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SUPPLEMENTARY MATERIAL

Synthesis of Norfijimycin A with Activity against *Mycobacterium tuberculosis*

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All **reactions** were carried out under an argon atmosphere and at room temperature (23 ^{o}C) unless the reaction was performed under aqueous conditions or unless otherwise specified. Reactions undertaken at –78 ^{o}C utilized a bath of dry ice and acetone. Reactions carried out at 0 ^{o}C employed a bath of water and ice. Anhydrous THF, CH_2Cl_2, DMF, and MeCN were obtained using a PureSolv® solvent purification system (water <10 ppm). Reactions were monitored by thin layer chromatography (TLC) on aluminium backed silica plates (Merck Silica Gel 60 F254). Visualisation of TLC plates was undertaken with an ultraviolet (UV) light at $\lambda = 254$ nm and staining with solutions of vanillin or phosphomolybdic acid (PMA), followed by exposure of the stained plates to heat. Silica flash column chromatography (Silica Gel 60 $40-63$ μm) was undertaken to purify crude reaction mixtures using solvents as specified. Separations were performed using a Biotage Isolera® purification system with a diode array detector and a fraction collector.

NMR spectra were obtained using a Bruker DRX 400 or DRX 500 at frequencies of 400 MHz or 500 MHz respectively in $CDCl_3$, CD_3OD, CD_3CN, or DMSO-d_6. Chemical shifts are reported in parts per million (ppm) and coupling constants in Hertz (Hz). The residual solvent peaks were used as internal standards ($CDCl_3$: δ_H = 7.26, δ_C = 77.16; CD_3OD: δ_H = 3.31, δ_C = 49.00; CD_3CN: δ_H = 1.94, δ_C = 118.26/1.32; DMSO-d_6: δ_H = 2.50, δ_C = 39.52 ppm).[1]. ^{1}H NMR data is reported as follows: Chemical shift values (ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), coupling constant(s) and relative integral. ^{13}C NMR spectra were obtained using a Bruker DRX 300, DRX 400, or DRX 500 at 75.5 MHz, 100.6 MHz, or 125.8 MHz in $CDCl_3$, CD_3OD, CD_3CN, or DMSO-d_6. ^{13}C NMR data is reported as chemical shift values (ppm). In the case of diastereomeric mixtures, the signals of the major diastereomer are reported unless otherwise noted.

Mass spectra were recorded on a Shimadzu 2020 (ESI) mass spectrometer operating in positive mode. High resolution mass spectra were recorded on a Bruker-Daltronics Apex Ultra 7.0T Fourier transform (FTICR) mass spectrometer.

Optical rotations were measured on a Perkin-Elmer 341 polarimeter at a wavelenght of 589 nm.

IR spectra were recorded on a Bruker ALPHA FT-IR-spectrometer using a diamond ATR unit.

Melting points were determined with a SRS Optimelt melting point apparatus and are uncorrected.

Preparative RP-HPLC was performed using a Waters 600 Multisolvent Delivery System and Waters 500 pump with 2996 photodiode array detector. Programmable wavelength detector operating at 210 – 300 nm. Compounds were purified using a XBridge BEH C_{18} 5 μm or a Sunfire C_{18} (19 × 150 mm or 30 × 150 mm) column operating at total flow rates of 32.0 mL·min^{-1} or 50.0 mL·min^{-1} respectively. A mobile phase of 0.1% trifluoroacetic acid in water (Solvent A) and 0.1% trifluoroacetic acid in acetonitrile (Solvent B) was used in all cases.

LC/MS was performed on a Shimadzu UPLC/MS instrument consisting of a LC-M20A pump and a SPD-M30A diode array detector coupled to a Shimadzu 2020 mass spectrometer (ESI) operating in positive mode. Separations on the UPLC/MS system were performed using a Waters Acquity UPLC BEH C_{18} 1.7 μm column (2.1 × 50 mm at a total flow rate of 0.60 mL·min^{-1}. Separations were performed using a mobile phase of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B).

General Procedures

General Procedure 1: **Solid phase peptide synthesis**

Preloading 2-chlorotrityl chloride resin: 2-Chlorotrityl chloride resin was swollen in dry DCM for 30 min then washed with DCM (5 × 1 mL). A solution of Fmoc-AA-OH and *i*Pr$_2$NEt (2.0 equiv. relative to resin functionalization) in DCM (final concentration 100 μM of amino acid) was added and the resin was shaken at rt for 16 h. The resin was washed with DMF (5 × 1 mL) and DCM (5 × 1 mL). The resin was capped by treating with a solution of DCM/CH$_3$OH/*i*Pr$_2$NEt (17:2:1 v/v/v, 1 mL) for 1 h and washed with DMF (5 × 1 mL), DCM (5 × 1 mL), and DMF (5 × 1 mL). The resin was subsequently submitted to iterative peptide assembly (Fmoc-SPPS).

Estimation of amino acid loading: The resin was treated with 20% piperidine/DMF (2 × 1 mL, 3 min) and 50 μL of the combined deprotection solution was diluted to 10 mL

using 20% piperidine/DMF in a volumetric flask. The UV absorbance of the resulting piperidine-fulvene adduct was measured (λ = 301 nm, ε = 7800 M^{-1} cm^{-1}) to estimate the amount of amino acid loaded onto the resin.

General Procedure 2: Iterative peptide assembly (Fmoc-SPPS)

General amino acid coupling: A solution of Fmoc-protected amino acid (4 equiv.), PyAOP (4 equiv.), HOAt (8 equiv.) and 4-methylmorpholine (NMM, 8 equiv.) in DMF (final concentration of resin-bound peptide 100 μM) was preactivated for 3 min before added to the resin. After 1 h (15 h in the case of *N*-methyl amino acids) the resin was washed with DMF (5 × 1 mL), DCM (5 × 1 mL) and DMF (5 × 1 mL).

Fmoc-deprotection: The resin was treated with 20% piperidine/DMF (2 × 1 mL, 3 min) and washed with DMF (5 × 1 mL), DCM (5 × 1 mL) and DMF (5 × 1 mL).

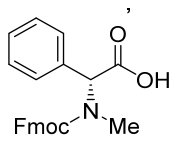
Capping: Acetic anhydride/pyridine (1:9 v/v, 1 mL)) was added to the resin. After 3 min the resin was washed with DMF (5 × 1 mL), DCM (5 × 1 mL) and DMF (5 × 1 mL).

Cleavage: TFA/TIS/H_2O (95:5:5 v/v/v, 3 mL) was added to the resin and shaken for 1 h. Then the resin was washed wih TFA/TIS/H_2O (95:5:5 v/v/v, 4 × 2 mL). The combined solutions were concentrated with a stream of N_2, purified by preparative RP-HPLC and analyzed by LC/MS (ESI+).

General Procedure 3: **Alloc-deprotection**: A solution of $Pd(PPh_3)_4$ (10 mol–%) and $PhSiH_3$ (25 equiv.) in DCM (final concentration of resin-bound peptide 100 μM) was added to the resin. After 15 min the resin was washed with DCM (10 × 3 mL), DMF (10 × 3 mL), DCM (10 × 3 mL), and DMF (10 × 3 mL).

General Procedure 4: **Allyl-deporotection**: A solution of $Pd(PPh_3)_4$ (0.8 equiv.) and $PhSiH_3$ (40 equiv.) in DCM (final concentration of resin-bound peptide 100 μM) was added to the resin. After 1 h the resin was washed with DCM (10 × 3 mL), DMF (10 × 3 mL), DCM (10 × 3 mL), and DMF (10 × 3 mL).

Experimental and analytical data



(*R*)-Fmoc-*N*(Me)-Phg-OH (7)

A suspension of (*R*)-*N*-methylphenylglycine (495 mg, 3.00 mmol) and FmocOSu (1.11 g, 3.30 mmol) in THF/sat. $NaHCO_3$ (20 mL, 1:1, v/v) was stirred at rt for 15 h. The reaction mixture was diluted with H_2O (10 mL), then washed with Et_2O (25 mL). Subsequently, HCl (1M) was added dropwise to reach pH = 3. The reaction mixture was extracted with ethyl acetate (3 × 20 mL), the combined organic extracts were dried over anhydrous Na_2SO_4, and the solvent was removed *in vacuo* yielding 1.10 g (95%) of the Fmoc-protected amino acid **7** as a colorless solid.

$[\alpha]_D^{25}$ = –72.3 (*c* 1.0, CH_2Cl_2).

m.p. 124.5 – 126 °C.

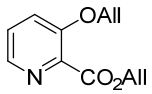
FTIR (ATR): $\tilde{\nu}$ = 2929, 1743, 1667, 1479, 1447, 1399, 1351, 1305, 757, 739, 704 cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.76 (d, *J* = 7.6 Hz, 2H), 7.60 (d, *J* = 6.6 Hz, 2H), 7.43 – 7.28 (m, 8H), 7.15 (br s, 1H), 6.15, 5.76 (rotamers, s, 1H), 4.58 – 4.44 (m, 2H), 4.30 (m, 1H), 2.78, 2.73, 2.68 (rotamers, s, 3H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 175.4, 144.0, 143.9, 141.5, 129.2, 129 0, 128.8, 127.9, 127.2, 125.20, 125.16, 120.1, 68.2, 62.7, 47.4, 31.2 ppm.

MS (ESI+): *m/z* (%): 410.1 (100) $[M + Na]^+$.

HRMS (ESI+): Calcd. for $[C_{24}H_{21}NO_4 + Na]$: *m/z* = 410.1363, found: 410.1365.



Allyl 3-*O*-Allyloxypicolinate

3-Hydroxypicolinic acid (3.06 g, 20.0 mmol) was added to a stirred mixture of NaH (60%, 2.00 g, 50.0 mmol) in DMF (100 mL) at rt and stirred for 20 min before allylbromide (3.62 mL, 42.0 mmol) was added and the resulting mixture was stirred for 15 h. After the addition of H_2O (2 mL), the solvent was removed *in vacuo* and the brown residue was taken up in sat. $NaHCO_3$ (100 mL) and extraced with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried over anhydrous Na_2SO_4 and the crude product was purified by column chromatography on silica [hexanes/EtOAc = 90:10 → 0:100 (12 CV)[a]] affording *the title compound* as a dark oil (2.52 g, 57%).

R_f = 0.58 (hexanes/EtOAc = 1:1).

FTIR (ATR): $\tilde{\nu}$ = 1732, 1578, 1445, 1300, 1193, 1139, 1102, 989, 801, 737 cm^{-1}.

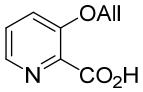
^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.24 (s, 1H), 7.34 – 7.29 (m, 2H), 6.03 – 5.99 (m, 2H), 5.42 (app t, J = 15.2 Hz, 2H), 5.27 (app t, J = 12.6 Hz, 2H), 4.86 – 4.84 (m, 2H), 4.61 (app br s, 2H) ppm.

^{13}C NMR (75.5 MHz, $CDCl_3$): δ = 164.7, 154.5, 141.4, 139.7, 132.1, 132.0, 126.9, 121.6, 119.1, 118.4, 69.7, 66.3 ppm.

MS (ESI+): *m/z* (%): 242.1 (100) $[M + Na]^+$.

HRMS (ESI+): Calcd. for $[C_{12}H_{13}NO_3 + Na]$: *m/z* = 242.0788, found:242.0788

[a] CV = column volumes



3-Allyloxypicolinic acid (12)

LiOH (320 mg, 13.4 mmol) was added to a solution of allyl 3-*O*-allyloxypicolinate (2.18 g, 9.95 mmol) in THF/H_2O (2:1, v/v) and the reaction mixture was stirred at rt for 15 h. After the addition of HCl (1M) to reach pH = 5, the solvent was removed *in vacuo* and the residue was purified by column chromatograhy on silica [CH_2Cl_2/MeOH = 95:5 → 85:15 (10 CV)] to yield the *title compound* as a colorless foam (1.71 g, 96%).

R_f = 0.34 (10% MeOH/CH_2Cl_2).

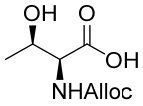
FTIR (ATR): $\tilde{\nu}$ = 3376, 1608, 1444, 1394, 1273, 1215, 1119, 982, 868, 803, 708, 667 cm^{-1}.

^{1}H NMR (500 MHz, CD_3OD, $CDCl_3$): δ = 8.12 (dd, J = 4.4, 1.4 Hz, 1H), 7.49 – 7.16 (m, 2H), 6.02 (ddt, J = 17.3, 10.4, 5.0 Hz, 1H), 5.44 (dq, J = 17.3, 1.7 Hz, 1H), 5.28 (dq, J = 10.4, 1.4 Hz, 1H), 4.66 (dt, J = 5.0, 1.7 Hz, 2H) ppm.

^{13}C NMR (125.8 MHz, CD_3OD, $CDCl_3$): δ = 168.4, 154.7, 141.7, 140.5, 132.7, 127.2, 122.8, 118.3, 70.1 ppm.

MS (ESI–): *m/z* (%): 178.6 (100) $[M - H]^-$, $[C_9H_9NO_3 - H]^-$.

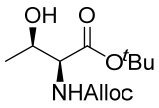
HRMS (ESI+): Calcd. for $[C_9H_9NO_3 + Na]$: *m/z* = 202.0475, found: 202.0475.



Alloc-Thr-OH

L-Threonine (**5**, 2.38 g, 20.0 mmol) was suspended in a THF/sat. $NaHCO_3$ (50 mL, 1:1 v/v) and cooled to 0 °C. Then allylchloro formate (2.13 mL, 20.0 mmol) was added dropwise and the mixture was stirred at this temperature for 10 min before the ice bath was removed and the reaction was allowed to stir at rt for 15 h. The reaction mixture was diluted with H_2O (50 mL), then washed with Et_2O (25 mL). Subsequently, HCl (5M) was added dropwise to reach pH = 1.5. The reaction mixture was extracted with ethyl acetate (3 × 100 mL), the combined organic extracts were dried over anhydrous Na_2SO_4, and the

solvent was removed *in vacuo* yielding 2.60g (64%) of the desired Alloc-protected amino acid as a colorless syrup which was used in the next step without further purification.



Alloc-Thr-O^{t}Bu (6)

A mixture of DIC (6.20 mL, 61.0 mmol), CuCl (226 mg, 2.28 mmol) and *tert*-BuOH (5.0 mL) was stirred at rt under the exclusion of light for 5 d. After dilution of the mixture with CH_2Cl_2 (25 mL), a solution of Alloc-Thr-OH (2.40 g, 11.8 mmol) in CH_2Cl_2 (15 mL) was added dropwise and the mixture was stirred at rt for 15 h. The mixture was filtered, washed with sat. $NaHCO_3$ (2 ×25 mL), and the organic phase was dried over anhydrous Na_2SO_4. After reomval of the solvent *in vacuo* the residue was purified by flash chromatography on silica [hexanes/EtOAc = 85:15 → 25:75 (10 CV)] furnishing **6** as a colorless syrup (2.20 g, 72%).

