# Supporting Information

## Synthesis of deuterated γ-linolenic acid and application for biological studies: metabolic tuning and Raman imaging

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### Contents

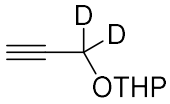
# Chemistry

## General

$^{1}H$, $^{2}H$ and $^{13}C$ NMR spectra were recorded on a JNM-ECA500 spectrometer. IR spectra were measured on Thermo Nicolet AVATAR 370 FT-IR. ESI-HRMS was taken on a Bruker Daltonics micrOTOF-QII-$_{RSL}$. Column chromatography was performed with silica gel 60 (40-100 μm) purchased from Kanto Chemical Co.. Gel permeation chromatography (GPC) was performed using LC-Forte/R (YMC Co., Ltd.).

## Synthesis of deuterated GLA derivatives

### Compound 2




To a stirred solution of trimethylsilylacetylene **1** (1.0 mL, 7.23 mmol) in dry THF (36 mL) was added *n*BuLi (4.7 mL, 7.23 mmol, 1.55 M in hexane) at -40 °C. The mixture was stirred at the same temperature for 30 min, and then $(CD_2O)_n$ (232 mg, 7.23 mmol) was added at the same temperature. Stirring was continued at 0 °C for 26 h, and then saturated aqueous $NH_4Cl$ solution was added to quench the reaction. The resulting mixture was extracted with $Et_2O$ twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo to yield an alcohol, which was used for the next reaction without further purification.

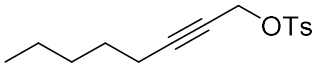
To a stirred solution of the above alcohol in dry DCM (36 mL) were added *p*-TsOH-$H_2O$ (138 mg, 0.723 mmol) and DHP (788 μL, 8.67 mmol) successively at room temperature. The mixture was stirred at the same temperature for 2 h, then further DHP (788 μL, 8.67 mmol) was added, and stirring was continued at the same temperature for 30 min. Saturated aqueous $NaHCO_3$ solution was added to quench the reaction, and the resulting mixture was extracted with DCM twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo to give the THP ether, which was used for the next reaction without further purification.

To a stirred solution of the above THP ether in dry THF (36 mL) was added TBAF (8.7 mL, 8.7 mmol, 1.0 M in THF) at 0 °C. The resulting mixture was stirred at the same temperature for 30 min, and then water was added. The resulting mixture was extracted with $Et_2O$ twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc:hexane = 1:9) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford the desired alkyne **2** (511 mg, 3.59 mmol, 50% in 3 steps) as a colorless oil.

$^{1}H$-NMR (500 MHz, $CDCl_3$): δ 4.81 (t, $J$ = 3.5 Hz, 1H), 3.80-3.85 (m, 1H), 3.50-3.55 (m, 1H), 2.39 (s, 1H), 1.78-1.85 (m, 1H), 1.70-1.76 (m, 1H), 1.49-1.65 (m, 4H). $^{2}H$-NMR (77 MHz, $CHCl_3$): δ 4.22 (d, $J$ = 3.5 Hz, 2H). $^{13}C$-NMR (126 MHz, $CDCl_3$): δ 96.8, 79.8, 74.1, 62.1, 53.5 (quintet, J = 22.8 Hz), 30.3, 25.4, 19.1. IR (neat, $cm^{-1}$): 3297, 2945, 2872, 1450, 1388, 1202, 1119, 1023, 895. HRMS (ESI):

Calculated for $C_8H_{10}D_2NaO_2$ $[M+Na]^+$, 165.0855: found, 165.0841.

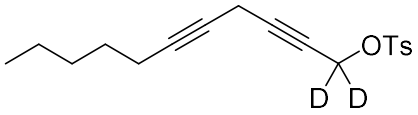
**Compound 4a**




To a stirred solution of 1-heptyne **3** (0.68 mL, 5.2 mmol) in dry THF (26 mL) was added *n*BuLi (3.7 mL, 5.7 mmol, 1.55 M in hexane) at -20 °C. The mixture was stirred at the same temperature for 1 h, and then $(CH_2O)_n$ (172 mg, 5.7 mmol) was added at the same temperature. Stirring was continued at room temperature for 15.5 h, and then saturated aqueous $NH_4Cl$ solution was added to quench the reaction. The resulting mixture was extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by short-path column chromatography (EtOAc:hexane = 1:1) to yield an alcohol, which was used for the next reaction without further purification.

To a stirred solution of the above alcohol in $H_2O$ (3.8 mL) were added 1-hexadecylimidazole[1] (152 mg, 0.52 mmol), $K_2CO_3$ (0.86 g, 6.24 mmol), and TsCl (1.49 g, 7.8 mmol) successively at room temperature. The resulting mixture was stirred at the same temperature for 26.5 h, and extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc:hexane = 1:9) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford **4a** (625 mg, 2.23 mmol, 43% in 2 steps) as a colorless oil. The analytical data were in good agreement with reported data.[2]

**Compound 5a**




To a stirred solution of tosylate **4a** (300 mg, 1.07 mmol) and alkyne **2** (152 mg, 1.07 mmol) in dry DMF (5.4 mL) were added $K_2CO_3$ (222 mg, 1.61 mmol), NaI (321 mg, 2.14 mmol), and CuI (224 mg, 1.18 mmol) successively at 0 °C. The mixture was stirred at room temperature for 15 h, then EtOAc was added, and the resulting mixture was filtered through a pad of Celite. The filtrate was washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by short-path column chromatography (EtOAc:hexane = 1:4) to afford a diyne, which was used for the next reaction without further purification.

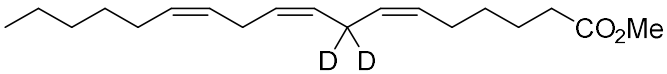
To a stirred solution of the above diyne in MeOH (5.4 mL) was added *p*-TsOH·$H_2O$ (20 mg, 0.105 mmol) at room temperature. The mixture was stirred at the same temperature for 1 h, and then saturated aqueous $NaHCO_3$ solution was added to quench the reaction. The resulting mixture was extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo* to yield an alcohol, which was used for the next reaction without further purification.

To a stirred solution of the above alcohol in $H_2O$ (0.77 mL) were added 1-hexadecylimidazole[1] (31

mg, 0.107 mmol), $K_2CO_3$ (177 mg, 1.28 mmol), and TsCl (306 mg, 1.61 mmol) successively at room temperature. The mixture was stirred at the same temperature for 21 h, and then extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc:hexane = 1:9 to 1:4) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford **5a** (195.2 mg, 0.609 mmol, 57% in 3 steps) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 7.82 (d, *J* = 8.0 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 3.04 (t, *J* = 2.4 Hz, 2H), 2.45 (s, 3H), 2.13 (tt, *J* = 7.3, 2.4 Hz, 2H), 1.45-1.51 (m, 2H), 1.27-1.36 (m, 4H), 0.89 (t, *J* = 7.2 Hz, 3H). $^2$H-NMR (77 MHz, $CHCl_3$): δ 4.66 (brs, 2H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 145.0, 133.1, 129.8, 128.1, 84.6, 81.4, 72.2, 71.8, 57.8 (quint, *J* = 24.0 Hz), 31.0, 28.3, 22.2, 21.6, 18.6, 13.9, 9.8. IR (neat, cm$^{-1}$): 2956, 2928, 2859, 1600, 1365, 1175, 1099, 953, 805, 735, 656. HRMS (ESI): Calculated for $C_{18}H_{20}D_2NaO_3S$ $[M+Na]^+$, 343.1307: found, 343.1322.

**Compound 6a**

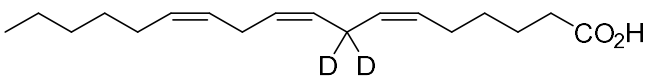



To a stirred solution of tosylate **5a** (188.2 mg, 0.587 mmol) and methyl 6-heptynoate (91 mg, 0.646 mmol) in dry DMF (5.9 mL) were added $K_2CO_3$ (122 mg, 0.881 mmol), NaI (176 mg, 1.17 mmol), and CuI (123 mg, 0.646 mmol) successively at 0 °C. The mixture was stirred at room temperature for 21 h, then EtOAc was added, and the resulting mixture was filtered through a pad of Celite. The filtrate was washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by short-path column chromatography (EtOAc:hexane = 1:9) to yield a triyne, which was used for the next reaction without further purification.

To a stirred solution of $Ni(OAc)_2$ (104 mg, 0.587 mmol) in dry MeOH (3.0 mL) under an $H_2$ atmosphere were added $NaBH_4$ (22 mg, 0.587 mmol) and ethylenediamine (0.16 ml, 2.35 mmol) at 0 °C. The mixture was stirred at the same temperature for 10 min, and then to it was added the above triyne in dry MeOH (2.9 mL). Stirring was continued at room temperature for 20 h, $Et_2O$ was added, and the resulting mixture was filtered through a pad of Celite. The filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc:hexane = 1:9) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford **6a** (96.8 mg, 0.329 mmol, 56% in 2 steps) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 5.30-5.42 (m, 6H), 3.67 (s, 3H), 2.80 (t, *J* = 6.6 Hz, 2H), 2.32 (t, *J* = 7.4 Hz, 2H), 2.03-2.10 (m, 4H), 1.62-1.68 (m, 2H), 1.26-1.42 (m, 8H), 0.89 (t, *J* = 6.9 Hz, 3H). $^2$H-NMR (77 MHz, $CHCl_3$): δ 2.78 (brs, 2H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 174.2, 130.5, 129.7, 128.5, 128.2, 128.1, 127.7, 51.6, 34.1, 31.6, 29.4, 29.2, 27.3, 27.0, 25.7, 25.1 (quint, *J* = 19.2 Hz), 24.7, 22.7, 14.2. IR (neat, cm$^{-1}$): 3007, 2925, 2852, 1742, 1437, 1192, 711. HRMS (ESI): Calculated for $C_{19}H_{30}D_2NaO_2$ $[M+Na]^+$, 317.2420: found, 317.2433.

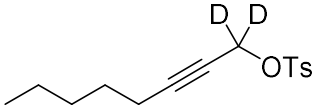
**8,8-$D_2$-GLA (7a)**




To a stirred solution of **6a** (60 mg, 0.204 mmol) in dry THF (2.0 mL) was added 1.0 M aqueous $LiOH \cdot H_2O$ solution (2.0 mL, 2.0 mmol) at room temperature. The mixture was stirred at the same temperature for 7 h, and then the pH was adjusted to ~3 by adding 1.0 M aqueous HCl solution at room temperature. The resulting mixture was extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc:hexane = 1:4) to afford the desired compound **7a** (56.5 mg, 0.201 mmol, 99%) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 5.31-5.43 (m, 6H), 2.80 (t, *J* = 6.6 Hz, 2H), 2.37 (t, *J* = 7.4 Hz, 2H), 2.03-2.12 (m, 4H), 1.63-1.69 (m, 2H), 1.25-1.46 (m, 8H), 0.89 (t, *J* = 6.9 Hz, 3H). $^2$H-NMR (77 MHz, $CHCl_3$): δ 2.78 (brs, 2H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 180.3, 130.6, 129.7, 128.6, 128.4, 128.1, 127.7, 34.1, 31.7, 29.5, 29.1, 27.4, 27.0, 25.8, 25.1 (quint, *J* = 19.2 Hz), 24.4, 22.7, 14.2. IR (neat**,** $cm^{-1}$): 3007, 2925, 2859, 1711, 1410, 1285, 936, 711. HRMS (ESI): Calculated for $C_{18}H_{28}D_2NaO_2$ $[M+Na]^+$, 303.2264: found, 303.2236.

**Compound 4b**



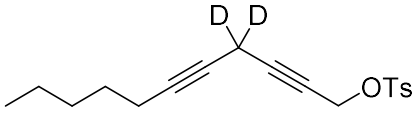

To a stirred solution of 1-heptyne **3** (1.3 mL, 10.0 mmol) in dry THF (50 mL) was added *n*BuLi (7.1 mL, 11.0 mmol, 1.55 M in hexane) at -20 °C. The mixture was stirred at the same temperature for 1 h, then $(CD_2O)_n$ (352 mg, 11.0 mmol) was added at the same temperature. Stirring was continued at room temperature for 18 h, and then saturated aqueous $NH_4Cl$ solution was added to quench the reaction. The resulting mixture was extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by short-path column chromatography (EtOAc:hexane = 1:1) to yield an alcohol, which was used for the next reaction without further purification.

To a stirred solution of the above alcohol in $H_2O$ (7.2 mL) were added 1-hexadecylimidazole[1] (293 mg, 1.0 mmol), $K_2CO_3$ (1.7 g, 12 mmol), and TsCl (2.9 g, 15 mmol) successively at room temperature. The mixture was stirred at the same temperature for 30 h, and then extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $MgSO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc:hexane = 1:9) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford **4b** (1.62 g, 5.74 mmol, 57% in 2 steps) as a colorless oil.

$^1$H-NMR (500 MHz, CDCl3): δ 7.82 (d, J = 8.0 Hz, 2H), 7.34 (d, J = 8.0 Hz, 2H), 2.45 (s, 3H), 2.06 (t, J = 6.9 Hz, 2H), 1.35-1.41 (m, 2H), 1.24-1.28 (m, 4H), 0.88 (t, J = 6.9 Hz, 3H). $^2$H-NMR (77 MHz, CHCl3): δ 4.68 (brs, 2H). $^{13}$C-NMR (126 MHz, CDCl3): δ 144.9, 133.5, 129.8, 128.1, 90.6,

71.8, 58.4 (quint, J = 24.0 Hz), 31.0, 27.8, 22.2, 21.7, 18.6, 14.0. IR (neat, $cm^{-1}$): 2959, 2927, 2862, 2250, 1742, 1597, 1365, 1178, 950, 808, 729, 652. HRMS (ESI): Calculated for $C_{15}H_{18}D_2NaO_3S$ $[M+Na]^+$, 305.1156: found, 305.1158.

**Compound 5b**

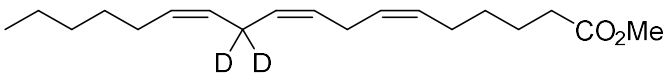



To a stirred solution of tosylate **4b** (300 mg, 1.06 mmol) and propargyl alcohol (63 μL, 1.06 mmol) in dry DMF (5.3 mL) were added $K_2CO_3$ (220 mg, 1.59 mmol), NaI (318 mg, 2.12 mmol), and CuI (223 mg, 1.17 mmol) successively at 0 °C. The mixture was stirred at room temperature for 20 h, EtOAc was added, and the resulting mixture was filtered through a pad of Celite. The filtrate was washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo* to yield a crude product, which was used for the next reaction without further purification.

To a stirred solution of the above alcohol in $H_2O$ (0.76 mL) were added 1-hexadecylimidazole[1] (31 mg, 0.106 mmol), $K_2CO_3$ (176 mg, 1.27 mmol), and TsCl (303 mg, 1.59 mmol) successively at room temperature. The mixture was stirred at the same temperature for 21 h, and then extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc:hexane = 1:9 to 1:4) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford the desired compound **5b** (288.9 mg, 0.902 mmol, 85% in 2 steps) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 7.81 (d, *J* = 8.0 Hz, 2H), 7.35 (d, *J* = 8.0 Hz, 2H), 4.69 (s, 2H), 2.45 (s, 3H), 2.13 (t, *J* = 7.2 Hz, 2H), 1.45-1.50 (m, 2H), 1.28-1.36 (m, 4H), 0.89 (t, *J* = 7.2 Hz, 3H). $^2$H-NMR (77 MHz, $CHCl_3$): δ 3.00 (brs, 2H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 145.0, 133.1, 129.8, 128.1, 84.6, 81.4, 72.2, 71.9, 58.2, 31.0, 28.3, 22.1, 21.6, 18.5, 13.9, 9.4 (quint, *J* = 20.4 Hz). IR (neat, $cm^{-1}$): 2959, 2921, 2859, 1745, 1600, 1364, 1192, 1171, 939, 815, 753, 659. HRMS (ESI): Calculated for $C_{18}H_{20}D_2NaO_3S$ $[M+Na]^+$, 343.1307: found, 343.1322.

**Compound 6b**

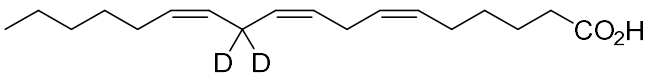



To a stirred solution of tosylate **5b** (245.8 mg, 0.767 mmol) and methyl 6-heptynoate (118 mg, 0.844 mmol) in dry DMF (7.7 mL) were added $K_2CO_3$ (159 mg, 1.15 mmol), NaI (230 mg, 1.53 mmol), and CuI (161 mg, 0.844 mmol) successively at 0 °C. The mixture was stirred at room temperature for 19 h, then EtOAc was added, and the resulting mixture was filtered through a pad of Celite. The filtrate was washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by short-path column chromatography (EtOAc:hexane = 1:9) to yield a triyne, which was used for the next reaction without further purification.

To a stirred solution of $Ni(OAc)_2$ (136 mg, 0.767 mmol) in dry MeOH (3.7 mL) under an $H_2$ atmosphere were added $NaBH_4$ (29 mg, 0.767 mmol) and ethylenediamine (0.21 ml, 3.07 mmol) at 0 °C. The mixture was stirred at the same temperature for 10 min. To the mixture was added the above triyne in dry MeOH (4.0 mL). Stirring was continued at room temperature for 24 h, then $Et_2O$ was added, and the resulting mixture was filtered through a pad of Celite. The filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc:hexane = 1:9) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford **6b** (150.3 mg, 0.51 mmol, 67% in 2 steps) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 5.31-5.42 (m, 6H), 3.67 (s, 3H), 2.80 (t, *J* = 5.4 Hz, 2H), 2.32 (t, *J* = 7.7 Hz, 2H), 2.03-2.10 (m, 4H), 1.62-1.68 (m, 2H), 1.27-1.41 (m, 8H), 0.89 (t, *J* = 7.2 Hz, 3H). $^2$H-NMR (77 MHz, $CHCl_3$): δ 2.78 (brs, 2H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 174.1, 130.5, 129.6, 128.3, 128.3, 128.1, 127.5, 51.5, 34.0, 31.6, 29.4, 29.2, 27.3, 26.9, 25.7, 25.1 (quint, *J* = 19.2 Hz), 24.6, 22.7, 14.1. IR (neat, cm$^{-1}$): 3004, 2921, 2848, 1738, 1431, 1164. HRMS (ESI): Calculated for $C_{19}H_{30}D_2NaO_2$ $[M+Na]^+$, 317.2420: found, 317.2443.

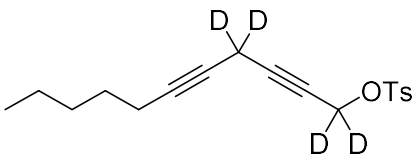
**11,11-$D_2$-GLA (7b)**




To a stirred solution of **6b** (58.8 mg, 0.20 mmol) in dry THF (2.0 mL) was added 1.0 M aqueous $LiOH·H_2O$ solution (2.0 mL, 2.0 mmol) at room temperature. The mixture was stirred at the same temperature for 8 h, and then the pH was adjusted to ~3 with 1.0 M aqueous HCl solution at room temperature. The resulting mixture was extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc:hexane = 1:4) to afford **7b** (50.7 mg, 0.181 mmol, 90%) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 5.31-5.42 (m, 6H), 2.80 (t, *J* = 5.4 Hz, 2H), 2.36 (t, *J* = 7.4 Hz, 2H), 2.03-2.11 (m, 4H), 1.63-1.69 (m, 2H), 1.25-1.44 (m, 8H), 0.89 (t, *J* = 6.9 Hz, 3H). $^2$H-NMR (77 MHz, $CHCl_3$): δ 2.79 (brs, 2H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 180.5, 130.6, 129.6, 128.5 (two peaks), 128.2, 127.6, 34.1, 31.7, 29.5, 29.1, 27.4, 27.0, 25.8, 25.1 (quint, J = 19.2 Hz), 24.4, 22.7, 14.2. IR (neat, cm$^{-1}$): 3004, 2925, 2852, 1711, 1413, 1271, 919. HRMS (ESI): Calculated for $C_{18}H_{28}D_2NaO_2$ $[M+Na]^+$, 303.2264: found, 303.2264.

**Compound 5c**




To a stirred solution of tosylate **4b** (300 mg, 1.06 mmol) and alkyne **2** (151 mg, 1.06 mmol) in dry

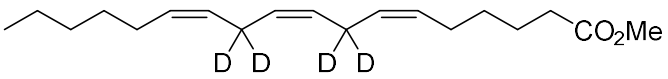
DMF (5.3 mL) were added $K_2CO_3$ (220 mg, 1.59 mmol), NaI (318 mg, 2.12 mmol), and CuI (223 mg, 1.17 mmol) successively at 0 °C. The mixture was stirred at room temperature for 30 h, then EtOAc was added, and the resulting mixture was filtered through a pad of Celite. The filtrate was washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by short-path column chromatography (EtOAc:hexane = 1:4) to yield a diyne, which was used for the next reaction without further purification.

To a stirred solution of the above diyne in MeOH (5.3 mL) was added *p*-TsOH·$H_2O$ (20 mg, 0.105 mmol) at room temperature. The mixture was stirred at the same temperature for 5 h, and then saturated aqueous $NaHCO_3$ solution was added to quench the reaction. The resulting mixture was extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo* to yield an alcohol, which was used for the next reaction without further purification.

To a stirred solution of the above alcohol in $H_2O$ (0.76 mL) were added 1-hexadecylimidazole[1] (31 mg, 0.106 mmol), $K_2CO_3$ (176 mg, 1.27 mmol), and TsCl (303 mg, 1.59 mmol) successively at room temperature. The mixture was stirred at the same temperature for 19 h, and then extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc:hexane = 1:9 to 1:4) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford **5c** (198.7 mg, 0.616 mmol, 58% in 3 steps) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 7.80 (d, $J$ = 8.0 Hz, 2H), 7.34 (d, $J$ = 8.0 Hz, 2H), 2.44 (s, 3H), 2.12 (t, $J$ = 7.2 Hz, 2H), 1.44-1.50 (m, 2H), 1.27-1.35 (m, 4H), 0.88 (t, $J$ = 6.9 Hz, 3H). $^2$H-NMR (77 MHz, $CHCl_3$): δ 4.66 (brs, 2H), 3.01 (brs, 2H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 145.0, 133.1, 129.7, 128.1, 84.6, 81.4, 72.2, 71.8, 57.7 (quint, $J$ = 24.0 Hz), 31.0, 28.3, 22.1, 21.6, 18.5, 13.9, 9.3 (quint, $J$ = 20.4 Hz). IR (neat, cm$^{-1}$): 2959, 2925, 2862, 2264, 1749, 1597, 1372, 1182, 943, 815, 725, 656. HRMS (ESI): Calculated for $C_{18}H_{18}D_4NaO_3S$ $[M+Na]^+$, 345.1433: found, 345.1460.

**Compound 6c**



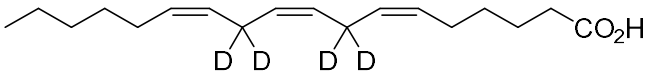

To a stirred solution of tosylate **5c** (179.4 mg, 0.556 mmol) and methyl 6-heptynoate (86 mg, 0.612 mmol) in dry DMF (5.6 mL) were added $K_2CO_3$ (115 mg, 0.834 mmol), NaI (167 mg, 1.11 mmol), and CuI (117 mg, 0.612 mmol) successively at 0 °C. The mixture was stirred at room temperature for 17 h, and then EtOAc was added. The resulting mixture was filtered through a pad of Celite. The filtrate was washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by short-path column chromatography (EtOAc:hexane = 1:9) to yield a triyne, which was used for the next reaction without further purification.

To a stirred solution of $Ni(OAc)_2$ (98 mg, 0.556 mmol) in dry MeOH (2.6 mL) under an $H_2$ atmosphere were added $NaBH_4$ (21 mg, 0.556 mmol) and ethylenediamine (0.15 ml, 2.22 mmol) at

0 °C. The mixture was stirred at the same temperature for 10 min. To the mixture was added the above triyne in dry MeOH (3.0 mL). Stirring was continued at room temperature for 18 h, then $Et_2O$ was added, and the resulting mixture was filtered through a pad of Celite. The filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc:hexane = 1:9) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford **6c** (125.8 mg, 0.424 mmol, 76% in 2 steps) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 5.31-5.42 (m, 6H), 3.67 (s, 3H), 2.32 (t, *J* = 7.7 Hz, 2H), 2.03-2.10 (m, 4H), 1.62-1.68 (m, 2H), 1.25-1.43 (m, 8H), 0.89 (t, *J* = 7.2 Hz, 3H). $^2$H-NMR (77 MHz, $CHCl_3$): δ 2.77 (brs, 4H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 174.1, 130.5, 129.7, 128.4, 128.2, 128.0, 127.6, 51.5, 34.0, 31.6, 29.4, 29.2, 27.3, 26.9, 25.1 (quint, J = 19.2 Hz, two peaks), 24.6, 22.7, 14.1. IR (neat, $cm^{-1}$): 3010, 2929, 2854, 1743, 1452, 1191, 1168, 848. HRMS (ESI): Calculated for $C_{19}H_{28}D_4NaO_2$ $[M+Na]^+$, 319.2546: found, 319.2543.

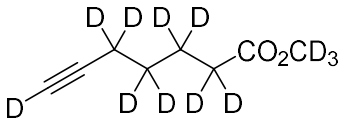
**8,8,11,11-D$_4$-GLA (7c)**




To a stirred solution of **6c** (64.2 mg, 0.217 mmol) in dry THF (2.2 mL) was added 1.0 M aqueous $LiOH·H_2O$ solution (2.2 mL, 2.2 mmol) at room temperature. The mixture was stirred at the same temperature for 14 h, and then the pH was adjusted to ~3 with 1.0 M aqueous HCl solution at room temperature. The resulting mixture was extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo. The residue was purified by column chromatography (EtOAc:hexane = 1:4) to afford compound **7c** (58.8 mg, 0.208 mmol, 96%) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 5.31-5.43 (m, 6H), 2.36 (t, *J* = 7.4 Hz, 2H), 2.03-2.11 (m, 4H), 1.63-1.69 (m, 2H), 1.27-1.46 (m, 8H), 0.89 (t, *J* = 6.9 Hz, 3H). $^2$H-NMR (77 MHz, $CHCl_3$): δ 2.79 (brs, 4H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 180.6, 130.6, 129.6, 128.5, 128.4, 128.1, 127.6, 34.1, 31.6, 29.5, 29.1, 27.4, 27.0, 25.1 (quint, *J* = 19.2 Hz, two peaks), 24.4, 22.7, 14.2. IR (neat, $cm^{-1}$): 33007, 2925, 2855, 1704, 1461, 1292, 933, 704. HRMS (ESI): Calculated for $C_{18}H_{26}D_4NaO_2$ $[M+Na]^+$, 305.2389: found, 305.2384.

**Compound 9**



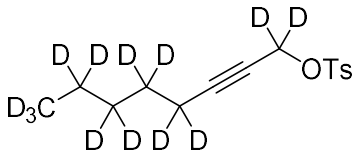

To a stirred solution of cyclohexene-d10 **8** (1.00 g, 10.84 mmol) in $CD_3OD$ (7.2 mL) and dry DCM (36 mL) was added anhydrous $NaHCO_3$ (273 mg, 3.25 mmol). The mixture was cooled to -78 °C, and bubbled with $O_3$ until the solution turned blue. Then, $N_2$ was passed through the solution until the blue color disappeared. To the resulting mixture was added benzene (11 mL), and the volume of the

solution was reduced to approximately 8 mL. Then, dry DCM (36 mL) $NEt_3$ (2.3 mL, 16.3 mmol) and $Ac_2O$ (3.1 mL, 32.5 mmol) were added at 0 °C. The resulting mixture was stirred at the same temperature for 15 min, then warmed to room temperature, stirred for another 2 h, and washed with aqueous 0.1 M hydrochloric acid, 10% aqueous solution of sodium hydroxide, water, and brine. The organic phase was dried over $Na_2SO_4$, filtered, and concentrated in vacuo to yield a crude product, which was used for the next reaction without further purification.

