Supplementary Information

Cyanide as a Primordial Reductant enables a Protometabolic Reductive Glyoxylate Pathway

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General experimental:

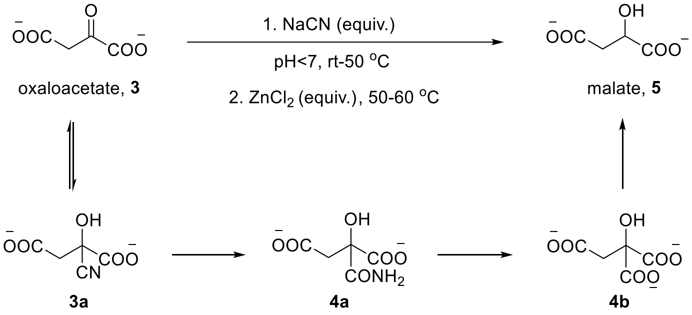
Reagents and solvents were purchased from commercial sources like Sigma-Aldrich, VWR International, Fischer Scientific, Acros Organics, Oakwood, TCI Chemicals, Alfa Aesar, Spectrum and Combi Blocks and used without further purification. Doubly ^{13}C labelled glyoxylic acid, 99%, [contains glycolic acid (3%) and oxalic acid (1.5 %)] was obtained from Cambridge Isotope Laboratories Inc. Carboxy succinate and 4-hydroxyketoglutarate (HKG) were synthesized by using known procedures. The ultrapure water used throughout the experiments was obtained from a laboratory water purifier Milli-Q®. Degassed water was used through-out all the experiments unless or otherwise stated. The 'degassed water' was prepared by sonicating for 10 minutes followed by purging argon/N_2 gas into the 'deionized water' for 20 min. and then subjecting the 'deionized water' to house-vacuum for 30 min. pH was measured using a Accumet Research AR25 pH meter. NMRs were recorded at 298 K on Bruker DPX 400, AV-500, Bruker DRX600 (cryoprobe), and AV-600 (equipped with a 5mm DCH cryoprobe); chemical shifts (δ) in parts per million (ppm), spin multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet), coupling constants (J) in Hertz (Hz), number of protons. Mass spectra were acquired using Agilent ESI-TOF or ThermoElectron Finnigan LTQ ion trap mass spectrometer.

Absolute yields of the reactions were calculated by ^{1}H NMR using an internal standard (t-BuOH). For the reactions where absolute conversion yields have been provided, it is based upon the ratio of integration of the ^{1}H NMR peaks of the product w.r.t. reactant/intermediate in the reaction mixture.

Notes

- All the reactions were performed in borosilicate glass vials (4 ml or 20 mL), and all the reactions involving cyanide were carefully carried out in a hood with strict precautions.
- Structures are drawn as carboxylates (or sodium salt) thought the SI, though there may be various degrees of protonated (carboxylic acid) versions of some tri- and tetra-carboxylic species at pH lower than 7.
- NMRs were recorded in two ways: Method 1) 550 µL of the reaction aliquot was directly taken in an NMR tube and a capillary filled with D_2O was inserted into the NMR tube (90% H_2O / 10% D_2O). Method 2) 100 µL of the reaction aliquot was dissolved in 500 µL of D_2O and this 600 µL solution was used for the NMR analysis (D_2O). These are represented as NMR was recorded in H_2O-D_2O (method 1) or in D_2O (method 2) throughout the SI. Identification of end-products or intermediates was by made NMR (and/or mass) analysis, either by comparison with literature values or by spiking with authentic samples.

Reaction of oxaloacetate 3 with sodium cyanide (NaCN):



Scheme S1: Reaction of oxaloacetate **3** with NaCN in H_2O showing the formation of malate **5** (Table S1 and Figures S1-S5).

Table S1: Reaction of oxaloacetate **3** with NaCN for the formation of malate. Identification of end-products (malate **5**) was by made by comparison with literature values and authentic samples.

Condition	**Oxaloacetate (conc.)**	**NaCN (equiv.)**	**$ZnCl_2$ (equiv.)**	**% conversion to 5[a]**	**NMR Figure number**
		Solutions at pH (unadjusted) in H_2O			
1	0.5M	1	1	68%	Figure S1
2	0.1M	1	1	68%	Figure S3
		Solution at pH 6.0 in 1M phosphate buffer			
3	0.5M	1	1	75%	Figure S4
		Solution at pH 4.2 in H_2O			
4	0.01M	10	1	n.d.	Figure S5

[a] % conversion based on the ^1H NMR integration of **5** (peaks referred as ●) w.r.t carboxymalate **4b** (peak referred as ●). Since carboxymalate was formed as a discrete product we used it as the basis for % yield conversion to malate. n.d. = not determined.

Example procedure (condition 1): In an appropriate glass vial equipped with a magnetic stir bar, sodium cyanide (98.0 mg, 1.0 equiv.) was carefully dissolved in deionized and degassed water (4.0 mL) at 0 °C in fume hood. To this homogeneous solution oxaloacetate **3** (264 mg, 1.0 equiv.) was added at 0° C. After addition of oxaloacetic acid, pH of the homogeneous colourless reaction mixture was kept unadjusted. The reaction mixture was stirred at room temperature and the progress of the reaction was monitored by ^1H-NMR or ^{13}C-NMR (H_2O-D_2O) over a period (Figure S1-S2). To enhance the rate of hydrolysis of the amide intermediate **4a** to carboxymalate **4b**, temperature of the reaction mixture was raised to 50 °C. Similarly, to enhance the rate of decarboxylation of carboxymalate **4b** to malate **5**, $ZnCl_2$ (1.0 equiv.) was added to the reaction mixture and heated at 50-60 °C. pH of the reaction mixture changed over the time (Figures S1-S2).

<u>Condition 1:</u> Sodium cyanide (98.0 mg, 1.0 equiv.), Oxaloacetate (264 mg, 0.5M, 1 equiv.), H_2O (4 mL).

- <u>After 30 min:</u> ^{1}H NMR (Figure **S1A**) showed formation of cyanohydrin **3a** as the major product (pH = 3.0).
- <u>After 15 days:</u> ^{1}H NMR (Figure **S1B**) showed formation of amide intermediate **4a** and carboxymalate **4b** as the major product (pH = 2.9).
- <u>After 43 days:</u> temperature of the reaction mixture was raised up to 50 ºC (pH = 4.0).
- <u>After 48 days:</u> ^{1}H NMR (Figure **S1C**) showed carboxymalate **4b** as the sole product (pH = 4.5).
- <u>After 48 days:</u> $ZnCl_2$ (1 equiv.) was added to the reaction mixture and stirring was continued at 50 ºC (pH = 3.2).
- <u>After 69 days:</u> temperature of the reaction mixture was raised up to 60 ºC (pH = 3.4).
- <u>After 71 days:</u> ^{1}H NMR (Figure **S1D**) showed malate as the major product (68% conversion w.r.t. carboxymalate **4b**) (pH = 3.5).
- <u>After 78 days:</u> Absolute yield of malate **5** formed was found to be 50% (calculated by ^{1}H NMR with an internal standard i.e., t-BuOH, pH = 3.5.
- <u>After 120 days:</u> Almost quantitative conversion of carboxymalate **4b** to malate **5** was observed (Figure **S2E**), pH = 3.7; however, it also showed some precipitates making absolute yield calculation at this stage less precise.

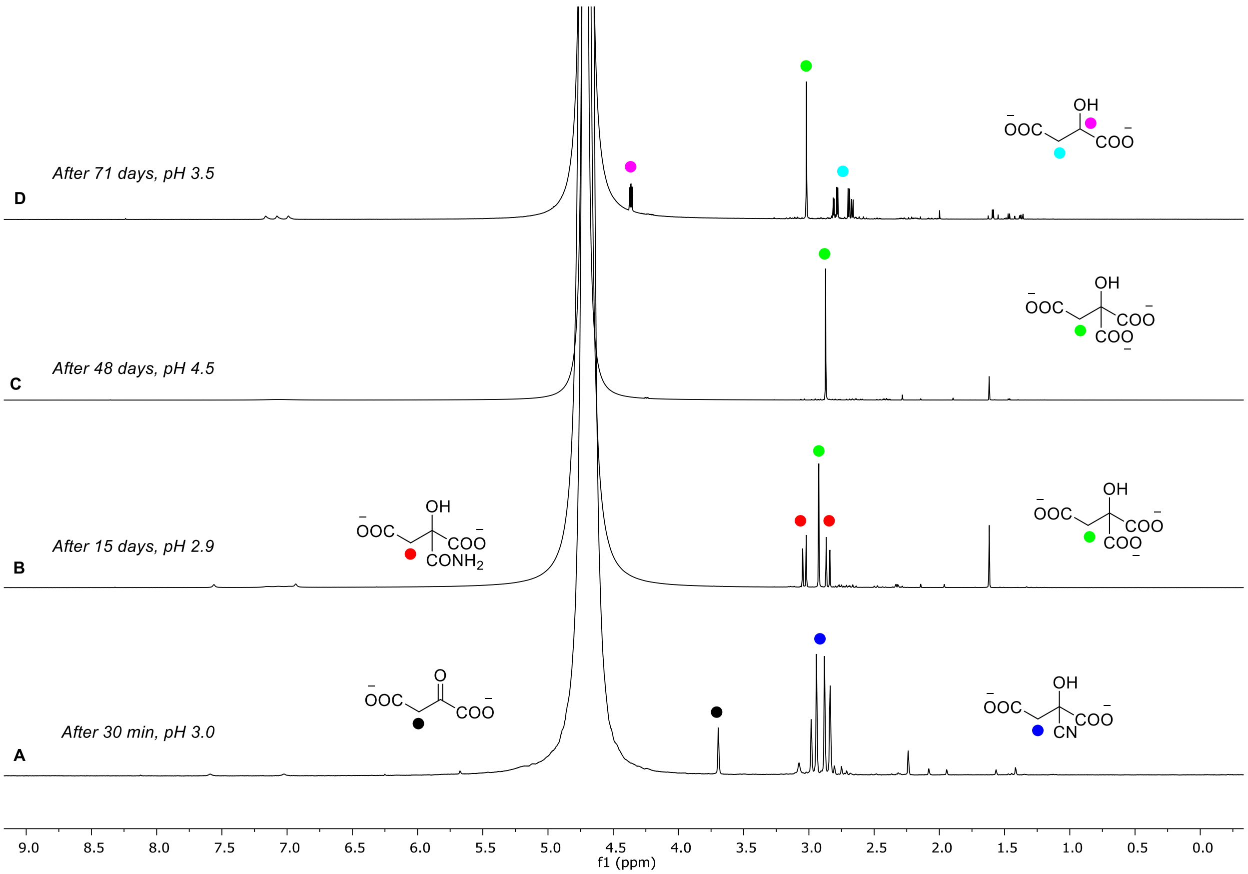


Figure S1: Stacked ^{1}H NMR (D_2O) of the reaction mixture showing progress of the reaction after 30 min (A), 15 days (B), 48 days (C) and 71 days (D). Conversion yields were calculated based upon the ^{1}H NMR peaks integration of product **5** (malate) i.e., peaks (~ 2.8 ppm) referred as ● w.r.t. carboxymalate **4b** peak (~ 3ppm) referred as ●. Since carboxymalate **4b** was formed as a discrete intermediate we used it as the basis for % yield conversion to malate **5**.

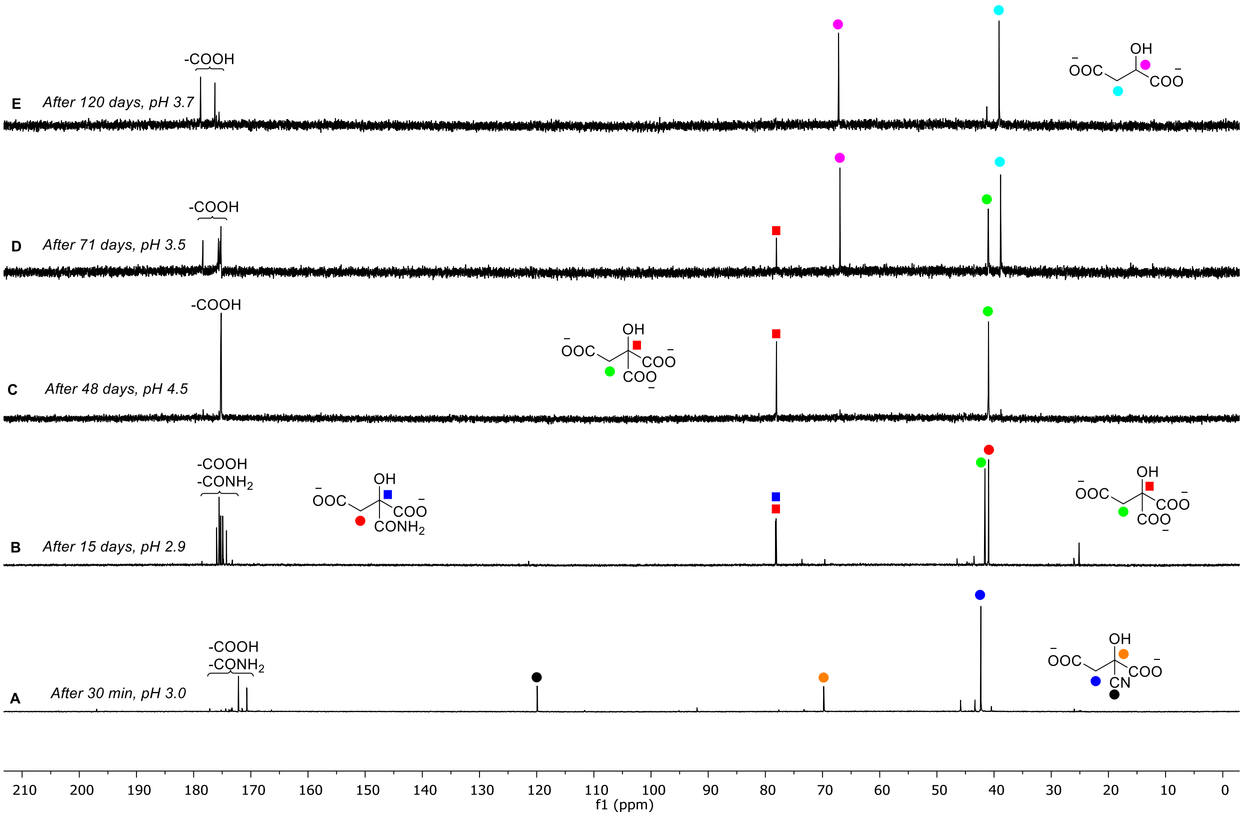


Figure S2: Stacked ^{13}C NMR (H_2O-D_2O) of the reaction mixture showing progress of the reaction after 30 min (A), 15 days (B), 48 days (C),71 days (D) and 120 days (E).

***Condition 2:* Sodium cyanide (9.8 mg, 1.0 equiv.), Oxaloacetate (26.4 mg, 0.1M, 1 equiv.), H_2O (2 mL).**

- After 30 min: ^{1}H NMR (Figure **S3A**) showed formation of cyanohydrin **3a** as the major product (pH = 3.2).
- After 21 days: ^{1}H NMR (Figure **S3B**) showed formation of amide intermediate **4a** and carboxymalate **4b** as the major product (pH = 3.5).
- After 21 days: temperature of the reaction mixture was raised up to 50 ºC (pH = 3.5).
- After 33 days: ^{1}H NMR (Figure **S3C**) showed carboxymalate **4b** as the sole product (pH = 4.2).
- After 33 days: $ZnCl_2$ (1 equiv.) was added to the reaction mixture and stirring was continued at 50 ºC (pH = 3.2).
- After 54 days: temperature of the reaction mixture was raised up to 60 ºC (pH = 3.4).
- After 56 days: ^{1}H NMR (Figure **S3D**) showed malate **5** as the major product (68% conversion w.r.t. carboxymalate **4b**) (pH = 3.5).

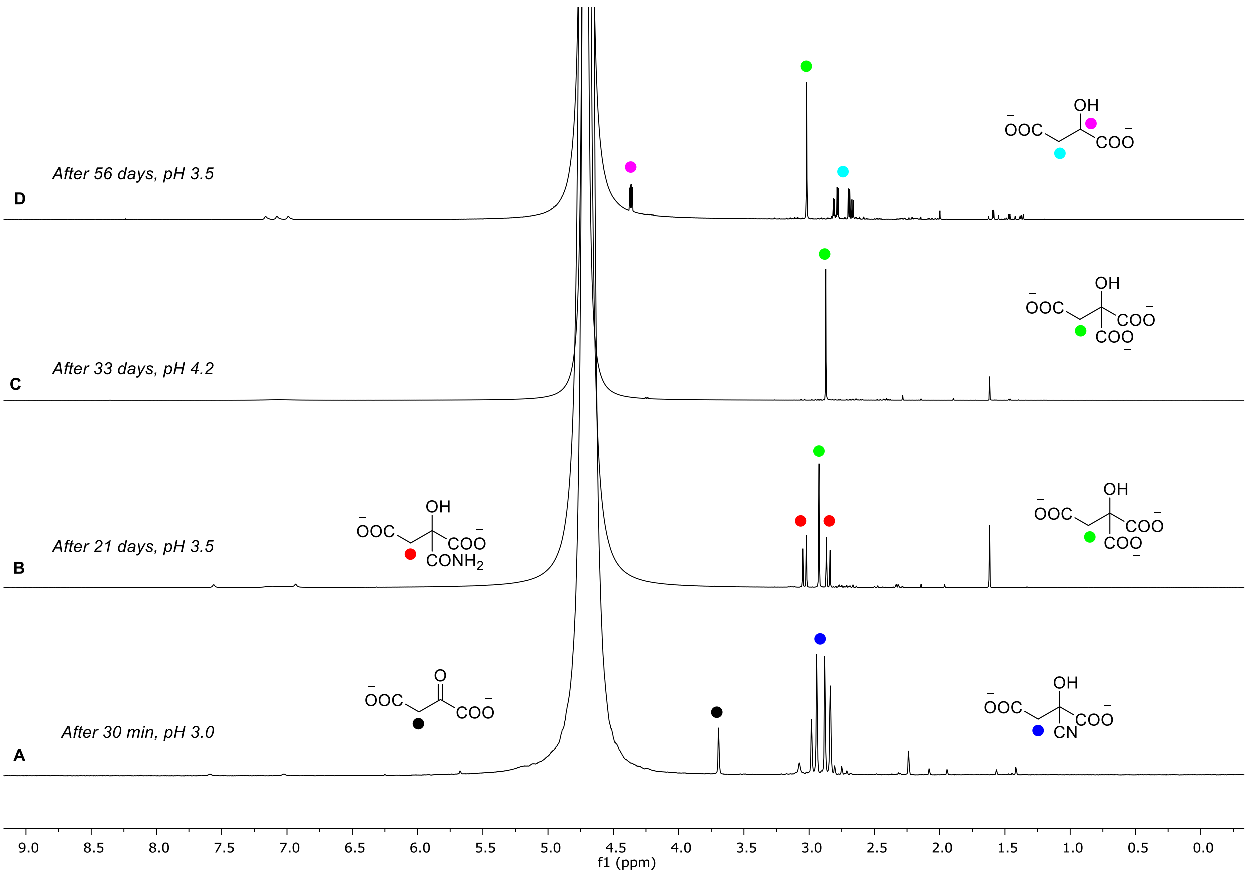


Figure S3: Stacked ^{1}H NMR (D_2O) of the reaction mixture showing progress of the reaction after 30 min (A), 21 days (B), 33 days (C) and 56 days (D). Conversion yields were calculated based upon the ^{1}H NMR peaks integration of product **5** (malate) i.e., peaks (~ 2.8 ppm) referred as ● w.r.t. carboxymalate **4b** peak (~ 3ppm) referred as ●.

Condition 3: Sodium cyanide (49.0 mg, 1.0 equiv.), Oxaloacetate (132 mg, 0.5M, 1 equiv.), 1M phosphate buffer (2 mL).

- After 5 min: ^{1}H NMR (Figure **S4A**) showed formation of cyanohydrin **3a** as the major product (pH = 6.0).
- After 12 days: ^{1}H NMR (Figure **S4B**) showed formation of amide intermediate **4a** and carboxymalate **4b** as the major product (pH = 6.0).
- After 21 days: temperature of the reaction mixture was raised up to 50 ºC (pH = 6.1).
- After 33 days: ^{1}H NMR (Figure **S4C**) showed carboxymalate **4b** as the sole product (pH = 6.2).
- After 33 days: ZnCl$_2$ (1 equiv.) was added to the reaction mixture and stirring was continued at 50 ºC (pH = 3.5).
- After 54 days: temperature of the reaction mixture was raised up to 60 ºC (pH = 3.6).
- After 56 days: ^{1}H NMR (Figure **S4D**) showed malate **5** as the major product (75% conversion w.r.t. carboxymalate **4b**) (pH = 3.8).

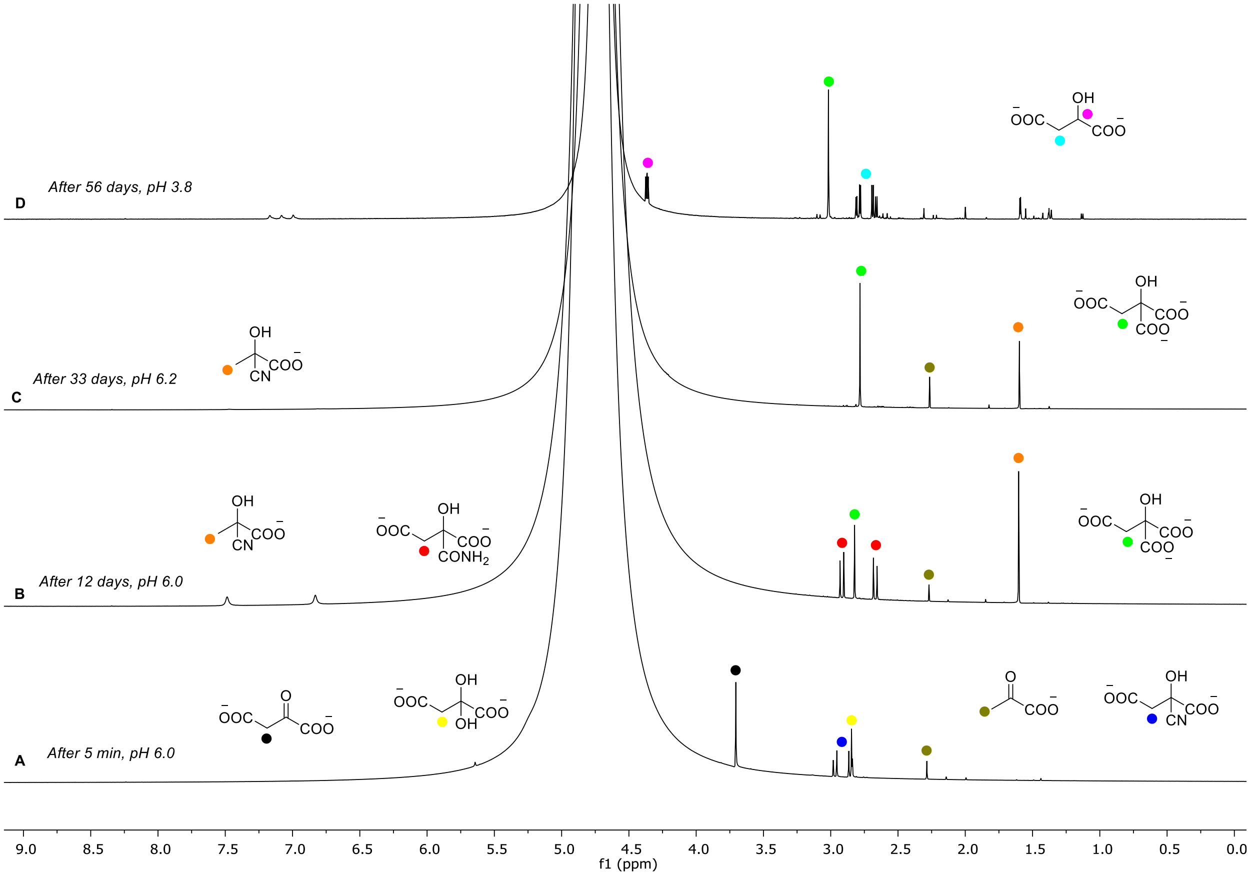


Figure S4: Stacked ^{1}H NMR (H$_2$O-D$_2$O) of the reaction mixture showing progress of the reaction after 5 min (A), 12 days (B), 33 days (C) and 56 days (D). Conversion yields were calculated based upon the ^{1}H NMR peaks integration of product **5** (malate) i.e., peaks (~ 2.8 ppm) referred as ● w.r.t. carboxymalate **4b** peak (~ 3ppm) referred as ●.

Condition 4: Sodium cyanide (25.0 mg, 1.0 equiv.), Oxaloacetate (6.6 mg, 0.01M, 1 equiv.), H_2O (5 mL).

- After 5 days: ^{1}H NMR (Figure **S5A**) showed formation of cyanohydrin **3a** and amide intermediate **4a** as the major products (pH = 4.2).
- After 15 days: ^{1}H NMR (Figure **S5B**) showed formation of carboxymalate **4b** along with amide **4a** and cyanohydrin **3a** intermediates (pH = 4.5).
- After 58 days: ^{1}H NMR (Figure **S5C**) showed formation of carboxymalate **4b** along with amide **4a** as the major product (pH = 5.8). Absolute combined yield of the carboxymalate **4b** and amide **4a** intermediate was found to be 91% (calculated by using the internal standard t-BuOH in a D_2O supressed ^{1}H NMR spectrum).

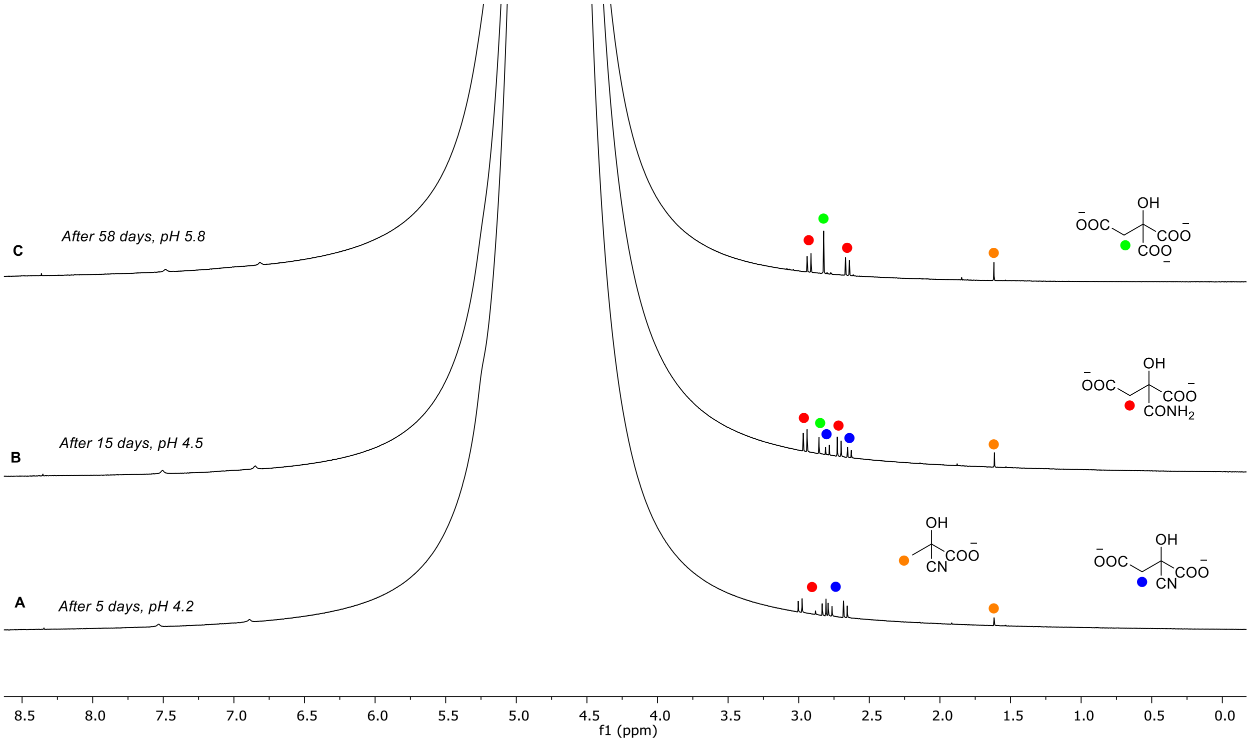
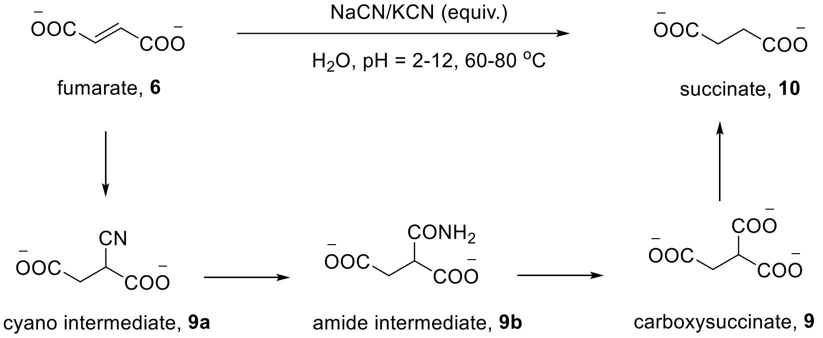


Figure S5: Stacked ^{1}H NMR (H_2O-D_2O) of the reaction mixture aliquots showing progress of the reaction after 5 days (A), 15 days (B), 58 days (C). Absolute yields were calculated based upon the ^{1}H NMR peaks integration of products (carboxymalate **4b** and amide **4a**) i.e., peaks (~ 2.8 ppm) w.r.t. internal standard (t-BuOH) used.

Reaction of fumarate 6 with cyanide (NaCN/KCN):



Scheme S2: Reaction of fumarate **6** with NaCN/KCN in H_2O showing the formation of succinate **10** (Table S2 and Figures S6-S8).

Table S2: Reaction of fumarate **6** or maleic acid with NaCN/KCN for the formation of succinate **10**. Identification of end-products (succinate **10**) was by made by comparison with literature values and authentic samples.[a]

Condition	Carboxylic acid (conc.)	$^-$CN source (equiv.)	Additive (equiv.)	pH	Temp. (°C)	Time (days)	% conversion to 10
1	fumarate (0.5M)	NaCN (1)	--	2.1	60	14	0%
2	fumarate (0.5M)	NaCN (1)	--	3.0	60	14	0%
3	fumarate (0.5M)	NaCN (1)	$MgCl_2$ (1)	3.0	60	14	0%
4	fumarate (0.5M)	NaCN (1)	--	9.0	60	14	0%
5	fumarate (0.5M)	KCN (2)	--	12.0	80	7	0%
6	maleate (0.5M)	NaCN (1)	--	2.5	60	14	0%
7	maleate (0.5M)	NaCN (1)	$MgCl_2$ (1)	2.1	60	14	0%
8	maleate (0.5M)	NaCN (1)	$ZnCl_2$ (1)	2.0	60	14	0%
9	fumarate (0.5M)	KCN (0.6)	--	4.2	60	7	8%
10	fumarate (1.0M)	KCN (0.5)	--	4.2	80	21	40%
11	fumarate (0.5M)	KCN (0.75)	--	4.3	80	19	20%
12	fumarate (0.5M)	KCN (1.0)	--	5.2	80	15	20%

[a] % conversion was calculated based on the ^{1}H NMR analysis product vs starting material remaining. All the reaction were performed in a 4 mL a glass vial with 1 mmol of carboxylic acid in H_2O at 60-80 °C.

Example procedure (table S2, condition 10): In a 4 mL glass vial equipped with a magnetic stir bar, fumaric acid **6** (116 mg, 1 mmol) was suspended in 1 mL of degassed milliQ water. After that 20 mg of NaOH (0.5 equiv.) was added to the reaction mixture and stirred for 5 minutes. Then 33 mg of KCN (0.5 equiv.) was carefully added to the reaction mixture and the reaction vial was immediately closed with cap and sealed with Teflon (to avoid any escape of cyanide in the form of HCN). pH of the reaction mixture was kept unadjusted (4.2, which was determined from another identical reaction taking into consideration the possible generation of HCN from the original reaction while measuring the pH). Reaction mixture was stirred at 80 °C and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR (100 µL reaction mixture in 500 µL of D_2O) with time (Figures S6-S7). The reaction mixture was cooled to 0 °C every time an aliquot was taken for the NMR analysis (to prevent escape of HCN). pH of the reaction mixture was measured every time NMR was taken and was observed to change over the course of time (Figures S6-S7).

Condition 10: Potassium cyanide (33 mg, 0.5 equiv.), Fumarate 6 (116 mg, 1.0M, 1 equiv.), NaOH (20 mg, 0.5 equiv.), H_2O (1 mL).

- After 5 min: ^{1}H NMR (Figure **S6A**) showed fumarate **6** only (pH = 4.2).
- After 2 days: ^{1}H NMR (Figure **S6B**) showed formation of the formation of succinate **10** (pH = 4.8).
- After 7 days: ^{1}H NMR (Figure **S6C**) showed increase in the formation of succinate **10** (pH = 5.2).
- After 14 days: ^{1}H NMR (Figure **S6D**) showed increase in the formation of succinate **10** (pH = 5.8).
- After 21 days: pH = 6.0, As per ^{1}H NMR (Figure **S6E**) analysis, relative yield of succinate **10** formed w.r.t. fumarate **6** was found to be 50%. Absolute yield of succinate **10** formed was found to be 40% (calculated by ^{1}H NMR with an internal standard i.e., t-BuOH).

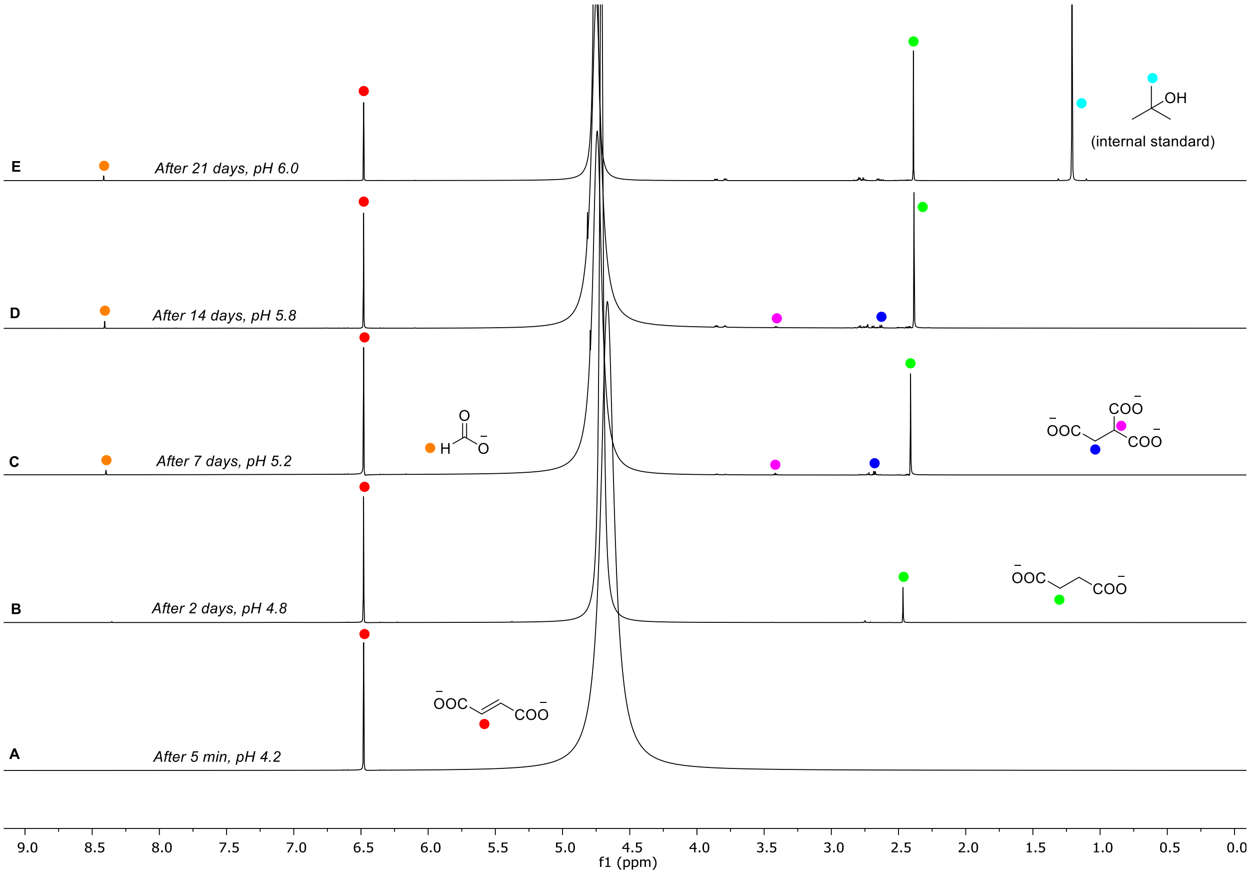


Figure S6: Stacked ^{1}H NMR (D_2O) of the reaction mixture showing progress of the reaction after 5 minutes (A), 2 days (B), 7 days (C), 14 days (D) and 21 days (D). Conversion yields were calculated based upon the ^{1}H NMR peaks integration of product (succinate **10**) i.e., peaks (~ 2.5 ppm) referred as ● w.r.t. fumarate **6** peak (~ 6.5 ppm) referred as ●. Absolute yield of succinate **10** formed was calculated by ^{1}H NMR with an internal standard i.e., t-BuOH.

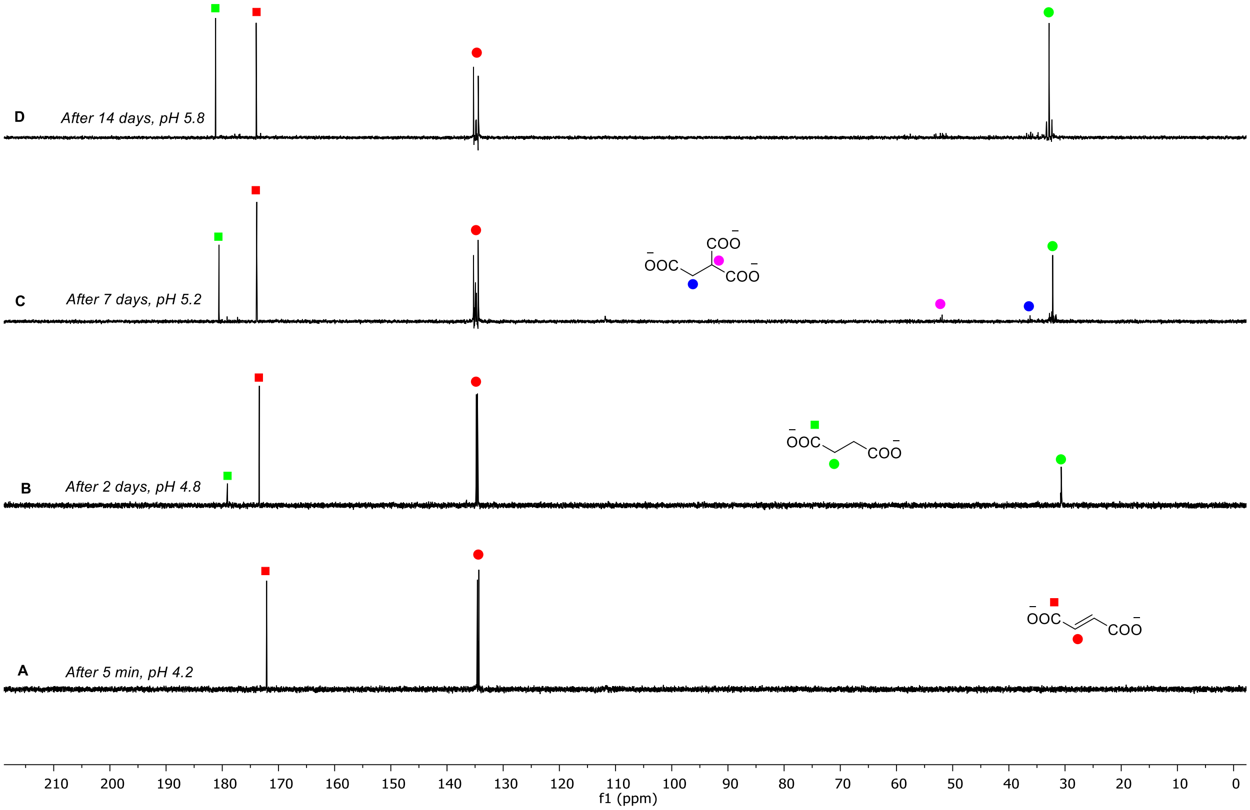


Figure S7: Stacked ^{13}C NMR (D_2O) of the reaction mixture showing progress of the reaction after 5 minutes (A), 2 days (B), 7 days (C), 14 days (D) and 21 days (D).

<u>Condition 12:</u> Potassium cyanide (65 mg, 1.0 equiv.), Fumarate 6 (116 mg, 0.5M, 1 equiv.), NaOH (20 mg, 0.5 equiv.), H_2O (2 mL).

- <u>After 5 min:</u> ^{1}H NMR (Figure **S8A**) showed fumarate **6** only (pH = 5.2).
- <u>After 5 days:</u> ^{1}H NMR (Figure **S8B**) showed increase in the formation of succinate **10** (pH = 5.6).
- <u>After 12 days:</u> pH = 6.0, As per ^{1}H NMR (Figure **S8C**) analysis, relative yield of succinate **10** formed w.r.t. fumarate **6** was found to be 17%. Absolute yield of succinate **10** formed was also found to be 17% (calculated by ^{1}H NMR with an internal standard i.e., t-BuOH).

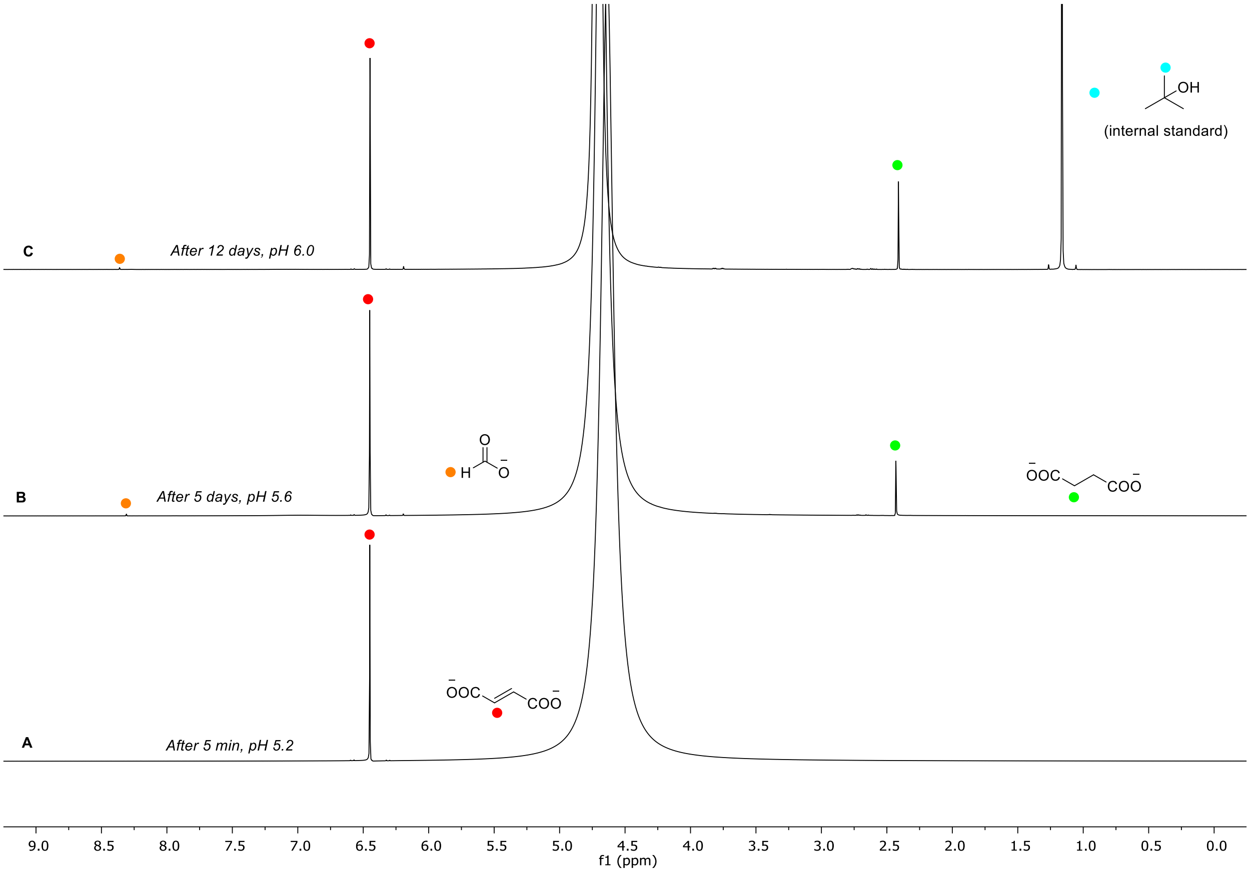
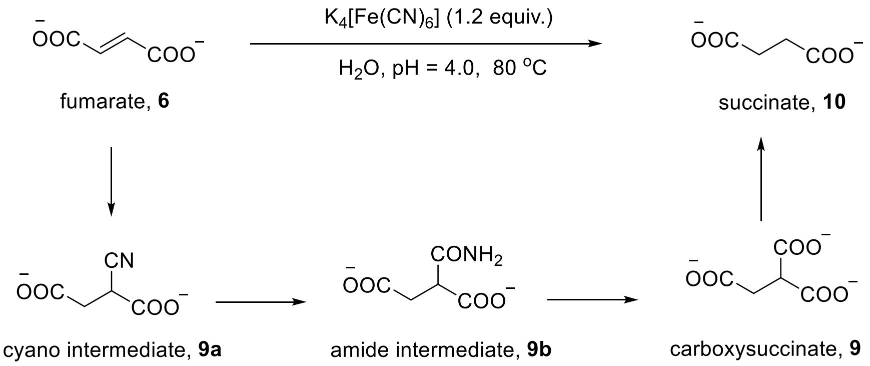


Figure S8: Stacked ^{1}H NMR (D_2O) of the reaction mixture showing progress of the reaction after 5 minutes (A), 5 days (B), and 12 days (C). Conversion yields were calculated based upon the ^{1}H NMR peaks integration of product (succinate **10**) i.e., peaks (~ 2.5 ppm) referred as ● w.r.t. fumarate **6** peak (~ 6.5 ppm) referred as ●. Absolute yield of succinate **10** formed was calculated by ^{1}H NMR with an internal standard i.e., t-BuOH.

Reaction of fumarate, 6 with potassium ferrocyanide as a CN⁻ ion source:



Scheme S3: Reaction of fumarate **6** with $K_4[Fe(CN)_6]$ in H_2O showing the formation of succinate **10** (Figure S9).

Reaction procedure: In a 4.0 mL glass vial equipped with a magnetic stir bar, fumaric acid, **6** (58 mg, 0.5 mmol, 1 equiv.) and $K_4[Fe(CN)_6]$ (254 mg, 0.6 mmol, 1.2 equiv.) were dissolved in deionized and degassed water (1.0 mL) and pH of the resulting mixture was adjusted to 4.0 with 5N NaOH solution. The resulting homogenous solution was heated to 80 °C and the reaction was monitored by ^1H NMR (NMR Method 2 in D_2O) (Figure S9).

Reaction Conditions:* *Potassium ferrocyanide (254 mg, 0.6 mmol, 1.2 equiv.), fumarate 6 (58 mg, 0.5 mmol), H_2O (1.0 mL).

- After 24 hours at 80 °C: showed the formation of succinate **10** (pH = 4.0) (figure **S9A**).
- After 6 days at 80 °C: % conversion to succinate **10** was 21% w.r.t fumarate **6** (pH = 4.2) (figure **S9B**).

