Supporting Information

Synthesis and Antimycobacterial Activity of 2,1’-Dihydropyridomycins

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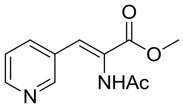
1. General Methods

All manipulations were conducted under an argon atmosphere using flame-dried glassware and standard syringe/septa and Schlenk techniques. Absolute solvents were purchased from Fluka (absolute over molecular sieves). Commercial chemicals were used without further purification. Solvents for extractions, flash column chromatography (FC) and thin layer chromatography (TLC) were purchased as commercial grade and distilled prior to use. TLC was performed on Merck TLC aluminum sheets (silica gel 60 F254). Spots were visualized with UV light (λ = 254 nm) or through staining with $Ce_2(SO_4)_3$/phosphomolybdic acid/H_2SO_4 (CPS), vanillin/H_2SO_4 or $KMnO_4$/K_2CO_3. Chromatographic purification of products by FC was performed using Fluka silica gel 60 for preparative column chromatography (particle size 40-63 µm).

NMR spectra were recorded on a Bruker Avance 400 MHz NMR spectrometer at 300 K. Chemical shifts (δ) are reported in ppm and are either referenced to the solvent signal as an internal standard (chloroform δ 7.26 ppm for ^{1}H and δ 77.00 ppm for ^{13}C spectra; DMSO-d_6 δ 2.50 ppm for ^{1}H and δ 39.43 ppm for ^{13}C spectra). Data are reported as follows: s = singlet, d = doublet, t = triplet, q = quartet, quint = quintet, sext = sextet, m = multiplet, br = broad signal, *J* = coupling constant in Hz. All ^{13}C-NMR spectra were measured with complete proton decoupling. ^{1}H- and ^{13}C-signals were assigned using two-dimensional correlation experiments (COSY, HMQC, HMBC). IR spectra were recorded on a Jasco FT/IR-6200 instrument as thin film. Optical rotations were measured on a Jasco P-1020 polarimeter operating at the sodium D line (λ = 589 nm) and are reported as follows: $[\alpha]_D^T$, concentration (*c* in g/100 mL) and solvent. Melting points were obtained in open capillary tubes using a Büchi melting point apparatus B-540 and are uncorrected. Mass spectra were recorded by the MS service of the Laboratory of Organic Chemistry (LOC) of the ETH Zürich; HRMS (ESI) spectra were measured on a Bruker Daltonics maxis (UHR-TOF) and HRMS (EI) on a Waters Micromass AutoSpec Ultima instrument.

2. Synthesis of Building Block 5

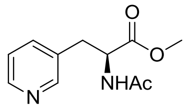


11

Ester 11: Pyridine-3-carbaldehyde (4.38 mL, 46.7 mmol, 1.00 eq.) was added to a mixture of *N*-acetylglycine (5.47 g, 46.7 mmol, 1.00 eq.) and NaOAc (4.21 g, 51.4 mmol, 1.10 eq.) followed by Ac_2O (24.8 mL, 243 mmol, 5.20 eq.) The dark brown mixture was stirred at 115 °C for 18 h. 10 mL MeOH were added (strongly exothermic!), in order to dilute the mixture, which was then poured into 40 mL MeOH containing 1.5 g NaOAc. The dark brown mixture was stirred at RT for72 h. It was then partitioned between sat. aq. Na_2CO_3 (20 mL) and $CHCl_3$ (40 mL) and the aq. phase was extracted with

$CHCl_3$ (3 x 20 mL). The combined org. extracts were concentrated *in vacuo* and the crude product was purified by FC (CH_2Cl_2/MeOH 3% → 10%) to yield **11** (8.06 g, 78%) as yellow crystals which were recrystallized from hexane/EtOAc.

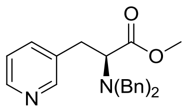
mp: 108-111 °C (Lit.: 110 °C, V. Busetti *et al.*, *J. Cryst. Spectrosc.* **1988**, *18*, 75-85)
^{1}H-NMR: (400 MHz, DMSO-d_6): δ 9.74 (s, 1H), 8.76 (s, 1H), 8.53 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.02 (dt, *J* = 8.0, 1.7 Hz, 1H), 7.45 (dd, *J* = 8.0, 4.8 Hz, 1H), 7.18 (s, 1H), 3.73 (s, 3H), 2.01 (s, 3H).
^{13}C-NMR: (100 MHz, $CDCl_3$): δ = 168.5, 165.5, 150.7, 149.8, 136.4, 130.4, 128.0, 125.5, 123.5, 53.1, 23.7.
IR: (neat, cm^{-1}): 3237, 2995, 2953, 1721, 1670, 1587, 1567, 1510, 1435, 1371, 1338, 1264, 1219, 1192, 1125, 1025, 985, 808, 764, 733, 705, 634, 609, 521.
HR-MS: (ESI): *m/z* calc. for $C_{11}H_{13}N_2O_3$ $[M+H]^+$ 221.0921, found 221.0920.



12

Ester 12: Olefin **11** (1.42 g, 6.45 mmol, 1.00 eq.) was dissolved in 75 mL freshly degassed MeOH and HBF_4 (50% in H_2O, 2.22 mL, 9.67 mmol, 1.50 eq.) was added. The solution was transferred into an autoclave and [Rh(COD)(*R*,*R*-DIPAMP)]BF_4 (4.88 mg, 6.45 µmol, 0.001 eq.) was added. The autoclave was pressurized with H_2 and subsequently vented 5 x before application of the final pressure of 5 bar. The mixture was heated to 50 °C and stirred for 18 h. It was then concentrated *in vacuo*, neutralized with sat. aq. Na_2CO_3 (15 mL) and extracted with $CHCl_3$ (4 x 20 mL). The crude product was purified by FC (CH_2Cl_2/MeOH 5%) to deliver **12** (1.22 g, 85%) as a yellow solid. The *ee* was determined by chiral HPLC (Daicel Chemical Industries, CHIRALPAK AD-H 0.46x15 cm column, isocratic hexane/iPrOH 9:1, 1.0 mL/min, t_R major: 10.67 min, t_R minor: 15.96 min).

R_f: 0.25 (CH_2Cl_2/MeOH 5%)
***ee*:** 87% (determined by HPLC analysis)
mp: 98-101 °C (Lit.: 101-103 °C, C. Döbler *et al.*, *Tetrahedron: Asymmetry* **1996**, *7*, 117-125)
$[\alpha]^{20}_D$: +100.3° (*c* 1.19, $CHCl_3$) (Lit.: +105.1, *c* 1.08, $CHCl_3$, *Tetrahedron: Asymmetry*, **1996**, *7*, 117-125)
^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.48 (dd, *J* = 4.82, 1.66 Hz, 1H), 8.34 (d, *J* = 1.96 Hz, 1H), 7.44 (dt, *J* = 7.82, 1.95 Hz, 1H), 7.22 (ddd, *J* = 7.81, 4.83, 0.63 Hz, 1H), 6.16 (d, *J* = 6.88 Hz, 1H), 4.90 (ddd, *J* = 7.54, 5.75, 1H), 3.74 (s, 3H), 3.18 (dd, *J* = 14.05, 5.84, 2H), 3.08 (dd, *J* = 14.05, 5.64, 2H), 1.99 (s, 3H).
^{13}C-NMR: (100 MHz, $CDCl_3$): δ 171.8, 169.8, 150.6, 148.7, 136.8, 131.8, 123.6, 53.0, 52.7, 35.3, 23.2.
IR: (neat, cm^{-1}): 3267, 3038, 2954, 1742, 1657, 1541, 1481, 1427, 1373, 1282, 1213, 1176, 1132, 1029, 802, 753, 714, 633, 597.
HR-MS: (ESI): *m/z* calc. for $C_{11}H_{15}N_2O_3$ $[M+H]^+$ 223.1077, found 223.1075.



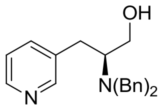
13

Ester 13: To acetamide **12** (350 mg, 1.58 mmol, 1.00 eq.) dissolved in 8.0 mL MeOH was added $SOCl_2$ (741 µl, 9.45 mmol, 5.00 eq.) at 0 °C. The solution was refluxed at 80 °C for 18 h. The mixture was concentrated and dissolved in toluene. The solvent was removed *in vacuo* and the yellow solid was portioned between $CHCl_3$ (10 mL) and sat. aq. Na_2CO_3 (5 mL). The aq. phase was extracted with $CHCl_3$ (4 x 15 mL) and the combined org. extracts were concentrated *in vacuo* to yield the free amino ester (crude, 243 mg, 86%) as an orange oil. ^{1}H- and ^{13}C-NMR spectra confirmed the complete transformation to the free amine **27**:

^{1}H-NMR: (400 MHz, CD_3OD): δ = 8.97 (s, 1H), 8.86 (d, *J* = 5.64 Hz, 1H), 8.68 (d, *J* = 8.08 Hz, 1H), 8.14 (dd, *J* = 8.02, 5.86 Hz, 1H), 4.59 (t, *J* = 7.04 Hz, 1H), 3.83 (s, 3H), 3.60 (dd, *J* = 14.69, 7.48 Hz, 2H), 3.51 (dd, *J* = 14.71, 6.58 Hz, 2H).
^{13}C-NMR: (101 MHz, CD_3OD): δ = 169.5, 149.5, 143.6, 142.0, 137.5, 128.8, 54.0, 53.9, 33.8.

To a solution of the above amino ester (234 mg, 1.30 mmol, 1.00 eq.) in 5 mL MeOH and 0.5 mL AcOH, benzaldehyde (791 µl, 7.79 mmol, 6.00 eq.), $NaCNBH_3$ (163 mg, 2.60 mmol, 2.00 eq.) and molecular sieves (4 Å) were added at RT and the suspension was stirred for 24 h. The mixture was neutralized with sat. aq. $NaHCO_3$ (5 mL) and extracted with Et_2O (3 x 10 mL). The combined org. extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by FC (hexane/EtOAc 4:1 → 7:3 → 0:1) to yield **13** (411 mg, 88%) as a colorless oil.

R_f: 0.21 (hexane/EtOAc 4:1)
$[\alpha]^{20}_D$: -88.16° (*c* 1.20, $CHCl_3$)
^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.49 (dd, *J* = 4.8, 1.7 Hz, 1H), 8.33 (d, *J* = 1.8 Hz, 1H), 7.31 – 7.18 (m, 11H), 7.14 (ddd, *J* = 7.8, 4.8, 0.8 Hz, 1H), 3.97 (d, *J* = 13.8 Hz, 2H), 3.79 (s, 3H), 3.66 (dd, *J* = 8.6, 6.8 Hz, 1H), 3.57 (d, *J* = 13.9 Hz, 2H), 3.05 (ddd, *J* = 22.9, 14.3, 7.7 Hz, 2H).
^{13}C-NMR: (100 MHz, $CDCl_3$): δ 172.5, 150.9, 147.9, 139.0, 136.8, 133.9, 128.8, 128.4, 127.3, 123.2, 62.0, 54.7, 51.4, 33.1.
IR: (neat, cm^{-1}): 3028, 2950, 2843, 1730, 1576, 1494, 1479, 1453, 1425, 1374, 1361, 1291, 1214, 1195, 1162, 1128, 1075, 1028, 990, 787, 747, 715, 699.
HR-MS: (ESI): *m/z* calc. for $C_{23}H_{25}N_2O_2$ $[M+H]^+$ 361.1911, found 361.1914.



14

Alcohol 14: Ester **13** (943 mg, 2.62 mmol, 1.00 eq.) was dissolved in 17 mL Et_2O and the solution was cooled to 0 °C. LAH (199 mg, 5.23 mmol, 2.00 eq.) was added and the suspension was stirred at 0 °C for 30 min and quenched with 2 mL H_2O, 2 mL 10% NaOH, 6 mL H_2O. The mixture was filtered, the filtrate was concentrated *in vacuo* and the residue purified by FC (hexane/EtOAc 2:3) to yield **14** (865 mg, 99%) as a colorless oil.

R_f: 0.26 (hexane/EtOAc 2:3)

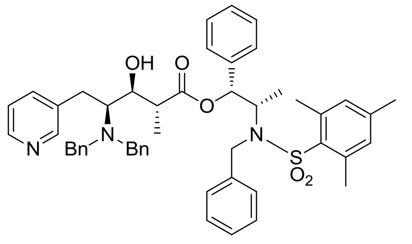
$[\alpha]^{20}_{D}$: + 26.33° (*c* 1.00, $CHCl_3$)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.38 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.32 (d, *J* = 1.8 Hz, 1H), 7.35 – 7.15 (m, 11H), 7.12 (ddd, *J* = 7.8, 4.8, 0.6 Hz, 1H), 3.87 (d, *J* = 13.3 Hz, 2H), 3.54 – 3.41 (m, 3H), 3.34 – 3.22 (m, 1H), 3.07 – 2.93 (m, 2H), 2.90 – 2.79 (m, 1H), 2.46 – 2.35 (m, 1H).

^{13}C-NMR: (100 MHz, $CDCl_3$): δ 150.4, 147.8, 138.8, 136.3, 134.8, 128.9, 128.6, 127.5, 123.4, 60.7, 60.3, 53.4, 29.2.

IR: (neat, cm^{-1}): 3304 (br.), 3061, 3028, 2930, 2834, 2804, 1578, 1494, 1480, 1453, 1425, 1363, 1129, 1044, 1028, 779, 746, 732, 714, 699.

HR-MS: (ESI): *m/z* calc. for $C_{22}H_{25}N_2O$ $[M+H]^+$ 333.1961, found 333.1952.



16

Ester 16: Alcohol **14** (40.0 mg, 332 µmol, 1.00 eq.) was dissolved in 1 mL CH_2Cl_2 and DMP (76.6 mg, 424 µmol, 1.50 eq.) was added at 0 °C. The suspension was stirred at 0 °C for 30 min and then diluted with Et_2O (1 mL). The reaction was quenched with 1 mL DMP workup solution (14 g sodium thiosulfate in 1 L 80% sat. aq. $NaHCO_3$) and the resulting mixture was stirred at 0 °C for 30 min. The aq. phase was extracted with Et_2O (3 x 5 mL). The combined org. extracts were washed with H_2O and brine (1 x 2 mL each), dried over $MgSO_4$ and concentrated *in vacuo* at 20 °C. The crude aldehyde **7** was dried at 10^{-3} mbar (RT) for 4 h. (1R,2S)-2-(N-benzyl-2,4,6-trimethylphenylsulfonamido)-1-phenylpropyl propionate (**15**) (75.1 mg, 157 µmol, 1.50 eq.) was dissolved in 1 mL CH_2Cl_2 and NEt_3 (54.4 µl, 392 µmol, 3.75 eq.) was added. The solution was cooled to -78 °C and dicyclohexylboron trifluoromethanesulfonate (112 mg, 345 µmol, 3.30 eq.) in 350 µl hexane was added dropwise during 15 min. The resulting solution was stirred at -78 °C for 3 h. Aldehyde **7** (34.5 mg, 104 µmol, 1.00 eq.) dissolved in 0.5 mL CH_2Cl_2 was added dropwise during 20 min and the solution was stirred at -78 °C for 3 h. The mixture was warmed very slowly to 0 °C and stirred for 1 h. The reaction was then quenched with 1 mL pH 7 buffer, and the mixture was diluted with 4.5 mL MeOH and stirred with 0.45 mL H_2O_2 (50%) at RT for 16 h. The org. solvents were removed *in vacuo* and the residue was taken up in CH_2Cl_2 (5 mL) and H_2O (5 mL). The aq. phase was extracted with CH_2Cl_2 (3 x 5 mL) and the combined org. extracts were dried over $MgSO_4$. The solvents were removed *in vacuo* and the residue was purified by FC (hexane/EtOAc 3:2) to yield **16** (71.1 mg, 73% over 2 steps) as a 5:1 mixture of isomers (**16** *vs.* all other isomers). The desired isomer **16** could be isolated from this mixture in pure form by FC with hexane/EtOAc 3:2 as eluent (50.7 mg, 52% over 2 steps). Analytical data are for pure isomer **16**.

R$_f$: 0.24 (hexane/EtOAc 3:2)

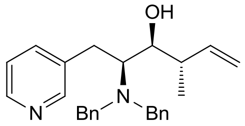
$[\alpha]^{20}_D$: + 20.22° (*c* 1.03, $CHCl_3$)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.48 (d, *J* = 1.7 Hz, 1H), 8.44 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.50 (dt, *J* = 7.8, 1.9 Hz, 1H), 7.38 – 7.05 (m, 19H), 6.89 – 6.81 (m, 4H), 5.80 (d, *J* = 4.0 Hz, 1H), 4.70 (d, *J* = 16.5 Hz, 1H), 4.51 (d, *J* = 16.5 Hz, 1H), 4.19 (d, *J* = 13.1 Hz, 2H), 4.16 – 4.08 (m, 1H), 3.44 (d, *J* = 13.5 Hz, 2H), 3.39 – 3.31 (m, 1H), 3.28 (d, *J* = 3.3 Hz, 1H), 3.13 – 2.99 (m, 3H), 2.93 – 2.84 (m, 1H), 2.48 (s, 6H), 2.27 (s, 3H), 1.13 (d, *J* = 7.0 Hz, 3H), 0.45 (d, *J* = 7.1 Hz, 3H).

^{13}C-NMR: (100 MHz, $CDCl_3$): δ 174.8, 150.6, 147.6, 142.5, 140.3, 139.7, 138.3, 138.2, 136.8, 135.7, 133.5, 132.1, 129.1, 128.4, 128.3, 127.9, 127.5, 127.2, 127.0, 125.9, 123.4, 78.4, 72.9, 59.1, 56.8, 55.6, 48.2, 42.6, 26.9, 22.9, 20.9, 13.1, 12.9.

IR: (neat, cm^{-1}): 3062, 3028, 2979, 2939, 1738, 1604, 1495, 1454, 1378, 1323, 1261, 1205, 1151, 1029, 1013, 931, 857, 751, 730, 699, 661, 568, 538.

HR-MS: (ESI): *m/z* calc. for $C_{50}H_{56}N_3O_5S$ $[M+H]^+$ 810.3935, found 810.3934.



18

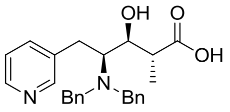
Olefin 18: Alcohol **14** (408 mg, 1.23 mmol, 1.00 eq.) was dissolved in 9 mL anhydrous DCM and DMP (781 mg, 1.84 mmol, 1.50 eq.) was added at 0 °C. The suspension was stirred at 0 °C for 30 min and then diluted with Et_2O (10 mL). The reaction was quenched with 8 ml DMP workup mix and the solution was stirred for 30 min at 0 °C. The aq. phase was extracted with Et_2O (3 x 15 mL). The combined org. extracts were washed with H_2O and brine (1 x 2 mL each), dried over $MgSO_4$ and concentrated *in vacuo* at 20 °C. The crude aldehyde **7** was dried at 10^{-3} mbar (RT) for 4 h. A solution of this aldehyde (370 mg, 1.12 mmol, 1.00 eq.) in 10 ml THF was added to $CrCl_2$ (1.10 g, 8.96 mmol, 8.00 eq.) which had been dried at 200 °C under vacuum for 25 min. This was followed by addition of crotylbromide (576 µl, 5.60 mmol, 5.00 eq.) and the mixture was stirred at RT for 3 h (color change from green to brown). The reaction was quenched with sat. aq. $NaHCO_3$ (5 mL) and extracted with EtOAc (3 x 10 mL). The combined org. extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by FC (hexane/EtOAc 1:4) to yield **18** as a yellow oil (308 mg, 67% over 2 steps, 15:1 ratio of isomers).

R$_f$: 0.26 (hexane/EtOAc 1:4)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.40 (dd, *J* = 4.7, 1.1 Hz, 1H), 8.34 (d, *J* = 1.8 Hz, 1H), 7.27 (d, *J* = 7.8 Hz, 1H), 7.22 – 7.00 (m, 11H), 5.52 – 5.40 (m, 1H), 5.04 (dd, *J* = 10.3, 1.4 Hz, 1H), 4.93 (d, *J* = 17.2 Hz, 1H), 3.78 (d, *J* = 14.1 Hz, 2H), 3.69 (d, *J* = 7.3 Hz, 1H), 3.54 (d, *J* = 14.1 Hz, 2H), 2.98 – 2.89 (m, 2H), 2.74 (dd, *J* = 17.9, 8.7 Hz, 1H), 2.23 – 2.10 (m, 1H), 0.78 (d, *J* = 6.8 Hz, 3H).

^{13}C-NMR: (100 MHz, $CDCl_3$): δ 151.0, 147.1, 139.8, 139.8, 136.9, 136.8, 128.7, 128.2, 126.9, 123.0, 117.7, 73.1, 60.2, 54.4, 42.6, 29.2, 16.8.

HR-MS: (ESI): *m/z* calc. for $C_{26}H_{31}N_2O$ $[M+H]^+$ 378.2431, found 387.2427.



5

Acid 5:

A) From ester 16: To a solution of **16** (176 mg, 217 µmol, 1.00 eq.) in 5.2 mL MeOH/THF/H_2O 3:2:2 was added LiOH·H_2O (45.6 mg, 1.09 mmol, 5.00 eq.) at RT. The clear solution was stirred at RT for 24 h and diluted with Et_2O (7 mL). The aq. phase was acidified to pH 2 (aq. HCl 1 M) and the cleaved auxiliary was extracted, leaving the product in the aq. phase. The latter was adjusted to pH 7 (sat. aq. $NaHCO_3$) and the product was extracted with $CHCl_3$ (4 x 15 mL). The org. phase was dried over $MgSO_4$ and concentrated *in vacuo* to yield **5** as a yellow, viscous resin (84.1 mg, 96%).

B) From olefin 18: To a solution of AD-mix α (1.51 g) and **18** (136 mg, 351 µmol, 1.00 eq.) in 3.8 mL *t*-BuOH/H_2O 1:1 was added methanesulfonamide (201 mg, 2.11 mmol, 6.00 eq.). The yellow suspension was stirred at RT for 3 d. 0.9 mL H_2O and 1.3 g Na_2SO_3 were added and the mixture was stirred for 30 min. The mixture was extracted with CH_2Cl_2 (3 x 10 mL), the org. phase was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was filtered through silica (CH_2Cl_2/MeOH 10%) to yield the crude diol (125 mg, 84%) as a white foam. This material (125 mg, 297 µmol, 1.00 eq.) was dissolved in 1.5 mL CH_2Cl_2 and $NaIO_4$ (254 mg, 1.19 mmol, 4.00 eq.) in 900 µL H_2O was added. The biphasic mixture was stirred at RT for 1 h. The aq. phase was extracted with Et_2O (3 x 5 mL) and concentrated *in vacuo*. The residue was dissolved in 2.88 mL *t*-BuOH/H_2O 4:1, 2,3-dimethylbutene (710 µl, 5.95 mmol, 20.0 eq.), $NaClO_2$ (134 mg, 1.49 mmol, 5.00 eq.) and NaH_2PO_4 (250 mg, 2.08 mmol, 7.00 eq., dissolved in H_2O) were added, and the clear biphasic mixture was stirred for 45 min at RT. 2 M HCl was added to pH 2 and the aq. phase was extracted with pentane (removal of scavenger). The aq. phase was adjusted to pH 7.5 ($NaHCO_3$) and extracted with $CHCl_3$ (4 x 10 mL). The org. phase was dried over $MgSO_4$ and concentrated to deliver **5** a yellow, viscous resin (64.3 mg, 45% from olefin **18**).

R_f: 0.08 (hexane/EtOAc 3:7)

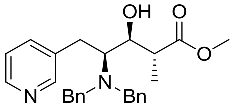
$[\alpha]^{20}_D$: +35.6° (*c* 0.540, $CHCl_3$)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.48 (s, 1H), 8.40 (d, *J* = 3.3 Hz, 1H), 7.53 (d, *J* = 7.9 Hz, 1H), 7.29 – 7.04 (m, 11H), 4.05 (d, *J* = 12.8 Hz, 2H), 3.46 (t, *J* = 5.6 Hz, 1H), 3.39 (d, *J* = 13.4 Hz, 2H), 3.04 – 2.89 (m, 3H), 2.80 – 2.64 (m, 1H), 0.84 (d, *J* = 7.1 Hz, 3H).

^{13}C-NMR: (100 MHz, $CDCl_3$): δ 177.7, 149.5, 146.3, 138.8, 137.8, 136.3, 129.2, 128.5, 127.4, 123.8, 73.0, 61.0, 55.1, 42.2, 28.6, 14.6.

IR: (neat, cm^{-1}): 3411, 3062, 3027, 2973, 2936, 2804, 1713, 1494, 1454, 1423, 1376, 1302, 1266, 1196, 1129, 1090, 1075, 1049, 1027, 1007, 983, 751, 700.

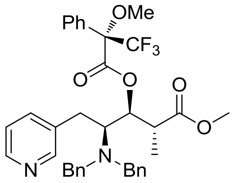
HR-MS: (ESI): *m/z* calc. for $C_{25}H_{29}N_2O_3$ $[M+H]^+$ 405.2173, found 405.2173.



22

Ester 22: Acid **5** (7.90 mg, 19.5 µmol, 1.00 eq.) was dissolved in 0.3 mL MeOH and 0.5 mL toluene. $TMSCHN_2$ (2.0 M in Et_2O, 10.7 µl, 21.5 µmol, 1.10 eq.) was added at 0 °C (after the last drop, the pale yellow color persisted) and the mixture was stirred at 0 °C for 5 min. The reaction was quenched with 3 drops of acetic acid and the solvents were removed *in vacuo*. The residue was purified by FC (hexane/EtOAc 2:3) to yield **22** (6.60 mg, 81%) as a colorless oil.

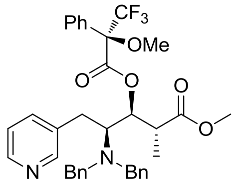
R_f:	0.25 (hexane/EtOAc 2:3)
$[\alpha]^{20}_D$:	+17.7° (*c* 1.39, $CHCl_3$)
^{1}H-NMR:	(400 MHz, $CDCl_3$): δ 8.57 (d, *J* = 1.8 Hz, 1H), 8.52 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.59 (dt, *J* = 7.8, 1.9 Hz, 1H), 7.36 – 7.22 (m, 11H), 4.09 (d, *J* = 13.1 Hz, 2H), 3.55 – 3.49 (m, 1H), 3.46 (s, 3H), 3.42 (d, *J* = 13.3 Hz, 2H), 3.16 – 3.05 (m, 2H), 3.04 – 2.96 (m, 1H), 2.90 – 2.81 (m, 1H), 0.81 (d, *J* = 7.1 Hz, 3H).
^{13}C-NMR:	(100 MHz, $CDCl_3$): δ 175.4, 150.6, 147.7, 139.1, 136.8, 135.8, 129.2, 128.5, 127.3, 123.4, 73.3, 59.7, 54.9, 51.5, 41.9, 28.5, 14.2.
IR:	(neat, cm^{-1}): 3259, 3027, 1733, 1453, 1424, 1262, 1195, 1166, 1129, 1092, 1075, 1028, 1013, 752, 732, 700.
HR-MS:	(ESI): *m/z* calc. for $C_{26}H_{30}N_2NaO_3$ $[M+Na]^+$ 441.2149, found 441.2136.



23

***S*-Mosher ester 23:** To a solution of **22** (6.60 mg, 15.8 µmol, 1.00 eq.) in 0.2 mL CH_2Cl_2 were added pyridine (3.80 µl, 47.3 µmol, 3.00 eq.), DMAP (6.70 mg, 55.2 µmol, 3.50 eq.) and (*R*)-(-)-MTPA-Cl (8.90 µl, 47.3 µmol, 3.00 eq.) at RT. The solution was stirred at RT for 24 h and (*R*)-(-)-MTPA-Cl (17.8 µl, 94.6 µmol, 6.00 eq.) was added. After 3 h, sat. aq. $NaHCO_3$ (1 mL) was added and the aq. phase was extracted with CH_2Cl_2 (3 x 3 mL). The org. phase was dried over $MgSO_4$ and concentrated *in vacuo*. The yellow residue was purified by FC (hexane/EtOAc 1:1) to deliver **23** as a colorless film (1.2 mg, 12%).

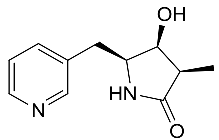
^{1}H-NMR:	(400 MHz, $CDCl_3$): δ 8.46 – 8.37 (m, 2H), 7.50 (dt, *J* = 7.9, 1.9 Hz, 1H), 7.44 (d, *J* = 7.3 Hz, 2H), 7.34 – 7.07 (m, 14H), 5.08 (dd, *J* = 10.2, 1.5 Hz, 1H), 3.84 (d, *J* = 13.3 Hz, 2H), 3.36 (s, 3H), 3.25 (dd, *J* = 10.1, 7.0 Hz, 1H), 3.19 (d, *J* = 13.3 Hz, 2H), 3.11 (s, 3H), 3.03 – 2.92 (m, 3H), 2.47 – 2.38 (m, 1H), 0.11 (d, *J* = 6.9 Hz, 3H).



24

***R*-Mosher ester 24:** To a stirred solution of (2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoic acid (6.00 mg, 25.6 µmol, 2.10 eq.), Et$_3$N (3.73 µl, 26.8 µmol, 2.20 eq.), and DMAP (3.30 mg, 26.8 µmol, 2.20 eq.) in 50 µl toluene were added 2,4,6-trichlorobenzoyl chloride (4.00 µl, 25.6 µmol, 2.10 eq.) and a solution of **22** (5.10 mg, 12.2 µmol, 1.00 eq.) dissolved in 100 µl toluene at RT. The white slurry was stirred at RT for 6 h and was heated to 45 °C for 18 h. The yellow mixture was concentrated *in vacuo* and purified by FC (hexane/EtOAc 1:1) to deliver **24** as a colorless film (0.8 mg, 10%).

^{1}H-NMR: (400 MHz, CDCl$_3$): δ 8.48 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.35 (t, *J* = 2.8 Hz, 1H), 7.62 (d, *J* = 8.1 Hz, 2H), 7.48 – 7.14 (m, 15H), 5.03 (dd, *J* = 9.9, 2.1 Hz, 1H), 3.75 (d, *J* = 13.5 Hz, 2H), 3.54 (s, 3H), 3.51 (s, 3H), 3.28 – 3.19 (m, 1H), 3.12 (d, *J* = 13.4 Hz, 2H), 2.96 (ddd, *J* = 9.6, 4.0, 2.0 Hz, 1H), 2.80 (dd, *J* = 13.9, 3.7 Hz, 1H), 2.16 (dd, *J* = 14.0, 9.7 Hz, 1H), 0.19 (d, *J* = 6.9 Hz, 3H).

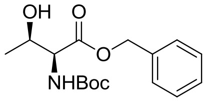


17

Lactam 17: Ester **22** (15.0 mg, 358 µmol, 1.00 eq.) was dissolved in 1 mL MeOH and Pd on charcoal (10%, 4.00 mg) was added under Ar. The atmosphere was exchanged with H$_2$ (1 bar) and the mixture was stirred at RT for 16 h. The suspension was filtered over celite, washed with MeOH and concentrated to yield **17** as a white solid (5.7 mg, 67%). For X-ray crystallography, **17** was crystallized from MeOH at RT by slow evaporation of the solvent.

R$_f$: 0.15 (CH$_2$Cl$_2$/MeOH 5%)
$[\alpha]^{20}_{D}$: +2.01° (*c* 0.570, MeOH)
^{1}H-NMR: (400 MHz, MeOD): δ 8.51 (d, *J* = 1.7 Hz, 1H), 8.40 (dd, *J* = 4.9, 1.5 Hz, 1H), 7.83 (dt, *J* = 7.7, 1.7 Hz, 1H), 7.39 (dd, *J* = 7.7, 4.9 Hz, 1H), 4.08 – 4.04 (m, 1H), 3.87 (ddd, *J* = 8.9, 6.1, 4.0 Hz, 1H), 3.09 (dd, *J* = 13.6, 8.8 Hz, 1H), 2.91 (dd, *J* = 13.6, 6.1 Hz, 1H), 2.54 (qd, *J* = 7.3, 5.1 Hz, 1H), 1.13 (d, *J* = 7.3 Hz, 3H).
^{13}C-NMR: (100 MHz, DMSO-d$_6$): δ 177.2, 150.3, 147.3, 136.7, 134.0, 123.3, 69.5, 58.3, 42.5, 31.7, 8.4.
IR: (neat, cm^{-1}): 3246, 2934, 1679, 1579, 1426, 1335, 1255, 1137, 1027, 994, 714, 638, 404.
HR-MS: (ESI): *m/z* calc. for $C_{11}H_{15}N_2O_2$ $[M+H]^+$ 207.1128, found 207.1132.

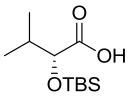
3. Synthesis of Building Blocks 6 and *epi*-6



9

Protected L-Thr 9: L-Thr (2.55 g, 21.4 mmol, 1.00 eq.) was dissolved in 75 mL 50% THF/H_2O. Na_2CO_3 (4.78 g, 45.1 mmol, 2.10 eq.) in 20 mL H_2O was added and the mixture was stirred at RT for 10 min. Boc_2O (5.92 mL, 25.8 mmol, 1.20 eq.) was added and the turbid mixture was stirred at RT for 14 h. It was then diluted with 15 mL H_2O and the pH was adjusted to 4 (aq. HCl 1 M). The aq. phase was extracted with EtOAc (3 x 15 mL), the pH was lowered to 3 (aq. HCl 1 M), NaCl was added to saturation and the aq. phase was again extracted with EtOAc (4 x 20 mL). The combined org. extracts were dried over $MgSO_4$ and concentrated *in vacuo* to yield L-Boc-Thr (3.79 g, 81% crude) as a colorless foam. The protected amino acid (3.79 g, 17.3 mmol, 1.00 eq.) and benzyl bromide (1.63 mL, 18.9 mmol, 1.05 eq.) were dissolved in 100 mL DMF at 0 °C. Cs_2CO_3 (2.93 g, 8.99 mmol, 0.52 eq.) was added and the suspension was stirred at RT for 20 h. H_2O (15 mL) was added and the mixture was extracted with EtOAc (3 x 20 mL). The combined org. extracts were washed with H_2O (1 x 10 mL) and brine (2 x 10 mL), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was purified by FC (hexane/EtOAc 9:1 → 3:2) to deliver **9** as a colorless oil (4.59 g, 86%).

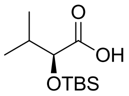
R_f: 0.31 (hexane/EtOAc 9:1)
$[\alpha]^{20}_{D}$: -14.45° (*c* 1.05, $CHCl_3$) (Lit.: -19.6°, C. W. Mosher and L. Goodman, *J. Org. Chem.* **1972***, 37*, 2928-2933)
^{1}H-NMR: (400 MHz, $CDCl_3$): δ 7.37-7.34 (m, 5H), 5.33 (br s , 1H, NH), 5.20 (q, *J* = 11.3 Hz, 2H), 4.35-4.27 (m, 2H), 2.07 (br s, 1H, OH), 1.44 (s, 9H), 1.23 (d, *J* = 6.32 Hz, 3H).
^{13}C-NMR: (100 MHz, $CDCl_3$): δ 171.3, 156.1, 128.6, 128.6, 128.4, 128.2, 80.1, 68.2, 67.2, 58.8, 28.3, 19.9.
IR: (neat, cm^{-1}): 3437, 2978, 2934, 1743, 1715, 1692, 1500, 1456, 1367, 1253, 1160, 112, 1066, 1000, 880, 752, 736, 698.
HR-MS: (ESI): *m/z* calc. for $C_{16}H_{23}NNaO_5$ $[M+Na]^+$ 332.1468, found 332.1471



8

(*R*)-TBS-protected alcohol 8: The compound was prepared according to I. Gonzáles *et al.*, *J. Chem. Soc., Perkin Trans. 1*, **1996**, 1427-1433 while the analytics are compared to: M. Kusakabe *et al.*, *J. Org. Chem*. **1989**, *54*, 2085-2091. (*R*)-2-Hydroxy-3-methylbutyric acid (305 mg, 2.58 mmol, 1.00 eq.), TBS-Cl (934 mg, 6.20 mmol, 2.40 eq.) and imidazole (1.07 g, 12.4 mmol, 4.80 eq) were dissolved in 3.5 mL DMF at RT and the mixture was stirred for 24 h. It was then diluted with EtOAc (65 mL), washed with sat. aq. citric acid, sat. aq. $NaHCO_3$ and brine (3 x 10 mL each), dried over $MgSO_4$ and concentrated. The resulting oil was dissolved in 22 mL MeOH and the solution was cooled to 0 °C. 650 mg K_2CO_3 in 8 mL H_2O were added and the mixture was stirred at RT for 2.5 h. The solution was adjusted to pH 4 (aq. HCl 1 M) and the aq. phase was extracted with EtOAc (3 x 10 mL). The combined org. extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The colorless oil was purified by FC (hexane/EtOAc 6:1 → 4:1) to deliver **8** as a colorless oil (339 mg, 57% over 2 steps).

