Supplementary Information

Synthesis and breakdown of universal metabolic precursors promoted by iron

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Content:

Materials and Methods

General information. All reactions were carried out in 10 mL Pyrex glass culture tubes under inert atmosphere (30 s argon purge) unless otherwise noted.

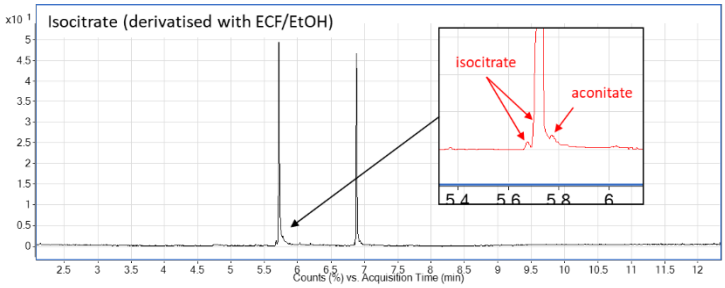
GC-MS analysis was performed on a GC System 7820A (G4320) coupled to an MSD block 5977E (G7036A). An Agilent High Resolution Gas Chromatography Column (PN 19091S – 433UI, HP – 5MS UI, 28 m×0.250 mm, 0.25 Micron, SN USD 489634H) was used. Hydrogen (99.999 % purity) was the carrier gas, supplied at a constant flow rate of 1.5 mL min^{-1}. Samples were prepared in ethyl acetate (200 µL sample volume). The analysis was carried out on a 1 µL injection volume (splitless mode). The injection port temperature was 250 °C, and the column oven temperature program was: 60 °C for 1 min, then increased to 310 °C with a 30 °C min^{-1} ramp, followed by a 3-min hold (total running time 12.33 min). The mass spectrometer was turned on after a 2-min delay and was operated at the electron ionization mode with quadrupole temperature of 150 °C. Data was acquired in the full-scan mode (50-500). Data analysis and integration were performed using *Agilent MassHunter Workstation v.B.06.00* software.

^{1}H NMR spectra were recorded on a Bruker Avance400 (400 MHz) spectrometer at ambient temperature in a H_2O:D_2O mixture (6:1) as solvent, with sodium 3-(trimethylsilyl)-1-propanesulfonate (DSS) as the internal standard (CH_3 peak at 0 ppm). Water suppression was achieved using the Bruker ZGESGP pulse program. Relaxation delay D1 was set to 87 s, with time domain size TD = 32768 and sweep width SWH = 4789.27 Hz (11.963 ppm). 32 scans were acquired for each sample. Integration was performed using *MestReNova v6.0.2* software.

Materials. Unless otherwise noted, all reagents and solvents were purchased from commercial suppliers and used without further purification. Hydroxyketoglutarate and oxopentenedioate were prepared using a literature procedure.[1] Water was obtained from a Milli-Q purification system (18 MΩcm) and was purged with argon before use. All glassware and stir bars were pre-washed with aqua regia, followed by distilled water and acetone, and oven dried to prevent any cross-contamination by metal salts.

Product identification. To facilitate GC-MS analysis, a literature derivatization procedure[2,3] was applied to the sample to convert carboxy groups to ethyl esters, hydroxy groups to ethyl carbonates, amino groups to ethyl carbamates, ketones to diethyl ketals, and aldehydes to acetals. A 700 µL aliquot of the reaction mixture was basified using ~50 mg solid KOH (Merck EMSURE) and centrifuged (6000 rpm, 3 min). To 600 µL of the supernatant was added EtOH (300 µL) and pyridine (40 µL), followed by ethyl chloroformate (ECF, 40 µL). After vortex mixing for 30 s, a second 40 µL portion of ECF was added and vortex mixing was continued for another 30 s. To this, $CHCl_3$ (200 µL) was added, followed by vortex mixing (10 s). Finally, saturated aqueous $NaHCO_3$ (600 µL) was added and the mixture was vortex mixed again for 10 s. The $CHCl_3$ layer was separated and dried over anhydrous Na_2SO_4. 50 µL of the dry $CHCl_3$ layer was added with 150 µL of ethyl acetate to a vial and subjected to GC-MS analysis.
Reaction products derivatized to ethyl esters were identified by comparing the mass spectra and retention times to those of analogously derivatized authentic samples, as shown below and also described elsewhere.[2]

N.b.: Unlike citrate,[2] isocitrate at 40 mM (a much higher concentration than the one used in this study) undergoes derivatisation-induced dehydration to aconitate in less than 1% yield. At lower concentrations, more representative of this study, dehydration is negligible (right).



Formate and acetate were determined using an NMR procedure and a GC-MS procedure, both reported in the literature.[3]

a) NMR procedure: to a 1.5 mL plastic microtube was added ~1 mL of the reaction mixture and ~50 mg solid KOH (Merck EMSURE). The resulting thick suspension was centrifuged at 10 000 rpm for 20 min. To 600 µL of the supernatant was added 100 µL of 0.05 M solution of internal standard (DSS in D_2O). The solution was analyzed by NMR using the Bruker ZGESGP pulse program, as described above.
b) GC-MS detection of formate and acetate as their amides with *N*-methylphenylethylamine: To a 120 µL aliquot of a reaction mixture in a 1.5 mL plastic microtube were added: 50 µL of 0.12 M solution of 1-hydroxybenzotriazole in H_2O, 75 µL of 0.08 M 1-ethyl-3-(3-dimethyl-aminopropyl)carbodiimide solution (EDC) in acetonitrile, and 75 µL of 0.06 M *N*-methylphenylethylamine (MPEA) in acetonitrile. The resulting mixture was vortex mixed for 30 s and incubated at 60 °C for 45 min. After cooling to room temperature, 200 µL of $CHCl_3$ was added to the mixture and vortex mixed for 30 s. The $CHCl_3$ layer was removed and dried over anhydrous $MgSO_4$. 50 µL of the $CHCl_3$ supernatant was added to a vial together with 150 µL of EtOAc, and analysed by GC-MS.

Product quantification and error analysis. Carboxylic acids and amino acids were quantified according to a literature procedure,[2] using 6-point calibration curves prepared from ECF/EtOH-derivatized solutions of authentic samples (0.006 M, 0.013 M, 0.020 M, 0.027 M, 0.033 M and 0.040 M) as described above.

Each data point was obtained from three independent measurements and the correlation line was obtained from the least-squares fitting (intercept = 0). Error bars on graphs are shown as ± standard deviation for each data point. For the linear fits, overall percentage error of the response factor corresponds to ± standard deviation for each slope value. For the polynomial fits, 95% confidence envelopes are shown.

Calibration lines for pyruvate, malate, fumarate, succinate, α-ketoglutarate, isocitrate, cis-aconitate, tricarballylate and alanine are identical to those we previously reported for the same analytical setup.[2]

Calibration lines for glyoxylate, glycolate, oxalate, malonate, levulinate, mesaconate, hydroxyketoglutarate/oxopentenedioate (HKG/OPD), glycine, aspartate and glutamate are shown below (Figures S2, S10).

Response factors corresponding to calibration lines for all the compounds detected by the GC-MS in this study are listed in the Table S1.

Yields were calculated by comparing the GC peak area against the calibration line. Each reaction was performed at least twice, and reported yields are an average of those two runs, with an error corresponding to ± standard deviation.

Formate and acetate were quantified by NMR with DSS as standard, following a procedure we reported before.[3] Equations corresponding to these calibration lines are listed in Table S1.

Hydroxyketoglutarate and oxopentenedioate (HKG/OPD) were quantified together (due to rapid interconversion of the two compounds during derivatization) as obtained in an aqueous reaction mixture (see Synthetic Procedures below). The concentration of HKG/OPD in the reaction mixture was determined by NMR (ZGESGP pulse program, 200 mL reaction mixture + 500 mL 0.0360 M solution of sodium fumarate in D_2O as internal standard) to be 0.0775 M. From this, 6 solutions were prepared by dilution, for subsequent ECF/EtOH derivatization and calibration on the GC-MS as described above for other carboxylic acids.
Mass spectra of all the compounds detected in this study through the derivatization with ECF/EtOH are shown in Figure S3.

Synthetic procedures

General procedure (qualitative metal screen): to a 10 mL Pyrex pressure tube were added sodium pyruvate (1 equiv, 0.1 mmol, 11 mg), sodium glyoxylate (2.0 equiv, 0.20 mmol, 22 mg), transition metal salt (2.0 equiv, 0.20 mmol, 40 mg of $MnCl_2{\cdot}4H_2O$ or 40 mg of $FeCl_2{\cdot}4H_2O$, or 48 mg of $CoCl_2{\cdot}6H_2O$, or 48 mg of $NiCl_2{\cdot}6H_2O$, or 34 mg of $CuCl_2{\cdot}6H_2O$, or 27 mg of $ZnCl_2$), and 3 mL of MilliQ water. The contents of the tube were flushed with argon. The tube was then sealed, and the reaction mixture stirred at 70 °C (1000 rpm, external heating block) for 3 h, followed by the KOH workup and ECF/EtOH derivatization, as described above.

General procedure (Fe^{2+}-promoted reactions): to a 10 mL Pyrex pressure tube were added sodium pyruvate (1 equiv, 0.20 mmol, 22 mg), sodium glyoxylate (2.0 equiv, 0.40 mmol, 44 mg), $FeCl_2{\cdot}4H_2O$ (2.0 equiv, 0.40 mmol, 80 mg), and 6 mL of MilliQ water. The contents of the tube were flushed with argon. The tube was then sealed, and the reaction mixture stirred at 70 °C (1000 rpm, external heating block) for up to 48 h, followed by the KOH workup and ECF/EtOH derivatization, as described above.

Reductive amination of glyoxylate/pyruvate network intermediates: to a 10 mL Pyrex pressure tube were added sodium pyruvate (1.0 equiv, 0.10 mmol, 11 mg), sodium glyoxylate (2.0 equiv, 0.20 mmol, 22 mg), $FeCl_2{\cdot}4H_2O$ (2.0 equiv, 0.20 mmol, 40 mg), and 3 mL of MilliQ water. The contents of the tube were flushed with argon. The tube was then sealed, and the reaction mixture stirred at 70 °C for 1 h (1000 rpm, external heating block). Then, to the reaction mixture were added hydroxylamine hydrochloride (6.0 equiv, 0.60 mmol, 42 mg) and Fe^0 powder (10 equiv, 1.0 mmol, 56 mg). The tube was sealed and stirring was continued at 70 °C for 1 h, followed by the KOH workup and ECF/EtOH derivatization, as described above.

Hydroxyketoglutarate: prepared using a literature procedure (*1*) from oxaloacetic acid (1.00 equiv, 0.902 mmol, 119 mg) in 9.88 mL of 1.0 M potassium phosphate buffer (pH 7.15) and glyoxylic acid (1.20 equiv, 1.08 mmol, added as 119 μL 50% w/w aq. solution). The reaction mixture was stirred for 3 h at 25 °C, yielding a 0.0775 M solution of hydroxyketoglutarate (86%, NMR yield). ^{1}H NMR (400 MHz, H_2O+D_2O) δ 4.26 (dd, J = 8.6, 3.4 Hz, 1H), 3.09 (dd, J = 17.8, 3.4 Hz, 1H), 2.95 (dd, J = 17.8, 8.5 Hz, 1H).

Time-point experiment (unlabeled compounds). The experiment was performed in two replicas, in parallel. 700 mL aliquots of the reaction mixtures were drawn at the following time points: 5 min (substrates added and vortex mixed for 30 s), 1 h, 3 h, 7 h, 10 h, 24 h, 48 h. The results were normalized against a "0 h" mixture of sodium pyruvate (1.0 equiv, 0.20 mmol, 22 mg) and sodium glyoxylate (2.0 equiv, 0.40 mmol, 44 mg) in water (3 mL), without Fe^{2+} added, and derivatized with ECF/EtOH using the procedure described above. Reported percentage values are scaled against the number of carbon atoms in each compound, to account for the total carbon mass balance of the system (Table S2). GC chromatograms of the time-point experiment are shown in Figure S4, and mass spectra of all compounds detected at t = 24 h (the highest complexity) are shown in Figure S5.
The reaction mixture pH change over time was measured with an AquaLytic AL10pH handheld pH meter and found to equal ~4.45 at t = "0 h" and ~5.65 at t = 24 h.

Time-point experiment (with pyruvate-2-^{13}C). The experiment was performed in two replicas, in parallel, using sodium pyruvate-2-^{13}C. 700 mL aliquots of the reaction mixtures were drawn at the following time points: 5 min (substrates added and vortex mixed for 30 s), 1 h, 3 h, 7 h, 10 h, 24 h, 48 h. The results were normalized against a "0 h" mixture of sodium pyruvate-2-^{13}C (1.0 equiv, 0.20 mmol, 22 mg) and sodium glyoxylate (2.0 equiv, 0.40 mmol, 44 mg) in water (3 mL), without Fe^{2+} added, derivatized with ECF/EtOH using the procedure described above. Reported percentage values are scaled against the number of carbon atoms in each compound, to account for the total carbon mass balance of the system (Table S4) and are shown in Figure S15.
The ^{13}C label present in pyruvate was found to be carried over to the following intermediates: levulinate, malonate, malate, fumarate, succinate, α-ketoglutarate, hydroxyketoglutarate/ oxopentenedioate, isocitrate, aconitate, tricarballylate, and acetate.
Oxalate, glycolate and formate remained label-free, meaning these compounds are derived from glyoxylate.
See Figure S16 for relevant GC chromatograms and mass spectra, and Figure S20 for a graphical depiction of the ^{13}C label propagation in the network. Additionally, see below for the detection of formate and acetate.

Detection of formate and acetate. Formate and acetate were detected in the reaction mixtures at the 48 h time-point, using NMR as well as GC-MS (derivatization with ECD/MPEA to amides[3]). See Product identification section for sample preparation details.
Results obtained for unlabeled starting materials are shown in Figure S7 (GC-MS) and Figure S8 (^{1}H NMR), for ^{13}C-labeled pyruvates – in Figure S18, Figure S25 and Figure S27; for $^{13}C_2$-labeled glyoxylate – in Figure S29.

Reductive amination of ketoacids with hydroxylamine. Reductive amination was performed according to the procedure described above on 0.10 mmol of each ketoacid (sodium glyoxylate: 11 mg; sodium pyruvate: 11 mg; oxaloacetic acid: 13 mg; α-ketoglutarate: 15 mg), 0.1 mmol of hydroxylamine hydrochloride (1 equiv, 7 mg) and 1.0 mmol of Fe(0) powder (10 equiv, 56 mg). The reaction was carried out at 100 °C over 16 h to ensure completion. Obtained chromatograms and mass spectra of ECF/EtOH derivatized reaction mixtures are shown in Figure S9. See Table 3 for product yields.

Detection of glycine, alanine, aspartic acid and glutamic acid in the Fe^{2+}-promoted reaction mixture. Reductive amination was performed according to the procedure described above. Following KOH workup and ECF/EtOH derivatization, four amino acids were detected as their

ethyl esters (glycine, alanine, aspartic acid and glutamic acid). A GC chromatogram of a typical reaction mixture as well as relevant mass spectra are shown in Figure S8. Mass spectra of authentic amino acids derivatized with ECF/EtOH are shown in Figure S11.

Control experiments with Fe^{2+} (without glyoxylate). Control experiments were performed on individual intermediates detected in the reaction network: glyoxylate, glycolate, pyruvate, oxalate, oxaloacetate, malonate, malate, fumarate, succinate, mesaconate, hydroxyketoglutarate/ oxopentenedioate (HKG/OPD), α-ketoglutarate, isocitrate and aconitate (Figure S13).
The general procedure was used (except for HKG/OPD): 1.0 equiv (0.1 mmol) of a chosen intermediate, $FeCl_2 \cdot 4H_2O$ (2.0 equiv, 0.20 mmol, 40 mg) and 3 mL of MilliQ water. The contents of the tube were flushed with argon. The tube was then sealed, and the reaction mixture stirred at 70 °C (1000 rpm, external heating block) for 16 h, followed by the KOH workup and ECF/EtOH derivatization, as described above.
HKG/OPD was prepared using the procedure described above. From the reaction mixture 3 mL were taken and combined with $FeCl_3 \cdot 6H_2O$ (2.0 equiv, 0.20 mmol, 54 mg). The contents of the tube were flushed with argon. The tube was then sealed, and the reaction mixture stirred at 70 °C (1000 rpm, external heating block) for 16 h, followed by the KOH workup and ECF/EtOH derivatization, as described above.

Control experiments with Fe^{2+} and glyoxylate. Control experiments with Fe^{2+} and glyoxylate were performed on individual intermediates detected in the reaction network: glycolate, pyruvate, oxalate, oxaloacetate, malonate, malate, fumarate, succinate, mesaconate, hydroxyketoglutarate/oxopentenedioate (HKG-OPD), α-ketoglutarate, isocitrate and aconitate (Figure S14).
The general procedure was used: 1 equiv (0.1 mmol) of a chosen intermediate, sodium glyoxylate (2.0 equiv, 0.20 mmol, 22 mg), $FeCl_2 \cdot 4H_2O$ (2.0 equiv, 0.20 mmol, 40 mg) and 3 mL of MilliQ water. The contents of the tube were flushed with argon. The tube was then sealed, and the reaction mixture stirred at 70 °C (1000 rpm, external heating block) for 16 h, followed by the KOH workup and ECF/EtOH derivatization, as described above.

Control experiments suggesting oxidation of malate to oxaloacetate. A control experiment was performed to trap oxaloacetate (undetectable via derivatization by ECF/EtOH) as aspartate, through *in situ* reductive amination with hydroxylamine and Fe(0).
Two experiments were performed: with Fe^{2+} and Fe^{3+}. Aspartic acid was detected in both cases, as shown in Figure S12 (see Figure S11 for an MS spectrum of an authentic sample of aspartate).
General reductive amination procedure was used: malic acid (1.0 equiv, 0.10 mmol, 13 mg) and iron salt (2.0 equiv, 0.20 mmol, 40 mg of $FeCl_2 \cdot 4H_2O$ or 54 mg of $FeCl_3 \cdot 6H_2O$), and 3 mL of MilliQ water. The contents of the tube were flushed with argon. The tube was then sealed, and the reaction mixture stirred at 70 °C for 1 h (1000 rpm, external heating block). Then, to the reaction mixture were added hydroxylamine hydrochloride (6.0 equiv, 0.60 mmol, 42 mg) and Fe^0 powder (10 equiv, 1.0 mmol, 56 mg). The tube was sealed and stirring was continued at 70 °C for 1 h, followed by the KOH workup and ECF/EtOH derivatization, as described above.
Another indirect proof of malate oxidation is the presence of acetate detected in the reaction mixture comprising of malic acid and iron salts heated at 70 °C over 16 h.
Oxaloacetate is unstable in solution in the presence of transition metal salts, and decarboxylates to pyruvate, which, in turn, decarboxylates to acetate, easily detected by NMR (Figure S19). General procedure was used: malic acid (1.0 equiv, 0.10 mmol, 13 mg) and iron salt (2.0 equiv, 0.20 mmol, 40 mg of $FeCl_2 \cdot 4H_2O$ or 54 mg of $FeCl_3 \cdot 6H_2O$), and 3 mL of MilliQ water. The contents of the

tube were flushed with argon. The tube was then sealed, and the reaction mixture stirred at 70 °C for 16 h (1000 rpm, external heating block) followed by the KOH workup and NMR sample preparation, as described above.

Control experiment: hydroxyketoglutarate/oxopentenedioate + Fe^{3+}. A control experiment was performed to evidence oxidative decarboxylations of HKG/OPD with Fe^{3+} species as oxidant. The results, highlighting the presence of fumarate and succinate, are shown in Figure S18.
HKG/OPD was prepared using the procedure described above. From the reaction mixture 3 mL were taken and combined with $FeCl_3 \cdot 6H_2O$ (2.0 equiv, 0.20 mmol, 54 mg). The contents of the tube were flushed with argon. The tube was then sealed, and the reaction mixture stirred at 70 °C (1000 rpm, external heating block) for 16 h, followed by the KOH workup and ECF/EtOH derivatization, as described above.

Additional ^{13}C labelling experiments.

1) *Experiment with sodium pyruvate-1-^{13}C*: the reaction was performed in two replicas, in parallel, following the general procedure described above. A 700 mL aliquot of each reaction mixture was drawn after 24 h and derivatized with ECF/EtOH, according to the procedure described above. After 48 h, another 700 mL aliquot from each reaction mixture was drawn and derivatized with ECD/MPEA, following the procedure described above, to detect formate and acetate. The ^{13}C label present in pyruvate was found to be carried over to the following intermediates: lactate, α-ketoglutarate, hydroxyketoglutarate/ oxopentenedioate, and mesaconate. See Figure S21 for the visualization of the ^{13}C label distribution in the network, and Figure S24 and S25 for relevant GC chromatograms and mass spectra.
2) *Experiment with sodium pyruvate-3-^{13}C*: the reaction was performed in two replicas, in parallel, following the general procedure described above. A 700 mL aliquot of each reaction mixture was drawn after 24 h and derivatized with ECF/EtOH, according to the procedure described above. After 48 h, another 700 mL aliquot from each reaction mixture was drawn and derivatized with ECD/MPEA, following the procedure described above, to detect formate and acetate. The ^{13}C label present in pyruvate was found to be carried over to the following intermediates: acetate, levulinate, malonate, lactate, fumarate, succinate, malate, α-ketoglutarate, isocitrate, aconitate, tricarballylate, hydroxyketoglutarate/ oxopentenedioate, and mesaconate. See Figure S22 for the visualization of the ^{13}C label distribution in the network, and Figure S26 and S27 for relevant GC chromatograms and mass spectra.
3) *Experiment with sodium glyoxylate-1,2-$^{13}C_2$*: the reaction was performed in a single replica, following the general procedure described above. A 700 mL aliquot of the reaction mixture was drawn after 24 h and derivatized with ECF/EtOH, according to the procedure described above. After 48 h, another 700 mL aliquot from each reaction mixture was drawn and derivatized with ECD/MPEA, following the procedure described above, to detect formate and acetate. The ^{13}C label present in pyruvate was found to be carried over to the following intermediates: formate, oxalate, glycolate, malonate, levulinate, fumarate, succinate, malate, α-ketoglutarate, hydroxyketoglutarate/ oxopentenedioate, isocitrate, aconitate, tricarballylate and mesaconate. See Figure S23 for the visualization of the ^{13}C label distribution in the network, and Figure S28 and S29 for relevant GC chromatograms and mass spectra.

Supplementary references

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2. K. B. Muchowska *et al.*, Metals promote sequences of the reverse Krebs cycle, *Nature Ecology & Evolution* **1**, 1716–1721 (2017).
3. S. J. Varma, K. B. Muchowska, P. Chatelain & J. Moran, Native iron reduces CO_2 to intermediates and end-products of the acetyl-CoA pathway, *Nature Ecology & Evolution* **2**, 1019–1024 (2018).

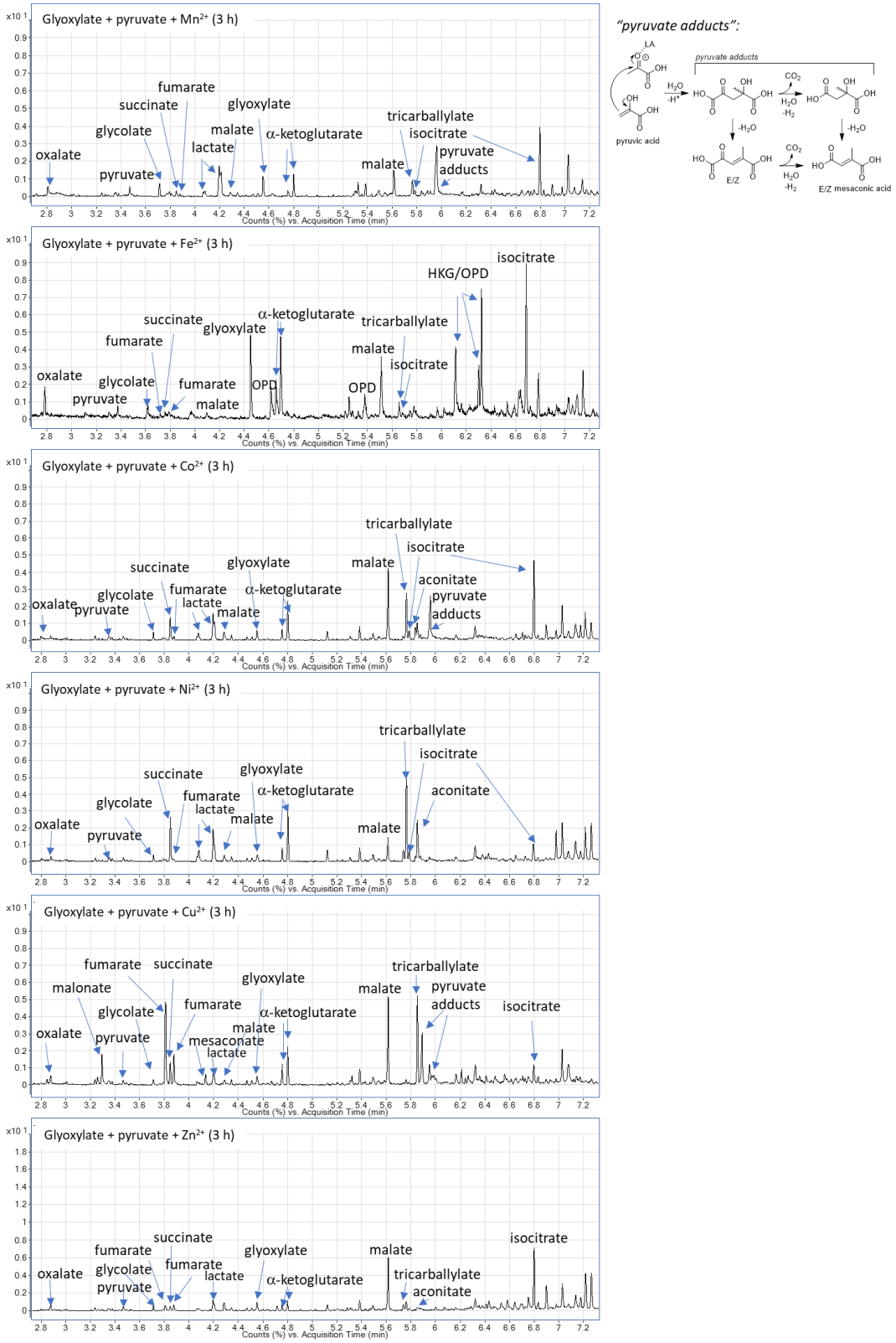


Figure S1.

GC chromatograms showing a reaction network arising from pyruvate and glyoxylate, promoted by transition metal ions at 70 ºC (qualitative screen).

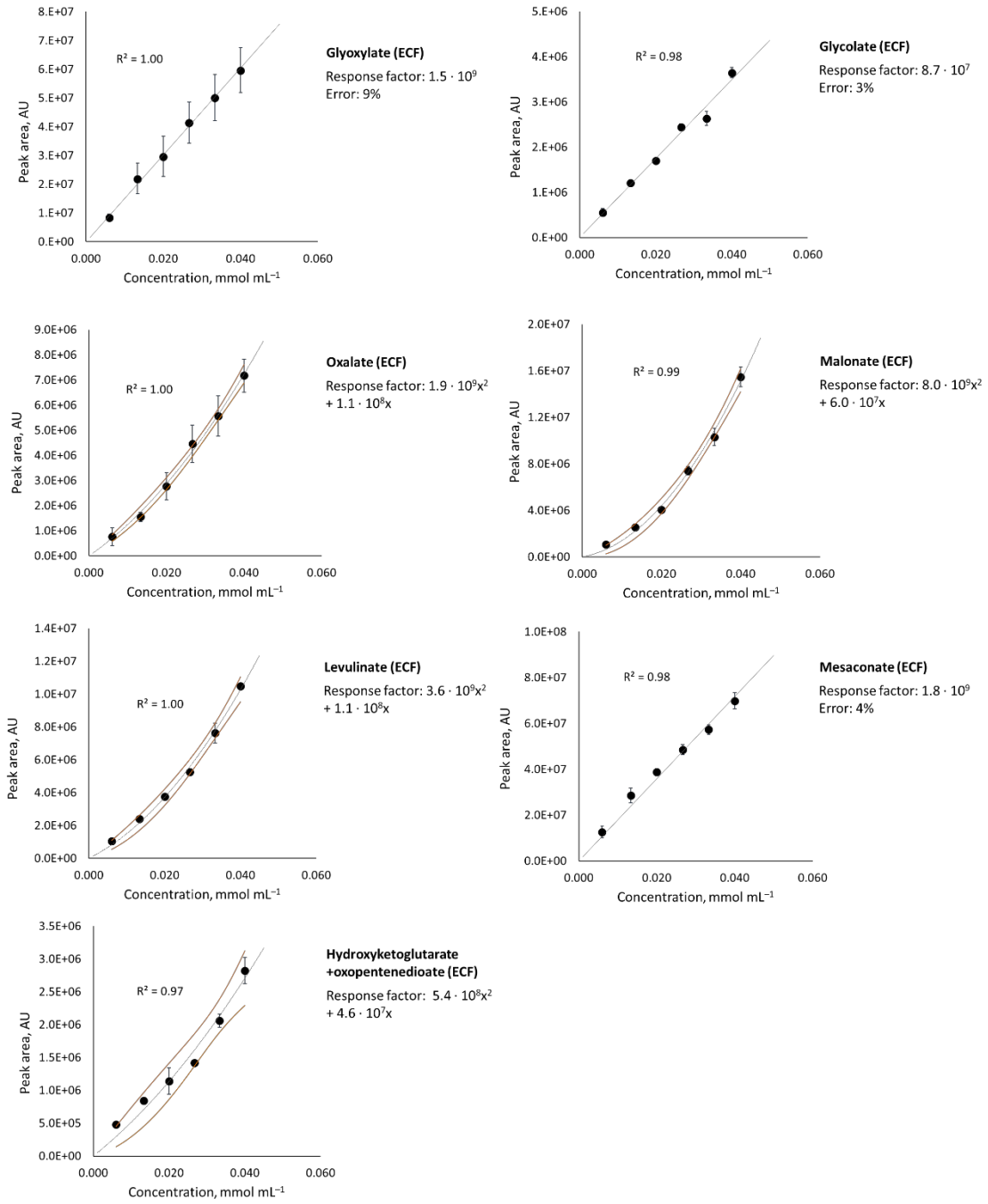
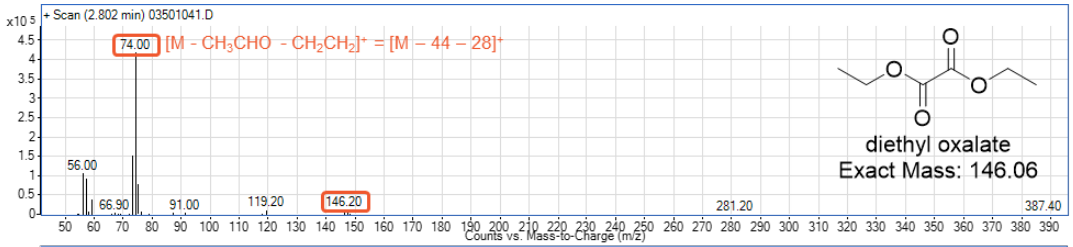
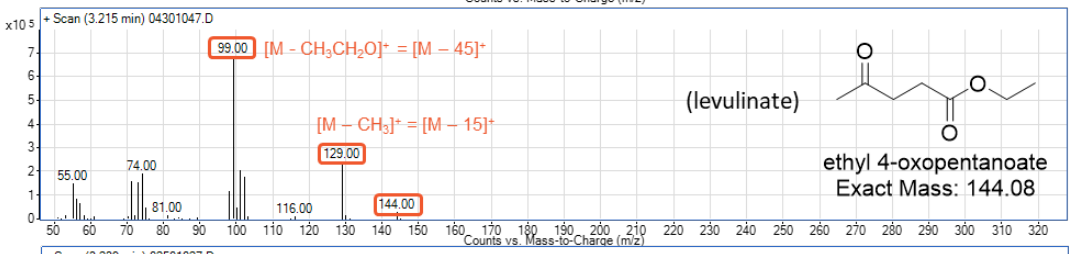
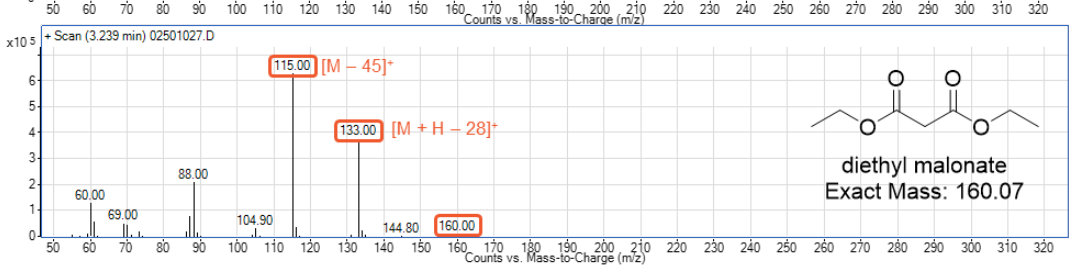


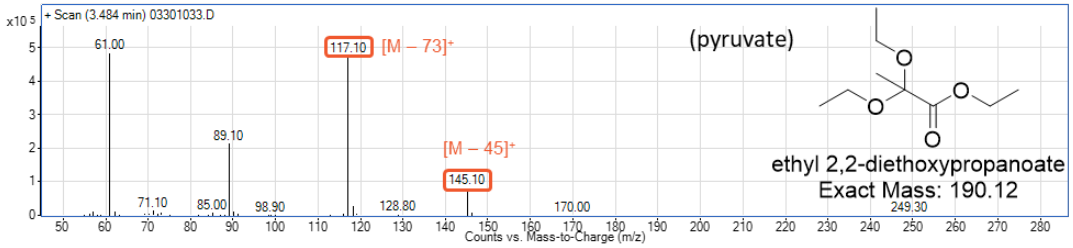
Figure S2.

Correlation between the concentration of an aqueous solution of carboxylic acids (glyoxylic, glycolic, oxalic, malonic, levulinic, mesaconic and hydroxyketoglutaric + oxopentenedioic) and the measured gas chromatography peak area. 95% confidence bounds computed for 2nd degree polynomial fits (*OriginPro*) are shown as orange lines.