To a stirred solution of the above crude product in $CD_3OD$ (5.5 mL) were added $K_2CO_3$ (3.0 g, 21.7 mmol) and dimethyl (1-diazo-2-oxopropyl)phosphonate (Ohira-Bestmann reagent, 1.65 mL, 10.8 mmol) successively at 0 °C. The mixture was stirred at the same temperature for 2 h, and then saturated aqueous $NH_4Cl$ solution was added to quench the reaction. The resulting mixture was extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc:hexane = 1:9) to afford compound **9** (1.24 g, 8.13 mmol, 75% in 3 steps) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): $\delta$ 3.67 (s), 1.94 (s). Small peaks of **H**-C≡C- and C(=O)O**Me** were observed. $^2$H-NMR (77 MHz, $CHCl_3$): δ 3.64 (brs, 3H), 2.30 (brs, 2H), 2.16 (brs, 2H), 1.94 (brs, 1H), 1.70 (brs, 2H), 1.51 (brs, 2H). $^{13}$C-NMR (126 MHz, $CDCl_3$): $\delta$ 173.9, 83.9 (H-C≡**C**-), 83.4 (brt, $J$ = 7.2 Hz), 68.7 (H-**C**≡C-), 68.4 (t, $J$ = 38.4 Hz), 51.5 (-COO**Me**), 50.8 (sept, $J$ = 22.8 Hz), 32.7 (quintet, $J$ = 19.2 Hz), 26.7 (quintet, $J$ = 19.2 Hz), 22.9 (quintet, $J$ = 19.2 Hz), 17.3 (quintet, $J$ = 19.2 Hz). IR (neat, cm$^{-1}$): 2925, 2855, 1735, 1296, 1081. HRMS (ESI): Calculated for $C_8D_{12}NaO_2$ $[M+Na]^+$, 175.1483: found, 175.1463.

**Compound 10**




To a stirred solution of **2** (100 mg, 0.703 mmol) in dry THF (2.5 mL) were added HMPA (0.15 mL, 0.844 mmol) and *n*BuLi (0.59 mL, 0.914 mmol, 1.55 M in hexane) successively at -78 °C. The mixture was stirred at 0 °C for 1 h, and then $C_5D_{11}Br$ (125 mg, 0.773 mmol) in dry THF (1.0 mL) was added at -78 °C. Stirring was continued at room temperature for 21 h, and then saturated aqueous $NH_4Cl$ solution was added to quench the reaction. The resulting mixture was extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by short-path column chromatography (EtOAc:hexane = 1:4) to yield the alkylated compound, which was used for the next reaction without further purification.

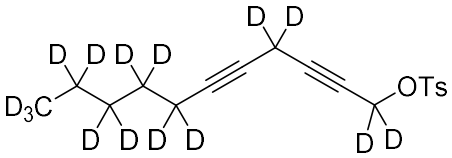
To a stirred solution of the above THP ether in MeOH (3.5 mL) was added *p*-TsOH·$H_2O$ (13 mg, 0.068 mmol) at room temperature. The mixture was stirred at the same temperature for 80 min, and then saturated aqueous $NaHCO_3$ solution was added to quench the reaction. The resulting mixture was

extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated in vacuo to yield an alcohol, which was used for the next reaction without further purification.

To a stirred solution of the above alcohol in $H_2O$ (0.5 mL) were added 1-hexadecylimidazole[1] (21 mg, 0.0703 mmol), $K_2CO_3$ (117 mg, 0.844 mmol), and TsCl (200 mg, 1.05 mmol) successively at room temperature. The mixture was stirred at the same temperature for 22 h, and then extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc:hexane = 1:9) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford **10** (86.2 mg, 0.294 mmol, 42% in 3 steps) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 7.81 (d, $J$ = 8.0 Hz, 2H), 7.34 (d, $J$ = 8.0 Hz, 2H), 2.44 (s, 3H). $^2$H-NMR (77 MHz, $CHCl_3$): δ 4.68 (brs, 2H), 2.01 (brs, 2H), 1.32 (brs, 2H), 1.20 (brs, 4H), 0.82 (brs, 3H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 144.9, 133.5, 129.8, 128.1, 90.7, 71.7, 58.4 (quintet, J = 24.0 Hz), 29.6 (quintet, J = 19.2 Hz), 26.6 (quintet, J = 19.2 Hz), 21.7, 20.9 (quintet, J = 19.2 Hz), 17.9 (quintet, J = 19.2 Hz), 12.8 (sept, J = 19.2 Hz). IR (neat, cm$^{-1}$): 2256, 2215, 2104, 1599, 1364, 1172, 940, 819, 724, 659. HRMS (ESI): Calculated for $C_{15}H_7D_{13}NaO_3S$ $[M+Na]^+$, 316.1841: found, 316.1825.

**Compound 11**




To a stirred solution of tosylate **10** (500 mg, 1.70 mmol) and alkyne **2** (242 mg, 1.70 mmol) in dry DMF (9.0 mL) were added $K_2CO_3$ (352 mg, 2.55 mmol), NaI (510 mg, 3.40 mmol), and CuI (356 mg, 1.87 mmol) successively at 0 °C. The mixture was stirred at room temperature for 21 h, then EtOAc was added, and the resulting mixture was filtered through a pad of Celite. The filtrate was washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by short-path column chromatography (EtOAc:hexane = 1:4) to give a diyne, which was used for the next reaction without further purification.

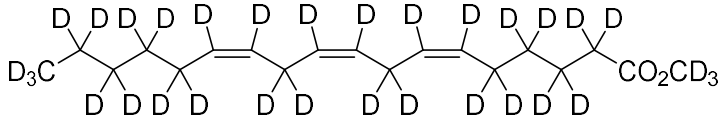
To a stirred solution of the above diyne in MeOH (9.0 mL) was added *p*-TsOH·$H_2O$ (32 mg, 0.17 mmol) at room temperature. The mixture was stirred at the same temperature for 2 h, and then saturated aqueous $NaHCO_3$ solution was added to quench the reaction. The resulting mixture was extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo* to afford an alcohol, which was used for the next reaction without further purification.

To a stirred solution of the above alcohol in $H_2O$ (1.2 mL) were added 1-hexadecylimidazole[1] (50 mg, 0.17 mmol), $K_2CO_3$ (282 mg, 2.04 mmol), and TsCl (486 mg, 2.55 mmol) successively at room temperature. The mixture was stirred at the same temperature for 19 h, and then extracted with EtOAc

twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc:hexane = 1:9 to 1:4) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford **11** (311.8 mg, 0.935 mmol, 55% in 3 steps) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 7.82 (d, $J$ = 8.6 Hz, 2H), 7.35 (d, $J$ = 8.6 Hz, 2H), 2.45 (s, 3H). $^2$H-NMR (77 MHz, $CHCl_3$): δ 4.67 (brs, 2H), 3.01 (brs, 2H), 2.08 (brs, 2H), 1.42 (brs, 2H), 1.24 (brs, 4H), 0.83 (brs, 3H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 145.1, 133.2, 129.9, 128.2, 84.7, 81.6, 72.2, 71.9, 57.9 (quintet, $J$ = 24.0 Hz), 29.7 (quintet, $J$ = 19.2 Hz), 27.2 (quintet, $J$ = 19.2 Hz), 21.7, 21.0 (quintet, $J$ = 19.2 Hz), 17.9 (quintet, $J$ = 19.2 Hz), 12.9 (sept, $J$ = 19.2 Hz), 9.5 (quintet, $J$ = 20.4 Hz). IR (neat, cm$^{-1}$): 2216, 2101, 1365, 1182, 957, 818, 735, 659. HRMS (ESI): Calculated for $C_{18}H_7D_{15}NaO_3S$ $[M+Na]^+$, 356.2129: found, 356.2125.

**Compound 12**

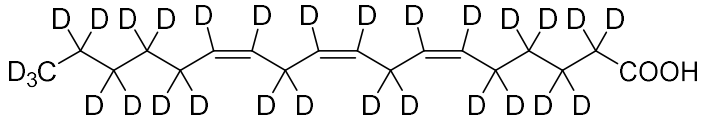



To a stirred solution of tosylate **11** (131.6 mg, 0.395 mmol) and **9** (66 mg, 0.435 mmol) in dry DMF (4.0 mL) were added $K_2CO_3$ (82 mg, 0.593 mmol), NaI (118 mg, 0.79 mmol), and CuI (83 mg, 0.435 mmol) successively at 0 °C. Stirring was continued at room temperature for 31 h, and then further alkyne **9** (33 mg, 0.217 mmol) in dry DMF (0.5 mL) was added. Stirring was continued at the same temperature for 13 h, then EtOAc was added, and the resulting mixture was filtered through a pad of Celite. The filtrate was washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by short-path column chromatography (EtOAc:hexane = 1:9) to give a triyne, which was used for the next reaction without further purification.

To a stirred solution of $Ni(OAc)_2$ (70 mg, 0.395 mmol) in $CD_3OD$ (2.0 mL) under a $D_2$ atmosphere were added $NaBD_4$ (17 mg, 0.395 mmol) and ethylenediamine (107 µl, 1.58 mmol) at 0 °C. The mixture was stirred at the same temperature for 10 min, and the above triyne in $CD_3OD$ (2.0 mL) was added. Stirring was continued at room temperature for 22 h, then $Et_2O$ was added, and the resulting mixture was filtered through a pad of Celite. The filtrate was concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc:hexane = 1:9) and then further purified by GPC ($CHCl_3$, 10 mL/min) to afford **12** (82.6 mg, 0.254 mmol, 64% in 2 steps) as a colorless oil.

$^1$H-NMR (500 MHz, $CDCl_3$): δ 3.66 (s). Small peak of methyl ester was observed. $^2$H-NMR (77 MHz, $CHCl_3$): δ 5.38 (brs, 6H), 3.64 (s, 3H), 2.76 (brs, 4H), 2.27 (brs, 2H), 2.03-1.97 (m, 4H), 1.59 (brs, 2H), 1.22-1.33 (m, 8H), 0.83 (brs, 3H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 174.3, 130.0 (t, $J$ = 22.8 Hz), 129.1 (t, $J$ = 22.8 Hz), 128.2-127.0 (m), 50.8 (sept, $J$ = 22.8 Hz), 33.3 (quint, $J$ = 19.2 Hz), 30.7-29.8 (m), 28.6-27.7 (m), 26.5-25.7 (m), 24.9 (quint, $J$ = 19.2 Hz), 24.0-23.3 (m), 21.8-21.1 (m), 13.0 (sept, $J$ = 19.2 Hz). IR (neat, cm$^{-1}$): 2209, 2098, 1738, 1278, 1088. HRMS (ESI): Calculated for $C_{19}D_{32}NaO_2$ $[M+Na]^+$, 347.4303: found, 347.4283.

**All-D-GLA**



To a stirred solution of **12** (30 mg, 0.0924 mmol) in dry THF (0.92 mL) was added 1.0 M aqueous $LiOH \cdot H_2O$ solution (2.2 mL, 2.2 mmol) at room temperature. The mixture was stirred at the same temperature for 16 h, and then the pH was adjusted to ~3 with 1.0 M aqueous HCl solution at room temperature. The resulting mixture was extracted with EtOAc twice. The combined organic phases were washed with brine, dried over $Na_2SO_4$, filtered, and concentrated *in vacuo*. The residue was purified by column chromatography (EtOAc:hexane = 1:4) to afford all-D-GLA (28.4 mg, 0.0923 mmol, quant.) as a colorless oil.

$^2$H-NMR (77 MHz, $CHCl_3$): δ 5.39 (brs, 6H), 2.76 (brs, 4H), 2.30 (brs, 2H), 1.92-2.04 (m, 4H), 1.60 (brs, 2H), 1.36-1.23 (m, 8H), 0.83 (brs, 3H). $^{13}$C-NMR (126 MHz, $CDCl_3$): δ 180.5, 130.0 (t, $J$ = 22.8 Hz), 129.0 (t, $J$ = 22.8 Hz), 128.2-127.0 (m), 33.3 (quintet, $J$ = 19.2 Hz), 30.6-29.8 (m), 28.6-27.6 (m), 26.6-25.6 (m), 24.9 (quintet, $J$ = 19.2 Hz), 23.6-22.8 (m), 21.8-21.1 (m), 13.0 (sept, $J$ = 19.2 Hz). IR (neat, cm$^{-1}$): 3035, 2212, 2098, 1704, 1410, 1289. HRMS (ESI): Calculated for $C_{18}D_{29}NaO_2$ $[M+Na]^+$, 330.3958: found, 330.3960.

# Biology

## Cell culture

VA-13 and WI-38 cells were maintained in Dulbecco's modified Eagle's medium supplemented with 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% heat-inactivated fetal bovine serum (FBS). Cells were grown in a humidified incubator at 37 °C under 5% $CO_2$/95% air.

## AlamarBlue assay

VA-13 or WI-38 cells (5 x $10^3$ cells/well, 100 μL) were suspended in fresh medium in a 96-well plate. After overnight incubation, the cells were treated with GLA derivatives in EtOH. After incubation for 48 h, 10 μL of AlamarBlue was added to each well. The cell viability was determined based on the increase of fluorescence (excitation 560 m/emission 590 nm) during a 2 h incubation. The fluorescence was measured with a microplate reader (Molecular Devices, SpectraMAX M2$^e$). Data are presented as mean ± S.D. (n = 3).

## Enzymatic oxidation of GLA and deuterated GLA

To confirm the effects of deuteration on GLA metabolism, we examined the oxidation of GLA by lipoxygenase according to the reported procedure using arachidonic acid (AA).[3] GLA and deuterated GLA **7a-c** (10 mM EtOH solution, final concentration: 500 μM) were reacted with soybean lipoxygenase (LOX, Sigma-Aldrich L7395) (2.5 μg/mL) in 100 mM sodium phosphate buffer (pH 7.4, 500 μL) with 50 μM diethylenetriamine-*N,N,N',N'',N''*-pentaacetic acid (DTPA) at 37 °C under air. After the indicated time, 50 μL of the reaction mixture was sampled and mixed with 50 μL of $CH_3CN$, and then analyzed by HPLC (Fig. S1). As shown in Fig. S1A, GLA showed several peaks after 5 min incubation with LOX. However, $d_4$-GLA (**7c**) showed very weak peaks in the same region even after 30 min incubation (Fig. S1B). We also examined GLA and deuterated GLA **7a-c** treated with LOX for 30 min (Fig. S1C) As a result, only $d_2$-GLA **7a** showed the peaks of metabolites in the same region as GLA, indicating that LOX selectively abstracts the proton at C11 carbon. In contrast, **7b** and **7c** having deuterium at C11 carbon are resistant to LOX metabolism. These results support the metabolic inhibition by deuteration of allylic methylenes of GLA in the same manner as AA.

HPLC system: Thermo Fisher UltiMate 3000
Type: GL Sciences Inertsil ODS-3 C18; size: 1.0 mm (ID)–150 mm (L) 3 μm (particle size); flow rate 50 μL/min; detector: UV 210 nm; eluent: mobile phase A, $H_2O$ with 0.1 % trifluoroacetic acid; mobile phase B, $CH_3CN$ with 0.1% trifluoroacetic acid; temperature 40 °C; injection volume 5 μL.
Analysis of lipid: Gradient curve: 50% B (0–5min), 50% to 95% B (5-35 min), 95% B (35–45 min), 95% to 50% B (45–55 min), 50% B (55–65 min); run time 65 min; RT = 28.3 min.

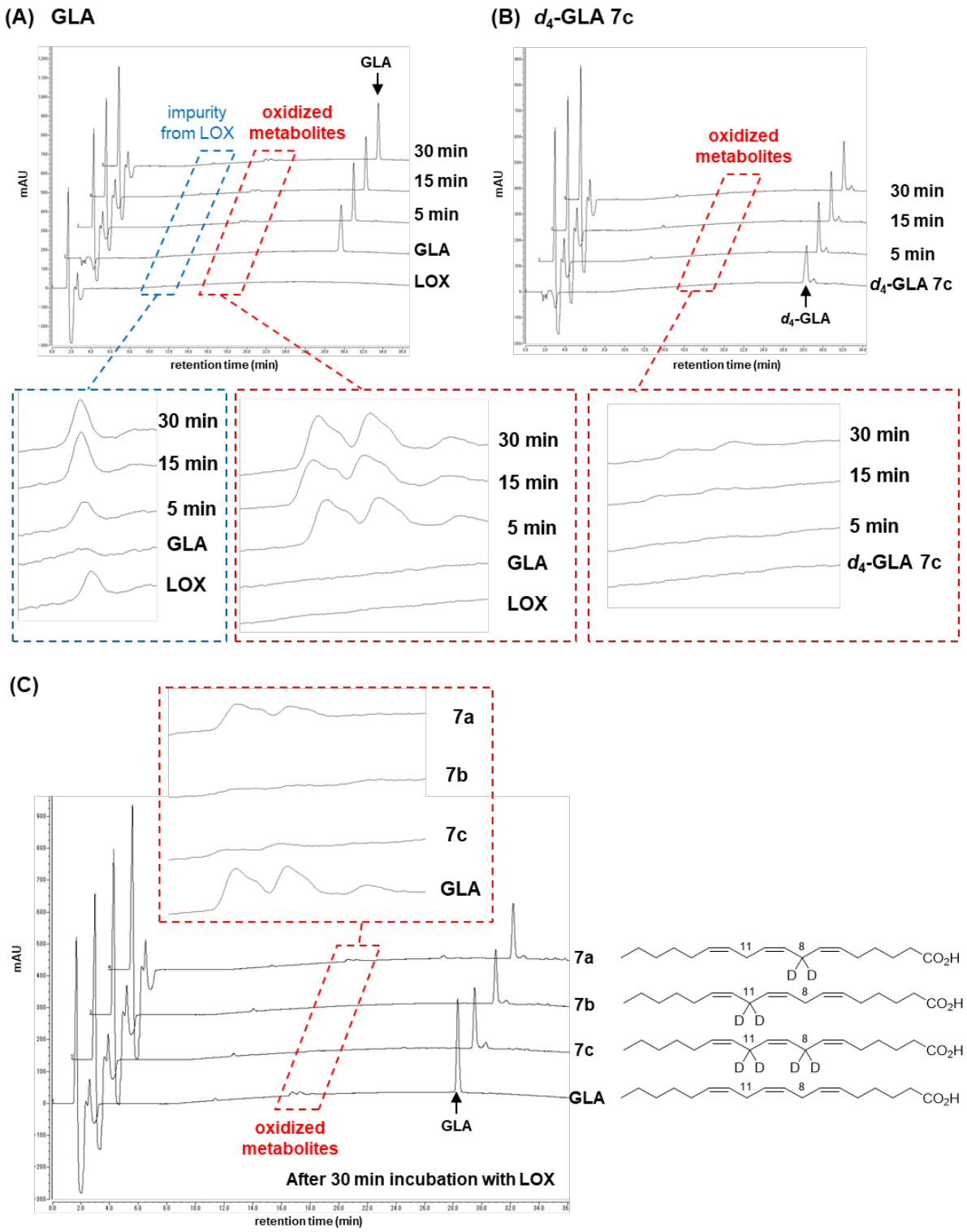



**Fig. S1** HPLC analysis of GLA and deuterated GLA **7a-c** reacted with lipoxygenase (LOX). (A, B) GLA or $d_4$-GLA **7c** was treated with LOX. After 5, 15, 30 min incubation, the reaction mixtures were analyzed by HPLC. (C) After 30 min reaction with LOX, GLA and deuterated GLA **7a-c** were analyzed by HPLC.

## Raman imaging

### Raman spectra of GLA and deuterated GLA

Raman spectra were obtained with a RAMAN-11 slit-scanning Raman microscope (Nanophoton, Japan), with excitation at 532 nm. Samples were placed on a quartz substrate during the measurements. The laser output was focused into the sample by a 60X/1.27 numerical aperture (NA) water immersion objective lens (CFI Plan Apo IR 60X WI, NIKON, Japan). The slit width of the spectrograph was 70 μm. The light intensity at the sample plane was calculated as 6.0 mW/μm$^2$ from the measured laser power at the sample position and the area of the illumination line. The exposure time for each line was 10 sec.

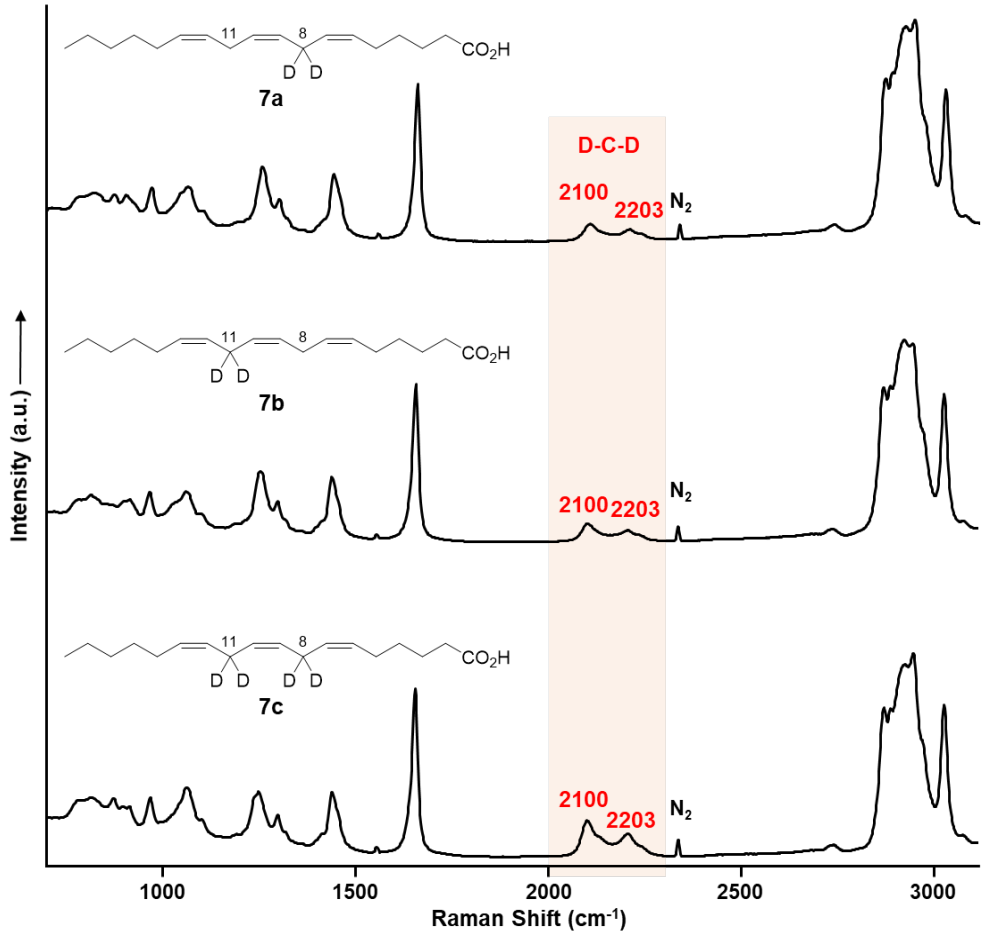



**Fig. S2** Raman Spectra of deuterated GLA **7a**-**7c**.

### Raman imaging of VA-13/WI-38 cells treated with all-D-GLA

VA-13/WI-38 cells (2.4 mL, 5.0 × 10$^4$ cells/mL) were placed on sterilized Φ25 mm quartz substrate in a tissue culture dish (TPP, Switzerland). After overnight incubation, 2.4 μL of all-D-GLA stock solution (100 mM in EtOH, final concentration: 100 μM) was added and incubation was continued for 6-48 hours. The medium was replaced with Tyrode's buffer (150 mM NaCl, 10 mM glucose, 10 mM HEPES, 4 mM KCl, 1 mM $MgCl_2$, 1 mM $CaCl_2$, 4 mM NaOH). The quartz substrate was used for

observation of Raman spectra with a RAMAN-11 slit-scanning Raman microscope, with excitation at 532 nm. The laser output was focused into the sample by a 60X/1.27 NA water immersion objective lens. The slit width of the spectrograph was 70 μm. The exposure time for each line was 10 sec. The light intensity at the sample plane was calculated as 6.0 mW/μm$^2$ from the measured laser power at the sample position and the area of the illumination line. To obtain Raman images, the Raman spectral data set was further processed using the singular value decomposition (SVD) technique for noise reduction.[4] We selected the several spectral regions (705-3105 cm$^{-1}$ for H-C-H/C=C-H bonds and cytochrome *c*, 1500-1800 cm$^{-1}$ for C=C bond, 2000-2300 cm$^{-1}$ for D-C-D bond) in the calculation procedure for SVD to avoid artifacts in constructed images. A modified polyfit technique[5] was then used at each pixel to determine the autofluorescence baseline signal, which was subtracted from the original Raman spectrum. Finally, Raman images were reconstructed using each vibrational band of interest. All data processing was performed by Raman Viewer image processing software (Nanophoton, Japan).

Cellular distribution of mitochondria was compared with those of all-D-GLA and lipid droplets (Figs. S3 and S4). All-D-GLA colocalized with lipid droplets but not mitochondria in VA-13 cells (Fig. S3).

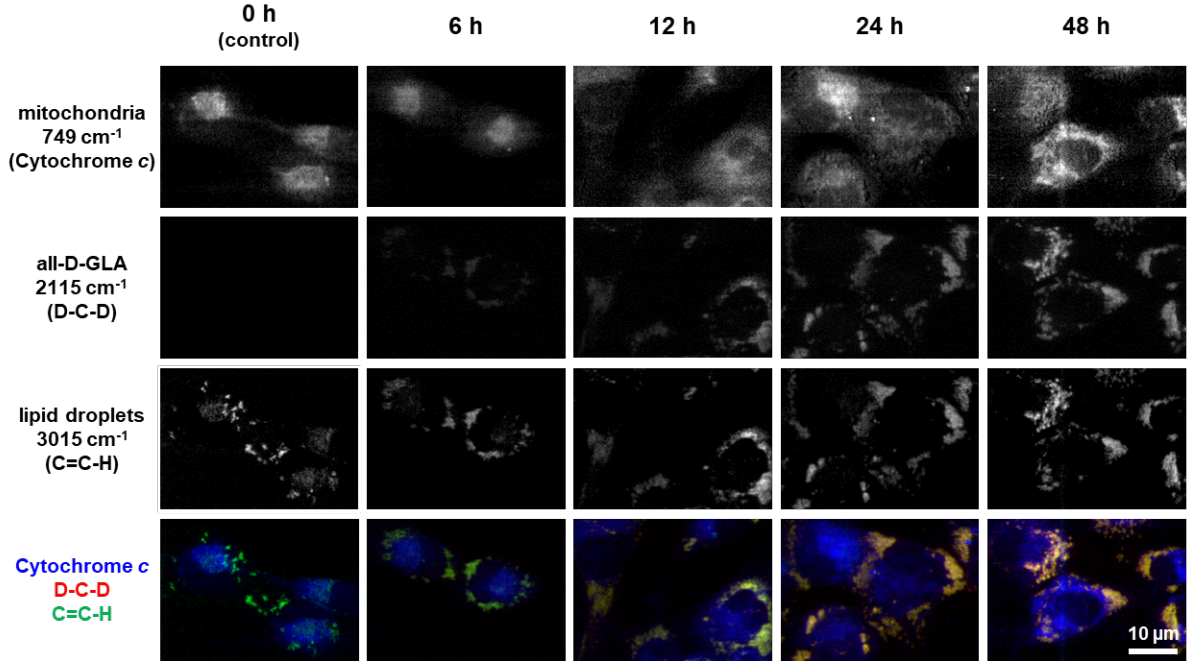



**Fig. S3** Comparison of Raman images of mitochondria, all-D-GLA, lipid droplets in VA-13 cells. VA-13 cells were treated with 100 μM all-D-GLA. The Raman signals at 749 cm$^{-1}$, 2115 cm$^{-1}$ and 3015 cm$^{-1}$ were assigned to the blue, red and green channels, respectively, and merged images were generated.

