Supplementary Information

Differential Peptidoglycan Recognition Assay Using Varied Surface Presentation

Elizabeth A. D'Ambrosio[1], Klare L. Bersch[1a)], Mackenzie L. Lauro[1b)], Catherine L. Grimes[*1,2]

Department of [1]Chemistry and Biochemistry and [2]Biological Sciences, University of Delaware, Newark, DE 19716

[a)] Present address: Prelude Therapeutics, Inc., 200 Powder Mill Road, Wilmington, DE 19803, United States

[b)] Present address: Merck & Company, Inc., Merck Animal Health, 126 East Lincoln Avenue, Rahway, NJ 07065, United States

To whom correspondence should be addressed: Catherine L. Grimes, Tel.: (302) 831-2985; Fax: (302) 831-6335; E-mail: cgrimes@udel.edu

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Materials

Primers were purchased from Eurofins Genomics. cDNA for human NLRP1 was obtained from the lab of Jenny Ting at UNC School of Medicine. Restriction endonucleases were purchased from New England Biolabs. Antibiotics were purchased from Gold Biotechnology. Expression host *E. coli* cells were purchased from Agilent Technologies. Alcohol terminated alkane thiol reagents were purchased from Sigma Aldrich. Carboxylic acid terminated alkane thiol reagents were purchased from Toronto research chemical. Gold sensor chips and 0.5% SDS were purchased from GE Health Care. All other chemicals were purchased from Sigma-Aldrich, unless otherwise indicated. Graphs were generated using GraphPad Prism 6.

Construction of expression plasmids

Residues 791-990 of human NLRP1 cDNA were PCR amplified to extract the LRR domain prior to insertion into the pGex-6p1 vector using the BamHI and XhoI restriction sites. Primers used for amplification are as follows:

Forward 5' TTTTTTGGATCCCCAGTCACAGATGCCTATTGG 3'

Reverse: 5' TTTTTTCTCGAGACTTGGTTTCCGTCTGC 3'

After transformation into DH5α cells selected colonies were purified using a mini-prep plasmid purification kit (Qiagen). Plasmid identity was verified by Sanger sequencing (Delaware biotechnology Institute sequencing and genotyping center). Sequenced plasmids were transformed into BL21 RIPL-codon plus cells (Agilent Technologies) for large scale expression.

NLRP1 Expression and Purification

NLRP1 LRR was expressed with an N-terminal Glutathione-S- transferase (GST) tag. Plasmids were transformed into BL21(DE3) competent cells, inoculated into 1 L LB and grown at 33°C with 100 μg ml^{-1} carbenicillin and 5 μg ml^{-}chloramphenicol antibiotics until OD 600nm reached 0.6. Protein expression was induced with 1mM isopropyl-1-thio-β-D-galactoside (IPTG) at 18°C for 15h. Cells were pelleted at 10,000 rpm for 15 min and stored at -80°C until use. A 2 L pellet was resuspended in 40 mL of 50 mM Tris buffer, pH 7.4 containing 150 mM NaCl, 1 mM EDTA with the addition of 2 protease inhibitor tablets (Roche). The resuspension was lysed using sonication with an amplitude setting of 50% pulsing 2 seconds on and 2 seconds off for 3 minutes of total sonication. Lysed cells were clarified by centrifugation at 14,500 rpm for 25 min. The clarified supernatant was applied to an equilibrated column containing GST Sepharose Fast Flow resin (GE Healthcare Life Sciences) and incubated at 4°C for 1.5 hrs. The column was washed with 6 column volumes of 50 mM Tris buffer, pH 7.4, containing 500 mM NaCl, 1 mM EDTA. Protein was eluted from the column by overnight incubation with Precession Protease (GE Healthcare Life Sciences) at 4°C in 50 mM Tris,150 mM NaCl, 1mM EDTA pH 7.4. After overnight incubation tag free LRR was eluted from the column. 20mM reduced glutathione was used to regenerate the resin prior to protein elution.

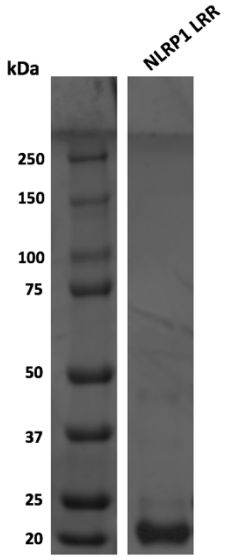


Figure S1. SDS-Page gel of NLRP1 LRR construct: GST tag was cleaved during purification using PreScission Protease. Expected MW of the tag free LRR is ~ 22 kDa.

Circular Dichroism Spectroscopy

CD spectra were taken with a JASCO J1500 CD Spectrometer with the protein solution contained in 0.1 cm path length cylindrical cell. Spectra were collected at 0.2 nm intervals over the wavelength range from 260 to 195 nm. All Spectra are the average of triplicate runs. CD spectra were taken in 10 mM sodium phosphate buffer 50 mM NaCl pH 7. Molar ellipticity [θ] was calculated using [θ] = θ/nCl where n is the number of amino acids (205) , C is the molar concentration (dM) and l is the pathlength (cm). The ellipticity was corrected using background buffer.

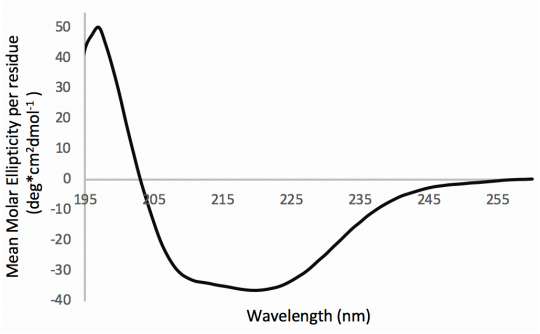


Figure S2. CD Spectra of tag free NLRP1 LRR domain. All spectra were run in triplicate in 10mM phosphate buffer, 50mM NaCl, pH 7. The spectra Indicates a well folded protein with a highly alpha helical secondary structure, characteristic of LRR domains and in agreement with crystallographic data.

TOF Mass Spectrometry analysis of NLRP1

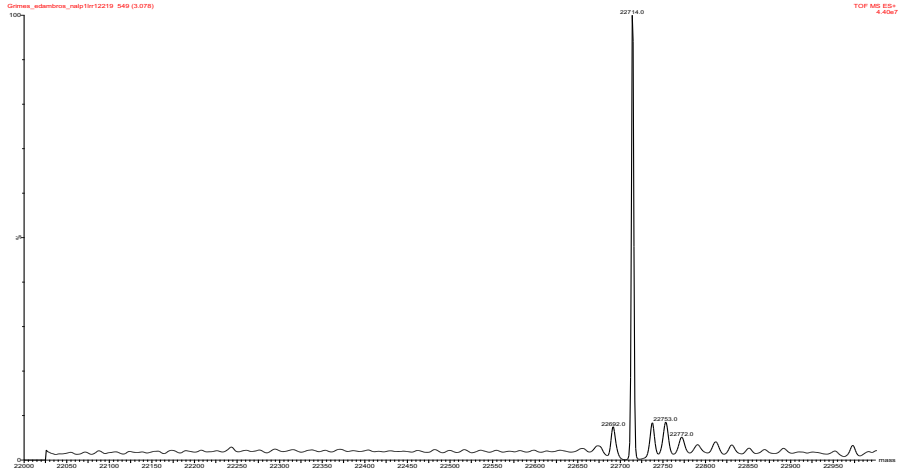


Figure S3. Mass conformation of purified NLRP1 LRR. All protein mass spectra were obtained using 30µM NALP1 LRR on a Waters Xevo G2-S QTof spectrometer in ESI+ mode equipped with a waters UPLC column. Data was deconvoluted using waters MassLynx software. Expected exact mass of tag free LRR construct= 22714.3 g/mol, observed exact mass= 22714 g/mol.

NOD2 Expression and purification

The Nod2 LRR plasmid was constructed as described previously reported from our lab[1]. The Nod2 LRR was expressed with an N-terminal Maltose binding protein and 6x histidine affinity tags according to our previous report. Briefly, the plasmid was transformed into BL21(DE3) competent cells and inoculated into 1 L LB and grown at 37°C with 100 mg/mL carbenicillin until OD 600nm reached 0.6. Protein expression was induced with 1mM isopropyl-1-thio-β-D-galactoside (IPTG) at 18°C for 18h. A 1 L pellet was resuspended in 30 mL of 50 mM sodium phosphate buffer, pH 7.4 containing 150 mM NaCl, and 10 mM imidazole with the addition of 2 protease inhibitor tablets (Roche). The resuspended pellet was lysed using sonication with an amplitude setting of 50% with pulsing 2s on 2s off for 3 min total. Lysed cells were clarified by centrifugation at 14000 rpm for 20 min and applied to Ni Sepharose 6 Fast Flow resin (GE Healthcare Life Sciences). The resin washed with 10 column volumes of 50 mM phosphate buffer, pH 7.4, containing 500 mM NaCl, 25 mM imidazole. Protein was eluted with 250 mM imidazole in 50 mM sodium phosphate buffer, pH 7.4 containing 150 mM NaCl, then dialyzed overnight into 2L 50 mM Tris buffer containing 150 mM NaCl, 1 mM EDTA, and, pH 7.4 prior to SPR analysis.

