

Electronic Supporting Information

**Synthesis of 6'-galactosyllactose, a deviant human milk oligosaccharide, with the aid of *Candida antarctica* lipase-B**

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Kinetic study of N435 deacetylation reaction with **1β**.

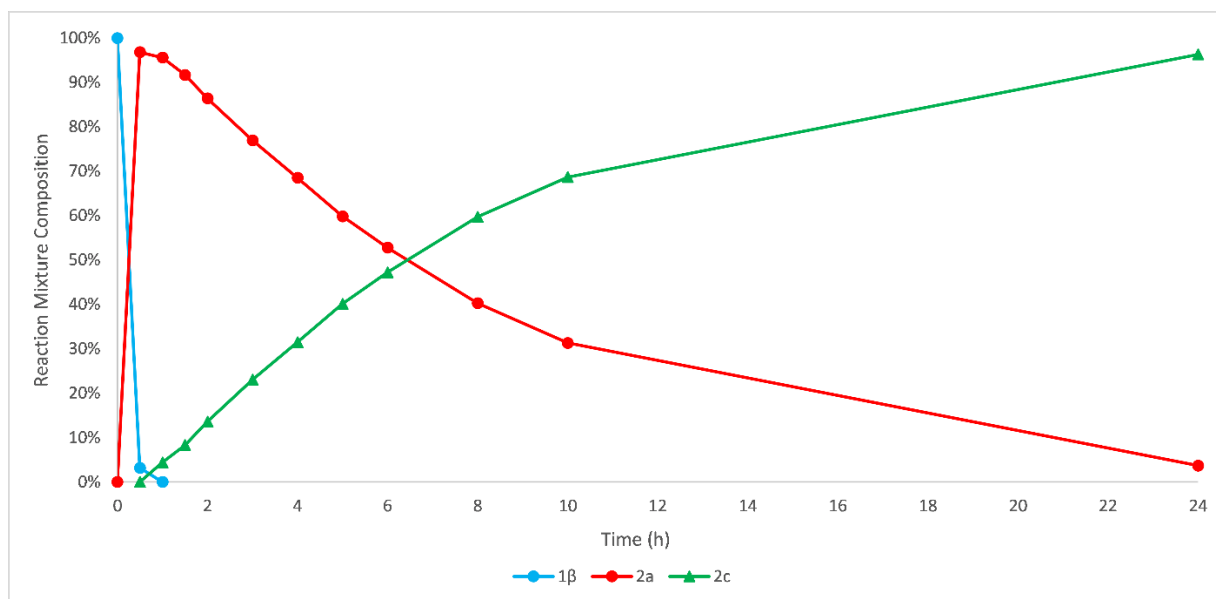
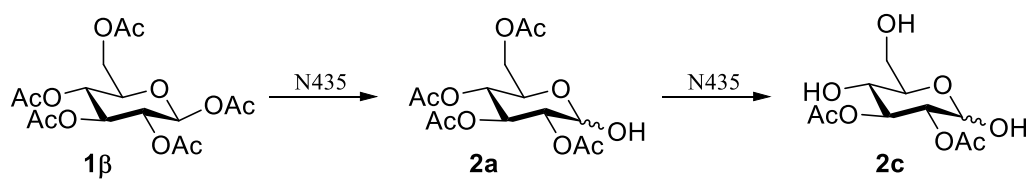
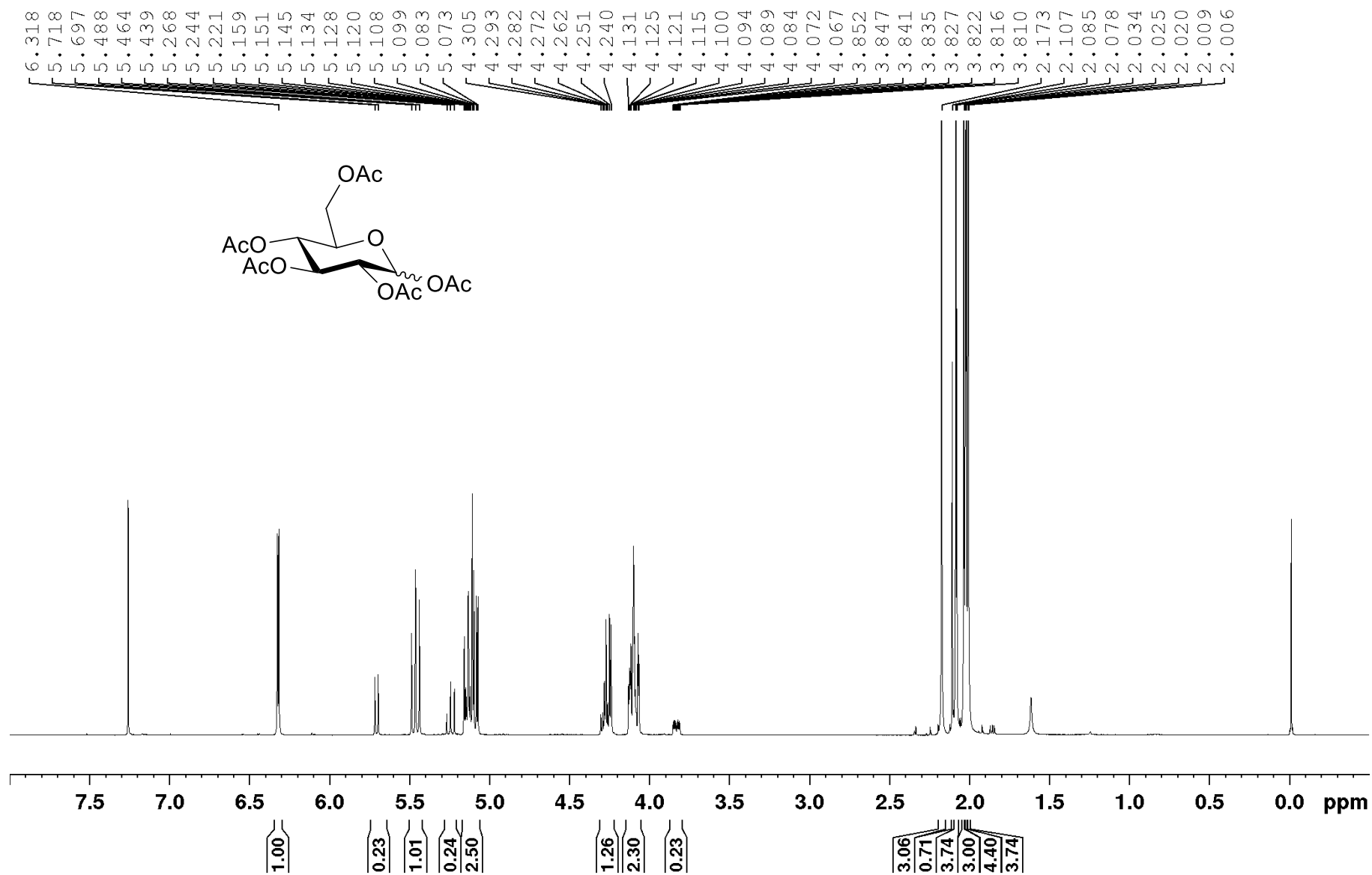


Figure S1 Reaction conditions: **1β** (100 mg), N435 (100 mg), MTBE (10 mL), *n*-BuOH (3.5 equiv, 82  $\mu$ L), 45  $^{\circ}$ C, 24 h



1,2,3,4,6-penta-O-acetyl-D-glucopyranoside **1** ( $\alpha$ : $\beta$  81:19)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )

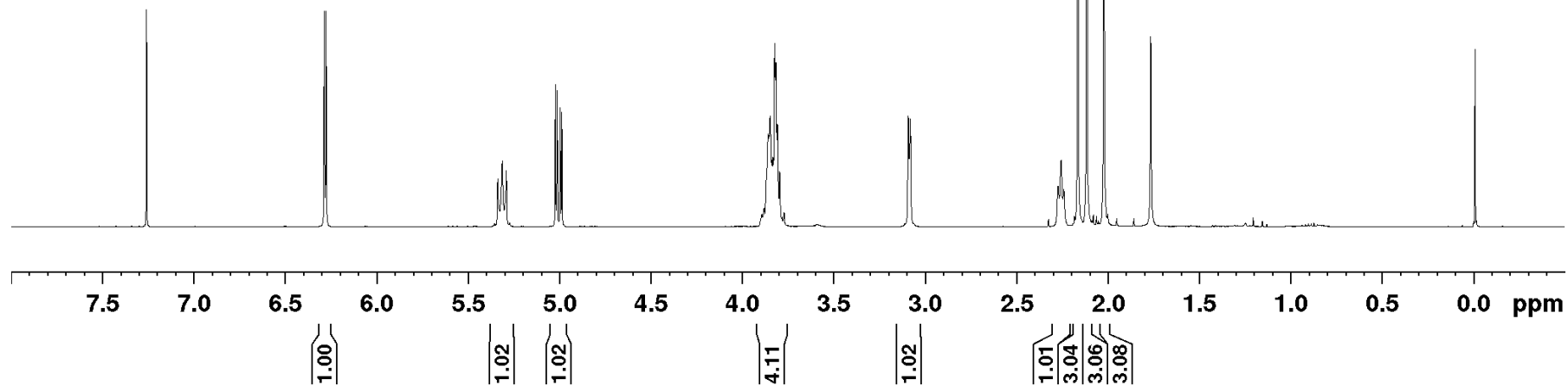
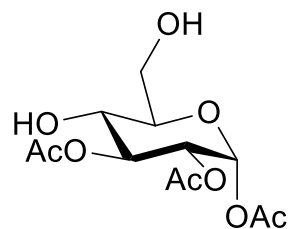


1,2,3-tri-O-acetyl- $\alpha$ -D-glucopyranoside **2**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )

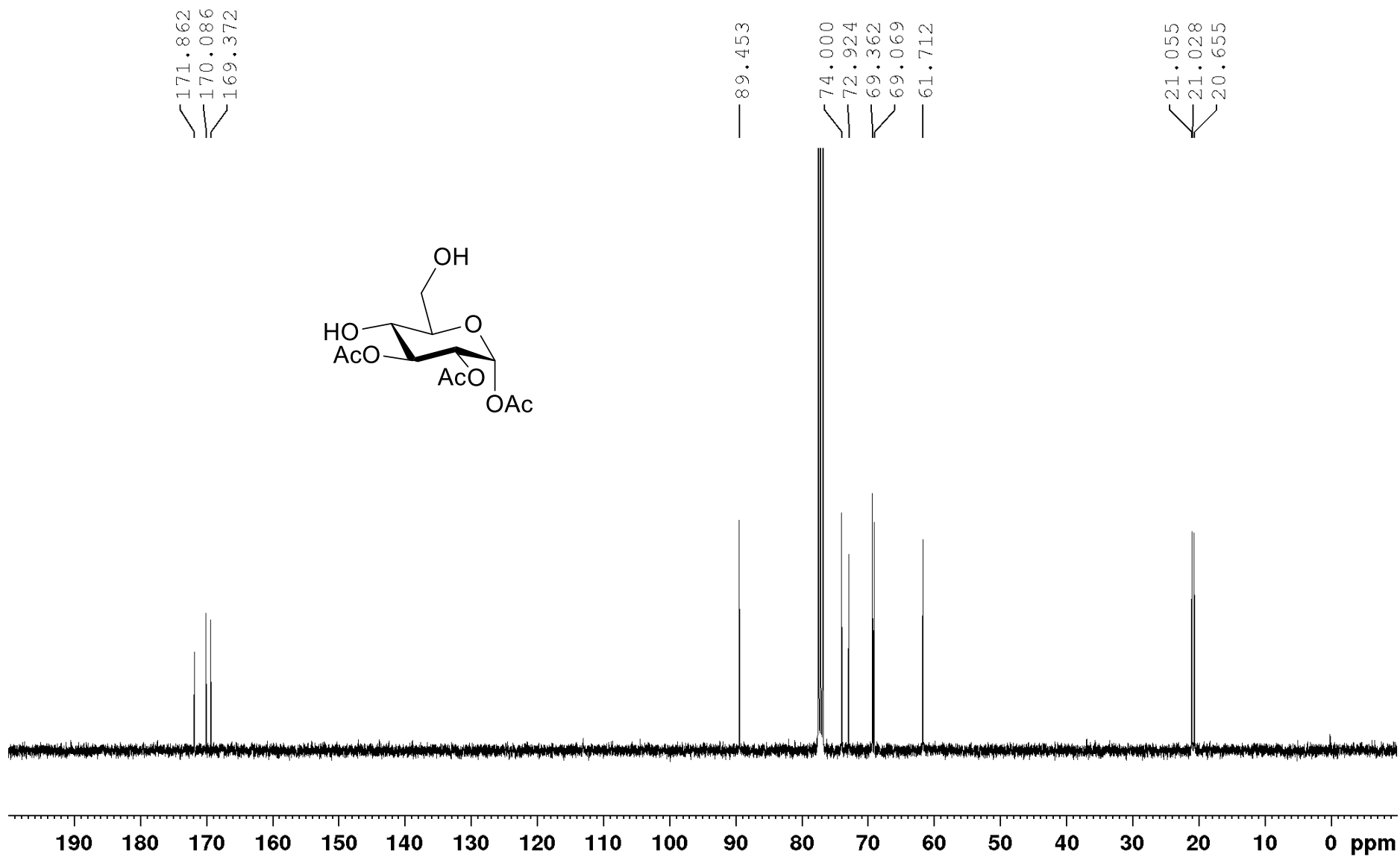
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5.313  
5.291  
5.023  
5.013  
4.997  
4.988

3.858  
3.847  
3.839  
3.833  
3.823  
3.816  
3.808  
3.796  
3.092  
3.081

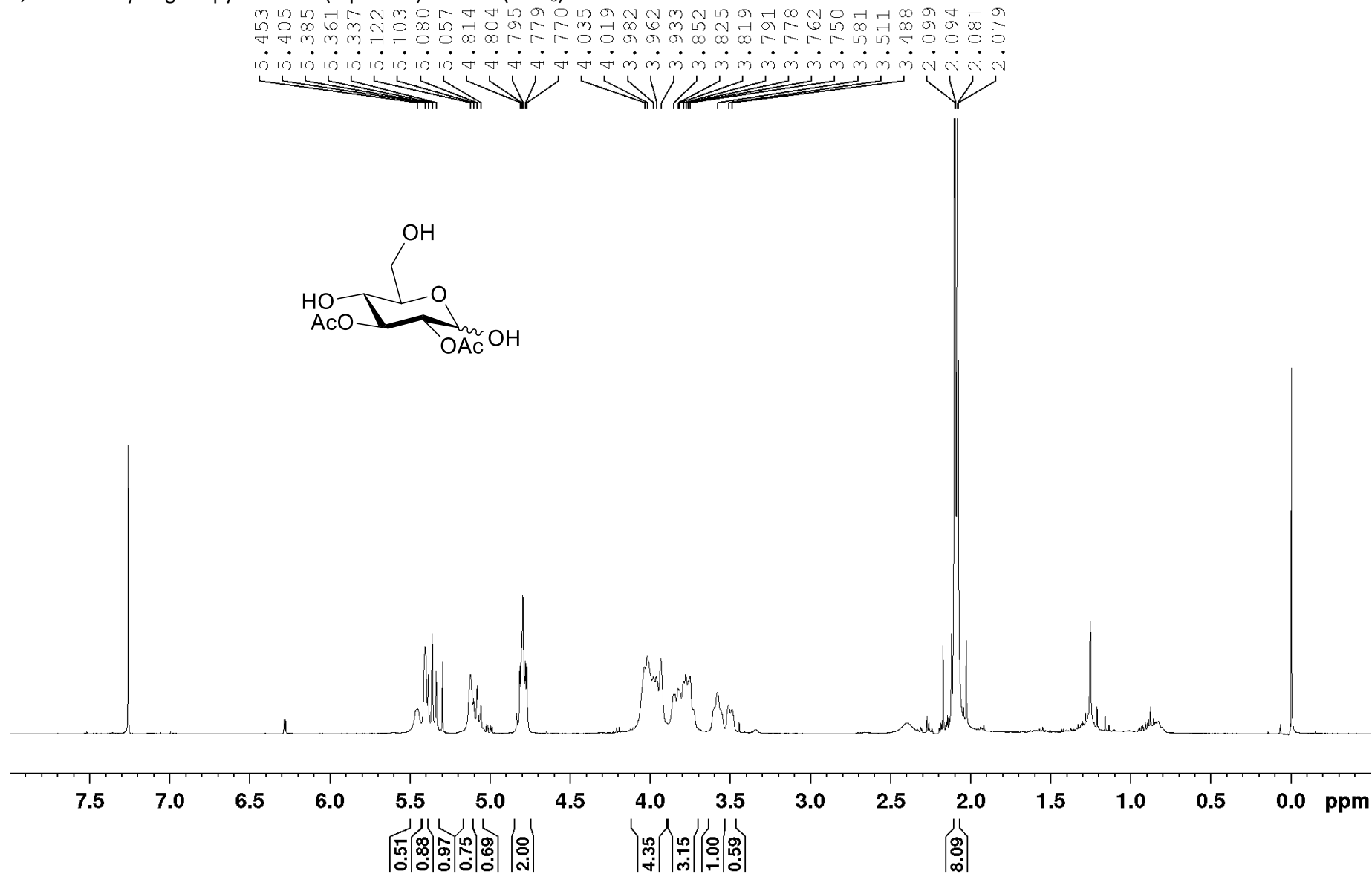
2.272  
2.164  
2.115  
2.022



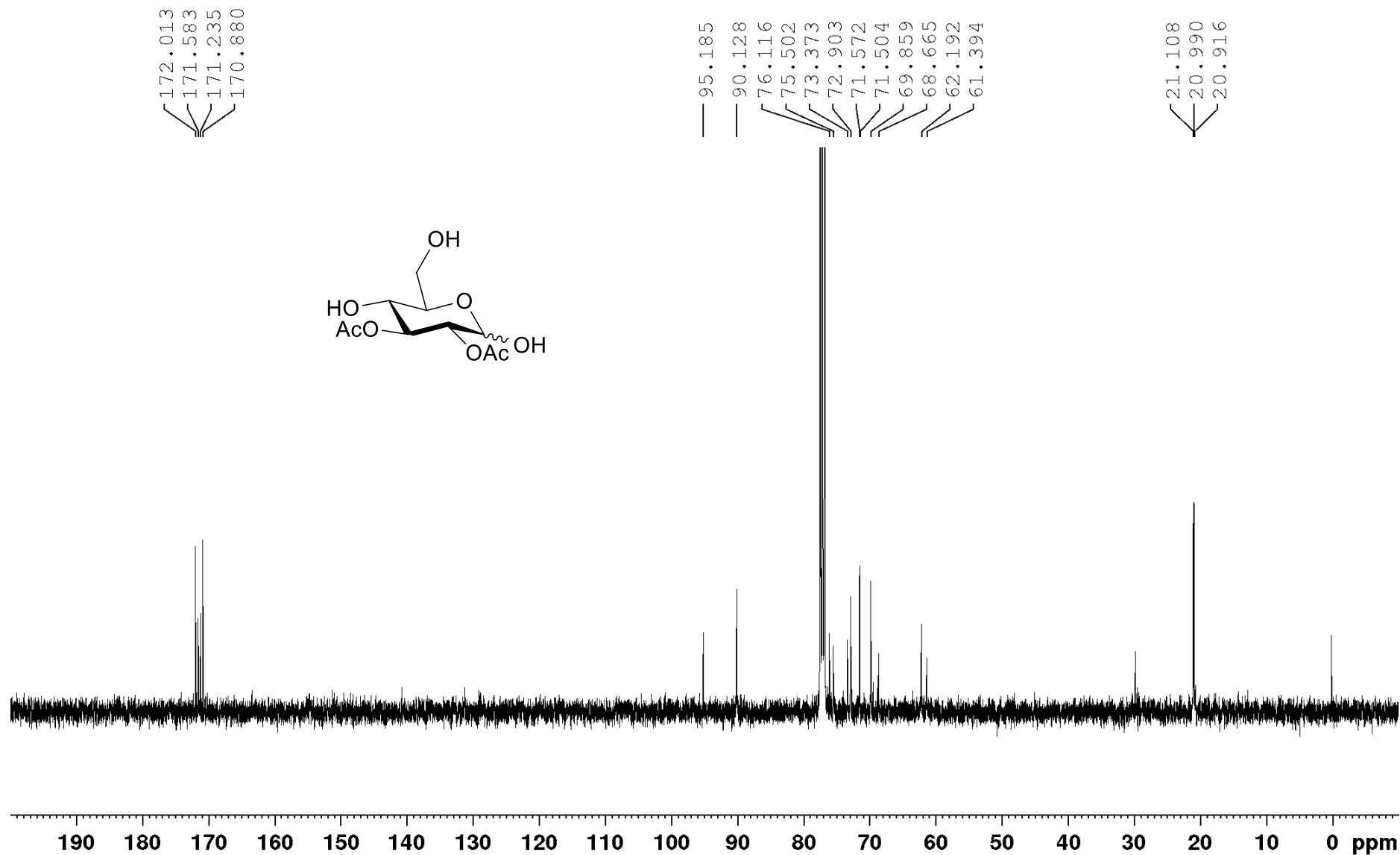
1,2,3-tri-O-acetyl- $\alpha$ -D-glucopyranoside **2**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )



2,3-di-O-acetyl-D-glucopyranose **2c** ( $\alpha:\beta$  63:37)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )

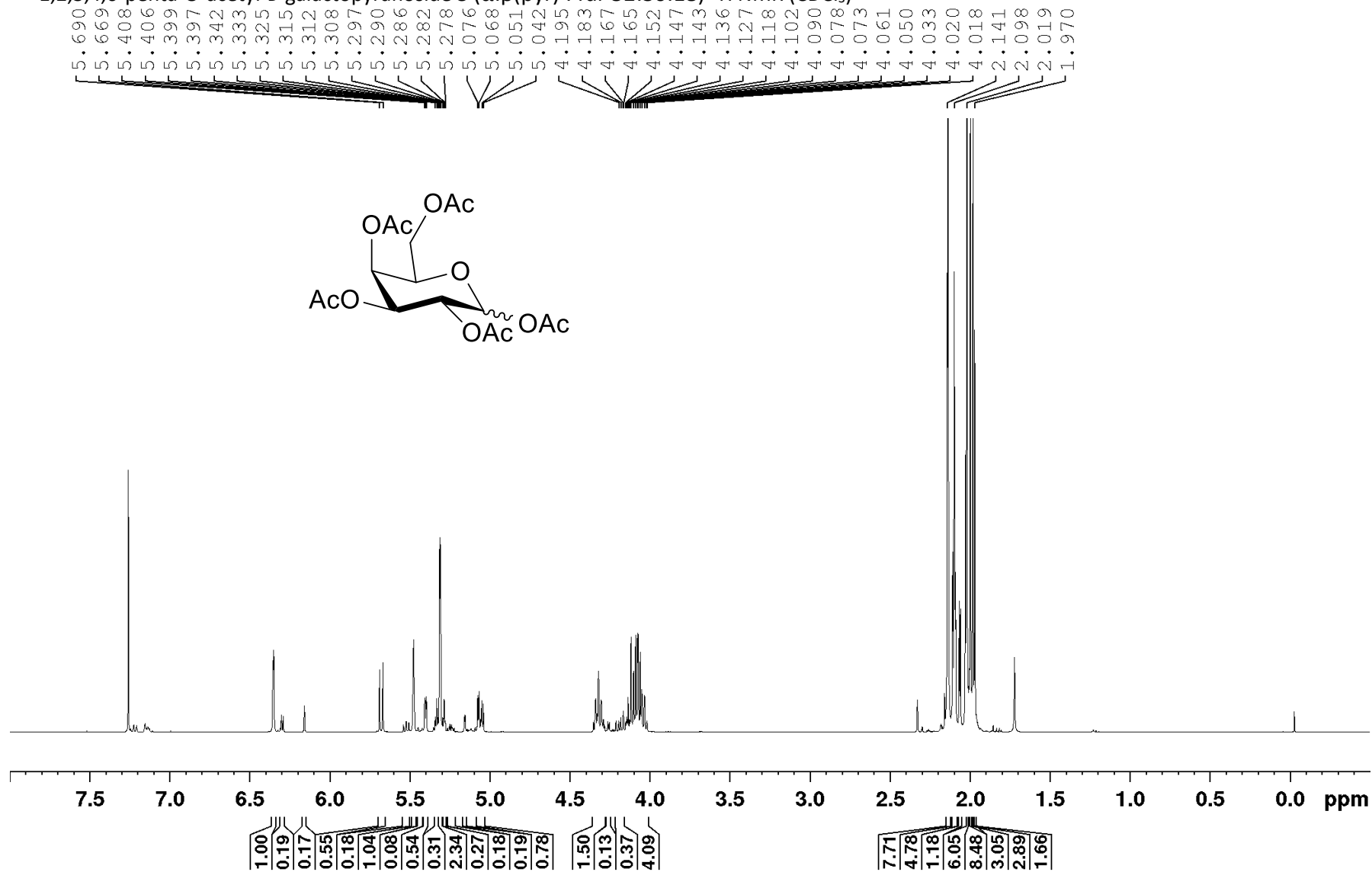


2,3-di-O-acetyl-D-glucopyranose **2c** ( $\alpha:\beta$  63:37)  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )

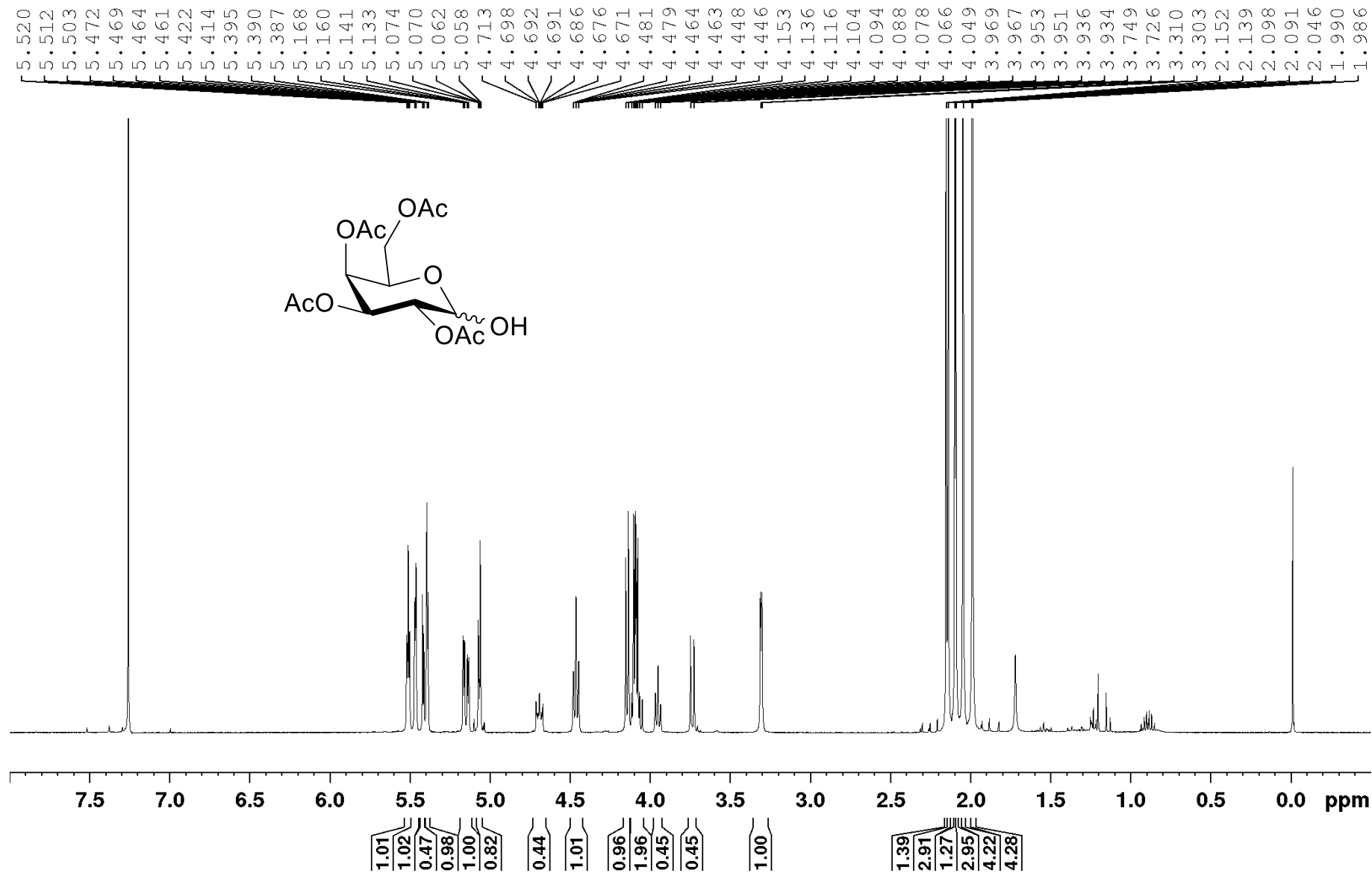




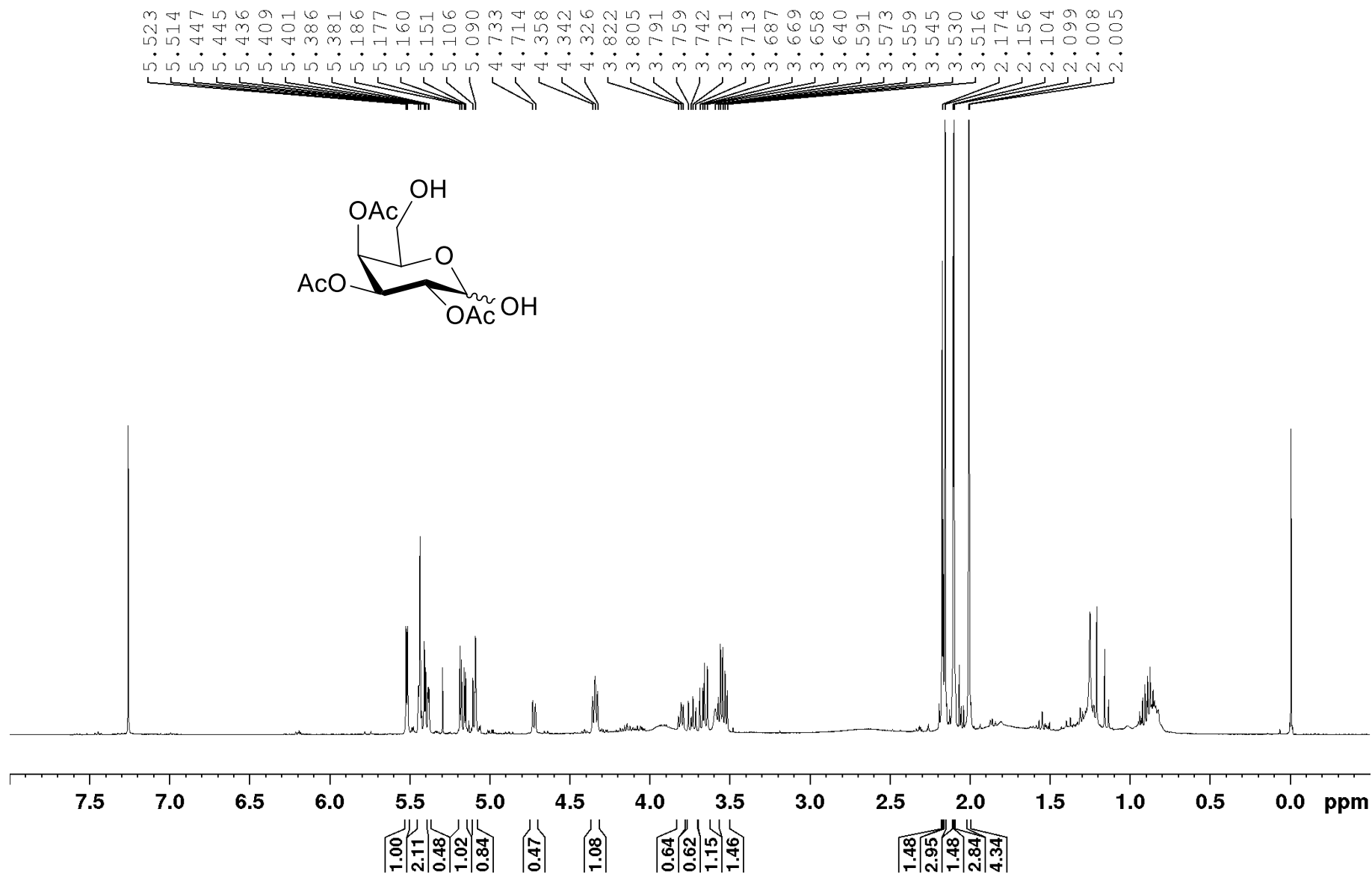
1,2,3,4,6-penta-O-acetyl-D-galactopyranoside **3** ( $\alpha:\beta(\text{pyr})$  : fur 52:30:18)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



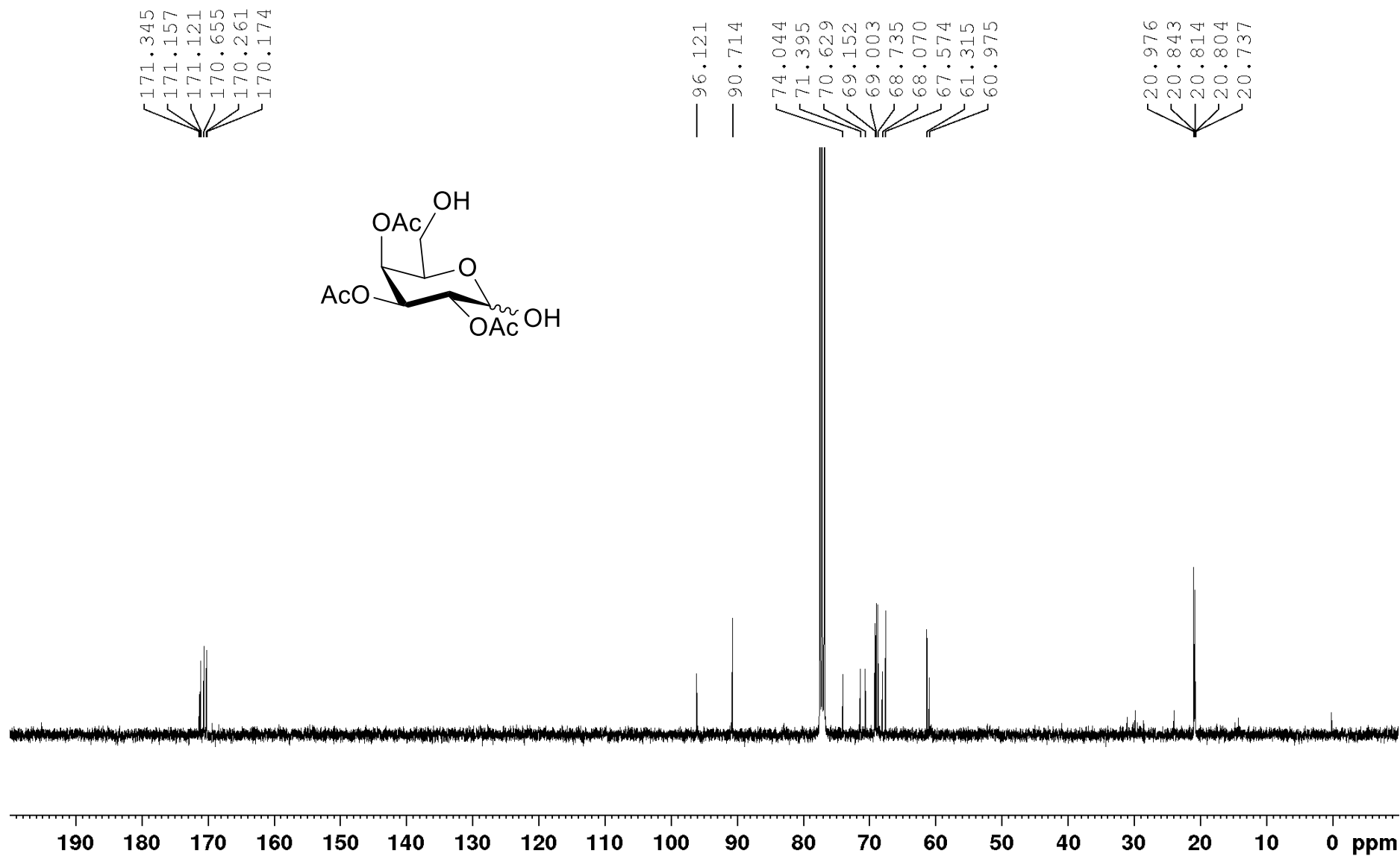
2,3,4,6-tetra-O-acetyl-D-galactopyranose 4 ( $\alpha:\beta$  69:31)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



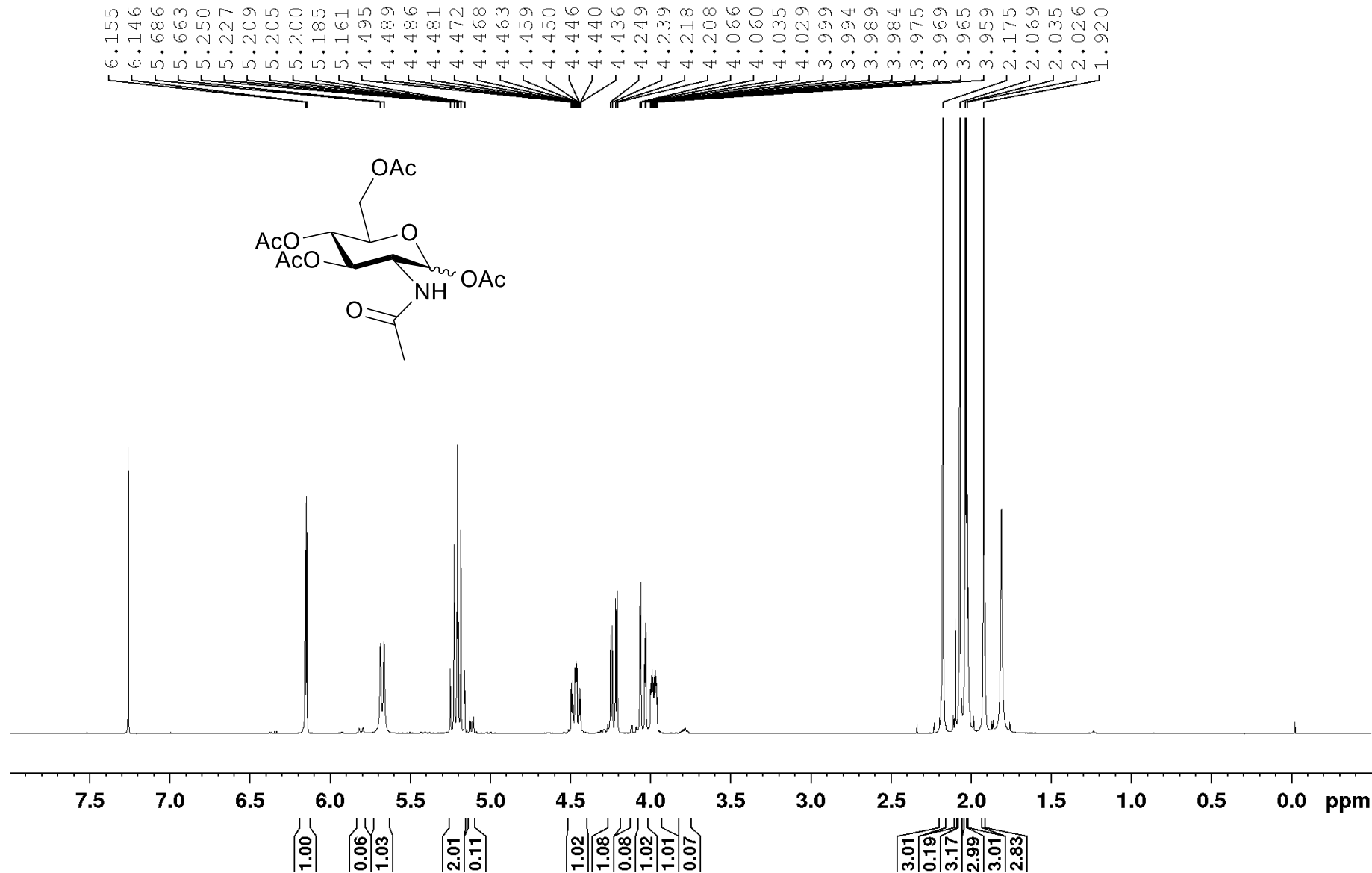
2,3,4-tri-O-acetyl-D-galactopyranoside **5a** ( $\alpha:\beta$  68:32)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



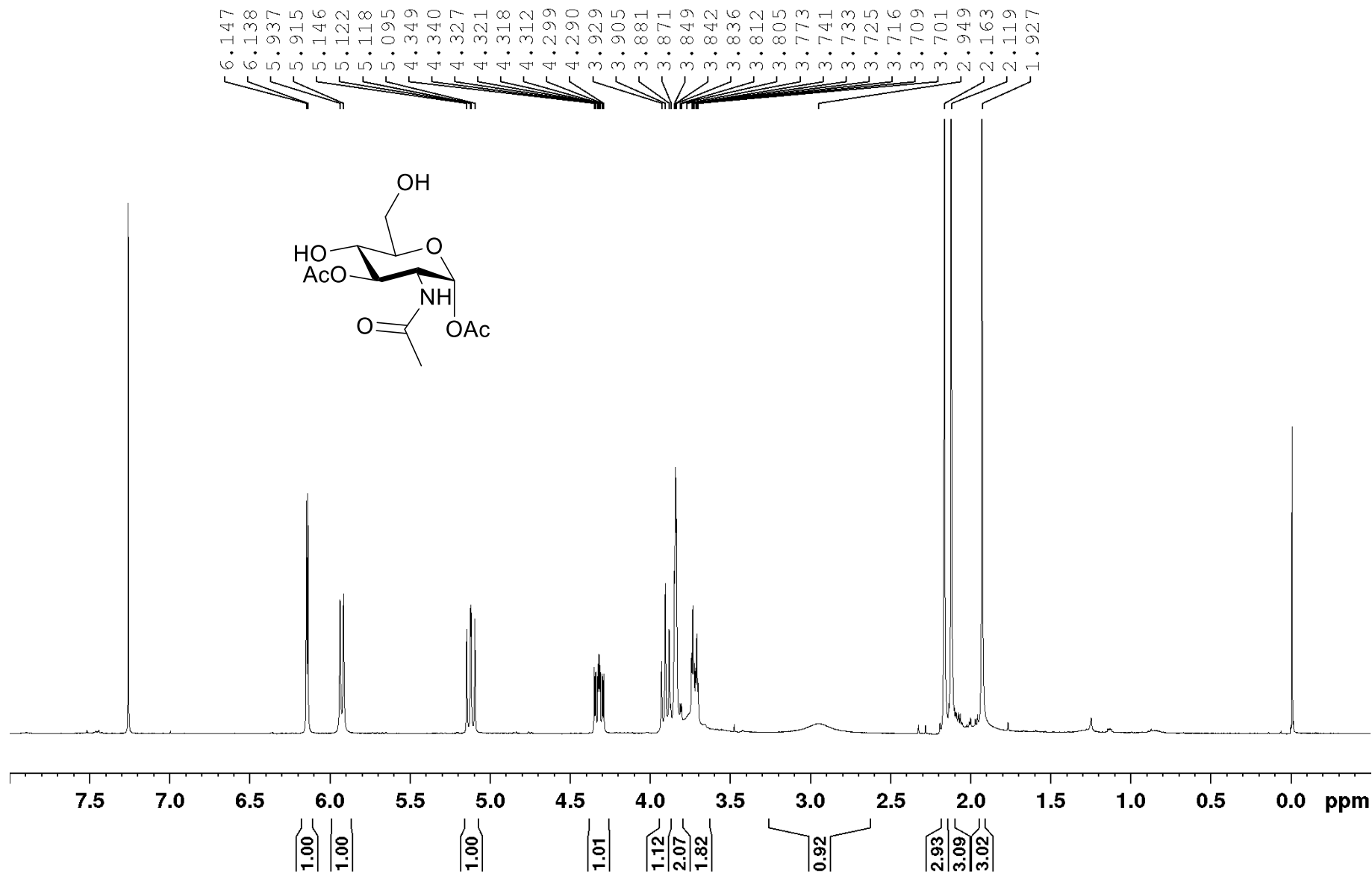
2,3,4-tri-O-acetyl-D-galactopyranoside **5a** ( $\alpha:\beta$  68:32)  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )



2-acetamido-1,3,4,6-tetra-O-acetyl-2-deoxy-D-glucopyranoside **6** ( $\alpha$ : $\beta$  95:5)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



2-acetamido-1,3-di-O-acetyl-2-deoxy- $\alpha$ -D-glucopyranoside **7**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )

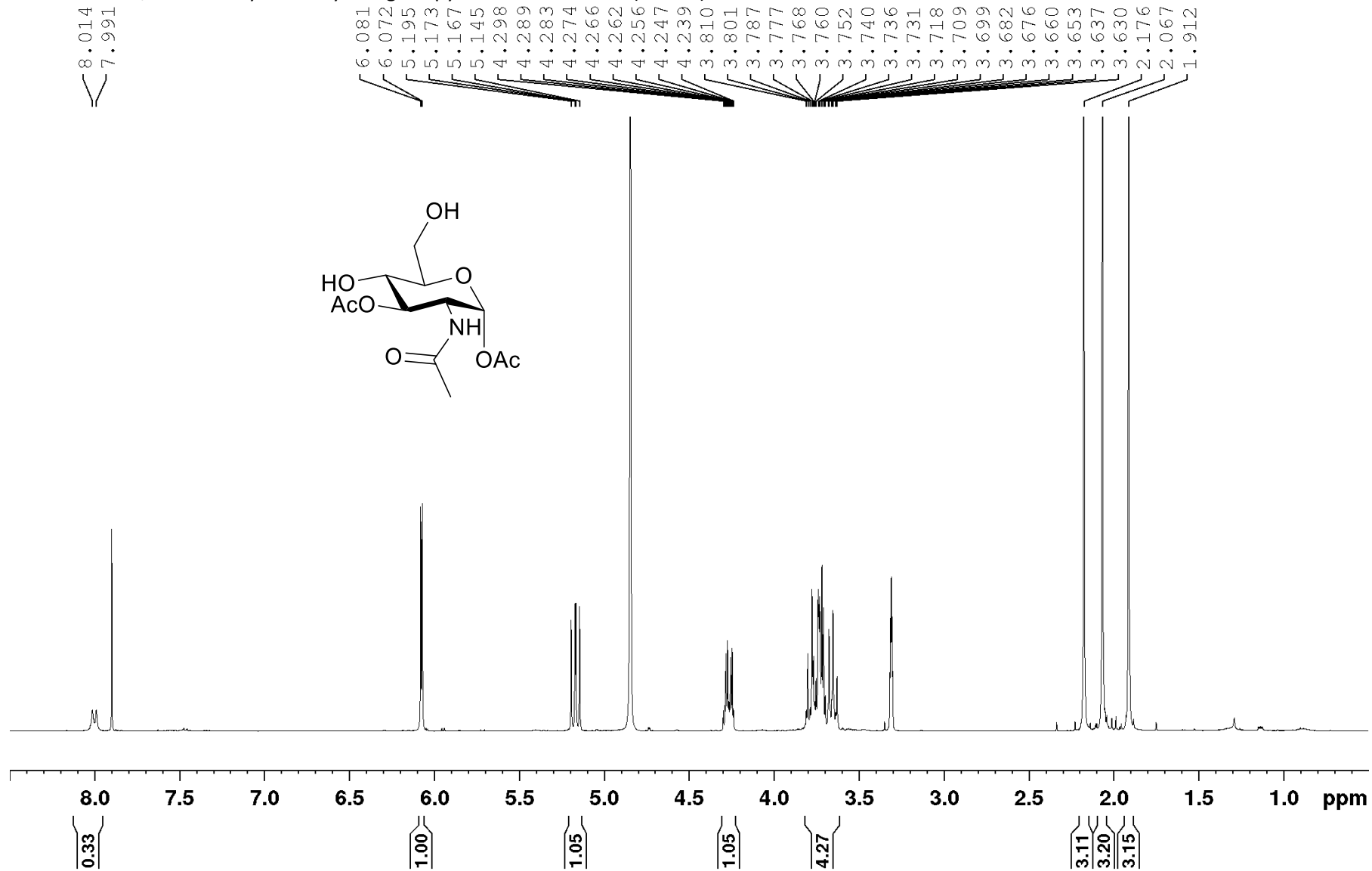


6.147  
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5.937  
5.915  
5.146  
5.122  
5.118  
5.095  
4.349  
4.340  
4.327  
4.321  
4.318  
4.312  
4.299  
4.290  
3.929  
3.905  
3.881  
3.871  
3.849  
3.842  
3.836  
3.812  
3.805  
3.773  
3.741  
3.733  
3.725  
3.716  
3.709  
3.701  
2.949  
2.163  
2.119  
1.927

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 ppm

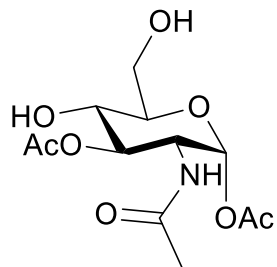
1.00  
1.00  
1.00  
1.01  
1.12  
2.07  
1.82  
0.92  
2.93  
3.09  
3.02

2-acetamido-1,3-di-O-acetyl-2-deoxy- $\alpha$ -D-glucopyranoside **7**  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )



2-acetamido-1,3-di-O-acetyl-2-deoxy- $\alpha$ -D-glucopyranoside **7**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )

172.533  
170.760  
169.536



91.127

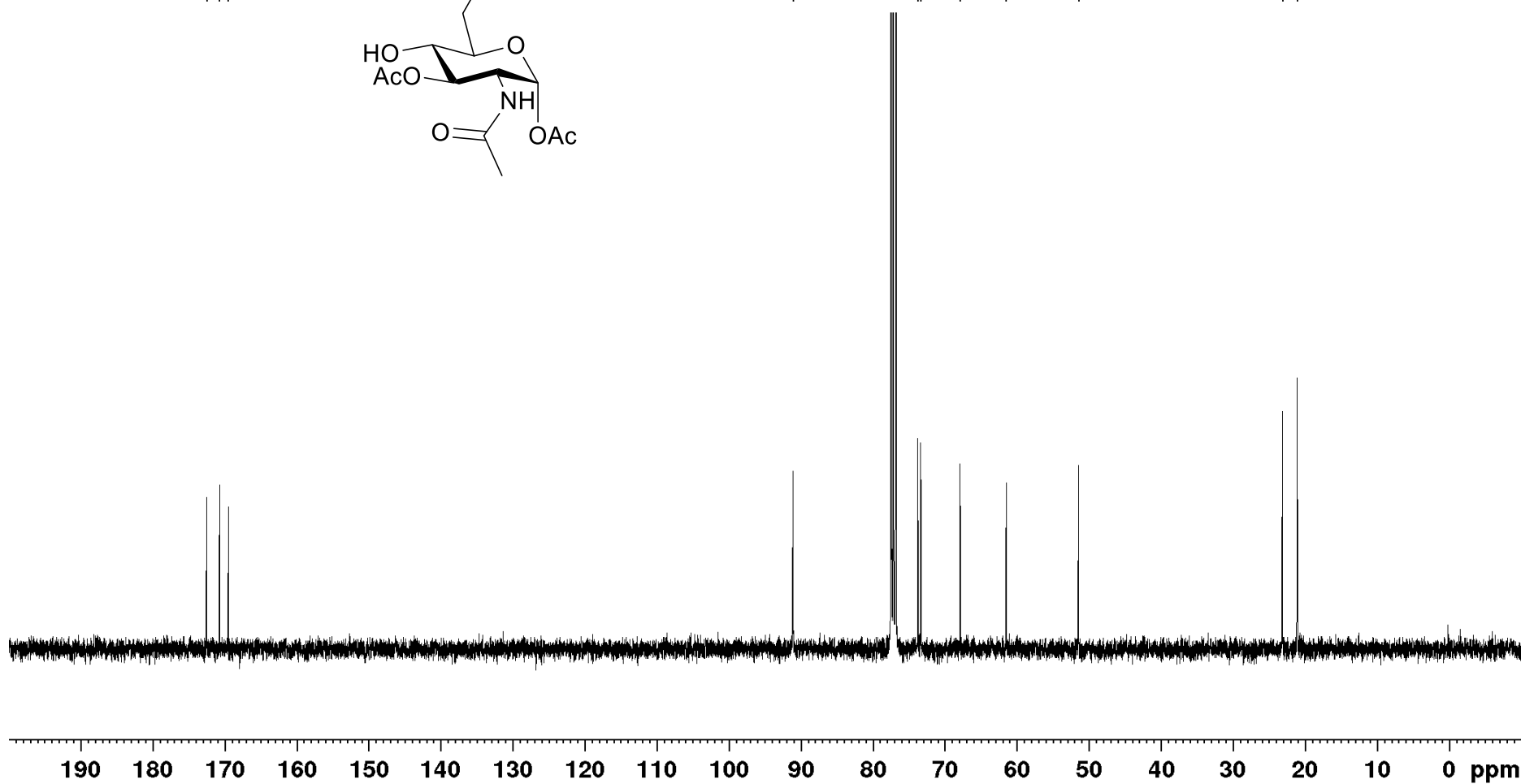
73.778  
73.357

67.896

61.506

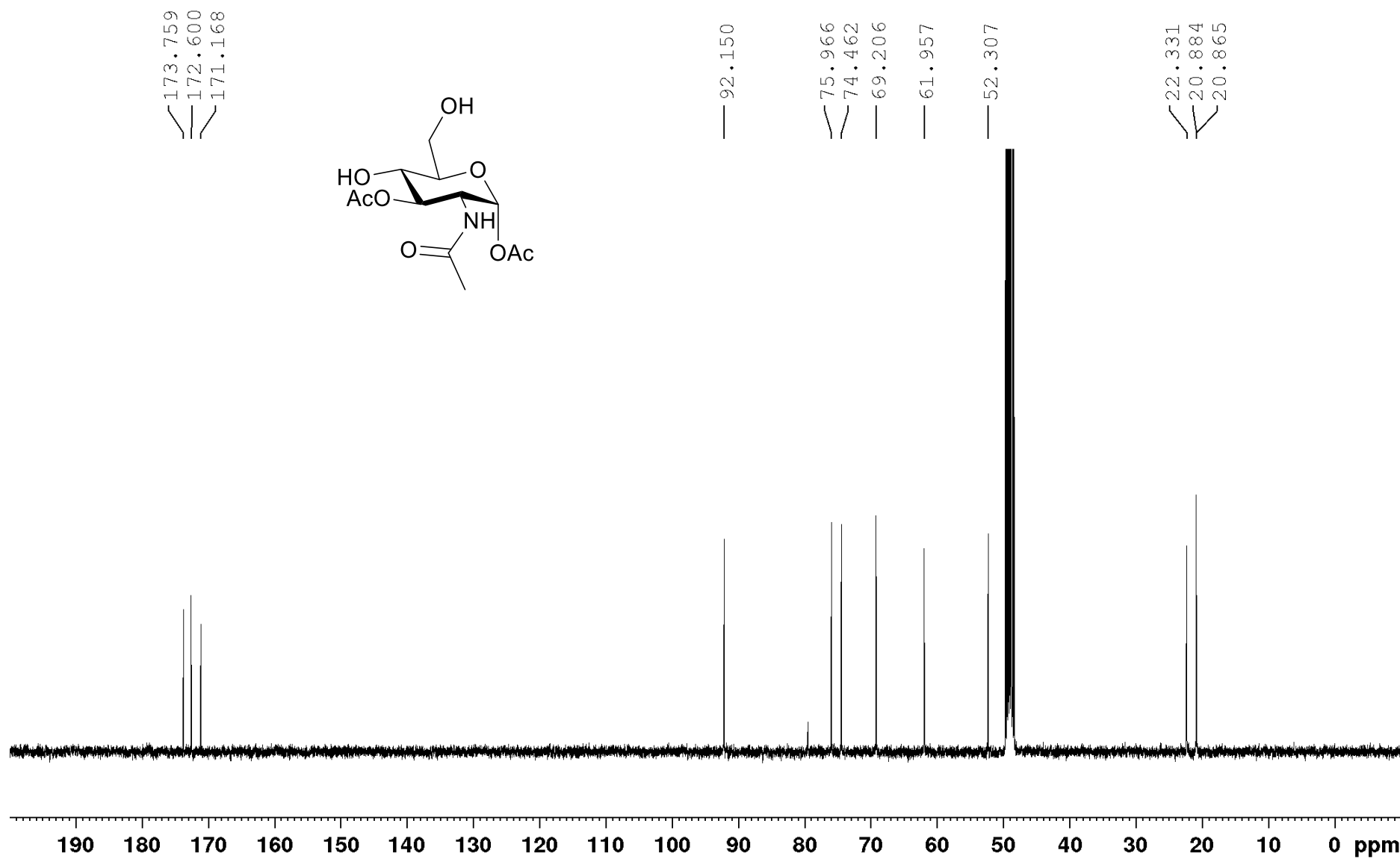
51.459

23.161  
21.120  
21.090

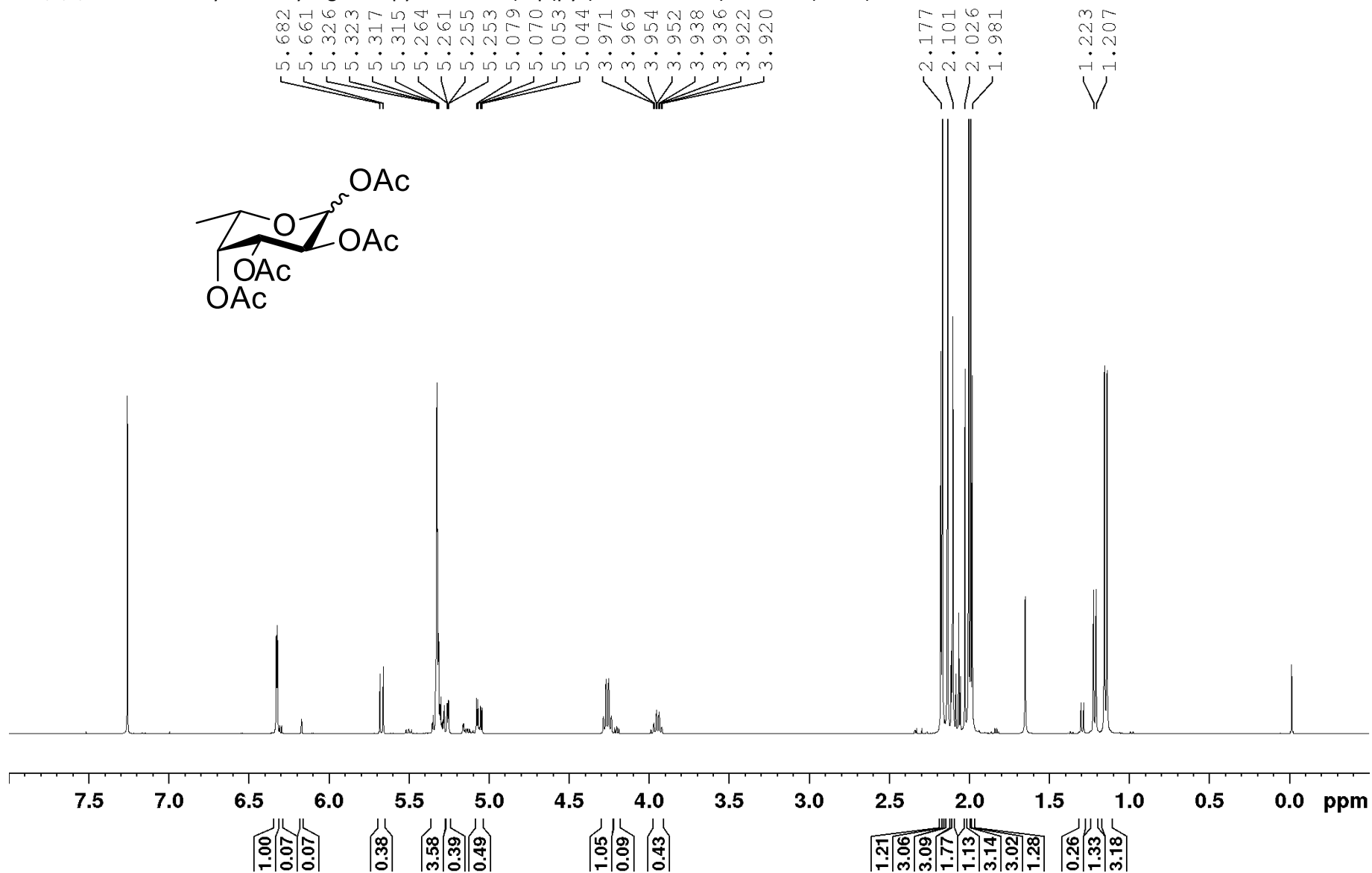




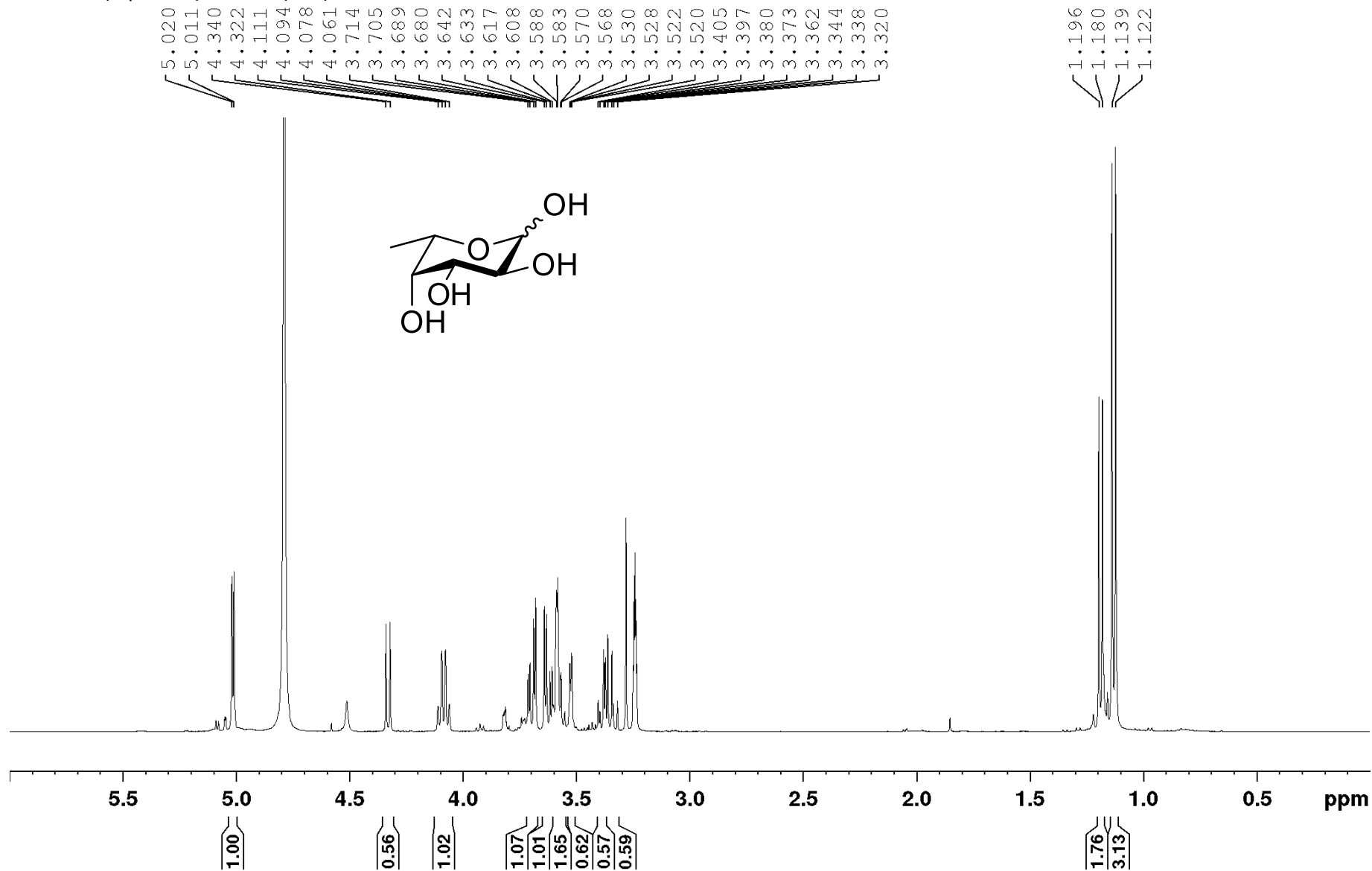
2-acetamido-1,3-di-O-acetyl-2-deoxy- $\alpha$ -D-glucopyranoside **7**  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )



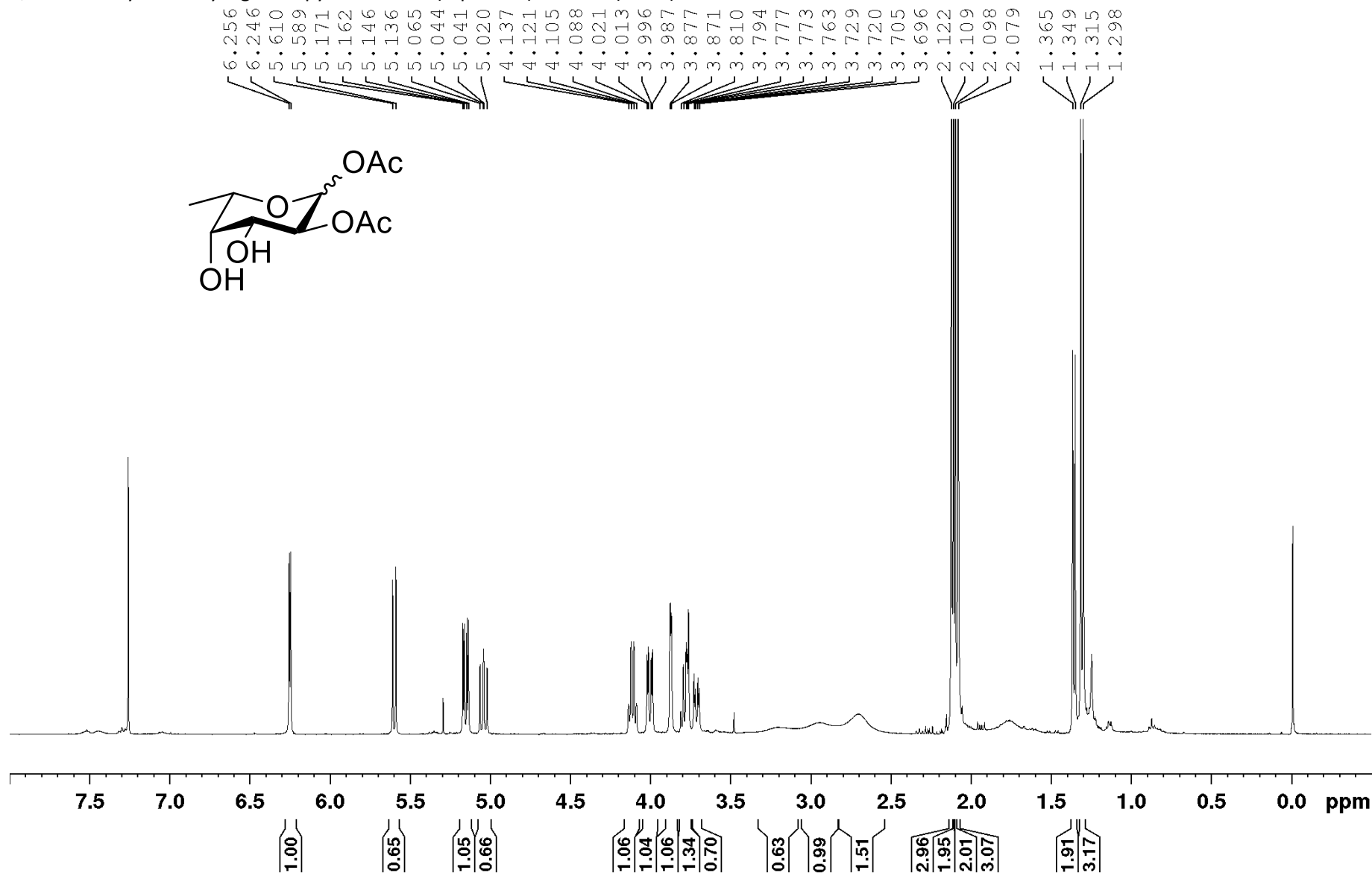
1,2,3,4-tetra-O-acetyl-6-deoxy-L-galactopyranoside **8** ( $\alpha:\beta(\text{pyr})$  : fur 66:25:9)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



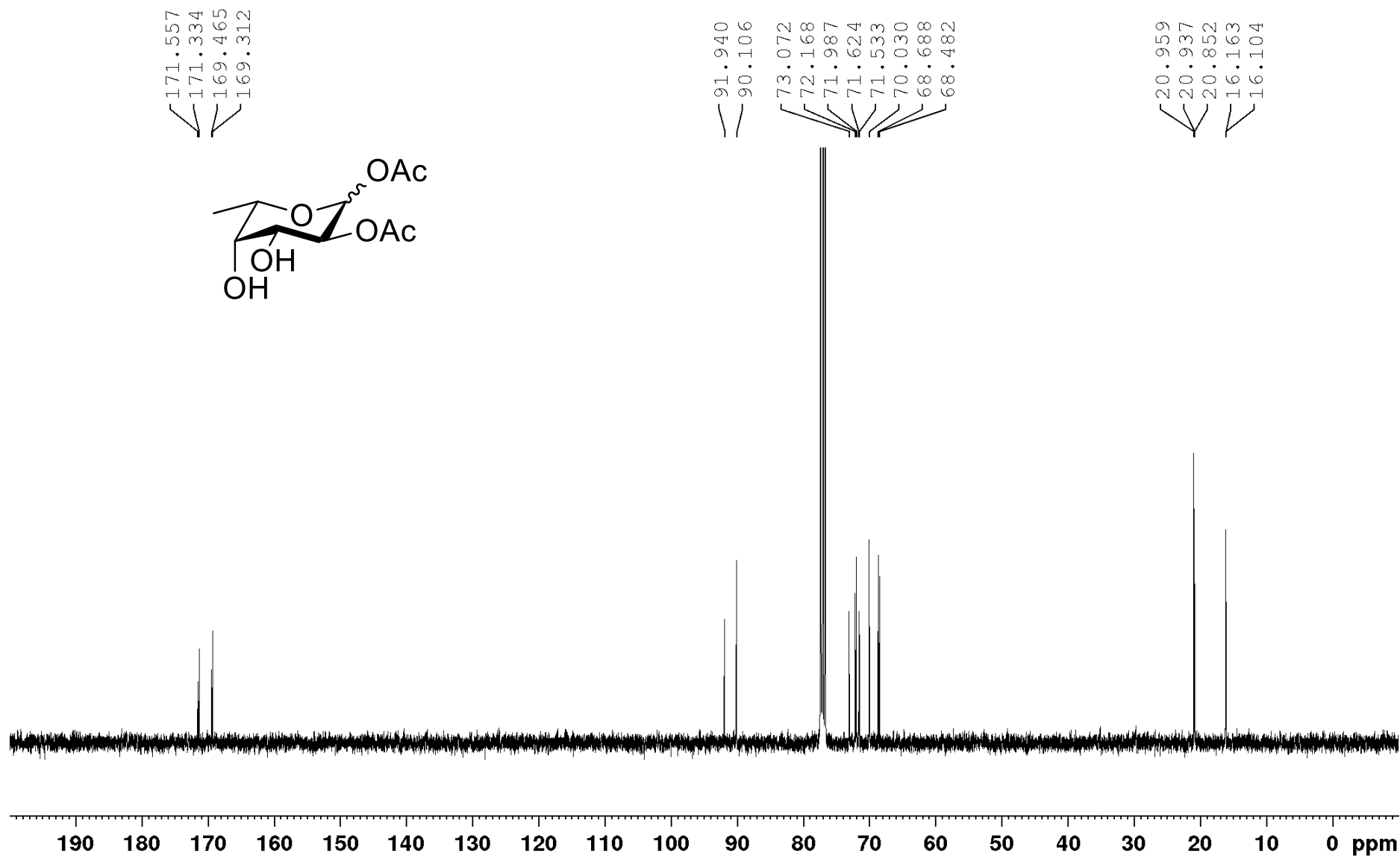
L-fucose 9 ( $\alpha:\beta$  64:36)  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )



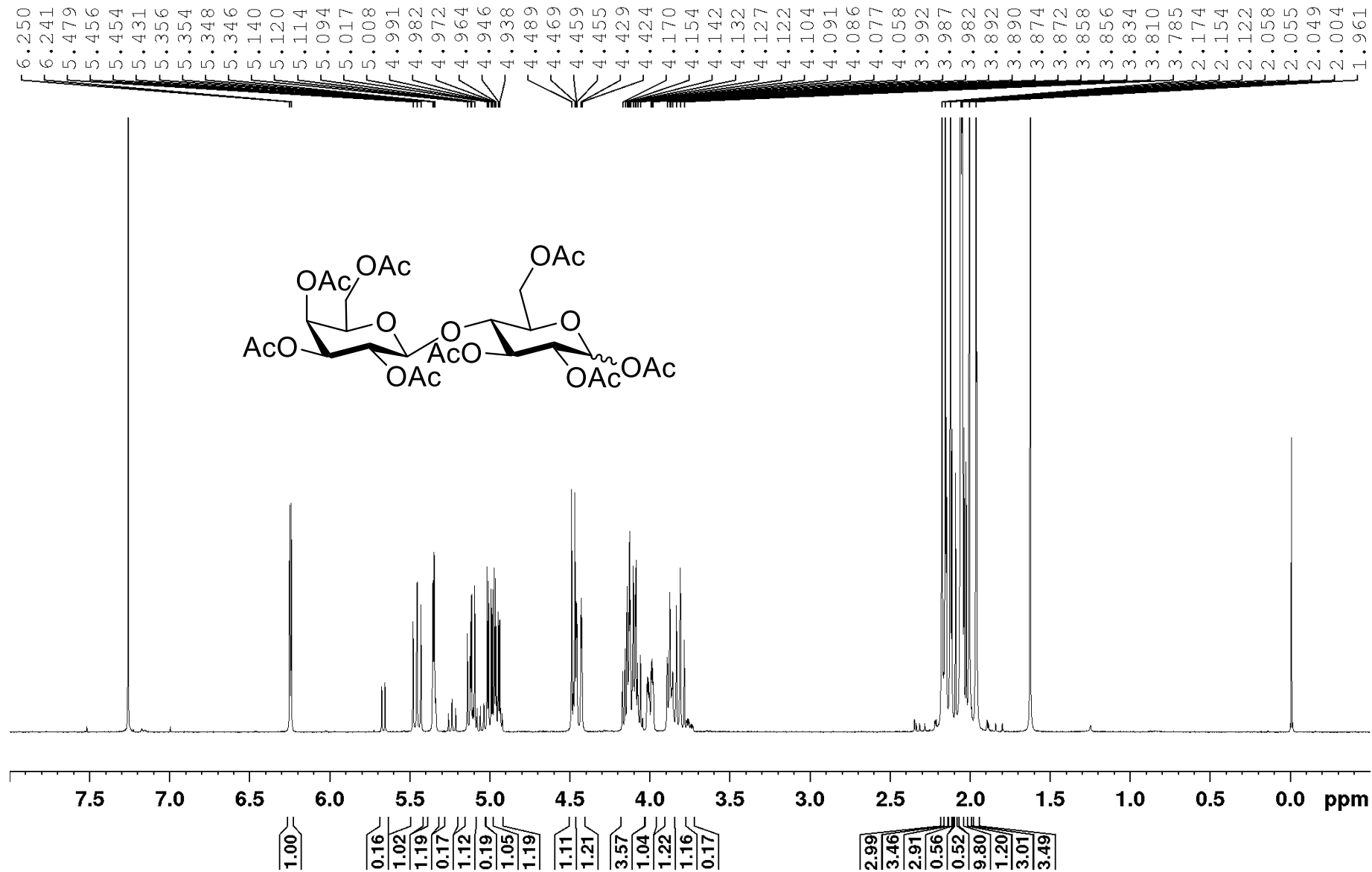
1,2-di-O-acetyl-6-deoxy-L-galactopyranoside **10** ( $\alpha$ : $\beta$  61:39)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



