

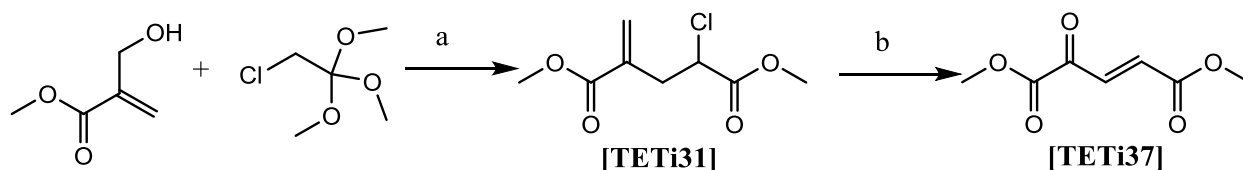
Synthesis of Molecules

General Procedures. All chemicals and reagents were used as received without further purification. Glassware was dried in an oven at 110°C and purged with a dry N₂ atmosphere prior to use. Unless otherwise mentioned, reactions were performed open to air. Reactions were monitored by TLC and visualized by a dual short/long wavelength UV lamp and or KMNO₄ or PMA stain. Flash column chromatography was performed using 230-400 mesh silica gel (Fisher). NMR spectra were recorded on a 500 MHz Bruker Ascend Avance III HD at room temperature. Chemical shifts for ¹H, and ¹³C NMR were reported as δ, part per million (ppm), and referenced to an internal deuterated solvent central line. Multiplicity and coupling constants (*J*) were calculated automatically on MestReNova 10.0, NMR processing software from Mestrelab Research. HRMS-ESI mass spectra were acquired on an Agilent Q-TOF. All reported compounds synthesized containing a stereocenter are racemic mixtures.

NOG (N-oxalylglycine) was purchased from Cayman Chemical. DMF (Dimethylfumarate) was purchased from Sigma-Aldrich. [TETi2M], 1,5-diethyl-2-methylidene pentanedioate was purchased from Enamine Ltd.

The syntheses of Dimethyl 2-chloro-4-methylenepentanedioate [TETi31] and Dimethyl (E)-4-oxopent-2-enedioate [TETi37] follow scheme I.

Scheme I



Reagent and condition (Scheme I): (a) Toluene, propionic acid, reflux, 4 h, 90% yield;(b) RuO₄, NaIO₄,CCl₄, CH₃CN, 20 °C, 4 h, 40% yield.

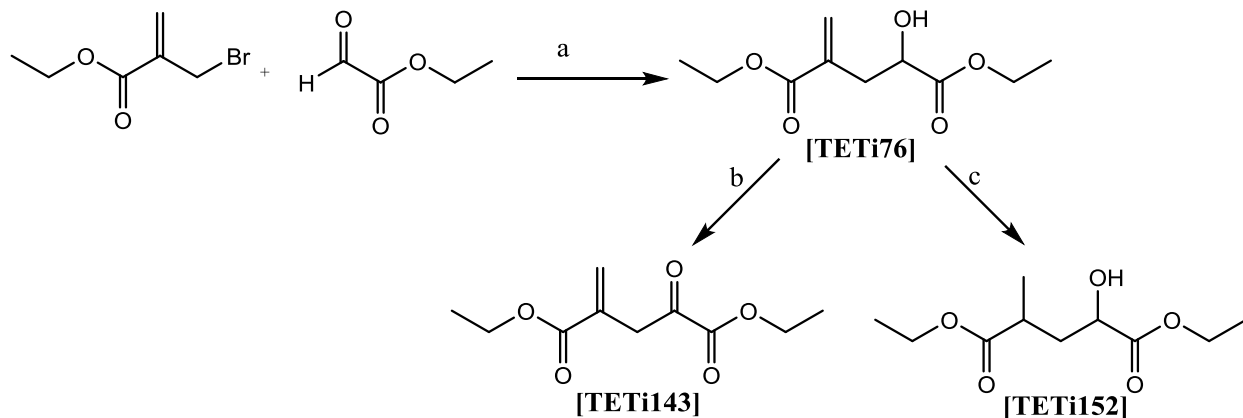
Synthesis of Dimethyl 2-chloro-4-methylenepentanedioate [TETi31]: Methyl 2-(hydroxymethyl)acrylate (5.0g, 43.06 mmol, d= 1.13 g/mL) and 2-chloro-1,1,1-trimethoxyethane (8.0 mL, 51.67 mmol, d= 1.15g/mL) were added together in a 200 mL round bottom flask with 100 mL anhydrous toluene and catalytic amount of propionic acid (1 mL, 4.31 mmol). The reaction

mixture was heated under reflux for 4 h. The completion of reaction was monitored by TLC. Methanol was slowly removed by azeotropic distillation with toluene (3x50 mL). The solution was then concentrated under reduced pressure. Flash silica gel chromatography (eluent ethyl acetate/hexanes) gave the desired compound as a yellow oil, yield 85%. ¹H NMR (500 MHz, CDCl₃) δ 6.34 (d, *J* = 1.3 Hz, 1H), 5.78 – 5.70 (m, 1H), 4.56 (ddd, *J* = 8.0, 6.4, 1.5 Hz, 1H), 3.80 – 3.74 (m, 6H), 3.08 (ddd, *J* = 14.2, 6.3, 1.4 Hz, 1H), 2.90 – 2.79 (m, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 169.78, 166.65, 134.71, 130.12, 54.96, 53.09, 52.29, 37.93

Dimethyl (E)-4-oxopent-2-enedioate [TETi37]: (1.0 g, 4.84 mmol) of [TETi31] was dissolved in 20 mL of CCl₄ and CH₃CN (1:1) in 100 mL round bottom flask. An aqueous solution of NaIO₄ (4.14 g, 19.36 mmol) was added to the reaction mixture followed by the drop wise addition of an aqueous solution of RuO₄ (0.5%, 10 mL, 0.48 mmol). The reaction mixture turned black and completion of the reaction was monitored by TLC. The reaction mixture was filtered through celite and the filtrate evaporated under reduced pressure. The residue was dissolved in dichloromethane (100 mL) and washed sequentially with sodium bisulfite (10% aqueous, w/v 50 ml) and followed by saturated brine solution (2x50 mL). The organic layer was separated, dried over the sodium sulfate, filtered and solvent evaporated under reduce pressure. The crude was redissolved in dichloromethane and purified by silica gel flash chromatography (eluted 5% Ethyl acetate: hexane) and afforded a yellow crystalline solid, yield 40%. ¹H NMR (500 MHz, CDCl₃) δ 7.63 (d, *J* = 16.1 Hz, 1H), 6.98 (d, *J* = 15.9 Hz, 1H), 3.94 (s, 3H), 3.85 (s, 3H). ¹³C NMR (126 MHz, CDCl₃) δ 182.29, 165.22, 161.09, 135.58, 134.22, 53.51, 52.74.

Several of our TETi compounds were prepared from commercially available Ethyl 2-(bromomethyl)acrylate via Barbier reaction with appropriate aldehyde or ketone. Other derivatives followed by either an oxidation or reduction protocol. The syntheses of Diethyl 2-hydroxy-4-methylenepentanedioate [TETi76], Diethyl 2-methylene-4-oxopentanedioate [TETi143], and Diethyl 2-hydroxy-4-methylpentanedioate [TETi152] follow scheme II.

Scheme II



Reagent and condition (scheme 2): (a) Zinc, acetic acid, CH₃CN, 20 °C, 12 h, 95% Yield; (b) Dess-Martin reagent, dichloromethane, 20 °C, 4 h, Yield 60%; (c) Pd/C, Ethanol, 30 Psi, 20 °C, 2 h, 90 % Yield.

General synthesis method for the Barbier Reaction: To a suspension of Zinc powder (2.5 equiv. mole ratio) in 50 mL dry acetonitrile was added acetic acid (1.5 equiv. mole ratio). Ethyl 2-(bromomethyl)acrylate (1.0 equiv. mole ratio) was added to the reaction mixture followed by the drop wise addition of respective aldehyde (1.1 equiv. mole ratio), ethyl glyoxylate for the synthesis of [TETi76]. The reaction mixture was stirred for 6-12 h with monitoring for completion of the reaction by TLC. The reaction mixture was filtered through celite and the filtrate evaporated under reduced pressure. The crude residue was redissolved in 100 ml of ethyl acetate and washed with 50 ml of 10% aqueous ammonium chloride solution. The organic layer was washed with once with brine solution (20%, 50 mL), separated, dried over sodium sulfate, filtered, and solvent removed under reduced pressure. The crude product was purified by flash silica gel column chromatography (eluent: 7-12% Ethyl acetate: hexanes).

Diethyl 2-hydroxy-4-methylenepentanedioate [TETi76]: Light yellow oil, obtained in 95% yield. ¹H NMR (500 MHz, CDCl₃) δ 6.29 (d, J = 1.3 Hz, 1H), 5.72 (q, J = 1.1 Hz, 1H), 4.42 – 4.37 (m, 1H), 4.23 (qd, J = 7.1, 5.5 Hz, 4H), 3.13 (d, J = 5.5 Hz, 1H), 2.86 (ddd, J = 14.2, 4.5, 1.2 Hz, 1H), 2.64 (ddd, J = 14.3, 8.1, 1.0 Hz, 1H), 1.31 (q, J = 7.2 Hz, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 174.26, 167.12, 135.83, 135.72, 128.48, 69.64, 61.73, 61.06, 37.25, 14.18.

General synthesis method for the Dess-Martin oxidation: The alcohol derivative (1.0 mole equiv.) was dissolved in dichloromethane (50 mL) in 100 mL round bottom flask and Dess-Martin-Periodinane (1.5mole equiv.) was added to the solution. The heterogeneous mixture was stirred at 20 °C for 4 h. The reaction were monitored by TLC for the complete consumption of hydroxyl derivative. The solvent were evaporated under reduced pressure and residue redissolved in ethyl acetate (100 ml). The organic layer was

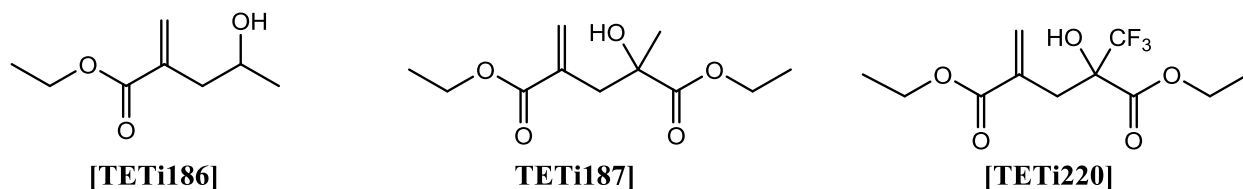
washed with aqueous 1:1 NaHCO₃ (10%) and Na₂S₂O₃ (10%) solution (2x 50 ml each). The organic layer was separated, washed with brine solution (50 mL), dried over the sodium sulfate, filtered and the solvent evaporated under reduced pressure. The crude was purified by flash silica gel chromatography (eluent: ethyl acetate/hexanes).

Diethyl 2-methylene-4-oxopentanedioate [TETi143]: White solid, yield 80%. ¹H NMR (500 MHz, CDCl₃) δ 6.39 (s, 1H), 5.70 (s, 1H), 4.33 (q, J = 7.1 Hz, 2H), 4.19 (q, J = 7.2 Hz, 2H), 3.82 (s, 2H), 1.36 (t, J = 7.2 Hz, 3H), 1.27 (t, J = 7.2 Hz, 3H).