+ Scan (2.802 min) 03501041.D
74.00 [M - CH3CHO - CH2CH2]+ = [M – 44 – 28]+
56.00
66.90
91.00
119.20
146.20
281.20
387.40
Counts vs. Mass-to-Charge (m/z)
diethyl oxalate
Exact Mass: 146.06


+ Scan (3.215 min) 04301047.D
99.00 [M - CH3CH2O]+ = [M – 45]+
[M – CH3]+ = [M – 15]+
129.00
55.00
74.00
81.00
116.00
144.00
Counts vs. Mass-to-Charge (m/z)
(levulinate)
ethyl 4-oxopentanoate
Exact Mass: 144.08


+ Scan (3.239 min) 02501027.D
115.00 [M – 45]+
133.00 [M + H – 28]+
60.00
69.00
88.00
104.90
144.80
160.00
Counts vs. Mass-to-Charge (m/z)
diethyl malonate
Exact Mass: 160.07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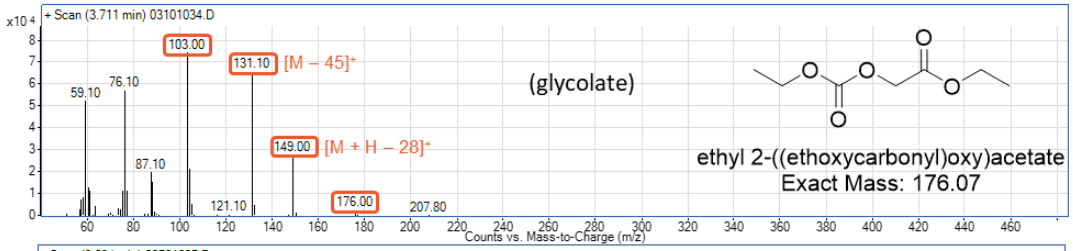
61.00
117.10 [M – 73]+
89.10
[M – 45]+
145.10
71.10
85.00
98.90
128.80
170.00
249.30
Counts vs. Mass-to-Charge (m/z)
(pyruvate)
ethyl 2,2-diethoxypropanoate
Exact Mass: 190.12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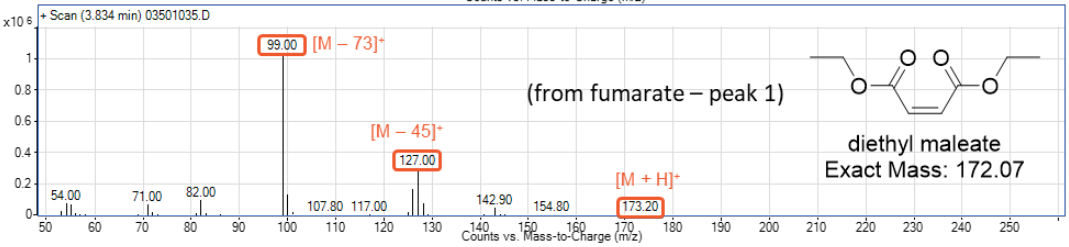
103.00
131.10 [M – 45]+
59.10
76.10
149.00 [M + H – 28]+
87.10
121.10
176.00
207.80
Counts vs. Mass-to-Charge (m/z)
(glycolate)
ethyl 2-((ethoxycarbonyl)oxy)acetate
Exact Mass: 176.07


+ Scan (3.834 min) 03501035.D
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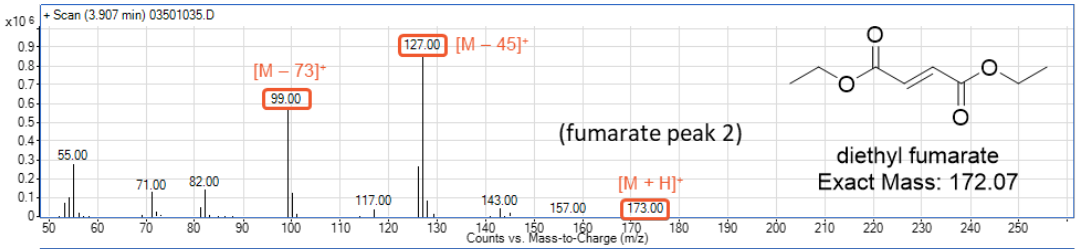
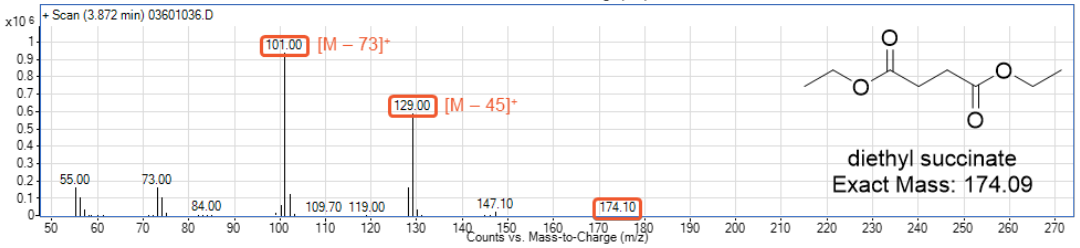
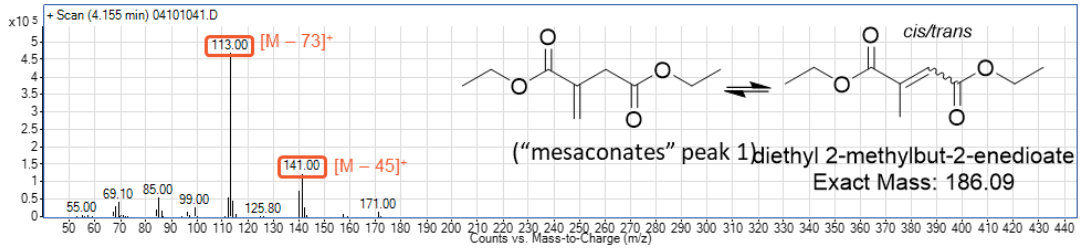
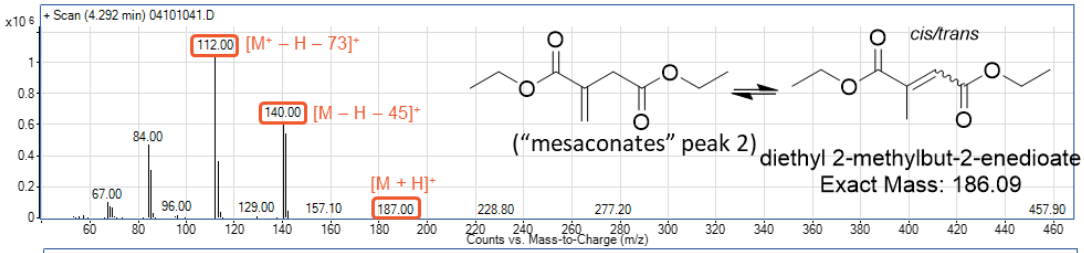
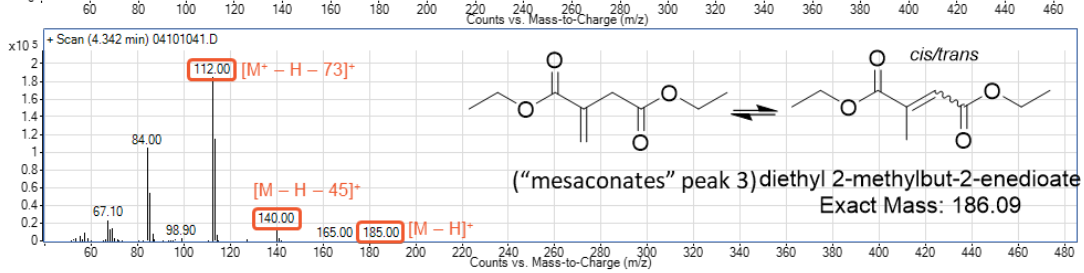
[M – 45]+
127.00
[M + H]+
54.00
71.00
82.00
107.80
117.00
142.90
154.80
173.20
Counts vs. Mass-to-Charge (m/z)
(from fumarate – peak 1)
diethyl maleate
Exact Mass: 172.07

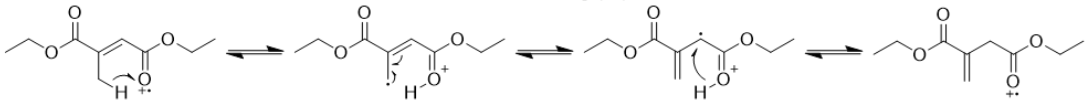

+ Scan (3.907 min) 03501035.D
127.00 [M – 45]+
[M – 73]+
99.00
(fumarate peak 2)
[M + H]+
173.00
diethyl fumarate
Exact Mass: 172.07
Counts vs. Mass-to-Charge (m/z)

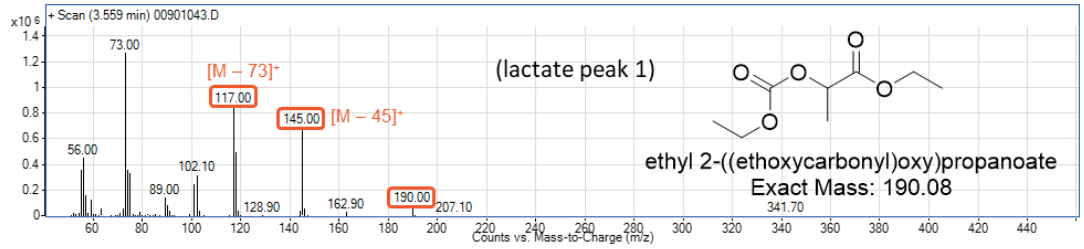

+ Scan (3.872 min) 03601036.D
101.00 [M – 73]+
129.00 [M – 45]+
diethyl succinate
Exact Mass: 174.09
174.10
Counts vs. Mass-to-Charge (m/z)

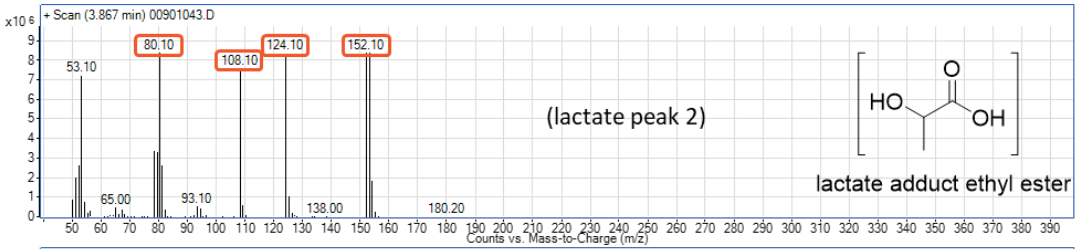

+ Scan (4.155 min) 04101041.D
113.00 [M – 73]+
141.00 [M – 45]+
cis/trans
("mesaconates" peak 1)
diethyl 2-methylbut-2-enedioate
Exact Mass: 186.09
Counts vs. Mass-to-Charge (m/z)

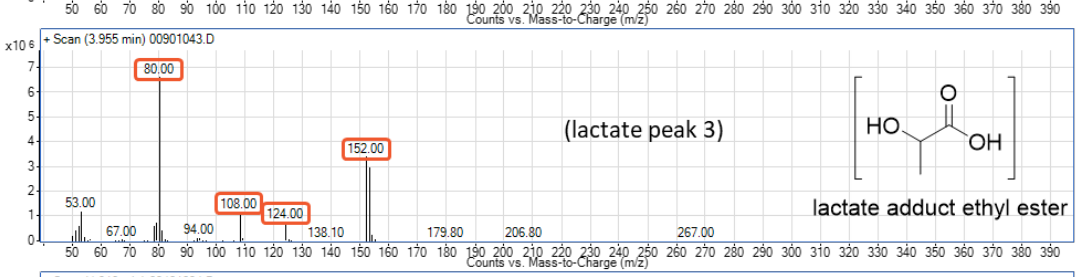

+ Scan (4.292 min) 04101041.D
112.00 [M+ – H – 73]+
140.00 [M – H – 45]+
[M + H]+
187.00
cis/trans
("mesaconates" peak 2)
diethyl 2-methylbut-2-enedioate
Exact Mass: 186.09
Counts vs. Mass-to-Charge (m/z)

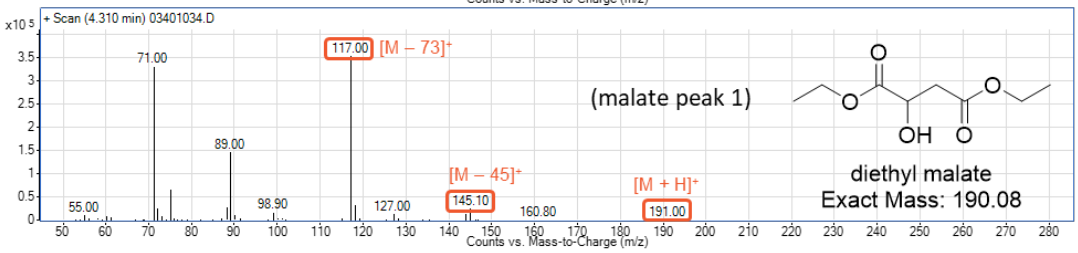

+ Scan (4.342 min) 04101041.D
112.00 [M+ – H – 73]+
[M – H – 45]+
140.00
185.00 [M – H]+
cis/trans
("mesaconates" peak 3)
diethyl 2-methylbut-2-enedioate
Exact Mass: 186.09
Counts vs. Mass-to-Charge (m/z)

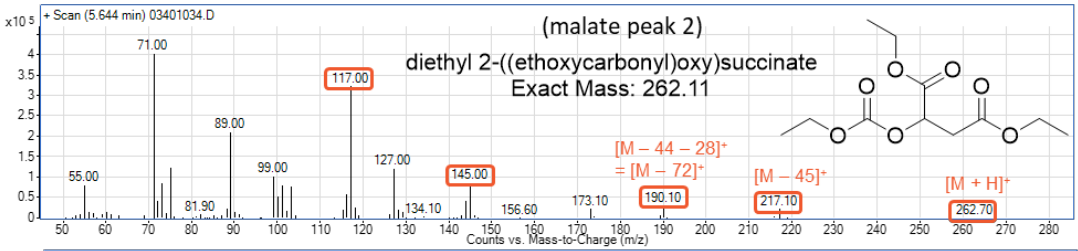


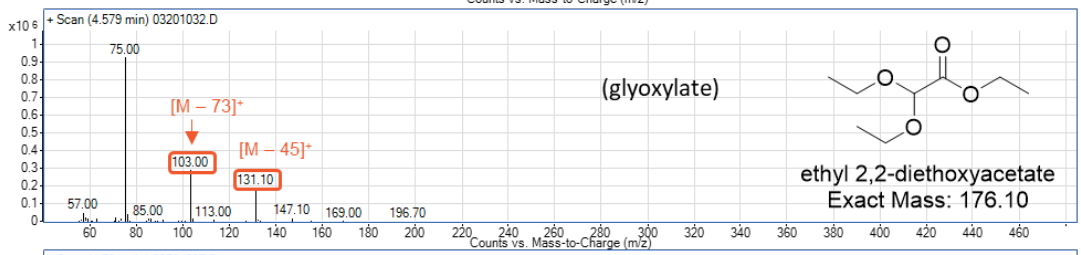

+ Scan (3.559 min) 00901043.D
73.00
[M – 73]+
117.00
145.00 [M – 45]+
(lactate peak 1)
ethyl 2-((ethoxycarbonyl)oxy)propanoate
Exact Mass: 190.08
190.00
Counts vs. Mass-to-Charge (m/z)

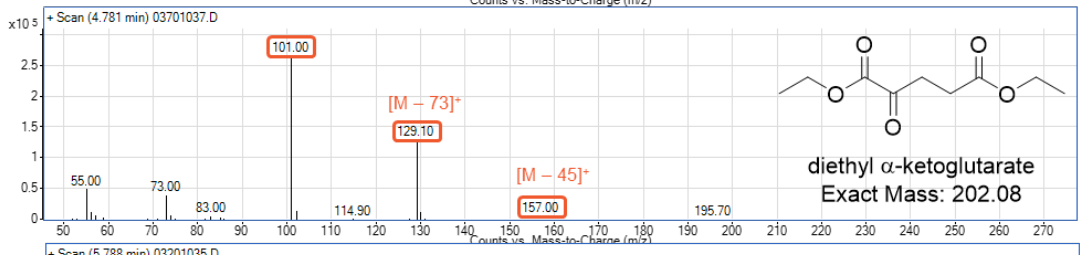

+ Scan (3.867 min) 00901043.D
(lactate peak 2)
lactate adduct ethyl ester
Counts vs. Mass-to-Charge (m/z)

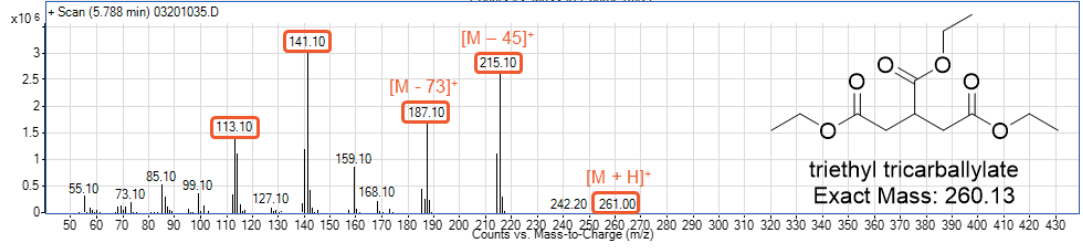

+ Scan (3.955 min) 00901043.D
(lactate peak 3)
lactate adduct ethyl ester
Counts vs. Mass-to-Charge (m/z)

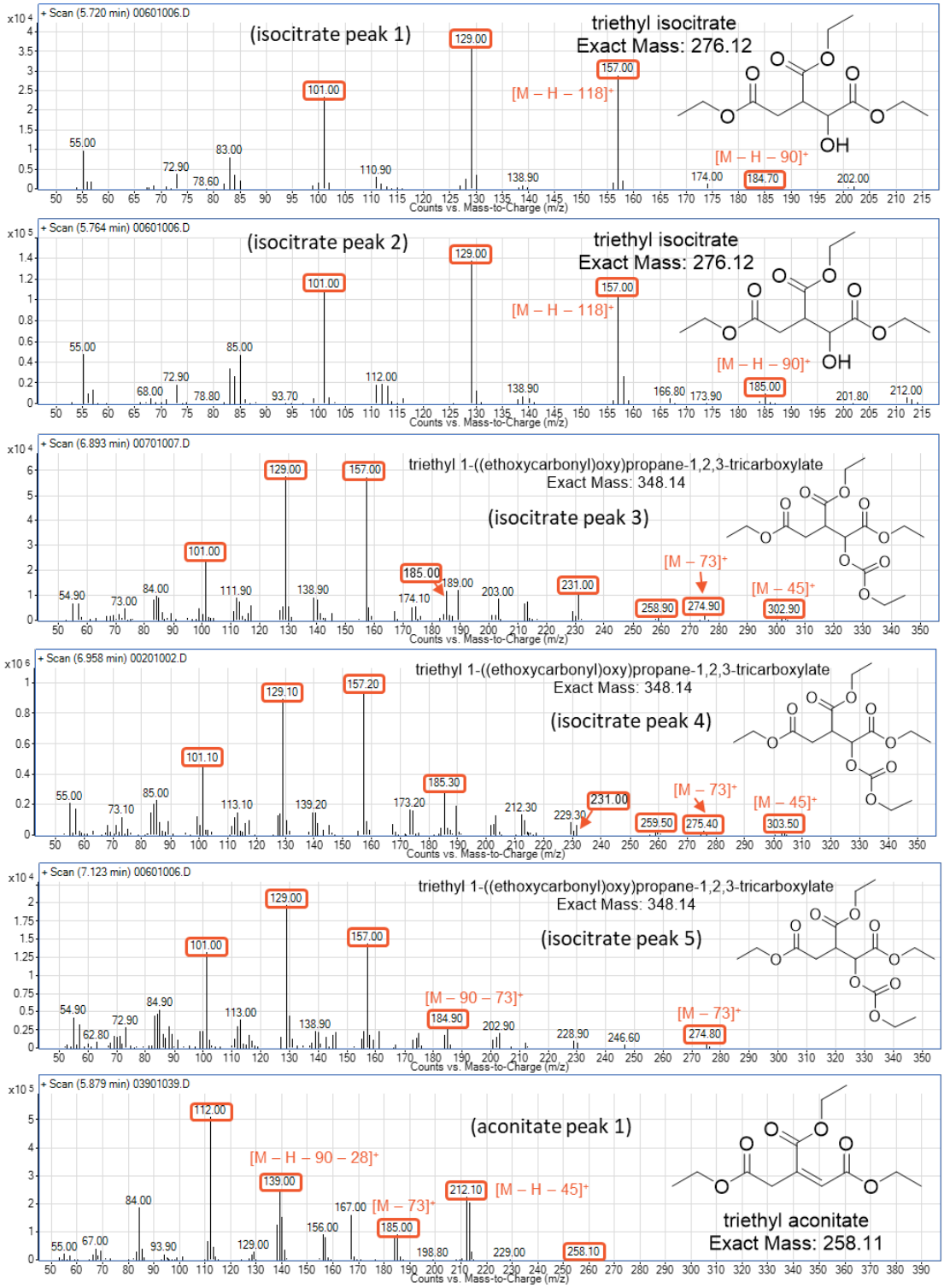

+ Scan (4.310 min) 03401034.D
(malate peak 1)
diethyl malate
Exact Mass: 190.08
Counts vs. Mass-to-Charge (m/z)


+ Scan (5.644 min) 03401034.D
(malate peak 2)
diethyl 2-((ethoxycarbonyl)oxy)succinate
Exact Mass: 262.11
Counts vs. Mass-to-Charge (m/z)


+ Scan (4.579 min) 03201032.D
(glyoxylate)
ethyl 2,2-diethoxyacetate
Exact Mass: 176.10
Counts vs. Mass-to-Charge (m/z)


+ Scan (4.781 min) 03701037.D
diethyl α-ketoglutarate
Exact Mass: 202.08
Counts vs. Mass-to-Charge (m/z)


+ Scan (5.788 min) 03201035.D
triethyl tricarballylate
Exact Mass: 260.13
Counts vs. Mass-to-Charge (m/z)


+ Scan (5.720 min) 00601006.D
(isocitrate peak 1)
triethyl isocitrate
Exact Mass: 276.12
[M – H – 118]+
[M – H – 90]+
+ Scan (5.764 min) 00601006.D
(isocitrate peak 2)
triethyl isocitrate
Exact Mass: 276.12
[M – H – 118]+
[M – H – 90]+
+ Scan (6.893 min) 00701007.D
triethyl 1-((ethoxycarbonyl)oxy)propane-1,2,3-tricarboxylate
Exact Mass: 348.14
(isocitrate peak 3)
[M – 73]+
[M – 45]+
+ Scan (6.958 min) 00201002.D
triethyl 1-((ethoxycarbonyl)oxy)propane-1,2,3-tricarboxylate
Exact Mass: 348.14
(isocitrate peak 4)
[M – 73]+
[M – 45]+
+ Scan (7.123 min) 00601006.D
triethyl 1-((ethoxycarbonyl)oxy)propane-1,2,3-tricarboxylate
Exact Mass: 348.14
(isocitrate peak 5)
[M – 90 – 73]+
[M – 73]+
+ Scan (5.879 min) 03901039.D
(aconitate peak 1)
[M – H – 90 – 28]+
[M – 73]+
[M – H – 45]+
triethyl aconitate
Exact Mass: 258.11
Counts vs. Mass-to-Charge (m/z)

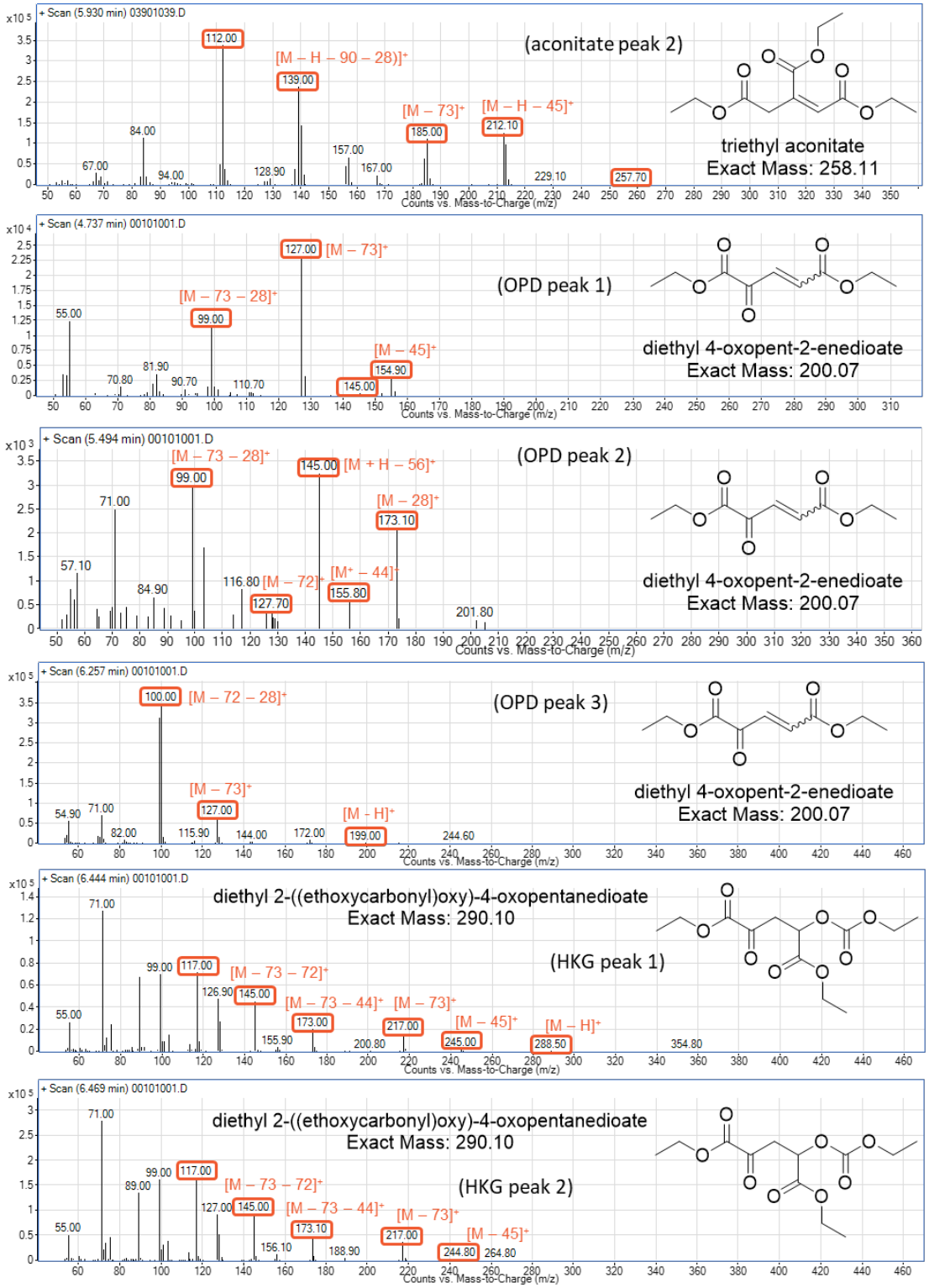


Figure S3.

Mass spectra of all ECF/EtOH-derivatized compounds detected in this study (authentic samples). Characteristic ("fingerprint") peaks are highlighted in red.

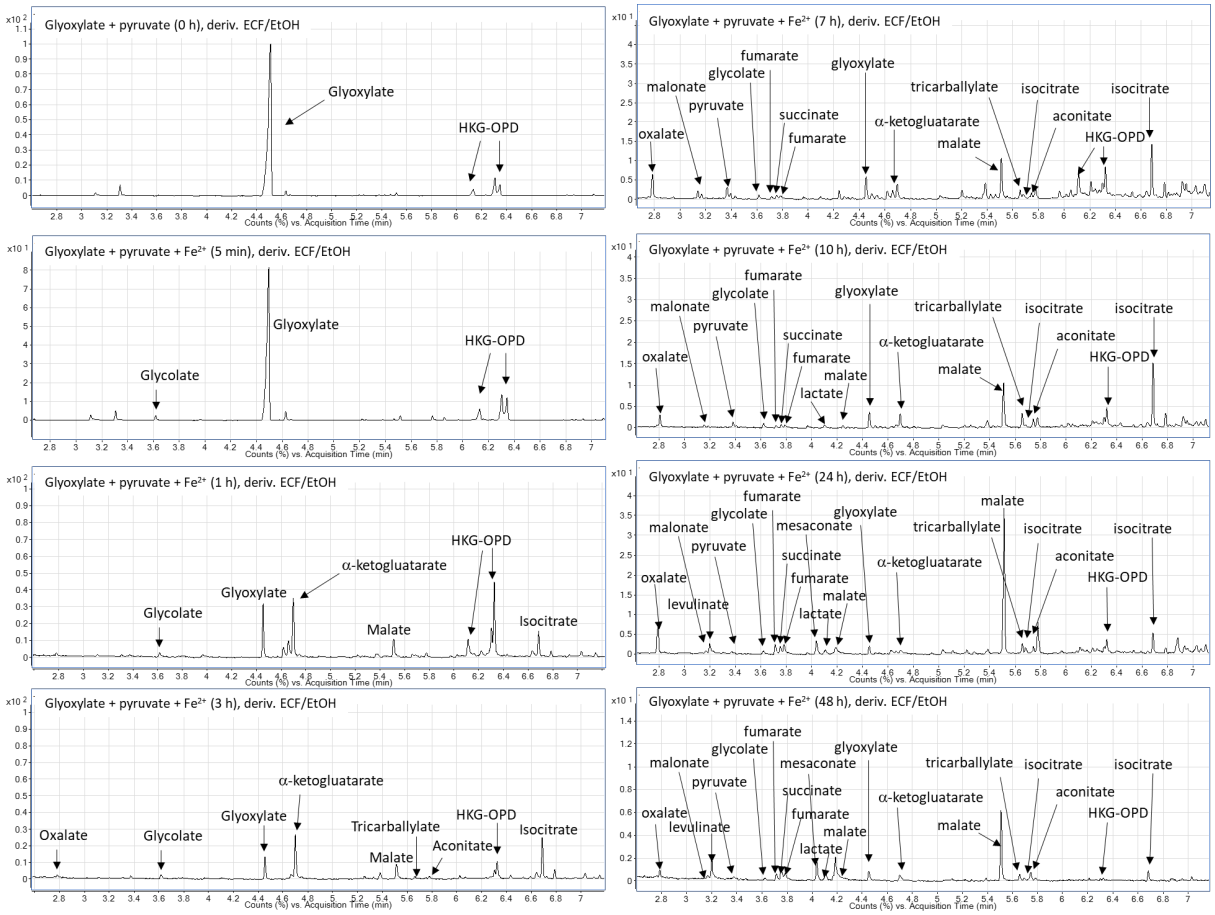
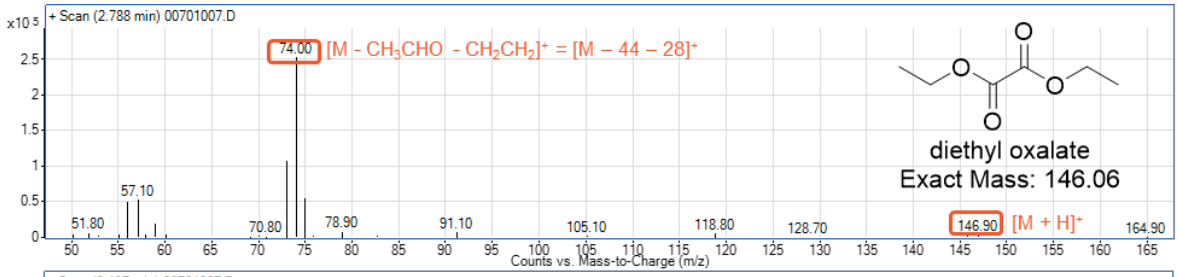
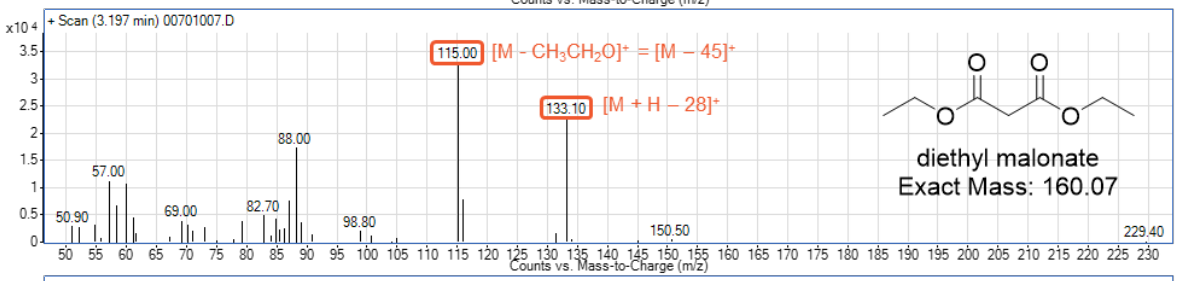
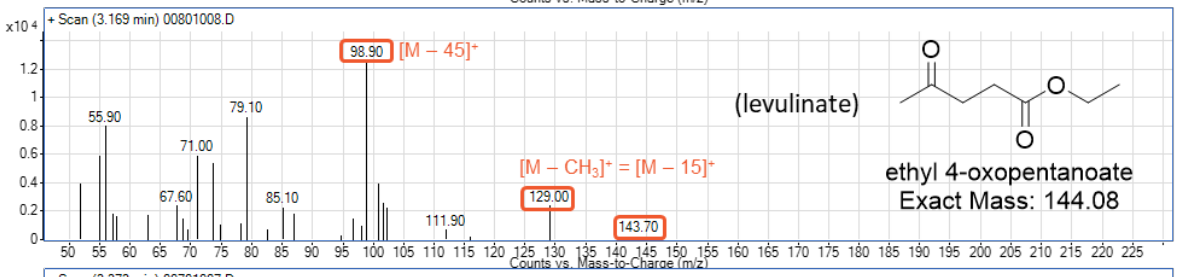


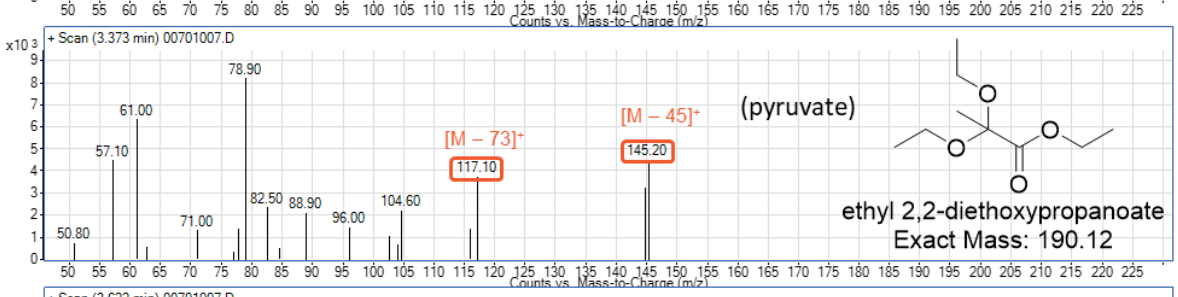
Figure S4.

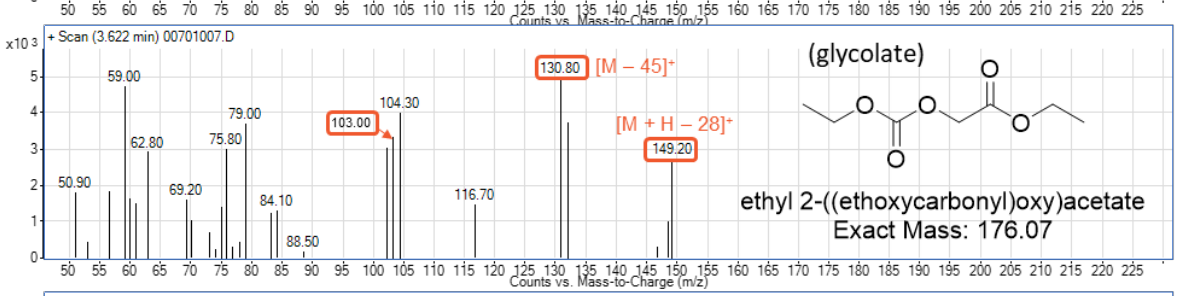
GC chromatograms showing evolution over time of a reaction network arising from pyruvate and glyoxylate, promoted by Fe^{2+} at 70 ºC. Peaks of major interest are captioned.

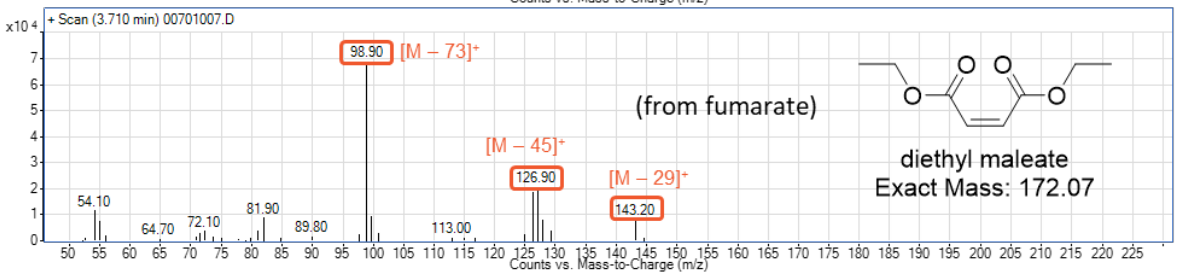

+ Scan (2.788 min) 00701007.D
74.00 [M - CH3CHO - CH2CH2]+ = [M – 44 – 28]+
57.10
51.80
70.80
78.90
91.10
105.10
118.80
128.70
146.90 [M + H]+
164.90
Counts vs. Mass-to-Charge (m/z)
diethyl oxalate
Exact Mass: 146.06

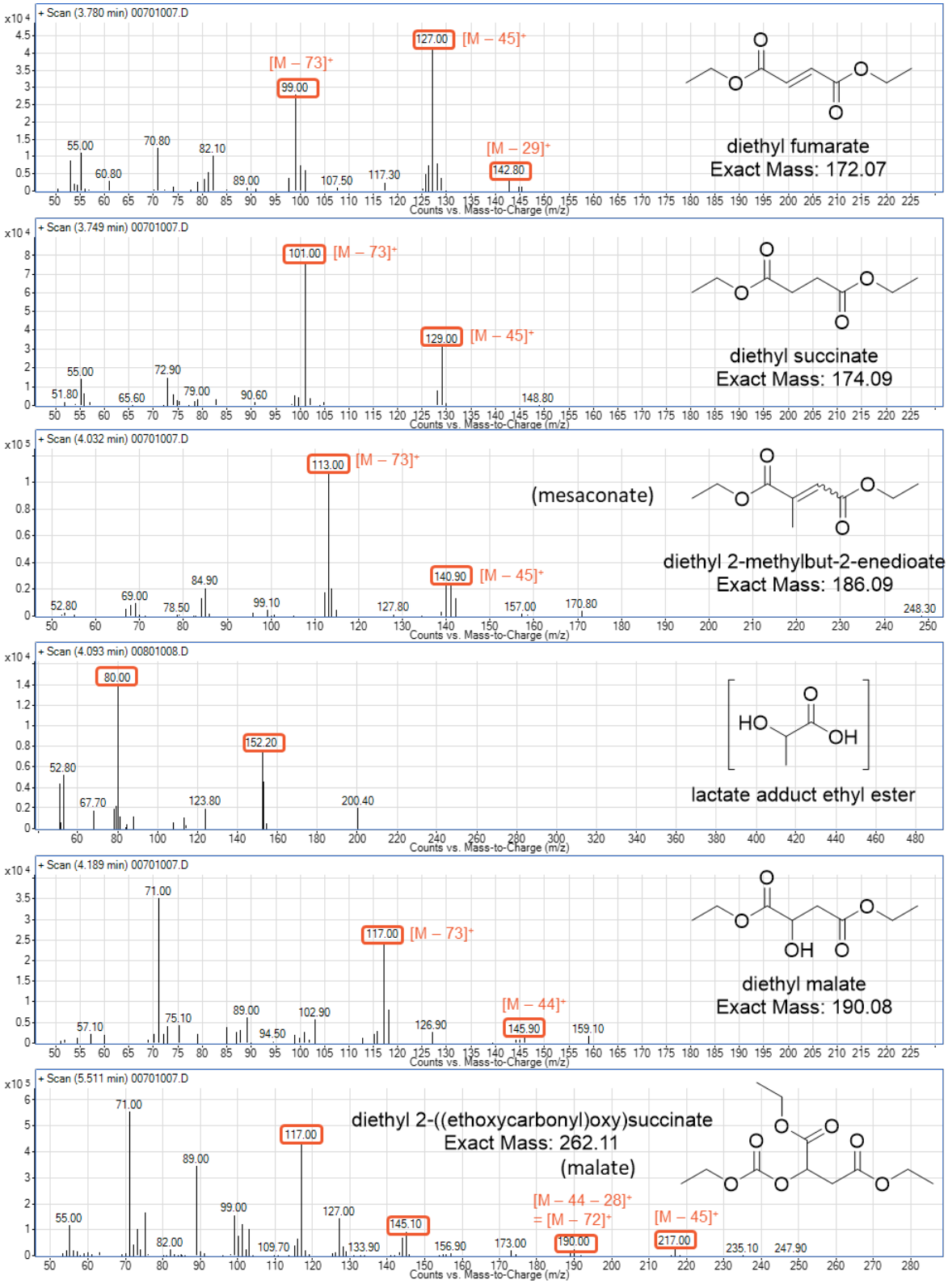

+ Scan (3.197 min) 00701007.D
115.00 [M - CH3CH2O]+ = [M – 45]+
133.10 [M + H – 28]+
88.00
57.00
50.90
69.00
82.70
98.80
150.50
229.40
Counts vs. Mass-to-Charge (m/z)
diethyl malonate
Exact Mass: 160.07

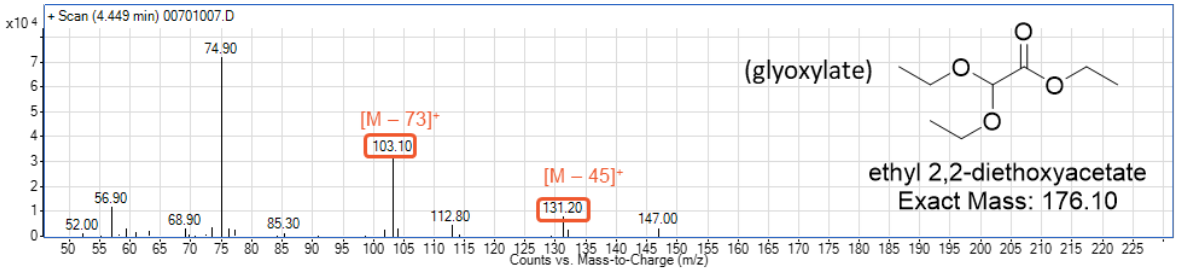

+ Scan (3.169 min) 00801008.D
98.90 [M – 45]+
55.90
79.10
71.00
67.60
85.10
111.90
[M – CH3]+ = [M – 15]+
129.00
143.70
Counts vs. Mass-to-Charge (m/z)
(levulinate)
ethyl 4-oxopentanoate
Exact Mass: 144.08

