Total Synthesis and Cytotoxicity Evaluation of Spirostanol Saponin Gitonin

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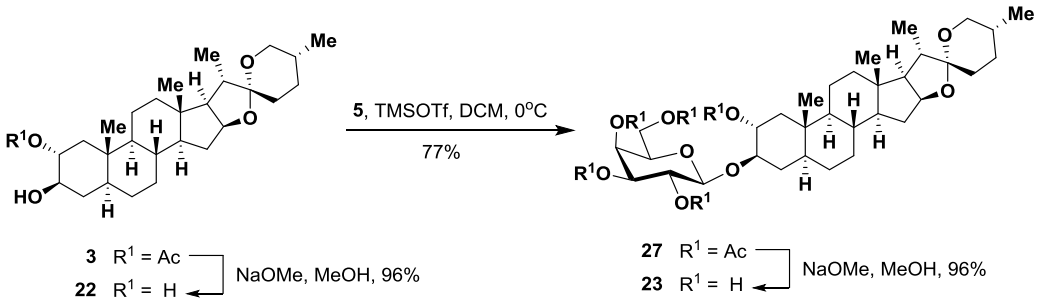
Contents

1. Synthesis of gitonin analogs for bioassay (**Scheme** S1-4)



Scheme S1. Synthesis of 2α-OH tigogenin derivative **22** and galactosylation derivative **23**

Synthesis of compound 22

To a solution of **3** (180 mg, 0.138 mmol) in MeOH (20 mL) was added 1 M NaOMe until pH of the solution reaches 9-10. After stirring at room temperature for 30 min, the reaction mixture neutralized with Dowex-50 (H^+) ion exchange resin. The mixture was filtered and the filtrate was concentrated. The residue was purified by silica gel chromatography (petroleum ether/EtOAc 3:1) to afford **22** as an amorphous solid (157 mg, 96%): $[\alpha]_D^{25}$ = –80 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, Pyridine-d_5): δ 5.61-5.11 (*br* s, 2H, OH), 4.51 (m, 1H, H-16), 4.04-3.97 (m, 1H, H-2), 3.85-3.78 (m, 1H, H-3), 3.56-3.44 (m, 2H, H-26), 2.26-2.19 (m, 1H), 2.06-1.95 (m, 1H), 1.95-1.84 (m, 1H), 1.83-1.72 (m, 2H), 1.70-0.60 (m, 20H), 0.96 (d, *J* = 7.2 Hz, 3H, Me-21), 0.82 (s, 3H, Me-19), 0.79 (s, 3H, Me-18), 0.65 (d, *J* = 5.6 Hz, 3H, Me-27); ^{13}C NMR (100 MHz, Pyridine-d_5): δ 109.4, 81.3, 76.9, 73.3, 67.1, 63.2, 56.6, 54.8, 46.7, 45.4, 42.2, 41.0, 40.4, 37.8, 37.4, 34.9, 32.6, 32.3, 32.0, 30.8, 29.5, 28.5, 21.7, 17.5, 16.8, 15.2, 13.9; HRMS(ESI): calcd. for $C_{27}H_{44}O_4Na^+$[M+Na]$^+$, 455.3131; found 455.3131.

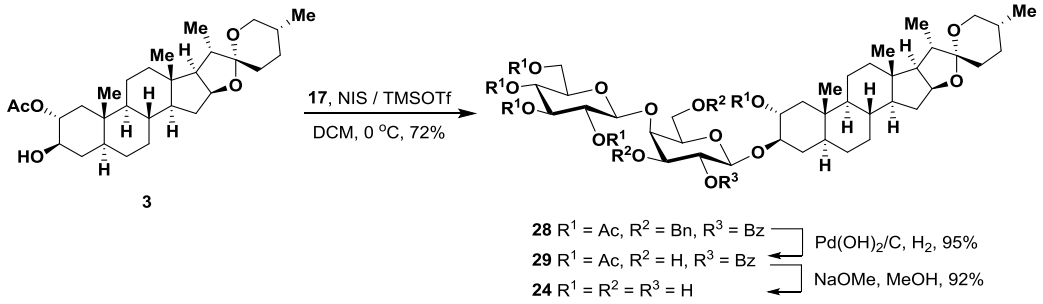
Synthesis of compound 27

The titled compound was prepared from compounds **3** (95 mg, 0.2 mmol) and **5** (118 mg, 0.24 mmol) by following a similar procedure to that for compound **17** to give **27** as a yellowish syrup (123 mg, 77%): $[\alpha]_D^{25}$ = – 30 (*c* 1.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.37 (d, *J* = 2.8 Hz, 1H, H-4^I), 5.14 (dd, *J* = 10.4, 8.0 Hz, 1H, H-2^I), 5.00 (dd, *J* = 10.8, 3.2 Hz, 1H, H-3^I), 4.96 (m, 1H, H-2), 4.53 (d, *J* = 8.0 Hz, 1H, H-1^I), 4.42-4.34 (m, 1H, H-16), 4.21-4.01 (m, 2H, H-6a^I and H-6b^I), 3.88 (t, *J* = 6.8 Hz, 1H, H-5^I), 3.63-3.58 (m, 1H, H-3), 3.49-3.47 (m, 2H, H-26), 2.17-1.99 (m, 15H, 5 × Ac), 1.91-1.84 (m, 1H), 1.78-0.67 (m, 24H), 0.97 (d, *J* = 6.8 Hz, 3H, Me-21), 0.90 (s, 3H, Me-19), 0.78 (d, *J* = 6.5 Hz, 3H, Me-27), 0.76 (s, 3H, Me-18); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.4, 170.32, 170.27, 170.2, 169.1, 109.3, 100.4, 81.3, 80.8, 77.2, 72.8, 71.0, 70.5, 69.3, 66.92, 66.86, 62.2, 61.2, 56.1, 54.2, 44.3, 42.4, 41.6, 40.5, 39.8, 37.1, 34.3, 33.5, 32.0, 31.7, 31.4, 30.3, 28.8, 27.9, 21.3, 20.8, 20.70, 20.67, 20.6, 17.1, 16.5, 14.5, 13.0; HRMS(ESI): calcd. for $C_{43}H_{64}O_{14}Na^+$[M+Na]$^+$, 827.4188; found 827.4186.

Synthesis of compound 23

The titled compound was prepared from compound **27** (117 mg, 0.15 mmol) by following a similar procedure to that for compound **1** to give **23** as a white foamy solid (83 mg, 96%): $[\alpha]_D^{25}$

= – 28 (*c* 1.0, CH_3OH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ 6.31-5.23 (*br* s, 5H, OH), 4.95 (d, *J* = 8.0 Hz, 1H, H-1^{I}), 4.53-4.41 (m, 5H), 4.19-4.08 (m, 2H), 3.97-3.91 (m, 1H, H-2), 3.87-3.81 (m, 1H, H-3), 3.56-3.43 (m, 2H, H-26), 2.25-0.48 (m, 25H), 1.08 (d, *J* = 6.8 Hz, 3H, Me-21), 0.77 (s, 3H, Me-19), 0.69 (s, 3H, Me-18), 0.65 (d, *J* = 4.8 Hz, 3H, Me-27); ^{13}C NMR (100 MHz, Pyridine-d_5): δ 109.0, 103.9, 84.9, 80.9, 77.0, 75.1, 72.1, 70.4, 70.0, 66.6, 62.8, 62.1, 56.1, 54.2, 45.5, 44.4, 41.7, 40.5, 39.8, 36.6, 34.4, 34.0, 32.0, 31.9, 31.6, 30.4, 29.0, 27.9, 21.2, 17.1, 16.4, 14.8, 13.2; HRMS(ESI): calcd. for $C_{33}H_{54}O_9Na^+$[M+Na]$^+$, 617.3660; found 617.3660.



Scheme S2. Synthesis of disaccharide derivative **24**

Synthesis of compound 28

The titled compound was prepared from compounds **3** (44 mg, 0.09 mmol) and **17** (95 mg, 0.11 mmol) by following a similar procedure to that for compound **21** to give **28** as a yellowish syrup (84 mg, 72%): $[\alpha]_D^{25}$ = – 52 (*c* 1.4, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.03-7.21 (m, 15H, Ph), 5.35 (dd, *J* = 10.0, 8.0 Hz, 1H, H-2^{I}), 5.23 (t, *J* = 9.6 Hz, 1H, H-3^{II}), 5.17 (d, *J* = 8.0 Hz, 1H, H-1^{II}), 5.12 (t, 1H, *J* = 9.2 Hz, H-4^{II}), 5.01 (dd, *J* = 9.6, 8.0 Hz, 1H, H-2^{II}), 4.92-4.83 (m, 1H, H-2), 4.65-4.54 (m, 4H, 2 PhC*H*$_2$), 4.52 (d, *J* = 8.0 Hz, 1H, H-1^{I}), 4.39-4.33 (m, 1H, H-16), 4.31 (d, 1H, *J* = 2.0 Hz, H-4^{I}), 4.23-4.19 (dd, *J* = 12.4, 4.4 Hz, 1H, H-6^{I}a), 4.06-4.10 (dd, *J* = 12.0, 2.0 Hz, 1H, H-6^{I}b), 3.79-3.75 (m, 1H, H-3^{I}), 3.67-3.53 (m, 5H), 3.35-3.47 (m, 2H, H-26), 1.96-2.23 (m, 15H, 5 × Ac), 1.87-0.85 (m, 24H), 0.97 (d, *J* = 7.2 Hz, 3H, Me-21), 0.80 (d, *J* = 6.4 Hz, 3H, Me-27), 0.77 (s, 3H, Me-19), 0.73 (s, 3H, Me-18), 0.63-0.55 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.54, 170.50, 170.23, 170.16, 169.5, 164.9, 137.4, 133.0, 130.3, 129.7, 128.5, 128.4 (d), 128.3, 127.9 (t), 127.8 (d), 127.7, 127.6 (t), 109.3, 100.1, 99.7, 80.8, 80.3, 79.9, 77.2, 73.6, 73.6, 72.7, 72.5, 72.0, 71.72, 71.67, 71.4, 70.3, 69.1, 68.6, 66.7, 62.1, 61.9, 56.1, 54.1, 44.2, 42.4, 41.6, 40.5, 39.9, 37.0, 34.3, 33.4, 32.0, 31.7, 31.4, 30.3, 28.8, 27.6, 21.1 (d), 20.7 (d), 20.6 (d), 17.1, 16.4, 14.5, 12.9; HRMS(ESI): calcd. for $C_{70}H_{90}O_{20}Na^+$[M+Na]$^+$, 1273.5917; found 1273.5915.

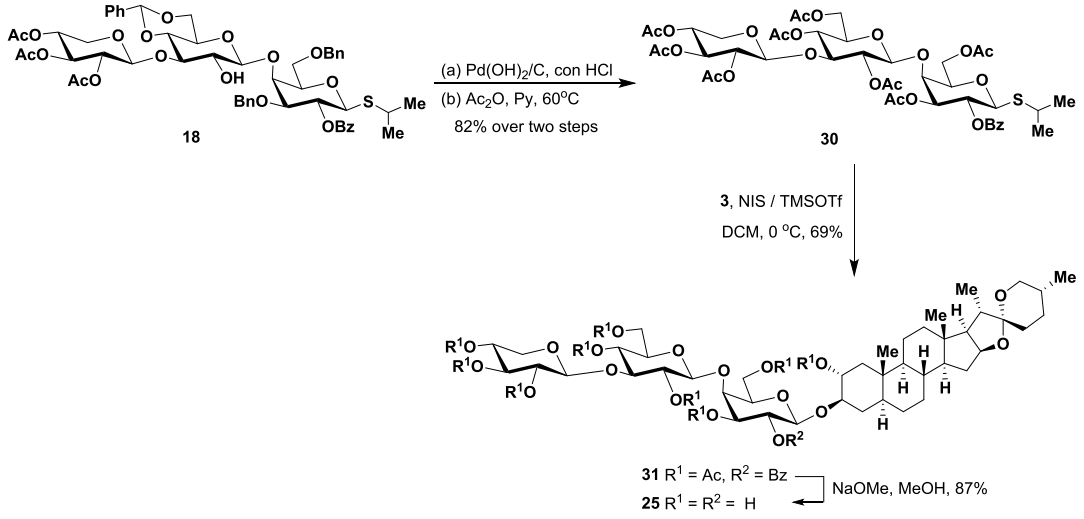
Synthesis of compound 29

The titled compound was prepared from **28** (84 mg, 0.067 mol) by following a similar procedure to that for compound **20** to give **29** as a white foamy solid (68 mg, 95%): $[\alpha]_D^{25}$ = + 39 (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.02-8.01 (m, 2H, Ph), 7.61-7.56 (m, 1H, Ph), 7.48-7.42 (m, 2H, Ph), 5.22 (d, *J* = 9.2 Hz, 1H, H-2^{I}), 5.19-5.00 (m, 3H), 4.91-4.84 (m, 2H), 4.62 (d, *J* = 7.6 Hz, 1H, H-1^{I}), 4.37-4.23 (m, 2H), 4.15 (d, *J* = 2.0 Hz, 1H, H-4^{I}), 4.03 (m, 1H), 4.84-4.73 (m, 3H), 3.68-3.56 (m, 3H), 3.46-3.32 (m, 2H, H-26), 3.03 (*br* s, 2H, OH), 2.24-1.98 (m, 15H, 5 × Ac),

1.88-0.81 (m, 24H), 0.94 (d, *J* = 7.6 Hz, 3H, Me-21), 0.79 (s, 3H, Me-19), 0.77 (d, *J* = 6.4 Hz, 3H, Me-27), 0.72 (s, 3H, Me-18), 0.68-0.59 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.5, 170.4, 170.2, 169.5, 169.4, 167.4, 133.5, 129.8 (d), 129.5, 128.4 (d), 109.3, 101.7, 99.9, 80.8, 80.7, 77.2, 75.5, 74.7, 73.9, 73.4, 73.1, 72.9, 72.1, 71.4, 68.5, 66.9, 65.6, 62.2, 59.6, 56.1, 54.2, 44.3, 42.4, 41.6, 40.5, 39.9, 37.1, 34.3, 33.8, 32.0, 31.7, 31.4, 30.3, 28.8, 27.7, 21.3, 21.2, 20.6 (d), 20.5, 17.1, 16.4, 14.5, 12.9; HRMS(ESI): calcd. for $C_{56}H_{78}O_{20}Na^+$[M+Na]$^+$, 1093.4978; found 1093.4981.

Synthesis of compound 24

The titled compound was prepared from **29** (55 mg, 0.051 mmol) by following a similar procedure to that for compound **1** to give **24** as a white foamy solid (36 mg, 92%): $[\alpha]_D^{25}$ = + 124 (*c* 0.3, CH_3OH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ 6.40-5.48 (*br* s, 8H, OH), 5.22 (d, *J* = 7.6 Hz, 1H, H-1^{II}), 4.86 (d, *J* = 7.6 Hz, 1H, H-1^{I}), 4.63-4.48 (m, 4H), 4.40 (t, *J* = 8.4 Hz, 1H), 4.25-3.91 (m, 8H), 3.93-3.84 (m, 1H), 3.81-3.77 (m, 1H), 3.54-3.41 (m, 2H, H-26), 2.19-2.11 (m, 1H), 1.97-1.82 (m, 2H), 1.78-1.69 (m, 2H), 1.56-0.74 (m, 19H), 1.07 (d, *J* = 7.2 Hz, 3H, Me-21), 0.75 (s, 3H, Me-19), 0.66 (s, 3H, Me-18), 0.63 (d, *J* = 5.2 Hz, 3H, Me-27), 0.59-0.46 (m, 1H); ^{13}C NMR (100 MHz, Pyridine-d_5): δ 109.4, 107.4, 103.7, 84.9, 81.3, 80.3, 78.9, 78.7, 76.1, 75.9, 75.4, 73.3, 72.4, 70.6, 67.0, 65.8, 63.3, 61.1, 56.5, 54.6, 45.9, 44.8, 42.2, 41.0, 40.2, 37.0, 34.8, 34.2, 32.4, 32.3, 32.0, 30.8, 29.4, 28.3, 21.6, 17.5, 16.8, 15.2, 13.6; HRMS(ESI): calcd. for $C_{39}H_{64}O_{14}Na^+$[M+Na]$^+$, 779.4188; found 779.4187.



Scheme S3. Synthesis of trisaccharide analogue **25**

Synthesis of compound 30

The titled compound was prepared from **18** (77 mg, 0.075 mmol) by following a similar procedure to that for compound **20** to give **30** as a white foamy solid (60 mg, 82%): $[\alpha]_D^{25}$ = - 22 (*c* 0.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01-7.99 (m, 2H), 7.60-7.58 (m, 1H), 7.48-7.45 (m, 2H, Ph), 5.41 (t, *J* = 9.6 Hz, 1H, H-2^{I}), 5.15-5.05 (m, 2H), 5.03-4.97 (m, 2H), 4.91-4.86 (m, 1H), 4.83-4.76 (m, 1H), 4.61 (d, *J* = 7.6 Hz, 1H, H-1^{III}), 4.60 (m, 1H), 4.46 (d, *J* = 8.0 Hz, 1H, H-1^{I}), 4.32 (dd, *J* = 12.0, 5.2 Hz, 1H), 4.22-4.08 (m, 5H), 3.89 (t, *J* = 9.2 Hz, 1H), 3.80 (t, *J* =5.2 Hz, 1H), 3.59 (d, *J* = 6.8 Hz, 1H), 3.41-3.36 (m, 1H), 3.26-3.20 (m, 1H, SC*H*), 2.30-2.03 (m, 24H, 8 × Ac),

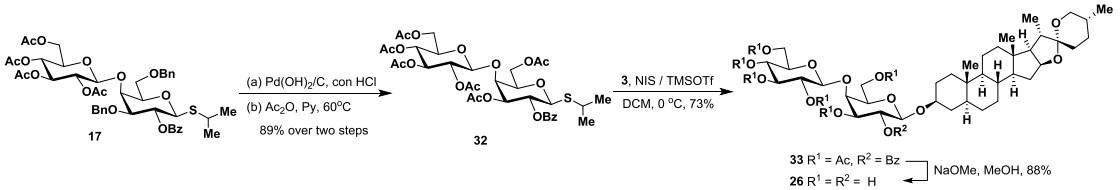
1.30-1.24 (m, 6H, 2 CH_3). ^{13}C NMR (100 MHz, $CDCl_3$): δ 171.1, 170.8, 170.4, 170.3, 170.1, 169.7, 169.5, 169.3, 165.2, 133.5, 130.0 (d), 129.8, 128.7 (d), 101.4, 101.3, 83.4, 80.2, 77.5, 76.0, 74.6, 74.4, 73.0, 71.9, 70.9, 70.3, 68.9, 68.4, 64.0, 62.3, 62.0, 35.2, 24.8, 23.9, 21.11, 21.05, 21.0 (d), 20.96, 20.94 (d), 20.8; HRMS(ESI): calcd. for $C_{43}H_{56}O_{23}SNa^+$[M+Na]$^+$, 995.2825; found 995.2825.

Synthesis of compound 31

The titled compound was prepared from compound **30** (117 mg, 0.12 mmol) and compound **3** (48 mg, 0.10 mmol) by following a similar procedure to that for compound **21** to afford **31** as a white foamy solid (95 mg, 69%): $[\alpha]_D^{25}$ = – 57 (*c* 0.5, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.01-7.98 (m, 2H), 7.60-7.58 (m, 1H), 7.47-7.43 (m, 2H, Ph), 5.26 (dd, *J* = 10.0, 8.0 Hz, 1H, H-2^{I}), 5.11-5.01 (m, 2H), 5.01-4.97 (m, 2H), 4.91-4.80 (m, 3H), 4.63 (d, *J* = 7.6 Hz, 1H, H-1^{III}), 4.59-4.53 (m, 2H), 4.39-4.34 (m, 1H, H-16), 4.30-4.04 (m, 6H), 3.89 (t, *J* = 9.2 Hz, 1H), 3.74 (t, *J* = 5.2 Hz, 1H), 3.62-3.56 (m, 2H), 3.49-3.32 (m, 3H), 2.30-1.94 (m, 27H, 9 × Ac), 1.87-0.82 (m, 24H), 0.94 (d, *J* = 6.8 Hz, 3H, Me-21), 0.78 (d, *J* = 6.4 Hz, 3H, Me-27), 0.77 (s, 3H, Me-19), 0.71 (s, 3H, Me-18), 0.68-0.59 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8, 170.5, 170.5, 170.2, 170.1, 169.9, 169.5, 169.3, 169.2, 165.8, 133.3, 129.7 (t), 128.4 (d), 109.2, 101.2, 100.5, 99.8, 80.8, 80.5, 79.7, 77.3, 73.5, 72.9, 72.7, 72.5, 71.9, 71.8, 70.9, 70.2, 70.0, 68.8, 68.7, 66.8, 63.2, 62.12, 62.08, 61.9, 56.1, 54.1, 44.2, 42.4, 41.6, 40.5, 39.8, 37.0, 34.3, 33.5, 32.0, 31.7, 31.3, 30.3, 28.8, 27.6, 21.1, 20.9 (d), 20.80, 20.77, 20.7 (d), 20.69, 20.5, 17.1, 16.4, 14.5, 12.9. HRMS(ESI): calcd. for $C_{69}H_{94}O_{28}Na^+$[M+Na]$^+$, 1393.5822; found 1393.5822.

Synthesis of compound 25

The titled compound was prepared from compound **31** (45 mg, 0.033 mmol) by following a similar procedure to that for compound **1** to afford **25** as a white foamy solid (26 mg, 87%): $[\alpha]_D^{25}$ = – 42 (*c* 0.7, CH_3OH); ^{1}H NMR (400 MHz, Pyridine-d_5): δ 6.65-6.24 (*br* s, 10H, OH), 5.22 (d, *J* = 7.6 Hz, 1H, H-1^{II}), 5.20 (d, *J* = 7.6 Hz, 1H, H-1^{III}), 4.86 (d, *J* = 7.6 Hz, 1H, H-1^{I}), 4.62 (d, *J* = 2.0 Hz, 1H, H-4^{I}), 4.62-4.45 (m, 3H), 4.37 (t, *J* = 8.8 Hz, 1H), 4.26-4.03 (m, 9H), 4.97-4.88 (m, 4H), 3.80-3.77 (m, 1H), 3.66-3.61 (m, 1H), 3.56-3.46 (m, 2H, H-26), 2.18-0.72 (m, 24H), 1.08 (d, *J* =6.8 Hz, 3H, Me-21), 0.77 (s, 3H, Me-19), 0.67 (s, 3H, Me-18), 0.65 (d, *J* = 4.8 Hz, 3H, Me-27), 0.61-0.51 (m, 1H); ^{13}C NMR (100 MHz, Pyridine-d_5): δ 109.4, 106.7, 106.1, 103.7, 87.3, 84.9, 81.3, 80.1, 78.3, 78.3, 75.9, 75.4, 75.2, 75.1, 73.1, 71.1, 70.6, 70.2, 67.5, 67.0, 63.2, 62.9, 61.0, 56.5, 54.6, 52.2, 45.9, 44.8, 42.2, 40.9, 40.2, 37.0, 34.8, 34.2, 32.4, 32.0, 30.8, 29.4, 28.3, 21.6, 17.5, 16.8, 15.2, 13.6; HRMS(ESI): calcd. for $C_{44}H_{72}O_{18}Na^+$[M+Na]$^+$, 911.4610; found 911.4613.



Scheme S4. Synthesis of disaccharide analogue **24**

Synthesis of compound 32

The titled compound was prepared from **17** (93 mg, 0.11 mmol) by following a similar procedure to that for compound **20** to give **32** as a colorless syrup (83 mg, 89%): $[\alpha]_D^{25}$ = + 6 (c 0.6, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, *J* = 7.6 Hz, 2H), 7.59-7.55 (m, 1H), 7.43 (d, *J* = 7.2 Hz, 2H, Ph), 5.41 (t, *J* = 10.0 Hz, 1H, H-2^{I}), 5.23 (t, *J* = 9.2 Hz, 1H, H-3^{II}), 5.15-5.02 (m, 3H, H-3^{I}, H-2^{II} and H-4^{II}), 4.61 (d, *J* = 9.6 Hz, 1H, H-1^{I}), 4.56 (d, *J* = 8.0 Hz, 1H, H-1^{II}), 4.30 (dd, *J* = 12.0, 4.8 Hz, 1H, H-6a^{I}), 4.23-4.09 (m, 4H, H-4^{I}, H-6a/b^{II} and H-6b^{I}), 3.80 (t, *J* = 5.6 Hz, 1H, H-5^{I}), 3.64 (dt, *J* = 6.8, 3.6 Hz, 1H, H-5^{II}), 3.20 (p, *J* = 6.4 Hz, 1H, SC*H*), 2.20 (s, 3H), 2.09 (s, 3H), 2.05-2.01 (12H, 4 × Ac), 1.28 (d, *J* = 6.8 Hz, 3H, CH_3), 1.24 (d, *J* = 6.8 Hz, 3H, CH_3). ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.6, 170.4, 170.3, 169.5, 169.4, 165.0, 133.3, 129.8 (d), 129.5, 128.5 (d), 101.2, 83.3, 75.8, 74.4, 74.3, 72.5, 71.7, 71.3, 68.5, 68.1, 63.6, 61.8, 35.1, 24.6, 23.7, 20.9, 20.8 (d), 20.7, 20.6 (d). HRMS(ESI): calcd. for $C_{34}H_{44}O_{17}SNa^+$ $[M+Na]^+$, 779.2191; found 779.2190.

