Supporting information

Synthesis of Deutrated-Isopentyl pyrophosphates for Chemo-Enzymatic Labelling Methods: GC-EI-MS based 1,2-Hydride shift in Epicedrol Biosynthesis

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Materials and Method:

General Methods. All reactions were carried out in flame-dried glassware under argon atmosphere. All reagents and other solvents were purchased from Sigma Aldrich were used without further purification. ACN was dried over the CaH_2. Flash column chromatography was performed using silica gel (60-120 and 100-200) purchased from Merck. Thin layer chromatography was carried out with Merck silica gel 60 - F-2014 and cellulose plates. The ^{1}H, ^{13}C and ^{31}P NMR spectrum were recorded 500, 125 and 202 MHz on AV- 500 and 400, 100 and 161 on AV- 400 respectively on Bruker Avance-II. $CDCl_3$, D_2O and TMS used as an internal standard ($CDCl_3$ at 7.27 ppm for ^{1}H, 77.00 ppm for ^{13}C and 4.75 ppm in D_2O for ^{1}H).

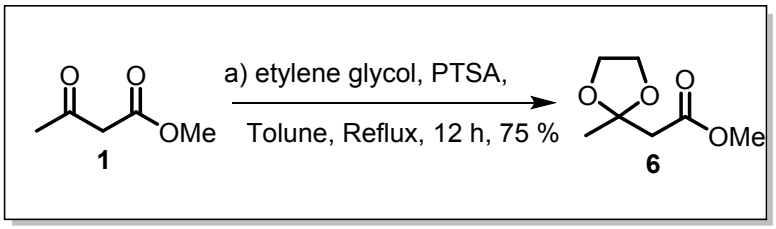
The cloning, expression and purification of Geranial pyrophosphate synthases (GPPS), farnesyl pyrophosphate synthases (FPPS, Genbank Acc. No. KF011939) and epi-cedrol synthase (EPCS, GenBank Acc.No. AF157059), we have reported earlier.[1-3]

General procedure for preparation of pyrophosphorylatio (A):

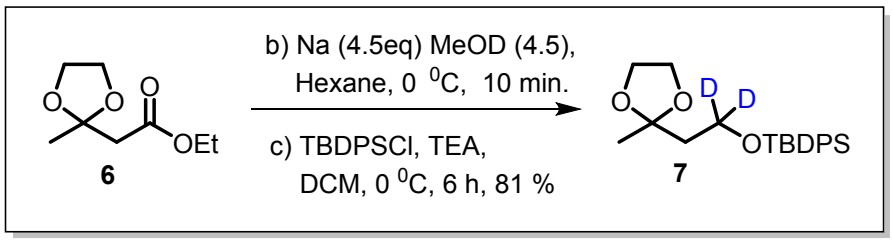
In a flame-dried round bottom flask equipped with magnetic stirrer. To a well stirred solution of 3.0 mmol of Tris-tetra-*n*-butylammonium hydrogen diphosphate in 25 mL ACN. Tosylate derivative **(9, 13, 16, 18)** which is dissolved in 5 mL ACN was slowly added in reaction mixture. The resulting reaction mixture was stirred at room temperature for 2 h. After compilation of reaction, the solvent was removed under reduced pressure to obtain a pale-yellow residue. The opaque residue was dissolved in minimum amount of 25 mM chromatographic buffer. The solution was passed through a column containing 30 equivalents of DOWEX AG 50W-X8 (100-200 mesh) cation-exchange resins (NH_4^+ form) and 25 mM chromatographic buffer as an eluent. The column was eluted with (2-column volume) of chromatographic buffer maintain the flow rate 5mL/min. Eluted was lyophilized to dryness to yield a fluffy, white solid. The solid was dissolved in a minimum amount of 50 mM chromatography buffer and loaded into a cellulose flash column. Fractions were analyzed on cellulose TLC plates, developed with sulfosalicylic acid-ferric chloride spray, product fraction are pooled, and concentrated by rotary evaporation at 40 °C. The material was transferred to a freeze-drying flask and lyophilized. The resulting white solid was collected and stored at -78 °C for future applications.

Preparation of deuterated Isoprenyl diphosphate

Synthesis of (1,1-^{2}H) IPP (2)[17, 22]



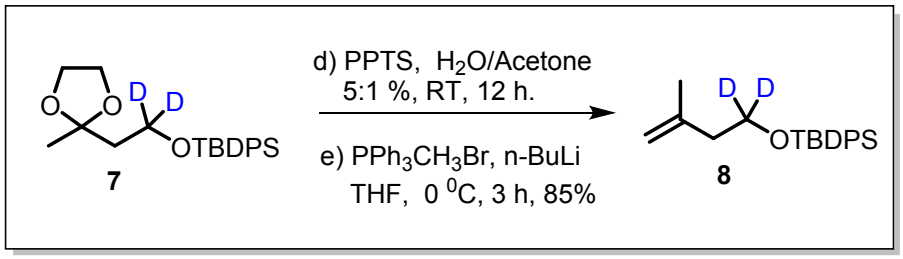
Methyl (2-methyl-1,3-dioxolan-2-yl) acetate (6): A solution of **1** (2 g, 15.1 mmol) and ethylene glycol (1.26 g, 22.5 mmol), PTSA (0.200 g, 0.15 mmol) was added in 100 mL of Toluene. The reaction medium was equipped with a Dean–Stark apparatus and then refluxed for 12h. The reaction was diluted using saturated $NaHCO_3$ and extracted using DCM × 3, combine organic layer were washed with brine and dried over Na_2SO_4, concentrated under reduced pressure. Purified by silica gel column chromatography 20 % ethyl acetate/pet to give **6** colorless liquid (1.95 g, 75 % yield). 1H NMR (200 MHz, $CDCl_3$) 1H NMR (400 MHz, $CDCl_3$) d = 3.89 (s, 4 H), 3.61 (s, 3 H), 2.59 (br. s., 2 H), 1.41 (br. s., 3 H) 13C NMR (100MHz, $CDCl_3$) δ 107.3, 64.5, 51.4, 43.7, 24.1; HRMS (ESI) calcd. For $C_7H_{12}O_4$ [M+1]: 160.0736 found 160.0735.



(1,1-^{2}H)- tert-butyl (2-(2-methyl-1,3-dioxolan-2-yl) ethoxy)diphenylsilane : (7) To stirred solution of **6** (0.500 g, 2.8 mmol), in dry hexane was added MeOD (0.426 g, 12 mmol) and Na (0.297g, 12 mmol) at 0°C. The

resulting solution was stirred vigorously for 10 min. The reaction was quenched by an aqueous solution of HCl and the aqueous layer was extracted using Et_2O × 3. Combined organic layered were washed with brine, dried over Na_2SO_4, concentrated under reduced pressure to give crude alcohol.

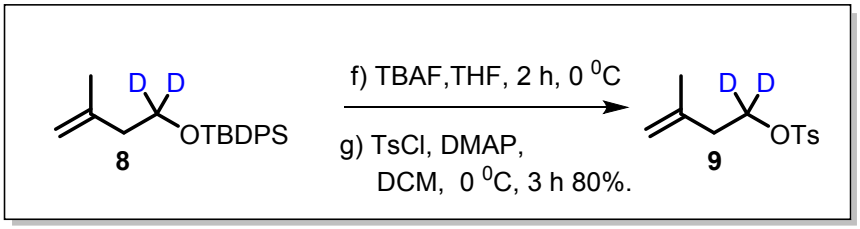
Crude alcohol (0.370 g, 2.7 mmol) is dissolved in dry 25 ml DCM in which TEA (0.545 g, 5.6 mmol) and TBDPSCl (0.98 g, 3.5 mmol) at 0 °C after 30 min reaction mixture allowed to warm at room temperature and stirred for 5 h. After completion of the reaction, a mixture was diluting with water and extracted with Et_2O×3. Combined organic layered were washed with brine, dried over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by silica gel column chromatography using 20 % ethyl acetate / pet ether to afforded **7** as a colorless liquid (0.865 g, 81 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 - 7.62 (m, 4 H), 7.50 - 7.35 (m, 6 H), 3.92 - 3.79 (m, 2 H), 1.95 (s, 2 H), 1.33 (s, 3 H), 1.06 (s, 9 H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.6, 133.9, 129.5, 127.6, 108.9, 64.4, 41.3, 29.7, 26.8, 24.4, 19.1. HRMS (ESI) calcd. For $C_5H_{10}D_2O_7P_2Na$ [M+Na]: 271.0080 found 271.0077.