R$_f$ = 0.79 (hexanes/EtOAc = 1:1).

[α]$_D^{25}$ = –26.9 (*c* 2.0, MeOH).

FTIR (ATR): $\tilde{\nu}$ = 2978, 1701, 1516, 1369, 1221, 1154, 1065, 993 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.93 (ddt, *J* = 17.4, 10.6, 5.7 Hz, 1H), 5.46 (d, *J* = 8.8 Hz, 1H), 5.39 – 5.28 (m, 1H), 5.22 (dq, *J* = 10.6, 1.4 Hz, 1H), 4.59 (dt, *J* = 5.7, 1.4 Hz, 2H), 4.26 (s, 1H), 4.19 (d, *J* = 8.8 Hz, 1H), 1.48 (s, 9H), 1.24 (d, *J* = 6.4 Hz, 3H) ppm.

^{13}C NMR (100.6 MHz, $CDCl_3$): δ = 170.3, 156.7, 132.8, 117.9, 82.8, 68.5, 66.1, 59.6, 28.2, 20.1 ppm.

MS (ESI+): *m/z* (%): 282.1 (100) $[M + Na]^+$.

HRMS (ESI+): Calcd. for $[C_{12}H_{21}NO_5 + Na]$: *m/z* = 282.1312, found: 282.1313.

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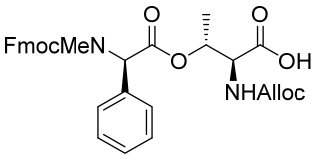


***O*-((*R*)-2-((((9*H*-fluoren-9-yl)methoxy)carbonyl)(methyl)amino)-2-phenylacetyl)-*N*-((allyloxy)carbonyl)-L-threonine (4)**

To a stirred solution of Alloc-Thr-O^{t}Bu (**6**, 337 mg, 1.30 mmol) and (*R*)-Fmoc-α-phenylsarcosine (**7**, 620 mg, 1.60 mmol) in CH_2Cl_2 (6.50 mL) was added EDC·HCl (380 mg, 2.00 mmol) and DMAP (16.0 mg, 0.10 mmol) at 0 °C and the mixture was stirred at this temperature for 3 h. After removal of the solvent by a stream of N_2, the residue was re-dissolved in EtOAc (50 mL) and washed with HCl (50 mL, 1 M), sat. $NaHCO_3$ (50 mL), and H_2O (50 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was removed *in vacuo*. The residue was purified by chromatography on silica [hexanes → 30% EtOAc/hexanes (18 CV)] yielding *tert*-butylester **8** as a colorless oil and a mixture of diastereomers (600 mg 73%, *dr* 66:34).

Ester **8** (300 mg, 477 µmol, *dr* 66:34) was treated with a mixture of TFA/CH_2Cl_2 (10 mL, 1:1, v/v) and stirred at rt for 15 min. The solvent was removed *in vacuo*, the residue was re-dissolved in MeCN/H_2O (10 mL, 1:1) and purified by preparative reversed-phase HPLC (Sunfire C_{18}, 30 × 150 mm) using an isokratic solvent combustion [MeCN/H_2O (0.1% TFA) = 55:45 total flow rate: 50 mL·min^{-1}] affording the desired diastereomer **4** (160 mg, 60%, *dr* > 99:1) and the minor component **9** (90.0 mg, 33%, *dr* 95:5) both as colorless oils.[b]

Preparative **RP-HPLC**: t_R =9.3 min (**9**), 10.2 min (**4**).

RP-LC/MS: t_R = 2.70 min (**9**), 2.73 min (**4**), MeCN/H_2O (0.1% HCO_2H) = 0:0 (0.00–0.30 min) → 100:0 (3.00 min), total flow rate: 0.60 mL·min^{-1}.

$[\alpha]_D^{25}$ = + 54.3 (*c* 0.4, MeOH).

FTIR (ATR): $\tilde{\nu}$ = 1649, 1450, 1149, 741, 691 cm^{-1}.

^{1}H NMR (500 MHz, Methanol-d_4): δ = 7.78 (d, *J* = 8.2 Hz, 2H), 7.61 (d, *J* = 7.2 Hz, 2H), 7.40 – 7.30 (m, 7H), 7.19 (br s, 1H), 7.03 (br s, 1H), 5.92, 5.60 (br s, 1H, rotamers), 5.90 – 5.84 (m, 1H), 5.52 (br s, 1H), 5.26 (d, *J* = 16.7 Hz, 1H), 5.14 (d, *J* = 10.8 Hz, 1H), 4.65 – 4.42 (m, 5H), 4.28 (m, 1H), 2.61 (s, 3H), 1.29 – 1.22 (m, 3H, rotamers) ppm.

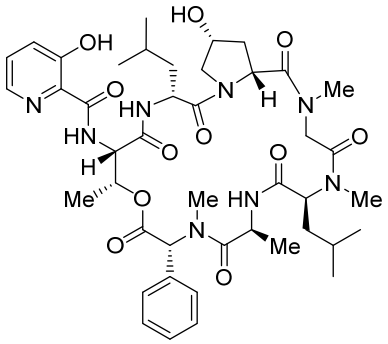
[b] crude *dr* 66:34 (**4**:**9**).

^{13}C NMR (125.8 MHz, Methanol-d_4): δ = 172.5, 170.7, 160.9, 158.7, 145.3, 142.7, 135.4, 134.1, 130.1, 129.9, 129.6, 128.8, 128.2, 126.0, 121.0, 117.8, 73.1, 69.0, 66.9, 63.8, 58.8, 48.5, 31.7, 17.1 ppm.

MS (ESI+): *m/z* (%): 595.3 (85) $[M + Na]^+$.

HRMS (ESI+): Calcd. for $[C_{32}H_{32}N_2O_8 + Na]$: *m/z* = 595.2051, found: 595.2052.

Norfijimyin A (3)



Fmoc-Ala-OH (9.34 mg, 30 µmol) and DIPEA (10.5 µL, 60 µmol) were dissolved in anhydrous CH_2Cl_2 (0.3 mL) and added to pre-swollen 2CTC-resin (12.5 mg). After 16 h the loading mixture was dicharged from the fritted syringe and the resin was washed with CH_2Cl_2 (5 × 1 mL), DMF (5 × 1 mL), and CH_2Cl_2 (5 × 1 mL). Then the resin was capped according to general procedure 1 and washed with CH_2Cl_2 (5 × 1 mL) and DMF (5 × 1 mL). After deprotection of the first amino acid (Ala) according to general procedure 2 the resin loading was determined to be 0.3 mmol·g^{-1} (24 µmol). The linear peptide was elongated using coupling conditions according to genral procedure 2 incorporating the commercially available amino acids Fmoc-*N*(Me)-Leu-OH, Fmoc-Sar-OH, Fmoc-D-*allo*-hydroxyproline, and Fmoc-D-Leu-OH. After deprotection of D-leucine using the standard protocol, ester bond containing fragment **4** was incoroprated.

Fragment **4** (32 mg, 56 µmol, 2.3 equiv.), PyAOP (29.2 mg, 56 µmol, 2.3 equiv,), HOAt (30.0 mg, 221 µmol, 9.2 equiv.), and NMM (12.2 µL, 106 µmol, 4.6 equiv.) were dissolved in anhydrous DMF (0.24 mL), and the resulting mixture was added to the resin. After 16 h resin-bound **11** was washed with DMF (5 × 1 mL), CH_2Cl_2 (5 × 1 mL), DMF (5 × 1 mL), and CH_2Cl_2 (5 × 1 mL).

Then, Alloc-protecting group was removed and the resin was washed according to general procedure 3 followed by coupling with 3-allyloxypicolinic acid (**12**) according to general

procedure 2.

O-allyl protecting group was cleaved off according to general procedure 4 followed by cleavage of the peptide from the solid-support accordig to general procedure 2.

10% Palladium on activated charcoal (12.8 mg, 12 µmol, 50 mol–%) was added to the a solution of the *N*-terminal protected crude linear depsipeptide in Methanol (5 mL). The mixture was degassed by bubbling a stream of N_2 into the solution. Then, a hydrogen-filled balloon was attached and the reaction mixture was stirred at rt for 12 h before it was filtered using a PTFE-syringe filter (0.22 µm pore size). The filter was thoroughly washed with MeOH (20 mL) and the solvent was removed by a stream of N_2.

The crude linear depsipeptide **13** was purified by reversed-phase preparative HPLC (30 × 150 mm) using a focused gradient [MeCN/H_2O (0.1% TFA) = 0:0 (0.00–1.00 min) → 25:75 (4.00 min) → 45:55 (18.00 min), total flow rate: 50.0 mL·min^{-1}, t_R = 6.5 – 9.0 min (broad peak)] affording **13** (8.2 mg, 41%) as a colorless lyophilisate.

13 (6.0 mg, 6.0 µmol) was dissolved in a mixture of CH_2Cl_2/DMF (1.2 mL, 1:1, v/v, final concentration 5 mM). HATU (4.6 mg, 12 µmol), HOAt (4.9 mg, 36 µmol) and *N*,*N*-diisopropylethylamine (4.2 µL, 24 µmol) were added and the reaction mixture was stirred at rt for 24 h [c] before the solvent was removed by a stream of N_2 and the crude cyclodepsipeptide was purified by preparative reversed-phase HPLC (19 × 150 mm) using a linear gradient [MeCN/H_2O (0.1% TFA) = 0:0 (0.00–1.00 min) → 60:40 (16.0 min), total flow rate: 32.0 mL·min^{-1}] affording two diastereomers (3.1 mg, 60% combined yield) as colorless lyophilisates (*epi*-**3**: 1.7 mg, 33%; **3**: 1.4 mg, 27%).

Preparative **RP-HPLC**: t_R =14.0 min (*epi*-**3**), 16.0 min (**3**).

RP-LC/MS: t_R = 4.83 min (*epi*-**3**), 5.05 min (**3**), MeCN/H_2O (0.1% HCO_2H) = 0:0 (0.00–0.50 min) → 100:0 (8.00 min), total flow rate: 0.60 mL·min^{-1}.

Norfijimycin A (**3**, mixture of conformers):[2]

^{1}H NMR (400 MHz, CD_3CN): δ = 9.61 (s), 8.60 (d, *J* = 7,9 Hz), 8.48 (d, *J* = 7.2 Hz), 8.04 (d, *J* = 3.6 Hz), 8.00 – 7.93 (m), 7.37 – 7.04(m), 5.88 (s), 5.68 (d, *J* = 11.3 Hz), 5.41 (br s), 5.29 (br s), 5.03 – 4.64 (m), 4.59 (br d, *J* = 8.0 Hz), 4.46 – 4.40 (m), 4.38 (s), 4.30 (br s),

[c] Aliquots were taken at 2, 5, 18 and 24 h and analyzed by LC/MS: After 2 h reaction time a small amount of the linear depsipeptide was cyclized and only one diastereomer (t_R = 5.05 min) was formed, presumably Norfijimycin A (**3**). After 5 h reaction time the amount of cyclic peptide was increased whereas a small amount of a second diastereomer (t_R = 4.83 min), presumably *epi*-**3**, was also detected. The final 1:1 ratio of **3** and *epi*-**3** was reached after 18 h. It is proposed that prolonged reaction time in the presence of coupling reagents led to increased epimerization.

4.25 (br d, *J* = 6.2 Hz), 4.19 (br s), 4.15 (br s), 4.11 – 4.05 (m), 4.03 (br d, *J* = 3.8 Hz), 4.01 – 3.99 (m), 3.98 (d, *J* = 4.8 Hz), 3.96 (br d, *J* = 4.7 Hz), 3.91 (dd, *J* = 11.6, 4.8 Hz), 3.88 (br s), 3.76 (dd, *J* = 8.6, 6.1 Hz), 3.67 – 3.62 (m), 3.54 (br d, *J* = 10.3 Hz), 3.41 (d, *J* = 5.1 Hz), 3.38 (d, *J* = 10.2 Hz), 3.36 (d, *J* = 11.5 Hz), 3.33 (d, *J* = 6.0 Hz), 3.16 (br s), 3.08 – 3.05 (m), 3.00 (br s), 2.92 (d, *J* = 17.2 Hz), 2.82 (d, *J* = 14.4 Hz), 2.76 (br s), 2.67 (br s), 2.21 (t, *J* = 7.6 Hz), 2.02 – 2.00 (m), 1.68 (p, *J* = 2.6 Hz), 1.50 – 1.47 (m), 1.42 – 1.31 (m), 1.25 (d, *J* = 7.8 Hz), 1.18 (br s), 1.12 (d, *J* = 7.0 Hz), 1.08 (br d, *J* = 6.4 Hz), 0.89 – 0.76 (m) ppm.

MS (ESI+): *m/z* (%): 865.8 (24) $[M + H]^+$ (*epi*-**3**), 865.8 (48) $[M + H]^+$ (**3**).

HRMS (ESI+): Calcd. for $[C_{43}H_{60}N_8O_{11} + Na]$: *m/z* = 887.4274, found: 887.4269 (**3**).

