Electronic Supplementary Material (ESI) for RSC Chemical Biology.

Evaluation of Kdo-8-N_3 Incorporation into Lipopolysaccharides of Various *Escherichia coli* strains

Zeynep Su Ziylan[1], Geert-Jan de Putter[1], Meike Roelofs[1], Jan Maarten van Dijl[2], Dirk-Jan Scheffers[3], Marthe T. C. Walvoort[1*]

[1] *Stratingh Institute for Chemistry, Faculty of Science and Engineering, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands*

[2] *Department of Medical Microbiology, University of Groningen, University Medical Center Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands*

[3] *Groningen Biomolecular Sciences and Biotechnology Institute, Faculty of Science and Engineering, University of Groningen, Nijenborgh 7, 9747 AG Groningen, The Netherlands*

* Corresponding author: m.t.c.walvoort@rug.nl

Supplementary Information

I. Additional Figures and Tables

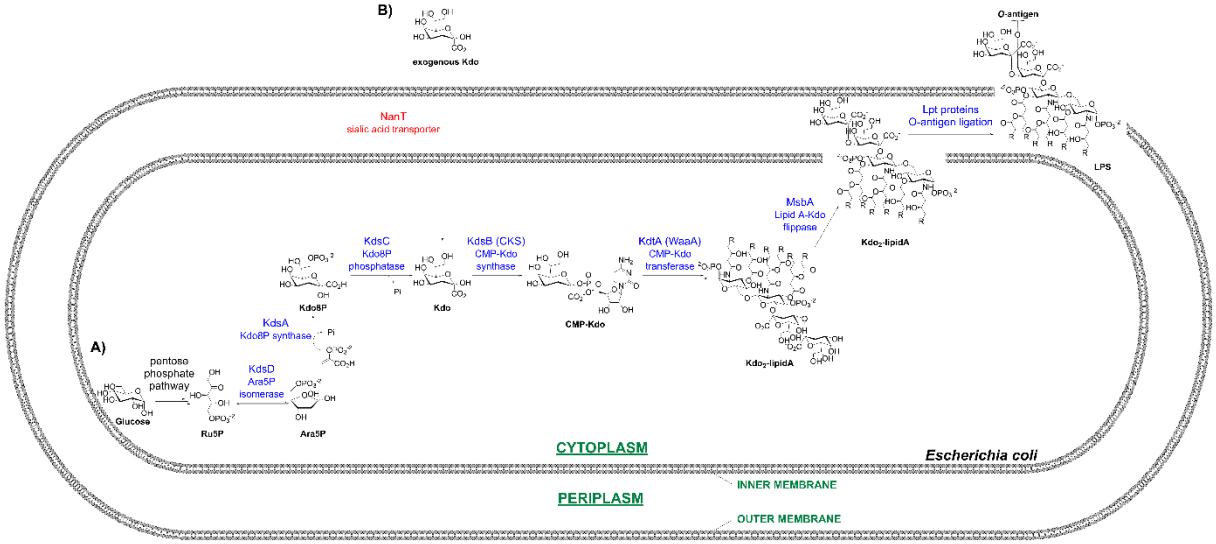


Figure S1. Representative scheme of the biosynthetic routes of Kdo and LPS in *Escherichia coli* cells. A: The *de novo* Kdo biosynthesis. B: The Kdo salvage pathway.

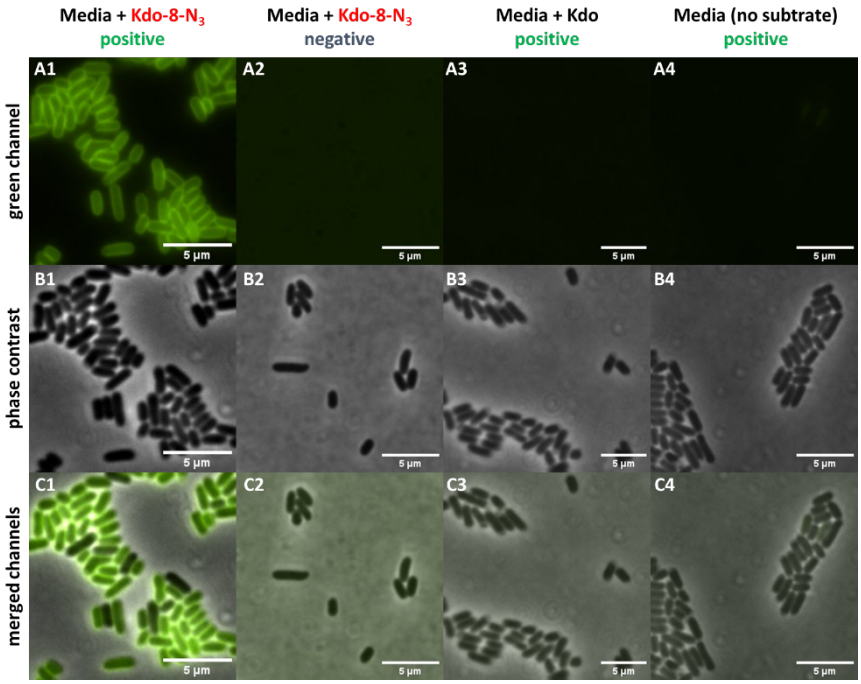


Figure S2. *E. coli* BW25113 cells grown in M9 medium: with Kdo-8-N_3 and clicked with FAM-DBCO (A1-C1); with Kdo-8-N_3 but not clicked with any fluorophore (A2-C2); with Kdo and clicked with FAM-DBCO (A3-C3); without the supplementation of Kdo or Kdo-8-N_3 but treated with FAM-DBCO (A4-C4). Cells were visualized with a Nikon Ti-E microscope using fluorescence optics (A1-A4: green channel), phase contrast (B1-B4), and co-localization of fluorescent and phase contrast images (C1-C4: merged channels).

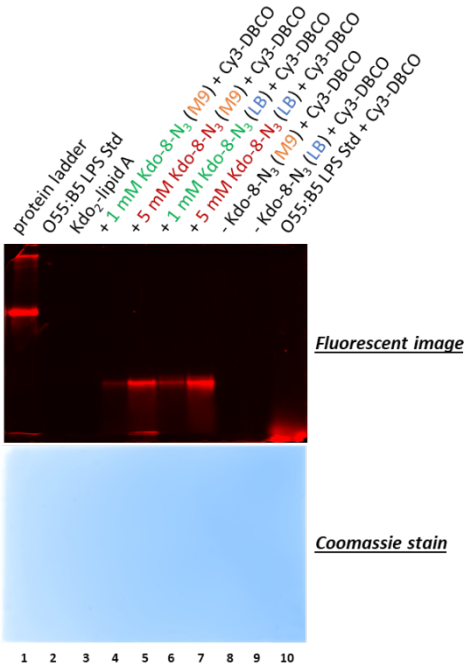


Figure S3. LPS separation on 16% Tris-Tricine SDS-PAGE: *E. coli* BW25113 cells grown with or without Kdo-8-N_3 (1 mM or 5 mM) in M9 minimal medium or the rich LB medium and clicked with Cy3-DBCO: Fluorescence image (top) of labeled LPS bands and Coomassie stained gel image (bottom) to visualize the protein content of the samples. The protein ladder (lane 1) was not observed with the Coomassie staining protocol due to the 40-fold dilution of the commercial stock solution, which rendered the final amounts of the reference too low to allow staining. No bands were observed on the lanes containing *E. coli* cell samples, indicating that the samples do not contain proteins, or that the amount is too low for detection.

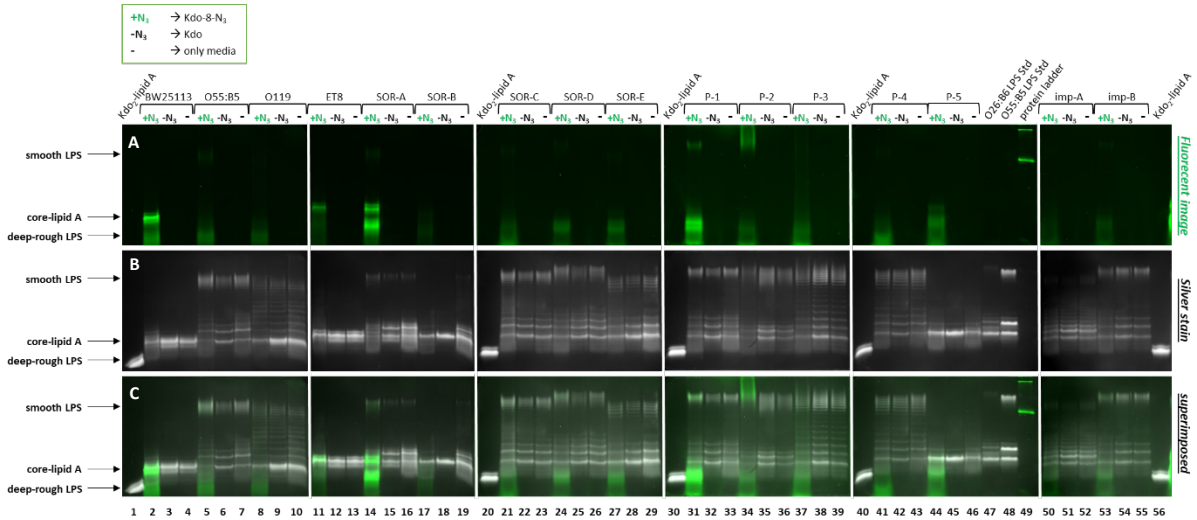


Figure S4. 16% Tris-Tricine SDS-PAGE gel images showing differential LPS labeling for the different *E. coli* strains when grown in LB with 5 mM Kdo-8-N_3 (+N_3), with 5 mM Kdo (**-N_3**) or without the supplementation of either substrate (**-**). A: Fluorescence images showing labeled structures. B: Silver-stained images showing total LPS. C: Superimposed gel images.

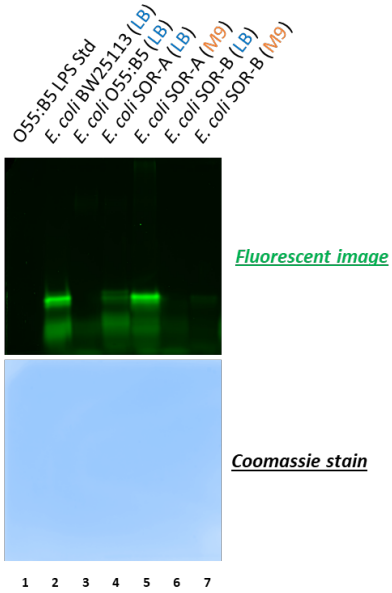


Figure S5. 16% Tris-Tricine SDS-PAGE LPS separation gel images for the selection of pathogenic *E. coli* strains visualized by microscopy.

Species (classification if known)	ID code (in this study)	Source/Origin
Escherichia coli K-12 BD792	BW25113	Leibniz Institute (DSM 27469)
Escherichia coli O55:K59(B5)	O55:B5	Leibniz Institute (DSM 4779)
Escherichia coli	O119	ATCC: The Global Bioresource Center
Escherichia coli ET12567 derivative	ET8	Zhu *et al.*[1]
Escherichia coli	SOR-A	UMCG (*Bacteremia sample)*
Escherichia coli	SOR-B	UMCG (*Bacteremia sample)*
Escherichia coli	SOR-C	UMCG (*Bacteremia sample)*
Escherichia coli	SOR-D	UMCG (*Bacteremia sample)*
Escherichia coli	SOR-E	UMCG (*Bacteremia sample)*
Escherichia coli	P-1	UMCG (*Clinical isolate*)
Escherichia coli	P-2	UMCG (*Clinical isolate characterized by Francis M. Cavallo*)
Escherichia coli	P-3	UMCG (*Clinical isolate characterized by Francis M. Cavallo*)
Escherichia coli	P-4	UMCG (*Clinical isolate characterized by Francis M. Cavallo*)
Escherichia coli	P-5	UMCG (*Clinical isolate characterized by Francis M. Cavallo*)
Escherichia coli	Imp-A	UMCG (*Clinical isolate from synovial fluid of a patient with an infected implant)*
Escherichia coli	Imp-B	UMCG (*Clinical isolate from synovial fluid of a patient with an infected implant)*

Table S1. List of the bacterial species and strains used in this work including the ID codes. (UMCG: University Medical Center Groningen).

