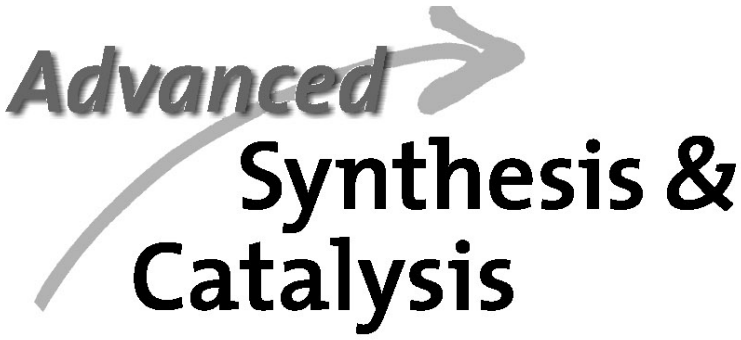

Supporting Information

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Borate as a Phosphate Ester Mimic in Aldolase-Catalyzed Reactions: Practical Synthesis of L-Fructose and L-Iminocyclitols

Masakazu Sugiyama, Zhangyong Hong, Lisa J. Whalen, William A. Greenberg*, and Chi-Huey Wong*

Department of Chemistry and the Skaggs Institute for Chemical Biology, The Scripps Research Institute, 10550 N. Torrey Pines Rd., La Jolla, CA, 92037, USA

Fax: (+1)-858-784-2409, email: wong@scripps.edu

General. Solvents, starting materials, and reagents were used as purchased without further purification. UV kinetic assays were performed on a Beckman DU-650 spectrophotometer. Proton NMR spectra (^{1}H NMR) were recorded at 600 MHz using a Bruker 5 mm DCH CryoProbe. Chemical shifts are expressed in parts per million (δ) and are referenced to residual protium in the NMR solvent: CD_2HOD, δ 3.31; DOH, δ 4.80. Carbon NMR (^{13}C NMR) spectra were recorded at 150 MHz using the same probe. Chemical shifts (δ ppm) are referenced to the carbon signal for the solvent: CD_3OD, δ 49.05; or an internal standard of CH_3CN (δ 1.39) or CH_3OH (δ 49.05). Optical rotation measurements were taken on a Perkin-Elmer 241 polarimeter with an aperture of 2 mm using a microcell with 1 cm path length. Aldehyde dehydrogenase, α-glycerophosphate dehydrogenase and triosephosphate isomerase were purchased from Sigma.

Expression and purification of RhaD. The *rhaD* [1] gene was amplified from chromosomal DNA of *E. coli* W3110 by PCR with following two primers; RhaD5Nde, 5'-cgcgcatatgcaaaacattactcagtcctgg-3', and RhaD3Xho, 5'-cggctcgagttacagcgccagcgcactggcgag-3'. The amplified 650 bp fragment was cloned into the *Nde*I and *Xho*I site of pETDuet vector (Novagene) to give pETDRhaD. *E. coli* BL21 (DE3) was transformed with pETDRhaD, and transformants were cultivated in LB medium containing carbenicillin (50 μg ml^{-1}) and IPTG (10 μM) at 37^{o}C for 16 h. The

recombinant RhaD was expressed in the soluble fraction, and appeared as a major band on SDS-PAGE. The cells were harvested by centrifuge, washed with saline, and used as whole cell catalysts. His-tagged RhaD was also overexpressed in *E. coli* JM109 harboring pQErham, and purified by Ni^{2+} affinity column chromatography as previously described. [2]