General synthesis method for Pd/C hydrogenation reduction: The olefin/methylene derivative (1 mole equiv.) was dissolved in 50 ml absolute ethanol and added to a 250 ml Parr hydrogenation bottle. Palladium hydroxide (20 wt % Pd (dry basis) on carbon (Aldrich, 212111, 0.05 mole equiv.). The reaction bottle was deoxygenated and flushed with N₂ (3x) prior to adding Hydrogen gas. The H₂ pressure was kept at 30-45 Psi and reaction shaken for 2 h until completion of the reaction. The reaction mixture were filtered through celite to remove catalyst and the filtrate evaporated under reduced pressure. The crude obtained compound was purified by flash silica gel chromatography (eluent: ethyl acetate/hexanes).

Diethyl 2-hydroxy-4-methylpentanedioate [TETi152]: Colorless oil, yield 60%. ¹H NMR (500 MHz, CDCl₃) δ 4.24 (tt, J = 7.6, 4.2 Hz, 2H), 4.21 – 4.17 (m, 1H), 4.17 – 4.10 (m, 2H), 2.74 (td, J = 14.2, 13.1, 5.5 Hz, 1H), 2.27 – 1.99 (m, 1H), 1.76 (dddd, J = 99.9, 13.8, 8.4, 4.0 Hz, 1H), 1.31 (t, J = 7.1 Hz, 3H), 1.28 – 1.25 (m, 3H), 1.22 (t, J = 6.8 Hz, 3H).

Figure I



[TETi186], [TETi187] and [TETi220] were prepared via the Barbier reaction using Ethyl 2-(bromomethyl)acrylate with acetaldehyde, ethyl pyruvate, or ethyl 3,3,3-trifluoropyruvate, respectively.

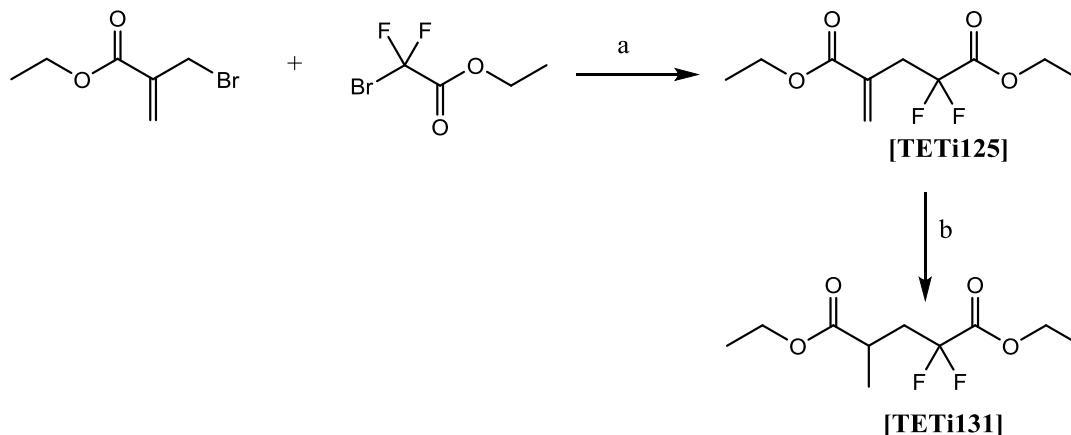
Ethyl 4-hydroxy-2-methylenepentanoate [TETi186]: ^1H NMR (500 MHz, CDCl_3) δ 6.26 (d, $J = 1.4$ Hz, 1H), 5.66 (t, $J = 1.3$ Hz, 1H), 4.23 (q, $J = 7.2$ Hz, 2H), 4.00 – 3.94 (m, 1H), 2.59 – 2.53 (m, 1H), 2.42 – 2.33 (m, 1H), 1.32 (td, $J = 7.1, 1.1$ Hz, 3H), 1.22 (dd, $J = 6.3, 1.1$ Hz, 3H). ^{13}C NMR (126 MHz, CDCl_3) δ 167.83, 137.82, 127.77, 66.98, 61.17, 42.14, 23.22, 14.31.

Diethyl 2-hydroxy-2-methyl-4-methylenepentanedioate [TETi187]: Colorless oil, yield 80%. ^1H NMR (500 MHz, CDCl_3) δ 6.27 (d, $J = 1.5$ Hz, 1H), 5.68 (d, $J = 1.4$ Hz, 1H), 4.27 – 4.15 (m, 4H), 3.62 (s, 1H), 2.85 (dd, $J = 13.9, 0.9$ Hz, 1H), 2.68 (dd, $J = 13.9, 1.0$ Hz, 1H), 1.44 (s, 3H), 1.30 (td, $J = 7.1, 5.9$ Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 175.89, 167.72, 135.81, 128.81, 122.89, 74.21, 61.74, 61.10, 41.83, 25.55, 14.14.

Diethyl 2-hydroxy-4-methylene-2-(trifluoromethyl)pentanedioate [TETi220]: Colorless oil co, yield 60%. ^1H NMR (500 MHz, CDCl_3) δ 6.32 (d, $J = 1.2$ Hz, 1H), 5.82 – 5.76 (m, 1H), 4.39 (s, 1H), 4.37 – 4.31 (m, 1H), 4.31 – 4.26 (m, 1H), 4.22 (tq, $J = 7.1, 3.6$ Hz, 2H), 3.12 (d, $J = 14.3$ Hz, 1H), 2.98 – 2.90 (m, 1H), 1.32 (dt, $J = 12.2, 7.1$ Hz, 6H). ^{13}C NMR (126 MHz, CDCl_3) δ 168.74, 167.40, 133.39, 130.23, 124.48, 122.20, 63.55, 61.48, 33.76, 33.75, 14.10, 13.91.

The syntheses of Diethyl 2,2-difluoro-4-methylenepentanedioate [TETi125] and [TETi131] follow **scheme III** and were achieved by coupling of ethyl 2-(bromomethyl)acrylate with ethyl bromodifluoroacetate in the presence of activated zinc powder and CuCN to provide [TETi125] as previously reported by Tomoko et al., J. Fluorine Chem. 1998,88, 99-103. [TETi131] was achieved by our general Pd catalyzed hydrogenation procedure.

Scheme III

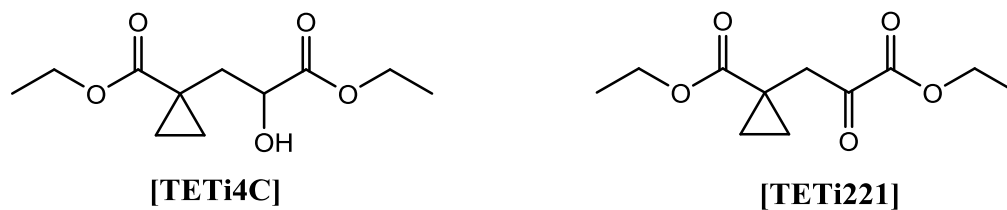


Reagent and condition (Scheme III): (a) Zn, CuCN, BrCF₂CO₂Et, THF, 48 h, 20 °C, 20% yield;
(b) Pd/C, Ethanol, 30 Psi, 20 °C, 2 h, 90 % Yield

Diethyl 2,2-difluoro-4-methylenepentanedioate [TETi125]: Colorless oil, yield 20%. ¹H NMR (500 MHz, CDCl₃) δ 4.32 (q, *J* = 7.2 Hz, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 2.82 – 2.74 (m, 1H), 2.64 (dtd, *J* = 22.1, 14.4, 7.9 Hz, 1H), 2.11 (dtd, *J* = 20.1, 14.4, 5.2 Hz, 1H), 1.36 (d, *J* = 7.2 Hz, 3H), 1.26 (t, *J* = 6.8 Hz, 6H). ¹³C NMR (126 MHz, CDCl₃) δ 165.96, 139.84, 131.18, 130.82, 125.44, 125.24, 114.56, 69.88, 69.01, 62.96, 61.28, 60.72, 60.65, 36.39, 36.20, 36.00, 33.71, 30.87, 29.68, 28.27, 14.21, 14.12, 13.90.

Diethyl 2,2-difluoro-4-methylpentanedioate [TETi131]: Colorless oil, yield 90%. ¹H NMR (500 MHz, CDCl₃) δ 4.32 (q, *J* = 7.2 Hz, 2H), 4.15 (q, *J* = 7.2 Hz, 2H), 2.82 – 2.74 (m, 1H), 2.64 (dtd, *J* = 22.1, 14.4, 7.9 Hz, 1H), 2.11 (dtd, *J* = 20.1, 14.4, 5.2 Hz, 1H), 1.36 (d, *J* = 7.2 Hz, 3H), 1.26 (t, *J* = 6.8 Hz, 6H).

Figure II

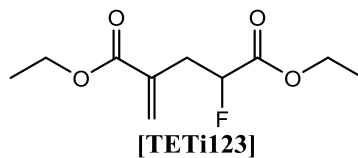


[TETi4C] was synthesized according to previously published literature.¹ Dess Martin oxidation of **[TETi4C]** according to our described general procedure gave **[TETi221]**.

Ethyl 1-(3-ethoxy-2-hydroxy-3-oxopropyl)cyclopropane-1-carboxylate [TETi4C]: ¹H NMR (500 MHz, CDCl₃) δ 4.42 (dt, *J* = 9.3, 4.5 Hz, 1H), 4.23 (dtd, *J* = 21.1, 7.1, 3.6 Hz, 2H), 4.12 (q, *J* = 7.1 Hz, 2H), 3.39 (d, *J* = 6.2 Hz, 1H), 2.09 (dd, *J* = 14.7, 4.2 Hz, 1H), 1.90 (dd, *J* = 14.7, 8.7 Hz, 1H), 1.30 (t, *J* = 7.1 Hz, 3H), 1.24 (t, *J* = 7.2 Hz, 3H), 0.91 (ddd, *J* = 9.7, 6.6, 3.8 Hz, 1H), 0.79 – 0.72 (m, 1H). ¹³C NMR (126 MHz, CDCl₃) δ 175.64, 174.94, 70.36, 61.64, 61.01, 39.12, 21.31, 16.20, 15.35, 14.27.

Ethyl 1-(3-ethoxy-2,3-dioxopropyl)cyclopropane-1-carboxylate [TETi221]: White solid, yield 80%. ¹H NMR (500 MHz, CDCl₃) δ 6.39 (s, 1H), 5.70 (s, 1H), 4.33 (q, *J* = 7.1 Hz, 2H), 4.19 (q, *J* = 7.2 Hz, 2H), 3.82 (s, 2H), 1.36 (t, *J* = 7.2 Hz, 3H), 1.27 (t, *J* = 7.2 Hz, 3H).