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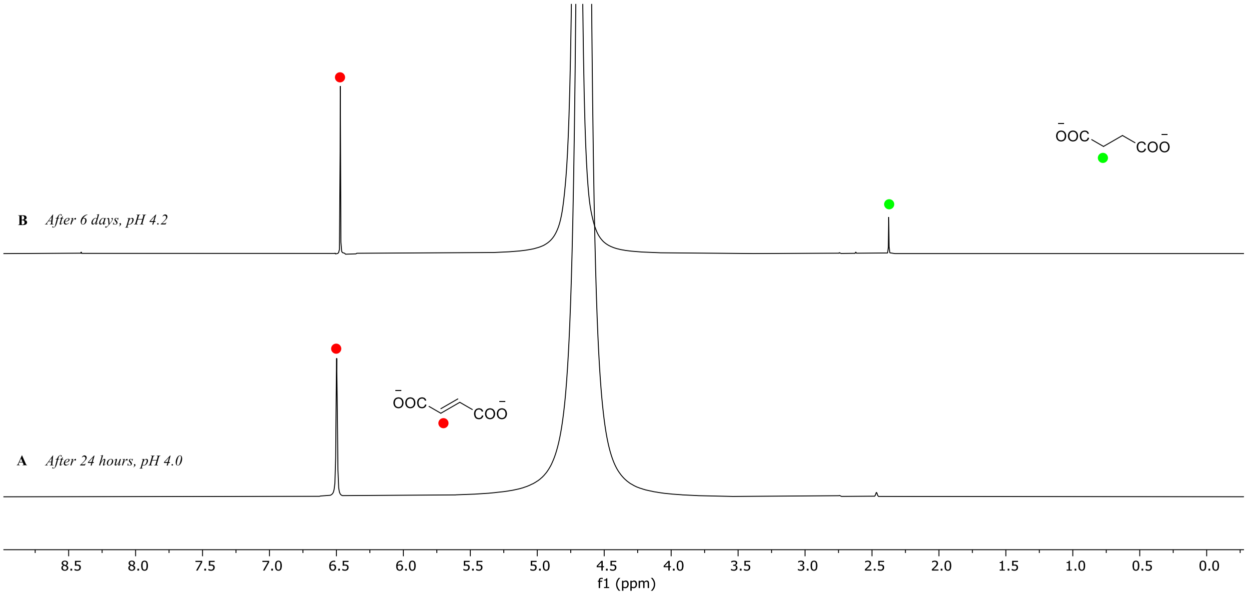
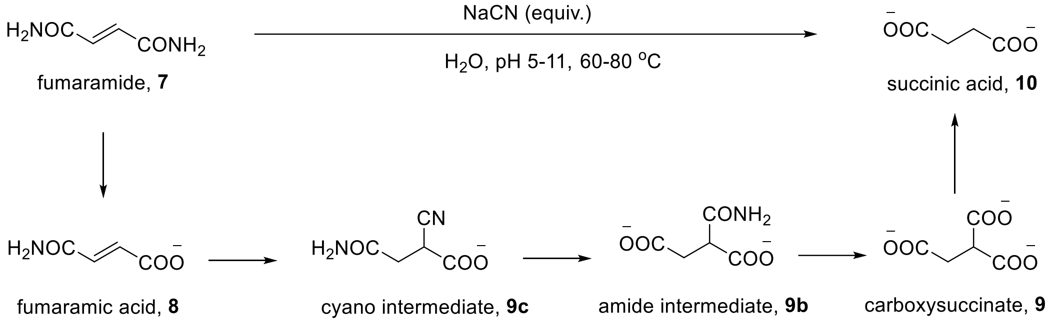


Figure S9: Stacked ^1H NMR of the reaction mixture (NMR method 2 in D_2O); reaction after 24 hours at 80 °C, pH 4.0 (A), reaction after 6 days at 80 °C, pH 4.2 (B). % Conversion is based on the ^1H NMR integration of the product succinate **10** (peak at 2.5 ppm, referred as ●) w.r.t to fumarate **6** (peak at 6.5 ppm, referred as ●)

Reaction of fumarate analogues with cyanide (NaCN/KCN):



Scheme S4: Reaction of fumaramide **7** with NaCN in H_2O showing the formation of succinate **10** (Table S3 and Figures S10-S15).

Table S3: Reaction of fumarate derivatives with NaCN for the formation of succinate. Identification of end-products (succinate **10**) was by made by comparison with literature values and authentic samples.[a]

Entry	**Carboxylic acid (conc.)**	**$^-$CN source (equiv.)**	**pH[b]**	**Temp. (°C)**	**Time (days)**	**% conversion to 10**
1	fumaramide (0.5M)	NaCN (1)	11.0	60	16	~5%
2	fumaramide (0.5M)	NaCN (1)	3.0	60	25	0%
3	fumaramide (0.5M)	NaCN (1)	6.0	60	14	~1%
4	fumaramide (0.5M)	NaCN (1)	11.0	80	11	69%[c]
5[d]	fumaramide (0.5M)	NaCN (1.5)	11.0	80	11	60%[c]
6[d]	fumaramide (0.5M)	NaCN (2)	11.0	80	11	47%[c]
7	maleamic acid (0.5M)	NaCN (1)	4.7	60	5	~1%
8	maleamic acid (0.5M)	NaCN (1)	9.0	60	16	~8%

[a] % conversion was calculated based on the ^{1}H NMR peaks integration of product vs formate/intermediates formed in the reaction mixture. All the reaction were performed in a 4 mL a glass vial with 1 mmol of carboxylic acid in H_2O at 60-80 °C. [b]pH indicated here is the starting pH of the reaction mixture; to enhance the rate of decarboxylation, pH of the reaction mixture was adjusted ~4-6 reaction at a later stage. [c]Yields are absolute yields (calculated by using an internal standard) calculated from identical reactions. [d]Precipitate formation was observed in the reactions when more than 1 equiv. of NaCN was used (which may affect the absolute yield calculation). % conversion was calculated based on the ^{1}H NMR peaks integration of product (succinate) vs the sum of starting material and intermediates formed in the reaction mixture.

Example procedure (table S3, entry 4): In a 4 mL glass vial equipped with a magnetic stir bar, fumaramide **7** (114 mg, 1 mmol) was suspended in 2 mL of degassed milliQ water. After that 49 mg of NaCN (1.0 equiv.) was added to the reaction mixture carefully. pH of the reaction mixture was kept unadjusted (11). Then the reaction mixture was stirred at 80 °C and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR (H_2O-D_2O) over a period (Figures S10-11). pH of the reaction mixture changed over the time (Figure S10). To enhance the rate of hydrolysis of the cyanide/amide intermediates to carboxysuccinate, pH of the reaction mixture was adjusted 4-6 and heating was continued at 80 °C.

***Condition 4:* Sodium cyanide (49 mg, 1.0 equiv.), Fumaramide (114 mg, 0.5M, 1 equiv.), H_2O (2 mL).**

- After 5 min: ^1H NMR was not recorded because of poor solubility of fumaramide **7** in H_2O at rt (pH = 11).
- After 3 days: ^1H NMR (Figure **S10A**) showed formation fumaramic acid **8** and the cyanide added intermediates with traces of succinate **10** (pH = 9.7).
- After 3 days: pH of the reaction mixture was adjusted to 6.0 with conc. HCl.
- After 6 days: pH of the reaction mixture jumped to 8.4 which was again adjusted to 6.0 with conc. HCl.
- After 7 days: ^1H NMR (Figure **S10B**) showed increase in the formation of succinate **10** (pH = 7.9).
- After 7 days: pH of the reaction mixture was adjusted to 6.0 with conc. HCl.
- After 10 days: ^1H NMR (Figure **S10C**) showed increase in the formation of succinate **10** (pH = 6.9).
- After 10 days: pH of the reaction mixture was adjusted to 4.0 with conc. HCl.
- After 11 days: pH = 4.4, As per ^1H NMR (Figure **S10D**) analysis, succinate **10** was the major and sole product formed in the reaction mixture (69% absolute yield).

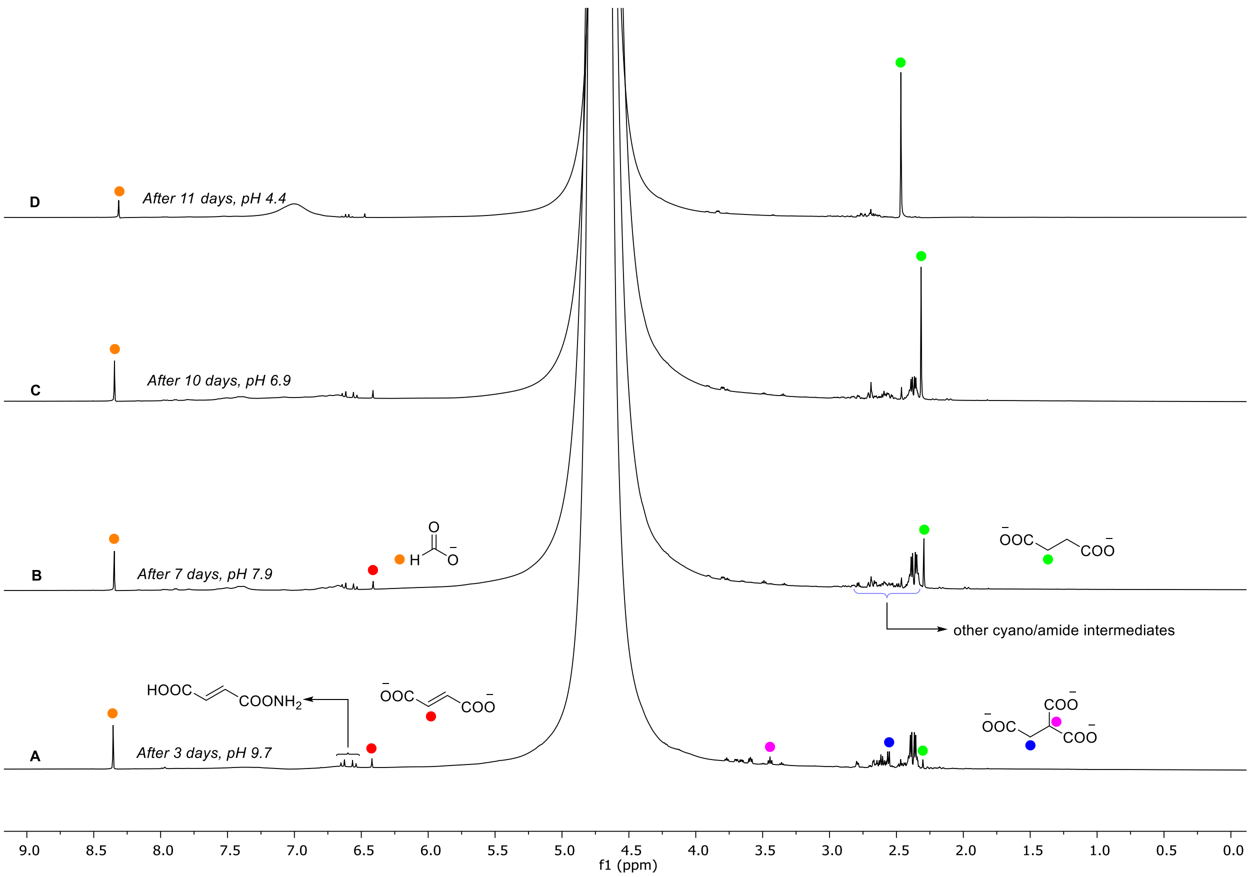


Figure S10: Stacked ^1H NMR (D_2O) of the reaction mixture aliquots showing progress of the reaction after 3 days (A), 7 days (B), 10 days (C) and 11 days (D). Absolute yield of succinate **10** formed was calculated by ^1H NMR integral from a similar reaction with an internal standard i.e., t-BuOH.

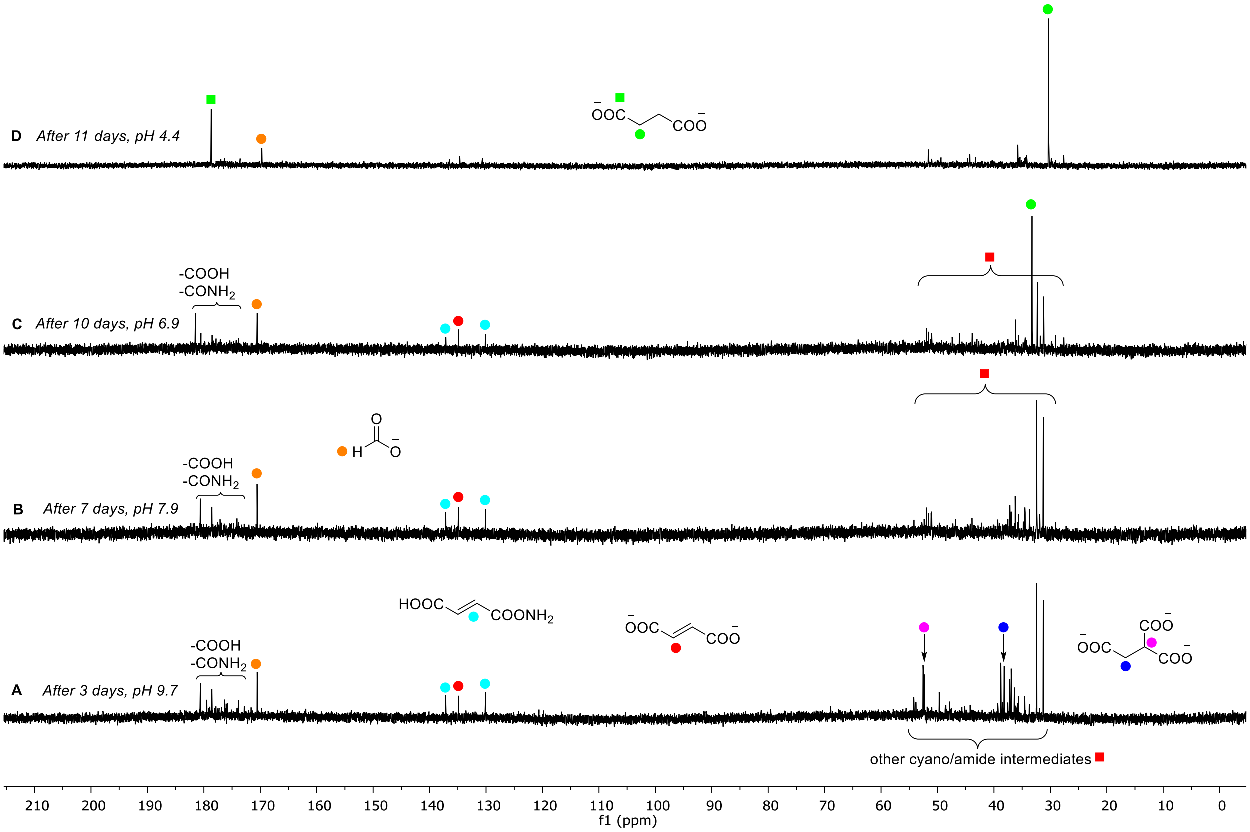


Figure S11: Stacked ^{13}C NMR (D_2O) of the reaction mixture aliquots showing progress of the reaction after 3 days (A), 7 days (B), 10 days (C) and 11 days (D).

Condition 6: Sodium cyanide (98 mg, 2.0 equiv.), Fumaramide 7 (114 mg, 0.5M, 1 equiv.), H_2O (2 mL).

- After 5 min: ^{1}H NMR was not recorded because of poor solubility of fumaramide **7** in H_2O at rt (pH = 11.5).
- After 3 days: ^{1}H NMR (Figure **S12A**) showed the formation of carboxy succinate **9** and the other cyanide added intermediates with traces of succinate (pH = 9.7).
- After 3 days: pH of the reaction mixture was adjusted to 6.0 with conc. HCl.
- After 6 days: pH of the reaction mixture jumped to 8.4 which was again adjusted to 6.0 with conc. HCl.
- After 7 days: ^{1}H NMR (Figure **S12B**) showed increase in the formation of succinate **10** (pH = 7.9).
- After 7 days: pH of the reaction mixture was adjusted to 6.0 with conc. HCl.
- After 10 days: ^{1}H NMR (Figure **S12C**) showed increase in the formation of succinate **10** (pH = 6.9).
- After 10 days: pH of the reaction mixture was adjusted to 4.0 with conc. HCl.
- After 11 days: pH = 4.4, As per ^{1}H NMR (Figure **S12D**) analysis, succinate **10** was the major and sole product formed in the reaction mixture (47% absolute yield).

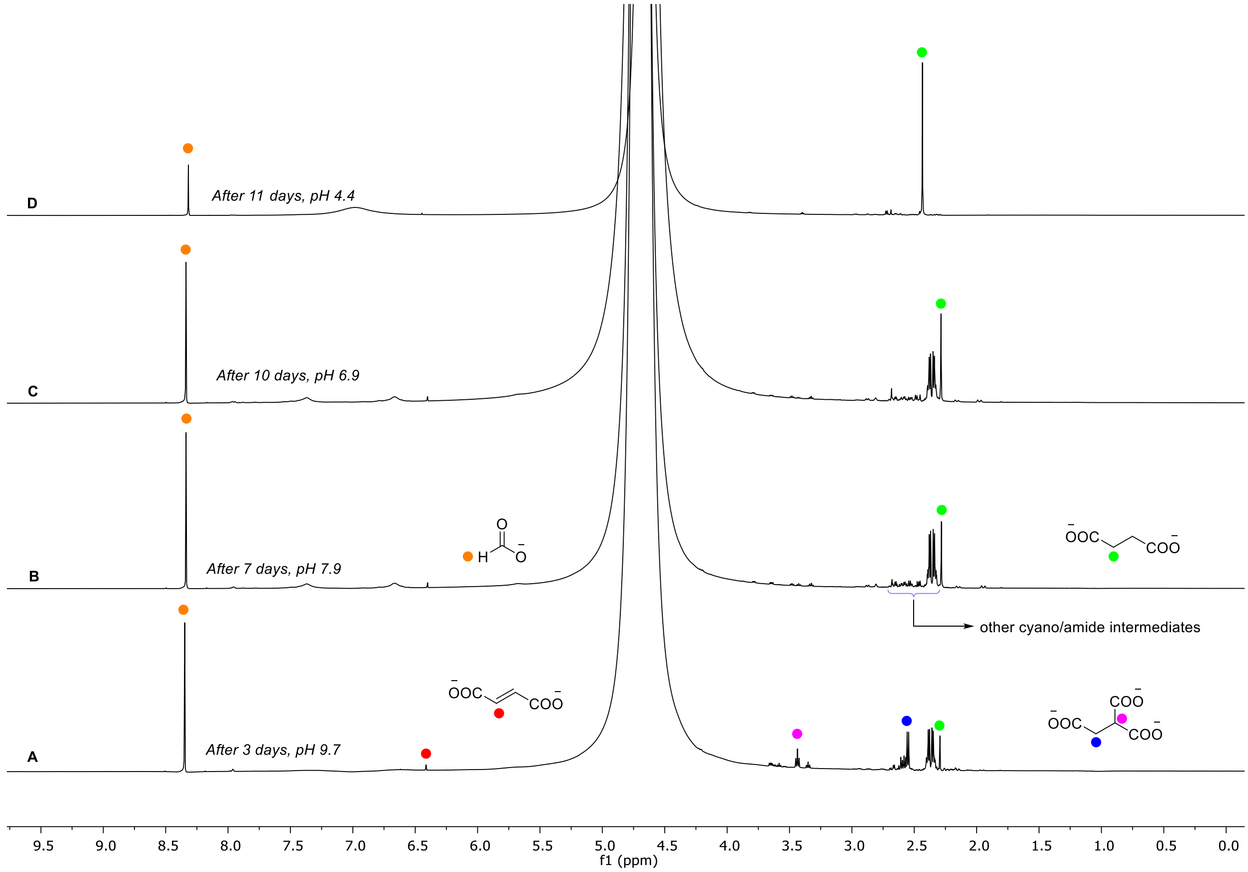


Figure S12: Stacked ^{1}H NMR (H_2O-D_2O) of the reaction mixture aliquots showing progress of the reaction after 3 days (A), 7 days (B), 10 days (C) and 11 days (D). Absolute yield of succinate **10** formed was calculated by ^{1}H NMR integral from a similar reaction with an internal standard i.e., t-BuOH.

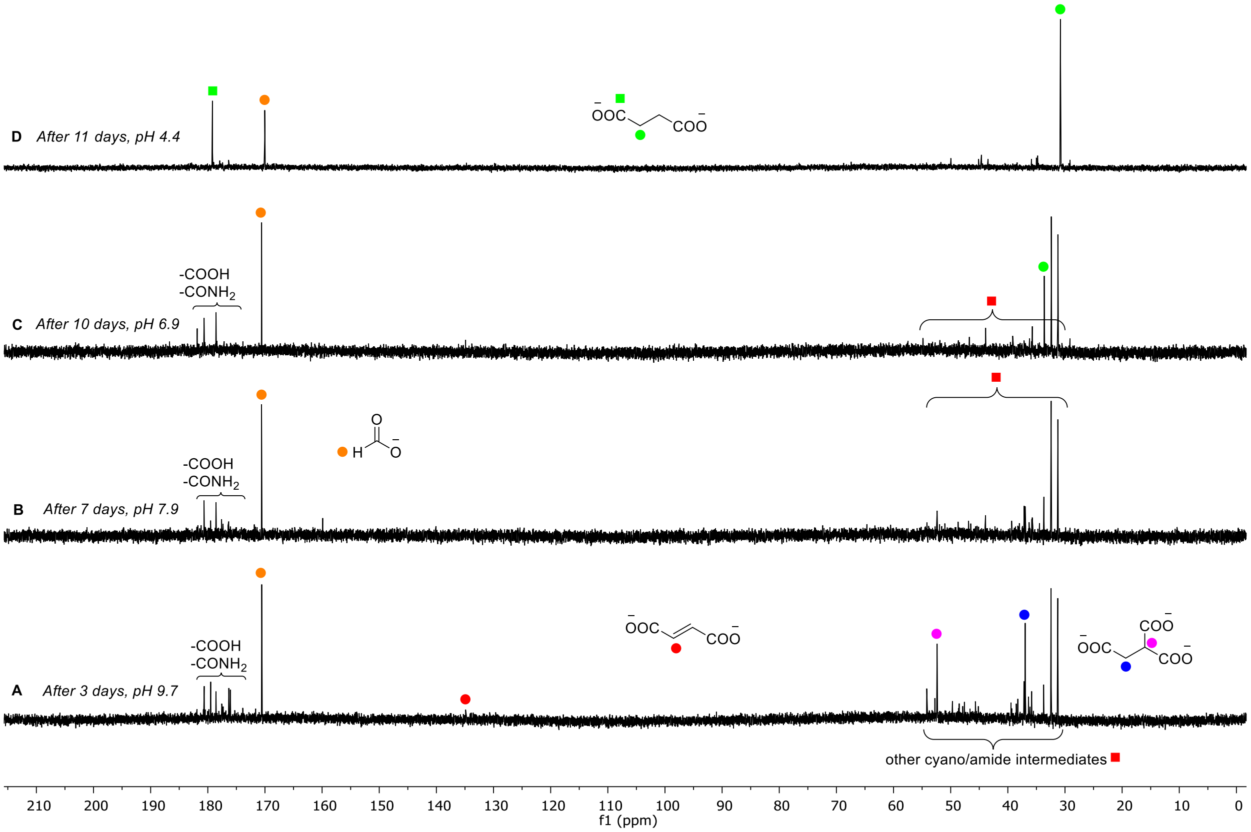


Figure S13: Stacked ^{13}C NMR (D_2O) of the reaction mixture aliquots showing progress of the reaction after 3 days (A), 7 days (B), 10 days (C) and 11 days (D).

Condition 8: ***Sodium cyanide (49 mg, 1.0 equiv.), Maleamate (115 mg, 0.5M, 1 equiv.), H_2O (2 mL).***

- After 5 min: pH = 9.0.
- After 3 days: ^{1}H NMR (Figure **S14A**) showed the formation of carboxy succinate **9** as the major intermediate (pH = 9.9).
- After 9 days: ^{1}H NMR (Figure **S14B**) showed the formation of succinate **10** along with other intermediates (pH = 9.7).
- After 16 days: ^{1}H NMR (Figure **S14C**) showed increase in the formation of succinate **10** (pH = 9.0). Conversion yield to succinate was found to be 8%.

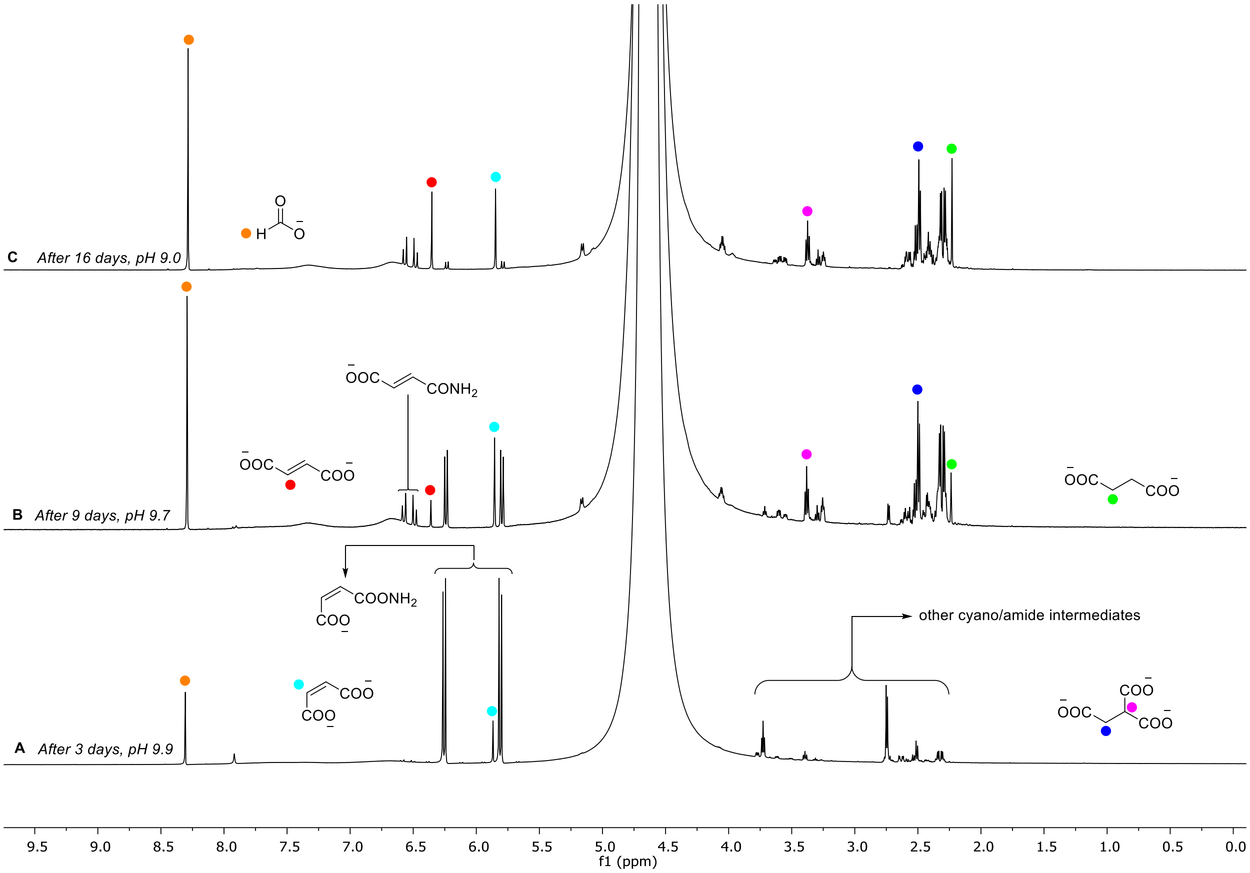


Figure S14: Stacked ^{1}H NMR (H_2O-D_2O) of the reaction mixture showing progress of the reaction after 3 days (A), 9 days (B), and 16 days (C). % conversion was calculated based on the ^{1}H NMR peaks integration of product (succinate) vs the sum of formate+intermediates formed in the reaction mixture.

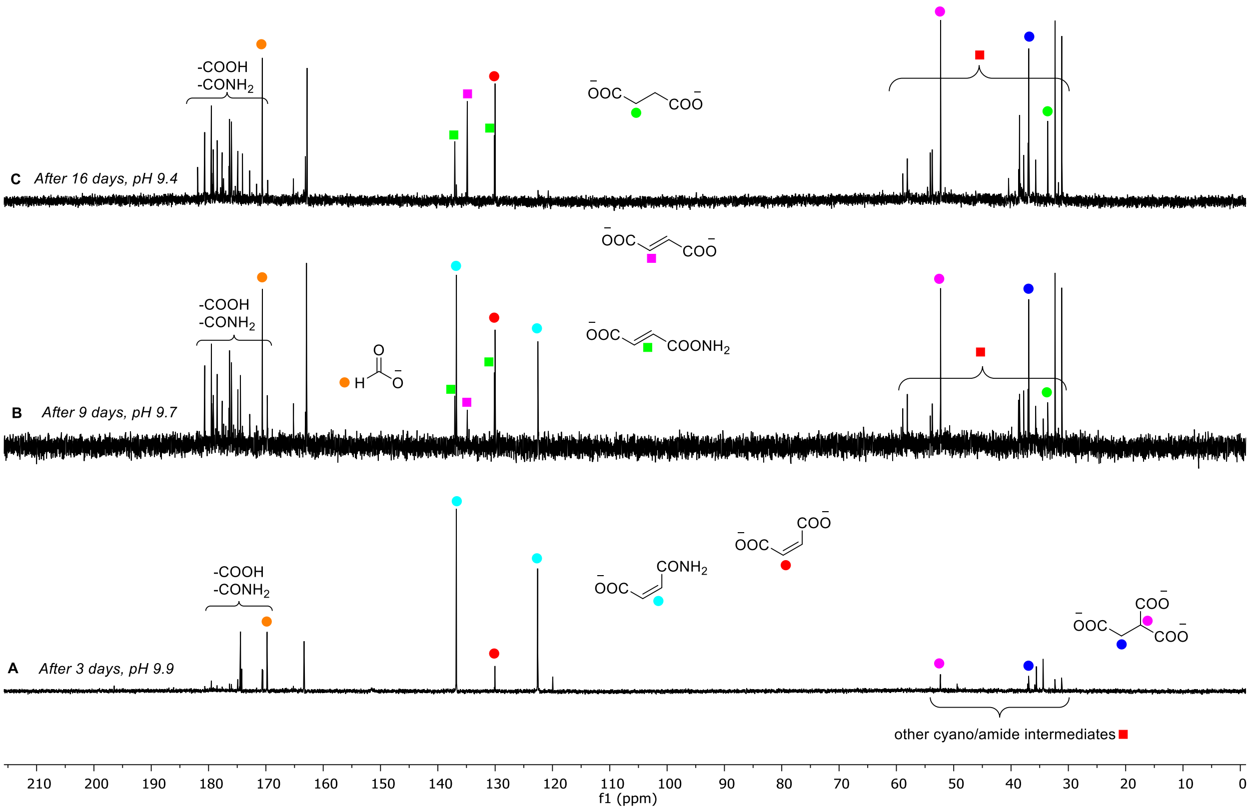
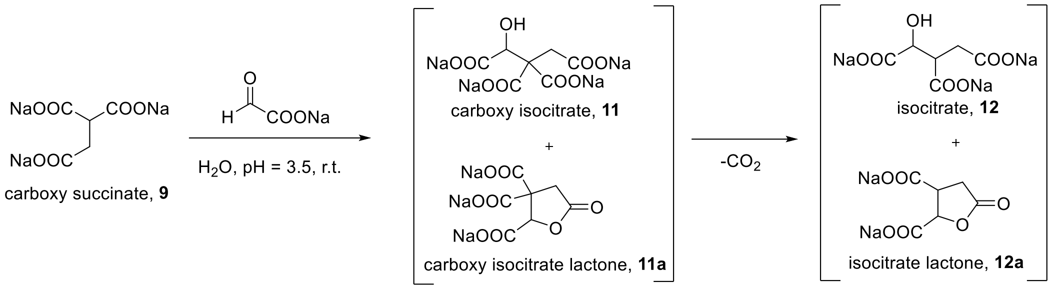


Figure S15: Stacked ^{13}C NMR (D_2O) of the reaction mixture aliquots showing progress of the reaction after 3 days (A), 9 days (B), and 16 days (C).

Reaction of carboxy succinate 9 with glyoxylate 1:



Scheme S5: Reaction of carboxy succinate **9** with glyoxylate **1** in H_2O showing the formation of isocitrate (Figures S16-S20).

Reaction procedure: In a 20 mL glass vial equipped with a magnetic stir bar, carboxysuccinate trisodium salt **9**[1] (456 mg, 2 mmol,1.0 equiv.) was dissolved in deionized and degassed water (4 mL). To this homogeneous solution, glyoxylic acid monohydrate (184 mg, 1.0 equiv.) was added. After addition of glyoxylic acid, pH of the homogeneous colourless reaction mixture was kept unadjusted (3.5). The reaction mixture was stirred at room temperature under argon atmosphere and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR over a period (Figures S16-S17). Once the formation of carboxy isocitrate **11** (with lactone **11a**) is complete, the rate of carboxylation (to give isocitrate and its corresponding lactone) was enhanced by raising the temperature of the reaction mixture to 50 ºC.

<u>Reaction Conditions:</u> Trisodium carboxy succinate 9 (456 mg, 1.0 equiv.), glyoxylic acid monohydrate (184 mg, 0.5M, 1 equiv.), H_2O (4 mL).

- <u>After 30 min:</u> pH = 3.5, ^{1}H NMR (Figure **S16A**) showed the starting materials along with the formation of traces of carboxy isocitrate **11**.
- <u>After 2h:</u> ^{1}H NMR showed 28% conversion of carboxy succinate **9** to carboxy isocitrate **11** (pH = 3 .6).
- <u>After 5h:</u> (Figure **S16B**) 45% conversion of carboxy succinate **9** to carboxy isocitrate **11** was observed (pH = 3.7).
- <u>After 24h:</u> ^{1}H NMR (Figure **S16C**) showed 65% conversion of carboxy succinate **9** to carboxy isocitrate **11** (pH = 4.2). Formation of corresponding lactone of carboxy isocitrate **11a** was also observed.
- <u>After 6 days:</u> pH = 4.5, ^{1}H NMR (Figure **S16D**) showed almost quantitative conversion of carboxy succinate **9** to carboxy isocitrate **11** and its corresponding lactone **11a** in almost 50:50 ratio. Decarboxylated products i.e., isocitrate **12** and isocitrate lactone **12a** were also observed in NMR. ESI-MS m/z calculated for **11** $C_8H_8O_{11}$ $[M+H]^+$ 281.00, found 281.00. ESI-MS m/z calculated for **11a** $C_8H_6O_{10}$ $[M+H]^+$ 263.00, found 263.00.
- <u>After 6 days:</u> The reaction mixture was heated at 50 °C to enhance the rate of decarboxylation.
- <u>After 7 days (24h at 50 °C):</u> ^{1}H NMR (Figure **S16E**) showed quantitative formation of isocitrate **12** diastereomers and isocitrate lactones **12a** (pH = 5.0). Absolute yield of the products formed was found to be 93% (Figure **S18**).
- <u>After 7 days:</u> ^{1}H NMR (Figure **S16F**) was recorded at pH = 7.0 (pH of NMR sample was adjusted with 5M NaOH).

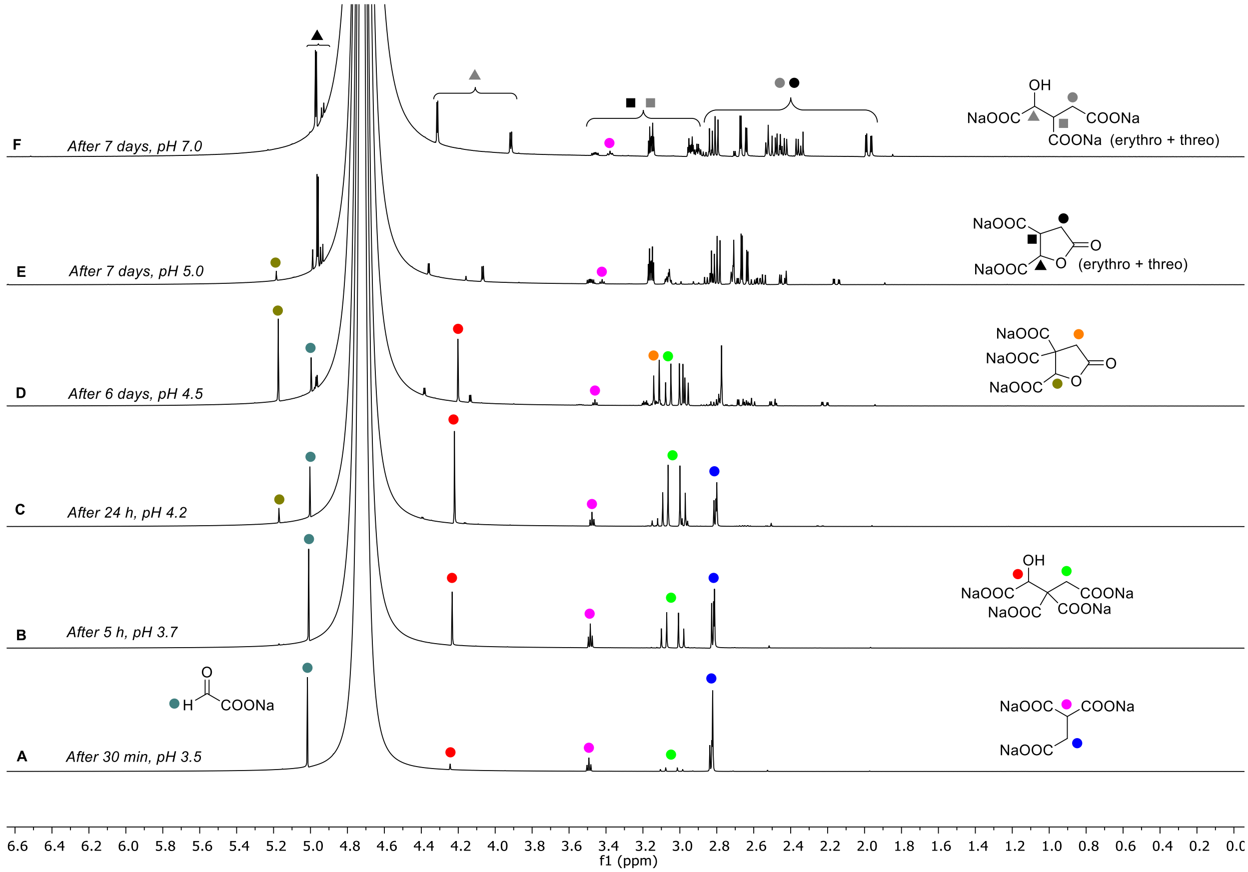


Figure S16: Stacked ^{1}H NMR (D_2O) showing progress of the reaction after 30 minutes (A), 5h (B), 24h (C), 6 days (D), 7 days (E) and 7 days at pH = 7.0 (F). % conversion was calculated based on the ^{1}H NMR peaks integration of products **12**, and **12a** vs carboxy succinate **9**.

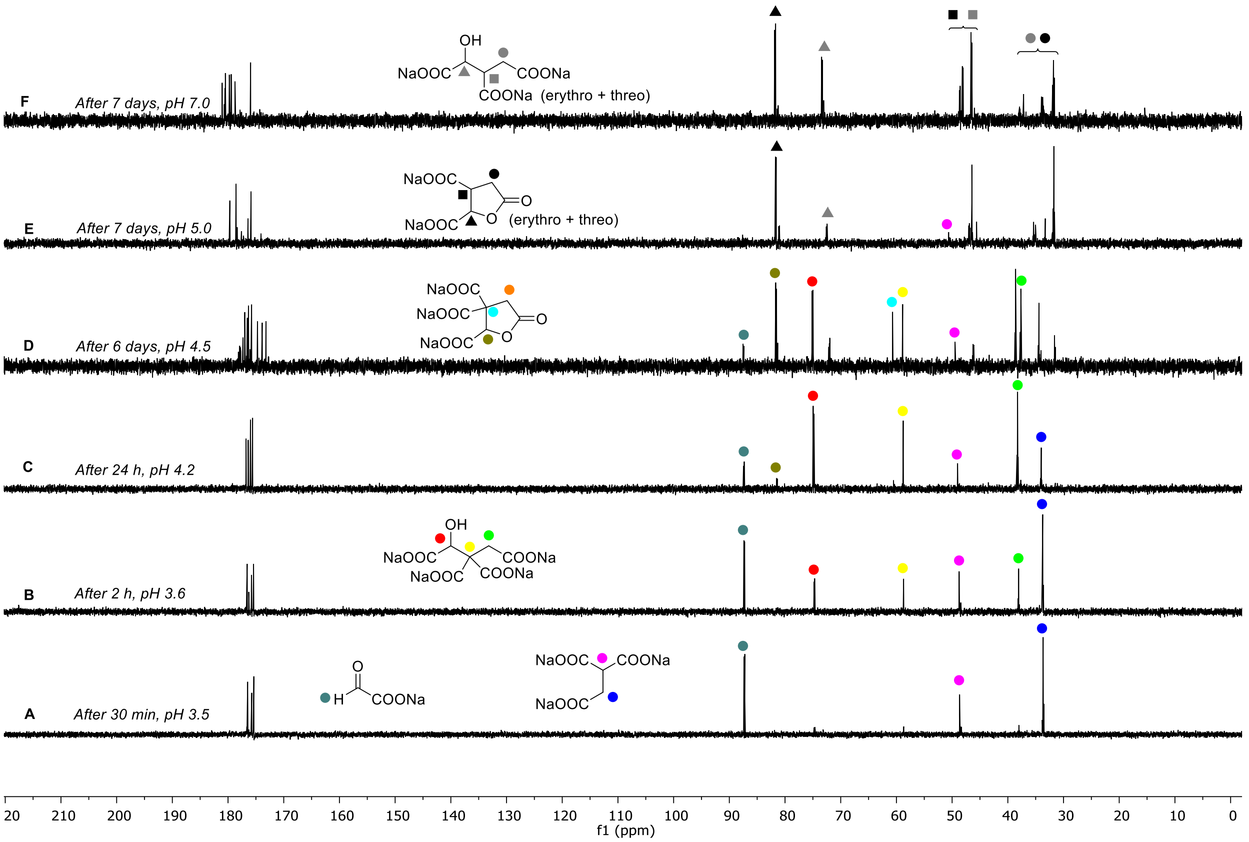


Figure S17: Stacked ^{13}C NMR (D_2O) showing progress of the reaction after 30 minutes (A), 2h (B), 24h (C), 6 days (D), 7 days (E) and 7 days at pH = 7.0 (F).

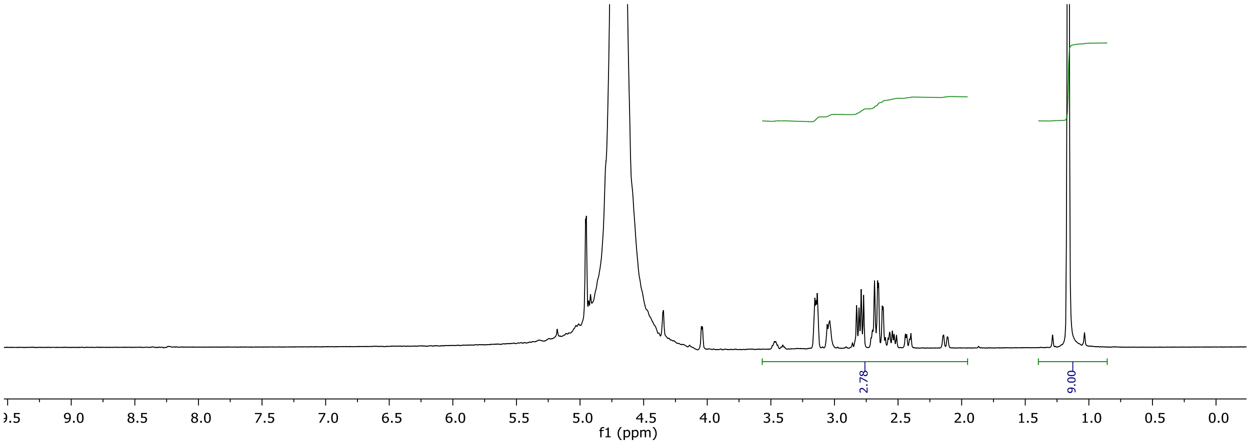


Figure S18: Absolute yield of the formation of isocitrate **12** and isocitrate lactone **12a** was 93% (calculated by ^{1}H NMR analysis in the presence of equimolar t-BuOH as an internal standard).

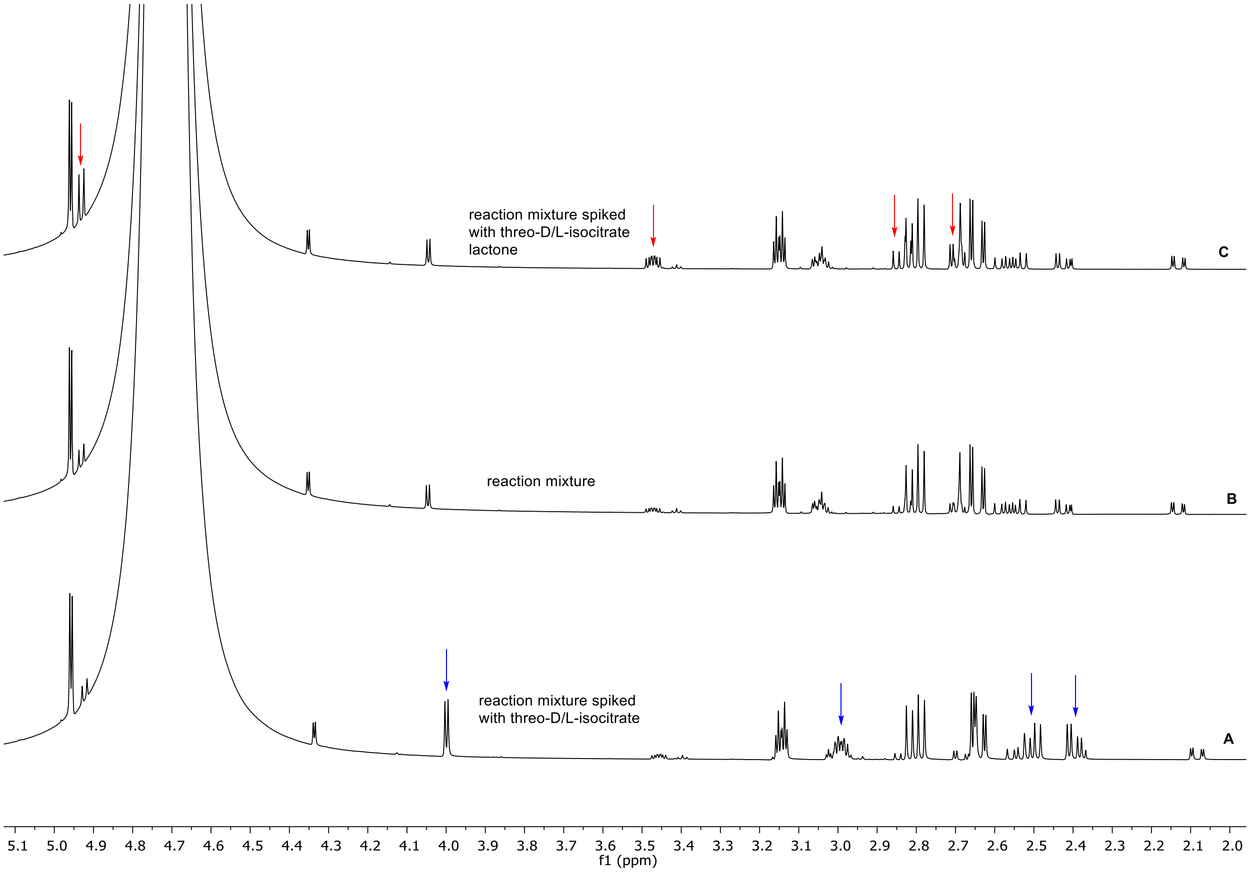


Figure S19: ^{1}H NMR (D_2O) of the reaction mixture spiked with commercially available *threo*-D/L-isocitrate **12** (A). ^{1}H NMR (H_2O-D_2O) of the reaction mixture (B). ^{1}H NMR (D_2O) of the reaction mixture spiked with commercially available *threo*-D/L-isocitrate lactone **12a** (C).