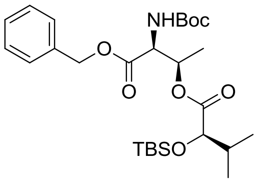
R_f: 0.25 (10% MeOH in CH_2Cl_2)
$[\alpha]^{20}_D$: +18.31° (*c* 0.942, CH_2Cl_2)
^{1}H-NMR: (400 MHz, $CDCl_3$) δ 4.05 (d, *J* = 4.0 Hz, 1H), 2.16 – 2.01 (m, 1H), 0.97 (d, *J* = 6.9 Hz, 3H), 0.95 – 0.91 (m, 12H), 0.09 (s, 6H).
^{13}C-NMR: (101 MHz, $CDCl_3$) δ 176.9, 76.7, 32.8, 25.7, 18.8, 16.7, -5.2.
HR-MS: (ESI): *m/z* calc. for $C_{11}H_{23}O_3Si$ $[M-H]^-$ 231.1422, found 231.1424.



ent-**8**

***(S)*-TBS-protected alcohol** ***ent*-8:** The compound was prepared according to I. Gonzáles *et al.*, *J. Chem. Soc., Perkin Trans. 1*, **1996**, 1427-1433. (*S*)-2-Hydroxy-3-methylbutyric acid (1.04 g, 8.80 mmol, 1.00 eq.), TBS-Cl (3.19 g, 21.1 mmol, 2.40 eq.) and imidazole (3.64 g, 42.3 mmol, 4.80 eq) were dissolved in 11 mL DMF at RT and the solution was stirred for 24 h. The mixture was diluted with EtOAc (200 mL), washed with sat. aq. citric acid, sat. aq. $NaHCO_3$ and brine (3 x 40 mL each), dried over $MgSO_4$ and concentrated *in vacuo*. The resulting oil was dissolved in 70 mL MeOH and the solution was cooled to 0 °C. 2 g K_2CO_3 in 24 mL H_2O were added and the mixture was stirred at RT for 2.5 h. The pH of the solution was adjusted to 4 (aq. HCl 1 M) and the aq. phase was extracted with EtOAc (3 x 20 mL). The combined org. extracts dried over $MgSO_4$ and concentrated *in vacuo*. The colorless oil was purified by FC (hexane/EtOAc 6:1 → 4:1) to deliver *ent*-**8** as a colorless oil (1.54 g, 75% over 2 steps).

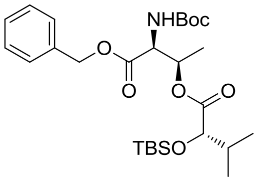
R_f: 0.28 (10% MeOH in CH_2Cl_2)
$[\alpha]^{20}_D$: -16.45° (*c* 0.811, CH_2Cl_2)
^{1}H-NMR: (400 MHz, $CDCl_3$): δ 10.09 (br. S, 1H), 4.06 (d, *J* = 4.0 Hz, 1H), 2.14-2.04 (m, 1H), 0.98 (d, *J* = 6.2 Hz, 3H), 0.94-0.93 (m, 12H, overlapping signals), 0.09 (s, 6H).
^{13}C-NMR: (100 MHz, $CDCl_3$): δ 176.8, 76.7, 32.8, 25.7, 18.7, 18.2, 16.7, -5.2.
HR-MS: (ESI): *m/z* calc. for $C_{11}H_{23}O_3Si$ $[M-H]^-$ 231.1422, found 231.1426.



25

(*R*)-TBS-ether 25: To a stirred solution of **8** (250 mg, 1.08 mmol, 1.00 eq.) and Et$_3$N (449 µl, 3.23 mmol, 3.00 eq.) in 10 mL toluene was added 2,4,6-trichlorobenzoyl chloride (210 µl, 1.35 mmol, 1.25 eq.). As the mixture became turbid (white precipitate) a solution of **9** (350 mg, 1.13 mmol, 1.05 eq.) in 5 mL toluene and DMAP (263 mg, 2.15 mmol, 2.00 eq.) was added at RT. The yellow slurry was stirred at RT for 18 h. Sat. aq. NaHCO$_3$ (3 mL) was added and the aq. phase was extracted with EtOAc (3 x 10 mL). The combined org. extracts were dried over MgSO$_4$ and concentrated *in vacuo*. The yellow residue was purified by FC (hexane/EtOAc 9.5:1) to deliver **25** as a colorless oil (411 mg, 73%).

R$_f$: 0.36, (hexane/EtOAc 9.5:1)
[α]$^{20}_D$: +44.4° (*c* 1.41, CHCl$_3$)
^{1}H-NMR: (400 MHz, CDCl$_3$) δ 7.39 – 7.29 (m, 5H), 5.48 (qd, *J* = 6.3, 2.5 Hz, 1H), 5.23 – 5.13 (m, 2H), 5.04 (d, *J* = 12.2 Hz, 1H), 4.46 (dd, *J* = 9.7, 2.4 Hz, 1H), 3.93 (d, *J* = 4.1 Hz, 1H), 2.00 – 1.89 (m, 1H), 1.46 (s, 9H), 1.30 (d, *J* = 6.4 Hz, 3H), 0.92 (s, 12H), 0.82 (d, *J* = 6.8 Hz, 3H), 0.03 (d, *J* = 6.7 Hz, 6H).
^{13}C-NMR: (100 MHz, CDCl$_3$) δ 172.2, 170.0, 155.9, 134.9, 128.6, 128.5, 128.4, 80.3, 76.5, 70.9, 67.6, 57.3, 32.7, 28.3, 25.7, 19.2, 18.3, 17.1, 16.4, -4.9, -5.4.
IR: (neat, cm^{-1}): 3027, 2934, 2805, 1715, 1496, 1455, 1423, 1302, 1266, 1208, 1129, 1075, 1048, 1027, 981, 751, 700, 500, 471, 435.
HR-MS: (ESI): *m/z* calc. for $C_{27}H_{45}NNaO_7Si$ [M+Na]$^+$ 546.2858, found 546.2857.



26

(*S*)-TBS-ether 26: To a stirred solution of *ent*-**8** (70.5 mg, 303 µmol, 1.00 eq.), Et$_3$N (169 µl, 1.21 mmol, 4.00 eq.) and DMAP (74.1 mg, 607 µmol, 2.00 eq.) in 2 mL toluene was added 2,4,6-trichlorobenzoyl chloride (71.2 µl, 455 µmol, 1.50 eq.). As the mixture became turbid (white precipitate) a solution of **9** (98.7 mg, 319 µmol, 1.05 eq.) dissolved in 2 mL toluene was added at RT. The yellow slurry was stirred at RT for 18 h. Sat. aq. NaHCO$_3$ (1 mL) was added and the aq. phase was extracted with EtOAc (3 x 5 mL). The combined org. extracts were dried over MgSO$_4$ and concentrated *in vacuo*. The yellow residue was purified by FC (hexane/EtOAc 9.5:1) to deliver **26** as a colorless oil (106 mg, 67%).

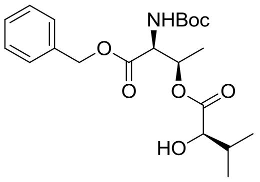
R$_f$: 0.41 (hexane/EtOAc 9.5:1)
[α]$^{20}_D$: +1.43 (*c* 1.36, CHCl$_3$)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 7.42 – 7.28 (m, 5H), 5.54 – 5.44 (m, 1H), 5.20 (d, *J* = 9.8 Hz, 1H), 5.16 (d, *J* = 12.2 Hz, 1H), 5.05 (d, *J* = 12.2 Hz, 1H), 4.47 (dd, *J* = 9.8, 1.7 Hz, 1H), 3.94 (d, *J* = 4.2 Hz, 1H), 2.03 – 1.92 (m, 1H), 1.45 (s, 9H), 1.30 (d, 3H), 0.92 – 0.90 (m, 12H, overlapping signals), 0.83 (d, *J* = 6.8 Hz, 3H), 0.03 (d, *J* = 6.8 Hz, 6H).

^{13}C-NMR: (101 MHz, $CDCl_3$): δ 172.2, 169.9, 155.9, 135.0, 128.6, 128.5, 128.4, 80.2, 76.6, 70.9, 67.6, 57.3, 32.8, 28.3, 25.7, 19.1, 18.2, 17.0, 16.5, -4.9, -5.4.

IR: (neat, cm^{-1}): 2959, 2931, 2858, 1751, 1722, 1500, 1457, 1386, 1367, 1314, 1251, 1163, 1143, 1112, 1083, 1066, 980, 861, 835, 778, 751, 678.

HR-MS: (ESI): *m/z* calc. for $C_{27}H_{46}NO_7Si$ $[M+H]^+$ 524.3038, found 524.3043.



6

Alcohol 6: To a solution of **25** (378 mg, 722 µmol, 1.00 eq.) in 10 mL THF was added HF•py (30%, 3.1 mL in 2 portions) at 0 °C. The solution was stirred at RT for 16 h. The reaction was quenched with sat. aq. $NaHCO_3$ (50 mL) and the solution extracted with EtOAc (3 x 15 mL). The combined org. extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by FC (hexane/EtOAc 4:1) to yield **6** as a colorless oil (254 mg, 86%).

R_f: 0.23 (hexane/EtOAc 4:1)

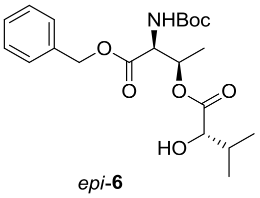
$[\alpha]^{20}_D$: +29.2° (*c* 0.765, $CHCl_3$)

^{1}H-NMR: (400 MHz, $CDCl_3$): δ 7.40 – 7.30 (m, 5H), 5.49 (qd, *J* = 6.2, 2.5 Hz, 1H), 5.24 – 5.13 (m, 2H), 5.07 (d, *J* = 12.1 Hz, 1H), 4.52 (dd, *J* = 9.6, 2.4 Hz, 1H), 3.96 (dd, *J* = 5.9, 3.2 Hz, 1H), 2.56 (d, *J* = 6.1 Hz, 1H), 2.01 – 1.89 (m, 1H), 1.46 (s, 9H), 1.33 (d, *J* = 6.4 Hz, 3H), 0.99 (d, *J* = 6.9 Hz, 3H), 0.76 (d, *J* = 6.8 Hz, 3H).

^{13}C-NMR: (101 MHz, $CDCl_3$): δ 173.8, 169.8, 155.8, 134.8, 128.7, 128.7, 128.4, 80.5, 75.1, 72.5, 67.8, 57.1, 31.9, 28.3, 18.9, 16.9, 15.5.

IR: (neat, cm^{-1}): 3460, 2974, 2936, 1740, 1717, 1501, 1456, 1384, 1368, 1346, 1315, 1282, 1248, 1213, 1164, 1136, 1085, 1063, 1031, 997, 698.

HR-MS: (ESI): *m/z* calc. for $C_{21}H_{31}NNaO_7$ $[M+Na]^+$ 432.1993, found 432.1984.

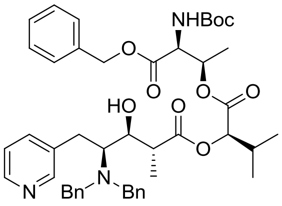


epi-**6**

Alcohol *epi*-6: To a solution of **26** (460 mg, 878 µmol, 1.00 eq.) in 12 mL THF was added HF•py (30%, 2 mL in 2 portions) at 0 °C. The solution was stirred at RT for 1 h. More HF•py (30%, 1 mL) was added and the mixture was stirred at RT for 16 h. It was then quenched with sat. aq. $NaHCO_3$ (55 mL) and extracted with EtOAc (3 x 15 mL). The combined org. extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by FC (hexane/EtOAc 4:1) to yield *epi*-**6** as a colorless oil (344 mg, 96%).

R$_f$:	0.21 (hexane/EtOAc 4:1)
$[\alpha]^{20}_D$:	+2.58° (*c* 1.91, $CHCl_3$)
^{1}H-NMR:	(400 MHz, $CDCl_3$): δ 7.41 – 7.29 (m, 5H), 5.50 (dd, *J* = 6.3, 2.2 Hz, 1H), 5.23 – 5.05 (m, 3H), 4.52 (dd, *J* = 9.6, 2.0 Hz, 1H), 3.76 (dd, *J* = 5.8, 3.4 Hz, 1H), 2.49 (d, *J* = 5.9 Hz, 1H), 1.98 (qd, *J* = 6.9, 3.4 Hz, 1H), 1.46 (s, 9H), 1.30 (d, *J* = 6.4 Hz, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.80 (d, *J* = 6.8 Hz, 3H).
^{13}C-NMR:	(100 MHz, $CDCl_3$): δ 173.8, 169.8, 155.8, 135.0, 128.7, 128.6, 128.5, 80.5, 74.5, 72.1, 67.7, 57.1, 31.9, 28.3, 18.8, 16.8, 15.7.
IR:	(neat, cm^{-1}): 3449, 2971, 2936, 1739, 1716, 1500, 1456, 1384, 1367, 1316, 1248, 1213, 1163, 1083, 1062, 1031, 996, 753, 698.
HR-MS:	(ESI): *m/z* calc. for $C_{21}H_{31}NNaO_7$ $[M+Na]^+$ 432.1993, found 432.1998.

4. Synthesis of Analogs 2 and 3

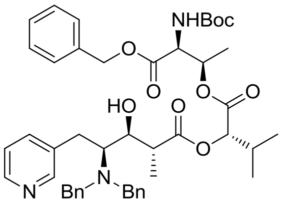


19

Ester 19: To a stirred solution of **5** (40.0 mg, 98.9 µmol, 1.00 eq.) and 2,4,6-trichlorobenzoyl chloride (27.1 µl, 173 µmol, 1.75 eq.) in 0.6 mL THF was added Et_3N (41.2 µl, 297 µmol, 3.00 eq.) at -78 °C. The mixture was stirred for 5 min and a solution of **6** (44.5 mg, 109 µmol, 1.10 eq.) and DMAP (15.7 mg, 129 µmol, 1.30 eq.) in 0.5 mL toluene was added at -78 °C. The clear solution was stirred at -78 °C for 30 min and then slowly warmed to -35 °C. The turbid mixture was stirred at that temperature for 43 h and allowed to warm to 0 °C for the last 25 min. The reaction was quenched at 0 °C with sat. aq. $NaHCO_3$ (2 mL). The aq. phase was extracted with EtOAc (3 x 10 mL) and the combined org. extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The yellow oil was purified by FC (hexane/EtOAc 3:2) to yield **19** as a colorless film (43.0 mg, 50%).

R$_f$:	0.24 (hexane/EtOAc 3:2)
$[\alpha]^{20}_D$:	+49.3° (*c* 1.12, $CHCl_3$)
^{1}H-NMR:	(400 MHz, $CDCl_3$): δ 8.45 (d, *J* = 1.7 Hz, 1H), 8.35 (dd, *J* = 4.7, 1.2 Hz, 1H), 7.51 (dt, *J* = 7.7, 1.7 Hz, 1H), 7.28 – 7.05 (m, 16H), 5.62 (d, *J* = 10.0 Hz, 1H), 5.46 (qd, *J* = 6.2, 2.5 Hz, 1H), 5.01 (d, *J* = 12.1 Hz, 1H), 4.93 (d, *J* = 12.1 Hz, 1H), 4.57 (d, *J* = 3.8 Hz, 1H), 4.40 (dd, *J* = 10.0, 2.4 Hz, 1H), 4.16 (br. s, 2H), 3.82 (d, *J* = 4.0 Hz, 1H), 3.39 – 3.26 (m, 3H), 3.22 – 2.97 (m, 3H), 2.77 – 2.64 (m, 1H), 2.10 (qd, *J* = 10.6, 6.8 Hz, 1H), 1.30 (s, 9H), 1.11 (d, *J* = 6.4 Hz, 3H), 0.87 (d, *J* = 6.9 Hz, 3H), 0.83 (d, *J* = 6.8 Hz, 3H), 0.16 (d, *J* = 6.8 Hz, 3H).

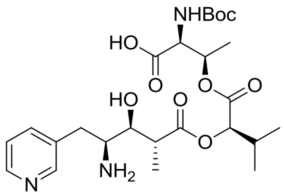
^{13}C-NMR: (100 MHz, $CDCl_3$): δ 175.8, 170.4, 169.0, 156.1, 150.8, 147.6, 140.1, 137.1, 135.8, 134.8, 129.5, 128.6, 128.5, 128.3, 127.1, 123.5, 80.2, 76.6, 73.9, 72.1, 68.0, 59.3, 57.2, 56.0, 42.1, 30.2, 28.3, 27.3, 18.9, 16.8, 16.8, 13.0.
IR: (neat, cm^{-1}): 3489, 2974, 2936, 2359, 1739, 1717, 1497, 1455, 1367, 1316, 1248, 1217, 1162, 1129, 1086, 1061, 987, 943, 753, 700.
HR-MS: (ESI): *m/z* calc. for $C_{46}H_{58}N_3O_9$ $[M+H]^+$ 796.4168, found 796.4166.



epi-**19**

Ester ***epi*-19:** To a stirred solution of **5** (34.0 mg, 84.1 µmol, 1.00 eq.) and 2,4,6-trichlorobenzoyl chloride (23.0 µl, 147 µmol, 1.75 eq.) in 0.5 mL THF was added Et_3N (35.1 µl, 252 µmol, 3.00 eq.) at -78 °C. The mixture was stirred for 5 min and a solution of *epi*-**6** (37.9 mg, 92.5 µmol, 1.10 eq.) and DMAP (13.4 mg, 109 µmol, 1.30 eq.) in 0.4 mL toluene was added at -78 °C. The clear solution was stirred at -78 °C for 30 min and then slowly warmed to -35 °C. The turbid mixture was stirred at that temperature for 45 h and then allowed to warm to 0 °C for the last 25 min. The reaction was quenched at 0 °C with sat. aq. $NaHCO_3$ (2 mL). The aq. phase was extracted with EtOAc (3 x 10 mL) and the combined org. extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The yellow oil was purified by FC (hexane/EtOAc 3:2) to yield *epi*-**19** as a colorless film (47.1 mg, 64%).

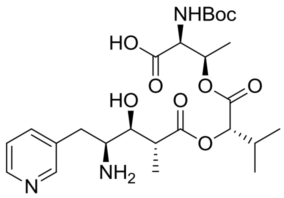
R_f: 0.21 (hexane/EtOAc 3:2)
$[\alpha]^{20}_D$: +45.0° (*c* 0.960, $CHCl_3$)
^{1}H-NMR: (400 MHz, $CDCl_3$): δ 8.42 (d, *J* = 1.7 Hz, 1H), 8.32 (dd, *J* = 4.8, 1.6 Hz, 1H), 7.50 – 7.43 (m, 1H), 7.27 – 7.03 (m, 16H), 5.43 – 5.33 (m, 1H), 5.25 (d, *J* = 9.5 Hz, 1H), 5.02 (d, *J* = 12.0 Hz, 1H), 4.93 (d, *J* = 12.0 Hz, 1H), 4.64 (d, *J* = 3.9 Hz, 1H), 4.37 (dd, *J* = 9.6, 2.5 Hz, 1H), 4.13 (d, *J* = 12.1 Hz, 2H), 3.62 (d, *J* = 4.0 Hz, 1H), 3.38 – 3.27 (m, 3H), 3.16 – 2.99 (m, 3H), 2.69 (dd, *J* = 8.4, 4.2 Hz, 1H), 2.07 – 1.92 (m, 1H), 1.30 (s, 9H), 1.13 (d, *J* = 6.4 Hz, 3H), 0.74 (d, *J* = 6.9 Hz, 3H), 0.67 (d, *J* = 6.8 Hz, 3H), 0.22 (d, *J* = 6.9 Hz, 3H).
^{13}C-NMR: (100 MHz, $CDCl_3$) δ 174.5, 169.7, 169.6, 155.9, 150.7, 147.5, 140.0, 136.9, 136.0, 134.8, 129.3, 128.6, 128.5, 128.4, 127.1, 123.4, 80.3, 75.6, 73.7, 72.4, 68.0, 58.8, 57.1, 55.8, 43.7, 29.9, 28.3, 26.9, 18.6, 16.7, 16.6, 13.2.
IR: (neat, cm^{-1}): 2975, 2935, 1736, 1497, 1455, 1423, 1368, 1311, 1251, 1215, 1164, 1129, 1086, 1062, 1028, 985, 937, 752, 700.,
HR-MS: (ESI): *m/z* calc. for $C_{46}H_{58}N_3O_9$ $[M+H]^+$ 796.4168, found 796.4163.



4

Amino acid 4: To a solution of **19** (41.2 mg, 51.8 µmol, 1.00 eq.) in 1 mL MeOH was added Pd on charcoal (10%, 22.0 mg, 20.7 µmol, 0.400 eq.) under Ar. The Ar was exchanged for H_2 (1 bar) and the mixture was stirred at RT for 5 h. The suspension was filtered through celite, the filter cake was washed with MeOH, and the filtrate was concentrated *in vacuo* to yield **4** as a white solid which was used crude in the next step (27.6 mg, quant.).

$[\alpha]^{20}_{D}$:	+15.3° (*c* 1.32, MeOH)
^{1}H-NMR:	(400 MHz, D_2O): δ 8.82 (d, *J* = 1.4 Hz, 1H), 8.78 (d, *J* = 5.6 Hz, 1H), 8.65 – 8.58 (m, 1H), 8.10 (dd, *J* = 8.0, 5.9 Hz, 1H), 5.61 – 5.44 (m, 1H), 4.96 (d, *J* = 4.2 Hz, 1H), 4.34 (d, *J* = 2.9 Hz, 1H), 4.09 – 3.99 (m, 1H), 3.88 (t, *J* = 5.7 Hz, 1H), 3.53 (dd, *J* = 15.0, 6.0 Hz, 1H), 3.27 (dd, *J* = 15.0, 8.7 Hz, 1H), 3.07 (p, *J* = 6.9 Hz, 1H), 2.28 – 2.15 (m, 1H), 1.45 (s, 9H), 1.33 (d, *J* = 6.4 Hz, 3H), 1.26 (d, *J* = 7.0 Hz, 3H), 0.95 (d, *J* = 6.9 Hz, 3H), 0.88 (d, *J* = 6.8 Hz, 3H).
^{13}C-NMR:	(100 MHz, D_2O): δ 174.7, 174.1, 170.7, 158.0, 147.6, 141.8, 140.8, 136.0, 127.7, 81.7, 77.8, 73.1, 71.4, 57.9, 53.3, 42.0, 32.5, 29.8, 27.6, 17.8, 16.3, 16.1, 13.4.
IR:	(neat, cm^{-1}): 3362, 2974, 2935, 2881, 1722, 1505, 1469, 1369, 1311, 1252, 1168, 1129, 1058, 992, 685, 549.
HR-MS:	(ESI): *m/z* calc. for $C_{25}H_{40}N_3O_9$ $[M+H]^+$ 526.2759, found 526.2756.

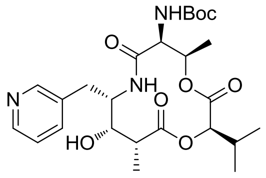


epi-**4**

Amino acid *epi*-4: To a solution *epi*-**19** (24.6 mg, 30.9 µmol, 1.00 eq.) in 0.8 mL MeOH was added Pd on charcoal (10%, 13.2 mg, 12.4 µmol, 0.400 eq.) under Ar. The Ar was exchanged for H_2 (1 bar) and the mixture was stirred at RT for 5 h. The suspension was filtered through celite, the filter cake was washed with MeOH, and the filtrate was concentrated *in vacuo* to yield *epi*-**4** as a white solid which was used crude in the next step (16.4 mg, quant.).

$[\alpha]^{20}_{D}$:	+12.6° (*c* 1.35, MeOH)
^{1}H-NMR:	(400 MHz, D_2O) δ 8.50 (d, *J* = 1.7 Hz, 2H), 7.88 (d, *J* = 7.9 Hz, 1H), 7.52 (dd, *J* = 7.8, 5.0 Hz, 1H), 5.36 (dt, *J* = 10.0, 5.9 Hz, 1H), 4.82 – 4.80 (m, 1H), 4.14 – 4.05 (m, 1H), 3.92 – 3.74 (m, 2H), 3.23 (dd, *J* = 14.5, 6.9 Hz, 1H), 3.08 (dd, *J* = 14.5, 7.6 Hz, 1H), 2.99 (p, *J* = 7.0 Hz, 1H), 2.28 – 2.14 (m, 1H), 1.45 – 1.43 (m, 1H), 1.42 (s, 9H), 1.22 (d, *J* = 7.1 Hz, 6H), 0.94 (t, *J* = 6.4 Hz, 6H).
^{13}C-NMR:	(100 MHz, D_2O): δ 175.6, 175.1, 170.6, 157.6, 148.7, 147.5, 139.0, 132.0, 124.9, 81.3, 78.1, 73.9, 70.8, 59.3, 53.5, 42.4, 32.8, 29.7 27.6, 17.7, 16.6, 16.3, 13.5.
IR:	(neat, cm^{-1}): 3401, 2975, 2937, 1720, 1596, 1501, 1389, 1250, 1171, 1131, 1055, 715.

HR-MS: (ESI): *m/z* calc. for $C_{25}H_{40}N_3O_9$ $[M+H]^+$ 526.2759, found 526.2752.



20

Protected amine 20: To a solution of DIEA (33.9 µl, 196 µmol, 2.60 eq.) and HATU (48.7 mg, 128 µmol, 1.70 eq.) in 30 mL CH_2Cl_2 and 0.3 mL DMF was added a solution of **4** (39.6 mg, 75.3 µmol, 1.00 eq.) in 20 mL CH_2Cl_2 and 0.2 mL DMF over a period of 4 h at RT (pale yellow color develops). The solution was stirred at RT for 18 h. Sat. aq. $NaHCO_3$ (2 mL) was added and the aq. phase was extracted with CH_2Cl_2 (3 x 5 mL). The combined org. extracts were dried over $MgSO_4$, concentrated *in vacuo*, and the residue was purified by FC (hexane/EtOAc 0.5:10) to deliver **20** as an orange film (24.1 mg, 63%).

R_f: 0.19 (hexane/EtOAc 05:10)

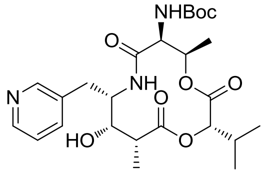
$[\alpha]^{20}_D$: -24.2° (*c* 1.21, MeOH)

^{1}H-NMR: (400 MHz, MeOD): δ 8.46 (d, *J* = 1.7 Hz, 1H), 8.37 (dd, *J* = 4.9, 1.4 Hz, 1H), 7.79 – 7.69 (m, 1H), 7.33 (dd, *J* = 7.7, 5.0 Hz, 1H), 5.27 – 5.12 (m, 1H), 4.68 (d, *J* = 5.1 Hz, 1H), 4.24 (d, *J* = 5.9 Hz, 1H), 4.08 (td, *J* = 7.6, 1.6 Hz, 1H), 3.63 (s, 1H), 3.06 – 2.91 (m, 2H), 2.59 (qd, *J* = 7.2, 1.0 Hz, 1H), 2.27 – 2.16 (m, 1H), 1.45 (s, 9H), 1.37 (d, *J* = 7.4 Hz, 3H), 1.29 (d, *J* = 6.5 Hz, 3H), 0.99 (d, *J* = 4.2 Hz, 3H), 0.97 (d, *J* = 4.0 Hz, 3H).

^{13}C-NMR: (100 MHz, MeOD): δ 178.4, 170.9, 169.3, 157.1, 151.1, 148.0, 139.3, 136.3, 125.0, 81.1, 79.1, 75.7, 70.9, 57.7, 56.8, 42.4, 36.4, 31.1, 28.7, 18.7, 18.0, 17.8, 15.0.

IR: (neat, cm^{-1}): 3350, 2974, 2935, 1744, 1717, 1673, 1503, 1459, 1388, 1370, 1251, 1169, 1049, 1023, 847, 558.

HR-MS: (ESI): *m/z* calc. for $C_{25}H_{38}N_3O_8$ $[M+H]^+$ 508.2653, found 508.2662.



epi-**20**

Protected amine *epi*-20: To a solution of DIEA (23.1 µl, 87.3 µmol, 2.60 eq.) and HATU (33.2 mg, 87.3 µmol, 1.70 eq.) in 20 mL CH_2Cl_2 and 0.2 mL DMF was added a solution of *epi*-**4** (27.0 mg, 51.4 µmol, 1.00 eq.) in 15 mL CH_2Cl_2 and 0.1 mL DMF over a period of 4 h at RT (pale yellow color develops). The solution was stirred at RT for 18 h. Sat. aq. $NaHCO_3$ (2 mL) was added and the aq. phase was extracted with CH_2Cl_2 (3 x 5 mL). The combined org. extracts were dried over $MgSO_4$, concentrated *in vacuo*, and the residue was purified by FC (hexane/EtOAc 0.5:10) to deliver *epi*-**20** as an orange film (20.5 mg, 79%).

R_f: 0.23 (hexane/EtOAc 05:10)

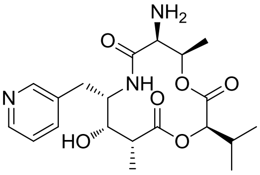
$[\alpha]^{20}_D$: -44.9° (*c* 1.03, MeOH)

^{1}H-NMR: (400 MHz, MeOD): δ 8.46 (d, *J* = 1.6 Hz, 1H), 8.38 (d, *J* = 3.9 Hz, 1H), 7.78 (d, *J* = 7.8 Hz, 1H), 7.35 (dd, *J* = 7.7, 5.0 Hz, 1H), 4.90 – 4.84 (m, 1H), 4.54 (d, *J* = 2.7 Hz, 1H), 4.35 (d, *J* = 4.6 Hz, 1H), 4.04 (t, *J* = 7.2 Hz, 1H), 3.65 (s, 1H), 3.05 (dd, *J* = 13.6, 6.4 Hz, 1H), 2.95 (dd, *J* = 13.6, 8.0 Hz, 1H), 2.44 (qd, *J* = 7.0, 2.0 Hz, 1H), 2.30 (dq, *J* = 13.5, 6.8 Hz, 1H), 1.45 (s, 9H), 1.31 (d, *J* = 7.2 Hz, 3H), 1.19 (d, *J* = 6.2 Hz, 3H), 1.07 (dd, *J* = 6.8, 4.4 Hz, 6H).

^{13}C-NMR: (100 MHz, MeOD): δ 175.5, 173.0, 169.5, 151.1, 148.1, 139.3, 136.2, 130.8, 125.2, 81.2, 79.8, 73.3, 72.1, 57.6, 56.9, 47.5, 37.0, 31.0, 28.7, 19.4, 17.8, 17.2, 13.1.

IR: (neat, cm^{-1}): 3423, 2973, 2933, 1722, 1671, 1492, 1369, 1252, 1169, 1051, 1020, 847, 771, 716, 608, 561, 535, 510, 446.

HR-MS: (ESI): *m/z* calc. for $C_{25}H_{38}N_3O_8$ $[M+H]^+$ 508.2653, found 508.2655.



21

Amine 21: To a solution of **20** (5.00 mg, 9.90 µmol, 1.00 eq.) in 0.6 mL CH_2Cl_2 was added TFA (75.4 µl, 985 mmol, 100 eq.) at 0 °C. The solution was stirred at RT for 3 h. The solvents were removed *in vacuo* to deliver **21** as a yellow oil which was used crude in the next step (10.0 mg, quant.).

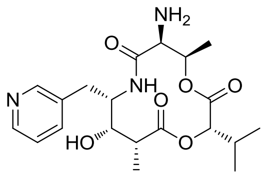
$[\alpha]^{20}_D$: -4.23° (*c* 0.965, MeOH)

^{1}H-NMR: (400 MHz, MeOD): δ 8.78 (s, 1H), 8.71 (d, *J* = 5.2 Hz, 1H), 8.51 – 8.44 (m, 1H), 7.94 (dd, *J* = 7.9, 5.7 Hz, 1H), 5.37 – 5.27 (m, 1H), 4.67 (d, *J* = 6.0 Hz, 1H), 4.32 (td, *J* = 7.2, 1.8 Hz, 1H), 4.04 (d, *J* = 5.7 Hz, 1H), 3.67 (t, *J* = 1.6 Hz, 1H), 3.24 (dd, *J* = 13.9, 7.0 Hz, 1H), 3.13 (dd, *J* = 13.9, 7.6 Hz, 1H), 2.65 (qd, *J* = 7.3, 1.2 Hz, 1H), 2.28 – 2.16 (m, 1H), 1.42 (d, *J* = 6.5 Hz, 3H), 1.38 (d, *J* = 7.4 Hz, 3H), 1.01 (d, *J* = 4.7 Hz, 3H), 0.99 (d, *J* = 4.8 Hz, 3H).

^{13}C-NMR: (100 MHz, MeOD): δ 178.6, 169.0, 166.9, 147.1, 144.7, 142.3, 130.8, 127.8, 79.6, 75.0, 69.0, 57.4, 55.3, 42.2, 36.8, 30.9, 18.5, 17.9, 17.7, 15.1.

IR: (neat, cm^{-1}): 3358, 2971, 2935, 1745, 1672, 1537, 1472, 1392, 1263, 1173, 1138, 1056, 837, 798, 723, 706, 600, 549, 508, 469, 458.

HR-MS: (ESI): *m/z* calc. for $C_{20}H_{30}N_3O_6$ $[M+H]^+$ 408.2129, found 408.2138.



epi-**21**

Amine ***epi*-21:** To a solution of *epi*-**20** (20.5 mg, 40.4 µmol, 1.00 eq.) in 2 mL CH_2Cl_2 was added TFA (309 µl, 4.04 mmol, 100 eq.) at 0 °C. The solution was stirred at RT for 2.4 h. The solvents were removed *in vacuo* to deliver *epi*-**21** as a pale yellow oil which was used crude in the next step (22.0 mg, quant.).

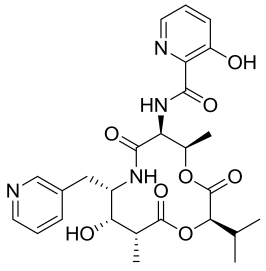
$[\alpha]^{20}_D$: -39.9° (*c* 0.820, MeOH)

^{1}H-NMR: (400 MHz, MeOD): δ 8.83 (s, 1H), 8.74 (d, *J* = 5.6 Hz, 1H), 8.57 (d, *J* = 8.1 Hz, 1H), 8.01 (dd, *J* = 8.0, 5.8 Hz, 1H), 5.11 – 5.01 (m, 1H), 4.55 (d, *J* = 3.0 Hz, 1H), 4.30 (dd, *J* = 12.6, 6.4 Hz, 1H), 4.22 (d, *J* = 4.8 Hz, 1H), 3.79 (d, *J* = 1.9 Hz, 1H), 3.25 (dd, *J* = 13.8, 7.1 Hz, 1H), 3.17 (dd, *J* = 13.9, 7.1 Hz, 1H), 2.57 (qd, *J* = 7.1, 2.3 Hz, 1H), 2.30 (dq, *J* = 13.3, 6.7 Hz, 1H), 1.34 (d, *J* = 7.2 Hz, 3H), 1.28 (d, *J* = 6.0 Hz, 3H), 1.12 – 0.99 (m, 6H).