Synthesis of compound 33

The titled compound was prepared from **32** (30 mg, 0.040 mmol) by following a similar procedure to that for compound **21** to give **33** as a white foamy solid (32 mg, 73%): $[\alpha]_D^{25}$ = - 9 (c 0.7, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (dd, *J* = 8.0, 1.2 Hz, 2H), 7.58-7.54 (m, 1H), 7.43 (d, *J* = 7.6 Hz, 2H), 5.30 (dd, *J* = 10.4, 8.0 Hz, 1H, H-2^{I}), 5.23 (t, *J* = 9.6 Hz, 1H, H-3^{II}), 5.09-5.00 (m, 3H, H-3^{I}, H-2^{II} and H-4^{II}), 4.63 (d, *J* = 8.0 Hz, 1H, H-1^{I}), 4.56 (d, *J* = 8.0 Hz, 1H, H-1^{II}), 4.39-4.29 (m, 2H, H-6a^{I} and H-16), 4.23 (dd, *J* = 11.6, 6.8 Hz, 1H, H-6b^{I}), 4.17-4.09 (m, 3H, H-4^{I}, H-6a/b^{II} and H-6b^{I}), 3.75 (t, *J* = 6.0 Hz, 1H, H-5^{I}), 3.63 (ddd, *J* = 10.0, 4.0, 2.8 Hz, 1H, H-5^{II}), 3.56-3.43 (m, 2H, H-3 and H-26a), 3.35 (t, *J* = 11.2 Hz, 1H, H-26b), 2.20 (s, 3H), 2.08 (s, 3H), 2.05 (s, 3H), 2.02-2.01 (9H, 3 × Ac), 1.93 (ddd, *J* = 12.0, 7.6, 5.2 Hz, 1H), 1.87-1.80 (m, 2H), 1.74-0.75 (m, 23H), 0.94 (d, *J* = 6.4 Hz, 3H, Me-21), 0.77 (d, *J* = 6.4 Hz, 3H, Me-23), 0.71 (s, 3H, Me-19), 0.66 (s, 3H, Me-18), 0.61-0.55 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 170.8, 170.5, 170.5, 170.4, 169.6, 169.3, 164.9, 133.2, 129.8 (d), 129.7, 128.4 (d), 109.3, 101.2, 99.8, 80.9, 79.5, 77.3, 74.3, 73.3, 72.6, 71.8, 71.7, 71.4, 69.9, 68.5, 66.9, 63.3, 62.2, 61.8, 56.3, 54.3, 44.7, 41.6, 40.6, 40.0, 36.9, 35.6, 35.1, 34.7, 32.2, 31.8, 31.4, 30.3, 29.1, 28.8, 28.6, 21.0, 20.9, 20.8 (d), 20.7, 20.6, 17.2, 16.5, 14.5, 12.2; HRMS (ESI): calcd. for $C_{58}H_{80}O_{20}Na^+$ $[M + Na]^+$, 1119.5135; found 1119.5133.

Synthesis of compound 26

The titled compound was prepared from compound **31** (18 mg, 0.016 mmol) by following a similar procedure to that for compound **1** to afford **26** as a white foamy solid (11 mg, 87%): $[\alpha]_D^{25}$ = - 41 (c 0.4, CH_3OH). ^{1}H NMR (400 MHz, Pyridine-d_5) δ 6.32-5.90 (*br* s, 7H, OH), 5.29 (d, *J* = 8.0 Hz, 1H, H-1^{I}), 4.89 (d, *J* = 7.6 Hz, 1H, H-1^{II}), 4.76-4.64 (m, 2H), 4.63-4.49 (m, 2H), 4.39 (dd, *J* = 9.2, 7.2 Hz, 1H, H-3^{II}), 4.33-4.17 (m, 4H), 4.17-3.99 (m, 4H), 3.96-3.88 (m, 1H), 3.62-3.46 (m, 3H, H-3, H-26a and H-26b), 2.09-1.91 (m, 3H), 1.83-1.76 (m, 2H), 1.73-1.50 (m, 9H), 1.46-0.73 (m, 14H), 1.13 (d, *J* = 6.8 Hz, 3H, Me-21), 0.69 (d, *J* = 5.2 Hz, 3H, Me-23), 0.64 (s, 3H, Me-18), 0.52 (t, *J* = 6.8 Hz, 1H). ^{13}C NMR (100 MHz, Pyridine-d_5) δ 109.0, 106.9, 102.3,

80.9, 79.9, 78.5, 78.3, 76.9, 75.7, 75.2, 75.0, 73.3, 72.1, 66.6, 62.9, 62.8, 60.8, 56.2, 54.2, 44.4, 41.7, 40.5, 39.9, 36.9, 35.6, 35.0, 34.6, 32.2, 31.9, 31.6, 30.4, 29.8, 29.0, 28.7, 21.0, 17.1, 16.4, 14.8, 12.0. HRMS (ESI): calcd. for $C_{39}H_{64}O_{13}SNa^{+}[M+Na]^{+}$, 763.4239; found 763.4237.

2. Bioassay experiment of gitonin and its derivatives

Cell Culture Assay: Cells were maintained in the RPMI 1640 medium containing 10% fetal bovine serum supplemented with *L*-glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. The leukemia cells were washed and re-suspended in the above medium to 3×10^4 cells/mL, and 196 μL of this cell suspension was placed in each well of a 96-well flat-bottom plate. The cells were incubated in 5% CO_2/air for 24 h at 37 ℃. After incubation, 4 μL of EtOH-H_2O (1:1) solution containing the sample was added to give the final concentrations of 0.01-10 μg/mL; 4 μL of EtOH-H_2O (1:1) was added into control wells. The cells were further incubated for 72 h in the presence of each agent, and then cell growth was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay procedure. The MTT assay was carried out according to a modified method of Sargent and Tayler as follows. After termination of the cell culture, 10 μL of 5 mg/mL MTT in phosphate buffered saline was added to every well and the plate was further re-incubated in 5% CO_2/air for 4 h at 37 ℃. The plate was then centrifuged to precipitate cells and formazan. An aliquot of 150 μL of the supernatant was removed from every well, and 175 μL of DMSO was added to dissolve the formazan crystals. The plate was mixed on a microshaker for 10 min and then read on a microplate reader at 550 nm. A dose-response curve was plotted and the concentration giving 50% inhibition (IC_{50}) was calculated.

