Exploratory Experiments on the Chemistry of the "Glyoxylate Scenario": Formation of Ketosugars from Dihydroxyfumarate.

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Supporting Information

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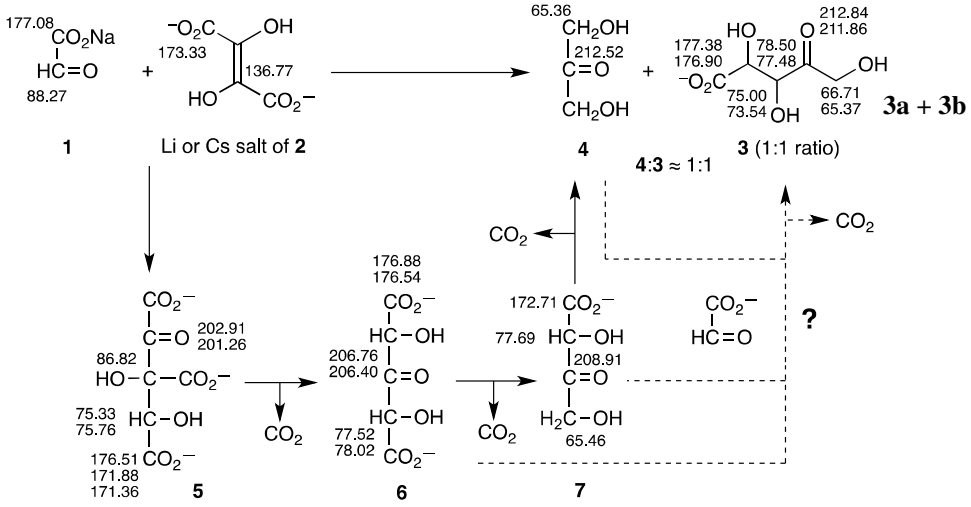
General experimental details:

Anhydrous lithium hydroxide (reagent grade) (#44241-0; batch # 10213CJ; CAS-1310-65-2); dihydroxyfumaric acid hydrate, 98%, solid; formalin, ACS grade, 37 wt % in H_2O, contains 10-15% MeOH as stabilizer; glycoaldehyde dimer, crystalline solid; (DL)-glyceraldehyde dimer, ≥ 97%, solid; 1,3-dihydroxyacetone dimer, 97%, solid; (S)-(+)-erythrulose hydrate (syrup); D-xylulose, 95%, syrup; D-ribulose, 95%, syrup; cesium carbonate, 99.995%; and sodium hydroxide, 97%, solid, 2,4-DNPH, 97% solid were obtained from *Sigma-Aldrich*. Glyoxylic acid monohydrate, 98%, solid was obtained from *Acros organics*. Doubly ^{13}C labeled glyoxylic acid, 99%, [contains glycolic acid (3%) and oxalic acid (1.5 %)] was obtained from Cambridge Isotope Laboratories Inc. Degassed water was used through out the experiments involving DHF. The 'degassed water' was prepared by purging N_2 gas into the 'deionized water' for 20 min. and then subjecting the 'deionized water' to house-vacuum for 30 min. This procedure was repeated 3 times. The organic solvents were all reagent grade.

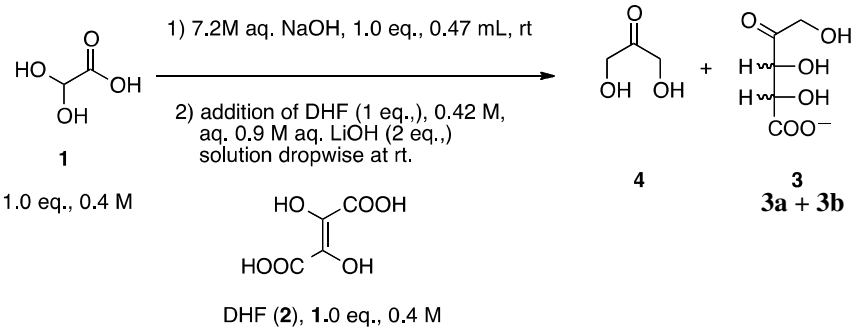
pH was measured using a *Accumet Research* AR25 pH meter; for smaller volumes *colorphast* pH-indicator strips (pH 0-14) were used. UV spectroscopy was measured on a *Cary* 1 C spectrophotometer (*Varian*). ^1H NMR and ^{13}C NMR spectra were recorded (at r.t. and at 4°C) with a Bruker WM 600 MHz spectrometer and on a Bruker DRX-600 equipped with a 5mm DCH cryoprobe. Samples for NMR studies were prepared by adding either 10% D_2O or a drop of DMSO-d_6. DMSO-d_6 was used as internal standard for the ^1H NMR spectra (s=singlet, d= doublet, t=triplet, br s=broad signal, q=quadruplt, m=multiplet); DMSO-d_6 (39.6 ppm) or D_2O for the ^{13}C NMR spectra. Chemical shifts δ values is expressed in *ppm* and coupling constants *J* in *Hz*. Multiplicities of carbon (CH_3, CH_2, CH and C) were determined by APT and DEPT ^{13}C NMR spectroscopy. High-resolution FAB-MS (ESI) was performed on a VG ZAB2 SEQ double focusing high-resolution mass spectrometer equipped with a cesium ion gun using a 3-nitrobenzyl alcohol/CsI matrix.

Abbreviations used: DHF = Dihydroxyfumarate; DHA = Dihydroxyacetone; 2,4-DNPH = 2,4-dinitrophenylhydrazine; P = pentulosonic acid; r.t. = Room temperature.

I. Reaction of dihydroxyfumaric acid (DHF) with glyoxylic acid



I.1. Reaction of DHF with glyoxylic acid in the presence of aq. LiOH/NaOH at r.t.



Scheme S1: Reaction of DHF with glyoxylic acid in the presence of aq. LiOH/NaOH at r.t.

Experimental procedure: The experiment was preformed according to the procedure described in the experimental section of the main paper. ^{13}C NMR (H_2O/D_2O) monitoring of the reaction of 0.4 M di-lithium salt of DHF with 0.4 M Na-glyoxylate (see Figure S1).

After 20 min., ^{13}C NMR (Figure S2) showed presence of dicarboxyl-dihydroxyacetone **6** as a major intermediate.
After 2 h, ^{13}C NMR (Figure S3) showed presence of monocarboxyl-dihydroxyacetone **7** as a major intermediate along with small amount of dihydroxyacetone **4** (DHA).
After 4 h, ^{13}C NMR (Figure S4) showed presence of **7**, **4**, and diastereomeric mixture of pentulosonic acid **3a** + **3b**.
After 24.0 h, ^{13}C NMR (Figure S5) showed presence of small amount of **7**, **4**, and diastereomeric mixture of **3**.
After 48 h, ^{13}C NMR (Figures S6 and S7) showed presence of **4** (and its hydrate), and diastereomeric mixture of **3a** + **3b**.

The reaction was also carried out 4°C, where after 24 h, predominant formation of the monocarboxyl-DHA (**7**) was observed (Figure S8).

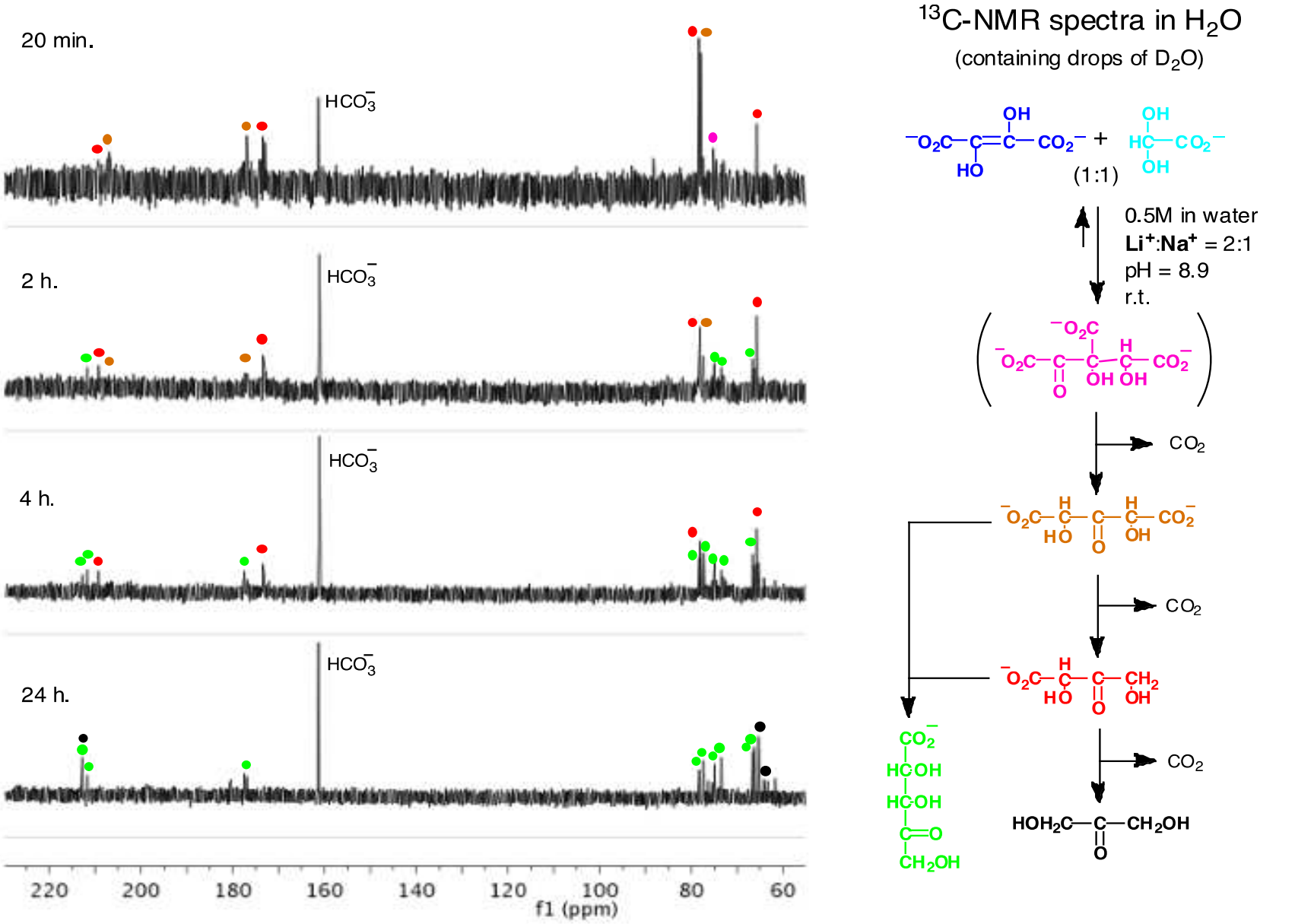


Figure S1. ^{13}C NMR (H_2O/D_2O) monitoring of the reaction of 0.4 M Li_2DHF with 0.4 M Na-glyoxylate, at r.t., over a 24 h period (shown is the expanded region of 60-220 ppm). Apart from the formation of dihydroxyacetone (**4**) (and its hydrate), the diastereomers of pentulosonic acid (**3a** + **3b**) were also observed.

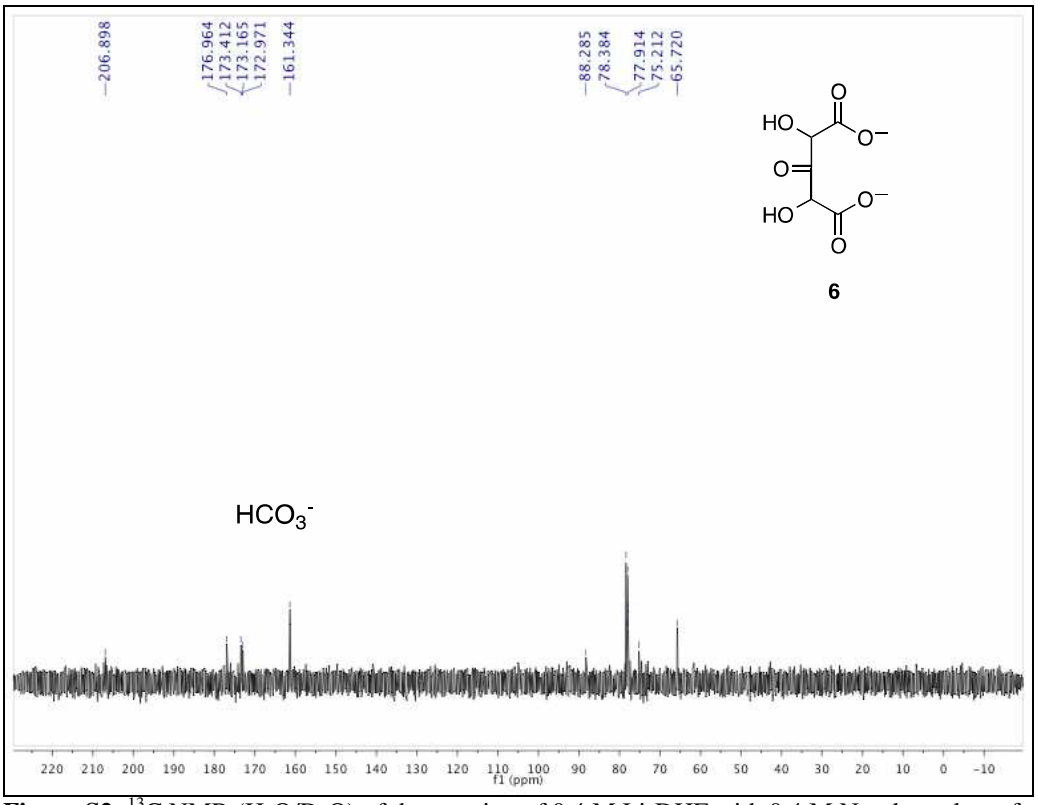


Figure S2: ^{13}C NMR (H_2O/D_2O) of the reaction of 0.4 M Li_2DHF with 0.4 M Na-glyoxylate after 20 min., at r.t. showing peaks for formationof **6** & **7**.

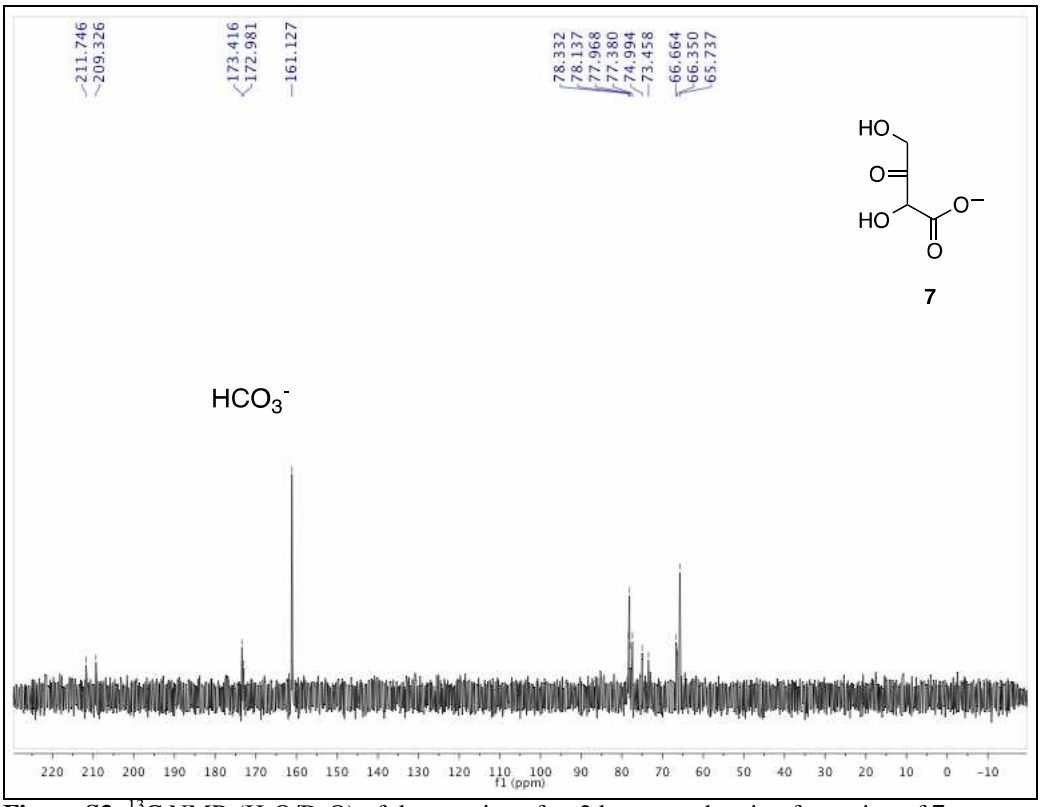


Figure S3: ^{13}C NMR (H_2O/D_2O) of the reaction after 2 h., at r.t. showing formation of **7**.

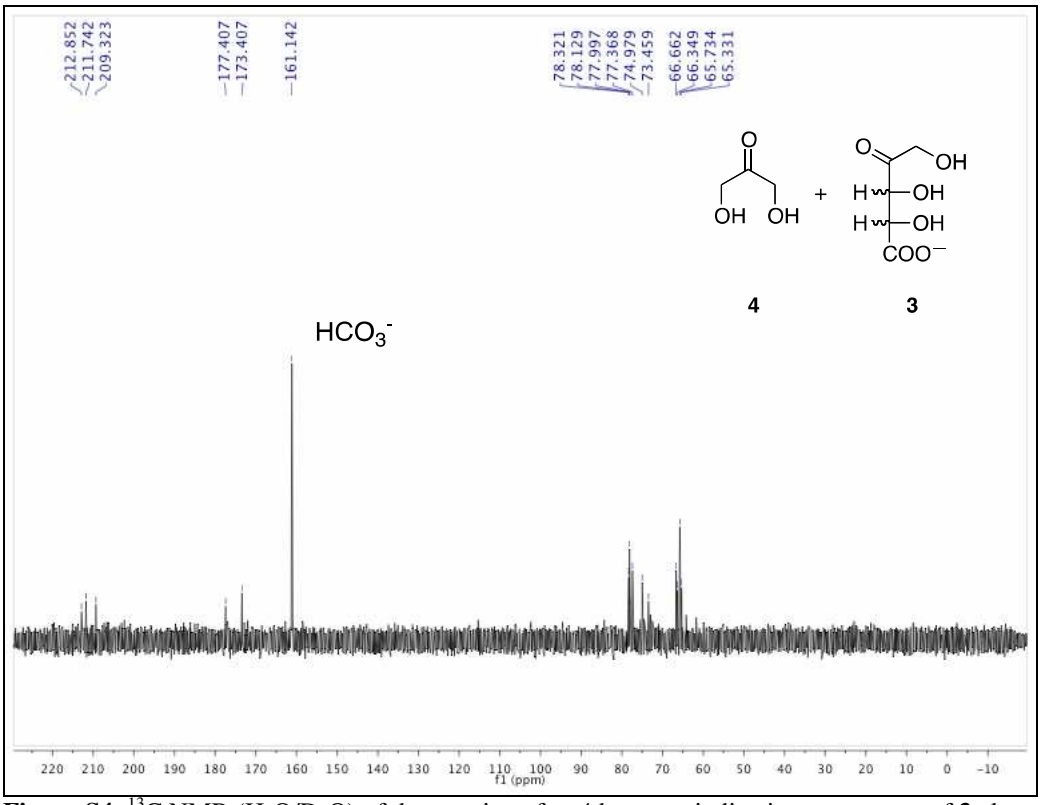


Figure S4: ^{13}C NMR (H_2O/D_2O) of the reaction after 4 h., at r.t. indicating emergence of **3** along with **4**.

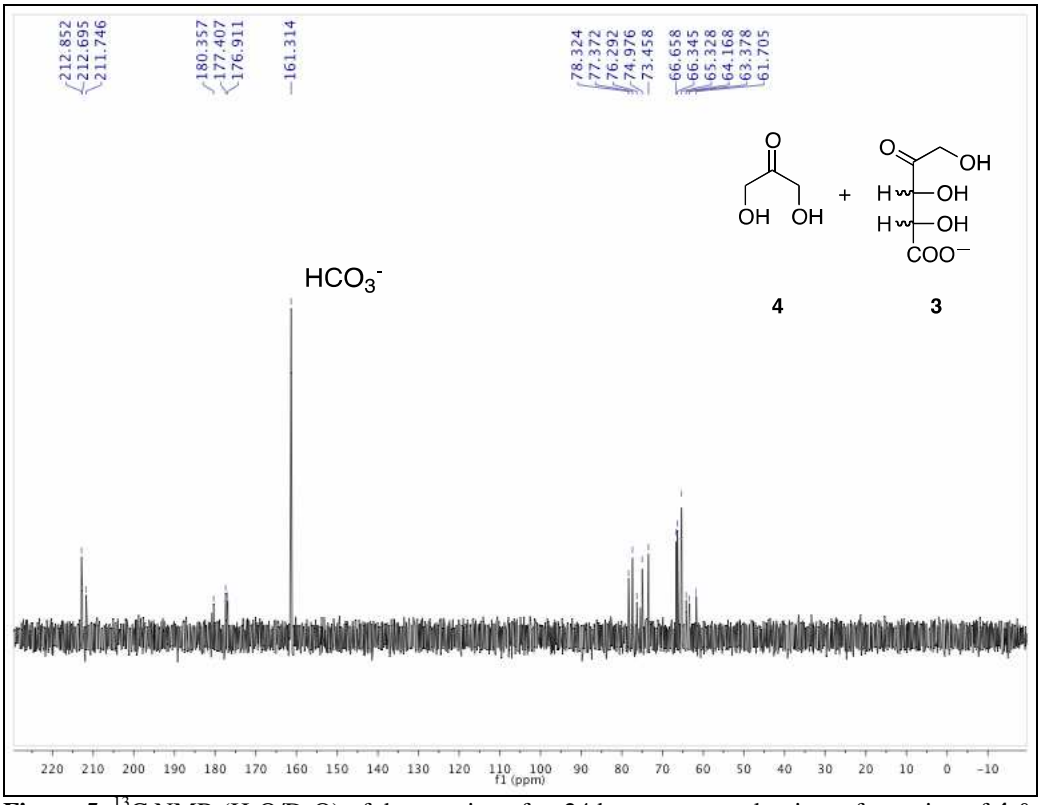


Figure 5: ^{13}C NMR (H_2O/D_2O) of the reaction after 24 h., at r.t. – predominant formation of **4** & **3**.

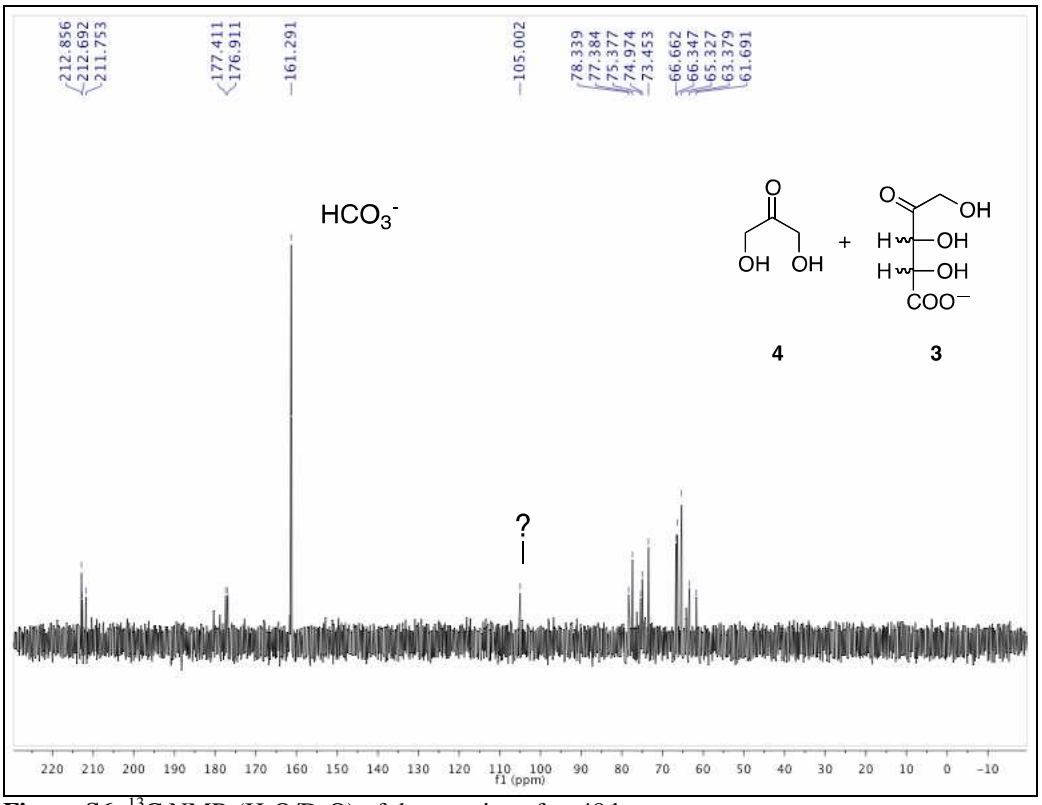


Figure S6: ^{13}C NMR (H_2O/D_2O) of the reaction after 48 h., at r.t.

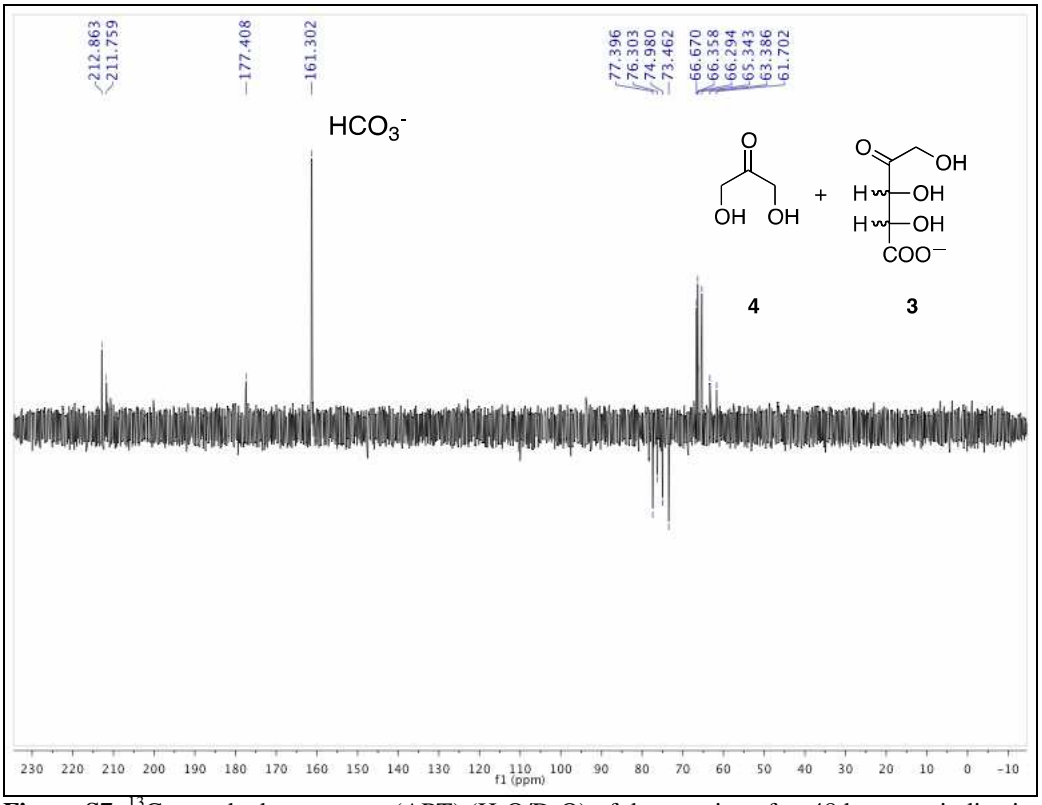


Figure S7: ^{13}C- attached proton test (APT) (H_2O/D_2O) of the reaction after 48 h., at r.t. indicating the multiplicities of the carbon (61-67 ppm – CH_2; 73-78 ppm – CH and 160-213 – quaternary carbons).

I.2. Reaction of DHF with glyoxylic acid in the presence of aq. LiOH/NaOH at **4** °C.

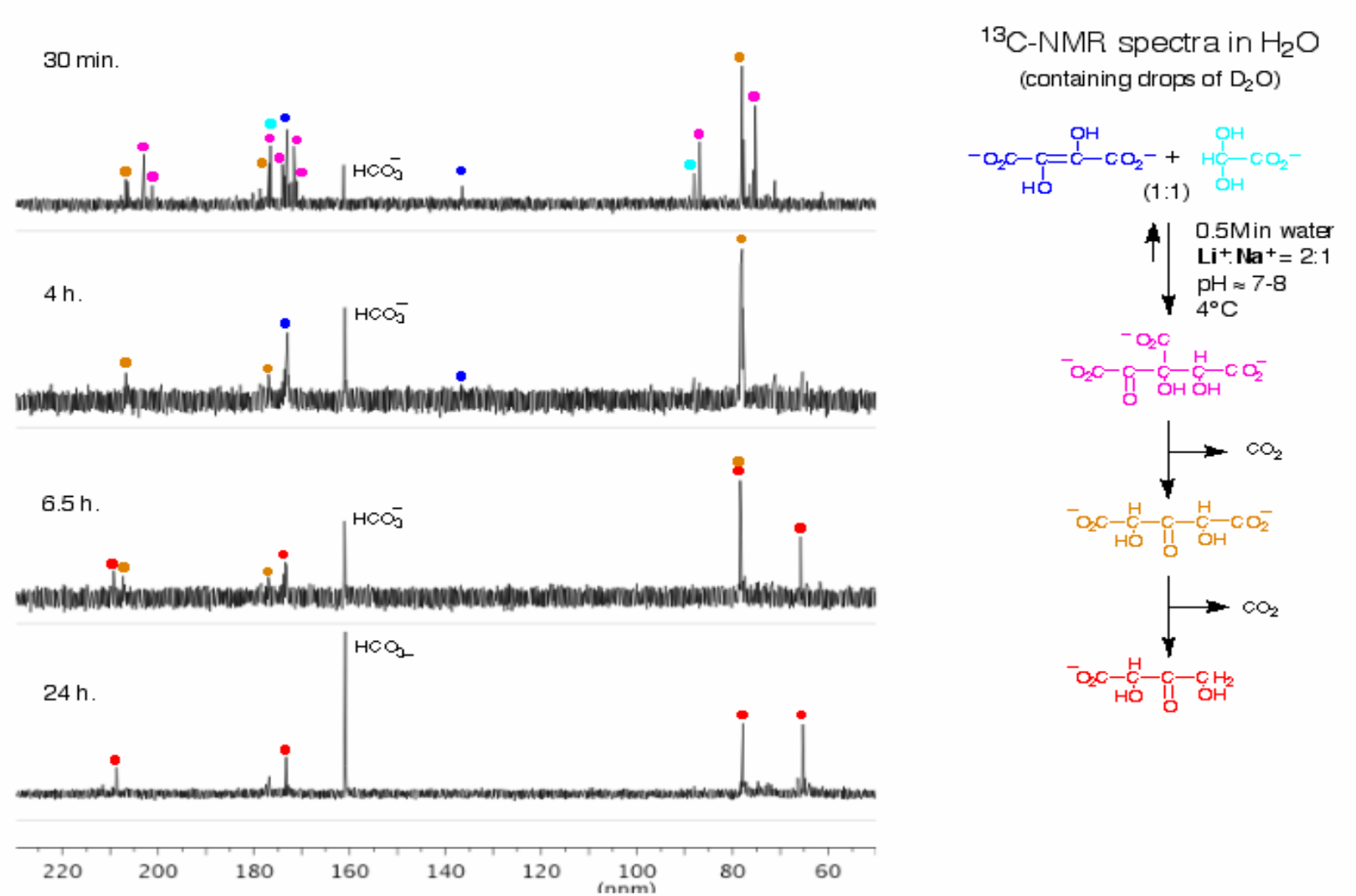


Figure S8. ^{13}C NMR (H_2O/D_2O) monitoring of the reaction of Li_2DHF with Na-glyoxylate at 4 °C. In 24 h the predominant formation of the monocarboxyl dihydroxyacetone intermediate (**7**) was observed (shown is the expanded region of 60-220 ppm).

I.3. Monitoring by UV-spectroscopy the difference in the rate of disappearance of the DHF by itself, and in the presence of glyoxylate.

1. DHF (500 mg, 1.0 eq., 3.38 mmol) was suspended in 3.75 mL degassed water under argon. To this 7.5 mL of aq. LiOH solution (0.9 M, 2.0 eq., 6.76 mmol) was added through syringe. Within 3 min. it became a clear solution. The concentration of DHF was 0.3 M; pH of the reaction was about 7-8 (pH paper).

2. DHF (500 mg, 1.0 eq., 3.38 mmol) and glyoxylic acid monohydrate (311 mg, 1.0 eq., 3.38 mmol) were taken in a round bottom flask. To this 7.5 mL of aq. LiOH solution (0.9 M, 3.0 eq., 10.134 mmol) was added through syringe at r.t. Within 3 min. it became a clear solution. The concentration of DHF and glyoxylic acid was 0.3 M each; pH of the solution was about 7-8.

From the above two solutions, 10 µL aliquot was taken (from each), diluted with 20 mL H_2O (degassed) and UV-spectrum[1] was measured immediately; the starting concentrations of both DHF and glyoxylate in this diluted solution would be 0.15 mM. For each individual measurement, fresh aliquots from the original solution (which was kept under argon) were taken.

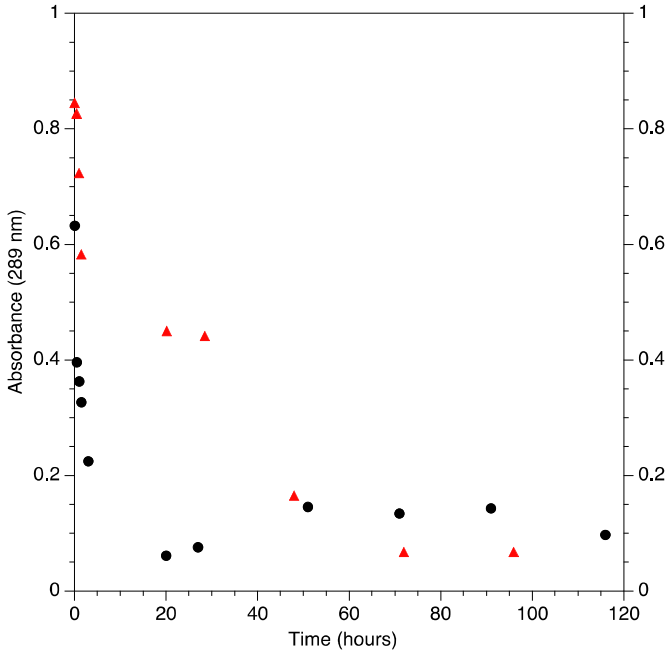
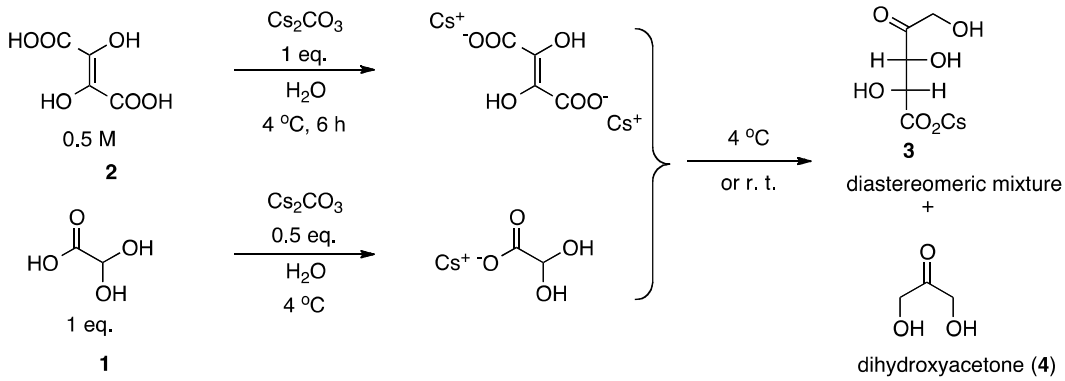


Figure S9. Monitoring the absorbance at 289 nm with time: comparison of Li_2DHF alone in water (red triangles) with Li_2DHF in the presence of glyoxylate in water (black dots).

The UV absorbance measurements in Figure S9 showed that Li_2DHF was consumed much faster in the presence of glyoxylate when compared to Li_2DHF alone.

1. DHF has an absorption at λ_{max} = 289 nm (ε = 9130 L.mol^{-1}.cm^{-1}). Hay, R. W., Harvie, S. J. *Aust. J. Chem.* **1965**, *18*, 1197-1209.

I.4. Reaction of DHF with glyoxylic acid in the presence of aq. Cs_2CO_3



Scheme S2

DHF (400 mg, 2.7 mmol, 1 eq.) was suspended in 1.2 mL H_2O (degassed) at 4 °C. Cs_2CO_3 (885 mg, 2.7 mmol, 1 eq.) was dissolved in 1.5 mL H_2O (4 °C) and added dropwise into the above suspension. It became a clear dark red/brown solution within 6 h at 4 °C.

Separately, glyoxylic acid (249 mg, 2.7 mmol, 1 eq.) was dissolved in 1.2 mL H_2O at 4 °C. Cs_2CO_3 (443 mg, 1.35 mmol, 0.5 eq.) was dissolved in 1.5 mL (4 °C) and added drop wise to this solution.

The above two solutions were mixed at 4 °C and gave a clear dark solution (pH 8.7).

The dark solution was divided into two portions.

- The first portion was kept at 4 °C (reaction I.4.a)
- The second portion was kept at r.t. (reaction I.4.b.)

All reactions were monitored by ^{13}C NMR (H_2O/D_2O). ^{13}C NMR spectra measurements (Figure S10) indicated that at 4 °C, the reaction proceeded slower - when compared to the reaction at r.t.; and ^{13}C NMR was able to detect all the intermediates (**5**, **6** and **7**) en route to the formation of pentulosnic acid (**3**) and dihydroxyacetone (**4**). In the r.t. reaction, by 20 hours (Figure S11), the complete conversion to **4** and **3** was observed, while at 4°C it takes almost 380 h to reach a similar stage (Figure S12).

As was the case with the reaction in the presence of aq. LiOH, the reaction of DHF with glyoxylate in the presence of aq. Cs_2CO_3 led to an approximately1:1 mixture of diastereomers of pentulosonic acid (**3a 3b**) as seen in figures S11 & S12.

I.4.a. Reaction of 0.5M DHF with 0.5 M glyoxylic acid in the presence of aq. Cs_2CO_3 at 4°C.

