List of Supplementary Information:

1. Extended Materials and Instrumentation

Materials:

All chemicals were purchased from Sigma Aldrich, ThermoFisher or ChemImpex and used without further purification unless otherwise noted. All solvents were reagent grade anhydrous and purchased from Sigma Aldrich. Unless otherwise noted, all reactions were performed in oven dried round bottom flasks equipped with rubber septa, positive pressure of nitrogen, and magnetic stirring. Deuterated NMR solvents were purchased from Cambridge Isotope Laboratories, Inc. Reactions were monitored by thin layer chromatography (TLC) with glass plates coated with silica gel (silica HD TLC plates, UV 254, 250 um, Sorbent Technologies) and visualized with shortwave 254 nm UV light or developed upon heating with p-Anisaldehyde or ninhydrin. Flash column chromatography was carried out on silica gel (60 A, 40-63 um, Sorbent Technologies). Semi-preparative HPLC was performed on an Agilent Series 1100 instrument using a Phenomenex Luna 5 um C18 100Å column (250 mm x 10 mm). Preparative HPLC purification was performed using a Waters 2767 sample manager with HPLC and SQD2 MS using a Sunfire Prep C18 OBD 5 um 19 x 1000 mm or 4.6 x 50 mm column.

Biological Tools:

Recombinant human PGLYRP-1, PGLYRP-3 and PGLYRP-4 were purchased from R&D Systems. Monoclonal mouse anti-human PGLYRP-1 was purchased from R&D Systems; monoclonal mouse anti-human PGLYRP-3 and PGLYRP-4 were purchased from Novus Biologicals. Cy3-conjugated goat anti-mouse IgG was used as the secondary antibody and was purchased from Invitrogen. Biotinylated-wheat germ agglutinin (WGA) was purchased Vector Laboratories. Cy3-conjugated streptavidin was used as the secondary antibody and was purchased

from Jackson ImmunoResearch Laboratories. MDP-antibody was provided by the Wang Laboratory. NagK was purchased from ProSpec.

Instrumentation:

NMR spectra were recorded on either a Bruker AVIII 400 MHz or AVIII 600 MHz spectrometer. High resolution mass spectrometry (HRMS-ESI) data were obtained on a Thermo Q-Exactive Orbitrap at the University of Delaware Mass Spectrometry Facility. Low resolution mass spectrometry (LRMS-ESI) data were obtained using an ACQUITY UPLC H-Class/SQD2 (University of Delaware).

2. Synthesis and Characterization of Microarray Components:

Preparation of Controls:

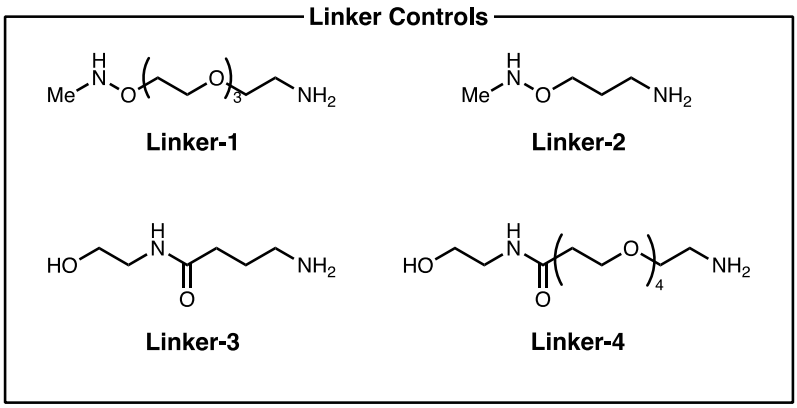


Figure S1: Structures of linker controls printed on the glycan array. Four linker controls were prepared to confirm that binding observed was due to the carbohydrate, not the linker moiety.

3,6,9,12-tetraoxa-2-azatetradecan-14-amine (**Linker-1**): Synthesized according to exact literature precedent. Spectral data agrees with literature reported values [39].

3-((methylamino)oxy)propan-1-amine (**Linker-2**): Synthesized according to exact literature precedent. Spectral data agrees with literature reported values[39].

4-amino-N-(2-hydroxyethyl)butanamide (**Linker-3**): Prepared by coupling of commercially available 4-azido butyrate NHS ester and ethanolamine. The corresponding amide formed a precipitate and was hydrogenated (Pd/C). HRMS-ESI. Calculated for $C_6H_{15}N_2O_2$ $[M+H]^+$ 147.1128; observed, 147.1124.

1-amino-N-(2-hydroxyethyl)-3,6,9,12-tetraoxapentadecan-15-amide (**Linker-4**): Prepared by coupling commercially available NHS-PEG4-azide and ethanolamine. The corresponding amide formed a precipitate and was hydrogenated (Pd/C). HRMS-ESI. Calculated for $C_{13}H_{29}N_2O_6$ $[M+H]^+$ 309.2020; observed, 309.2022.

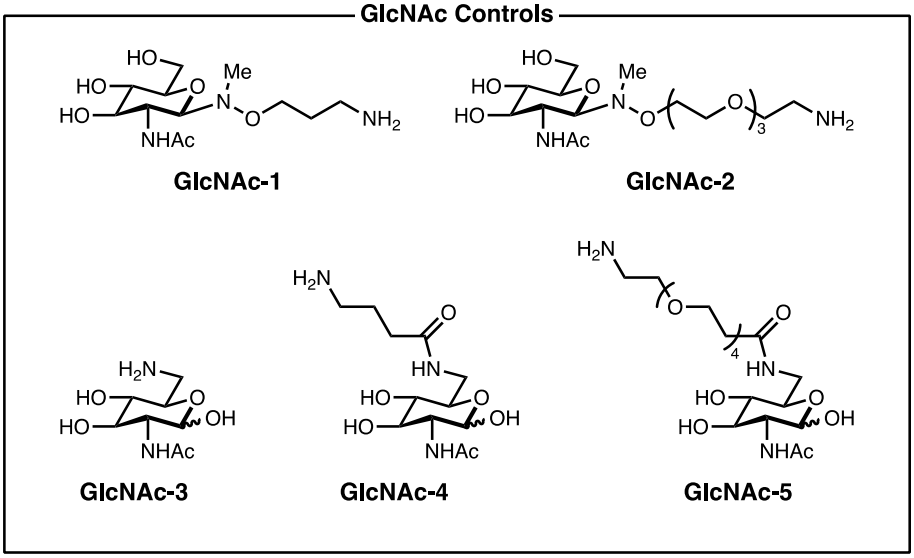


Figure S2: Structures of GlcNAc controls printed on the glycan array. As a control, *N*-acetylglucosamine (GlcNAc) derivatives were prepared and immobilized in order to ensure specificity for the MurNAc portion, not the GlcNAc residue of disaccharides.

N-((2R,3R,4R,5S,6R)-2-((3-aminopropoxy)(methyl)amino)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)acetamide (**GlcNAc-1**): *N*-Acetyl glucosamine (0.023 g, 0.10 mmol, 1 equiv.) and **Linker-2** (0.340 g, 1.04 mmol, 10 equiv.) were dissolved in 1M sodium acetate buffer (pH 4.5) (0.91 mL). The solution was allowed to stir at room temperature for 3 d. After which, the reaction mixture was lyophilized and the crude product was purified on a

Waters mass-directed auto-purification system using a HILIC column with a mobile phase of 100-80% acetonitrile in water with 0.1% formic acid over 5 min. Following lyophilization, **GlcNAc-1** was obtained as a white powder (0.016 g, 51%). ^{1}H NMR (400 MHz, D_2O) δ 4.04 (d, J = 9.7 Hz, 1H), 3.89 – 3.55 (m, 4H), 3.49 (dt, J = 8.9, 6.2 Hz, 1H), 3.43 – 3.23 (m, 3H), 2.96 (dd, J = 8.6, 6.4 Hz, 2H), 2.62 (s, 3H), 1.91 (s, 3H), 1.87 – 1.71 (m, 2H). ^{13}C NMR (101 MHz, D_2O) δ 174.07, 91.43, 77.37, 75.35, 69.5, 68.96, 60.62, 52.03, 38.85, 36.95, 25.72, 22.21. HRMS-ESI. Calculated for $C_{12}H_{26}N_3O_6$ $[M+H]^+$ 308.18161; observed, 308.18080.

N-((2R,3R,4R,5S,6R)-2-((2-(2-(2-(2-aminoethoxy)ethoxy)ethoxy)ethoxy)(methyl)amino)-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-3-yl)acetamide (**GlcNAc-2**): *N*-Acetyl glucosamine (0.010 g, 0.05 mmol, 1 equiv.) and **Linker-1** (0.117 g, 0.26 mmol, 5.7 equiv.) were dissolved in 1M sodium acetate buffer (pH 4.5) (0.45 mL). The solution was allowed to stir at room temperature for 48 h. After which, the reaction mixture was lyophilized and the crude product was purified on a Waters mass-directed auto-purification system using a HILIC column with a mobile phase of 100-70% acetonitrile in water with 0.1% formic acid over 12.5 min. Following lyophilization, **GlcNAc-2** was obtained as a white powder (0.004 g, 18%). ^{1}H NMR (600 MHz, D_2O) δ 4.08 (d, J = 9.7 Hz, 1H), 3.84 – 3.68 (m, 4H), 3.68 – 3.48 (m, 17H), 3.42 – 3.22 (m, 3H), 3.06 (s, 3H), 2.59 (s, 3H), 1.88 (d, J = 6.0 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 170.94, 91.52, 77.32, 75.35, 71.05, 69.63, 69.53, 69.47, 69.42, 68.51, 66.35, 60.74, 52.34, 39.10, 38.65, 22.27. HRMS-ESI. Calculated for $C_{17}H_{36}N_3O_9$ $[M+H]^+$ 426.24461; observed, 426.24390.

N-((2S,3R,4R,5S,6R)-6-(aminomethyl)-2,4,5-trihydroxytetrahydro-2H-pyran-3-yl)acetamide (**GlcNAc-3**): Prepared from 6-azido-*N*-acetylglucosamine[2] via hydrogenation (10% Pd/C). ^{1}H NMR (600 MHz, methanol-d_4) (Anomers – α 1.00 : β 0.34) δ 5.13 (d, *J* = 3.3 Hz, 1H), 4.65 (d, *J*

= 8.3 Hz, 1H), 4.03 – 3.98 (m, 2H), 3.86 (dd, *J* = 10.7, 3.3 Hz, 1H), 3.75 – 3.69 (m, 1H), 3.65 – 3.58 (m, 1H), 3.53 – 3.46 (m, 2H), 3.41 – 3.33 (m, 2H), 3.27 – 3.20 (m, 2H), 3.04 (dd, *J* = 13.0, 8.5 Hz, 2H), 2.00 (s, 3H), 1.99 (s, 3H). ^{13}C NMR (151 MHz, methanol-d_4) δ 172.90, 172.41, 95.83, 91.24, 74.01, 72.72, 72.33, 72.03, 70.73, 67.45, 57.32, 54.36, 40.74, 40.71, 21.52, 21.21. HRMS-ESI. Calculated for $C_8H_{17}N_2O_5$ $[M+H]^+$ 221.1132; observed, 221.1131.

N-(((2R,3S,4R,5R)-5-acetamido-3,4,6-trihydroxytetrahydro-2H-pyran-2-yl)methyl)-4-aminobutanamide (**GlcNAc-4**): Prepared in 2 steps from **GlcNAc-3** by coupling commercially available N_3-C3-NHS ester. The resulting azide was hydrogenated. HRMS-ESI. Calculated for $C_{12}H_{24}N_3O_6$ $[M+H]^+$ 306.1660; observed, 306.1660.

N-(((2R,3S,4R,5R)-5-acetamido-3,4,6-trihydroxytetrahydro-2H-pyran-2-yl)methyl)-1-amino-3,6,9,12-tetraoxapentadecan-15-amide (**GlcNAc-5**): Prepared in 2 steps from **GlcNAc-3** by first coupling commercially available azido-PEG_4-NHS ester. The resulting azide was hydrogenated. HRMS-ESI. Calculated for $C_{19}H_{38}N_3O_{10}$ $[M+H]^+$ 468.2552; observed, 468.2563.

Preparation of Muramyl Dipeptide (MDP) Derivatives:

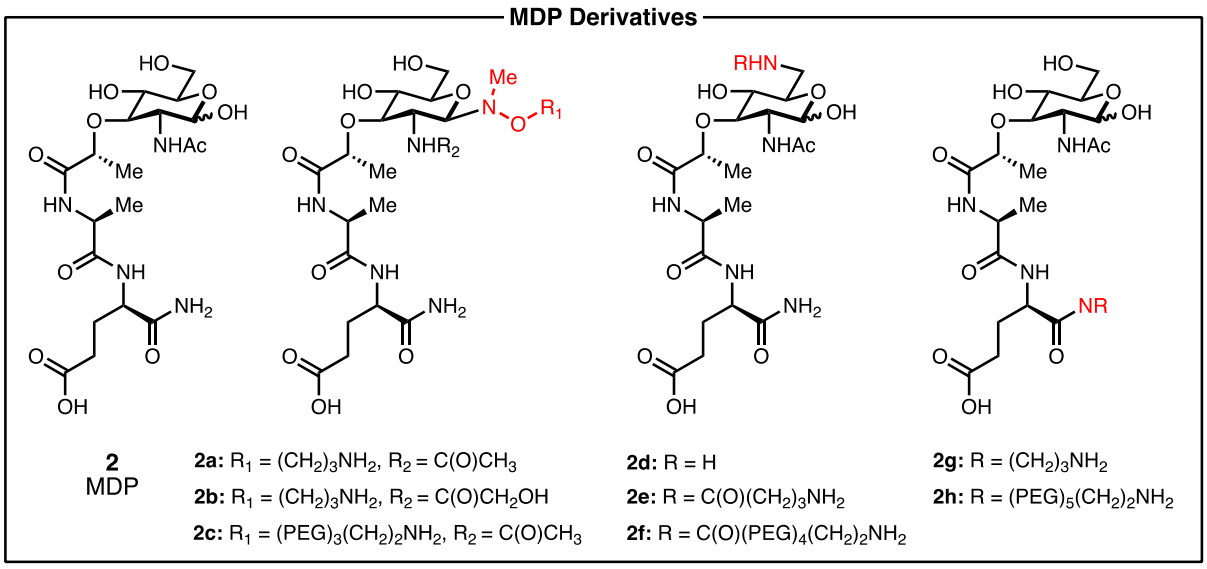


Figure S3: Structures of MDP derivatives printed on the glycan array.

(R)-4-((S)-2-((R)-2-(((2R,3R,4R,5S,6R)-3-acetamido-2-((3-aminopropoxy) (methyl) amino)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propanamido) propanamido)-5-amino-5-oxopentanoic acid (**2a**): Synthesized according to exact literature precedent. Spectral data agrees with literature reported values.[48]

(R)-5-amino-4-((S)-2-((R)-2-(((2R,3R,4R,5S,6R)-2-((3-aminopropoxy)(methyl)amino)-5-hydroxy-3-(2-hydroxyacetamido)-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propanamido)propanamido)-5-oxopentanoic acid (**2b**): In accordance with the literature procedure[1] commercially available *N*-glycoyl MDP was coupled to **Linker-3** in 1 M sodium

acetate buffer (pH 4.5). HRMS-ESI. Calculated for $C_{23}H_{43}N_6O_{12}$ $[M+H]^+$ 595.2933; observed, 595.2944.

(R)-4-((S)-2-((R)-2-(((2R,3R,4R,5S,6R)-3-acetamido-2-((2-(2-(2(aminoethoxy)ethoxy) ethoxy)ethoxy)(methyl)amino)-5-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propanamido)propanamido)-5-amino-5-oxopentanoic acid (**2c**): Synthesized according to exact literature precedent. Spectral data agrees with literature reported values.[48]

(S)-4-((S)-2-((R)-2-(((2S,3R,4R,5R,6R)-3-acetamido-6-(aminomethyl)-2,5 dihydroxytetrahydro-2H-pyran-4-yl)oxy)propanamido)propanamido)-5-amino-5-oxopentanoic acid (**2d**): Synthesized according to exact literature precedent. Spectral data agrees with literature reported values [40].

(4R)-4-((2S)-2-((2R)-2-(((3R,5R,6R)-3-acetamido-6-((4-aminobutanamido)methyl)-2,5-dihydroxytetrahydro-2H-pyran-4-yl)oxy)propanamido)propanamido)-5-amino-5-oxopentanoic acid (**2e**): **2d** (69 mg, 0.141 mmol, 1 equiv.) and sodium carbonate (45 mg, 0.422 mmol, 3 equiv.) were suspended in anhydrous DMSO (5 mL) with stirring under nitrogen. N_3-C3-NHS ester (35 mg, 0.155 mmol, 1.1 equiv.) added portion wise over 1 h. The solution was allowed to stir at room temperature for 16 hrs. After which time, the reaction mixture was filtered and condensed. The residue was purified via HPLC C18 column chromatography using a Waters mass-directed auto-purification system with a mobile phase of 5-95% acetonitrile in water (0.1% formic acid). Following lyophilization, **2e.1** was obtained as a white solid (73 mg, 86% yield).

