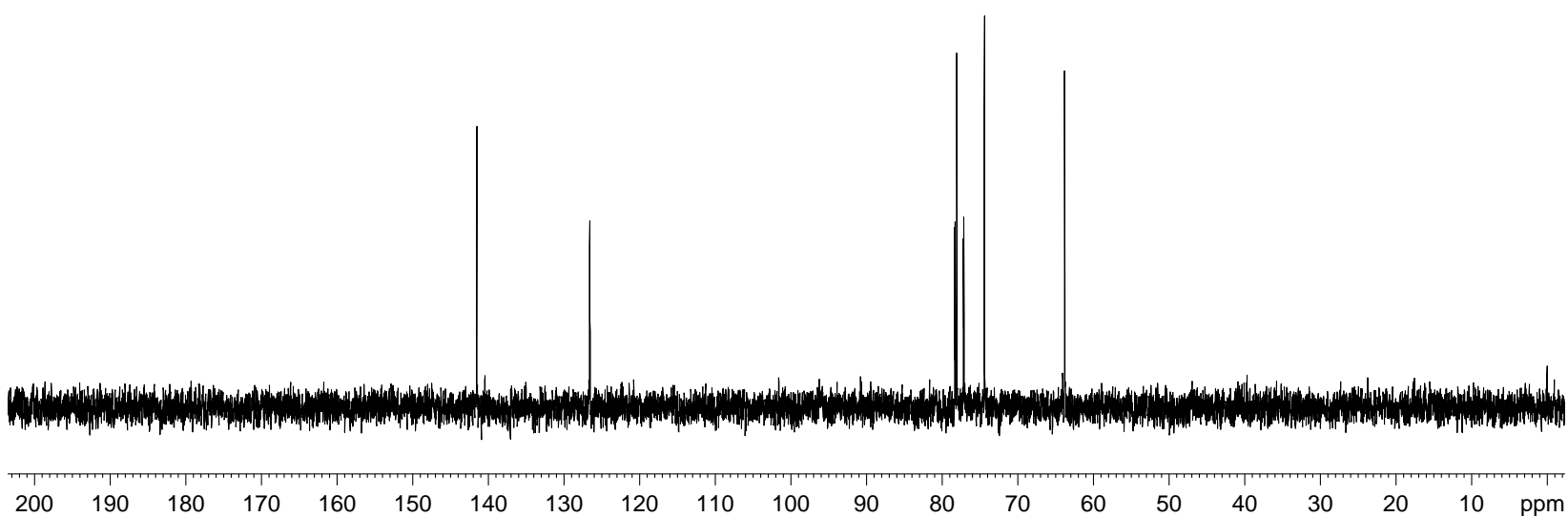
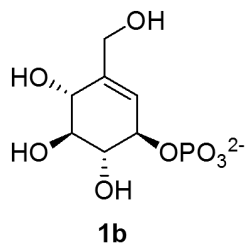


Figure S9. <sup>13</sup>C NMR spectrum of compound 1b



2.854

```
Current Data Parameters
NAME      JTY-1-98-1
EXPNO    1
PROCNO   1

F2 - Acquisition Parameters
Date_    20080131
Time     15.16
INSTRUM  DFX300
PROBHD   5 mm QNP 1H/1
PULPROG  zgpg30
TD       16384
SOLVENT  D2O
NS       128
DS       2
SWH      11574.074 Hz
FIDRES   0.706425 Hz
AQ       0.7078388 sec
RG       20642.5
DW       43.200 usec
DE       6.00 usec
TE       298.2 K
D1       2.0000000 sec
d11      0.0300000 sec
DELTA    1.89999998 sec
TD0      1

===== CHANNEL f1 =====
NUC1     31P
P1       7.65 usec
PL1      -3.00 dB
SFO1     121.4960659 MHz

===== CHANNEL f2 =====
CPDPRG2  waltz16
NUC2     1H
PCPD2    80.00 usec
PL2      -3.00 dB
PL12     17.55 dB
PL13     17.55 dB
SFO2     300.1312005 MHz

F2 - Processing parameters
SI       32768
SF       121.4947551 MHz
WDW      EM
SSB      0
LB       3.00 Hz
GB       0
PC       1.40
```

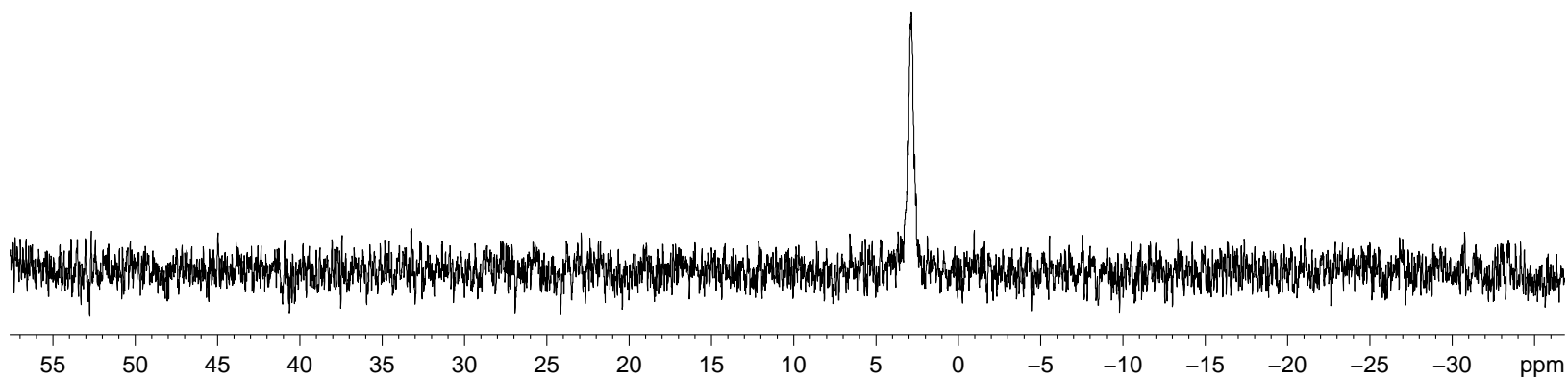
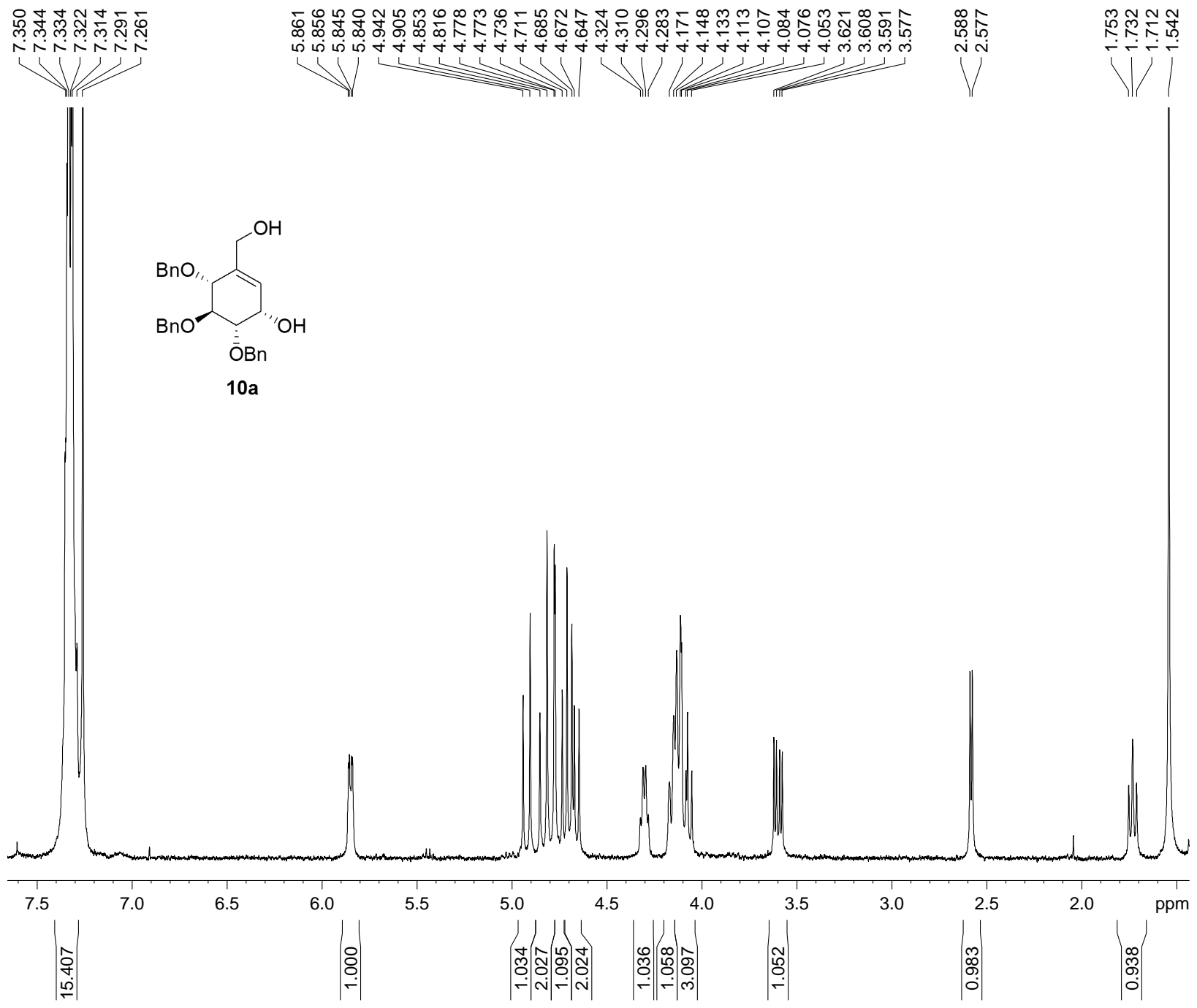