^{13}C-NMR: (100 MHz, MeOD): δ 175.4, 169.0, 166.4, 148.9, 143.3, 140.8, 140.6, 128.2, 79.7, 73.8, 70.2, 56.0, 55.7, 37.9, 36.5, 31.0, 19.3, 17.8, 16.9, 13.0.

IR: (neat, cm^{-1}): 2973, 2933, 1735, 1673, 1526, 1473, 1282, 1201, 1135, 1069, 837, 798, 757, 722, 483, 470, 458, 444, 409.

HR-MS: (ESI): *m/z* calc. for $C_{20}H_{29}N_3NaO_6$ $[M+Na]^+$ 430.1949, found 430.1938.



2

Dihydropyridomycin 2: To a solution of 3-hydroxypyridine-2-carboxylic acid (1.50 mg, 10.8 µmol, 1.10 eq.), HATU (4.48 mg, 11.8 µmol, 1.20 eq.) and DIEA (5.10 µl, 29.5 µmol, 3.00 eq.) in 100 µl MeCN was added a solution of **21** (4.00 mg, 9.82 µmol, 1.00 eq.) in 0.4 mL MeCN at RT. The mixture was stirred at RT for 18 h. The mixture was diluted with CH_2Cl_2 (1 mL) and sat. aq. $NaHCO_3$ (0.5 mL). The aq. phase was extracted with CH_2Cl_2 (3 x 2 mL) and the combined org. extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The remaining green oil was purified by FC (CH_2Cl_2/MeOH 5%) to yield **2** as a colorless film (2.7 mg, 52% over 2 steps). The samples prepared for biological testing were purified by reversed phase HPLC (Symmetry® C18 5 µm 19x100 mm column, gradient: 30% → 100% MeCN in H_2O in 14 min, flow: 25 mL/min, room temperature, t_R = 6.88 min) to a purity >98%.

R_f: 0.25 (CH_2Cl_2/MeOH 5%)

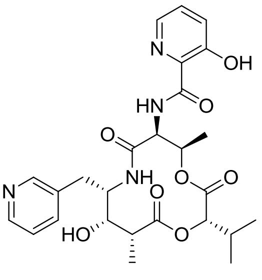
$[\alpha]^{20}_D$: -29.7° (*c* 0.110, MeOH)

^{1}H-NMR: (500 MHz, DMSO-d$_{6}$): δ 11.80 (s, 1H), 8.37 (d, *J* = 1.8 Hz, 1H), 8.30 (d, *J* = 4.7 Hz, 1H), 8.21 – 8.16 (m, 2H), 8.10 (d, *J* = 9.4 Hz, 1H), 7.58 (dd, *J* = 8.5, 4.4 Hz, 1H), 7.55 (dt, *J* = 7.8, 1.9 Hz, 1H), 7.46 (dd, *J* = 8.5, 1.3 Hz, 1H), 7.07 (dd, *J* = 7.7, 4.9 Hz, 1H), 5.29 (p, *J* = 6.4 Hz, 1H), 4.81 – 4.64 (m, 3H), 4.15 – 4.03 (m, 1H), 3.62 (d, *J* = 9.1 Hz, 1H), 2.86 (dd, *J* = 13.5, 6.0 Hz, 1H), 2.77 (dd, *J* = 13.4, 8.7 Hz, 1H), 2.66 – 2.56 (m, 1H), 2.19 – 2.06 (m, *J* = 6.7 Hz, 1H), 1.28 (d, *J* = 7.3 Hz, 3H), 1.21 (d, *J* = 6.5 Hz, 3H), 0.92 (dd, *J* = 6.8, 2.0 Hz, 6H)

^{13}C-NMR: (126 MHz, DMSO-d$_{6}$): δ 176.2, 168.1, 167.5, 157.7, 150.8, 147.6, 140.6, 137.0, 134.0, 130.8, 130.2, 126.8, 123.4, 77.5, 73.9, 68.8, 56.1, 53.3, 41.4, 35.2, 29.8, 18.5, 17.7, 17.4, 15.0.

IR: (neat, cm^{-1}): 3370, 2971, 2938, 1745,1650, 1522, 1450, 1386, 1296, 1254, 1168, 1062, 810, 778, 715, 656.

HR-MS: (ESI): *m/z* calc. for $C_{26}H_{33}N_{4}O_{8}$ [M+H]$^{+}$ 529.2293, found 529.2305.



3

Dihydropyridomycin 3: To a solution of 3-hydroxypyridine-2-carboxylic acid (6.20 mg, 44.5 µmol, 1.10 eq.), HATU (18.5 mg, 48.6 µmol, 1.20 eq.) and DIEA (21.0 µl, 122 µmol, .3.00 eq.) in 0.4 mL MeCN (dark green) was added a solution of *epi*-**21** (16.5 mg, 40.5 µmol, 1.00 eq.) in 1.7 mL MeCN at RT. The mixture was stirred at RT for 24 h. The mixture was diluted with CH_2Cl_2 (2 mL) and sat. aq. $NaHCO_3$ (1 mL). The aq. phase was extracted with CH_2Cl_2 (3 x 5 mL) and the combined org. extracts were dried over $MgSO_4$ and concentrated *in vacuo*. The remaining orange oil was purified by FC (CH_2Cl_2/MeOH 5%) to yield **3** as a colorless film (12.0 mg, 56% over 2 steps). The samples prepared for biological testing were purified by reversed phase HPLC (Symmetry® C18 5 µm 19x100 mm column, gradient: 30% → 100% MeCN in H_2O in 14 min, flow: 25 mL/min, room temperature, t_R = 6.78 min) to a purity >98%.

R_f: 0.30 (CH_2Cl_2/MeOH 5%)

$[\alpha]^{20}_{D}$: -60.5° (*c* 0.110, MeOH)

^{1}H-NMR: (500 MHz, DMSO-d$_{6}$): δ 11.84 (s, 1H), 8.58 (d, *J* = 6.4 Hz, 1H), 8.44 (d, *J* = 1.8 Hz, 1H), 8.30 (dd, *J* = 4.8, 1.6 Hz, 1H), 8.18 (dd, *J* = 4.3, 1.2 Hz, 1H), 7.65 (dt, *J* = 7.8, 1.8 Hz, 1H), 7.60 (d, *J* = 3.4 Hz, 1H), 7.56 (dd, *J* = 8.5, 4.4 Hz, 1H), 7.45 (dd, *J* = 8.5, 1.2 Hz, 1H), 7.21 (dd, *J* = 7.7, 4.8 Hz, 1H), 5.49 (s, 1H), 5.02 (p, *J* = 5.9 Hz, 1H), 4.88 (dd, *J* = 6.9, 5.4 Hz, 1H), 4.54 (d, *J* = 5.3 Hz, 1H), 4.05 (q, *J* = 7.6 Hz, 1H), 3.63 (d, *J* = 4.1 Hz, 1H), 2.85 (qd, *J* = 13.5, 7.2 Hz, 2H), 2.50 – 2.44 (m, 1H), 2.28 – 2.17 (m, 1H), 1.23 (d, *J* = 7.1 Hz, 3H), 1.09 (d, *J* = 6.2 Hz, 3H), 1.01 (dd, *J* = 6.7, 4.6 Hz, 6H).

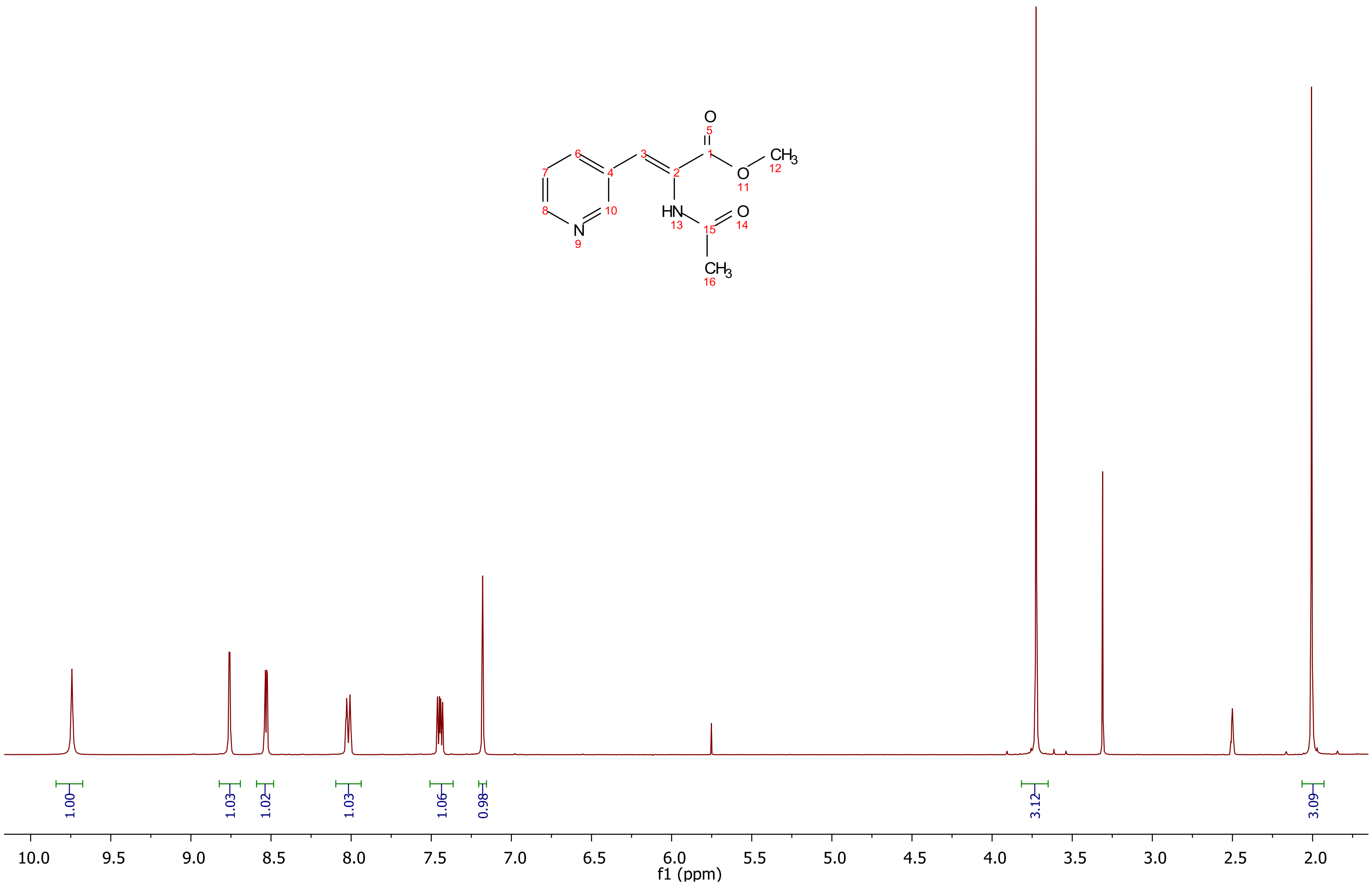
^{13}C-NMR: (126 MHz, DMSO-d$_{6}$): δ 174.0, 168.3, 168.2, 166.7, 157.7, 150.8, 147.8, 140.7, 137.1, 134.6, 130.9, 130.2, 126.8, 123.7, 78.1, 71.7, 69.7, 55.2, 54.0, 45.8, 36.0, 29.8, 19.1, 17.9, 16.6, 13.1.

IR: (neat, cm^{-1}): 3373, 2977, 2942, 1732, 1650, 1510, 1450, 1293, 1257, 1062, 1026, 1013, 811, 783, 717, 662, 589.

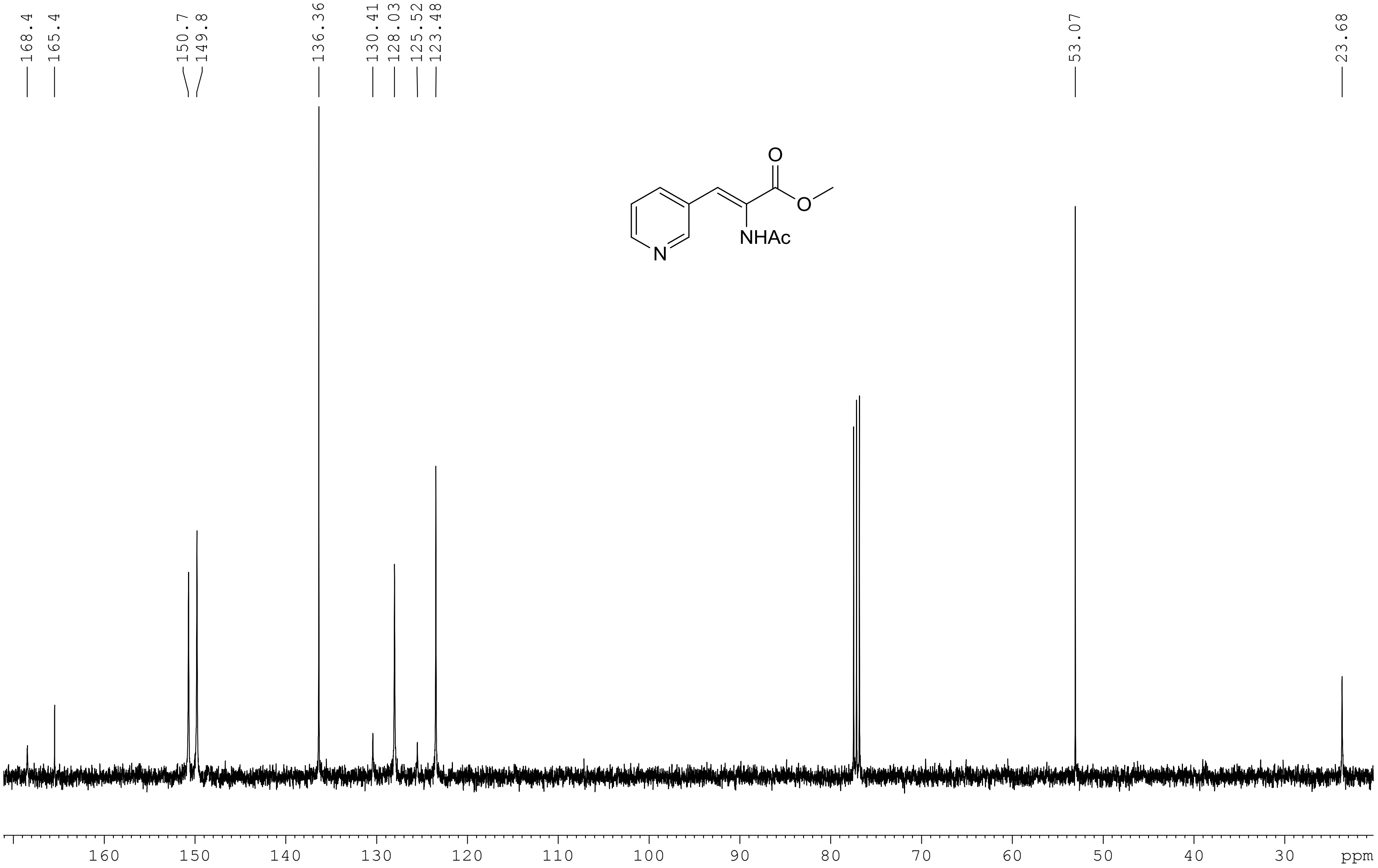
HR-MS: (ESI): *m/z* calc. for $C_{26}H_{33}N_4O_8$ $[M+H]^+$ 529.2293, found 529.2281.

5. ^{1}H- and ^{13}C-NMR Spectra of New Comp

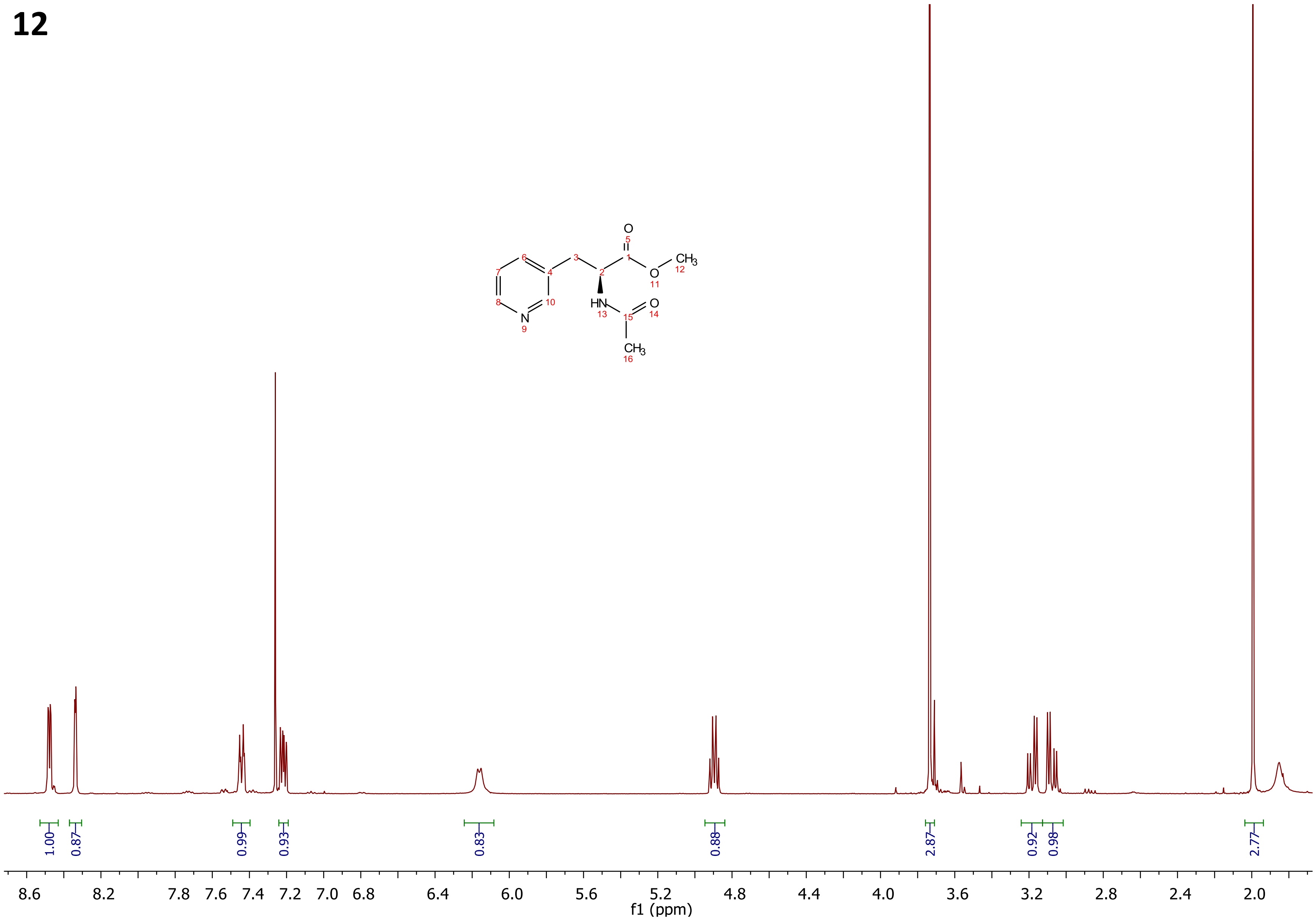
11


O
5
1
CH3
12
O
11
6
3
2
7
4
8
10
N
9
HN
13
15
O
14
CH3
16
1.00
1.03
1.02
1.03
1.06
0.98
3.12
3.09
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
f1 (ppm)

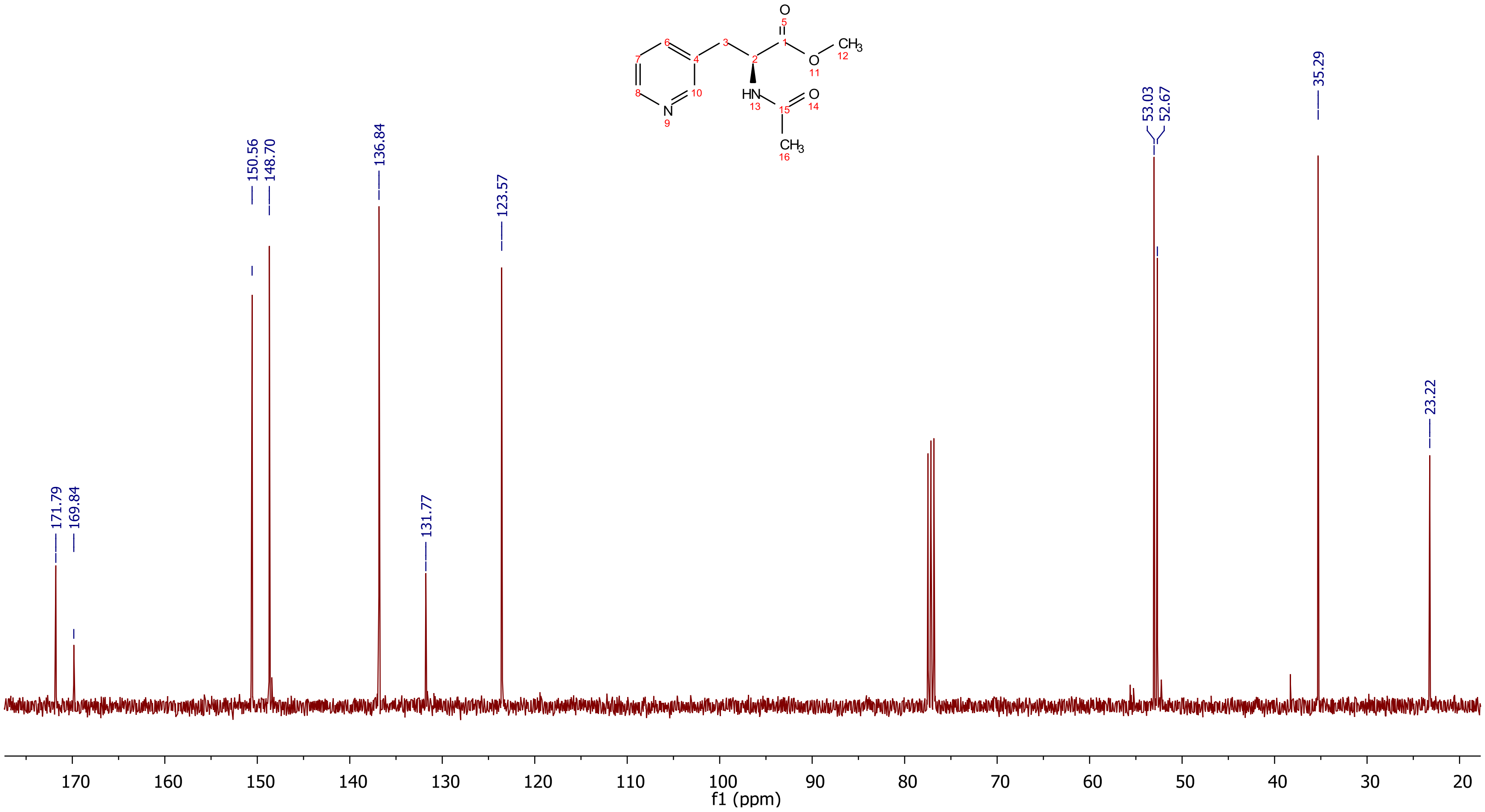
11


168.4
165.4
150.7
149.8
136.36
130.41
128.03
125.52
123.48
53.07
23.68
O
O
N
NHAc
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

12

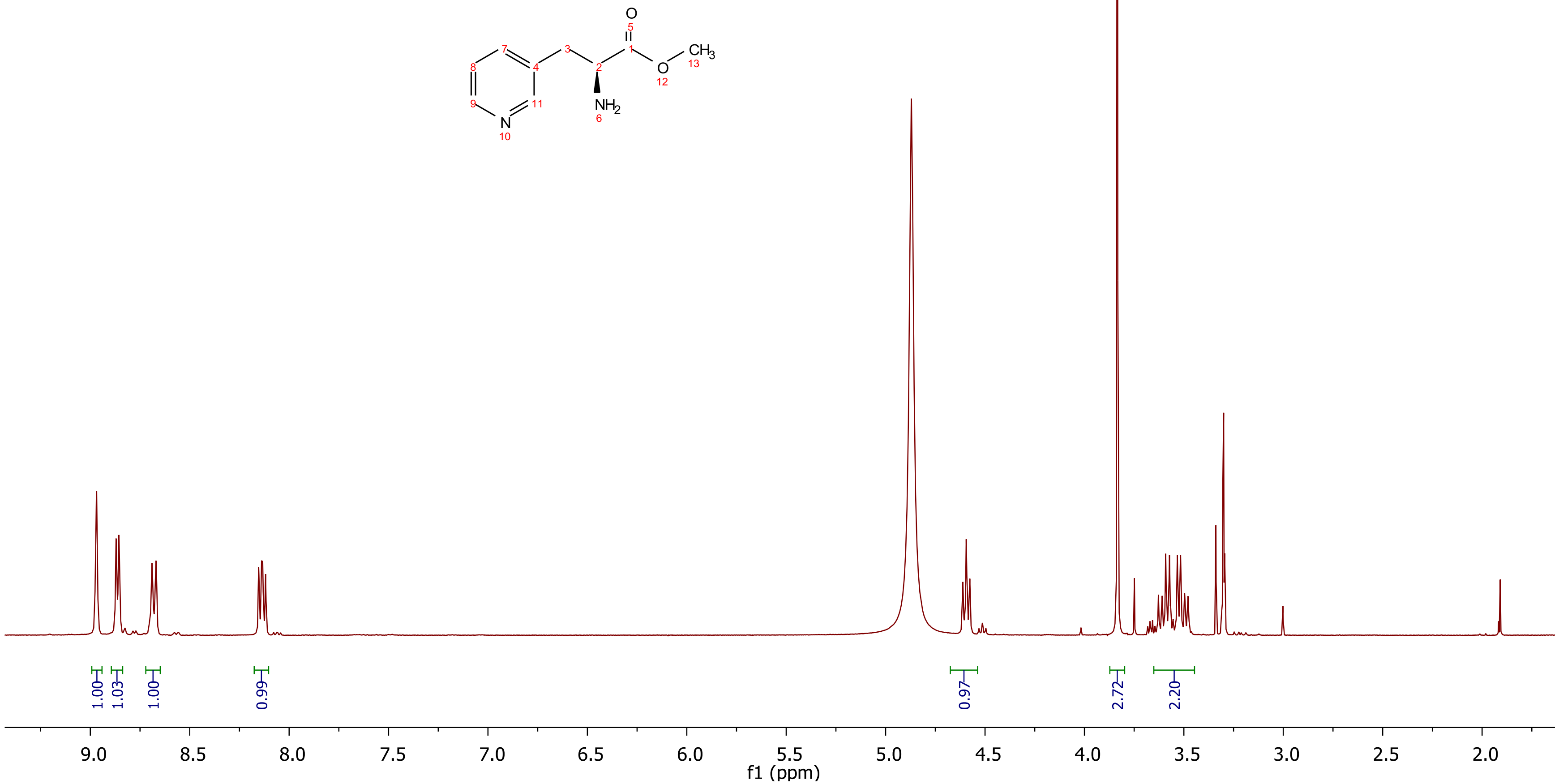

O
CH3
O
HN
O
N
CH3
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
1.00
0.87
0.99
0.93
0.83
0.88
2.87
0.92
0.98
2.77
8.6
8.2
7.8
7.6
7.4
7.2
7.0
6.8
6.4
6.0
5.6
5.2
4.8
4.4
4.0
3.6
3.2
2.8
2.4
2.0
f1 (ppm)

12

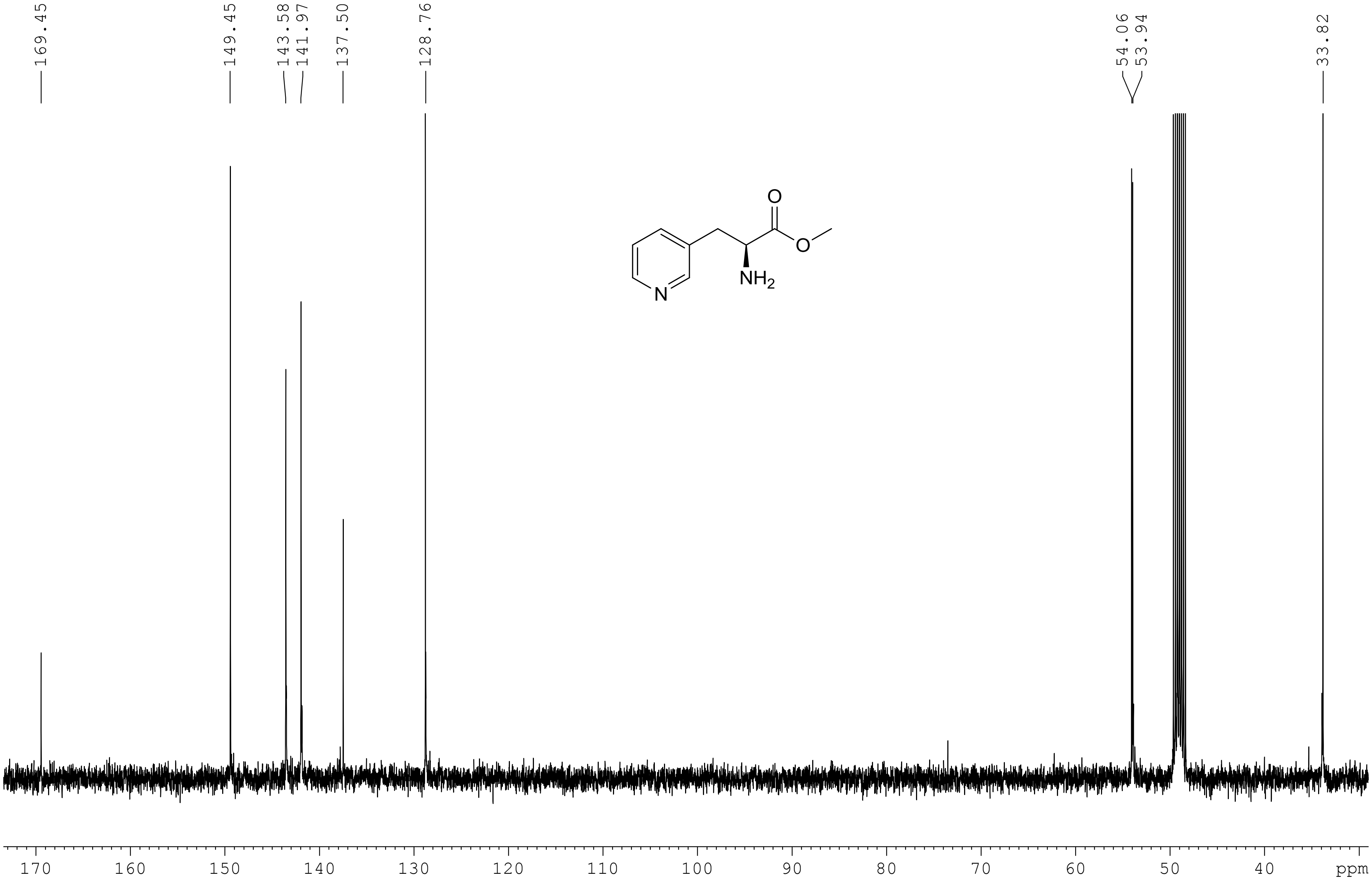


O
5
CH3
1
12
6
3
7
4
2
O
11
8
10
HN
O
N
13
15
14
9
CH3
16
171.79
169.84
150.56
148.70
136.84
131.77
123.57
53.03
52.67
35.29
23.22
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

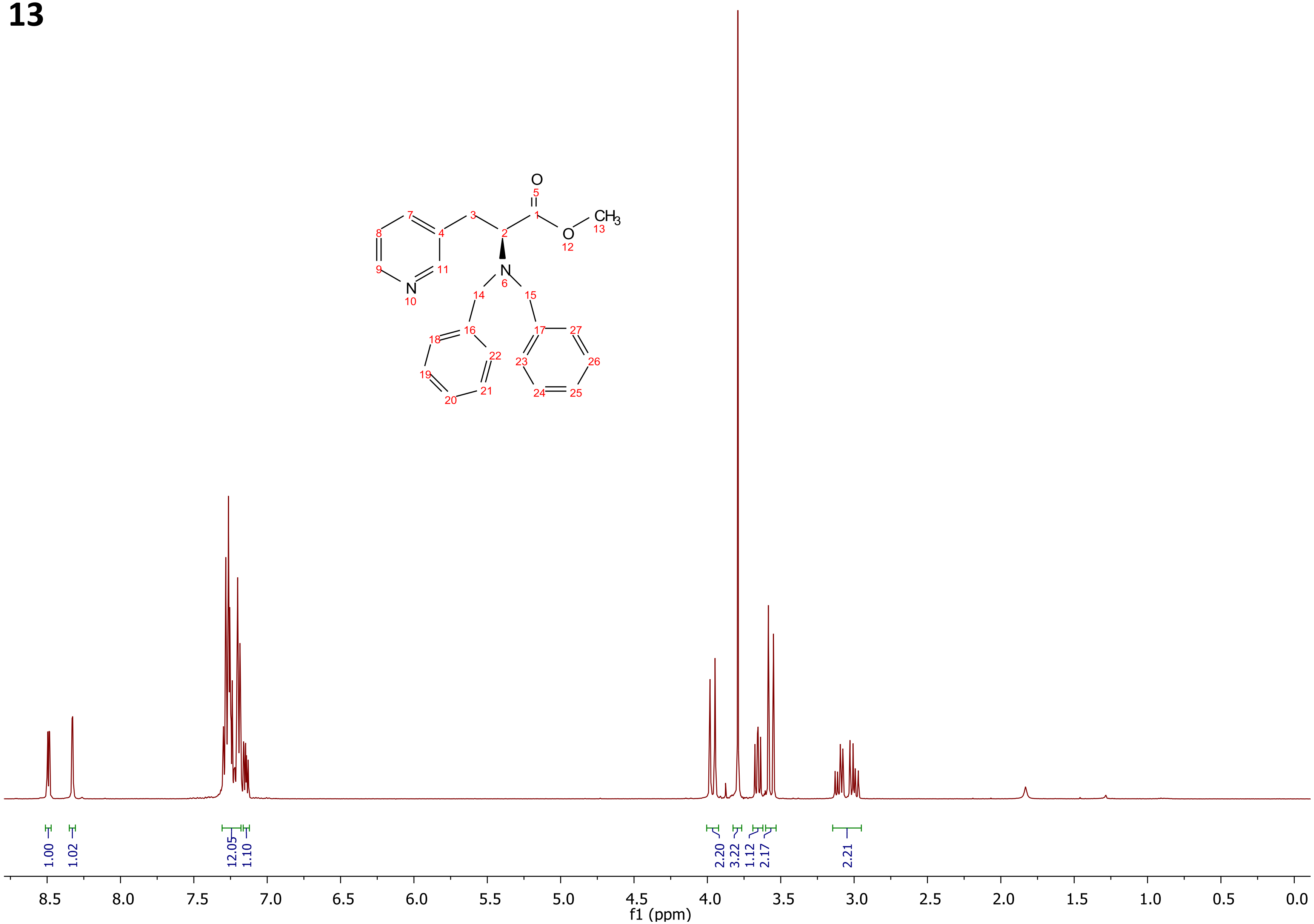
27 (crude)


O
5
1
CH3
13
O
12
2
3
7
8
4
9
11
N
10
NH2
6
1.00
1.03
1.00
0.99
0.97
2.72
2.20
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
f1 (ppm)

