Isolation and Identification of a Urinary Biomarker for Lung Cancer: 27-Nor-5β-cholestane-3α,7α,12α,24R,25S pentol glucuronide and its Deuterated Analog

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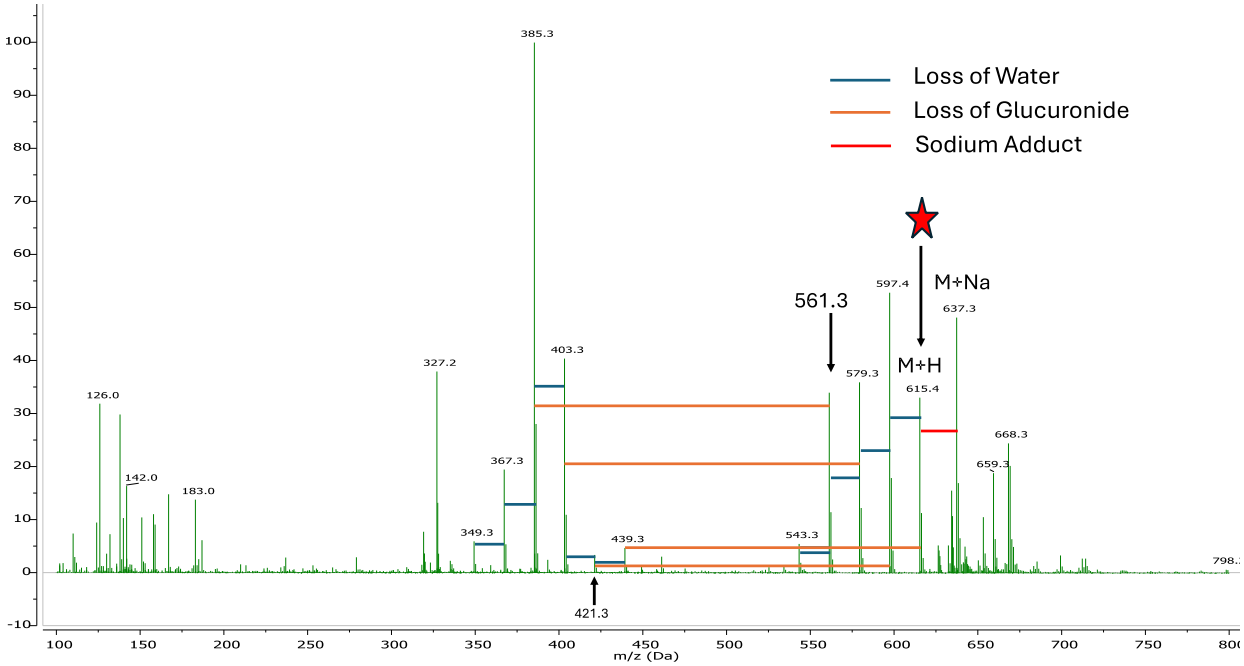
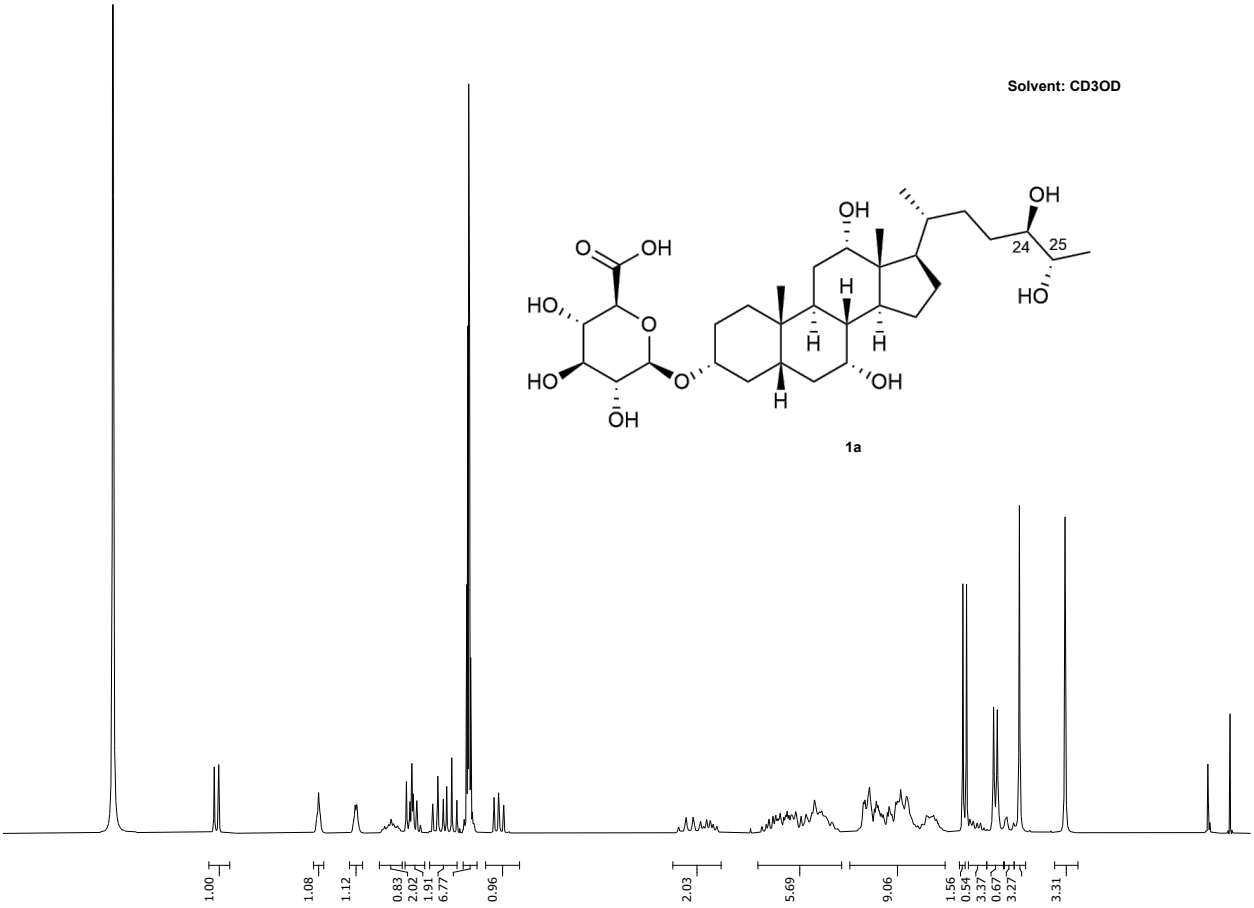
Supplemental Information

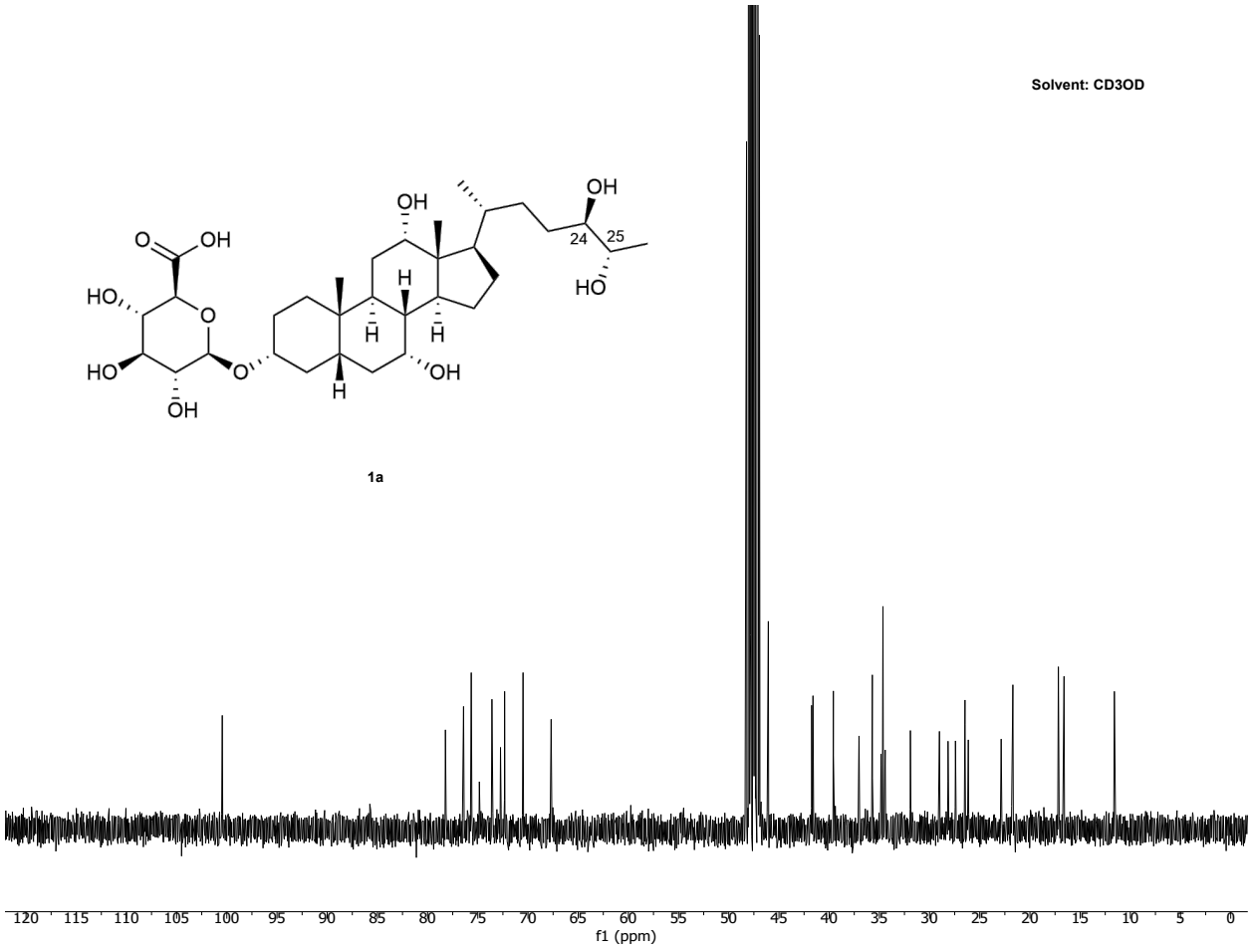
MS Spectrum of "561+" isolated from human urine



Proton and Carbon NMR Spectra

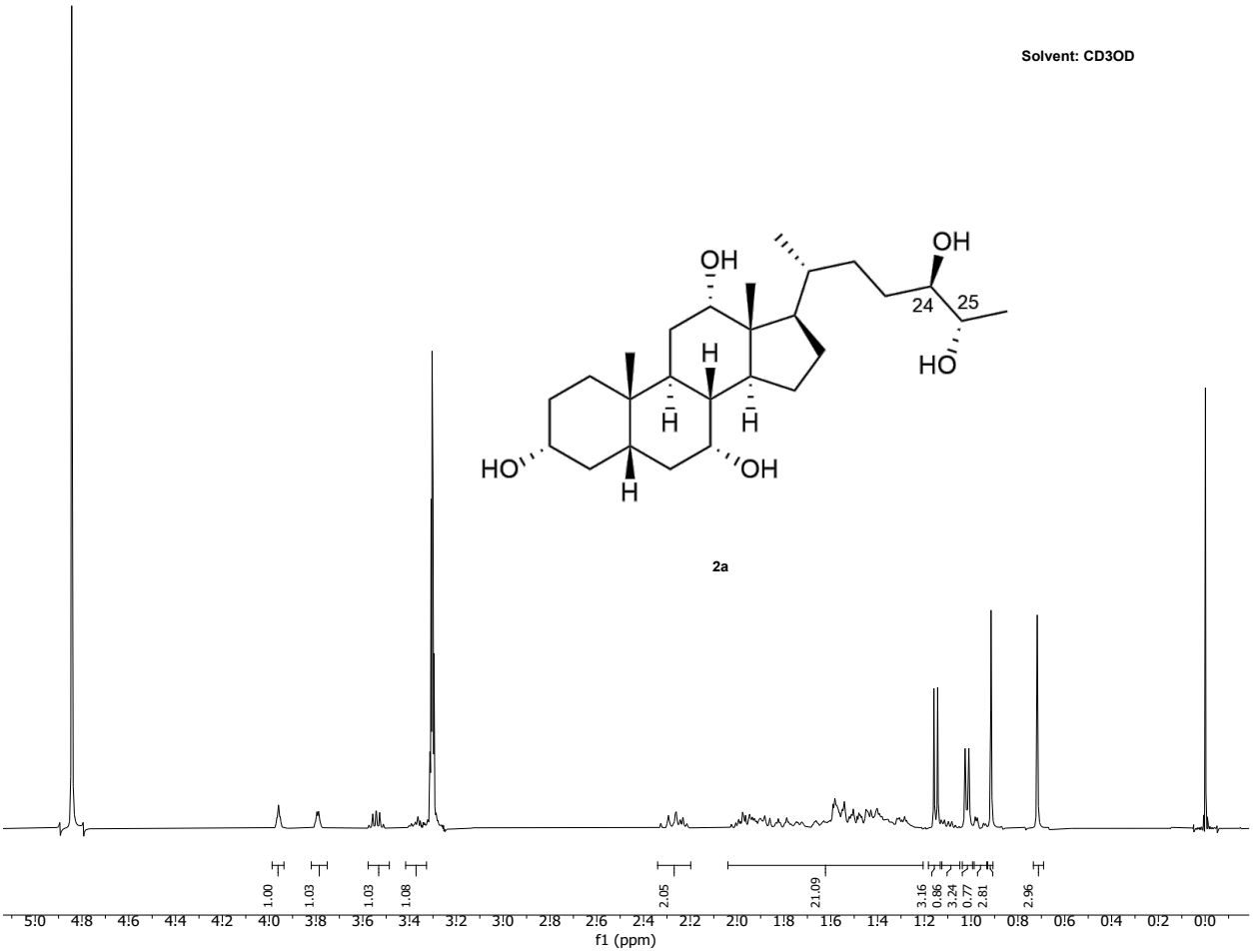
Compound 1a



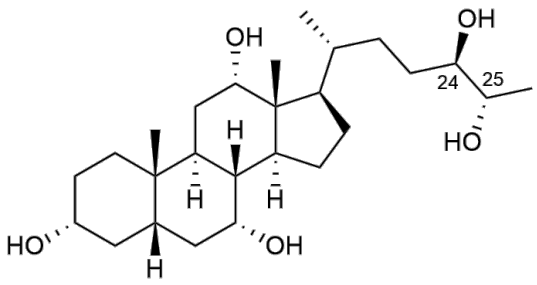

Solvent: CD3OD
OH
O
OH
HO
O
HO
OH
O
H
H
H
OH
H
OH
24
25
HO
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
f1 (ppm)

1a

Compound 2a

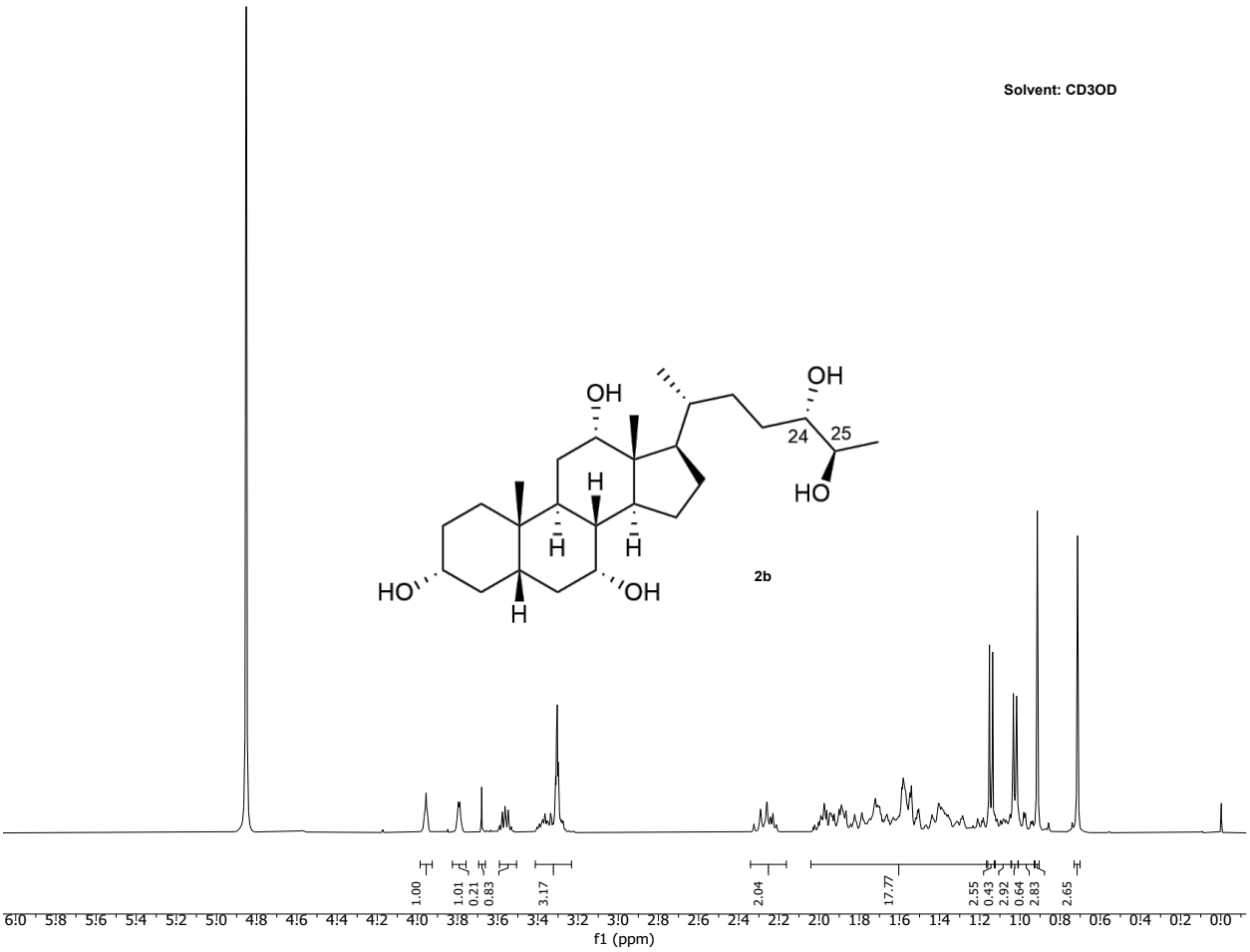

Solvent: CD3OD
OH
OH
24
25
HO
H
H
H
HO
OH
H
2a
1.00
1.03
1.03
1.08
2.05
21.09
3.16
0.86
3.24
0.77
2.81
2.96
5:0 4:8 4:6 4:4 4:2 4:0 3:8 3:6 3:4 3:2 3:0 2:8 2:6 2:4 2:2 2:0 1:8 1:6 1:4 1:2 1:0 0:8 0:6 0:4 0:2 0:0
f1 (ppm)