Antimicrobial Screening: Resazurin Assay for *Mtb*

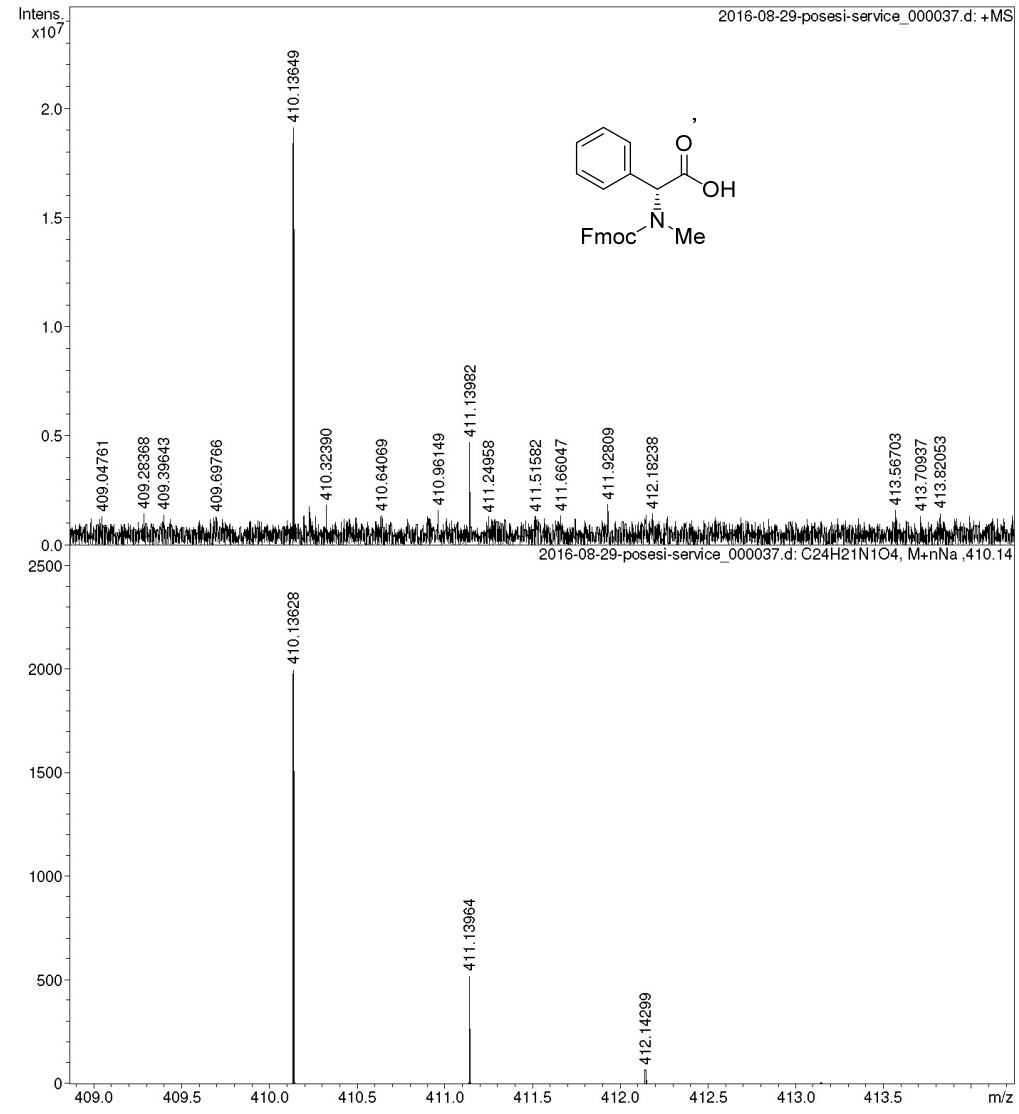
The compounds were originally stored as 10 mM stock solutions in 100% DMSO. Two fold serial dilutions of the compounds were made in a 96 well plate using Middlebrook 7H9 medium supplemented with ADC (0.5% v/v glycerol and 0.05% v/v Tween-80). *M. tuberculosis* H37Rv was grown to mid-exponential phase to an OD_{600} of 0.6–0.8 in 7H9 media at 37 °C. On the day of the assay, culture was diluted to an OD_{600} of 0.002 and 100 µl of bacterial suspension was added to the 96 well plate containing 100 µL of the diluted compounds. The plate was incubated for 5 days at 37 °C in a humidified incubator and 30 µL of Resazurin (0.02% w/v) and 12.5 µL of Tween-80 was added to each well and incubated for further 24 h. On day 6, the fluorescence was read using a BMG Labtech Polarstar plate reader (excitation 530 nm and emission 590 nm). The results are presented as *M. tuberculosis* survival as a percentage of negative control.

Mass Spectra and Chromatograms

Generic Display Report

Analysis Info

Acquisition Date	26/08/2016 4:26:44 PM		
Analysis Name	D:\Data\nick-2016-files\ESI_Positive\08-August\2016-08-29\2016-08-29-posesi-service_000037.d		
Method	1MW Positive ESI	Operator	
Sample Name	AS4.100	Instrument	apex-Ultra
Comment	MeOH 1M TOF delay 0.0008s, Q1 300 m/z		



Bruker Compass DataAnalysis 4.0 printed: 26/08/2016 4:31:38 PM Page 1 of 1

Generic Display Report

Analysis Info

Acquisition Date	29/08/2016 12:16:05 PM		
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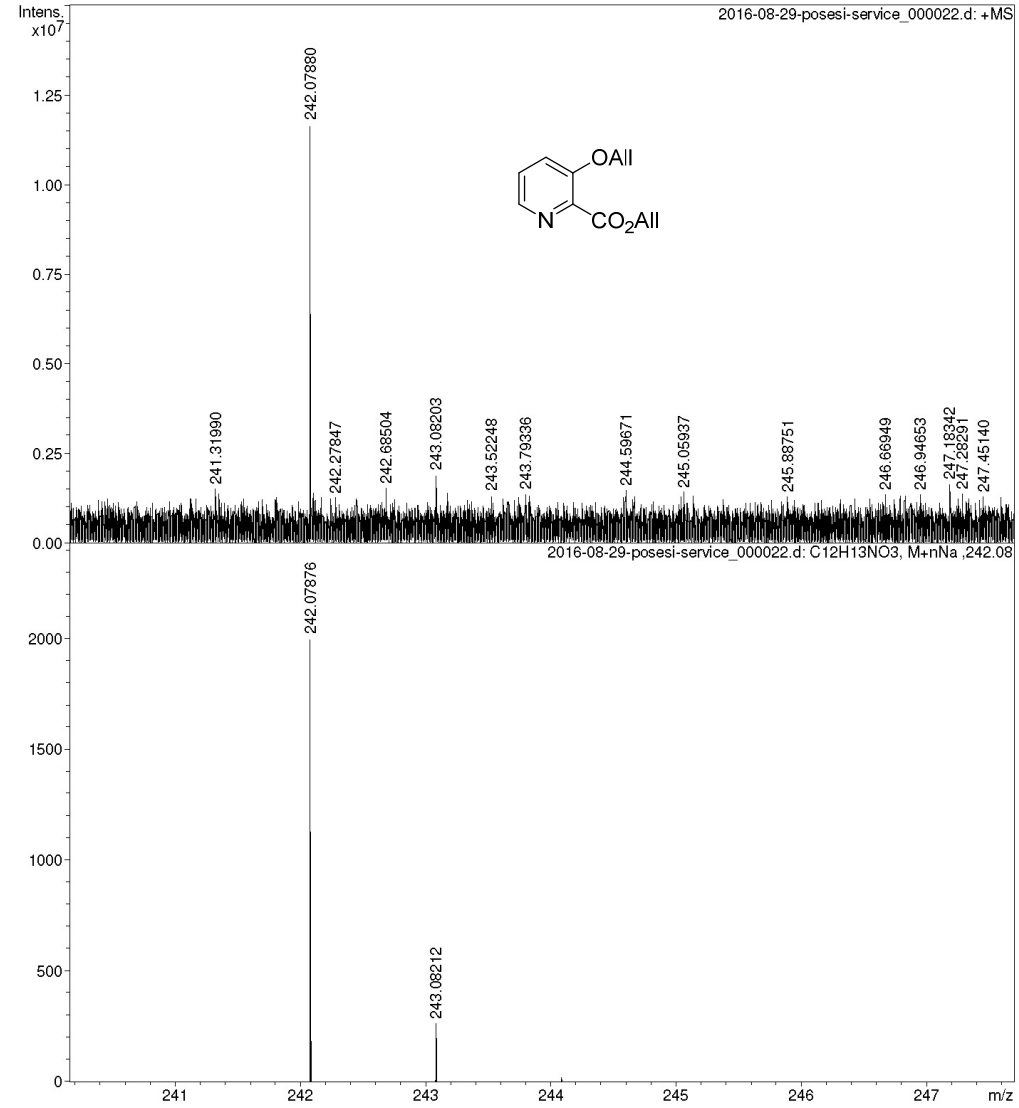
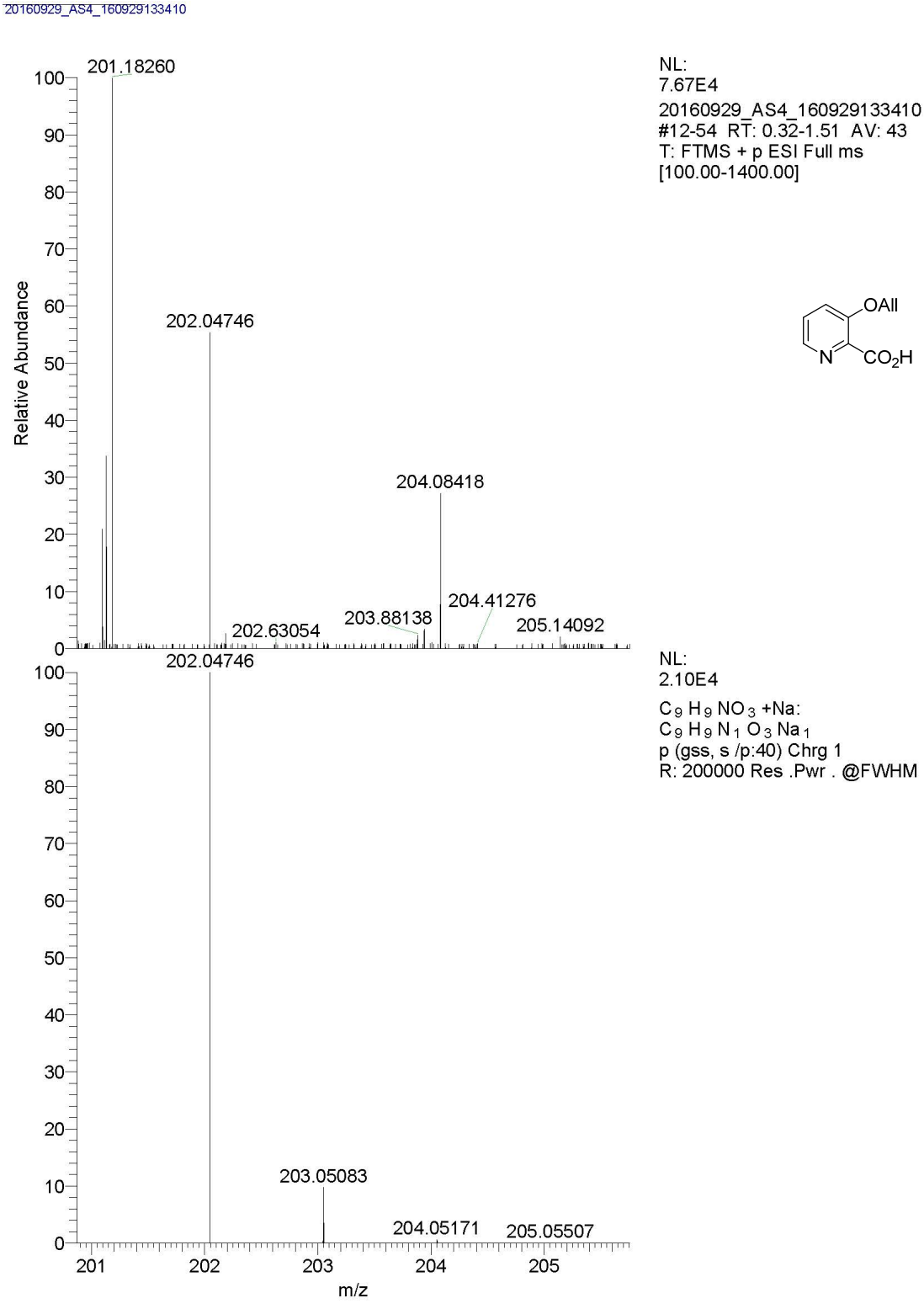
Method	1MW Positive ESI	Operator	
Sample Name	AS4.102	Instrument	apex-Ultra
Comment	MeOH 1M TOF delay 0.0006s, Q1 300 m/z		



Bruker Compass DataAnalysis 4.0 printed: 29/08/2016 12:19:54 PM Page 1 of 1


20160929_AS4_160929133410
201.18260
202.04746
204.08418
204.41276
203.88138
202.63054
205.14092
Relative Abundance
NL:
7.67E4
20160929_AS4_160929133410
#12-54 RT: 0.32-1.51 AV: 43
T: FTMS + p ESI Full ms
[100.00-1400.00]
OAll
N
CO2H
202.04746
203.05083
204.05171
205.05507
m/z
NL:
2.10E4
C9 H9 NO3 +Na:
C9 H9 N1 O3 Na1
p (gss, s /p:40) Chrg 1
R: 200000 Res .Pwr . @FWHM