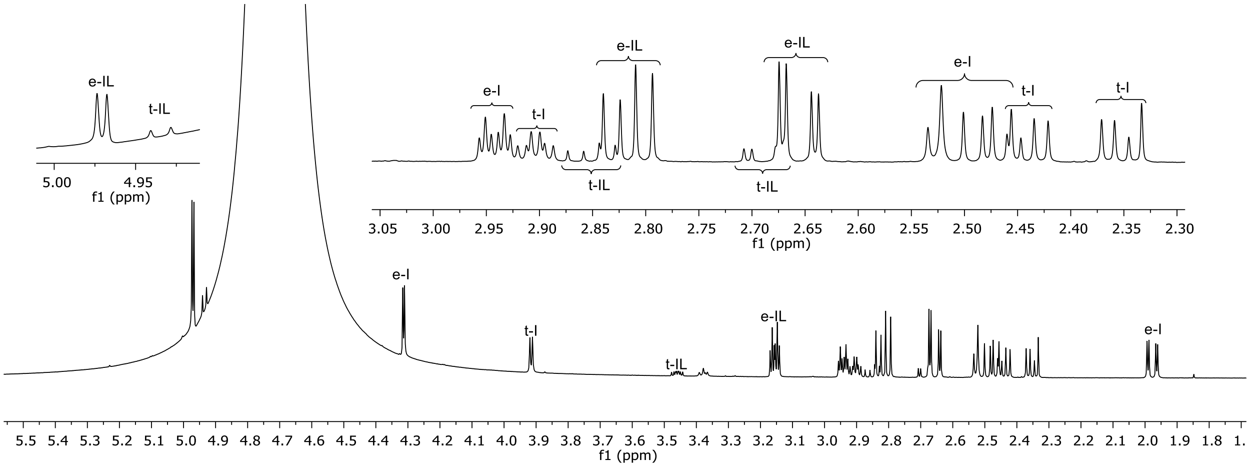
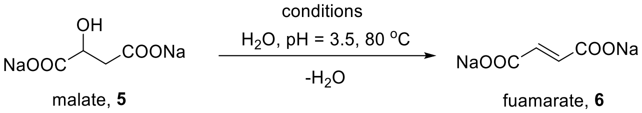


Figure S20: ^{1}H NMR (D_2O) of the reaction mixture showing the assignment of peaks related to diastereomers of isocitrate **12** and isocitrate lactone **12a**. e-IL: *erythro*-isocitrate lactone, t-IL: *threo*-isocitrate lactone, e-I: *erythro*-isocitrate, t-I: *threo*-isocitrate (in the ratio of 43:7:29:21 respectively).

Dehydration of malate 5 to fumarate 6:



Scheme S6: Dehydration of malate **5** in the presence of different ammonium salts (Figures S21-S22).

Table S4: Attempts for the dehydration of malate **5** to fumarate **6**. Identification of end-products (fumarate **6**) was by made by comparison with literature values and authentic samples.

Condition	**Reaction conc.**	**Additive (equiv.)**	**pH**	**Temp. (°C)**	**Time (days)**	**% conversion to 6**[a]
1	0.5M	$NaHSO_3$ (5)	2.7	80	35	70%
2	0.5M	NH_4HCO_2 (5)	2.5	80	53	80%
3	0.5M	NH_4OAc (5)	3.5	80	53	90%
4	0.5M	NH_4OAc (2)	3.0	80	32	50%
5	0.5M	NH_4Cl (5)	3.5	80	50	70%

[a] % conversion was calculated based on the ^1H NMR analysis product vs starting material remaining. All the reaction were performed in a 4 mL a glass vial starting with 1 mmol of malate **5** in H_2O 80 °C (wet-dry cycles, 1 cycle/24h).

Example procedure (Condition 3): In a 4 mL glass vial, malic acid **5** (134 mg, 1 mmol, 1.0 equiv.) was dissolved in deionized and degassed water (2 mL). To this homogeneous solution ammonium acetate (385 mg, 5.0 equiv.) was added. After addition of ammonium acetate, pH of the homogeneous colourless reaction mixture was adjusted to 3.5 with conc. HCl. The reaction mixture was then placed at 80 °C (without cap) and the progress of the reaction was monitored by ^1H-NMR or ^{13}C-NMR over a period (Figures S21-22). To record the NMR of the reaction mixture, the reaction mixture was dissolved in 2.0 mL of milliQ water and then a fraction of reaction mixture (550 uL) was taken in an NMR tube (with D_2O in capillary). After recording the NMR, the sample was again transferred to the reaction vial and heated again for the next cycle. One cycle comprises of 24 h. Where NMR was not recorded, 2 mL of H_2O was added to the reaction mixture and heating was continued for the next cycle.

<u>Condition 3:</u> ammonium acetate (385 mg, 5.0 equiv.), malic acid 5 (134 mg, 0.5M, 1 equiv.), H_2O (2 mL).

- <u>After 5 min:</u> pH = 3.5.
- <u>After 2 days:</u> ^{1}H NMR (Figure **S21A**) showed majorly the starting material with traces of dehydrated product (pH = 3.6).
- <u>After 9 days:</u> ^{1}H NMR (Figure **S21B**) showed good amount of dehydrated product (pH =3 .3).
- <u>After 23 days:</u> ^{1}H NMR (Figure **S21C**) showed fumarate **6** as the major product (68% conversion w.r.t. malate **5**) (pH = 3.2).
- <u>After 53 days:</u> ^{1}H NMR (Figure **S21D**) showed fumarate **6** as the sole product (pH = 3.4). Absolute yield of fumarate **6** formed was found to be 50% (calculated by ^{1}H NMR with an internal standard i.e., t-BuOH).

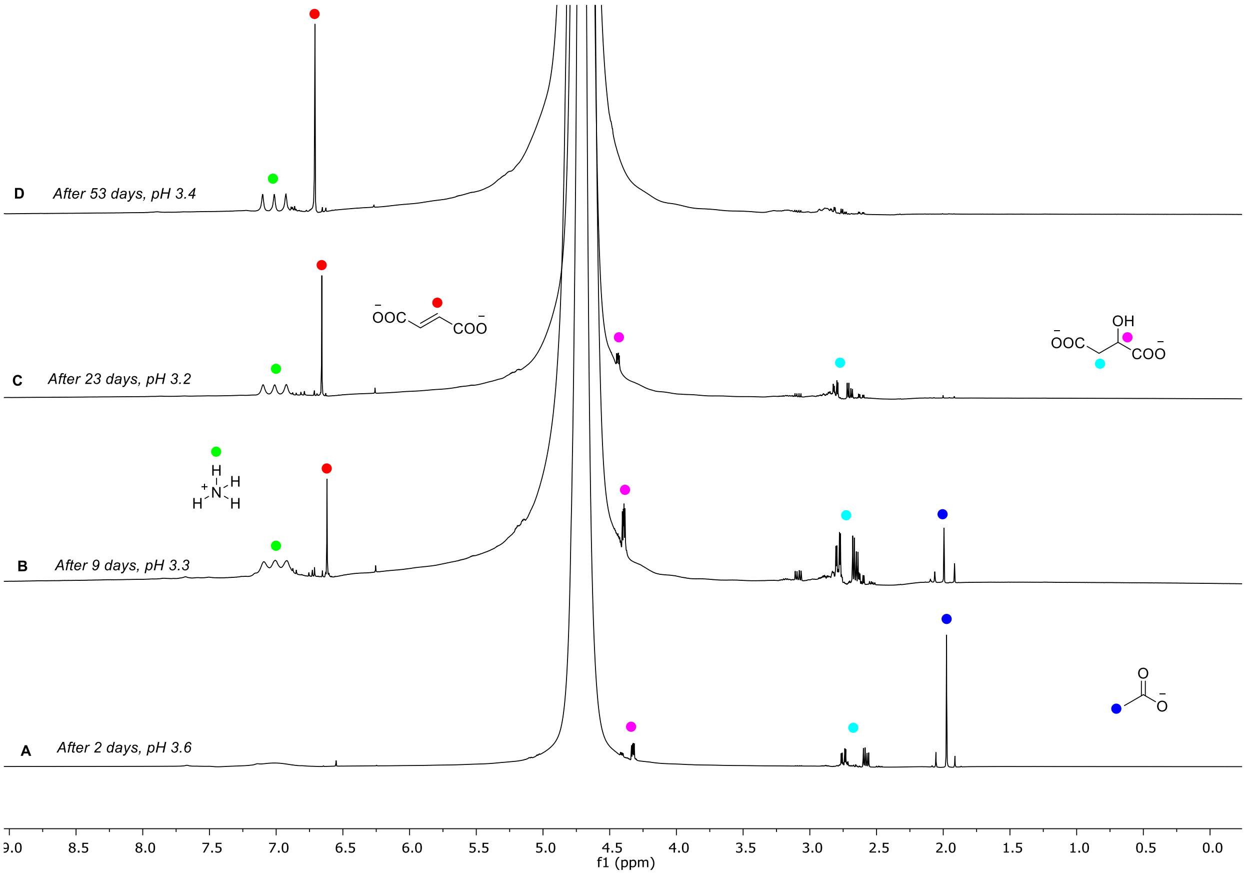


Figure S21: Stacked ^{1}H NMR (D_2O) showing progress of the reaction after 2 days (A), 9 days (B), 23 days (C), and 53 days (D). % conversion was calculated based on the ^{1}H NMR peaks integration of product **6** vs malate **5**.

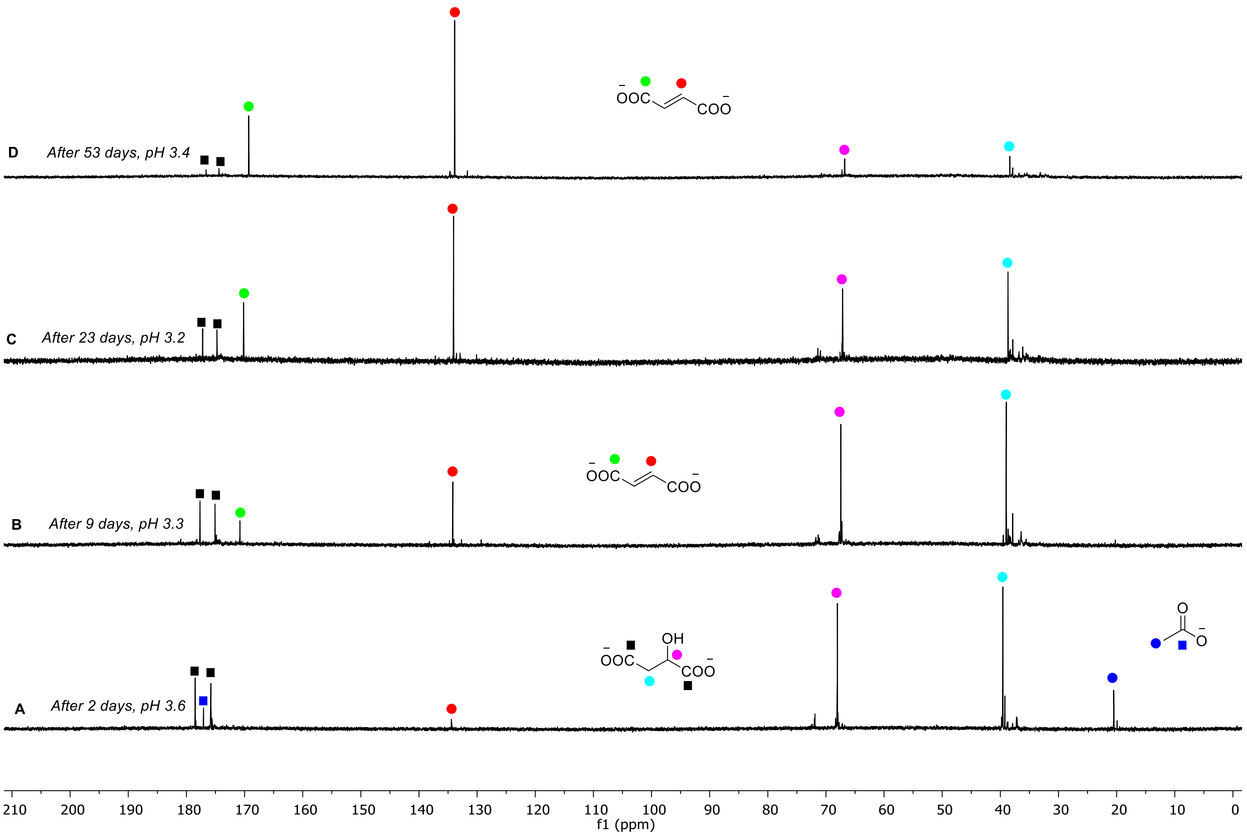
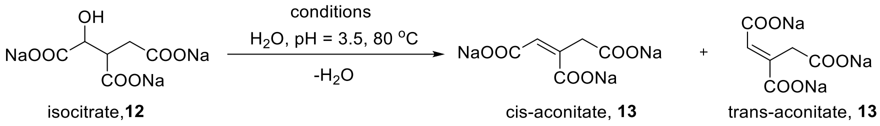


Figure S22: Stacked ^{13}C NMR (D_2O) showing progress of the reaction after 2 days (A), 9 days (B), 23 days (C), and 53 days (D).

Dehydration of isocitrate 12 to aconitate 13 (also includes the results from decarboxylative-dehydration of carboxy-isocitrate to aconitate):



Scheme S7: Dehydration of isocitrate **12** to aconitate **13** (Figures S23-S24).

Table S5: Attempts for the dehydration of isocitrate **12** to aconitate. Identification of end-products (aconitate **13**) was by made by comparison with literature values and authentic samples.

Condition	**Reaction conc.**	**Additive (equiv.)**	**pH**	**Temp. (°C)**	**Time (days)**	**% conversion to 13**[a]
1 [b]	0.25M	NH_4OAc (2)	2.7	80	14	<1%
2 [b]	0.25M	--	1.9	80	14	<1%
3 [b]	0.25M	$Ca(OH)_2$ (2)	13.0	80	9	~3%
4[c]	0.5M	$ZnCl_2$ (1.5)	4.5	80	50	9%
5[d]	0.5M	$ZnCl_2$ (1.5)	4.5	80	36	14%[f]
6[e]	0.1M	$ZnCl_2$ (1.5)	4.5	80	30	5%[f]

[a] % conversion was calculated based on the ^{1}H NMR analysis product vs starting material remaining. All of the reactions were performed in a 4 mL a glass vial starting with 0.25 mmol of isocitric acid in H_2O (1 mL) 80 °C. [b]These reaction were dry heated at 80 °C using the wet dry protocol (same as used for malate dehydration reactions). [c]Reaction was started with carboxy-isocitrate produced from the reaction of carboxy-succinate and glyoxylate. [d]Reaction was started with isocitrate produced from the reaction of carboxy-succinate and glyoxylate. [e]Reaction was started with commercial isocitrate (D/L-isocitrate). [f]Yields are absolute yields (calculated by ^{1}H NMR using an internal standard).

Example procedure (Condition 5): In a 4 mL glass vial, isocitric acid **12** produced from a reaction of carboxy-succinate **9** and glyoxylate **1** (1 mL of 0.5M reaction mixture, 0.5 mmol, 1.0 equiv.) was taken (from scheme S5, Figure S16). To this homogeneous solution $ZnCl_2$ (100 mg, 1.5 equiv.) was added. After addition of $ZnCl_2$, pH of the homogeneous colourless reaction mixture was adjusted to 4.5 with conc. HCl. The reaction mixture was then placed at 80 °C in the closed vial and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR over a period (Figures S23-24).

Condition 5: $ZnCl_2$ (100 mg, 1.5 equiv.), isocitrate 12 (0.5 mmol, 1 equiv.), H_2O 1 mL.

- After 5 min: pH = 4.5, ^{1}H NMR (Figure **S23A**) showed majorly the starting material **12** in open chain and lactone form with small amount of carboxy-succinate **9** and glyoxylate (probably regenerated by retro-aldol reaction).
- After 16 days: ^{1}H NMR (Figure **S23B**) showed the formation of *cis*- and *trans*-aconitate **13** (9% overall conversion w.r.t. isocitrate **12**) (pH = 5.0), pH was readjusted to 4.5.
- After 36 days: ^{1}H NMR (Figure **S23C**) showed formation of *cis*- and *trans*-aconitate **13** in 14% absolute yield (calculated by using an internal standard) (pH = 4.5).
- After 16 days, we observed the formation of succinate **10** in the reaction mixture (Figure **S23B**). This succinate **10** can come either from the decarboxylation of carboxy-succinate **9** which was present in the reaction mixture in a small amount, or it can come from the retro-aldol of isocitrate **12**. After 16 days, NMR data did not show the presence of carboxy-succinate **9**, so we hypothesize that if the formation of succinate **10** increases after 16 days it will come from the retro-aldol of isocitrate **12** only. With this in mind, we continued the reaction mixture under same conditions and monitored the reaction mixture after 36 days. From 16 to 36 days, we observed an increase in the formation of succinate **10**. This confirmed that some retro-aldol of iso-citrate **12** is happening simultaneously (along with dehydration) to give succinate **10**.

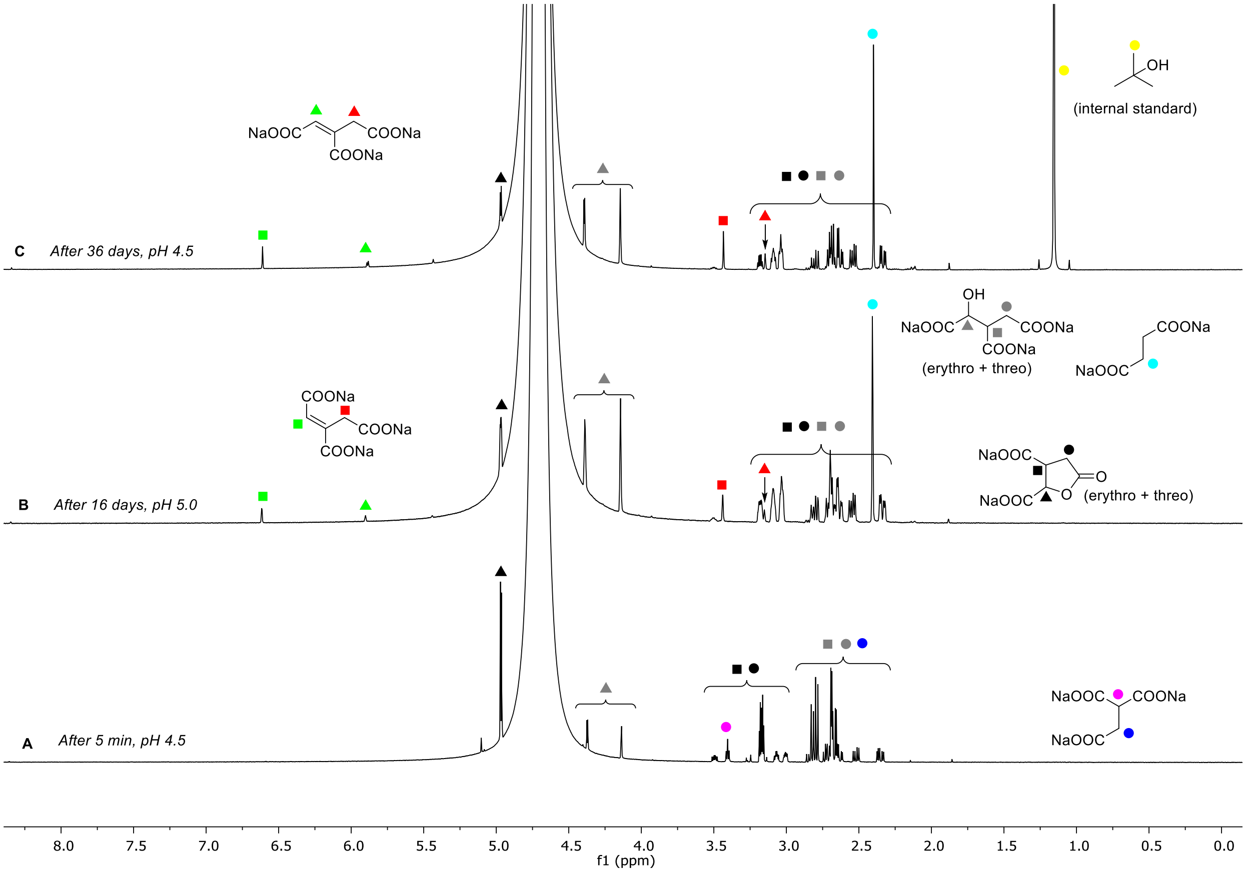


Figure S23: Stacked ^{1}H NMR (D_2O) showing progress of the reaction after 5 min (A), 16 days (B), 36 days (C). % conversion was calculated based on the ^{1}H NMR peaks integration (relative) of products **13** vs starting materials **12** and side products **10**. Absolute yield of aconitate **13** formed was calculated by ^{1}H NMR integral of product **13** w.r.t. an internal standard i.e., t-BuOH. Succinate **10** formation was confirmed by spiking experiment with authentic succinate **10**.

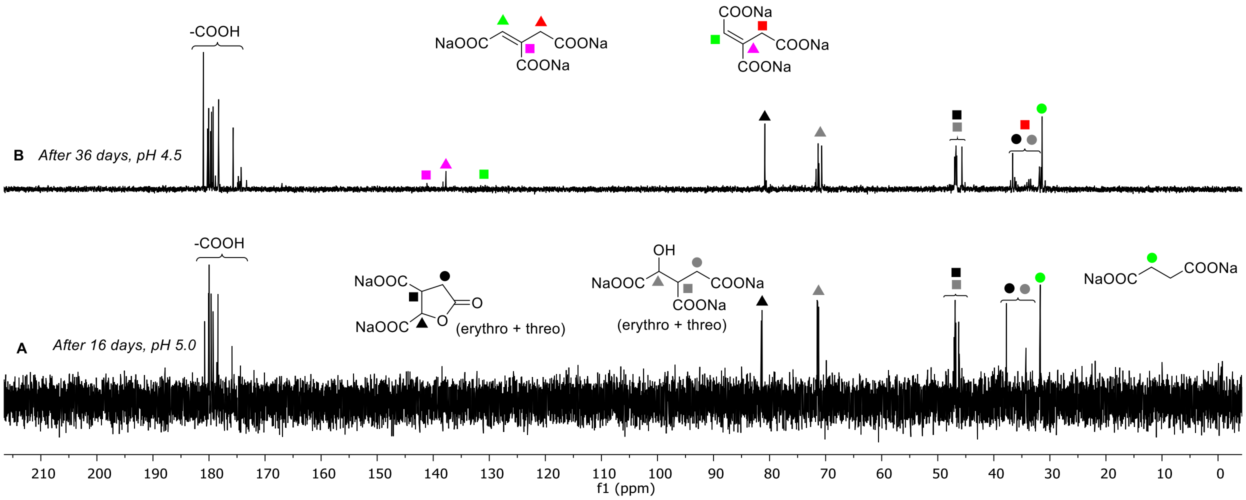


Figure S24: Stacked ^{13}C NMR (D_2O) showing progress of the reaction after 16 days (A), 36 days (B).

<u>Condition 6:</u> ZnCl$_2$ (21 mg, 1.5 equiv.), commercial D/L-threo-isocitrate trisodium salt 12 (28 mg, 0.1 mmol, 1 equiv.), H$_2$O 1 mL.

- <u>After 5 min:</u> pH = 4.5, ^{1}H NMR (Figure **S25A**) showed only the starting material **12** in open chain.
- <u>After 16 days:</u> pH = 4.6, ^{1}H NMR (Figure **S25B**) showed formation of aconitate **13**.
- <u>After 52 days:</u> ^{1}H NMR (Figure **S25C**) showed formation of *cis*- and *trans*-aconitate **13** in 7% absolute yield (calculated by using an internal standard) (pH = 4.7).
- After 16 days, we observed a little formation of succinate **10** in the reaction mixture (Figure **S25B**). This succinate **10** can come only from the retro-aldol of isocitrate **12**. With time, we observed an increase in the formation of succinate **10**. This confirmed that some retro-aldol of iso-citrate **12** is happening simultaneously (along with dehydration) to give succinate **10**.
- We started this reaction with commercially available *threo* D/L-isocitrate **12** (Figure **S25A**). However, the NMR after 16 days showed the presence of other diastereomer (erythro) also. This kind of isomerization is only possible from the retroaldol-aldol or dehydration-hydration reactions, which is supported by the formation of aconitate **13** and succinate **10** in the reaction mixture.

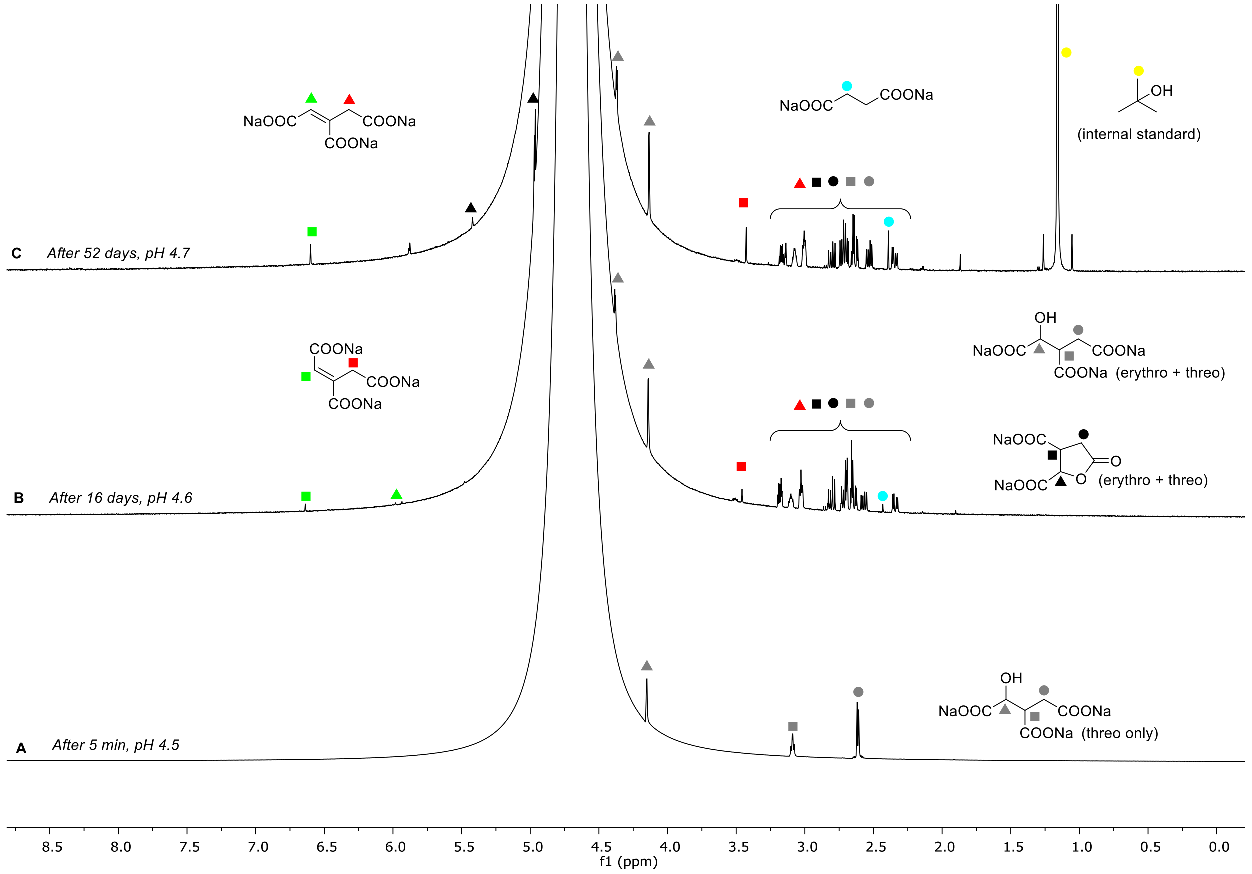
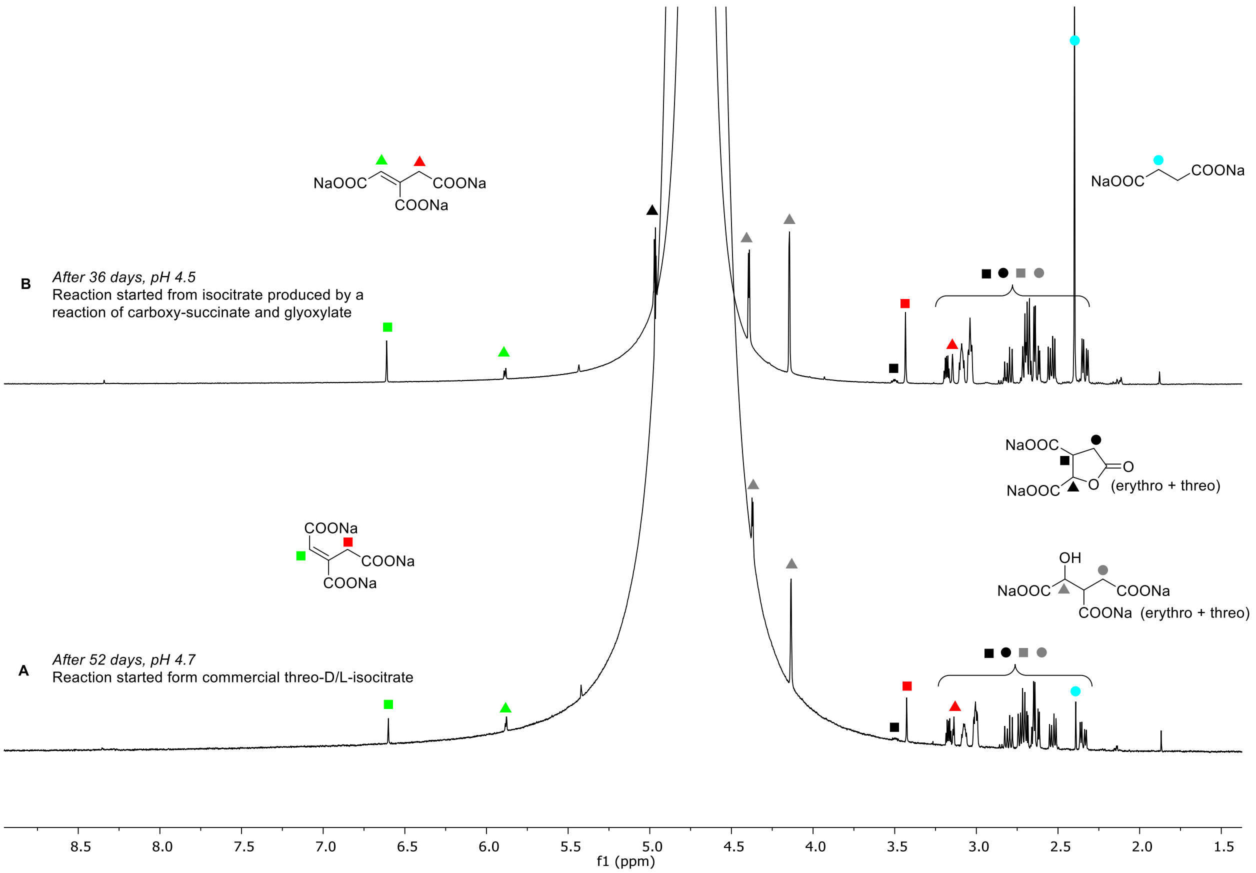


Figure S25: Stacked ^{1}H NMR (H$_2$O-D$_2$O) showing progress of the reaction after 5 min (A), 16 days (B), and 52 days (C). Absolute yield of aconitate **13** formed was calculated by ^{1}H NMR integral of product **13** w.r.t. an internal standard i.e., t-BuOH. Succinate **10** formation was confirmed by spiking experiment with authentic succinate **10**.


B After 36 days, pH 4.5
Reaction started from isocitrate produced by a reaction of carboxy-succinate and glyoxylate

A After 52 days, pH 4.7
Reaction started form commercial threo-D/L-isocitrate

Figure S26: ^{1}H NMR (H_2O-D_2O) showing the similarly of the reactions starting from the commercial D/L-isocitrate **12** (A) and isocitrate **12** (*threo*, *erythro* mixture with corresponding lactones) produced from the reaction of carboxy-succinate **9** and glyoxylate **1** (B).

Interestingly, the reactions starting from the commercial *threo*-D/L-isocitrate **12** and synthetic isocitrate **12** (*threo*, *erythro* mixture with corresponding lactones) produced from the reaction of carboxy-succinate **9** and glyoxylate **1** produced similar kind of products after heating at 80 °C in the presence of $ZnCl_2$ (Figure S26). These results clearly shows that the isomerization of isocitrate **12** diastereomers is happening under the experimental conditions, and this type of isomerization is indicative of dehydration-hydration and retroaldol-aldol reactions. However, the formation of aconitate **13** and succinate **10** was observed more when we started from the synthesized isocitrate **12** (from the reaction of carboxy-succinate **9** and glyoxylate) in comparison to when we started from the commercial isocitrate **12**. This could be possible because of the formation of favourable conformations (for dehydration and retro-aldol reactions) when we started from the diastereomeric mixture of isocitrate **12** (from the reaction of carboxy-succinate **9** and glyoxylate).

While we did observe the retro-aldol reaction of isocitrate to succinate, however, glyoxylate was not detected under these conditions and it suggested a competing (Cannizzaro) reaction. To test this, we performed a control reaction starting from succinate and glyoxylate in the presence of zinc, which showed the formation of glycolate (via Cannizzaro reaction). We did not see any peaks related to oxalate (another product of Cannizzaro reaction) because that can make zinc oxalate which is highly insoluble in H_2O, and we observed lot of precipitation formation in the reaction mixture (see figures S85-86 for more details).

Experimental procedure (Condition 4): In a 4 mL glass vial, carboxy-isocitric acid **11** (with lactone **11a**) produced from a reaction mixture of carboxy-succinate **9** and glyoxylate **1** (0.25 mmol, 1.0 equiv.) in deionized and degassed water (1 mL). To this homogeneous solution $ZnCl_2$ (50 mg, 1.5 equiv.) was added. After addition of $ZnCl_2$, pH of the homogeneous colourless reaction mixture was adjusted to 4.5 with conc. HCl. The reaction mixture was then placed at 80 °C in the closed vial and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR over a period (Figure S27).

Condition 4: ***$ZnCl_2$ (50 mg, 1.5 equiv.), carboxy-isocitrate 12 (0.25 mmol, 1 equiv.), H_2O (1 mL).***

- After 5 min: pH = 4.5, ^{1}H NMR (Figure **S27A**) showed majorly the starting material **12** in open chain and lactone form with small amount of carboxy-succinate **9** and glyoxylate (probably regenerated by retro-aldol reaction).
- After 5 days: ^{1}H NMR (Figure **S27B**) showed majorly isocitrate **12** diastereomers and corresponding lactones with traces of aconitate **13** (pH = 4.8).
- After 21 days: ^{1}H NMR (Figure **S27C**) showed increased amount of dehydrated product **13** (pH = 5.0). pH was readjusted to 4.5.
- After 53 days: ^{1}H NMR (Figure **S27D**) showed formation of *cis-* and *trans*-aconitate **13** in 9% overall conversion w.r.t. carboxy-isocitrate **11** and **11a** (pH = 5.0).

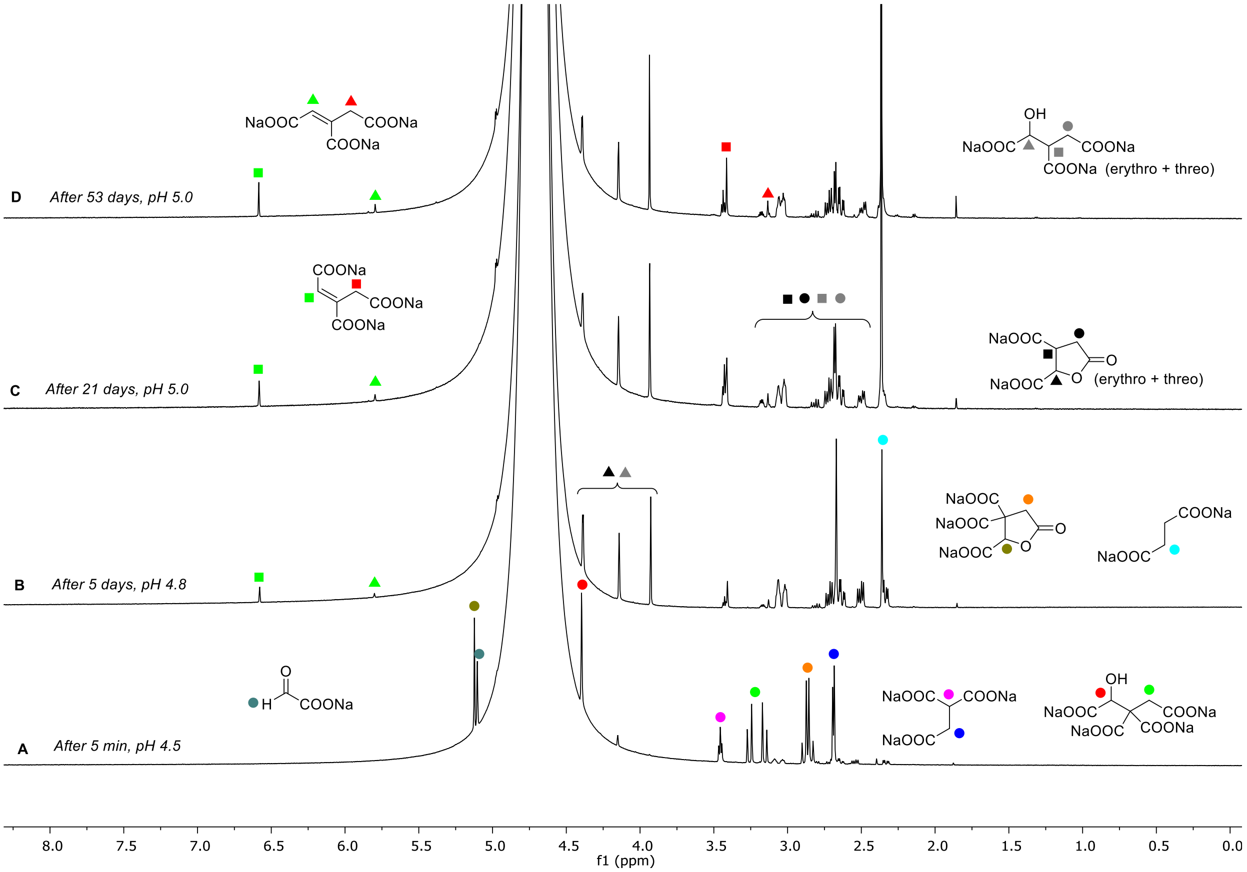
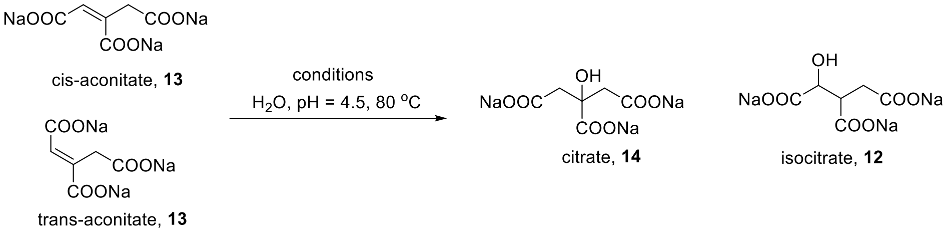


Figure S27: Stacked ^{1}H NMR (D_2O) showing progress of the reaction after 5 min (A), 5 days (B), 21 days (C), and 53 days (D). % conversion was calculated based on the ^{1}H NMR peaks integration of products **13** vs starting materials and side products.

Hydration of aconitate 13 to citrate 14 (and isocitrate 12):



Scheme S8: Hydration of aconitate **13** to citrate **14** (Figures S28-29).

Table S6: Attempts for the hydration of aconitate **13** to citrate **14**. Identification of end-products (citrate **14**/isocitrate **12**) was made by comparison with literature values and authentic samples.[a]

Condition	**Reaction conc.**	**Additive (equiv.)**	**pH**	**Temp. (°C)**	**Time (days)**	**% conversion to 12+14**
1	0.5M	$Ca(OH)_2$ (2.0)	4.2	80	5	<1%
2	0.5M	$MgCl_2$(2.0)	4.0	80	5	<1%
3	0.5M	$ZnCl_2$ (1.5)	4.0	80	5	~3%
4	0.5M	$ZnCl_2$ (1.5)	5.0	80	5	~5% (14 major)
5	0.5M	$ZnCl_2$ (1.5)	5.0	80	78	55% (12 major)
6[b]	0.5M	$ZnCl_2$ (1.5)	3.0	80	5	~2%
7[b]	0.5M	$ZnCl_2$ (1.5)	7.0	80	5	<1%
8[b]	0.5M	$ZnCl_2$ (1.5)	9.0	80	5	<1%

[a] % conversion was calculated based on the ^{1}H NMR analysis product vs starting material remaining. All the reaction were performed in a 4 mL a glass vial starting with 0.5 mmol of aconitate in H_2O (1 mL) 80 °C. [b]These reactions were monitored for 2 weeks; however, didn't show good formation of citrate/isocitrate (hence stopped monitoring after 2 weeks).

Example procedure (Condition 4-5): In a 4 mL glass vial, *trans*-aconitic acid **13** (0.5 mmol, 1.0 equiv.) was dissolved in deionized and degassed water (1 mL). To this homogeneous solution $ZnCl_2$ (100 mg, 1.5 equiv.) was added. After addition of $ZnCl_2$, pH of the homogeneous colourless reaction mixture was adjusted to 4.5 with conc. HCl. The reaction mixture was then placed at 80 °C in the closed vial and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR over a period (Figures S28-29).

Condition 4: $ZnCl_2$ (100 mg, 1.5 equiv.), trans-aconitate 13 (87 mg, 0.5 mmol, 1 equiv.), H_2O 1 mL.

- After 16 h: pH = 4.5, ^{1}H NMR (Figure **S28A**) showed only some isomerization of *trans*-aconitate to *cis*-aconitate.
- After 7 days: pH = 4.8, ^{1}H NMR (Figure **S28B**) showed a little formation of citrate **14**.
- After 21 days: pH = 5.2, ^{1}H NMR (Figure **S28C**) showed no increase in the formation of citrate **14**, however, isocitrate formation **12** was observed. pH of the reaction mixture was readjusted to 4.8.
- After 58 days: pH = 5.0, ^{1}H NMR (Figure **S28D**) showed good amount of isocitrate **12**.
- After 78 days: ^{1}H NMR (Figure **S28E**) showed an increase in the formation of isocitrate **12** (55% conversion w.r.t. aconitate **13**) (pH = 5.0).

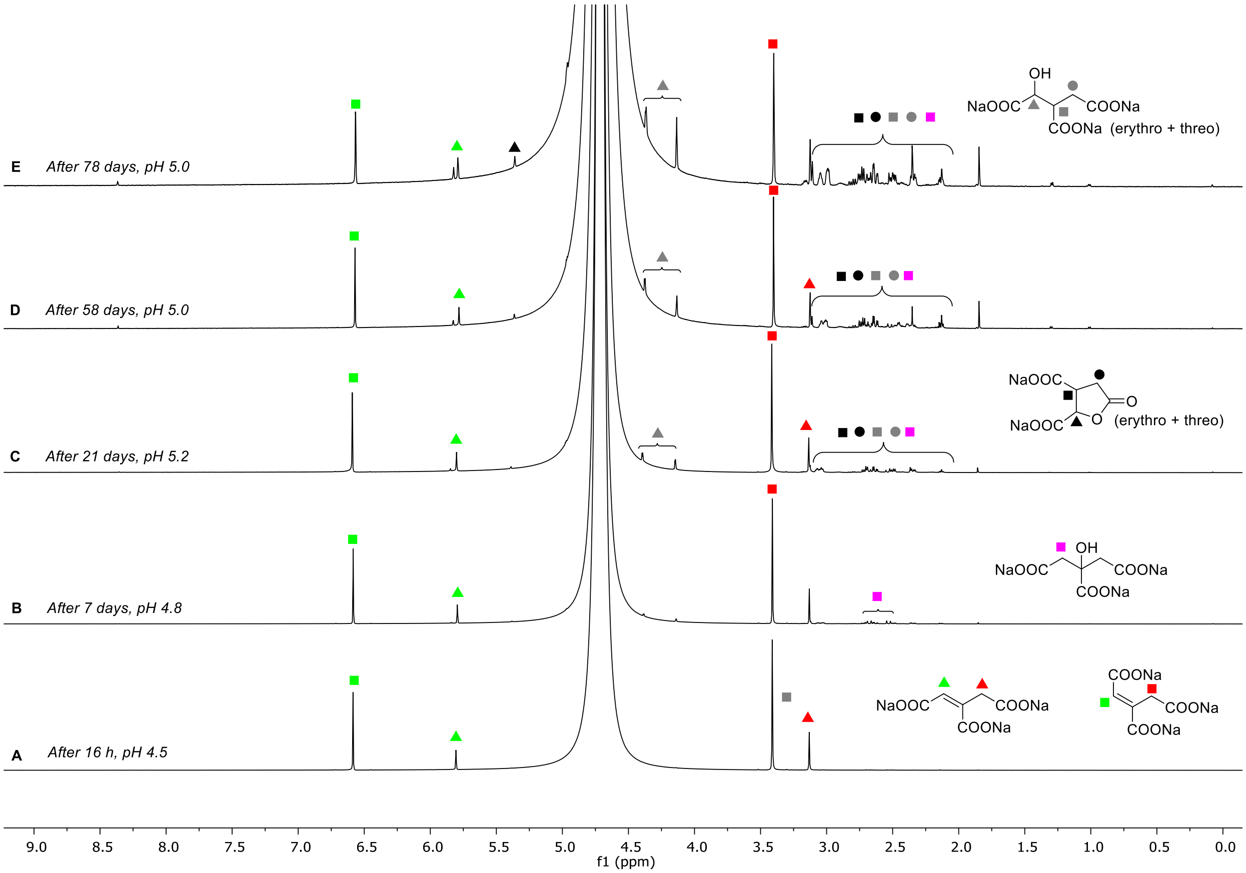


Figure S28: Stacked ^{1}H NMR (D_2O) showing progress of the reaction after 16 h (A), 7 days (B), 21 days (C), 58 days (D), and 78 days (E). % conversion was calculated based on the ^{1}H NMR peaks integration (relative) of products **12** vs starting materials and side products.

