

Supplementary Information

for

Improved one-pot multienzyme (OPME) systems for synthesizing UDP-uronic acids and glucuronides

Table of Contents

Materials for enzyme cloning, expression, purification, and activity assays.....	S2
Cloning, expression, and purification of <i>Arabidopsis thaliana</i> glucuronokinase (AtGlcAK).....	S2–S3
pH profile of AtGlcAK.....	S3–S4
EDTA and metal cation effects on the activity of AtGlcAK.....	S4
Substrate specificity of AtGlcAK.....	S5
Kinetics assays of AtGlcAK.....	S5–S6
Cloning, expression, and purification of <i>Arabidopsis thaliana</i> USP-sugar pyrophosphorylase (AtUSP).....	S6–S7
LC-MS analysis of AtUSP activity.....	S7
General methods for compound purification and characterization	S7
Chemical synthesis of D-ManA 3	S7–S8
Chemical synthesis of L-IdoA 4	S8–S9
General methods for enzymatic synthesis and charcoal purification of uronic acid-1-phosphates (5–7).....	S9–S10
One-pot three-enzyme synthesis of UDP-GlcA (9).....	S10
One-pot three-enzyme synthesis of UDP-GalA (10).....	S10–S11
Sequential one-pot multienzyme (OPME) synthesis of heparosan oligosaccharides (14–16).....	S11–S12
References.....	S13
NMR spectra comparison of synthetic and commercial L-IdoA (4).....	S14
NOE H-1 anomers for synthetic L-IdoA (4)	S15
COSY spectra for synthetic L-IdoA (4).....	S16
HSQC spectra for synthetic L-IdoA (4).....	S17
HRMS spectra for synthetic L-IdoA (4).....	S18
¹ H and ¹³ C NMR spectra for compounds 5–7 , 9 , 10 , and 14–16	S19–S26

List of Figures

Figure S1. SDS-PAGE analysis of AtGlcAK expression and purification.....	S3
Figure S2. pH profile of AtGlcAK.....	S4
Figure S3. The effect of EDTA and divalent metal cations on the activity of AtGlcAK.....	S4
Figure S4. Time course of AtGlcAK.....	S4

List of Tables

Table S1. Substrate specificity of AtGlcAK.....	S5
Table S2. Apparent kinetic parameters of AtGlcAK.....	S6
Table S3. Comparisons of the obtained and the reported NMR data for compounds 5–7	S12
Table S4. Comparisons of the obtained and the reported NMR data for 9 and 10	S13

Materials for enzyme cloning, expression, purification, and activity assays

The cDNA library of *Arabidopsis thaliana* was purchased from AMS Biotechnology (Lake Forest, CA, USA). Restriction enzymes including *Nde*I and *Bam*HI were purchased from New England BioLabs (Beverly, MA, USA). Vectors pET15b and pET22b(+) were purchased from Novagen (EMD Biosciences Inc., Madison, WI, USA). Herculase-enhanced DNA polymerase was purchased from Stratagene (La Jolla, CA, USA). T4 DNA ligase and 1 kb DNA ladder were from Promega (Madison, WI, USA). Ni²⁺-nitrilotriacetic acid (NTA) agarose, QIAprep spin miniprep kit and QIAquick gel extraction kit were purchased from Qiagen (Valencia, CA, USA). Bicinchoninic acid (BCA) protein assay kit was from Pierce Biotechnology, Inc. (Rockford, IL, USA). *Escherichia coli* DH5 α electrocompetent cells and BL21 (DE3) chemically competent cells were purchased from Invitrogen (Carlsbad, CA, USA). Glucuronic acid (GlcA), glucose (Glc), galactose (Gal), mannose (Man), xylose (Xyl), arabinose (Ara), ethylenediaminetetraacetic acid (EDTA), adenosine 5'-triphosphate (ATP), magnesium chloride, manganese chloride, calcium chloride, cobalt chloride, copper chloride, zinc chloride, nickel sulfate, and uridine 5'-triphosphate (UTP) were purchased from Sigma Aldrich (Saint Louis, MO, USA).

Cloning, expression, and purification of *Arabidopsis thaliana* glucuronokinase (AtGlcAK)

Full length *Arabidopsis thaliana* Glucuronokinase (EC 2.7.1.43) (AtGlcAK) was cloned as a C-His₆-tagged fusion protein in pET22b(+) vector from an *Arabidopsis thaliana* cDNA library. Primer used were: forward 5' ACGCGTCGACATGGATCCGAATTCCACGG 3' (*Sal*I restriction site is underlined) and reverse 5' CCGCTCGAGTAAGGTCTGAATGTCAGAATCATTC 3' (*Xho*I restriction site is underlined). Polymerase chain reaction (PCR) were performed in a total volume of 50 μ L containing 200 ng of cDNA library, 0.2 μ M of each of forward and reverse primers, 5 μ L of 5 \times Herculase II buffer, 0.2 mM of dNTP mixture, and 5 U of Herculase II DNA polymerase. The reaction was performed at an annealing temperature of 58 $^{\circ}$ C for 32 cycles. The PCR products were digested with *Sal*I and *Xho*I, purified, and ligated with pET22b+ vector predigested with *Sal*I and *Xho*I at 16 $^{\circ}$ C for overnight. The ligated product was transformed into electrocompetent *E. coli* DH5 α cells. Selected clones were grown for minipreps and positive clones were verified by restriction mapping and DNA sequencing performed by Davis Sequencing Facility. The DNA sequence of the insert matched to that reported (GenBank gene accession number: NM_111030).

The plasmid with the correct insert was transformed into *E. coli* BL21 (DE3) chemically competent cells for protein expression. *E. coli* cells harboring the pET22b-*AtGlcAK* plasmid were cultured in LB medium (10 g L⁻¹ tryptone, 5 g L⁻¹ yeast extract, and 10 g L⁻¹ NaCl) containing ampicillin (100 μ g mL⁻¹) at 37 $^{\circ}$ C with vigorous shaking at 250 rpm in a C25KC incubator shaker (New Brunswick Scientific, Edison, NJ) until the OD_{600 nm} of the culture reached 0.8–1.0. Overexpression of the targeted protein was achieved by adding 0.15 mM of isopropyl-1-thio- β -D-galactopyranoside (IPTG) for induction followed by incubation at 18 $^{\circ}$ C for 20 hr with vigorous shaking at 250 rpm.

His₆-tagged protein was purified from cell lysate using Ni²⁺-NTA affinity column. To obtain cell lysate, cells were harvested by centrifugation at 4,000 rpm (Sorvall) at 4 $^{\circ}$ C for 2 h. The cell pellet was resuspended in lysis buffer (pH 8.0, 100 mM Tris-HCl containing 0.1% Triton X-100). Lysozyme (100 μ g mL⁻¹) and DNase I (5 μ g mL⁻¹) were added to the cell suspension. The mixture was incubated at 37 $^{\circ}$ C for 1 h with vigorous shaking (200 rpm). Cell lysate was obtained as the supernatant by centrifugation at 12,000 rpm (Sorvall) at 4 $^{\circ}$ C for 20 min. Purification is performed by loading the supernatant onto a nickel-nitrilotriacetic acid (Ni²⁺-NTA)

column pre-equilibrated with 10 column volumes of binding buffer (5 mM imidazole, 0.5 M NaCl, 50 mM Tris-HCl, pH 7.5). The column was wash with 10 column volumes of binding buffer and 10 column volumes of washing buffer (40 mM imidazole, 0.5 M NaCl, 50 mM Tris-HCl, pH 7.5). Protein of interest was eluted with Tris-HCl (pH 7.5, 50 mM) containing imidazole (200 mM) and NaCl (0.5 M). The fractions containing the purified enzyme were collected and dialyzed against Tris-HCl buffer (pH 7.5, 20 mM) containing 10% glycerol and 0.25 M NaCl. Dialyzed proteins were stored at 4 °C. Alternatively, fractions containing purified enzyme were dialyzed against Tris-HCl buffer (pH 7.5, 20 mM) and freeze dried.

As shown in **Figure S1** by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis, the molecular weight of the obtained recombinant protein was around 42 kDa, matched well to the calculated molecular mass of 42.2 kDa. One-step Ni²⁺-NTA column purification was sufficient to provide fractions with good purity. The fractions containing the purified enzyme were collected and dialyzed against Tris-HCl buffer (pH 7.5, 20 mM) containing 10% glycerol. Additional glycerol was added to the dialyzed protein samples to a final concentration of 30% glycerol and the samples were stored at -20 °C. Alternatively, fractions containing purified enzyme were dialyzed against Tris-HCl buffer (pH 7.5, 20 mM) and lyophilized. By average, 65 mg of purified protein was obtained from 1 liter of cell culture.

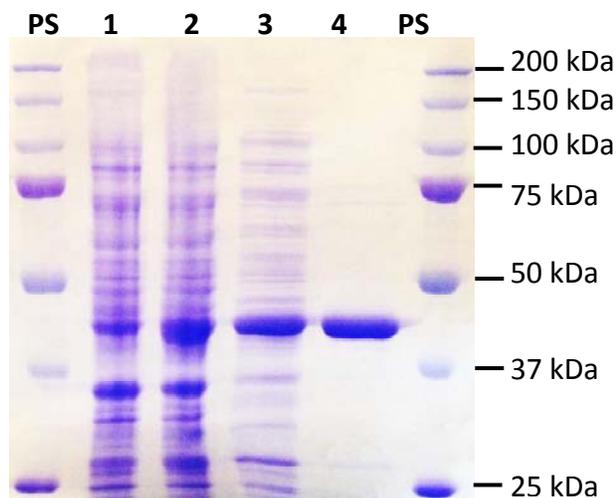


Figure S1. SDS-PAGE analysis of AtGlcAK expression and purification. Lanes: PS, Protein standard; 1, whole cell before induction; 2, whole cell after induction; 3, cell lysate; 4, purified AtGlcAK.

pH profile of AtGlcAK

Typical enzymatic assays for pH profile studies were performed in a total volume of 20 μ L containing GlcA (10 mM), ATP (10 mM), MgCl₂ (20 mM) and AtGlcAK (412 ng) in various buffers (200 mM) with pH varying from 5.0 to 10.0 (MES buffer for pH 5.0 and 6.0; MOPS buffer for pH 7.0–8.0; and CAPS buffer for pH 9.0 and 10.0). All reactions were allowed to proceed for 15 min at 37 °C. The reaction mixture was quenched by boiling in water bath for 1 min followed by adding 20 μ L of ice cold 95% (v/v) ethanol. The samples were then centrifuged at 13,000 rpm for 2 min, and kept on ice until analyzed by a Beckman Coulter P/ACE MDQ Capillary Electrophoresis (CE) system equipped with a UV detector. A 50 cm capillary tubing (75 μ m I.D., Beckman Coulter) was used. Assays were run at 25 kV with sodium borate buffer (25 mM, pH 9.8) for 22 min. Percent conversions were calculated from the ratios of ATP and ADP, which were determined by UV absorbance at 254 nm. All assays were carried out in duplicate, and standard deviations were used to represent errors.

As shown in **Figures S2**, AtGlcAK has a relatively narrow active pH range with an optimal pH of 7.5. This is in agreement with a previous report.¹ The activity dropped fast below 7.0 or above 8.0. No activity was detected at pH value below 5.0 or above 10.0.

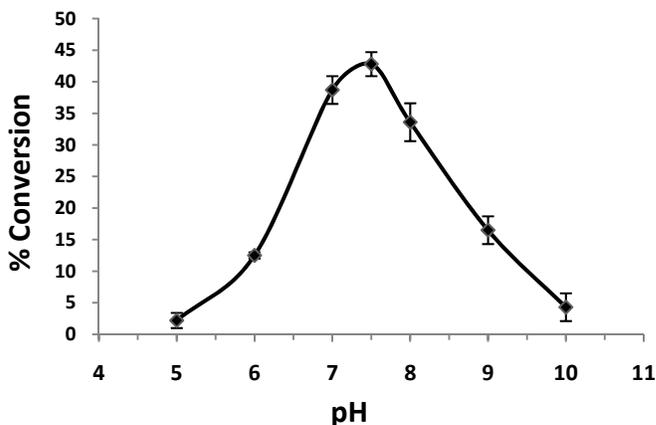


Figure S2. pH profile of AtGlcAK.

Ethylenediaminetetraacetic acid (EDTA) and metal effects on the activity of AtGlcAK

EDTA (5 mM), 20 mM of various divalent metal salts (CaCl₂, CoCl₂, CuSO₄, MnCl₂, ZnCl₂) were mixed in a MOPS buffer (pH 7.5, 100 mM) to analyze their effects on the kinase activity of AtGlcAK (412 ng) in 20 μ L total volume containing 10 mM of ATP and GlcA. Reaction without EDTA or metal ions was used as a control. Reactions were quenched and assayed using the same method as those for pH profile. All reactions were performed in duplicate, and standard deviations were used to represent errors.

As shown in **Figure S3**, a divalent metal cation is required for the activity of AtGlcAK. In the presence of EDTA, AtGlcAK showed no kinase activity towards GlcA. Among divalent metal cations tested, Mg²⁺ was shown to be the best for the activity of AtGlcAK. Medium activities were obtained in the presence of Mn²⁺, Ca²⁺, or Co²⁺. However, Cu²⁺ and Zn²⁺ were not effective in assisting the AtGlcAK activity.