Generic Display Report

Analysis Info

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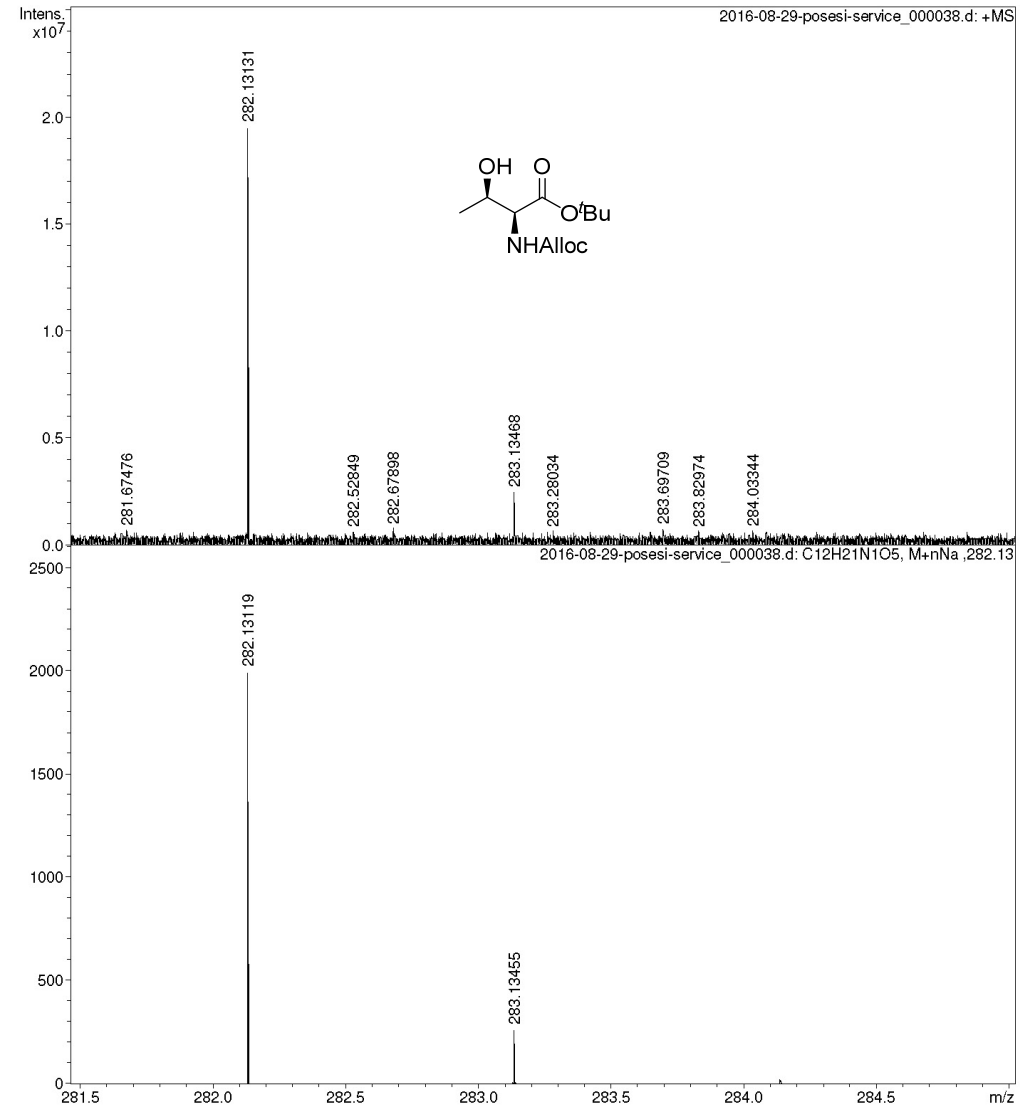
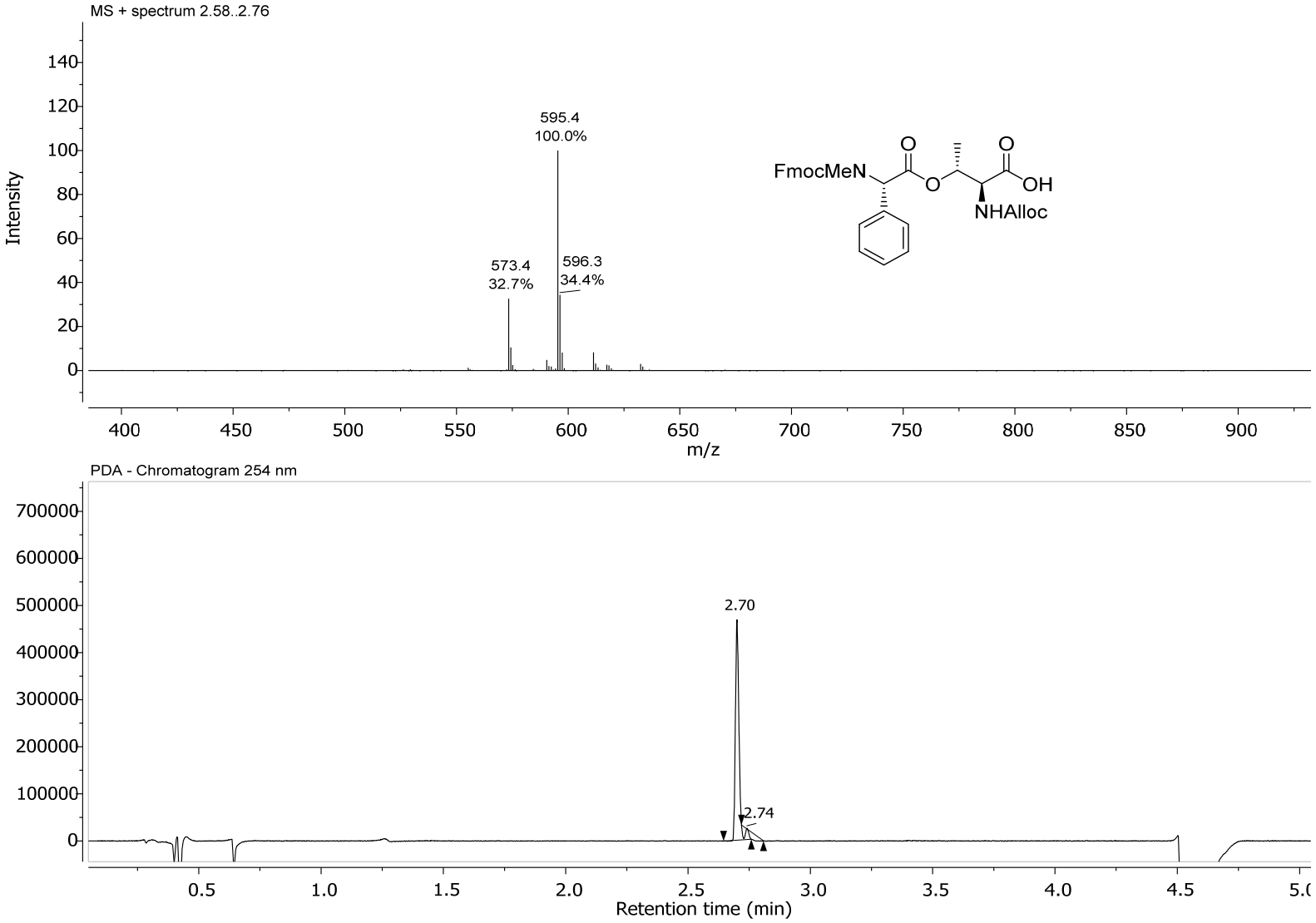
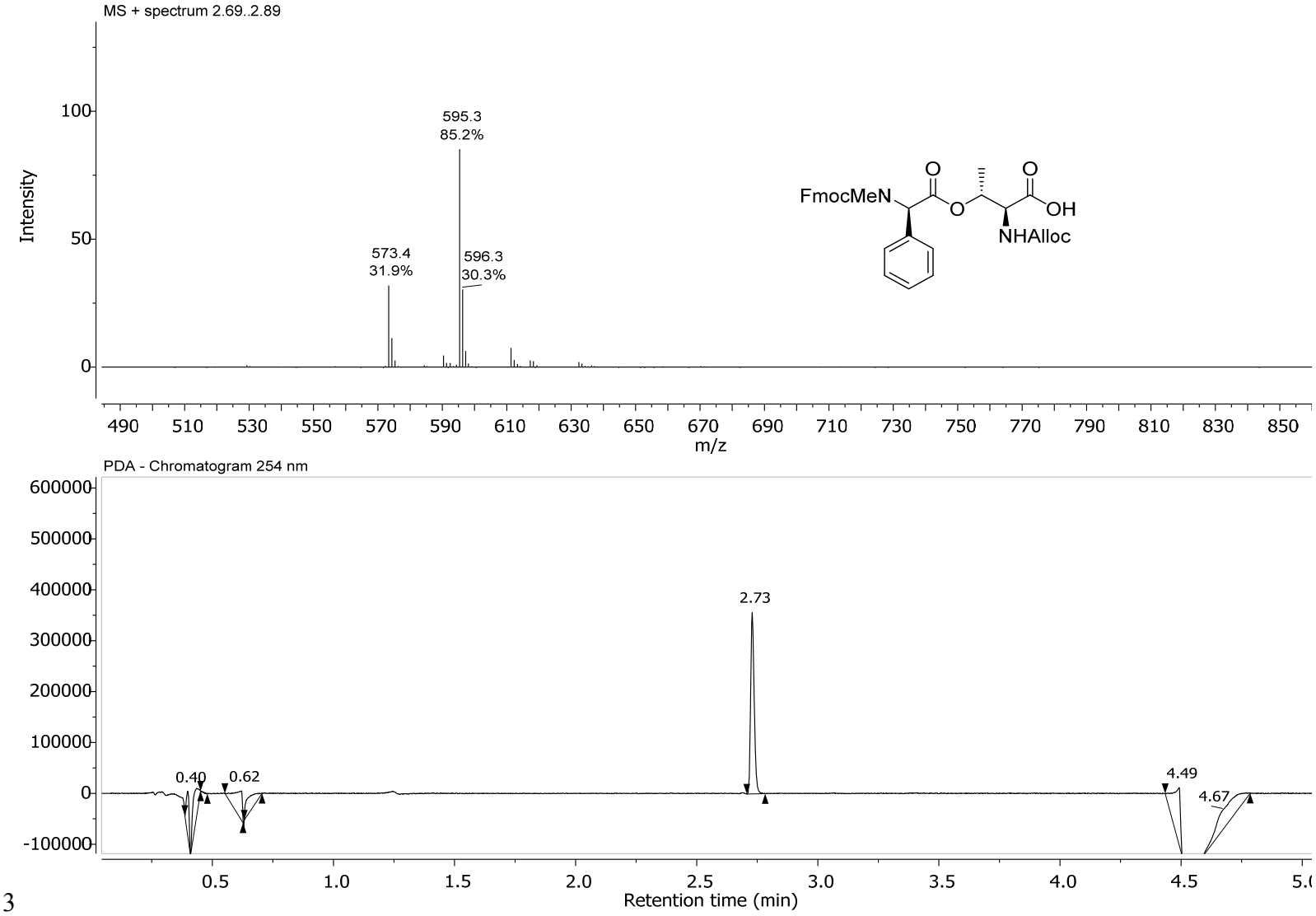
Analysis Name	D:\Data\nick-2016-files\ESI_Positive\08-August\2016-08-29\2016-08-29-posesi-service_000038.d		
Method	1MW Positive ESI	Operator	
Sample Name	AS4.116	Instrument	apex-Ultra
Comment	MeOH 1M TOF delay 0.0008s, Q1 300 m/z		




MS + spectrum 2.58..2.76
Intensity
595.4
100.0%
573.4
32.7%
596.3
34.4%
FmocMeN
O
O
O
OH
NHAlloc
m/z
PDA - Chromatogram 254 nm
2.70
2.74
Retention time (min)

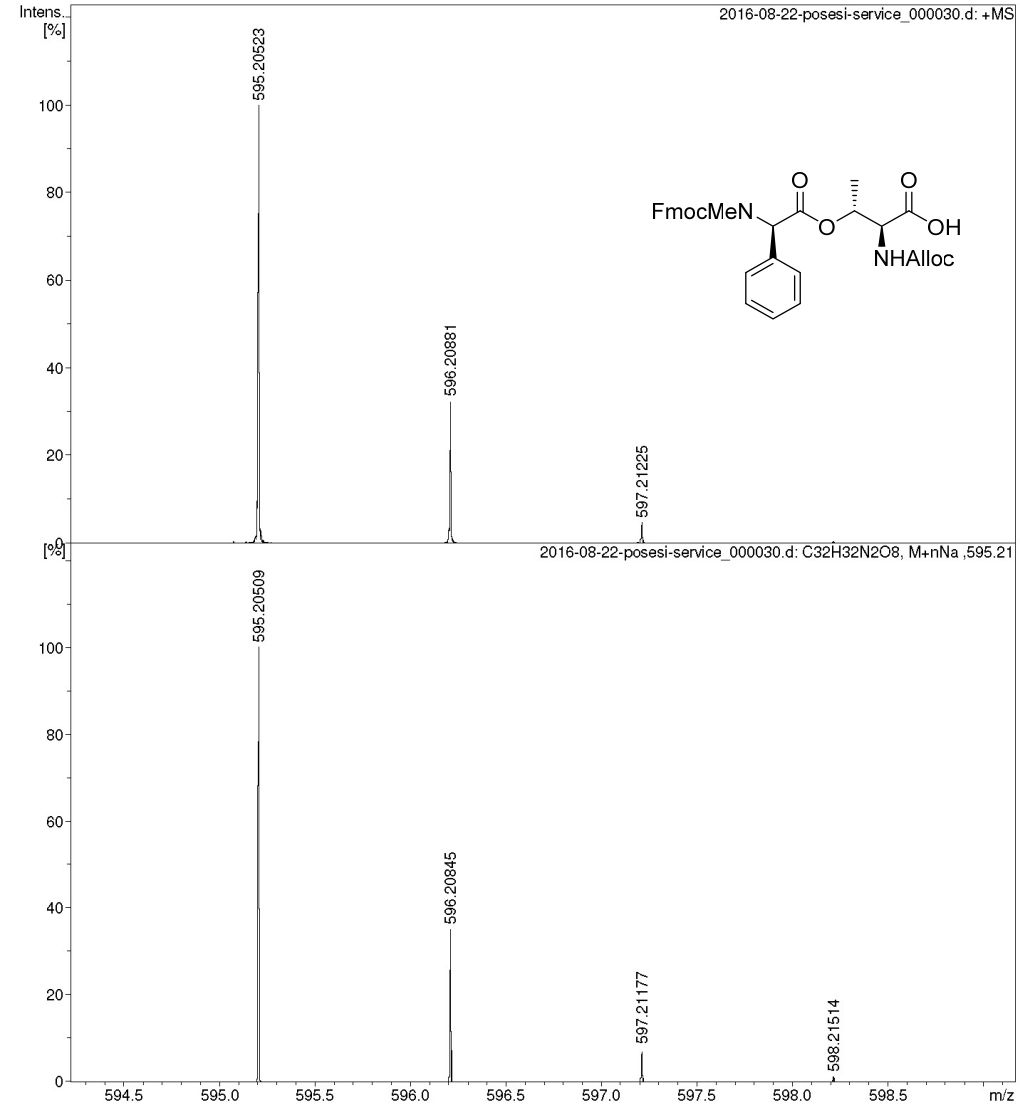

MS + spectrum 2.69..2.89
Intensity
100
50
0
595.3
85.2%
573.4
31.9%
596.3
30.3%
FmocMeN
O
O
O
OH
NHAlloc
490 510 530 550 570 590 610 630 650 670 690 710 730 750 770 790 810 830 850
m/z
PDA - Chromatogram 254 nm
600000
500000
400000
300000
200000
100000
0
-100000
2.73
0.40
0.62
4.49
4.67
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5
Retention time (min)