Solvent: CD3OD

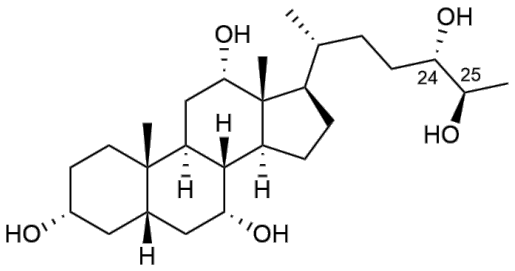


2a

Compound 2b

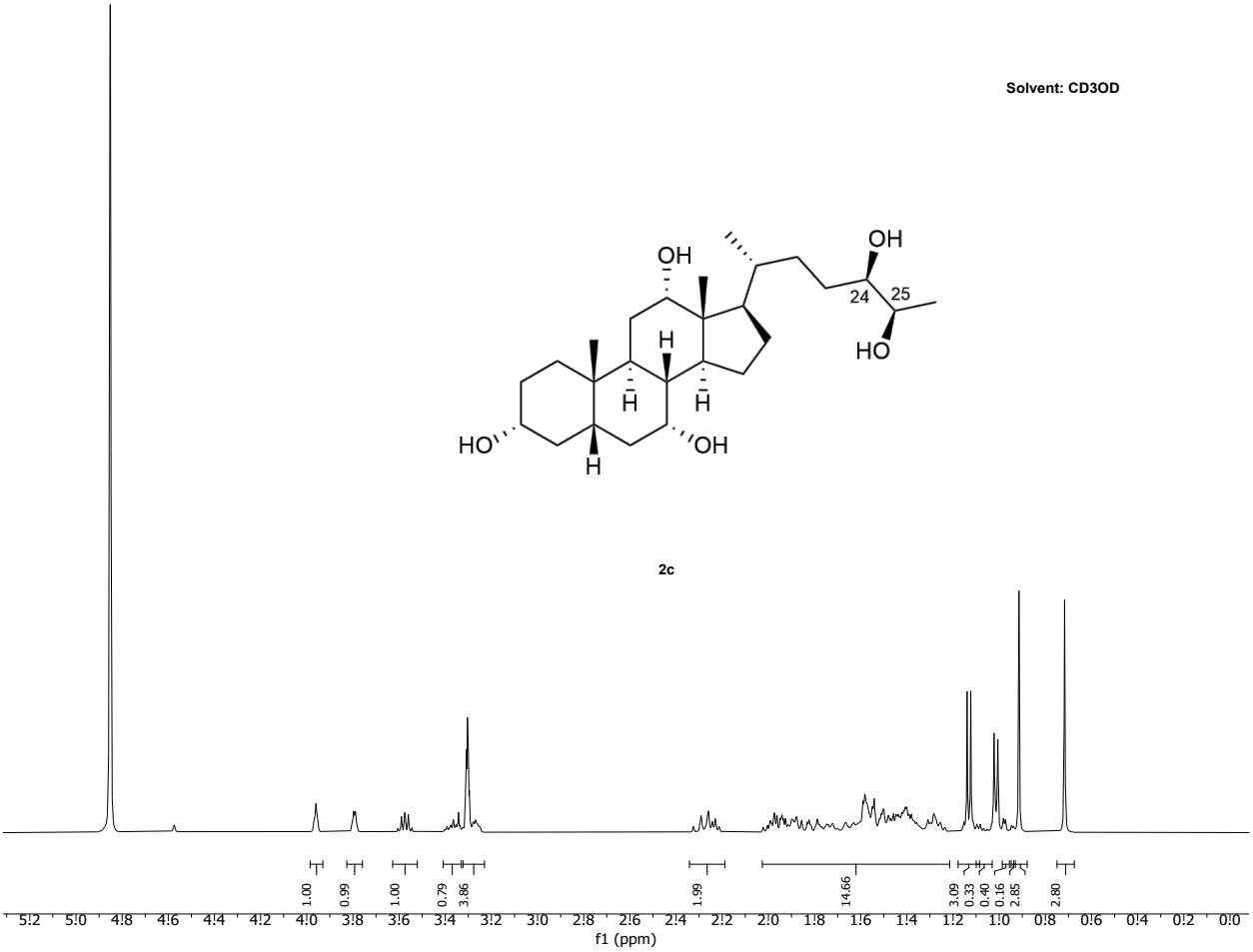

Solvent: CD3OD
OH
OH
24
25
HO
H
H
H
HO
OH
H
2b
1.00
1.01
0.21
0.83
3.17
2.04
17.77
2.55
0.43
2.92
0.64
2.83
2.65
6:0 5:8 5:6 5:4 5:2 5:0 4:8 4:6 4:4 4:2 4:0 3:8 3:6 3:4 3:2 3:0 2:8 2:6 2:4 2:2 2:0 1:8 1:6 1:4 1:2 1:0 0:8 0:6 0:4 0:2 0:0
f1 (ppm)

Solvent: CD3OD

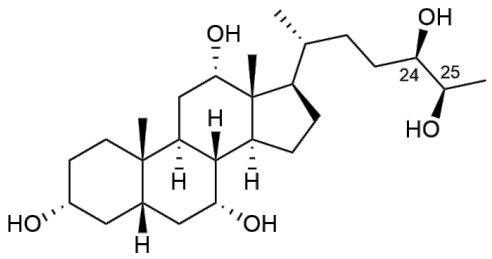


2b

Compound 2c

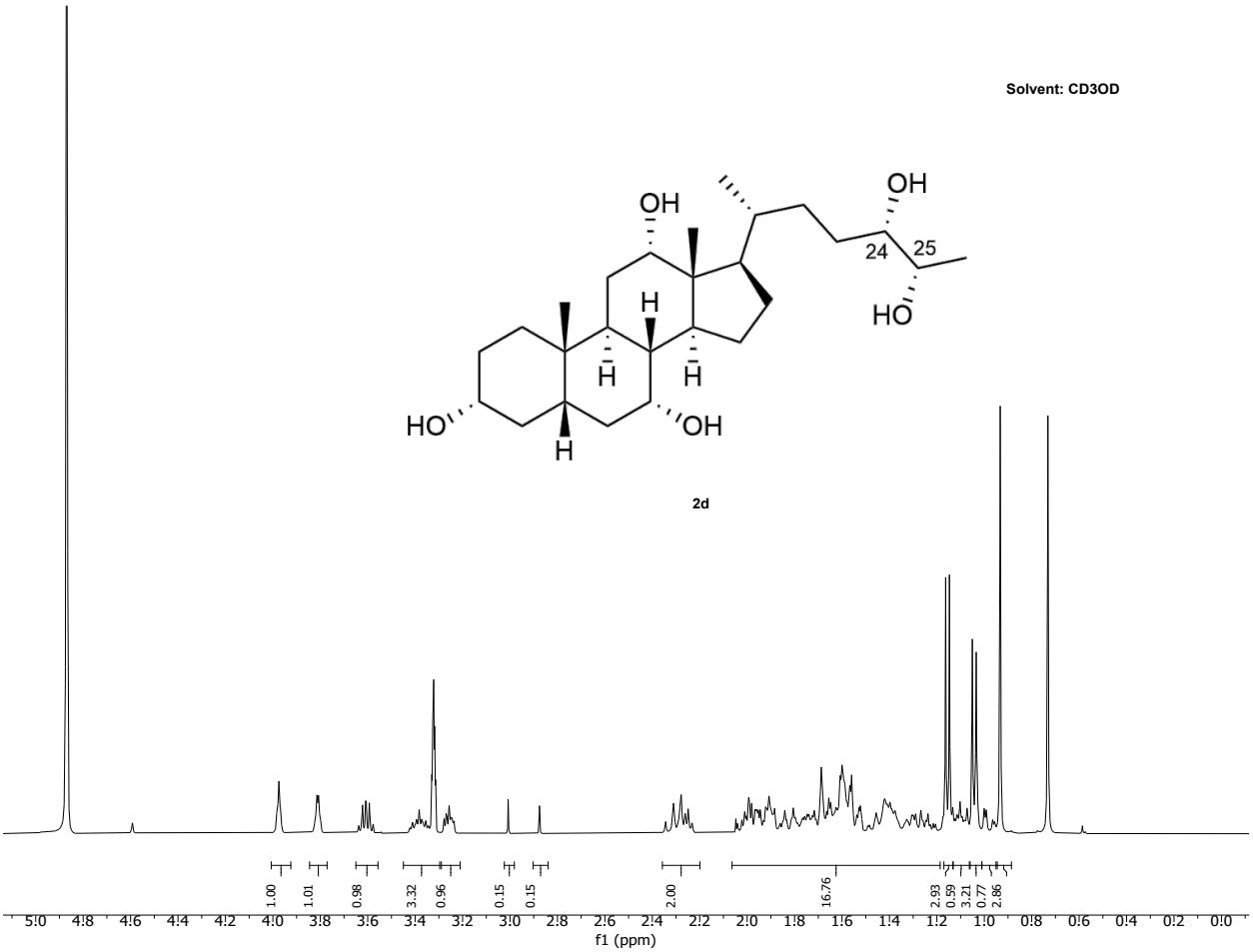

Solvent: CD3OD
OH
OH
24
25
HO
H
H
H
HO
OH
H
2c
1.00
0.99
1.00
0.79
3.86
1.99
14.66
3.09
0.33
0.40
0.16
2.85
2.80
5:2
5:0
4:8
4:6
4:4
4:2
4:0
3:8
3:6
3:4
3:2
3:0
2:8
2:6
2:4
2:2
2:0
1:8
1:6
1:4
1:2
1:0
0:8
0:6
0:4
0:2
0:0
f1 (ppm)

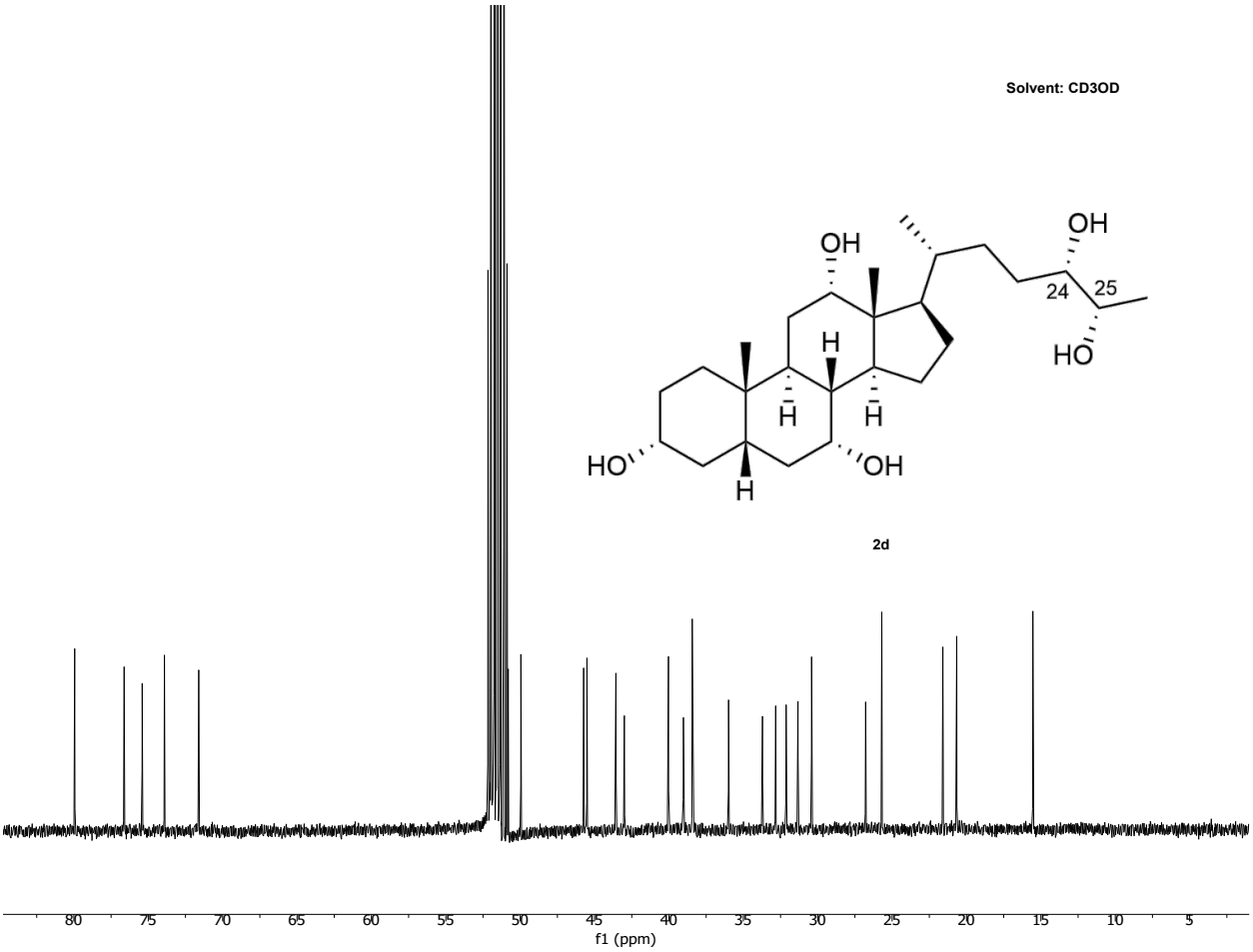
Solvent: CD3OD



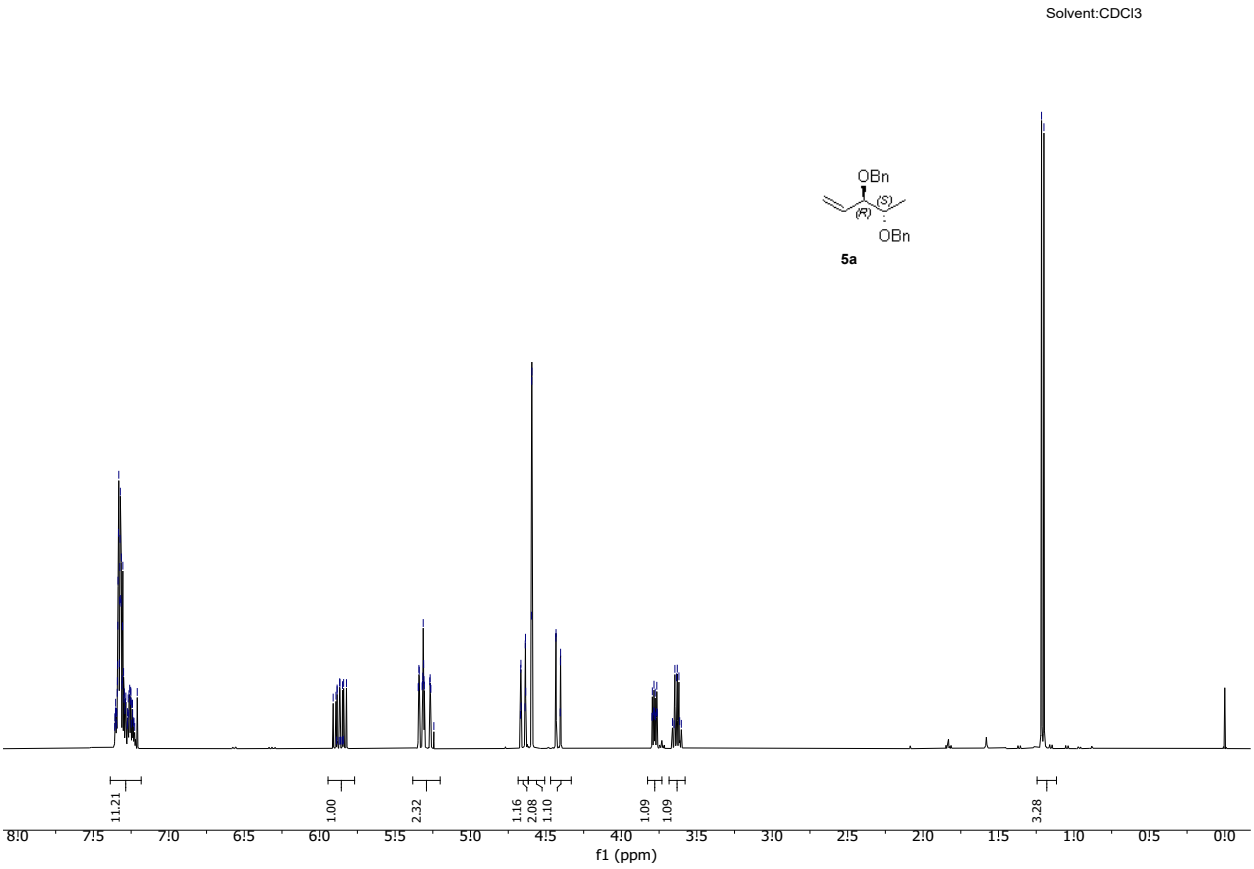
2c

Compound 2d

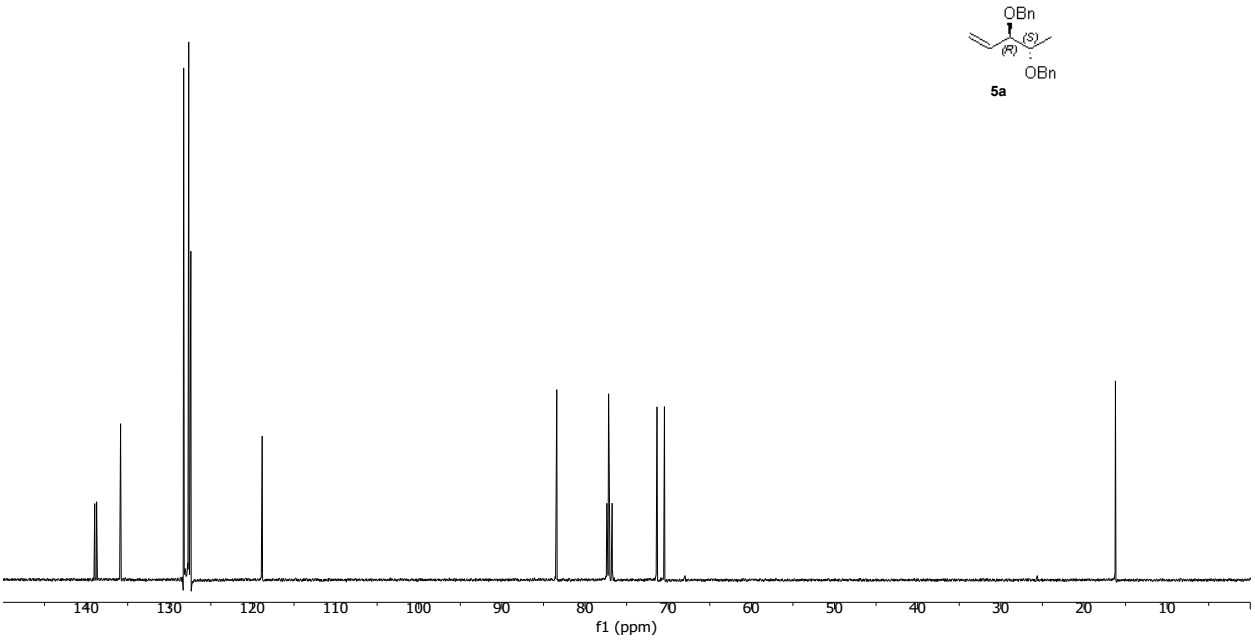

Solvent: CD3OD
OH
OH
24
25
HO
H
H
H
HO
OH
H
2d
1.00
1.01
0.98
3.32
0.96
0.15
0.15
2.00
16.76
2.93
0.59
3.21
0.77
2.86
5:0
4:8
4:6
4:4
4:2
4:0
3:8
3:6
3:4
3:2
3:0
2:8
2:6
2:4
2:2
2:0
1:8
1:6
1:4
1:2
1:0
0:8
0:6
0:4
0:2
0:0
f1 (ppm)