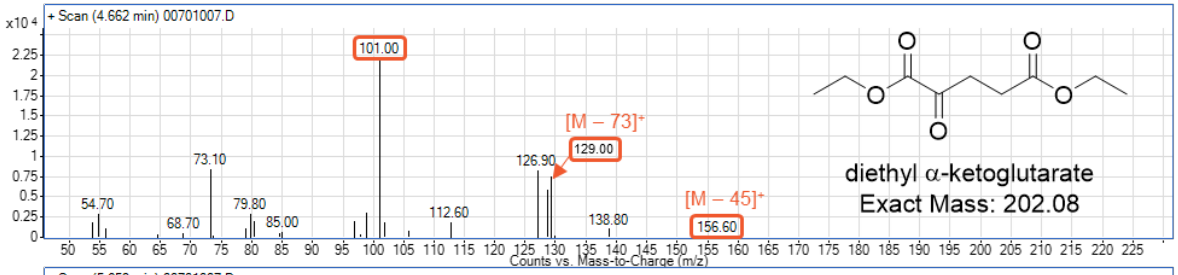

+ Scan (3.373 min) 00701007.D
78.90
61.00
57.10
50.80
71.00
82.50
88.90
96.00
104.60
[M – 73]+
117.10
[M – 45]+
145.20
Counts vs. Mass-to-Charge (m/z)
(pyruvate)
ethyl 2,2-diethoxypropanoate
Exact Mass: 190.12

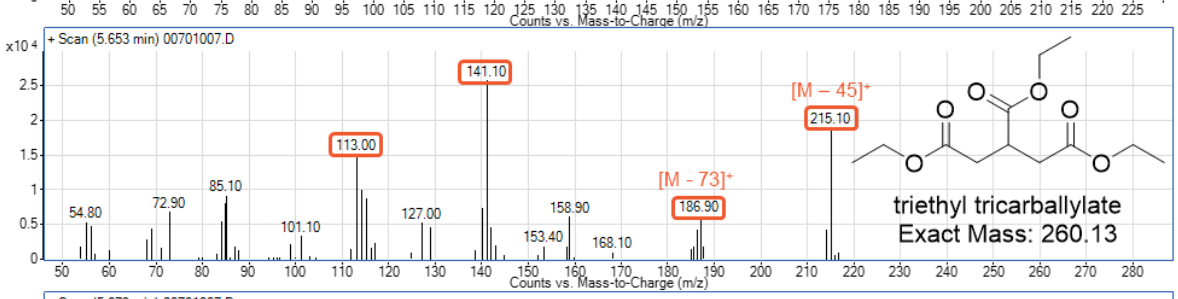

+ Scan (3.622 min) 00701007.D
59.00
62.80
79.00
75.80
50.90
69.20
84.10
88.50
103.00
104.30
116.70
130.80 [M – 45]+
[M + H – 28]+
149.20
Counts vs. Mass-to-Charge (m/z)
(glycolate)
ethyl 2-((ethoxycarbonyl)oxy)acetate
Exact Mass: 176.07

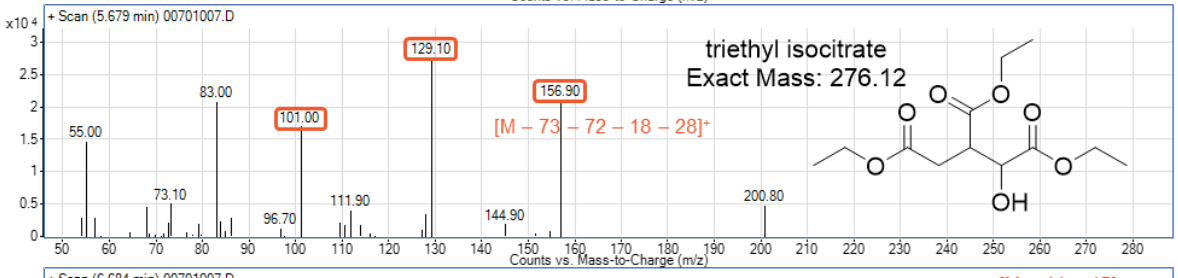

+ Scan (3.710 min) 00701007.D
98.90 [M – 73]+
54.10
64.70
72.10
81.90
89.80
113.00
[M – 45]+
126.90
[M – 29]+
143.20
Counts vs. Mass-to-Charge (m/z)
(from fumarate)
diethyl maleate
Exact Mass: 172.07

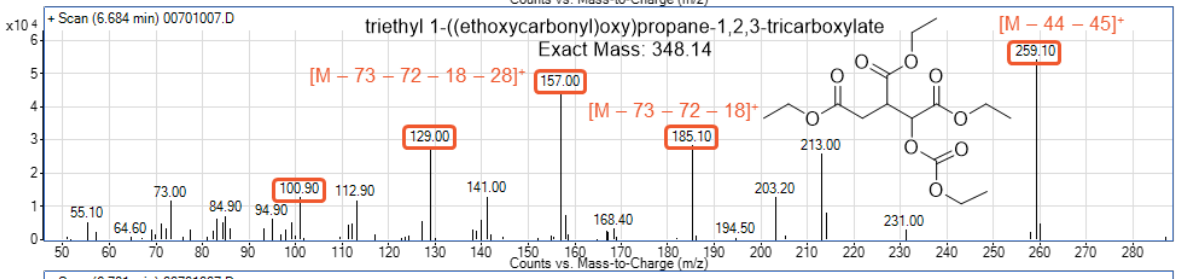

+ Scan (3.780 min) 00701007.D
127.00 [M – 45]+
[M – 73]+
99.00
[M – 29]+
142.80
55.00
70.80
82.10
60.80
89.00
107.50
117.30
diethyl fumarate
Exact Mass: 172.07
Counts vs. Mass-to-Charge (m/z)
+ Scan (3.749 min) 00701007.D
101.00 [M – 73]+
129.00 [M – 45]+
55.00
72.90
51.80
65.60
79.00
90.60
148.80
diethyl succinate
Exact Mass: 174.09
Counts vs. Mass-to-Charge (m/z)
+ Scan (4.032 min) 00701007.D
113.00 [M – 73]+
(mesaconate)
140.90 [M – 45]+
84.90
69.00
52.80
78.50
99.10
127.80
157.00
170.80
248.30
diethyl 2-methylbut-2-enedioate
Exact Mass: 186.09
Counts vs. Mass-to-Charge (m/z)
+ Scan (4.093 min) 00801008.D
80.00
152.20
52.80
67.70
123.80
200.40
lactate adduct ethyl ester
Counts vs. Mass-to-Charge (m/z)
+ Scan (4.189 min) 00701007.D
71.00
117.00 [M – 73]+
[M – 44]+
145.90
89.00
102.90
75.10
57.10
94.50
126.90
159.10
diethyl malate
Exact Mass: 190.08
Counts vs. Mass-to-Charge (m/z)
+ Scan (5.511 min) 00701007.D
71.00
117.00
89.00
diethyl 2-((ethoxycarbonyl)oxy)succinate
Exact Mass: 262.11
(malate)
[M – 44 – 28]+
= [M – 72]+
[M – 45]+
55.00
99.00
127.00
145.10
82.00
109.70
133.90
156.90
173.00
190.00
217.00
235.10
247.90
Counts vs. Mass-to-Charge (m/z)

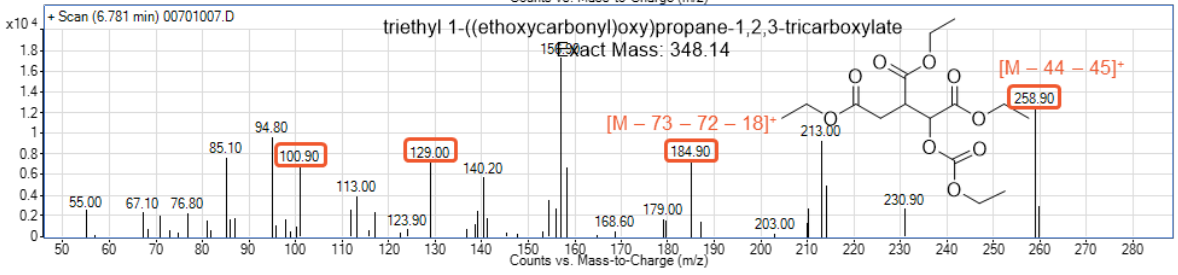

+ Scan (4.449 min) 00701007.D
(glyoxylate)
[M – 73]+
[M – 45]+
ethyl 2,2-diethoxyacetate
Exact Mass: 176.10
Counts vs. Mass-to-Charge (m/z)


+ Scan (4.662 min) 00701007.D
[M – 73]+
[M – 45]+
diethyl α-ketoglutarate
Exact Mass: 202.08
Counts vs. Mass-to-Charge (m/z)


+ Scan (5.653 min) 00701007.D
[M – 45]+
[M - 73]+
triethyl tricarballylate
Exact Mass: 260.13
Counts vs. Mass-to-Charge (m/z)


+ Scan (5.679 min) 00701007.D
triethyl isocitrate
Exact Mass: 276.12
[M – 73 – 72 – 18 – 28]+
OH
Counts vs. Mass-to-Charge (m/z)


+ Scan (6.684 min) 00701007.D
triethyl 1-((ethoxycarbonyl)oxy)propane-1,2,3-tricarboxylate
Exact Mass: 348.14
[M – 73 – 72 – 18 – 28]+
[M – 73 – 72 – 18]+
[M – 44 – 45]+
Counts vs. Mass-to-Charge (m/z)


+ Scan (6.781 min) 00701007.D
triethyl 1-((ethoxycarbonyl)oxy)propane-1,2,3-tricarboxylate
Exact Mass: 348.14
[M – 73 – 72 – 18]+
[M – 44 – 45]+
Counts vs. Mass-to-Charge (m/z)

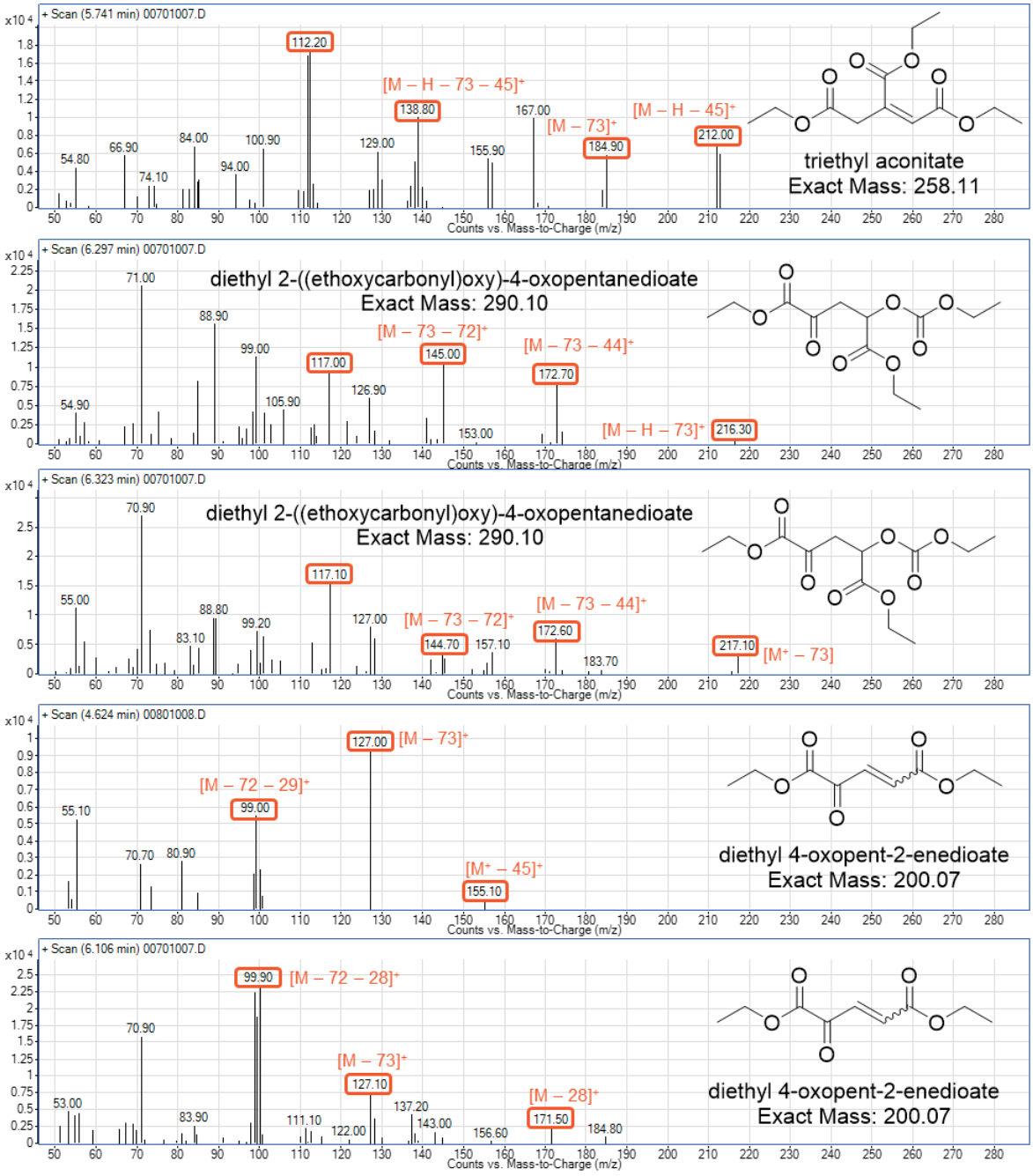


Figure S5.

Mass spectra of all ECF/EtOH-derivatized compounds detected at t = 24 h in the reaction network arising from pyruvate and glyoxylate, promoted by Fe^{2+} at 70 ºC. Characteristic ("fingerprint") peaks are highlighted in red.

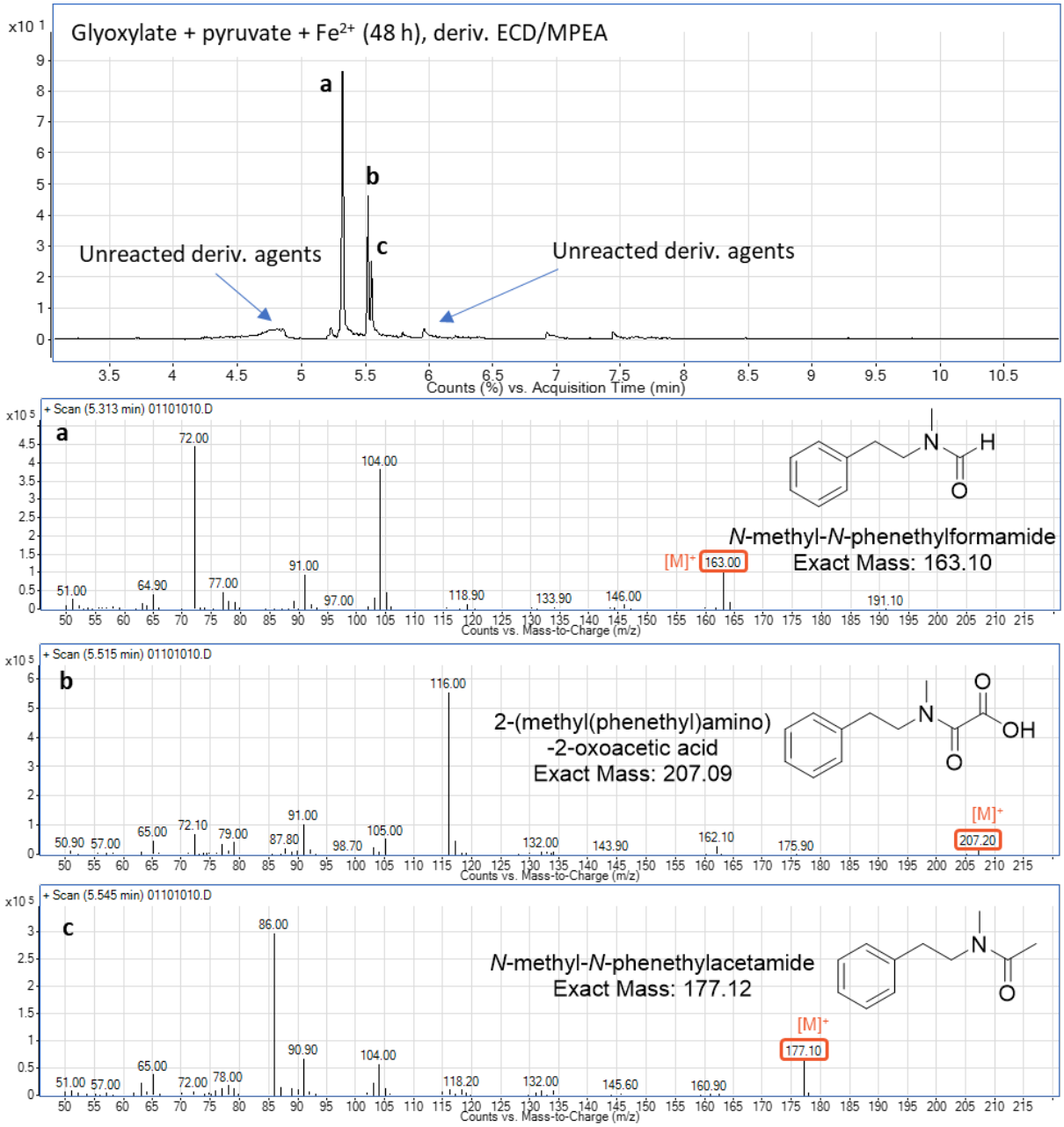


Figure S6

GC chromatogram and mass spectra confirming the presence of formate, oxalate and acetate, detected as their *N*-methyl-*N*-phenethylamides, after derivatization of a typical reaction mixture with ECD/MPEA after 48 h reaction time.

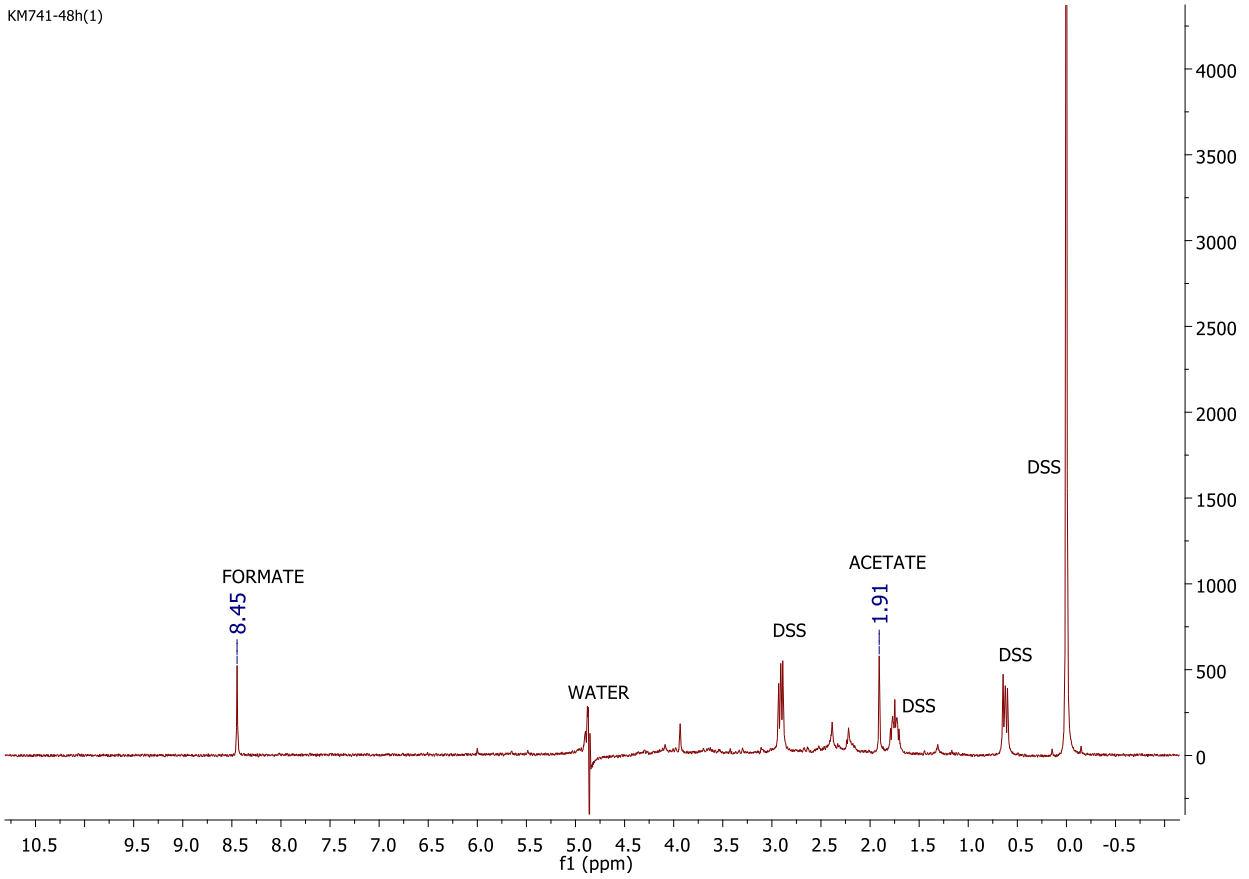


Figure S7.

1H NMR spectrum of a typical reaction mixture after 48 h, confirming the presence of formate and acetate (DSS was used as standard).

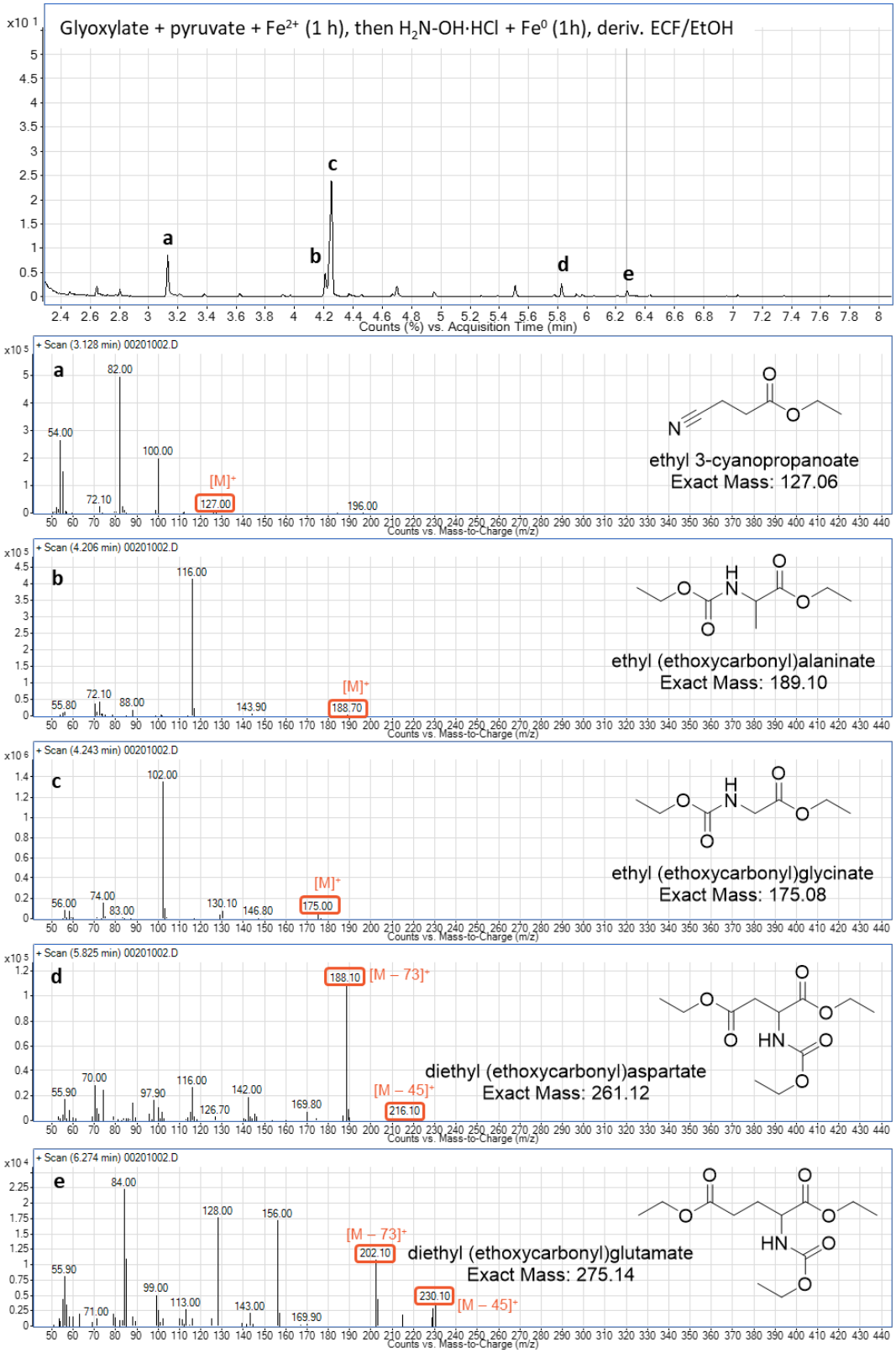
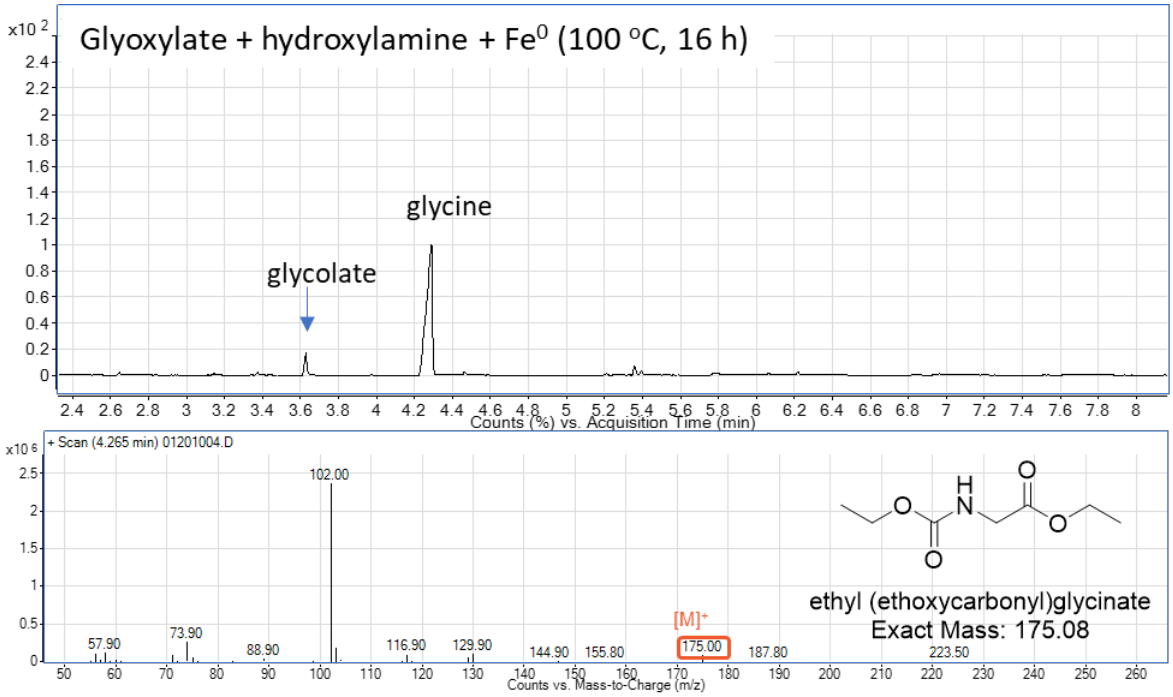
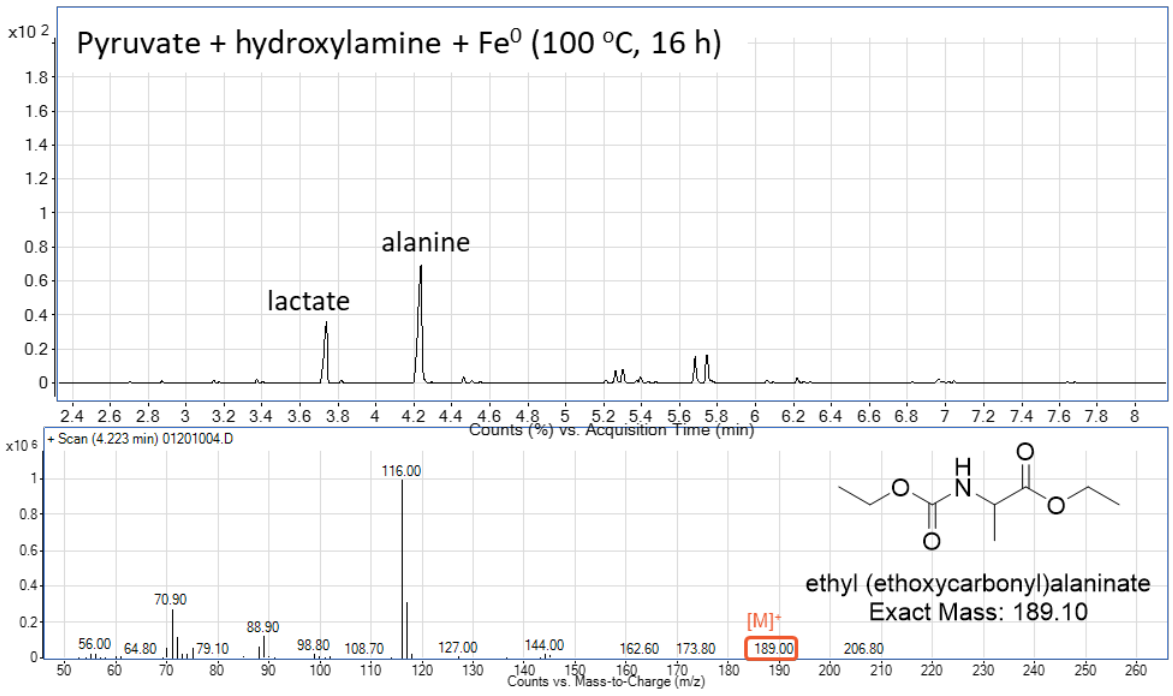


Figure S8.

GC chromatogram and mass spectra confirming the presence of glycine, alanine, aspartic acid and glutamic acid, after derivatization of a reaction mixture with ECF/EtOH.


Glyoxylate + hydroxylamine + Fe0 (100 °C, 16 h)
glycine
glycolate
Counts (%) vs. Acquisition Time (min)
+ Scan (4.265 min) 01201004.D
102.00
[M]+
175.00
ethyl (ethoxycarbonyl)glycinate
Exact Mass: 175.08
Counts vs. Mass-to-Charge (m/z)


Pyruvate + hydroxylamine + Fe0 (100 °C, 16 h)
alanine
lactate
Counts (%) vs. Acquisition Time (min)
+ Scan (4.223 min) 01201004.D
116.00
[M]+
189.00
ethyl (ethoxycarbonyl)alaninate
Exact Mass: 189.10
Counts vs. Mass-to-Charge (m/z)

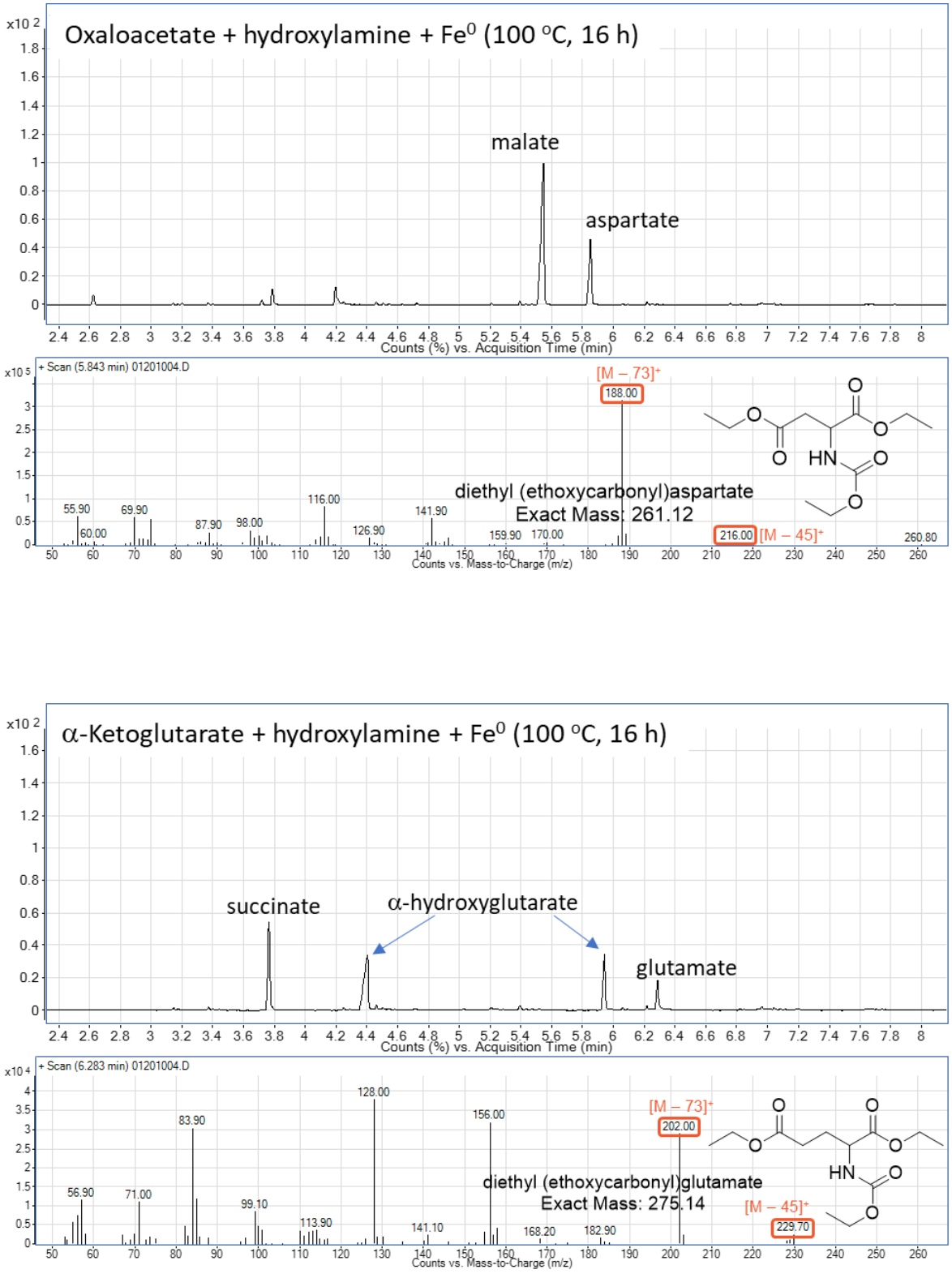


Figure S9.

GC chromatograms and mass spectra of ECF/EtOH-derivatized amino acids arising from different ketoacids, hydroxylamine and Fe^0 at 100 °C after 16 h.

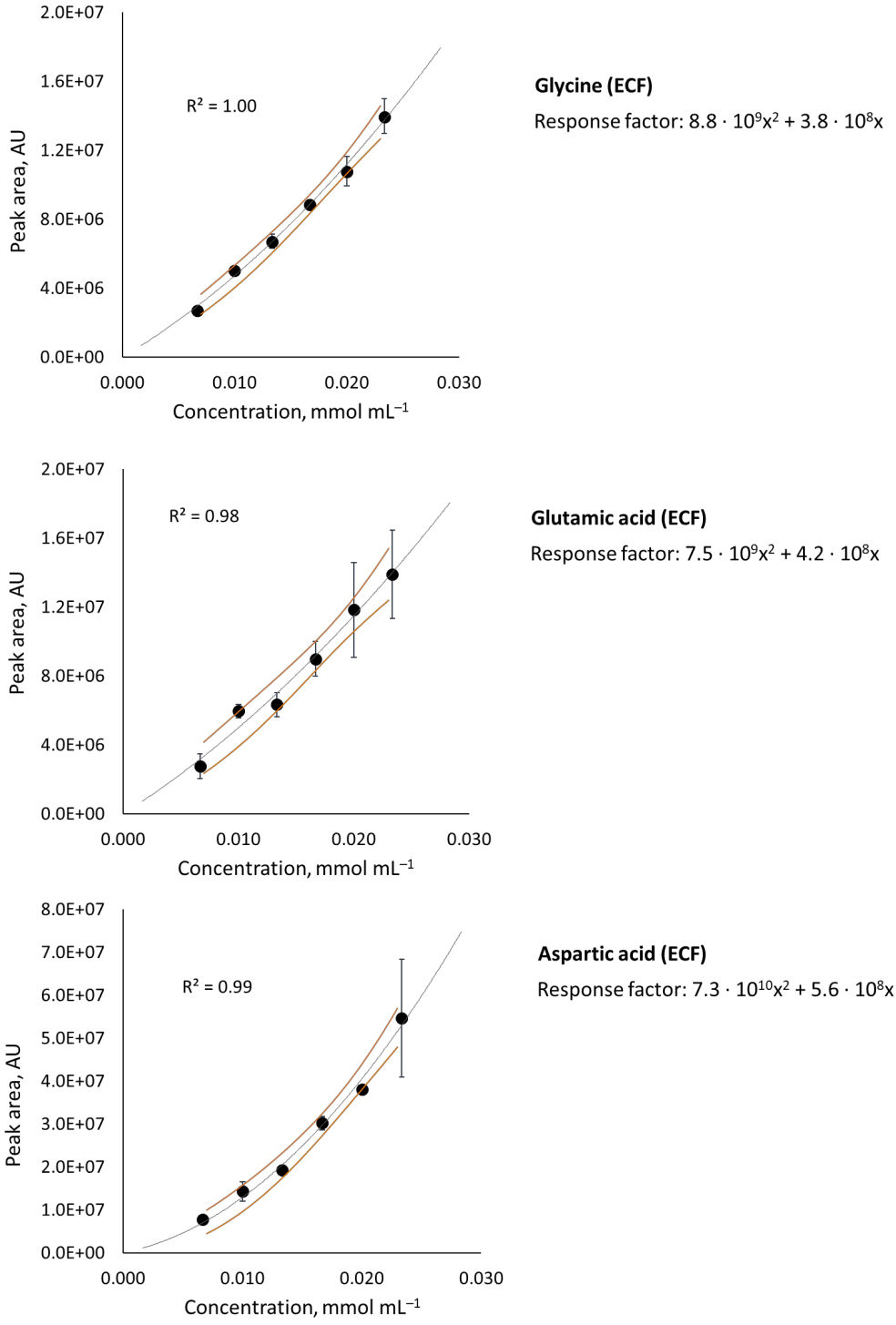


Figure S10.

Correlation between the concentration of an aqueous solution of amino acids (glycine, aspartic acid and glutamic acid) and the measured gas chromatography peak area. 95% confidence bounds computed for 2nd degree polynomial fits (*OriginPro*) are shown as orange lines.