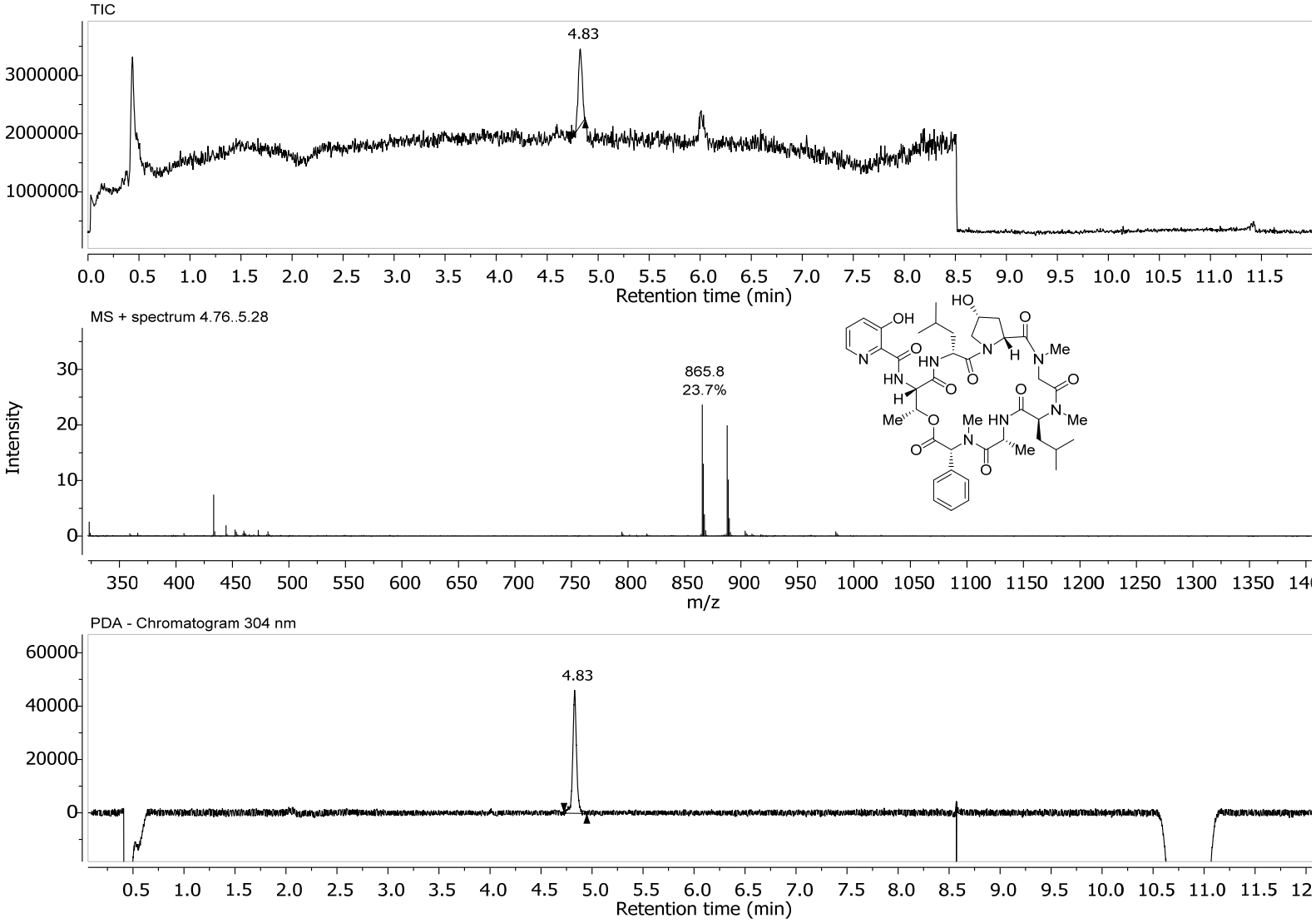
3

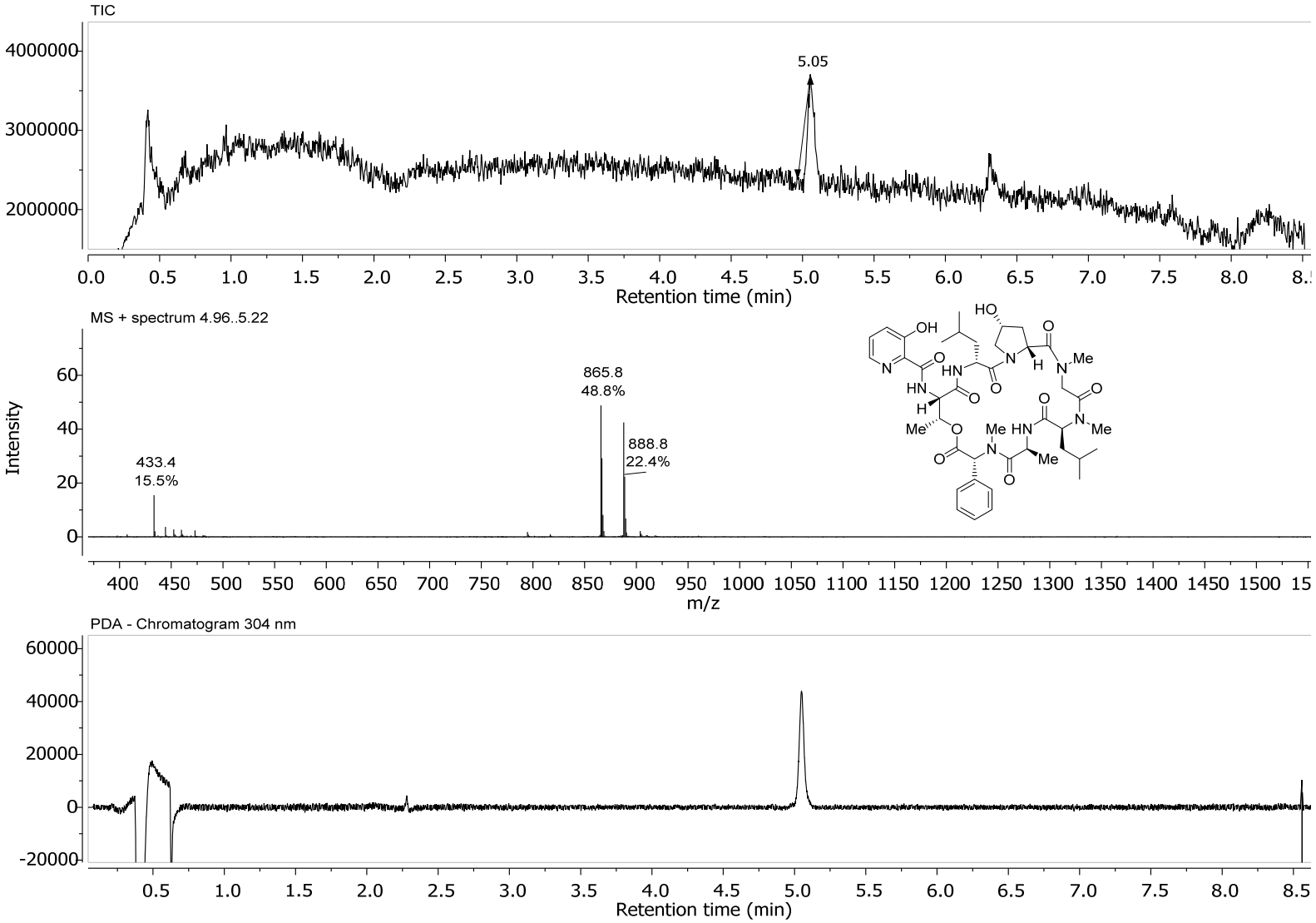
Generic Display Report

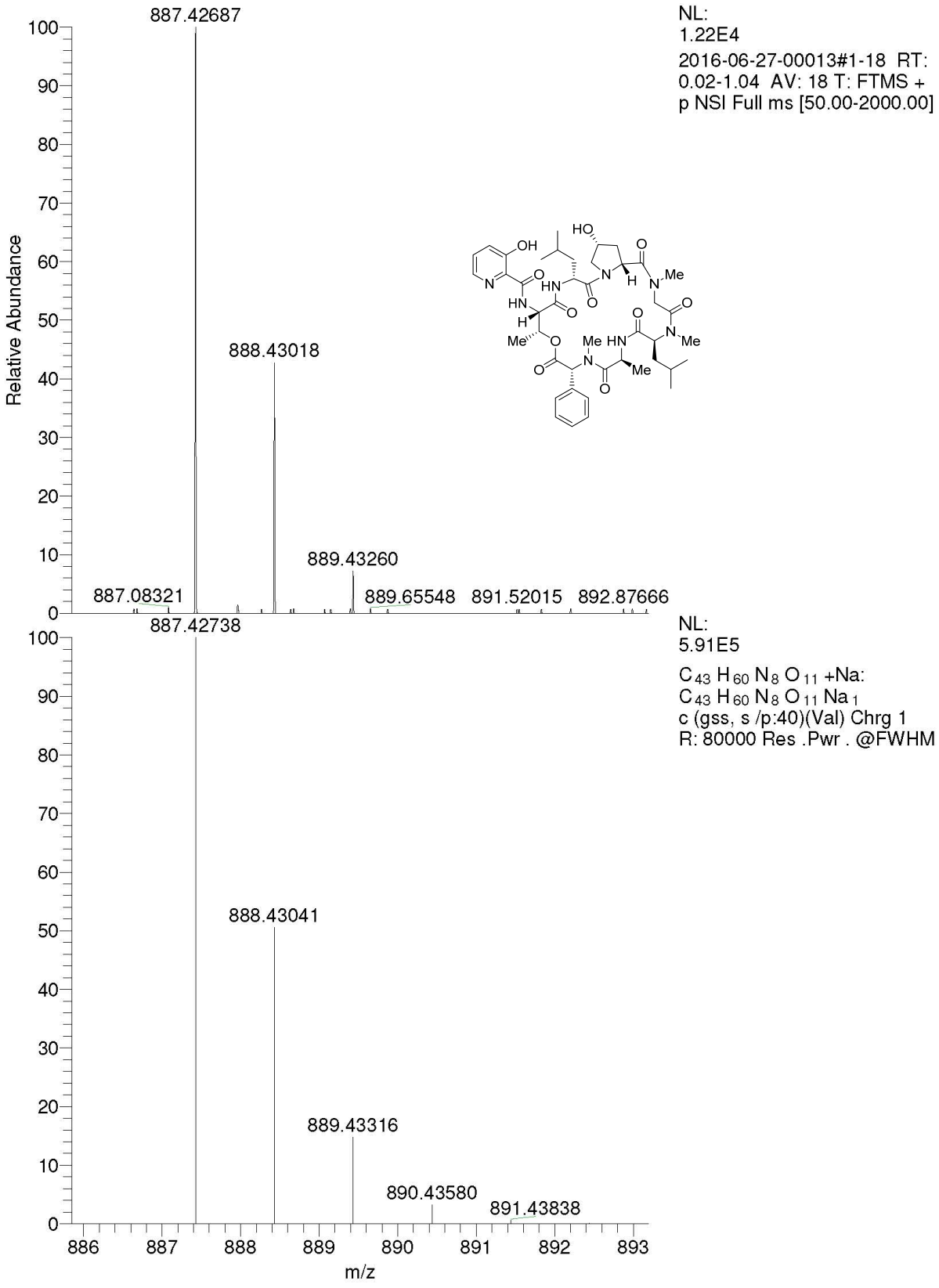
Analysis Info

		Acquisition Date	22/08/2016 3:19:38 PM
Analysis Name	D:\Data\nick-2016-files\ESI_Positive\08-August\2016-08-22\2016-08-22-posesi-service_000030.d		
Method	1MW Positive ESI	Operator	
Sample Name	AS5.14	Instrument	apex-Ultra
Comment	MeOH 1M TOF delay 0.0006s, Q1 300 m/z		