Solvent: CD3OD
OH
OH
24
25
HO
H
H
H
HO
OH
H
2d
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
f1 (ppm)

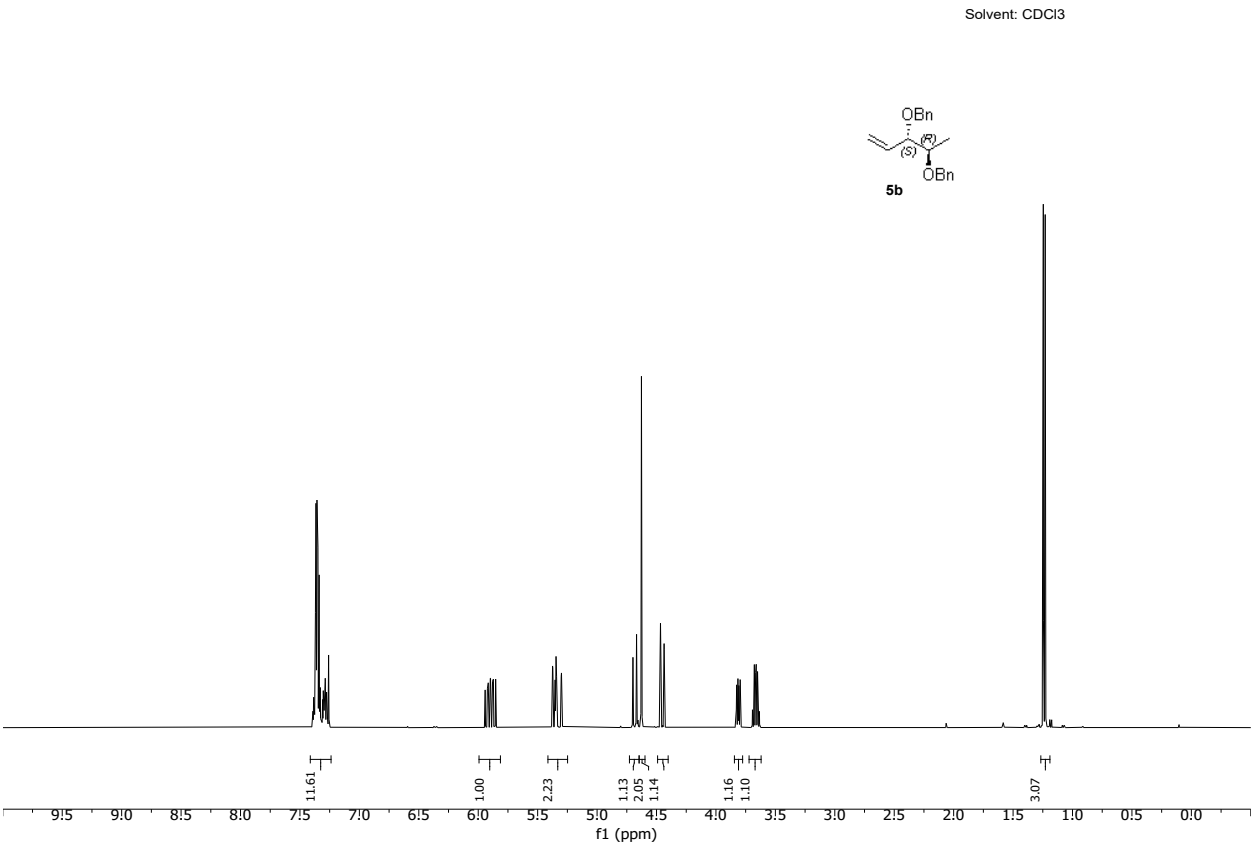
Compound 5a


Solvent:CDCl3
OBn
(S)
(R)
OBn
5a
11.21
1.00
2.32
1.16
2.08
1.10
1.09
1.09
3.28
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

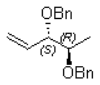
Solvent: CDCl3



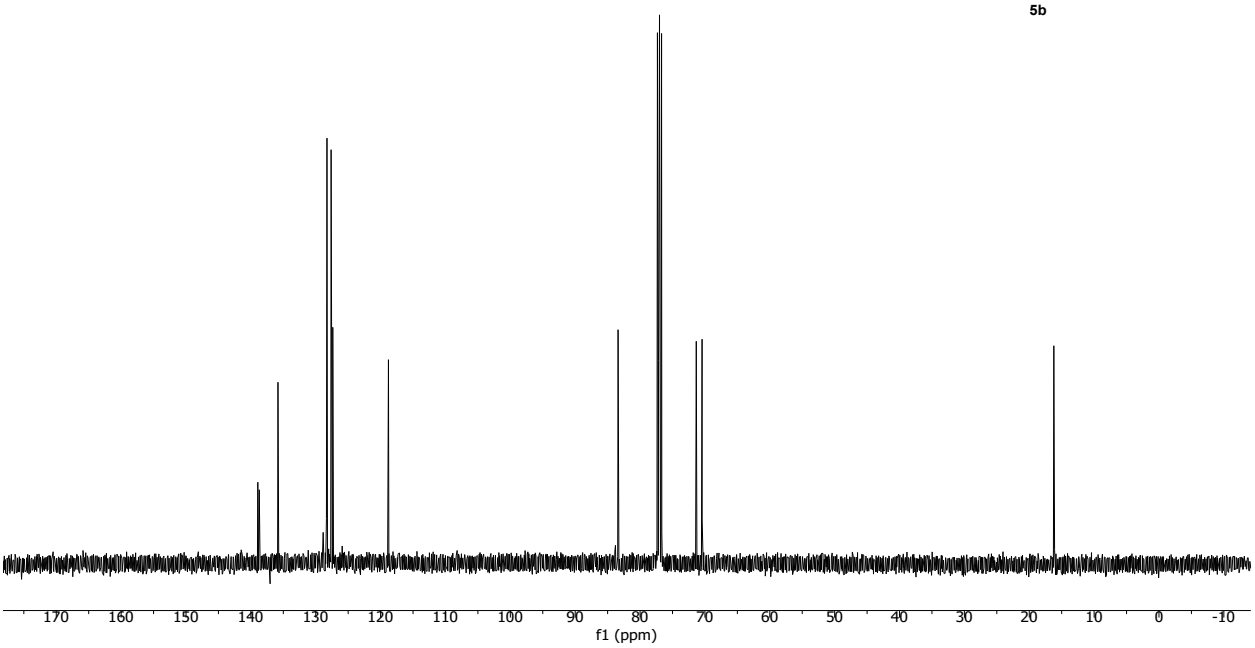
Compound 5b


Solvent: CDCl3
OBn
(S)
(R)
OBn
5b
11.61
1.00
2.23
1.13
2.05
1.14
1.16
1.10
3.07
9!5
9!0
8!5
8!0
7!5
7!0
6!5
6!0
5!5
5!0
4!5
4!0
3!5
3!0
2!5
2!0
1!5
1!0
0!5
0!0
f1 (ppm)

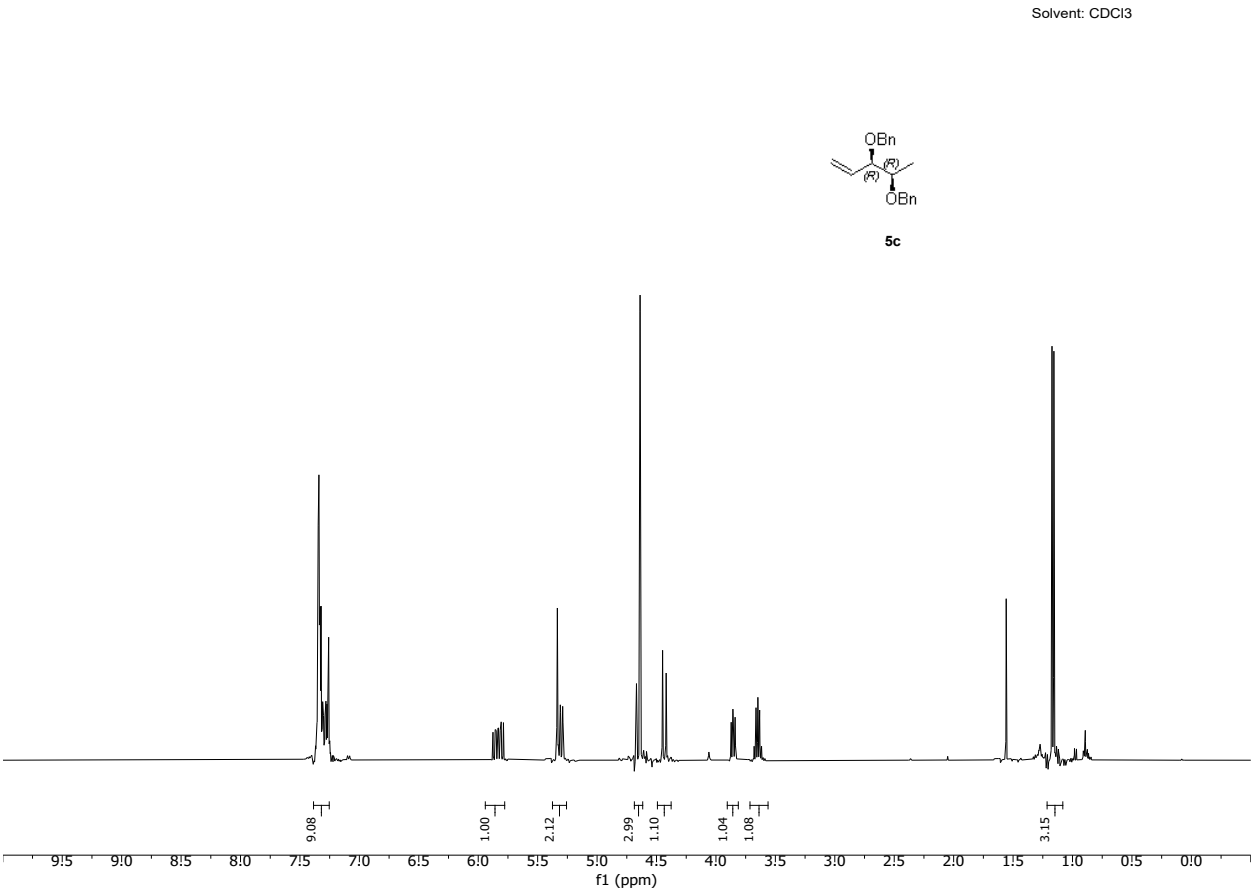
Solvent: CDCl3



5b



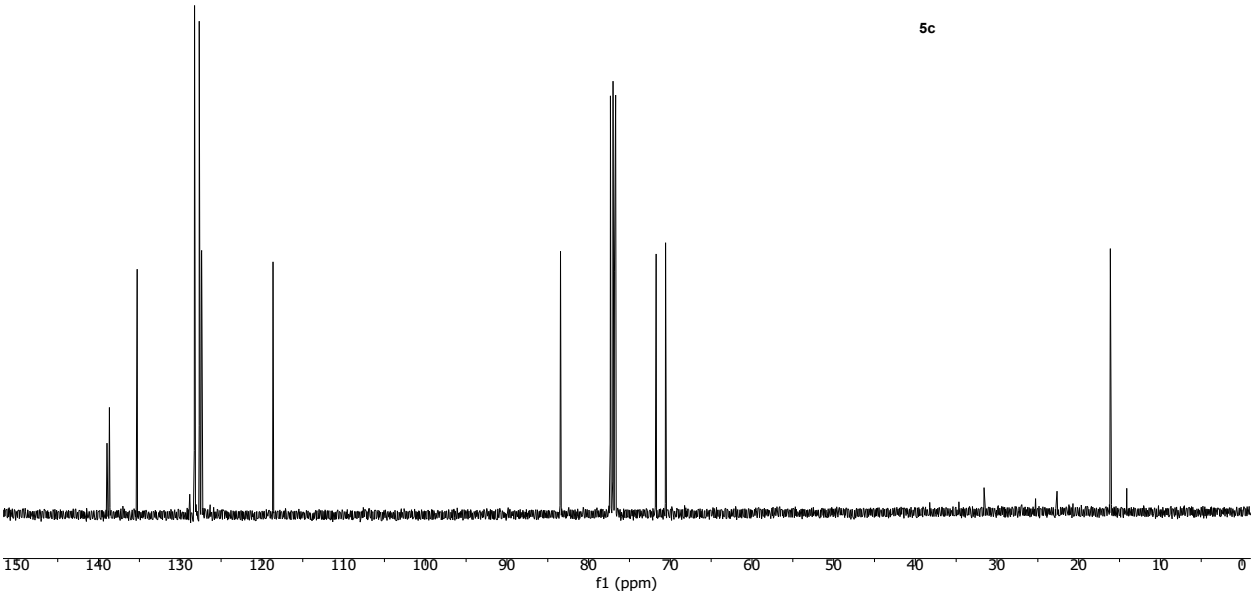
Compound 5c


Solvent: CDCl3
OBn
(R)
(R)
OBn
5c
9.08
1.00
2.12
2.99
1.10
1.04
1.08
3.15
9!5 9!0 8!5 8!0 7!5 7!0 6!5 6!0 5!5 5!0 4!5 4!0 3!5 3!0 2!5 2!0 1!5 1!0 0!5 0!0
f1 (ppm)

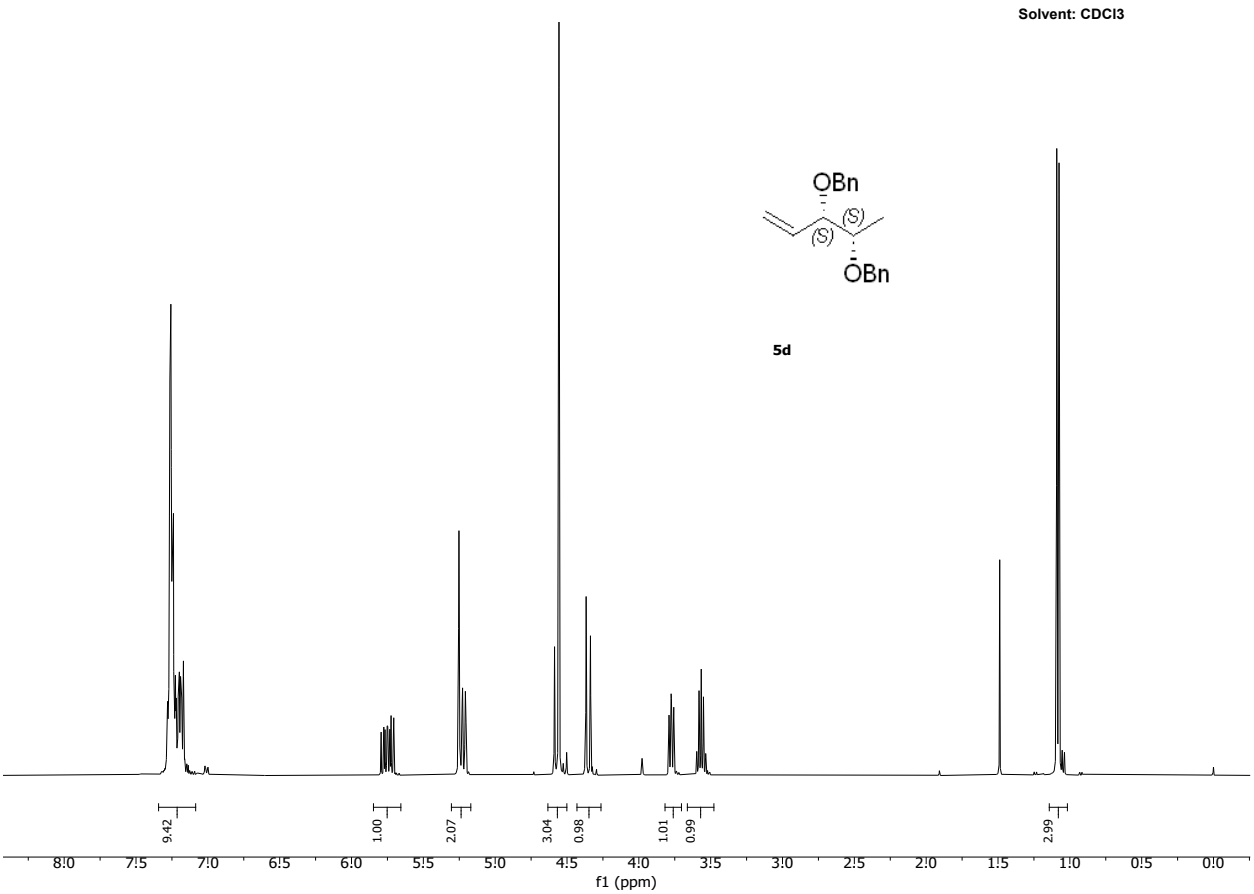
Solvent CDCl3



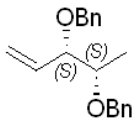
5c



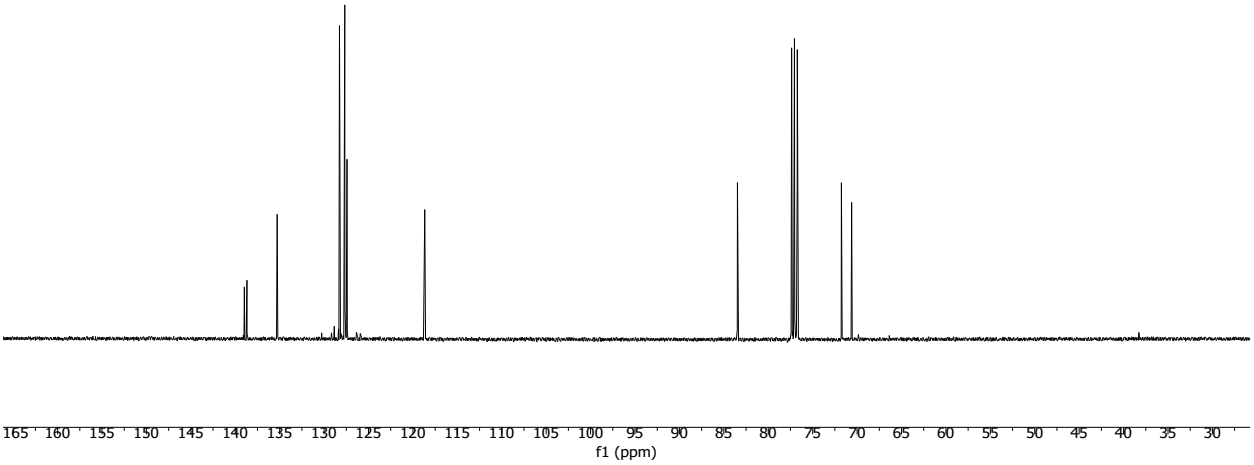
Compound 5d


Solvent: CDCl3
OBn
(S)
(S)
OBn
5d
9.42
1.00
2.07
3.04
0.98
1.01
0.99
2.99
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