Figure S10. <sup>31</sup>P NMR spectrum of compound **1b**



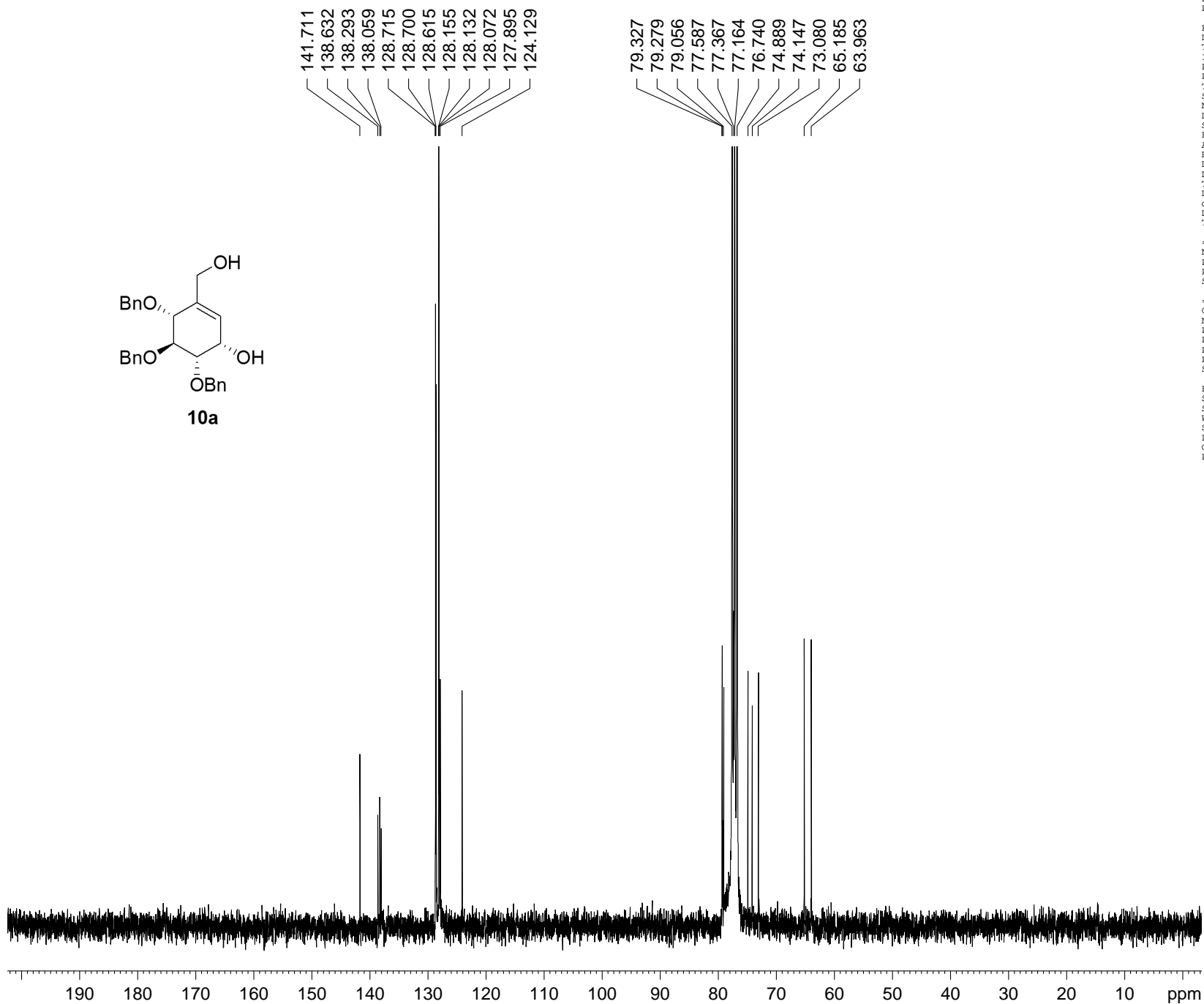
Current Data Parameters  
NAME JTY-1-109-06  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20080219  
Time 17.00  
INSTRUM DRX300  
PROBHD 5 mm BBO BB-1H  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 17  
DS 2  
SWH 4496.403 Hz  
FIDRES 0.068610 Hz  
AQ 7.2876530 sec  
RG 2580.3  
DW 111.200 usec  
DE 158.64 usec  
TE 298.0 K  
D1 1.6000002 sec  
TD0 1

==== CHANNEL f1 =====  
NUC1 1H  
P1 11.00 usec  
PL1 0.00 dB  
SFO1 300.1319508 MHz

F2 - Processing parameters  
SI 32768  
SF 300.1300060 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

Figure S11. <sup>1</sup>H NMR spectrum of compound 10a



```
Current Data Parameters
NAME      JTY-1-109-06c
EXPNO    1
PROCNO   1

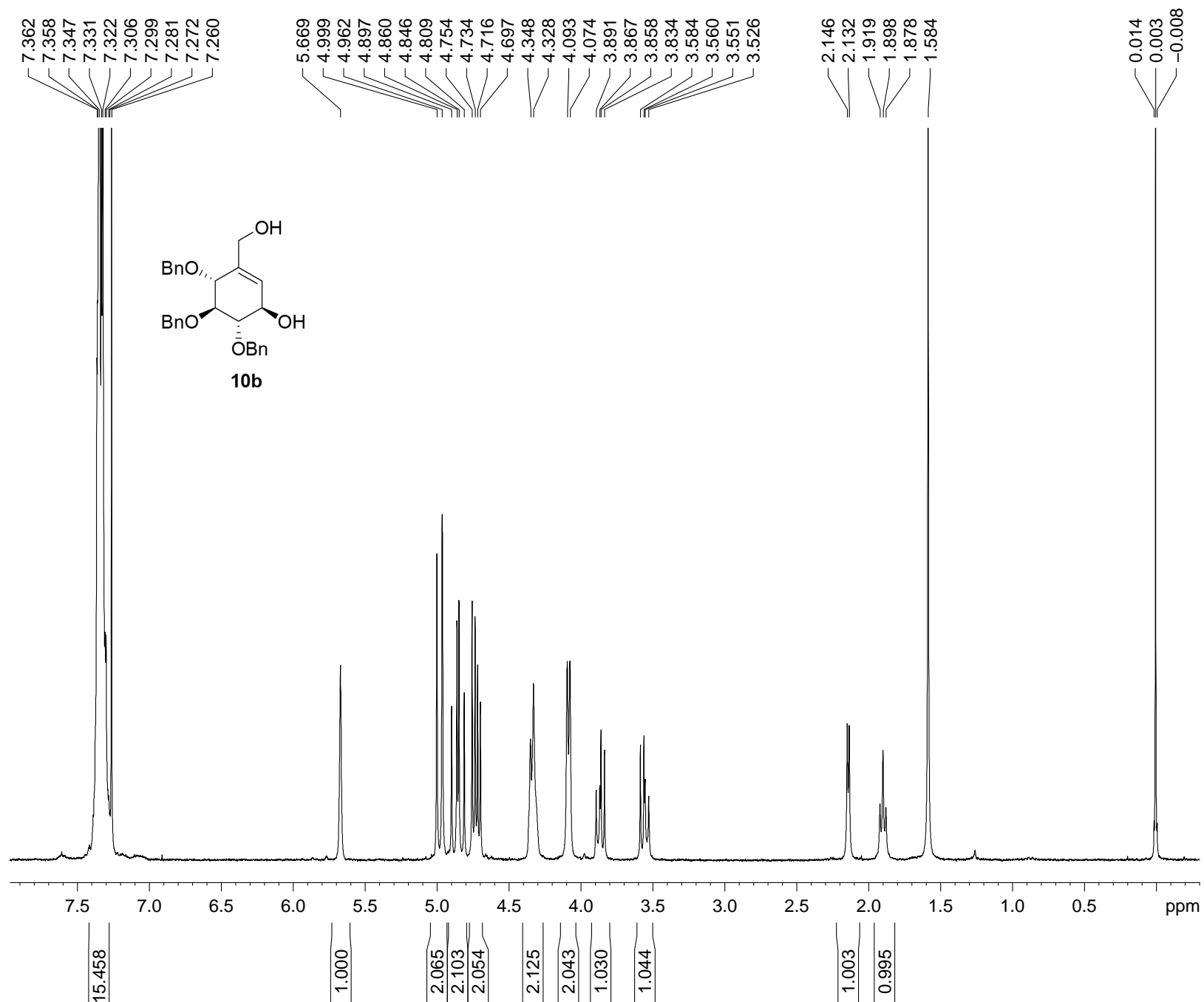
F2 - Acquisition Parameters
Date_    20080219
Time     22.00
INSTRUM  DRX300
PROBHD   5 mm BBO BB-1H
PULPROG  zgpg30
TD       65536
SOLVENT  CDCl3
NS       28490
DS       8
SWH      22727.273 Hz
FIDRES   0.346791 Hz
AQ       1.4418420 sec
RG       10321.3
DW       22.000 usec
DE       6.00 usec
TE       298.0 K
D1       0.3000001 sec
d11      0.03000000 sec
DELTA    0.2000002 sec
TD0      1

===== CHANNEL f1 =====
NUC1     13C
P1       8.95 usec
PL1      0.00 dB
SFO1     75.4783145 MHz

===== CHANNEL f2 =====
CPDPRG2  waltz16
NUC2     1H
PCPD2    85.00 usec
PL2      0.00 dB
PL12     19.00 dB
PL13     19.00 dB
SFO2     300.1312005 MHz

F2 - Processing parameters
SI       32768
SF       75.4677363 MHz
WDW      EM
SSB      0
LB       1.00 Hz
GB       0
PC       1.40
```