Preparation of Self Assembled Monolayers for Surface Plasmon Resonance Assays

Conditions for preparing the SPR chip, mixed-SAM and running the SPR assay were adapted from Grimes et al. and Lauro et al[1–3]. A BIAcore 3000 SPR was docked with functionalized cassettes described below. Gold chips (GE Healthcare Life Sciences) were submerged in a mixture of thiols (1% mole fraction of hexa (ethylene glycol)-carboxylic acid (EG)6CO2H)- terminated thiol in tri(ethylene glycol) ((EG)3OH)-terminated thiol) (2 mM total concentration) for 17 hours. The chip was removed from the solution, rinsed with ethanol, and dried under nitrogen prior to being mounted onto a cassette following the manufacturer's instructions (SIA Kit, GE Healthcare Life Sciences). Filtered and degassed PBS-EP was used as the running buffer for all SPR binding experiments. Solutions of *N*-hydroxysuccinimide (NHS) (0.10 M) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide (EDC) (0.4 M) for amide coupling were prepared in distilled, deionized water and used immediately. All amine functionalized compounds (20 mg/mL) were dissolved in 25 mM phosphate buffer, pH 8.0 to a final concentration of 2 mg/ml. 1 M Ethanolamine, pH 8.5 was used to cap all unreacted activated carboxylic acids and cap the control lane.

<u>Surface Plasmon Resonance</u>

Small Molecule Immobilization and Binding Experiments

All amine functionalized compounds we immobilized on the activated SAM using a flow rate of 5 µL/min. Fresh solutions 0.1 M NHS and 0.4 M EDC in distilled water were prepared. The surface was equilibrated with PBS-EP and activation of the surface carboxylic acid groups into NHS esters was achieved by passing a mixture of 0.05 M NHS and 0.20 M EDC in water over the surface for 7 min (the mixture is prepared by the automated robotics of the BIAcore 3000 using the DILUTE command). The surface was washed with PBS-EP (2 min) and the solution of 1 was injected to flow cell 1, a solution of 2 was injected to flow cell, the solution of 3 was injected to flow cell 3 and the ethanolamine solution was injected to flow cell 4 for 7 min. After amide bond formation and ethanolamine capping, all flow cells were washed with SDS (10 mg/mL) for 3 min. A flow rate of 3 µL/min was used to inject protein over the chip surface for a total of 20 min. The chip was then washed with PBS-EP for 10 min, SDS for 3 min and PBS-EP for 10 min.

Data Analysis

The binding of the NLRP1 LRR to the various chip-tethered ligands was recorded after 1,170 s in resonance units (RU). All ligand activated flow cells were corrected for background binding by subtracting the RU of flow cell 4 (Ethanolamine). All sensograms and binding curves were generated using the non-linear one site binding model from GraphPad Prism 6.

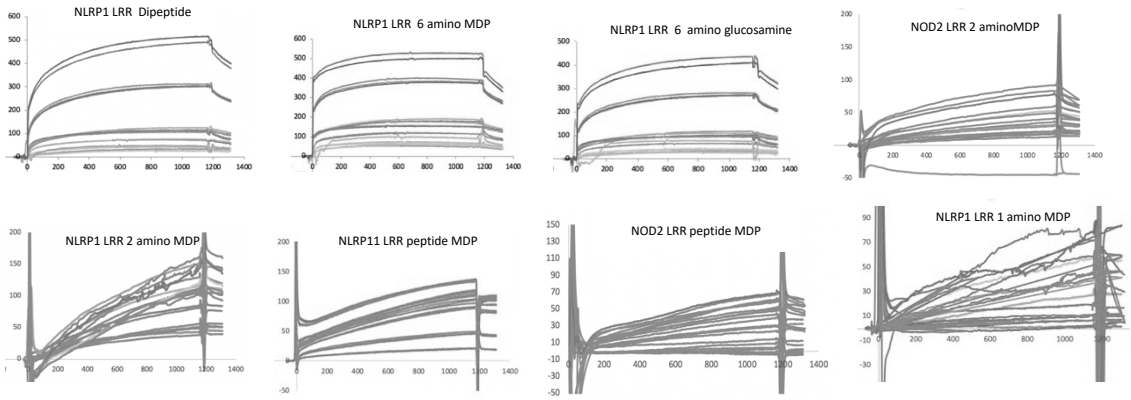


Figure S4. Raw SPR curves for binding of NOD2 and NLRP1 to MDP derivatives. These sensograms do generate binding curves, shown in figures 1c, figure 3b-d (main text) and figure S5.

NOD2 SPR curves

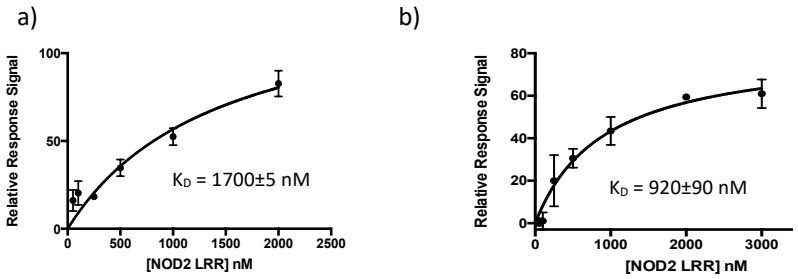


Figure S5. SPR binding curves for purified NOD2 LRR to MDP tethered at a) C2 position and b) D-isoglutamine peptide established by SPR. Experimental methods and data analysis are described in above.

BSA negative control sensograms

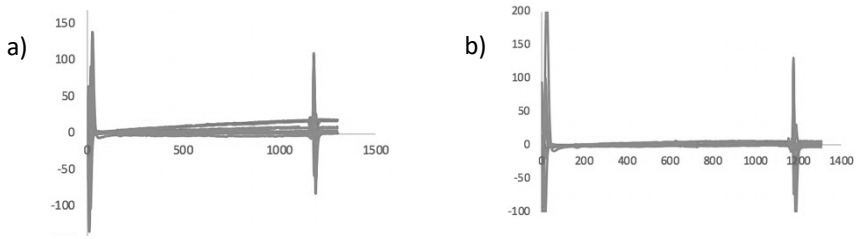


Figure S6. Raw sensograms of BSA applied to a subset of the peptidoglycan library. No specific binding is detected for BSA to these ligands. a) 2 amino MDP b) 6 amino MDP

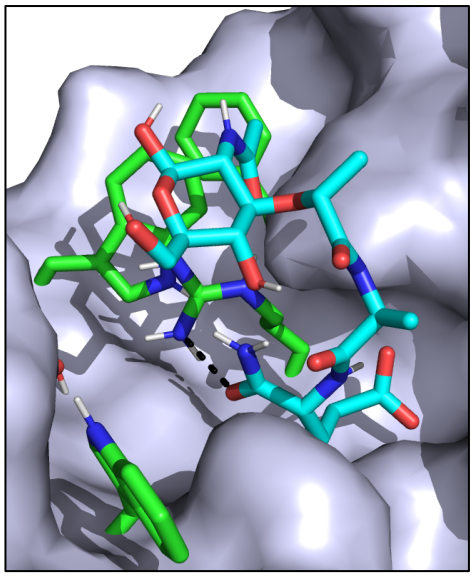


Figure S7. AutoDock model of MDP and NOD2 showing the formation of a potential hydrogen bonding interaction between the R877 residue on NOD2 and the terminal amide of MDP

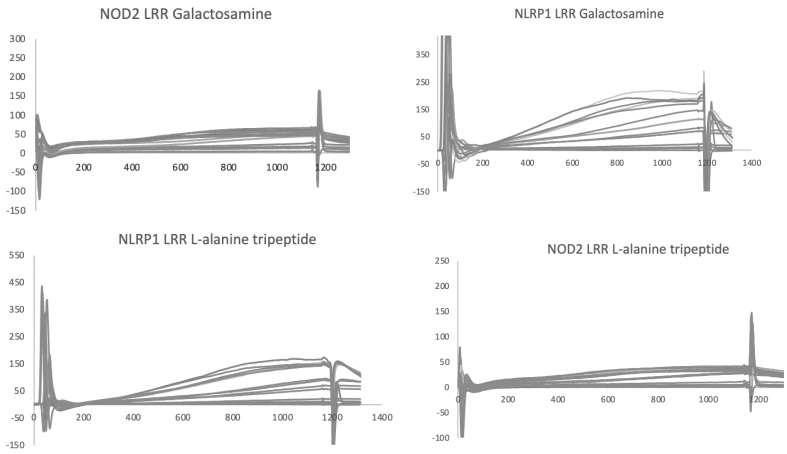


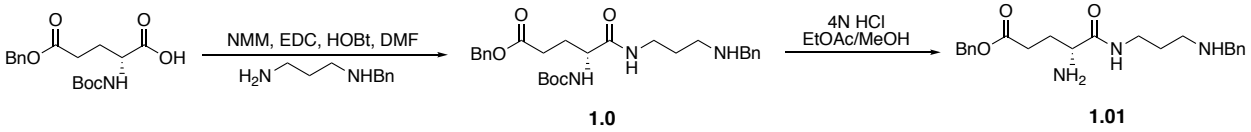
Figure S8. Raw SPR sensograms for NOD2 and NLRP1 to L-alanine tripeptide and galactosamine. These sensograms do not generate binding curves; increase in response units is representative of increasing concentration being applied to the sensor surface.

