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# Supplementary information

## A closer look at ligand specificity for cellular activation of NOD2 with synthetic muramyl dipeptide analogues

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## Biochemistry Materials and Methods

***In vitro* HEK-Blue™ h/mNOD2 reporter cell assay**

HEK cells expressing the human or mouse NOD2 receptor and carrying the NF-κB SEAP reporter gene (InvivoGen) were used according to the manufacturer's instructions. Briefly, to the HEK-Blue NOD2 or Null2 cells adhered in 96-well plates, 20 µL of the tested compounds (i.e., MDP analogues) at various concentrations were added and incubated for 16-18 h at 37 °C (total volume: 200 µL per well). Endotoxin-free deionized water was used as the negative control. For SEAP detection, 20 µL of supernatant from each well was collected and added to 180 µL of QUANTI-blue substrate (InvivoGen) in another 96-well microtiter plate. The mixture was incubated at 37 °C and SEAP activity was assessed by OD650 nm measurement. All MDP analogues were reconstituted in endotoxin-free water. Results are representative of three independent biological triplicates as means ± standard error (SE).

**LC-MS/MS quantification of intracellular uptake of MDP analogues in HEK293T cells**

HEK293T cells (American Type Culture Collection; ATCC, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco/BRL; Burlington, ON, Canada) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco/BRL; Burlington, ON, Canada) and 1% Pen Strep antibiotics (life technologies corporation, Grand Island, NY, USA ) in 37 °C at 100% humidity in 5% $CO_2$.

For cellular uptake assay, the respective MDP analogue (20 µM) was added to the attached HEK293T cells (2.8 × 10000 cells/mL) at 37 °C for 6 h or 16 h. At the indicated incubation period, culture media were removed, and cells were washed with PBS twice prior to snap-freeze and stored at −80 °C. To lyse the cells, ice-cold 90% methanol (LC–MS grade Optima, Fisher Scientific) was added to the frozen cell pellets, followed by boiling at 90 °C in a heating block to ensure sufficient lysis. The mixtures were then centrifuged at 15,000 *g* for 15 min to collect soluble lysates for drying in a SpeedVac concentrator. Dried samples were reconstituted in 50 µl $H_2O$ (LC–MS grade Optima, Fisher Scientific), and vortexed for 15 min before centrifugation at 15,000 *g* for 15 min. The soluble fraction was subjected to LC-MS analysis.

LC-MS/MS analysis was performed using Vanquish 3000 HPLC coupled to Orbitrap Exploris 120 MS (Thermo Fisher Scientific) in the positive mode. Separation was performed with a Synergy™ hydro-RP 80 Å LC column (4.6 x 150 mm, 4 µm, Phenomenex) with solvent A (LC-MS grade water with 0.05% formic acid) and solvent B (LC-MS acetonitrile with 0.05% formic acid) at a flow rate of 0.5 mL/min and the following conditions: initial 1% B; 1-5% B for 2.5 min, 5-80% B for 7.5 min, 80% B for 2.5 min, 90-5% B for 1 min and finally, 1% B for 4 min. MS/MS spectra were collected with Data Dependent Acquisition in selective ion monitoring mode targeting the exact mass of MDP analogues at 30% HCD. Peak area (AUC) was extracted from MS raw files using Xcalibur Processing Setup Quan (version 4.2.47, Thermo Scientific). Results are shown as means ± standard error (SE).

**Overexpression and Purification of His-MBP-NAGK protein**

*E.coli* BL21 Rosetta(DE3) for His-MBP-NAGK overexpression was a gift from Veit Hornung's lab.[1] The previous protocol for His-MBP-NAGK overexpression and purification was followed. Briefly, cells were induced with 0.3 mM IPTG at $OD_{600}$ ~1.0 for 4 h at 37 °C before harvesting by centrifugation (5000 *g*, 20 min, 4 °C). Cell pellets were lysed by resuspension in 20 mL ice-cold lysis buffer (20 mM HEPES, pH 7.5, 300 mM NaCl, 10 mM imidazole, 1 mM β-Me, 5% glycerol, 1% triton-X) and sonication (30% Amp,10 s on and 10 s off, 10 min in total). The soluble lysate was collected following centrifugation of the lysate at 12, 000 rpm for 20 min at 4 °C, and loaded onto 3 mL pre-equilibrated HisPur™ Cobalt resin (Thermo Fisher Scientific) for affinity purification. The loaded resin was washed sequentially with 30 mL of lysis buffer, 30 mL high-salt buffer (20 mM HEPES, pH 7.5, 1M NaCl, 10 mM imidazole, 1 mM β-Me, 5% glycerol), and wash buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 10 mM imidazole, 1 mM β-Me, 5% glycerol), followed by eluting His-MBP-NAGK protein with 15 mL of elution buffer (20 mM HEPES, pH 7.5, 150 mM NaCl, 250 mM imidazole, 1 mM β-Me, 5% glycerol). Fractions containing the target His-MBP-NAGK protein were pooled and concentrated with a 10 kD MWCO Amicon Ultra Centrifuge Filter Device (Millipore).

**Tev protease cleavage of MBP tag in His-MBP-NAGK to afford NAGK protein**

TEV protease was purchased from Sigma-Aldrich for MBP cleavage from His-MBP-NAGK protein to yield tag-free NAGK protein. Tev protease was incubated with the His-MBP-NAGK protein (50:1 w/w, 2 mL total) in a dialysis bag (10 kD MWCO)

for overnight dialysis in 2 L buffer (20 mM HEPES, pH 7.5, 50 mM NaCl, 2 mM DTT) at 4 °C. The mixture was then loaded onto 3 mL pre-equilibrated HisPur™ Cobalt resin for collection of the flowthrough fraction that contains the target NAGK protein, which was further concentrated with a 10 kD MWCO Amicon Filter Device to afford NAGK protein at 0.7 mg/mL.

**LC-MS analysis of *in vitro* phosphorylation of MDP analogues by NAGK**

The respective MDP analogue (2 mM) was incubated with ATP (5 mM) and NAGK (3.6 μM) in a total of 200 μL reaction buffer (50 mM Tris-HCl, pH=8.0, 10 mM $MgCl_2$) for incubation at 37 °C with shaking at 200 rpm. At the desired time points (i.e., 5, 15, 30, 60, 90, 120 min), 10 μL of the reaction mixture was collected, diluted 100-fold with $dH_2O$, and subjected to LC-MS analysis.

LC-MS analysis was performed using Vanquish Core HPLC-Orbitrap Exploris 120 system, equipped with a Phenomenex Luna Omega Polar C18 column (100 x 4.6mm, 3 μm, 100 Å) with polar C18 SecurityGuard cartridges. Sample was separated in a 16-min gradient (buffer A: 0.05% formic acid in $H_2O$, buffer B: 0.05% formic acid in acetonitrile, flow rate at 500 μL/min; 1% B in the first 3 min, 1-50% B from 3-8 min, 50% B maintained for 2 min, increased to 80% in 1 min and maintained for 4 min, then lowered to 1% in 0.5 min, equilibrated for 1.5 min).

**ADP-Glo™ kinase luminescence assay**

The respective MDP analogue (2 mM) was incubated with ATP (5 mM) and NAGK (3.6 μM) in a total of 200 μL of reaction buffer (50 mM Tris-HCl, pH=8.0, 10 mM $MgCl_2$) for incubation at 37 °C with shaking at 200 rpm. At the desired time points (i.e., 5, 15, 30, 60, 90, 120, 240 min), 10 μL of the reaction mixture was collected and diluted 5-fold with reaction buffer. ADP-Glo™ kinase assay (Promega, V6930) was used according to the manufacturer's instructions to measure ADP concentration in each sample. Luminescence was measured with Tecan Infinite M Plex pro multimode microplate reader with Tecan i-control 2.0 version software.

***In vitro* RAW264.7 cell-based assay**

RAW264.7 cells (American Type Culture Collection; ATCC, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle Medium (DMEM) (Gibco/BRL; Burlington, ON, Canada) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Gibco/BRL; Burlington, ON, Canada) and 1% Pen Strep antibiotics (life technologies corporation, Grand Island, NY, USA ) in 37 °C at 100% humidity in 5% $CO_2$.

For treatment, RAW264.7 cells (1.0 × 10000 cells/well) were plated into 24-well plates for overnight attachment and treated with respective MDP analogues (20 μM) for 24 h with the aid of lipofectamine™ (Thermo Fisher Scientific). Following treatment, cell supernatants were collected for ELISA analysis. Cytokine TNF-α (Biolegend, Cat # 430901) was measured according to the manufacturer's instructions. Results were shown as means ± standard error (SE).

**RT-qPCR for cytokine gene expression analysis in THP1**

Wildtype and $NAGK^{-/-}$ THP1 cells were obtained from Veit Hornung's lab.[1] For the treatment of either cell lines with MDP or GMDP, cells were seeded in complete RPMI at $1.5 \times 10^5$ cells/mL. The cells were treated with 200 μM of MDP or GMDP with Lipofectamine 3000 with modifications to the manufacturer's instructions. Briefly, for every well with 1 mL of cells, 3 μL of Lipofectamine 3000 reagent was mixed with 20 μL of incomplete RPMI. The compounds to be tested (MDP or GMDP) were each diluted in 20 μL of incomplete RPMI in a separate tube to a final concentration of 200 μM while distilled water added to incomplete RPMI was used as the negative control. The diluted compound was added to the tube containing diluted Lipofectamine 3000 and allowed to complex by incubating at RT for 15 min. The mixture was then added to the seeded cells and incubated for 24 h at 37 °C with 5% $CO_2$.

The treated cells were harvested for total RNA extraction according to manufacturer's protocol (New England Biolabs, US). RNA amount was normalized to 500 ng and reverse transcribed using ProtoScript II First Strand cDNA Synthesis Kit (New England Biolabs, US). The synthesized cDNA was used as the qPCR template to determine the expression levels of IL8 and TNFα normalized to housekeeping gene ACTB using the following primer pairs:

hIL8-F: 5’-AACTTTCAGAGACAGCAGAGCA-3’
hIL8-R: 5’-TGGTTCTTTCCGGTGGTTTC-3’

hTNFa-F: 5’-TGGGATCATTGCCCTGTGAG-3’
hTNFa-R: 5’-GGTGTCTGAAGGAGGGGGTA-3’

hACTB-F: 5’-CTCGCCTTTGCCGATCC-3’
hACTB-R: 5’-TCTCCATGTCGTCCCAGTTG-3’

qPCR was performed using SsoAdvanced Universal SYBR Green Supermix (BioRad, US) according to the recommended protocols.

**Molecular Docking Using Autodock-Vina**

For the docking of MDP and analogues to rabbit NOD2_LRR, the protein sequence (residues S764 to S1010) was downloaded from UniProt (accession number: G1T469) and submitted for homology modeling by AlphaFold2. Molecular dockings were simulated in a 30 × 20 × 30 Å grid box around the concave binding region of the LRR domain according to the rabbit NOD2 crystal structure (PDB: 5IRN).

For GlcNAc kinase (NAGK) docking, the protein model was obtained from UniProt (PDB: 2CH5) and served as the protein receptor for docking simulations of MDP and GMDP. The structures of both MDP and GMDP were derived from their respective SMILES notation and converted into individual PDB files using OpenBabel. MDP or GMDP was allowed to dock flexibly to the human NAGK binding pocket[2] in a 30 × 30 × 30 Å grid box.

All ligands and receptors used in this study were converted to their respective PDBQT files using OpenBabelGUI after removing water and other atoms (HETATM) and adding hydrogen atoms to the polar atoms. All receptor-ligand complexes were analyzed using BIOVIA Discovery Studio Visualizer.

**Statistics**

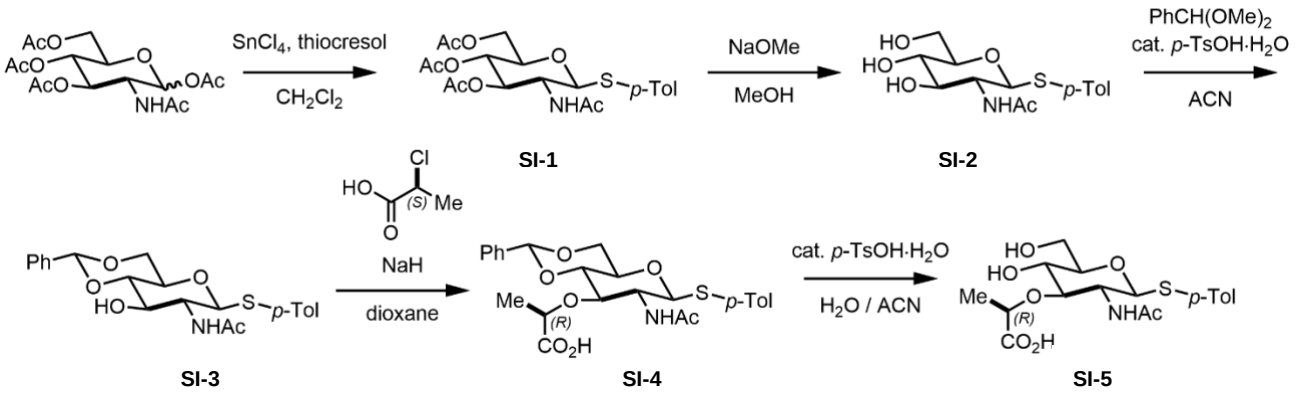
Unless otherwise indicated, statistical significance was determined by one-way. The exact number of replicates (*n*) is indicated within the figure legends. Data plotting and statistical analysis were performed using GraphPad Prism 9.

## General Synthetic Procedures

Water was purified with a Millipore Milli-Q system (Merck K. Ga. Co., Darmstadt, Germany). Chemical reagents and solvents were obtained from commercial sources (Millipore-Sigma, TCI, Alfa-Aesar, or Fluorochem). Cold temperatures were maintained using the following conditions: 0 - 5 °C, ice-water bath; -78 °C, acetone-dry ice bath. Normal phase column chromatography was carried out with Grace Davisil® LC60A 40-63 micron silica gel. Preparative HPLC was performed on an Agilent 1260 Infinity II equipped with a 21.2 x 250 mm C18 column using a 10 mL/min flow rate. Removal of solvents was done with Buchi R-100 rotary evaporator equipped with a Vacuubrand® MD-1C diaphragm vacuum pump and an Eyela CCA-1110 chiller. Final muropeptide products were lyophilized from water/acetonitrile to ensure an accurate weight.

NMR spectra were recorded on a Bruker Avance 400 spectrometer (400 MHz for $^{1}$H NMR, 101 MHz for $^{13}$C NMR). Spectral measurements of <10 mg muropeptide final products were performed using a Shigemi BMS-005B NMR tube. Spectra were recorded using $CDCl_3$, MeOH-$d_4$, DMSO-$d_6$, or $D_2O$. $^{1}$H and $^{13}$C signal positions (δ) are reported in parts per million from tetramethylsilane (δ 0) and were measured relative to the signal of the solvent ($^{1}$H NMR: $CDCl_3$ δ 7.26, DMSO-$d_6$ δ 2.50; MeOH-$d_4$ δ 3.31; $^{13}$C NMR: $CDCl_3$ δ 77.16, DMSO-$d_6$ δ 39.52; MeOH-$d_4$ δ 49.00). When $D_2O$ was used, about 0.2 µL DMSO was spiked in as an internal standard (δ 2.71 for $^{1}$H NMR and δ 39.39 for $^{13}$C NMR). Coupling constants (*J* values) are reported in Hertz (Hz). 1H NMR spectral data are tabulated in the order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br., broad), coupling constant, number of protons. Only selected diagnostic peaks are assigned.

High-resolution mass spectra were measured using a Themo-Fisher Vanquish Core HPLC-Orbitrap Exploris 120 system.




To a stirred solution of glucosamine pentaacetate (38.76 g, 99.5 mmol, 1.0 eq) in 350 mL $CH_2Cl_2$ was charged *p*-thiocresol (14.8 g,119 mmol, 1.2 eq) followed by $SnCl_4$ (8.1 mL, 69.7 mmol, 0.7 eq). The mixture was refluxed for 4 days under inert atmosphere and then cooled to room temperature. The reaction was carefully quenched to a stirred biphasic mixture of 1 L sat. aq. $NaHCO_3$ and 500 mL EtOAc (*note*: upper organic layer contained suspended product solids and lower aqueous layer contained suspended inorganic material; it was not possible to cleanly separate the layers). The aqueous layer was discarded, the organic material was washed with 3 x 100 mL $H_2O$, and the mixture was concentrated to dryness. The residue was stirred with 180 mL 1 : 1 : 1 v/v hexane : EtOAc : MeOH to obtain a mobile slurry. The slurry was then filtered and the cake was rinsed with 20 mL 1 :1 v/v $Et_2O$ : hexane. The solids were dried to afford thioglycoside **SI-1** (34.03 g, 75.0 mmol, 75% yield) as a white solid.

$^1$H NMR (400 MHz, DMSO-$d_6$, 301 K, δ): 8.08 (d, *J* = 9.2 Hz, 1H), 7.33 (d, *J* = 7.6 Hz, 2H), 7.15 (d, *J* = 7.6 Hz, 2H), 5.12 (t, *J* = 9.7 Hz, 1H), 4.97 (d, *J* = 10.4 Hz, 1H), 4.80 (t, *J* = 9.7 Hz, 1H), 4.14 (dd, *J* = 12.0, 5.0 Hz, 1H), 4.04 (d, *J* = 12.0 Hz, 1H), 3.90 (m, 1H), 3.82 (q, *J* = 9.9 Hz, 1H), 2.28 (s, 3H), 2.00 (s, 3H), 1.97 (s, 3H), 1.91 (s, 3H), 1.80 (s, 3H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$, 301 K, δ): 170.0, 169.6, 169.3, 169.2, 137.1, 131.5, 129.6, 129.2, 85.2, 74.5, 73.6, 68.5, 62.0, 52.1, 22.7, 20.6, 20.5, 20.4, 20.3.

HRMS (ESI-TOF, m/z): calc'd 454.1530 for $C_{21}H_{28}NO_8S$ ($[M + H]^+$); found 454.1533.

To a stirred 0 - 5 °C suspension of 9.70 g thioglycoside **SI-1** (21.4 mmol, 1.0 eq) in 175 mL MeOH and 25 mL $CH_2Cl_2$ was charged 1 M NaOMe in MeOH (11.8 mL; 11.8 mmol, 0.55 eq) (*note*: reaction became a clear solution and then a hazy white suspension). After 3 hours, the reaction was neutralized by charging 10 g Amberlite IR-120 $H^+$. The mixture was filtered, and copious MeOH was applied to rinse forward the gelatinous precipitated product. The filtrate was concentrated and co-evaporated with PhMe to afford triol **SI-2** (6.90 g, 21.1 mmol, quantitative yield) as a white solid.

$^1$H NMR (400 MHz, DMSO-$d_6$, 301 K, δ): 7.84 (d, *J* = 9.2 Hz, 1H), 7.31 (d, *J* = , 7.8 Hz, 2H), 7.11 (d, *J* = , 7.8 Hz, 2H), 5.06 (br. s, 2H), 4.67 (d, *J* = 10.3 Hz, 1H), 4.59 (br. s, 1H), 3.69 (d, *J* = 11.5 Hz, 1H), 3.57 (d, *J* = 9.8 Hz, 1H), 3.46 (m, 1H), 3.33 (t, *J* = 8.8 Hz, 1H), 3.14 (m, 2H), 2.26 (s, 3H), 1.83 (s, 3H) (*note*: peak at 3.46 ppm obscured by $H_2O$ peak).

$^{13}$C NMR (101 MHz, DMSO-$d_6$, 301 K, δ): 169.6, 136.3, 132.1, 130.3, 129.9, 87.1, 81.6, 75.9, 70.8, 61.5, 54.9, 23.5, 21.0.

HRMS (ESI-TOF, m/z): calc'd for $C_{15}H_{22}NO_5S^+$ ($[M + H]^+$) 328.1213; found 328.1208.

To a rbf was charged triol **SI-2** (7.03 g, 21.5 mmol, 1.0 eq), 90 mL ACN, benzaldehyde dimethyl acetal (12.8 mL, 85.9 mmol, 4.0 eq), and *p*-TsOH·$H_2O$ (164 mg, 0.86 mmol, 0.04 eq). The mixture was stirred at reflux for 40 hours under inert atmosphere and cooled to room temperature. The mixture was distilled to about 40 mL pot volume. With stirring, 20 mL $Et_2O$ was charged, and the mixture was cooled to 0 - 5 °C. The resulting mobile slurry was filtered and rinsed with 30 mL

1:1 v/v $Et_2O$ : hexane. The solids were dried to afford benzylidene acetal **SI-3** (8.57 g, 20.6 mmol, 96% yield) as a white solid (*note*: NMR data indicate a ca. 55 :45 mixture of epimers).

$^1$H NMR (400 MHz, DMSO-$d_6$, 301 K, δ): 7.98 & 7.84 (d, *J* = 8.1 Hz & d, *J* = 8.9 Hz, 1H), 7.37 (m, 4H), 7.15 & 7.11 (d, *J* = 7.7 Hz, & d, *J* = 7.5 Hz, 2H), 5.60 & 5.40 (s & d, *J* = 2.2 Hz, 1H), 5.06 (m, 1H) 4.88 & 4.67 (d, *J* = 9.4 Hz & d, *J* = 10.3 Hz, 1H), 4.59 & 4.19 (t, *J* = 4.5 Hz & d, *J* = 9.2 Hz, 1H), 3.69 & 3.57 (m, 2H), 3.45 & 3.34 (m, 1H), 3.15 (m, 1H), 2.28 & 2.26 (s & s, 3H), 1.85 & 1.83 (s & s, 3H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$, 301 K, δ): 169.3, 169.1, 137.7, 136.5, 135.9, 134.6, 131.7, 130.7, 130.4, 129.8, 129.6, 129.5, 129.2, 128.9, 128.1, 126.4, 100.7, 86.8, 86.7, 81.1, 80.9, 75.5, 71.7, 70.3, 70.0, 67.7, 61.1. 55.0, 54.4, 23.1, 20.6

HRMS (ESI-TOF, m/z): calc'd for $C_{22}H_{26}NO_5S^+$ ($[M + H]^+$) 416.1526; found 416.1522.

Under inert atmosphere, a stirred suspension of **SI-3** (4.12 g, 9.92 mmol, 1.0 eq) in 200 mL 1,4-dioxane was cooled in an ice-water bath. (*S*)-2-chloropropionic acid (4.3 mL, 49.6 mmol, 5.0 eq) was charged, and then sodium hydride (2.78 g, 69.4 mmol, 7.0 eq) was carefully charged in portions over 10 minutes (*note*: the solvent partially froze during addition of reagents). The resulting thick paste was heated in a 70 °C bath to obtain a mobile slurry. After 36 hours, the mixture was cooled to room temperature to obtain a thick paste. 50 mL $Et_2O$ was charged, and the resulting mobile slurry was quenched into a stirred solution of 200 mL 10% NaCl + 2% citric acid. The slurry was then filtered, and the solids were rinsed with copious $H_2O$ followed by 50 mL $Et_2O$. The solids were dried to afford carboxylic acid **SI-4** (4.80 g, 9.85 mmol, quantitative yield) as a white solid (*note*: although the starting material was an epimeric mixture, NMR data indicate the product is a single isomer).

$^1$H NMR (400 MHz, DMSO-$d_6$, 301 K, δ): 7.94 (d, *J* = 8.5 Hz, 1H), 7.40 (m, 5H), 7.32 (d, *J* = 8.0 Hz, 2H), 7.15 (d, *J* = 8.0 Hz, 2H), 5.68 (s, 1H), 4.98 (d, *J* = 10.2 Hz, 1H), 4.21 (m, 2H), 3.73 (m, 2H), 3.62 (t, *J* = 9.2 Hz, 1H), 3.48 (m, 1H), 2.28 (s, 3H), 1.84 (s, 3H), 1.24 (d, *J* = 6.8 Hz, 3H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$, 301 K, δ): 174.0, 169.4, 137.6, 136.7, 131.0, 130.0, 129.6, 128.8, 128.2, 125.8, 100.0, 86.5, 81.1, 79.0, 75.5, 69.5, 67.6, 53.5, 23.1, 20.6, 18.8.

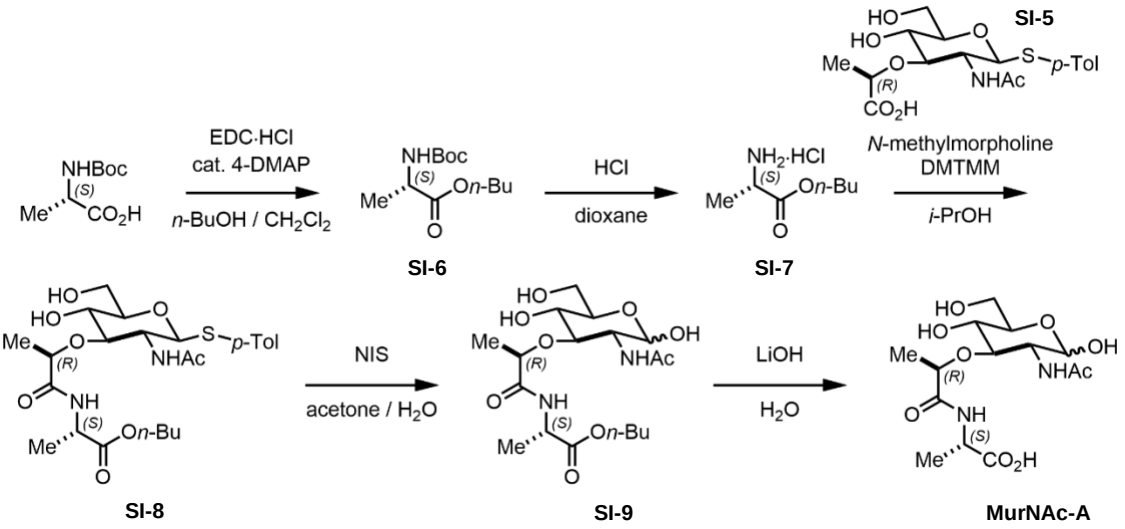
HRMS (ESI-TOF, m/z): calc'd for $C_{25}H_{30}NO_7S^+$ ($[M + H]^+$) 488.1737; found 488.1730.

To a rbf was charged **SI-4** (2.42 g, 4.96 mmol, 1.0 eq), *p*-TsOH·$H_2O$ (95 mg, 0.50 mmol, 0.1 eq), 50 mL $H_2O$, and 50 mL ACN. The resulting slurry was heated in a 85 °C bath for 12 hours and then cooled to room temperature (*note*: changes from slurry to solution as the reaction proceeds; the reaction was accompanied by partial formation of a δ-lactone impurity). The mixture was distilled to about 25 mL pot volume and then diluted with 15 mL $H_2O$. The mixture was stirred in a 85 °C bath for a further 6 hours (*note*: TLC indicated essentially full hydrolysis of the δ-lactone impurity) and then cooled to room temperature. The mixture was distilled to about 10 mL pot volume to obtain a thick slurry and diluted with 10 mL EtOAc to obtain a mobile slurry. The slurry was filtered and rinsed forward with 15 mL EtOAc. The solids were dried to afford diol **SI-5** (1.51 g, 3.79 mmol, 76% yield) as a white solid.

$^1$H NMR (400 MHz, DMSO-$d_6$, 301 K, δ): 12.43 (br s, 1H), 7.78 (d, *J* = 6.6 Hz, 1H), 7.32 (d, *J* = 5.3 Hz, 2H), 7.12 (d, *J* = 5.3 Hz, 2H), 5.33 (br s, 1H), 4.75 (d, *J* = 7.9 Hz, 1H), 4.34 (q, *J* = 4.8 Hz, 1H), 3.68 (d, *J* = 10.6 Hz, 1H), 3.55 (m, 1H), 3.45 (m, 3H), 3.25 (t, *J* = 8.1 Hz, 1H), 3.20 (m, 1H), 2.27 (s, 3H), 1.81 (s, 3H), 1.28 (d, *J* = 4.8 Hz, 3H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$, 301 K, δ): 174.8, 169.3, 136.0, 131.5, 130.0, 129.5, 86.5, 83.0, 80.9, 75.4, 70.3, 60.7, 53.2, 23.1, 20.6, 19.0.

HRMS (ESI-TOF, m/z): calc'd for $C_{18}H_{26}NO_7S^+$ ($[M + H]^+$) 400.1424; found 400.1416.




To a stirred 0 - 5 °C solution of Boc-(*S*)-Ala-OH (620 mg, 3.28 mmol, 1.0 eq), 4-DMAP (40 mg, 0.33 mmol, 0.1 eq), and *n*-BuOH (1.8 mL, 19.7 mmol, 6.0 eq) in 14 mL $CH_2Cl_2$ was charged EDC·HCl (849 mg, 4.43 mmol, 1.35 eq). After 3 hours, 10 mL 10% NaCl + 2% citric acid was charged and the bulk of the $CH_2Cl_2$ was distilled off. 20 mL EtOAc was charged, the mixture was extracted, and the aqueous material was discarded. The organic extract was washed with brine, dried ($MgSO_4$), filtered, and concentrated. The residue was purified by column chromatography (10 g silica, 15% v/v EtOAc in hexane), to afford ester **SI-6** (789 mg, 3.22 mmol, 98% yield) as an oil.

$^1$H NMR (400 MHz, $CDCl_3$ 301 K, δ): 5.08 (m, 1H), 4.27 & 3.61 (p, *J* = 6.5 Hz & t, *J* = 6.0 Hz, 1H), 4.11 (m, 2H), 1.60 (m, 2H), 1.41 (s, 9H), 1.35 (t, *J* = 7.0 Hz, 3H), 1.35 (m, 2H), 0.90 (t, *J* = 7.3 Hz, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$, 301 K, δ): 173.3, 155.0, 79.6, 65.0, 62.5, 49.2, 34.8, 30.5, 28.2, 18.9, 18.8, 18.6, 13.7, 13.5.

HRMS (ESI-TOF, m/z): we were unable to observe the expected $H^+$ or $Na^+$ adducts by ESI-MS.

To a rbf charged with **SI-6** (789 mg, 3.22 mmol, 1.0 eq) was charged 4 M HCl in 1,4-dioxane (6.4 mL, 25.6 mmol, 8.0 eq), and the flask was swirled to homogenize. After 4 hours, volatiles were removed and the residue was co-evaporated with 1,4-dioxane and then $CHCl_3$ to afford hydrochloride salt **SI-7** (581 mg, 3.20 mmol, quantitative yield) as an oil which crystallized on standing.

$^1$H NMR (400 MHz, $CDCl_3$, 301 K, δ): 8.68 (br s, 3H), 4.18 (br m, 2H), 3.75 & 3.63 (m & m, 1H), 1.71 (br, m, 3H), 1.62 (br. m, 2H), 1.36 (br. m, 2H), 0.91 (t, *J* = 5.7 Hz, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$, 301 K, δ): 170.2, 66.4, 49.6, 30.5, 19.1, 16.3, 13.7.

HRMS (ESI-TOF, m/z): calc'd for $C_7H_{16}NO_2^+$ ($[M + H]^+$) 146.1176; found 146.1174.

To a rbf charged with carboxylic acid **SI-5** (80 mg, 0.20 mmol, 1.0 eq) was charged a solution of hydrochloride salt **SI-7** (51 mg, 0.28 mmol, 1.4 eq) in 2 mL *i*-PrOH, and the mixture was stirred. To the resulting clear solution was charged *N*-methylmorpholine (35 µL, 0.32 mmol, 1.6 eq) and DMTMM (77 mg, 0.28 mmol, 1.4 eq) (*note*: a slurry formed as the reaction progressed). After 2.5 hours, 3 mL $H_2O$ was charged and the pot volume was reduced to about 2.5 mL. The resulting thick slurry was filtered and rinsed with 2 mL $H_2O$. The solids were dried to obtain **SI-8** (60 mg, 0.11 mml, 57% yield) as a white solid.

$^{1}$H NMR (400 MHz, MeOH-$d_4$, 301 K, δ): 7.39 (d, *J* = 7.6 Hz, 2H), 7.12 (d, *J* = 7.6 Hz, 2H), 4.66 (d, *J* = 10.2 Hz, 1H), 4.35 (q, *J* = 7.0 Hz, 1H), 4.15 (m, 3H), 3.91 (m, 1H), 3.88 (d, *J* = 11.8 Hz, 1H), 3.70 (dd, *J* = 11.8, 5.4 Hz, 1H), 3.44 (m, 2H), 2.31 (s, 3H), 1.95 (s, 3H), 1.63 (m, 2H), 1.41 (m, 7H), 0.95 (t, *J* = 7.3 Hz, 3H).

$^{13}$C NMR (101 MHz, MeOH-$d_4$, 301 K, δ): 175.6, 174.1, 173.4, 138.7, 133.0, 131.8, 130.6, 89.0, 85.7, 82.1, 79.4, 70.6, 66.2, 62.8, 55.4, 49.5, 31.7, 23.2, 21.1, 20.1, 19.6, 17.4, 14.0.

HRMS (ESI-TOF, m/z): calc'd for $C_{25}H_{39}N_2O_8S^+$ ([M + H]$^+$) 527.2422; found 527.2408.

To a stirred solution of **SI-8** (60 mg, 114 µmol, 1.0 eq) in 2.3 mL 3:1 v/v acetone : $H_2O$ was charged *N*-iodosuccinimide (32 mg, 143 µmol, 1.25 eq) in portions over 1.5 hours. After a further 1 hour, the reaction was treated with aqueous $NaHS_2O_3$ until the brown colour disappeared. The mixture was then concentrated and co-evaporated with *i*-PrOH. The residue was purified by column chromatography (13% v/v MeOH in EtOAc) to afford lactol **SI-9** (48 mg, 114 µmol, quantitative yield).

$^{1}$H NMR (400 MHz, MeOH-$d_4$, 301 K, δ): 5.12 (d, *J* = 3.4 Hz; H-1α), 4.55 (d, *J* = 8.3 Hz; H-1β), 4.37 (q, *J* = 7.3 Hz, 1H), 4.33 (q, *J* = 6.7 Hz; lactoyl C*H*α), 4.26 (q, *J* = 6.7 Hz; lactoyl C*H*β), 4.15 (m, 2H), 3.90 (dd, *J* = 10.3, 3.4 Hz; H-2α), 3.87 (dd, *J* = 12.0, 2.1 Hz, H-6aβ), 3.82 (ddd, *J* = 10.0, 5.0, 2.2 Hz; H-5α), 3.80 (dd, *J* = 11.7, 2.2 Hz; H-6aα), 3.73 (m; H-2β), 3.72 (dd, *J* = 11.7, 5.0 Hz; H-6bα), 3.71 (m, H-6bβ), 3.64 (dd, *J* = 10.3, 9.0 Hz; H-3α), 3.49 (t, *J* = 9.3 Hz; H-4α), 3.45 (t, *J* = 9.0 Hz; H-3β), 3.39 (t, *J* = 9.3 Hz; H-4β), 3.28 (m; H-5β), 1.95 & 1.94 (s & s, 3H), 1.64 (m, 2H), 1.41 (m, 8H), 0.95 (t, *J* = 7.4 Hz, 3H) (*note*: the product contained ca. 0.1 eq succinimide as an impurity as indicated by the singlet at 2.69 ppm; the $HD_2COD$ peak partly obscures the H-5β peak at 3.28 ppm).

$^{13}$C NMR (101 MHz, MeOH-$d_4$, 301 K, δ): 176.1, 176.0, 174.24, 174.18, 174.15, 173.4, 97.2 (C-1β), 92.5 (C-1α), 83.9 (C-3β), 80.9 (C-3α), 78.8 (lactoyl *C*Hβ), 78.5 (lactoyl *C*Hα), 77.9 (C-5β), 73.2 (C-5α), 71.5 (C-4α), 71.1 (C-4β), 66.2, 62.74 (C-6β), 62.67 (C-6α), 58.0 (C-2β), 55.4 (C-2α), 49.53, 49.49, 31.74, 31.73, 23.1, 22.9, 20.1, 19.7, 19.6, 17.43, 17.36, 14.0.

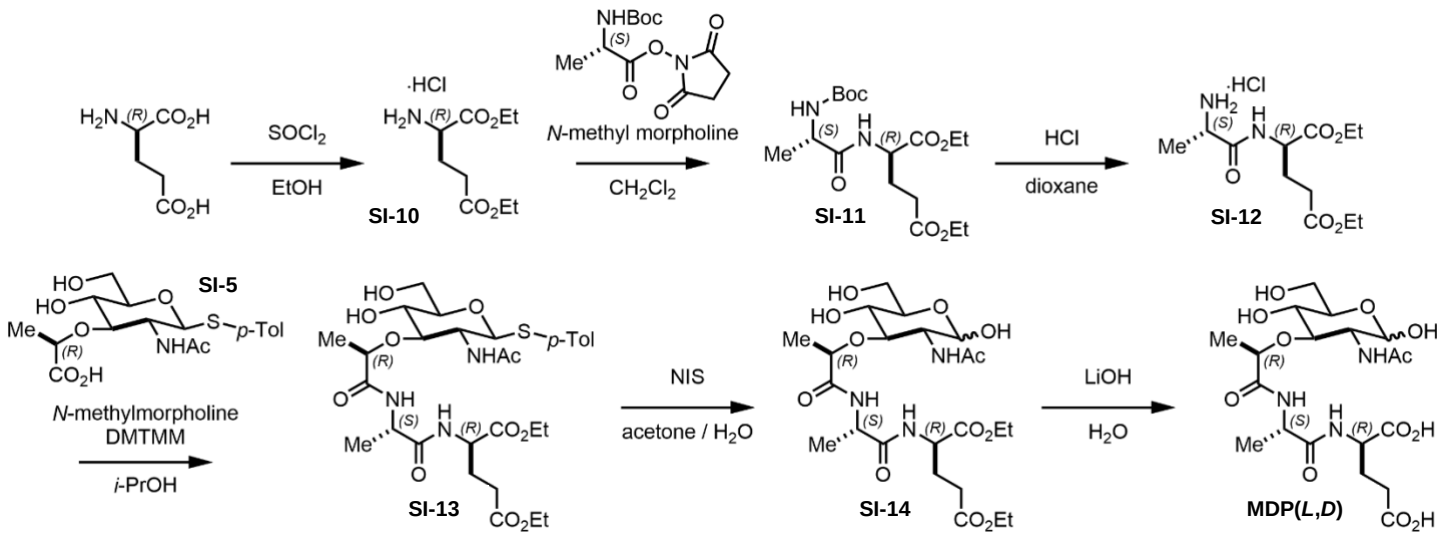
HRMS (ESI-TOF, m/z): calc'd for $C_{18}H_{33}N_2O_9^+$ ([M + H]$^+$) 421.2181; found 421.2173.

To a rbf containing **SI-9** (38 mg, 0.090 mmol, 1.0 eq) was charged 100 µL $H_2O$. The reaction was cooled in an ice/water bath and then 0.5 M LiOH (315 µL, 0.16 mmol, 1.75 eq) was charged and the mixture was swirled to homogenize. After 35 minutes, the mixture was quenched to a final pH of ~3 by adding 2 M HCl (85 µL, 0.17 mmol, 1.9 eq). The crude reaction mixture was chromatographed on 5 g C18 silica gel (0 to 50% MeOH in $H_2O$). Fractions containing the product were lyophilized to afford 12.7 mg of a red solid. This material was further purified by preparative HPLC (0 to 20% ACN in 0.1% TFA) to afford **MurNAc-A** (3.3 mg, 9.1 µmol, 10% yield) as a white solid.

$^{1}$H NMR (400 MHz, $D_2O$, 301 K, δ): 5.15 (d, *J* = 3.4 Hz; H-1α), 4.67 (d, *J* = 8.4 Hz; H-1β), 4.33 (q, *J* = 7.4 Hz; 1H), 4.28 (q, *J* = 6.7 Hz, lactoyl C*H*α), 4.21 (q, *J* = 6.7 Hz, lactoyl C*H*β), 3.95 (dd, *J* = 10.4, 3.4 Hz; H-2α), 3.90 (dd, *J* = 12.4, 2.1 Hz; H-6aβ), 3.87 (ddd, *J* = 9.9, 5.0, 2.1 Hz; H-5α), 3.84 (dd, *J* = 12.4, 2.1 Hz; H-6aα), 3.79 (dd, *J* = 12.4, 5.0 Hz; H-6bα), 3.77 (m; H-2β), 3.75 (dd, *J* = 12.4, 5.4 Hz; H-6bβ), 3.69 (dd, *J* = 10.0, 9.0 Hz; H-3α), 3.57 (t, *J* = 9.7 Hz; H-4α), 3.52 (m; H-4β), 3.50 (m; H-3β), 3.48 (m; H-5β), 1.972 & 1.968 (s & s, 3H), 1.44 (d, *J* = 7.4 Hz, 3H), 1.38 (d, *J* = 6.8 Hz, 3H).

$^{13}$C NMR (101 MHz, $D_2O$, 301 K, δ): 176.9, 176.8, 176.4, 176.2, 175.0, 174.7, 95.6 (C-1β), 91.6 (C-1α), 83.2 (C-3β), 80.2 (C-3α), 78.8 (lactoyl *C*Hβ), 78.5 (lactoyl *C*Hα), 76.4 (C-5β), 72.2 (C-5α), 69.8 (C4-α), 69.5 (C-4β), 61.4 (C-6β), 61.2 (C-6α), 56.8 (C-2β), 54.3 (C-2α), 49.1, 22.9, 22.6, 19.2, 19.1, 16.69, 16.67.

HRMS (ESI-TOF, m/z): calc'd for $C_{14}H_{25}N_2O_9^+$ ([M + H]$^+$) 365.1555; found 365.1548.




To a stirred 0 - 5 °C suspension of (*R*)-glutamic acid (2.21 g, 15 mmol, 1.0 eq) in 40 mL EtOH was carefully charged 8.0 mL thionyl chloride in portions over 5 minutes to afford a clear solution. The mixture was heated in a 40 °C bath for 13 hours and then cooled to room temperature. The residue was co-evaporated with ethanol and then lyophilized from ACN / $H_2O$ to afford hydrochloride salt **SI-10** (3.57 g, 14.9 mmol, quantitative yield) as a white solid.

$^1$H NMR (400 MHz, DMSO-$d_6$, 301 K, δ): 8.79 (br. s., 3H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.06 (q, *J* = 7.1 Hz, 2H), 3.99 (m, 1H), 2.55 (m, 1H), 2.46 (m, 1H), 2.07 (q, *J* = 7.1 Hz, 2H), 1.23 (t, *J* = 7.1 Hz, 3H), 1.19 (t, *J* = 7.1 Hz, 3H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$, 301 K, δ): 171.7, 169.0, 61.8, 60.1, 51.1, 29.1, 25.1, 14.1, 13.9.

HRMS (ESI-TOF, m/z): calc'd for $C_9H_{18}NO_4^+$ ($[M + H]^+$) 204.1230; found 204.1227.

To a stirred solution of hydrochloride salt **SI-10** (1.77 g, 7.38 mmol, 1.0 eq) and Boc-(*S*)-Ala-OSu (2.76 g, 10.3 mmol, 1.4 eq) in 30 mL $CH_2Cl_2$ was charged *N*-methylmorpholine (1.22 mL, 11.1 mmol, 1.5 eq). After 3 hours, the mixture was diluted with EtOAc, washed with 10% NaCl + 2% citric acid, washed with brine, dried ($Na_2SO_4$), and concentrated. The residue was purified by column chromatography (25 g silica, 1 : 1 v/v EtOAc : hexane) to afford dipeptide **SI-11** (2.67 g, 7.13 mmol, 97% yield) as an oil.

$^1$H NMR (400 MHz, $CDCl_3$, 301 K, δ): 6.87 (br. d, *J* = 5.6 Hz, 1H), 5.01 (br. s, 1H), 4.56 (dt, *J* = 5.2, 8.0 Hz, 1H), 4.19 (m, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 2.36 (m, 2H), 2.20 (m, 1H), 1.98 (m, 1H), 1.44 (s, 9H), 1.35 (d, *J* = 7.1 Hz, 3H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$, 301 K, δ): 172.8, 172.7, 171.8, 155.6, 80.4, 61.8, 60.8, 51.7, 50.3, 30.3, 28.4, 27.4, 18.3, 14.3, 14.2.

HRMS (ESI-TOF, m/z): calc'd 375.2126 for $C_{17}H_{31}N_2O_7$ ($[M + H]^+$); found 375.2131.