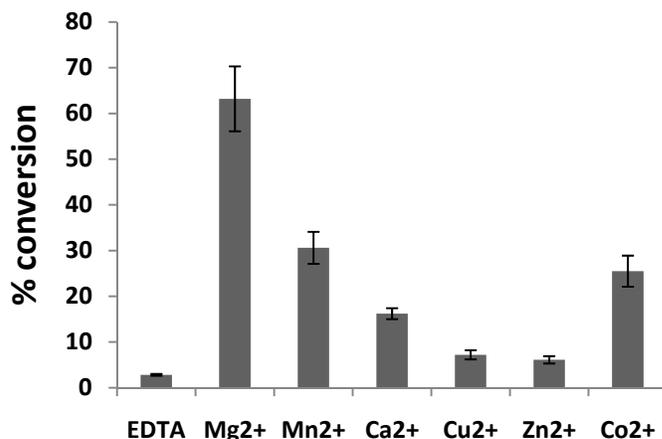


Figure S3. The effect of EDTA and divalent metal cations on the activity of AtGlcAK.

Substrate specificity of AtGlcAK

Substrate specificity assays were performed under two conditions: low enzyme concentration assays are performed in 20 μL reaction mixture containing ATP (10 mM), sugar substrate (10 mM), MgCl_2 (20 mM), MOPS (100 mM, pH 7.5) and AtGlcAK (412 ng) at 37 $^\circ\text{C}$ for 15 min, while high enzyme concentration assays are performed in 20 μL reaction mixture containing 10 mM ATP, sugar substrate (10 mM), MgCl_2 (20 mM), MOPS (100 mM, pH 7.5) and AtGlcAK (2.1 μg) at 37 $^\circ\text{C}$ for 60 min. The reactions were quenched and assayed with the same CE method as those for pH profile. All reactions were performed in duplicates.

The product formation was also monitored by LC-MS using a LC-2010A_{XL} High Performance Liquid Chromatography (HPLC) system linked with a LCMS-2020 mass spectrometer (Shimadzu Scientific instrument Inc., Columbia, MD). Liquid nitrogen was used as nitrogen gas source. A Shimadzu C18 column (5 μm particle size, 4.6 mm \times 50 mm) was used to clean up the reaction. Mobile phase consists of 30% acetonitrile (ACN) in water. The flow rate was set at 0.8 mL min^{-1} . Each run was set for 6 minutes and mass spectrometer was set to scan the range from 190 to 600 Da per second.

As shown in **Table S1**, it is very obvious that the carboxylic acid at C6 of the sugar substrate is critical for binding to the AtGlcAK. Only those acidic monosaccharides can be phosphorylated by AtGlcAK with detectable yields.

Table S1. Substrate specificity of AtGlcAK

Substrate	Percentage conversion of ATP (%)	
	Low Enzyme Conc.	High Enzyme Conc.
GlcA	61.5	N.A.
GalA	6.2	15.3
IdoA	5.8	11.7
Glc	N.D.	< 5
Gal	N.D.	N.D.
Man	N.D.	N.D.
Xyl	N.D.	<5
Ara	N.D.	N.D.

N.A., Not Assayed; N.D., Not Detected.

Kinetics assays of AtGlcAK

Kinetic parameters of GlcA were assayed in duplicate in reaction mixture of 20 μL containing 100 mM MES buffer (pH 7.5), MgCl_2 (20 mM), ATP (10 mM), different concentration of GlcA (0.5, 1.0, 2.0, 5.0, 10.0, and 20.0 mM), and AtGlcAK (206 ng). All reactions were allowed to proceed at 37 $^\circ\text{C}$ for 15 min. Reactions with no GlcA was used as negative controls. The apparent kinetic parameters were obtained by fitting the experimental data (average values of duplicate assays) into Michaelis-Menten equation using Grafit 5.0.

All assays were performed in a Beckman Coulter P/ACETM MDQ Capillary Electrophoresis System (Fullerton, CA, USA) equipped with a UV detector. Reactions were stopped by adding 20 μL of ice-cold ethanol, centrifuged at 13,000 rpm for 2 min, and kept on ice until aliquots of 15 μL were transferred in to micro sample vial and subjected to CE analysis. ATP samples with higher concentrations are further diluted before injection. An eCAPTM Capillary Tubing (50 cm effective length, 75 μm I.D., 375 μm O.D.) from Beckman Coulter was used to separate ATP

and ADP in a sodium borate buffer (25 mM, pH 9.8) under 25 kV voltages. Sample injections were achieved by pressurizing sample vial to 0.5 psi for 6 sec. Separations were achieved within 22 min and percent conversions were calculated from the ratios of sugar nucleotides and nucleotide triphosphates, which were determined by UV absorbance at 254 nm.

Enzyme amount was adjusted so that with 10 mM of ATP and GlcA, at 15 min the reaction rate is still in its linear range and the ATP conversion is relatively high. The reaction rate started to slow down after 20 min (**Figure S4**). The activity of AtGlcAK towards GalA and IdoA was too low to obtain kinetic data. Therefore, only the parameters for ATP and GlcA were obtained (**Table S2**). These results differ slightly from those reported previously for GlcA ($k_{cat}/K_m = 11.9 \text{ mM}^{-1} \text{ s}^{-1}$) and ATP ($k_{cat}/K_m = 15.3 \text{ mM}^{-1} \text{ s}^{-1}$),² which may be due to the difference in the experimental methods and conditions used.

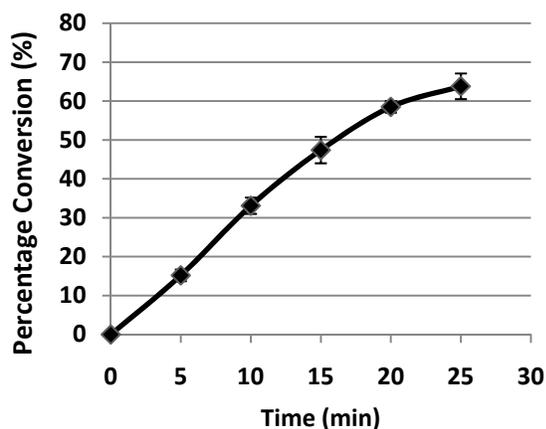


Figure S4. Time course of AtGlcAK.

Table S2. Apparent kinetic parameters of AtGlcAK.

	$k_{cat} (\text{s}^{-1})$	$K_m (\text{mM})$	$k_{cat}/K_m (\text{mM}^{-1} \text{ s}^{-1})$
ATP	26.8	1.1	24.4
GlcA	25.4	1.3	19.5

Cloning, expression, and purification of *Arabidopsis thaliana* UDP-sugar pyrophosphorylase (AtUSP).

Full length *Arabidopsis thaliana* UDP-sugar pyrophosphorylase (EC 2.7.7.64) (AtUSP)³ was cloned from the *Arabidopsis thaliana* cDNA library in pET15b vector as an N-His₆-tagged fusion protein. Primers used were: forward: CCGCTCGAGATGGCTTCTACGGTTGATTC (*Xho*I restriction site is underlined) and reverse: CGGGATCCTCAATCTTCAACAGAAAATTTGC (*Bam*HI restriction site is underlined). It was also cloned in pET22b(+) vector as a C-His₆-tagged fusion protein. Primers used were: forward: CGGGATCCGATGGCTTCTACGGTTGATTC (*Bam*HI restriction site is underlined) and reverse: CCCAAGCTTATCTTCAACAGAAAATTTGCCAG (*Hind*III restriction site is underlined). Positive clones were verified by restriction mapping and DNA sequencing performed by Davis Sequencing Facility. The DNA sequence of the insert matched to that reported for AtUSP (GenBank accession number: NM_124635.3). Proteins were overexpressed in *Escherichia coli* BL21 (DE3) using Luria-Bertani (LB) media similar to that described above for AtGlcAK. The expression level of N-His₆-tagged AtUSP was better compared to the C-His₆-

tagged one. Therefore, only the N-His₆-tagged one was expressed, purified, and used for synthesis. The N-His₆-tagged recombinant protein was purified from cell lysate using Ni²⁺-NTA affinity column similar to that described above for AtGlcAK. According to SDS-PAGE analysis, the recombinant protein of around 68 kDa (calculated molecular mass 68.7 kDa) can be obtained effectively in high purity. The fractions containing the purified enzyme were collected and dialyzed against Tris-HCl buffer (pH 7.5, 20 mM) containing 10% glycerol. Dialyzed proteins were stored at -20 °C. Alternatively, fractions containing purified enzyme were dialyzed against Tris-HCl buffer (pH 7.5, 20 mM) and lyophilized. On average, 87 mg of purified protein was obtained from 1 liter of cell culture.

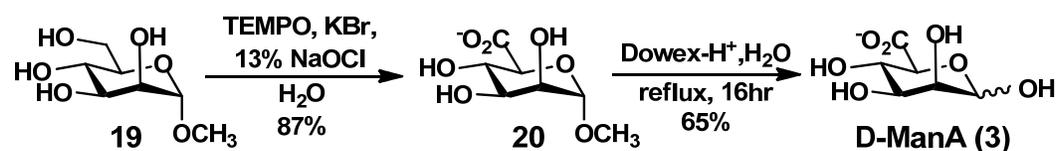
LC-MS analysis of AtUSP activity

One-pot three-enzyme reaction system was set up to detect AtUSP activity. The reaction system contained Tris-HCl (100 mM, pH 7.5), MgCl₂ (20 mM), D-GlcA (5 mM), ATP (6 mM), UTP (6.5 mM), AtGlcAK (0.4 mg mL⁻¹), AtUSP (0.4 mg mL⁻¹) and PmPpA (0.3 mg mL⁻¹). Reaction total volume is 15 μL. The peak of 579.15 represents [M - H]⁻ of UDP-GlcA. The reaction did go to completion with a 1 : 1.2 : 1.3 ratio of GlcA:ATP:UTP.

General methods for compound purification and characterization

Chemicals were purchased and used without further purification. ¹H NMR and ¹³C NMR spectra were recorded on a Varian Mercury 600 or a Bruker 800 Avance III NMR spectrometer. High resolution electrospray ionization (ESI) mass spectra were obtained in negative mode using Thermo Electron LTQ-Orbitrap mass spectrometer. Silica gel 60 Å (Sorbent Technologies) was used for flash column chromatography. Thin-layer chromatography (TLC) was performed on silica gel plates 60 GF254 (Sorbent Technologies) using anisaldehyde sugar stain for detection. Gel filtration chromatography was performed with a column (100 cm × 2.5 cm) packed with BioGel P-2 Fine resins (Bio-Rad). GlcAβProN₃ **13**⁴ was synthesized as described previously. NanK_ATCC55813,⁵ PmGlmU,⁶ BLUSP,⁷ BiGalK,⁸ and PmPpA⁶ were overexpressed as reported.

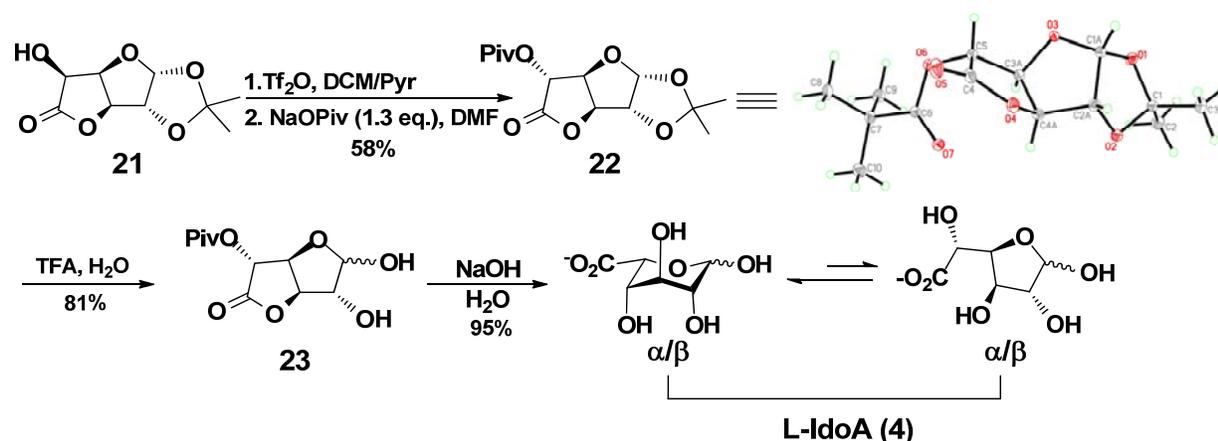
Synthesis of D-mannuronic acid (D-ManA, **3**)



Compound **19** (500 mg, 2.57 mmol), TEMPO (4 mg, 0.026 mmol), and potassium bromide (153 mg, 1.28 mmol) were dissolved in water (7 mL). The reaction mixture was then cooled in ice bath followed by an addition of pre-chilled solution pH = 10 aqueous sodium hypochlorite (12–15%, 8.6 mL). The reaction was allowed to react for 1.5 hr and pH (pH = 10) was maintained by adding 2 M of sodium hydroxide solution. Once the reaction was completed as indicated by TLC, the reaction was neutralized by adding hydrochloric acid (4 M). The reaction mixture was concentrated and passed through a silica gel column (EtOAc:MeOH:H₂O = 6:2:1) and then a Bio-Gel P2 column to desalt to afford compound **20** (467 mg, 87%). ¹H NMR (600 MHz, D₂O) δ = 4.82 (d, *J* = 1.6, 1H), 3.95–3.90 (m, 1H), 3.89 (d, *J* = 8.7, 1H), 3.85–3.74 (m, 2H), 3.43 (s, 3H). ¹³C NMR (151 MHz, D₂O) δ = 176.68, 100.83, 72.78, 70.29, 69.69, 68.71, 54.90.

Compound **20** (455 mg, 2.2 mmol) was dissolved in water (15 mL) and Dowex-H resin (2 g) was added. The reaction mixture was refluxed for 16 hr and was neutralized by adding 2 M of sodium hydroxide. The resin was filtered out. The solution was concentrated and passed through silica gel column (EtOAc:MeOH:H₂O = 6:3:1.5) to afford compound **3** (277 mg, 65%) ¹H NMR (600 MHz, D₂O) δ = 5.22 (d, *J*=2.0, 0.65H), 4.91 (d, *J* = 1.0, 0.35H), 4.08 (d, *J* = 9.2, 0.65H), 3.93 (d, *J* = 3.2, 0.35H), 3.92–3.63 (m, 3H). ¹³C NMR (151 MHz, D₂O) δ = 176.94, 176.20, 93.86, 93.55, 76.30, 72.86, 72.82, 71.05, 70.37, 70.04, 68.93, 68.51. HRMS (ESI) *m/z* calcd for C₆H₁₀O₇ (M-H) 193.0354, found 193.0356.