In WI-38 cells, all-D-GLA mainly localized in lipid droplets at first, however a diffuse distribution in the cytoplasm was also observed during 48 h incubation (Fig. S4). Therefore, it is difficult to confirm all-D-GLA is not incorporated into mitochondria in WI-38 cells. GLA could be also used to synthesize membrane phospholipids in the same manner as other PUFAs. Due to the limitation of

resolution, it is difficult to discriminate between mitochondria and other organelles. Therefore, there is the possibility that GLA may be incorporated into mitochondria in WI-38 cells.

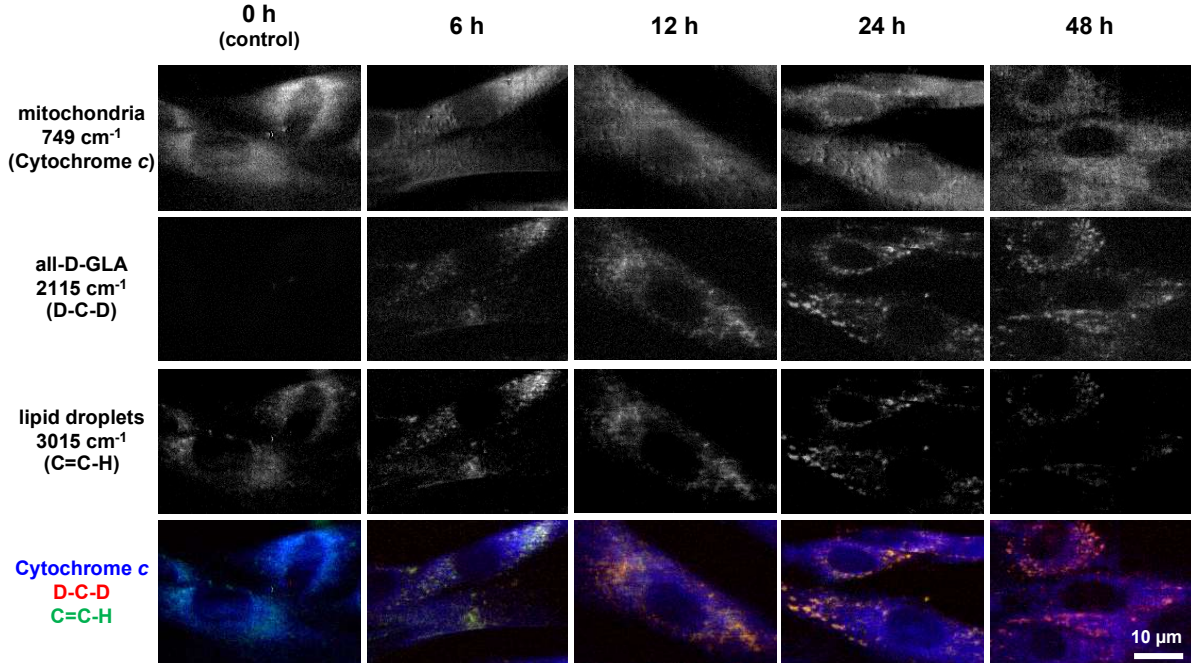



**Fig. S4** Comparison of Raman images of mitochondria, all-D-GLA, lipid droplets in WI-38 cells. WI-38 cells were treated with 100 μM all-D-GLA. The Raman signals at 749 $cm^{-1}$, 2115 $cm^{-1}$ and 3015 $cm^{-1}$ were assigned to the blue, red and green channels, respectively, and merged images were generated.

In Fig. S5A, average Raman spectra in lipid droplets region of five VA-13/WI-38 were obtained by removing the water background. To show the changes clearly, the Raman peak intensities of D-C-D, H-C-H, and C=C-H were calculated as the difference of the peak top and peak bottom intensity (Fig. S6). In VA-13 cells, all-D-GLA accumulated into lipid droplets in proportion to incubation time (Fig. S6A). In contrast, in WI-38 cells, the accumulation of all-D-GLA reached a plateau around 12-24 h, and the signals from endogenous lipids decreased gradually (Fig. S6B). These data indicate the limited uptake of GLA and replacement of endogenous fatty acids by all-D-GLA in WI-38 cell. These differences in lipid droplets might be responsible for tumor-selective cytotoxicity of GLA.

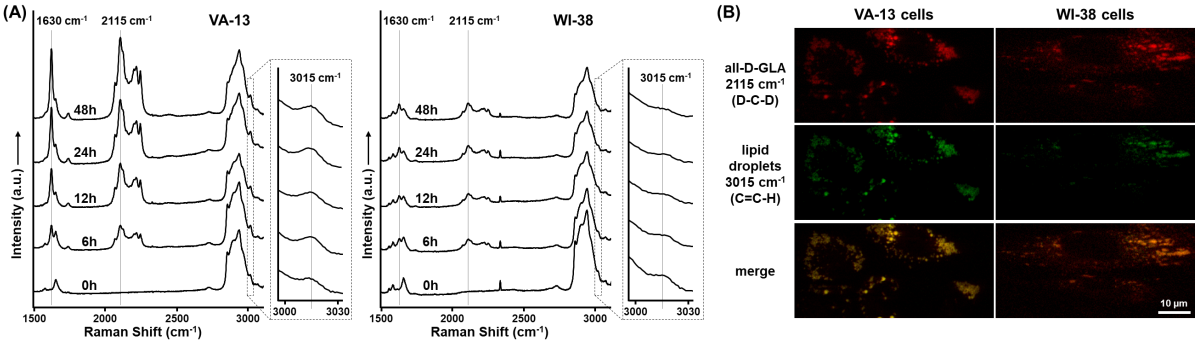



**Fig. S5** (A) Average Raman spectra in the lipid droplets region (3 μm × 3.8 μm: 10 × 10 pixels) of five VA-13 and WI-38 cells treated with 100 μM all-D-GLA. (B) Comparison of Raman images of all-D-GLA and lipid droplets in VA-13 and WI-38 cells. VA-13 and WI-38 cells were treated with 100 μM all-D-GLA for 48 hr. The Raman signals at 2115 $cm^{-1}$ and 3015 $cm^{-1}$ were assigned to the red and green channels, respectively, and merged images were generated.

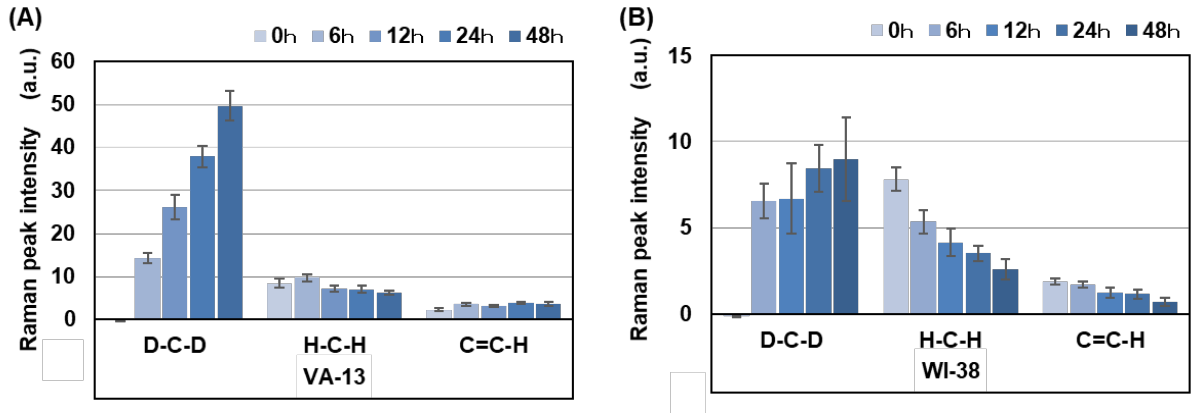



**Fig. S6** Raman peak intensities of D-C-D (2115 $cm^{-1}$), H-C-H (2854 $cm^{-1}$), and C=C-H (3015 $cm^{-1}$) in the lipid droplets region of VA-13 (A) and WI-38 cells (B) treated with 100 μM all-D-GLA. Error bars indicate the standard error of the mean (n = 5).

## References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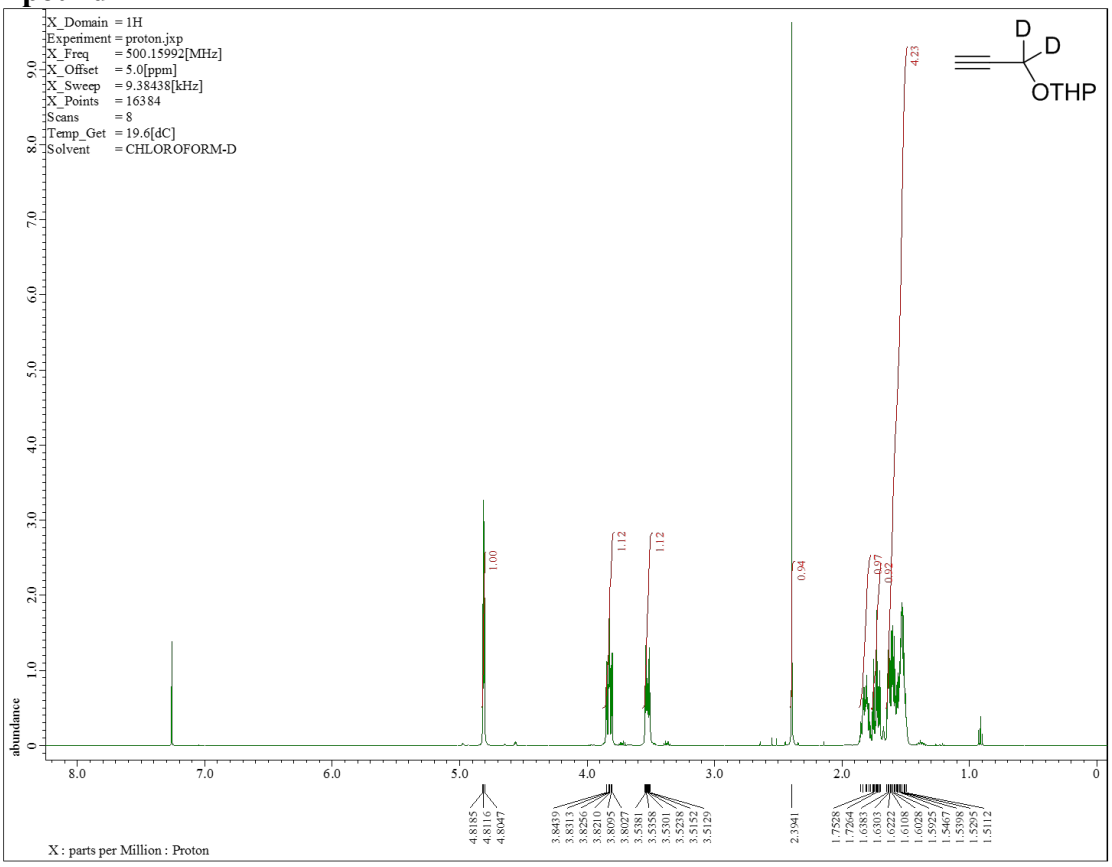
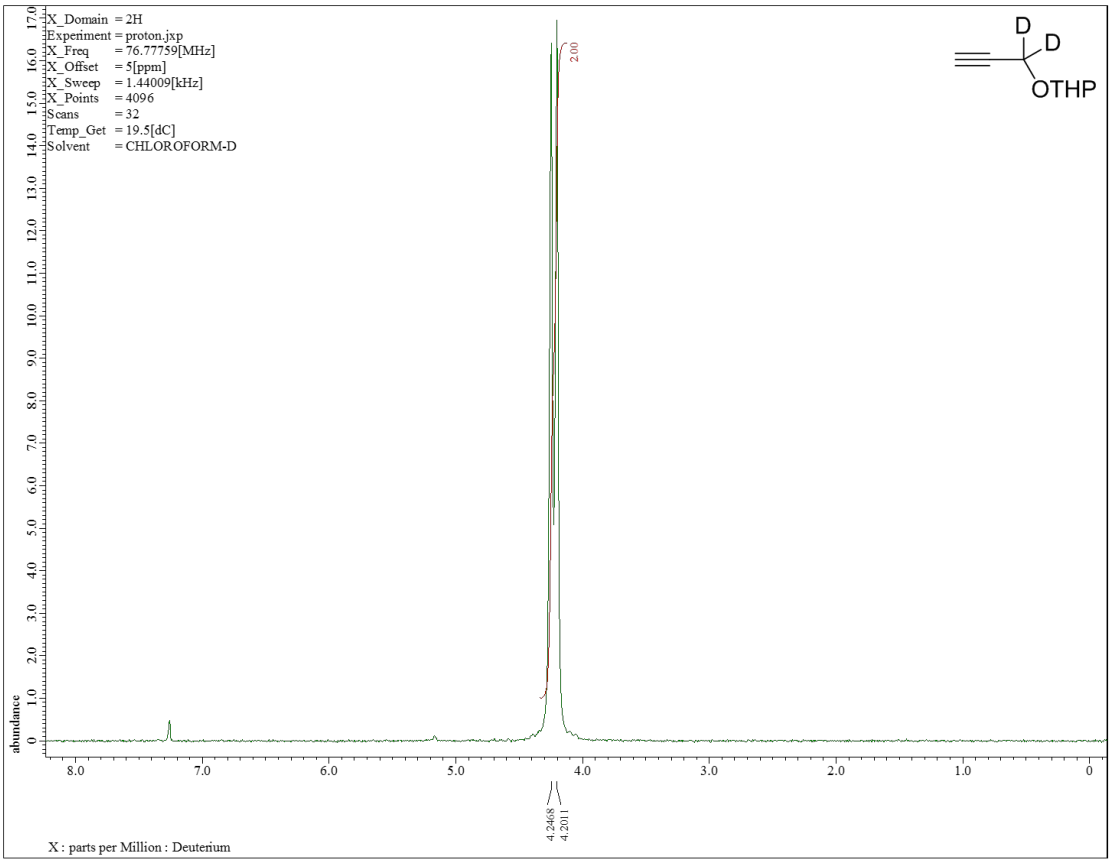
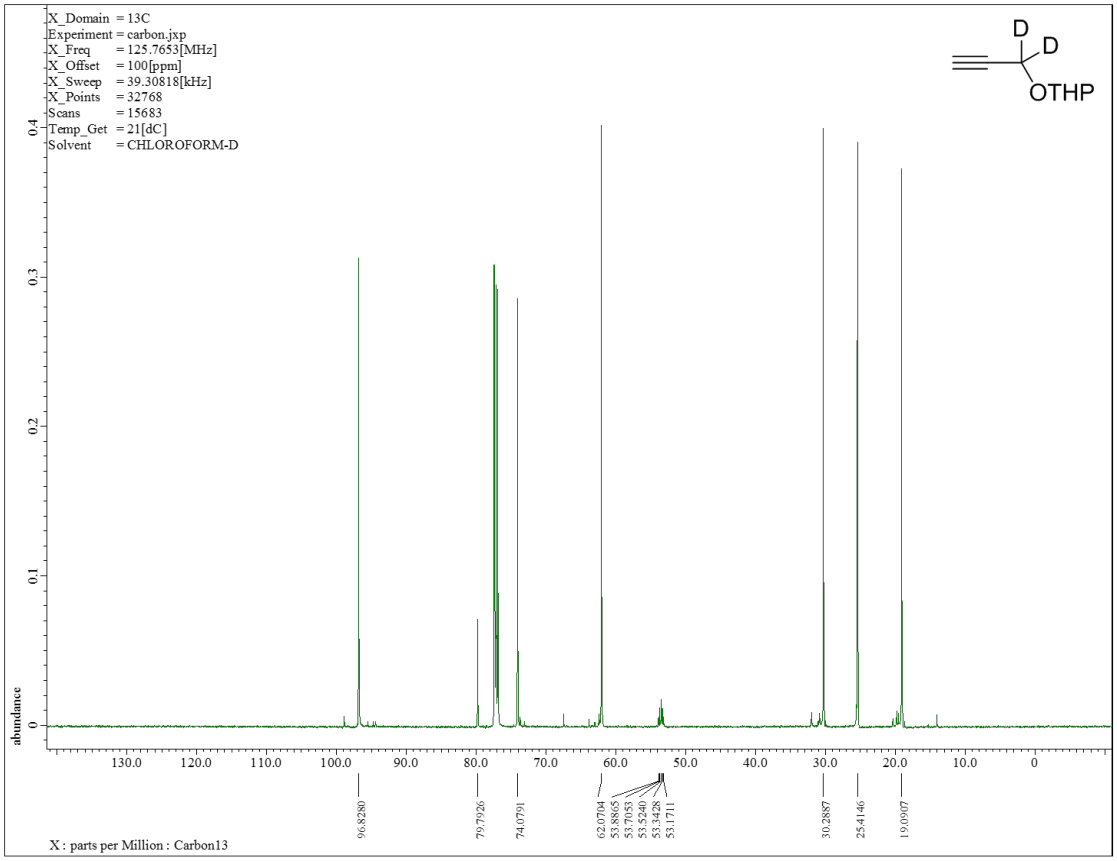
# NMR spectra