Expression and purification of DHA kinase. For an enzyme-coupled assay of DHA, His-tagged DHA kinase was prepared as follows. The *dhaK* gene [3] was amplified from chromosomal DNA of *Citrobacter braakii* ATCC 6750 (formerly named as *C. freundii*) by PCR with the following two primers; pETDhaK-SNde, 5'-ggcccatatgtctcaattctttttaaccaacgc-3', and pETDhaK-ASXho, 5'-cgcctcgaggcccagctcactctccgctagcgctttaaacacc-3'. The amplified 1.6 kbp fragment was cloned into *Nde*I and *Xho*I site of pET26 vector (Novagene) to give pETDhaK. *E. coli* BL21 (DE3) was transformed by pETDhak, and transformants were cultivated in LB medium containing kanamycin (50 μg ml^{-1}) at 37°C overnight. One milliliter of the broth was seeded into LB-kanamycin medium (100 ml), and cultivated at 30°C. ITPG (final concentration at 1 mM) was added when OD_{600} reached 0.5-0.7, and incubated for another 4 h. The recombinant DhaK was expressed in the soluble fraction, and appeared as a major band on SDS-PAGE. His-tagged DhaK was purified by Ni^{2+} affinity

column chromatography. Cells were suspended in Binding buffer containing Hepes-NaOH (50 mM, pH 8.0), NaCl (500 mM), imidazole (20 mM), and β-mercaptoethanol (1 mM), and disrupted by sonication. After removing debris by centrifuge, the soluble fraction was applied onto Ni^{2+} affinity column, washed with binding buffer, and His-tagged DhaK was eluted with Elution buffer (Binding buffer containing 250 mM imidazol). The eluant was collected and dialyzed against buffer containing Hepes-NaOH (10 mM, pH 7.5), NaCl (100 mM), and β-mercaptoethanol (2 mM).

DhaK activity was determined spectrophotometrically at 30^{o}C by monitoring the decrease of absorbance at 340 nm under the following conditions. Tris-HCl (50 mM, pH 8.0), DHA (1 mM), ATP (1 mM), NADH (0.25 mM), dithiothieitol (1 mM), $MgCl_2$ (2.5 mM), α-glycerophosphate dehydrogenase (6.4 U ml^{-1}), and triosephosphate isomerase (11 U ml^{-1}).

Synthesis of sugar phosphates. DHAP was synthesized as described by Jung *et al.* [4] L-Rhamnulose 1-phosphate and L-fructose 1-phosphate were synthesized from DHAP and DL-lactaldehyde or DL-glyceraldehyde, respectively, by aldol condensation using *E. coli* BL21 (DE3) cells harboring pETDRhaD as a catalyst according to the methods of Liu *et al.* [5] and Alajarin *et al.* [6]

RhaD activity assays. Retroaldol activities for L-rhamnulose 1-phosphate and L-fructose 1-phosphate were determined spectrophotometrically at 25^{o}C by monitoring the decrease of an absorbance at 340 nm under the following conditions. Tris-HCl (50 mM, pH 7.5), KCl (50 mM), sugar phosphate (1 mM), NADH (0.25 mM), α-glycerophosphate dehydrogenase (6.4 U ml^{-1}), triosephosphate isomerase (11 U ml^{-1}).

Retroaldol activitiy assays for L-rhamnulose or L-fructose in the presence of borate were also performed as following, sodium borate (100 mM, pH7.6), KCl (50 mM), DhaK (0.75 U ml^{-1}), ATP (1 mM), NADH (0.25 mM), dithiothieitol (1 mM), $MgCl_2$ (2.5 mM), α-glycerophosphate dehydrogenase (6.4 U ml^{-1}), triosephosphate isomerase (11 U ml^{-1}) and RhaD. It was confirmed that DHA can be detected in the presence of borate under this condition.

Aldol condensation activities for L-rhamnulose 1-phosphate or L-fructose 1-phosphate formation were determined as following. Aldol reactions were performed in 300 μl Tris-HCl buffer (50 mM, pH 7.5) containing KCl (50 mM), DHAP (20 mM), DL-lactaldehyde (80 mM) or DL-glyceraldehyde (80 mM), and RhaD. At certain intervals, 50 μl aliquots were withdrawn and quenched with percholic acid (15 μl, 7% (w/v)). After 30 min 10 μl of NaOH (1M) was added followed by the addition of 175 μl of Tris-HCl (50 mM, pH7.5). Thus obtained neutralized mixtures were assayed for

DHAP by a coupled enzyme assay as follows, Tris-HCl (50 mM, pH 7.5), NADH (0.25 mM), α-glycerophosphate dehydrogenase (6.4 U ml^{-1}), triosephosphate isomerase (11 U ml^{-1}), and diluted DHAP mixtures. After incubation at room temperature for 5 min, the decrease of absorbance at 340 nm was monitored.