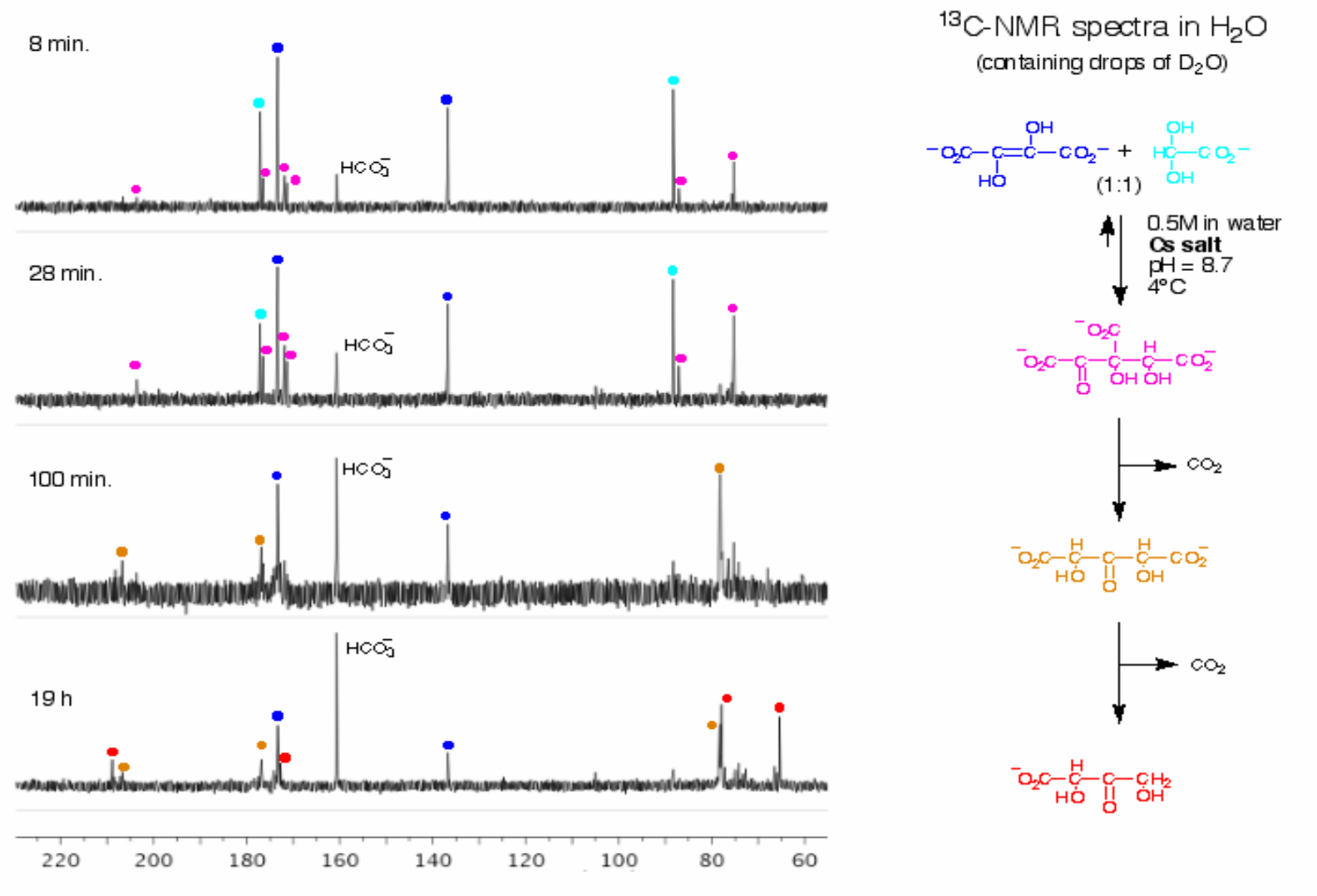


Figure S10. ^{13}C NMR monitoring of the reaction of 0.5 M di-cesium salt of DHF with 0.5M Cs-glyoxylate at 4 °C (shown is the expanded region of 60-220 ppm). There were no signals below the 60 ppm region.

I.4.b. Reaction of 0.5M DHF with 0.5M glyoxylic acid in the presence of aq. Cs_2CO_3 at r.t.

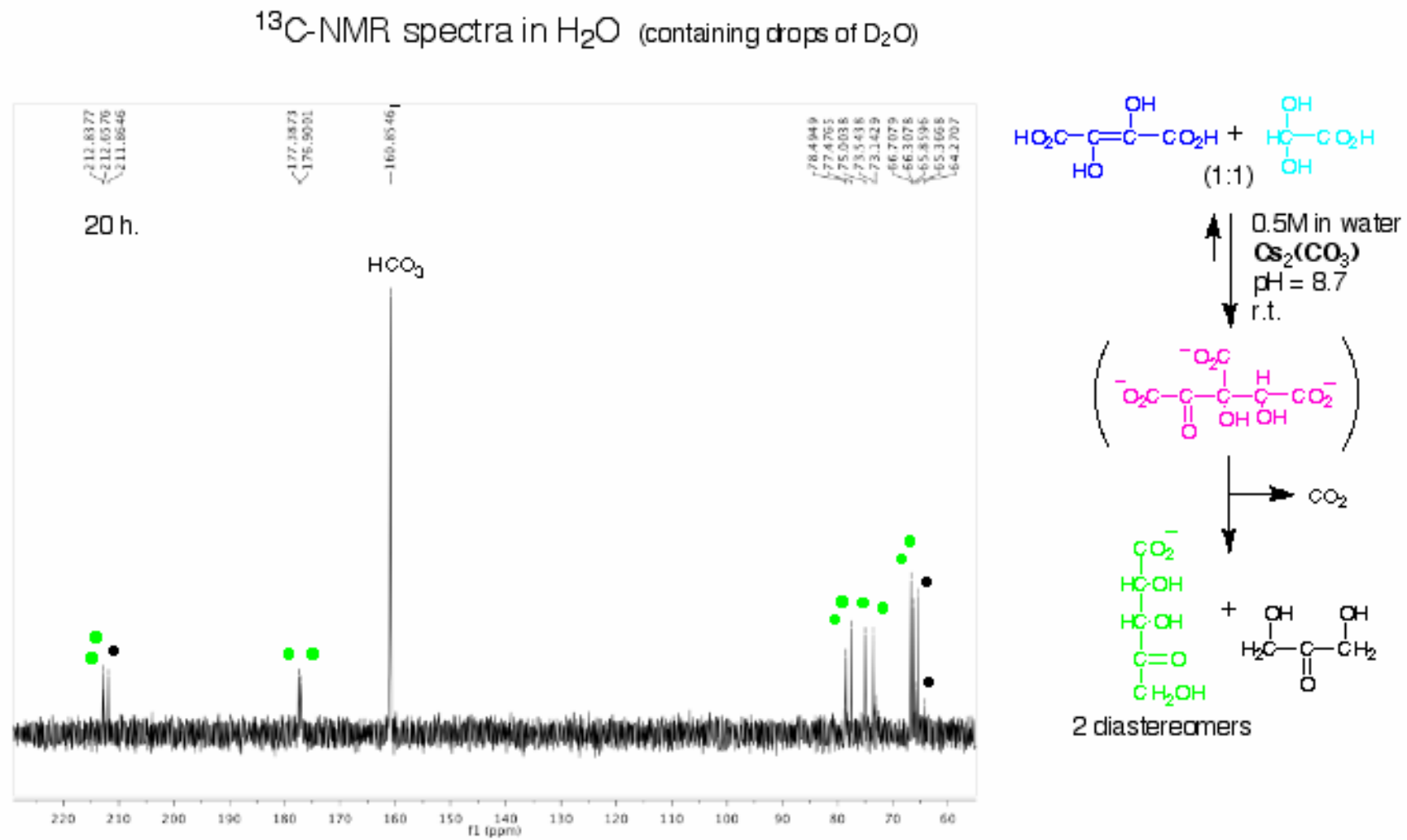


Figure S11: ^{13}C NMR of the reaction mixture showing the final formation of the two (≈ 1:1) diastereomers of pent-4-ulosonic acid (**3**) and dihydroxyacetone (**4**) in an almost 1:1 ratio, at 20 h (RT).

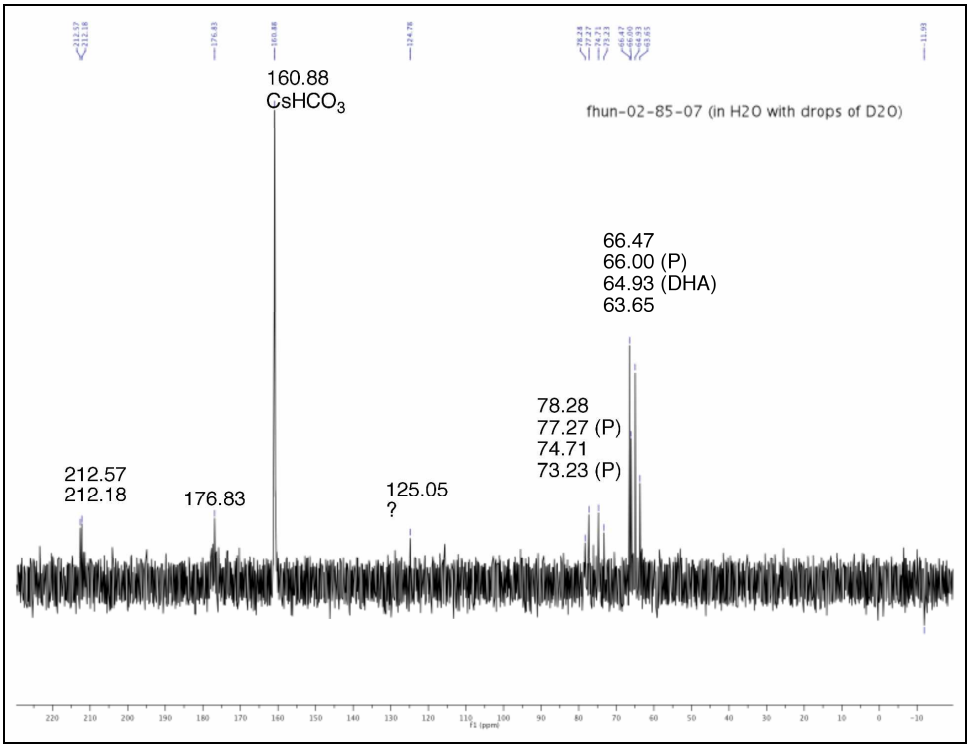


Figure S12: ^{13}C NMR (H_2O/D_2O) of reaction I.4.b. at 4 °C, (380 h); peak at 125 is attributed to CO_2. P = pentulosonic acid.

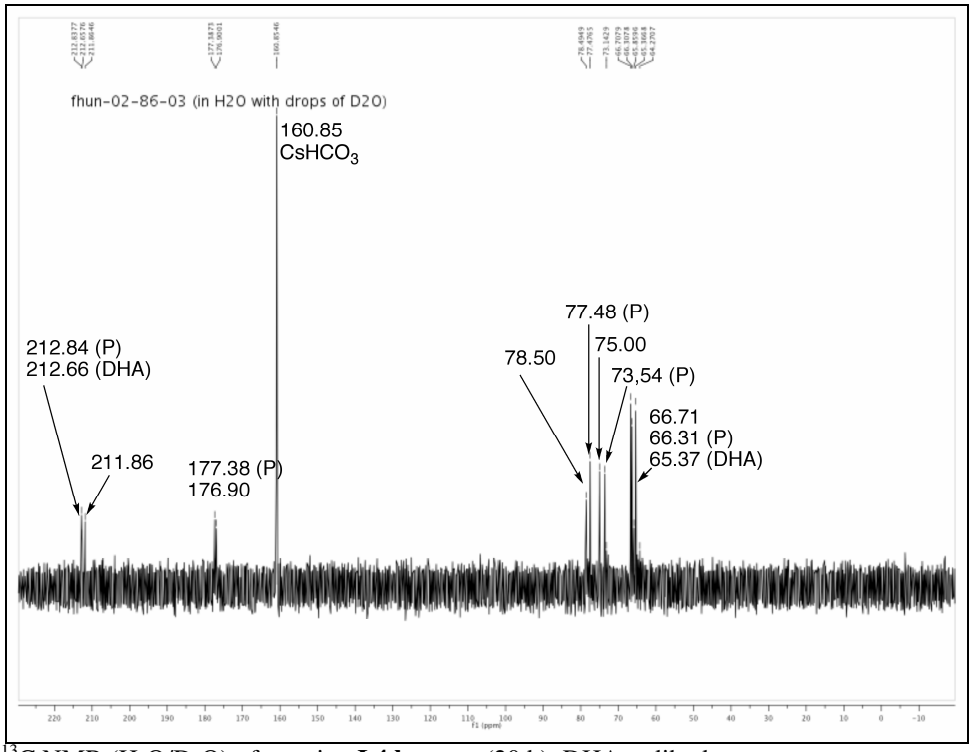
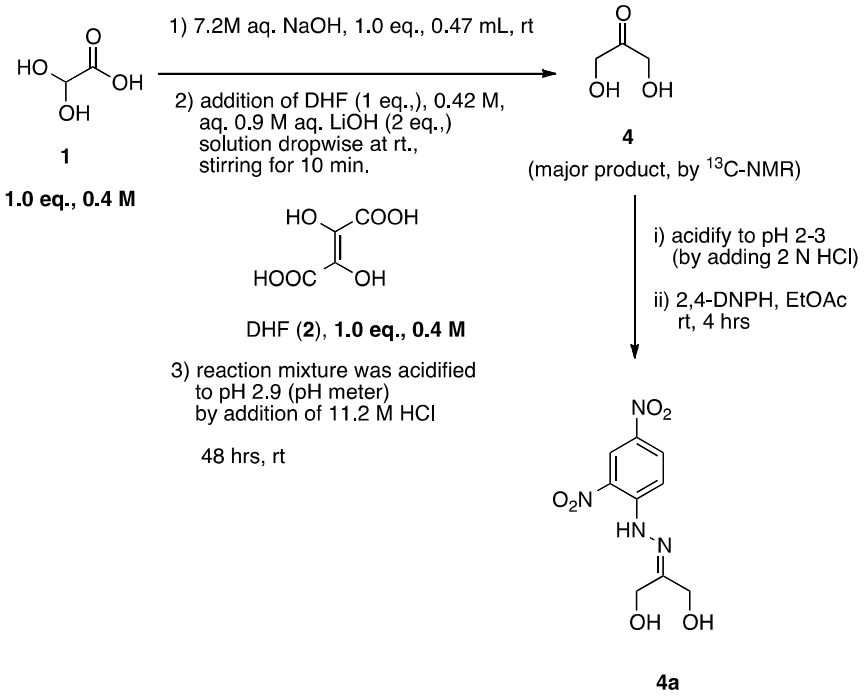


Figure S13: ^{13}C NMR (H_2O/D_2O) of reaction **I.4.b.** at r.t. (20 h). DHA = dihydroxyacetone.

I.5. Identification of dihydroxyacetone (formed from reaction of dilithium salt of DHF and Na salt of glyoxylic acid) as its 2,4-DNP derivative.



Scheme S3

DHF (500 mg, 1 eq., 3.378 mmol) was placed in a round bottom flask and kept under vacuum for 10 min. To this 7.5 mL of 0.9 M aq. LiOH solution was added through syringe at r.t. under nitrogen atmosphere and stirred at until it became a clear solution (approximately 3-5 min.). pH of the reaction mixture 7-8 (by pH paper).

Glyoxylic acid monohydrate (331 mg, 1.0 eq.) was taken into round bottom flask under nitrogen atmosphere and to this was added freshly prepared 7.2 M aqueous NaOH solution (0.47 mL, 1.0 eq.). Immediately heat was generated and was cooled to r.t. before treating with DHF dilithium salt.

After the preparation of both salts, aq.'DHF dilithium salt' solution was added drop wise to aq. sodium glyoxylate solution at r.t. under nitrogen atmosphere and stirring was continued at the same temperature. After the addition, the reaction became clear solution and pH of the resulting reaction mixture was 8.3. After 10 min., the reaction mixture was acidified to pH 2.9 (monitored by pH meter) at the r.t. by drop wise addition approximately 0.1 mL of 11.2 M aq. HCl, and continued stirring for 48 hrs. After 48 h, pH of the reaction mixture became 7.1 (by pH meter). Reaction mixture was kept nitrogen atmosphere (and not exposed) for 48 hrs. After 48 h, 0.4 mL of aliquot was taken from the reaction mixture [total volume of the reaction mixture was 8.1 mL] through syringe and ^{13}C NMR was measured in H_2O/D_2O (Figure S14), which indicated the predominant formation of dihydroxyacetone. *The same sample was spiked (Figure S16) with 0.3 M aq. authentic dihydroxyacetone (Figure S15).*

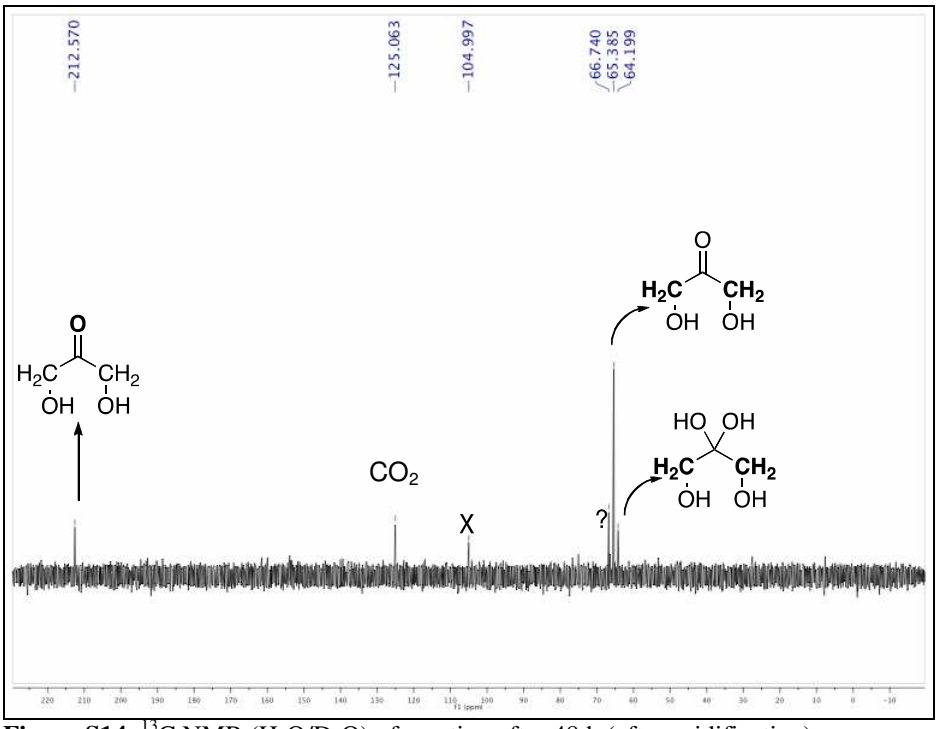


Figure S14: ^{13}C NMR (H_2O/D_2O) of reaction after 48 h (after acidification).

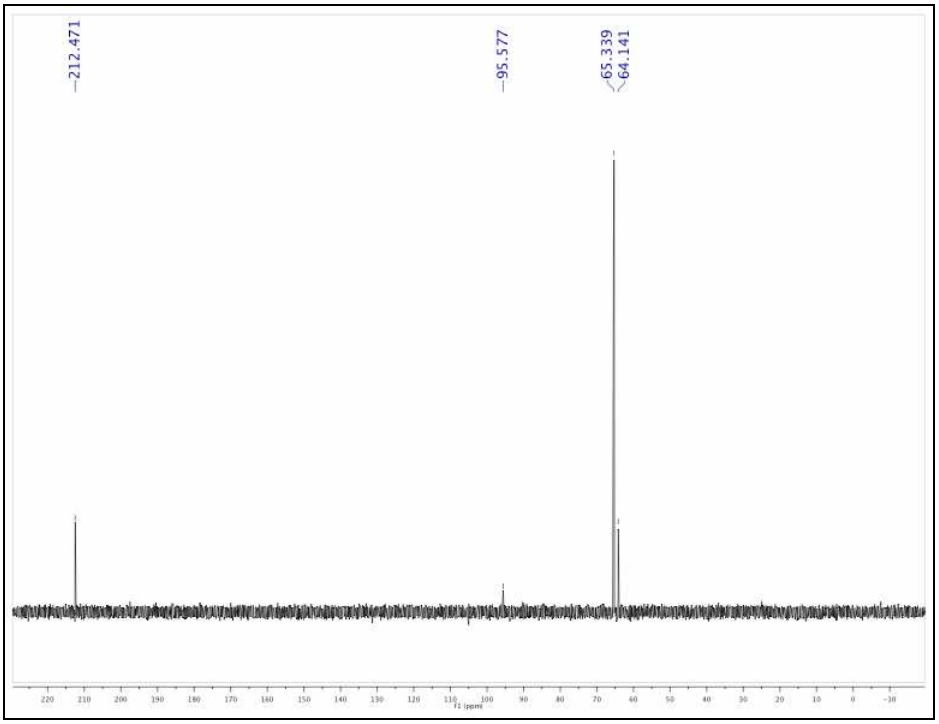


Figure S15: ^{13}C NMR (drops of D_2O for locking) of authentic dihydroxyacetone 0.3 M in water.

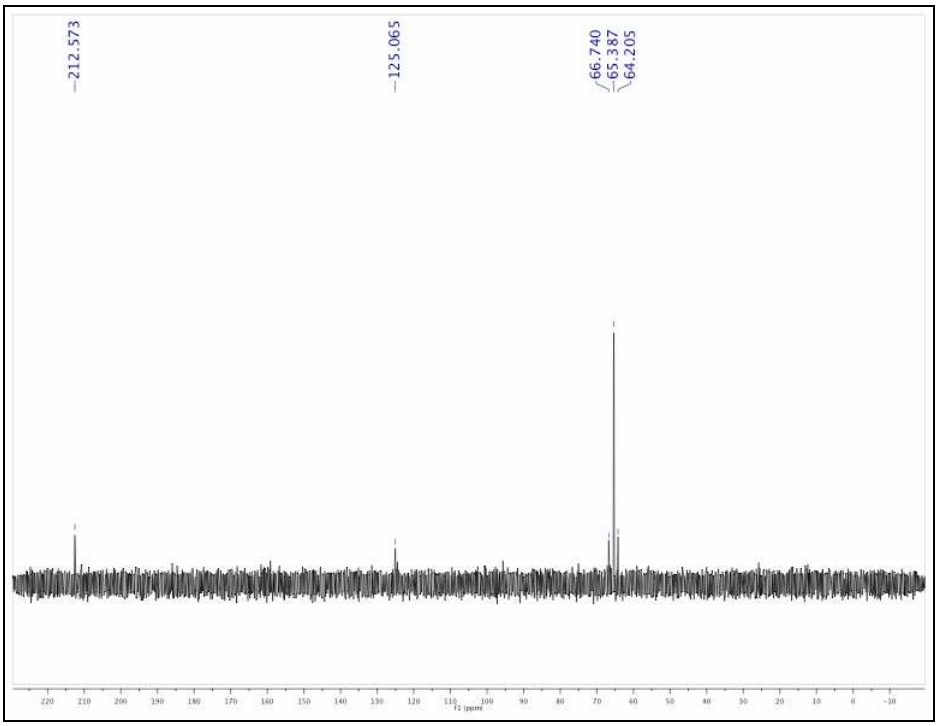


Figure S16: ^{13}C NMR (2 drops of D_2O for locking) of the sample from Figure S14, spiked with authentic dihydroxyacetone from Figure S15.

Derivatization with 2,4-dinitrophenylhydrazine: 8.1 mL (0.4 mL from NMR tube was also used) of reaction mixture was acidified to pH 2-3 by adding 0.2 ml of 2N HCl; to this acidified mixture was added 2,4-DNPH (1.1 eq., 736 mg, assuming 1,3-dihydroxyacetone has formed with 100% yield) and EtOAc (10 mL) at r.t. After 4.0 hrs stirring at room, 40 mL of EtOAc and 10 mL of water were added to the reaction mixture and water layer was extracted with EtOAc (3 x 40 ml). The combined EtOAc extracts was washed with water (2 x 50 mL), brine, dried (Na_2SO_4) and concentrated (rota vap.) at r.t. to give a dark yellow solid residue. The residue was dissolved in 5 mL EtOAc, adsorbed on silica gel and purified by column chromatography (silica gel, 3.0 cm diameter, 15 cm length, EtOAc/hexane (60:40 to 70:30)) to give the 1,3-dihydroxyacetone hydrazone (164.4 mg, 18% from 500 mg of DHF) as crystalline yellow solid.
TLC: (EtOAc/hexane; 50:40): R_f 0.20.M.p.: 164-165 °C (from column purified) (lit.[2] m.p. 163-164 °C). ^1H NMR (300 MHz, DMSO-d_6): δ 13.14 (s, N*H*), 8.85 (d, *J* = 2.4 Hz, 1H, aromatic *H*3), 8.34 (dd, *J* = 9.6, 2.4 Hz, 1H, aromatic *H*5), 7.92 (d, *J* = 9.6 Hz, 1H, aromatic *H*6), 6.38 (s, 1H, O*H*), 5.29 (t, *J* = 6.0 Hz, 1H, O*H*), 4.53 (s, 2H, CH_2), 4.09 (d, *J* = 6.0 Hz, 2H, CH_2). ^{13}C NMR (75 MHz, DMSO-d_6): δ 158.3, 144.7, 136.6, 129.8, 128.9 (aromatic); 123.1 (-N=*C*); 115.6 (aromatic), 63.3 (*C*H_2), 60.3 (*C*H_2). TOF MS (pos., in MeOH): 271 (7, $[M+H]^+$), 293 (89, $[M+Na]^+$), 253 (11, $[M-H_2O+H]$), 563 (14, $[2M+Na]^+$). TOF MS (Neg., in MeOH): 269 (100, M-H). *Yield of dihydroxyacetone: 20.2%* (factoring into account the yield of dihydroxyacetone hydrazone prepared from authentic dihydroxyacetone by this method was 89%).

2. Collatz.; Neuberg. *Biochem. Z.*, **1932**, *255*, 271; 1 eq. of 2,4-DNPH was used in the authentic dihydroxyacetone to hydrazone derivatization under biphasic conditions.

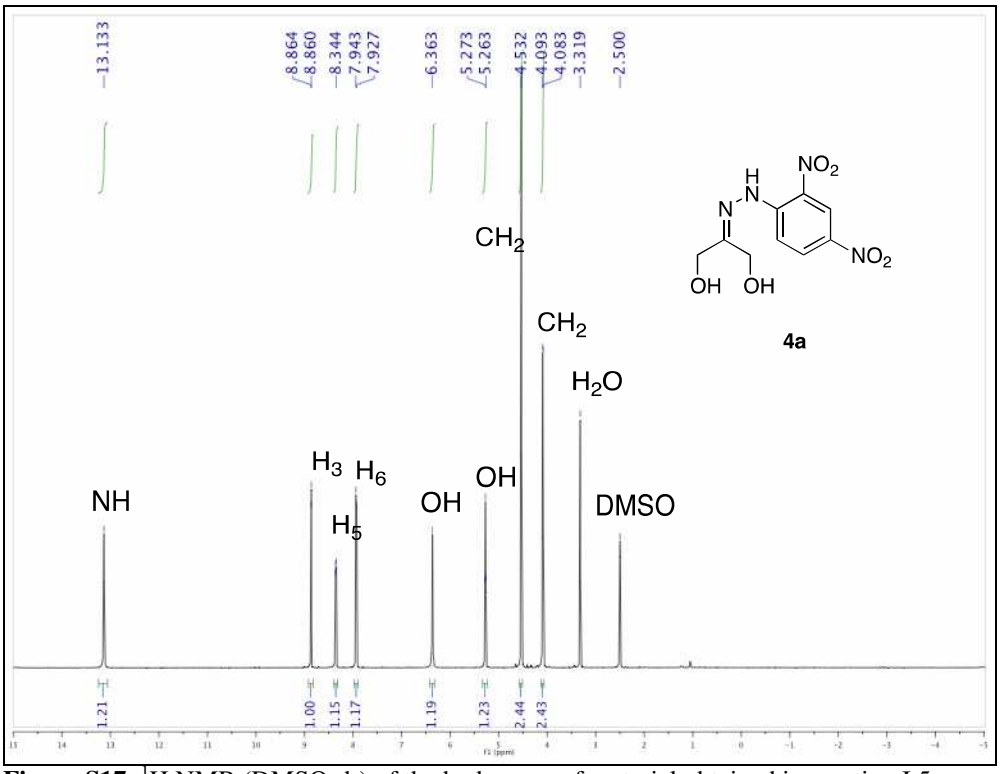


Figure S17: ^{1}H NMR (DMSO-d_6) of the hydrazone of material obtained in reaction I.5.

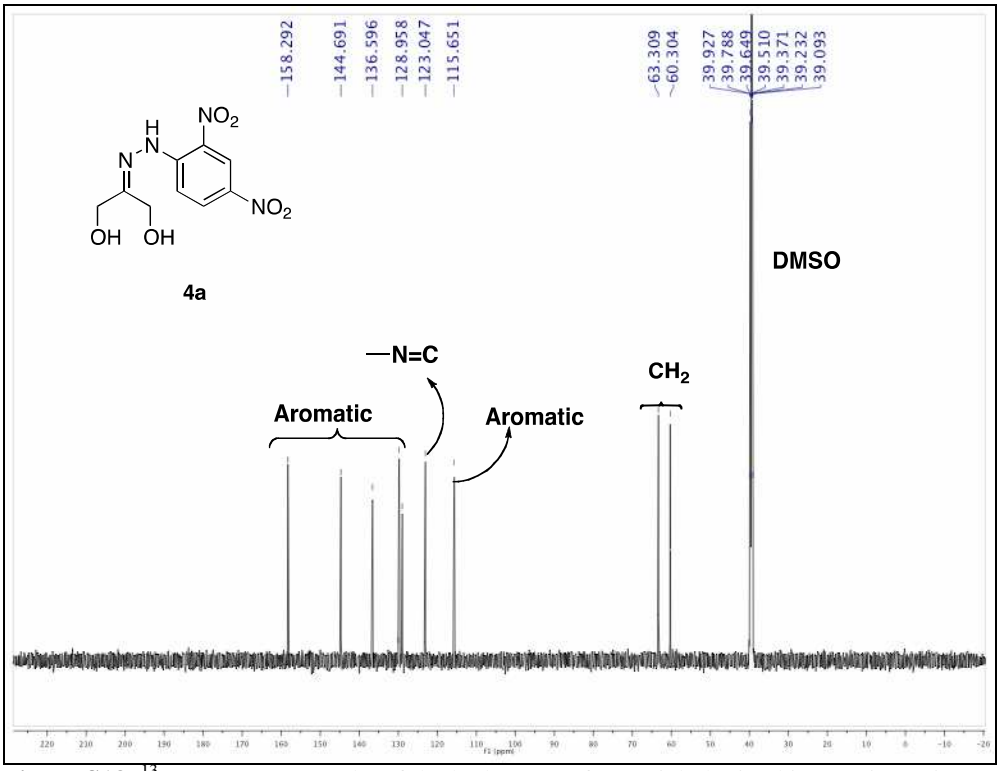


Figure S18: ^{13}C NMR (DMSO-d_6) of the hydrazone of material obtained in reaction I.5.

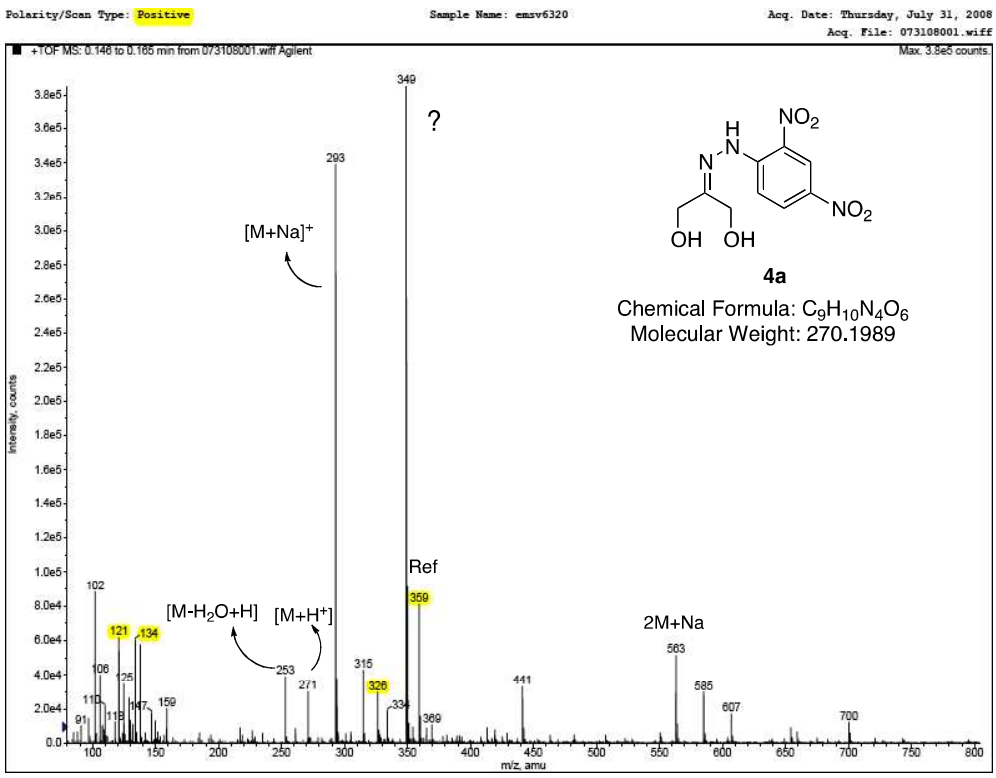


Figure S19: MALDI-TOF-MS (Pos., MeOH) of the hydrazone of dihydroxyacetone obtained in reaction I.5.

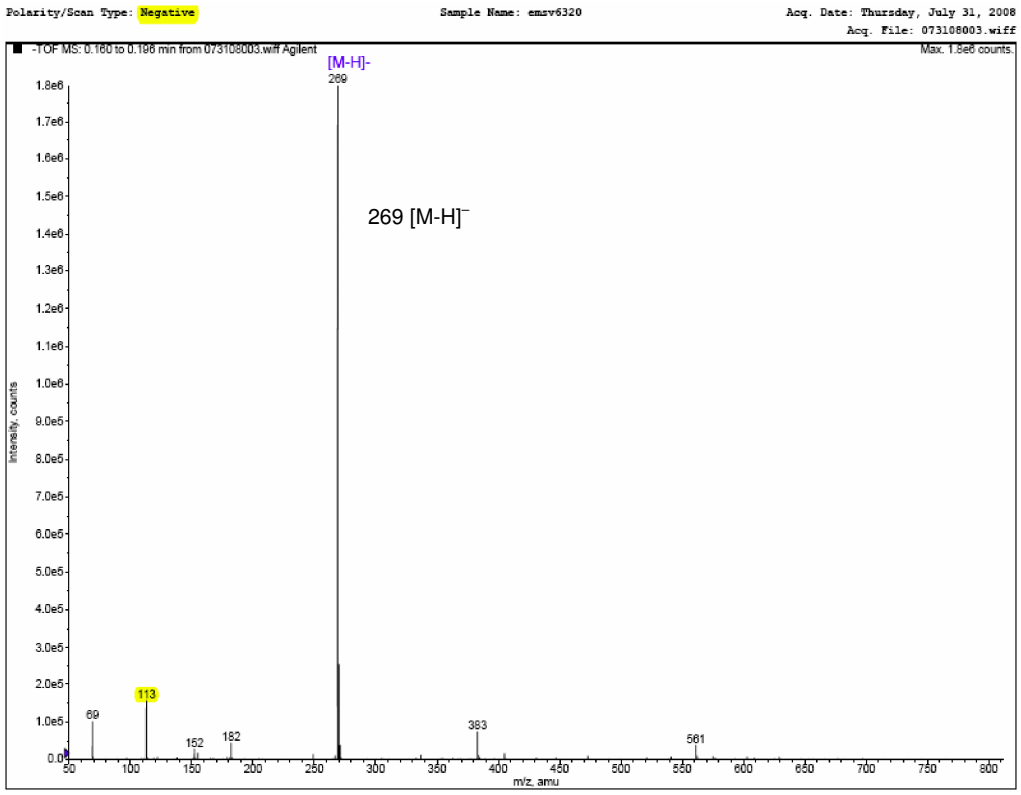
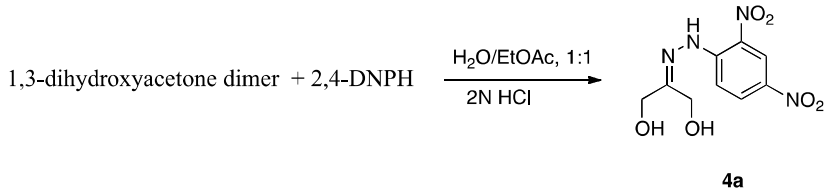


Figure S20: MALDI-TOF MS (Neg., in MeOH) of the hydrazone of dihydroxyacetone obtained in reaction I.5.

I.6. Hydrazone formation by reaction of authentic 1,3-dihydroxyacetone with 2,4-DNP:



4a

Scheme S4

1,3-dihydroxyacetone dimer (90 mg, 1mmol) was dissolved in 3 mL of H_2O, acidified to pH 2 by adding 2N HCl (0.5 ml). To this solution, 2,4-DNPH (396 mg, 2 mole equiv.) and EtOAc (3.5 mL) were added at r.t. and continued stirring at the same temperature. After 5 h, 20 mL of EtOAc and 10 mL of water were added to the reaction mixture and water layer was extracted with EtOAc (3 x 20 ml). The combined EtOAc extracts were washed once with cold saturated aqueous $NaHCO_3$, brine, dried (Na_2SO_4) and concentrated (rota vap.) at r.t. to give the crude product as dark yellow solid. The residue was dissolved in 5 mL EtOAc, adsorbed on silica gel and purified by column chromatography (silica gel) using EtOAc/hexane (60:40) as eluent to give the 1,3-dihydroxyacetone hydrazone (243 mg, 89.6%) as yellow solid. M.p.: 165-166 °C (lit.[2] m.p. 163-164 °C). TLC: (EtOAc/hexane, 50:50): R_f 0.20. ^{1}H NMR (600 MHz, DMSO-d_6): δ 8.85 (d, *J* = 2.4 Hz, 1H, aromatic *H*3), 8.36 (dd, *J* = 9.6, 2.4 Hz, 1H, aromatic *H*5), 7.94 (d, *J* = 9.6 Hz, 1H, aromatic *H*6), 6.37 (s, 1H, O*H*), 5.28 (t, *J* = 6.0 Hz, 1H, O*H*), 4.53 (s, 2H, CH_2), 4.09 (d, *J* = 6.0 Hz, 2H, CH_2). ^{13}C NMR (150 MHz, DMSO-d_6): δ 158.3, 144.7, 136.6, 129.8, 128.9 (aromatic); 123.1 (-N=*C*); 115.6 (aromatic), 63.3 (*C*H_2), 60.3 (*C*H_2). ESI (pos., in MeOH): 271 (100, $[M+H]^+$), 293 (66, $[M+Na]^+$), 253 (93, $[M-H_2O+H]$), 563 (17, $[2M+Na]^+$).

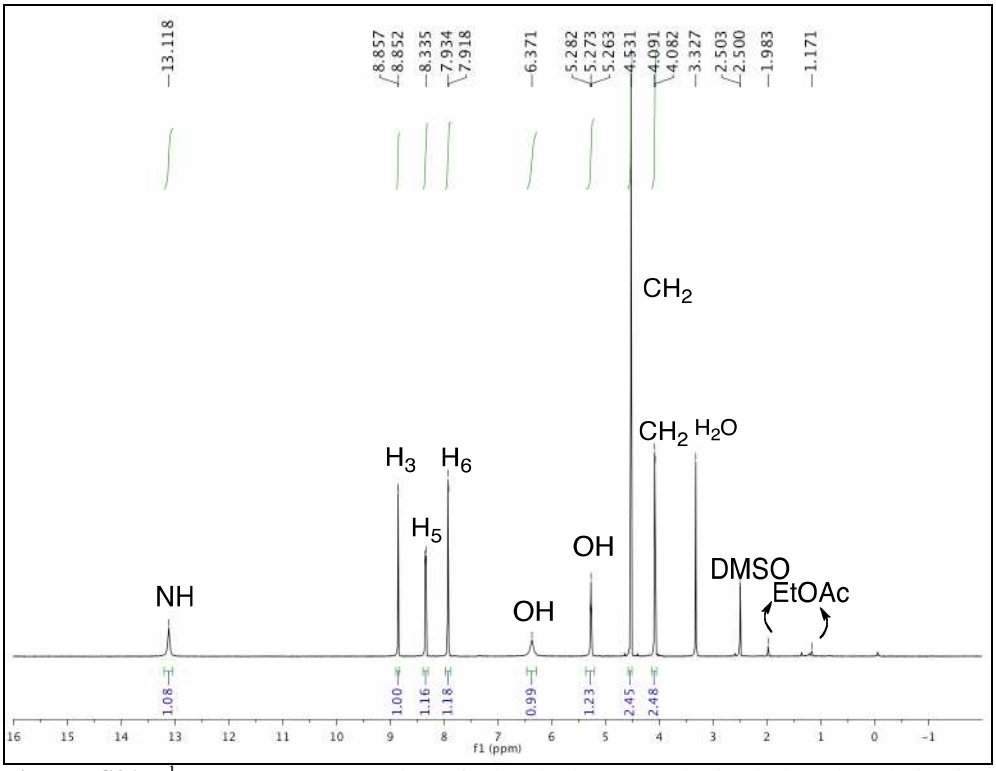


Figure S21: ^{1}H NMR (DMSO-d_6) of the hydrazone obtained by the derivatization of authentic 1,3-dihydroxyacetone under biphasic reaction conditions.