## Compound 2

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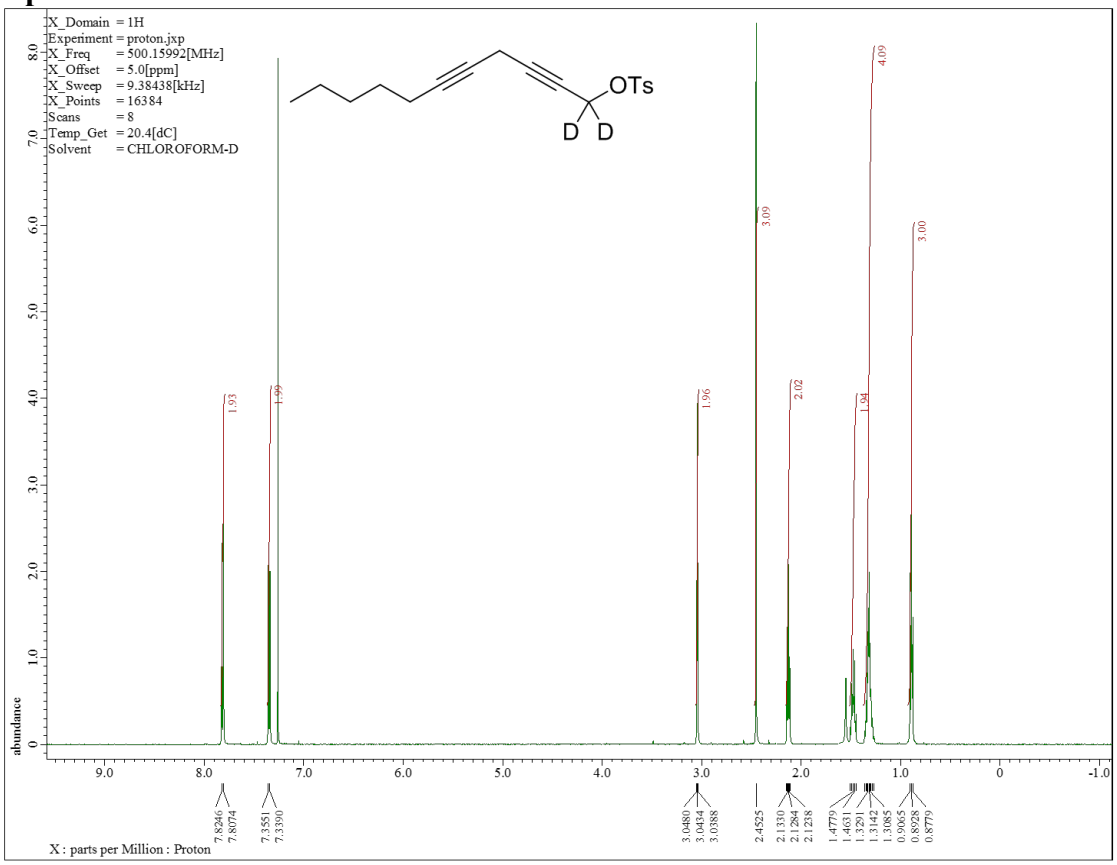
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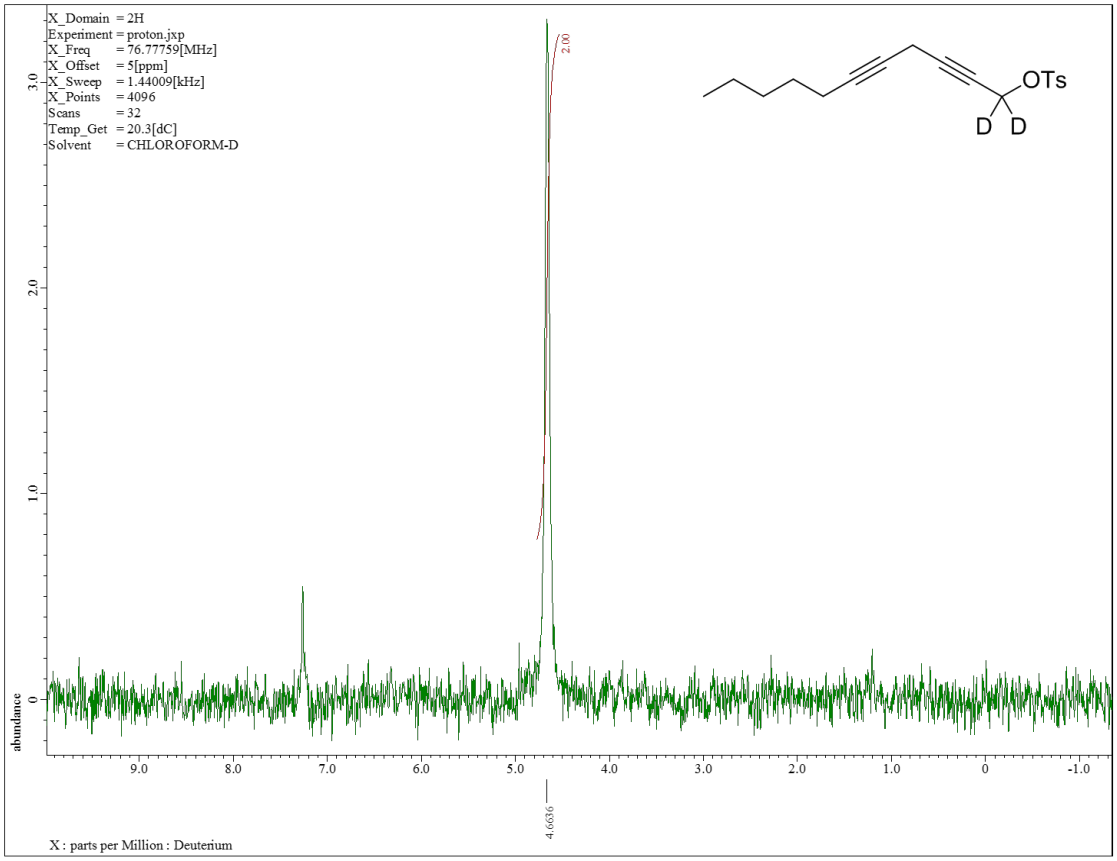
D D
abundance
X : parts per Million : Proton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OTs
D D
abundance
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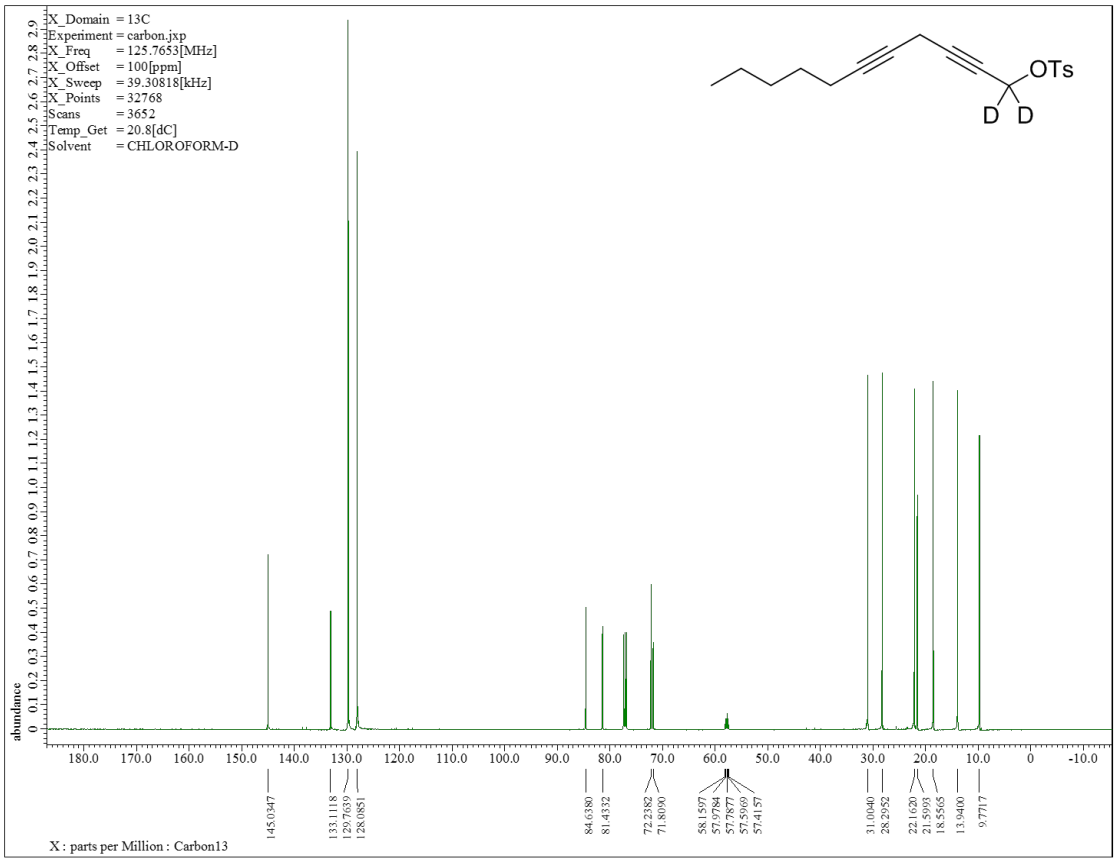
X : parts per Million : Deuterium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81.4332
72.2382
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57.9784
57.7877
57.5969
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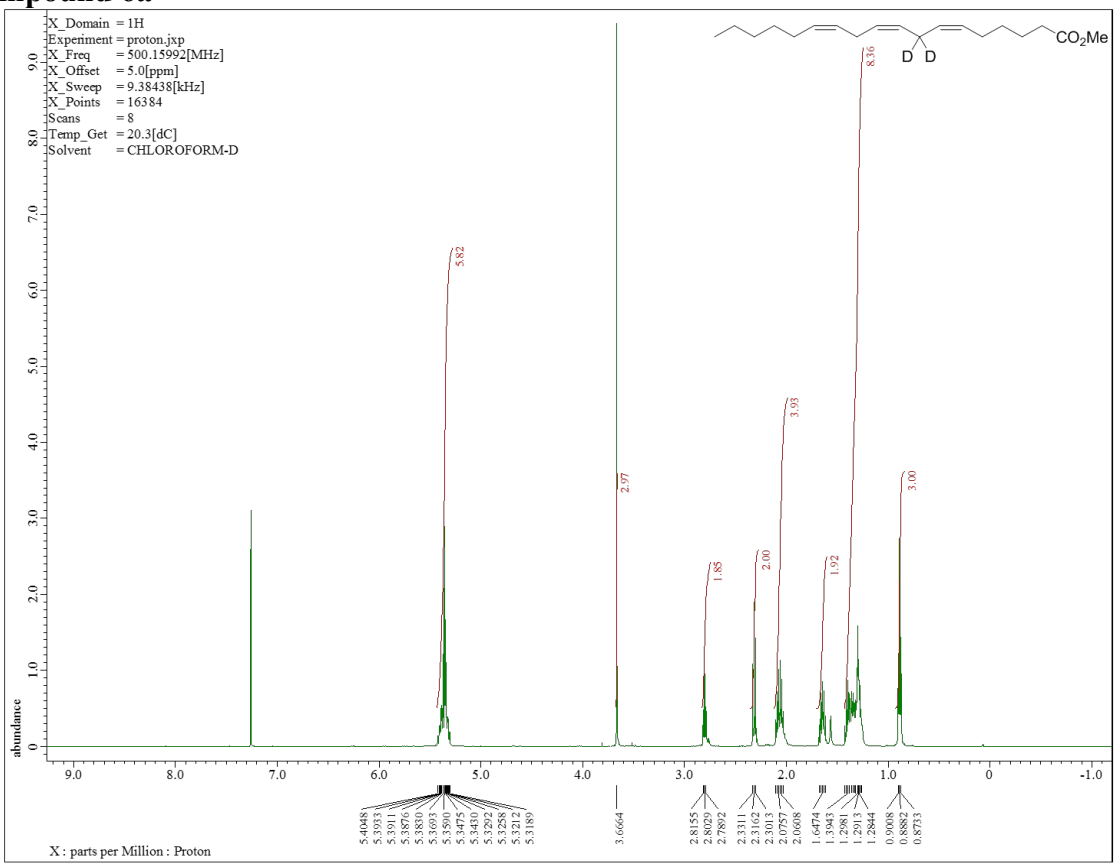
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3.93
1.92
3.00
abundance
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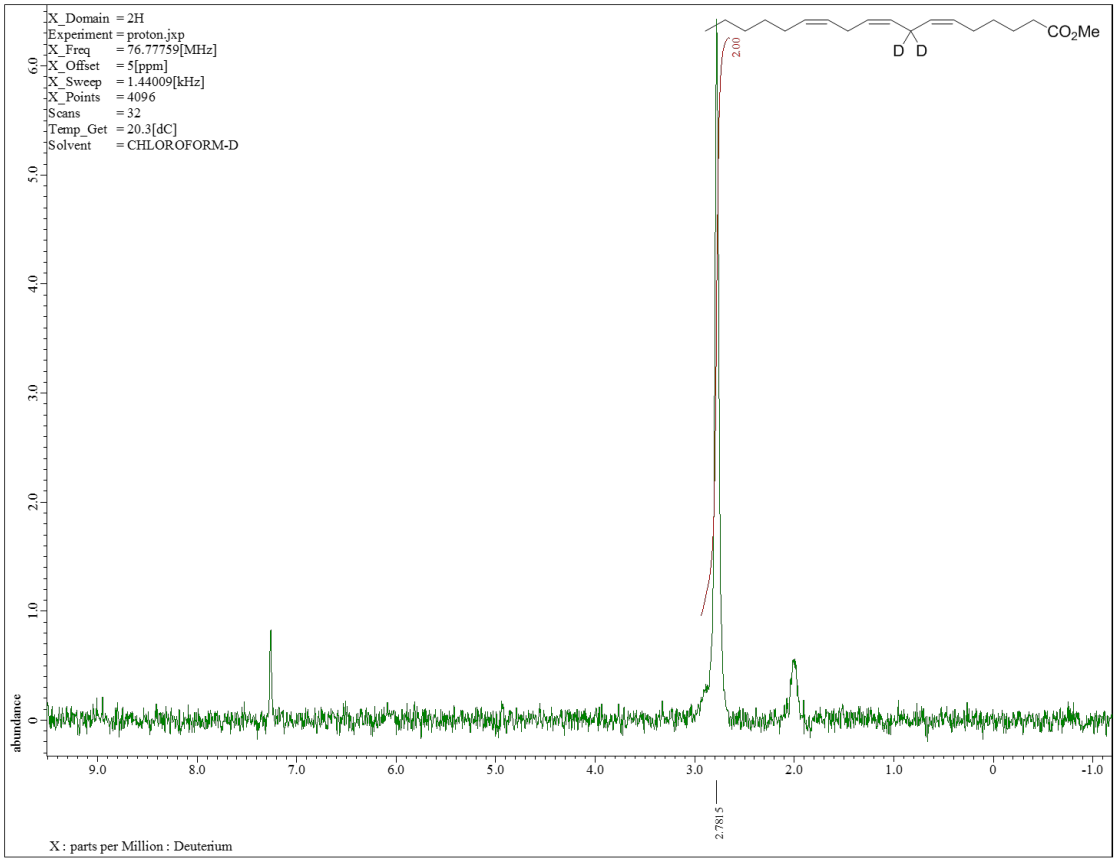
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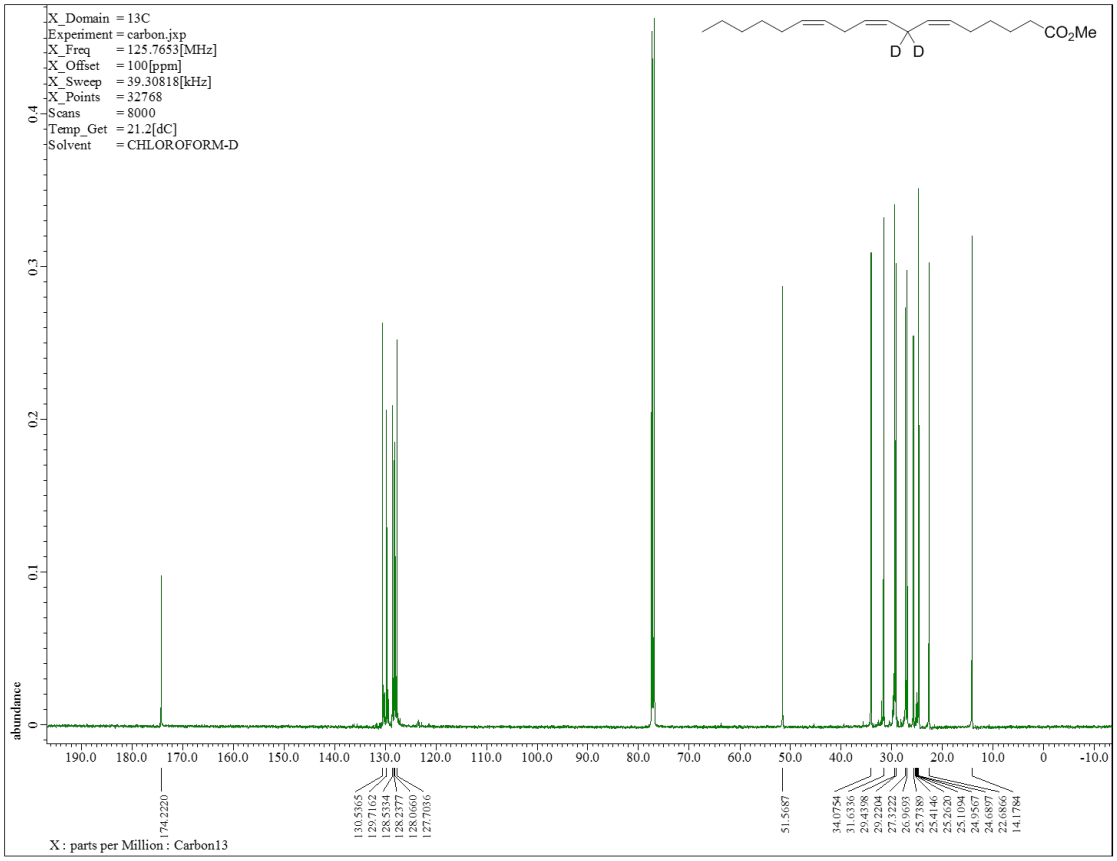
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D D
abundance
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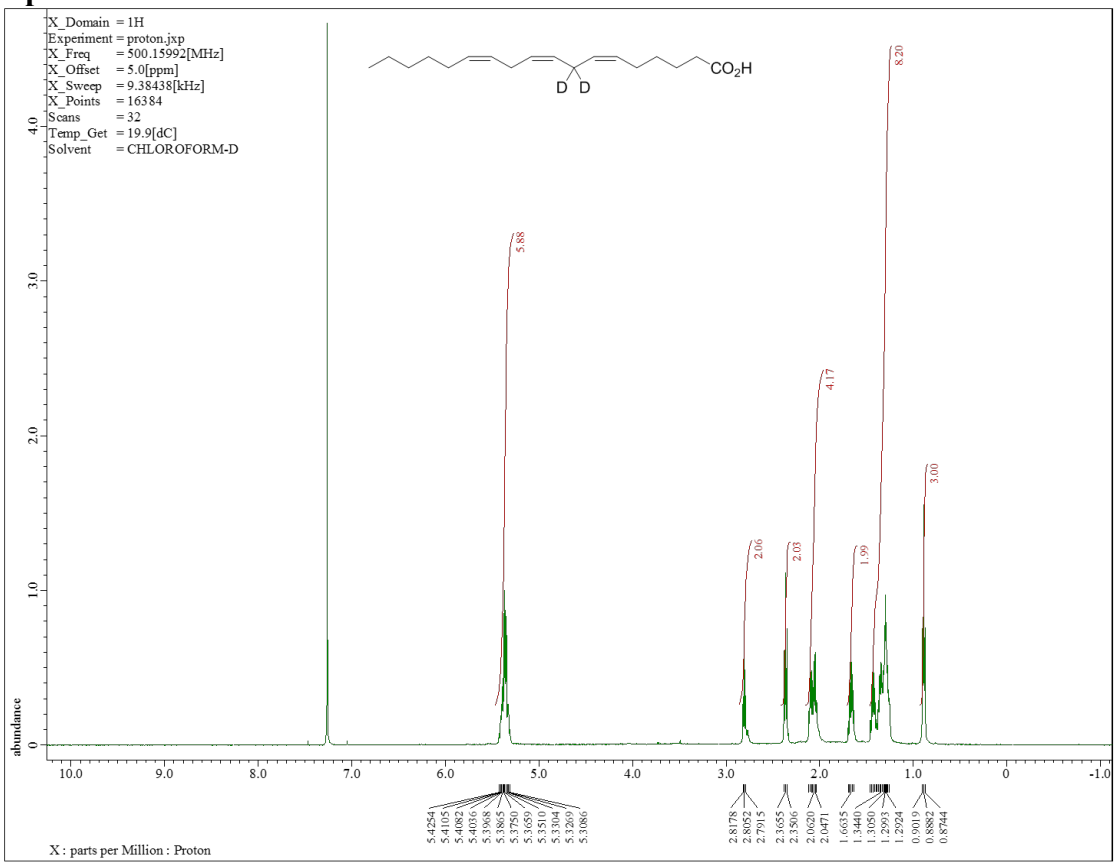
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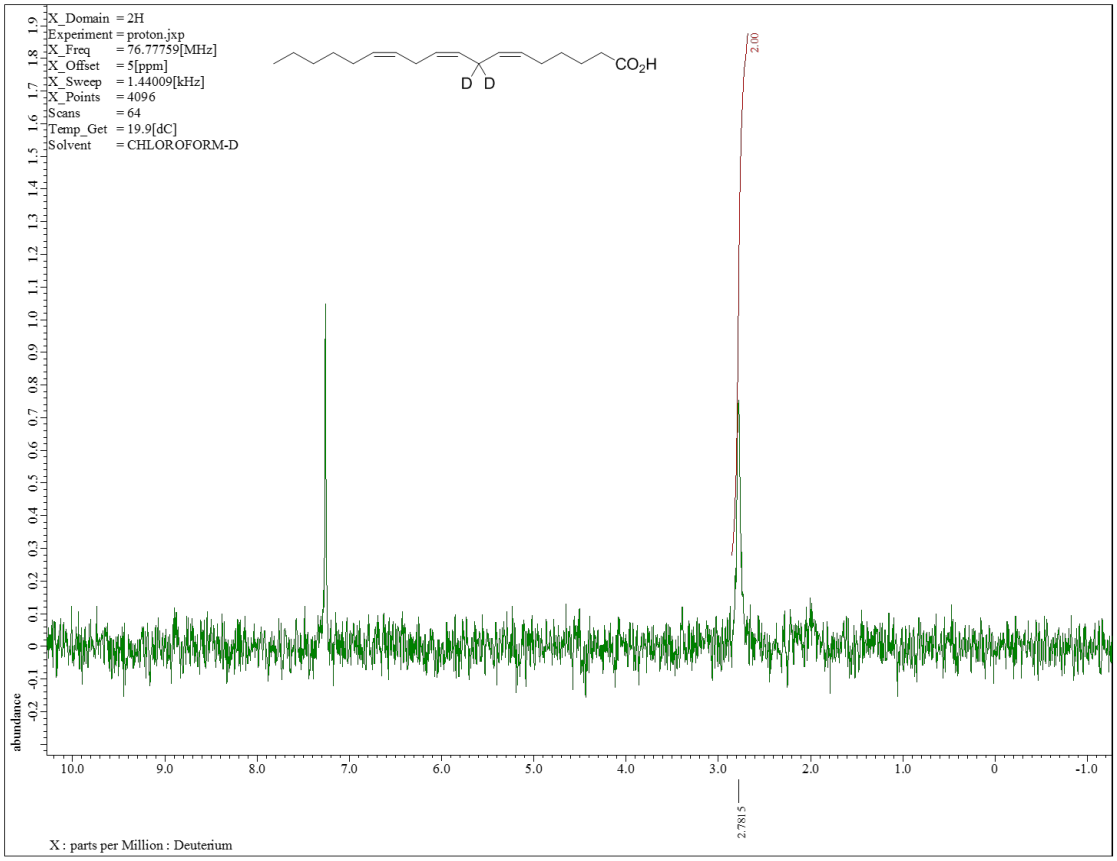
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CO2H
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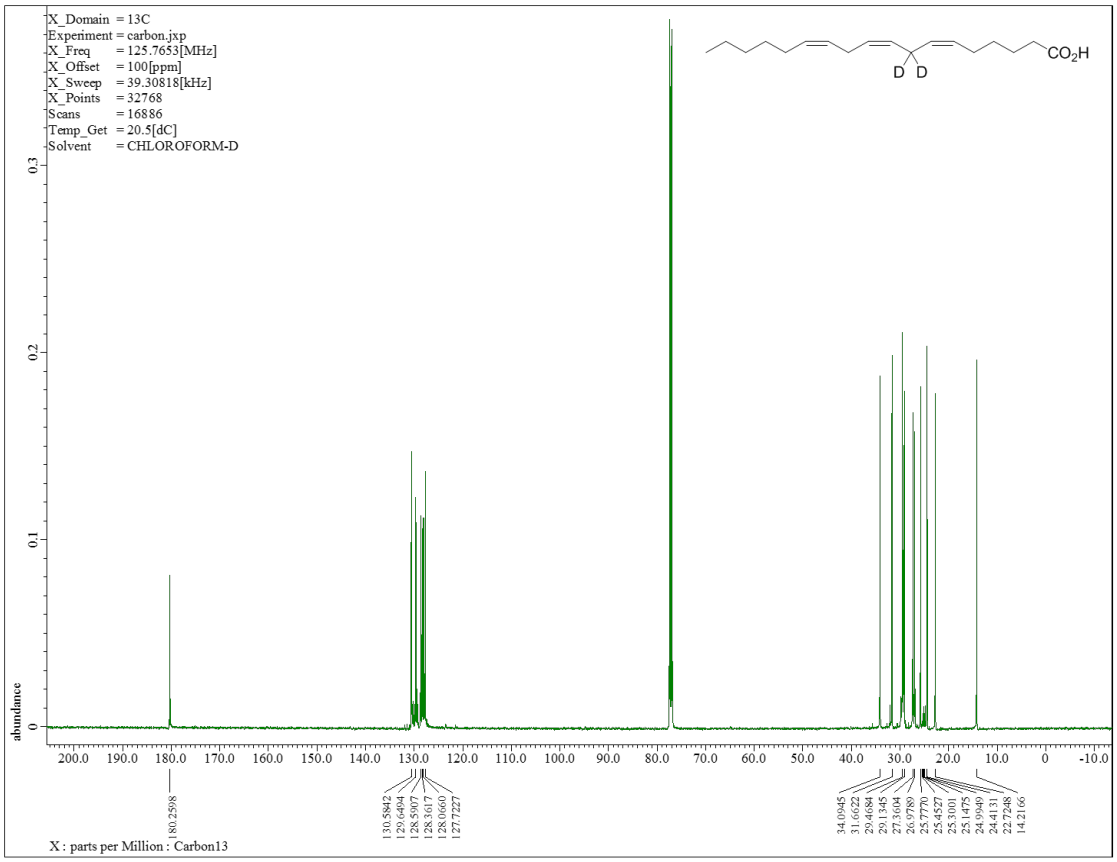
abundance
X : parts per Million : Deuterium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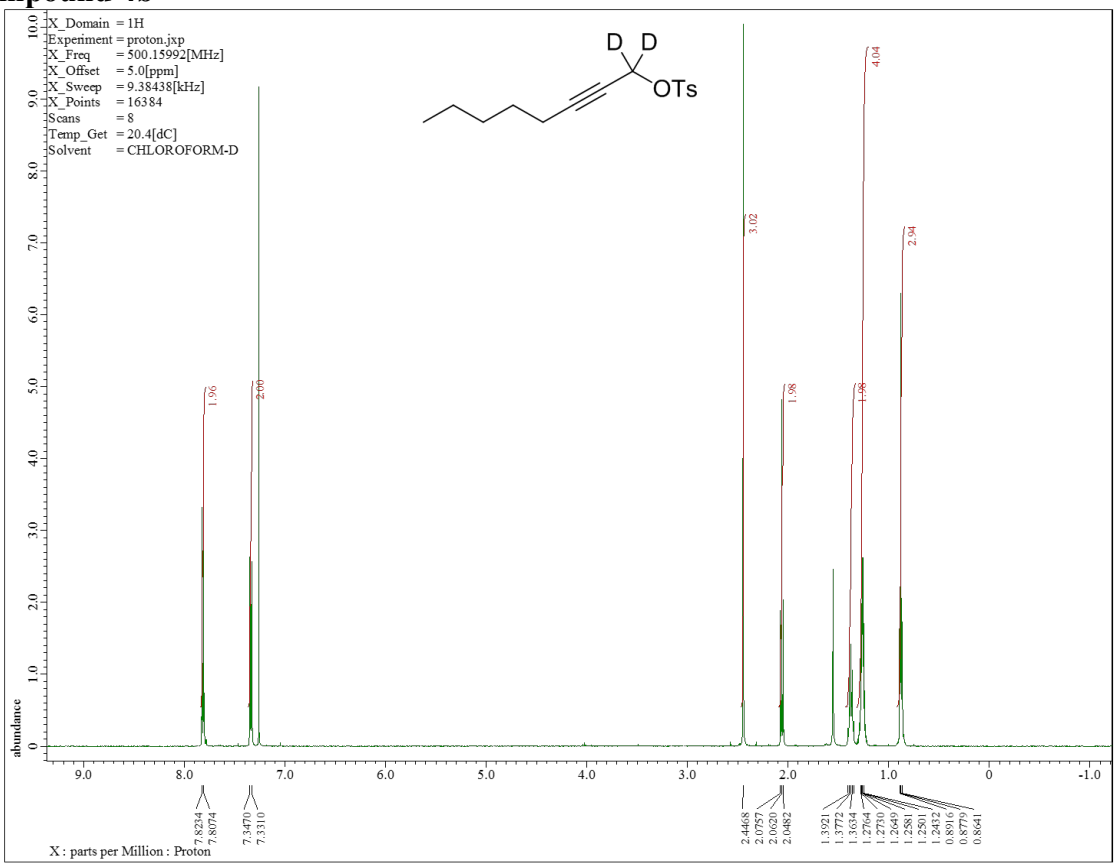
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abundance
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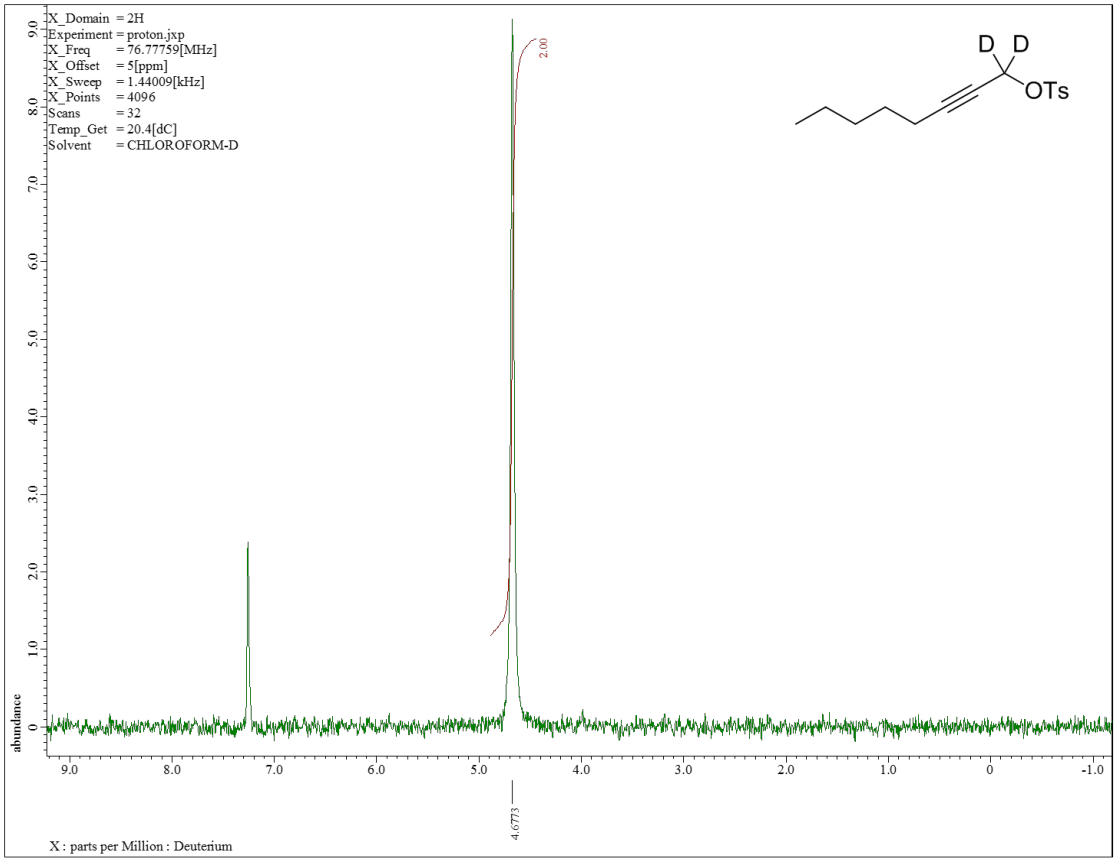
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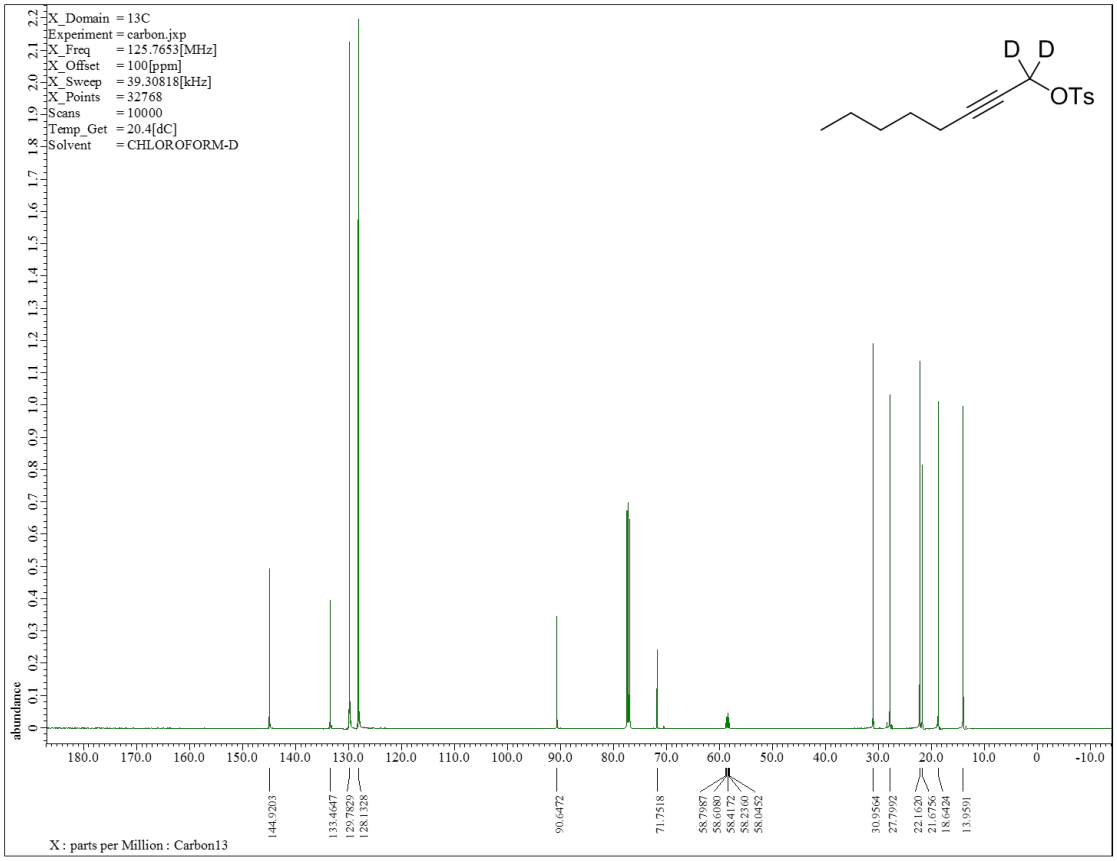
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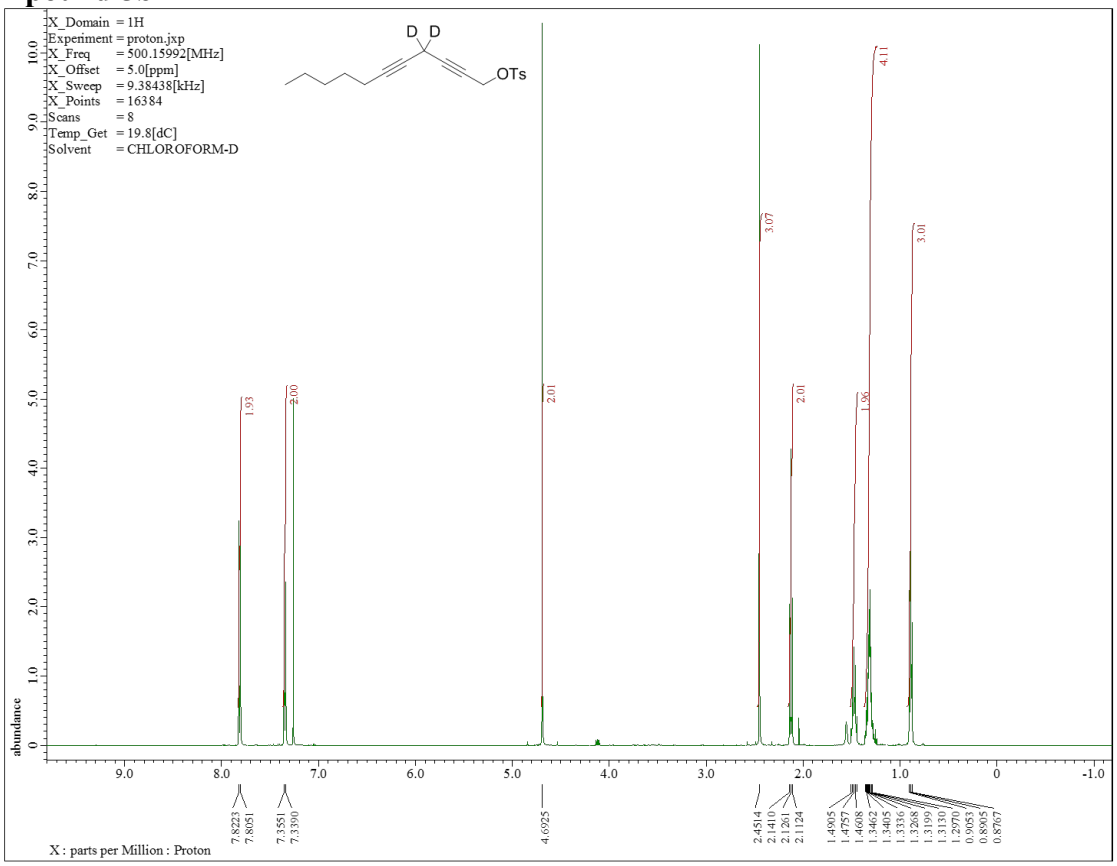
## Compound 5b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D D
OTs
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2.01
3.07
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1.96
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abundance
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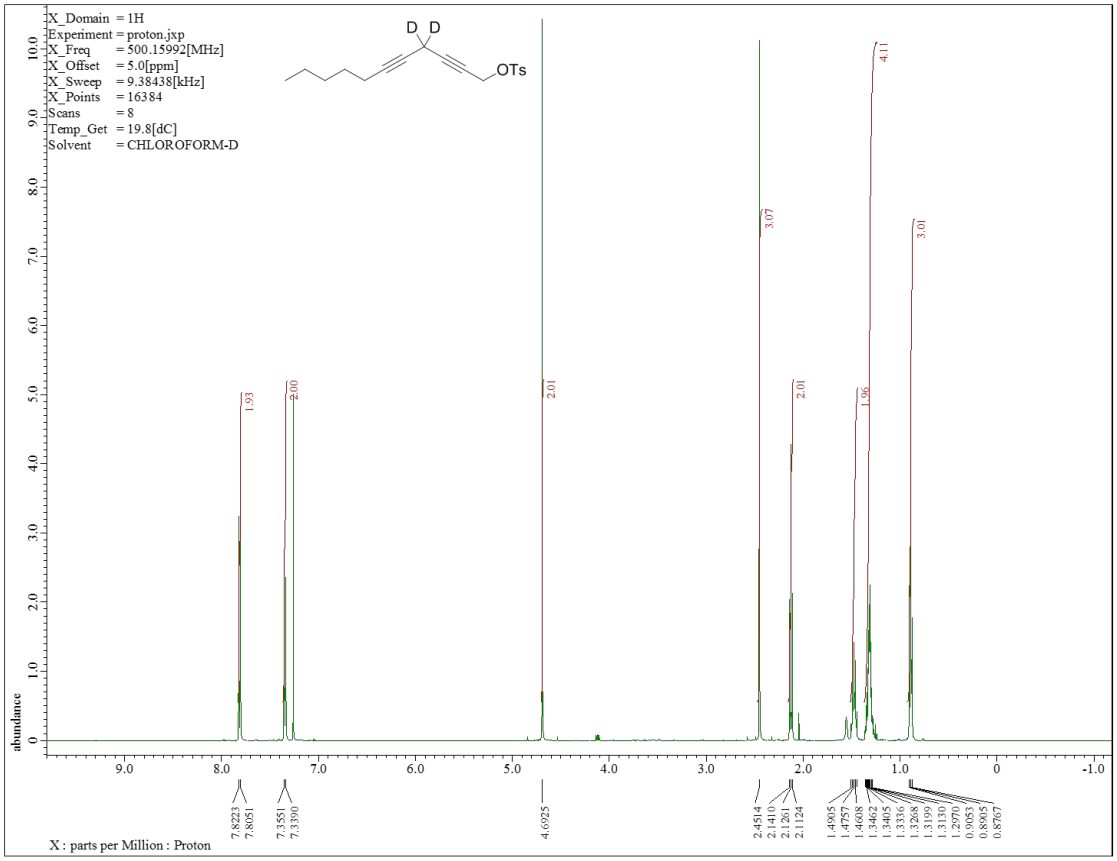
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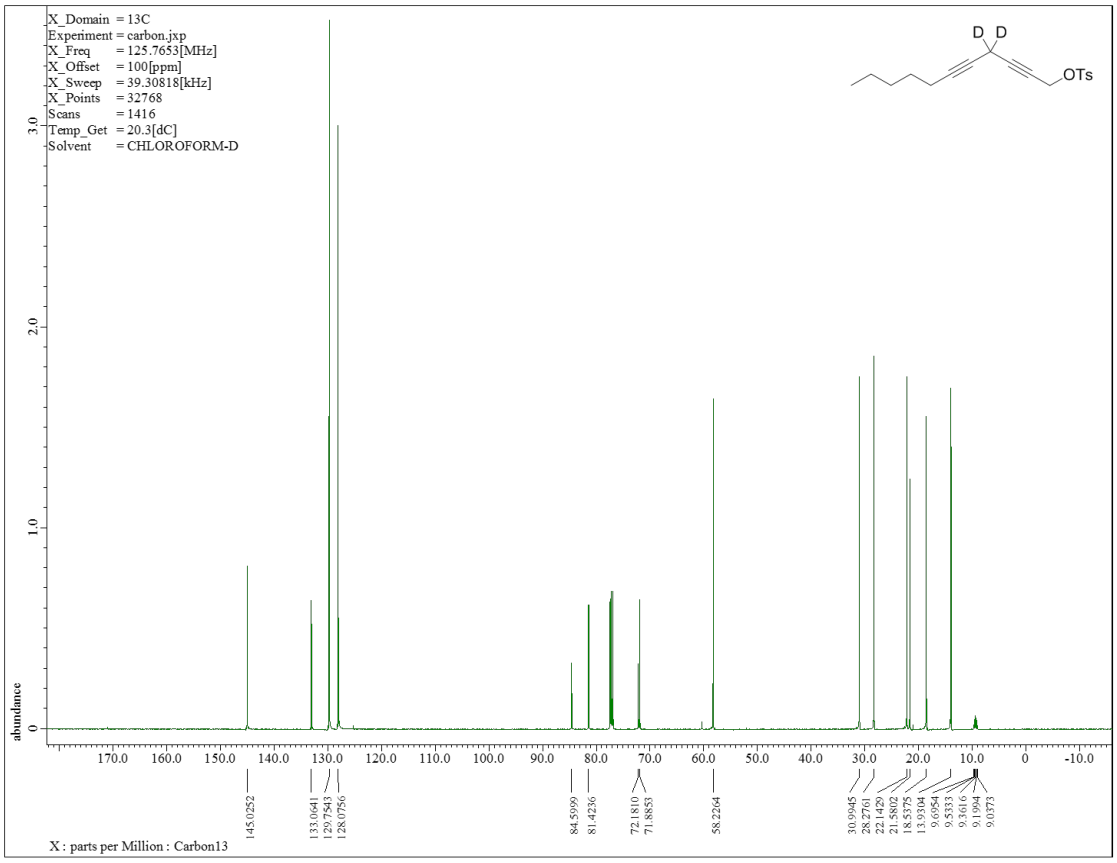
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OTs
abundance
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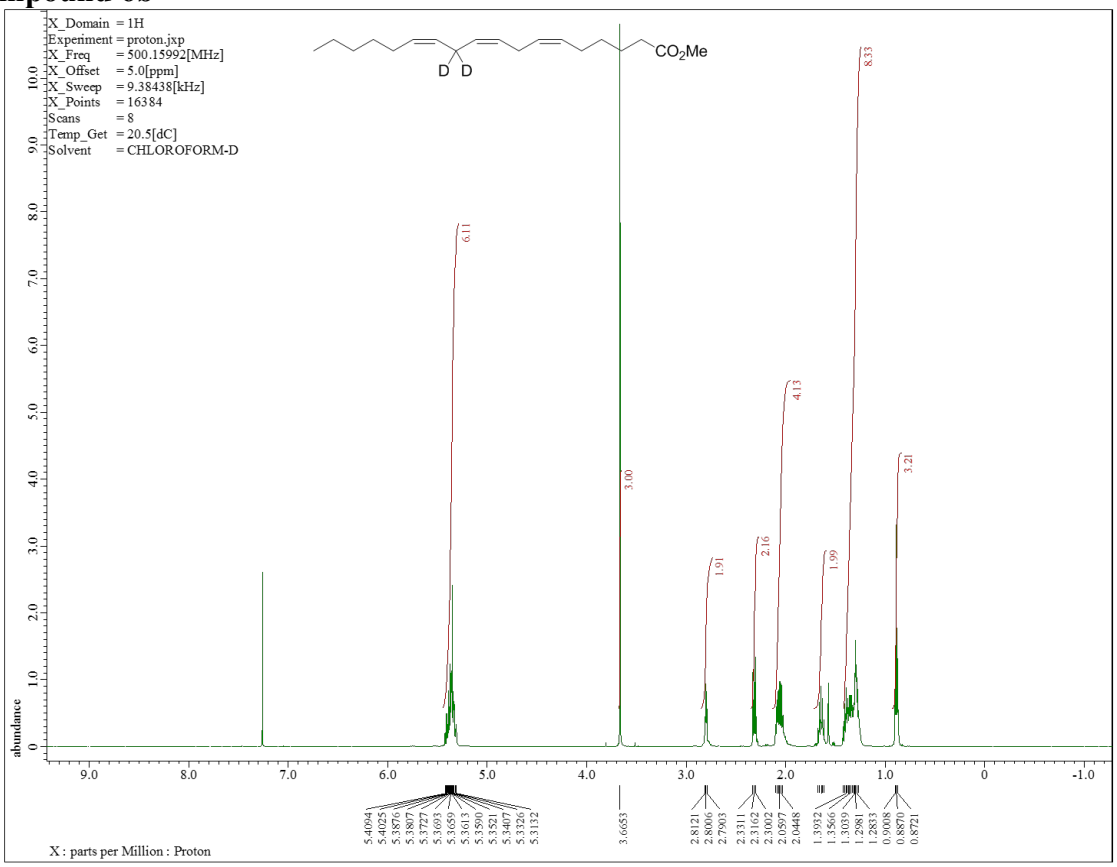
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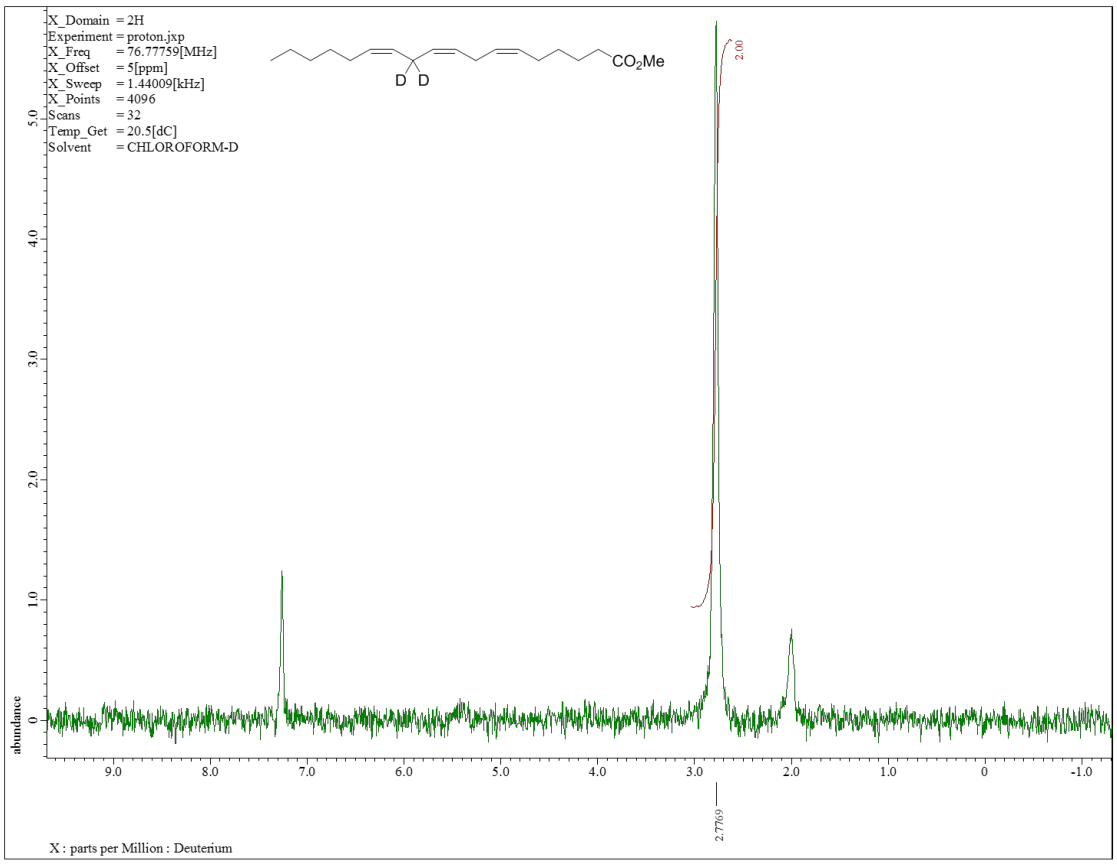
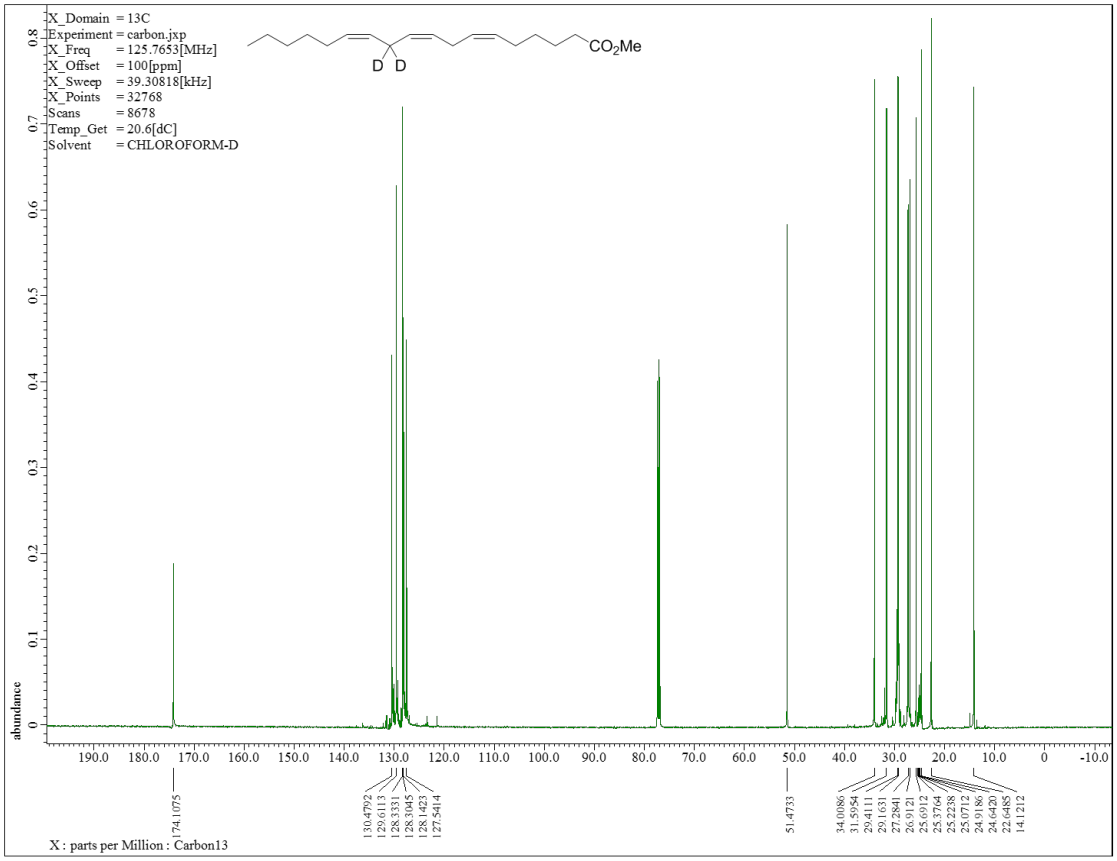
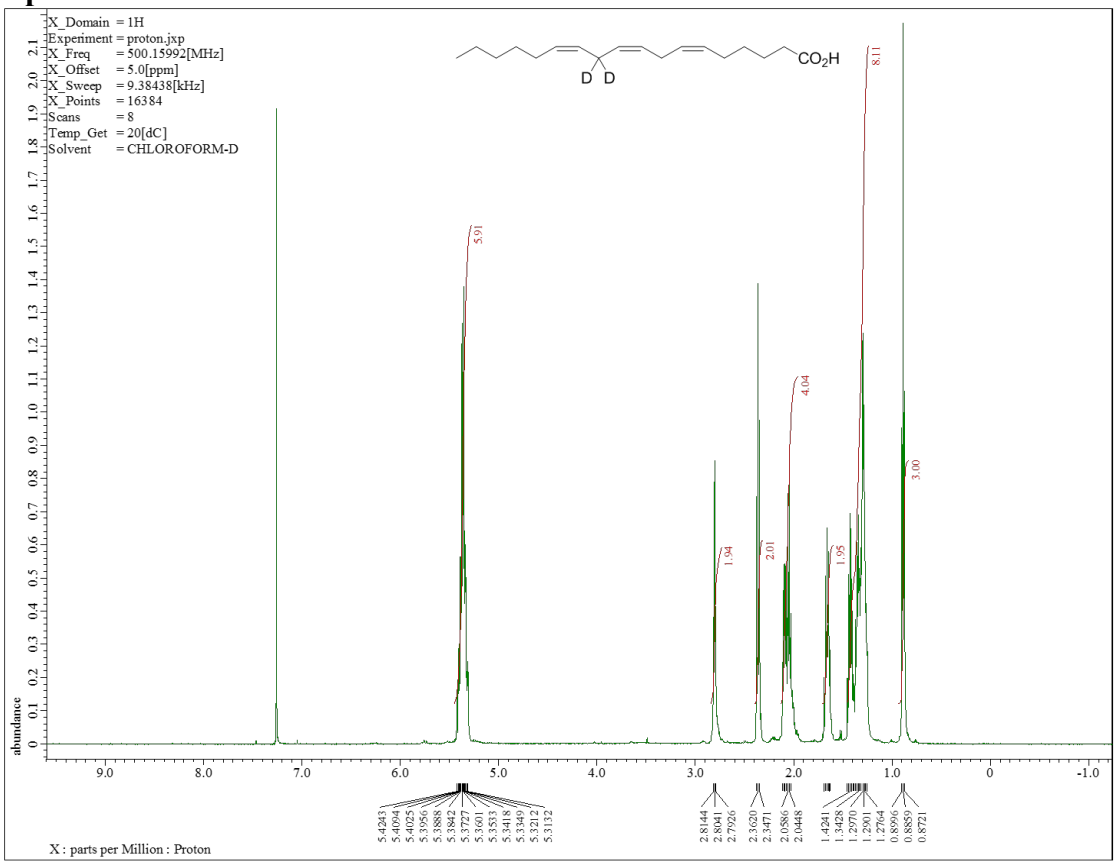
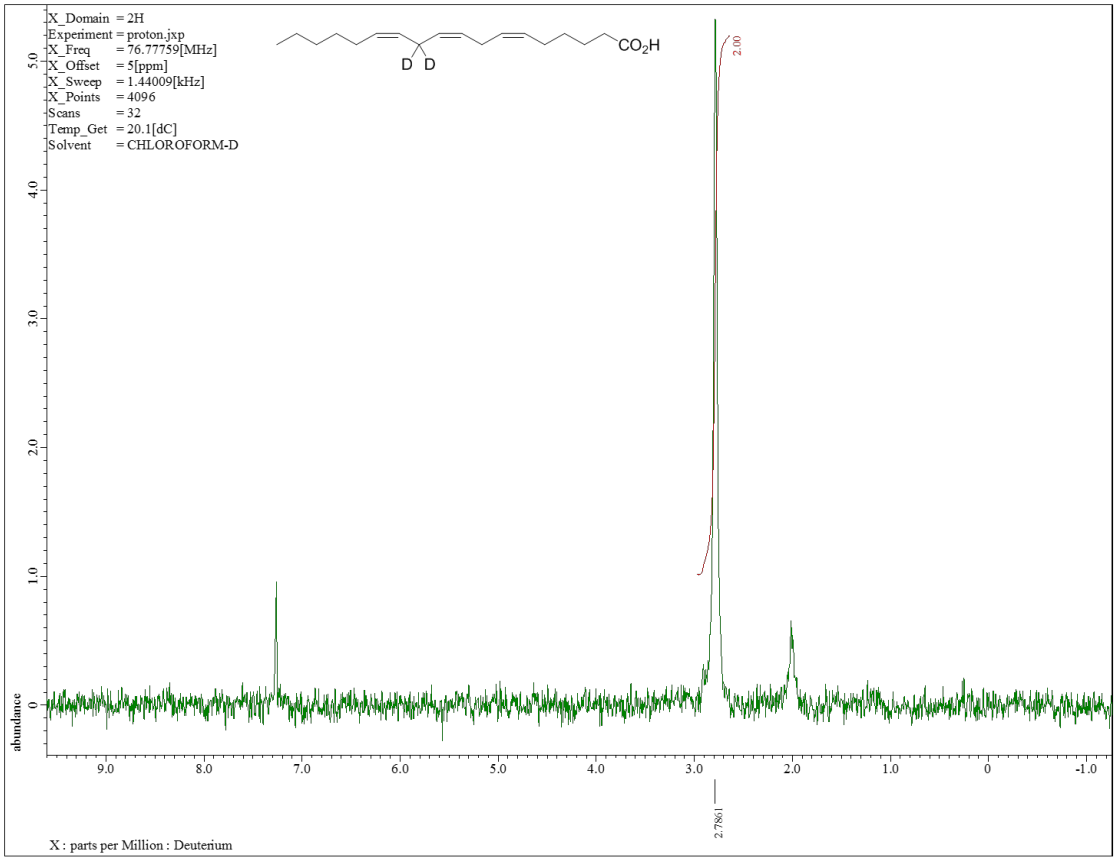
abundance
X : parts per Million : Proton