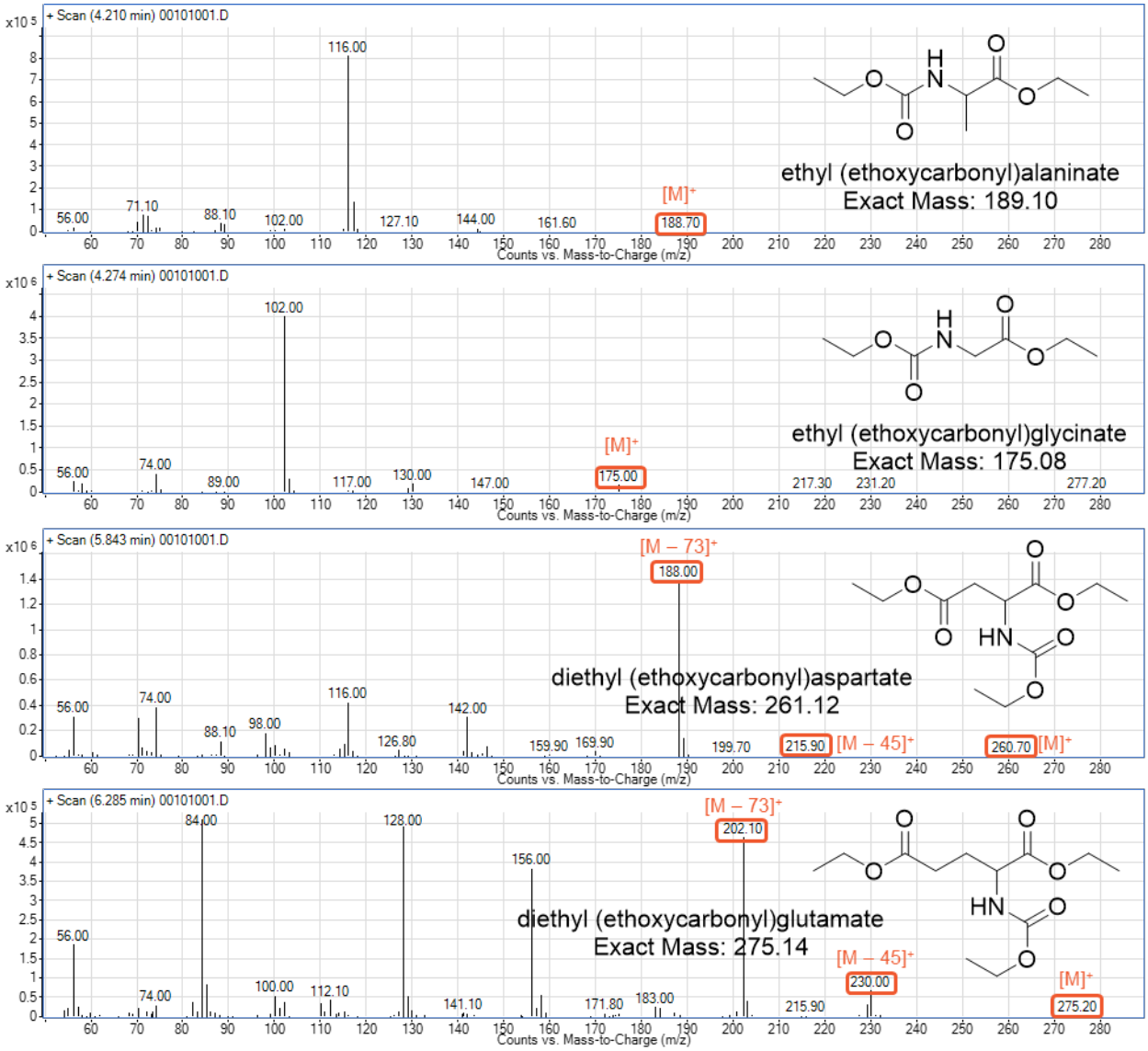


Figure S11.

Mass spectra of authentic samples of glycine, alanine, aspartic acid and glutamic acid, after derivatization with ECF/EtOH.

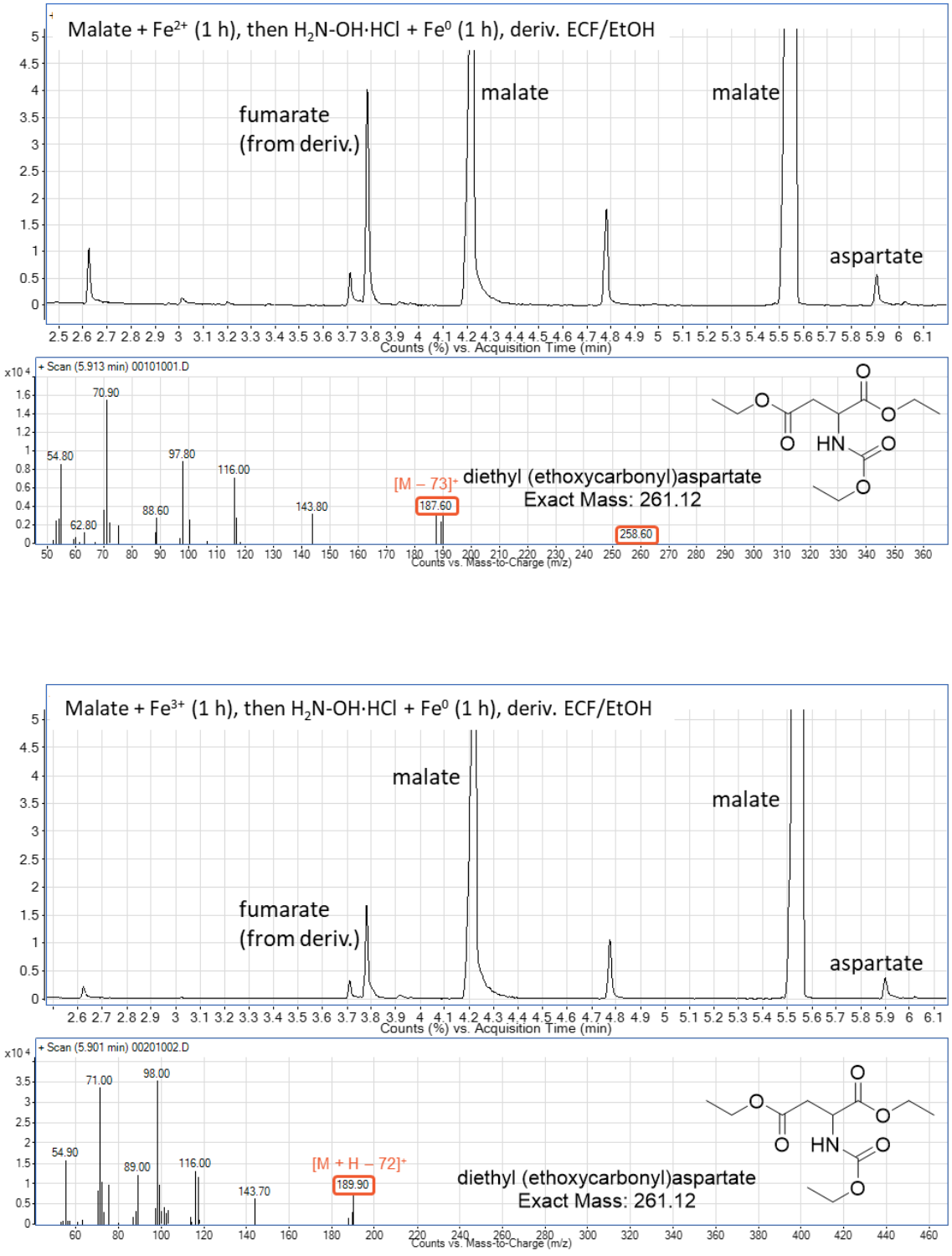
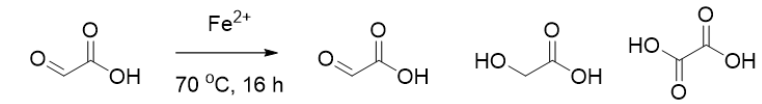
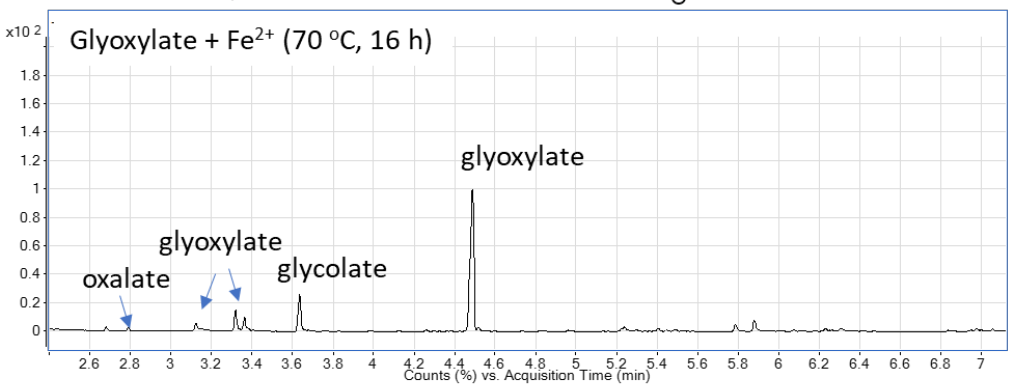
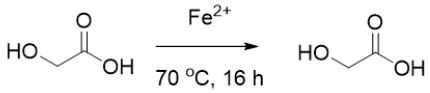


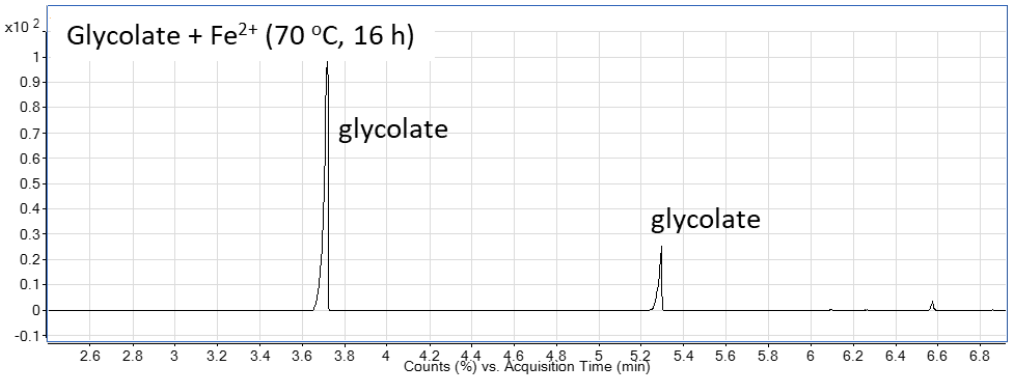
Figure S12

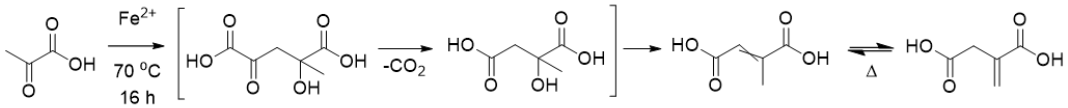
GC chromatograms of control reactions (malate + Fe^{2+} or Fe^{3+}, then reductive amination with hydroxylamine), after derivatization of the reaction mixture with ECF/EtOH.

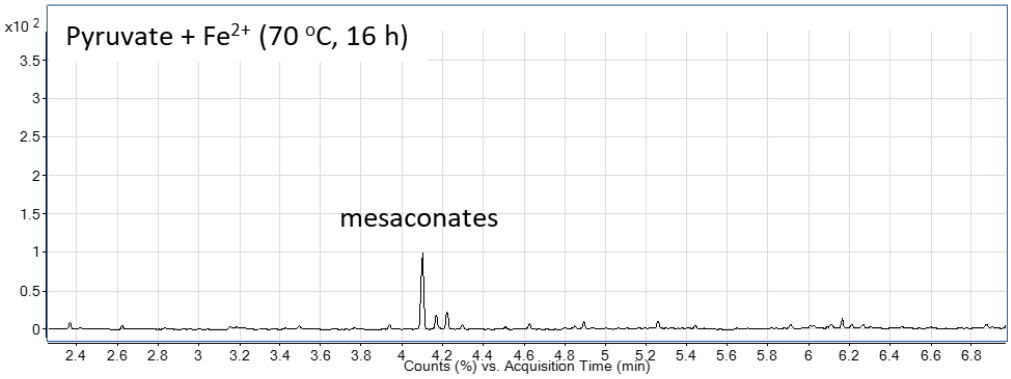

Fe2+
70 °C, 16 h

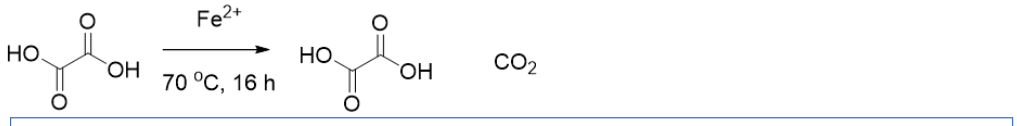

Glyoxylate + Fe2+ (70 °C, 16 h)
glyoxylate
glyoxylate
glycolate
oxalate
Counts (%) vs. Acquisition Time (min)

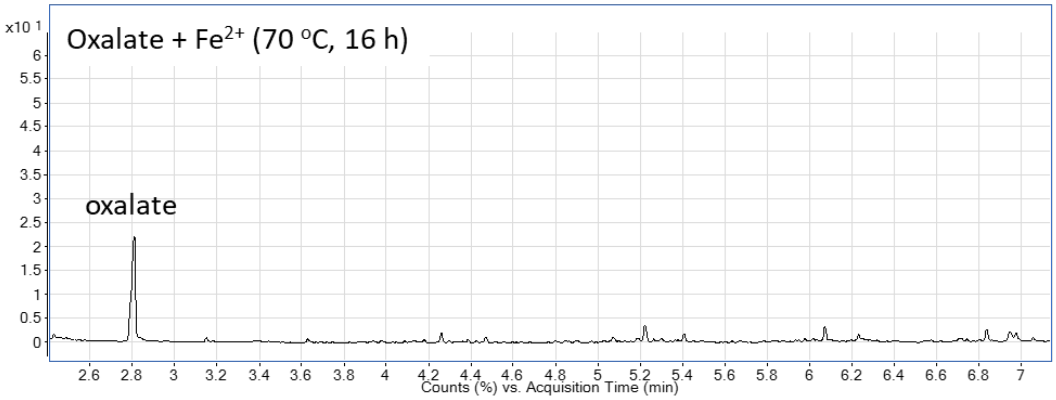

Fe2+
70 °C, 16 h

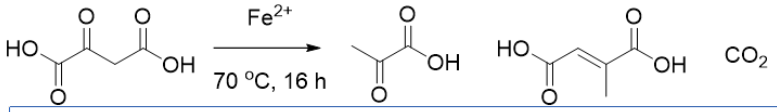

Glycolate + Fe2+ (70 °C, 16 h)
glycolate
glycolate
Counts (%) vs. Acquisition Time (min)

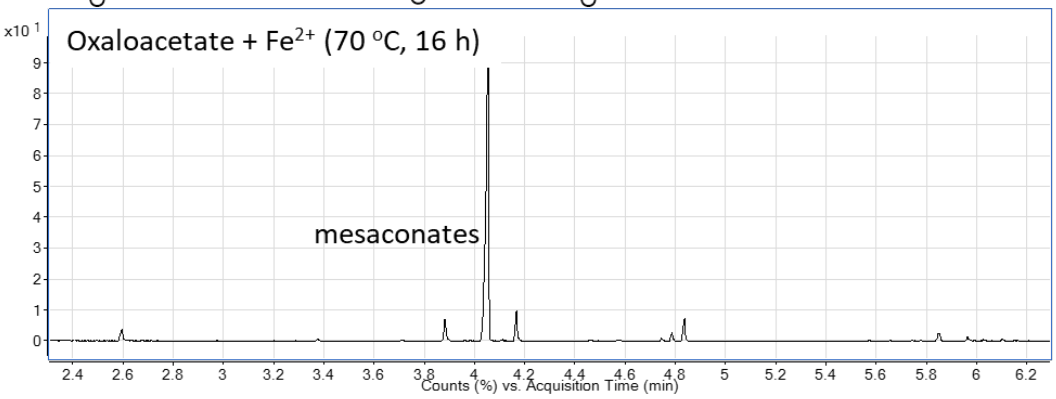

Fe2+
70 °C
16 h
-CO2
Δ

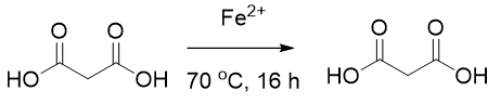

Pyruvate + Fe2+ (70 °C, 16 h)
mesaconates
Counts (%) vs. Acquisition Time (min)

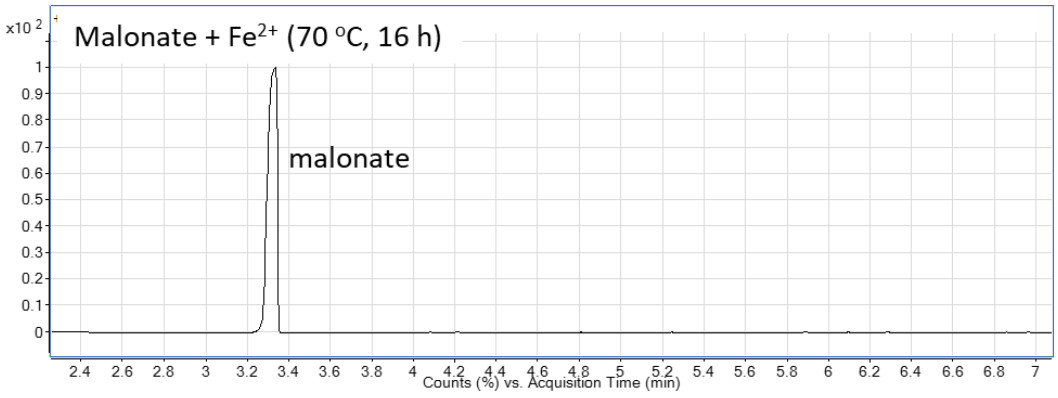

Fe2+
70 °C, 16 h
CO2

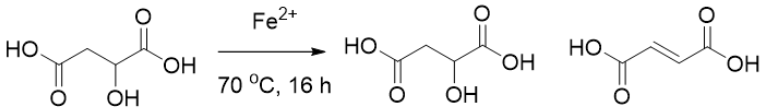

Oxalate + Fe2+ (70 °C, 16 h)
oxalate
Counts (%) vs. Acquisition Time (min)

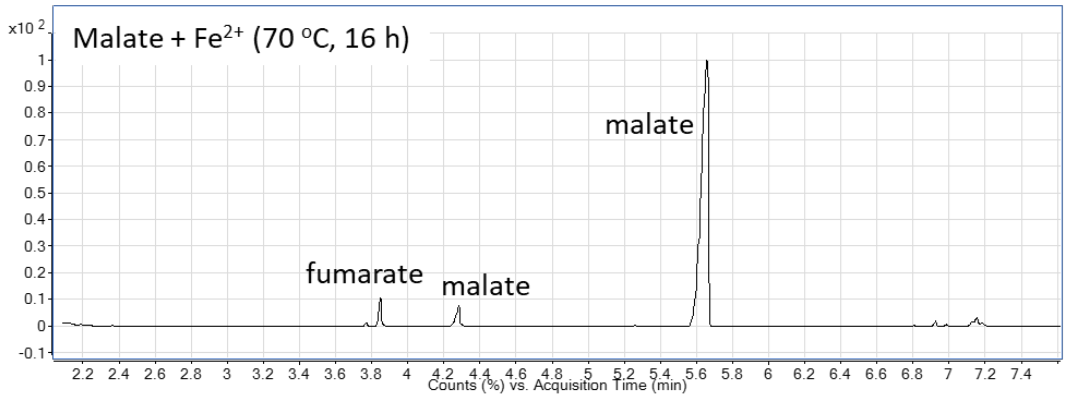

Fe2+
70 °C, 16 h
CO2

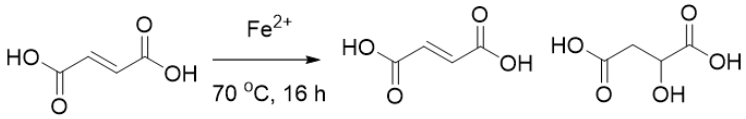

Oxaloacetate + Fe2+ (70 °C, 16 h)
mesaconates
Counts (%) vs. Acquisition Time (min)

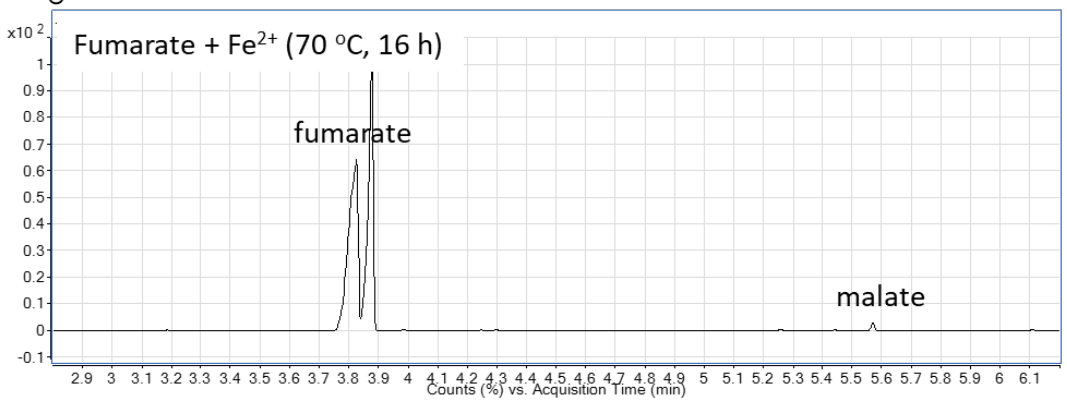

Fe2+
70 °C, 16 h

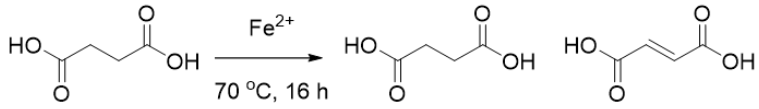

Malonate + Fe2+ (70 °C, 16 h)
malonate
Counts (%) vs. Acquisition Time (min)

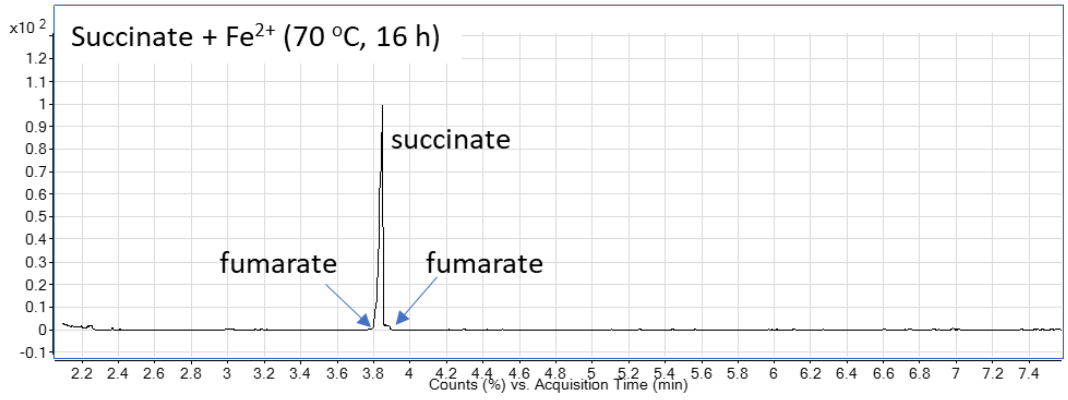

HO
O
OH
O
OH
Fe2+
70 °C, 16 h
HO
O
OH
O
OH
HO
O
OH
O

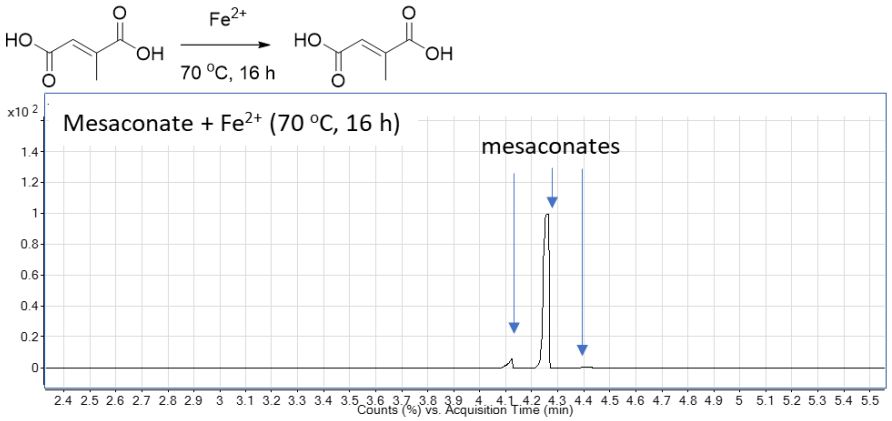

Malate + Fe2+ (70 °C, 16 h)
malate
fumarate
malate
Counts (%) vs. Acquisition Time (min)

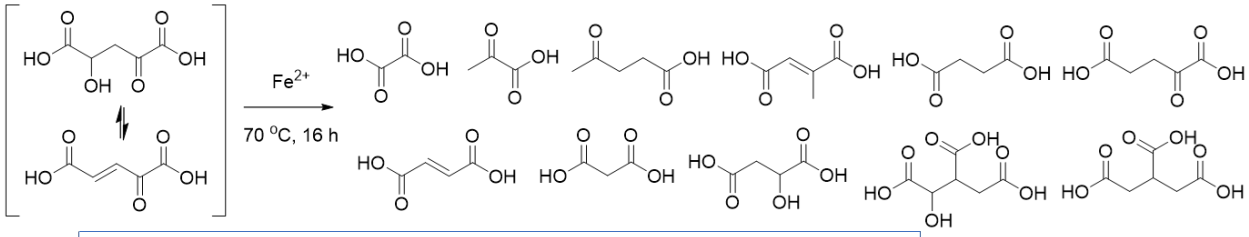

HO
O
OH
O
Fe2+
70 °C, 16 h
HO
O
OH
HO
O
OH
O
OH

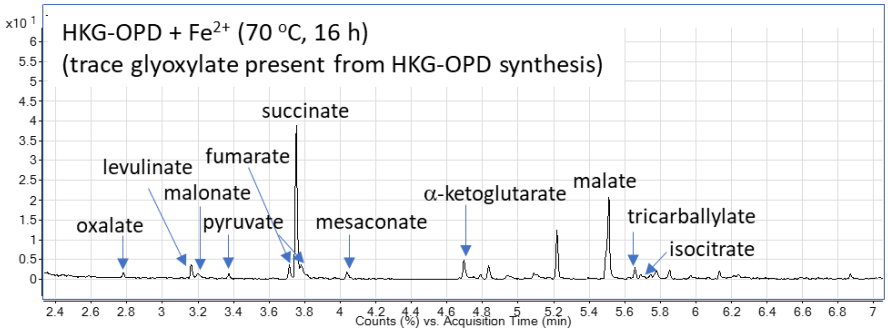

Fumarate + Fe2+ (70 °C, 16 h)
fumarate
malate
Counts (%) vs. Acquisition Time (min)

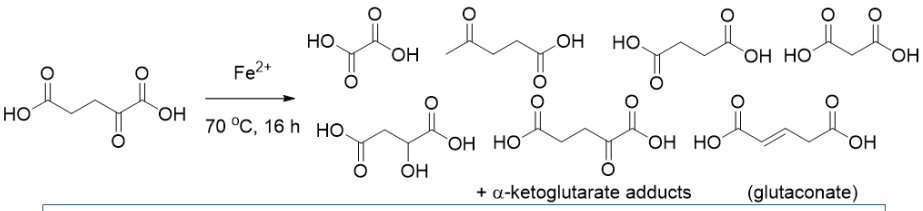

HO
O
OH
O
Fe2+
70 °C, 16 h
HO
O
OH
HO
O
OH
O

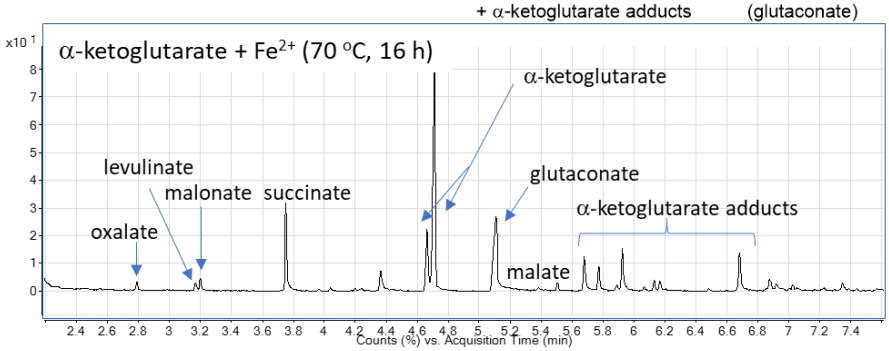

Succinate + Fe2+ (70 °C, 16 h)
succinate
fumarate
fumarate
Counts (%) vs. Acquisition Time (min)


Fe2+
70 °C, 16 h
Mesaconate + Fe2+ (70 °C, 16 h)
mesaconates
Counts (%) vs. Acquisition Time (min)


Fe2+
70 °C, 16 h


HKG-OPD + Fe2+ (70 °C, 16 h)
(trace glyoxylate present from HKG-OPD synthesis)
succinate
levulinate
fumarate
malonate
α-ketoglutarate
malate
oxalate
pyruvate
mesaconate
tricarballylate
isocitrate
Counts (%) vs. Acquisition Time (min)


Fe2+
70 °C, 16 h
+ α-ketoglutarate adducts
(glutaconate)


α-ketoglutarate + Fe2+ (70 °C, 16 h)
α-ketoglutarate
levulinate
malonate
succinate
glutaconate
α-ketoglutarate adducts
oxalate
malate
Counts (%) vs. Acquisition Time (min)

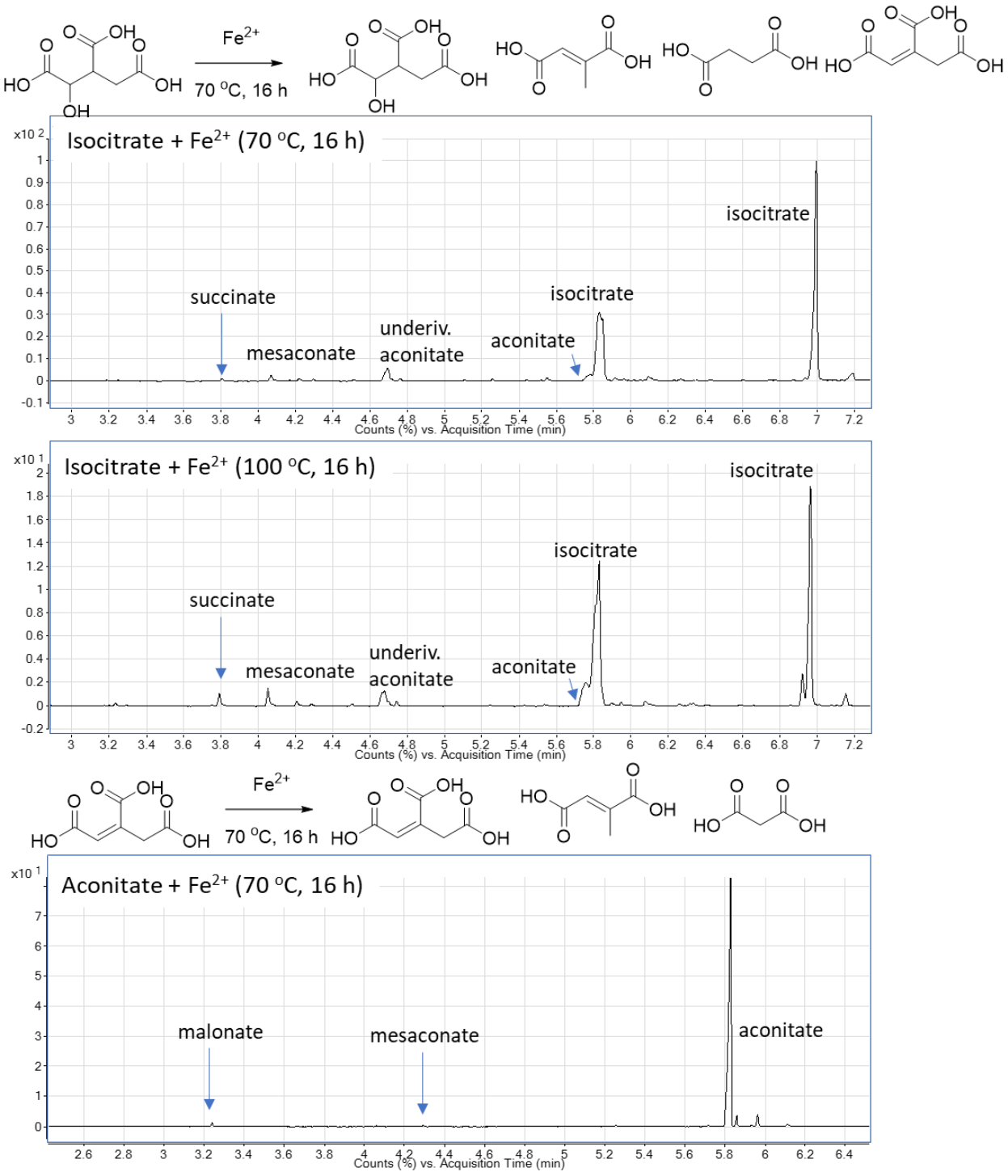
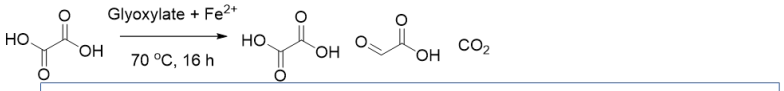
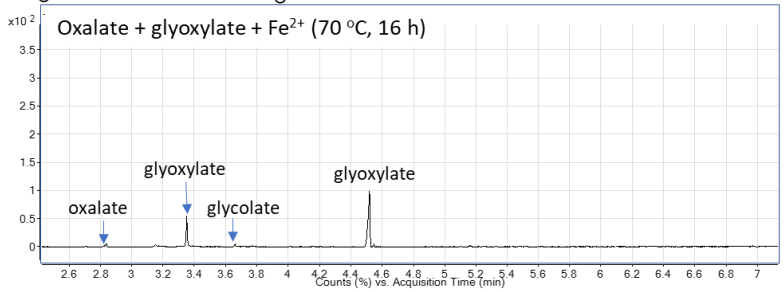
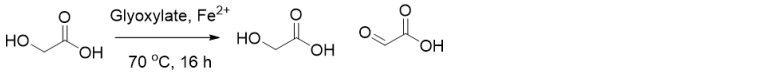
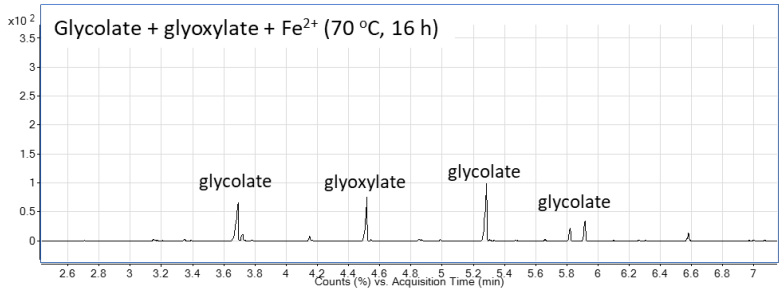


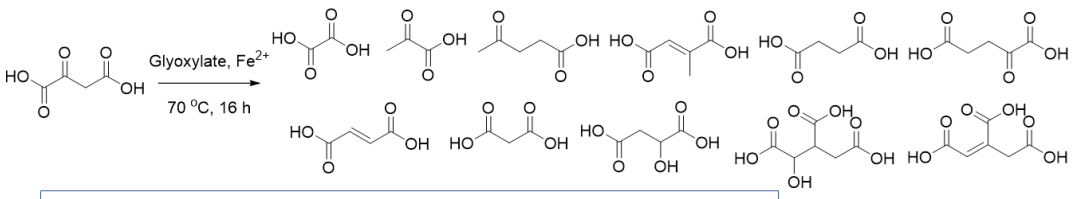
Figure S13.
Reaction scheme and GC chromatograms of control reactions (intermediate + Fe^{2+}), after derivatization of the reaction mixture with ECF/EtOH.