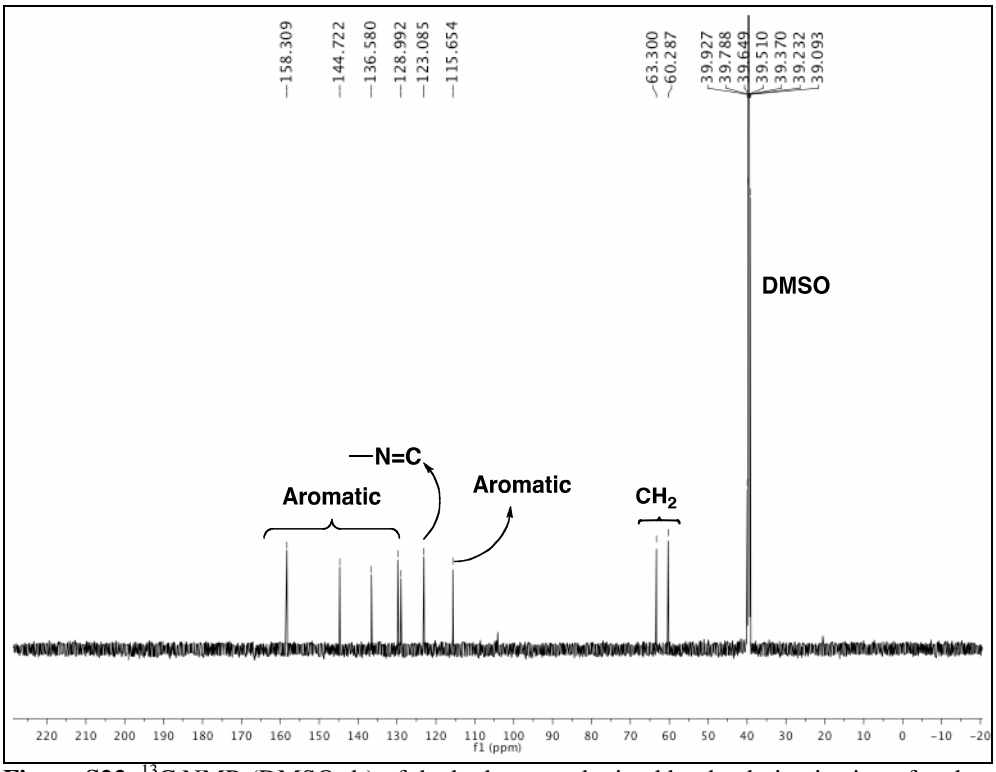


Figure S22: ^{13}C NMR (DMSO-d_6) of the hydrazone obtained by the derivatization of authentic 1,3-dihydroxyacetone under biphasic reaction conditions.

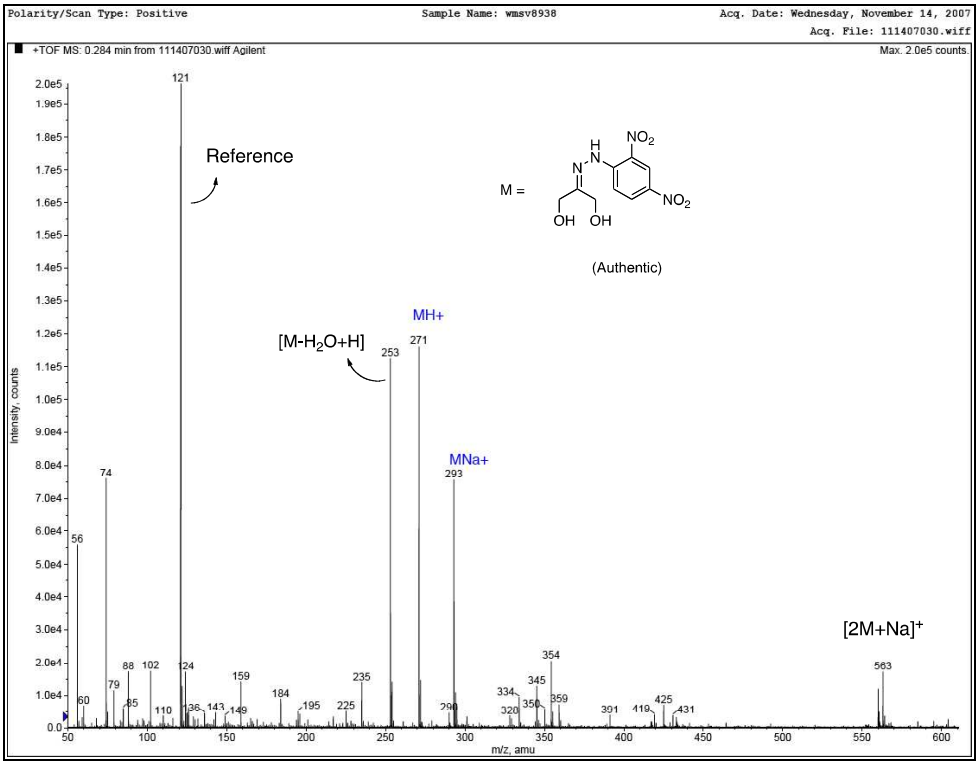
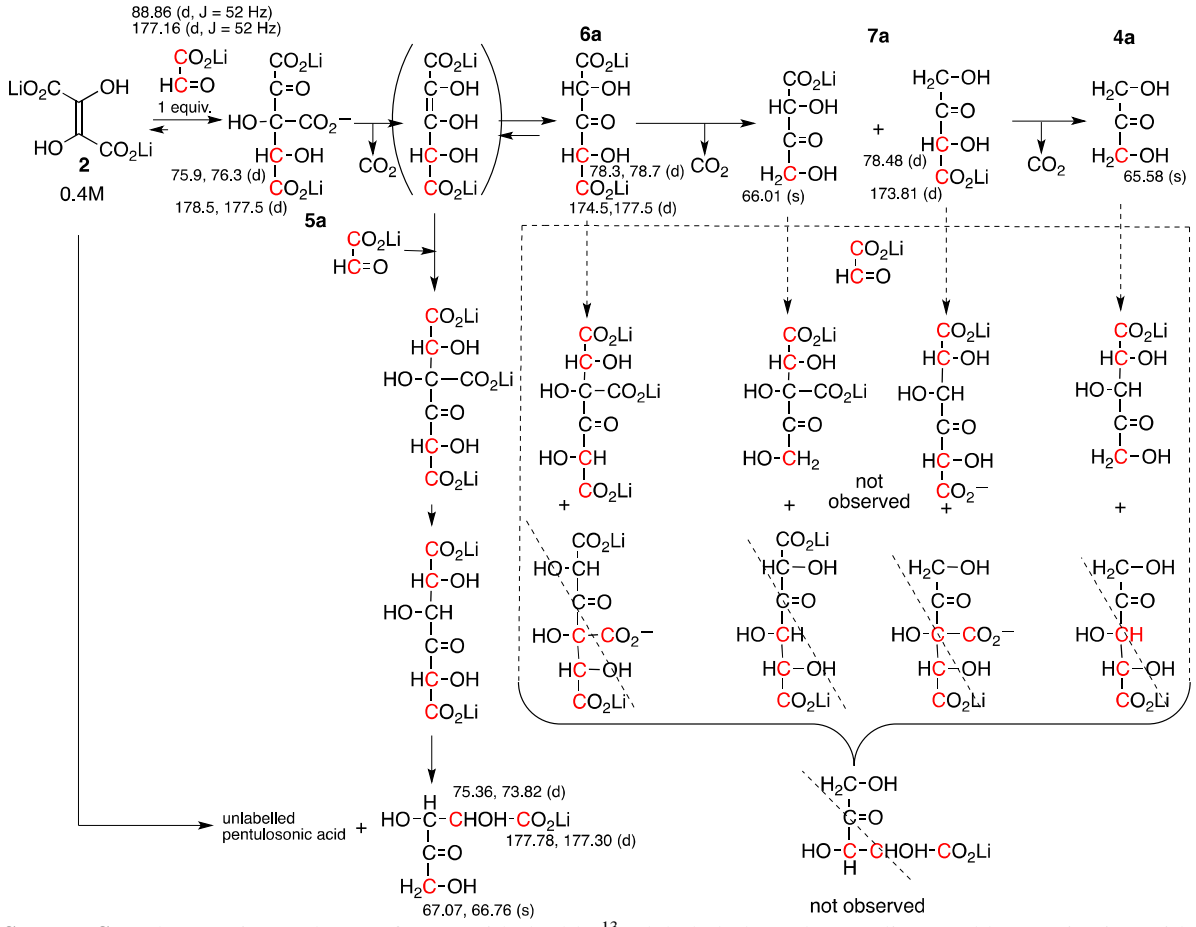


Figure S23: MALDI-TOF (Pos., in MeOH) of the hydrazone obtained by the derivatization of authentic 1,3-dihydroxyacetone under biphasic reaction conditions.

I.7. Reactions of DHF with doubly ^{13}C-labeled glyoxylate in aqueous medium:

I.7.a. Reaction of doubly ^{13}C-labeled glyoxylate with unlabeled DHF (0.4 M, 4°C). DHF (25 mg, 1.0 eq, 0.17 mmol) was taken into 5 mL sample vial, kept under vacuum for 10 minutes, and cooled to 4 °C. To this 0.425 mL of 1.18 M LiOH (3 eq) precooled solution was added through syringe at 4 °C under nitrogen atmosphere. The mixture became a clear brown (DHF salt) solution within a minute.

To the solid ^{13}C labeled glyoxylic acid (1 equiv., contains glycolic acid (3%) and oxalic acid (1.5 %)) was added the above DHF salt solution drop wise at 4 °C under nitrogen atmosphere. After the addition, the reaction mixture became a clear solution. The reaction mixture was kept at 4 °C and monitored by ^{13}C NMR (Figure S24). After 8 days, the reaction mixture was left at r.t. and continued monitoring (Figure S24).



Scheme S5: The reaction pathway of DHF with doubly ^{13}C-labeled glyoxylate as discerned by monitoring with ^{13}C NMR spectroscopy.

Within 10 min at 4 °C: ^{13}C-signals for glyoxylate has disappeared (and does not reappear over the course of the reaction). Adduct **5a** and dicarboxyl-DHA **6a** were the major species observed. Bunch of minor signals ≈ 78 and between 71-75 (all doublets; $J \approx 45$ Hz) were observed - indicative of minor intermediates.

After 22.0 h at 4 °C: Monocarboxyl-DHA was the predominant species with dicarboxyl-DHA. Bunch of signals ≈ 78 and between 71-75 (all doublets; $J \approx 45$ Hz) were still present.

Pentulosonic acid was seen (66/67 ppm).

After 48.0 hat 4 °C: Intensities for dicarboxyl-DHA peaks decreased; peaks at 70-75 & 66/67 ppm increased in intensity.

After 3 days: Peak intensities for 76/78 decreased, 66/72-75 increased.

After 6 days at 4 °C: 73-75 (4 doublets); two d at 66 increased. singlets at 65 decrease; peaks at 76-78 almost disappeared; 74-75 increased; 66 and 65 were present. 4 major CO_2H (doublets). Formate peak (171.9 ppm) was observed.

After 8 days at 4°C: sample was brought to r.t. then monitored after 5 days, 7 days, 12 days: doublets at 73.1, 74.7, 75.4; singlet at 67.03, 65.36. Four CO_2H groups (all doublets) Formate was observed at 171.9 (singlet).

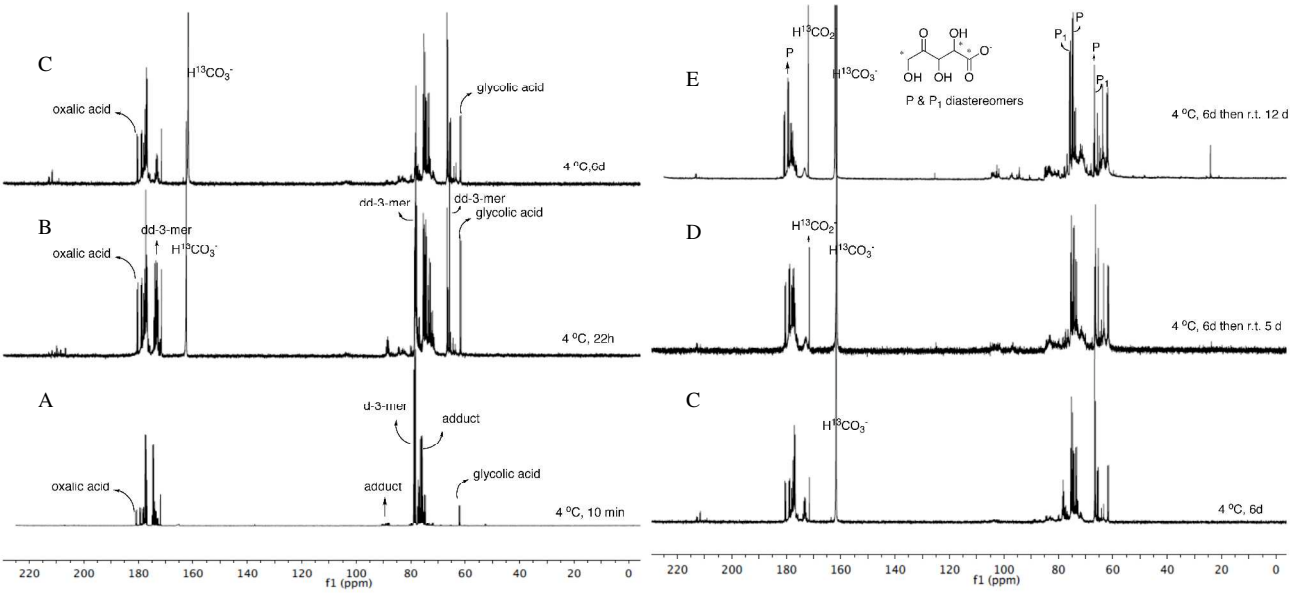


Figure S24: ^{13}C NMR (150 MHz, D_2O drops) spectra documenting the course of reaction (DHF with 1 eq. of ^{13}C labeled glyoxylate) at 4 °C 8 days and then at r.t. for 12 days (d-3mer = dicarboxyl-DHA, dd-3-mer = monocarboxyl-DHA).

I.7.b. Reaction of Li_2DHF with doubly ^{13}C labeled glyoxylate (0.4 M, r.t.). DHF (25 mg, 1.0 eq, 0.17 mmol) was taken into 5 mL sample vial and cooled to 4 °C. To this 0.425 mL of 1.18 M LiOH (3 eq) precooled solution was added through syringe at 4 °C under nitrogen atmosphere. The reaction mixture became a clear brown solution within a minute.

To the solid doubly ^{13}C labeled glyoxylic acid (1 equiv.) was added DHF salt solution drop wise at 4 °C under nitrogen atmosphere. After the addition, the reaction mixture became a clear solution. The reaction mixture was kept at r.t. and monitored by ^{13}C NMR (added drops of D_2O; Figure S25).

Within 10 min: ^{13}C-signals for glyoxylate has disappeared (and does not reappear over the course of the reaction).

After 30 min., minor signals at 71.9, 72.77, 73.83, 74.93 (all doublets; J = 45 Hz). However, the monocarboxyl-DHA does not appear until 1 h, while DHA does not appear until 3 h.

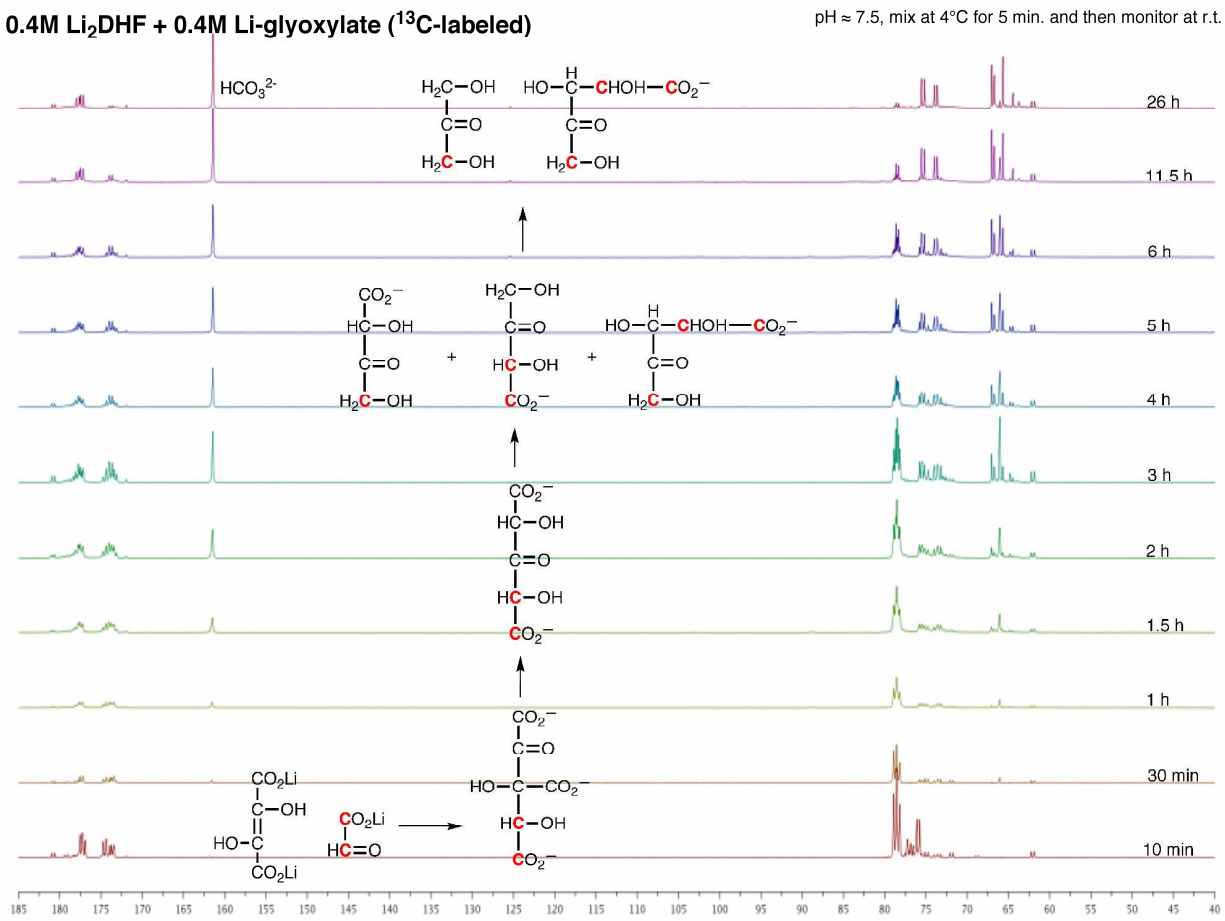


Figure S25: ^{13}C NMR (150 MHz, D_2O drops) spectra documenting the course of reaction of 0.4M DHF with 1 eq. of ^{13}C labeled glyoxylate (0.4 M) at r.t.

I.7.c. Reaction of Li_2DHF with ^{13}C labeled glyoxylate under dilute condition (0.04 M): DHF (25 mg, 1.0 eq, 0.17 mmol) was taken into 5 mL sample vial and cooled to 4 °C. To this 4.25 mL of 0.12 M LiOH (3 eq) precooled solution was added through syringe at 4 °C under nitrogen atmosphere resulting in a clear brown solution. To the solid ^{13}C labeled glyoxylic acid (1 equiv.) was added the DHF salt solution, drop-wise, at 4 °C under nitrogen atmosphere. After the addition, the reaction mixture became a clear solution. The reaction mixture was kept at r.t. and monitored by ^{13}C NMR (added drops of D_2O; Figure S26).

10 min.: Peaks for dicarboxyl-DHA, adduct **5a** and unreacted glyoxylate were present.

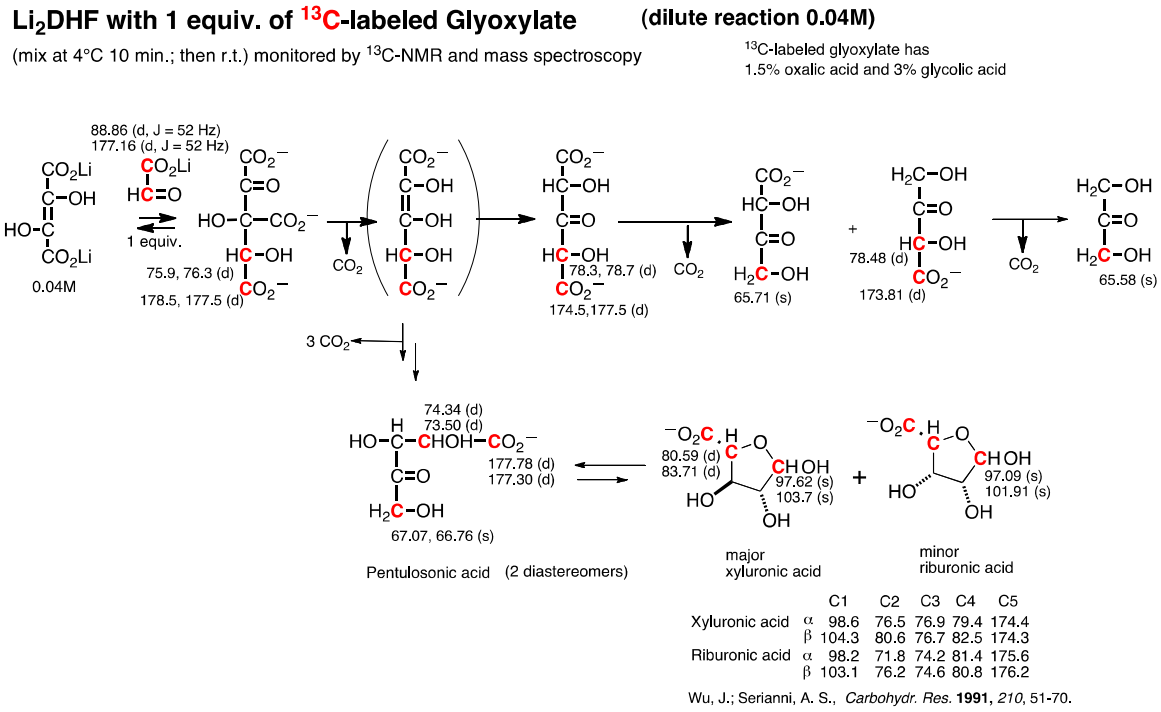
24h: Pentulosonic acid was the major component; *comparatively little DHA* (with ^{13}C-label) *was formed.* New peaks at ppm 80.6 (d), 83.9 (d), 97.62 (s), 103.7 (s) were seen. Formate (≈ 171); 177.5 (2 major doublets and 2 minor doublets). HCO_3^- was observed.

2 days and 3 days: same as above; mass (3 d): (ESI, +ve) 174 (pentulosonic acid + Li), 180 (pentulosonic-Li + Li), 215 and 221; (ESI, –ve): 166 (pentulosonic acid – H); Figure S27).

7 days: Two diastereomers of pentulosonic acid plus DHA. Also, xyluornic acid[3] (major) and riburonic acid[3] (minor) were formed.

After 11 days: Formate peak has increased; peak at ≈ 171 ppm.

3. Wu, J., Serianni, A. S. *Carbohydr. Res.* **1991**, *210*, 51-70.



Scheme S6: Reaction of DHF and ^{13}C-labeled glyoxylate under dilute (0.04M) conditions.

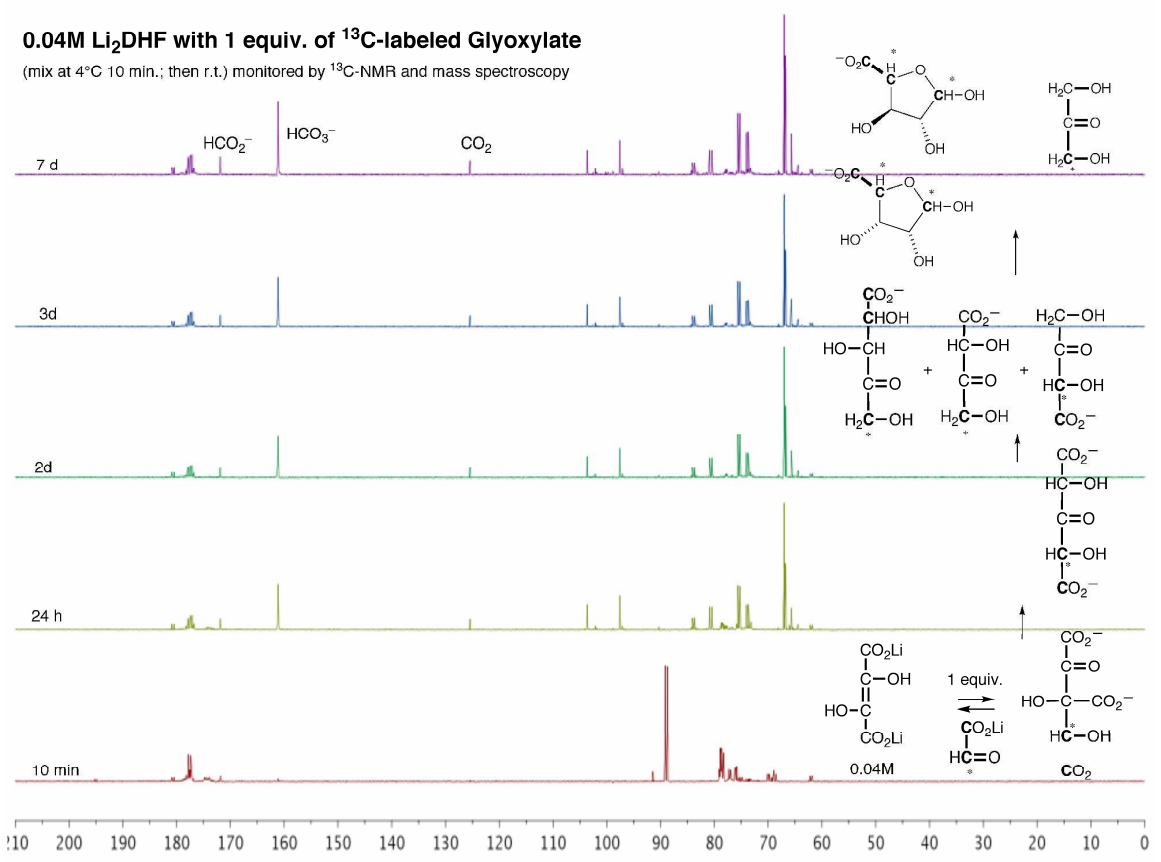


Figure S26: ^{13}C NMR (150 MHz, D_2O drops) spectra documenting the course of reaction [DHF with 1 eq. of ^{13}C labeled glyoxylate (0.04 M)] at r.t.

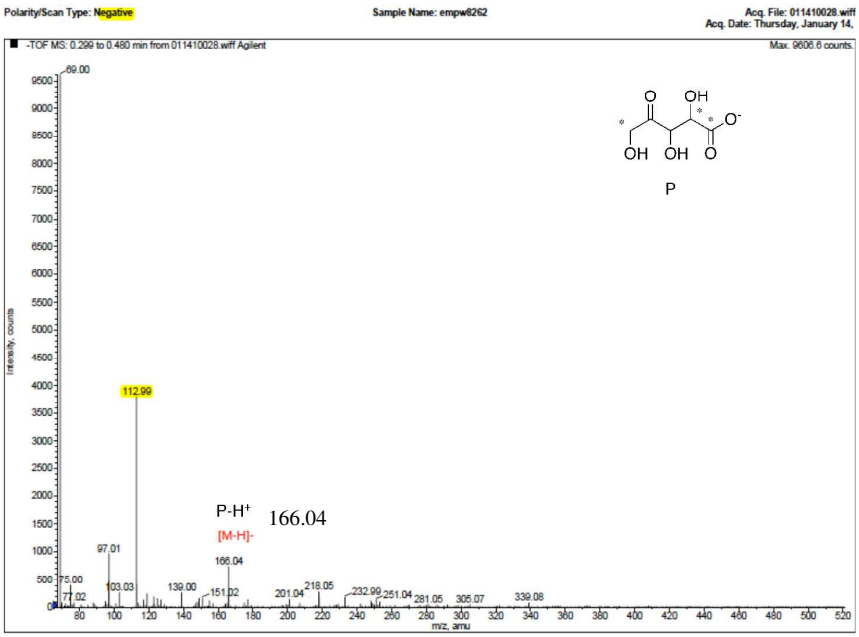


Figure S27: Mass spectrum (ESI, Neg.,) of DHF with 1 eq. of ^{13}C labeled glyoxylate (0.04 M) at r.t., 3days.

I.8. Preparation of dicarboxyl-DHA and reaction with glyoxylate at 4 °C and r.t. DHF (500 mg, 1.0 eq, 3.38 mmol) was taken into round bottom flask and kept in vacuum for 10 min then cooled to 4 °C. To this 7.5 mL of 0.9 M LiOH (2.0 eq) precooled solution was added through syringe at 4 °C under nitrogen atmosphere. The reaction mixture became a clear brown solution with in a minute. Glyoxylic acid (310 mg, 1.0 eq, 3.38 mmol) was taken into 5 mL sample vial. To this 3.6 M NaOH (1.0 eq, 0.94 mL) solution was added through syringe at r.t. The reaction mixture became a clear solution, which was cooled to 4 °C. To the aqueous solution of 'DHF dilithium salt' was added drop wise aq. Glyoxylate solution at 4 °C under nitrogen atmosphere. After the addition, the reaction mixture became a clear solution. After 3 h, the reaction mixture by ^{13}C NMR showed dicarboxyl-DHA as a major product (pH 8.29). At this time one more equivalent of glyoxylate was added and the reaction mixture was divided into two parts. First part of reaction mixture was kept at 4 °C and second part of reaction mixture was kept at r.t., and were monitored by ^{13}C NMR (in H_2O/D_2O).

Monitored at 4 °C, 18 h: peaks were observed at 78.4, 88.7, 173.5, 177.5, 207.5 (seems to have monocarboxyl-DHA); 44h: some pentulosonic acid observed; 4 days: pentulosonic acid formed as a major product; little DHA was observed (Figure S28).

Monitored at r.t., 18h: pentulosonic acid formation was observed; 44 h: pentulosonic acid formed as a major product; DHA also observed; contains minor peaks at 63.77, 65.70, 68.04, 76.69, 80.39; 4 days: mass analysis (ESI, neg.): 163 (pentulosonic acid-H^+); 193; 215; 237 (Figure S29 & S30).

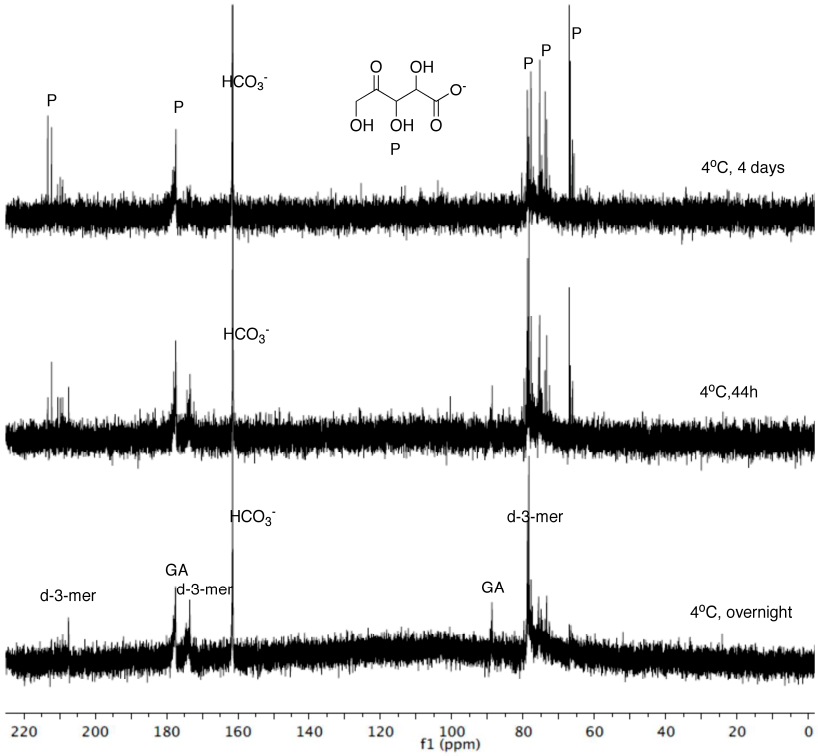


Figure S28: ^{13}C NMR (150 MHz, D_2O drops) spectra documenting the course of reaction of dicarboxyl-dihydroxyacetone with 1 eq. of glyoxylate, at 4 °C (d-3-mer = dicarboxyl-dihydroxyacetone).

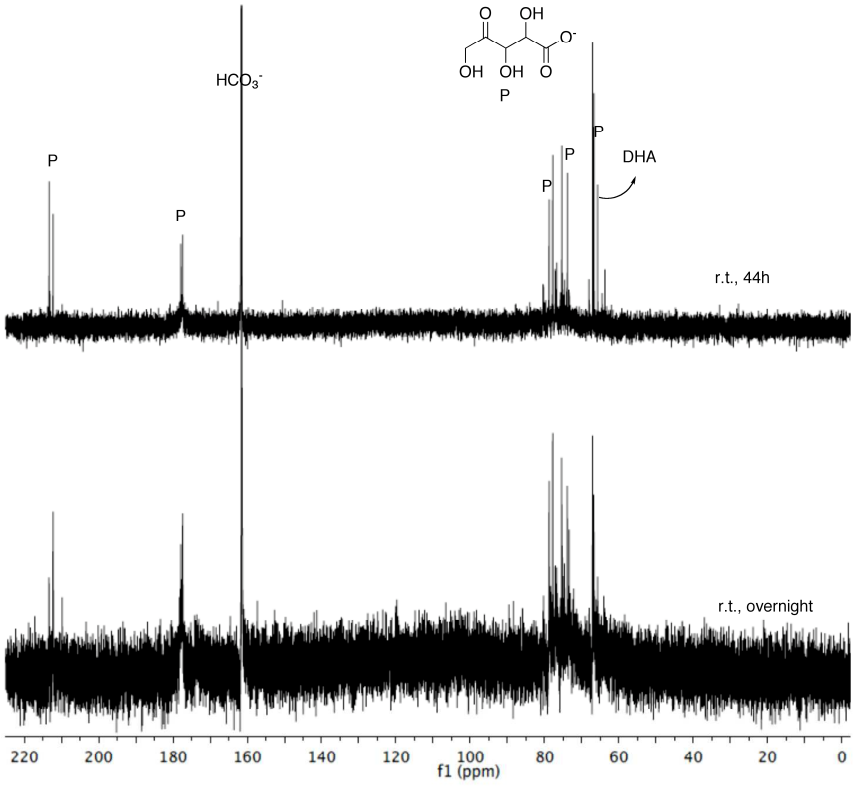


Figure S29: ^{13}C NMR (150 MHz, D_2O drops) spectra documenting the course of reaction of dicarboxyl-dihydroxyacetone with 1 eq. of glyoxylate, at r.t.

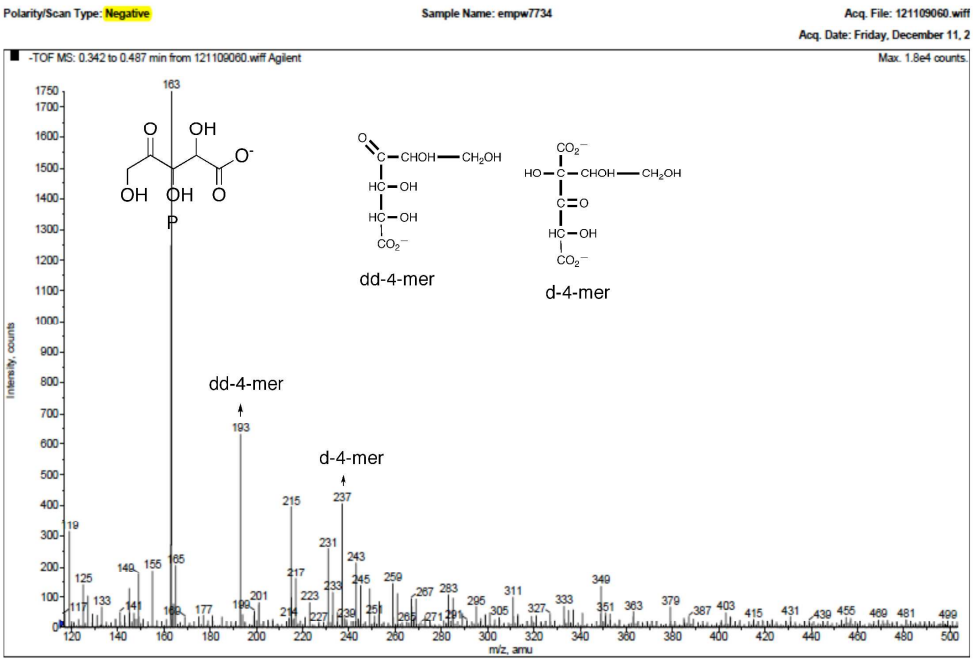


Figure S30: Mass spectrum (ESI, pos.) of dicarboxyl dihydroxyacetone + glyoxylate reaction mixture (4 d, r.t.).

I.9. Preparation of monocarboxyl-DHA and reaction with 1.0 eq. of glyoxylate at r.t.: DHF (500 mg, 1.0 eq, 3.38 mmol) was taken into round bottom flask and kept in vacuum for 10 min then cooled to 4 °C. To this 7.5 mL of 0.9 M LiOH (2.0 eq) precooled solution was added through syringe at 4 °C under nitrogen atmosphere, resulting in a clear brown solution with in a minute. Glyoxylic acid (310 mg, 1.0 eq, 3.38 mmol) was taken into 5 mL sample vial. To this 3.6 M NaOH (1.0 eq) solution was added through syringe at r.t. The reaction mixture became a clear solution, which was cooled to 4 °C. To the aqueous solution of 'DHF dilithium salt' was added drop wise the aq. glyoxylate solution at 4 °C under nitrogen atmosphere. After the addition, the reaction mixture became a clear solution. After 24 h at 4 °C, the reaction mixture (by ^{13}C NMR) showed monocarboxyl-DHA as a major product. One equivalent of glyoxylate was added and the reaction mixture was left at r.t. and monitored by ^{13}C NMR (in H_2O/D_2O) by taking 0.5 mL into NMR tube (Figure S31).

24 h: No glyoxylate present; no monocarboxyl-DHA; pentulosonic acid peaks present.

2 days: pentulosonic acid peaks present; peaks at 76 intensity increased; peaks at 62, 63.7, 65, 73, 76, 98.8, 209.7, 212.2, 213.3, 214.7. Mass (ESI, pos.) showed signals for 91 (M_{DHA} +H), 173 ($M_{pentulose}$+Na), 195 ($M_{hexulosonic\ acid}$+H), Mass (ESI, neg.): 163 ($M_{pentulosonic\ acid}$-H), 193 ($M_{hexulosonic\ acid}$-H)

6 days: Pentulosonic acid peaks were present; peak intensities increased at ~67, ~73, 85, 100, 172, 213, 214; lots of acid peaks observed.

30 days: Major peaks at 62, 72, 75, 77, 180.7 and peaks at 62-85, 94, 103.8, 171, 176-178, 180, 213. Mass (ESI, pos.) showed signals for 91 (M_{DHA} +H), 173 ($M_{pentulose}$ +Na), 195 ($M_{hexulosonic\ acid}$ +H), 203 ($M_{hexulose}$ + Na); 217 ($M_{heptulose}$ + Na). Mass (ESI, -ve): 193 ($M_{hexulosonic\ acid}$-H). MALDI TOF (THAP matrix): 343 ($M_{tetra\text{-}d\text{-}7\text{-}mer}$–$CO_2$+H), 387 ($M_{tetra\text{-}d\text{-}7\text{-}mer}$ +H), 401 ($M_{iso\text{-}d\text{-}6\text{-}mer}$+H), 357 ($M_{dd\text{-}6\text{-}mer}$+H), 403(?) [Figures S33 and S34].