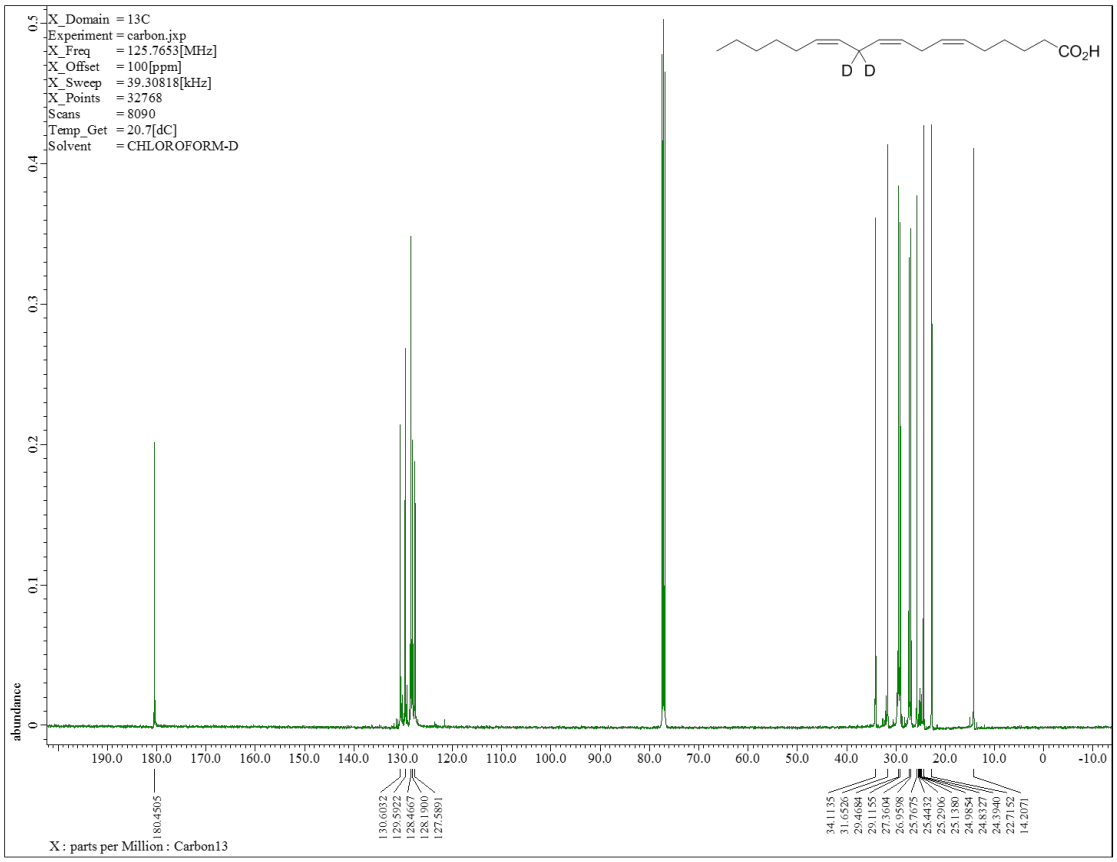

X_Domain = 2H
Experiment = proton.jxp
X_Freq = 76.77759[MHz]
X_Offset = 5[ppm]
X_Sweep = 1.44009[kHz]
X_Points = 4096
Scans = 32
Temp_Get = 20.5[dC]
Solvent = CHLOROFORM-D
D D
CO2Me
2.00
2.7769
abundance
X : parts per Million : Deuterium


X_Domain = 13C
Experiment = carbon.jxp
X_Freq = 125.7653[MHz]
X_Offset = 100[ppm]
X_Sweep = 39.30818[kHz]
X_Points = 32768
Scans = 8678
Temp_Get = 20.6[dC]
Solvent = CHLOROFORM-D
D D
CO2Me
abundance
X : parts per Million : Carbon13