Aldol condensation activities for L-rhamnulose or L-fructose formation in the presence of borate were determined by measuring the concentration of the products by HPLC. The reactions were performed at 25^{o}C in 200 μl of sodium borate buffer (100 mM, pH7.6), KCl (50 mM), DL-lactaldehyde (100 mM) or DL-glyceraldehyde (100 mM), DHA (50 mM), and RhaD. At certain intervals, 40 μl aliquots were taken and quenched with 160 μl H_2SO_4 (6 mM), and produced sugars were determined by HPLC analysis.

HPLC analysis. HPLC analysis was performed on Hitachi L-7000 series system with a refractive index detector Shodex RI-71 (Showa Denko). Sugars and sugar phosphate concentrations were determined with a Shodex Sugar SC1101 column (Showa Denko). Distilled water was used as isocratic eluent at the flow rate of 1 ml min^{-1}, and column temperature was set at 80^{o}C. An HP87X-H column (Bio-Rad) was also used to determine L-rhamnulose and L-fructose with H_2SO_4 (6 mM) as isocratic eluent at the flow rate of 0.6 ml mim^{-1}, and column temperature was set at 50^{o}C.

Effects of borate and DHA concentration on L-fructose synthesis. To examine the effect of borate concentration on L-fructose synthesis (Fig. 2a), the reactions were performed as follows, Sodium borate buffer (pH 7.6, concentration was varied from 10 to 400 mM), DHA (50 mM), DL-glyceraldehyde (100 mM), *E. coli* BL21 (DE3) cells harboring pETDRhaD (50 mg ml^{-1}), toluene (1% (v/v)), 37., 2 h. DHA concentration was also varied as follows (Fig2b), Sodium borate buffer (200 mM, pH 7.6), DHA (10 to 200 mM), DL-glyceraldehyde (100 mM), *E. coli* BL21 (DE3) cells harboring pETDRhaD (50 mg ml^{-1}), toluene (1% (v/v)), 37^{o}, 2 h. After the incubation, L-fructose concentration was determined by HPLC analysis.

One-pot synthesis of L-rhamnulose. Ozone gas was injected into a solution containing methanol (30 ml), CH_2Cl_2 (100 ml), and 3-butene-2-ol (5.12 g, 71.1 mmol) at -80^{o}C until the color of the reaction solution became pale blue. After bubbling nitrogen to remove excess ozone, the ozonide was treated with dimethyl sulfide (10 ml) for 30 min at -80^{o}C and for 3h at room temperature. After concentrating the solvent by bubbling in nitrogen at room temperature overnight, thus obtained solution was used directly (approximately 6.6 M DL-lactaldehyde as determined by HPLC or enzymatic assay using aldehyde dehydrogenase[7]). To 125 μl of DL-lactaldehyde solution (0.83 mmol), 1M sodium borate buffer (2 ml, final concentration 200 mM), 1M DHA (0.5 ml, 0.5 mmol), toluene

(10 μl) were added and filled to 10 ml with water. To this reaction mixture, *E. coli* BL21 (DE3) cells harboring pETDRhaD (0.2 g by wet weight) was suspended. The reaction mixture was shaken at 37°C for 16 h, and cells were removed by centrifuge. The reaction mixture was lyophilized and purified using chromatography on silica gel with ethyl acetate/methanol/water (40/10/5) as eluent. Fractions containing L-rhamnulose were collected and concentrated. A second round of silica gel chromatography with ethyl acetate/methanol (40/10) gave L-rhamnulose (36 mg, 0.22 mmol, 53% based on L-lactaldehyde) after evaporation. ^{1}H NMR and ^{13}C NMR was identical to authentic L-rhamnulose[5] and 6-deoxy-D-fructose[8] as previously reported.