3. Copies of NMR Spectra

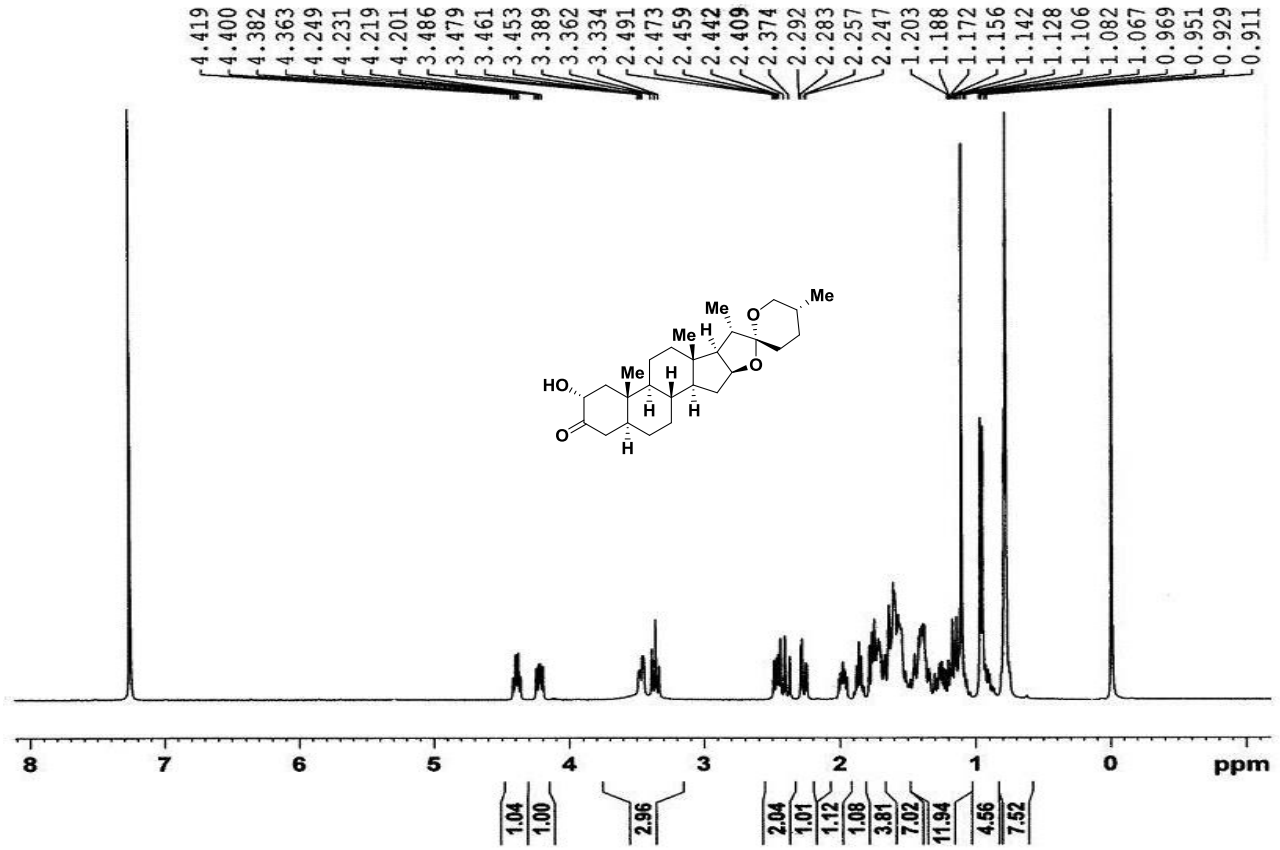


Figure 1. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **11**

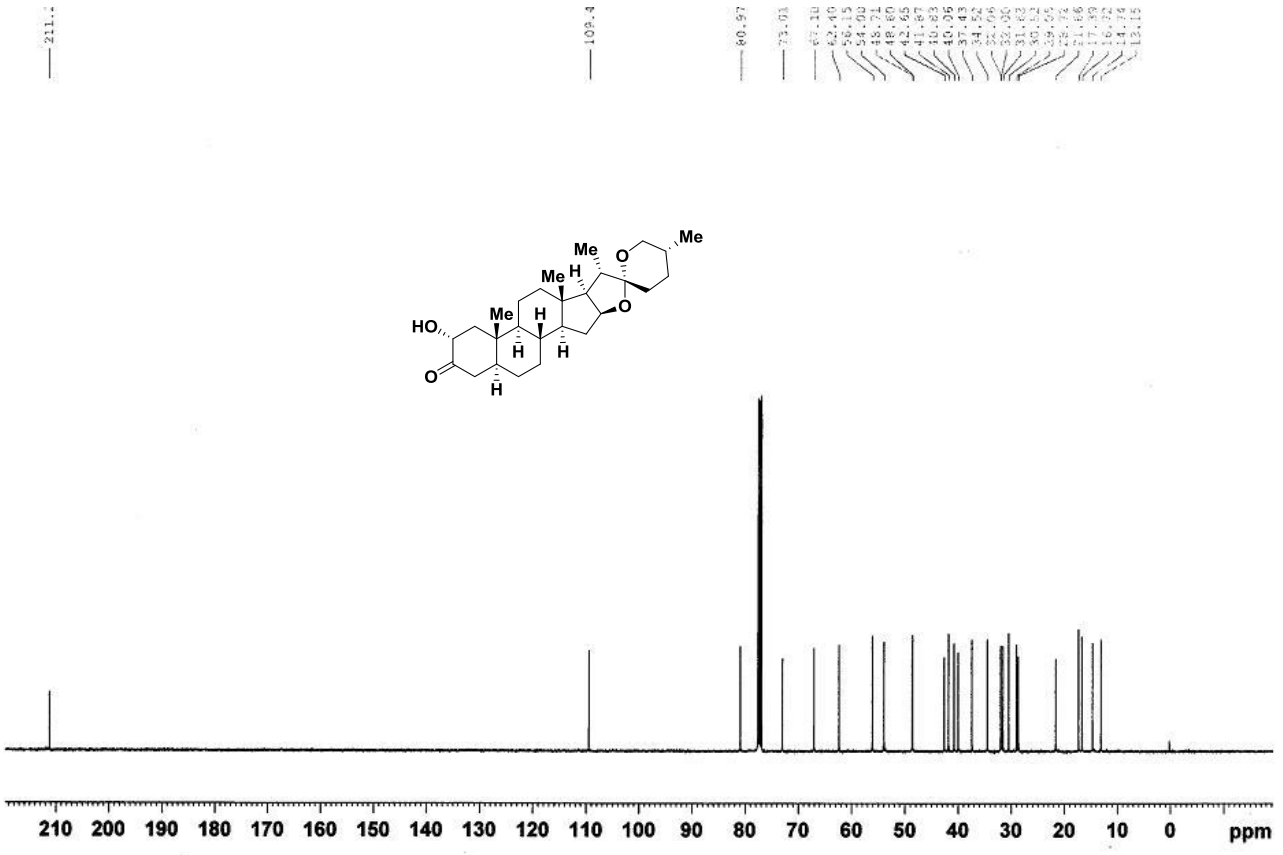


Figure 2. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **11**

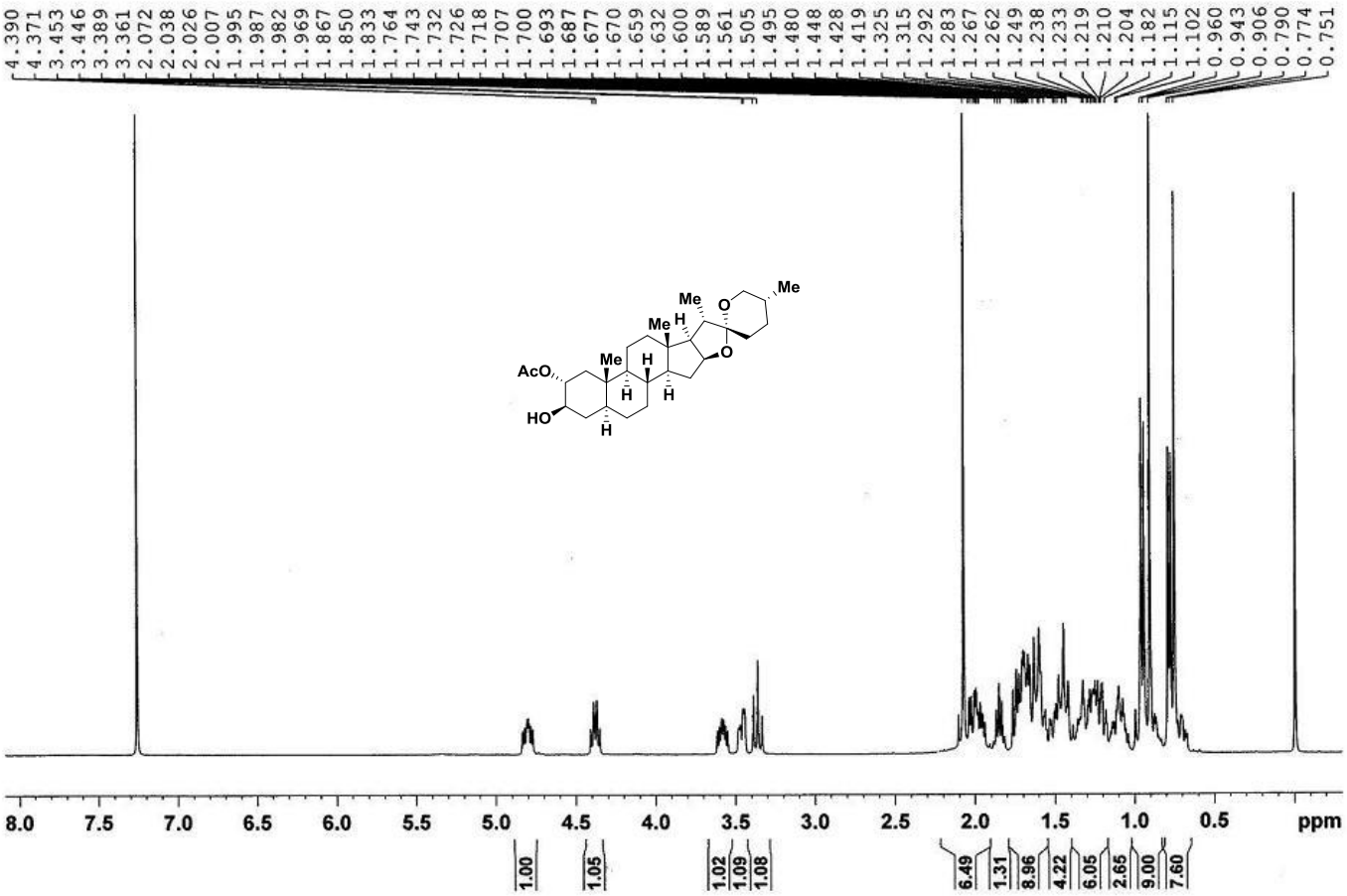


Figure 3. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **3**

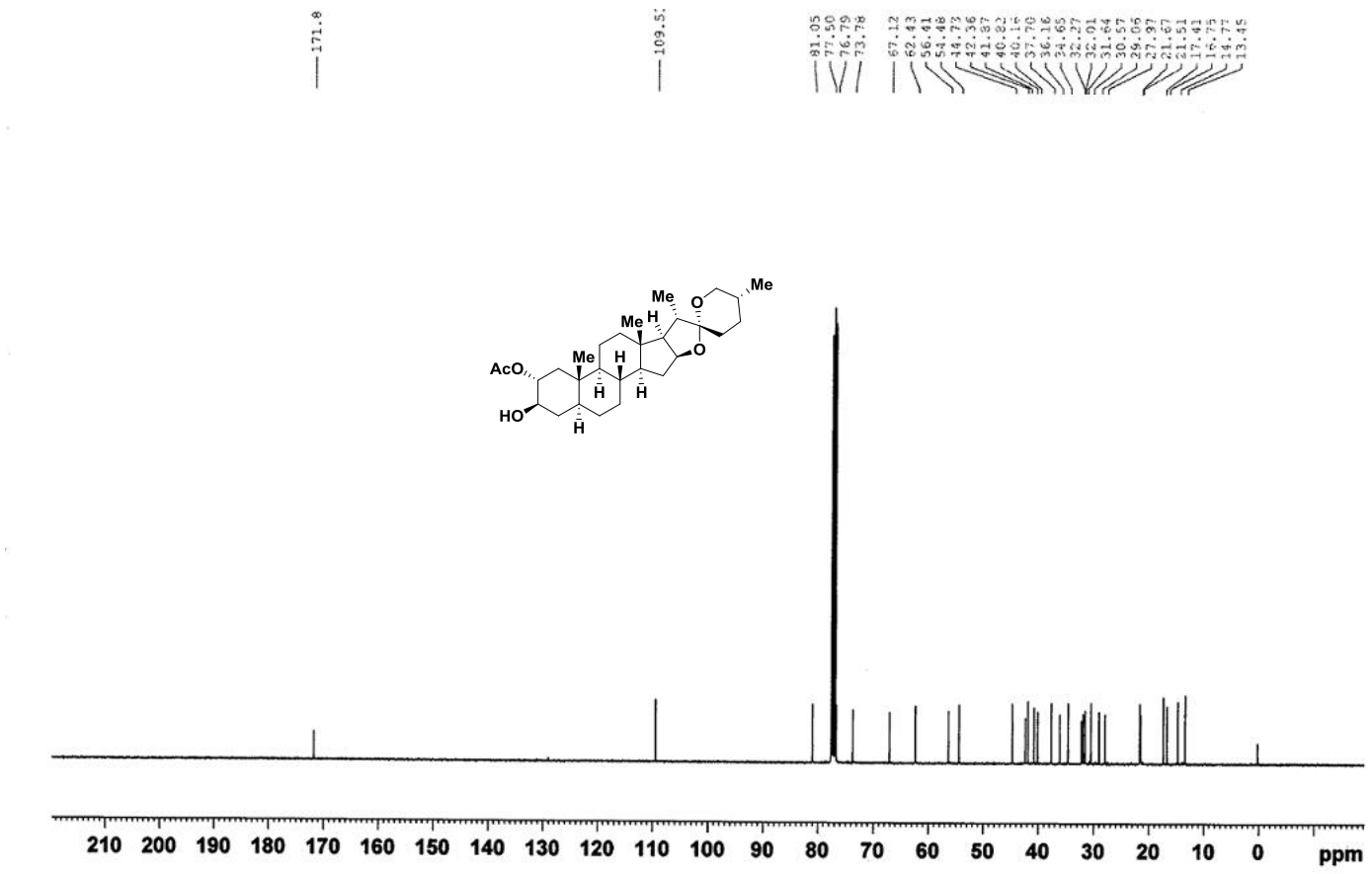


Figure 4. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **3**

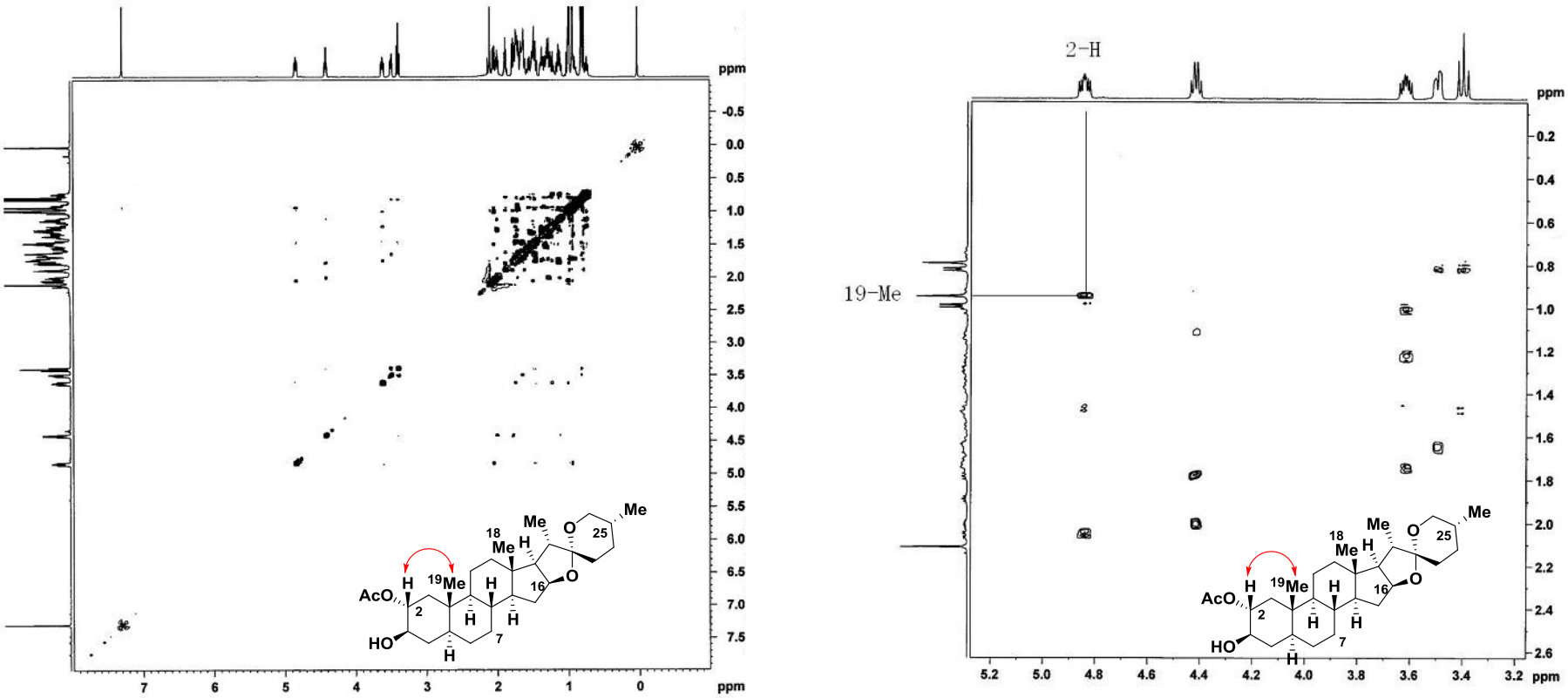


Figure 5. NOESY (400 MHz, $CDCl_3$) spectrum of compound **3**

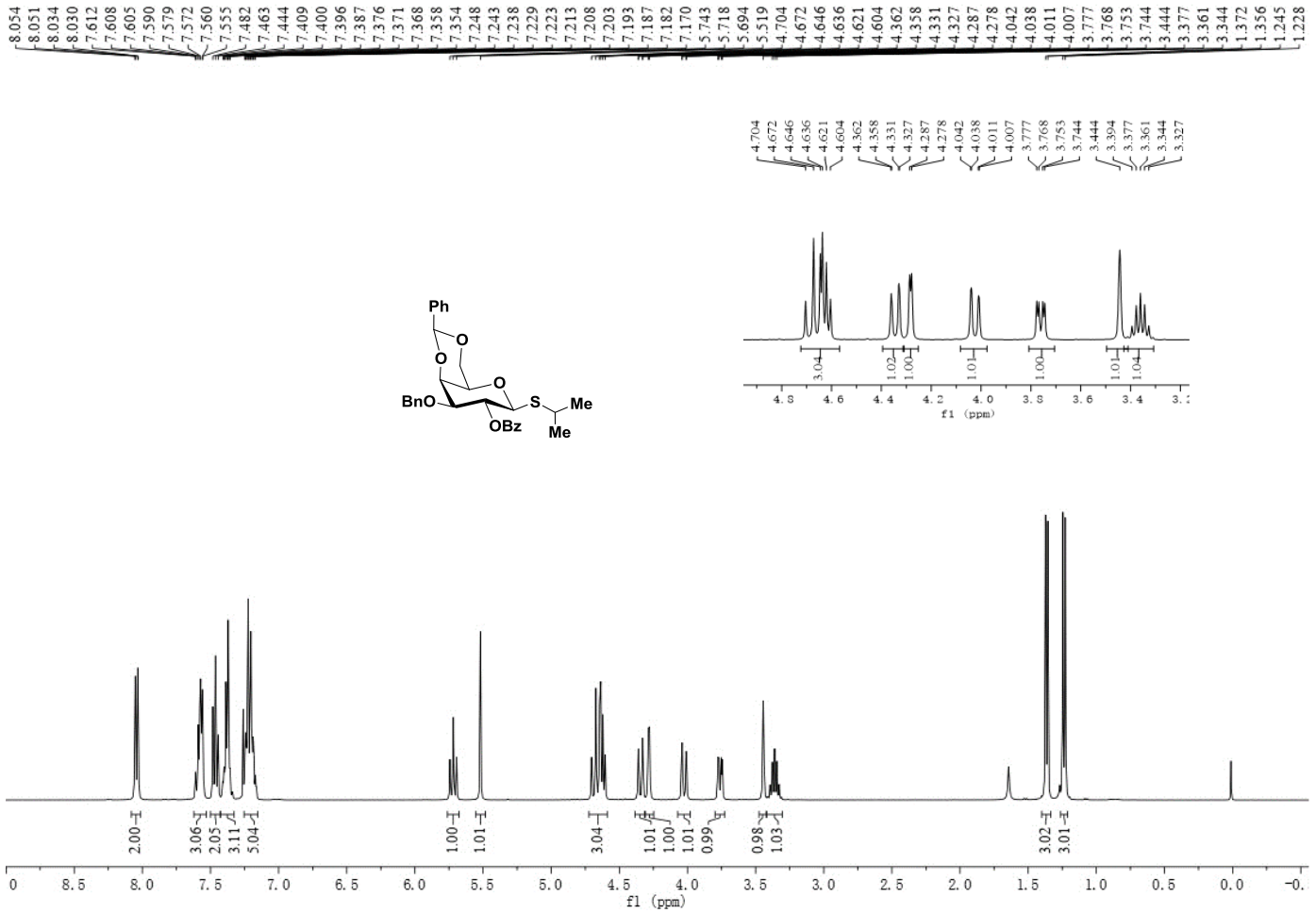


Figure 6. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **14**

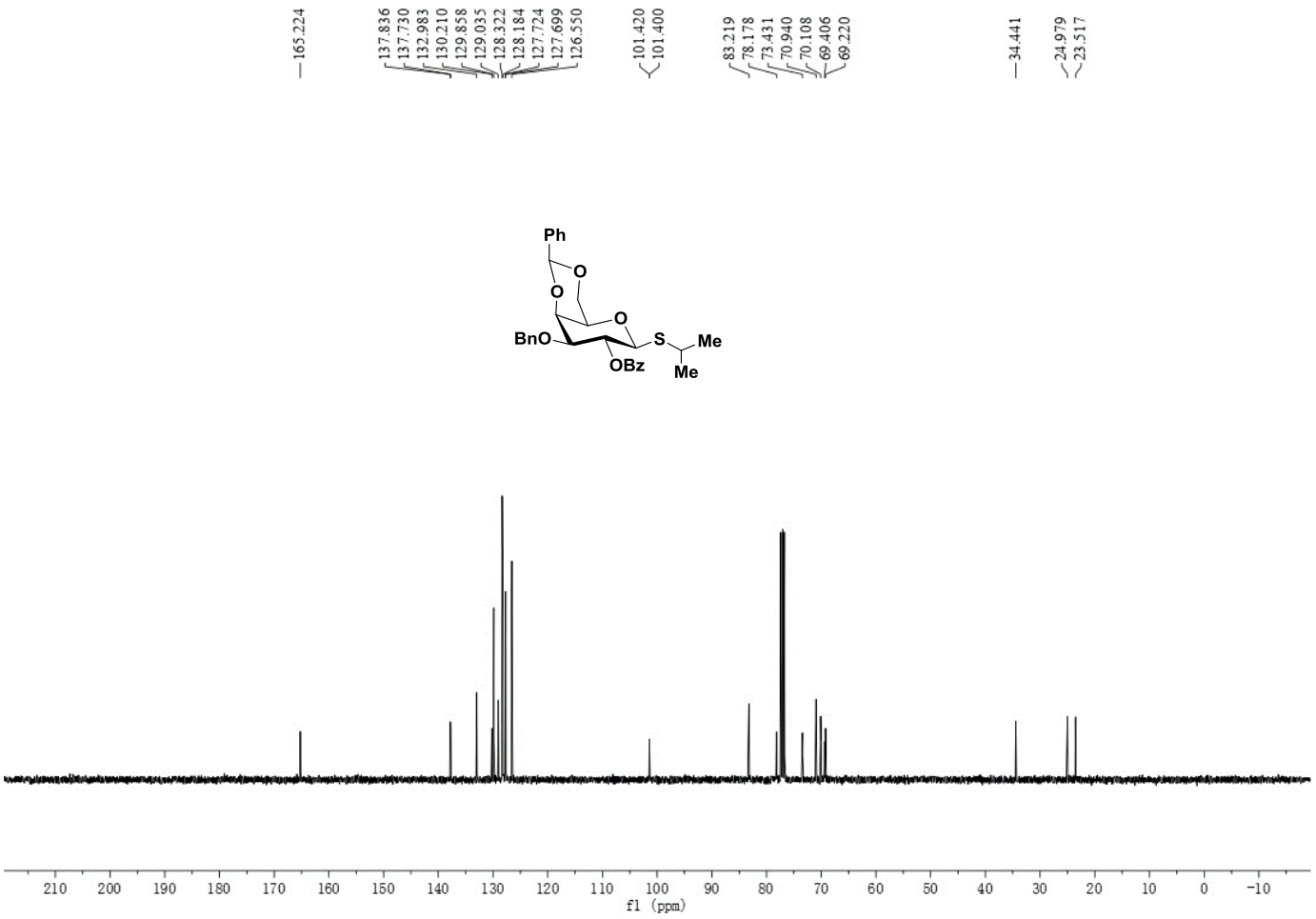


Figure 7. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **14**

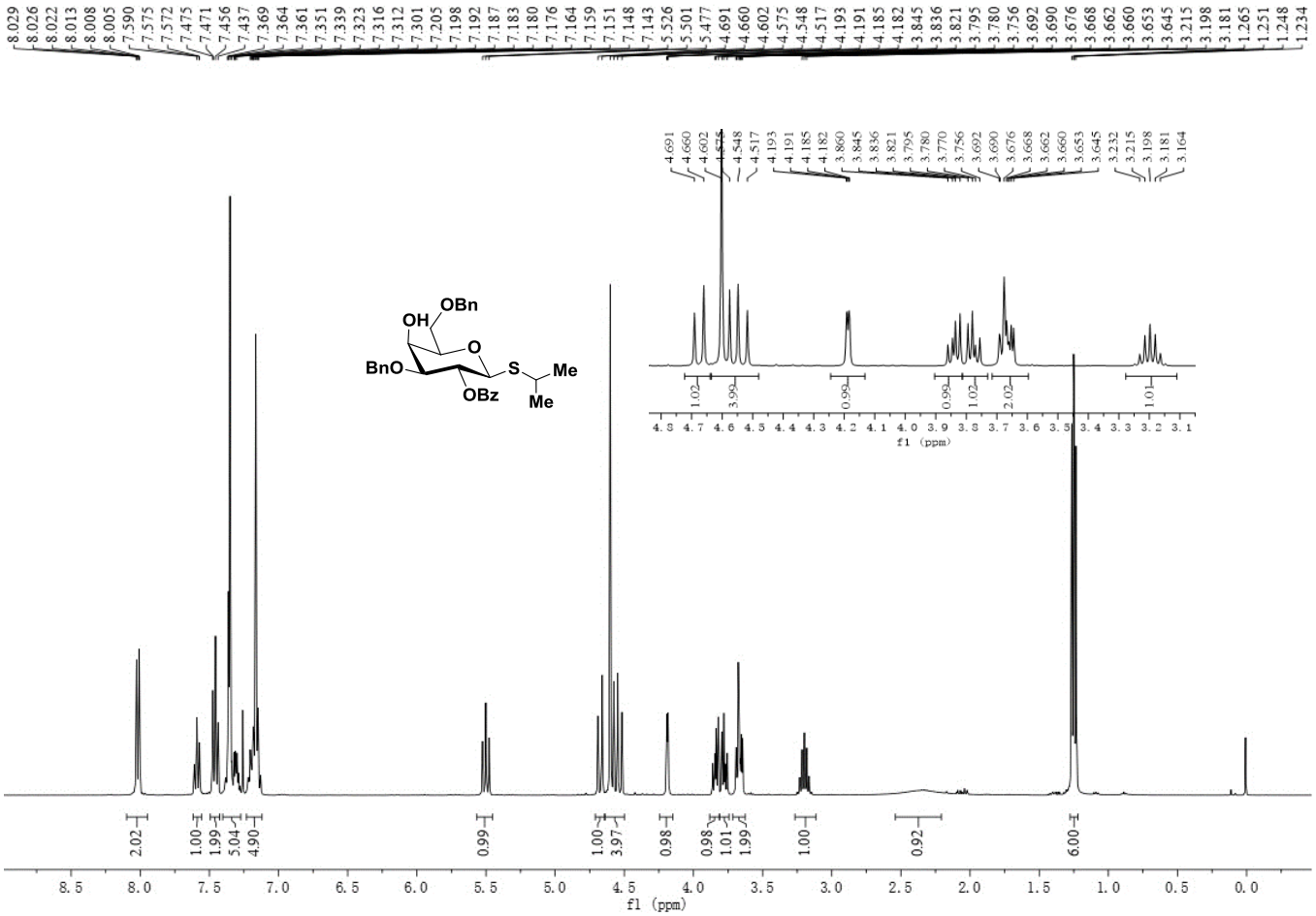