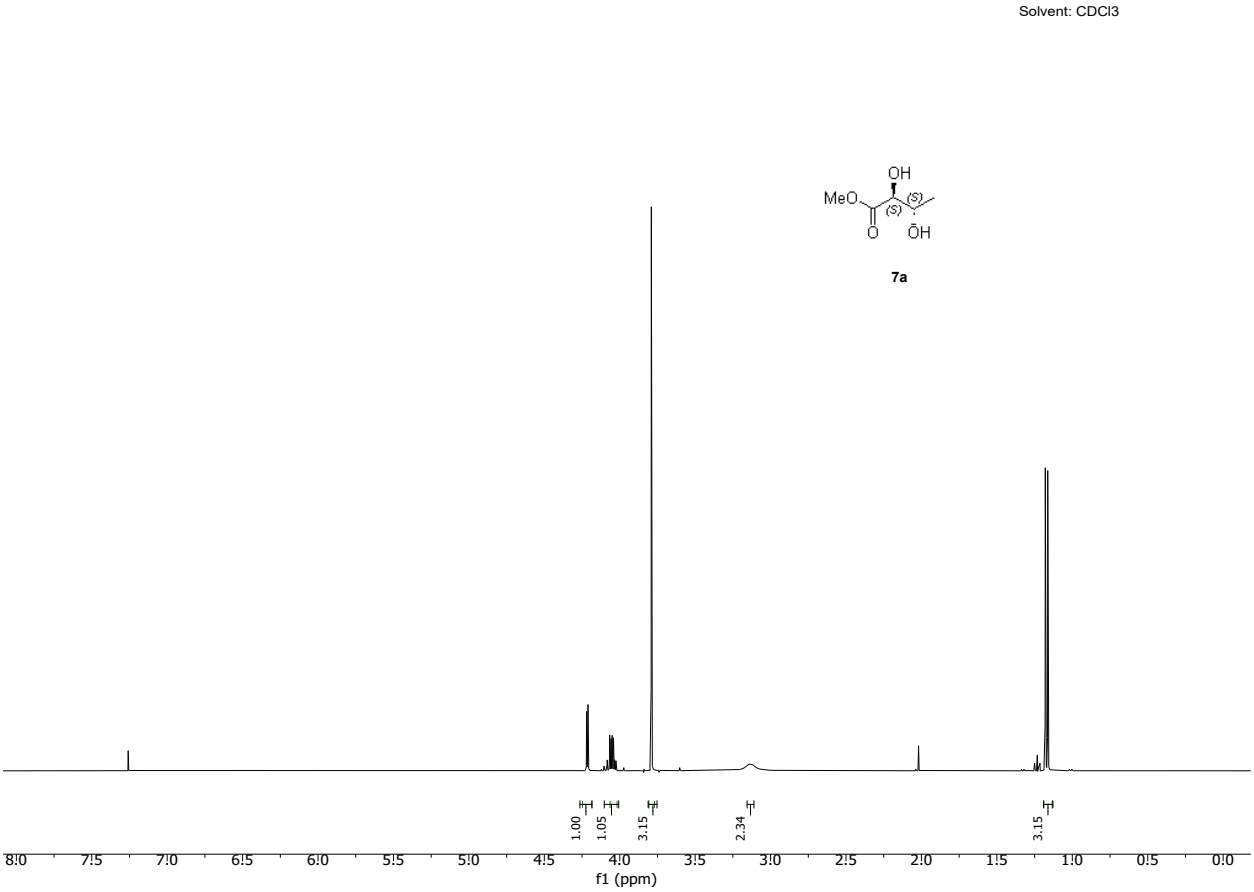
Solvent: CDCl3



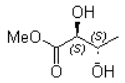
5d



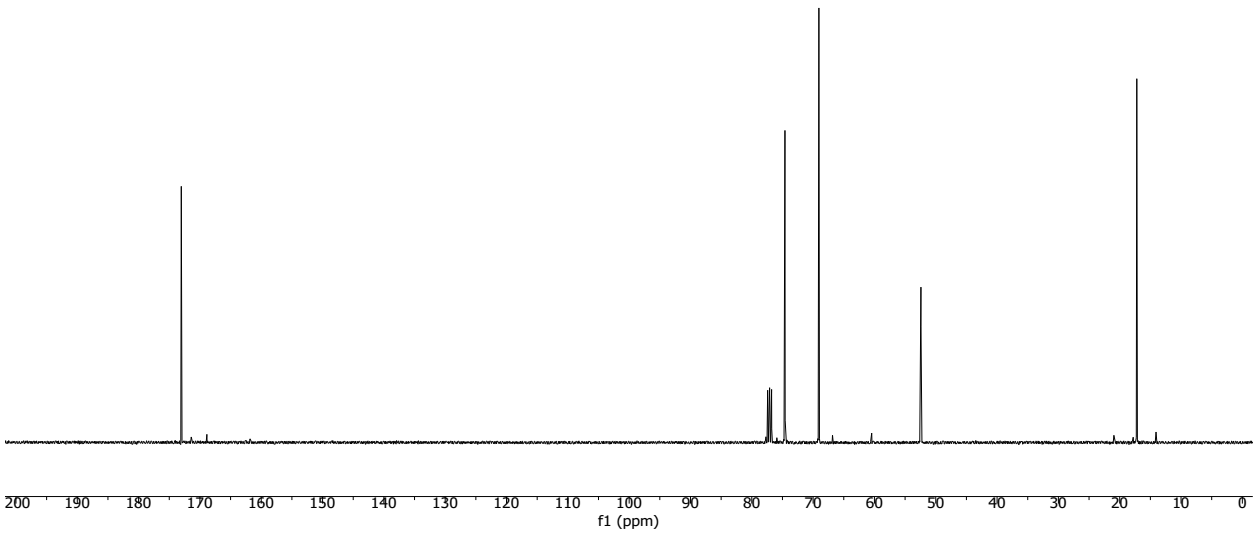
Compound 7a


Solvent: CDCl3
OH
MeO
(S)
(S)
O
OH
7a
1.00
1.05
3.15
2.34
3.15
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

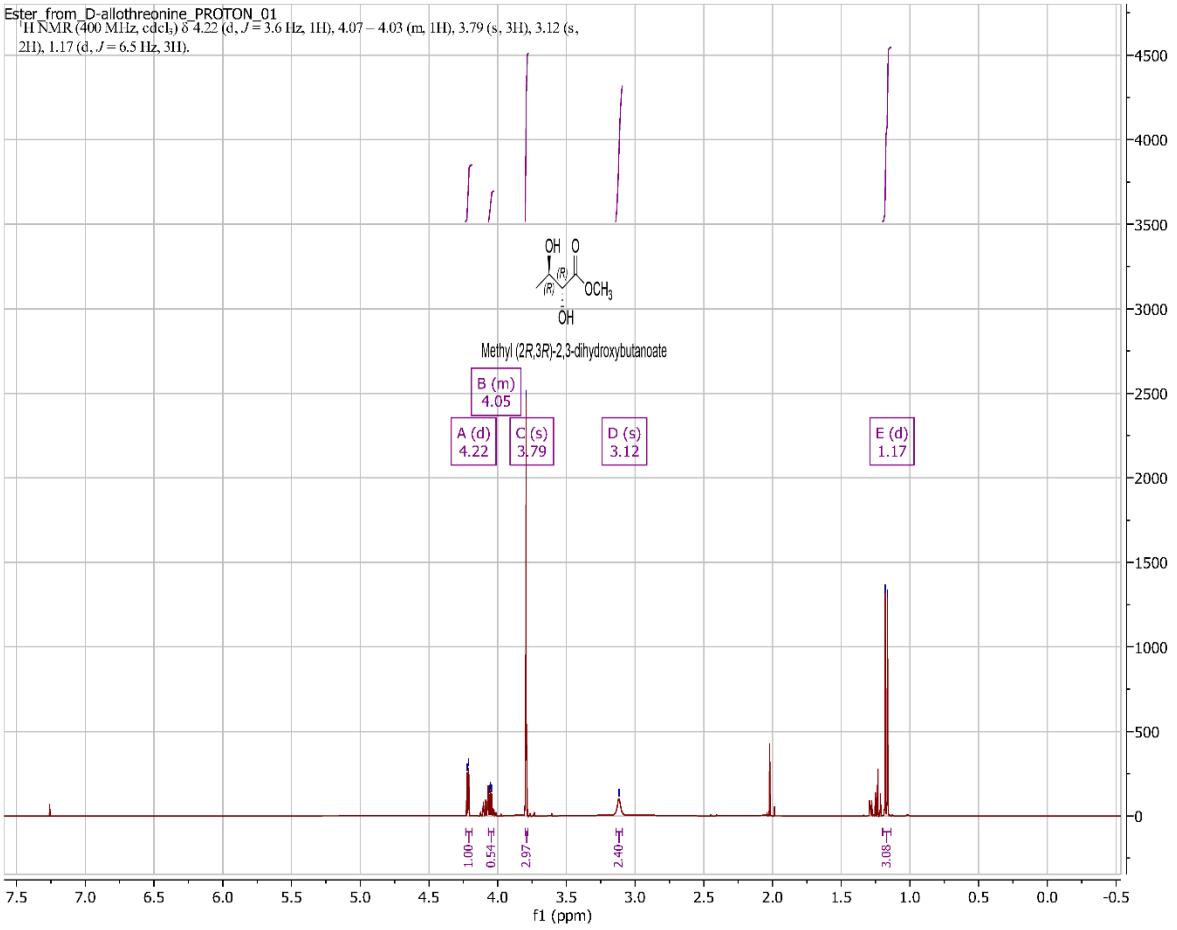
Solvent: CDCl3

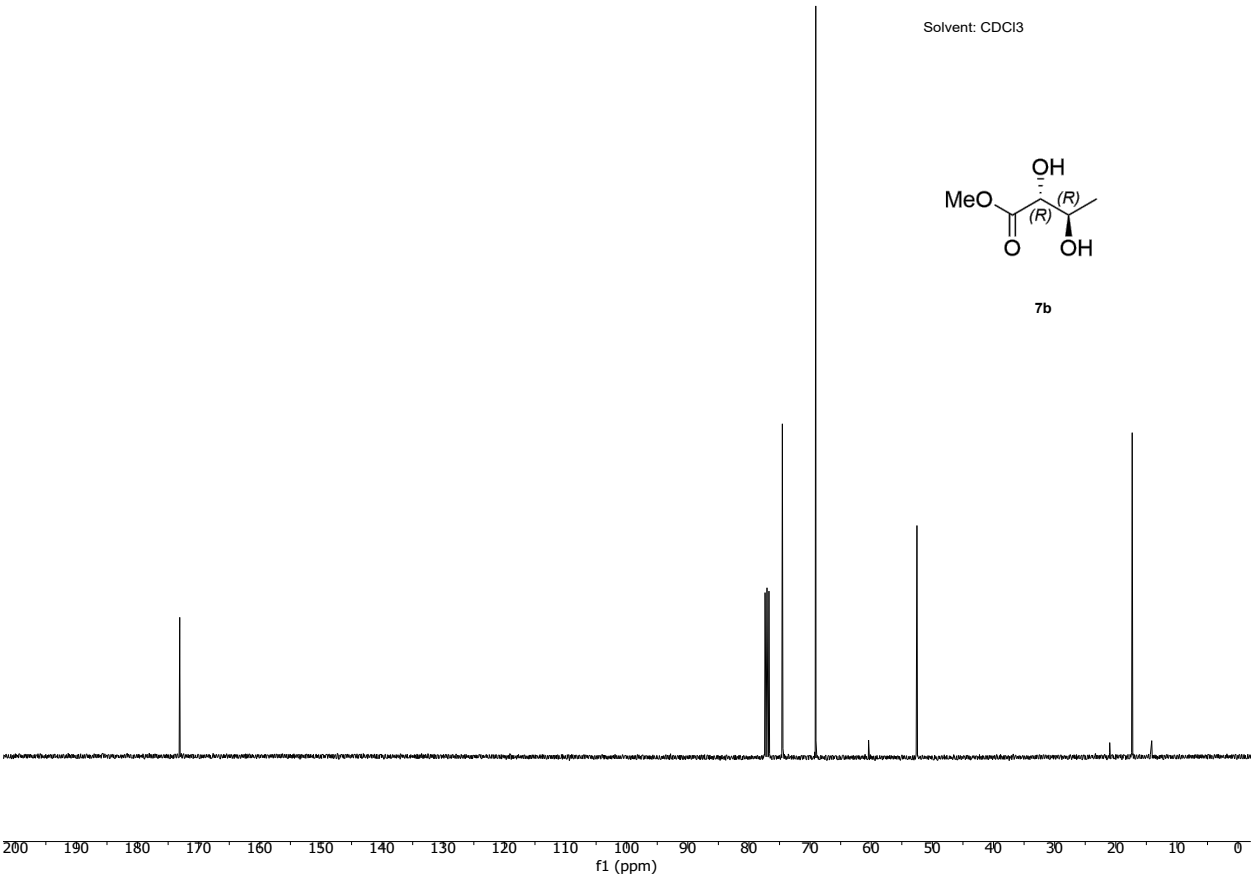


7a

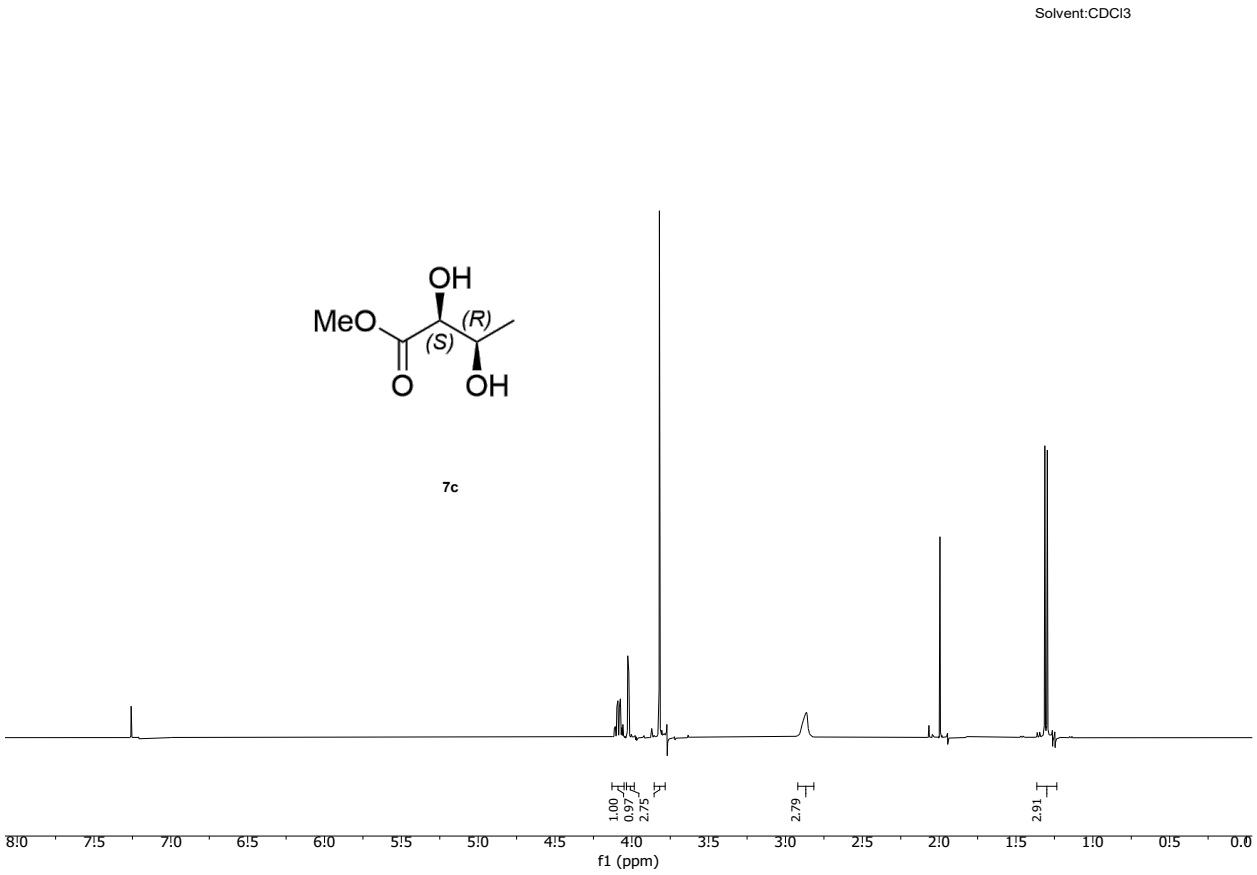


Compound 7b

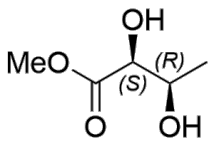

Ester_from_D-allothreonine_PROTON_01
^{1}H NMR (400 MHz, cdcl$_3$) δ 4.22 (d, J = 3.6 Hz, 1H), 4.07 – 4.03 (m, 1H), 3.79 (s, 3H), 3.12 (s, 2H), 1.17 (d, J = 6.5 Hz, 3H).
Methyl (2R,3R)-2,3-dihydroxybutanoate
B (m) 4.05
A (d) 4.22
C (s) 3.79
D (s) 3.12
E (d) 1.17
1.00
0.54
2.97
2.40
3.08
f1 (ppm)