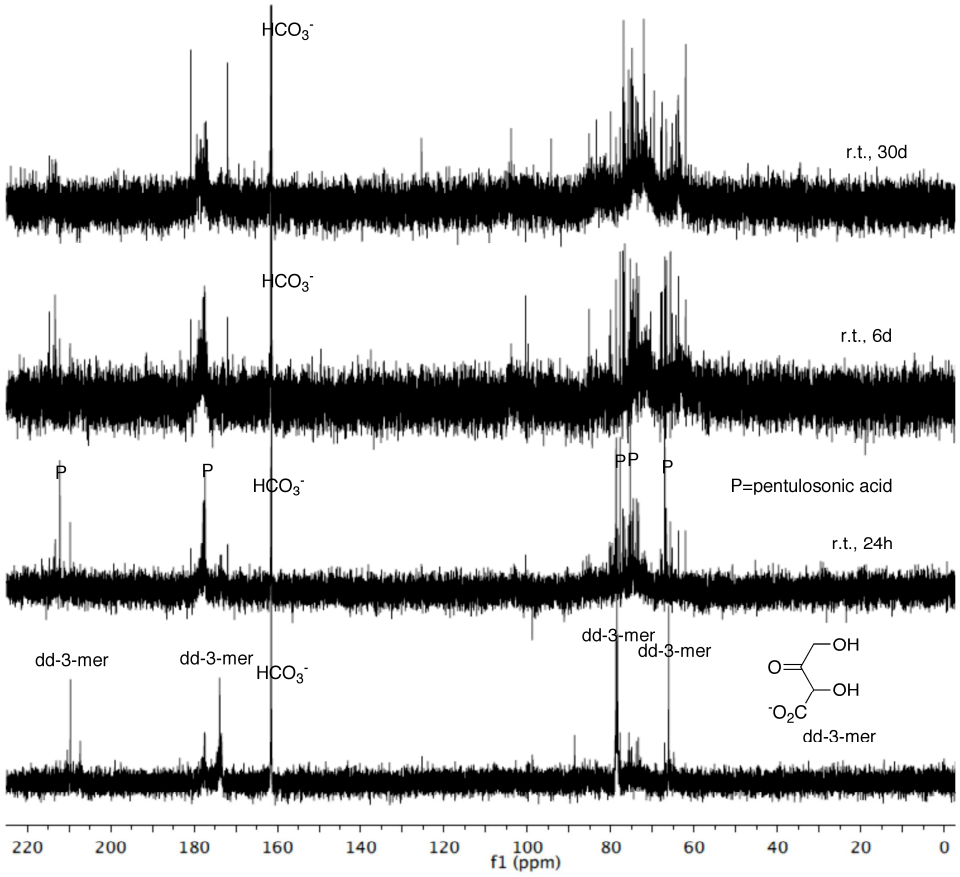


Figure S31: ^{13}C NMR (150 MHz, D_2O drops) spectra documenting the course of reaction (monocarboxyl dihydroxyacetone with 1 eq. of glyoxylate) at r.t. (dd-3-mer = monocarboxyl-dihydroxyacetone).

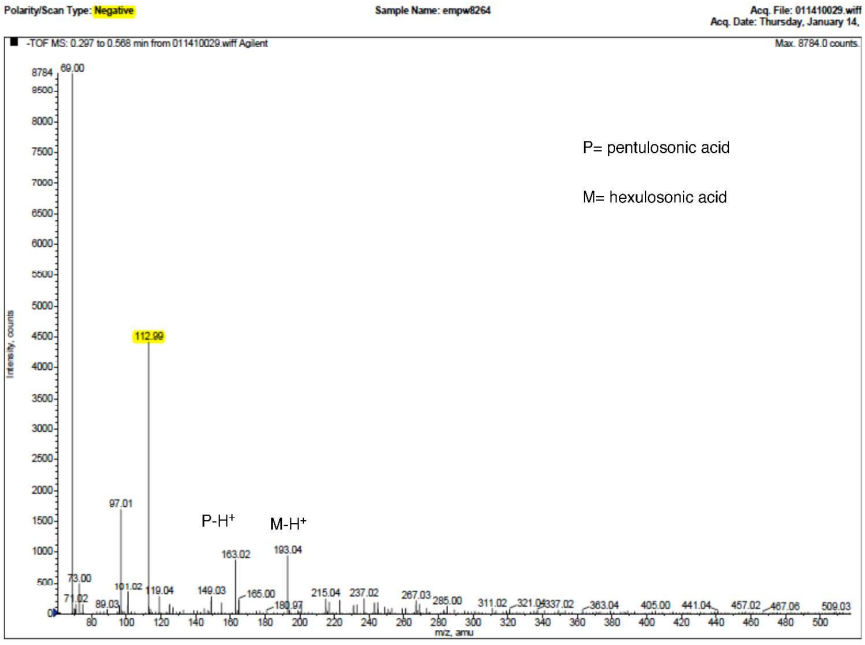


Figure S32: Mass spectrum (ESI, neg.,) of monocarboxyl-DHA + glyoxylate (1.0 eq) reaction mixture (2 d, r.t.)

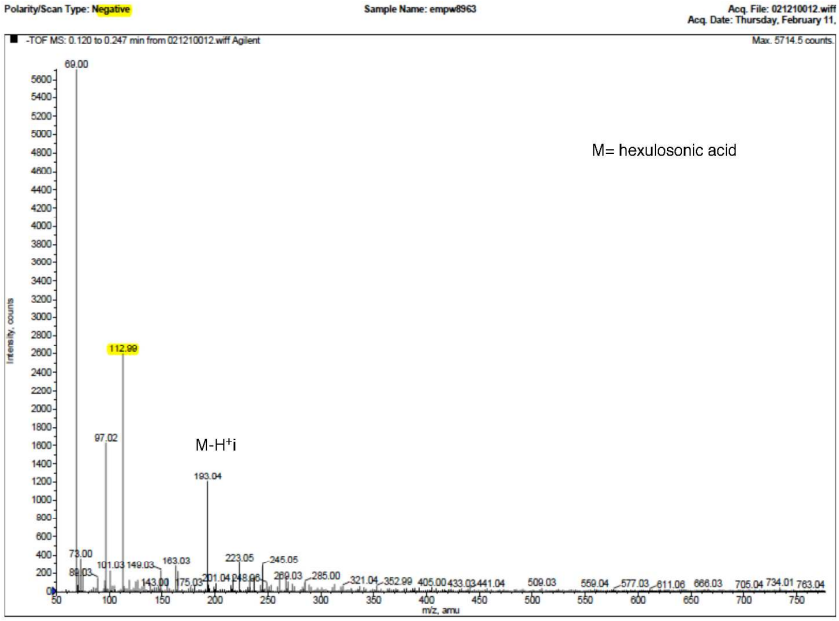


Figure S33: Mass spectrum (ESI, neg.) of monocarboxyl-DHA + glyoxylate (1.0 eq) reaction mixture (30 d, r.t.).

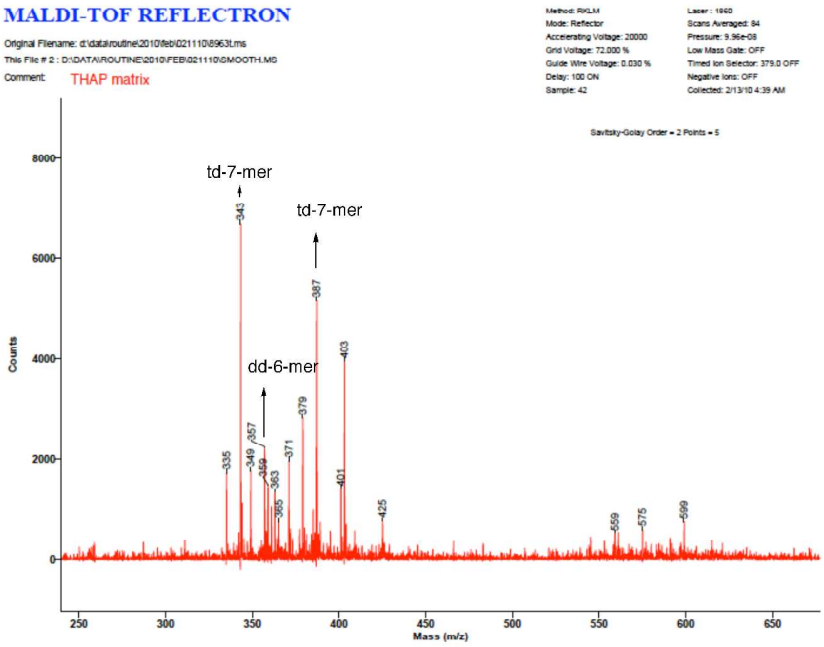


Figure S34: Mass spectrum (MALDI TOF, THAP matrix) of monocarboxyl-DHA + glyoxylate (1.0 eq) reaction mixture (30 d, r.t.).

I.10. Reaction of dihydroxyacetone with ^{13}C-labeled-glyoxylate: Doubly ^{13}C labeled glyoxylic acid (15.8 mg, 1.0 eq, 0.168 mmol) was taken into 5 mL sample vial and cooled to 4 °C. To this 0.85 mL of 0.2 M NaOH (1 eq) precooled solution was added through syringe at 4 °C under nitrogen atmosphere. The reaction mixture became a clear solution. To the dihydroxyacetone (solid, 2.0 eq.) was added the ^{13}C labeled glyoxylate solution drop-wise at 4 °C under nitrogen atmosphere. After the addition, the reaction mixture became a clear solution. The pH of the reaction mixture was adjusted to 9.96 with 1N NaOH. The reaction mixture was kept at r.t. and monitored by ^{13}C NMR (added drops of D_2O).

24 h: unreacted glyoxylate; unreacted DHA & hydrated DHA and pentulosonic acid (2 diastereomers)

3d: more pentulosonic acid; small amount of di-adduct; unreacted glyoxylate and DHA present.

10 d: more pentulosonic acid was formed; slightly more di-adduct was formed; unreacted glyoxylate and DHA were present.

30 d: glyoxylate disappeared. Unreacted DHA was present. Pentulosonic acid was major; more di-adduct was formed. Also seen were minor signals assignable to xyluronic acid.

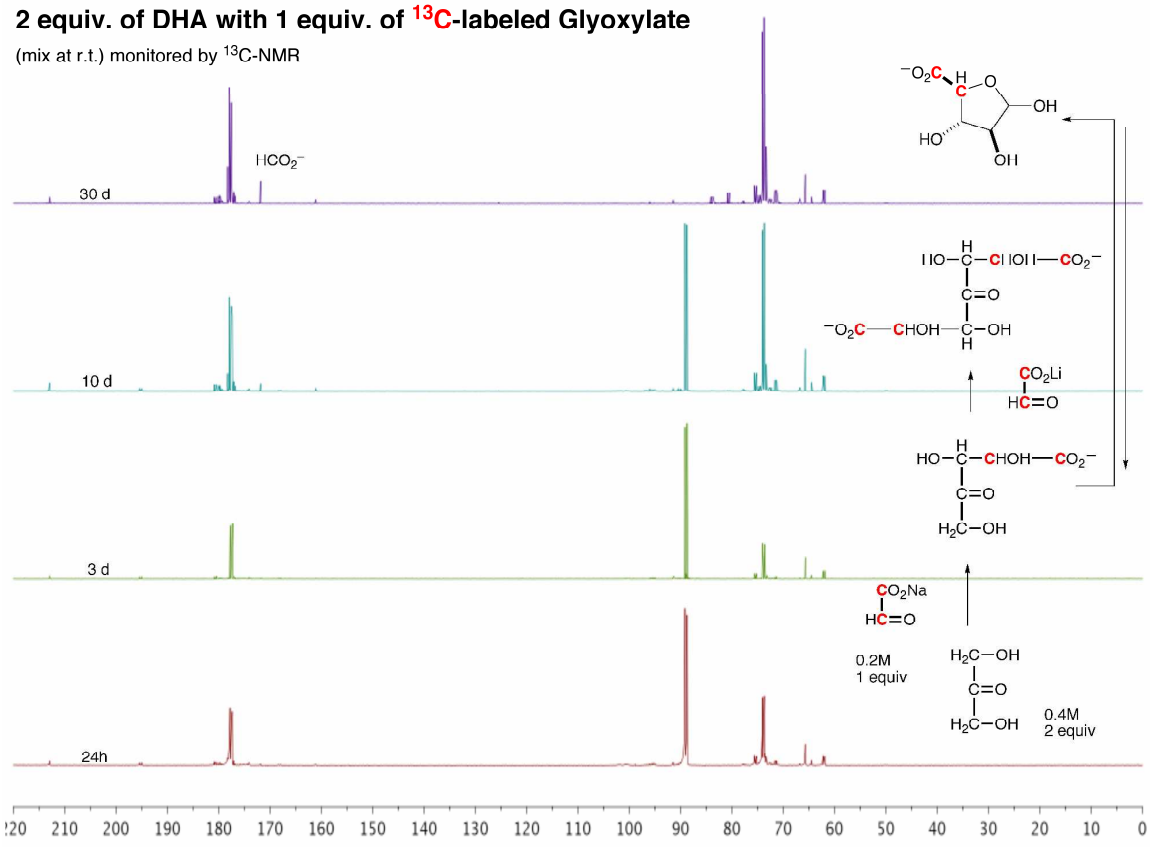
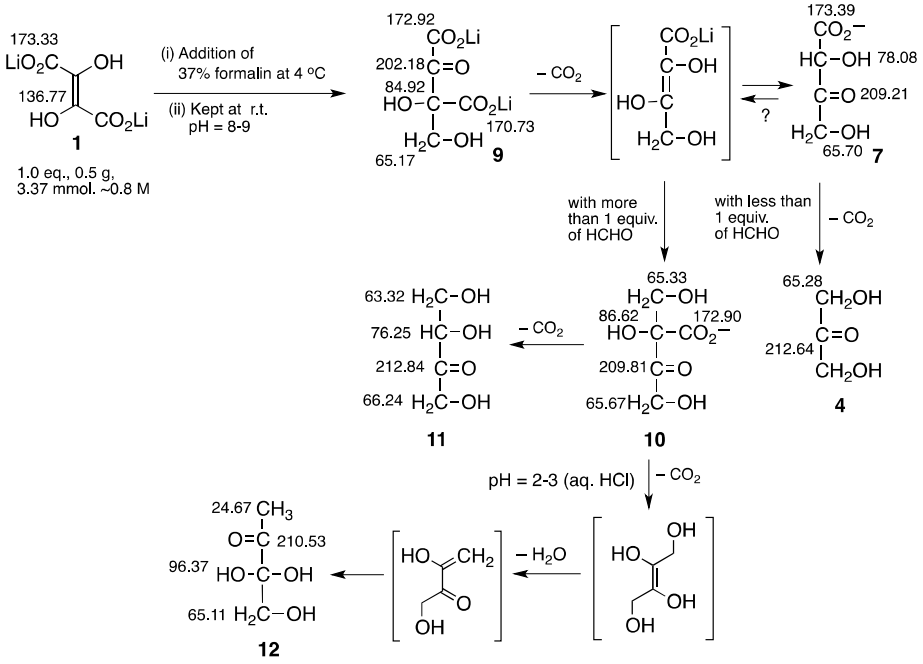


Figure S35: ^{13}C NMR (150 MHz, D_2O drops) spectra documenting the course of reaction (DHA (2.0 eq.) with 1 eq. of ^{13}C labeled glyoxylate (0.2 M) at r.t.

II. Reactions of Dihydroxyfumarate (DHF) with aldehydes in aqueous medium.

All of the reactions were followed by ^{13}C NMR, and, intermediates and end-products were identified based on ^{13}C NMR data (and where available by comparison, and spiking, with authentic samples).

II.a. <u>Reaction of DHF with formaldehyde:</u>



Scheme S7: Reaction of DHF with formaldehyde leads to predominant formation of tetrulose as followed by ^{13}C NMR spectroscopy; acidification leads to the formation of 2,3-diketobutanol (1-hydroxy-2,3-butandione).

II.a.1. *<u>Reaction of DHF with 2.0 eq of formaldehyde:</u>* To a cold (4 °C) aq. solution of 1.0 eq., of DHF dilithium salt (0.8 M), 1.0 eq of 37% formalin in water (~1 eq, 0.30 mL, Aldrich, contains 10-15% MeOH as stabilizer) was added in one portion under nitrogen atmosphere at 4 °C. The reaction mixture became a clear solution and the pH of the resulting solution was 8-9 (by pH paper). After ~ 2 min., the 'reaction flask' was placed at r.t. and monitored by ^{13}C NMR.

After 1 h at r.t., ^{13}C NMR showed peaks for second aldolization product **10** at ppm 209.81 (keto), 172.90 (CO_2Li, with bigger intensity), 86.62 (quaternary carbon, C-OH), 65.67, 65.32 (CH_2OH) [Figures S36, S37 and S38].

After over night (16 h) at r.t., ^{13}C NMR and ^{13}C NMR-APT showed peaks for 'tetrulose' at ppm 212.89, 76.25, 66.24, 63.32 with high intensity and less intense peaks were observed at 209.78, 172.88, 86.60, 65.63 and 65.32 [Figure S39].

Even after 24 h, ^{13}C NMR showed similar peaks as observed at 16 h [Figures S40 and S41].

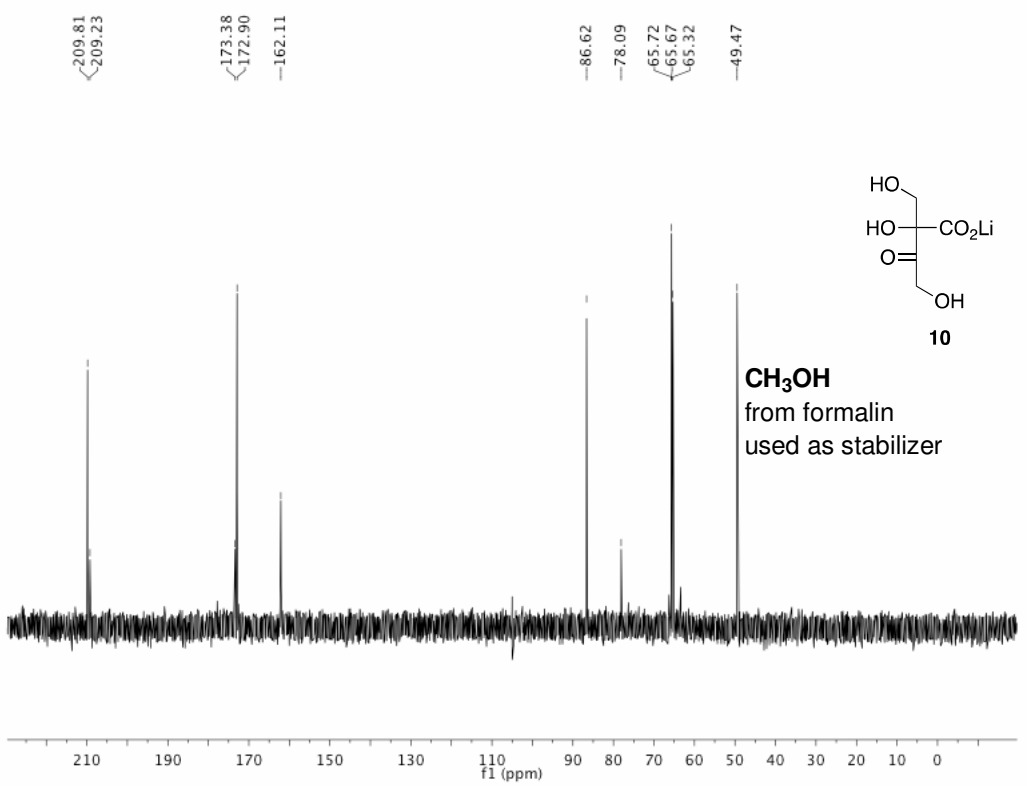


Figure S36. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde after 1 h at r.t.

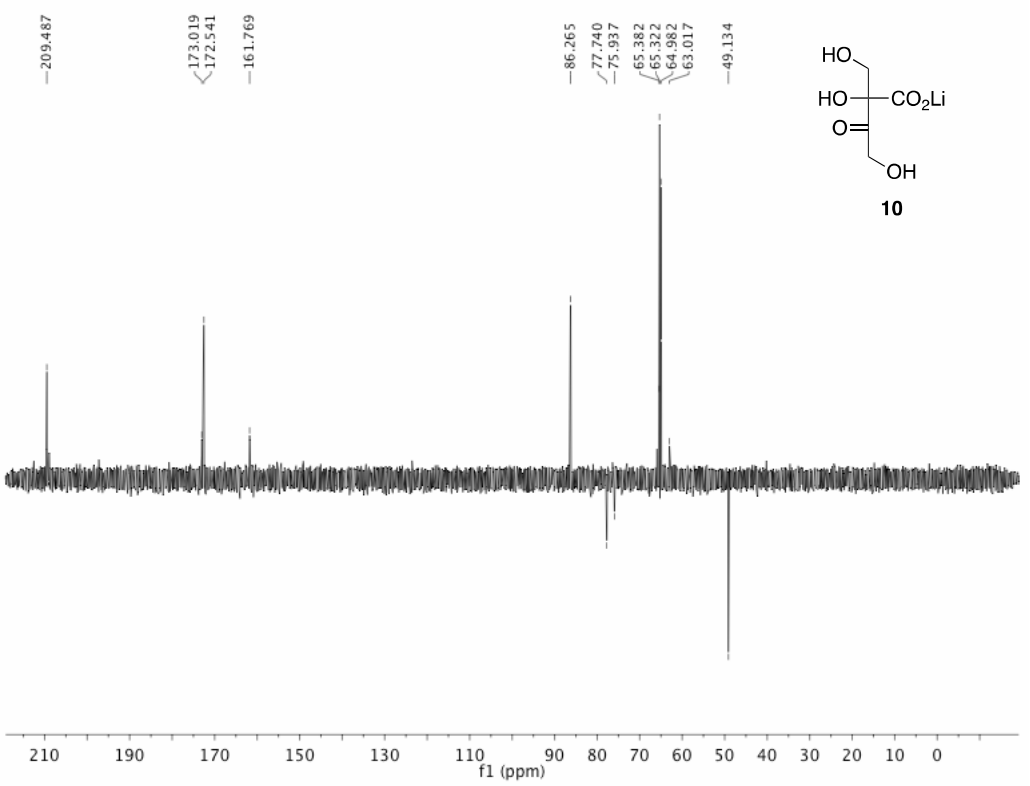


Figure S37. ^{13}C NMR-APT (H_2O/D_2O) of the reaction of DHF and formaldehyde after 1 h at r.t.

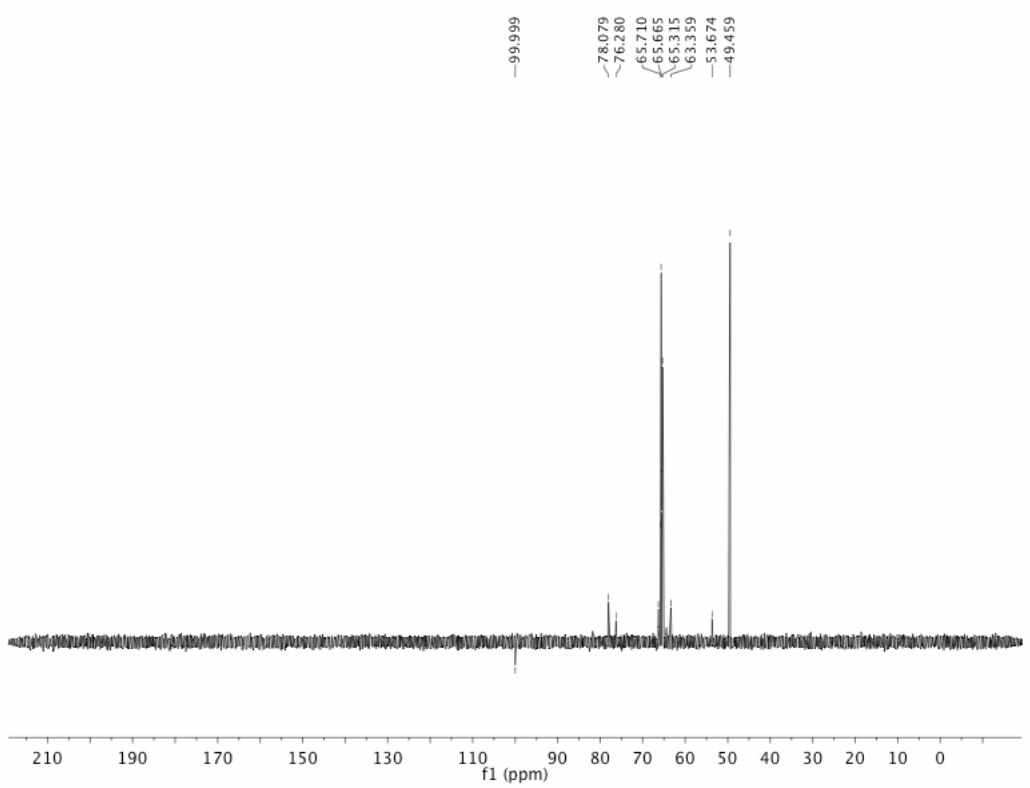


Figure S38. QNP-DEPT45 (H_2O/D_2O) of the reaction of DHF and formaldehyde after 1 h at r.t.

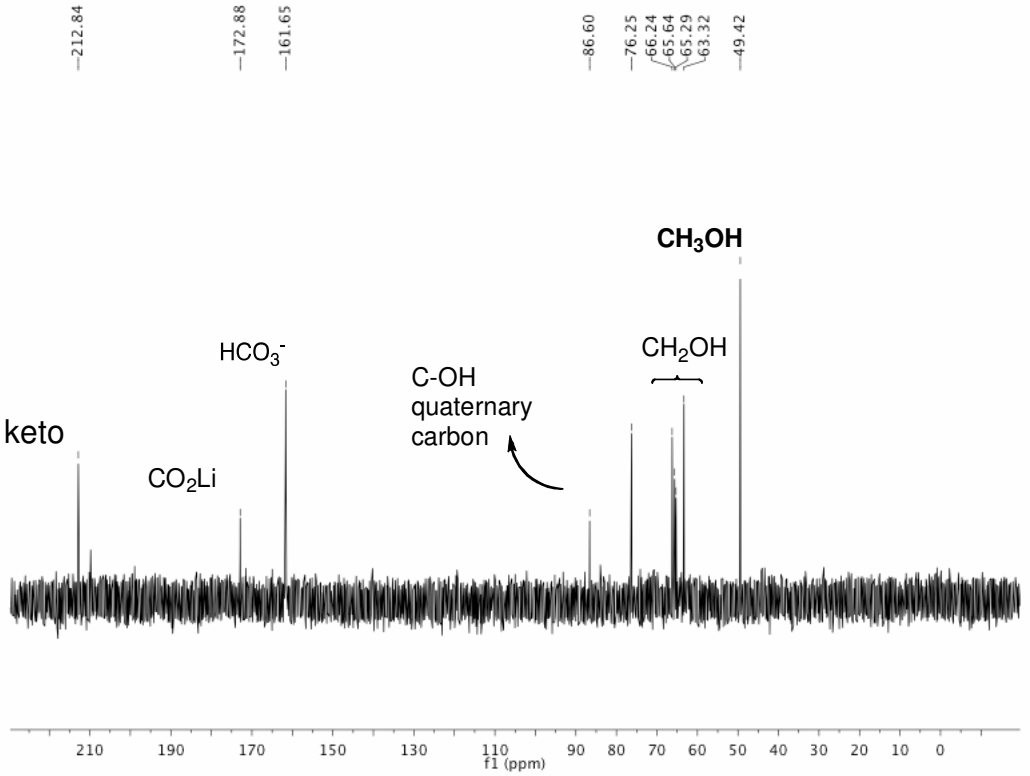


Figure S39. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde after 16 h at r.t.

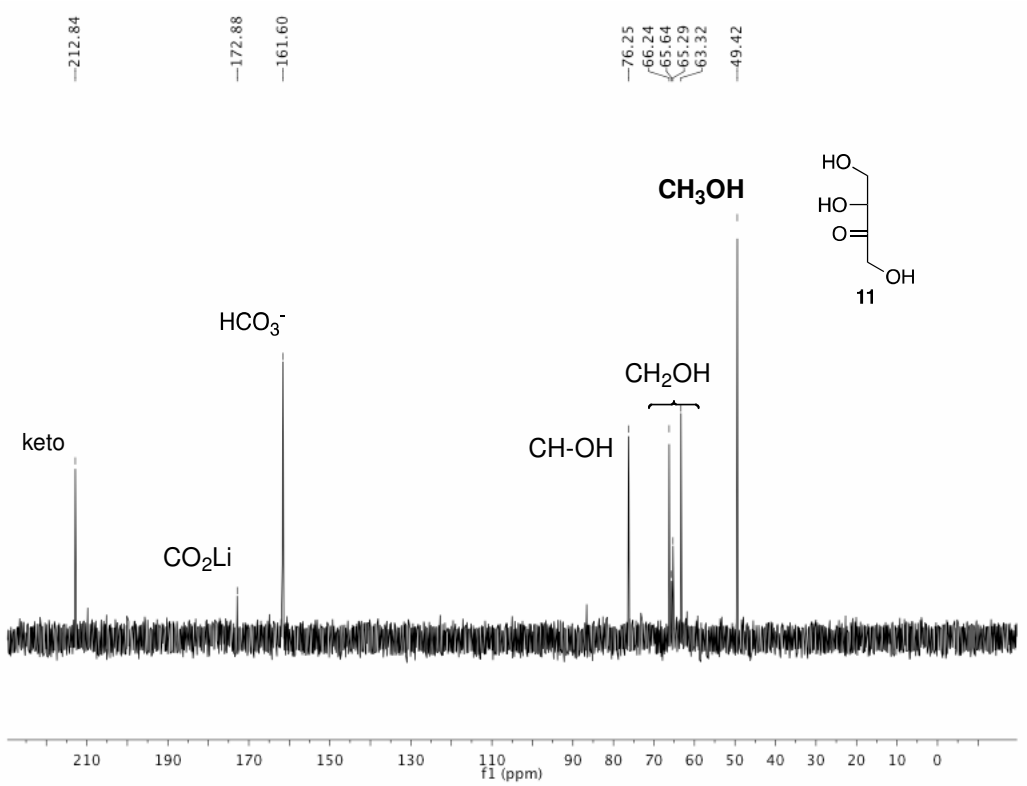


Figure S40. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde after 24 h at r.t.

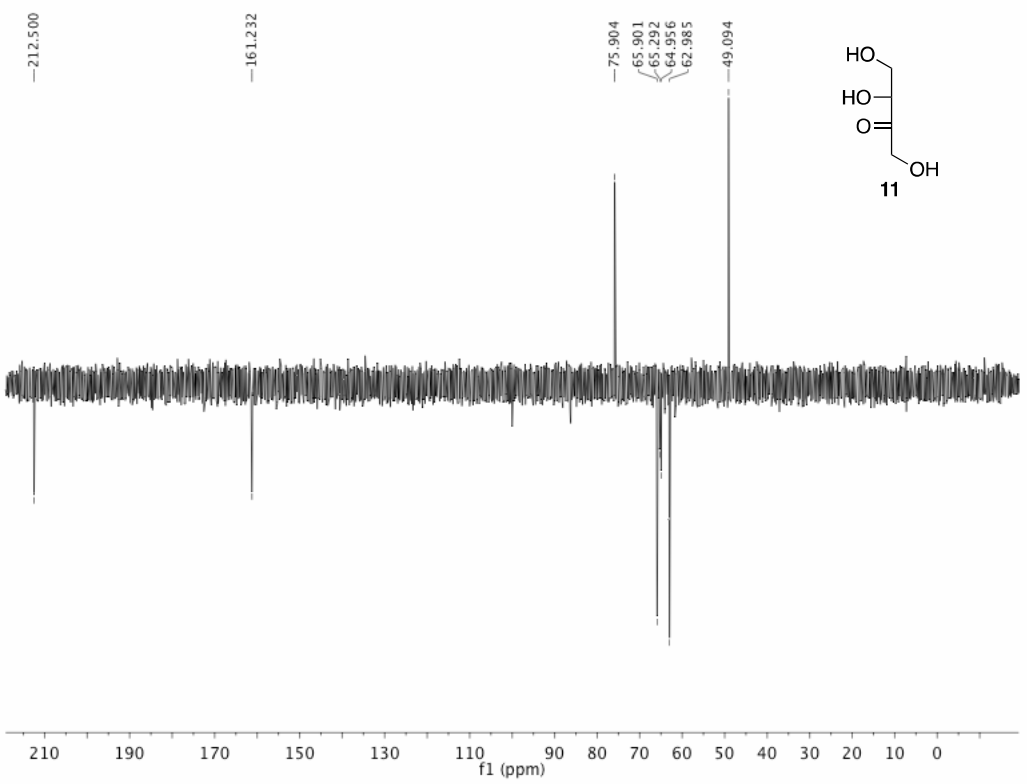


Figure S41. ^{13}C NMR-APT (H_2O/D_2O) of the reaction of DHF and formaldehyde after 24 h at r.t.

II.a.2. *Reaction of DHF with 2.0 eq of formaldehyde followed by acidification to pH 2-3:* The reaction was performed as described earlier (section II.a.1). After 30 min., at r.t., 0.7 mL of aliquot was transferred to sample vial and acidified to pH 2-3 (by pH paper) by adding 6N HCl (immediately gas was evolved). ^{13}C NMR was measured after 1 h, over night and 24 h.

After 1 h at r.t., ^{13}C NMR showed peaks at 210.52 (keto, less intensity), 207.99 (keto, more intensity), 171.64 (COOH, more intensity), 96.35 (hydrated keto, less intensity), 85.22 (quaternary carbon, more intensity), 75.50 (CH-OH, less intensity), 66.09 (CH_2-OH, less intensity), 65.89 (CH_2OH, more intensity), 65.08 (CH_2OH, more intensity), 64.32 (CH_2OH, less intensity) and 24.65 (CH_3C=O, less intensity) [Figure S42 and S43].

After over night (16.0 h) at r.t., ^{13}C NMR showed peaks at 210.52 (keto, more intensity), 96.36 ((hydrated keto, more intensity), 65.10 ((CH_2OH, more intensity) and 24.66 (CH_3C=O, more intensity). These peaks can be assigned for compound **12** [Figures S44, S45 and S46].

After 24 h at r.t., ^{13}C NMR showed peaks for derivative **12** [Figure S47].

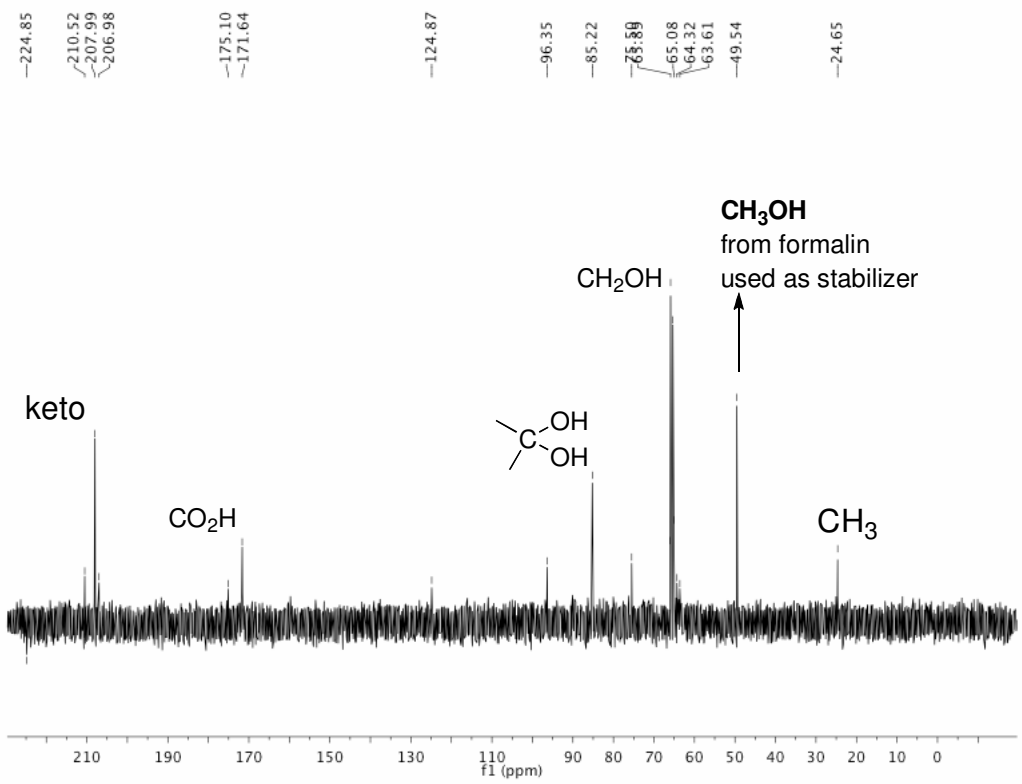


Figure S42. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde (after acidification), 1 h at r.t.

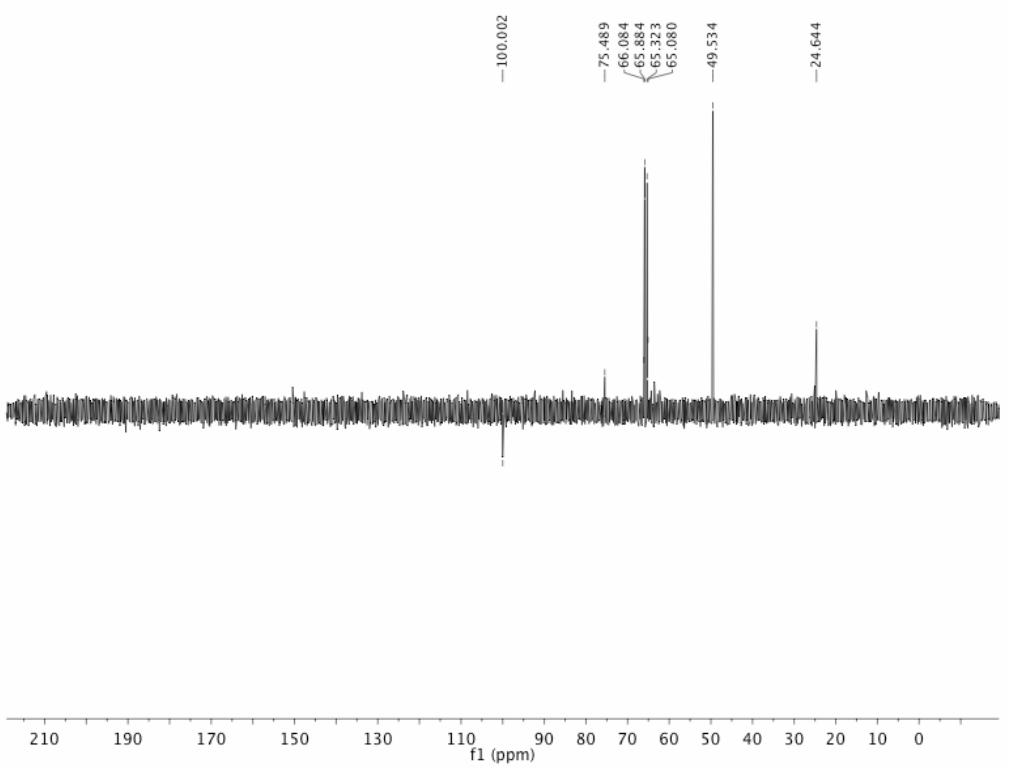


Figure S43. ^{13}C NMR-APT (H_2O/D_2O) of the reaction of DHF and formaldehyde (after acidification), 1 h at r.t.

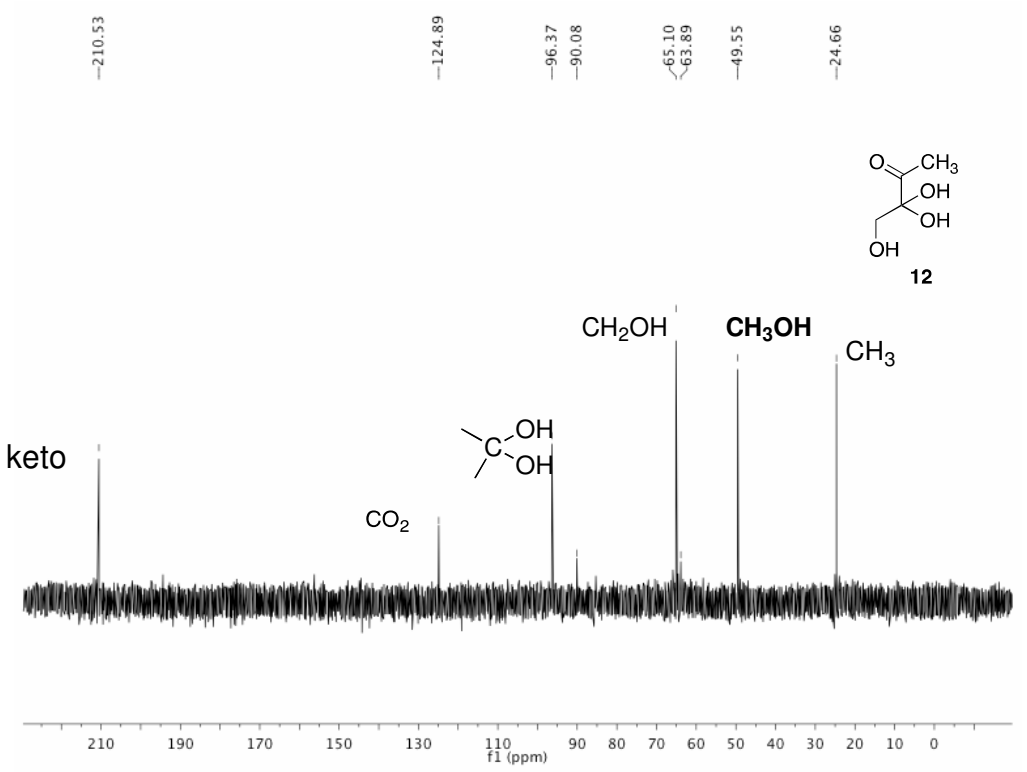


Figure S44. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde (after acidification),16 h at r.t.