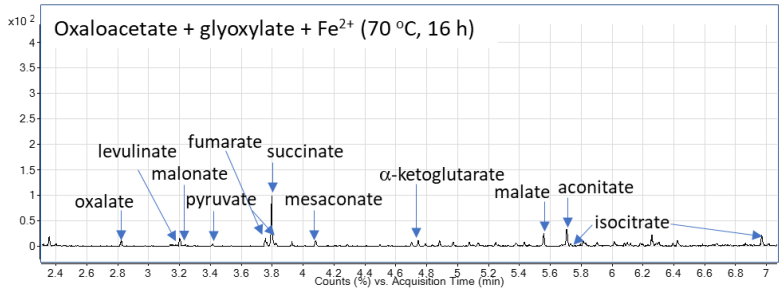

Glyoxylate + Fe2+
70 °C, 16 h
CO2

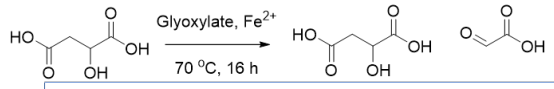

Oxalate + glyoxylate + Fe2+ (70 °C, 16 h)
oxalate
glyoxylate
glycolate
glyoxylate
Counts (%) vs. Acquisition Time (min)

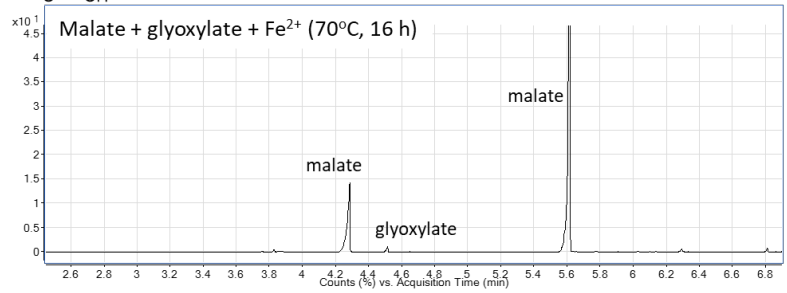

Glyoxylate, Fe2+
70 °C, 16 h

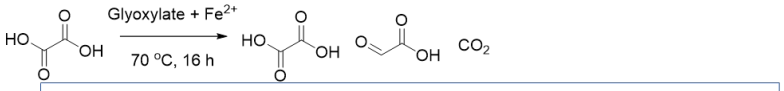

Glycolate + glyoxylate + Fe2+ (70 °C, 16 h)
glycolate
glyoxylate
glycolate
glycolate
Counts (%) vs. Acquisition Time (min)

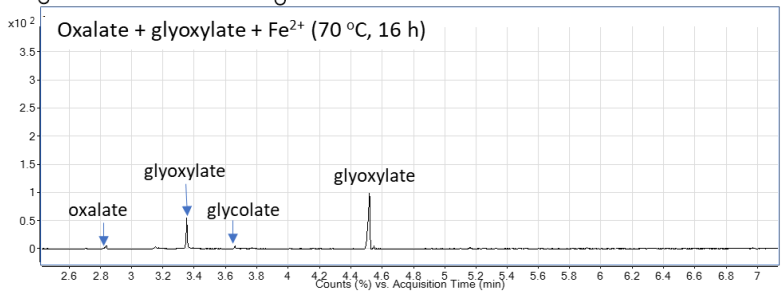

Glyoxylate, Fe2+
70 °C, 16 h

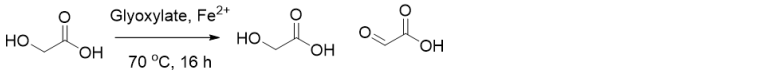

Oxaloacetate + glyoxylate + Fe2+ (70 °C, 16 h)
levulinate
fumarate
succinate
malonate
α-ketoglutarate
oxalate
pyruvate
mesaconate
malate
aconitate
isocitrate
Counts (%) vs. Acquisition Time (min)

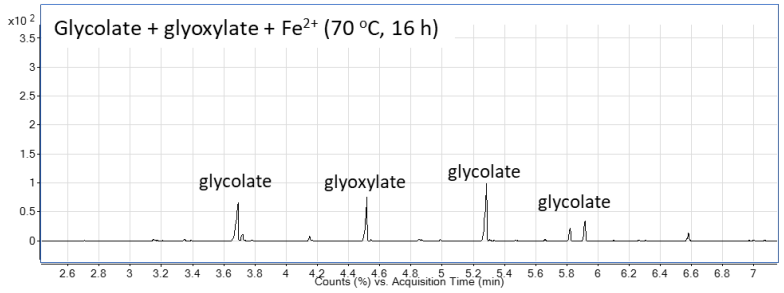

Glyoxylate, Fe2+
70 °C, 16 h

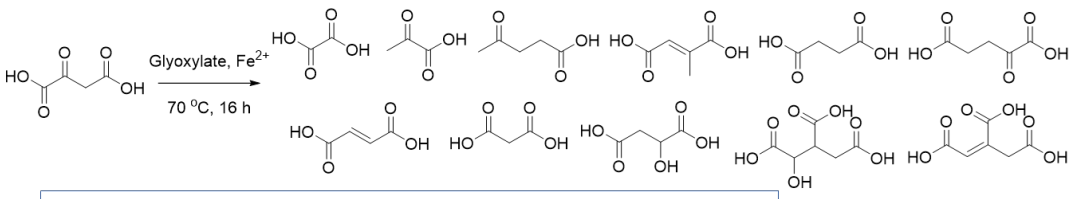

Malate + glyoxylate + Fe2+ (70°C, 16 h)
malate
malate
glyoxylate
Counts (%) vs. Acquisition Time (min)

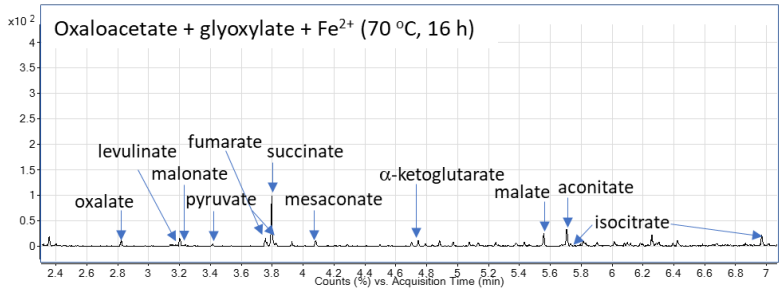

Glyoxylate + Fe2+
70 °C, 16 h
CO2

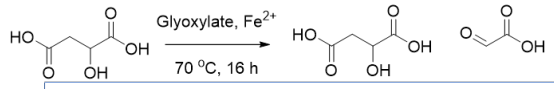

Oxalate + glyoxylate + Fe2+ (70 °C, 16 h)
oxalate
glyoxylate
glycolate
glyoxylate
Counts (%) vs. Acquisition Time (min)

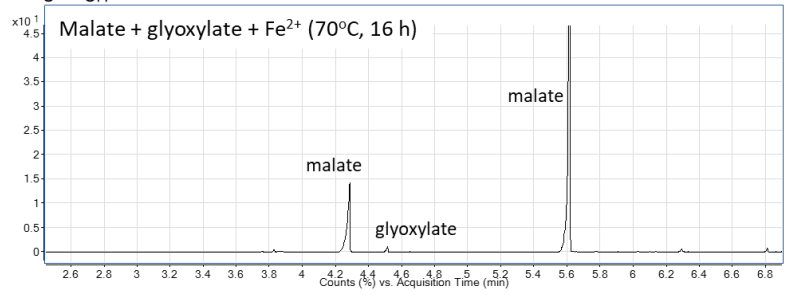

Glyoxylate, Fe2+
70 °C, 16 h


Glycolate + glyoxylate + Fe2+ (70 °C, 16 h)
glycolate
glyoxylate
glycolate
glycolate
Counts (%) vs. Acquisition Time (min)


Glyoxylate, Fe2+
70 °C, 16 h


Oxaloacetate + glyoxylate + Fe2+ (70 °C, 16 h)
levulinate
fumarate
succinate
malonate
α-ketoglutarate
oxalate
pyruvate
mesaconate
malate
aconitate
isocitrate
Counts (%) vs. Acquisition Time (min)


Glyoxylate, Fe2+
70 °C, 16 h


Malate + glyoxylate + Fe2+ (70°C, 16 h)
malate
malate
glyoxylate
Counts (%) vs. Acquisition Time (min)

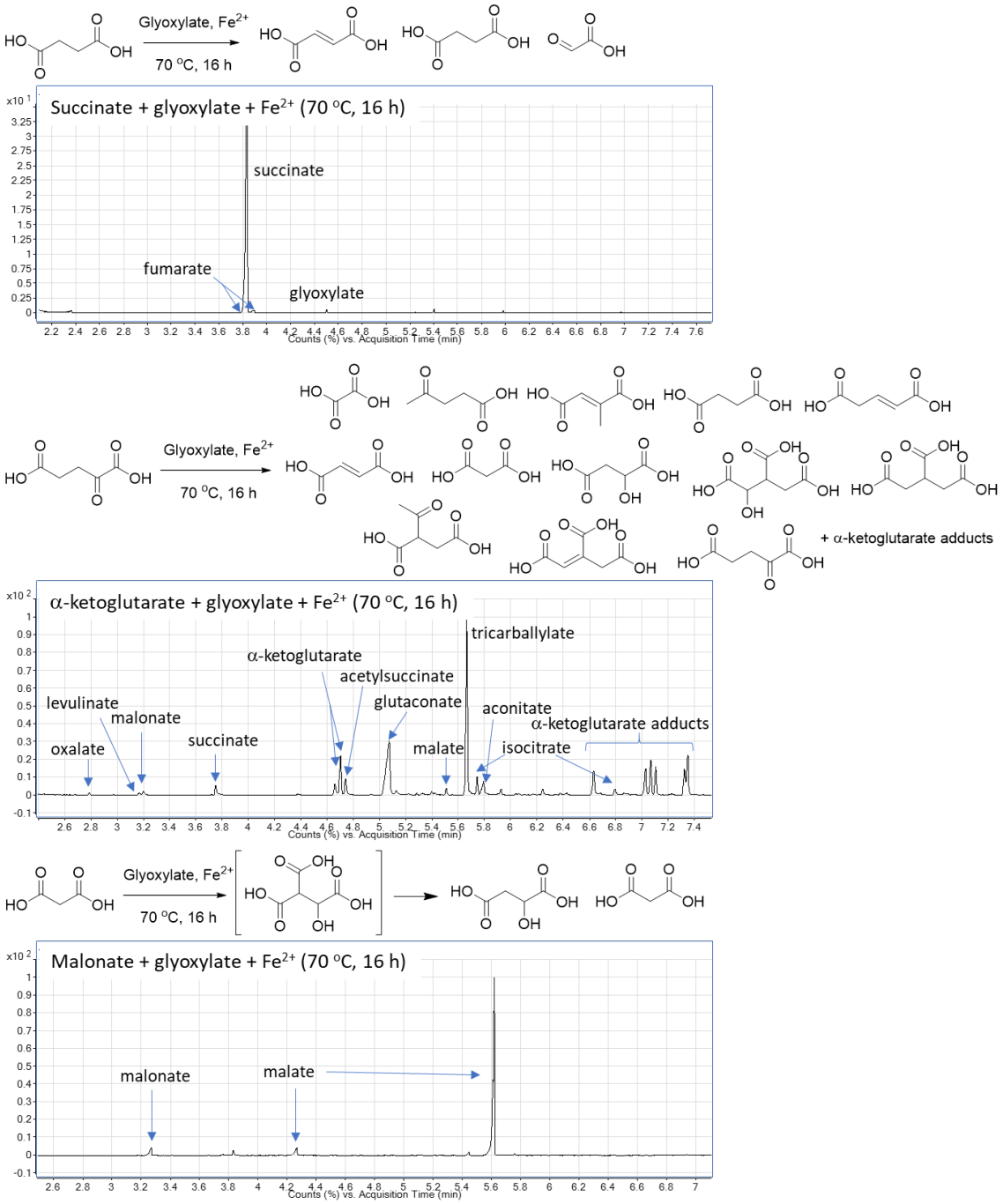


Figure S14.

Reaction scheme and GC chromatograms of control reactions (intermediate + 1 equiv glyoxylate + Fe^{2+}), after derivatization of the reaction mixture with ECF/EtOH.

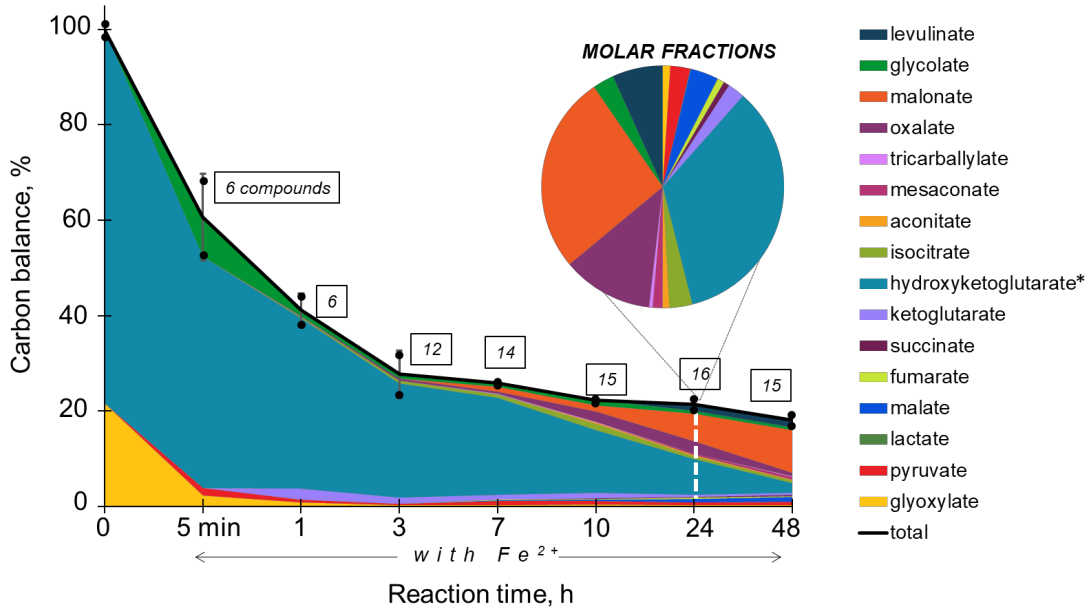
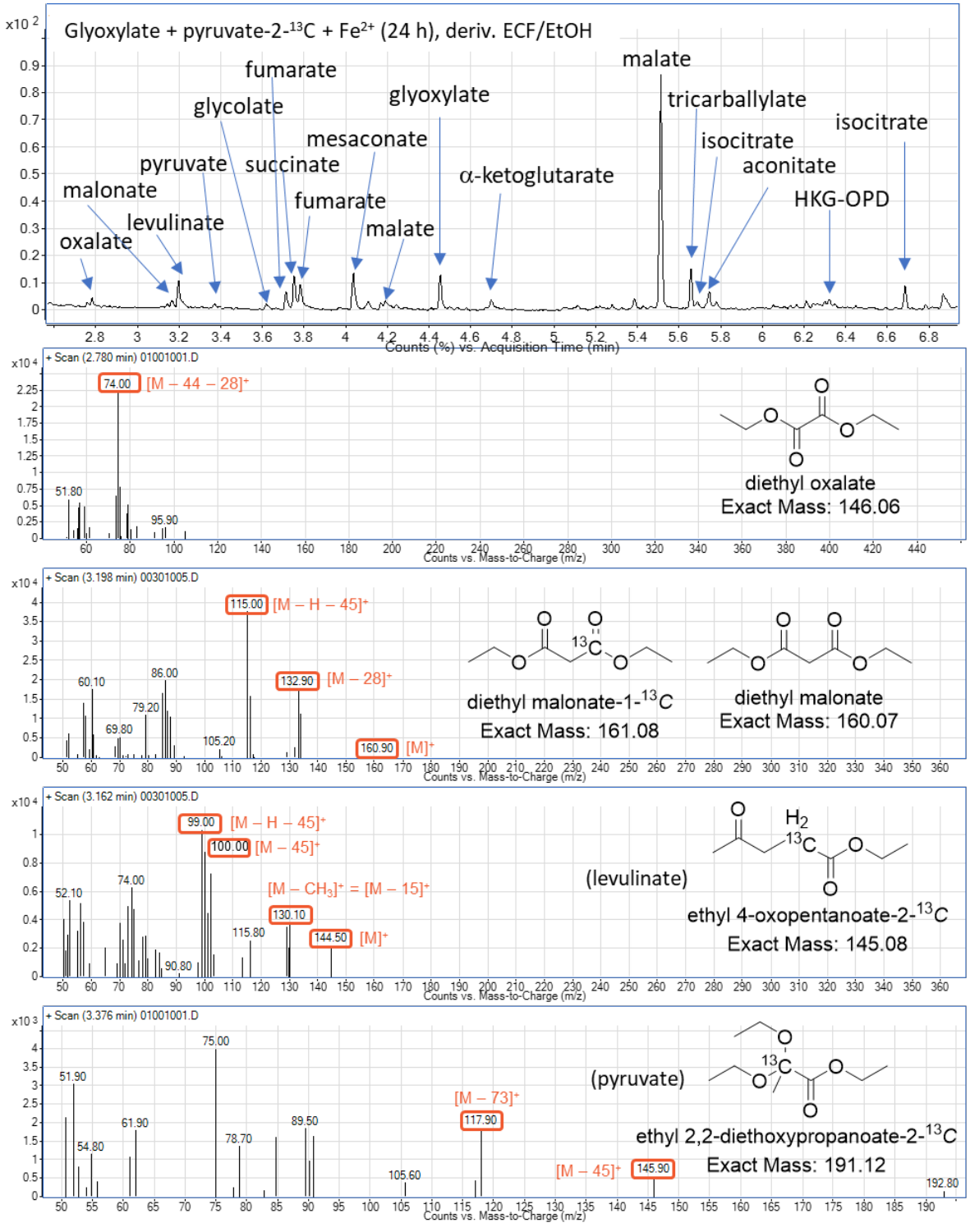
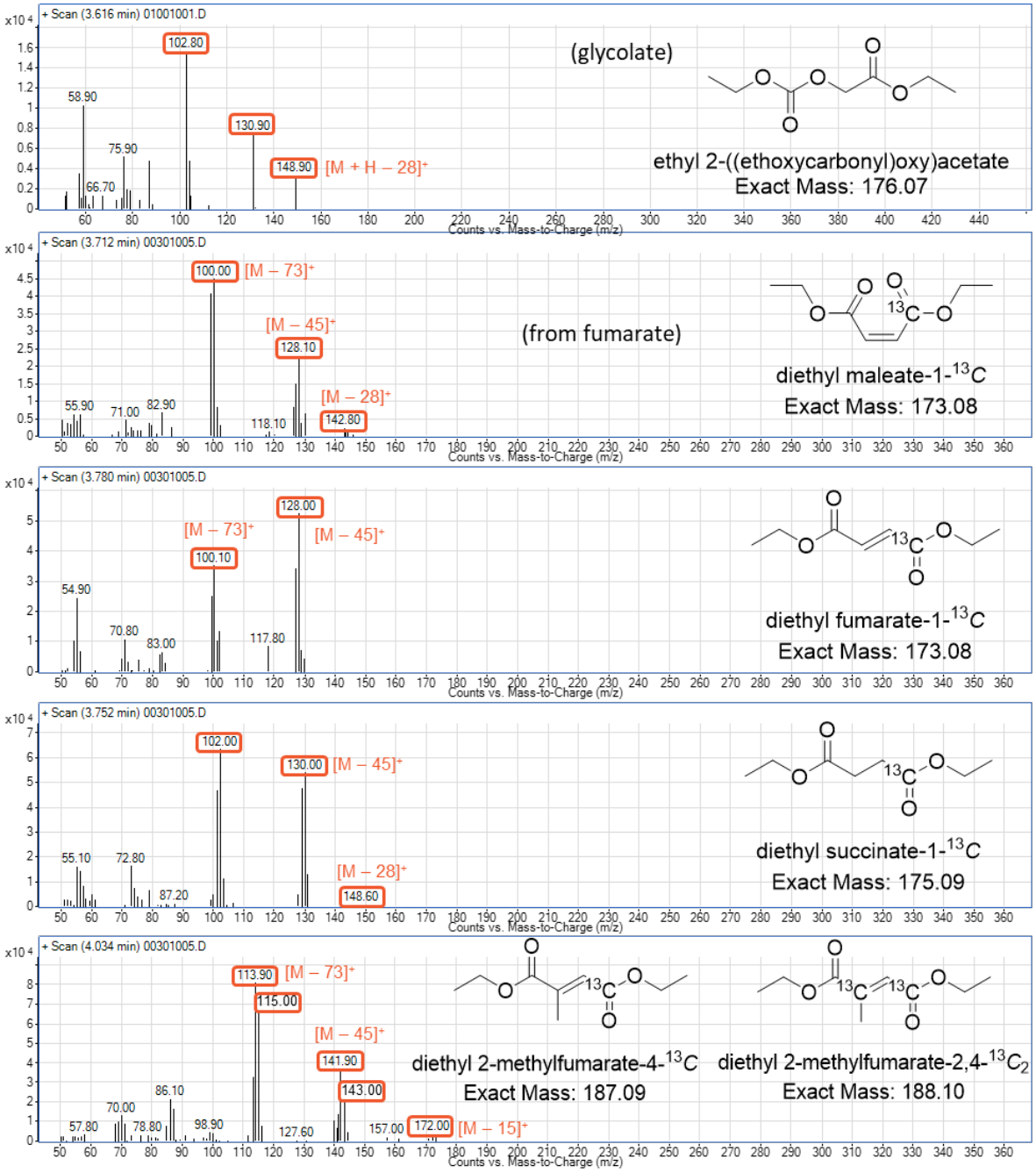
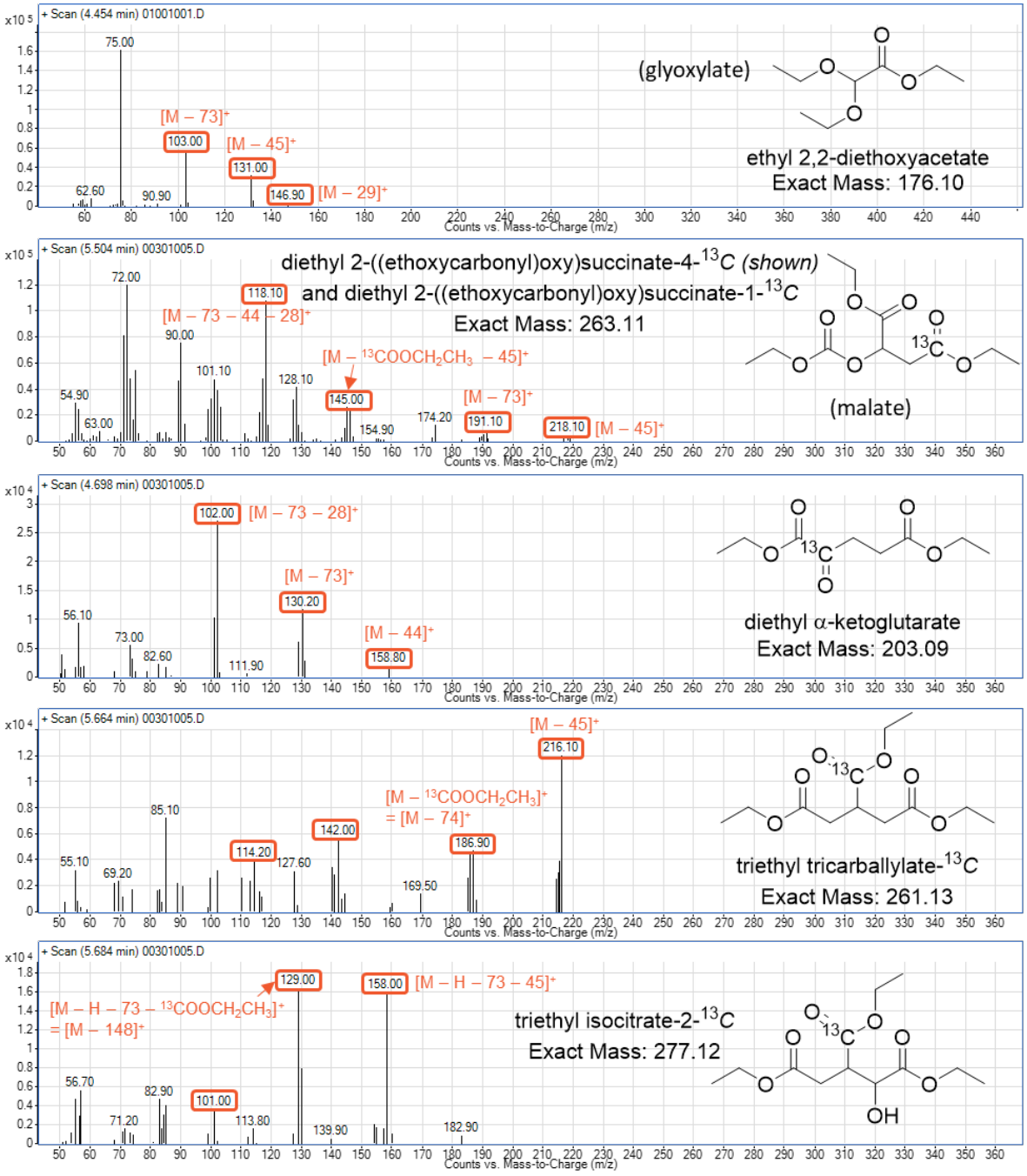


Figure S15.

Evolution over time of a reaction network arising from pyruvate-2-^{13}C and glyoxylate, promoted by Fe^{2+} at 70 ºC. Carbon balance refers to the % of carbon atoms observed in solution relative to 0 h and is reported as the average of two independent runs. Product distribution at 24 h is shown in a pie chart. Error bars correspond to the standard deviation. *Values for hydroxyketoglutarate also include oxopentanedioate.


Glyoxylate + pyruvate-2-13C + Fe2+ (24 h), deriv. ECF/EtOH
malonate
oxalate
levulinate
pyruvate
fumarate
glycolate
succinate
mesaconate
fumarate
malate
glyoxylate
α-ketoglutarate
malate
tricarballylate
isocitrate
aconitate
isocitrate
HKG-OPD
Counts (%) vs. Acquisition Time (min)
+ Scan (2.780 min) 01001001.D
74.00 [M – 44 – 28]+
51.80
95.90
diethyl oxalate
Exact Mass: 146.06
Counts vs. Mass-to-Charge (m/z)
+ Scan (3.198 min) 00301005.D
115.00 [M – H – 45]+
132.90 [M – 28]+
160.90 [M]+
60.10
86.00
79.20
69.80
105.20
diethyl malonate-1-13C
Exact Mass: 161.08
diethyl malonate
Exact Mass: 160.07
Counts vs. Mass-to-Charge (m/z)
+ Scan (3.162 min) 00301005.D
99.00 [M – H – 45]+
100.00 [M – 45]+
[M – CH3]+ = [M – 15]+
130.10
144.50 [M]+
52.10
74.00
115.80
90.80
(levulinate)
ethyl 4-oxopentanoate-2-13C
Exact Mass: 145.08
Counts vs. Mass-to-Charge (m/z)
+ Scan (3.376 min) 01001001.D
75.00
51.90
54.80
61.90
78.70
89.50
105.60
[M – 73]+
117.90
[M – 45]+
145.90
192.80
(pyruvate)
ethyl 2,2-diethoxypropanoate-2-13C
Exact Mass: 191.12
Counts vs. Mass-to-Charge (m/z)


+ Scan (3.616 min) 01001001.D
102.80
58.90
130.90
75.90
148.90
[M + H – 28]+
66.70
Counts vs. Mass-to-Charge (m/z)
(glycolate)
ethyl 2-((ethoxycarbonyl)oxy)acetate
Exact Mass: 176.07
+ Scan (3.712 min) 00301005.D
100.00
[M – 73]+
[M – 45]+
128.10
[M – 28]+
142.80
55.90
71.00
82.90
118.10
Counts vs. Mass-to-Charge (m/z)
(from fumarate)
diethyl maleate-1-13C
Exact Mass: 173.08
+ Scan (3.780 min) 00301005.D
128.00
[M – 73]+
[M – 45]+
100.10
54.90
70.80
83.00
117.80
Counts vs. Mass-to-Charge (m/z)
diethyl fumarate-1-13C
Exact Mass: 173.08
+ Scan (3.752 min) 00301005.D
102.00
130.00
[M – 45]+
[M – 28]+
148.60
55.10
72.80
87.20
Counts vs. Mass-to-Charge (m/z)
diethyl succinate-1-13C
Exact Mass: 175.09
+ Scan (4.034 min) 00301005.D
113.90
[M – 73]+
115.00
[M – 45]+
141.90
143.00
86.10
70.00
57.80
78.80
98.90
127.60
157.00
172.00
[M – 15]+
Counts vs. Mass-to-Charge (m/z)
diethyl 2-methylfumarate-4-13C
Exact Mass: 187.09
diethyl 2-methylfumarate-2,4-13C2
Exact Mass: 188.10


+ Scan (4.454 min) 01001001.D
75.00
[M – 73]+
103.00
[M – 45]+
131.00
62.60
90.90
146.90
[M – 29]+
(glyoxylate)
ethyl 2,2-diethoxyacetate
Exact Mass: 176.10
Counts vs. Mass-to-Charge (m/z)
+ Scan (5.504 min) 00301005.D
diethyl 2-((ethoxycarbonyl)oxy)succinate-4-13C (shown)
and diethyl 2-((ethoxycarbonyl)oxy)succinate-1-13C
Exact Mass: 263.11
72.00
118.10
[M – 73 – 44 – 28]+
90.00
[M – 13COOCH2CH3 – 45]+
101.10
128.10
54.90
145.00
[M – 73]+
63.00
154.90
174.20
191.10
218.10
[M – 45]+
(malate)
Counts vs. Mass-to-Charge (m/z)
+ Scan (4.698 min) 00301005.D
102.00
[M – 73 – 28]+
[M – 73]+
130.20
56.10
73.00
82.60
[M – 44]+
111.90
158.80
diethyl α-ketoglutarate
Exact Mass: 203.09
Counts vs. Mass-to-Charge (m/z)
+ Scan (5.664 min) 00301005.D
[M – 45]+
216.10
[M – 13COOCH2CH3]+
= [M – 74]+
85.10
142.00
114.20
186.90
55.10
69.20
127.60
169.50
triethyl tricarballylate-13C
Exact Mass: 261.13
Counts vs. Mass-to-Charge (m/z)
+ Scan (5.684 min) 00301005.D
129.00
158.00
[M – H – 73 – 45]+
[M – H – 73 – 13COOCH2CH3]+
= [M – 148]+
triethyl isocitrate-2-13C
Exact Mass: 277.12
56.70
82.90
101.00
71.20
113.80
139.90
182.90
OH
Counts vs. Mass-to-Charge (m/z)

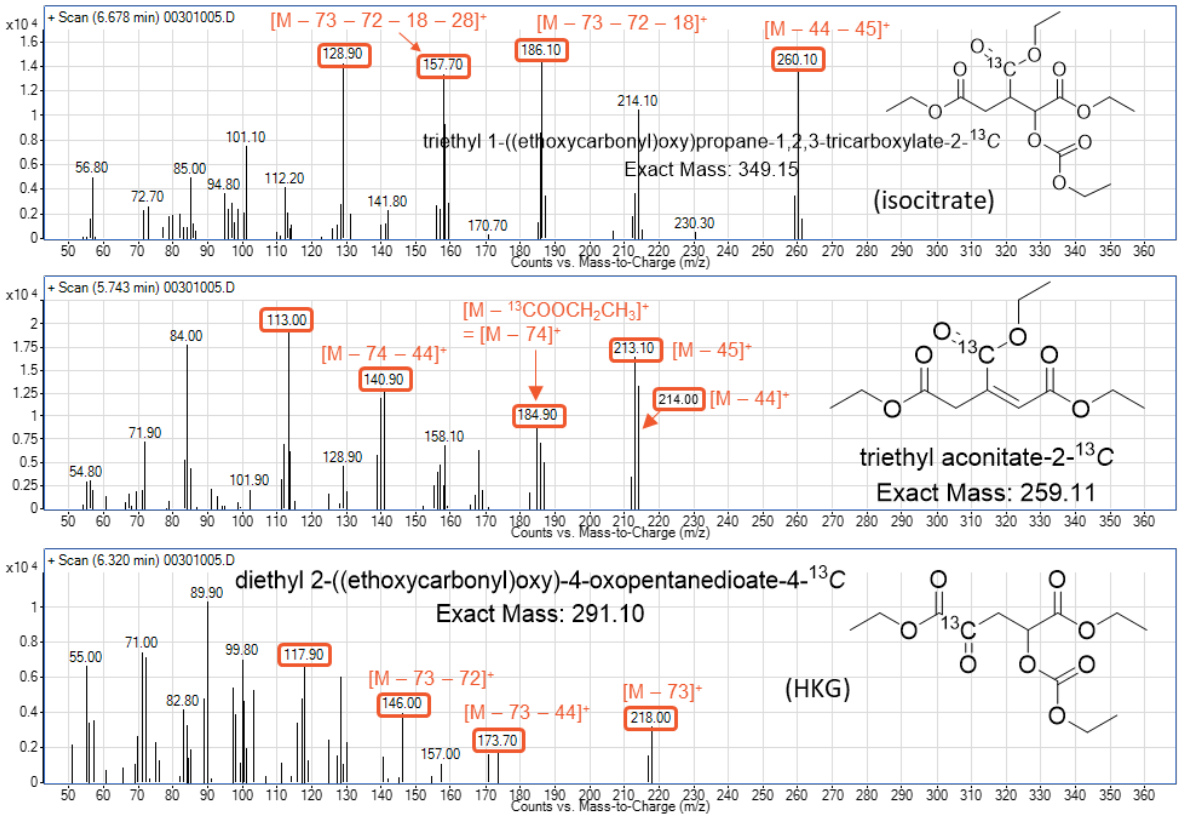


Figure S16.

GC chromatogram and mass spectra of ECF/EtOH-derivatized compounds detected at t = 24 h in the reaction network arising from pyruvate-2-^{13}C and glyoxylate, promoted by Fe^{2+} at 70 ºC. Characteristic ("fingerprint") peaks are highlighted in red.

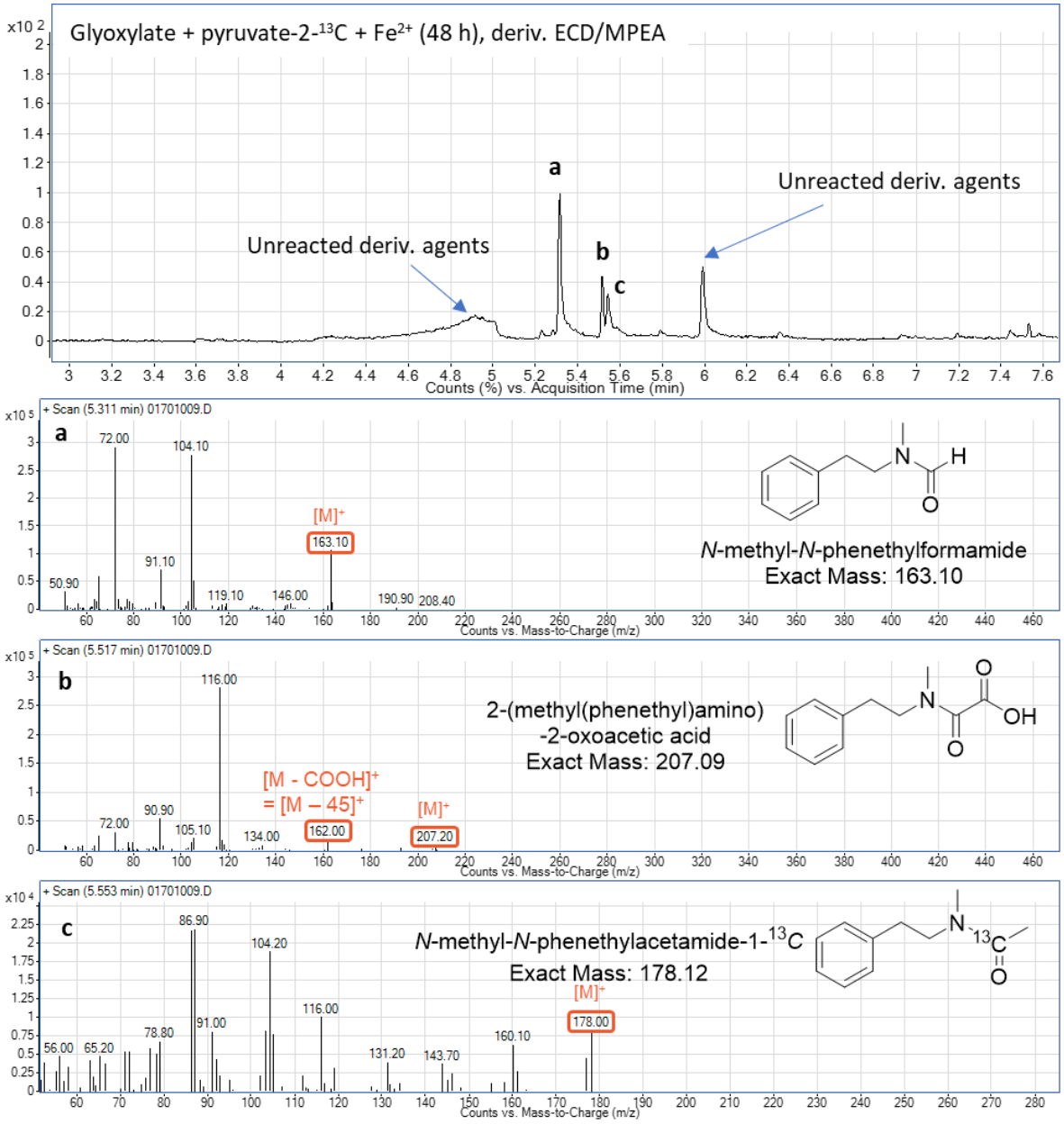


Figure S17.

GC chromatogram and mass spectra confirming the presence of formate, oxalate and acetate, detected as their *N*-methyl-*N*-phenethylamides, after derivatization of a typical ^{13}C-labeled reaction mixture (pyruvate-1-^{13}C + glyoxylate) with ECD/MPEA after 48 h reaction time.

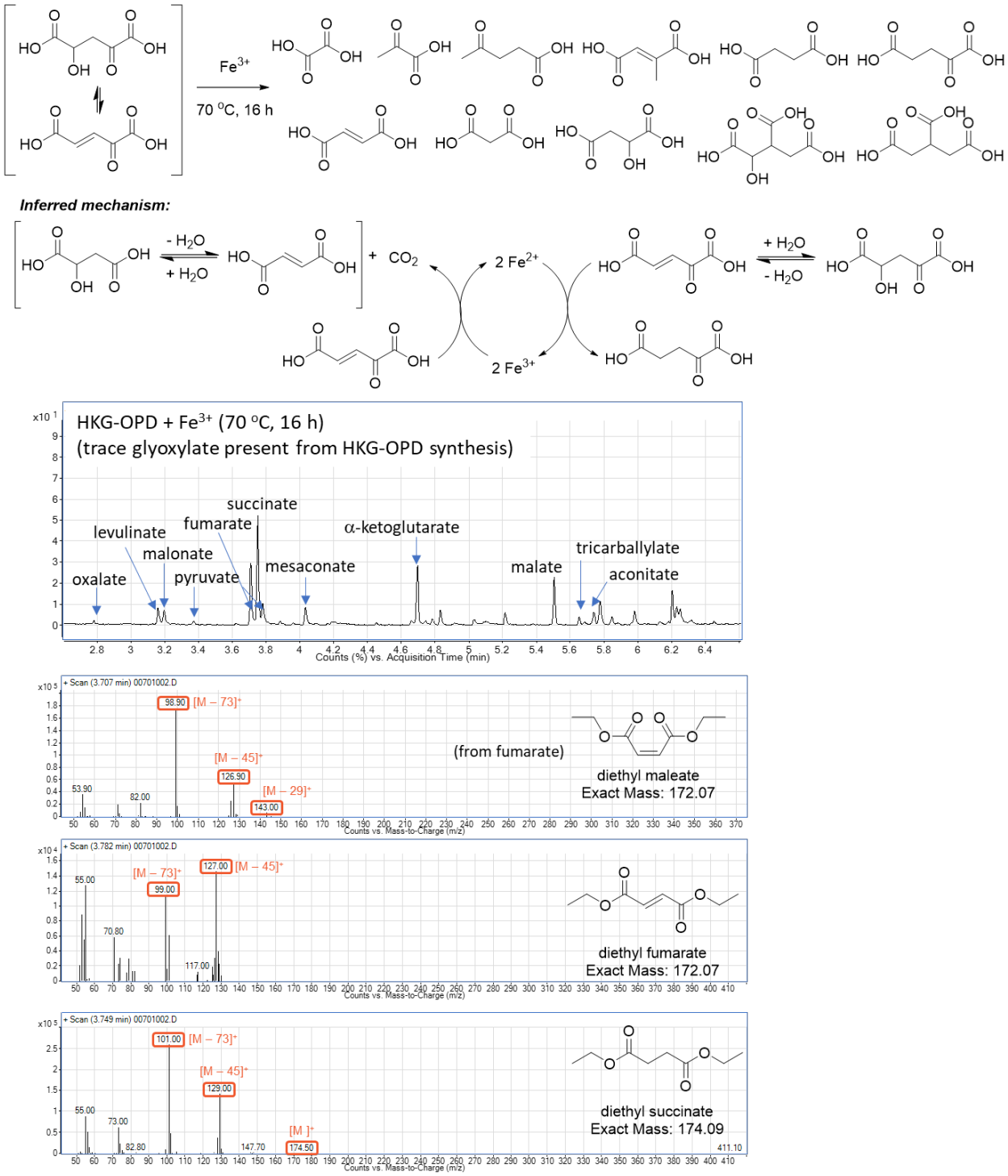


Figure S18.