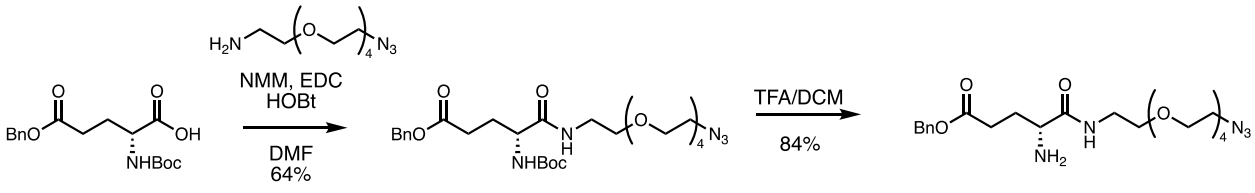
2e.1 (50 mg, 0.08 mmol, 1 equiv.) was dissolved in THF (0.4 mL), water (1.6 mL), and acetic acid (0.2 mL). 10% Pd/C (9 mg, 0.008 mmol, 0.1 equiv.) was added. The flask was evacuated 3X and filled with hydrogen via balloon. The solution was allowed to stir under H_2 with monitoring by LCMS until completion (20 h). The reaction mixture was filtered then condensed. The product was purified via HPLC C18 column chromatography on a Waters auto purification system, 0-45% acetonitrile in water (with 0.1% formic acid) over 4 minutes at 20 mL/min to yield **2e** as white solid (43 mg, 0.074 mmol, 89%). ^{1}H NMR (600 MHz, D_2O) δ 5.09 (d, J = 3.5 Hz, 1H), 4.60 (d, J = 8.4 Hz, 0H), 4.25 (d, J = 7.3 Hz, 1H), 4.24 – 4.15 (m, 2H), 3.88 (dd, J = 10.5, 3.5 Hz, 1H), 3.85 (ddd, J = 9.6, 5.7, 3.2 Hz, 1H), 3.71 (t, J = 9.4 Hz, 0H), 3.63 (t, J = 9.7 Hz, 1H), 3.57 (dd, J = 14.0, 2.4 Hz, 0H), 3.47 (dtd, J = 21.0, 13.2, 11.9, 4.8 Hz, 2H), 3.42 – 3.34 (m, 1H), 2.96 (t, J = 7.7 Hz, 2H), 2.62 (s, 3H), 2.36 (dt, J = 11.0, 5.4 Hz, 2H), 2.21 (td, J = 7.6, 3.6 Hz, 2H), 2.05 (h, J = 7.3 Hz, 1H), 1.93 – 1.87 (m, 5H), 1.87 (d, J = 7.1 Hz, 1H), 1.85 (s, 2H), 1.37 (dd, J = 7.2, 3.5 Hz, 3H), 1.31 (t, J = 6.0 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 181.35, 179.71, 176.49, 175.75, 175.50, 175.30, 175.29, 175.07, 174.36, 174.07, 94.94, 90.91, 82.01, 79.19, 77.90, 77.64, 74.57, 73.78, 71.22, 70.61, 70.27, 69.84, 56.07, 53.66, 53.55, 53.53, 49.72, 49.69, 40.04, 39.91, 38.89, 38.80, 33.72, 32.48, 27.75, 27.69, 25.06, 23.08, 22.28, 22.05, 18.65, 16.78, 16.77. HRMS-ESI. Calculated for $C_{23}H_{41}N_6O_{11}$ $[M+H]^+$ 577.2828; observed, 577.2811.

4R)-4-((2S)-2-((2R)-2-(((3R,5R,6R)-3-acetamido-6-(17-amino-3-oxo-6,9,12,15- tetraoxa-2-azaheptadecyl)-2,5-dihydroxytetrahydro-2H-pyran-4- yl)oxy) propanamido) propanamido)-5-amino-5-oxopentanoic acid (**2f**): **2d** (69 mg, 0.14 mmol, 1 equiv.) and sodium carbonate (45 mg, 0.42 mmol, 3 equiv.) were suspended in anhydrous DMSO (2 mL) with stirring under nitrogen. Azido-PEG$_4$-NHS ester (60 mg, 0.16 mmol, 1.1 equiv.) was added portion wise over 1 h. The

solution was allowed to stir at room temperature for 16 hrs. After which time, the reaction mixture was filtered and condensed. The residue was purified via HPLC C18 column chromatography using a Waters mass-directed auto-purification system with a mobile phase of 5-95% acetonitrile in water (0.1% formic acid). Following lyophilization, **2f.1** was obtained as a white solid (87 mg, 81% yield).

2f.1 (50 mg, 0.07 mmol, 1 equiv.) was dissolved in THF (0.4 mL), water (1.6 mL) and acetic acid (0.2 mL). 10% Pd/C (7 mg, 0.007 mmol, 0.1 equiv.) was added and the flask was evacuated 3X and filled with hydrogen via balloon. The solution was allowed to stir under H_2 with monitoring by LCMS until completion (20 h).). The reaction mixture was filtered then condensed. The product was purified via HPLC C18 column chromatography on a Waters auto purification system, 0-45% acetonitrile in water (with 0.1% formic acid) over 4 minutes at 20 mL/min to yield **2f** as white solid (24 mg, 0.032 mmol, 49%). HRMS-ESI. Calculated for $C_{30}H_{55}N_6O_{15}$ $[M+H]^+$ 739.3720; observed, 739.3713.

(*4R)-4-((2S)-2-((2R)-2-(((3R,5S,6R)-3-acetamido-2,5-dihydroxy-6-(hydroxymethyl) tetrahydro-2H-pyran-4-yl)oxy)propanamido)propanamido)-5-((3-aminopropyl)amino)-5-oxopentanoic acid* (**2g**): Synthesized according to exact literature precedent. Spectral data agrees with literature reported values.[18]



Scheme 1:

Peptide: Prepared in 2 steps from commercially available Boc-D-Glu(OBn)-OH. ^{1}H NMR (600 MHz, methanol-d4) δ 7.48 – 7.30 (m, 5H), 5.17 (s, 2H), 3.96 – 3.84 (m, 1H), 3.74 – 3.46 (m, 18H), 3.43 – 3.35 (m, 3H), 2.58 (td, J = 7.5, 3.1 Hz, 2H), 2.17 (td, J = 7.7, 6.5 Hz, 2H). ^{13}C NMR (151 MHz, methanol-d4) δ 172.01, 168.07, 128.20, 128.00, 127.98, 70.18, 70.16, 70.14, 70.10, 69.79, 69.68, 68.74, 66.36, 52.33, 50.38, 48.02, 47.87, 47.73, 47.59, 47.45, 47.30, 47.16, 39.21, 28.84, 26.23.

(20R)-20-((2S)-2-((2R)-2-(((3R,4R,5S,6R)-3-acetamido-2,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propanamido)propanamido)-1-amino-19-oxo-3,6,9,12,15-pentaoxa-18-azatricosan-23-oic acid (**2h**): Protected intermediate[4] (0.073 g, 0.13 mmol, 1 equiv.) and peptide (0.096 g, 0.16 mmol, 1.2 eq) were dissolved in DMF (2.6 mL) at 0℃. HBTU (0.058 g, 0.15 mmol, 1.2 equiv.) and DIPEA (0.085 mL, 0.52 mmol, 4 equiv.) were added and the solution was overnight. Once complete, the reaction mixture was diluted with DCM and washed with 1N HCl, saturated $NaHCO_3$ and brine. The combined organic phases were dried over Na_2SO_4 and condensed. The crude product was purified by column chromatography, eluting with 0-7% MeOH in DCM to afford the desired product (0.09g, 69%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.53 – 7.25 (m, 23H), 7.25 – 7.01 (m, 4H,), 6.30 (d, J = 7.9 Hz, 1H), 5.56 (s, 1H), 5.09 (s, 2H), 4.94 (d, J = 3.8 Hz, 1H), 4.72 (d, J = 11.8 Hz, 1H), 4.50 (d, J = 11.8 Hz, 1H), 4.43 (td, J = 8.2, 4.8 Hz, 1H), 4.36 – 4.16 (m, 3H), 4.09 (q, J = 6.7 Hz, 1H), 3.94 – 3.31 (m, 36H), 2.55 – 2.34 (m, 2H), 2.20 (tt, J = 13.0, 6.3 Hz, 1H), 2.02 – 1.86 (m, 4H), 1.38 (dd, J = 11.0, 6.9 Hz, 8H). ^{13}C NMR (101 MHz, $CDCl_3$) δ 173.61, 173.34, 172.11, 171.09, 170.79, 137.02, 136.67, 135.70, 129.08, 128.72, 128.59, 128.40, 128.33, 128.29, 128.20, 126.00, 125.94, 101.44, 97.43, 81.43, 77.36, 77.04, 76.72,

70.49, 70.39, 70.36, 70.26, 70.13, 69.98, 69.90, 69.70, 68.83, 66.53, 63.11, 55.65, 53.47, 53.09, 52.62, 50.62, 49.67, 43.64, 39.29, 30.44, 27.24, 23.46, 19.41, 17.50, 12.54, 0.03.

Intermediate-2 (0.023 g, 0.02 mmol, 1 equiv.) was dissolved in THF (0.5 mL) and water (1.7 mL). 20% $Pd(OH)_2$ (0.032 g, 0.043 mmol, 2 equiv.) was added. The flask was evacuated 3X and filled with hydrogen via balloon. The solution was allowed to stir under H_2 with monitoring by LCMS until completion (20 h). The reaction mixture was filtered then condensed. The product was purified via HPLC C18 column chromatography on a Waters mass-directed auto purification system, 0-15% acetonitrile in water (with 0.1% formic acid) using a C18 column. Following lyophilization, **2h** was obtained as a white solid (0.006 g, 35%). ^{1}H NMR (400 MHz, D_2O) δ 5.03 – 5.01 (m, 1H), 4.12 (tq, J = 13.4, 6.7 Hz, 5H), 3.86 – 3.40 (m, 45H), 3.28 (q, J = 5.7 Hz, 2H), 3.11 – 3.05 (m, 2H), 2.12 (t, J = 7.5 Hz, 3H), 1.86 – 1.75 (m, 7H), 1.30 (dd, J = 7.2, 2.5 Hz, 5H,), 1.24 (dd, J = 6.8, 3.4 Hz, 5H). ^{13}C NMR (101 MHz, D_2O) δ 175.71, 175.47, 174.85, 174.10, 173.85, 173.57, 99.98, 94.80, 90.83, 82.43, 79.51, 77.68, 75.59, 71.37, 69.55, 69.50, 69.42, 69.39, 69.35, 68.82, 68.65, 66.28, 60.57, 60.36, 56.01, 53.72, 53.60, 49.60, 39.00, 38.83, 27.76, 22.08, 21.85, 18.56, 16.67. HRMS-ESI. Calculated for $C_{29}H_{54}N_5O_{15}$ $[M+H]^+$ 712.36109; observed, 712.36145.

Preparation of Muramyl Tripeptide (MTP) Derivatives:

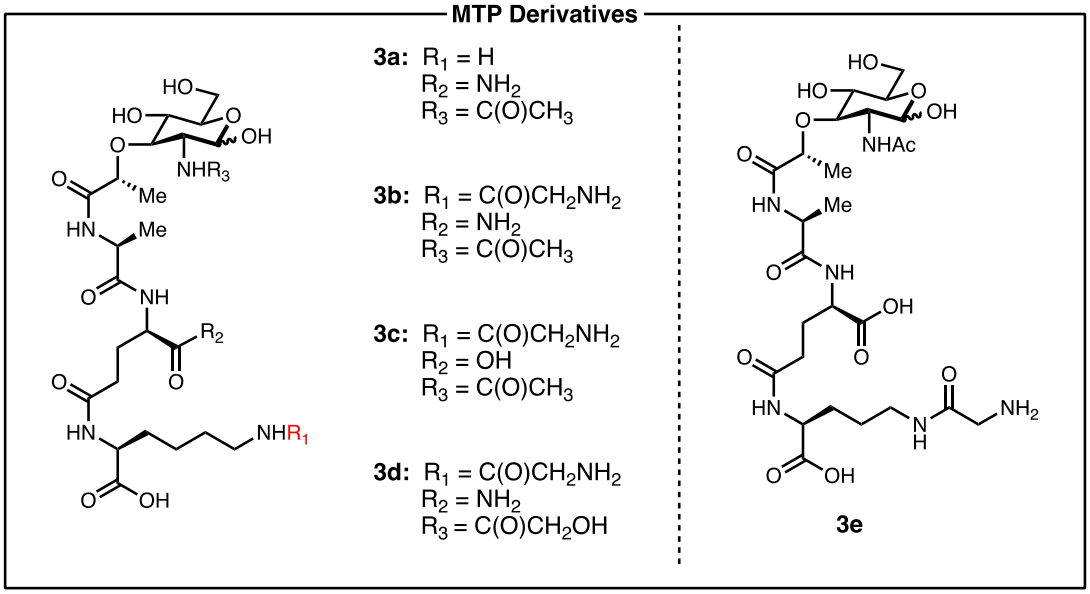


Figure S4: Structures of MTP derivatives printed on the glycan array.

General procedure #1: To a solution of protected muramic acid[5] (1.0 equiv.) in anhydrous DMF (0.1 M) was added the tripeptide (1.0 equiv.). NMM (3.0 equiv.) and HATU (1.2 equiv.) was added and the solution was stirred at RT with monitoring by TLC. Upon completion, solvent was removed *in vacuo*. The resulting residue was dissolved in EtOAc and washed with 1N HCl then brine. The combined organic phases were dried over Na_2SO_4 and condensed. When further purification was needed, column chromatography (often 5-8% methanol in DCM) provided quality protected carbohydrate substrates. The protected substrates were globally deprotected (often treatment with TBAF to remove silyl protecting groups) and hydrogenated (Pd/C). Purification (reverse-phase HPLC) gave highly pure products.

((R)-4-((S)-2-((R)-2-(((3R,4R,5S,6R)-3-acetamido-2,5-dihydroxy-6- (hydroxymethyl) tetrahydro-2H-pyran-4-yl)oxy)propanamido)propanamido)-5- amino-5-oxopentanoyl)-L-lysine (**3a**): Prepared following general procedure #1. ^{1}H NMR (600 MHz, D_2O) δ 8.48 (s, 1H), 5.13 – 5.09 (m, 1H), 4.63 (dd, *J* = 8.4, 1.6 Hz, 1H), 4.25 (dtd, *J* = 14.3, 9.1, 5.9 Hz, 2H), 4.20 – 4.10 (m, 2H), CH(Lys)), 3.91 (dd, *J* = 10.5, 3.5 Hz, 1H), 3.88 – 3.69 (m, 7H), 3.66 (td, *J* = 9.6, 8.8, 1.7 Hz, 1H), 3.56 – 3.45 (m, 2H), 3.43 (ddd, *J* = 9.7, 4.7, 2.7 Hz, 1H), 2.95 (t, *J* = 7.6 Hz, 2H), 2.35 (t, *J* = 7.7 Hz, 2H), 2.17 – 2.08 (m, 1H), 2.00 – 1.91 (m, 5H), 1.82 – 1.73 (m, 1H), 1.64 (ddt, *J* = 14.0, 10.4, 7.3 Hz, 3H), 1.40 – 1.30 (m, 7H). ^{13}C NMR (151 MHz, D_2O) δ 178.56, 175.96, 175.92, 175.71, 175.12, 174.37, 174.35, 174.27, 174.00, 169.93, 94.93, 90.96, 82.53, 79.63, 77.73, 75.74, 71.54, 68.98, 68.76, 60.74, 60.55, 56.16, 54.83, 53.69, 53.02, 49.75, 39.26, 31.87, 30.94, 26.97, 26.94, 22.24, 22.15, 18.66, 16.59. HRMS-ESI. Calculated for $C_{25}H_{45}N_6O_{12}$ $[M+H]^+$ 621.30172; observed, 621.30652.

N^2-((R)-4-((S)-2-((R)-2-(((3R,4R,5S,6R)-3-acetamido-2,5-dihydroxy-6- (hydroxymethyl) tetrahydro-2H-pyran-4-yl)oxy)propanamido)propanamido)-5- amino-5-oxopentanoyl)-N^6-glycyl-L-lysine (**3b**): Prepared following general procedure #1. ^{1}H NMR (600 MHz, D_2O) δ 4.67 (s, 5H), 4.30 – 4.21 (m, 1H), 4.09 (dd, *J* = 8.1, 5.2 Hz, 1H), 3.89 (s, 1H), 3.85 (s, 1H), 3.83 (s, 1H), 3.88 – 3.60 (m, 2H), 3.56 – 3.40 (m, 1H), 3.19 – 3.14 (m, 1H), 2.34 (t, *J* = 7.6 Hz, 1H), 2.13 (tt, *J* = 13.2, 6.0 Hz, 1H), 1.93 (d, *J* = 3.4 Hz, 2H), 1.72 (ddt, *J* = 21.3, 13.3, 7.0 Hz, 1H), 1.62 (dq, *J* = 15.0, 7.8 Hz, 1H), 1.50 – 1.43 (m, 1H), 1.38 (dd, *J* = 7.2, 4.1 Hz, 2H), 1.35 – 1.23 (m, 3H). ^{13}C NMR (151 MHz, D2O) δ 179.12, 175.97, 175.90, 175.68, 175.11, 174.27, 174.01, 170.88, 167.73, 94.94, 90.96, 82.51, 79.60, 78.04, 77.73, 75.74, 71.55, 69.01, 68.78, 60.74, 60.55, 58.65, 56.15, 55.20, 53.69, 53.07, 49.75, 49.73, 42.53, 40.45, 39.13, 33.77, 31.92, 31.14, 27.87, 26.95, 22.43, 22.24,

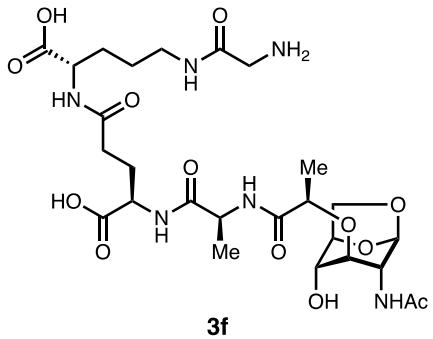
22.02, 18.65, 16.60. HRMS-ESI. Calculated for $C_{27}H_{48}N_7O_{13}$ $[M+H]^+$ 678.3305; observed, 678.3317.

((R)-4-((S)-2-((R)-2-(((3R,4R,5S,6R)-3-acetamido-2,5-dihydroxy-6- (hydroxymethyl) tetrahydro-2H-pyran-4-yl)oxy)propanamido)propanamido)-4- carboxybutanoyl)-L-lysine (**3c**): Prepared following general procedure #1. ^{1}H NMR (600 MHz, D_2O) δ 5.10 (d, *J* = 3.5 Hz, 1H), 4.69 (s, 1H), 4.35 (dd, *J* = 9.4, 4.9 Hz, 1H), 4.27 – 4.13 (m, 3H), 3.92 (dd, *J* = 10.4, 3.5 Hz, 1H), 3.88 – 3.60 (m, 7H), 3.59 – 3.39 (m, 5H), 3.04 – 2.87 (m, 2H), 2.37 (t, *J* = 7.4 Hz, 2H), 2.12 (dtd, *J* = 15.2, 7.7, 4.9 Hz, 1H), 1.98 – 1.87 (m, 4H), 1.85 – 1.77 (m, 1H), 1.69 (ddd, *J* = 14.1, 9.3, 5.8 Hz, 1H), 1.67 – 1.52 (m, 2H), 1.38 (dd, *J* = 7.2, 3.3 Hz, 3H), 1.36 – 1.26 (m, 5H). ^{13}C NMR (151 MHz, D_2O) δ 177.81, 177.67, 175.81, 175.58, 175.02, 174.27, 174.00, 172.52, 94.91, 90.99, 82.55, 79.71, 78.07, 77.77, 71.53, 68.91, 68.76, 60.86, 56.19, 54.13, 53.72, 53.08, 49.98, 39.26, 30.80, 30.72, 26.77, 26.20, 22.02, 22.01, 18.65, 16.70. HRMS-ESI. Calculated for $C_{25}H_{44}N_5O_{13}$ $[M+H]^+$ 622.2930; observed, 622.2920.