(1,1-^{2}H)-*tert*-Butyl((3-methylbut-3-en-1-yl) oxy) diphenylsilane (8): To a solution of **7** (0.500 g, 1.3 mmol) in acetone and water (5:1) at room temperature PPTS (0.022 g, 0.13 mol) was added and stirred reaction mixture for 12 h. The reaction mixture diluted using saturated $NaHCO_3$ and extracted with Et_2O × 3. Combined organic layer were dried over Na_2SO_4, concentrated under reduced pressure to give a crude ketone.

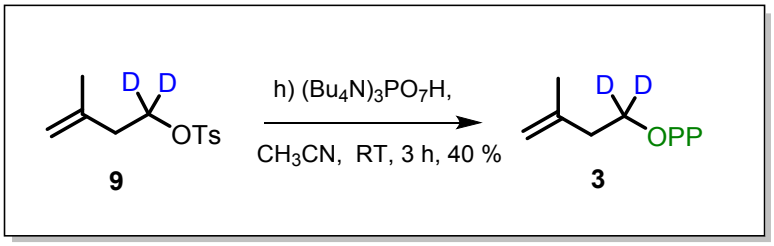
Methyltriphenylphosphonium bromide (0.935 g, 2.5 mmol) was dissolved in anhydrous THF (30 mL) and cooled to 0 °C under nitrogen atmosphere prior to the slow addition of *n*-BuLi (1.5 mL, 2.51 mmol). The resulting Pale-yellow suspension was stirred for 30 min then slowly addition of crude ketone (0.399 g, 1.25 mmol) dissolved in dry THF (5 mL). The mixture was stirred at room temperature for 3 h. After completion of the reaction, a mixture was quench using the addition of saturated NH_4Cl and extracted with Et_2O × 3. Combined organic layer were washed with brine, dried over Na_2SO_4, concentrated under reduced pressure. purified by silica gel column chromatography using 10 % ethyl acetate/pet ether to give colorless liquid **8** (0.376 g, 85 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 - 7.67 (m, 4 H), 7.44 - 7.37 (m, 6 H),

4.76 (s, 1 H), 4.69 (s, 1H), 2.6 (s, 2H), 1.69 (s, 3 H), 1.06 (s, 9 H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 141.6, 135.1, 134.8, 129.6, 127.7, 115.8, 38.1, 26.5, 20.2, 19.0. HRMS (ESI) calcd. for $C_{21}H_{26}{}^2H_2ONaSi$ [M+Na]: 349.1933, found: 349.1934.



(1,1-^{2}H)-3-Methylbut-3-en-1-yl 4-methylbenzenesulfonate (9): A stirred solution of **8** (0.350 g, 1.07 mmol) in THF (5 mL) was treated with TBAF (1.6 mL, 1.61 mmol) for 2 h at room temperature. The progress of the reaction was monitored by TLC. After completion of the reaction, a mixture was diluted using saturated $NaHCO_3$ and extracted with DCM × 3. Combined organic layer were washed with brine dried over the Na_2SO_4. In the filtrate solution of crude alcohol (0.90 g, 1.07 mmol) DMAP (0.162 g, 1.61 mmol) and TsCl (0.300 g, 1.61 mmol) were added at 0 °C. The resulting solution was stirred at room temperature for 10 h. The reaction mixture was quenched using saturated NH_4Cl. The aqueous layer was extracted with DCM ×3. Combined organic layer were washed with brine dried over anhydrous Na_2SO_4, concentrated under reduce pressure. The crude product was purified by silica gel column chromatography (100 % DCM) to give **9** yellow oil (0.250 g 80 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.93 (d, J = 7.9 Hz, 2 H), 7.42 (d, J = 8.5 Hz, 2 H), 5.12 (s, 1 H), 4.95 (s, 1 H), 2.50 (s, 3 H),

2.01 (s, 2 H), 1.88 (s, 3 H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 146.8, 141.7, 130.2, 127.0, 115.8, 77.3, 76.7, 38.1, 21.8, 20.2. HRMS (ESI) calcd. $C_{12}H_{14}{}^2H_2O_3NaS$ [M+Na]: 265.0843, found: 265.0848.

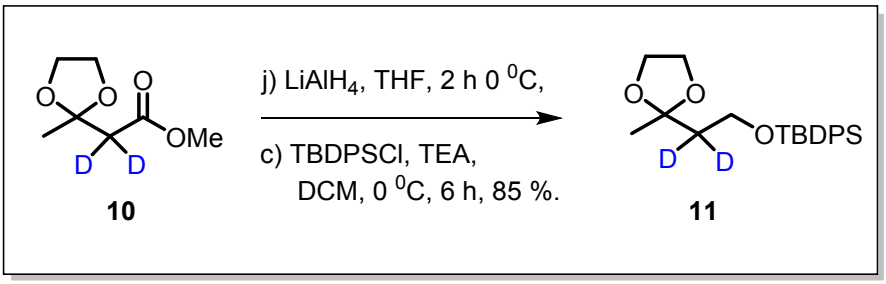


(1,1-^{2}H) IPP (2): To a stirred solution of tris-tetra-*n*-butylammonium hydrogen diphosphate (1.1 g, 1.23 mmol) in anhydrous ACN (20 mL) **9** (0.100g, 0.41 mmol) in ACN (5 mL) was added under inert conditions. The resulting solution was stir for 3h at RT. Further Purification of the product, use a general procedure for preparation of pyrophosphorylatio **A** (S3). After purification to give white solid **1** (0.040 g 40 % yield). ^{1}H NMR (500 MHz, (500 MHz, D_2O) δ 4.81 - 4.78 (m, 2 H), 2.90 (s, 2 H), 2.02 (s, 3H), ^{13}C NMR (125 MHz, (500 MHz, D_2O) δ 141.7, 115.4, 37.9 (d, $J_{c,p}$ = 5.80 Hz), 20.1. ^{31}P NMR (202 MHz, D_2O) δ -10.39 (1 P, d, $J_{P,P}$ = 19.7 Hz P1), -6.49 (1 P, d, $J_{P,P}$ = 19.7 Hz P2), HRMS (ESI) calcd. For $C_5H_{10}D_2O_7P_2Na$ [M+Na]: 271.0080 found 271.0076.

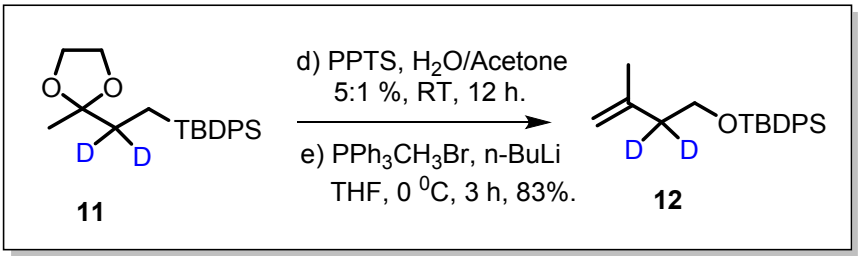
Synthesis of (2,2-^{2}H) IPP (3):

(2,2-^{2}H) Methyl (2-methyl-1,3-dioxolan-2-yl) acetate (10): The neat solution of **1** (1 g, 7.6 mmol) and D_2O (0.4 g, 23 mmol) was added and stirred reaction mixture further 12 h. Exchange proton of active methylene was determined using NMR. After the complete exchange of hydrogen, the solution was extracted with DCM × 3. Combined organic layer were dried over Na_2SO_4, concentrated under reduced pressure to give colorless oil