Synthetic Procedures

Materials and General Procedures

All reagents were purchased from Sigma Aldrich, Fisher Scientific, Alfa Aesar or Invitrogen and used without further purification, unless otherwise noted. Anhydrous Solvents were reagent grade and were further dried when necessary. Unless otherwise noted, all reactions were performed in oven dried flasks equipped with rubber septa, positive pressure of nitrogen, and magnetic stirring. NMR solvents were purchased from Cambridge Isotope Laboratories, Inc. Flash chromatography was carried out on silica gel (60 Å, 40-63 μm), purchased from Sorbent Technologies. Reactions were monitored by electrospray ionization liquid chromatography mass spectrometry (ESI LC-MS) and thin layer chromatography (TLC) in which glass plates coated with silica gel (250 μm, Silica Gel HL, Sorbent Technologies) were used and visualized with shortwave 254 nm UV light or developed upon heating with p-anisaldehyde or ninhydrine. Preparative HPLC purification was performed on a Waters 2767 Sample Manager with HPLC and SQD2 MS using a Sunfire® Prep C18 OBD 5μm 19x100mm or 4.6x50mm columns. All NMR spectra were recorded on Bruker AV 400 MHz and AV III 600 MHz spectrometers. Proton chemical shifts were recorded in parts per million (ppm) on the δ scale, downfield from tetramethylsilane (TMS) and referenced from an internal standard S38 of residual protium in the NMR solvents (CHCl3: δ 7.26, D2HCOD 3.30). Data for 13C NMR were reported in ppm downfield from tetramethylsilane and referenced based on the chemical shift from the carbon resonances of the solvent (CDCl3: δ 77.16, CD3OD 49.0). NMR data were reported as follows: chemical shift, multiplicity (s = singlet d = doublet, t = triplet, q = quartet, m = multiplet, br = broad), coupling constant in Hz, integration, and assignment based on two dimensional COSY, HSQC and HMBC experiments. High-resolution mass spectra (HR-MS), ESI mode, were obtained on a Thermo Q-Exactive Orbitrap at the Mass Spectroscopy Facility at the Department of Chemistry, University of Delaware. Purity of novel compounds was assessed quantitatively through analytical HPLC/MS on a Waters 2767 Sample Manager with HPLC and SQD2 MS using a Sunfire® C18 5μm 6x50mm column.

Scheme 1



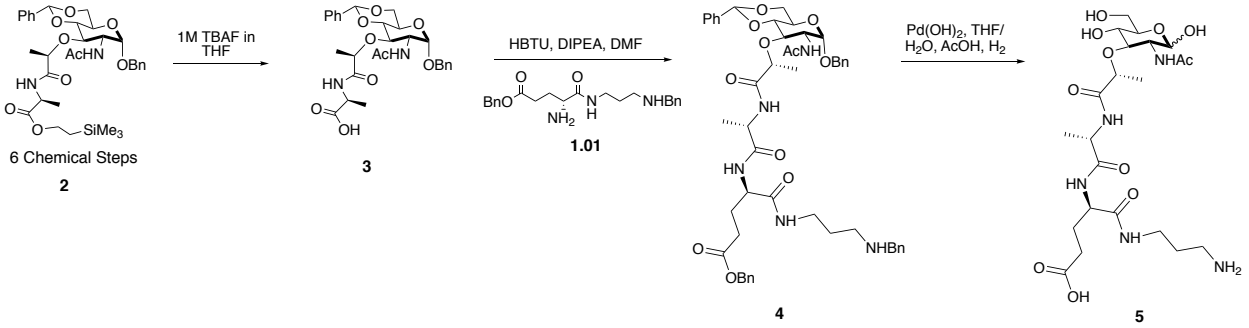
1.0 **1.01**

(*R*)-benzyl 5-((3-(benzylamino)propyl)amino)-4-((*tert*-butoxycarbonyl)amino)-5-oxopentanoate *(compound 1.0):* To a solution of Boc-D-isolgu(OBn)-OH (0.2 g, 0.59 mmol, 1 eq) in 5.9 mL of DMF at 0˚C was added EDC (0.083 g, 0.59 mmol, 1.0 eq), HOBt (0.087 g, 0.65 mmol, 1.1 eq), and *N*-Methylmorpholine (0.071 mL, 1.1 eq). After 5 min, *N*-1-benzylpropane-1,3-diamine (0.097 mL, 0.59 mmol, 1 eq) was added and stirred at room temperature overnight. The organic layer was diluted in DCM, washed, dried and purified via column chromatography (10-20% MeOH in DCM) to yield the product (0.136 g, 48%). ^{1}H NMR (400 MHz, Methanol-*d4*) δ 7.54 – 7.29 (m, 9H, 5-Benzyls), 5.15 (s, 2H, CH_2-Bn-γ-isoglu), 4.15 (s,

2H, CH_2-Bn-linker), 4.02 (dd, J = 9.0, 5.3 Hz, 1H, α-C*H*-isoglu), 3.4 (CH_2-Linker, *covered by MeOD*), 3.06 (t, J = 7.1 Hz, 2H, CH_2-Linker), 2.57 – 2.44 (m, 2H, CH_2-γ-D-isoglu), 2.09 (dq, J = 13.7, 7.2 Hz, 1H, C*H*-β-D-isoglu), 1.90 (h, J = 6.8 Hz, 3H, C*H*-β-D-isoglu, CH_2-Linker), 1.46 (s, 9H, Boc-(CH_3)$_3$). ^{13}C NMR (101 MHz, MeOD) δ 174.70, 172.76, 156.55, 136.12, 131.62, 129.49, 129.19, 128.88, 128.15, 127.82, 123.74, 79.38, 66.04, 54.26, 51.32, 44.47, 35.27, 29.97, 27.29, 26.67, 26.27. LRMS-ESI [M+H]= 484.20

(*R*)-benzyl 4-amino-5-((3-(benzylamino)propyl)amino)-5-oxopentanoate *(compound 1.01).* 1.0 (0.136 g, 0.28 mmol, 1 eq) was dissolved in 6 mL of 4N HCl in ethyl acetate and 1 mL of MeOH was added at 0˚ C and stirred for 2hours. Once complete the reaction was condensed and azeotroped three times with ethyl acetate to remove HCl. The residue was titrated with MeOH and ether then collected to yield the deprotected product (0.085 g, 73%). ^{1}H NMR (400 MHz, Methanol-*d4*) δ 7.60 – 7.32 (m, 10H, 2-Benzyls), 5.18 (s, 2H, CH_2-Bn-γ-isoglu), 4.31 – 4.14 (m, 2H, CH_2-Bn-linker), 3.97 (t, J = 6.5 Hz, 1H, α-C*H*-isoglu), 3.44 – 3.35 (m, 2H, CH_2-Linker, *covered by MeOD*) , 3.12 (dd, J = 8.5, 6.9 Hz, 2H, CH_2-Linker), 2.63 – 2.48 (m, 2H, CH_2-γ-D-isoglu), 2.18 (tq, J = 10.1, 7.3 Hz, 2H, CH_2-β-D-isoglu), 1.97 (h, J = 6.9 Hz, 2H, CH_2-Linker). ^{13}C NMR (101 MHz, MeOD) δ 171.93, 169.10, 135.88, 131.10, 129.59, 129.37, 128.94, 128.20, 128.01, 66.42, 52.33, 51.16, 44.82, 36.03, 28.86, 26.21, 25.87. HRMS-ESI: $C_{22}H_{30}N_3O_3$+ [M+H]= expected, 384.22817; observed, 384.22721.

Scheme 2



Compound **2 ((*S*)-2-(trimethylsilyl)ethyl 2-((*R*)-2-(((2*R*,4a*R*,6*S*,7*R*,8*R*,8a*S*)-7-acetamido-6-(benzyloxy)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-yl)oxy)propanamido)propanoate)** was synthesized over 6 chemical steps as previously reported[4].