Synthesis of L-Iduronic acid (L-IdoA, **4**)



Compound **21**⁹ (5.178 g, 23.95 mmol) was dissolved in dichloromethane (40 mL) and pyridine (23.95 mmol, 1.9 mL). The reaction mixture cooled in dry ice acetone bath and trifluoromethanesulfonic anhydride (31.14 mmol, 5.20 mL) was added dropwisely. The temperature was maintained for 1 hr between -35 °C to -25 °C and then left overnight. The reaction mixture was washed 2 M of HCl (3 × 100 mL), saturated sodium bicarbonate (1 × 100 mL), and brine (1 × 100 mL). The organic layer was collected, and dried with magnesium sulfate, filtered over celite, and concentrated until dryness. The crude product was dissolved in DMF (20 mL) and the reaction mixture was cooled -15 °C in sodium chloride ice bath. Sodium pivalate (31.14 mmol, 3.865 g) was added to reaction mixture and allowed to react overnight. The solvent was then concentrated from the reaction mixture and passed through silica gel column (Hex: EtOAc = 4:1) to afford compound **22** (4.2 g, 58.2%). Flaky white crystals formed spontaneously in the collected fractions upon solvent evaporation in the fume hood. ¹H NMR (600 MHz, CDCl₃) δ = 5.95 (d, *J* = 3.7, 1H), 5.10 (d, *J* = 3.9, 1H), 4.89–4.78 (m, 2H), 4.73 (d, *J* = 3.9, 1H), 1.48 (s, 3H), 1.34 (s, 3H), 1.22 (s, 9H). ¹³C NMR (151 MHz, CDCl₃) δ = 177.47, 170.93, 113.24, 106.27, 85.38, 82.34, 81.91, 73.26, 38.76, 27.28, 27.01, 26.68.

Compound **22** (4.19 g, 14 mmol) was dissolved in trifluoroacetic acid:water (13.5 mL : 1.5 mL) solution. The reaction mixture was allowed to react until completion and then extracted with EtOAc: Brine (150:100 mL). The organic layer was collected and dried with magnesium sulfate and filtrated over celite. The organics were concentrated and passed through silica gel column (Hex: EtOAc = 1:1–0:1) to afford compound **23** (2.94 g, 81%). ¹H NMR (600 MHz, DMSO) δ = 5.20 (br, 0.9H), 5.13 (d, *J* = 2.9, 0.2H), 4.95 (d, *J* = 1.0, 0.6H), 4.93–4.80 (m, 1.8H), 4.71 (dd, *J*

= 6.8, 2.8, 0.2H), 4.07 (t, J = 4.4, 0.2H), 4.04 (br, 0.80H), 3.38 (br, O-H), 1.18 (s, 9H). ^{13}C NMR (151 MHz, DMSO) δ = 176.96, 171.97, 102.76, 86.00, 81.39, 76.57, 75.56, 38.06, 26.56.

Compound **23** (2.064 g, 8 mmol) was dissolved in 1 M sodium hydroxide (8 mL) at room temperature. In addition (4 mL) of 2 M of sodium hydroxide were added to raise the pH to ~10.5 and the reaction was left for two hours in which TLC analysis indicated quantitative yield. The reaction mixture was neutralized with DOWEX-H resin and the resin was filtered out. The solution was concentrated and passed through silica gel column (EtOAc: MeOH: H₂O = 6:3:1.5) to afford compound **4** (1.47 g, 95%). NMR analysis indicates that the compound exists as a mixture of anomers of both pyranose and furanose in D₂O. α -anomer pyranose: ^1H NMR (600 MHz, D₂O) δ = 4.94 (d, J = 6.3, 1H), 4.42 (d, J = 5.0, 1H), 3.78 (dd, J = 8.0, 5.0, 1H), 3.62 (t, J = 8.0, 1H), 3.78 (dd, J = 8.0, 6.3, 1H). ^{13}C NMR (151 MHz, D₂O) δ = 176.33–176.13 (COO⁻), 93.90, 72.51, 72.50, 71.20, 70.71. β -anomer pyranose: ^1H NMR (600 MHz, D₂O) δ = 5.05 (d, J = 1.5, 1H), 4.33 (d, J = 1.9, 1H), 4.11 (m, 1H), 3.91 (m, 1H), 3.67–3.63 (m, 1H). ^{13}C NMR (151 MHz, D₂O) δ = 176.33–176.13 (COO⁻), 92.11, 74.92, 69.81, 69.38, 69.1.1 HRMS (ESI) m/z calcd for C₆H₁₀O₇(M-H) 193.0354, found 193.0354.

General methods for enzymatic synthesis of uronic acid-1-phosphates (5–7).

Uronic acid (30–65 mg), ATP (1.5 eq.) were dissolved in water in a 15 mL centrifuge tube containing MOPS buffer (100 mM, pH 7.0) and MgCl₂ (10 mM). After the addition of appropriate amount of AtGlcAK (1–1.5 mg) per uronic acid substrate except for D-GalA in which AtGlcAK (15–20 mg) was used. water was then added to bring the total volume to 10 mL. The reaction was carried out by incubating the solution in an isotherm incubator for 12 to 36 hr at 37 °C with gentle shaking. Product formation was monitored by TLC (*n*-propanol:H₂O:NH₄OH = 7:4:2 by volume) with *p*-anisaldehyde sugar staining. The reaction was stopped by adding the same volume of ice-cold ethanol and incubating at 4 °C for 30 min and then centrifuged. The supernatant was collected and concentrated and passed through a BioGel P-2 gel filtration column followed by charcoal purification to obtain pure product. α -D-Galacturonopyranosyl-1-phosphate (GalA-1-P) was also synthesized using *Bifidobacterium infantis* galactokinase (BiGalK) according to the procedure previously reported.⁸

Charcoal purification of sugar-1-phosphates.

To a 50 mL centrifuge tube, 2 g of charcoal were added. Ethanol (absolute ethanol or 90% + ethanol) 30 mL was added to the tube, and it was mixed thoroughly by inverting the tube. The tube was then centrifuged at 12,000 rpm for 30 min and the supernatant was decanted. The process was repeated in which the ethanol was added to wash the charcoal, centrifuged, and decanted. Water 30 ml was added to tube and it was mixed thoroughly by inverting the tube followed by centrifugation for 30 min and supernatant was decanted. The tube was left at room temperature for 30 min to evaporate any residual of ethanol. Crude sugar-1-phosphate (10–100 mg) was added to tube with 30 ml of water and the tube was mixed thoroughly. The tube was then put into shaker to shake for 1–2 hr at 37 °C. The tube was centrifuged 12,000 rpm for 30 min to spin down the charcoal and the supernatant was collected and passed through filter to remove any charcoal particles. The filtered solution was then lyophilized to give pure white solids sugar-1-phosphates.

α -D-Glucuronopyranosyl-1-phosphate (GlcA-1-P, 5). 80 mg. Yield, quantitative; white solid.

^1H NMR (600 MHz, D_2O) δ = 5.46 (dd, J = 7.4, 3.5, 1H), 4.11 (d, J = 10.2, 1H), 3.75 (t, J = 9.5, 1H), 3.54 (ddd, J = 9.8, 3.4, 2.0, 1H), 3.49 (t, J = 9.7, 1H). ^{13}C NMR (151 MHz, D_2O) δ = 176.79, 93.96, 72.57, 72.42, 71.70, 71.47. HRMS (ESI) m/z calcd for $\text{C}_6\text{H}_{11}\text{O}_{10}\text{P}$ (M-H) 273.0017, found 273.0016.

α -D-Galacturonopyranosyl-1-phosphate (GalA-1-P, 6). 20 mg, yield, 31% (using AtGlcAK)

and 117 mg, yield: 92% (using BiGalK); white solid. ^1H NMR (600 MHz, D_2O) δ 5.55 (dd, J = 7.0, 3.7, 1H), 4.46 (d, J = 1.4, 1H), 4.31 (dd, J = 3.4, 1.4, 1H), 3.96 (dd, J = 10.3, 3.4, 1H), 3.82 (ddd, J = 10.3, 3.6, 2.4, 1H). ^{13}C NMR (151 MHz, D_2O) δ 175.68, 94.51, 72.33, 70.67, 69.36, 68.15. HRMS (ESI) m/z calcd for $\text{C}_6\text{H}_{11}\text{O}_{10}\text{P}$ (M-H) 273.0017, found 273.0016.

α -D-Mannuronopyranosyl-1-phosphate (ManA-1-P, 7). 65 mg. Yield, 95%; white solid

^1H NMR (600 MHz, D_2O) δ 5.37 (dd, J = 8.6, 1.4, 1H), 4.11 (d, J = 10.1, 1H), 3.97–3.94 (m, 2H), 3.78 (dd, J = 10.5, 8.6, 1H). ^{13}C NMR (151 MHz, D_2O) δ = 177.26, 95.06, 72.82, 70.77, 69.84, 68.82. HRMS (ESI) m/z calcd for $\text{C}_6\text{H}_{11}\text{O}_{10}\text{P}$ (M-H) 273.0017, found 273.0014.

One-pot three-enzyme (OP3E) synthesis of uridine 5'-diphospho- α -D-glucuronic acid (UDP-GlcA, 9).

Glucuronic acid (63 mg), ATP (1.5 eq.), and UTP (1.5 eq.) were dissolved in water in a 15 mL centrifuge tube containing MES buffer (100 mM, pH 6.5) and MgCl_2 (10 mM). After the addition of appropriate amount of AtGlcAK (3 mg), BLUSP (4 mg), PmPpA (2.5 mg). Water was then added to bring the total volume of reaction mixture to 10 ml. The reaction was carried out by incubating the solution in an isotherm incubator for 36 hr at 37 °C with gentle shaking. Product formation was monitored by TLC (EtOH:H₂O = 7:3) with *p*-anisaldehyde sugar staining. The reaction was stopped by adding the same volume of ice-cold ethanol and incubating at 4 °C for 30 min and then centrifuged. The supernatant was collected and passed through a BioGel P-2 gel filtration column to obtain the desired product. Additional silica gel column (EtOAc: MeOH: H₂O = 7:3:1) and BioGel P-2 gel filtration [NH_4HCO_3 (aq.), 50 mM] column was applied when necessary to achieve further purification to afford UDP-GlcA 9 (white solid; 151 mg, 80%).

^1H NMR (600 MHz, D_2O) δ = 7.95 (d, J = 8.1, 1H), 6.04–5.96 (m, 2H), 5.65 (dd, J = 7.5, 3.5, 1H), 4.42–4.35 (m, 2H), 4.34–4.23 (m, 2H), 4.24–4.18 (m, 1H), 4.17 (d, J = 10.2, 1H), 3.80 (t, J = 9.5, 1H), 3.62 (dt, J = 9.8, 3.2, 1H), 3.54 (dd, J = 10.2, 9.2, 1H). ^{13}C NMR (151 MHz, D_2O) δ = 176.31, 166.15, 151.77, 141.50, 102.61, 95.27, 88.25, 83.2 (d, J = 9.2 Hz), 73.70, 72.99, 72.52, 71.79, 71.27, 69.59, 64.89. HRMS (ESI) m/z calcd for $\text{C}_{15}\text{H}_{22}\text{N}_2\text{O}_{18}\text{P}_2$ (M-H) 579.0270, found 579.0285.

An alternative OP3E synthesis of uridine 5'-diphospho- α -D-glucuronic acid (UDP-GlcA, 9).

An alternative OP3E system was used to synthesize UDP-GlcA (9) by replacing BLUSP by AtUSP. For this, glucuronic acid (50 mg), ATP (1.5 eq.), and UTP (1.5 eq.) were dissolved in water in a 15 mL centrifuge tube containing Tris buffer (100 mM, pH 7.5) and MgCl_2 (10 mM). After the addition of appropriate amount of AtGlcAK (4 mg), AtUSP (3 mg), PmPpA (2.5 mg). The reaction was carried out by incubating the solution in an isotherm incubator for 48 hr at 37 °C with gentle shaking. The product purification was the same as described above to obtain UDP-GlcA 9 (white solid; 85 mg, 57 %).

One-pot three-enzyme (OP3E) synthesis of uridine 5'-diphospho- α -D-galacturonic acid (UDP-GalA, **10).** Galacturonic acid monohydrate (32 mg), ATP (2 eq.) and UTP (1.2 eq.) were dissolved in water in a 15 mL centrifuge tube containing TRIS buffer (100 mM, pH 7.0) and MgCl₂ (10 mM). After the addition of appropriate amount of BiGalK (7 mg), AtUSP (5 mg), and PmPpA (4 mg). Water then was added to bring the total volume of reaction mixture to 10 mL. The reaction was carried out by incubating the solution in an isotherm incubator for 30 hr at 37 °C with gentle shaking. Product formation was monitored by TLC (EtOH:H₂O = 7:3, by volume) with *p*-anisaldehyde sugar staining. The reaction was stopped by adding the same volume of ice-cold ethanol and incubating at 4 °C for 30 min and then centrifuged. The supernatant was collected and passed through a BioGel P-2 gel filtration column to obtain the desired product. Additional silica gel column (EtOAc:MeOH:H₂O = 7:3:1) and BioGel P-2 gel filtration [NH₄HCO₃ (aq), 50 mM] column was applied when necessary to achieve further purification to afford UDP-GalA **9** (white solid; 34 mg, 39%). ¹H NMR (800 MHz, D₂O) δ = 7.95 (d, *J*=8.1, 1H), 6.09–5.85 (m, 2H), 5.68 (dd, *J* = 6.8, 3.3, 1H), 4.64 (br, 1H), 4.39–4.34 (m, 2H), 4.33 (d, *J* = 2.6, 1H), 4.28 (br, 1H), 4.26–4.21 (m, 1H), 4.17 (ddd, *J* = 11.7, 5.2, 2.7, 1H), 3.99 (dd, *J* = 10.3, 3.4, 1H), 3.83 (dt, *J* = 10.4, 2.7, 1H). ¹³C NMR (201 MHz, D₂O) δ = 173.41, 165.76, 151.35, 141.10, 102.18, 95.12, 87.85, 82.75 (d, *J* = 9.0 Hz), 73.32, 71.79, 69.82, 69.13, 68.55, 67.42, 64.44. HRMS (ESI) *m/z* calcd for C₁₅H₂₂N₂O₁₈P₂ (M-H) 579.0270, found 579.0247.