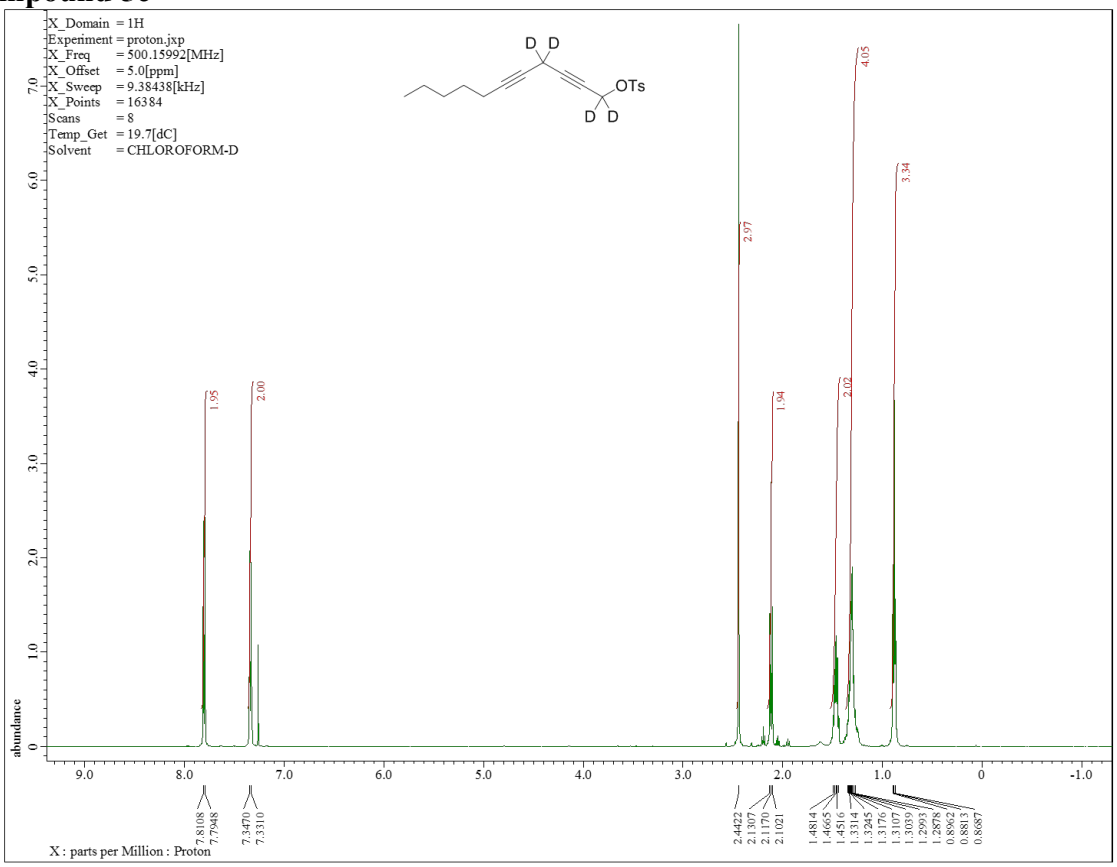
## Compound 7b

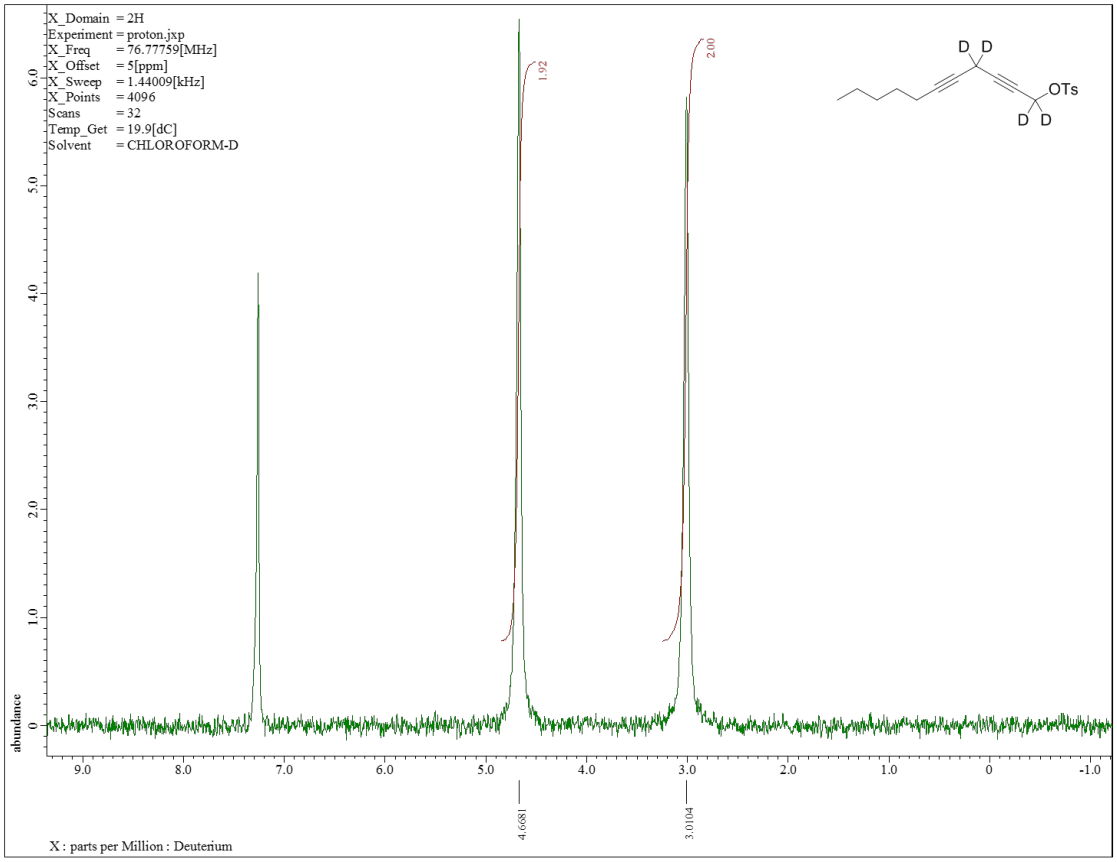

X_Domain = 1H
Experiment = proton.jxp
X_Freq = 500.15992[MHz]
X_Offset = 5.0[ppm]
X_Sweep = 9.38438[kHz]
X_Points = 16384
Scans = 8
Temp_Get = 20[dC]
Solvent = CHLOROFORM-D
D D
CO2H
5.91
8.11
4.04
3.00
1.94
2.01
1.95
abundance
X : parts per Million : Proton

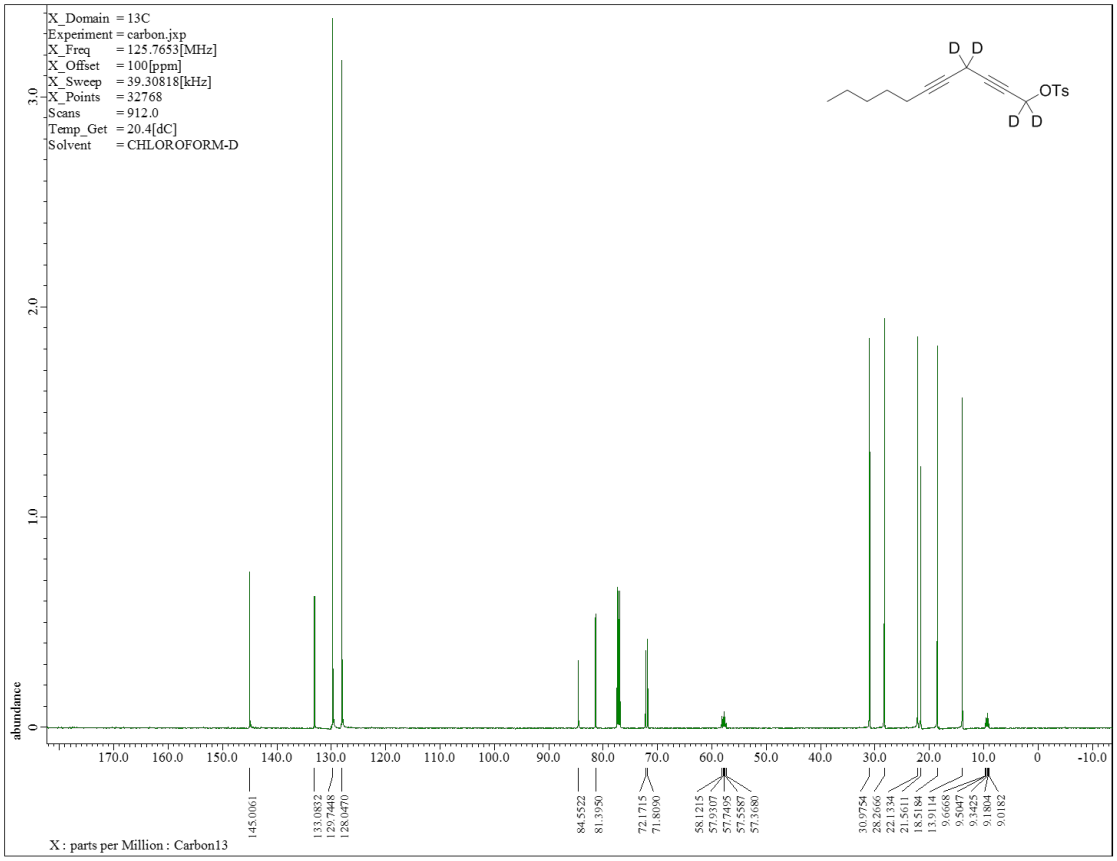

X_Domain = 2H
Experiment = proton.jxp
X_Freq = 76.77759[MHz]
X_Offset = 5[ppm]
X_Sweep = 1.44009[kHz]
X_Points = 4096
Scans = 32
Temp_Get = 20.1[dC]
Solvent = CHLOROFORM-D
CO2H
D D
2.00
2.7861
abundance
X : parts per Million : Deuterium


X_Domain = 13C
Experiment = carbon.jxp
X_Freq = 125.7653[MHz]
X_Offset = 100[ppm]
X_Sweep = 39.30818[kHz]
X_Points = 32768
Scans = 8090
Temp_Get = 20.7[dC]
Solvent = CHLOROFORM-D
CO2H
D D
180.4505
130.6032
129.5922
128.4467
128.1900
127.5891
34.1135
31.6526
29.4684
29.1155
27.3604
26.9598
25.7675
25.4432
25.2906
25.1380
24.9854
24.8327
24.3940
22.7152
14.2071
abundance
X : parts per Million : Carbon13

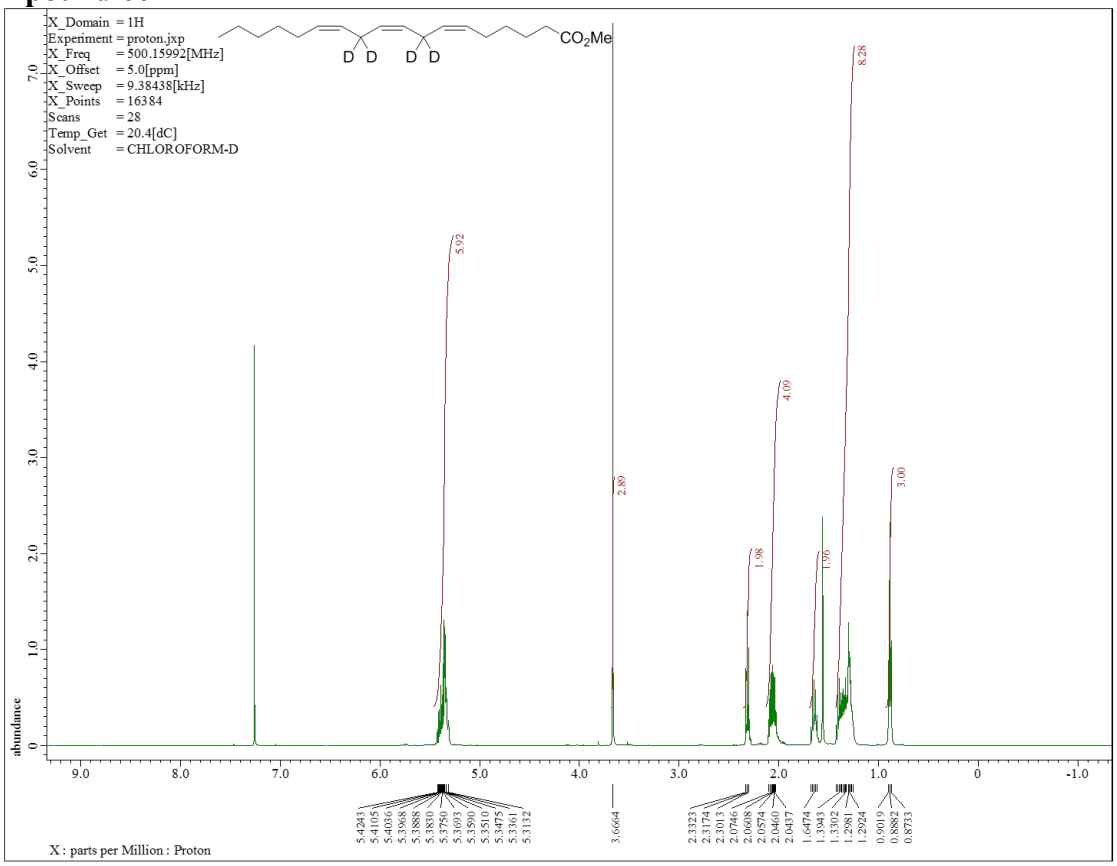
## Compound 5c

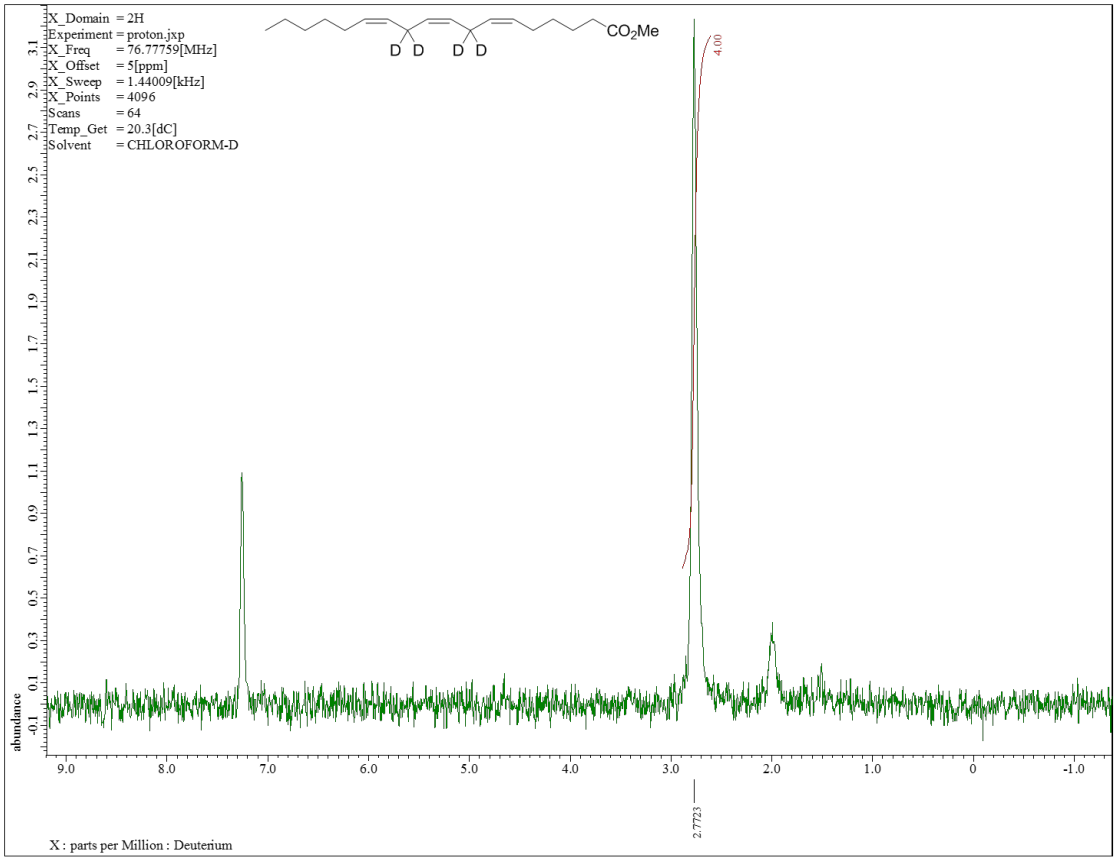

X_Domain = 1H
Experiment = proton.jxp
X_Freq = 500.15992[MHz]
X_Offset = 5.0[ppm]
X_Sweep = 9.38438[kHz]
X_Points = 16384
Scans = 8
Temp_Get = 19.7[dC]
Solvent = CHLOROFORM-D
D D
OTs
D D
1.95
2.00
2.97
1.94
2.02
4.05
3.34
7.8108
7.7948
7.3470
7.3310
2.4422
2.1307
2.1170
2.1021
1.4814
1.4665
1.4516
1.3314
1.3245
1.3176
1.3107
1.3039
1.2993
1.2878
0.8962
0.8813
0.8687
abundance
X : parts per Million : Proton

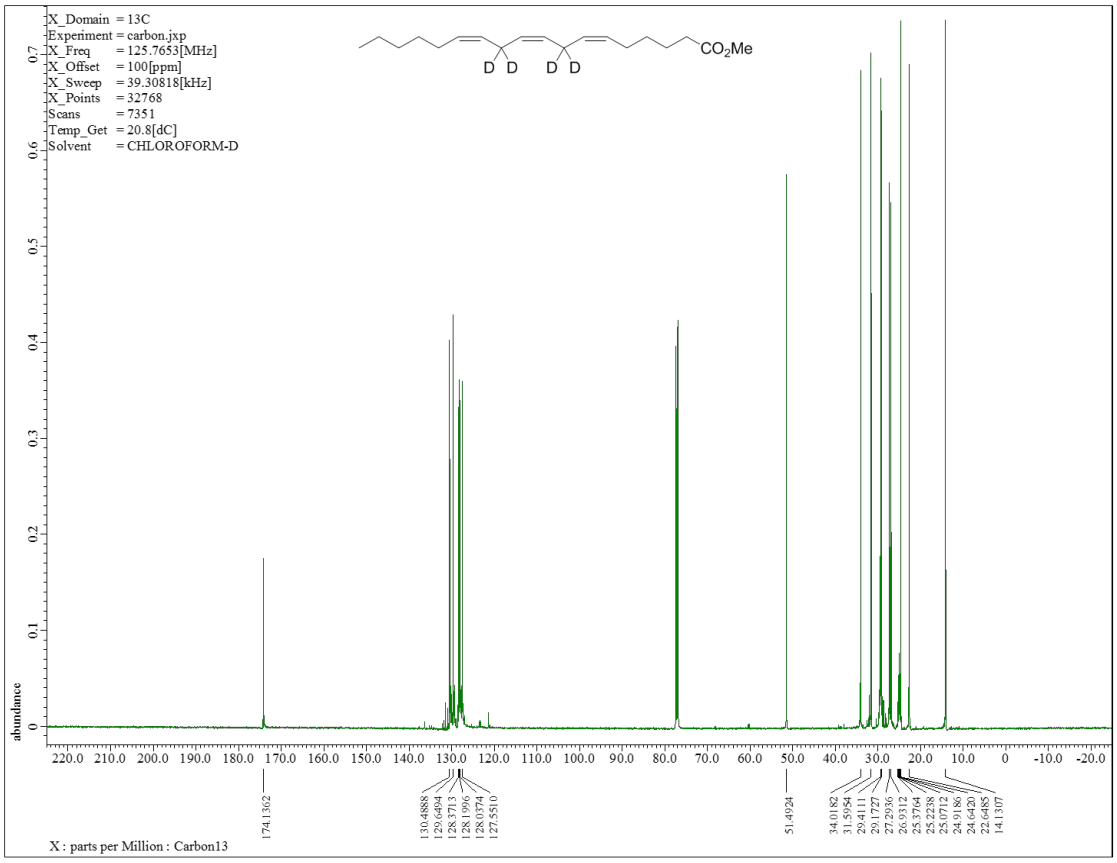

X_Domain = 2H
Experiment = proton.jxp
X_Freq = 76.77759[MHz]
X_Offset = 5[ppm]
X_Sweep = 1.44009[kHz]
X_Points = 4096
Scans = 32
Temp_Get = 19.9[dC]
Solvent = CHLOROFORM-D
D D
OTs
D D
1.92
2.00
4.6681
3.0104
abundance
X : parts per Million : Deuterium


X_Domain = 13C
Experiment = carbon.jxp
X_Freq = 125.7653[MHz]
X_Offset = 100[ppm]
X_Sweep = 39.30818[kHz]
X_Points = 32768
Scans = 912.0
Temp_Get = 20.4[dC]
Solvent = CHLOROFORM-D
D D
OTs
D D
abundance
145.0061
133.0832
129.7448
128.0470
84.5522
81.3950
72.1715
71.8090
58.1215
57.9307
57.7495
57.5587
57.3680
30.9754
28.2666
22.1334
21.5611
18.5184
13.9114
9.6668
9.5047
9.3425
9.1804
9.0182
X : parts per Million : Carbon13

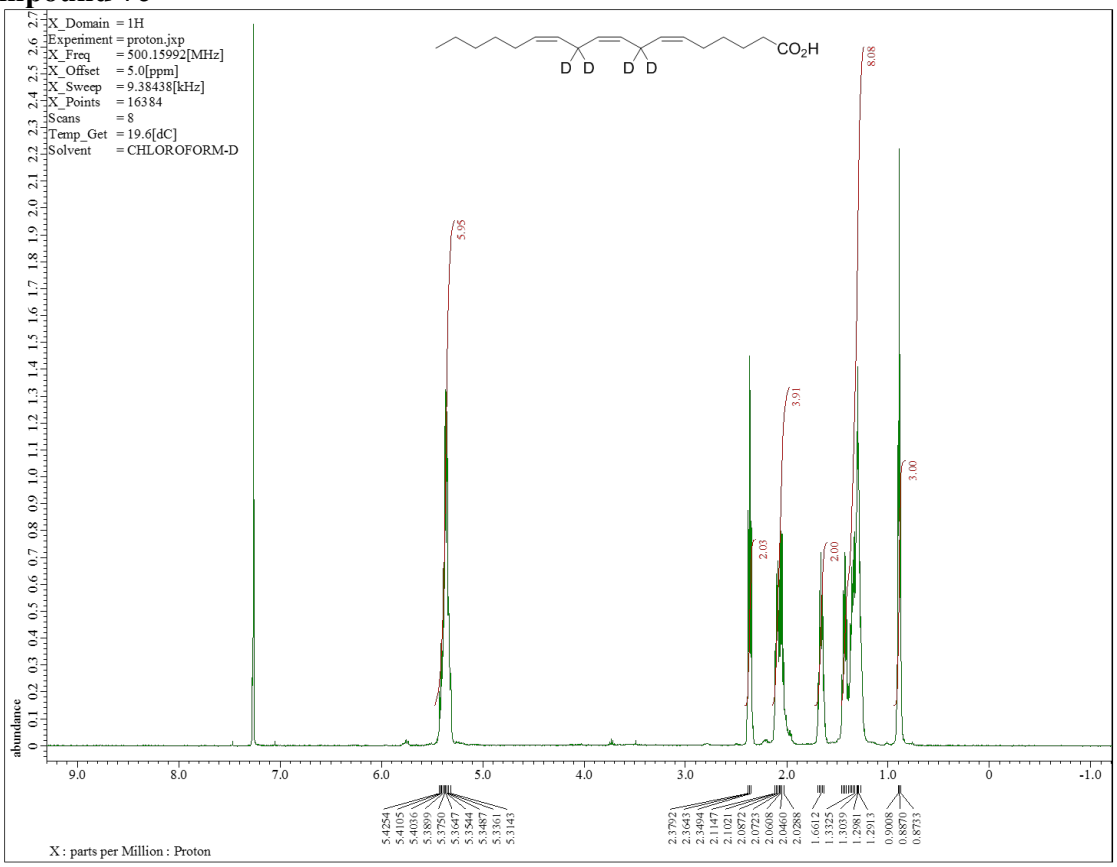
## Compound 6c

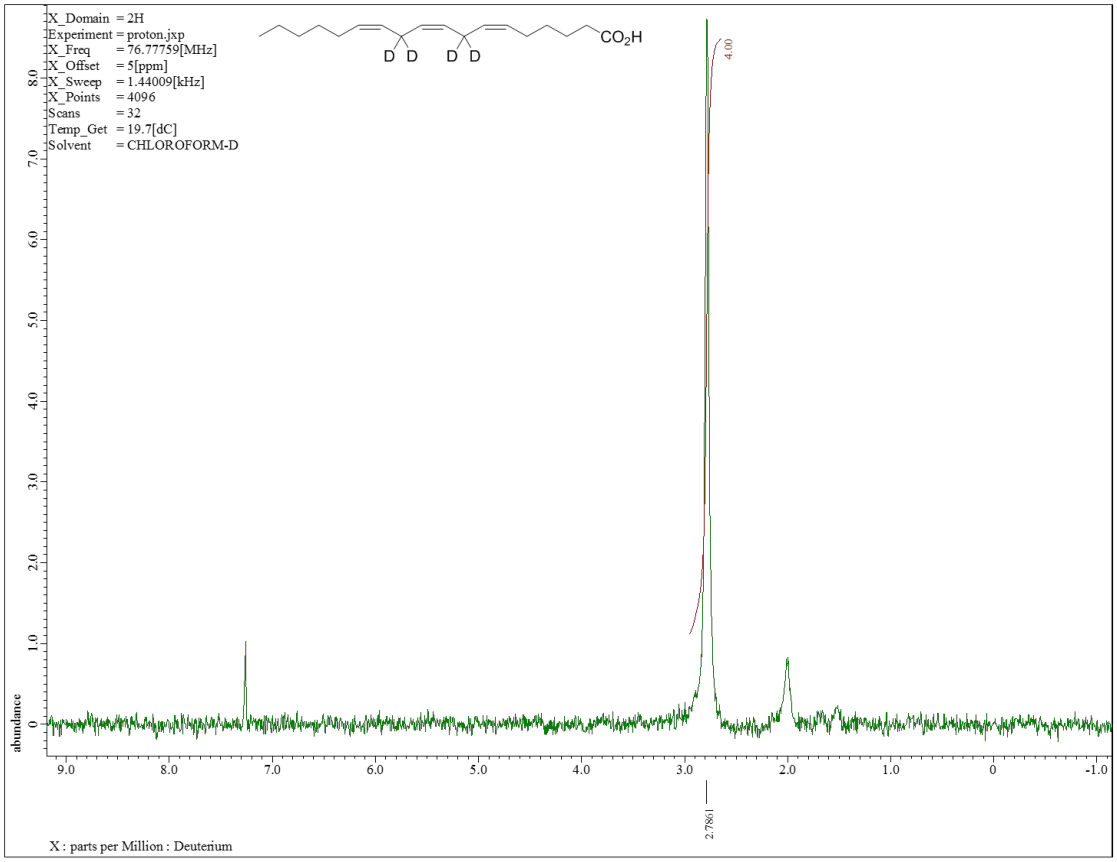

X_Domain = 1H
Experiment = proton.jxp
X_Freq = 500.15992[MHz]
X_Offset = 5.0[ppm]
X_Sweep = 9.38438[kHz]
X_Points = 16384
Scans = 28
Temp_Get = 20.4[dC]
Solvent = CHLOROFORM-D
D D D D
CO2Me
5.92
2.89
1.98
4.09
1.96
8.28
3.00
abundance
5.4243
5.4105
5.4036
5.3968
5.3888
5.3830
5.3750
5.3693
5.3590
5.3510
5.3475
5.3361
5.3132
3.6664
2.3323
2.3174
2.3013
2.0746
2.0698
2.0574
2.0460
2.0437
1.6474
1.3943
1.3302
1.2981
1.2924
0.9019
0.8882
0.8733
X : parts per Million : Proton

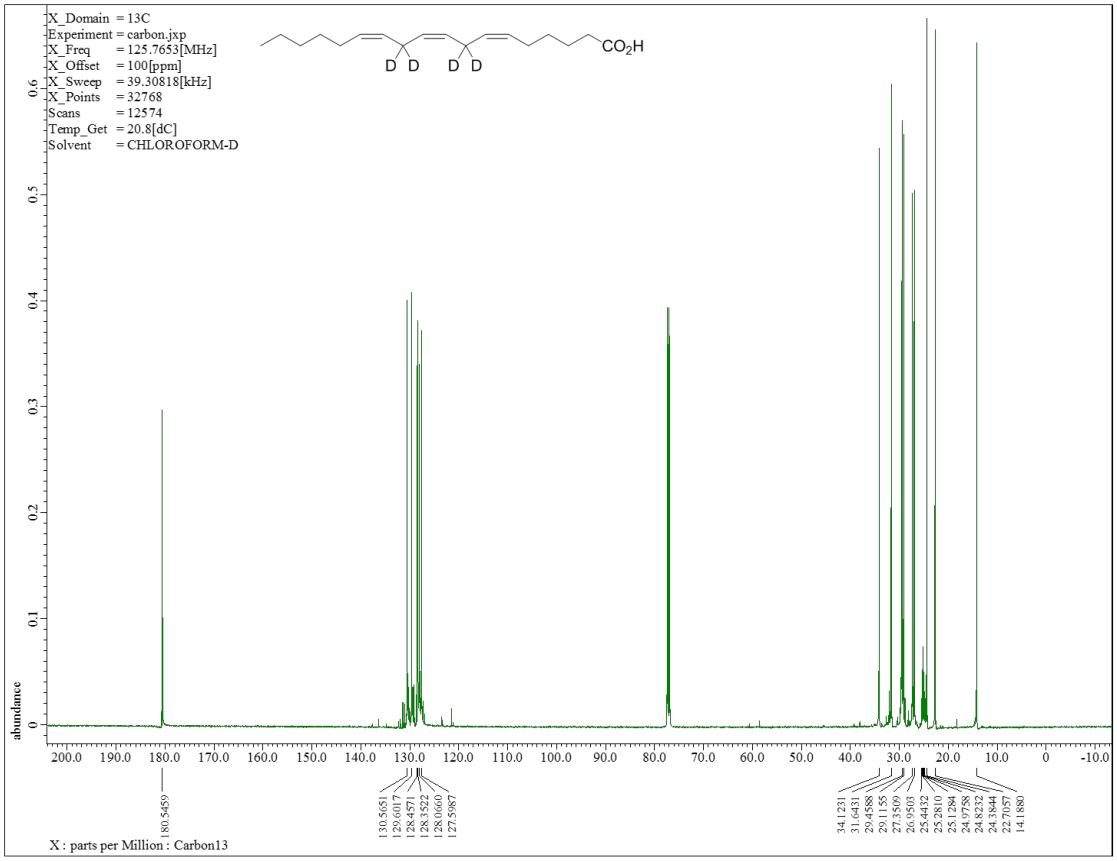

X_Domain = 2H
Experiment = proton.jxp
X_Freq = 76.77759[MHz]
X_Offset = 5[ppm]
X_Sweep = 1.44009[kHz]
X_Points = 4096
Scans = 64
Temp_Get = 20.3[dC]
Solvent = CHLOROFORM-D
D D D D
CO2Me
4.00
2.7723
abundance
X : parts per Million : Deuterium


