Supporting Information

Azetidine and *N*-Carboxylic Azetidine Iminosugars as Amyloglucosidase Inhibitors: Synthesis, Glycosidase Inhibitory Activity and Molecular Docking Studies.

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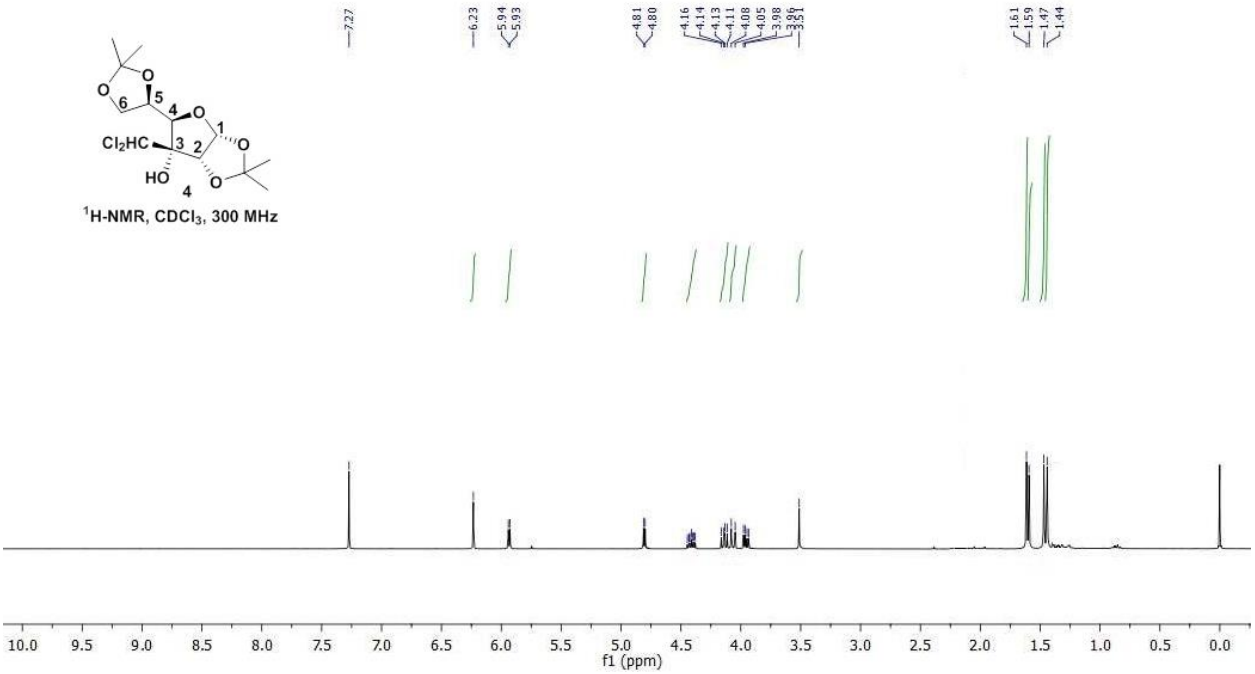
Contents

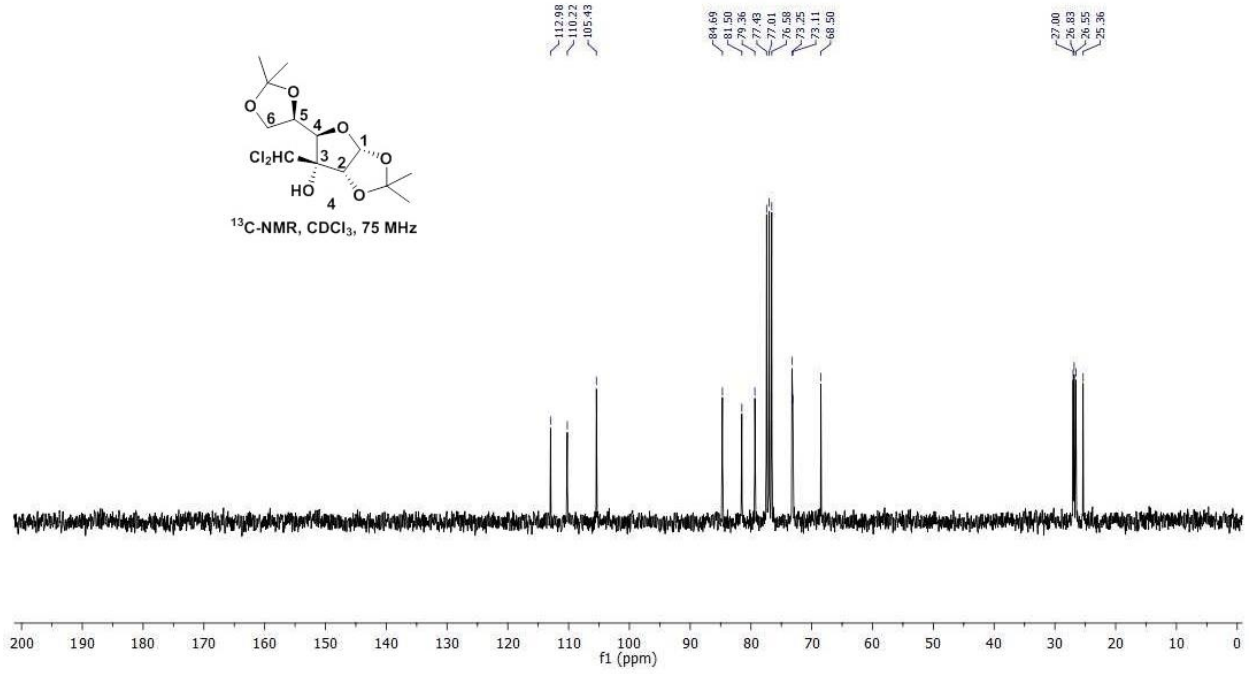
Note:
The ^{1}H and ^{13}C NMR spectra of compounds **12, 13, 14** and **15** shows doubling of the signals due to presence of *N*-Cbz group.

1,2;5,6-Di-*O*-isopropylidene-3-*C*-(dichloromethyl)-α-D-allofuranose (4)

^{1}H-NMR, $CDCl_3$, 300 MHz

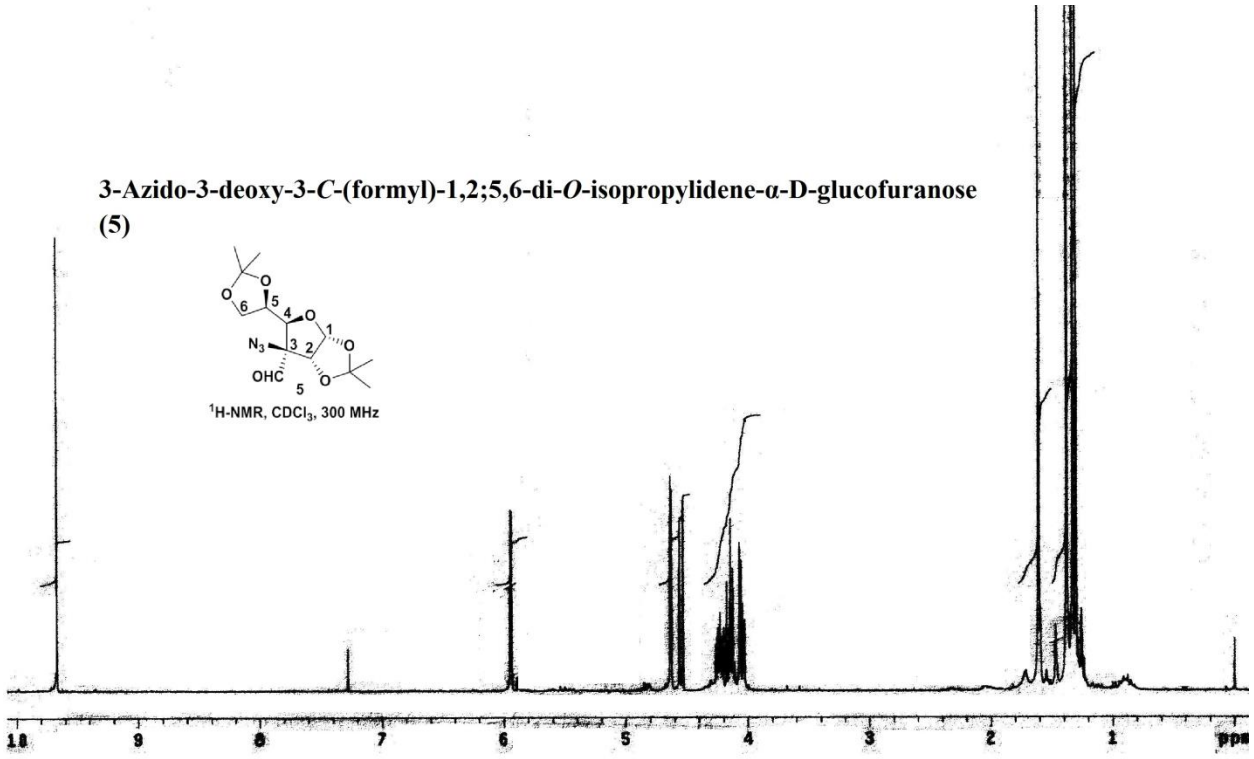


^{13}C-NMR, $CDCl_3$, 75 MHz

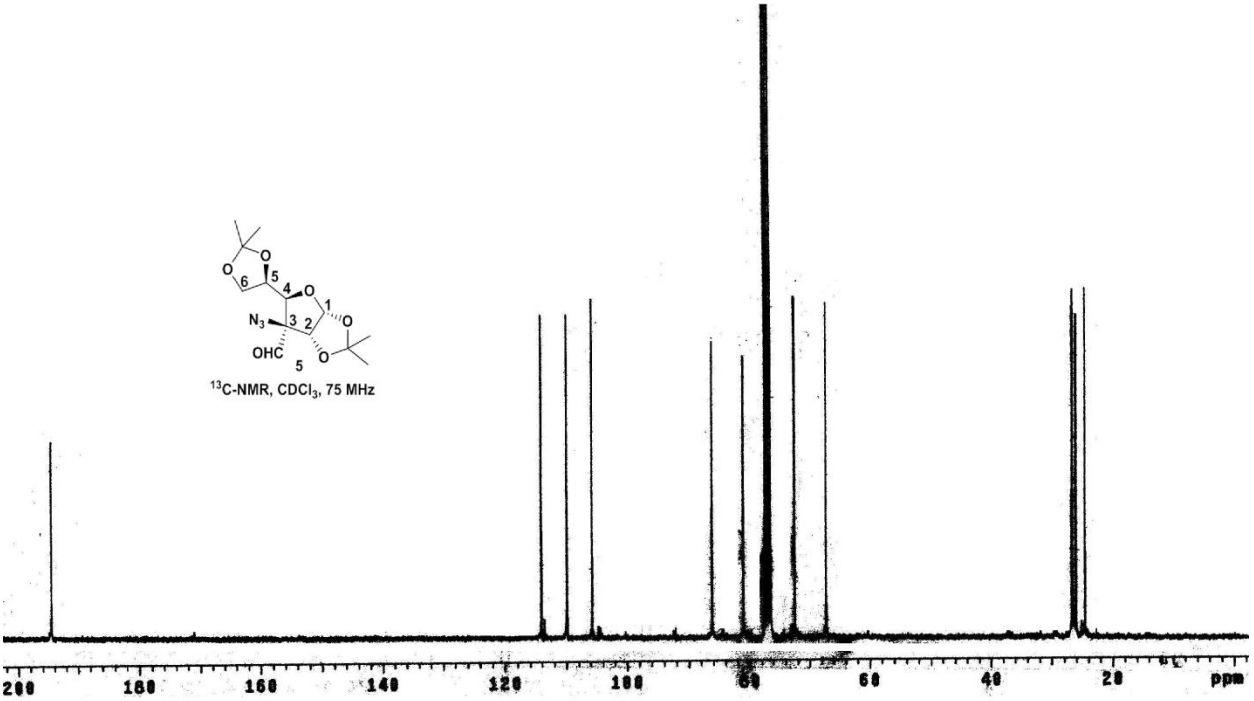


3-Azido-3-deoxy-3-*C*-(formyl)-1,2;5,6-di-*O*-isopropylidene-α-D-glucofuranose (5)

^{1}H-NMR, $CDCl_3$, 300 MHz

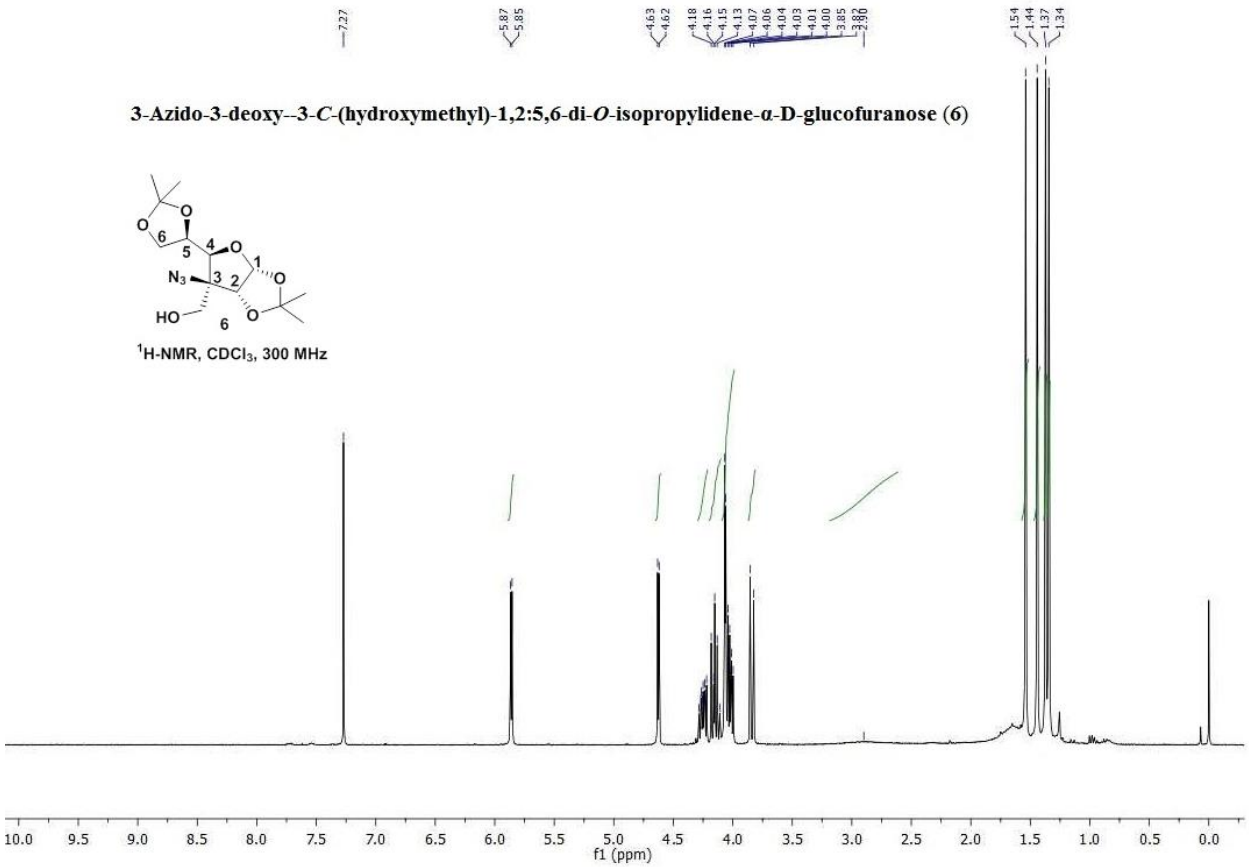


^{13}C-NMR, $CDCl_3$, 75 MHz

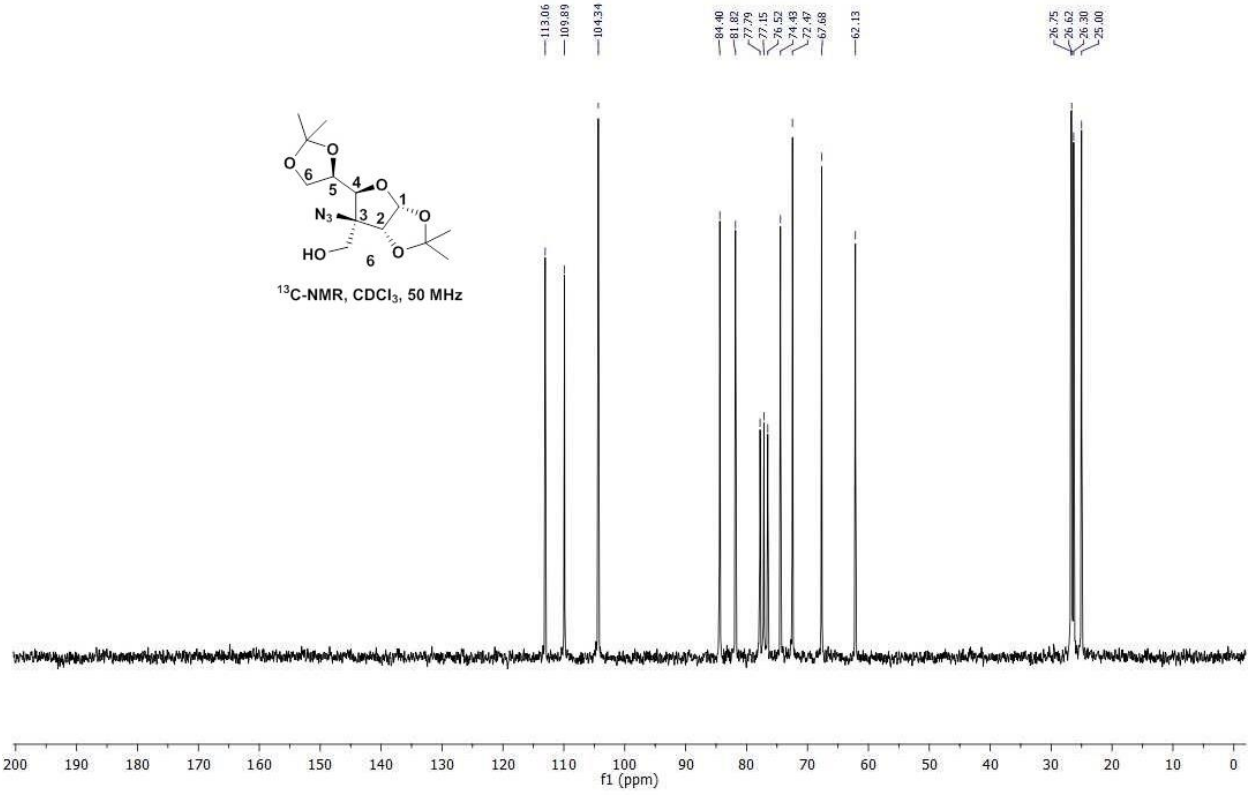


3-Azido-3-deoxy--3-*C*-(hydroxymethyl)-1,2:5,6-di-*O*-isopropylidene-*α*-D-glucofuranose (6)