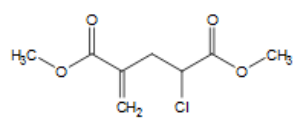
Figure III



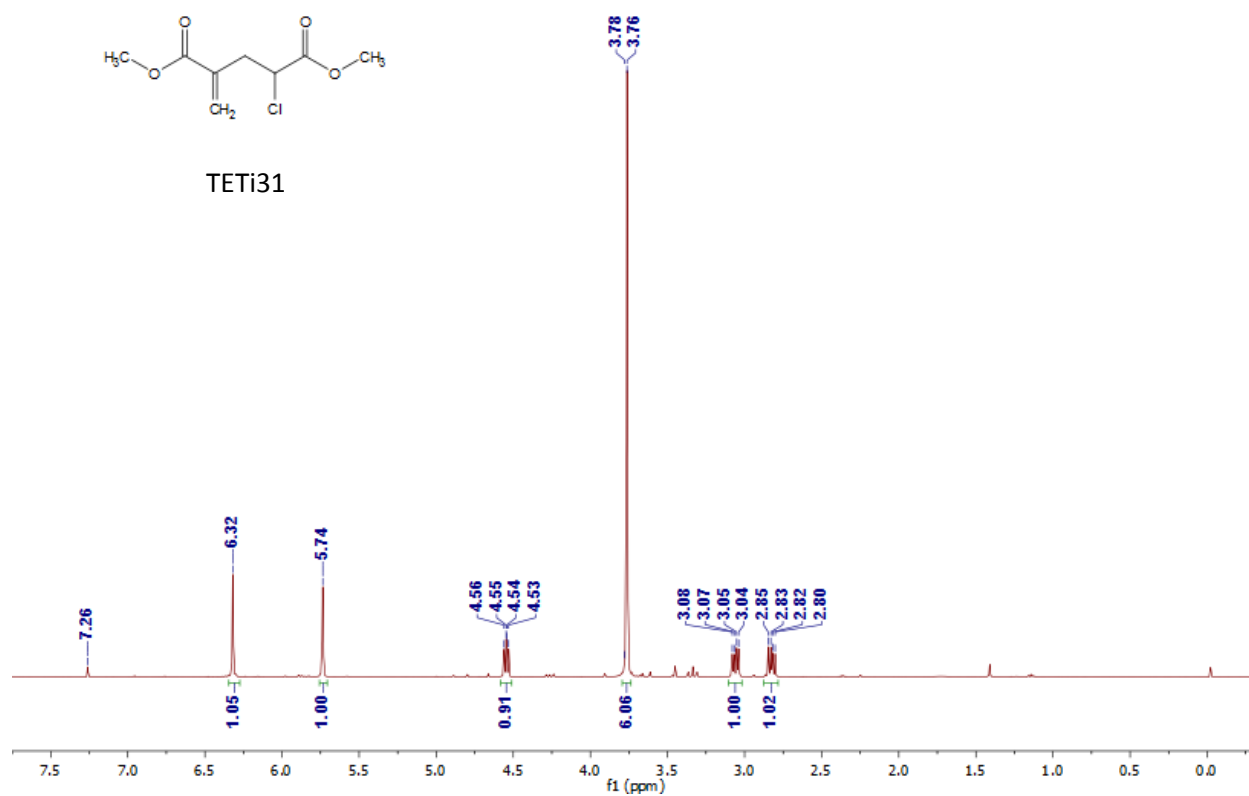
[TETi123] was prepared as described.²

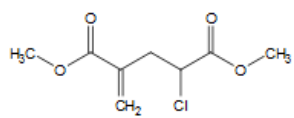
Spectra:

1. ¹H and ¹³C Spectra for all the Intermediate and final compounds Synthesized.
2. HRMS Spectra for the all the new compounds (**TETi**).

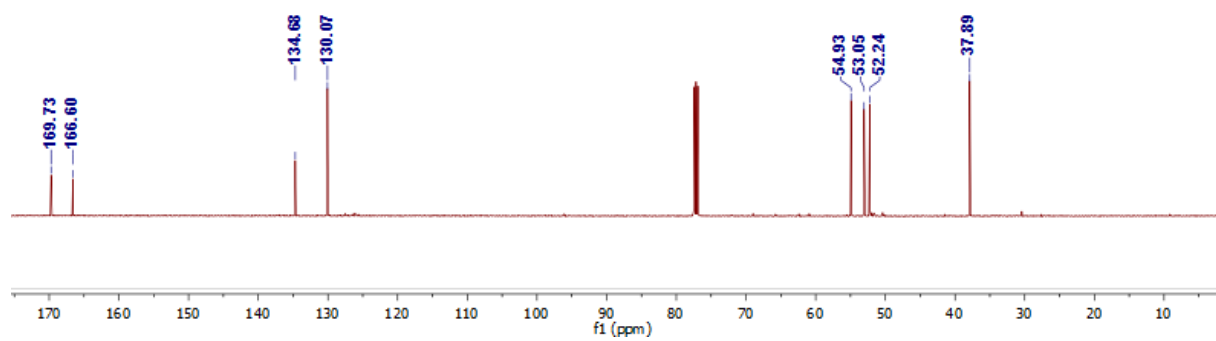


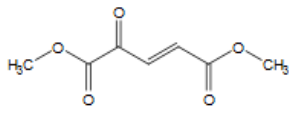
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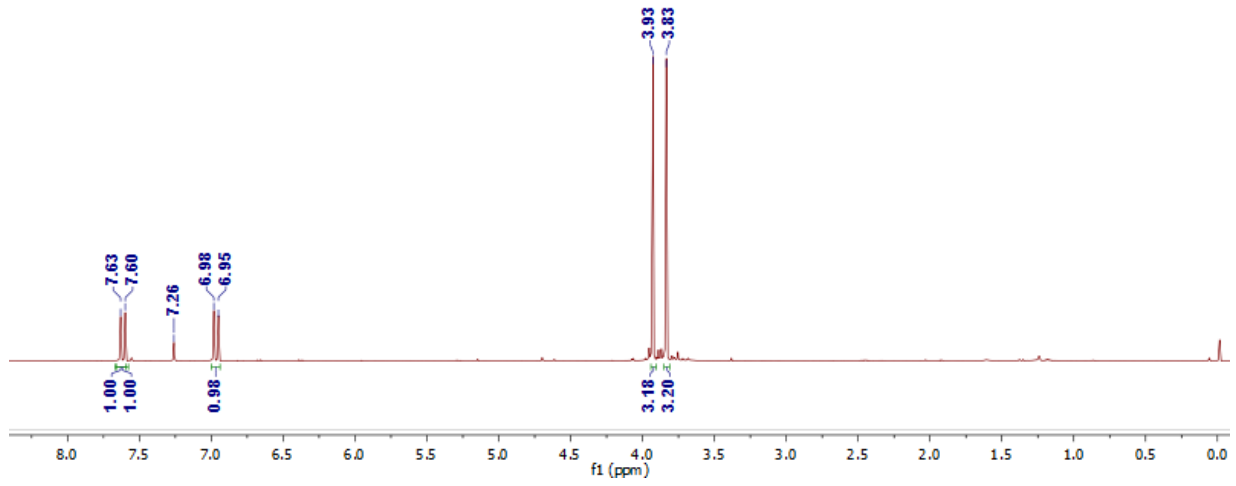


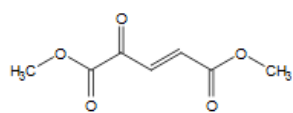
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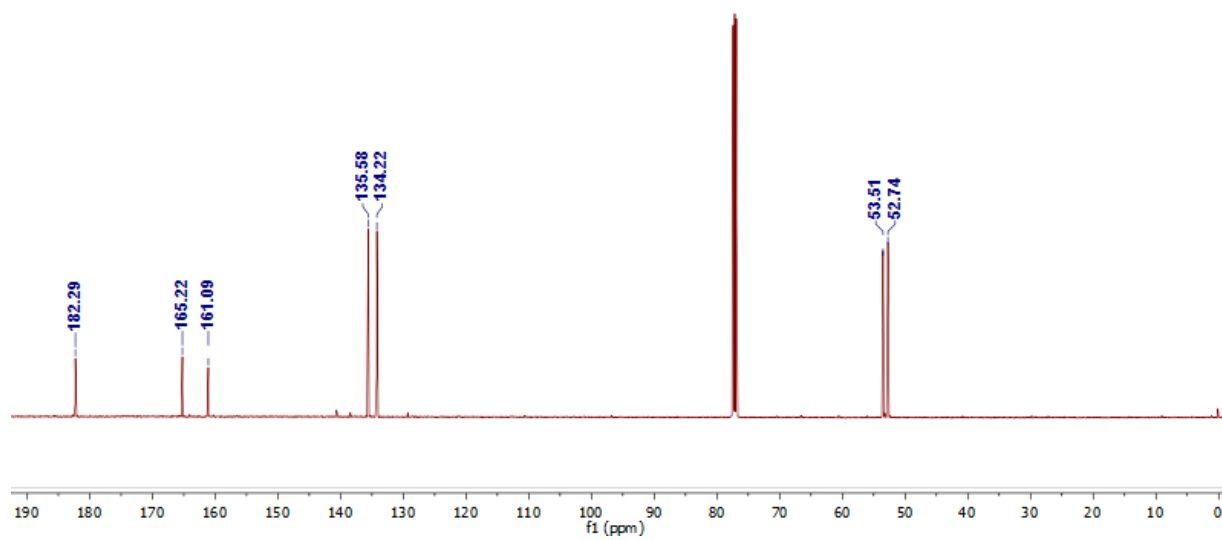


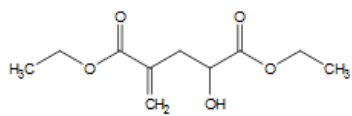
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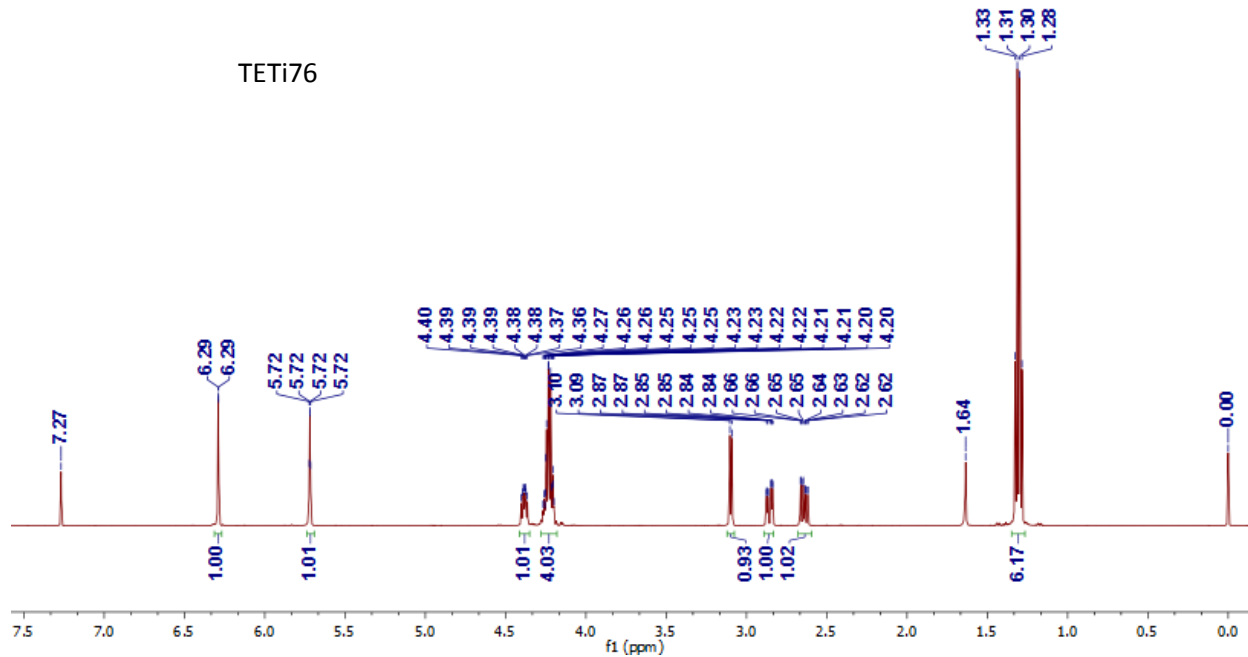