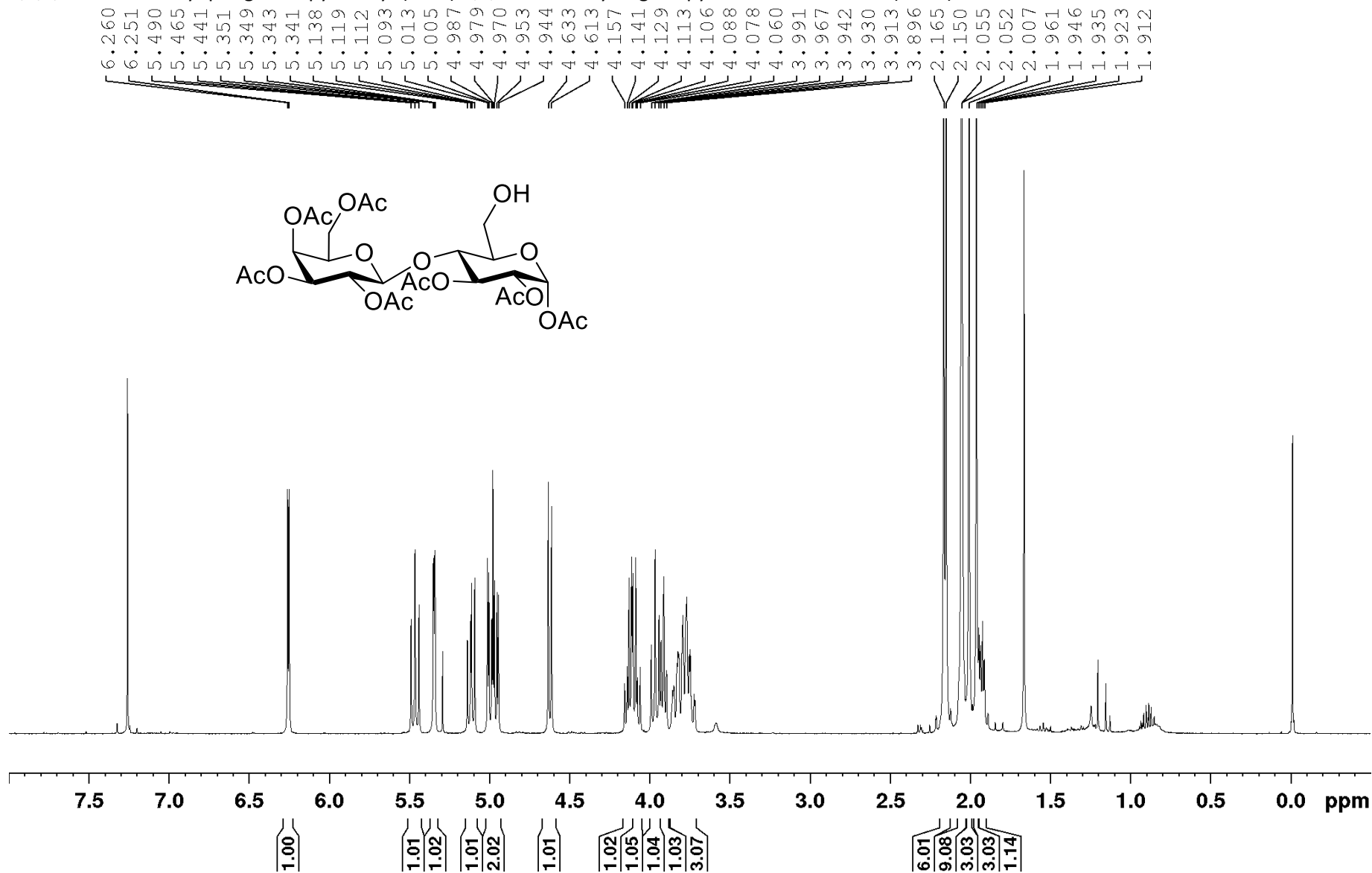
1,2-di-O-acetyl-6-deoxy-L-galactopyranoside **10** ( $\alpha:\beta$  61:39)  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )



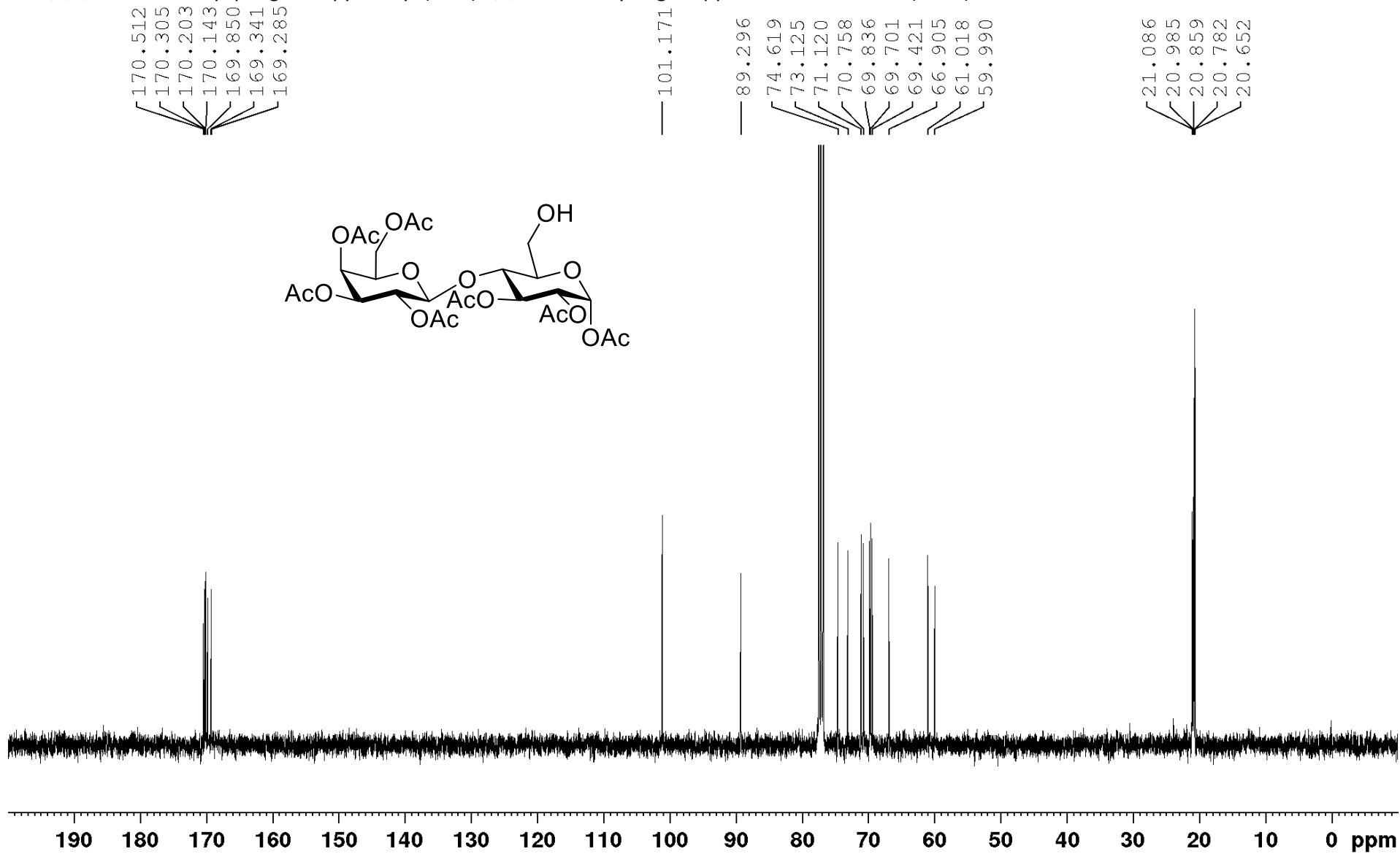
2,3,4,6-tetra-O-acetyl- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-1,2,3,6-tetra-O-acetyl-D-glucopyranoside **11** ( $\alpha$ : $\beta$  86:14)  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl-(1→4)-1,2,3-tri-O-acetyl-D-glucopyranoside **12** <sup>1</sup>H NMR (CDCl<sub>3</sub>)

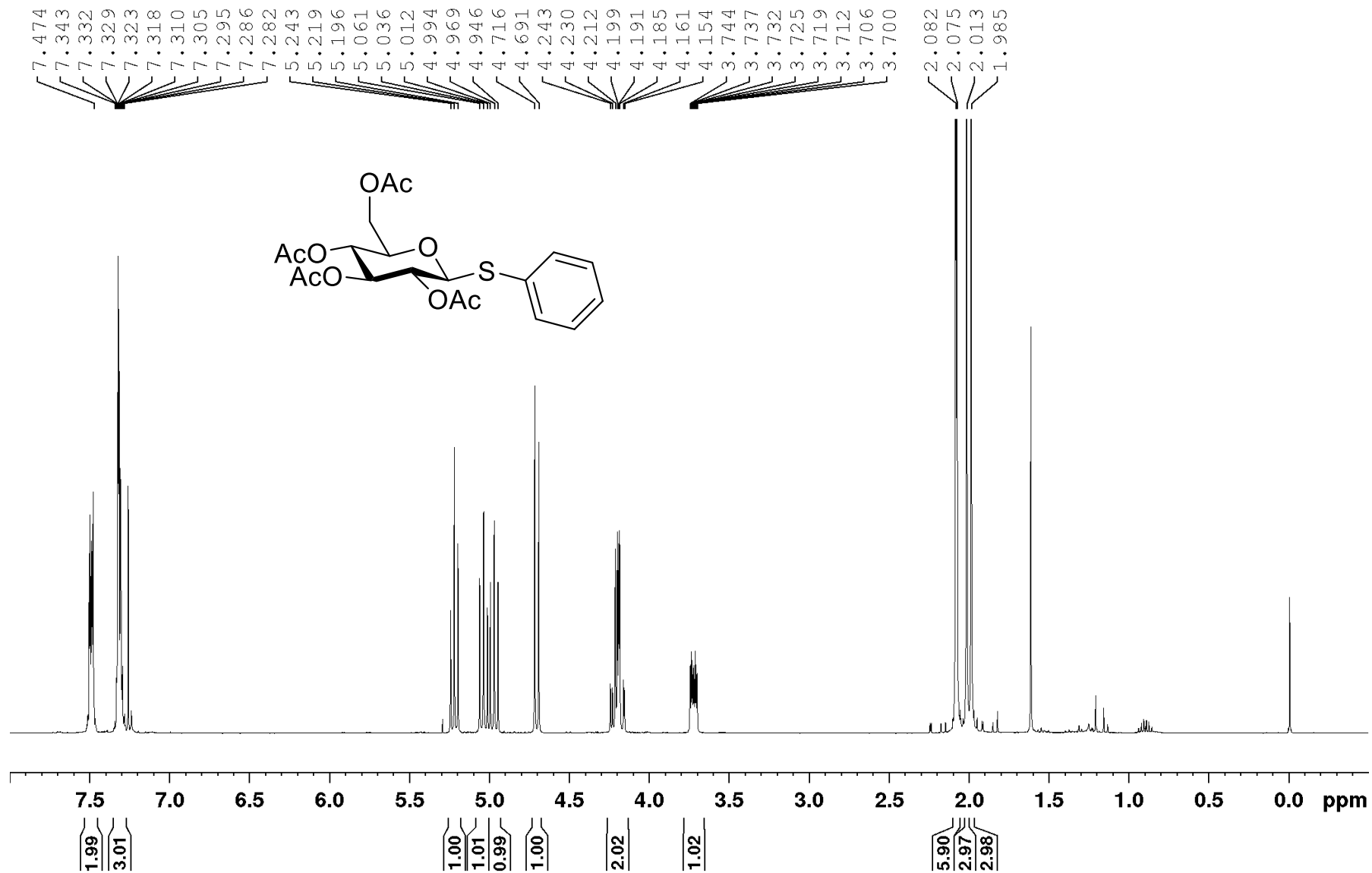


2,3,4,6-tetra-O-acetyl- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-1,2,3-tri-O-acetyl-D-glucopyranoside **12**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )

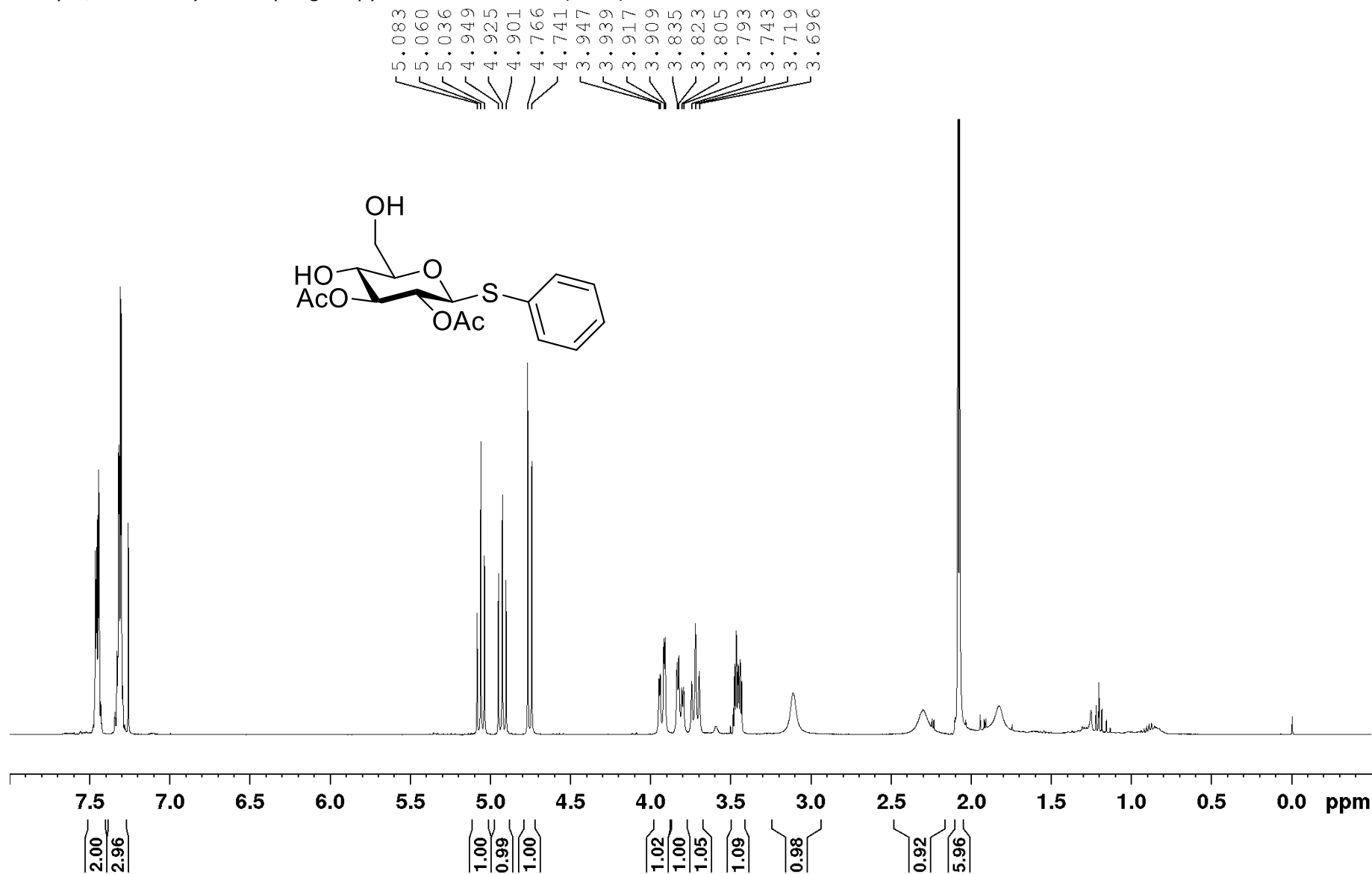




Phenyl 2,3,4,6-tetra-O-acetyl-1-thio- $\beta$ -D-glucopyranoside **13**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



Phenyl 2,3-di-O-acetyl-1-thio-β-D-glucopyranoside **14** <sup>1</sup>H NMR (CDCl<sub>3</sub>)



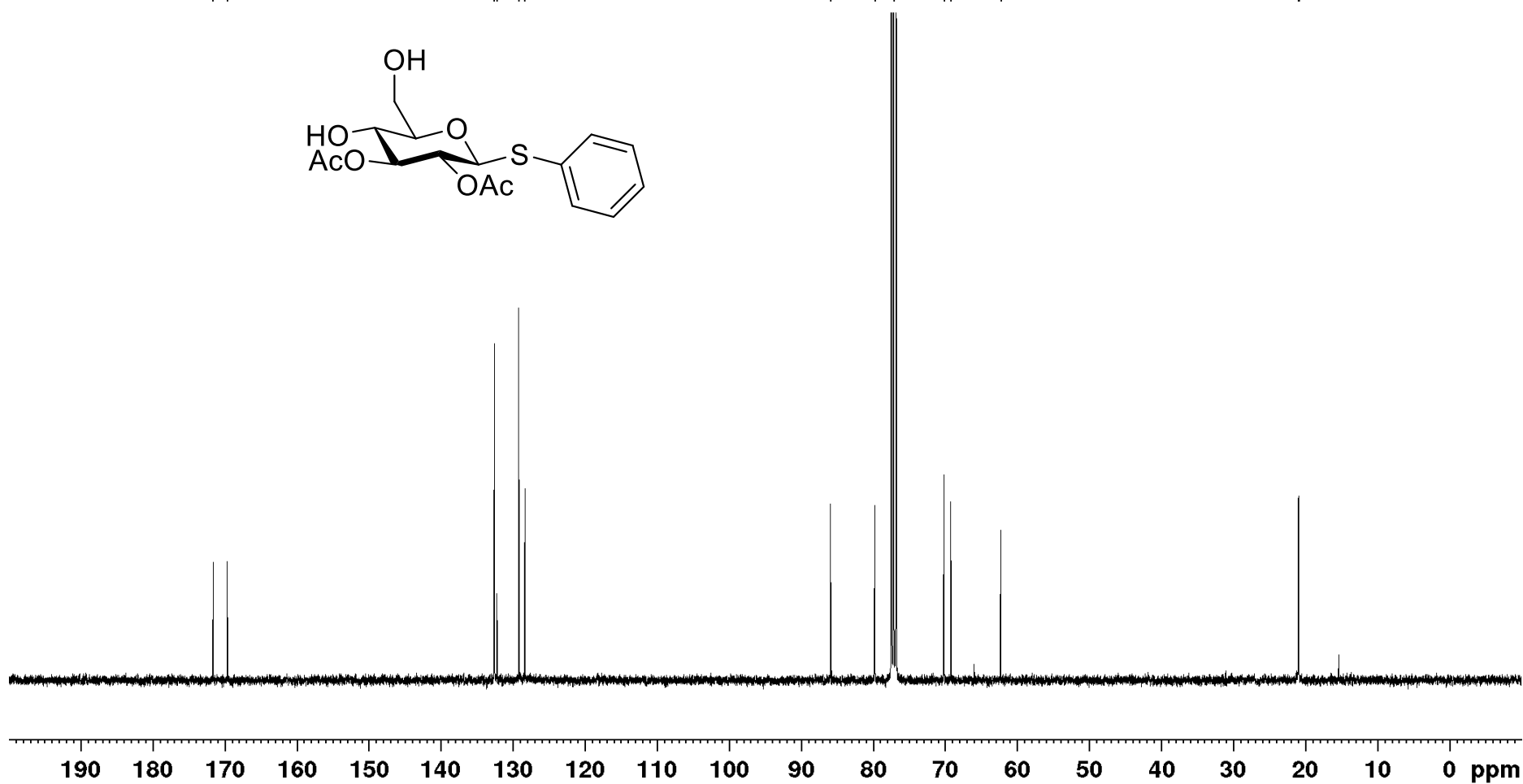
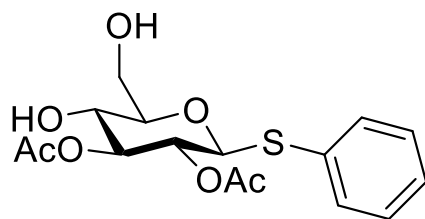
Phenyl 2,3-di-O-acetyl-1-thio- $\beta$ -D-glucopyranoside **14**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )

171.647  
169.699

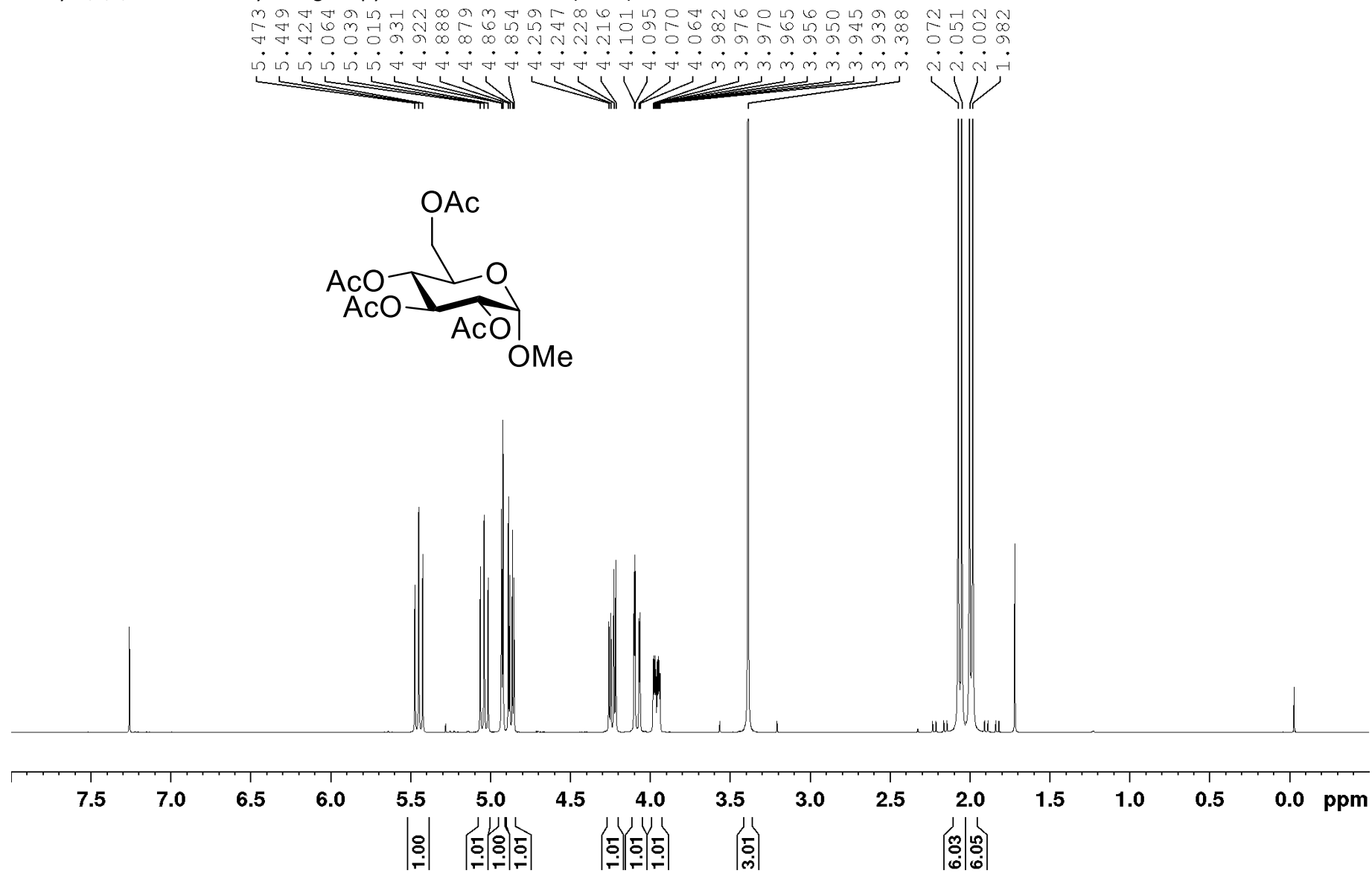
132.620  
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128.379

85.919  
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77.156  
70.179  
69.213  
62.305