Reaction scheme and GC chromatograms of a control reaction (HKG/OPD + Fe^{3+}), after derivatization of the reaction mixture with ECF/EtOH.

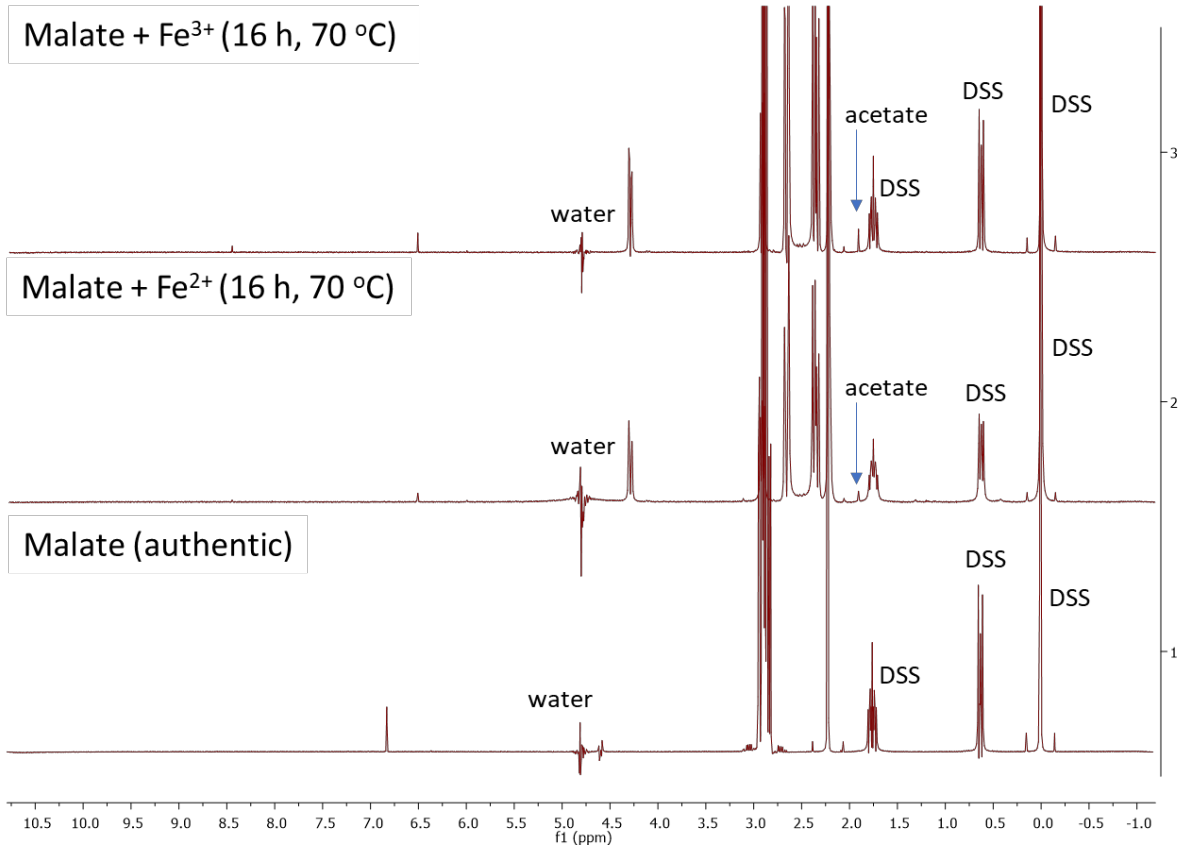


Figure S19

^{1}H NMR of control reactions (malate + Fe^{2+} or Fe^{3+}, 70 °C for 16 h), highlighting the presence of acetate (oxidation of malate to oxaloacetate, then Fe^{2+}(or Fe^{3+})-promoted decarboxylation to pyruvate and subsequently to acetate). DSS was used as standard.

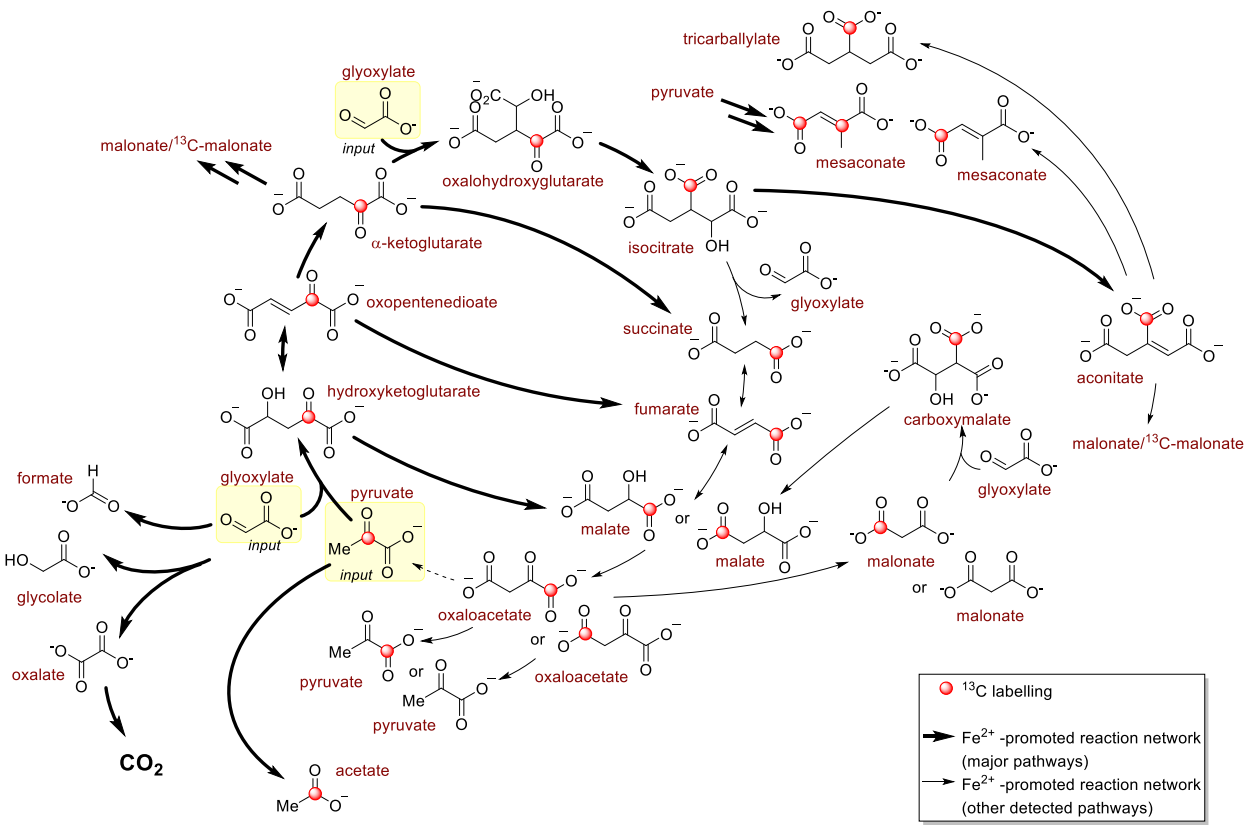


Figure S20

Propagation of the ^{13}C label in the Fe^{2+}-promoted reaction network arising from glyoxylate and pyruvate-2-^{13}C at 70 ºC.

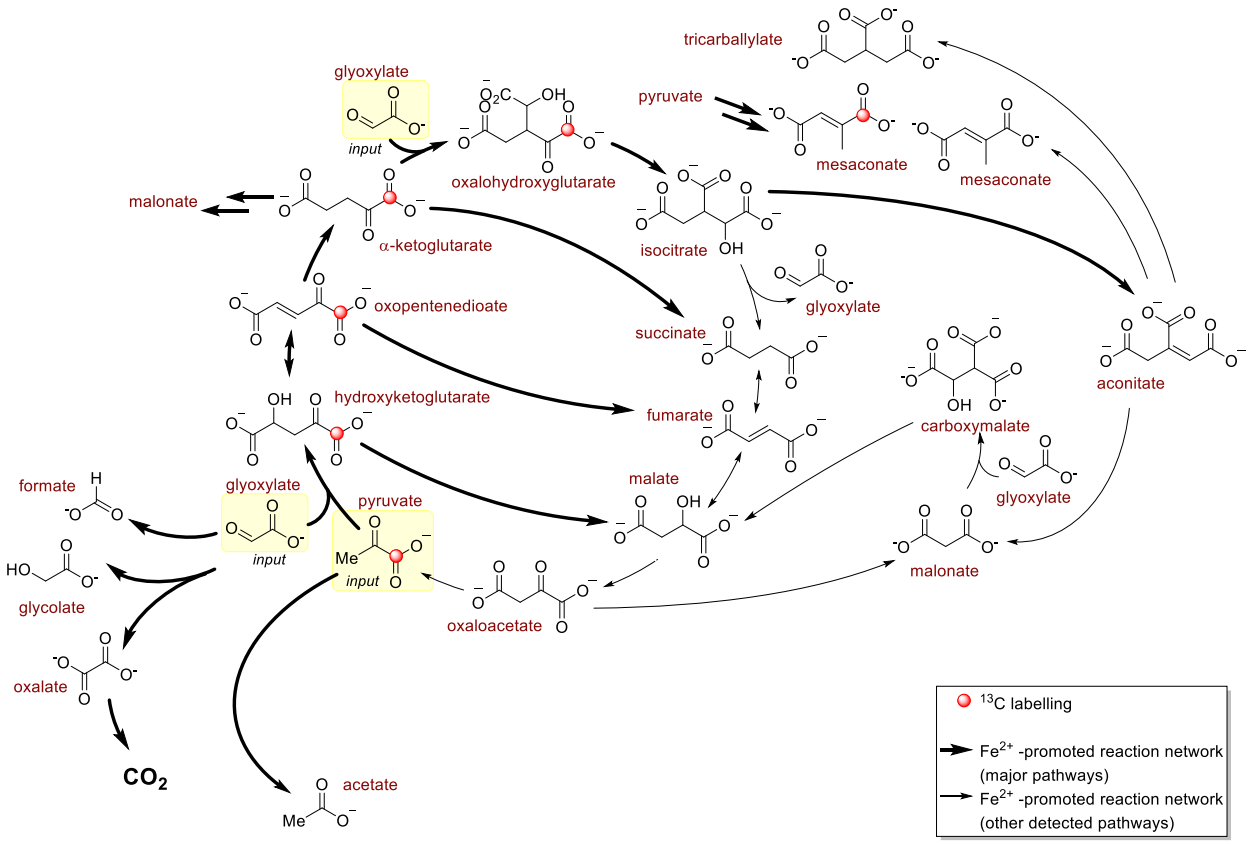


Figure S21

Propagation of the ^{13}C label in the Fe^{2+}-promoted reaction network arising from glyoxylate and pyruvate-1-^{13}C at 70 °C.

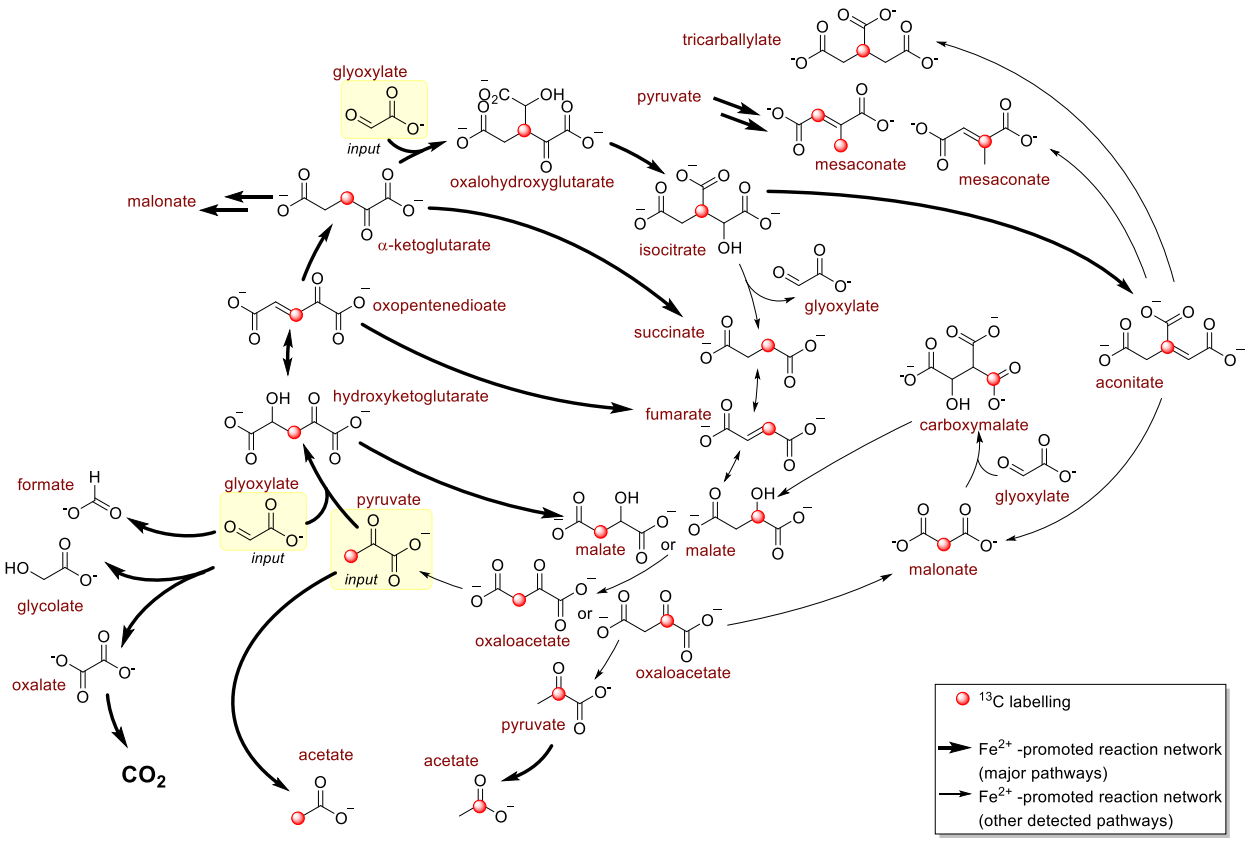


Figure S22

Propagation of the ^{13}C label in the Fe^{2+}-promoted reaction network arising from glyoxylate and pyruvate-3-^{13}C at 70 ºC.

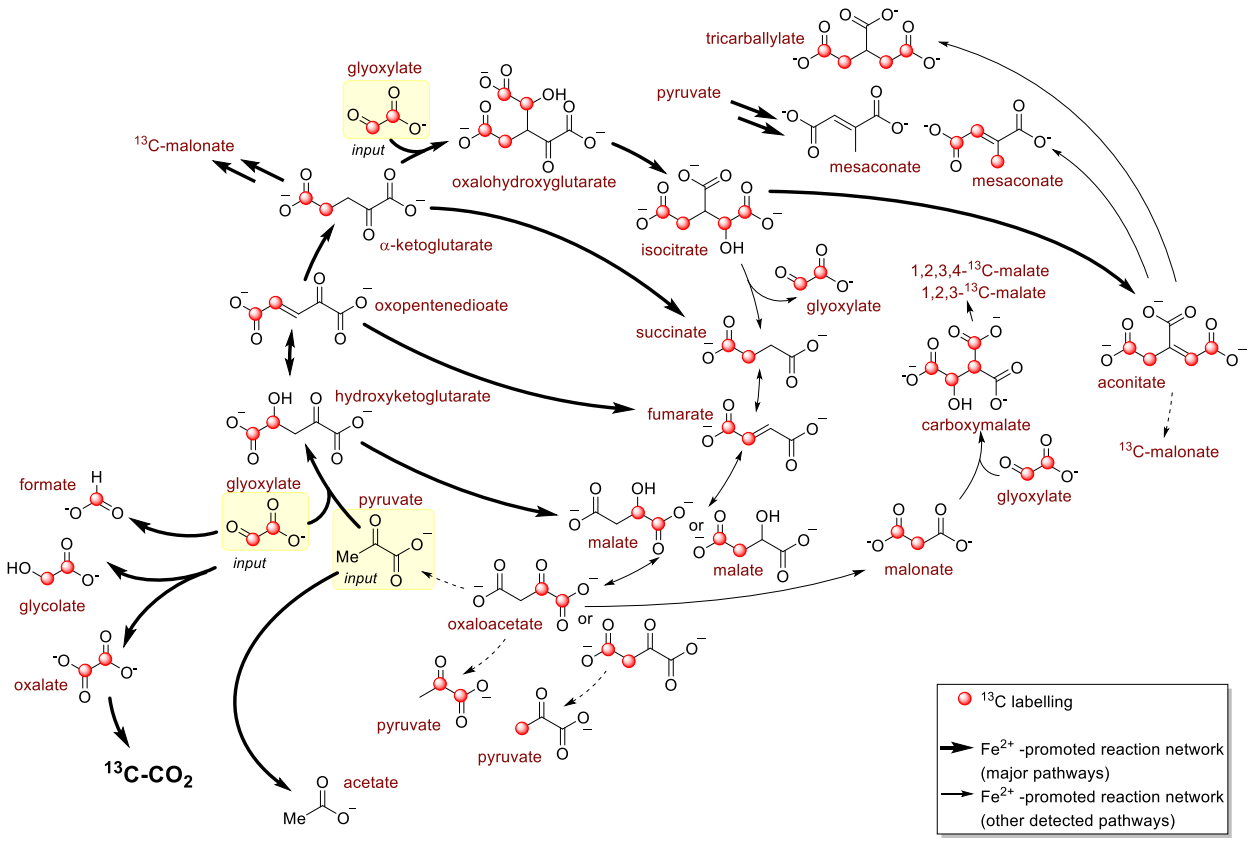
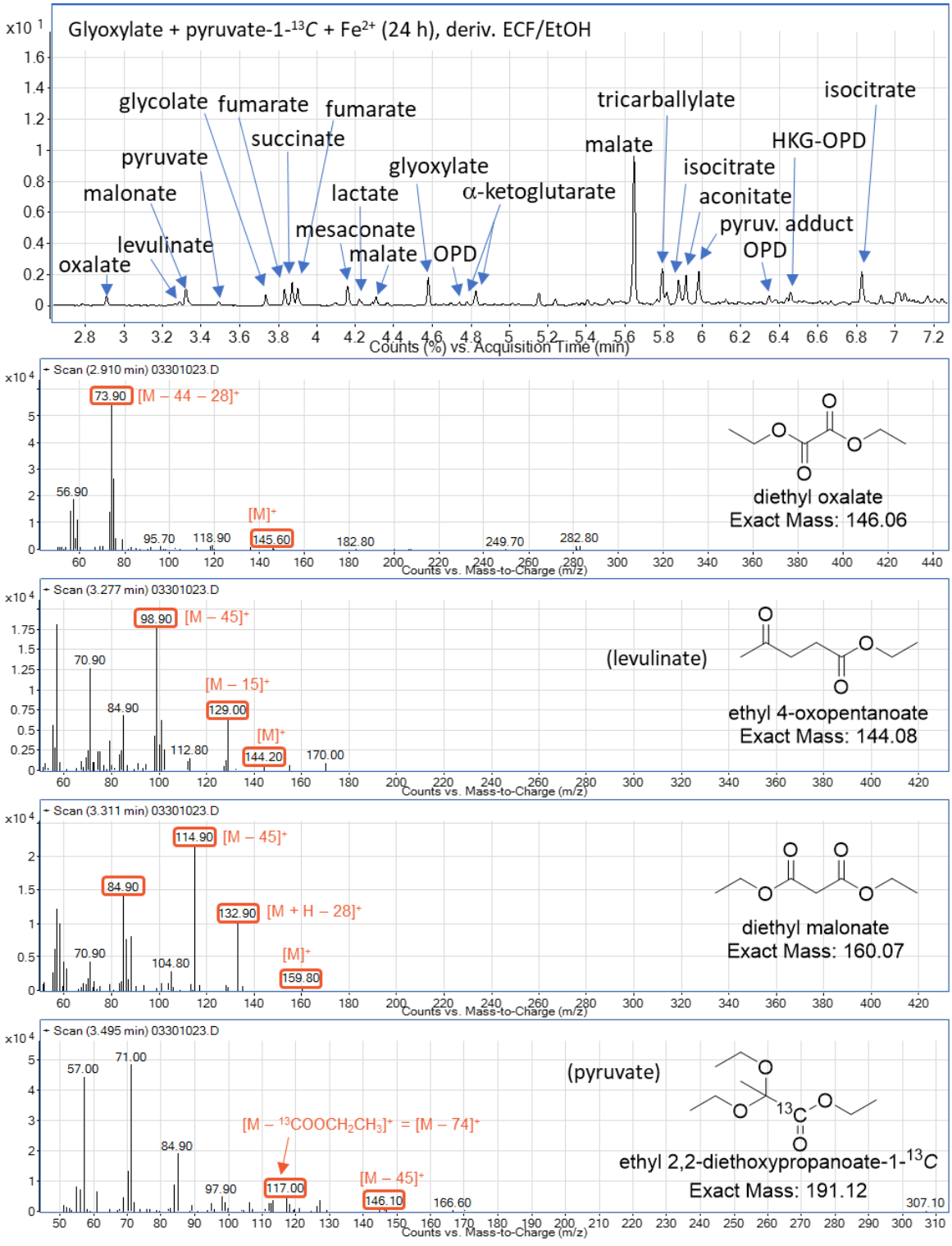
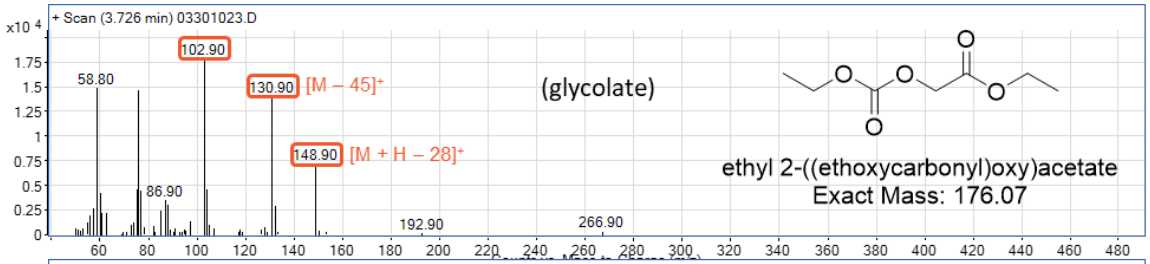
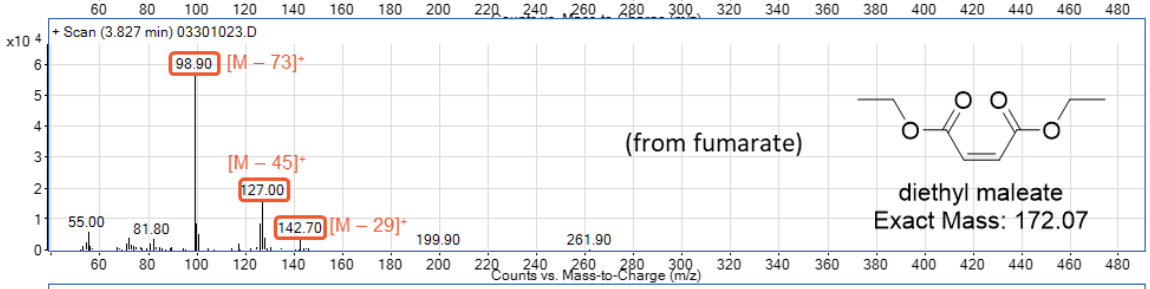


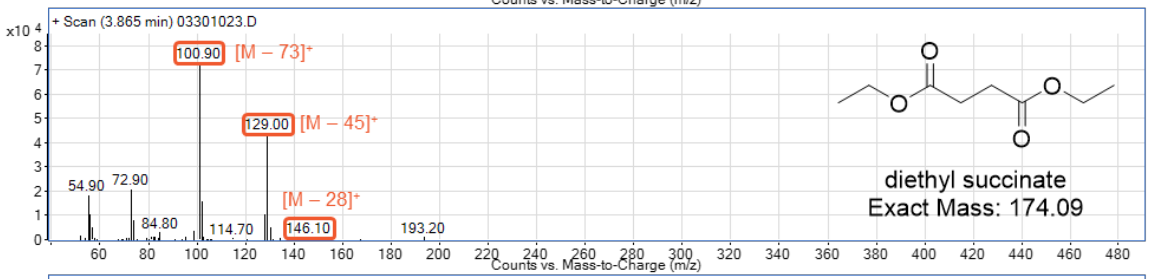
Figure S23

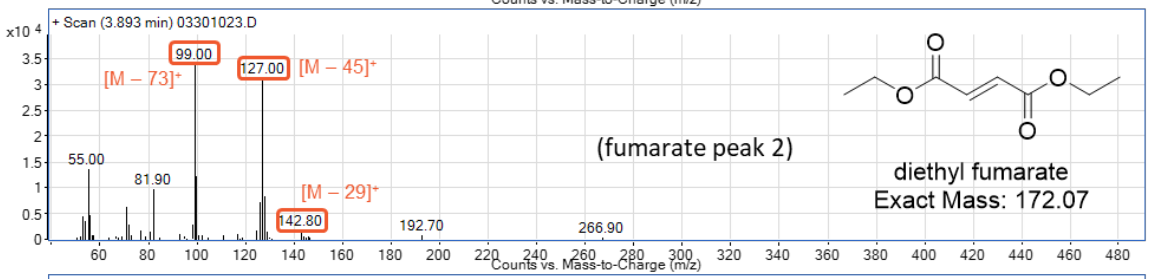
Propagation of the ^{13}C label in the Fe^{2+}-promoted reaction network arising from glyoxylate and glyoxylate-1,2-$^{13}C_2$ at 70 ºC.

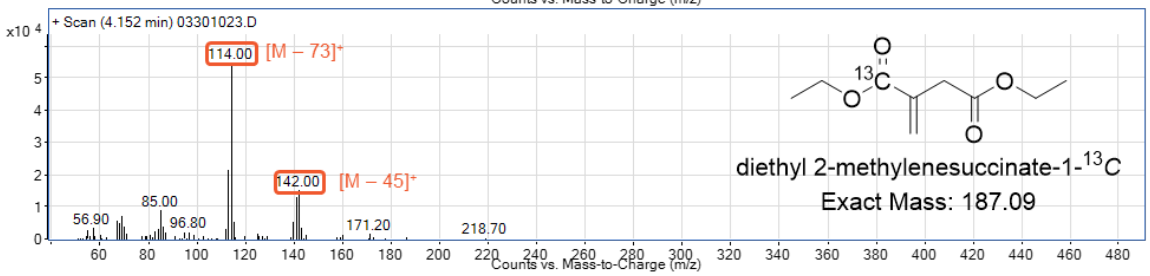

Glyoxylate + pyruvate-1-13C + Fe2+ (24 h), deriv. ECF/EtOH
glycolate
fumarate
fumarate
succinate
pyruvate
malonate
levulinate
oxalate
lactate
mesaconate
malate
glyoxylate
OPD
α-ketoglutarate
tricarballylate
malate
isocitrate
aconitate
pyruv. adduct
OPD
HKG-OPD
isocitrate
Counts (%) vs. Acquisition Time (min)
Scan (2.910 min) 03301023.D
73.90 [M – 44 – 28]+
56.90
[M]+
145.60
95.70
118.90
182.80
249.70
282.80
diethyl oxalate
Exact Mass: 146.06
Counts vs. Mass-to-Charge (m/z)
Scan (3.277 min) 03301023.D
98.90 [M – 45]+
70.90
[M – 15]+
129.00
84.90
[M]+
144.20
112.80
170.00
(levulinate)
ethyl 4-oxopentanoate
Exact Mass: 144.08
Counts vs. Mass-to-Charge (m/z)
Scan (3.311 min) 03301023.D
114.90 [M – 45]+
84.90
132.90 [M + H – 28]+
70.90
104.80
[M]+
159.80
diethyl malonate
Exact Mass: 160.07
Counts vs. Mass-to-Charge (m/z)
Scan (3.495 min) 03301023.D
57.00
71.00
[M – 13COOCH2CH3]+ = [M – 74]+
84.90
97.90
117.00
[M – 45]+
146.10
166.60
307.10
(pyruvate)
ethyl 2,2-diethoxypropanoate-1-13C
Exact Mass: 191.12
Counts vs. Mass-to-Charge (m/z)

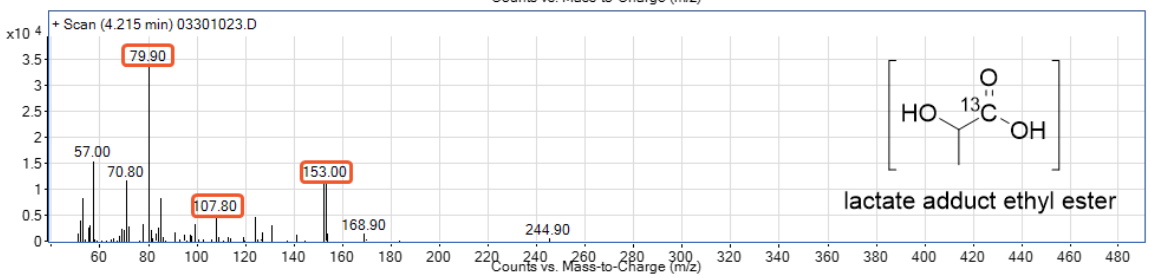

+ Scan (3.726 min) 03301023.D
102.90
58.80
130.90 [M – 45]+
148.90 [M + H – 28]+
86.90
192.90
266.90
(glycolate)
ethyl 2-((ethoxycarbonyl)oxy)acetate
Exact Mass: 176.07

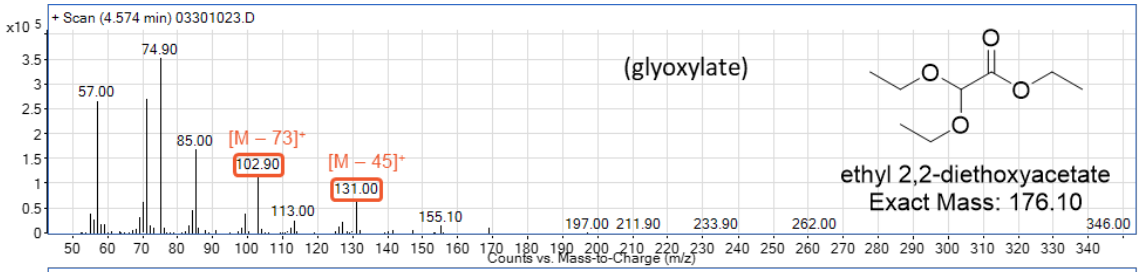

+ Scan (3.827 min) 03301023.D
98.90 [M – 73]+
[M – 45]+
127.00
142.70 [M – 29]+
55.00
81.80
199.90
261.90
(from fumarate)
diethyl maleate
Exact Mass: 172.07
Counts vs. Mass-to-Charge (m/z)

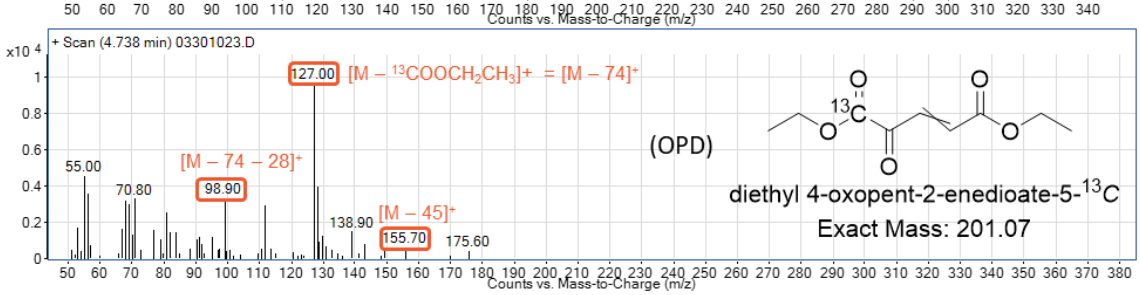

+ Scan (3.865 min) 03301023.D
100.90 [M – 73]+
129.00 [M – 45]+
[M – 28]+
54.90
72.90
84.80
114.70
146.10
193.20
diethyl succinate
Exact Mass: 174.09
Counts vs. Mass-to-Charge (m/z)

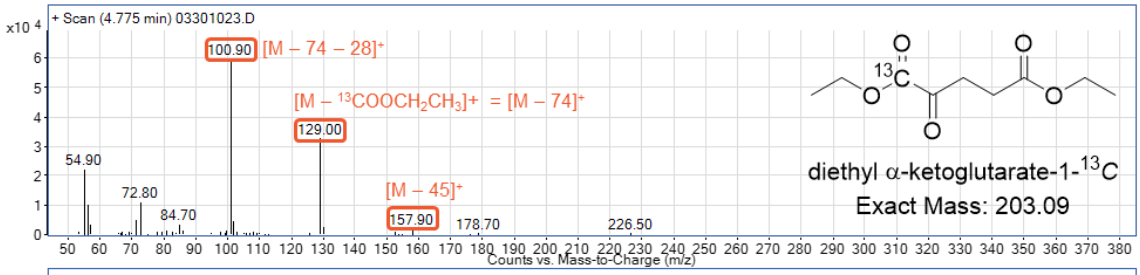

+ Scan (3.893 min) 03301023.D
99.00
[M – 73]+
127.00 [M – 45]+
[M – 29]+
142.80
55.00
81.90
192.70
266.90
(fumarate peak 2)
diethyl fumarate
Exact Mass: 172.07
Counts vs. Mass-to-Charge (m/z)

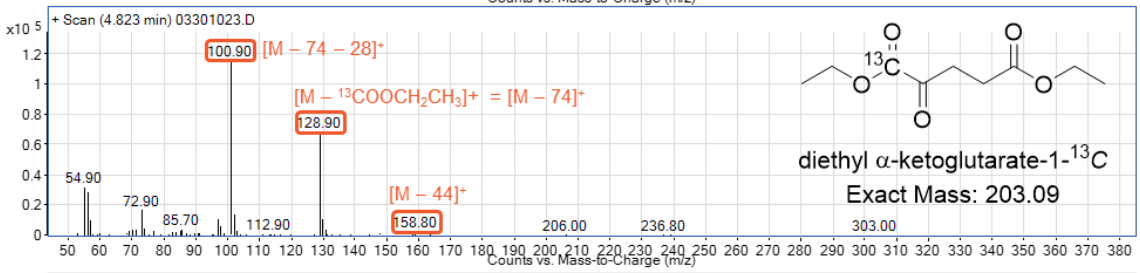

+ Scan (4.152 min) 03301023.D
114.00 [M – 73]+
142.00 [M – 45]+
85.00
56.90
96.80
171.20
218.70
diethyl 2-methylenesuccinate-1-13C
Exact Mass: 187.09
Counts vs. Mass-to-Charge (m/z)

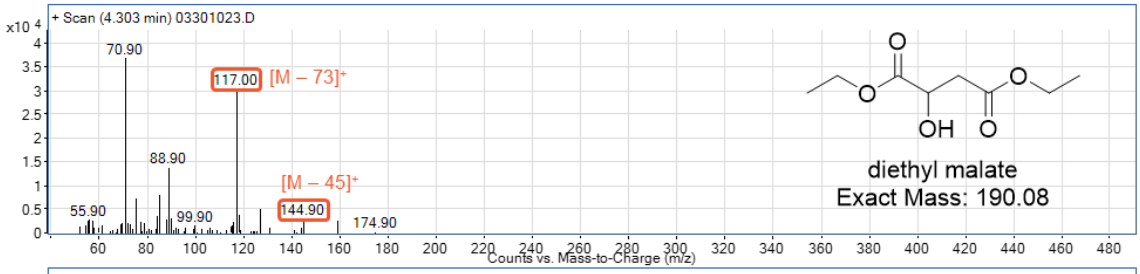

+ Scan (4.215 min) 03301023.D
79.90
57.00
70.80
153.00
107.80
168.90
244.90
lactate adduct ethyl ester
Counts vs. Mass-to-Charge (m/z)

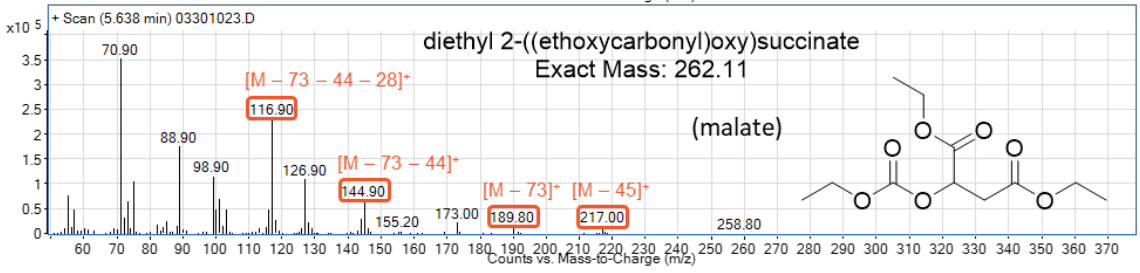

+ Scan (4.574 min) 03301023.D
74.90
57.00
85.00
[M – 73]+
102.90
[M – 45]+
131.00
113.00
155.10
197.00 211.90 233.90 262.00 346.00
Counts vs. Mass-to-Charge (m/z)
(glyoxylate)
ethyl 2,2-diethoxyacetate
Exact Mass: 176.10


+ Scan (4.738 min) 03301023.D
127.00 [M – 13COOCH2CH3]+ = [M – 74]+
55.00
[M – 74 – 28]+
98.90
70.80
138.90
[M – 45]+
155.70
175.60
Counts vs. Mass-to-Charge (m/z)
(OPD)
diethyl 4-oxopent-2-enedioate-5-13C
Exact Mass: 201.07


+ Scan (4.775 min) 03301023.D
100.90 [M – 74 – 28]+
[M – 13COOCH2CH3]+ = [M – 74]+
129.00
54.90
72.80
84.70
[M – 45]+
157.90
178.70
226.50
Counts vs. Mass-to-Charge (m/z)
diethyl α-ketoglutarate-1-13C
Exact Mass: 203.09


+ Scan (4.823 min) 03301023.D
100.90 [M – 74 – 28]+
[M – 13COOCH2CH3]+ = [M – 74]+
128.90
54.90
72.90
85.70
112.90
[M – 44]+
158.80
206.00
236.80
303.00
Counts vs. Mass-to-Charge (m/z)
diethyl α-ketoglutarate-1-13C
Exact Mass: 203.09


+ Scan (4.303 min) 03301023.D
70.90
117.00 [M – 73]+
88.90
55.90
99.90
[M – 45]+
144.90
174.90
Counts vs. Mass-to-Charge (m/z)
diethyl malate
Exact Mass: 190.08


+ Scan (5.638 min) 03301023.D
diethyl 2-((ethoxycarbonyl)oxy)succinate
Exact Mass: 262.11
70.90
[M – 73 – 44 – 28]+
116.90
88.90
98.90
126.90
[M – 73 – 44]+
144.90
155.20
173.00
[M – 73]+
189.80
[M – 45]+
217.00
258.80
Counts vs. Mass-to-Charge (m/z)
(malate)

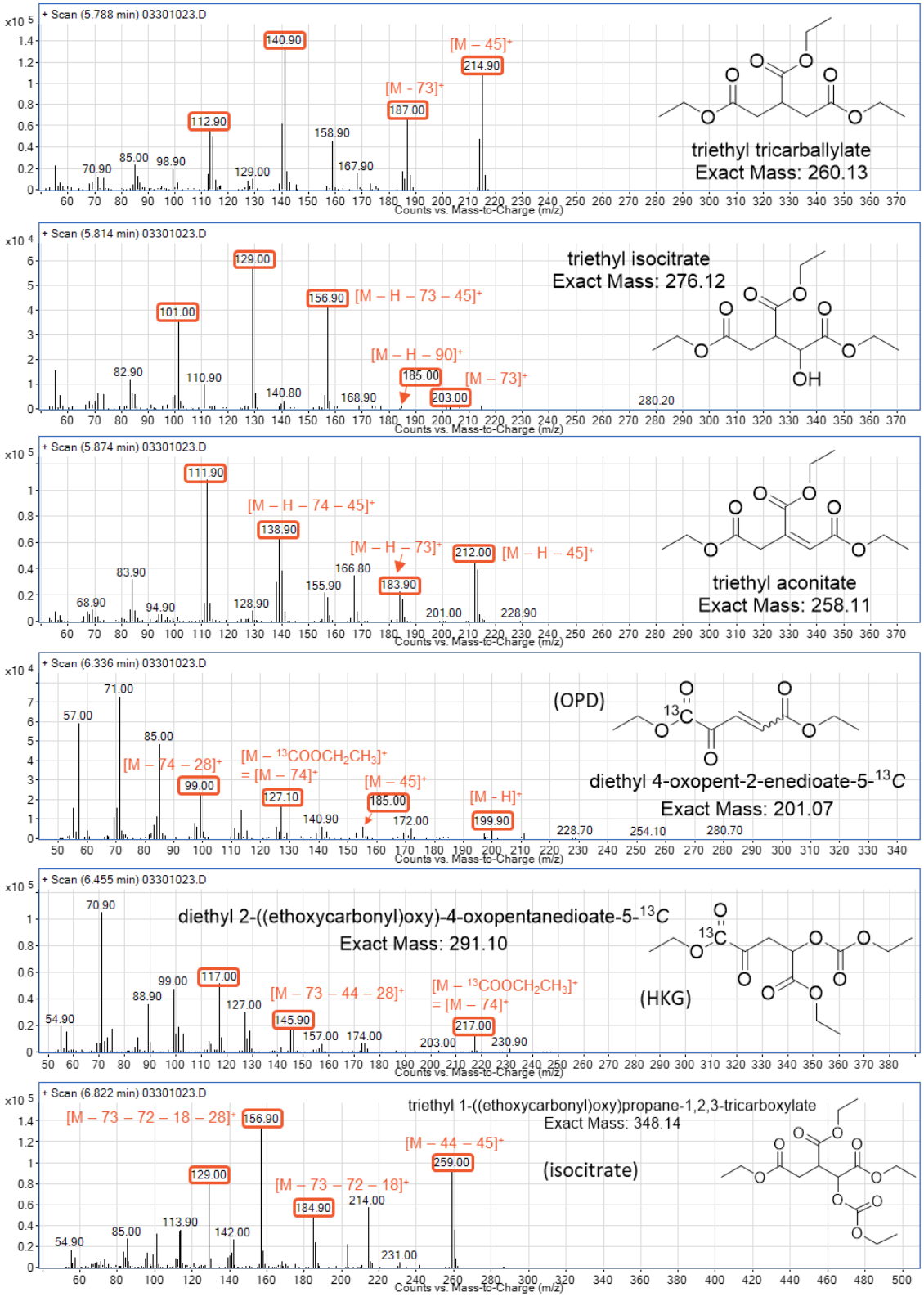


Figure S24.
GC chromatogram and mass spectra of ECF/EtOH-derivatized compounds detected at t = 24 h in the reaction network arising from pyruvate-1-^{13}C and glyoxylate, promoted by Fe^{2+} at 70 ºC. Characteristic ("fingerprint") peaks are highlighted in red.