N^2-((R)-5-amino-4-((S)-2-((R)-2-(((3R,4R,5S,6R)-2,5-dihydroxy-3-(2-hydroxyacetamido) -6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propanamido)propanamido)-5-oxopentanoyl)-N^6-glycyl-L-lysine (**3d**): Prepared following general procedure #1. ^{1}H NMR (600 MHz, D_2O) δ 5.15 (d, *J* = 3.6 Hz, 1H), δ 4.74 – 4.72 (m, 1H), 4.32 – 4.19 (m, 3H), 4.17 (q, *J* = 6.7 Hz, 1H), 4.12 – 3.94 (m, 3H), 3.92 – 3.67 (m, 3H), 3.72 (s, 2H), 3.61 – 3.48 (m, 2H), 3.44 (ddd, *J* = 9.9, 5.7, 2.1 Hz, 1H), 3.20 (t, *J* = 6.9 Hz, 2H), 2.36 (td, *J* = 8.1, 7.6, 3.5 Hz, 2H), 2.14 (tt, *J* = 13.4, 7.7 Hz, 1H), 1.99 – 1.89 (m, 1H), 1.83 (dq, *J* = 10.2, 6.3, 5.0 Hz, 1H), 1.75 – 1.65 (m, 1H), 1.50 (p, *J* = 7.7 Hz, 2H), 1.39 – 1.30 (m, 8H). ^{13}C NMR (151 MHz, D_2O) δ 175.99, 175.93, 175.82, 175.66, 175.33, 175.22, 175.13, 174.96, 174.80, 166.67, 163.11, 162.87, 94.67, 90.90, 82.02, 79.03, 77.71, 77.31,

75.74, 71.57, 69.06, 68.71, 61.09, 60.95, 60.74, 60.51, 55.93, 53.32, 52.89, 52.87, 49.78, 49.72, 40.39, 39.16, 31.48, 30.05, 27.69, 26.79, 22.38, 18.56, 16.55. HRMS-ESI. Calculated for $C_{27}H_{48}N_7O_{14}$ $[M+H]^+$ 694.3254; observed, 694.3254.

N^2-(((R)-2-(((3R,4R,5S,6R)-3-acetamido-2,5-dihydroxy-6- (hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propanoyl)-L-alanyl)-N^5-((S)-4-(2- aminoacetamido)-1-carboxybutyl)-D-glutamine (**3e**): Prepared following general procedure #1. ^{1}H NMR (600 MHz, D_2O) δ 5.12 (d, *J* = 3.5 Hz, 1H), 4.64 (d, *J* = 8.4 Hz, 0H), 4.31 – 4.23 (m, 2H), 4.20 (dd, *J* = 7.7, 5.0 Hz, 1H), 4.14 (dd, *J* = 8.5, 5.0 Hz, 1H), 3.89 (ddd, *J* = 29.6, 11.4, 2.8 Hz, 1H), 3.83 (s, 1H), 3.82 – 3.74 (m, 1H), 3.74 (s, 2H), 3.73 – 3.64 (m, 1H), 3.57 – 3.41 (m, 1H), 3.22 (t, *J* = 6.7 Hz, 2H), 2.26 (t, *J* = 7.9 Hz, 2H), 2.09 (dd, *J* = 14.1, 6.4 Hz, 1H), 1.95 – 1.86 (m, 4H), 1.78 (dd, *J* = 13.5, 5.5 Hz, 1H), 1.64 (dq, *J* = 14.9, 7.9 Hz, 1H), 1.51 (p, *J* = 7.2 Hz, 2H), 1.41 – 1.32 (m, 6H). 13C NMR (151 MHz, D2O) δ 175.71, 174.06, 166.74, 94.95, 90.95, 82.36, 79.54, 78.07, 77.79, 75.74, 71.54, 69.05, 68.86, 60.74, 60.55, 56.13, 54.51, 54.28, 53.70, 49.61, 40.43, 38.99, 32.07, 28.84, 27.91, 27.73, 24.83, 22.24, 22.01, 18.66, 16.96. HRMS-ESI. Calculated for $C_{26}H_{45}N_6O_{14}$ $[M+H]^+$ 665.29155; observed, 665.29836.



3f

Figure S6: Structure of anhydromuropeptide (L-Ala-D-Glu-L-OrnGly).

General procedure #2: To a solution of protected anhydro-muramic acid [41,42] (1.0 equiv.) in anhydrous THF (0.1 M) was added the dipeptide (1.5 equiv.). NMM (3.0 equiv.) and HATU (1.2 equiv.) was added and the solution was stirred at RT with monitoring by TLC. Upon completion, solvent was removed *in vacuo*. The resulting residue was dissolved in EtOAc and washed with 1N HCl then brine. The combined organic phases were dried over Na_2SO_4 and condensed. When further purification was needed, column chromatography (often 5-8% methanol in DCM) provided quality protected carbohydrate substrates. The protected anhydromuropeptides were treated with 4N HCl (to remove the trityl protecting group) and subsequently hydrogenated. Purification (reverse-phase HPLC) gave highly pure products.

N^2-(((R)-2-(((1R,2S,3R,4R,5R)-4-acetamido-2-hydroxy-6,8-dioxabicyclo[3.2.1]octan-3-yl)oxy)propanoyl)- L-alanyl)-N^5-((S)-4-(2- aminoacetamido)-1-carboxybutyl)- D-glutamine (**3f**): Prepared following general procedure #2. ^{1}H NMR (600 MHz, D_2O) δ 8.41 (s, 1H), 5.44 (d, *J* = 1.7 Hz, 1H), 4.64 (s, 1H), 4.36 (q, J = 7.1 Hz, 1H), 4.26 – 4.21 (m, 1H), 4.15 (dd, J = 7.4, 4.5 Hz, 2H), 4.10 (dd, *J* = 8.3, 4.8 Hz, 1H), 3.88 (d, *J* = 2.0 Hz, 1H), 3.84 (d, *J* = 1.9 Hz, 1H), 3.77 (dd, *J* = 7.8, 5.8 Hz), 3.73 (s, 2H), 3.39 (p, *J* = 1.6 Hz, 1H), 3.21 (t, *J* = 6.7 Hz, 2H), 2.27 – 2.20 (m, 2H), 2.07 (dt, *J* = 14.8, 7.5 Hz, 1H), 1.96 (s, 3H), 1.90 (dt, *J* = 14.9, 7.4 Hz, 1H), 1.75 (s, 1H), 1.62 (dq, *J* = 15.0, 7.8 Hz, 1H), 1.50 (h, *J* = 7.5 Hz, 2H), 1.39 (d, *J* = 7.2 Hz, 3H), 1.34 (d, *J* = 6.8 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 178.86, 175.49, 174.86, 173.88, 173.62, 166.79, 99.97, 78.43, 75.92, 68.23, 65.22, 54.83, 54.58, 49.46, 40.46, 39.04, 32.17, 29.03, 28.07, 24.85, 21.83, 18.02, 17.16. HRMS-ESI. Calculated $C_{26}H_{43}N_6O_{13}$ $[M+H]^+$ 647.28881; observed, 647.28814.

Preparation of GlcNAc-MurNAc Tripeptide (GMTri-Peptide) Derivatives:

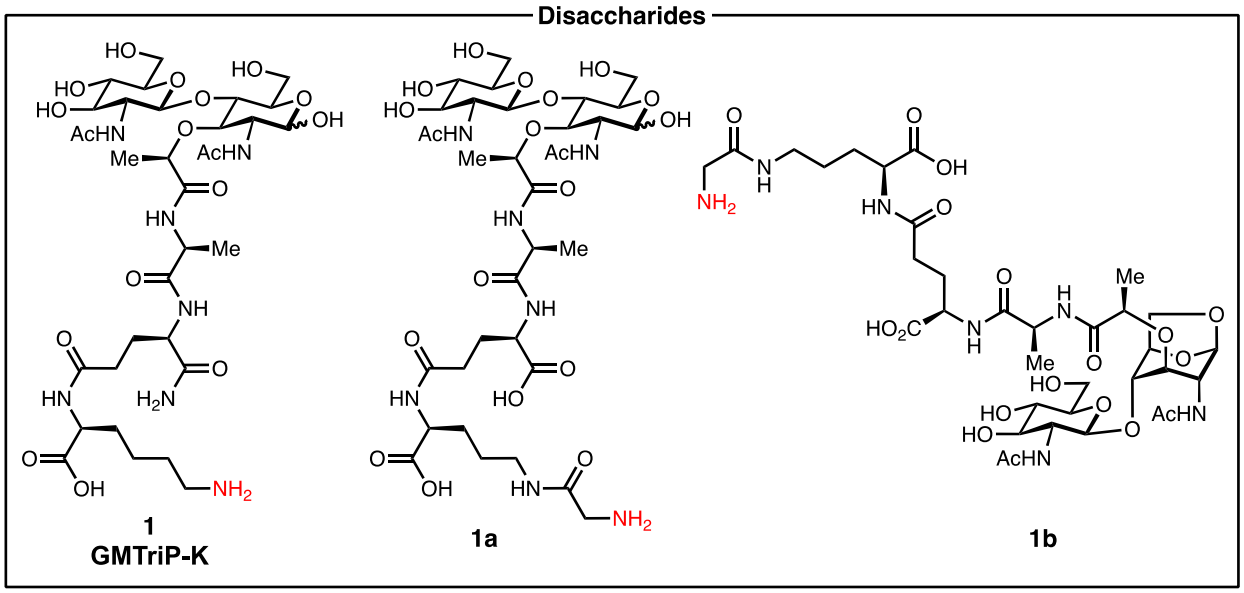


Figure S7: Structures of disaccharide fragments printed on the glycan array.

(S)-2-((R)-4-((S)-2-((R)-2-(((3R,4R,5S,6R)-3-acetamido-5-(((2S,3R,4R,5S,6R)-3-acetamido- 4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-2-hydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-4-yl)oxy)propanamido)propanamido)-5-amino-5-oxopentanamido)-6-aminohexanoic acid (**GMTriP-K, 1**): Synthesized according to literature precedent.[3] Spectral data agrees with literature reported values.

N^2-(((2R)-2-(((3R,5S,6R)-3-acetamido-5-(((2S,3R,4R,5S,6R)-3-acetamido-4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-2-hydroxy-6- (hydroxymethyl) tetrahydro-2H-pyran-4-yl)oxy)propanoyl)- L-alanyl)-N^5-((S)-4- (2-aminoacetamido)-1-carboxybutyl)- D-

glutamine (**1a**): Prepared according to a modification to literature[3] procedure. ^{1}H NMR (600 MHz, D_2O) δ 5.20 (d, *J* = 3.5 Hz, 1H), 4.59 (d, *J* = 8.4 Hz, 0H), 4.55 – 4.46 (m, 2H), 4.39 (dt, *J* = 16.0, 7.3 Hz, 1H), 4.33 – 4.24 (m, 1H), 4.27 (s, 1H), 3.92 – 3.75 (m, 5H), 3.75 – 3.63 (m, 5H), 3.61 – 3.48 (m, 2H), 3.41 – 3.34 (m, 2H), 3.23 (t, *J* = 6.8 Hz, 3H), 3.10 – 3.03 (m, 1H), 2.35 (t, *J* = 10.1 Hz, 2H), 2.14 (dt, *J* = 13.4, 6.6 Hz, 1H), 2.00 (d, *J* = 11.2 Hz, 4H), 1.98 – 1.89 (m, 4H), 1.84 (d, *J* = 7.0 Hz, 0H), 1.71 (dt, *J* = 16.0, 8.5 Hz, 1H), 1.57 (dq, *J* = 17.0, 9.9, 8.1 Hz, 4H), 1.41 – 1.31 (m, 6H). ^{13}C NMR (101 MHz, D2O) δ 193.21, 174.59, 166.70, 162.82, 144.15, 120.71, 113.24, 100.40, 90.13, 75.73, 75.40, 73.43, 71.14, 70.13, 60.98, 49.78, 47.72, 40.26, 38.68, 31.52, 28.18, 27.90, 26.95, 24.66, 21.99, 18.06, 16.71. HRMS-ESI. Calculated for $C_{34}H_{58}N_7O_{19}$ $[M+H]^+$ 868.37092; observed, 868.37750.

N^2-(((R)-2-(((1R,2S,3R,4R,5R)-4-acetamido-2-(((2S,3R,4R,5S,6R)-3-acetamido- 4,5-dihydroxy-6-(hydroxymethyl)tetrahydro-2H-pyran-2-yl)oxy)-6,8-dioxabicyclo[3.2.1]octan-3-yl)oxy)propanoyl)- L-alanyl)-N^5-((S)-4-(2- aminoacetamido)-1-carboxybutyl)- D-glutamine (**1b**): ^{1}H NMR (600 MHz, D_2O) δ 5.40 (s, 1H), 4.66 (d, *J* = 6.1 Hz, 1H), 4.62 (d, *J* = 8.4 Hz, 1H), 4.34 (q, *J* = 7.0 Hz, 1H), 4.23 (d, *J* = 7.9 Hz, 1H), 4.14 (q, *J* = 6.7 Hz, 1H), 3.94 (d, *J* = 14.0 Hz, 2H), 3.85 (d, *J* = 12.2 Hz, 1H), 3.79 – 3.68 (m, 5H), 3.57 – 3.50 (m, 2H), 3.45 – 3.37 (m, 2H), 3.22 (t, *J* = 6.5 Hz, 2H), 2.30 (d, *J* = 8.0 Hz, 2H), 2.18 – 2.09 (m, 1H), 2.02 (d, *J* = 11.6 Hz, 6H), 1.97 (s, 1H), 1.82 (s, 1H), 1.68 (s, 1H), 1.54 (d, *J* = 8.7 Hz, 2H), 1.38 (d, *J* = 6.9 Hz, 3H), 1.34 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 175.44, 175.02, 174.41, 173.70, 166.76, 163.28, 163.05, 162.81, 100.59, 99.91, 77.20, 76.11, 76.01, 73.61, 73.34, 69.82, 64.82, 60.63, 55.54, 49.43, 48.86, 40.40, 38.87, 31.76, 28.14, 26.97, 24.83, 22.35, 21.93, 18.00, 16.99. HRMS-ESI. Calculated for $C_{34}H_{56}N_7O_{18}$ $[M+H]^+$ 850.36036; observed, 850.36544.

3. Glycan Microarray Fabrication:

A total of 110 array components were printed in duplicate on 3-D hydrogel NHS-activated slides (3-D Hydrogel Coating (H), Schott Minifab, Phoenix, AZ) using a NanoPrintT 2 LM60-2 arrayer (ArrayIt, Sunnyvale, CA). Print buffer for each component was composed of 150 mM phosphate buffer, pH 8.5. Four SMP2 microspotting pins (ArrayIt, Sunnyvale, CA) in a 2x2 printhead arrangement were used. Each slide contains 16 arrays in a 2×8 format. Humidity level was maintained at ~60% in the arraying chamber during the print. Slides were kept in a humidified atmosphere overnight to facilitate reaction between amine-modified glycans and the NHS-activated slide surface. Following the print, printed slides were vacuum sealed and stored at -20 °C until time of use.

4. Glycan Microarray Validation:

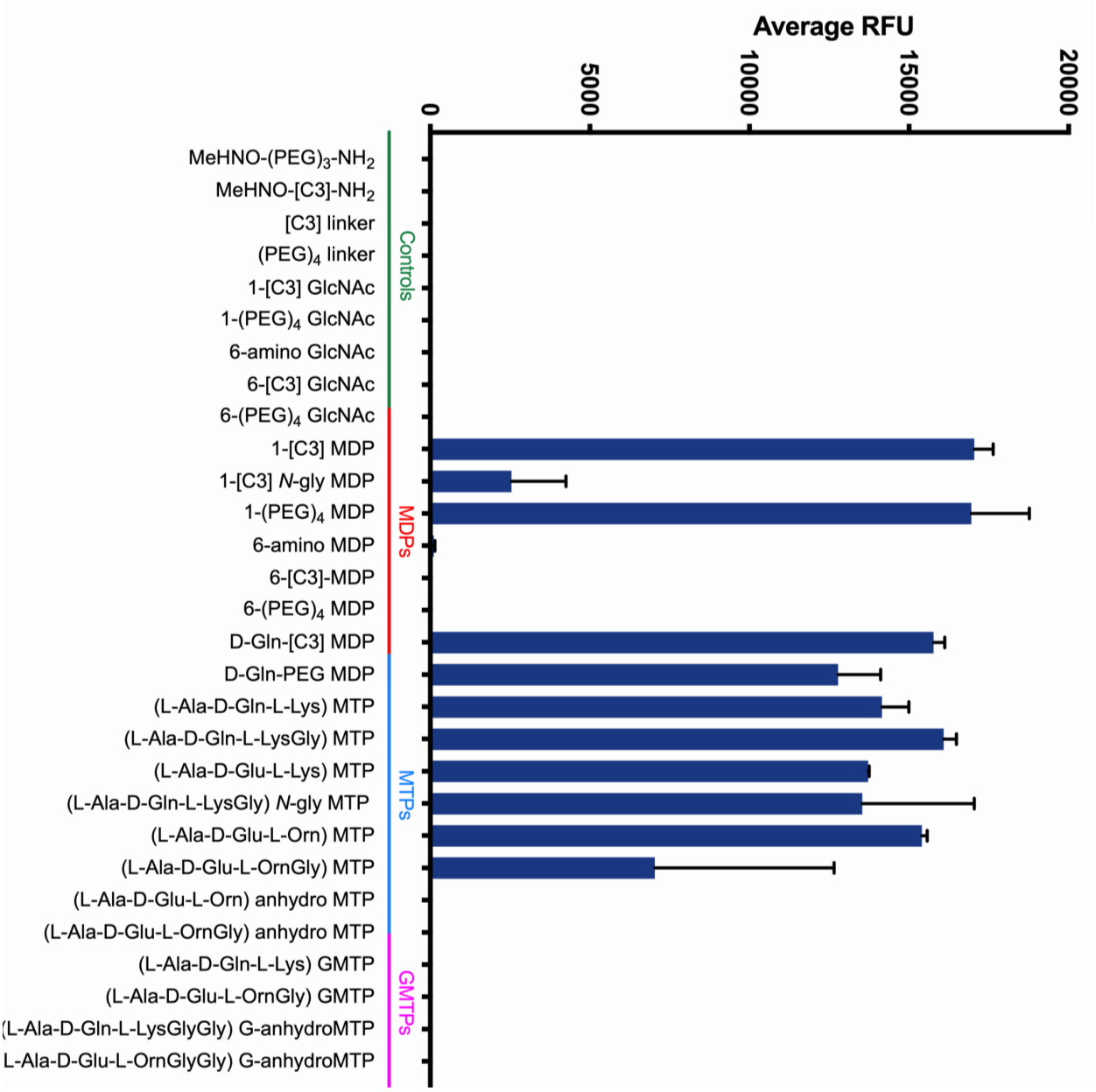
To validate the array, we first used the MDP-specific monoclonal antibody 2E7 which was generated by Wang et al.[43] by immunizing mice with MDP conjugated to BSA via an ethylenediamine linker, then purified and the second used a well-known lectin, wheat germ agglutinin (WGA) that recognizes GlcNAc polymers [44]. To define the binding pocket of antibody 2E7 for MDP, we analyzed its binding specificity using a the PGN array (Supplementary Figure S8A. Briefly, the arrays were incubated for 2 hours with 10 μg/mL of 2E7. This revealed that the binding specificity of 2E7 to linked MDP derivatives was affected by the orientation of MDP on the array surface and also affected by the location of the linker in our library synthesis. For example, compounds linked via the C6 position of carbohydrates did not bind to the antibody. We found that 2E7 recognizes MDP fragments tethered to the array surface through C1 and the D-

isoglutamine position, and MTP bound at the γ-carboxylic acid. Notably, the antibody did not bind to the *N*-acetylglucosamine moiety alone nor the disaccharide fragments, neither did it bind when fragments were attached via C6. These data show the specific binding of 2E7 to MDP-like and MTP-like fragments. Importantly, the binding specificity for 2E7 observed in the array matches that of the original characterization of the antibody, which used an ELISA-based method to show that 2E7 binds to MDP, but not *N*-acetylglucosamine or tracheal cytotoxin [43].