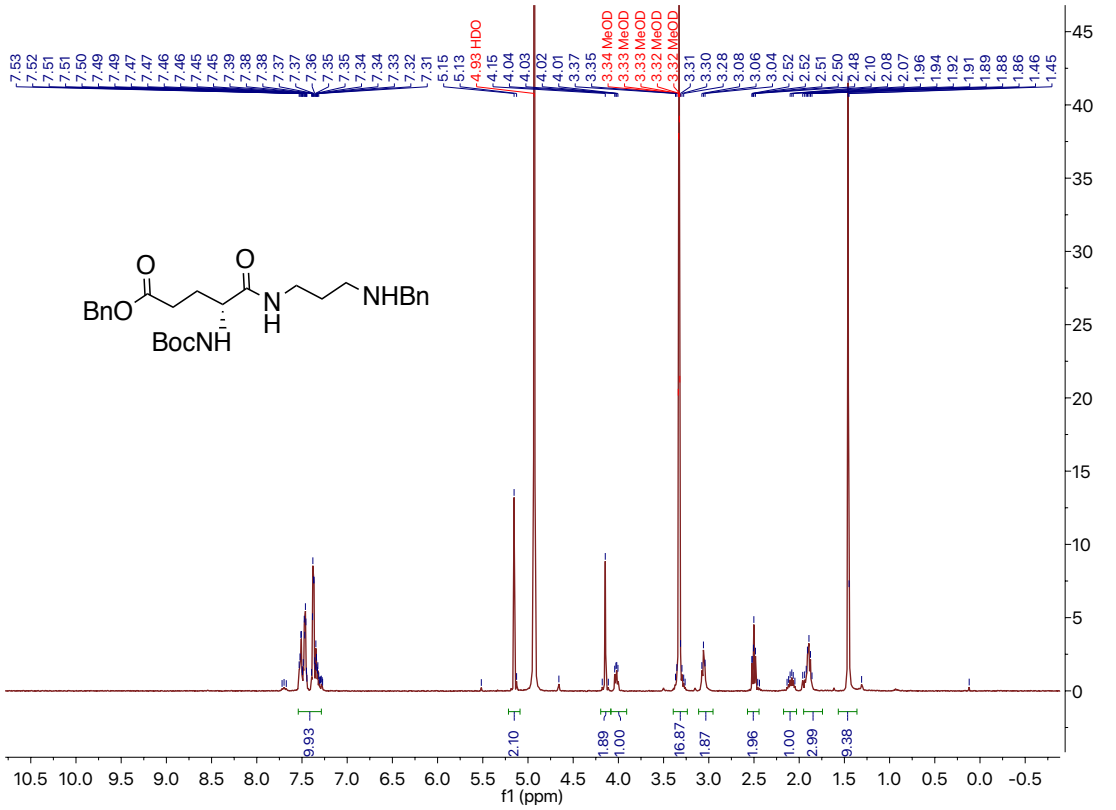
(*S*)-2-((*R*)-2-(((2*R*,4a*R*,6*S*,7*R*,8*R*,8a*S*)-7-acetamido-6-(benzyloxy)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-yl)oxy)propanamido)propanoic acid *(compound 3)* (0.140 g, 0.2 mmol, 1 eq) of **2** was dissolved in 4.6 mL of THF on activated 4 Å molecular sieves. 1M TBAF in THF (0.93 mL, 0.93 mmol, 4 eq) was added and left to stir for 1.5 hours at room temperature. The reaction was then condensed and directly subjected to column chromatography with 0-10% MeOH in DCM with 0.1% acetic acid to yield the pure product (0.063g, 54%). Note: prior to column crude product can be diluted in ethyl acetate and washed with 1N HCl to aid in removing the excess TBAF. ^{1}H NMR (600 MHz, Methanol-*d4*) δ 7.51 – 7.25 (m, 10H, Acetal-Ph, Benzyl), 5.63 (s, 1H, Acetal-C*H*), 4.91 (d, J = 3.7 Hz, 1H, $H_{1\alpha}$), 4.75 – 4.67 (m, 1H, C*H*-Ph), 4.59 – 4.51 (m, 1H, C*H*-Ph), 4.40 – 4.28 (m, 1H, α-C*H*), 4.26 – 4.06 (m, 3H, α-C*H,* $H_{2\alpha}$, $H_{4\alpha}$), 3.93 – 3.71 (m, 4H, $H_{3\alpha.}$ $H_{5\alpha,}$ $2H_{6\alpha}$), 1.90 (d, J = 15.1 Hz, 3H, Acetate-CH_3), 1.40 – 1.33 (m, 3H, β-

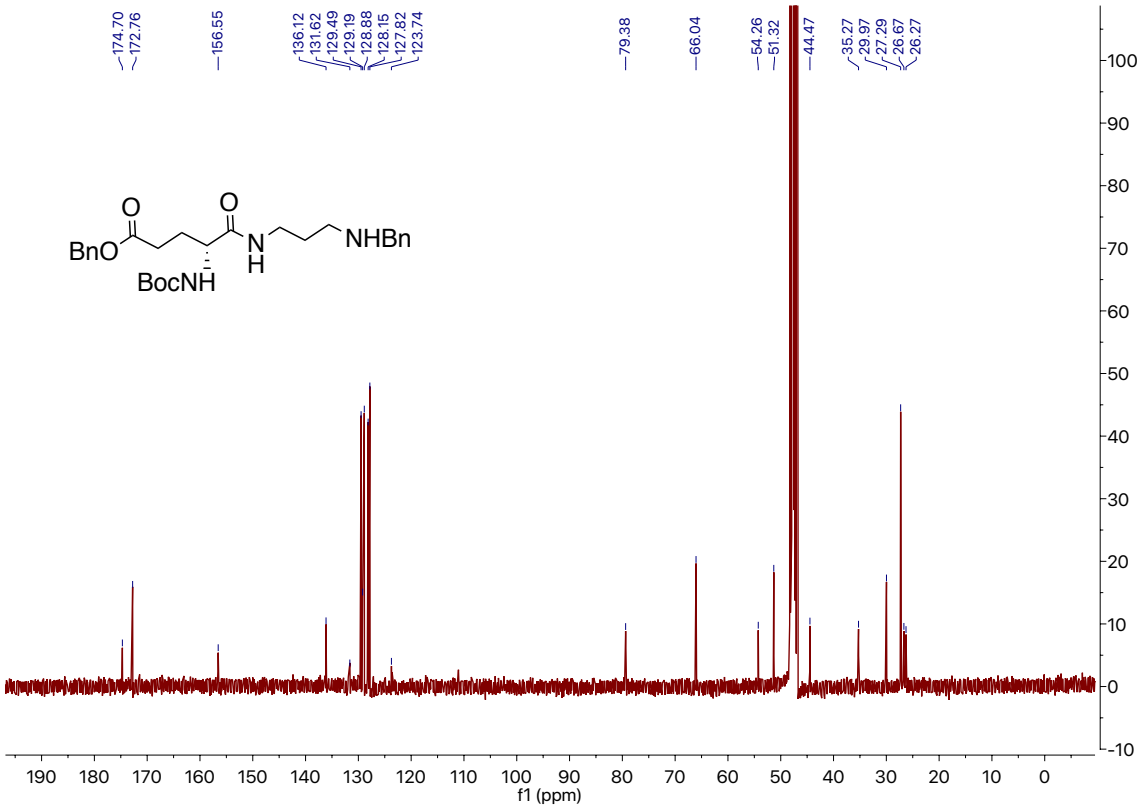
CH_3), 1.33 – 1.26 (m, 3H, β-CH_3). ^{13}C NMR (151 MHz, MeOD) δ 174.54, 173.99, 172.11, 137.67, 137.32, 128.52, 128.06, 127.95, 127.73, 127.58, 127.41, 125.87, 101.42, 96.92, 81.85, 77.24, 76.40, 69.41, 68.42, 63.06, 53.49, 48.02, 47.87, 47.73, 47.59, 47.45, 47.31, 47.16, 21.43, 18.22, 16.40. LRMS [M+H]= 543.18