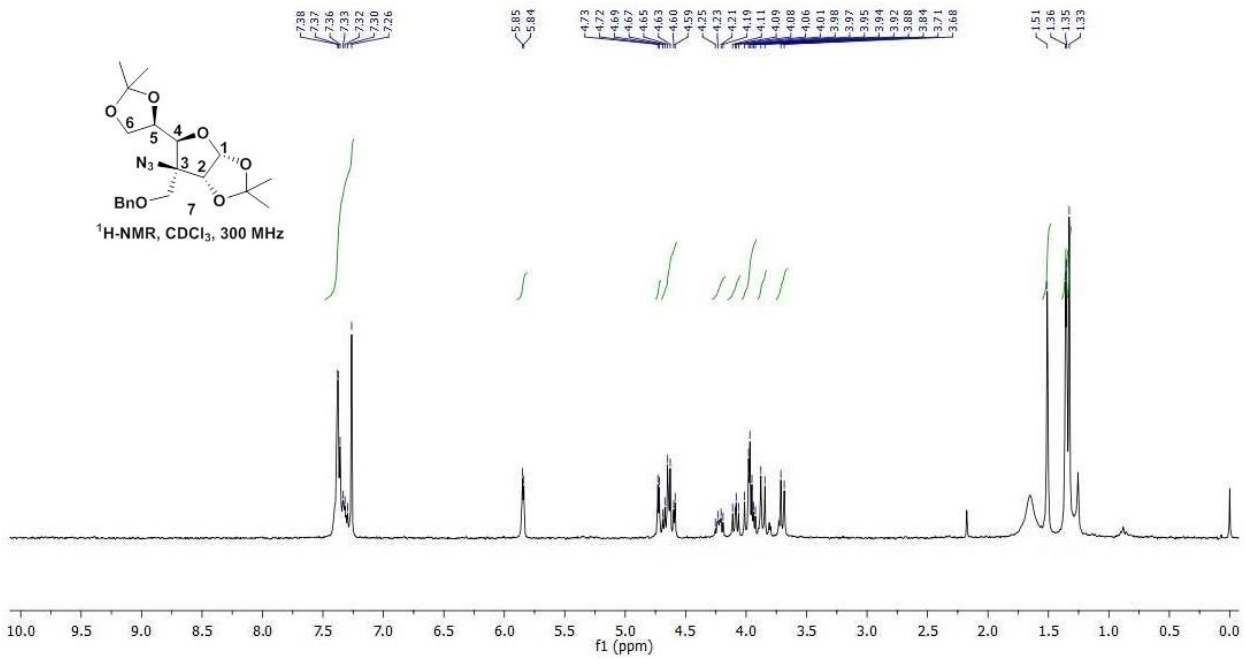
^{1}H-NMR, $CDCl_3$, 300 MHz

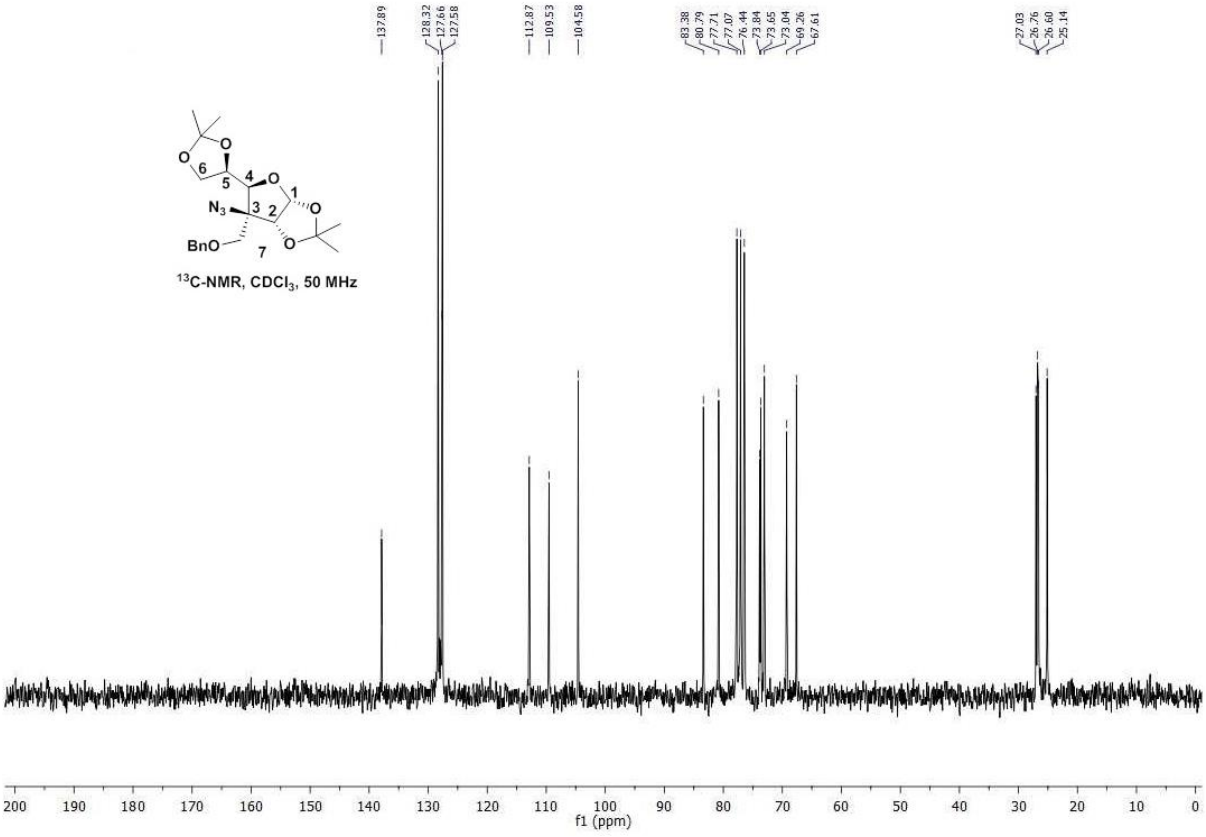


^{13}C-NMR, $CDCl_3$, 50 MHz



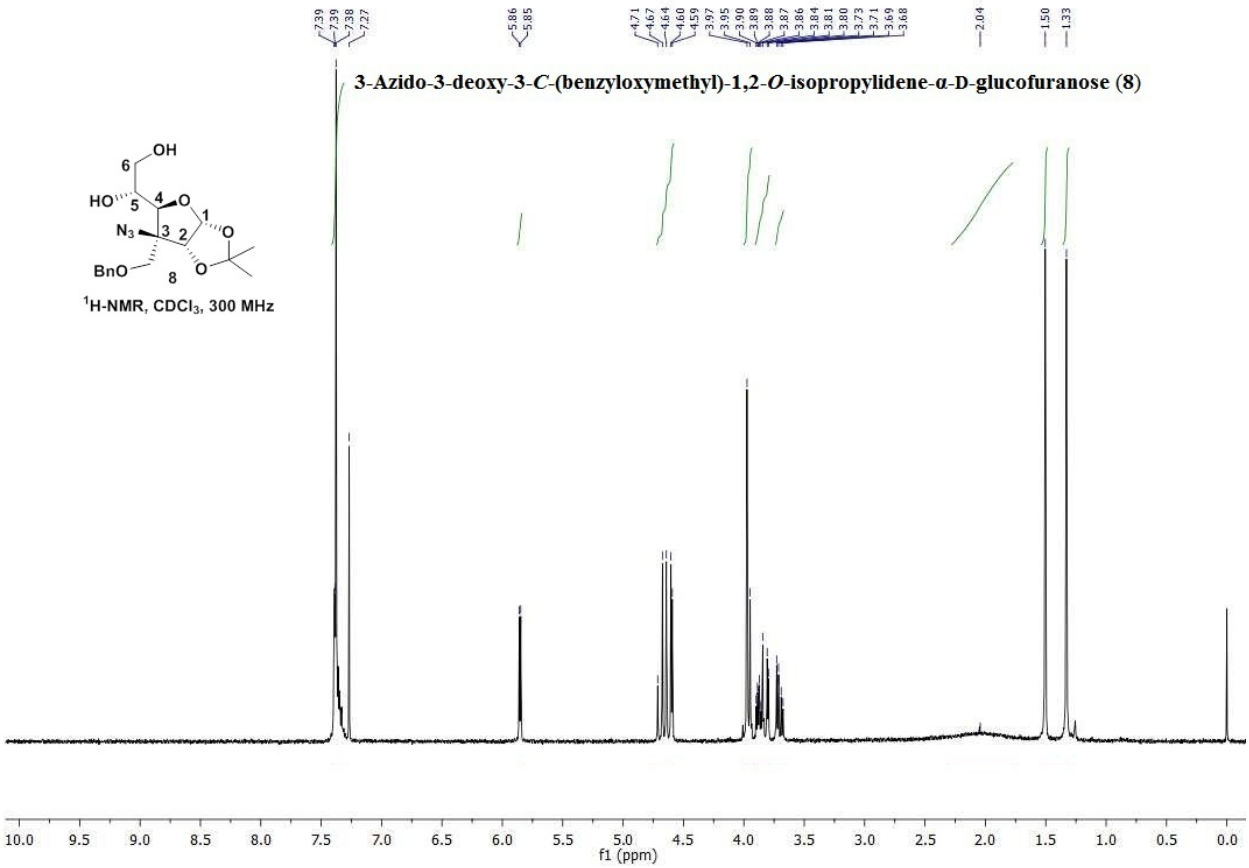
3-Azido-3-deoxy-3-*C*-(benzyloxymethyl)-1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose (7)



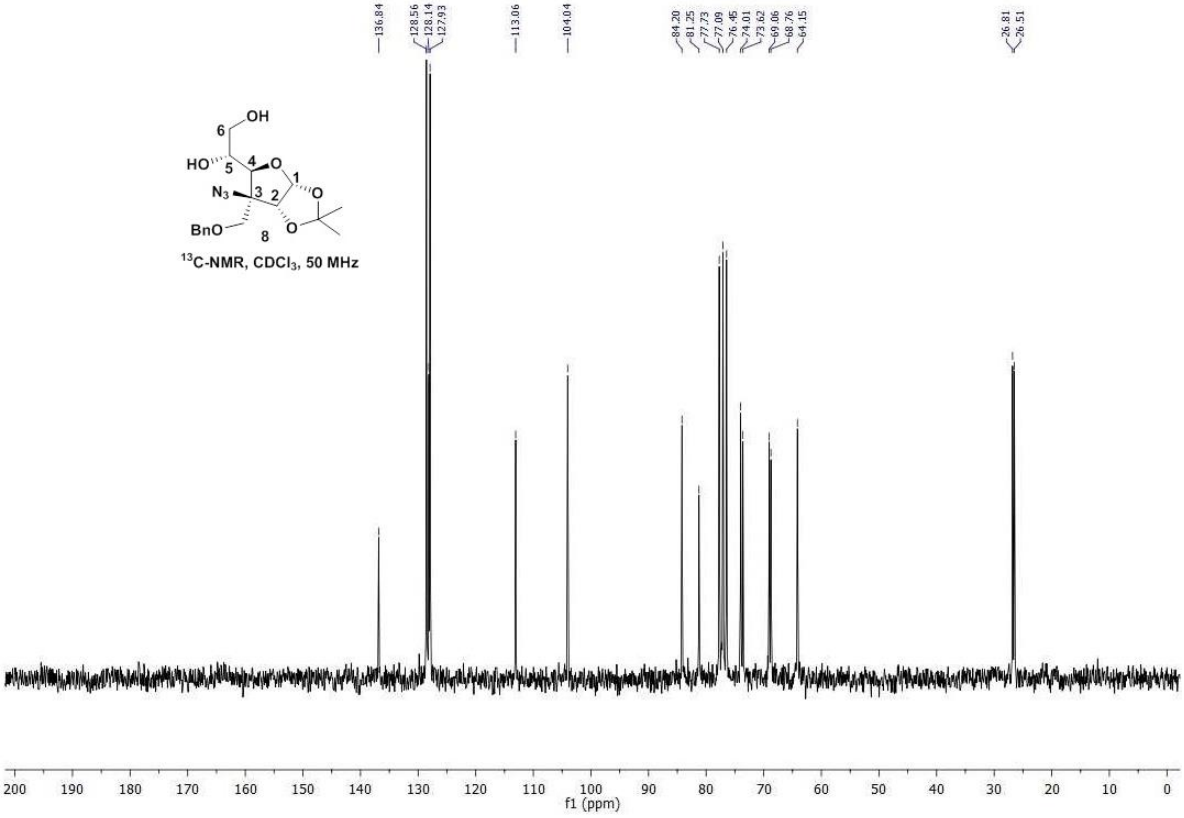


3-Azido-3-deoxy-3-*C*-(benzyloxymethyl)-1,2-*O*-isopropylidene-α-D-glucofuranose (8)

^{1}H-NMR, $CDCl_3$, 300 MHz

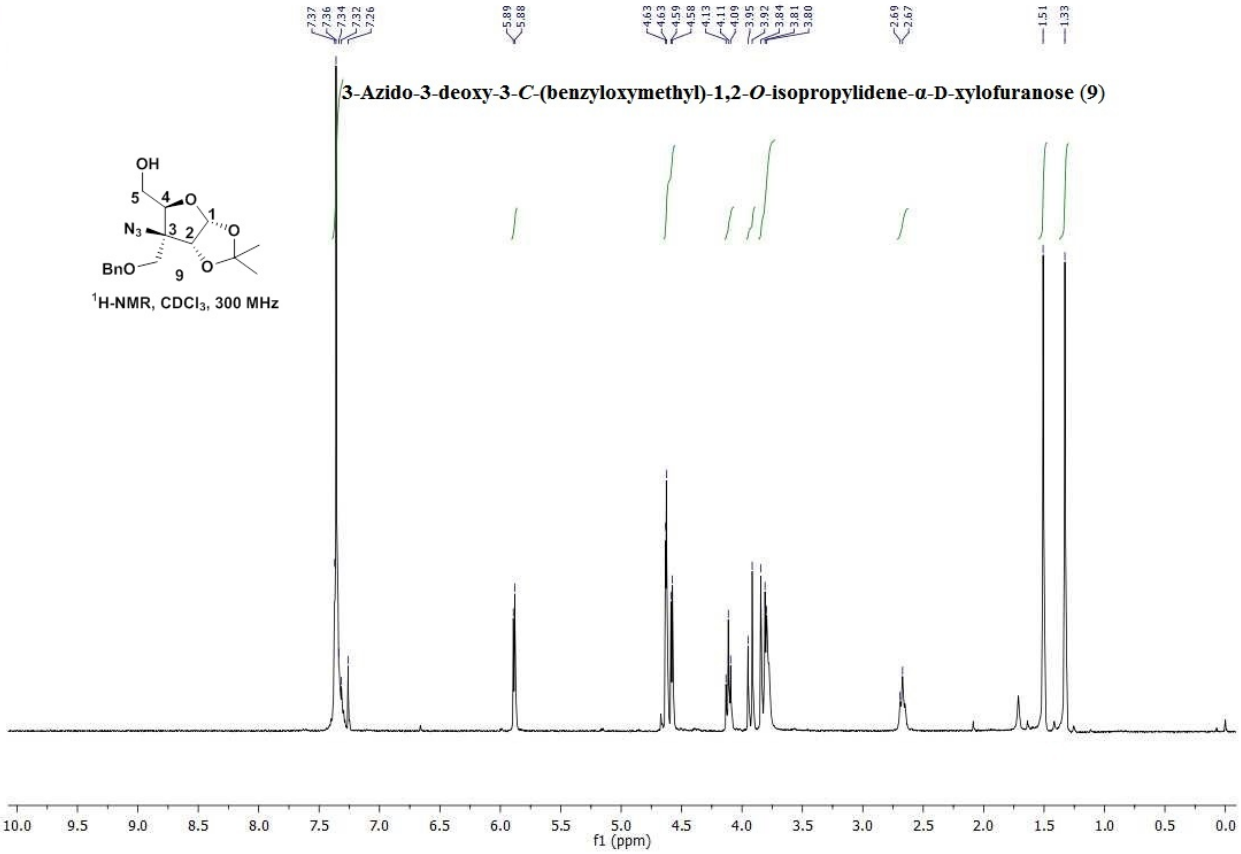


^{13}C-NMR, $CDCl_3$, 50 MHz

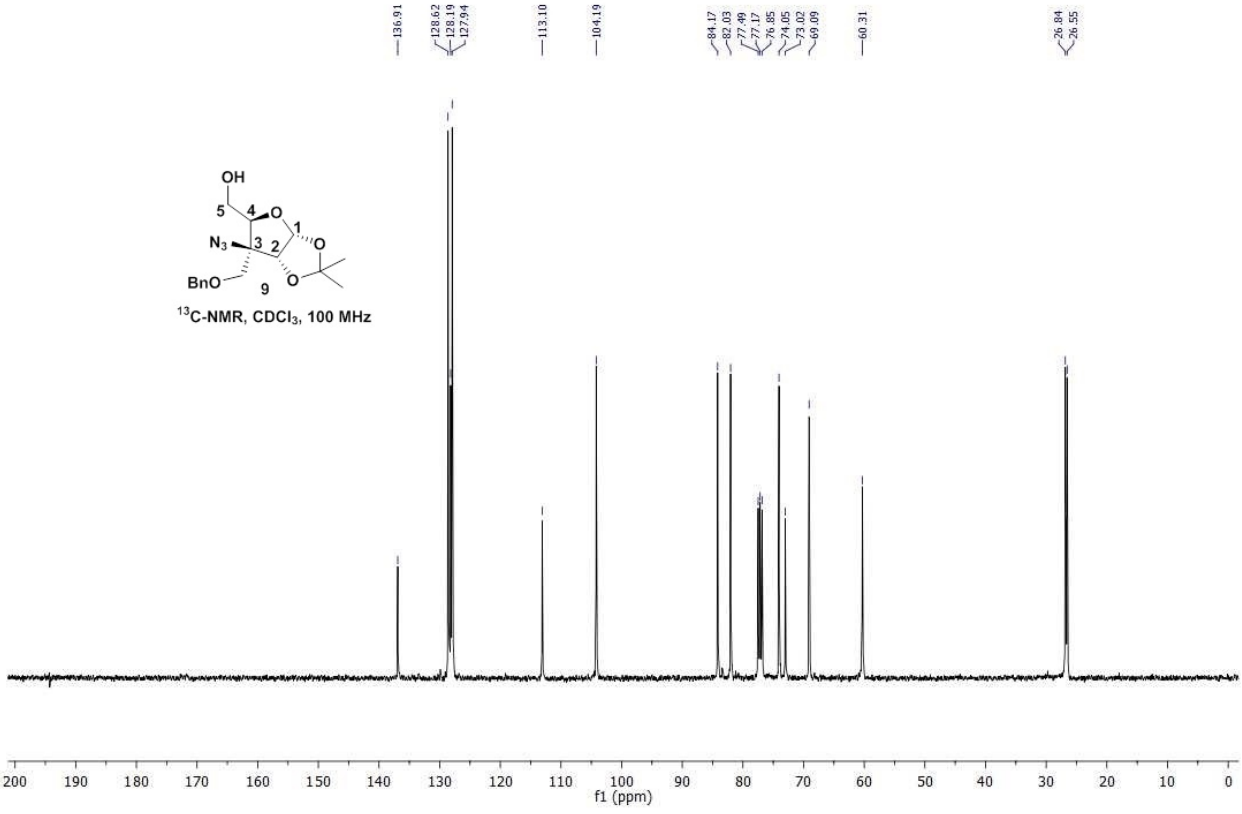


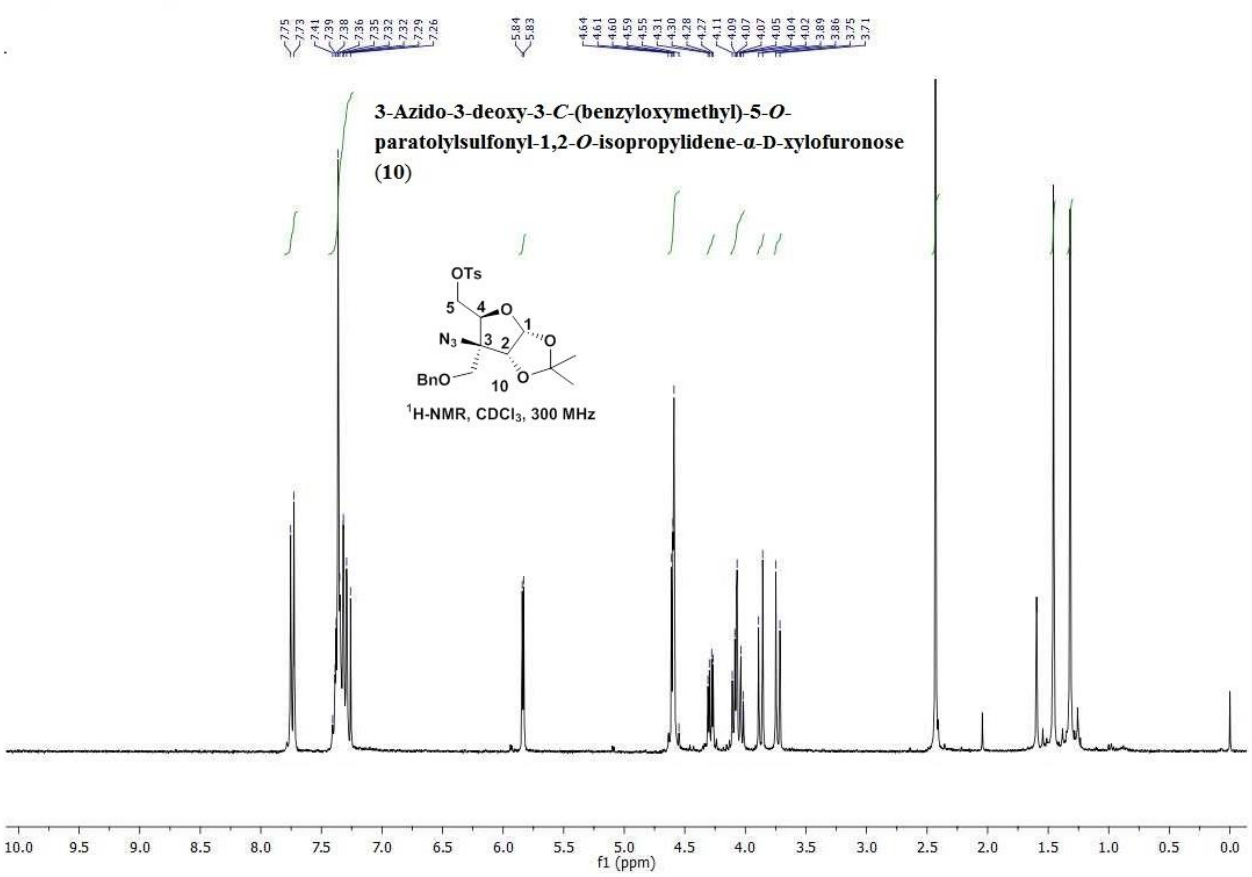
3-Azido-3-deoxy-3-*C*-(benzyloxymethyl)-1,2-*O*-isopropylidene-α-D-xylofuranose (9)