Figure 8. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **15**

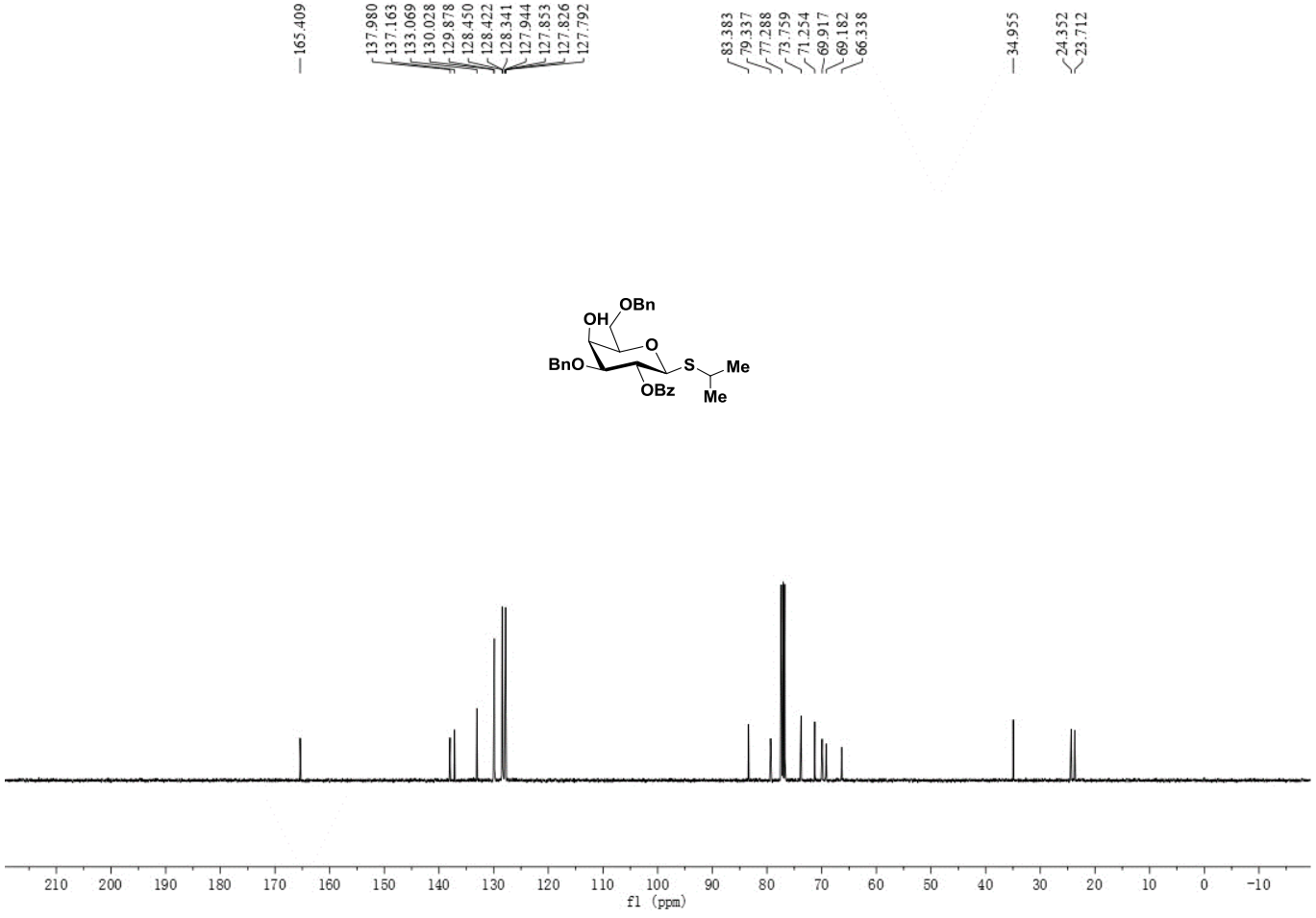


Figure 9. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **15**

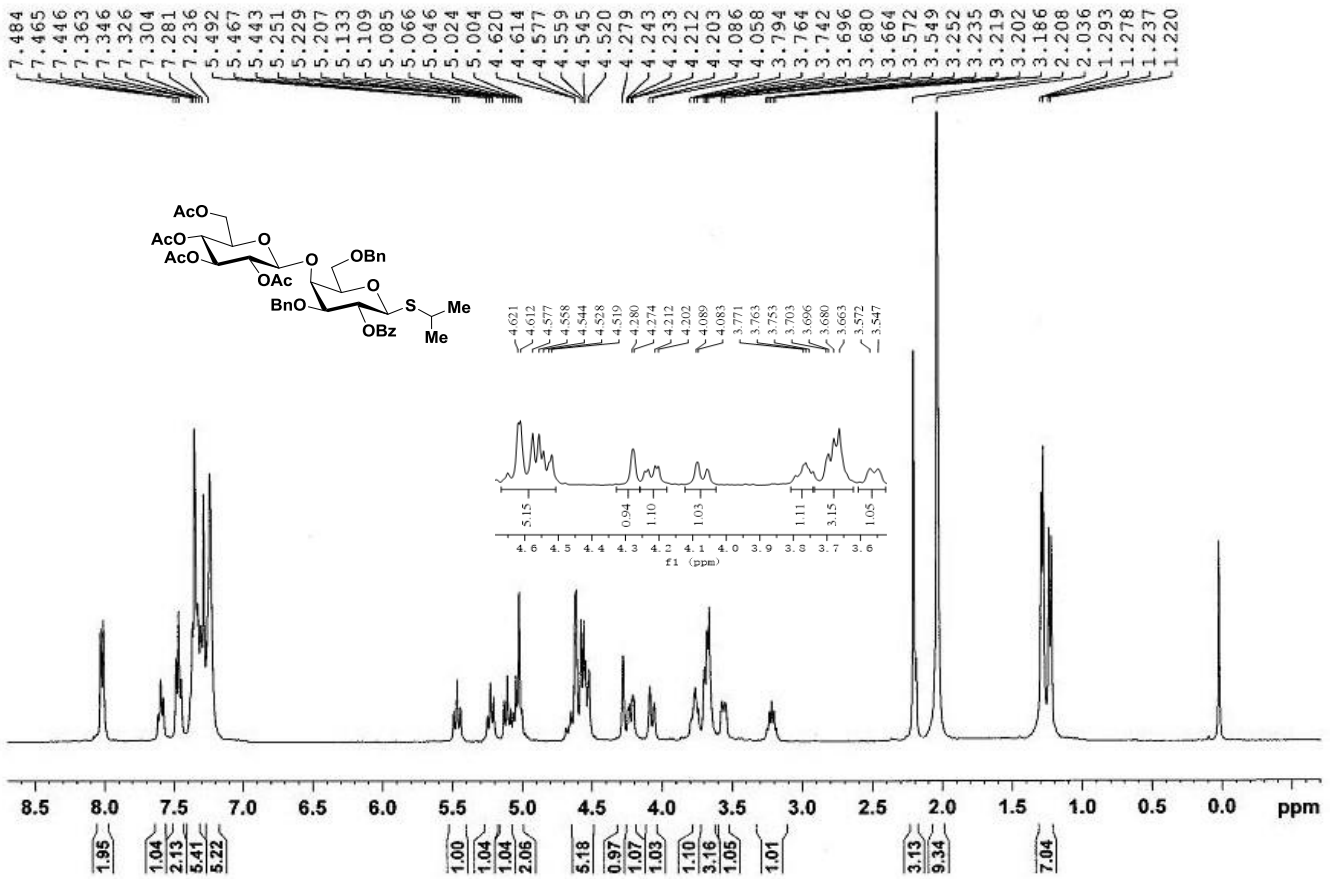


Figure 10. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **17**

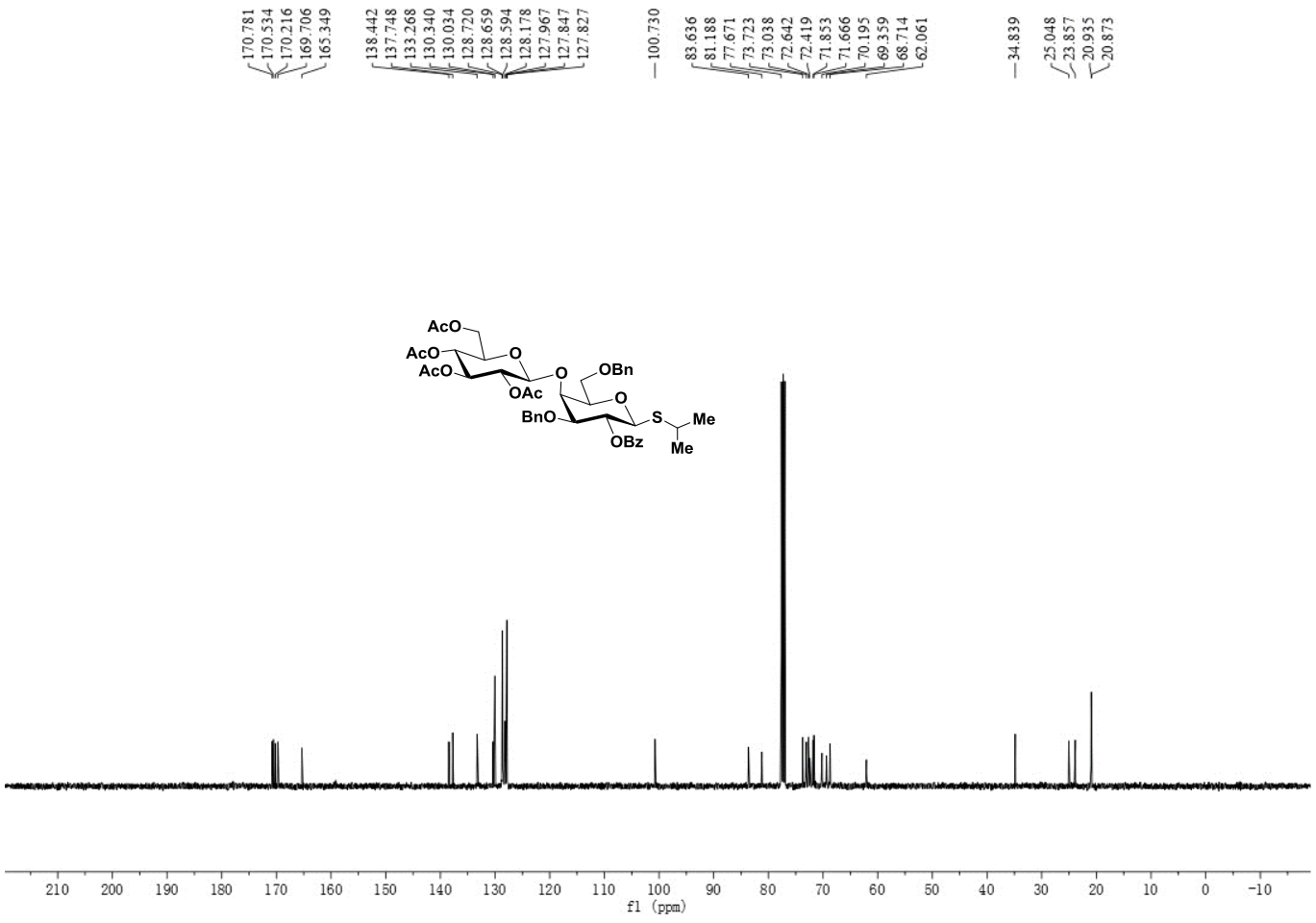


Figure 11. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **17**

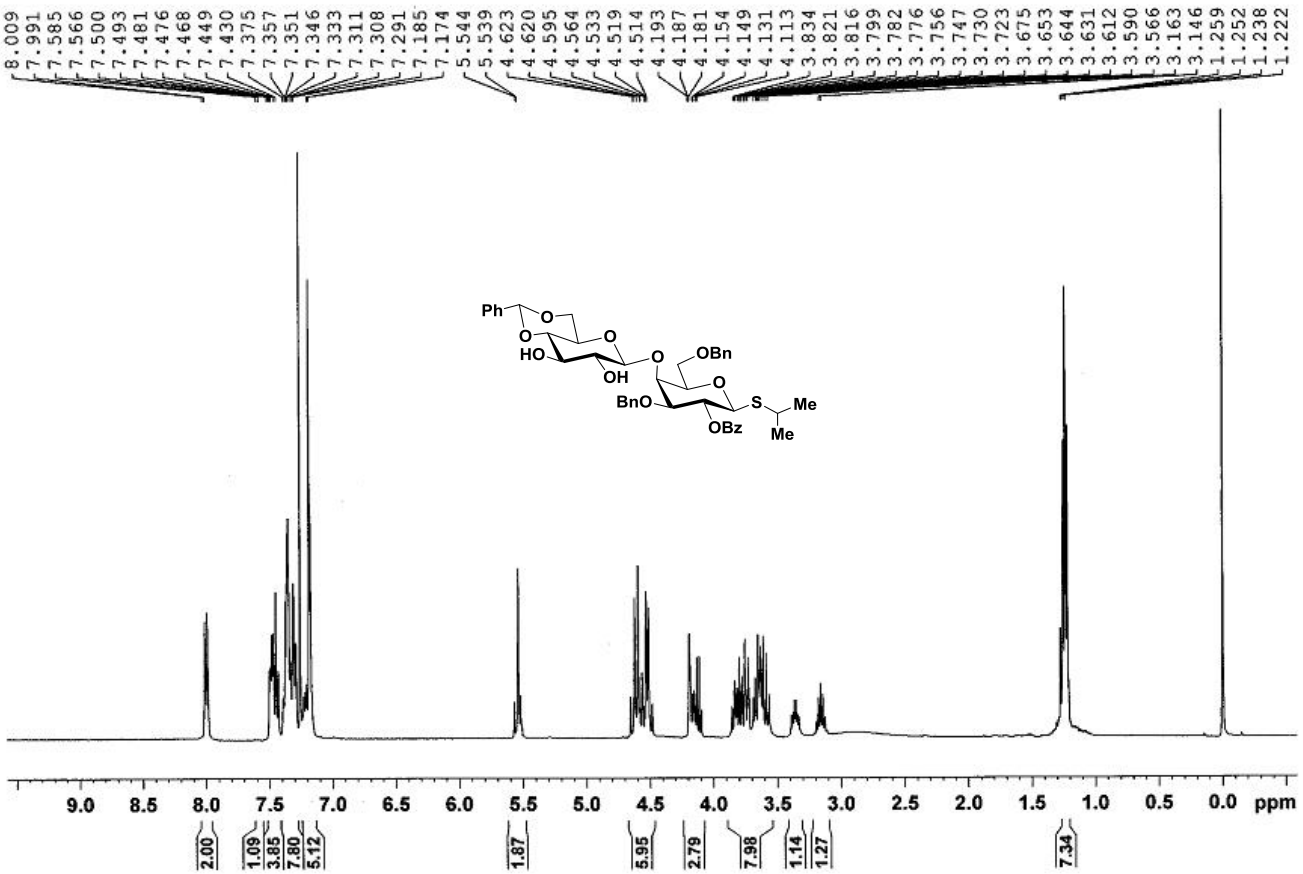


Figure 12. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **4**

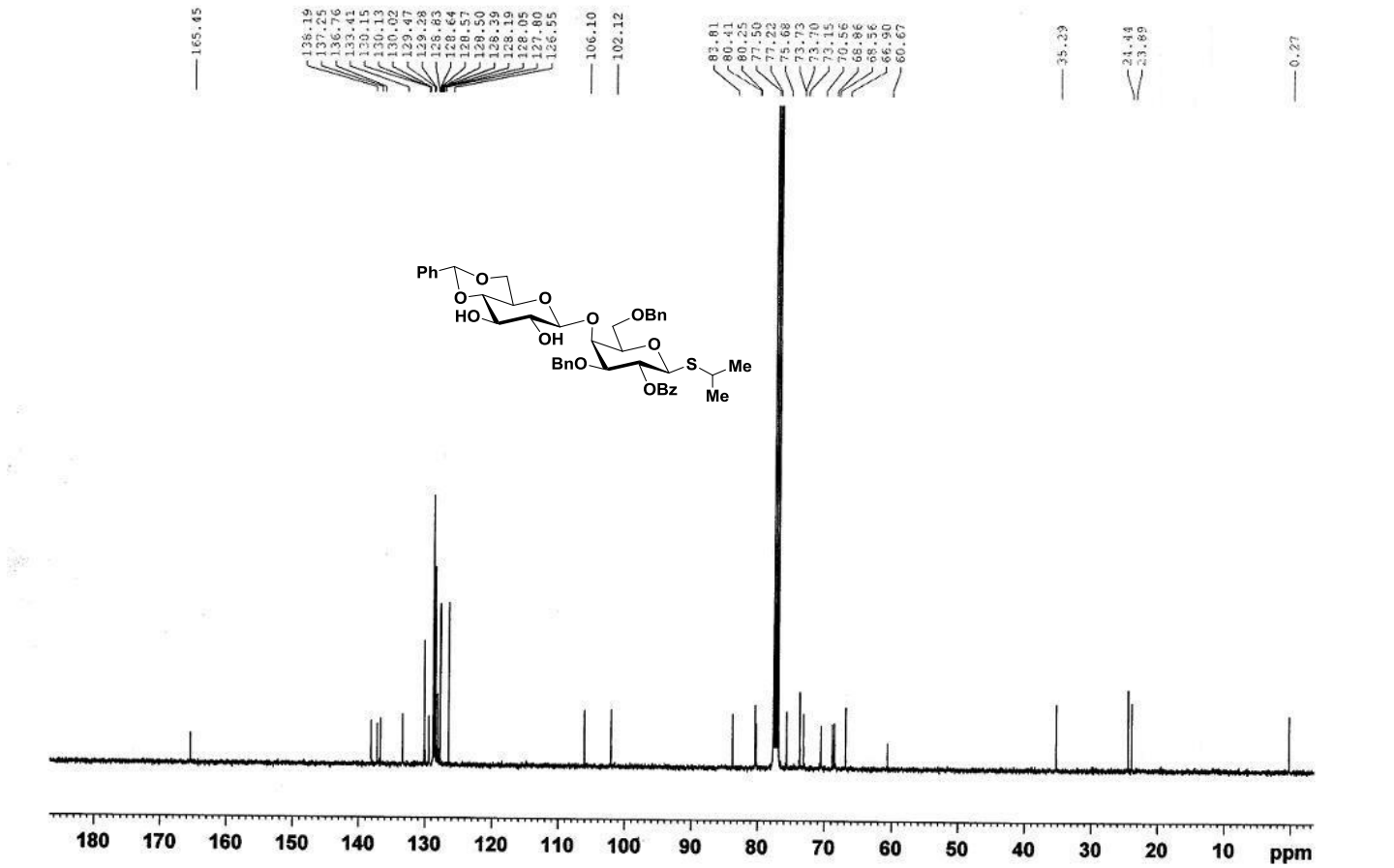


Figure 13. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **4**

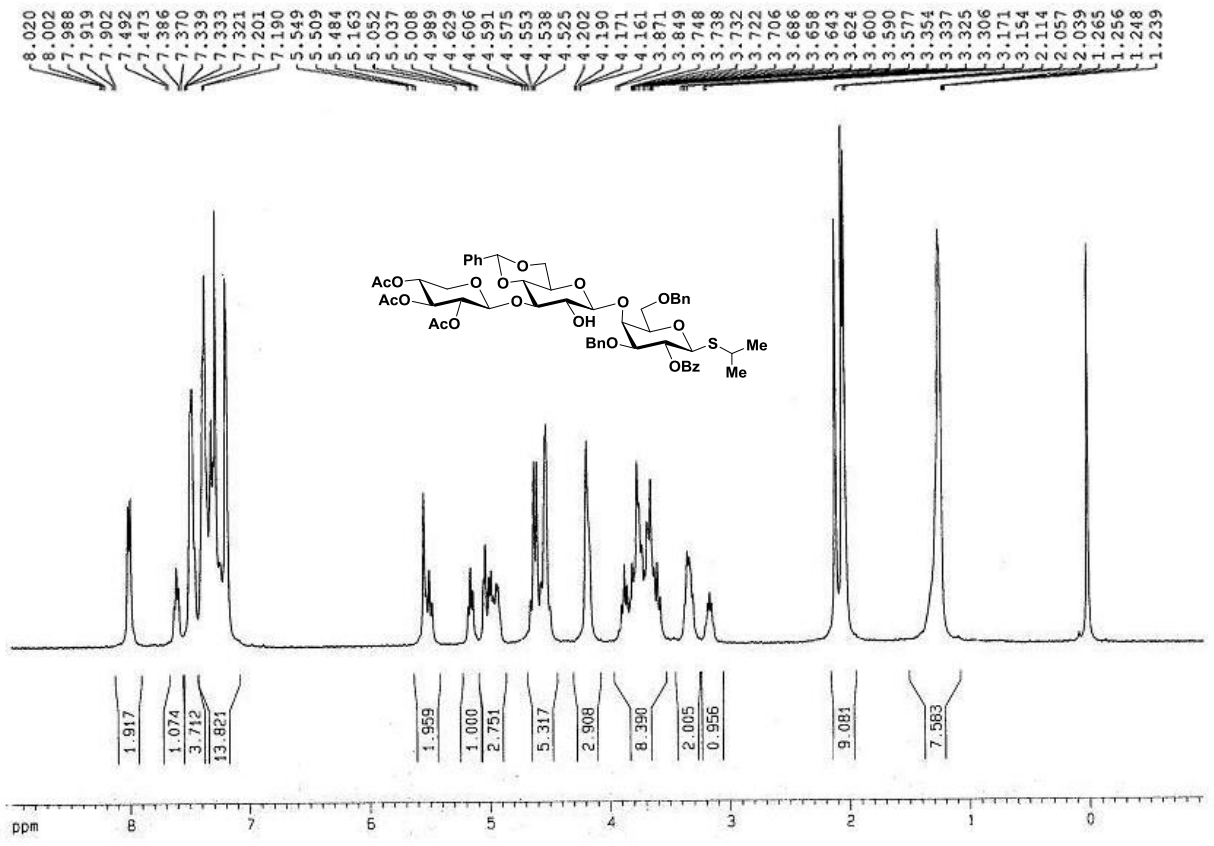


Figure 14. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **18**

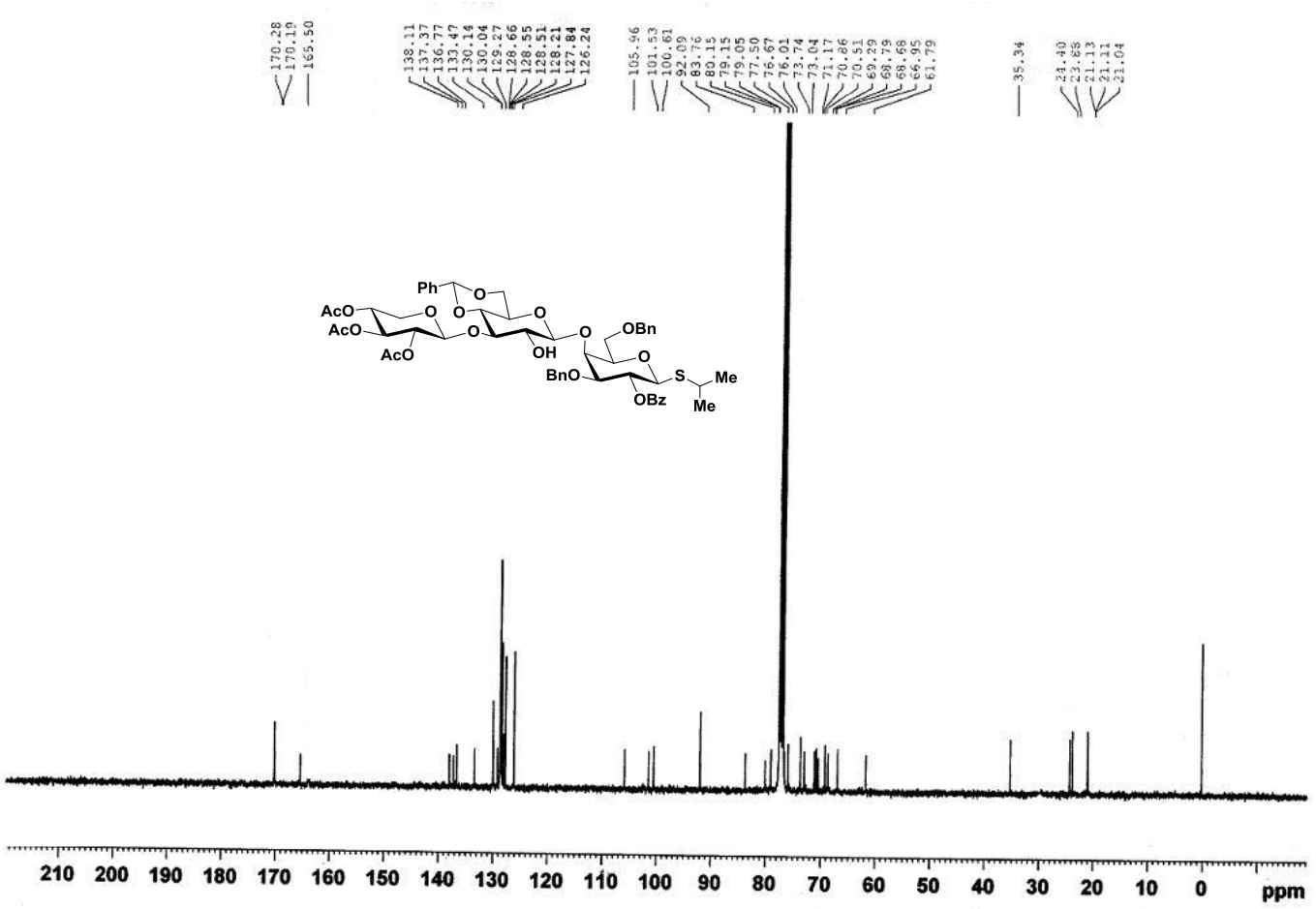