The rbf containing **SI-11** (2.67 g, 7.13 mmol, 1.0 eq) was cooled in an ice/water bath and 4 M HCl in 1,4-dioxane (18 mL, 71.3 mmol, 10.0 eq) was charged. The mixture was swirled to obtain a clear solution. After 3 hours, the mixture was concentrated and co-evaporated with 1,4-dioxane. The residue was suspended in 20 mL 1 :1 v/v ACN : $H_2O$ and minor white debris was filtered off ($H_2O$ rinse). The filtrate was lyophilized to afford hydrochloride salt **SI-12** (1.80 g, 5.80 mmol, 81% yield) as an oil.

$^{1}$H NMR (400 MHz, DMSO-$d_6$, 301 K, δ): 9.05 (d, *J* = 7.5 Hz, 1H), 8.34 (br. s, 3H), 4.27 (dt, *J* = 5.0, 8.3 Hz, 1H), 4.09 (q, *J* = 7.1 Hz, 2H), 4.03 (q, *J* = 7.1 Hz, 2H), 3.87 (m, 1H), 2.37 (t, *J* = 7.2 Hz, 2H), 2.02 (m, 1H), 1.88 (m, 1H), 1.38 (d, *J* = 6.7 Hz, 3H), 1.18 (t, *J* = 7.1 Hz, 3H), 1.16 (t, *J* = 7.1 Hz, 3H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$, 301 K, δ): 172.1, 171.2, 169.9, 60.9, 60.1, 51.4, 48.2, 29.8, 26.0, 17.4, 14.1, 14.1.

HRMS (ESI-TOF, m/z): calc'd for $C_{12}H_{23}N_2O_5$ $[M + H]^+$ 275.1601; found 275.1598.

To a stirred solution of carboxylic acid **SI-5** (399 mg, 1.0 mmol, 1.0 eq), hydrochloride salt **SI-12** (559 mg, 1.8 mmol, 1.8 eq), and *N*-methylmorpholine (242 µL, 2.2 mmol, 2.2 eq) in 10 mL *i*-PrOH was charged DMTMM (415 mg, 1.5 mmol, 1.5 eq) (*note*: becomes a white suspension as the reaction proceeds). After 5 hours, the slurry was filtered, and the cake was rinsed with 5 mL *i*-PrOH. The solids were dried to afford amide **SI-13** (488 mg, 0.74 mmol, 74% yield) as a white solid.

$^{1}$H NMR (400 MHz, MeOH-$d_4$, 301 K, δ): 7.39 (d, *J* = 8.1 Hz, 2H), 7.12 (d, *J* = 8.1 Hz, 2H), 4.68 (d, *J* = 10.4 Hz, 1H), 4.41 (dd, *J* = 5.2, 9.2 Hz, 1H), 4.36 (q, *J* = 7.1 Hz, 1H), 4.25 (q, *J* = 6.7 Hz, 1H), 4.14 (q, *J* = 7.2 Hz, 2H), 4.12 (q, *J* = 7.2 Hz, 2H), 3.89 (m, 1H), 3.87 (dd, *J* = 2.1, 12.1 Hz, 1H), 3.70 (dd, *J* = 5.6, 12.1 Hz, 1H), 3.46 (m, 2H), 3.30 (m, 1H), 2.40 (t, *J* = 7.5 Hz, 2H), 2.31 (s, 3H), 2.18 (m, 1H), 1.96 (s, 3H), 1.95 (m, 1H), 1.40 (d, *J* = 7.1 Hz, 3H), 1.37 (d, *J* = 6.7 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 6H) (*note*: the $HD_2COD$ peak obscures the peak at 3.30 ppm).

$^{13}$C NMR (101 MHz, MeOH-$d_4$, 301 K, δ): 175.5, 175.0, 174.2, 173.4, 172.9, 138.7, 132.9, 132.0, 130.6, 89.1, 84.9, 82.1, 78.8, 70.9, 62.8, 62.5, 61.7, 55.4, 53.0, 50.2, 31.2, 27.6, 23.2, 21.1, 19.4, 18.3, 14.5, 14.4.

HRMS (ESI-TOF, m/z): calc'd for $C_{30}H_{46}N_3O_{11}S$ $[M + H]^+$ 656.2848; found 656.2833.

A stirred 50 mM solution of thioglycoside **SI-13** (47 mg, 72 µmol, 1.0 eq) in 3 :1 v/v acetone : $H_2O$ was cooled to 0 - 5 °C, and then *N*-iodosuccinimide (18.5 mg, 82 µmol, 1.15 eq) was charged. After 2 hours, the reaction was quenched with 200 µL saturated aqueous $NaHS_2O_3$, concentrated, and co-evaporated with *i*-PrOH. The residue was purified by column chromatography (17.5 g silica, 10% MeOH in $CH_2Cl_2$ then 12.5% MeOH in EtOAc) to afford lactol **SI-14** (23 mg, 41.9 µmol, 58% yield) as a white solid.

$^{1}$H NMR (400 MHz, MeOH-$d_4$, 301 K, δ): 5.13 (d, *J* = 3.3 Hz; H-1α), 4.59 (d, *J* = 8.4 Hz; H-1β), 4.44 (dd, *J* = 9.1, 5.1 Hz, 1H), 4.37 (q, *J* = 6.8 Hz; lactoyl C*H*), 4.36 (q, *J* = 7.2 Hz; 1H), 4.21 to 4.07 (m, 4H), 3.90 (dd, *J* = 10.4, 3.3 Hz; H-2α), 3.86 (dd, *J* = 12.2, 2.1 Hz; H-6aβ), 3.81 (ddd; *J* = 9.9, 5.1, 2.2 Hz; H-5α), 3.79 (dd, *J* = 11.7, 2.2 Hz; H-6aα), 3.73 (dd, *J* = 11.7, 5.1 Hz; H-6bα), 3.71 (m; H-2β), 3.70 (dd, *J* = 12.2, 5.1 Hz; H-6bβ), 3.66 (dd, *J* = 10.3, 8.8 Hz; H-3α), 3.49 (t, *J* = 9.3 Hz; H-4α), 3.46 (m; H-3β), 3.45 (m; H-4β), 3.32 (m; H-5β), 2.42 (t, *J* = 7.3 Hz, 2H), 2.19 (m, 1H), 1.97 & 1.956 (s & s, 3H), 1.963 (m, 1H) 1.40 (m, 6H), 1.25 (m, 6H) (*note*: residual EtOAc is present; the $HD_2COD$ peak obscures the C-5β peak at 3.32 ppm).

$^{13}$C NMR (101 MHz, MeOH-$d_4$, 301 K, δ): 176.0, 175.8, 175.1, 175.0, 174.3, 174.2, 173.4, 173.1, 172.9, 97.2 (C-1β), 92.4 (C-1α), 83.0 (C-3β), 80.4 (C-3α), 78.3 (lactoyl *C*Hβ), 78.2 (lactoyl *C*Hα), 77.8 (C-5β), 73.2 (C-5α), 71.5 (C-4α), 71.3 (C-4β), 62.56 (C-6β), 62.53 (C-6α), 61.75, 61.72, 61.5, 57.9 (C-2β), 55.4 (C-2α), 53.0, 50.3, 31.2, 27.6 27.5, 23.2, 22.9, 19.7, 19.6, 18.4, 18.3, 14.49, 14.45 (*note*: the peaks at 77.8 (C-5β) & 57.9 ppm (C-2β) are not clearly visible and are observed indirectly by HSQC correlation).

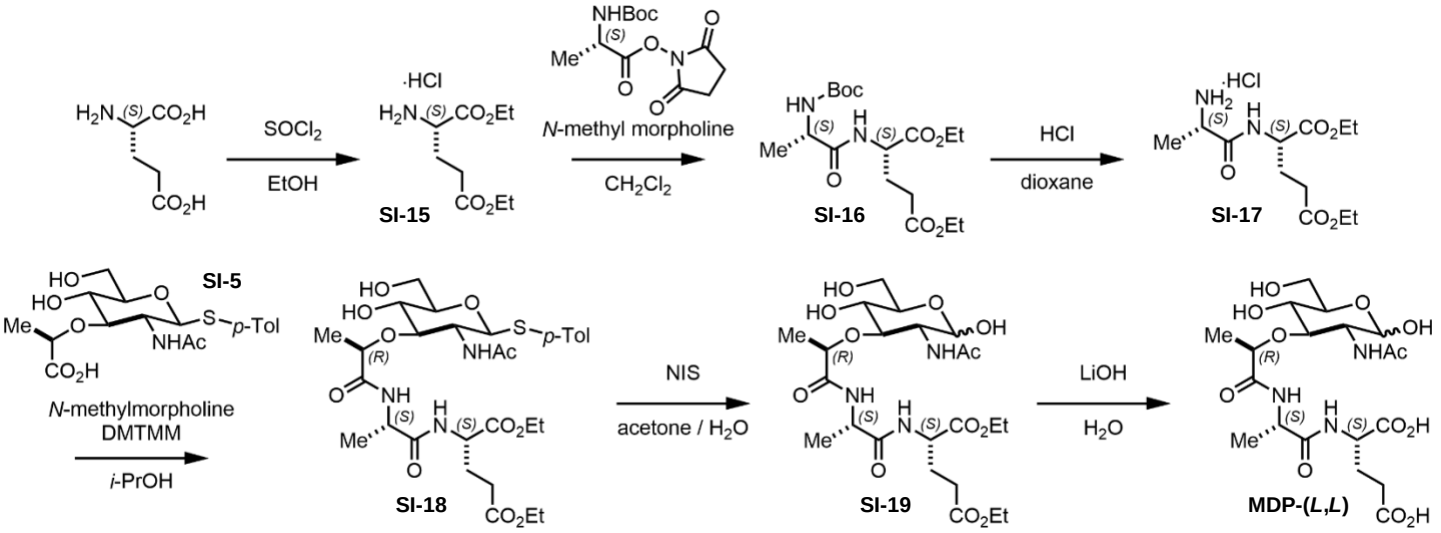
HRMS (ESI-TOF, m/z): calc'd for $C_{23}H_{40}N_3O_{12}^+$ ($[M + H]^+$) 550.2607; found 550.2592.

To a rbf charged with **SI-14** (23 mg, 41.9 µmol, 1.0 eq) was charged 419 µL $H_2O$. The mixture was cooled to 0 - 5 °C, 0.5 M LiOH (335 µL, 168 µmol, 4.0 eq) was charged, and the mixture was swirled to homogenize. After 2 hours, the mixture was neutralized with Amberlite IR-120 $H^+$, filtered, and concentrated. Purification of the crude material by preparative HPLC (0 to 20% ACN in 0.1% TFA) afforded **MDP(*L*,*D*)** (4.9 mg, 9.9 µmol, 24% yield) as a white solid.

$^{1}$H NMR (400 MHz, $D_2O$, 301 K, δ): 5.16 (d, *J* = 3.4 Hz; H-1α), 4.66 (H-1β), 4.45 (dd, *J* = 9.2, 4.7 Hz, 1H), 4.293 (q, *J* = 7.1 Hz, 1H) 4.292 (q, *J* = 6.8 Hz; lactoyl C*H*α), 4.22 (q, *J* = 6.8 Hz; lactoyl CHβ), 3.95 (dd, *J* = 10.4, 3.4 Hz; H-2β), 3.90 (dd, *J* = 12.5, 1.9 Hz; H-6aβ), 3.87 (m; H-5α), 3.84 (dd, *J* = 12.1, 1.9 Hz; H-6aα), 3.79 (dd; *J* = 12.1, 4.7 Hz; H-6bα), 3.78 (m; H-2β), 3.75 (dd, *J* = 12.5, 5.4 Hz; H-6bβ), 3.69 (t; *J* = 9.6 Hz; H-3α), 3.57 (t, *J* = 9.5 Hz; H-4α), 3.52 (dd, *J* = 11.2, 10.1 Hz; H-4β), 3.50 (m; H-3β), 3.46 (m; H-5β), 2.46 (t, *J* = 7.0 Hz, 2H), 2.23 (m, 1H), 2.00 (m, 1H), 1.97 & 1.96 (s & s, 3H), 1.42 (d, *J* = 7.1 Hz, 3H), 1.37 (d, *J* = 6.8 Hz, 3H).

$^{13}$C NMR (101 MHz, $D_2O$, 301 K, δ): 177.68, 177.66, 176.4, 176.1, 175.6, 175.5, 174.9, 174.7, 95.6 (C-1β), 91.6 (C-1α), 83.1 (C-3β), 80.2 (C-3α), 78.7 (lactoyl *C*Hβ), 78.4 (lactoyl *C*Hα), 76.4 (C-5β), 72.2 (C-5α), 69.7 (C-4α), 69.5 (C-4β), 61.4 (C-6β), 61.2 (C-6α), 56.8 (C-2β), 54.3 (C-2α), 52.7, 50.3, 50.2, 30.6, 26.5, 22.9, 22.6, 19.2, 17.42, 17.41.

HRMS (ESI-TOF, m/z): calc'd for $C_{19}H_{32}N_3O_{12}^+$ ([M + H]$^+$) 494.1980; found 494.1970.




To a stirred 0 - 5 °C suspension of (*S*)-glutamic acid (2.21 g, 15.0 mmol, 1.0 eq) in 40 mL EtOH was carefully charged 8.0 mL thionyl chloride in portions over 5 minutes to obtain a clear solution. The mixture was then heated in a 40 °C bath for 21 hours and then cooled to room temperature. The mixture was concentrated and co-evaporated with ethanol and then ACN. The residue the lyophilized from ACN / $H_2O$ to afford hydrochloride salt **SI-15** (3.55 g, 14.8 mmol, quantitative yield) as a white solid.

$^{1}$H NMR (400 MHz, DMSO-$d_6$, 301 K, δ): 8.75 (br. s, 3H, 4.18 (q, *J* = 7.1 Hz, 2H), 4.06 (q, *J* = 7.1 Hz, 2H), 3.99 (t, *J* = 6.4 Hz, 1H), 2.55 (m, 1H), 2.46 (m, 1H), 2.06 (q, *J* = 7.1 Hz, 2H), 1.22 (t, *J* = 7.1 Hz, 3H), 1.18 (t, *J* = 7.1 Hz, 3H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$, 301 K, δ): 171.7, 169.0, 61.8, 60.2, 51.1, 29.1, 25.1, 14.1, 13.9.

HRMS (ESI-TOF, m/z): calc'd for $C_9H_{18}NO_4^+$ ([M + H]$^+$) 204.1230; found 204.1228.

To a stirred solution of hydrochloride salt **SI-15** (1.36 g, 5.67 mmol, 1.0 eq) and Boc-(*S*)-Ala-OSu (2.13 g, 7.94 mmol, 1.4 eq) in 30 mL $CH_2Cl_2$ was charged *N*-methylmorpholine (940 µL, 8.51 mmol, 1.5 eq). After 3 hours, the mixture was diluted with EtOAc, washed with 10% NaCl + 2% citric acid, washed with brine, dried ($Na_2SO_4$), and concentrated. The residue was purified by column chromatography (20 g silica, 1:1 v/v EtOAc : hexane) to afford dipeptide **SI-16** (1.85 g, 4.94 mmol, 87% yield).

$^{1}$H NMR (400 MHz, $CDCl_3$, 301 K, δ): 6.93 (br. d, *J* = 5.9 Hz, 1H), 5.22 (br. s, 1H), 4.52 (dt, *J* = 5.1, 8.1 Hz, 1H), 4.14 (m, 1H), 4.13 (q, *J* = 7.1 Hz, 2H), 4.06 (q, *J* = 7.1 Hz, 2H), 2.32 (m, 2H), 2.15 (m, 1H), 1.93 (m, 1H), 1.38 (s, 9H), 1.30 (d, *J* = 7.1 Hz, 3H), 1.22 (q, *J* = 7.1 Hz, 3H), 1.18 (q, *J* = 7.1 Hz, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$, 301 K, δ): 172.8, 171.6, 155.5, 79.9, 61.6, 60.7, 51.6, 50.1, 30.2, 28.3, 27.3, 18.3, 14.1, 14.1 (*note*: based on HMBC correlations, there are two overlapping peaks at 172.8 ppm).

HRMS (ESI-TOF, m/z): calc'd for $C_{17}H_{31}N_2O_7^+$ $[M + H]^+$ 375.2126; found 375.2123.

The rbf containing 1.85 g **SI-16** (4.94 mmol, 1.0 eq) was cooled in an ice/water bath and 4 M HCl in 1,4-dioxane (12.4 mL, 49.4 mmol, 10.0 eq) was charged. The mixture was swirled to obtain a clear solution. After 3 hours, the mixture was concentrated and co-evaporated with 1,4-dioxane. The residue was suspended in 20 mL 1 : 1 v/v ACN : $H_2O$ and minor white debris was filtered off ($H_2O$ rinse). The filtrate was lyophilized to afford hydrochloride salt **SI-17** (1.43 g, 4.60 mmol, 93% yield) as an oil.

$^{1}$H NMR (400 MHz, DMSO-$d_6$, 301 K, δ): 8.99 (d, *J* = 7.2 Hz, 1H), 8.32 (s, 3H), 4.29 (q, *J* = 6.8 Hz, 1H), 4.06 (m, 4H), 3.90 (m, 1H), 2.42 (t, *J* = 7.3 Hz, 2H), 2.01 (m, 1H), 1.86 (m, 1H), 1.37 (d, *J* = 6.8 Hz, 3H), 1.17 (q, *J* = 7.0 Hz, 6H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$, 301 K, δ): 172.1, 171.2, 169.9, 60.9, 60.1, 51.5, 48.0, 29.9, 25.9, 17.1, 14.2, 14.0.

HRMS (ESI-TOF, m/z): calc'd for $C_{12}H_{23}N_2O_5$ $[M + H]^+$ 275.1601; found 275.1597.

To a stirred solution of **SI-5** (200 mg, 0.50 mmol, 1.0 eq), **SI-17** (272 mg, 0.70 mmol, 1.4 eq), and *N*-methylmorpholine (88 µL, 0.80 mmol, 1.6 eq) in 5 mL *i*-PrOH was charged DMTMM (194 mg, 0.70 mmol, 1.4 eq) (*note*: becomes a white slurry as the reaction proceeds). After 14 hours, the mixture was concentrated to ~3 mL pot volume, cooled to 0 - 5 °C, and filtered. The solids were rinsed with 1 mL ice-cold *i*-PrOH and dried to afford amide **SI-18** (218 mg, 0.33 mol, 67% yield) as a white solid.

$^{1}$H NMR (400 MHz, MeOH-$d_4$, 301 K, δ): 8.36 (d, *J* = 7.9 Hz, 1H), 7.72 (d, *J* = 6.5 Hz, 1H), 7.39 (d, *J* = 8.1 Hz, 2H), 7.11 (d, *J* = 8.1 Hz, 2H), 4.69 (d, *J* = 10.4 Hz, 1H), 4.45 (m, 1H), 4.33 (m, 1H), 4.26 (q, *J* = 6.7 Hz, 1H), 4.14 (q, *J* = 7.1 Hz, 2H), 4.11 (q, *J* = 7.1 Hz, 2H), 3.90 (m, 1H), 3.87 (dd, *J* = 2.2, 12.1 Hz, 1H), 3.70 (dd, *J* = 5.6, 12.1 Hz, 1H), 3.47 (m, 1H), 3.33 (dd, *J* = 2.0, 5.6 Hz, 1H), 2.42 (d, *J* = 7.5 Hz, 2H), 2.30 (s, 3H), 2.17 (m, 1H), 1.96 (s, 3H), 1.95 (m, 1H), 1.41 (d, *J* = 7.1 Hz, 3H), 1.37 (d, *J* = 6.5 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.23 (t, *J* = 7.1 Hz, 3H).

$^{13}$C NMR (101 MHz, MeOH-$d_4$, 301 K, δ): 175.6, 175.2, 174.2, 173.4, 173.0, 138.6, 132.8, 132.0, 130.6, 89.1, 84.9, 82.0, 78.8, 70.9, 62.8, 62.5, 61.7, 55.4, 53.1, 50.3, 31.2, 27.7, 23.2, 21.1, 19.5, 18.1, 14.5, 14.4.

HRMS (ESI-TOF, m/z): calc'd for $C_{30}H_{46}N_3O_{11}S$ $[M + H]^+$ 656.2848; found 656.2833.

A stirred 50 mM solution of **SI-18** (62 mg, 95 µmol, 1.0 eq) in 3 : 1 v/v acetone : $H_2O$ was cooled to 0 - 5 °C and then *N*-iodosuccinimide (27 mg, 109 µmol, 1.15 eq) was charged. After 2 hours, the mixture was quenched with 300 µL $NaHS_2O_3$, concentrated and co-evaporated with *i*-PrOH. The crude residue was purified by column chromatography (17.5 g silica, 10% MeOH in $CH_2Cl_2$ then 12.5% MeOH in EtOAc) to afford lactol **SI-19** (31 mg, 56 µmol, 59% yield) as an oil.

$^{1}$H NMR (400 MHz, MeOH-$d_4$, 301 K, δ): 5.12 (d, *J* = 3.3 Hz; H-1α), 4.62 (dd, *J* = 1.8, 8.3 Hz; H-1β), 4.45 (dd, *J* = 5.1, 9.0 Hz, 1H), 4.37 (q, *J* = 7.2 Hz, 1H), 4.34 (q, *J* = 6.8 Hz; lactoyl C*H*), 4.17 (q, *J* = 7.1 Hz, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.92 (dd, *J* = 3.4, 10.5 Hz; H-2α), 3.87 (dd, *J* = 12.1, 2.2 Hz; H-6aβ), 3.82 (ddd, *J* = 9.9, 5.0, 2.2 Hz; H-5α), 3.80 (dd, *J* = 2.2, 10.4 Hz; H-6aα), 3.73 (dd, *J* = 11.8, 5.0 Hz; H-6bα), 3.72 (m; H-2β), 3.72 (m; H-6bβ), 3.66 (dd, *J* = 10.3, 9.0 Hz; H-3α), 3.49 (t, *J* = 9.3 Hz; H-4α), 3.48 (m; H-3β), 3.46 (t, *J* = 9.0 Hz; H-4β), 3.34 (m; H-5β), 2.44 (t, *J* = 7.4 Hz, 2H), 2.18 (m, 1H), 1.97 & 1.956 (s & s, 3H), 1.962 (m, 1H), 1.40 (d, *J* = 7.2 Hz, 3H), 1.39 (d, *J* = 6.8 Hz, 3H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H) (*note*: methanol is present as a contaminant and obscures the H-5β peak at 3.34 ppm).

$^{13}$C NMR (101 MHz, MeOH-$d_4$, 301 K, δ): 175.9, 175.0, 174.4, 173.4, 172.9, 97.2 (C-1β), 92.5 (C-1α), 82.9 (C-3β), 80.6 (C-3α), 78.3 (lactoyl *C*Hα), 77.7 (C-5β), 73.1 (C-5α), 71.4, 62.5 (C-6α), 61.7, 61.6, 57.9 (C-2β), 55.2 (C-2α), 53.0, 50.1, 31.2, 27.5, 22.9, 19.6, 18.2, 14.5, 14.4 (*note*: the peaks at 97.2 (C-1β), 82.9 (C-3β), 77.7 (C-5β), and 57.9 (C-2β) are not clearly visible and are detected indirectly through HSQC correlation; the peaks of C-6β and lactoyl *C*Hβ are presumed to overlap with the corresponding α anomer peaks and are not assigned).

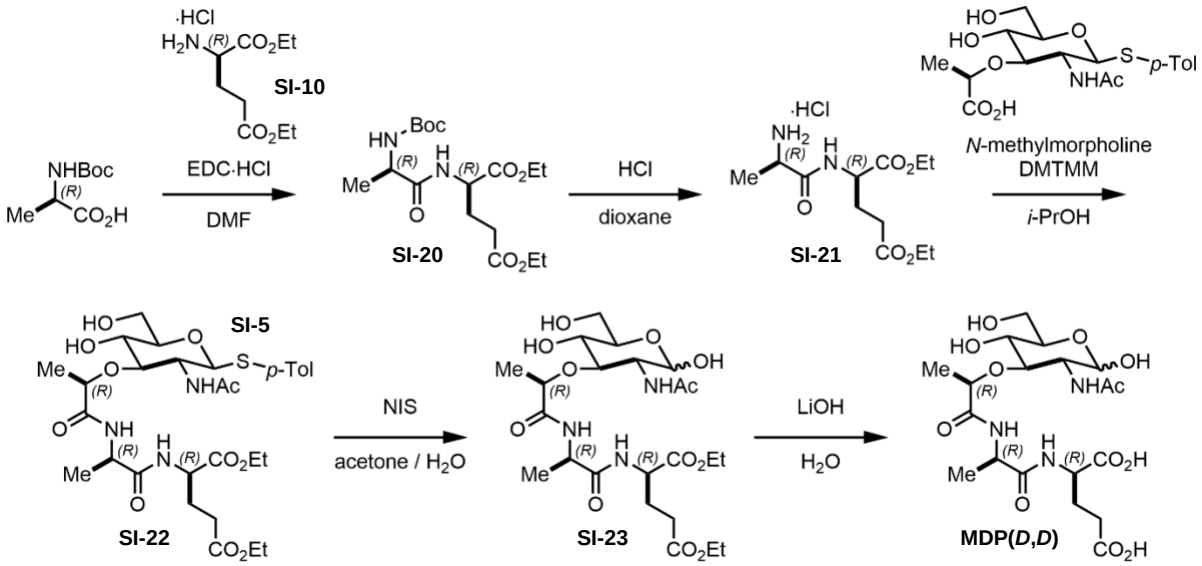
HRMS (ESI-TOF, m/z): calc'd for $C_{23}H_{40}N_3O_{12}^+$ ([M + H]$^+$) 550.2607; found 550.2596.

To a rbf charged with **SI-19** (31 mg, 56 µmol, 1.0 eq) was charged 560 µL $H_2O$ and the mixture was cooled to 0 - 5 °C. 0.5 M LiOH (448 µL, 224 µmol, 4.0 eq) was charged, and the mixture was swirled to homogenize. After 2 hours, the mixture was neutralized with Amberlite IR-120 $H^+$, filtered, and concentrated. The crude residue was purified by preparative HPLC (0 to 20% ACN in 0.1% TFA) to afford **MDP(*L*,*L*)** (4.5 mg, 9.1 µmol, 16% yield) as a white solid.

$^1$H NMR (400 MHz, $D_2O$, 301 K, δ): 5.15 (d, *J* = 3.4 Hz; H-1α), 4.67 (d, *J* = 8.4 Hz; H-1β), 4.43 (dd, *J* = 5.1, 9.1 Hz, 1H), 4.277 (q, *J* = 7.2 Hz, 1H), 4.275 (q, *J* = 6.8 Hz; lactoyl C*H*α), 4.22 (q, *J* = 6.8 Hz; lactoyl C*H*β), 3.95 (dd, *J* = 10.4, 3.4 Hz; H-2α), 3.90 (dd, *J* = 12.4, 2.0 Hz; H-6aβ), 3.87 (m; H-5α), 3.84 (dd, *J* = 12.5, 2.1 Hz; H-6aα), 3.79 (dd, *J* = 12.5, 5.0 Hz; H-6bα), 3.78 (m; H-2β), 3.75 (dd, *J* = 12.2, 5.2 Hz; H-6bβ), 3.69 (dd, *J* = 10.0, 9.0 Hz; H-3α), 3.57 (t, *J* = 9.6 Hz; H-4α), 3.52 (t. *J* = 10.6 Hz; H-4β), 3.49 (m; H-3β), 3.47 (m; H-5β), 2.50 (t, *J* = 7.2 Hz, 2H), 2.22 (m, 1H), 2.01 (m, 1H), 1.972 & 1.967 (s & s, 3H), 1.41 (d, *J* = 7.2 Hz, 3H), 1.37 (d, *J* = 6.8 Hz, 3H).

$^{13}$C NMR (101 MHz, $D_2O$, 301 K, δ): 177.72, 177.71, 176.4, 176.2, 175.6, 175.5, 174.9, 174.6, 95.6 (C-1β), 91.6 (C-1α), 83.1 (C-3β), 80.3 (C-3α), 78.8 (lactoyl *C*Hβ), 78.5 (lactoyl *C*Hα), 76.4 (C-5β), 72.2 (C-5α), 69.6 (C-4α), 69.5 (C-4β), 61.4 (C-6β), 61.2 (C-6α), 56.8 (C-2β), 54.3 (C-2α), 52.7, 50.1, 30.6, 26.5, 22.9, 22.6, 19.3, 17.2.

HRMS (ESI-TOF, m/z): calc'd for $C_{19}H_{32}N_3O_{12}^+$ ([M + H]$^+$) 494.1980; found 494.1972.




To a stirred solution of Boc-(*R*)-Ala-OH (1.57 g, 8.30 mmol, 1.0 eq), hydrochloride salt **SI-10** (2.47 g, 10.3 mmol, 1.25 eq), and *N*-methylmorpholine (1.28 mL, 11.6 mmol, 1.4 eq) in 40 mL DMF was charged EDC·HCl (2.23 g, 11.6 mmol, 1.4 eq). After 3 hours, the mixture was diluted with EtOAc, washed with 10% NaCl + 2% citric acid, washed with brine, dried ($Na_2SO_4$), filtered, and concentrated. The residue was purified by column chromatography (40 g silica, 45 to 50% v/v EtOAc in hexane) to afford dipeptide **SI-20** (1.32 g, 3.51 mmol, 42%) as an oil.

$^1$H NMR (400 MHz, $CDCl_3$, 301 K, δ): 6.87 (br. s, *J* = 6.0 Hz, 1H), 5.14 (br. m, 1H), 4.55 (dt, *J* = 5.1, 8.0 Hz, 1H), 4.15 (m, 1H), 4.15 (q, *J* = 7.1 Hz, 2H), 4.08 (q, *J* = 7.1 Hz, 2H), 2.34 (m, 2H), 2.16 (m, 1H), 1.95 (m, 1H), 1.40 (s, 9H), 1.32 (d, *J* = 7.0 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.20 (t, *J* = 7.1 Hz, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$, 301 K, δ): 172.8, 172.7, 171.6, 155.5, 80.1, 61.7, 60.7, 51.7, 50.1, 30.3, 28.3, 27.4, 18.3, 14.21, 14.17.

HRMS (ESI-TOF, m/z): calc'd 375.2126 for $C_{17}H_{31}N_2O_7$ ($[M + H]^+$); found 375.2130.

The rbf containing **SI-20** (1.32 g, 3.51 mmol, 1.0 eq) was cooled in an ice/water bath and 4 M HCl in 1,4-dioxane (8.8 mL, 35.1 mmol, 10.0 eq) was charged. The mixture was swirled to obtain a clear solution. After 3 hours, the mixture was concentrated and co-evaporated with 1,4-dioxane. The residue was dissolved in 15 mL 1 : 1 v/v ACN : $H_2O$ and lyophilized to afford hydrochloride salt **SI-21** (695 mg, 2.24 mmol, 64% yield) as an oil.

$^1$H NMR (400 MHz, DMSO-$d_6$, 301 K, δ): 9.05 (d, *J* = 7.2 Hz 1H), 8.37 (br. s, 3H), 4.27 (m, 1H), 4.05 (m, 4H), 3.91 (q, *J* = 6.8 Hz, 1H), 2.42 (t, *J* = 7.5 Hz, 2H), 2.00 (m, 1H), 1.86 (m, 1H), 1.37 (d, *J* = 6.8 Hz, 3H), 1.16 (t, *J* = 7.1 Hz, 6H).

$^{13}$C NMR (101 MHz, DMSO-$d_6$, 301 K, δ): 172.1, 171.2, 169.9, 60.8, 60.0, 51.5, 48.0, 29.9, 25.8, 17.1, 14.1, 14.0.

HRMS (ESI-TOF, m/z): calc'd for $C_{12}H_{23}N_2O_5$ $[M + H]^+$ 275.1601; found 275.1599.

To a stirred solution of carboxylic acid **SI-5** (200 mg, 0.50 mmol, 1.0 eq), hydrochloride salt **SI-21** (272 mg, 0.70 mmol, 1.4 eq), and *N*-methylmorpholine (88 µL, 0.80 mmol, 1.6 eq) in 5 mL *i*-PrOH was charged DMTMM (194 mg, 0.70 mmol, 1.4 eq) (*note*: reaction becomes a thick white slurry as the reaction proceeds). After 14 hours, the mixture was concentrated to ~3 mL pot volume, cooled to 0 - 5 °C, and filtered. The solids were rinsed with 1 mL ice-cold *i*-PrOH and dried to afford amide **SI-22** (242 mg, 0.37 mol, 74% yield) as a white solid.

$^{1}$H NMR (400 MHz, MeOH-$d_4$, 301 K, δ): 7.41 (d, *J* = 8.1 Hz, 2H), 7.12 (d, *J* = 8.1 Hz, 2H), 4.91 (m, 1H), 4.48 (dd, *J* = 5.0, 9.3 Hz, 1H), 4.39 (q, *J* = 7.2 Hz, 1H), 4.19 (q, *J* = 7.1 Hz, 3H), 4.12 (dq, *J* = 7.1, 1.6 Hz, 2H), 3.87 (dd, *J* = 2.0, 12.0 Hz, 1H), 3.68 (dd, *J* = 5.6, 12.0 Hz, 1H), 3.67 (m, 1H), 3.42 (t, *J* = 9.0 Hz, 1H), 3.33 (dd, *J* = 2.0, 5.6 Hz, 1H), 3.30 (m, 1H), 2.46 (m, 2H), 2.31 (s, 3H), 2.21 (m, 1H), 1.99 (m, 1H), 1.95 (s, 3H), 1.39 (d, *J* = 7.1 Hz, 3H), 1.36 (d, *J* = 6.7 Hz, 3H), 1.28 (t, *J* = 7.1 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H).

$^{13}$C NMR (101 MHz, MeOH-$d_4$, 301 K, δ): 176.0, 175.3, 174.3, 173.8, 173.0, 138.9, 133.6, 131.1, 130.6, 87.7, 84.5, 82.0, 78.6, 70.8, 62.8, 62.5, 61.7, 56.3, 53.2, 49.9, 31.3, 27.5, 23.4, 21.1, 19.9, 18.2, 14.53, 14.49.

HRMS (ESI-TOF, m/z): calc'd for $C_{30}H_{46}N_3O_{11}S$ $[M + H]^+$ 656.2848; found 656.2829.

A stirred 50 mM solution of thioglycoside **SI-22** (76 mg, 116 µmol, 1.0 eq) in 3 : 1 v/v acetone : $H_2O$ was cooled to 0 - 5 °C and then *N*-iodosuccinimide (30 mg, 133 µmol, 1.15 eq) was charged. After 3 hours, the mixture was quenched with 300 µL $NaHS_2O_3$, concentrated, and co-evaporated with *i*-PrOH. The crude residue was purified by column chromatography (15 g silica, 10% MeOH in $CH_2Cl_2$ followed by 12.5% MeOH in EtOAc) to afford lactol **SI-23** (29 mg, 53 µmol, 45% yield) as a white solid.

$^{1}$H NMR (400 MHz, MeOH-$d_4$, 301 K, δ): 5.16 (d, *J* = 3.3 Hz; H-1α) 4.71 (d, *J* = 8.1 Hz; H-1β), 4.48 (m; lactoyl C*H*α), 4.47 (m, 1H), 4.43 (m, 1H), 4.34 (q, *J* = 6.8 Hz; lactoyl C*H*β), 4.21 to 4.07 (m, 4H), 3.89 (dd, *J* = 10.5, 3.3 Hz; H-2β), 3.86 (dd, *J* = 12.0, 2.1 Hz; H-6aβ), 3.79 (m; H-5α), 3.78 (dd, *J* = 11.7, 2.1 Hz; H-6aα), 3.72 (dd, *J* = 11.7, 5.1 Hz; H-6bα), 3.69 (dd, *J* = 11.7, 5.1 Hz; H-6bβ), 3.67 (m; H-2β), 3.66 (dd, *J* = 10.0, 9.0 Hz; H-3α), 3.59 (dd, *J* = 9.9, 8.7 Hz; H-3β), 3.52 (t, *J* = 9.2 Hz; H-4α), 3.47 (t, *J* = 9.2 Hz; H-4β), 3.34 (m; H-5β), 2.46 (t, *J* = 7.3 Hz, 2H), 2.20 (m, 1H), 1.987 (m, 1H), 1.985 & 1.97 (s & s, 3H), 1.40 (m, 6H), 1.26 (m, 6H) (*note*: methanol is present as a contaminant and obscures the H-5β peak at 3.34 ppm).

$^{13}$C NMR (101 MHz, MeOH-$d_4$, 301 K, δ): 176.3, 176.0, 175.2, 175.1, 174.4, 173.6, 173.0, 172.98, 96.7 (C-1β), 92.3 (C-1α), 82.9 (C-3β), 79.7 (C-3α), 77.8 (lactoyl *C*Hβ), 77.7 (C-5β), 77.5 (lactoyl *C*Hα), 73.2 (C-5α), 71.8 (C-4α), 71.1 (C-4β), 62.52, 62.50 (C-6β), 62.4 (C-6α), 61.7, 57.9 (C-2β), 55.0 (C-2α), 53.1, 49.9, 31.3, 27.4, 23.3, 23.0, 19.90, 19.86, 18.4, 14.5, 14.4.

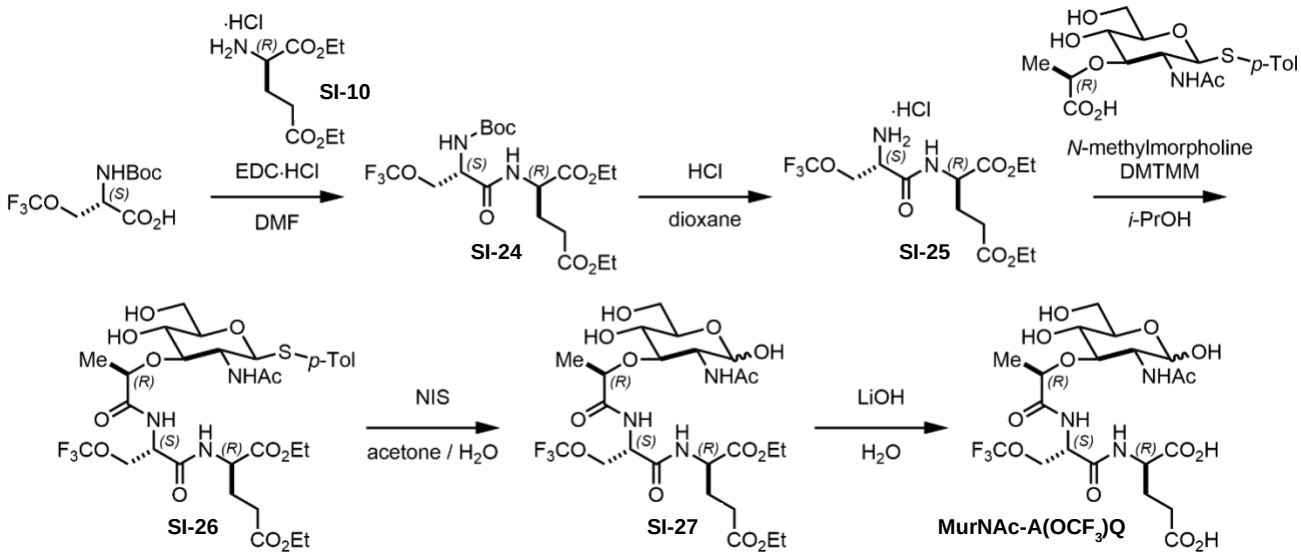
HRMS (ESI-TOF, m/z): calc'd for $C_{23}H_{40}N_3O_{12}^+$ ($[M + H]^+$) 550.2607; found 550.2598.

To a rbf charged with **SI-23** (29 mg, 53 µmol, 1.0 eq) was charged 530 µL $H_2O$. The mixture was cooled to 0 - 5 °C, 0.5 M LiOH (424 µL, 212 µmol, 4.0 eq) was charged, and the mixture was swirled to homogenize. After 1 hour, the mixture was neutralized with Amberlite IR-120 $H^+$, filtered, and concentrated. The crude residue was purified by preparative HPLC (0 to 20% ACN in 0.1% TFA) to afford **MDP(*D*,*D*)** (4.1 mg, 8.3 µmol, 16% yield) as a white solid.

$^{1}$H NMR (400 MHz, $D_2O$, 301 K, δ): 5.20 (d, *J* = 3.4 Hz, H-1α), 4.75 (m; H-1β), 4.44 (dd, *J* = 5.0, 9.2 Hz, 1H), 4.41 (q, *J* = 6.8 Hz; lactoyl C*H*α), 4.35 (q, *J* = 7.1 Hz, 1H), 4.28 (q, *J* = 6.8 Hz; lactoyl C*H*β), 3.91 (dd, *J* = 10.5, 3.4 Hz; H-2α), 3.90 (dd, *J* = 12.4, 2.4 Hz; H-6aβ), 3.85 (ddd, *J* = 9.9, 5.0, 2.1 Hz; H-5α), 3.83 (dd, *J* = 12.3, 2.1 Hz; H-6aα), 3.77 (dd, *J* = 12.3, 5.0 Hz; H-6bα), 3.74 (dd, *J* = 12.3, 5.4 Hz; H-6bβ), 3.69 (dd, *J* = 10.1, 9.0 Hz; H-3α), 3.68 (dd, *J* = 9.6, 8.3 Hz; H-2β), 3.59 (dd, *J* = 9.8, 8.3 Hz; H-3β), 3.58 (dd, *J* = 9.8, 9.0 Hz; H-4α), 3.52 (dd, *J* = 9.9, 8.4 Hz; H-4β), 3.47 (m, H-5β), 2.52 (t, *J* = 7.3 Hz, 1H), 2.23 (m, 1H), 2.04 (m, 1H), 2.01 & 1.99 (s & s, 3H), 1.41 (d, *J* = 7.1 Hz, 3H), 1.37 (d, *J* = 6.8 Hz, 3H) (*note*: the HDO peak obscures the C-1β peak at 4.75 ppm).

$^{13}$C NMR (101 MHz, $D_2O$, 301 K, δ): 177.7, 176.2, 176.0, 175.6, 175.4, 175.3, 175.1, 174.9, 95.2 (C-1β), 91.4 (C-1α), 82.2 (C-3β), 79.1 (C-3α), 77.6 (lactoyl *C*Hβ), 77.4 (lactoyl *C*Hα), 76.4 (C-5β), 72.2 (C-5α), 70.1 (C-4α), 69.7 (C-4β), 61.4 (C-6β), 61.1 (C-6α), 56.9 (C-2β), 54.1 (C-2α), 52.7, 50.0, 30.6, 26.4, 26.3, 23.0, 22.7, 19.22, 19.21, 17.3.

HRMS (ESI-TOF, m/z): calc'd for $C_{19}H_{32}N_3O_{12}^+$ ($[M + H]^+$) 494.1980; found 494.1971.




To a stirred solution of trifluoromethoxy-Boc-(*S*)-Ala-OH[3] (117 mg, 0.428 mmol, 1.0 eq), hydrochloride salt **SI-10** (185 mg, 0.771 mg, 1.8 eq), oxyma (30 mg, 0.214 mmol, 0.5 eq), and *N*-methylmorpholine (94 µL, 0.856 mmol, 2.0 eq) in 4.3 mL DMF was charged EDC·HCl (115 mg, 0.599 mmol, 1.4 eq). After 14.5 hours, the mixture was diluted with EtOAc, washed with pH 2 buffer, washed successively with pH 10 buffer (ensuring complete removal of oxyma by TLC), and washed with brine. The organic extract was dried ($Na_2SO_4$), filtered, and concentrated. The crude product was purified by column chromatography (10 g silica, 20 to 30% v/v EtOAc in hexane) to afford dipeptide **SI-24** (131 mg, 0.286 mmol, 67% yield) as an oil.

$^1$H NMR (400 MHz, $CDCl_3$, 301 K, δ): 7.12 (m, 1H), 5.38 (m, 1H), 4.58 (dd, *J* = 7.8, 5.0 Hz, 1H), 4.47 (m, 1H), 4.34 (m, 1H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.14 (dd, *J* = 9.9, 3.0 Hz, 1H), 4.11 (q, *J* = 7.1 Hz, 2H), 2.36 (m, 2H), 2.20 (m, 1H), 1.99 (m, 1H), 1.44 (s, 9H), 1.24 (m, 6H).

$^{13}$C NMR (101 MHz, $CDCl_3$, 301 K, δ): 172.9, 171.5, 168.4, 155.4, 121.5, 81.1, 66.5, 61.9, 60.9, 53.2, 52.0, 30.1, 28.3, 27.2, 14.2, 14.1.

HRMS (ESI-TOF, m/z): calc'd for$C_{18}H_{30}F_3N_2O_8^+$ $[M + H]^+$ 459.1949; found 459.1951.