X_Domain = 13C
Experiment = carbon.jxp
X_Freq = 125.7653[MHz]
X_Offset = 100[ppm]
X_Sweep = 39.30818[kHz]
X_Points = 32768
Scans = 7351
Temp_Get = 20.8[dC]
Solvent = CHLOROFORM-D
D D D D
CO2Me
174.1362
130.4888
129.6494
128.3713
128.1996
128.0374
127.5510
51.4924
34.0182
31.5954
29.4111
29.1727
27.2936
26.9312
25.3764
25.2238
25.0712
24.9186
24.6420
22.6485
14.1307
abundance
X : parts per Million : Carbon13

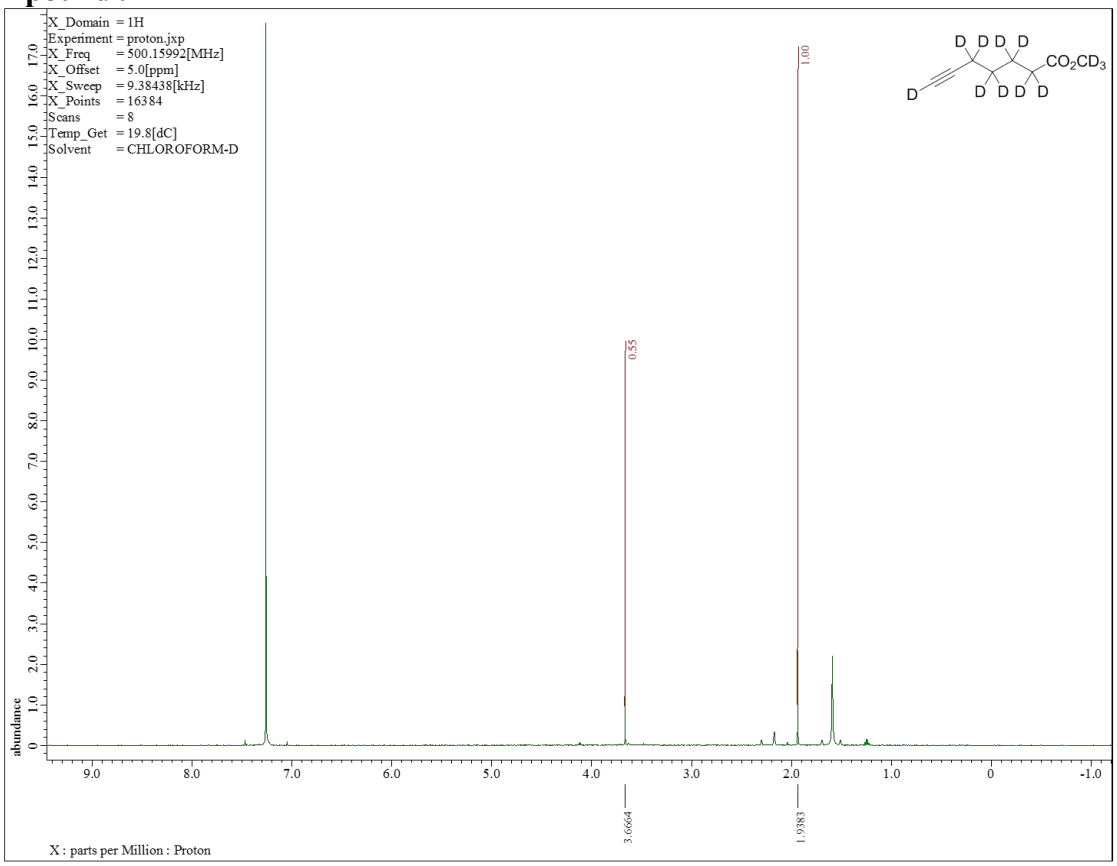
## Compound 7c

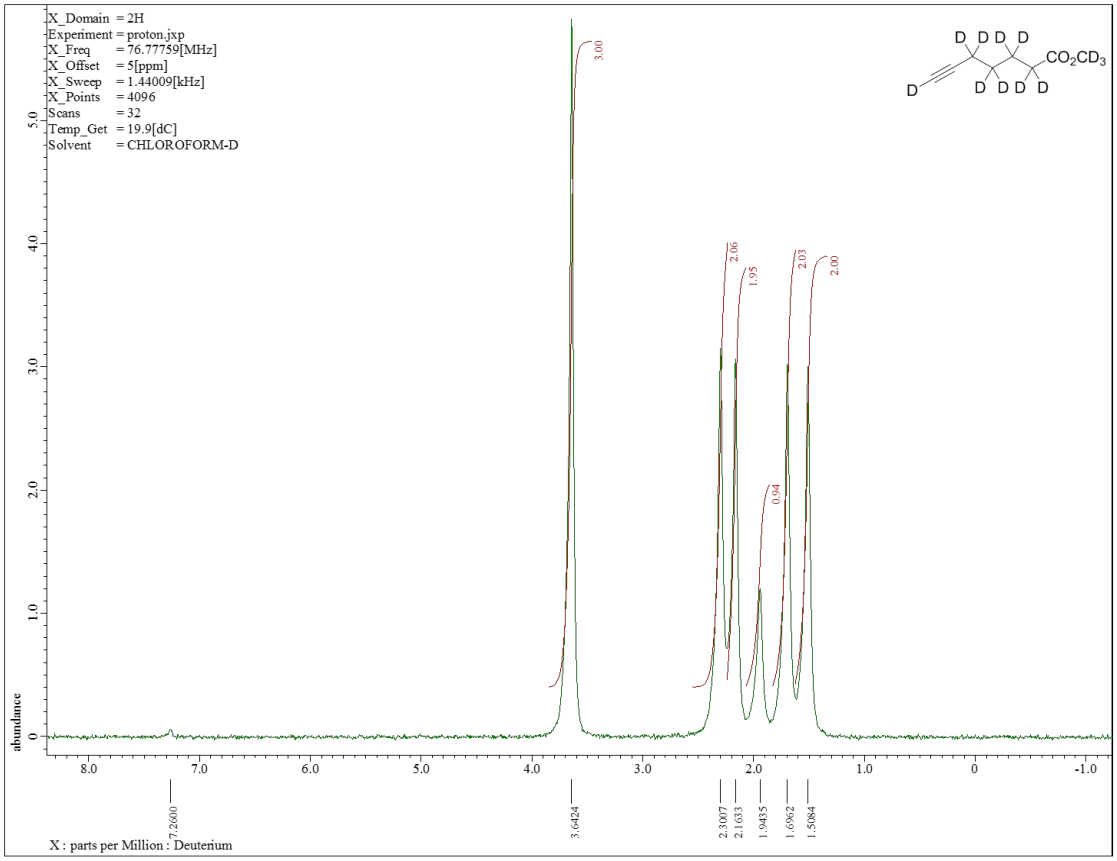

X_Domain = 1H
Experiment = proton.jxp
X_Freq = 500.15992[MHz]
X_Offset = 5.0[ppm]
X_Sweep = 9.38438[kHz]
X_Points = 16384
Scans = 8
Temp_Get = 19.6[dC]
Solvent = CHLOROFORM-D
D D D D
CO2H
5.95
8.08
3.91
3.00
2.03
2.00
abundance
X : parts per Million : Proton

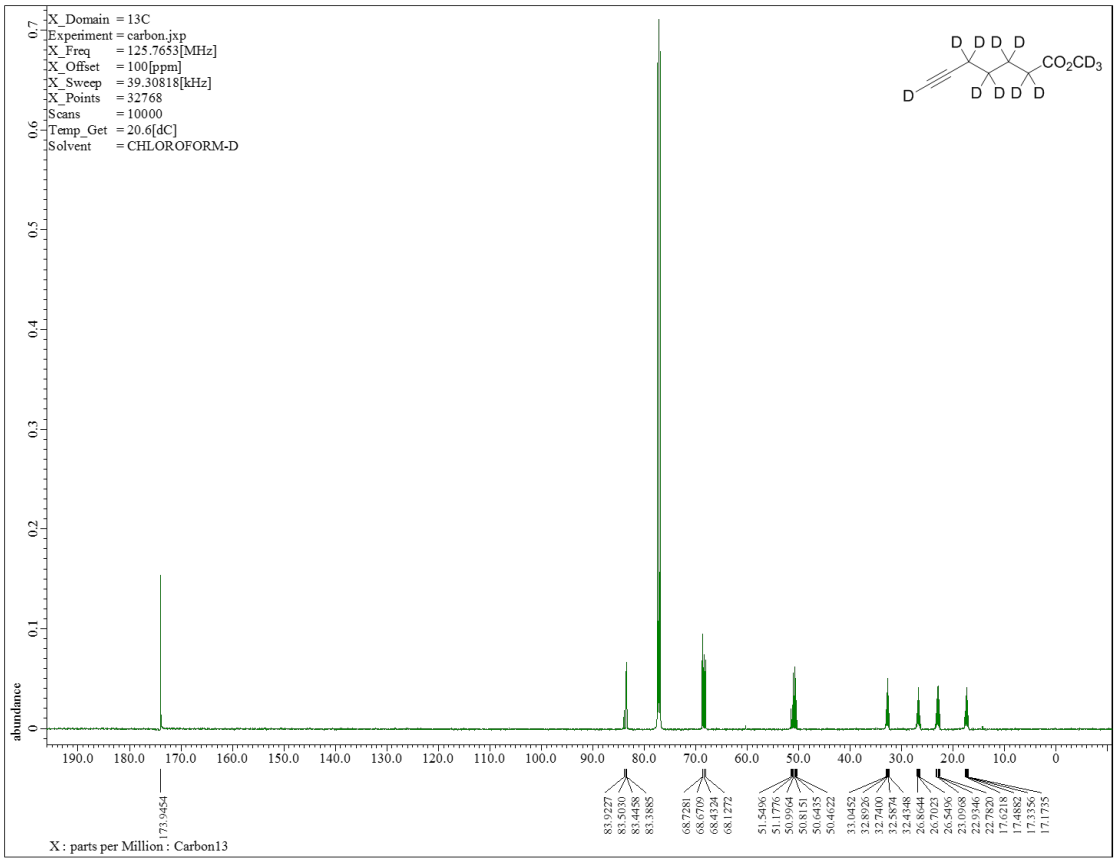

X_Domain = 2H
Experiment = proton.jxp
X_Freq = 76.77759[MHz]
X_Offset = 5[ppm]
X_Sweep = 1.44009[kHz]
X_Points = 4096
Scans = 32
Temp_Get = 19.7[dC]
Solvent = CHLOROFORM-D
D D D D
CO2H
4.00
2.78861
abundance
X : parts per Million : Deuterium


X_Domain = 13C
Experiment = carbon.jxp
X_Freq = 125.7653[MHz]
X_Offset = 100[ppm]
X_Sweep = 39.30818[kHz]
X_Points = 32768
Scans = 12574
Temp_Get = 20.8[dC]
Solvent = CHLOROFORM-D
CO2H
D D D D
abundance
180.5459
130.5651
129.6017
128.4571
128.3522
128.0060
127.5987
34.1231
31.6431
29.4588
29.1155
27.3509
26.9503
25.4432
25.2810
25.1284
24.9758
24.8232
24.3844
22.7057
14.1880
X : parts per Million : Carbon13

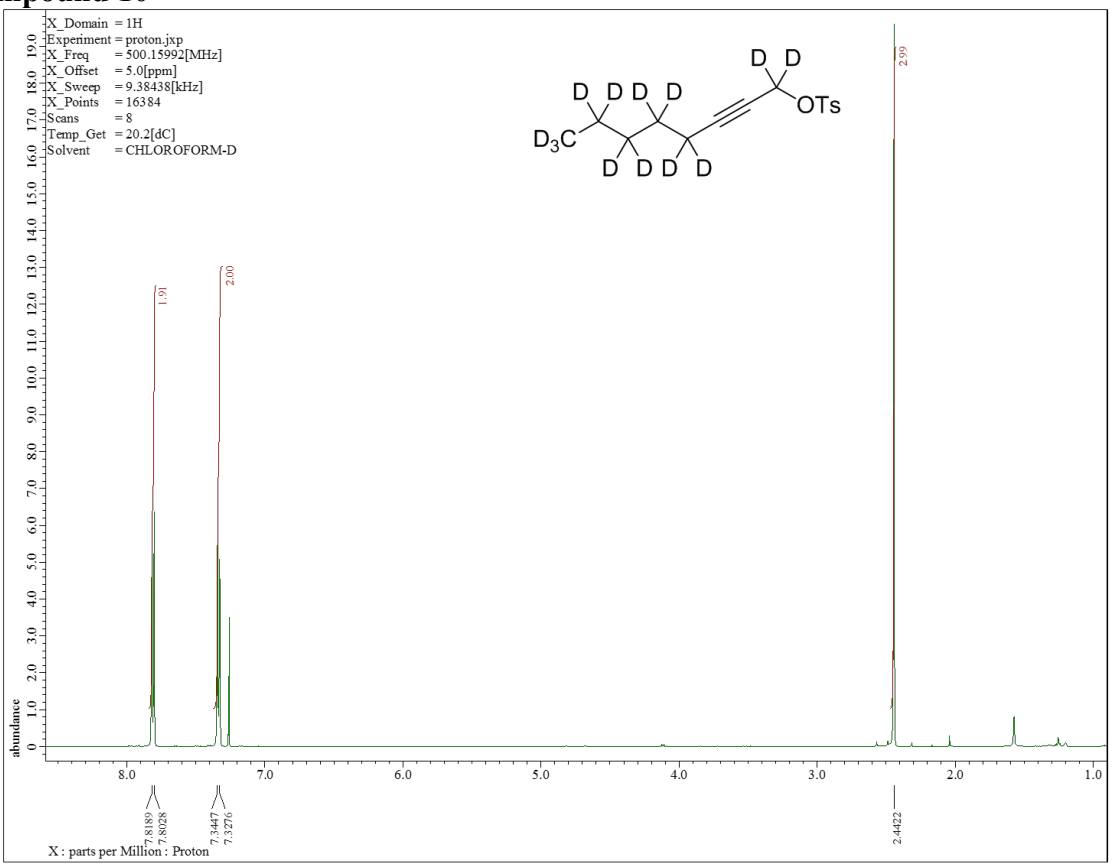
## Compound 9

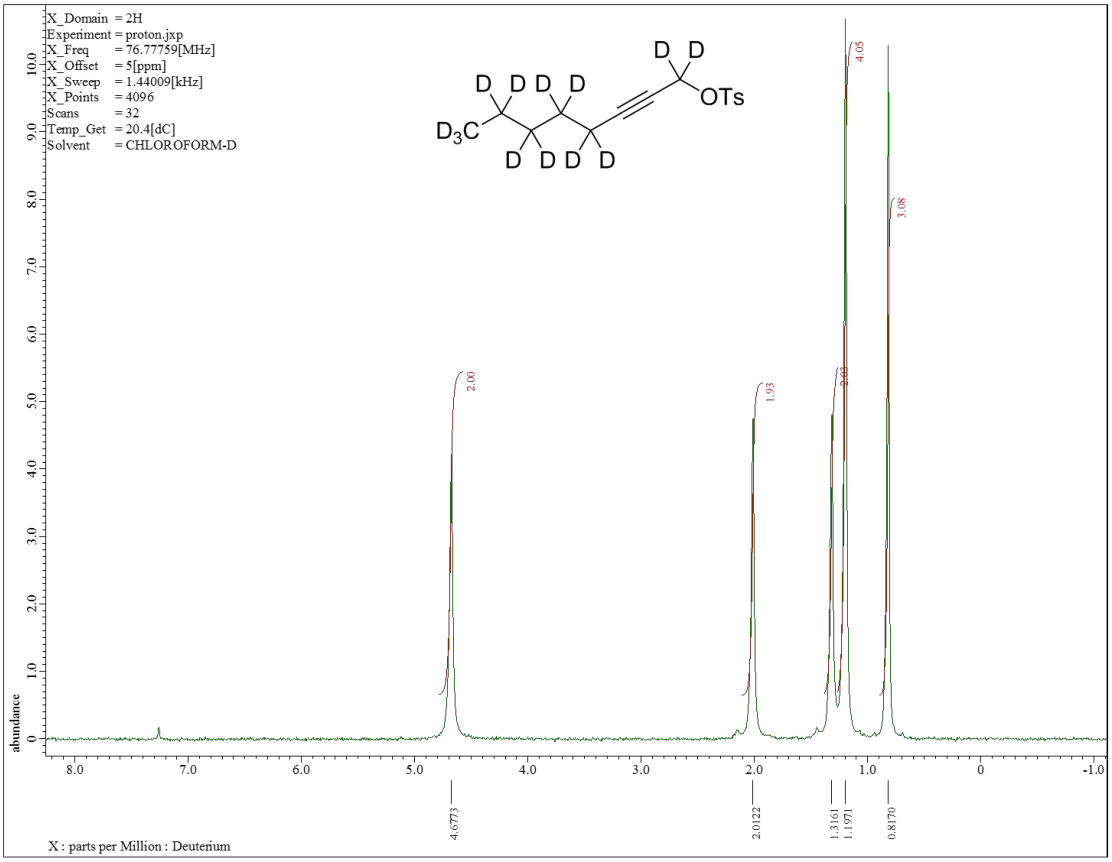

X_Domain = 1H
Experiment = proton.jxp
X_Freq = 500.15992[MHz]
X_Offset = 5.0[ppm]
X_Sweep = 9.38438[kHz]
X_Points = 16384
Scans = 8
Temp_Get = 19.8[dC]
Solvent = CHLOROFORM-D
D D D D
CO2CD3
D
D D D D
1.00
0.55
abundance
3.6664
1.9383
X : parts per Million : Proton

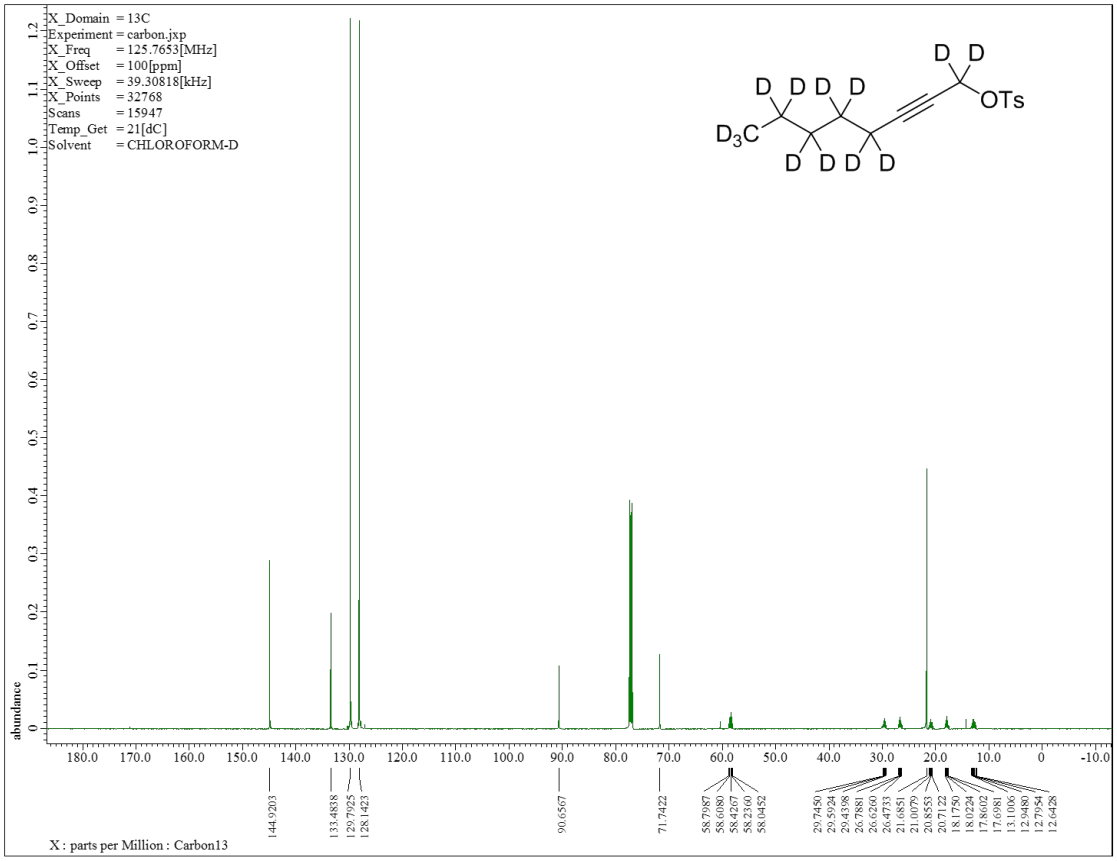

X_Domain = 2H
Experiment = proton.jxp
X_Freq = 76.77759[MHz]
X_Offset = 5[ppm]
X_Sweep = 1.44009[kHz]
X_Points = 4096
Scans = 32
Temp_Get = 19.9[dC]
Solvent = CHLOROFORM-D
3.00
2.06
1.95
0.94
2.03
2.00
abundance
X : parts per Million : Deuterium
7.2600
3.6424
2.3007
2.1633
1.9435
1.6962
1.5084
D D D D
CO2CD3
D
D D D D


X_Domain = 13C
Experiment = carbon.jxp
X_Freq = 125.7653[MHz]
X_Offset = 100[ppm]
X_Sweep = 39.30818[kHz]
X_Points = 32768
Scans = 10000
Temp_Get = 20.6[dC]
Solvent = CHLOROFORM-D
abundance
X : parts per Million : Carbon13
173.9454
83.9227
83.5030
83.4498
83.3885
68.7281
68.6709
68.4324
68.1272
51.5496
51.1776
50.9964
50.8151
50.6455
50.4622
33.0452
32.8926
32.7400
32.5874
32.4348
26.8644
26.7023
26.5496
23.0968
22.9346
22.7820
17.6218
17.4882
17.3356
17.1735
D D D D
CO2CD3
D
D D D D

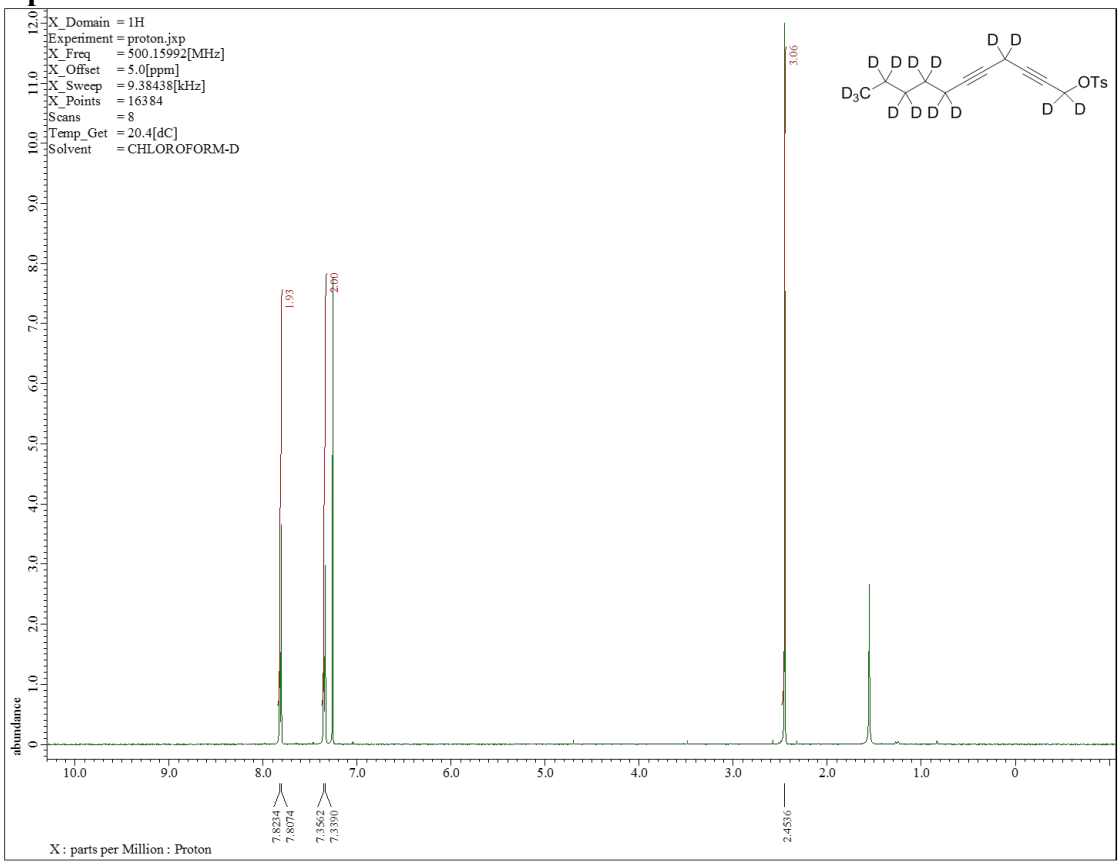
## Compound 10

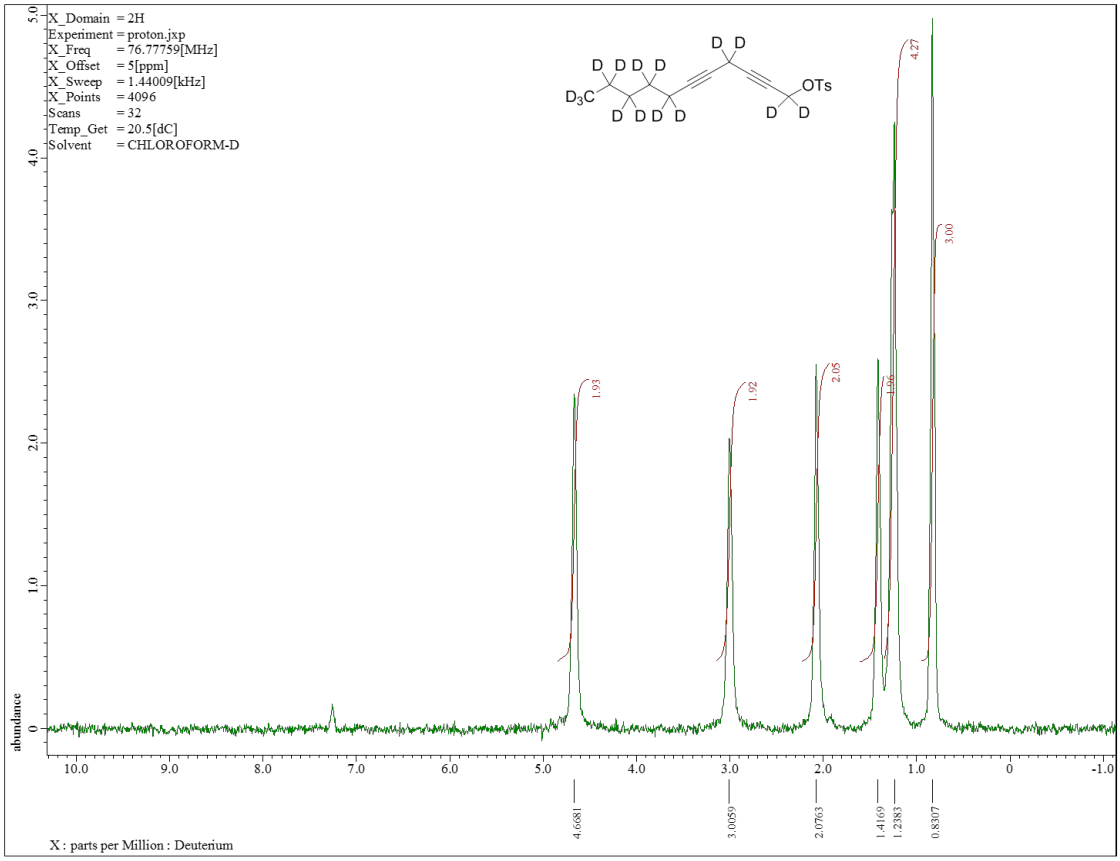

X_Domain = 1H
Experiment = proton.jxp
X_Freq = 500.15992[MHz]
X_Offset = 5.0[ppm]
X_Sweep = 9.38438[kHz]
X_Points = 16384
Scans = 8
Temp_Get = 20.2[dC]
Solvent = CHLOROFORM-D
D D
D D D D
D3C
D D D D
OTs
1.91
2.00
2.99
abundance
7.8189
7.8028
7.3447
7.3276
2.4422
X : parts per Million : Proton