Figure 15. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **18**

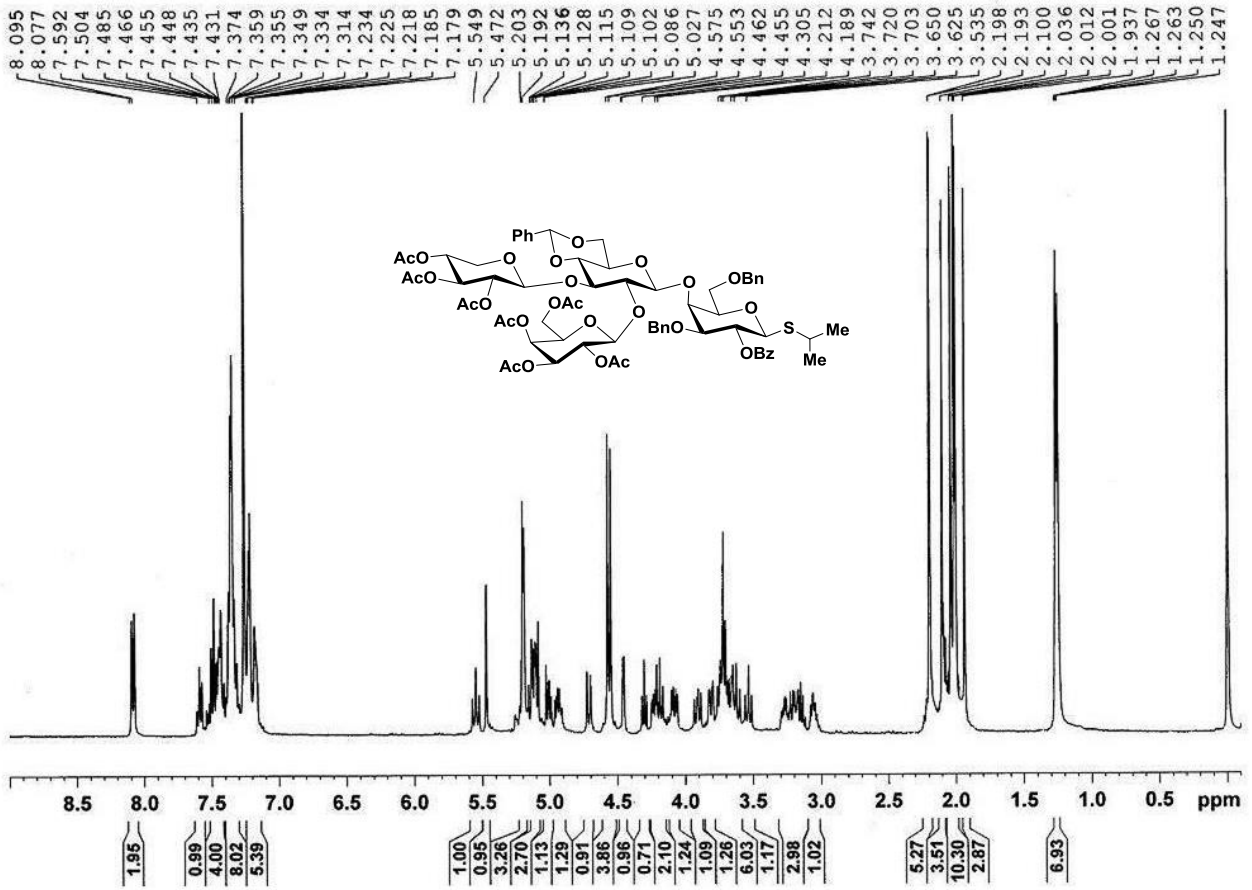


Figure 16. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **2**

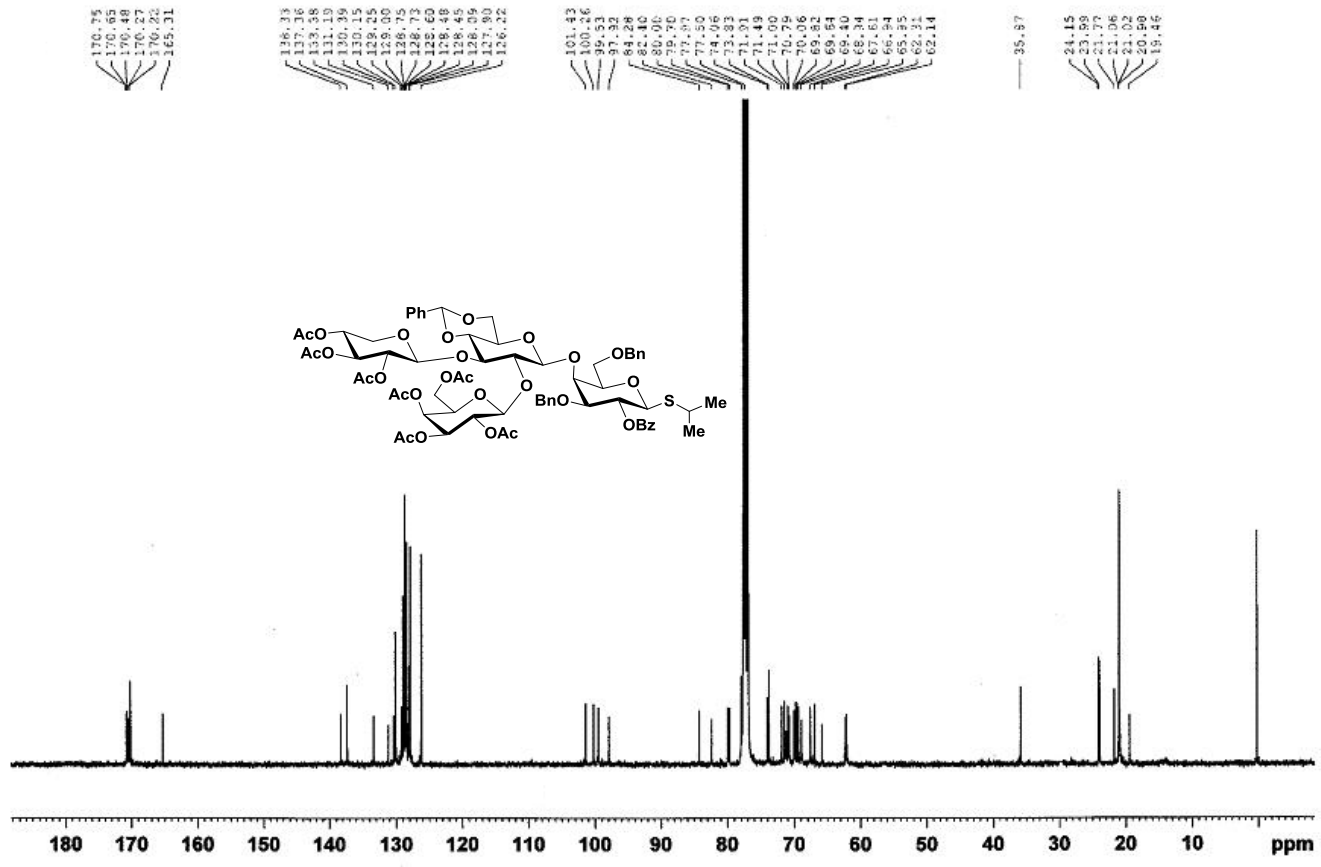


Figure 17. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **2**

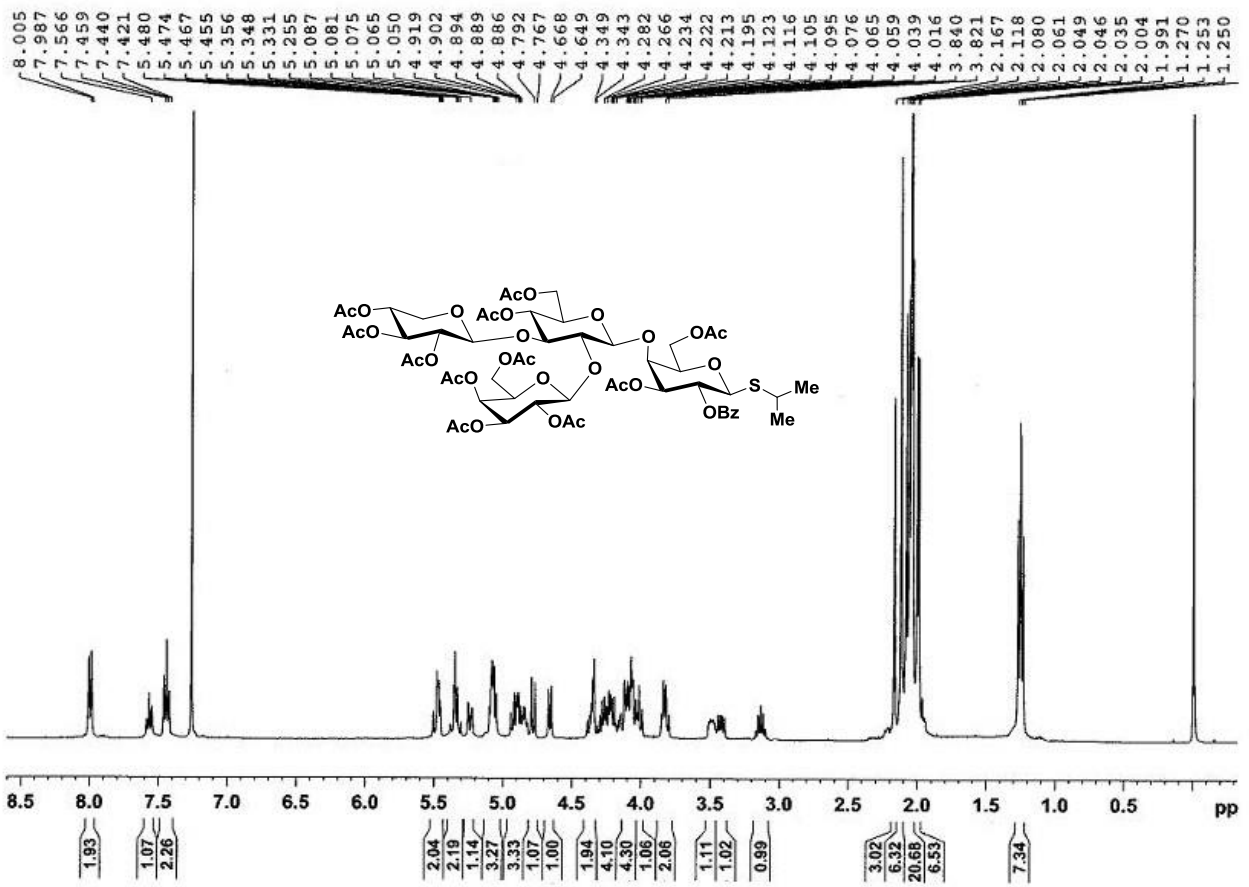


Figure 18. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **20**

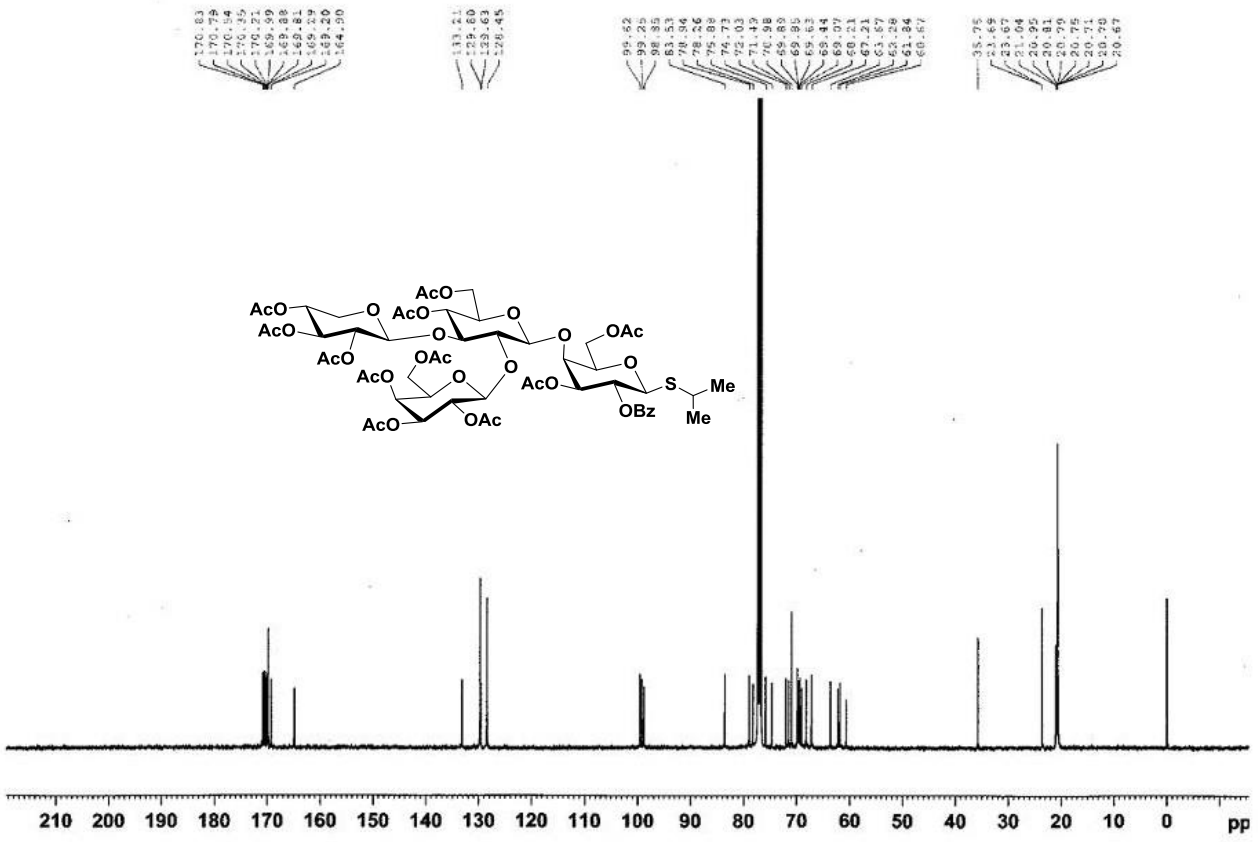


Figure 19. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **20**

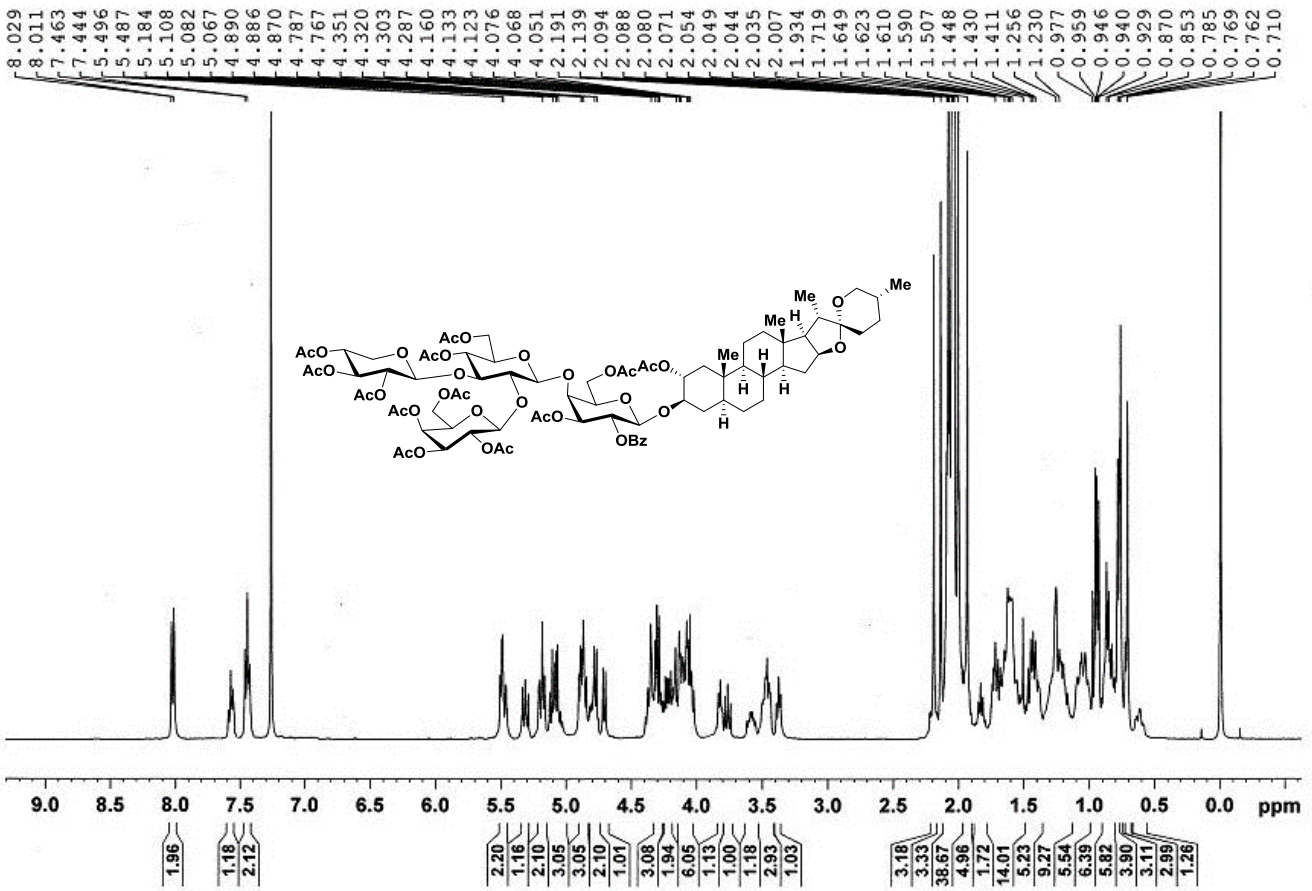


Figure 20. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **21**

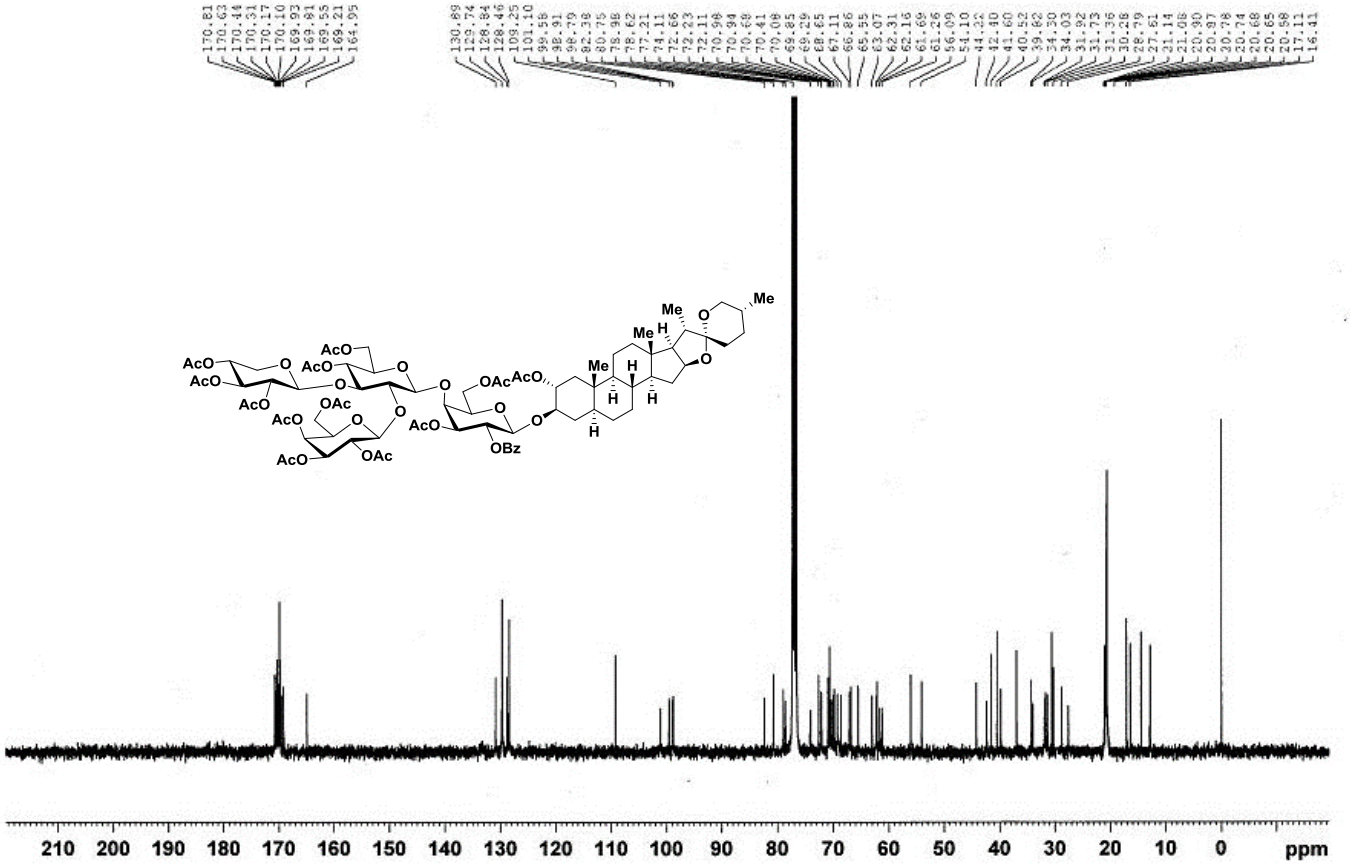


Figure 21. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **21**

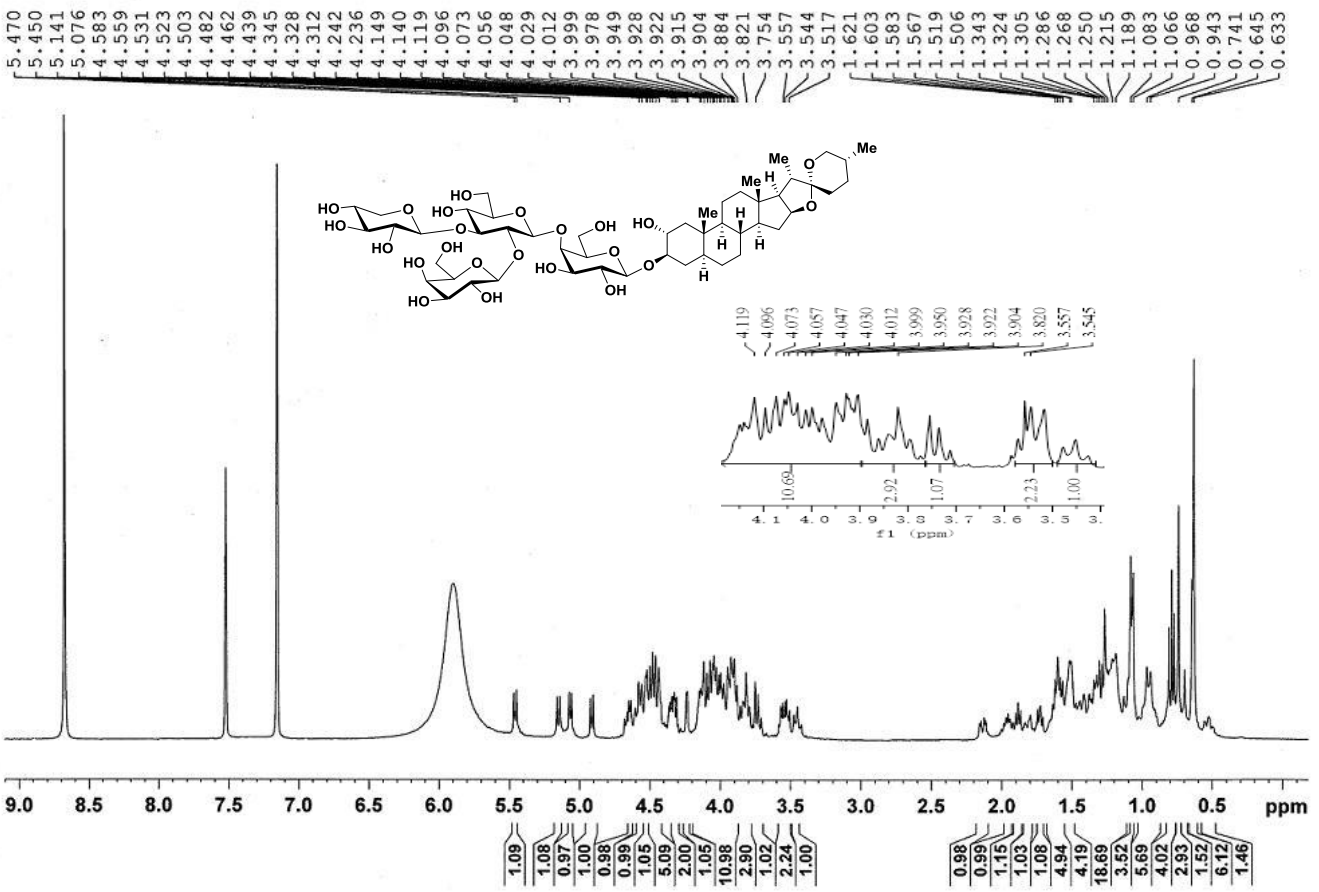


Figure 22. ^{1}H NMR (400 MHz, Pyridine-d_5) spectrum of Gitonin (**1**)