A rbf charged with **SI-24** (131 mg, 0.286 mmol, 1.0 eq) was cooled in an ice bath and then 4 M HCl in 1,4-dioxane (715 µL, 2.86 mmol, 10 eq) was charged. After 3 hours, the mixture was concentrated, co-evaporated with 1,4-dioxane, and co-evaporated with $CHCl_3$ to afford hydrochloride salt **SI-25** (123 mg; nominally 0.311 mmol, 109% yield) (*note*: note some 1,4-dioxane and an unidentified impurity are present as contaminants).

$^1$H NMR (400 MHz, $CDCl_3$, 301 K, δ): 8.88 (br s, 1H), 8.32 (br s, 1H), 5.02 (br s, 1H), 4.66 (br s, 1H), 4.59 (br s, 1H), 4.18 (m, 2H), 4.08 (m, 2H), 2.38 (br s, 2H), 2.19 (br s, 1H), 2.04 (br s, 1H), 1.22 (m, 6H) (*note*: most peaks have substantial peak broadening).

$^{13}$C NMR (101 MHz, $CDCl_3$, 301 K, δ): 172.7, 172.2, 166.0, 121.2 (q, *J* = 257 Hz), 65.9, 62.3, 60.7, 52.5, 52.3, 30.1, 26.8, 14.1, 14.0.

HRMS (ESI-TOF, m/z): calc'd for $C_{13}H_{22}F_3N_2O_6^+$ $[M + H]^+$ 359.1424; found 359.1420.

To a stirred solution of carboxylic acid **SI-5** (153 mg, 0.383 mmol, 1.0 eq), hydrochloride salt **SI-25** (143 mg, 0.460 mmol, 1.2 eq), and *N*-methylmorpholine (63 µL, 0.575 mmol, 1.5 eq) in 4 mL *i*-PrOH was charged DMTMM (149 mg, 0.537 mmol,

1.4 eq) (*note*: the initial clear solution becomes a white suspension). After 13.5 hours, the mixture was concentrated. 7 mL $H_2O$ was charged, and the mixture was stirred to obtain a mobile slurry. The mixture was cooled in an ice bath, filtered, and rinsed with 1 mL $H_2O$. The solids were dried to obtain amide **SI-26** (230 mg, 0.311 mmol, 81% yield) as a tan solid.

$^1$H NMR (400 MHz, MeOH-$d_4$, 301 K, δ): 7.39 (d, *J* = 8.0 Hz, 2H), 7.12 (d, *J* = 8.0 Hz, 2H), 4.71 (t, *J* = 5.5 Hz, 1H), 4.70 (d, *J* = 10.8 Hz, 1H), 4.48 (dd, *J* = 9.1, 4.9 Hz, 1H), 4.32 (m, 3H), 4.13 (m, 4H), 3.86 (m, 2H), 3.69 (dd, *J* = 12.1, 5.6 Hz, 1H), 3.48, (m, 2H), 2.41 (t, *J* = 7.4 Hz, 2H), 2.31 (s, 3H), 2.19 (m, 1H), 1.97 (m, 1H), 1.96 (s, 3H), 1.37 (d, *J* = 6.5 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H), 1.24 (t, *J* = 7.0 Hz, 3H).

$^{13}$C NMR (101 MHz, MeOH-$d_4$, 301 K, δ): 175.8, 174.2, 173.5, 172.7, 170.3, 138.7, 133.0, 131.9, 130.6, 123.0 (q, *J* = 254 Hz), 88.9, 84.5, 82.0, 78.3, 70.8, 67.4, 62.8, 62.6, 61.7, 55.5, 53.2, 53.0, 31.0, 27.5, 23.2, 21.1, 19.4, 14.5, 14.4.

HRMS (ESI-TOF, m/z): calc'd for $C_{31}H_{45}F_3N_3O_{12}S^+$ $[M + H]^+$ 740.2671; found 740.2658.

To as stirred suspension of thioglycoside **SI-26** (75 mg, 0.102 mmol, 1.0 eq) in 2.0 mL 3:1 v/v acetone : $H_2O$ was charged *N*-iodosuccinimide (26.3 mg, 0.117 mmol, 1.15 eq). After 40 minutes, the reaction was quenched with 250 µL saturated aqueous $NaHS_2O_3$ and aged overnight in a 4 °C refrigerator. The mixture was the concentrated and co-evaporated with *i*-PrOH. The crude material was purified by column chromatography (8 to 15% v/v MeOH in $CH_2Cl_2$) to afford lactol **SI-27** (23 mg, 36 µmol, 36% yield) as an oil which solidifies on standing.

$^1$H NMR (400 MHz, MeOH-$d_4$, 301 K, δ): 5.17 (d, *J* = 3.4 Hz; H-1α), 4.76 (t, *J* = 5.7 Hz, 1H), 4.57 (d, *J* = 8.1 Hz; H-1β), 4.49 (m, 1H), 4.48 (q, *J* = 6.7 Hz; lactoyl C*H*α), 4.41 (q, *J* = 6.7 Hz; lactoyl C*H*β), 4.31 (m, 2H), 4.18 (q, *J* = 7.1 Hz, 2H), 4.13 (q, *J* = 7.1 Hz, 2H), 3.86 (dd, *J* = 10.5, 3.4 Hz; H-2α), 3.83 (m; H-6aβ), 3.80 (ddd, *J* = 9.6, 5.0, 2.2 Hz; H-5α), 3.79 (dd; *J* = 11.6, 2.2 Hz; H-6aα), 3.71 (dd; *J* = 11.6, 5.0 Hz; H-6bα), 3.67 (m; H-6bβ), 3.662 (m; H-2β), 3.660 (dd, *J* = 10.1, 8.8 Hz; H-3α), 3.50 (t, *J* = 9.3 Hz; H-4α), 3.463 (m; H-4β), 3.458 (m; H-3β), 3.30 (m; H-5β), 2.42 (t, *J* = 7.3 Hz, 2H), 2.20 (m, 1H), 1.98 & 1.967 (s & s, 3H), 1.965 (m, 1H), 1.39 (q, *J* = 6.7 Hz, 3H), 1.27 (t, *J* = 7.1 Hz, 3H), 1.24 (t, *J* = 7.1 Hz, 3H) (*note*: the $HD_2COD$ peak obscures the H-5β peak at 3.30 ppm).

$^{13}$C NMR (101 MHz, MeOH-$d_4$, 301 K, δ): 176.6, 176.3, 174.5, 174.2, 174.2, 173.5, 172.7, 172.7, 170.2, 170.2, 131.2 (q, *J* = 256 Hz), 122.9 (q, *J* = 255 Hz), 97.3 (C-1β), 92.3 (C-1α), 82.7 (C-3β), 79.8 (C-3α), 77.9 (C-5β), 77.8 (lactoyl *C*Hβ), 77.7 (lactoyl *C*Hα), 73.2 (C-5α), 71.7 (C-4α), 71.3 (C-4β), 67.6, 67.5, 62.7 (C-6β), 62.63 (C-6α), 62.62, 61.7, 58.1 (C-2β), 55.4 (C-2α), 53.3, 53.2, 53.03, 53.00, 31.0, 27.5, 23.1, 22.9, 19.7, 19.6, 14.5, 14.4.

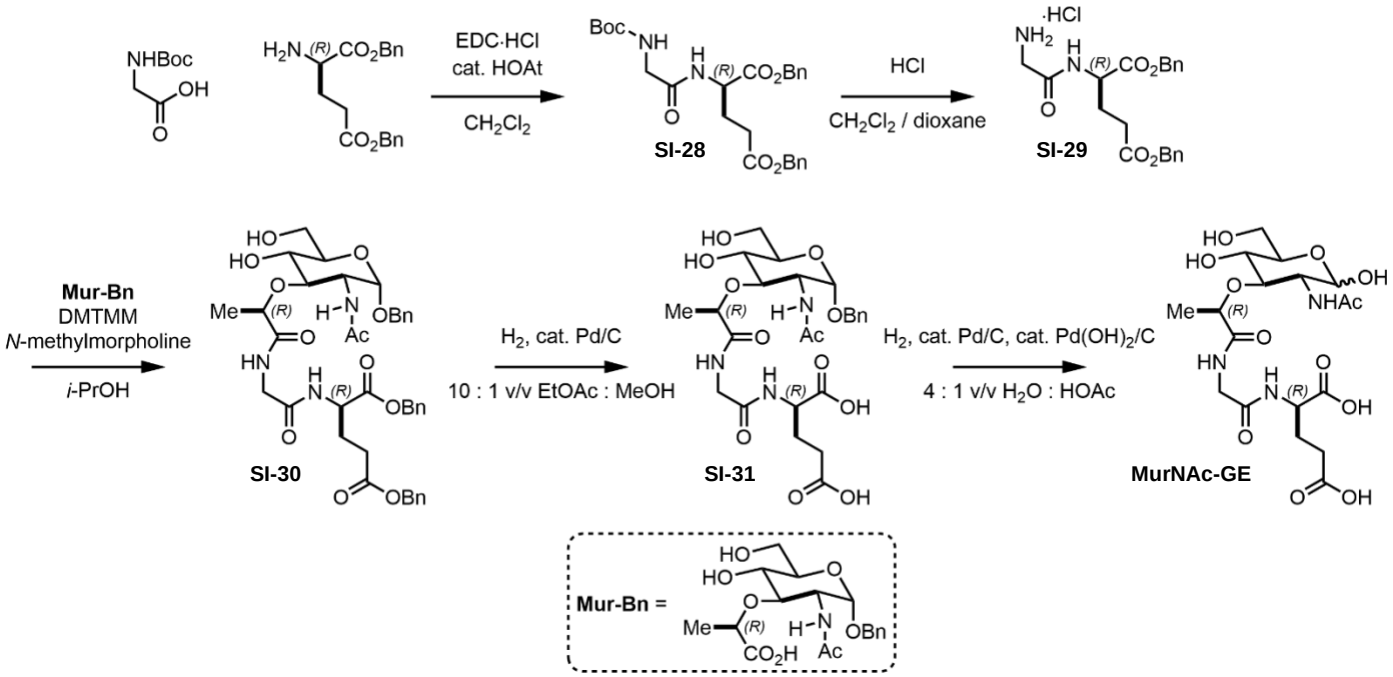
HRMS (ESI-TOF, m/z): calc'd for $C_{24}H_{39}F_3N_3O_{13}^+$ $[M + H]^+$ 634.2429; found 634.2412.

To a solution of **SI-27** (23 mg, 36 µmol, 1.0 eq) in 350 µL $H_2O$ was charged 0.5N aqueous LiOH (288 µL, 144 µmol, 4.0 eq). After 1 hour, the pH was adjusted to 3 with citric acid and the mixture was concentrated. Purification by preparative HPLC (0 to 25% ACN in 0.1% TFA) afforded **MurNAc-A(OCF$_3$)E** (4.6 mg, 8.0 µmol, 22% yield).

$^1$H NMR (400 MHz, $D_2O$, 301 K, δ): 5.18 (d, *J* = 3.4 Hz; H-1α), 4.75 (m, 1H), 4.67 (d, *J* = 8.4 Hz; H-1β), 4.51 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.39 (m, 3H), 3.93 (dd, *J* = 10.4, 3.4 Hz; H-2α), 3.90 (dd, *J* = 12.0, 1.9 Hz; H-6aβ), 3.86 (ddd, *J* = 9.9, 5.0, 2.1 Hz; H-5α), 3.83 (dd, *J* = 12.0, 2.1 Hz, H-6aα), 3.78 (dd, *J* = 12.1, 5.0 Hz; H-6bα), 3.77 (m; H-6bβ), 3.76 (m; H-2β), 3.72 (dd, *J* = 10.4, 9.3 Hz; H-3α), 3.58 (t, *J* = 9.4 Hz; H-4α), 3.54 (m; H-4β), 3.535 (m; H-3β), 3.47 (m; H-5β), 2.48 (t, *J* = 7.2 Hz, 2H), 2.25 (m, 1H), 2.02 (m, 1H), 1.98 & 1.96 (s & s, 3H), 1.39 (d, *J* = 6.6 Hz, 3H) (*note*: the HDO peak partly obscures the peak at 4.75 ppm).

$^{13}$C NMR (101 MHz, $D_2O$, 301 K, δ): 177.58, 177.57, 176.6, 176.3, 175.1, 175.0, 174.8, 170.6, 121.9 (q, *J* = 255 Hz), 95.6 (C-1β), 91.5 (C-1α), 82.5 (C-3β), 79.6 (C-3α), 78.1 (lactoyl *C*Hβ), 77.9 (lactoyl *C*Hα), 76.4 (C-5β), 72.2 (C-5α), 69.8 (C-4α), 69.6 (C-4β), 66.8, 61.4 (C-6β), 61.2 (C-6α), 56.7 (C-2β), 54.3 (C-2α), 52.82, 52.77, 30.4, 26.3, 22.9, 22.6, 19.2, 19.1 (*note*: the O*C*F$_3$ peak of the minor β anomer was too weak to be identified).

HRMS (ESI-TOF, m/z): calc'd for $C_{20}H_{31}F_3N_3O_{13}^+$ $[M + H]^+$ 578.1803; found 578.1790.




To a stirred solution of Boc-Gly-OH (64 mg, 0.363 mmol, 1.0 eq), (*R*)-glutamic acid dibenzyl ester (137 mg, 0.418 mmol, 1.15 eq), and HOAt (20 mg, 0.145 mmol, 0.4 eq) in 3.6 mL $CH_2Cl_2$ was charged EDC·HCl (80 mg, 0.418 mmol, 1.15 eq). After 21 hours, the mixture was concentrated to dryness. The residue was diluted with EtOAc, washed twice with pH 11 buffer, washed with pH 2 buffer, washed with brine, dried ($Na_2SO_4$), filtered, and concentrated. The crude material was purified by column chromatography (15 then 60% EtOAc in hexane) to afford **SI-28** (60 mg, 0.124 mmol, 34% yield) as an oil.

$^1$H NMR (400 MHz, $CDCl_3$, 301 K, δ): 7.33 (m, 10H), 6.88 (d, *J* = 7.8 Hz, 1H), 5.17 (br s, 1H), 5.15 (s, 2H), 5.10 (d, *J* = 12.3 Hz, 2H), 5.07 (d, *J* = 12.3 Hz, 2H), 4.68 (td, *J* = 7.9, 5.0 Hz, 1H), 3.78 (m, 2H), 2.40 (m, 2H), 2.24 (m, 1H), 2.03 (m, 1H), 1.44 (s, 9H).

$^{13}$C NMR (101 MHz, $CDCl_3$, 301 K, δ): 172.6, 171.5, 169.7, 135.8, 135.2, 128.8, 128.7, 128.6, 128.41, 128.38, 128.37, 80.4, 67.5, 66.6, 51.7, 44.4, 30.2, 28.4, 27.3.

HRMS (ESI-TOF, m/z): calc'd for $C_{26}H_{33}N_2O_7^+$ $[M + H]^+$ 485.2282; found 485.2276.

To a stirred solution of **SI-28** (60 mg, 0.124 mmol, 1.0 eq) in 2 mL $CH_2Cl_2$ was charged 4 M HCl in 1,4-dioxane (0.31 mL, 1.25 mmol, 10 eq). After 15 hours, the mixture was concentrated to dryness and co-evaporated with $CHCl_3$ to afford hydrochloride salt **SI-29** as an oil. To a stirred solution of α-*O*1-benzyl muramic acid[4] (35 mg, 0.092 mmol, 1.0 eq), **SI-29** (0.124 mmol, 1.35 eq assuming 100% yield in Boc deprotection), *N*-methylmorpholine (25 µL, 0.23 mmol, 2.5 eq) in 1.8 mL *i*-PrOH was charged DMTMM (29 mg, 0.106 mmol, 1.15 eq). After 6 hours, the mixture was concentrated to dryness. The residue was diluted with EtOAc, washed twice with pH 2 buffer, washed twice with sat. aq $NaHCO_3$, washed with brine, dried ($Na_2SO_4$), filtered, and concentrated. The resulting gummy solids were triturated with 3 mL $Et_2O$ to afford **SI-30** (44 mg, 59 µmol, 64% yield) as a semisolid.

$^1$H NMR (400 MHz, MeOH-*d*$_4$, 301 K, δ): 7.33 (m, 17H), 5.14 (s, 2H), 5.08 (s, 2H), 4.84 (m, 1H), 4.71 (d, *J* = 12.1 Hz), 4.57 (dd, *J* = 8.6, 5.1 Hz, 1H), 4.48 (d, *J* = 12.1 Hz, 1H), 4.44 (q, *J* = 6.7 Hz, 1H), 3.98 (dd, *J* = 10.3, 3.2 Hz, 1H), 3.96 (d, *J* = 16.8 Hz, 1H), 3.91 (d, *J* = 16.8 Hz, 1H), 3.83 (d, *J* = 12.0 Hz, 1H), 3.72 (dd, *J* = 12.0, 5.1 Hz, 1H), 3.69 (m, 1H), 3.67 (m, 1H), 3.52 (t, *J* = 9.1 Hz, 1H), 2.43 (t, *J* = 7.3 Hz, 2H), 2.18 (m, 1H), 1.99 (m, 1H), 1.83 (s, 3H), 1.38 (d, *J* = 6.7 Hz, 3H) (*note*: the H-1 peak at 4.84 ppm is obscured by the HDO peak).

$^{13}$C NMR (101 MHz, MeOH-$d_4$, 301 K, δ): 176.9, 173.9, 173.6, 172.8, 171.6, 138.8, 137.4, 137.1, 129.6, 129.6, 129.5, 129.4, 129.4, 129.3, 129.2, 129.2, 128.8, 97.5, 80.3, 78.5, 74.1, 72.0, 70.1, 68.0, 67.4, 62.5, 54.4, 53.1, 42.8, 31.1, 27.6, 22.8, 19.7.

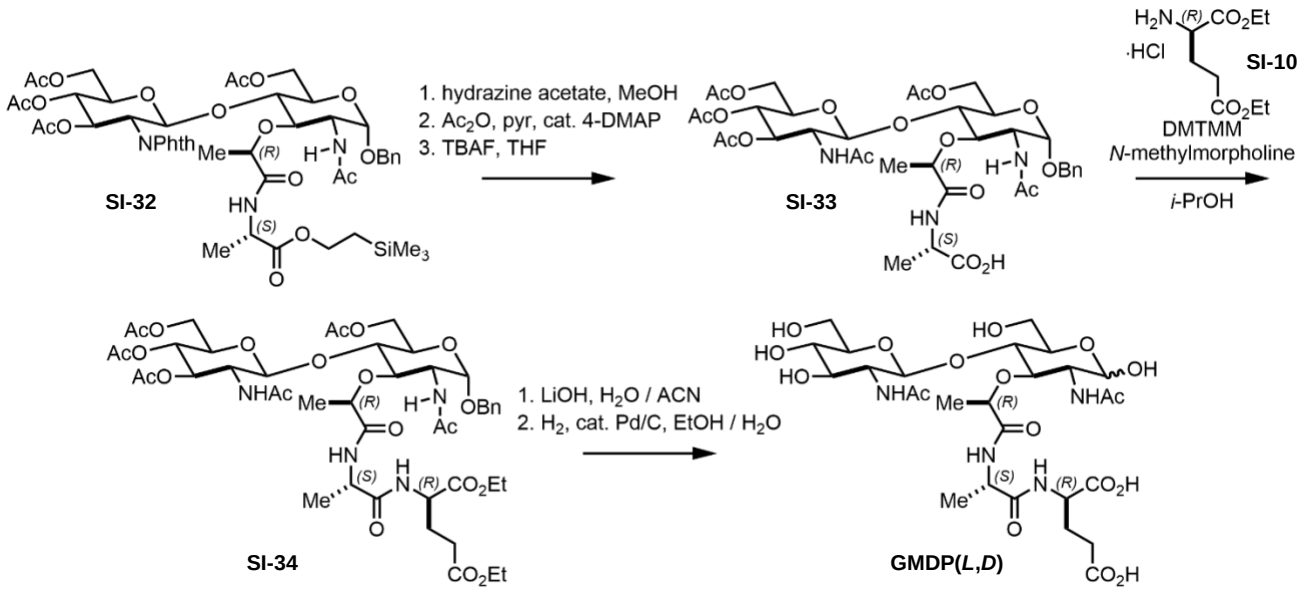
HRMS (ESI-TOF, m/z): calc'd for $C_{39}H_{48}N_3O_{12}^+$ $[M + H]^+$ 750.3233; found 750.3229.

**SI-30** (44 mg, 59 µmol, 1.0 eq) was hydrogenated in 5 mL 10 : 1 v/v EtOAc : MeOH with 40 mg Pd/C and $H_2$ (balloon) with vigorous agitation. After 2 hours, MS indicated complete cleavage of the two benzyl ester groups to afford **SI-31**. The reaction mixture was filtered through Celite, rinsed forward with MeOH, and concentrated. The residue was hydrogenated in 2 mL 4 : 1 v/v $H_2O$ : HOAc with 20 mg Pd/C, 20 mg $Pd(OH)_2$/C, and $H_2$ (balloon) with vigorous agitation. After 2.5 hours, the mixture was filtered through a syringe filter, rinsed forward with MeOH, and concentrated. Purification of the crude by preparative HPLC (0 to 20% v/v ACN in 0.1% aq. TFA) afforded **MurNAc-GE** (11.5 mg, 24 µmol, 41% yield) as a white solid.

$^1$H NMR (400 MHz, $D_2O$, 301 K, δ): 5.15 (d, *J* = 3.4 Hz; H-1α) 4.72 (d, *J* = 8.4 Hz; H-1β), 4.49 (dd, *J* = 9.1, 5.0 Hz, 1H), 4.34 (q, *J* = 6.8 Hz; lactoyl C*H*α), 4.26 (q, *J* = 6.8 Hz; lactoyl C*H*β), 4.04 (d, *J* = 16.9 Hz, 1H), 3.97 (dd, *J* = 10.4, 3.4 Hz; H-2α), 3.91 (d, *J* = 16.9 Hz, 1H), 3.89 (m; H-6aβ), 3.87 (m; H-5α), 3.84 (dd, *J* = 12.3, 2.2 Hz; H-6aα), 3.79 (dd, *J* = 12.3, 5.0 Hz, H-6bα), 3.75 (m; H-6bβ), 3.73 (H-2β), 3.72 (dd, *J* = 10.2, 8.9 Hz; H-3α), 3.57 (t, *J* = 9.3 Hz; H-4α), 3.56 (m; H-3β), 3.53 (t, *J* = 8.5 Hz; H-4β), 3.47 (ddd; *J* = 9.7, 5.3, 2.0 Hz; H-5β), 2.49 (t, *J* = 7.2 Hz, 2H), 2.24 (m, 1H), 2.02 (m, 1H), 1.97 & 1.96 (s & s, 3H), 1.40 (d, *J* = 6.8 Hz, 3H).

$^{13}$C NMR (101 MHz, $D_2O$, 301 K, δ): 177.7, 177.2, 177.0, 175.4, 175.1, 174.8, 171.71, 171.68, 95.4 (C-1β), 91.6 (C-1α), 82.9 (C-3β), 80.1 (C-3α), 78.6 (lactoyl *C*Hβ), 78.3 (lactoyl *C*Hα), 76.3 (C-5β), 72.2 (C-5α), 69.9 (C-4α), 69.6 (C-4β), 61.4 (C-6β), 61.2 (C-6α), 57.0 (C-2β), 54.2 (C-2α), 52.6, 42.61, 42.57, 30.6, 26.4, 22.9, 22.6, 19.3, 19.2.

HRMS (ESI-TOF, m/z): calc'd for $C_{18}H_{30}N_3O_{12}^+$ $[M + H]^+$ 480.1824; found 480.1819.




To a stirred solution of disaccharide **SI-32**[5] (6.66 g, 6.58 mmol, 1.0 eq) in 150 mL MeOH was charged 2 M hydrazine acetate in MeOH (17 mL, 34 mmol, 5.2 eq), and the mixture was heated in a 71 °C bath. After 3 hours, additional 2 M hydrazine acetate solution (6 mL, 12 mmol, 1.8 eq) was charged. After 7 hours, the heating bath was adjusted to 45 °C, and the mixture was aged an additional 11 hours. The mixture was then concentrated to dryness and co-evaporated with toluene and then pyridine. The residue was stirred with 75 mL pyridine. 25 mL $Ac_2O$ was charged followed by 50 mg 4-DMAP. After 6 hours, the mixture was concentrated to dryness and co-evaporated with toluene. The residue was diluted with EtOAc, washed twice with pH 2 buffer, washed with sat. aq. $NaHCO_3$, washed with brine, dried ($Na_2SO_4$), filtered, and concentrated to dryness. The crude material was purified by column chromatography (150 mg silica, 60 then 80% EtOAc in hexane) to afford a white solid (6.67 g; nominally 113% yield) (*note*: $^1$H NMR indicated a mixture of the product and EtOAc). This material was stirred in 150 mL THF to obtain a clear solution and then 1 M TBAF in THF was charged (16.4 mL, 16.4 mmol, 2.5 eq). After 1.5 hours, the mixture was concentrated to dryness and diluted with EtOAc. To remove *n*-$Bu_4N$ salts, the mixture was washed five times with 0.5% aqueous citric acid (*note*: the first two washes were back-extracted with EtOAc to minimize product loss). The pooled organic extract was washed with brine, dried ($Na_2SO_4$), filtered, and concentrated to dryness. The crude solids were suspended in 20 mL $Et_2O$ and stirred to obtain a mobile slurry. The slurry was filtered and the cake was washed with 20 mL $Et_2O$. The solids were dried under vacuum to obtain carboxylic acid **SI-33** (3.31 g, 4.01 mmol, 61% yield over 3 steps) as a white solid.

$^1$H NMR (400 MHz, $CDCl_3$, 301 K, δ): 7.37 (m, 3H), 7.31 (m, 3H), 6.94 (d, *J* = 6.4 Hz, 1H), 6.24 (d, *J* = 9.6 Hz, 1H), 5.14 (d, *J* = 3.4 Hz, 1H), 5.10 (t, *J* = 9.4 Hz, 1H), 5.05 (t, *J* = 9.8 Hz, 1H), 4.64 (d, *J* = 12.0 Hz, 1H), 4.51 (q, *J* = 7.3 Hz, 1H), 4.50 (d, *J* = 12.0 Hz, 1H), 4.45 (q, *J* = 6.8 Hz, 1H), 4.38 (d, *J* = 8.3 Hz, 1H), 4.33 (dd, *J* = 12.3, 3.9 Hz, 1H), 4.27 (dd, *J* = 12.1, 3.8 Hz, 1H), 4.16 (dd, *J* = 12.1, 1.2 Hz, 1H), 4.11 (d, *J* = 9.6 Hz, 1H), 4.07 (dd, *J* = 12.3, 2.1 Hz, 1H), 3.98 (ddd, *J* = 10.6, 6.7, 3.6 Hz, 1H), 3.75 (m, 2H), 3.58 (m, 2H), 2.15 (s, 3H), 2.05 (s, 3H), 2.03 (s, 3H), 2.02 (s, 3H), 1.97 (s, 3H), 1.95 (s, 3H), 1.47 (d, *J* = 7.3 Hz, 3H), 1.39 (d, *J* = 6.8 Hz, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$, 301 K, δ): 174.5, 174.4, 171.7, 171.6, 171.5, 171.0, 170.8, 169.5, 137.4, 128.6, 128.1, 127.9, 100.3, 96.6, 77.0, 76.3, 75.6, 72.7, 72.0, 70.2, 69.6, 68.4, 62.6, 61.8, 54.6, 53.8, 48.3, 23.1, 23.0, 21.1, 20.73, 20.71, 20.67, 18.9, 17.8.

HRMS (ESI-TOF, m/z): calc'd for $C_{37}H_{52}N_3O_{18}^+$ $[M + H]^+$ 826.3240; found 826.3229.

To a stirred solution of carboxylic acid **SI-33** (46 mg, 56 µmol, 1.0 eq), hydrochloride salt **SI-10** (27 mg, 111 µmol, 2.0 eq) and *N*-methylmorpholine (18 µL, 167 µmol, 3.0 eq) in 1.25 mL *i*-PrOH was charged DMTMM (19 mg, 70 µmol, 1.25 eq).

After 30 minutes a thick slurry had formed, so an additional 0.6 mL *i*-PrOH was charged. After 12 hours, kicker charges of hydrochloride salt **SI-10** (20 mg, 84 µmol, 1.5 eq), *N*-methylmorpholine (15 µmol, 2.5 eq), DMTMM (12 mg, 42 µmol, 0.75 eq), and 0.4 mL *i*-PrOH were added. After an additional 22 hours, the mixture was concentrated to dryness. The residue was diluted with EtOAc. The mixture was washed twice with pH 2 buffer, washed with sat. aq. $NaHCO_3$, and washed with brine. The organic extract was dried ($Na_2SO_4$), filtered, and concentrated. The crude material was purified by chromatography (8 g silica, 6% v/v MeOH in $CH_2Cl_2$) to obtain amide **SI-34** (47 mg, 47 µmol, 83% yield) as a semisolid.

$^1$H NMR (400 MHz, $CDCl_3$, 301 K, δ): 7.38 to 7.23 (m, 7H), 7.14 (d, *J* = 7.1 Hz, 1H), 7.04 (d, *J* = 7.7 Hz, 1H), 6.63 (d, *J* = 8.6 Hz, 1H), 5.16 (t, *J* = 9.9 Hz, 1H), 5.05 (d, *J* = 3.4 Hz, 1H), 5.03 (t, *J* = 9.6 Hz, 1H), 4.59 (d, *J* = 12.2 Hz, 1H), 4.53 (d, *J* = 8.3 Hz, 1H), 4.46 (d, *J* = 12.2 Hz, 1H), 4.44 (m, 1H), 4.43 (m, 1H), 4.42 (q, *J* = 6.7 Hz, 1H), 4.31 (dd, *J* = 12.3, 4.5 Hz, 1H), 4.25 (dd, *J* = 11.8, 2.1 Hz, 1H), 4.18 (d, *J* = 11.8 Hz, 1H), 4.12 (q, *J* = 7.1 Hz, 2H), 4.09 (q, *J* = 7.1 Hz, 2H), 4.04 to 3.89 (m, 3H), 3.77 (m, 2H), 3.60 (dt, *J* = 10.7, 4.5 Hz, 1H), 3.55 (ddd, *J* = 9.9, 4.2, 2.2 Hz, 1H), 2.37 to 2.17 (m, 2H), 2.10 (s, 3H), 2.09 (m, 1H), 2.01 (s, 3H), 2.00 (s, 3H), 1.98 (s, 3H), 1.93 (s, 3H), 1.901 (m, 1H), 1.897 (s, 3H), 1.39 (d, *J* = 7.1 Hz, 3H), 1.36 (d, *J* = 6.7 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H), 1.22 (t, *J* = 7.1 Hz, 3H).

$^{13}$C NMR (101 MHz, $CDCl_3$, 301 K, δ): 174.3, 172.7, 172.4, 171.7, 171.3, 171.2, 171.0, 170.8, 170.6, 169.4, 137.3, 128.6, 128.1, 127.8, 99.7, 96.7, 76.6, 75.9, 75.8, 72.5, 72.0, 70.0, 69.2, 68.5, 62.7, 61.8, 61.6, 60.7, 54.8, 53.2, 51.8, 49.1, 30.3, 27.2, 23.2, 23.1, 21.0, 20.72, 20.71, 20.65, 19.1, 18.1, 14.3, 14.2.

HRMS (ESI-TOF, m/z): calc'd for $C_{46}H_{67}N_4O_{21}^+$ $[M + H]^+$ 1011.4292; found 1011.4287.

To a stirred suspension of SI-34 (47 mg, 47 µmol, 1.0 eq) in 400 µL ACN was charged 0.5 M aq. LiOH (930 µL, 465 µmol, 10 eq). After 1.5 hours, the pH was adjusted to 4 by charging 2 M aq. HCl (419 µL, 419 µL, 9.0 eq). The mixture was concentrated to dryness and co-evaporated with EtOH. The residue was hydrogenated in 1.5 mL 1 : 1 v/v EtOH : H2O with 40 mg Pd/C and H2 (balloon). After 14 hours, the mixture was filtered through Celite, rinsed forward with MeOH, and concentrated to dryness. The crude product was purified by preparative HPLC (0 to 30% v/v ACN in 0.1% aq. TFA) to afford GMDP(L, D) (20.7 mg, 29.7 µmol, 64% yield over 2 steps) as a white solid.

1H NMR (400 MHz, D2O, 301 K, δ): 5.26 (d, J = 2.3 Hz; H-1α), 4.63 (d, J = 7.8 Hz; H-1β), 4.61 (q, J = 6.7 Hz; lactoyl CHα), 4.53 (d, J = 8.1 Hz; H-1'), 4.48 (dd, J = 8.7, 5.3 Hz, 1H), 4.46 (q, J = 6.8 Hz; lactoyl CHβ), 4.31 (q, J = 7.1 Hz, 1H), 3.94 (d, J = 12.3 Hz; H-6a'), 3.870 (m; H-4α), 3.863 (m; H-5α), 3.862 (m; H-6aβ), 3.85 (m; H-4β), 3.804 (m; H-6aα), 3.798 (m; H-2α), 3.76 (m; H-6b'), 3.74 (m; H-2'), 3.724 (m; H-3α), 3.717 (m; H-6bα), 3.70 (m; H-6bβ), 3.58 (m; H-3β), 3.558 (m; H-2β), 3.5555 (m; H-3'), 3.47 (m; H-5β), 3.43 (m; H-5'), 3.42 (m; H-4'), 2.47 (t, J = 7.0 Hz, 2H), 2.23 (m, 1H), 2.04 (s, 3H), 2.00 (m, 1H), 1.97 & 1.95 (s & s, 3H), 1.44 (d, J = 7.1 Hz, 3H), 1.40 (d, J = 6.8 Hz, 3H).

13C NMR (101 MHz, D2O, 301 K, δ): 177.6, 177.56, 176.3, 175.62, 175.60, 175.57, 175.55, 175.3, 175.2, 174.8, 101.11 (C-1'α), 101.07 (C-1'β), 95.8 (C-1β), 90.8 (C-1α), 79.9 (C-3β), 78.6 (lactoyl CHβ), 78.1 (lactoyl CHα), 76.8 (C-3α), 76.7 (C-5'), 76.2 (C-4α), 75.83 (C-5β), 75.80 (C-4β), 74.22 (C-3'α), 74.19 (C-3'β), 71.7 (C-5α), 70.9 (C-4'), 61.8 (C-6'), 60.6 (C-6β), 60.4 (C-6α), 56.7 (C-2'α), 56.6 (C-2'β), 56.4 (C-2β), 54.3 (C-2α), 52.7, 50.6, 50.3, 30.7, 26.8, 26.7, 22.9, 22.8, 22.7, 18.9, 18.6, 17.5, 17.3.

HRMS (ESI-TOF, m/z): calc'd for C17H45N4O17+ [M + H]+ 697.2774; found 697.2764.

## References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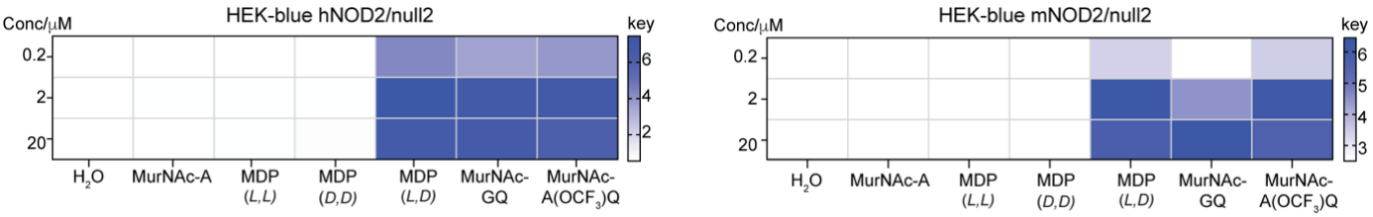



**Figure S1**. Evaluation of MDP analogues in HEK-blue hNOD2 and mNOD2 reporter assay. OD650nm was measured and normalized against the background in Null2 parental cells.

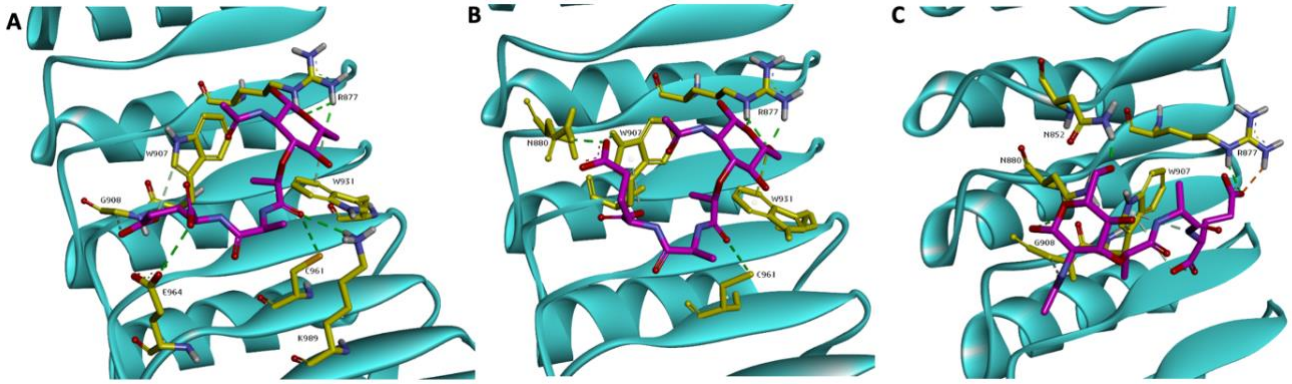



**Figure S2. Molecular docking of NOD2-LRR with MDP-*(L,D)* (A), MDP-*(L,L)* (B), and MDP-*(D,D)* (C).** Carbon atoms of the amino acid residues that interact with MDP analogues are highlighted in yellow.

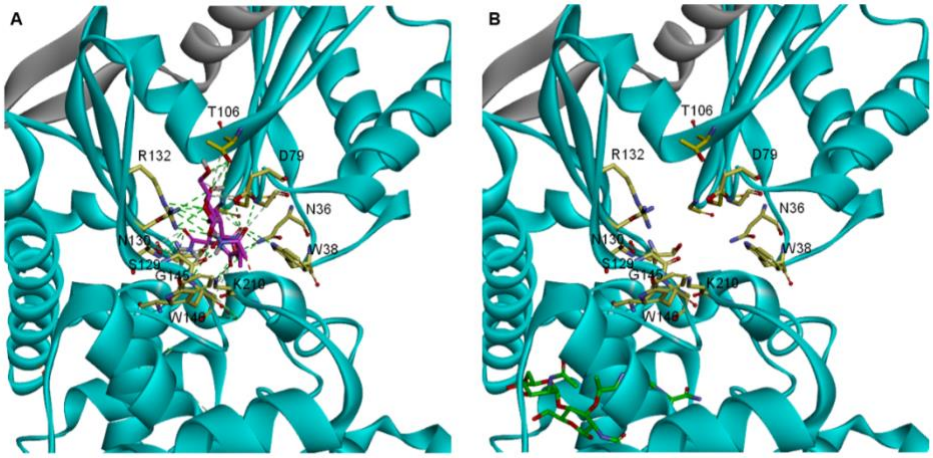



**Figure S3.** Molecular docking shows binding of MDP (**A**, magenta) at the GlcNAc binding pocket of human NAGK protein model (cyan & grey ribbons) but GMDP (**B**, green) is unable to bind at the same site. Carbon atoms of the amino acid residues that interact with MDP are highlighted in yellow.

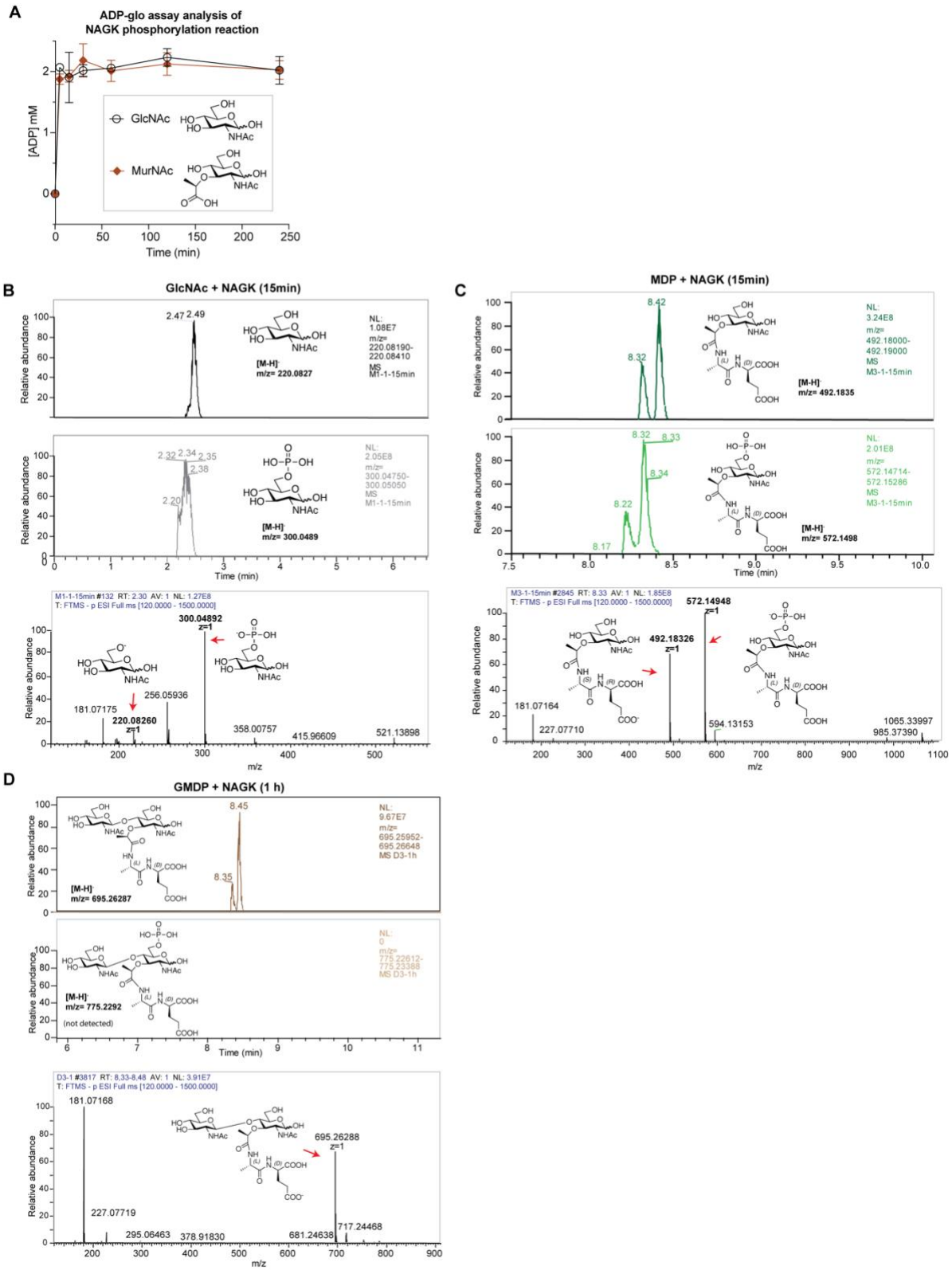



**Figure S4.** In vitro assays to evaluate NAGK phosphorylation. The respective substrate (2 mM) was incubated with ATP (5 mM) and NAGK (3.6 µM) at 37 °C. At the desired time points (i.e., 5, 15, 30, 60, 90, 120, 240 min) samples were collected for subsequent analysis. Triplicates were performed for each time point of each substrate. A) ADP-GloTM assay shows that

GlcNAc and MurNAc are efficiently phosphorylated by NAGK in vitro; B-D) LC-MS analysis revealed that GMDP is not a suitable substrate for NAGK phosphorylation. Extracted ion chromatogram (EIC) and MS1 spectra were shown.