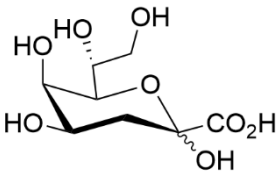
II. Experimental Procedures

General Procedures

All solvents used were of commercial grade and used without further purification unless stated otherwise. Dry solvents were generated by an MBraun SPS 800 solvent purification system or dried over activated (by heating *in vacuo*) molecular sieves 4Å (Merck, Germany). Column chromatography was performed manually with silica (Standard Silica 60M, 0.04 – 0.063 mm, 230 – 400 mesh, Macherey-Nagel GmbH, Germany) or by an automated column chromatography instrument; Reveleris flash column chromatography system (purchased from Buchi). Solvents used for workup and column chromatography were of technical or HPLC grade from Boom, Biosolve, or Honeywell and used as purchased. Solvents were removed by rotary evaporation under reduced pressure at 40 °C, unless specified otherwise. Reagents were purchased from Sigma-Aldrich, Acros, TCI Europe, or CarboSynth and used without further purification. Reaction temperature refers to the temperature of the heating or cooling baths equipped with a stirring bar. Reaction pH values were measured using a portable pH meter (Mettler Toledo, S2-K, 30207950) equipped with an Inlab Micro pro ISM electrode (Mettler Toledo, 51344163) or with indicator sticks (pH 0-14, VWR International, H0011400075). Reactions were monitored by TLC analysis on Merck silica gel 60/Kieselguhr F254 and spots were visualized by UV light, or spraying with orcinol stain (180 mg orcinol, 10 mL 85% H_3PO_4, 5 mL EtOH, and 85 mL H_2O) or with Seebach's stain (2.5 g phosphomolybdic acid, 1 g $Ce(SO_4)_2$, 6 mL H_2SO_4 and 94 mL H_2O), or with Ninhydrin stain (1.5 g ninhydrin, 3 mL acetic acid and 100 mL *n*-butanol) followed by heating with a heat gun.

^1H and ^{13}C NMR spectra were recorded on a Varian Agilent 400-MR (400/100 MHz) or Bruker (600/151 MHz). Chemical shifts are given in ppm with the solvent resonance as an internal standard ($CDCl_3$: δ 7.26 for ^1H, δ 77 for ^{13}C, CD_3OD: δ 3.31 for ^1H, δ 49 for ^{13}C and D_2O: δ 4.79 for ^1H). All individual signals were assigned using 2D NMR spectroscopy: HH-gCOSY, gHSQC. Data are reported as follows: chemical shifts (δ), multiplicity (s = singlet, d = doublet, dd = double doublet, ddd = double double doublet, t = triplet, q = quartet, m = multiplet,), coupling constants *J* (Hz), and integration. High-resolution mass measurements were performed on an LTQ Orbitrap XL spectrometer (Thermo Fisher Scientific) with an ESI ionization source.

Chemical Synthesis of Kdo



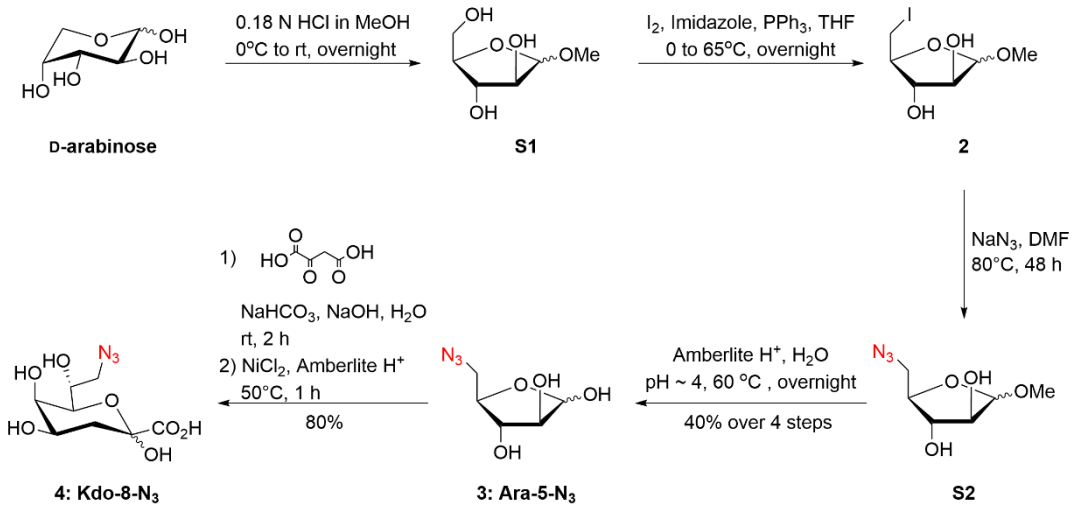
3-Deoxy-α/β-D-*manno*-oct-2-ulosonic acid (1: Kdo)

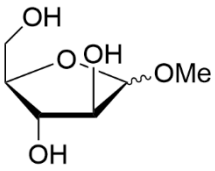
To a solution of $NaHCO_3$ (48 mg, 0.05 eq) in distilled water (3 mL) was added 10 M aqueous NaOH at 0 °C until pH 12 (as monitored by pH indicator sticks). Oxaloacetic acid (1.5 g, 11.36 mmol, 1 eq) in distilled water (7 mL) and 10 M aq. NaOH (~4 mL) were added simultaneously dropwise over a period of 25 min, while keeping the pH above 12 (as monitored by pH meter). The pH was stabilized at 12.45 after addition was complete. Commercial D-arabinose (2.2 g, 14.76 mmol, 1.3 eq) was dissolved in distilled water (7 mL) and added dropwise into the reaction mixture over a period of 10 min. During the addition the pH was adjusted to 12 and stabilized at 12.08 after the addition was complete. The reaction was left to stir at ambient temperature for 2 h. After then, $NiCl_2$ (29 mg, 0.02 eq) was added into the reaction mixture and the reaction mixture was heated to 50 °C. Amberlite IR120 (H^+) resin was added in portions over a period of 1 h until pH was stable at 5.41 for at least 15 min. The reaction mixture was filtered, and the filtrate was applied to an anion exchange column (freshly prepared HCO_3^- resin). The product was eluted with a gradient of $NaHCO_3$ solution (0.5 M to 2 M). Combined fractions were

neutralized using Amberlite IR120 (H^+), filtered and then concentrated *in vacuo* to give the product as a transparent foaming syrup (0.98 g, 4.09 mmol, 36%, 4:1 Kdo/4-epi-Kdo, including 11% pyruvate).

NMR data is in accordance with the reported data[2]. The major α-anomer product is reported: ^{1}H NMR (600 MHz, D_2O, HH-COSY, HSQC, HMBC) δ 4.07 (dq, *J* = 12.0, 5.1, 4.1 Hz, 1H, H-4), 4.02 (dd, *J* = 2.0, 1.0 Hz, 1H, H-5), 3.89 (ddd, *J* = 10.9, 5.3, 2.5 Hz, 1H, H-7), 3.82 (d, *J* = 10.2 Hz, 1H, H-6), 3.81 – 3.76 (m, 1H, H-8'), 3.63 (dd, *J* = 12.0, 5.8 Hz, 1H, H-8"), 1.99 (t, *J* = 12.6 Hz, 1H, H-3'), 1.89 (dd, *J* = 13.1, 5.2 Hz, 1H, H-3"). ^{13}C NMR (151 MHz, D_2O) δ 176.7 (C-1), 96.4 (C-2), 71.1 (C-6), 69.2 (C-7), 66.6 (C-5), 66.2 (C-4), 63.0 (C-8), 33.6 (C-3). HR-MS (ESI): m/z: calcd for $C_8H_{13}O_8$ $[M - H]^-$ 237.0616, found 237.0617.

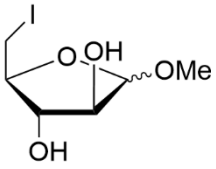
Chemical Synthesis of Kdo-8-N₃





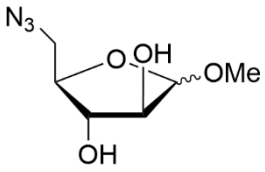
Methyl α/β-D-arabinofuranoside (S1)

D-Arabinose (5 g, 33.3 mmol, 1 eq) was suspended in dry methanol (124 mL) under N_2 and 32 mL of a freshly prepared methanolic HCl solution (0.94 M, 2 mL acetyl chloride in 30 mL MeOH) was added dropwise at 0 °C over a period of 20 min (final concentration 0.18 N HCl). The reaction was stirred overnight at ambient temperature. The reaction was quenched by the addition of solid $NaHCO_3$ until pH ~7. The resulting suspension was filtered, and the filtrate was concentrated *in vacuo*. Purification by column chromatography (silica gel, ethyl acetate/methanol, 3/1, v/v) yielded the title compound **S1** as a colorless syrup (4.88 g, 29.73 mmol, 89%, 2:1 α/β). TLC: R_f = 0.5 (ethyl acetate/methanol, 5/1, v/v). ^{1}H NMR (400 MHz, CD_3OD, HH-COSY, HSQC) δ 4.76 (d, *J* = 1.6 Hz, 1H, H-1α), 4.74 (d, *J* = 4.4 Hz, 0.5H, H-1β), 3.98 – 3.94 (m, 1H, H-2β, H-3β), 3.93 (dd, *J* = 3.7, 1.6 Hz, 1H, H-2α), 3.90 (dd, *J* = 5.8, 3.7 Hz, 1H, H-4α), 3.83 (dd, *J* = 6.2, 3.6 Hz, 1H, H-3α), 3.80 – 3.77 (m, 0.5H, H-4β), 3.75 (dd, *J* = 11.9, 3.3 Hz, 1H, H-5'α), 3.63 (dd, *J* = 11.9, 5.4 Hz, 1H, H-5"α), 3.63-3.55 (m, 0.5H, 3.55, H-5'β), (dd, *J* = 11.7, 7.2 Hz, 1H, H-5"β), 3.41 (s, 1.5H, OCH3), 3.37 (s, 3H, OCH3). ^{13}C NMR (101 MHz, CD_3OD) δ 110.6 (C-1α), 104.0 (C-1β), 85.5 (C-4α), 84.4 (C-4β), 83.3 (C-2α), 79.0 (C-3β), 78.7 (C-3α), 76.8 (C-2β), 65.5 (C-5β), 63.0 (C-5α), 55.5 (OCH3), 55.2 (OCH3). HR-MS (ESI): m/z: calcd for $C_6H_{11}O_5$ $[M-H]^-$ 163.0612, found 163.0611.



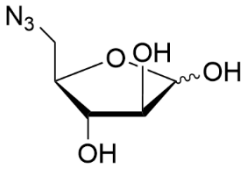
Methyl 5-deoxy-5-iodo-α/β-D-arabinofuranoside (2)

O-Methyl-α/β-D-arabinofuranose (4.88 g, 29.73 mmol, 1 eq) was dissolved in anhydrous THF (100 mL) under N_2 and cooled down to 0 °C. To the cooled solution was added imidazole (5.26 g, 77.29 mmol, 2.6 eq), iodine (9.81 g, 38.65 mmol, 1.3 eq) and triphenylphosphine (9.36 g, 35.67 mmol, 1.2 eq) in the respective order. The reaction mixture was stirred at 65 °C overnight. The reaction mixture was left to cool to ambient temperature and then extracted with toluene/water. The aqueous layer was collected and concentrated *in vacuo*. Purification by column chromatography (silica gel, heptane/ethyl acetate, 1/2, v/v) yielded the title compound **2** as a brown syrup (5.08 g, 18.54 mmol, 63%, 5:2 α/β). TLC: R_f = 0.43 (chloroform/acetone, 3/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.94 (d, *J* = 1.8 Hz, 1H, H-1α), 4.84 (d, *J* = 4.6 Hz, 0.4H, H-1β), 4.15 (dd, *J* = 2.6, 1.2 Hz, 1H, H-2α), 4.11 (dd, *J* = 7.1, 4.6 Hz, 0.4H, H-2β), 4.05-3.99 (m, 0.4H, H-4β), 4.03 (ddd, *J* = 6.0, 4.2 Hz, 1H, H-4α), 3.93 (t, *J* = 6.4 Hz, 0.4H, H-3β), 3.90 (dd, *J* = 4.3, 2.5 Hz, 1H, H-3α), 3.46 (s, 1.2H, OCH3), 3.45 – 3.37 (m, 0.4H, H-5'β), 3.41 (s, 3H, OCH3), 3.35 (dd, *J* = 10.4, 5.7 Hz, 1.4H, H-5'α, H-5"β), 3.30 (d, *J* = 6.5 Hz, 1H, H-5"α). ^{13}C NMR (101 MHz, $CDCl_3$) δ 108.93 (C-1α), 102.0 (C-1β), 84.3 (C-4α), 81.6 (C-4β), 81.2 (C-3α), 81.0 (C-2α), 80.6 (C-3β), 78.6 (C-2β), 55.7 (OCH3), 55.3 (OCH3), 8.2 (C-5β), 6.7 (C-5α). HR-MS (ESI): m/z: calcd for $C_6H_{11}IO_4Na$ $[M + Na]^+$ 296.9594, found 296.9594.