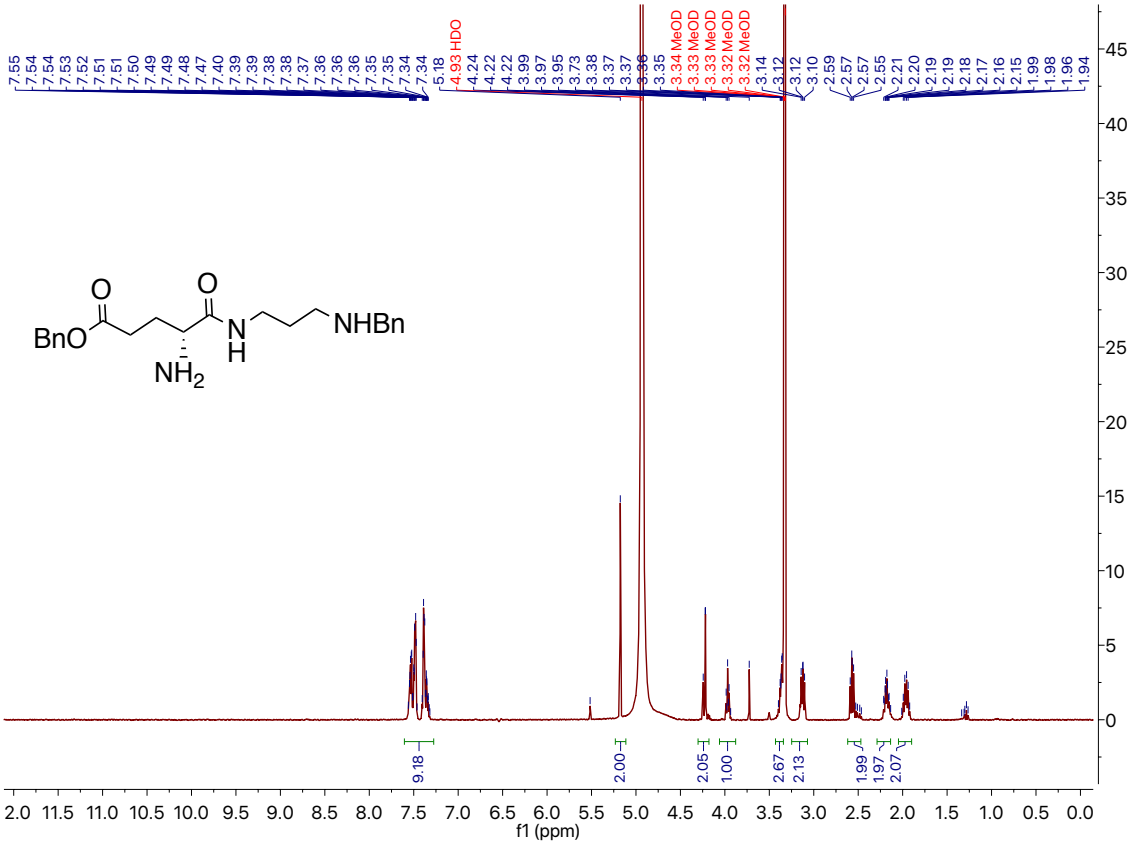
(4*R*)-benzyl 4-((2*S*)-2-((2*R*)-2-(((2*R*,4a*R*,6*S*,7*R*,8a*S*)-7-acetamido-6-(benzyloxy)-2-phenylhexahydropyrano[3,2-*d*][1,3]dioxin-8-yl)oxy)propanamido)propanamido)-5-((3-(benzylamino)propyl)amino)-5-oxopentanoate *(compound 4): 3* (0.101 g, 0.187 mmol, 1 eq) was dissolved in 1.86 mL of DMF with **1.01** (0.086 g, 0.22 mmol, 1.2 eq) at 0˚C then HBTU (0.083 g, 0.22 mmol, 1.2 eq) and DIPEA (0.146 mL, 0.75 mmols, 4 eq) was added. The reaction was stirred at room temperature overnight until complete. The crude product was diluted in organic solvent and washed with 1 N HCl, saturated bicarbonate and brine then dried and condensed. The crude was purified with 5% MeOH in DCM to give the pure product (0.092 g, 55%). ^{1}H NMR (400 MHz, DMSO-*d6*) δ 8.26 (d, J = 8.1 Hz, 1H, N*H*), 8.16 (d, J = 8.5 Hz, 1H, N*H*), 7.47 – 7.23 (m, 22H, 4-Benzyls), 5.68 (s, 1H, Acetal-C*H*), 5.07 (s, 2H, CH_2-OBn), 4.85 (d, J = 3.7 Hz, 1H, $H_{1\alpha}$), 4.71 (d, J = 12.5 Hz, 1H, C*H*-Ph), 4.51 (d, J = 12.5 Hz, 1H, C*H*-Ph), 4.36 – 4.08 (m, 5H, α-C*H*, α-C*H* α-C*H-D-isoglu,* C*H*-Benzyl''), 4.08 – 3.94 (m, 1H, $H_{2\alpha}$), 3.85 – 3.65 (m, 56H, $H_{4\alpha}$, $H_{5\alpha,}$ $2H_{6\alpha,}$ $H_{3\alpha,}$ C*H*-Benzyl'), 3.09 (d, J = 7.0 Hz, 2H, CH_2-Linker), 2.60 (s, 2H, CH_2-Linker), 2.34 (t, J = 7.8 Hz, 2H, CH_2-γ-D-isoglu), 1.98 (td, J = 13.6, 5.4 Hz, 1H, C*H*-β-D-isoglu), 1.80 (s, 3H, Acetate- CH_3), 1.74 (dt, J = 15.8, 8.0 Hz, 1H, C*H*-β-D-isoglu), 1.69 – 1.55 (m, 2H, CH_2-Linker), 1.20 (dd, J = 16.0, 6.9 Hz, 6H, 2 β-CH_3). ^{13}C NMR (101 MHz, DMSO) δ 172.62, 172.52, 172.26, 170.09, 138.00, 137.98, 136.57, 128.88, 128.74, 128.72, 128.57, 128.47, 128.37, 128.14, 128.08, 126.34, 100.74, 99.99, 97.44, 81.22, 76.64, 69.17, 65.98, 63.29, 53.50, 52.20, 40.57, 40.36, 40.15, 39.94, 39.73, 39.60, 39.52, 39.31, 30.43, 27.64, 23.06, 19.31, 18.96. LRMS [M+H]= 908.46.

(4*R*)-4-((2*S*)-2-((2*R*)-2-(((3*R*,5*S*,6*R*)-3-acetamido-2,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-4-yl)oxy)propanamido)propanamido)-5-((3-aminopropyl)amino)-5-oxopentanoic acid *(compound 5):* 4 (0.034 g, 0.037 mmol, 1 eq) was dissolved in 0.308 mL THF then 3.08 mL water followed by 0.2 mL of acetic acid to get the starting material suspended. 20% $Pd(OH)_2$ (0.052 g, 0.071 mmol, 2 eq) was added and the flask was evacuated three times and filled with hydrogen then left to stir overnight. The reaction was filter and condensed. Crude product was purified by an 800 mg C18 plug using 0-5% acetonitrile and no acid to yield the final product (17.2 mg, 84%). Mixture of two isomers present α/β: 1 to 0.59 ratio. 1H NMR (600 MHz, Methanol-d4) δ 4.98 (d, J = 3.4 Hz, 1H, $H_{1\alpha}$), 4.48 (d, J = 8.3 Hz, 1H, $H1_{\beta}$), 4.22 – 4.10 (m, 5H, αCH-isolglu, αCH-propionic, αCH-alanine), 3.88 (ddd, J = 10.5, 6.8, 3.4 Hz, 1H, $H_{2\alpha}$), 3.80 – 3.55 (m, 6H, $H_{3\alpha}$, $H_{5\alpha}$, 2 $H_{6\alpha}$, $H_{2\beta}$, $H_{4\beta}$, $H_{5\beta}$, 2 $H_{6\beta}$), 3.44 – 3.32 (m, 2H, $H_{4\alpha}$, $H_{3\beta}$), 3.2 (CH_2-Linker), 2.84 (td, J = 7.3, 1.7 Hz, 3H, CH_2-Linker), 2.31 (t, J = 7.4 Hz, 3H, γ-CH_2-isoglu), 2.09 (dq, J = 13.3, 7.6 Hz, 2H, β-CH-isoglu), 1.87 (m, J = 6.6 Hz, 5H, β-CH-isoglu, Acetate-CH_3), 1.75 (p, J = 6.7 Hz, 3H, CH_2-linker), 1.32 (dd, J = 7.1, 3.7 Hz, 6H, β-CH_3), 1.29 (dd, J = 6.7, 3.3 Hz, 6H, β-CH_3). 13C NMR (101 MHz, MeOD) δ 174.88 (carbonyl), 174.08 (carbonyl), 173.27 (carbonyl), 172.05 (carbonyl), 91.25 ($C_{1\alpha}$), 79.68 ($C_{1\beta}$), 77.68, 76.68 (α-$CH_{peptide}$), 71.79, 69.61, 61.16, 53.70 ($C_{2\alpha}$), 53.11 (α-$CH_{peptide}$), 49.44 (α-$CH_{peptide}$), 36.67 (CH_2-Linker), 35.40 (CH_2-Linker), 27.20 (CH_2-Linker), 26.30 (β-CH_2-D-isoglu), 21.78, 21.48 (Acetate-CH_3), 18.19 (β-CH_3), 16.38 (β-CH_3). HRMS-ESI: $C_{22}H_{40}N_5O_{11}$+ [M+H]= expected, 550.27188; observed, 550.27154.