Figure S12. 13C NMR spectrum of compound 10a



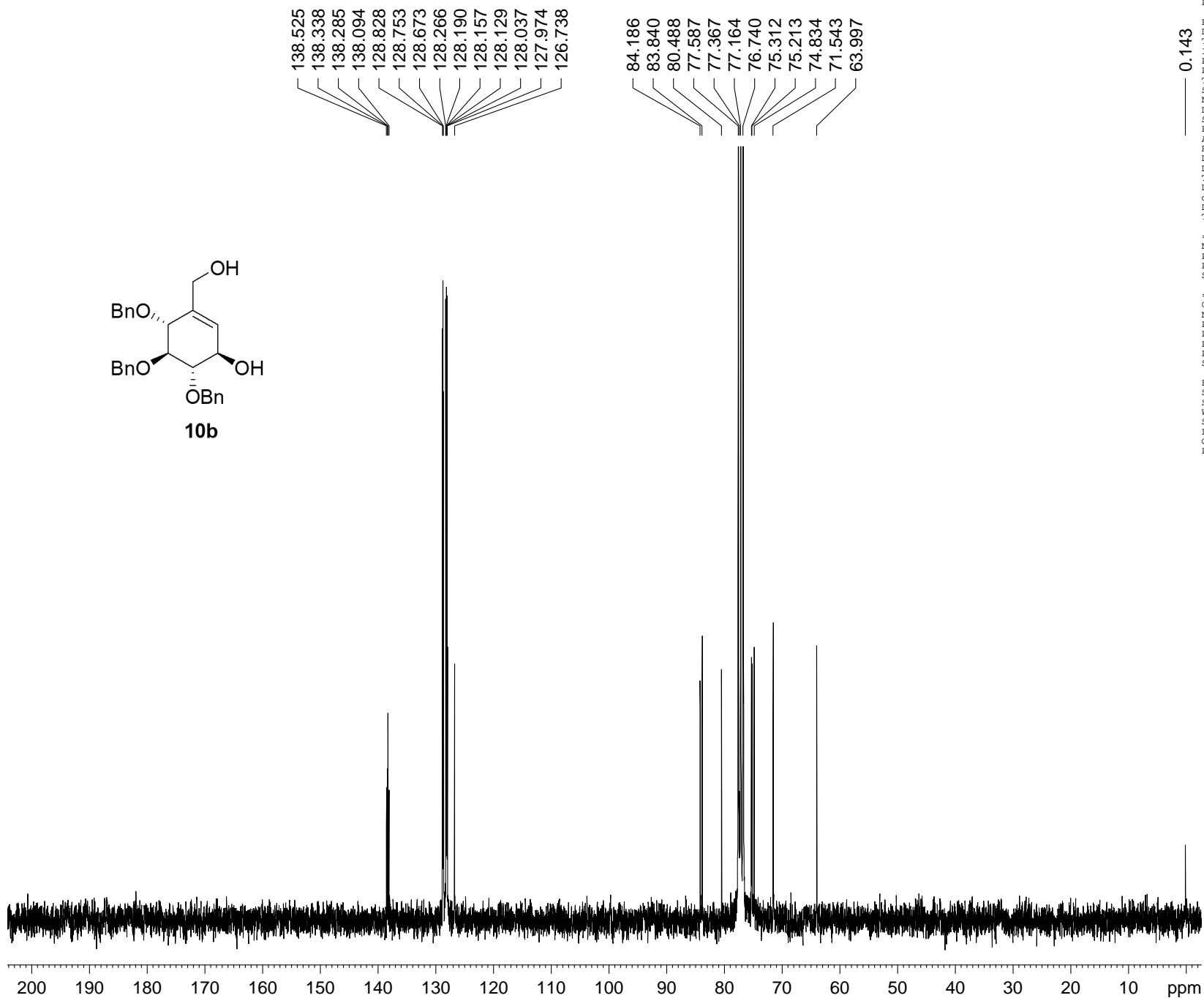
Current Data Parameters  
NAME JTY-1-59-05  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20070718  
Time\_ 10.06  
INSTRUM DRX300  
PROBHD 5 mm Multinucl  
PULPROG zg30  
TD 65536  
SOLVENT CDCl3  
NS 14  
DS 2  
SWH 4496.403 Hz  
FIDRES 0.068610 Hz  
AQ 7.2876530 sec  
RG 1149.4  
DW 111.200 usec  
DE 158.64 usec  
TE 300.0 K  
D1 1.6000002 sec  
TD0 1

==== CHANNEL f1 =====  
NUC1 1H  
P1 11.00 usec  
PL1 0.00 dB  
SFO1 300.1319508 MHz

F2 - Processing parameters  
SI 32768  
SF 300.1300062 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

Figure S13. <sup>1</sup>H NMR spectrum of compound 10b



```
Current Data Parameters
NAME      JTY-1-59-05c
EXPNO     2
PROCNO    1

F2 - Acquisition Parameters
Date_     20070718
Time      12.12
INSTRUM   DRX300
PROBHD    5 mm Multinucl
PULPROG   zgpg30
TD         65536
SOLVENT   CDCl3
NS         4000
DS         8
SWH        22727.273 Hz
FIDRES     0.346791 Hz
AQ         1.4418420 sec
RG         10321.3
DW         22.000 usec
DE         6.00 usec
TE         295.0 K
D1         0.3000001 sec
d11        0.03000000 sec
DELTA     0.2000002 sec
TDO        1

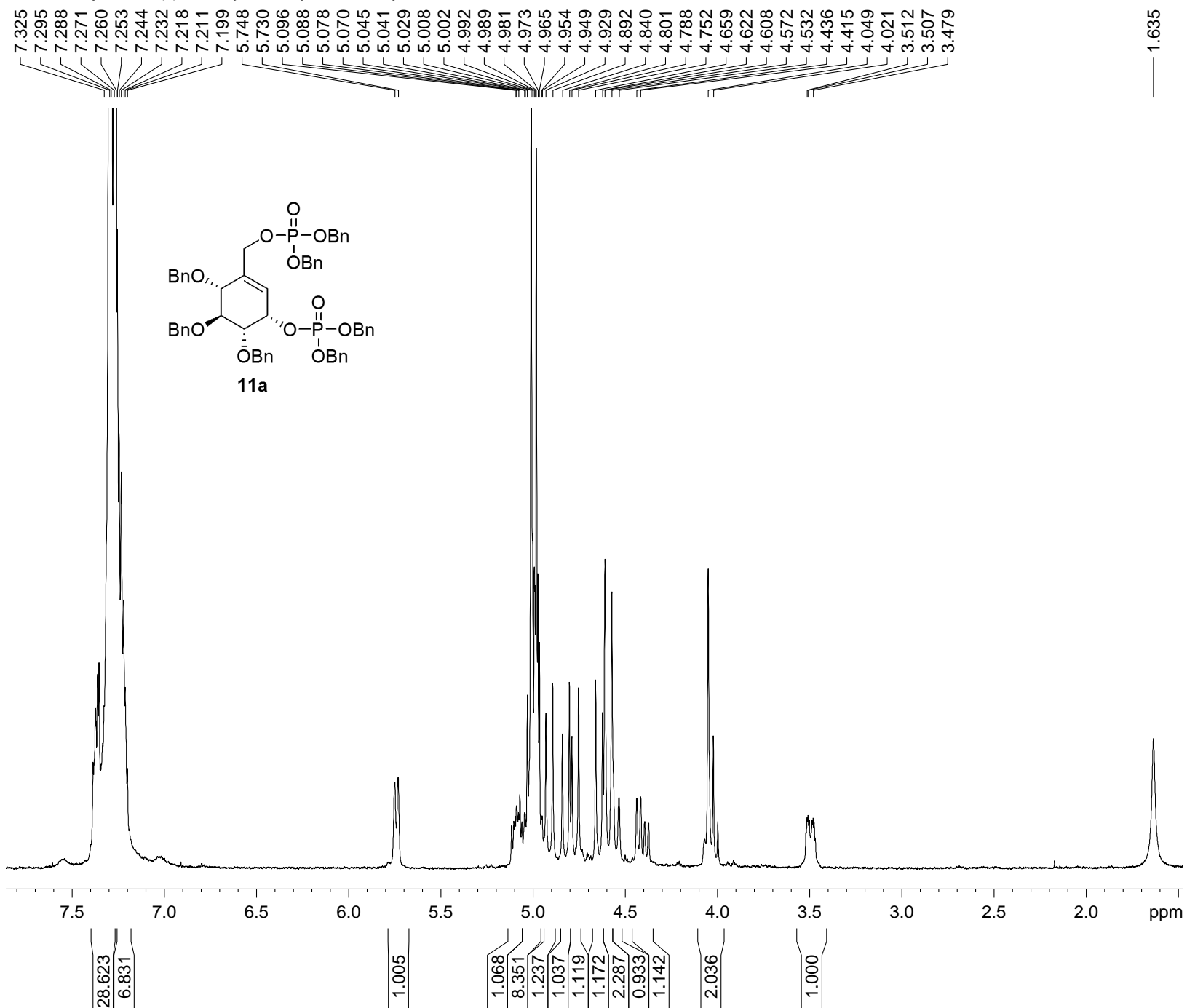
===== CHANNEL f1 =====
NUC1       13C
P1         8.95 usec
PL1        0.00 dB
SFO1       75.4783145 MHz

===== CHANNEL f2 =====
CPDPRG2    waltz16
NUC2       1H
PCPD2      85.00 usec
PL2        0.00 dB
PL12       19.00 dB
PL13       19.00 dB
SFO2       300.1312005 MHz

F2 - Processing parameters
SI         32768
SF         75.4677384 MHz
WDW        EM
SSB        0
LB         1.00 Hz
GB         0
PC         1.40
```