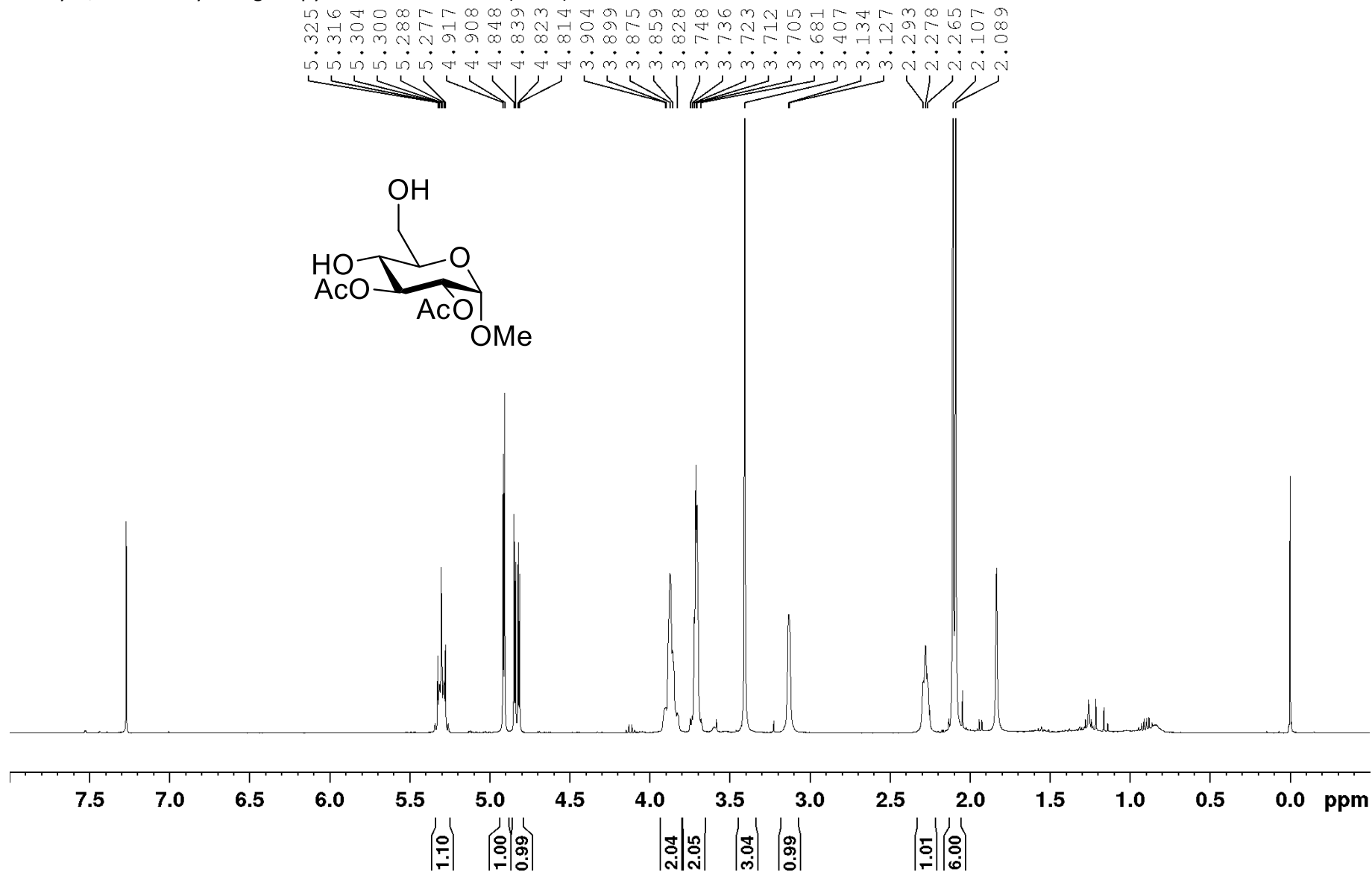
20.981  
20.925



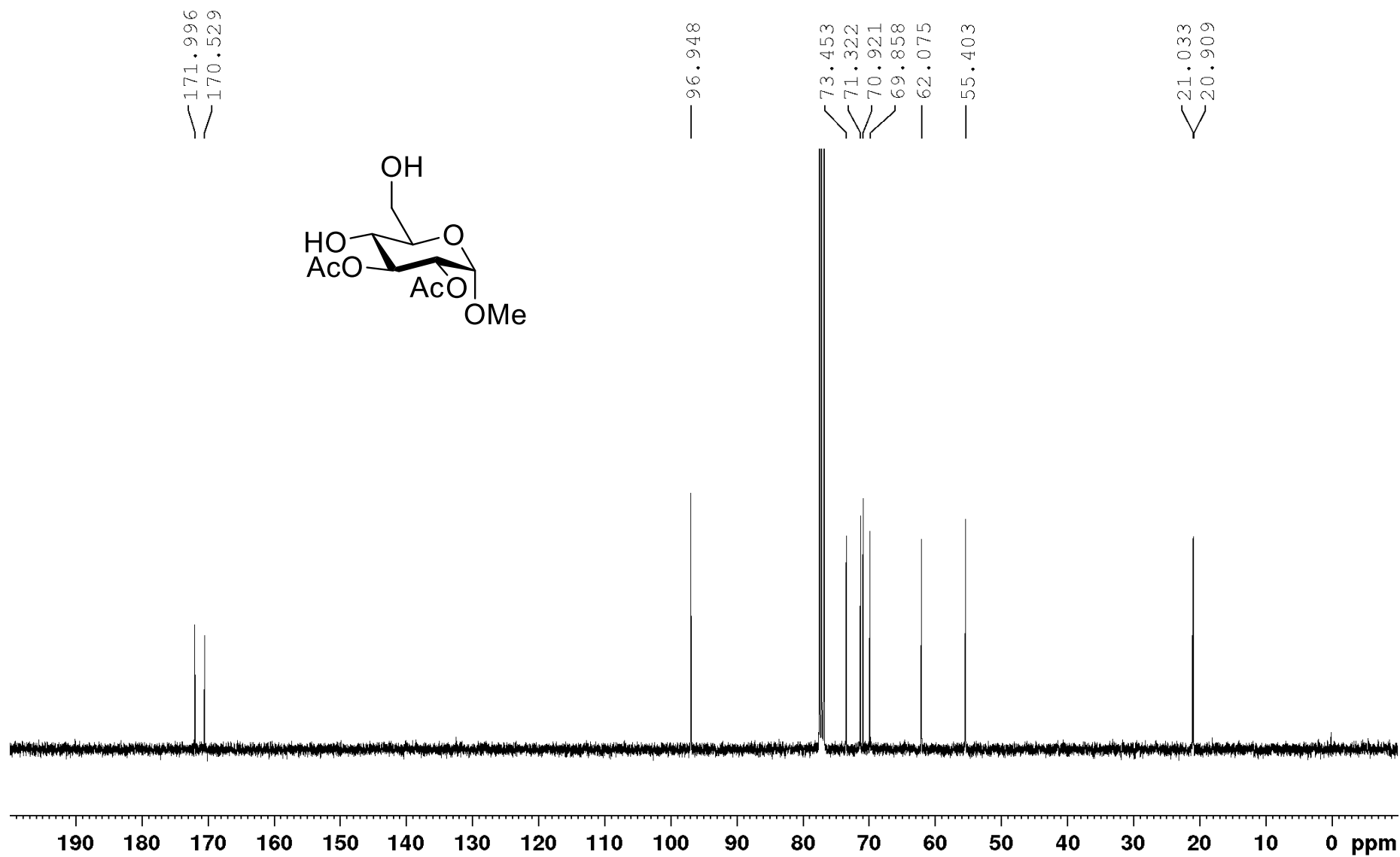
Methyl 2,3,4,6-tetra-O-acetyl- $\alpha$ -D-glucopyranoside **15**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



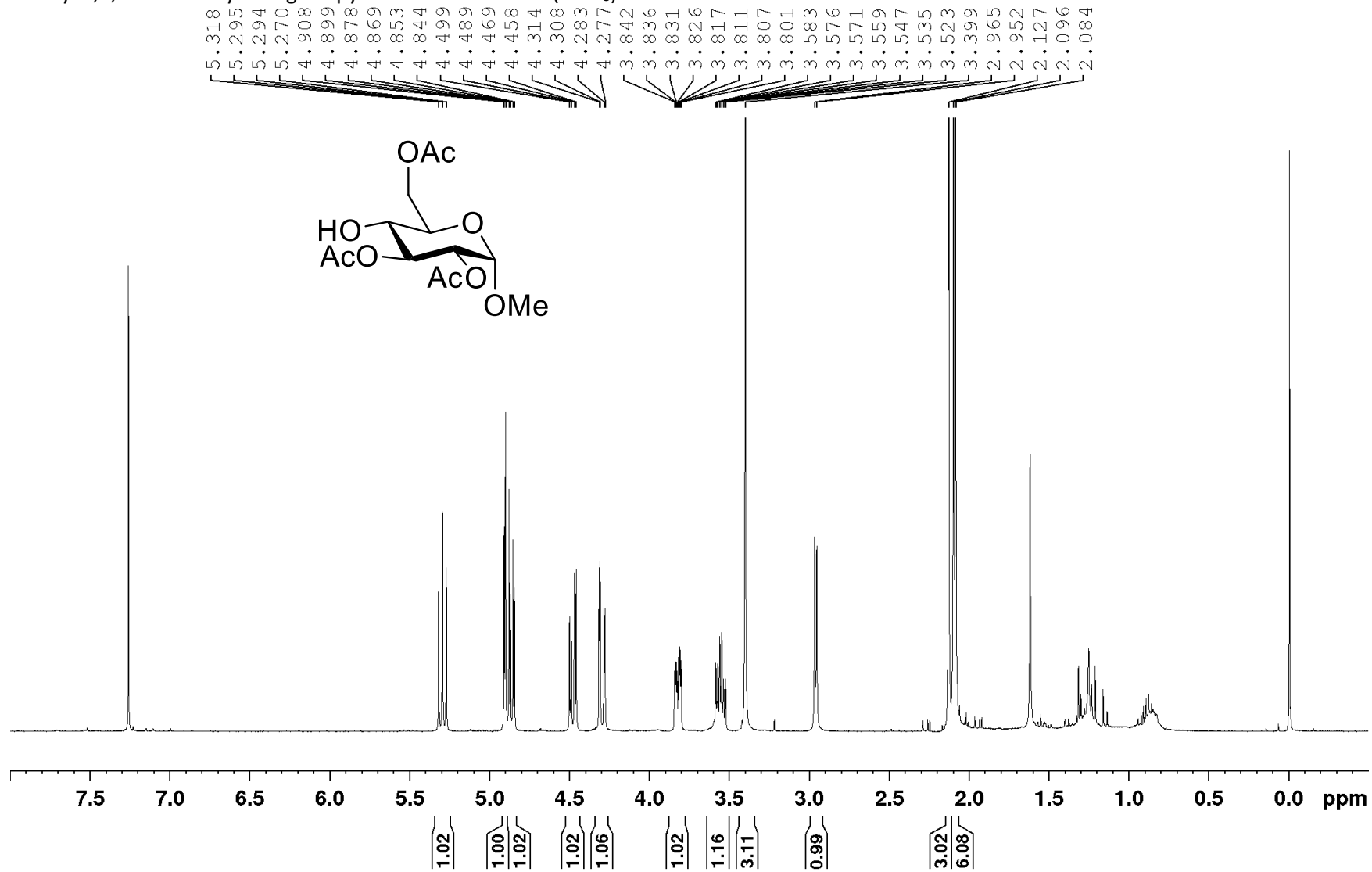
Methyl 2,3-di-O-acetyl- $\alpha$ -D-glucopyranoside **16**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



Methyl 2,3-di-O-acetyl- $\alpha$ -D-glucopyranoside **16**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )



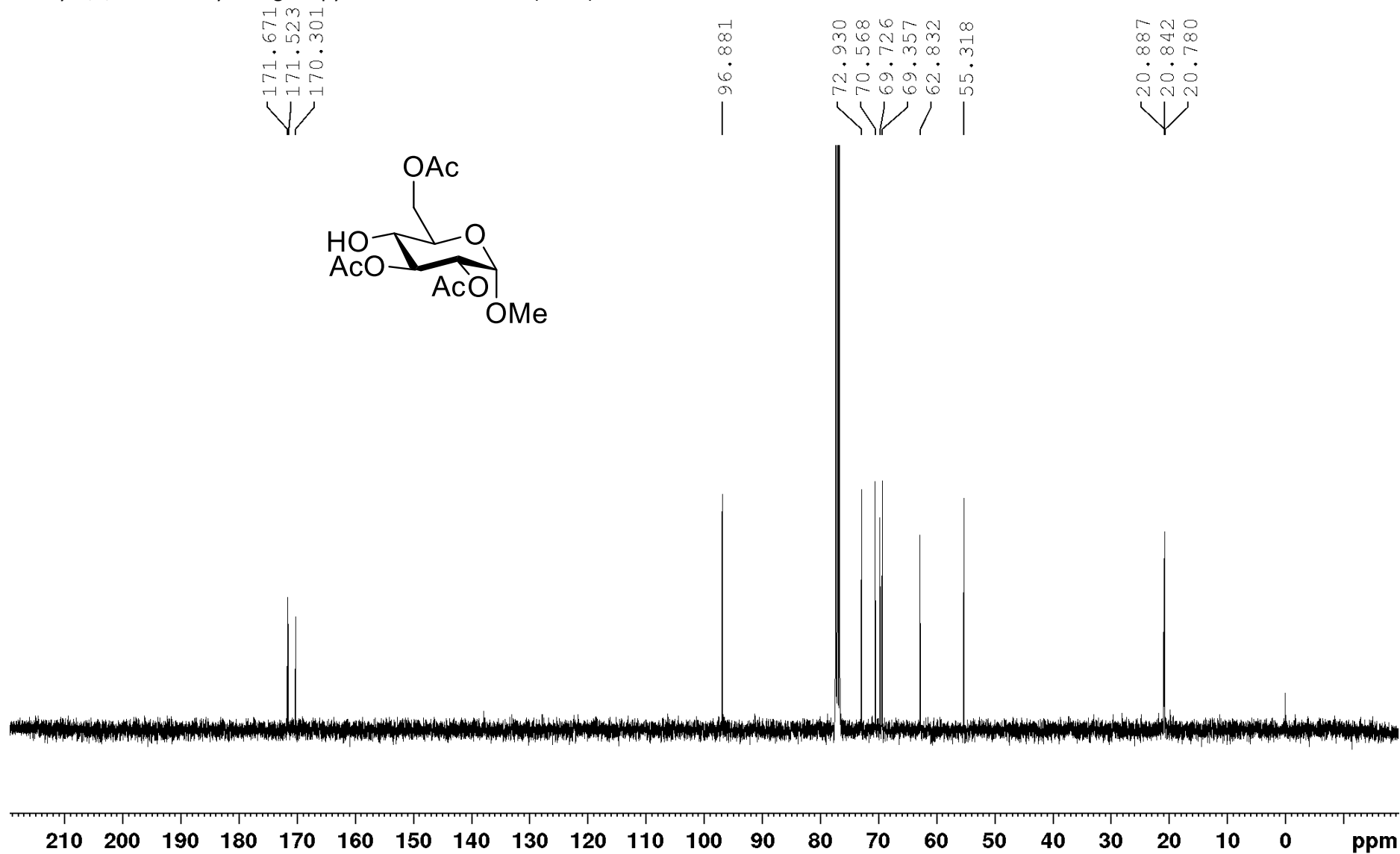
Methyl 2,3,6-tri-O-acetyl- $\alpha$ -D-glucopyranoside **17**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



- 5.318
- 5.295
- 5.294
- 5.270
- 4.908
- 4.899
- 4.878
- 4.869
- 4.853
- 4.844
- 4.499
- 4.489
- 4.469
- 4.458
- 4.314
- 4.308
- 4.283
- 4.277
- 3.842
- 3.836
- 3.831
- 3.826
- 3.817
- 3.811
- 3.807
- 3.801
- 3.583
- 3.576
- 3.571
- 3.559
- 3.547
- 3.535
- 3.523
- 3.399
- 2.965
- 2.952
- 2.127
- 2.096
- 2.084

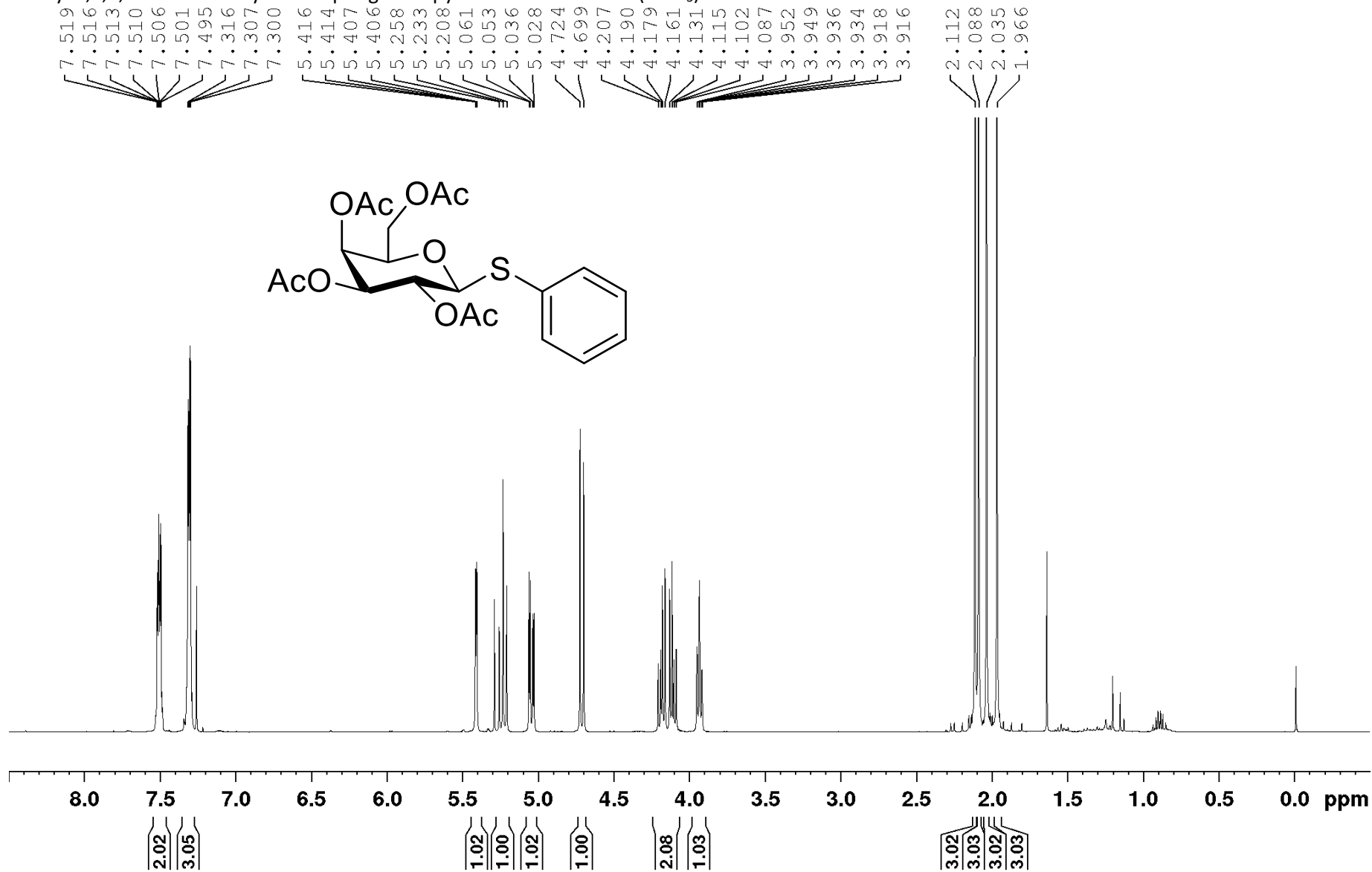
- 1.02
- 1.00
- 1.02
- 1.02
- 1.06
- 1.02
- 1.16
- 3.11
- 0.99
- 3.02
- 6.08

Methyl 2,3,6-tri-O-acetyl- $\alpha$ -D-glucopyranoside **17**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )

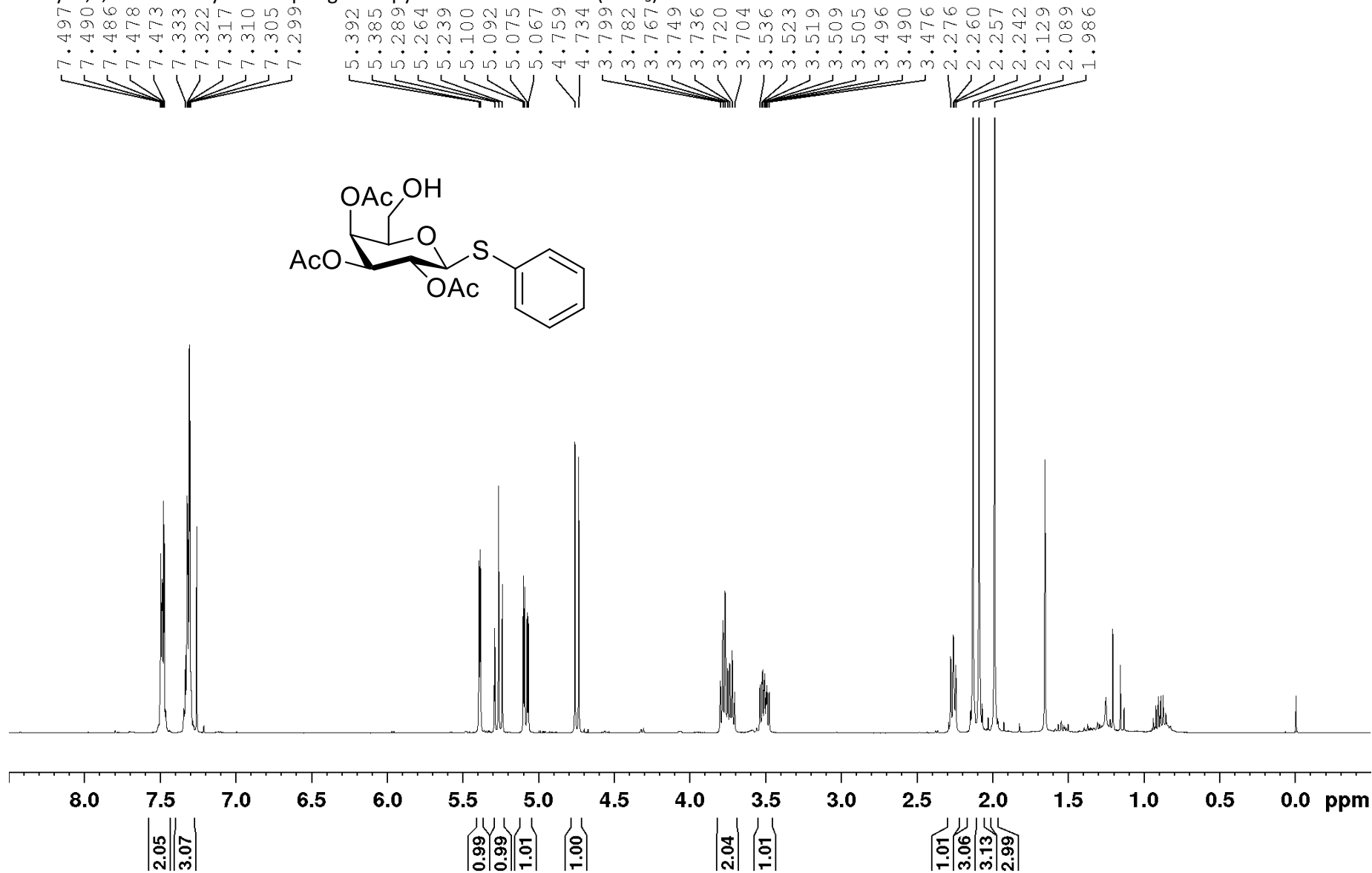




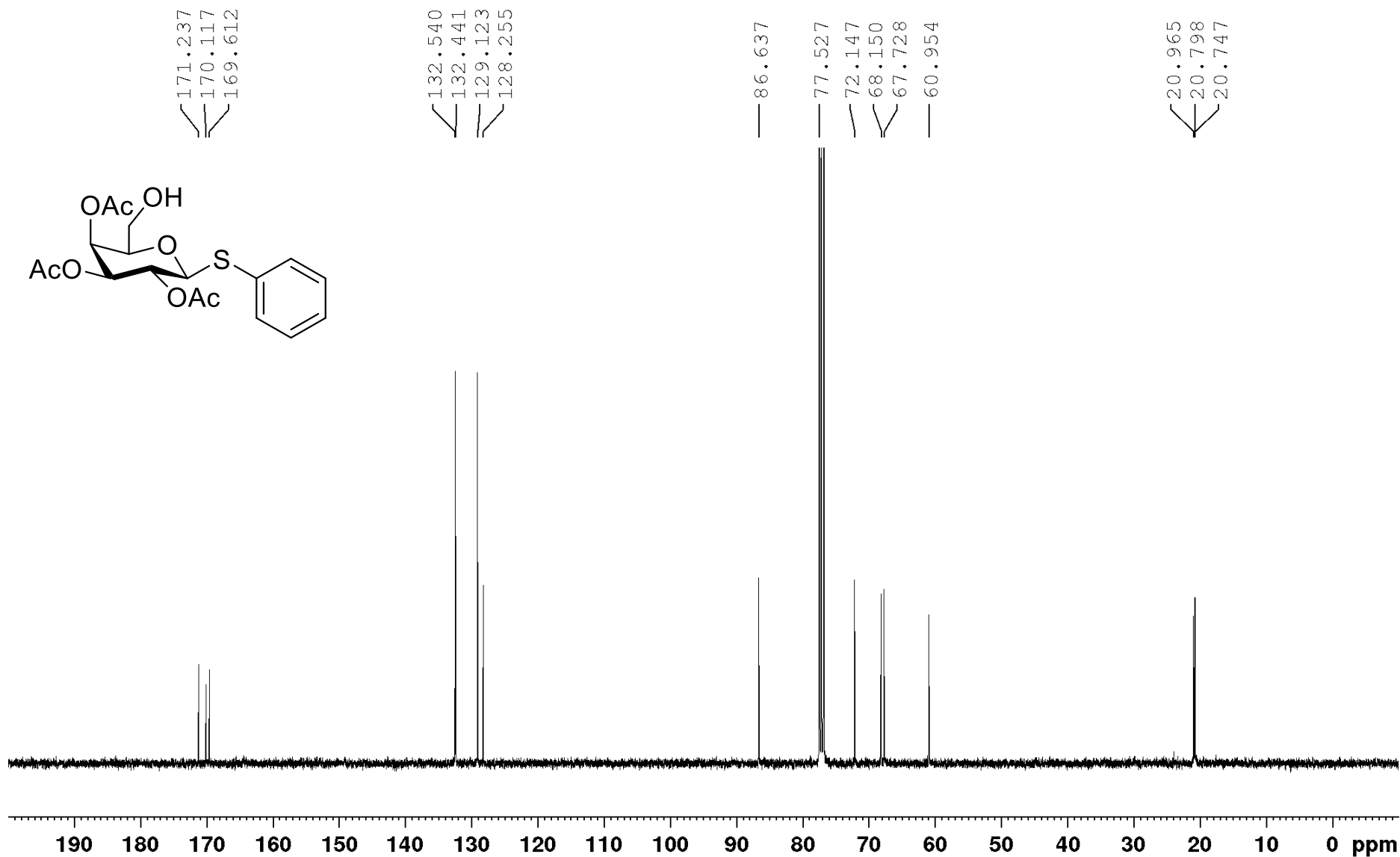
Phenyl 2,3,4,6-tetra-O-acetyl-1-thio- $\beta$ -D-galactopyranoside **18**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