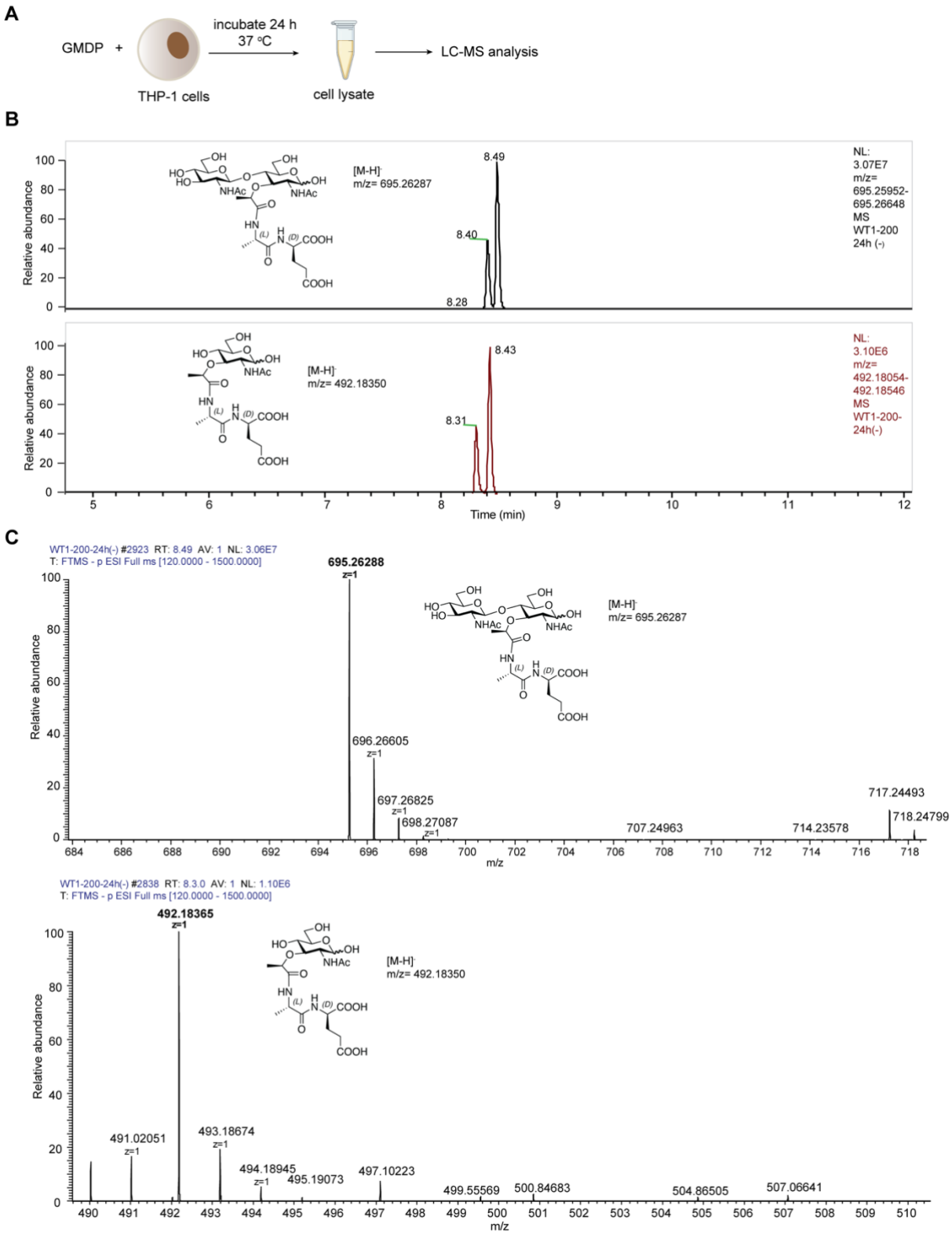
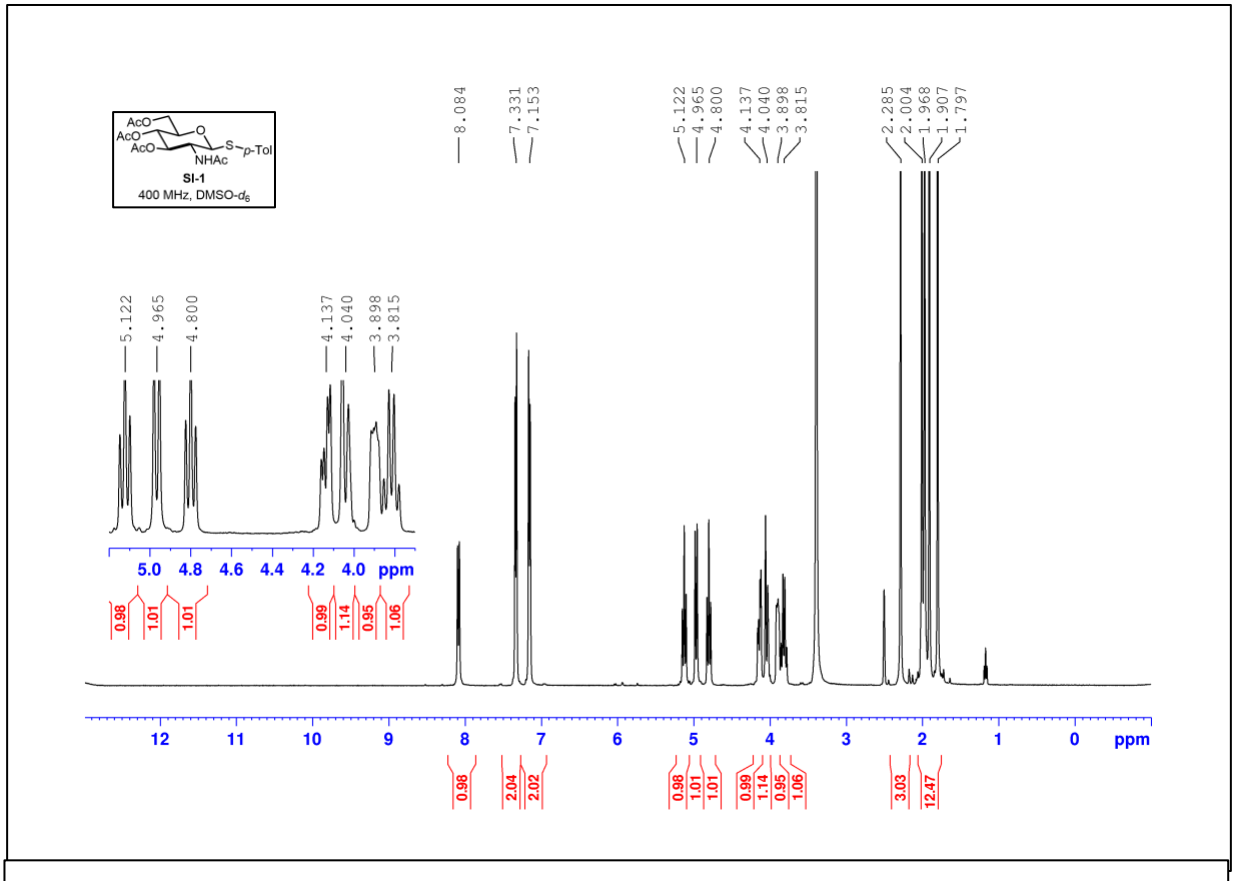



**Figure S5.** LC-MS analysis identified cytosolic MDP in THP-1 cells treated with GMDP. Wildtype THP-1 cells were treated with GMDP (200 μm) via lipofectamine delivery and subsequently incubated at 37°C for 24h before harvested and lysed for LC-MS analysis. Duplicates were performed. The result suggests potential host glycosidase cleavage of disaccharide

muropeptide GMDP that yields monosaccharide MDP, a suitable substrate for subsequent NAGK phosphorylation and NOD2 activation.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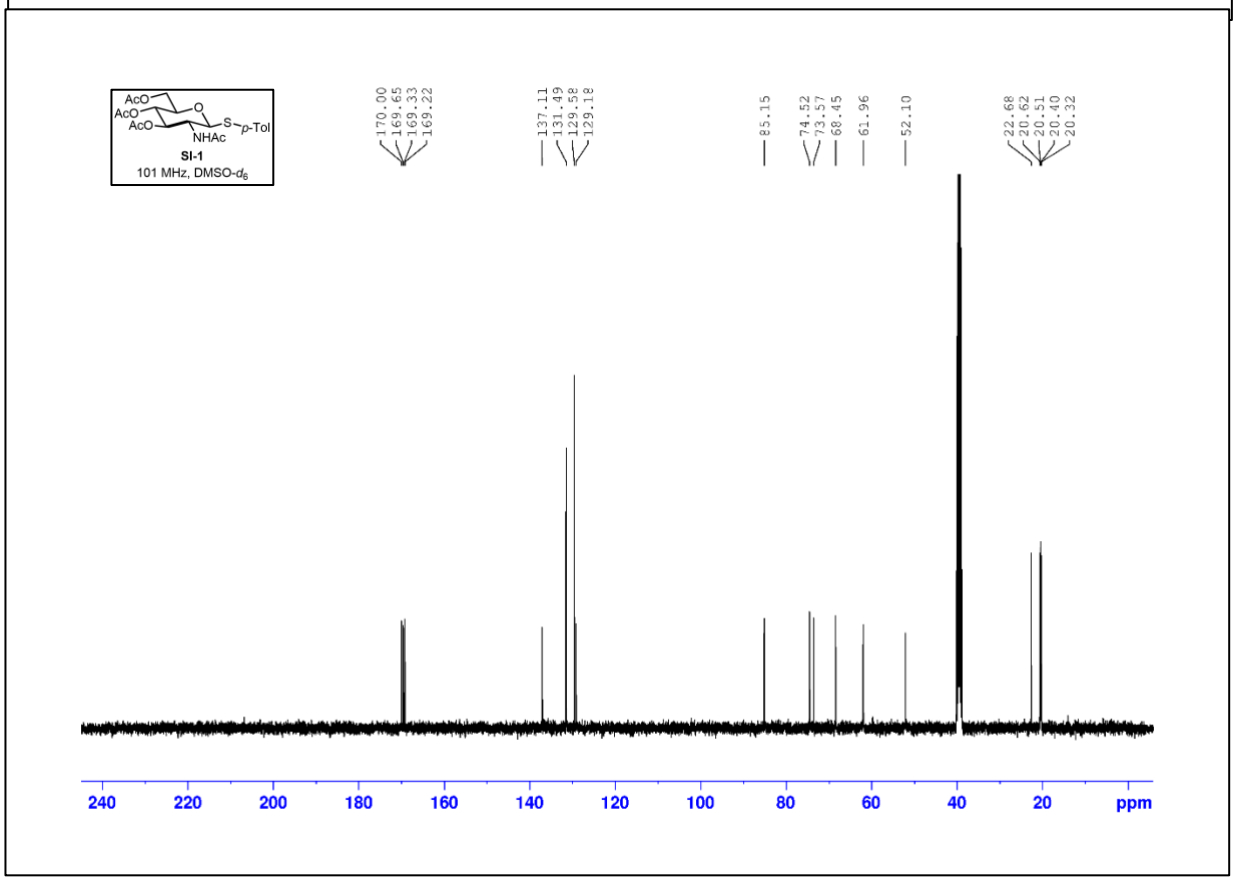
AcO
AcO
O
S
p-Tol
NHAc
SI-1
400 MHz, DMSO-d6
8.084
7.331
7.153
5.122
4.965
4.800
4.137
4.040
3.898
3.815
2.285
2.004
1.968
1.907
1.797
0.98
2.04
2.02
0.98
1.01
1.01
0.99
1.14
0.95
1.06
3.03
12.47
ppm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AcO
AcO
O
S
p-Tol
NHAc
SI-1
101 MHz, DMSO-d6
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169.65
169.33
169.22
137.11
131.49
129.58
129.18
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74.52
73.57
68.45
61.96
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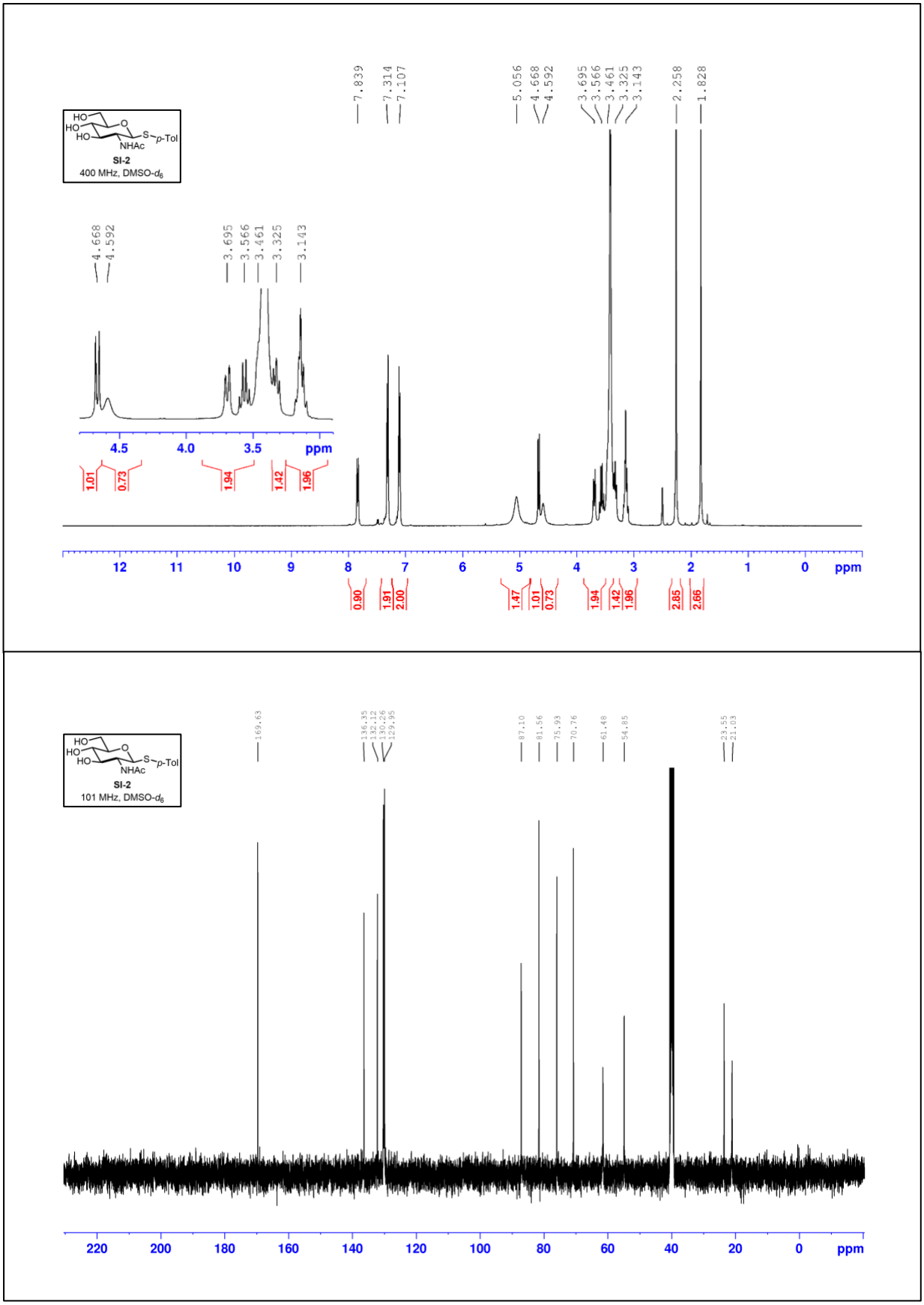
22.68
20.62
20.51
20.40
20.32
ppm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3.566
3.461
3.325
3.143
2.258
1.828
ppm
SI-2
101 MHz, DMSO-$d_6$
169.63
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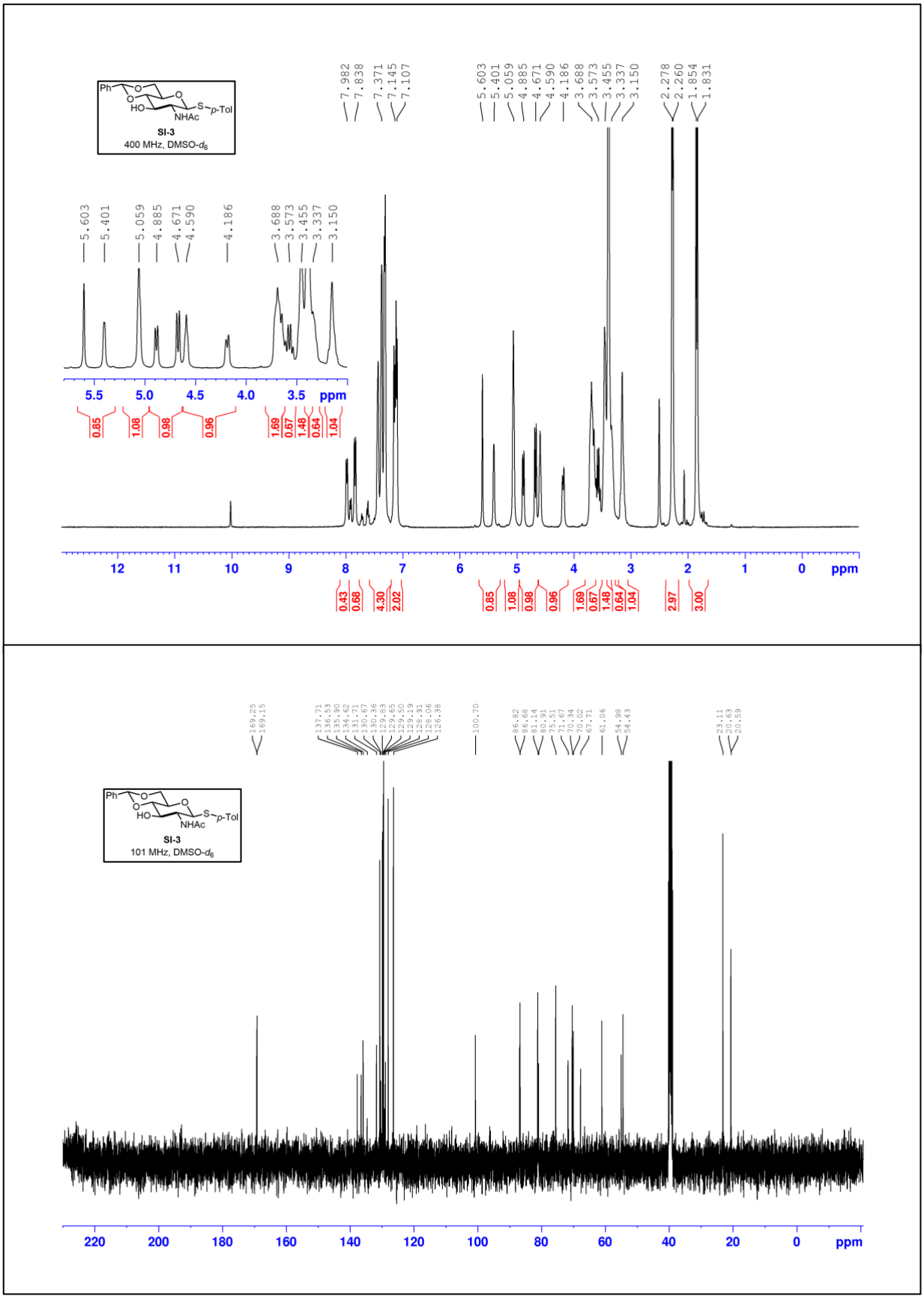
21.03
ppm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400 MHz, DMSO-$d_6$
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7.145
7.107
5.603
5.401
5.059
4.885
4.671
4.590
4.186
3.688
3.573
3.455
3.337
3.150
2.278
2.260
1.854
1.831
ppm
SI-3
101 MHz, DMSO-$d_6$
169.25
169.15
137.71
136.53
135.90
134.62
131.71
130.67
130.36
129.83
129.65
129.50
129.19
128.91
128.06
126.38
100.70
86.82
86.68
81.14
80.91
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71.44
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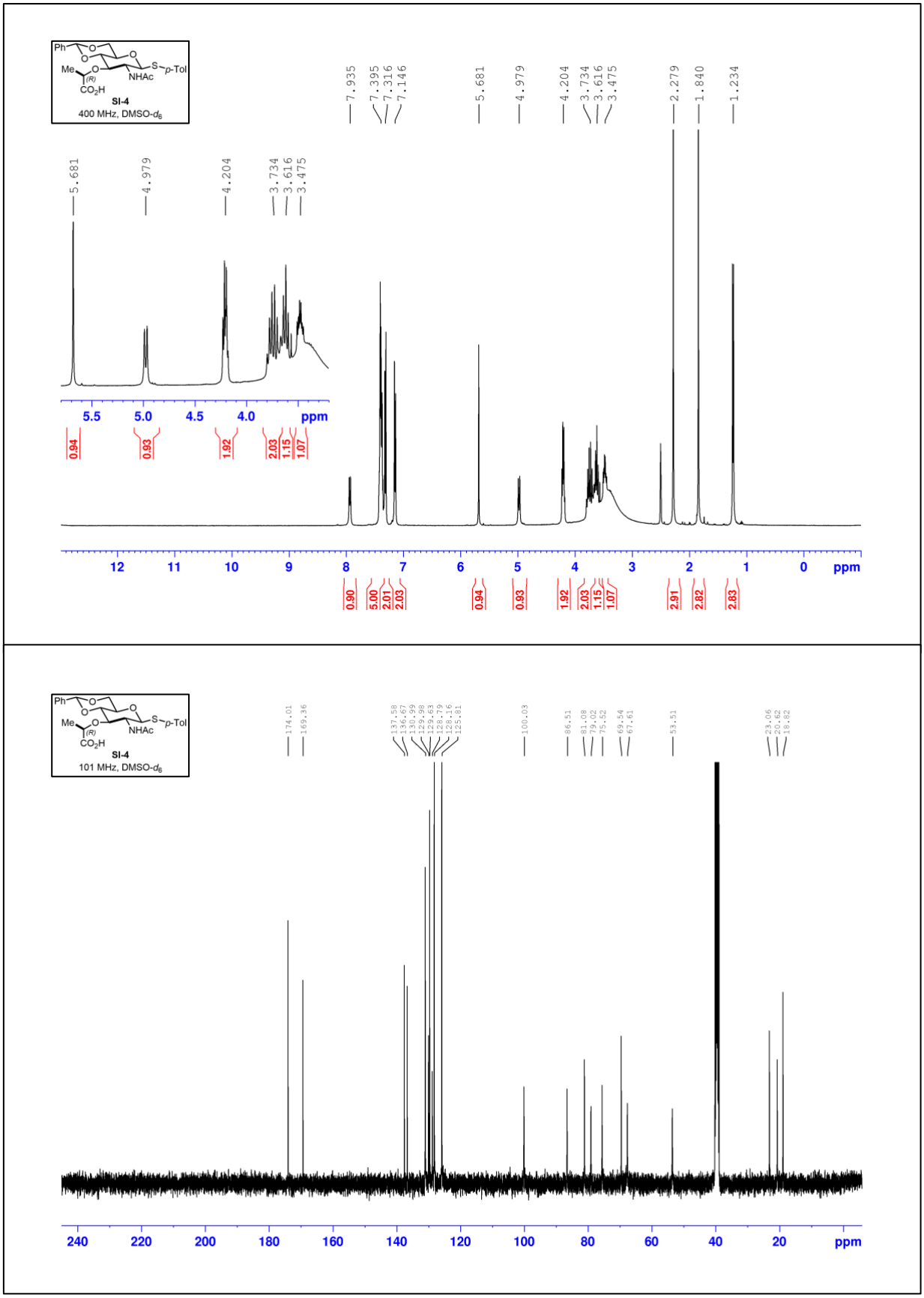
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4.204
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3.616
3.475
2.279
1.840
1.234
ppm
SI-4
101 MHz, DMSO-$d_6$
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137.58
136.67
130.99
129.98
129.63
128.79
128.16
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81.08
79.02
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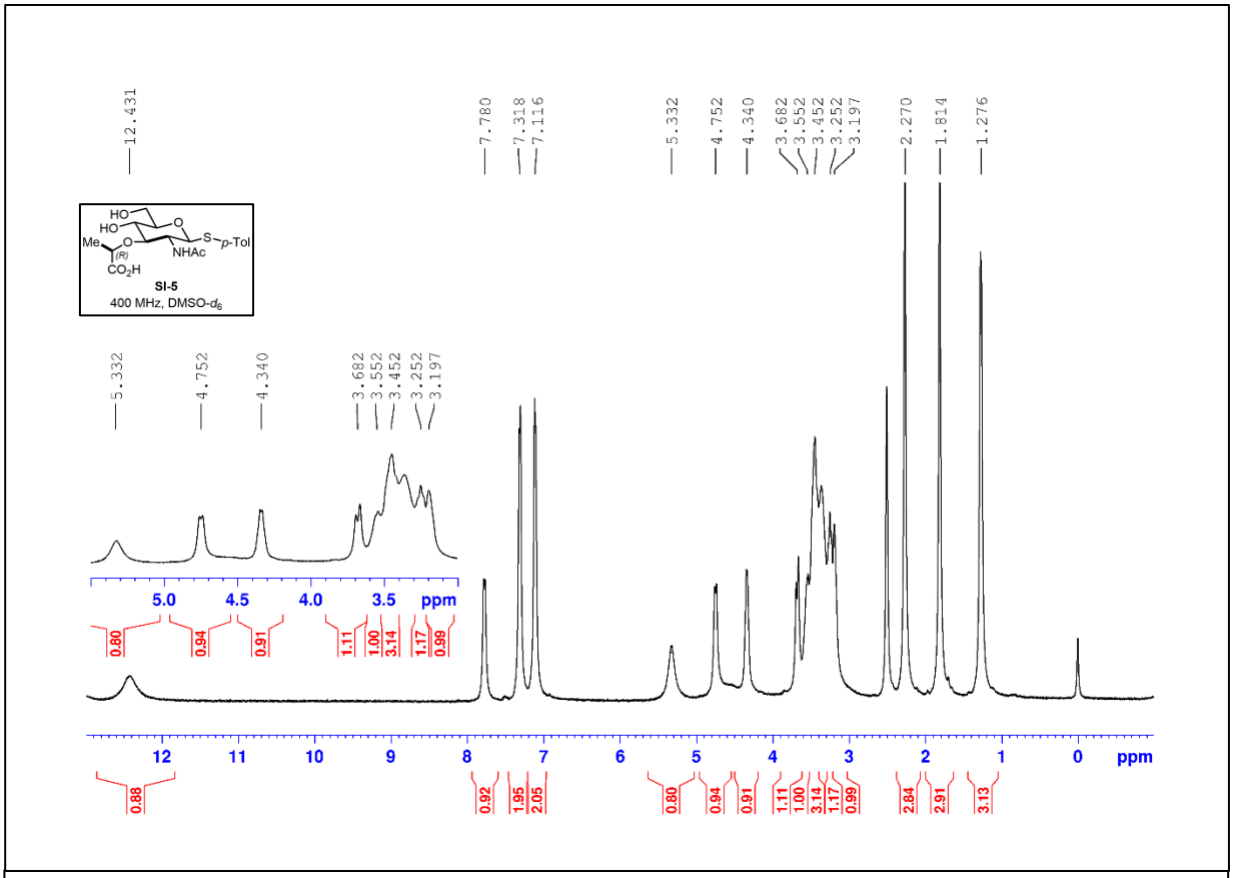
53.51
23.06
20.82
18.82
ppm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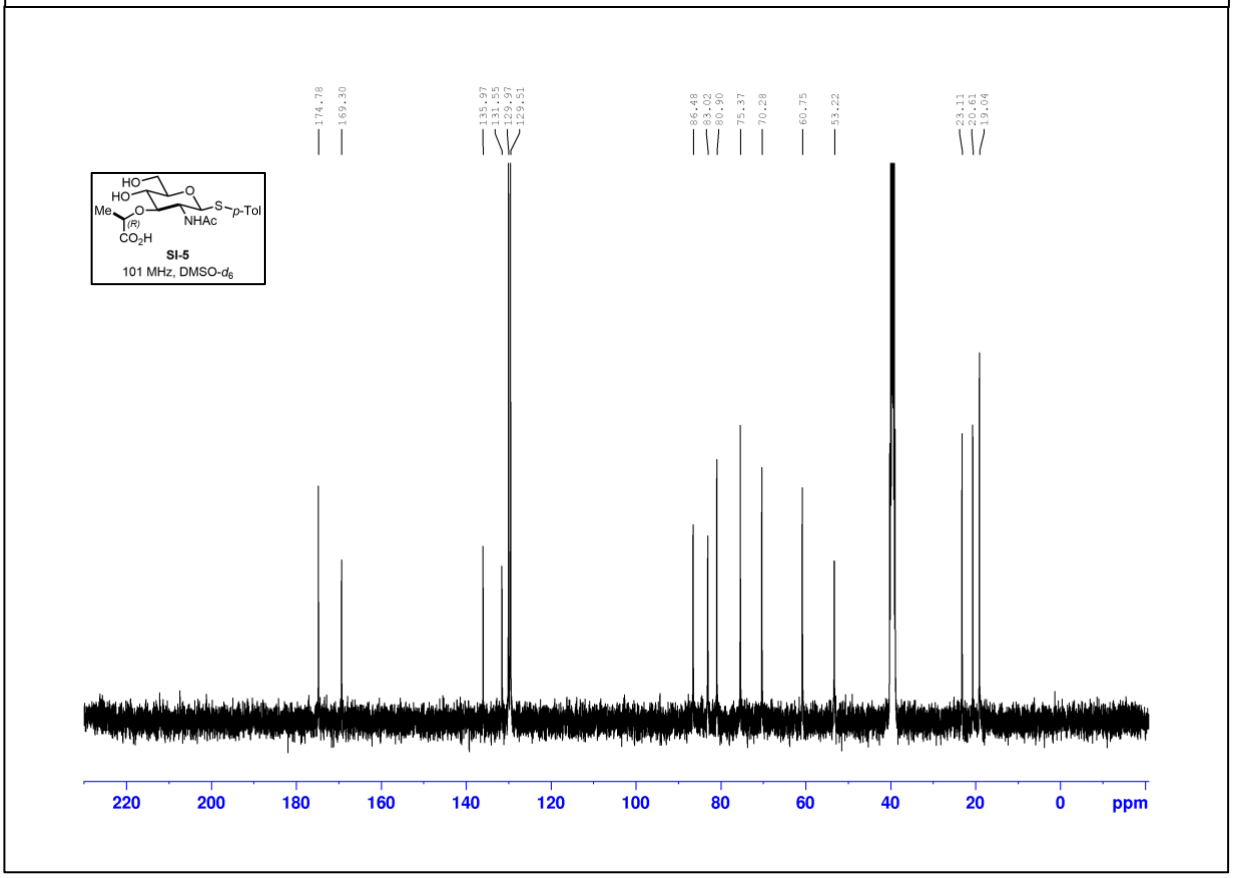
7.780
7.318
7.116
5.332
4.752
4.340
3.682
3.552
3.452
3.252
3.197
2.270
1.814
1.276
HO
HO
Me
O
(R)
CO2H
O
S
p-Tol
NHAc
SI-5
400 MHz, DMSO-d6
5.0
4.5
4.0
3.5
ppm
0.80
0.94
0.91
1.11
1.00
3.14
1.17
0.99
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.88
0.92
1.95
2.05
0.80
0.94
0.91
1.11
1.00
3.14
1.17
0.99
2.84
2.91
3.13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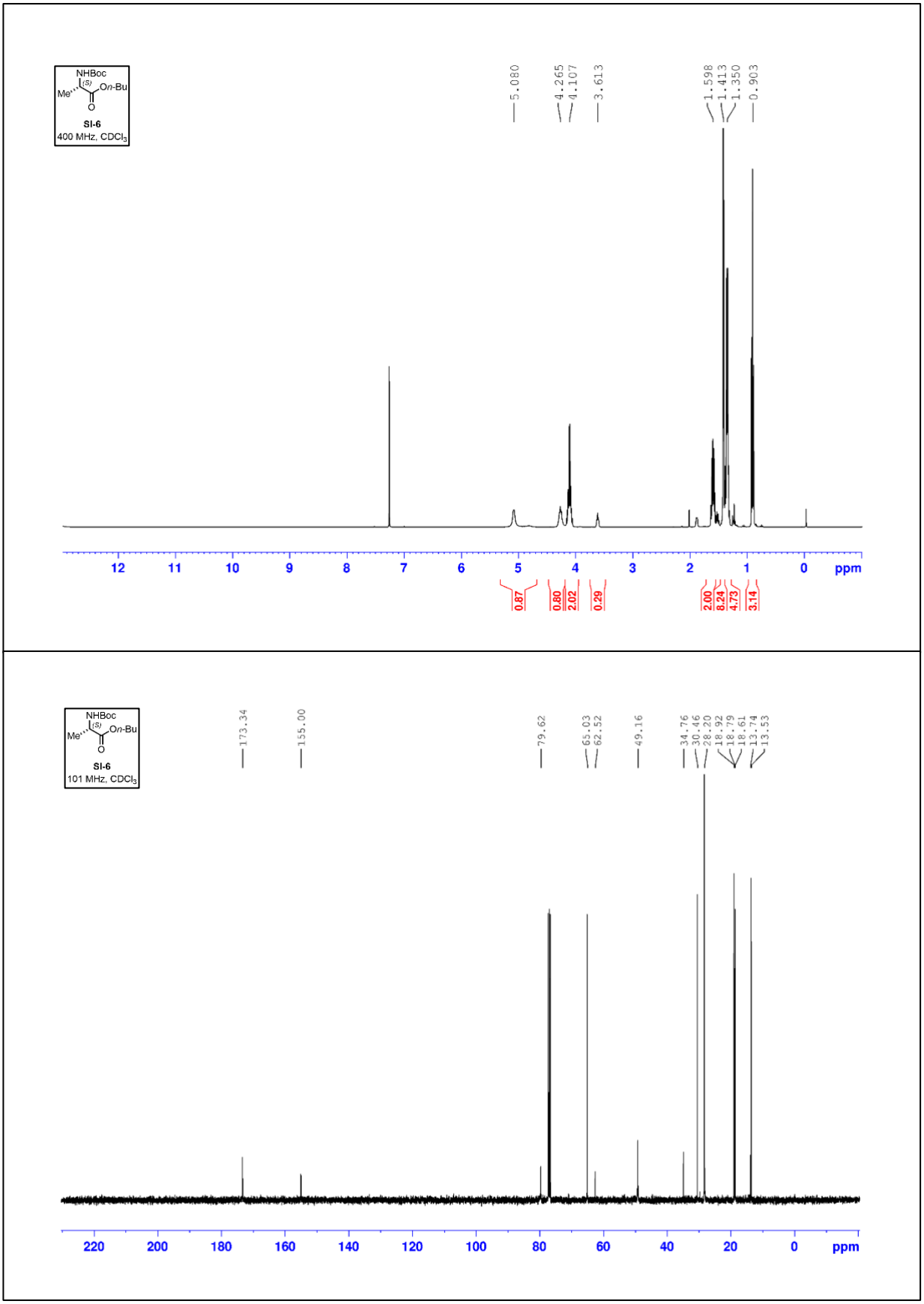
135.97
131.55
130.97
129.51
86.48
83.02
80.90
75.37
70.28
60.75
53.22
23.11
20.61
19.04
HO
HO
Me
O
(R)
CO2H
O
S
p-Tol
NHAc
SI-5
101 MHz, DMSO-d6
220
200
180
160
140
120
100
80
60
40
20
0
ppm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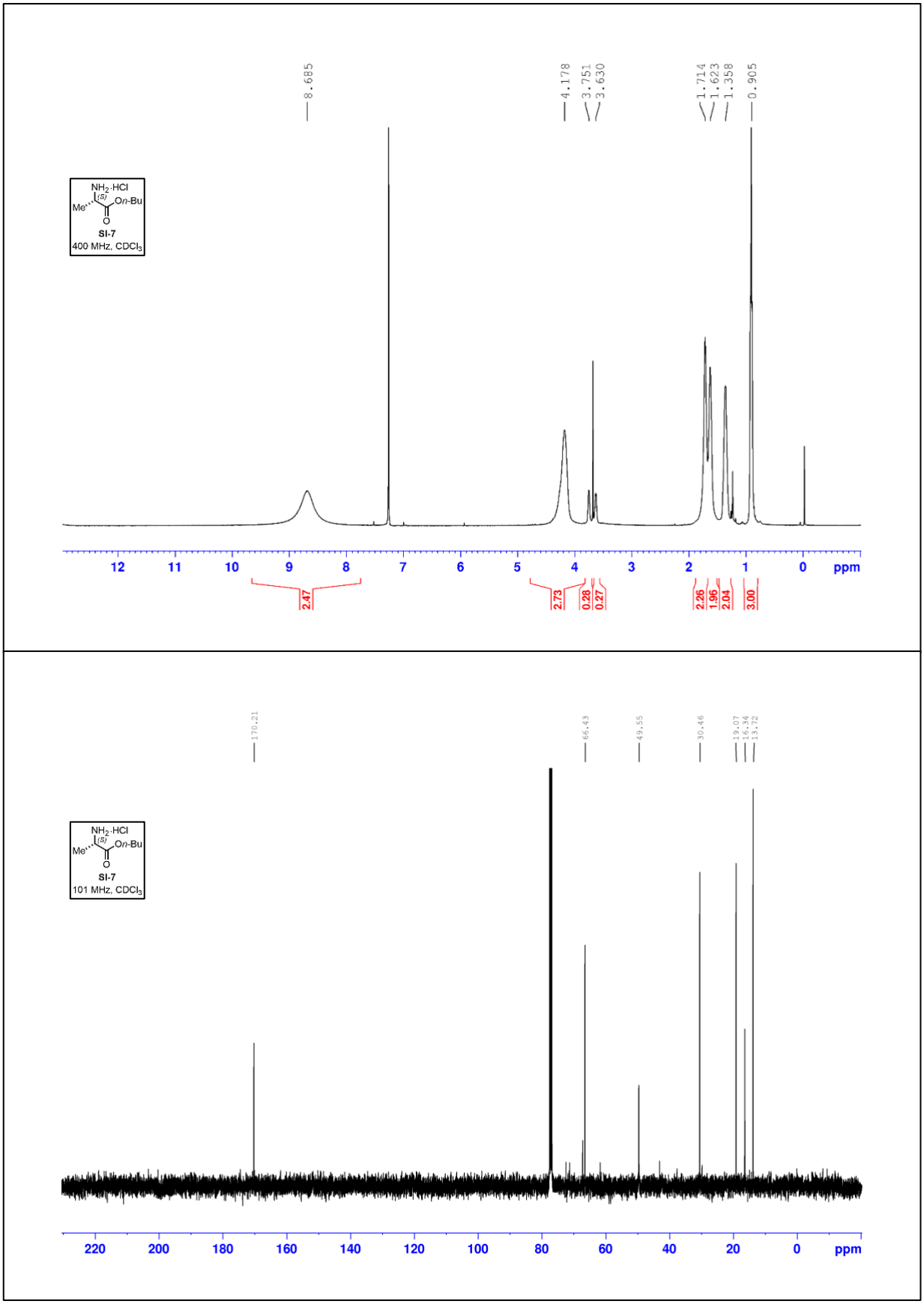
Me
(S)
O
O
n-Bu
SI-6
400 MHz, CDCl3
5.080
4.265
4.107
3.613
1.598
1.413
1.350
0.903
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.87
0.80
2.02
0.29
2.00
8.24
4.73
3.14
NHBoc
Me
(S)
O
O
n-Bu
SI-6
101 MHz, CDCl3
173.34
155.00
79.62
65.03
62.52
49.16
34.76
30.46
28.20
18.92
18.79
18.61
13.74
13.53
220
200
180
160
140
120
100
80
60
40
20
0
ppm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1.358
0.905
NH2·HCl
Me
(S)
On-Bu
O
SI-7
400 MHz, CDCl3
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
2.47
2.73
0.28
0.27
2.26
1.96
2.04
3.00
170.21
66.43
49.55
30.46
19.07
16.34
13.72
NH2·HCl
Me
(S)
On-Bu
O
SI-7
101 MHz, CDCl3
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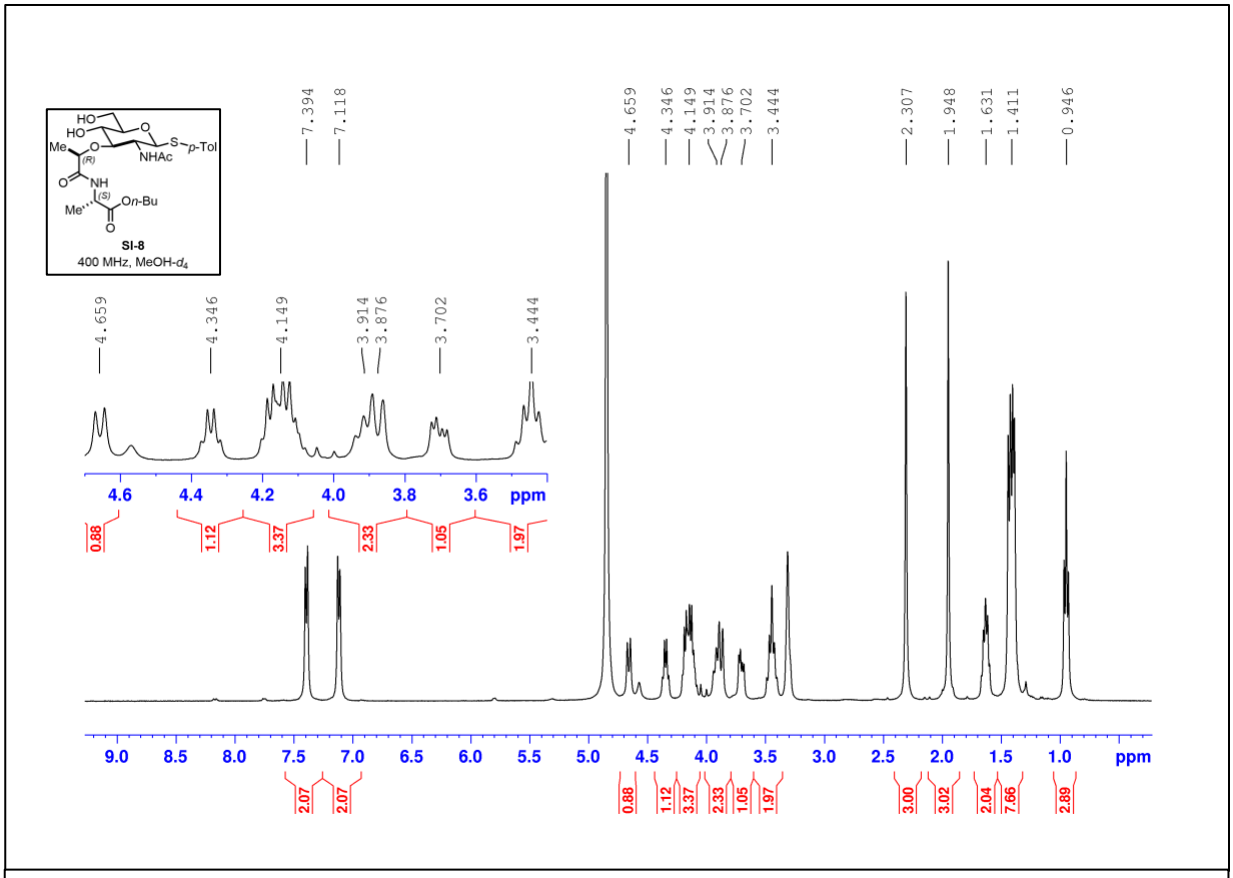
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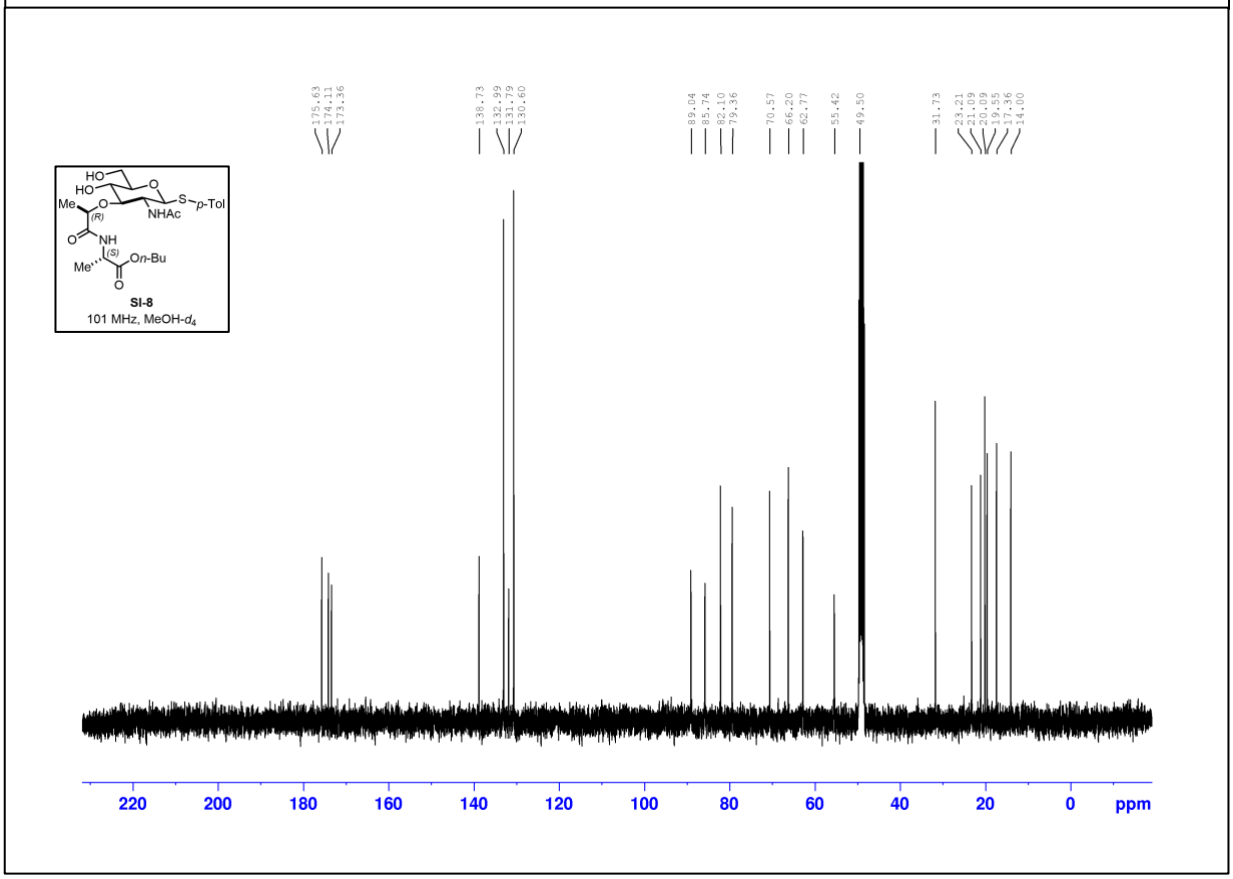
ppm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101 MHz, MeOH-$d_4$
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174.11
173.36
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132.99
131.79
130.60
89.04
85.74
82.10
79.36
70.57
66.20
62.77
55.42
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31.73
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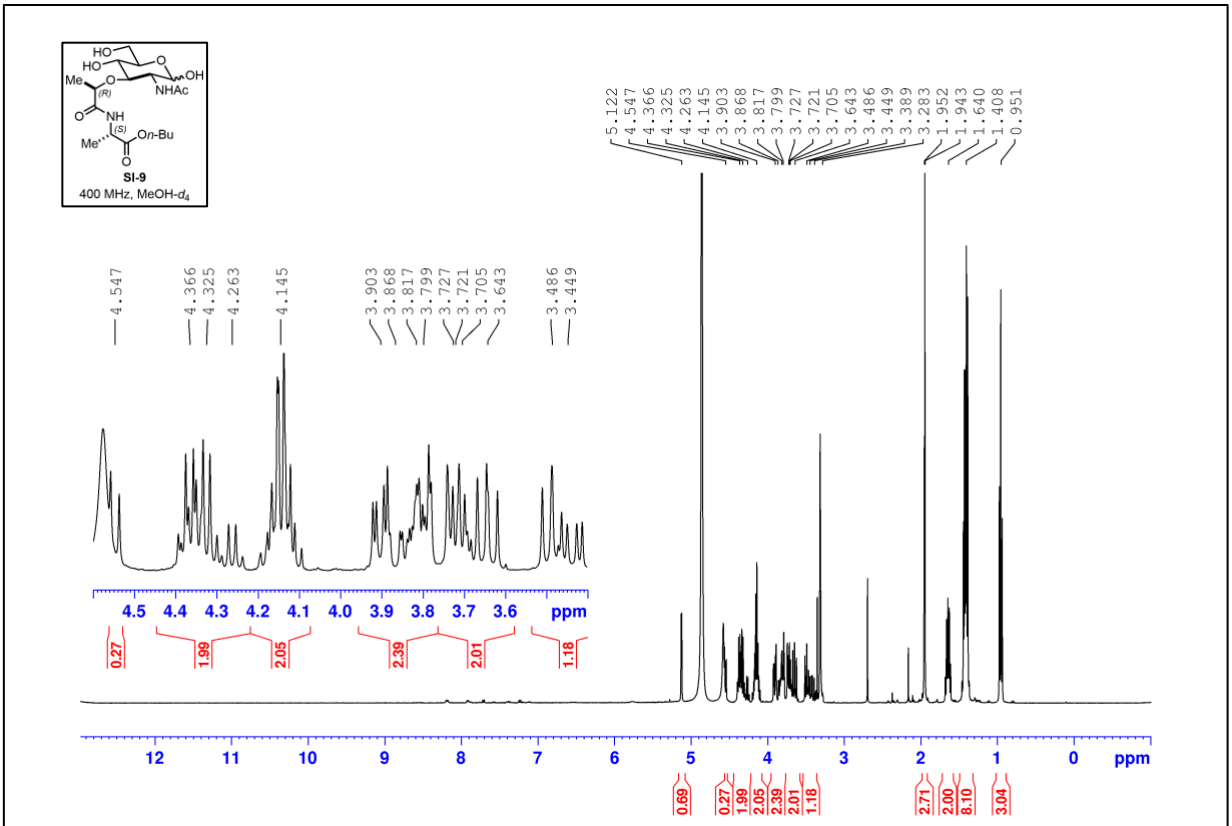
21.09
20.09
19.55
17.36
14.00
ppm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4.263
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3.868
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3.705
3.643
3.486
3.449
3.389
3.283
1.952
1.943
1.640
1.408
0.951
ppm
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0.27
1.99
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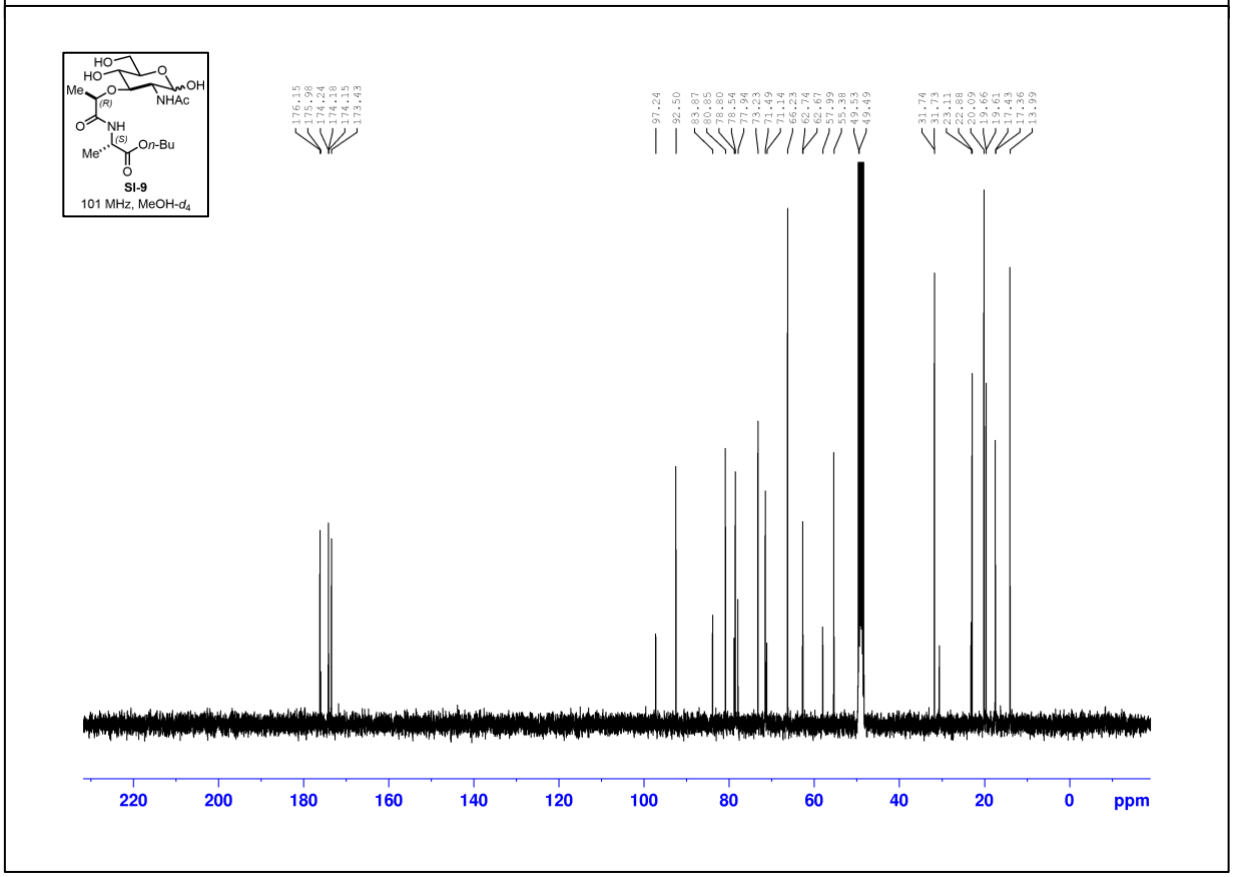
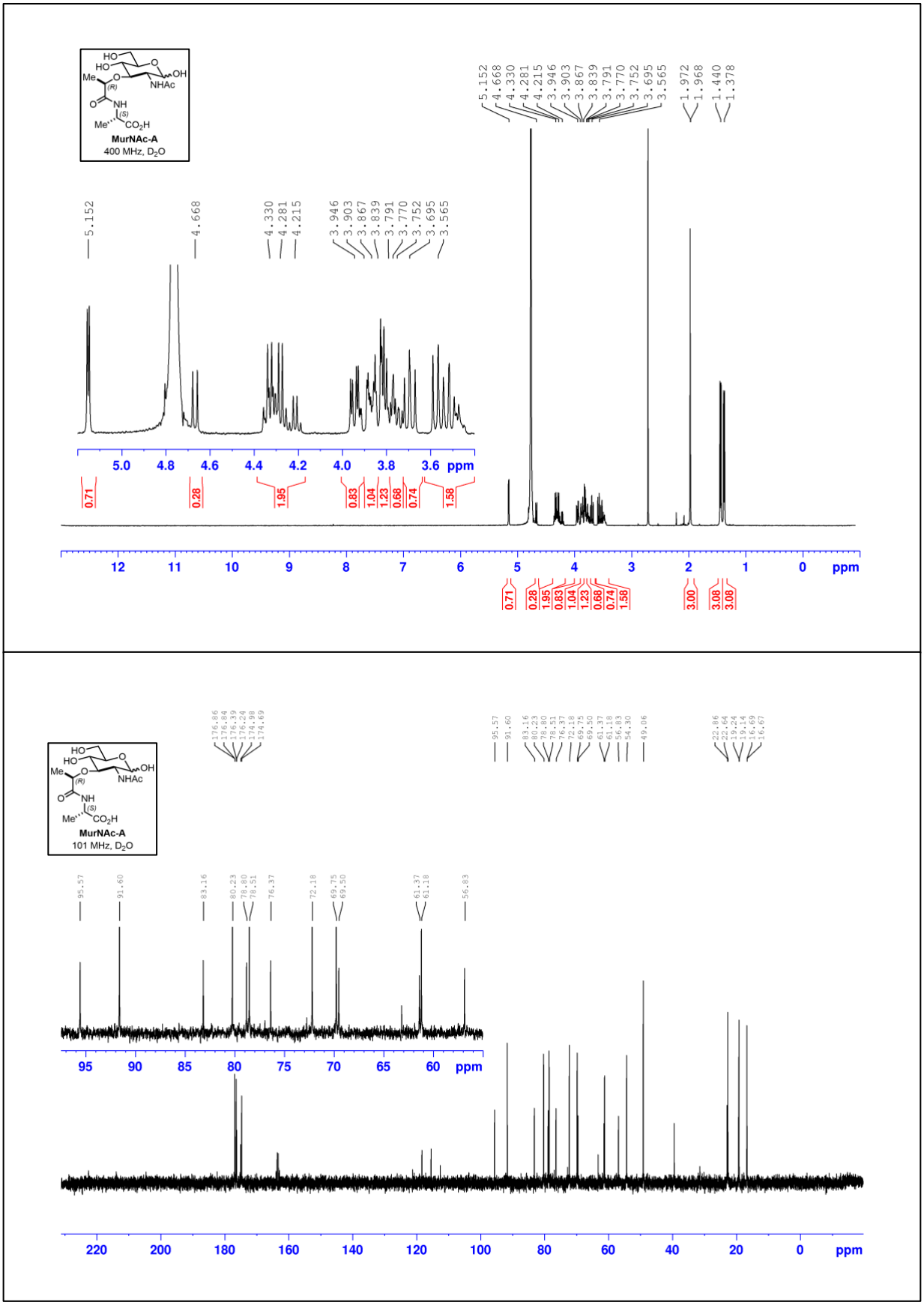
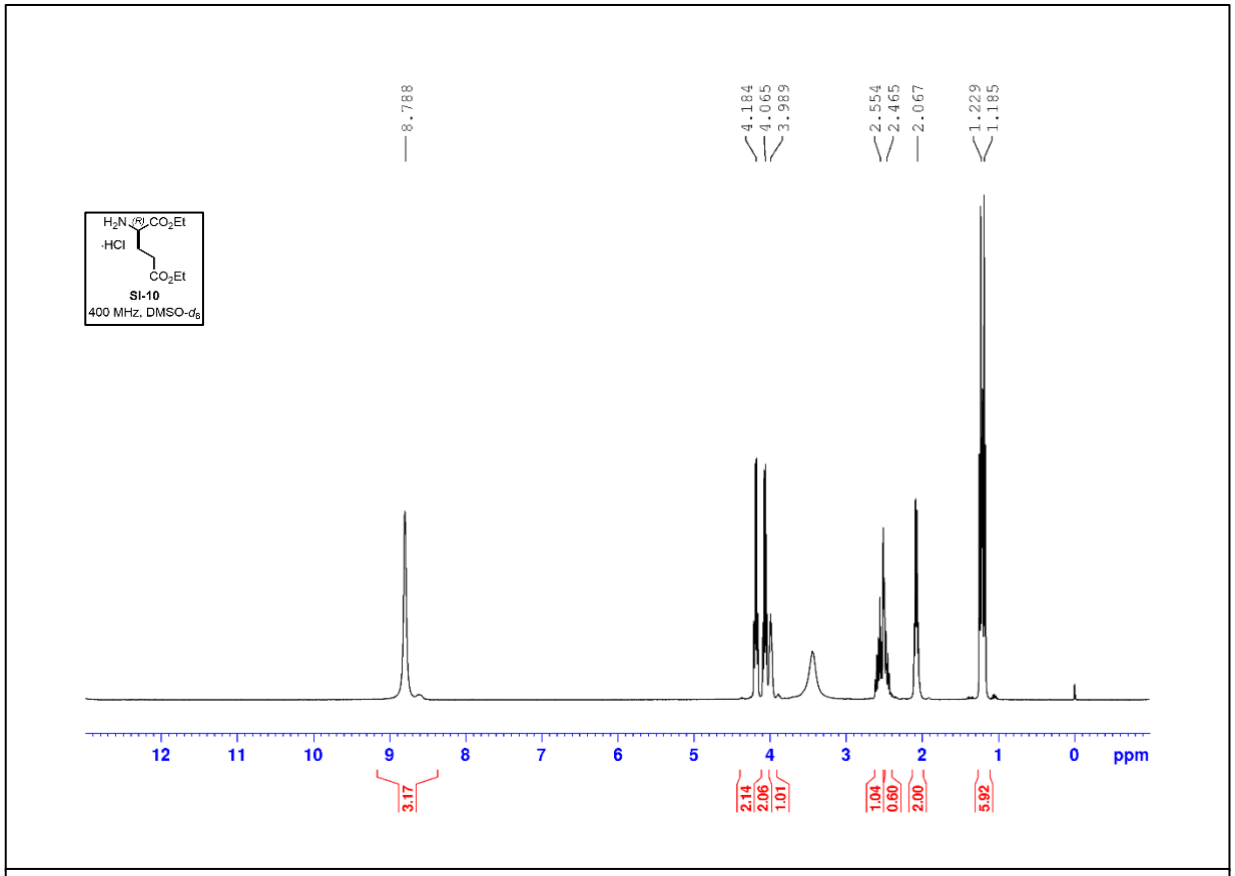
2.39
2.01
1.18
2.71
2.00
8.10
3.04