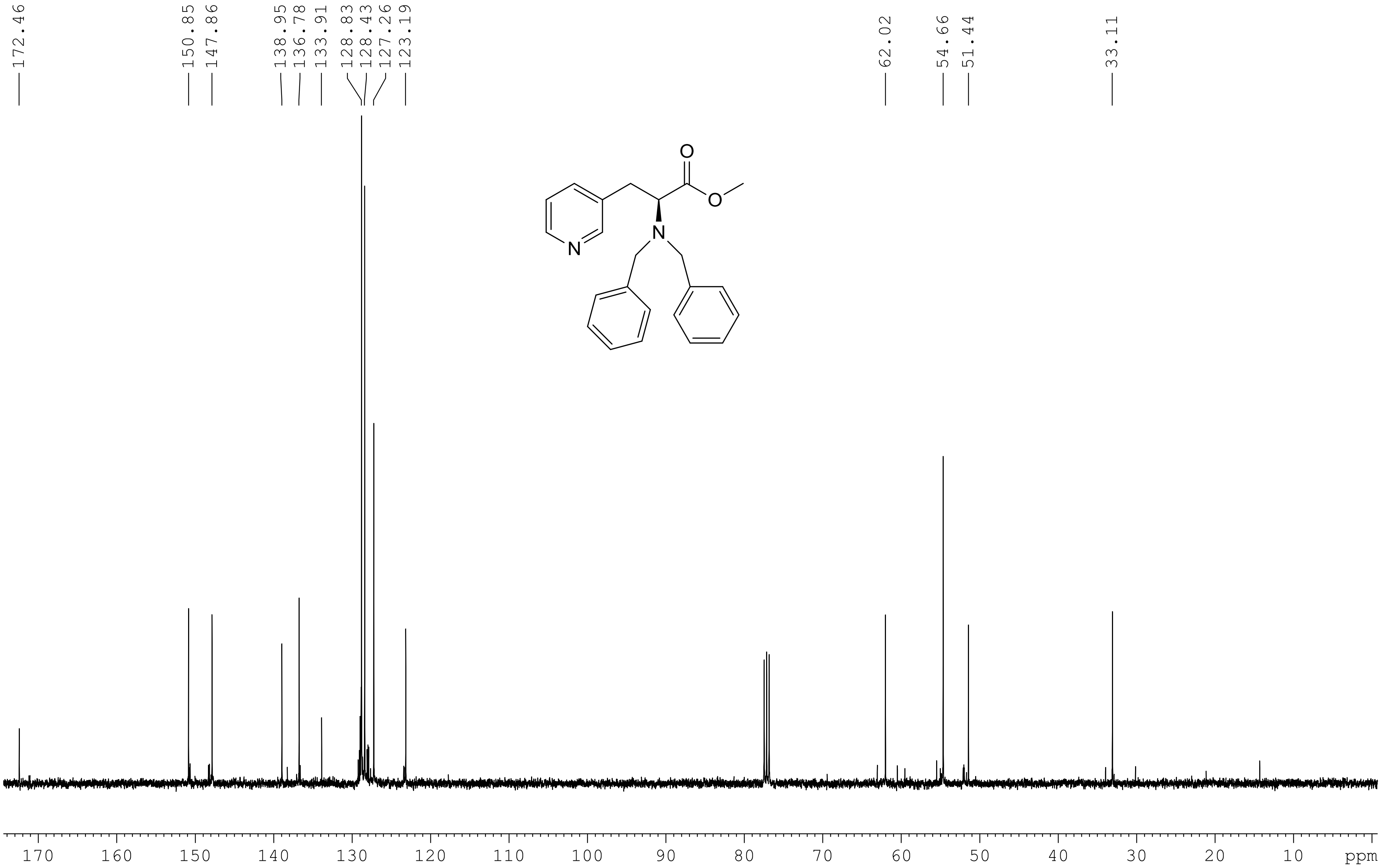
27 (crude)


169.45
149.45
143.58
141.97
137.50
128.76
54.06
53.94
33.82
O
O
N
NH2
170
160
150
140
130
120
110
100
90
80
70
60
50
40
ppm

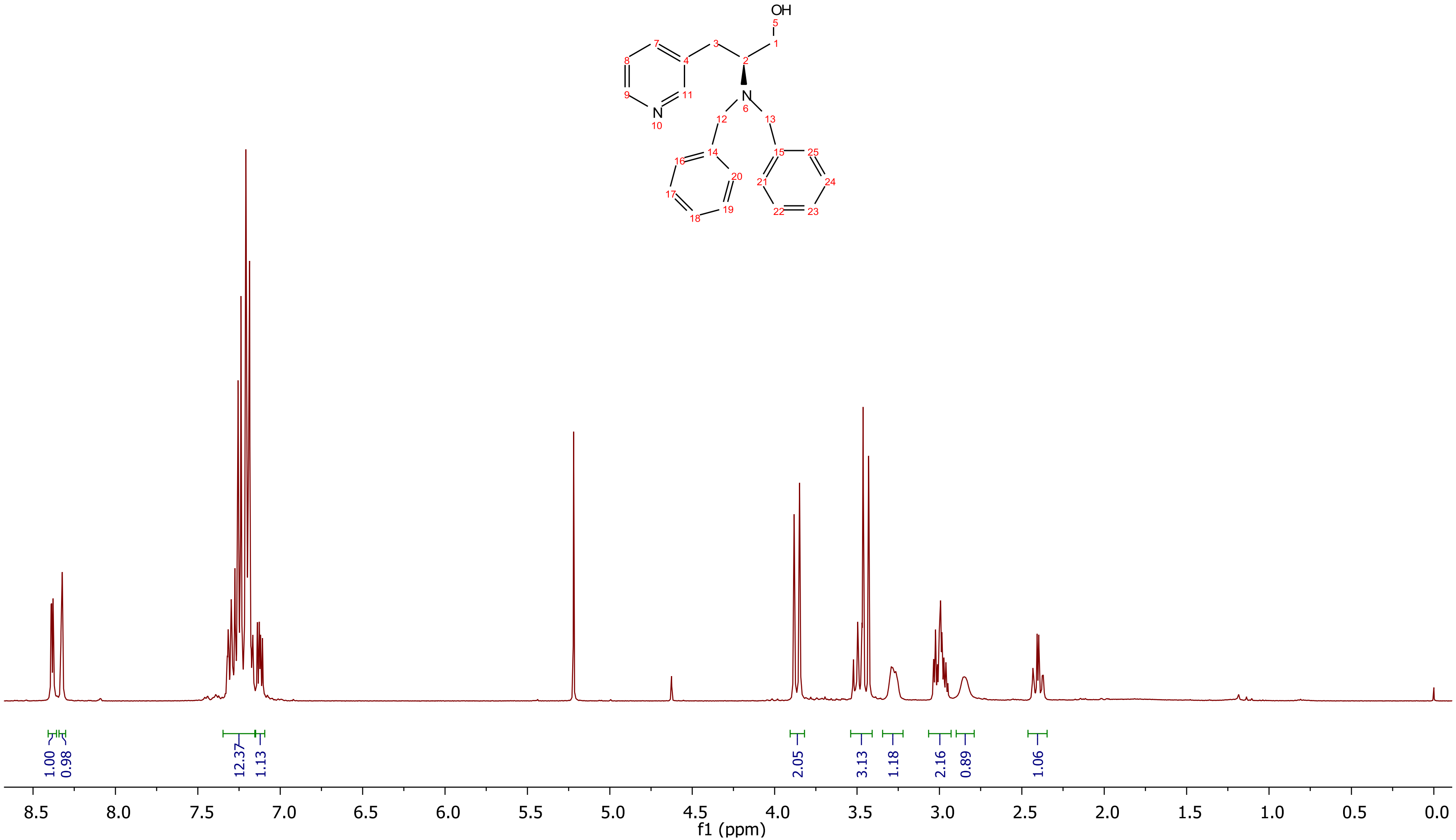
13


O
5
1
CH3
13
O
12
3
2
7
8
4
9
11
N
10
N
6
14
15
16
18
22
19
20
21
17
27
23
26
24
25
1.00
1.02
12.05
1.10
2.20
3.22
1.12
2.17
2.21
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)

13

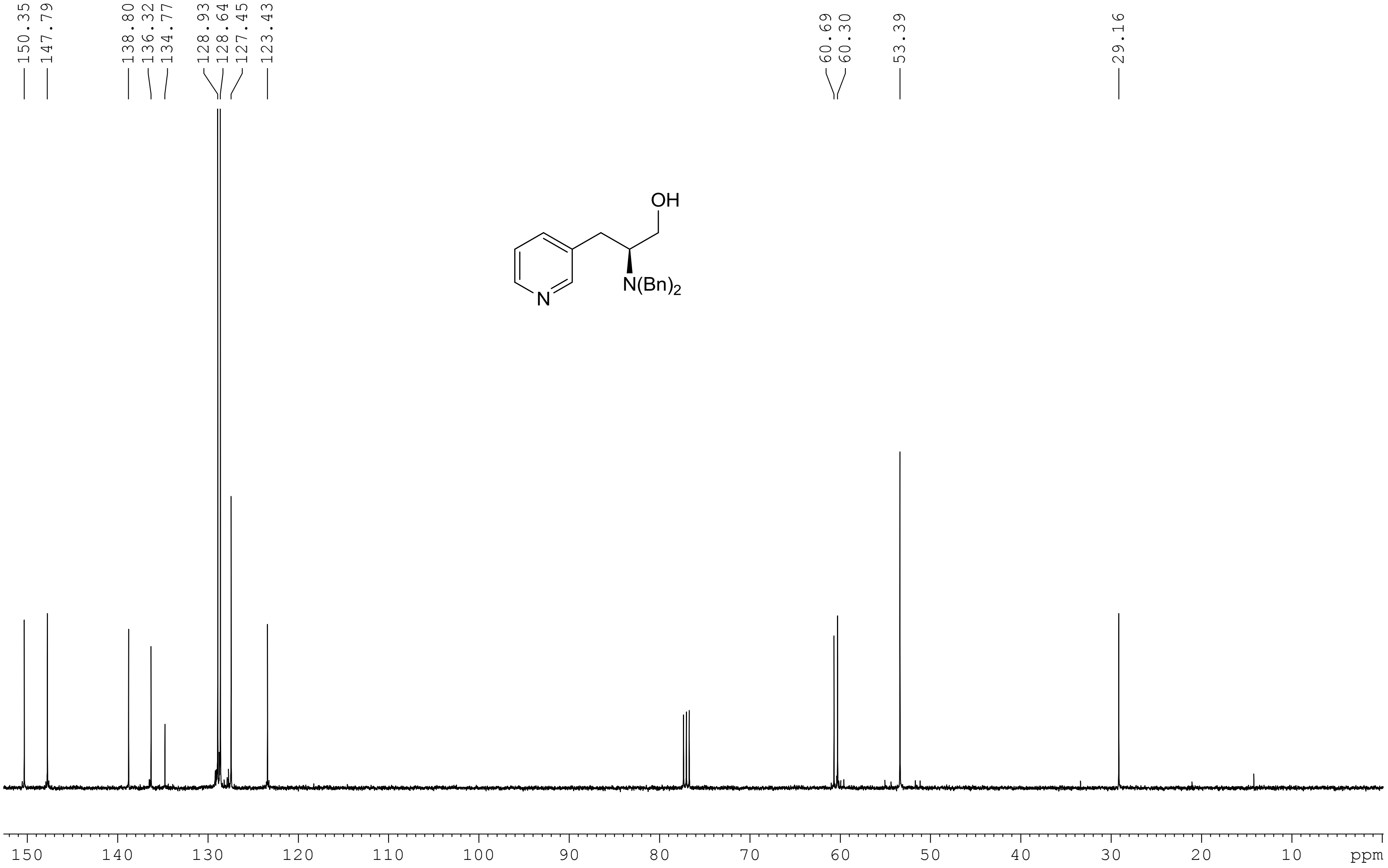

172.46
150.85
147.86
138.95
136.78
133.91
128.83
128.43
127.26
123.19
62.02
54.66
51.44
33.11
O
O
N
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

14

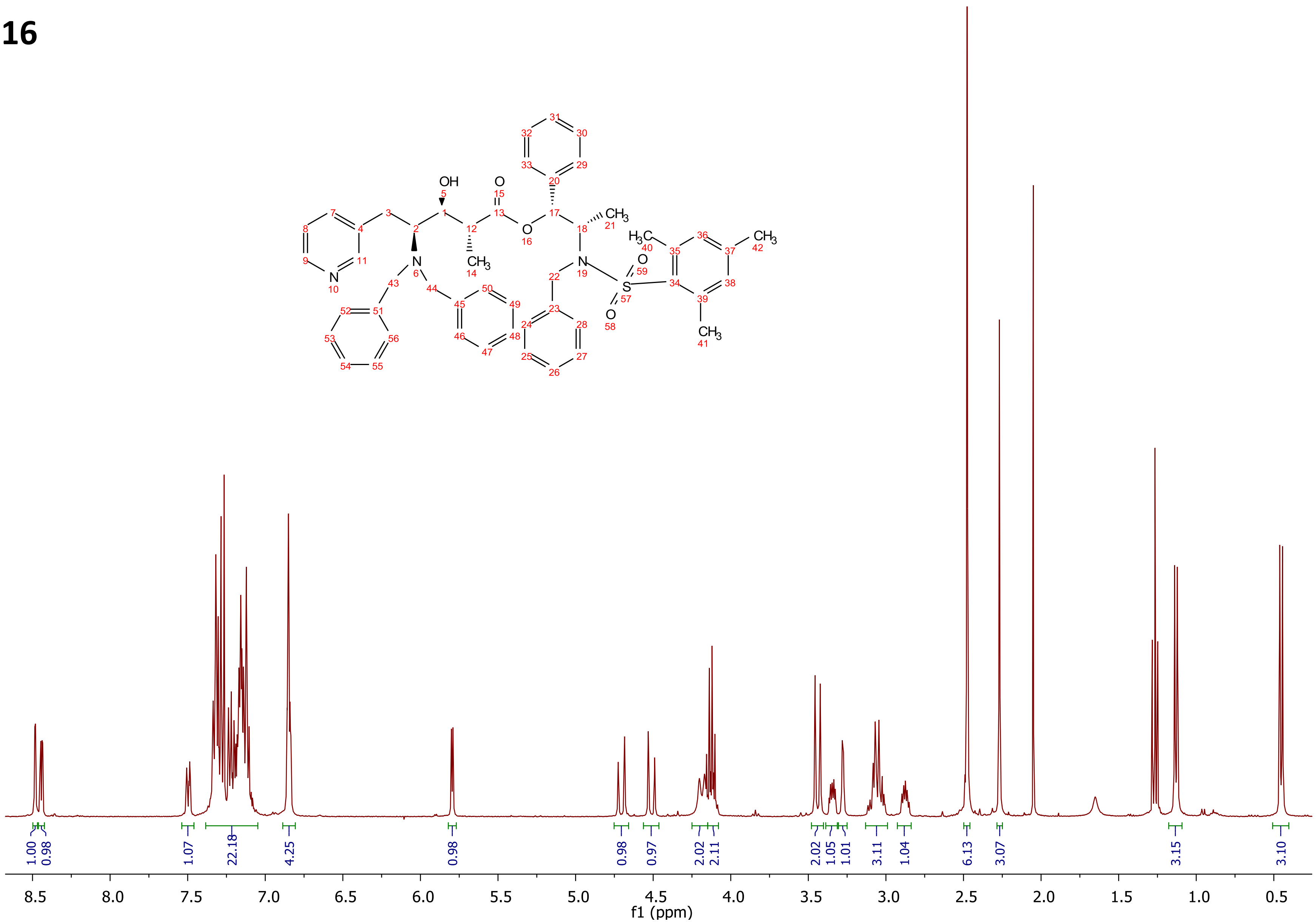


OH
N
N
1.00
0.98
12.37
1.13
2.05
3.13
1.18
2.16
0.89
1.06
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

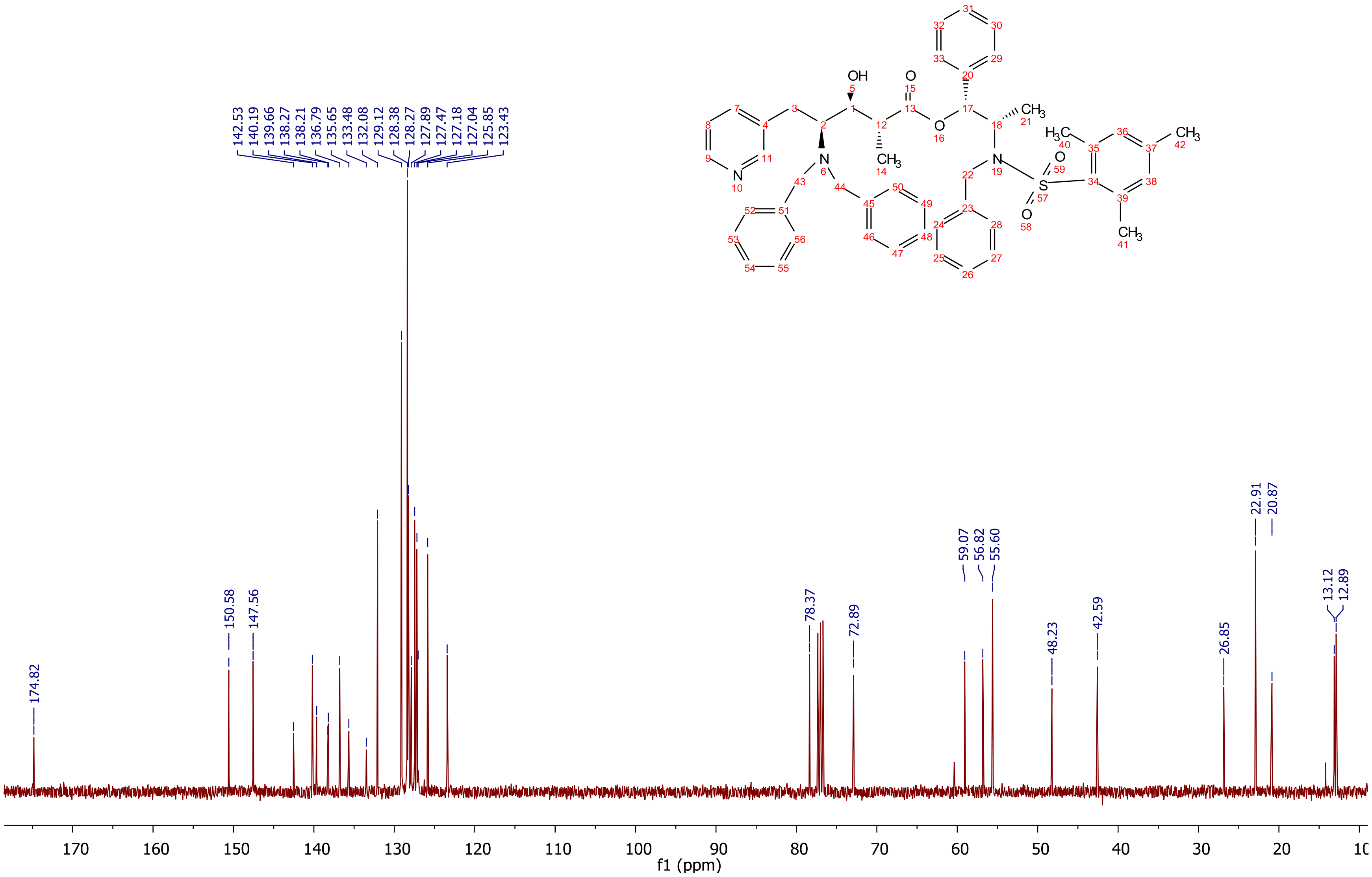
14


150.35
147.79
138.80
136.32
134.77
128.93
128.64
127.45
123.43
60.69
60.30
53.39
29.16
OH
N
N(Bn)$_2$
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

16

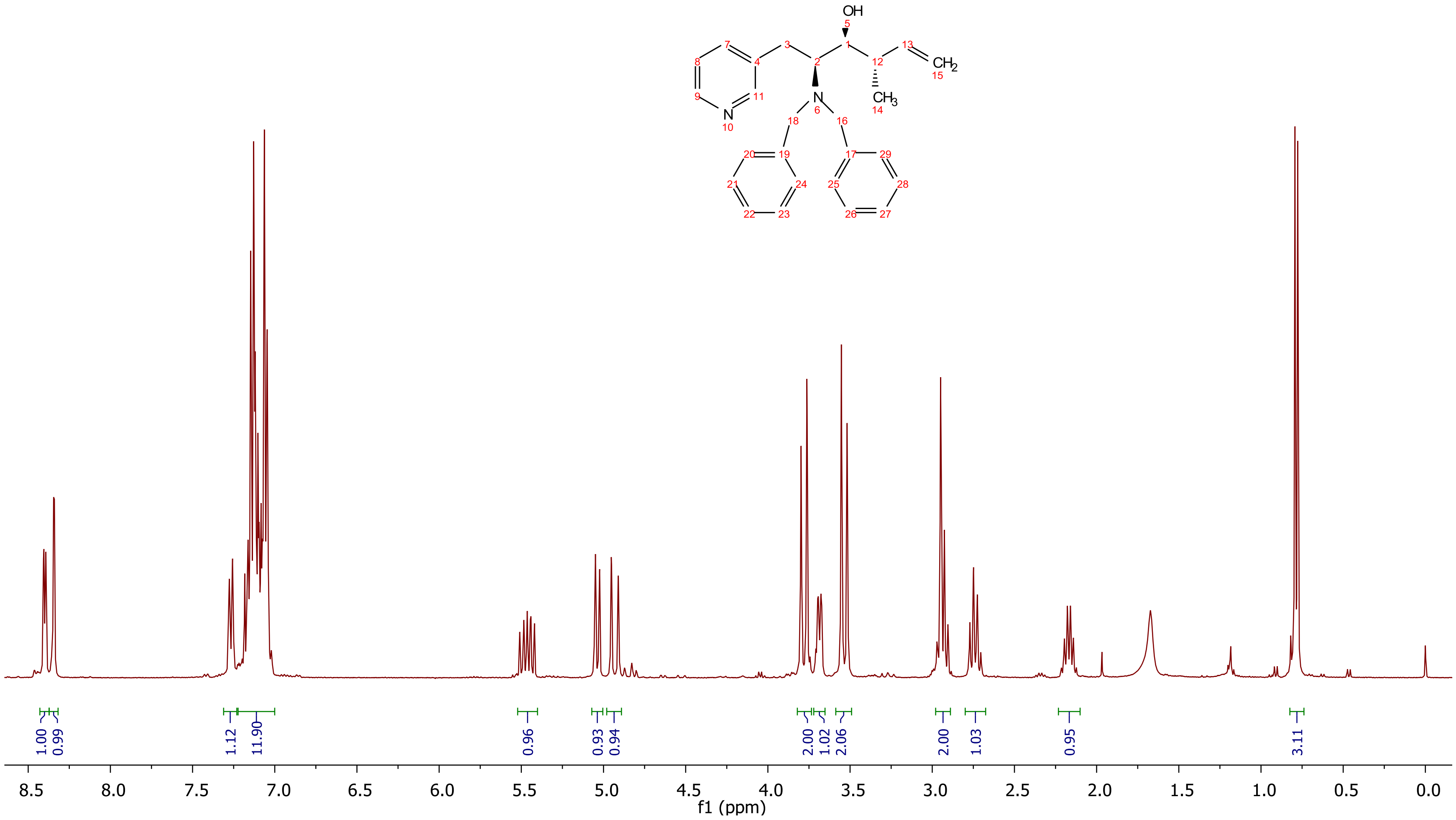

OH
O
CH3
N
O
N
H3C
CH3
S
O
O
CH3
CH3
1.00
0.98
1.07
22.18
4.25
0.98
0.98
0.97
2.02
2.11
2.02
1.05
1.01
3.11
1.04
6.13
3.07
3.15
3.10
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

16

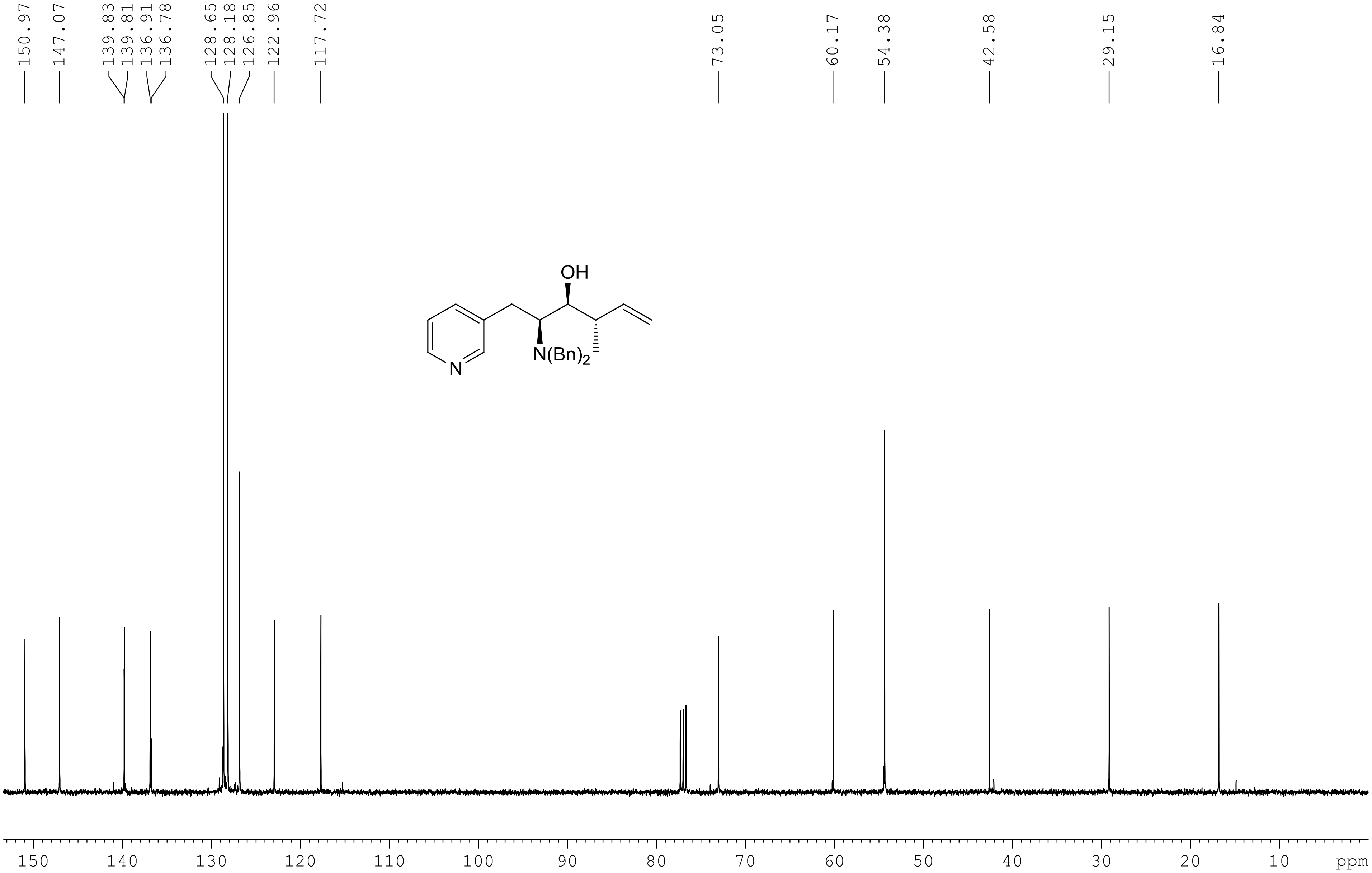


142.53
140.19
139.66
138.27
138.21
136.79
135.65
133.48
132.08
129.12
128.38
128.27
127.89
127.47
127.18
127.04
125.85
123.43
174.82
150.58
147.56
78.37
72.89
59.07
56.82
55.60
48.23
42.59
26.85
22.91
20.87
13.12
12.89
OH
O
N
O
CH3
CH3
H3C
CH3
N
O
S
O
CH3
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

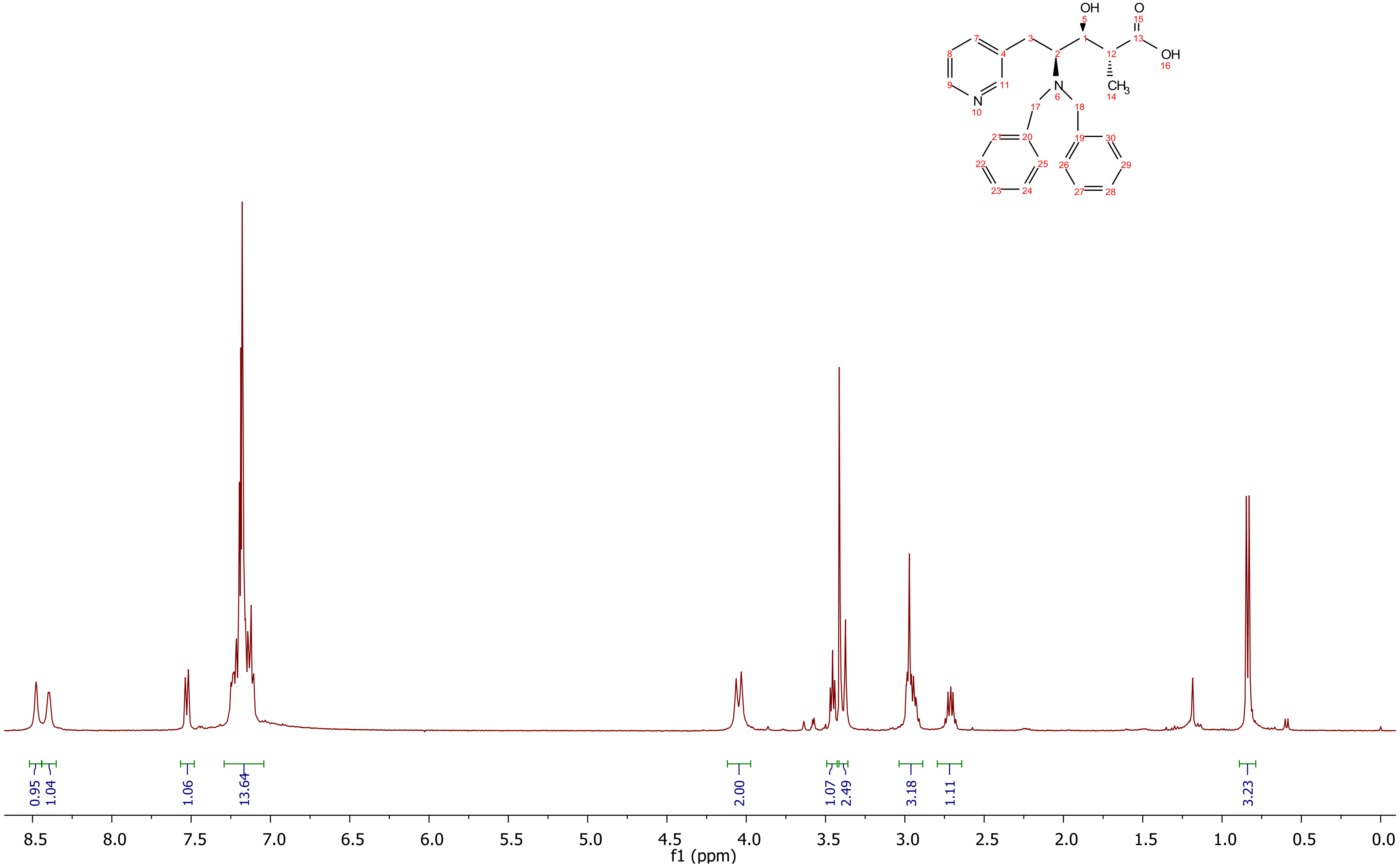
18


OH
CH2
CH3
N
N
1.00
0.99
1.12
11.90
0.96
0.93
0.94
2.00
1.02
2.06
2.00
1.03
0.95
3.11
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)

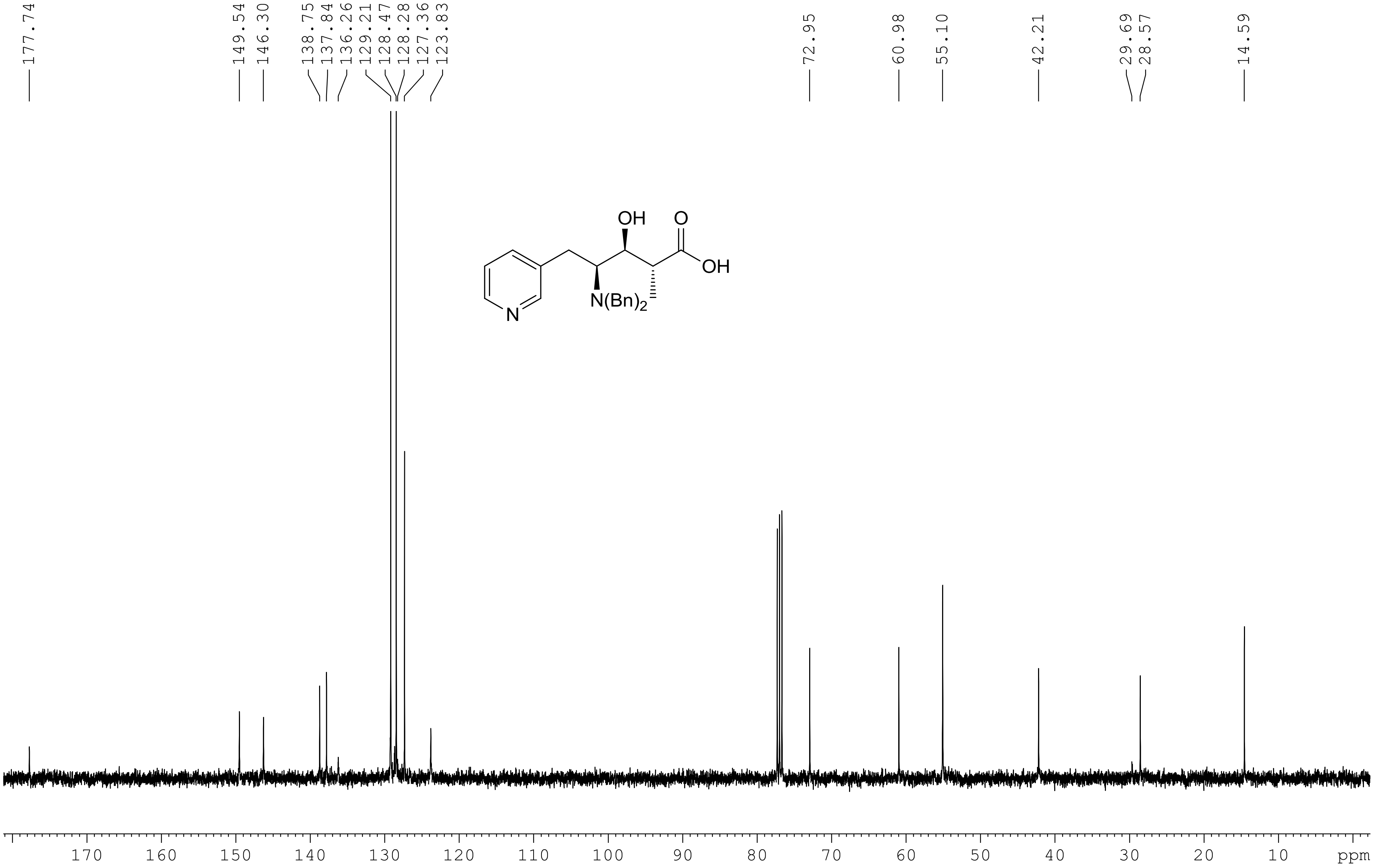
18


150.97
147.07
139.83
139.81
136.91
136.78
128.65
128.18
126.85
122.96
117.72
73.05
60.17
54.38
42.58
29.15
16.84
OH
N(Bn)$_2$
N
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