To further validate the array, the lectin, wheat-germ-agglutinin (WGA) (10 μg/mL), which binds specifically to GlcNAc residues was used. WGA bound to each compound shown in Fig. 1a that has a GlcNAc, either in mono-saccharide or di-saccharide form but it did not bind to MurNAc monosaccharide compounds (Supplemental text, Fig. S8B). Thus, in addition to validation the array, WGA contributed another feature to the PGN array, as it is a powerful tool for characterizing and screening lectins, such a as WGA. WGA had been previously shown in elegant NMR studies to bind N-acetylglucosamine N-acetylmuramic dipeptide (GMDiP) [45]; the PGN array here validated those results and clearly demonstrated that the lectin did not bind anhydro-disaccharide (Fig.1, 1b) or monosaccharide muramic acid (Fig.1) containing fragments (Fig. SI 8B).

We use multiple methods to ensure quality of arrays. A slide from each print batch was profiled with the lectin (e.g., WGA) and monoclonal antibodies (see main text for experimental details with MDP-specific monoclonal antibody, 2E7). Together, these data showed specific and broad-spectrum coverage of array components suggesting each spot was deposited successfully on the slide, and signifying that the arrays were a high-quality tool for further experimentation. As another assessment of quality, individual slides were scanned before experimentation (at full gain settings) to identify any missing spots or defects. Microarray slides were scanned using at GenePix 4000B microarray scanner (Molecular Devices, Sunnyvale, CA). Data analysis was performed

using GenePix Pro 7 software (Molecular Devices, Sunnyvale, CA). Missing spots were flagged and excluded from analysis. Background fluorescence was subtracted from median fluorescence, and values were averaged for duplicate spots. Data was processed using excel and GraphPad Prism 9 software.



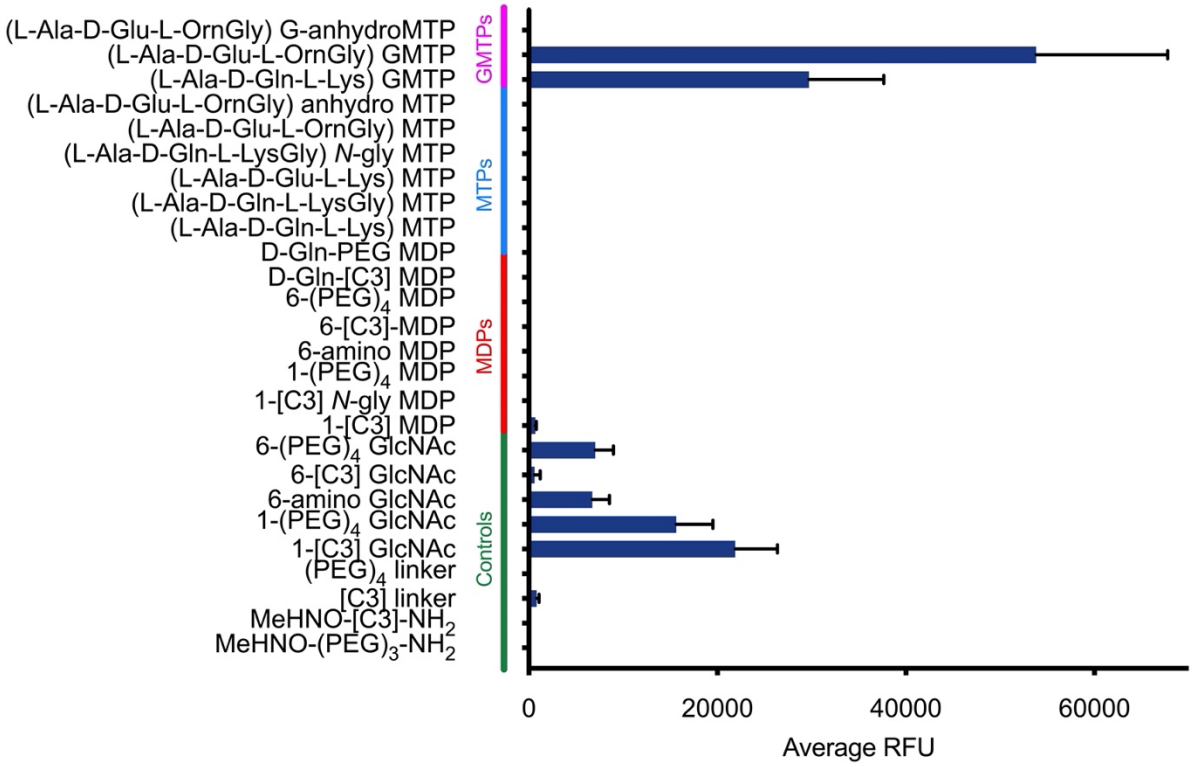


Figure S8: Array validation. A. Bar graphs showing signals in relative fluorescence units (RFUs) for 2E7 (10 ug/mL) on PGN array. B. Bar graphs showing signals in relative fluorescence units (RFUs) for WGA (10 ug/mL) on our 110-component array. Components which did not show significant binding and not relevant to the analysis were excluded for clarity. See Table for the complete list of compounds analyzed; all components showing any signal are depicted on the graph above.

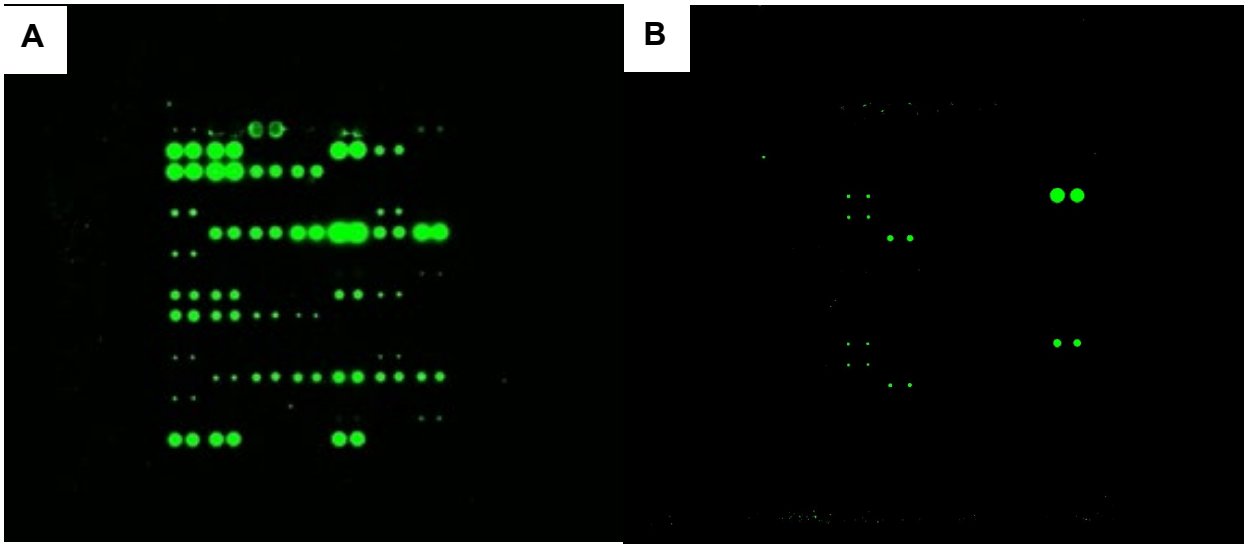
Array Incubation with WGA: Biotinylated-wheat germ agglutinin (WGA) was purchased from Vector Laboratories: 50 uL of 10 ug/mL biotinylated-WGA applied to individual wells in duplicate. The slide was enclosed in a ProPlate 16-well module (Grace BioLabs, Bend, OR) and sealed with adhesive film before incubating at 37 °C for 1 h. The arrays were then washed thoroughly (3x 200 uL PBST) and patted dry. Cy3-conjugated streptadividin (Jackson ImmunoResearch Laboratories) was used as the fluorescently-labelled secondary antibody, and

was diluted 500-fold and applied to each subarray. The slide was incubated for 1 h at 24 °C. Arrays were rinsed with PBST, and the 16-well module was removed. The slide was fully submerged in water for 5 min and then slide was dried by centrifugation (1000 RPM for 5 min) prior to scanning.

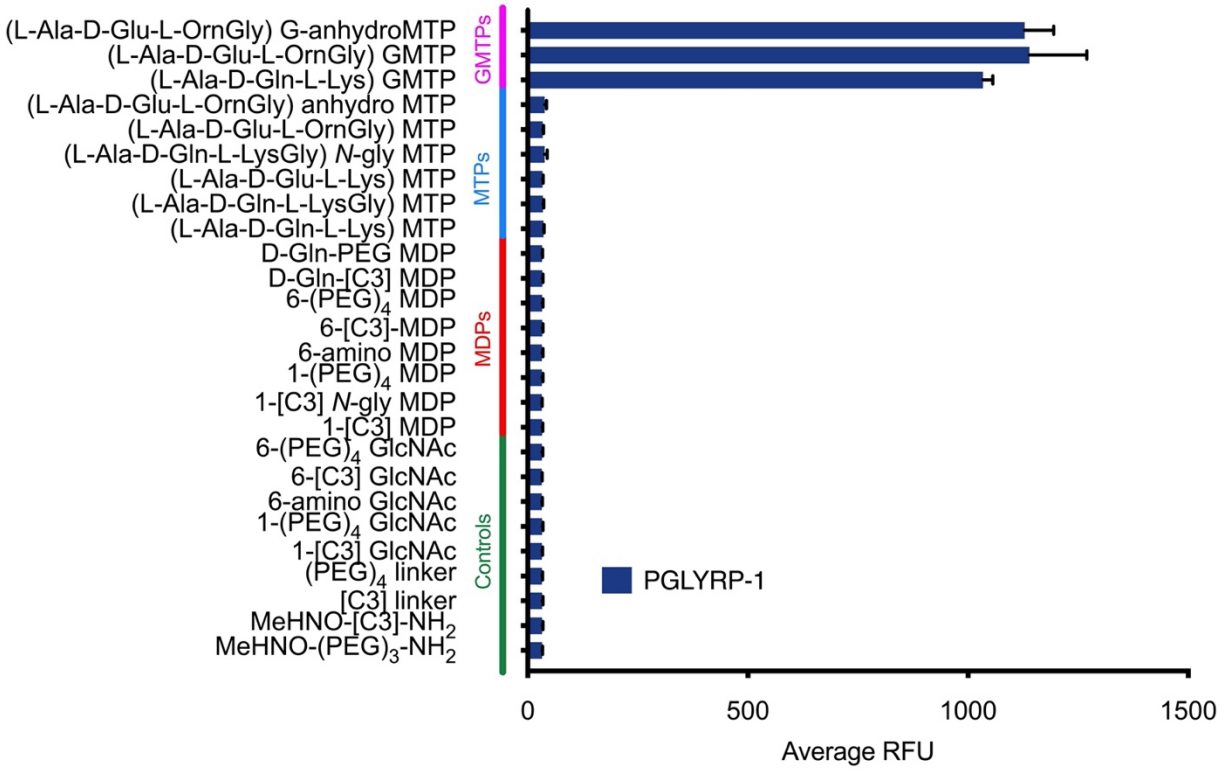
5. Application of Innate Immune Receptors to the Peptidoglycan Array

Initial Binding Profile: To assess the binding profiles of PGLYRP-1, PGLYRP-3 and PGLYRP-4 (R&D Systems, Minneapolis, MN), the proteins were individually applied to pre-blocked subarrays at a concentration of 10 ug/mL in duplicate. The slide was enclosed in a ProPlate 16-well module (Grace Bio-Labs, Bend, OR) and sealed with adhesive film before incubating for 12 h at 4 °C. After the initial incubation, the protein was removed and the wells were washed (3X 200 uL PBST) and patted dry. Each well was incubated with 50 uL of the appropriate primary antibody diluted 1:100 for 2 h at 24 °C. After washing the wells (3X 200 uL PBST), Cy3-conjugated goat anti-mouse IgG (Invitrogen) was used as the secondary antibody. Each well was incubated with 50 uL of 1:500 diluted of the secondary antibody at 24 °C for 1 h. The secondary was removed and the wells were washed 3X 200 uL PBST. The 16-well module was removed, and the slide was fully submerged in water for 5 min. The slide was dried by centrifugation (5 min at 1000 RPM) prior to scanning. The microarray slides were scanned using a GenePix 4000B Scanner (Molecular Devices, Sunnyvale, CA). The binding of PGLYRP-1 to the PGN-array was recorded in average fluorescence units (RFU). Error is standard deviation of the technical replicates in each experiment. To assure rigorous analysis, each protein was assayed at least three different times, on three different microarrays.

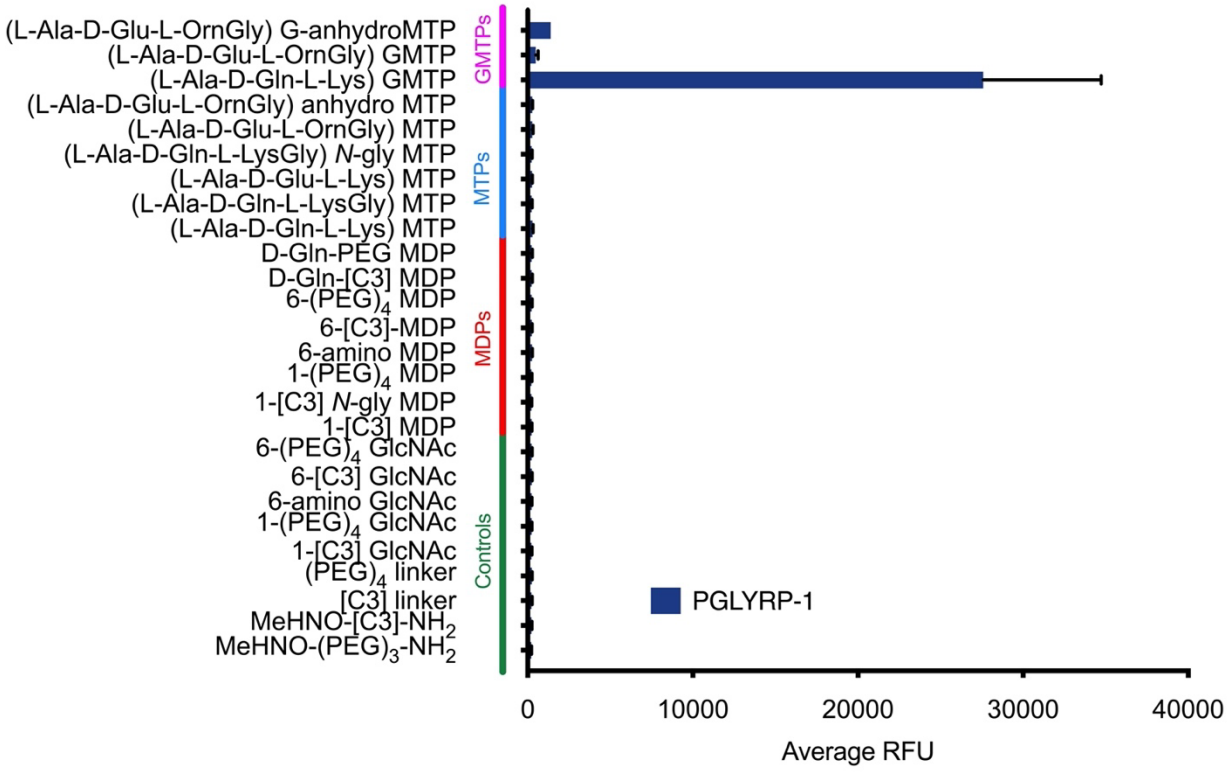
6. Binding Analysis of Peptidoglycan Recognition Proteins to the PGN Array:



A

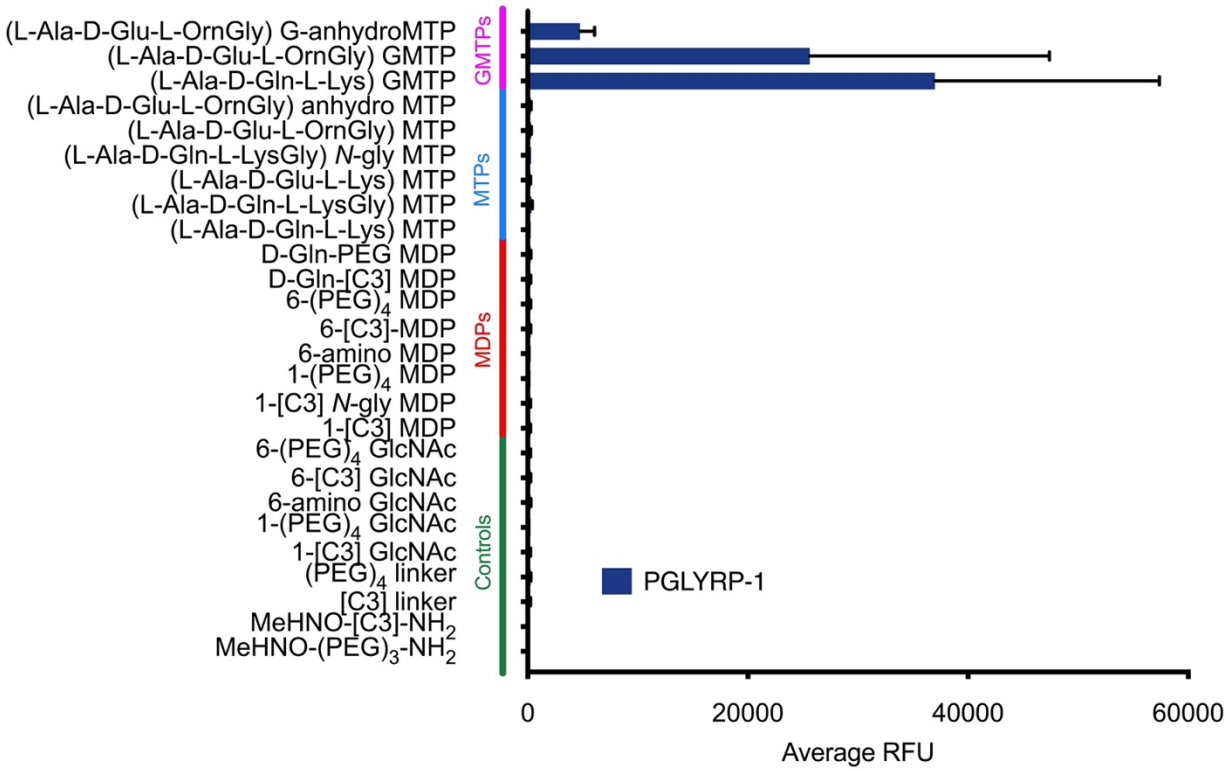


B


(L-Ala-D-Glu-L-OrnGly) G-anhydroMTP
(L-Ala-D-Glu-L-OrnGly) GMTP
(L-Ala-D-Gln-L-Lys) GMTP
(L-Ala-D-Glu-L-OrnGly) anhydro MTP
(L-Ala-D-Glu-L-OrnGly) MTP
(L-Ala-D-Gln-L-LysGly) N-gly MTP
(L-Ala-D-Glu-L-Lys) MTP
(L-Ala-D-Gln-L-LysGly) MTP
(L-Ala-D-Gln-L-Lys) MTP
D-Gln-PEG MDP
D-Gln-[C3] MDP
6-(PEG)4 MDP
6-[C3]-MDP
6-amino MDP
1-(PEG)4 MDP
1-[C3] N-gly MDP
1-[C3] MDP
6-(PEG)4 GlcNAc
6-[C3] GlcNAc
6-amino GlcNAc
1-(PEG)4 GlcNAc
1-[C3] GlcNAc
(PEG)4 linker
[C3] linker
MeHNO-[C3]-NH2
MeHNO-(PEG)3-NH2
GMTPs
MTPs
MDPs
Controls
PGLYRP-1
0
10000
20000
30000
40000
Average RFU

475

C


(L-Ala-D-Glu-L-OrnGly) G-anhydroMTP
(L-Ala-D-Glu-L-OrnGly) GMTP
(L-Ala-D-Gln-L-Lys) GMTP
(L-Ala-D-Glu-L-OrnGly) anhydro MTP
(L-Ala-D-Glu-L-OrnGly) MTP
(L-Ala-D-Gln-L-LysGly) N-gly MTP
(L-Ala-D-Glu-L-Lys) MTP
(L-Ala-D-Gln-L-LysGly) MTP
(L-Ala-D-Gln-L-Lys) MTP
D-Gln-PEG MDP
D-Gln-[C3] MDP
6-(PEG)4 MDP
6-[C3]-MDP
6-amino MDP
1-(PEG)4 MDP
1-[C3] N-gly MDP
1-[C3] MDP
6-(PEG)4 GlcNAc
6-[C3] GlcNAc
6-amino GlcNAc
1-(PEG)4 GlcNAc
1-[C3] GlcNAc
(PEG)4 linker
[C3] linker
MeHNO-[C3]-NH2
MeHNO-(PEG)3-NH2
GMTPs
MTPs
MDPs
Controls
PGLYRP-1
0
20000
40000
60000
Average RFU

476

Figure S10A-C: Biological replicates of PGLYRP-1 application to the glycan array: PGLYRP-1 (10 μg/mL) was applied to the array for 12 h at 4 °C. Protein was removed, and arrays were thoroughly washed before primary anti-PGLYRP-1 was applied. The array was incubated for 4h at 24 °C following which the arrays were washed and dried again. Cy3-conjugated anti-IgG was used as the secondary antibody. The arrays were scanned GenePix 4000B microarray scanner and data analysis was performed using GenePix Pro 7 software. Error bars represent standard error between technical replicates.

7. Determination of Apparent Dissociation Constant for PGLYRP-1 to GMTriP-K:

By plotting phase changes as a function of varying ligand concentration (from 1 ug/mL to 200 ug/mL; 8 concentrations in total) and fitting the resulting points to a simple single site binding model given by

$$y = \frac{y_{\max}(x)}{K_D + (x)} \tag{1-2}$$

where y is the observed, calculated fluorescence change, ymax is the observed, maximal, calculated fluorescence change, x is the PGLYRP-1 concentration (ug/mL) and K_D is the equilibrium dissociation constant. By incubating samples for 12 hours, it can be reasonably implied that the observed binding systems are in a state of dynamic equilibrium. The apparent Kd's were calculated for GMTriP-K and found to be: 50 uM print concentration: Kd = 5.763 to 34.40 and 200 uM print concentration: Kd = 9.791 to 42.14. Error represents standard deviation from the mean. The experiment was conducted in technical replicate.

8. Characterization of the Kinase, NagK, with GMTriP-K

GlcNAc, MurNAc and MDP controls (1 um) (control) or GMTriP-K (1 uM) (disaccharide) was incubated with NagK (1.0 ug) in slick tubes using 100 uL reaction buffer (50 mM bis-Tris-propane buffer, 1.2 mM ATP, 0.5 mM $MgCl_2$). The reaction was incubated at room temperature for 12 h. Mass spectrometry samples were prepared by removing 10 µL of the reaction mixture and adding it into 90µL MeOH to precipitate out the enzyme. The solution was centrifuged at 12,000 rpm for 5 minutes. The supernatant was subjected to high-resolution LCMS, ESI negative mode. Data is shown below. All positive controls (monosaccharides) showed expected M/Z signals. Disaccharide containing fragments were not observed to have been phosphorylated.

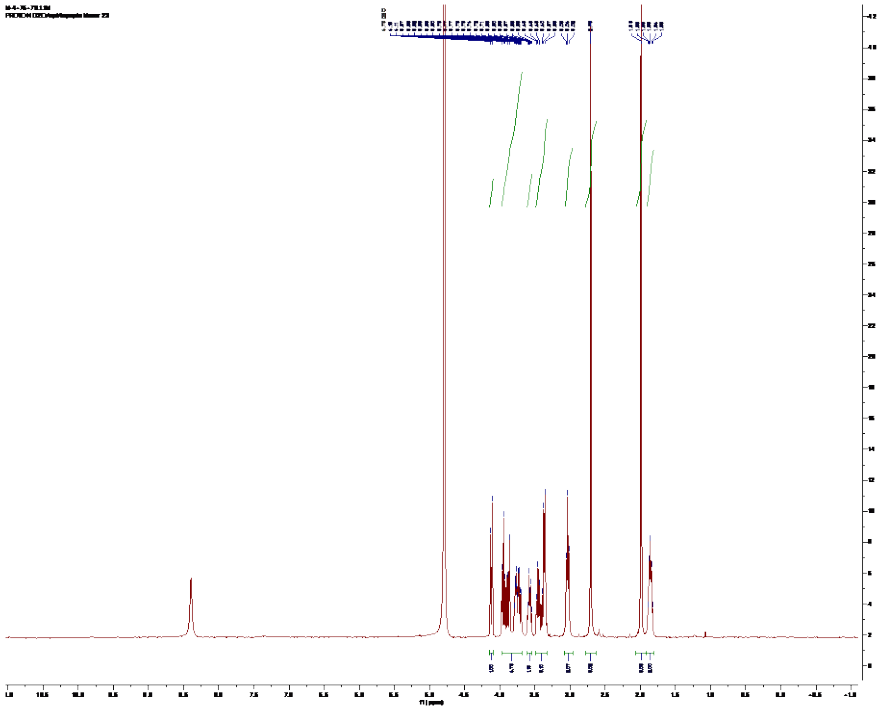
Substrate	**Theoretical m/z $[M-H]^-$**	**Experimental m/z $[M-H]^-$**	**Mass Accuracy (ppm)**
N-Acetyl Glucosamine (C8H16NO9P)	300.0490	300.0489	-0.3333
N-Acetyl muramic acid (C11H20NO11P)	372.0701	372.0706	1.3438
Muramyl dipeptide (C19H33N4O14P)	571.1658	571.1661	0.5252
Muramyl tripeptide (DAP) (C26H44N5O18P)	744.2346	744.2366	2.6873

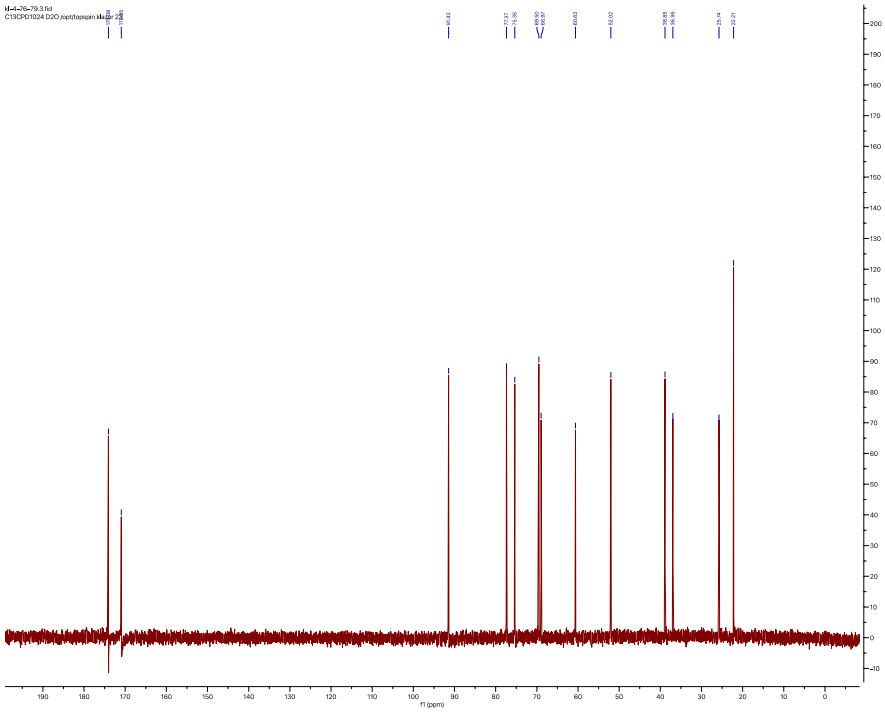
Muramyl tripeptide (Lys) (C25H45N6O15P)	699.2608	699.2626	2.5741
GMMP (C22H38N3O17P)	646.1866	Not Observed	
GMDP (C27H46N5O19P)	774.2452	Not Observed	
GMTP(Orn) (C34H58N7O22P)	946.3300	Not Observed	

Figure S13: High Resolution Mass Spectrum (HRMS) Analysis of Phosphorylated peptidoglycan fragments with the human kinase, NAGK.

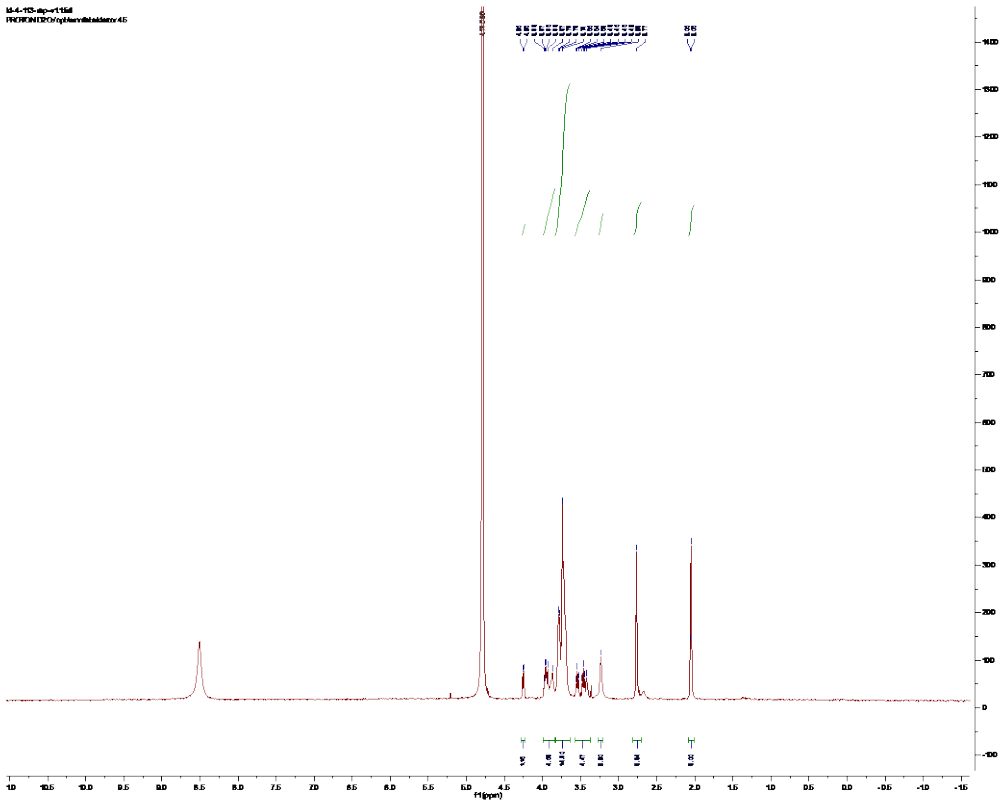
9. 1H and 13C NMR Spectra

Compound **GlcNAc-1** ^{1}H NMR and ^{13}C Spectra (D_2O)





520 Compound **GlcNAc-2** ^{1}H NMR and ^{13}C Spectra (D_2O)



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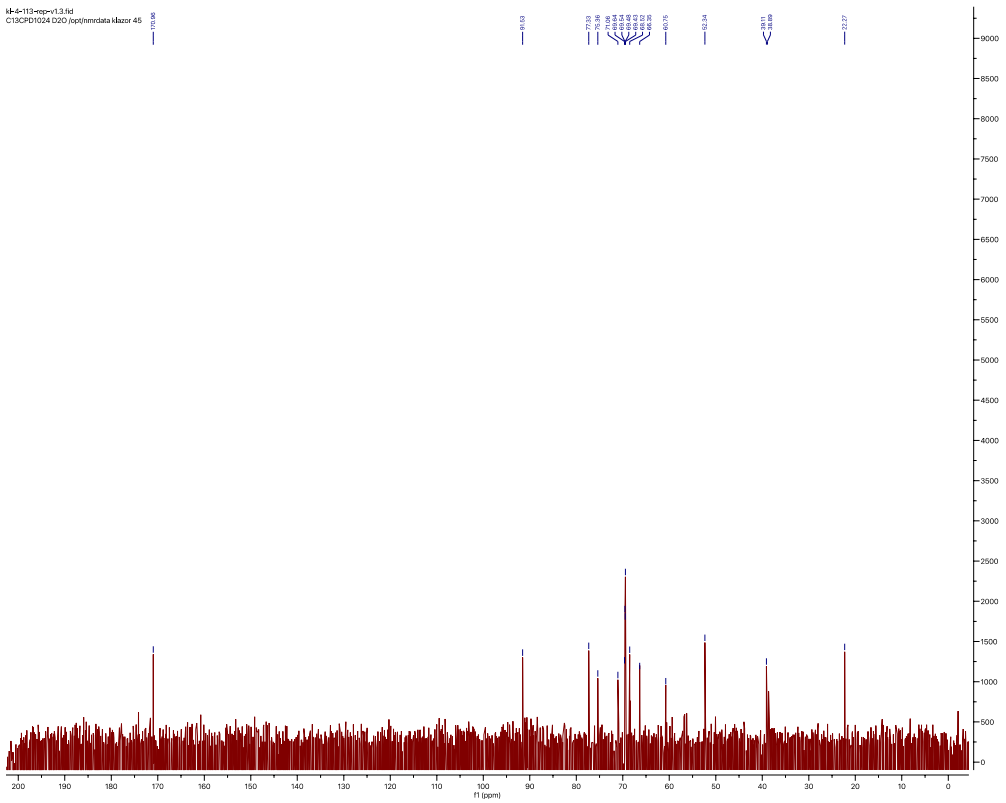
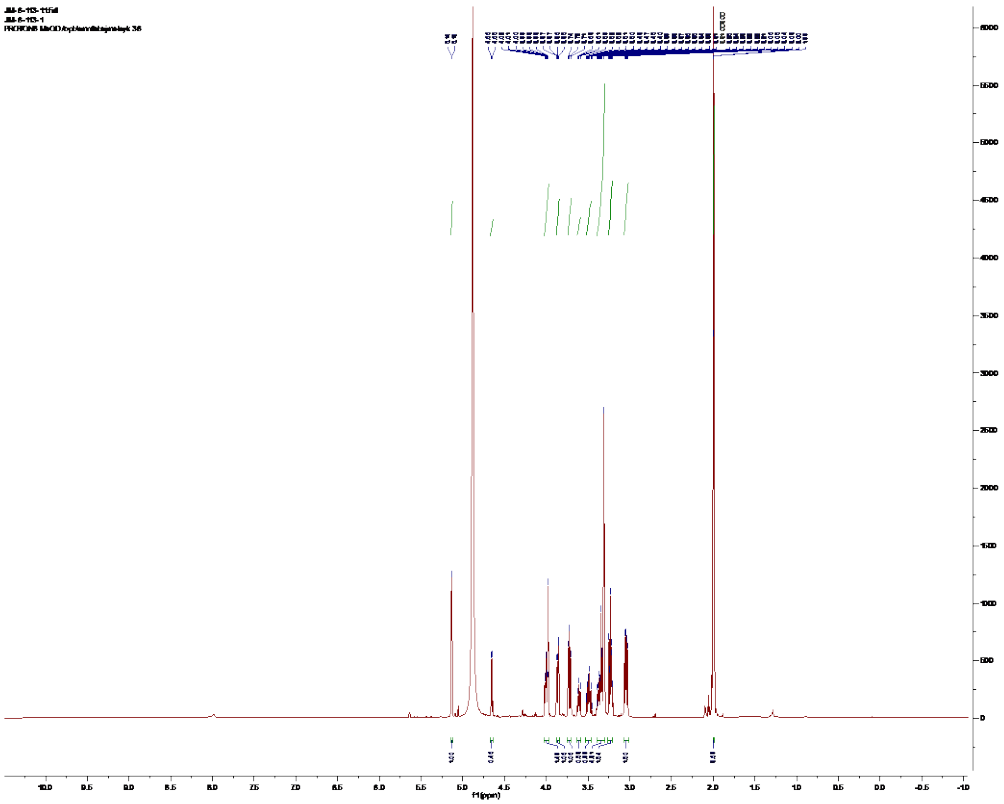
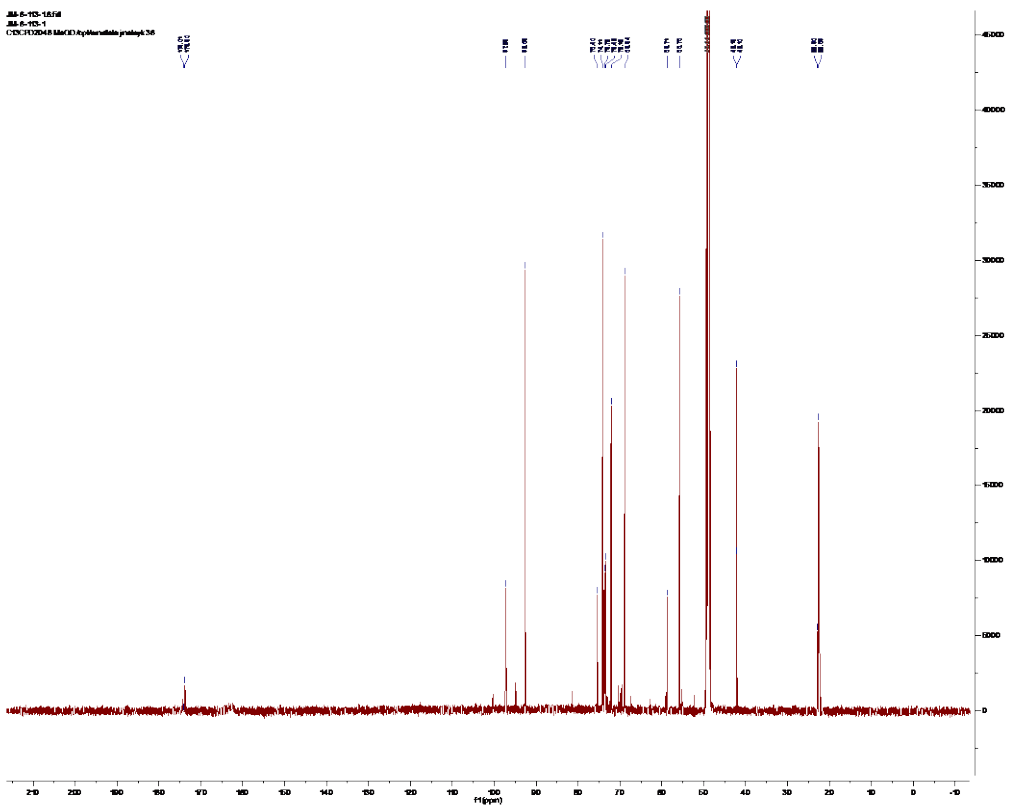