[1] H NMR and [13]C NMR Spectra



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NH2
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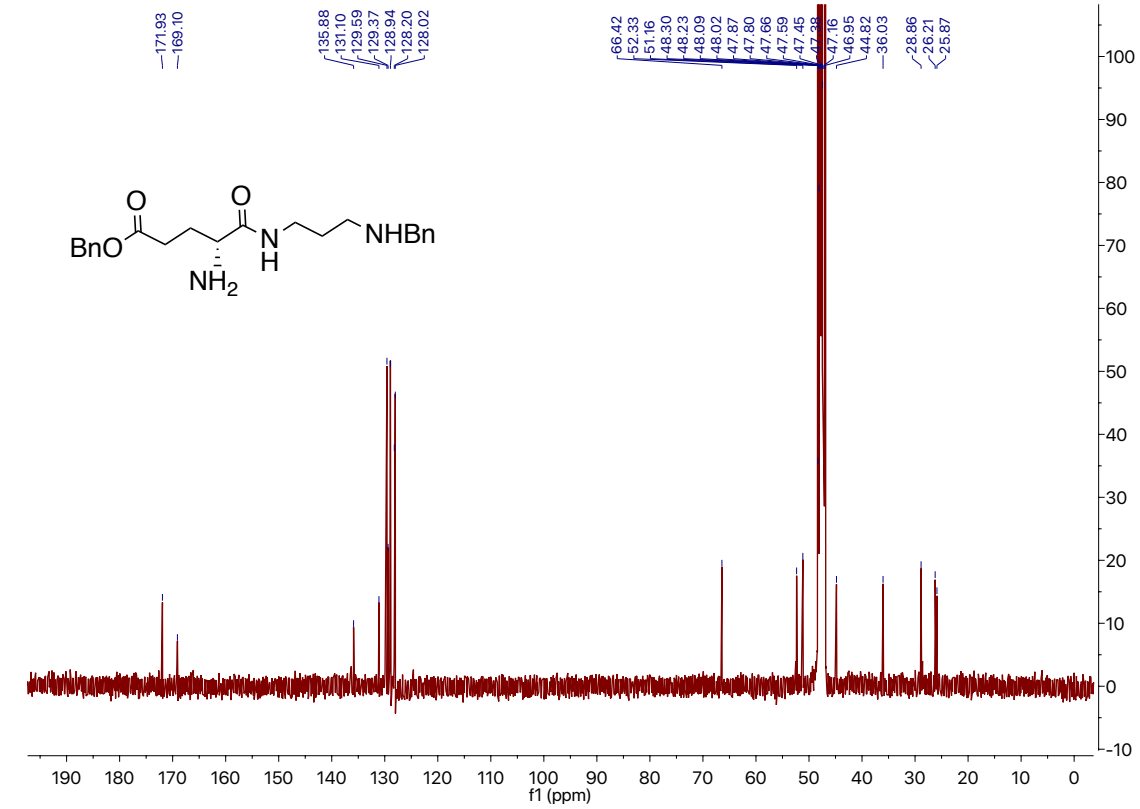
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H
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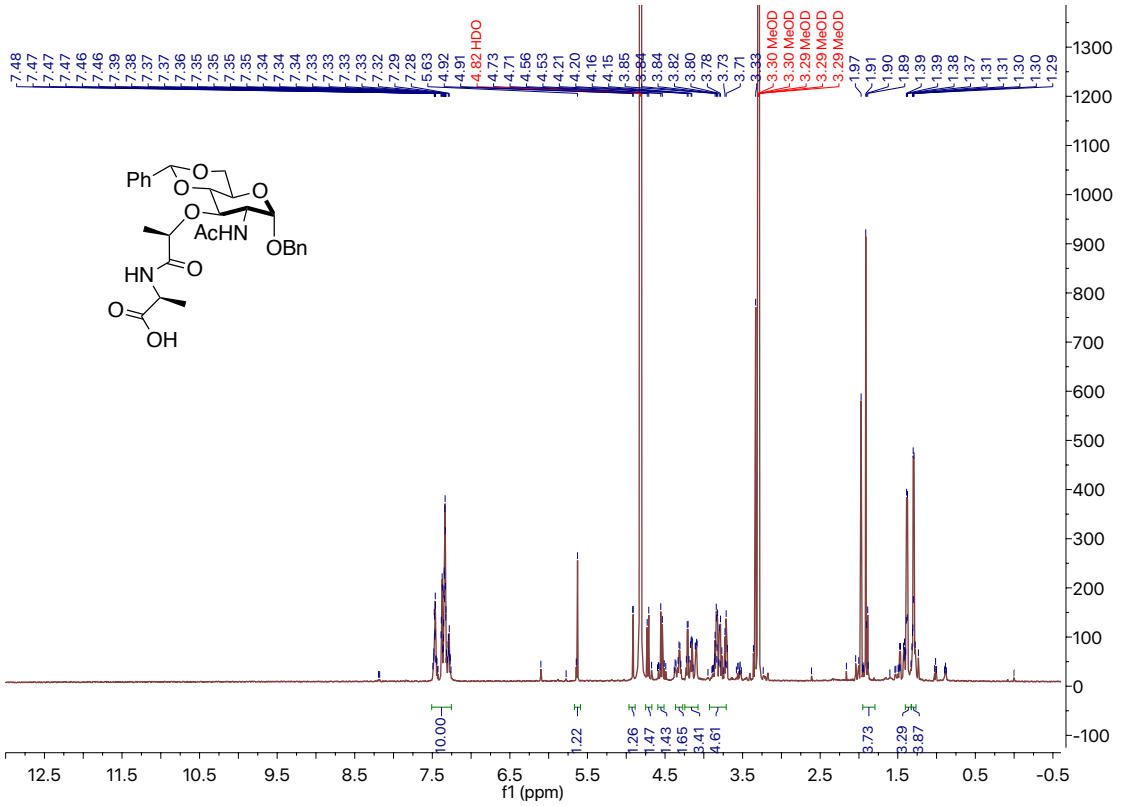
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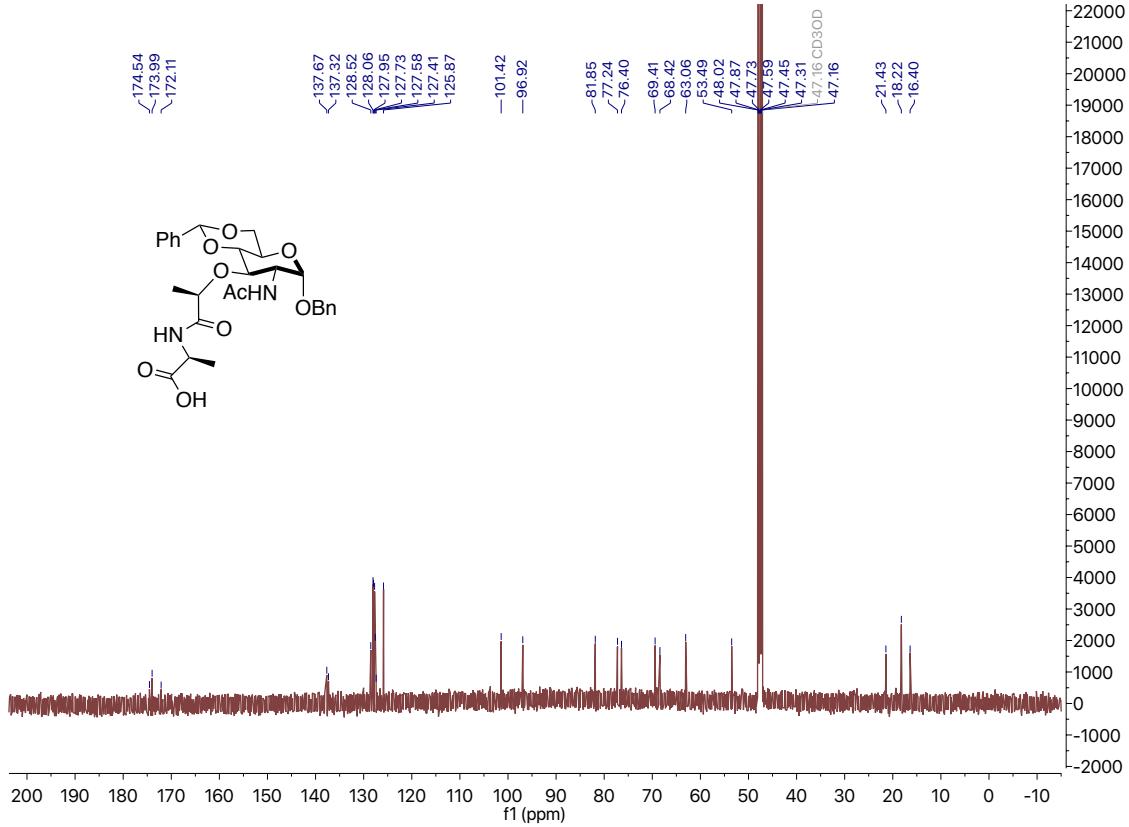
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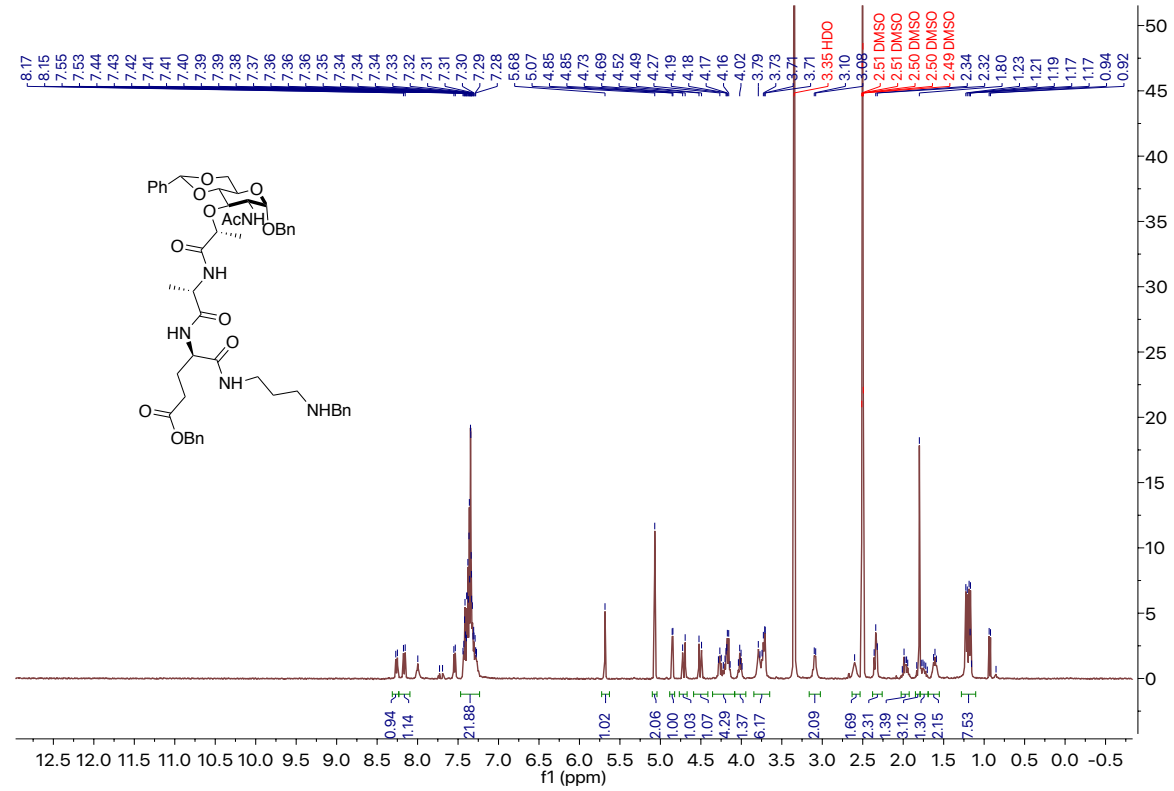
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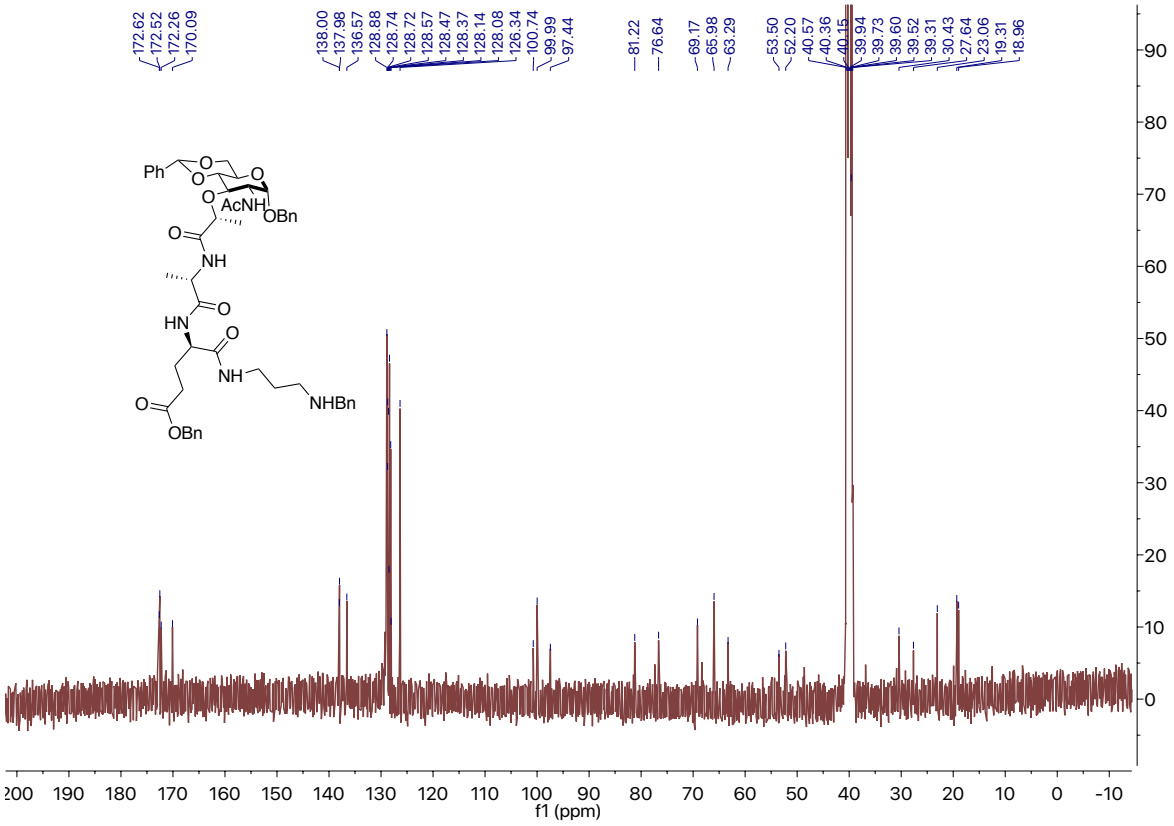
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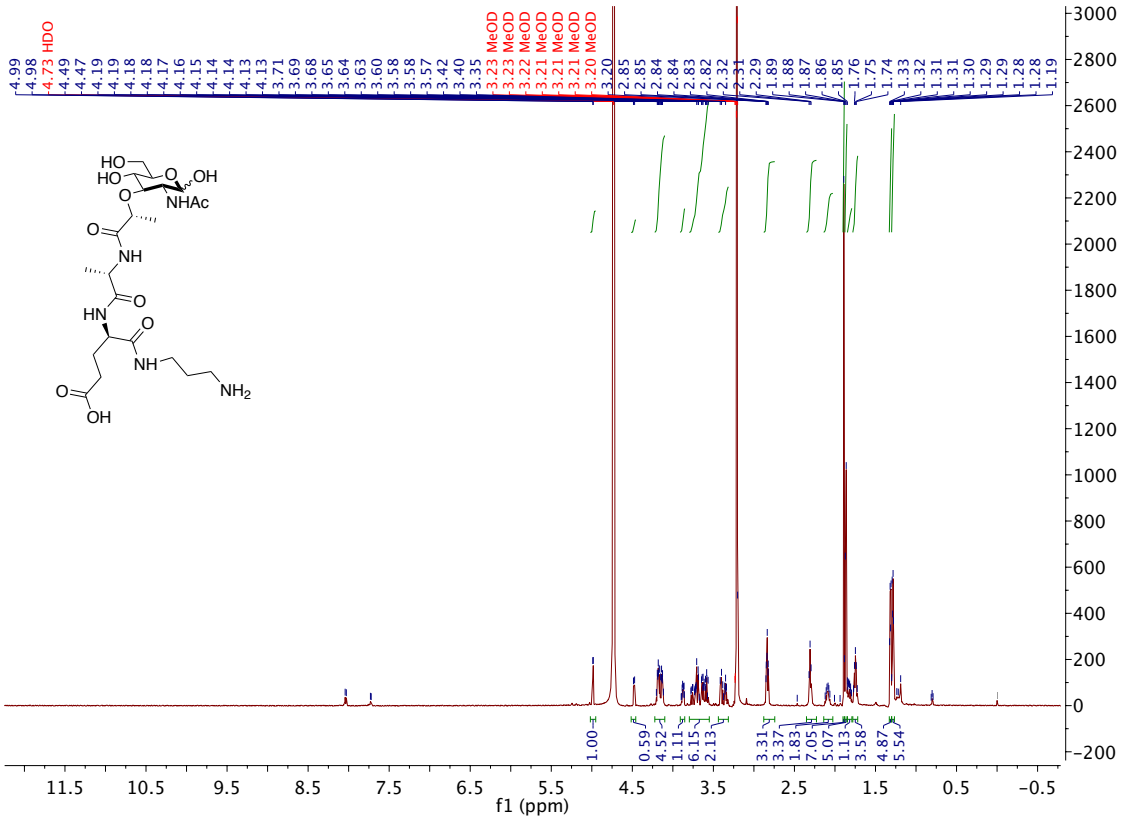
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3.40
3.35
3.23 MeOD
3.23 MeOD
3.22 MeOD
3.21 MeOD
3.21 MeOD
3.21 MeOD
3.20 MeOD
3.20
2.85
2.85
2.84
2.84
2.83
2.82
2.32
2.31
2.29
1.89
1.88
1.87
1.86
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1.28