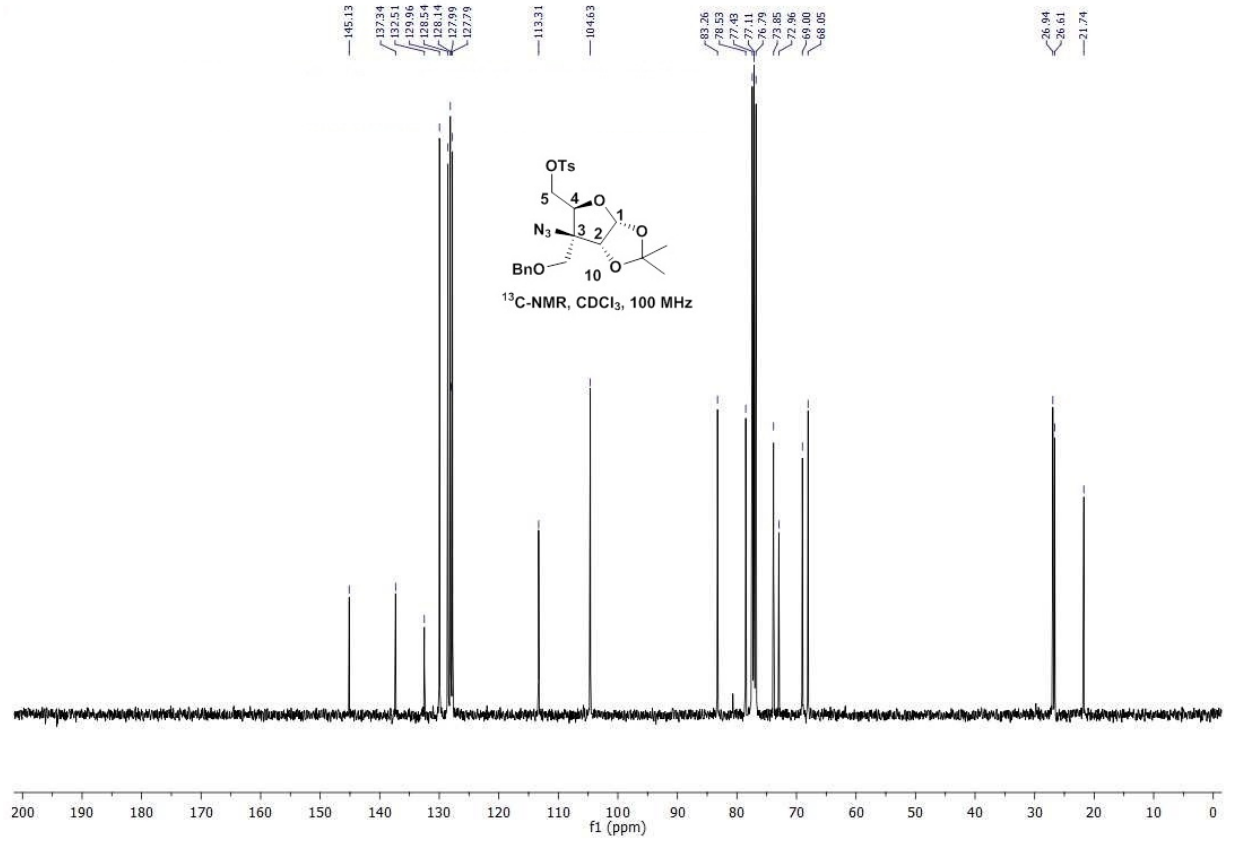
^{1}H-NMR, $CDCl_3$, 300 MHz

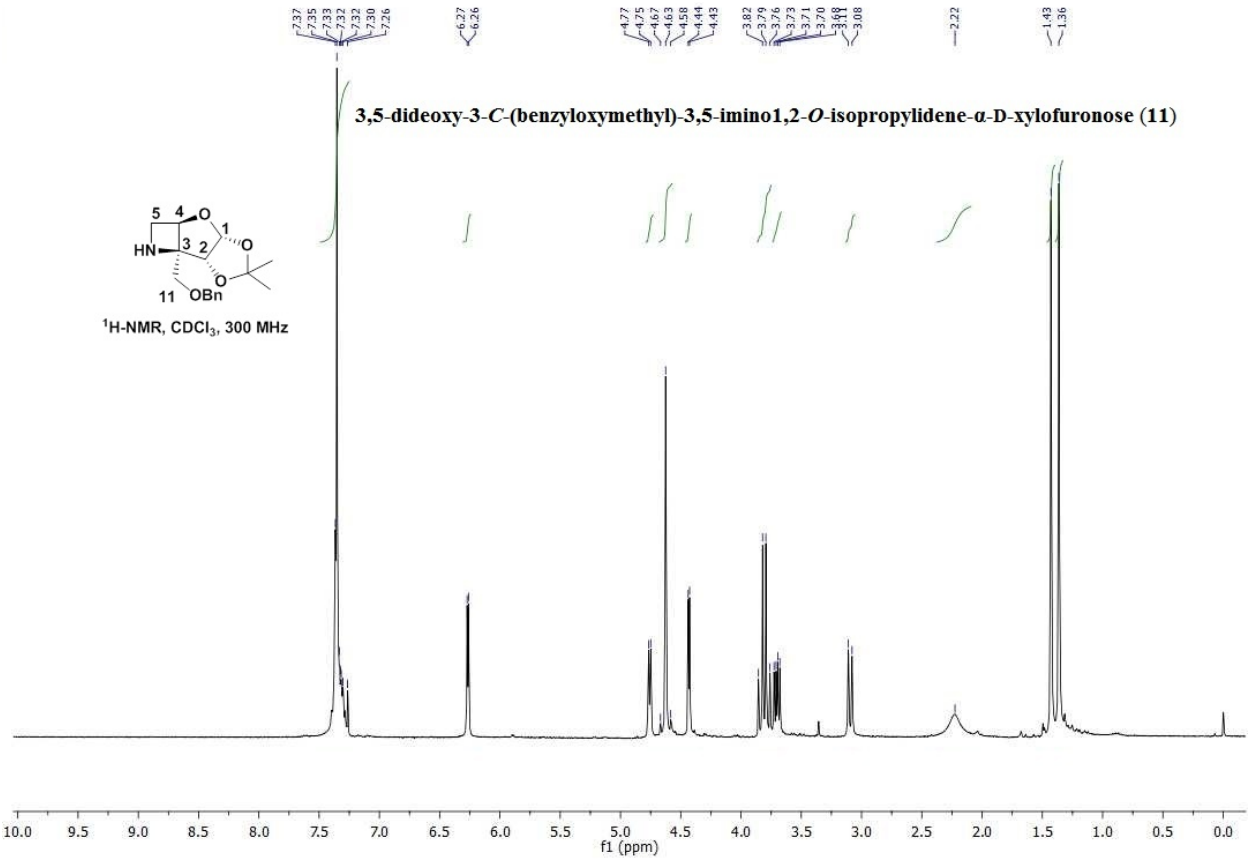


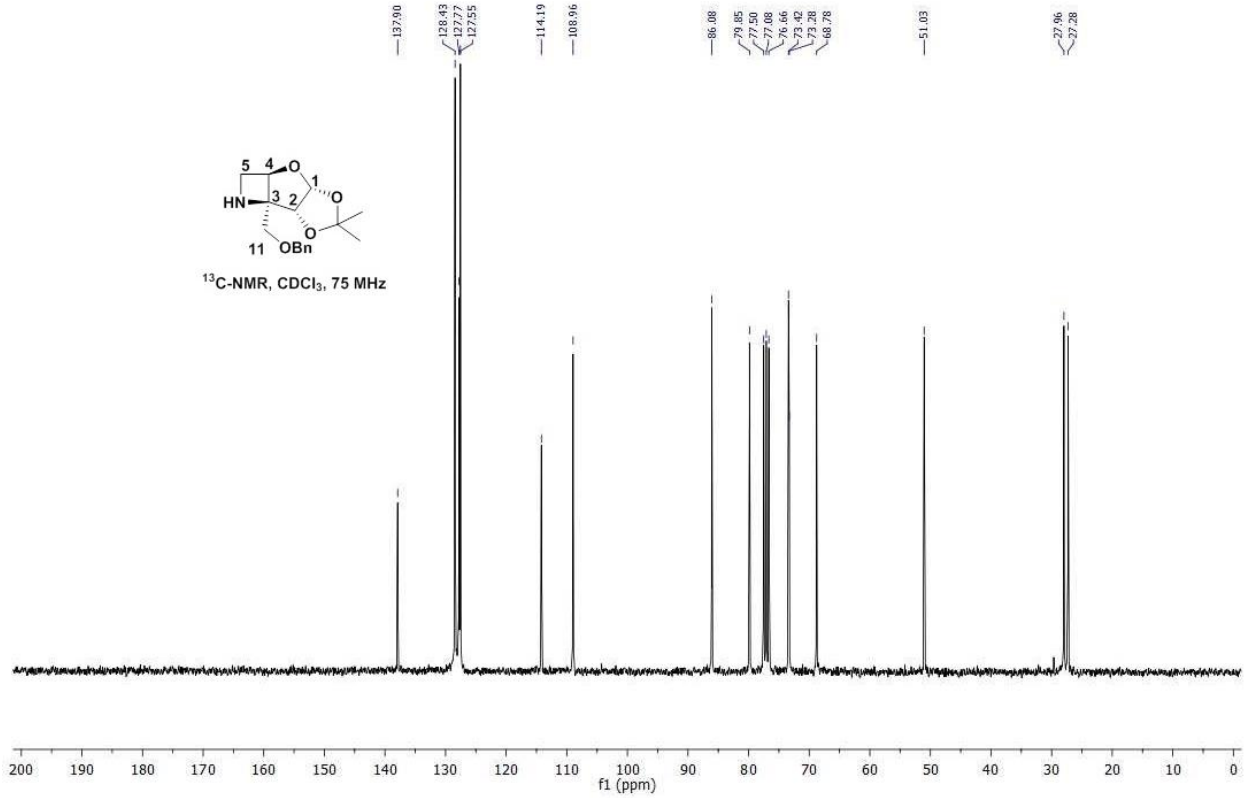
^{13}C-NMR, $CDCl_3$, 100 MHz

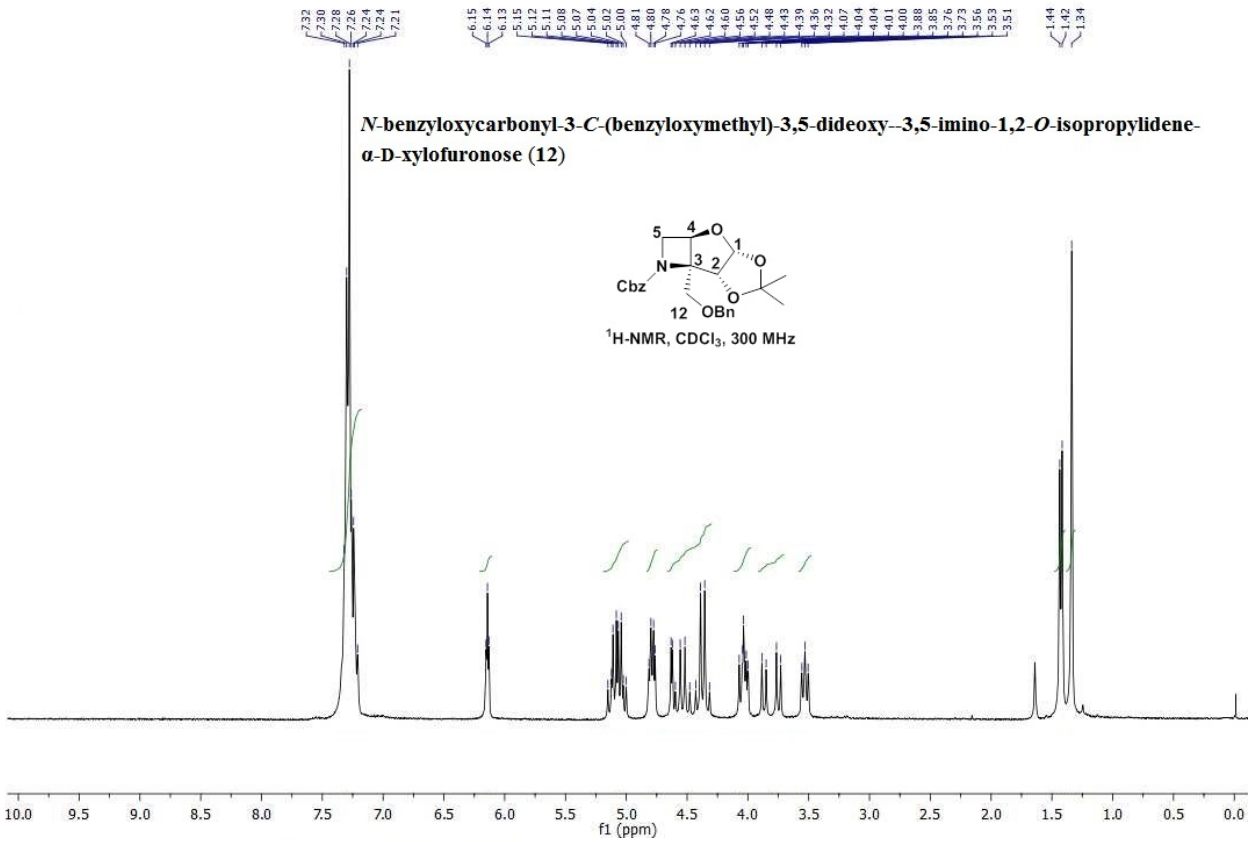


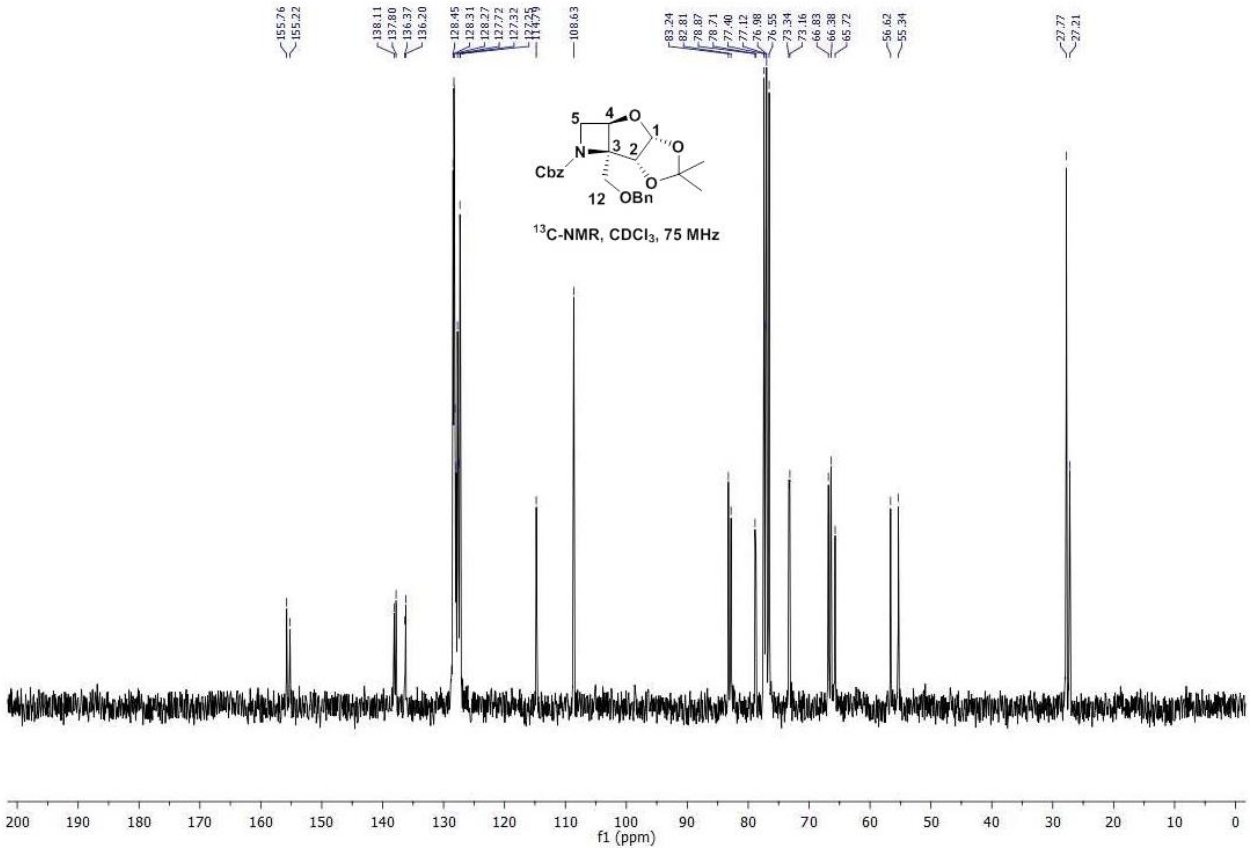

3-Azido-3-deoxy-3-C-(benzyloxymethyl)-5-O-paratolylsulfonyl-1,2-O-isopropylidene-α-D-xylofuronose (10)
OTs
BnO
N3
1H-NMR, CDCl3, 300 MHz
f1 (ppm)


OTs
BnO
N3
13C-NMR, CDCl3, 100 MHz
f1 (ppm)


7.37
7.35
7.33
7.32
7.32
7.30
7.26
6.27
6.26
4.77
4.75
4.67
4.63
4.58
4.44
4.43
3.82
3.79
3.76
3.73
3.71
3.70
3.68
3.61
3.08
2.22
1.43
1.36
3,5-dideoxy-3-C-(benzyloxymethyl)-3,5-imino1,2-O-isopropylidene-α-D-xylofuronose (11)
HN
OBn
1H-NMR, CDCl3, 300 MHz
f1 (ppm)

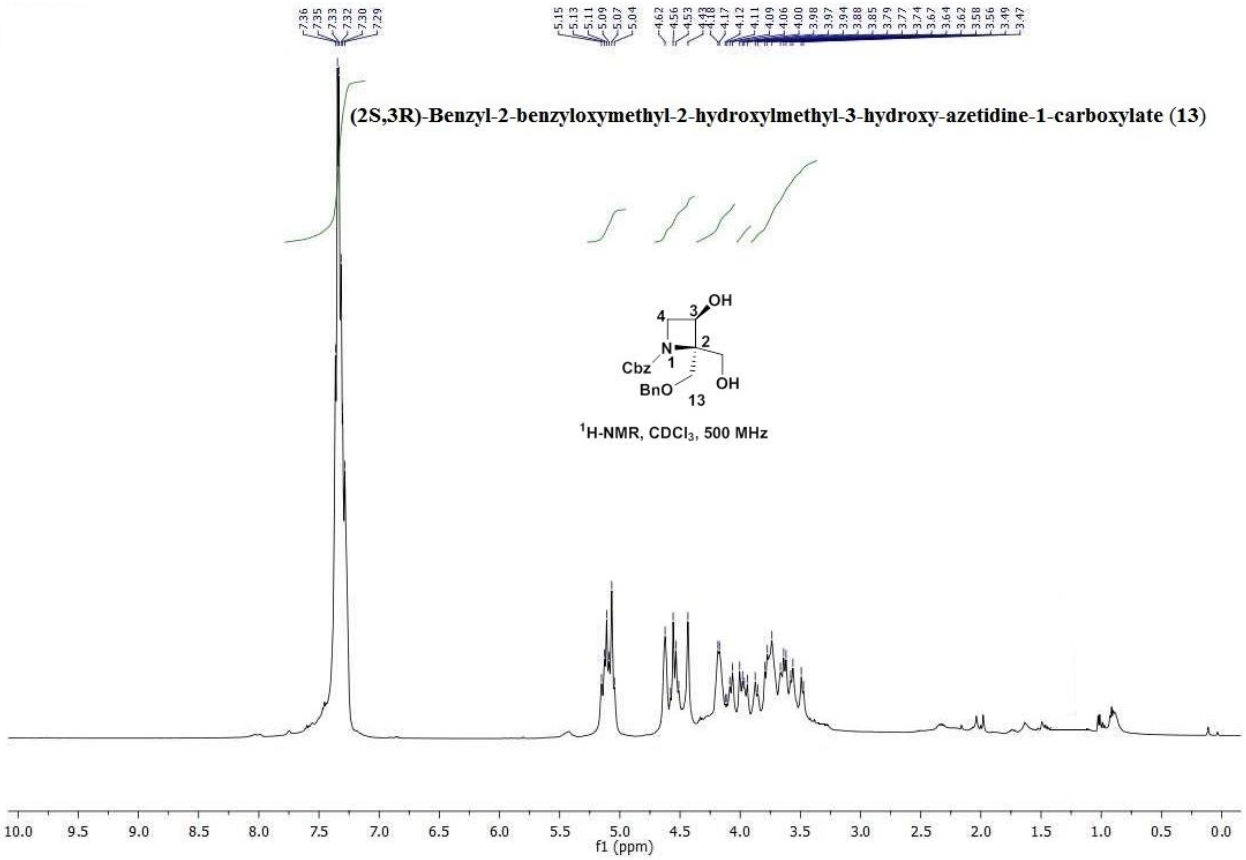

137.90
128.43
127.77
127.55
114.19
108.96
86.08
79.85
77.50
77.08
76.66
73.42
73.28
68.78
51.03
27.96
27.28
HN
OBn
13C-NMR, CDCl3, 75 MHz
f1 (ppm)


7.32
7.30
7.28
7.26
7.24
7.24
7.21
6.15
6.14
6.13
5.15
5.12
5.11
5.08
5.07
5.04
5.02
5.00
4.81
4.80
4.78
4.76
4.63
4.62
4.60
4.56
4.52
4.48
4.43
4.39
4.36
4.32
4.07
4.04
4.04
4.01
4.00
3.88
3.85
3.76
3.73
3.56
3.53
3.51
1.44
1.42
1.34
N-benzyloxycarbonyl-3-C-(benzyloxymethyl)-3,5-dideoxy--3,5-imino-1,2-O-isopropylidene-α-D-xylofuronose (12)
Cbz
OBn
12
1H-NMR, CDCl3, 300 MHz
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)