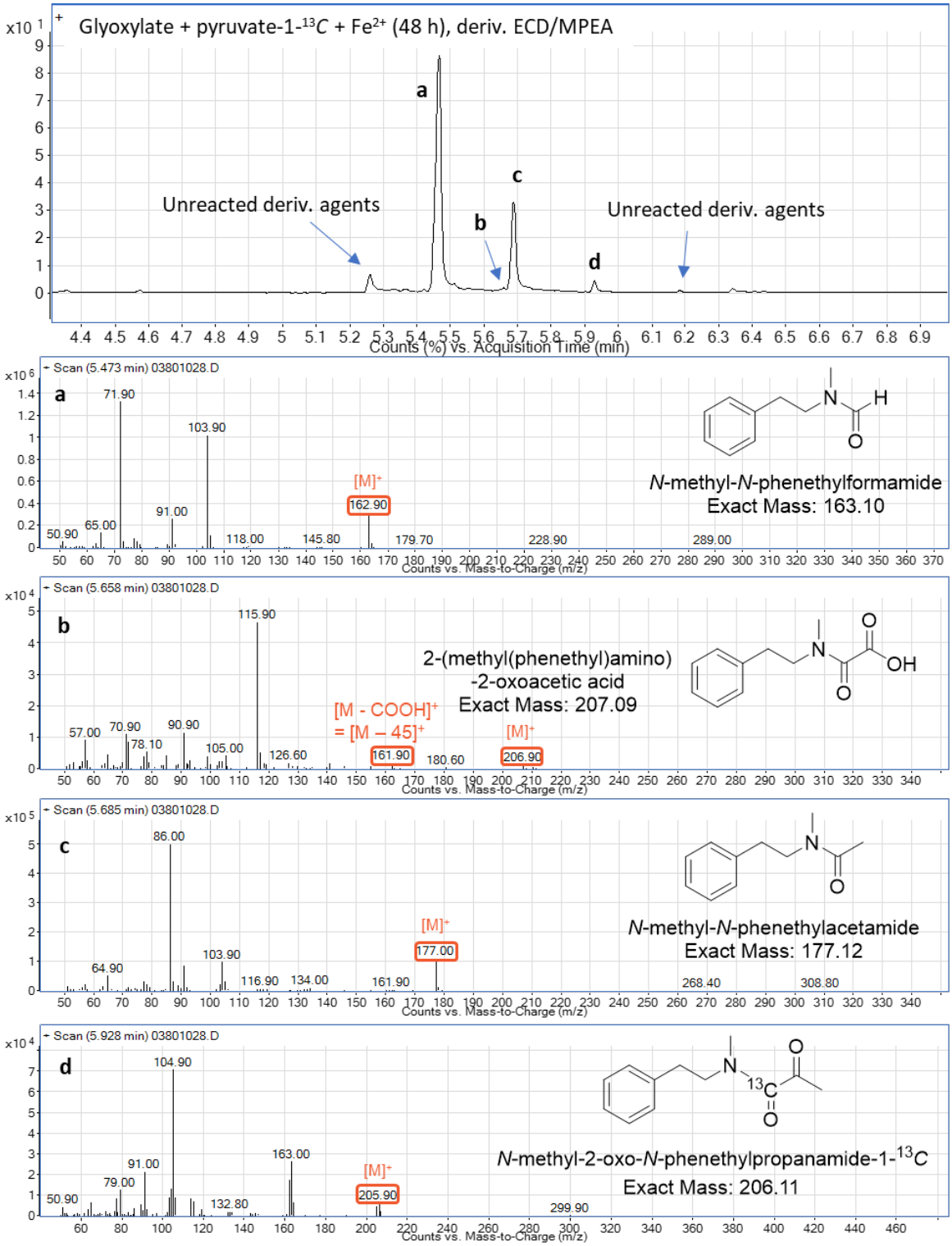
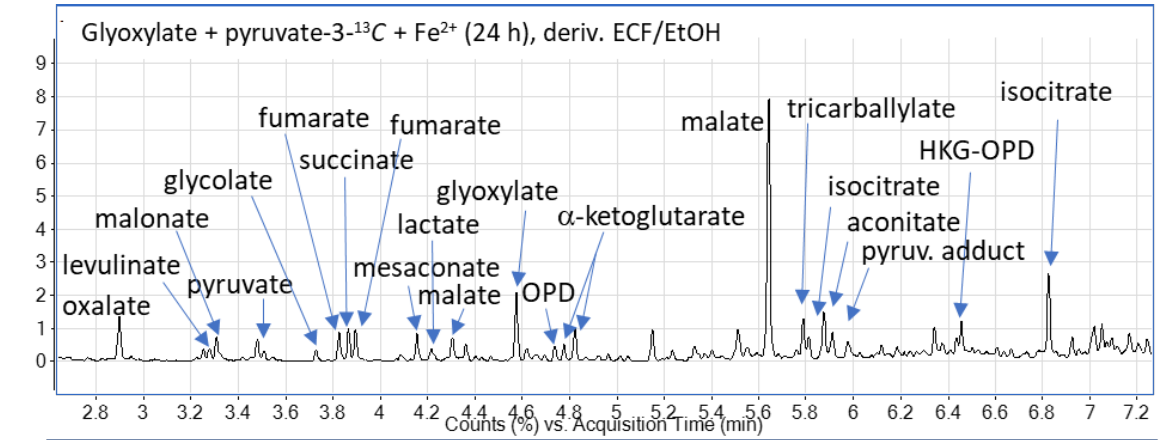
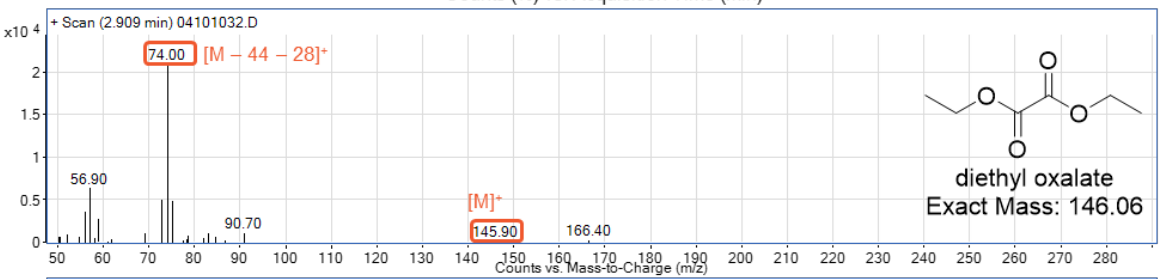
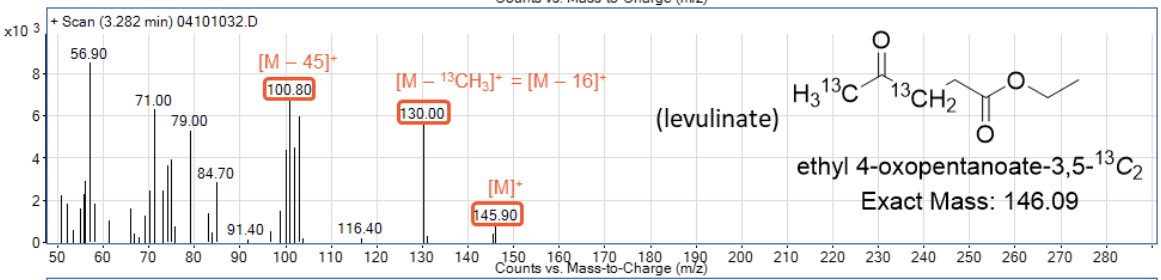
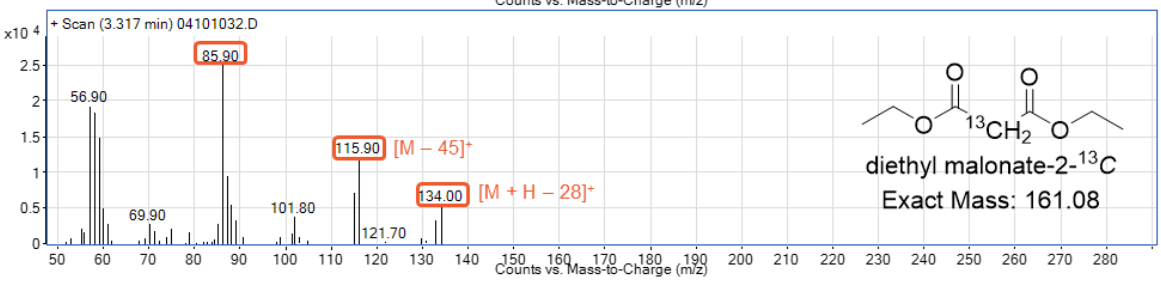


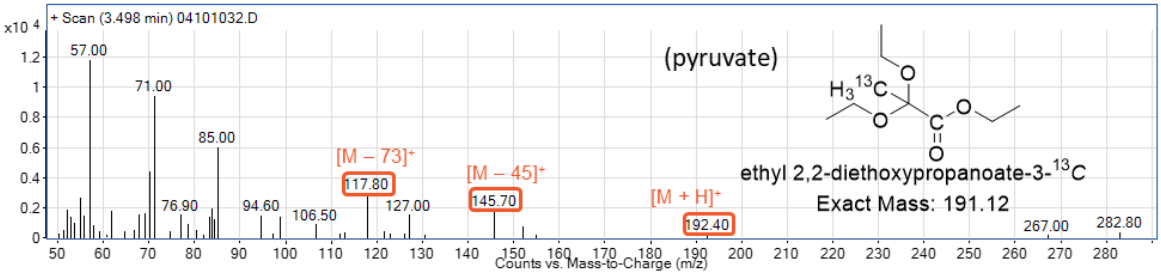
Figure S25.
GC chromatogram and mass spectra confirming the presence of formate, oxalate and acetate, detected as their *N*-methyl-*N*-phenethylamides, after derivatization of a typical reaction mixture (pyruvate-1-^{13}C + glyoxylate) with ECD/MPEA after 48 h reaction time.

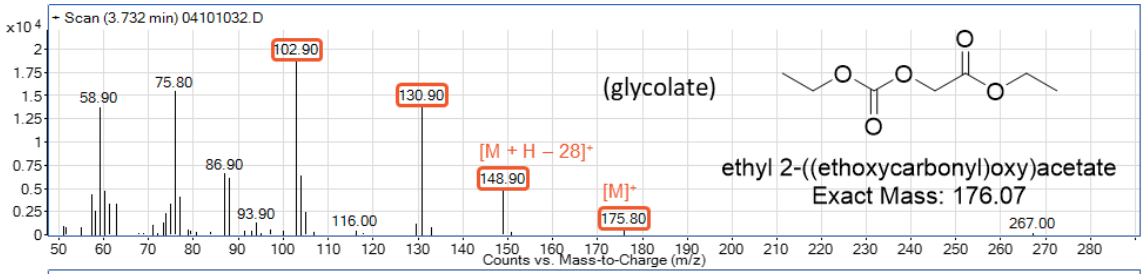

Glyoxylate + pyruvate-3-13C + Fe2+ (24 h), deriv. ECF/EtOH
oxalate
levulinate
malonate
glycolate
pyruvate
fumarate
succinate
fumarate
lactate
mesaconate
malate
glyoxylate
OPD
α-ketoglutarate
malate
tricarballylate
isocitrate
aconitate
isocitrate
HKG-OPD
pyruv. adduct
Counts (%) vs. Acquisition Time (min)

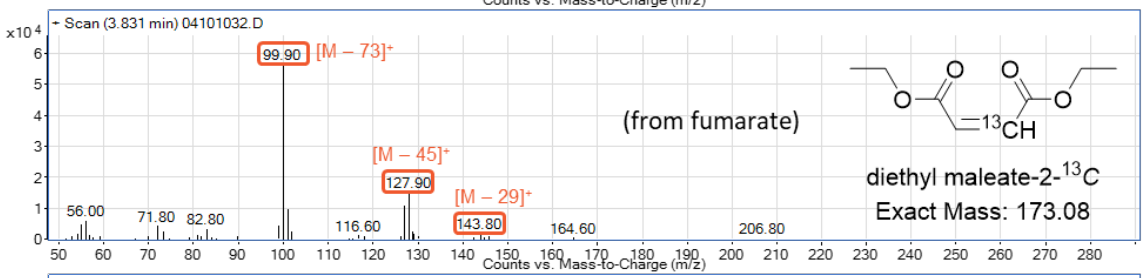

+ Scan (2.909 min) 04101032.D
74.00 [M – 44 – 28]+
56.90
90.70
[M]+
145.90
166.40
diethyl oxalate
Exact Mass: 146.06
Counts vs. Mass-to-Charge (m/z)

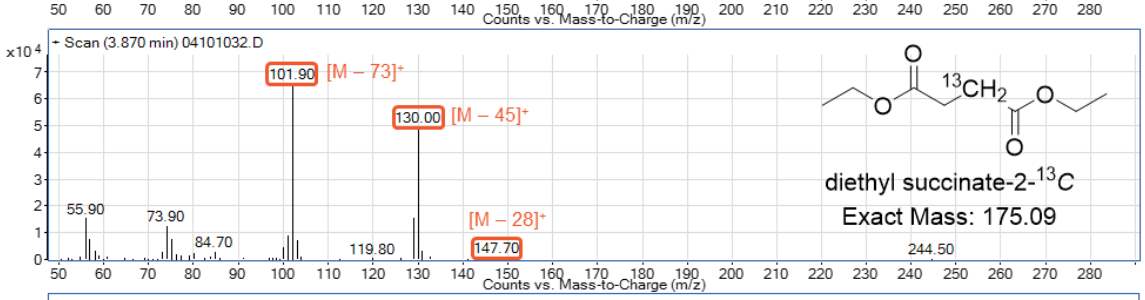

+ Scan (3.282 min) 04101032.D
56.90
71.00
79.00
84.70
91.40
[M – 45]+
100.80
[M – 13CH3]+ = [M – 16]+
130.00
116.40
[M]+
145.90
(levulinate)
ethyl 4-oxopentanoate-3,5-13C2
Exact Mass: 146.09
Counts vs. Mass-to-Charge (m/z)

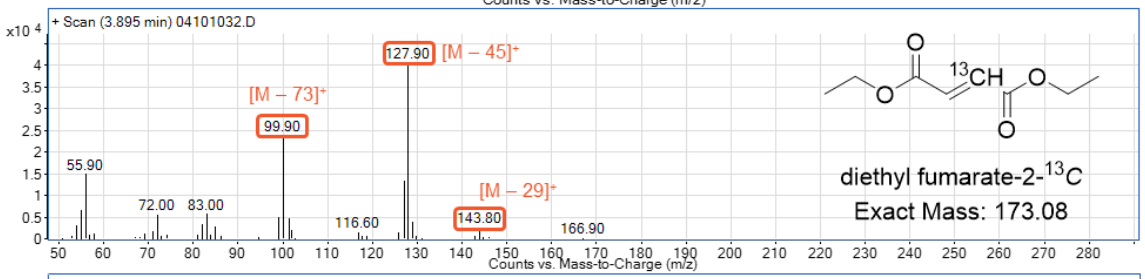

+ Scan (3.317 min) 04101032.D
85.90
56.90
69.90
101.80
115.90 [M – 45]+
121.70
134.00 [M + H – 28]+
diethyl malonate-2-13C
Exact Mass: 161.08
Counts vs. Mass-to-Charge (m/z)

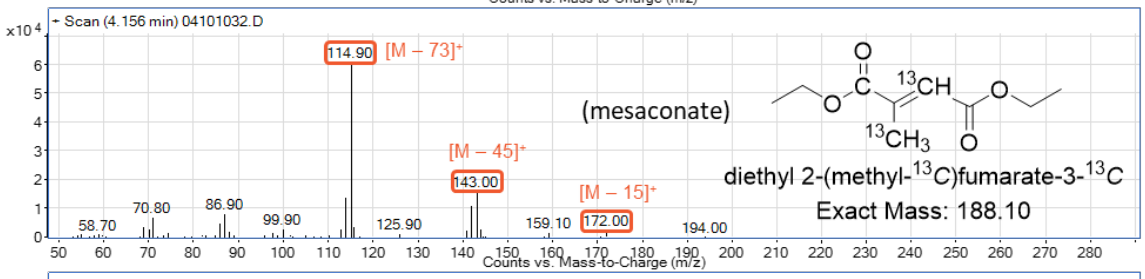

+ Scan (3.498 min) 04101032.D
57.00
71.00
85.00
76.90
94.60
106.50
[M – 73]+
117.80
127.00
[M – 45]+
145.70
[M + H]+
192.40
267.00
282.80
(pyruvate)
ethyl 2,2-diethoxypropanoate-3-13C
Exact Mass: 191.12
Counts vs. Mass-to-Charge (m/z)

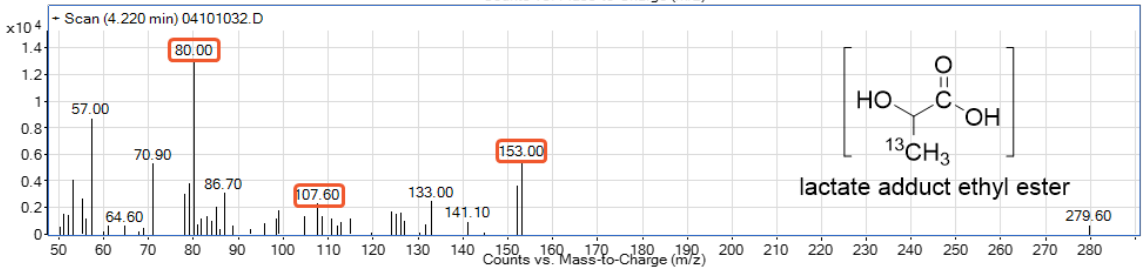

Scan (3.732 min) 04101032.D
102.90
75.80
58.90
130.90
86.90
148.90
[M + H – 28]+
[M]+
175.80
93.90
116.00
267.00
(glycolate)
ethyl 2-((ethoxycarbonyl)oxy)acetate
Exact Mass: 176.07
Counts vs. Mass-to-Charge (m/z)

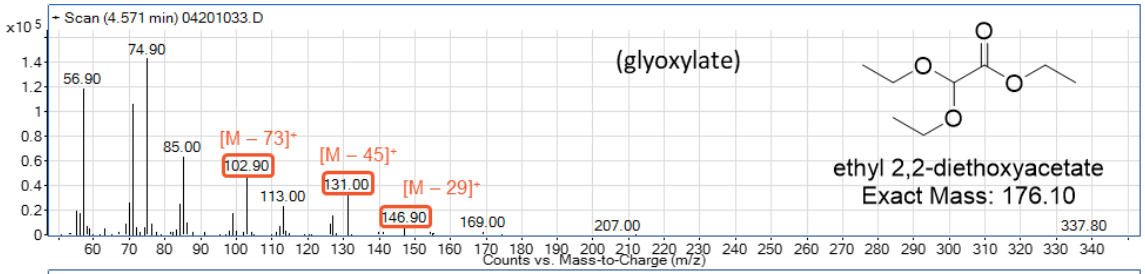

Scan (3.831 min) 04101032.D
99.90
[M – 73]+
[M – 45]+
127.90
[M – 29]+
143.80
56.00
71.80
82.80
116.60
164.60
206.80
(from fumarate)
diethyl maleate-2-13C
Exact Mass: 173.08
Counts vs. Mass-to-Charge (m/z)

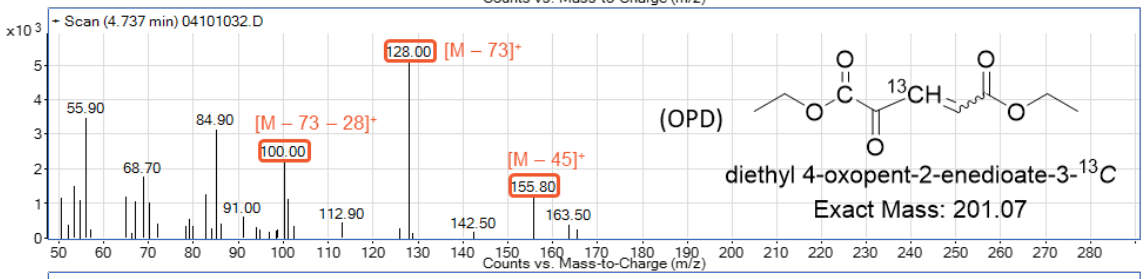

Scan (3.870 min) 04101032.D
101.90
[M – 73]+
130.00
[M – 45]+
55.90
73.90
84.70
[M – 28]+
119.80
147.70
244.50
diethyl succinate-2-13C
Exact Mass: 175.09
Counts vs. Mass-to-Charge (m/z)

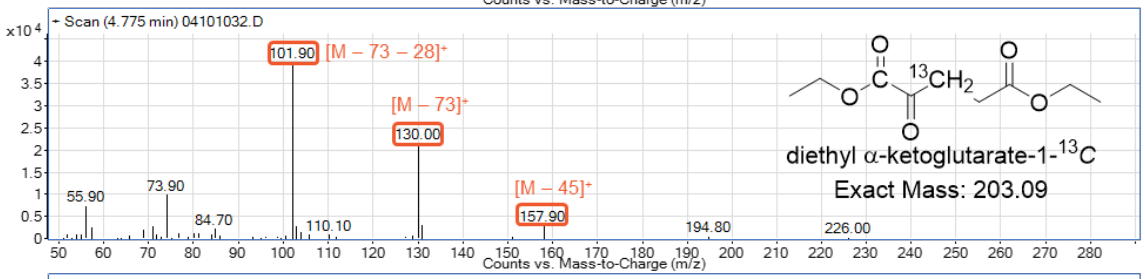

Scan (3.895 min) 04101032.D
127.90
[M – 45]+
[M – 73]+
99.90
55.90
72.00
83.00
[M – 29]+
116.60
143.80
166.90
diethyl fumarate-2-13C
Exact Mass: 173.08
Counts vs. Mass-to-Charge (m/z)

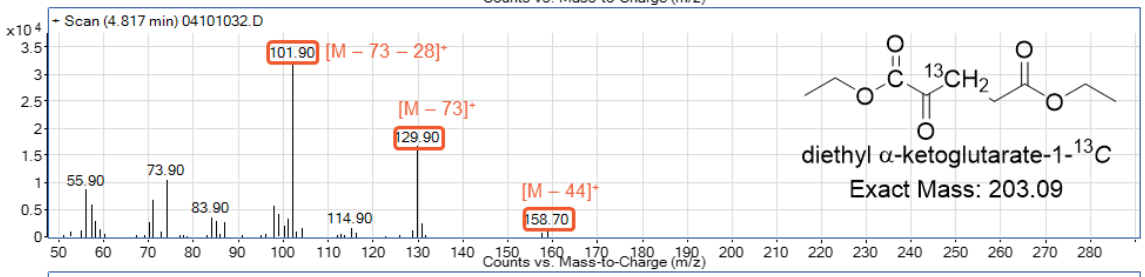

Scan (4.156 min) 04101032.D
114.90
[M – 73]+
[M – 45]+
143.00
[M – 15]+
70.80
86.90
58.70
99.90
125.90
159.10
172.00
194.00
(mesaconate)
diethyl 2-(methyl-13C)fumarate-3-13C
Exact Mass: 188.10
Counts vs. Mass-to-Charge (m/z)

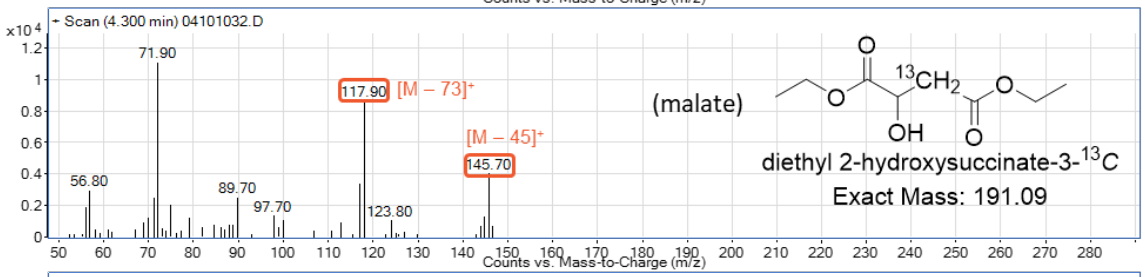

Scan (4.220 min) 04101032.D
80.00
57.00
70.90
153.00
86.70
107.60
133.00
141.10
64.60
279.60
lactate adduct ethyl ester
Counts vs. Mass-to-Charge (m/z)

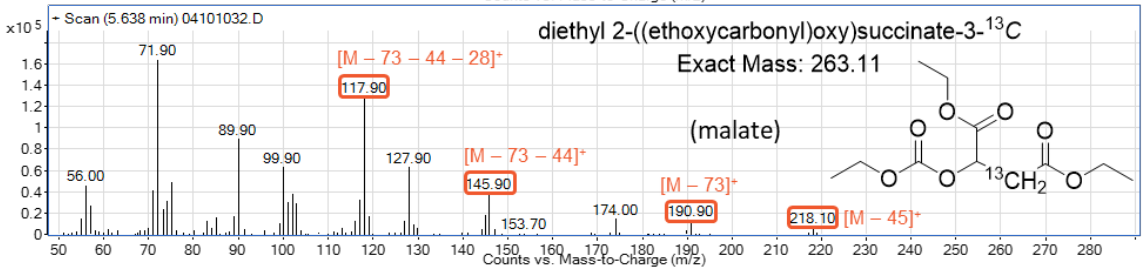

Scan (4.571 min) 04201033.D
(glyoxylate)
ethyl 2,2-diethoxyacetate
Exact Mass: 176.10
[M – 73]+
[M – 45]+
[M – 29]+
Counts vs. Mass-to-Charge (m/z)


Scan (4.737 min) 04101032.D
(OPD)
diethyl 4-oxopent-2-enedioate-3-13C
Exact Mass: 201.07
[M – 73]+
[M – 73 – 28]+
[M – 45]+
Counts vs. Mass-to-Charge (m/z)


Scan (4.775 min) 04101032.D
diethyl α-ketoglutarate-1-13C
Exact Mass: 203.09
[M – 73 – 28]+
[M – 73]+
[M – 45]+
Counts vs. Mass-to-Charge (m/z)


Scan (4.817 min) 04101032.D
diethyl α-ketoglutarate-1-13C
Exact Mass: 203.09
[M – 73 – 28]+
[M – 73]+
[M – 44]+
Counts vs. Mass-to-Charge (m/z)


Scan (4.300 min) 04101032.D
(malate)
diethyl 2-hydroxysuccinate-3-13C
Exact Mass: 191.09
[M – 73]+
[M – 45]+
Counts vs. Mass-to-Charge (m/z)


Scan (5.638 min) 04101032.D
diethyl 2-((ethoxycarbonyl)oxy)succinate-3-13C
Exact Mass: 263.11
(malate)
[M – 73 – 44 – 28]+
[M – 73 – 44]+
[M – 73]+
[M – 45]+
Counts vs. Mass-to-Charge (m/z)

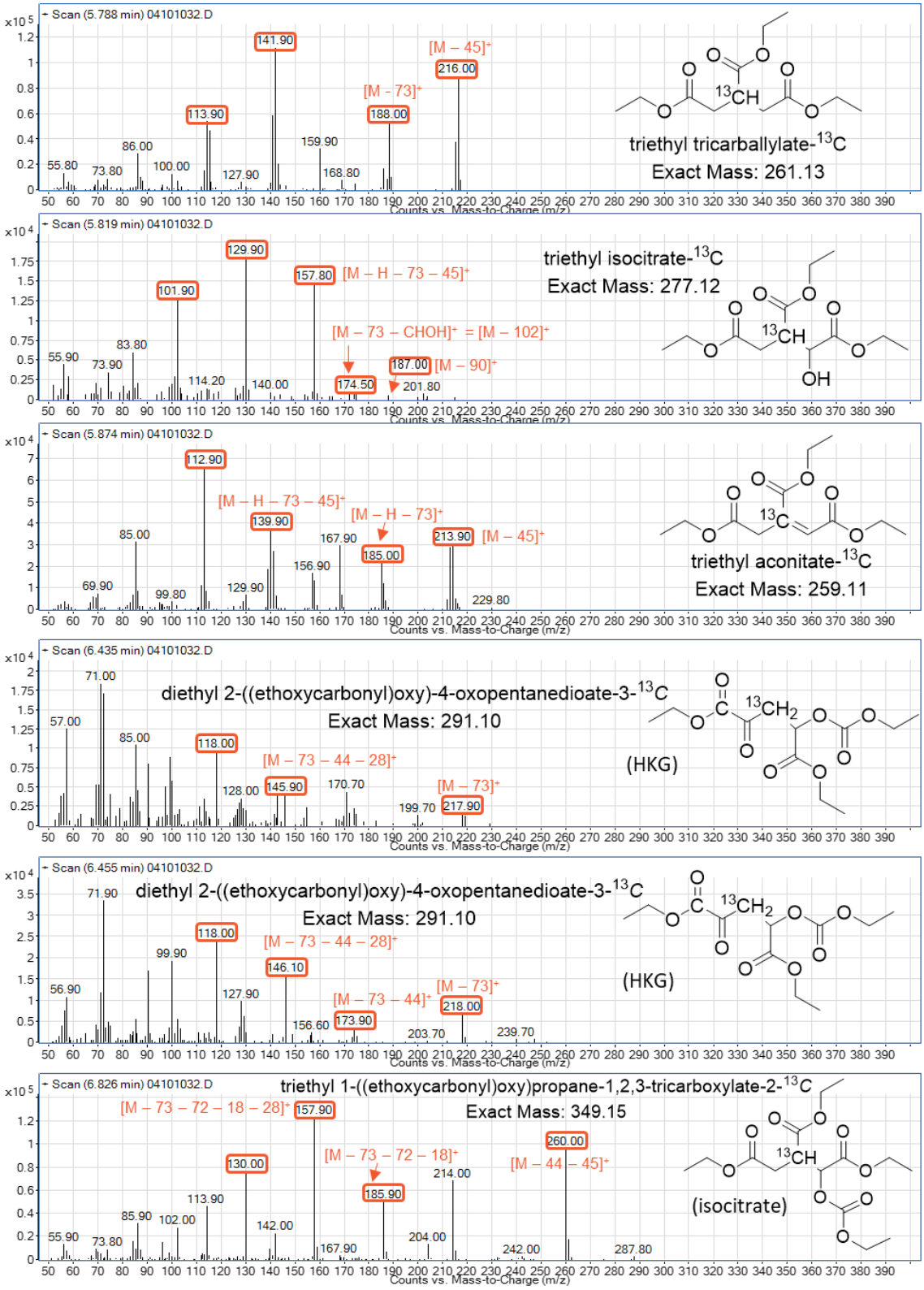


Figure S26.
GC chromatogram and mass spectra of ECF/EtOH-derivatized compounds detected at t = 24 h in the reaction network arising from pyruvate-3-^{13}C and glyoxylate, promoted by Fe^{2+} at 70 ºC. Characteristic ("fingerprint") peaks are highlighted in red.