SI-9
101 MHz, MeOH-$d_4$
176.15
175.98
174.24
174.18
174.15
173.43
97.24
92.50
83.87
80.85
78.80
78.54
73.64
73.23
71.49
71.14
66.23
62.74
62.67
57.99
57.71
49.53
49.49
31.74
31.73
23.11
22.88
20.09
19.66
19.41
17.43
17.36
13.99
ppm


MurNAc-A
400 MHz, D2O
5.152
4.668
4.330
4.281
4.215
3.946
3.903
3.867
3.839
3.791
3.770
3.752
3.695
3.565
1.972
1.968
1.440
1.378
ppm
MurNAc-A
101 MHz, D2O
176.86
176.84
176.49
176.24
174.98
174.69
95.57
91.60
83.16
80.23
78.80
78.51
76.37
72.18
69.75
69.50
61.37
61.18
56.83
54.30
49.06
22.86
22.64
19.24
19.14
16.69
16.67
ppm


8.788
4.184
4.065
3.989
2.554
2.465
2.067
1.229
1.185
SI-10
400 MHz, DMSO-$d_6$
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
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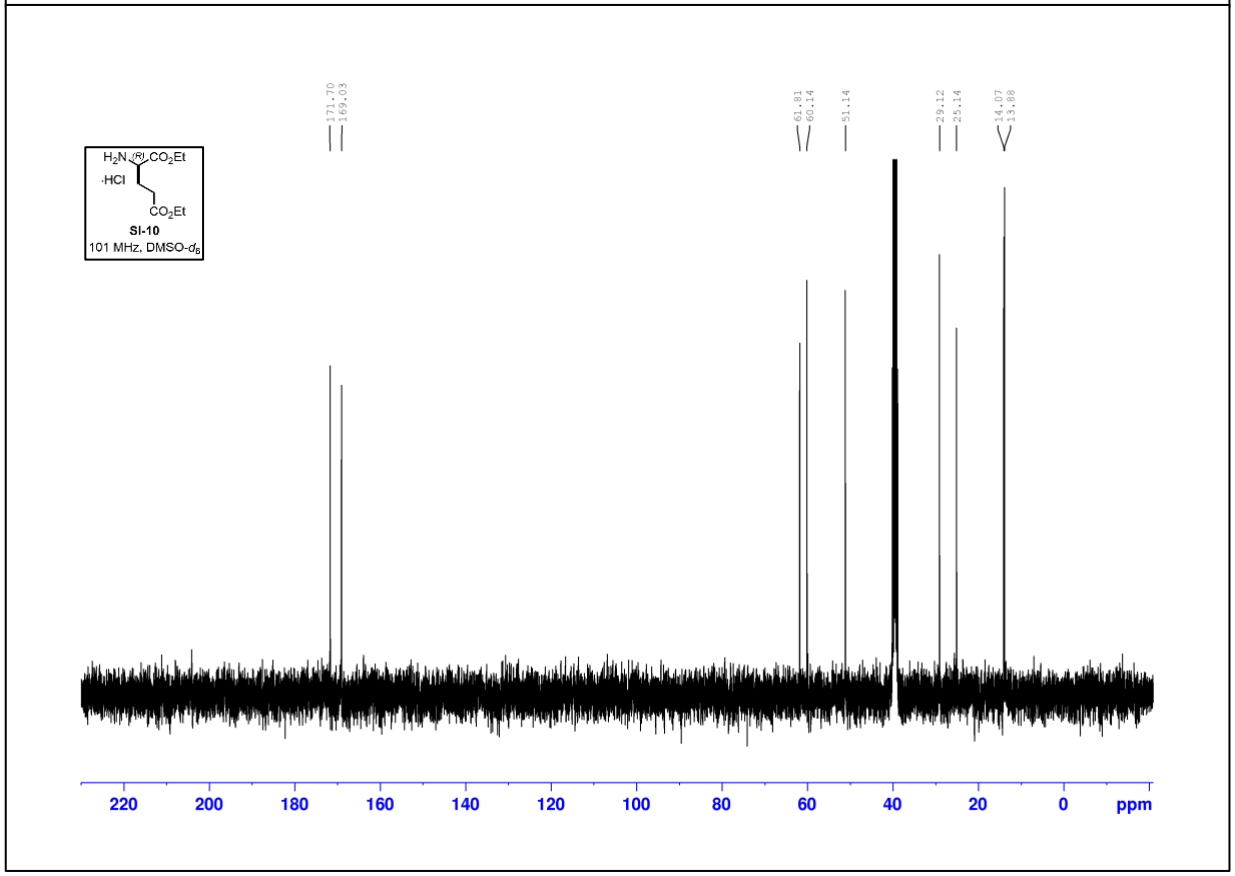
2.14
2.06
1.01
1.04
0.60
2.00
5.92


171.70
169.03
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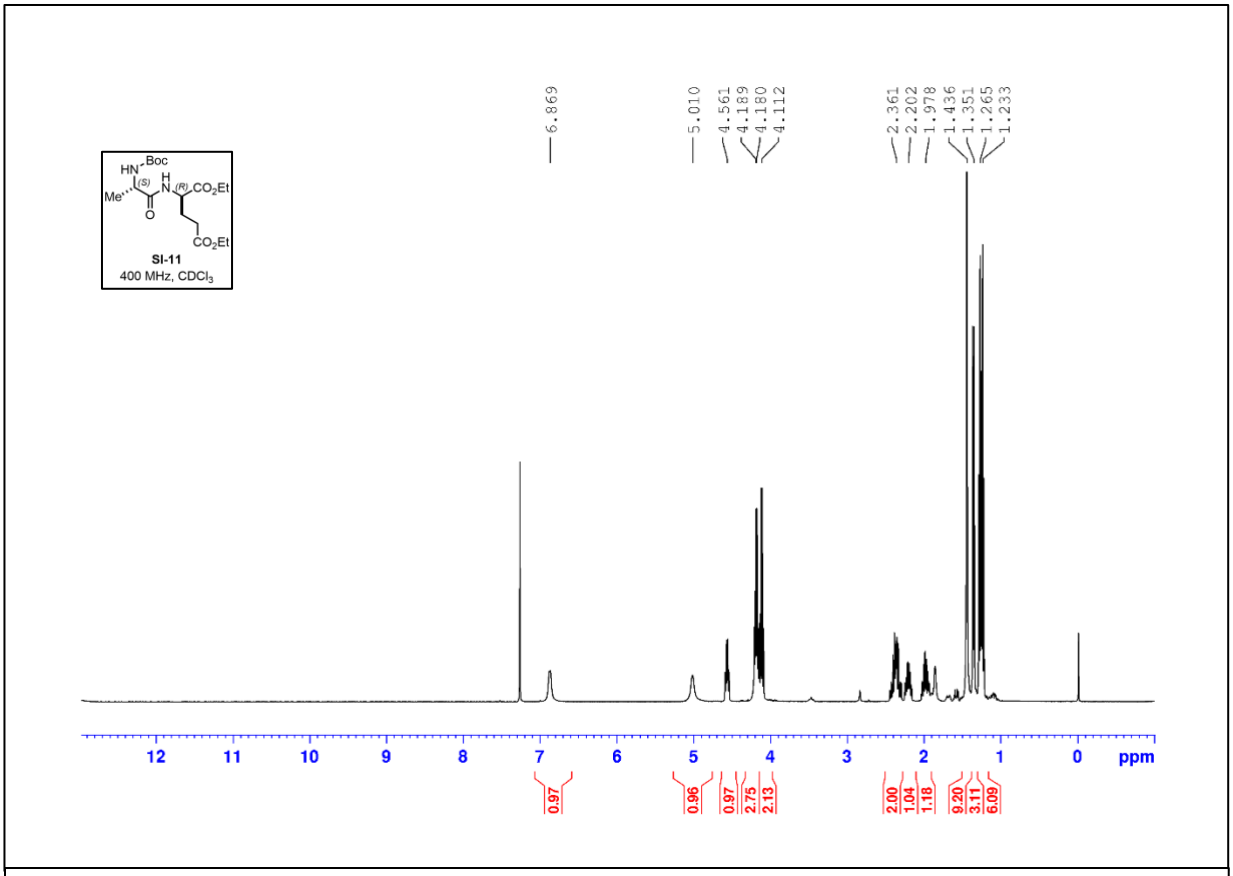
51.14
29.12
25.14
14.07
13.88
SI-10
101 MHz, DMSO-$d_6$
220 200 180 160 140 120 100 80 60 40 20 0 ppm


SI-11
400 MHz, CDCl3
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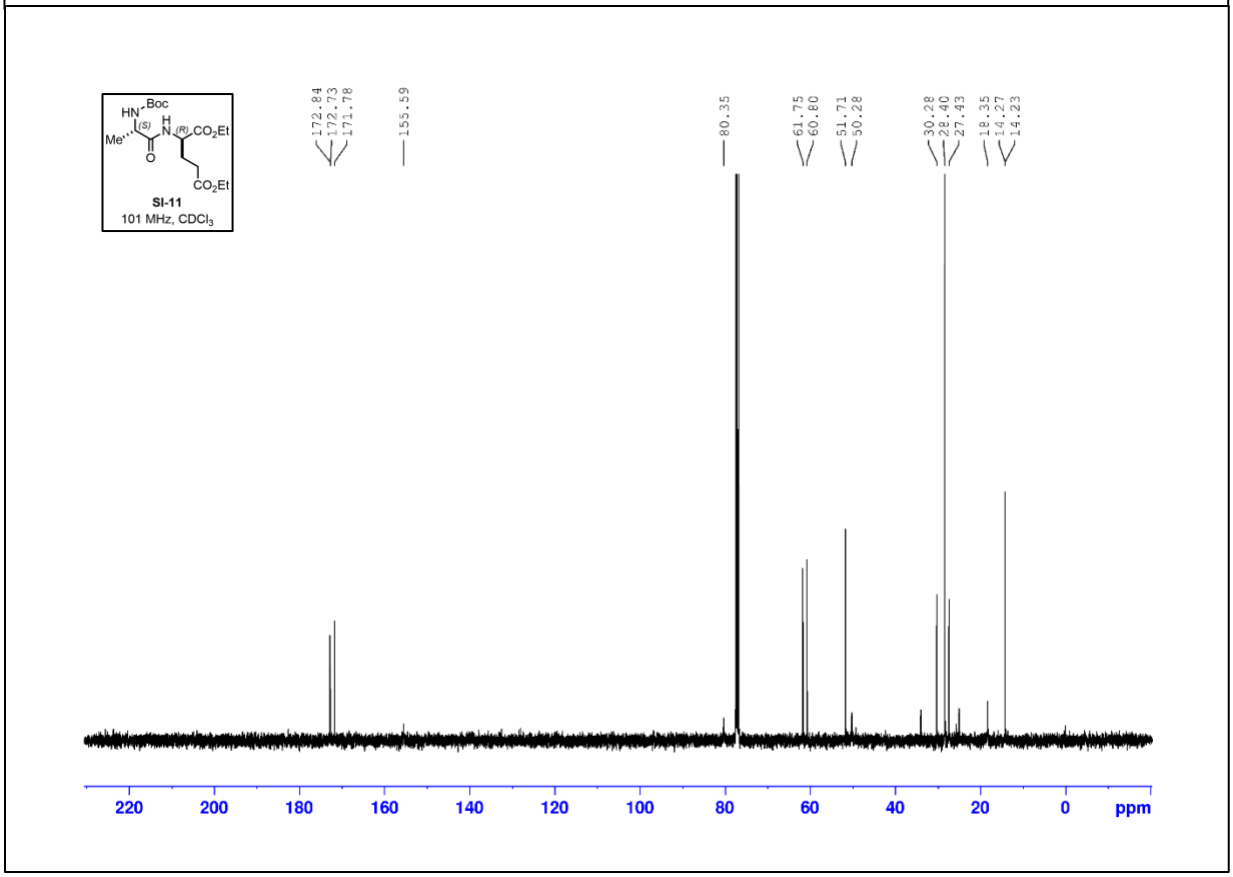
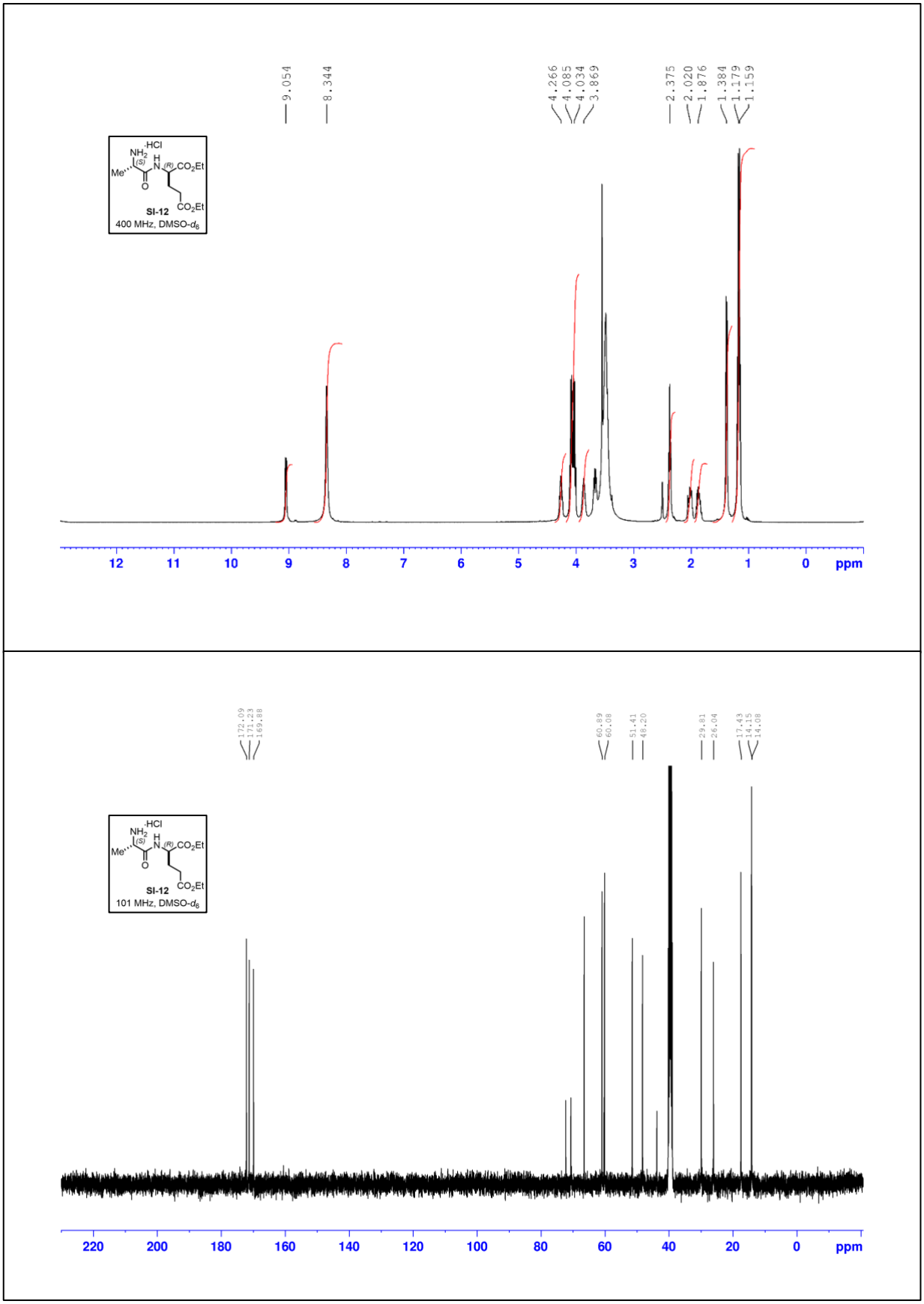
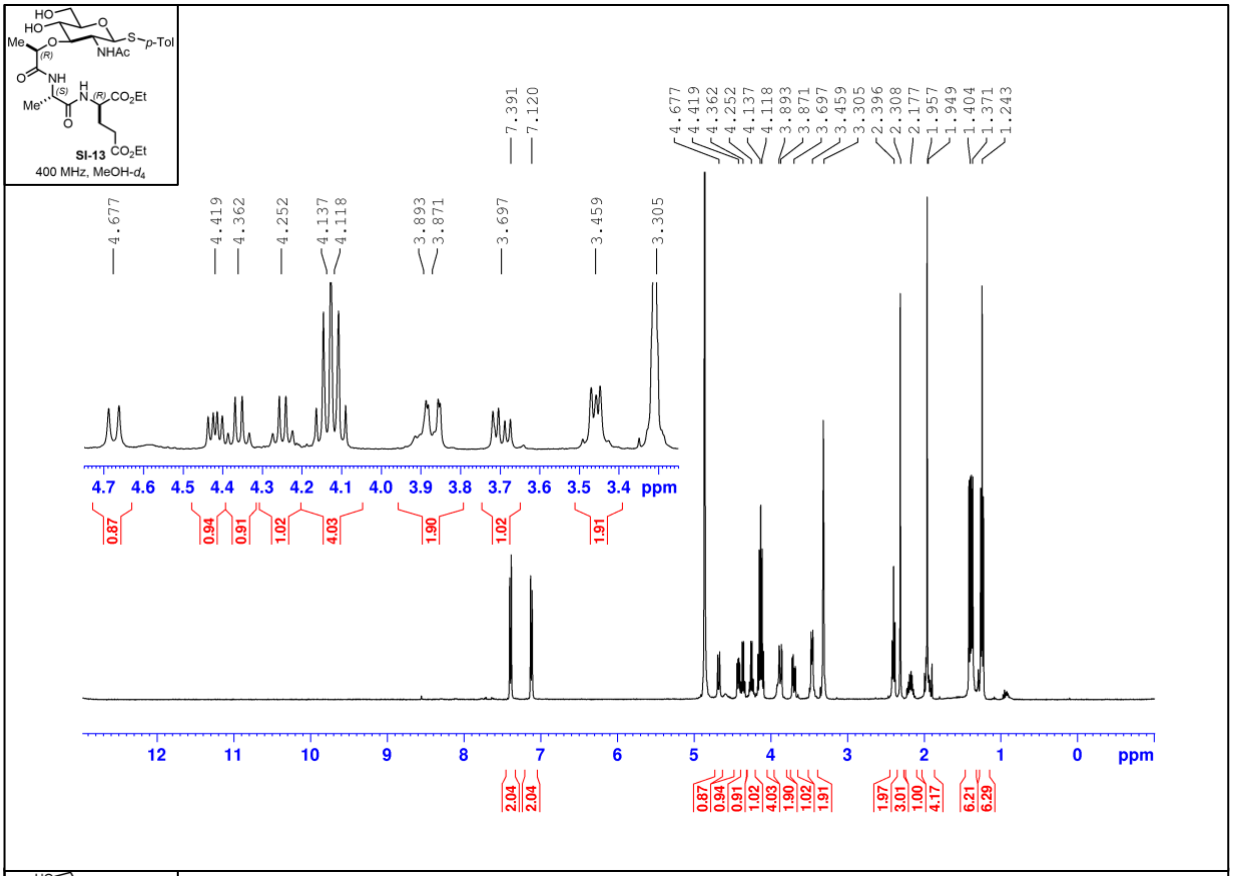
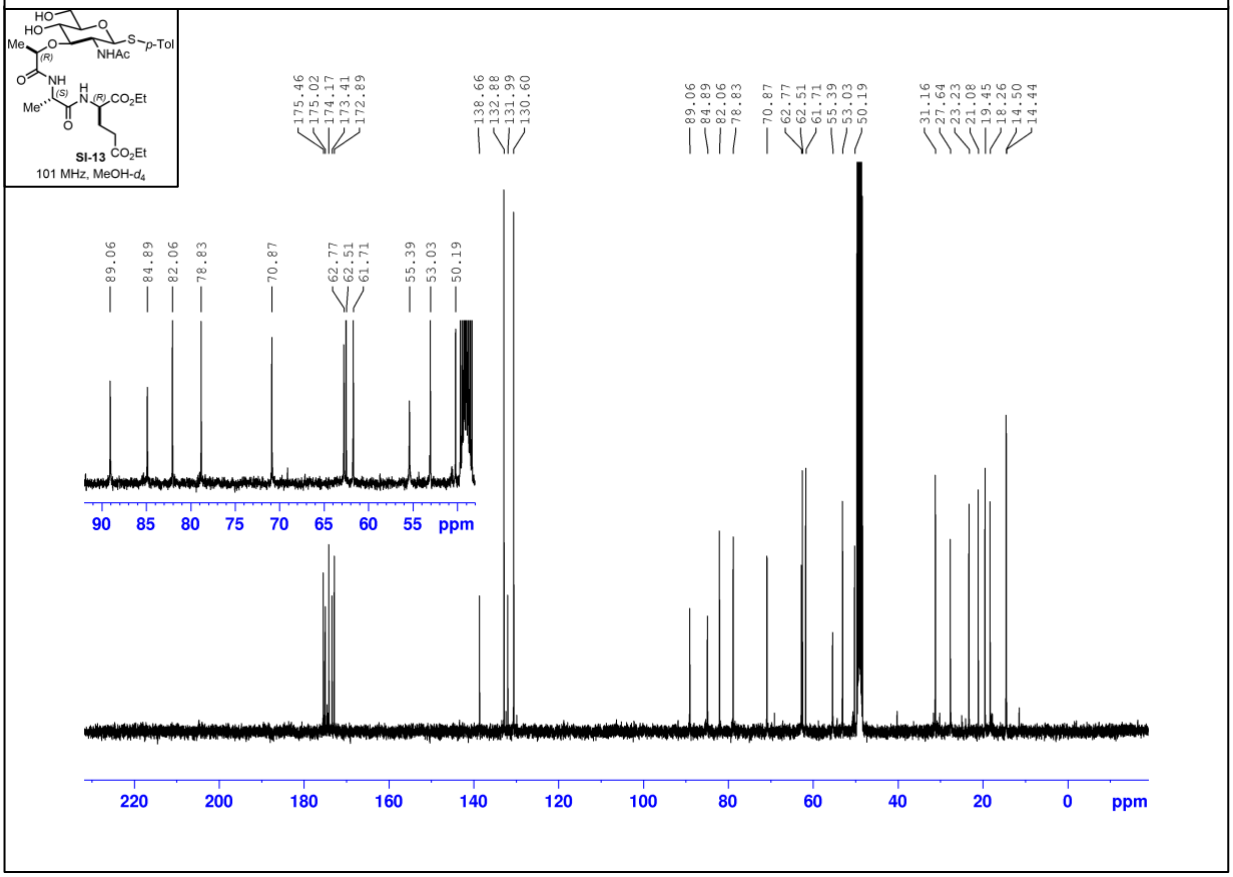
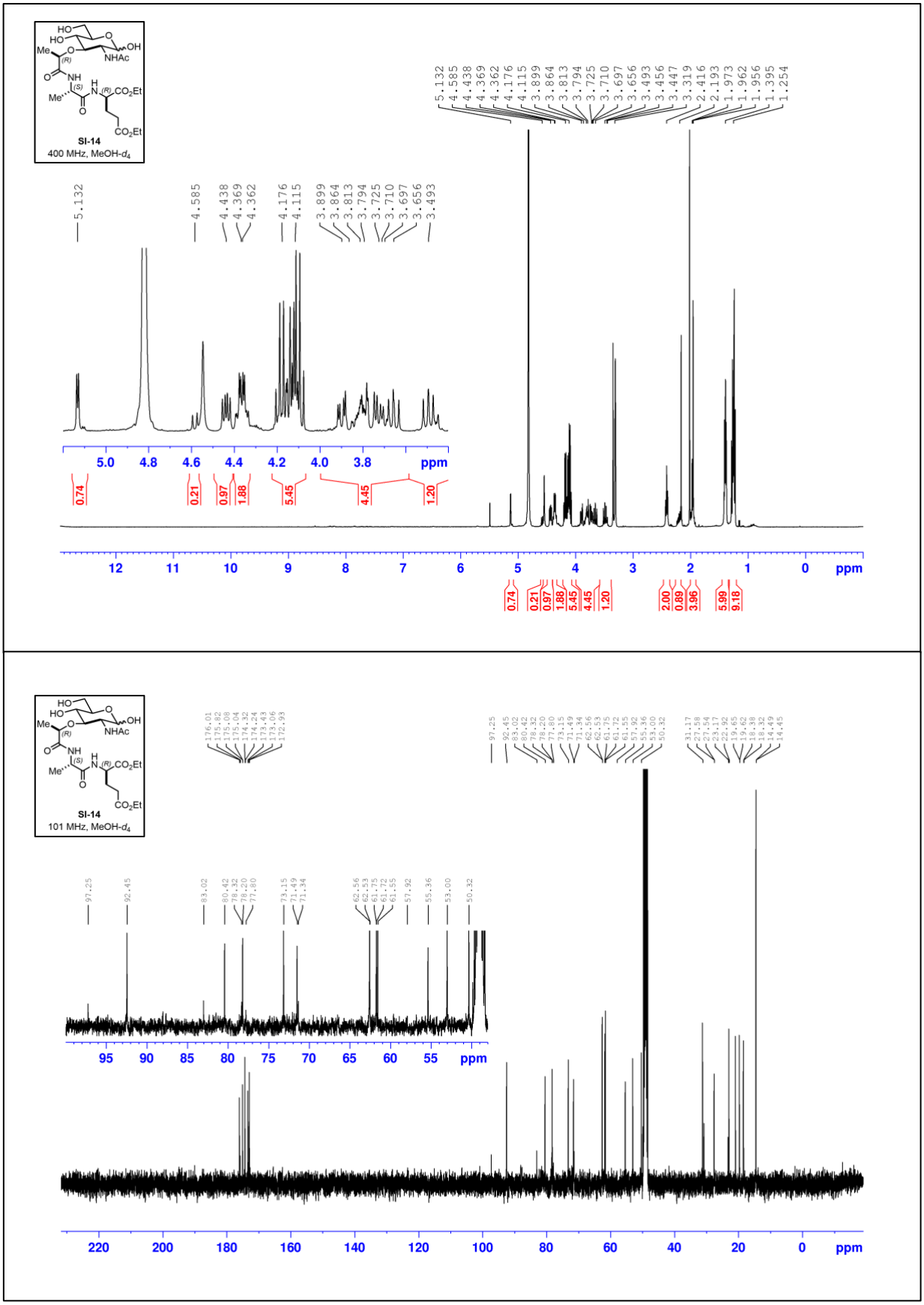
5.010
4.561
4.189
4.180
4.112
2.361
2.202
1.978
1.436
1.351
1.265
1.233
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.97
0.96
0.97
2.75
2.13
2.00
1.04
1.18
9.20
3.11
6.09