Chemo-enzymatic synthesis of L-iminocyclitols from azido aldehydes and DHA in the presence of borate. For the synthesis of iminocyclitol **1**, to a solution of 3-azido-2-hydroxy-propionaldehyde (173 mg, 1.5 mmol) and DHA (135 mg, 1.5 mmol) in sodium borate buffer (200 mM, 15 ml, pH7.6), toluene (100 μl) was added and *E. coli* BL21 (DE3) cells harboring pETDRhaD (0.3 g by wet weight) was suspended. For the synthesis of iminocyclitol **2**, to a solution of azido-acetaldehyde (43 mg, 0.5 mmol) and DHA (90 mg, 1.0 mmol) in sodium borate buffer (200 mM, 10 ml, pH7.6), toluene (100 μl) was added and *E. coli* BL21 (DE3) cells harboring pETDRhaD (0.2 g by wet weight) was suspended. The reaction mixture was shaken at 37°C for 6 h and cells were removed

by centrifuge. For the synthesis of iminocyclitols **3**, to a solution of 2-azido-3-hydroxy-propionaldehyde (173 mg, 1.5 mmol) and DHA (135 mg, 1.5 mmol) in borate buffer (200 mM, 15 ml, pH7.6), toluene (200 µl) was added and RhaD-expressing *E. coli* cells (0.3 g) were used. For the synthesis of iminocyclitol **4**, to a solution of N-(2-azido-3-oxo-propyl)-acetamide (155 mg, 1.0 mmol) and DHA (90 mg, 1.0 mmol) in borate buffer (200 mM, 10 ml, pH7.6), toluene (100 µl) was added and RhaD-expressing *E. coli* cells (0.2 g) were used. The reaction mixture was shaken at 37^{o}C for 6 h and cells were removed by centrifuge.

General procedure for synthesis and isolation of L-iminocyclitols

After lyophilization of the enzymatic reaction, the remaining residue was dissolved in MeOH and Iatrobeads™ 6RS-8060 silica gel (Mitsubishi Kagaku Iatron, Inc.) (3 mL) was added. Following evaporation of the solvent, the azidoketones were isolated by flash chromatography over Iatrobeads™, using the following solvent systems: **1**: 90:10 CH_2Cl_2:MeOH, 80:20 CH_2Cl_2:MeOH, then 60:40 CH_2Cl_2:MeOH; **2**: 19:1 EtOAc:MeOH; **3**: 90:10 CH_2Cl_2:MeOH then 85:15 CH_2Cl_2:MeOH; **4**: 90:10 CH_2Cl_2:MeOH, 85:15 CH_2Cl_2:MeOH, then 80:20 CH_2Cl_2:MeOH. Each isolated azidoketone was then dissolved in MeOH (1.5 mL) and 10% Pd/C was added (50 mg per mmol substrate). The reaction was pressurized to 50 psi H_2 and allowed to stir for 12 h,

at which time TLC indicated consumption of starting material. The Pd catalyst was removed by filtration over Celite and the reaction was concentrated. Flash chromatography of the residue over Iatrobeads™ using 6:4:1:0.1 CH_2Cl_2:MeOH:H_2O:NH_4OH provided iminocyclitols 1-4. Isolated yields over 2 steps, based on azido aldehyde starting material, were as follows: **1**: 17%; **2**: 4%; **3a,b**: 3%; **4**: 9%.

Optical rotation data: 1: $[\alpha]^{23}_D = +30^o$ (c = 3, MeOH) (lit: + 28^o, c = 1, MeOH); **2**: $[\alpha]^{23}_D = -13^o$ (c = 0.5, MeOH) (lit: -18^o, c = 1.5, MeOH), **3a/3b mixture**: $[\alpha]^{23}_D = -37^o$ (c = 0.6, MeOH) (**3a** lit: -23.6^o, c = 2, H_2O, **3b** lit: -52.7^o, c = 0.28, H_2O), **4**: $[\alpha]^{23}_D = -11^o$ (c = 0.8, MeOH) (lit for enantiomer: $+12.8^o$, c = 0.43, MeOH).