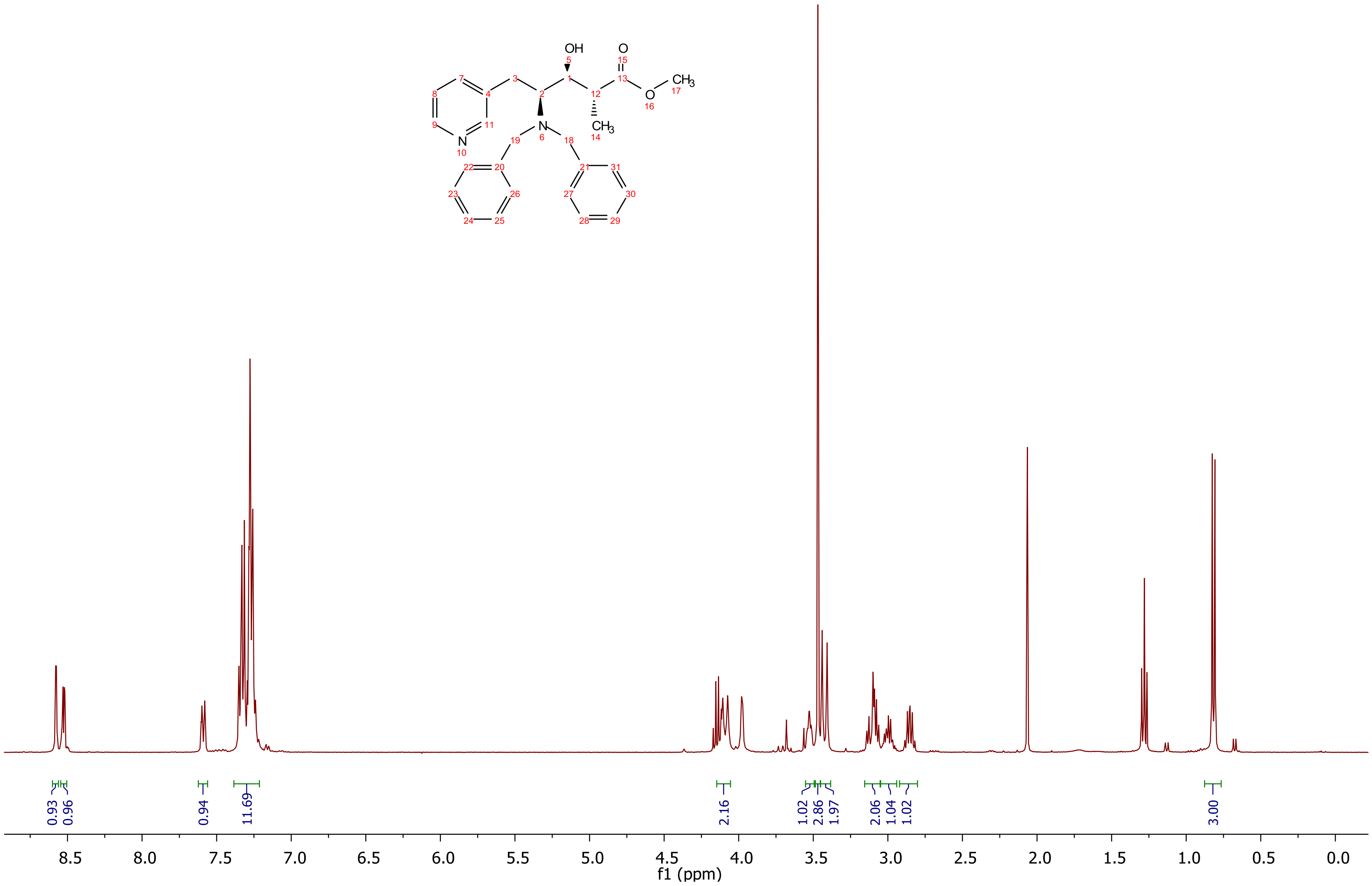
5


OH
O
OH
N
N
CH3
0.95
1.04
1.06
13.64
2.00
1.07
2.49
3.18
1.11
3.23
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)

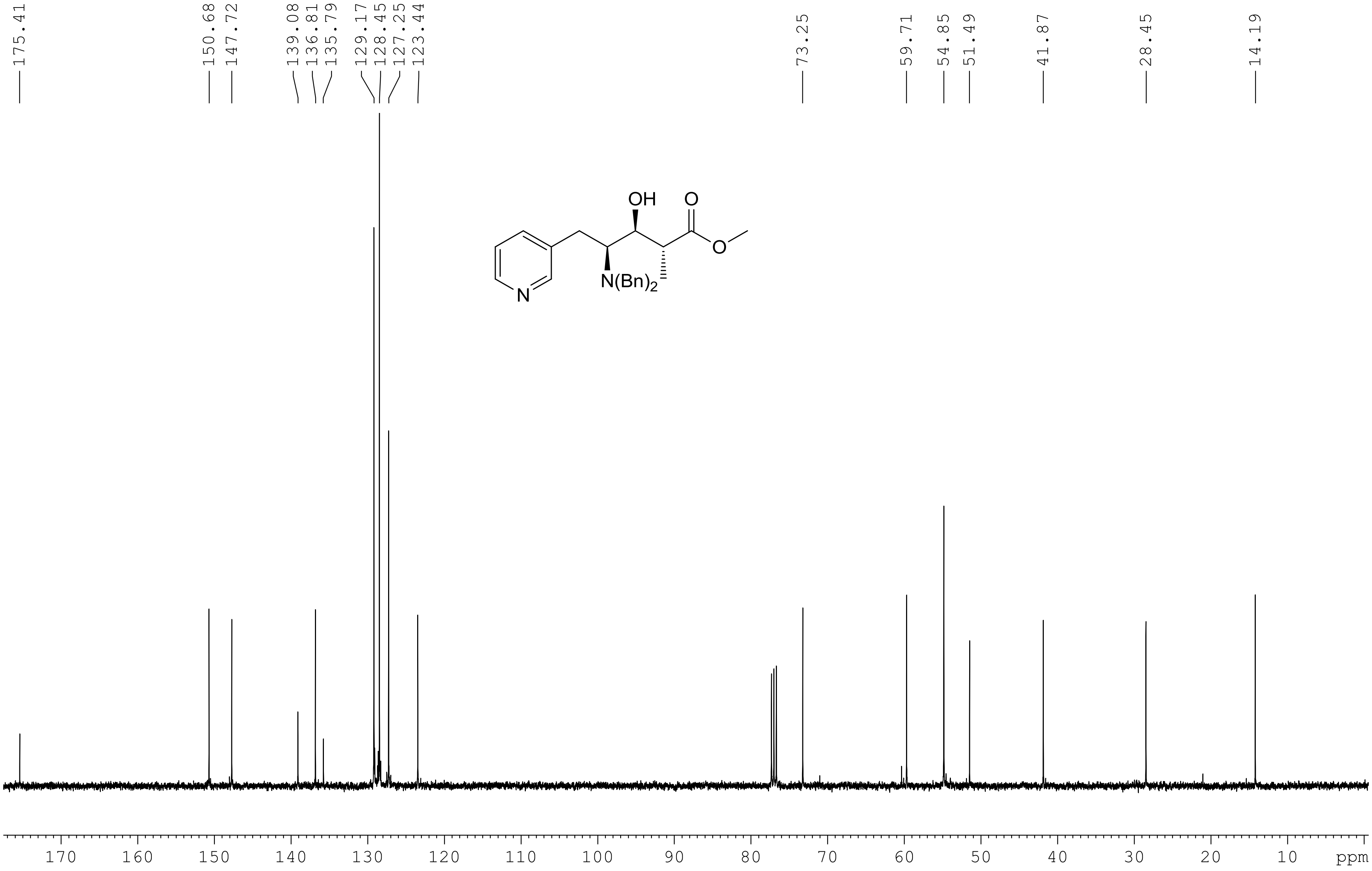
5


177.74
149.54
146.30
138.75
137.84
136.26
129.21
128.47
128.28
127.36
123.83
72.95
60.98
55.10
42.21
29.69
28.57
14.59
OH
O
OH
N
N(Bn)$_2$
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

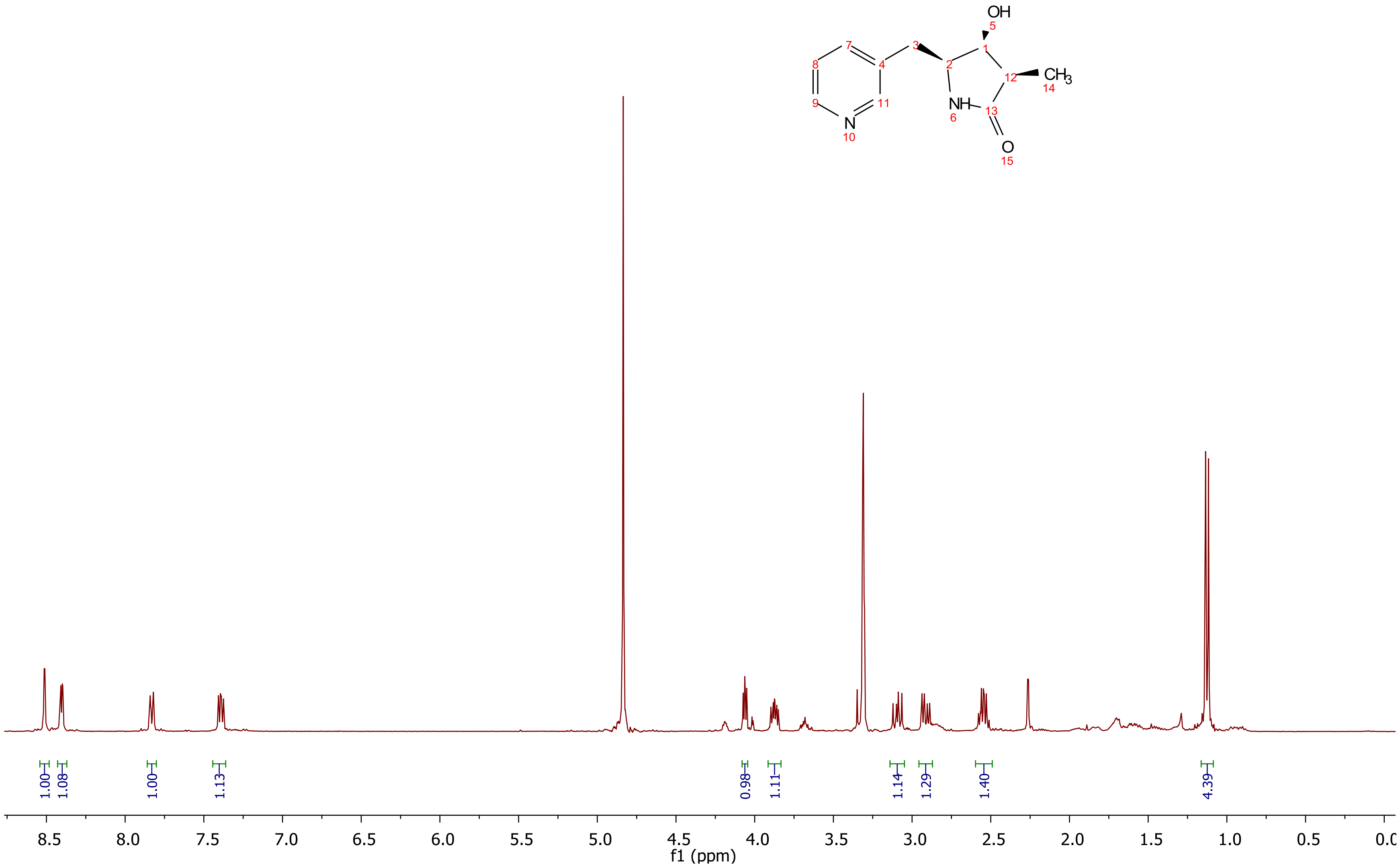
22


OH
O
CH3
O
CH3
N
N
0.93
0.96
0.94
11.69
2.16
1.02
2.86
1.97
2.06
1.04
1.02
3.00
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)

22

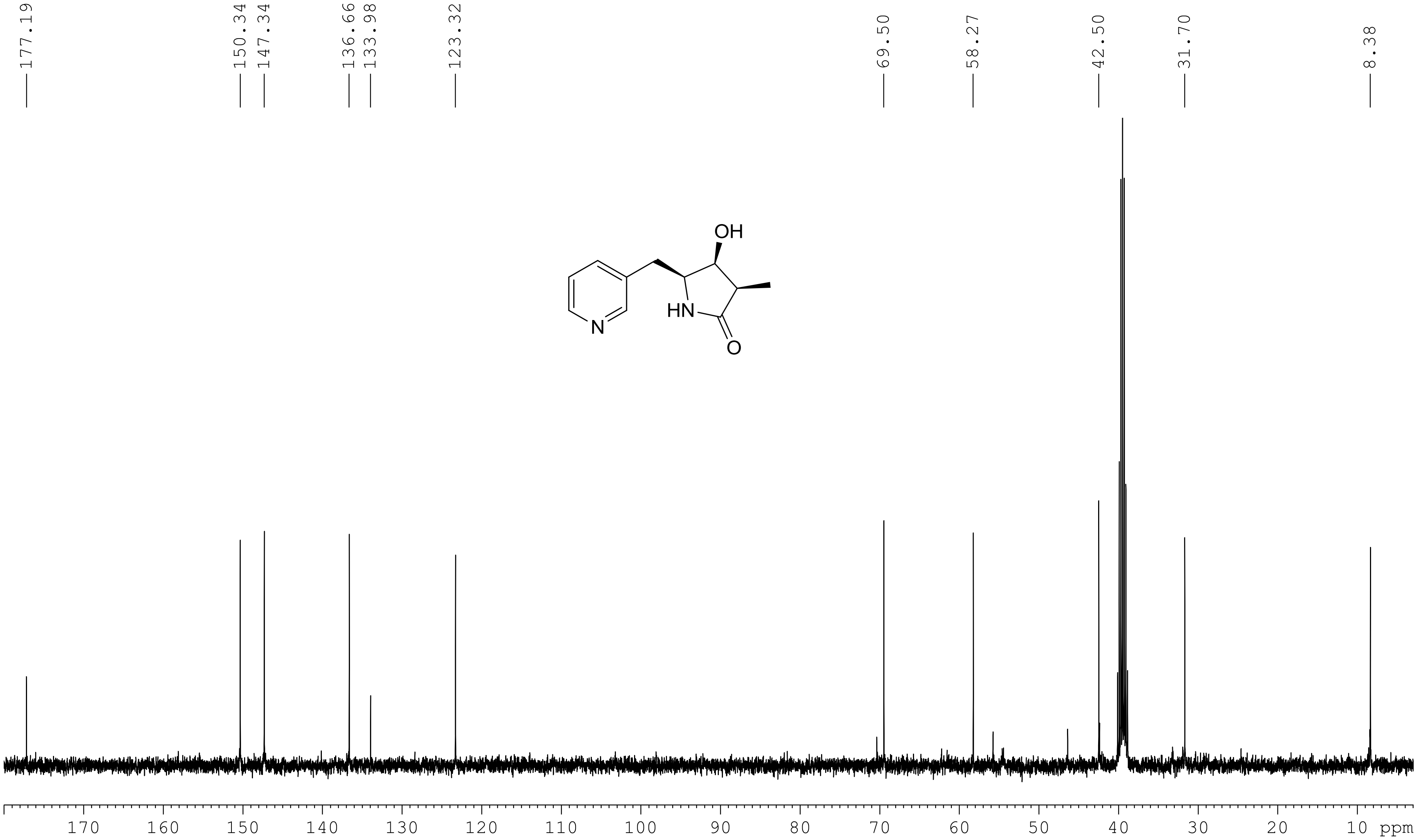

175.41
150.68
147.72
139.08
136.81
135.79
129.17
128.45
127.25
123.44
73.25
59.71
54.85
51.49
41.87
28.45
14.19
OH
O
O
N
N(Bn)$_2$
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

17

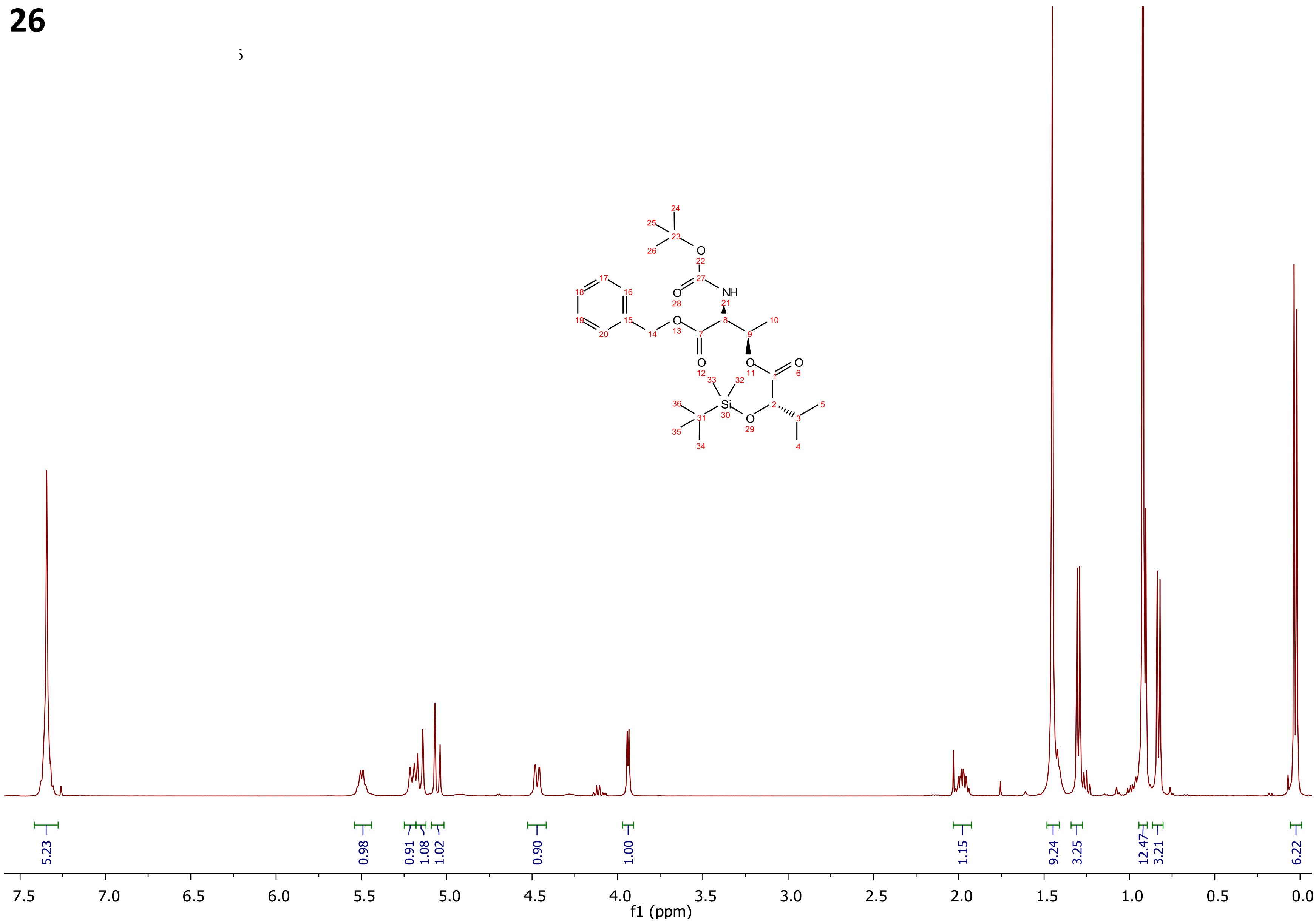


OH
CH3
NH
N
O
1.00
1.08
1.00
1.13
0.98
1.11
1.14
1.29
1.40
4.39
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

17

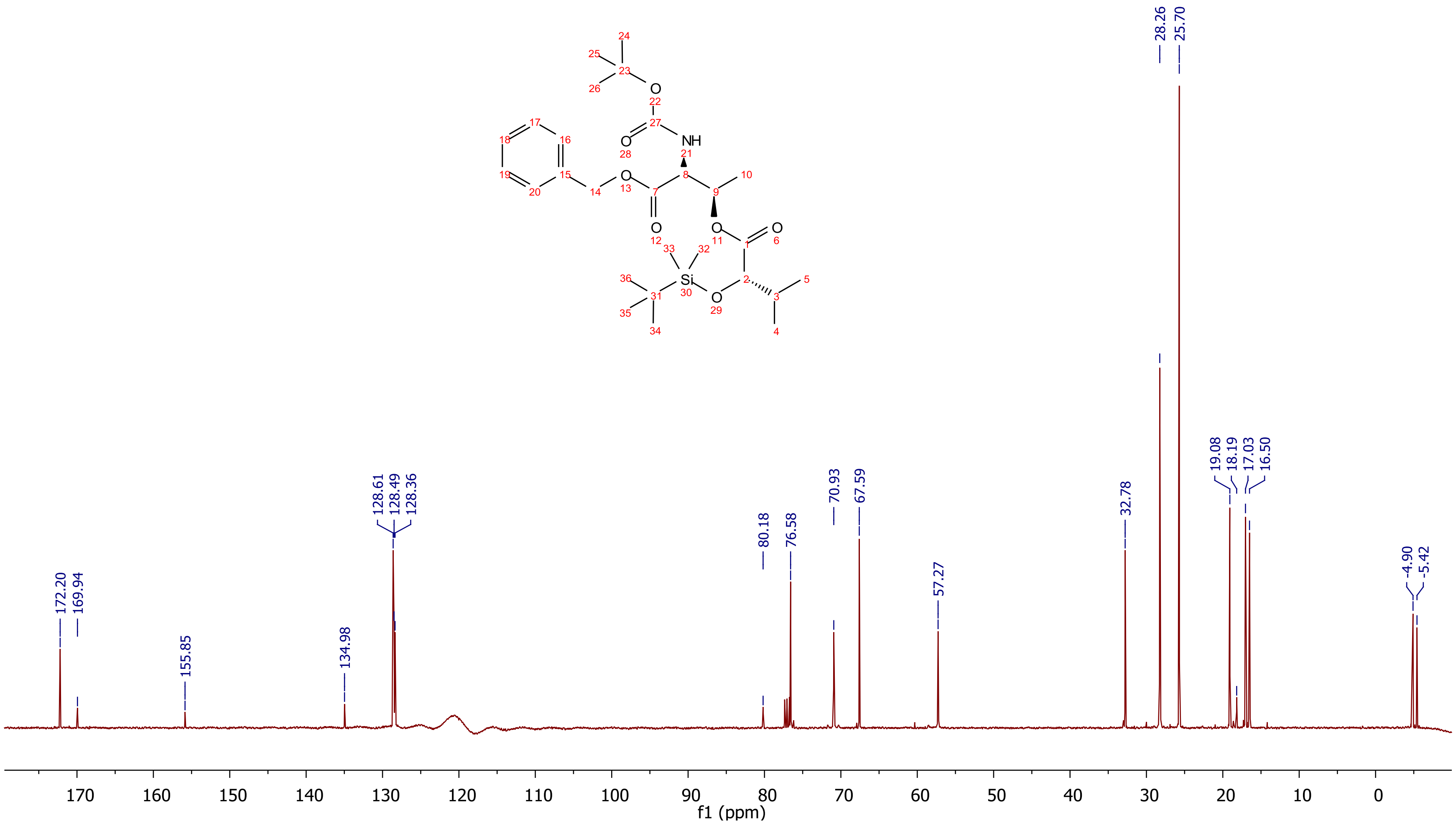

177.19
150.34
147.34
136.66
133.98
123.32
69.50
58.27
42.50
31.70
8.38
OH
HN
N
O
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10 ppm

26

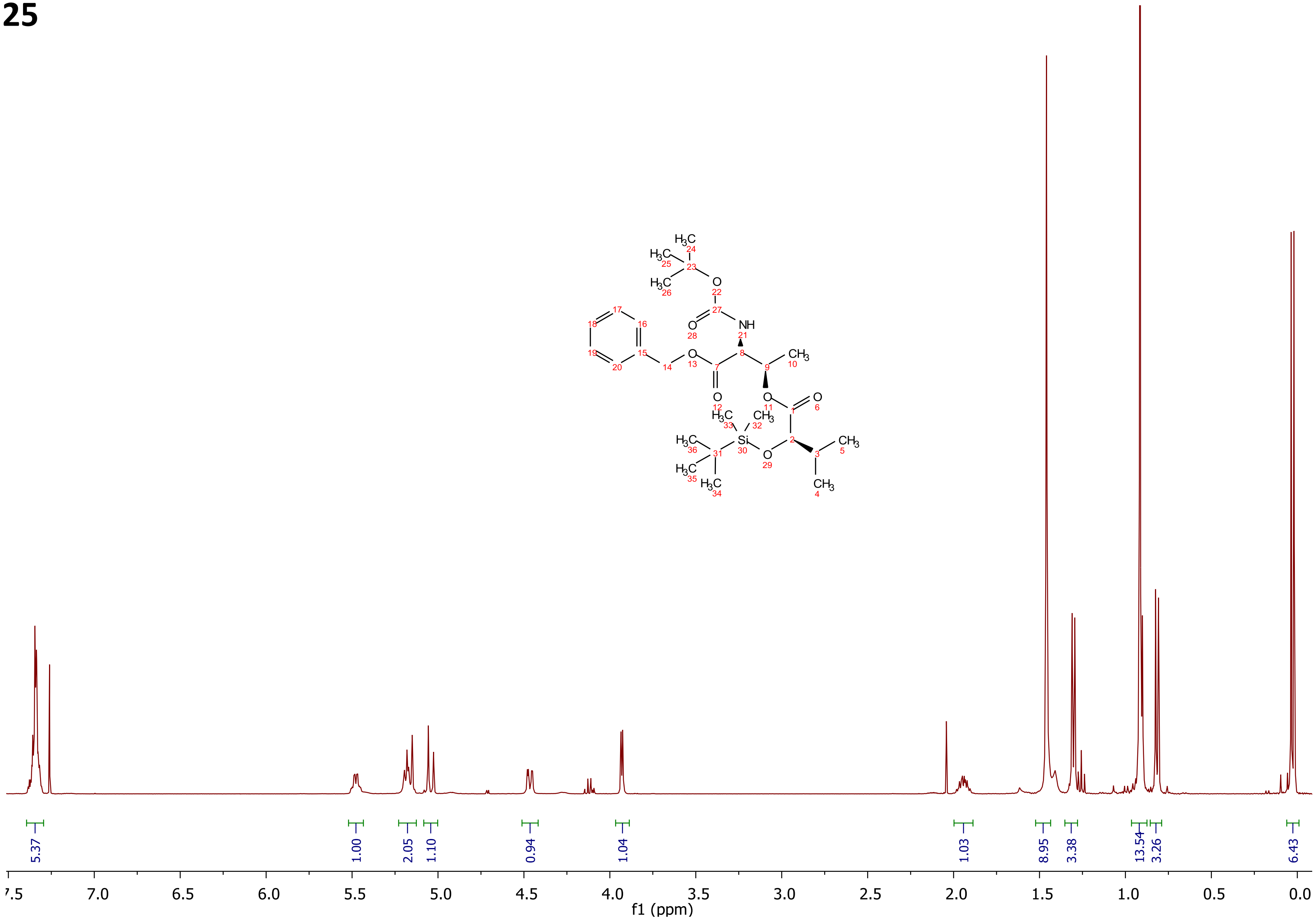


O
NH
O
O
O
O
O
O
Si
5.23
0.98
0.91
1.08
1.02
0.90
1.00
1.15
9.24
3.25
12.47
3.21
6.22
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

26

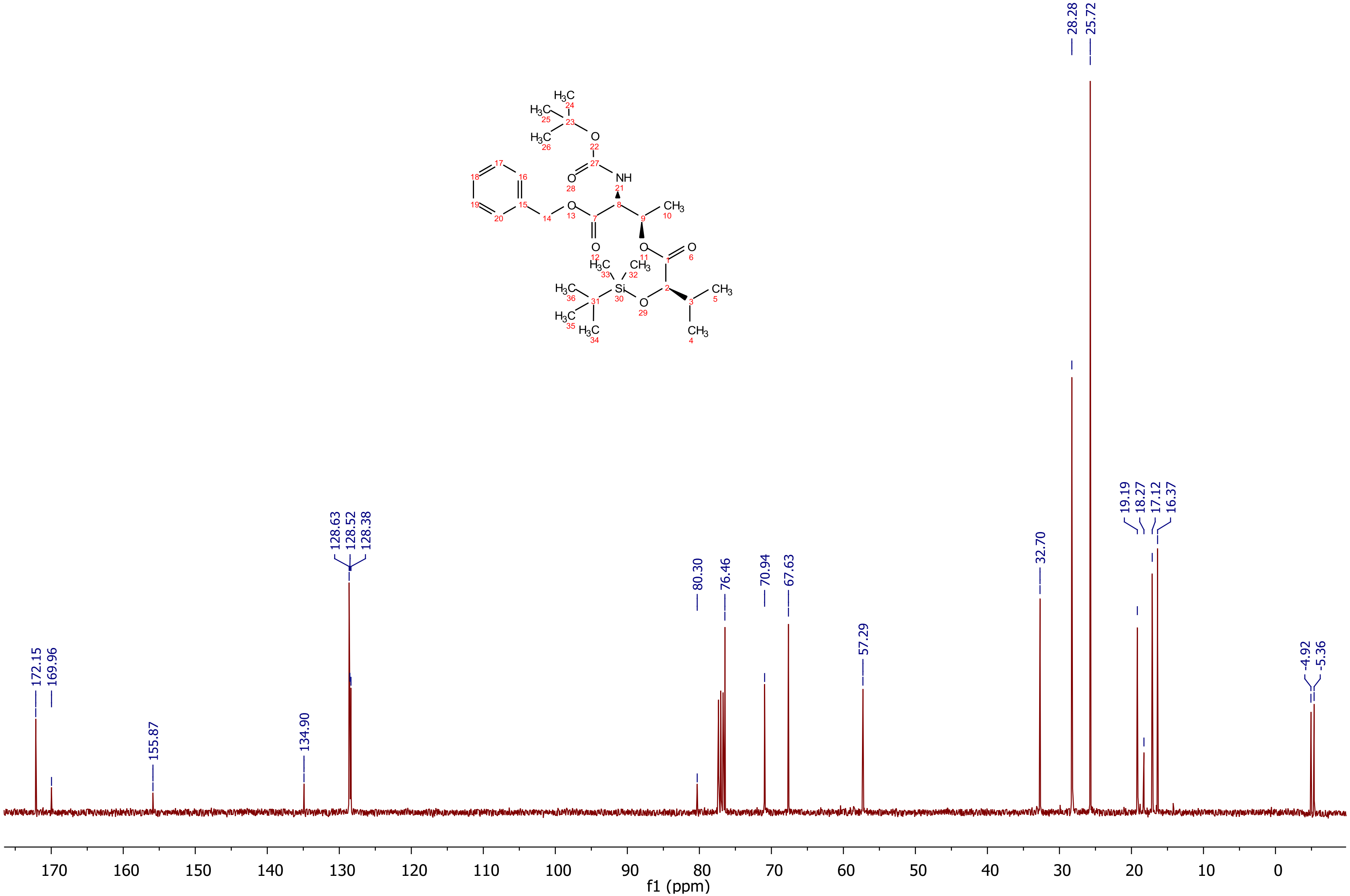

172.20
169.94
155.85
134.98
128.61
128.49
128.36
80.18
76.58
70.93
67.59
57.27
32.78
28.26
25.70
19.08
18.19
17.03
16.50
-4.90
-5.42
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)
NH
O
Si

25

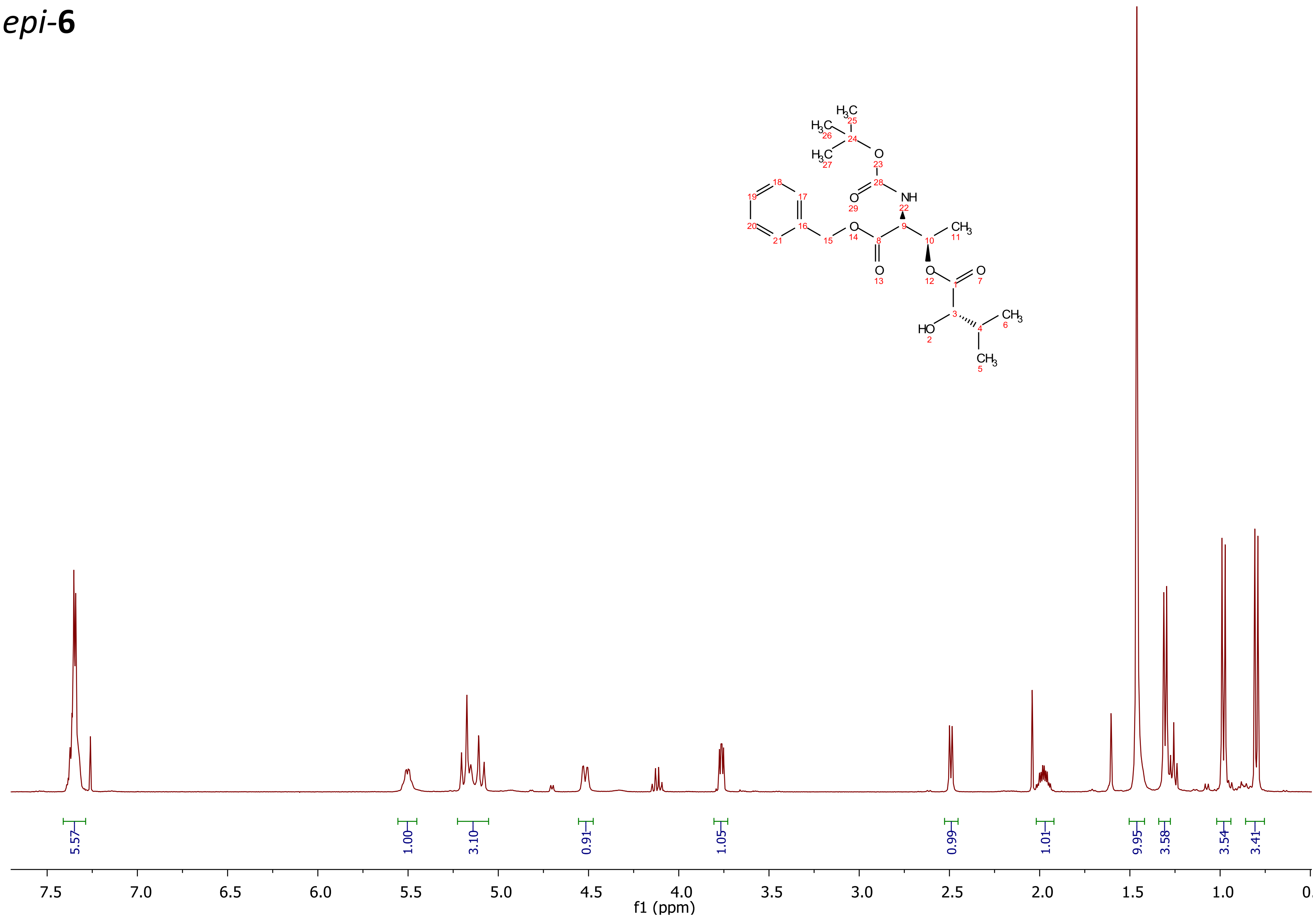


H3C
H3C
H3C
O
NH
O
O
O
CH3
O
O
O
H3C
CH3
H3C
Si
O
CH3
H3C
H3C
CH3
5.37
1.00
2.05
1.10
0.94
1.04
1.03
8.95
3.38
13.54
3.26
6.43
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