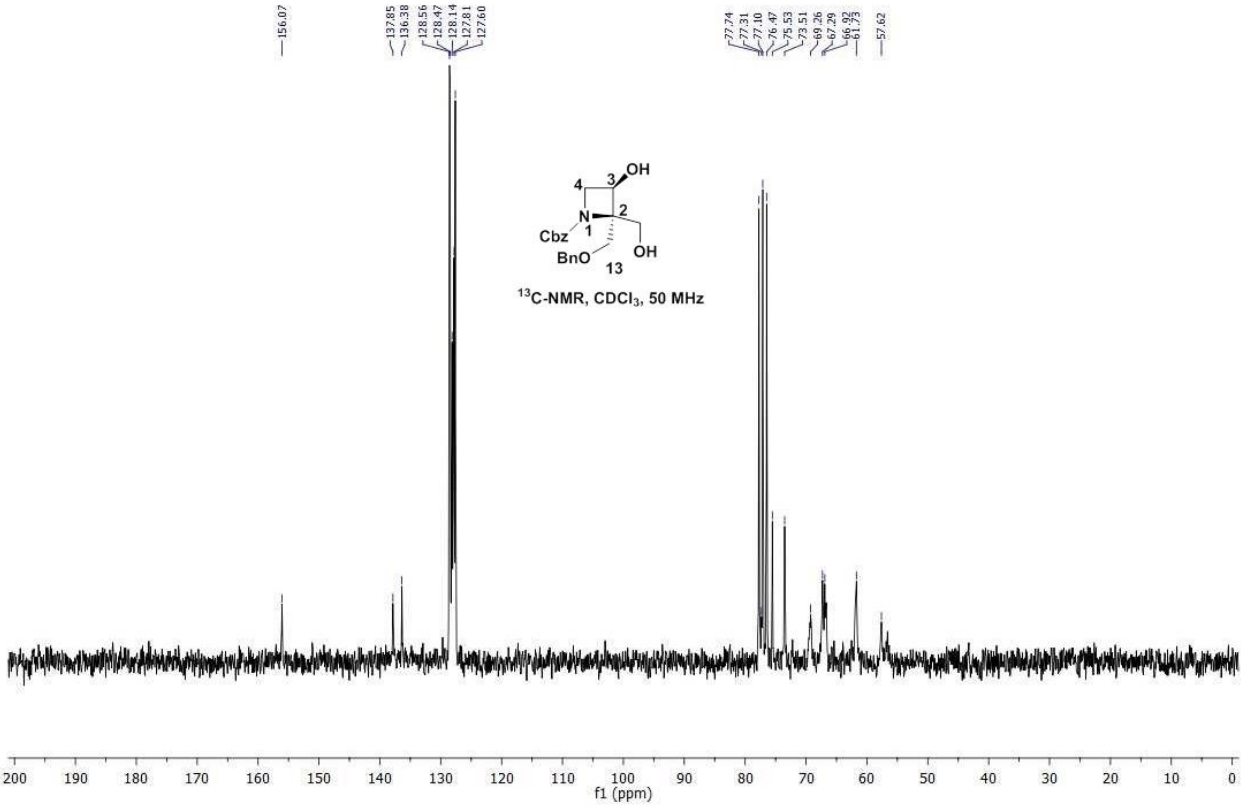

155.76
155.22
138.11
137.80
136.37
136.20
128.45
128.31
128.27
127.72
127.32
108.63
83.24
82.81
78.87
78.71
77.40
77.12
76.98
76.55
73.34
73.16
66.83
66.38
65.72
56.62
55.34
27.77
27.21
Cbz
OBn
12
13C-NMR, CDCl3, 75 MHz
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

(2S,3R)-Benzyl-2-benzyloxymethyl-2-hydroxylmethyl-3-hydroxy-azetidine-1-carboxylate (13)

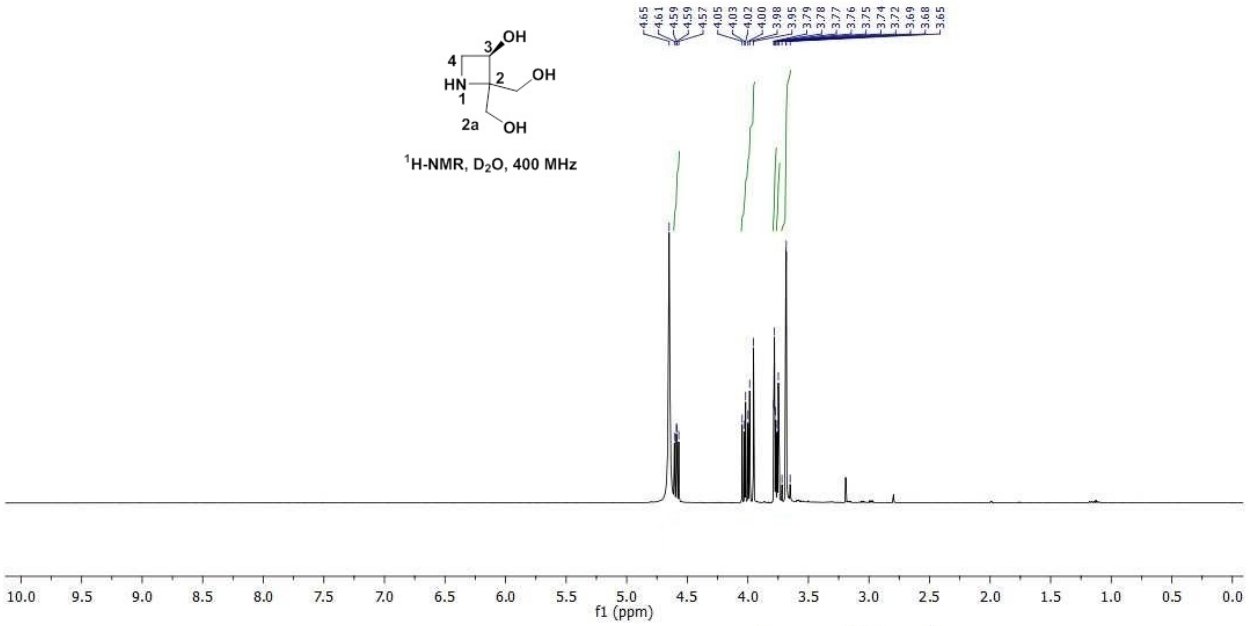
^{1}H-NMR, $CDCl_3$, 500 MHz



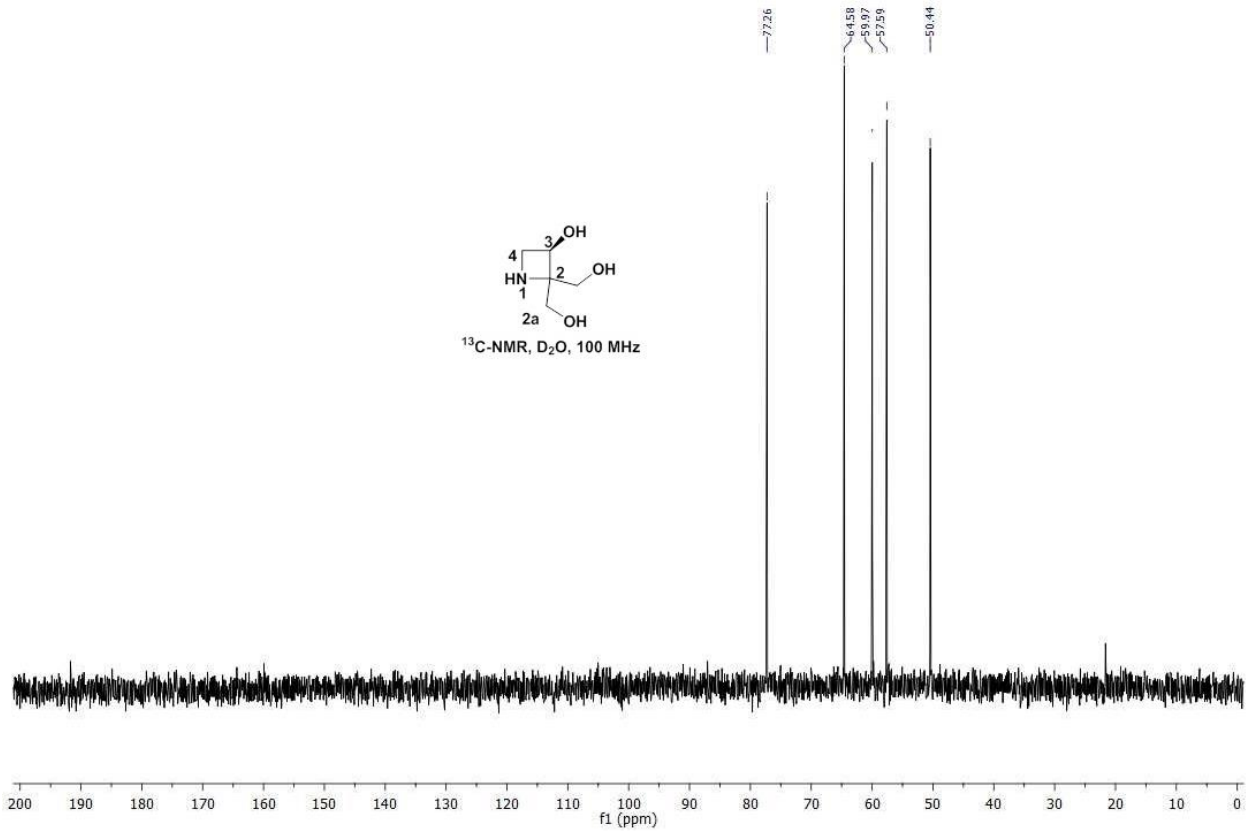
^{13}C-NMR, $CDCl_3$, 50 MHz



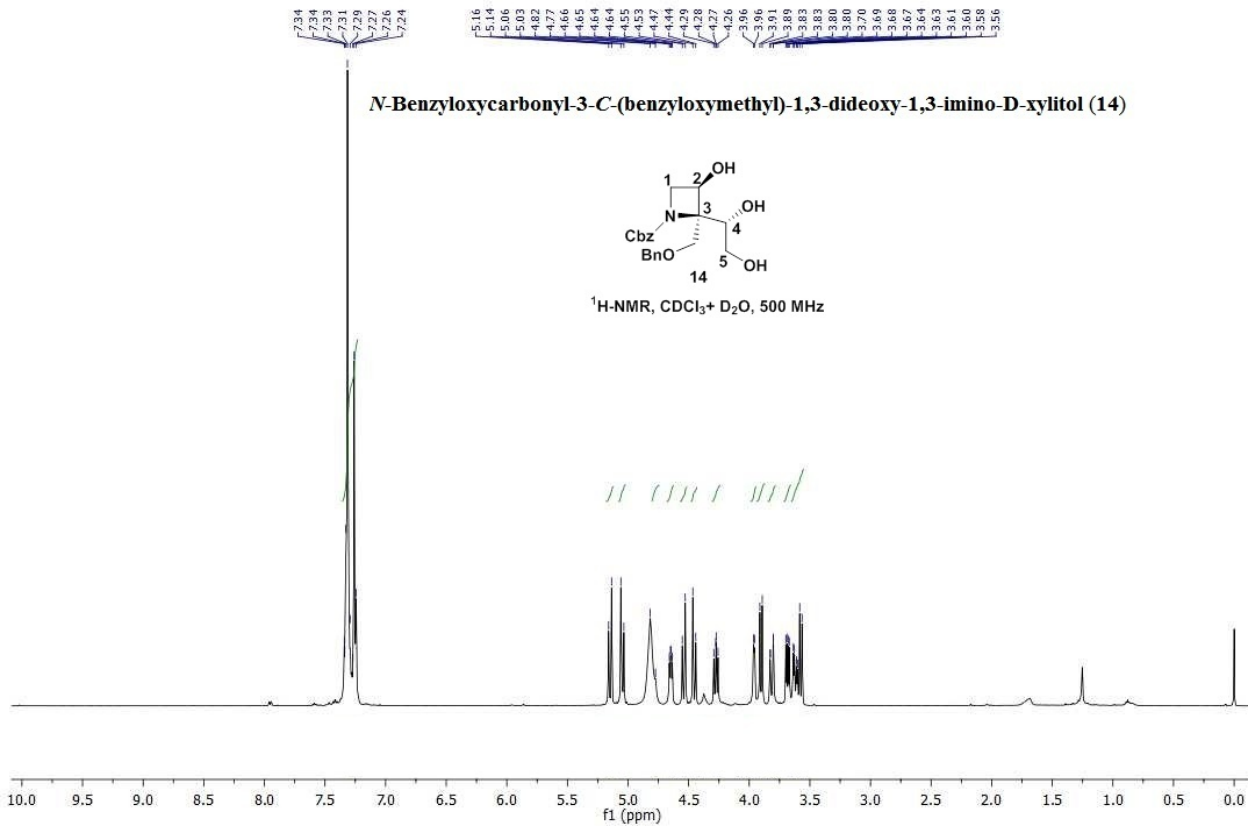
(3R)-2,2-Bis(hydroxymethyl)-azetidin-3-ol (2a)