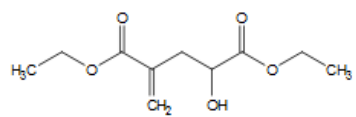
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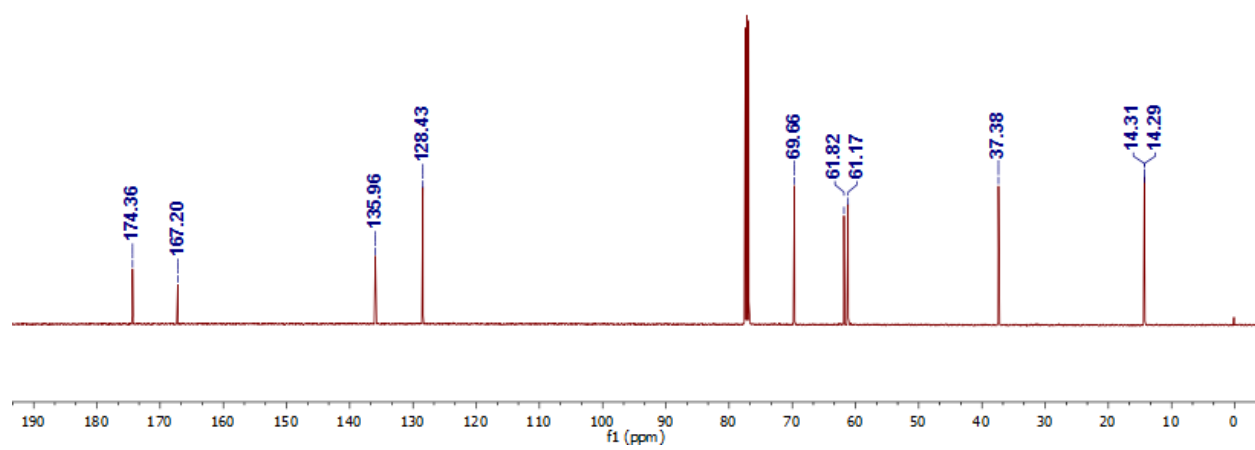


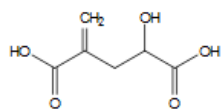
TETI76



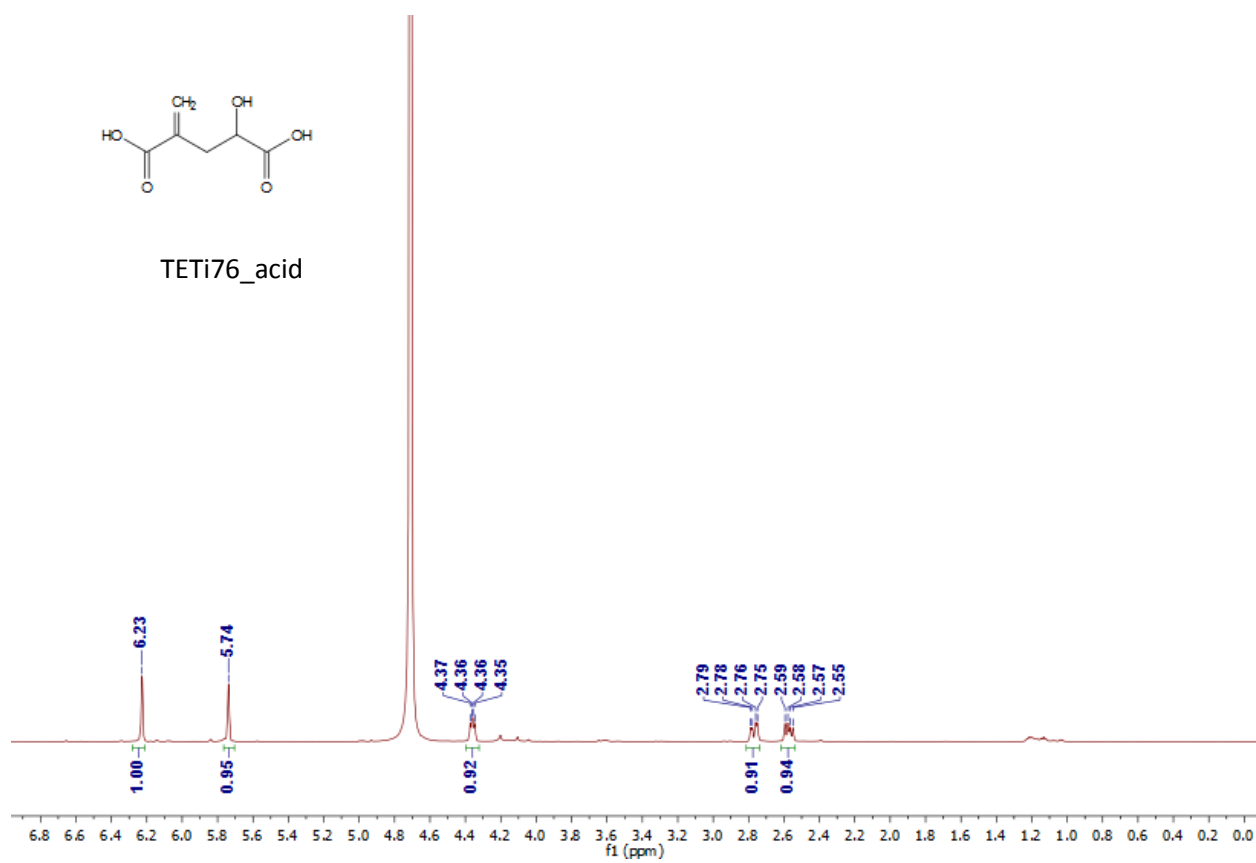


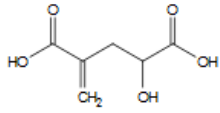
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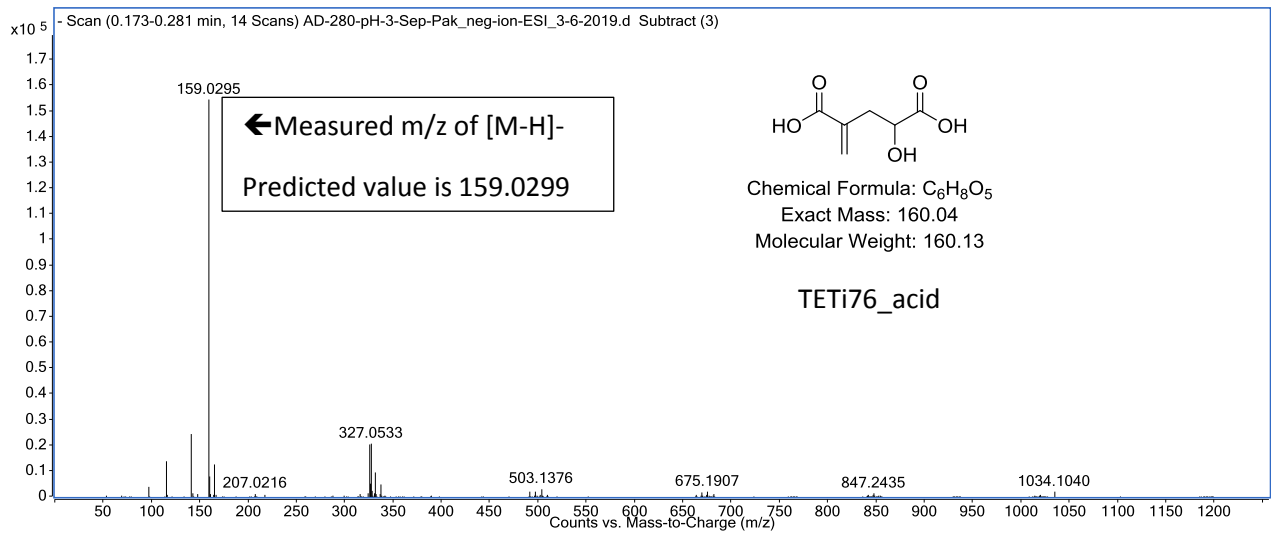
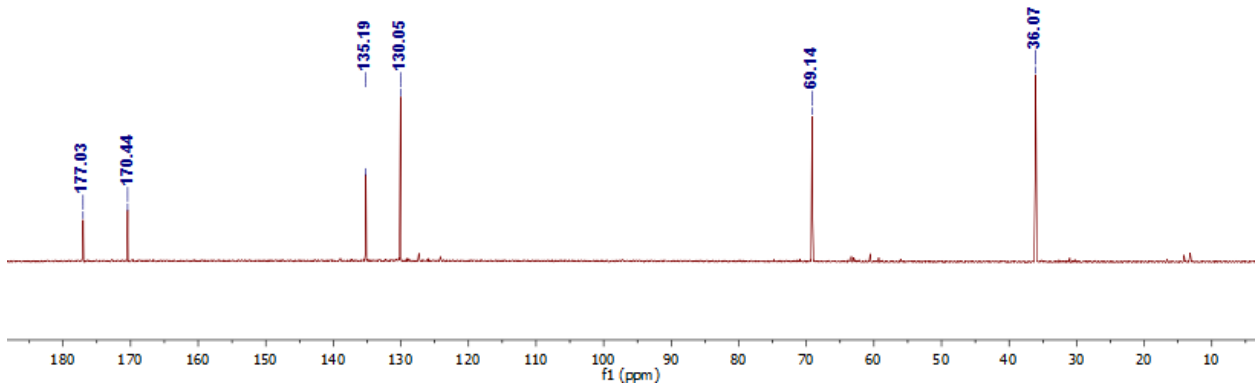


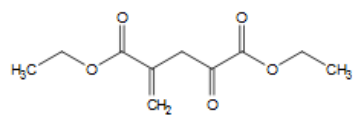
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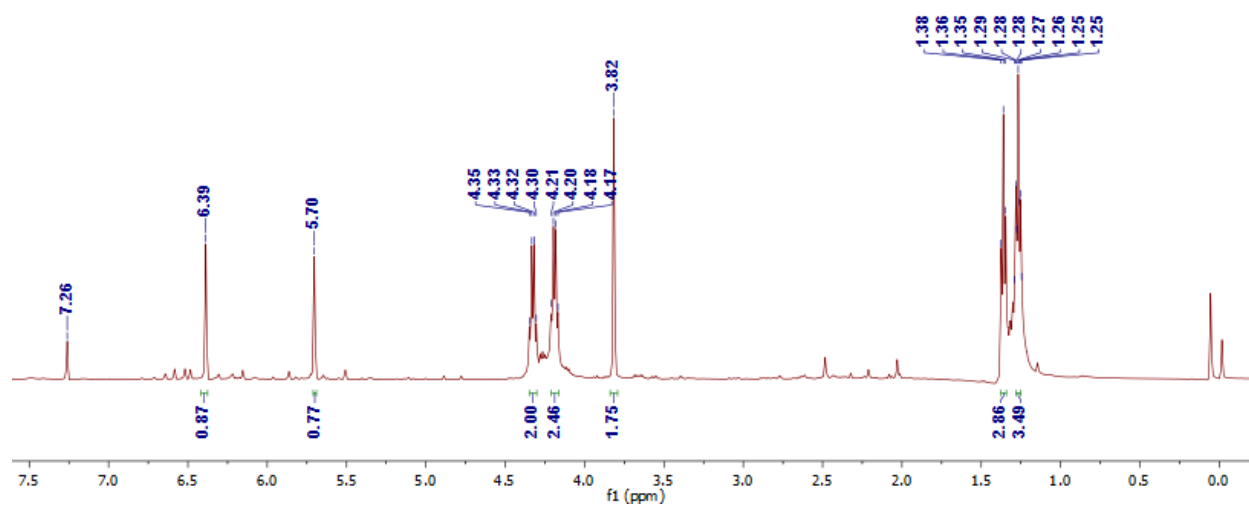