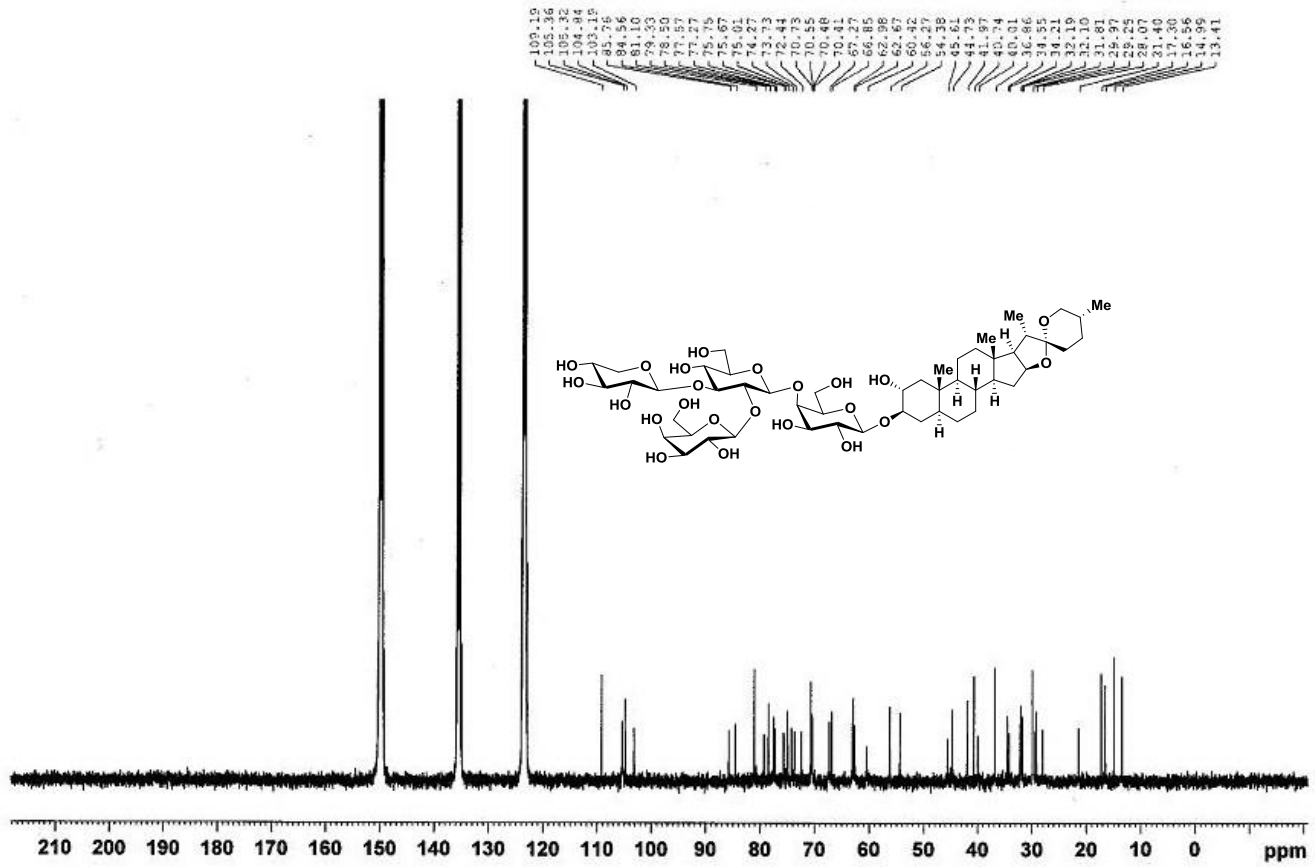


Figure 23. ^{13}C NMR (100 MHz, Pyridine-d_5) spectrum of Gitonin **1**

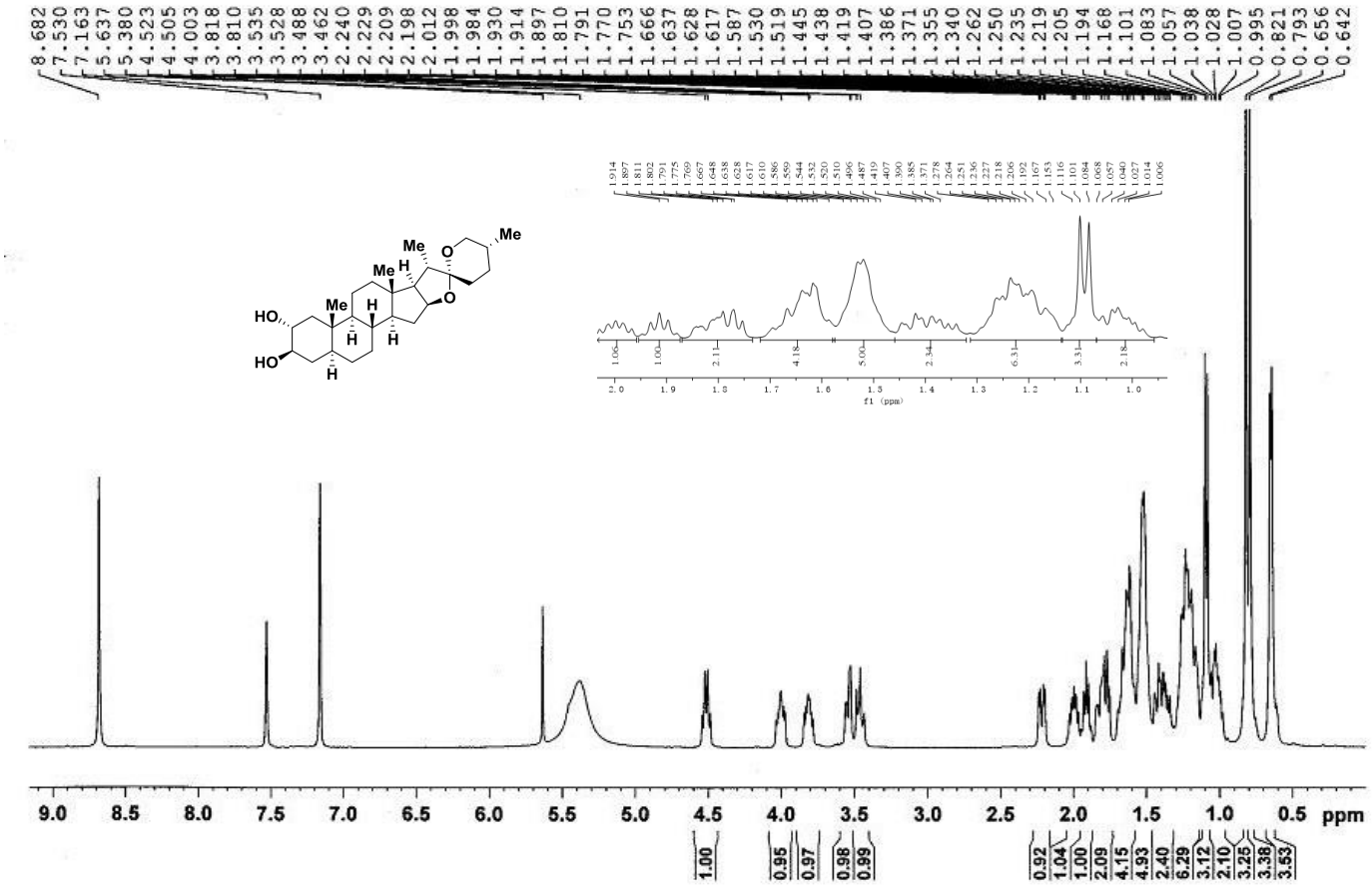


Figure 24. ^{1}H NMR (400 MHz, Pyridine-d_5) spectrum of compound **22**

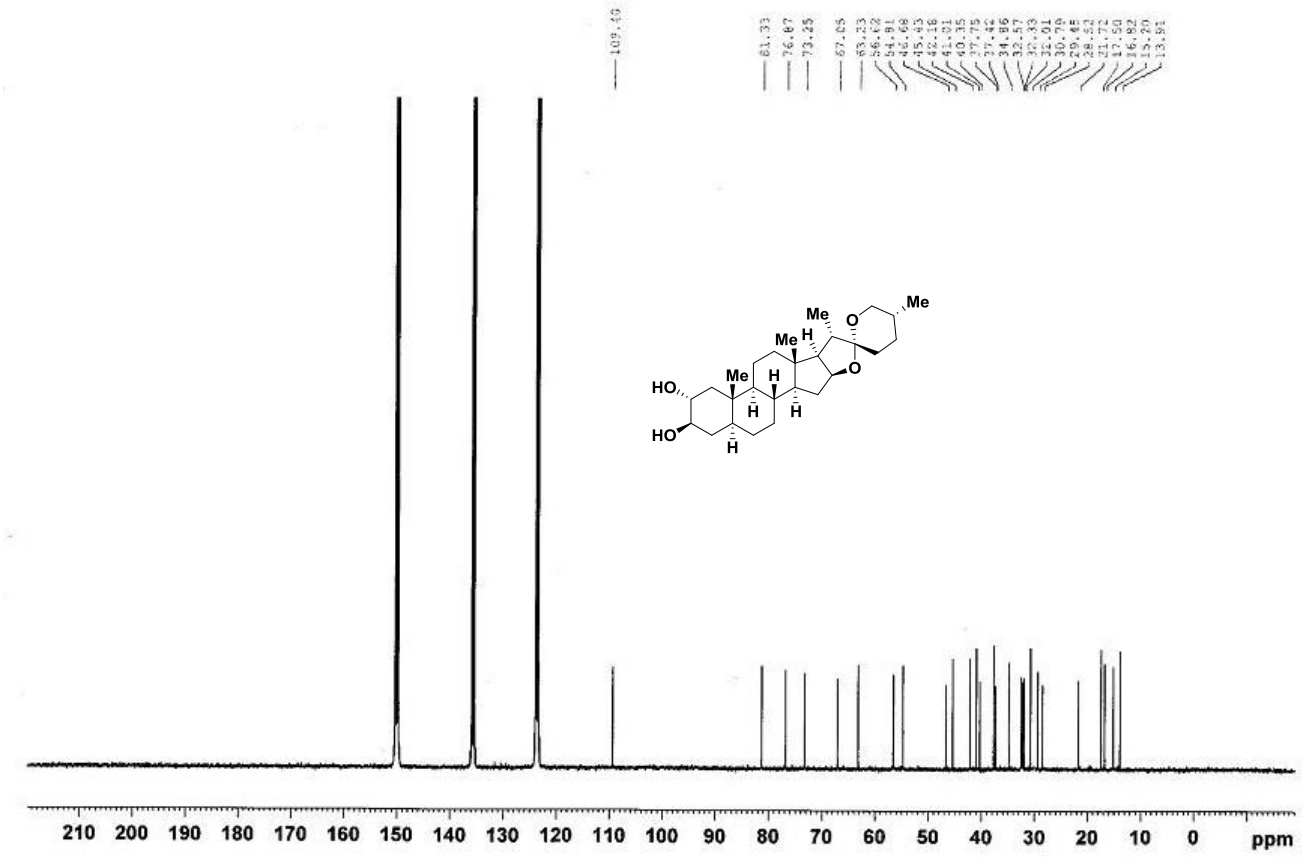


Figure 25. ^{13}C NMR (100 MHz, Pyridine-d_5) spectrum of compound **22**

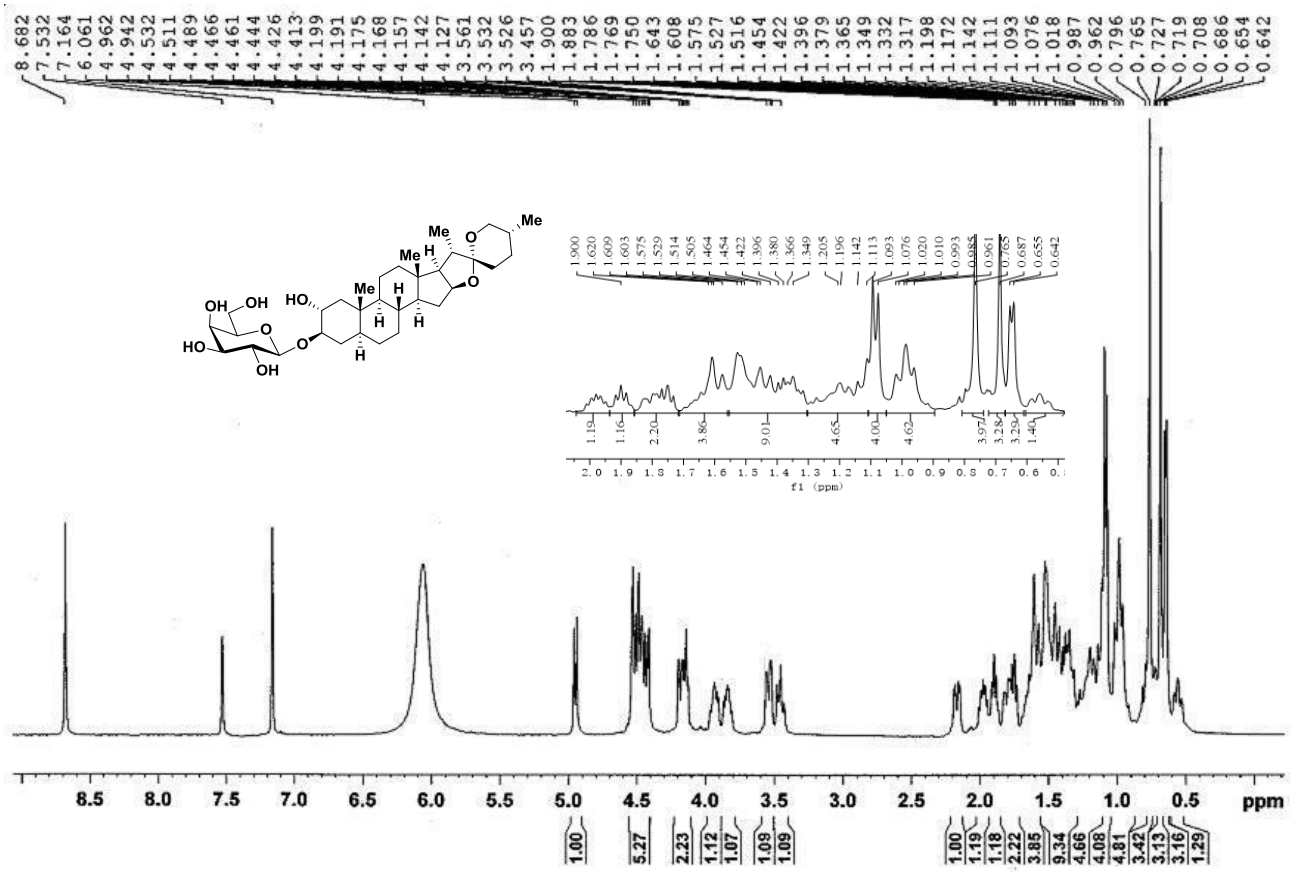


Figure 26. ^{1}H NMR (400 MHz, Pyridine-d_5) spectrum of compound **23**

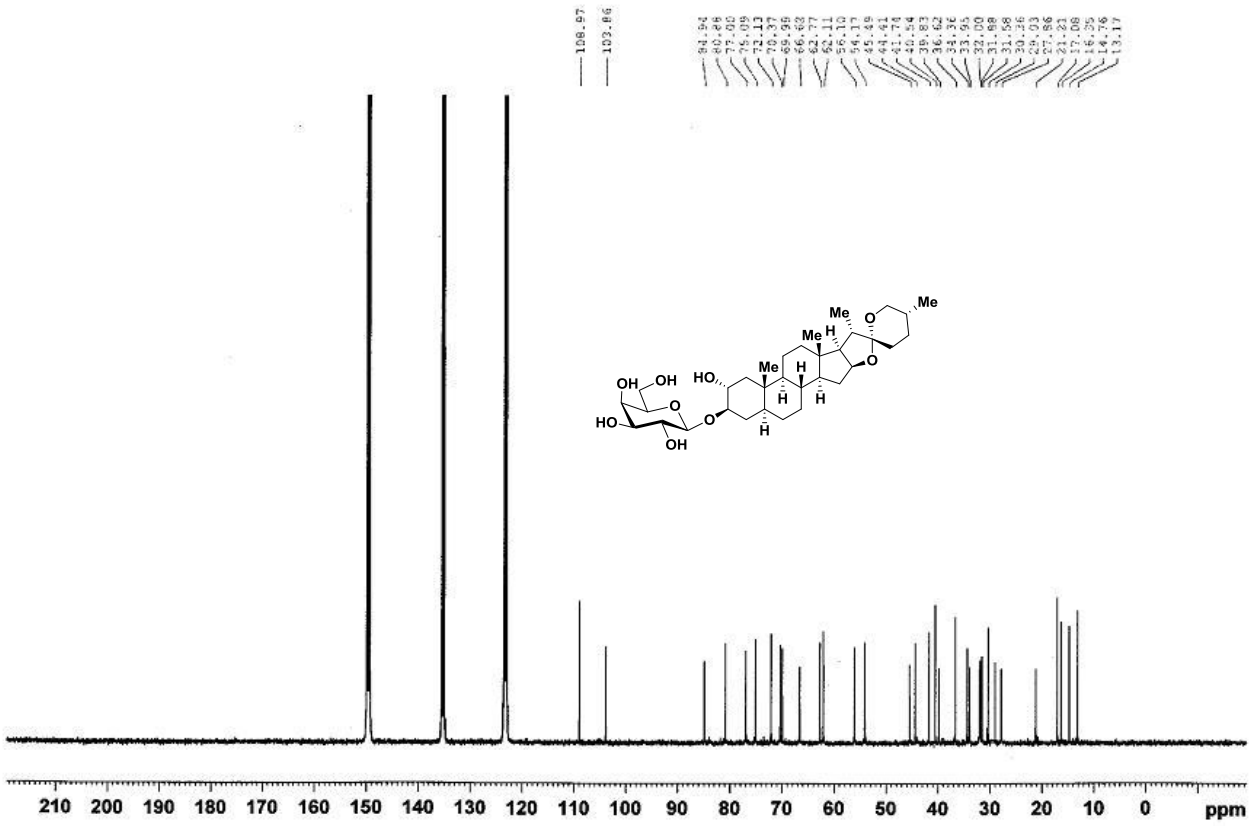


Figure 27. ^{13}C NMR (100 MHz, Pyridine-d_5) spectrum of compound **23**

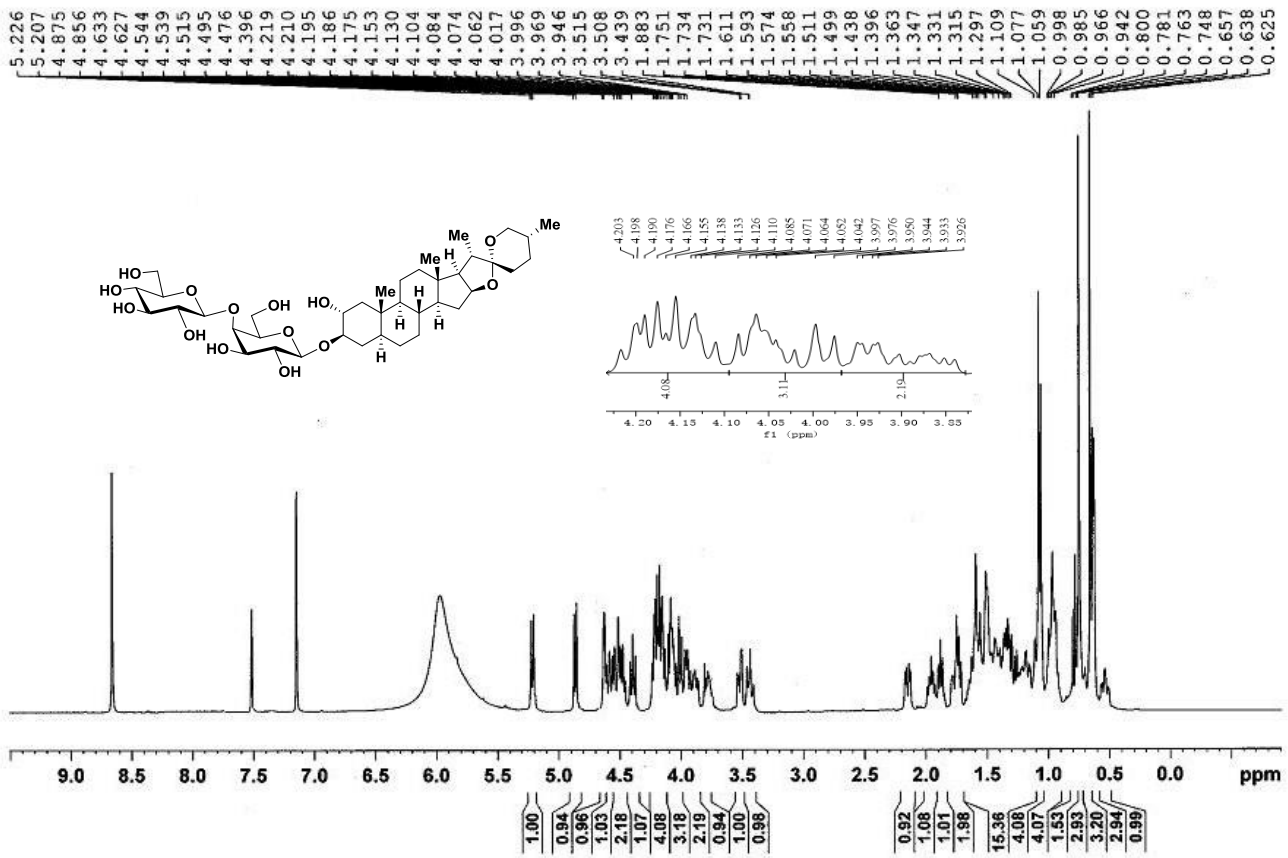


Figure 28. ^{1}H NMR (400 MHz, Pyridine-d_5) spectrum of compound **24**

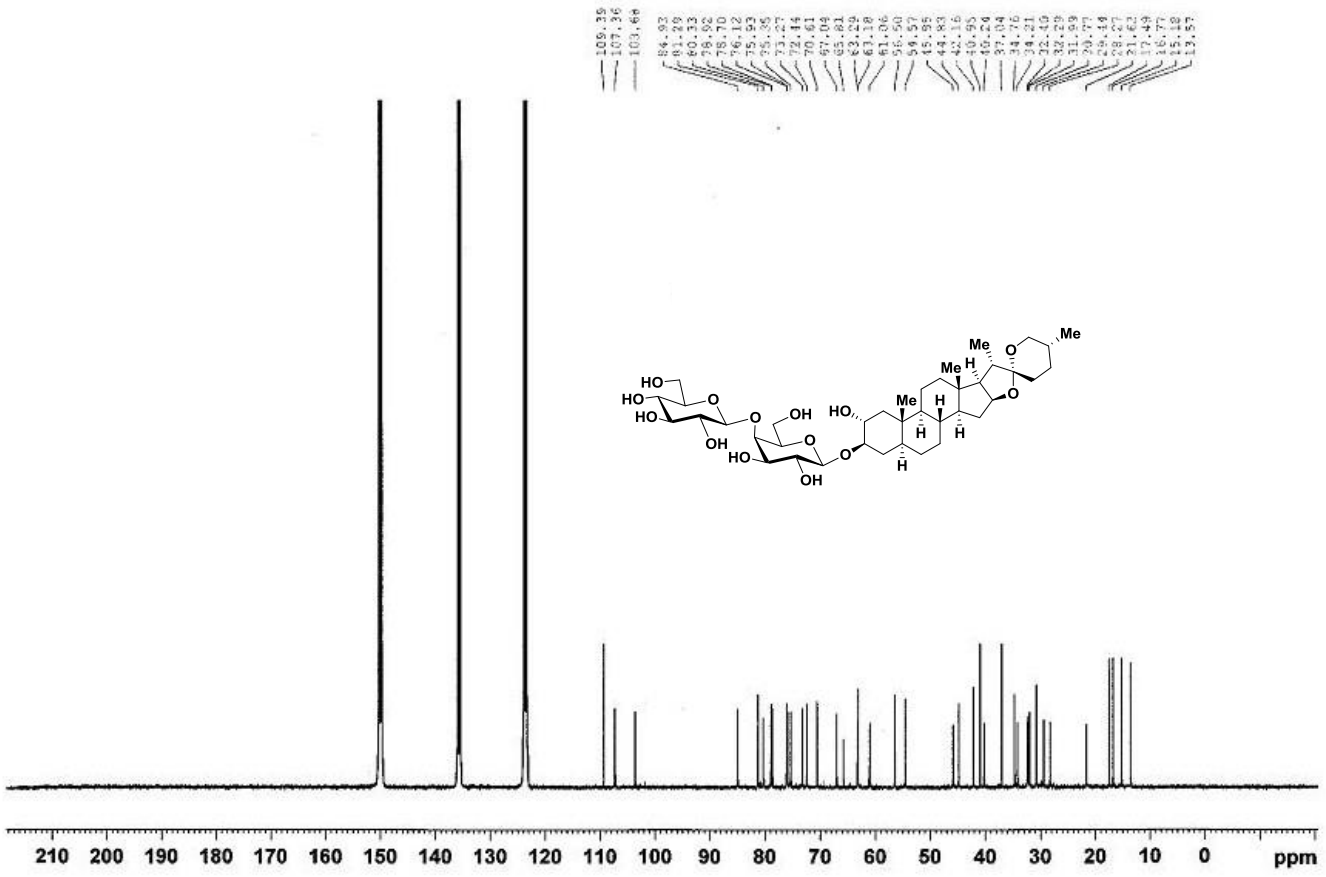


Figure 29. ^{13}C NMR (100 MHz, Pyridine-d_5) spectrum of compound **24**

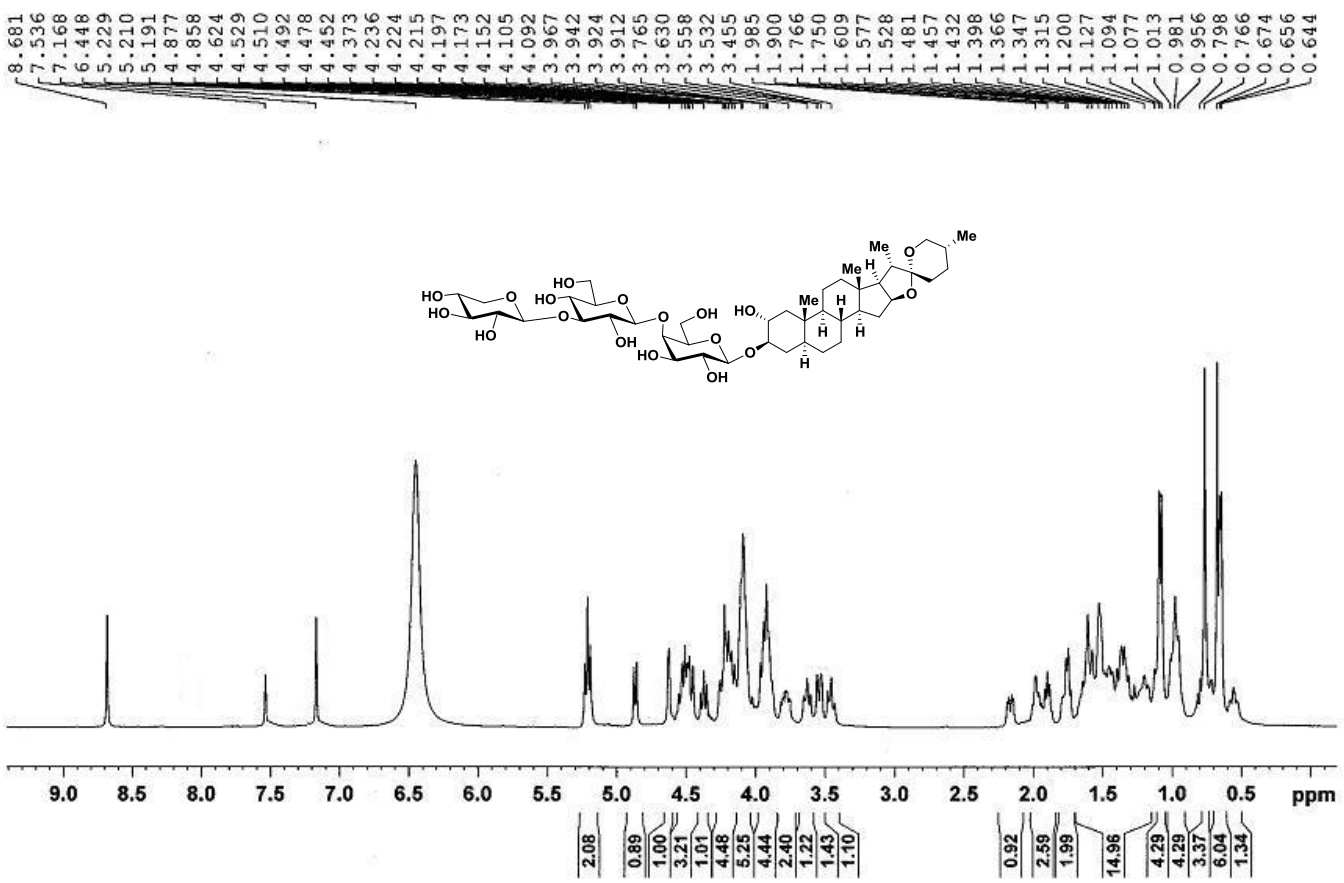


Figure 30. ^{1}H NMR (400 MHz, Pyridine-d_5) spectrum of compound **25**

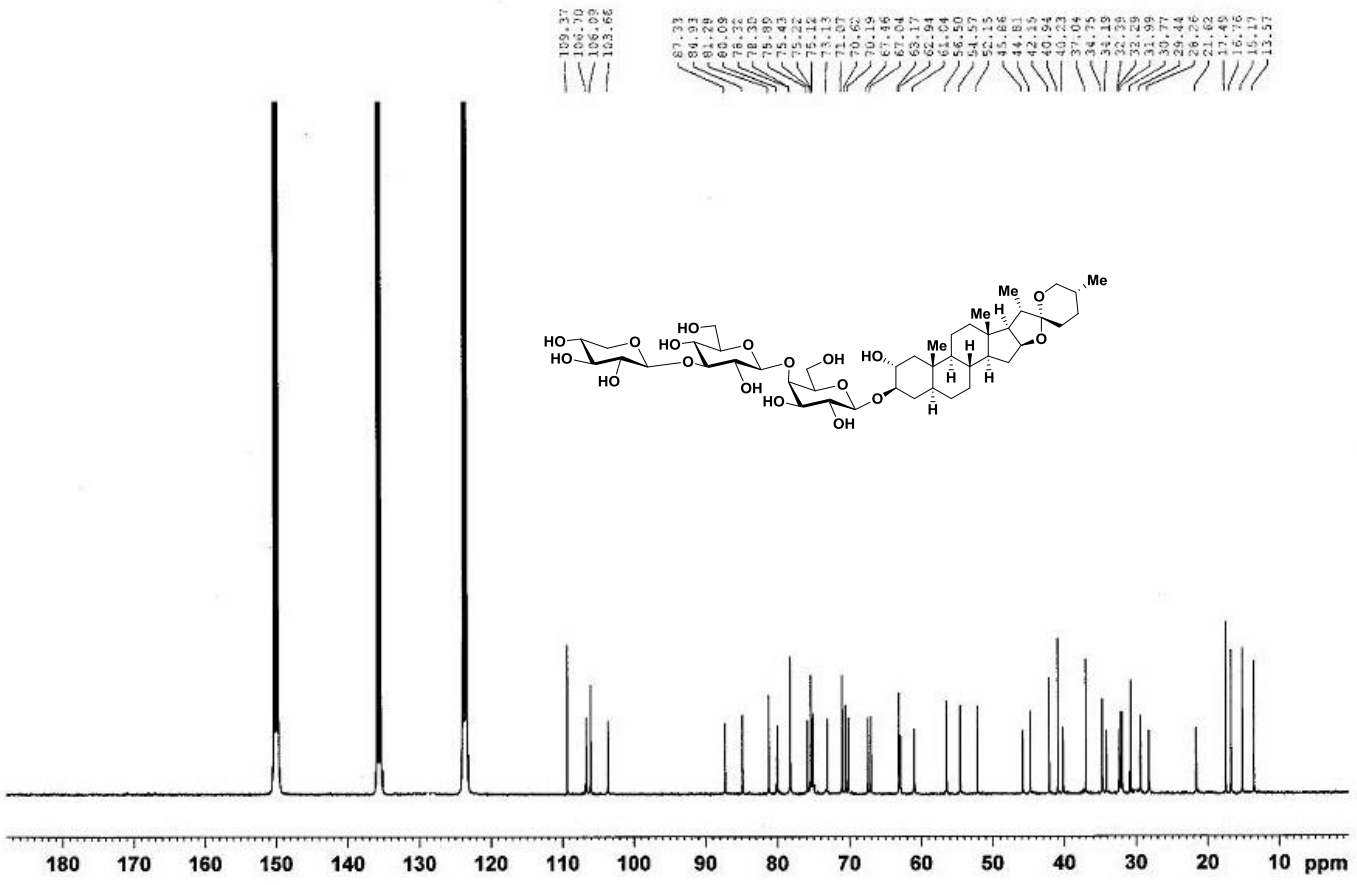


Figure 31. ^{13}C NMR (100 MHz, Pyridine-d_5) spectrum of compound **25**

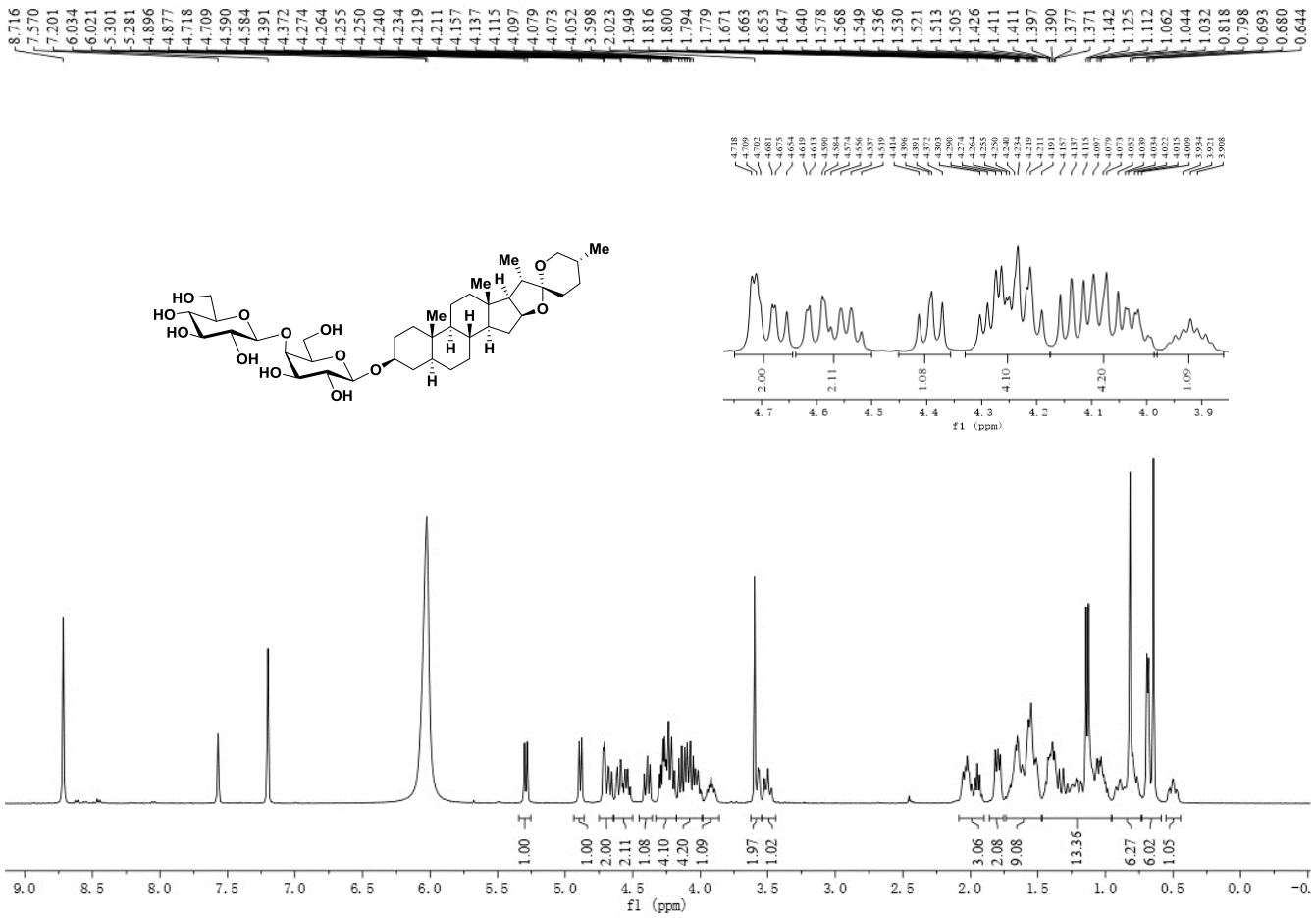


Figure 32. ^{1}H NMR (400 MHz, Pyridine-d_5) spectrum of compound **26**

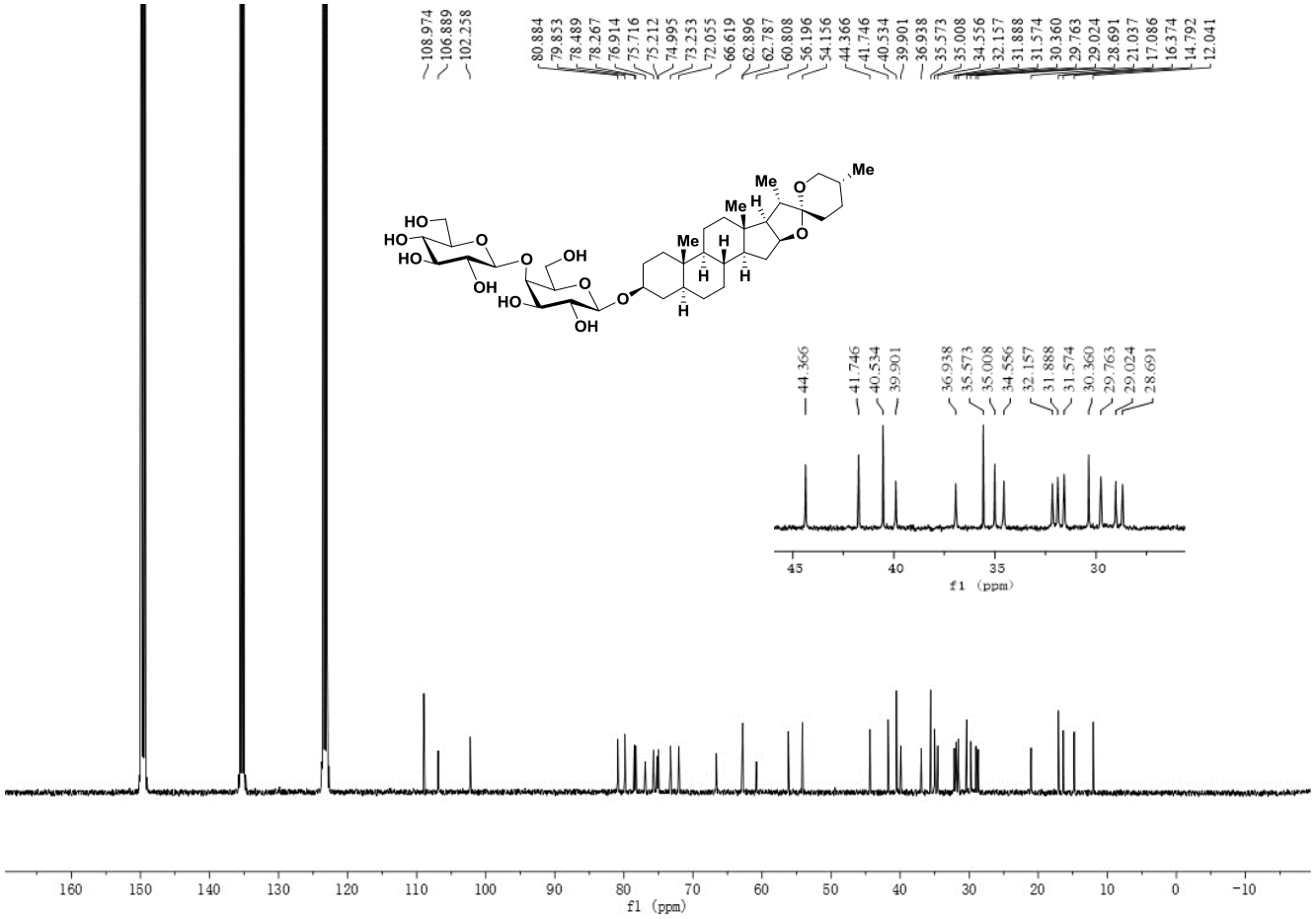


Figure 33. ^{13}C NMR (100 MHz, Pyridine-d_5) spectrum of compound **26**