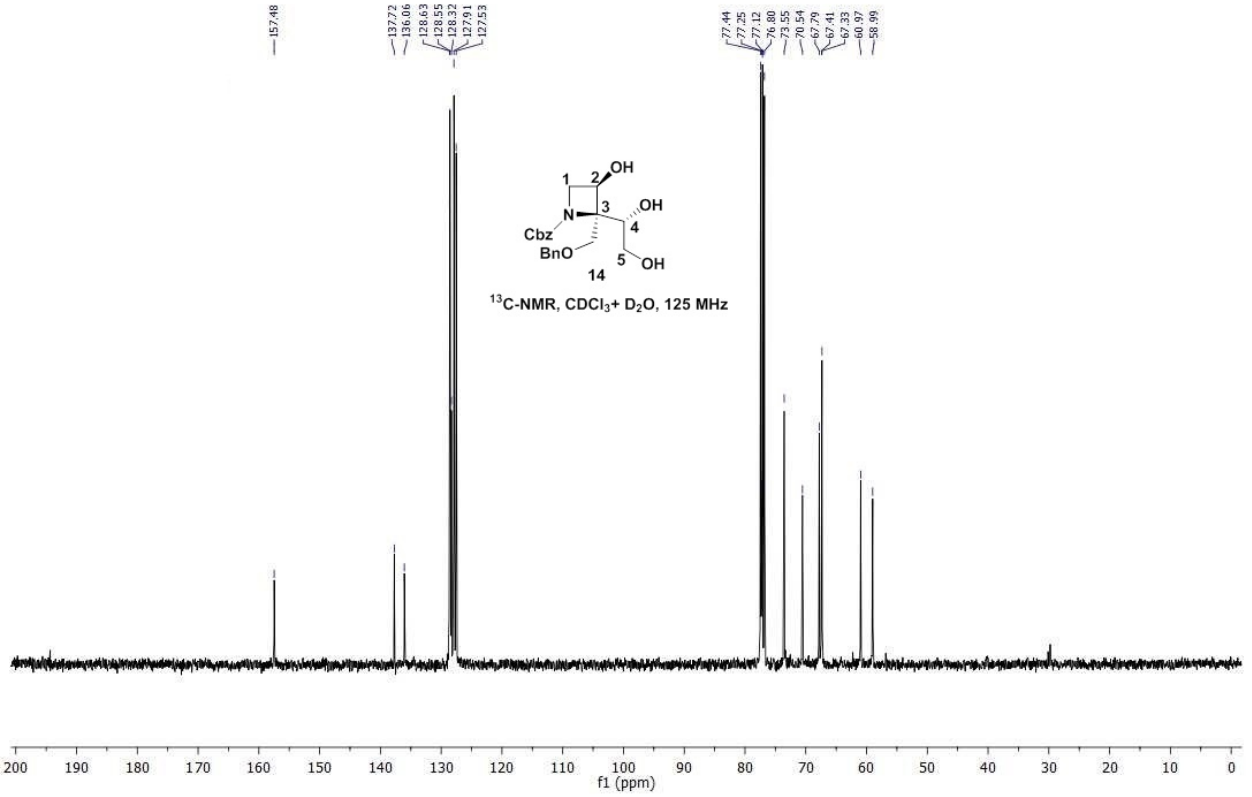


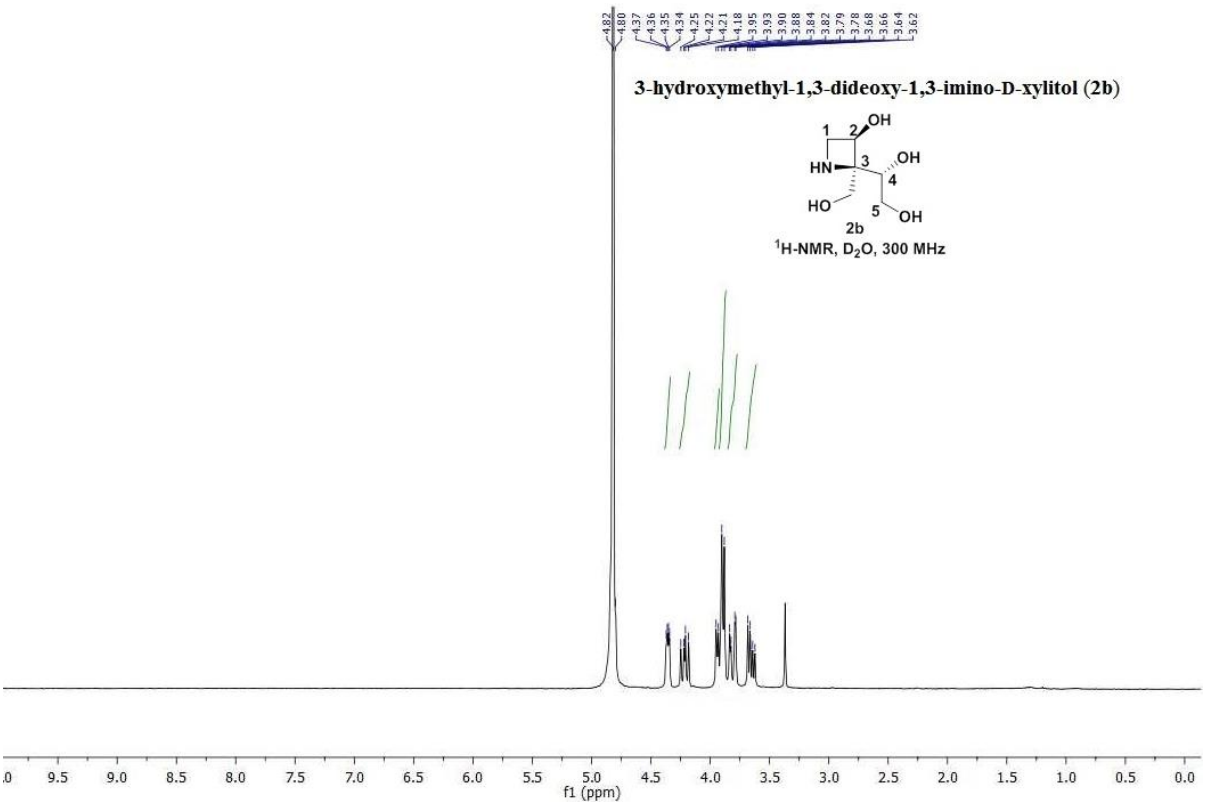
^{1}H-NMR, D_2O, 400 MHz

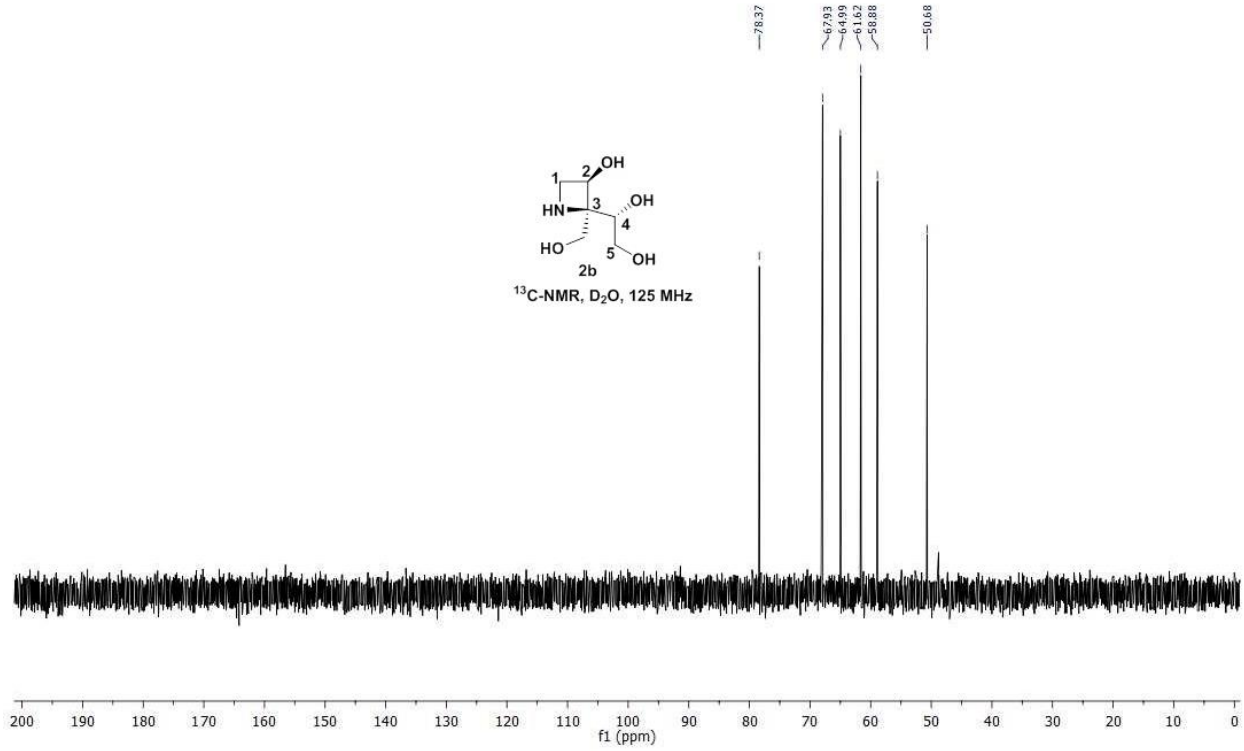


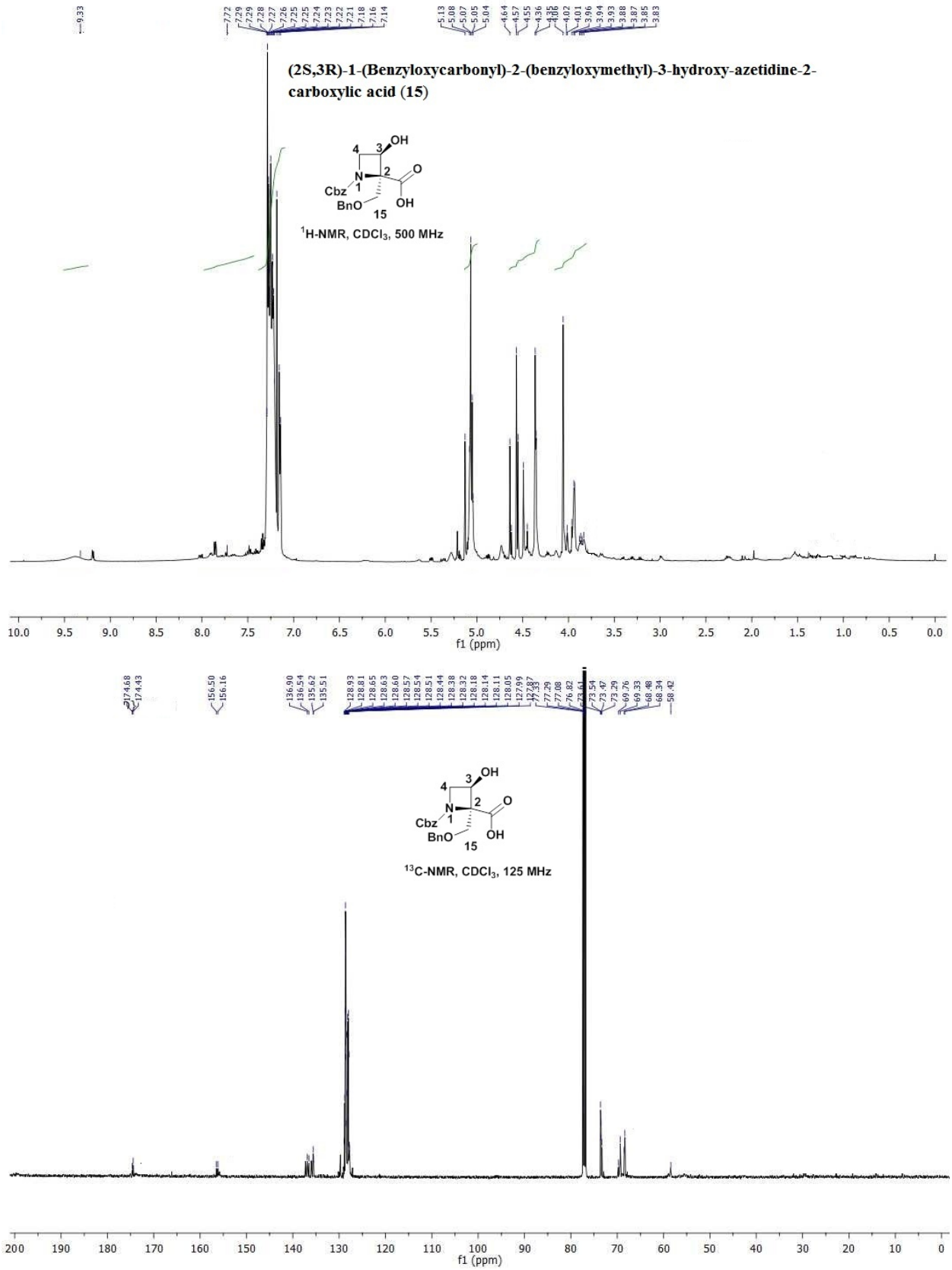
^{13}C-NMR, D_2O, 100 MHz


7.34
7.34
7.33
7.31
7.29
7.27
7.26
7.24
5.16
5.14
5.06
5.03
4.82
4.77
4.66
4.65
4.64
4.64
4.55
4.53
4.47
4.44
4.29
4.28
4.27
4.26
3.96
3.96
3.91
3.89
3.83
3.83
3.80
3.80
3.70
3.69
3.68
3.67
3.64
3.63
3.61
3.60
3.58
3.56
N-Benzyloxycarbonyl-3-C-(benzyloxymethyl)-1,3-dideoxy-1,3-imino-D-xylitol (14)
OH
1
2
3
OH
N
4
Cbz
BnO
5
OH
14
1H-NMR, CDCl3+ D2O, 500 MHz
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)

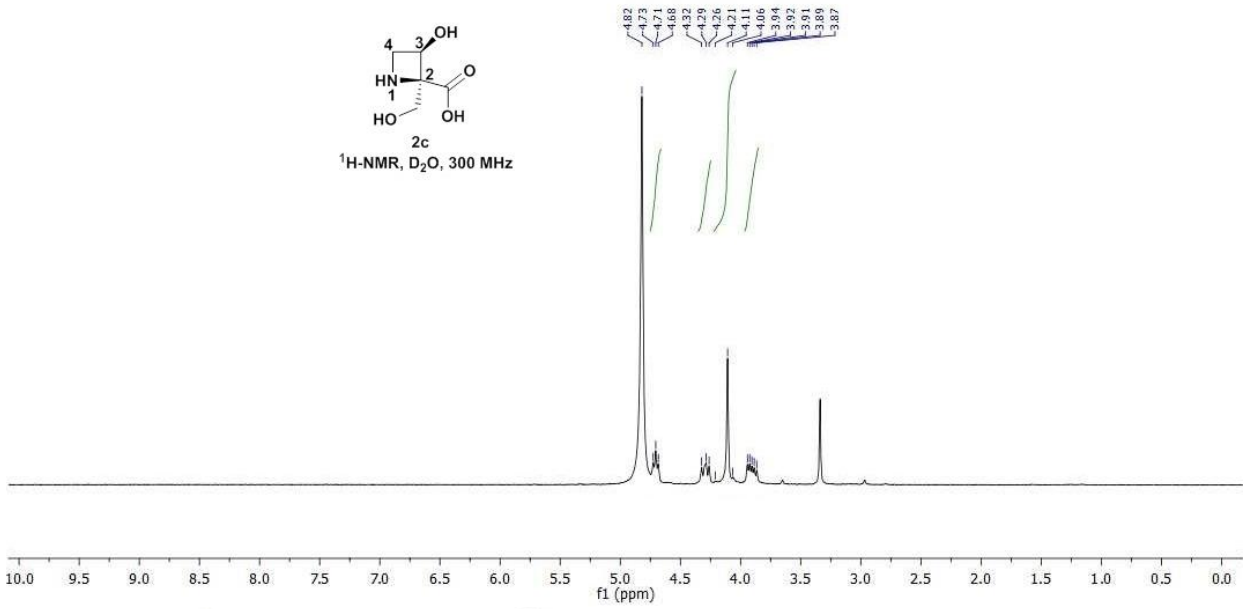

157.48
137.72
136.06
128.63
128.55
128.32
127.91
127.53
77.44
77.25
77.12
76.80
73.55
70.54
67.79
67.41
67.33
60.97
58.99
OH
1
2
3
OH
N
4
Cbz
BnO
5
OH
14
13C-NMR, CDCl3+ D2O, 125 MHz
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

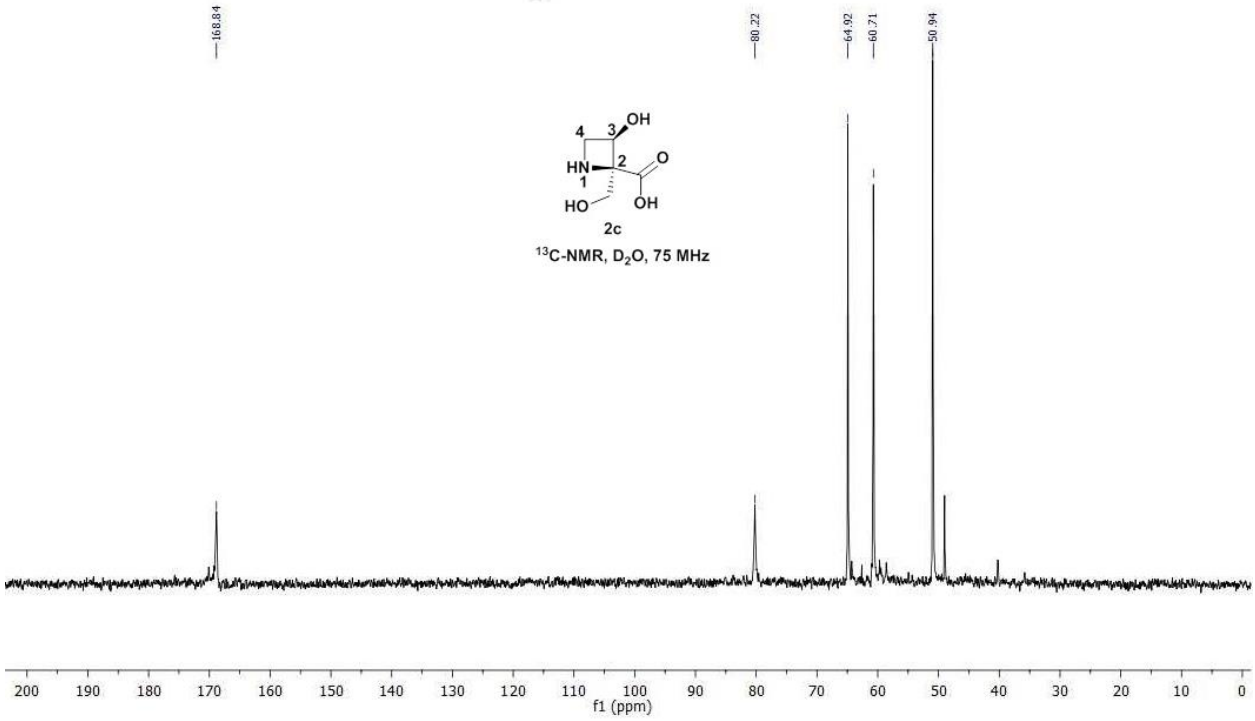

4.82
4.80
4.37
4.36
4.35
4.34
4.25
4.22
4.21
4.18
3.95
3.93
3.90
3.88
3.84
3.82
3.79
3.78
3.68
3.66
3.64
3.62
3-hydroxymethyl-1,3-dideoxy-1,3-imino-D-xylitol (2b)
1
2
OH
HN
3
OH
4
HO
5
OH
2b
1H-NMR, D2O, 300 MHz
f1 (ppm)


78.37
67.93
64.99
61.62
58.88
50.68
1
2
OH
HN
3
OH
4
HO
5
OH
2b
13C-NMR, D2O, 125 MHz
f1 (ppm)

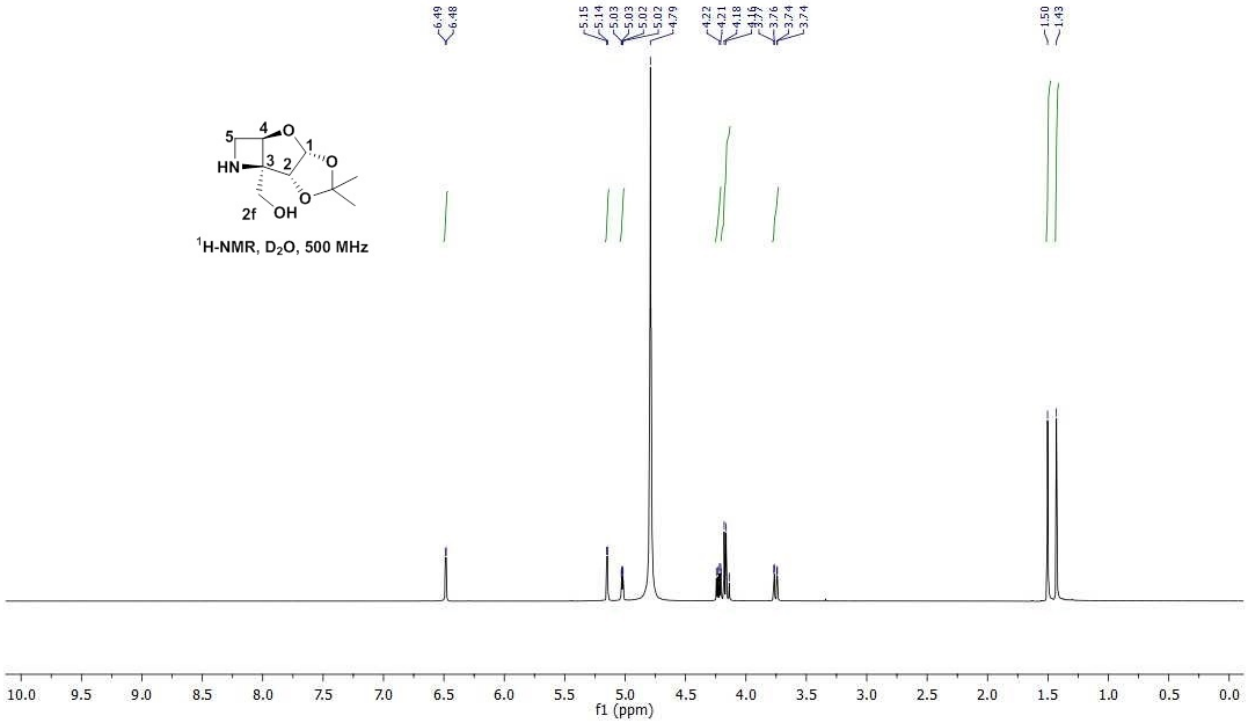

(2S,3R)-1-(Benzyloxycarbonyl)-2-(benzyloxymethyl)-3-hydroxy-azetidine-2-carboxylic acid (15)
Cbz
BnO
OH
15
^{1}H-NMR, CDCl$_3$, 500 MHz
f1 (ppm)
^{13}C-NMR, CDCl$_3$, 125 MHz
f1 (ppm)

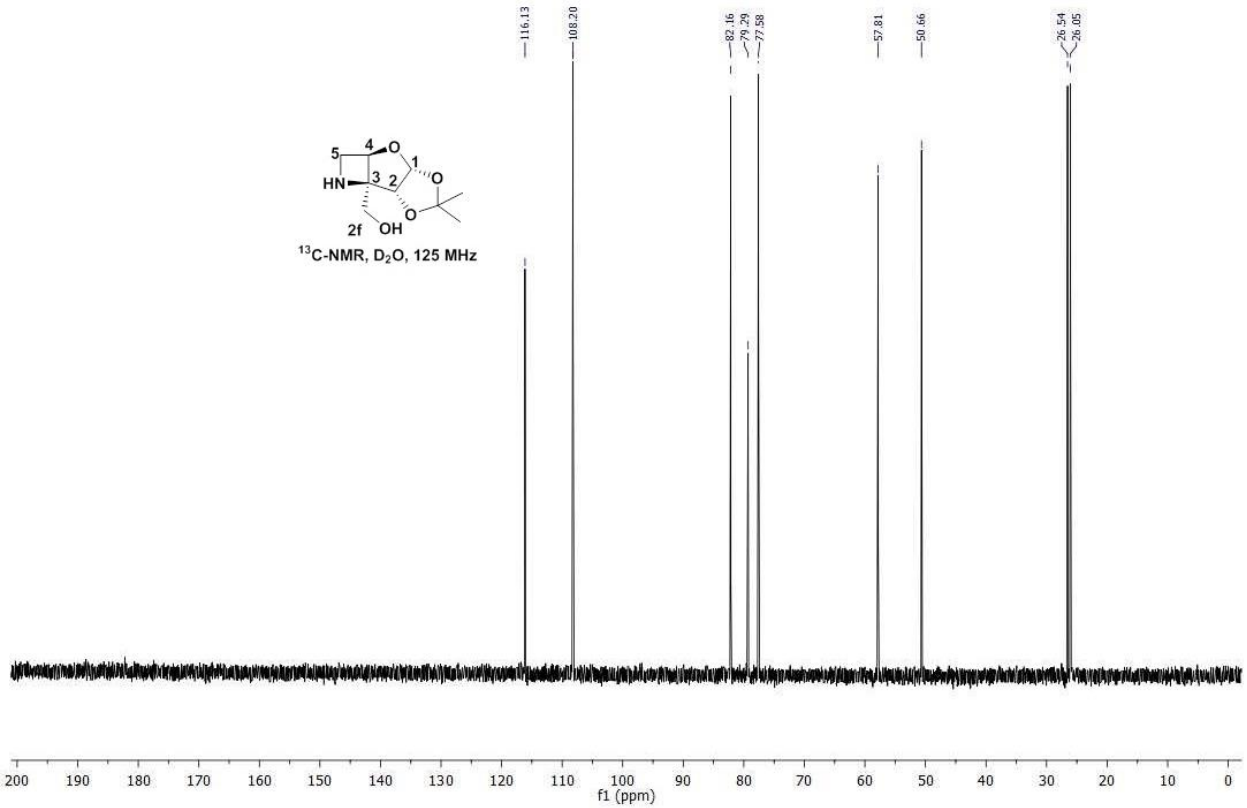
(2S,3R)- 2-(hydroxymethyl)-3-hydroxy-azetidine-2-carboxylic acid (2c)





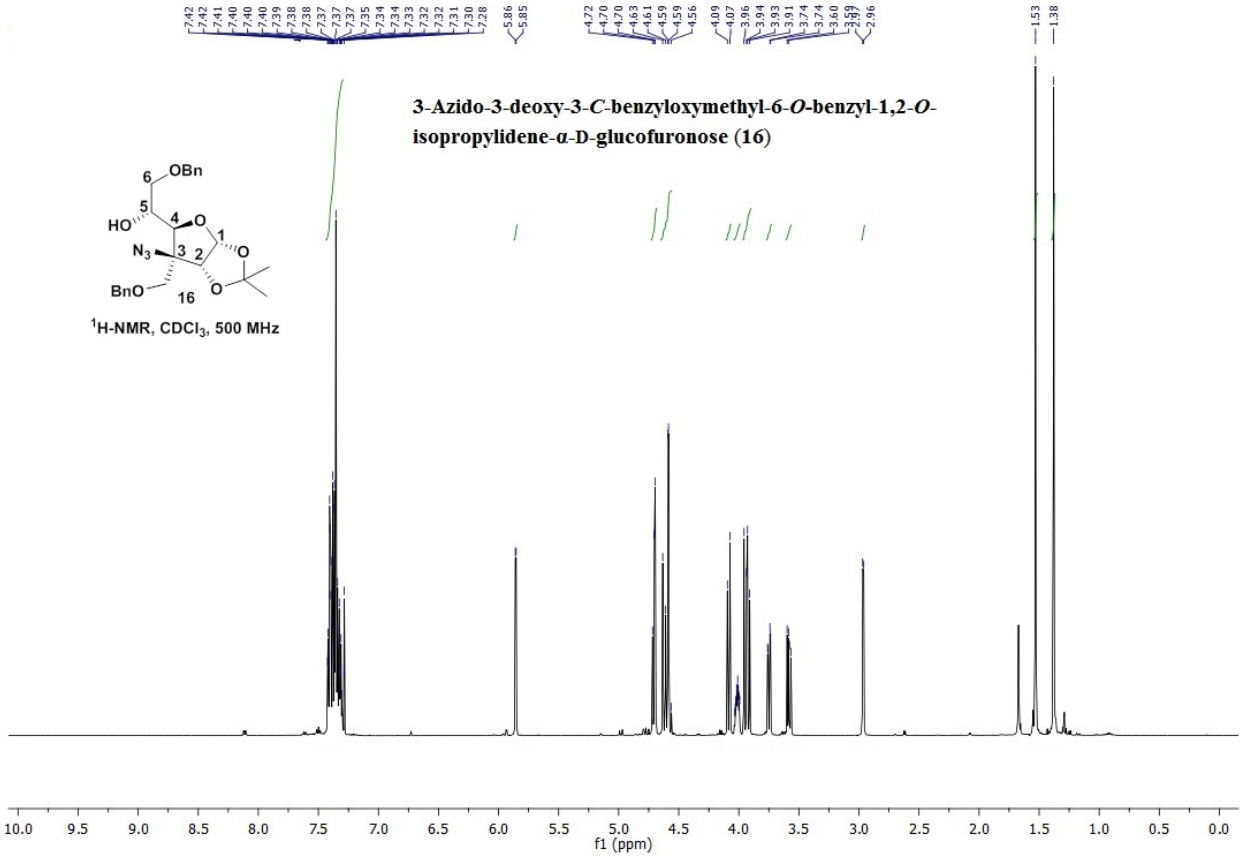
3,5-dideoxy-3-*C*-(hydroxymethyl)-3,5-imino1,2-di-*O*-isopropylidene-α-D-xylofuronose (2f)