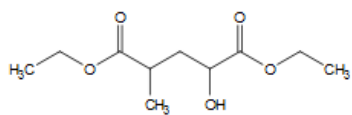
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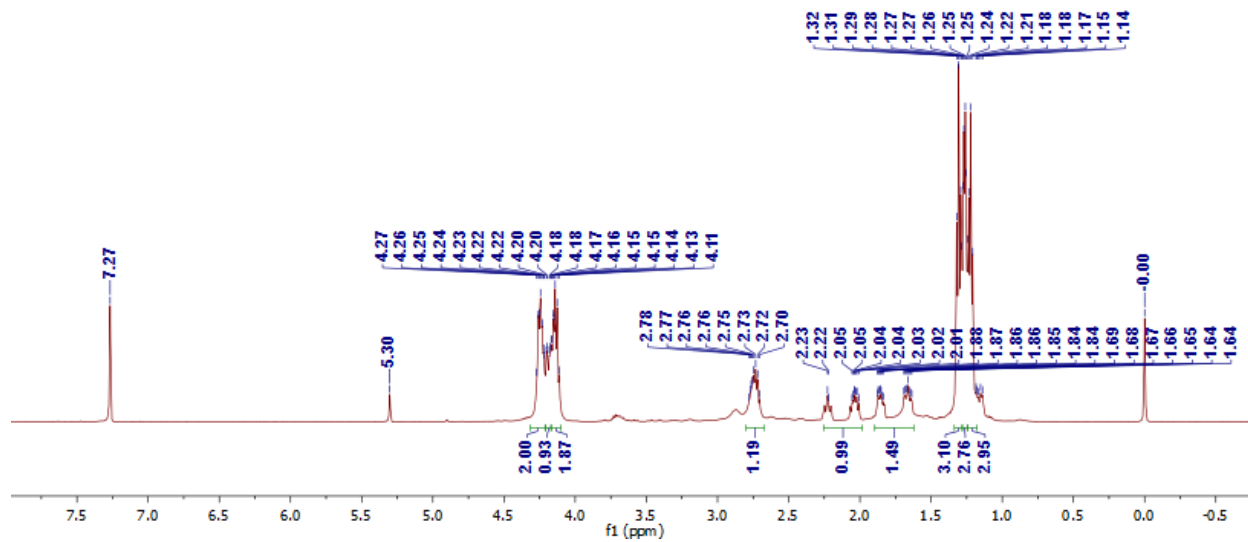


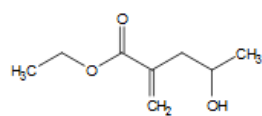
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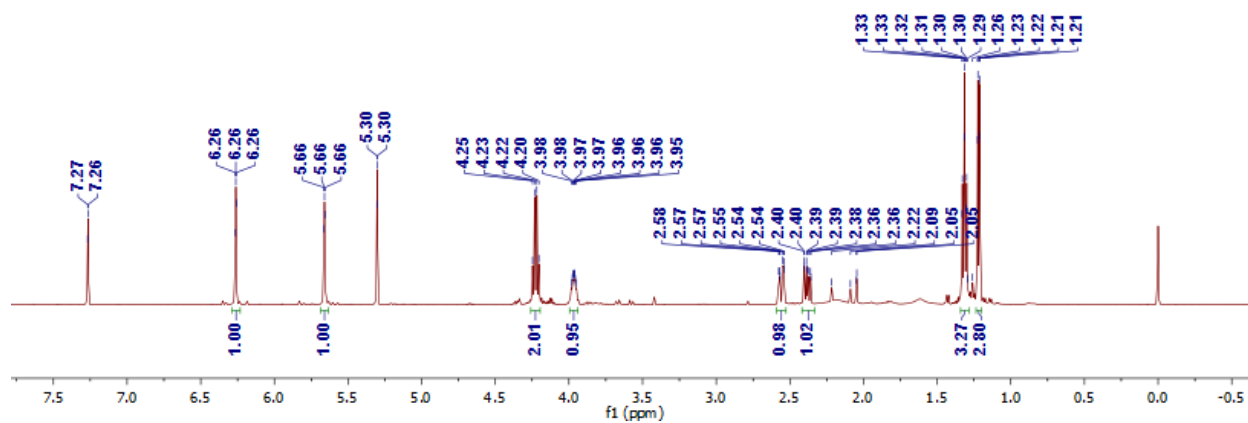


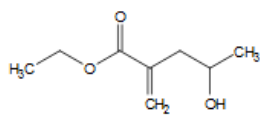
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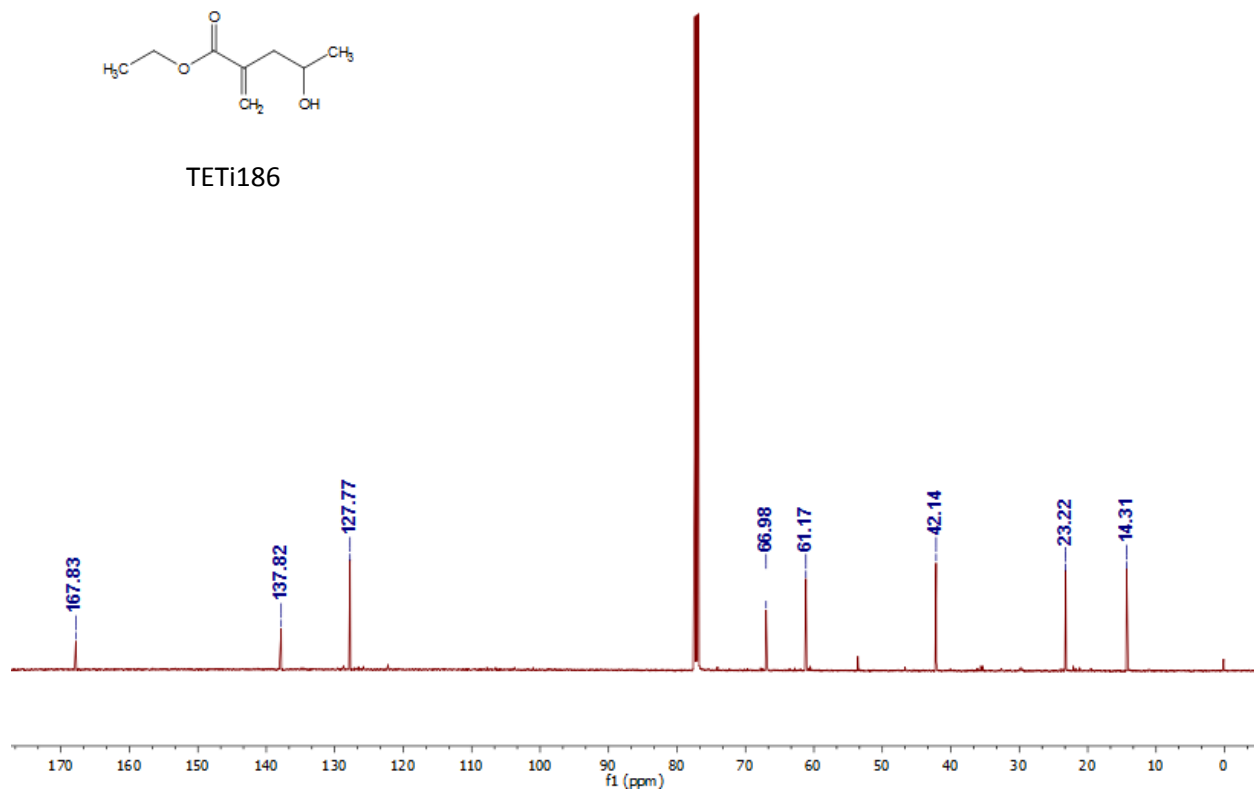


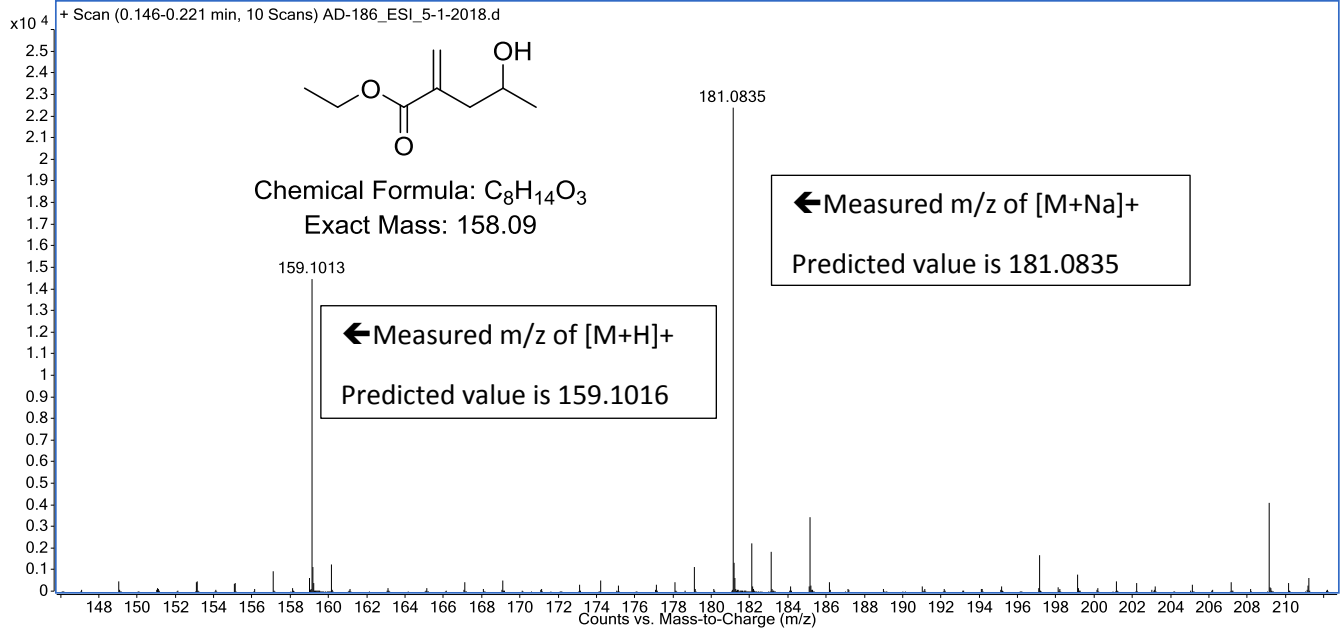
TETi186

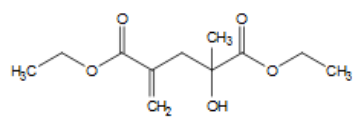




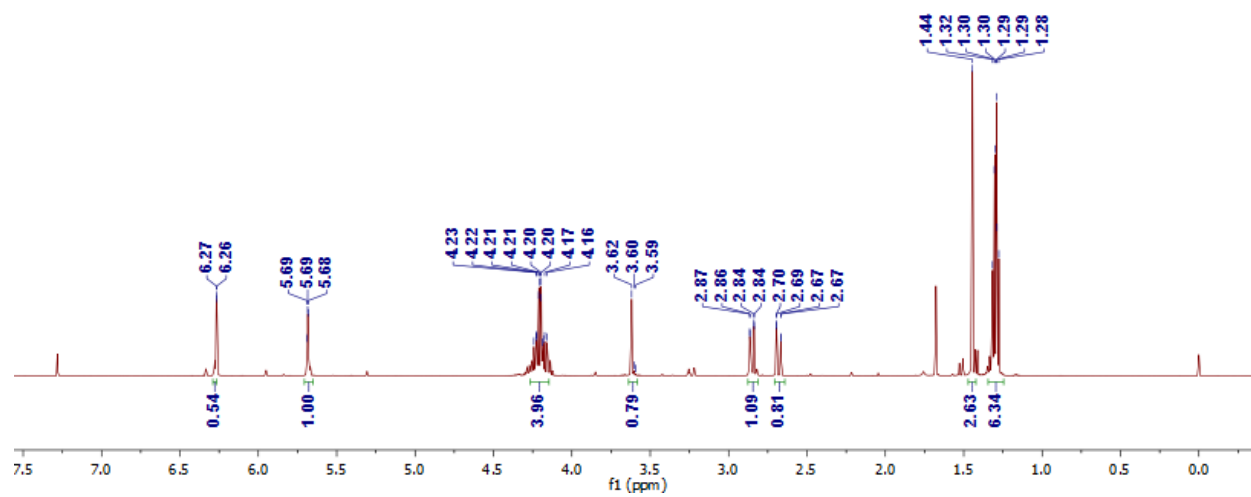
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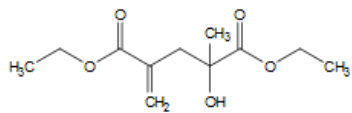