A solution of Deuterated ethyl acetoacetate (1g, 7.5 mmol) and ethylene glycol (0.7 g, 11.3 mmol) PTSA (0.120 g, 0.75 mmol) were added in toluene. The reaction medium was equipped with a Dean–Stark apparatus and then refluxed until no more water was collected 12 h. The reaction was quenched using saturated $NaHCO_3$ and extracted using DCM × 3. Combined organic layer were washed with brine, dried and over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by silica gel column chromatography in 20 % ethyl acetate/pet ether to give **10** as a colorless liquid (0.957 g 72 % yield). ^{1}H NMR (400 MHz, $CDCl_3$) 1.41 (s, 3H), 3.61 (s, 3H), 3.81 (s, 4H). HRMS (ESI) calcd. For $C_7H_{10}D_2O_2Na$ [M+Na]: 162.0861 found 162.0865



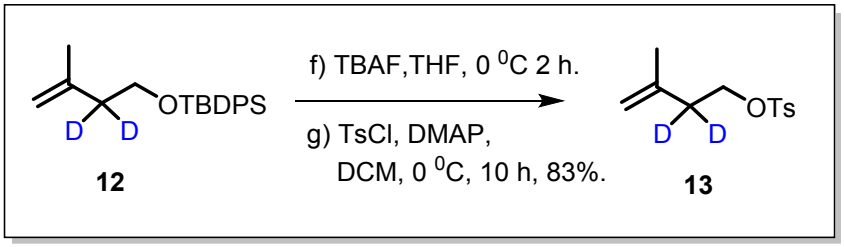
(2,2-^{2}H)- 1, 3-Dioxolane, 2-[2-[[(1,1-dimethylethyl)diphenylsilyl]oxy] ethyl]-2-methyl- (11): To stirred solution of $LiAH_4$ (0.327g, 1 8.5 mmol) in dry THF was added solution of **10** (0.800 g, 4.5, mmol) in THF at 0 °C. After the 2h reaction mixture was quenched using saturated Na_2SO_4 and filtered through celite pad and dried over Na_2SO_4. Concentrated under reduced pressure to give crude alcohol (0.500 g). This crude product was dissolved in dry DCM were added TEA (0.74 g, 7.4 mol) and TBDPSCl (1.5 g, 5.5 mol) at 0 °C. After 30 min reaction mixture allowed to warm to room temperature stirred for 5 h then reaction mixture dilute with water and extracted with DCM × 3. Combined organic layer were washed with brine and dried over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by silica gel column chromatography in 20 % ethyl acetate/pet ether to give **11** (1.1 g, 85% yield). ^{1}H NMR (400MHz, $CDCl_3$) 7.72 - 7.68 (m, 4 H), 7.44 - 7.37 (m, 6 H), 4.1 (s, 2 H), 3.92 - 3.81 (m, 2 H), 1.33 (s, 3 H), 1.06 (s, 9 H), ^{13}C NMR (100 MHz, $CDCl_3$) 135.5, 133.9, 129.5, 127.6, 108.9, 64.4, 62.4, 26.8, 24.3, 19.1. HRMS (ESI) calcd. For $C_{22}H_{28}{}^2H_2O_3NaSi$ [M+Na]: 395.2090, found: 395.2093.



(2,2-^{2}H)- tert-butyl(2-(2-methyl-1,3-dioxolan-2-yl)ethoxy)diphenylsilane (12): To a solution of **11** (0.900 g, 2.4 mmol) in acetone and water (5:1) at room temperature PTSA (0.041.g, 0.24 mmol) was added in one portion and stirred for 12 h. The reaction mixture was diluted using saturated $NaHCO_3$ and extracted with Et_2O × 3. Combined organic layer were dried over Na_2SO_4 concentrated under reduced pressure to give a crude deprotected ketone. Used this product without further purification.

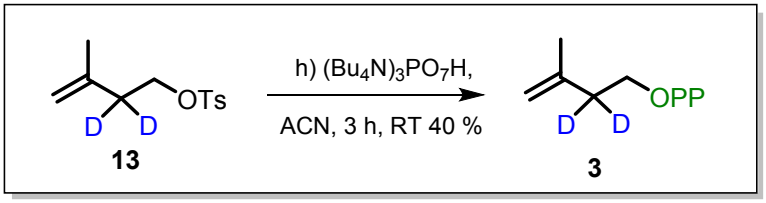
Methyltriphenylphosphonium bromide (1.2 g, 3.6 mmol) was dissolved in anhydrous THF (30 mL) and cooled to 0°C under inert atmosphere prior to the slow addition of *n*-BuLi (2.2 g, 3.6 mmol) The resulting Pale-yellow suspension was stirred at for 30 min and then slowly addition of ketone (0.600 g, 3.36 mmol) dissolved in anhydrous THF (5 mL). The mixture was stirred at room temperature for 3 h. the Progress of the reaction was monitored by TLC. On completion, the reaction was quenched by addition of NH_4Cl. The reaction mixture was extracted using Et_2O × 3. The crude product was purified by silica gel column chromatography in 10 % ethyl acetate/pet ether to give **12** as a colorless liquid (0.589 g, 83% yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.72 - 7.67 (m, 4 H), 7.44 - 7.37 (m, 6 H), 4.76 (s, 1 H), 4.69 (s, 1 H),

3.77 (s, 2 H), 1.69 (s, 3 H), 1.06 (s, 9 H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 142.9, 135.9, 135.6, 134.0, 129.5, 127.6, 111.7, 62.6, 30.9, 29.7, 26.8, 22.7, 19.2. HRMS (ESI) calcd. for $C_{21}H_{26}{}^2H_2ONaSi$ [M+Na$^+$]: 349.1933, found: 349.1931.

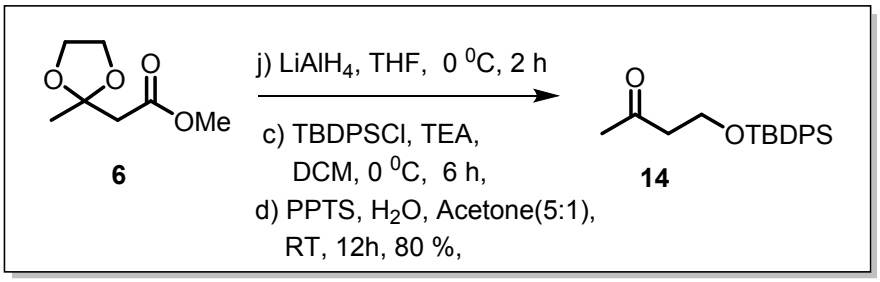


(2,2-^{2}H)-3-Methylbut-3-en-1-yl 4-methylbenzenesulfonate (13): A stirred solution of **12** (0.450 g, 1.3 mmol) in THF (5 mL) was treated with TBAF (2.2 mL, 2.2mmol) for 2 h at room temperature. The progress of the reaction was monitored by TLC. After completions of reaction, a mixture was diluted using saturated $NaHCO_3$ and extracted with DCM × 3. Combined organic layer were washed with brine and dried over Na_2SO_4. The filtrate solution of crude alcohol (0.100g, 1.1 mmol) was added DMAP (0.293 g, 1.7 mmol) and TsCl (0.323 g, 1.7 mmol) at 0 °C. The resulting mixture was stirred at room temperature for 10 h and the reaction was quenched by addition of NH_4Cl. The aqueous layer was extracted with DCM × 3. Combined organic layer were washed with brine and dried over anhydrous Na_2SO_4 and concentrated in rotatory vacuum. The crude product was purified by silica gel column chromatography (100 % DCM) to give **13** pale yellow oil (0.250 g, 83 %) yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J = 7.8

Hz, 2 H), 7.35 (d, J = 7.8 Hz, 2 H), 4.79 - 4.68 (s, 2H), 4.14 - 4.09 (2, 14 H), 2.45 (s, 3 H), 1.66 (s, 3 H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.7, 140.0, 133.1, 129.8, 127.9, 113.1, ,68.4, 29.6, 22.2, 21.6. HRMS (ESI) calcd. $C_{12}H_{14}{}^{2}H_2O_3NaS$ [M+Na]: 265.0843, found: 265.0846.