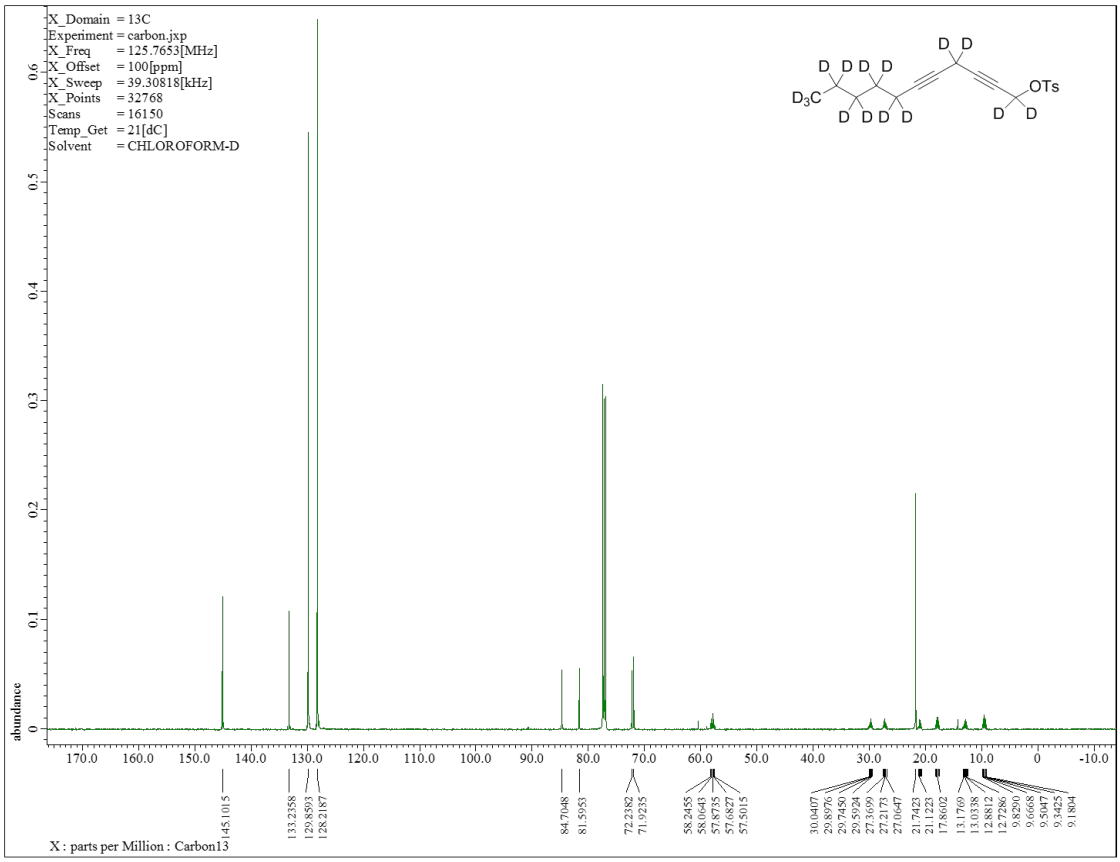

X_Domain = 2H
Experiment = proton.jxp
X_Freq = 76.77759[MHz]
X_Offset = 5[ppm]
X_Sweep = 1.44009[kHz]
X_Points = 4096
Scans = 32
Temp_Get = 20.4[dC]
Solvent = CHLOROFORM-D
D D
D D D D
D3C
D D D D
OTs
2.00
1.93
2.03
4.05
3.08
abundance
4.6773
2.0122
1.3161
1.1971
0.8170
X : parts per Million : Deuterium


X_Domain = 13C
Experiment = carbon.jxp
X_Freq = 125.7653[MHz]
X_Offset = 100[ppm]
X_Sweep = 39.30818[kHz]
X_Points = 32768
Scans = 15947
Temp_Get = 21[dC]
Solvent = CHLOROFORM-D
D D
D D D D
D3C
D D D D
OTs
abundance
144.9203
133.4838
129.7925
128.1423
90.6567
71.7422
58.7987
58.6080
58.4267
58.2360
58.0452
29.7450
29.5924
29.4398
26.7881
26.6270
26.4733
21.6851
21.0079
20.8553
20.7122
18.1750
18.0224
17.8602
17.6981
13.1006
12.9480
12.7954
12.6428
X : parts per Million : Carbon13

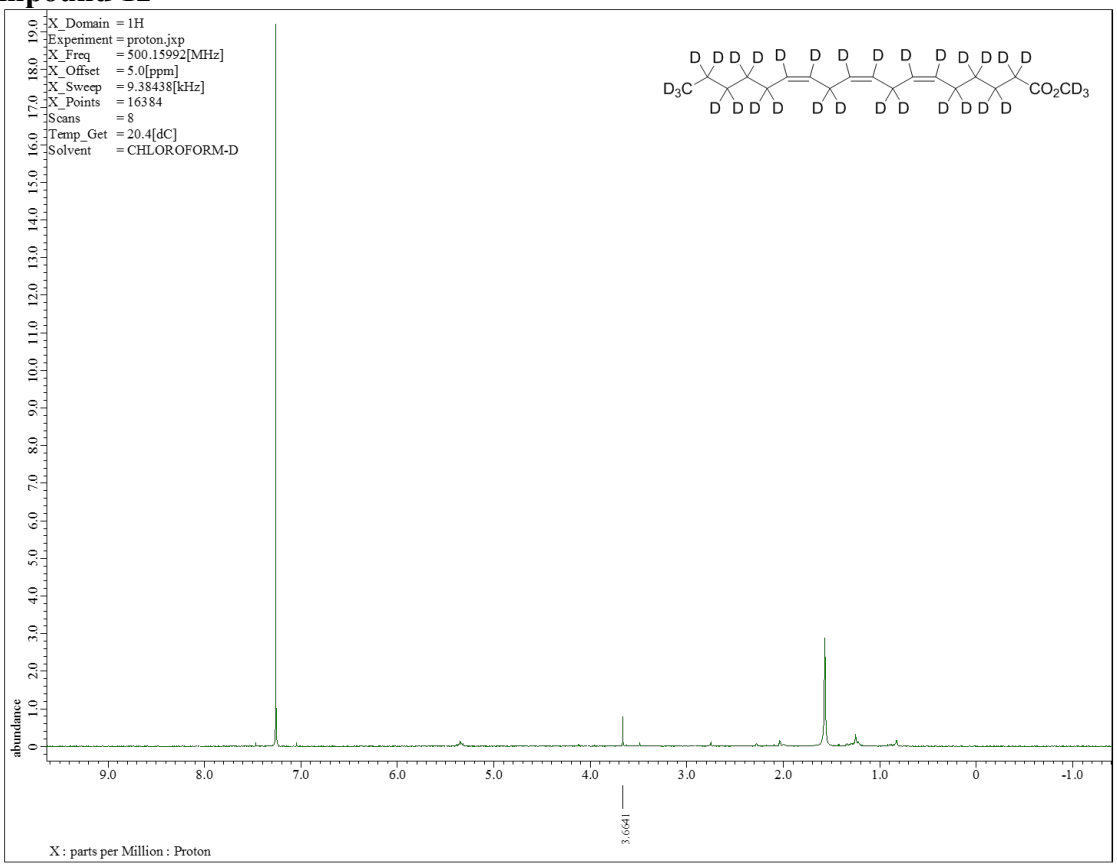
## Compound 11

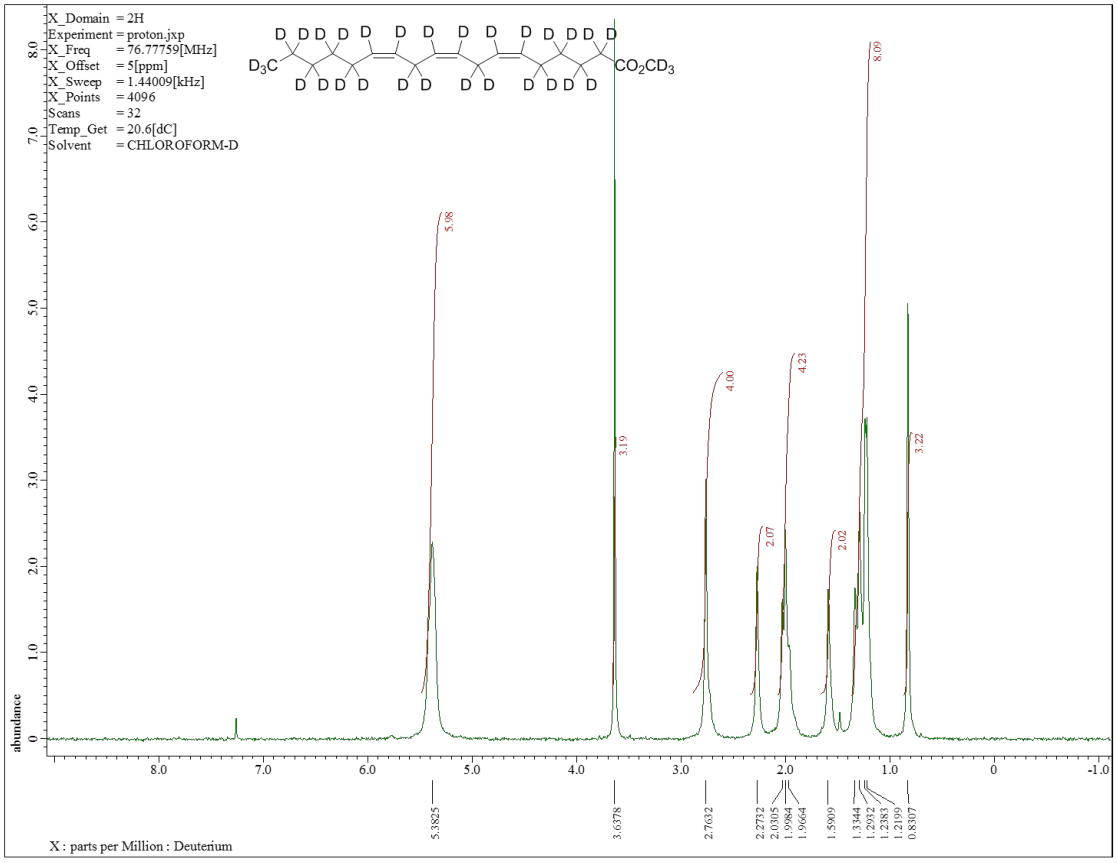

X_Domain = 1H
Experiment = proton.jxp
X_Freq = 500.15992[MHz]
X_Offset = 5.0[ppm]
X_Sweep = 9.38438[kHz]
X_Points = 16384
Scans = 8
Temp_Get = 20.4[dC]
Solvent = CHLOROFORM-D
D D
D D D D
D3C
D D D D
OTs
D D
1.93
2.00
3.06
abundance
7.8254
7.8074
7.3562
7.3390
2.4536
X : parts per Million : Proton

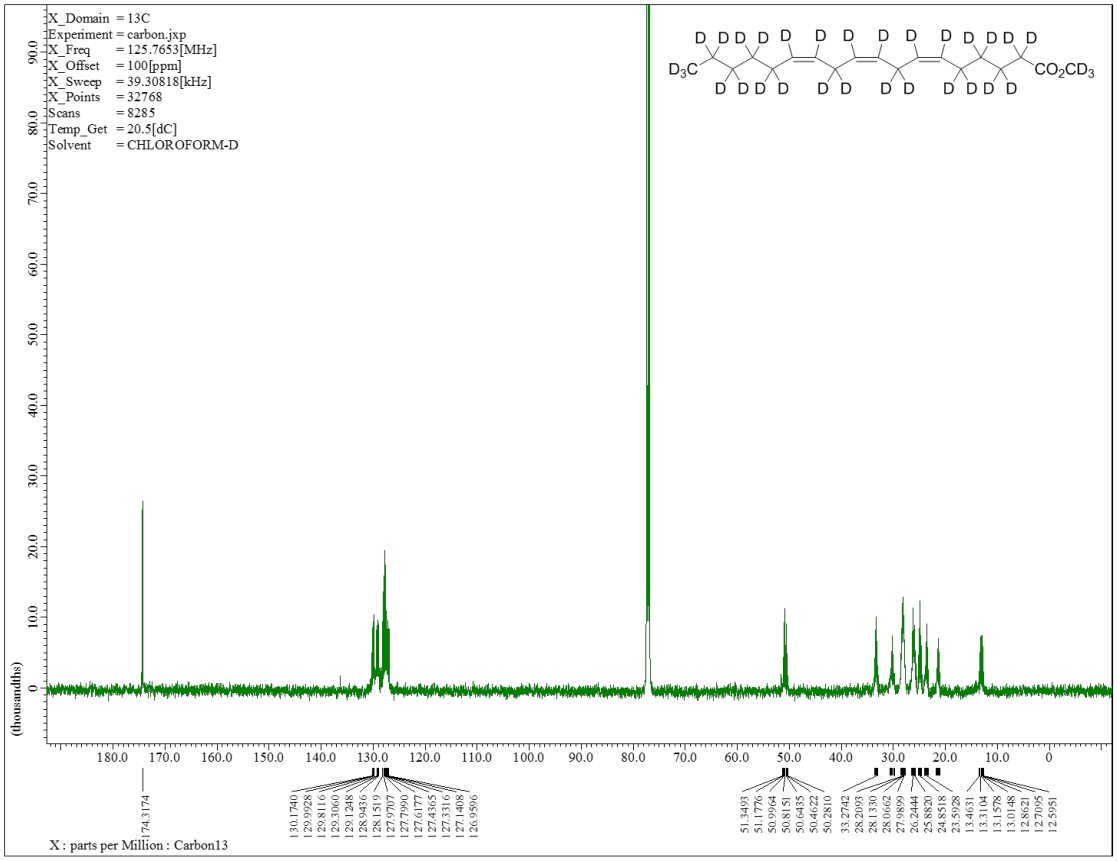

X_Domain = 2H
Experiment = proton.jxp
X_Freq = 76.77759[MHz]
X_Offset = 5[ppm]
X_Sweep = 1.44009[kHz]
X_Points = 4096
Scans = 32
Temp_Get = 20.5[dC]
Solvent = CHLOROFORM-D
D D
D D D D
D3C
D D D D
OTs
D D
4.27
3.00
2.05
1.93
1.92
1.46
abundance
10.0 9.0 8.0 7.0 6.0 5.0 4.0 3.0 2.0 1.0 0 -1.0
4.6681
3.0059
2.0763
1.4169
1.2383
0.8307
X : parts per Million : Deuterium


X_Domain = 13C
Experiment = carbon.jxp
X_Freq = 125.7653[MHz]
X_Offset = 100[ppm]
X_Sweep = 39.30818[kHz]
X_Points = 32768
Scans = 16150
Temp_Get = 21[dC]
Solvent = CHLOROFORM-D
D D
D D D D
D3C
D D D D
OTs
D D
abundance
170.0 160.0 150.0 140.0 130.0 120.0 110.0 100.0 90.0 80.0 70.0 60.0 50.0 40.0 30.0 20.0 10.0 0 -10.0
145.1015
133.2358
129.8593
128.2187
84.7048
81.5953
72.2382
71.9235
58.2455
58.0643
57.8735
57.6827
57.5015
30.0407
29.8976
29.7450
29.5924
27.3699
27.2173
27.0647
21.7423
21.1223
17.8602
13.1769
13.0338
12.8812
12.7286
9.8290
9.6668
9.5047
9.3425
9.1804
X : parts per Million : Carbon13

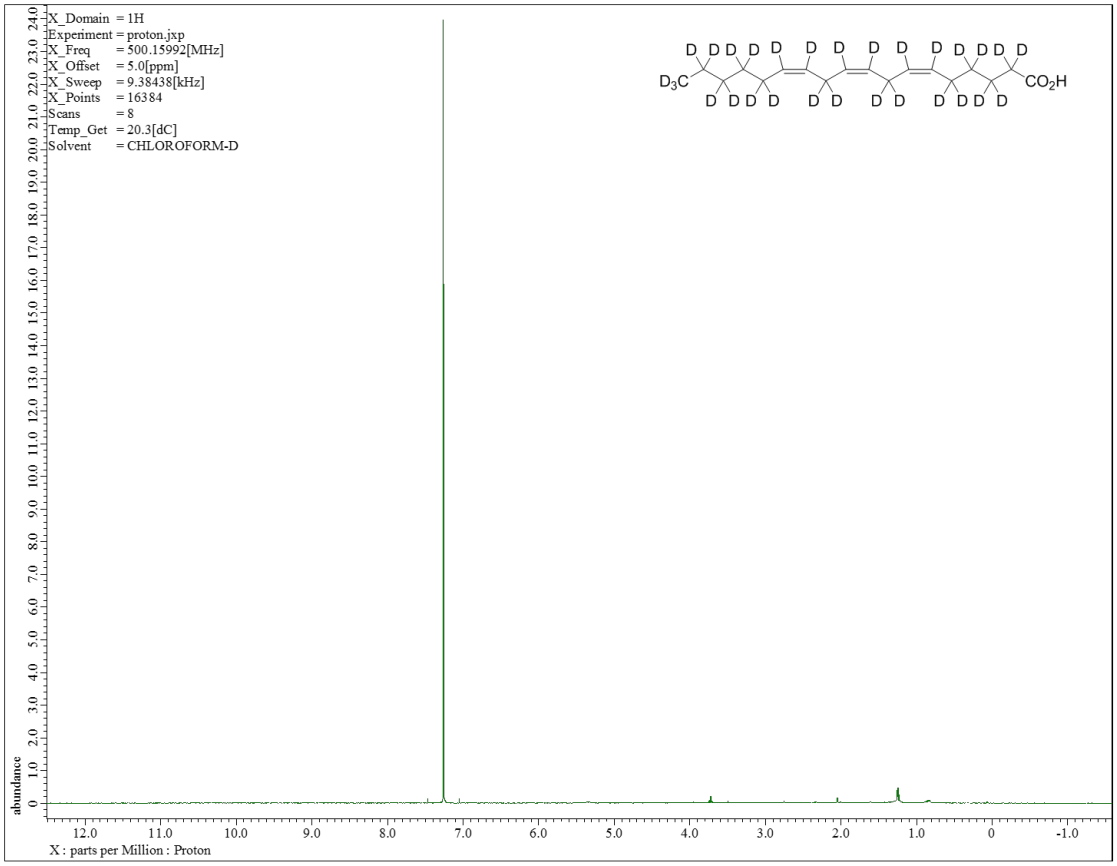
## Compound 12

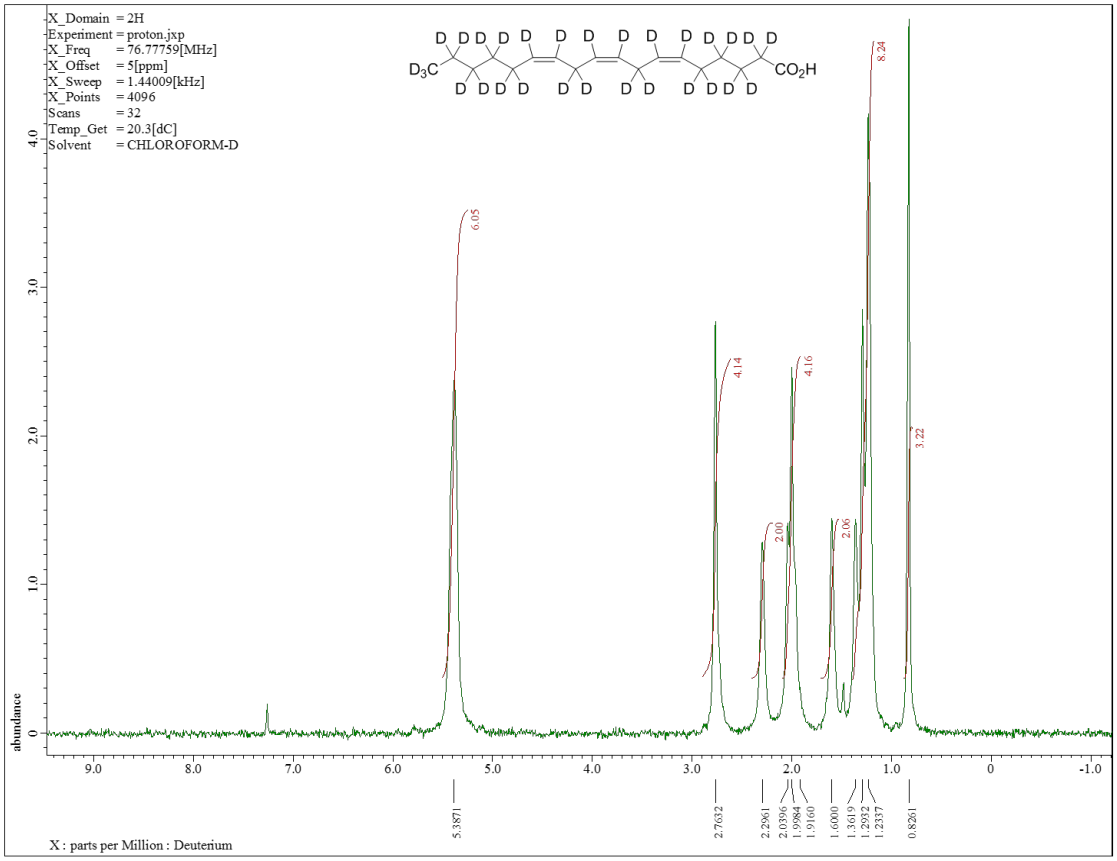

X_Domain = 1H
Experiment = proton.jxp
X_Freq = 500.15992[MHz]
X_Offset = 5.0[ppm]
X_Sweep = 9.38438[kHz]
X_Points = 16384
Scans = 8
Temp_Get = 20.4[dC]
Solvent = CHLOROFORM-D
abundance
3.6641
X : parts per Million : Proton

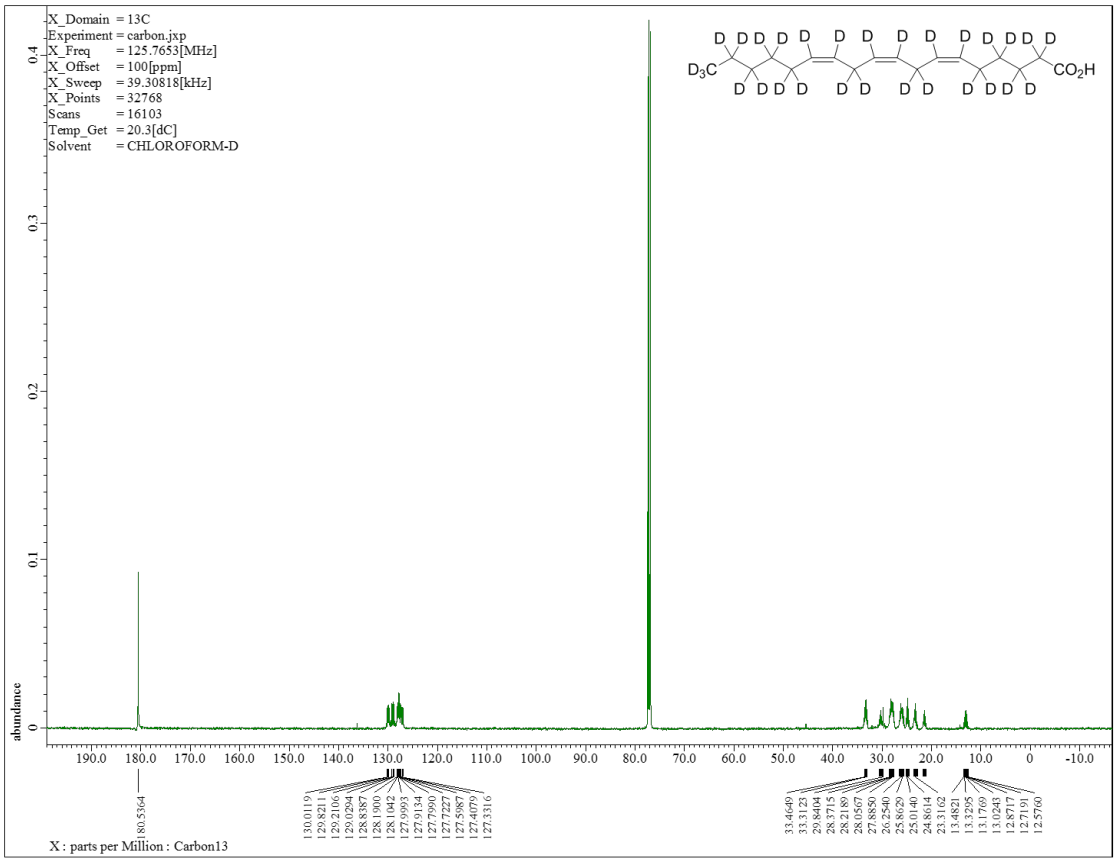

X_Domain = 2H
Experiment = proton.jxp
X_Freq = 76.77759[MHz]
X_Offset = 5[ppm]
X_Sweep = 1.44009[kHz]
X_Points = 4096
Scans = 32
Temp_Get = 20.6[dC]
Solvent = CHLOROFORM-D
5.98
3.19
4.00
2.07
4.23
2.02
8.09
3.22
abundance
5.3825
3.6378
2.7632
2.2732
2.0315
1.9984
1.9664
1.5909
1.3344
1.2932
1.2383
1.2199
0.8307
X : parts per Million : Deuterium


X_Domain = 13C
Experiment = carbon.jxp
X_Freq = 125.7653[MHz]
X_Offset = 100[ppm]
X_Sweep = 39.30818[kHz]
X_Points = 32768
Scans = 8285
Temp_Get = 20.5[dC]
Solvent = CHLOROFORM-D
(thousandths)
X : parts per Million : Carbon13

## all-D-GLA


X_Domain = 1H
Experiment = proton.jxp
X_Freq = 500.15992[MHz]
X_Offset = 5.0[ppm]
X_Sweep = 9.38438[kHz]
X_Points = 16384
Scans = 8
Temp_Get = 20.3[dC]
Solvent = CHLOROFORM-D
abundance
X : parts per Million : Proton


X_Domain = 2H
Experiment = proton.jxp
X_Freq = 76.77759[MHz]
X_Offset = 5[ppm]
X_Sweep = 1.44009[kHz]
X_Points = 4096
Scans = 32
Temp_Get = 20.3[dC]
Solvent = CHLOROFORM-D
D3C
CO2H
6.05
4.14
2.00
4.16
2.06
8.24
3.22
abundance
X : parts per Million : Deuterium
5.3871
2.7632
2.2961
2.0396
1.9984
1.9160
1.6000
1.3619
1.2932
1.2337
0.8261


X_Domain = 13C
Experiment = carbon.jxp
X_Freq = 125.7653[MHz]
X_Offset = 100[ppm]
X_Sweep = 39.30818[kHz]
X_Points = 32768
Scans = 16103
Temp_Get = 20.3[dC]
Solvent = CHLOROFORM-D
D3C
CO2H
abundance
X : parts per Million : Carbon13
180.5364
130.0119
129.8211
129.2106
129.0294
128.8387
128.1900
128.1042
127.9993
127.9134
127.7990
127.7227
127.5987
127.4079
127.3316
33.4649
33.3123
29.8684
28.3715
28.2189
28.0567
27.8850
26.2540
25.8629
25.0140
24.8614
23.3162
13.4821
13.3295
13.1769
13.0243
12.8717
12.7191
12.5760