Sequential one-pot multienzyme (OPME) synthesis of heparosan oligosaccharides (14–16).

GlcNAc α 1–4GlcA β ProN₃ (14**).** GlcA β ProN₃ **13** (50 mg, 1 eq.), *N*-acetylglucosamine (1.5 eq.), ATP (1.8 eq.) and UTP (1.8 eq.) were dissolved in water in a 50 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 7.5) and MgCl₂ (10 mM). After the addition of appropriate amount of NanK_ATCC55813 (2 mg), PmGlmU (4 mg), PmPpA (1.5 mg), and PmHS2 (1 mg), water was added to bring the concentration of GlcA β ProN₃ **1** to 10 mM. The reaction was carried out by incubating the solution in an isotherm incubator for 24 hr at 37 °C with gentle shaking. Product formation was monitored by TLC (EtOAc:MeOH:H₂O = 4:2:1 by volume) with *p*-anisaldehyde sugar staining. The reaction was stopped by adding the same volume of ice-cold ethanol and incubating at 4 °C for 30 min and then centrifuged. The supernatant was collected and passed through a BioGel P-2 gel filtration column and was further purified by silica gel column (EtOAc:MeOH:H₂O = 5:2:1) to afford disaccharide GlcNAc α 1–4GlcA β ProN₃ **14** (86 mg, 81%; white solid). ¹H NMR (600 MHz, D₂O) δ = 5.39 (d, *J* = 3.7, 1H), 4.45 (d, *J* = 8.0, 1H), 3.95 (m, 1H), 3.87 (dd, *J* = 10.7, 3.8, 1H), 3.83–3.61 (m, 8H), 3.47 (t, *J* = 9.6, 1H), 3.44 (t, *J* = 6.7, 2H), 3.29 (t, *J* = 8.7, 1H), 2.03 (s, 3H), 1.88 (m, 2H). ¹³C NMR (151 MHz, D₂O) δ = 174.98, 174.30, 102.17, 96.78, 76.80, 76.49, 75.75, 73.35, 71.80, 70.64, 69.60, 67.36, 60.03, 53.61, 47.78, 28.16, 21.85. HRMS (ESI) *m/z* calcd for C₁₇H₂₈N₄O₁₂ (M-H) 479.1631, found 479.1634.

GlcA β 1–4GlcNAc α 1–4GlcA β ProN₃ (15**).** Disaccharide GlcNAc α 1–4GlcA β ProN₃ **14** (40 mg, 1 eq.), glucuronic acid (1.5 eq), ATP (1.8 eq) UTP (1.5 eq) were dissolved in water in a 15 mL centrifuge tube containing MES buffer (100 mM, pH 6.5) and MgCl₂ (10 mM). After the addition of appropriate amount of AtGlcAK (3 mg), BLUSP (4 mg), PmPpA (1.5 mg), PmHS2 (1.4 mg), water was added to bring the volume of the reaction mixture to 10 mL. The reaction was carried out by incubating the solution in an isotherm incubator for 16 hr at 37 °C with gentle shaking. Product formation was monitored by TLC (EtOAc: MeOH: H₂O = 5:3:1.5) with *p*-anisaldehyde sugar staining. The reaction was stopped by adding the same volume of ice-cold ethanol and incubating at 4 °C for 30 min and then centrifuged. The supernatant was collected

and passed through a BioGel P-2 gel filtration column to obtain the desired product. The trisaccharide was further purified by silica gel column chromatography (EtOAc:MeOH:H₂O = 6:3:1) to afford GlcA β 1-4GlcNAc α 1-4GlcA β ProN₃ **15** (54 mg, quant; white solid). ¹H NMR (600 MHz, D₂O) δ 5.38 (d, *J* = 3.6, 1H), 4.51 (d, *J* = 7.9, 1H), 4.45 (d, *J* = 8.0, 1H), 4.00–3.92 (m, 1H), 3.93 – 3.61 (m, 11H), 3.57–3.48 (m, 2H), 3.45 (t, *J* = 6.7, 2H), 3.37 (t, *J* = 8.3, 1H), 3.29 (t, *J* = 8.7, 1H), 2.04 (s, 3H), 1.89 (p, *J* = 6.4, 2H). ¹³C NMR (151 MHz, D₂O) δ 175.20, 174.81, 174.24, 102.23, 102.16, 96.64, 78.37, 76.63, 76.50, 76.01, 75.05, 73.34, 72.81, 71.65, 70.52, 69.10, 67.37, 59.30, 53.22, 47.78, 28.16, 21.85. HRMS (ESI) *m/z* calcd for C₂₃H₃₆N₄O₁₈ (M-H) 655.1952, found 655.1932.

GlcNAc α 1-4GlcA β 1-4GlcNAc α 1-4GlcA β ProN₃ (16) Trisaccharide GlcA β 1-4GlcNAc α 1-4GlcA β ProN₃ **15** (6.3 mg, 1 eq.), *N*-acetylglucosamine (1.5 eq.), ATP (1.8 eq.), and UTP (1.8 eq.) were dissolved in water in a 15 mL centrifuge tube containing Tris-HCl buffer (100 mM, pH 7.5) and MgCl₂ (10 mM). After the addition of appropriate amount of NanK_ATCC55813 (1 mg), PmGlmU (1 mg), PmPpA (1.5 mg), and PmHS2 (0.8 mg), water was added to bring the volume of the reaction mixture to 10 mL. The reaction was carried out by incubating the solution in an isotherm incubator for 16 hr at 37 °C with gentle shaking. Product formation was monitored by TLC (EtOAc:MeOH:H₂O = 5:3:1.5) with *p*-anisaldehyde sugar staining. The reaction was stopped by adding the same volume of ice-cold ethanol and incubating at 4 °C for 30 min and then centrifuged. The supernatant was collected and passed through a Bio-Gel P-2 gel filtration column to obtain the desired product. The tetrasaccharide was further purified HPLC chromatography to afford GlcNAc α 1-4GlcA β 1-4GlcNAc α 1-4GlcA β ProN₃ **20** (8.1 mg, quant; white solid). ¹H NMR (800 MHz, D₂O) δ = 5.36 (d, *J* = 3.4, 1H), 5.33 (d, *J* = 3.4, 1H), 4.56 (d, *J* = 7.9, 1H), 4.49 (d, *J* = 8.0, 1H), 4.05 (d, *J* = 9.7, 1H), 4.01 (d, *J* = 9.7, 1H), 3.96–3.86 (m, 4H), 3.83–3.66 (m, 13H), 3.59 (d, *J* = 10.0, 1H), 3.50 (t, *J* = 9.6, 1H), 3.43 (t, *J* = 6.6, 2H), 3.36 (t, *J* = 8.7, 1H), 3.30 (t, *J* = 8.7, 1H), 2.03 (s, 3H), 2.02 (s, 3H), 1.87 (p, *J* = 6.5, 2H). ¹³C NMR (201 MHz, D₂O) δ = 174.33, 174.28, 172.36, 172.32, 102.25, 102.15, 97.41, 97.25, 78.09, 76.45, 76.12, 75.86, 75.71, 74.44, 74.21, 73.15, 73.05, 72.14, 70.84, 70.36, 69.22, 68.69, 67.47, 59.74, 59.02, 53.42, 53.18, 47.66, 28.11, 21.76(2C). HRMS (ESI) *m/z* calcd for C₃₁H₄₉N₅O₂₃ (M-H) 858.2746, found 858.2723.

Table S3. Comparisons of the obtained and the reported ¹H NMR chemical shifts (δ) and coupling constants (*J*, Hz) for compounds **5**–**7**. "–", not available or not compared. No reported NMR data were found for compound **7**. ^aRef. 10, ^bRef. 8.

Compound	H-1	H-2	H-3	H-4	H-5	C-1	C-2	C-3	C-4	C-5	C-6
5	5.46	3.53	3.75	3.48	4.11	94.0	71.5	72.5	71.9	72.4	176.8
^a 5	5.30	3.35	3.62	3.32	3.99	93.0	71.4	71.5	71.5	72.4	176.6
6	5.55	3.82	3.96	4.31	4.46	95.1	68.2	70.7	69.4	72.3	175.7
^b 6	5.49	3.77	3.90	4.25	4.40	94.6	68.0	70.6	68.3	72.3	175.8
7	5.36	3.95	3.95	3.78	4.10	95.1	70.8	69.8	68.8	72.8	177.3
	<i>J</i> _{p,H1}	<i>J</i> _{1,2} ; <i>J</i> _{p,H2}	<i>J</i> _{2,3}	<i>J</i> _{3,4}	<i>J</i> _{4,5}	<i>J</i> _{p,C1}	<i>J</i> _{p,C2}			<i>J</i> _{p,C5}	
5	7.4	3.5; 2.2	9.5	9.5	10.2	5.7	7.4			–	
^a 5	7.3	3.4; –	9.5	9.5	9.9	4.9	7.3			1.8	
6	7.0	3.7; 2.4	10.3	3.4	1.5	–	–			–	
^b 6	6.4	3.6; –	10.0	2.8	–	–	–			–	
7	8.6	1.8; –	9.7	9.7	10.0	4.4	7.1			–	

Table S4. Comparisons of the obtained and the reported ^1H NMR chemical shifts (δ) and coupling constants (J , Hz) for compounds **9** and **10**. "–", not available or not compared. ^aRef. 11, ^bRef. 12.

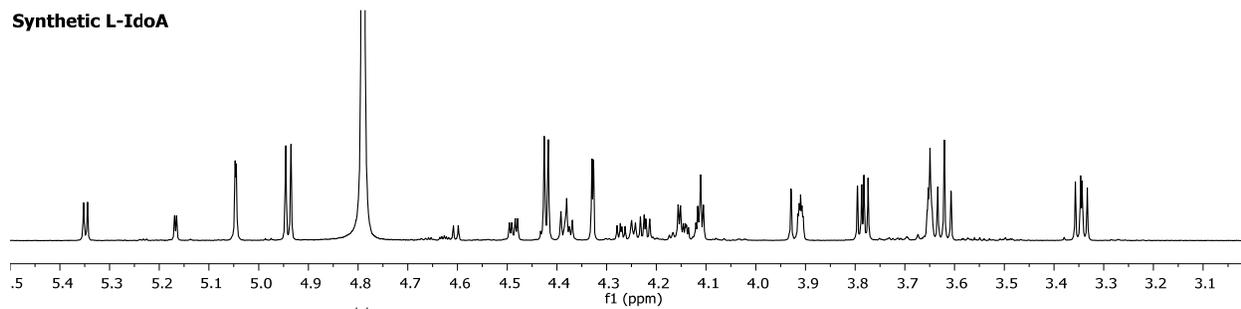
Compound	H-1	H-2	H-3	H-4	H-5	H-6''	H-5''	H-1'	H-2'3'	H-4'	H-5a'	C-5b'
9	5.65	3.62	3.80	3.54	4.17	7.95	6.00	6.00	4.38	4.31	4.26	4.20
^a 9	5.65	3.61	3.81	3.53	4.17	8.0	6.00	6.00	–	–	–	–
10	5.68	3.83	3.99	4.33	4.64	7.95	5.97	5.96	4.35	4.28	4.23	4.17
^b 10	5.73	3.84	3.97	4.30	4.47	7.93	5.96	5.97	4.34	4.27	4.20	4.14
	$J_{p,H1}$	$J_{1,2}; J_{p,H2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$	$J_{5'',6'}$						
9	7.5	3.2; 3.2	9.5	9.5	10.2	8.1	–	–	–	–	–	–
^a 9	7.5	3.3; 3.2	9.8	9.4	10.1	8.1	–	–	–	–	–	–
10	6.8	3.3; –	10.3	3.4	–	8.1	–	–	–	–	–	–
^b 10	–	–	10.2	3.0	2.4	8.4	–	–	–	–	–	–

References:

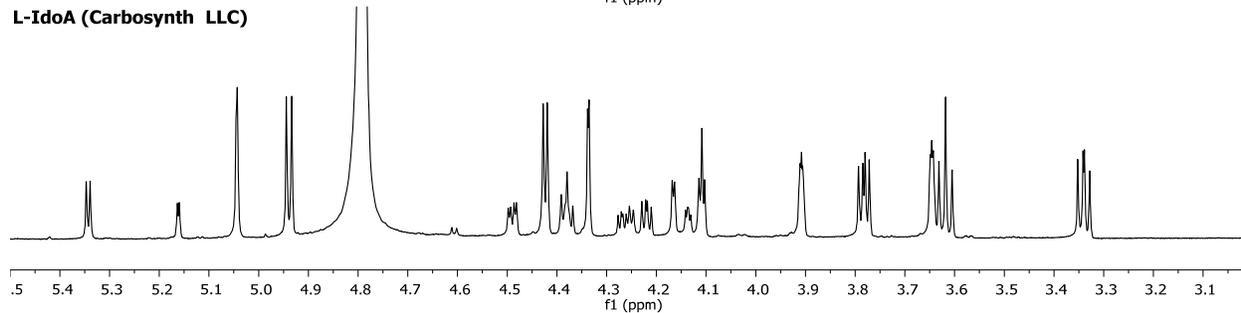
1. A. M. Pieslinger, M. C. Hoepflinger and R. Tenhaken, *Ana. Biochem.*, 2009, **388**, 254–259.
2. A. M. Pieslinger, M. C. Hoepflinger and R. Tenhaken, *J. Biol. Chem.*, 2010, **285**, 2902–2910.
3. L. A. Litterer, J. A. Schnurr, K. L. Plaisance, K. K. Storey, J. W. Gronwald and D. A. Somers, *Plant Phys. Biochem.*, 2006, **44**, 171–180.
4. Y. Chen, Y. Li, H. Yu, G. Sugiarto, V. Thon, J. Hwang, L. Ding, L. Hie and X. Chen, *Angew. Chem. Int. Ed.*, 2013, **52**, 11852–11856.
5. Y. Li, H. Yu, Y. Chen, K. Lau, L. Cai, H. Cao, V. K. Tiwari, J. Qu, V. Thon, P. G. Wang and X. Chen, *Molecules*, 2011, **16**, 6396–6407.
6. Y. Chen, V. Thon, Y. Li, H. Yu, L. Ding, K. Lau, J. Qu, L. Hie and X. Chen, *Chem. Commun.*, 2011, **47**, 10815–10817.
7. M. M. Muthana, J. Qu, Y. Li, L. Zhang, H. Yu, L. Ding, H. Malekan and X. Chen, *Chem. Commun.*, 2012, **48**, 2728–2730.
8. L. Li, Y. Liu, W. Wang, J. Cheng, W. Zhao and P. Wang, *Carbohydr. Res.*, 2012, **355**, 35–39.
9. D. Matsuura, T. Mitsui, T. Sengoku, M. Takahashi and H. Yoda, *Tetrahedron*, 2008, **64**, 11686–11696.
10. A. Heeres, H. A. van Doren, K. F. Gotlieb and I. P. Bleeker, *Carbohydr. Res.*, 1997, **299**, 221–227.
11. S. D. Breazeale, A. A. Ribeiro and C. R. H. Raetz, *J. Biol. Chem.*, 2002, **277**, 2886–2896.
12. T. Bülter, T. Schumacher, D.-J. Namdjou, R. Gutiérrez Gallego, H. Clausen and L. Elling, *ChemBioChem*, 2001, **2**, 884–894.

NMR spectra comparison of synthetic and commercial (Carbosynth LLC) L-IdoA (4).

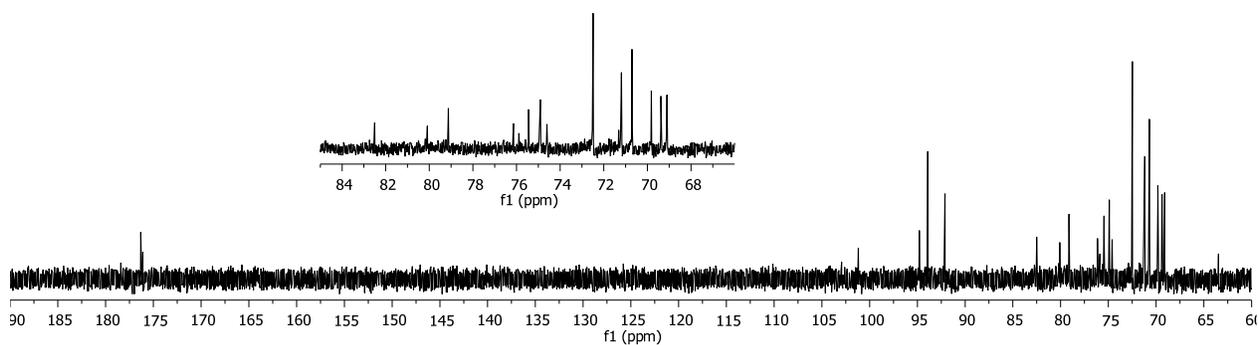
Synthetic L-IdoA



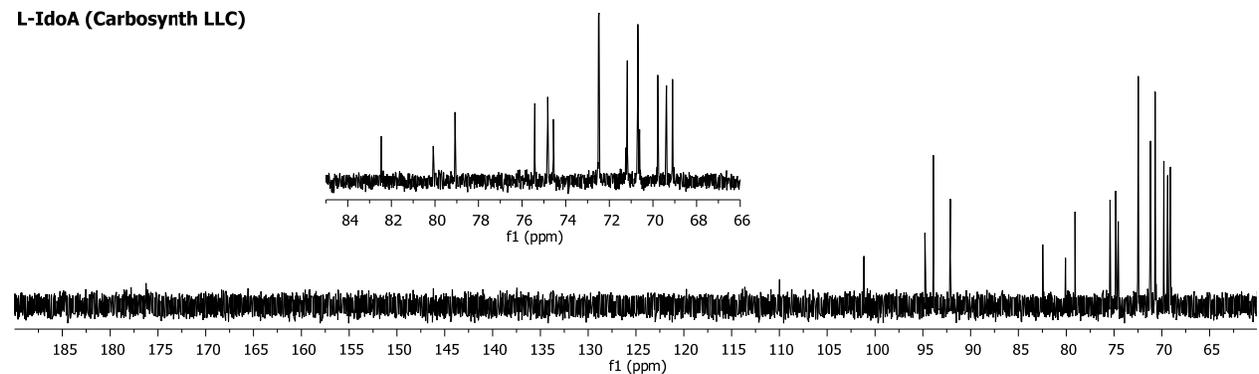
L-IdoA (Carbosynth LLC)



Synthetic L-IdoA

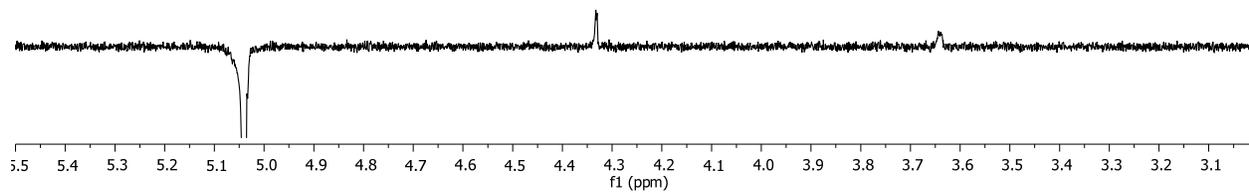


L-IdoA (Carbosynth LLC)

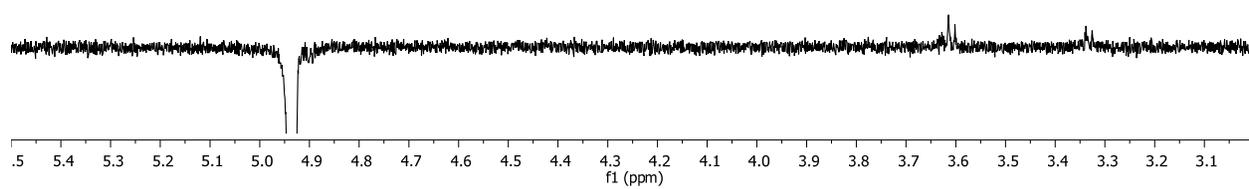


NOE H-1 anomers for synthetic L-IdoA (4)

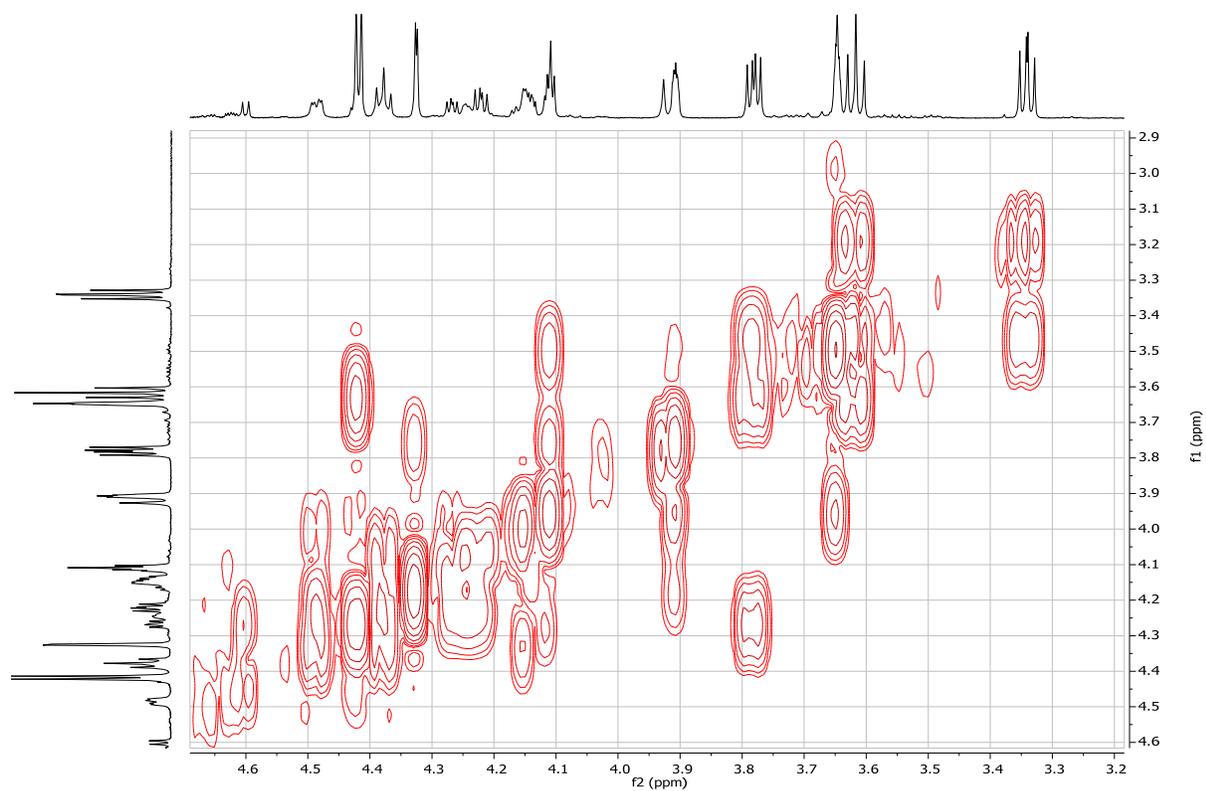
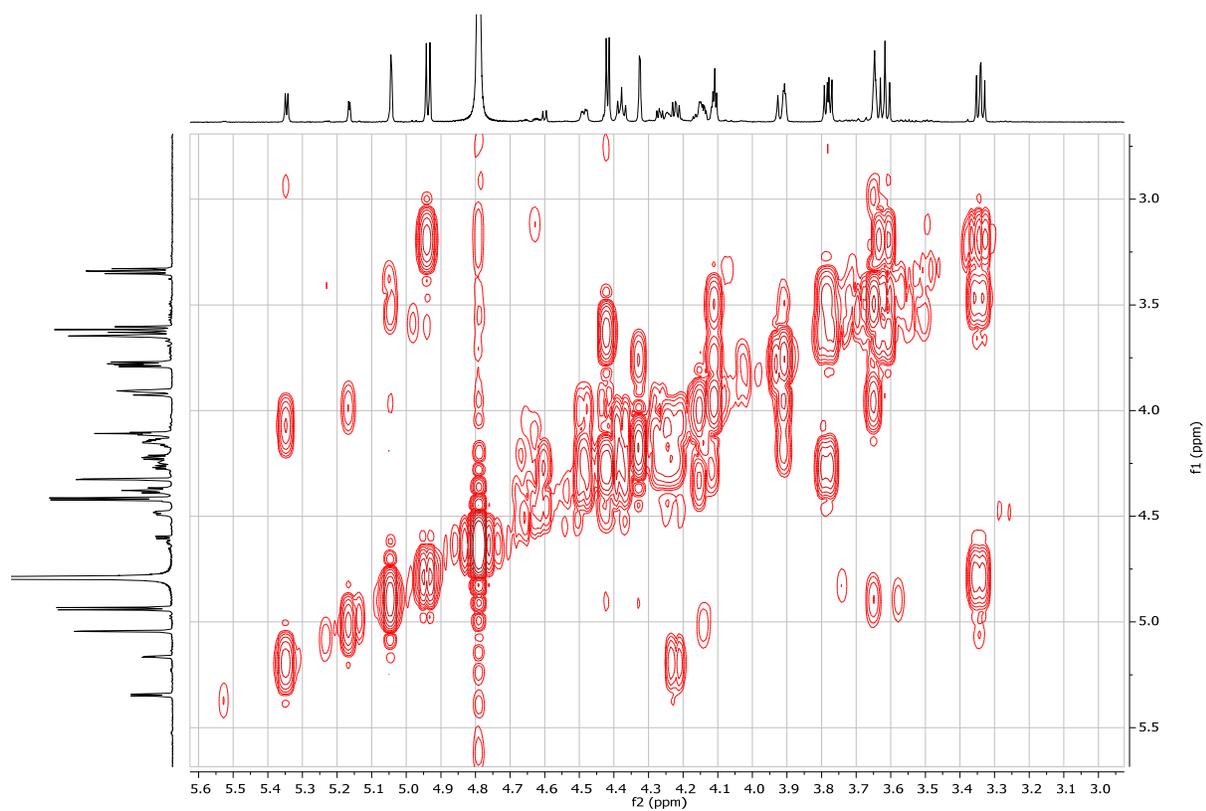
L-IdoA NOE H1 beta anomer