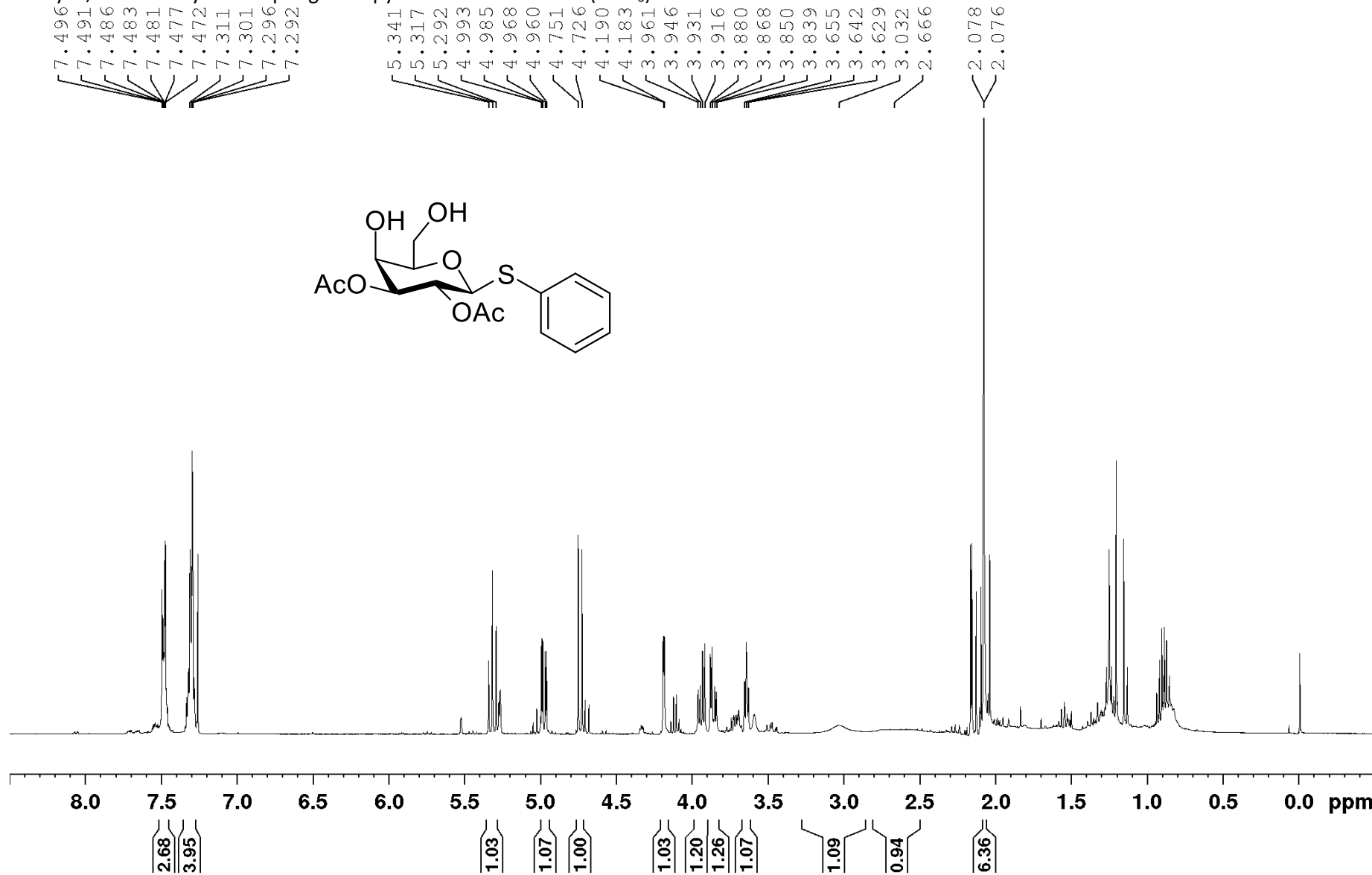
Phenyl 2,3,4-tri-O-acetyl-1-thio-β-D-galactopyranoside **19** <sup>1</sup>H NMR (CDCl<sub>3</sub>)



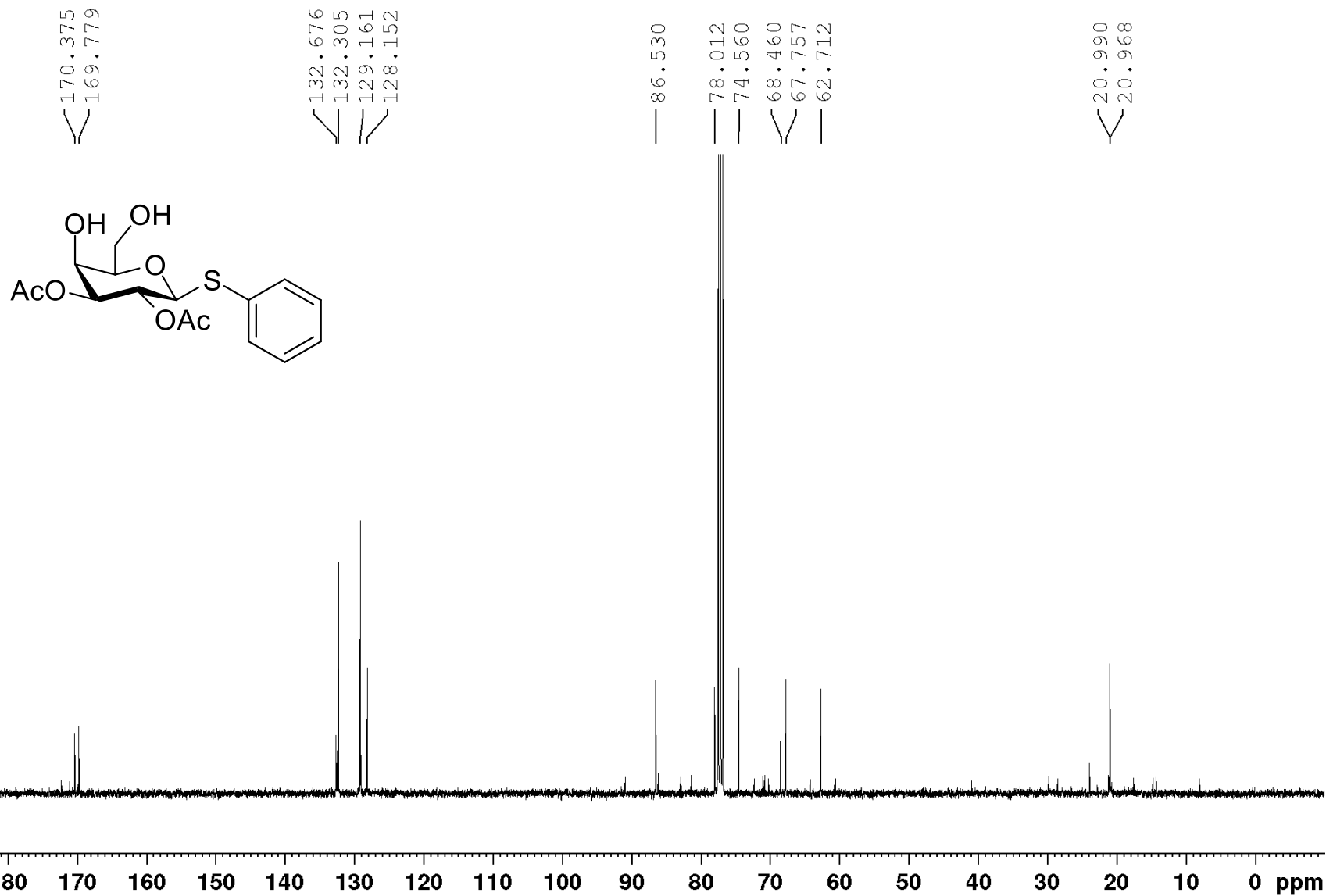
Phenyl 2,3,4-tri-O-acetyl-1-thio-β-D-galactopyranoside **19** <sup>13</sup>C NMR (CDCl<sub>3</sub>)



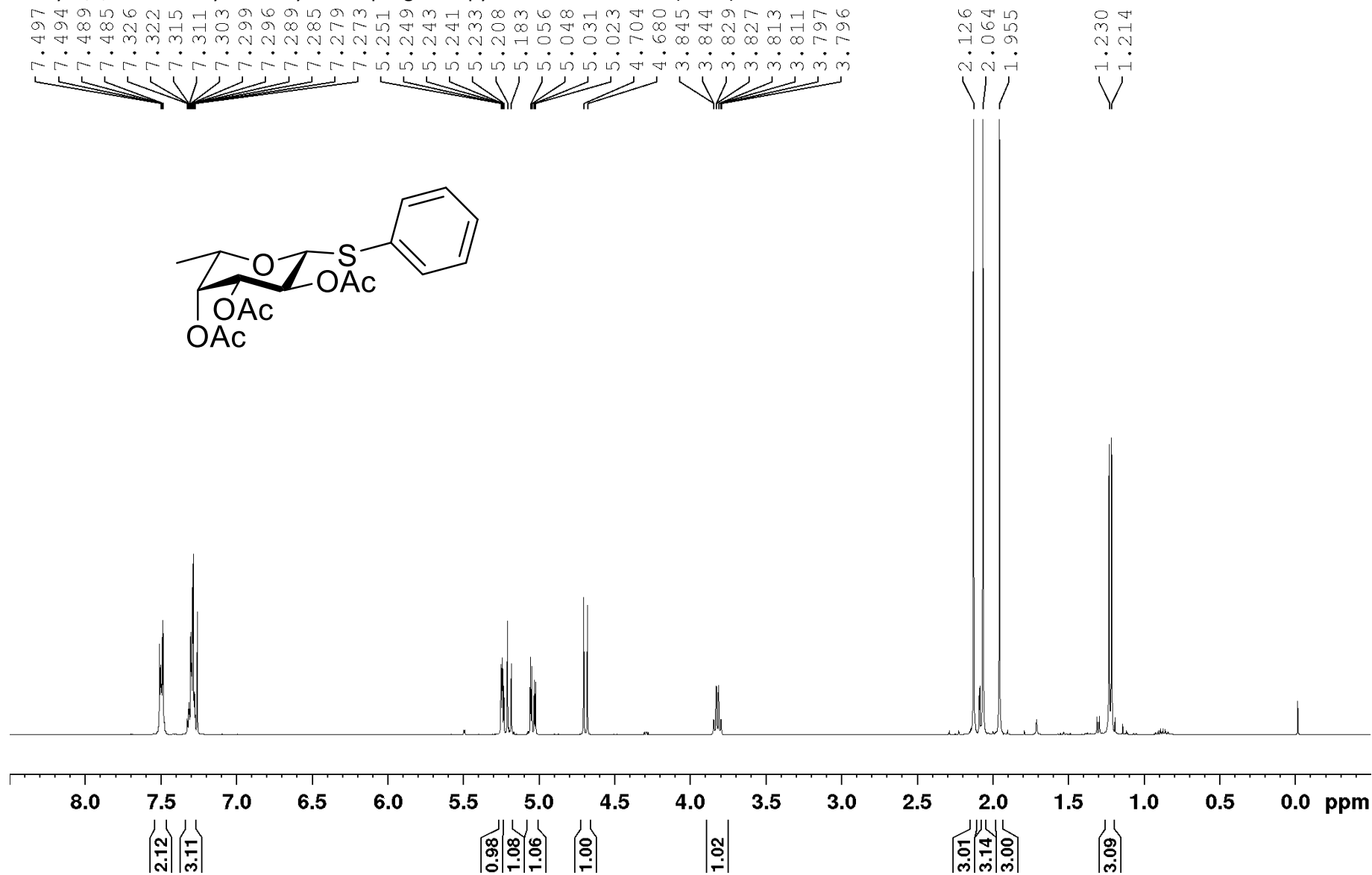
Phenyl 2,3-di-O-acetyl-1-thio-β-D-galactopyranoside **19a** <sup>1</sup>H NMR (CDCl<sub>3</sub>)



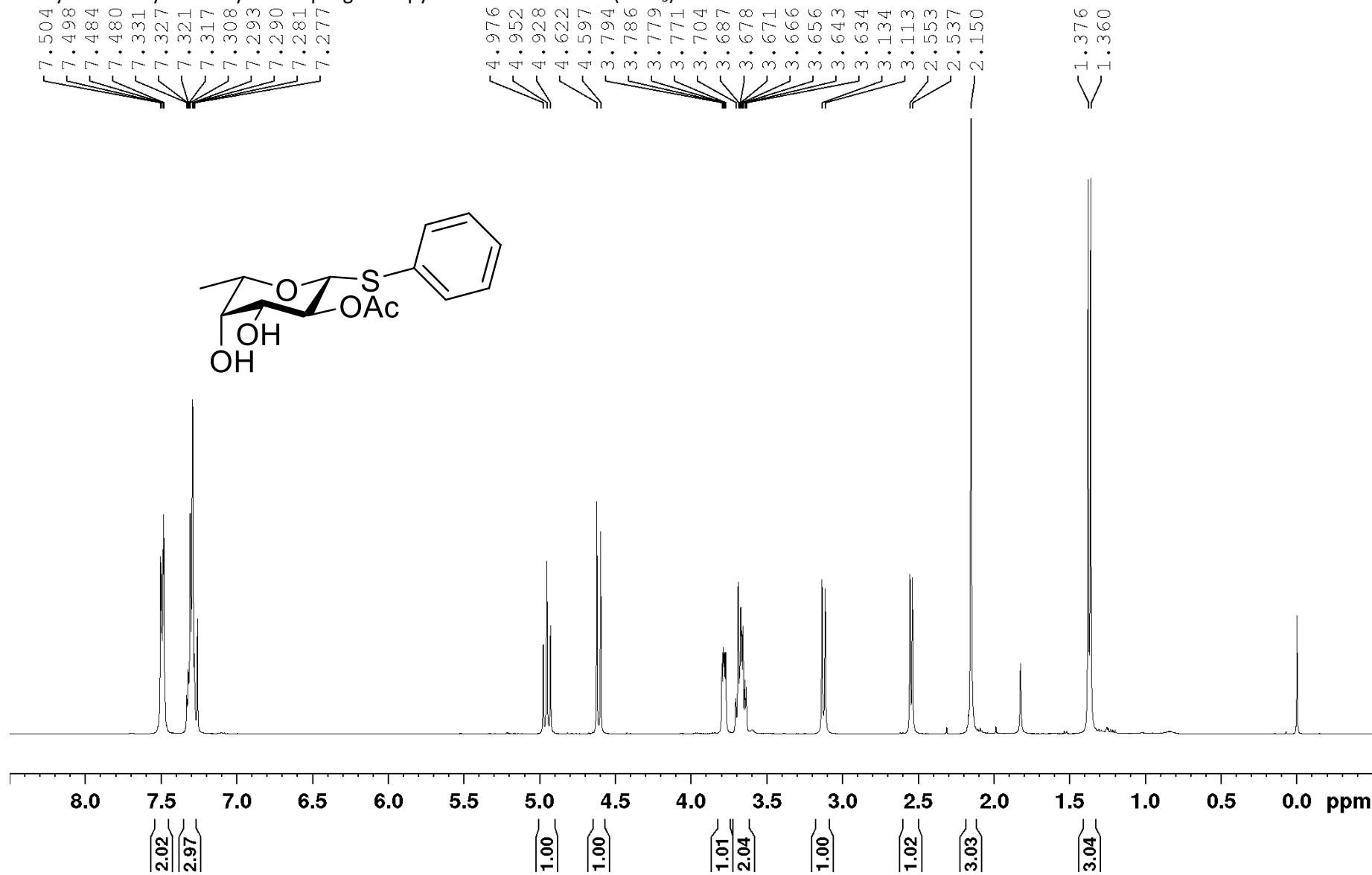
Phenyl 2,3-di-O-acetyl-1-thio-β-D-galactopyranoside **19a** <sup>13</sup>C NMR (CDCl<sub>3</sub>)



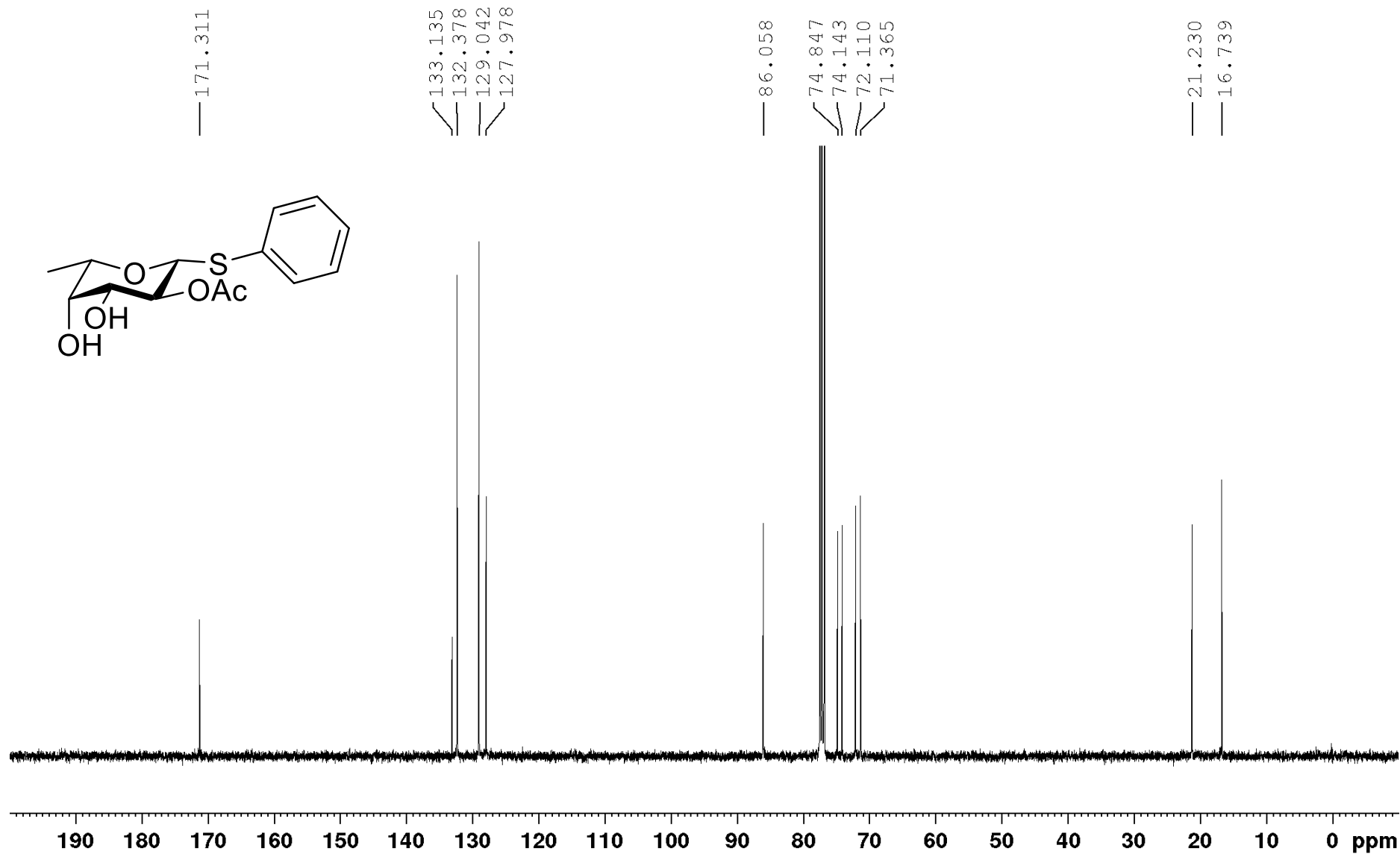
Phenyl 2,3,4-tri-O-acetyl-6-deoxy-1-thio- $\beta$ -L-galactopyranoside **20**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



Phenyl 2-O-acetyl-6-deoxy-1-thio-β-L-galactopyranoside **21** <sup>1</sup>H NMR (CDCl<sub>3</sub>)

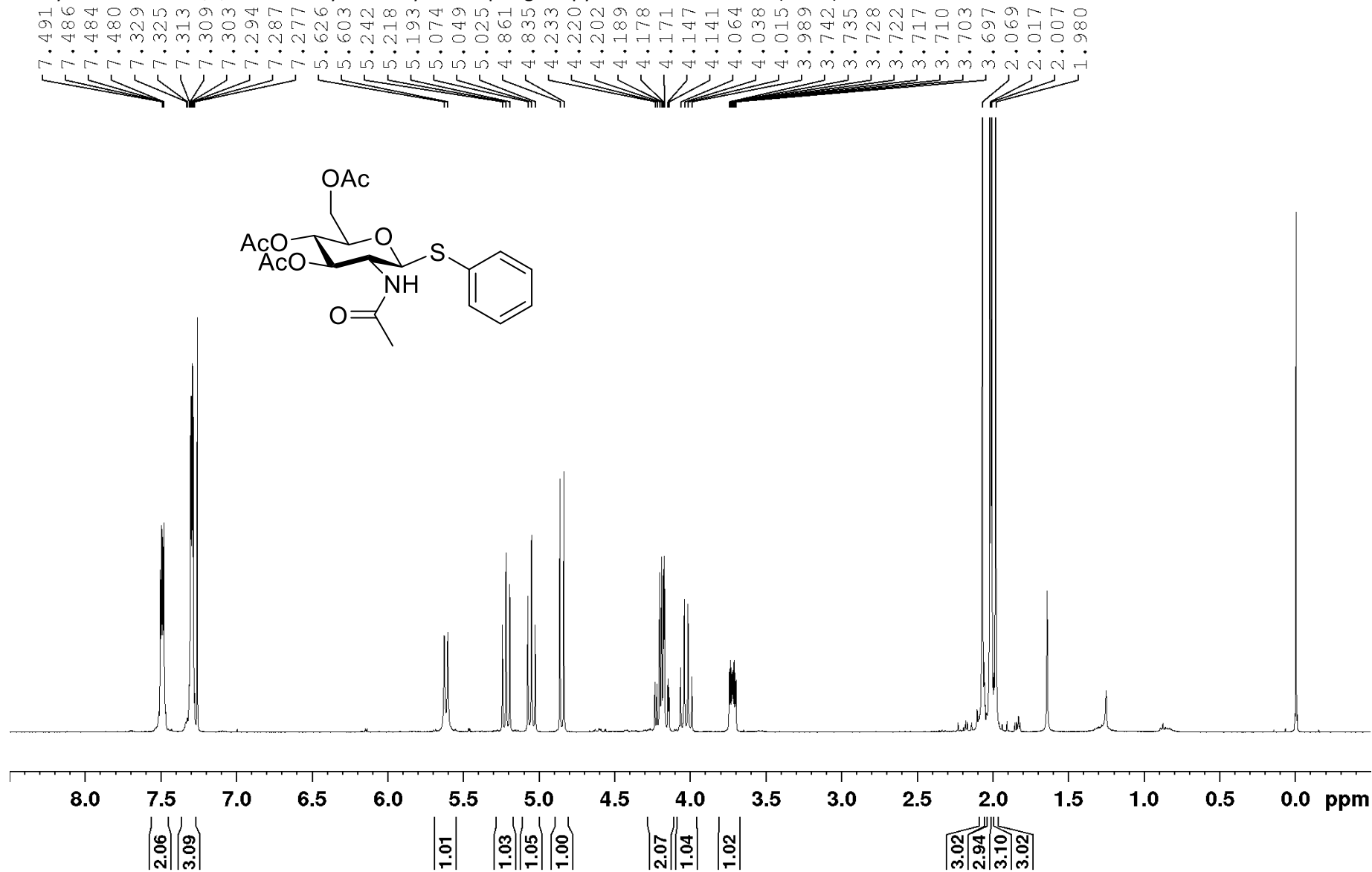


Phenyl 2-O-acetyl-6-deoxy-1-thio- $\beta$ -L-galactopyranoside **21**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )

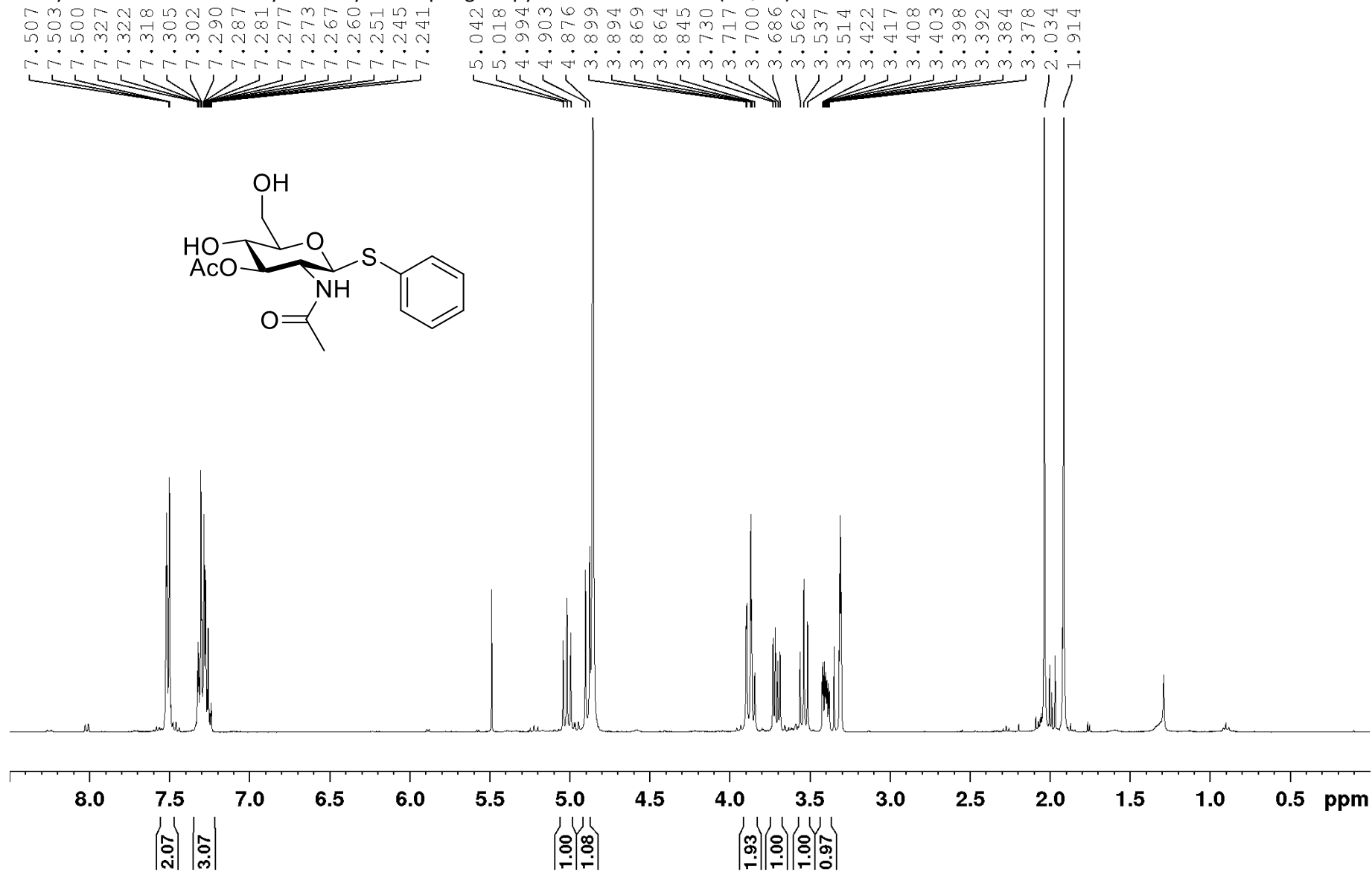




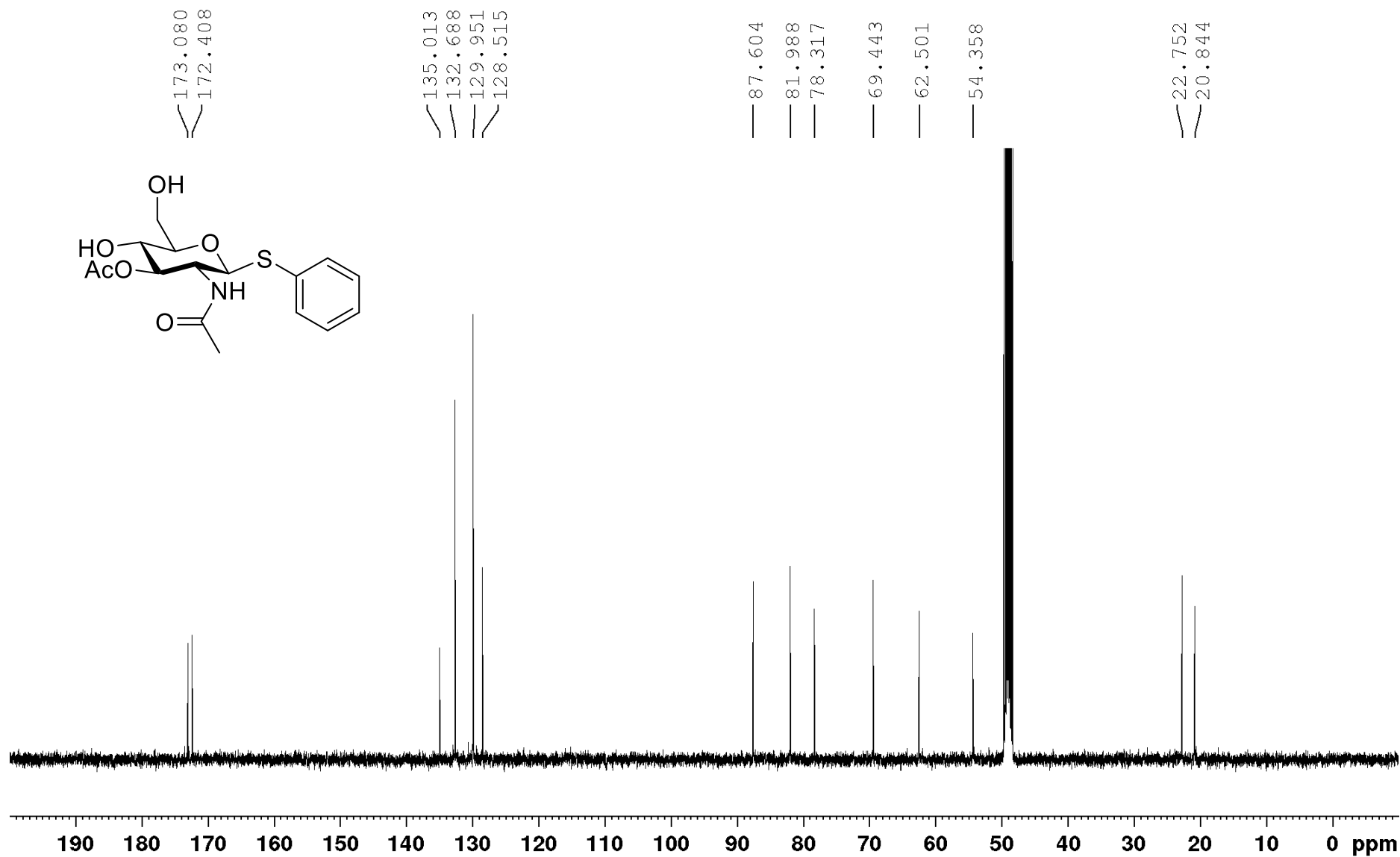
Phenyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside **22** <sup>1</sup>H NMR (CDCl<sub>3</sub>)