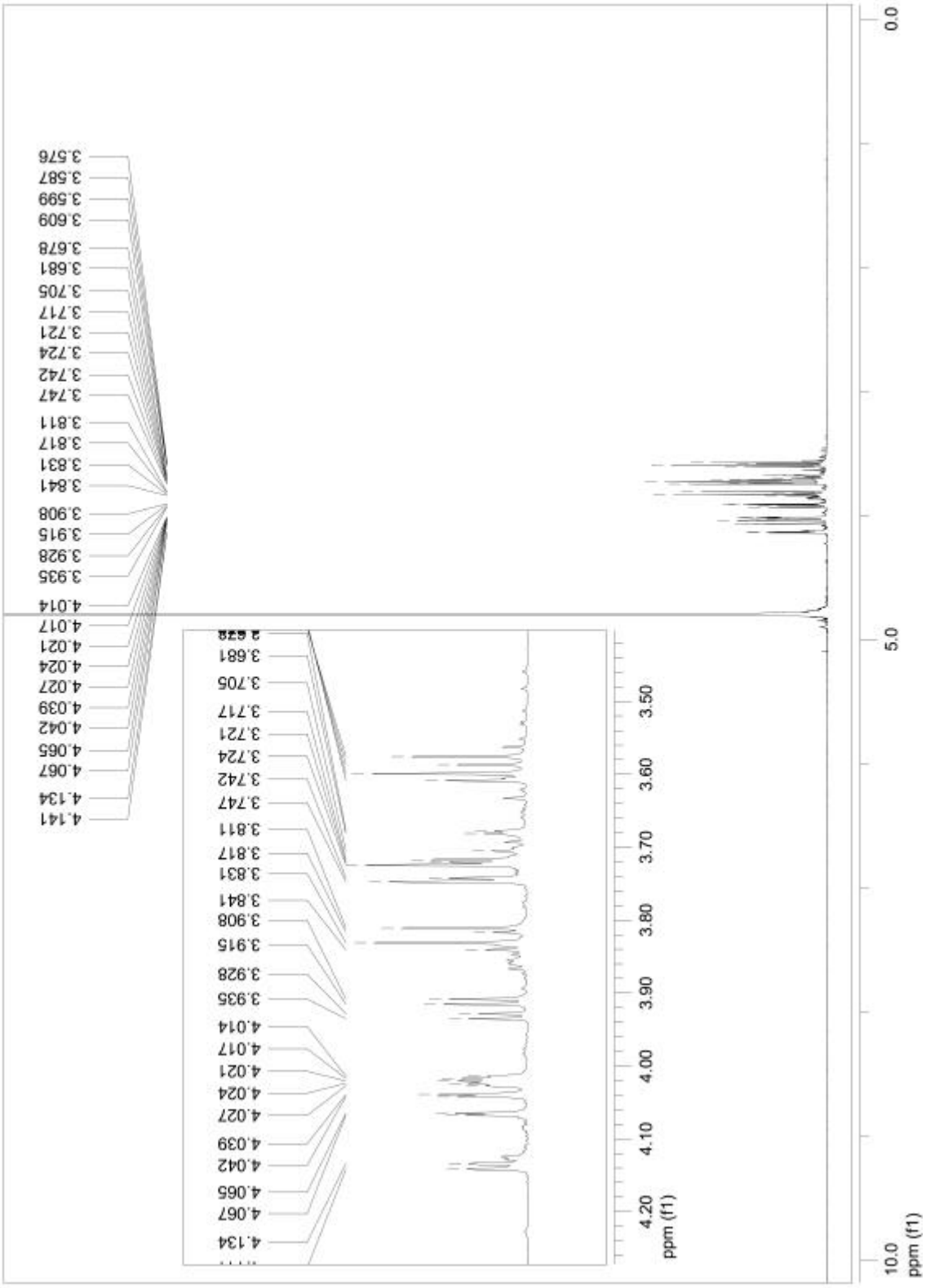

Figure S1. ^{1}H NMR spectra of synthesized L-fructose.