(2,2-^{2}H) IPP (3): To a solution of tris-tetra-*n*-butylammonium hydrogen diphosphate (1.1 g, 1.36 mmol) in anhydrous ACN (20 ml) was added a solution of **13** (0.110 g, 0.45mmol) in ACN (3 ml) under inert conditions. The resulting solution was stir for 2 h. Further purification of product to use general procedure for preparation of pyrophosphorylation (**A**). After purification to give white solid (0.038 g, 34 % yield). ^{1}H NMR (500 MHz, D_2O) δ 4.47 (m, 2 H), 2.18 (s, 2 H), 2.02 (s, 3 H), ^{13}C NMR (125 MHz, D_2O) δ 143.8, 111.5, 64.0, (d, $J_{C,\,P}$ = 4.1 Hz) 21.6, ^{31}P NMR (202 MHz, D_2O) δ 10.44 (1 P, d, $J_{P,\,P}$ = 19.8 Hz, P1), -7.30 (1 P, d, $J_{P,\,P}$ = 19.8 Hz, P2). HRMS (ESI) calcd. For $C_5H_{10}D_2O_7P_2Na$ [M+Na]: 171.0080 found 171.0077.

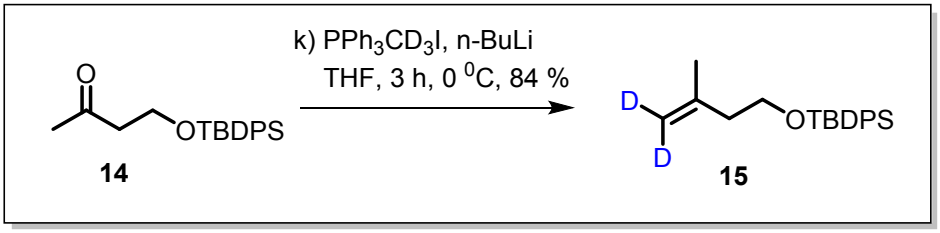


1, 3-Dioxolane, 2-[2-[[(1,1-dimethylethyl)diphenylsilyl]oxy]ethyl]-2-methyl- (14): To stirred solution of $LiAlH_4$ (0.210 g, 5.8 mmol) in dry THF was added solution of **6** (500 g, 2.9 mmol) in THF at 0 °C. After 2 h reaction mixture was quenched using saturated Na_2SO_4 filtered through a celiet pad and dried over Na_2SO_4. Concentrated under reduced pressure give crude alcohol.

Crude alcohol (0.300 g, 10.6 mmol) dissolved in dry DCM were added TEA (0.44 g, 4.4 mmol) and TBDPSCl (0.927 g, 3.4 mmol) at 0 °C. After 30 min reaction mixture allowed to warm to room temperature stirred for 6 h. The reaction mixture dilutes with water, extracted with Et_2O × 3. Combined organic layer were washed with brine and dried over Na_2SO_4, concentrated under reduced pressure, to gate crude TBDPS protected ether. To a solution of crude TBDPS ether (0.700 g, 1.8 mmol) In acetone and water (5:1) at room temperature PTSA (0.032 g, 0.18 mmol) was added in one portion and stirred for 12 h. The reaction mixture diluted using saturated $NaHCO_3$ and extracted with Et_2O × 3. Combined organic layer were dried over Na_2SO_4, concentrated under reduced. Crude product was purified by silica gel column chromatography in 20 % ethyl acetate / pet ether to give **14** (80 %

Yield), ^{1}H NMR (500MHz ,$CDCl_3$) δ 7.67 (dd, J = 1.5, 7.9 Hz, 4 H), 7.49 - 7.36 (m, 6 H), 3.95 (t, J = 6.3 Hz, 2 H), 2.65 (t, J = 6.3 Hz, 2 H), 2.20 (s, 3 H), 1.04 (s, 9 H) ^{13}C NMR (500 MHz, $CDCl_3$) δ 207.9, 135.5, 133.4, 129.7, 127.7, 59.7, 46.3, 30.7, 26.8, 19.1 HRMS (ESI) calcd. For $C_{21}H_{28}OSi$ [M+1]: 324.1909 found 324.1905.

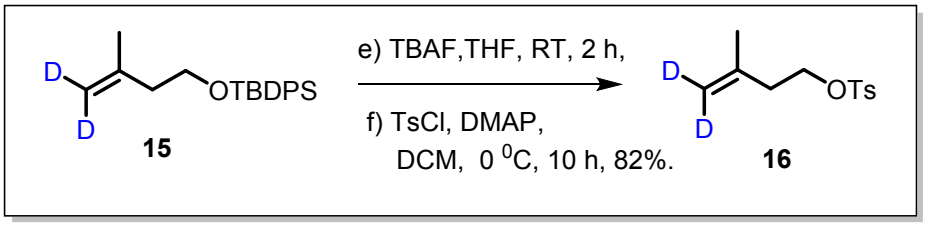
Synthesis of (4,4-^{2}H) IPP (4)



(4,4-^{2}H)-*tert*-Butyl((3-methylbut-3-en-1-yl)oxy)diphenylsilane (15):

To stirred solution of ($^{2}H_3$)-methyltriphenylphosphonium iodide (1.09 g, 3.0 mmol) was dissolved in anhydrous THF (25 mL) and cooled at 0 °C under inert atmosphere prior to the slow addition of *n*-BuLi (1.8 g, 3.3 mol). The resulting Pale-yellow suspension was stirred at for 30 min and slow addition of ketone (0.500 g, 1.5 mmol) dissolved in anhydrous THF (5 mL). The mixture was stirred at room temperature for 3h. After completion of the reaction was quenched, using saturated NH_4Cl. The reaction mixture was extracted using Et_2O × 3. Combined organic layer were dried over Na_2SO_4, concentrated under reduced pressure. A Crude product was purified by silica gel column chromatography in 10 % ethyl acetate/pet ether to give clear liquid (0.506 84 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.71 - 7.66 (m, 4 H),

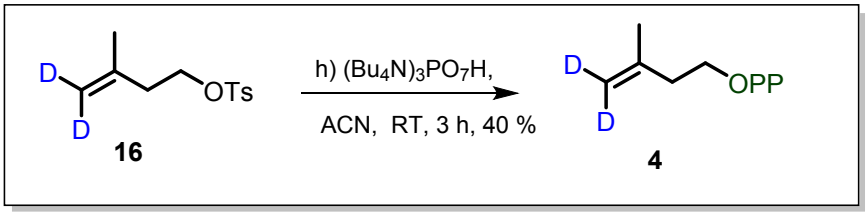
7.46 - 7.36 (m, 6 H), 3.77 (t, J = 6.8 Hz, 3 H), 2.28 (t, J = 6.8 Hz, 2 H), 1.68 (s, 3 H), 1.05 (s, 9 H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.6, 134.0, 129.5, 127.6, 62.7, 40.8, 29.7, 26.8, 19.2. HRMS (ESI) calcd. for $C_{21}H_{26}{}^2H_2ONaSi$ [M+Na]: 349.1933, found: 349.1937.



(4,4-^{2}H)-3-Methylbut-3-en-1-yl 4-methylbenzenesulfonate (16): A stirred solution of **15** (0.400 g, 1.2 mol) in THF (5 mL) was treated with TBAF (1.8 g, 1.8 mmol) for 2 h at room temperature. After completion of reaction add saturated $NaHCO_3$ and extracted with DCM × 3. The combined organic layers were washed with brine dried over the Na_2SO_4.

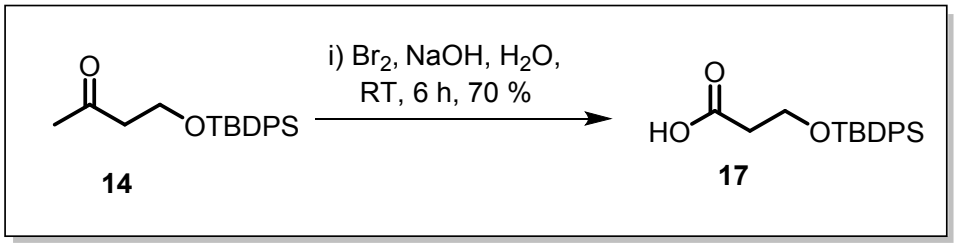
The filtrate solution of a crude alcohol (0.098g, 1.01 mmol) was added DMAP (0.183 g, 1.13 mmol) and TsCl (0.288 g, 1.5 mmol) the reaction mixture at 0 °C. The resulting solution was stirred at room temperature for 10 h and the reaction was diluted using saturated NH_4Cl. The aqueous layer was extracted with DCM × 3. Combined organic layer were washed with brine, dried over Na_2SO_4, concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (DCM) to give pale yellow oil **16** (0.243 g, 82 %) yield. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (d, J = 8.3 Hz, 2 H), 7.35 (d, J = 8.3 Hz, 2 H), 4.13 (t, J = 6.8 Hz, 2 H), 2.45 (s, 3 H), 2.35 (t, J = 6.8 Hz, 2

H), 1.66 (s, 3 H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.7, 139.9, 133.1, 129.8, 127.9, 68.5, 36.6, 22.2, 21.6. HRMS (ESI) calcd. $C_{12}H_{14}{}^2H_2O_3NaS$ [M+Na]: 265.0843, found: 265.0841.