kk-4-113-rep-v1.3.fid
C13CPD1024 D2O /opt/nmrdata kkazor 45
175.96
89.63
77.33
76.96
76.64
69.84
69.43
69.03
68.52
66.26
60.15
52.94
39.71
38.89
22.37
f1 (ppm)

522

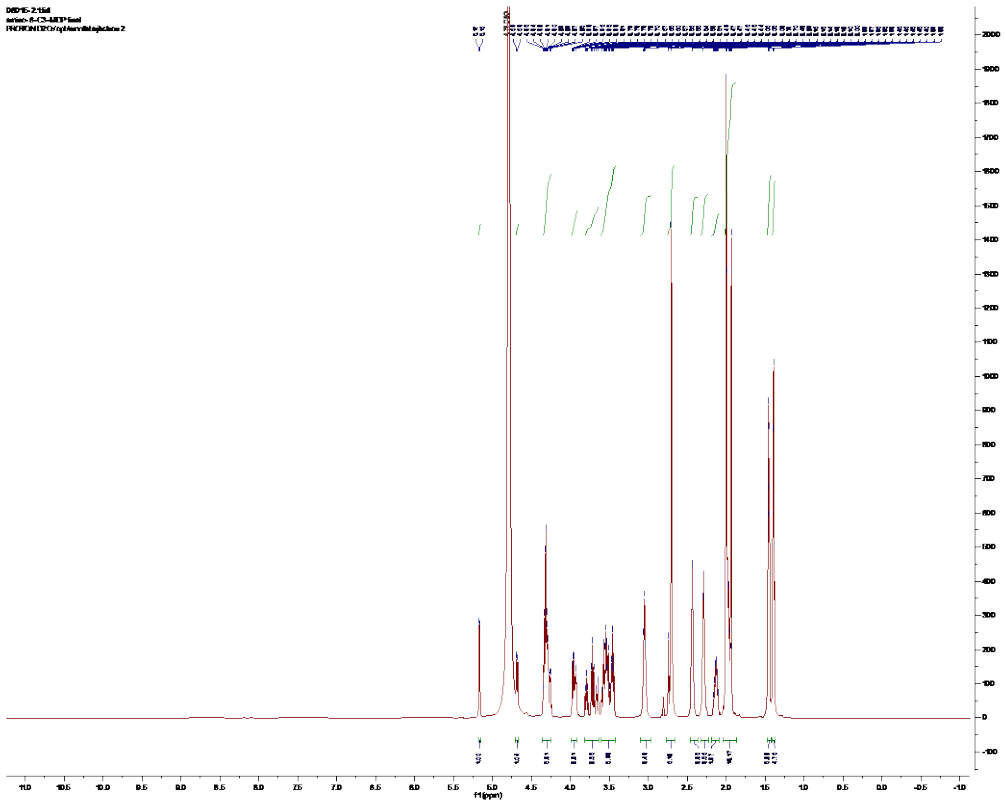
Compound **GlcNAc-3** ^{1}H NMR and ^{13}C Spectra (MeOD)

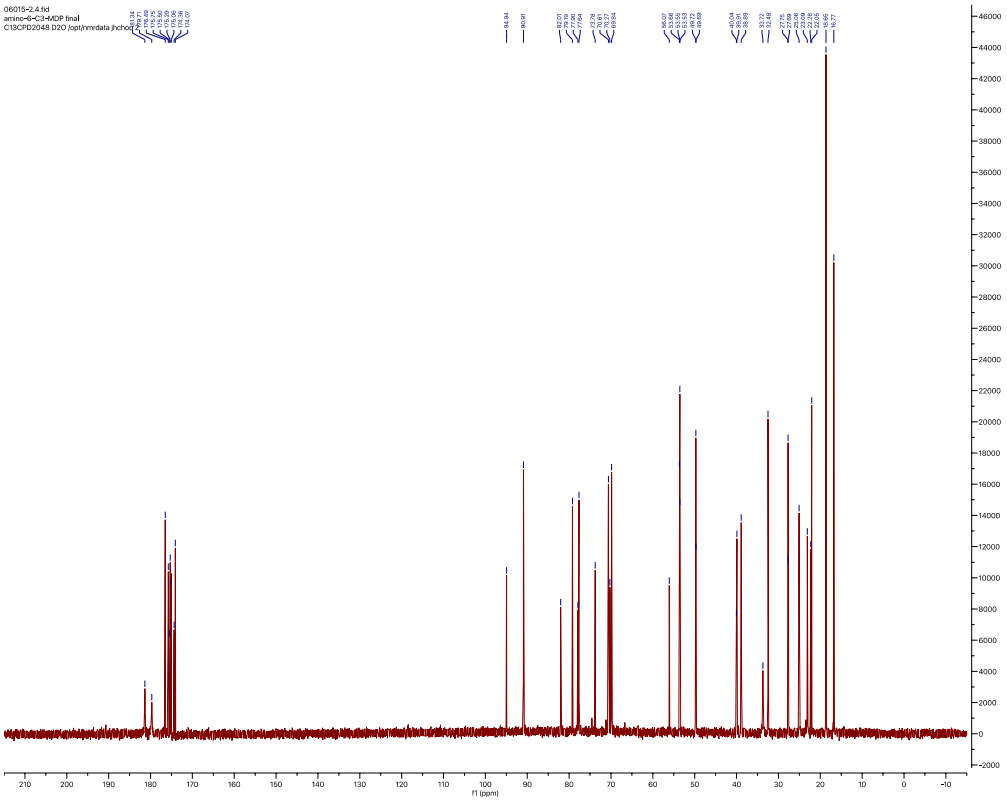




525

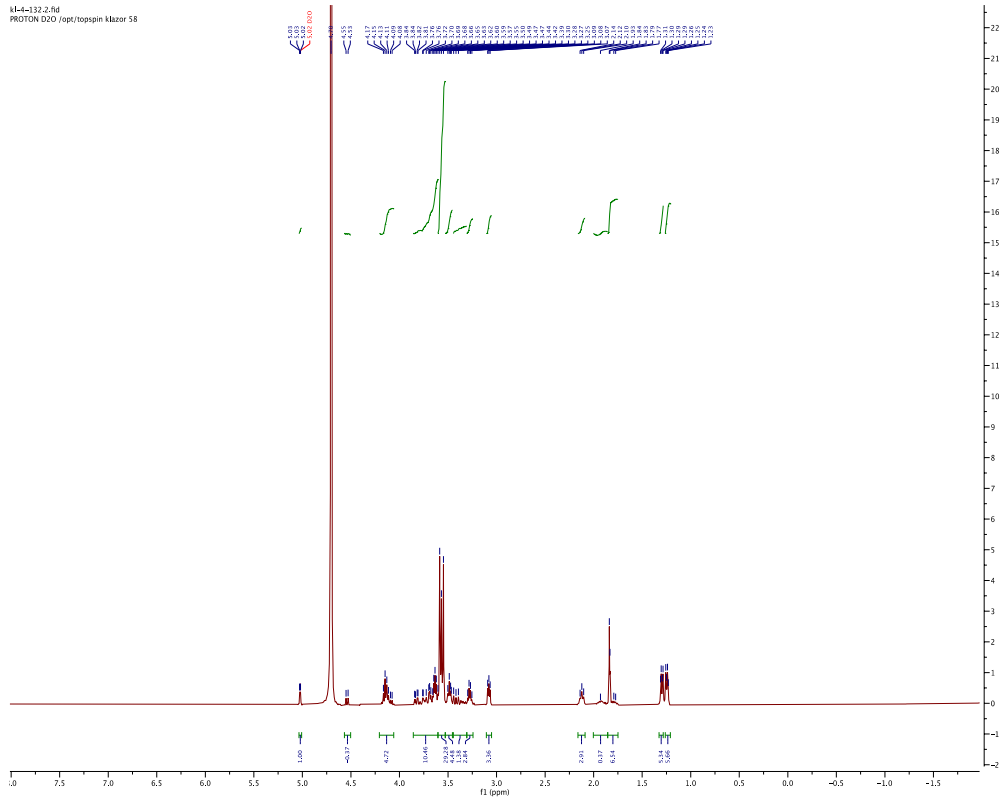
Compound **2e** ^{1}H NMR and ^{13}C Spectra (D_2O)




06015-2.4.fid
amino-6-C3-ADP final
C13CPD2048 D2O /opt/nmrdata jhchoi
f1 (ppm)

528

529 Compound **2h** ^{1}H NMR and ^{13}C Spectra (D_2O)



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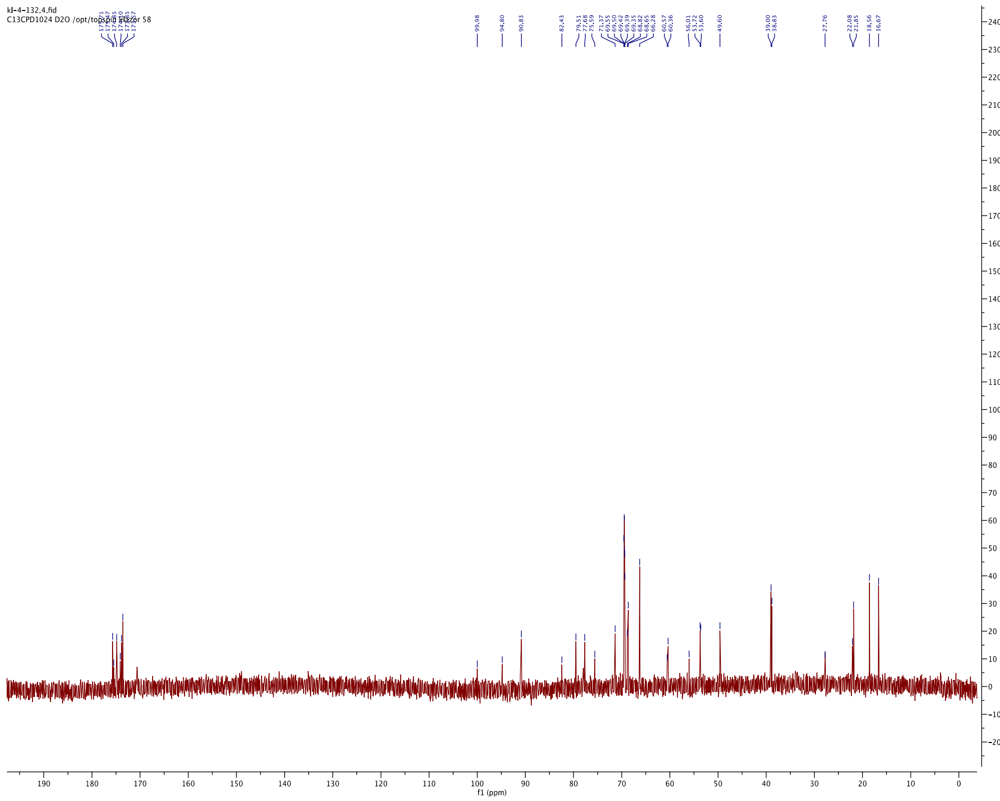
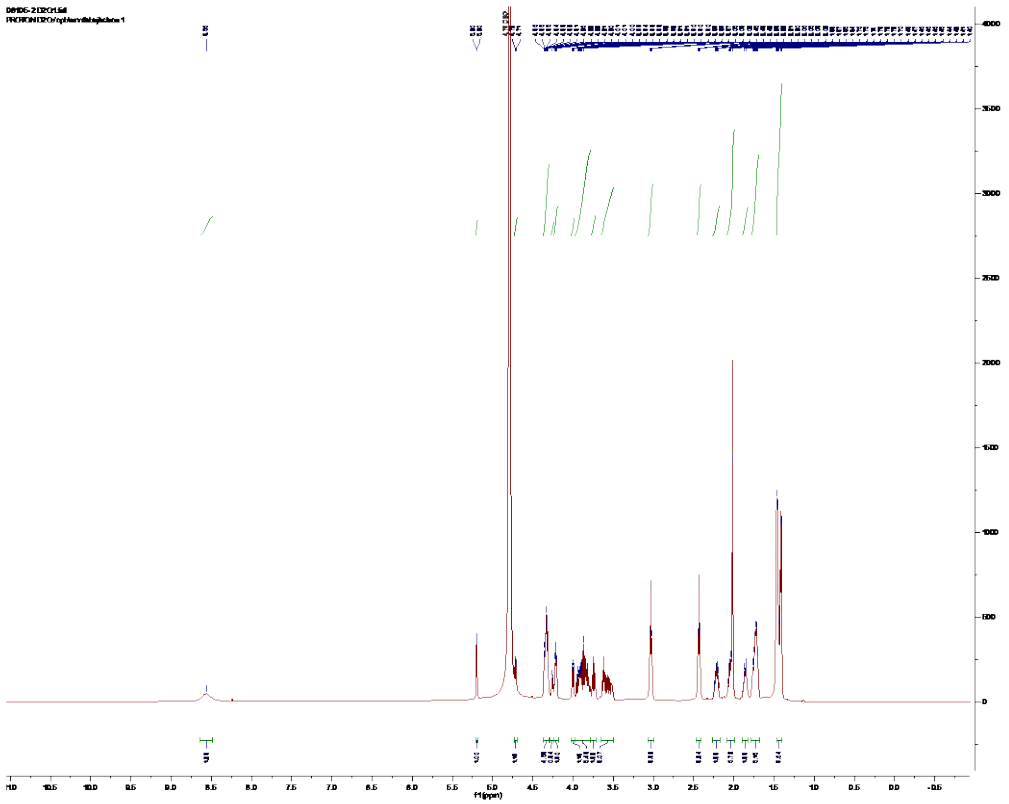