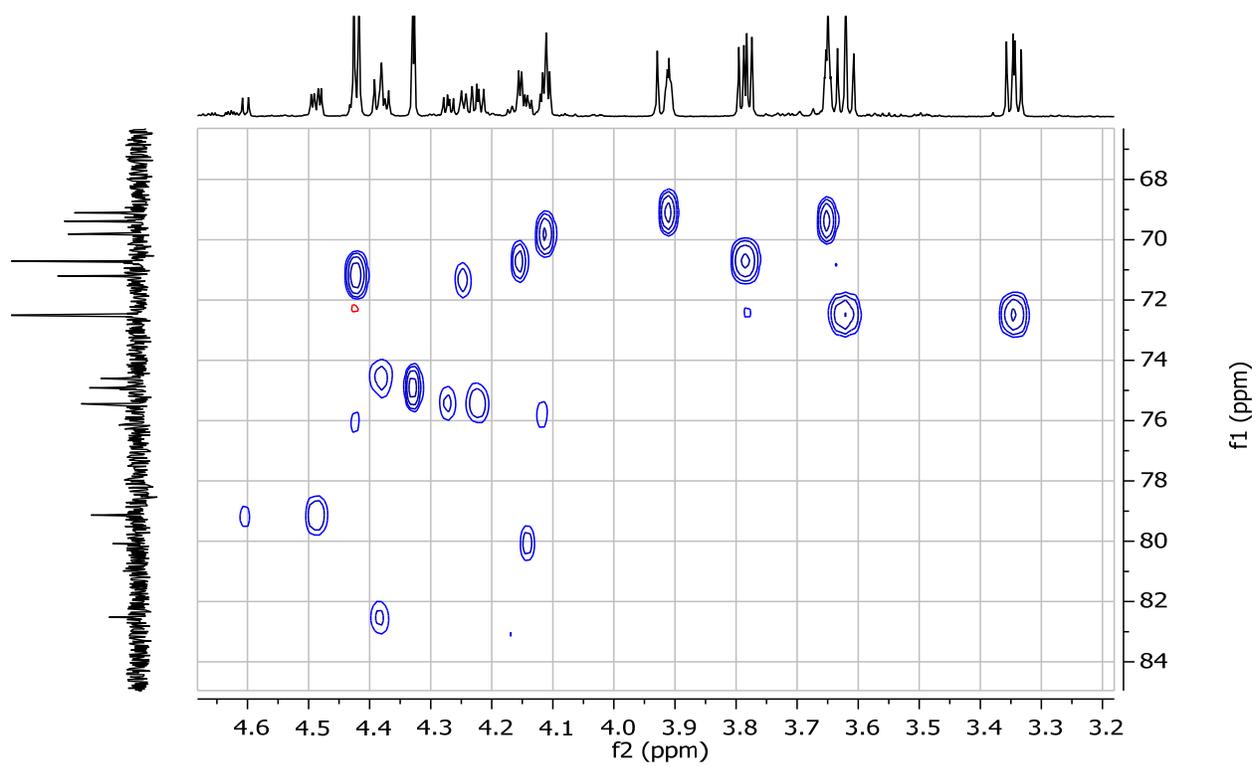
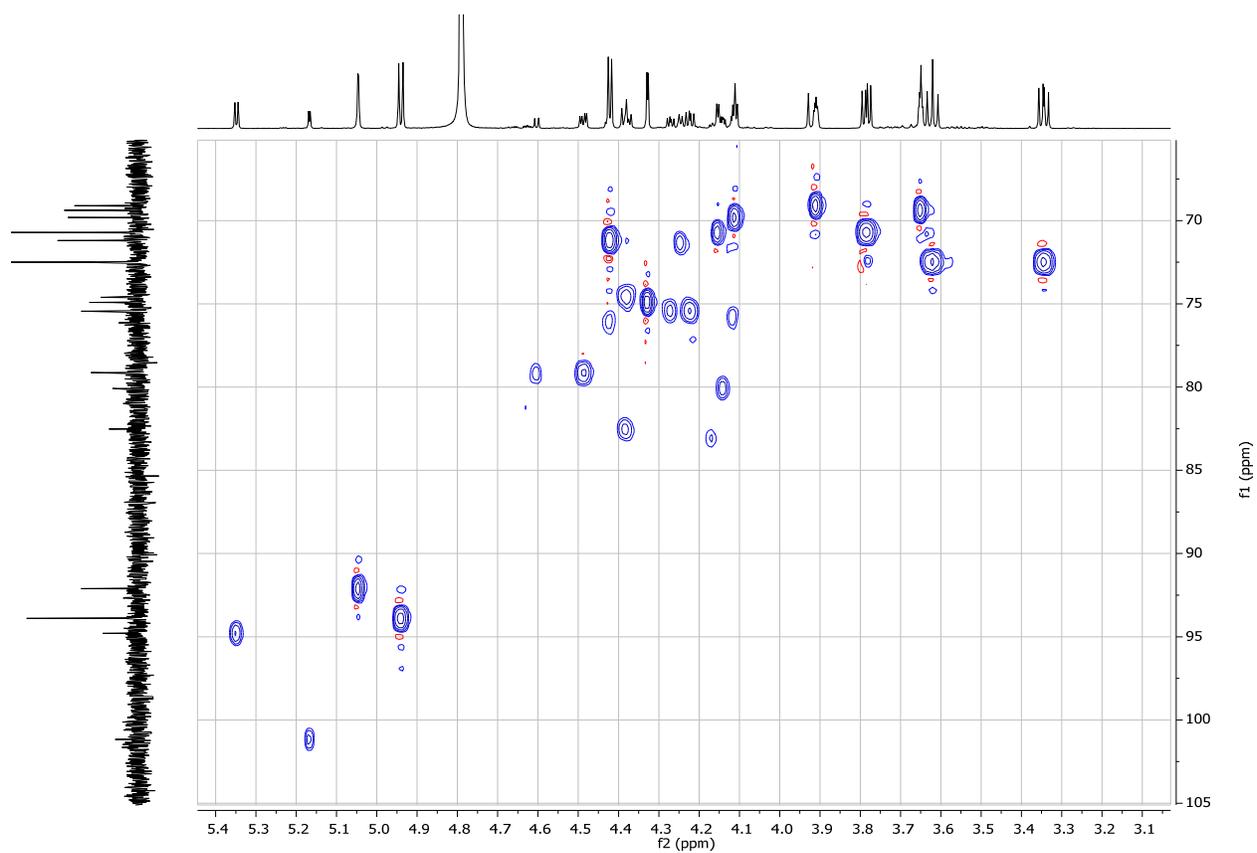
L-IdoA NOE H1 alpha anomer



COSY spectra for synthetic L-IdoA (4)

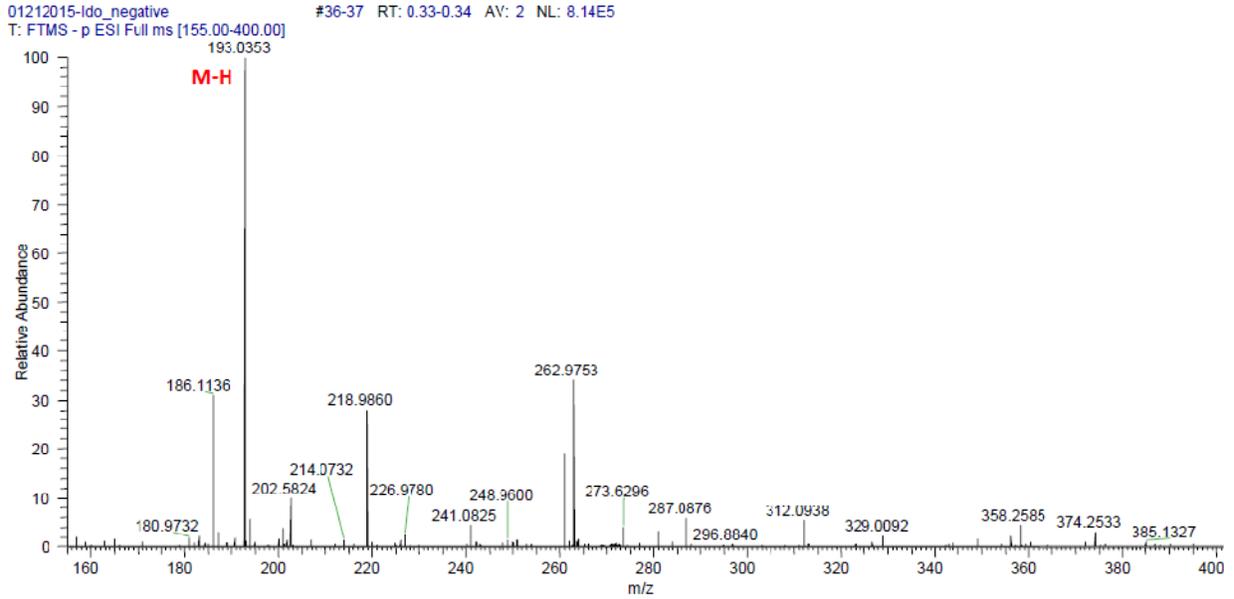


HSQC spectra for synthetic L-IdoA (4)

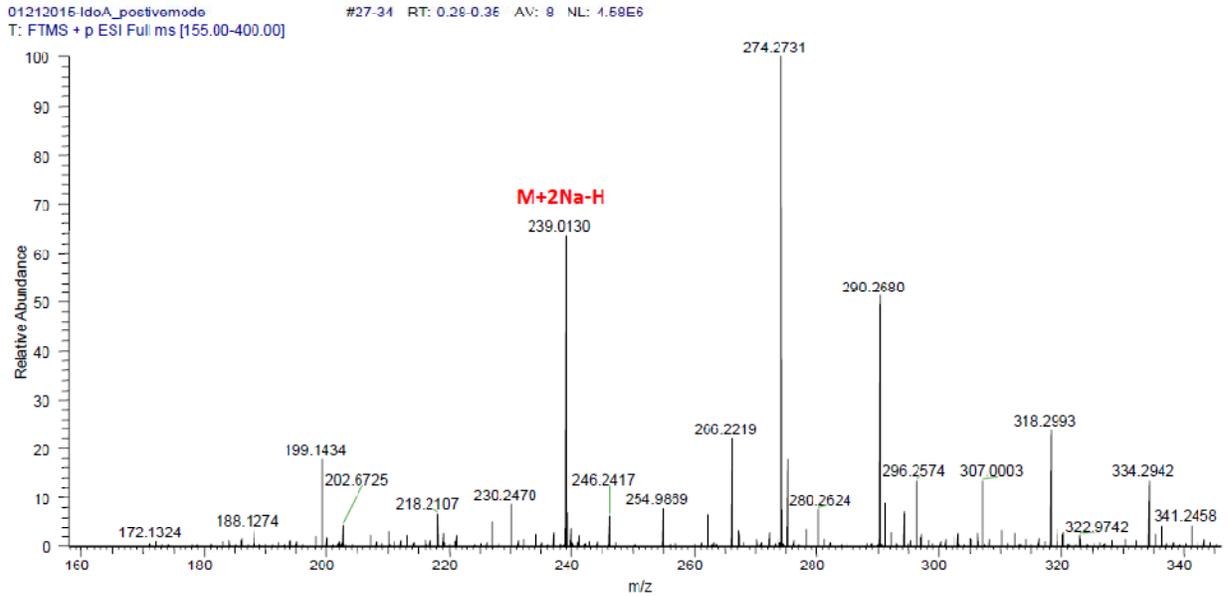


HRMS spectra for synthetic L-IdoA (4)

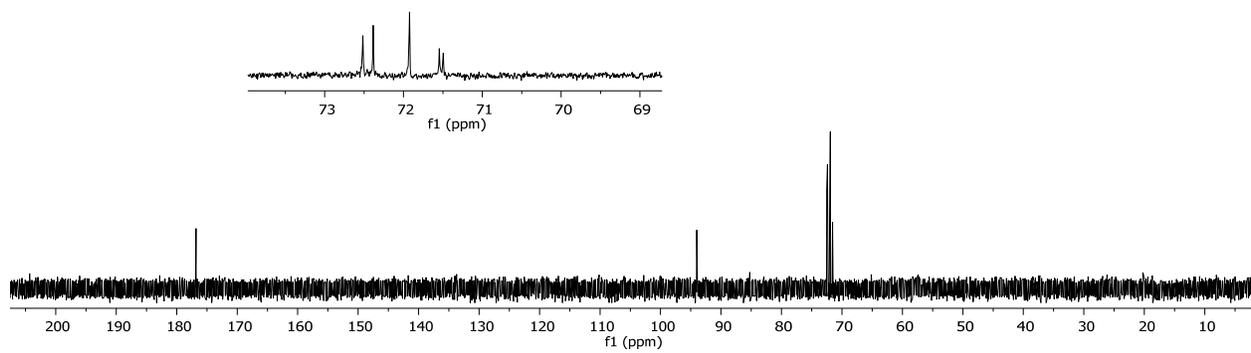
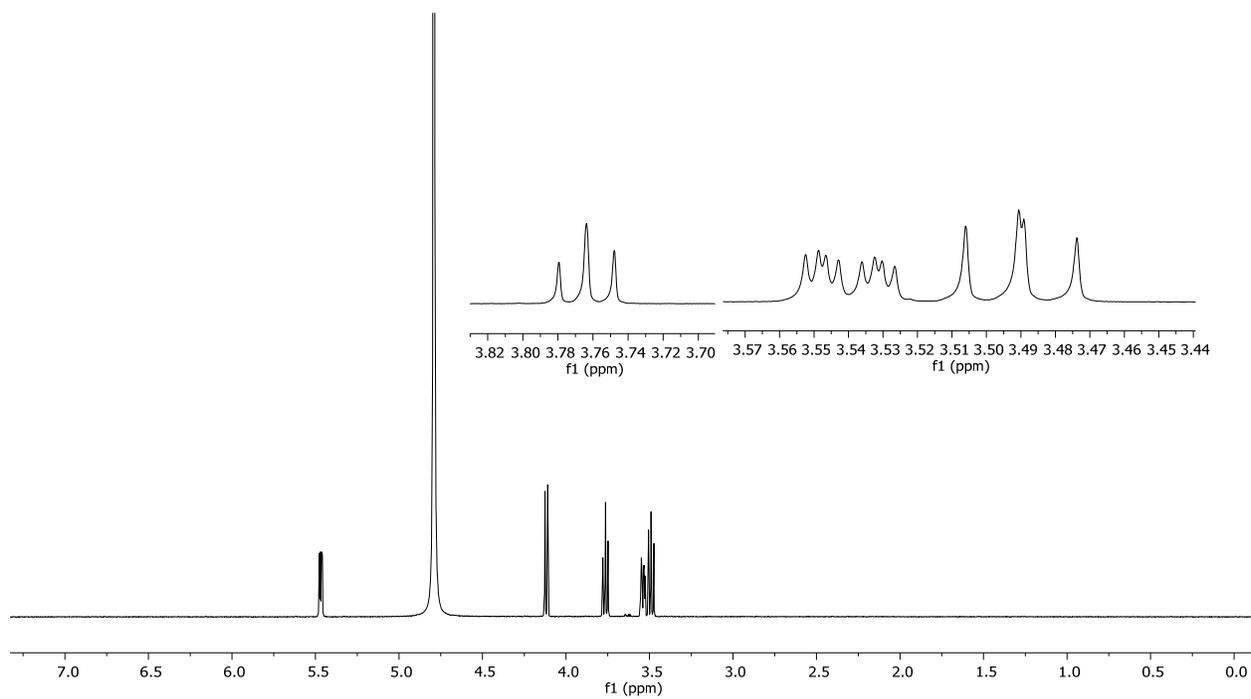
(-) Mode