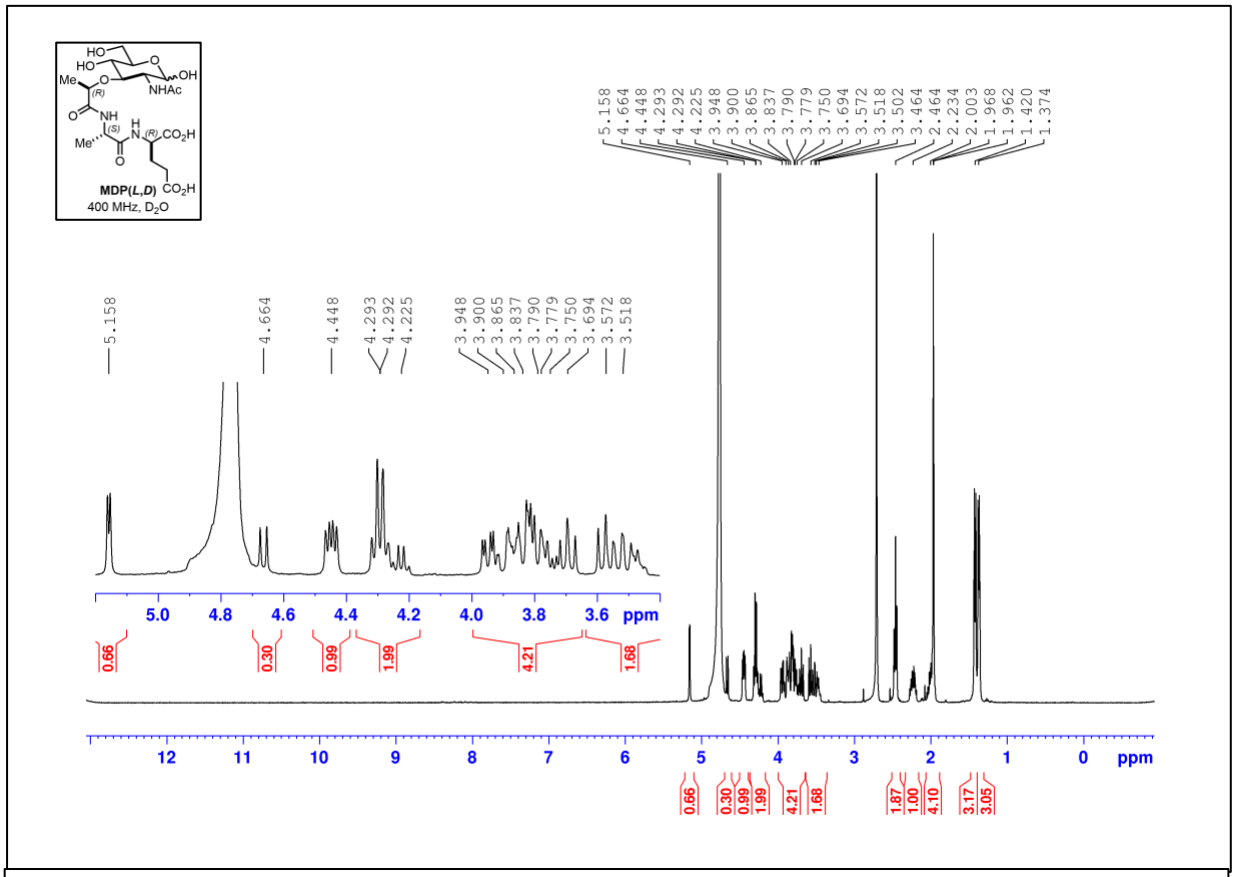

SI-11
101 MHz, CDCl3
172.84
172.73
171.78
155.59
80.35
61.75
60.80
51.71
50.28
30.28
28.40
27.43
18.35
14.27
14.23
220
200
180
160
140
120
100
80
60
40
20
0
ppm

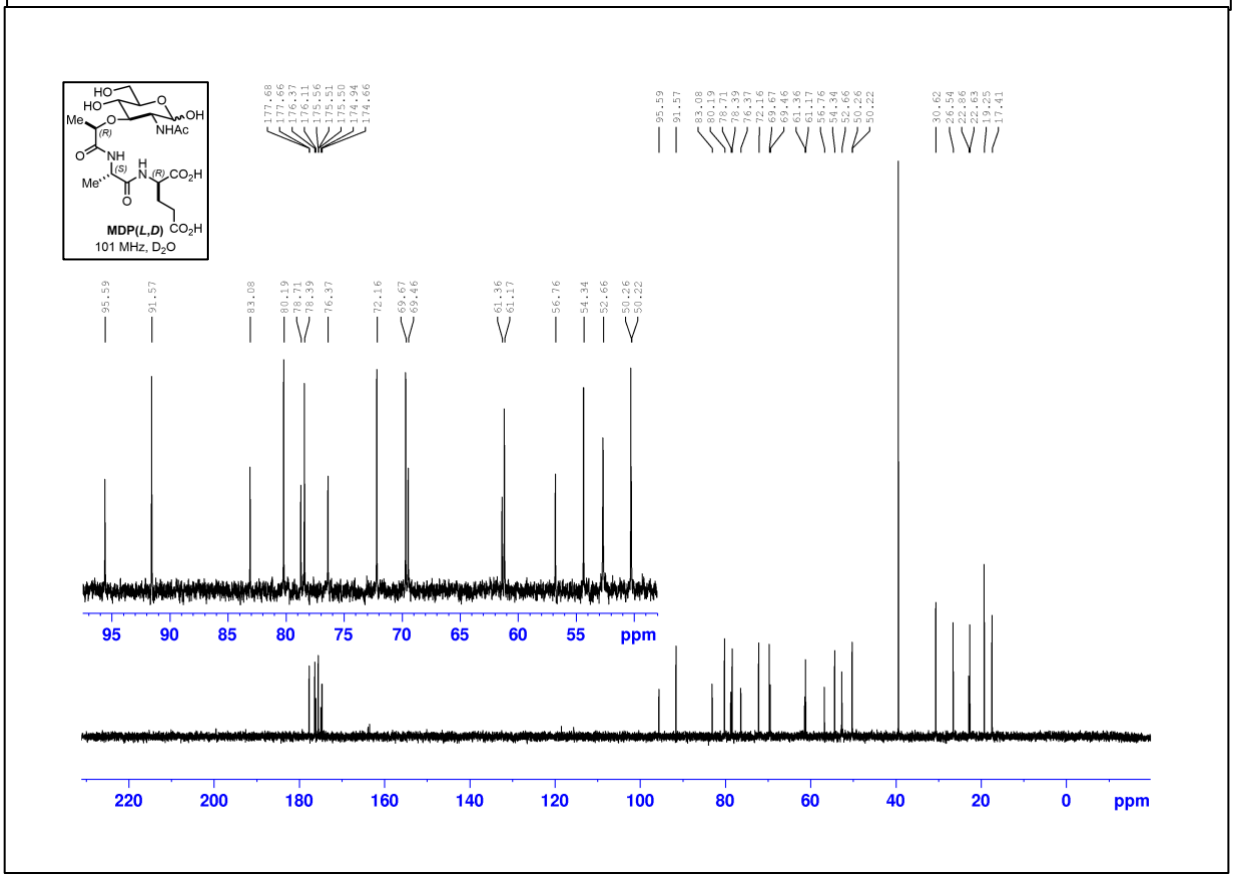

9.054
8.344
4.266
4.085
4.034
3.869
2.375
2.020
1.876
1.384
1.179
1.159
·HCl
SI-12
400 MHz, DMSO-$d_6$
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
172.09
171.23
169.88
60.89
60.08
51.41
48.20
29.81
26.04
17.43
14.15
14.08
·HCl
SI-12
101 MHz, DMSO-$d_6$
220 200 180 160 140 120 100 80 60 40 20 0 ppm

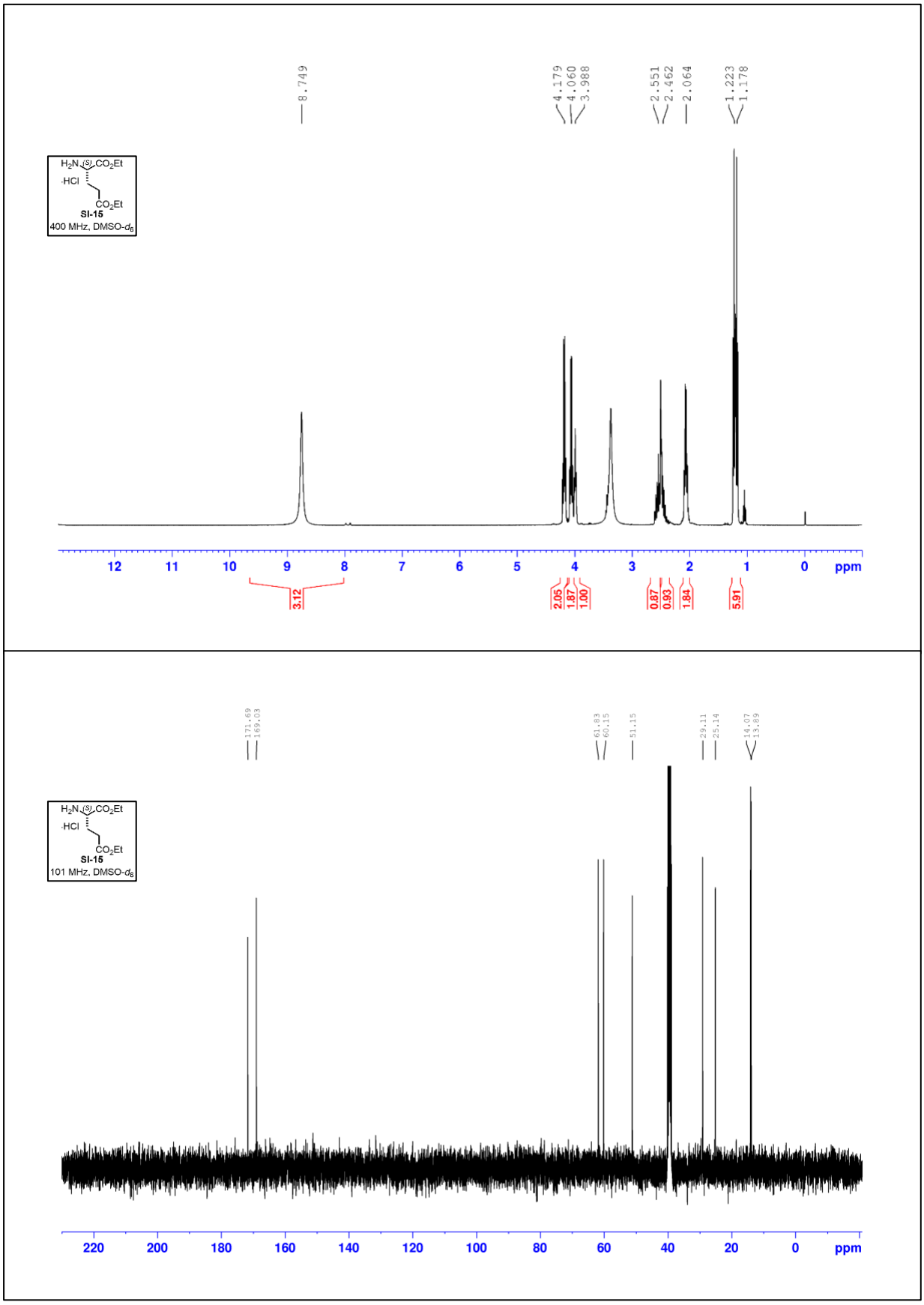

SI-13
400 MHz, MeOH-$d_4$
4.677
4.419
4.362
4.252
4.137
4.118
3.893
3.871
3.697
3.459
3.305
7.391
7.120
2.396
2.308
2.177
1.957
1.949
1.404
1.371
1.243
ppm

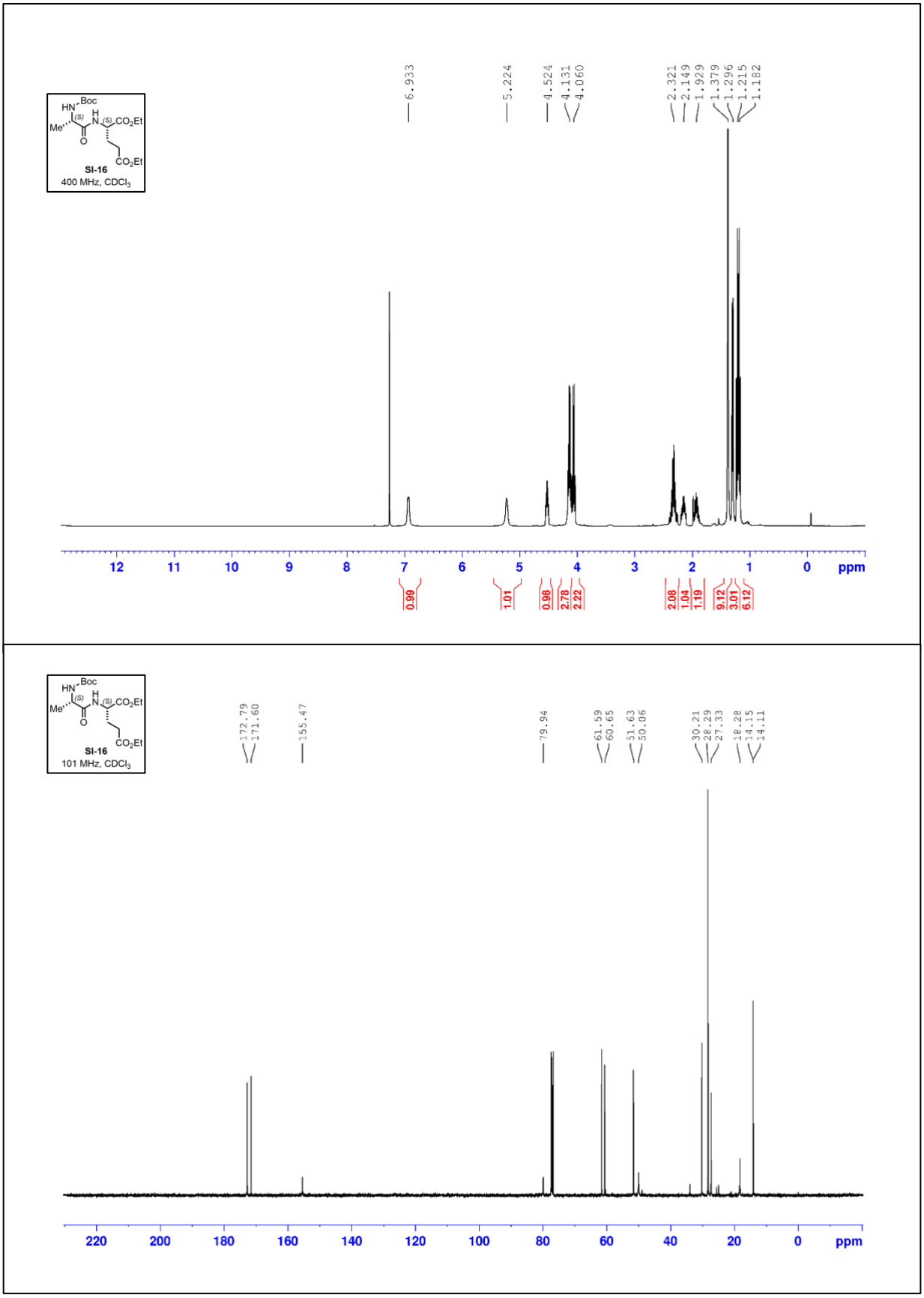

SI-13
101 MHz, MeOH-$d_4$
175.46
175.02
174.17
173.41
172.89
138.66
132.88
131.99
130.60
89.06
84.89
82.06
78.83
70.87
62.77
62.51
61.71
55.39
53.03
50.19
31.16
27.64
23.23
21.08
19.45
18.26
14.50
14.44
ppm

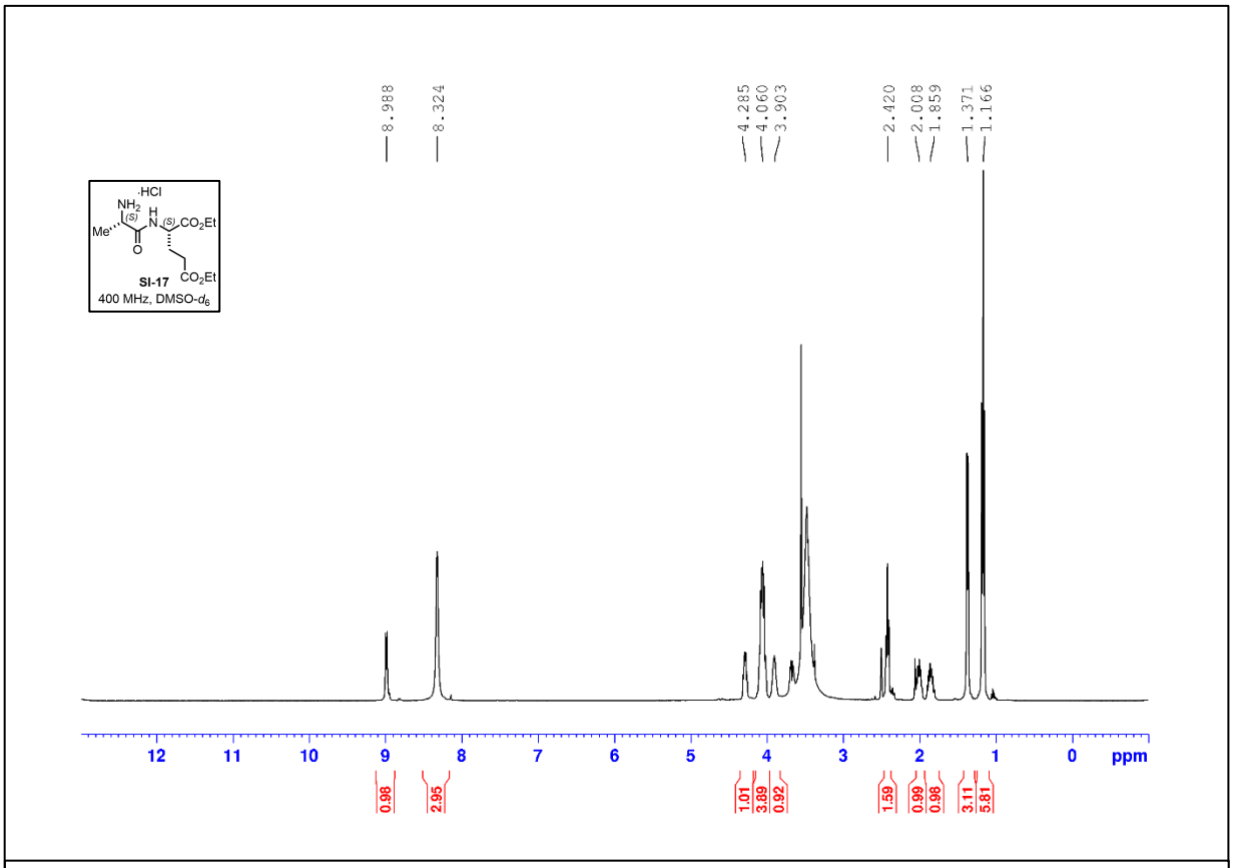

SI-14
400 MHz, MeOH-$d_4$
5.132
4.585
4.438
4.369
4.362
4.176
4.115
3.899
3.864
3.813
3.794
3.725
3.710
3.697
3.656
3.493
3.456
3.447
3.319
2.416
2.193
1.973
1.962
1.956
1.395
1.254
ppm
0.74
0.21
0.97
1.88
5.45
4.45
1.20
2.00
0.89
3.96
5.99
9.18
SI-14
101 MHz, MeOH-$d_4$
176.01
175.82
175.08
175.04
174.32
174.24
173.43
173.06
172.93
97.25
92.45
83.02
80.42
78.32
78.20
77.80
73.15
71.49
71.34
62.56
62.53
61.75
61.72
61.55
57.92
55.36
53.00
50.32
31.17
27.58
27.54
23.17
22.92
19.65
19.62
18.38
18.32
14.49
14.45
ppm

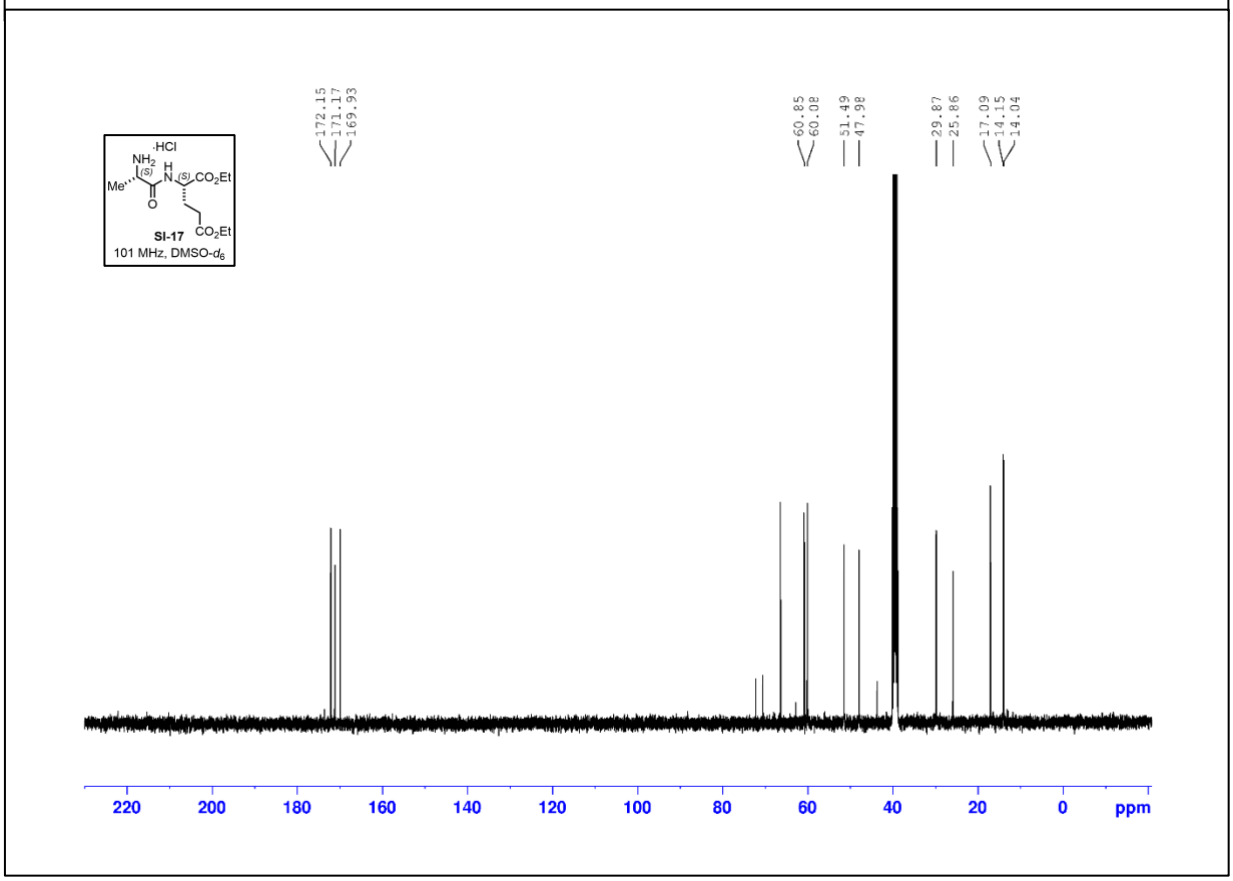

MDP(L,D)
400 MHz, D2O
5.158
4.664
4.448
4.293
4.292
4.225
3.948
3.900
3.865
3.837
3.790
3.779
3.750
3.694
3.572
3.518
3.502
3.464
2.464
2.234
2.003
1.968
1.962
1.420
1.374
ppm
0.66
0.30
0.99
1.99
4.21
1.68
1.87
1.00
4.10
3.17
3.05

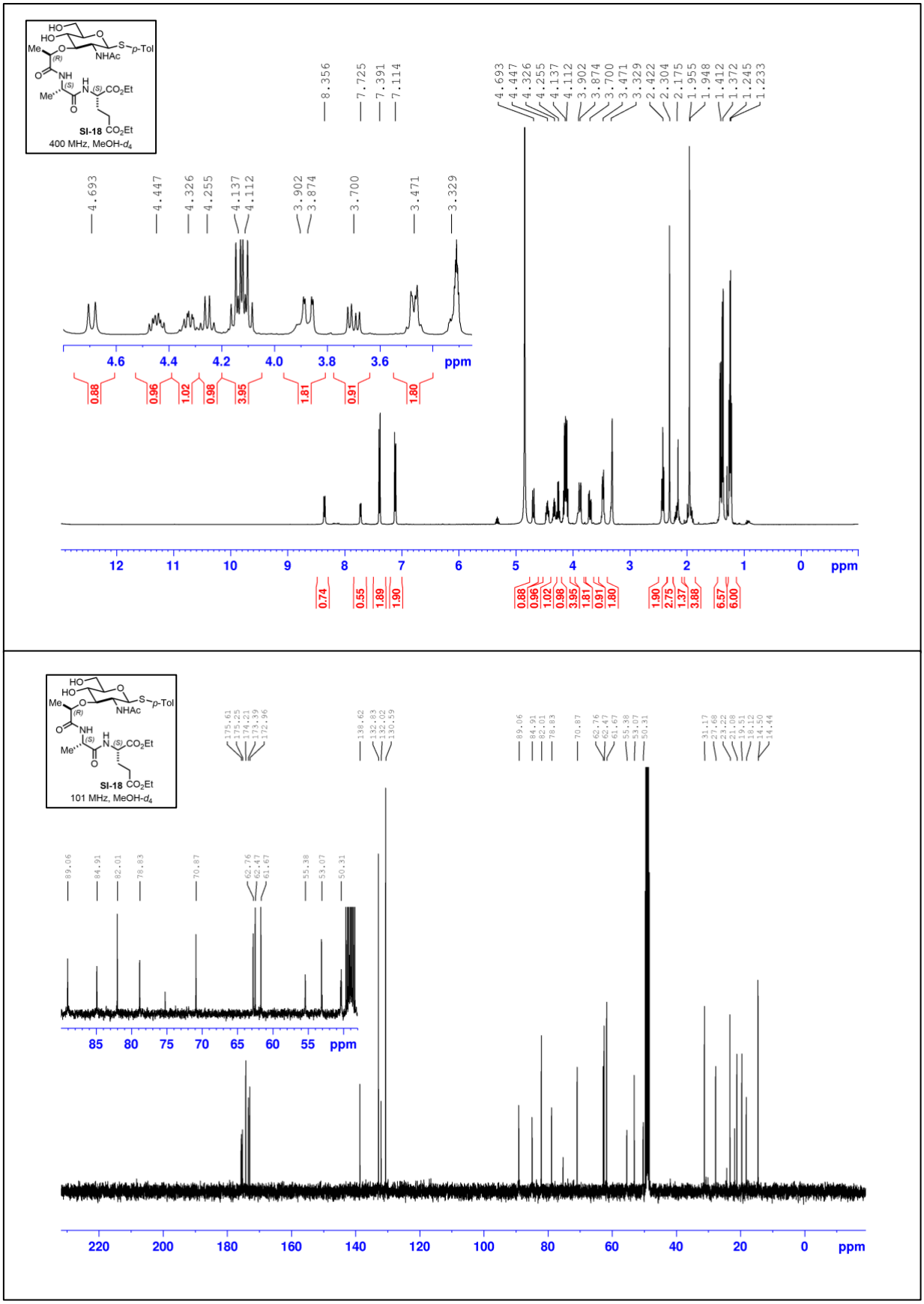

MDP(L,D)
101 MHz, D2O
177.68
177.66
176.37
176.11
175.66
175.51
175.50
174.94
174.66
95.59
91.57
83.08
80.19
78.71
78.39
76.37
72.16
69.67
69.46
61.36
61.17
56.76
54.34
52.66
50.26
50.22
30.62
26.54
22.86
22.63
19.25
17.41
ppm

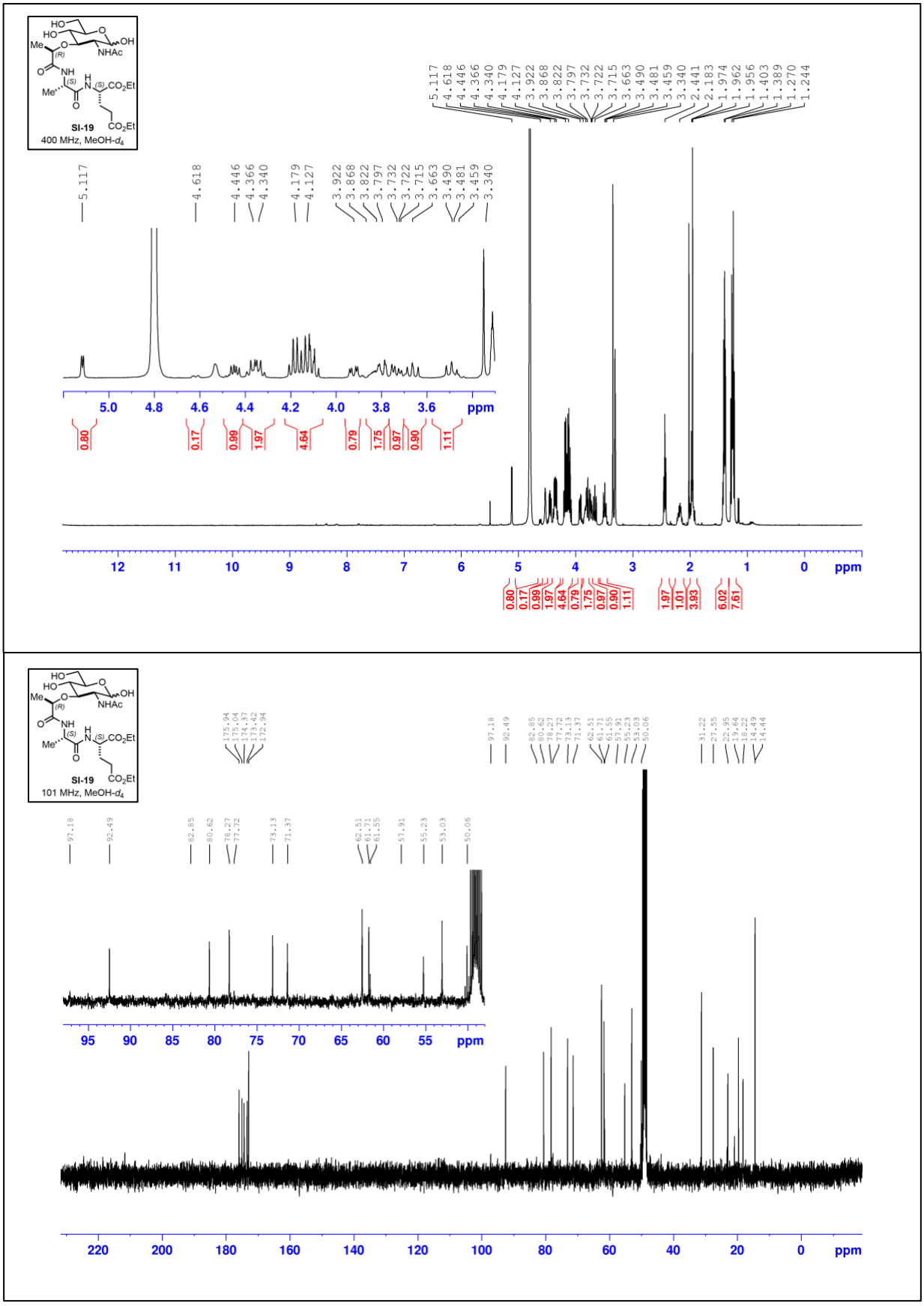

8.749
4.179
4.060
3.988
2.551
2.462
2.064
1.223
1.178
SI-15
400 MHz, DMSO-$d_6$
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
3.12
2.05
1.87
1.00
0.87
0.93
1.84
5.91
171.69
169.03
61.83
60.15
51.15
29.11
25.14
14.07
13.89
SI-15
101 MHz, DMSO-$d_6$
220 200 180 160 140 120 100 80 60 40 20 0 ppm

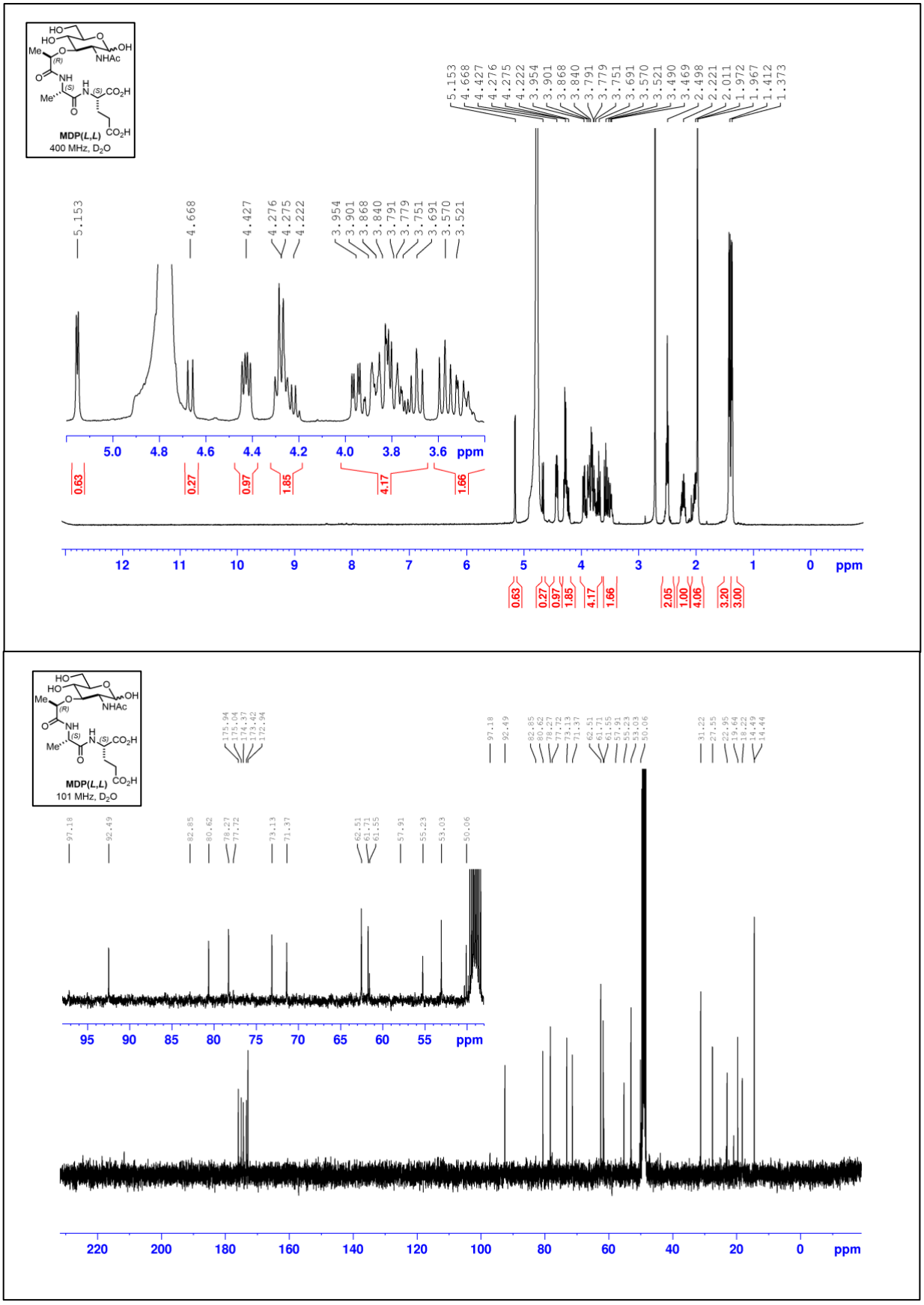

SI-16
400 MHz, CDCl3
6.933
5.224
4.524
4.131
4.060
2.321
2.149
1.929
1.379
1.296
1.215
1.182
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.99
1.01
0.98
2.78
2.22
2.08
1.04
1.19
9.12
3.01
6.12
SI-16
101 MHz, CDCl3
172.79
171.60
155.47
79.94
61.59
60.65
51.63
50.06
30.21
28.29
27.33
18.28
14.15
14.11
220
200
180
160
140
120
100
80
60
40
20
0
ppm

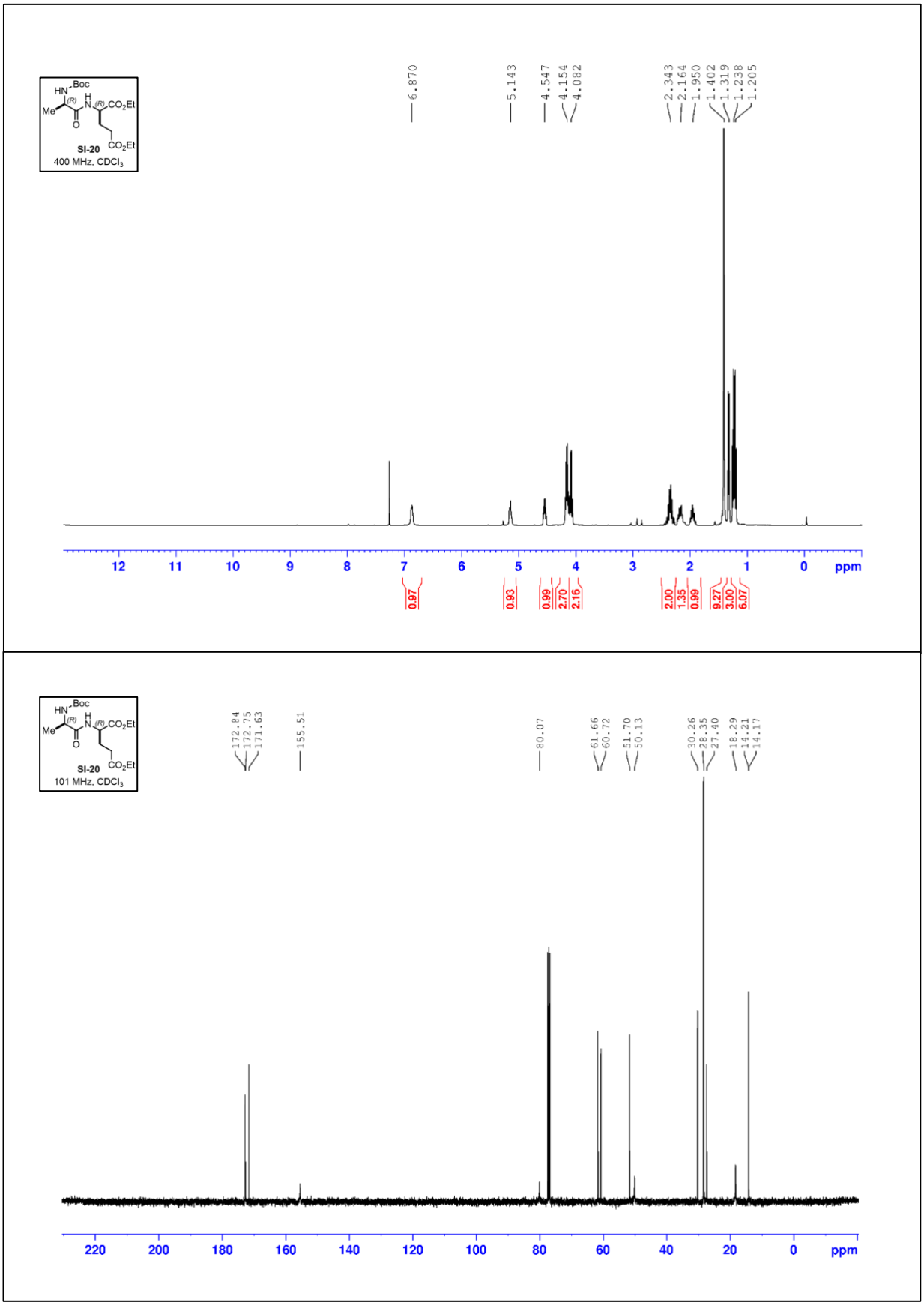

8.988
8.324
4.285
4.060
3.903
2.420
2.008
1.859
1.371
1.166
·HCl
SI-17
400 MHz, DMSO-$d_6$
12 11 10 9 8 7 6 5 4 3 2 1 0 ppm
0.98
2.95
1.01
3.09
0.92
1.59
0.99
0.98
3.11
5.81

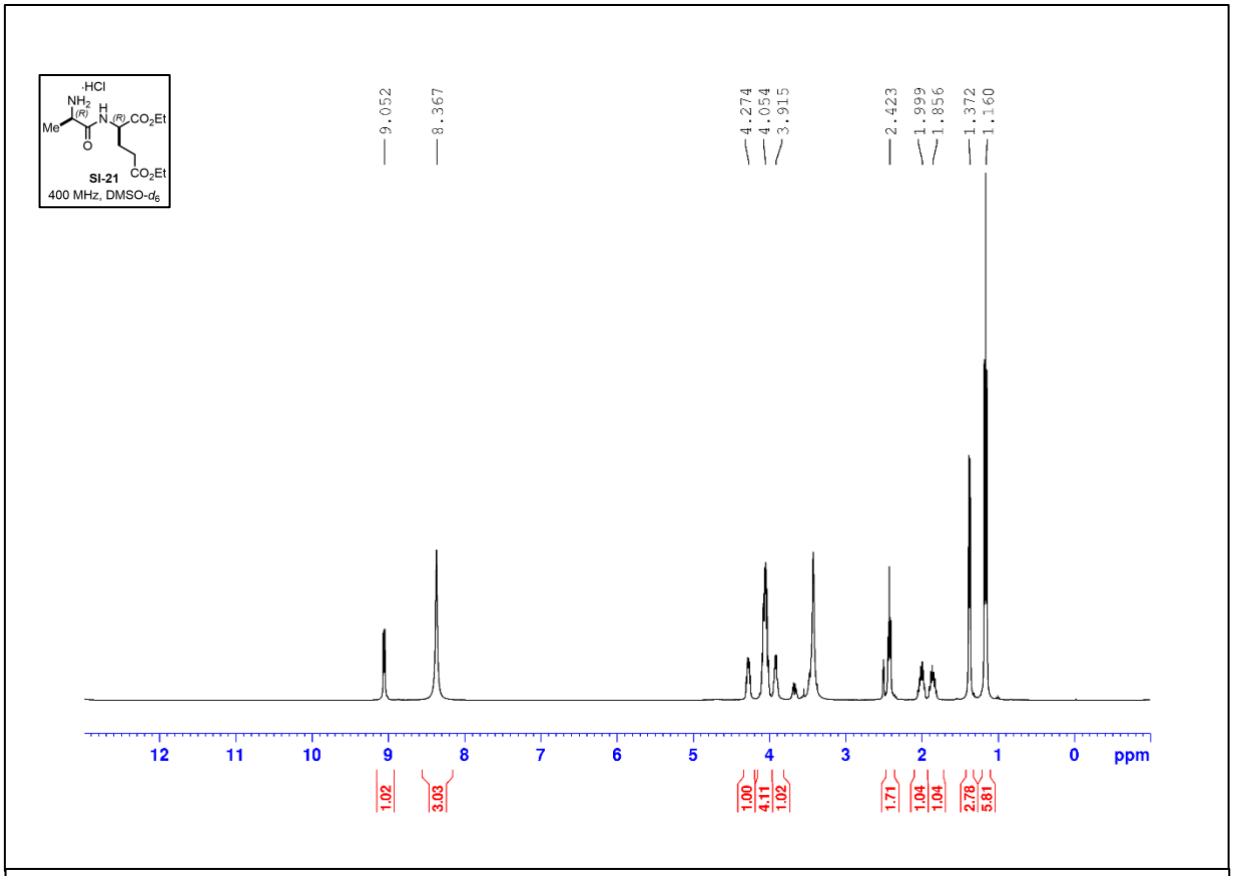

172.15
171.17
169.93
60.85
60.08
51.49
47.98
29.87
25.86
17.09
14.15
14.04
·HCl
SI-17
101 MHz, DMSO-$d_6$
220 200 180 160 140 120 100 80 60 40 20 0 ppm

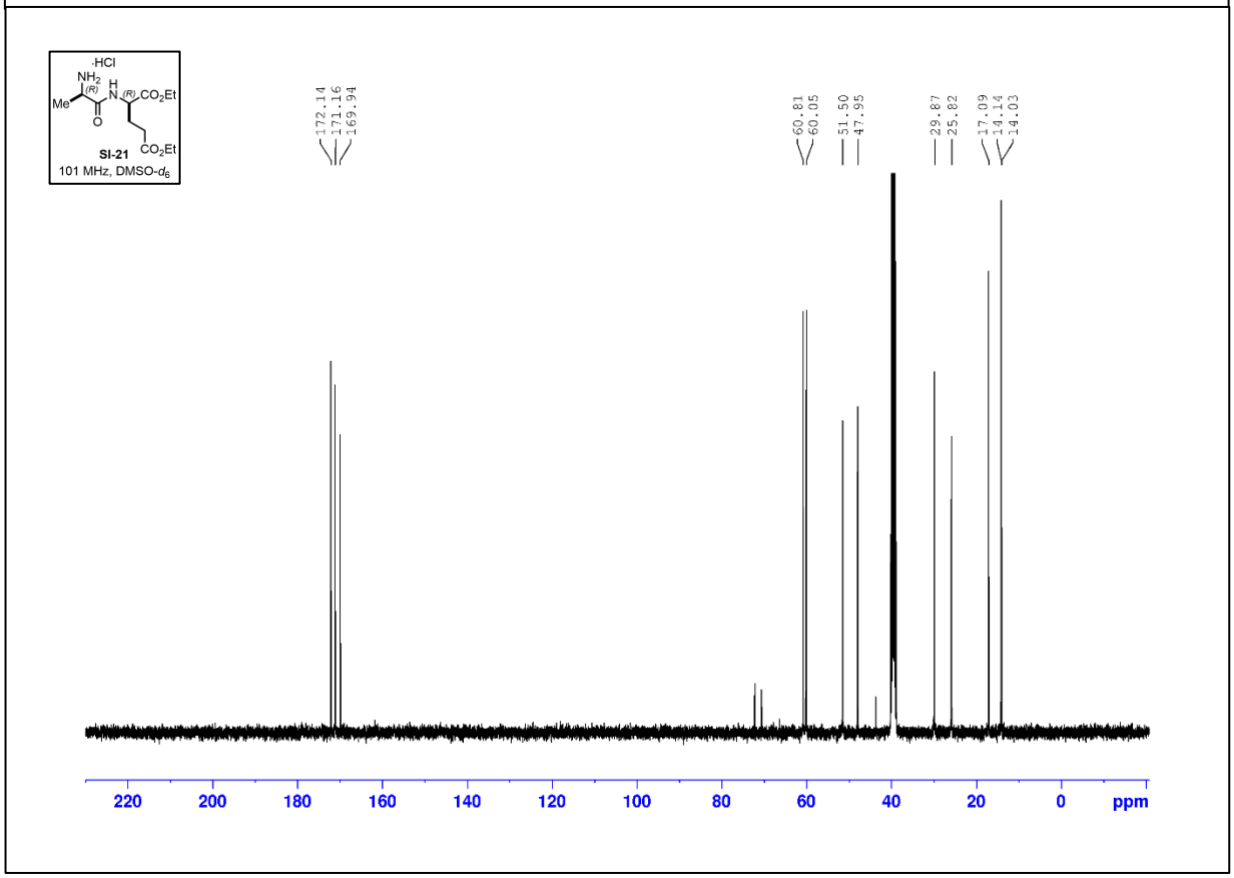

SI-18
400 MHz, MeOH-$d_4$
8.356
7.725
7.391
7.114
4.693
4.447
4.326
4.255
4.137
4.112
3.902
3.874
3.700
3.471
3.329
2.422
2.304
2.175
1.955
1.948
1.412
1.372
1.245
1.233
ppm
SI-18
101 MHz, MeOH-$d_4$
175.61
175.25
174.21
173.39
172.96
138.62
132.83
132.02
130.59
89.06
84.91
82.01
78.83
70.87
62.76
62.47
61.67
55.38
53.07
50.31
31.17
27.68
23.22
21.08
19.51
18.12
14.50
14.44
ppm