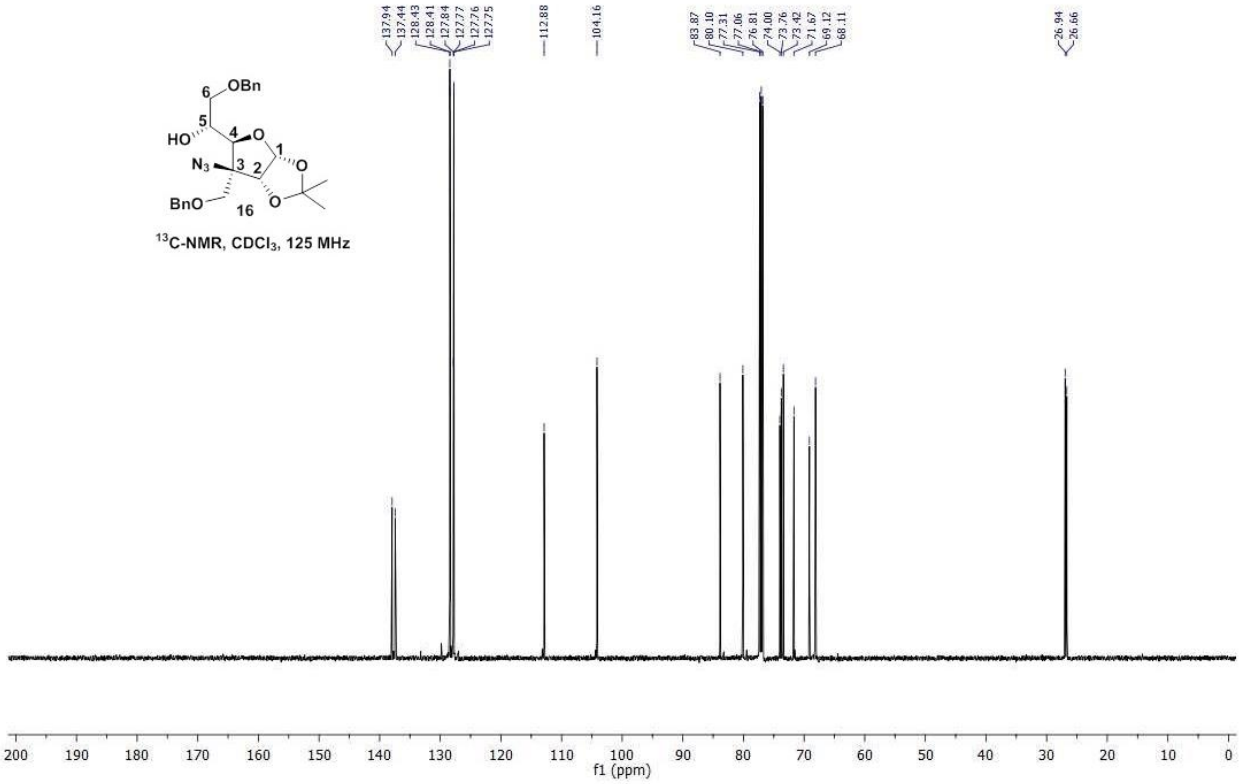


3-Azido-3-deoxy-3-*C*-benzyloxymethyl-6-*O*-benzyl-1,2-*O*-isopropylidene-α-D-glucofuronose (16)

^{1}H-NMR, $CDCl_3$, 500 MHz

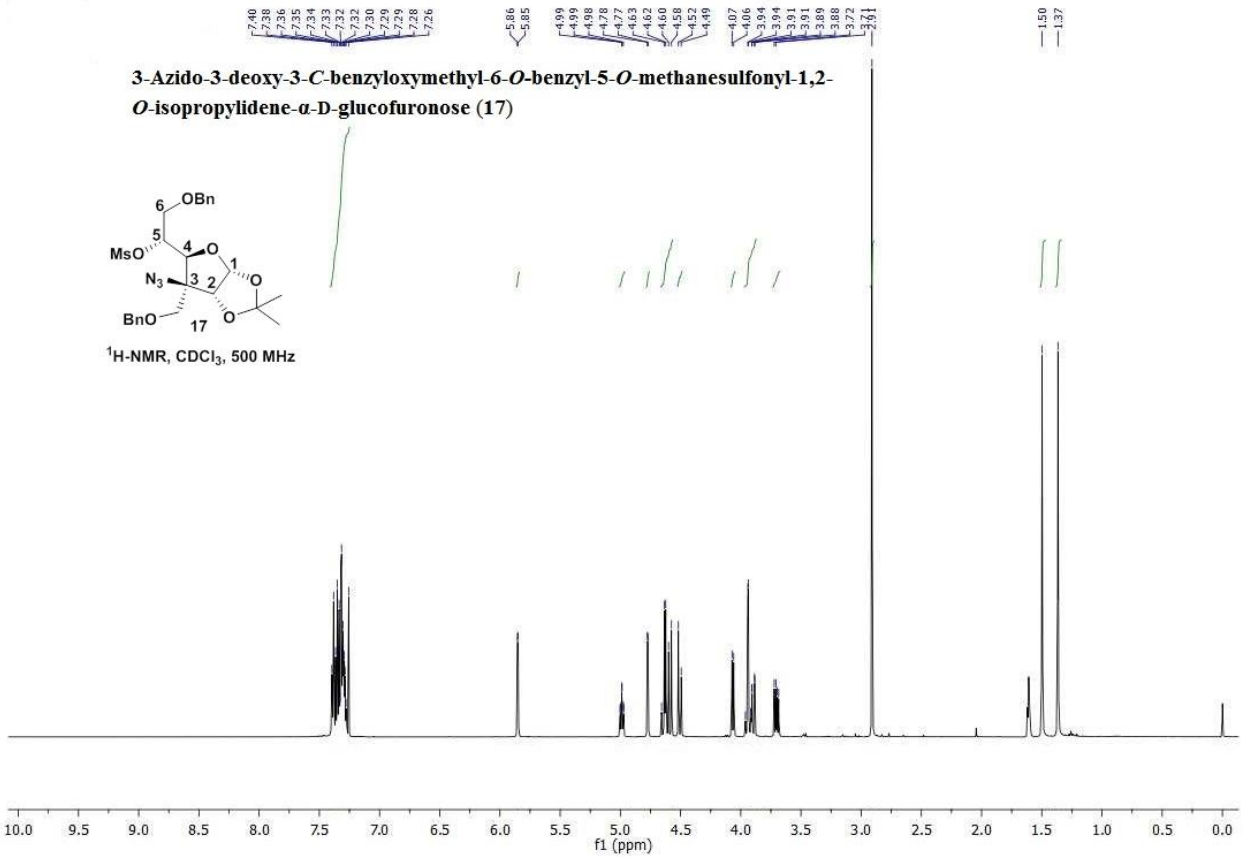


^{13}C-NMR, $CDCl_3$, 125 MHz

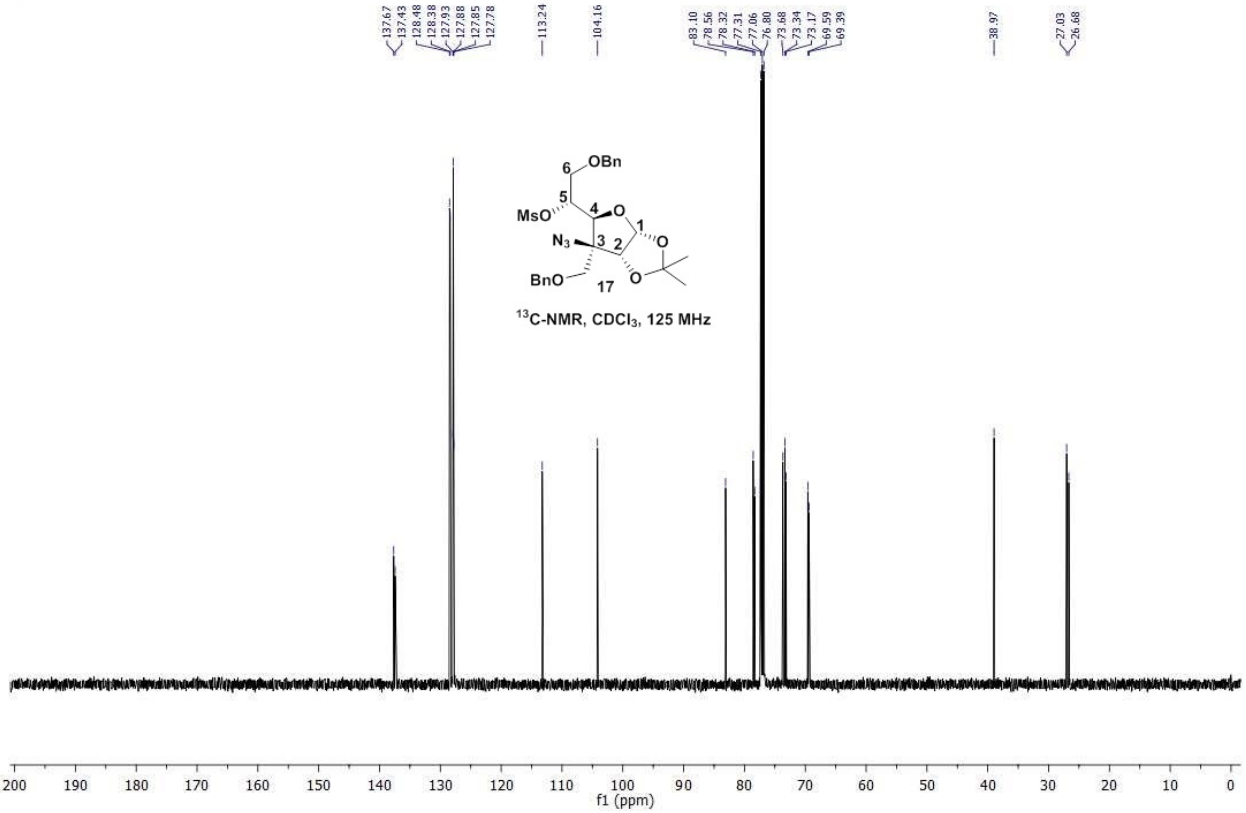


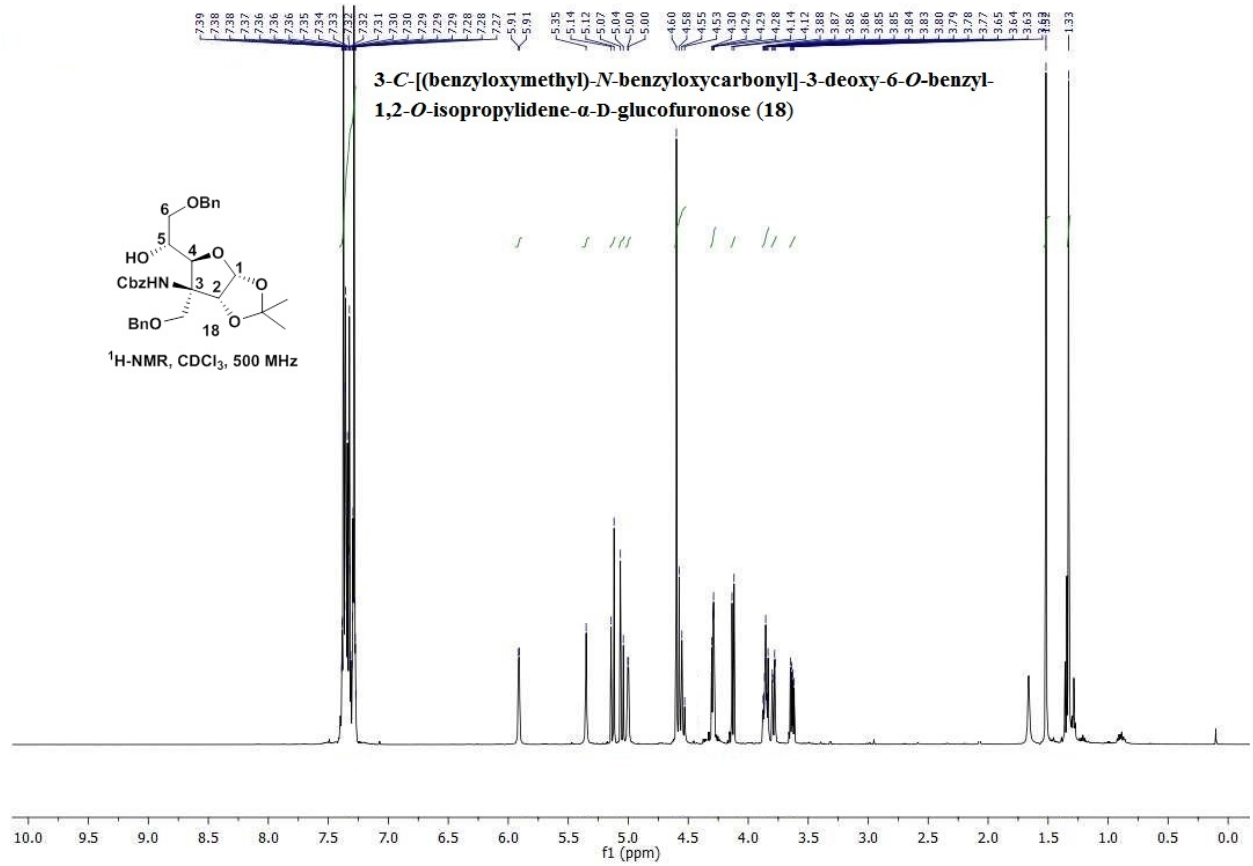
3-Azido-3-deoxy-3-*C*-benzyloxymethyl-6-*O*-benzyl-5-*O*-methanesulfonyl-1,2-*O*-isopropylidene-α-D-glucofuronose (17)

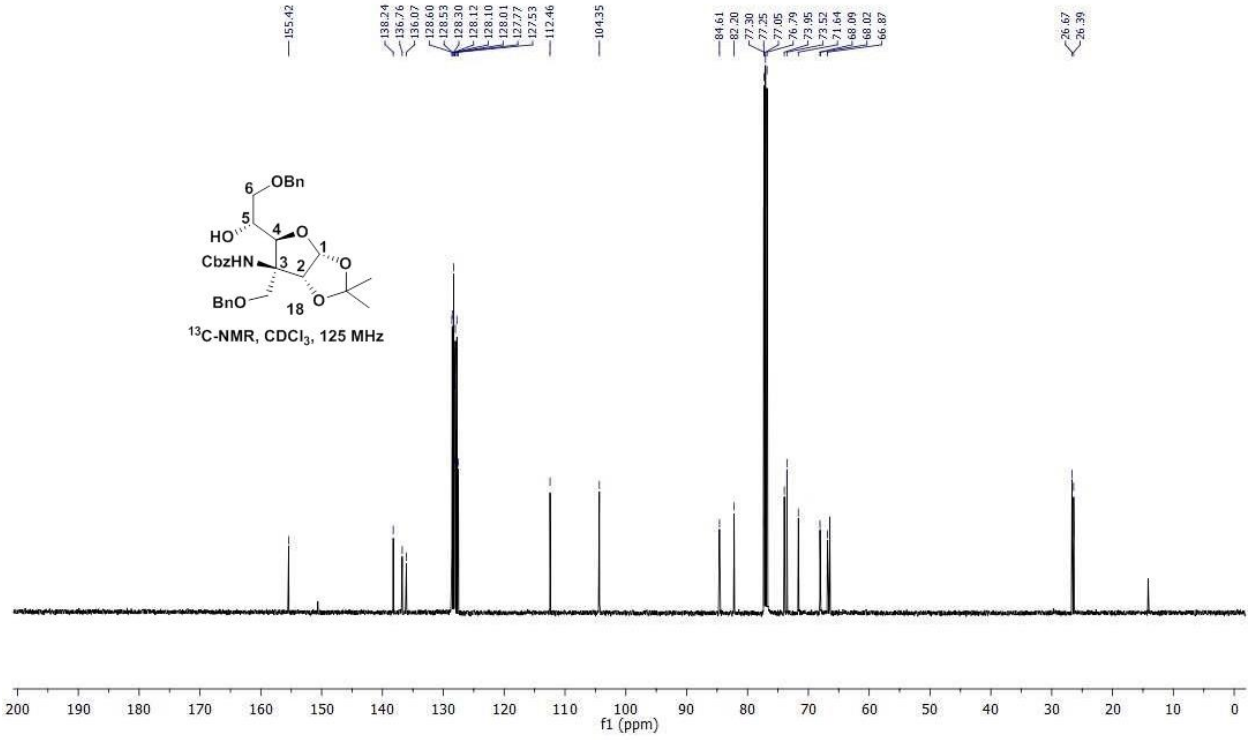
^{1}H-NMR, $CDCl_3$, 500 MHz

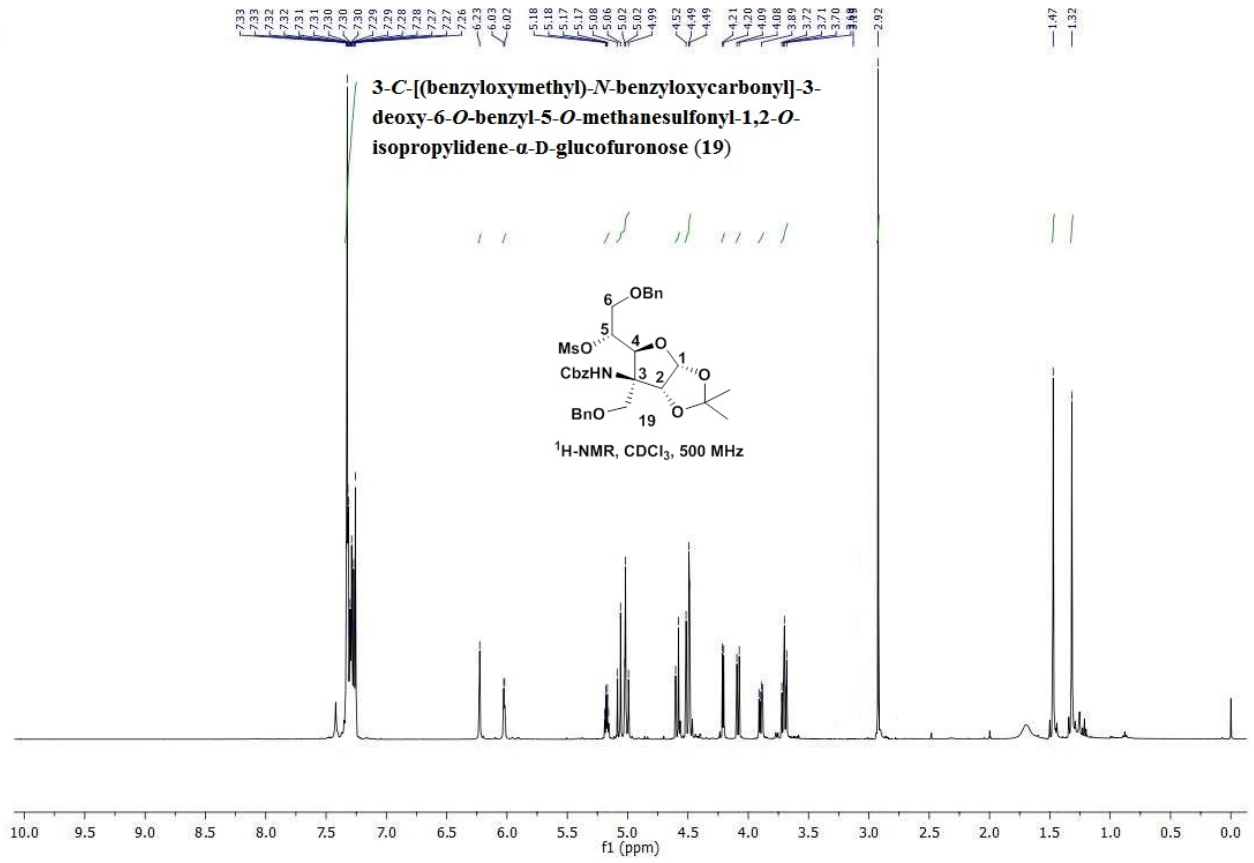


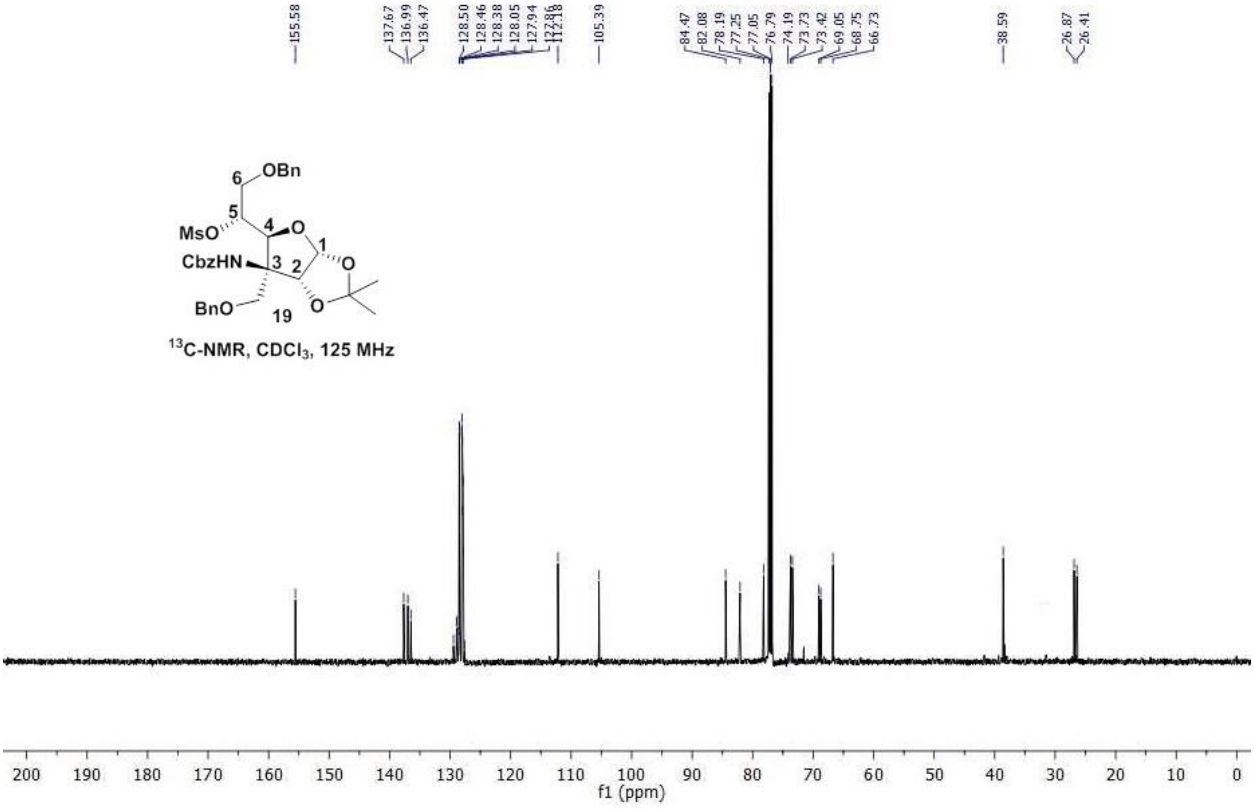
^{13}C-NMR, $CDCl_3$, 125 MHz



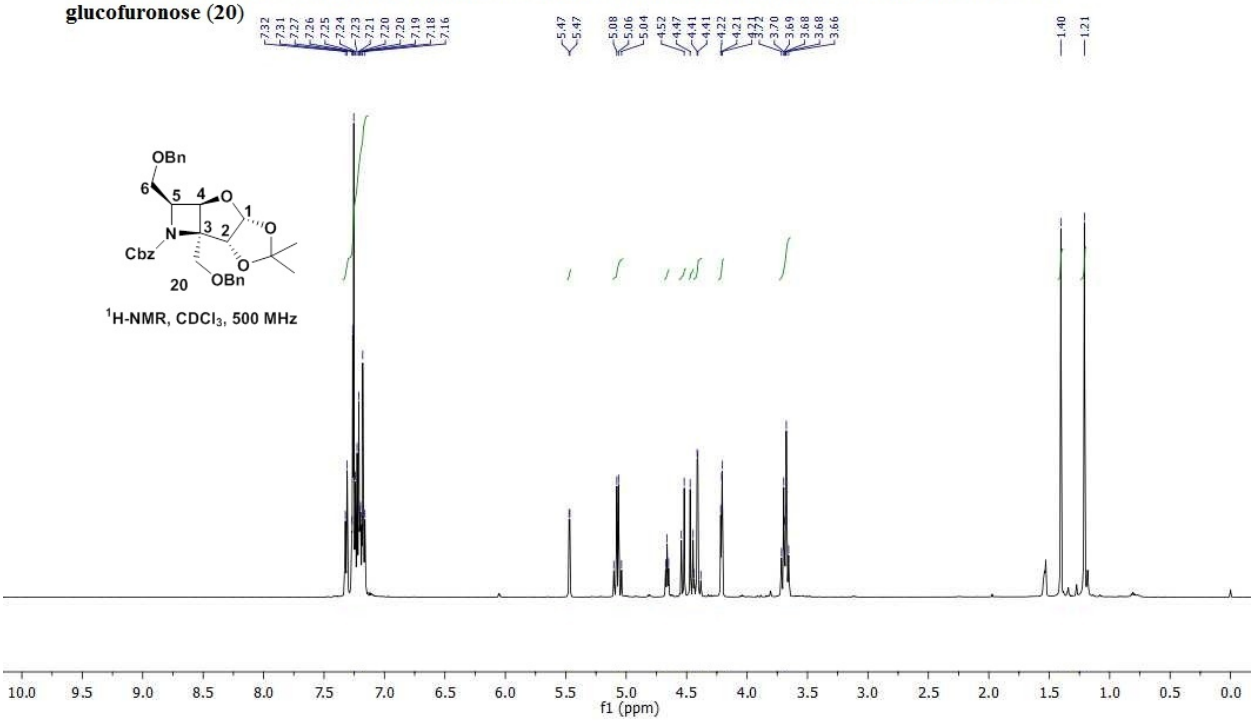

3-C-[(benzyloxymethyl)-N-benzyloxycarbonyl]-3-deoxy-6-O-benzyl-1,2-O-isopropylidene-α-D-glucofuronose (18)
OBn
HO
CbzHN
BnO
18
1H-NMR, CDCl3, 500 MHz
f1 (ppm)