(4,4-^{2}H) IPP (4): To a solution of tris-tetra-*n*-butylammonium hydrogen diphosphate **(**1.3 g, 1.47 mmol) in anhydrous ACN (25 mL) was added a solution of **16** (0.120g, 0.49 mmol) in ACN (5 mL) under inert condition. The resulting solution was stir for 3 h. Further Purification of product to use general procedure for preparation of pyrophosphorylation **A (S-3)**. After purification to give white solid (0.041 g, 38 %) yield. ^{1}H NMR (500 MHz, D_2O) δ 4.00 (q, J = 6.4 Hz, 2 H), 2.33 (t, J = 6.7 Hz, 2 H), 1.71 (s, 3 H), ^{13}C NMR (125 MHz, D_2O) δ 143.7, 64.0, (d, $J_{C,P}$ = 5.72 Hz) 37.8, (d, $J_{C,P}$ = 7.63 Hz) 37.7, 21.6, ^{31}P NMR (202 MHz, D_2O) δ -10.39 (1 P, d, $J_{P,P}$ = 19.78 Hz, P1), - 6.55 (1 P, d, $J_{P,P}$ = 19.78 Hz, P2). HRMS (ESI) calcd. For $C_5H_{10}D_2O_7P_2Na$ [M+Na]: 271.0080 found 271.0083.

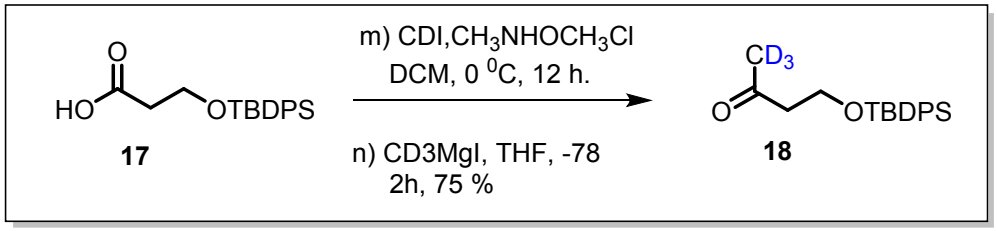
Synthesis of (3,3,3-^{2}H) IPP:



4-((tert-butyldiphenylsilyl) oxy) butan-2-one (17): To a solution of **14** (1 g, 2.7 mmol) in acetone and H_2O (5:1) at room temperature PTSA (0.046 g, 0.27 mmol) was added in one portion and stirred for 12 h. The reaction mixture was diluted using saturated $NaHCO_3$ and extracted with diethyl ether. Combined organic layer were dried over Na_2SO_4 and concentrated under reduced pressure to give crude ketone used for next step without purification.

Bromine (1.7g 10.7 mmol) was added dropwise over five minutes to an aqueous solution of NaOH (0.24 g 6.3mmol, 3.10 M aq). After 30 minutes, the resulting pale orange solution was transferred to RB containing crude ketone (0.720 mg, 2.4 mmol) in dioxane as a solvent. The pale-yellow mixture was allowing to stir for 5 h. Then the reaction mixture was quenched using $Na_2S_2O_3$. The mixture allowed stirring for 20 minutes. Acidified with 20 % H_2SO_4 and then extracted with DCM (3 x 2 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to afford crude acid. The residue was purified by column chromatography in 30 % ethyl acetate/pet ether to

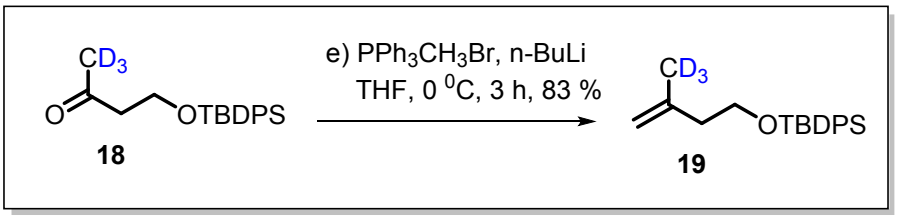
give acid **17** (0.620 g 70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.69 (d, *J* = 6.7 Hz, 4 H), 7.47 - 7.38 (m, 6 H), 3.97 (t, *J* = 6.1 Hz, 2 H), 2.63 (t, *J* = 6.1 Hz, 2 H), 1.04 (s, 9 H), ^{13}C NMR (100 MHz, $CDCl_3$) δ 178.1, 135.5, 133.3, 129.7, 127.7, 59.5, 37.6, 31.36, 26.7 HRMS (ESI) calcd. For $C_{19}H_{24}O_3SiNa$ [M+Na]: 328.1392 found 328.1397.



(4,4,4-^{2}H)- 4-(tert-Butyldiphenylsilyloxy)-2-butanone (19): 4 To the stair solution of 1,1′-Carbonyldiimidazole (0.300 g, 2.2 mmol) was added in over a period of 15 min to a solution of **17** (0.500 g, 14.9 mmol) in DCM (25 mL) at 0 C and then stirred at for 1 h. *N, O*-dimethylhydroxylamine hydrochloride (0.200 g, 3 mmol) was then added and the resulting reaction mixture was stirred 12 h. The reaction mixture was then diluted with diethyl ether and filtered. The filtrate was washed with 5 % aq. citric acid and brine, dried over Na_2SO_4 and concentrated under vacuum to give crude product used without further purification.

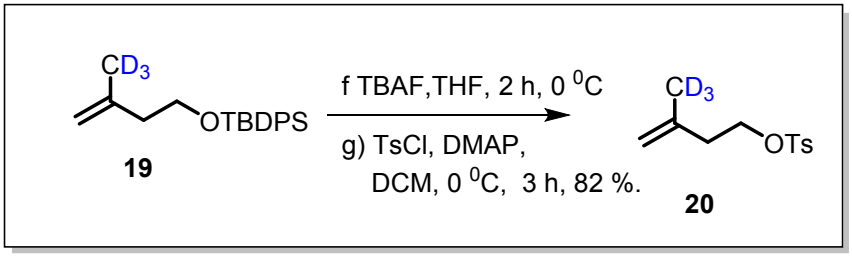
CD_3MgBr (3M 0.420 mL, 1.5 mmol) was added dropwise to a solution of starting solution of crude amide (0.410 g, 1.2 mmol) in dry THF (30 mL) at −78 °C for 1h. Then the reaction mixture was gradually warmed to 0 °C and stirred for 2 h. The reaction was quenched by slow

addition of saturated NH_4Cl solution and extracted using EtOAc × 3. The combined organic layers were washed with brine, dried over Na_2SO_4, and concentrated under vacuum. Purification by flash column chromatography in 20 % ethyl acetate/pet ether to give **18** (0.380 g 75%) yield. ^1H NMR (400 MHz, $CDCl_3$) δ 7.68 (d, *J* = 6.8 Hz, 4 H), 7.46 - 7.35 (m, 6 H), 3.96 (t, *J* = 6.1 Hz, 2 H), 2.66 (t, *J* = 6.1 Hz, 2 H), 1.06 (s, 9 H), ^{13}C NMR (101 MHz, $CDCl_3$) δ 208.0, 135.5, 133.4, 129.7, 127.7, 77.3, 76.7, 59.7, 46.3, 29.7, 26.7. HRMS (ESI) calcd. For $C_{22}H_{23}D_3O_2SiNa$ [M+Na]: 352.1788 found 352.1786.