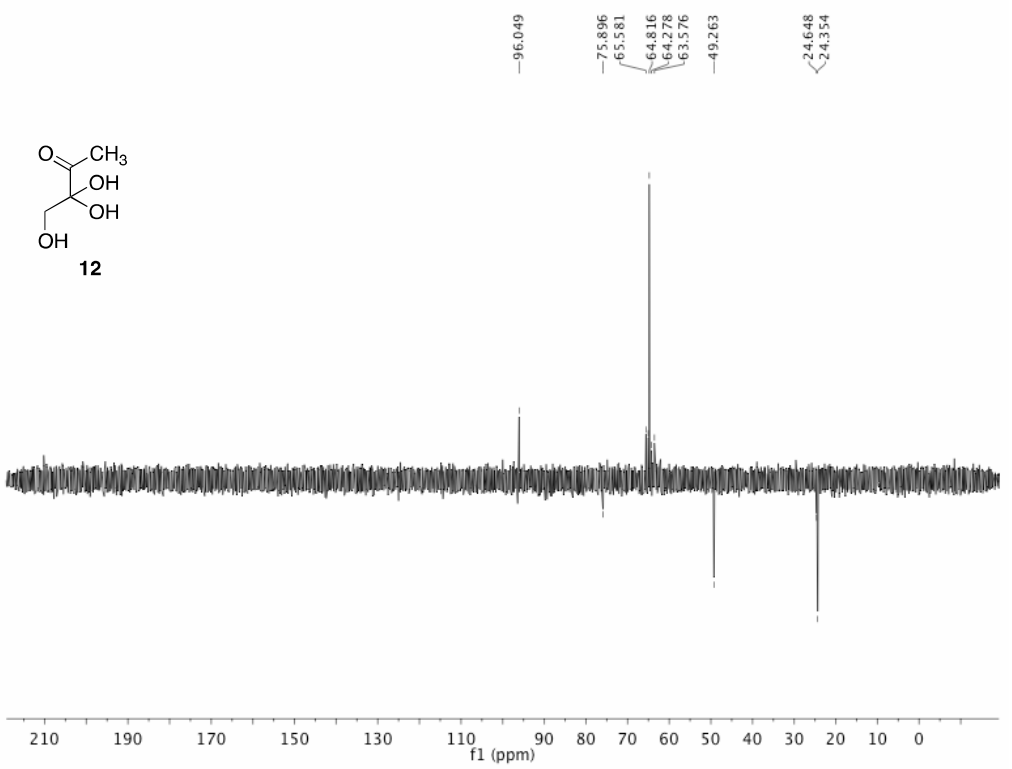


Figure S45. ^{13}C NMR-APT (H_2O/D_2O) of the reaction of DHF and formaldehyde (after acidification),16 h at r.t.

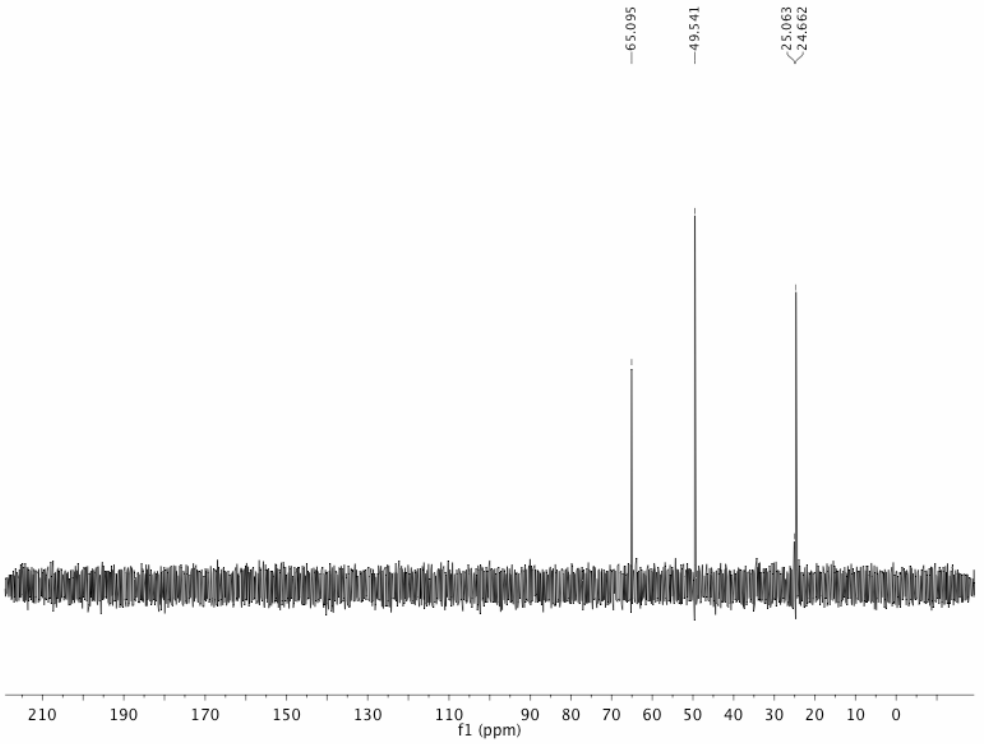


Figure S46. QNP-DEPT45 (H_2O/D_2O) of the reaction of DHF and formaldehyde (after acidification), 16 h at r.t.

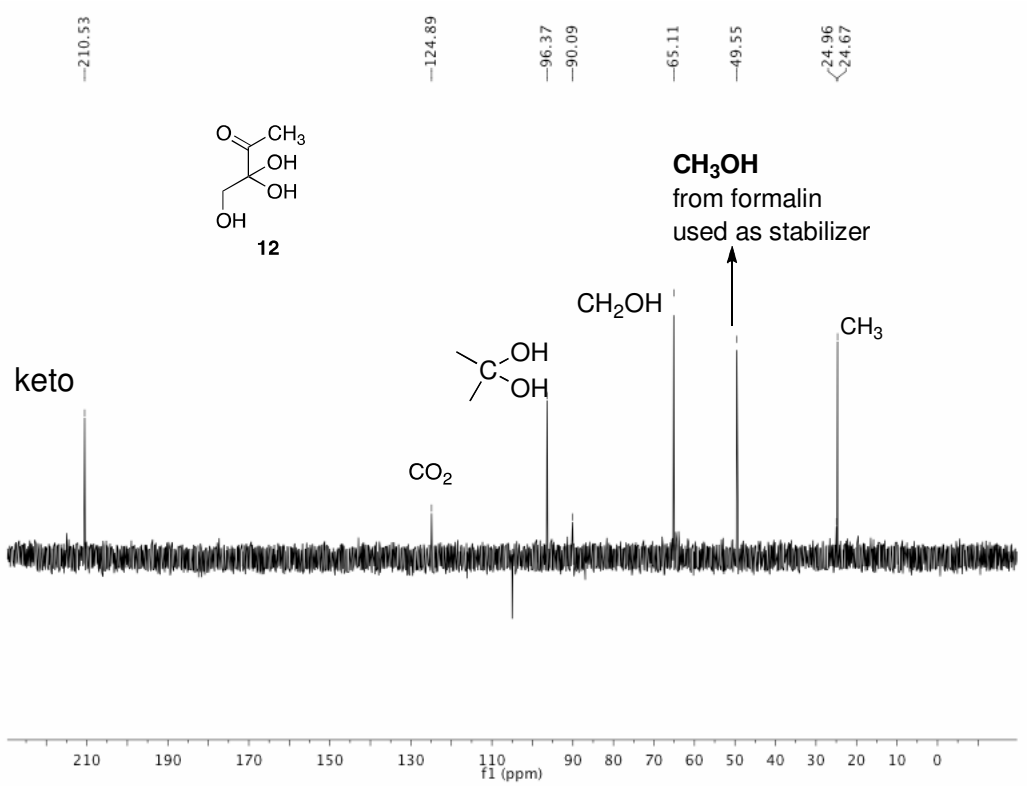


Figure S47. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde (after acidification), 24 h at r.t.

II.a.3. *Reaction of DHF with 1.0 eq of formaldehyde:* To a cold (4 °C) aq. solution of 1.0 eq., of DHF dilithium salt (0.8 M), 1.0 eq of 37% formalin in water (0.15 mL, Aldrich, contains 10-15% MeOH as stabilizer MeOH) was added in one portion under nitrogen atmosphere at 4 °C. The reaction mixture became a clear solution and the pH of the resulting solution was 8-9 (by pH paper). The reaction was monitored by ^{13}C NMR at 4 °C and r.t.

At 4 °C, after 30 min. ^{13}C NMR showed peaks for first aldol adduct **9** at ppm 65.16 (*C*H_2OH), 84.91 [quaternary, -*C*(OH)(CO_2^-)], 170.73, 172.91 (*C*O_2^-), 202.18 (*C*=O) [Figure S48].

After 3.5 h at 4 °C, ^{13}C NMR showed peaks for first aldol adduct **9**, and decarboxylated product **7** with almost similar intensities. ^{13}C NMR gave peaks for intermediate **7** at ppm 65.19 (*C*H_2OH), 77.96 (*C*HOH), 173.39 (*C*O_2^-), 208.9 (*C*=O) [Figure S49].

After 20 h at 4 °C, ^{13}C NMR showed peaks for decarboxylated intermediate **7** as the major product [Figure S50].

At r.t., after 30 min. ^{13}C NMR showed peaks for first decarboxylated intermediate (monocarboxyl-dihydroxyacetone) **7** and first aldol adduct **9** [Figure S51].

After 3.5 h and 6.5 h at r.t., ^{13}C NMR showed major peaks for monocarboxyl-dihydroxyacetone **7** and minor peaks for dihydroxyacetone (**4**) [Figures S52 and S53].

After 20 h at r.t., ^{13}C NMR showed major peaks for dihydroxyacetone [Figure S54].

After 3 days at r.t., ^{13}C NMR showed major peaks for dihydroxyacetone (**4**) [Figure S55].

In both cases minor peaks attributable to DHF dimer were seen, but seem to vanish with time (e.g. Figures S54 and S55).

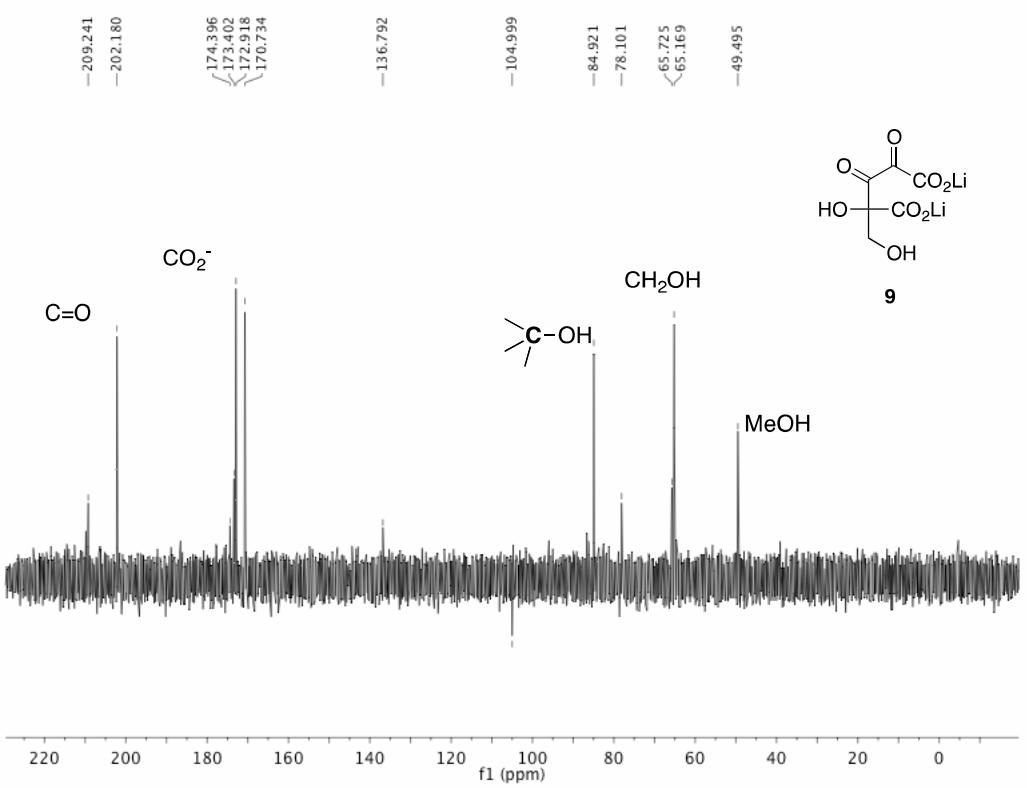


Figure S48. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde (1:1), after 30 min at 4 °C.

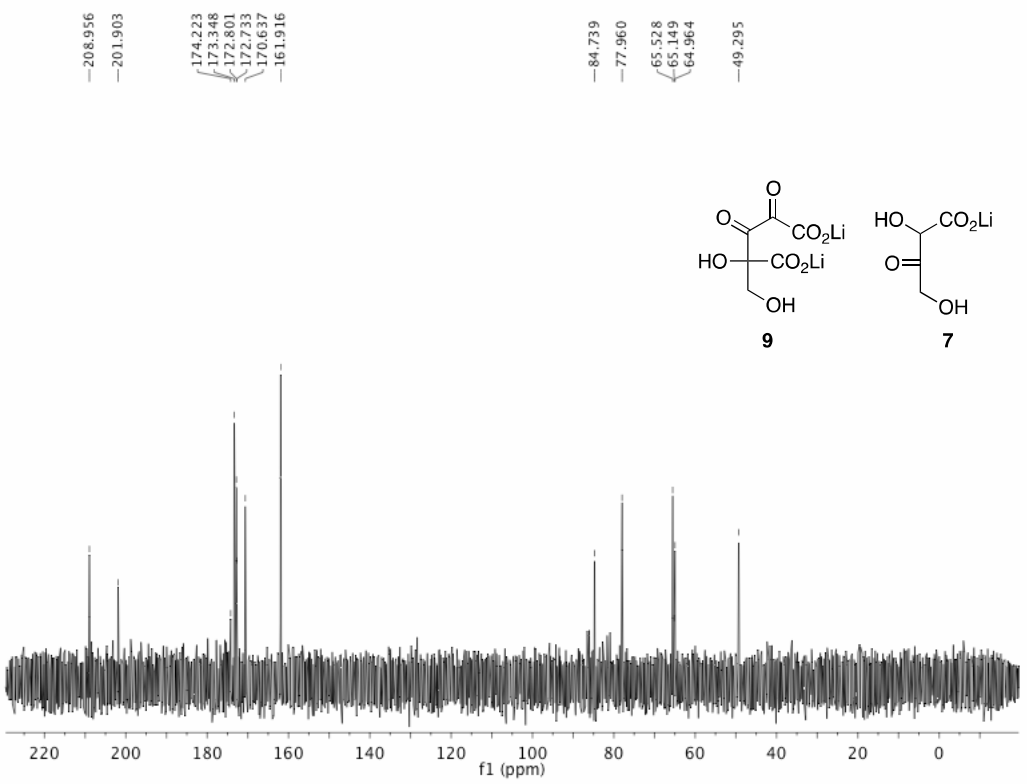


Figure S49. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde (1:1), after 3.5 h at 4 °C.

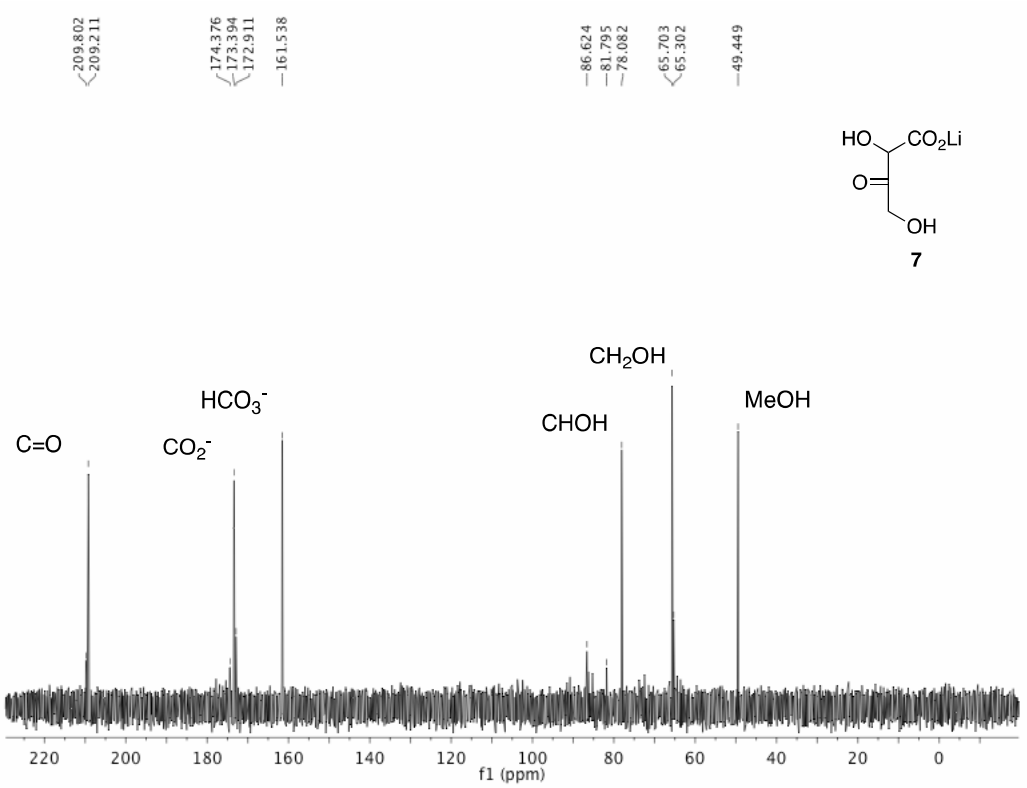


Figure S50. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde (1:1), after 20 h at 4 °C.

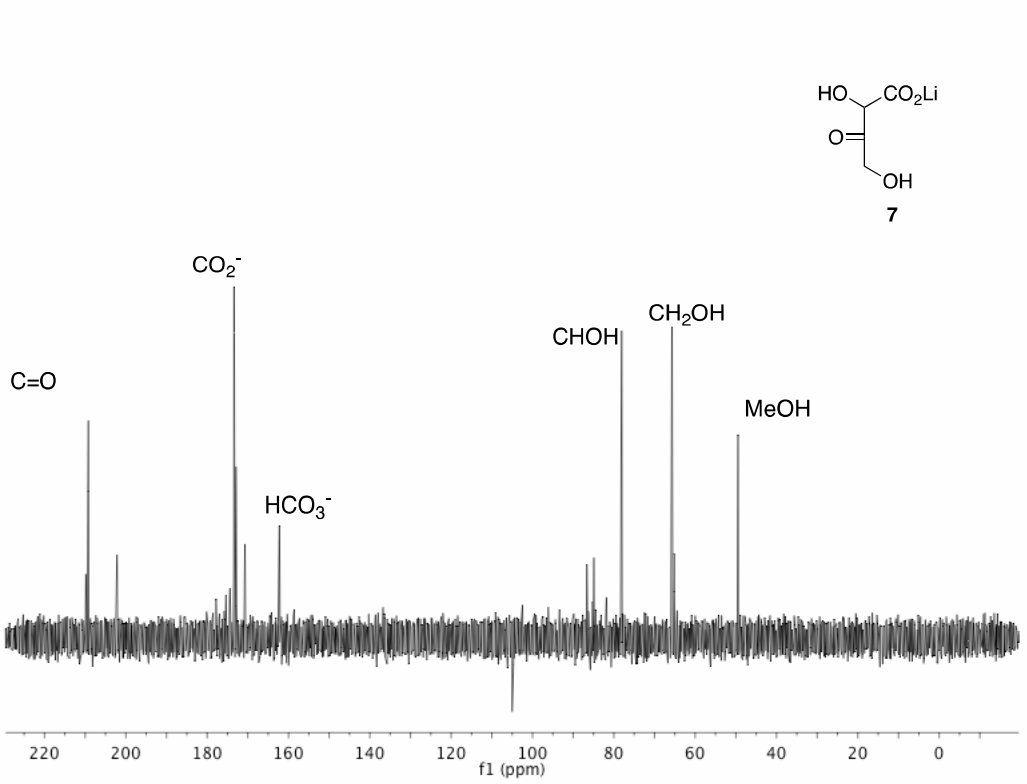


Figure S51. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde (1:1), 30 min at 4 °C, and then 30 min at r.t.

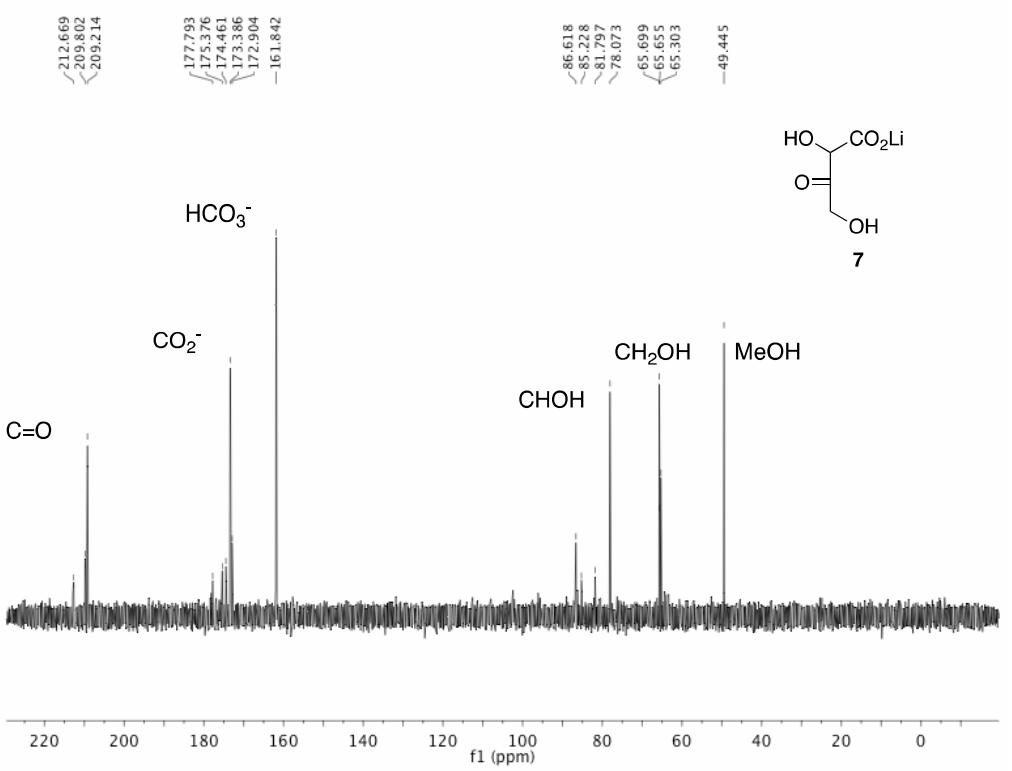


Figure S52. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde (1:1), 30 min at 4 °C, and then 3.5 h at r.t.

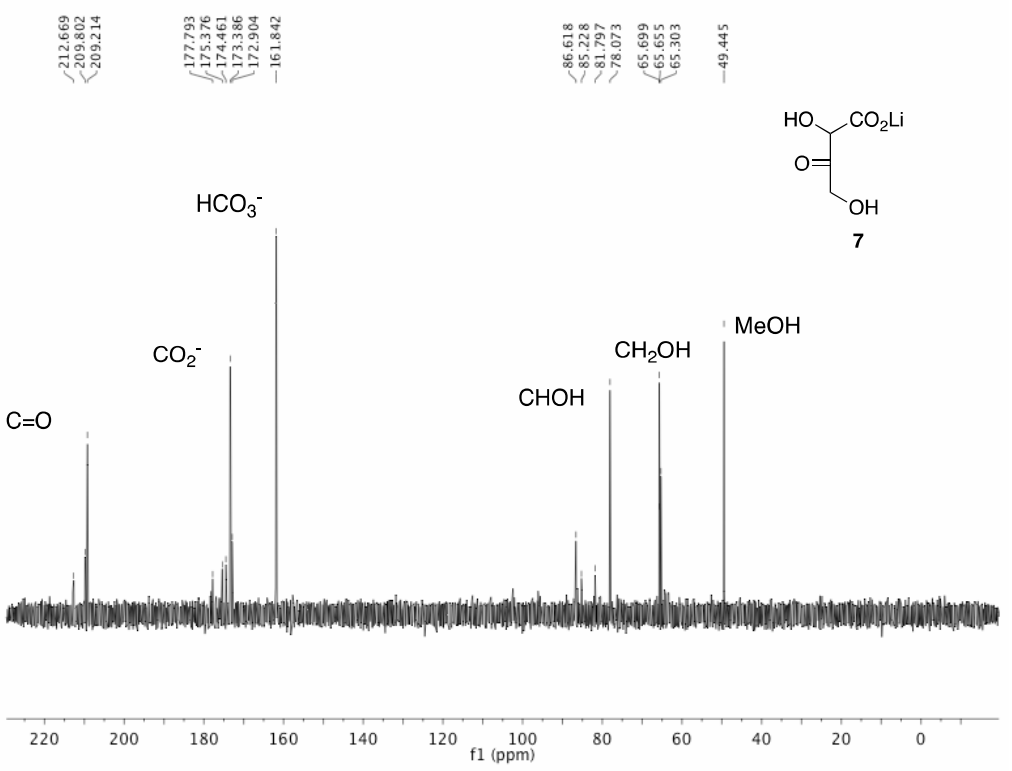


Figure S53. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde (1:1), 30 min at 4 °C, and then 6.5 h at r.t.

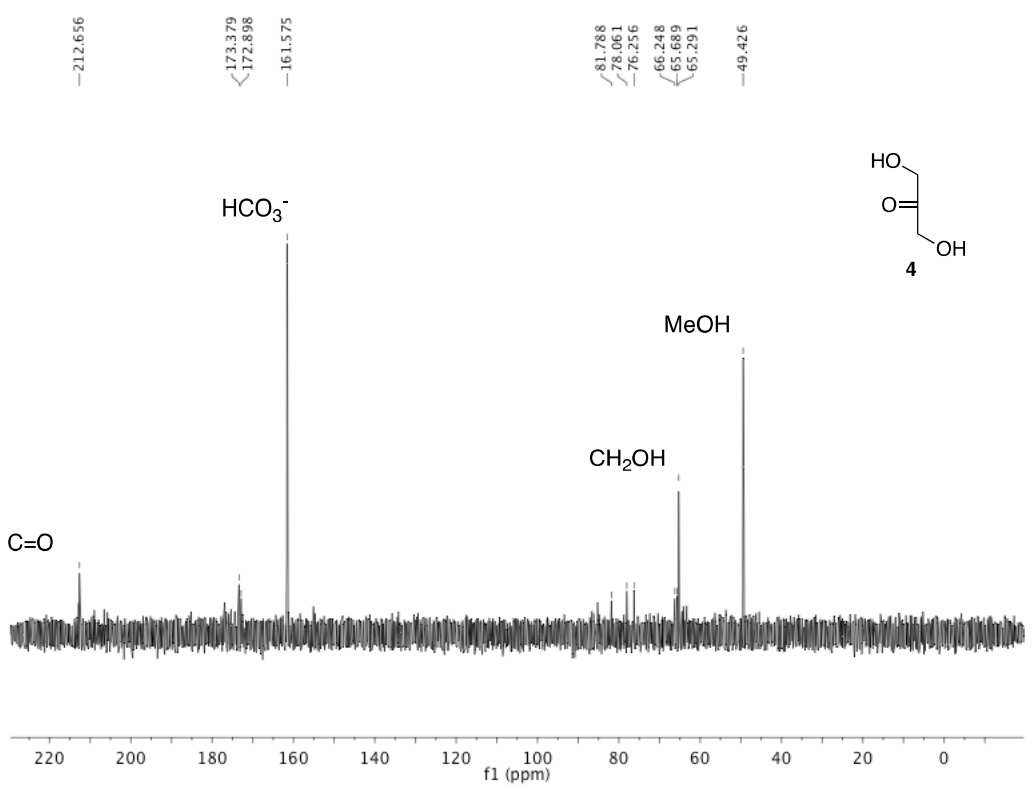


Figure S54. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde (1:1), 30 min at 4 °C, and 20 h at r.t.

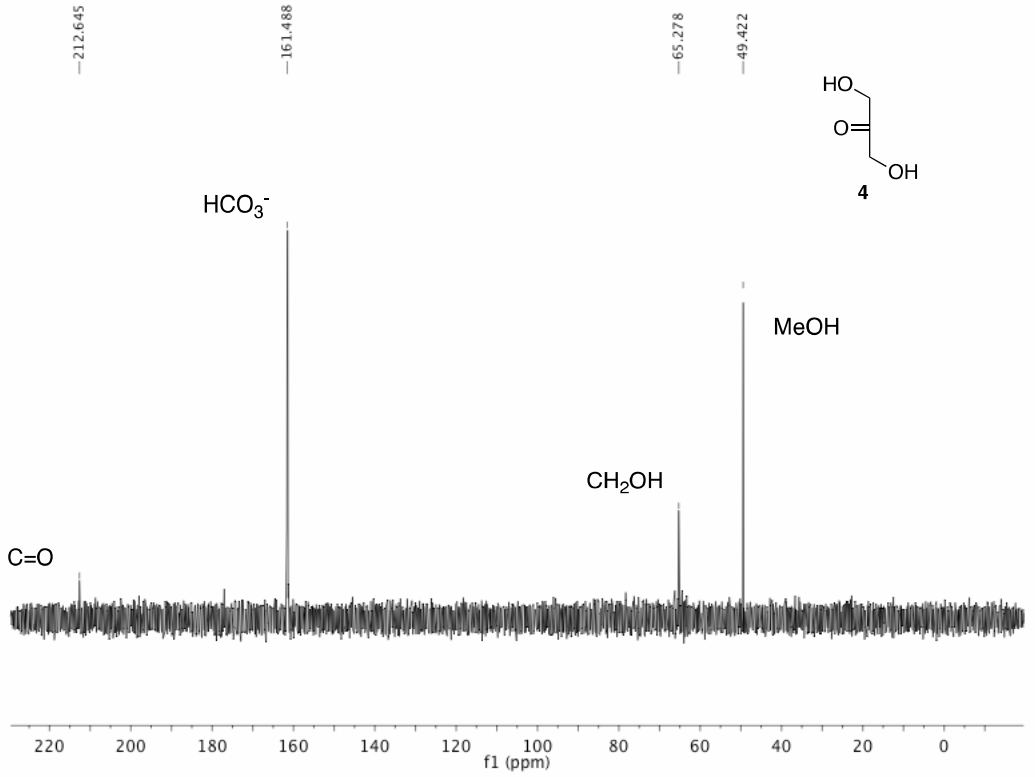
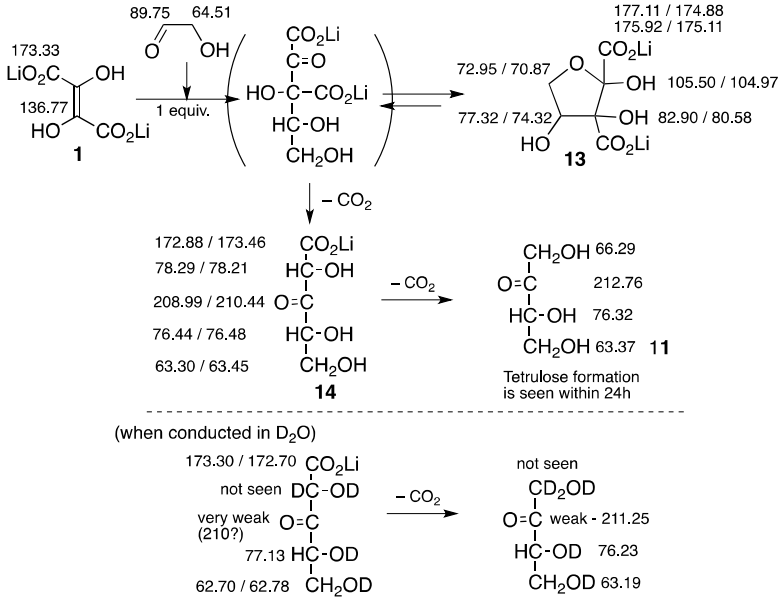


Figure S55. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF and formaldehyde (1:1), 30 min at 4 °C, and then 3 days at r.t.

II.b. Reaction of DHF with glycolaldehyde:



Scheme S8: Reaction of 1.0 equivalent of DHF with 1.0 equivalent of glycolaldehyde leading to formation of tetrulose.

II.b.1. Reaction of DHF (0.5 M) with glycolaldehyde (preliminary attempts)*:* The experiment was performed according to the procedure described in the experimental section of the main paper. The reaction mixture was monitored by ^{13}C NMR.

After 1.0 h at 4 °C: After 1 h at 4 °C, 0.6 mL of aliquot was taken from the reaction mixture through syringe and ^{13}C NMR was measured (at 4 °C) by adding 2 drops of D_2O. The ^{13}C NMR showed peaks for aldol product **13** and DHF peaks were not observed. ^{13}C NMR for cyclic product (**13**) at ppm 72.95, 70.97 (CH_2-O); 77.32, 74.32 (CH-OH); 82.90, 80.58 [(-C(OH)(CO_2^-)]; 105.50, 104.97 [(O-C(OH)(CO_2^-)]; 177.11, 174.99; 175.92, 175.11 (CO_2^-) as diastereomeric mixture.

After 1h at 4 °C and 1h at r.t.: The above NMR sample was kept at r.t. for 1 h and again ^{13}C NMR was measured. ^{13}C NMR showed presence of first decarboxylated product (**14**), tetrulose **11** and peak for HCO_3^-. ^{13}C NMR data for first decarboxylated product (**14**, as diastereomeric mixture, ppm): 63.30, 63.45 (*C*H_2-O); 76.44, 76.49 (*C*H-OH); 79.29, 79.21 [(*C*H(OH)CO_2^-)]; 172.99, 173.46 (*C*O_2^-); 209.99, 210.44 (*C*=O); ^{13}C NMR data for tetrulose (**11**, ppm): 212.8 (*C*=O); 161.38 (H*C*O_3^-); 72.28 (*C*H-OH); 66.26, 63.34 (*C*H_2OH).

After overnight at r.t.: After the decarboxylation had begun at r.t., the 'reaction flask' was kept at r.t. for overnight. After overnight, ^{13}C NMR showed presence of only tetrulose (Figure S56). ^{13}C NMR (150 MHz, drops of D_2O): δ 212.8 (keto), 161.38 (HCO_3^-), 72.28 (*C*H-OH), 66.26, 63.34 (*C*H_2OH) [Figure S56].

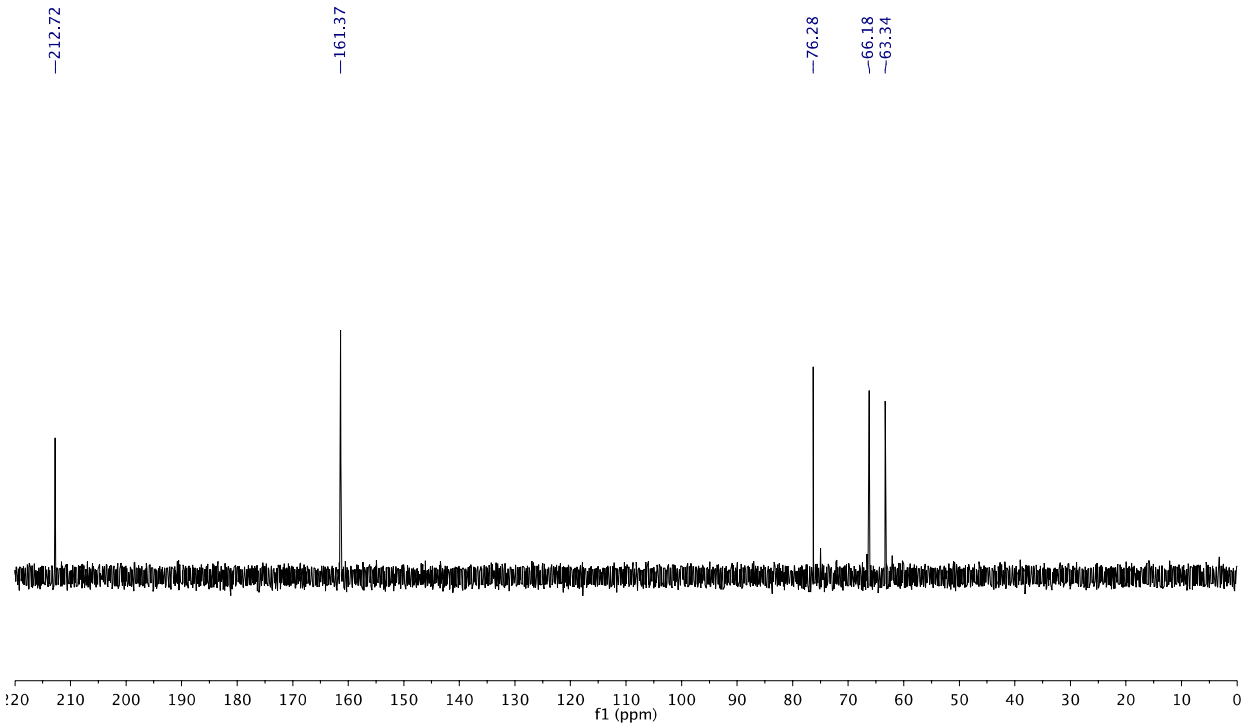


Figure S56. ^{13}C NMR (H_2O/D_2O) spectrum of the reaction mixture of DHF and glycolaldehyde (1:1), overnight (≈ 20h) at r.t.

II.b.2. Reaction of DHF (0.3 M) with glycolaldehyde at 4 °C*:* To a cooled (4 °C) aqueous solution of 'DHF dilithium salt (0.3 M)' was added aq. glycolaldehyde solution (0.3 M solution in water, stored for over night at r.t. to convert dimer to monomer) at 4 °C under nitrogen atmosphere and monitored by ^{13}C NMR (at 4 °C, Figure S57).

After 20 min. at 4 °C: 0.6 mL of aliquot was taken from the reaction mixture through syringe and ^{13}C NMR was measured (at 4 °C) by adding 2 drops of D_2O. The ^{13}C NMR showed peaks for cyclic aldol product **13** as mixture of diastereomers. ^{13}C NMR (ppm): 70.92, 72.95 (CH_2O); 74.34, 77.32 (CH-OH); 82.82, 80.58 [(-C(OH)(CO_2^-)]; 105.51, 104.93 [(O-C(OH)(CO_2^-)]; 177.11, 175.93; 175.10, 174.89 (CO_2^-) as diastereomeric mixture.

After 2.0 h at 4 °C: ^{13}C NMR showed peaks for one major diastereomer of 'first aldol adduct' and another as minor diastereomer. The difference in the peak intensities of the diastereomers of intermediate **13** implied that one diastereomer was either equilibrating to the other diastereomer or decarboxylating faster than other diastereomer. By comparison with NMR at 24.0 h, it seems that one of the diastereomers is decarboxylating at a faster rate. ^{13}C NMR data for diastereomer **13a** (faster decarboxylating diastereomer, ppm): 72.95 (CH_2O); 77.32 (CHOH); 82.82 [(-C(OH)(CO_2^-)]; 104.93 [(O-C(OH)(CO_2^-)]; 175.10, 175.93 (CO_2^-). 13C NMR data for Diastereomer **13b** (slower decarboxylating diastereomer, ppm): 70.92 (CH_2-O); 74.34 (CH-OH); 80.58 [(-C(OH)(CO_2^-)]; 105.51 [(O-C(OH)(CO_2^-)]; 177.11, 174.89 (CO_2^-).