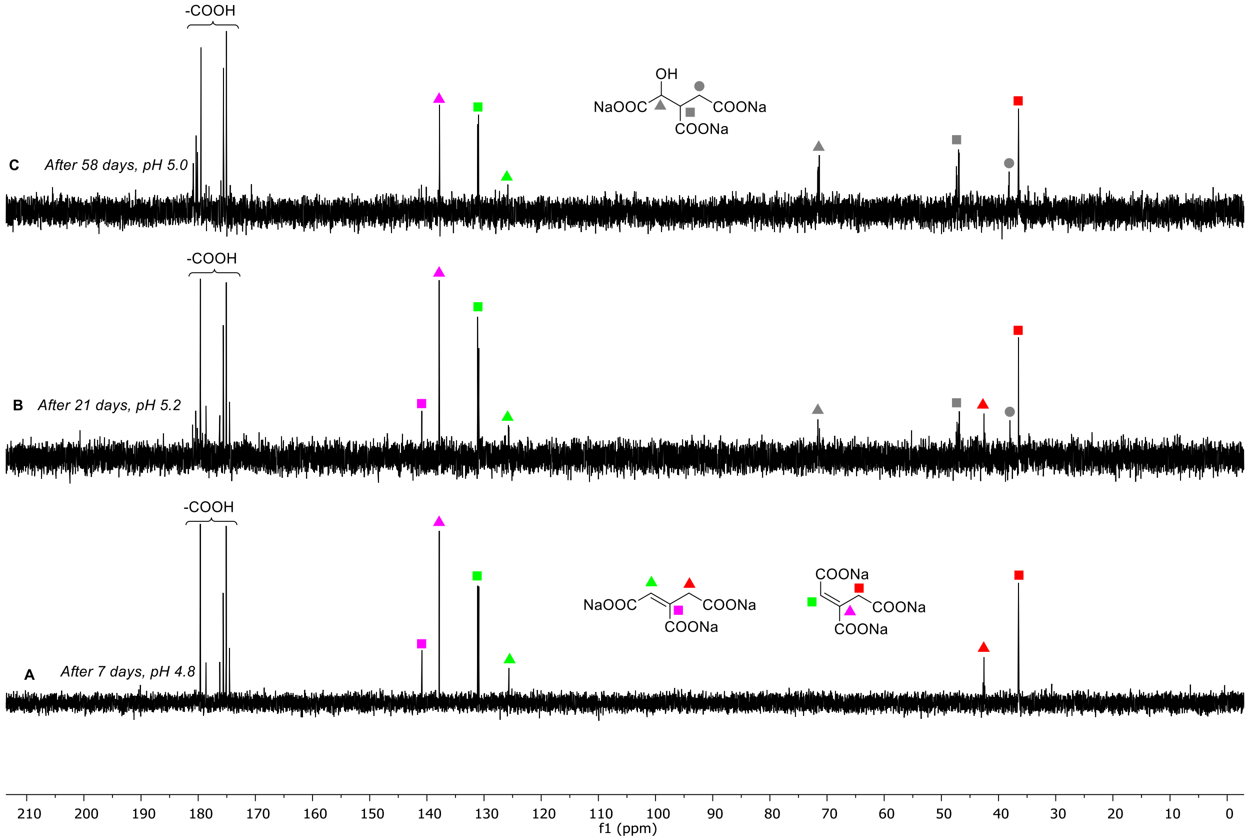
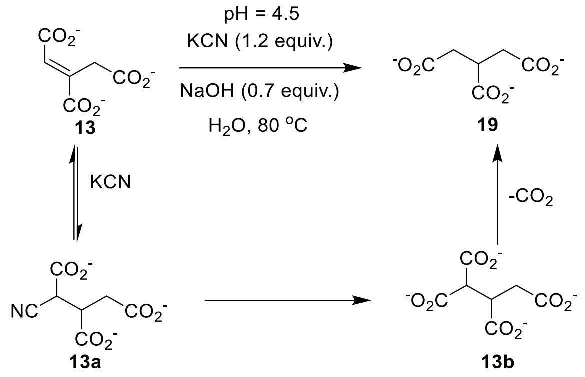


Figure S29: Stacked ^{13}C NMR (D_2O) showing progress of the reaction after 7 days (A), 21 days (B), and 58 days (C).

Reaction of *trans*-aconitate 13 with potassium cyanide (KCN):



Scheme S9: Reaction of *trans*-aconitate **13** with KCN to tricarballylic acid **19** (Figure S30).

Reaction procedure: In a 4.0 mL glass vial equipped with a magnetic stir bar, *trans*-aconitic acid **13** (87 mg, 0.5 mmol) was dissolved in deionized and degassed water (1.0 mL, 0.5 M). To this homogeneous solution, NaOH (14 mg, 0.35 mmol, 0.7 eq) was added and stirred at room temperature for 10 min and then KCN (39 mg, 0.6 mmol, 1.2 equiv.) was added carefully in a fume hood. pH of the resulting reaction mixture was adjusted to pH 4.5 with 5M HCl solution. The reaction mixture was stirred at 80 °C for 6 days and the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR (NMR method 1 in H_2O-D_2O) (% conversion of *trans*-aconitate to tricarballylic acid was found to be 16%) (Figure S30).

***Reaction conditions:* trans-aconitate 13 (87 mg, 0.5 mmol), potassium cyanide (39 mg, 0.6 mmol, 0.7 equiv.), and H_2O (1.0 mL).**

- After 18 days: pH = 4.5, ^{1}H NMR (Figure **S30**) showed the formation of tricarballylic acid **19**. % conversion to tricarballylic acid **19** was found to be 16%.

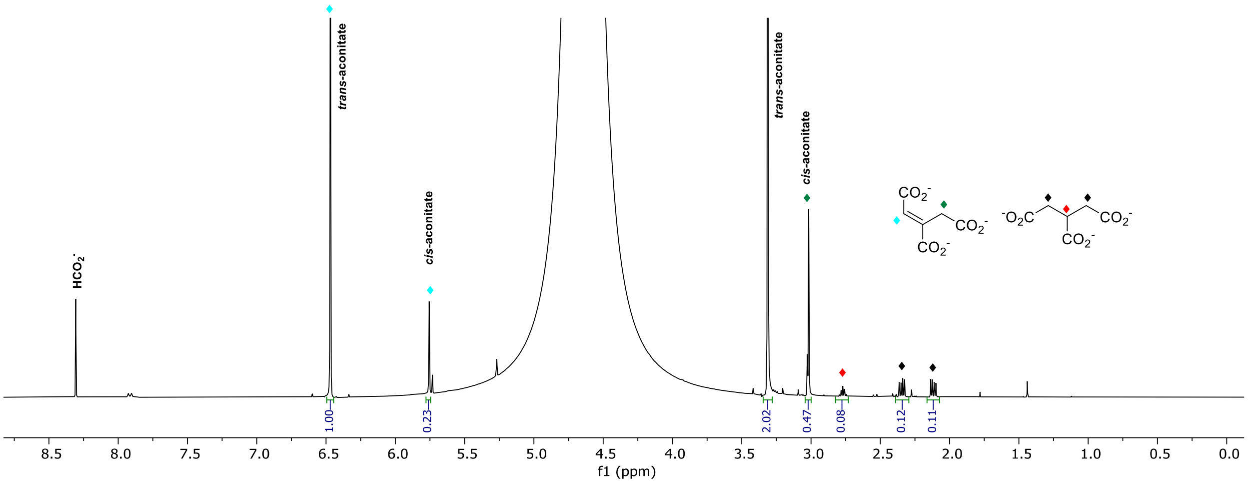
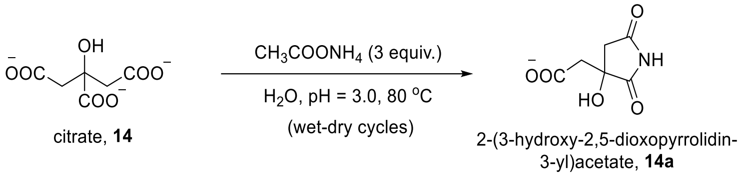


Figure S30: ^{1}H NMR of the reaction mixture aliquots (NMR method 1 in H_2O-D_2O) after 18 days at pH = 4.5. % Conversion yields were calculated based upon the ^{1}H NMR peaks integration of product (tricarballylic acid, **19**) i.e., peaks (~ 2.8 ppm) referred as ♦ w.r.t. *trans*-aconitate, **13** peak (~ 6.5 ppm) referred as ♦. (% conversion of *trans*-aconitate to tricarballylic acid is 16%.

Reaction of citrate 14 with ammonium acetate:

We explored a reaction of citric acid **14** in the presence of ammonium acetate to see if citrate can be dehydrated to aconitate **13**. We didn't observe aconitate formation under these conditions; however, an another compound was formed (quantitative conversion) which was characterized as 2-(3-hydroxy-2,5-dioxopyrrolidin-3-yl)acetic acid (Scheme S10). Formation of 2-(3-hydroxy-2,5-dioxopyrrolidin-3-yl)acetic acid **14a** was confirmed by NMR and mass spectroscopic techniques.



Scheme S10: Reaction of citrate **14** in the presence of different ammonium acetate (Figures S31-32).

Reaction procedure: In a 4 mL glass vial, citric acid **14** (192 mg, 1 mmol, 1.0 equiv.) was dissolved in deionized and degassed water (2 mL). To this homogeneous solution ammonium acetate (231 mg, 3.0 equiv.) was added. After addition of ammonium acetate, pH of the homogeneous colourless reaction mixture was adjusted to 2.8 with conc. HCl or 5M NaOH. The reaction mixture was then placed at 80 ^{o}C (without cap) and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR over a period (Figures 31-32). To record the NMR of the reaction mixture, the reaction mixture was dissolved in 2.0 mL of milliQ water and then a fraction of reaction mixture (550 uL) was taken in an NMR tube (with D_2O in capillary). After recording the NMR, the sample was again transferred to the reaction vial and heated again for the next cycle. One cycle comprises of 24 h. The days when NMR was not recorded, 2 mL of H_2O was added to the reaction mixture and heating was continued for the next cycle.

***Reaction Condition:* ammonium acetate (231 mg, 3.0 equiv.), citrate (192 mg, 0.5M, 1 equiv.), H_2O (2 mL).**

- After 10 min: ^{1}H NMR (Figure **S31A**) showed majorly citric acid **14** peaks only along with acetate peak (pH = 2.8).
- After 7 days: ^{1}H NMR (Figure **S31B**) showed appearance of peaks corresponding to the formation of 2-(3-hydroxy-2,5-dioxopyrrolidin-3-yl)acetic acid **14a** besides citric acid **14** peaks (pH = 2.9).
- After 15 days: ^{1}H NMR (Figure **S31C**) showed quantitative conversion of citric acid to 2-(3-hydroxy-2,5-dioxopyrrolidin-3-yl)acetic acid **14a** (pH = 3.0). ESI-MS m/z calculated for **14a** $C_6H_7NO_5$ $[M-H]^-$ 172.03, found 172.06.

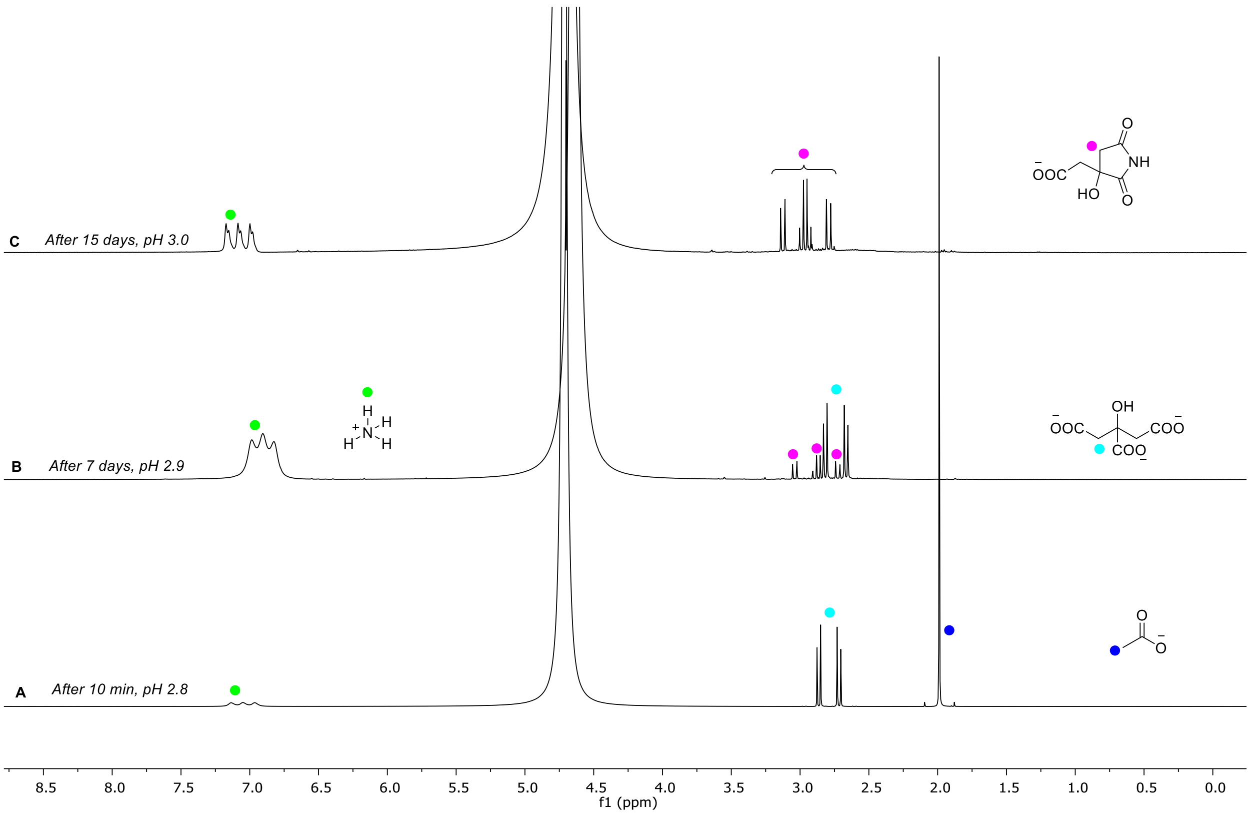


Figure S31: Stacked ^{1}H NMR (H_2O-D_2O) showing progress of the reaction after 10 min (A), 7 days (B), and 15 days (C).

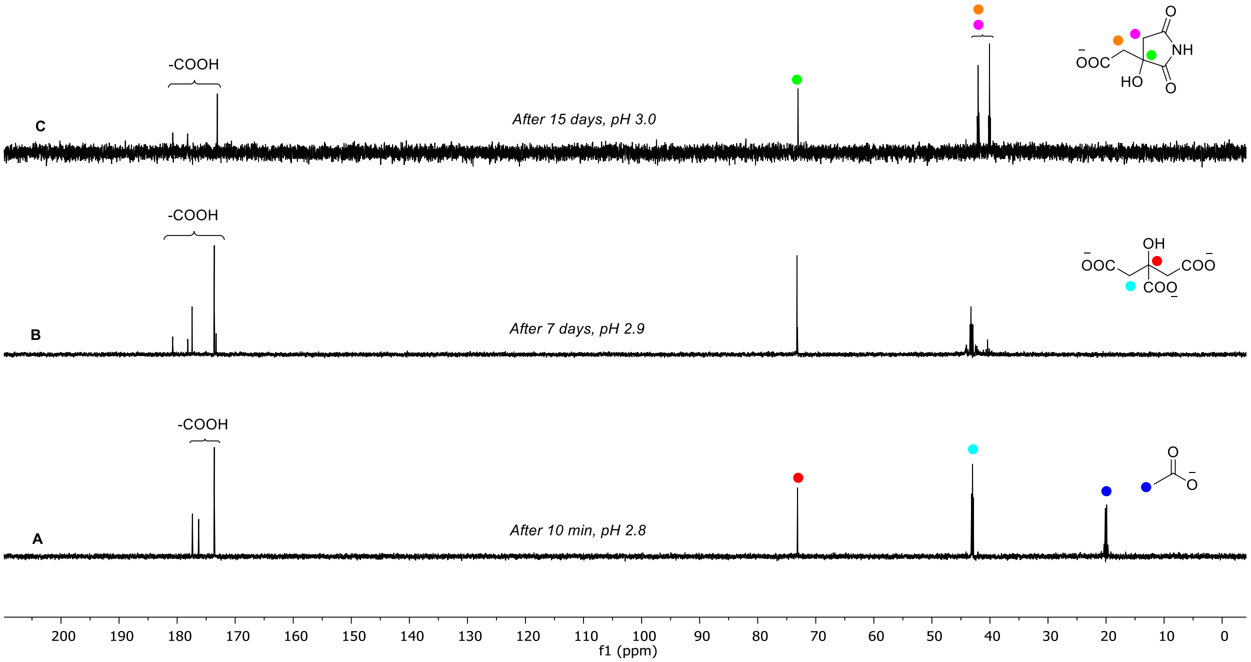
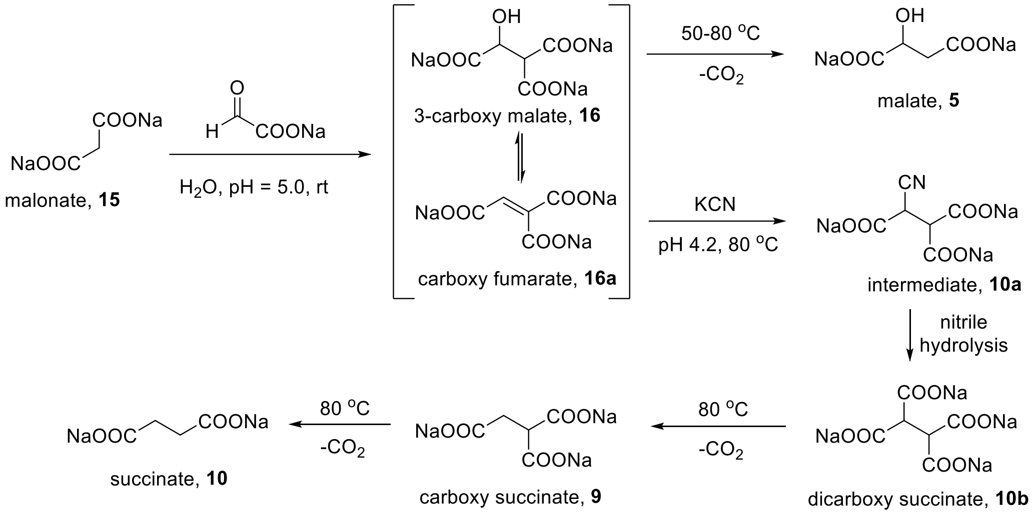


Figure S32: Stacked ^{13}C NMR (H_2O-D_2O) showing progress of the reaction after 10 min (A), 7 days (B), and 15 days (C).

Reaction of malonate 15 with glyoxylate 1 showing the formation of malate and succinate 10 via decarboxylative and cyanide mediated reductive pathways:



Scheme S11: Reaction of malonate **15** with glyoxylate in H_2O showing the formation of malate **5** and succinate **10** via decarboxylative and cyanide mediated reductive pathways (Figures S33-S36).

Reaction procedure for 3-carboxy malate and carboxy fumarate synthesis (step I): In a 20 mL glass vial equipped with a magnetic stir bar, malonic acid **15** (208 mg, 2 mmol, 1.0 equiv.) was dissolved in deionized and degassed water (3 mL). To this homogeneous solution glyoxylic acid monohydrate **1** (184 mg, 1.0 equiv.) was added. After addition of glyoxylic acid, pH of the homogeneous colourless reaction mixture was adjusted to 5.0 with 5M NaOH (~ 1 mL). The reaction mixture was stirred at 50 °C under argon atmosphere and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR over a period (Figures 33-34). After 24h, ^{1}H NMR analysis of the reaction mixture showed the formation of carboxy malate **16** and carboxy fumarate **16a** (almost 50:50). This reaction mixture was directly used for the other reactions (malate and succinate formation).

Reaction procedure for the formation of malate (step IIa): 1 mL of the above reaction mixture taken in a 4 mL glass vial equipped with a stir bar and $ZnCl_2$ (100 mg, 1.5 equiv) was added to it. pH of the reaction mixture was with kept unadjusted (3.5) and the reaction mixture was stirred at 50-80 °C. Progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR over a period (Figure 35).

Reaction procedure for the formation of succinate (step IIb): 1 mL of the above reaction mixture (0.5M, containing mixture of 3-carboxy malate and carboxy fumarate) taken in a 4 mL glass vial equipped with a stir bar and KCN (40 mg, 1.2 equiv) was added to it. pH of the reaction mixture was with kept adjusted to 4.2 (with 5M HCl) and the reaction mixture was stirred at 80 °C. Progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR over a period (Figure 36).

Reaction Conditions (Step I): malonate 15 (208 mg, 1.0 equiv.), glyoxylic acid monohydrate (184 mg, 0.5M, 1 equiv.), H_2O (4 mL).

- After 10 min: pH = 5.0, ^{1}H NMR (Figure **S33A**) showed the starting materials along with the formation of traces of carboxy malate.
- After 24h: ^{1}H NMR (Figure **S33B**) showed formation of carboxy malate **16** and carboxy fumarate **16a** (almost 1:1) as the major product with a little amount of starting material remaining (pH = 5.2).
- After 24h, the reaction mixture was cooled and this reaction mixture was directly used for next reactions (step IIa, and IIb).

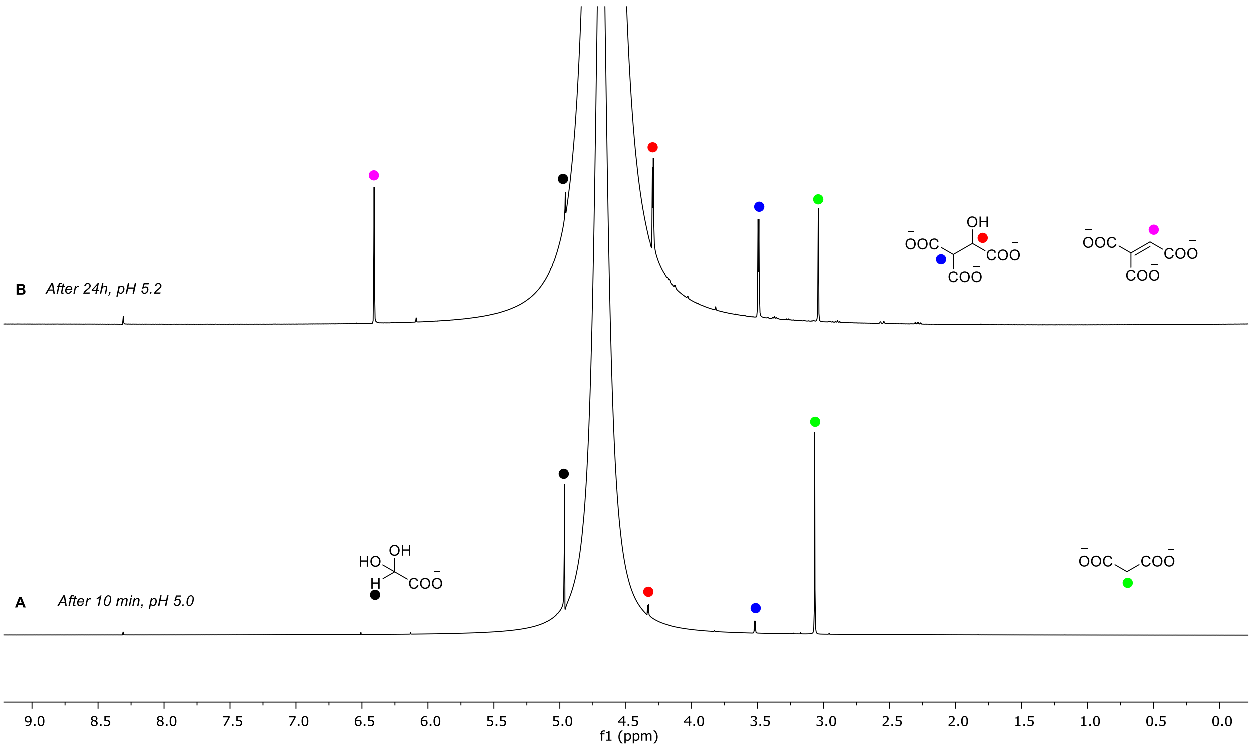


Figure S33: Stacked ^{1}H NMR (H_2O-D_2O) showing progress of the reaction after 10 minutes (A), and 24h (B).

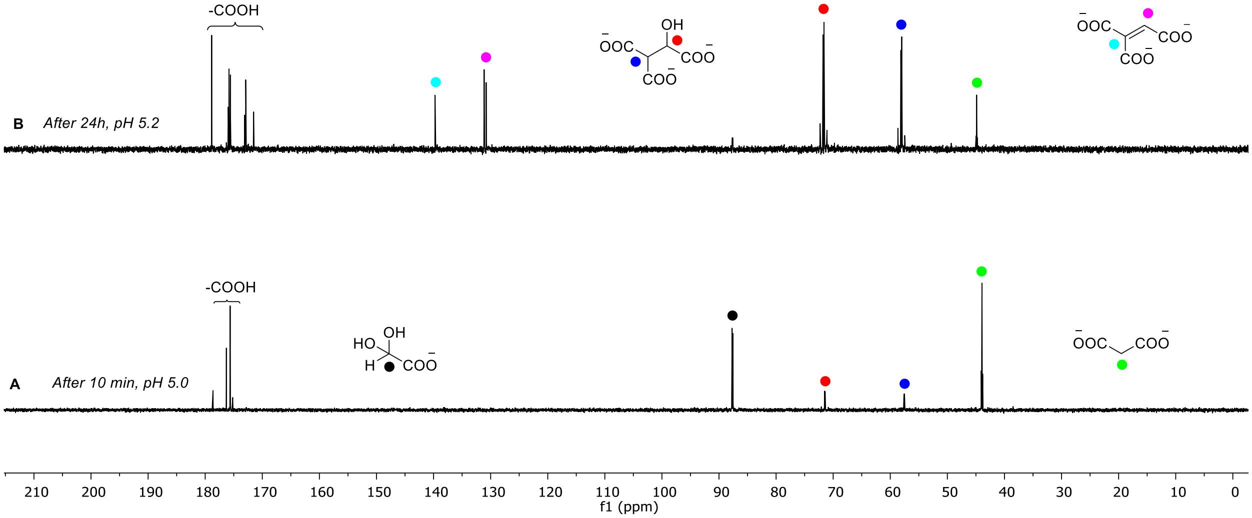


Figure S34: Stacked ^{13}C NMR (H_2O-D_2O) showing progress of the reaction after 10 minutes (A), and 24h (B).

Reaction Conditions (Step IIa): carboxy malate 16 from step I (0.5 mmol, 1 mL, 1.0 equiv.), $ZnCl_2$ (100 mg, 1.5 equiv.).

- After 5 min: pH = 3.5, ^{1}H NMR (Figure **S35A**) showed carboxy malate **16**, carboxy fumarate **16a** and malonate **15** peaks.
- After 24h: ^{1}H NMR showed formation of malate **5**, Figure **S35B** (pH = 3.6). Heating at 50 °C was continued for 8 days.
- After 8 days: Temperature of the reaction mixture was raised to 80 °C (to increase the rate of decarboxylation reaction), pH = 3.8.
- After 15 days: ^{1}H NMR showed malate **5** and malonate **15** (produced through retro-aldol) only, Figure **S35C** (pH = 5.2). Absolute yield of malate formed was found to be 18% (calculated by using internal standard in ^{1}H NMR experiment).

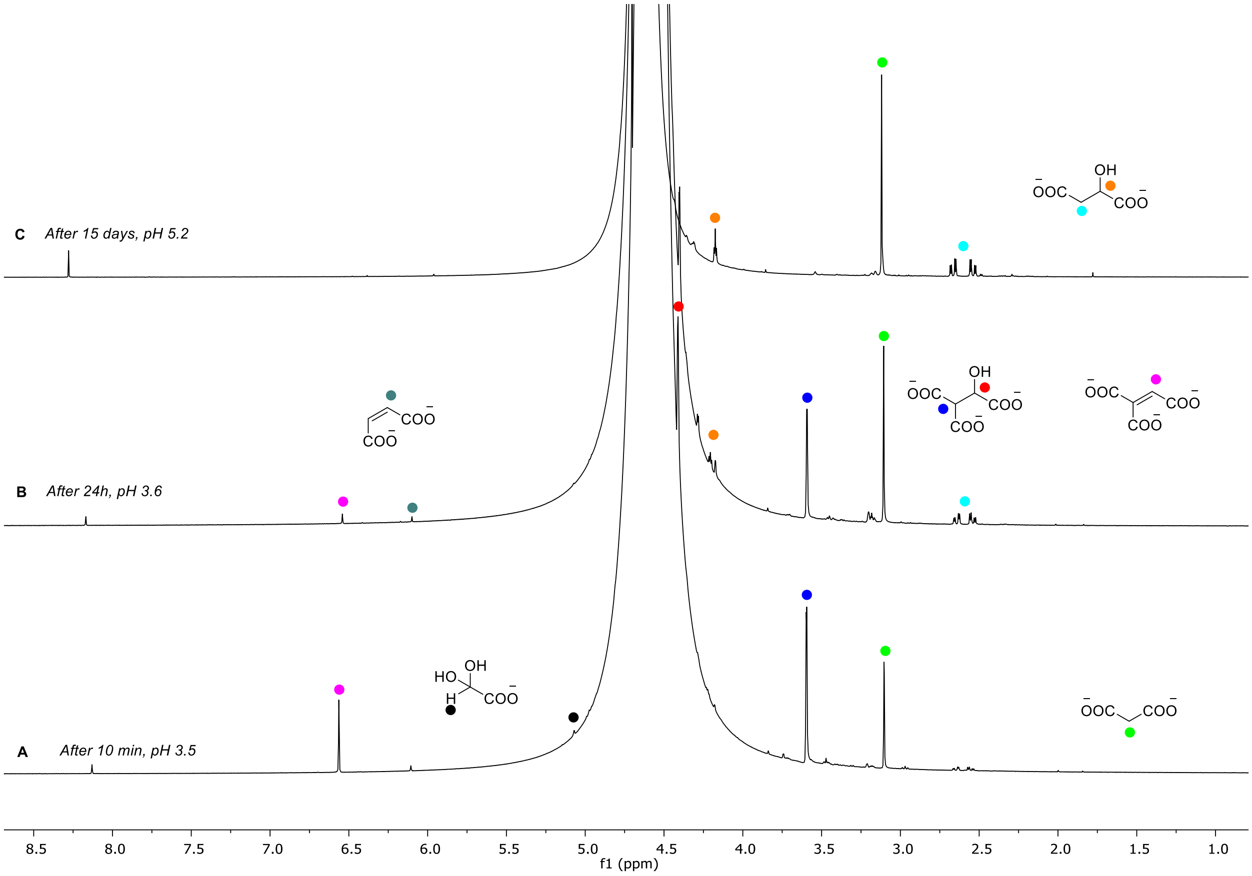


Figure S35: Stacked ^{1}H NMR (H_2O-D_2O) showing progress of the reaction after 10 minutes (A), and 24h (B), and 15 days (C).

<u>Reaction Conditions (Step IIb: </u> carboxy fumarate 16a from step I (0.5 mmol, 1 mL, 1.0 equiv.), KCN (40 mg, 1.2 equiv.).

- <u>After 5 min:</u> pH = 4.2, ^{1}H NMR (Figure **S36A**) showed carboxy malate **16**, carboxy fumarate **16a** and malonate **15** peaks.
- <u>After 24h:</u> ^{1}H NMR showed formation of succinate **10** along with several intermediates (nitriles, amides and carboxylates), Figure **S36B** (pH = 7.0). pH of the reaction mixture was again adjusted to 4.5 with 5M HCl.
- <u>After 60h:</u> ^{1}H NMR showed succinate **10** as the major product formed in the reaction mixture with some malonate **15** (produced through retro-aldol), Figure **S36C** (pH = 5.2). Absolute yield of succinate **10** formed was found to be 40% (calculated by using internal standard in ^{1}H NMR experiment).

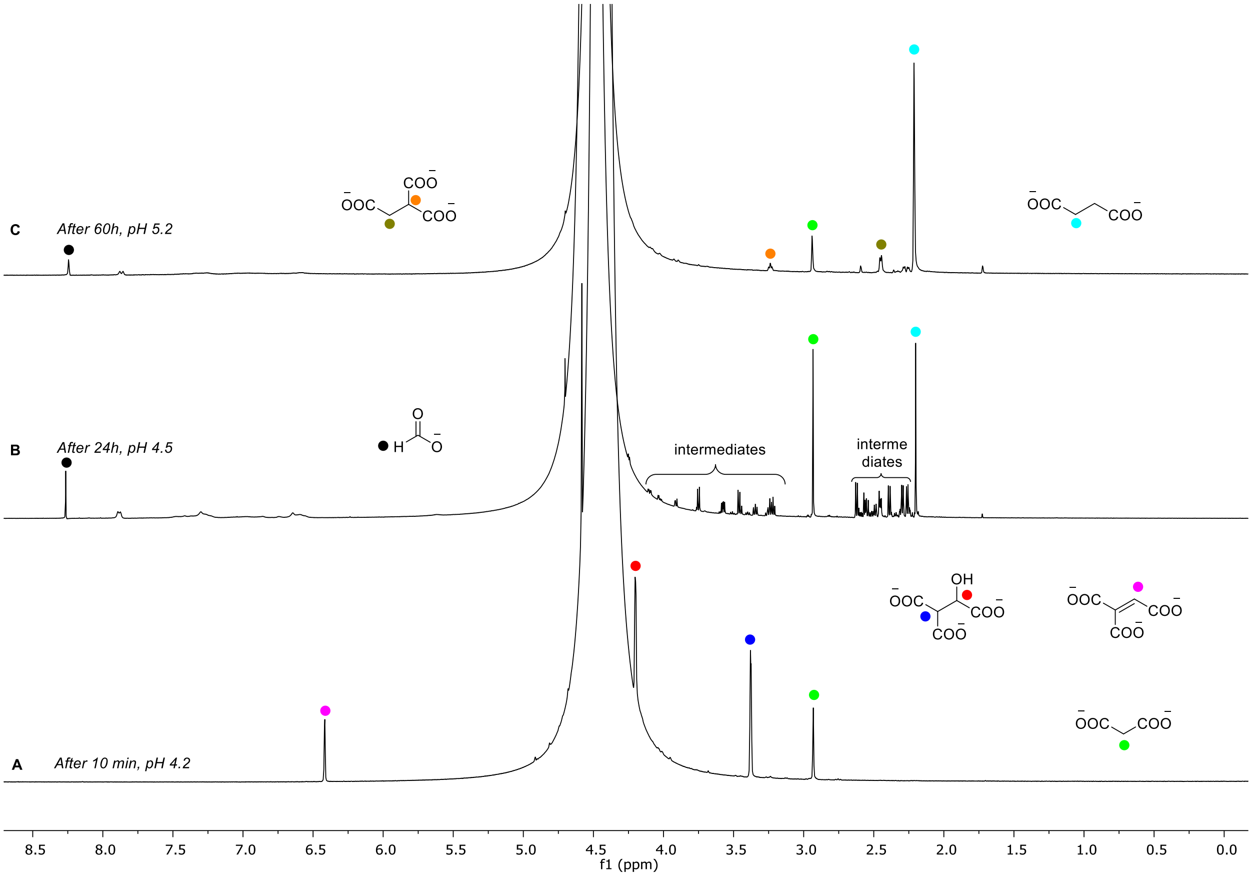
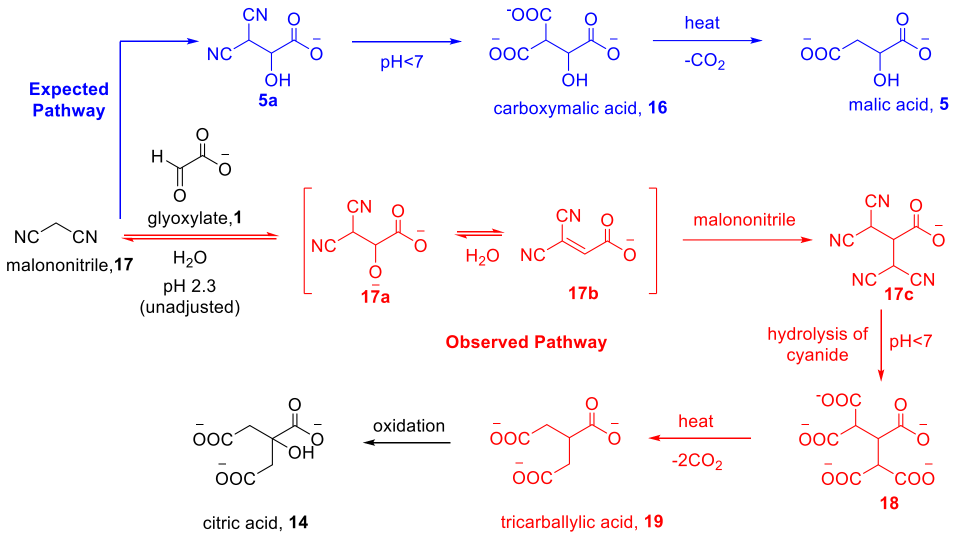


Figure S36: Stacked ^{1}H NMR (H_2O-D_2O) showing progress of the reaction after 10 minutes (A), and 24h (B), and 60h (C).

Reaction of malononitrile 17 with glyoxylate:



Scheme S12: Expected and observed pathways from a reaction of malononitrile **17** with glyoxylate **1** in H_2O (Figures S37-39).

Reaction procedure: In a 20 mL glass vial equipped with a magnetic stir bar, malononitrile **17** (132.0 mg, 2 mmol, 2.0 equiv.) was dissolved in deionized and degassed water (4.0 mL). To this homogeneous solution glyoxylic acid monohydrate **1** (92 mg, 1.0 equiv.) was added. After addition of glyoxylic acid, pH of the homogeneous colourless reaction mixture was kept unadjusted (~2.2). The reaction mixture was stirred at room temperature and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR (H_2O-D_2O) over a period (Figures S37-38). After formation of pentacarboxylic acid **18** (8 days) as the major product, temperature of the reaction mixture was raised to 80 °C (24 h) for the final decarboxylation to form tricarballylic acid **19**.

When a similar reaction was performed with 1:1 equivalent of malononitrile and glyoxylate, 0.5 equiv. of glyoxylate was consumed to form the pentacarboxylic acid product **18** and another half equiv. remained unreacted in the reaction mixture. For more details see figure S39.

We also confirmed the formation of the product **18** by using ^{13}C(2)-labelled glyoxylate.

Reaction Conditions: ***malononitrile 17 (132 mg, 2.0 equiv.), glyoxylic acid monohydrate (92 mg, 1 equiv.), H_2O (4 mL).***

- After 5 min: pH = 2.2, ^{1}H NMR (Figure **S37A**) showed majorly the starting materials.
- After 24 h: ^{1}H NMR (Figure **S37B**) showed the formation of various intermediates via hydrolysis of nitriles as ammonia formation was observed in the NMR spectrum (pH = 3.3).
- After 8 days: ^{1}H NMR (Figure **S37C**) showed the formation of pentacarboxylic acid **18** as the major product (pH = 4.8).
- After 8 days: The reaction mixture was heated at 80 °C (to facilitate decarboxylation) for 24h.
- After 9 days: ^{1}H NMR (Figure **S37D**) showed tricarballylic acid **19** as the major product (pH = 6.8). Absolute yield of the tricarballylic acid **19** formed was found to be 33% (calculated by ^{1}H NMR with an internal standard i.e., t-BuOH).

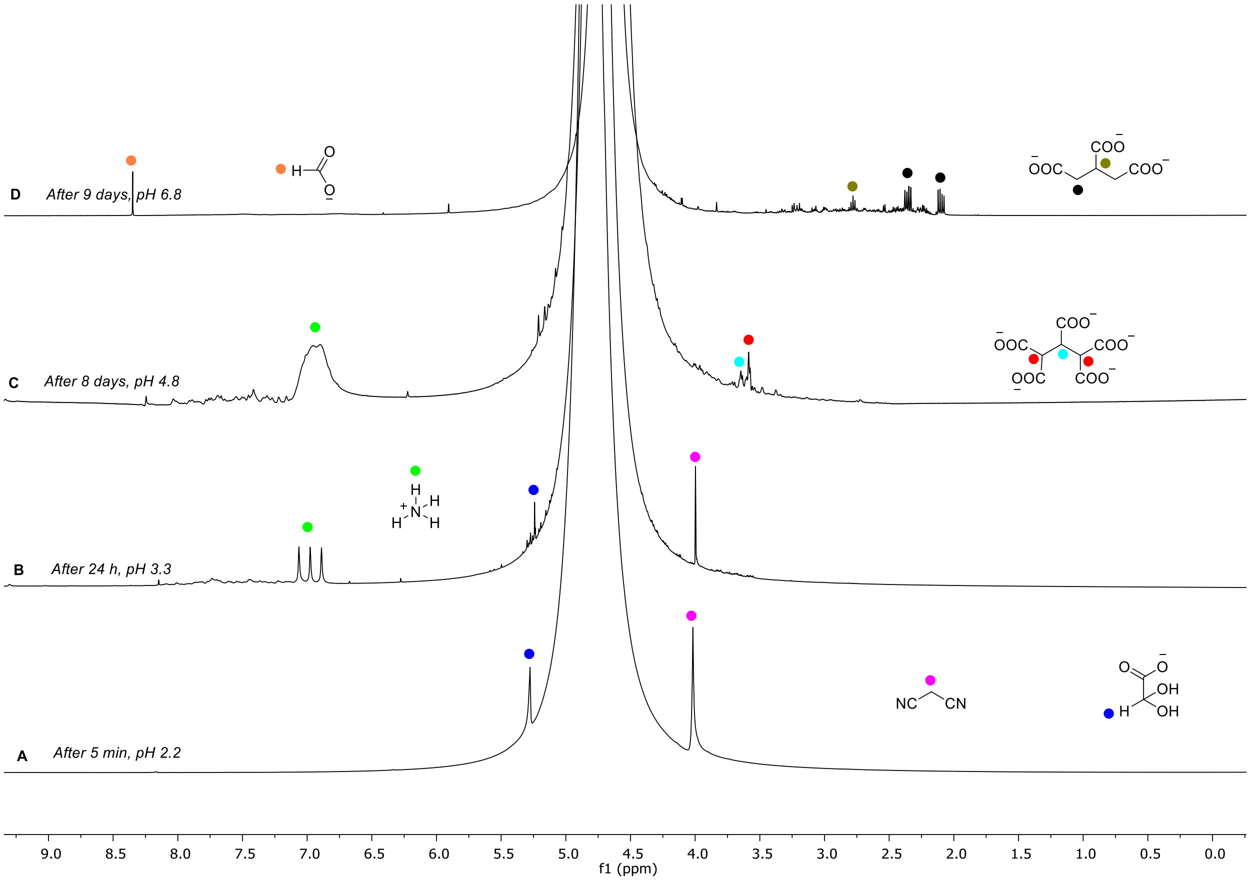


Figure S37: Stacked ^{1}H NMR (H_2O-D_2O) showing progress of the reaction after 5 min (A), 24h (B), 8 days (C), and 9 days (D). Absolute yield of tricarballylic acid **19** was calculated by ^{1}H NMR using internal standard (t-BuOH).

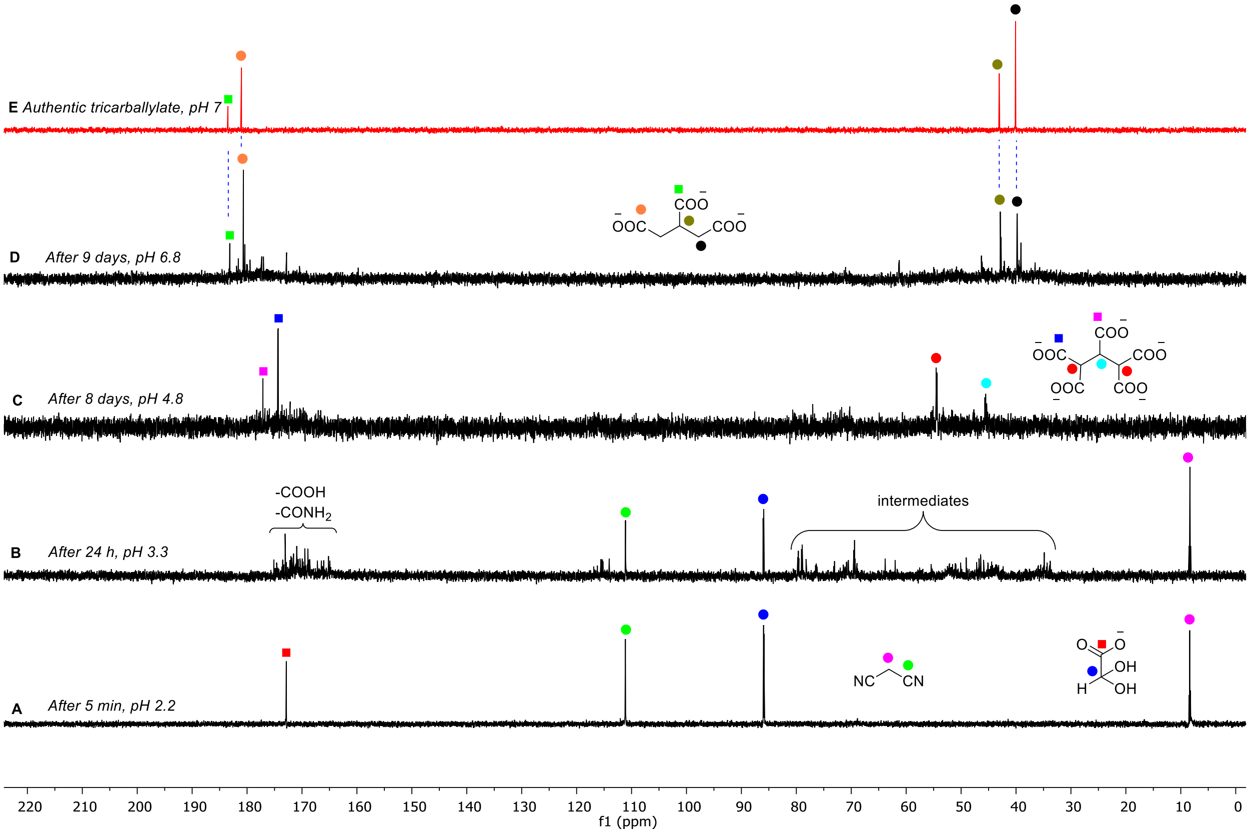


Figure S38: Stacked ^{13}C NMR (H_2O-D_2O) showing progress of the reaction after 5 min (A), 24h (B), 8 days (C), and 9 days (D) compared with authentic tricarballylic acid **19** at pH = 7.0 (E).

***Reaction Conditions:* malononitrile 17 (132 mg, 1.0 equiv.), glyoxylic acid monohydrate (184 mg, 1 equiv.), H_2O (4 mL).**

- After 5 min: pH = 2.0, ^{1}H NMR (Figure **S39A**) showed majorly the starting materials.
- After 20 h: ^{1}H NMR (Figure **S39B**) showed the starting materials along with the formation of various intermediates via hydrolysis of nitriles to release ammonia (pH =3.3).
- After 44 h: ^{1}H NMR (Figure **S39C**) showed complex mixture of various intermediates (pH = 4.0).
- After 8 days: ^{1}H NMR (Figure **S39D**) showed pentacarboxylic acid **18** as the major product along with some unreacted glyoxylate (pH = 4.5).

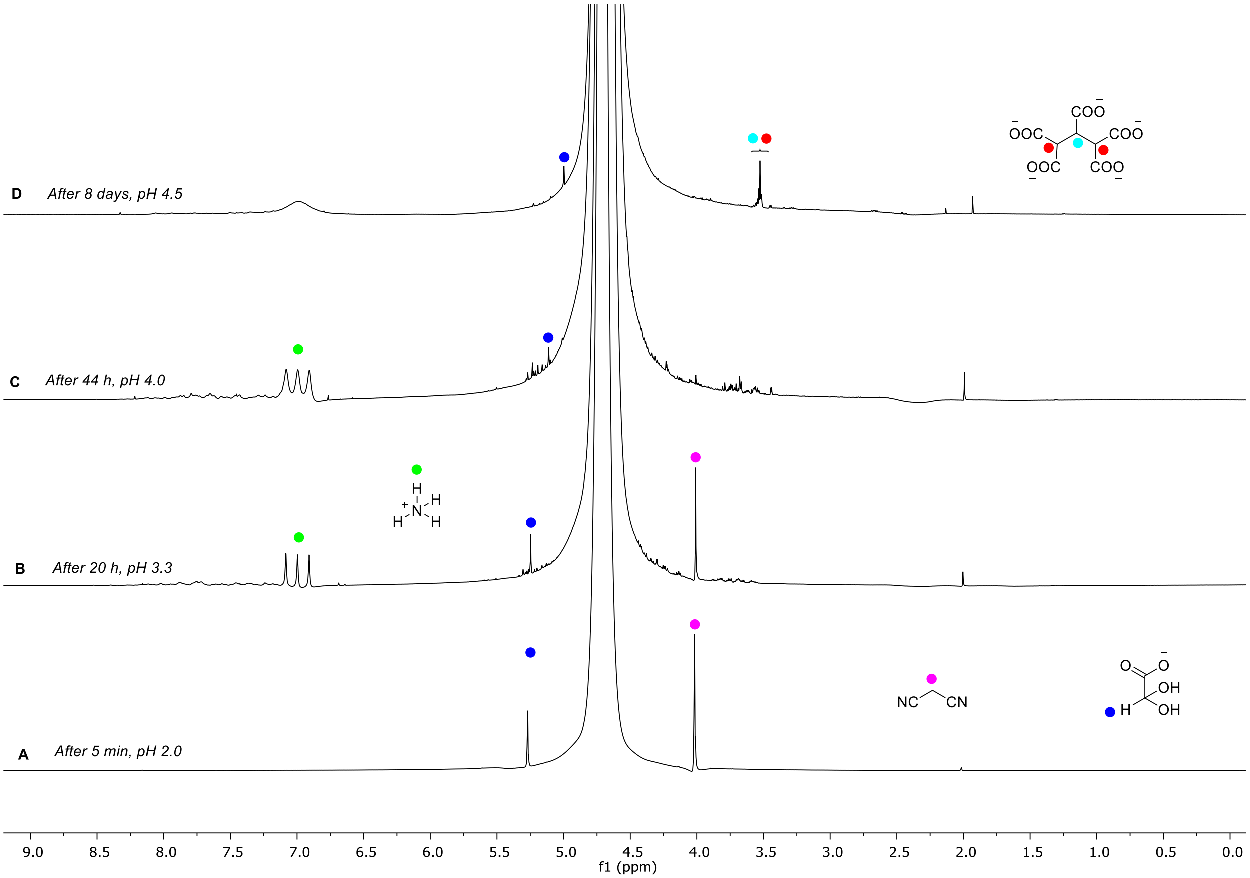
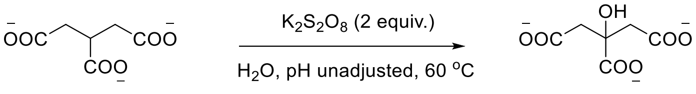


Figure S39: Stacked ^{1}H NMR (H_2O-D_2O) showing progress of the reaction after 5 min (A), 20h (B), 44h (C), and 8 days (D).