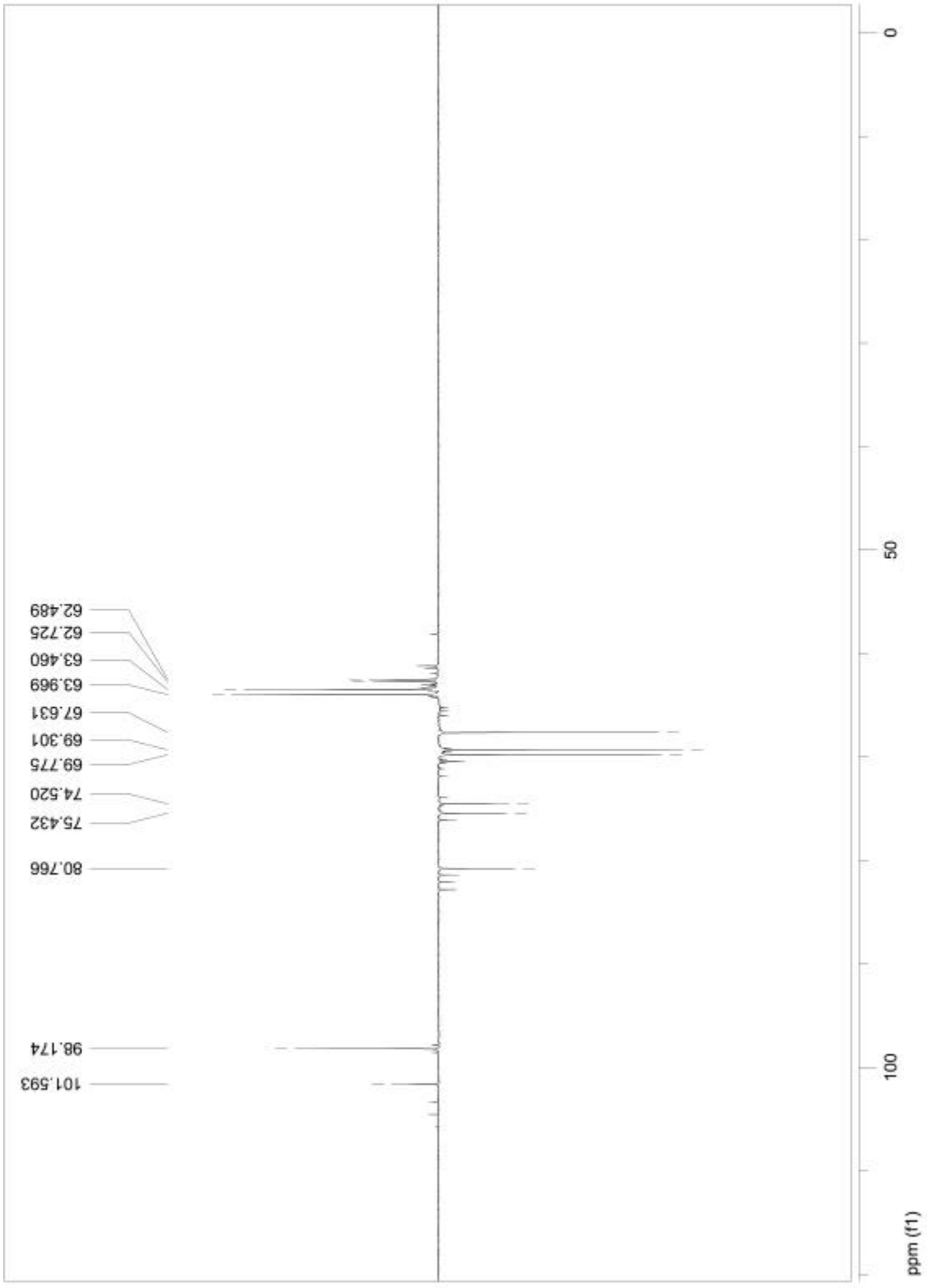

Figure S2. ^{13}C NMR spectra of synthesized L-fructose.