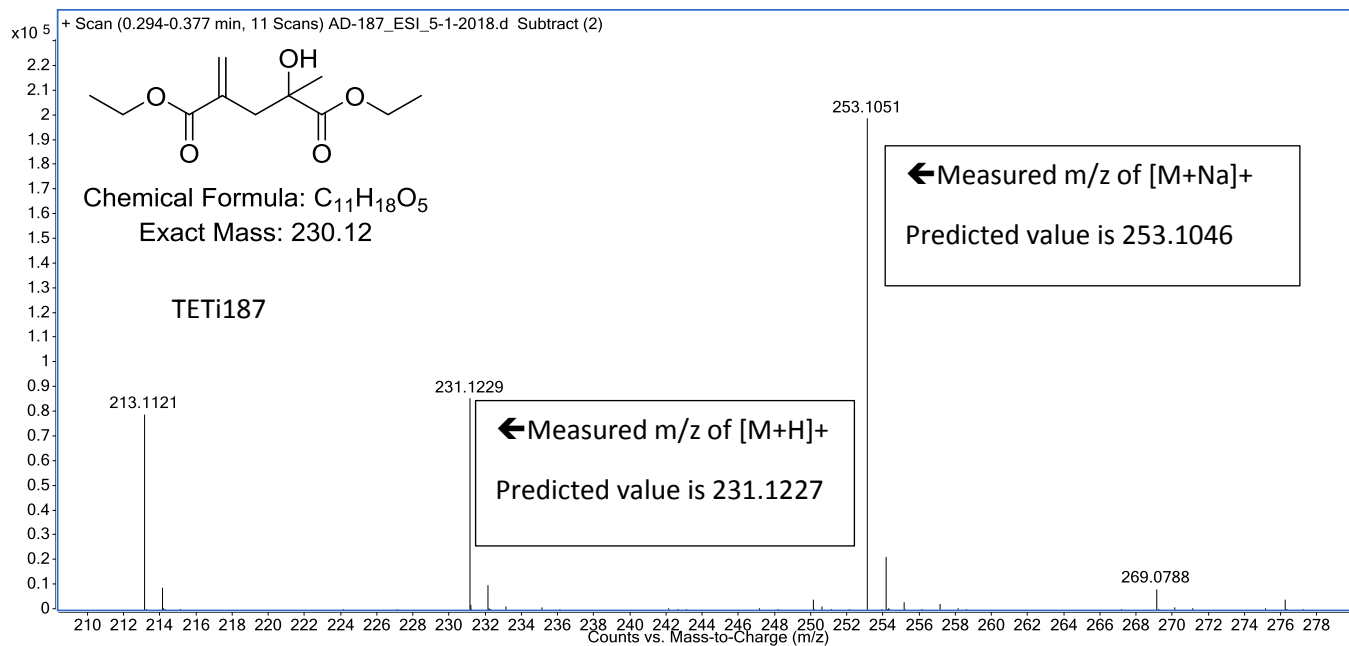
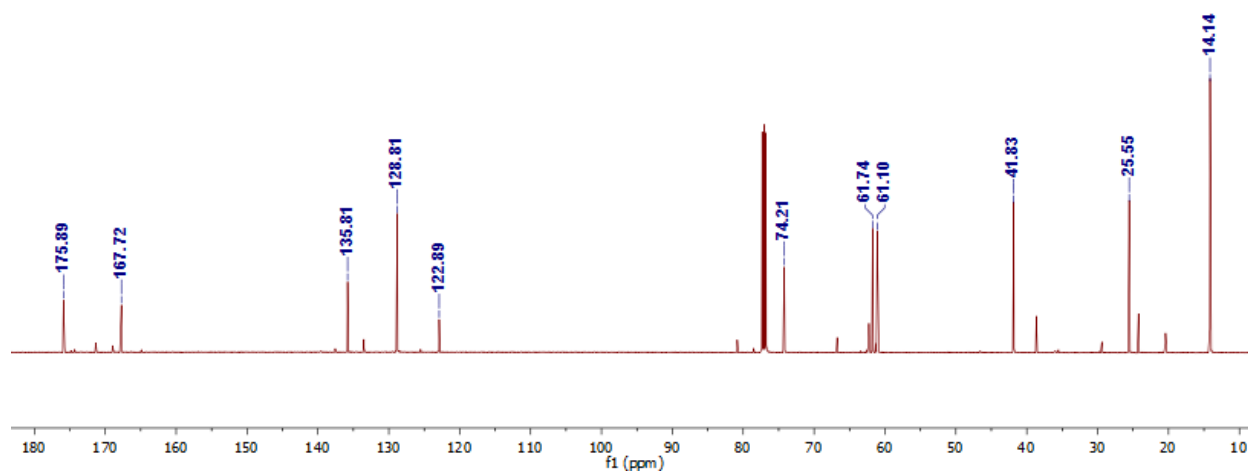
TETi187



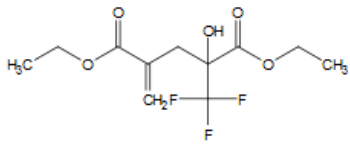
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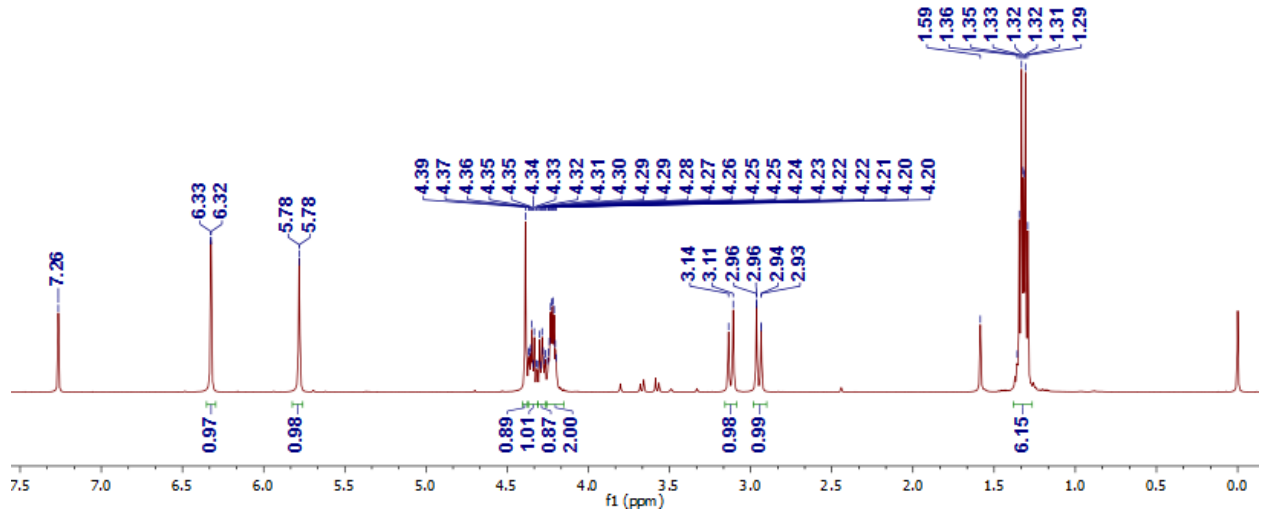
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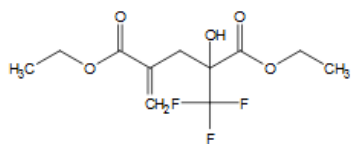
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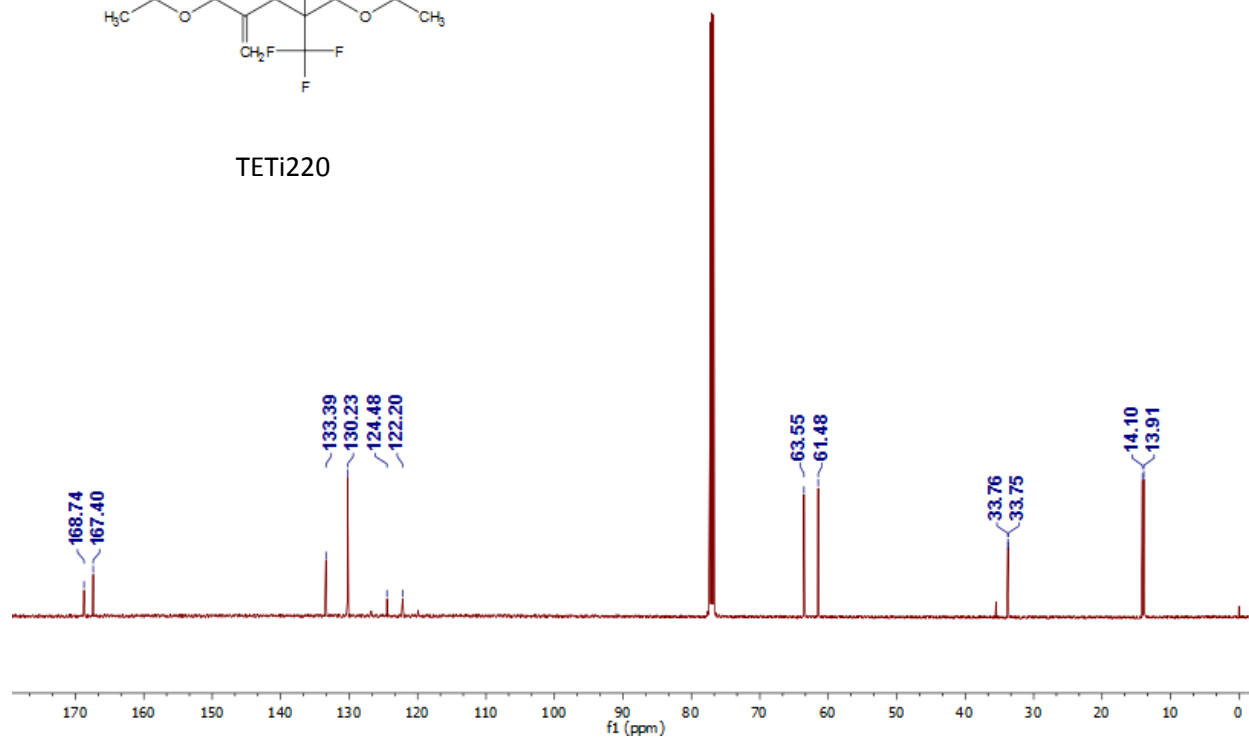
TETi220