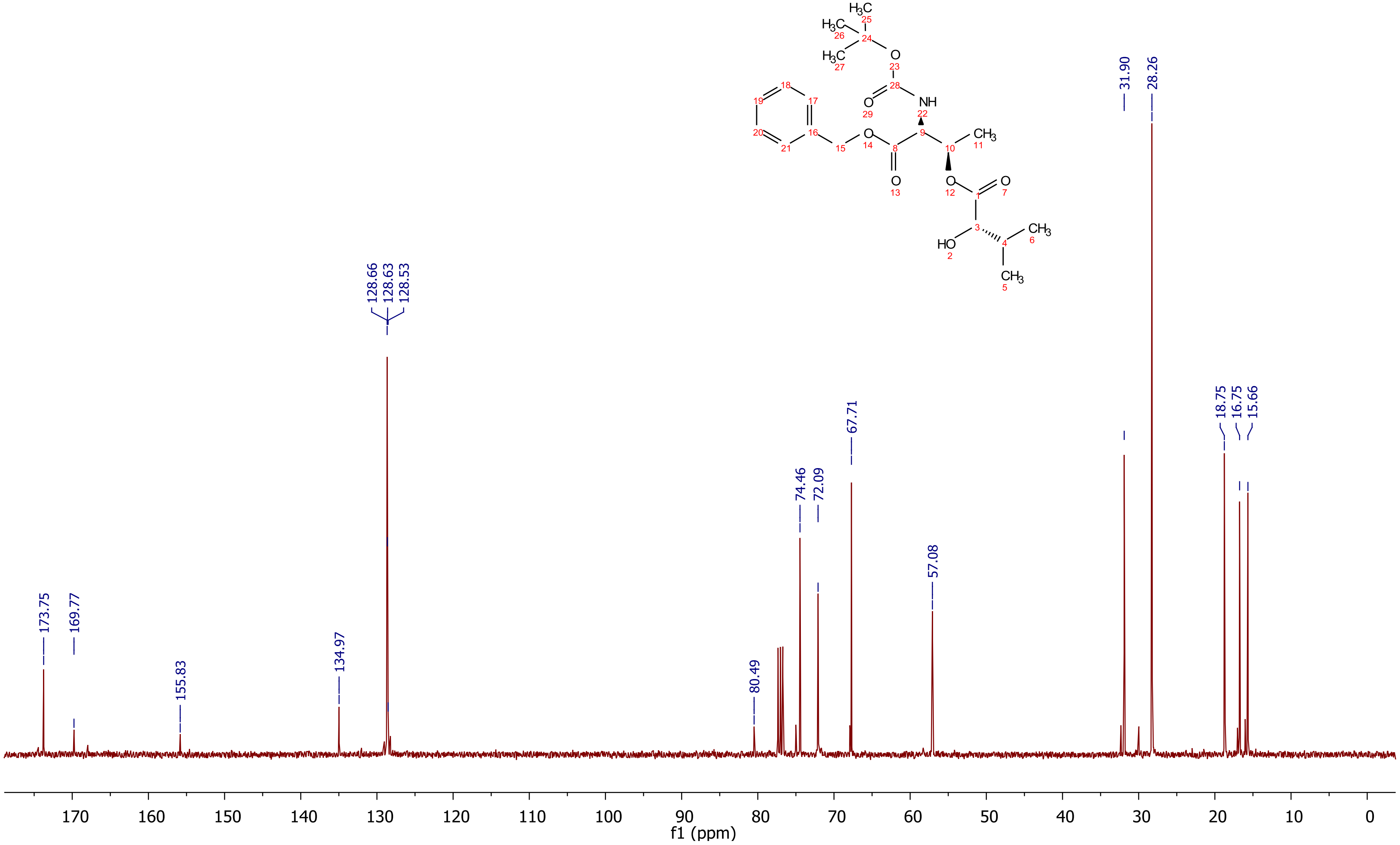
25


172.15
169.96
155.87
134.90
128.63
128.52
128.38
80.30
76.46
70.94
67.63
57.29
32.70
28.28
25.72
19.19
18.27
17.12
16.37
-4.92
-5.36
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)
H3C
H3C
H3C
O
O
NH
O
O
O
CH3
O
O
H3C
CH3
Si
O
H3C
H3C
H3C
CH3
CH3

epi-**6**

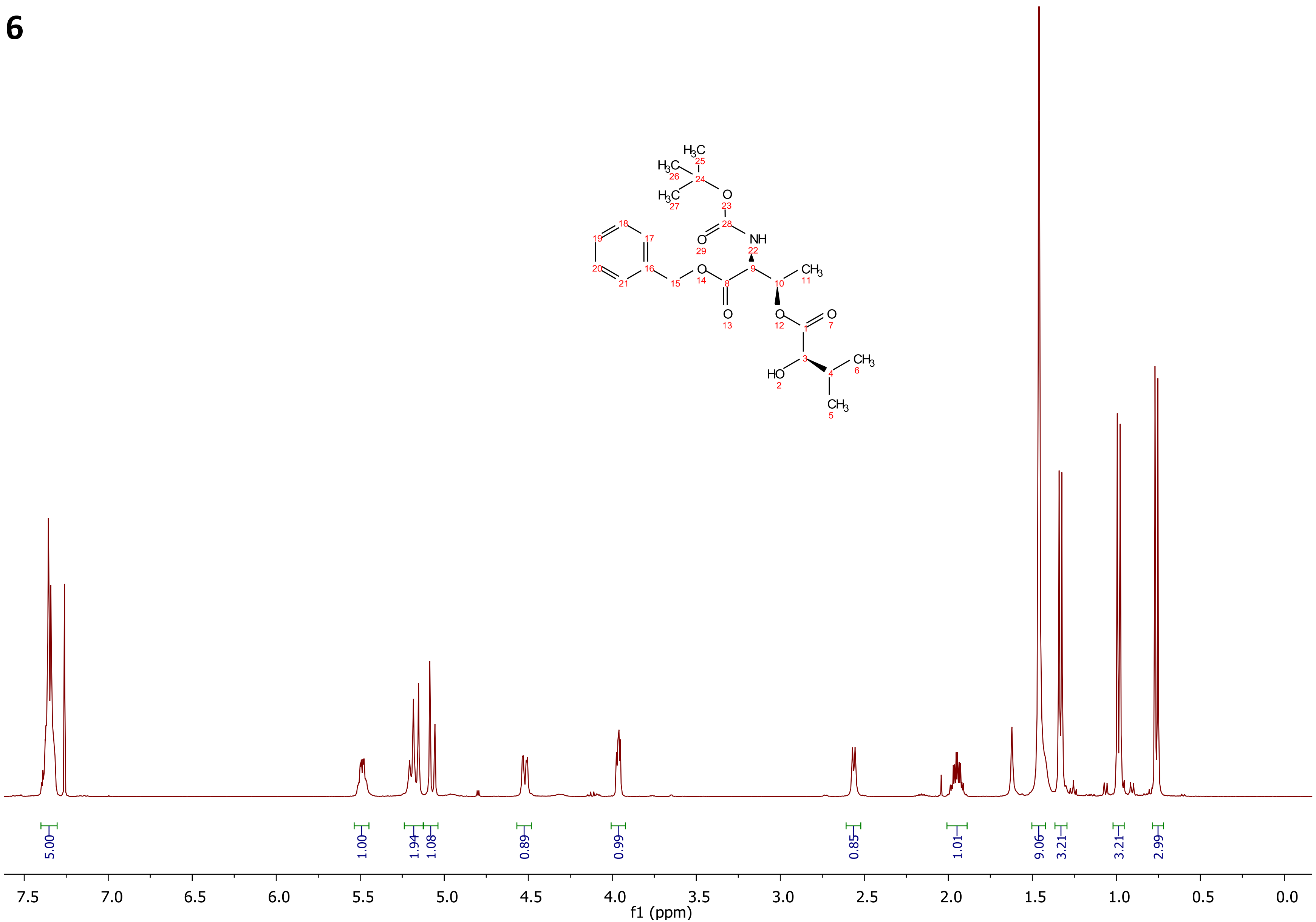

5.57
1.00
3.10
0.91
1.05
0.99
1.01
9.95
3.58
3.54
3.41
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.
f1 (ppm)

epi-**6**

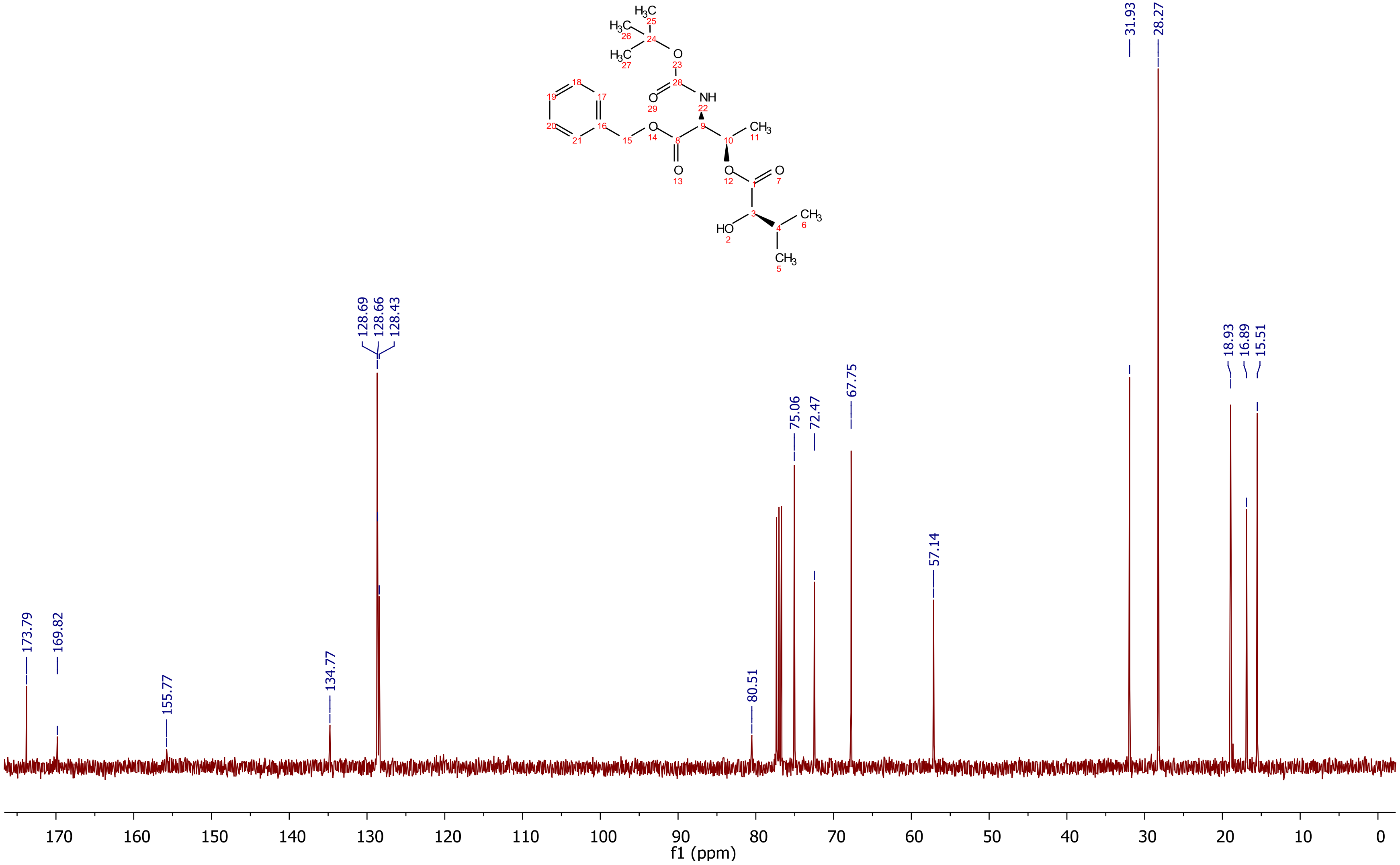


H3C 25
H3C 26
H3C 27
24
O 23
28
O 29
NH 22
9
10
CH3 11
16
17
18
19
20
21
15
O 14
8
O 13
O 12
1
O 7
3
HO 2
4
CH3 6
CH3 5
173.75
169.77
155.83
134.97
128.66
128.63
128.53
80.49
74.46
72.09
67.71
57.08
31.90
28.26
18.75
16.75
15.66
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

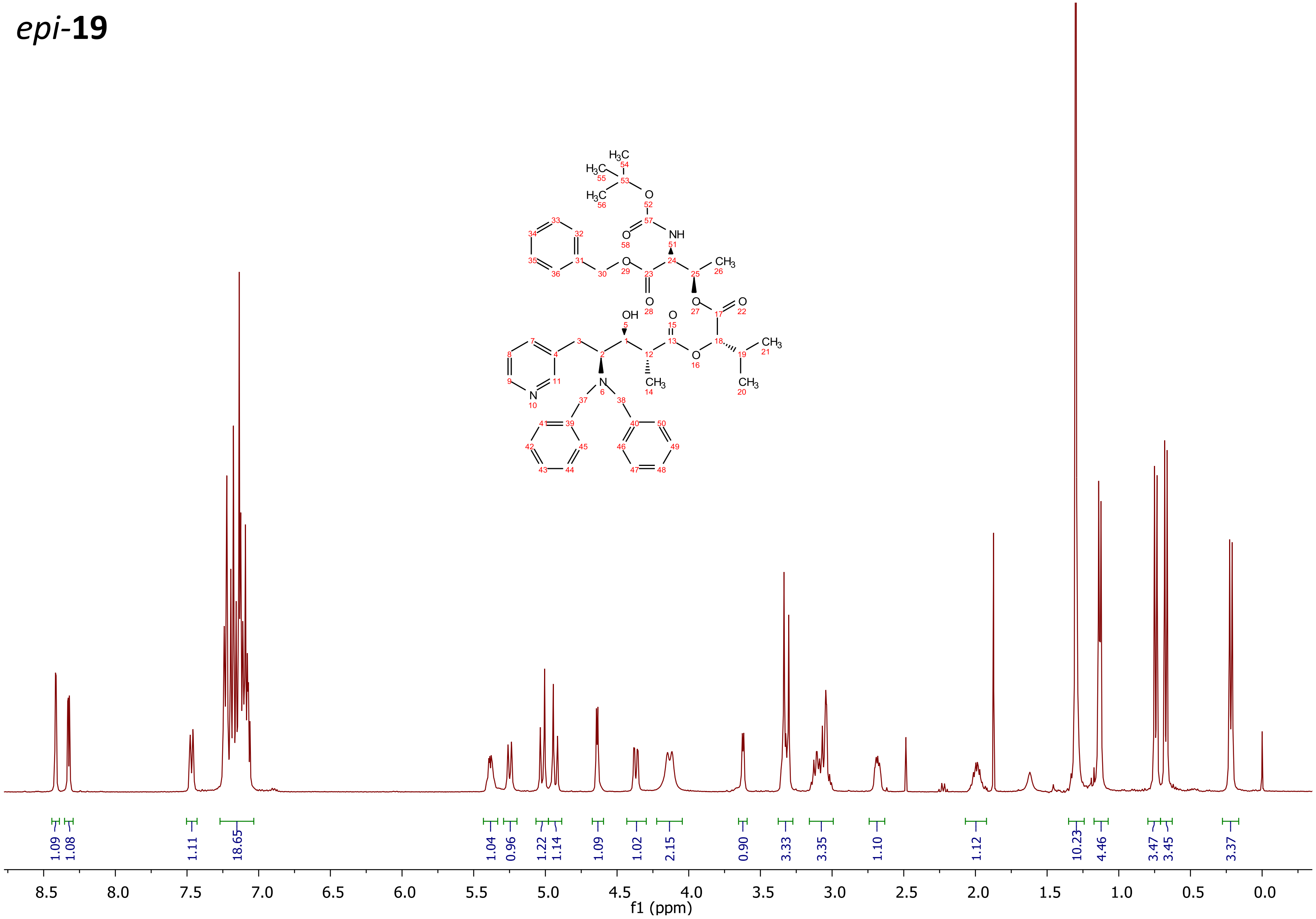
6


H3C
25
H3C
26
24
O
23
H3C
27
28
O
29
NH
22
18
17
19
20
16
21
15
O
14
8
O
13
9
10
CH3
11
O
12
1
O
7
3
HO
2
4
CH3
6
CH3
5
5.00
1.00
1.94
1.08
0.89
0.99
0.85
1.01
9.06
3.21
3.21
2.99
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)

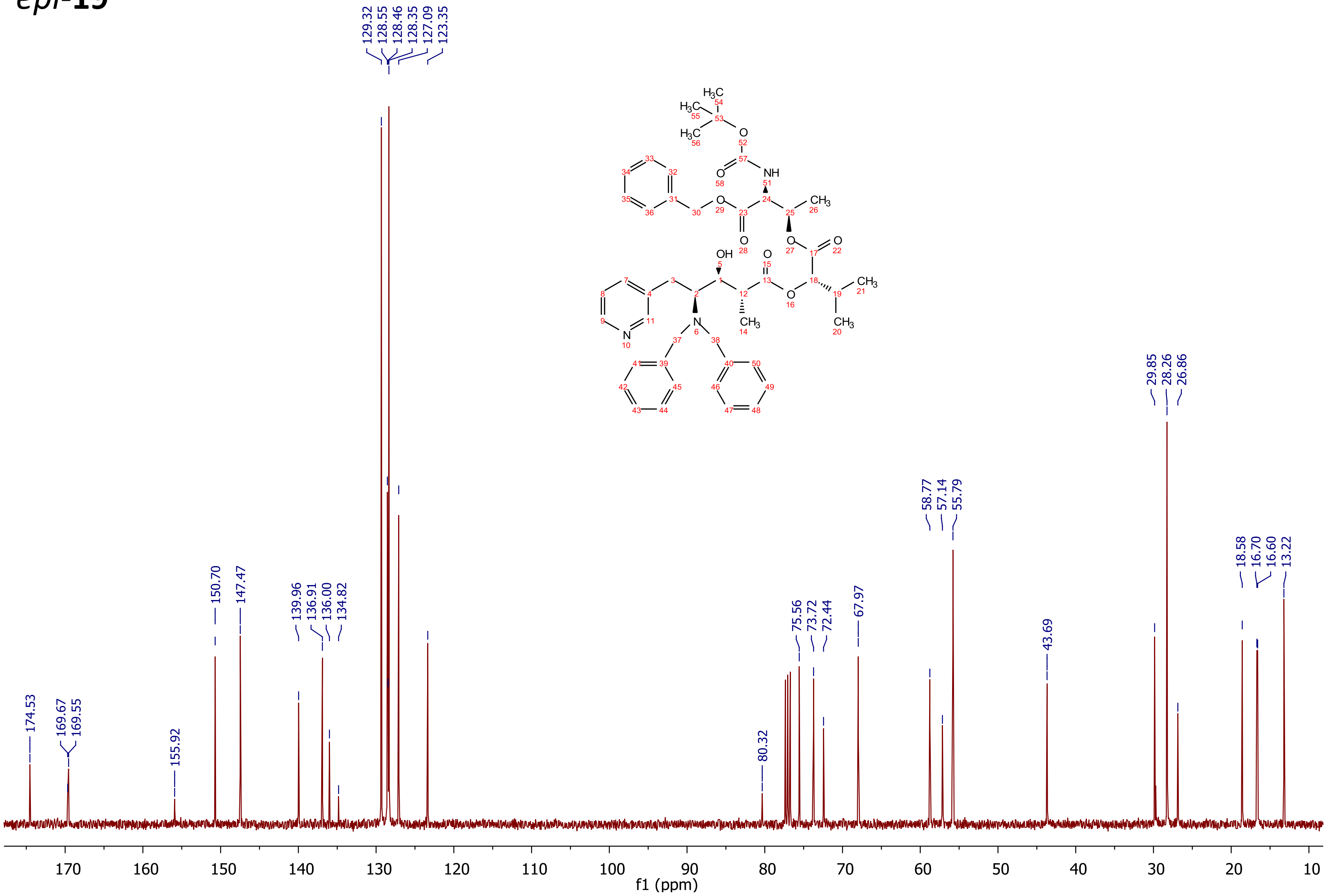
6


173.79
169.82
155.77
134.77
128.69
128.66
128.43
80.51
75.06
72.47
67.75
57.14
31.93
28.27
18.93
16.89
15.51
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)
H3C
H3C
H3C
O
NH
O
O
CH3
O
O
O
HO
CH3
CH3

epi-**19**

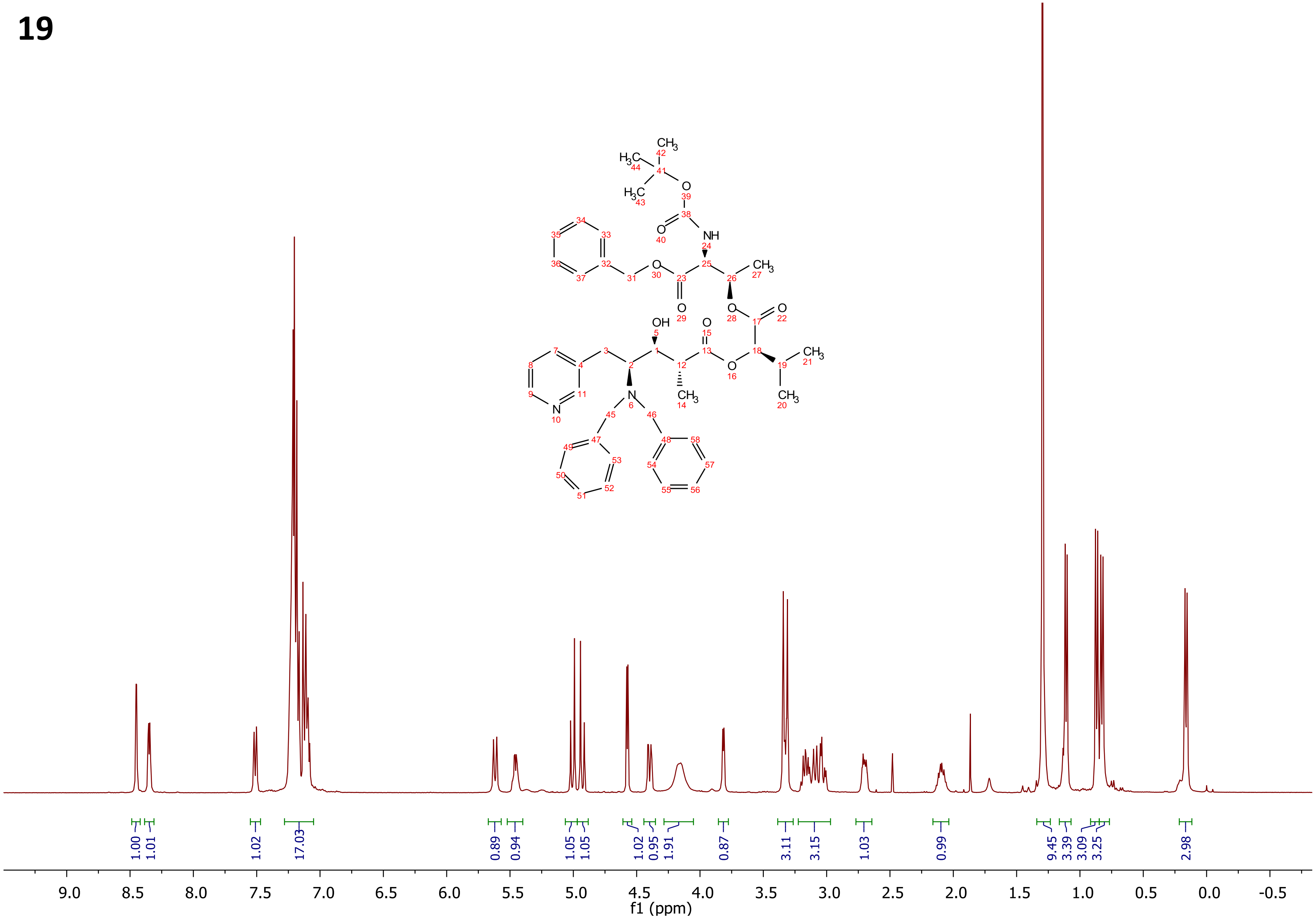

H3C
H3C
H3C
O
O
NH
O
CH3
O
O
O
OH
O
CH3
N
N
CH3
CH3
1.09
1.08
1.11
18.65
1.04
0.96
1.22
1.14
1.09
1.02
2.15
0.90
3.33
3.35
1.10
1.12
10.23
4.46
3.47
3.45
3.37
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

epi-**19**



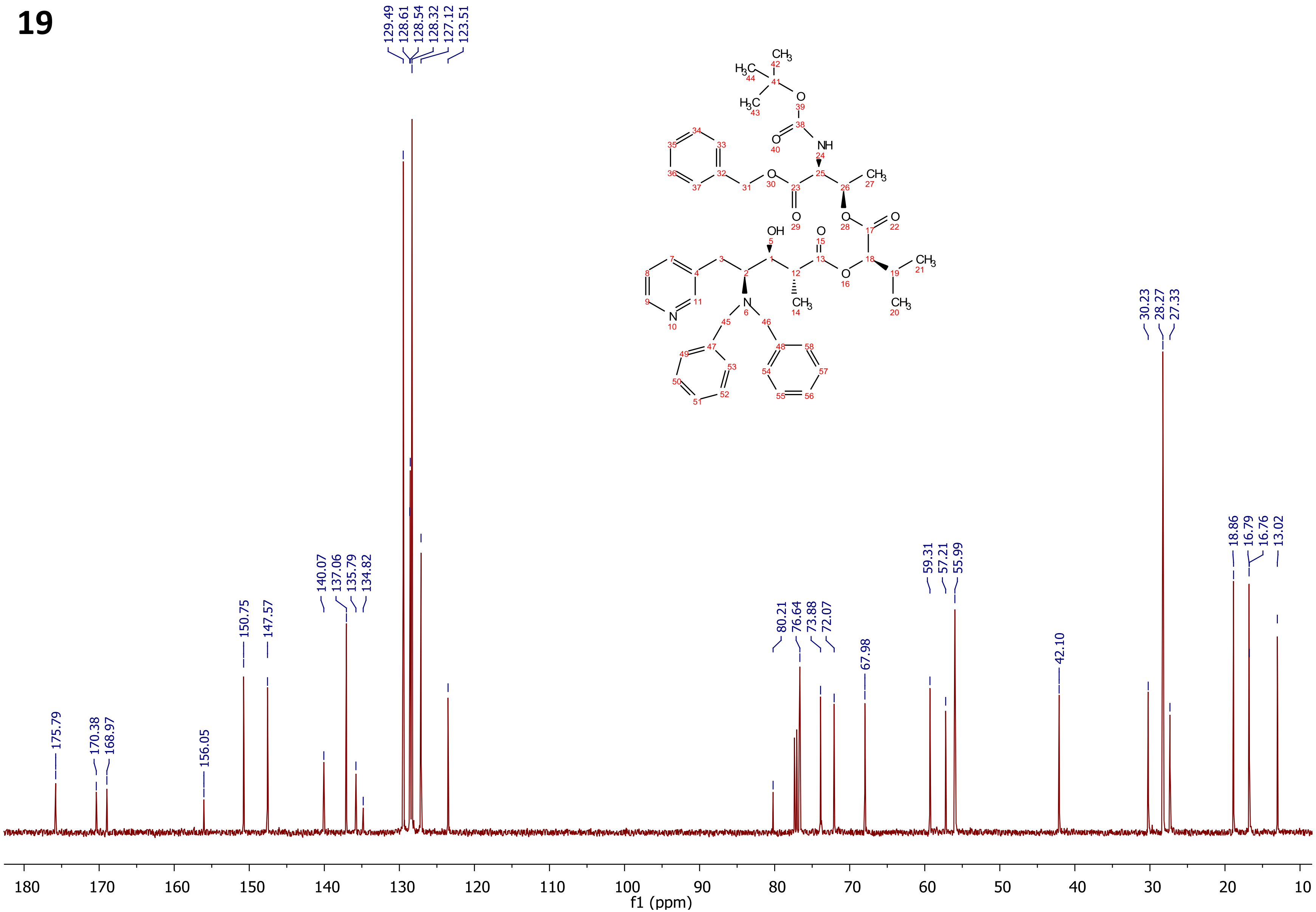
129.32
128.55
128.46
128.35
127.09
123.35
174.53
169.67
169.55
155.92
150.70
147.47
139.96
136.91
136.00
134.82
80.32
75.56
73.72
72.44
67.97
58.77
57.14
55.79
43.69
29.85
28.26
26.86
18.58
16.70
16.60
13.22
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)
H3C
H3C
H3C
O
O
NH
O
CH3
O
O
OH
O
O
O
CH3
N
N
CH3
CH3
CH3

19

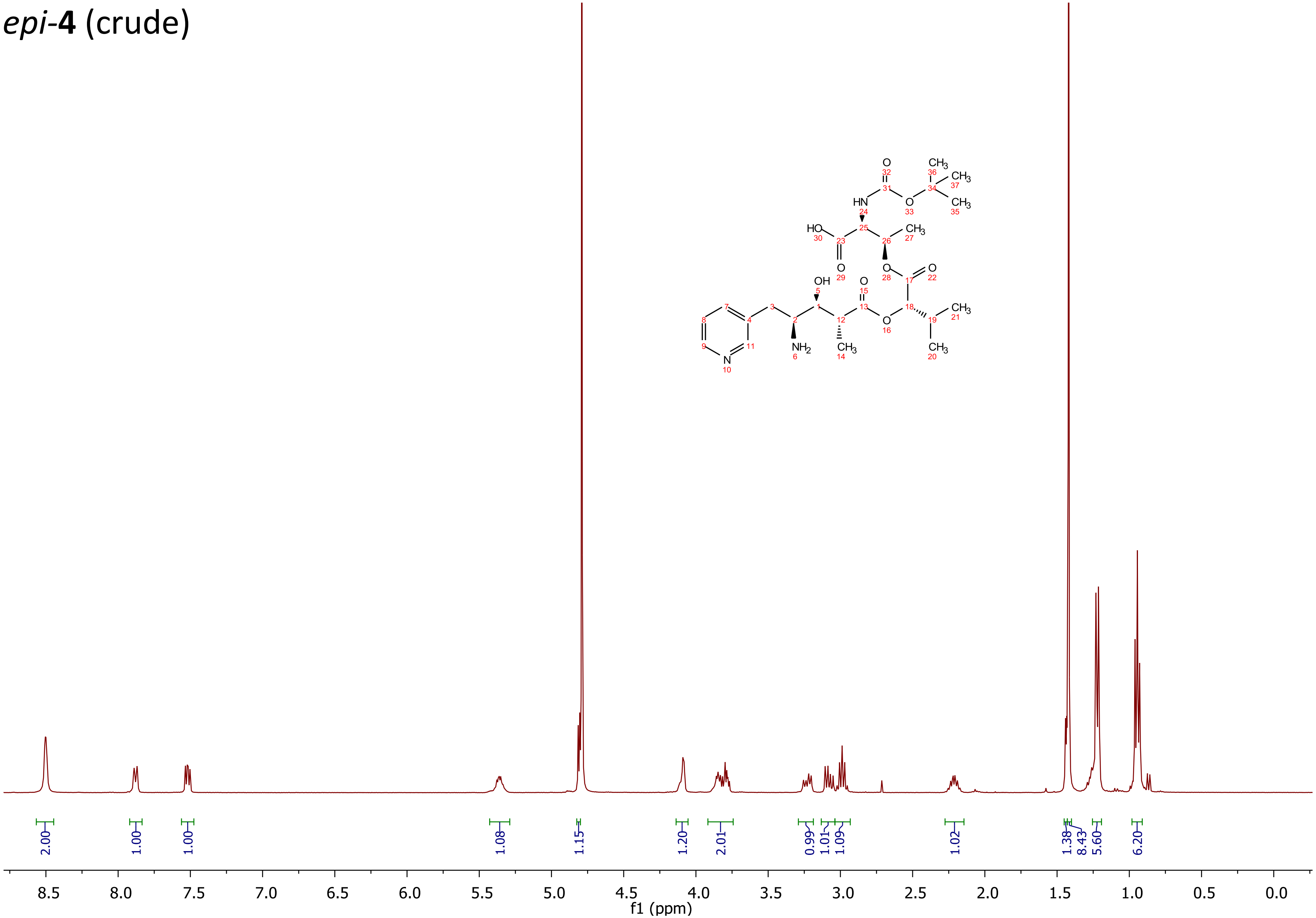


CH3
H3C
H3C
O
NH
O
O
CH3
O
O
O
OH
O
CH3
N
N
CH3
CH3
1.00
1.01
1.02
17.03
0.89
0.94
1.05
1.05
1.02
0.95
1.91
0.87
3.11
3.15
1.03
0.99
9.45
3.39
3.09
3.25
2.98
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
f1 (ppm)

19


129.49
128.61
128.54
128.32
127.12
123.51
175.79
170.38
168.97
156.05
150.75
147.57
140.07
137.06
135.79
134.82
80.21
76.64
73.88
72.07
67.98
59.31
57.21
55.99
42.10
30.23
28.27
27.33
18.86
16.79
16.76
13.02
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)
CH3
H3C
H3C
O
O
NH
O
O
CH3
O
O
OH
O
O
N
CH3
N
CH3
CH3
CH3

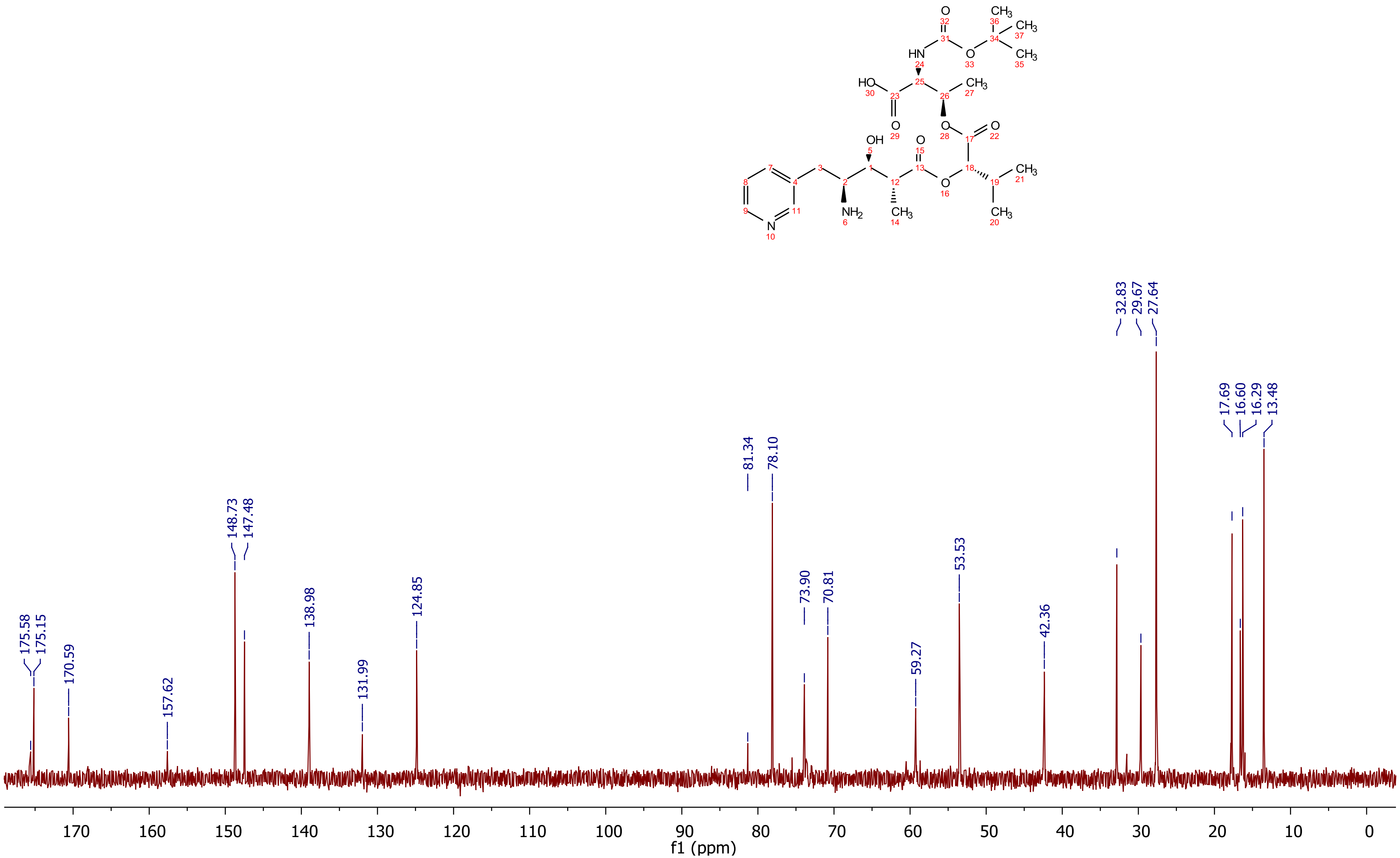
epi-**4** (crude)