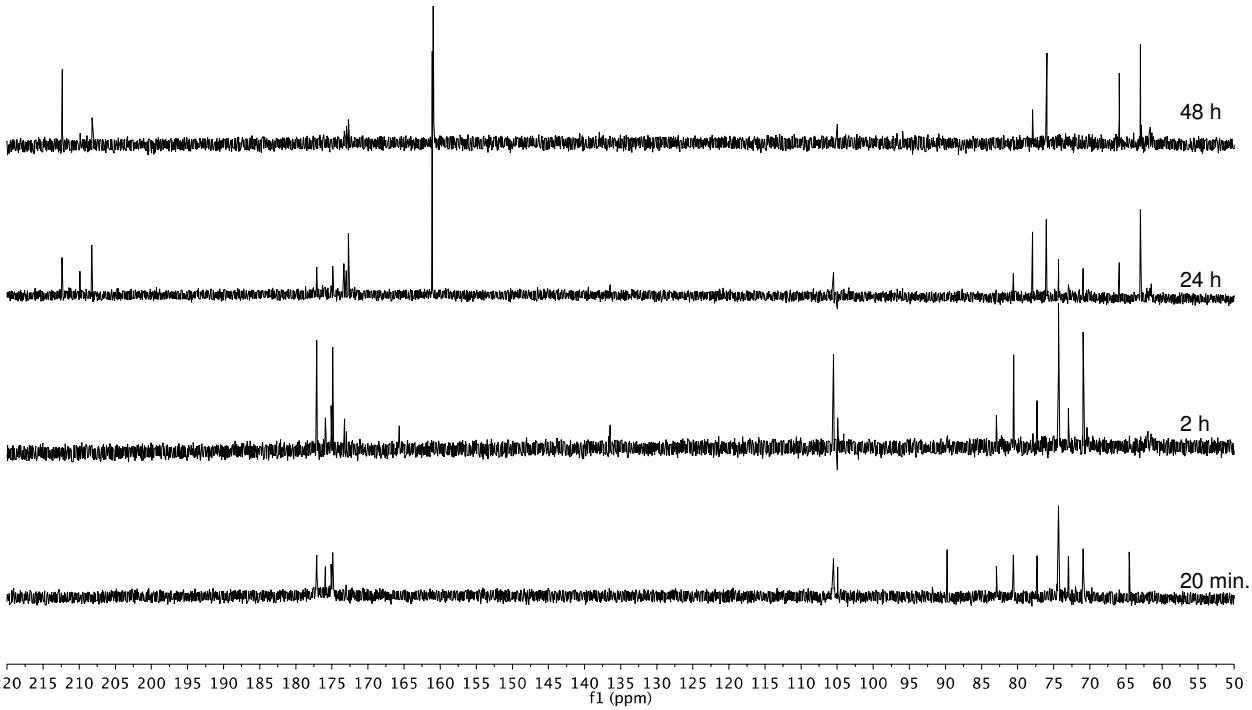


Figure S57. ^{13}C NMR spectroscopy monitoring of reaction of DHF (0.3 M) with 1 equiv of glycolaldehyde at 4 °C; Comparison of ^{13}C NMR spectra after 20 min. at 4 °C and after 24 h at 4 °C showed difference in rate of decarboxylation of first aldol adduct **13**.

After 24.0 h at 4 °C: ^{13}C NMR showed peaks for 'first aldol adduct' (**13b**, slower decarboxylated diastereomer) at ppm 70.92, 74.34, 80.58, 105.51, 177.11, 174.89; for first decarboxylated intermediate **14** at ppm 63.00, 62.9, 76.04 (CH_2OH); 75.99, 76.04, 77.95, 77.91 (CHOH); 172.71, 173.34 (CO_2^-), 209.93, 208.28 (C=O); for tetrulose at 61.50 (CH_2OH); 65.94 (CH_2OH); 75.99 (CHOH); 212.40 (C=O).

After 48.0 h at 4 °C: After 48.0 h at 4 °C, ^{13}C NMR showed peaks for tetrulose at ppm 62.98, 65.91, 75.97, 212.37 and first decarboxylated intermediate with small intensities. First aldol adduct was not observed by ^{13}C NMR.

II.b.3. *Reaction of DHF (0.3 M) with glycolaldehyde at r.t.:* To a cooled (4 °C) aqueous solution of 'DHF dilithium salt (0.3 M)' was added aq. glycolaldehyde solution (0.5 M solution in water, stored for over night at r.t. for hydrolysis) at 4 °C under nitrogen atmosphere and shifted the reaction mixture to r.t. The reaction was monitored by ^{13}C NMR (scanned at r.t.). After 1.0 hr, ^{13}C NMR showed presence of 'first aldol adduct (**13b**)' as a single diastereomer, first decarboxylated intermediate (**14**) and tetrulose (**11**). After 24.0 h at r.t., ^{13}C NMR showed presence of only tetrulose (**11**).

II.b.4. *Reaction of DHF (0.1 M) with glycolaldehyde at r.t.* Reaction of DHF with glycolaldehyde under 0.1 M concentration also gave tetrulose (same as 0.5 M and 0.3 M reactions) as discerned by ^{13}C NMR monitoring of the reaction mixture at 1.0 h, 3.5 h and 20.0 h.

Treatment of reaction mixture with IR-120 (H^+) resin at r.t.: In the process of isolating 'tetrulose' formed in the reaction of DHF (3.37 mmol, 1.0 eq, 0.5 M) and glycolaldehyde (1.0

eq) at r.t., The reaction mixture was treated (after 16 h at r.t., ^{13}C NMR showed presence of tetrulose) with IR-120 (H^+) resin. After 45 min at r.t., the reaction mixture was filtered from resin and the filtrate was analyzed by ^{13}C NMR by adding drops of D_2O. ^{13}C NMR of the filtrate showed presence of tetrulose at ppm 63.19, 66.16 (CH_2OH); 76.16 (CHOH); 212.56 (C=O) along with some other unknown peaks (may be octulose) at ppm 59.65, 61.73, 61.90, 62.25 (may be CH_2OH); 70.06, 70.20, 70.30 (may be CH_2-O); 71.30, 71.42 (CHOH); 71.67, 71.81 (may be CH_2O-) 71.93, 73.18, 73.38, 74.29, 75.02, 75.46, 76.43, 77.43, 77.34, 80.32, 81.56, 83.28 (CH-OH); 103.18, 103.51, 103.62, 103.67 (quaternary).

<u>*Attempts to isolate tetrulose from the reaction mixture by column chromatography:*</u> As an alternative method, purification of tetrulose by column chromatography was attempted. After the confirmation of tetrulose (by ^{13}C NMR, 24 h at r.t.) in the reaction mixture, water was removed by rotovap (at r.t.). The resulting half white solid material was dissolved in MeOH, centrifuged and the supernatant layer was separated from the solid material and concentrated to give a yellow solid material. This material was analyzed by ^{13}C NMR by dissolving in D_2O. ^{13}C NMR showed presence of tetrulose and bicarbonate. ^{13}C NMR of the other solid light brown color material from centrifugation showed presence of only bicarbonate. Then the crude 'yellow solid material' was subject to column chromatography (silica gel column chromatography (1.0 cm x 8 cm), MeOH/EtOAc (5:95)) furnished tetrulose **11** (52%) as thick yellow syrupy liquid. ^{13}C NMR, ^{13}C NMR-APT and ^{1}H NMR in MeOD of this yellow syrup showed presence of tetrulose (as major compound) along with some other (may be octulose) small, but, intense peaks. Mass spectrum also showed presence of tetrulose (M+Li) at 127. In an alternative batch (batch 2) tetrulose was isolated by column chromatography. The spectral data obtained from this batch was similar to the previous batch and also similar to the authentic tetrulose (Figure S58), confirming the presence of tetrulose.

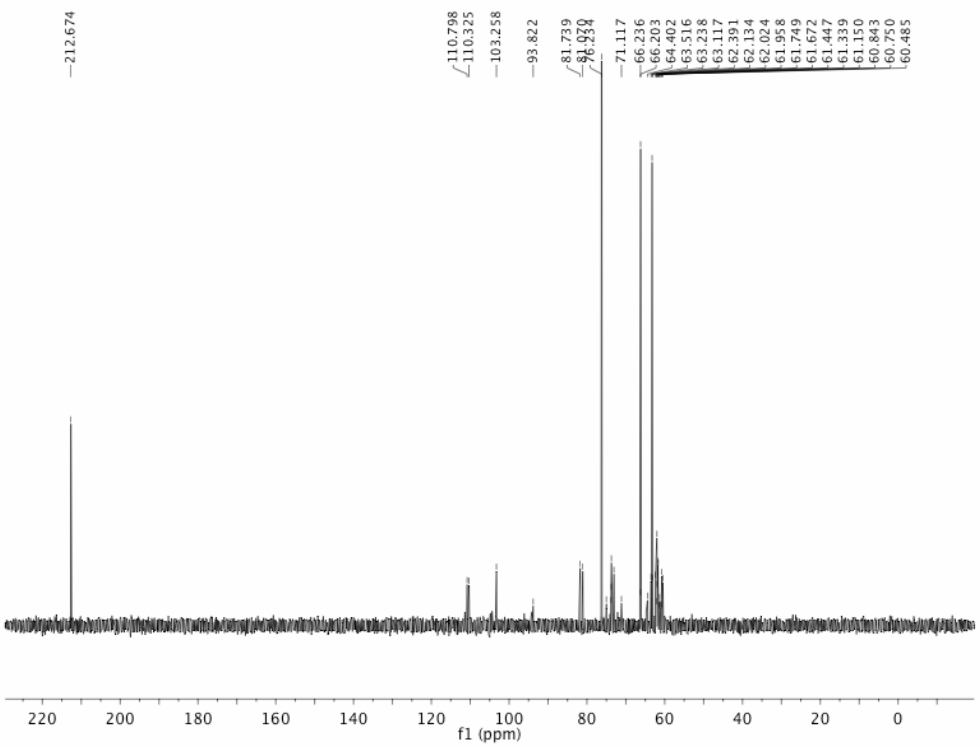


Figure S58. ^{13}C NMR (D_2O) of commercial (+)-erythrulose; this sample seems to be contaminated with higher aldoses derived from erythrulose.

Monitoring of the reaction of DHF with glycolaldehyde at 4 °C in "D_2O" under very dilute concentration: Glycolaldehyde solution was prepared by dissolving in D_2O. Li_2DHF was in D_2O was prepared by adding LiOH solution (prepared by dissolving in D_2O) to solid Li_2DHF. Reactions were carried out, as described above, at 0.4 M, 0.1 M, 0.04 M and 0.01 M concentrations at r.t.

^{13}C NMR of the 0.4 M sample after 20.0 h at r.t. showed deuterated tetrulose (**11a**) as the end product (Figure S59). ^{13}C NMR gave the peaks at ppm 63.18 [CH_2OH (C-4)], 76.23 [CHOH(C-3)] and remaining carbons [CH_2OH(C-1)] and C=O were not observed (coupled further with deuterium at C-1 carbon and hence not observed) due to deuterium exchange at the C-1 carbon during the decarboxylation step.

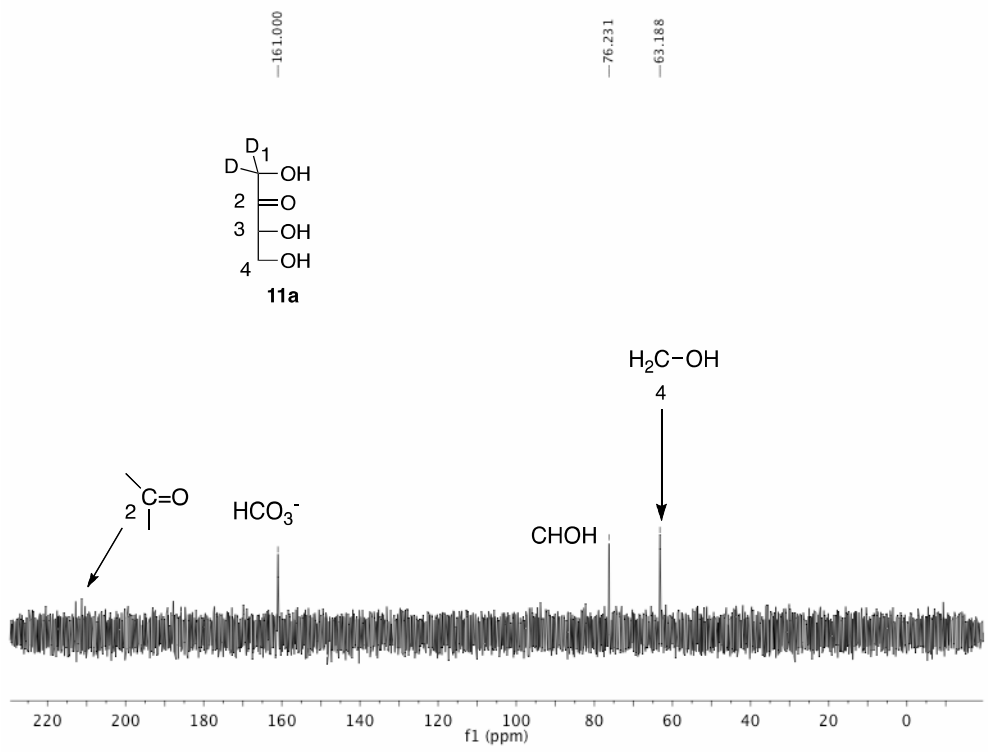
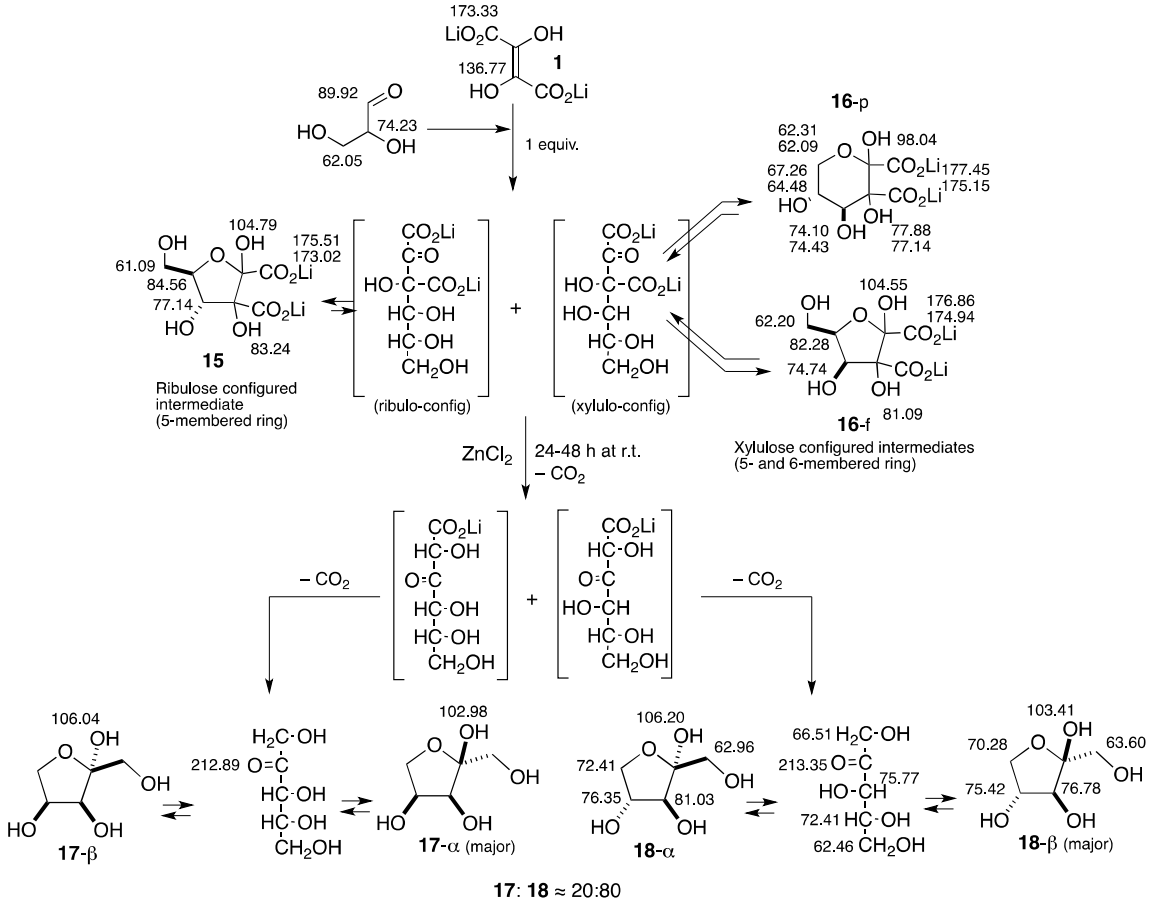


Figure S59. ^{13}C NMR of DHF + glycolaldehyde reaction conducted in D_2O after 20 h at r.t.

II.c. Reaction of DHF with Glyceraldehyde:

Table S1: Exploratory conditions for the reaction of 0.33M Li_2DHF with 1 equiv. glyceraldehyde (unless otherwise noted). Identification of end-products (pentuloses) was by comparison with literature values and authentic samples.

S.No	Conditions	Observations (^{13}C NMR)
1	Mixed reactants at 4°C. Wait for 2.0 h (end pH ≈ 10.7); then kept at r.t. for 46 h (end pH 8.30)	At 10 min. (4°C) pyranosyl intermediate **16-p** major. After 3 h at r.t. pyranosyl and furanosyl intermediates (**16-p**, **16-f**) were seen. By 20 h, decarboxylation has set in. At 46 h, only pentuloses were seen.
2	Mixed reactants at 4°C (pH ≈ 10.7); then kept at 4 °C for 18 days	The reaction was slowed down with pyranosyl intermediate persisting even after 18 days. Furanosyl intermediate was seen as minor component. Formation of xylulose was observed at 18 days.
3	Mixed reactants at 4°C. Waited for 6 h (end pH ≈ 9) and adjusted pH to ≈ 1 (by addition of 11.8N HCl). Kept at 4°C for 16 h, then at r.t. for 5 days.	After 15 min and 4 h pyranosyl intermediate was predominant. After 16 h (4°C) pyranosyl intermediate was still the major species. At 7 h (RT) diastereomers of pyranosyl and furanosyl intermediates were seen. After 1, 3 and 5 days at r.t. pyranosyl (major) and furanosyl (minor) intermediates were seen. No further progress.
4	Mixed reactants at 4°C. Wait for 3.5 h (end pH ≈ 11.4) and adjusted pH to 4.68 (by addition of 3N HCl). Kept at r.t. for 48 h.	At 5 min. and 3.5 h formation of a (major) pyranosyl intermediate; which slowly converted to end-product by decarboxylation; the predominant peaks were assignable to β-xylulose (**18-β**).
5	DHF (0.37 M, 1 equiv.) + glyceraldehyde (1 equiv.) + 2 equiv (0.4M) Cs_2CO_3. Mixed at 4°C and kept for 5 h (pH 7-8); then kept at r.t. for 68 h.	After 5 h, only s.m. observed. By 19 h a clear solun. was formed and xylulose was observed. After 68 h **18-β** was the major product.
6	Mixed reactants at 4°C. Waited for 3.5 h (end pH ≈ 8-9); added 2.0 equiv. $MgCl_2$ (pH ≈ 7-8) and brought to r.t. and kept for 26 h.	After 30 min. pyranosyl intermediate was major; addition of $MgCl_2$ (in 6 h at r.t.) resulted in a violet solution; only bicarbonate peak was seen. At 26 h pentulose formation was observed with xylulose as the major product.
7	Mixed reactants at 4°C. Waited for 3.5 h (end pH ≈ 8-9); adjusted pH (3N HCl) at 4°C to pH≈ 1-2; added 2.0 equiv. $MgCl_2$ (pH ≈ 2) and brought to r.t. and kept for 2 days.	After 1 h at 4°C, pyranosyl intermediate major; At r.t. (1 h, 18h & 2 days) pyranosyl intermediate was the only compound observed. Pentuloses were not observed.
8	Mix reactants at 4°C. Waited for 3.5h (end pH ≈ 11.4) and added 2.0 equiv of $ZnCl_2$ (pH ≈ 4.62) and kept at r.t. for 19 h (end pH ≈ 5.63)	At 5 min. and 3.5 h formation of a (major) pyranosyl intermediate; addition of $ZnCl_2$ initiated a violet ppt. formation. NMR peaks assignable to xylulose, ribulose and CO_2.
9	Mixed reactants at 4°C. Waited for 2.0 h (end pH ≈ 8-9); added 0.2 equiv. $ZnCl_2$ (pH ≈ 7) and kept at 4°C overnight. Brought to r.t. and kept for 27 h.	After 30 min. pyranosyl intermediate was major; overnight, pyranosyl intermediate was still major with little decarboxylation. At 27 h (RT) pentulose formation was observed with xylulose as the major product.
10	Mixed reactants at 4°C along with 2.0 equiv. of $ZnCl_2$. Waited for 1.5 h (end pH ≈ 5); kept for 1 day at 4°C.	White ppt. was formed on addition of $ZnCl_2$. After 1.5 h and 1 day, peaks of glyceraldehyde, HCO_3^- and CO_2 were seen. Addition of $ZnCl_2$ at the beginning seems to have precipitated DHF out of the reaction.
11	Mixed reactants at 4°C. Waited for 3.5 h (end pH ≈ 8-9); adjusted pH (3N HCl) at 4°C to pH≈ 1-2; added 2.0 equiv. $ZnCl_2$ (pH ≈ 2) and brought to r.t. and kept for 2 days.	After 1 h at 4°C, pyranosyl intermediate major; At r.t. (1 h, 18h & 2 days) pyranosyl intermediate was the major compound observed. After 2 days, new peaks seen – but pentuloses were not formed.

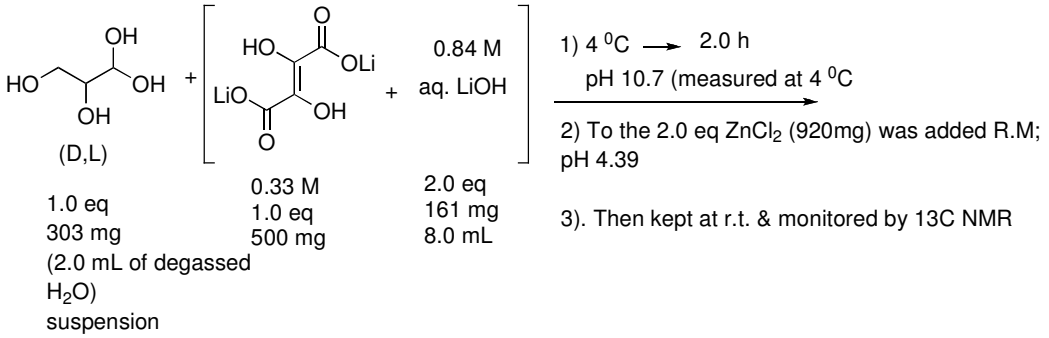


Scheme S9: Formation of pentuloses from the reaction of DHF with glyceraldehyde as discerned by ^{13}C NMR spectroscopy.

II.c.1. *Use of $ZnCl_2$ to investigate the pathway of the formation of pentuloses*. A suspension of 0.33 M glyceraldehyde in water was treated with 0.33 M of Li_2-DHF at 4°C for 2 h (pH ≈ 10.7). Then, 2.0 equiv of $ZnCl_2$ was added (which lowered the pH to 4.39). The reaction mixture was kept at r.t. and monitored by ^{13}C NMR spectroscopy.

After the completion of the reaction, the violet suspension was centrifuged, the yellow supernatant was separated and concentrated in vacuo to afford a syrup. ^{13}C, ^{1}H NMR and mass-spectra of this crude was clean (Figure S60, S61, S62 and S63) which showed the formation of only the two pentuloses **18** and **19**. Comparison with authentic samples or ribulose and xylulose (Figure S64) allowed assignment of the different forms of the two pentuloses. Integration of the relevant peaks determined the ratio of the pentuloses formed: 78% of xylulose and 22% of ribulose. Spiking with authentic xylulose and ribulose confirmed the pentulose formation.

II.c.2. *Preparative formation of pentuloses by reaction of DHF with glyceraldehyde:*



The experiment was set up according to the procedure described in the experimental section of the main paper; corresponding NMR spectra are shown in figures S60, S61, S62, S63 and S64.

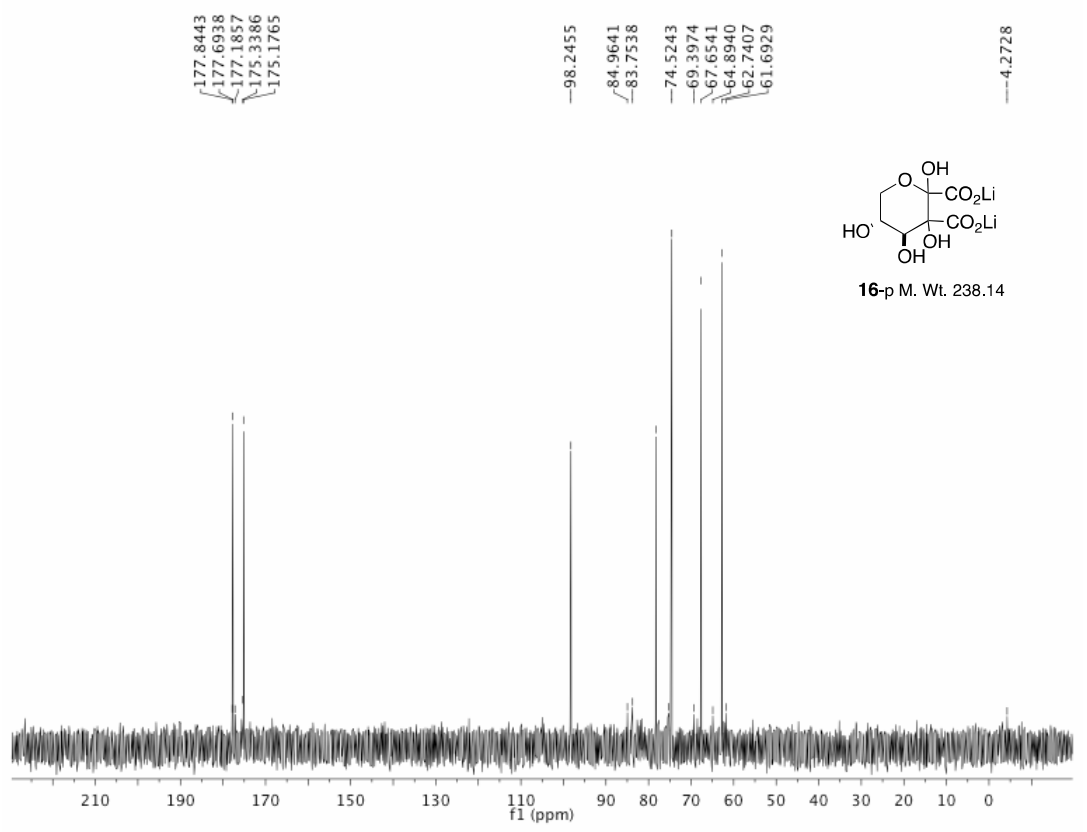


Figure S60: ^{13}C NMR (150 MHz, drops of D_2O) of reaction mixture after 2 h at 4 °C (0.33 M Li_2DHF + 0.33 M glyceraldehyde in degassed H_2O), documenting the formation of the pyranosyl adduct **16-p** as the major adduct.

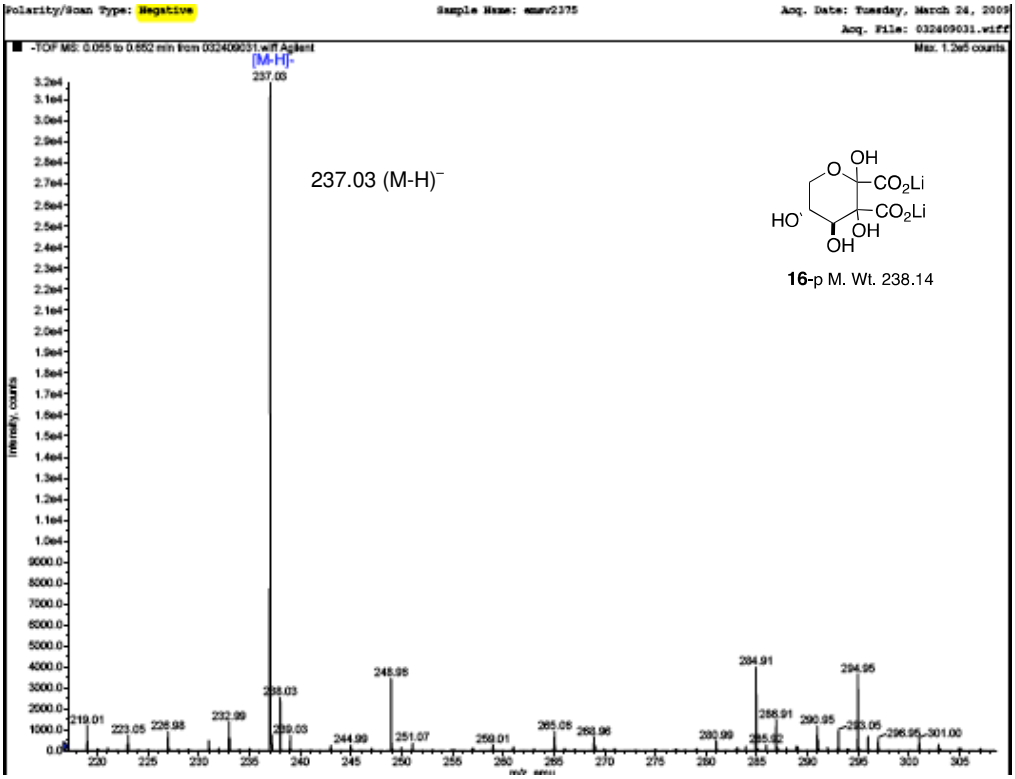


Figure S61. ESI (neg., MeOH) of the 'half white solid material obtained by lyophilization of filtrate from IR-120 (H^+) treatment of DHF-glyceraldehyde reaction at 4 °C for 4 h.

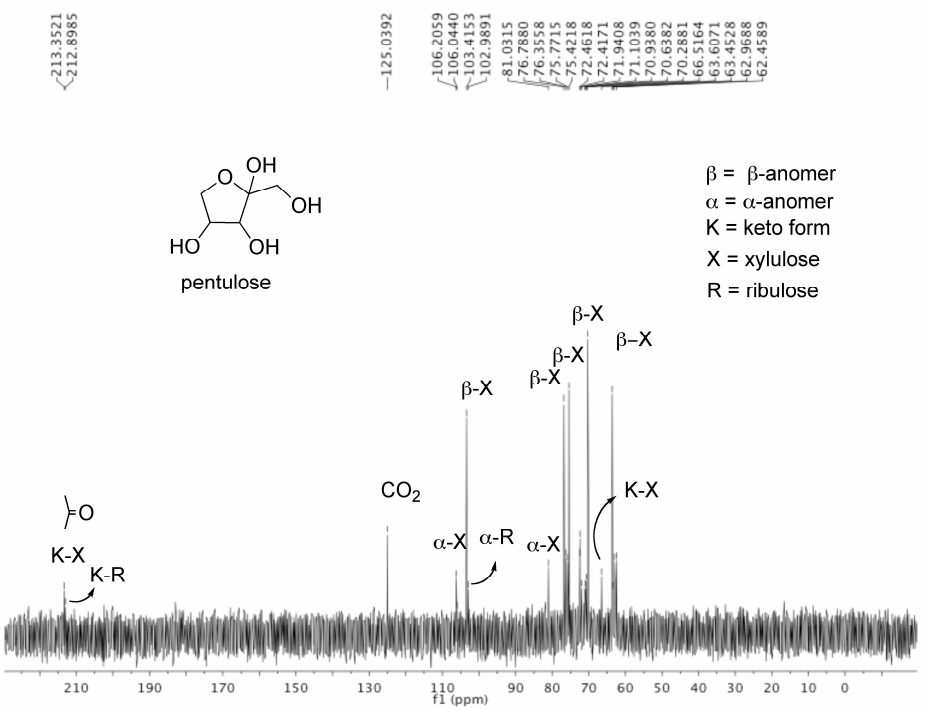


Figure S62: ^{13}C NMR spectrum (150 MHz, drops of D_2O) of reaction mixture supernatant after overnight at r.t. (0.33 M Li_2DHF + 0.33 M glyceraldehyde in degassed H_2O + $ZnCl_2$).

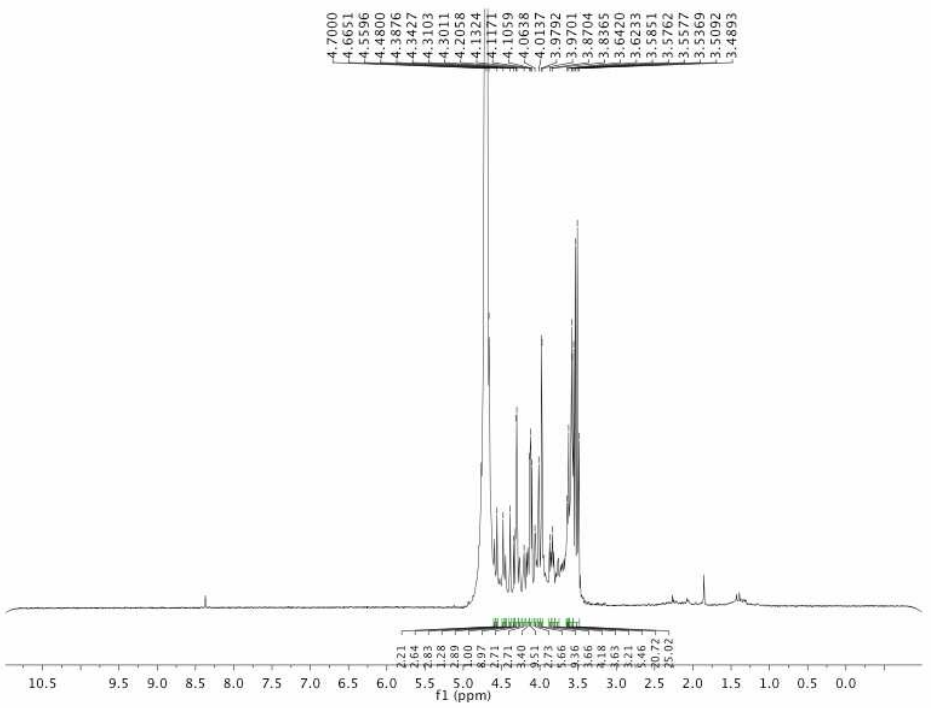


Figure S63: ^{1}H NMR spectrum (600 MHz, D_2O) of crude product (0.33 M Li_2DHF + 0.33 M glyceraldehyde in degassed H_2O + $ZnCl_2$).

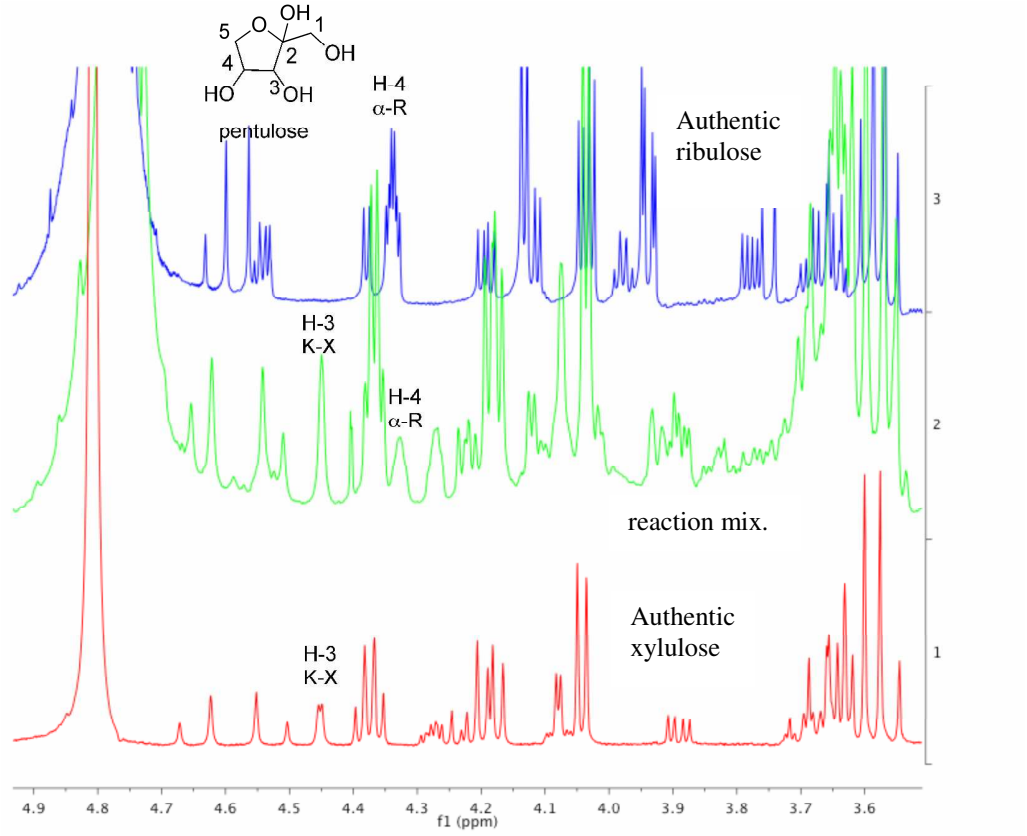


Figure S64: Comparison of ^{1}H NMR spectra of (a) authentic ribulose (blue), (b) crude product from reaction mixture (green) and (c) authentic xylulose (red).

II.c.3. *Estimation of pentuloses yield in glyceraldehyde and Li_2DHF reaction*.

Glyceraldehyde (0.33 M, 1 eq, 303 mg) was suspended in degassed water (at 4°C), which gave a yellowish suspension. To this yellow suspension, at 4 °C, a solution of Li_2DHF [0.33 M, prepared by addition of 2.0 eq. of aq. LiOH (0.84 M solution, 161 mg) to solid DHF (1.0 eq, 500 mg) kept at 4°C; the Li_2DHF solution was dark brown in color] was added. The resulting solution became pale yellow over a period of 5 minutes and pH was 10.7 (at 4 °C). Reaction was kept at 4 °C for 2 h; the reaction mixture was added to 2.0 eq of $ZnCl_2$ (920 mg) at 4°C. The reaction mixture acquires a dark violet color. After complete addition, the reaction mixture was left at r.t. for 24 h; the pH of the mixture (after the immediate addition and bringing to r.t.) was 4.39. Formation of a suspension was observed after 30 min. of mixing and after overnight, more precipitation was observed. The suspension was violet in color, while the supernatant was pale yellow in color. The reaction mixture was centrifuged and the pale yellow supernatant was separated and 0.1 mL of supernatant was diluted with 0.5 mL of D_2O and mixed with 1.3 mg (9.02 μmol) of sodium benzoate and analyzed by ^{1}H NMR (Figure S65). The integration of H-3 of keto form xylulose (which corresponds to 19% of xylulose) and H-4 of α-ribulose (which corresponds to 63% ribulose). Therefore, from the spectrum of crude sample, the integral value (0.35) of the H-3 of xylulose keto form corresponds to 19%, the total integration for xylulose (all forms totaling 100%) was calculated to be a value of 1.842. The integral value (0.2) of the H-4 of ribulose α-anomer corresponds to 63%, total integration for ribulose (all forms totaling 100%) was calculated to be a value of 0.317. Total integration for pentulose (xylulose and ribulose) present in the sample was 2.159 (1.842 + 0.317). From the comparison of sodium benzoate one proton (~7.47) integration (equal to 9.02 μmol) with total pentulose integration was 2.159. from which pentulose present in 0.1 mL R.M. as 19.47 μmol was calculated, which implied 1.97 mol (57%) in 10 mL total R.M.