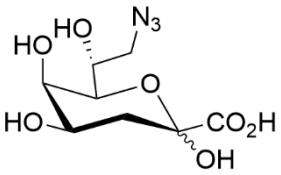
Methyl 2,3-di-*O*-acetyl-5-azido-5-deoxy-α/β-D-arabinofuranoside (S2)

To a solution of compound **2** (5.08 g, 18.54 mmol, 1 eq) in DMF (96 mL) was added NaN_3 (4.84 g, 74.31 mmol, 4 eq). The reaction was stirred at 80 °C for 48 h. The reaction was allowed to cool down to ambient temperature and the solvent was removed *in vacuo*. Purification by column chromatography (silica gel, pentane/ethyl acetate, 1/2, v/v) yielded the title compound as a brown syrup (3.47 g, 18.34 mmol, 99%, 5:1 α/β). The α-anomer was isolated separately and used in the next step. TLC: R_f = 0.5 (chloroform/acetone, 3/1, v/v). ^{1}H NMR (400 MHz, $CDCl_3$, HH-COSY, HSQC) δ 4.92 (s, 1H, H-1α), 4.16 (td, *J* = 3.8, 2.6 Hz, 1H, H-4α), 4.04 (d, *J* = 1.2 Hz, 1H, H-2α), 3.89 (d, *J* = 2.6 Hz, 1H, H-3α), 3.65 (t, *J* = 3.8 Hz, 2H, H-1α), 3.41 (s, 3H, OCH_3). ^{13}C NMR (101 MHz, $CDCl_3$) δ 109.3 (C-1), 85.0 (C-4), 79.9 (C-2), 78.8 (C-3), 55.2 (OCH_3), 52.8 (C-5). HR-MS (ESI): m/z: calcd for $C_6H_{11}N_3O_4Na$ $[M + Na]^+$ 212.0642, found 212.0639.



5-Azido-5-deoxy-α/β-D-arabinose (3: Ara-5-N_3)

Compound **S2** (0.69 g, 3.65 mmol, 1 eq) was dissolved in water (8.2 mL) and the solution was heated up to 70 °C. Amberlite IR120 (H^+) resin was added gradually into the reaction mixture and the pH was adjusted to 3. The reaction was left to stir at 70 °C overnight. Then the solvent was removed *in vacuo*. Purification by column chromatography (silica gel, dichloromethane/methanol, 95/5, v/v) yielded the title compound **3** as a brown syrup (0.46 g, 2.63 mmol, 72%, 2:1 α/β). TLC: R_f = 0.48 (dichloromethane/acetone, 8/1, v/v). ^{1}H NMR (400 MHz, CD_3OD, HH-COSY, HSQC) δ 5.20 (d, *J* = 4.5 Hz, 0.5H, H-1β), 5.14 (d, *J* = 2.6 Hz, 1H, H-1α), 4.13 – 4.07 (ddd, *J* = 9.6, 6.8, 3.4 Hz, 1H, H-4α), 3.98 (t, *J* = 6.5 Hz, 0.5H, H-4β), 3.91 (dd, *J* = 4.6, 2.6 Hz, 1.5H, H-2β, H-2α), 3.83 (dd, *J* = 6.6, 4.7 Hz, 1H, H-3α), 3.81 – 3.77 (m, 0.5H, H-3β), 3.49 (dd, *J* = 13.2, 3.4 Hz, 1H, H-5'α), 3.42 – 3.37 (m, 2H, H-5"α, H-5'β, H-5"β). ^{13}C NMR (101 MHz, CD_3OD) δ 103.3 (C-1α), 97.4 (C-1β), 83.7 (C-2α), 83.0 (C-4α), 82.0 (C-3β), 78.7 (C-3α), 78.3 (C-2β), 77.3 (C-4β), 54.9 (C-5β), 53.2 (C-5α). IR ν_{max}/cm^{-1} = 2104.8 (conj. CN). HR-MS (ESI): m/z: calcd for $C_5H_9N_3O_4Na$ $[M + Na]^+$ 198.0485, found 198.0483.



8-Azido-3,8-dideoxy-α/β-D-manno-oct-2-ulosonic acid (4: Kdo-8-N_3)

To a solution of $NaHCO_3$ (9.1 mg, 0.06 eq) in distilled water (0.7 mL) was added 10 M aqueous NaOH at 0 °C until pH 13.50. Oxaloacetic acid (250 mg, 1.89 mmol, 1 eq) in distilled water (0.9 mL) and 10 M aq. NaOH (0.6 mL) were added simultaneously dropwise over a period of 25 min, while keeping the pH around 12-13 (as monitored by pH meter). The pH was stabilized at 12.35 after addition was complete. Compound **3** (431 mg, 2.46 mmol, 1.3 eq) was dissolved in distilled water (1 mL) and added dropwise into the reaction mixture over a period of 10 min. During the addition the pH was adjusted to 13 and stabilized at 12.96 after the addition was complete. The reaction was left to stir at ambient temperature for 2 h. After then, $NiCl_2$ (5 mg, 0.04 eq) was added into the reaction mixture and the reaction mixture was heated to 50 °C. Amberlite IR120 (H^+) resin was added in portions over a period on 1 h until pH was stable at 4.47 for 15 min. The reaction mixture was filtered and the filtrate was applied to an anion exchange column (freshly prepared HCO_3^- resin). Product was eluted with a gradient of $NaHCO_3$ solution (0.5 M to 2 M). Combined fractions were neutralized using Amberlite IR120 (H^+), filtered and then concentrated *in vacuo* to give the title product as a highly viscous syrup (397 mg, 1.51 mmol, 80%, 4:1 Kdo-8-N_3/4-*epi*-Kdo-8-N_3, including 4% pyruvate). Data is reported for the major α-anomer: ^{1}H NMR (600 MHz, D_2O, HH-COSY, HSQC, HMBC): δ 4.07 (ddd, *J* = 11.7, 5.0, 2.9 Hz, 1H, H-4), 4.04 – 3.96 (d, *J* = 3.0 Hz 1H, H-7), 4.03-3.96 (m, 1H, H-5), 3.83 (dd, *J* = 9.2, 1.2 Hz, 1H, H-6), 3.61 (dd, *J* = 13.2, 2.6 Hz, 1H, H-8'), 3.44 (dd, *J* = 13.1, 6.0 Hz, 1H, H-8"), 1.99 (t, *J* = 12.5 Hz, 1H, H-3'), 1.85 (dd, *J* = 13.0, 5.1 Hz, 1H, H-3"). ^{13}C NMR (151 MHz, D_2O) δ 176.5 (C-1), 96.4 (C-2), 71.6 (C-6), 68.1 (C-7), 66.4 (C-5), 66.2 (C-4), 53.7 (C-8), 33.6 (C-3). IR ν_{max}/cm^{-1} = 2105.6 (conj. CN). HR-MS (ESI): m/z: calcd for $C_8H_{12}N_3O_7$ $[M - H]^-$ 262.0681, found 262.0681.

III. Materials and Methods

Bacterial strains

Bacterial strains used in this project are: *E. coli* BW25113 (*E. coli* K-12 derivative, Leibniz Institute; DSM 27469), *E. coli* O55:B5 (*E. coli* O55:K59(B5), Leibniz Institute; DSM 4779), *E. coli* O119 (ATCC: The Global Bioresource Center), *E. coli* ET8 (*E. coli* ET12567 derivative obtained from Zhu *et al*.[1]) and clinical isolates of *E. coli* obtained from the strains collection at the University Medical Center Groningen (UMCG). Clinical *E. coli* isolates listed here as SOR-A, SOR-B, SOR-C, SOR-D, SOR-E, P-1, P-2, P-3, P-4, P-5, imp-A and imp-B were obtained from independent blood cultures or from synovial fluid of parients with infected implants (see Table S1 for details). All strains were preserved at -70 °C in lysogeny broth (LB) containing 25% glycerol.

Materials

Lysogeny broth (LB Broth, Miller) used for bacterial growth was commercially obtained in powder form from Sigma-Aldrich (L3522). Sterile Dubecco's Phosphate Buffered Saline, PBS (1x), used in click reactions or in microscopy experiments was commercially obtained from Gibco. The following commercial click reagents were used in the corresponding click reactions: FAM-DBCO (FAM DBCO, 6-isomer, Lumiprobe, 551F0), Cy3-DBCO (Cyanine3-DBCO, Lumiprobe, E10F0), TAMRA-DBCO (TAMRA DBCO, 5-isomer, Lumiprobe, 4F77P) and FAM-Alkyne (FAM alkyne, 5-isomer, Lumiprobe, B41B0). All reagents were prepared as stock solutions in DMSO (2.5 mM) and stored at -20°C. The protein ladder used during SDS-PAGE was purchased from Bio-Rad (Precision Plus Protein™ Dual Color Standards; 1610374). LPS standards were purchased from Sigma-Aldrich: O26:B6 LPS Std (Lipopolysacharides from Escherichia coli O26:B6, ≥10,000 EU/mg, purified by phenol extraction; L8274), O55:B5 LPS Std

(Lipopolysacharides from Escherichia coli O55:B5, purified by phenol extraction; L2880), and Kdo_2-lipidA (KLA, ≥90% (HPLC); SML2430). Commercial Kdo-8-N_3 was purchased from Click Chemistry Tools (Kdo Azide, >90% purity, CAS 1380099-68-2, catalog #1241-10).

Bacterial growth media and growth conditions

The strains were grown either in lysogeny broth (LB (Miller), containing 10 g/L tryptone, 5 g/L yeast extract, 10 g/L NaCl, pH 7.5, adjusted using 6 M HCl and 6 M NaOH) or M9 minimal medium (M9, containing 0.3% KH_2PO_4, 0.6% Na_2HPO_4, 0.5% NaCl, 0.1% NH_4Cl, 2 mM $MgSO_4$, 0.1 mM $CaCl_2$, supplemented with 0.2% of maltose, sterilized through a filter) as indicated below. Overnight cultures were typically prepared by inoculation of LB medium and incubation at 37 °C at 220 rpm.

Growth experiments

Growth experiments were performed in a microplate reader (BioTek^R, Synergy H1) using 200 µL as the final volume per well of a 96 well-plate. An overnight liquid culture was used to inoculate fresh LB or M9 medium containing 5 mM of Kdo or Kdo-8-N_3 or an equivalent amount of sterile MilliQ water to an OD_{600} of 0.05. The prepared plate was then incubated at 37 °C with continuous shaking for 24 h. OD_{600} measurements were recorded every 5 minutes and recorded. The growth analyses were done using BioTek^R Gen5 Software. Each experiment was repeated in triplicate and the average of these measurements was used to depict the graphs along with the calculated standard deviations.

Copper-free click reaction

Overnight cultures were inoculated into 1 mL of fresh LB or M9 medium containing 1 mM or 5 mM of Kdo or Kdo-8-N_3 or an equivalent amount of sterile MilliQ water to an OD_{600} of 0.05. The liquid cultures were incubated at 37 °C at 220 rpm for 16 h, then pelleted at 7000 rpm for 2 min, and washed three times using fresh medium. The cell pellets corresponding to an OD_{600} of 0.3 were resuspended in fresh M9 medium and treated with the appropriate fluorophore to a final concentration of 0.25 mM in 20 µL. The reaction was performed at 37 °C for 1 h in the dark. After the reaction, the samples were pelleted at 7000 rpm for 2 min and washed three times using fresh M9 medium. The resulting cell pellets were prepared either for SDS-PAGE or fluorescence microscopy.