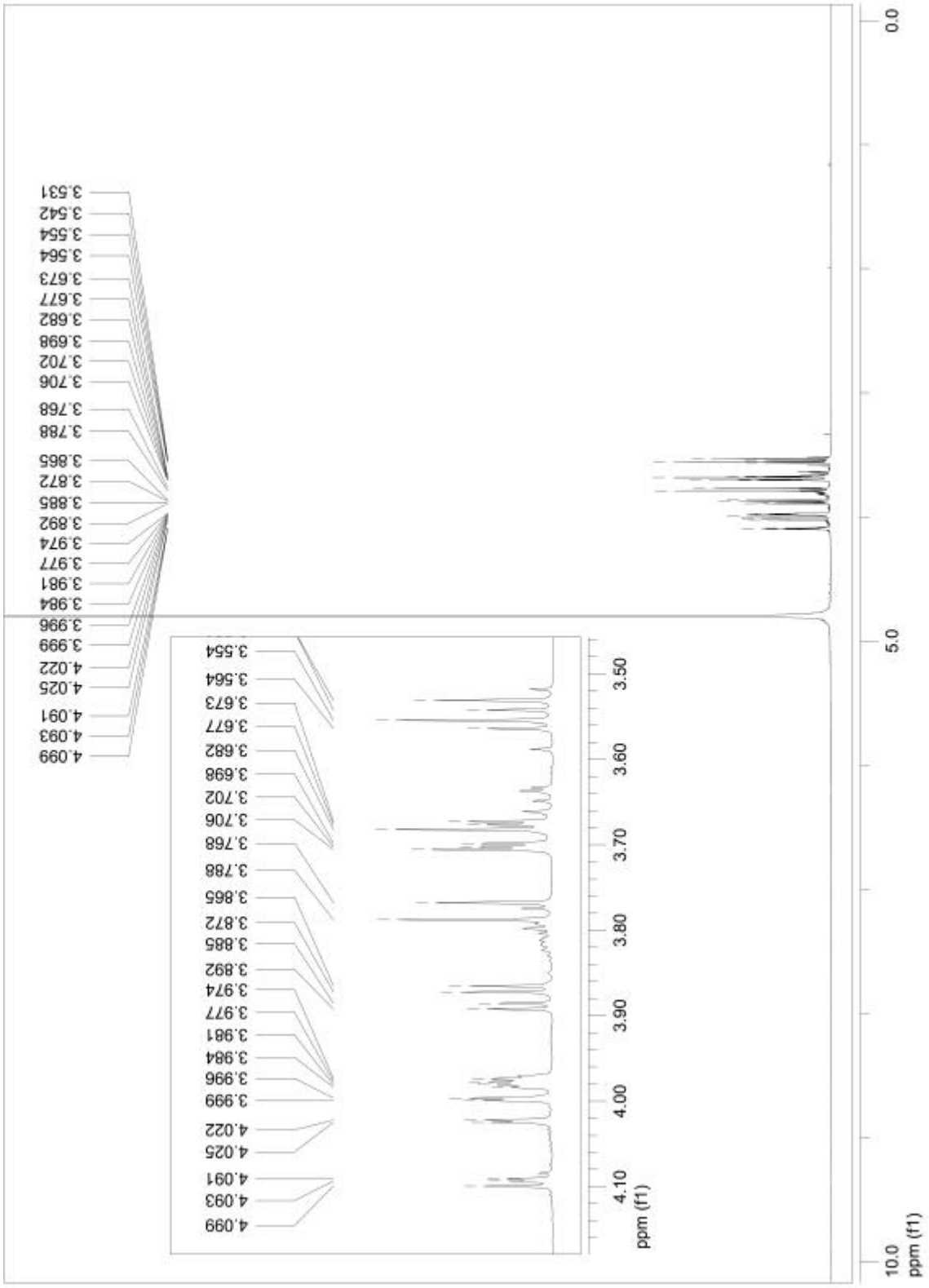

Figure S3. ^{1}H NMR spectra of authentic D-fructose.