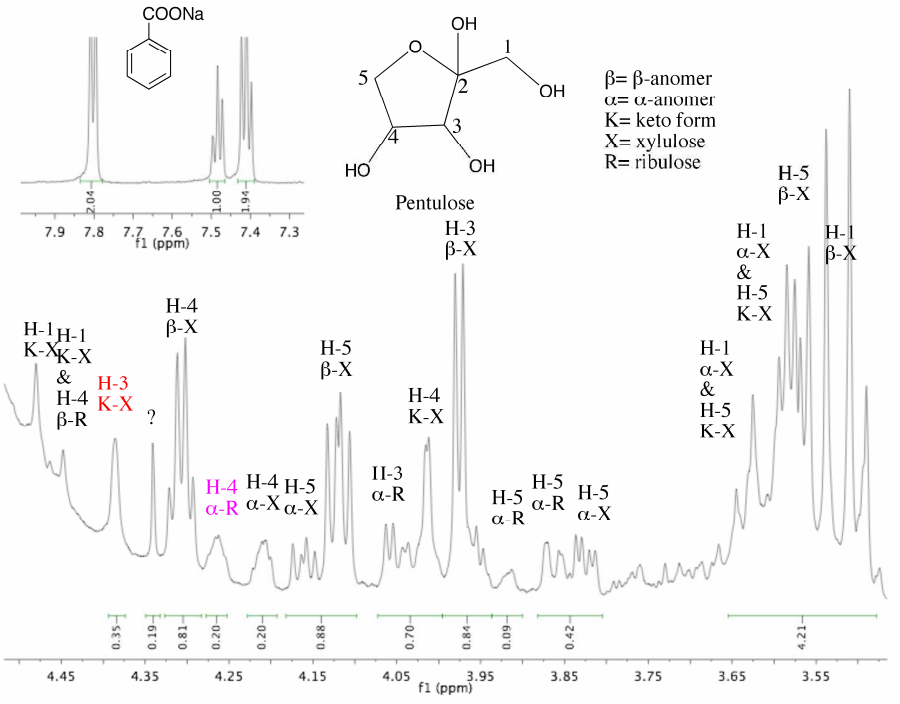


Figure S65: Expansion from 3.50 to 4.50 ppm ^{1}H NMR (600 MHz, D_2O) of crude product (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O + after 2h at 4°C treated with $ZnCl_2$ (2.0 eq) + left at r.t. 24h + 0.1 mL of R.M. diluted with 0.5 mL of D_2O and 1.3 mg of sodium benzoate)

II.c.4. *Exploratory reactions of glyceraldehyde with Li_2DHF to see effect of reaction time and temperature effect on xylulose and ribulose ratio.*

Li_2DHF was prepared as usual and treated with glyceraldehyde at 4 °C (The reaction mixture was divided into 5 parts). ^{13}C NMR was taken immediately after 10 minutes of mixing (Figure S66; NMR scanning time was for 1 hr about 1400 scans) - therefore this NMR represents the reaction mixture at 1 hour at 4°C; This spectrum showed predominantly the six-membered ring intermediate (62.74, 67.65, 74.53, 78.29, 98.24, 175.17 and 177.69) and with peaks for only one five-membered ring intermediate (61.09, 77.14, 83.24, 84.56, 104.79, 173.02 and 175.51) (may be erythro-configured primary adduct) not the same that we had seen in previous experiments.

Part 1: The reaction mixture was treated immediately with $ZnCl_2$ (2.0 eq.) and then kept at r.t for 24 h (this converts cleanly the intermediates to the pentuloses); the reaction mixture (1 mL) was centrifuged and the pale yellow filtrate was separated and concentrated to dryness to give syrup (yellow in color). This was dissolved in 1 ml of D_2O; small amount of un-dissolved white particles were separated by centrifugation and the clear solution (0.1 mL) was diluted with 0.5 mL of D_2O and analyzed by ^{1}H NMR (Figure S67 and S68). ^{1}H NMR at this stage showed that the ratio between xylulose and ribulose was 76:24.

Part 2: The reaction mixture was continued to be kept at 4 °C; after 48 h a 1-hour ^{13}C NMR (1400 scans) was taken (Figure S69). This now showed the six-membered and the 5-membered ring intermediate (may be erythro-configured primary adduct) to be intact; while there was the formation of a *newer* 5-membered ring with more intensity (62.04, 74.76, 81.11, 82.29, 104.56, 174.94 and 176.87) (may be threo-configured primary adduct). After 48 h at 4 °C, $ZnCl_2$ was added and allowed to stand at r.t for 24 h; the reaction mixture (1 mL) was centrifuged and the pale yellow filtrate was separated and concentrated to dryness to give syrup (yellow in color). This was dissolved in 1 ml of D_2O; small amount of un-dissolved white particles were separated by centrifugation and the clear solution (0.1 mL) was diluted with 0.5 mL of D_2O and analyzed by ^{1}H NMR (Figure S70 and S71). 1H NMR at this stage revealed that the ratio between xylulose and ribulose was 78:22.

Part 3: The reaction mixture was kept at r.t.; After 3 h a 1-hour ^{13}C NMR (1400 scans) was taken (Figure S72). This now showed the six-membered and the 5-membered ring intermediate (may be erythro-configured primary adduct) to be intact; while there was the formation of a NEWER 5-membered ring (62.04, 74.76, 81.11, 82.29, 104.56, 174.94 and 176.87) (may be *threo*-configured primary adduct). After 48 h at 4°C, $ZnCl_2$ was added and allowed to stand at r.t for 24 h; the reaction mixture (1 mL) was centrifuged and the pale yellow filtrate was separated and concentrated to dryness to give syrup (yellow in color). This was dissolved in 1 ml of D_2O; small amount of un-dissolved white particles were separated by centrifugation and the clear solution (0.1 mL) was diluted with 0.5 mL of D_2O and analyzed by ^{1}H NMR (Figure S73). ^{1}H NMR at this stage revealed that the ratio between xylulose and ribulose was 77:23.

Part 4: The reaction mixture was kept at r.t for 23 h; after that it was monitored by ^{13}C NMR (1h, 1400 scans) (Figure S74). They showed that 6-membered ring intermediate (present from initial mixing) was still there, and the two 5-membered ring intermediates were equal in intensity now, and was the same as the one seen from previous experiments and the one that was seen from the 48 h at 4 °C (part 2) experiment. $ZnCl_2$ was added after 24 h following above mentioned procedure. ^{1}H NMR (Figure S75) at this stage revealed that the ratio between xyluolose and ribulose was 73:27.

Part 5: The reaction mixture was kept at r.t. for 47 h, after that it was monitored by ^{13}C NMR (1h, 1400 scans) (Figure S76), which showed pentulose formation and absence of 6-membered ring intermediate and two 5-membered ring intermediates. $ZnCl_2$ was added after 24 h following above mentioned procedure. ^{1}H NMR (Figure S77) at this stage revealed that the ratio between xyluolose and ribulose was 75: 25.

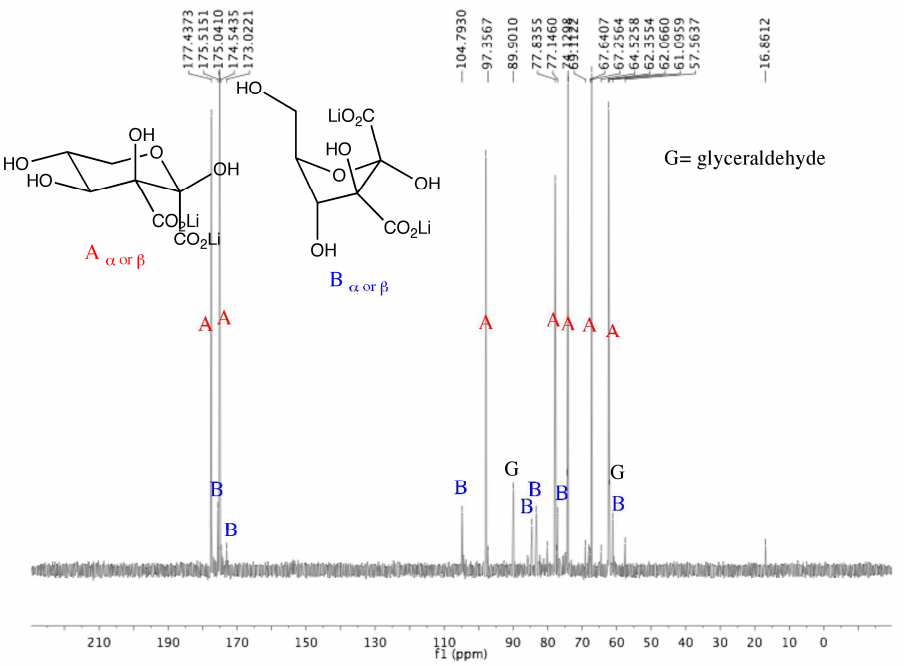


Figure S66: ^{13}C NMR (150 MHz, drops of D_2O) of reaction mixture (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O) after 10 min. (1 h NMR running time) at 4 °C (1400 scans).

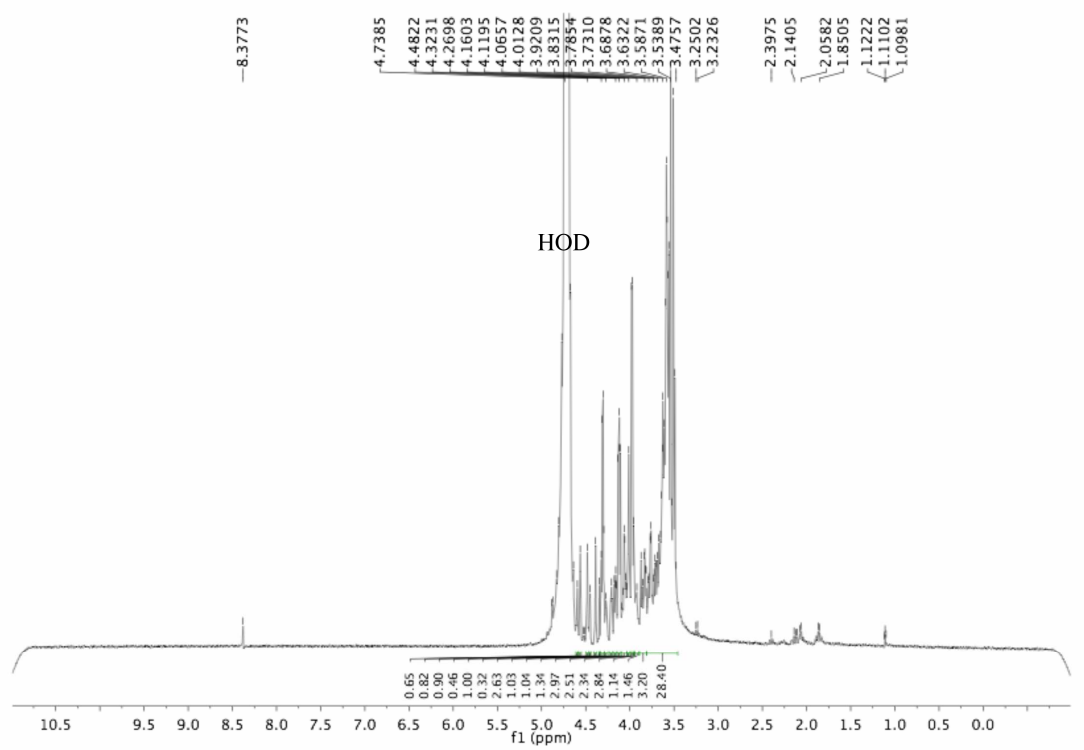


Figure S67: From Part 1 - ^{1}H NMR (600 MHz, D_2O) of crude product (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O + after 10 min. treated with $ZnCl_2$ (2.0 eq) + left at r.t., 24h + concentrated to dryness and re-dissolved in D_2O)

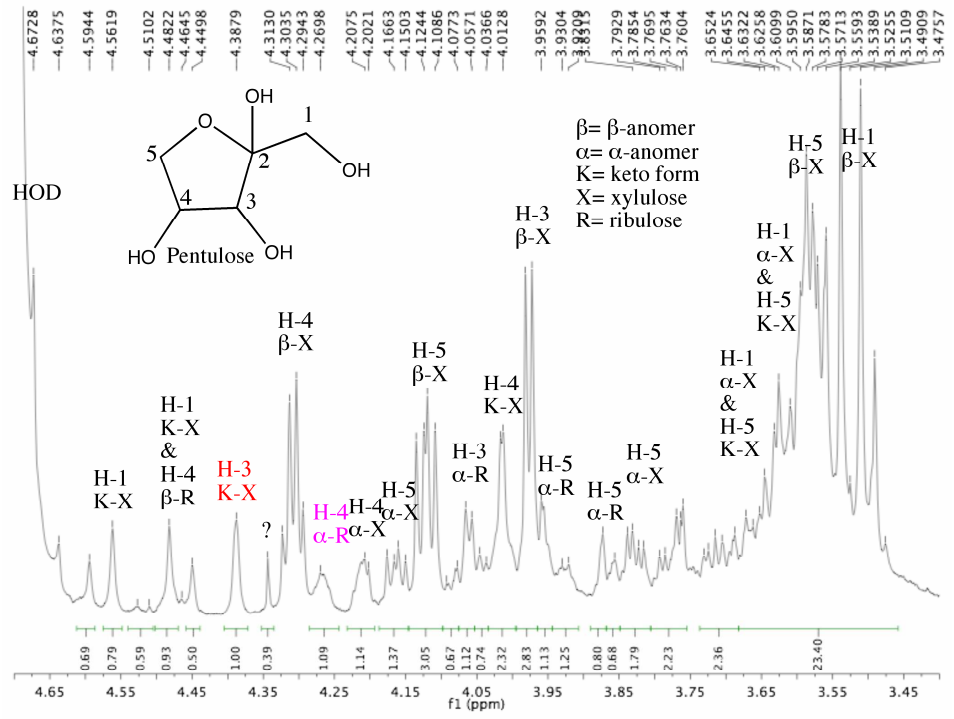


Figure S68: From Part 1 - Expansion of Figure S67 from 3.40 to 4.70 ppm

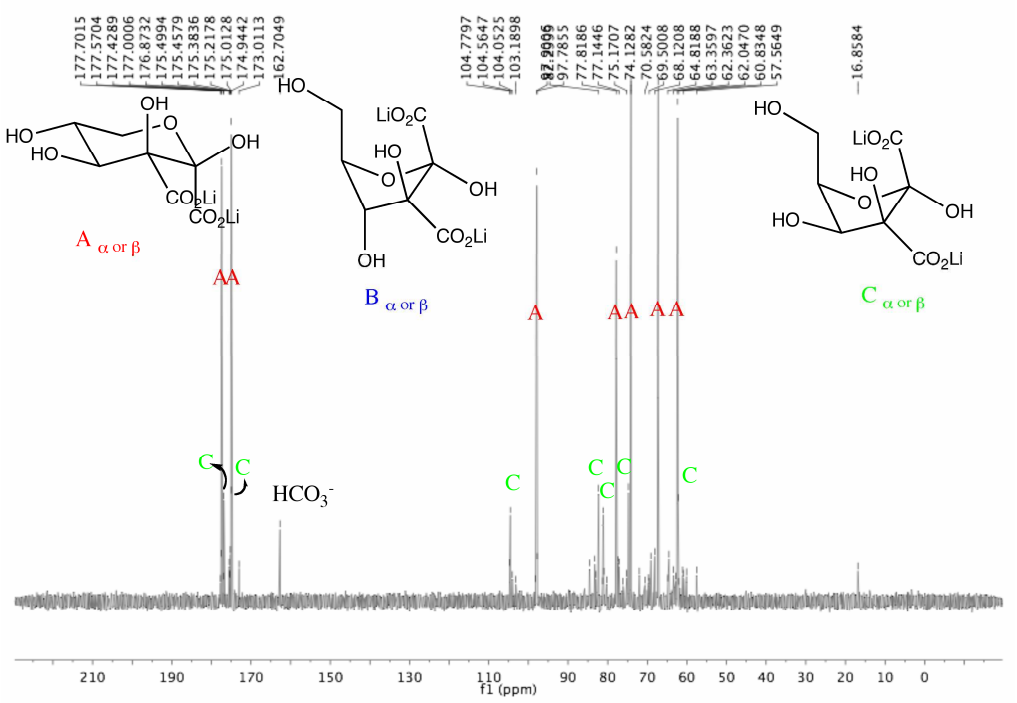


Figure S69: From Part 2 - ^{13}C NMR (150 MHz, drops of D_2O) of reaction mixture (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O) after 47 h (1h NMR running time) at 4 °C.

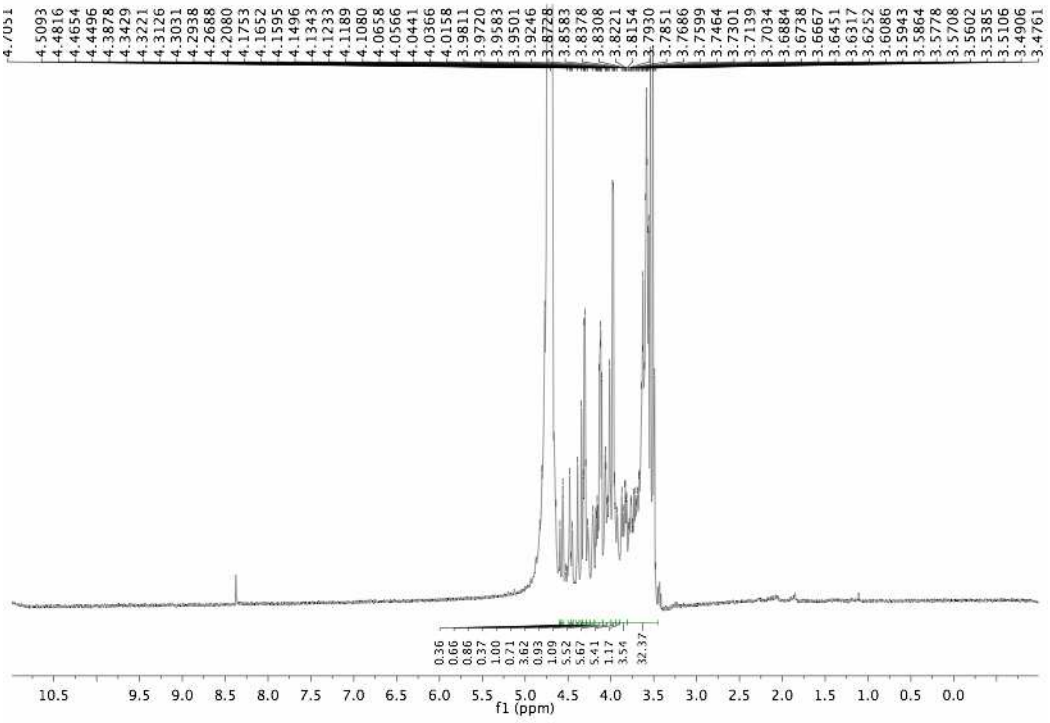


Figure S70: From Part 2 - ^{1}H NMR (600 MHz, D_2O) of crude product (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O + after 48 h at 4 °C treated with $ZnCl_2$ (2.0 eq) + left at r.t., 24 h + concentrated to dryness)

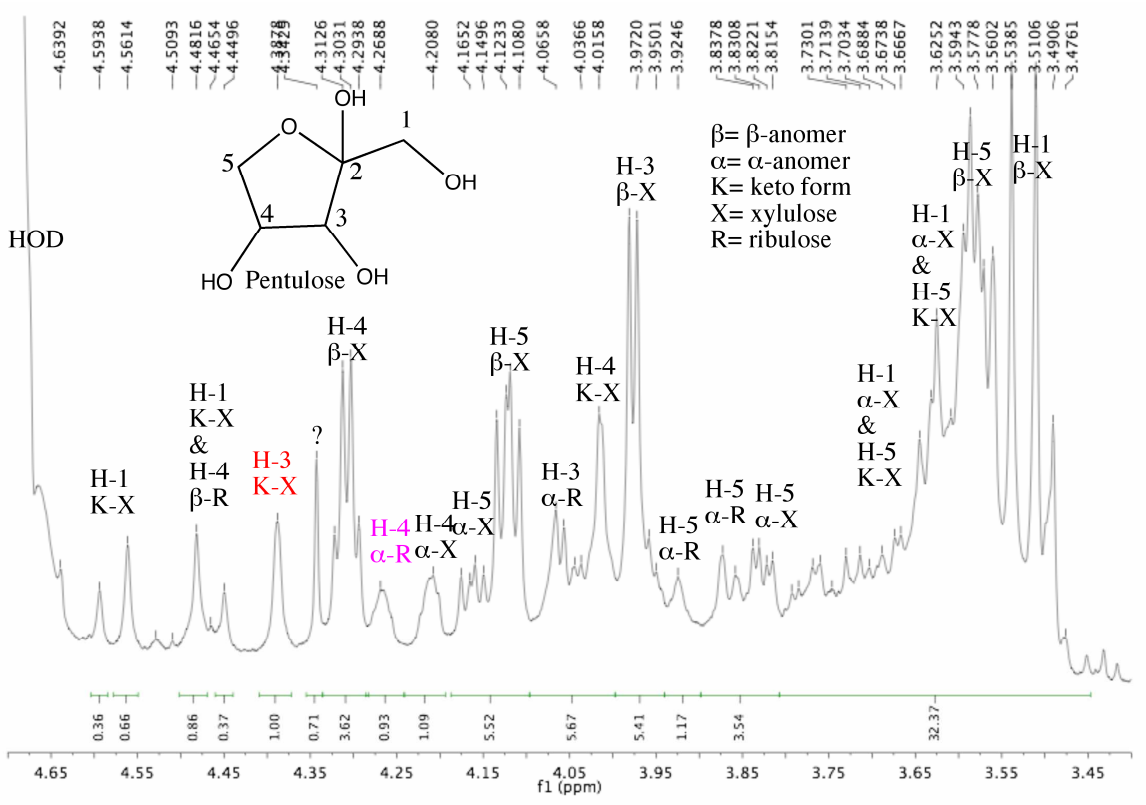


Figure S71: Expansion of Figure S70 from 3.40 to 4.70 ppm.

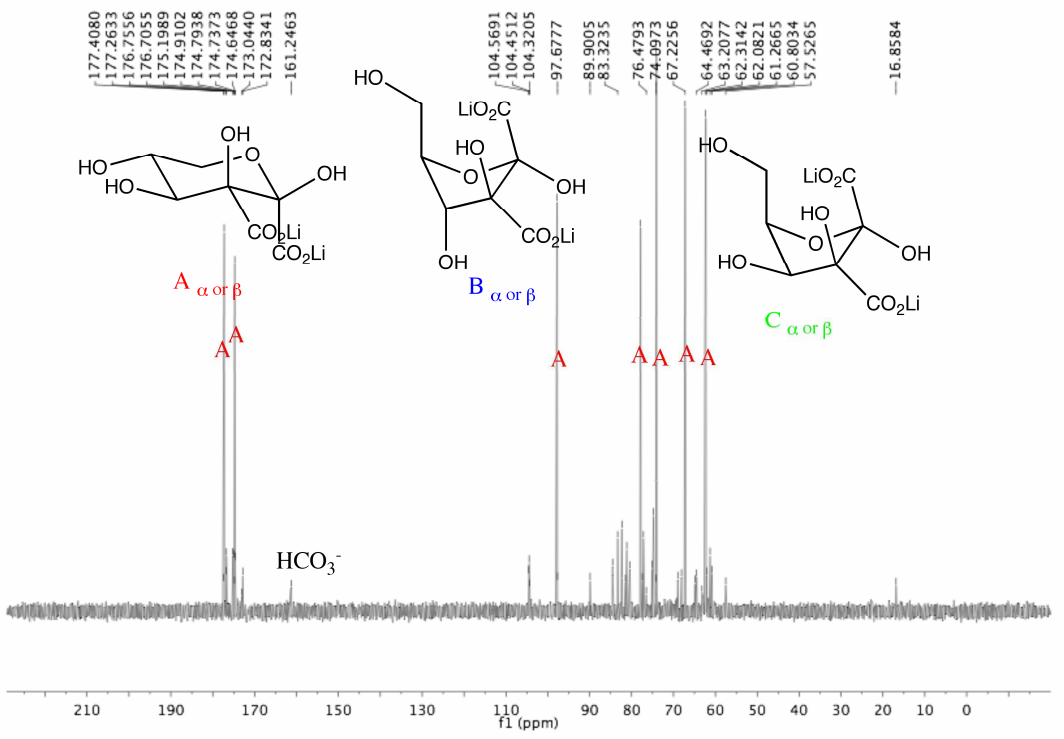


Figure S72: From Part 3 - ^{13}C NMR (150 MHz, drops of D_2O) of reaction mixture (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O) after 3h at r.t. (1h NMR running time).

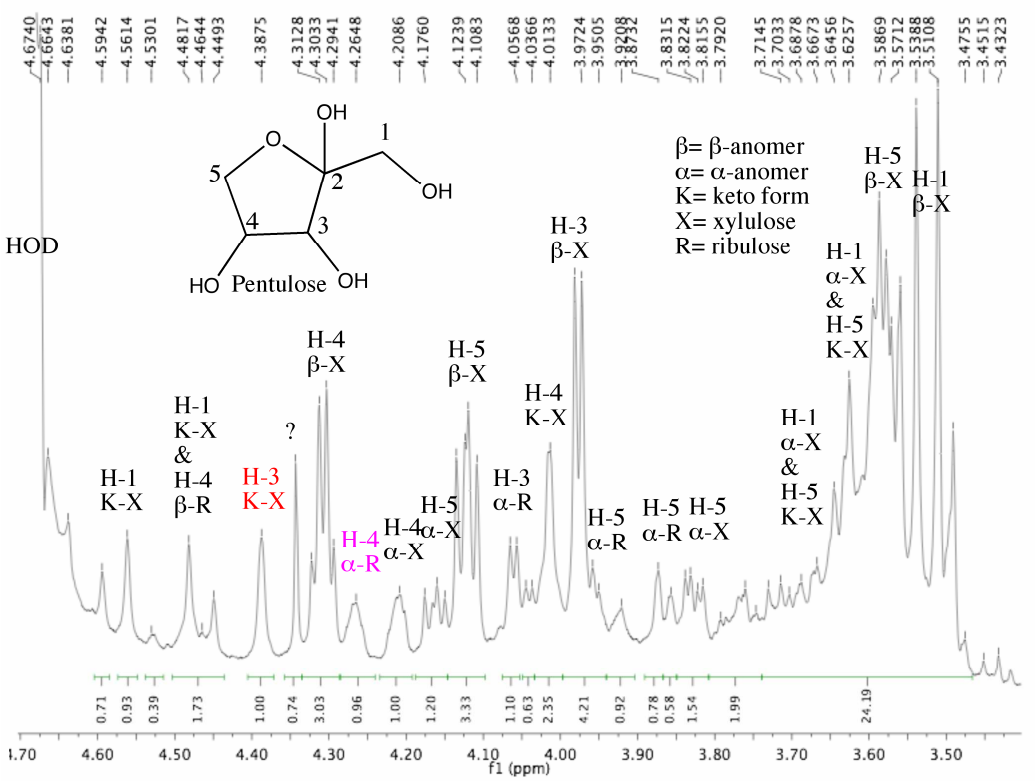


Figure S73: From Part 3 - Expansion from 3.40 to 4.70 ppm; ^{1}H NMR (600 MHz, D_2O) of crude product (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O & after 4 h at r.t. treated with $ZnCl_2$ (2.0 eq) & left at r.t. 24 h + concentrated to dryness).

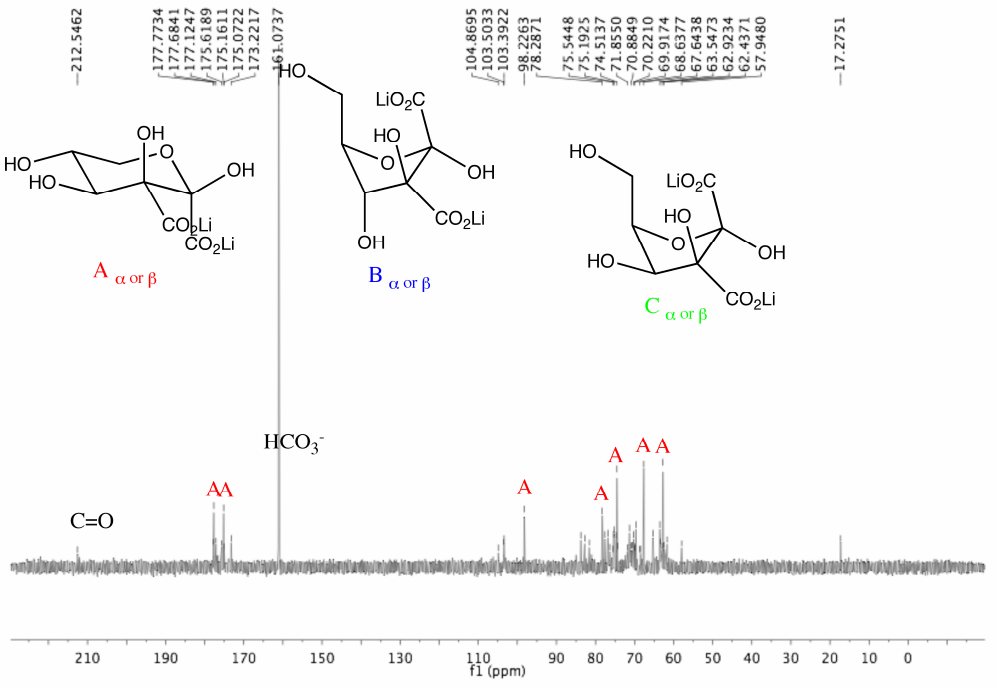


Figure S74: From Part 4 - ^{13}C NMR (150 MHz, drops of D_2O) of reaction mixture (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O) after 23 h (1h NMR running time) at r.t.

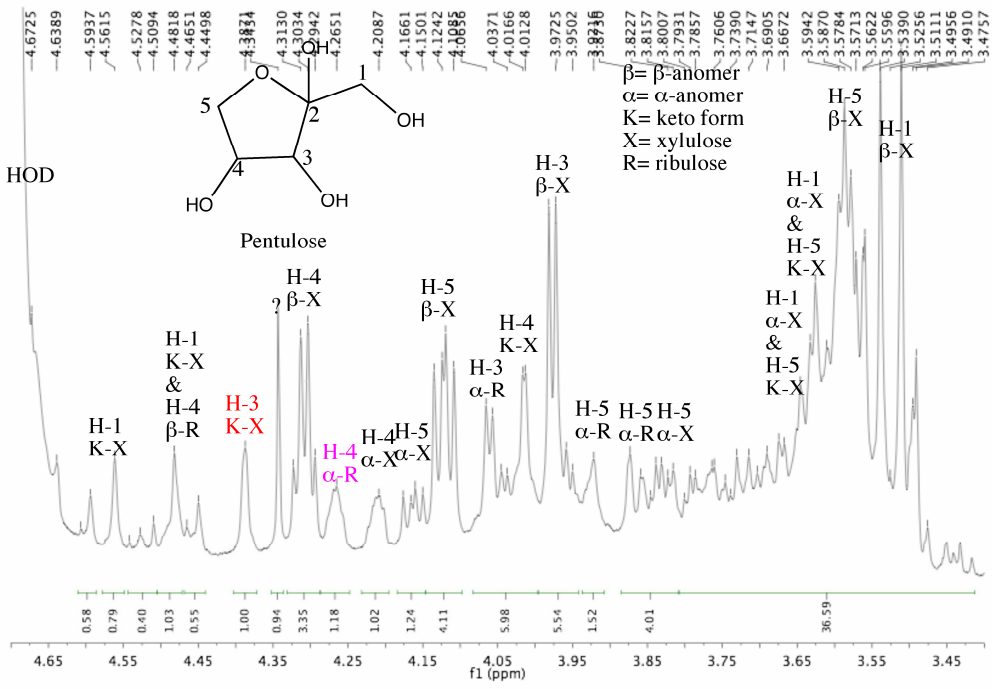


Figure S75: From Part 4 - Expansion from 3.40 to 4.70 ppm; ^{1}H NMR (600 MHz, D_2O) of crude product (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O & after 24 h at r.t. treated with $ZnCl_2$ (2.0 eq) & left at r.t. 24 h & concentrated to dryness and redissolved in D_2O).

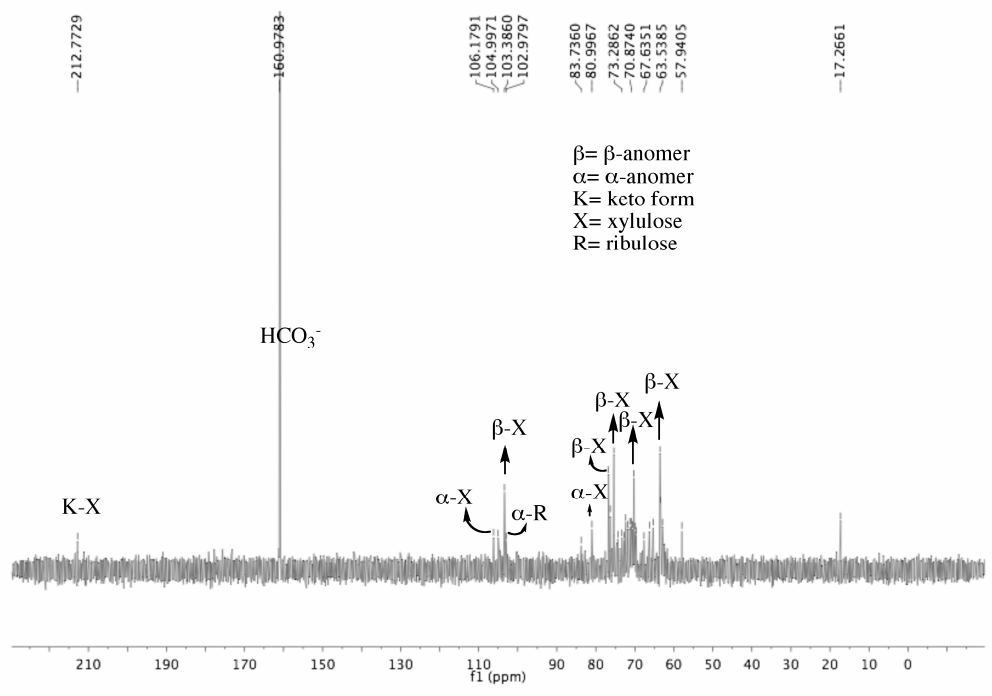


Figure S76: From Part 5 - ^{13}C NMR (150 MHz, drops of D_2O) of reaction mixture (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O) after 47 h (+1h NMR running time) at r.t.

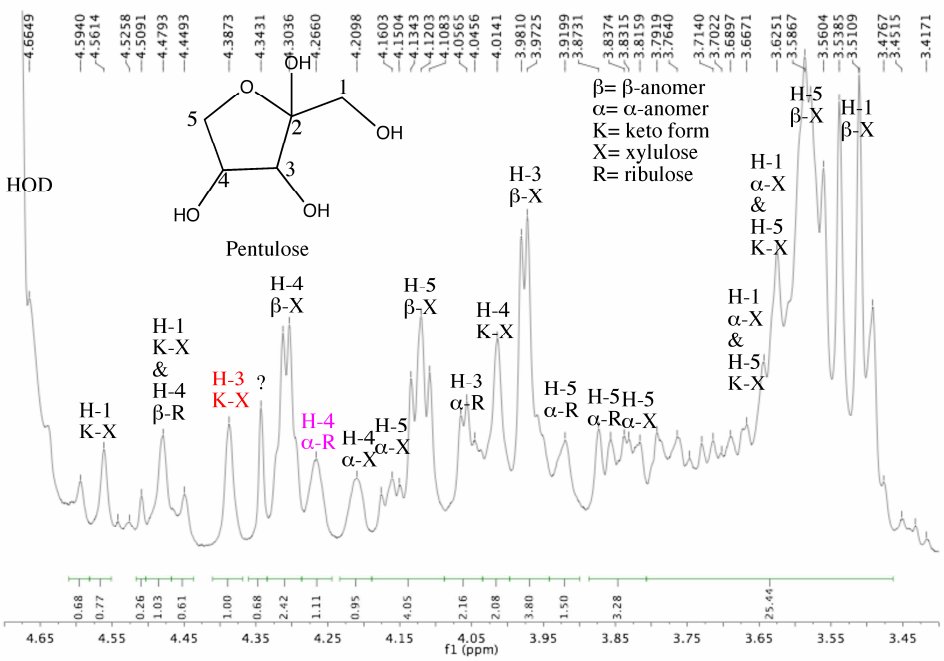


Figure S77: From Part 5 - Expansion from 3.40 to 4.70 ppm; ^{1}H NMR (600 MHz, D_2O) of crude product (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O + after 48 h at r.t. treated with $ZnCl_2$ (2.0 eq) + left at r.t. 24 h + concentrated to dryness).

II.c. 5. *Xylulose and ribulose ratio estimation from r.t. reaction*

Glyceraldehyde (0.33 M, 1 eq, 303 mg) was suspended in degassed water (at r.t.). It was a yellowish suspension. To the above suspension, at r.t., a solution of Li_2DHF (0.33 M, prepared by addition of 2.0 eq. of aq. LiOH (0.84 M solution, 80 mg) to solid DHF (1 eq, 250 mg) kept at 4 °C; the Li_2DHF solution was dark brown in color) was allowed to attain r.t. then added. The resulting reaction mixture turns into pale yellow solution over a period of 5 minutes; pH of the reaction mixture was 10.7 (at r.t.). Reaction was kept at r.t. for 24 h; To the reaction mixture was added 2.0 eq of $ZnCl_2$ (460 mg). The reaction mixture acquires a dark violet color. After complete addition, the reaction mixture was left at r.t.; the pH of the mixture was 4.39. The reaction mixture was left at r.t. overnight. Formation of a suspension was observed after 30 min. of mixing and after overnight, more precipitation was seen. The suspension was violet in color, while the supernatant was pale yellow in color. The reaction mixture was centrifuged and the pale yellow filtrate was separated and checked by ^{13}C NMR and concentrated to dryness to give syrup (yellow in color). A small portion of this syrup was dissolved in 0.5 ml D_2O; small amount of un-dissolved white particles were separated by centrifugation and the clear solution was analyzed by ^{1}H NMR (Figure S78) and xylulose and ribulose ratio was found to be 2:1 (66:33).

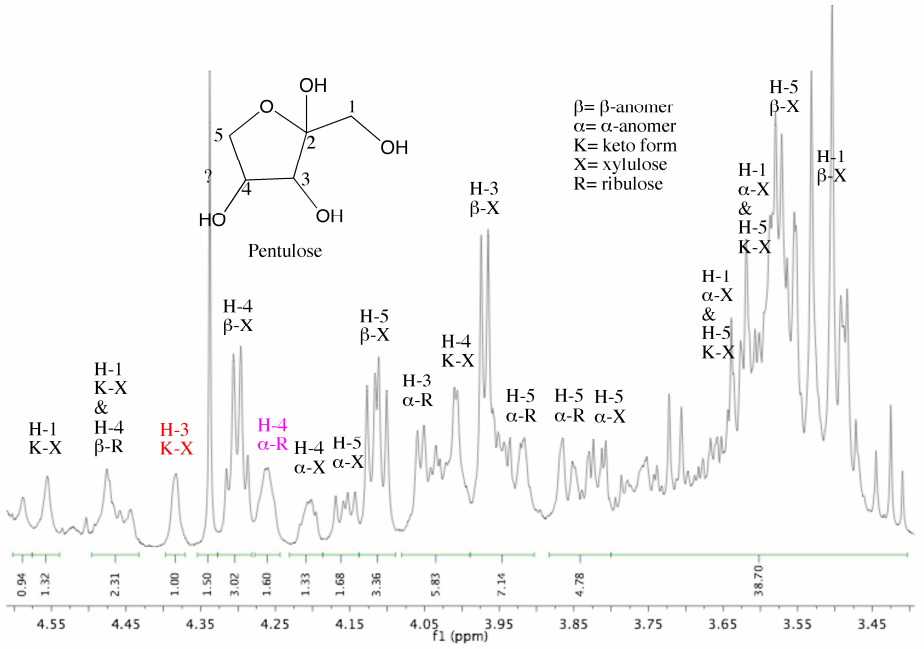
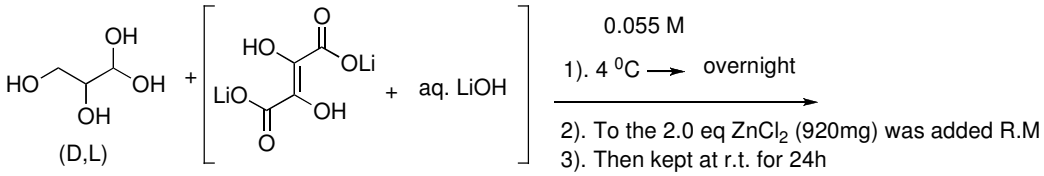


Figure S78: ^{1}H NMR (600 MHz, D_2O) of crude product (0.33 M Li_2DHF + 0.33 M Glyceraldehyde in degassed H_2O + after 24h at r.t. treated with $ZnCl_2$ (2.0 eq) + left at r.t. 24 h + concentrated to dryness and re-dissolved in D_2O)

II.c.6. *Study of effect of dilution on xylulose and ribulose ratio (0.055M)*



1.0 eq
50.5 mg
0.561 mmol
(2.0 mL of degassed H_2O)
suspension

1.0 eq
83.3 mg
0.562 mmol

2.0 eq
26.95 mg
1.125 mmol
8.0 mL

This reaction was conducted as described previously but under high dilution (0.055 M) conditions. The results of this reaction showed (Figure S79) no change in xylulose and ribulose ratio under high dilution conditions.