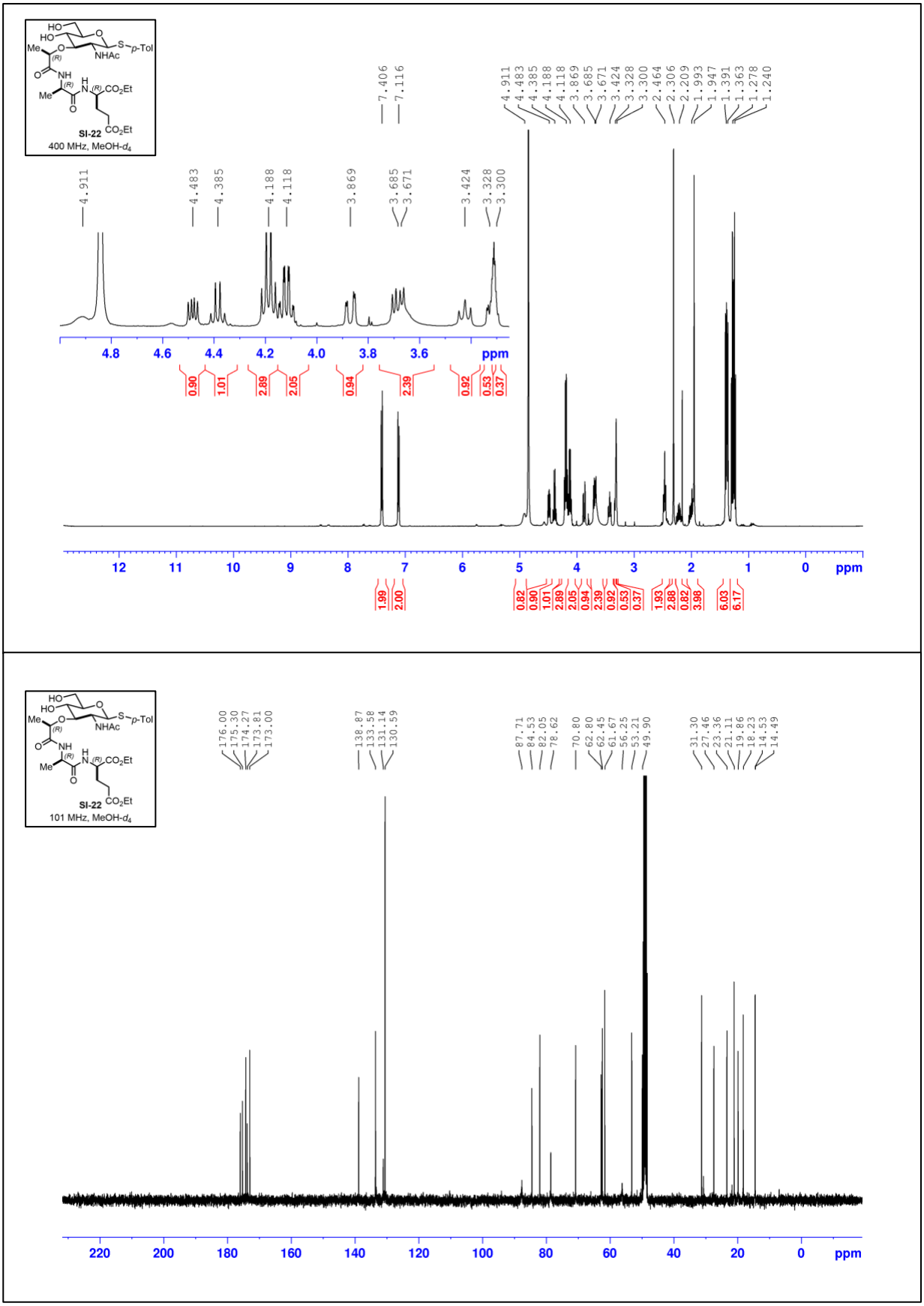

SI-19
400 MHz, MeOH-$d_4$
SI-19
101 MHz, MeOH-$d_4$

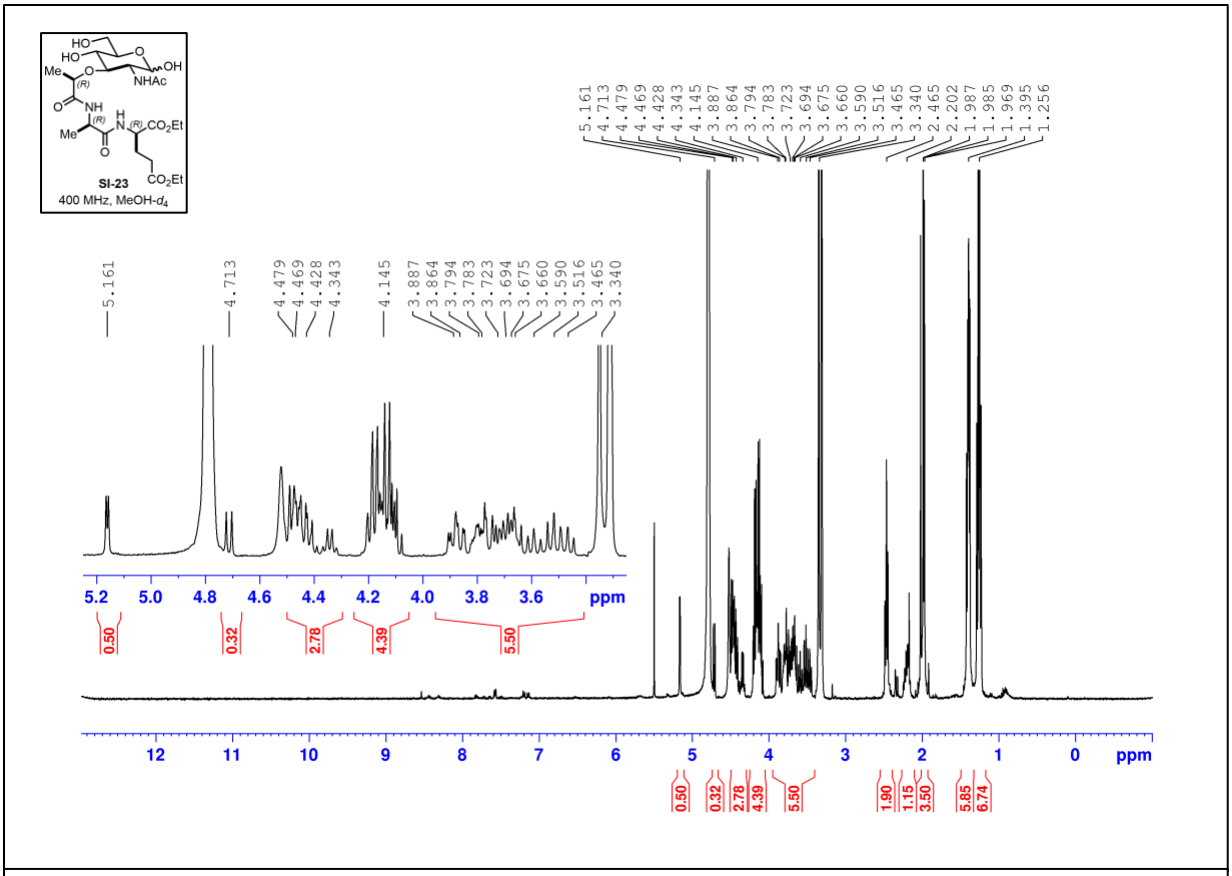

MDP(L,L)
400 MHz, D2O
MDP(L,L)
101 MHz, D2O
ppm

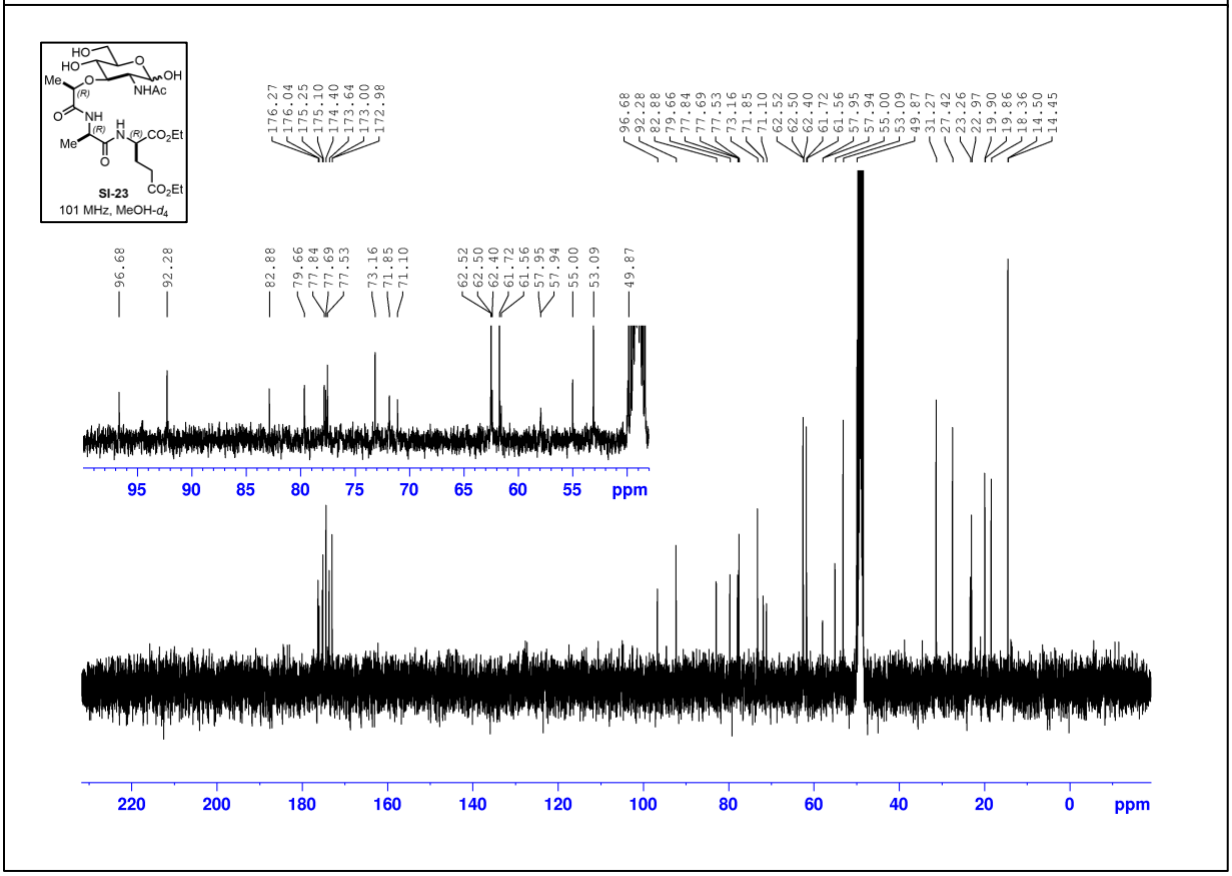

SI-20
400 MHz, CDCl3
6.870
5.143
4.547
4.154
4.082
2.343
2.164
1.950
1.402
1.319
1.238
1.205
ppm
0.97
0.93
0.99
2.70
2.16
2.00
1.35
0.99
9.27
3.00
6.07
SI-20
101 MHz, CDCl3
172.84
172.75
171.63
155.51
80.07
61.66
60.72
51.70
50.13
30.26
28.35
27.40
18.29
14.21
14.17
ppm

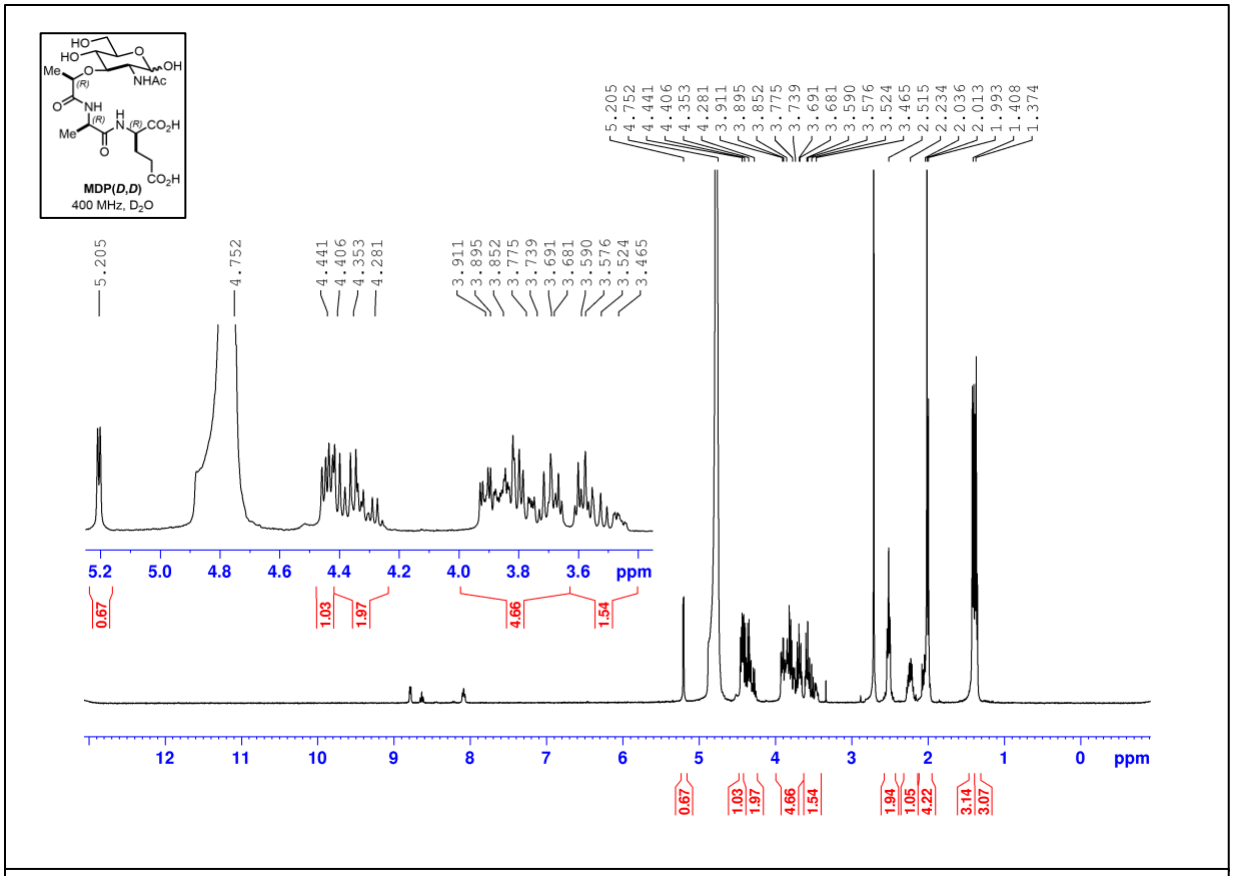

·HCl
NH2
(S)
H
N
(R)
CO2Et
Me
O
CO2Et
SI-21
400 MHz, DMSO-d6
9.052
8.367
4.274
4.054
3.915
2.423
1.999
1.856
1.372
1.160
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
1.02
3.03
1.00
4.11
1.02
1.71
1.04
1.04
2.78
5.81

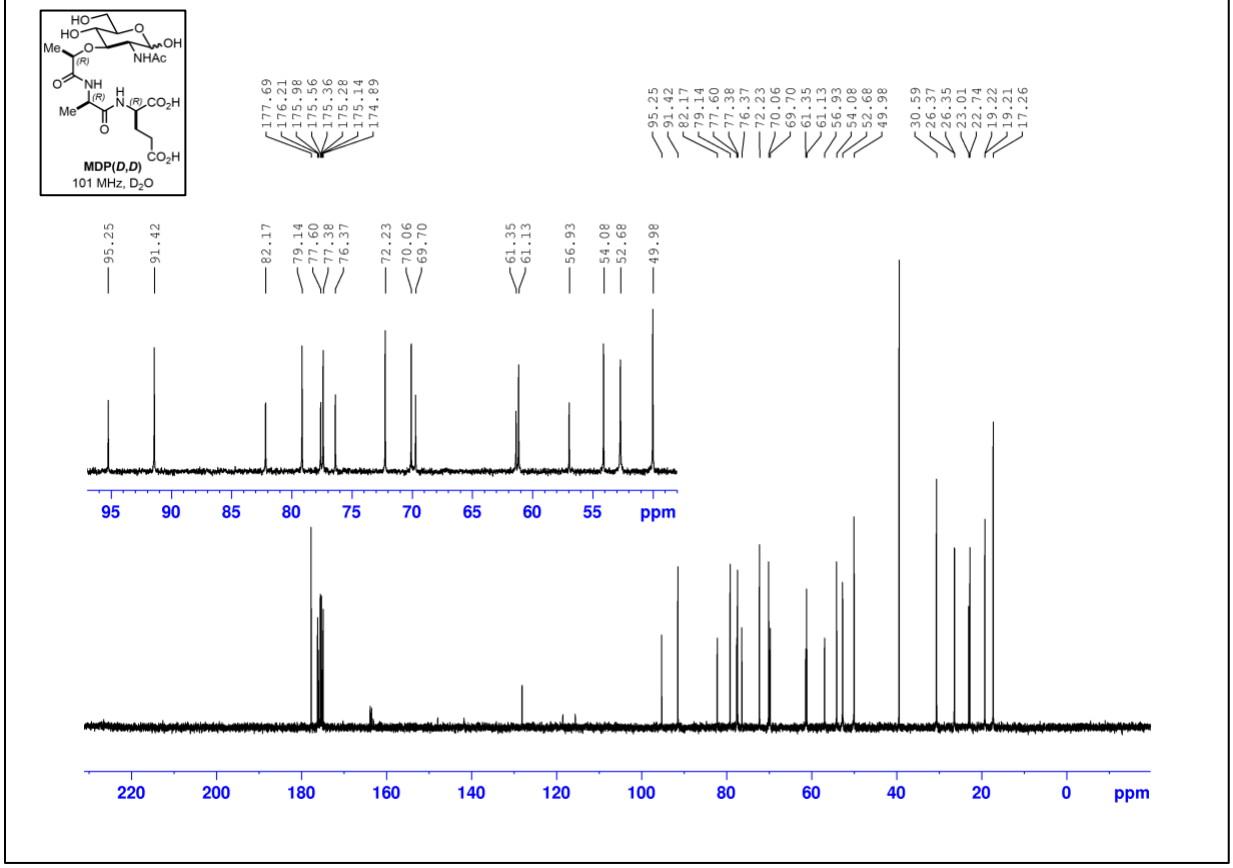

·HCl
NH2
(S)
H
N
(R)
CO2Et
Me
O
CO2Et
SI-21
101 MHz, DMSO-d6
172.14
171.16
169.94
60.81
60.05
51.50
47.95
29.87
25.82
17.09
14.14
14.03
220
200
180
160
140
120
100
80
60
40
20
0
ppm

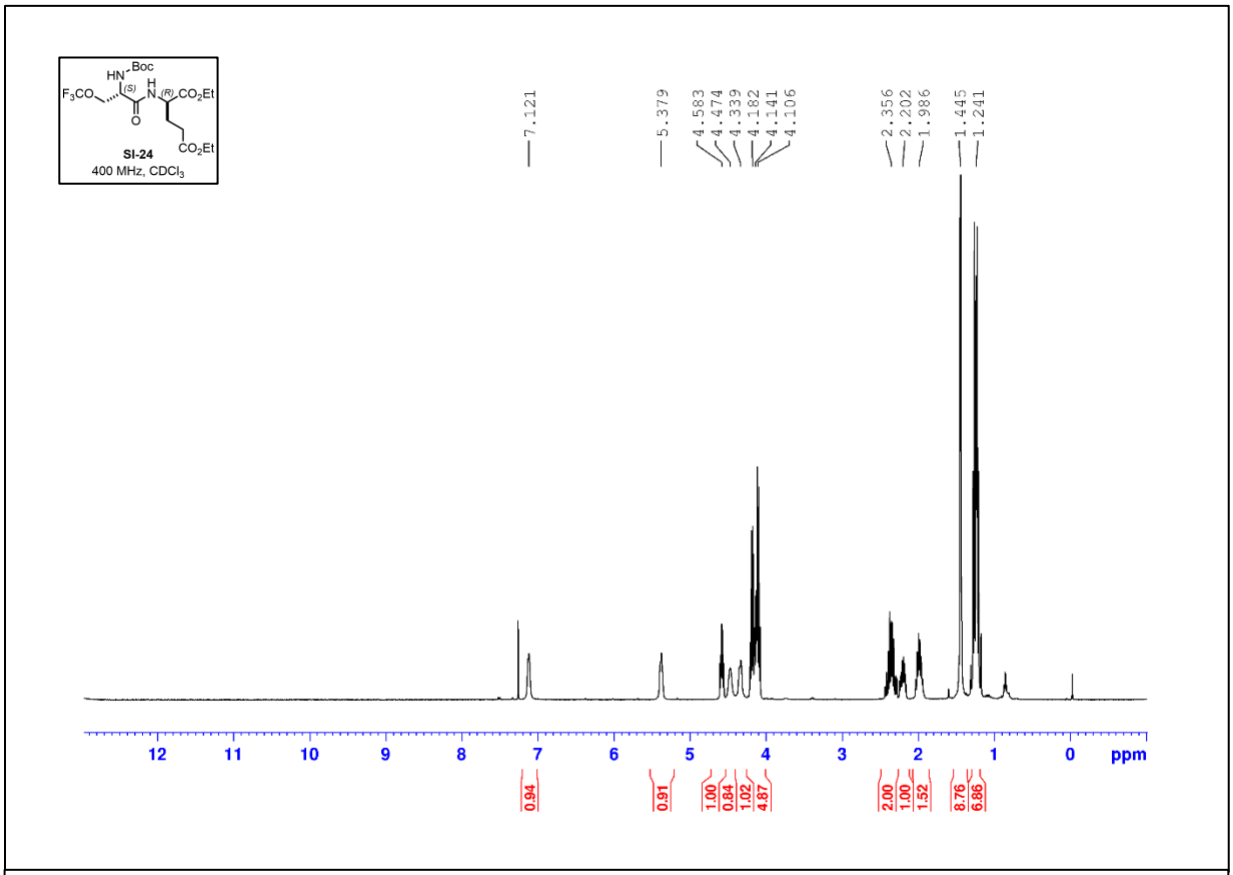

SI-22
400 MHz, MeOH-$d_4$
7.406
7.116
4.911
4.483
4.385
4.188
4.118
3.869
3.685
3.671
3.424
3.328
3.300
2.464
2.306
2.209
1.993
1.947
1.391
1.363
1.278
1.240
ppm
SI-22
101 MHz, MeOH-$d_4$
176.00
175.30
174.27
173.81
173.00
138.87
133.58
131.14
130.59
87.71
84.53
82.05
78.62
70.80
62.80
62.45
61.67
56.25
53.21
49.90
31.30
27.46
23.36
21.11
19.86
18.23
14.53
14.49
ppm

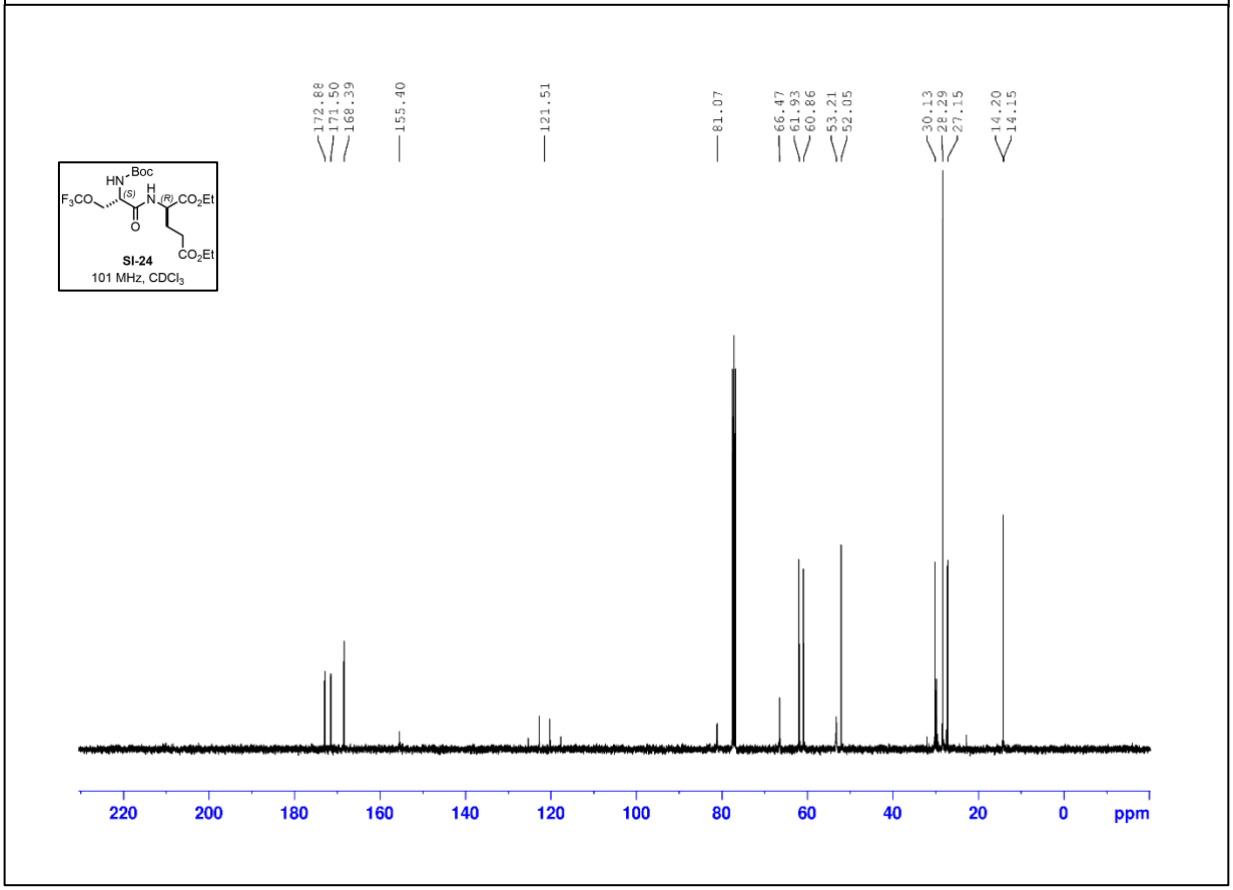

SI-23
400 MHz, MeOH-$d_4$
5.161
4.713
4.479
4.469
4.428
4.343
4.145
3.887
3.864
3.794
3.783
3.723
3.694
3.675
3.660
3.590
3.516
3.465
3.340
2.465
2.202
1.987
1.985
1.969
1.395
1.256
ppm
0.50
0.32
2.78
4.39
5.50
1.90
1.15
3.50
5.85
6.74

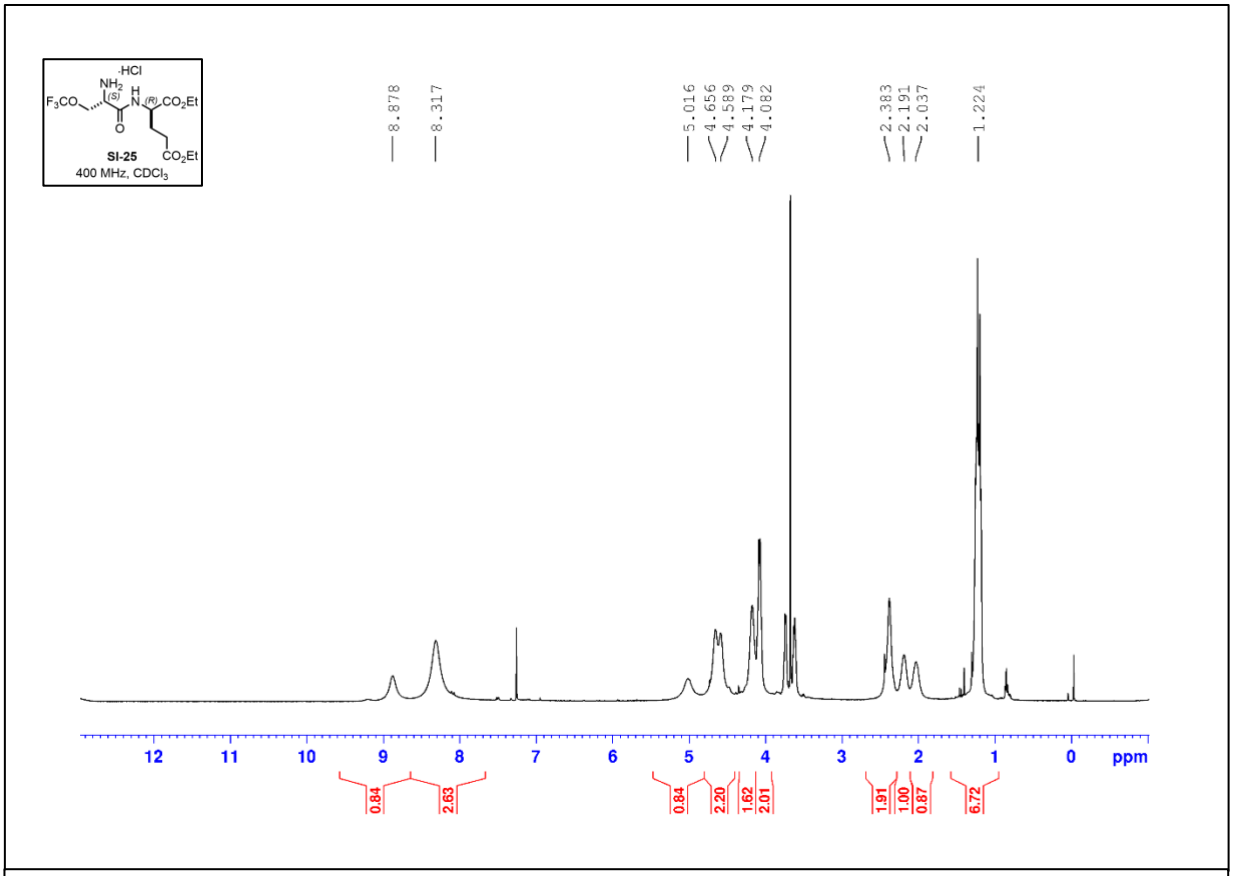

SI-23
101 MHz, MeOH-$d_4$
176.27
176.04
175.25
175.10
174.40
173.64
173.00
172.98
96.68
92.28
82.88
79.66
77.84
77.69
77.53
73.16
71.85
71.10
62.52
62.50
62.40
61.72
61.56
57.95
57.94
55.00
53.09
49.87
31.27
27.42
23.26
22.97
19.90
19.86
18.36
14.50
14.45
ppm

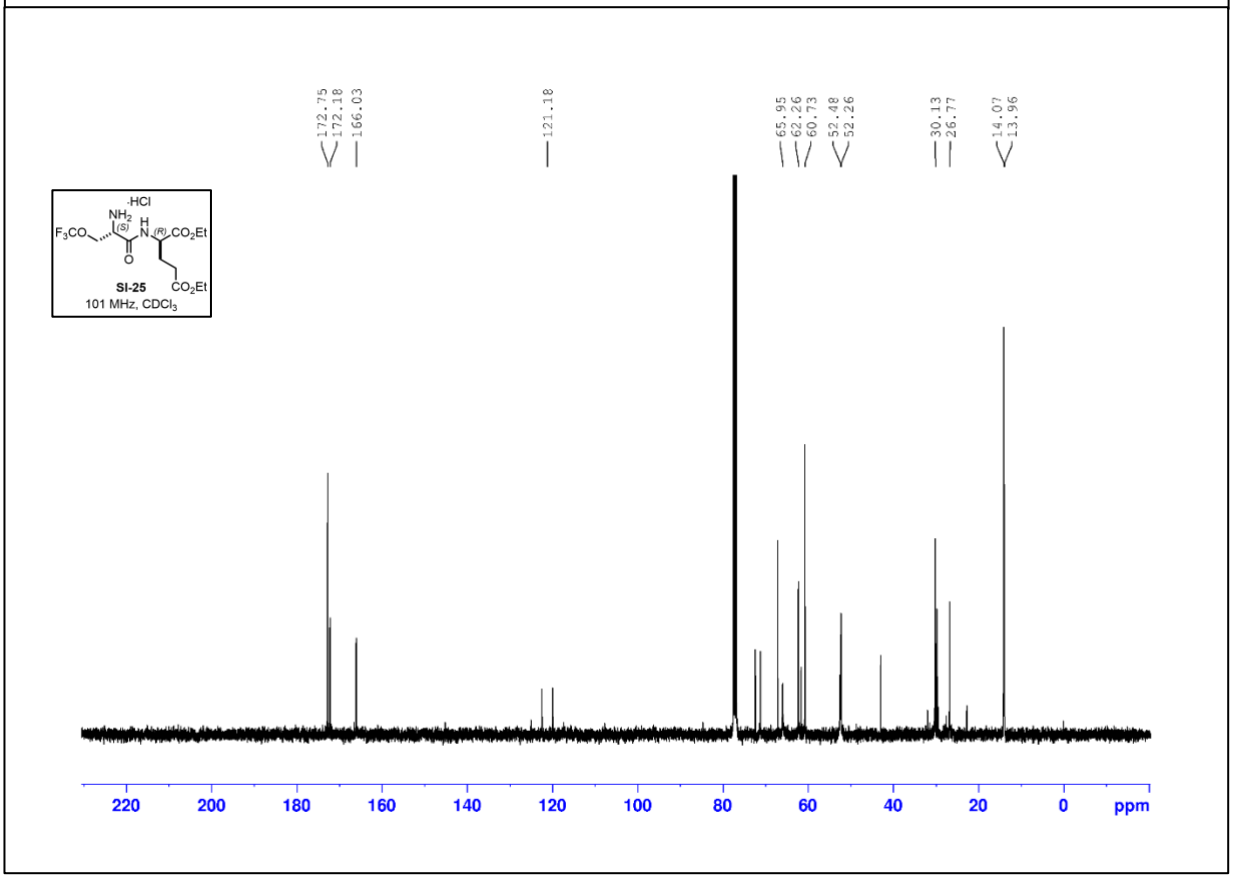

MDP(D,D)
400 MHz, D2O
5.205
4.752
4.441
4.406
4.353
4.281
3.911
3.895
3.852
3.775
3.739
3.691
3.681
3.590
3.576
3.524
3.465
2.515
2.234
2.036
2.013
1.993
1.408
1.374
ppm

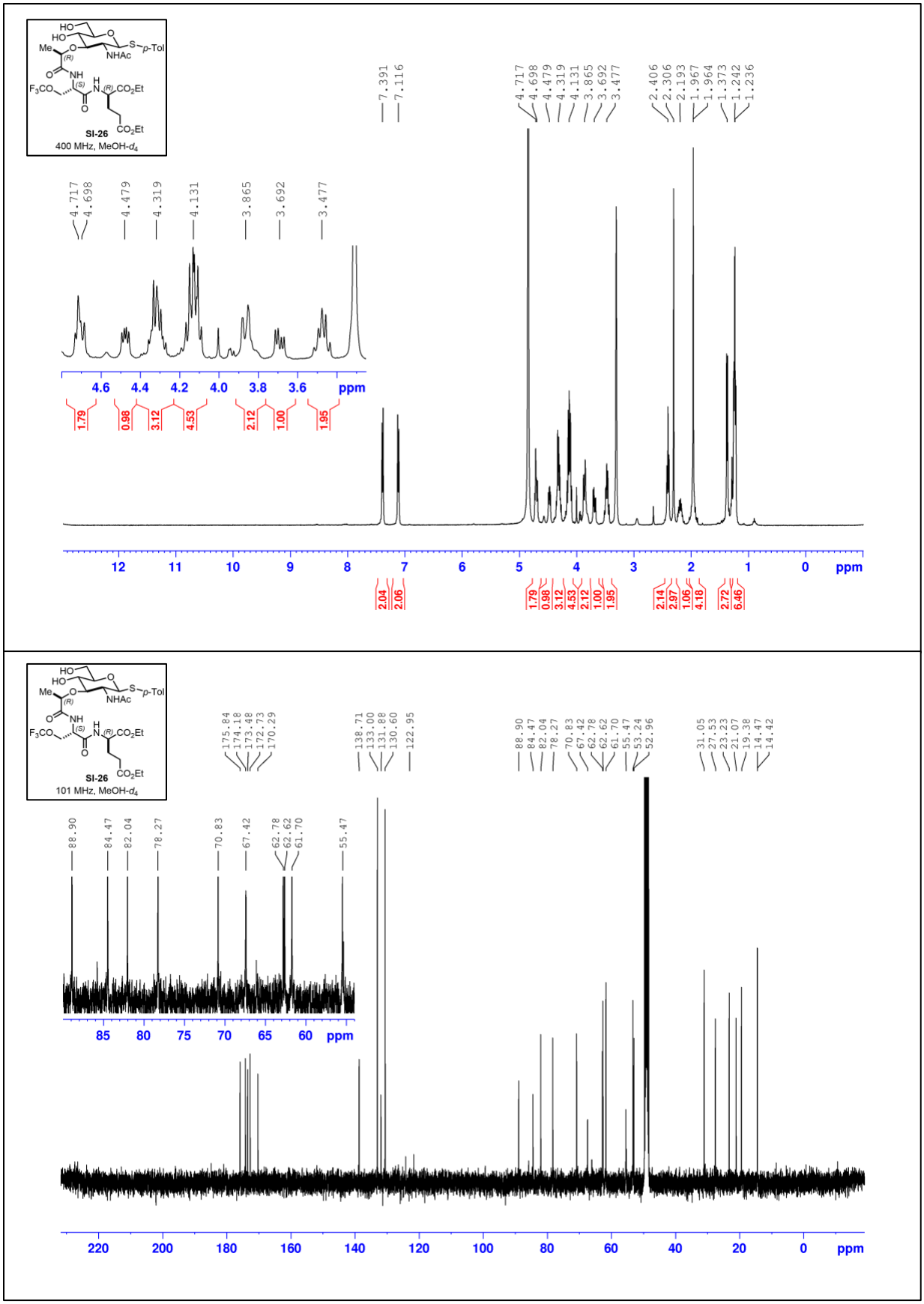

MDP(D,D)
101 MHz, D2O
177.69
176.21
175.98
175.56
175.36
175.28
175.14
174.89
95.25
91.42
82.17
79.14
77.60
77.38
76.37
72.23
70.06
69.70
61.35
61.13
56.93
54.08
52.68
49.98
30.59
26.37
26.35
23.01
22.74
19.22
19.21
17.26
ppm

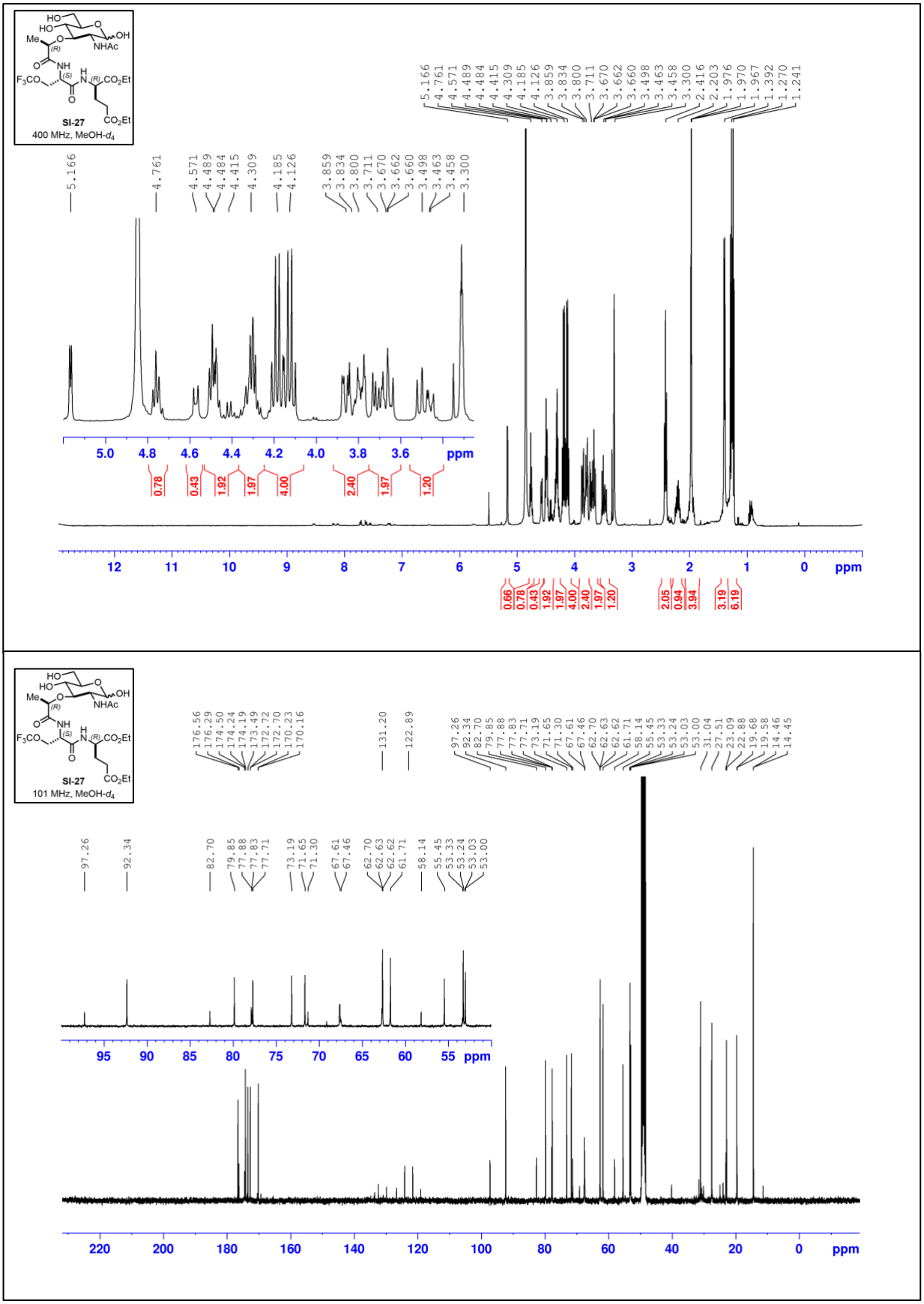

SI-24
400 MHz, CDCl3
7.121
5.379
4.583
4.474
4.339
4.182
4.141
4.106
2.356
2.202
1.986
1.445
1.241
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.94
0.91
1.00
0.84
1.02
4.87
2.00
1.00
1.52
8.76
6.86