1.19
HO
HO
O
OH
O
NHAc
O
NH
O
HN
O
NH
NH2
O
OH
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0.59
4.52
1.11
6.15
2.13
3.31
3.37
1.83
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3.58
4.87
5.54
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10.5
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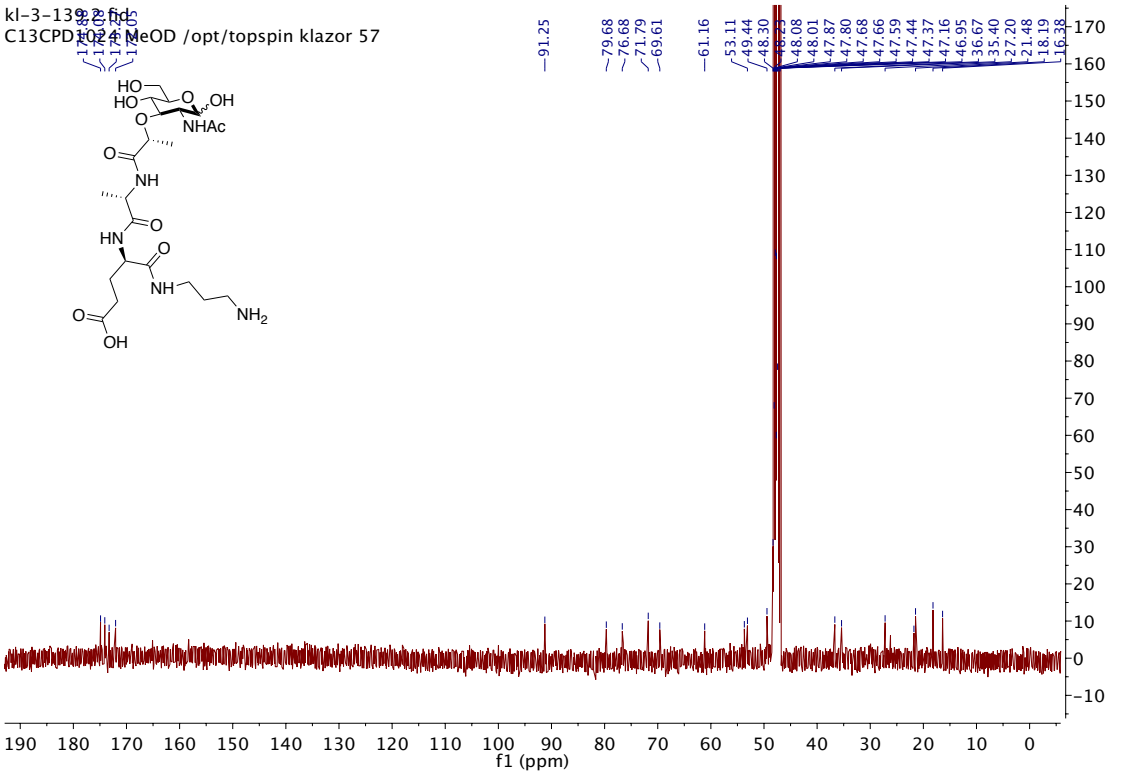
f1 (ppm)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C13CPD 1024 MeOD /opt/topspin klazor 57
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71.79
69.61
61.16
53.11
49.44
48.30
48.23
48.08
48.01
47.87
47.80
47.68
47.66
47.59
47.44
47.37
47.16
46.95
36.67
35.40
27.20
21.48
18.19
16.38
HO
HO
O
OH
O
NHAc
O
NH
O
HN
O
NH
NH2
O
OH
190
180
170
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100
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80
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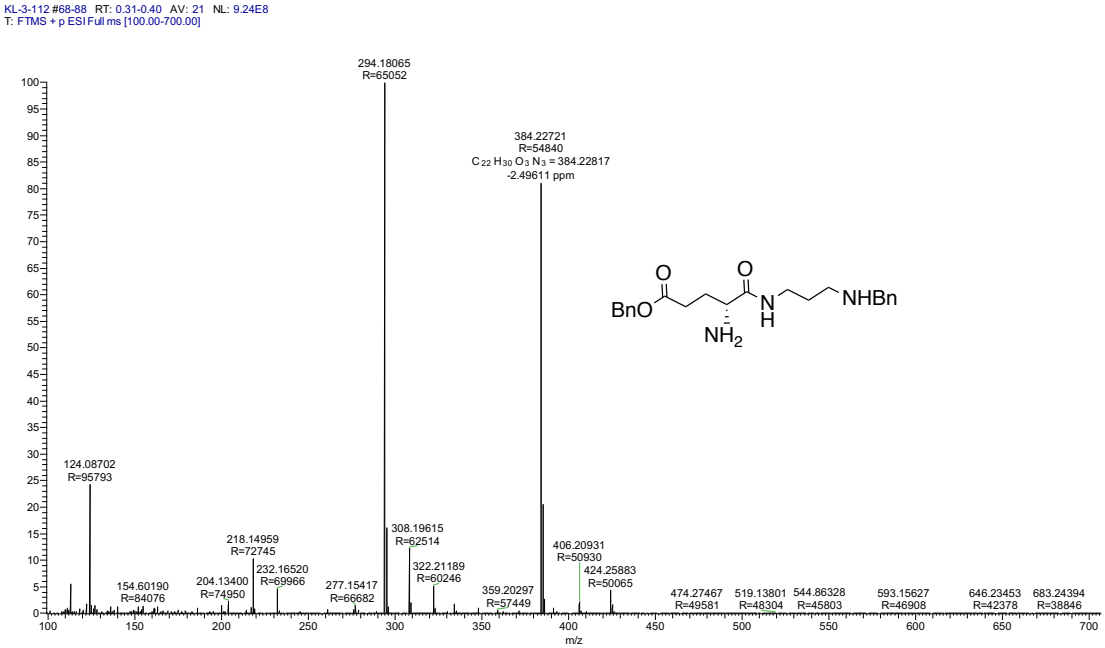
40
30
20
10
0
f1 (ppm)