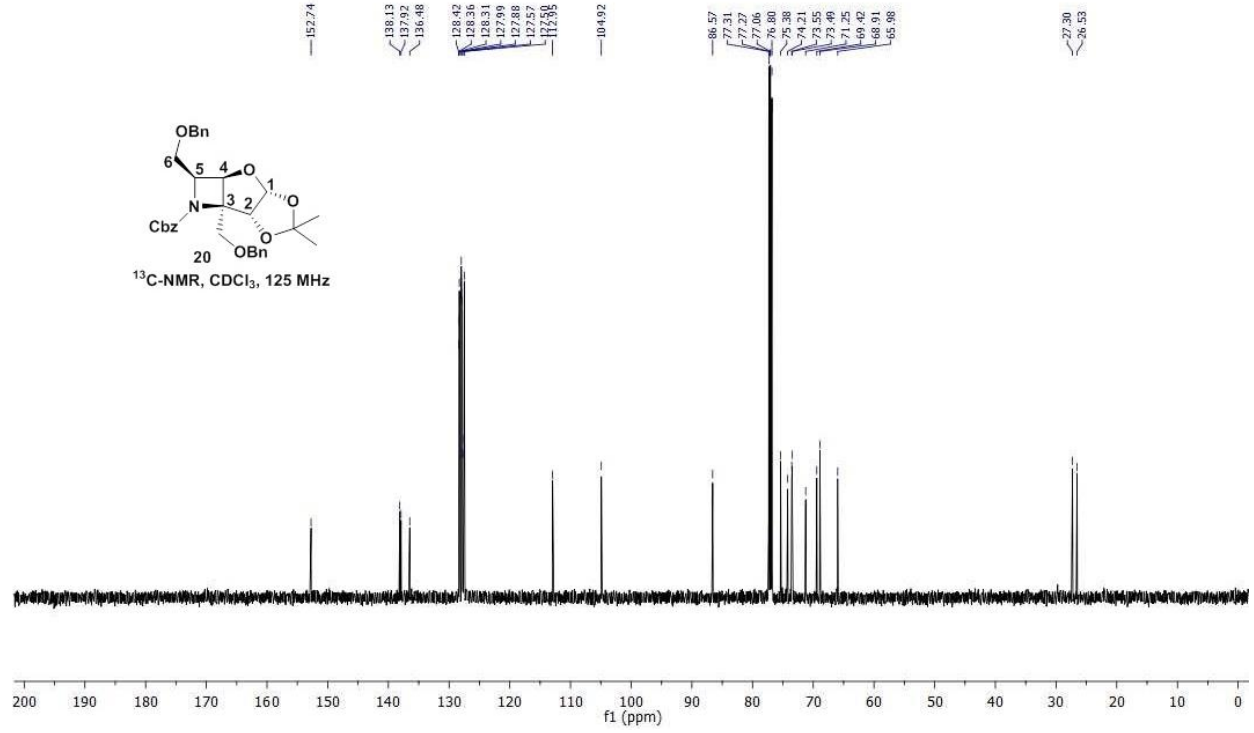

OBn
HO
CbzHN
BnO
18
13C-NMR, CDCl3, 125 MHz
f1 (ppm)


3-C-[(benzyloxymethyl)-N-benzyloxycarbonyl]-3-deoxy-6-O-benzyl-5-O-methanesulfonyl-1,2-O-isopropylidene-α-D-glucofuronose (19)
6
OBn
5
4
O
1
MsO
CbzHN
3
2
O
BnO
19
O
1H-NMR, CDCl3, 500 MHz
f1 (ppm)

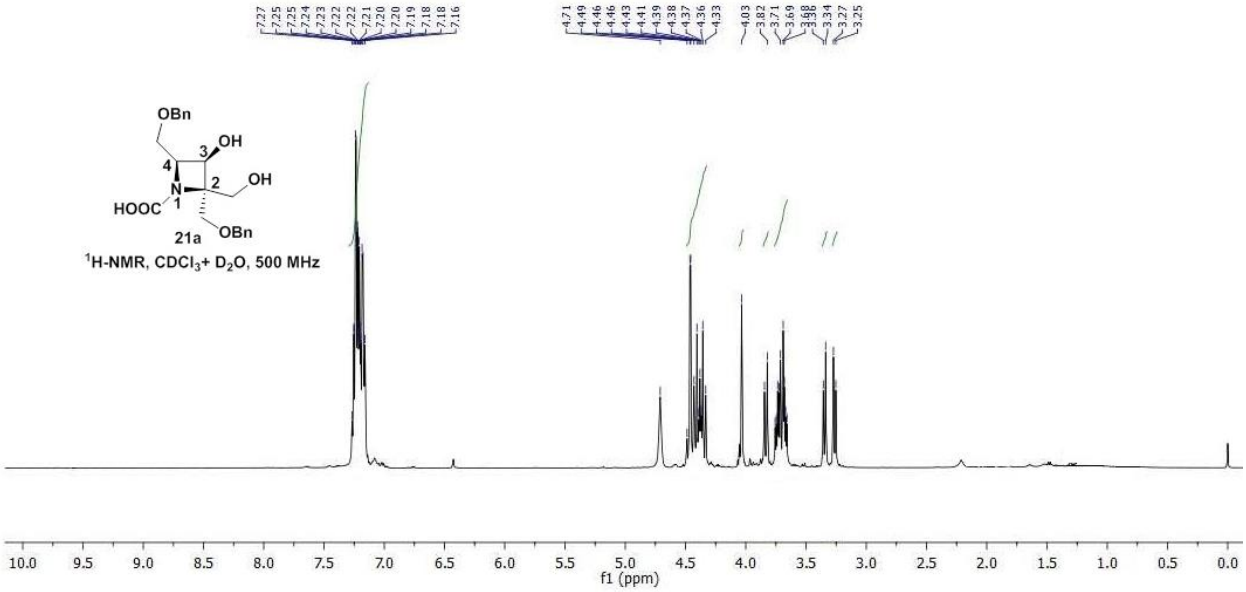

6
OBn
5
4
O
1
MsO
CbzHN
3
2
O
BnO
19
O
13C-NMR, CDCl3, 125 MHz
f1 (ppm)

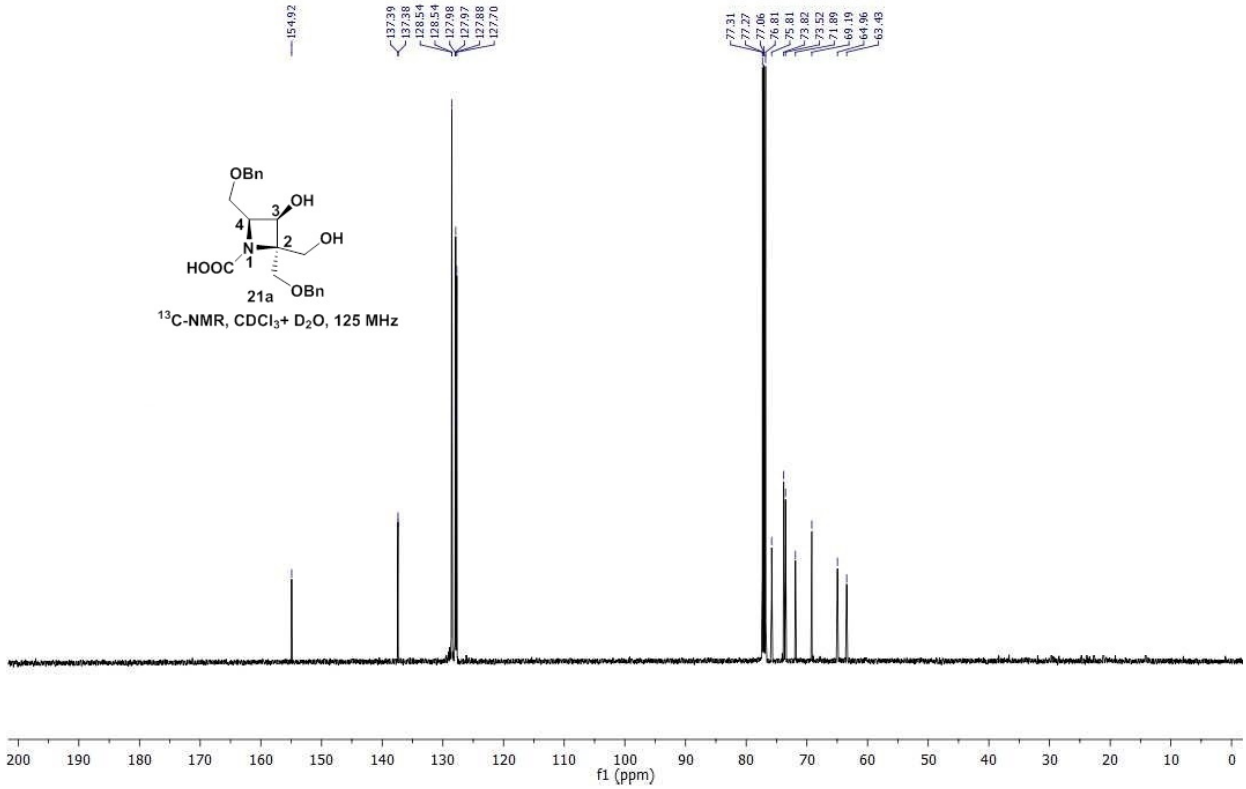
***N*-benzyloxycarbonyl-3-*C*-(benzyloxymethyl)-6-*O*-benzyl-3,5-dideoxy--3,5-imino-1,2-*O*-isopropylidene-α-D-glucofuronose (20)**



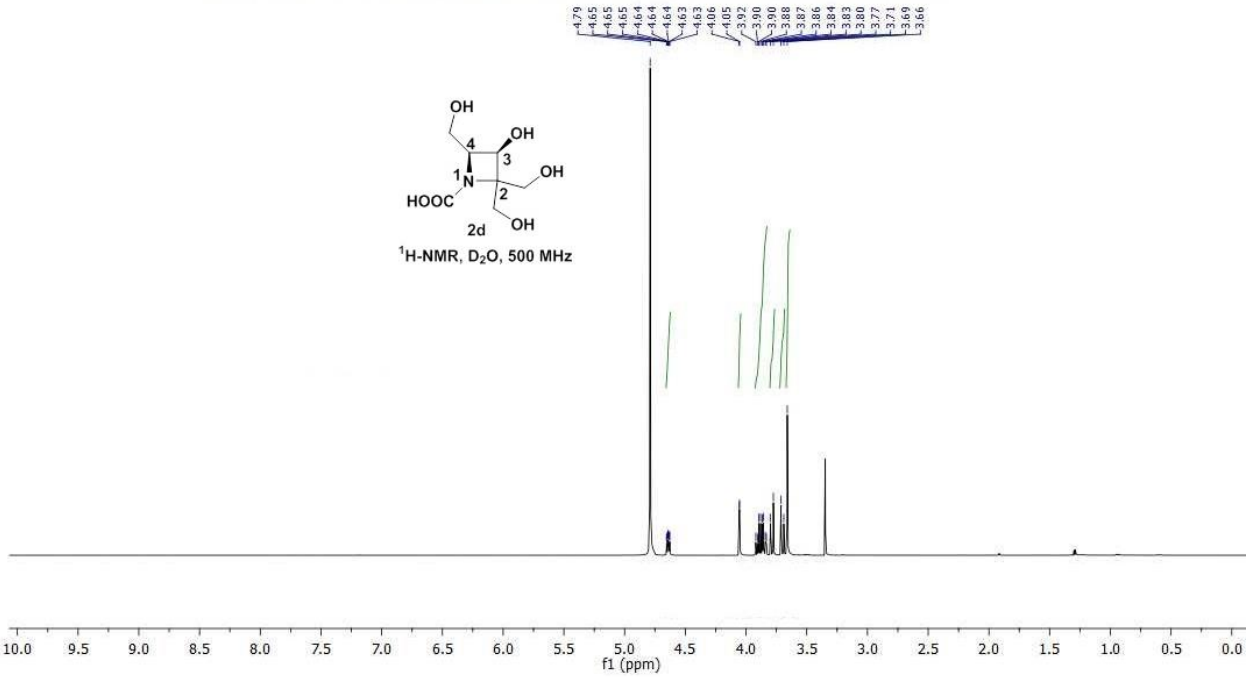


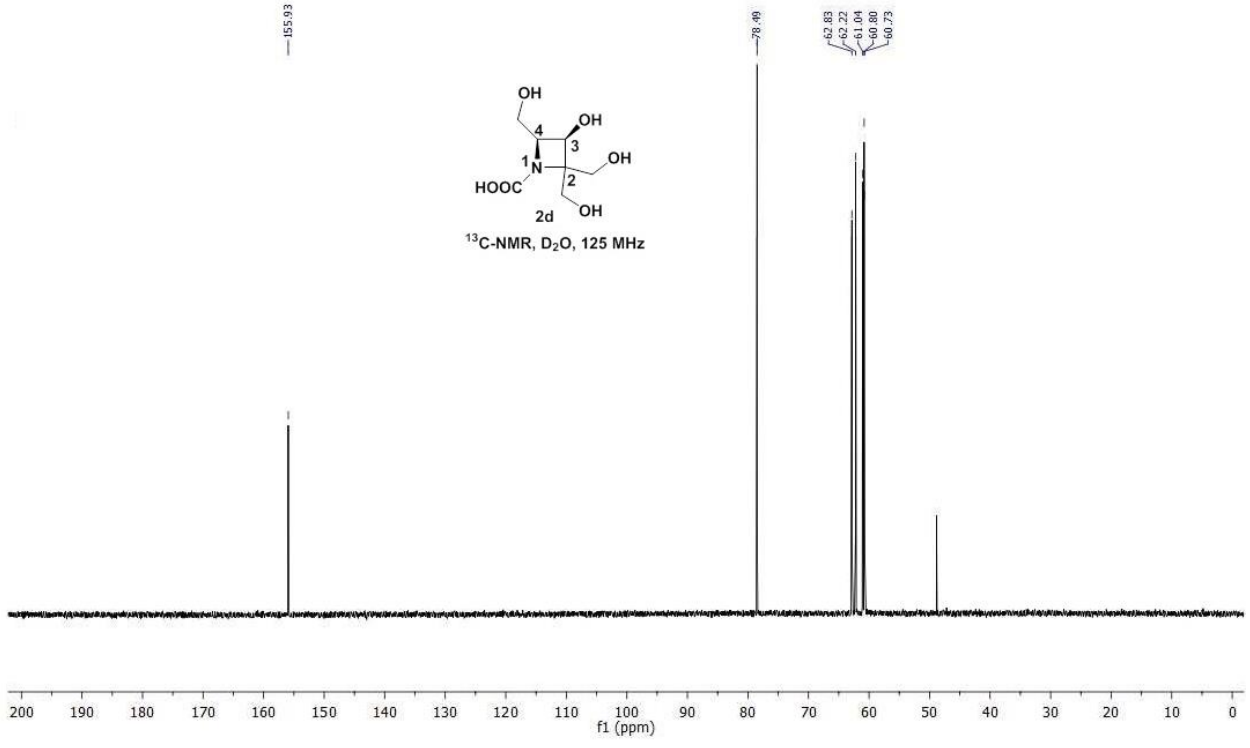
(2*S*,3*R*,4*S*)-2,4-Bis(benzyloxymethyl)-2-hydroxymethyl-3-hydroxy-azetidine-1-carboxylic acid (21a)

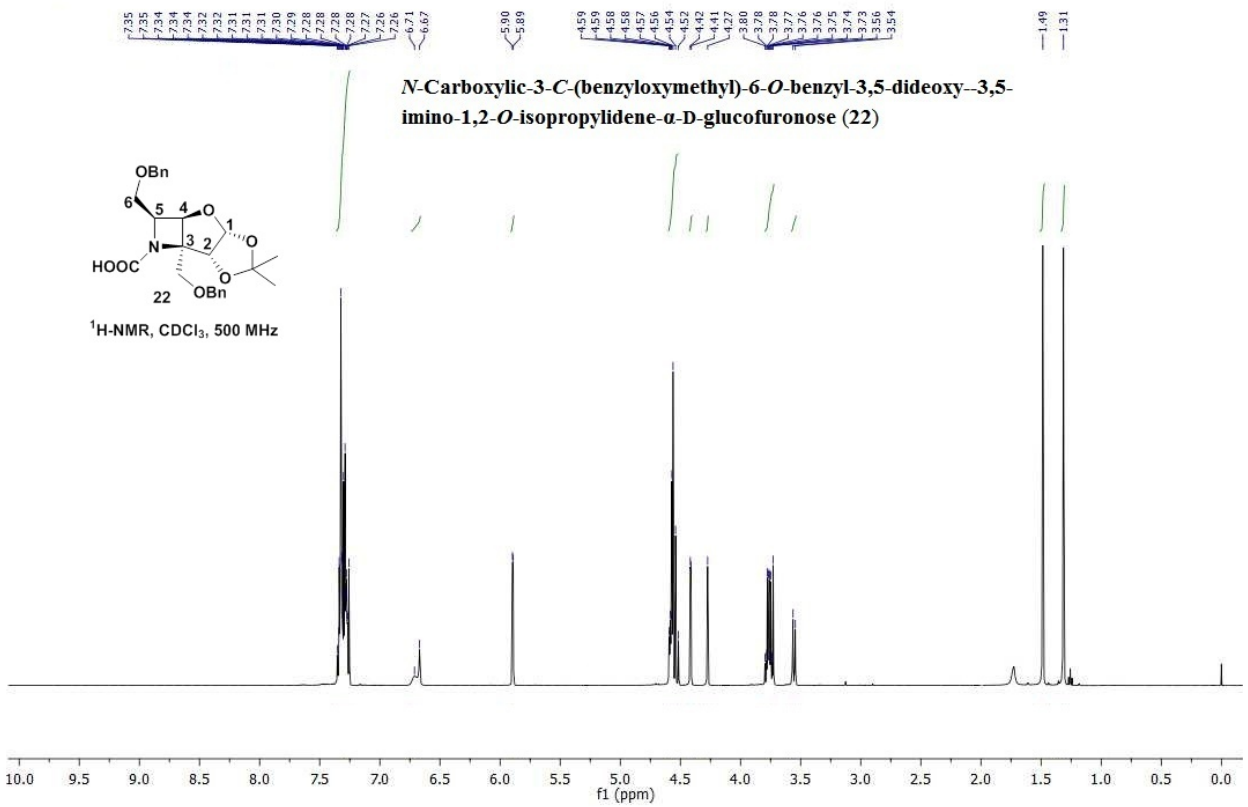


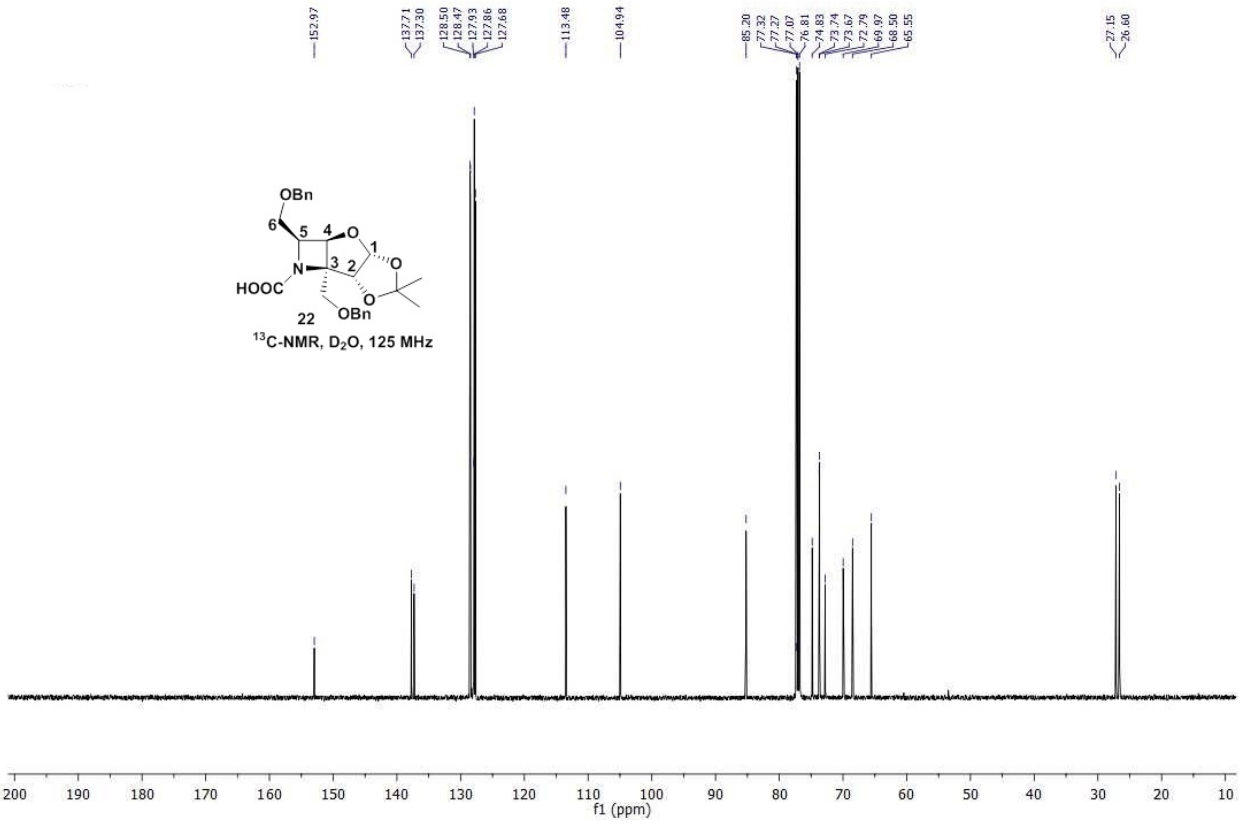


(3*R*,4*S*)-3-Hydroxy-2,2,4-tris(hydroxymethyl)-azetidine-1-carboxylic acid (2d)