Solvent: CDCl3
OH
MeO
(R)
(R)
O
OH
7b
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

Compound 7c

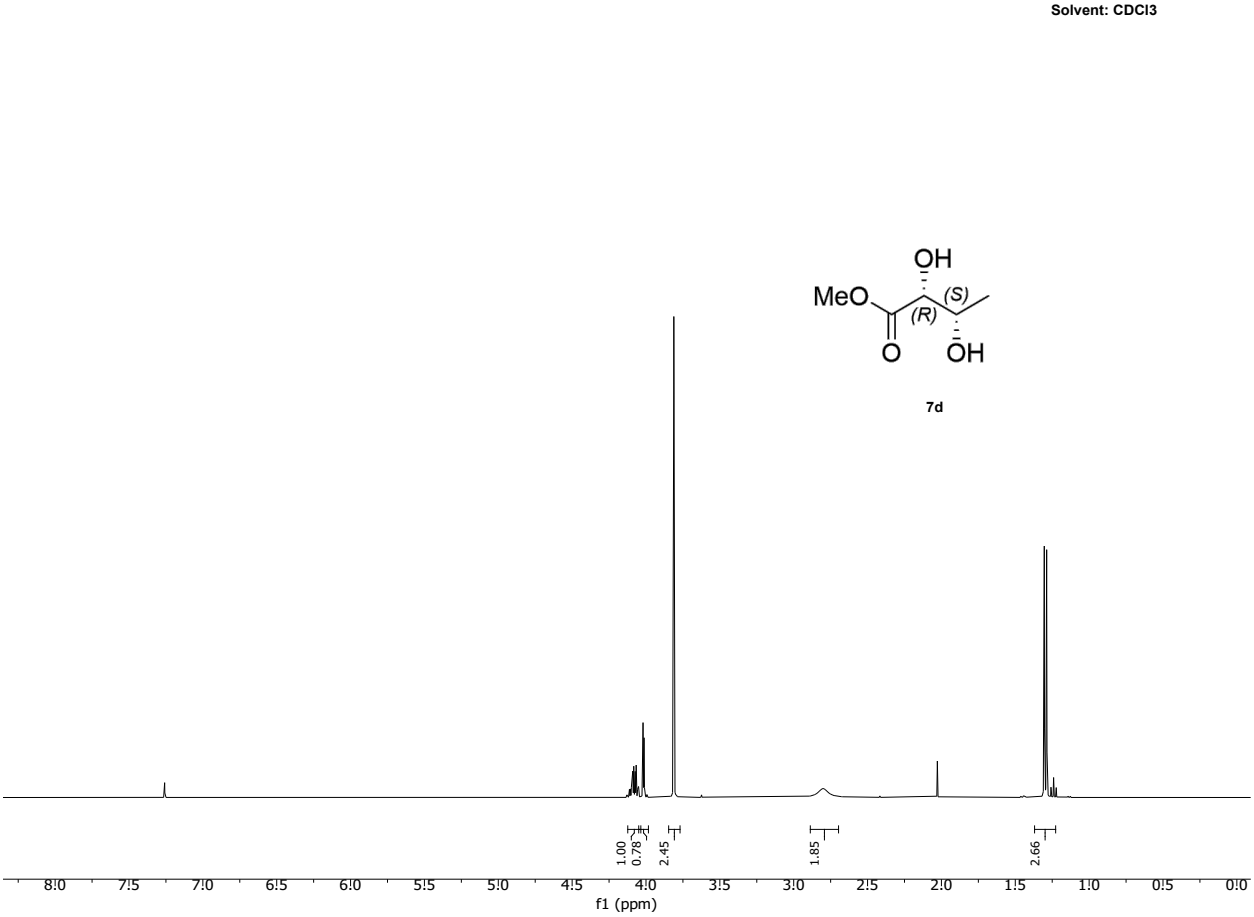

Solvent:CDCl3
OH
MeO
(R)
(S)
O
OH
7c
1.00
0.97
2.75
2.79
2.91
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

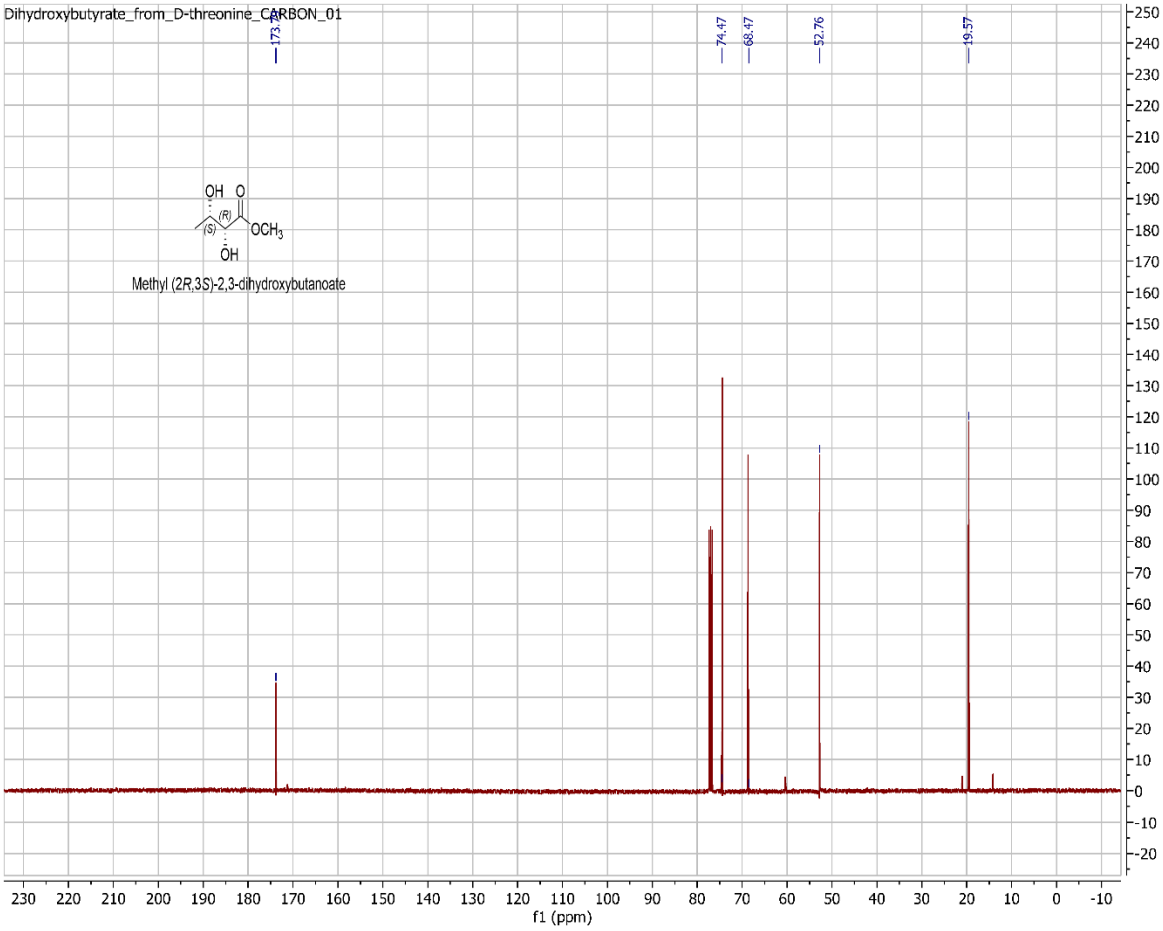
Solvent:CDCl3



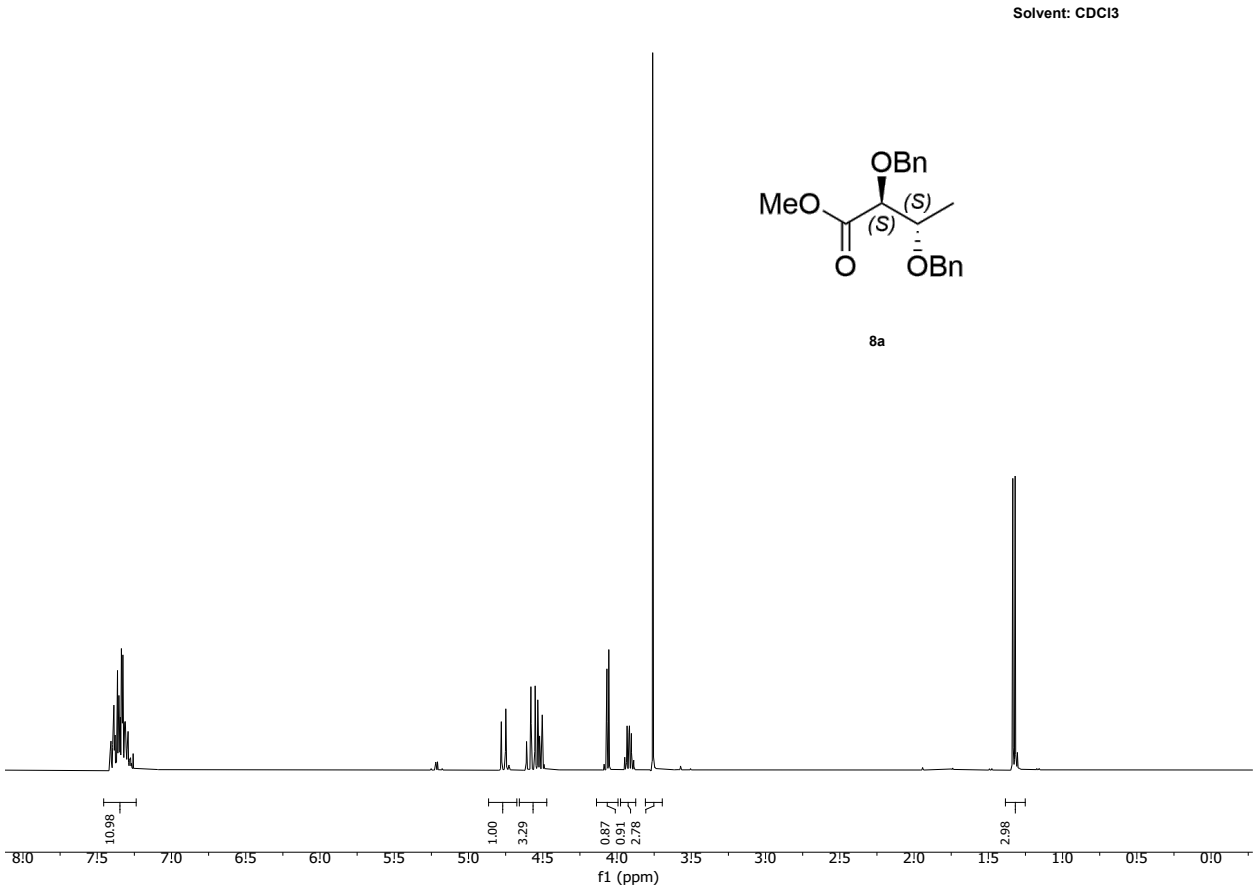
7c

Compound 7d

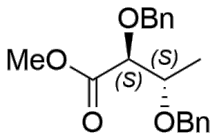

Solvent: CDCl3
OH
MeO
(S)
(R)
O
OH
7d
1.00
0.78
2.45
1.85
2.66
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


Dihydroxybutyrate_from_D-threonine_CARBON_01
173.72
74.47
68.47
52.76
19.57
OH O
(R)
(S)
OCH3
OH
Methyl (2R,3S)-2,3-dihydroxybutanoate
f1 (ppm)

Compound 8a

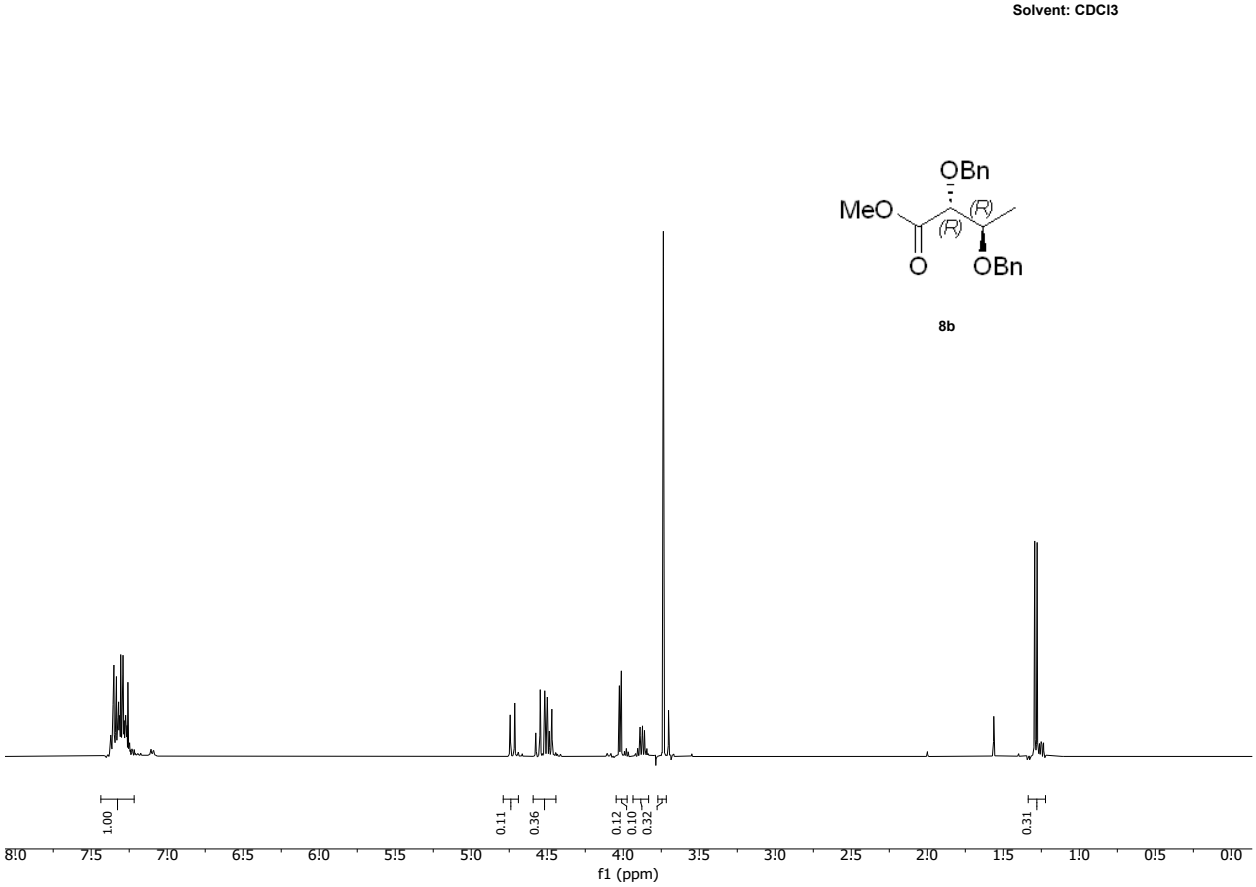

Solvent: CDCl3
OBn
MeO
(S)
(S)
O
OBn
8a
10.98
1.00
3.29
0.87
0.91
2.78
2.98
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Solvent: CDCl3

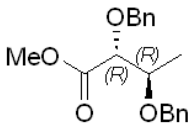


8a

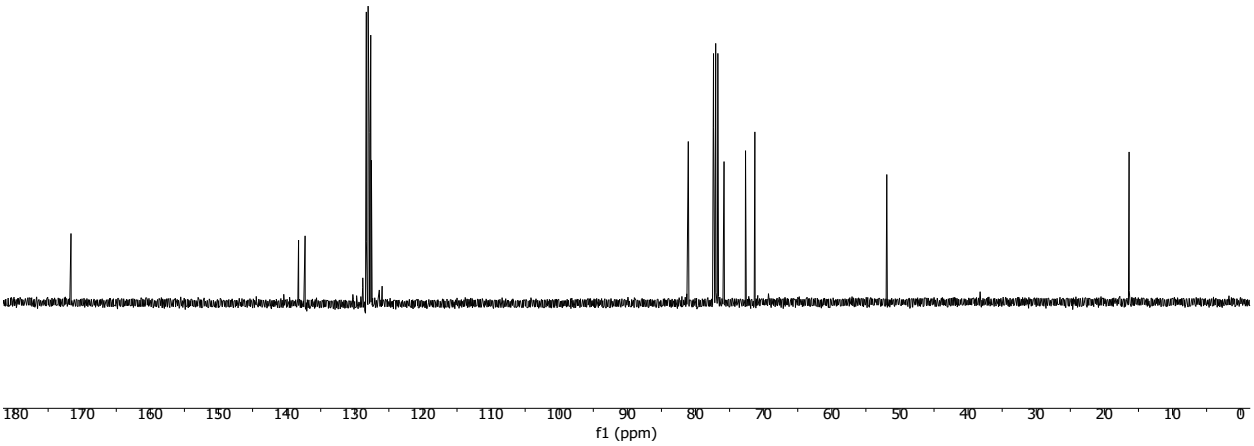
Compound 8b


Solvent: CDCl3
OBn
MeO
(R)
(R)
O
OBn
8b
1.00
0.11
0.36
0.12
0.10
0.32
0.31
8!0
7!5
7!0
6!5
6!0
5!5
5!0
4!5
4!0
3!5
3!0
2!5
2!0
1!5
1!0
0!5
0!0
f1 (ppm)