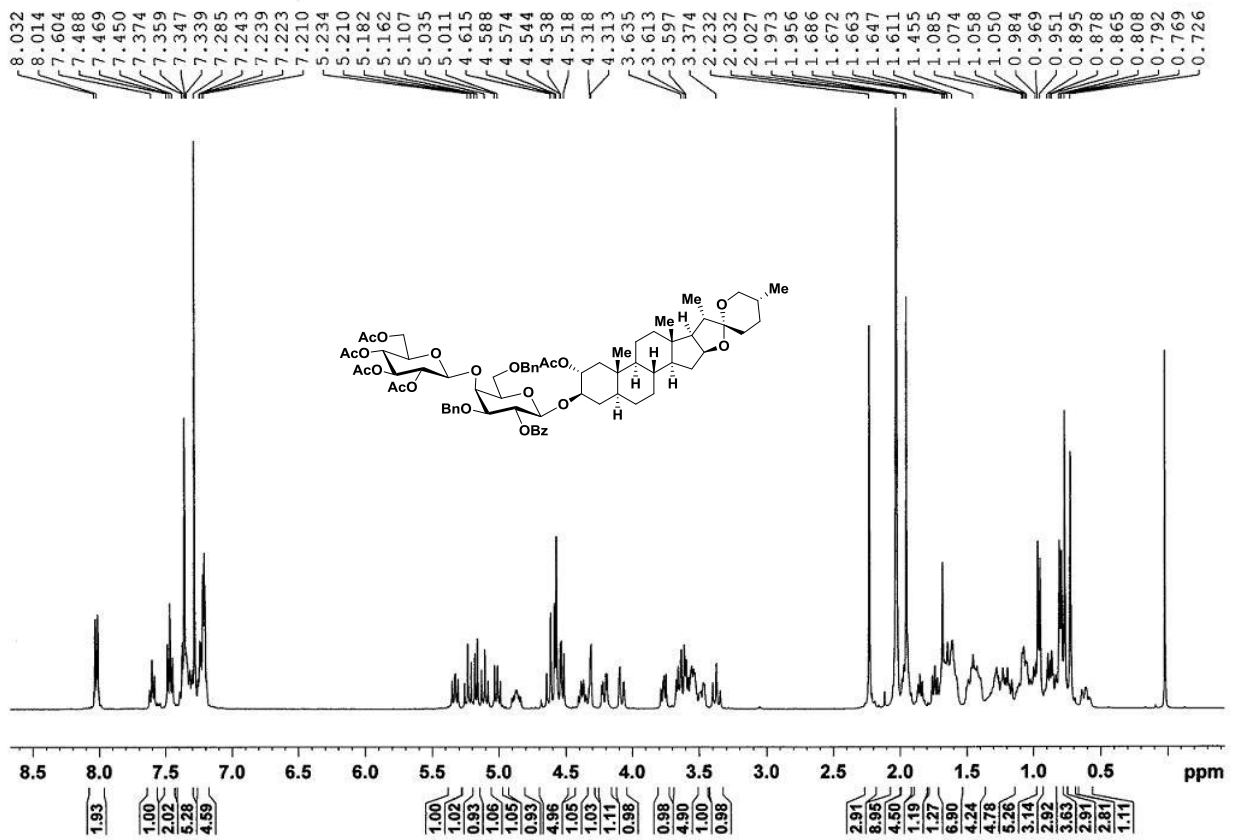


Figure 36. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **28**

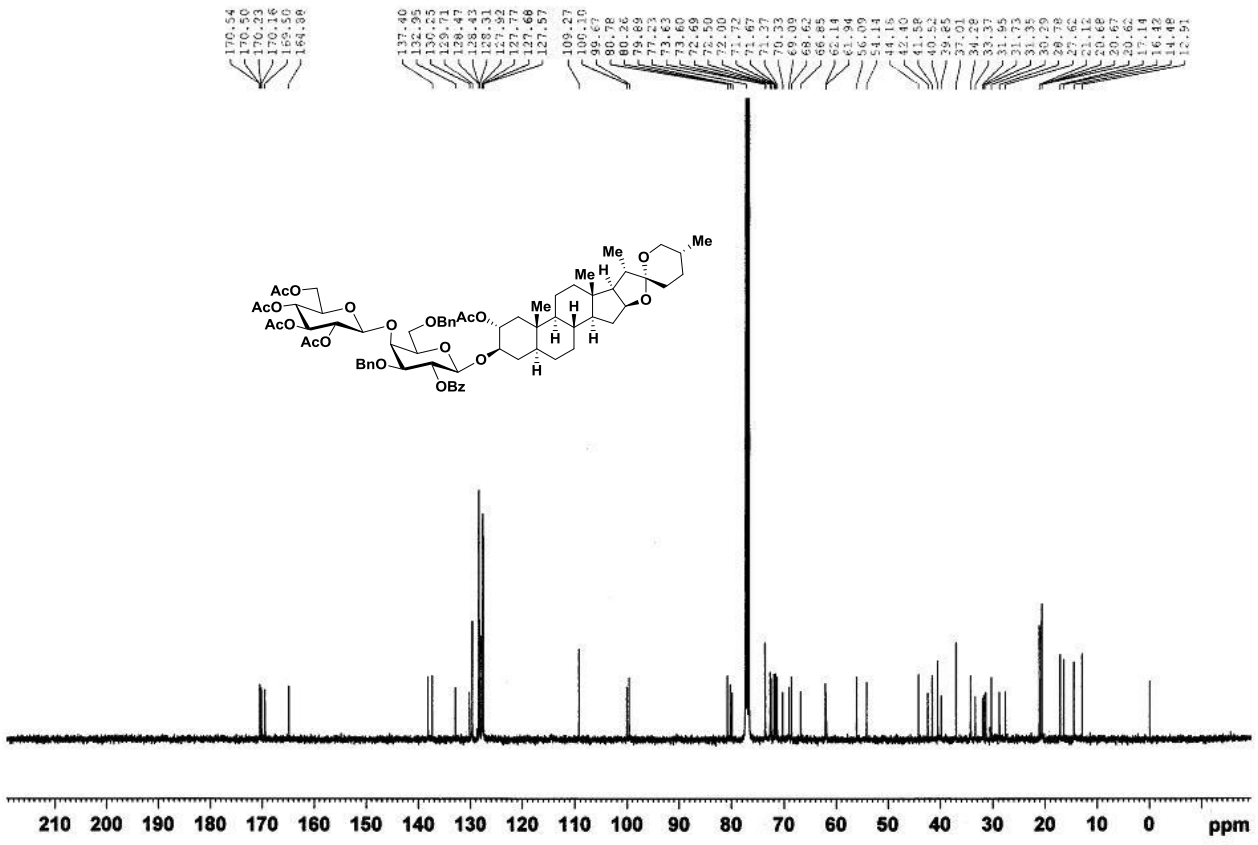


Figure 37. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **28**

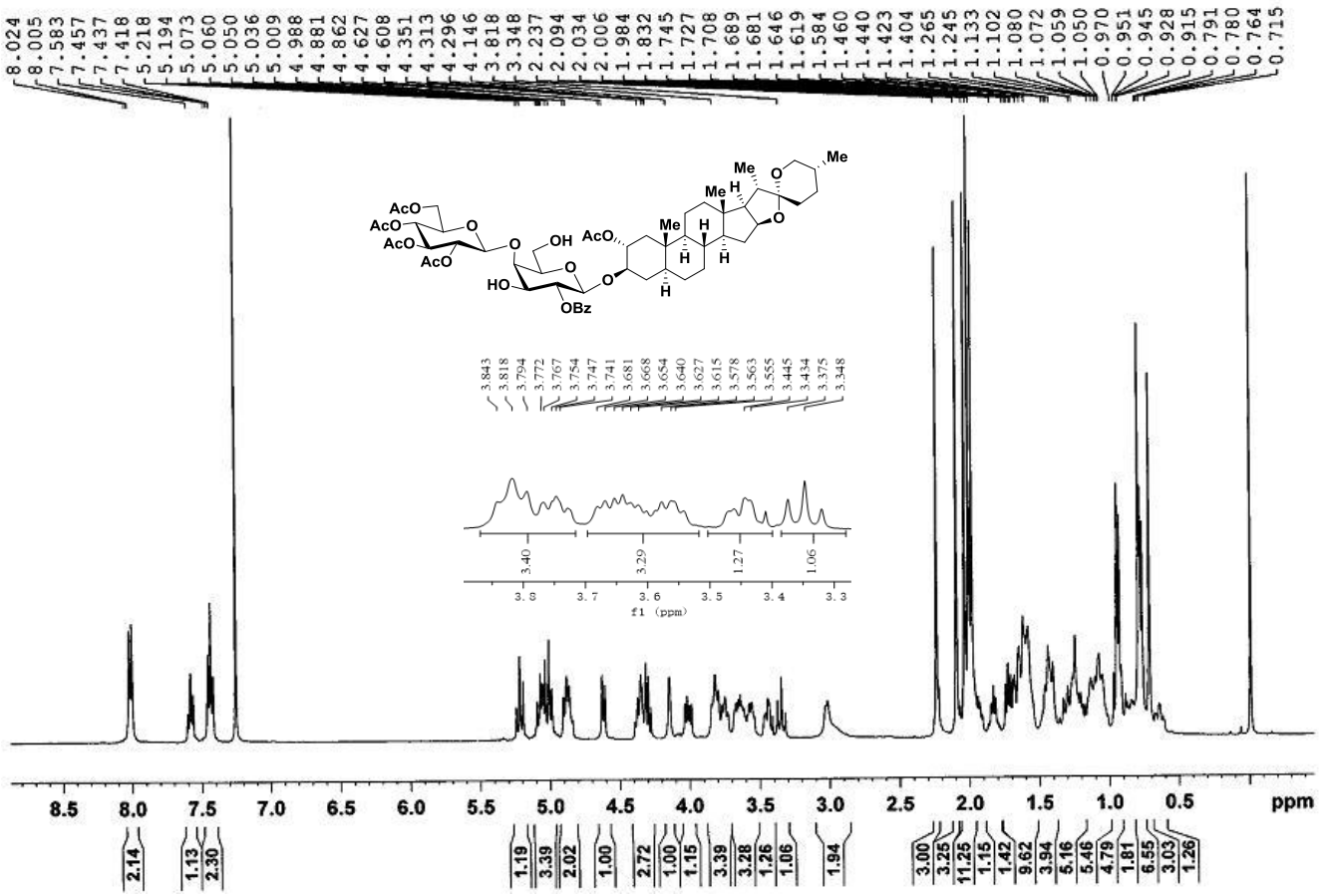


Figure 38. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **29**

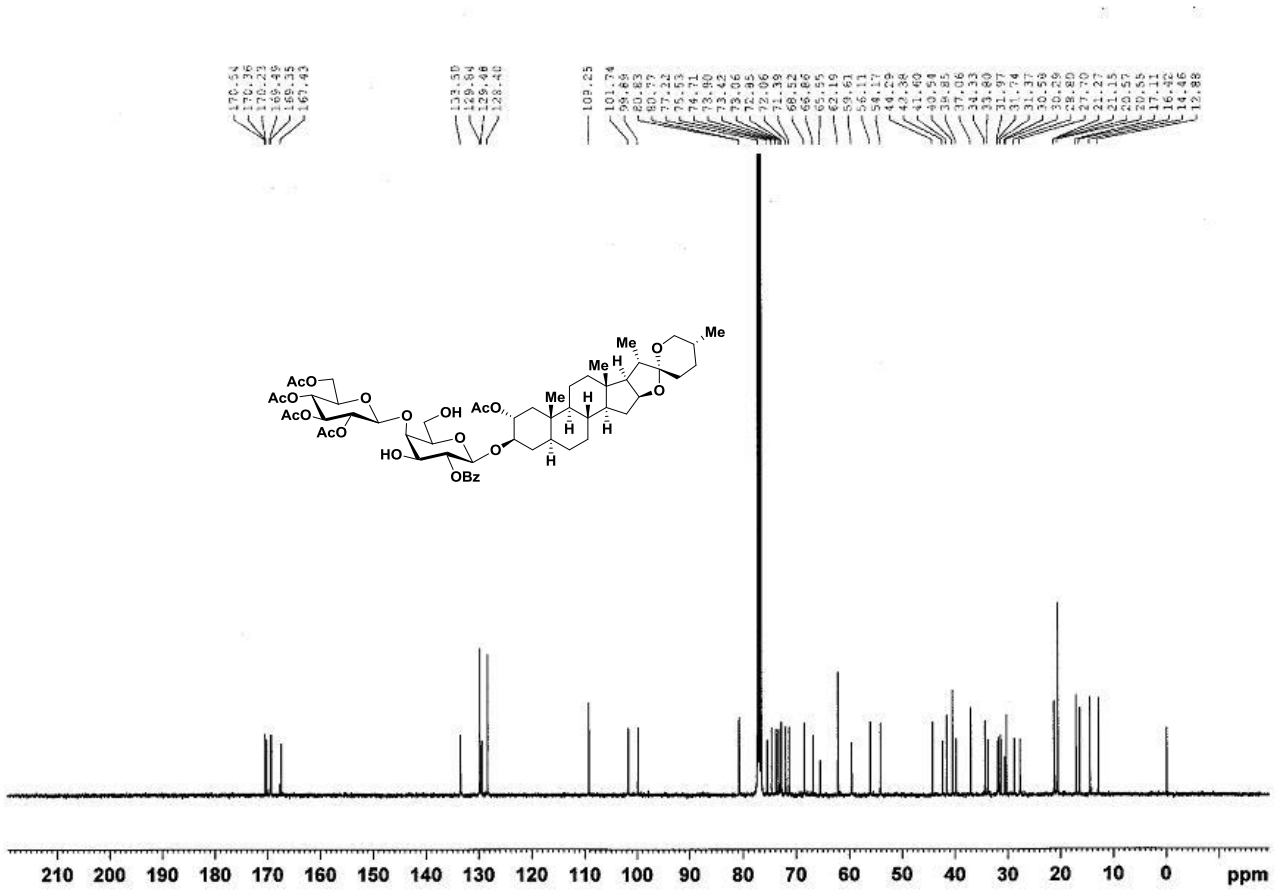


Figure 39. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **29**

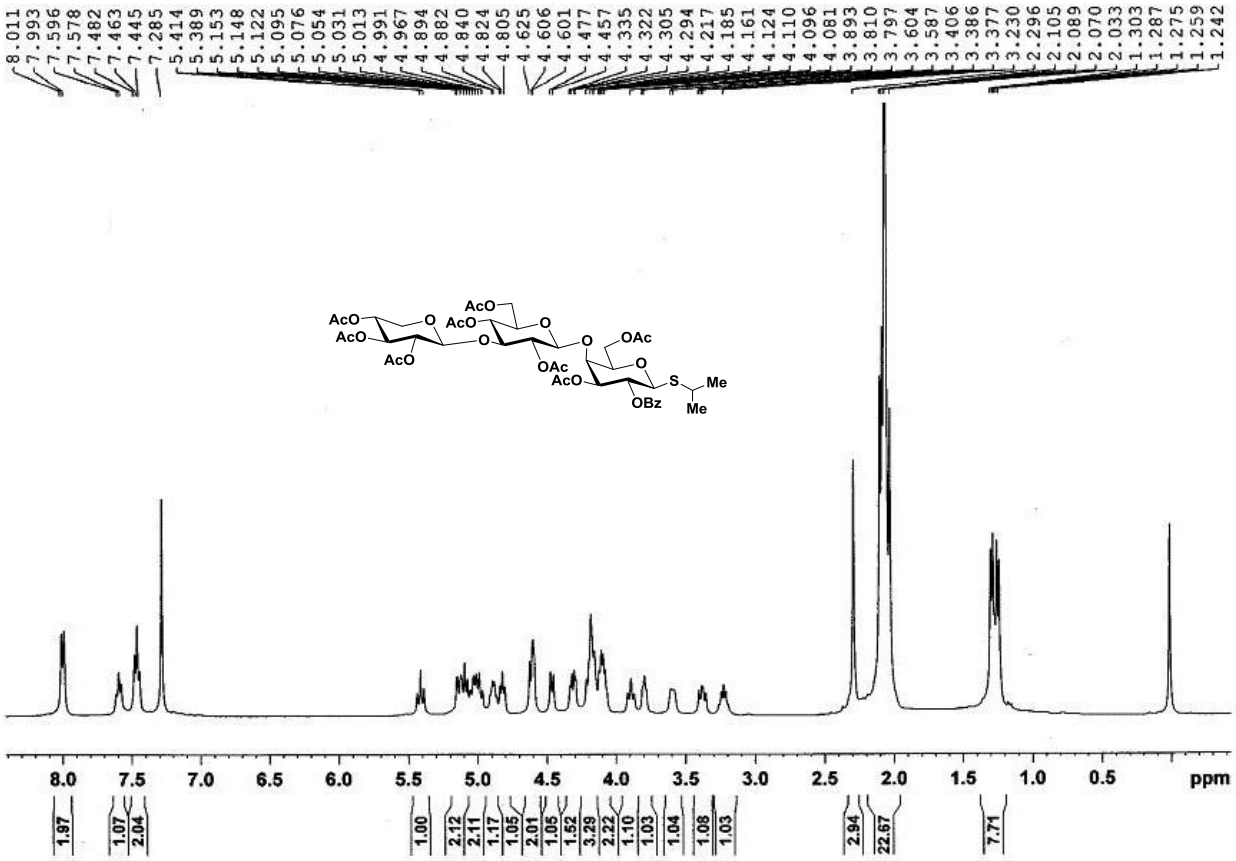


Figure 40. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **30**

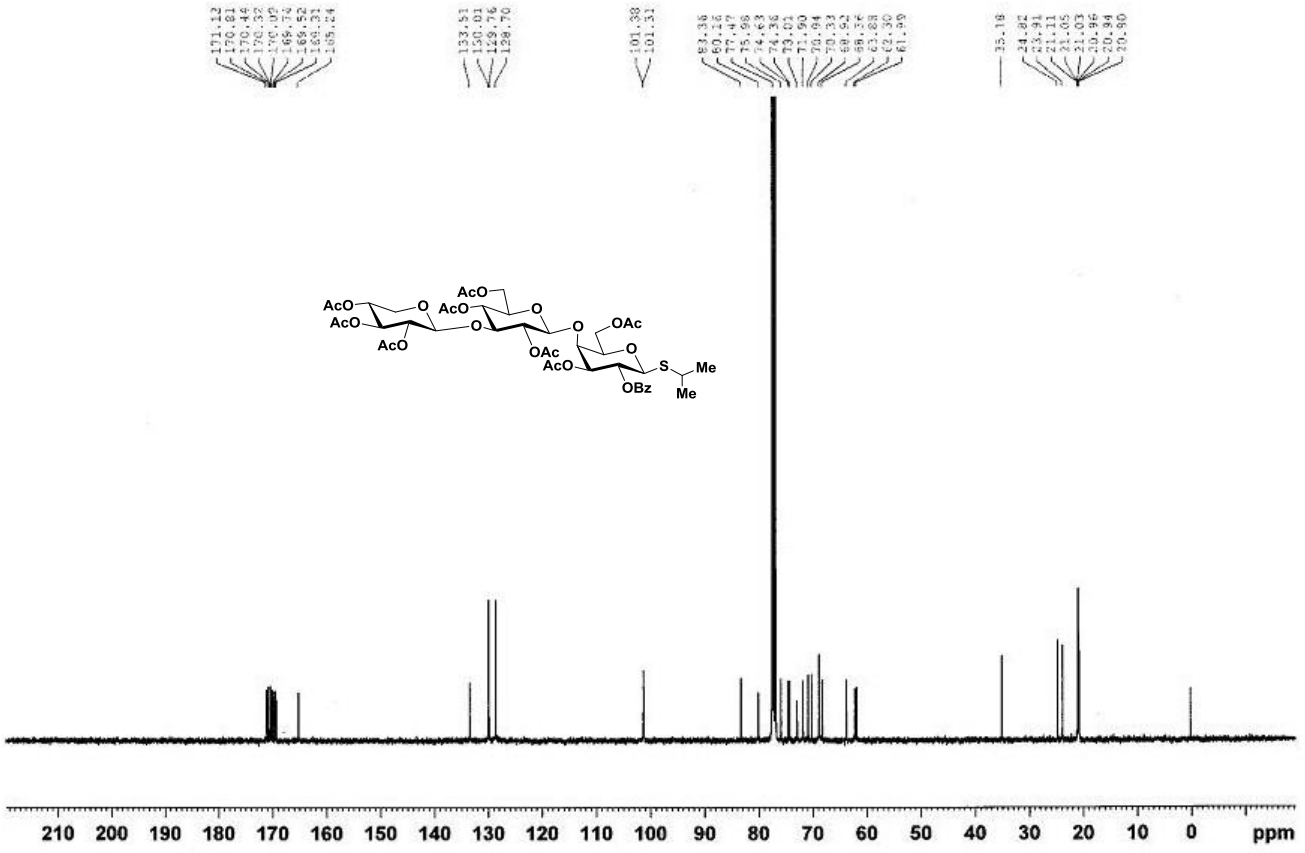


Figure 41. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **30**

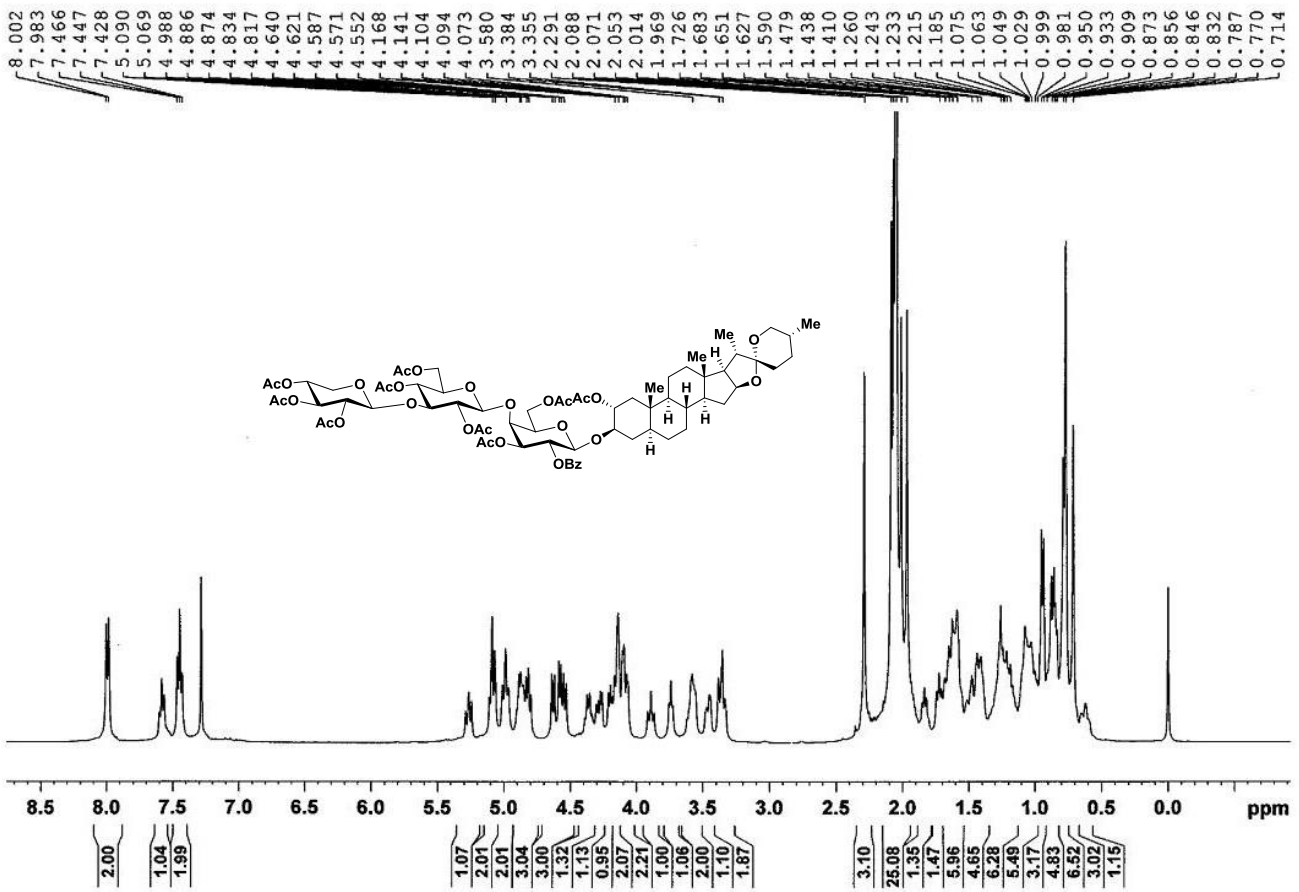


Figure 42. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **31**

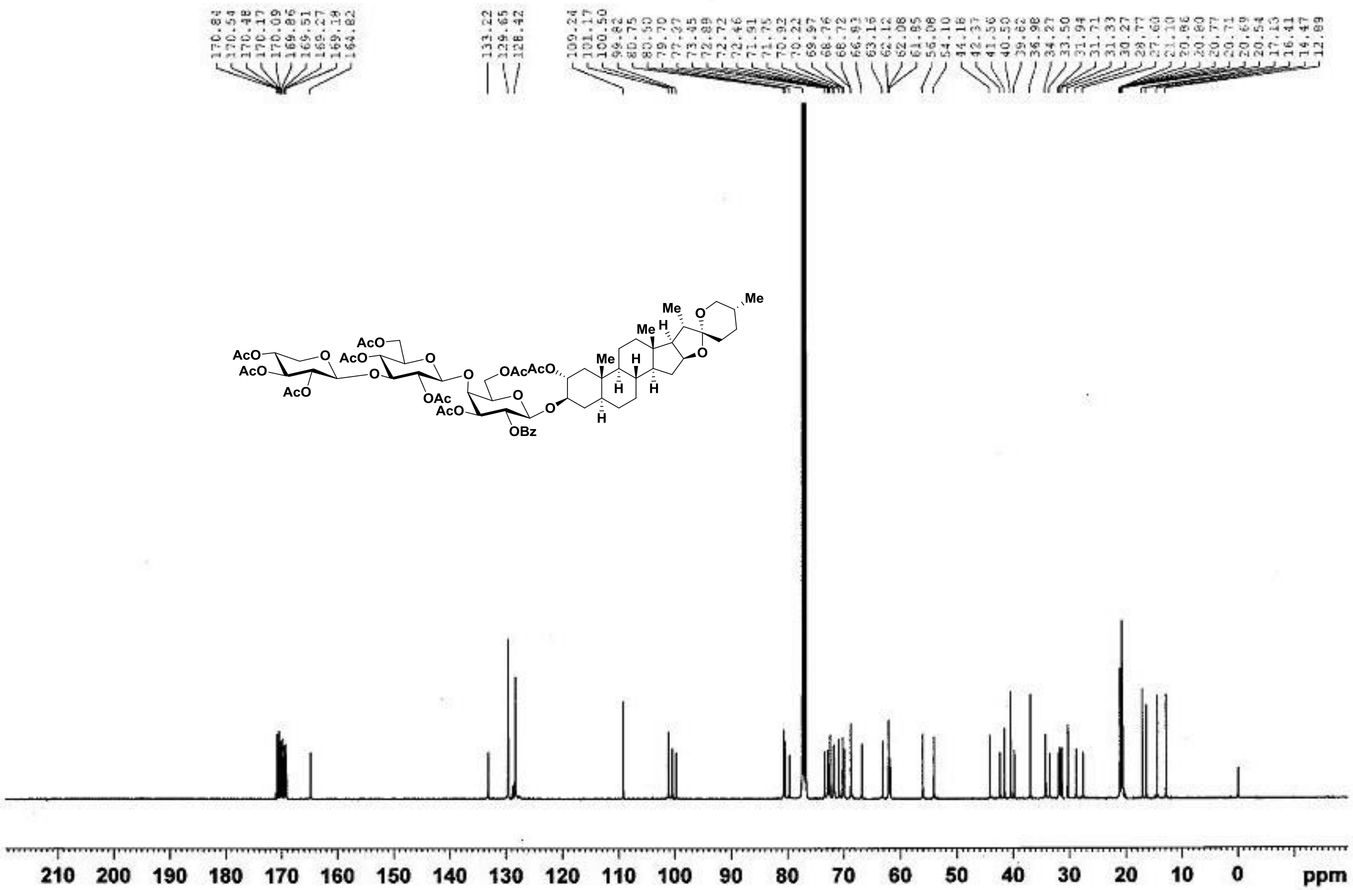


Figure 43. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **31**

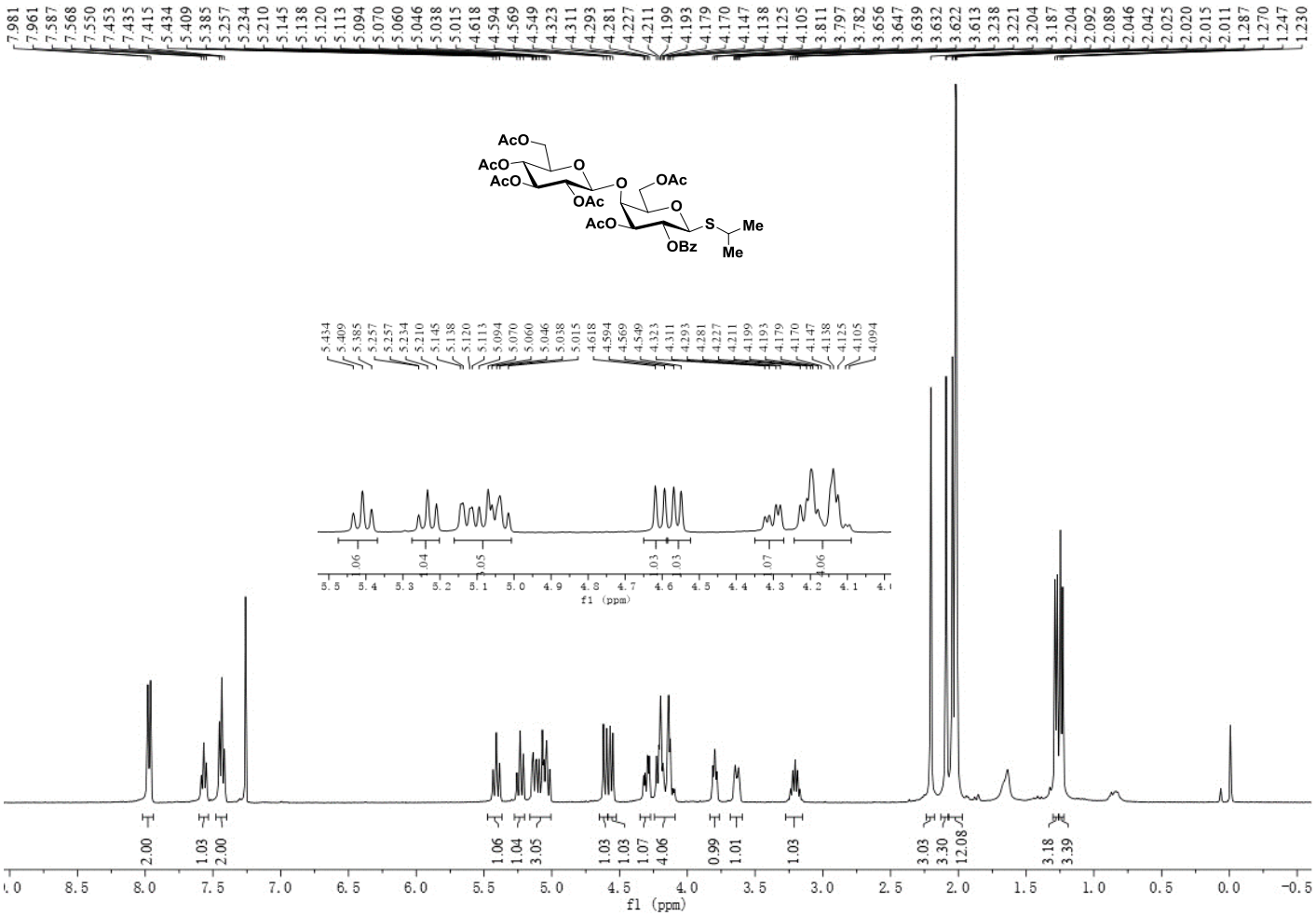


Figure 44. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **32**

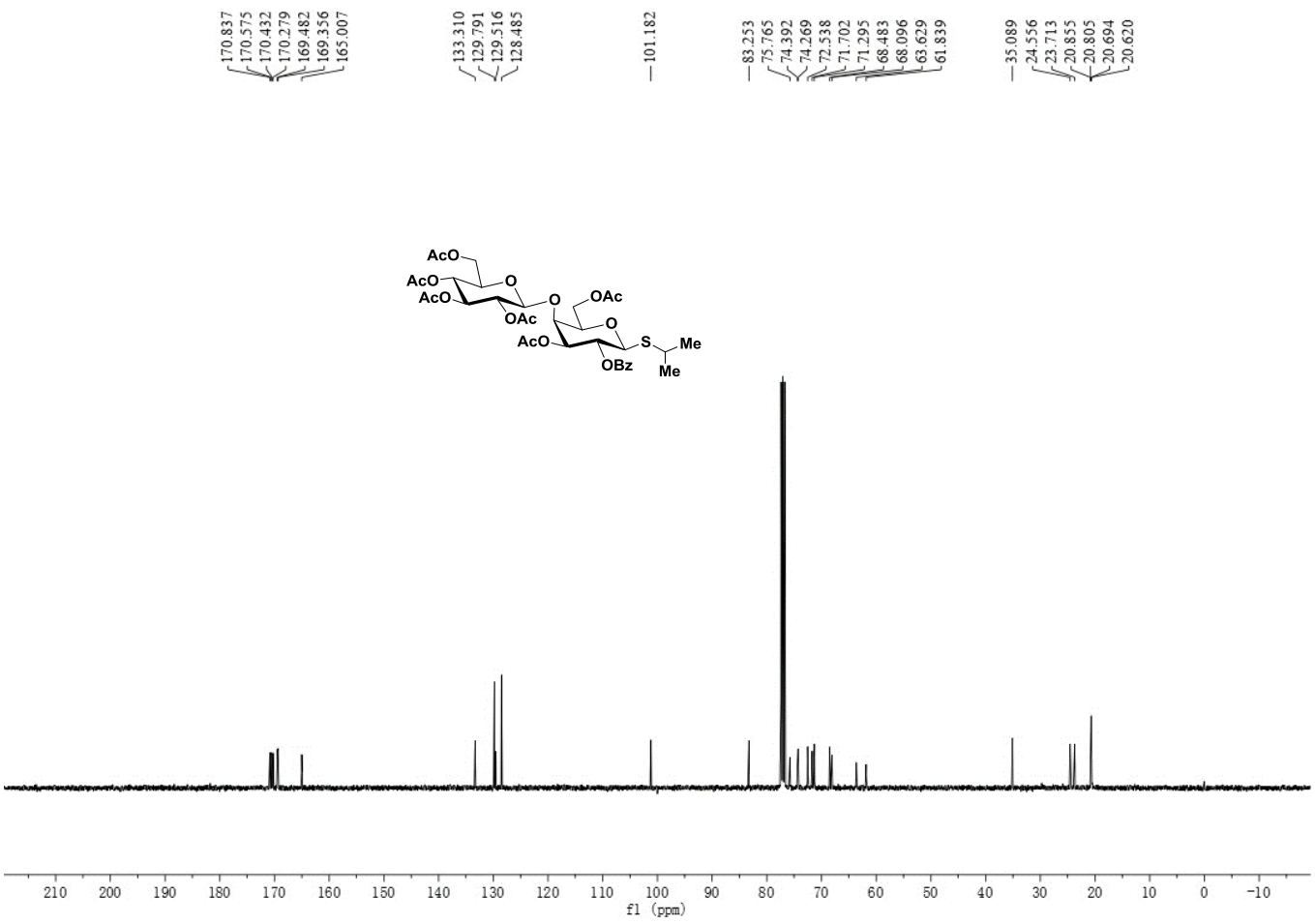


Figure 45. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **32**

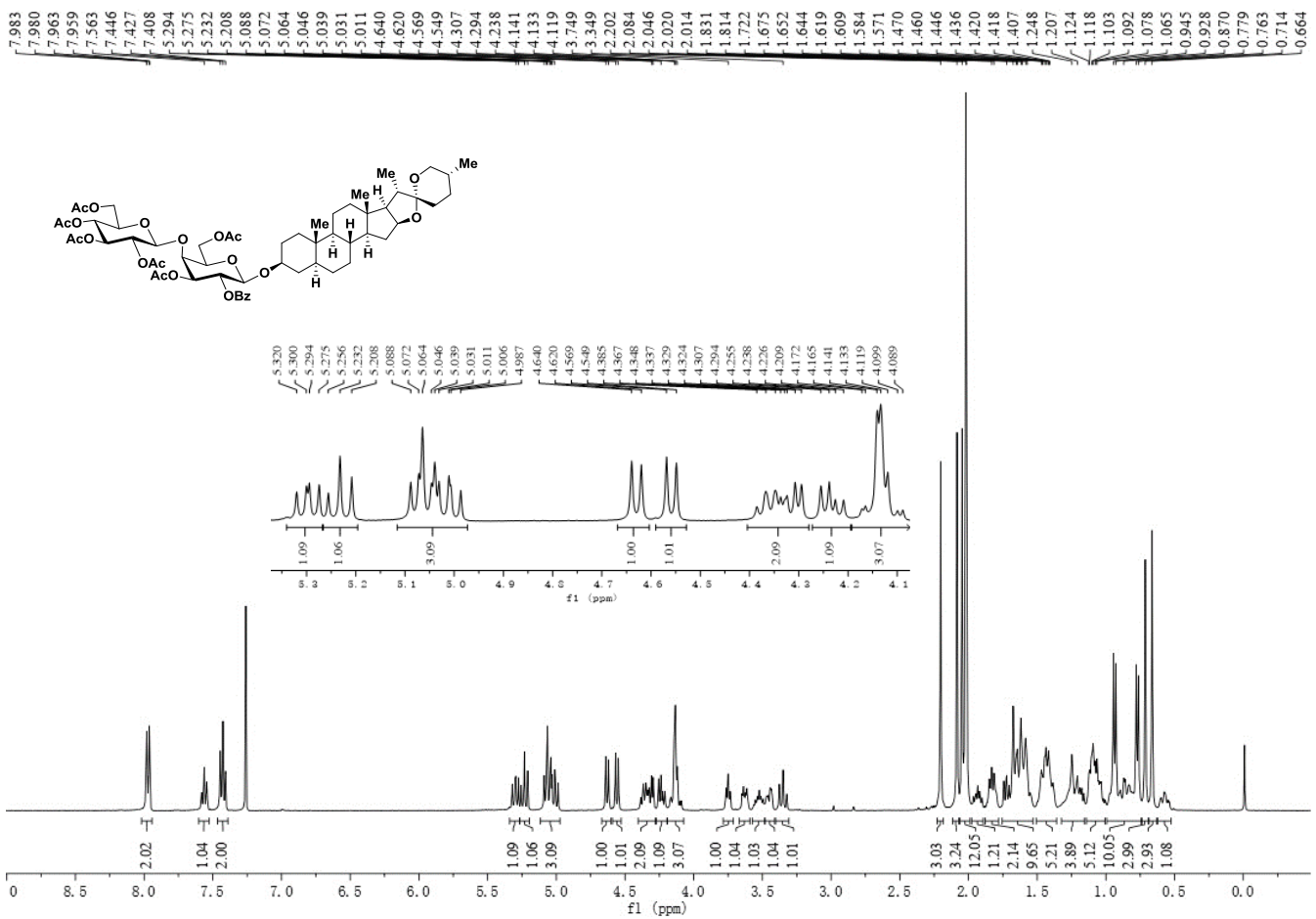


Figure 46. ^{1}H NMR (400 MHz, $CDCl_3$) spectrum of compound **33**

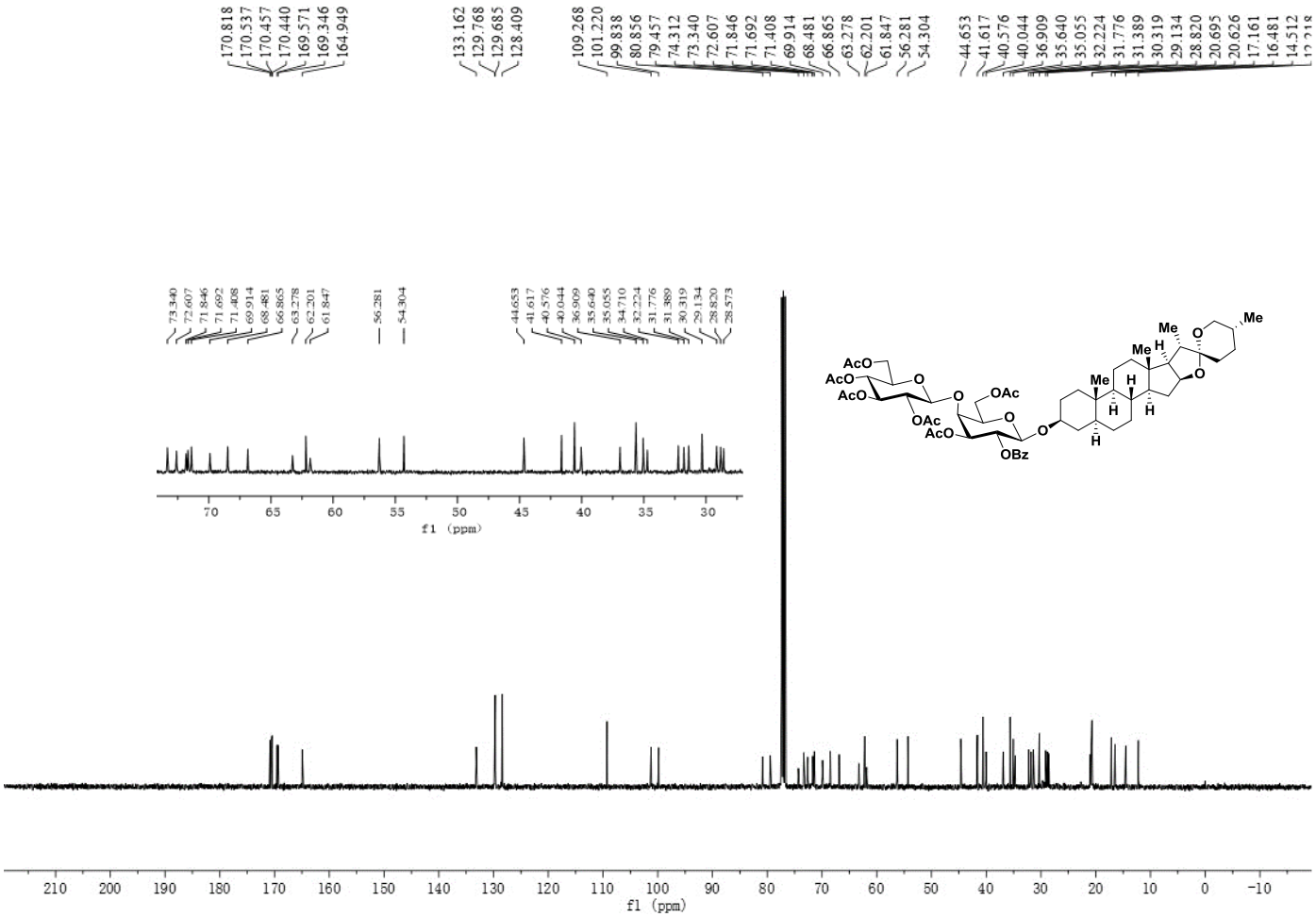