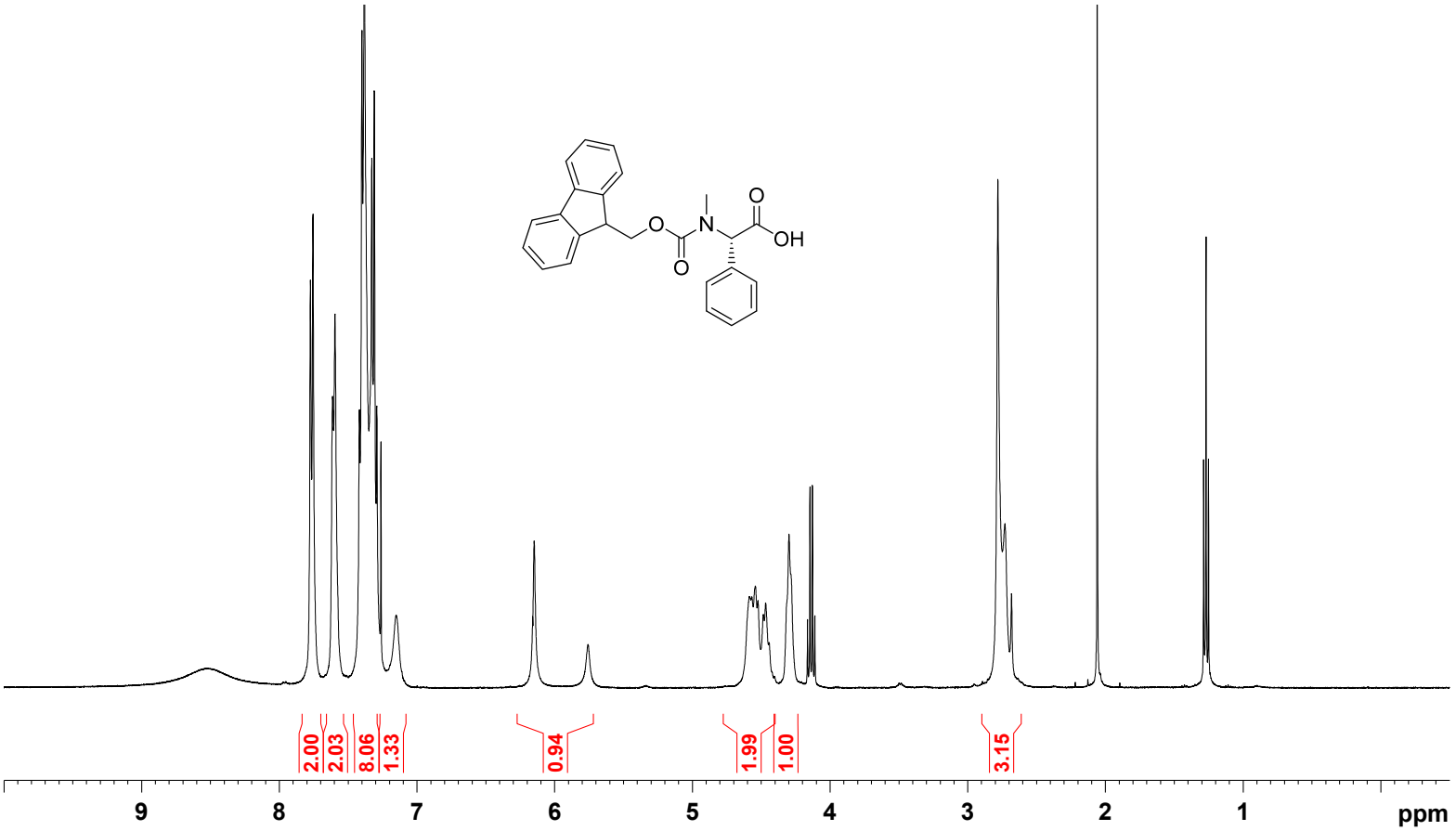


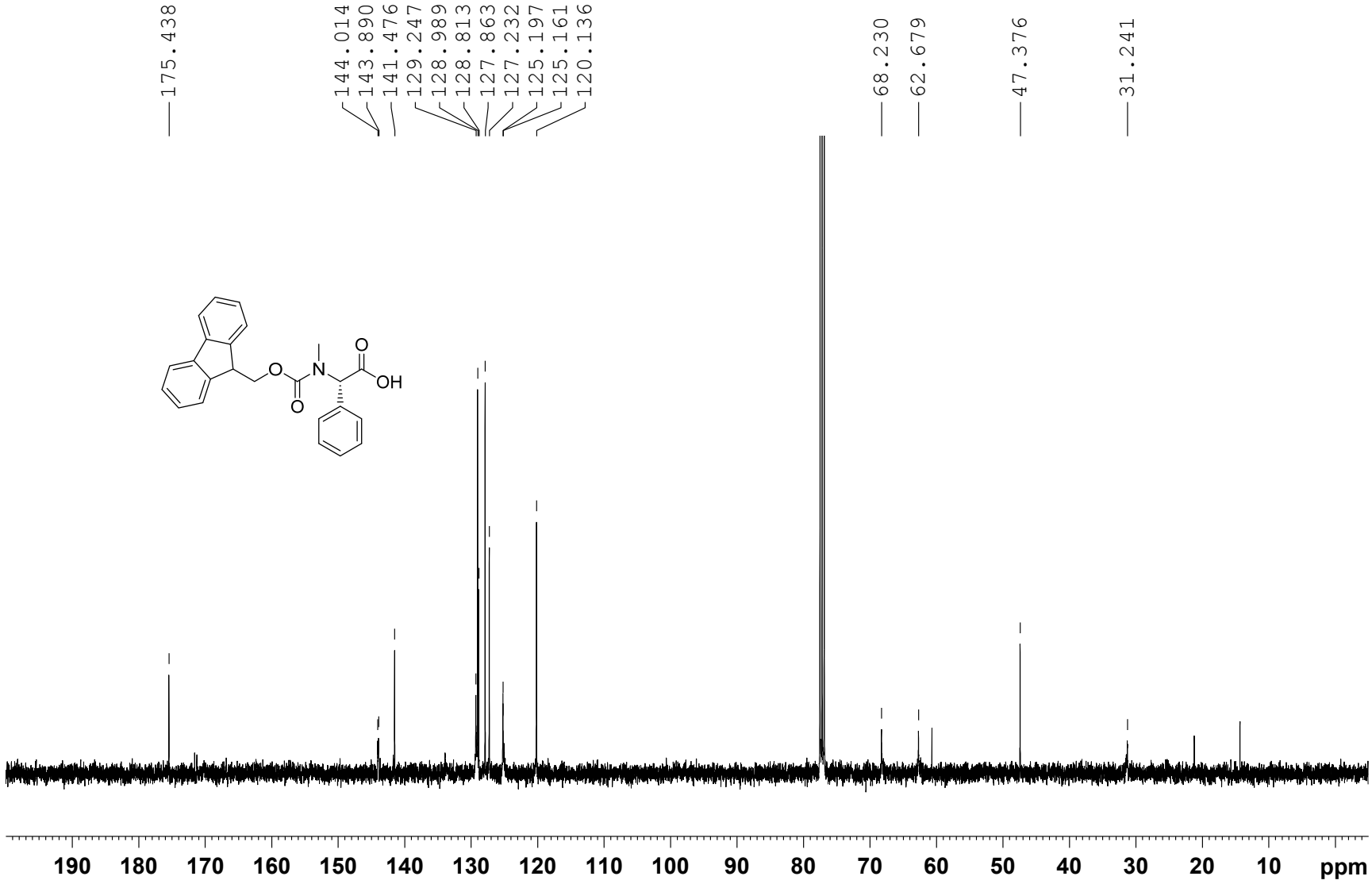

TIC
4.83
3000000
2000000
1000000
0.0 0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0 10.5 11.0 11.5
Retention time (min)
MS + spectrum 4.76..5.28
865.8
23.7%
Intensity
30
20
10
0
350 400 450 500 550 600 650 700 750 800 850 900 950 1000 1050 1100 1150 1200 1250 1300 1350
m/z
PDA - Chromatogram 304 nm
4.83
60000
40000
20000
0
0.5 1.0 1.5 2.0 2.5 3.0 3.5 4.0 4.5 5.0 5.5 6.0 6.5 7.0 7.5 8.0 8.5 9.0 9.5 10.0 10.5 11.0 11.5
Retention time (min)

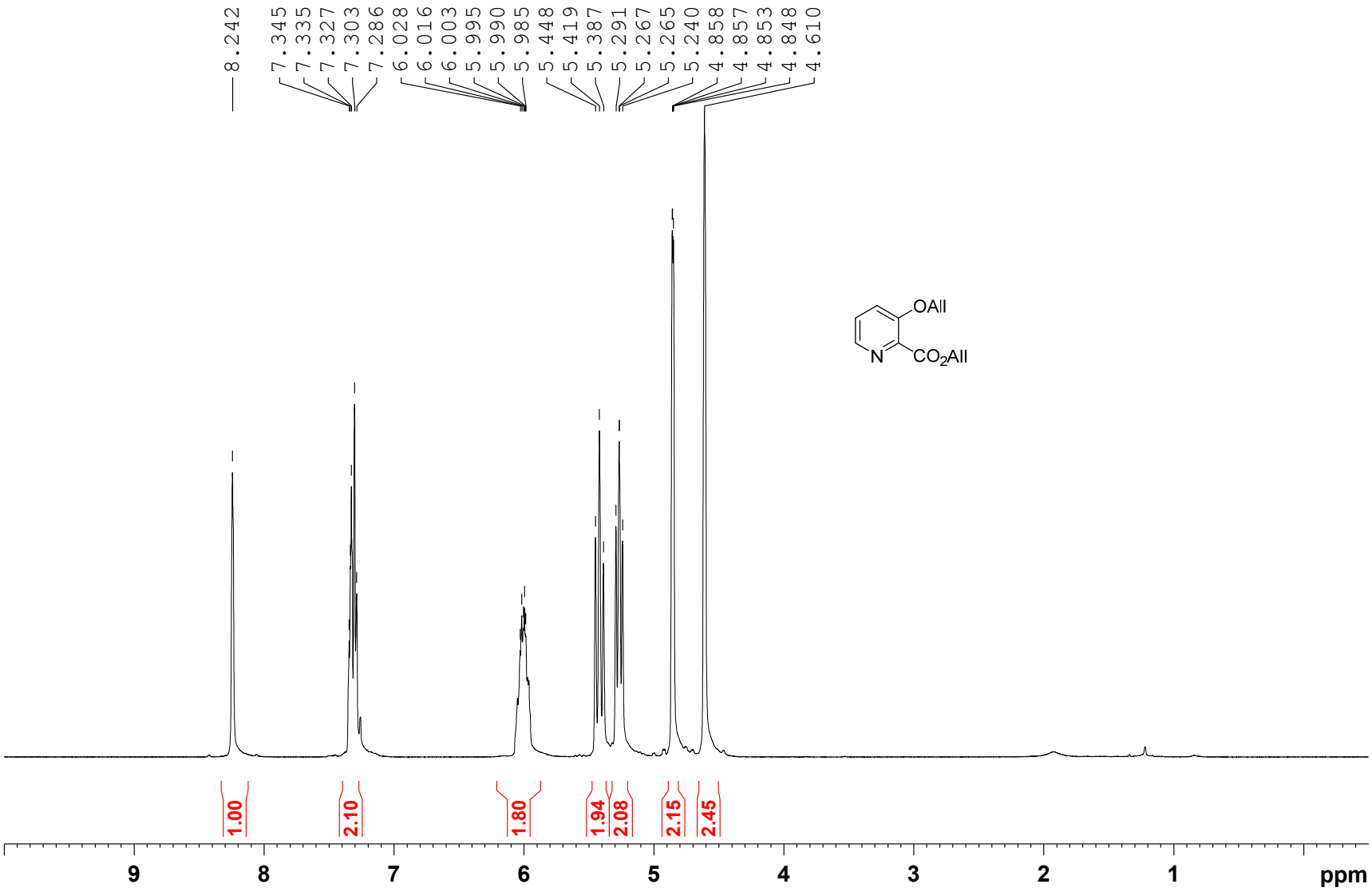

TIC
5.05
Retention time (min)
MS + spectrum 4.96..5.22
865.8
48.8%
888.8
22.4%
433.4
15.5%
Intensity
m/z
PDA - Chromatogram 304 nm
Retention time (min)

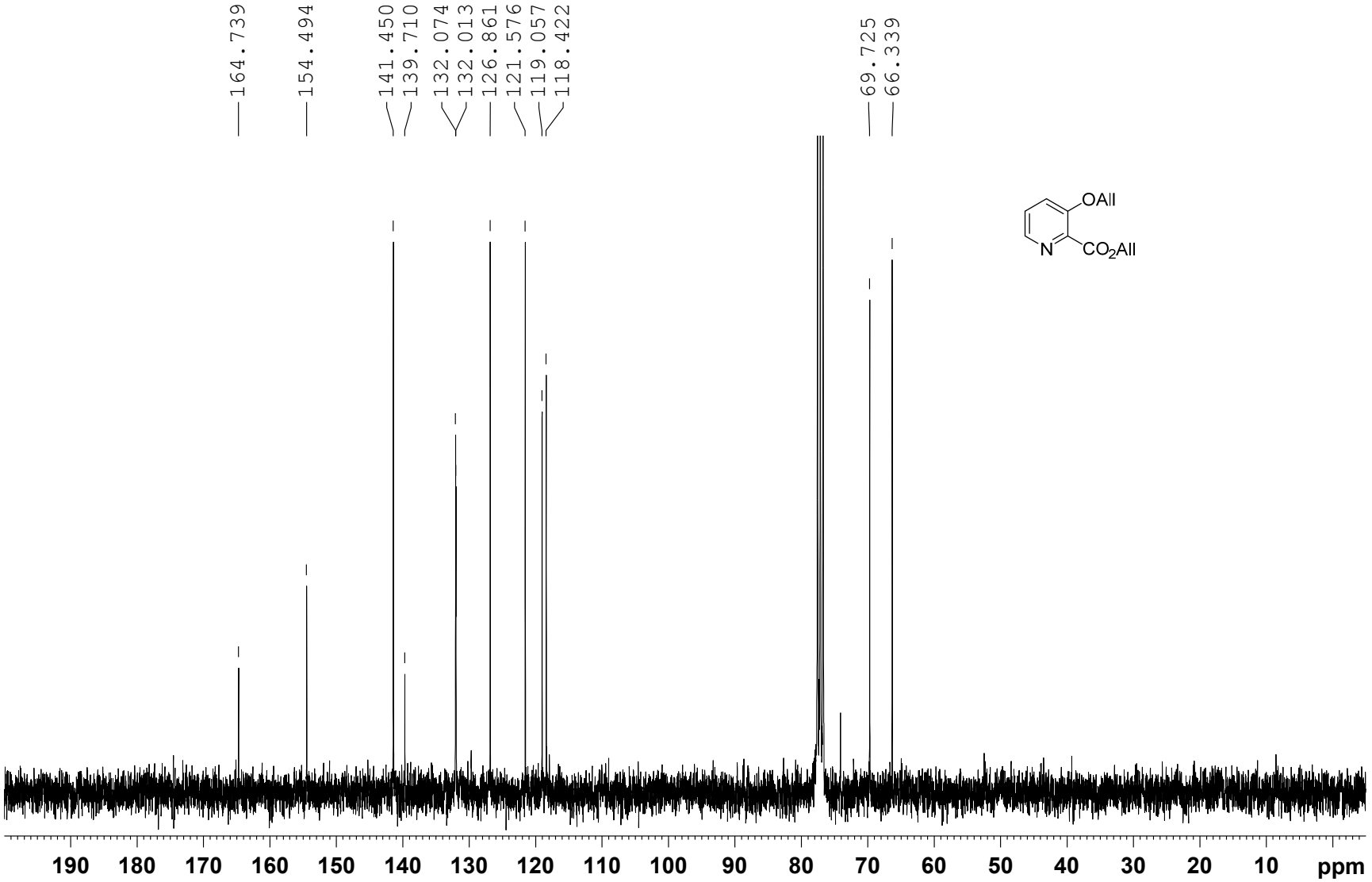

887.42687
888.43018
889.43260
887.08321
889.65548
891.52015
892.87666
NL:
1.22E4
2016-06-27-00013#1-18 RT:
0.02-1.04 AV: 18 T: FTMS +
p NSI Full ms [50.00-2000.00]
Relative Abundance
887.42738
888.43041
889.43316
890.43580
891.43838
NL:
5.91E5
C43 H60 N8 O11 +Na:
C43 H60 N8 O11 Na1
c (gss, s /p:40)(Val) Chrg 1
R: 80000 Res .Pwr . @FWHM
m/z