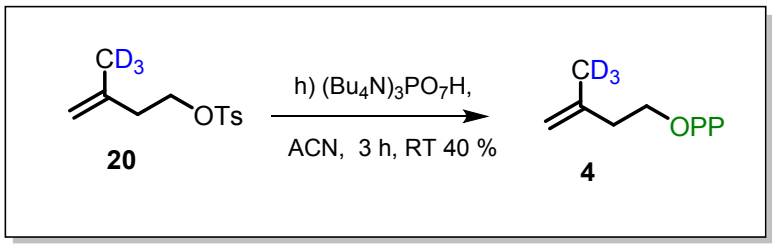
(3,3,3-^{2}H)-*tert*-Butyl((3-methylbut-3-en-1-yl)oxy)diphenylsilane (19): Methyltriphenylphosphonium bromide (0.760 g, 2.1 mmol) was dissolved in anhydrous THF (25 mL) and cooled to 0 °C under inert atmosphere prior to the slow addition of *n*-BuLi (1.3 mL, 2.1 mmol) The resulting Pale-yellow suspension was stirred for 30 min and then slowly addition of ketone **18** (0.350 g, 1.0 mmol) dissolved in anhydrous THF (5 mL). The mixture was stirred at room temperature for 3 h. The progress of the reaction was monitored by TLC. On completion, the reaction was quenched by addition NH_4Cl. The reaction mixture was

extracted using diethyl ether. The crude product was purified by silica gel column chromatography in 10 % ethyl acetate/pet ether to give **19** colorless liquid (0.589 g, 83%) yield. 1H NMR (400MHz, $CDCl_3$) δ 7.68 (d, *J* = 6.7 Hz, 4 H), 7.48 - 7.35 (m, 6 H), 4.75 (s 1 H), 4.69 (s, 1 H), 3.77 (t, *J* = 7.0 Hz, 2 H), 2.29 (t, *J* = 6.7 Hz, 2 H), 1.05 (s, 9 H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 135.6, 134.0, 129.5, 127.6, 111.7, 62.7, 40.8, 29.7, 26.8. HRMS (ESI) calcd. For $C_{21}H_{25}D_3O_3SiNa$ [M+Na]: 350.1995 found 350.1994.



(3,3,3-^{2}H)-3-Methylbut-3-en-1-yl 4-methylbenzenesulfonate (20): A stirred solution of **19** (0.270 g, 0.8 mol) in THF (5 mL) was treated with TBAF (1.2 g, 1.2 mmol) for 2 h at room temperature. The progress of the reaction was monitored by TLC. After completions of reaction, diluted using saturated $NaHCO_3$ extracted with DCM. The combined organic layers were washed with brine dried over the Na_2SO_4. The filtrate solution of crude alcohol (0.070 g, 0.76 mmol) was added DMAP (0.178 g, 1.1 mmol) and TsCl (0.209 g, 1.1 mmol) in a reaction mixture at 0 °C. The resulting solution was stirred at room temperature for 3 h. The reaction was quenched by addition of saturated NH_4Cl. The aqueous

layer was extracted with DCM × 3. Combined organic layer was washed with brine and dried over anhydrous Na_2SO_4, concentrated under reduced pressure. The crude product was purified by silica gel column chromatography (100% DCM) to give **20** yellow oil (0.176 % 83 % yield). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.80 (d, J = 8.0 Hz, 1 H), 7.35 (d, J = 8.0 Hz, 1 H), 4.79 (m 2H,) 4.13 (t, J = 6.9 Hz, 2 H), 2.46 (s, 3 H) 2.35 (t, J = 6.9 Hz, 2 H), ^{13}C NMR (125 MHz, $CDCl_3$) δ 144.7, 140.3, 133.1, 129.8, 127.9, 113.1, 68.5, 36.7, 21.6. HRMS (ESI) calcd. For $C_{12}H_{13}D_3O_3Na$ [M+Na]: 243.0906 found 243.0904.

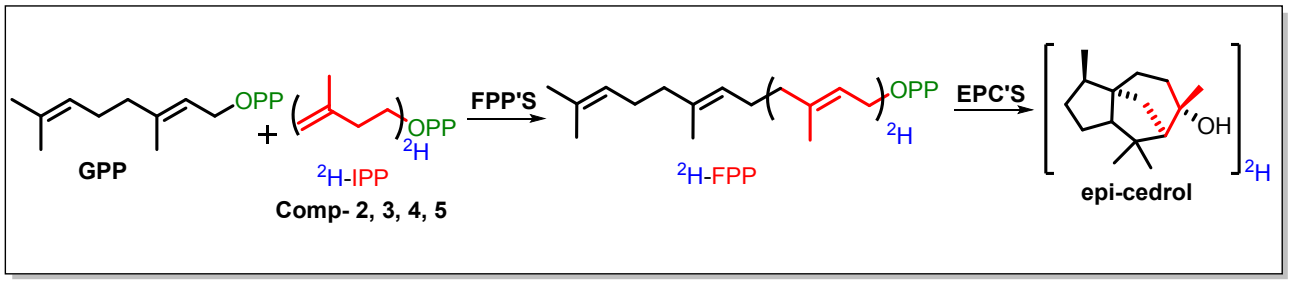


(3,3,3-^{2}H) IPP: To the stirred solution of Tris-tetra-*n*-butylammonium hydrogen diphosphate **(**1.2 g, 1.13 mmol) in anhydrous ACN (25 mL) was added a solution of **20** (0.105g, 0.41 mmol) in ACN (5 mL) under inert conditions. The resulting solution was stir for 3 h. Further Purification of product to use general procedure for preparation of pyrophosphorylatio **A (S-3)**. After purification to give white solid **4** (0.038 g, 36% yield. ^{1}H NMR (500 MHz, D_2O) δ 4.80 (dd, J = 1.5, 9.5 Hz, 2 H), 4.01 (q, J = 6.4 Hz, 2 H), 2.34 (t, J = 6.6 Hz, 2 H), ^{13}C NMR (125 MHz, D_2O) δ 143.8, 111.5, 64.1, (d, $J_{C,\ P}$ = 4.7 Hz) 37.8 (d, $J_{C,\ P}$ = 7.63 Hz), ^{31}P NMR (202 MHz, D_2O) δ -10.46 (1 P, d, $J_{P,\ P}$ = 19.8 Hz,

P1), -7.25 (1 P, d, $J_{P,\ P}$ = 19.8 Hz, P2), HRMS (ESI) calcd. For $C_5H_9D_3O_7P_2Na$ [M+Na]: 272.0144 found 172.0139. HRMS (ESI) calcd. For $C_5H_{10}D_2O_7P_2Na$ [M+Na]: 271.0080 found 271.0077.

Enzymatic assay:

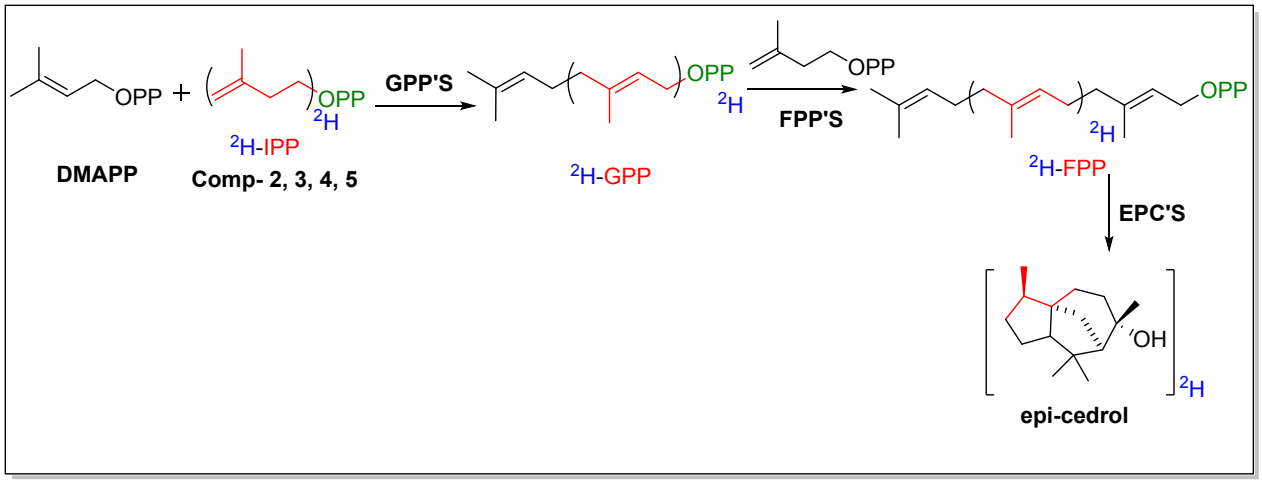
1)