531

532 Compound **3a** ^{1}H NMR and ^{13}C Spectra (D_2O)



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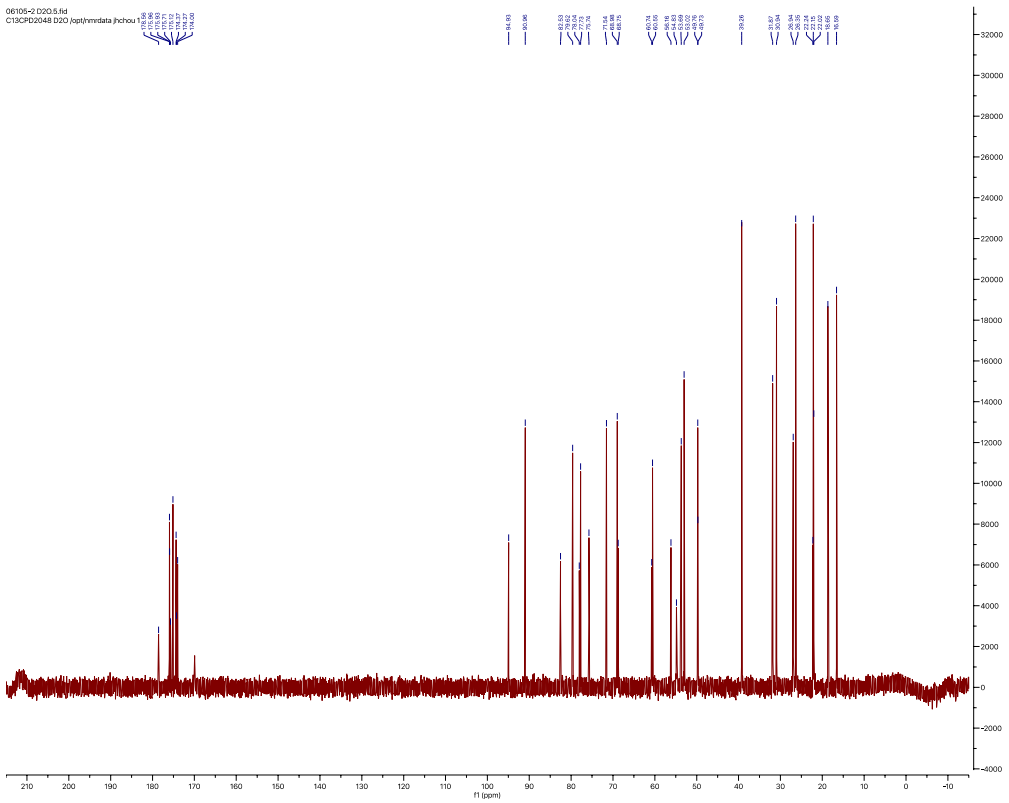
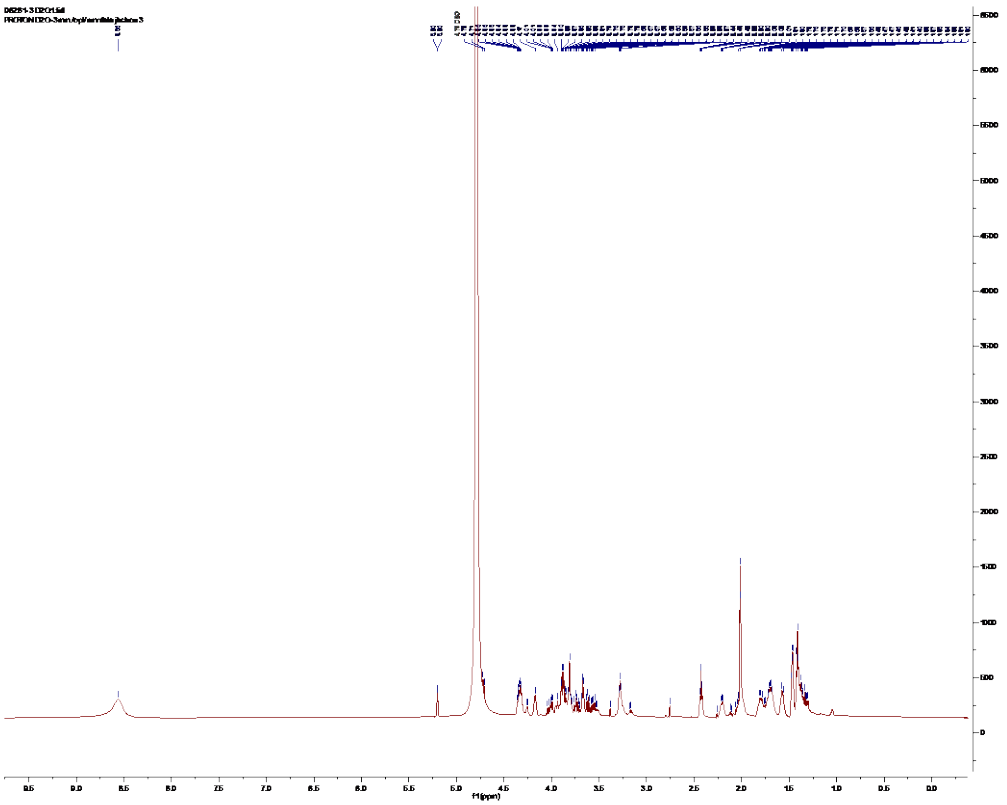
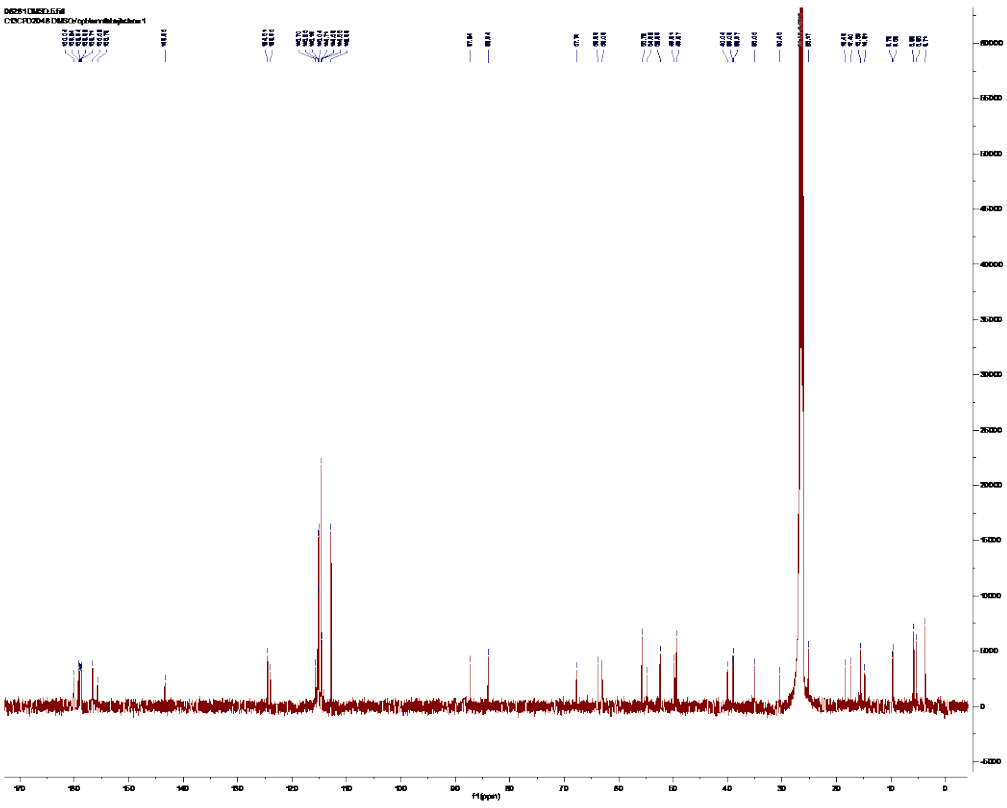


534

535 Compound **3b** ^{1}H NMR (D_2O) and ^{13}C Spectra (DMSO)

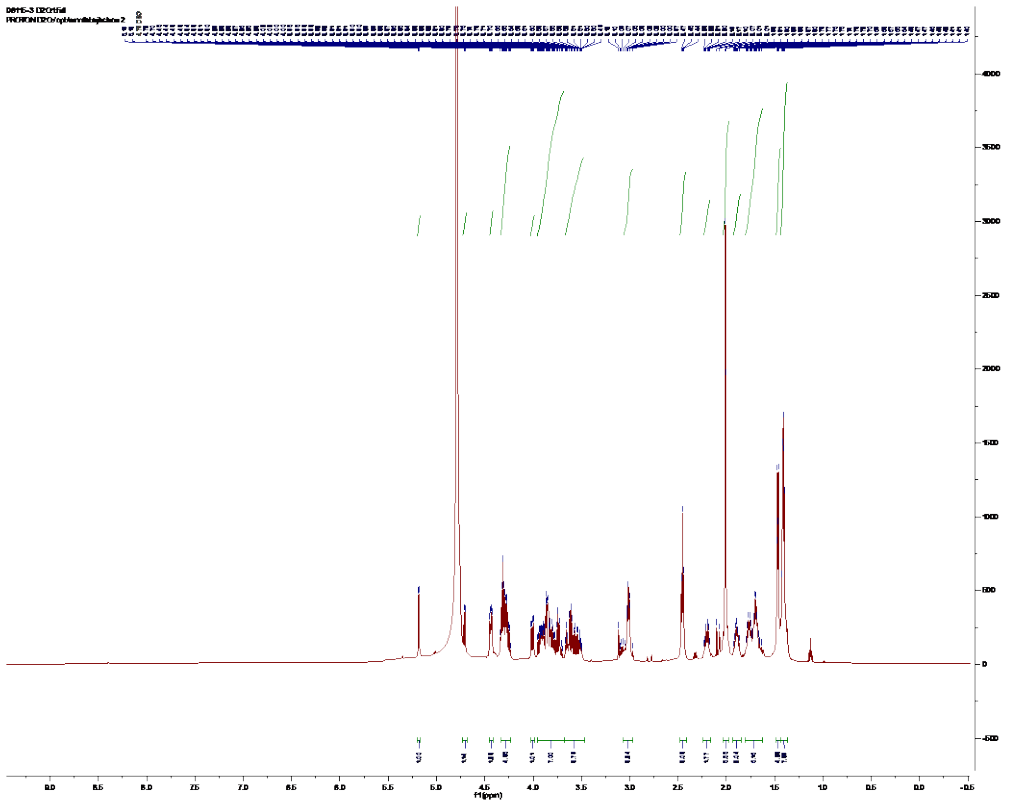


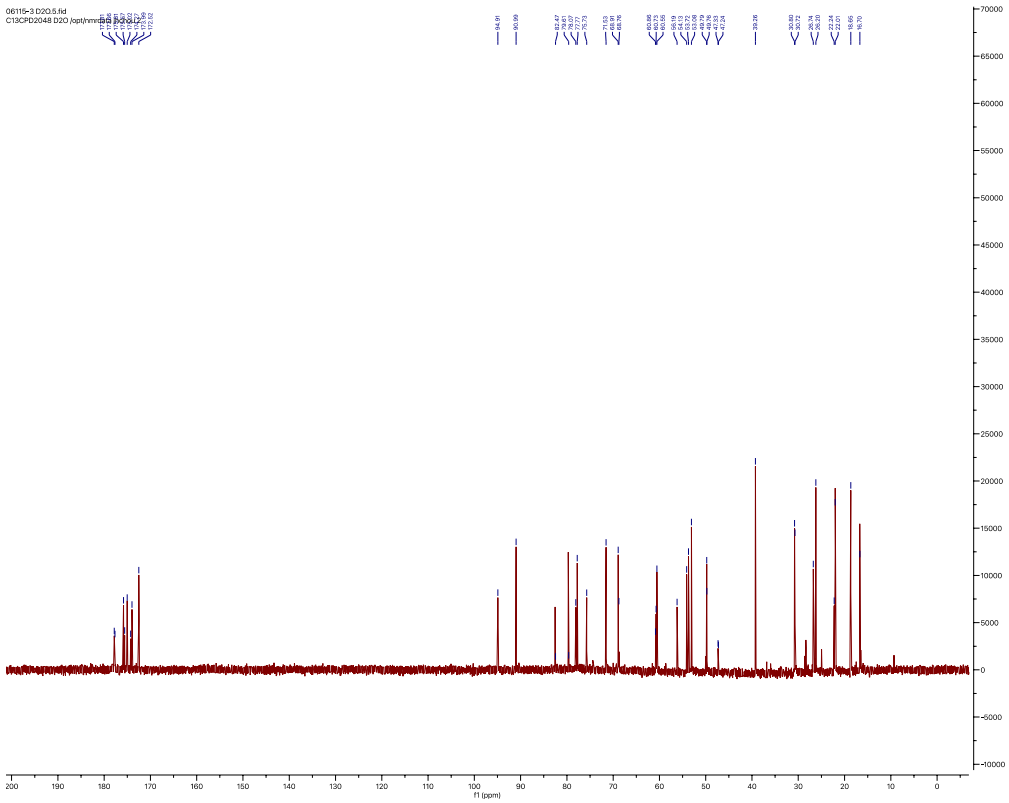
536



537

Compound **3c** ^{1}H NMR and ^{13}C Spectra (D_2O)

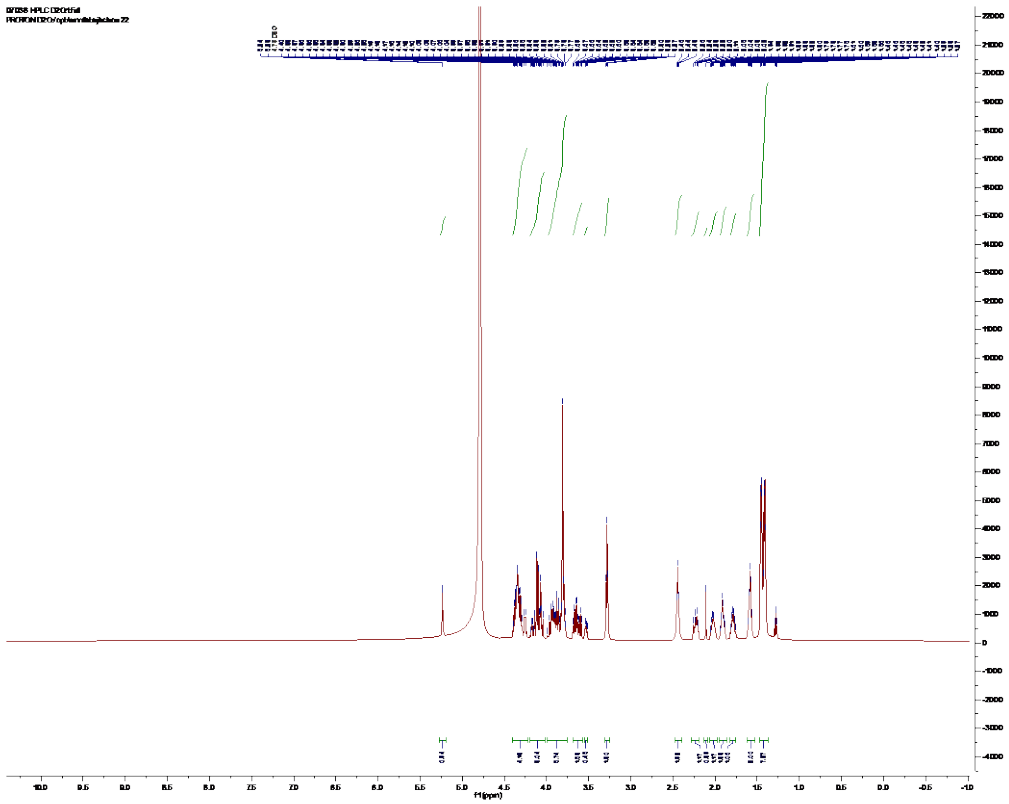




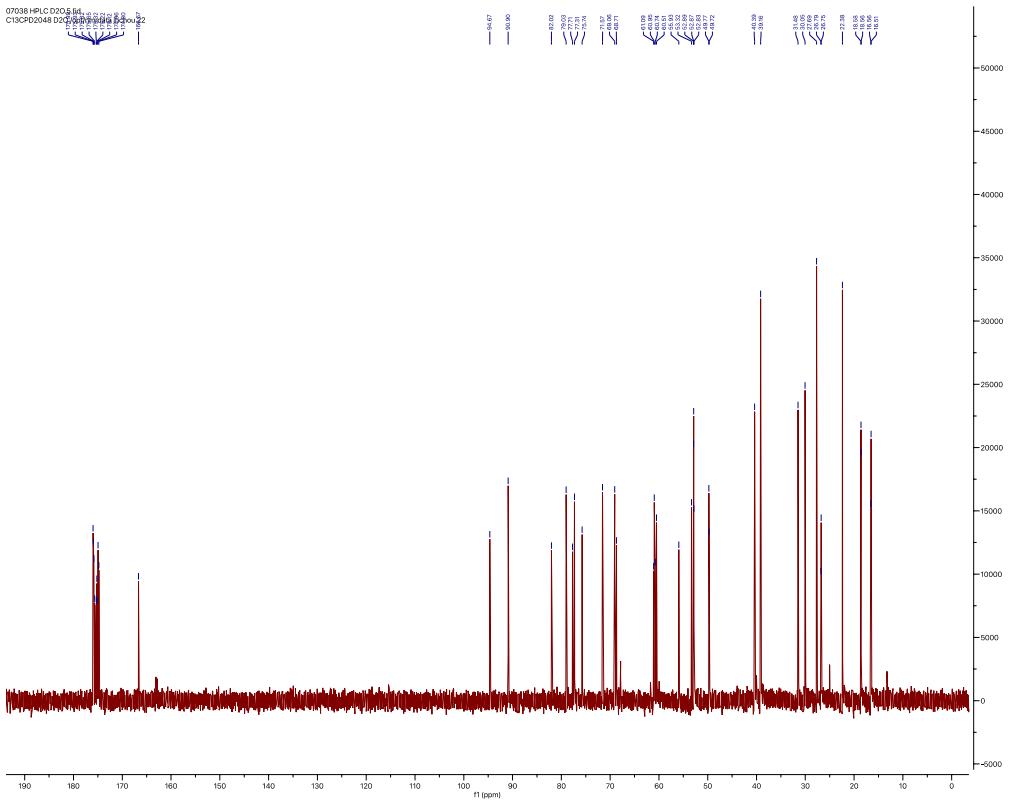
540

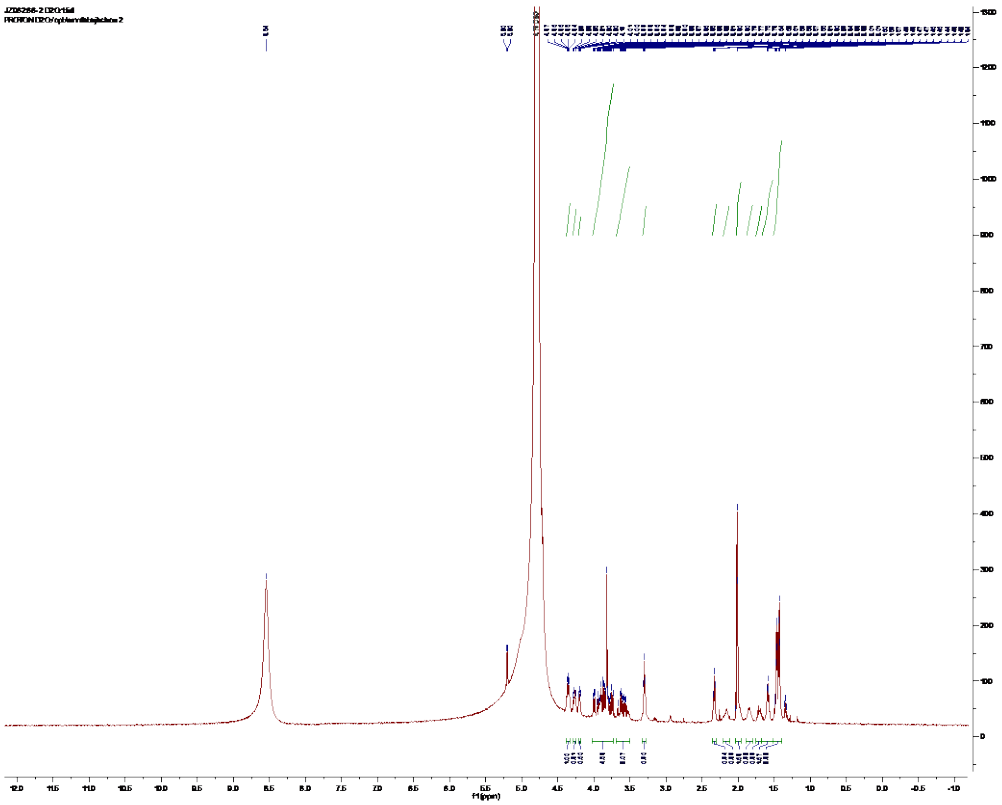
541

Compound **3d** ^{1}H NMR and ^{13}C Spectra (D_2O)