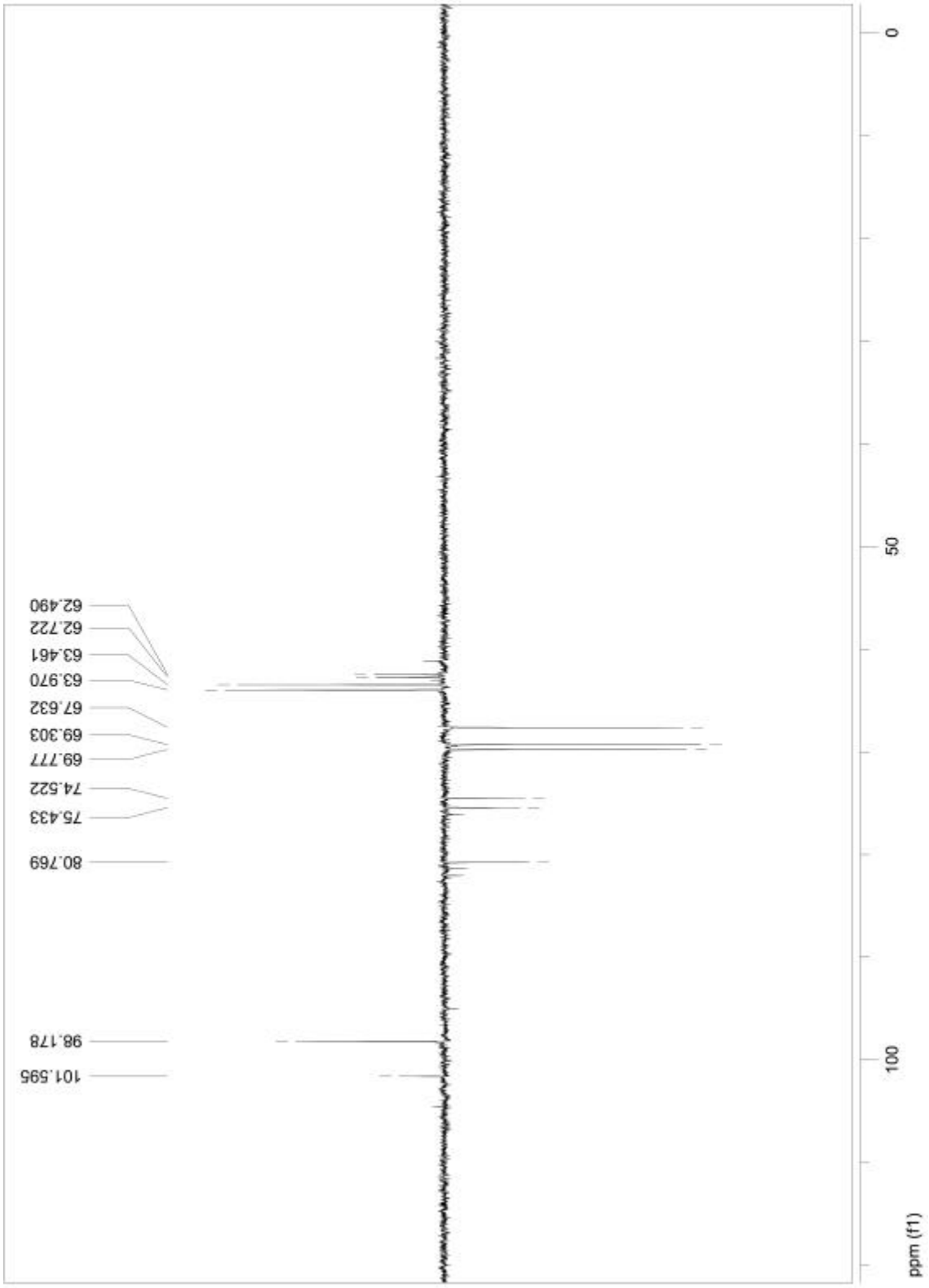

Figure S4. ^{13}C NMR spectra of authentic D-fructose.

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