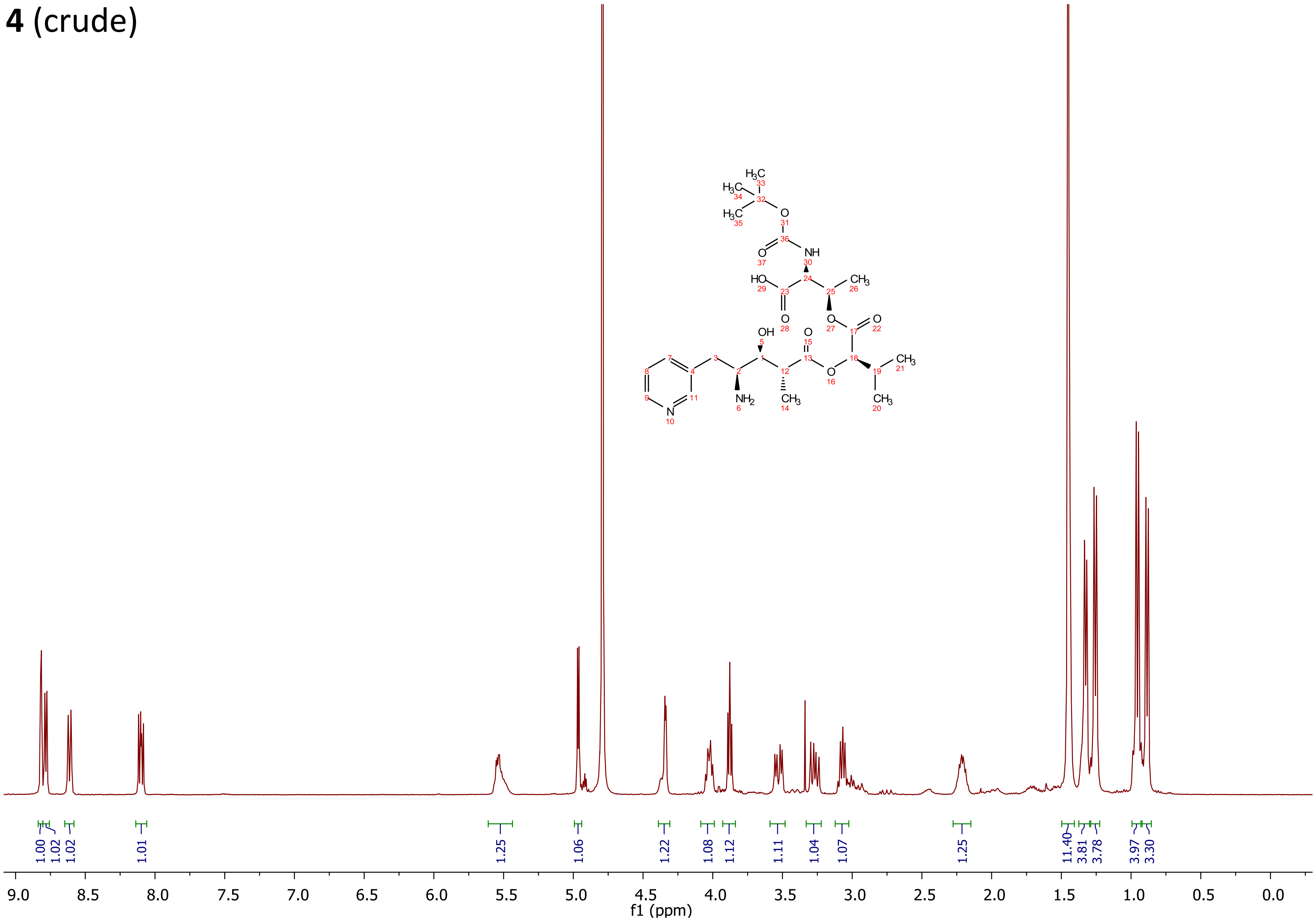
2.00
1.00
1.00
1.08
1.15
1.20
2.01
0.99
1.01
1.09
1.02
1.38
8.43
5.60
6.20
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)
HO
HN
OH
NH2
N
O
CH3

epi-**4** (crude)

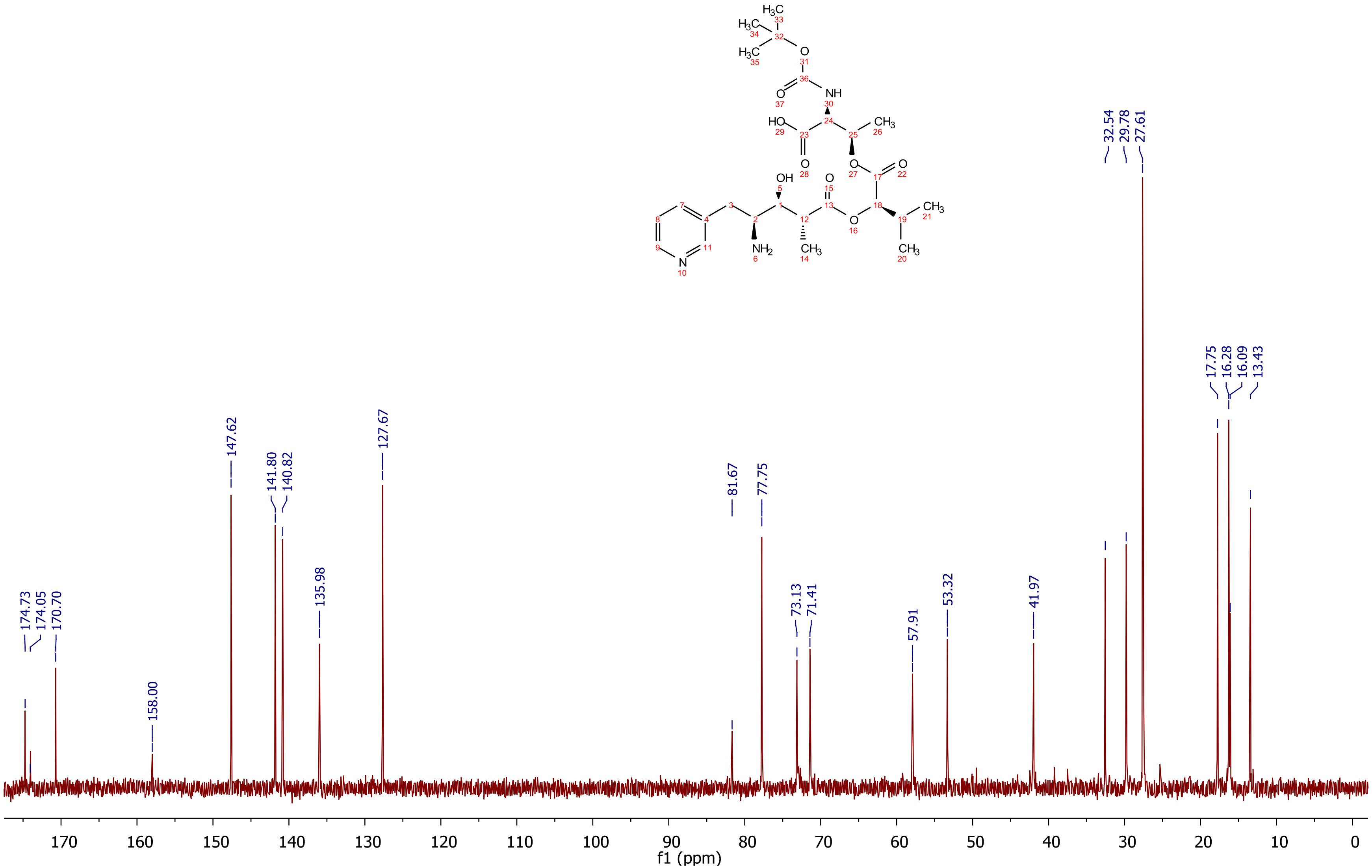
3


O
CH3
CH3
CH3
HN
O
HO
CH3
O
O
O
OH
O
CH3
O
N
NH2
CH3
CH3
175.58
175.15
170.59
157.62
148.73
147.48
138.98
131.99
124.85
81.34
78.10
73.90
70.81
59.27
53.53
42.36
32.83
29.67
27.64
17.69
16.60
16.29
13.48
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

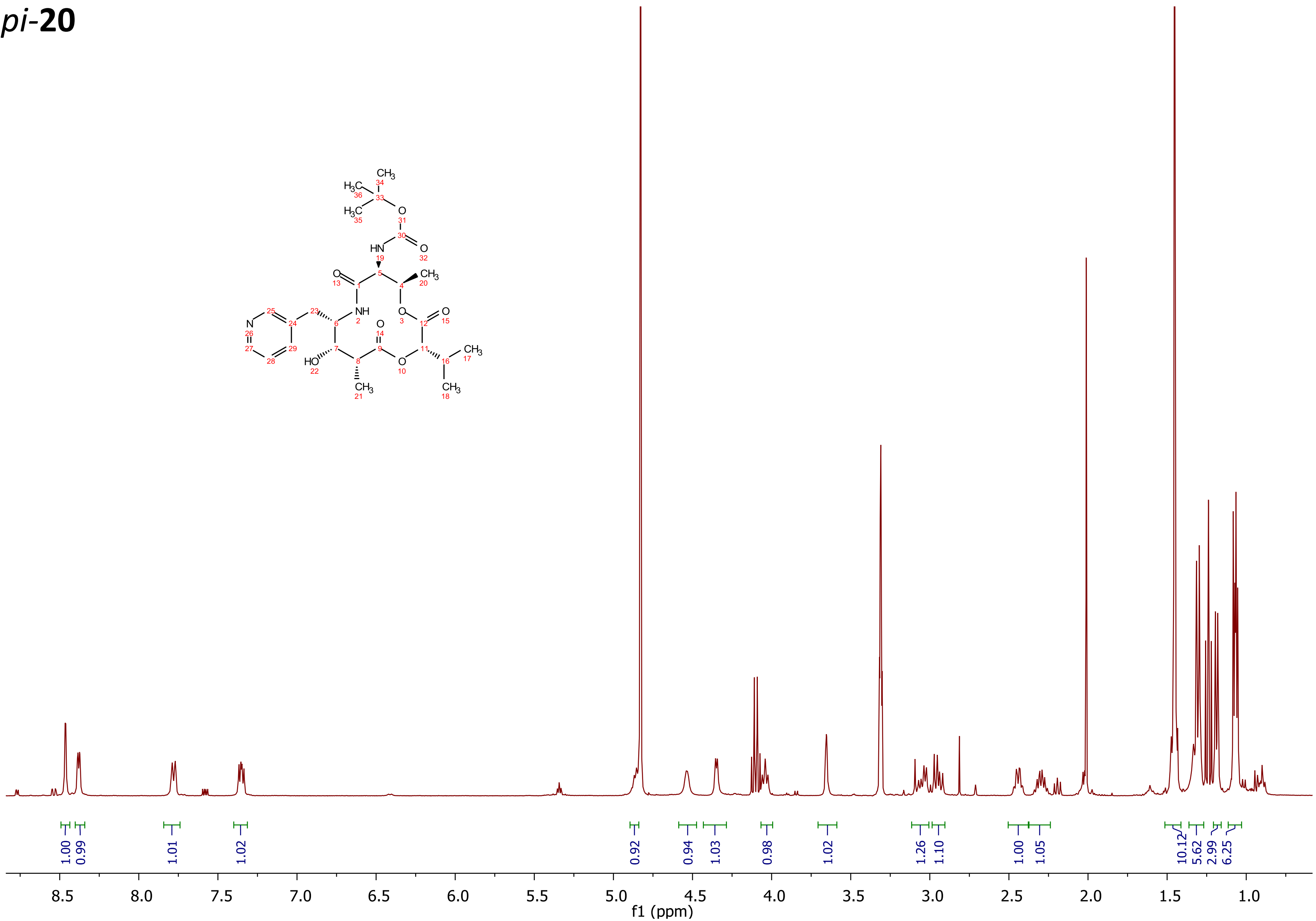
4 (crude)


1.00
1.02
1.02
1.01
1.25
1.06
1.22
1.08
1.12
1.11
1.04
1.07
1.25
11.40
3.81
3.78
3.97
3.30
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)
H3C
H3C
H3C
O
O
NH
HO
CH3
O
O
O
OH
O
NH2
CH3
O
CH3
CH3
N

4 (crude)

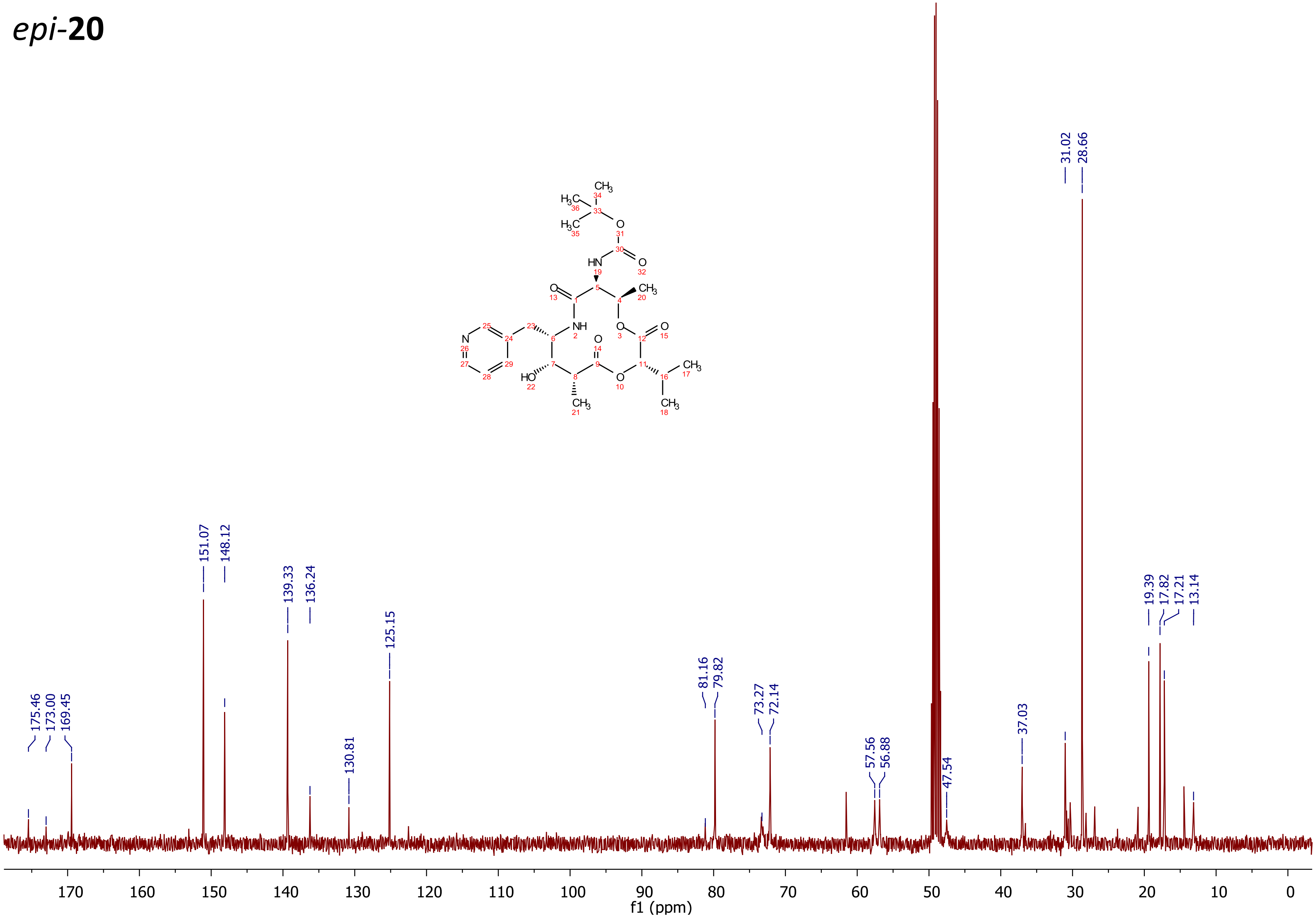

H3C
H3C
H3C
O
O
NH
HO
CH3
O
O
O
OH
O
O
CH3
NH2
CH3
CH3
N
174.73
174.05
170.70
158.00
147.62
141.80
140.82
135.98
127.67
81.67
77.75
73.13
71.41
57.91
53.32
41.97
32.54
29.78
27.61
17.75
16.28
16.09
13.43
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

epi-**20**

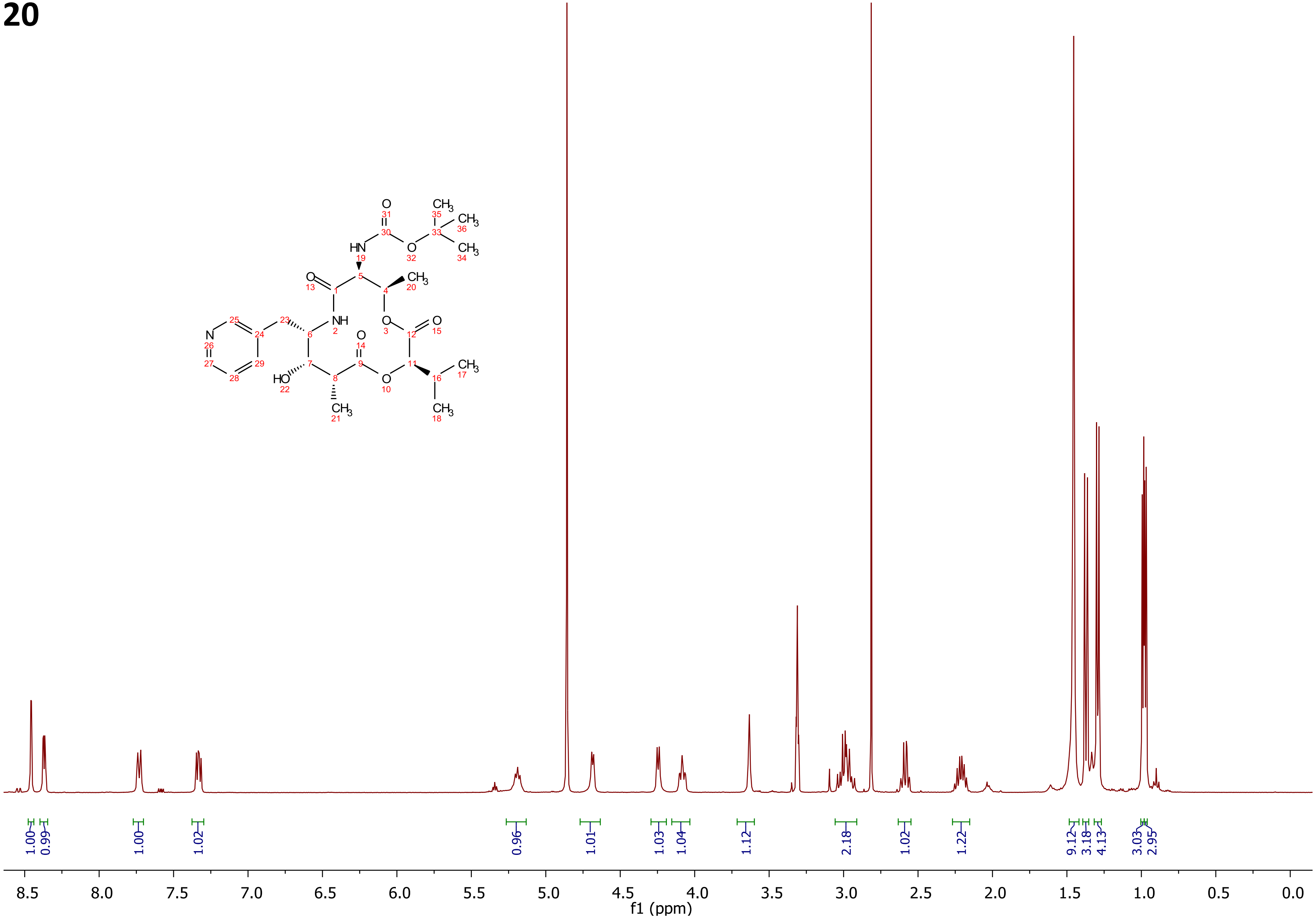


CH3
H3C
H3C
O
HN
O
O
CH3
N
NH
O
O
O
HO
O
O
CH3
CH3
CH3
1.00
0.99
1.01
1.02
0.92
0.94
1.03
0.98
1.02
1.26
1.10
1.00
1.05
10.12
5.62
2.99
6.25
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)

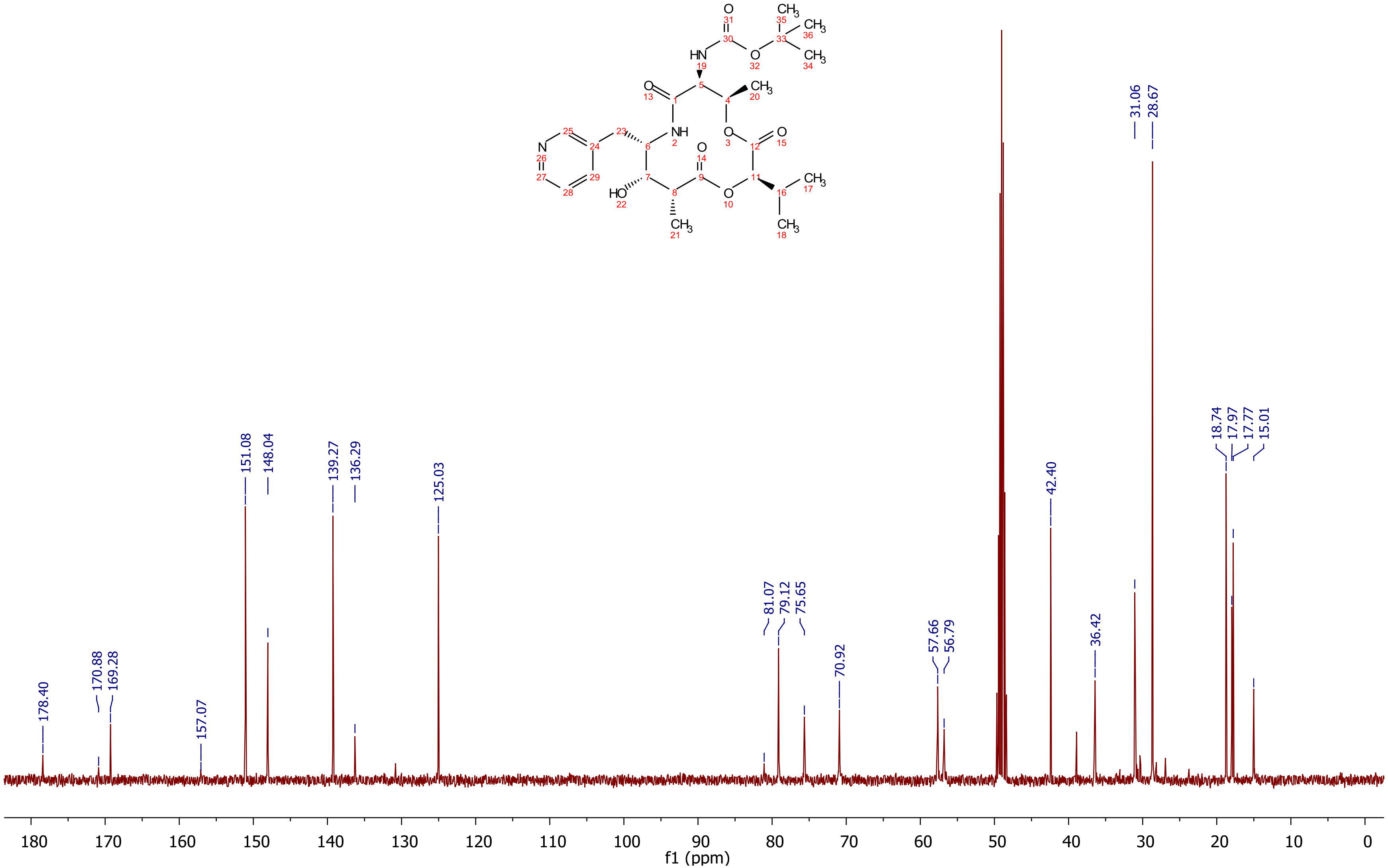
epi-**20**


175.46
173.00
169.45
151.07
148.12
139.33
136.24
130.81
125.15
81.16
79.82
73.27
72.14
57.56
56.88
47.54
37.03
31.02
28.66
19.39
17.82
17.21
13.14
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)
CH3
H3C
H3C
O
HN
O
O
CH3
O
NH
N
O
O
O
HO
O
CH3
CH3
CH3

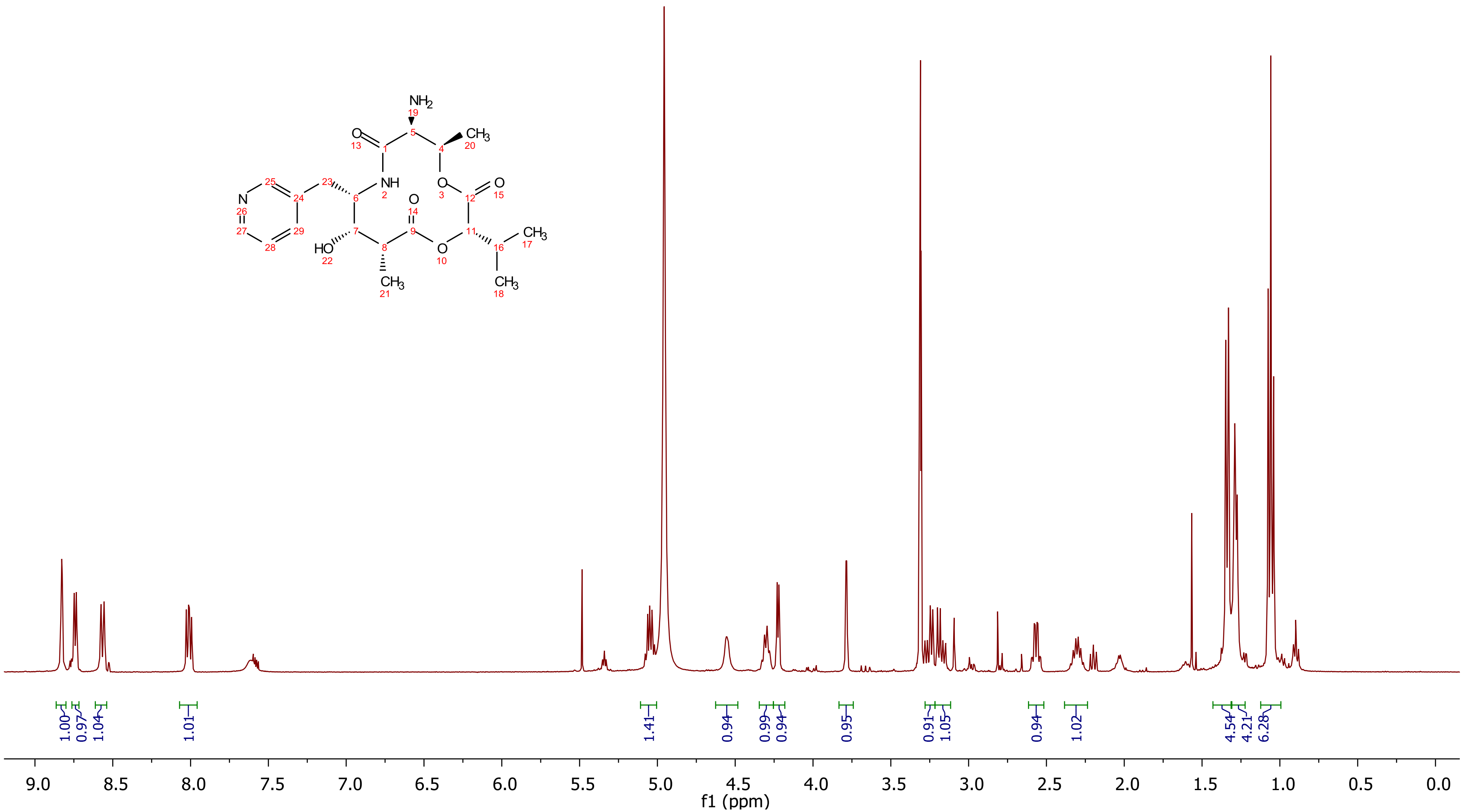
20


1.00
0.99
1.00
1.02
0.96
1.01
1.03
1.04
1.12
2.18
1.02
1.22
9.12
3.18
4.13
3.03
2.95
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)

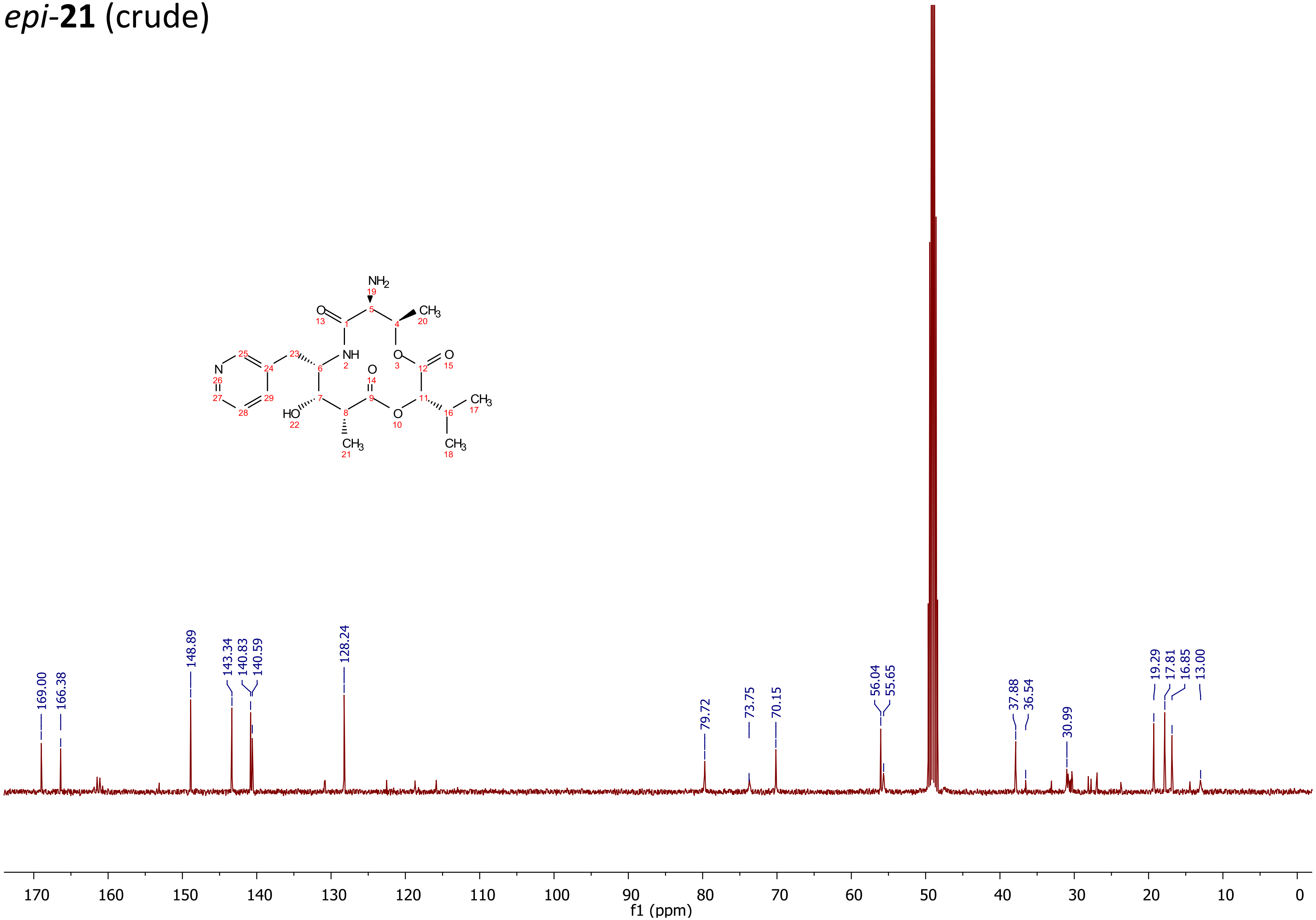
20


178.40
170.88
169.28
157.07
151.08
148.04
139.27
136.29
125.03
81.07
79.12
75.65
70.92
57.66
56.79
42.40
36.42
31.06
28.67
18.74
17.97
17.77
15.01
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)
O
CH3
CH3
CH3
HN
O
O
CH3
O
NH
N
O
O
O
HO
O
CH3
CH3
CH3

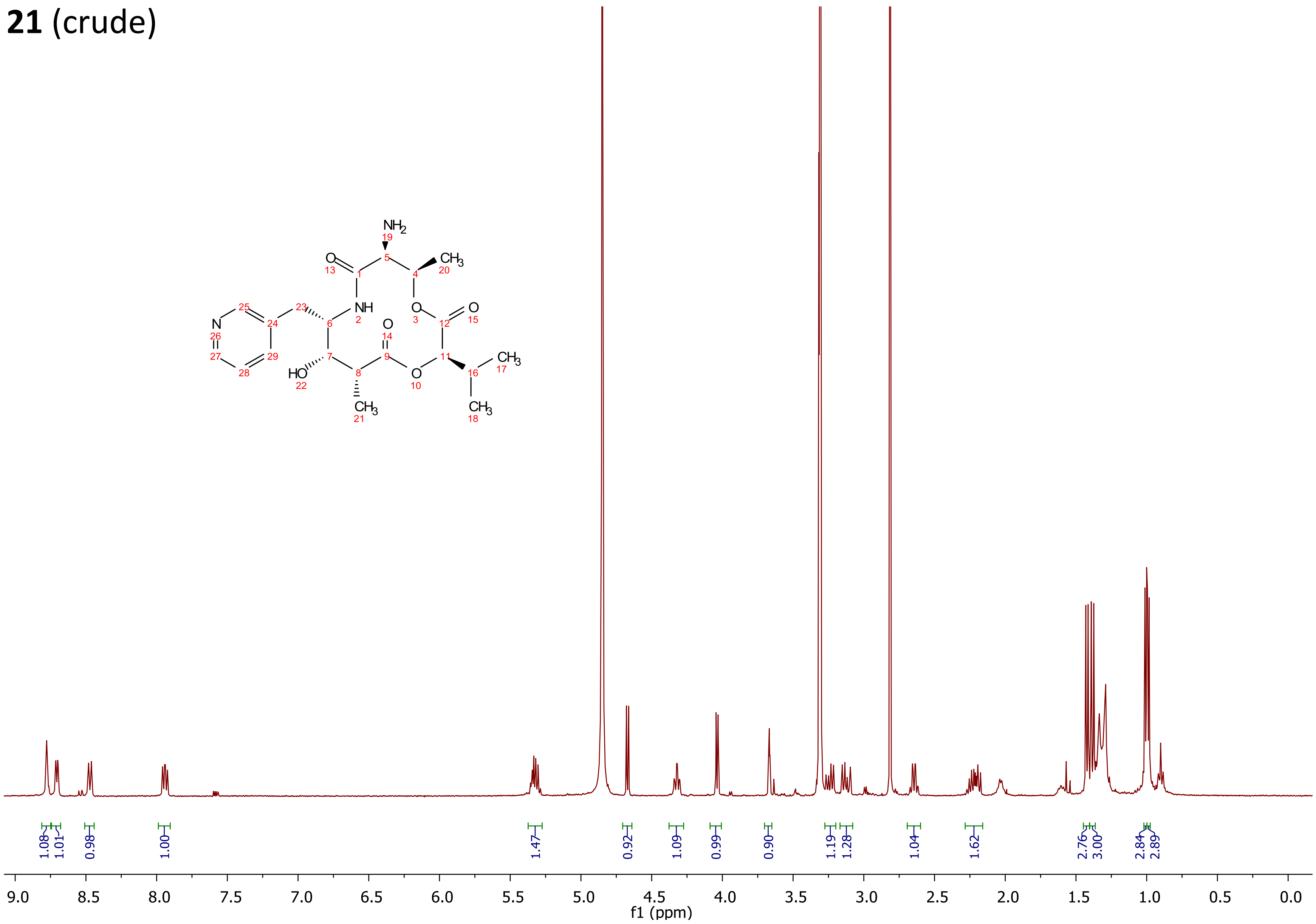
epi-**21** (crude)