Oxidation of tricarballylic acid 19 to citric acid 14:



tricarballylic acid, **19**

citric acid, **14**

Scheme S13: Persulfate mediated oxidation of tricarballylic acid **19** to citric acid **14** in aqueous medium (Figures S40-41).

Reaction procedure: In a 4 mL glass vial equipped with a magnetic stir bar, tricarballylic acid **19** (176.0 mg, 1 mmol, 1.0 equiv.) was dissolved in deionized and degassed water (2.0 mL). To this homogeneous solution $K_2S_2O_8$ (540 mg, 2.0 equiv.) was added. pH of the reaction mixture was kept unadjusted (~1.2). The reaction mixture was stirred at room temperature and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR (D_2O) over a period (Figures S40-41).

Persulfate-mediated oxidation of tricarballylic acid **19** to citric acid **14** was also observed at moderately acidic pHs (3-5).

<u>Reaction Conditions:</u> tricarballylic acid 19 (176 mg, 1.0 equiv.), $K_2S_2O_8$ (540 mg, 2 equiv.), H_2O (2 mL).

- <u>After 5 min:</u> pH = 1.2, ^{1}H NMR (Figure **S40A**) showed only tricarballylic acid **19** (starting material) peaks.
- <u>After 3 days:</u> ^{1}H NMR (Figure **S40B**) showed the formation citric acid **14** (pH = 2.0).
- <u>After 6 days:</u> ^{1}H NMR (Figure **S40C**) showed increase in the formation of citric acid **14** (pH = 2.2).
- <u>After 10 days:</u> ^{1}H NMR (Figure **S40D**) showed further increase in the formation of citric acid **14**. % conversion yield of the citric acid **14** formed was found to be 33% (w.r.t. remaining tricarballylic acid **19**), pH = 2.5.

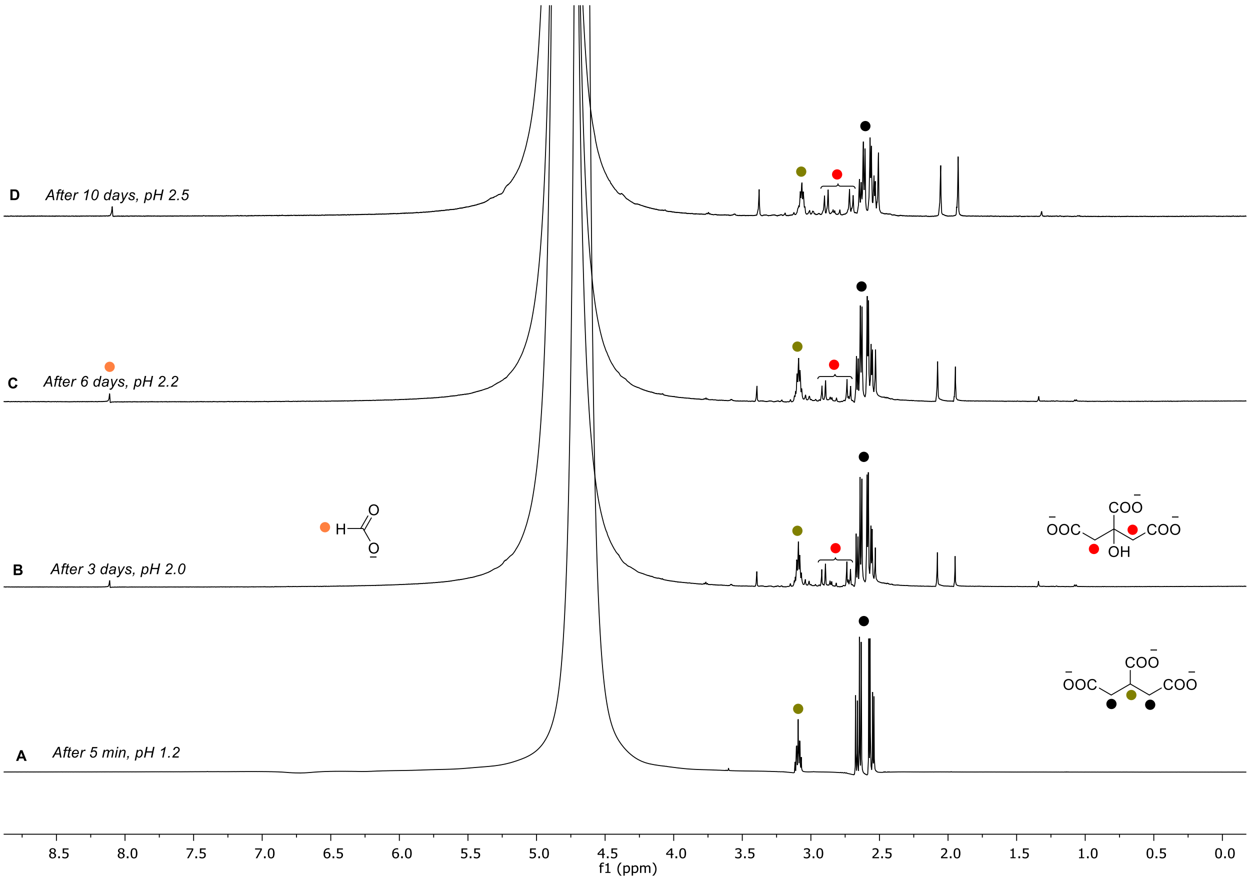


Figure S40: Stacked ^{1}H NMR (D_2O) showing progress of the reaction after 5 min (A), 3 days (B), 6 days (C), and 10 days (D).

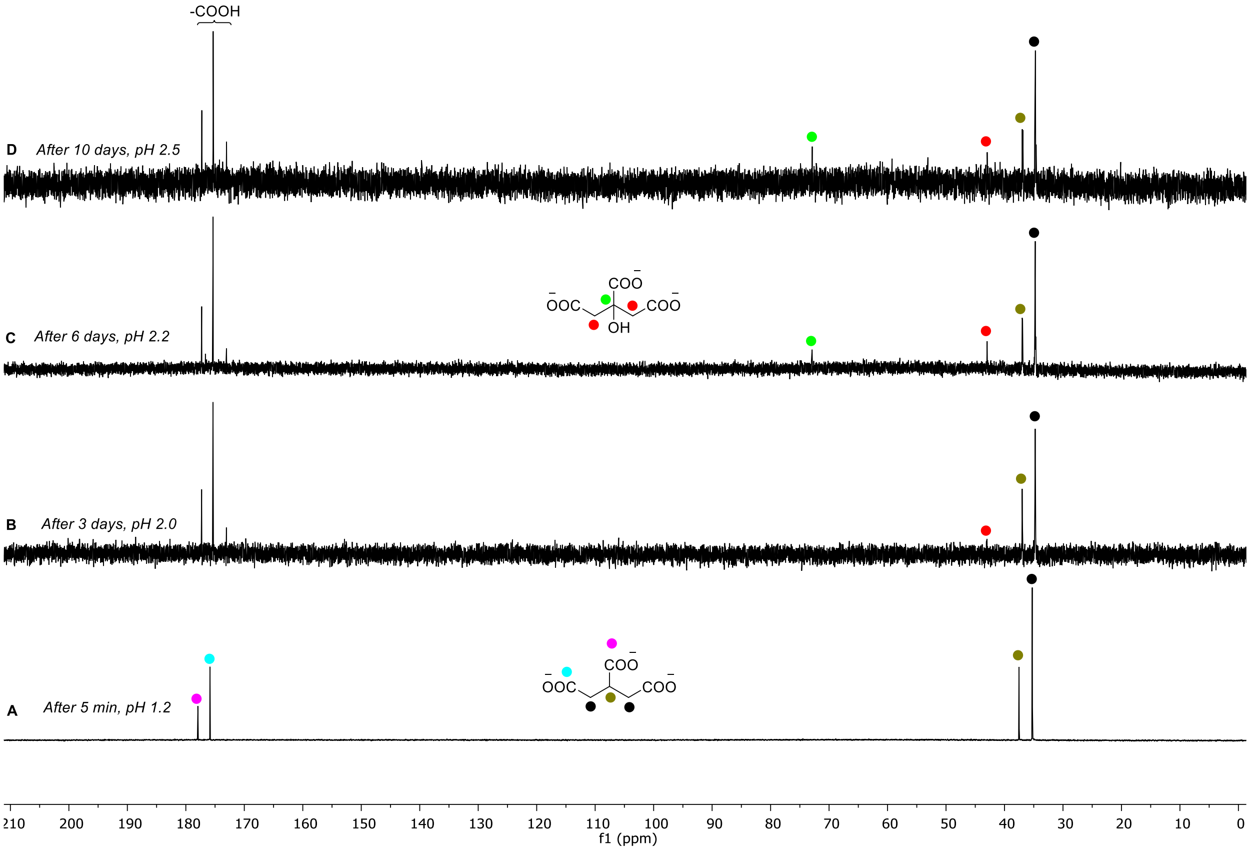
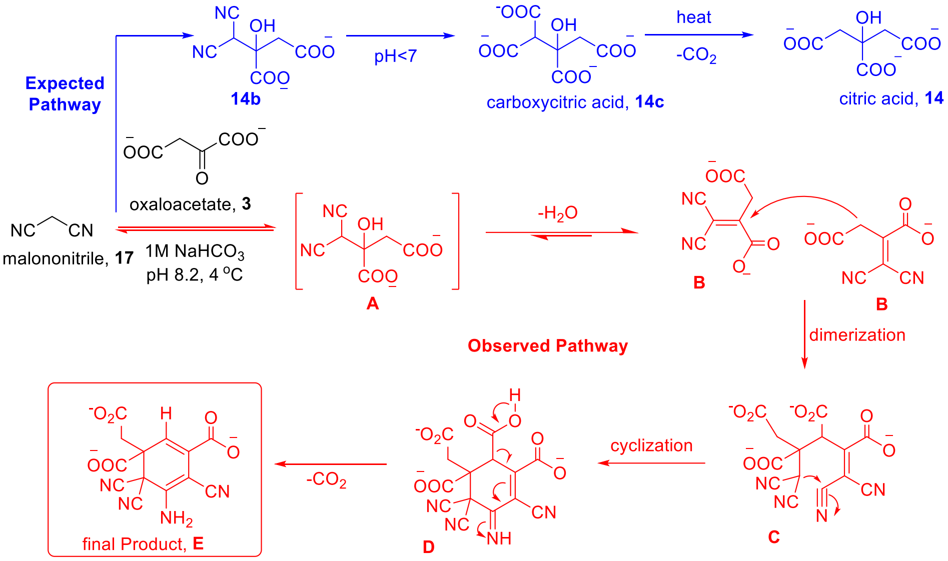


Figure S41: Stacked ^{13}C NMR (D_2O) showing progress of the reaction after 5 min (A), 3 days (B), 6 days (C), and 10 days (D).

Reaction of malononitrile 17 with oxaloacetate 3:



Scheme S14: Expected and observed pathways from a reaction of malononitrile **17** with oxaloacetate **3** in aqueous medium (Figures S42-43).

Reaction procedure: In a 20 mL glass vial equipped with a magnetic stir bar, malononitrile **17** (132.0 mg, 2 mmol, 1.0 equiv.) was dissolved in 1M $NaHCO_3$ (10.0 mL). To this homogeneous solution, oxaloacetatic acid **3** (264 mg, 1.0 equiv.) was added. After addition of oxaloacetic acid **3**, pH of the homogeneous colourless reaction mixture was kept unadjusted (~8.2). The reaction mixture was stirred at 4 °C in a cold room (a similar reaction was also carried out at r.t. which gave similar results; however, the product **E** formed is not very stable for longer time i.e., 3-4 days at r.t.) and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR (H_2O-D_2O) over a period (Figures S42-43).

A set of similar reactions at acidic pH (3-6) were also carried out; however, the reaction mixtures were very complex (formation of product **E** was observed as a major product along with other unknown products) when the reactions were carried out at acidic pH. We did not make further efforts to characterize the compounds formed in those reactions.

Reaction Conditions: ***malononitrile 17 (132 mg, 1.0 equiv.), oxaloacetate 3 (264 mg, 1 equiv.), 1M NaHCO$_3$ (10 mL).***

- After 20 min: pH = 8.2, ^{1}H NMR (Figure **S42A**) showed the quick formation of the product **E** along with some starting material.
- After 3 days: ^{1}H NMR (Figure **S42B**) showed almost quantitative formation of the product **E** (pH = 8.8).
- After 10 days: ^{1}H NMR (Figure **S42C**) showed the that the product **E** formed is very stable at 4 ºC (pH = 8.9). When stored at r.t., slow degradation (unknown compound) of the product was observed.
- ESI-MS m/z calculated for **E** $C_{13}H_8N_4O_6$ $[M+H]^+$ 317.0517, found 371.0537.

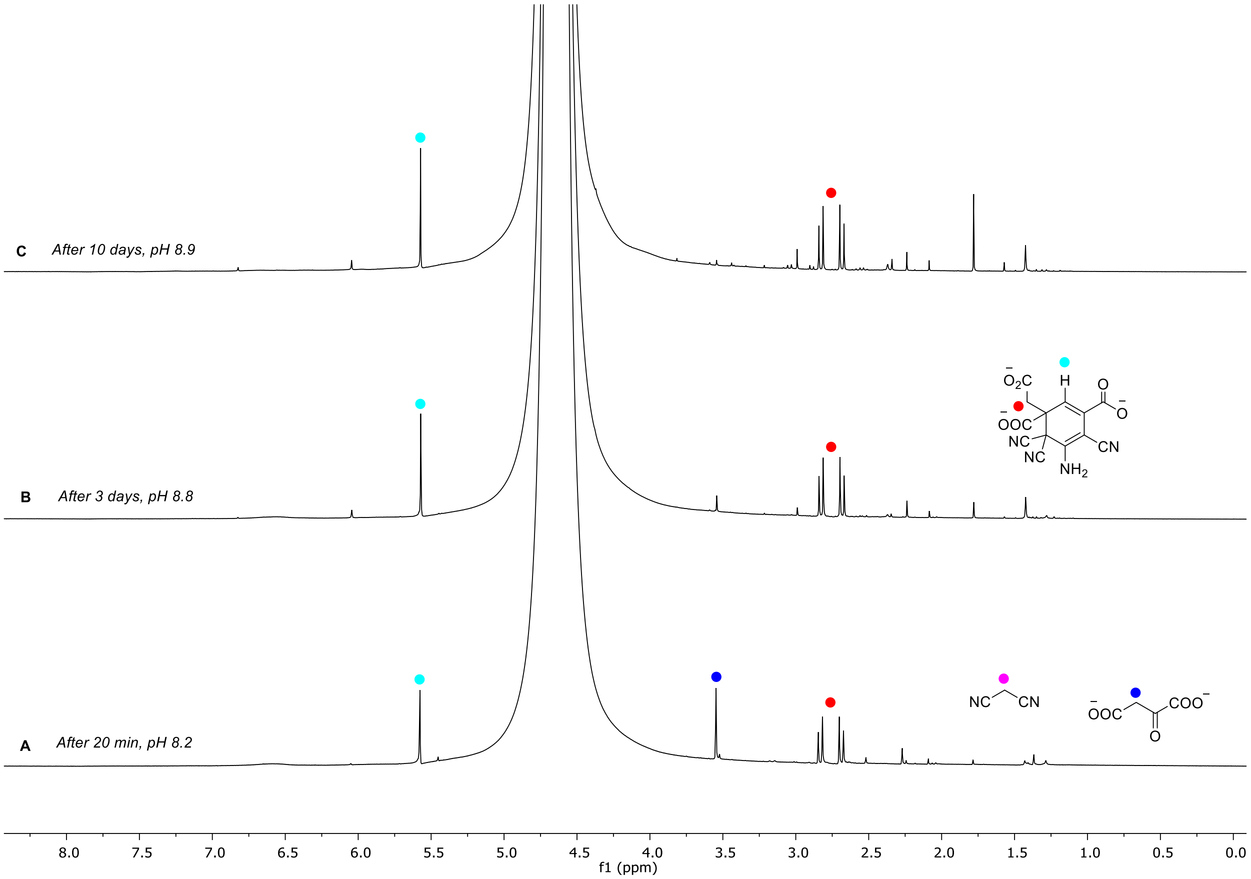


Figure S42: Stacked ^{1}H NMR (H_2O-D_2O) showing progress of the reaction after 20 min (A), 3 days (B), and 10 days (C).

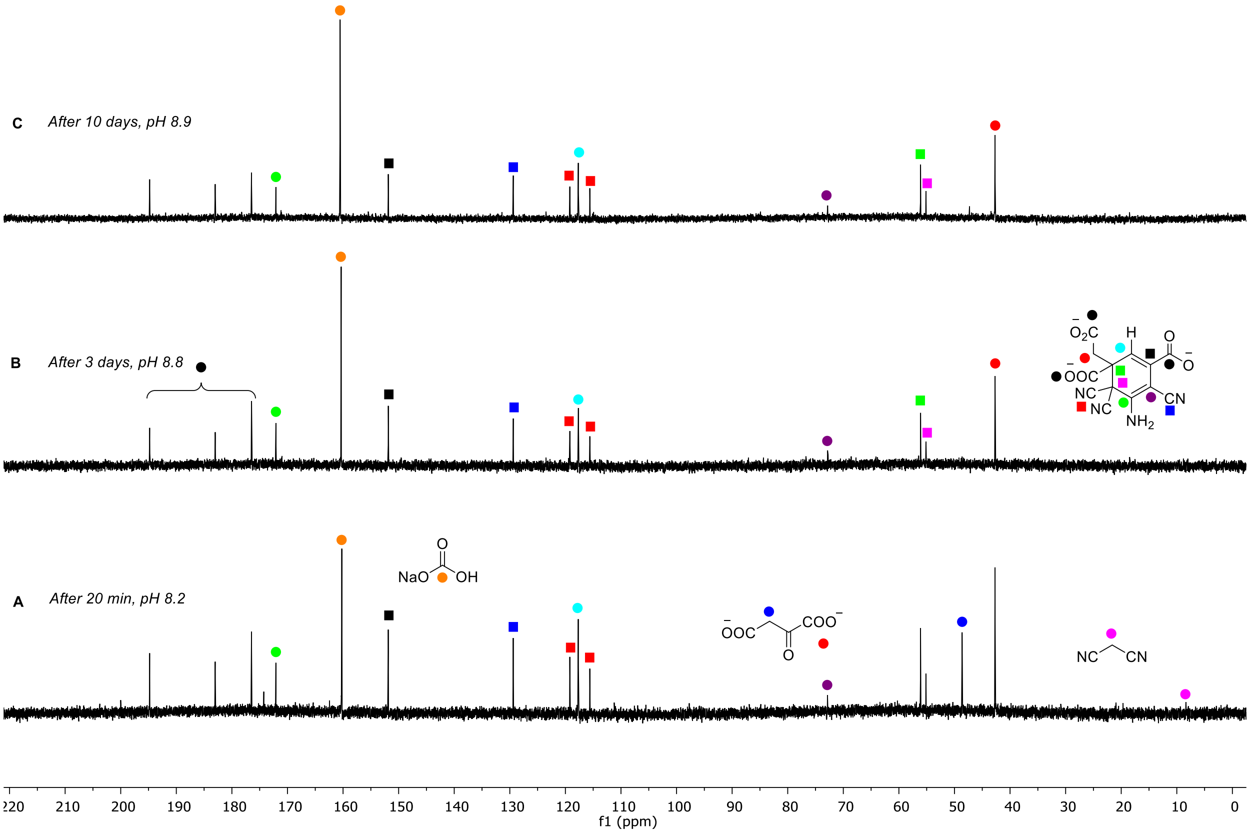
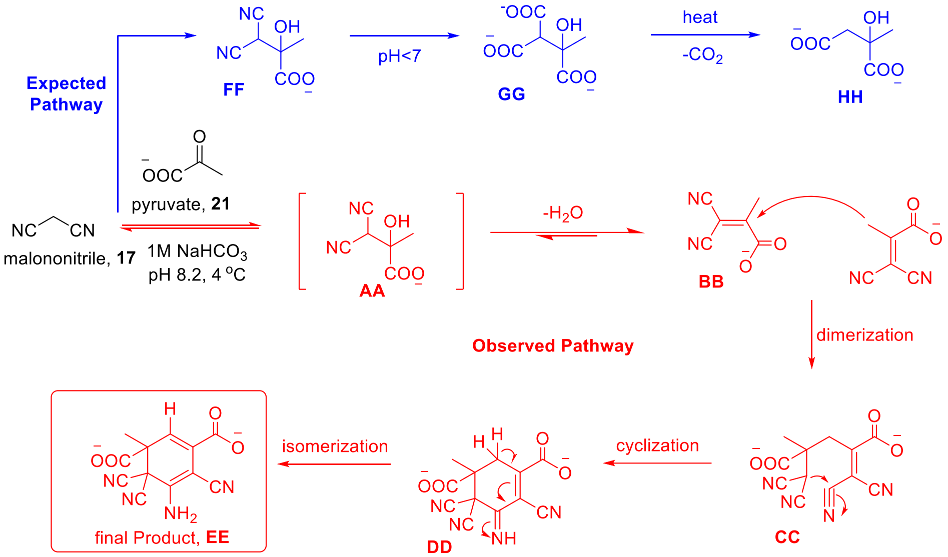


Figure S43: Stacked ^{13}C NMR (H_2O-D_2O) showing progress of the reaction after 20 min (A), 3 days (B), and 10 days (C). Assignment of product peaks was made via combined information from HSQC, H-coupled ^{13}C NMR, DEPT and Chemdraw predictions.

Reaction of malononitrile 17 with pyruvate 21:



Scheme S15: Expected and observed pathways from a reaction of malononitrile **17** with pyruvate **21** in aqueous medium (Figures S44-45).

Reaction procedure: In a 20 mL glass vial equipped with a magnetic stir bar, malononitrile **17** (132.0 mg, 2 mmol, 1.0 equiv.) was dissolved in 1M $NaHCO_3$ (10.0 mL). To this homogeneous solution sodium pyruvate **21** (220 mg, 1.0 equiv.) was added. After addition of sodium pyruvate **21**, pH of the homogeneous colourless reaction mixture was kept unadjusted (~8.2). The reaction mixture was stirred at 4 °C in a cold room (a similar reaction was also carried out at r.t. which gave similar results; however, the product **EE** formed is not very stable for longer time i.e., 3-4 days at r.t.) and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR (H_2O-D_2O) over a period (Figures S44-45).

A set of similar reactions at acidic pH (3-6) were also carried out; however, the reaction mixtures were very complex (formation of product **EE** was observed as a major product along with other unknown products) when the reactions were carried out at acidic pH. We did not make further efforts to characterize the compounds formed in those reactions.

Reaction Conditions: malononitrile 17 (132 mg, 1.0 equiv.), sodium pyruvate 21 (220 mg, 1 equiv.), 1M $NaHCO_3$ (10 mL).

- After 20 min: pH = 8.2, ^{1}H NMR (Figure **S44A**) showed the quick formation of the product **EE** along with some starting material.
- After 24h: ^{1}H NMR (Figure **S44B**) showed almost quantitative formation of the product **EE** (pH = 8.5).
- After 5 days: ^{1}H NMR (Figure **S44C**) showed the that the product **EE** formed is very stable at 4 ºC (pH = 8.4). When stored at rt, slow degradation (unknown compound) of the product was observed.

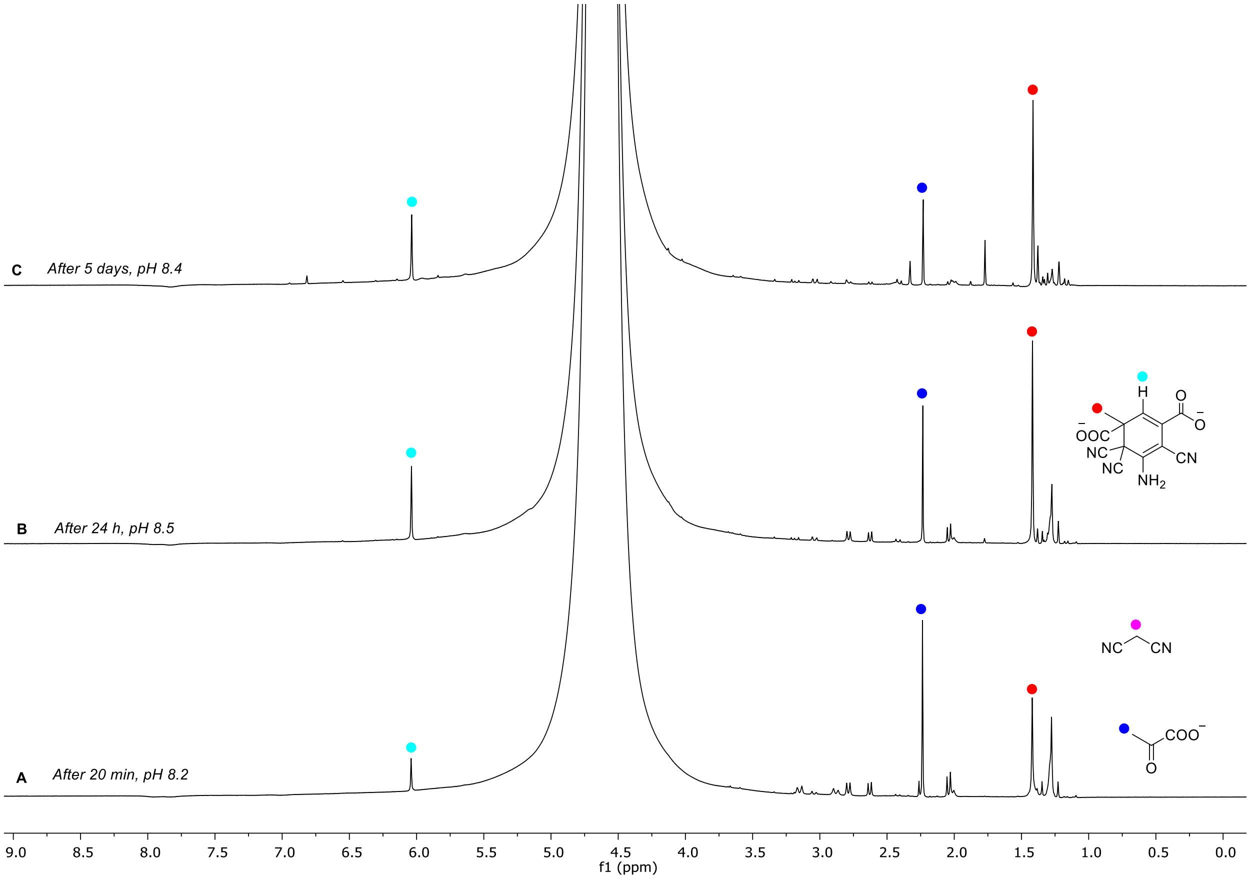


Figure S44: Stacked ^{1}H NMR (H_2O-D_2O) showing progress of the reaction after 20 min (A), 24h (B), and 5 days (C).

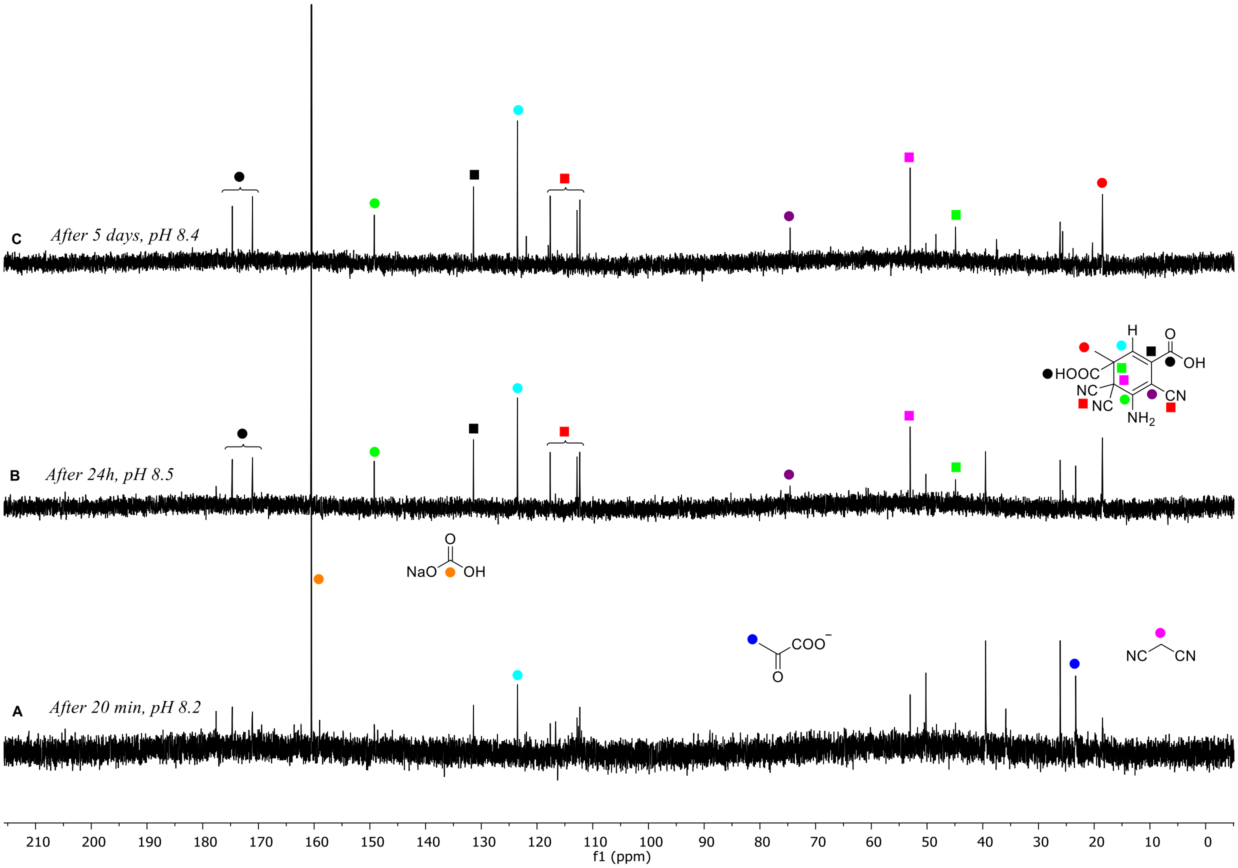
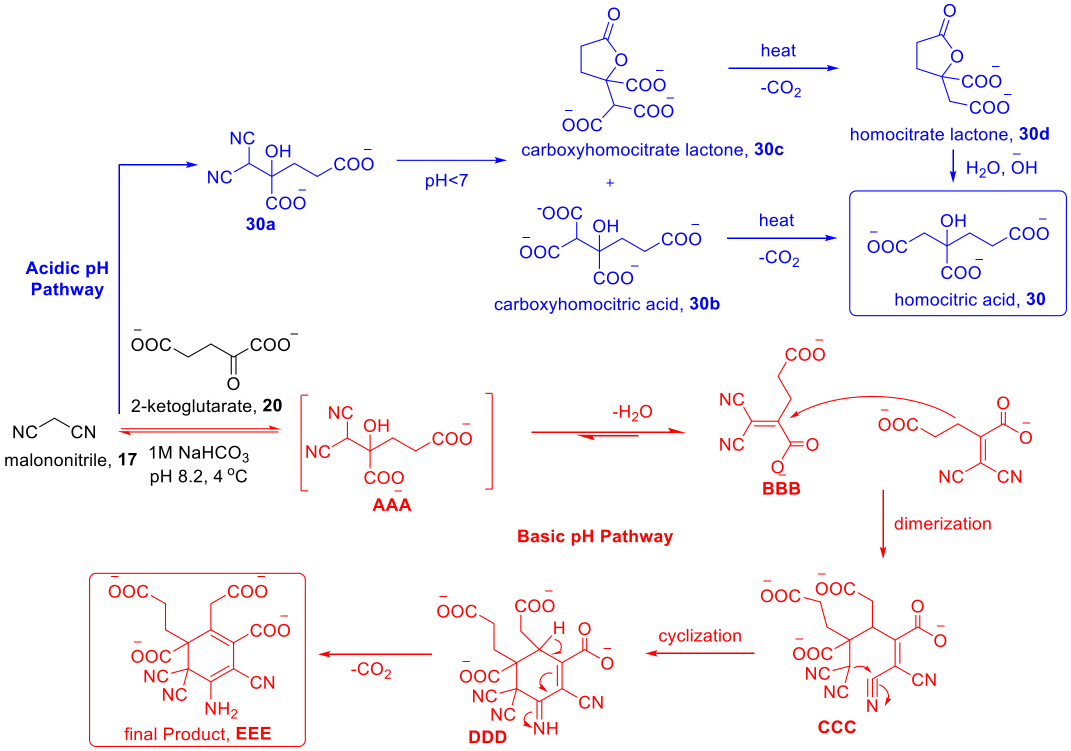


Figure S45: Stacked ^{13}C NMR (H_2O-D_2O) showing progress of the reaction after 20 min (A), 24h (B), and 5 days (C). Assignment of product peaks was made via combined information from H-coupled ^{13}C NMR, DEPT and Chemdraw predictions.

Reaction of malononitrile 17 with 2-ketoglutarate 20:



Scheme S16: Alternative pathways from a reaction of malononitrile **17** with 2-ketoglutarate **20** in basic (Figures S46-47) and acidic (Figures 48-49) aqueous medium.

Reaction procedure:

Basic Medium (pH>7), condition 1: In a 20 mL glass vial equipped with a magnetic stir bar, malononitrile **17** (132.0 mg, 2 mmol, 1.0 equiv.) was dissolved in 1M $NaHCO_3$ (10.0 mL). To this homogeneous solution 2-ketoglutaric acid **20** (292 mg, 1.0 equiv.) was added. After addition of 2-ketoglutaric acid **20**, pH of the homogeneous colourless reaction mixture was kept unadjusted (~8.2). The reaction mixture was stirred at 4 °C in a cold room (a similar reaction was also carried out at r.t. which gave similar results; however, the product formed **EEE** is not very stable for longer time i.e., 3-4 days at r.t.) and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR (H_2O-D_2O) over a period (Figures S46-47).

Acidic Medium (pH<7), condition 2: In a 20 mL glass vial equipped with a magnetic stir bar, malononitrile **17** (132.0 mg, 2 mmol, 1.0 equiv.) was dissolved in deionized and degassed H_2O (10.0 mL). To this homogeneous solution 2-ketoglutaric acid **20** (292 mg, 1.0 equiv.) was added. After addition of 2-ketoglutaric acid **20**, pH of the homogeneous colourless reaction mixture was kept unadjusted (~2.0) or adjusted at 3-4 (with 5M NaOH). The reaction mixture was stirred at r.t. and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR (H_2O-D_2O) over a period (Figure S48). After formation (almost quantitative) of carboxyhomocitric acid lactone **30c** (8 days) as the sole product, temperature of the reaction mixture was raised to 80 °C (24 h) for the final decarboxylation to form homocitric acid lactone **30d** which was finally hydrolysed to homocitrate **30** (Figures S48-51).

<u>Reaction Condition 1:</u> malononitrile 17 (132 mg, 1.0 equiv.), 2-ketoglutaric acid 20 (292 mg, 1 equiv.), 1M $NaHCO_3$ (10 mL).

- <u>After 20 min:</u> pH = 8.2, ^{1}H NMR (Figure **S46A**) showed the quick formation of the aldol adduct of malononitrile **17** and 2-ketoglutarate **20**.
- <u>After 1h:</u> ^{1}H NMR (Figure **S46B**) showed a little formation of the final product **EEE** (pH = 8.2).
- <u>After 24h:</u> ^{1}H NMR (Figure **S46C**) showed almost quantitative conversion to the product. The final product formed is somewhat stable at 4 °C (pH = 8.3). However, when stored at rt, degradation (unknown compound) of the product was observed.

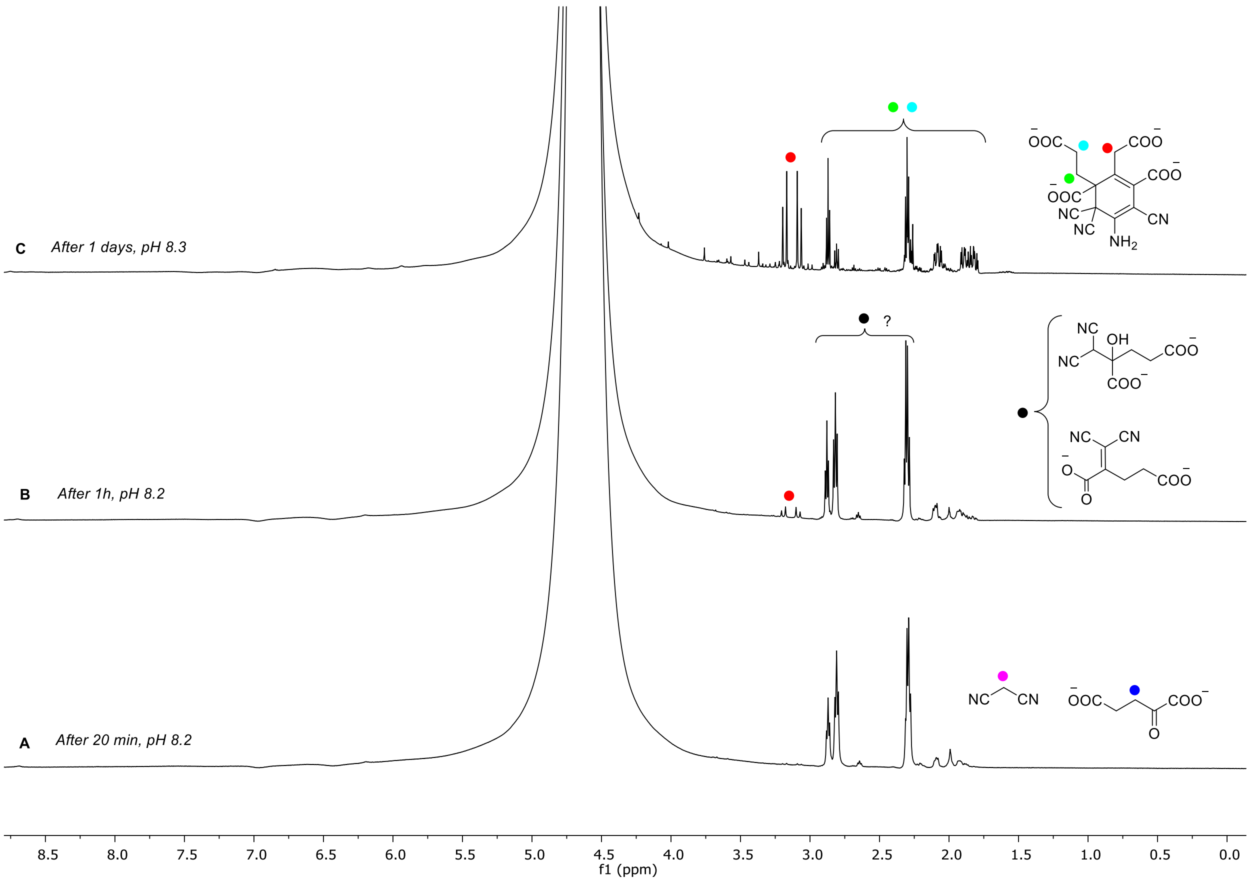


Figure S46: Stacked ^{1}H NMR (H_2O-D_2O) showing progress of the reaction after 20 min (A), 1h (B), and 24h (C).

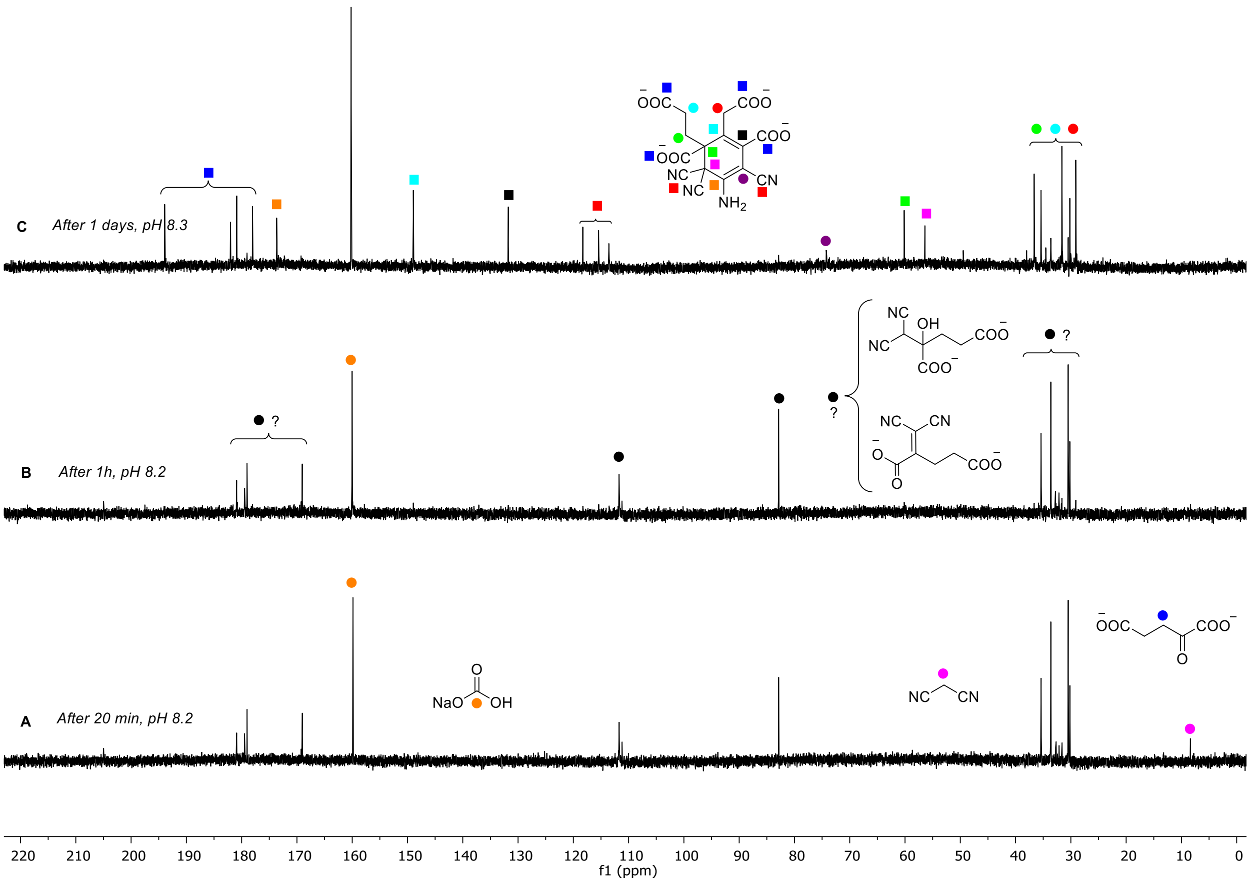


Figure S47: Stacked ^{13}C NMR (H_2O-D_2O) showing progress of the reaction after 20 min (A), 1h (B), and 24h (C). Assignment of product peaks was made via combined information from H-coupled ^{13}C NMR, DEPT and Chemdraw predictions.

Reaction Condition 2: malononitrile 17 (132 mg, 1.0 equiv.), 2-ketoglutaric acid 21 (292 mg, 1 equiv.), H_2O (10 mL).

- After 5 min: pH = 1.8, ^{1}H NMR (Figure **S48A**) showed the starting materials i.e., malononitrile **17** and 2-ketoglutarate **20**.
- After 18h: ^{1}H NMR (Figure **S48B**) showed the formation of carboxy-homocitrate lactone **30c** as the major product (pH = 3.5).
- After 4 days: ^{1}H NMR (Figure **S48C**) showed quantitative conversion to the carboxy-homocitrate lactone **30c** which is very stable at r.t. in aqueous solution (pH = 3.9).
- After 4 days: The reaction mixture was heated at 80 °C (for 24 h) to facilitate the decarboxylation of carboxy-homocitrate lactone **30c** to homocitrate lactone **30d**.
- After 5 days: pH = 6.8, the reaction mixture showed the formation of homocitrate lactone **30d** as the major product (confirmed with spiking experiment, Figure **S50**) along with homocitrate **30** as the minor product (Figure **S50D**). Other minor products detected in the ^{1}H NMR (after heating at 80 °C for 24 h) were malonate **15** and 2-ketoglutarate **20**, which were produced via the retro-aldol cleavage of carboxy-homocitrate **30b**. Absolute yield of homocitrate **30** produced in this reaction (after saponification with 10% aq. NaOH, see Figure **S51**) was found to be 50% (calculated by ^{1}H NMR peak integration of product **30** w.r.t. internal standard used).
- A similar decarboxylation reaction in the presence of $ZnCl_2$ (1.0 equiv., to increase the rate of decarboxylation) was also tried; however, in presence of zinc, mostly retro-aldol cleavage of carboxy-homocitrate **30b** to malonate **15** and 2-ketoglutarate **20** was observed.