Copper-catalyzed click reaction

An overnight culture of *E. coli* BW25113 was used to inoculate 1 mL of M9 medium containing 5 mM of Kdo-8-N_3 or an equivalent amount of sterile MilliQ water to an OD_{600} of 0.05. The liquid cultures were incubated at 37 °C at 220 rpm for 16 h, then pelleted at 7000 rpm for 2 min, and washed three times using fresh medium. The cell pellets corresponding to an OD_{600} of 0.3 were resuspended in 10 µl PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4, pH 7.4), and were treated with 1 mM $CuSO_4$, 1 mM THPTA, and 0.25 mM FAM-Alkyne, followed by 20 mM NaAsc in a total volume of 20 µL. The samples were incubated in the dark at 37 °C (no shaking) for 20 min. Afterwards, the cells were washed three times with PBS buffer. The resulting cell pellets were prepared either for SDS-PAGE or fluorescence microscopy.

Fluorescence imaging microscopy

The cell pellets corresponding to an OD_{600} of 0.3 in 1 mL were resuspended in 50 µL PBS buffer and 6 µL of each sample were mounted on a 1% agarose pad on a microscopy slide. The samples were imaged using a Nikon Ti-E microscope (Nikon Instruments, Tokyo, Japan) equipped with a Hamamatsu Orca Flash 4.0 camera. Images were acquired with Nikon Instruments Elements 4.10 software and processed using ImageJ 1.52p.[3]

SDS-PAGE analysis and LPS visualization

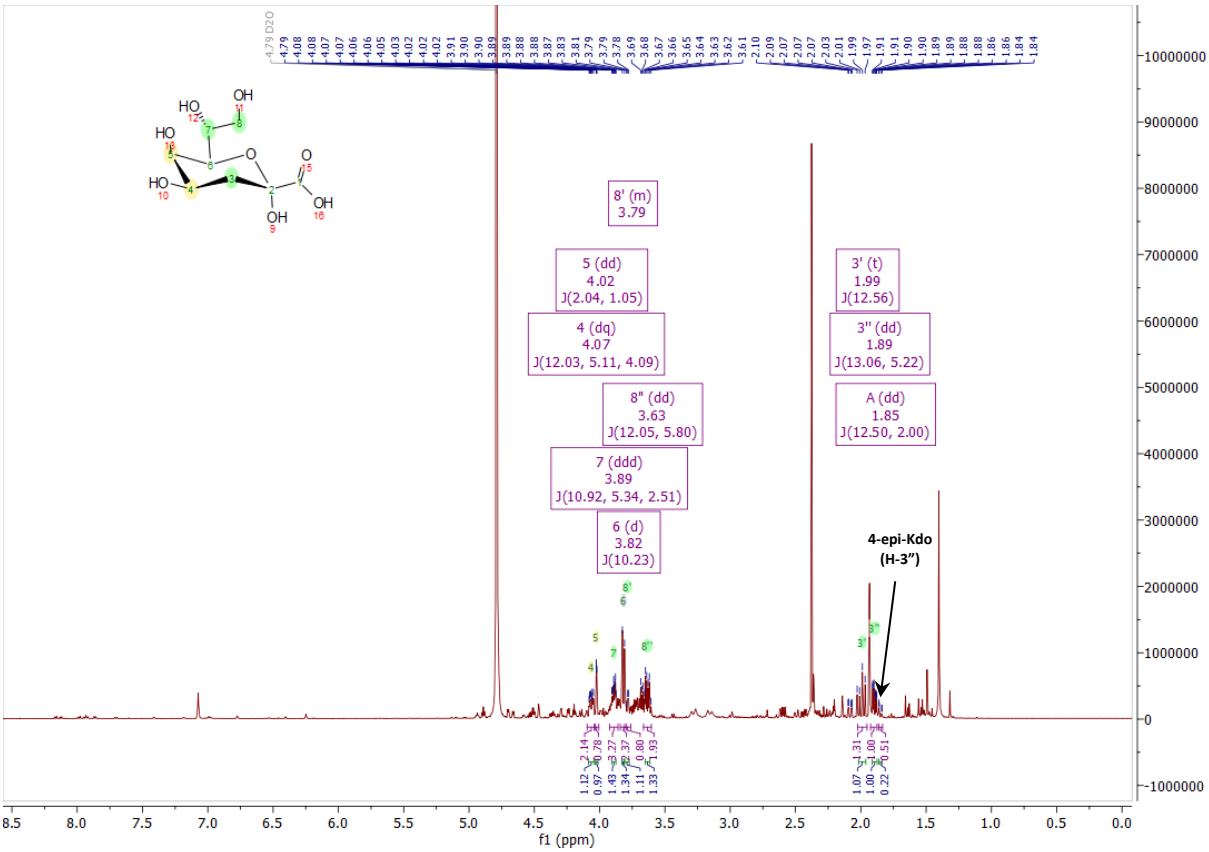
Sample preparation: Cell pellets corresponding to an OD_{600} of 0.3 in 1 mL were resuspended in 50 µL Tricine SDS sample buffer (4% β-mercaptoethanol, 200 mM Tris-HCl, 2% SDS, 0.04% Coomassie Blue in a 40% glycerol solution in MilliQ), and boiled at 100 °C for 10 min. After the samples were cooled down to ambient temperature, *Streptomyces griseus* protease (Type XIV, ≥3.5 units/mg solid, Sigma-Aldrich) was added to the samples to a final concentration of 3.3 mg/mL in a total volume of 60 µL, and the samples were incubated overnight at 55 °C. Next day, the samples were vortexed.

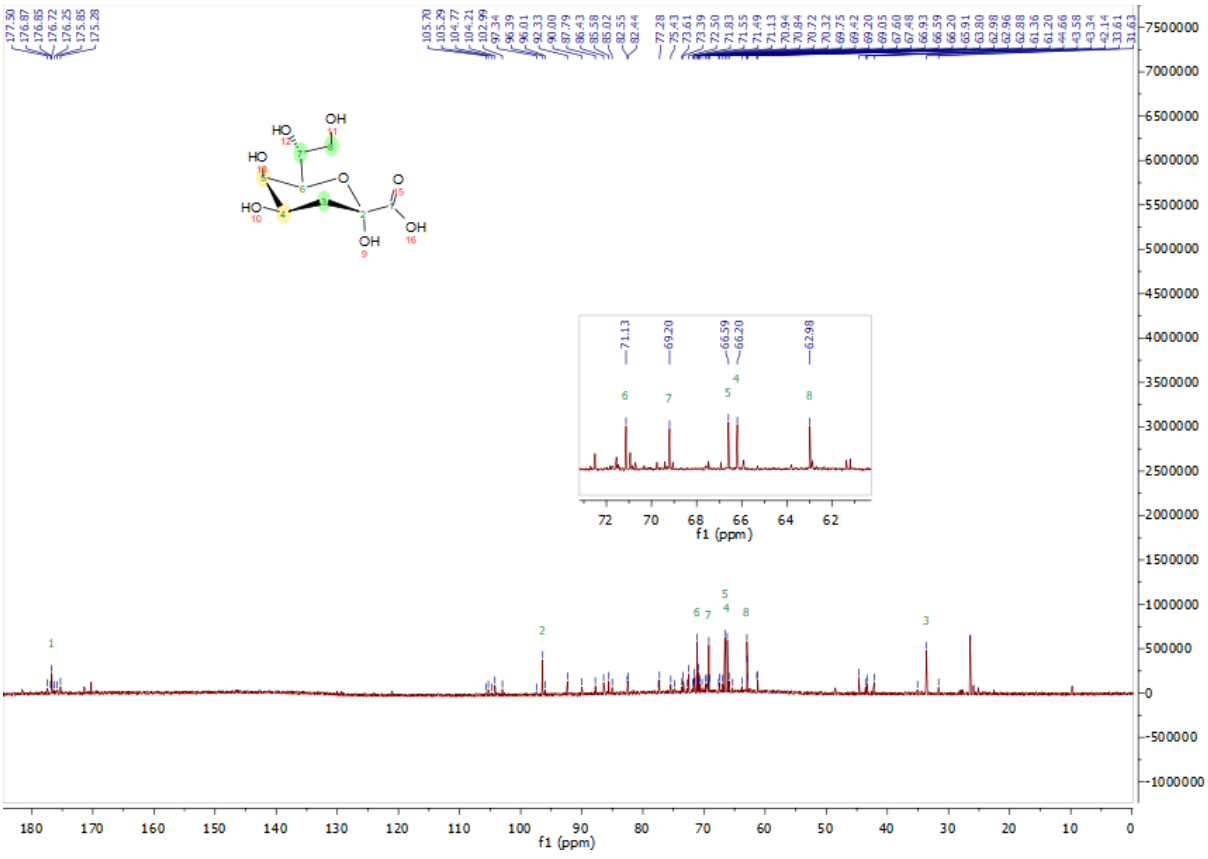
16% Tris-Tricine PAGE for LPS separation: The loading of samples was adjusted in a way where each well contained a portion of the sample that equals to an OD_{600} of 0.025. The gels were run approximately at 20 mA for ~5 h. The in-gel fluorescence was analyzed on Typhoon FLA 9500 (GE Healthcare) in appropriate channels; Channel 1 (green): Ex= 488 nm, Em= 524 nm, or Channel 2 (red): Ex= 555 nm, Em= 569 nm.

Gel staining protocols and imaging for total LPS: The gels were stained using either the staining protocol of Pro-Q™ Emerald 300 Lipopolysaccharide Gel Stain Kit (P20495, Thermo Fischer Scientific), or a silver staining protocol with slight modifications to the previously described method[4]: The gels were incubated overnight in 50 mL fixing solution (40% ethanol, 5% AcOH in water) with gentle shaking. Next day, the gels were incubated in oxidizing solution (2.1 g sodium periodate, 120 mL ethanol, 15 mL AcOH in 165 mL water) for 10 min with gentle shaking, then washed with fresh water for 15 min. The washing step was repeated 2 times. The gels were then incubated in the freshly prepared silver staining solution (151.6 mg NaOH, 1.32 g silver nitrate, 2.9 mL of 25% NH_4OH solution in 200 mL water) for 10 min with gentle shaking and subsequently washed with water 3 times in 15 min intervals. The LPS bands were developed using the developing solution (20 mg citric acid, 200 µL of 37% formaldehyde solution in 200 mL water) for 3 to 5 min and the development was stopped by incubating the gels in 7% AcOH solution. The images of stained gels were taken on a GelDoc™ EZ Imager (Bio-Rad).