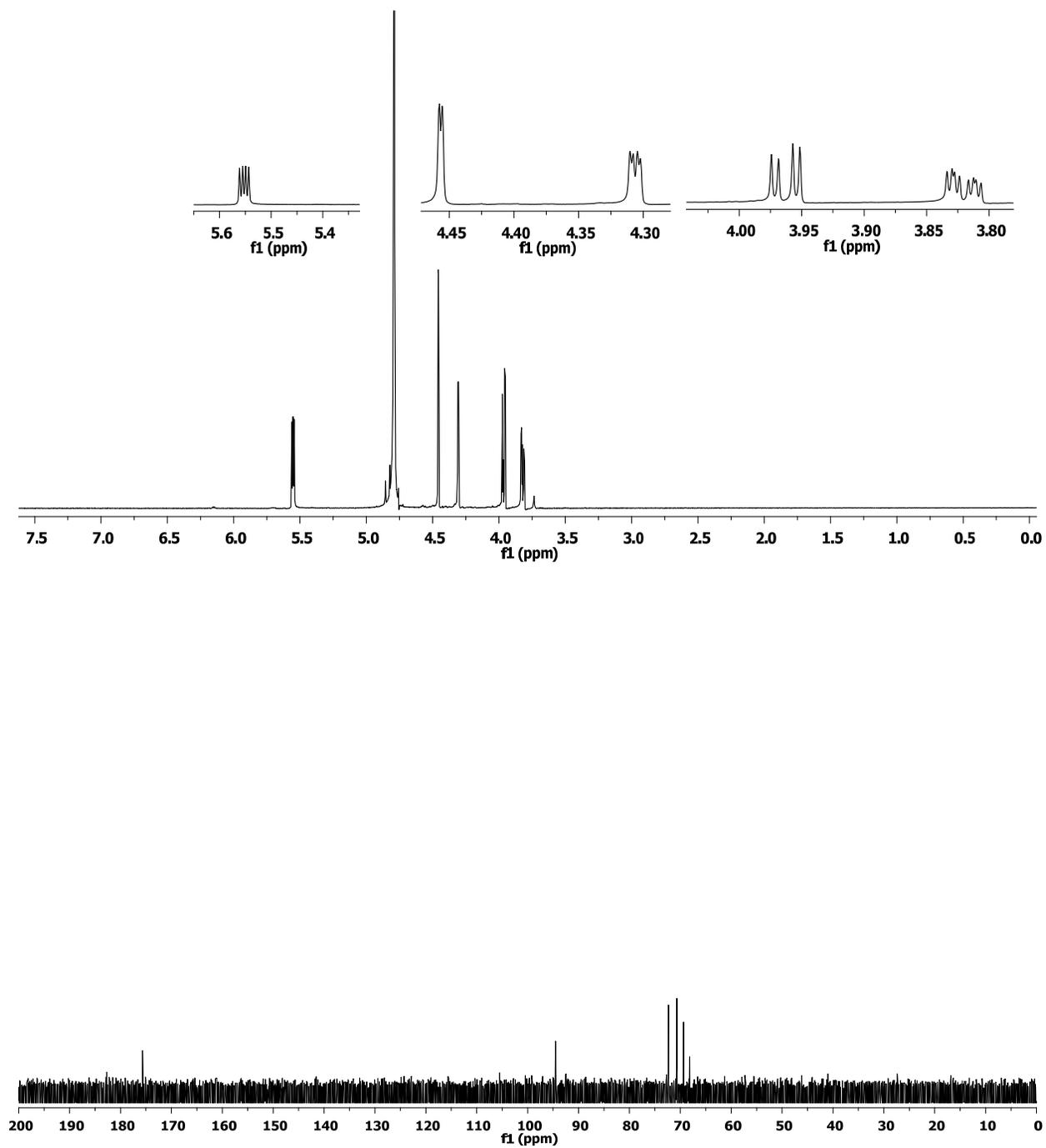
(+) Mode



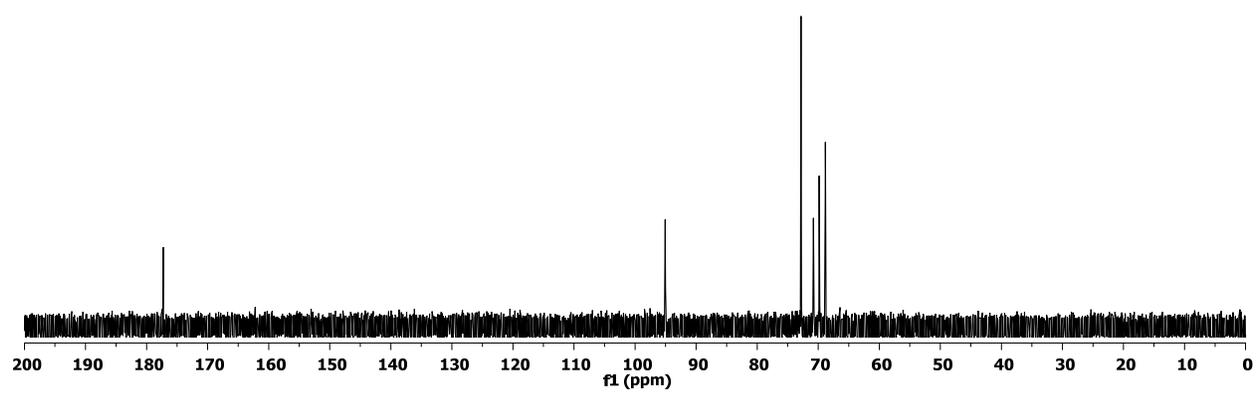
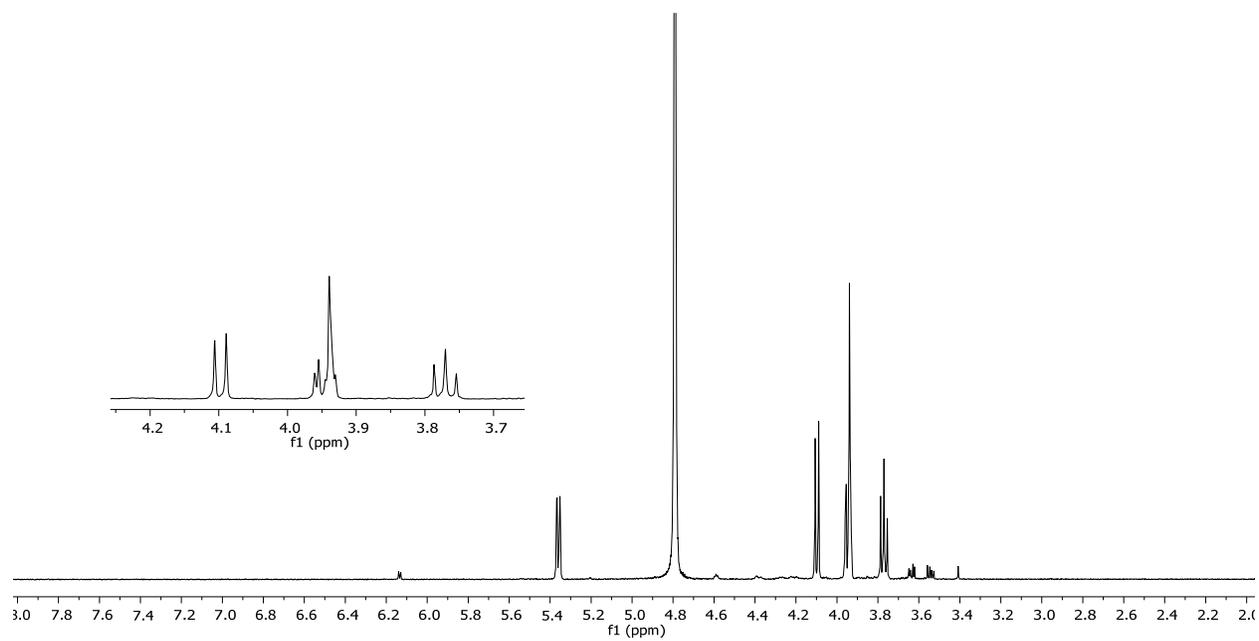
^1H and ^{13}C NMR spectra for GlcA-1-P (5)



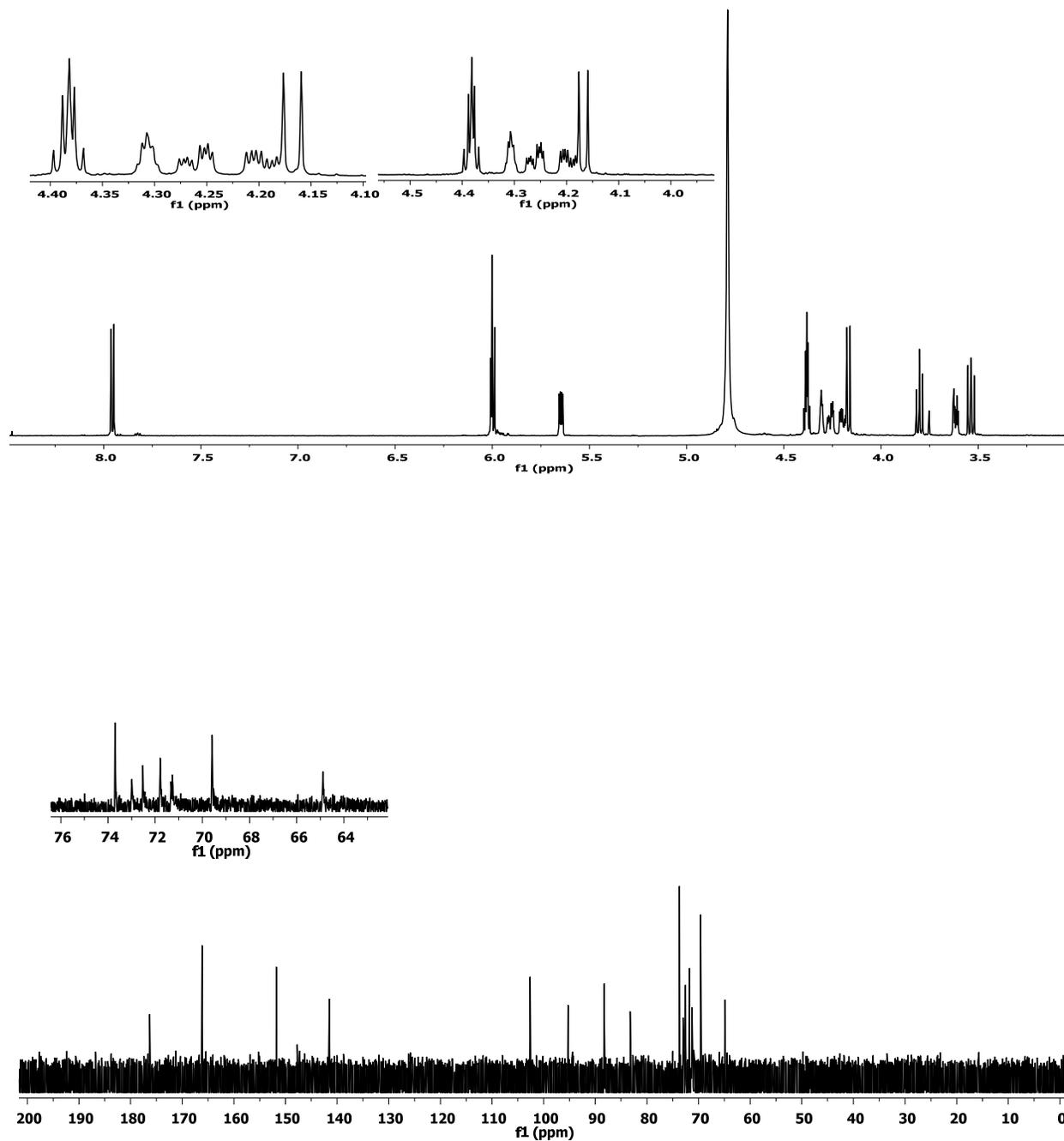
^1H and ^{13}C NMR spectra for GalA-1-P (6)