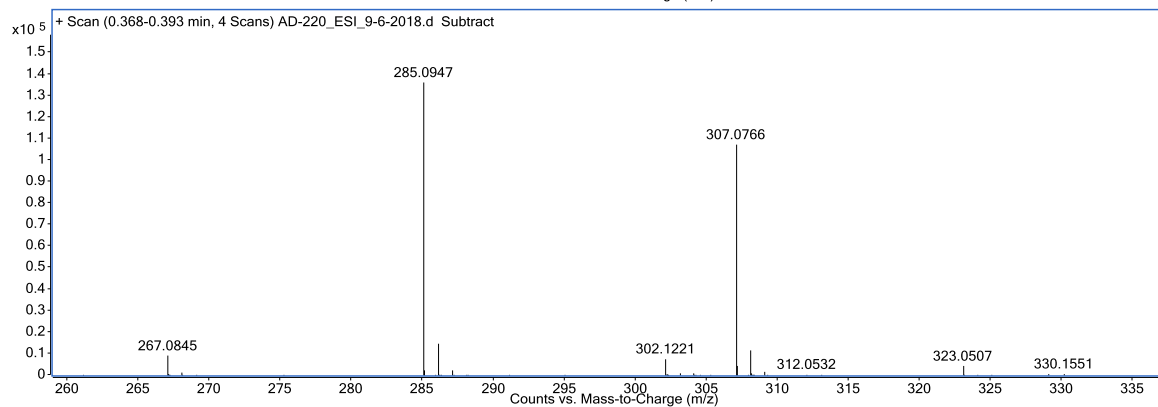
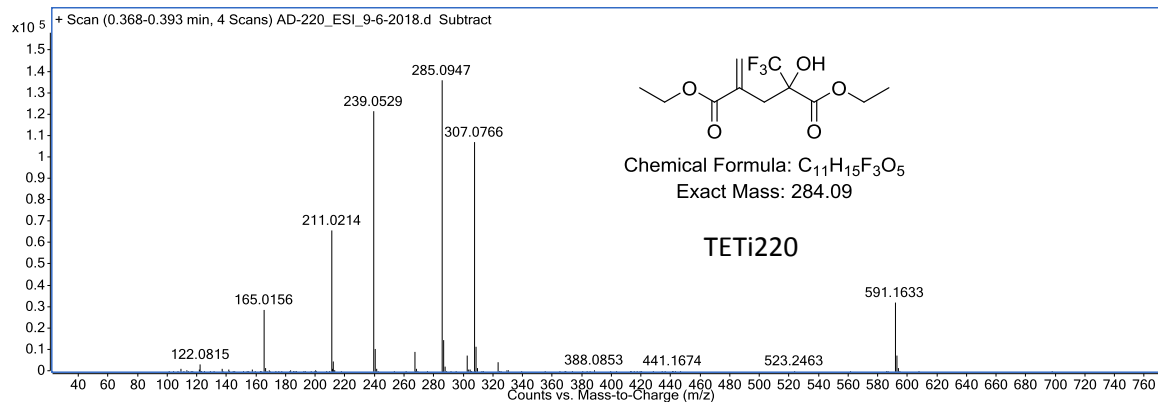


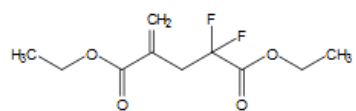
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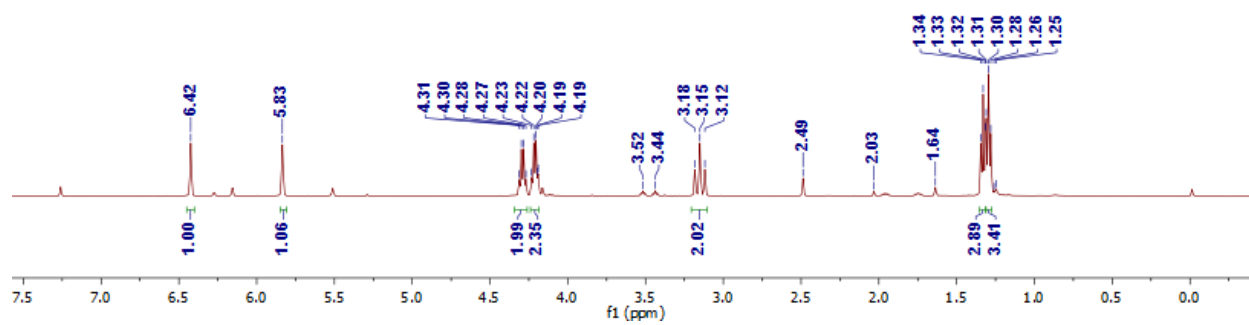
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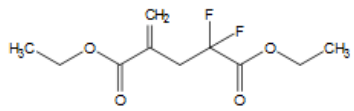




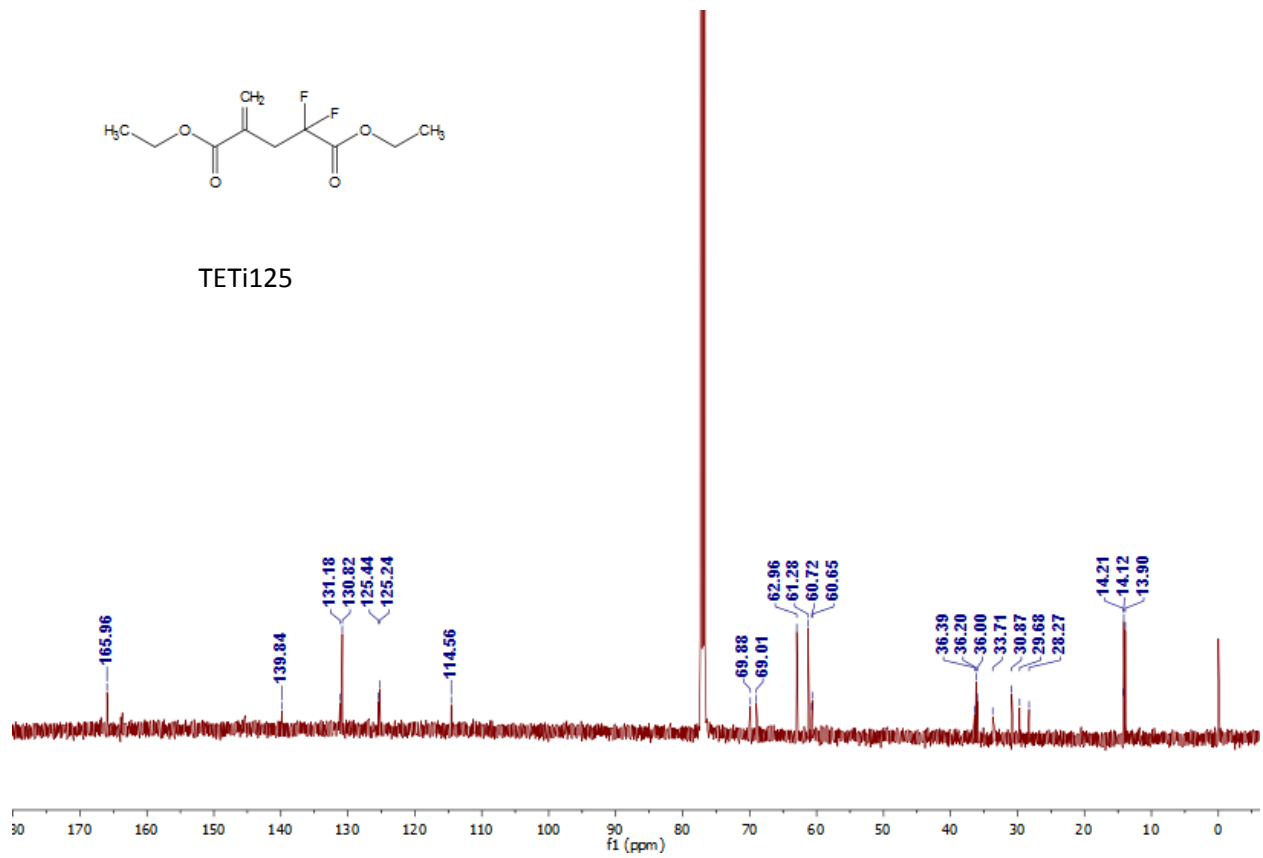


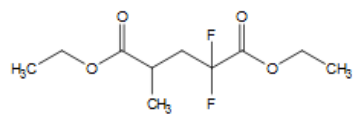
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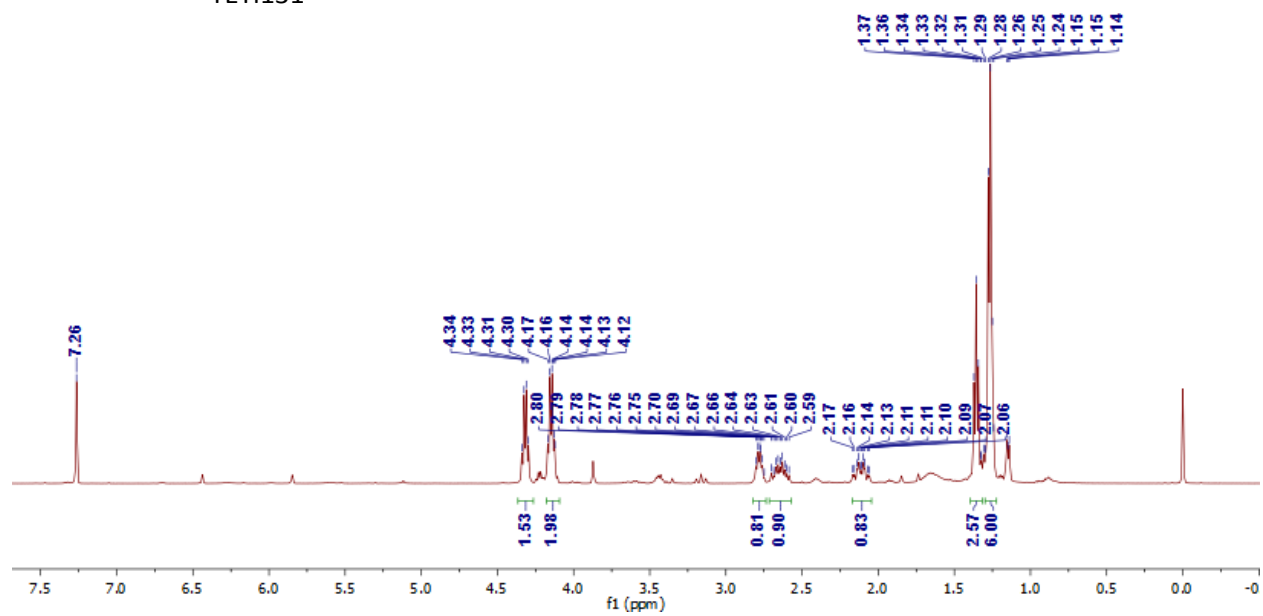