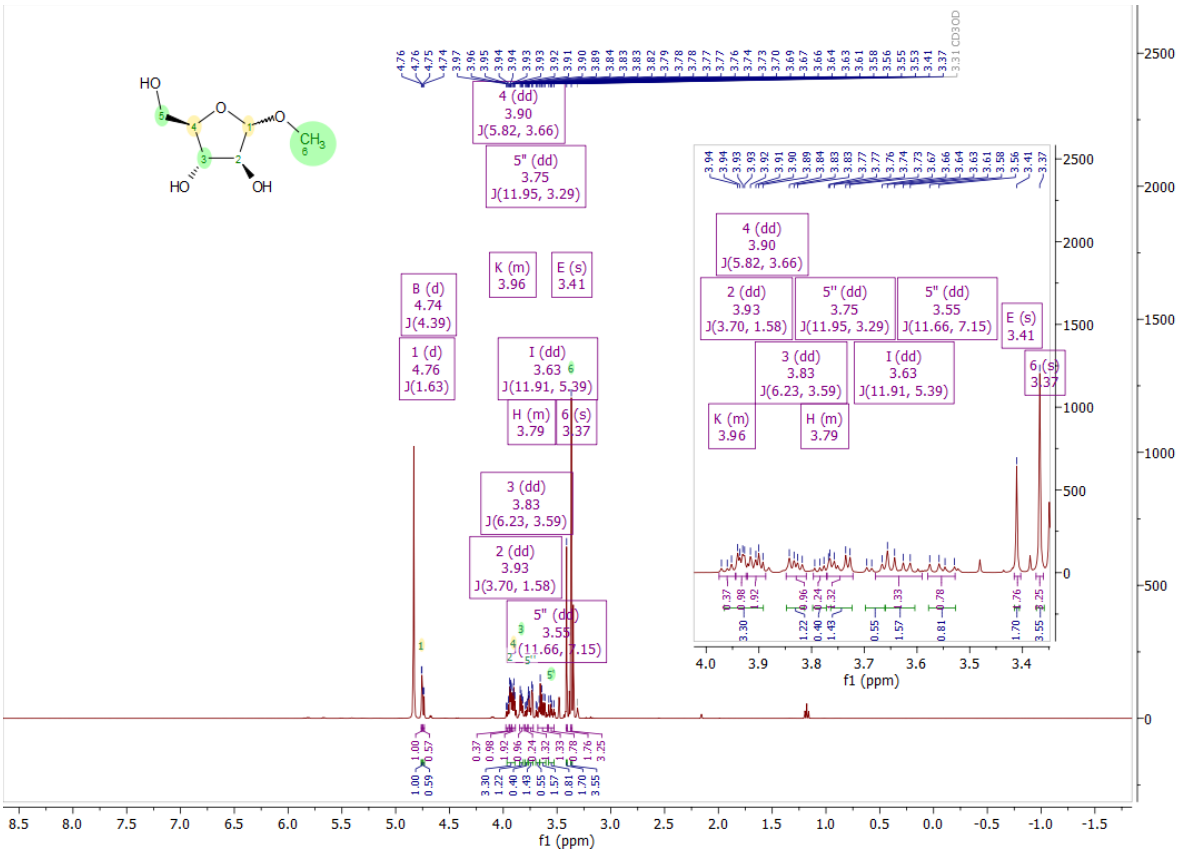
IV. NMR Spectra

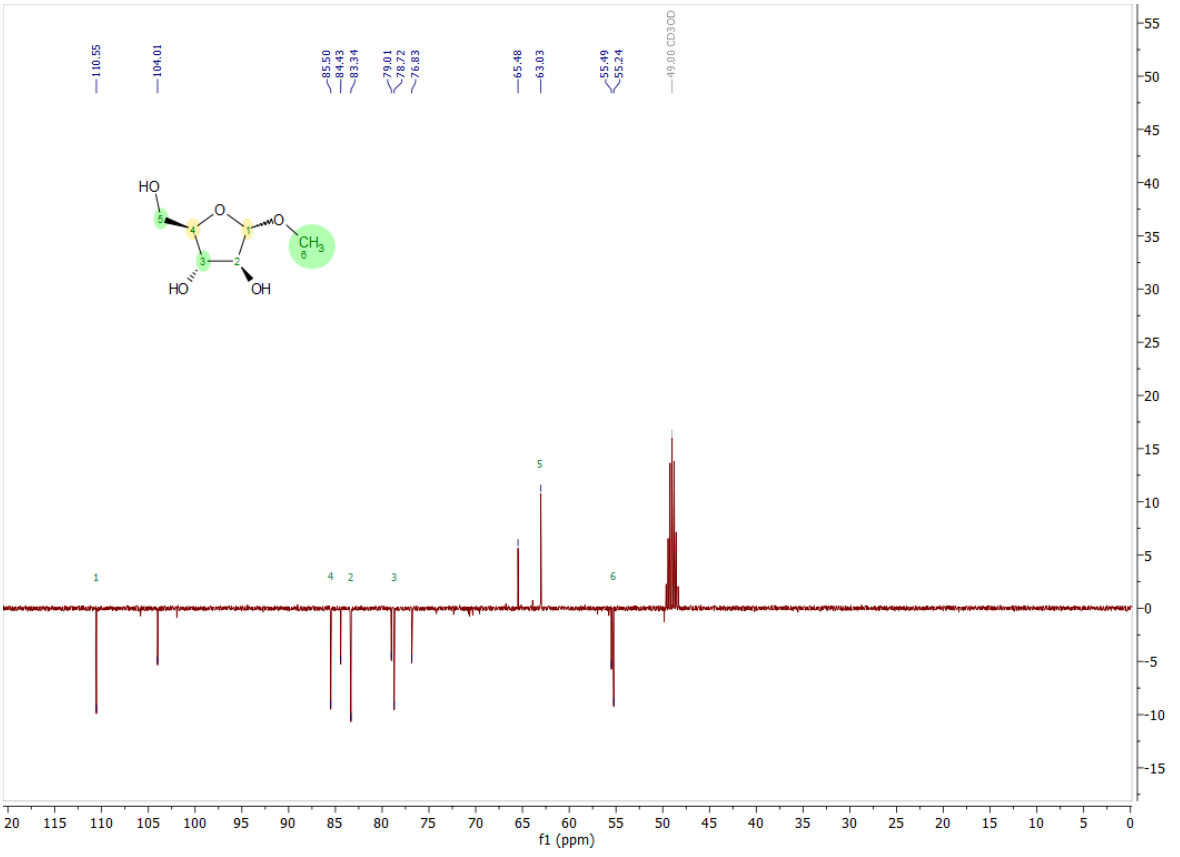
Compound 1:



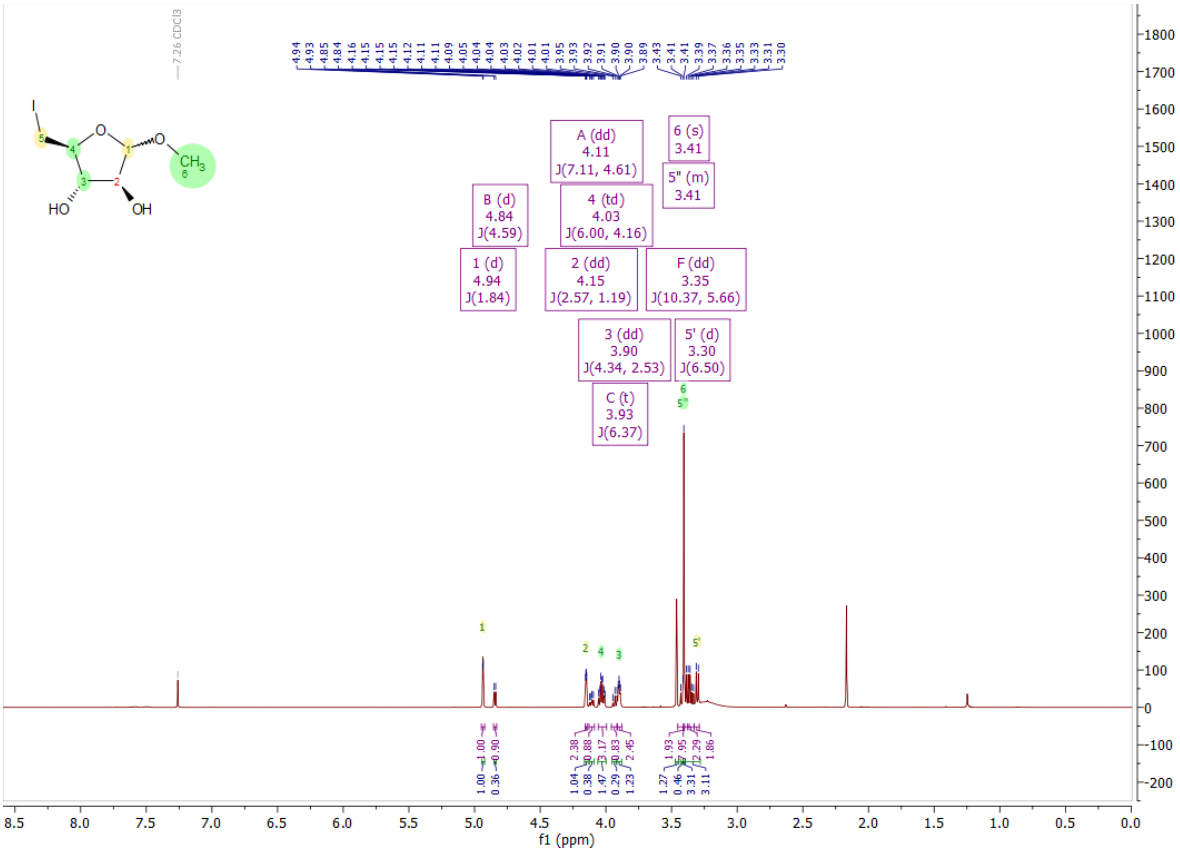


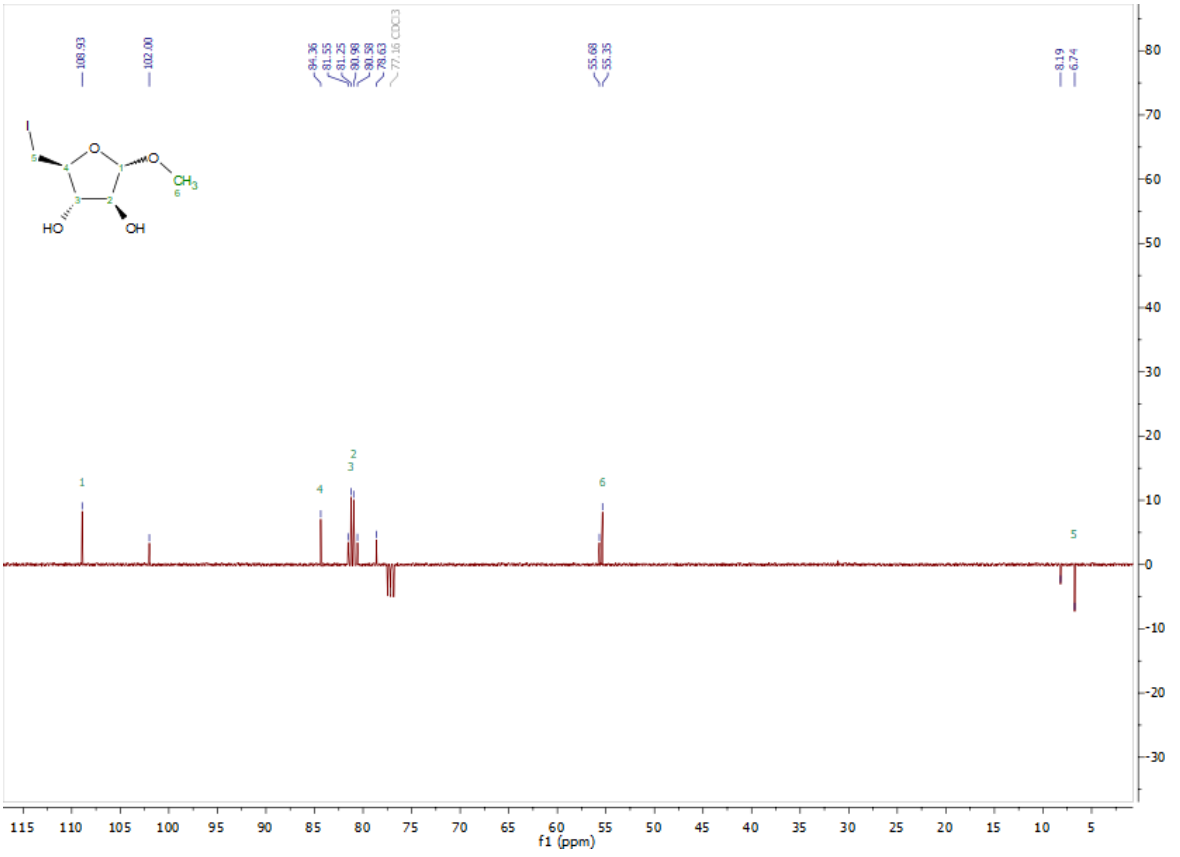
Compound S1:


HO
O
O
CH3
HO
OH
4 (dd)
3.90
J(5.82, 3.66)
5" (dd)
3.75
J(11.95, 3.29)
K (m)
3.96
E (s)
3.41
B (d)
4.74
J(4.39)
1 (d)
4.76
J(1.63)
I (dd)
3.63
J(11.91, 5.39)
H (m)
3.79
6 (s)
3.37
3 (dd)
3.83
J(6.23, 3.59)
2 (dd)
3.93
J(3.70, 1.58)
5" (dd)
3.55
J(11.66, 7.15)
f1 (ppm)