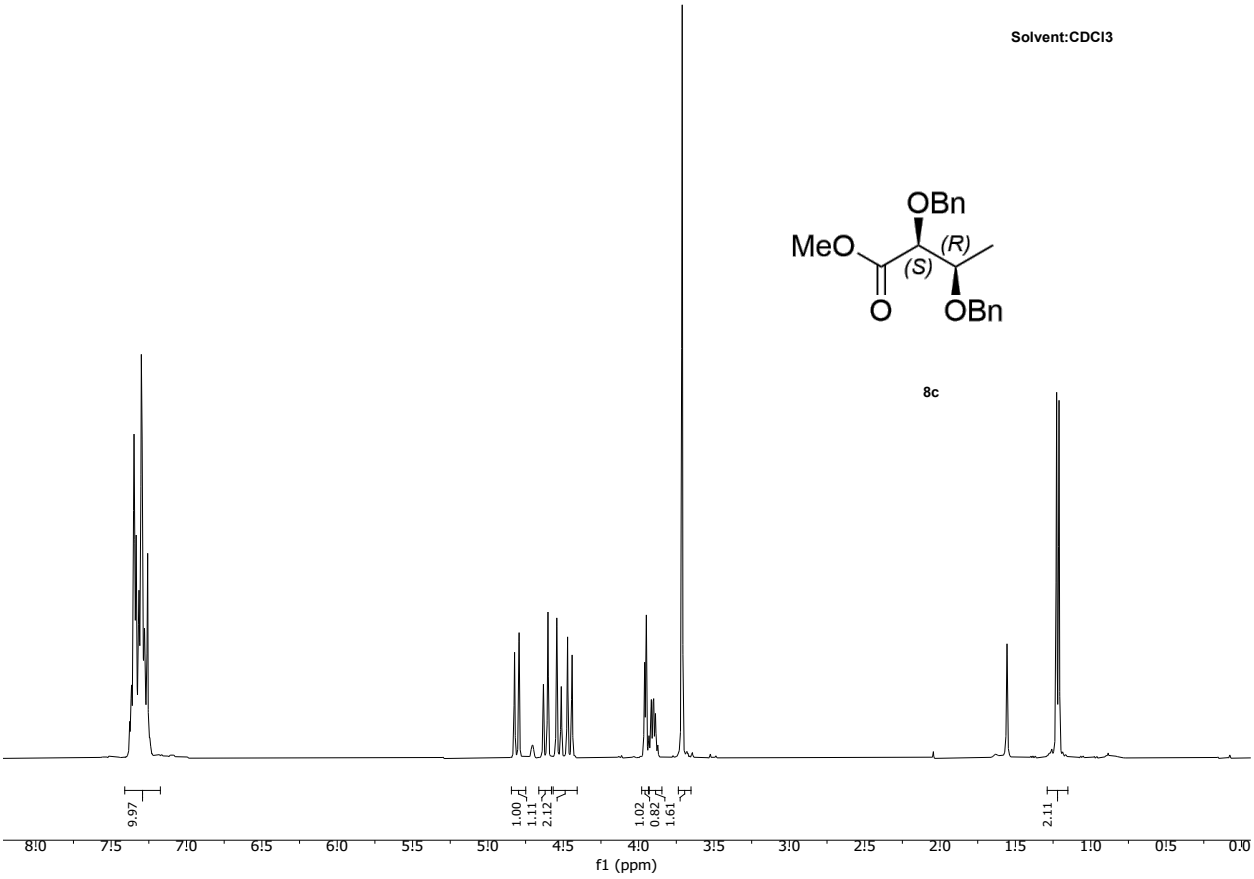
Solvent: CDCl3



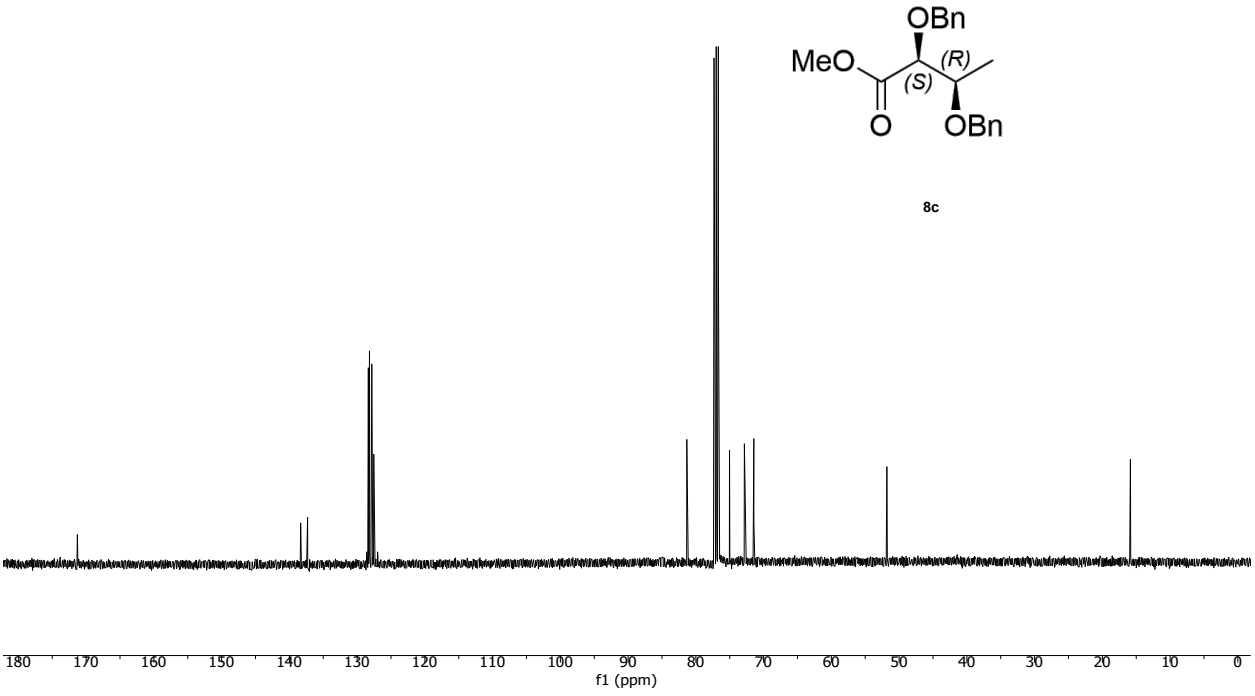
8b



Compound 8c

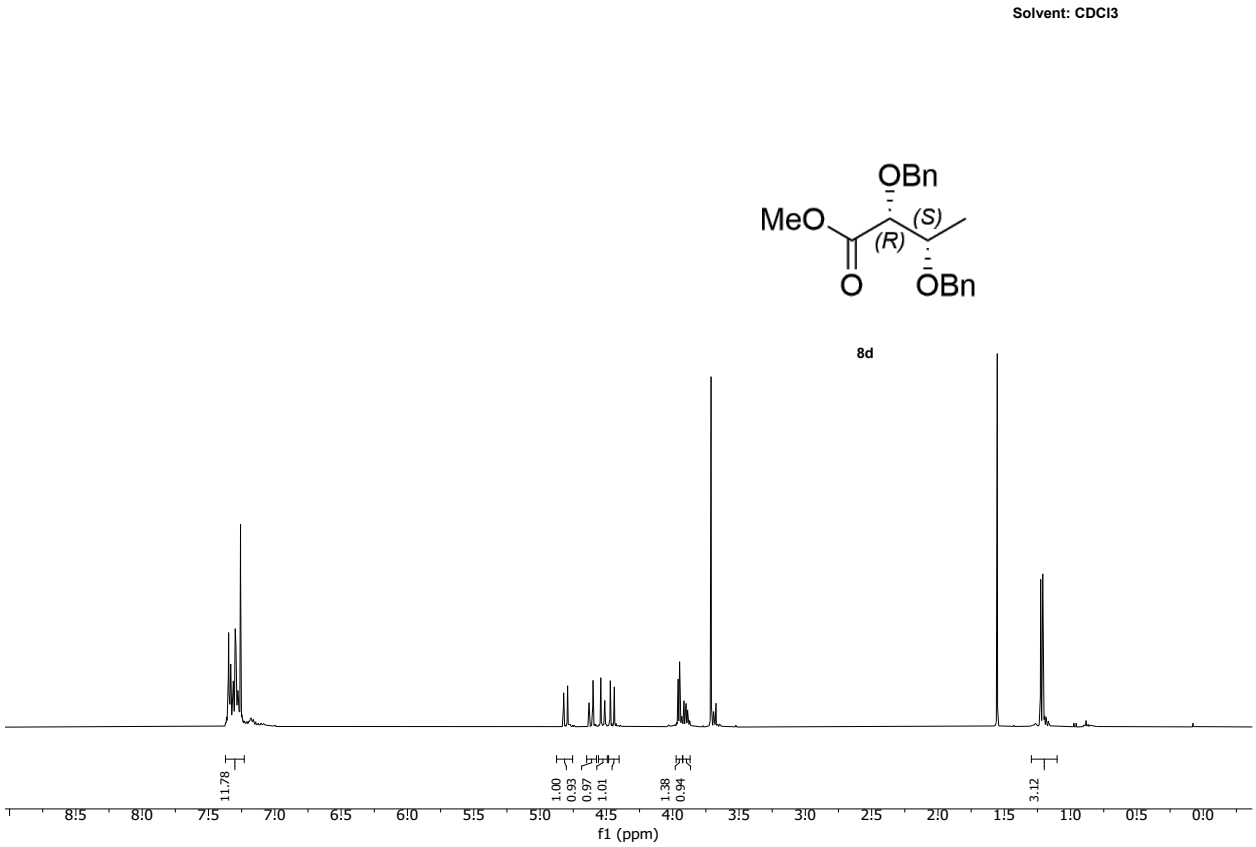

Solvent:CDCl3
OBn
MeO
(R)
(S)
O
OBn
8c
9.97
1.00
1.11
2.12
1.02
0.82
1.61
2.11
8!0
7!5
7!0
6!5
6!0
5!5
5!0
4!5
4!0
3!5
3!0
2!5
2!0
1!5
1!0
0!5
0!0
f1 (ppm)

Solvent:CDCl3

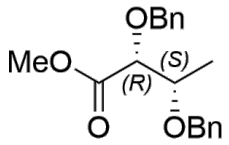


8c

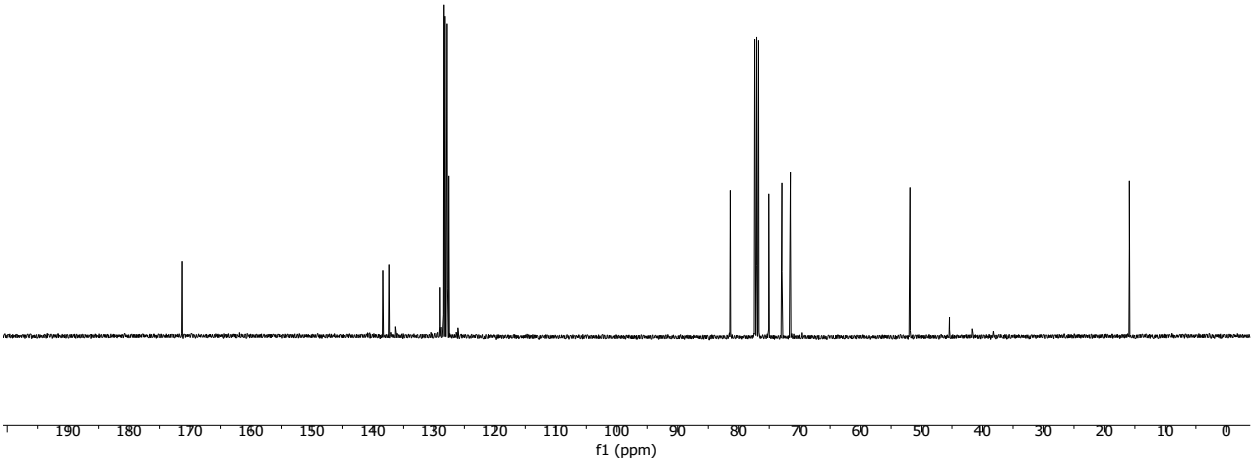
Compound 8d


Solvent: CDCl3
OBn
MeO
(S)
(R)
O
OBn
8d
11.78
1.00
0.93
0.97
1.01
1.38
0.94
3.12
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

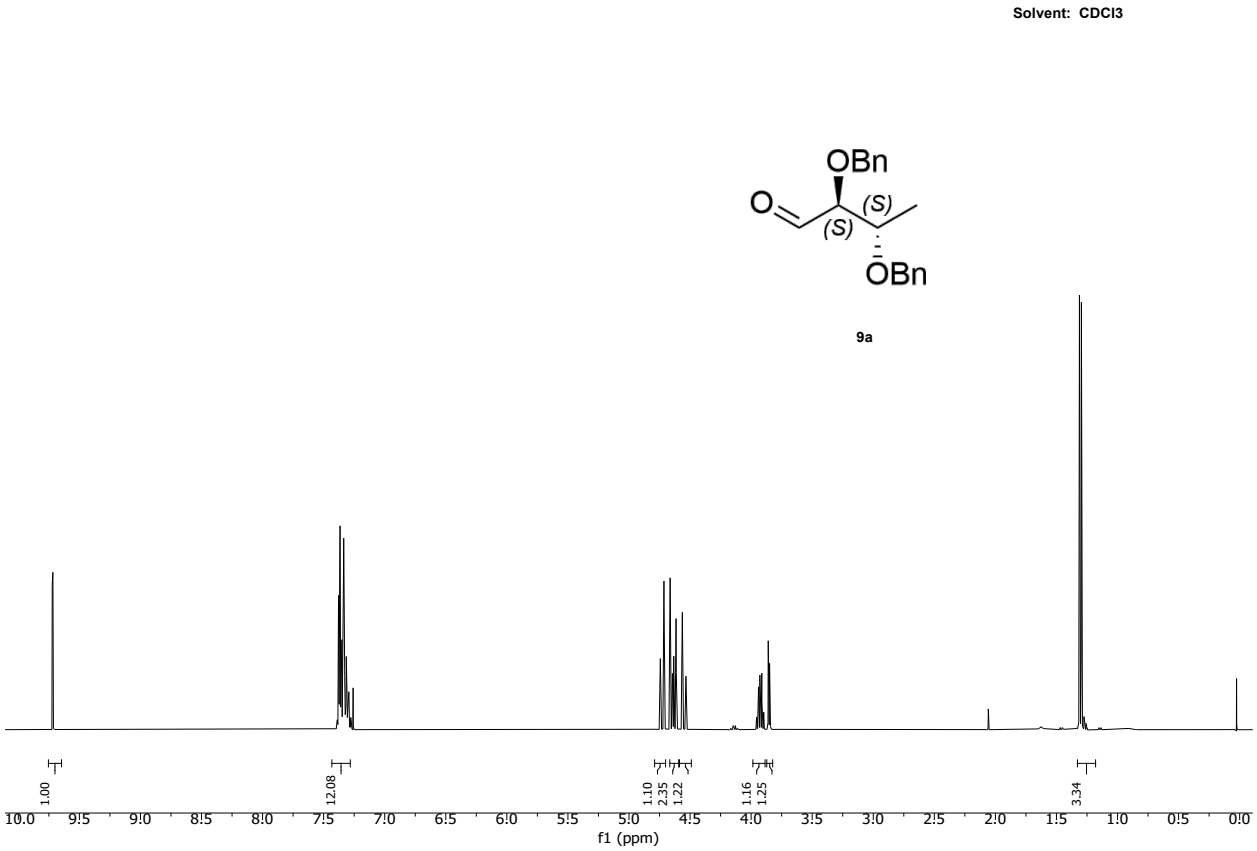
Solvent: CDCl3



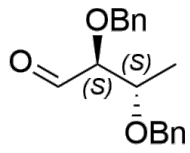
8d



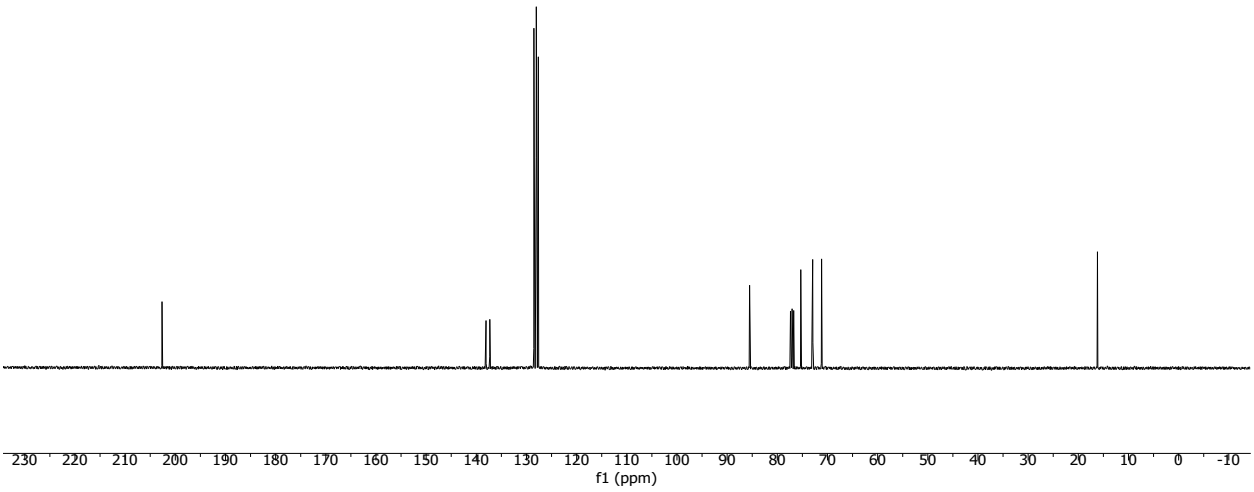
Compound 9a


Solvent: CDCl3
OBn
O
(S)
(S)
OBn
9a
1.00
12.08
1.10
2.35
1.22
1.16
1.25
3.34
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