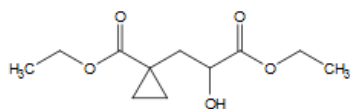
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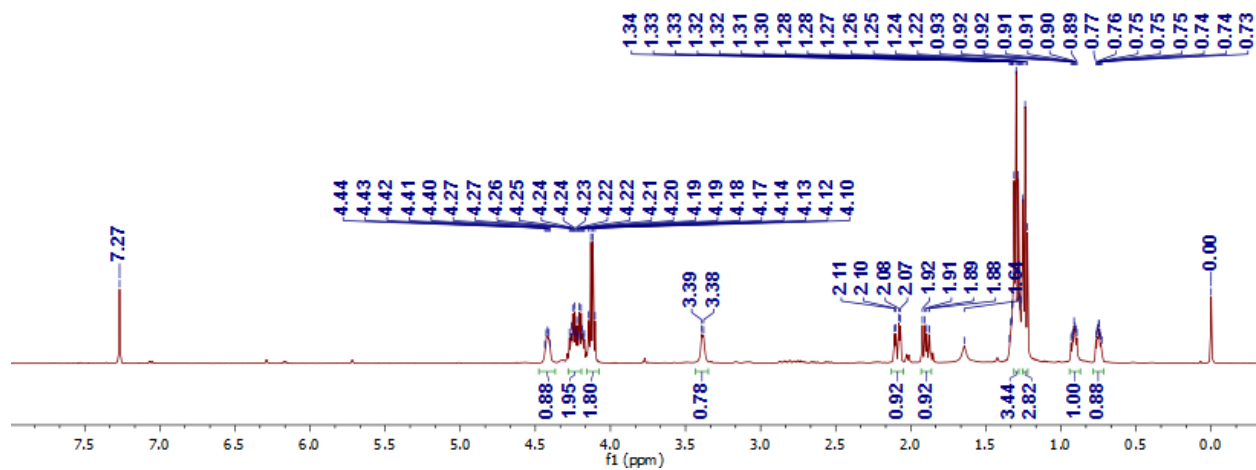


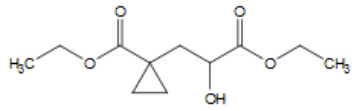
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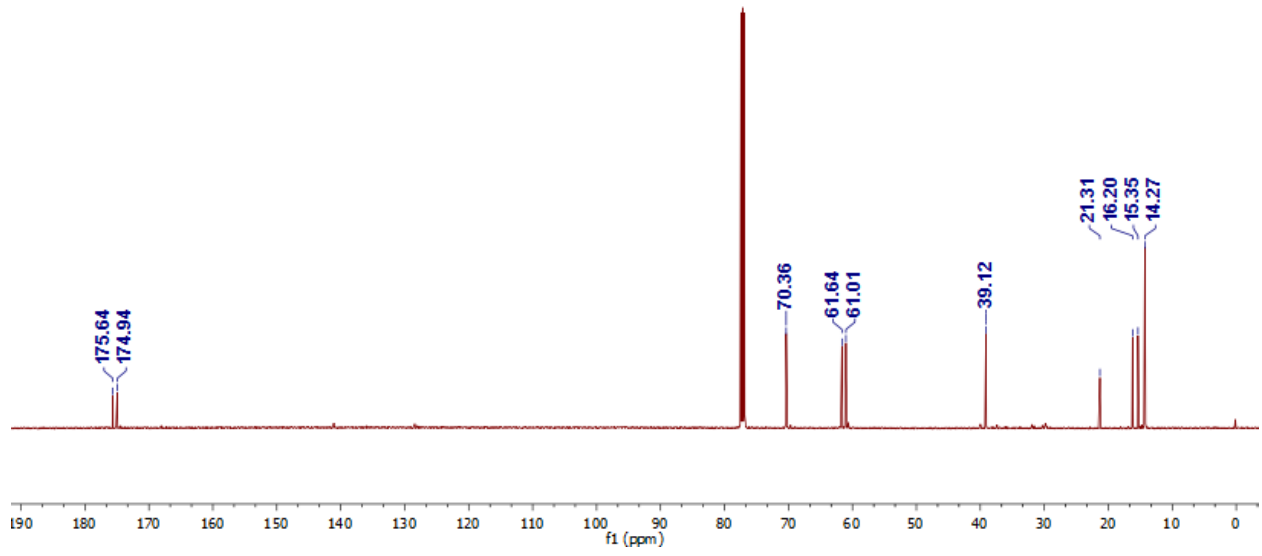


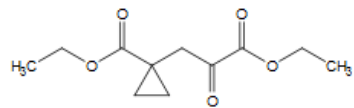
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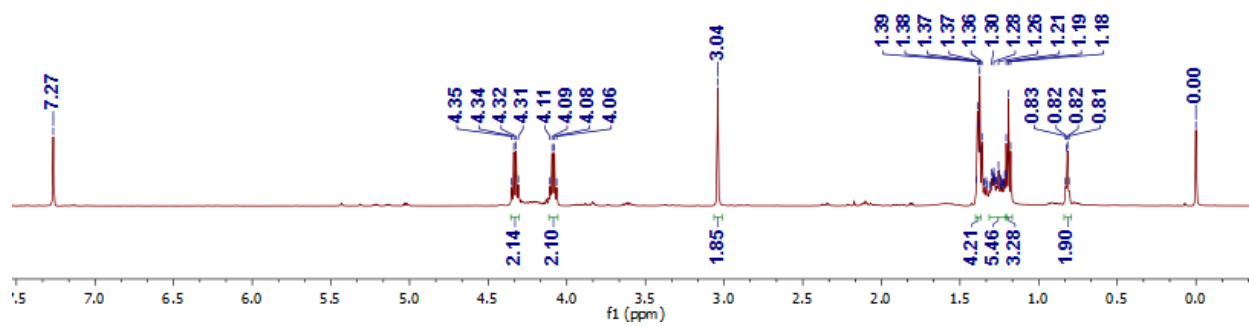


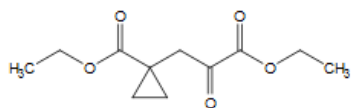
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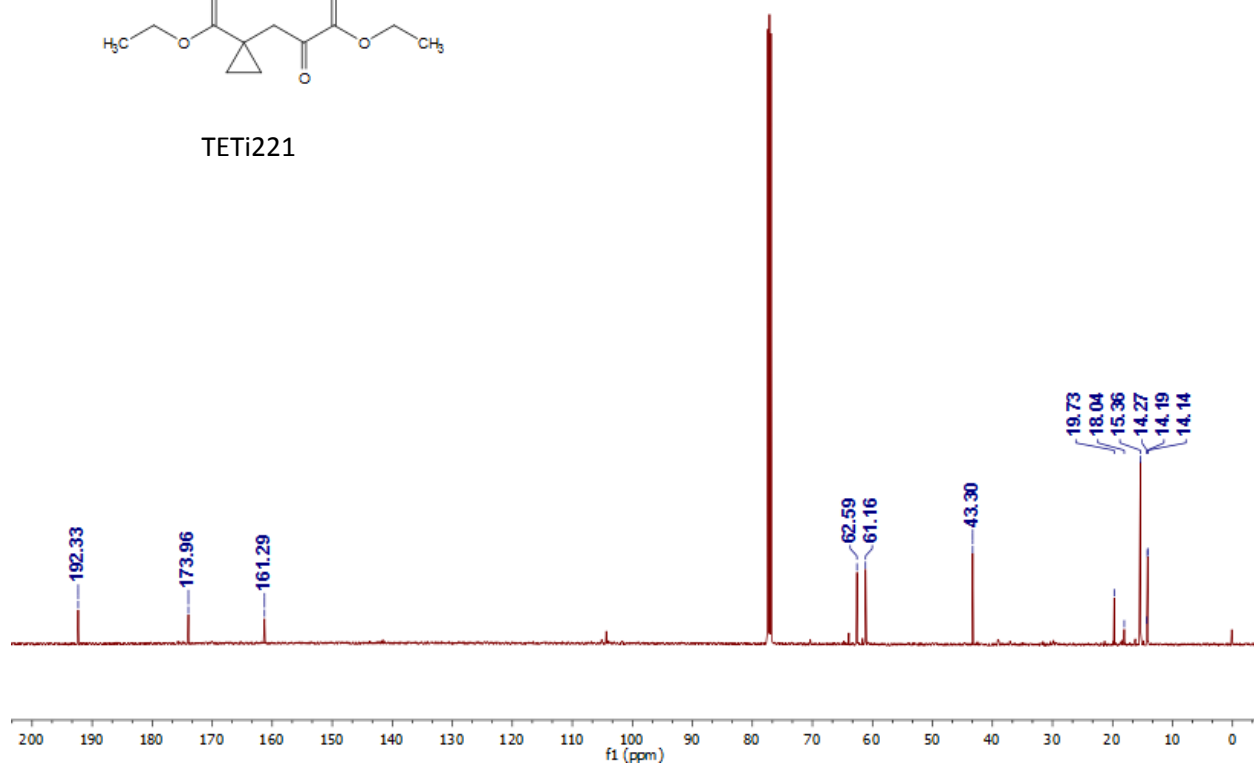


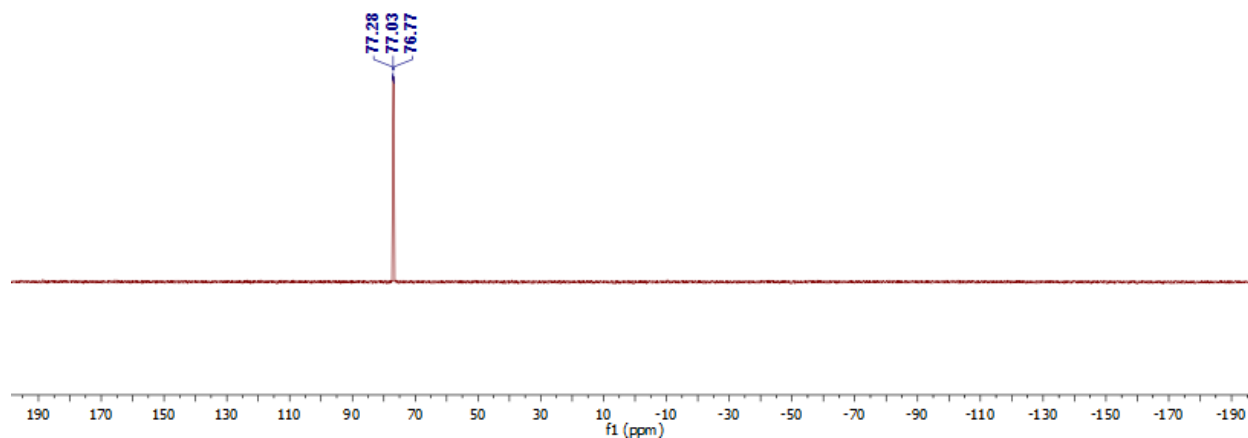
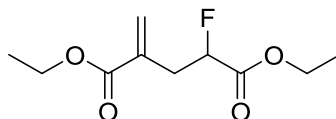
TETi221





TETI221





References

1. Xinjun Liu, Han Chen, Erik Laurini, Yang Wang, Valentina Dal Col, Paola Posocco, Fabio Ziarelli, Maurizio Fermeglia, Cheng-Cal Zhang, Sabrina Prici, and Ling Peng. "2-Difluoromethylene-4-methylenepentanoic Acid, A Paradoxical Probe Able To Mimic the Signaling Role of 2-Oxoglutaric Acid in Cyanobacteria" *Organic Letters*, **2011**, Vol.13, No.11, 2924-2927.
2. Xinjun Liu, Yang Wang, Erik Laurini, Paola Posocco, Han Chen, Fabio Ziarelli, Annick Janicki, Fanqi Qu, Maurizio Fermeglia, Sabrina Prici, Cheng-Cal Zhang, and Ling Peng. "Structural Requirements of 2-Oxoglutaric Acid Analogues To Mimic Its Signaling Function" *Organic Letters*, **2013**, Vol. 15, No. 18, 4662-4665.