Figure S14. <sup>13</sup>C NMR spectrum of compound 10b





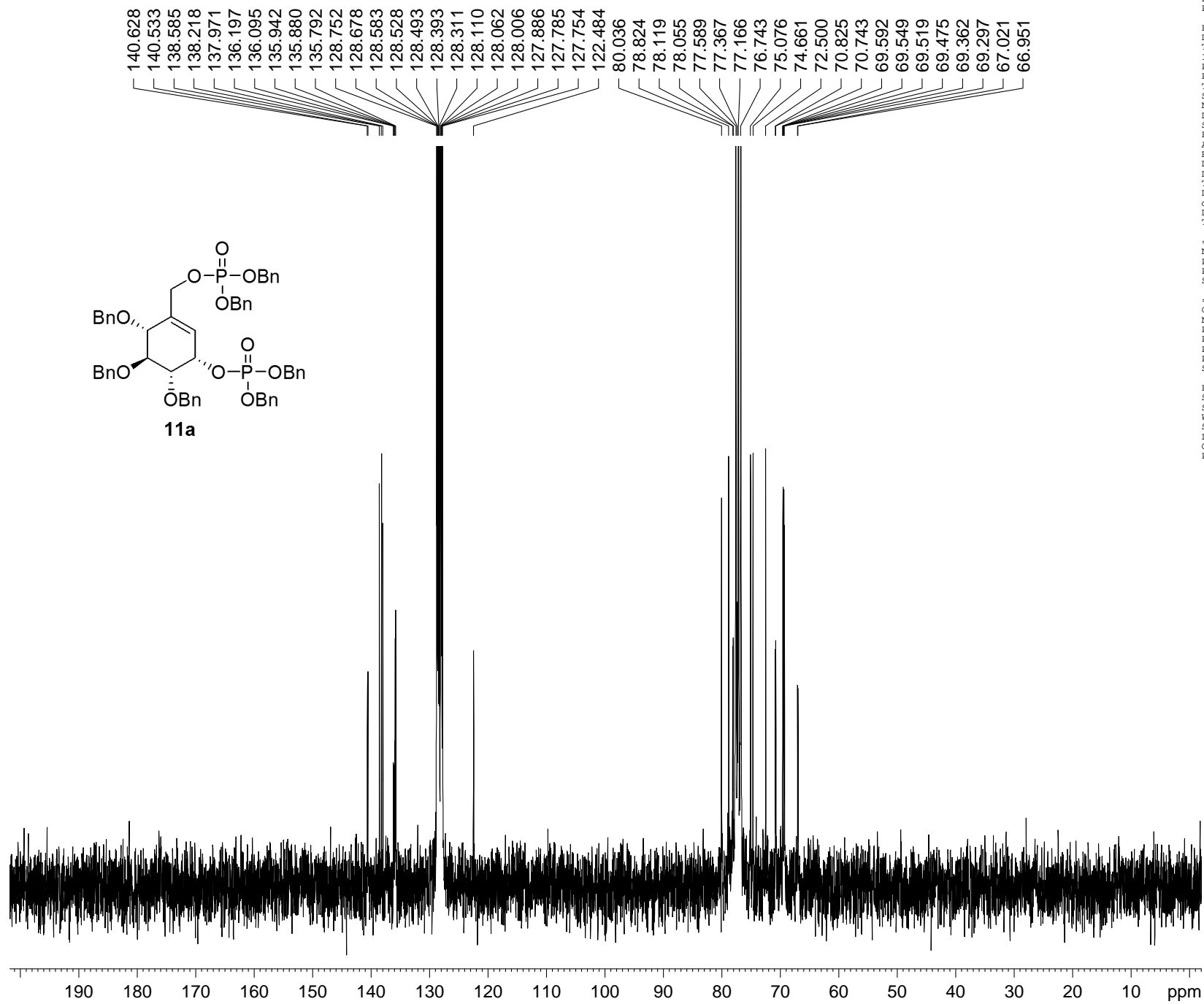
Current Data Parameters  
NAME JTY-1-112-10  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20080306  
Time 14.38  
INSTRUM DRX300  
PROBHD 5 mm BBO BB-1H  
PULPROG zg30  
TD 65536  
SOLVENT D2O  
NS 9  
DS 2  
SWH 4496.403 Hz  
FIDRES 0.068610 Hz  
AQ 7.2876530 sec  
RG 574.7  
DW 111.200 usec  
DE 158.64 usec  
TE 298.0 K  
D1 1.79999995 sec  
TDO 1

==== CHANNEL f1 =====  
NUC1 1H  
P1 11.00 usec  
PL1 0.00 dB  
SFO1 300.1319508 MHz

F2 - Processing parameters  
SI 32768  
SF 300.1300061 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

Figure S15. <sup>1</sup>H NMR spectrum of compound 11a



Current Data Parameters  
NAME JTY-1-112-10c  
EXPNO 2  
PROCNO 1

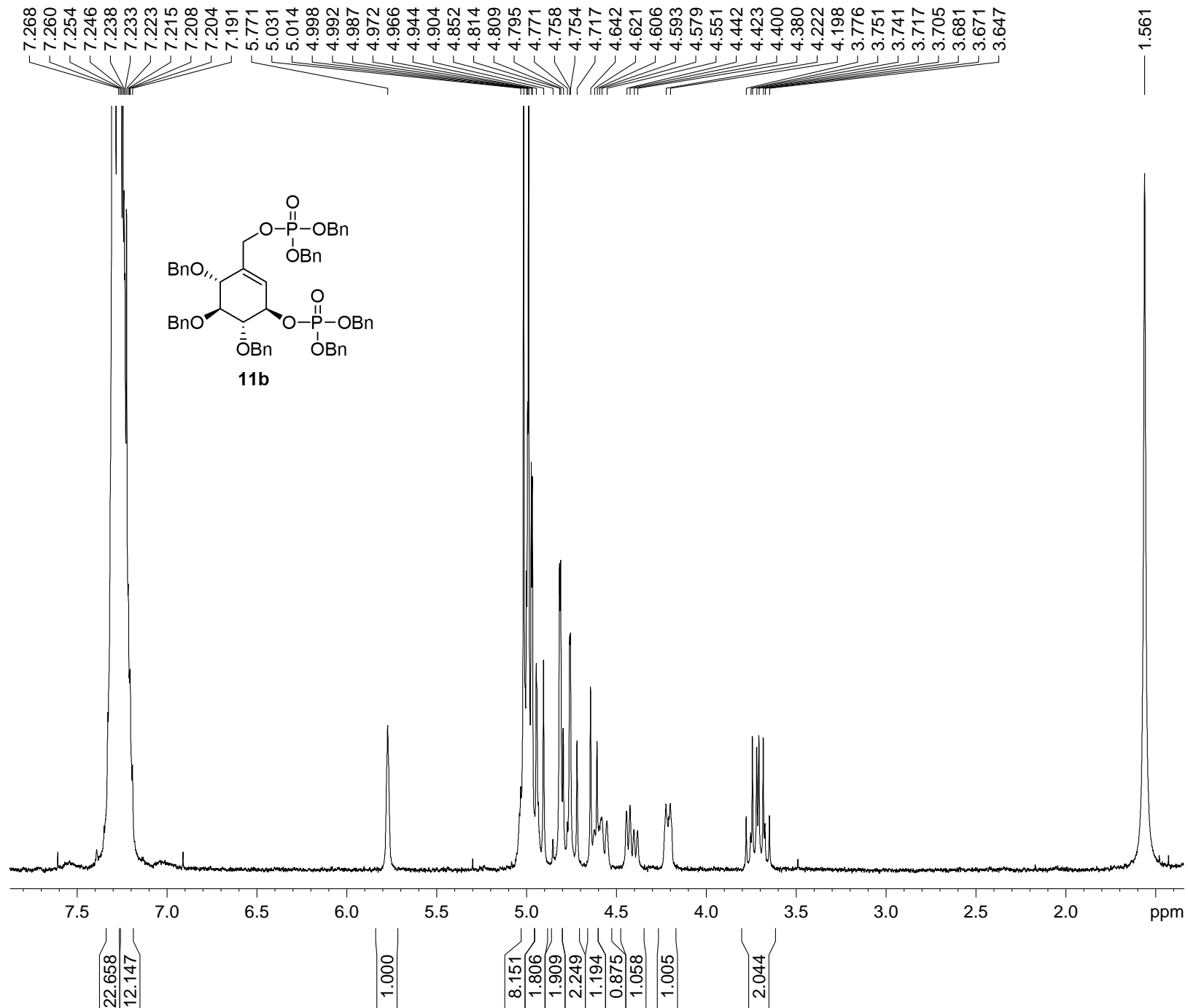
F2 - Acquisition Parameters  
Date\_ 20080306  
Time 17.25  
INSTRUM DRX300  
PROBHD 5 mm BBO BB-1H  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 2840  
DS 8  
SWH 22727.273 Hz  
FIDRES 0.346791 Hz  
AQ 1.4418420 sec  
RG 10321.3  
DW 22.000 usec  
DE 6.00 usec  
TE 298.0 K  
D1 0.3000001 sec  
d11 0.0300000 sec  
DELTA 0.2000002 sec  
TDO 1