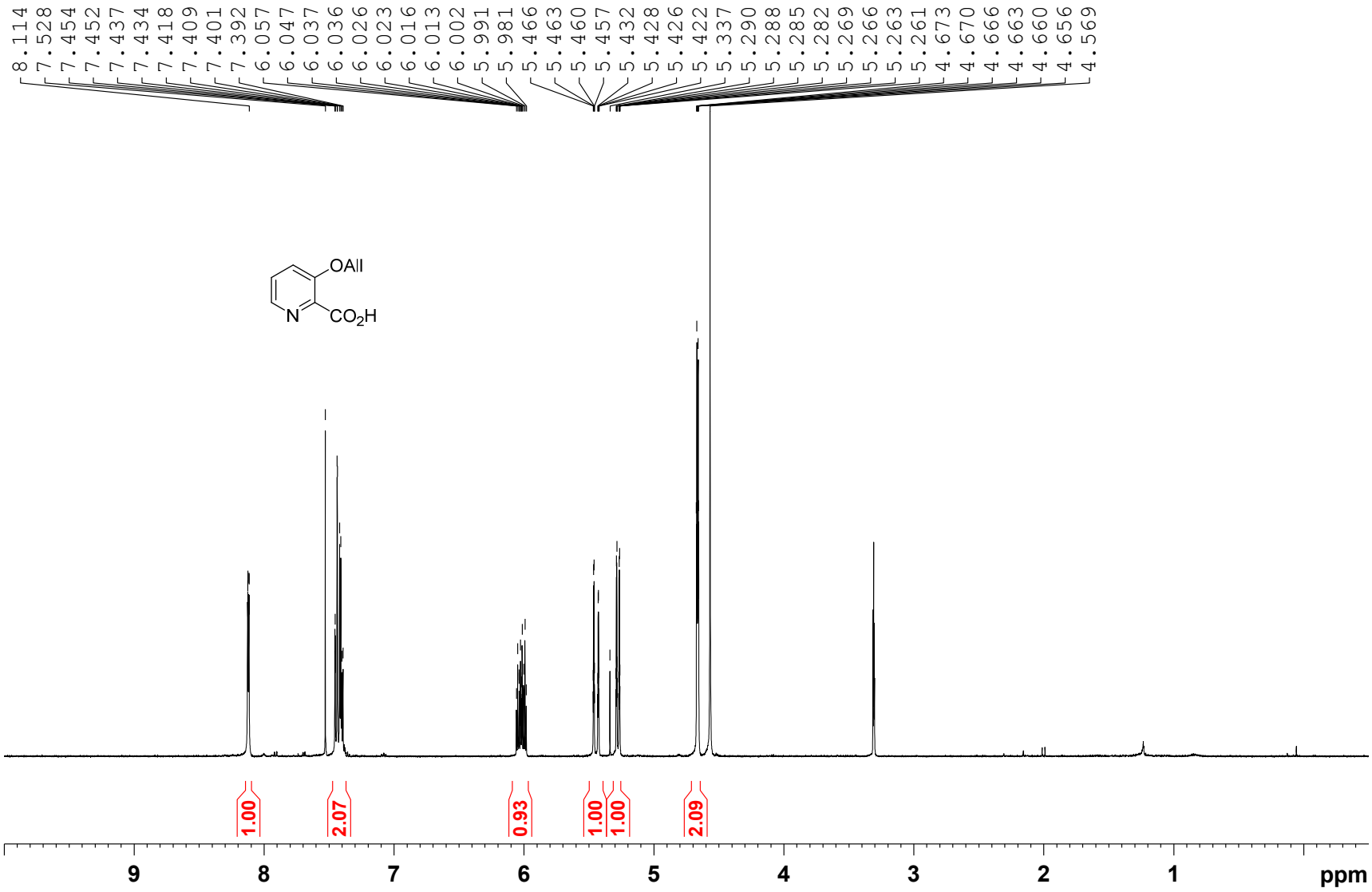
NMR spectra

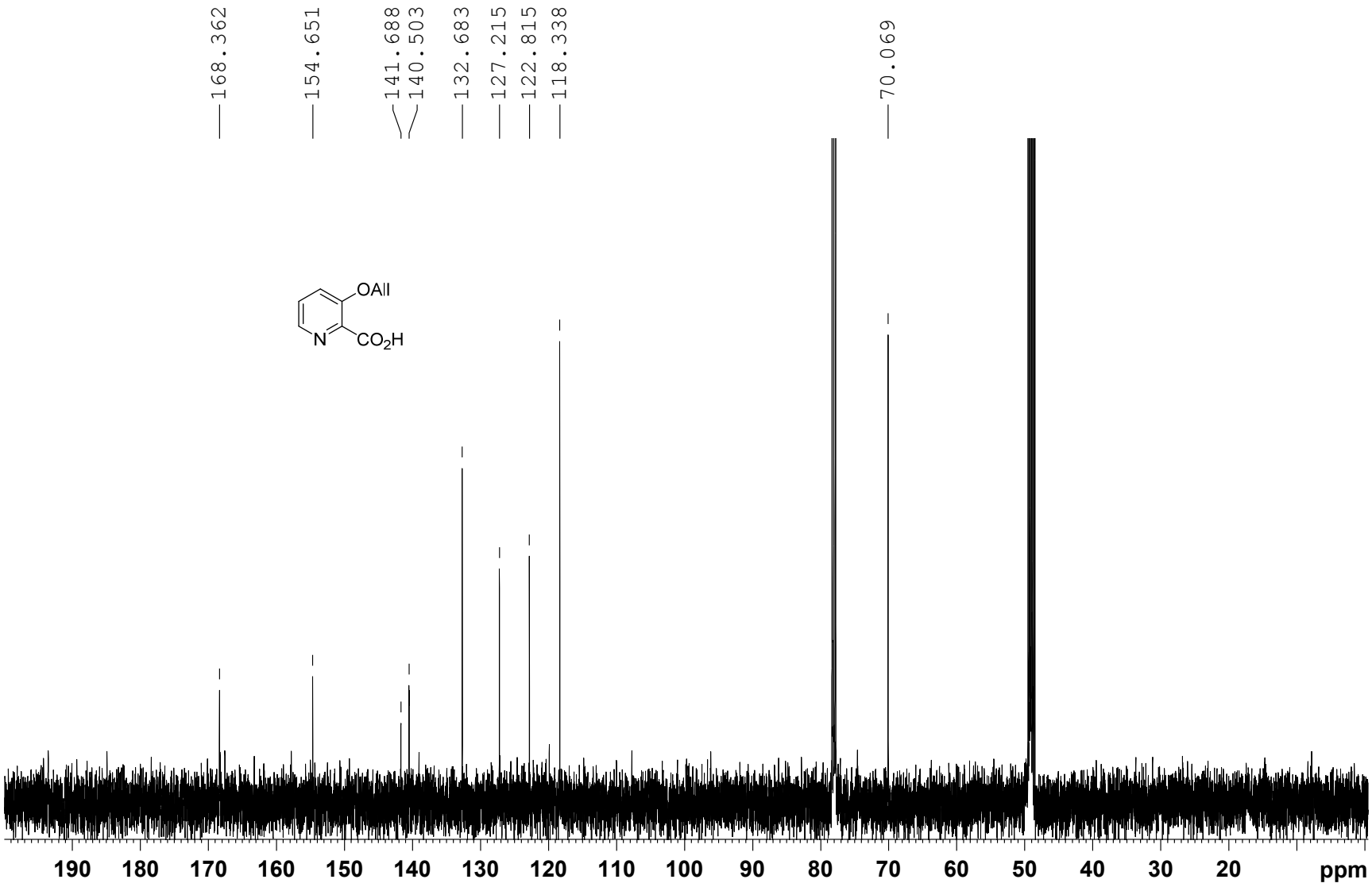


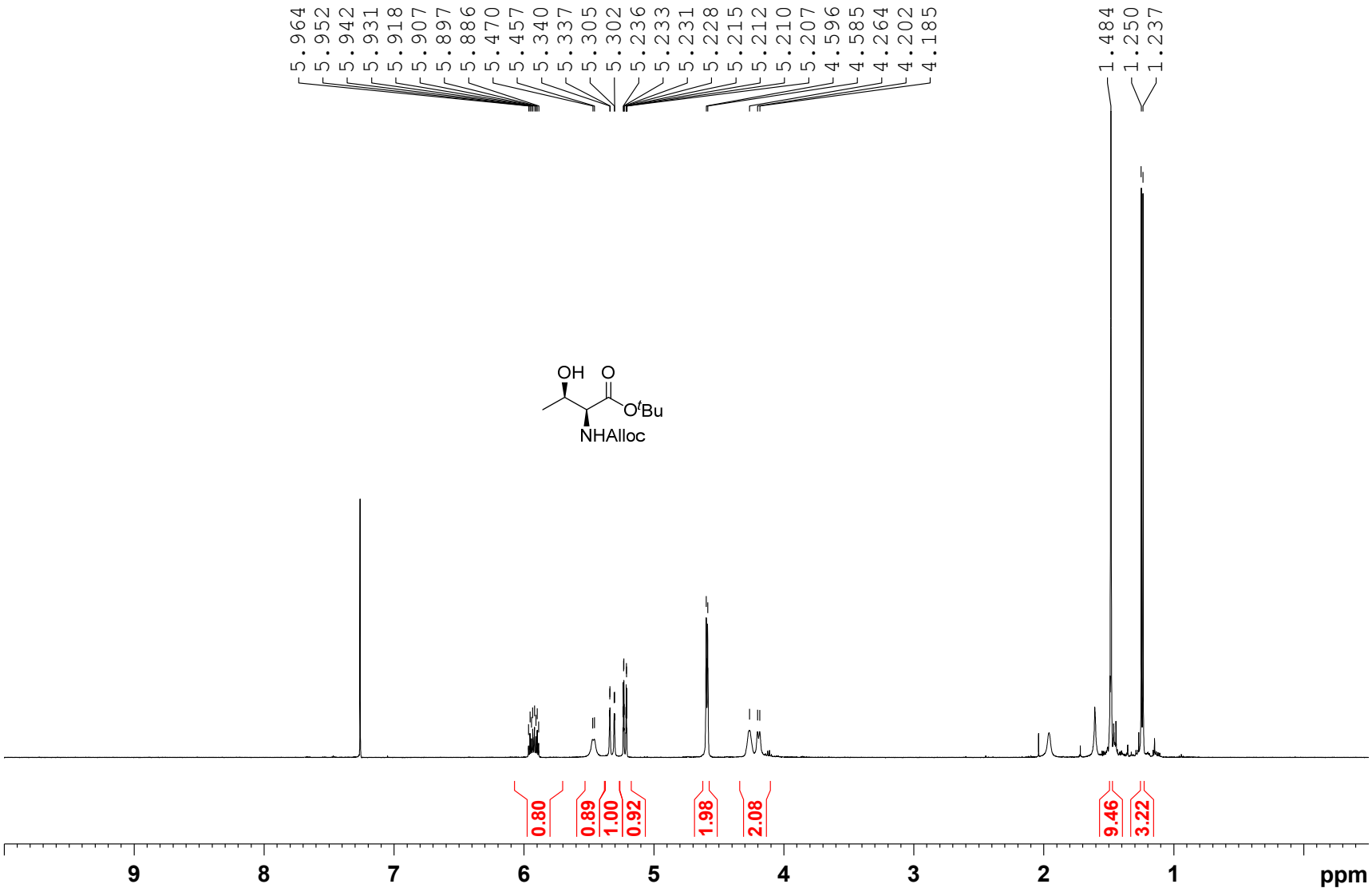

175.438
144.014
143.890
141.476
129.247
128.989
128.813
127.863
127.232
125.197
125.161
120.136
68.230
62.679
47.376
31.241
O
N
O
O
OH
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

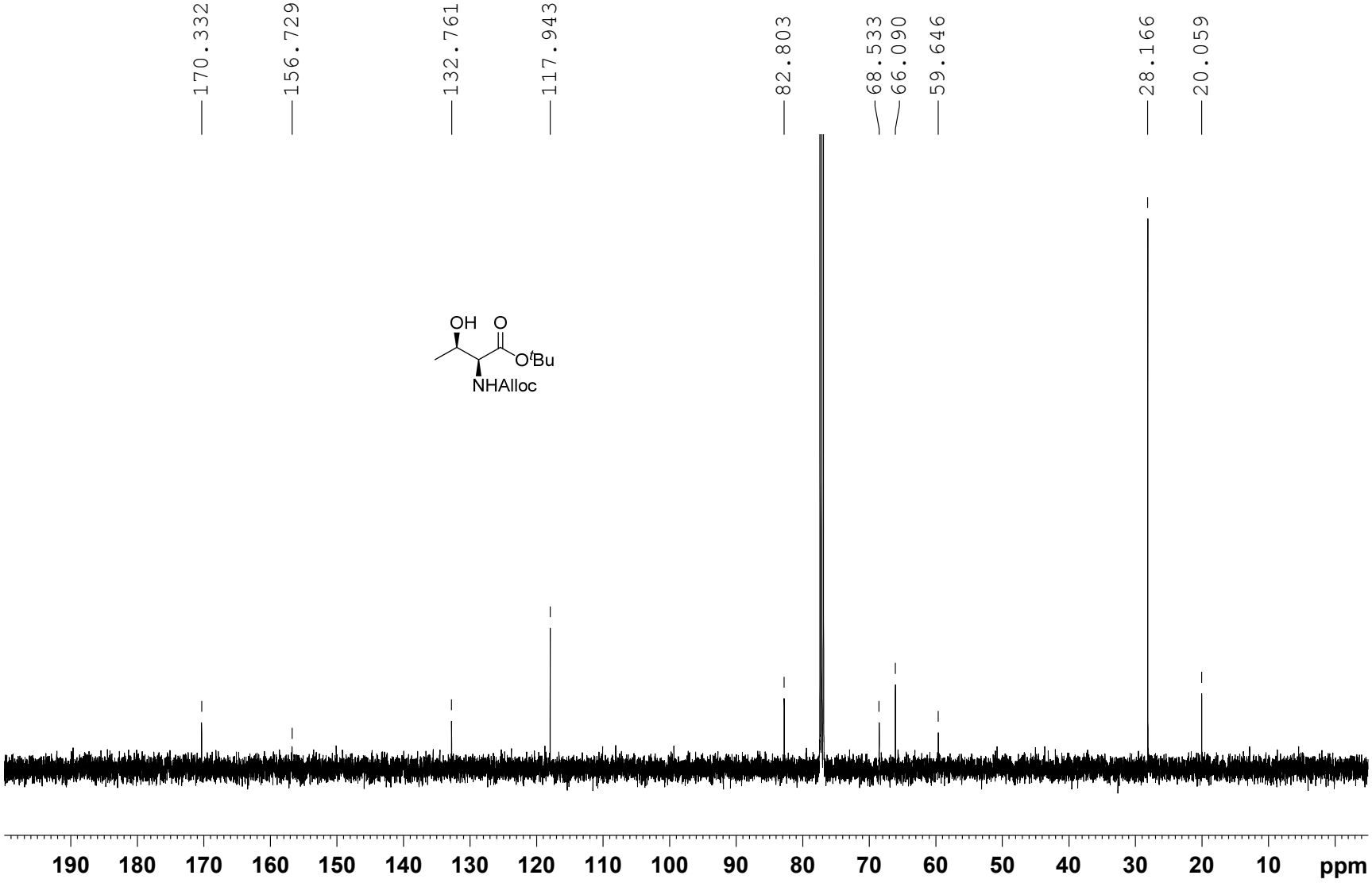

8.242
7.345
7.335
7.327
7.303
7.286
6.028
6.016
6.003
5.995
5.990
5.985
5.448
5.419
5.387
5.291
5.267
5.265
5.240
4.858
4.857
4.853
4.848
4.610
OAll
N
CO2All
1.00
2.10
1.80
1.94
2.08
2.15
2.45
9
8
7
6
5
4
3
2
1
ppm