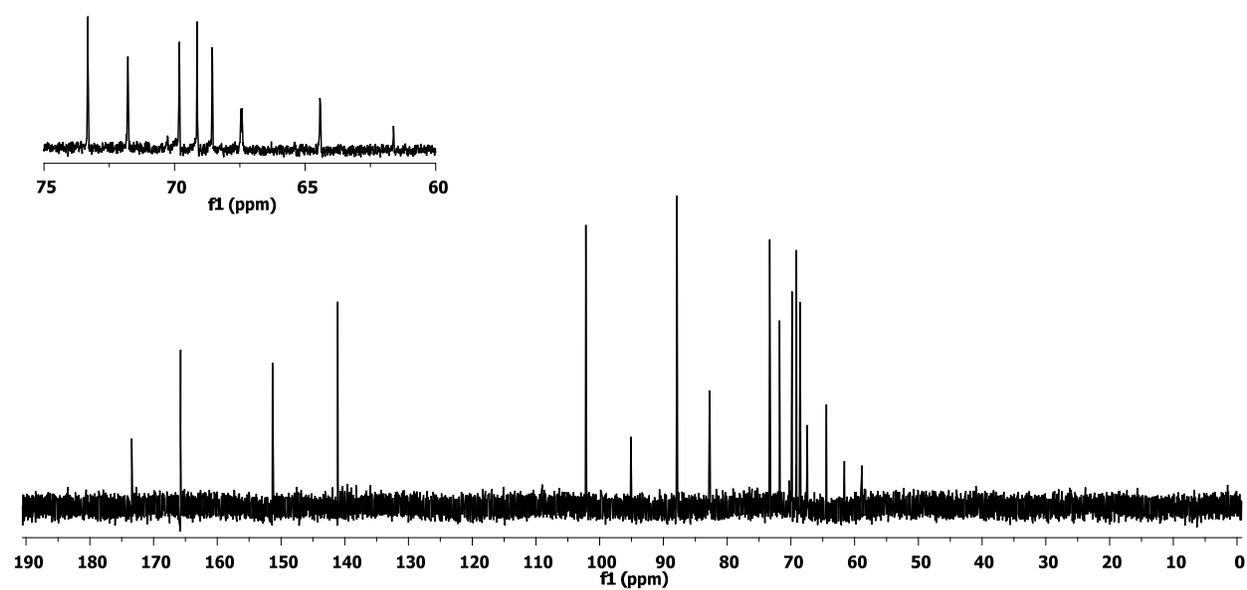
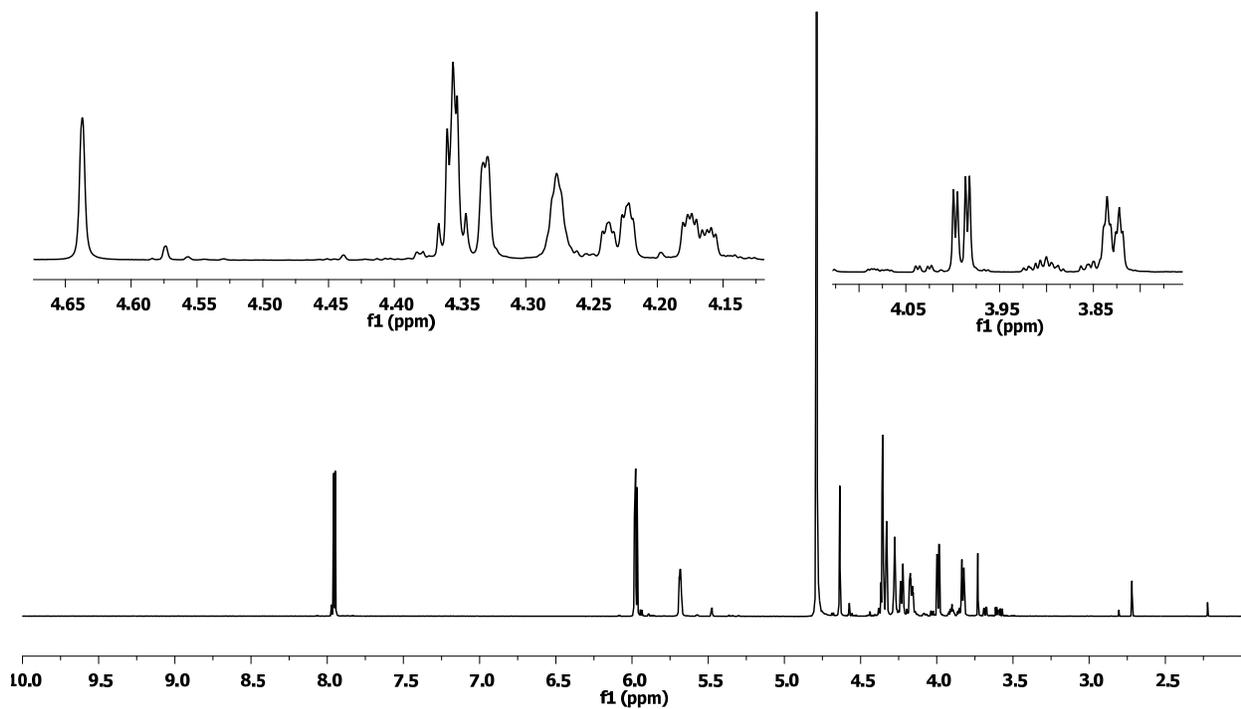
^1H and ^{13}C NMR spectra for ManA-1-P (7)



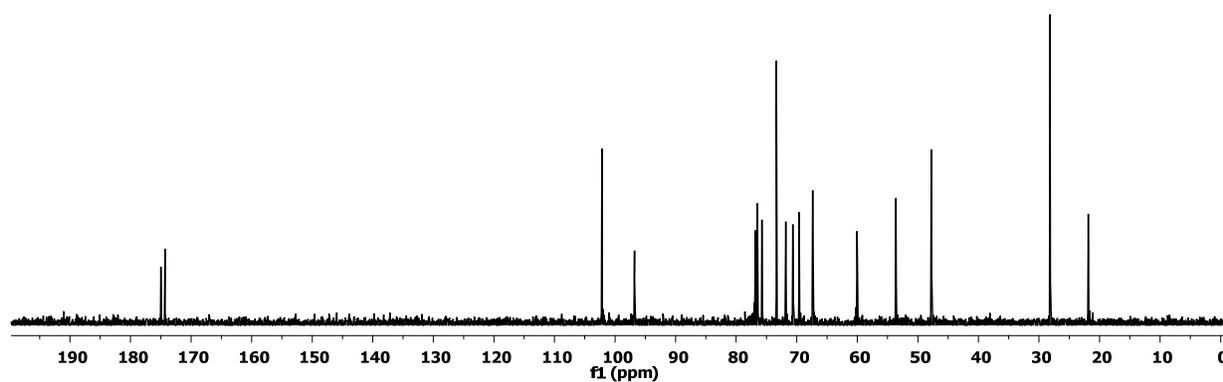
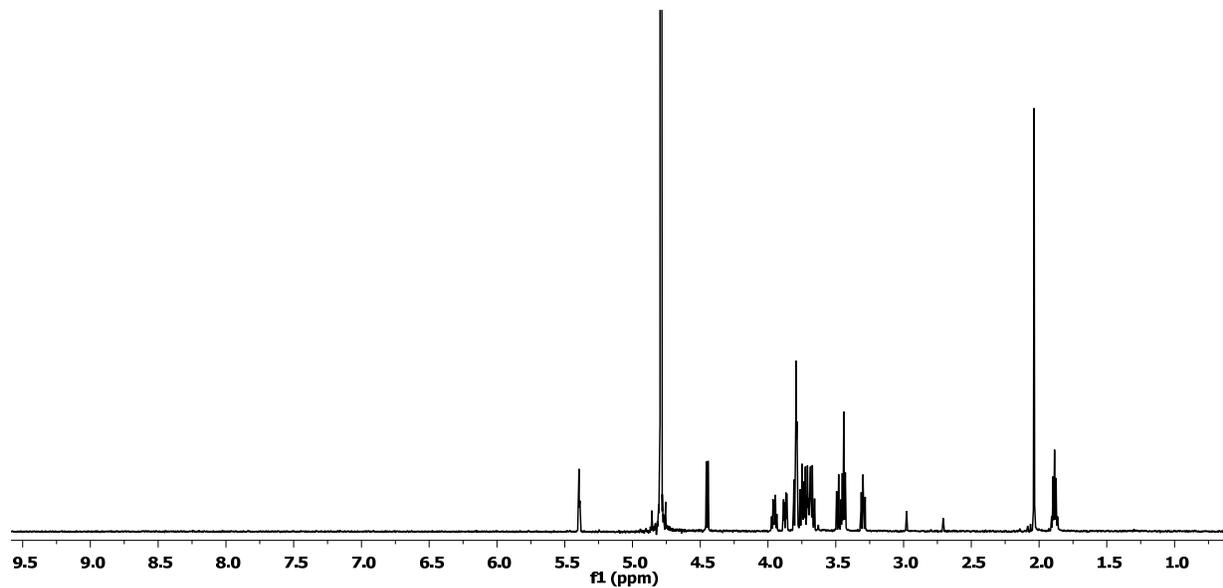
^1H and ^{13}C NMR spectra for UDP-GlcA (9)



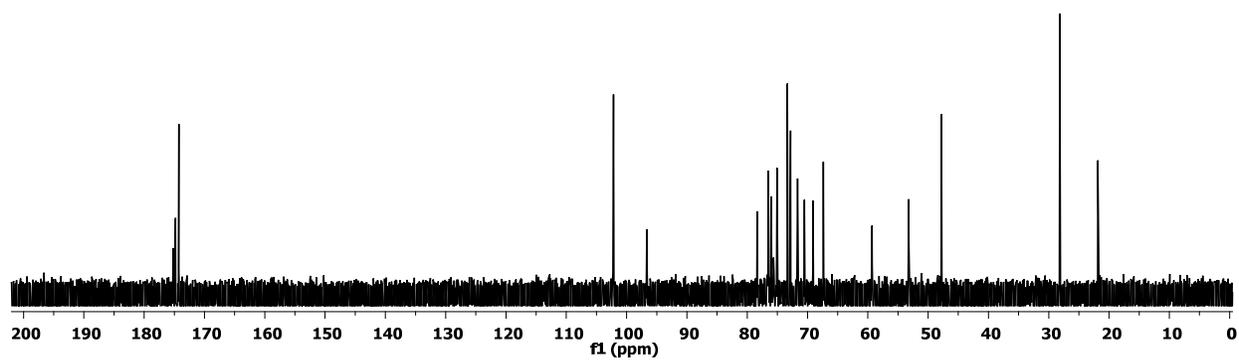
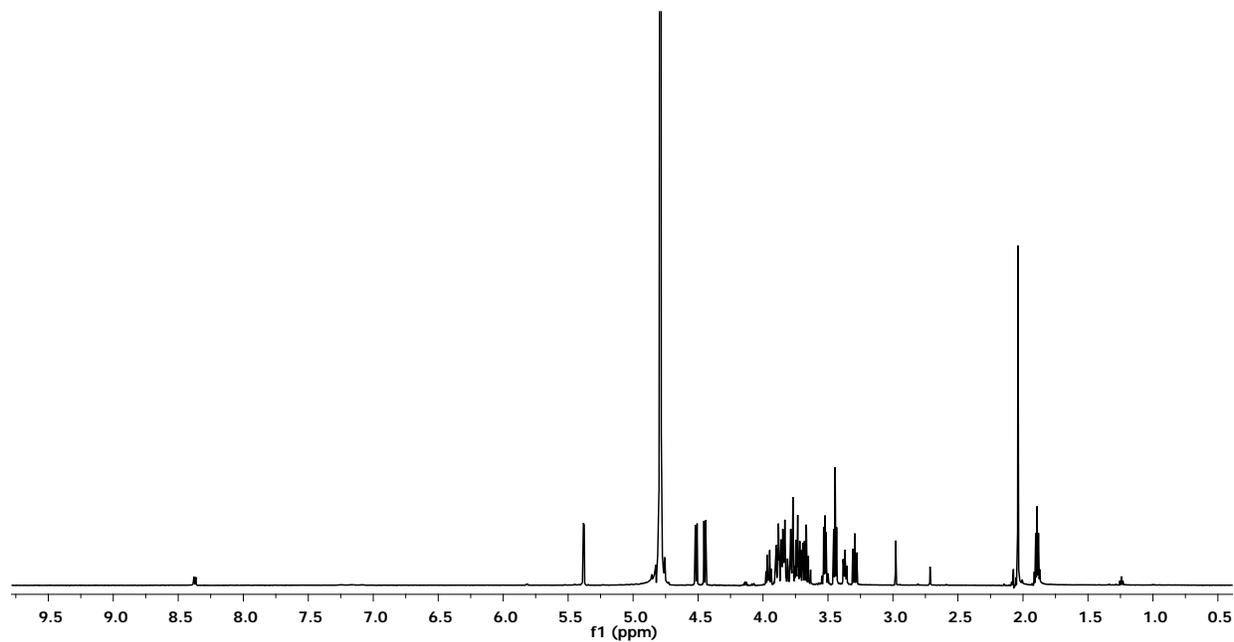
^1H and ^{13}C NMR spectra for UDP-GalA (10)



^1H and ^{13}C NMR spectra for GlcNAc α 1-4GlcA β ProN $_3$ (14)



^1H and ^{13}C NMR spectra for GlcA β 1-4GlcNAc α 1-4GlcA β ProN $_3$ (15)



^1H and ^{13}C NMR spectra for GlcNAc α 1-4GlcA β 1-4GlcNAc α 1-4GlcA β ProN $_3$ (16)