==== CHANNEL f1 =====  
NUC1 13C  
P1 8.95 usec  
PL1 0.00 dB  
SF01 75.4783145 MHz

==== CHANNEL f2 =====  
CPDPRG2 waltz16  
NUC2 1H  
PCPD2 85.00 usec  
PL2 0.00 dB  
PL12 19.00 dB  
PL13 19.00 dB  
SFO2 300.1312005 MHz

F2 - Processing parameters  
SI 32768  
SF 75.4677385 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.20

Figure S16. <sup>13</sup>C NMR spectrum of compound 11a



Current Data Parameters  
 NAME JTY-1-111-04  
 EXPNO 1  
 PROCNO 1

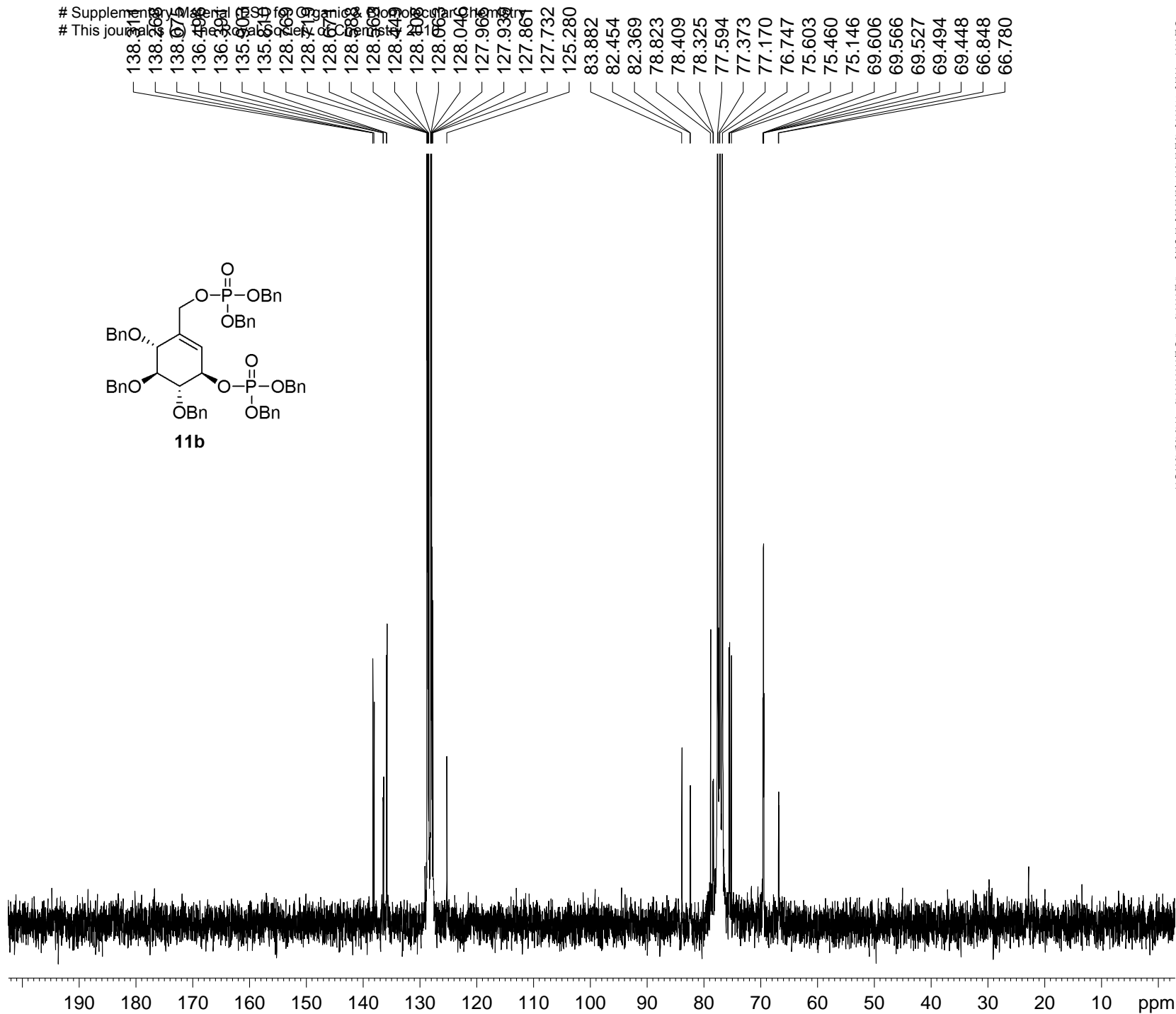
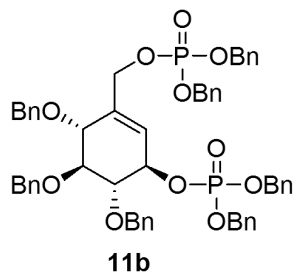
F2 - Acquisition Parameters  
 Date\_ 20080317  
 Time\_ 16.21  
 INSTRUM DRX300  
 PROBH 5 mm BBO BB-1H  
 PULPROG zg30  
 TD 6536  
 SOLVENT CDCl3  
 NS 23  
 DS 2  
 SWH 4496.403 Hz  
 FIDRES 0.068610 Hz  
 AQ 7.2876530 sec  
 RG 1824.6  
 DW 111.200 usec  
 DE 158.64 usec  
 TE 298.0 K  
 DI 1.79999995 sec  
 TDO 1

==== CHANNEL f1 =====  
 NUCL1 1H  
 P1 11.00 usec  
 PL1 0.00 dB  
 SFO1 300.1319508 MHz

F2 - Processing parameters  
 SI 32768  
 SF 300.1300066 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00

Figure S17. <sup>1</sup>H NMR spectrum of compound 11b

# Supplementary Material for Organic Chemistry  
# This journal article



Current Data Parameters  
NAME JTY-1-66-04c  
EXPNO 1  
PROCNO 1

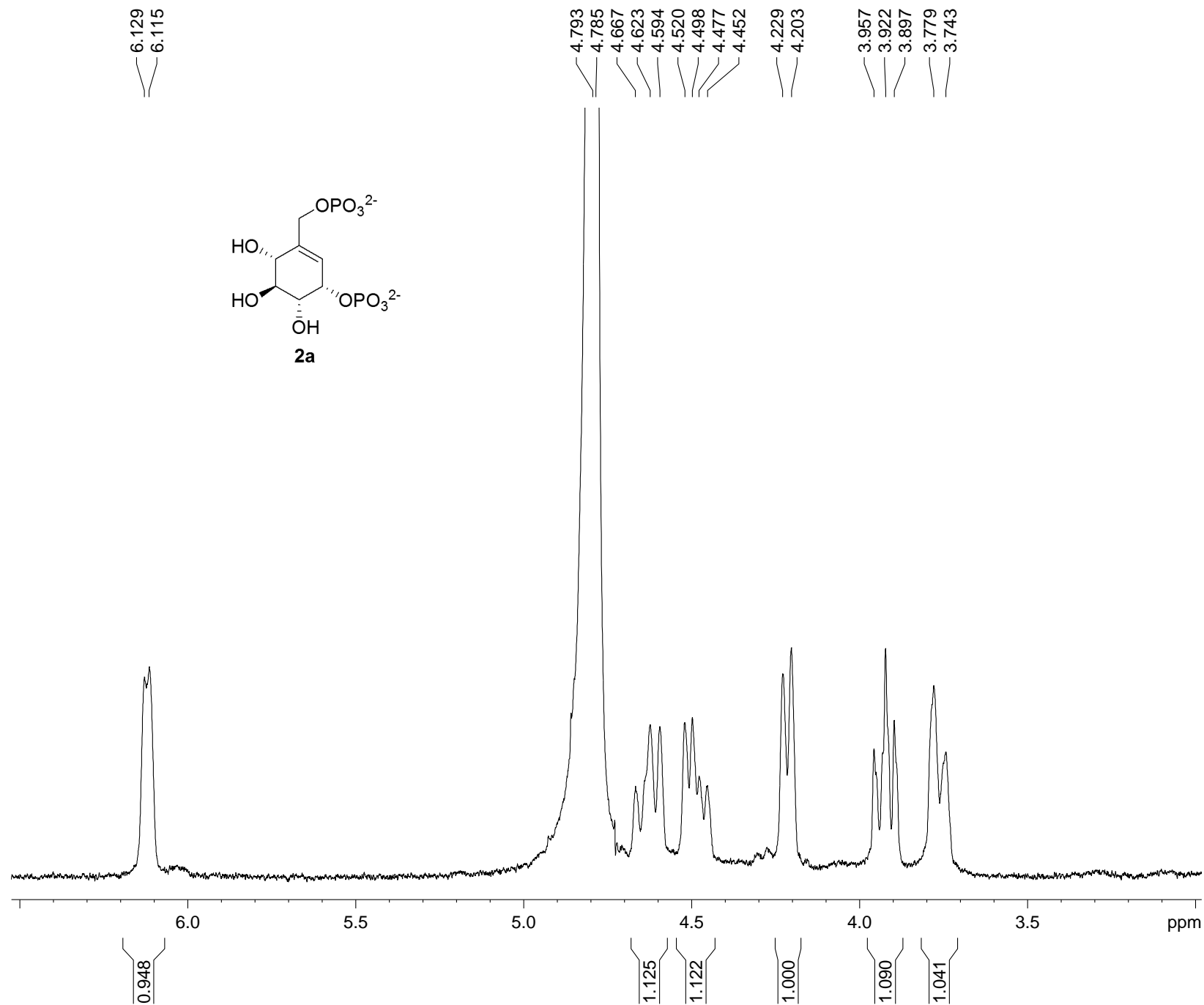
F2 - Acquisition Parameters  
Date\_ 20070821  
Time 21.39  
INSTRUM DRX300  
PROBHD 5 mm BBO BB-1H  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 8734  
DS 8  
SWH 22727.273 Hz  
FIDRES 0.346791 Hz  
AQ 1.4418420 sec  
RG 5792.6  
DW 22.000 usec  
DE 6.00 usec  
TE 297.8 K  
D1 0.3000001 sec  
d11 0.0300000 sec  
DELTA 0.2000002 sec  
TDO 1