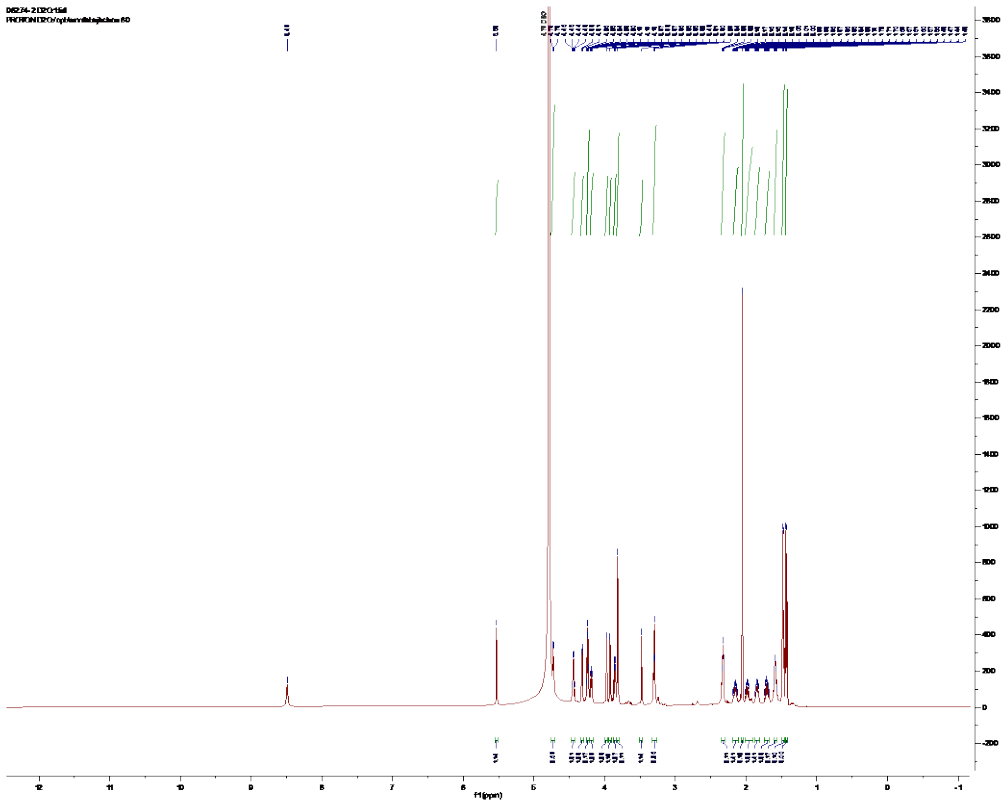
Compound **3e** ^{1}H NMR and ^{13}C Spectra (D_2O)





547

548 Compound **3f** ^{1}H NMR and ^{13}C Spectra (D_2O)



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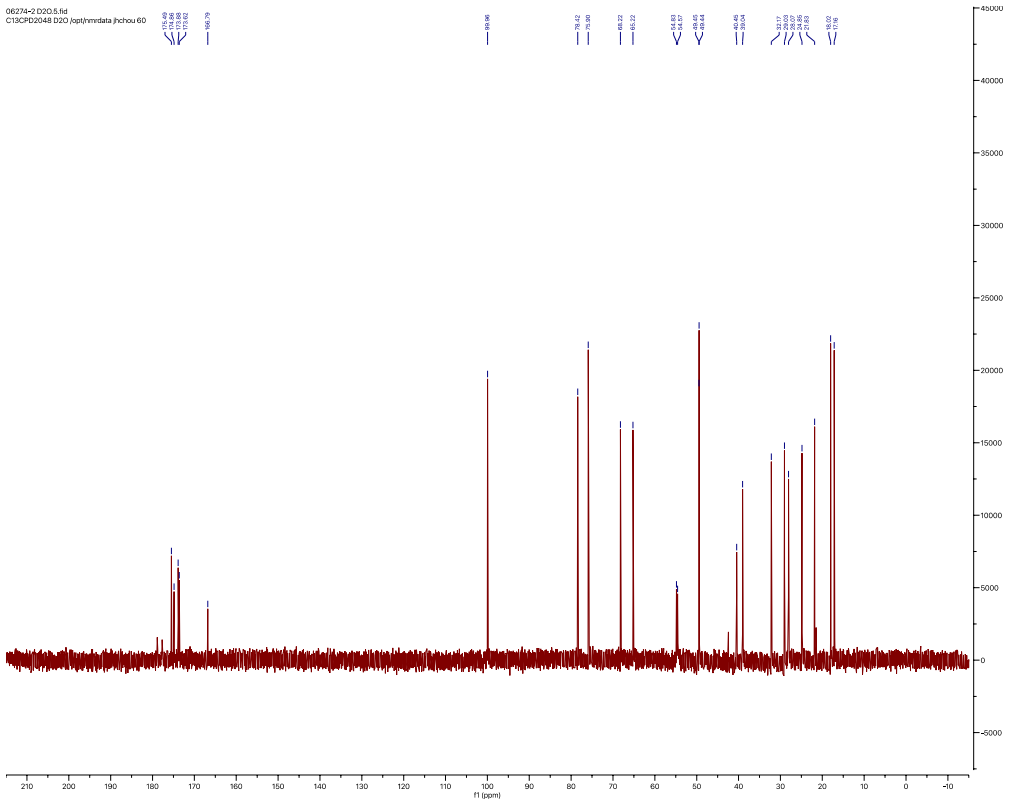
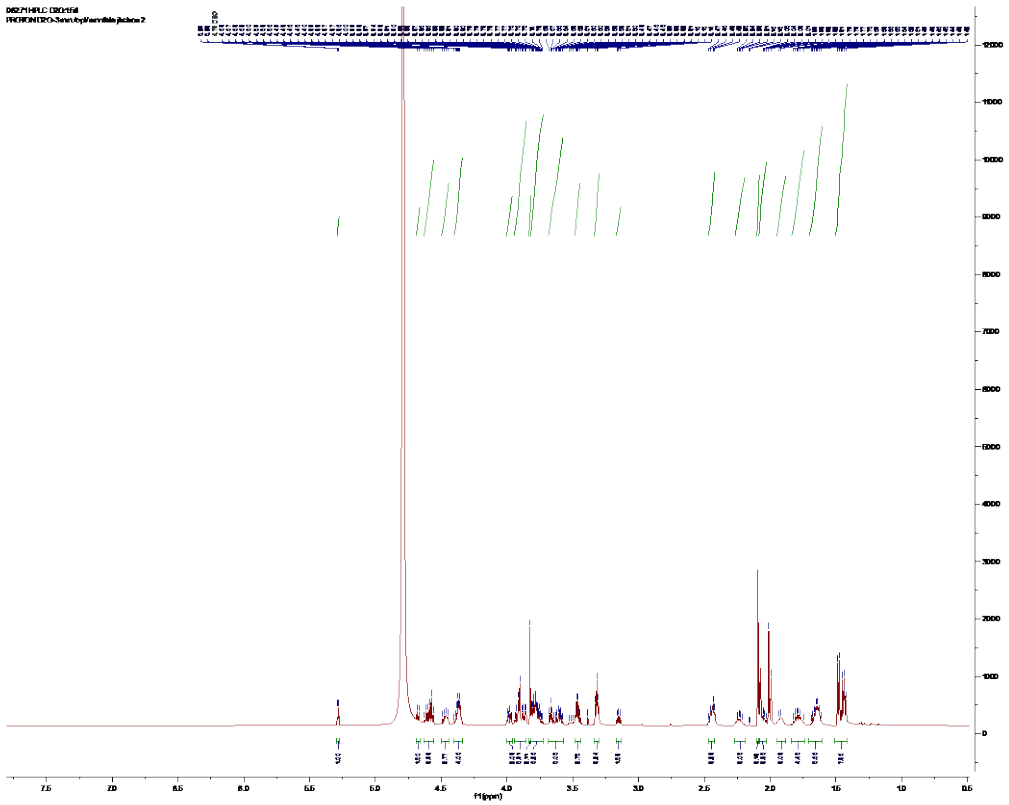


550

551 Compound **1a** ^{1}H NMR and ^{13}C Spectra (D_2O)



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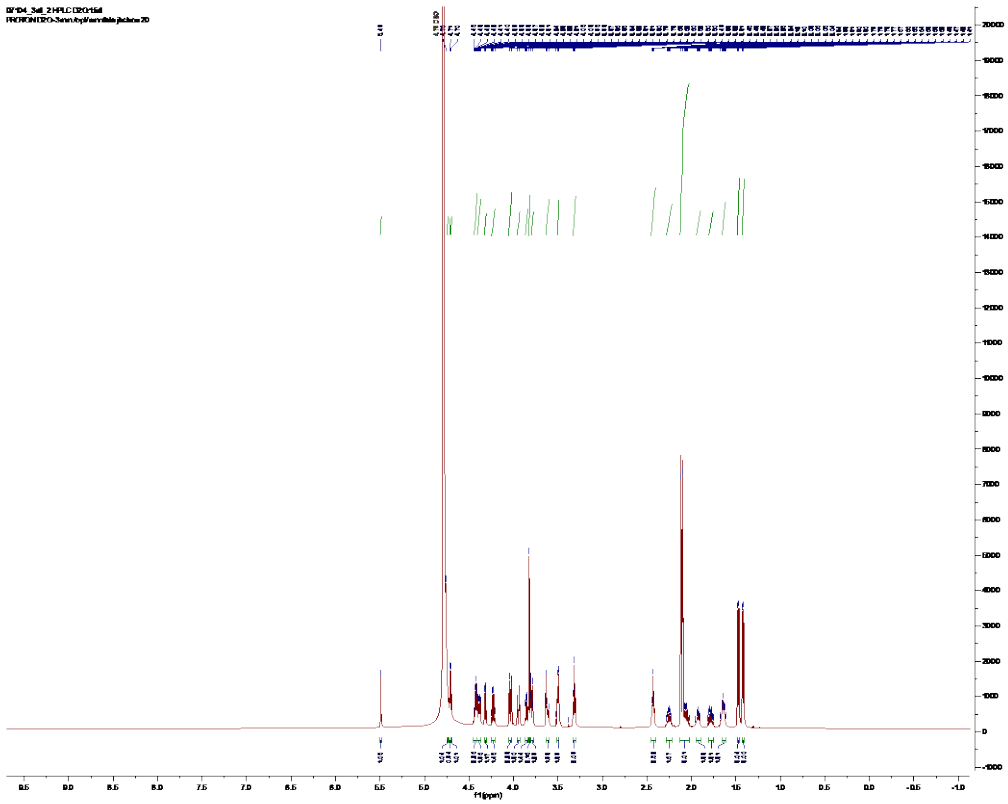
553 Compound **1b** ^{1}H NMR and ^{13}C Spectra (D_2O)



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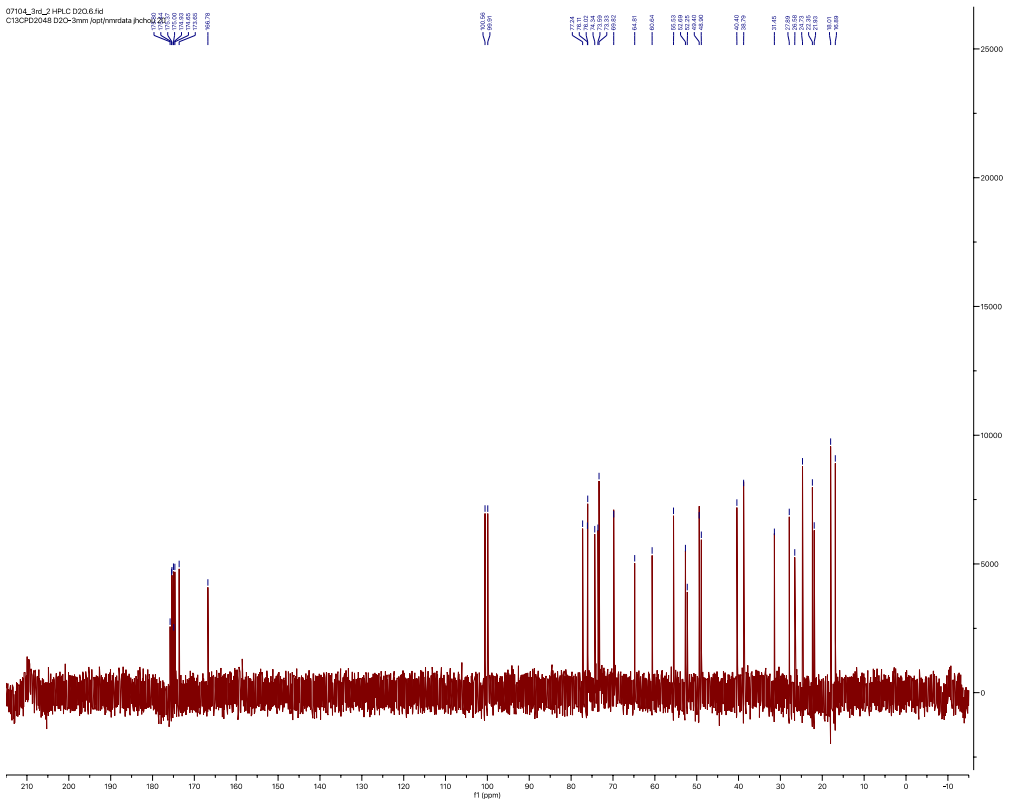

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558

559

10. Supplementary Tables

Table S1 (*Presented as a csv file)*

Full list of array components.

Table S2 (*Presented as a excel file)*

DESeq2 analysis of gene expression in wild type BMBMs stimulated versus stimulated with GMTriP-K for 18 hours.

Table S3 *(Presented as a excel file)*

DESeq2 analysis of gene expression in PGLYRP-1 -/- BMBMs stimulated versus unstimulated with GMTriP-K for 18 hours.

Table S4 *(Presented as a excel file)*

DESeq2 analysis of gene expression of GMTriP-K stimulated BMDNs from *Pglyrp-1*-/- vs wild type mice.

Table S5 (*(Presented as a excel file)*

DESeq2 analysis of gene expression of GMTriP-K stimulated BMDNs from *Nod2* -/- vs wild type mice.

Table S6 (*(Presented as a excel file)*

Hierarchical clustering of genes that were induced more than 4-fold by GMTriP-K or MDP in wild type or PGLYRP-1 -/- BMDMs.

584

585 **Table S7** *(Presented as a excel file)*

586 Hierarchical clustering of gene expression in GMTriP-K stimulated wild type and PGLYRP1-,

587 NOD2-, and GEF-H1-deficient BMDMs.

588

589 **Table S8** (*(Presented as excel file)*

590 Hierarchical clustering of gene expression in MDP stimulated wild type and PGLYRP1-, NOD2,

591 and GEF-H1-deficient BMDMs.

592

593 **Table S9**

594 Colocalization analysis of confocal microscopy of PGLYRP-1, Sec61β, and GM130 expression

595 in HEK 293T cells.

PGLYRP-1/SEC61b/GM130 colocalization/ %of RO1 material colocalized/Pearson's coefficient in ROI volume							
Cell	% SEC61b	% PGLYRP-1	P-Co		% PGYRP-1	% GM130	P-Co
1	39.44	59.07	0.8527		5.3	35.79	0.3748
2	67.49	76.46	0.8619		4.55	36.54	0.291
3	54.58	45.11	0.685		1.45	35.17	0.1222
4	62.36	58.24	0.9144		4.74	8.8	0.4273
5	81.07	69.6	0.7632		22.41	21.99	0.634
6	32.06	45.98	0.682		12.84	9.5	0.472
7	38.46	45.17	0.7272		16.26	11	0.447
8	62.31	57.74	0.7606		14.24	14.91	0.5004
9	53.06	66.23	0.794		21.82	9.46	0.5505
10	49.08	70.84	0.7496		25.86	19.91	0.6791
Mean	53.99	59.44	0.7791		12.95	20.31	0.4498

STD	14.96	11.37	0.07691		8.668	11.59	0.1627
SEM	4.73	3.596	0.02432		2.741	3.666	0.05147

596

597 **Table S10**

598 Colocalization analysis of confocal microscopy PGLYRP-1, NOD2, and GM130 expression in

599 HEK 293T cells.

PGLYRP-1/NOD2 colocalization/ %of RO1 material colocalized/Pearson's coefficient in ROI volume

Cell	%NOD2	% GM130	P-Co		%PG1colocalized	% NOD2 colocalized	P-Co		%PGLTYRP-1	%GM130	P-Co
1	0.93	26.09	0.2182		41.65	36.99	0.525		9.82	38.1	0.5201
2	2.16	31.56	0.1136		48.78	14.02	0.569		12.28	33.68	0.3821
3	0.68	30.12	0.2292		36.32	5.32	0.4523		16.16	55.1	0.5883
4	2.76	77.24	0.1779		41.9	13.68	0.571		9.48	60.05	0.5447
5	1.8	28.97	0.3516		63.21	19.75	0.5148		13.26	28.96	0.7733
6	2.05	63.28	0.1804		50.87	15.25	0.4747		14.8	61.21	0.606
7	2.76	69.21	0.1545		48.92	17.42	0.4938		8.96	35.29	0.4549
8	0.52	11.18	0.1582		7.51	7.43	0.3747		12.38	53.33	0.575
9	2.07	58.64	0.2072		65.06	18	0.4932		9.67	57.53	0.4226
10	2.6	42.43	0.2054		77.23	21.25	0.4888		10.12	39.22	0.3727
11	3.26	53.17	0.2909		67.84	24.53	0.5636		13.85	52.31	0.5922
12	4.2	64.37	0.2444		63.45	22.2	0.5224		17.97	59.91	0.5682
Mean	2.149	46.36	0.211		51.06	17.99	0.5036		12.4	47.89	0.5333
STD	1.08	20.74	0.06441		18.49	8.254	0.05548		2.915	11.89	0.1127

SEM	0.3117	5.988	0.0185			5.337	2.383	0.01602		0.8414	3.431	0.03253

600

601

602 **Table S11** *(Presented as a excel file)*

603 Expression of genes in the heatmap Fig. 6b (Gene expression was normalized and scaled)

604

605 **Table S12** *(Presented as a excel file)*

606 List of clustered genes Fig. 6b.

607

608 **Table S13** *(Presented as a excel file)*

609 Differential gene expression in active UC vs healthy colon tissue samples.

610

611 **Table S14** *(Presented as a excel file)*

612 Differential gene expression in inactive UC vs healthy colon tissue samples.

613

614 **Table S15** *(Presented as word file)*

615 List of key resources

616

617