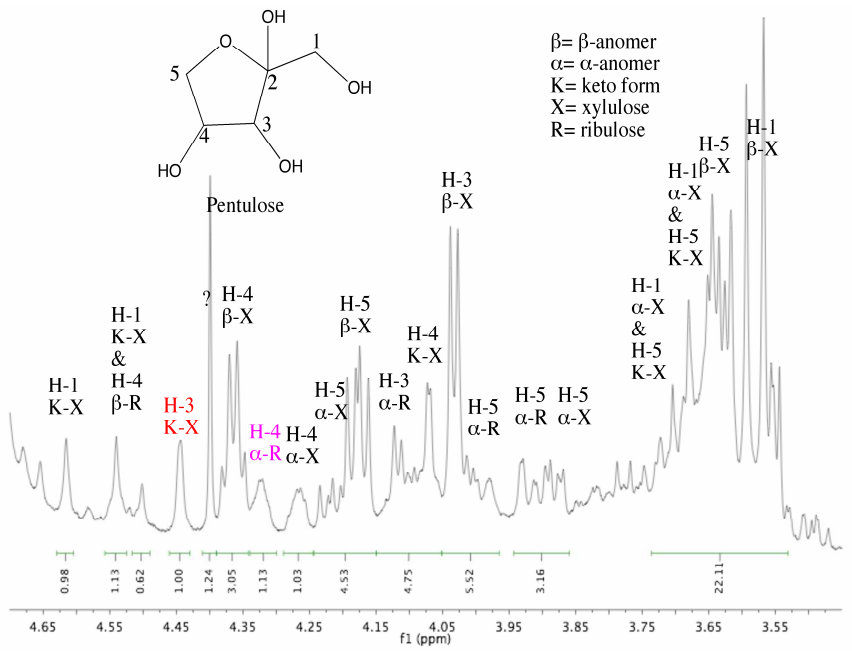


Figure S79: ^{1}H NMR (500 NMR, drops of D_2O) of reaction mixture (0.055 M Li_2DHF + 0.055 M glyceraldehyde in degassed H_2O) + after 24 h at 4 °C treated with $ZnCl_2$ (2.0 eq) + left at r.t. 24 h + concentrated to dryness and re-dissolved in D_2O).

II.c.7. *Checking the stability of six-membered intermediate:*

Six-membered intermediate **16-p** was prepared as described before. Stability of the intermediate was monitored at 4 °C over a period of 18 days (by ^{13}C NMR). It was observed that six-membered intermediate was stable for 7 days and slow conversion was observed at 18 days (Figure S80).

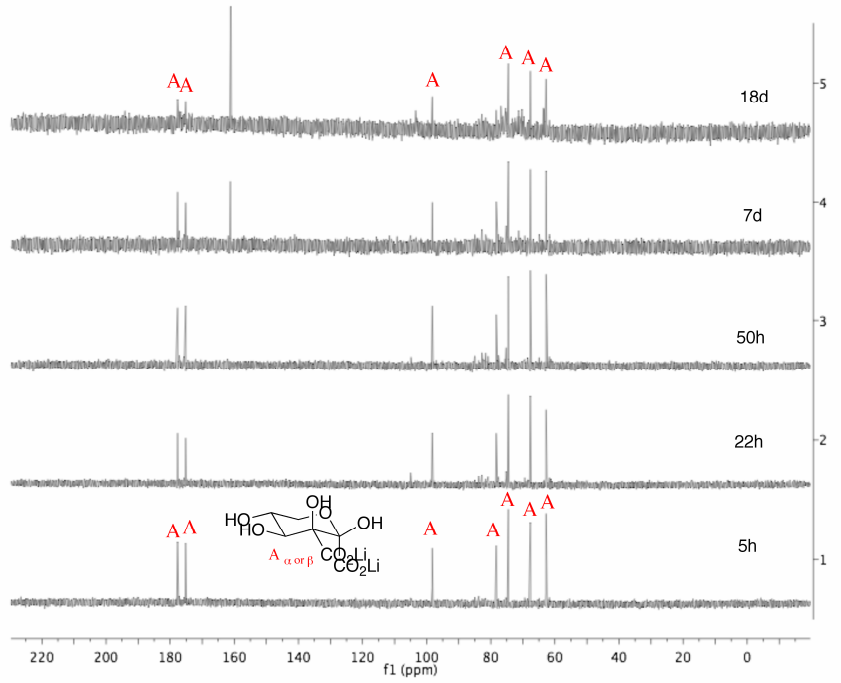


Figure S80: Stability of six-membered intermediate **16-p** (A) over the time of 18 days at 4 °C.

II.c.8. *Spiking of crude reaction mixture with authentic xylulose and ribulose.*

Crude pentulose was prepared as described before. A sample was prepared by dissolving the residue in D_2O. This sample was first spiked with ribulose (Figure S81) and then with xylulose (Figure S82), respectively.

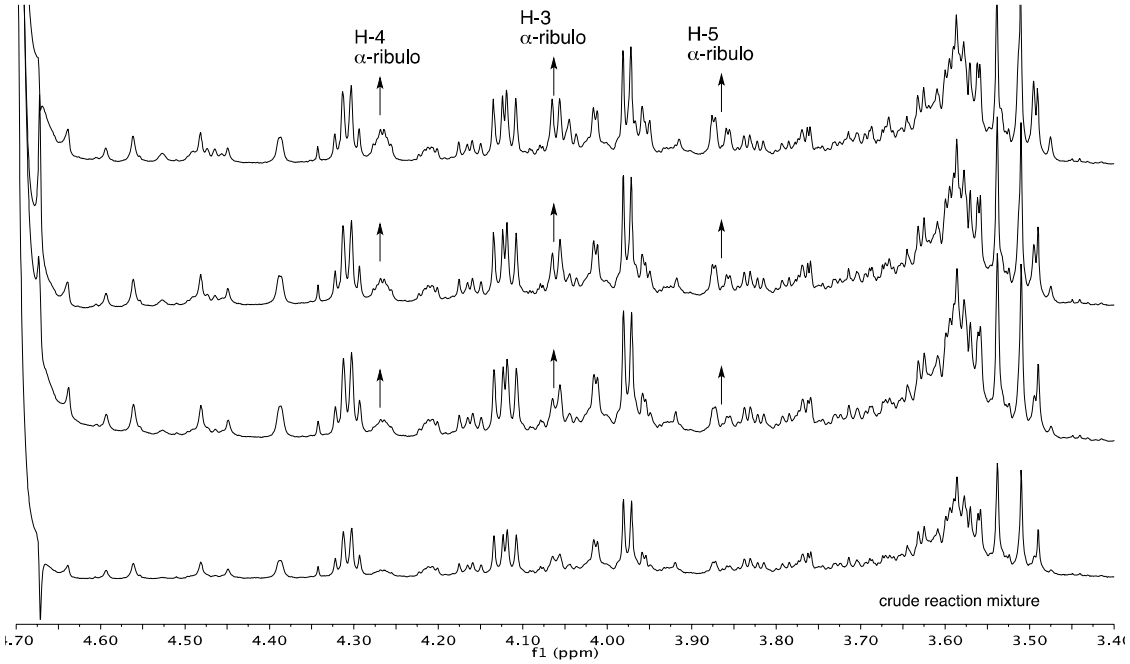


Figure S81: ^{1}H NMR (D_2O) of the sample spiked with ribulose (increase in the α-ribulose peaks intensity indicated with an arrow).

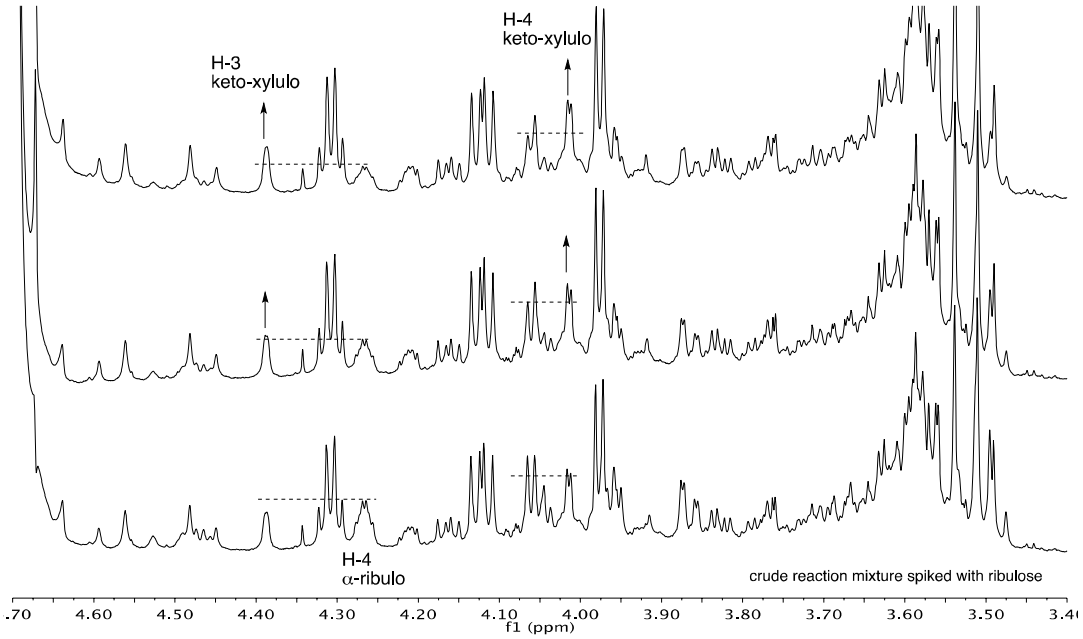
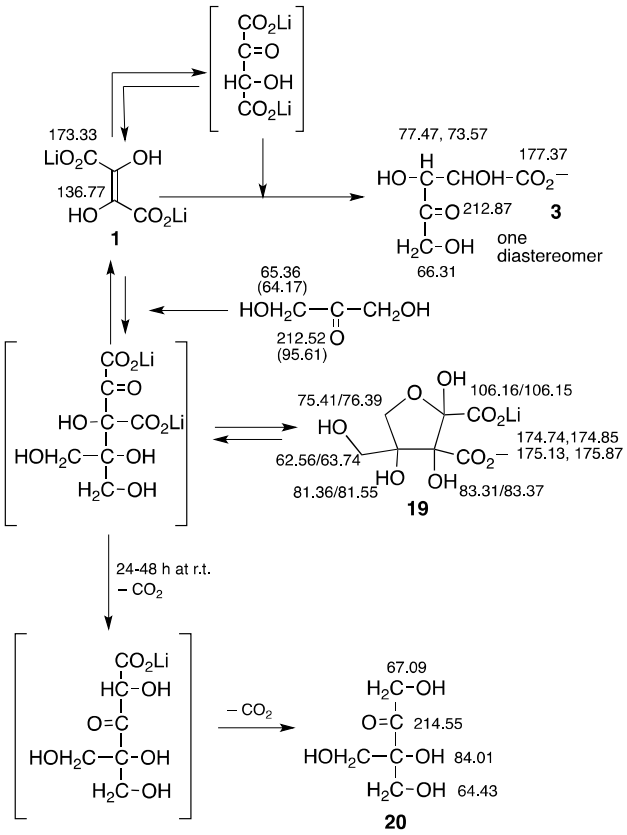


Figure S82: ^{1}H NMR (D_2O) of the sample spiked with xylulose (increase in H-4 keto-xylulose peak intensity indicated with an arrow).

III. Reaction of DHF with dihydroxyacetone:



Scheme S10: Reaction of dihydroxyacetone with DHF as discerned by ^{13}C NMR spectroscopy.

An aqueous solution of 'DHF dilithium salt' (1.0 eq, 0.35 M, 9.58 mL) was added in one portion to aq. dihydroxyacetone[4] (2.0 M in water, 1.0 eq, 1.68 mL) at 4 °C under nitrogen atmosphere. After the addition, the reaction became very clear solution and pH of the resulting mixture was 9.5 (after 2 h). The reaction mixture was monitored by ^{13}C NMR at 4 °C.

After 1 h at 4 °C: After 1h at 4 °C, 0.5 mL of aliquot was taken from the reaction mixture through syringe and ^{13}C NMR was measured (at 4 °C) by adding 2 drops of D_2O for lock. The ^{13}C NMR (Figure S83) and ^{13}C NMR APT showed peaks for aldol adduct (Table S2) and unreacted DHF and dihydroxyacetone.

After 5 h at 4 °C: ^{13}C NMR showed similar peaks as at 1 h. But the peaks intensity of DHF in peaks slightly less than the one at 1 h.

After 3 days at 4 °C: ^{13}C NMR showed similar peaks as at 5 h. But the peaks intensity of DHF in peaks slightly less than the one at 5 h (Figure S84).

After 30 days at 4 °C: ^{13}C NMR showed pentulosonic acid (major product from reaction of DHF with itself), unreacted DHA along with substituted tetrulose (Table S3).

[4] Dihydroxyacetone solution was left overnight to ensure that all dimeric forms were converted to the monomeric hydrated form of dihydroxyacetone (as judged by ^{13}C NMR spectroscopy).

Table S2. ^{13}C NMR values (in ppm) of first aldol adduct **19**.

S. No	Carbon	δ in ppm
1	*C*H_2OH	62.56, 63.74
2	*C*H_2-O-	75.41, 76.39
3	-*C*(OH)(CH_2OH)	81.36, 81.55
4	-*C*(OH)(CO_2^-), quaternary	83.31, 83.37
5	-O*C*(OH)(CO_2^-), quaternary	106.16, 106.65
6	CO_2^-	174.74, 174.85, 175.13, 175.87

Table S3. Comparison of ^{13}C NMR values (ppm) of substituted tetrulose **20** with reported values.

S. No	Carbon	Observed values δ in ppm (drops of D_2O)	Reported[5] values δ in ppm (D_2O)
1	*C*H_2OH (**C5** and **C4**)	64.43	64.5
2	*C*H_2OH (**C1**)	67.09	67.0
3	[*C*(OH)(CH_2OH)$_2$] (**C3**)	84.01	83.9
4	*C*=O (**C2**)	214.55	214.5

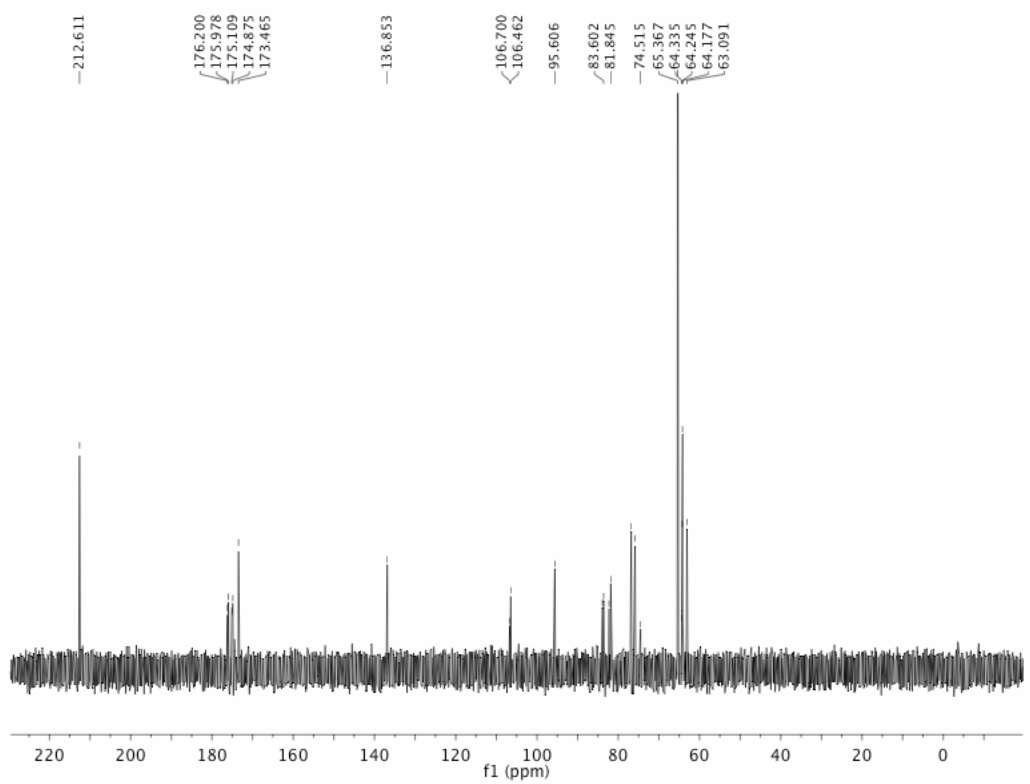


Figure S83. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF with dihydroxyacetone, after 30 min at 4 °C

[5] Simonov, A. N.; Matvenk, L. G.; Pestunova, O. P.; Parmon, V. N.; Komandrova, N.; Denisenko, V. A.; Vas'kovskii, V. E. *Kinetic and Catalysis* **2007**, *48*, 550-555.

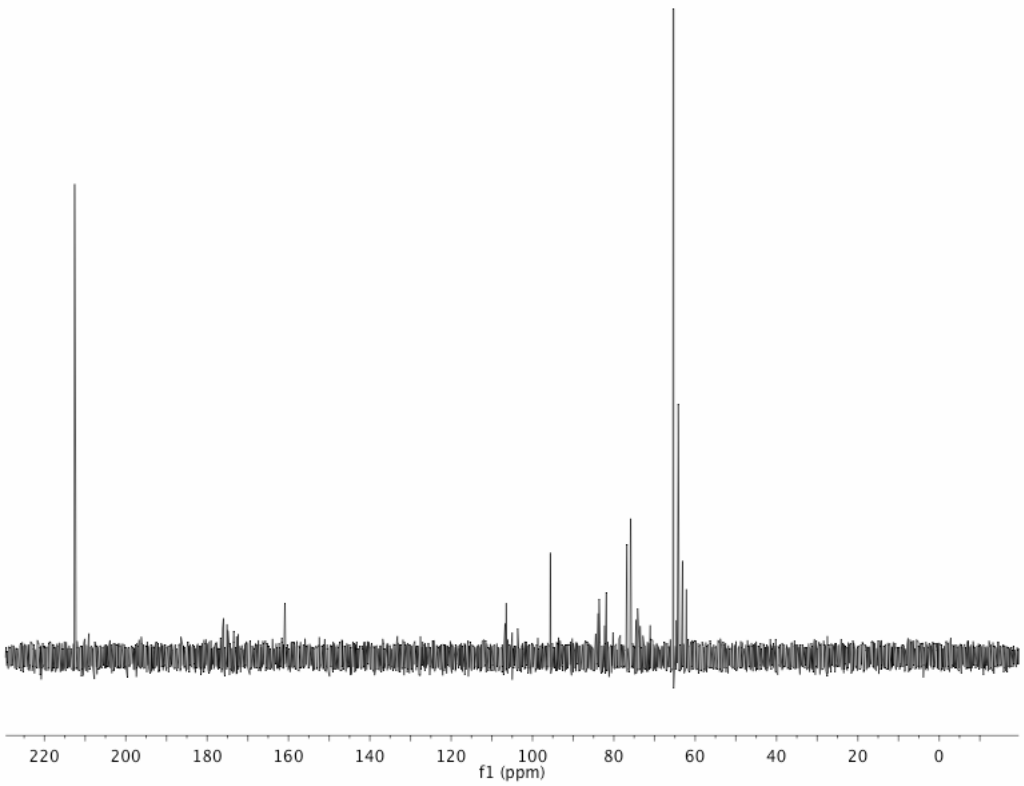


Figure S84. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF with dihydroxyacetone, after 72 h at 4 °C.

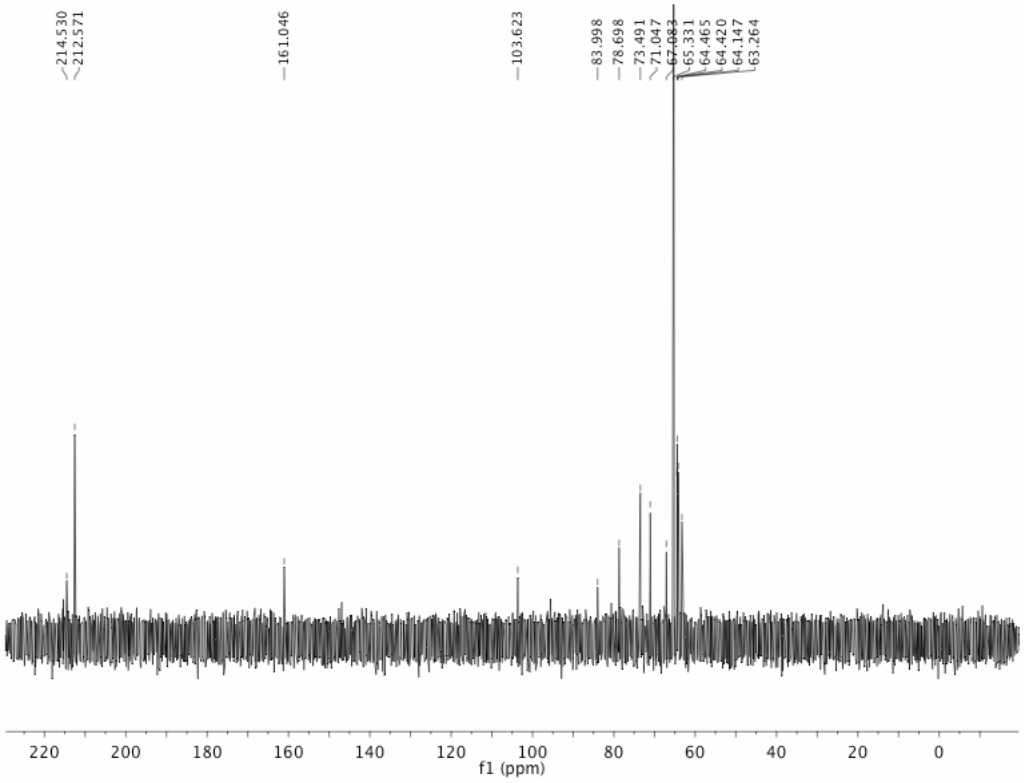


Figure S85. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF with dihydroxyacetone, after 24 h at 4 °C and 4 days at r.t.

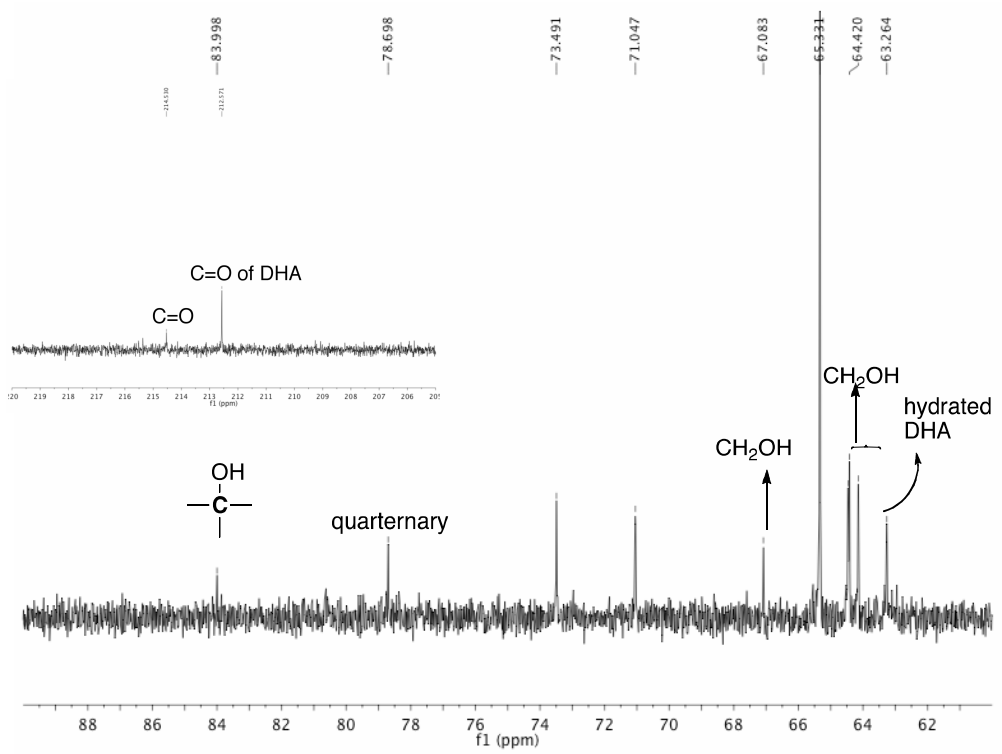


Figure S86. Expansion of ^{13}C NMR of Figure S85.

IV. <u>Reaction of DHF with acetone</u>: An aqueous solution of 'DHF dilithium salt' (1.0 eq, 0.35 M) was added in one portion to acetone (2.0 eq.) at 4 °C under nitrogen atmosphere. After the addition, the reaction became very clear solution and pH of the resulting mixture was 9.0 (after 30 min.) The reaction mixture was monitored by ^{13}C NMR at 4 °C. After 16 h at 4 °C, ^{13}C NMR showed presence of only DHF dimer and acetone (Figure S87).

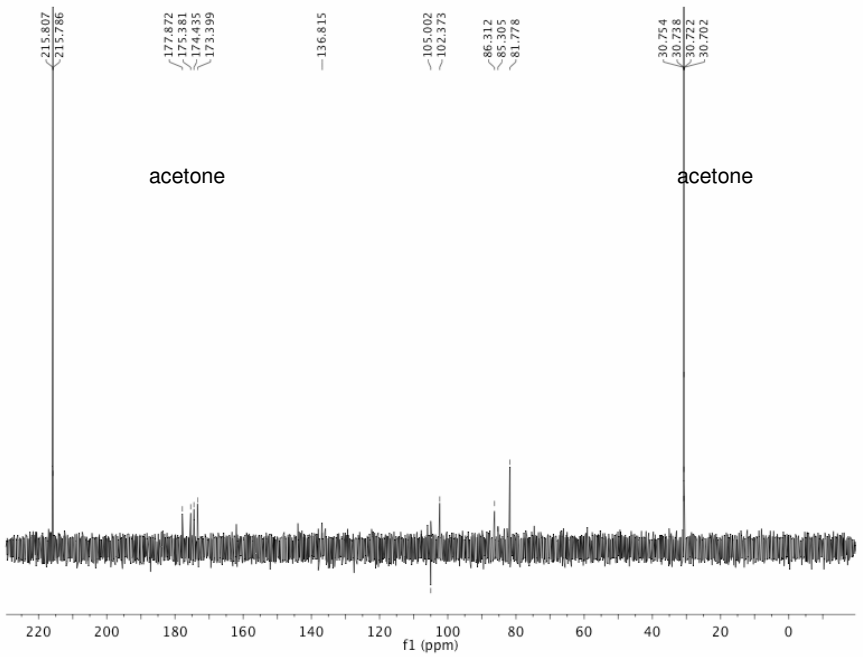


Figure S87. ^{13}C NMR (H_2O/D_2O) of the reaction of DHF with acetone (1: 2 ratio), after 16 h at 4 °C

V. <u>Reaction of DHF with dihydroxyacetone and glyceraldehyde (1:1)</u>: An aqueous solution of 'DHF dilithium salt' (1.0 eq, 0.35 M) was added in one portion to a suspension containing dihydroxyacetone[4] (1.0 eq.) and glyceraldehyde (1.0 eq.) at 4 °C under nitrogen atmosphere. After the addition, the reaction became very clear solution and pH of the resulting mixture was 9.0 (after 30 min.) The reaction mixture at 4 °C was monitored by ^{13}C NMR. After 2 h at 4 °C, ^{13}C NMR showed presence of predominantly DHF-glyoxylate adduct and unreacted dihydroxyacetone (Figure S88).

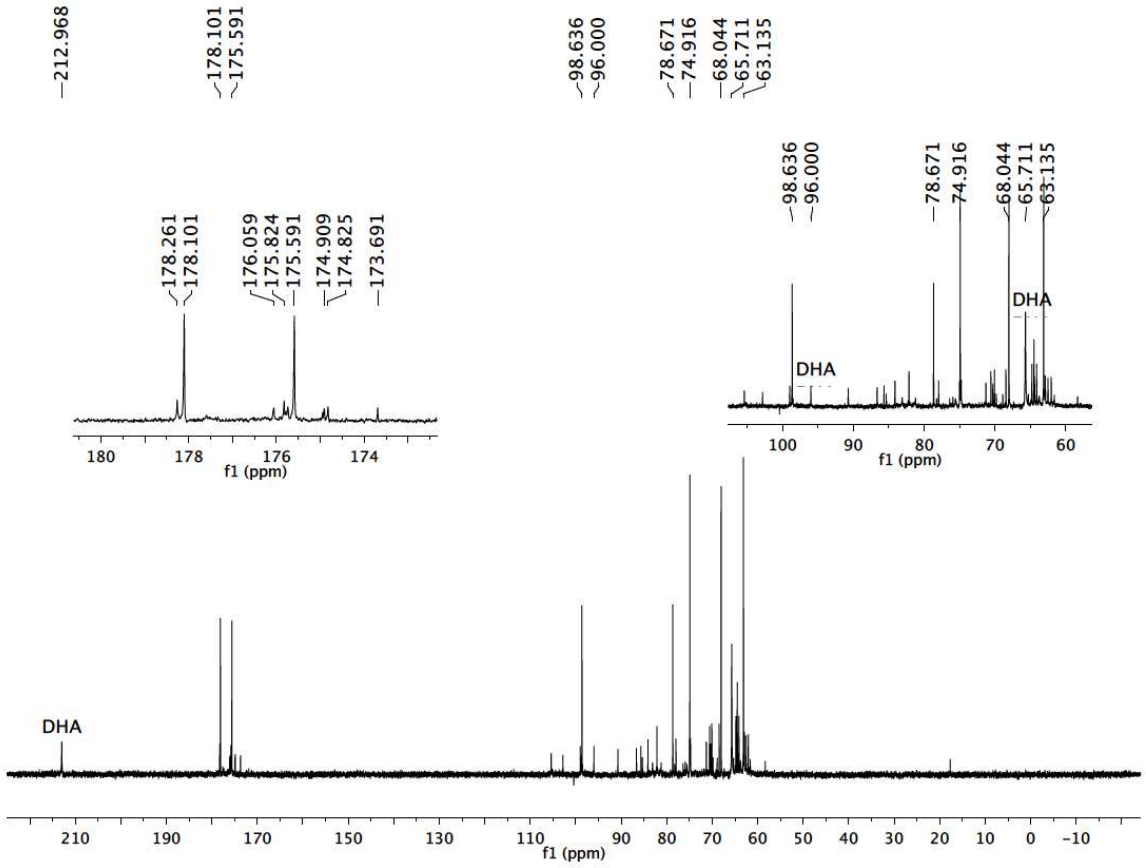


Figure S88. ^{13}C NMR (H_2O/D_2O) of the reaction of Li_2DHF with dihydroxacetone and glyceraldehyde (1: 1 ratio), after 2 h at 4 °C. Peaks corresponding to the Li_2DHF-dihydroxyacetone adduct, **19**, are not present. Compare to Figure S60 on page 50 (Li_2DHF-glyceraldehyde adduct) and Figure S83 on page 66 (Li_2DHF-dihydroxyacetone adduct).

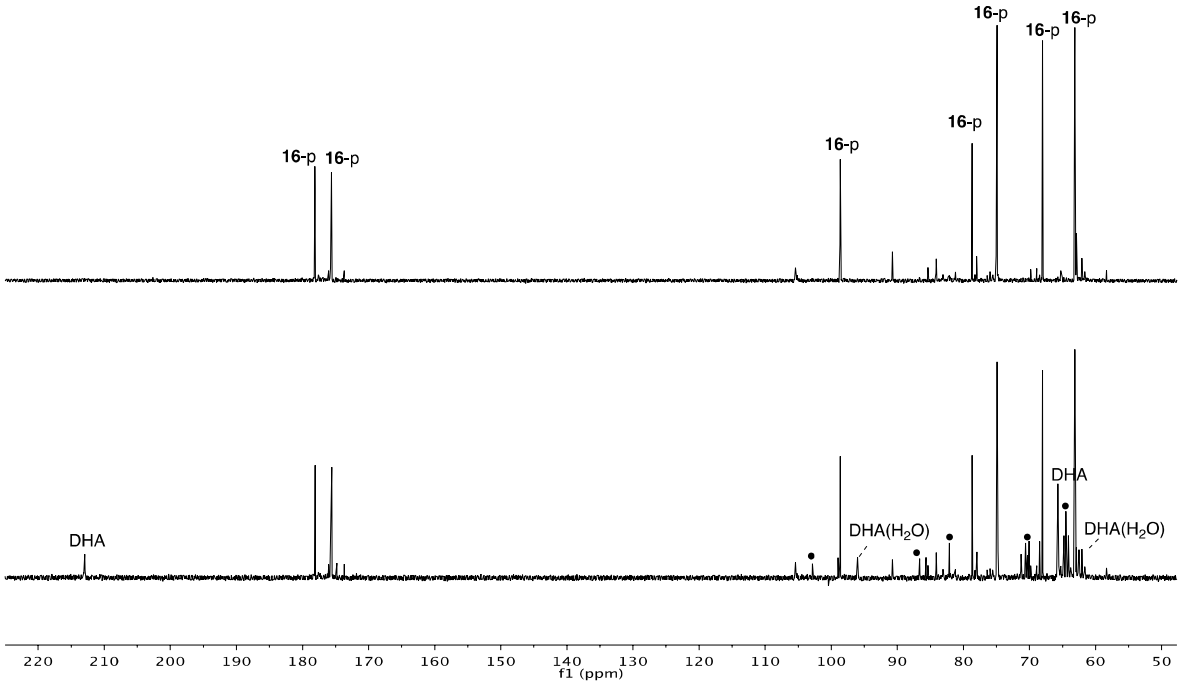


Figure S89. ^{13}C NMR (H_2O/D_2O) comparison of the reaction of (top) Li_2DHF with glyceraldehyde and (bottom) Li_2DHF with dihydroxacetone and glyceraldehyde (1: 1 ratio), after 2 h at 4 °C. The 'extra peaks' marked with (•) are best ascribed to products arising from the reaction of dihydroxyacetone with glyceraldehyde under the mixing conditions.

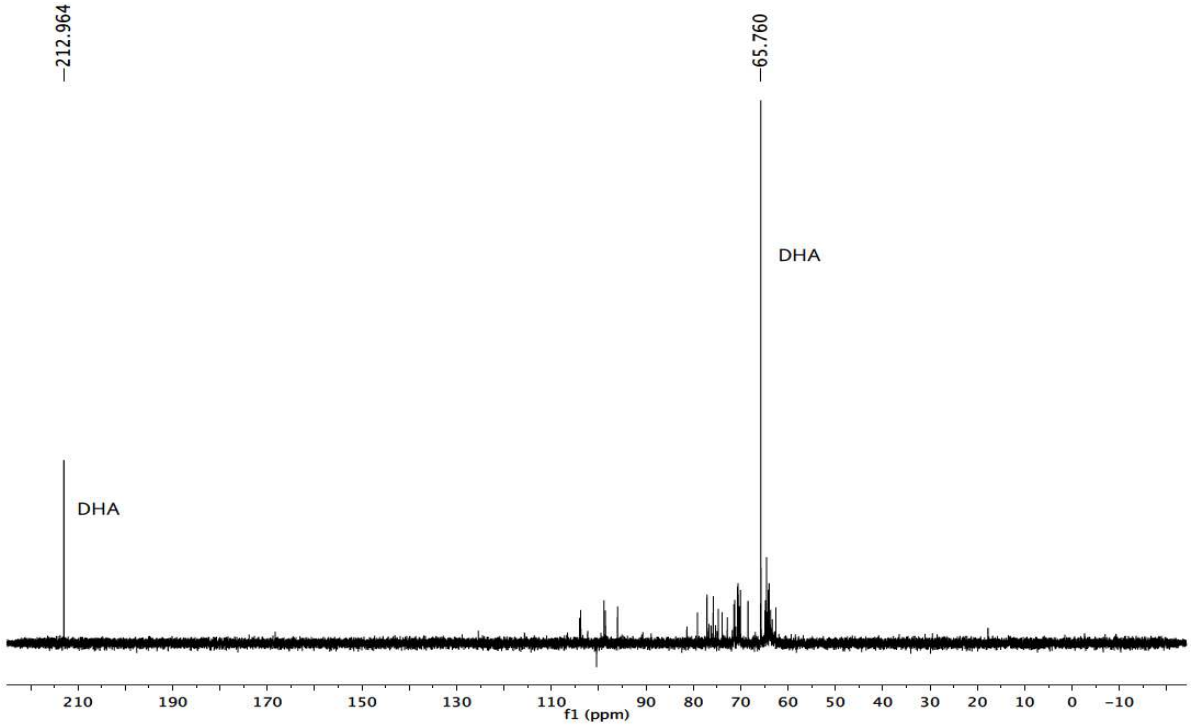


Figure S90. ^{13}C NMR (H_2O/D_2O) of the reaction of Li_2DHF with dihydroxacetone and glyceraldehyde (1: 1 ratio), after addition of $ZnCl_2$ after 24 h at 4 °C.

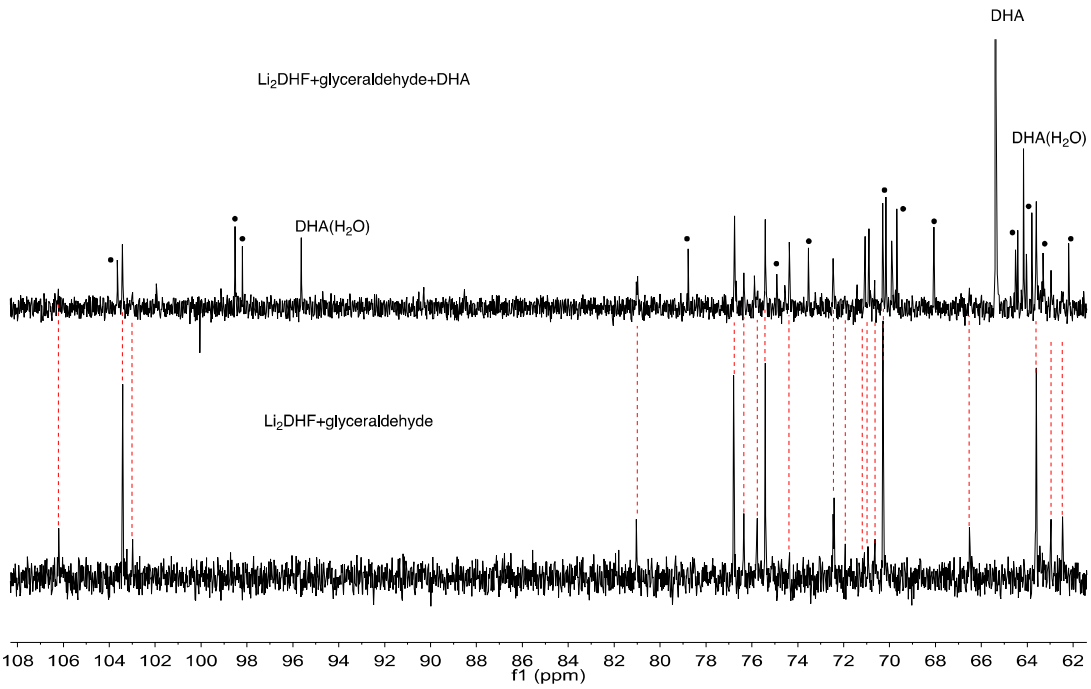


Figure S91. ^{13}C NMR (H_2O/D_2O) spectral comparison of the reaction of (top) Li_2DHF with glyceraldehyde + dihydroxyacetone, and (bottom) Li_2DHF with glyceraldehyde (1: 1 ratio), after $ZnCl_2$ addition, 24 h at 4 °C (with the red-dashed-lines pointing to the common product signals belonging to the pentuloses). The 'extra peaks' are marked with (•).