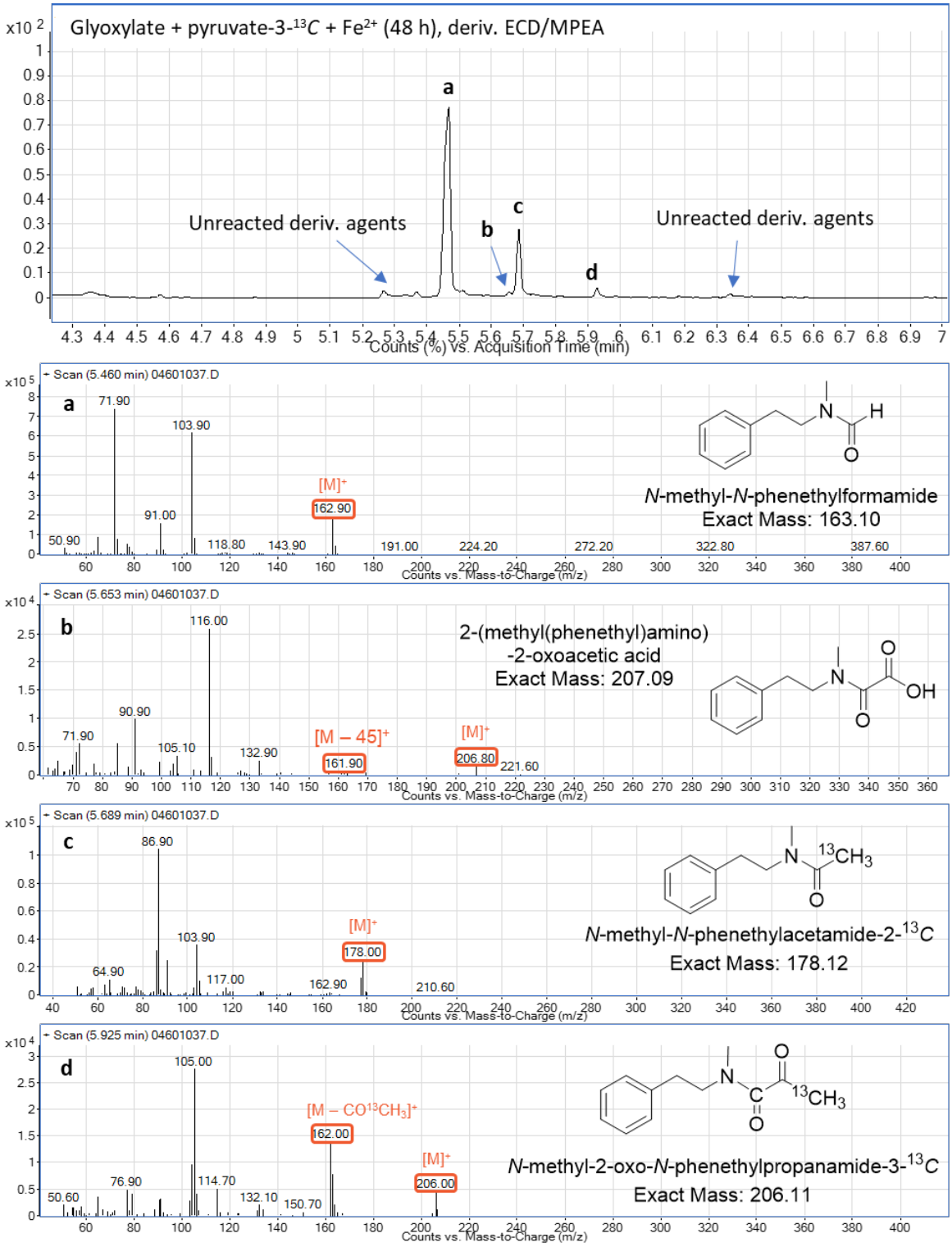
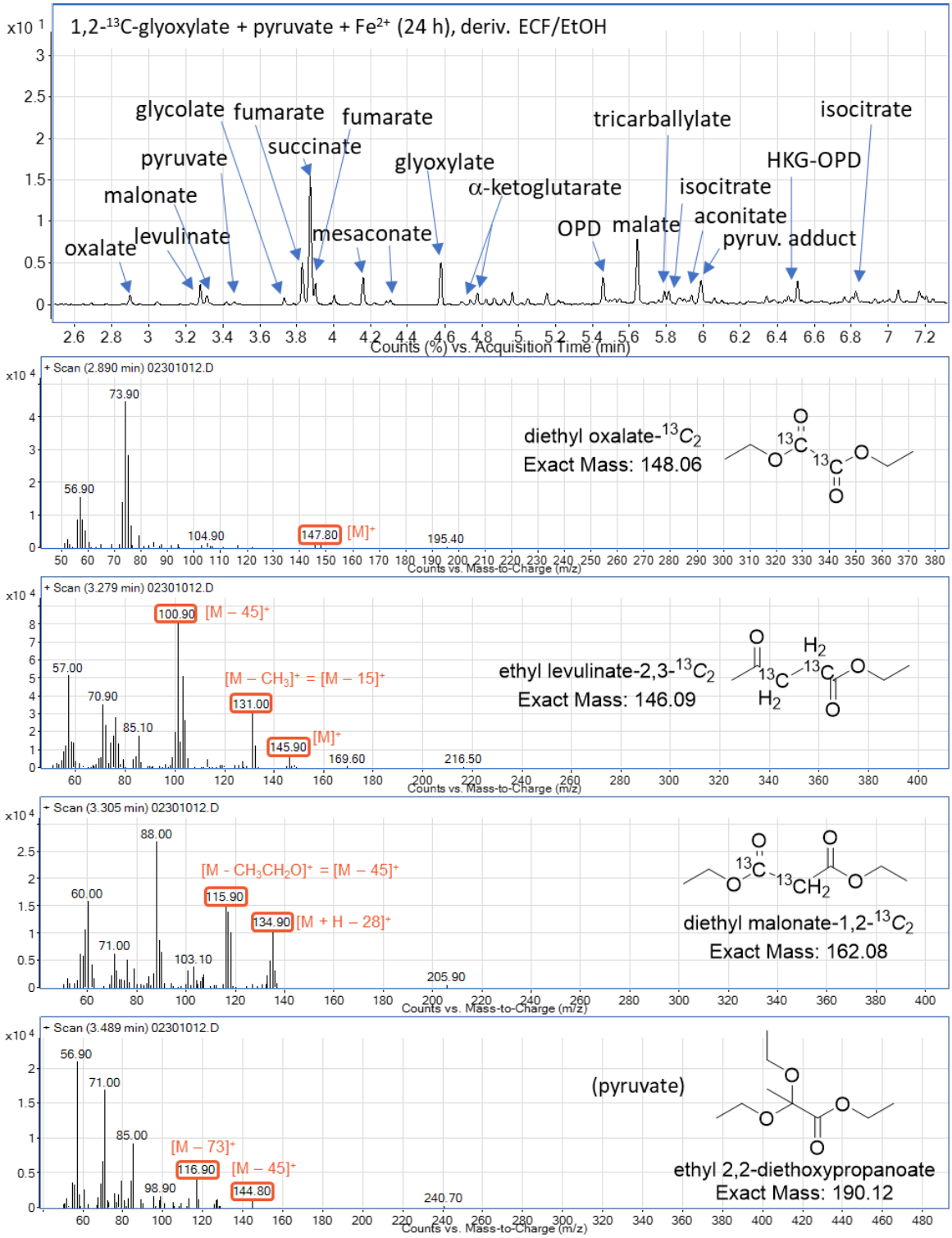
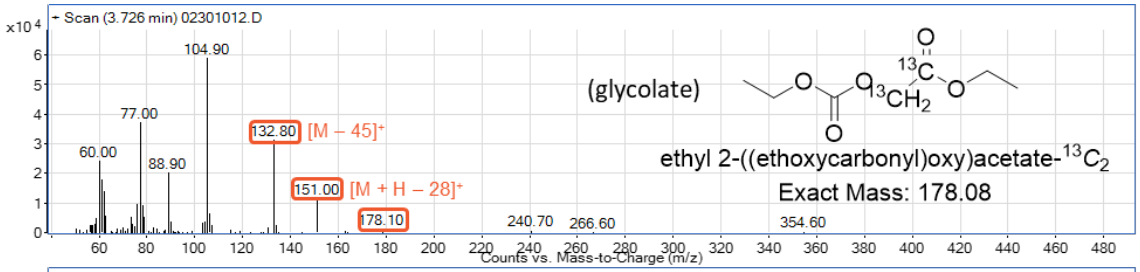
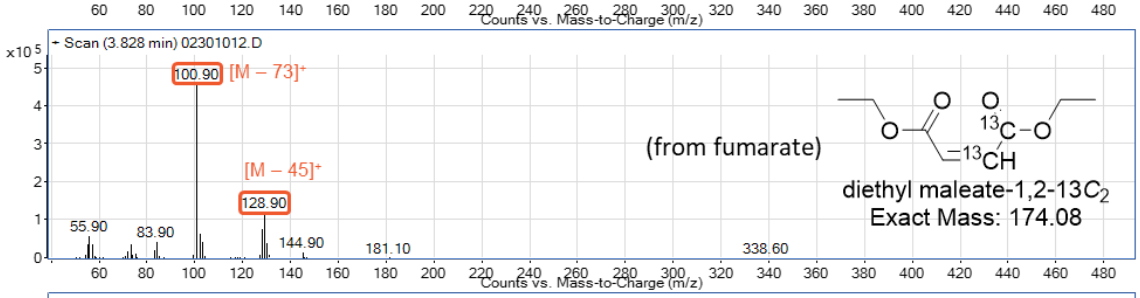
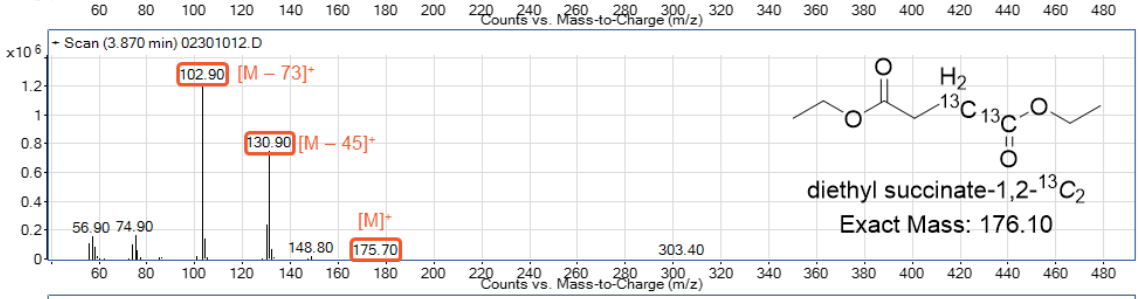


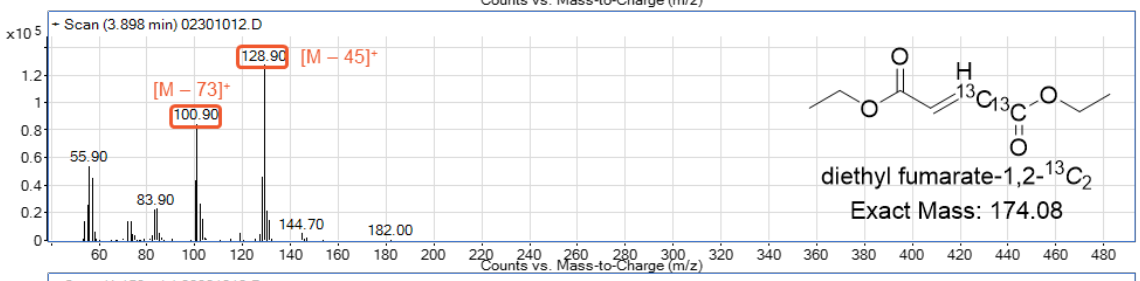
Figure S27.
GC chromatogram and mass spectra confirming the presence of formate, oxalate and acetate, detected as their *N*-methyl-*N*-phenethylamides, after derivatization of a typical reaction mixture (pyruvate-3-^{13}C + glyoxylate) with ECD/MPEA after 48 h reaction time.

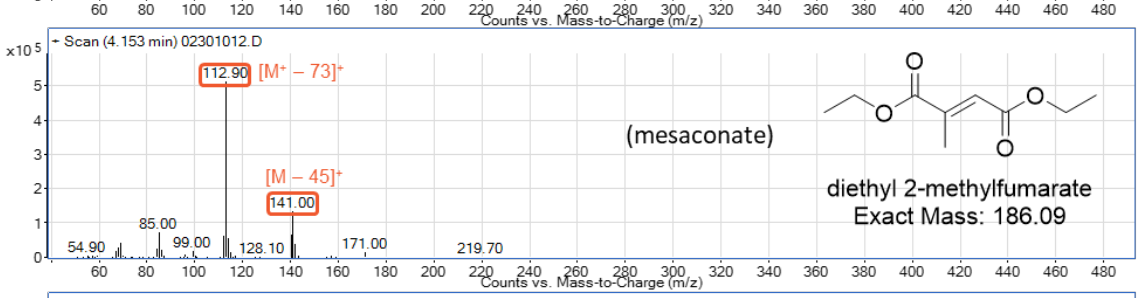

1,2-13C-glyoxylate + pyruvate + Fe2+ (24 h), deriv. ECF/EtOH
glycolate
fumarate
fumarate
succinate
pyruvate
glyoxylate
tricarballylate
isocitrate
HKG-OPD
malonate
α-ketoglutarate
isocitrate
aconitate
OPD
malate
pyruv. adduct
oxalate
levulinate
mesaconate
Counts (%) vs. Acquisition Time (min)
+ Scan (2.890 min) 02301012.D
73.90
56.90
104.90
147.80 [M]+
195.40
diethyl oxalate-13C2
Exact Mass: 148.06
Counts vs. Mass-to-Charge (m/z)
+ Scan (3.279 min) 02301012.D
100.90 [M – 45]+
57.00
70.90
85.10
[M – CH3]+ = [M – 15]+
131.00
145.90 [M]+
169.60
216.50
ethyl levulinate-2,3-13C2
Exact Mass: 146.09
Counts vs. Mass-to-Charge (m/z)
+ Scan (3.305 min) 02301012.D
88.00
[M - CH3CH2O]+ = [M – 45]+
60.00
115.90
134.90 [M + H – 28]+
71.00
103.10
205.90
diethyl malonate-1,2-13C2
Exact Mass: 162.08
Counts vs. Mass-to-Charge (m/z)
+ Scan (3.489 min) 02301012.D
56.90
71.00
85.00
[M – 73]+
116.90
[M – 45]+
98.90
144.80
240.70
(pyruvate)
ethyl 2,2-diethoxypropanoate
Exact Mass: 190.12
Counts vs. Mass-to-Charge (m/z)

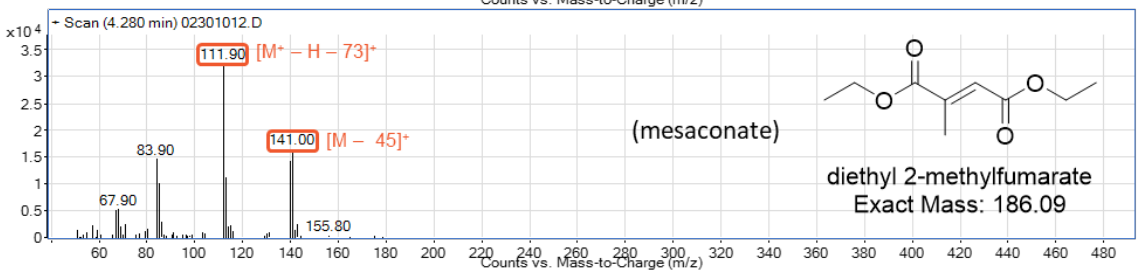

Scan (3.726 min) 02301012.D
104.90
77.00
132.80 [M – 45]+
60.00
88.90
151.00 [M + H – 28]+
178.10
240.70
266.60
354.60
Counts vs. Mass-to-Charge (m/z)
(glycolate)
ethyl 2-((ethoxycarbonyl)oxy)acetate-13C2
Exact Mass: 178.08

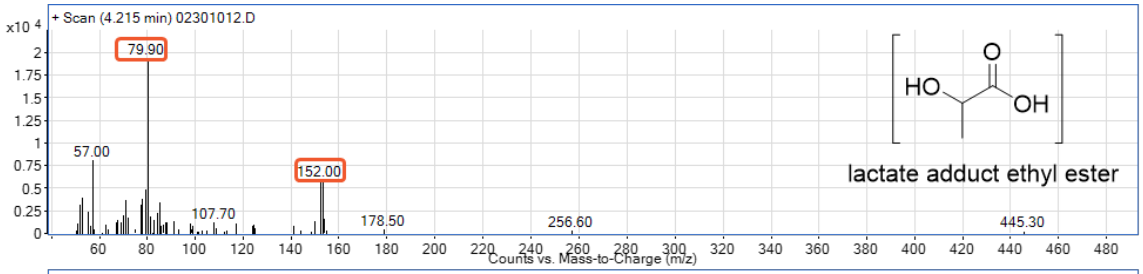

Scan (3.828 min) 02301012.D
100.90 [M – 73]+
[M – 45]+
128.90
55.90
83.90
144.90
181.10
338.60
Counts vs. Mass-to-Charge (m/z)
(from fumarate)
diethyl maleate-1,2-13C2
Exact Mass: 174.08

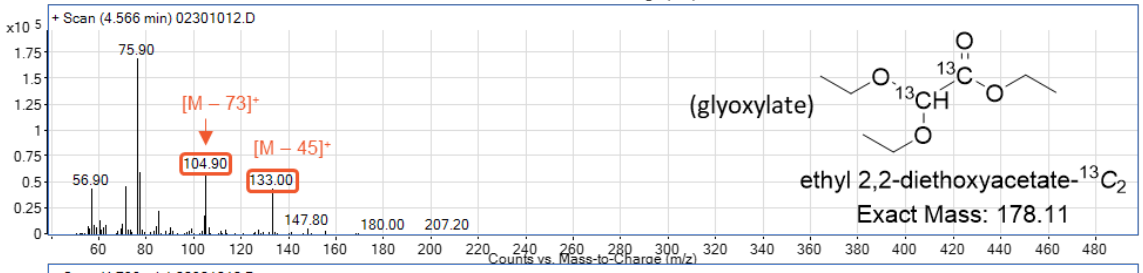

Scan (3.870 min) 02301012.D
102.90 [M – 73]+
130.90 [M – 45]+
56.90
74.90
[M]+
148.80
175.70
303.40
Counts vs. Mass-to-Charge (m/z)
diethyl succinate-1,2-13C2
Exact Mass: 176.10

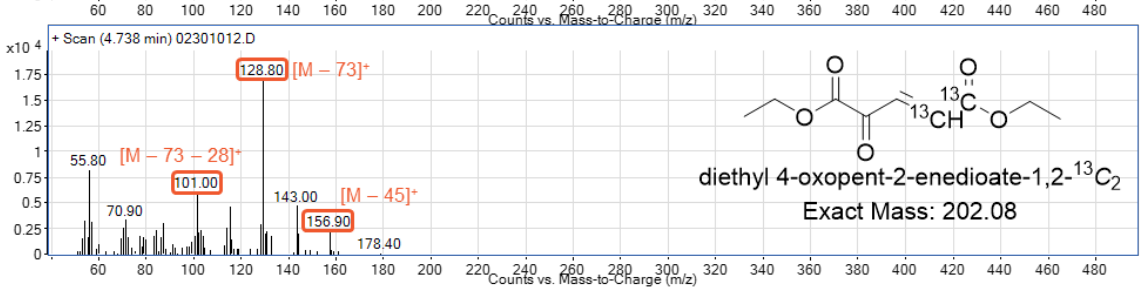

Scan (3.898 min) 02301012.D
128.90 [M – 45]+
[M – 73]+
100.90
55.90
83.90
144.70
182.00
Counts vs. Mass-to-Charge (m/z)
diethyl fumarate-1,2-13C2
Exact Mass: 174.08

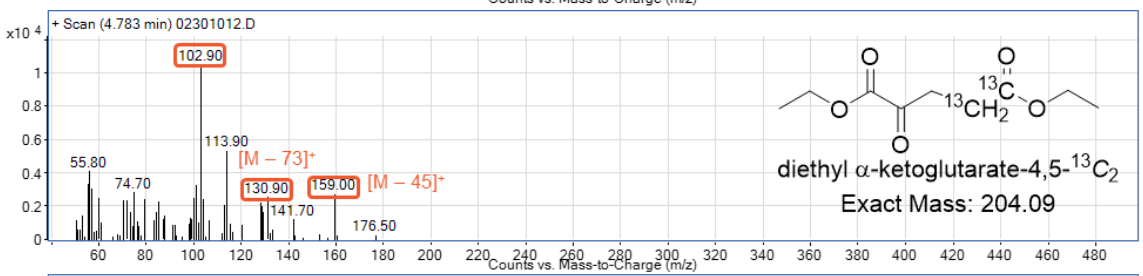

Scan (4.153 min) 02301012.D
112.90 [M+ – 73]+
[M – 45]+
141.00
85.00
54.90
99.00
128.10
171.00
219.70
Counts vs. Mass-to-Charge (m/z)
(mesaconate)
diethyl 2-methylfumarate
Exact Mass: 186.09

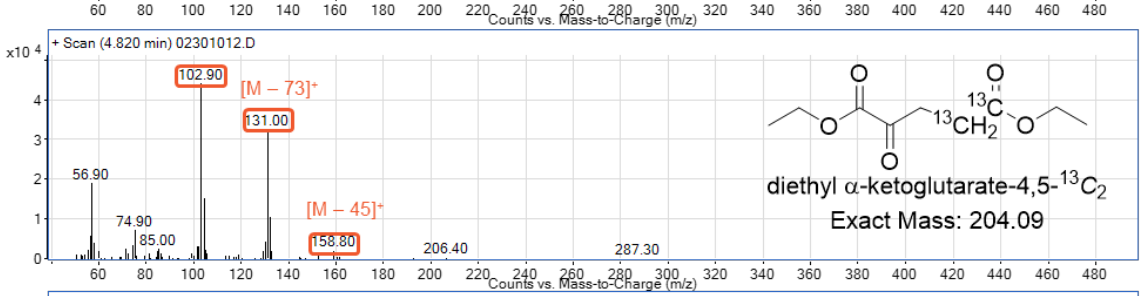

Scan (4.280 min) 02301012.D
111.90 [M+ – H – 73]+
141.00 [M – 45]+
83.90
67.90
155.80
Counts vs. Mass-to-Charge (m/z)
(mesaconate)
diethyl 2-methylfumarate
Exact Mass: 186.09

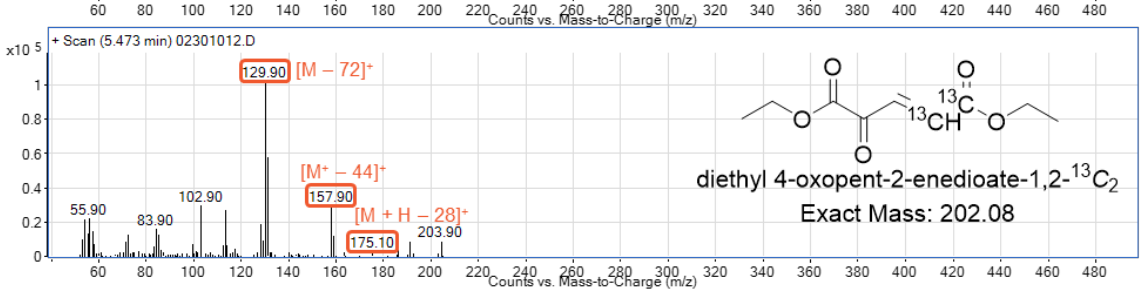

+ Scan (4.215 min) 02301012.D
79.90
57.00
152.00
107.70
178.50
256.60
445.30
HO
OH
lactate adduct ethyl ester
Counts vs. Mass-to-Charge (m/z)


+ Scan (4.566 min) 02301012.D
75.90
[M – 73]+
[M – 45]+
104.90
133.00
56.90
147.80
180.00
207.20
(glyoxylate)
ethyl 2,2-diethoxyacetate-13C2
Exact Mass: 178.11
Counts vs. Mass-to-Charge (m/z)


+ Scan (4.738 min) 02301012.D
128.80
[M – 73]+
55.80
[M – 73 – 28]+
101.00
143.00
[M – 45]+
70.90
156.90
178.40
diethyl 4-oxopent-2-enedioate-1,2-13C2
Exact Mass: 202.08
Counts vs. Mass-to-Charge (m/z)


+ Scan (4.783 min) 02301012.D
102.90
113.90
[M – 73]+
55.80
74.70
130.90
159.00
[M – 45]+
141.70
176.50
diethyl α-ketoglutarate-4,5-13C2
Exact Mass: 204.09
Counts vs. Mass-to-Charge (m/z)


+ Scan (4.820 min) 02301012.D
102.90
[M – 73]+
131.00
56.90
[M – 45]+
74.90
85.00
158.80
206.40
287.30
diethyl α-ketoglutarate-4,5-13C2
Exact Mass: 204.09
Counts vs. Mass-to-Charge (m/z)


+ Scan (5.473 min) 02301012.D
129.90
[M – 72]+
[M+ – 44]+
157.90
102.90
55.90
83.90
[M + H – 28]+
175.10
203.90
diethyl 4-oxopent-2-enedioate-1,2-13C2
Exact Mass: 202.08
Counts vs. Mass-to-Charge (m/z)

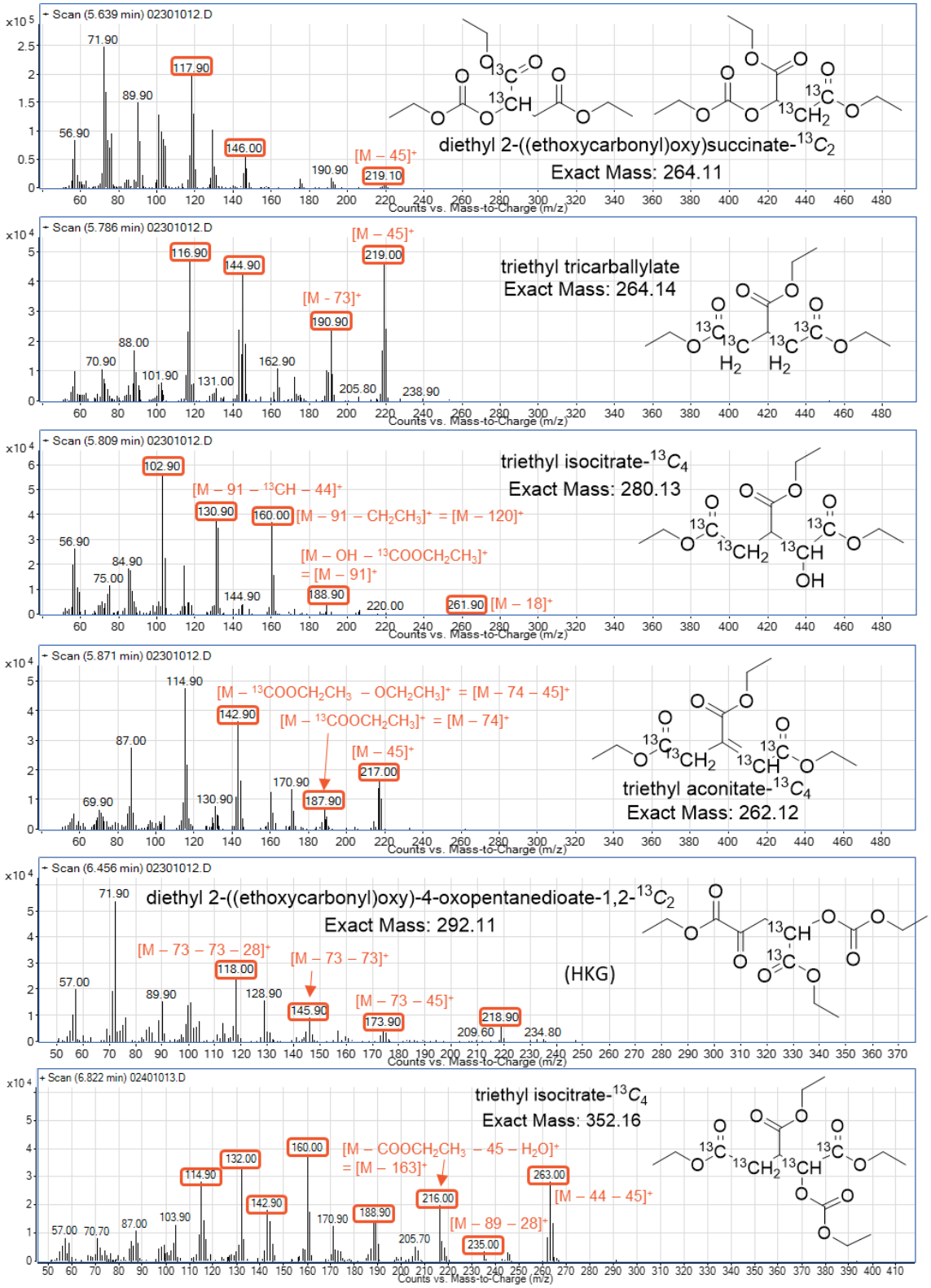


Figure S28.
GC chromatogram and mass spectra of ECF/EtOH-derivatized compounds detected at t = 24 h in the reaction network arising from pyruvate and glyoxylate-1,2-$^{13}C_2$, promoted by Fe^{2+} at 70 ºC. Characteristic ("fingerprint") peaks are highlighted in red.

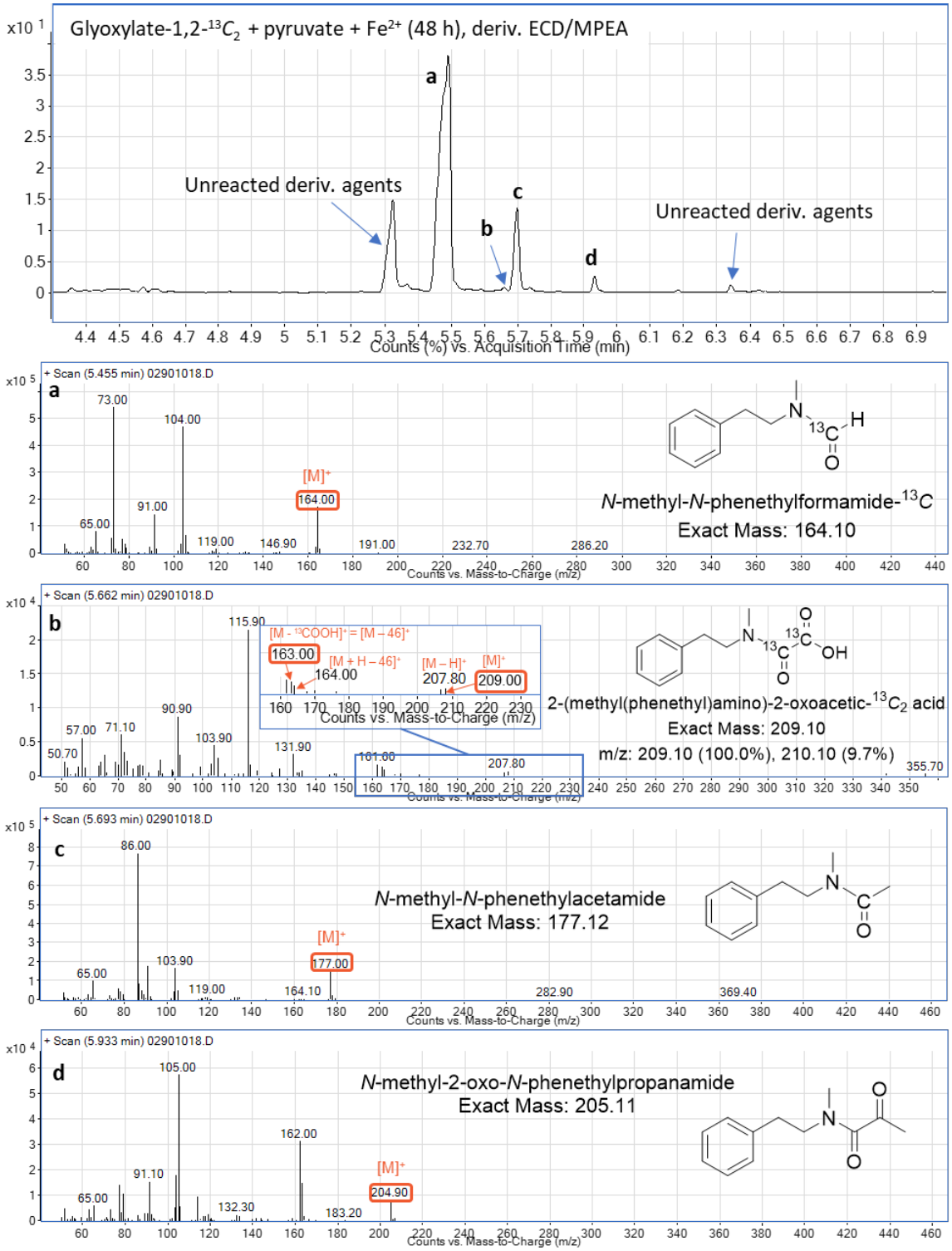


Figure S29.
GC chromatogram and mass spectra confirming the presence of formate, oxalate and acetate, detected as their *N*-methyl-*N*-phenethylamides, after derivatization of a typical reaction mixture (pyruvate + glyoxylate-1,2-$^{13}C_2$) with ECD/MPEA after 48 h reaction time.

Table S1.

Response factors corresponding to calibration lines ($c = ax^2 + bx$, where c = concentration in mmol mL^{-1}, x = peak area, and *a*, *b* = response factors) for all the compounds detected as their ethyl esters on the GC-MS in this study. Calibration lines were obtained in at least 3 replicas, with % error corresponding to the standard deviation of *b* (linear fit). *For polynomial curves, see Figure S2 and Figure S10 for 95% confidence bounds (nonlinear fit, *OriginPro*).

Intermediate	**Response factors**		**Error (on *b*), %**
	a	***b***	
Carboxylic acids detected on the GC-MS			
Oxalic	1871637755	105933343	*
Malonic	7966254029	59730553	*
Glycolic	-	87414654	3
Levulinic	3551346017	114690195	*
Glyoxylic	-	1512276534	9
Pyruvic	-	100121708	6
Lactic	-	18684986864	*single run*
Malic	-	7404200671	3
Fumaric	-	5504902362	6
Succinic	-	3013611468	8
α-Ketoglutaric	-	1230698200	4
α-Hydroxyglutaric	-	2397763078	*single run*
HKG/OPD	1871637755	105933343	*
Isocitric	-	1706650152	7
Aconitic	-	2264986318	11
Mesaconic	-	1790780000	4
Tricarballylic	-	3181106809	4
Amino acids detected on the GC-MS			
Glycine	8766531636	384533833.4	*
Alanine	-	3811220283	1
Aspartic acid	73528291403	556120932.3	
Glutamic acid	7524177005	424126900.3	*
Carboxylic acids detected by NMR			
Acetic acid (2.19 ppm)	-	40.97509	5
Formic acid (8.46 ppm)	-	18.26024	8

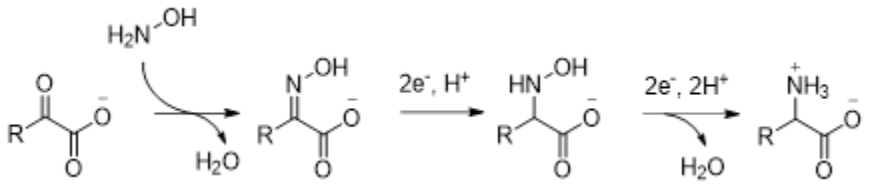
Table S2.

Carbon balance of the glyoxylate-pyruvate reaction network calculated as mmol of carbon atoms retained in a typical reaction mixture (see: Synthetic procedures) at t = 0 h to t = 48 h time points. Reported values are an average obtained from two independent runs. Errors correspond to ±standard deviation. "100%" corresponds to the material recovered from an aqueous solution of starting materials (SM) without Fe^{2+} added. *n. d.* = not determined.

Intermediate	**#C**	**0 h**	**5 min**	**1 h**	**3 h**	**7 h**	**10 h**	**24 h**	**48 h**
		C in reaction mixture, mmol mL^{-1}							
SM: Glyoxylate	**2**	0.140 ± 0.016	0.046 ± 0.013	0.002 ± 0.001	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.001	0.001 ± 0.000
SM: Pyruvate	**3**	0.002 ± 0.000	0.003 ± 0.000	0.002 ± 0.001	0.002 ± 0.001	0.006 ± 0.001	0.005 ± 0.000	0.003 ± 0.000	0.004 ± 0.001
Lactate	3	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Malate	4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.004 ± 0.000	0.004 ± 0.002
Fumarate	4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
Succinate	4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
a-Keto-glutarate	5	0.000 ± 0.000	0.001 ± 0.000	0.009 ± 0.002	0.009 ± 0.005	0.007 ± 0.000	0.003 ± 0.000	0.002 ± 0.001	0.002 ± 0.000
HKG/OPD	5	0.417 ± 0.026	0.258 ± 0.018	0.183 ± 0.014	0.121 ± 0.001	0.096 ± 0.002	0.079 ± 0.009	0.042 ± 0.001	0.017 ± 0.001
Isocitrate	6	0.000 ± 0.000	0.000 ± 0.000	0.003 ± 0.000	0.007 ± 0.002	0.011 ± 0.000	0.010 ± 0.000	0.004 ± 0.001	0.005 ± 0.000
Aconitate	6	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.002 ± 0.000
Mesaconate	5	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Tricarbally-late	6	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.002 ± 0.000	0.003 ± 0.000
Oxalate	2	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
Malonate	3	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.003 ± 0.002	0.007 ± 0.000	0.006 ± 0.002	0.017 ± 0.007	0.006 ± 0.001
Glycolate	2	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.003 ± 0.001	0.002 ± 0.001	0.018 ± 0.004	0.030 ± 0.001
Levulinate	5	0.003 ± 0.000	0.013 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.004 ± 0.001	0.004 ± 0.000	0.003 ± 0.000	0.004 ± 0.001
Sum, mmol		**0.56 ± 0.01**	**0.32 ± 0.03**	**0.20 ± 0.02**	**0.15 ± 0.01**	**0.14 ± 0.00**	**0.12 ± 0.01**	**0.10 ± 0.00**	**0.09 ± 0.01**
Sum, mol%		**100% ± 2%**	**56% ± 6%**	**36% ± 3%**	**26% ± 2%**	**24% ± 0%**	**21% ± 2%**	**18% ± 1%**	**16% ± 1%**
Acetate	2	*n. d.*							0.009 ± 0.000
Formate	1								0.012 ± 0.001

Table S3.

Products of the reaction of ketoacids with hydroxylamine hydrochloride (1 equiv) and Fe^0 (10 equiv) at 100 °C over 16 h. Reported values are an average obtained from two independent runs. Errors correspond to ±standard deviation.



Ketoacid	Products (%)	
	Amination	**Reduction**
Glyoxylic	Glycine (63 ± 9%)	Glycolic acid (26 ± 11%)
Pyruvic	Alanine (2 ± 0%)	Lactic acid (< 1 %)
Oxaloacetic	Aspartic acid (17 ± 1%)	Malic acid (7 ± 1%)
α-Ketoglutaric	Glutamic acid (9 ± 2%)	α-hydroxyglutaric acid (11 ± 0%)

Table S4.

Carbon balance of the glyoxylate-pyruvate-2-^{13}C reaction network calculated as mmol of carbon atoms retained in a typical reaction mixture (see: Synthetic procedures) at t = 0 h to t = 48 h time points. Reported values are an average obtained from two independent runs. Errors correspond to ±standard deviation. "100%" corresponds to the material recovered from an aqueous solution of starting materials (SM) without Fe^{2+} added. Grey shading denotes compounds with a detected ^{13}C label.

Intermediate	#C	0 h	5 min	1 h	3 h	7 h	10 h	24 h	48 h
		C in reaction mixture, mmol mL^{-1}							
SM: Glyoxylate	**2**	0.100 ± 0.001	0.010 ± 0.000	0.004 ± 0.000	0.001 ± 0.001	0.001 ± 0.000	0.002 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
SM: Pyruvate	**3**	0.000 ± 0.000	0.007 ± 0.002	0.003 ± 0.000	0.001 ± 0.000	0.004 ± 0.001	0.003 ± 0.000	0.003 ± 0.000	0.003 ± 0.000
Lactate	3	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Malate	4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.001	0.004 ± 0.001	0.004 ± 0.001
Fumarate	4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
Succinate	4	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
α-Keto-glutarate	5	0.000 ± 0.000	0.000 ± 0.000	0.011 ± 0.001	0.006 ± 0.001	0.004 ± 0.000	0.005 ± 0.000	0.002 ± 0.000	0.002 ± 0.000
HKG/OPD	5	0.361 ± 0.008	0.224 ± 0.037	0.164 ± 0.016	0.111 ± 0.024	0.094 ± 0.003	0.061 ± 0.007	0.034 ± 0.007	0.010 ± 0.001
Isocitrate	6	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.002 ± 0.001	0.003 ± 0.000	0.006 ± 0.000	0.003 ± 0.000	0.002 ± 0.000
Aconitate	6	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.001 ± 0.000	0.001 ± 0.000
Mesaconate	5	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
Tricarbally-late	6	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.002 ± 0.000
Oxalate	2	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.000 ± 0.000	0.001 ± 0.001
Malonate	3	0.000 ± 0.000	0.000 ± 0.000	0.001 ± 0.000	0.002 ± 0.000	0.002 ± 0.001	0.010 ± 0.003	0.012 ± 0.005	0.004 ± 0.000
Glycolate	2	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.005 ± 0.001	0.006 ± 0.000	0.026 ± 0.004	0.041 ± 0.006
Levulinate	5	0.000 ± 0.000	0.038 ± 0.011	0.006 ± 0.001	0.004 ± 0.002	0.003 ± 0.000	0.005 ± 0.001	0.003 ± 0.000	0.003 ± 0.000
Sum, mmol		**0.46 ± 0.01**	**0.28 ± 0.05**	**0.19 ± 0.02**	**0.13 ± 0.03**	**0.12 ± 0.00**	**0.10 ± 0.00**	**0.10 ± 0.01**	**0.08 ± 0.01**
Sum, mol%		**100% ± 2%**	**49% ± 9%**	**33% ± 3%**	**22% ± 5%**	**21% ± 0%**	**18% ± 0%**	**17% ± 1%**	**15% ± 1%**