===== CHANNEL f1 =====  
NUC1 13C  
P1 8.95 usec  
PL1 0.00 dB  
SF01 75.4783145 MHz

===== CHANNEL f2 =====  
CPDPRG2 waltz16  
NUC2 1H  
PCPD2 85.00 usec  
PL2 0.00 dB  
PL12 19.00 dB  
PL13 19.00 dB  
SFO2 300.1312005 MHz

F2 - Processing parameters  
SI 32768  
SF 75.4677378 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40

Figure S18. <sup>13</sup>C NMR spectrum of compound 11b



Current Data Parameters  
NAME JTY-2-39-19  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20090224  
Time 11.21  
INSTRUM DRX300  
PROBHD 5 mm BBO BB-1H  
PULPROG zg30  
TD 65536  
SOLVENT D2O  
NS 32  
DS 2  
SWH 4496.403 Hz  
FIDRES 0.068610 Hz  
AQ 7.2876530 sec  
RG 1149.4  
DW 111.200 usec  
DE 158.64 usec  
TE 298.5 K  
D1 1.79999995 sec  
TD0 1

==== CHANNEL f1 =====  
NUC1 1H  
P1 11.00 usec  
PL1 0.00 dB  
SFO1 300.1319508 MHz

F2 - Processing parameters  
SI 32768  
SF 300.1299723 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

Figure S19. <sup>1</sup>H NMR spectrum of compound 2a

Current Data Parameters  
NAME JTY-2-39-19c  
EXPNO 1  
PROCNO 1

F2 - Acquisition Paramet  
Date\_ 20090225  
Time 9.24  
INSTRUM DRX300  
PROBHD 5 mm BBO BB-1H  
PULPROG zgpg30  
TD 65536  
SOLVENT CDC13  
NS 31808  
DS 8  
SWH 22727.273  
FIDRES 0.346791  
AQ 1.4418420  
RG 2298.8  
DW 22.000  
DE 6.00  
TE 298.5  
D1 0.30000001  
d11 0.03000000  
DELTA 0.20000002  
TDO 1

==== CHANNEL f1 ====  
NUC1 13C  
P1 8.95  
PL1 0.00  
SFO1 75.4783145

==== CHANNEL f2 ====  
CPDPRG2 waltz16  
NUC2 1H  
PCPD2 85.00  
PL2 0.00  
PL12 19.00  
PL13 19.00  
SFO2 300.1312005

F2 - Processing paramete  
SI 32768  
SF 75.4675478  
WDW EM  
SSB 0  
LB 1.00  
GB 0  
PC 1.40

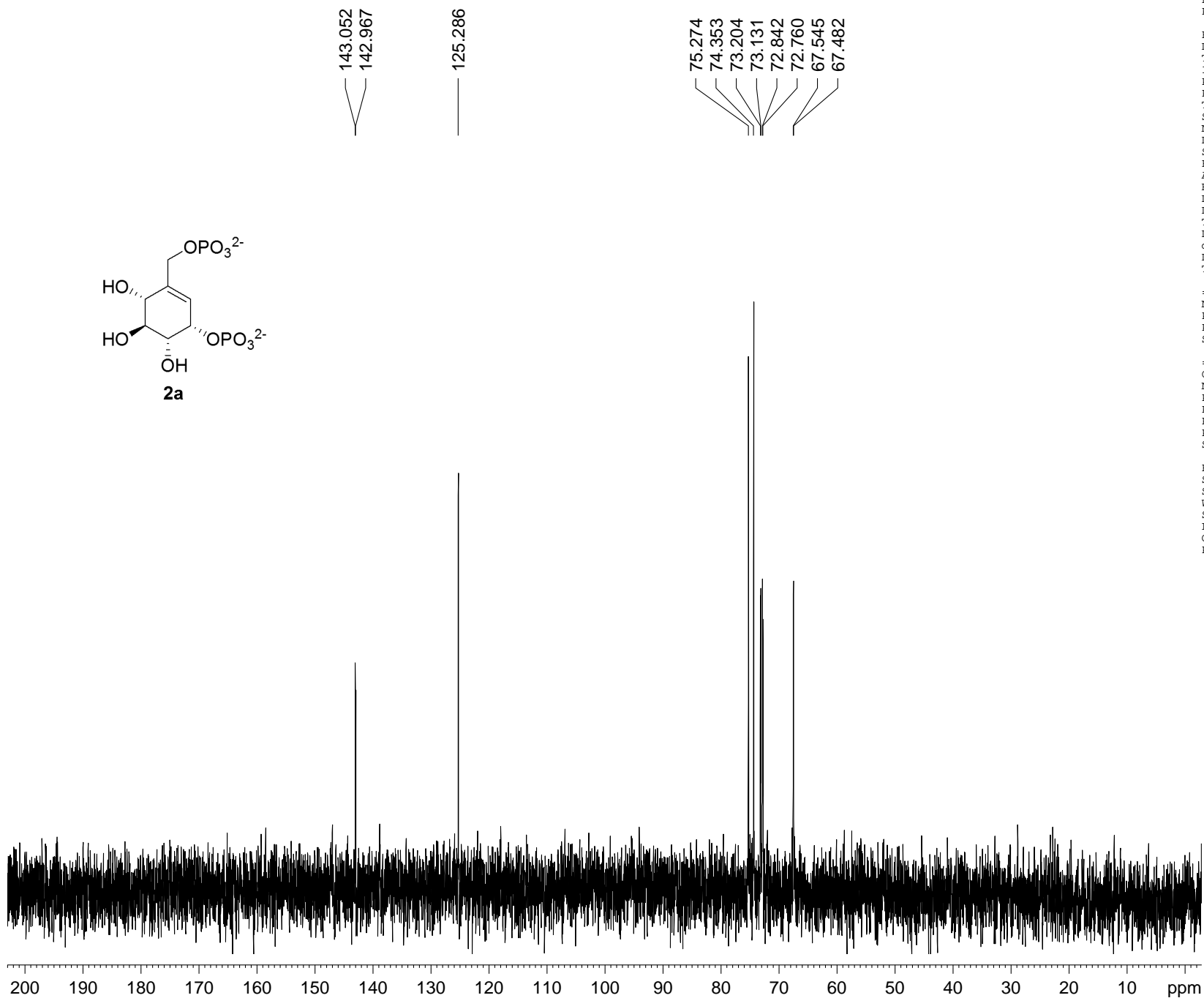
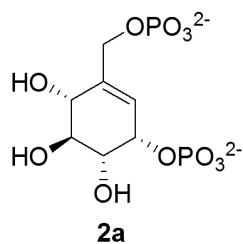


Figure S20. <sup>13</sup>C NMR spectrum of compound 2a



1.228  
1.220

Current Data Parameters  
NAME JTY-2-39-1  
EXPNO 3  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20090226  
Time 12.01  
INSTRUM DPX300  
PROBHD 5 mm QNP 1H/1  
PULPROG zgpg30  
TD 16384  
SOLVENT D2O  
NS 128  
DS 2  
SWH 11574.074 Hz  
FIDRES 0.706425 Hz  
AQ 0.7078388 sec  
RG 16384  
DW 43.200 usec  
DE 6.00 usec  
TE 298.2 K  
D1 2.0000000 sec  
d11 0.0300000 sec  
DELTA 1.8999998 sec  
TDO 1

===== CHANNEL f1 =====  
NUC1 31P  
P1 7.65 usec  
PL1 -3.00 dB  
SFO1 121.4960659 MHz

===== CHANNEL f2 =====  
CPDPRG2 waltz16  
NUC2 1H  
PCPD2 80.00 usec  
PL2 -3.00 dB  
PL12 17.55 dB  
PL13 17.55 dB  
SFO2 300.1312005 MHz

F2 - Processing parameters  
SI 32768  
SF 121.4947576 MHz  
WDW EM  
SSB 0  
LB 3.00 Hz  
GB 0  
PC 1.40