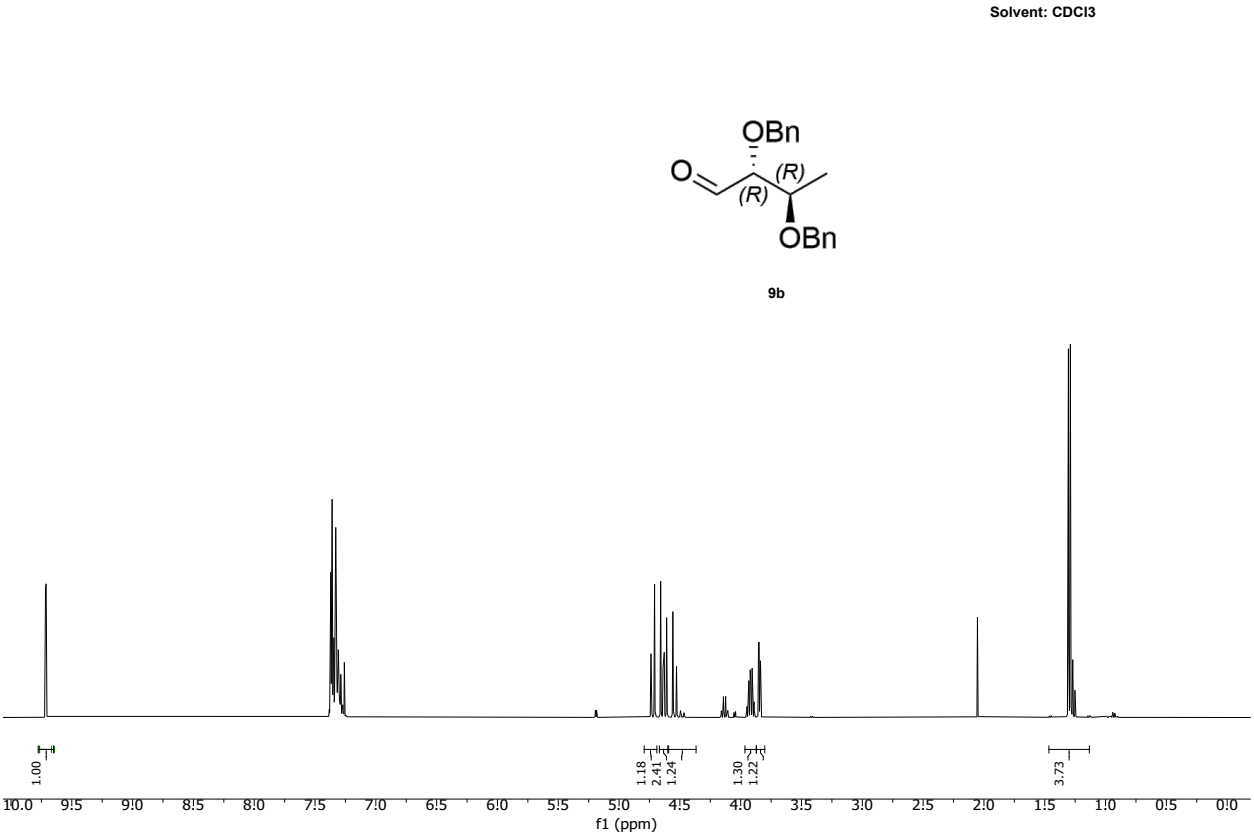
Solvent: CDCl3



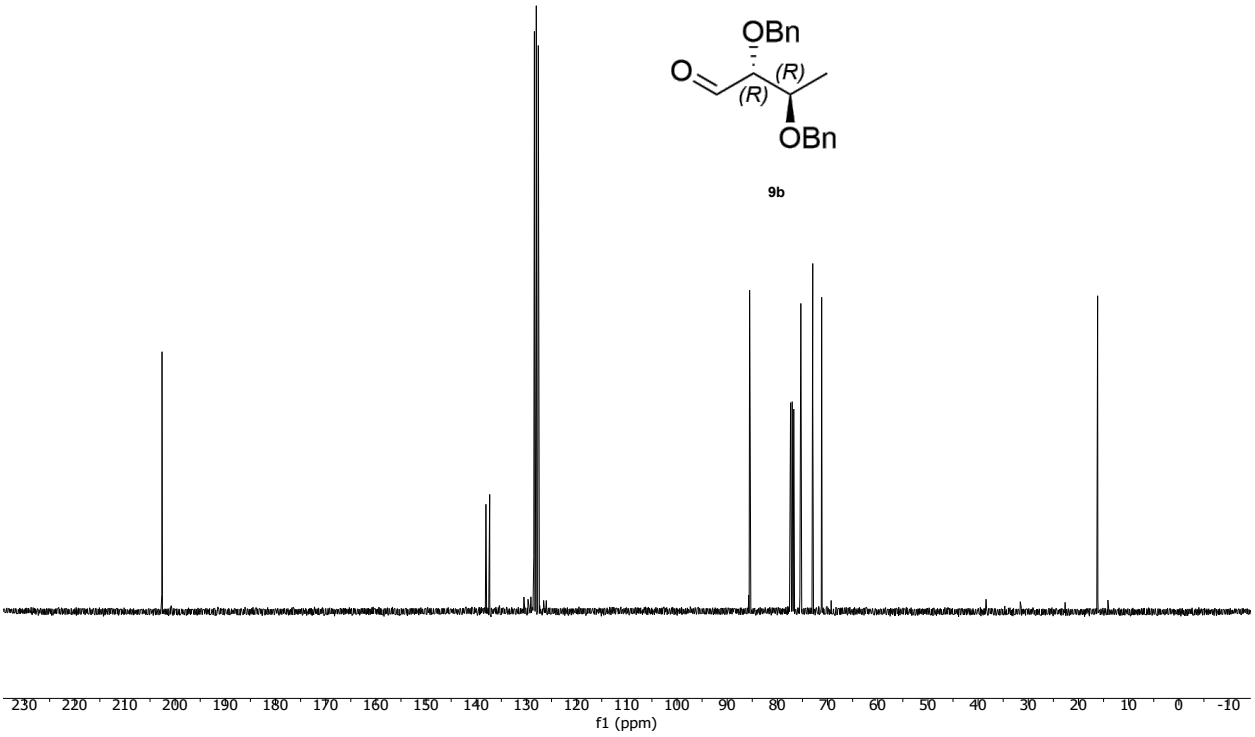
9a



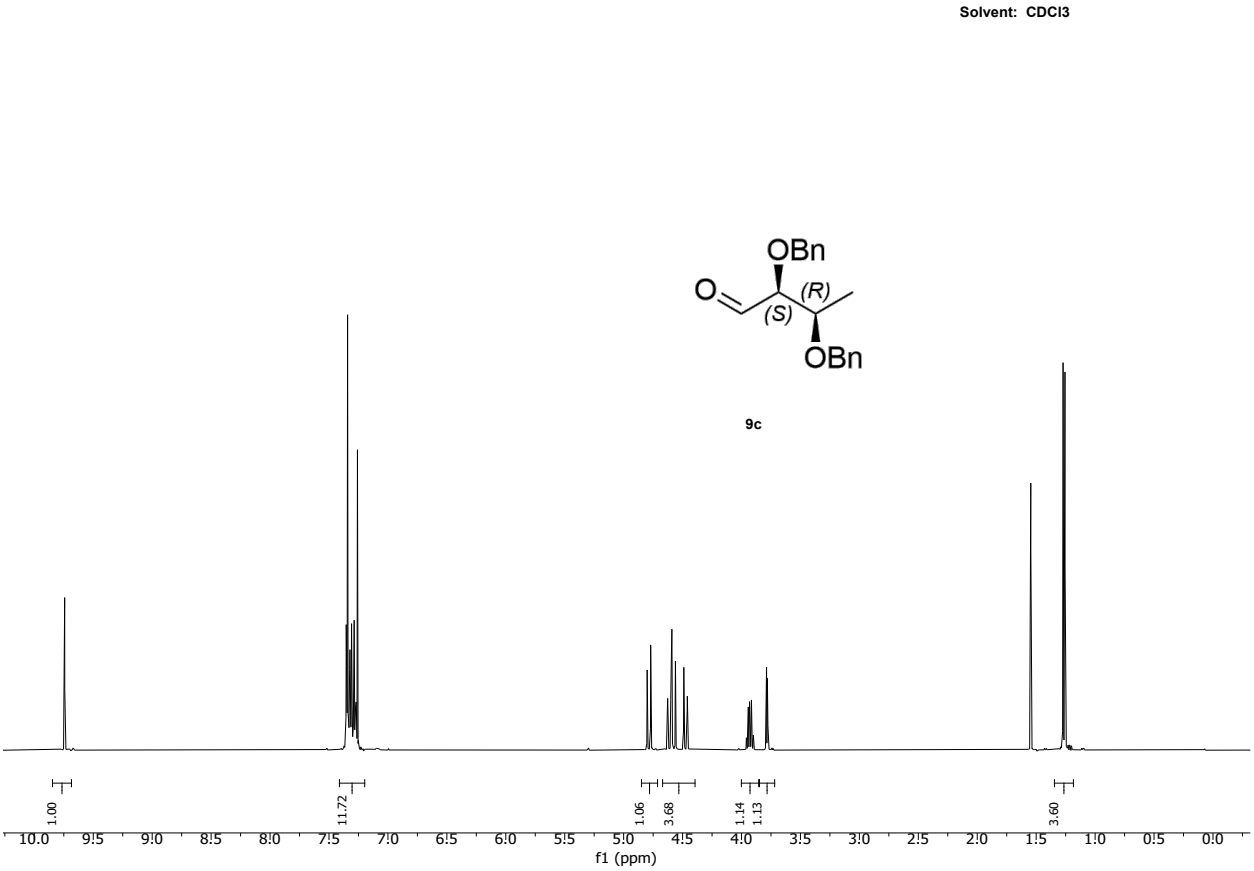
Compound 9b


Solvent: CDCl3
OBn
O
(R)
(R)
OBn
9b
1.00
1.18
2.41
1.24
1.30
1.22
3.73
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

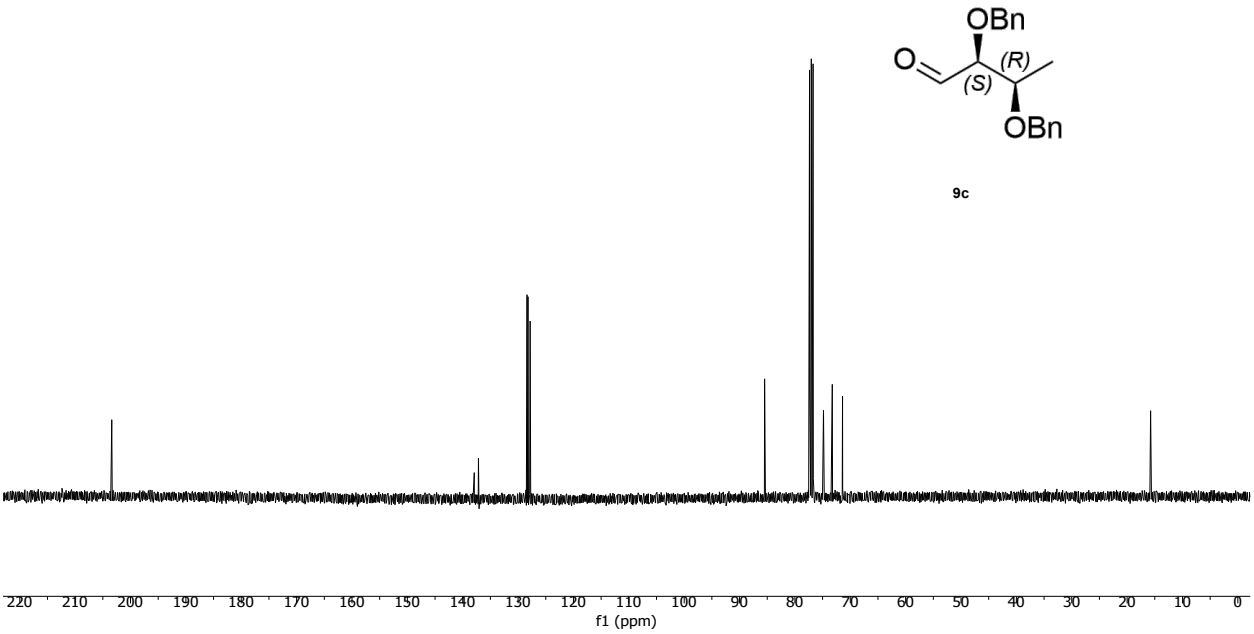
Solvent: CDCl3



Compound 9c

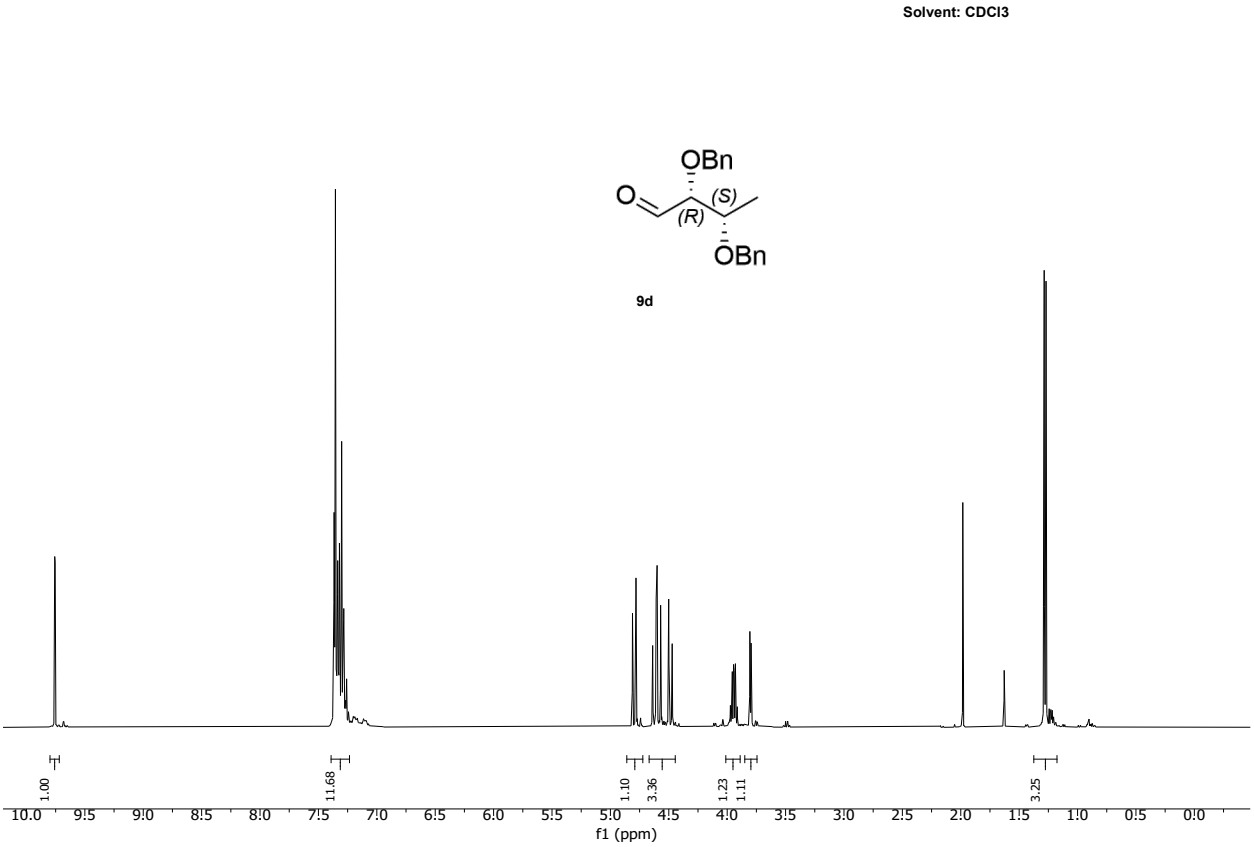

Solvent: CDCl3
OBn
O
(R)
(S)
OBn
9c
1.00
11.72
1.06
3.68
1.14
1.13
3.60
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

Solvent: CDCl3

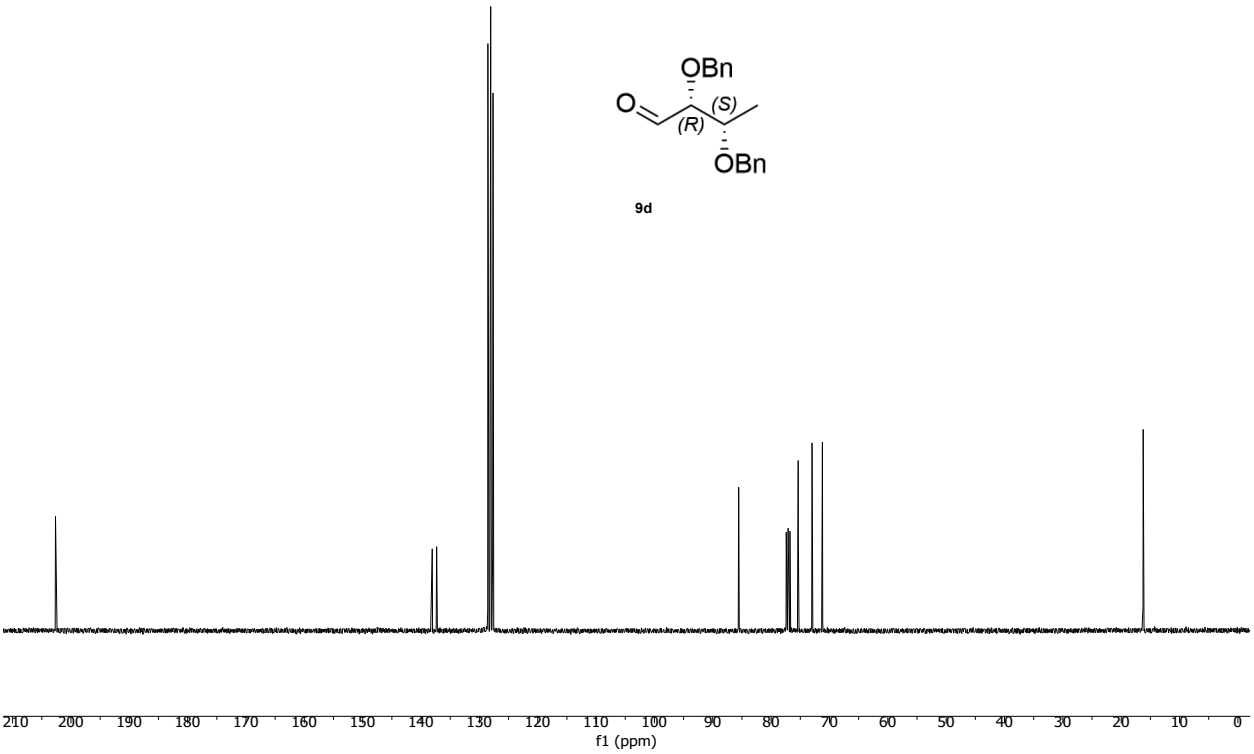


9c

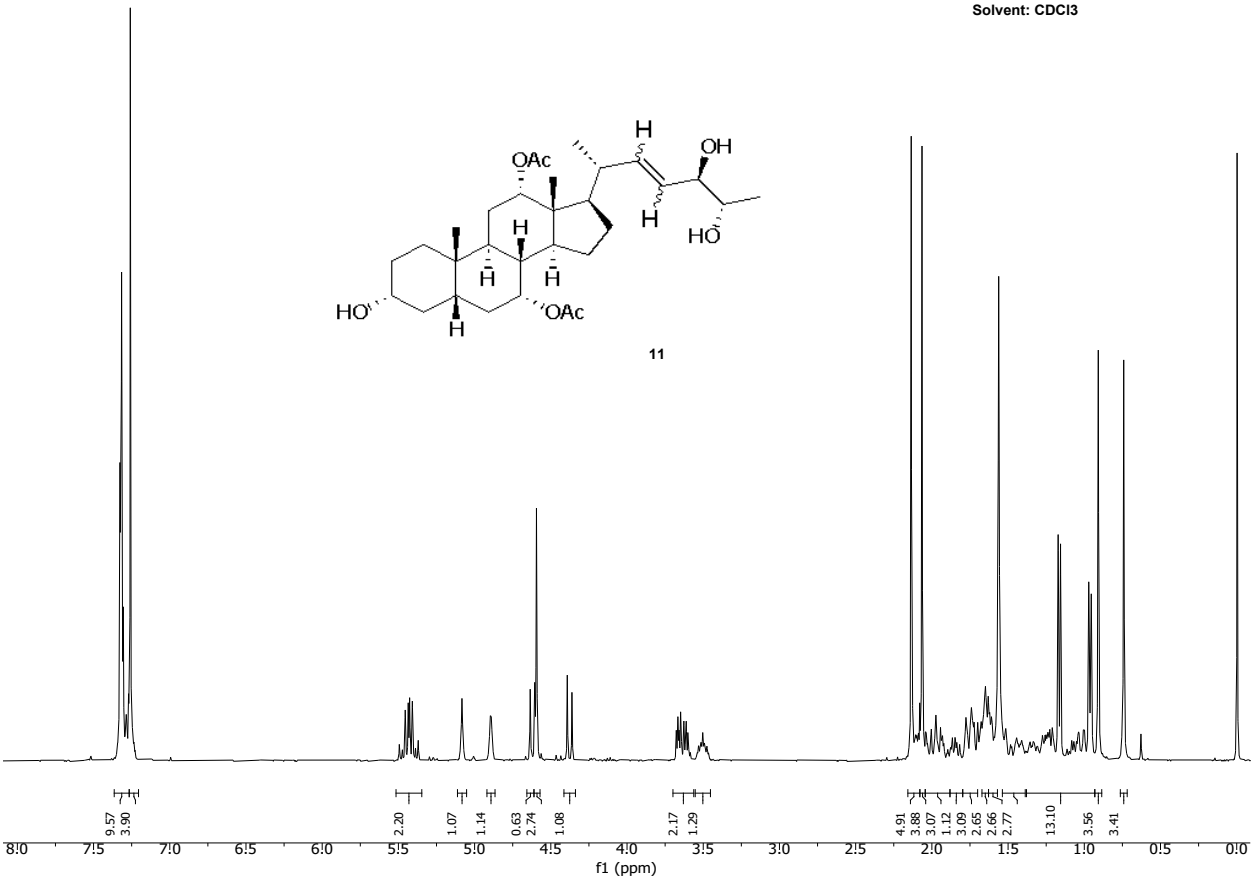
Compound 9d


Solvent: CDCl3
OBn
O
(S)
(R)
OBn
9d
1.00
11.68
1.10
3.36
1.23
1.11
3.25
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