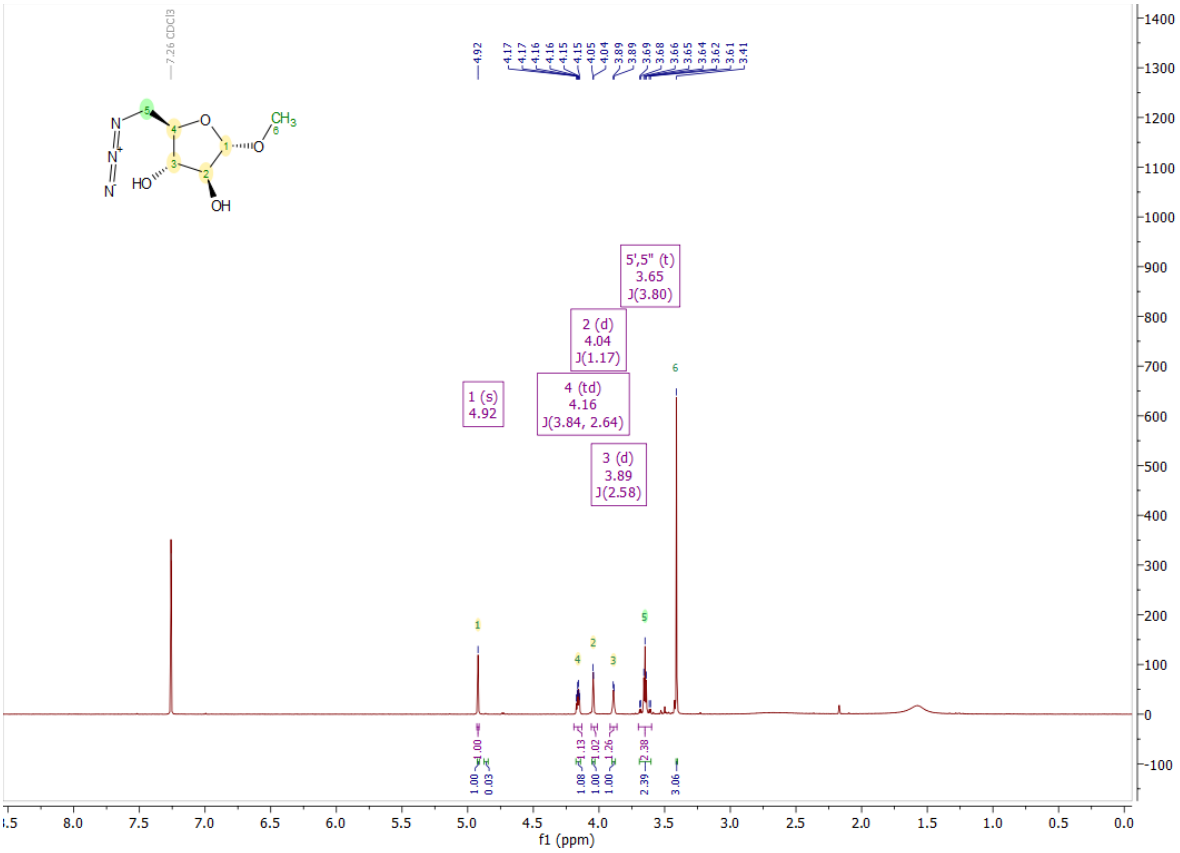

110.55
104.01
85.50
84.43
83.34
79.01
78.72
76.83
65.48
63.03
55.49
55.24
49.00 CD3OD
HO
O
O
CH3
HO
OH
f1 (ppm)

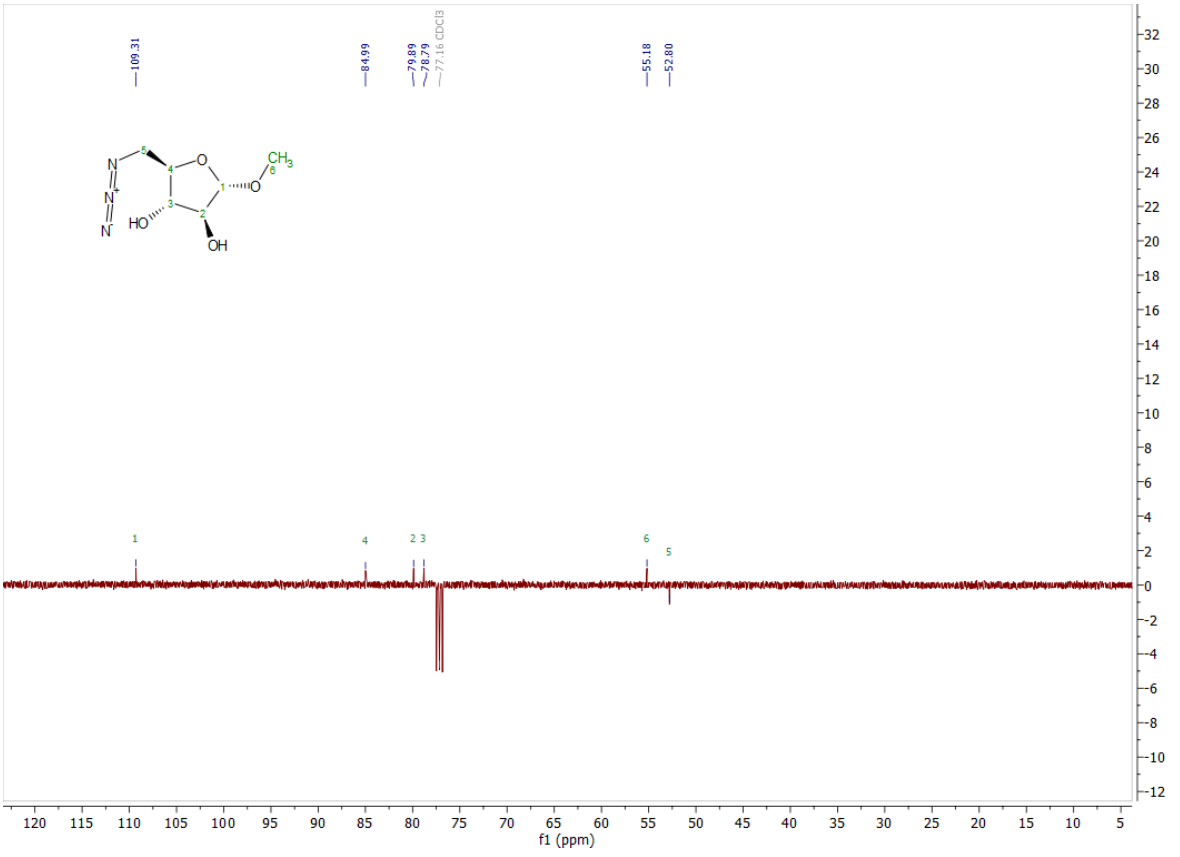
Compound 2:


A (dd)
4.11
J(7.11, 4.61)
6 (s)
3.41
5'' (m)
3.41
B (d)
4.84
J(4.59)
4 (td)
4.03
J(6.00, 4.16)
1 (d)
4.94
J(1.84)
2 (dd)
4.15
J(2.57, 1.19)
F (dd)
3.35
J(10.37, 5.66)
3 (dd)
3.90
J(4.34, 2.53)
5' (d)
3.30
J(6.50)
C (t)
3.93
J(6.37)
f1 (ppm)

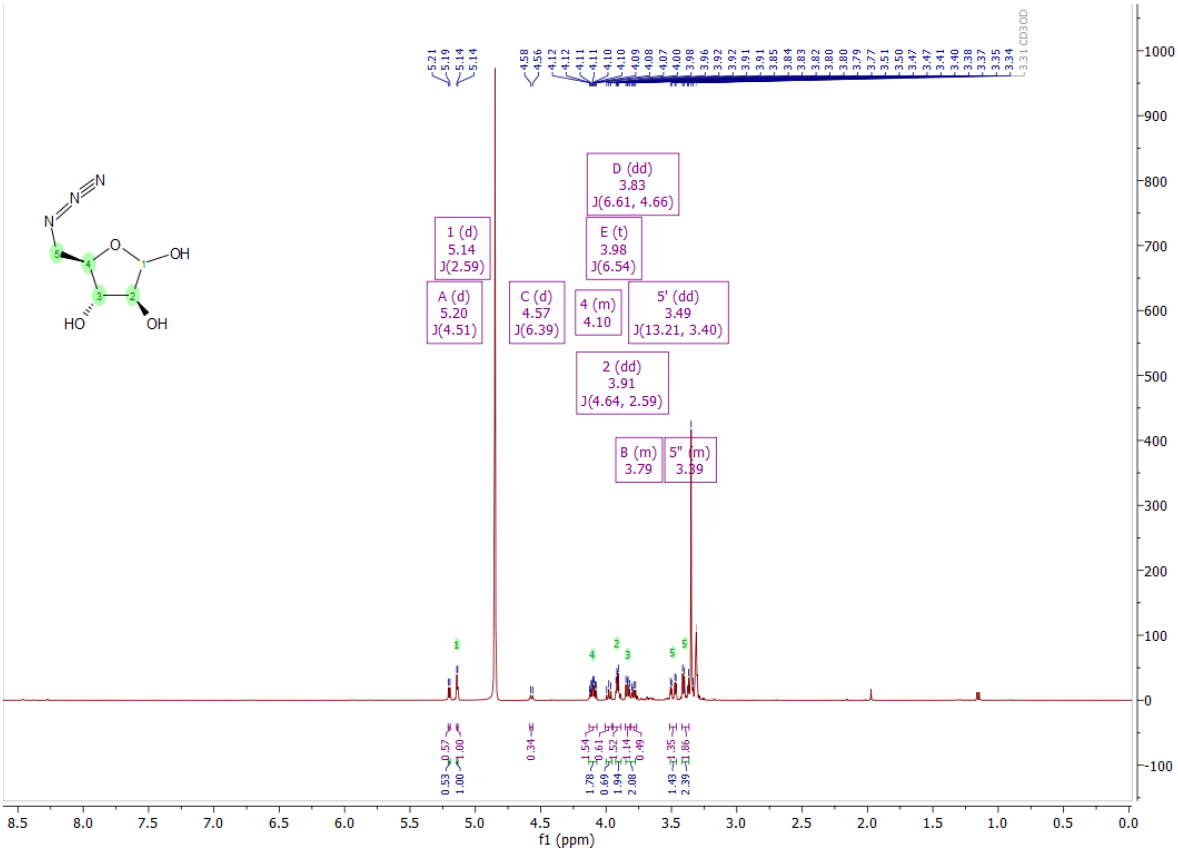

f1 (ppm)

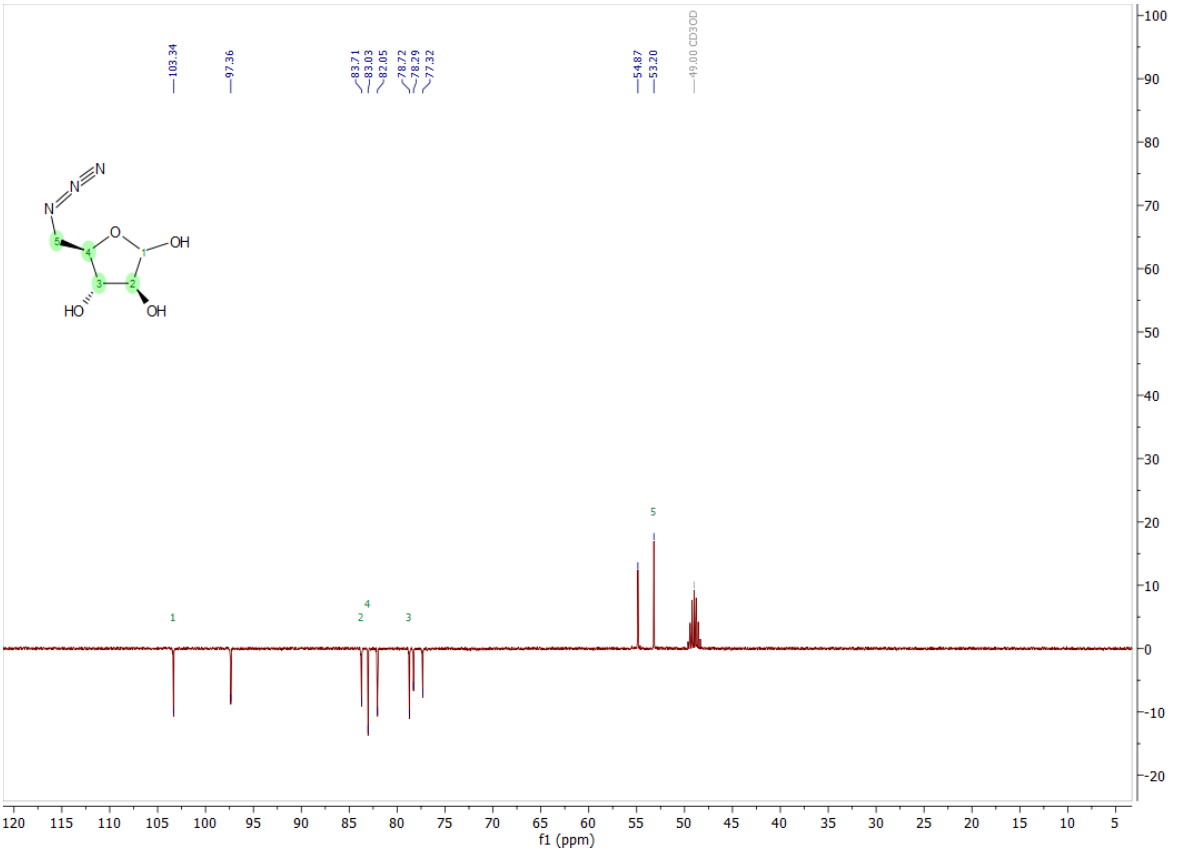
Compound S2:


7.26 CDCl3
4.92
4.17
4.17
4.16
4.16
4.15
4.15
4.05
4.04
3.89
3.89
3.69
3.68
3.66
3.65
3.64
3.62
3.61
3.41
5',5" (t)
3.65
J(3.80)
2 (d)
4.04
J(1.17)
1 (s)
4.92
4 (td)
4.16
J(3.84, 2.64)
3 (d)
3.89
J(2.58)
1.00
1.00
0.03
1.08
1.13
1.00
1.02
1.00
1.26
2.39
2.38
3.06
f1 (ppm)

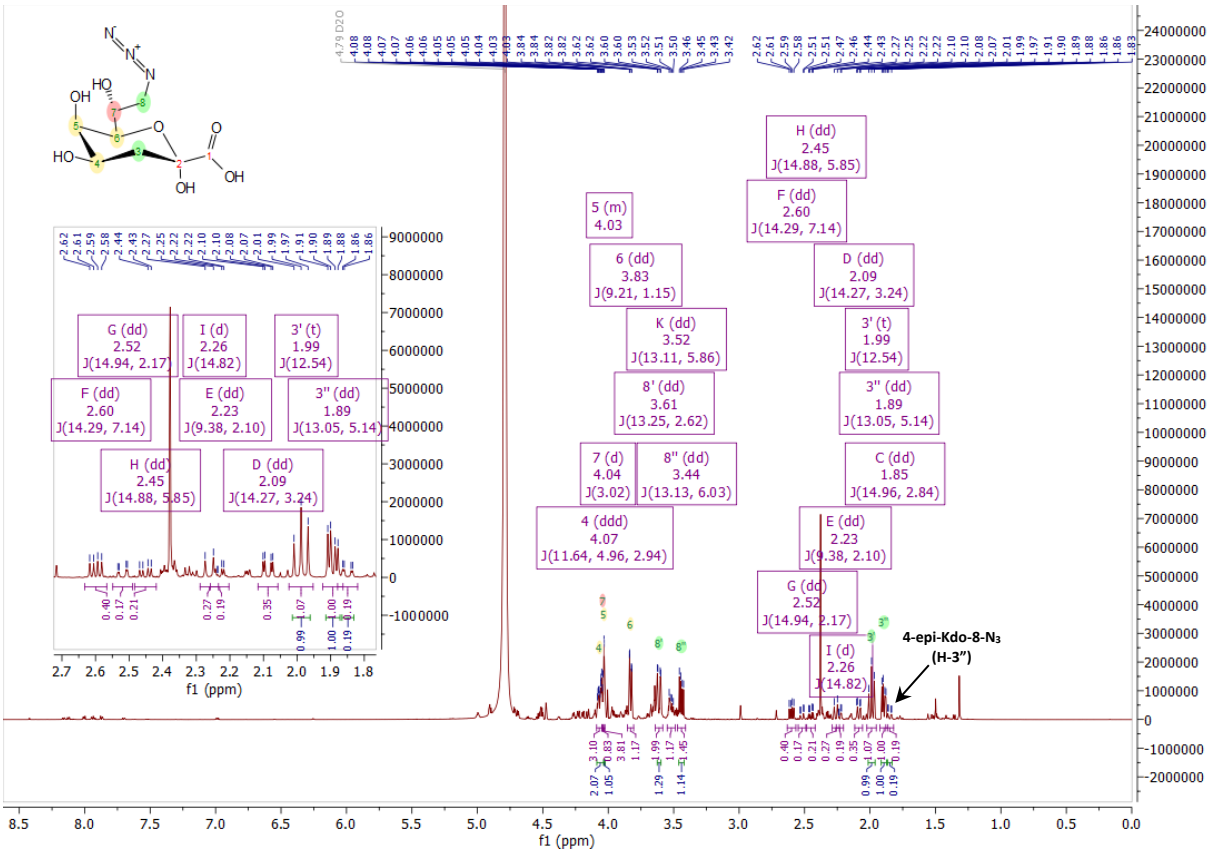

109.31
84.99
79.89
78.79
77.16 CDCl3
55.18
52.80
f1 (ppm)

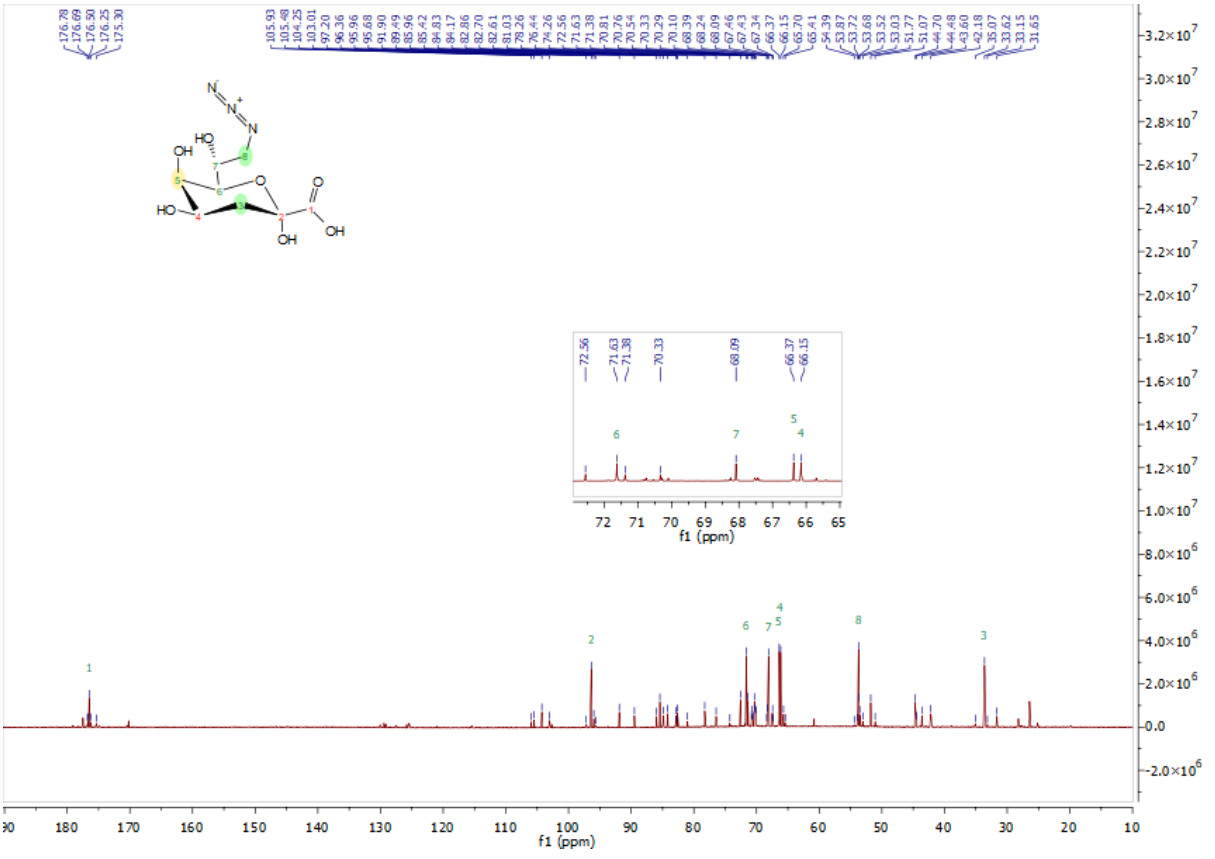
Compound 3:


1 (d)
5.14
J(2.59)
A (d)
5.20
J(4.51)
C (d)
4.57
J(6.39)
D (dd)
3.83
J(6.61, 4.66)
E (t)
3.98
J(6.54)
4 (m)
4.10
5' (dd)
3.49
J(13.21, 3.40)
2 (dd)
3.91
J(4.64, 2.59)
B (m)
3.79
5" (m)
3.39
f1 (ppm)


103.34
97.36
83.71
83.03
82.05
78.72
78.29
77.32
54.87
53.20
49.00 CD3OD
f1 (ppm)

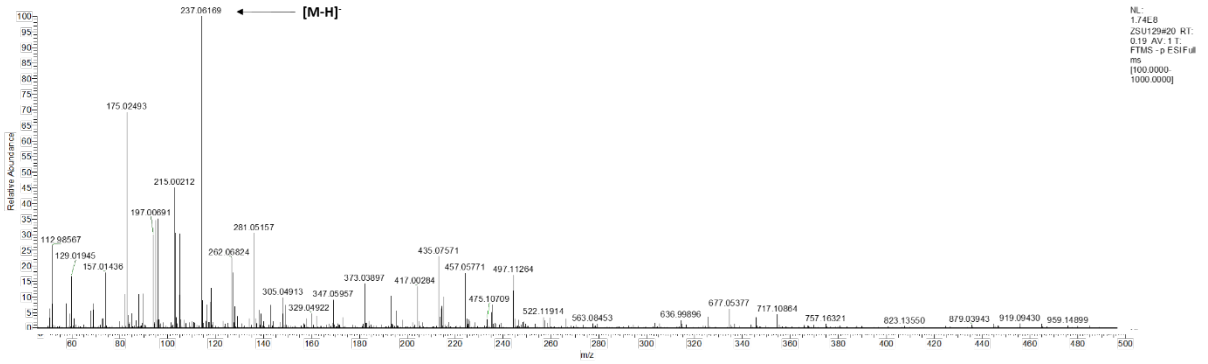
Compound 4:


H (dd)
2.45
J(14.88, 5.85)
F (dd)
2.60
J(14.29, 7.14)
5 (m)
4.03
6 (dd)
3.83
J(9.21, 1.15)
D (dd)
2.09
J(14.27, 3.24)
K (dd)
3.52
J(13.11, 5.86)
3' (t)
1.99
J(12.54)
8' (dd)
3.61
J(13.25, 2.62)
3" (dd)
1.89
J(13.05, 5.14)
7 (d)
4.04
J(3.02)
8" (dd)
3.44
J(13.13, 6.03)
C (dd)
1.85
J(14.96, 2.84)
4 (ddd)
4.07
J(11.64, 4.96, 2.94)
E (dd)
2.23
J(9.38, 2.10)
G (dd)
2.52
J(14.94, 2.17)
I (d)
2.26
J(14.82)
4-epi-Kdo-8-N3
(H-3")
f1 (ppm)