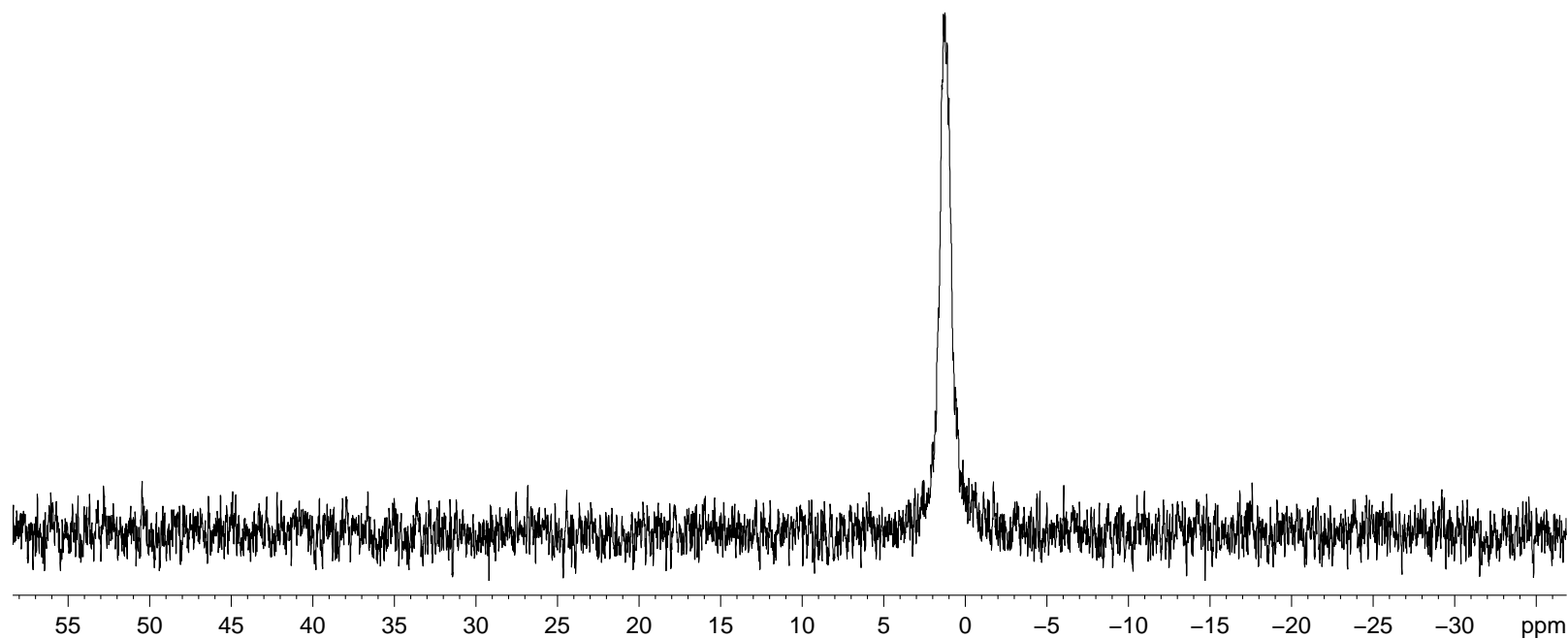
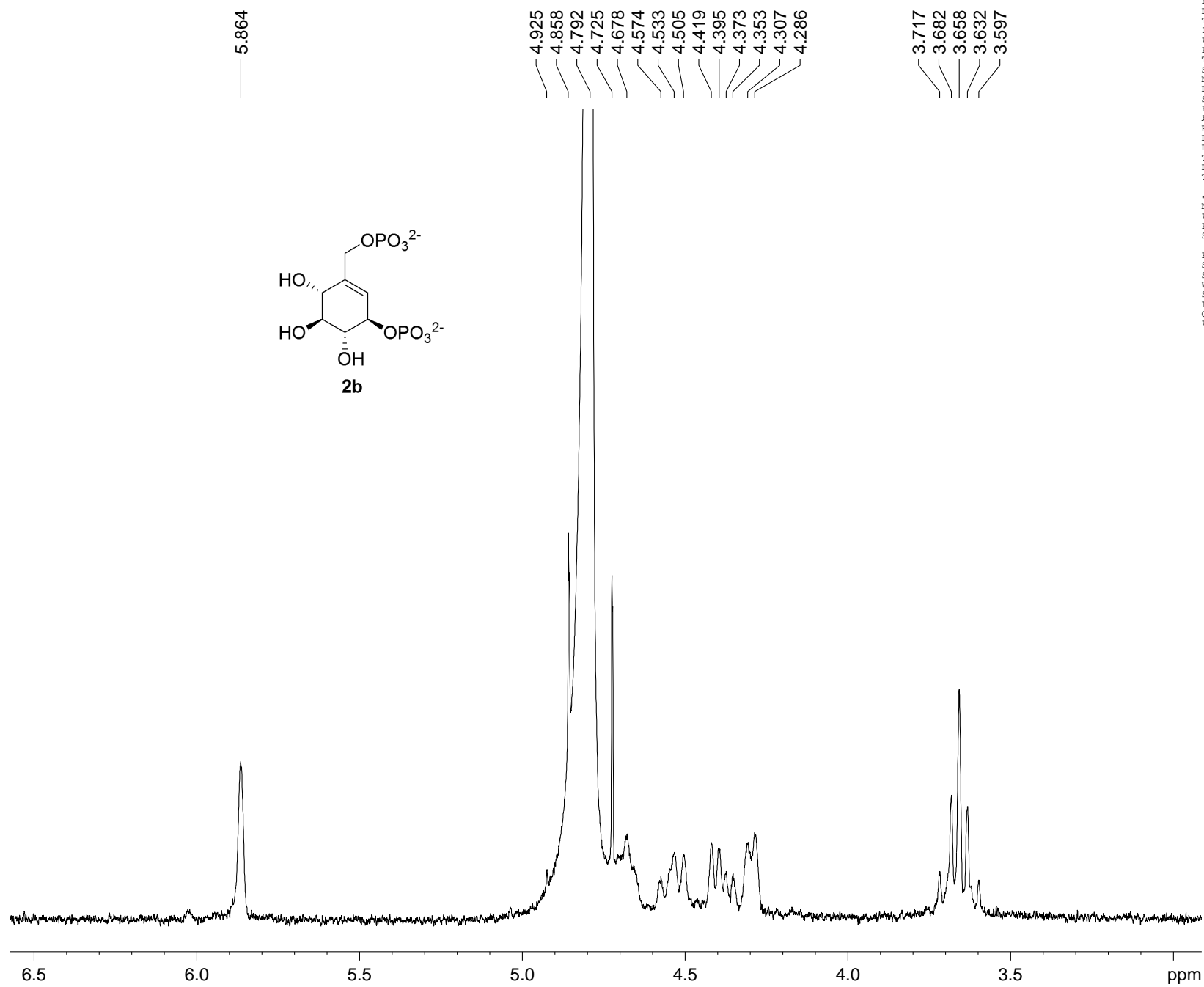


Figure S21. <sup>31</sup>P NMR spectrum of compound 2a



```
Current Data Parameters
NAME      JTY-1-69-45
EXPNO    1
PROCNO   1

F2 - Acquisition Parameters
Date_    20080701
Time     23.46
INSTRUM  DRX300
PROBHD   5 mm BBO BB-1H
PULPROG  zg30
TD       65536
SOLVENT  D2O
NS       32
DS       2
SWH      4496.403 Hz
FIDRES   0.068610 Hz
AQ       7.2876530 sec
RG       2580.3
DW       111.200 usec
DE       158.64 usec
TE       297.9 K
D1       1.79999995 sec
TD0      1

===== CHANNEL f1 =====
NUC1     1H
P1       11.00 usec
PL1      0.00 dB
SFO1     300.1319508 MHz

F2 - Processing parameters
SI       32768
SF       300.1299718 MHz
WDW      EM
SSB      0
LB       0.30 Hz
GB       0
PC       1.00
```

Figure S22. <sup>1</sup>H NMR spectrum of compound **2b**



Current Data Parameters  
NAME JTY-1-69-31c  
EXPNO 1  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20070914  
Time 9.12  
INSTRUM DRX300  
PROBHD 5 mm BBO BB-1H  
PULPROG zgpg30  
TD 65536  
SOLVENT CDCl3  
NS 19000  
DS 8  
SWH 22727.273 Hz  
FIDRES 0.346791 Hz  
AQ 1.4418420 sec  
RG 2580.3  
DW 22.000 usec  
DE 6.00 usec  
TE 295.6 K  
D1 0.3000001 sec  
d11 0.03000000 sec  
DELTA 0.2000002 sec  
TDO 1

===== CHANNEL f1 =====  
NUC1 13C  
P1 8.95 usec  
PL1 0.00 dB  
SF01 75.4783145 MHz

===== CHANNEL f2 =====  
CPDPRG2 waltz16  
NUC2 1H  
PCPD2 85.00 usec  
PL2 0.00 dB  
PL12 19.00 dB  
PL13 19.00 dB  
SFO2 300.1312005 MHz

F2 - Processing parameters  
SI 32768  
SF 75.4675247 MHz  
WDW EM  
SSB 0  
LB 1.00 Hz  
GB 0  
PC 1.40