In typical enzyme activity was assayed using A 250 µL enzymatic reaction mixture containing Tris-Assay buffer pH 8.5 (25mM Tris-HCl, 2.5mM Dithiothretrol, 10 mM $MgCl_2$,10% glycerol), GPP (150 µM), 100 µM ^{2}H-IPP (C1-^{2}H2, C2-^{2}H, C4-^{2}H2, C5-^{2}H3), FPPS (25 µg) incubated at 30 ºC for 2 h at 60 rpm (in wet bath, Brunswick, Eppendorf). After that epicedrol synthase (EPCS, 25 µg) enzyme was added in each assay and keep it for 1 h incubation at 30 ºC at 60 rpm. All the enzymatic reaction was stopped by an addition of 10µl of absolute ethanol (95%) followed by vortex it for 30 secs, was extracted with hexane (3×50 mL), hexane layer was removed by passing it with N_2 gas. labeled Epicedrol was identified by GC-MS analysis by using Agilent Technology 5975-7890 GC-MS system with a HP-5MS capillary

column (30m x 0.250 mm x 0.25 µ coating of 5% phenyl methyl siloxane). Injections were made cool on-column at 40ºC with oven programming from 40°C (50°C y min) to 50°C (5-min hold), then 10°Cy min to 250°C, then 50ºCy min to 300ºC. Separations were made under a constant flow of 1 ml He/min. Mass spectral data were collected at 70 eV and analyzed by using MSD Chem station software.

2)



A 250 µL enzymatic reaction mixture containing Tris-assay buffer pH 8.5 (25mM Tris-HCl,2.5mM Dithiothretrol, 10mM $MgCl_2$, 10% glycerol), purified GPPS (50.2µg), 100 µM ^{2}H-IPP (C1-$^2H_2$, C2-$^2H_2$, C4-$^2H_2$, C5-$^2H_3$), 150 µM DMAPP incubated at 30 ºC for 6 h at 60 rpm (in wet bath, Brunswick, Eppendorf). After that again IPP (100 µM) then FPPS (25 µg), EPCS (25 µg) both the enzymes were added in each assay and keep it for 2h incubation at 30 ºC at 60 rpm. All the enzymatic reaction was stopped by an addition of 10µl of absolute ethanol (95%) followed by vortex it for 30 secs, was extracted with

hexane (3×50 mL), hexane layer was removed by passing it with N_2 gas. labeled Epicedrol was identified by GC-MS analysis by using Agilent Technology 5975-7890 GC-MS system with a HP-5MS capillary column (30m x 0.250 mm x 0.25 μ coating of 5% phenyl methyl siloxane). Injections were made cool on-column at 40ºC with oven programming from 40°C (50°C y min) to 50°C (5-min hold), then 10°Cy min to 250°C, then 50ºCy min to 300ºC. Separations were made under a constant flow of 1 ml He/min. Mass spectral data were collected at 70 eV and analyzed by using MSD Chem station software.

References:

17. S.S. Shinde, A. Minami, Z. Chen, T. Tokiwano, T. Toyomasu, N. Kato, T. Sassa, H. Oikawa, *J. Antibiotics*., **2017**, 70, 632

22.V. A. Weller, M. D. Distefano, *J. Am. Chem. Soc.*, **1998**, 120, 7975.

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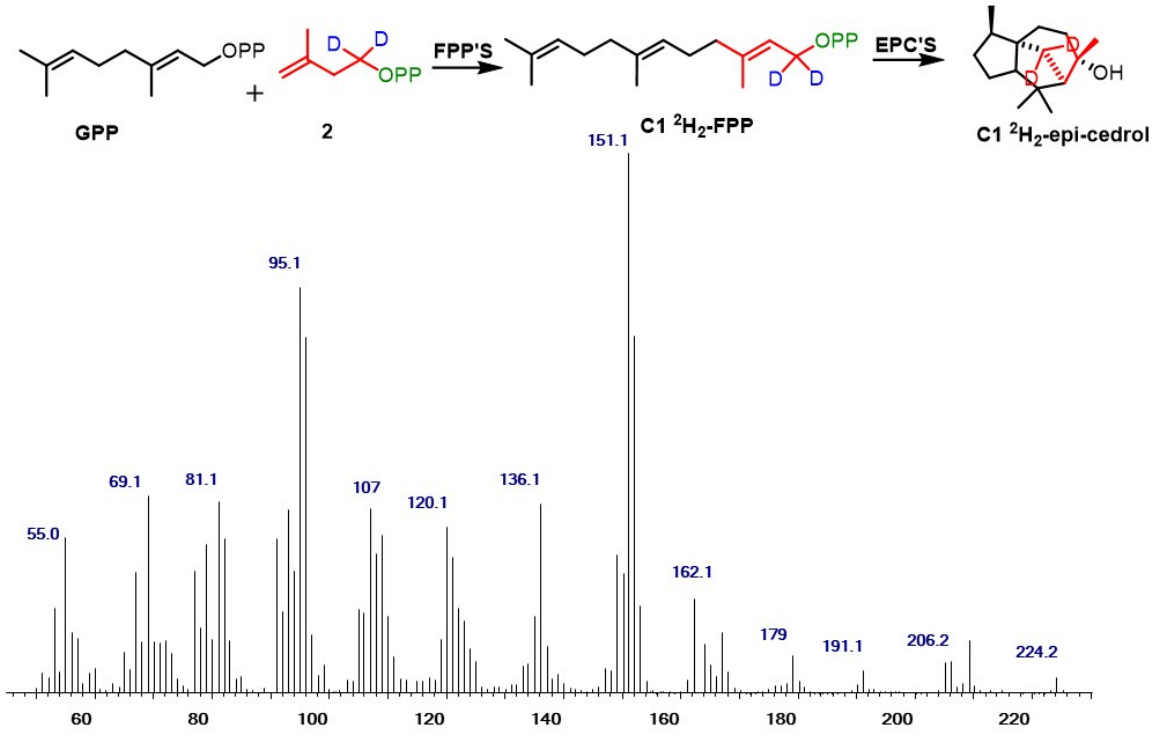
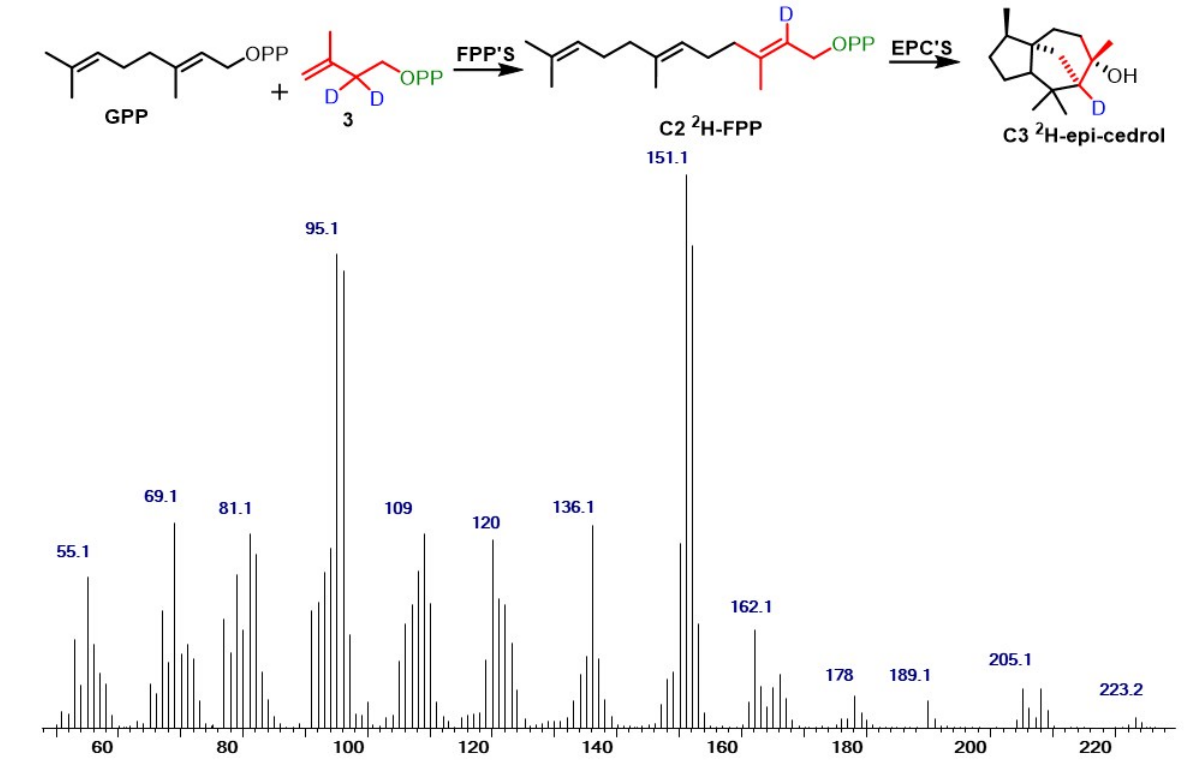
GC-MS of frist unit labling of FPP.