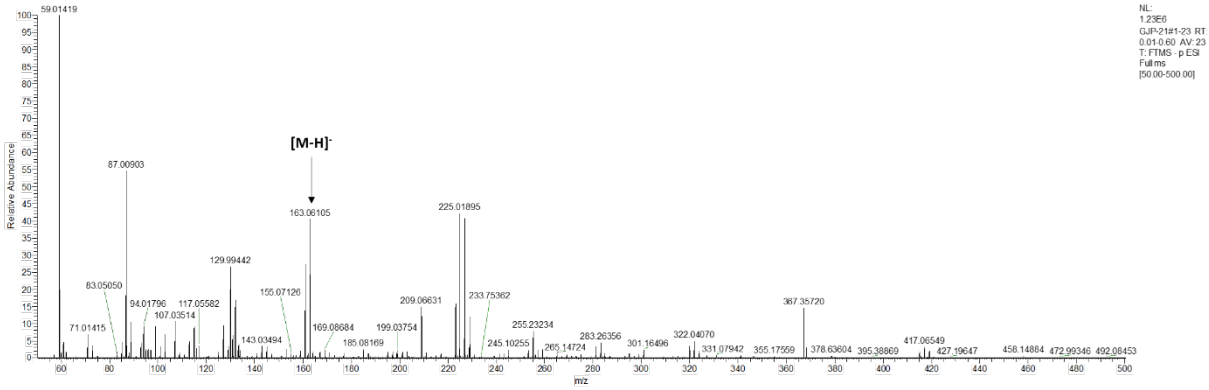

f1 (ppm)

V. HRMS Spectra

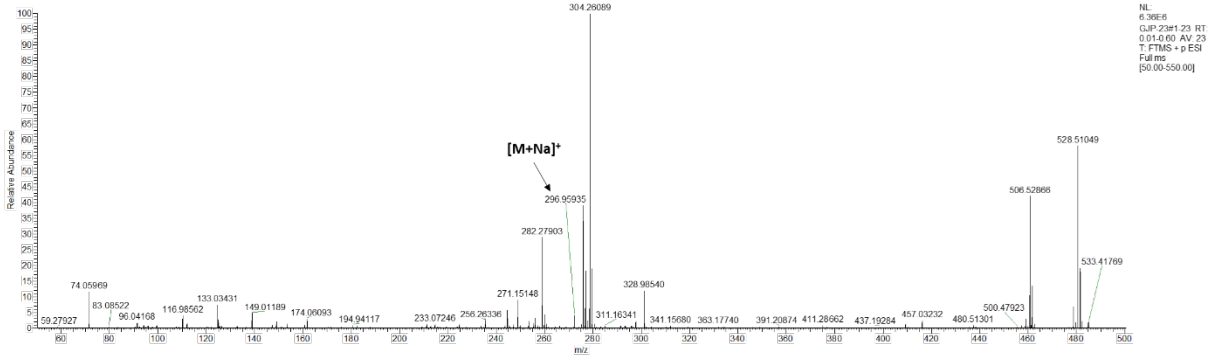
Compound 1:



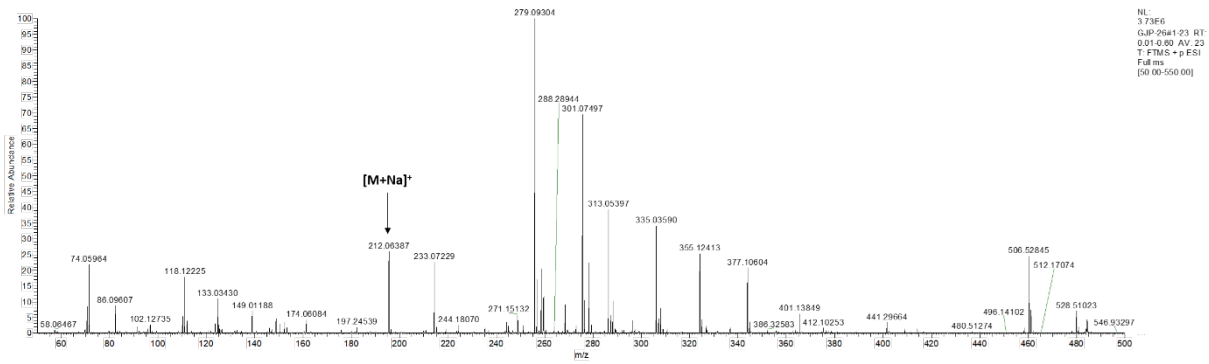
Compound S1:



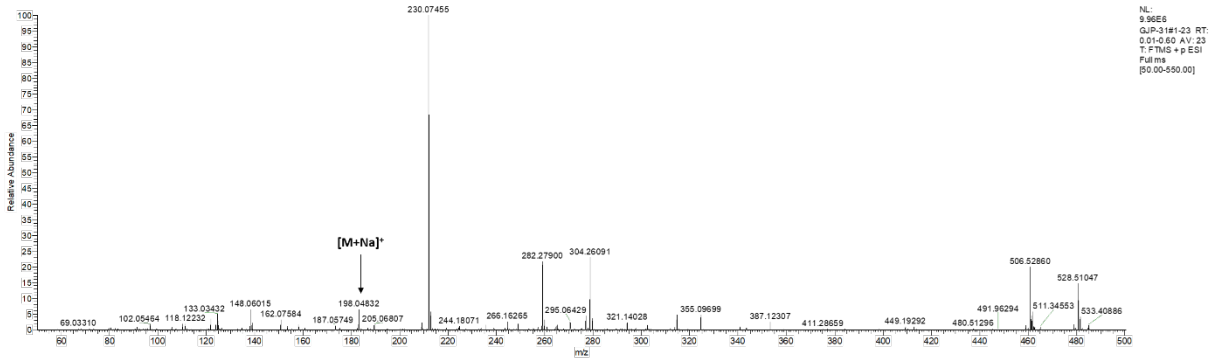
Compound 2:



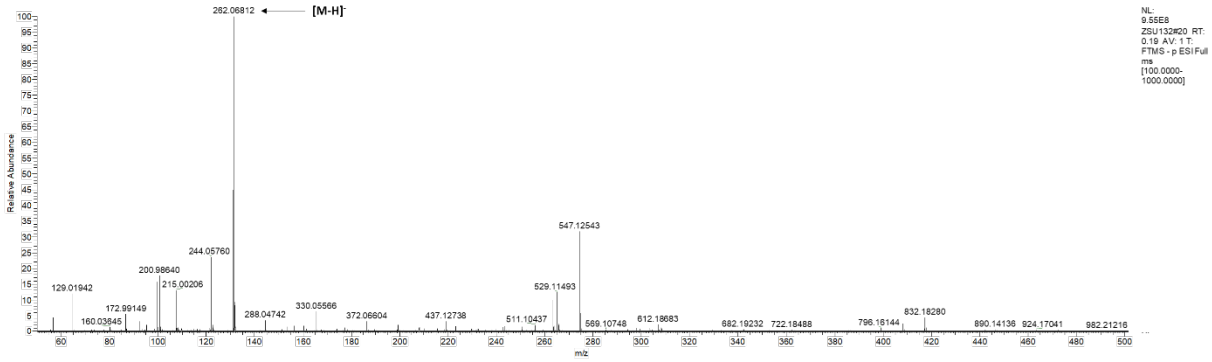
Compound S2:



Compound 3:

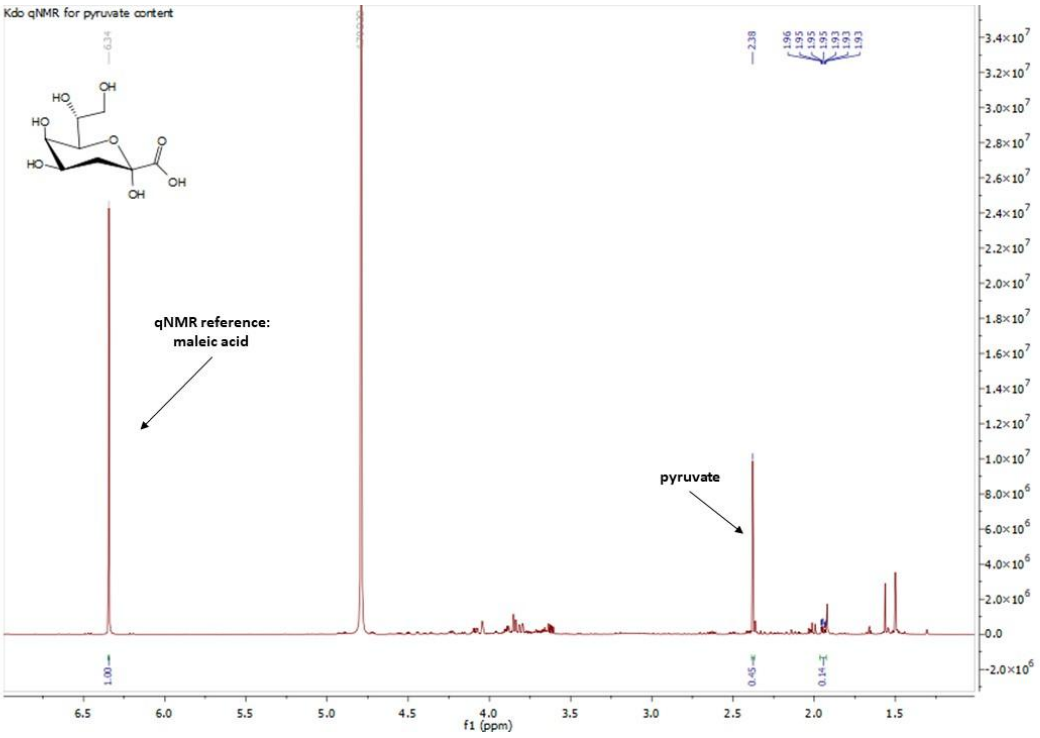


Compound 4:

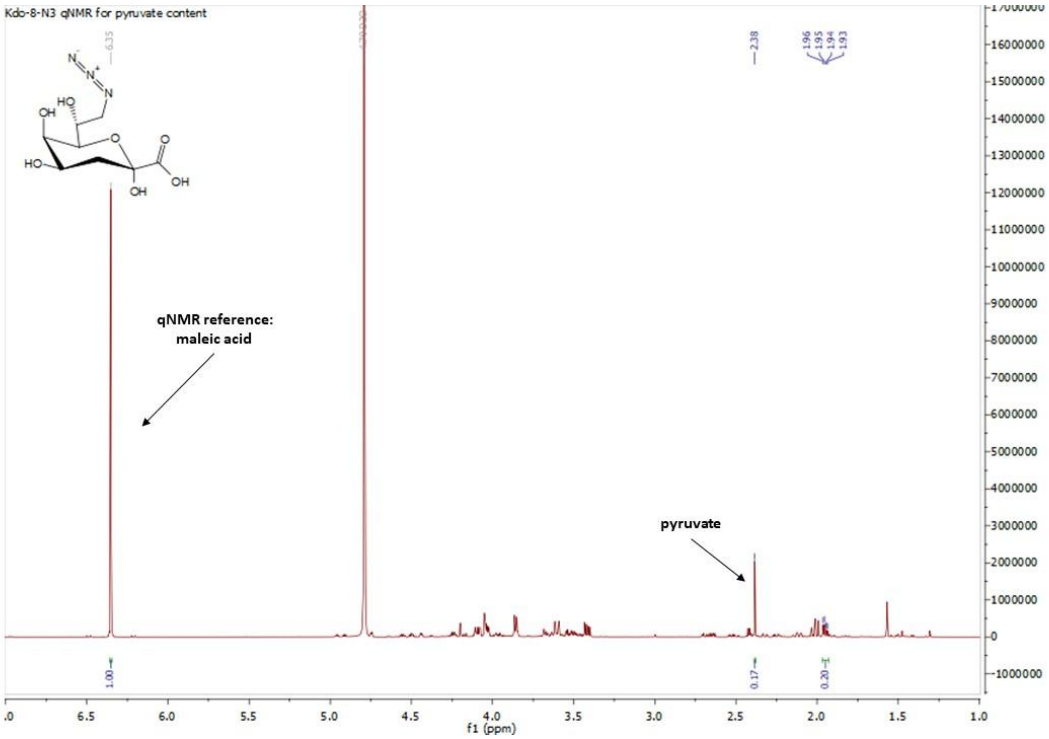


VI. qNMR Spectra

Compound 1:



Compound 4:



VII. References

1 H. Zhu, J. Swierstra, C. Wu, G. Girard, Y. H. Choi, W. Van Wamel, S. K. Sandiford and G. P. van Wezel, *Microbiology*, 2014, **160**, 1714–1726.

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3 C. A. Schneider, W. S. Rasband and K. W. Eliceiri, *Nat. Methods*, 2012, **9**, 671–675.

4 C.-M. Tsai and C. E. Frasch, *Anal. Biochem.*, 1982, **119**, 115–119.