139.969  
139.885  
128.113

78.372  
78.306  
77.893  
77.333  
77.283  
74.000  
67.286  
67.225

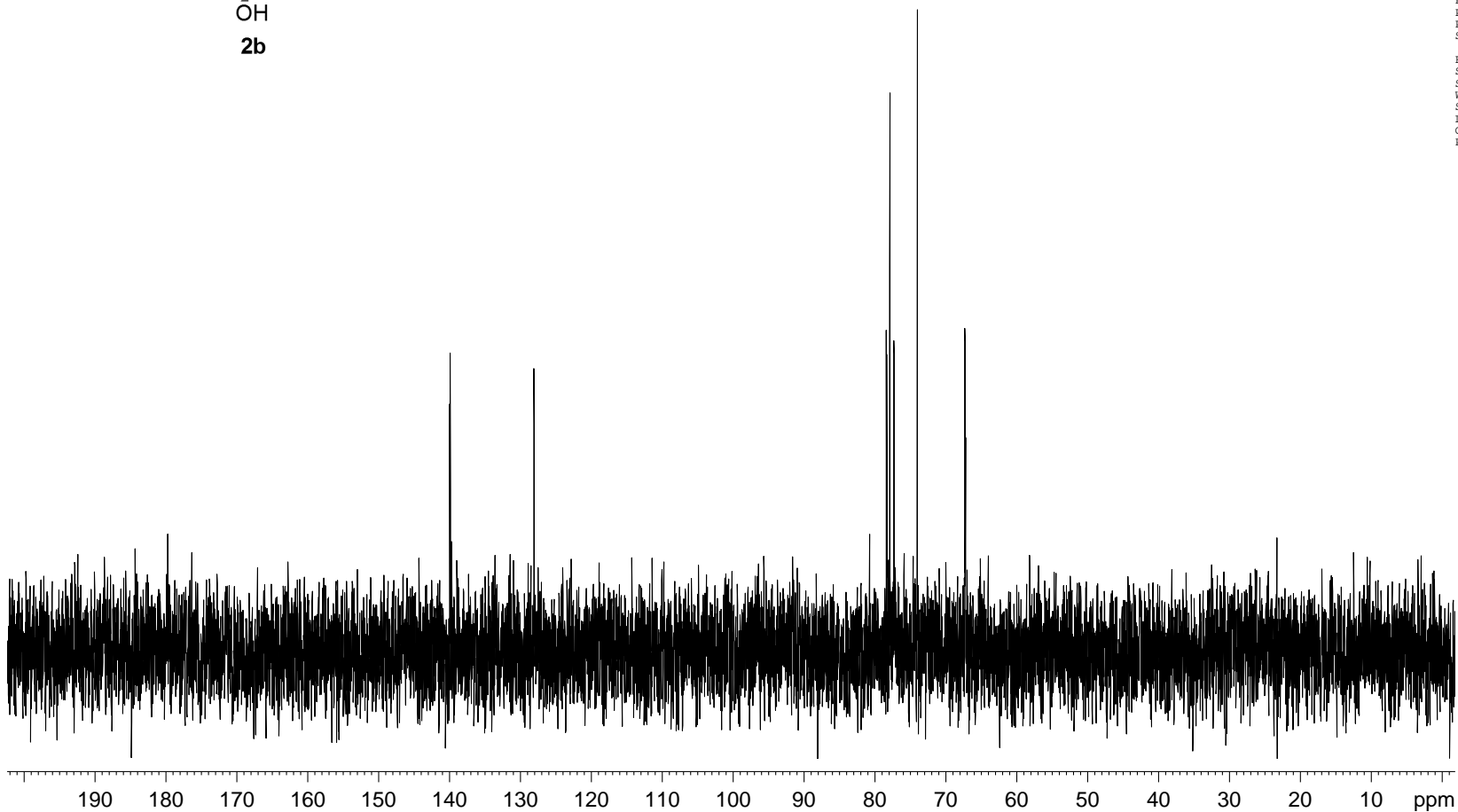
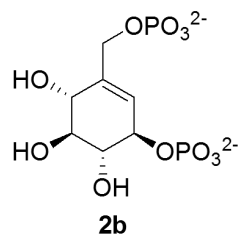
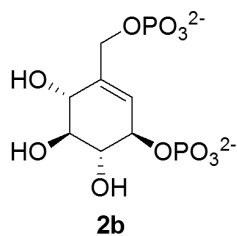


Figure S23. <sup>13</sup>C NMR spectrum of compound 2b



1.376  
0.963

```
Current Data Parameters
NAME      JTY-1-69-01
EXPNO    1
PROCNO   1

F2 - Acquisition Parameters
Date_    20080702
Time     15.45
INSTRUM  DPX300
PROBHD   5 mm QNP 1H/1
PULPROG  zgpg30
TD       16384
SOLVENT  D2O
NS       128
DS       2
SWH      11574.074 Hz
FIDRES   0.706425 Hz
AQ       0.7078388 sec
RG       26008
DW       43.200 usec
DE       6.00 usec
TE       298.2 K
D1       2.00000000 sec
d11      0.03000000 sec
DELTA    1.89999998 sec
TD0      1

===== CHANNEL f1 =====
NUC1     31P
P1       7.65 usec
PL1      -3.00 dB
SFO1     121.4960659 MHz

===== CHANNEL f2 =====
CPDPRG2  waltz16
NUC2     1H
PCPD2    80.00 usec
PL2      -3.00 dB
PL12     17.55 dB
PL13     17.55 dB
SFO2     300.1312005 MHz

F2 - Processing parameters
SI       32768
SF       121.4947599 MHz
WDW      EM
SSB      0
LB       3.00 Hz
GB       0
PC       1.60
```

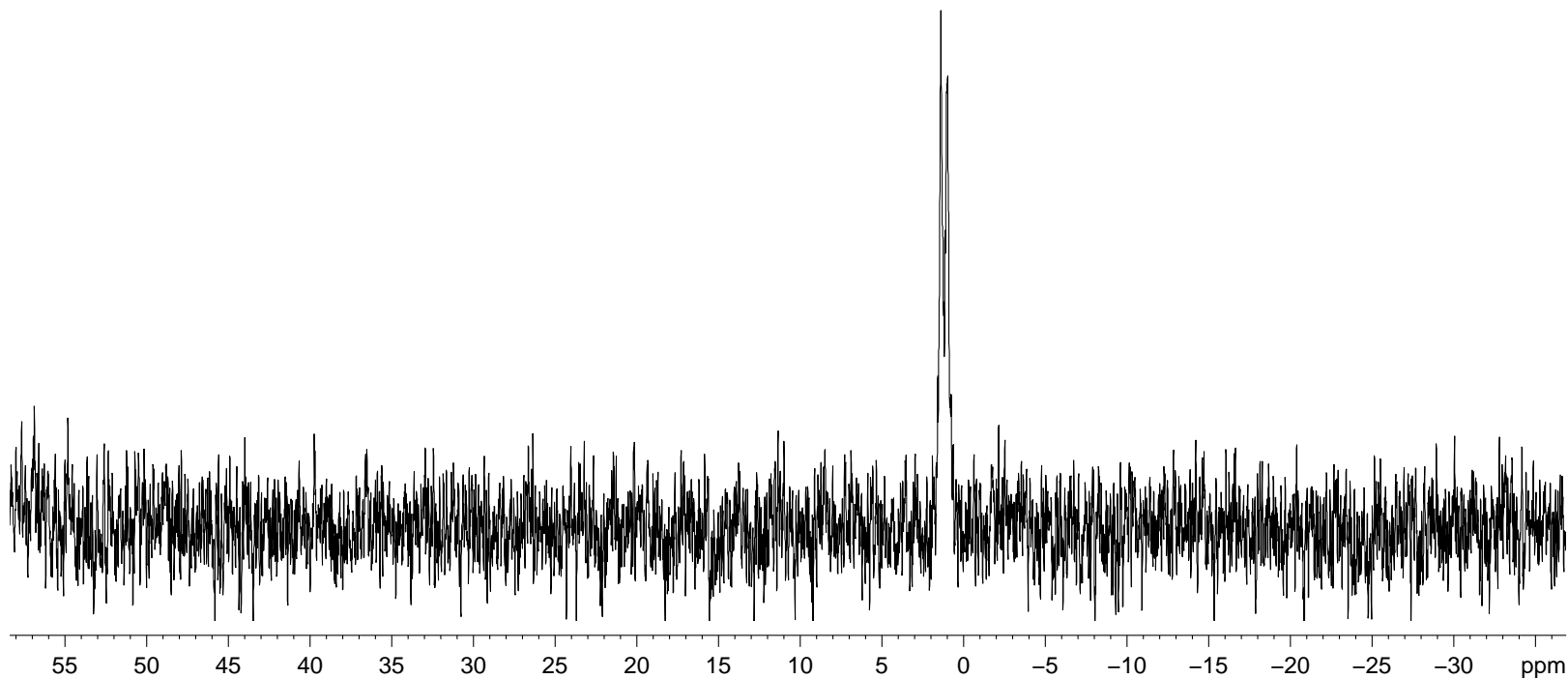


Figure S24.  $^{31}\text{P}$  NMR spectrum of compound **2b**

## References

1. M. S. Paget, L. Chamberlin, A. Atrih, S. J. Foster and M. J. Buttner, *J Bacteriol*, 1999, **181**, 204-211.
2. B. Gust, G. L. Challis, K. Fowler, T. Kieser and K. F. Chater, *Proc Natl Acad Sci U S A*, 2003, **100**, 1541-1546.
3. Y. Yu, L. Bai, K. Minagawa, X. Jian, L. Li, J. Li, S. Chen, E. Cao, T. Mahmud, H. G. Floss, X. Zhou and Z. Deng, *Appl Environ Microbiol*, 2005, **71**, 5066-5076.
4. Y. Sun, X. Zhou, J. Liu, K. Bao, G. Zhang, G. Tu, T. Kieser and Z. Deng, *Microbiology*, 2002, **148**, 361-371.
5. T. Smokvina, P. Mazodier, F. Boccard, C. J. Thompson and M. Guerineau, *Gene*, 1990, **94**, 53-59.
6. H. Xu, Y. Zhang, J. Yang, T. Mahmud, L. Bai and Z. Deng, *Chem Biol*, 2009, **16**, 567-576.