Figure 47. ^{13}C NMR (100 MHz, $CDCl_3$) spectrum of compound **33**

4. Comparison of selected ^{1}H NMR and ^{13}C NMR data of natural and synthetic gitonin.

	No.	^{1}H-Natural[a]	^{1}H-Our work[b]	$\Delta\delta = \delta_a - \delta_b$	^{13}C-Natural[a]	^{13}C-Our work[b]	$\Delta\delta = \delta_a - \delta_b$
	18	0.79	0.78	0.01	16.6	16.6	0
	19	0.69	0.68	0.01	13.5	13.4	0.1
	21	1.11 (7.0)	1.11 (6.8)	0	15.0	15.0	0
	27	0.70 (6.3)	0.69 (6.4)	0.01	17.3	17.3	0
3-O-Gal	1	4.91 (7.8)	4.92 (8.0)	-0.01	103.3	103.2	0.1
	2				72.5	72.4	0.1
	3				75.8	75.8	0
	4				79.4	79.3	0.1
	5				75.7	75.7	0
	6				60.5	60.4	0.1
Glc	1	5.15 (8.0)	5.15 (8.0)		105.2	105.3	0
	2				81.2	81.1	0.1
	3				86.1	85.9	0.2
	4				70.5	70.4	0.1
	5				77.6	77.6	0
	6				63.0	63.0	0
Gal	1	5.48 (7.8)	5.47 (8.0)	0.01	105.3	105.4	-0.1
	2				73.7	73.7	0
	3				74.4	74.3	0.1
	4				70.5	70.6	-0.1
	5				77.2	77.3	-0.1
	6				62.6	62.7	-0.1
Xyl	1	5.09 (7.6)	5.08 (7.2)	0.01	104.9	104.9	0
	2				75.1	75.0	0.1
	3				78.5	78.5	0
	4				70.8	70.7	0.1
	5				67.3	67.3	0

[a] Only selected ^{1}H NMR data was provided in the isolation paper. (*Phytochemistry* **1996,** *42*, 1417-1422) Spectra were recorded at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) in Pyridine-d_5.

[b] Spectra were recorded at 400 MHz (^{1}H NMR) and 100 MHz (^{13}C NMR) in Pyridine-d_5.