a)



b)

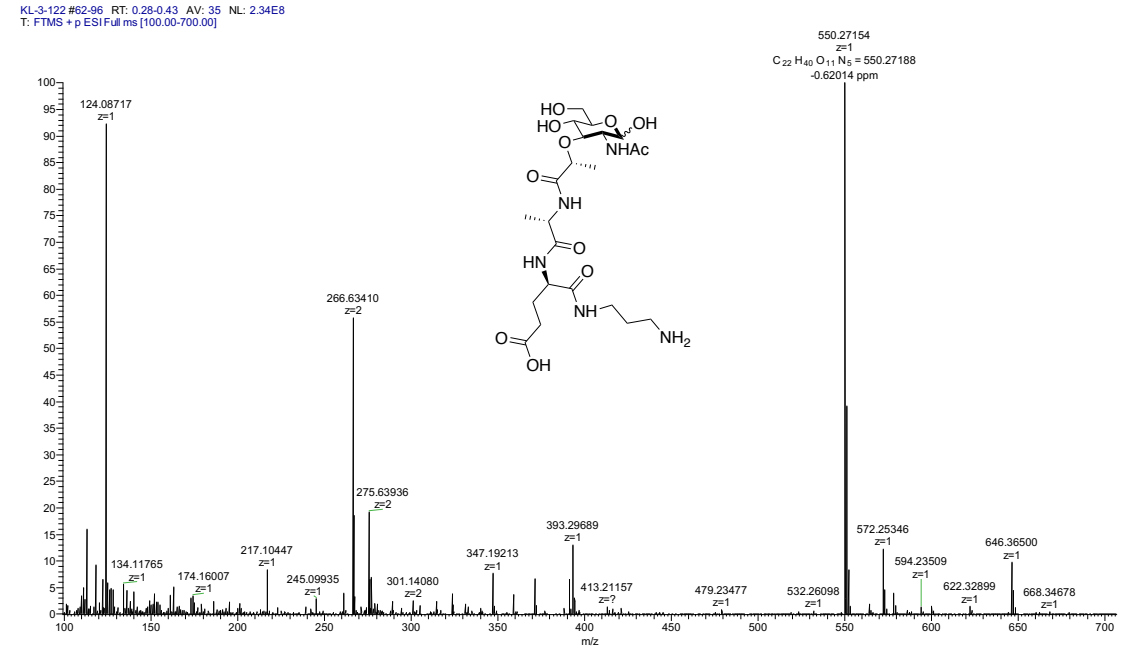


Figure S8. High resolution mass spectra of a) compound and b) compound **6**.

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