NH2
CH3
O
NH
O
O
O
O
N
HO
O
CH3
CH3
CH3
1.00
0.97
1.04
1.01
1.41
0.94
0.99
0.94
0.95
0.91
1.05
0.94
1.02
4.54
4.21
6.28
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)

epi-**21** (crude)

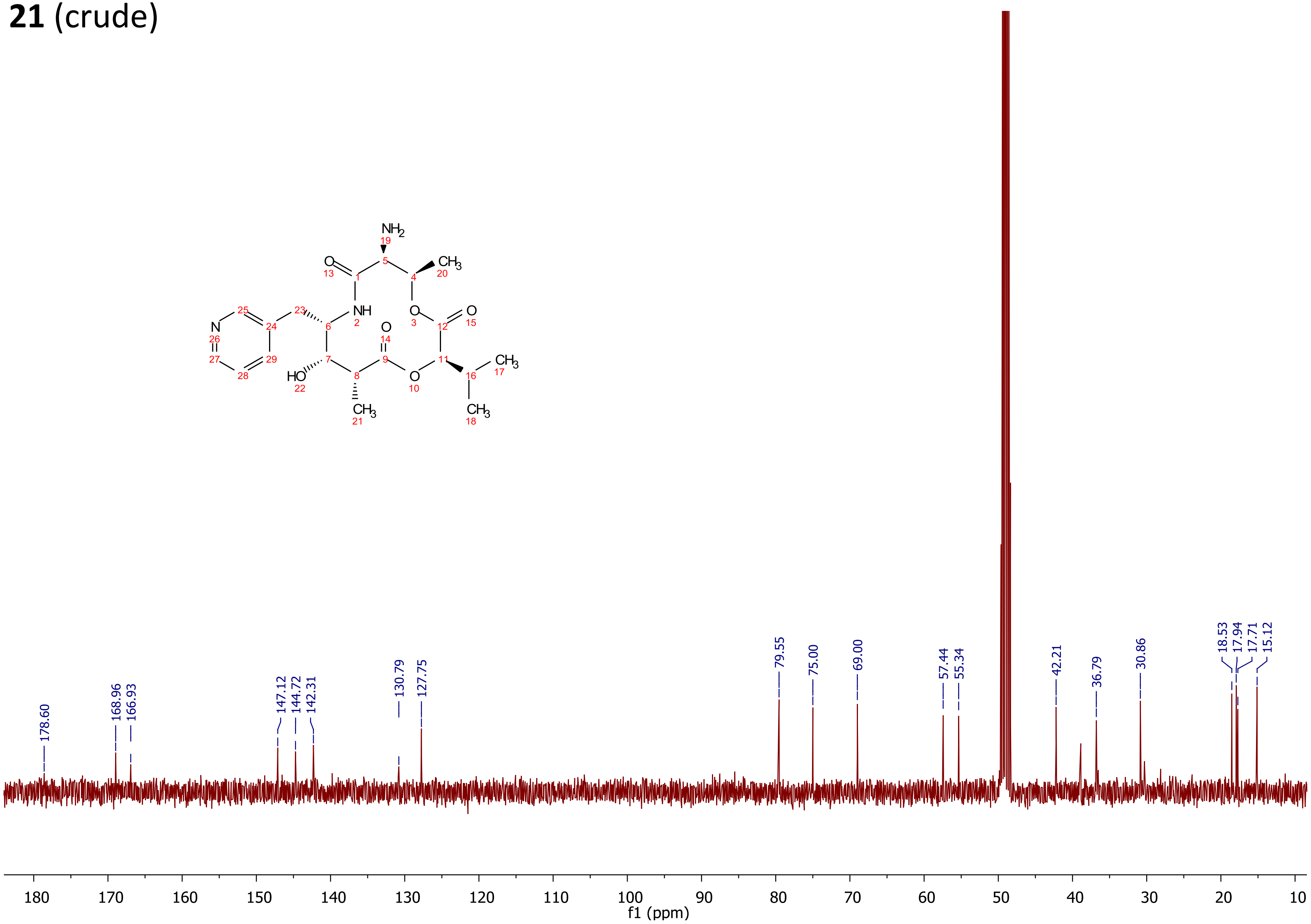

NH2
CH3
O
NH
O
O
N
HO
CH3
CH3
CH3
169.00
166.38
148.89
143.34
140.83
140.59
128.24
79.72
73.75
70.15
56.04
55.65
37.88
36.54
30.99
19.29
17.81
16.85
13.00
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

21 (crude)

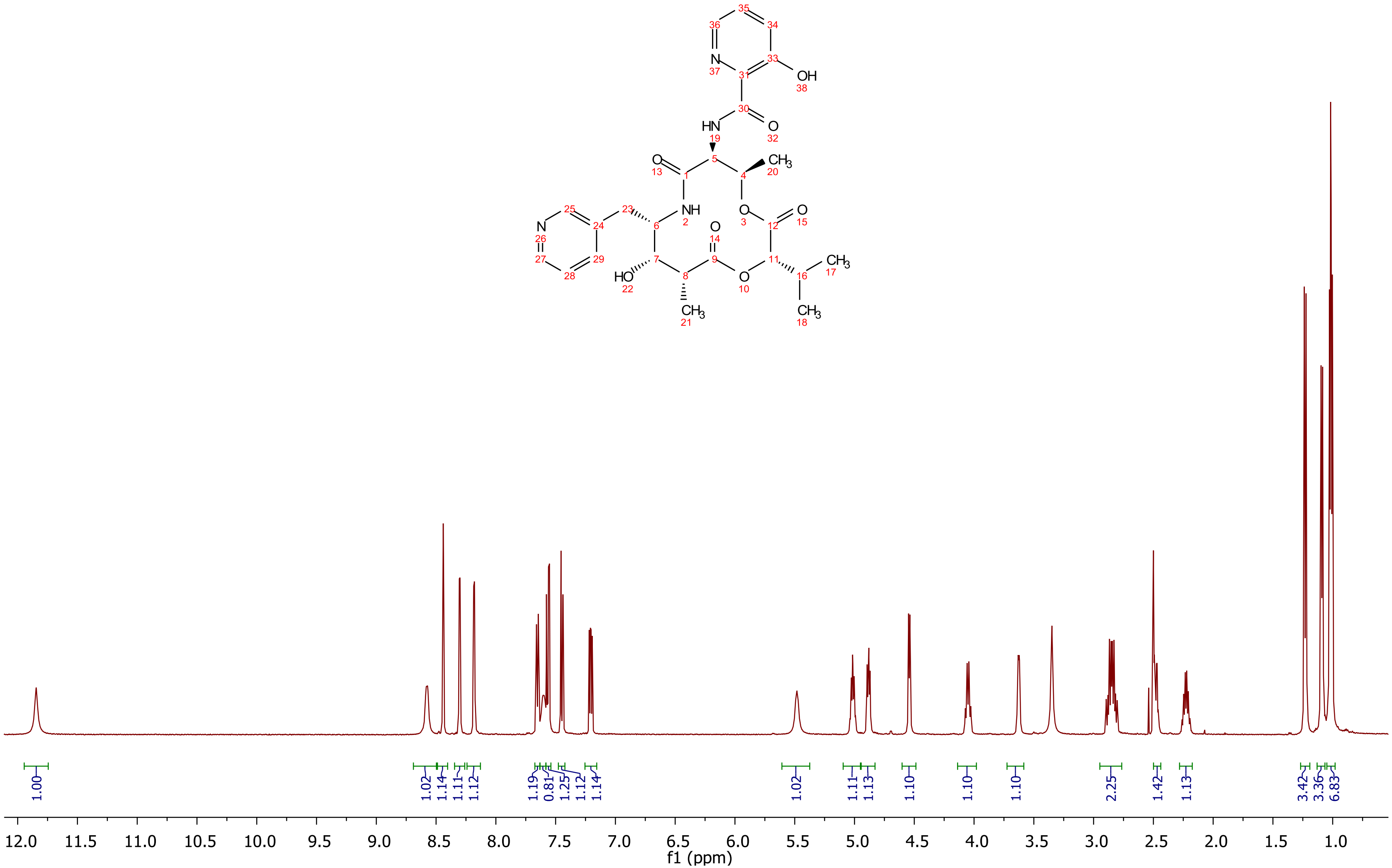


1.08
1.01
0.98
1.00
1.47
0.92
1.09
0.99
0.90
1.19
1.28
1.04
1.62
2.76
3.00
2.84
2.89
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

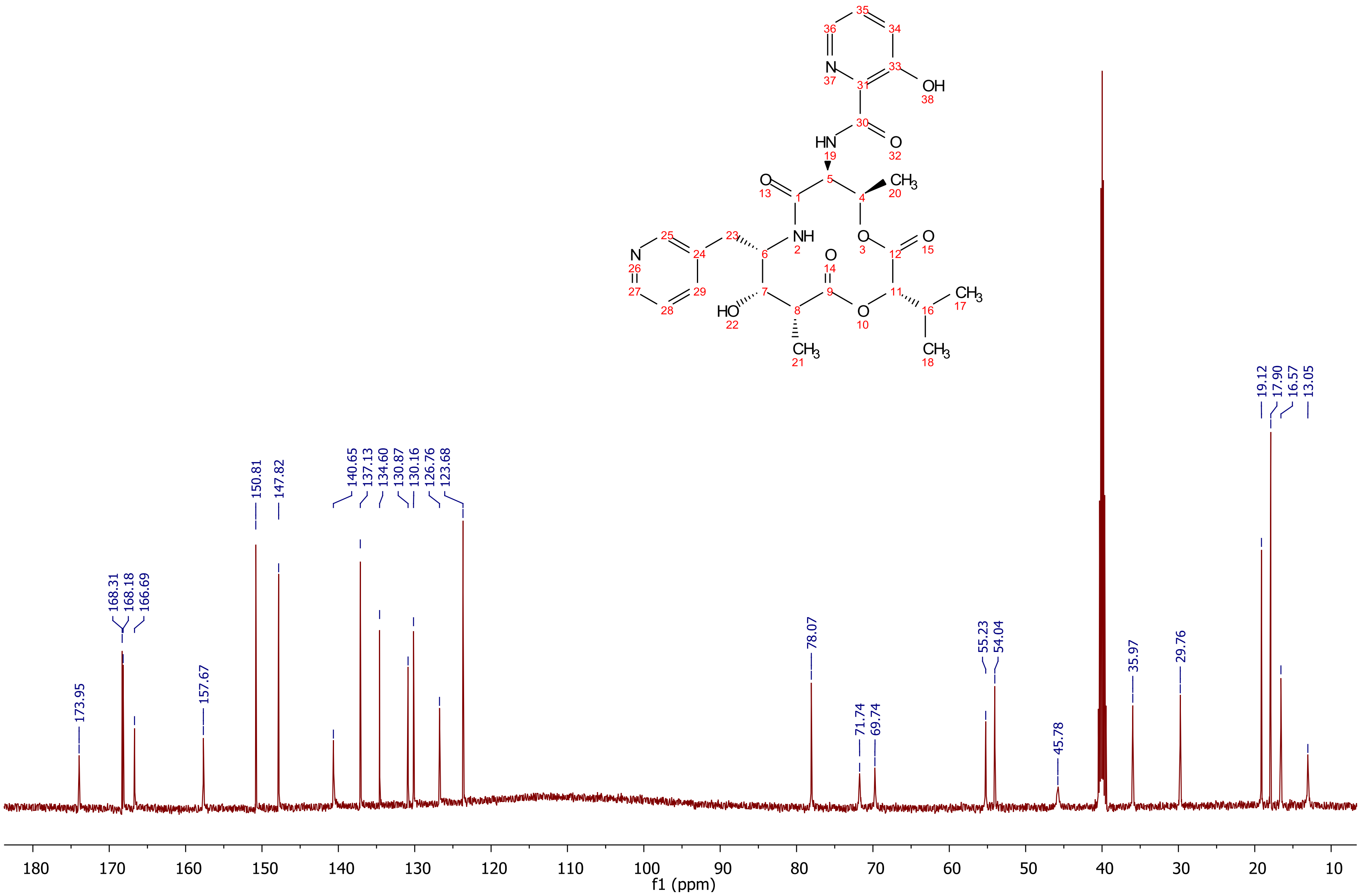
21 (crude)


NH2
19
O
13
1
5
4
CH3
20
NH
2
O
3
O
15
12
25
23
24
6
N
26
27
29
28
O
14
9
7
HO
22
8
CH3
21
O
10
11
16
CH3
17
CH3
18
178.60
168.96
166.93
147.12
144.72
142.31
130.79
127.75
79.55
75.00
69.00
57.44
55.34
42.21
36.79
30.86
18.53
17.94
17.71
15.12
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)

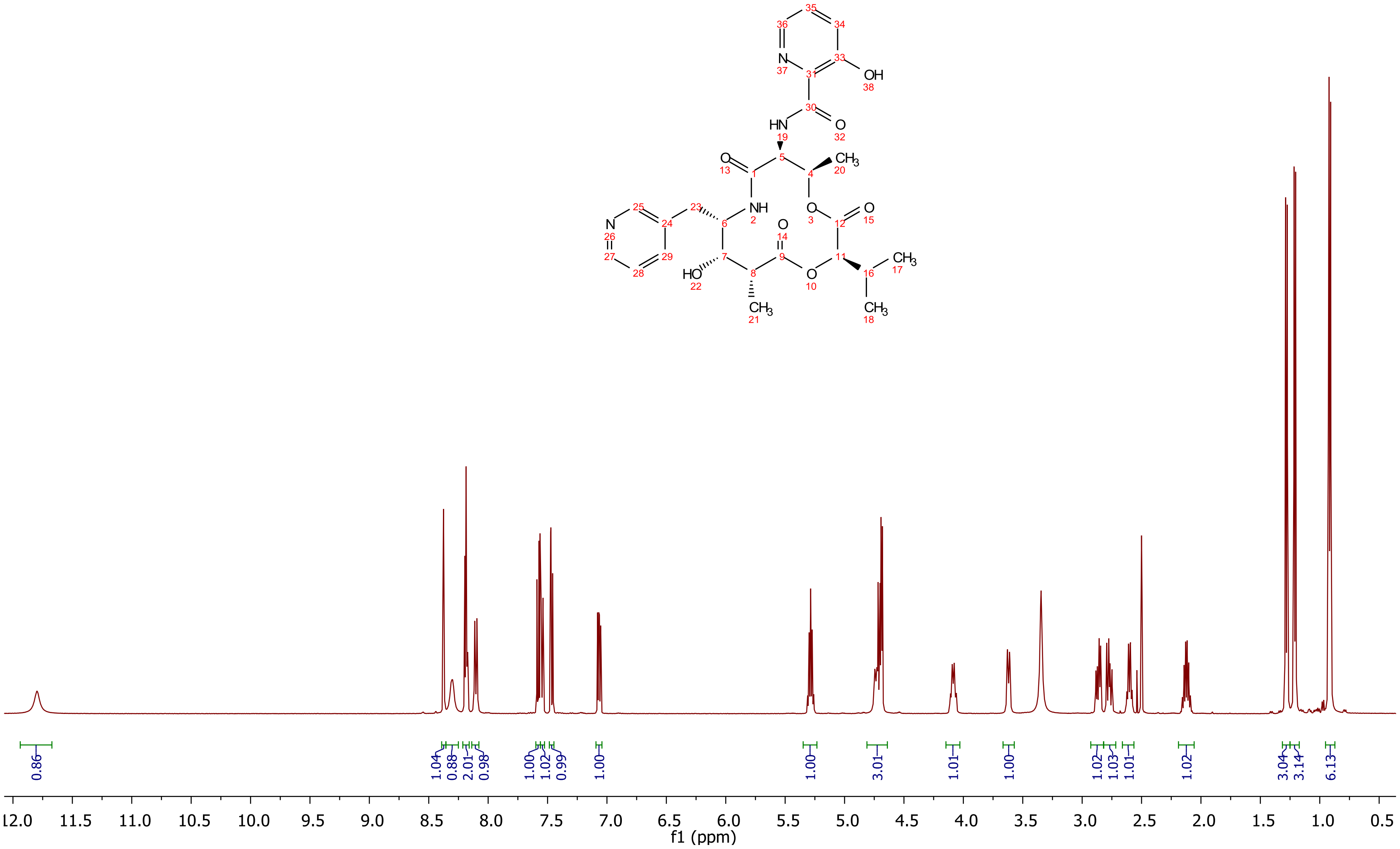
3


OH
HN
O
CH3
NH
N
HO
1.00
1.02
1.14
1.11
1.12
1.19
0.81
1.25
1.12
1.14
1.02
1.11
1.13
1.10
1.10
1.10
2.25
1.42
1.13
3.42
3.36
6.83
12.0
11.5
11.0
10.5
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
f1 (ppm)

3


173.95
168.31
168.18
166.69
157.67
150.81
147.82
140.65
137.13
134.60
130.87
130.16
126.76
123.68
78.07
71.74
69.74
55.23
54.04
45.78
35.97
29.76
19.12
17.90
16.57
13.05
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)
OH
HN
O
CH3
NH
N
HO

2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HN
O
CH3
NH
O
HO
CH3
CH3
CH3
N
N
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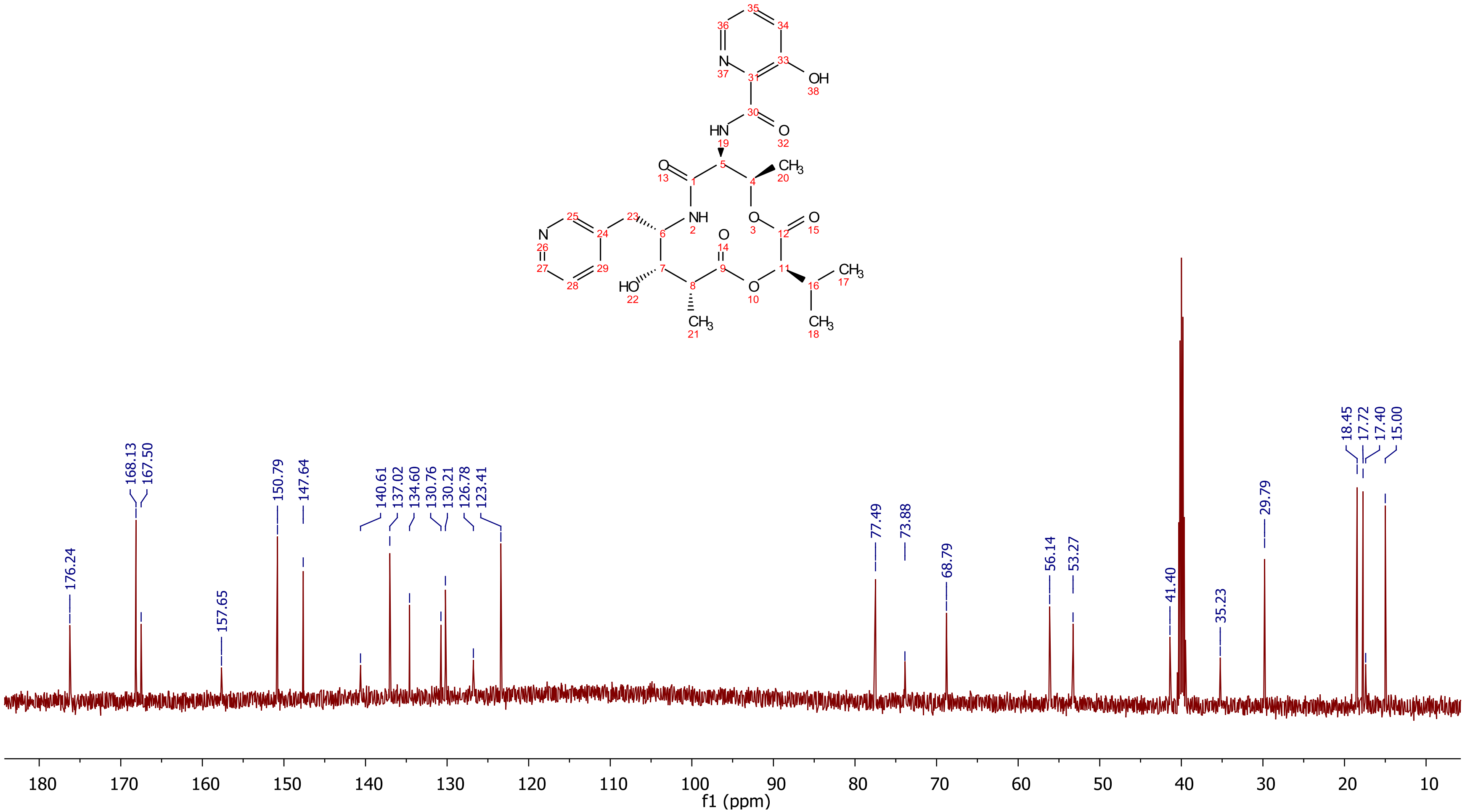
0.98
1.00
1.02
0.99
1.00
1.00
3.01
1.01
1.00
1.02
1.03
1.01
1.02
3.04
3.14
6.13
12.0
11.5
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f1 (ppm)

2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168.13
167.50
157.65
150.79
147.64
140.61
137.02
134.60
130.76
130.21
126.78
123.41
77.49
73.88
68.79
56.14
53.27
41.40
35.23
29.79
18.45
17.72
17.40
15.00
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
f1 (ppm)
OH
HN
NH
HO
N
O
CH3

6. Biological Evaluation

*Determination of the minimal inhibitory concentration (MIC) of **1**, **2**, and **3**:*[1]

The drug susceptibility of Mycobacterium tuberculosis strain H37Rv was determined using the resazurin microtitre assay (REMA) (Palomino JC, Martin A, Camacho M, Guerra H, Swings J, Portaels F, Resazurin microtiter assay plate: simple and inexpensive method for detection of drug resistance in Mycobacterium tuberculosis, *Antimicrob Agents Chemother* **2002**, *46*, 2720-2722). Briefly, bacteria were diluted from frozen stocks to an OD600 of 0.0001, and grown in a 96-well plate in the presence of serial compound dilutions. After 10 generations (7 days for M. tuberculosis) bacterial viability was determined using 10 µL of resazurin (0.025 % (w/v), and calculated as a percentage of resazurin turnover in the absence of compound. The MIC was determined as the minimal concentration of compound that caused background resazurin reduction

Steady state kinetics and inhibition of InhA:[1]

Inhibition of InhA activity was investigated using InhA(S94A) at 60 nM. Kinetic parameters were determined by following NADH oxidation every min for 30 min at 340 nm using a TECAN FL200 spectrophotometer. All reactions were performed at 25°C in 30 mM PIPES (pH 6.5), 150 mM NaCl and 10% glycerol in a 384 well plate. After addition of variable concentrations of NADH, reactions were initiated by adding 2-trans-dodecenoyl-CoA to a final concentration of 40 µM (250 µM in one experiment). Steady state Km for NADH was determined by measuring enzyme kinetics at different NADH concentrations (0 – 800 µM). NADH Km and pyridomycin and analogs Ki's were determined by measuring enzyme kinetics with both different NADH concentrations (33, 40, 50, 66, 75, 100, 150 µM) and different pyridomycin and analog concentrations (0, 1.25, 2.5, 5, 10, 20, 30 and 40 ug/mL). Firstly the rate of NADH oxidation was determined by linear regression of the 340 nm absorbance values (enzyme velocity) using Graphpad Prism. Then the data was analyses to determine NADH km and compound Ki values (competitive inhibition model).

[1] R. C. Hartkoorn, C. Sala, J. Neres, F. Pojer, S. Magnet, R. Mukherjee, S. Uplekar, S. Boy-Rottger, K.-H. Altmann, S. T. Cole, *EMBO Mol. Med.* **2012**, *4*, 1032-1042.

7. Crystallographic Data for 17 (CCDC 905295)

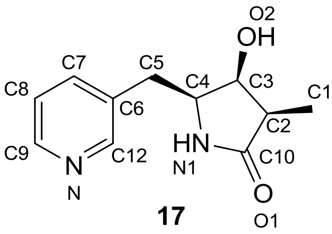
The crystal structure of compound **17** is deposited at the Cambridge Crystallographic Data Centre with the CCDC reference number 905295.

Table 1. Crystal data and structure refinement for **17** (905295).

Identification code	905295
Empirical formula	C11 H14 N2 O2

Formula weight	206.24
Temperature	100(2) K
Wavelength	1.54178 A
Crystal system, space group	Orthorhombic, P2(1)2(1)2(1)
Unit cell dimensions	a = 4.7626(3) A alpha = 90 deg. b = 8.9582(6) A beta = 90 deg. c = 23.7884(19) A gamma = 90 deg.
Volume	1014.92(12) A^3
Z, Calculated density	4, 1.350 Mg/m^3
Absorption coefficient	0.769 mm^-1
F(000)	440
Crystal size	0.13 x 0.02 x 0.01 mm
Theta range for data collection	3.72 to 67.18 deg.
Limiting indices -	3<=h<=5, -9<=k<=10, -26<=l<=27
Reflections collected / unique	5482 / 1686 [R(int) = 0.0858]
Completeness to theta	= 67.18 93.5 %
Absorption correction	None
Max. and min. transmission	0.9939 and 0.9086
Refinement method	Full-matrix least-squares on F^2
Data / restraints / parameters	1686 / 0 / 192
Goodness-of-fit on F^2	0.873
Final R indices [I>2sigma(I)]	R1 = 0.0574, wR2 = 0.1438
R indices (all data)	R1 = 0.0850, wR2 = 0.1640
Absolute structure parameter	-0.2(6)
Largest diff. peak and hole	0.235 and -0.289 e.A^-3

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters (A^2 x 10^3) for 905295.
U(eq) is defined as one third of the trace of the orthogonalized Uij tensor.



17

	x	y	z	U(eq)
O(1)	-5307(6)	-4179(3)	-2578(1)	39(1)
O(2)	-6768(5)	-3236(3)	-1022(1)	38(1)
N(1)	-7490(7)	-2169(4)	-2172(1)	32(1)
C(1)	-7597(11)	-5964(5)	-1654(2)	42(1)
C(2)	-8835(9)	-4477(5)	-1828(2)	32(1)
C(3)	-9203(9)	-3324(5)	-1367(2)	34(1)

C(4)	-9456(9)	-1872(5)	-1702(2)	32(1)
C(5)	-8598(9)	-453(5)	-1395(2)	36(1)
C(6)	-10607(9)	16(4)	-937(2)	32(1)
C(7)	-12521(9)	1173(4)	-1020(2)	35(1)
C(8)	-14268(9)	1614(5)	-581(2)	38(1)
C(9)	-14117(9)	877(5)	-80(2)	36(1)
C(10)	-7006(8)	-3628(4)	-2242(2)	33(1)
N	-12308(8)	-257(4)	15(1)	40(1)
C(12)	-10597(9)	-646(5)	-413(2)	37(1)

Table 3. Bond lengths [A] and angles [deg] for 905295.

O(1)-C(10)	1.240(5)
O(2)-C(3)	1.422(5)
O(2)-H(2)	1.09(5)
N(1)-C(10)	1.338(5)
N(1)-C(4)	1.483(5)
N(1)-H(1)	0.97(5)
C(1)-C(2)	1.515(6)
C(1)-H(1A)	1.06(4)
C(1)-H(1B)	1.00(6)
C(1)-H(1C)	0.99(6)
C(2)-C(3)	1.518(6)
C(2)-C(10)	1.519(5)
C(2)-H(2A)	1.05(4)
C(3)-C(4)	1.531(6)
C(3)-H(3)	1.04(4)
C(4)-C(5)	1.522(6)
C(4)-H(4)	1.05(4)
C(5)-C(6)	1.510(5)
C(5)-H(5A)	1.01(4)
C(5)-H(5B)	0.99(5)
C(6)-C(12)	1.379(6)
C(6)-C(7)	1.395(6)
C(7)-C(8)	1.392(6)
C(7)-H(7)	0.94(4)
C(8)-C(9)	1.363(6)
C(8)-H(8)	0.98(6)
C(9)-N	1.351(6)
C(9)-H(9)	1.00(5)
N-C(12)	1.351(5)
C(12)-H(12)	0.98(5)
C(3)-O(2)-H(2)	114(3)
C(10)-N(1)-C(4)	112.2(4)
C(10)-N(1)-H(1)	130(3)
C(4)-N(1)-H(1)	117(3)
C(2)-C(1)-H(1A)	117(2)
C(2)-C(1)-H(1B)	111(3)

H(1A)-C(1)-H(1B)	105(4)
C(2)-C(1)-H(1C)	109(3)
H(1A)-C(1)-H(1C)	107(4)
H(1B)-C(1)-H(1C)	108(4)
C(1)-C(2)-C(3)	116.4(3)
C(1)-C(2)-C(10)	113.3(3)
C(3)-C(2)-C(10)	101.2(3)
C(1)-C(2)-H(2A)	115(2)
C(3)-C(2)-H(2A)	106(2)
C(10)-C(2)-H(2A)	104(2)
O(2)-C(3)-C(2)	111.2(3)
O(2)-C(3)-C(4)	108.5(3)
C(2)-C(3)-C(4)	102.1(3)
O(2)-C(3)-H(3)	108(2)
C(2)-C(3)-H(3)	116(2)
C(4)-C(3)-H(3)	111(2)
N(1)-C(4)-C(5)	110.0(3)
N(1)-C(4)-C(3)	101.0(3)
C(5)-C(4)-C(3)	116.0(4)
N(1)-C(4)-H(4)	114(2)
C(5)-C(4)-H(4)	106(2)
C(3)-C(4)-H(4)	110(2)
C(6)-C(5)-C(4)	114.1(3)
C(6)-C(5)-H(5A)	110(2)
C(4)-C(5)-H(5A)	108(2)
C(6)-C(5)-H(5B)	112(2)
C(4)-C(5)-H(5B)	107(3)
H(5A)-C(5)-H(5B)	105(3)
C(12)-C(6)-C(7)	116.7(4)
C(12)-C(6)-C(5)	122.0(4)
C(7)-C(6)-C(5)	121.2(4)
C(8)-C(7)-C(6)	119.7(4)
C(8)-C(7)-H(7)	120(2)
C(6)-C(7)-H(7)	120(2)
C(9)-C(8)-C(7)	119.1(4)
C(9)-C(8)-H(8)	122(3)
C(7)-C(8)-H(8)	119(3)
N-C(9)-C(8)	123.0(4)
N-C(9)-H(9)	115(3)
C(8)-C(9)-H(9)	122(3)
O(1)-C(10)-N(1)	125.6(4)
O(1)-C(10)-C(2)	126.4(4)
N(1)-C(10)-C(2)	108.0(3)
C(9)-N-C(12)	116.8(4)
N-C(12)-C(6)	124.6(4)
N-C(12)-H(12)	114(2)
C(6)-C(12)-H(12)	121(2)

Symmetry transformations used to generate equivalent atoms: 0

Table 4. Anisotropic displacement parameters (A^2 x 10^3) for 905295.
The anisotropic displacement factor exponent takes the form:
-2 pi^2 [h^2 a*^2 U11 + ... + 2 h k a* b* U12]

	U11	U22	U33	U23	U13	U12
O(1)	34(2)	46(2)	38(2)	0(1)	4(1)	4(1)
O(2)	24(2)	51(2)	38(2)	-1(2)	-6(1)	-3(1)
N(1)	31(2)	35(2)	31(2)	1(2)	3(2)	1(2)
C(1)	38(3)	39(2)	49(3)	7(2)	7(2)	-3(2)
C(2)	25(2)	36(2)	36(2)	3(2)	4(2)	-4(2)
C(3)	16(2)	49(2)	37(2)	-1(2)	-4(2)	-3(2)
C(4)	16(2)	46(2)	34(2)	-2(2)	7(2)	0(2)
C(5)	25(2)	45(3)	39(2)	-1(2)	7(2)	-3(2)
C(6)	25(2)	38(2)	34(2)	-4(2)	4(2)	-4(2)
C(7)	29(2)	37(2)	38(2)	-3(2)	0(2)	-3(2)
C(8)	28(2)	44(2)	42(3)	-5(2)	-5(2)	0(2)
C(9)	25(2)	48(2)	36(2)	-7(2)	2(2)	-1(2)
C(10)	24(2)	40(2)	36(2)	0(2)	-3(2)	-2(2)
N	36(2)	51(2)	34(2)	0(2)	7(2)	0(2)
C(12)	35(2)	39(2)	38(2)	-3(2)	4(2)	-2(2)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters (A^2 x 10^3) for 905295.

	x	y	z	U(eq)
H(2)	-6770(110)	-4020(60)	-670(20)	63(15)
H(2A)	-10780(90)	-4550(40)	-2031(15)	28(10)
H(1A)	-7420(90)	-6790(50)	-1972(16)	34(10)
H(5A)	-8400(80)	370(40)	-1680(15)	24(10)
H(4)	-11550(80)	-1710(40)	-1825(14)	28(10)
H(5B)	-6670(100)	-620(50)	-1251(17)	40(13)
H(3)	-10920(80)	-3470(40)	-1105(15)	23(9)
H(1)	-7010(100)	-1330(50)	-2411(18)	49(13)
H(1B)	-5650(130)	-5840(50)	-1506(19)	54(14)
H(9)	-15350(110)	1130(50)	250(20)	57(14)
H(1C)	-8770(120)	-6390(60)	-1350(20)	71(17)
H(12)	-9360(100)	-1500(50)	-328(17)	44(12)
H(7)	-12660(80)	1640(40)	-1374(16)	25(10)
H(8)	-15680(130)	2390(60)	-650(20)	70(16)

Table 6. Torsion angles [deg] for 905295.

C(1)-C(2)-C(3)-O(2)	45.4(5)
C(10)-C(2)-C(3)-O(2)	-77.9(4)
C(1)-C(2)-C(3)-C(4)	160.9(3)
C(10)-C(2)-C(3)-C(4)	37.7(4)
C(10)-N(1)-C(4)-C(5)	143.6(4)
C(10)-N(1)-C(4)-C(3)	20.5(4)
O(2)-C(3)-C(4)-N(1)	82.0(4)
C(2)-C(3)-C(4)-N(1)	-35.5(4)
O(2)-C(3)-C(4)-C(5)	-36.9(5)
C(2)-C(3)-C(4)-C(5)	-154.4(3)
N(1)-C(4)-C(5)-C(6)	175.3(3)
C(3)-C(4)-C(5)-C(6)	-70.9(5)
C(4)-C(5)-C(6)-C(12)	79.5(5)
C(4)-C(5)-C(6)-C(7)	-102.2(5)
C(12)-C(6)-C(7)-C(8)	0.8(6)
C(5)-C(6)-C(7)-C(8)	-177.5(4)
C(6)-C(7)-C(8)-C(9)	-1.5(6)
C(7)-C(8)-C(9)-N	1.0(6)
C(4)-N(1)-C(10)-O(1)	-174.8(4)
C(4)-N(1)-C(10)-C(2)	3.6(5)
C(1)-C(2)-C(10)-O(1)	26.6(6)
C(3)-C(2)-C(10)-O(1)	152.0(4)
C(1)-C(2)-C(10)-N(1)	-151.8(4)
C(3)-C(2)-C(10)-N(1)	-26.4(4)
C(8)-C(9)-N-C(12)	0.2(6)
C(9)-N-C(12)-C(6)	-1.0(6)
C(7)-C(6)-C(12)-N	0.4(6)
C(5)-C(6)-C(12)-N	178.8(4)

Symmetry transformations used to generate equivalent atoms: 0