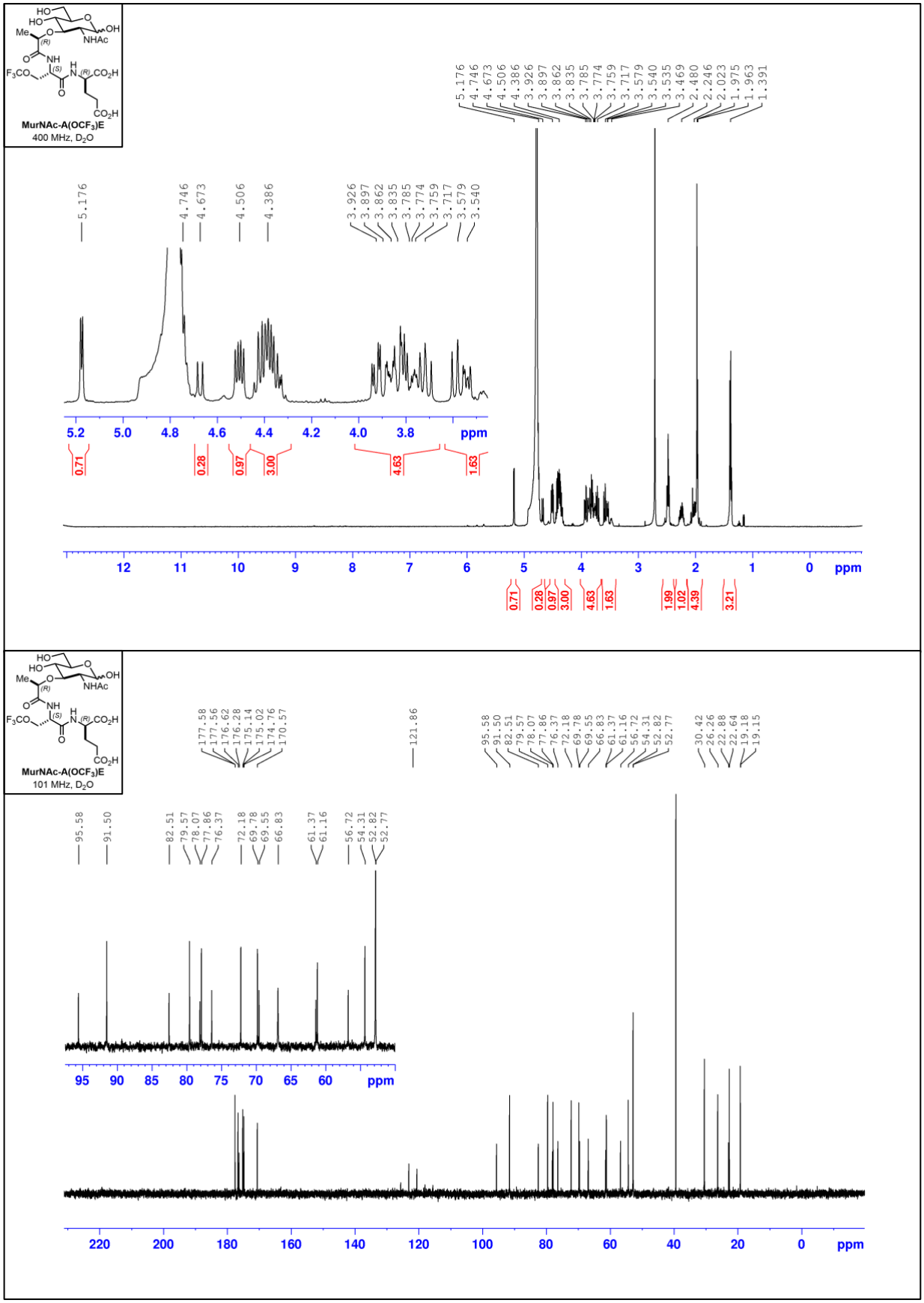

SI-24
101 MHz, CDCl3
172.88
171.50
168.39
155.40
121.51
81.07
66.47
61.93
60.86
53.21
52.05
30.13
28.29
27.15
14.20
14.15
220
200
180
160
140
120
100
80
60
40
20
0
ppm

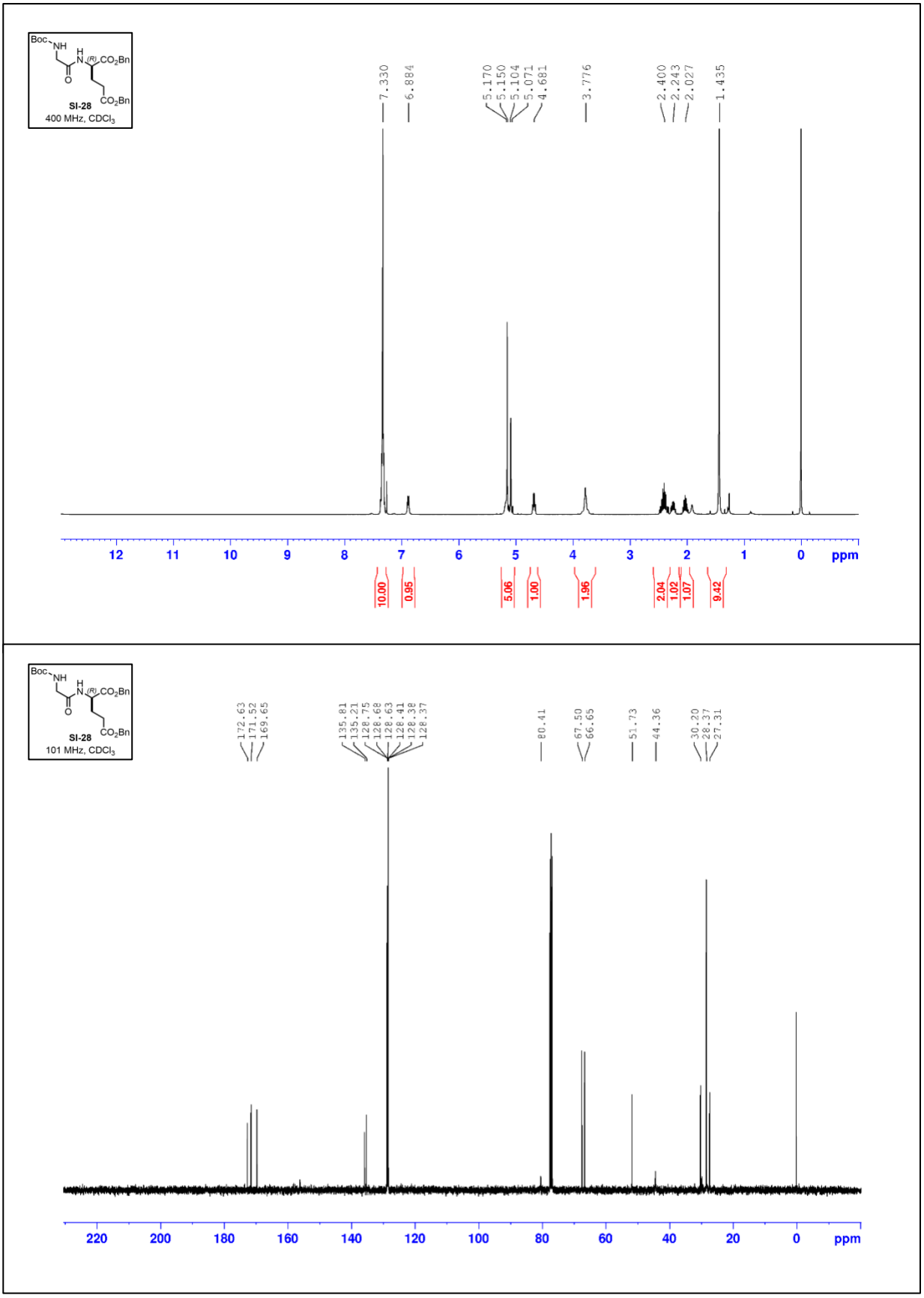

·HCl
SI-25
400 MHz, CDCl3
8.878
8.317
5.016
4.656
4.589
4.179
4.082
2.383
2.191
2.037
1.224
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.84
2.63
0.84
2.20
1.62
2.01
1.91
1.00
0.87
6.72

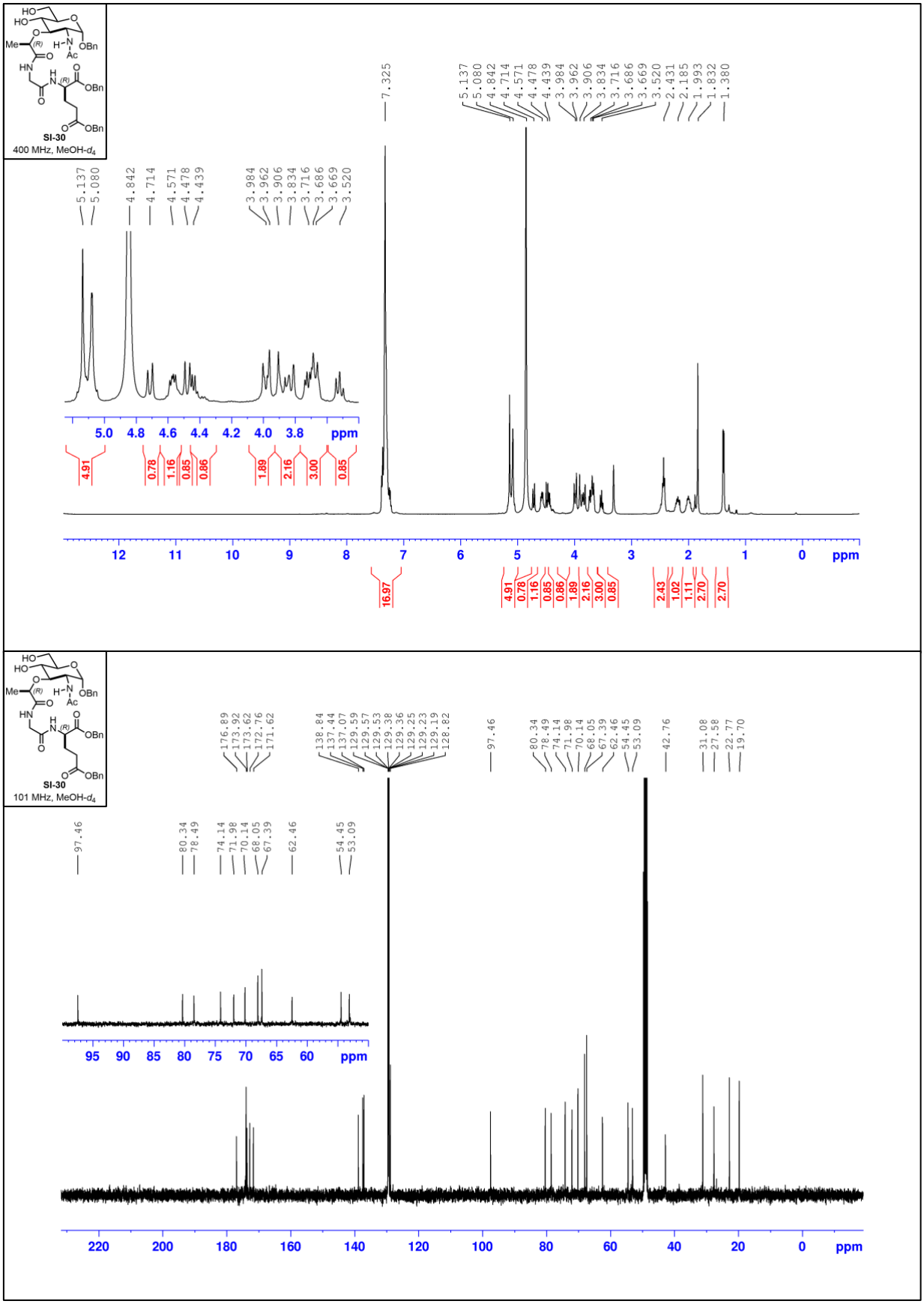

·HCl
SI-25
101 MHz, CDCl3
172.75
172.18
166.03
121.18
65.95
62.26
60.73
52.48
52.26
30.13
26.77
14.07
13.96
220
200
180
160
140
120
100
80
60
40
20
0
ppm

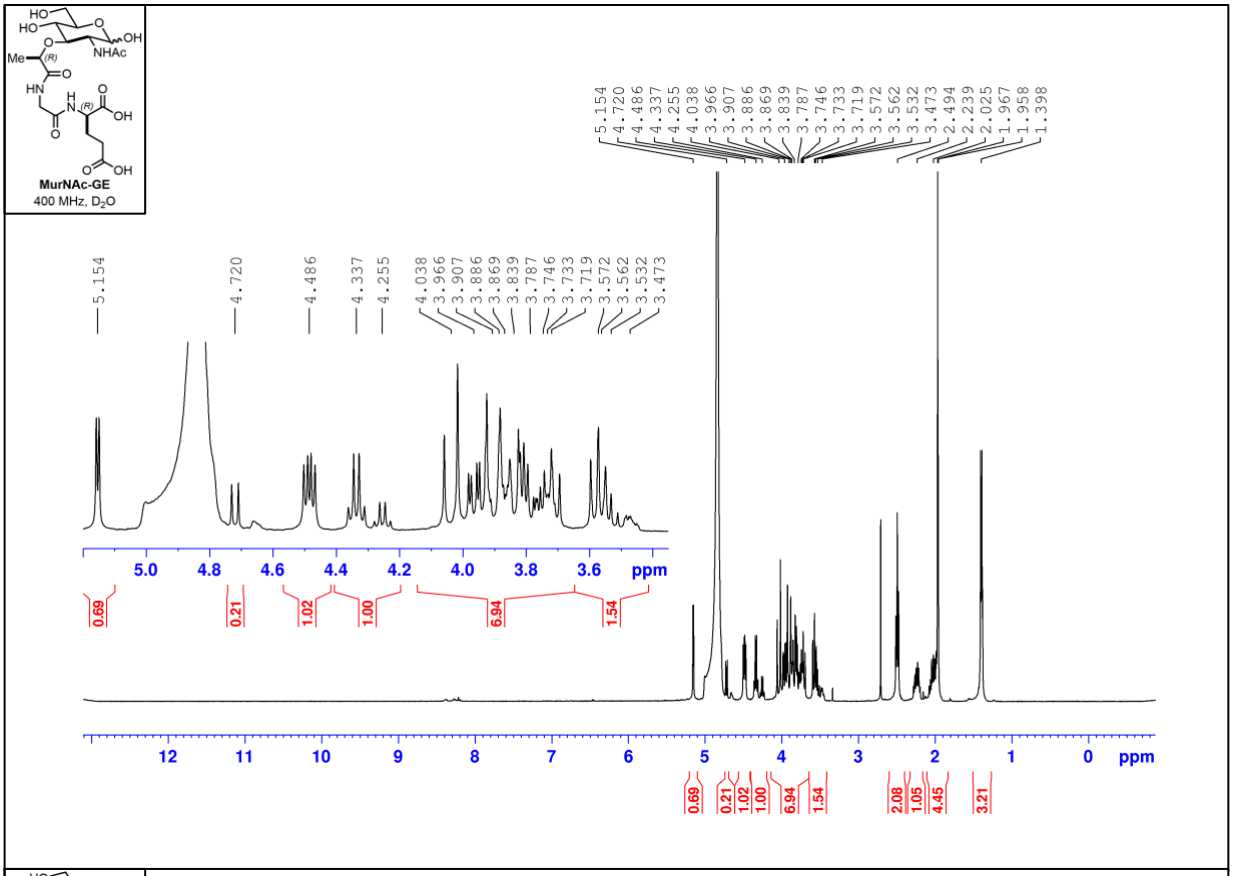

SI-26
400 MHz, MeOH-$d_4$
SI-26
101 MHz, MeOH-$d_4$

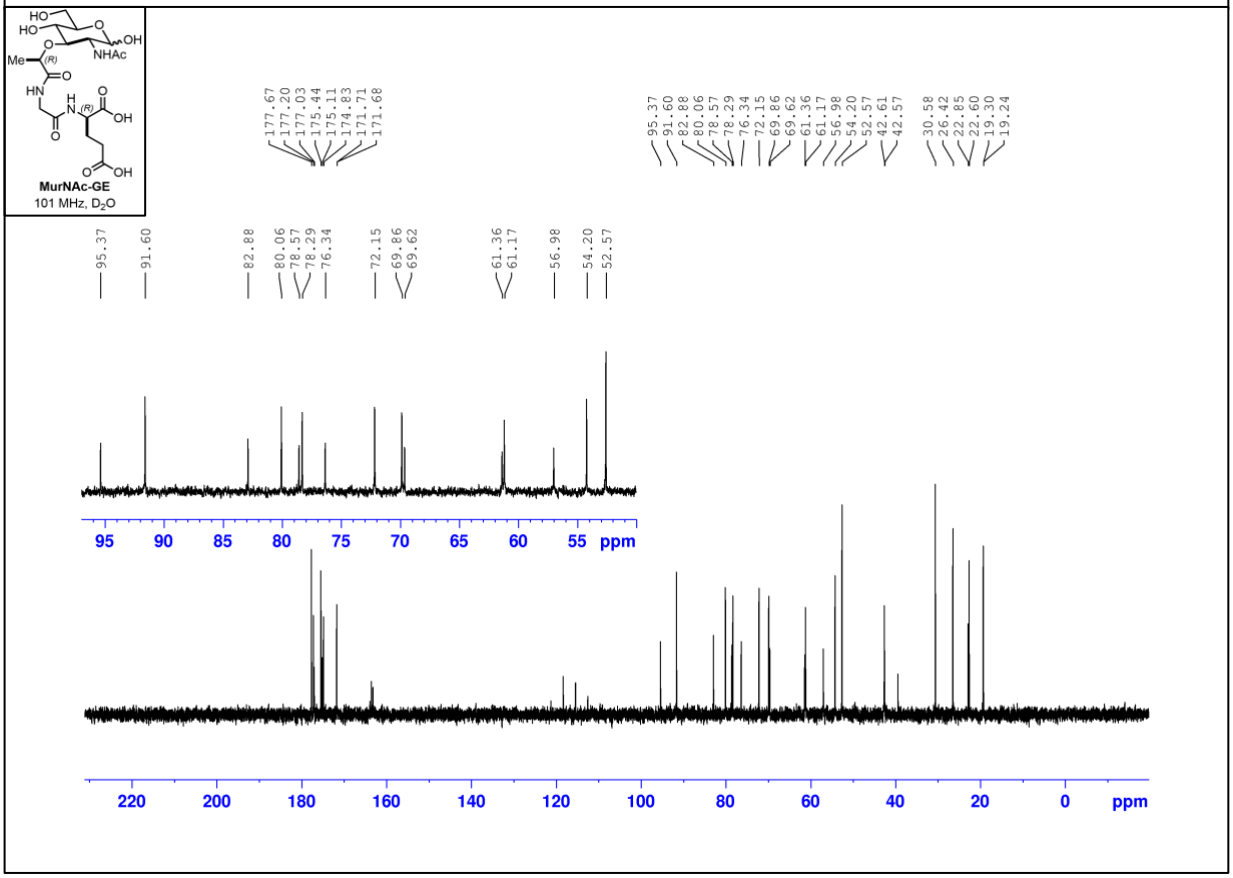

SI-27
400 MHz, MeOH-$d_4$
SI-27
101 MHz, MeOH-$d_4$
ppm

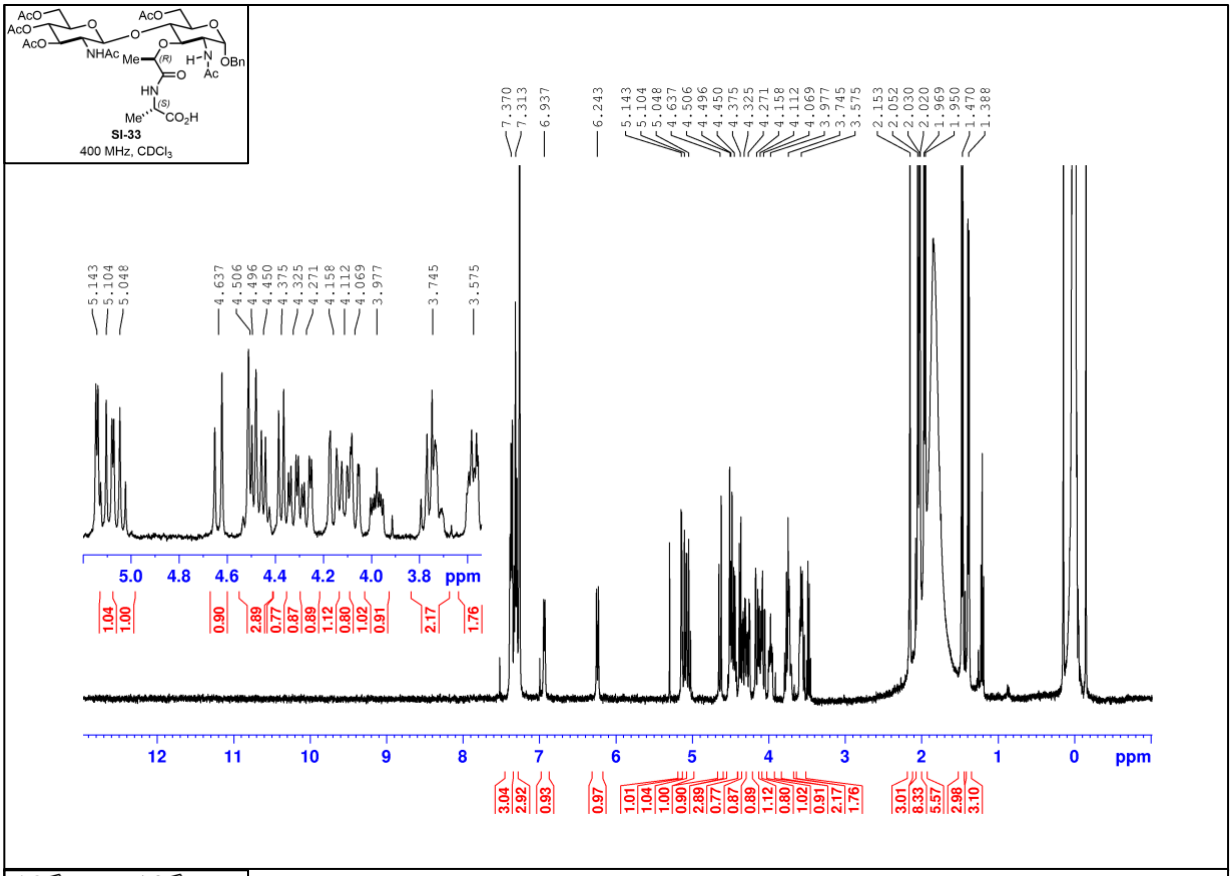

MurNAc-A(OCF3)E
400 MHz, D2O
5.176
4.746
4.673
4.506
4.386
3.926
3.897
3.862
3.835
3.785
3.774
3.759
3.717
3.579
3.540
3.535
3.469
2.480
2.246
2.023
1.975
1.963
1.391
ppm
0.71
0.28
0.97
3.00
4.63
1.63
1.99
1.02
4.39
3.21
MurNAc-A(OCF3)E
101 MHz, D2O
177.58
177.56
176.62
176.28
175.14
175.02
174.76
170.57
121.86
95.58
91.50
82.51
79.57
78.07
77.86
76.37
72.18
69.78
69.55
66.83
61.37
61.16
56.72
54.31
52.82
52.77
30.42
26.26
22.88
22.64
19.18
19.15
ppm

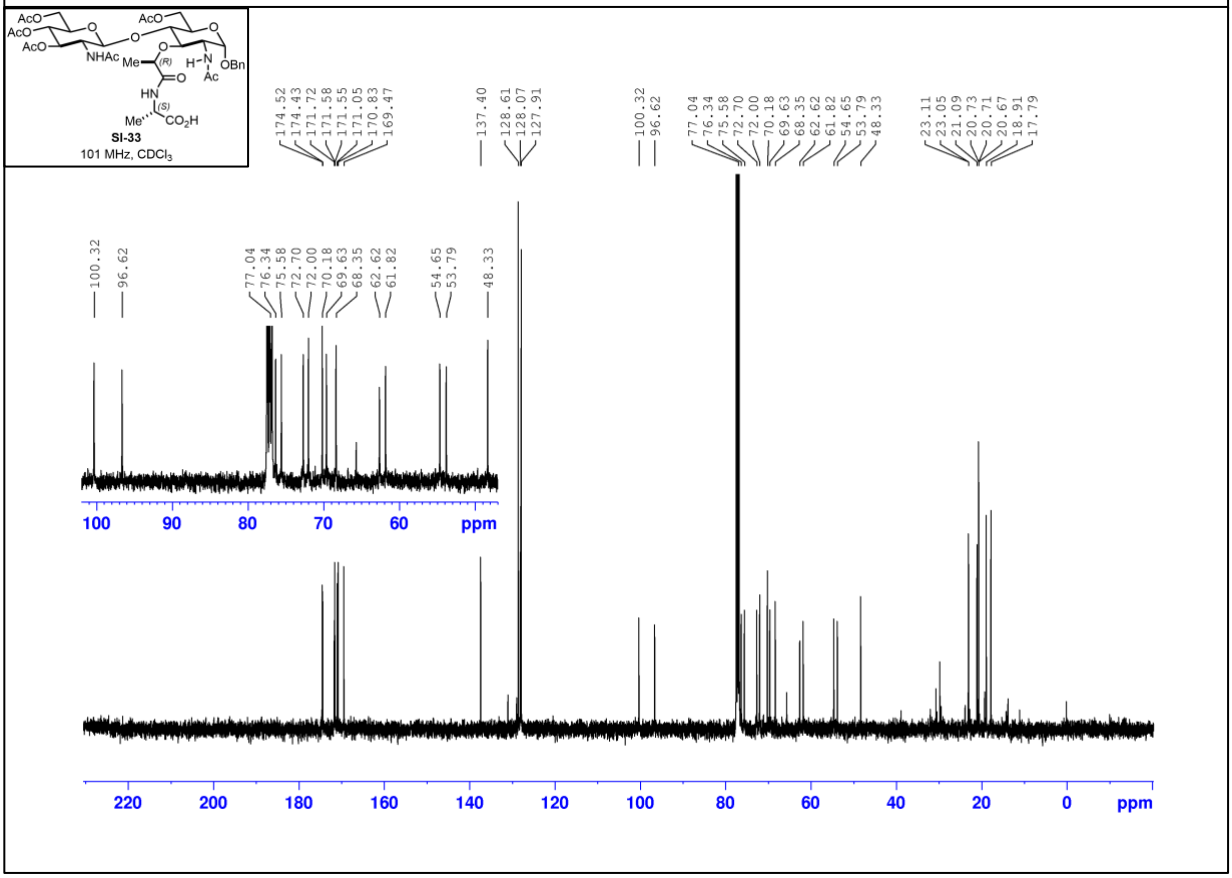

SI-28
400 MHz, CDCl3
7.330
6.884
5.170
5.150
5.104
5.071
4.681
3.776
2.400
2.243
2.027
1.435
ppm
10.00
0.95
5.06
1.00
1.96
2.04
1.02
1.07
9.42
SI-28
101 MHz, CDCl3
172.63
171.52
169.65
135.81
135.21
128.75
128.68
128.63
128.41
128.38
128.37
80.41
67.50
66.65
51.73
44.36
30.20
28.37
27.31
ppm

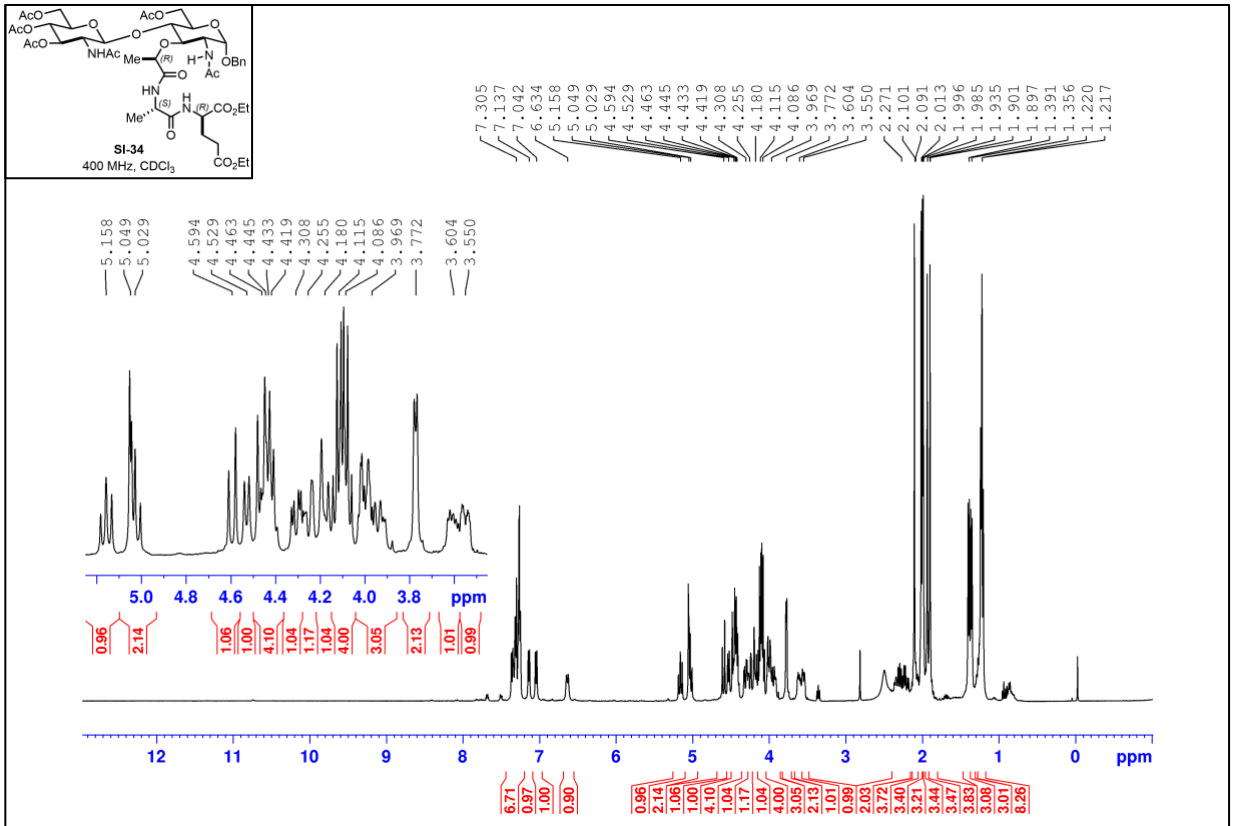

SI-30
400 MHz, MeOH-$d_4$
SI-30
101 MHz, MeOH-$d_4$
ppm

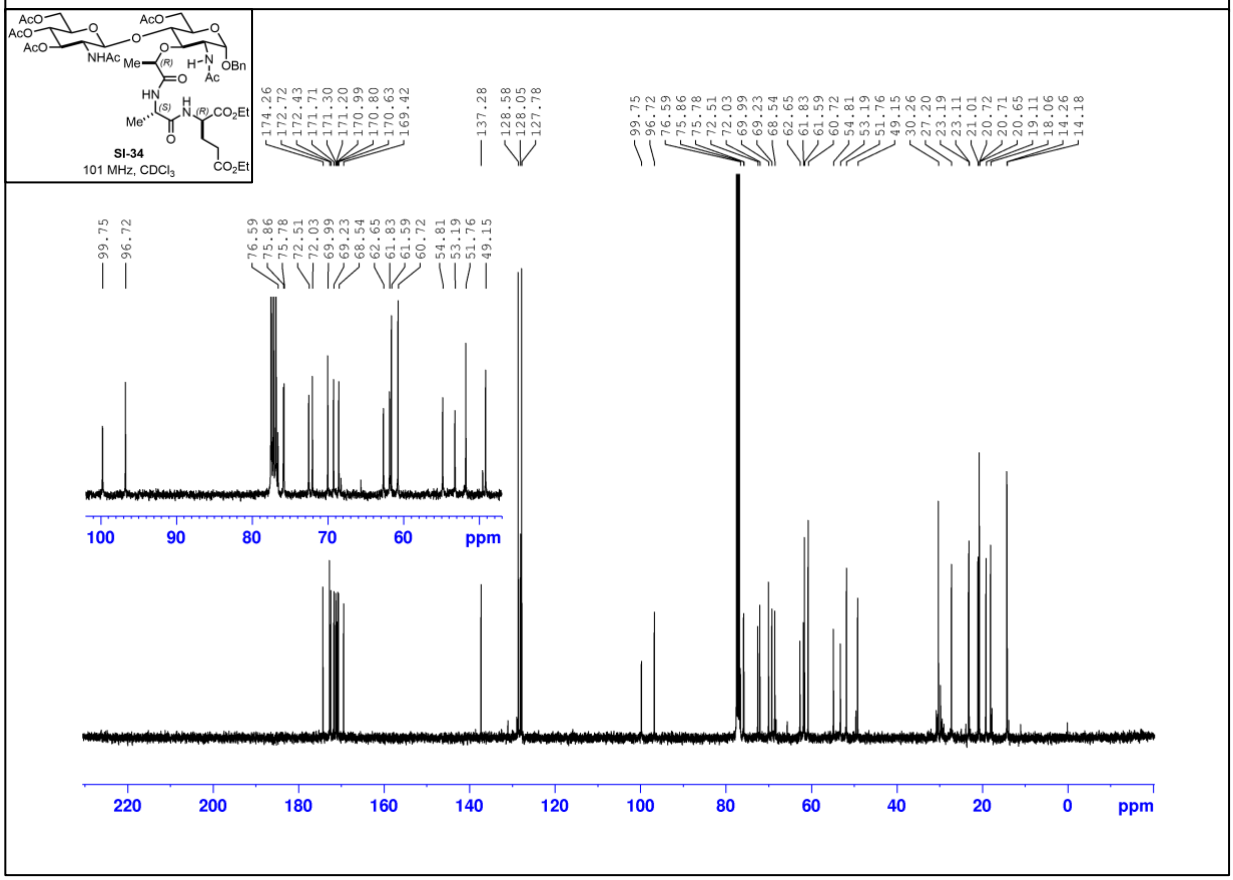

MurNAc-GE
400 MHz, D2O
5.154
4.720
4.486
4.337
4.255
4.038
3.966
3.907
3.886
3.869
3.839
3.787
3.746
3.733
3.719
3.572
3.562
3.532
3.473
2.494
2.239
2.025
1.967
1.958
1.398
ppm
0.69
0.21
1.02
1.00
6.94
1.54
2.08
1.05
4.45
3.21

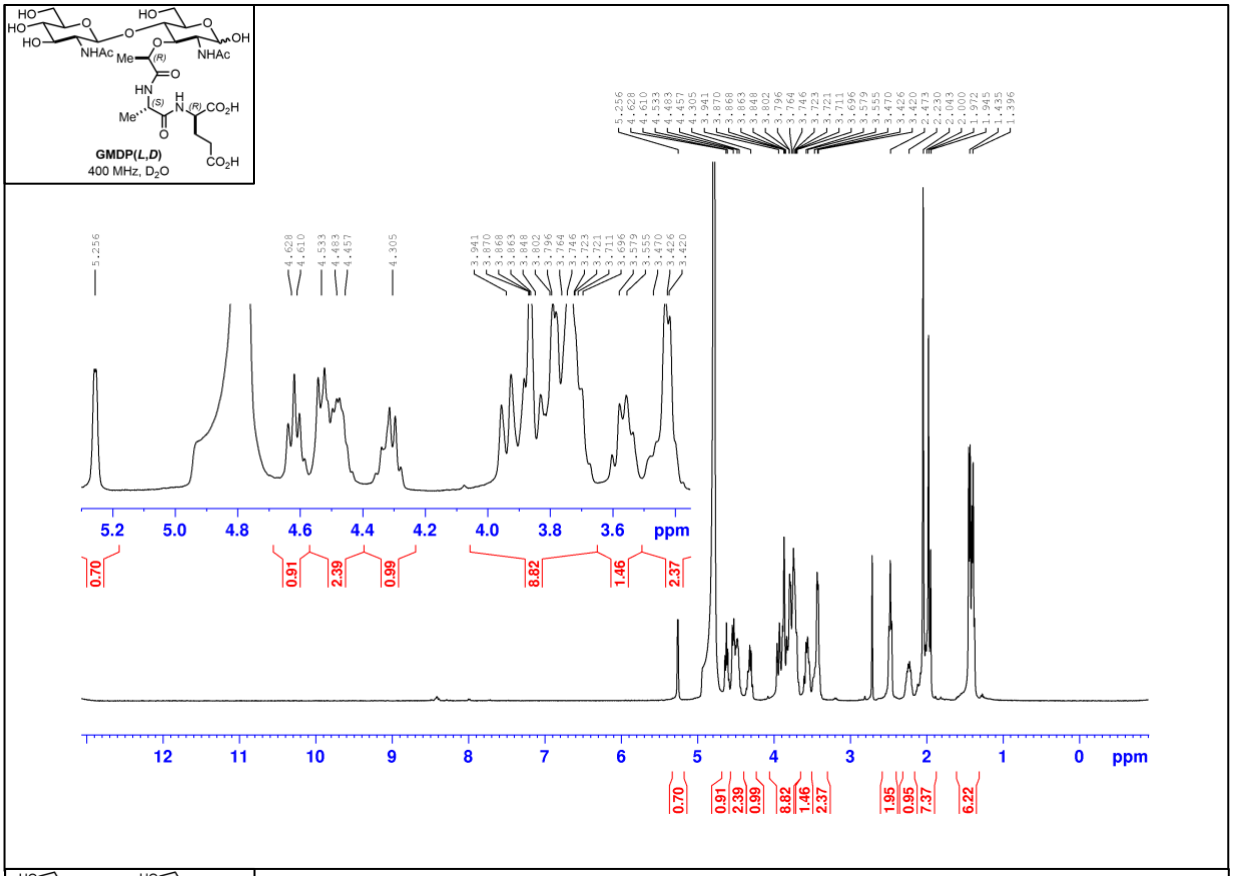

MurNAc-GE
101 MHz, D2O
177.67
177.20
177.03
175.44
175.11
174.83
171.71
171.68
95.37
91.60
82.88
80.06
78.57
78.29
76.34
72.15
69.86
69.62
61.36
61.17
56.98
54.20
52.57
42.61
42.57
30.58
26.42
22.85
22.60
19.30
19.24
ppm

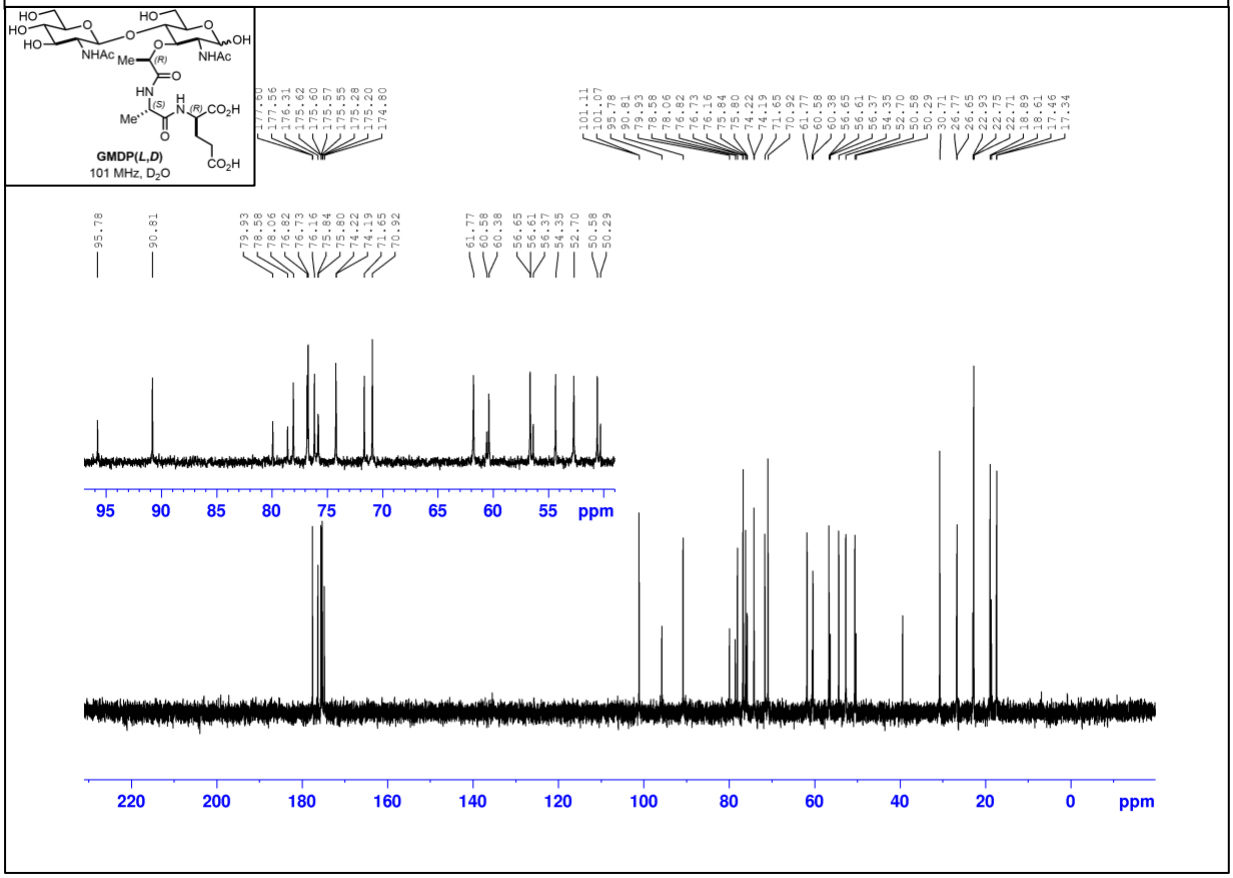

SI-33
400 MHz, CDCl3
7.370
7.313
6.937
6.243
5.143
5.104
5.048
4.637
4.506
4.496
4.450
4.375
4.325
4.271
4.158
4.112
4.069
3.977
3.745
3.575
2.153
2.052
2.030
2.020
1.969
1.950
1.470
1.388
ppm


SI-33
101 MHz, CDCl3
174.52
174.43
171.72
171.58
171.55
171.05
170.83
169.47
137.40
128.61
128.07
127.91
100.32
96.62
77.04
76.34
75.58
72.70
72.00
70.18
69.63
68.35
62.62
61.82
54.65
53.79
48.33
23.11
23.05
21.09
20.73
20.71
20.67
18.91
17.79
ppm


SI-34
400 MHz, CDCl3
7.305
7.137
7.042
6.634
5.158
5.049
5.029
4.594
4.529
4.463
4.445
4.433
4.419
4.308
4.255
4.180
4.115
4.086
3.969
3.772
3.604
3.550
2.271
2.101
2.091
2.013
1.996
1.985
1.935
1.901
1.897
1.391
1.356
1.220
1.217
ppm


SI-34
101 MHz, CDCl3
174.26
172.72
172.43
171.71
171.30
171.20
170.99
170.80
170.63
169.42
137.28
128.58
128.05
127.78
99.75
96.72
76.59
75.86
75.78
72.51
72.03
69.99
69.23
68.54
62.65
61.83
61.59
60.72
54.81
53.19
51.76
49.15
30.26
27.20
23.19
21.01
20.72
20.71
20.65
19.11
18.06
14.26
14.18
ppm


GMDP(L,D)
400 MHz, D2O
5.256
4.628
4.610
4.533
4.483
4.457
4.305
3.941
3.870
3.868
3.863
3.848
3.802
3.796
3.764
3.746
3.723
3.721
3.711
3.696
3.579
3.555
3.470
3.426
3.420
2.473
2.230
2.043
2.000
1.972
1.945
1.435
1.396
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
ppm
0.70
0.91
2.39
0.99
8.82
1.46
2.37
12
11
10
9
8
7
6
5
4
3
2
1
0
ppm
0.70
0.91
2.39
0.99
8.82
1.46
2.37
1.95
0.95
7.37
6.22


GMDP(L,D)
101 MHz, D2O
177.60
177.56
176.31
175.62
175.60
175.57
175.55
175.28
175.20
174.80
101.11
101.07
95.78
90.81
79.93
78.58
78.06
76.82
76.73
76.16
75.84
75.80
74.22
74.19
71.65
70.92
61.77
60.58
60.38
56.65
56.61
56.37
54.35
52.70
50.58
50.29
30.71
26.77
26.65
22.93
22.75
22.71
18.89
18.61
17.46
17.34
95
90
85
80
75
70
65
60
55
ppm
220
200
180
160
140
120
100
80
60
40
20
0
ppm