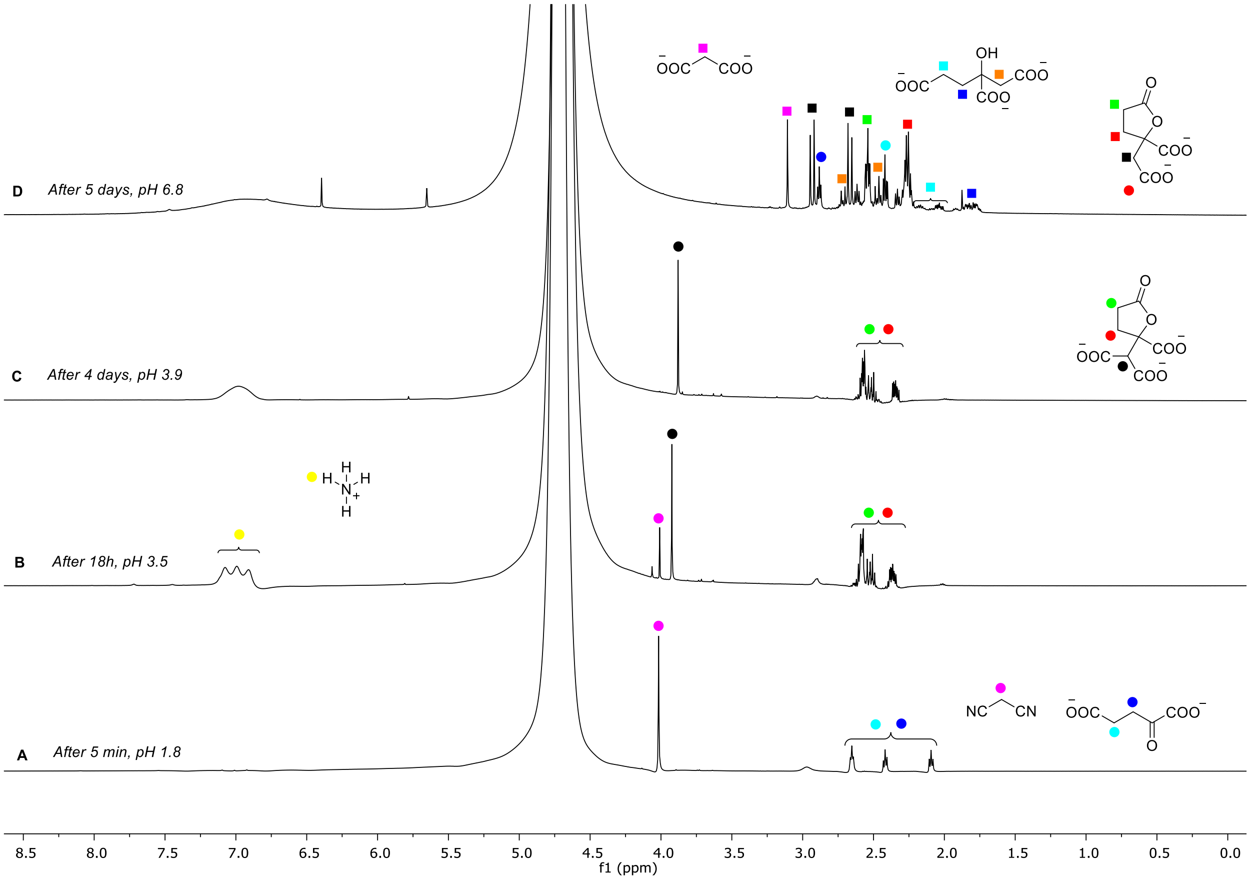


Figure S48: Stacked ^{1}H NMR (H_2O-D_2O) showing progress of the reaction after 5 min (A), 18h (B), 4 days (C) and 5 days (D).

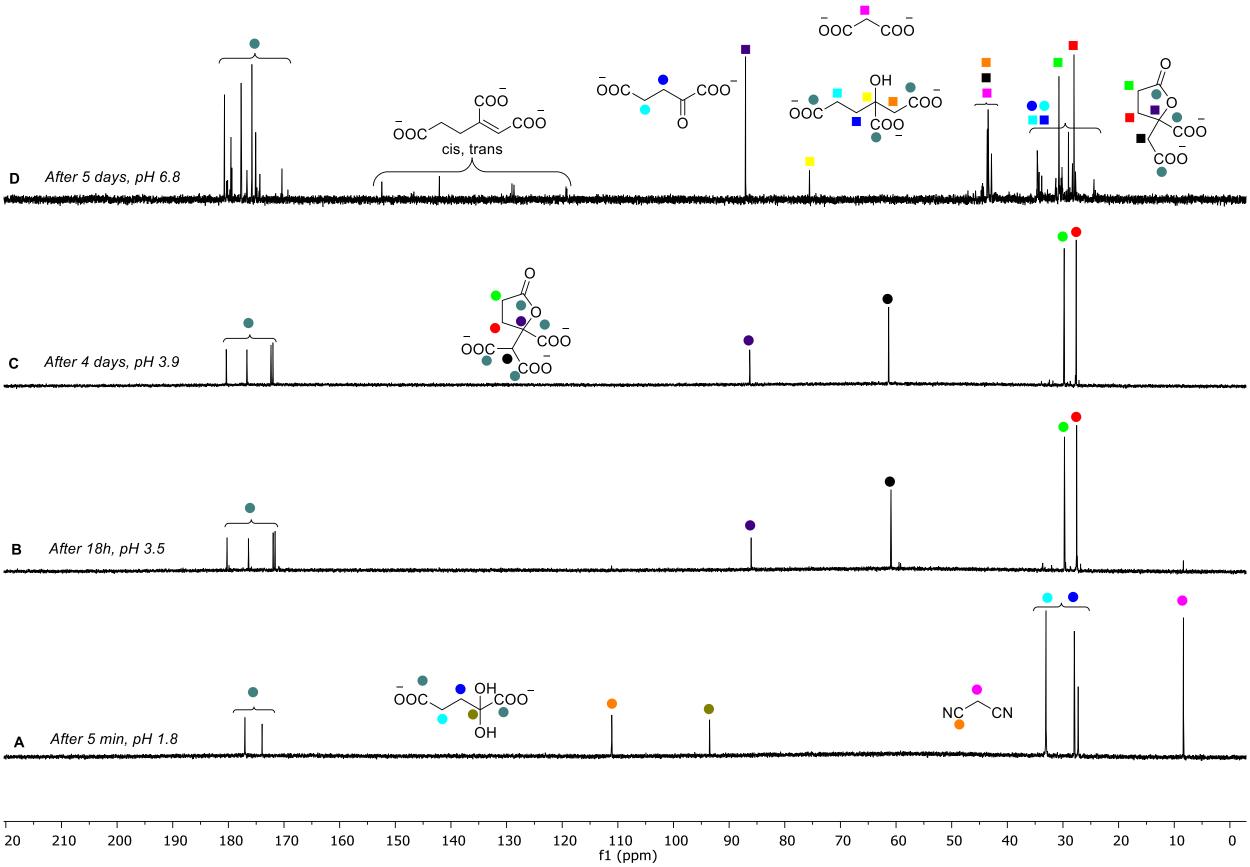


Figure S49: Stacked ^{13}C NMR (H_2O-D_2O) showing progress of the reaction after 5 min (A), 18h (B), 4 days (C) and 5 days (D).

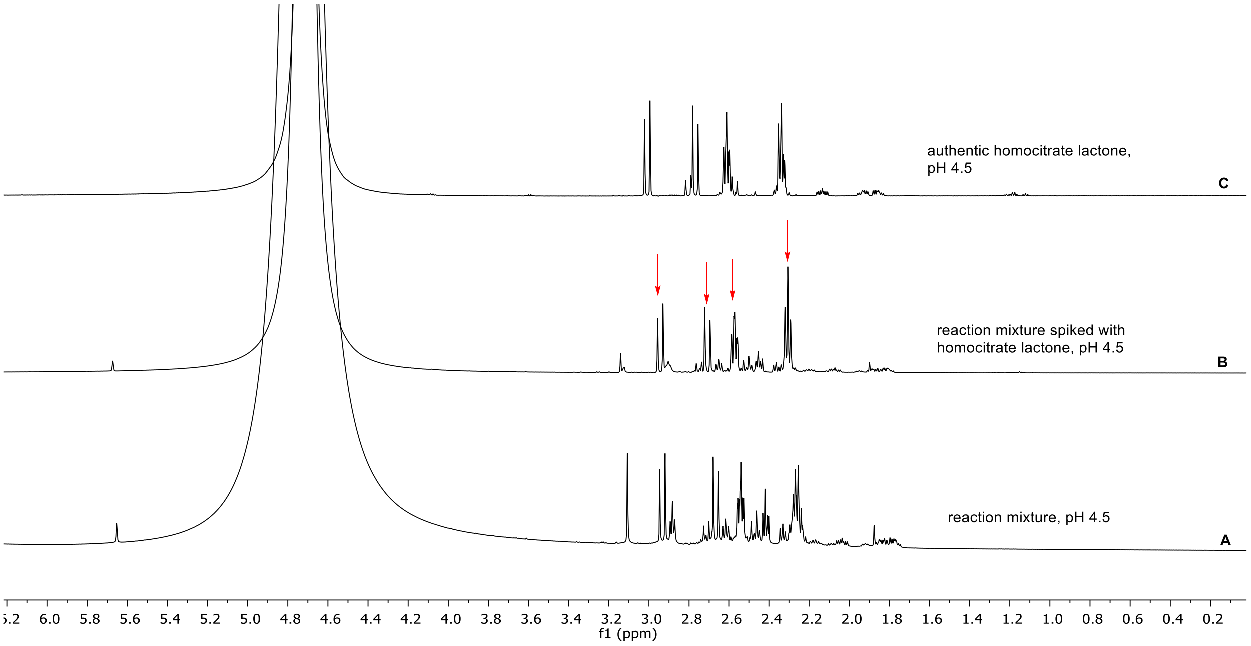


Figure S50: ^{1}H NMR (D_2O) of the reaction mixture spiked with authentic homocitrate lactone **30d** at pH = 4.5 (B). ^{1}H NMR (D_2O) of the reaction mixture (A). ^{1}H NMR (D_2O) of authentic homocitrate lactone **30d** at pH = 4.5 (C).

Saponification of homocitrate lactone 30d to homocitrate 30: In a 4 mL glass vial equipped with a magnetic stir bar, homocitrate lactone **30d** produced after the decarboxylation reaction (200 µL of 0.5 M reaction mixture) was dissolved in 800 µL of 10% aq. NaOH (D_2O was used instead of H_2O). This homogenous reaction mixture was stirred at 50 °C for 2h. After that, the reaction mixture was cooled, and pH of the reaction mixture was adjusted to 8.0 with conc. HCl. ^{1}H NMR of the reaction mixture showed complete hydrolysis of homocitrate lactone to homocitrate (Figure S51B). Absolute yield of homocitrate **30** produced in this reaction (after saponification) was found to be 50% (calculated by ^{1}H NMR peak integration of product **30** w.r.t. internal standard used).

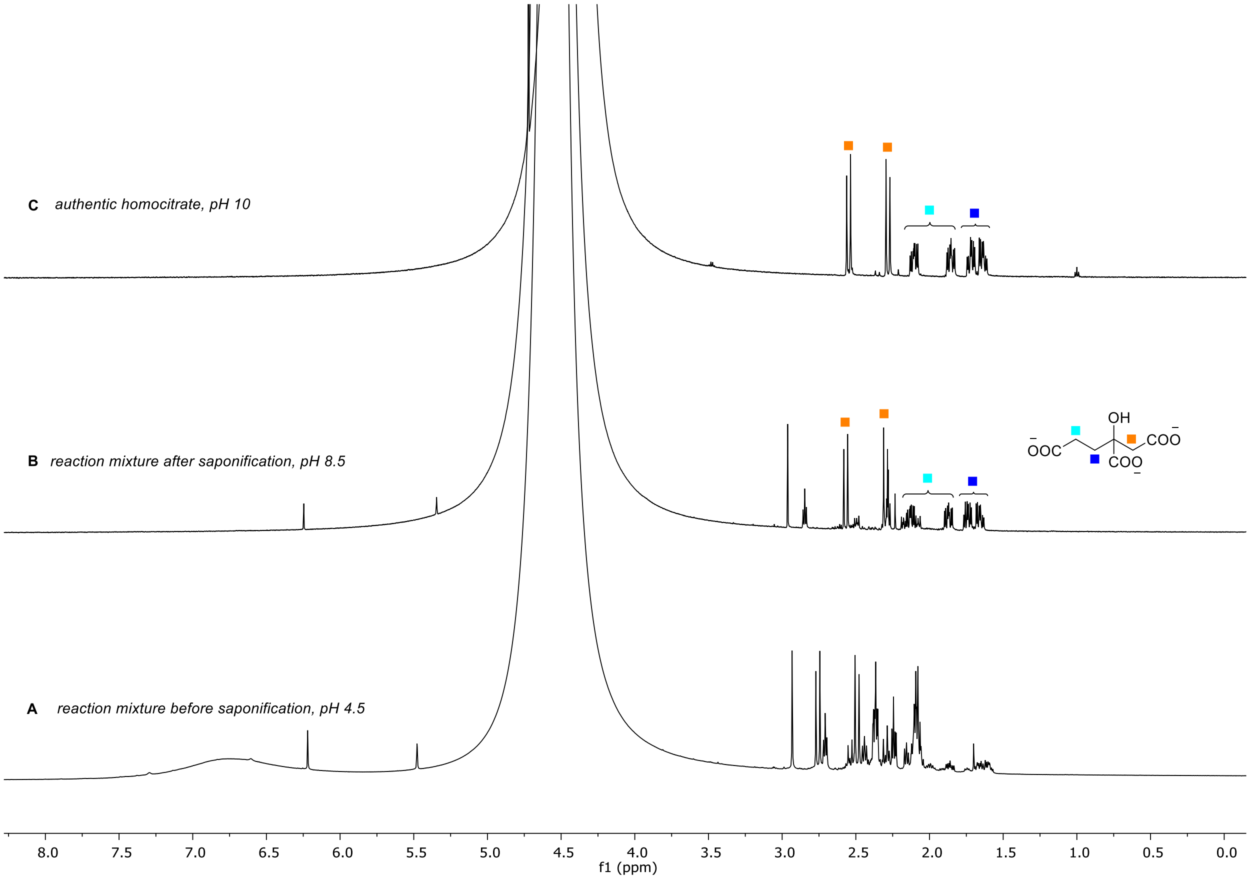
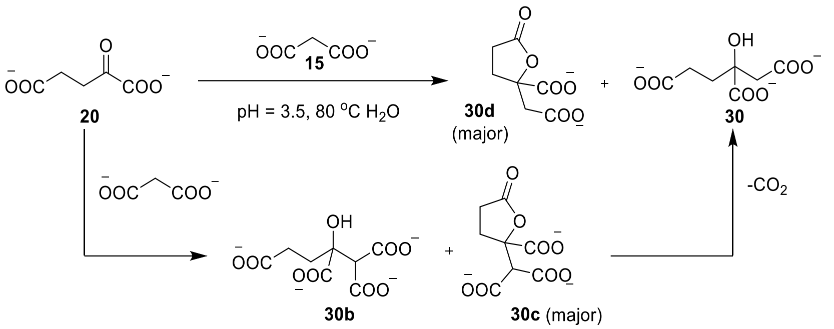


Figure S51: ^{1}H NMR (D_2O) of the reaction mixture before saponification (A). ^{1}H NMR (D_2O) of the reaction mixture after saponification (B). ^{1}H NMR (D_2O) of authentic homocitrate **30** produced by hydrolysis of commercial homocitrate lactone **30d** (C).

Reaction of α-ketoglutarate, 20 with malonic acid, 15 at 80 ºC



Scheme S17: Reaction of α-ketoglutarate **20** with malonate **15** at 80 ºC, pH = 3.5 in H_2O (Figures S52-54).

Reaction procedure: In a 4.0 mL glass vial equipped with a magnetic stir bar, α-ketoglutarate, **20** (73 mg, 0.5 mmol, 1 equiv.) and malonic acid, **15** (52 mg, 0.5 mmol, 1.0 equiv.) were dissolved in deionized and degassed water (1.0 mL) and pH of the resulting mixture was adjusted to 3.5 with 5N NaOH solution. The resulting homogenous solution was heated to 80 ºC and the reaction was monitored by ^{1}H and ^{13}C NMR (NMR method 1 in H_2O-D_2O for Figure S52A and S52C, NMR method 2 in D_2O for Figure S52B).

***Reaction conditions:* α-ketoglutarate (73 mg, 0.5 mmol), malonate (52 mg, 0.5 mmol, 1 equiv.) and H_2O (1.0 mL).**

- After 2 days at 80 ºC, showed the formation of homocitrate lactone **30d** (pH = 3.6) (Figure **52B**).
- After 4 days at 80 ºC, showed the % conversion of homocitrate lactone **30d** is 74% w.r.t α-ketoglutarate, **20** and absolute yield is 45% (pH = 3.9) (Figure **52C**).

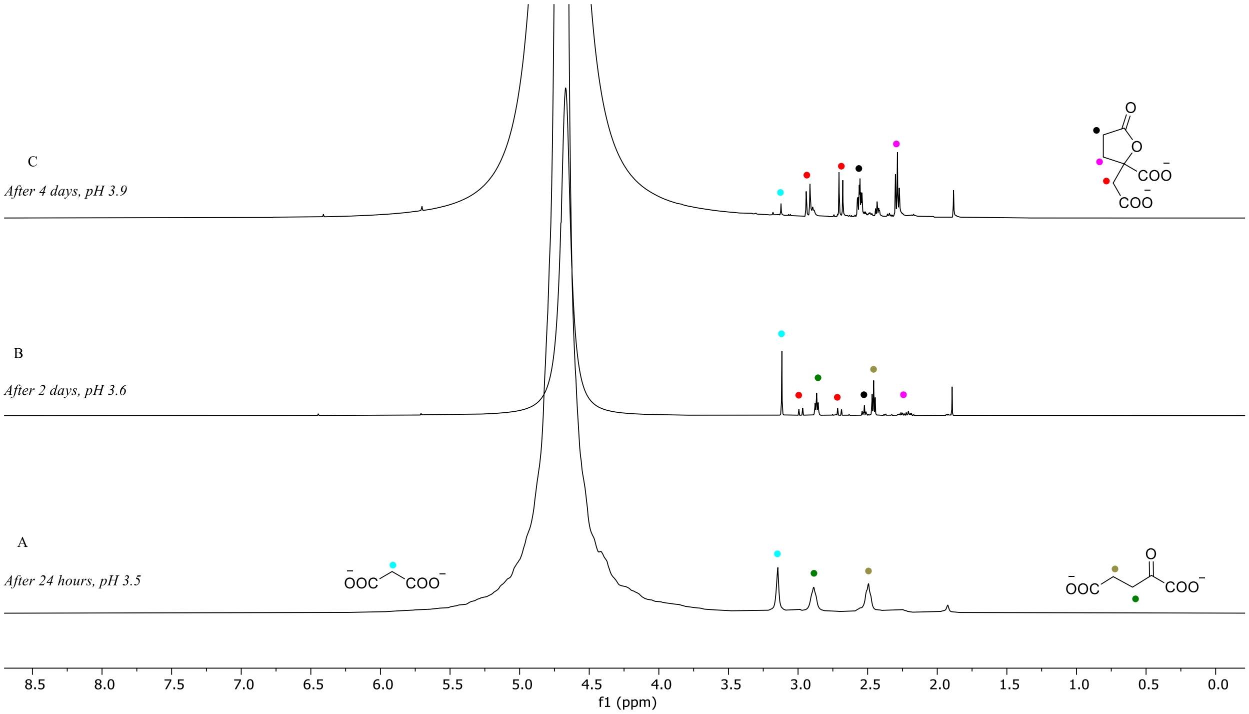


Figure S52: Stacked ^{1}H NMR of the reaction mixture (NMR method 2 in D_2O for Figure S52A and S52C, method 1 in H_2O-D_2O for Figure S52B); reaction after 24 hours at 80 ºC, pH 3.5 (A), reaction after 2 days at 80 ºC, pH 3.6 (B). reaction after 4 days at 80 ºC, pH = 3.9 (C). % Conversion is based on the ^{1}H NMR integration of the product homocitrate lactone **30d** (peak at 2.3 ppm, referred as ●) w.r.t to α-ketoglutarate, **20** (peak at 2.4 ppm, referred as ●)

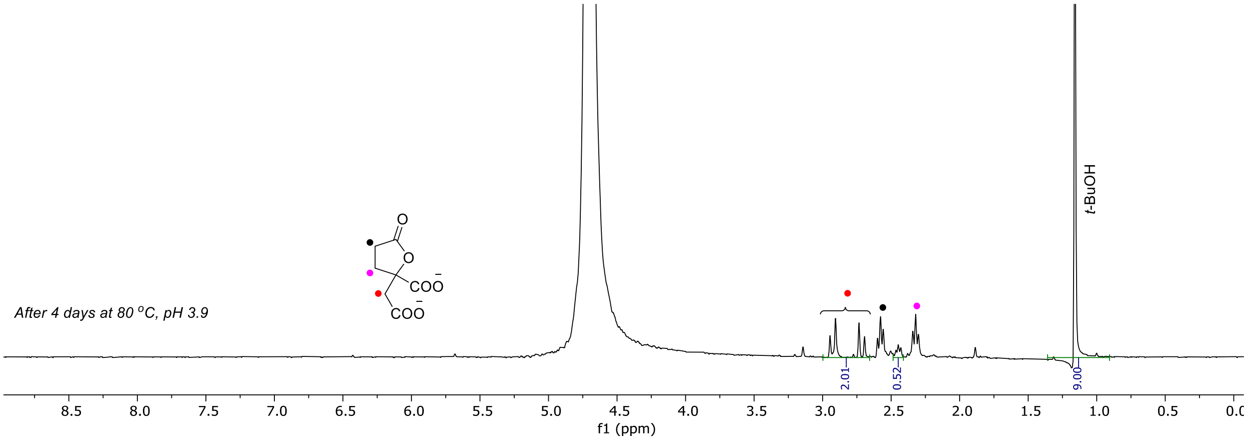


Figure S53: ^{1}H NMR of the reaction mixture (0.277M) (NMR method 2 in D_2O containing 0.0125 mmol of *t*-BuOH)). Absolute yield of homocitrate lactone **30d** was found to be 45%.

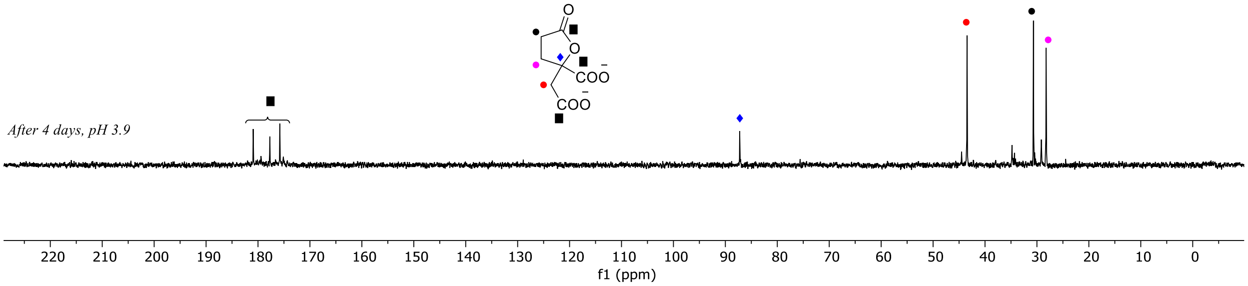
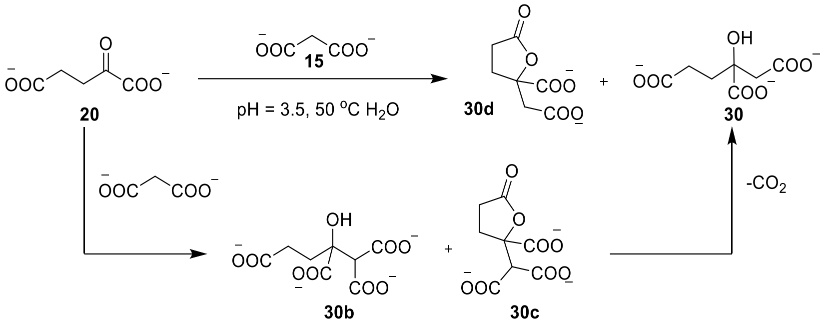


Figure S54: ^{13}C NMR of the reaction mixture (NMR method 1 in H_2O-D_2O) after 4 days at 80 ºC, pH = 3.9.

Reaction of α-ketoglutarate 20 with malonic acid 15 at 50 ºC



Scheme S18: Reaction of α-ketoglutarate **20** with malonate **15** at 50 ºC, pH = 3.5 in H_2O (Figures S55-56).

Procedure: In a 4.0 mL glass vial equipped with a magnetic stir bar, α-ketoglutarate, **20** (73 mg, 0.5 mmol, 1 equiv.) and manonic acid, **15** (52 mg, 0.5 mmol, 1.0 equiv.) were dissolved in deionized and degassed water (1.0 mL) and pH of the resulting mixture was adjusted to 3.5 with 5N NaOH solution. The resulting homogenous solution was heated to 50 ºC and the reaction was monitored by ^{1}H and ^{13}C NMR (NMR method 1 in H_2O-D_2O) (Figures S55-56).

***Reaction conditions:* α-ketoglutarate (73 mg, 0.5 mmol), malonate (52 mg, 0.5 mmol, 1 equiv.) and H_2O (1.0 mL).**

- After 4 days at 80 ^{o}C, showed the formation of homocitrate lactone **30d** (pH = 3.8) (Figure **S55B**).
- After 8 days at 80 ^{o}C, showed the % conversion of homocitrate lactone **30d** is 24% w.r.t α-ketoglutarate, **20** (pH = 3.9) (Figure **S55C**).

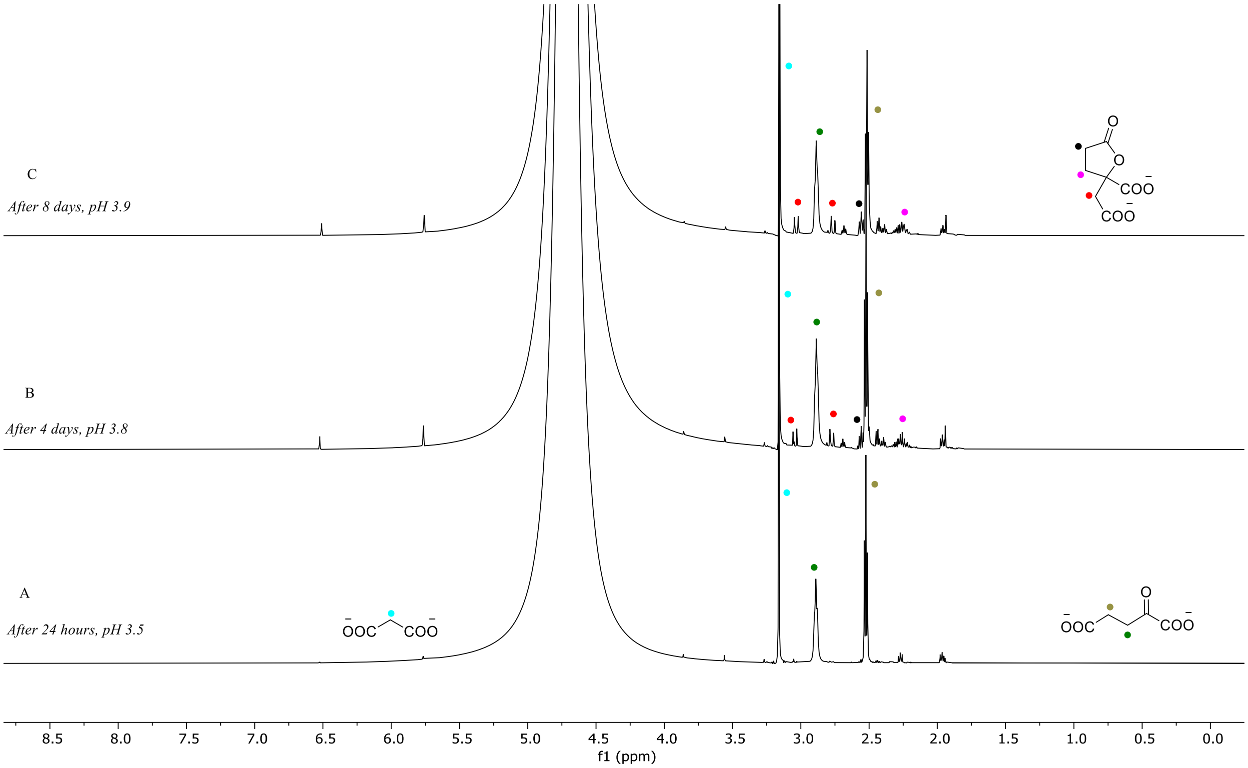


Figure S55: Stacked ^{1}H NMR of the reaction mixture (NMR method 1 in H_2O-D_2O). reaction after 24 hours at 50 ^{o}C, pH 3.5 (A), reaction after 4 days at 50 ^{o}C, pH 3.8 (B). reaction after 8 days at 50 ^{o}C, pH 3.9 (C). % Conversion is based on the ^{1}H NMR integration of the product homocitrate lactone **30d** (peak at 2.3 ppm, referred as ●) w.r.t to α-ketoglutarate, **20** (peak at 2.4 ppm, referred as ●).

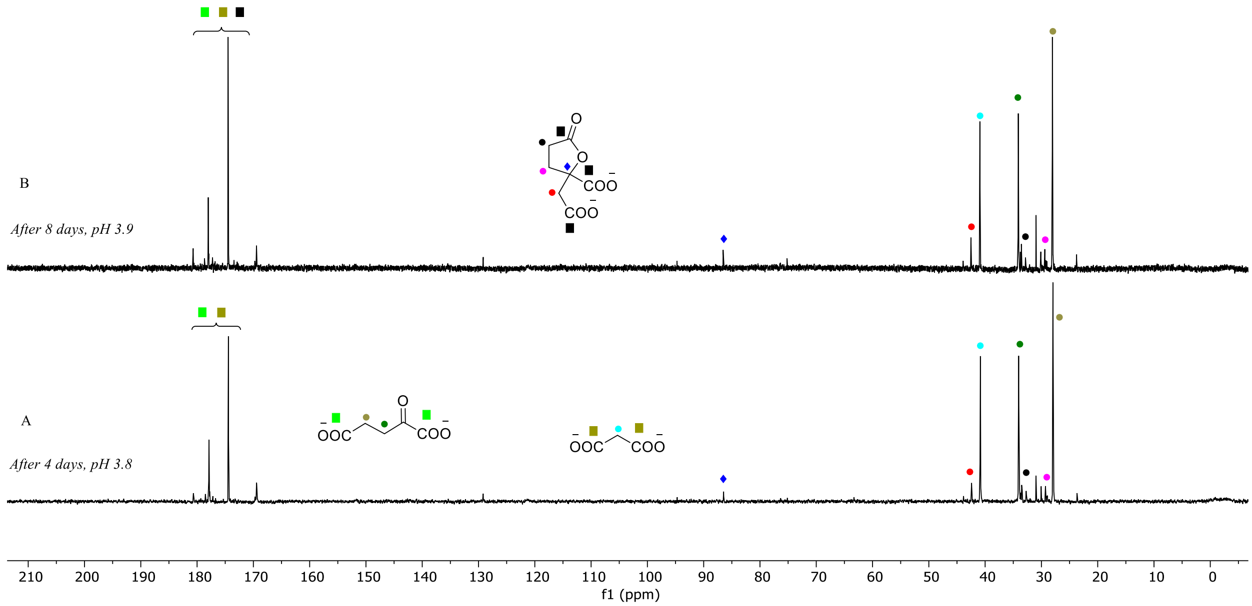
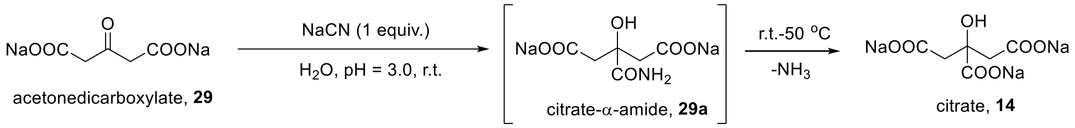


Figure S56: Stacked ^{13}C NMR of the reaction mixture (NMR method 1 in H_2O-D_2O). reaction after 4 days at 50 ºC, pH 3.8 (A). reaction after 8 days at 50 ºC, pH = 3.9 (B).

Acetonedicarboxylate (β-ketoglutarate) 29 to citrate 14:



Scheme S19: Reaction of acetonedicarboxylic acid **29** with NaCN in H_2O showing the formation of citric acid **14** (Figures S57-S58).

Reaction procedure: In an appropriate glass vial (4 mL) equipped with a magnetic stir bar, sodium cyanide (49.0 mg, 1.0 equiv.) was carefully dissolved in deionized and degassed water (2.0 mL) or buffer at 0 °C in fume hood. To this homogeneous solution acetonedicarboxylic acid **29** (146 mg, 1.0 equiv.) was added at 0° C. After addition of acetonedicarboxylic acid, pH of the homogeneous colourless reaction mixture was kept unadjusted. The reaction mixture was stirred at room temperature and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR over a period (Figures S57-58). To enhance the rate of hydrolysis of the amide intermediate **29a** to citrate **14**, temperature of the reaction mixture was raised to 50 °C. pH of the reaction mixture changed over the time (Figures S57-58).

<u>Reaction condition:</u> NaCN (49 mg, 1.0 equiv.), acetonedicarboxylic acid 29 (146 mg, 0.5M, 1 equiv.), H_2O (2 mL).

- <u>After 10 min:</u> ^{1}H NMR (Figure **S57A**) showed starting material peaks along with the formation of cyanohydrin intermediate (pH = 3.3).
- <u>After 14 days:</u> ^{1}H NMR (Figure **S57B**) showed formation of citrate-α-amide **29a** intermediate as the major product along with other minor products (acetone and citramalate-α-amide) coming from mono- and di-decarboxylation of acetonedicarboxylic acid **29** (pH = 4.7).
- <u>After 25 days:</u> temperature of the reaction mixture was raised up to 50 °C (pH 4.8).
- <u>After 30 days:</u> ^{1}H NMR (Figure **S57C**) showed citric acid **14** as the major product and citramalate as the minor product (pH = 5.3). Absolute yield of citrate was found to be 65% (calculated by using an internal standard in ^{1}H NMR experiment).

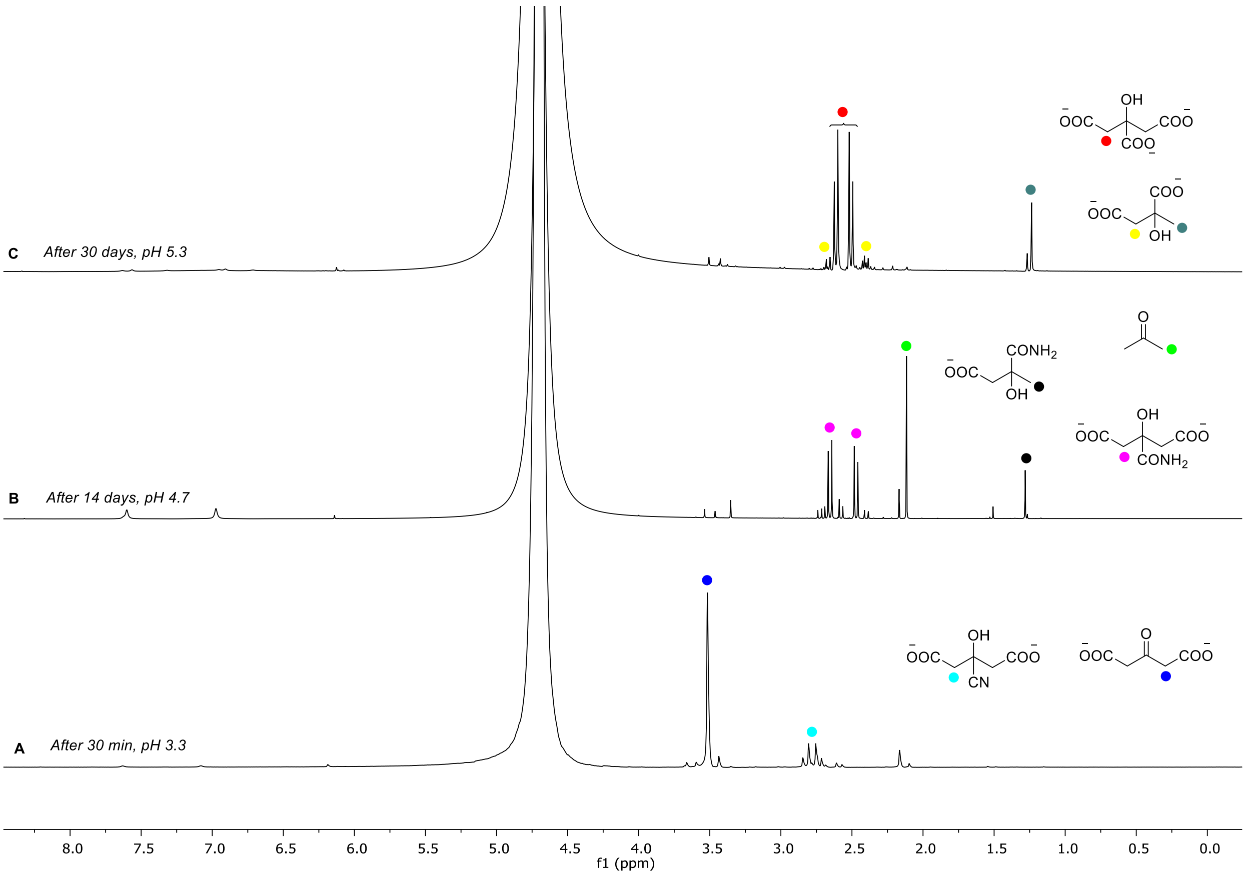


Figure S57: Stacked ^{1}H NMR (H_2O-D_2O) showing progress of the reaction after 30 min (A), 14 days (B), and 30 days (C).

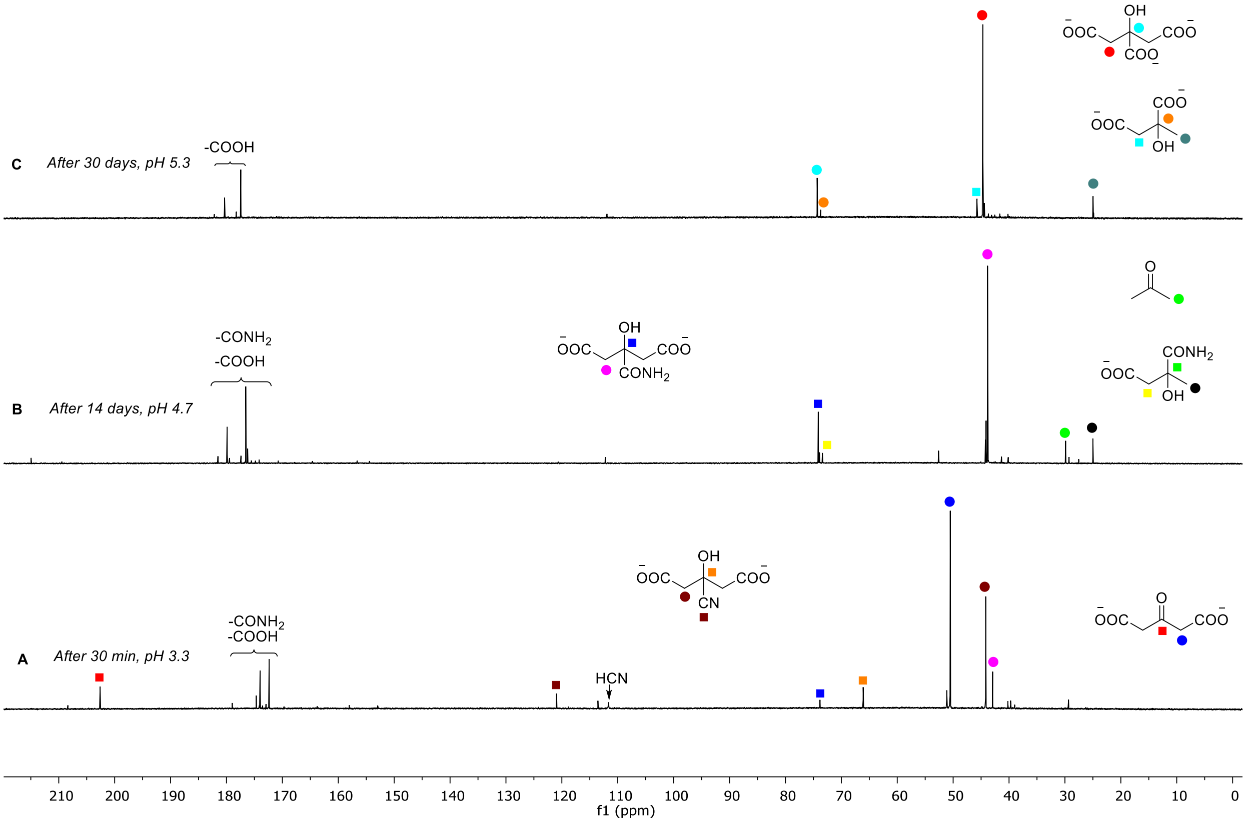
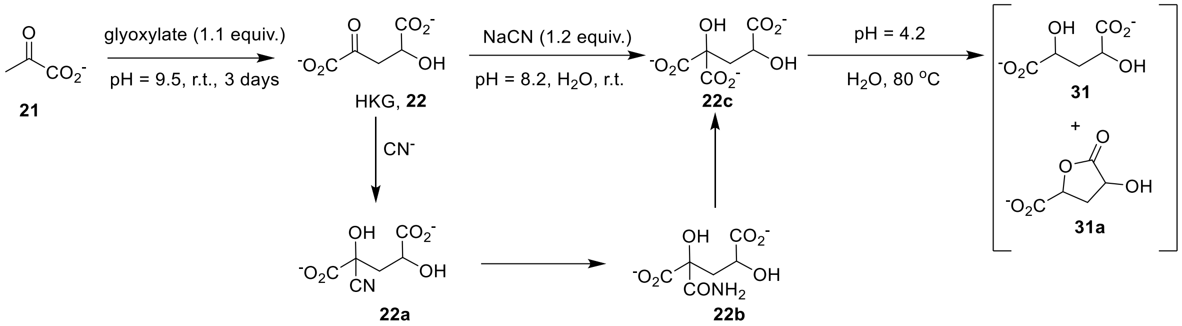


Figure S58: Stacked ^{13}C NMR (H_2O-D_2O) showing progress of the reaction after 30 min (A), 14 days (B), and 30 days (C).

Reaction of maloyl formate (HKG) 22 with NaCN:



Scheme S20: Reaction of maloyl formate **22** with NaCN (Figure S59-S61)

Reaction procedure for maloyl formate (HKG), 22 synthesis (step-I)[2]

In an appropriate glass vial equipped with a magnetic stir bar, pyruvate (220.0 mg, 2.0 mmol, 1.0 equiv.), and glyoxylic acid monohydrate (202 mg, 2.2 mmol, 1.1 equiv.) were dissolved in deionized and degassed water (4.0 mL). pH of the homogenous reaction mixture was adjusted to 9.5 with 5M NaOH and stirred at room temperature for 3-4 days to produce HKG, **22** (68%) (Figure S59).

Reaction procedure for diol-dicarboxylate 31 synthesis (step-II): In a 4.0 mL glass vial equipped with a magnetic stir bar, 1.0 mL of the above reaction mixture containing HKG, **22** (1.0 mL, 0.3 mmol) was taken. To this solution, NaCN (19.6 mg, 0.4 mmol, 1.2 eq) was dissolved carefully. pH of the resulting homogeneous reaction mixture was adjusted to pH 8.2 with 5M HCl solution. The reaction mixture was stirred at room temperature for 21 hours to obtain the tricarboxylate **22c** (Figure S60A).

After observing the complete conversion to tricarboxylate **22c**, pH of the reaction mixture was adjusted to 4.2 with 5M HCl. The resulting homogenous solution was heated to 80 °C and the reaction was monitored by ^{1}H-NMR (NMR method 1 in H_2O-D_2O) (Figure S61, 62B-C).

<u>Reaction conditions for step-I:</u> pyruvate 21 (220 mg, 2.0 mmol), glyoxylic acid monohydrate (202 mg, 2.2 mmol, 1.1 equiv.), and H_2O (4 mL).

- <u>After 3 days:</u> ^{1}H NMR (Figure **S59**) showed the formation of HKG **22**. Absolute yield of HKG **22** w.r.t. *t*-BuOH is 68%.

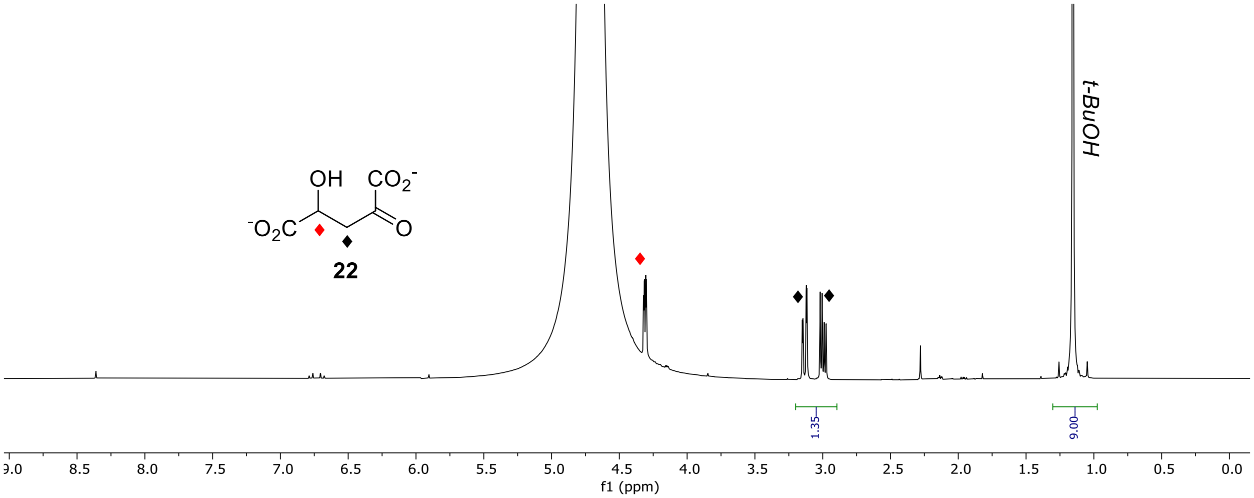


Figure S59: ^{1}H NMR of the reaction mixture after 3 days at pH = 9.5; (NMR method 2 in D_2O containing equimolar amount (0.05 mmol) of *t*-BuOH w.r.t. pyruvate **21**). Absolute yields were calculated based upon the ^{1}H NMR peaks integration of product (HKG **22**) i.e., peaks (~ 3.2 ppm) referred as ♦ w.r.t. *t*-BuOH peak (~ 1.2 ppm). Yield of HKG, **22** is 68%.

<u>Reaction conditions for step-II:</u> HKG 22 (1.0 mL, 0.3 mmol), NaCN (19.6 mg, 0.4 mmol, 1.2 equiv.)

- <u>After 21 hours at room temperature:</u> ^{1}H and ^{13}C NMR (Figure **S60A**) showed formation of tricarboxylate, **22c** as the major product (pH = 8.2).
- <u>After 21 hours:</u> pH of the reaction mixture was adjusted to 4.3 with 5M HCl. Then, the reaction mixture was heated to 80 ^{o}C.
- <u>After 48 hours:</u> ^{1}H and ^{13}C NMR showed formation of a mixture of lactone, **31a** as a major product (pH = 4.3) (Figure **S60B**). ESI-MS m/z calculated for **31** $C_5H_8O_6$ [M-H]- 163.0248, found 163.0246, calculated for **31a** $C_5H_6O_5$ [M-H]- 145.0142, found 145.0142.
- <u>After 8 days:</u> ^{1}H and ^{13}C NMR showed formation of a mixture of lactone, **31a** as a minor product and dicarboxylated compound, **31** (as an enantiomer) as a major product (27%) (pH = 4.5) (Figure **S60C**, **S61**, **S62B**).