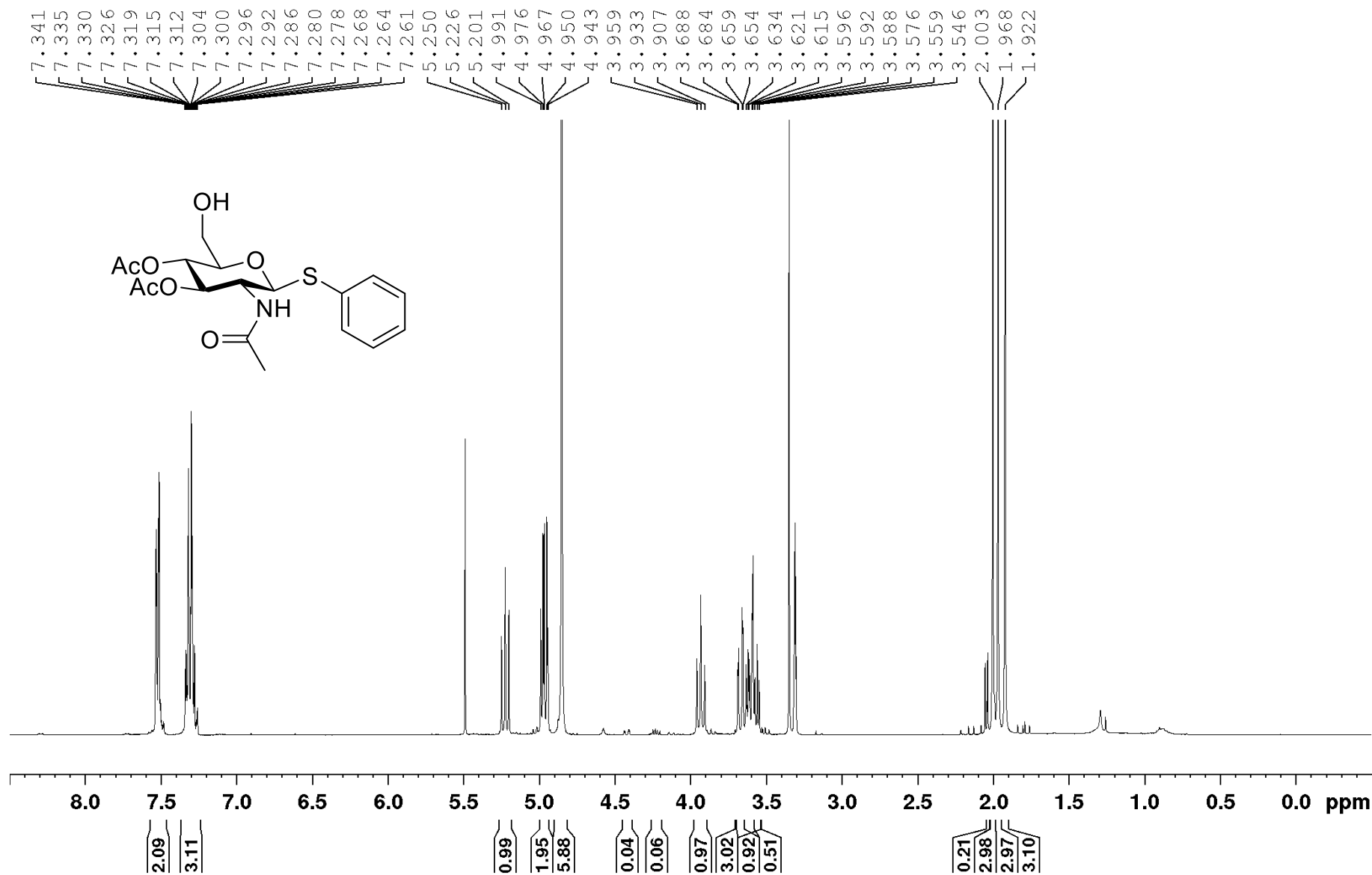
Phenyl 2-acetamido-3-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside **23** <sup>1</sup>H NMR (CD<sub>3</sub>OD)



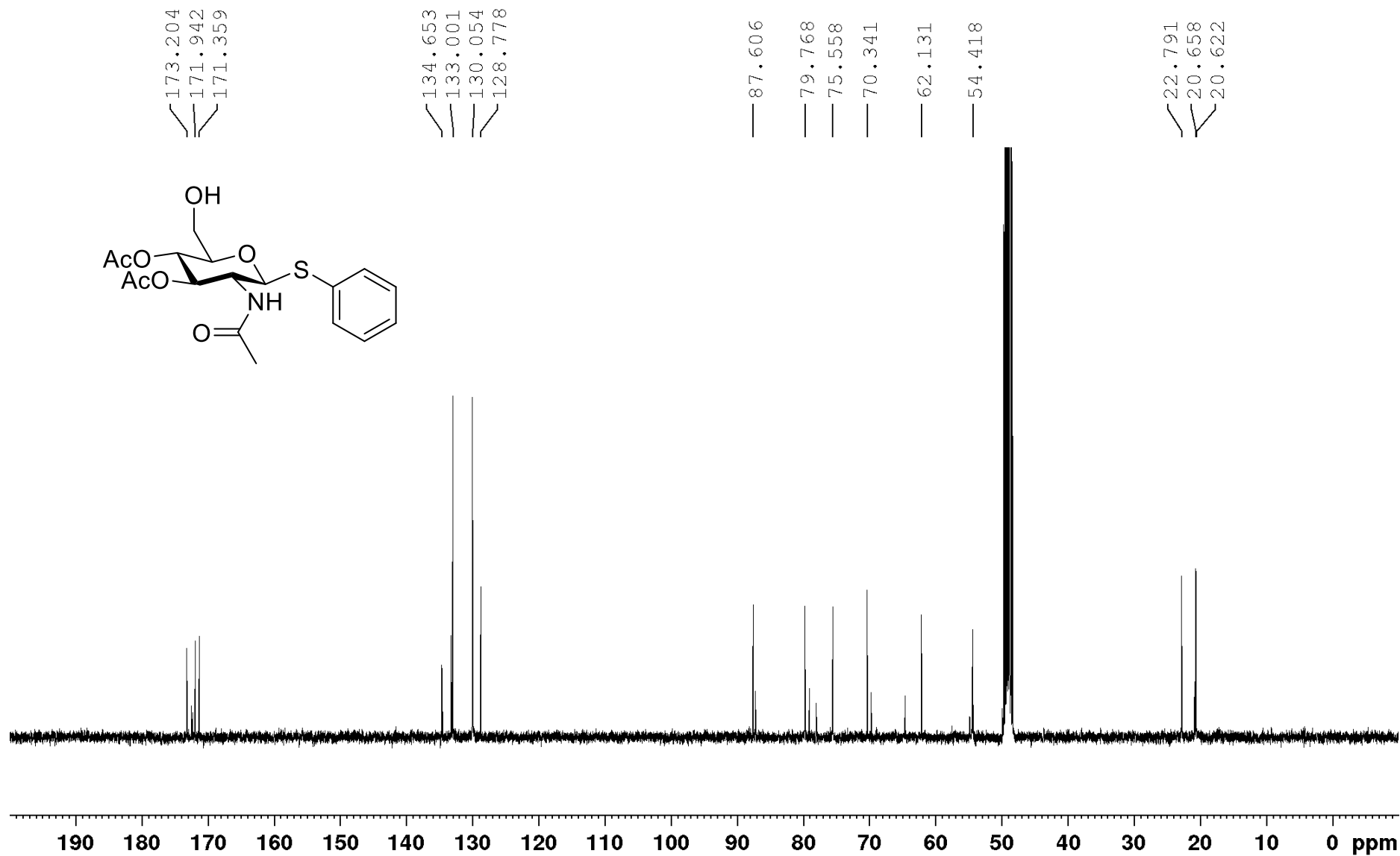
Phenyl 2-acetamido-3-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside **23** <sup>13</sup>C NMR (CD<sub>3</sub>OD)



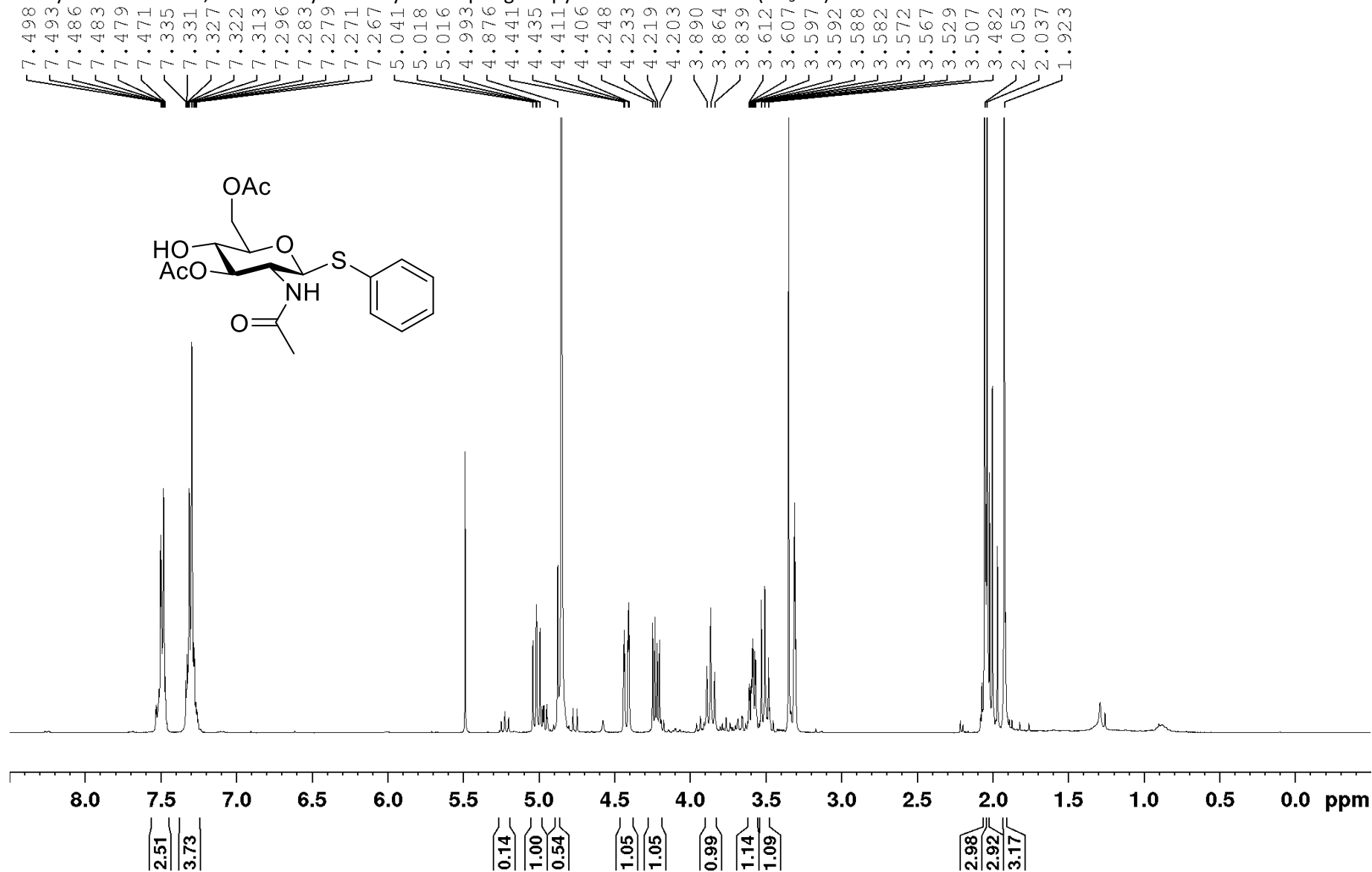
Phenyl 2-acetamido-3,4-di-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside **24** <sup>1</sup>H NMR (CD<sub>3</sub>OD)



Phenyl-2-acetamido-3,4-di-O-acetyl-2-deoxy-1-thio- $\beta$ -D-glucopyranoside **24**  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )



Phenyl 2-acetamido-3,6-di-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside **25** <sup>1</sup>H NMR (CD<sub>3</sub>OD)



Phenyl-2-acetamido-3,6-di-O-acetyl-2-deoxy-1-thio-β-D-glucopyranoside **25** <sup>13</sup>C NMR (CD<sub>3</sub>OD)

173.093  
172.517  
172.307

134.464  
133.227  
129.898  
128.768

87.221

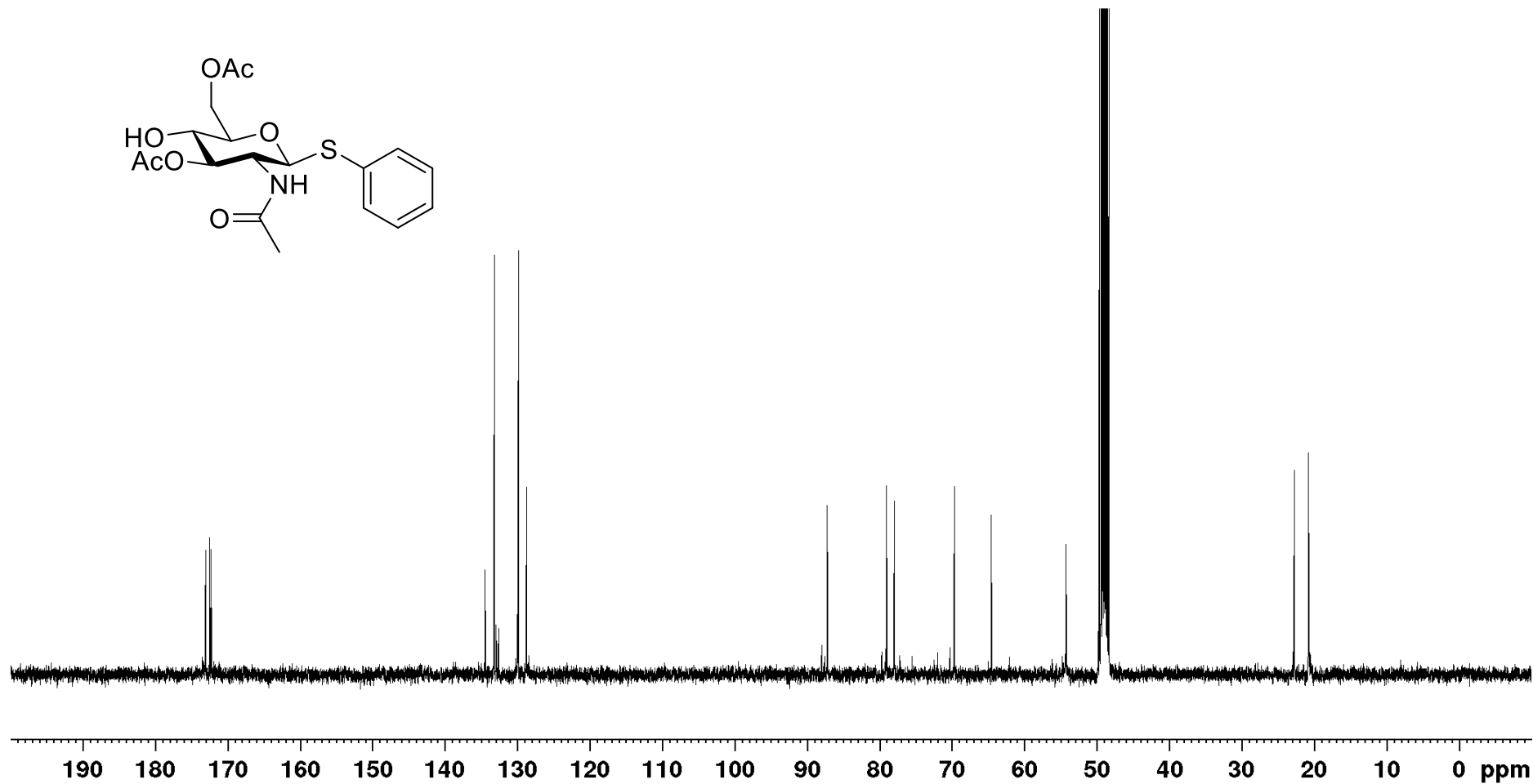
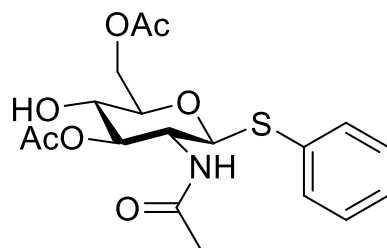
79.028  
77.990

69.700

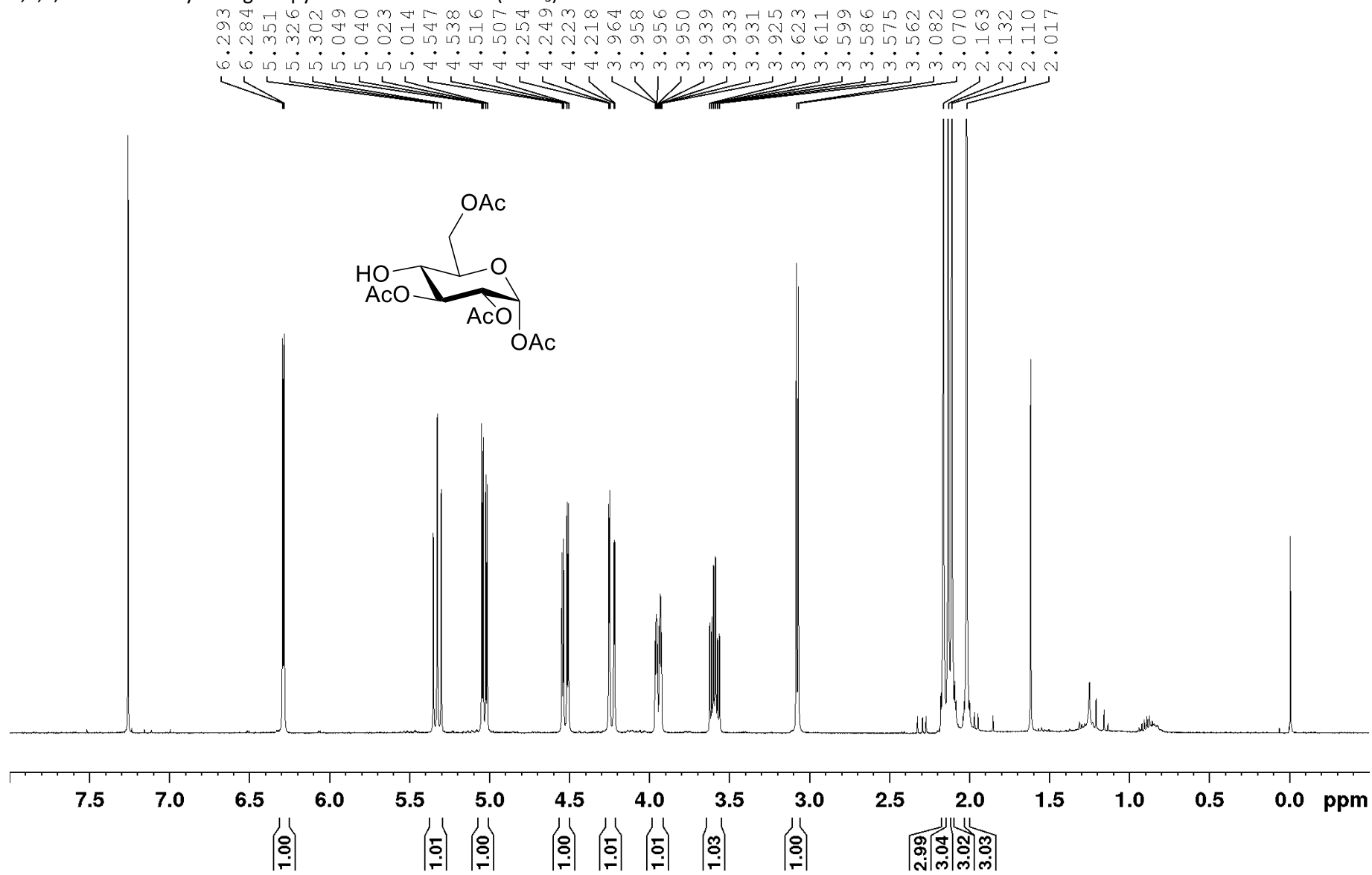
64.578

54.258

22.751  
20.805  
20.759



1,2,3,6-tetra-O acetyl- $\alpha$ -D-glucopyranoside **26**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )





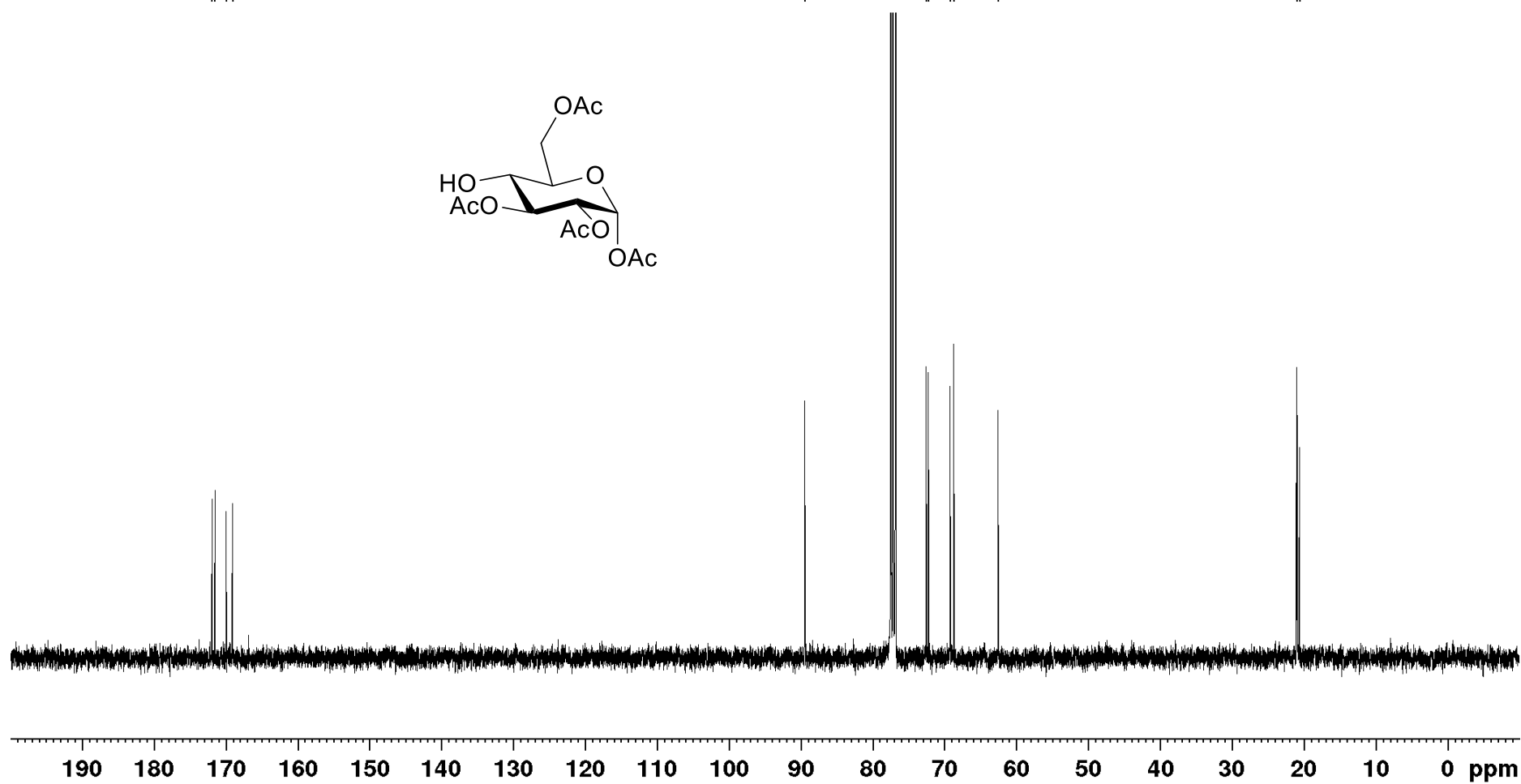
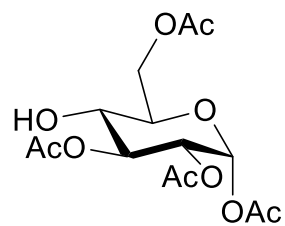
1,2,3,6-tetra-O acetyl- $\alpha$ -D-glucopyranoside **26**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )

171.954  
171.566  
169.996  
169.071

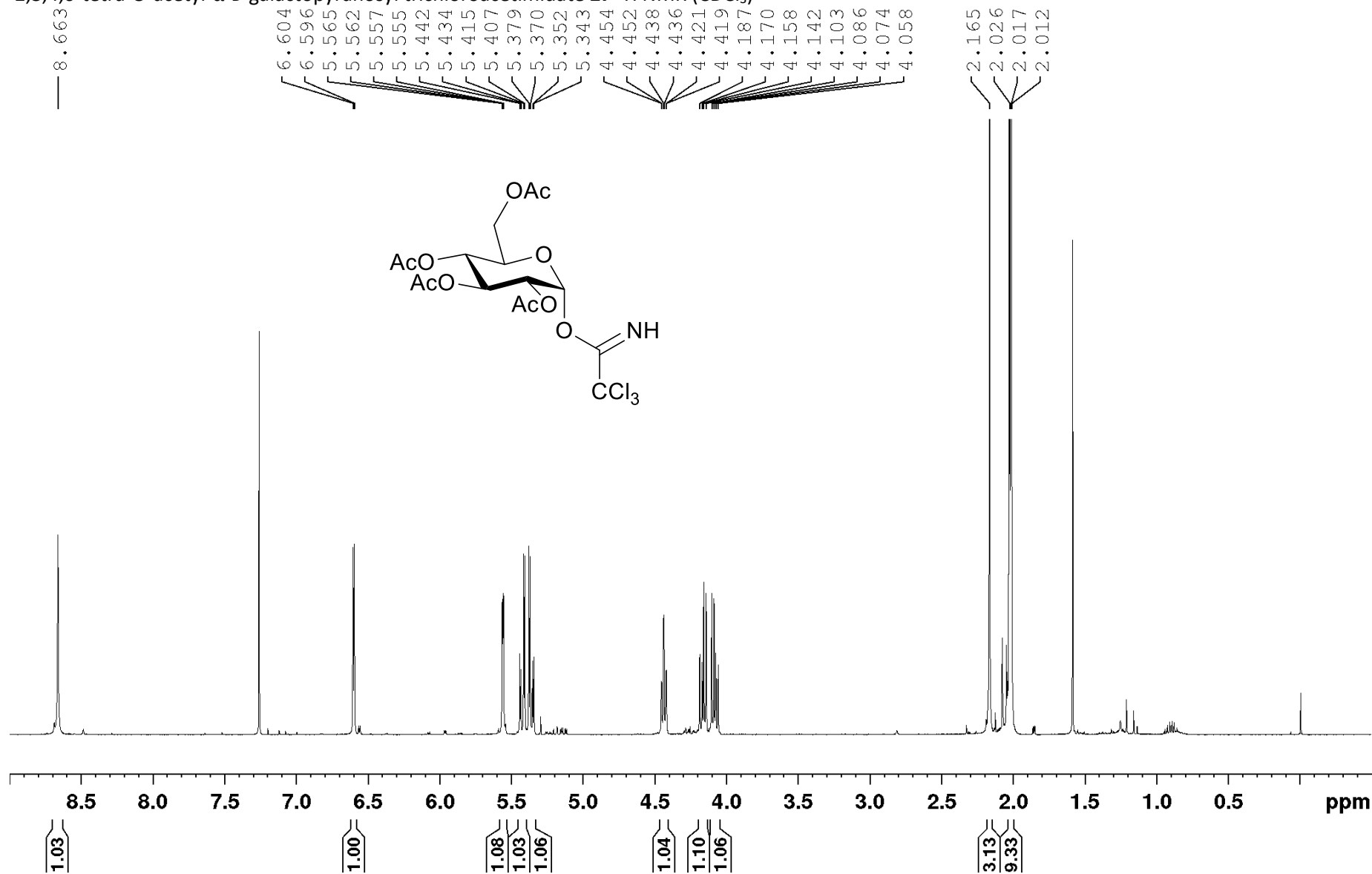
89.454

72.583  
72.247  
69.248  
68.726  
62.558

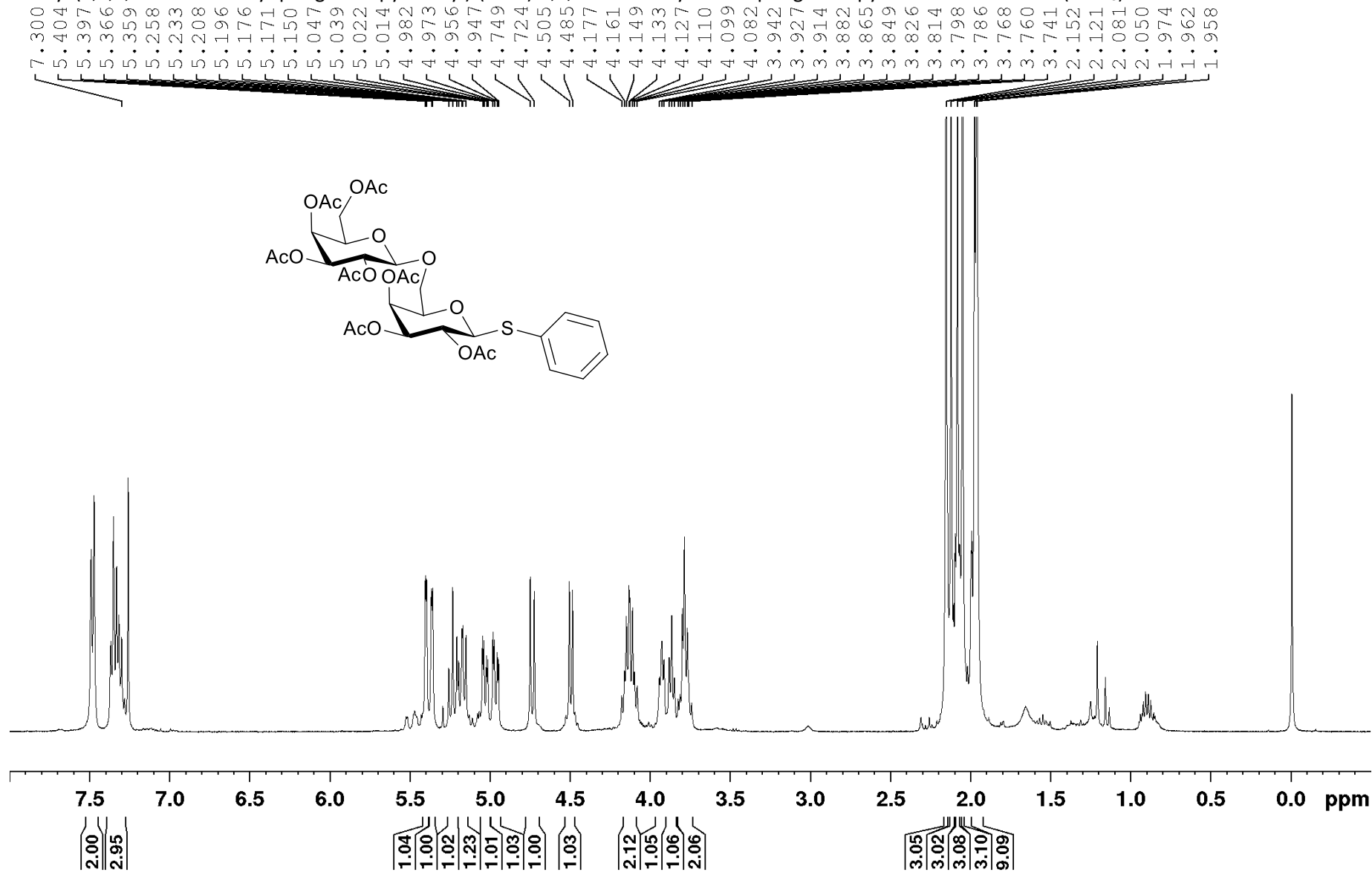
21.048  
21.009  
20.953  
20.635



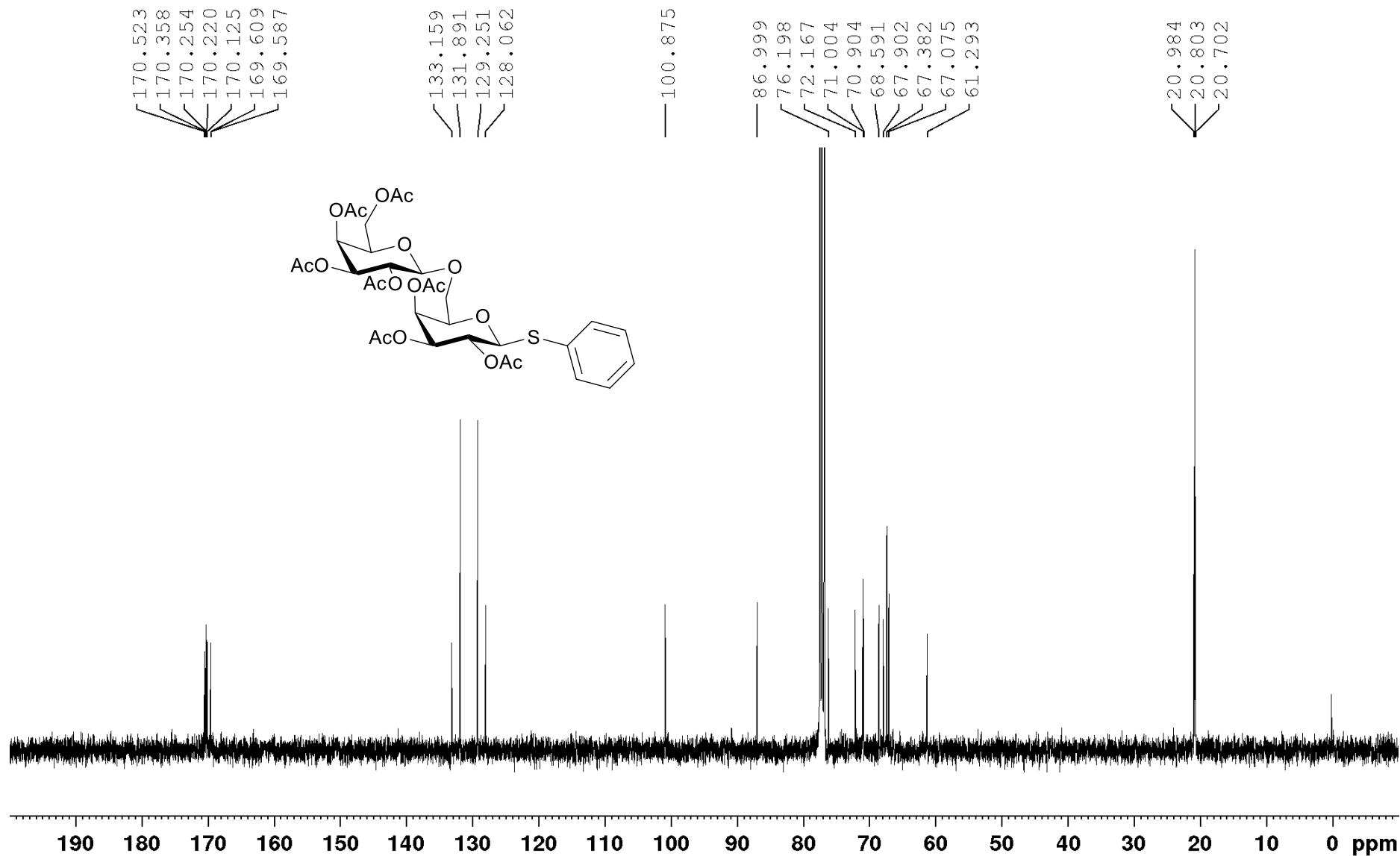
2,3,4,6-tetra-O-acetyl- $\alpha$ -D-galactopyranosyl trichloroacetimidate **27**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



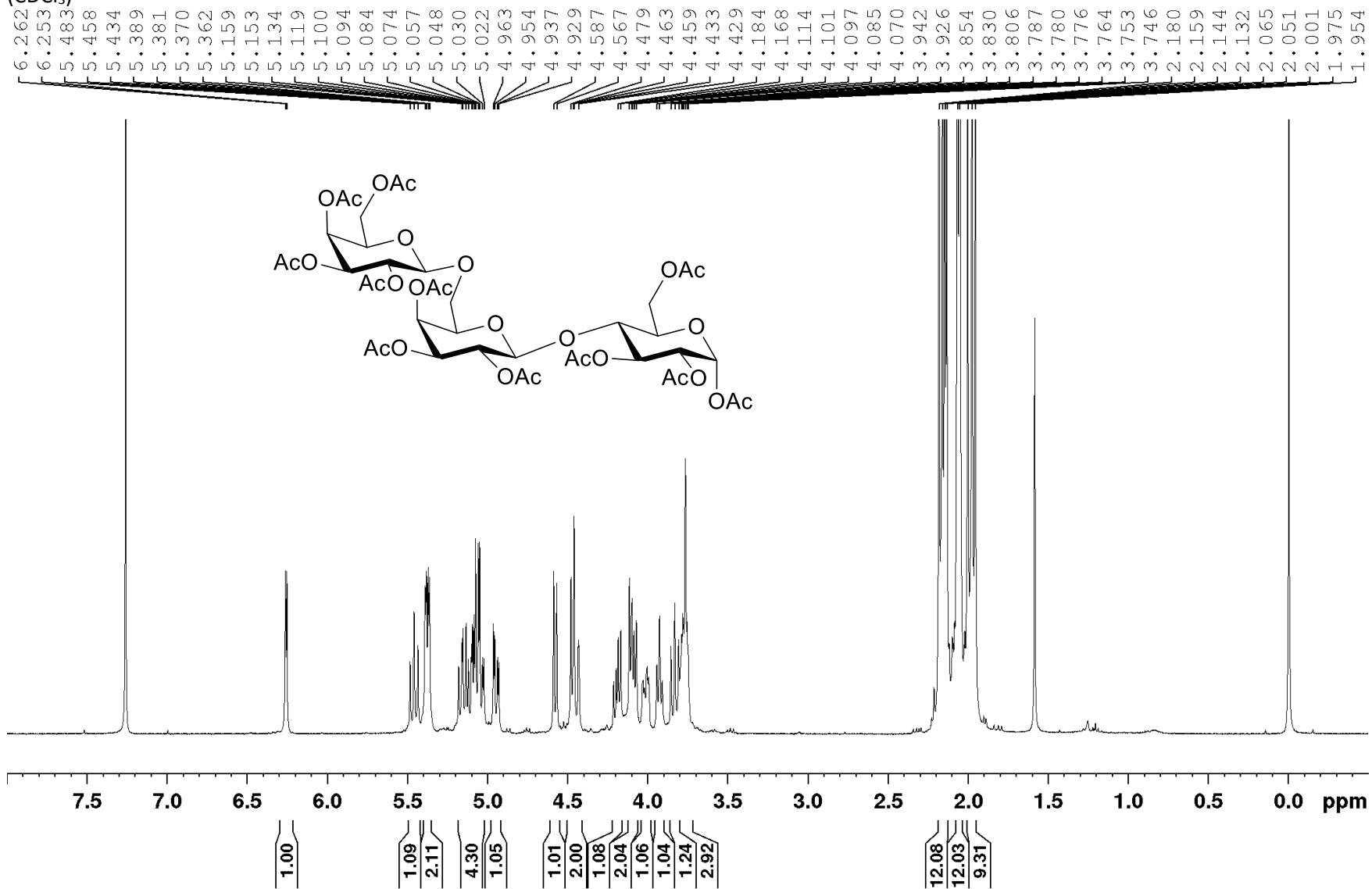
Phenyl (2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-(1→6)-2,3,4-tri-O-acetyl-1-thio-β-D-galactopyranoside **28** <sup>1</sup>H NMR (CDCl<sub>3</sub>)



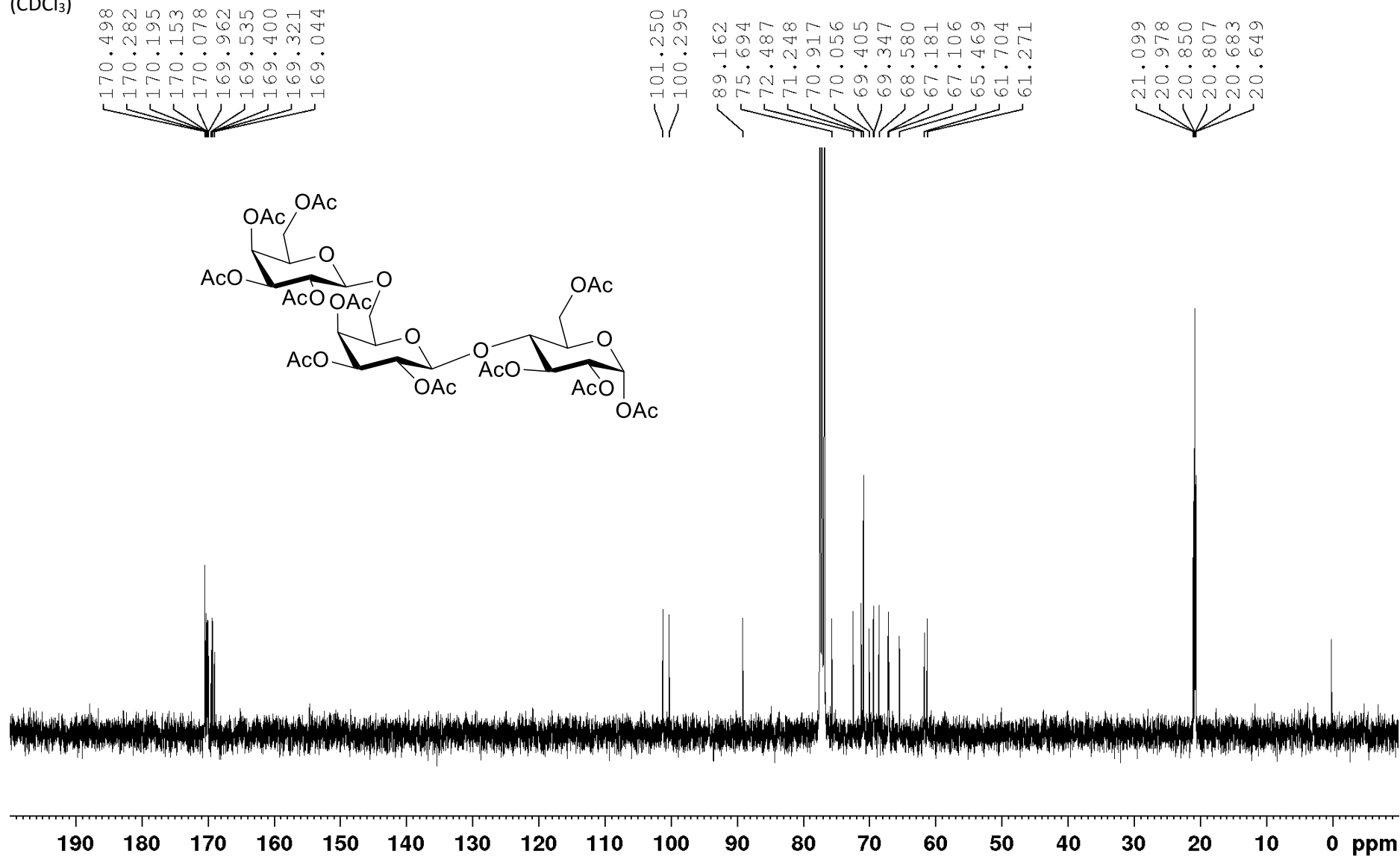
Phenyl (2,3,4,6-tetra-O-acetyl- $\beta$ -D-galactopyranosyl)-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl-1-thio- $\beta$ -D-galactopyranoside **28**  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )



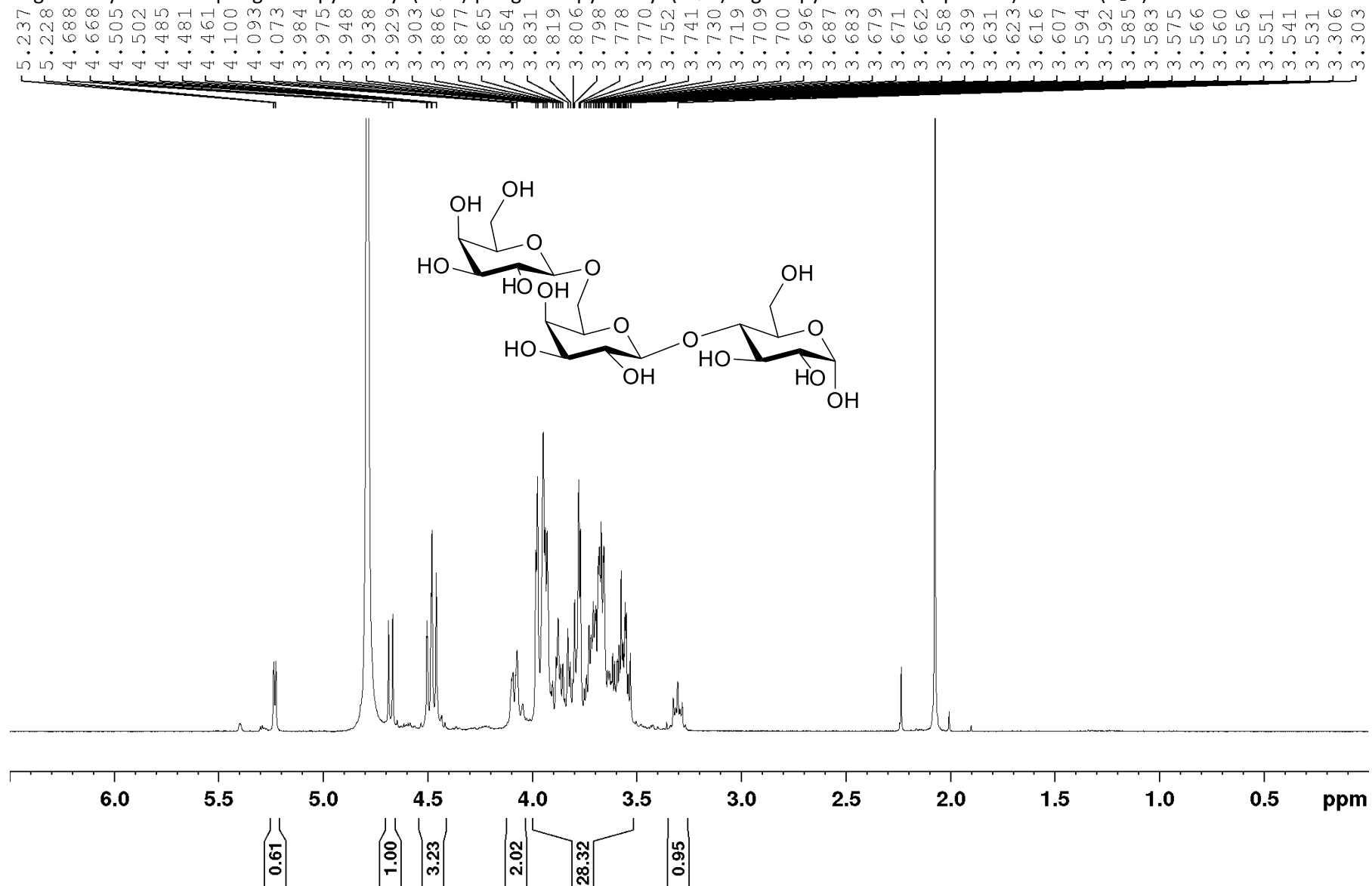
2,3,4,6-tetra-O-acetyl- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-1,2,3,6-tetra-O-acetyl- $\alpha$ -D-glucopyranoside **29**  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )



2,3,4,6-tetra-O-acetyl- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 6)-2,3,4-tri-O-acetyl- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-1,2,3,6-tetra-O-acetyl- $\alpha$ -D-glucopyranoside **29**  $^1\text{H}$  NMR (CDCl<sub>3</sub>)



6'-galactosyllactose or  $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose **30** ( $\alpha$ : $\beta$  38:62)  $^1\text{H}$  NMR ( $\text{D}_2\text{O}$ )



6'-galactosyllactose or  $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 6)- $\beta$ -D-galactopyranosyl-(1 $\rightarrow$ 4)-D-glucopyranose **30** ( $\alpha$ : $\beta$  38:62)  $^{13}\text{C}$  NMR ( $\text{D}_2\text{O}$ )

104.025  
103.836  
103.799  
96.376  
92.501  
80.132  
79.870  
75.799  
75.369  
75.228  
74.683  
74.428  
73.291  
73.276  
73.114  
72.313  
71.738  
71.516  
71.477  
70.643  
69.722  
69.312  
69.144  
61.678  
60.843  
60.693