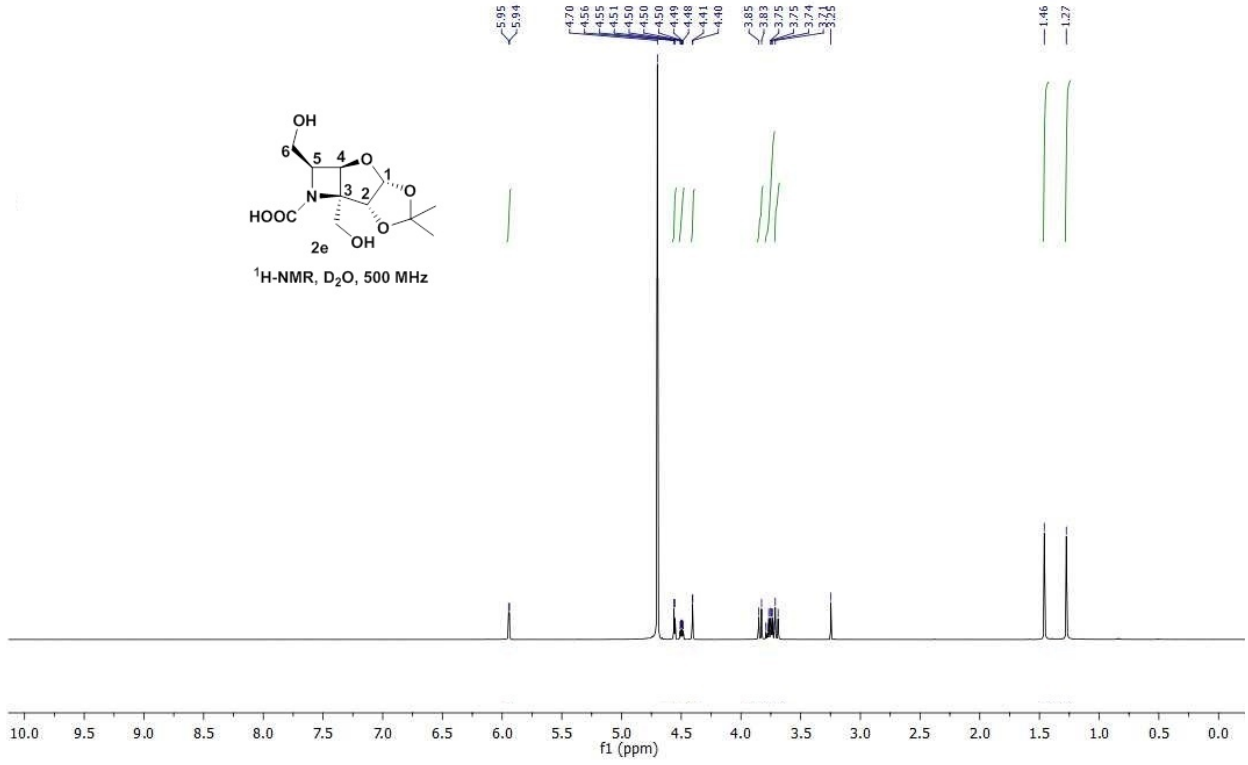




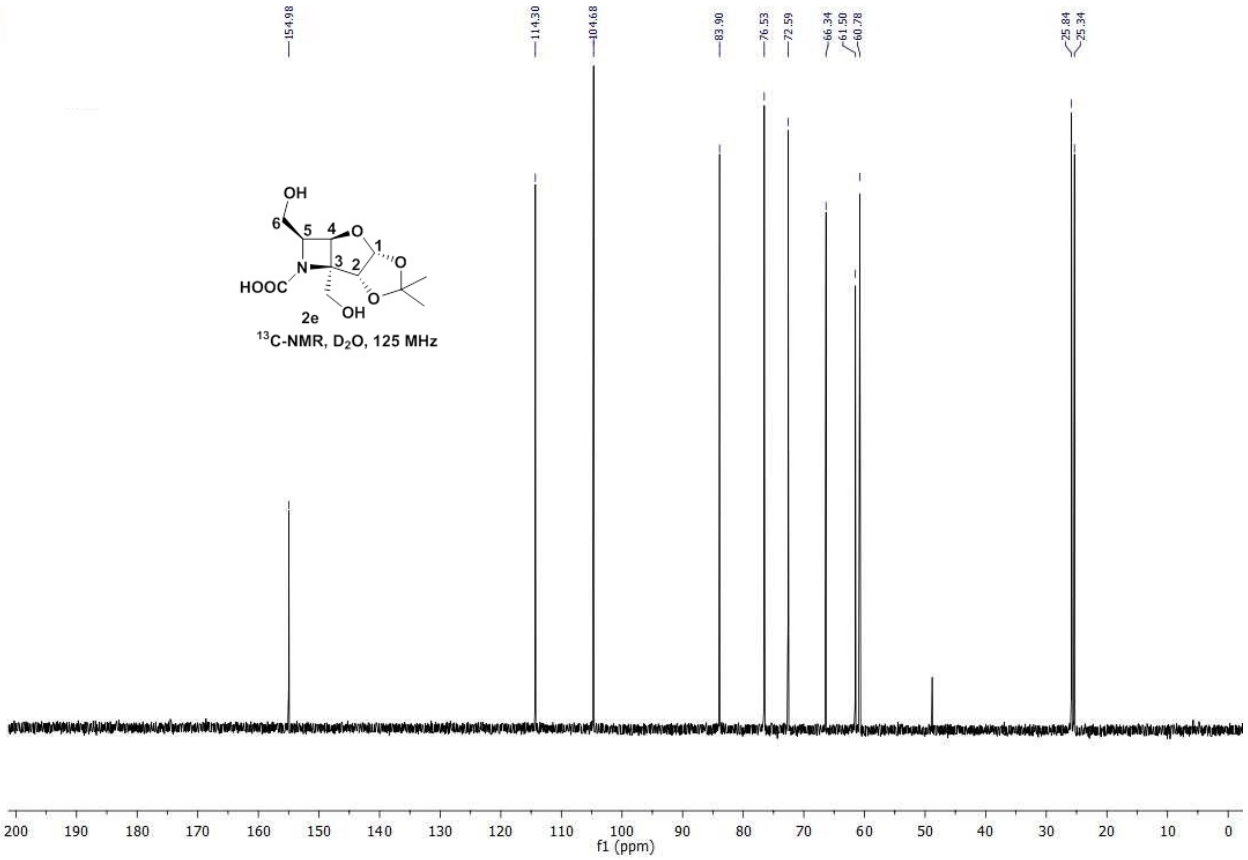

N-Carboxylic-3-C-(benzyloxymethyl)-6-O-benzyl-3,5-dideoxy--3,5-imino-1,2-O-isopropylidene-α-D-glucofuronose (22)
OBn
6
5
4
O
1
N
3
2
O
HOOC
O
22
OBn
1H-NMR, CDCl3, 500 MHz
f1 (ppm)


OBn
6
5
4
O
1
N
3
2
O
HOOC
O
22
OBn
13C-NMR, D2O, 125 MHz
f1 (ppm)

***N*-Carboxylic-3-*C*-(hydroxymethyl)-3,5-dideoxy--3,5-imino-1,2-*O*-isopropylidene-α-D-glucofuronose (2e)**



^{1}H-NMR, D_2O, 500 MHz



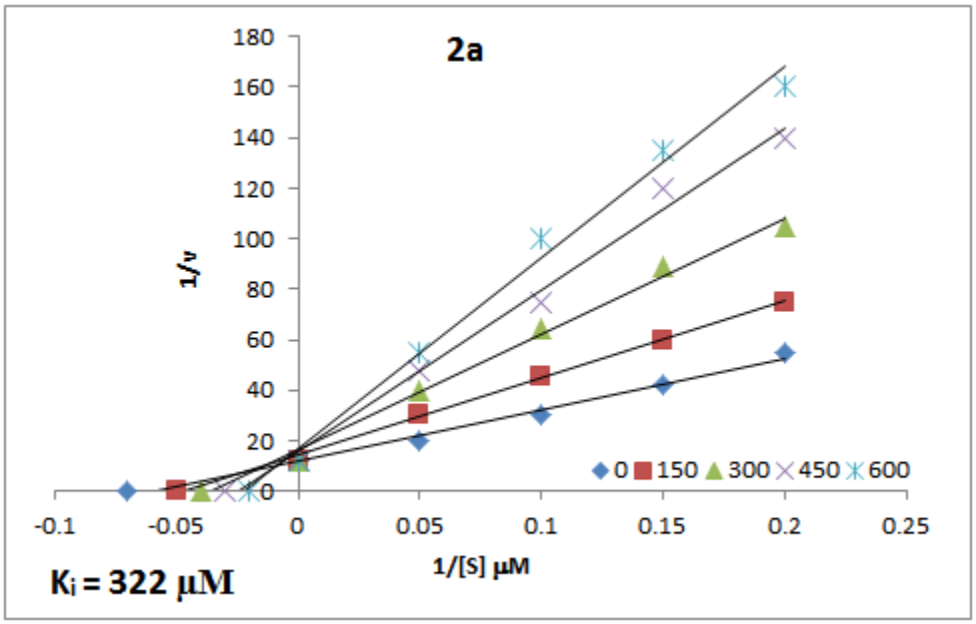
^{13}C-NMR, D_2O, 125 MHz

General Procedure for glycosidase inhibition assay:

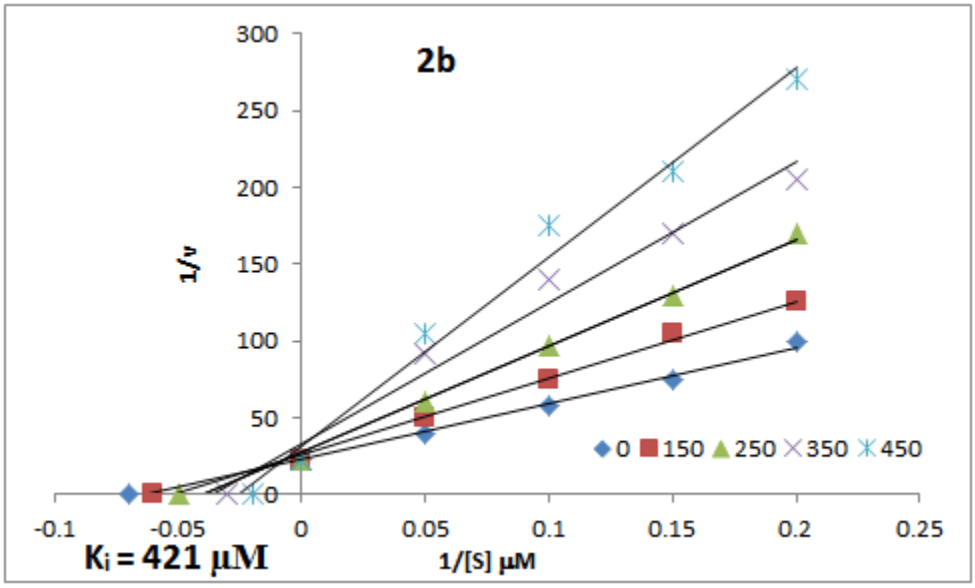
The substrates *p*-nitrophenyl-*α*-D-glucopyranoside, *p*-nitrophenyl-*β*-D-glucopyranoside, *p*-nitrophenyl-*α*-D-galactopyranoside, *p*-nitrophenyl-*β*-D-galactopyranoside, *p*-nitrophenyl-α-D-mannopyranoside and *p*-nitrophenyl-*β*-maltoside were procured from sigma chemicals. The inhibition assay with compound was performed by measuring the residual hydrolytic activities of the glycosidases with 2 mM concentration of *p*-nitrophenyl-glycopyranoside prepared in citrate buffer (0.025 M, pH 4.0) and used for assay. The test compound was pre-incubated with the enzyme, buffered at its optimal pH, for 1 h at 37 °C (for α- galactosidase at 60 °C and for amyloglucosidase 50 °C). The enzyme reaction was initiated by the addition of 100 µL of substrate. Reaction was terminated with the addition of 0.05 M Borate buffer (pH 9.8) and absorbance of the liberated *p*-nitrophenol was measured at 420 nm with Molecular Devices Spectramax M5 mulitmode plate reader. Controls were run simultaneously in the absence of test compound. One unit of glycosidase activity is defined as the amount of enzyme that hydrolyzed 1 µmol of *p*-nitrophenol per minute under assay condition.

Lineweaver Burk Plots:

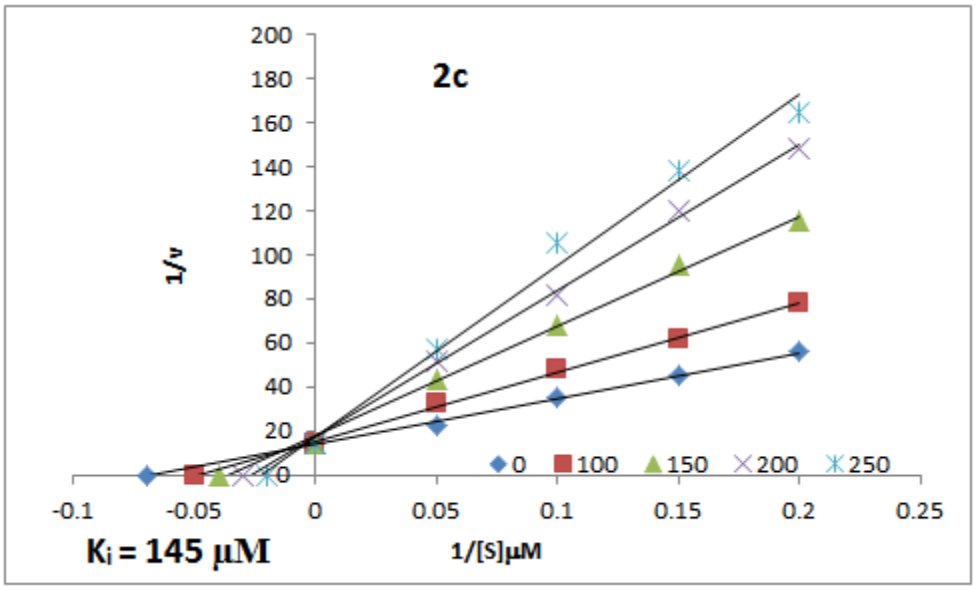
Lineweaver Burk plot of **2a** with amyloglucosidase from Aspergillus niger



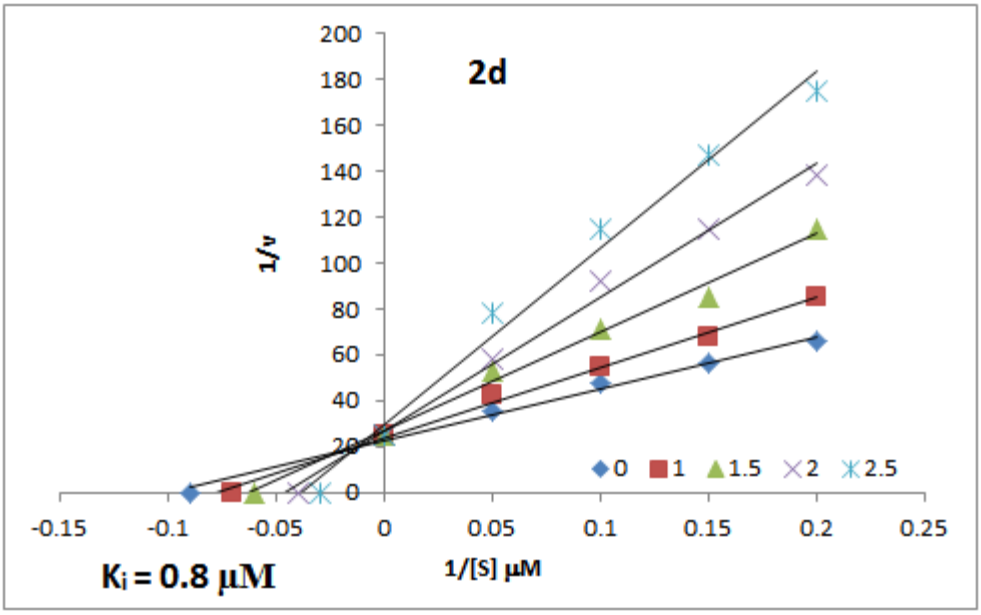
Lineweaver Burk plot of **2b** with amyloglucosidase from Aspergillus niger



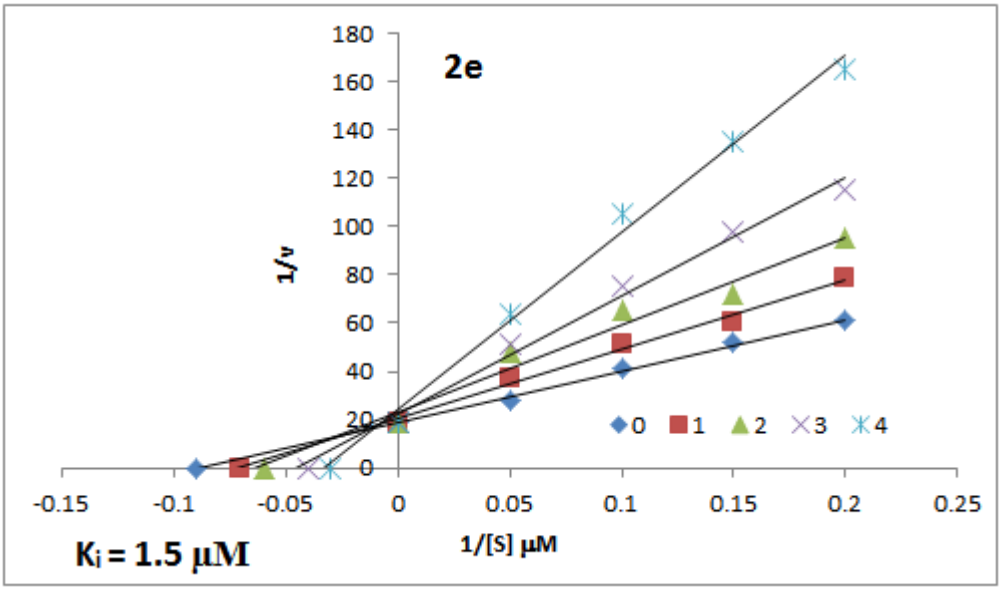
Lineweaver Burk plot of **2c** with amyloglucosidase from Aspergillus niger



Lineweaver Burk plot of **2d** with amyloglucosidase from Aspergillus niger



Lineweaver Burk plot of **2e** with amyloglucosidase from Aspergillus niger



Lineweaver Burk plot of **2f** with amyloglucosidase from Aspergillus niger