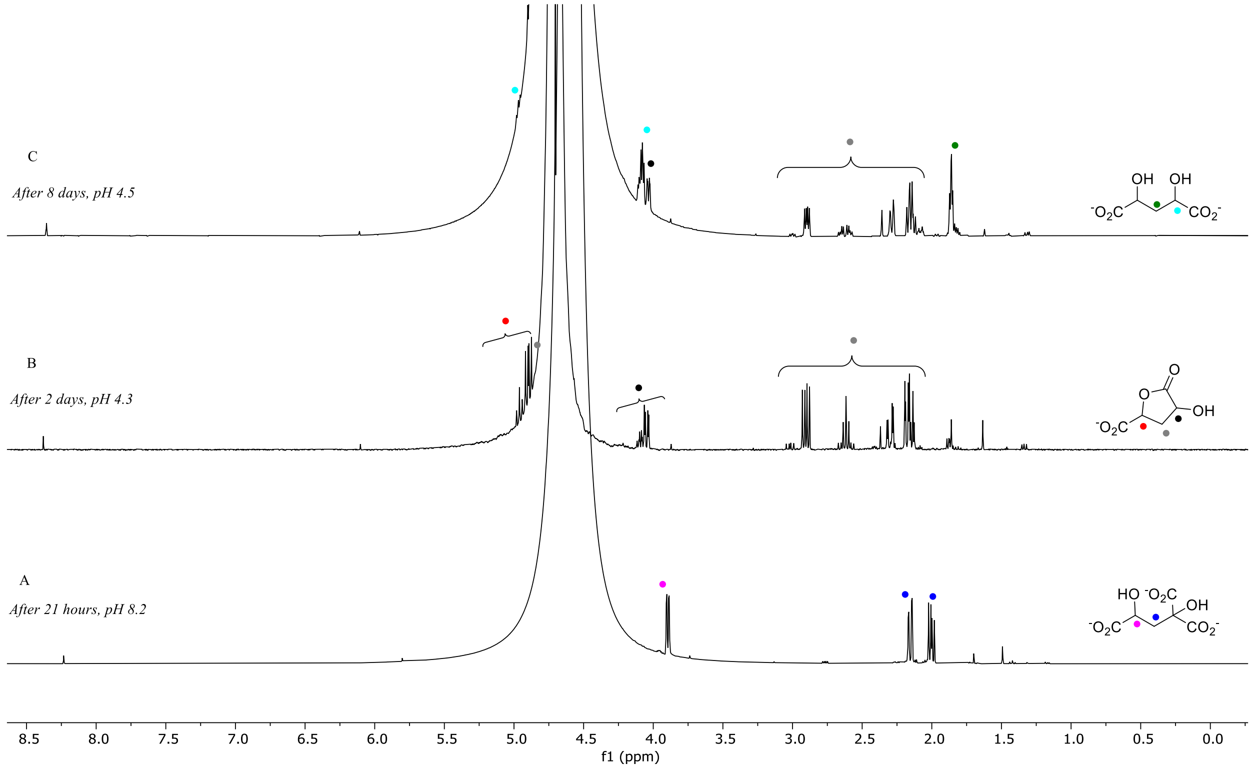


Figure S60: Stacked ^{1}H NMR of the reaction mixture (NMR method 1 in H_2O-D_2O for A and C; NMR method 2 in D_2O for B); reaction after 48 hours at pH 8.8 (A), reaction after 48 hours at 80 ^{o}C, pH 4.3 (B), reaction after 8 days at 80 ^{o}C, pH 4.5 (C).

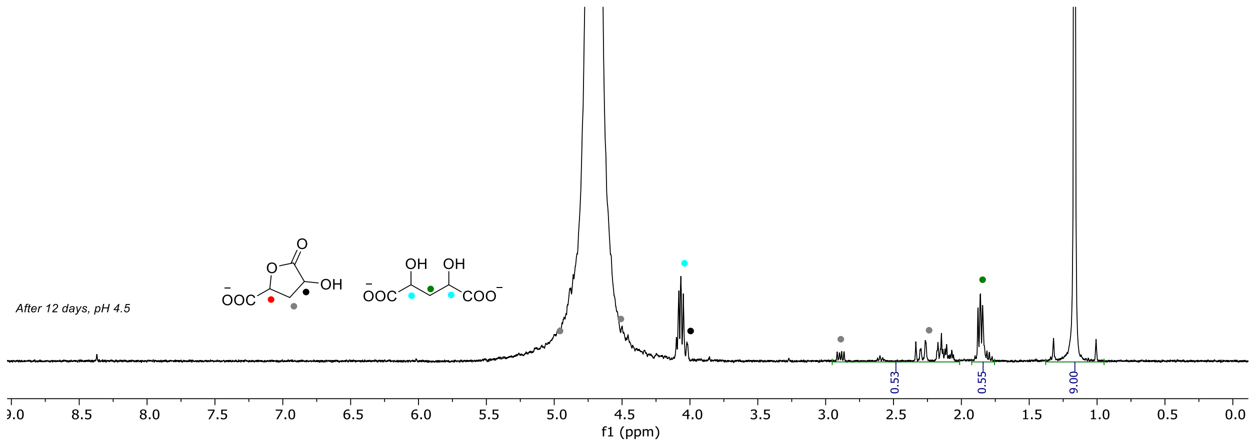


Figure S61: ^{1}H NMR of the reaction mixture (NMR method 2 in D_2O containing equimolar amount (0.022 mmol) of *t*-BuOH w.r.t HKG); reaction after 12 days at pH 4.5. Yield of the symmetrical diol, **31** is 27% (based on the ^{1}H NMR integration of the product symmetrical diol **31** (peak at ~1.6 ppm, referred as ●) w.r.t. *t*-BuOH (peak at ~1.2 ppm)) and yield of mixture of lactones (diastereomers) is 26% (based on the ^{1}H NMR integration of the product lactone **31a** (peaks from 3.0-2.0 ppm, referred as ●) w.r.t. *t*-BuOH (peak at ~1.2 ppm))

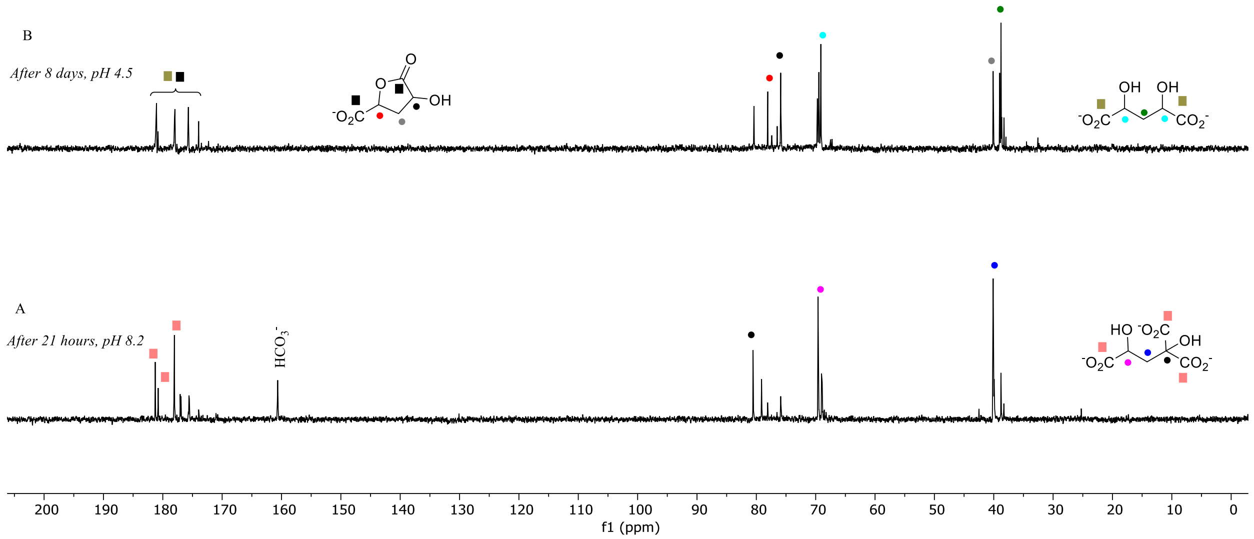
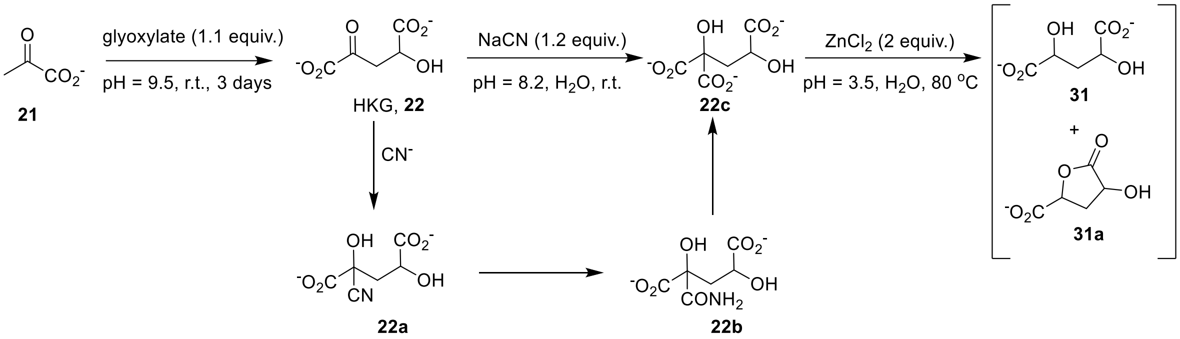


Figure S62: Stacked ^{13}C NMR of the reaction mixture (NMR method 1 in H_2O-D_2O); reaction after 21 hours at pH 8.2 (A), reaction after 8 days at 80 °C, pH = 4.5 (B)

Reaction of maloyl formate (HKG), 22 with NaCN and $ZnCl_2$ (2 equiv.):



Scheme S21: Reaction of maloyl formate **22** with NaCN and $ZnCl_2$ (2 equiv.) (Figures S63-S64).

Reaction procedure for the synthesis of diol-dicarboxylate 31 and lactone 31a (step-II): In a 4.0 mL glass vial equipped with a magnetic stir bar, 1.0 mL of the step 1 reaction mixture containing HKG **22** (1.0 mL, 0.3 mmol, from Scheme S20) was taken. To this solution, NaCN (19.6 mg, 0.4 mmol, 1.2 equiv.) was dissolved carefully. pH of the resulting homogeneous reaction mixture was adjusted to pH 8.2 with 5M HCl solution. The reaction mixture was stirred at room temperature for 21 hours.

After observing the complete conversion to tricarboxylate **22c**, anhyd. $ZnCl_2$ (45.4 mg, 0.6 mmol, 2 equiv.) was added and adjusted the pH to 3.5 with 5M HCl. The resulting homogenous solution was heated to 80 °C and the reaction was monitored by ^{1}H-NMR (NMR method 1 in H_2O-D_2O) (Figures S63-64).

***Reaction conditions for step-II:* HKG 22 (1.0 mL, 0.3 mmol), NaCN (19.6 mg, 0.4 mmol, 1.2 equiv.), and anhyd. $ZnCl_2$ (45.4 mg, 0.6 mmol, 2 equiv.)**

- After 21 hours at room temperature: ^{1}H and ^{13}C NMR (Figure **S63A**) showed formation of tricarboxylate **22c,** as the major product (pH = 8.2)
- After 21 hours: $ZnCl_2$ (45.4 mg, 0.6 mmol, 2 equiv.) was added and adjusted the pH to 3.5 with 5M HCl. Then, the reaction mixture was heated to 80 °C
- After 6 days: ^{1}H and ^{13}C NMR (Figure **S63B**) showed formation of lactone, **31a** as a major product (as a mixture of diastereomer) (pH = 3.5) (Yield of the reaction was not calculated because of the precipitation with $ZnCl_2$)

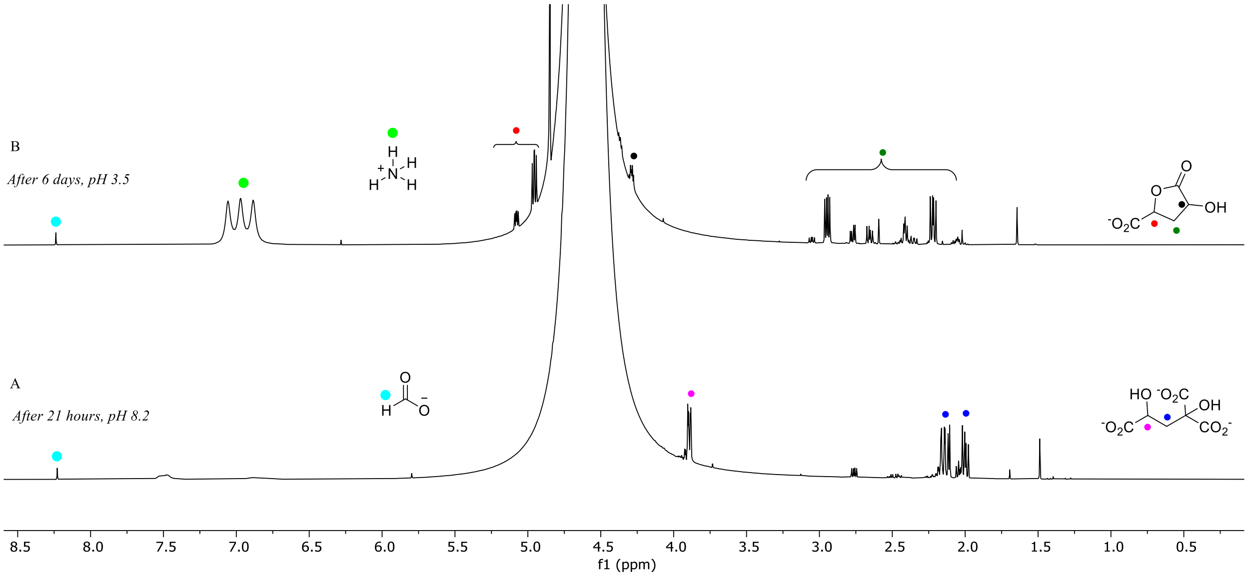


Figure S63: Stacked ^{1}H NMR of the reaction mixture (NMR method 1 in H_2O-D_2O); reaction after 21 hours at pH 8.2 (A), reaction with $ZnCl_2$ (2 eq.) after 6 days at 80 °C, pH = 3.5 (B).

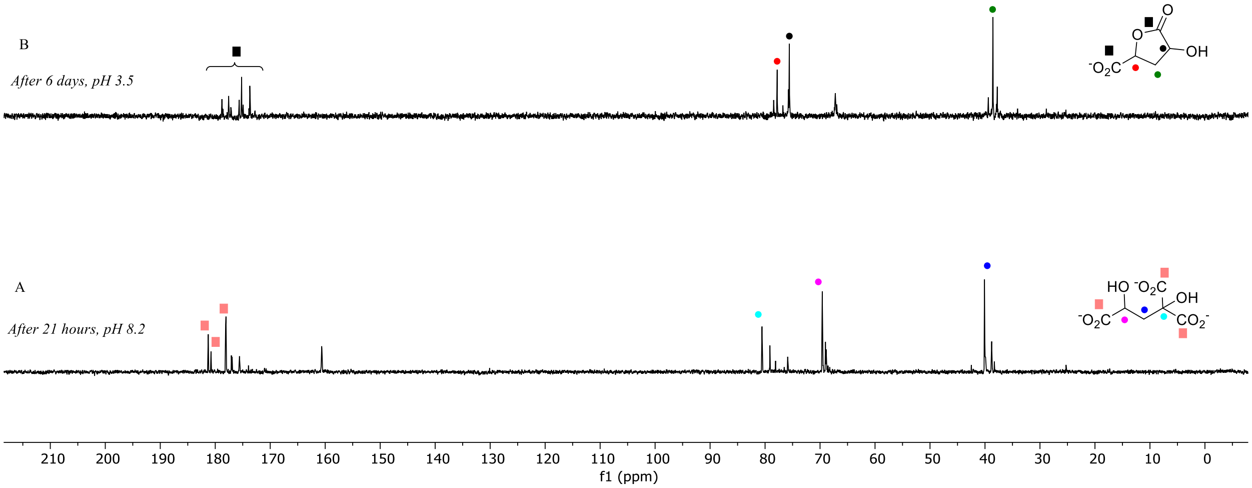
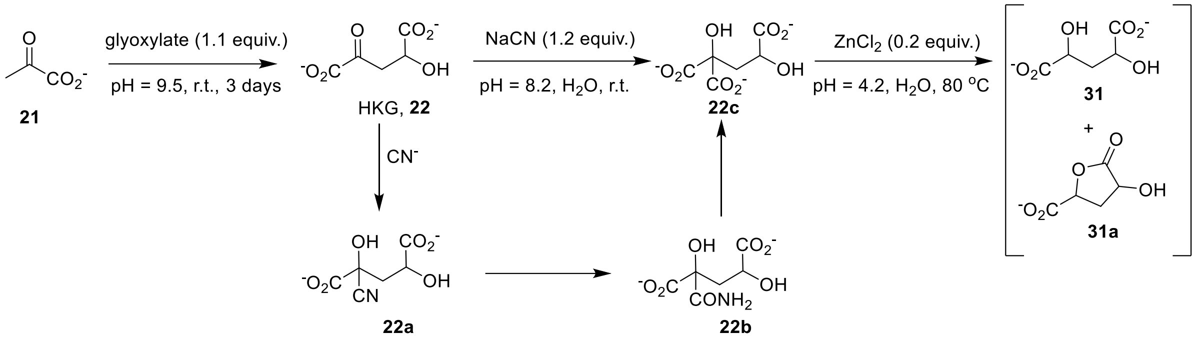


Figure S64: Stacked ^{13}C NMR of the reaction mixture (NMR method 1 in H_2O-D_2O); reaction after 21 hours at pH 8.2 (A), reaction with $ZnCl_2$ (2 eq.) after 6 days at 80 °C, pH 3.5 (B).

Reaction of maloyl formate (HKG) 22 with NaCN and $ZnCl_2$ (0.2 equiv.):



Scheme S22: Reaction of maloyl formate **22** with NaCN and $ZnCl_2$ (0.2 equiv.) (Figure S65-S66).

Reaction procedure for the synthesis of diol-dicarboxylate 31 and lactone 31a (step-II): In a 4.0 mL glass vial equipped with a magnetic stir bar, 1.0 mL of the step 1 reaction mixture containing HKG **22** (1.0 mL, 0.3 mmol, from Scheme S20). To this solution, NaCN (19.6 mg, 0.4 mmol, 1.2 eq) was dissolved carefully. pH of the resulting homogeneous reaction mixture was adjusted to pH 8.2 with 5M HCl solution. The reaction mixture was stirred at room temperature for 21 hours.

After observing the complete conversion to tricarboxylate **22c**, anhyd, $ZnCl_2$ (5.45 mg, 0.06 mmol, 0.2 equiv.) was added and adjusted the pH to 4.2 with 5M HCl. The resulting homogenous solution was heated to 80 °C and the reaction was monitored by ^{1}H-NMR (NMR method 1 in H_2O-D_2O) (Figures S65-S66).

<u>Reaction conditions for step-II:</u> HKG 22 (1.0 mL, 0.3 mmol), NaCN (19.6 mg, 0.4 mmol, 1.2 equiv.), and anhyd. $ZnCl_2$ (5.45 mg, 0.06 mmol, 0.2 equiv.)

- After 21 hours at room temperature: ^{1}H and ^{13}C NMR (Figure **S65A**) showed formation of tricarboxylate, **22c** as the major product (pH = 8.2).
- After 21 hours: $ZnCl_2$ (5.45 mg, 0.06 mmol, 0.2 eq.) was added and adjusted the pH to 4.2 with 5M HCl. Then, the reaction mixture was heated to 80 °C.
- After 2 days: ^{1}H and ^{13}C NMR (Figure **S65B**) showed formation of a mixture of lactone,**31a** and dicarboxylated compound, **31** (as an enantiomer) (pH = 4.3).
- After 8 days: ^{1}H and ^{13}C NMR (Figure **S65C**) showed formation of a mixture of lactone, **31a** as a minor product and dicarboxylated compound, **31** (as an enantiomer) as a major product (pH = 4.5) (Yield of the reaction was not calculated because of the precipitation with $ZnCl_2$).

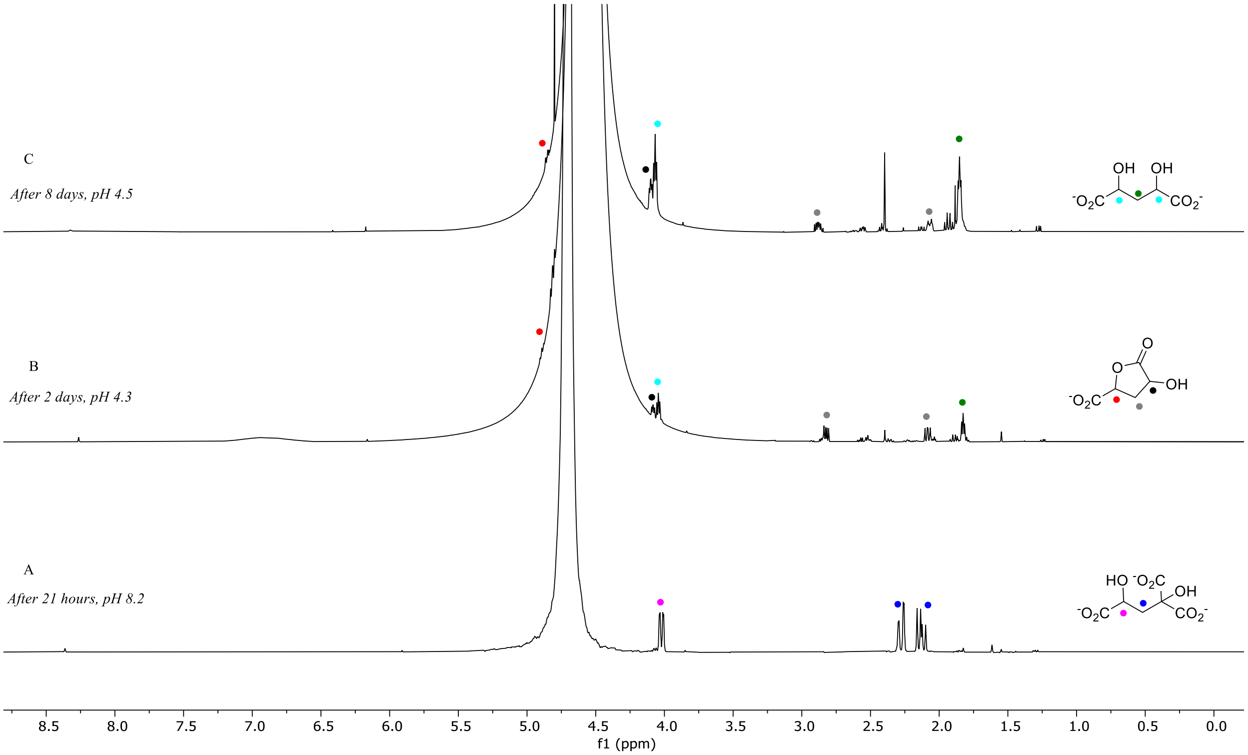


Figure S65: Stacked ^{1}H NMR of the reaction mixture (NMR method 2 in D_2O for A; NMR method 1 in H_2O-D_2O for B and C); reaction after 21 hours at pH 8.2 (A), reaction with $ZnCl_2$ (0.2 equiv.) after 2 days at 80 °C, pH 4.3 (B), reaction with $ZnCl_2$ (0.2 equiv.) after 8 days at 80 °C, pH = 4.5 (C).

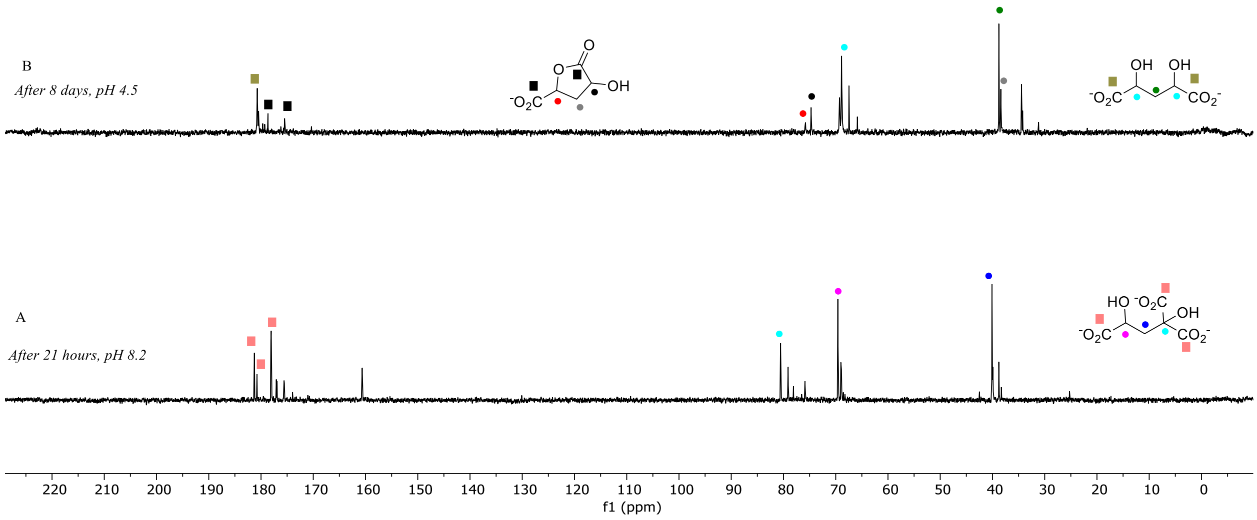
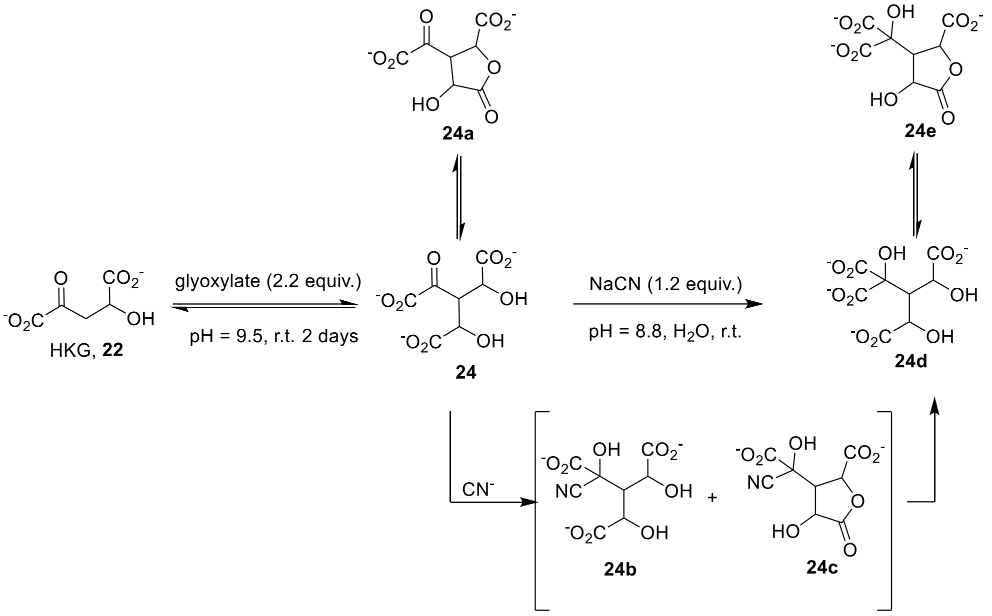


Figure S66: Stacked ^{13}C NMR of the reaction mixture (NMR method 1 in H_2O-D_2O); reaction after 21 hours at pH 8.2 (A), reaction with $ZnCl_2$ (0.2 equiv.) after 8 days at 80 ºC, pH = 4.5 (B).

Reaction of di-HKG 24 with NaCN:



Scheme S23: Reaction of di-HKG **24** with NaCN at pH = 8.8, r.t. in H_2O (Figures S67-68)

Reaction procedure for di-HKG 24 synthesis (step-I): In a 4.0 mL glass vial equipped with a magnetic stir bar, 1.0 mL of HKG prepared solution (1.0 mL, 0.226 mmol, 1.0 equiv.) (as described in step 1, scheme S20) was taken. To this solution, glyoxylic acid monohydrate (46.0 mg, 0.5 mmol, 2.2 equiv.) was dissolved. pH of the homogenous reaction mixture was adjusted to 9.5 with 5M NaOH and stirred at room temperature for 2 days to form a mixture of di-HKG **24** and lactone **24a** in 90% conversion (Figures S67-68).

Reaction procedure for tetracarboxylate 24d and lactone 24e synthesis (Step-II): In a 4.0 mL glass vial equipped with a magnetic stir bar, 0.4 mL of above solution containing a mixture of di-HKG **24** and lactone **24a** (0.4 mL, 0.3 mmol, 1 equiv.) was taken in deionized and degassed water (0.2 mL). To this solution, NaCN (17.64 mg, 0.36 mmol, 1.2 equiv.) was carefully dissolved. pH of the resulting mixture was adjusted to 8.5 with 5N NaOH solution. The resulting homogenous solution was stirred at room temperature and the reaction was monitored by ^{1}H and ^{13}C NMR (NMR method 1 in H_2O-D_2O) (Figures S69-70).

<u>Reaction conditions for step-I:</u> HKG (1.0 mL, 0.226 mmol), and glyoxylic acid monohydrate (46 mg, 0.5 mmol, 2.2 equiv.)

- <u>After 2 days at r.t</u>., showed the formation of di-HKG **24** and its lactone **24a** (pH = 9.5) % conversion to di-HKG, **24** and lactone, **24a** is 90%. (Figure **S67-68**).

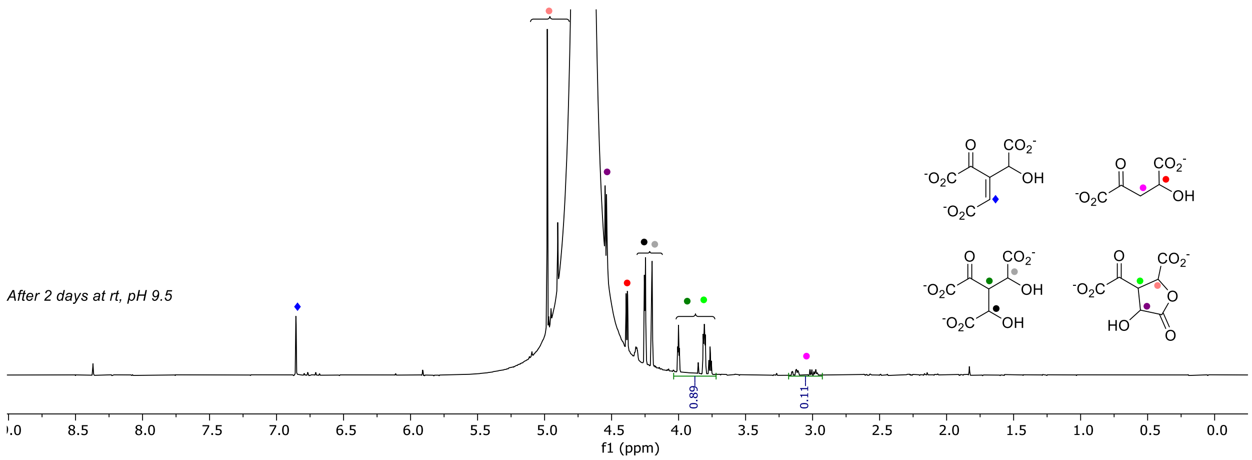


Figure S67: ^{1}H NMR of the reaction mixture after 2 days at pH = 9.5; (NMR method 1 in H_2O-D_2O). % conversion was calculated based upon the ^{1}H NMR peaks integration of a mixture of product (di-HKG **24**) and lactone i.e., peaks (3.7-4.0 ppm) referred as ● ● w.r.t. HKG **22** (3.2-2.9 ppm) referred as ●. % conversion to di-HKG, **24** and lactone, **24a** is 90%.

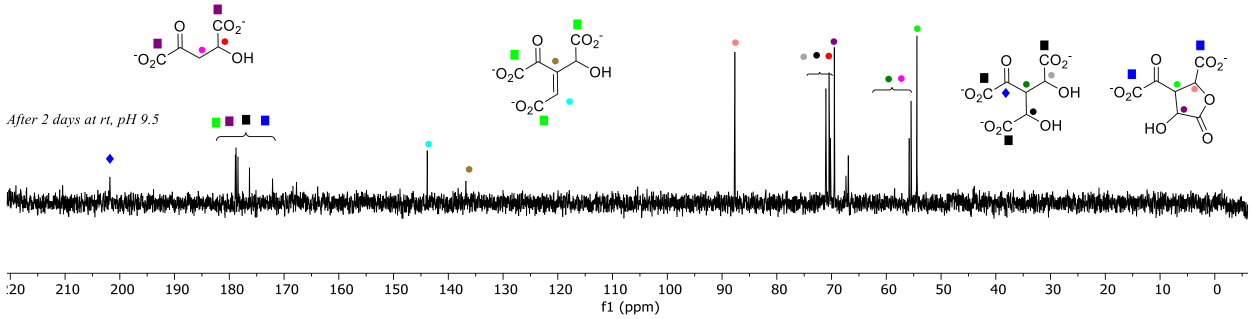


Figure S68: ^{13}C NMR of the reaction mixture after 2 days at pH = 9.5; (NMR method 1 in H_2O-D_2O).

***Reaction conditions for step-II:* di-HKG 24 reaction mixture (0.4 mL, 0.3 mmol), NaCN (17.64 mg, 0.36 mmol, 1.2 equiv.) and H_2O (0.2 mL)**

- After 24 hours at r.t., showed the formation of cyanohydrins **24b** and **24c** (pH = 8.8) (Figure **S69-70A**).
- After 7 days at r.t., showed the formation of a mixture of tetracarboxylate, **24d** and lactone, **24e** (pH = 8.9). Yield of the reaction was not calculated because of the complexity (Figure **S69-70A**).
- Stirring the reaction further at pH = 4.0, 80 ºC, increased the complexity in NMR analysis.
- Assignment of the product structures is approximate as there was assigned based on the previous reaction's NMR pattern knowledge and Chemdraw predictions.

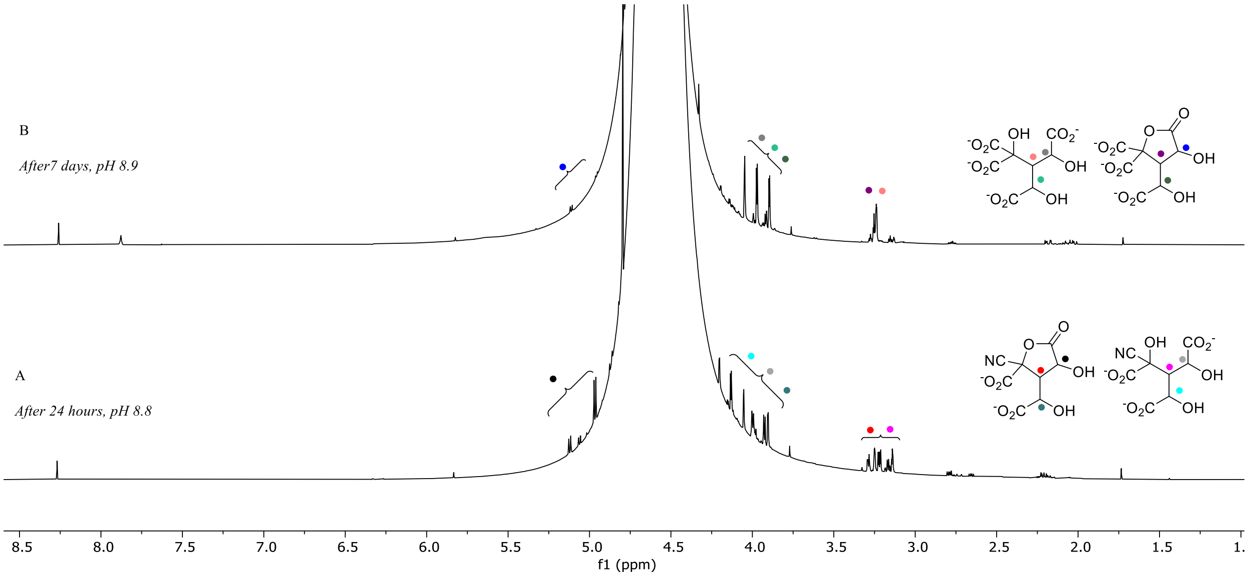


Figure S69: Stacked ^{1}H NMR of the reaction mixture (NMR method 1 in H_2O-D_2O). reaction after 24 hours at pH = 8.8 (A), reaction after 7 days, pH = 8.9 (B).

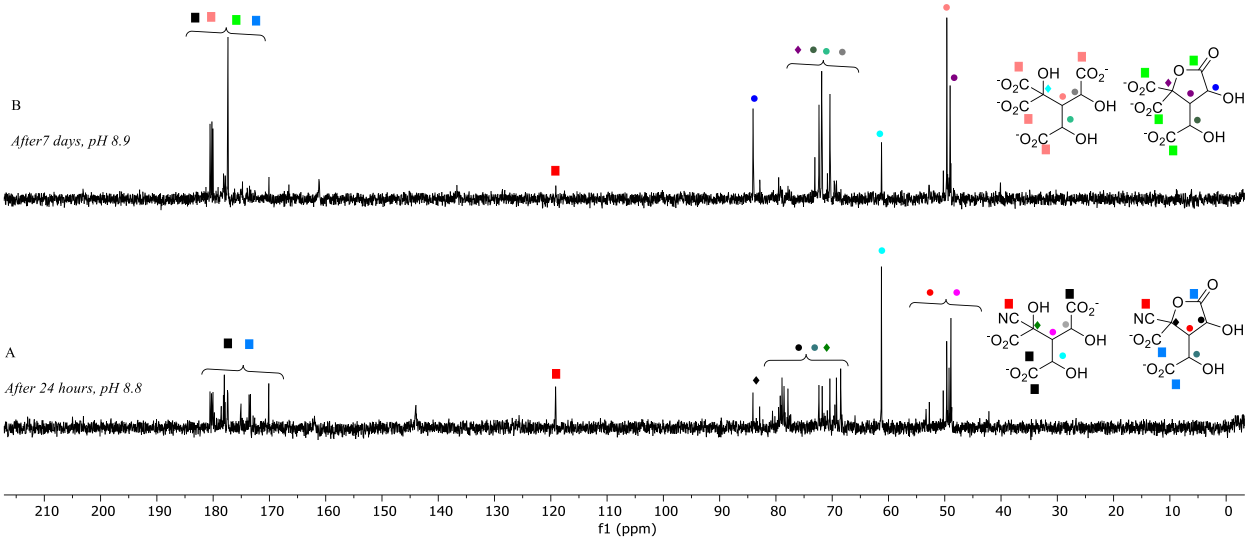
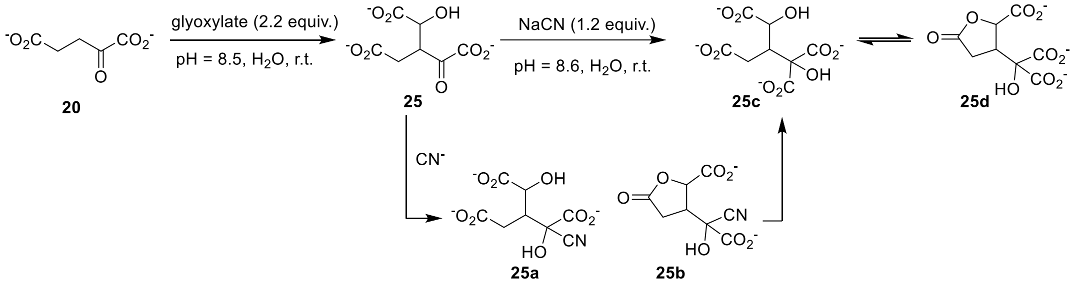


Figure S70: Stacked ^{13}C NMR of the reaction mixture (NMR method 1 in H_2O-D_2O). reaction after 24 hours at pH = 8.8 (A), reaction after 7 days, pH = 8.9 (B).

Reaction of isocitroyl formate, 25 with NaCN:



Scheme S24: Reaction of isocitroyl formate **25** with NaCN at pH = 8.6, r.t. in H_2O (Figures S71-72)

Reaction procedure for isocitroyl formate 25 synthesis (step-I): In a 4.0 mL glass vial equipped with a magnetic stir bar, α-ketoglutarate **20** (73 mg, 0.5 mmol, 1.0 equiv.), and glyoxylic acid monohydrate (46 mg, 0.5 mmol, 2.2 equiv.) were dissolved in deionized and degassed water (1.0 mL). pH of the homogenous reaction mixture was adjusted to 8.5 with 5M NaOH and stirred at room temperature for 2 days to form a mixture of *threo*- and *erythro*- isomers of isocitroyl formate **25** in 95% conversion (Figures S71-72).

Reaction procedure for step-II (reaction of isocitroyl formate 25 with NaCN): (step 2) In a 4.0 mL glass vial equipped with a magnetic stir bar, 0.4 mL of above reaction mixture containing 0.49M of isocitroyl formate **25** (mixture of *threo* and *erythro* isomers) (0.4 mL, 0.2 mmol, 1 equiv.) was taken in deionized and degassed water (0.6 mL). To this, NaCN (12 mg, 0.24 mmol, 1.2 equiv.) was carefully added and dissolved. pH of the resulting mixture was adjusted to 8.5 with 5N HCl solution. The resulting homogenous solution was heated to room temperature and the reaction was monitored by ^{1}H and ^{13}C NMR (NMR method 1 in H_2O-D_2O) (Figures S73-S74).

***Reaction conditions for step-I:* α-ketoglutarate 20 (73 mg, 0.5 mmol, 1.0 equiv.), glyoxylic acid monohydrate (46 mg, 0.5 mmol, 2.2 equiv.), and H_2O (1.0 mL)**

- After 2 days at r.t., showed the formation of isocitroyl formate **25** (pH = 8.5) % conversion to isocitroyl formate **25** w.r.t. α-ketoglutarate is 95%. (Figure **S71-72**).

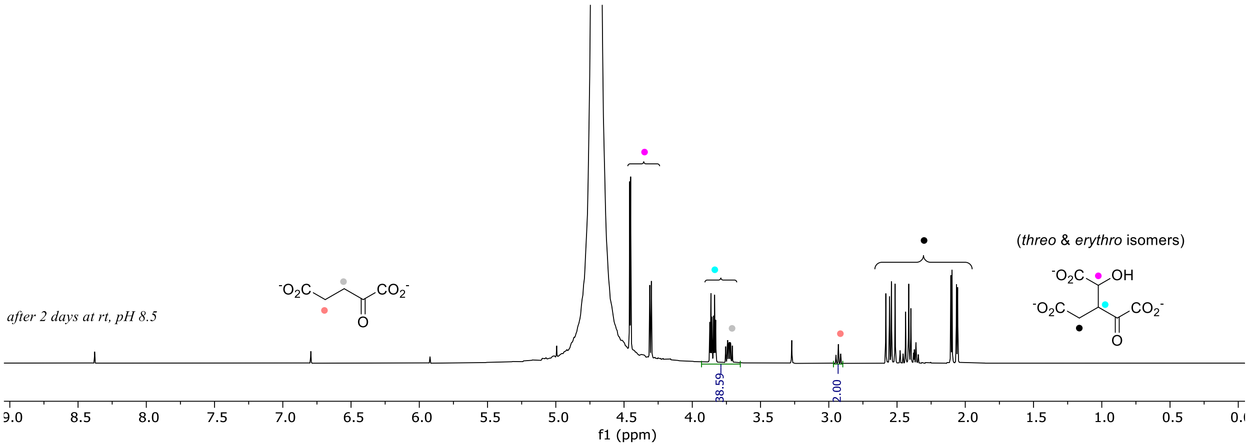


Figure S71: ^{1}H NMR of the reaction mixture after 2 days at pH = 5.5; (NMR method 2 in D_2O). % conversion was calculated based upon the ^{1}H NMR peaks integration of a mixture of product (mixture of *threo*- and *erythro* isocitroyl formate isomers, **25**) (3.9-3.65 ppm) referred as ● w.r.t. α-ketoglutarate (~2.9 ppm) referred as ●. % conversion to isocitroyl formate **25** is 95%.

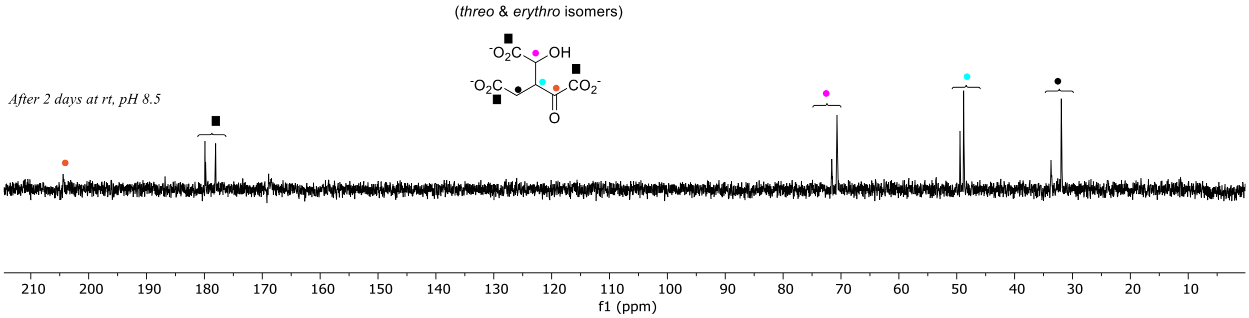


Figure S72: ^{13}C NMR of the reaction mixture after 2 days at pH 5.5 (NMR method 2 in D_2O).

<u>Reaction conditions for step-II:</u> isocitroyl formate 25 reaction mixture (0.4 mL, 0.2 mmol), NaCN (12 mg, 0.24 mmol, 1.2 equiv.), and H_2O (0.6 mL)

- <u>After 10 min at r.t.,</u> ^{1}H NMR showed the formation of cyanohydrin, **25a** and lactone **25b** (pH = 8.6) (Figure **S73A**).
- <u>After 48 hours at r.t.</u>, showed the formation of a mixture of tetracarboxylate, **25c** and lactone, **25d**. (pH = 8.5). Yield of the reaction was not calculated because of the complexity. (Figure **S73B**).
- Reaction did not progress further on stirring for more time or increasing the temperature to 80 °C and lowering the pH to 4.0.
- Assignment of the product structures is approximate as there was assigned based on the previous reaction's NMR pattern knowledge and Chemdraw predictions.

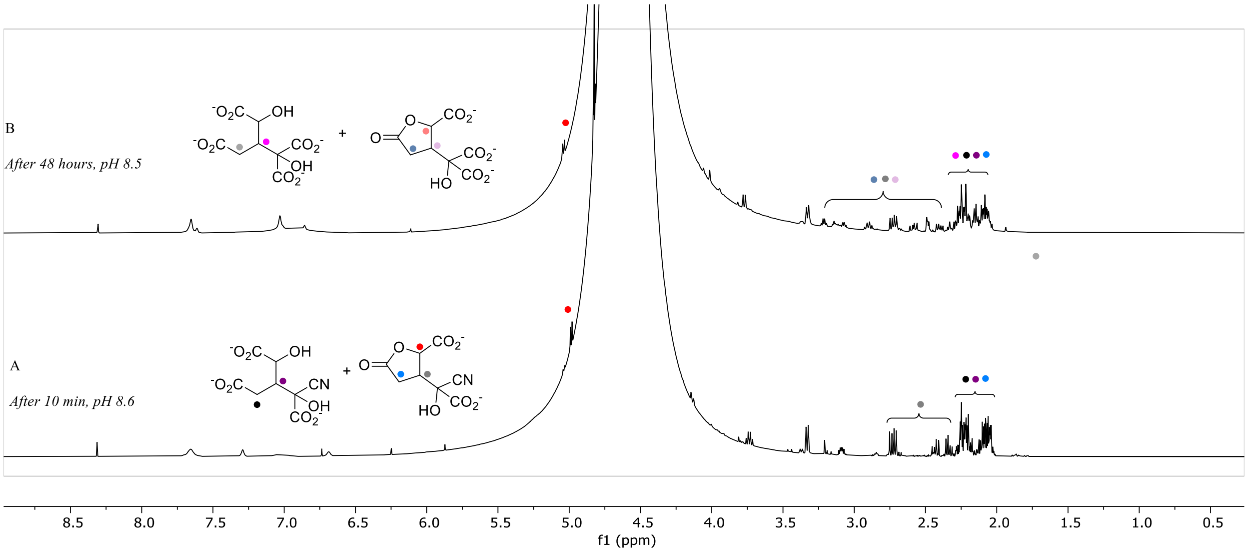


Figure S73: Stacked ^{1}H NMR of the reaction mixture (NMR method 1 in H_2O-D_2O). reaction after 10 min at pH = 8.6 (A), reaction after 48 hours, pH = 8.5 (B).