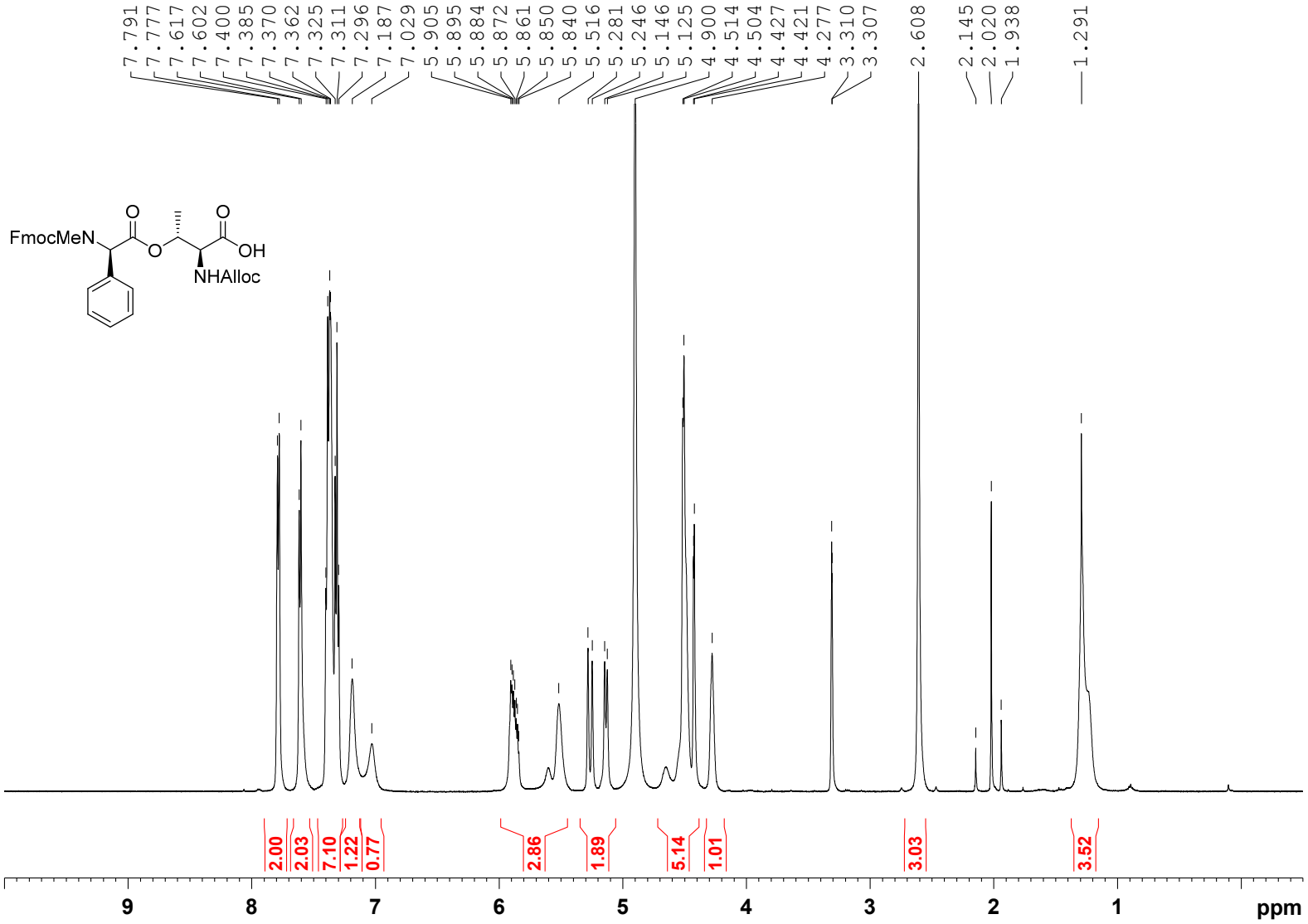

164.739
154.494
141.450
139.710
132.074
132.013
126.861
121.576
119.057
118.422
69.725
66.339
OAll
N
CO2All
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm

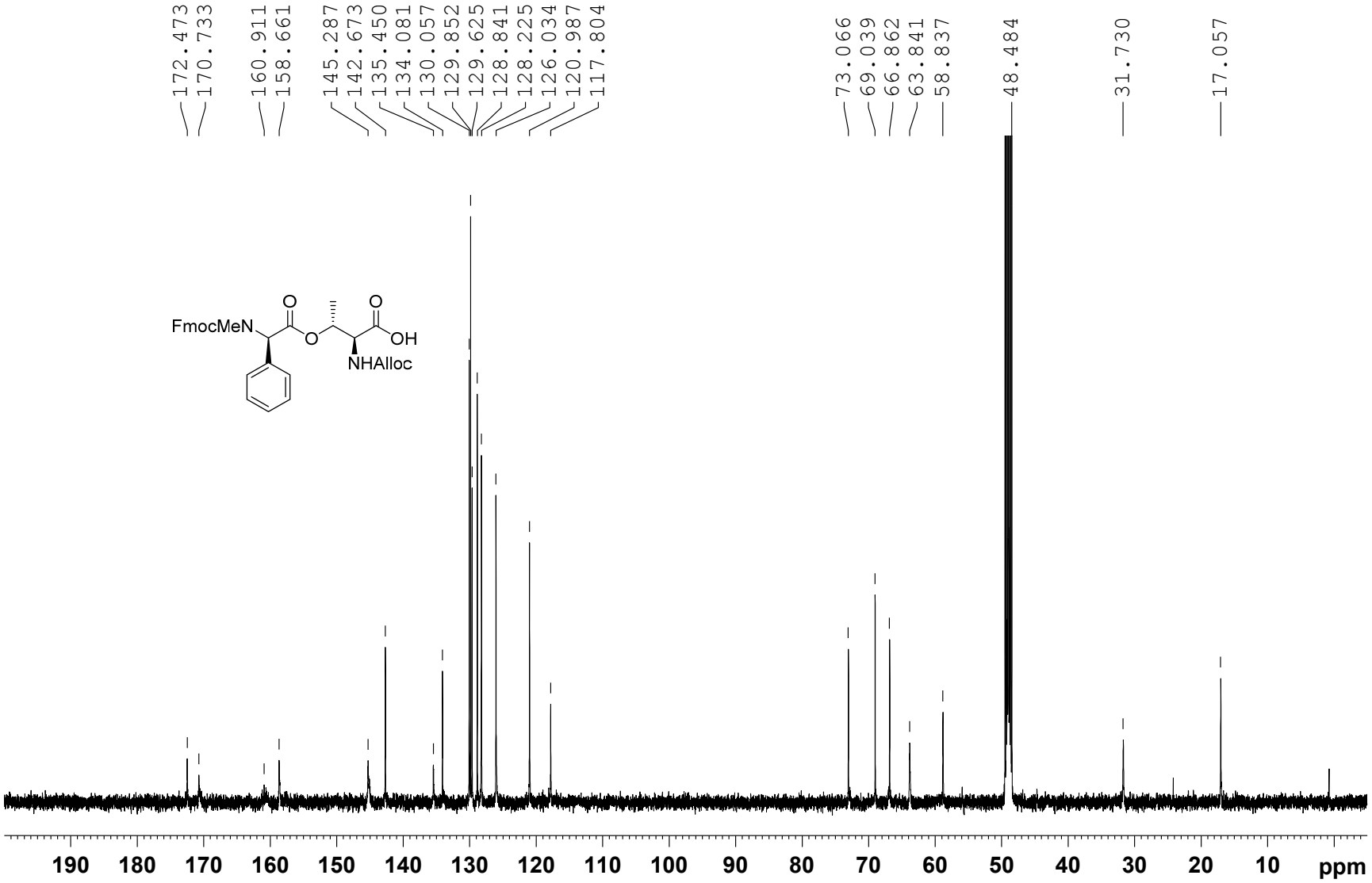

8.114
7.528
7.454
7.452
7.437
7.434
7.418
7.409
7.401
7.392
6.057
6.047
6.037
6.036
6.026
6.023
6.016
6.013
6.002
5.991
5.981
5.466
5.463
5.460
5.457
5.432
5.428
5.426
5.422
5.337
5.290
5.288
5.285
5.282
5.269
5.266
5.263
5.261
4.673
4.670
4.666
4.663
4.660
4.656
4.569
OAll
N
CO2H
1.00
2.07
0.93
1.00
1.00
2.09
9
8
7
6
5
4
3
2
1
ppm


168.362
154.651
141.688
140.503
132.683
127.215
122.815
118.338
70.069
OAll
N
CO2H
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


5.964
5.952
5.942
5.931
5.918
5.907
5.897
5.886
5.470
5.457
5.340
5.337
5.305
5.302
5.236
5.233
5.231
5.228
5.215
5.212
5.210
5.207
4.596
4.585
4.264
4.202
4.185
1.484
1.250
1.237
OH
O
O^{t}Bu
NHAlloc
0.80
0.89
1.00
0.92
1.98
2.08
9.46
3.22
9
8
7
6
5
4
3
2
1
ppm


170.332
156.729
132.761
117.943
82.803
68.533
66.090
59.646
28.166
20.059
OH
O
O
tBu
NHAlloc
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm


7.791
7.777
7.617
7.602
7.400
7.385
7.370
7.362
7.325
7.311
7.296
7.187
7.029
5.905
5.895
5.884
5.872
5.861
5.850
5.840
5.516
5.281
5.246
5.146
5.125
4.900
4.514
4.504
4.427
4.421
4.277
3.310
3.307
2.608
2.145
2.020
1.938
1.291
FmocMeN
O
O
O
OH
NHAlloc
2.00
2.03
7.10
1.22
0.77
2.86
1.89
5.14
1.01
3.03
3.52
9
8
7
6
5
4
3
2
1
ppm


172.473
170.733
160.911
158.661
145.287
142.673
135.450
134.081
130.057
129.852
129.625
128.841
128.225
126.034
120.987
117.804
73.066
69.039
66.862
63.841
58.837
48.484
31.730
17.057
FmocMeN
O
O
O
OH
NHAlloc
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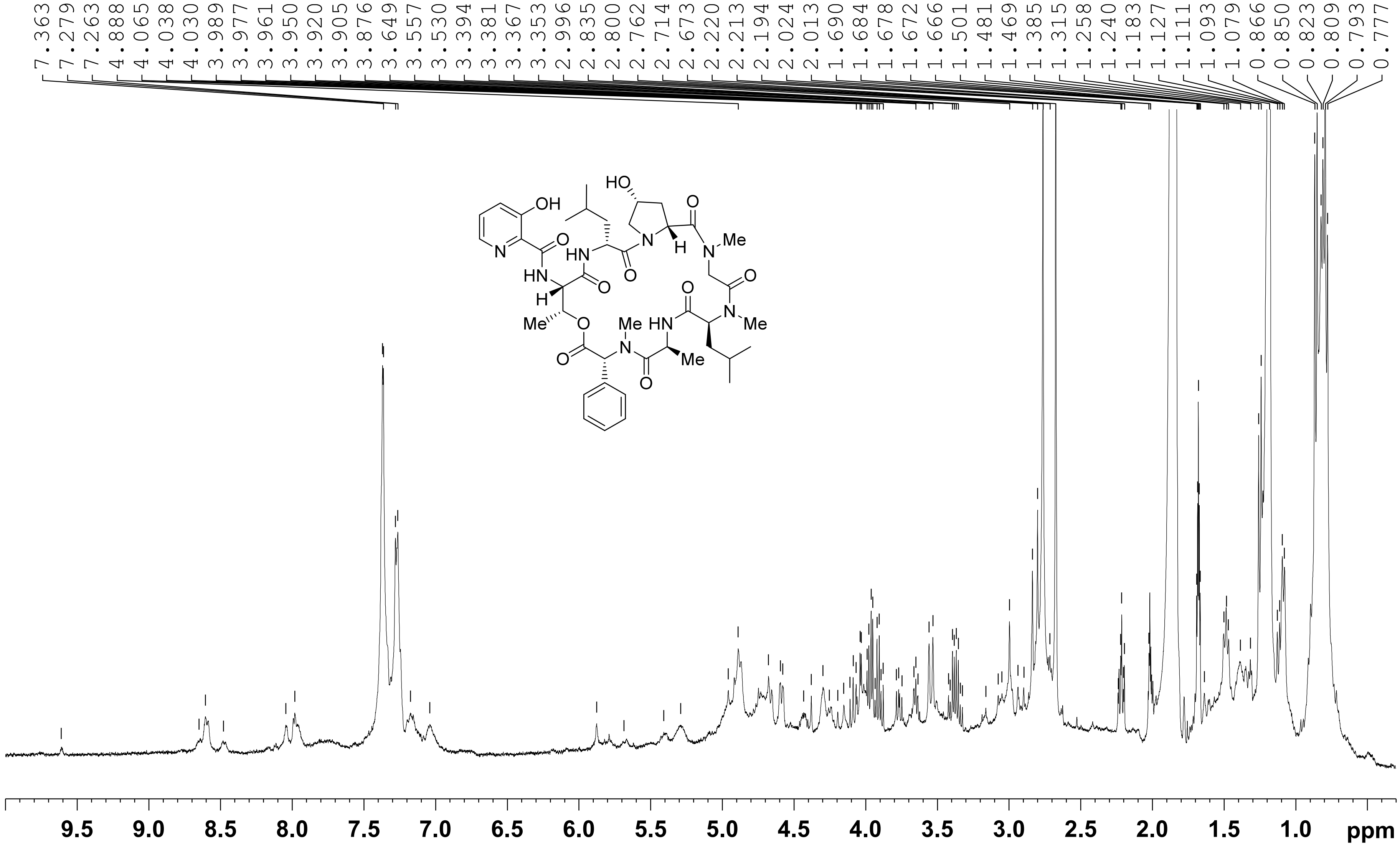
90
80
70
60
50
40
30
20
10
ppm


7.363
7.279
7.263
4.888
4.065
4.038
4.030
3.989
3.977
3.961
3.950
3.920
3.905
3.876
3.649
3.557
3.530
3.394
3.381
3.367
3.353
2.996
2.835
2.800
2.762
2.714
2.673
2.220
2.213
2.194
2.024
2.013
1.690
1.684
1.678
1.672
1.666
1.501
1.481
1.469
1.385
1.315
1.258
1.240
1.183
1.127
1.111
1.093
1.079
0.866
0.850
0.823
0.809
0.793
0.777
OH
N
O
HN
H
Me
HO
HN
O
N
H
N
Me
O
O
N
Me
O
Me
N
HN
O
Me
O
O
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
ppm

References

[1] a) G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw, K. I. Goldberg, *Organometallics* **2010**, *29*, 2176–2179; b) H. E. Gottlieb, V. Kotlyar, A. Nudelman, *J. Org. Chem.* **1997**, *62*, 7512–7515.

[2] P. Sun, K. N. Maloney, S.-J. Nam, N. M. Haste, R. Raju, W. Aalbersberg, P. R. Jensen, V. Nizet, M. E. Hensler, W. Fenical, *Bioorg. Med. Chem.* **2011**, *19*, 6557–6562.