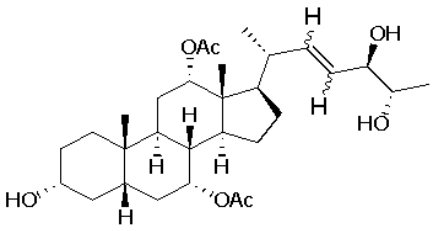
Solvent: CDCl3



Compound 11

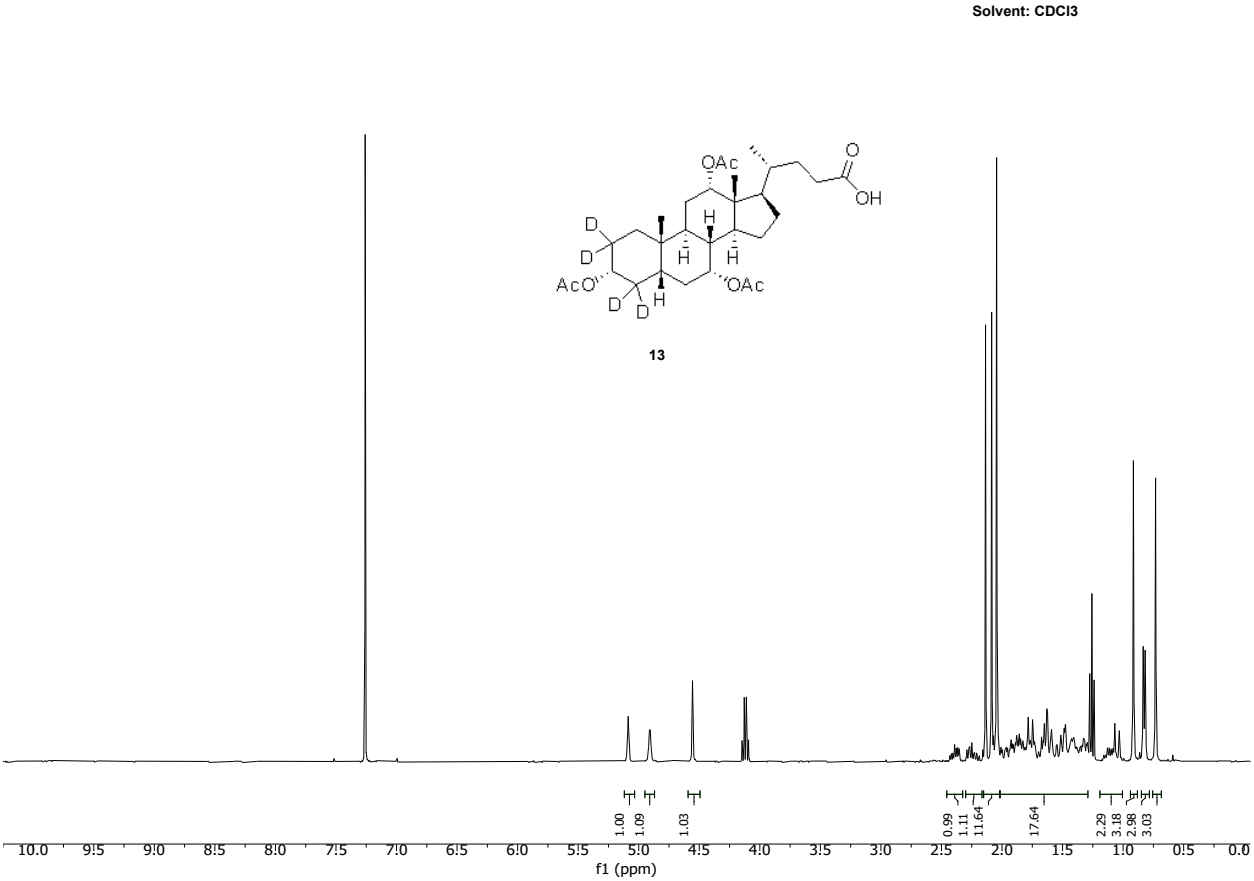

Solvent: CDCl3
OAc
H
OH
H
HO
H
H
H
HO
OAc
H
11
9.57
3.90
2.20
1.07
1.14
0.63
2.74
1.08
2.17
1.29
4.91
3.88
3.07
1.12
3.09
2.65
2.66
2.77
13.10
3.56
3.41
8:0
7:5
7:0
6:5
6:0
5:5
5:0
4:5
4:0
3:5
3:0
2:5
2:0
1:5
1:0
0:5
0:0
f1 (ppm)

Solvent: CDCl3

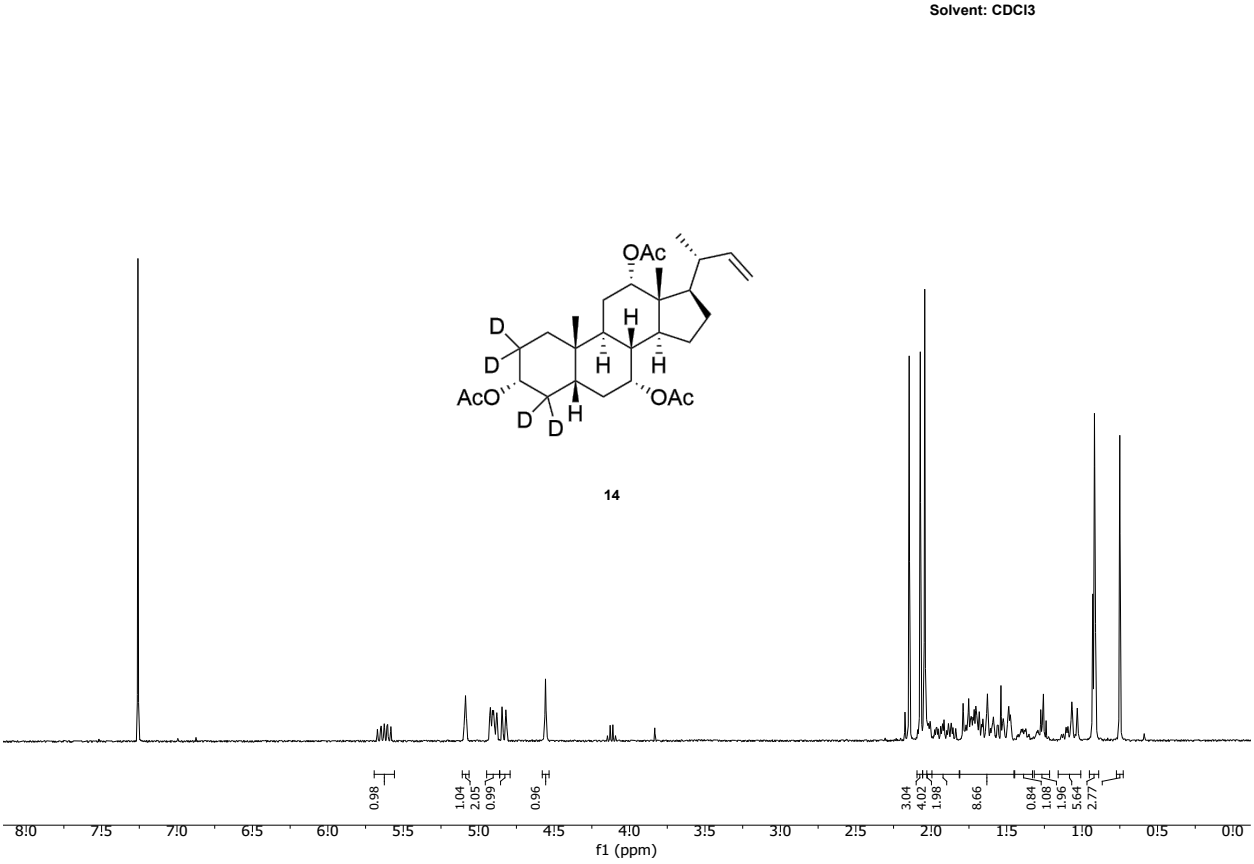


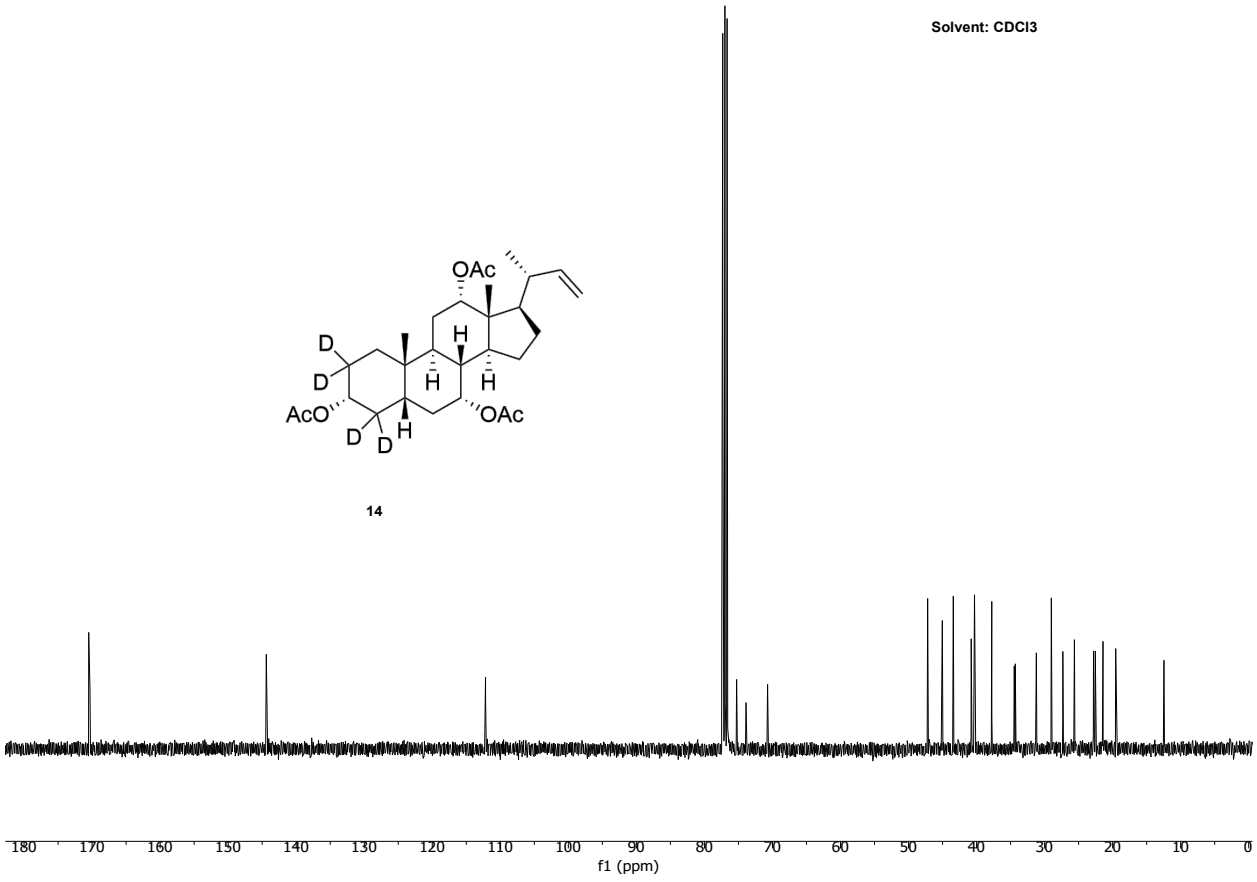
11

Compound 13


Solvent: CDCl3
OAc
O
OH
D
D
H
H
H
AcO
OAc
D
D
H
13
1.00
1.09
1.03
0.99
1.11
11.64
17.64
2.29
3.18
2.98
3.03
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

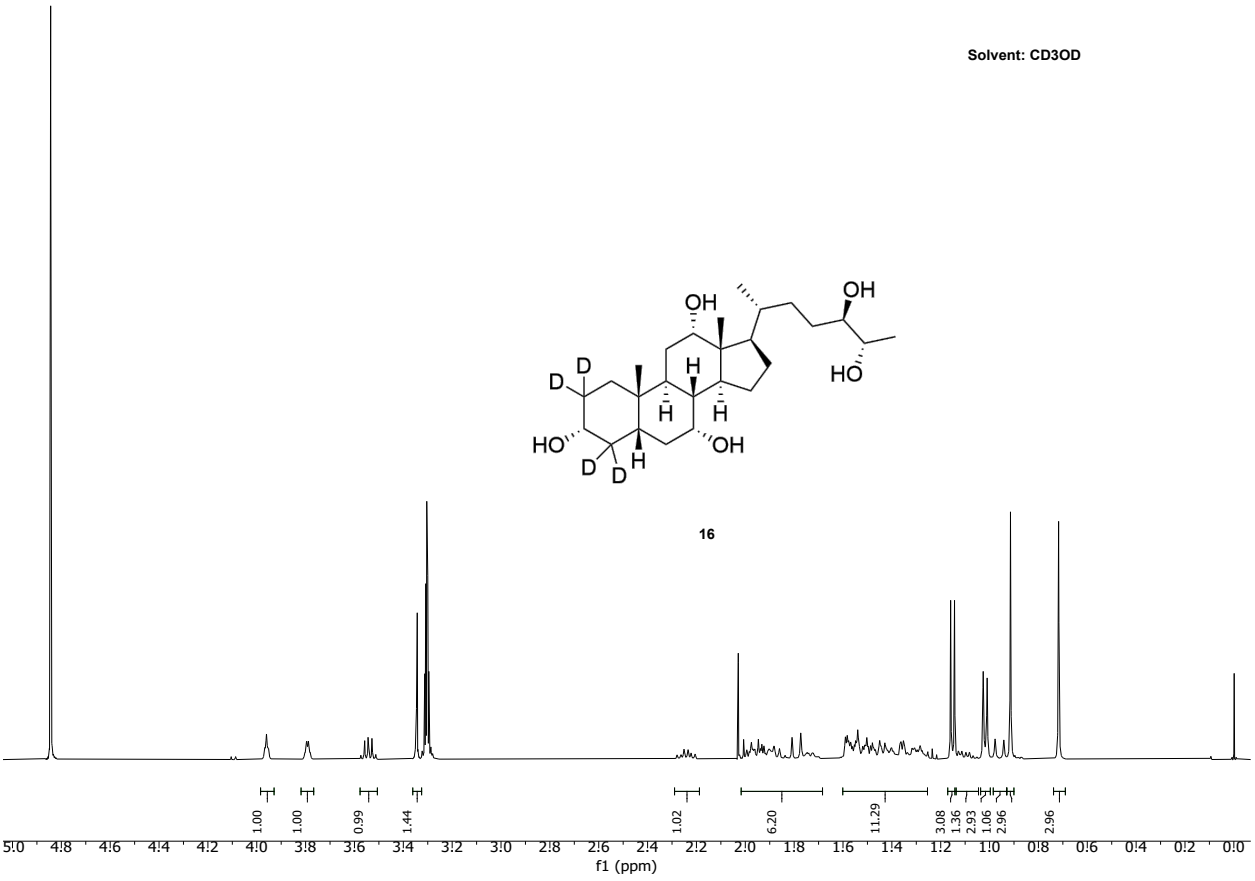
Compound 14


Solvent: CDCl3
OAc
D
D
H
H
H
AcO
OAc
D
D
H
14
0.98
1.04
2.05
0.99
0.96
3.04
4.02
1.98
8.66
0.84
1.08
1.96
5.64
2.77
8!0
7!5
7!0
6!5
6!0
5!5
5!0
4!5
4!0
3!5
3!0
2!5
2!0
1!5
1!0
0!5
0!0
f1 (ppm)

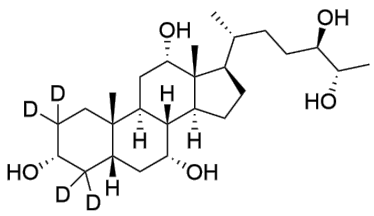

Solvent: CDCl3
OAc
D
D
H
H
H
AcO
OAc
D
D
H
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

14

Compound 16


Solvent: CD3OD
OH
OH
HO
D
D
H
H
H
HO
OH
D
D
H
16
1.00
1.00
0.99
1.44
1.02
6.20
11.29
3.08
1.36
2.93
1.06
2.96
2.96
5:0
4:8
4:6
4:4
4:2
4:0
3:8
3:6
3:4
3:2
3:0
2:8
2:6
2:4
2:2
2:0
1:8
1:6
1:4
1:2
1:0
0:8
0:6
0:4
0:2
0:0
f1 (ppm)

Solvent: CD3OD



16