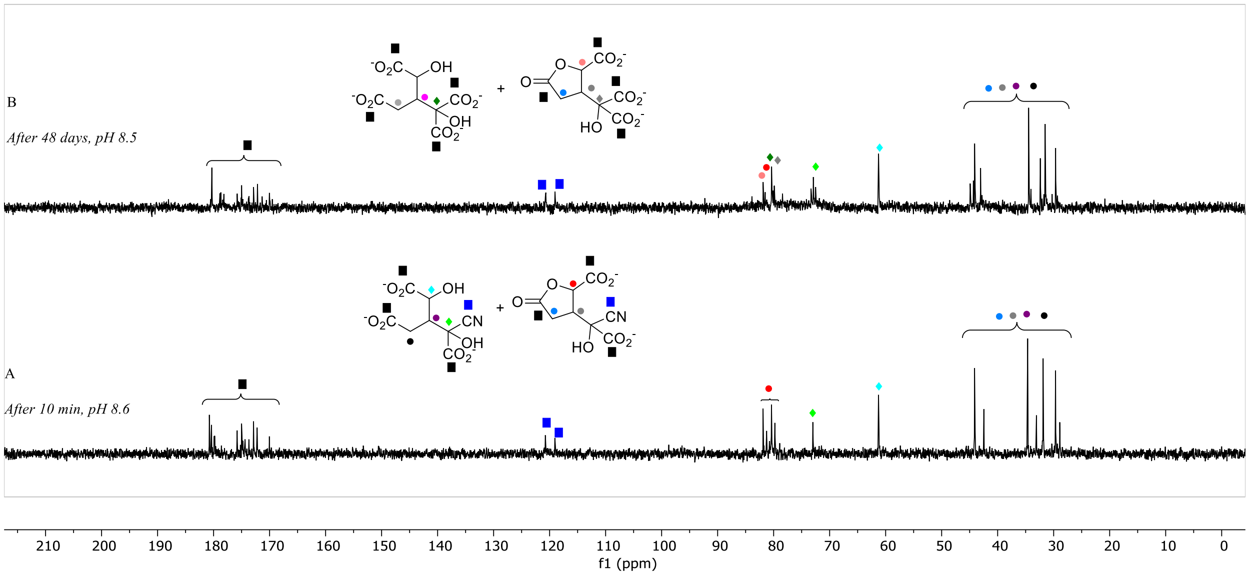
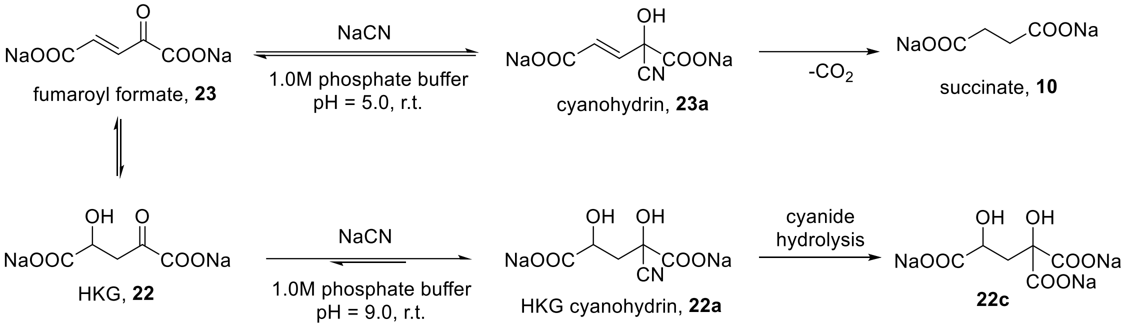


Figure S74: Stacked ^{13}C NMR of the reaction mixture (NMR method 1 in H_2O-D_2O). reaction after 10 min at pH = 8.6 (A), reaction after 48 hours, pH = 8.5 (B).

Fumaroyl formate 23 to succinate 10:



Scheme S25: Reaction of fumaroyl formate **23** with NaCN in 1M phosphate buffer (also works in H_2O) showing the formation of succinate **10** via cyanide mediated pathways (Figures S75-S78).

Reaction procedure for synthesis of fumaroyl formate 23 from HKG 22 (step I): In a 4 mL glass vial equipped with a magnetic stir bar, 2 mL of 0.5 M HKG **22** (produced by known procedure in 1.0 M phosphate buffer)[2] solution was taken, and pH of the reaction mixture was adjusted to 8.0 with 5M NaOH. The reaction mixture was stirred at 60 ^{o}C for 24h. After 24h, ^{1}H NMR analysis of the reaction mixture showed the formation of fumaroyl formate **23** (almost 1:2 ratio with HKG), see NMR figure **S75A**. This reaction mixture was directly used for the further reactions (fumaroyl formate **23** to succinate **10**).

Reaction procedure for fumaroyl formate 23 to 22b (step IIa): 1 mL of the above reaction mixture (0.5 M, containing mixture of fumaroyl formate **23** and HKG **22** (approximately 1:2 ratio) taken in a 4 mL glass vial equipped with a stir bar and NaCN (25 mg, 1.0 equiv) was added to it. pH of the reaction mixture was adjusted to 9.0 (with 5M NaOH) and the reaction mixture was stirred at rt. Progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR over a period (Figure S75-76).

Reaction procedure for fumaroyl formate to succinate (step IIb): 1 mL of the above reaction mixture (0.5 M, containing mixture of fumaroyl formate **23** and HKG **22** (approximately 1:2 ratio) taken in a 4 mL glass vial equipped with a stir bar and NaCN (25 mg, 1.0 equiv) was added to it. pH of the reaction mixture was adjusted to 5.0 (with 5M HCl) and the reaction mixture was stirred at rt. Progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR over a period (Figure S77-78).

<u>Reaction Conditions (Step IIa:</u> fumaroyl formate 23 from step I (0.5 mmol, 1 mL, 1.0 equiv.), NaCN (25 mg, 1.0 equiv.), pH = 9.0.

- <u>After 3 days:</u> ^{1}H NMR showed formation of product **22b** as a sole product, Figure **S75B** (pH = 9.0).
- <u>After 6 days:</u> ^{1}H NMR didn't show any remarkable change from day 3-6, Figure S**75C** (pH = 9.0).

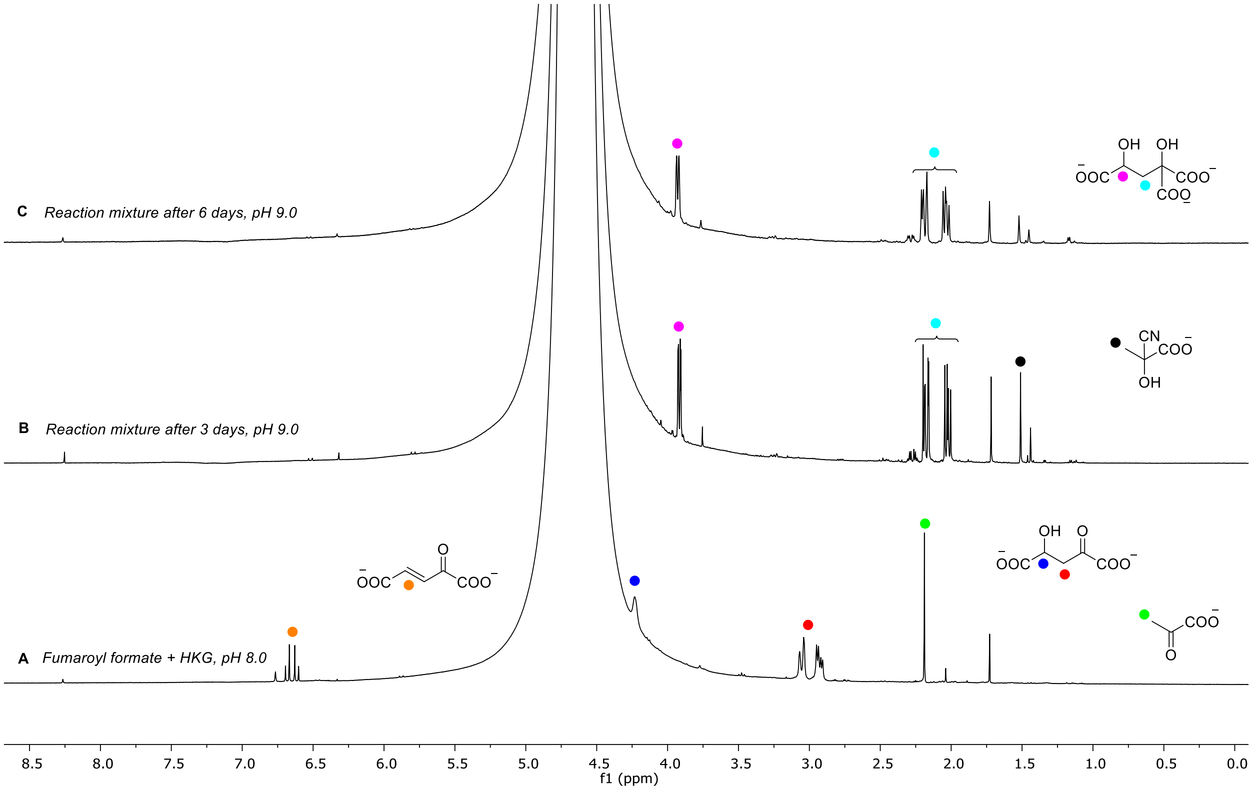


Figure S75: Stacked ^{1}H NMR (H_2O-D_2O) showing starting material i.e., from step I (A), and progress of the reaction after 3 days (B), and 6 days (C).

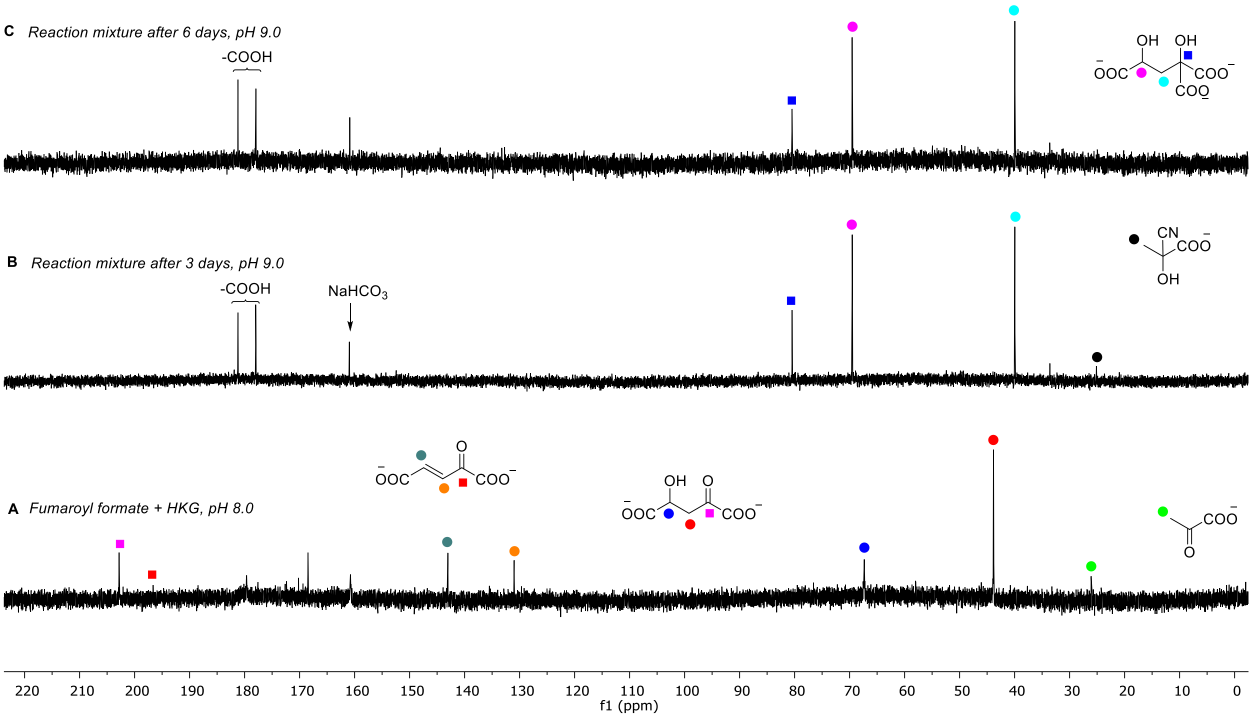


Figure S76: Stacked ^{13}C NMR (H_2O-D_2O) showing starting material i.e., from step I (A), and progress of the reaction after 3 days (B), and 6 days (C).

<u>Reaction Conditions (Step IIb:</u> fumaroyl formate 23 from step I (0.5 mmol, 1 mL, 1.0 equiv.), NaCN (25 mg, 1.0 equiv.), pH = 5.0.

- <u>After 3 days:</u> ^{1}H NMR showed formation of product **22b**, succinate **10** and pyruvate cyanohydrin, Figure **S77B** (pH = 5.0).
- <u>After 6 days:</u> ^{1}H NMR didn't show any remarkable change from day 3-6, Figure **S77C** (pH = 5.0). Conversion ratio of succinate **10** was found to be 15% (calculated based upon ^{1}H NMR peak integration of all the molecules). Formation of succinate was also confirmed by ^{1}H NMR spiking (with authentic succinate) experiment (Figure **S78D**).

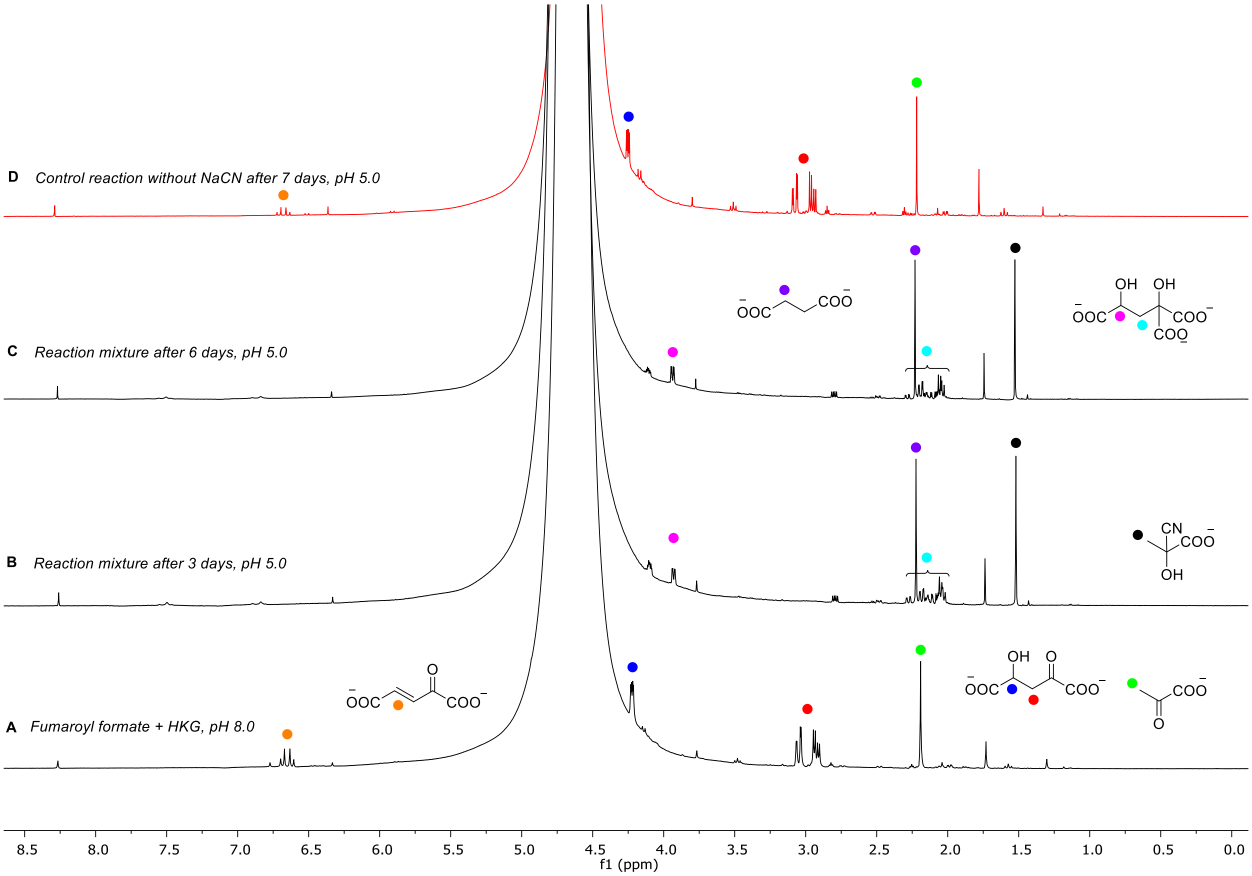


Figure S77: Stacked ^{1}H NMR (H_2O-D_2O) showing starting material i.e., from step I (A), and progress of the reaction after 3 days (B), and 6 days (C) compared with a control reaction in absence of NaCN at pH = 5.0 in 1.0M phosphate buffer after 7 days showing no or very little formation of succinate **10** (D).

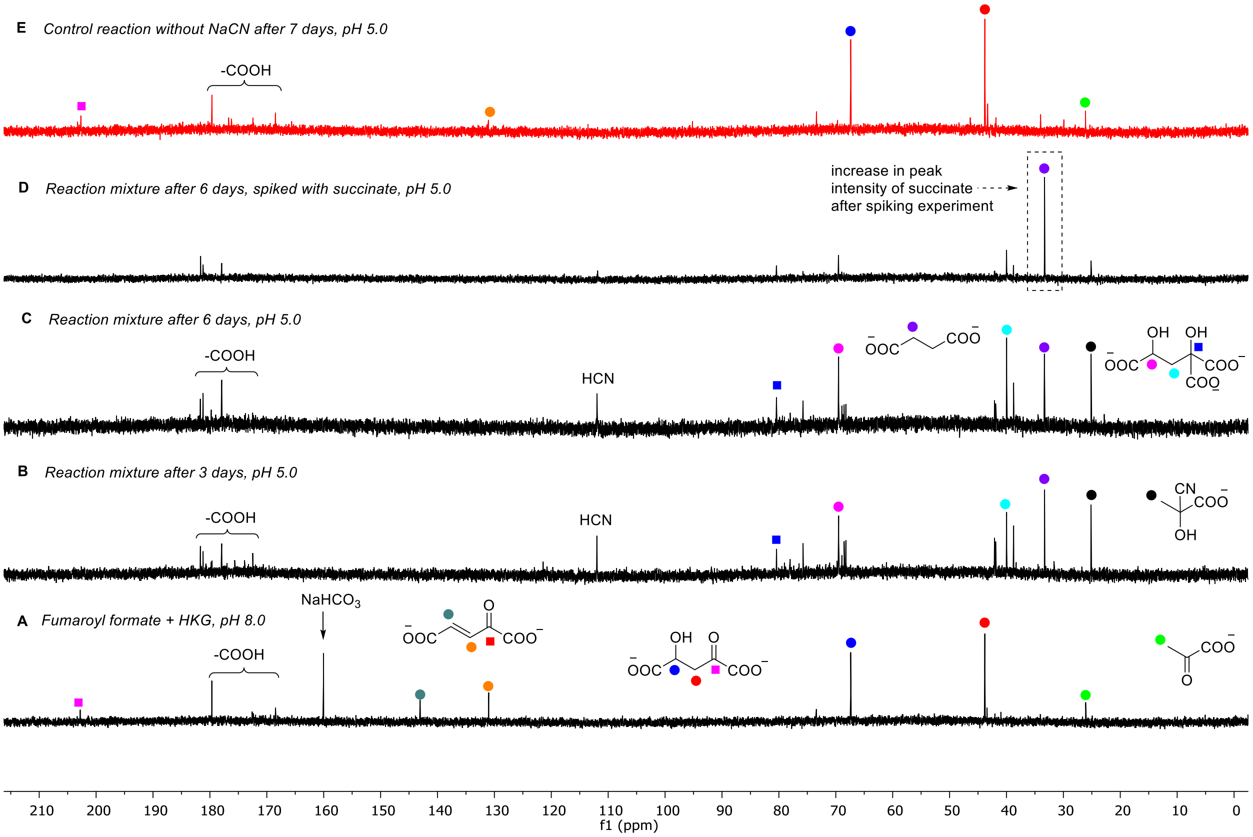
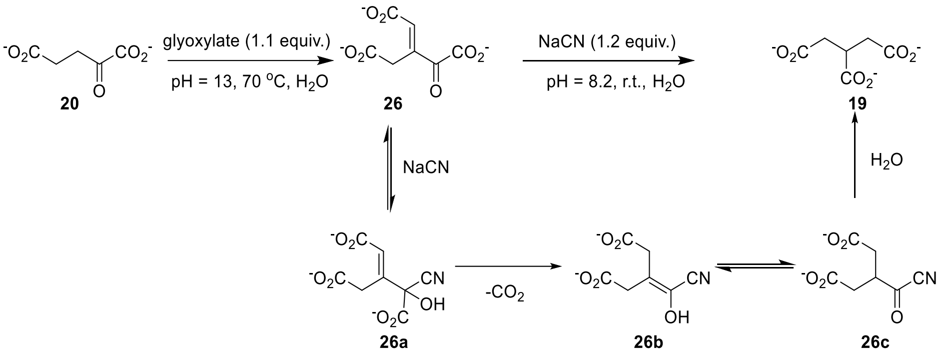


Figure S78: Stacked ^{13}C NMR (H_2O-D_2O) showing starting material i.e., from step I (A), and progress of the reaction after 3 days (B), and 6 days (C), reaction mixture after 6 days spiked with authentic succinate (D), compared with a control reaction in absence of NaCN at pH = 5.0 in 1.0M phosphate buffer after 7 days showing no or very little formation of succinate **10** (E).

Reaction of aconitoyl formate, 26 with sodium cyanide (NaCN) under basic conditions:



Scheme S26: Reaction of aconitoyl formate **26** with NaCN at pH = 8.2, r.t. in H_2O (Figures S81-S83).

Reaction procedure for aconitoyl formate 26 synthesis (step-I): In an appropriate glass vial equipped with a magnetic stir bar, α-ketoglutarate **20** (590.0 mg, 4.0 mmol, 1.0 equiv.), and glyoxylic acid monohydrate (405 mg, 4.4 mmol, 1.1 equiv.) were dissolved in deionized and degassed water (16.0 mL). pH of the homogenous reaction mixture was adjusted to 13 with 5M NaOH (~4.0 mL) and heated to 70 °C for 2 hours (Figures S79-80). We used the high pH for this reaction to reduce the time of reaction. This reaction also works at pH 8-9; however, it takes longer time at pH 8-9.

Reaction procedure for tricarballylic acid synthesis (step-II): In a 4.0 mL glass vial equipped with a magnetic stir bar, 0.4 mL of the above reaction mixture containing 0.5M of aconitoyl formate **26** (0.4 mL, 0.2 mmol) was taken in deionized and degassed water (0.6 mL). To this, sodium cyanide (12.0 mg, 0.24 mmol, 1.2 equiv.) was carefully added and dissolved in fume hood. pH of the homogeneous reaction mixture was adjusted to pH 8.2 with 5M HCl and 5M NaOH. The reaction mixture was stirred at room temperature and the progress of the reaction was monitored by ^{1}H-NMR or ^{13}C-NMR (NMR method 1 in H_2O-D_2O) over a period (Figures S81-S82).

***Reaction conditions for step-I:* α-ketoglutarate 20 (590 mg, 4.0 mmol, 1.0 equiv.), glyoxylic acid monohydrate (405 mg, 4.4 mmol, 1.1 equiv.), and H_2O (16.0 mL)**

- After 2 hours at 70 ^{o}C., showed the formation of aconitoyl formate **26** (pH = 8.2) Absolute yield of aconitoyl formate **26** w.r.t. *t*-BuOH is 97%. (Figures **S79-80**).

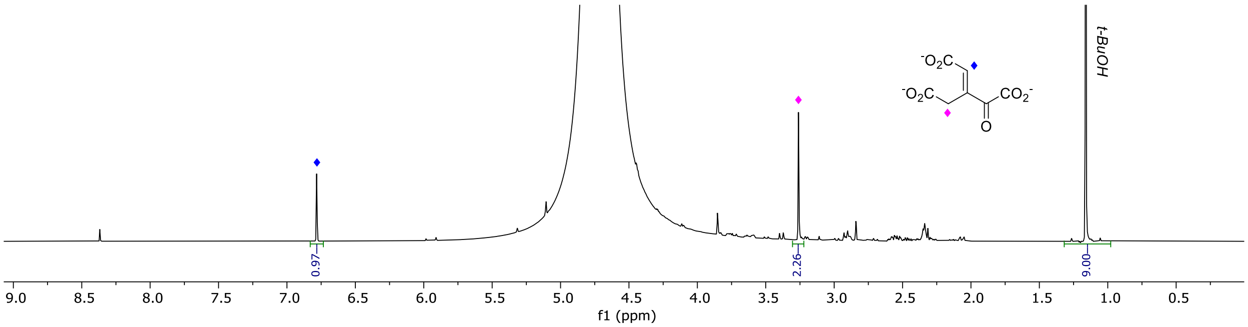


Figure S79: ^{1}H NMR of the reaction mixture after 2 hours at pH = 13; (NMR method 2 in D_2O containing equimolar amount (0.02 mmol) of *t*-BuOH w.r.t. 2-ketoglutarate **20**). Absolute yields were calculated based upon the ^{1}H NMR peaks integration of product (aconitoyl formate **26**) i.e., peaks (~ 6.8 ppm) referred as ♦ w.r.t. *t*-BuOH peak (~ 1.2 ppm). Yield of aconitoyl formate, **26** is 97%.

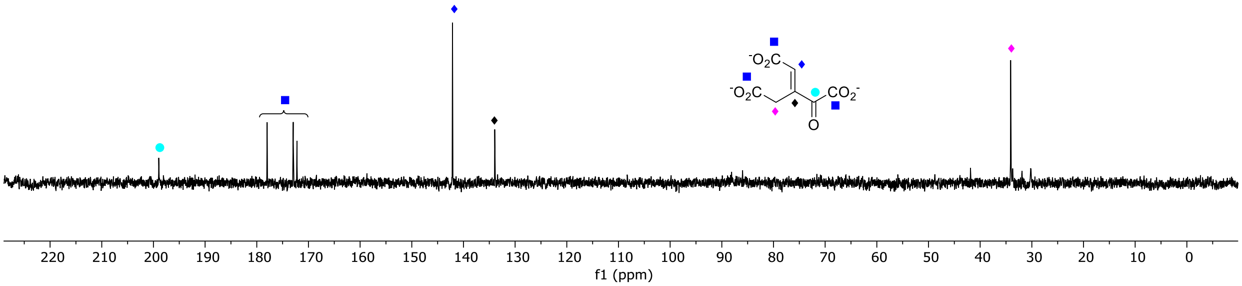


Figure S80: ^{13}C NMR of the reaction mixture after 12 hours at pH = 13 (NMR method 2 in D_2O).

***Reaction conditions for step-II:* aconitoyl formate 26 reaction mixture (0.4 mL, 0.2 mmol), NaCN (12 mg, 0.24 mmol, 1.2 equiv.) and H_2O (0.6 mL), pH = 8.2**

- After 5 min: ^{1}H and ^{13}C NMR (Figure **S81B**) showed formation of cyanohydrin, **26a** as the major product (pH = 8.2).
- After 3 days: ^{1}H and ^{13}C NMR (Figure **S81C**) showed formation of tricarballylic acid, **19** (pH = 8.5) (absolute yield is 26%) (Figure S82).

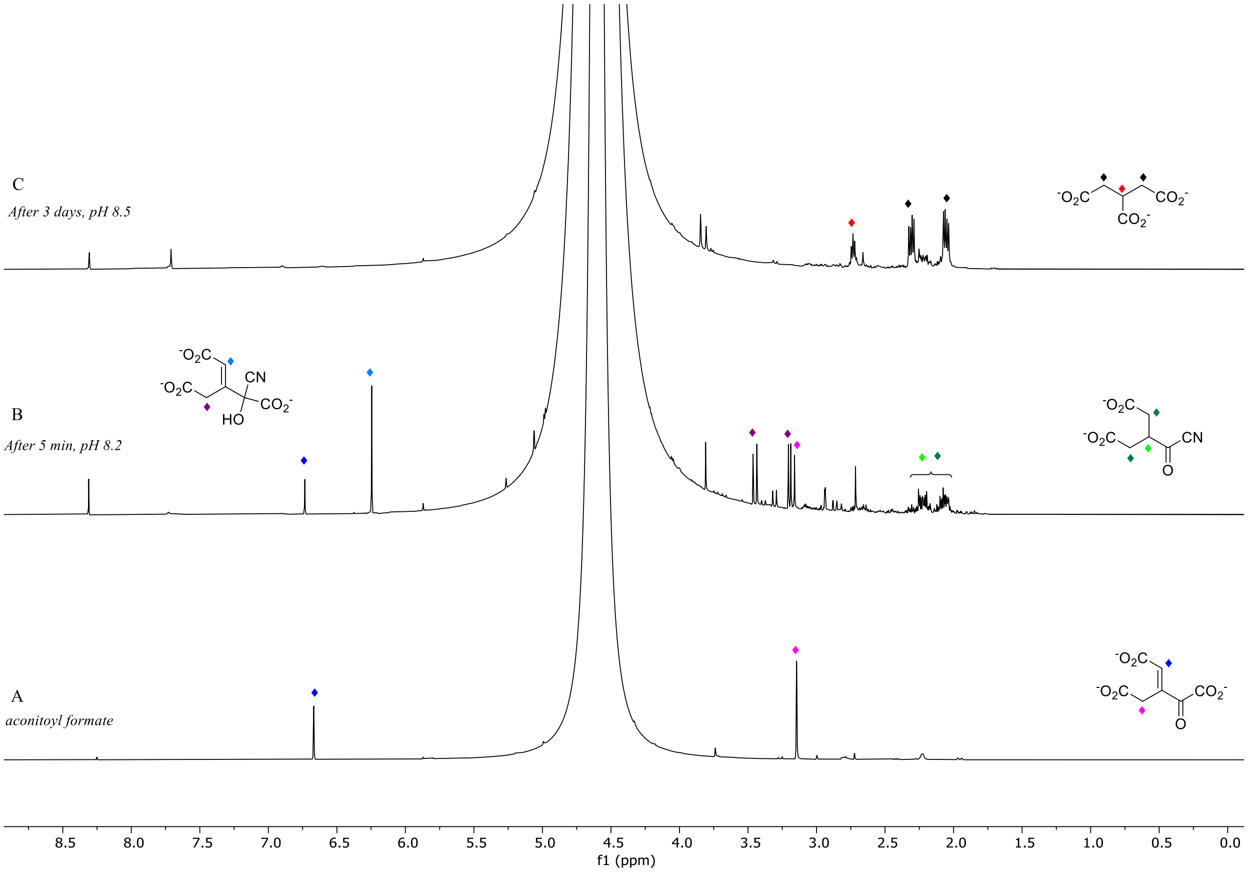


Figure S81: Stacked ^{1}H NMR NMR method 1 in H_2O-D_2O) of the starting material aconitoyl formate, **26** (A), reaction mixture after 5 min at pH = 8.2 (B), and reaction mixture after 3 days at pH = 8.5 (C).

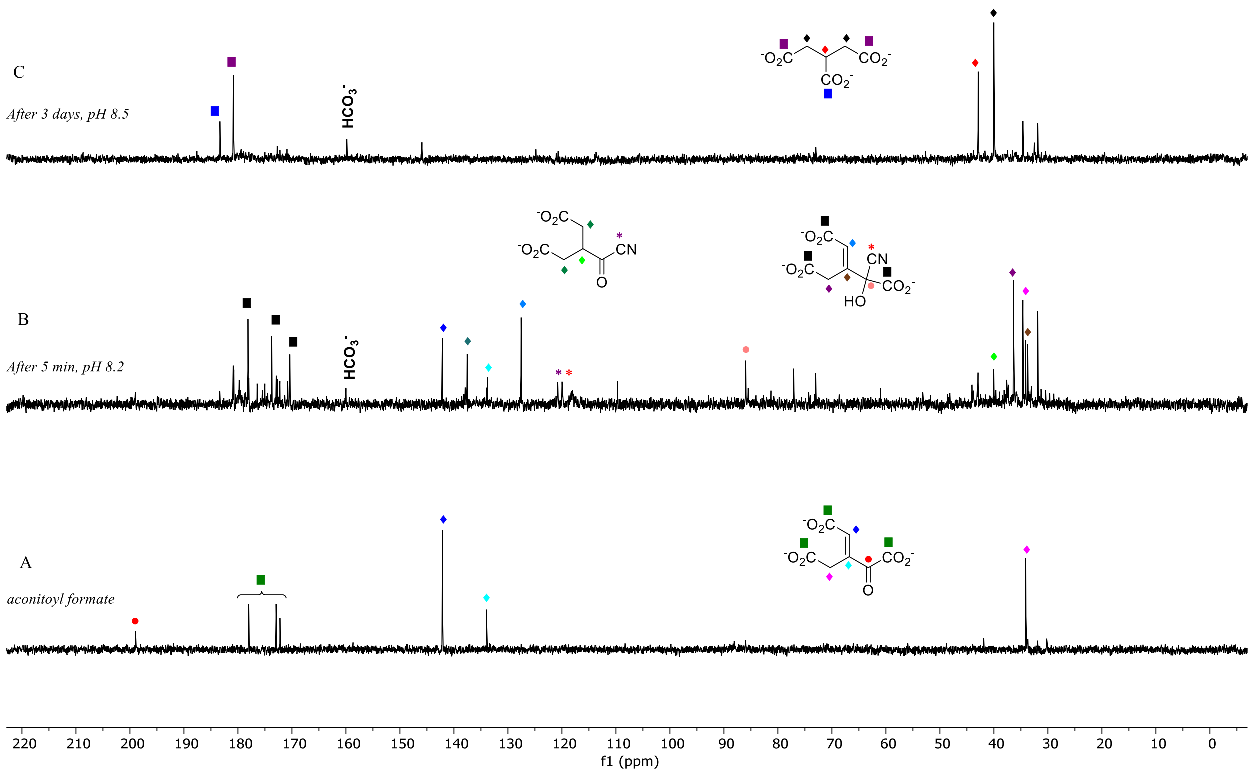


Figure S82: Stacked ^{13}C NMR (NMR method 1 in H_2O-D_2O) of the starting material aconitoyl formate, **26** (A), reaction mixture after 5 min at pH = 8.2 (B), and reaction mixture after 3 days at pH = 8.5 (C).

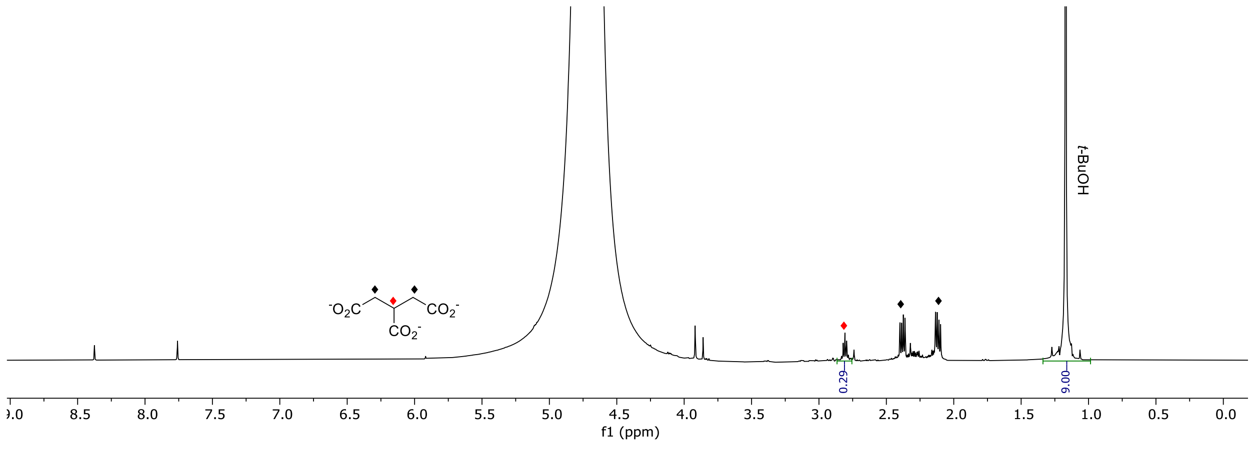
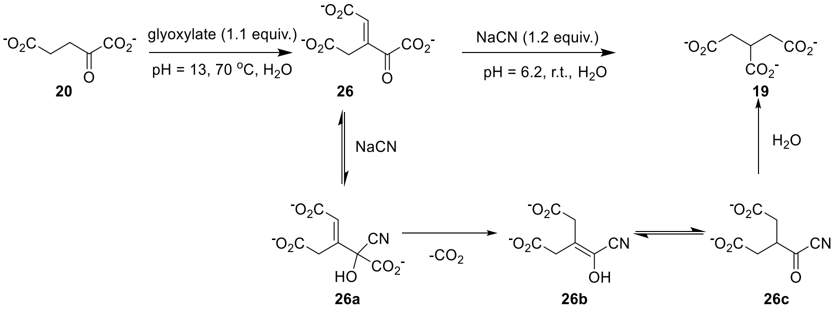


Figure S83: ^{1}H NMR of the reaction after 3 days at pH 8.5. (NMR method 2 in D_2O containing equal amount (0.02 mmol) of *t*-BuOH). Absolute yields were calculated based upon the ^{1}H NMR peaks integration of product (tricarballylic acid, **19**) i.e., peaks (~ 2.8 ppm) referred as ♦ w.r.t. *t*-BuOH peak (~ 1.2 ppm).

Reaction of aconitoyl formate, 26 with sodium cyanide (NaCN) under acidic conditions:



Scheme S27: Reaction of aconitoyl formate **26** with NaCN at pH = 6.2, r.t. in H_2O (Figure S84).

Reaction procedure for tricarballylic acid synthesis: In a 4.0 mL glass vial equipped with a magnetic stir bar, 0.4 mL of the reaction mixture containing 0.5M of aconitoyl formate **26** (0.4 mL, 0.2 mmol, from Scheme S26, step-I) was taken in deionized and degassed water (0.6 mL). To this, sodium cyanide (12.0 mg, 0.24 mmol, 1.2 equiv.) was carefully added and dissolved in fume hood. pH of the homogeneous reaction mixture was adjusted to pH 6.2 with 5M HCl solution. The reaction mixture was stirred at room temperature for 24 hours and the progress of the reaction was monitored by ^{1}H-NMR (NMR method 1 in H_2O-D_2O) over a period (Figure S84B).

***<u>Reaction conditions for step-II:</u>* aconitoyl formate 26 reaction mixture (0.4 mL, 0.2 mmol), NaCN (12 mg, 0.24 mmol, 1.2 equiv.) and H_2O (0.6 mL), pH = 6.2**

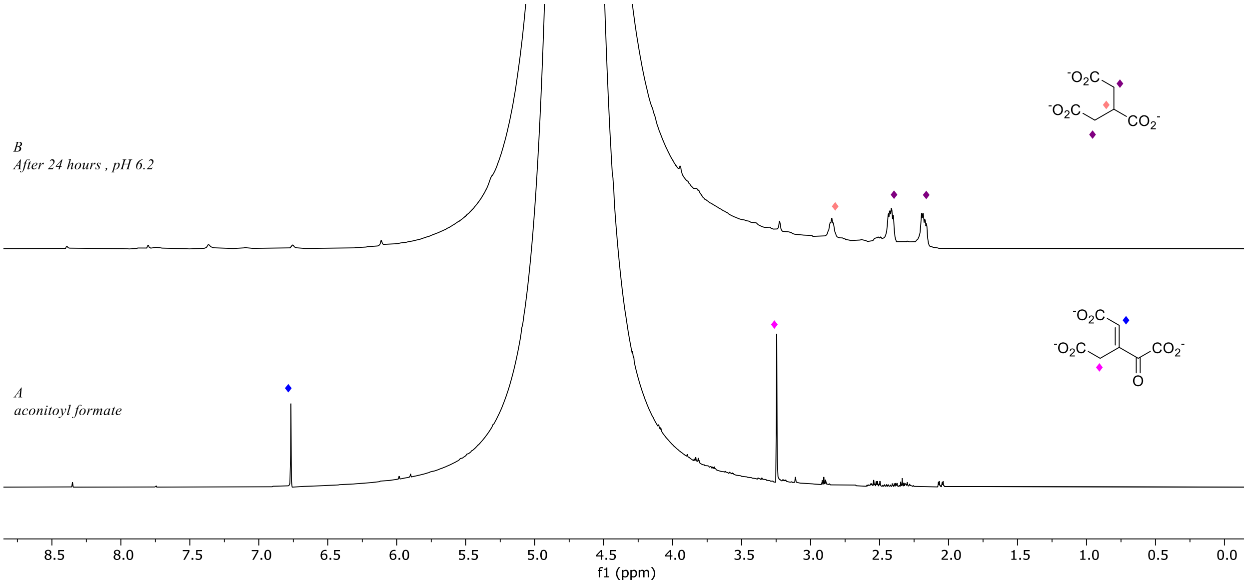
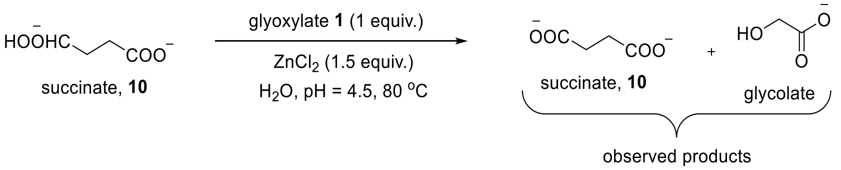


Figure S84: Stacked ^{1}H NMR (NMR method 1 in H_2O-D_2O) of the starting material aconitoyl formate, **26** (A), and reaction mixture after 24 hours at pH = 6.2 (B).

Control reaction of succinate 10 with glyoxylate 1 in presence of $ZnCl_2$:

During dehydration reaction of isocitrate to aconitate (Scheme S7) in the presence of $ZnCl_2$, we observed succinate formation (Figures S23 and S25). Formation of succinate under these conditions hinted the retro-aldol fragmentation of isocitrate to succinate and glyoxylate. However, we did not see any glyoxylate peak in the ^1H NMR or ^{13}C NMR spectra. We hypothesized that glyoxylate may not be stable under these conditions (with $ZnCl_2$, 80 °C, pH 4.5) and undergo Cannizzaro reaction to form glycolate and oxalate, which may form zinc oxalate and zinc glycolate. Zinc oxalate is insoluble in water and hence will precipitate out (similar may be the case with zinc glycolate under these conditions). Indeed, we observed precipitates in the reaction mixture.

We conducted a control reaction starting from succinate and glyoxylate in the presence of $ZnCl_2$ at 80 °C. This reaction showed no glyoxylate after 5 days, only glycolate and succinate were seen in the reaction mixture (based on NMR spectral data) along with precipitate formation after 5 days. This control experiment suggests that if glyoxylate is produced from the retro-aldol of isocitrate, it is not stable under the experimental conditions and transforms to other compounds like oxalate and glycolate.



Scheme S28: Control reaction of succinate **10** with glyoxylate **1** in the presence of $ZnCl_2$ (Figures S85-86).

Reaction procedure: In a 4 mL glass vial, succinic acid **10** (118 mg, 1 mmol, 1.0 equiv.) and glyoxylic acid monohydrate **1** (92 mg, 1 mmol, 1.0 equiv.) were dissolved in deionized and degassed water (2 mL). To this solution $ZnCl_2$ (200 mg, 1.5 equiv.) was added. After addition of $ZnCl_2$, pH of the homogeneous colourless reaction mixture was adjusted to 4.5 with 5M NaOH. The reaction mixture was then sonicated and purged with argon (2x5 min) placed at 80 °C and the progress of the reaction was monitored by ^1H-NMR or ^{13}C-NMR over a period (Figures S85-86).

***Reaction Condition:* glyoxylate (92 mg, 1.0 equiv.), succinate (118 mg, 0.5M, 1 equiv.), $ZnCl_2$ (200 mg, 1.5 equiv.), H_2O (2 mL).**

- After 10 min: ^{1}H NMR (Figure **S85A**) showed starting succinate **10** and glyoxylate **1** (pH = 4.5).
- After 5 days: ^{1}H NMR (Figure **S85**) showed formation of glycolate along with starting succinate (pH = 4.7).

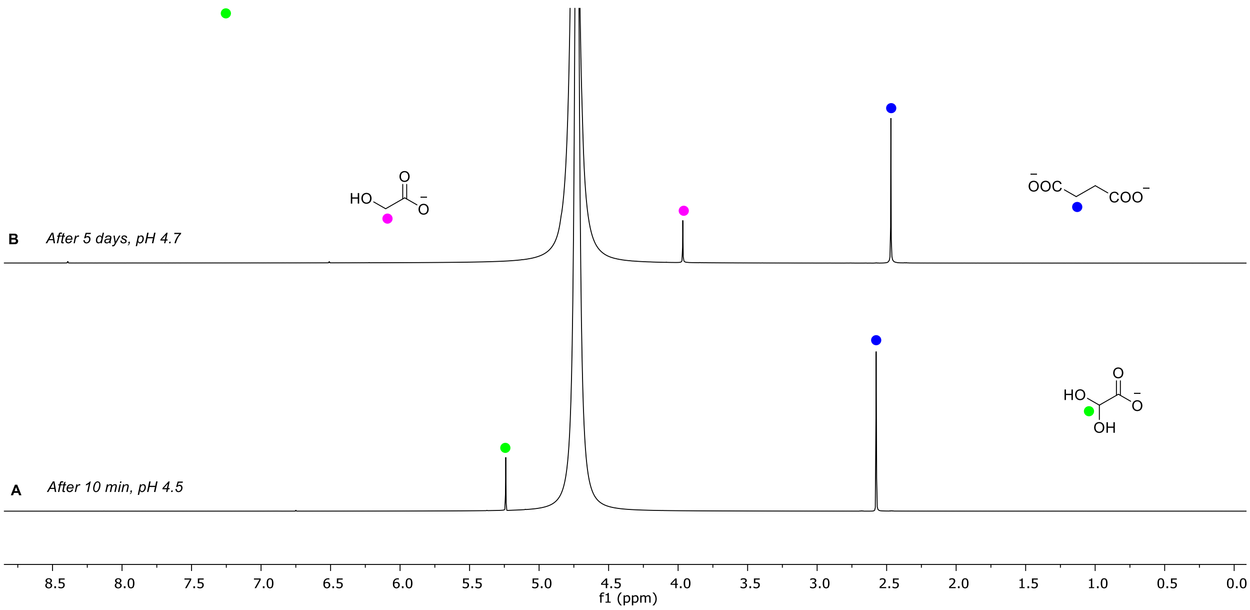


Figure S85: Stacked ^{1}H NMR (D_2O) showing progress of the reaction after 10 min (A), 5 days.

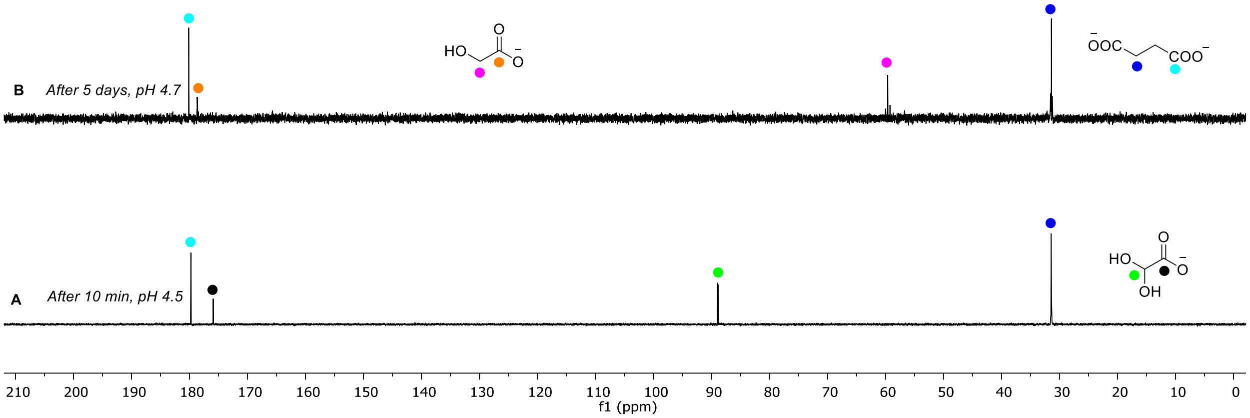


Figure S86: Stacked ^{13}C NMR (D_2O) showing progress of the reaction after 10 min (A), 5 days.

References

1. Nair, G.; Roberts, J. D. *Org. Lett.* **2003**, *20*, 3699-3701.
2. Stubbs, R. T.; Yadav, M.; Krishnamurthy, R.; Springsteen, G. *Nat. Chem.* **2020**, *12*, 1016-1022.