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EPC'S
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81.1
109
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162.1
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223.2
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80
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160
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200
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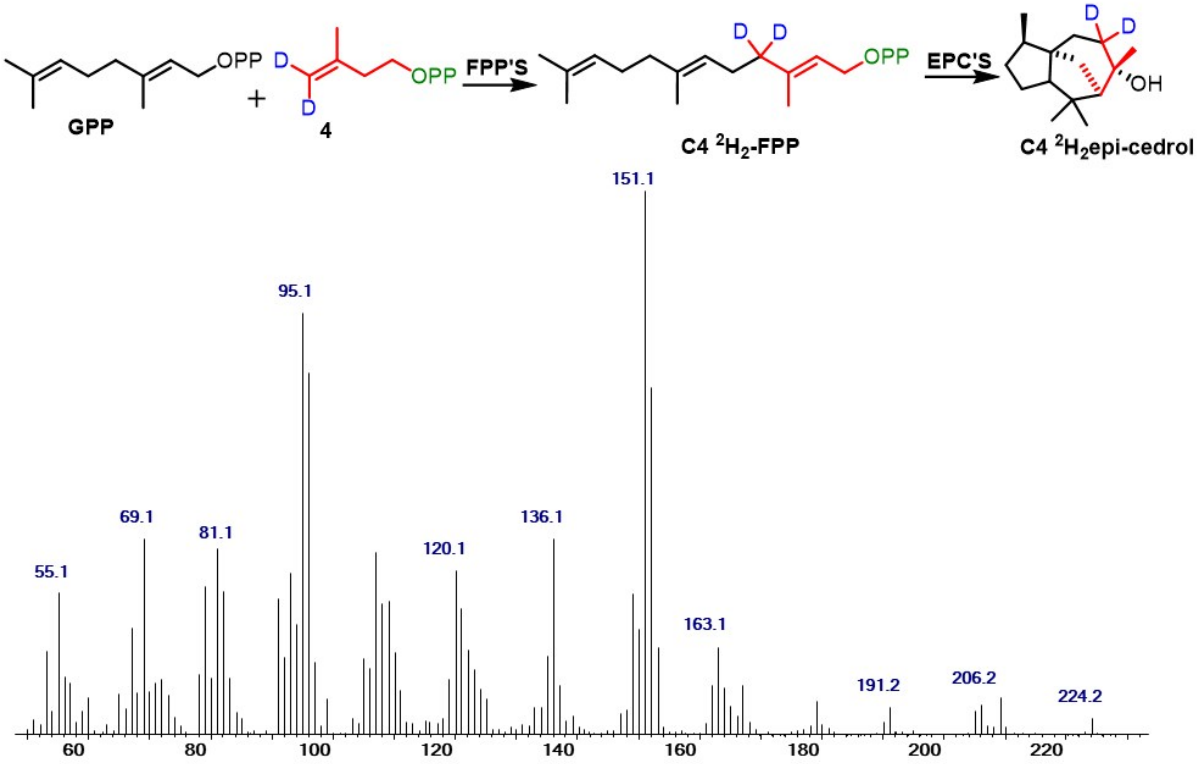
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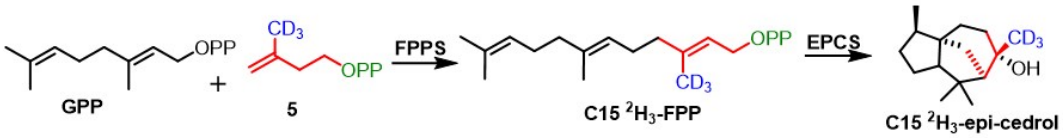
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C15 2H3-FPP
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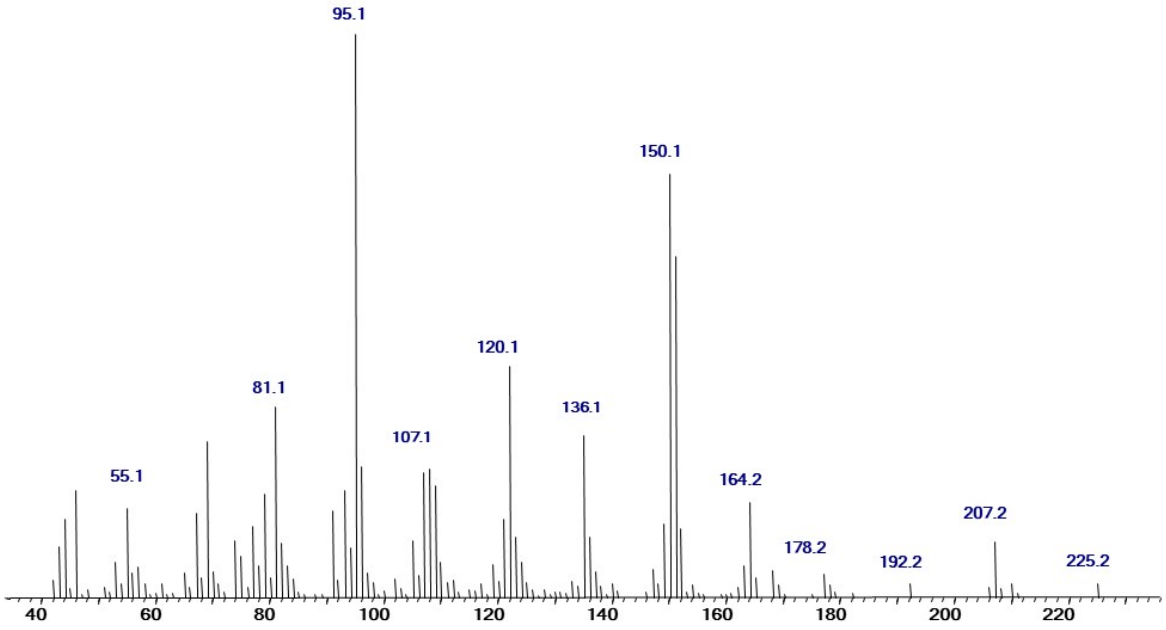
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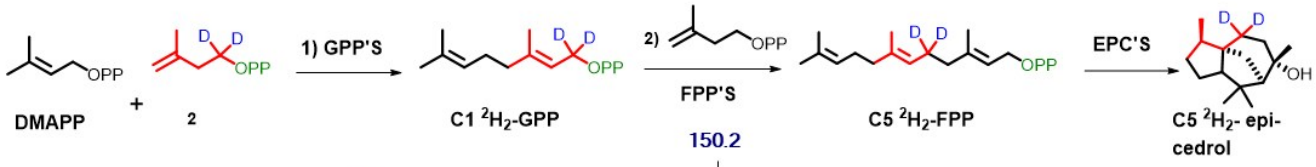
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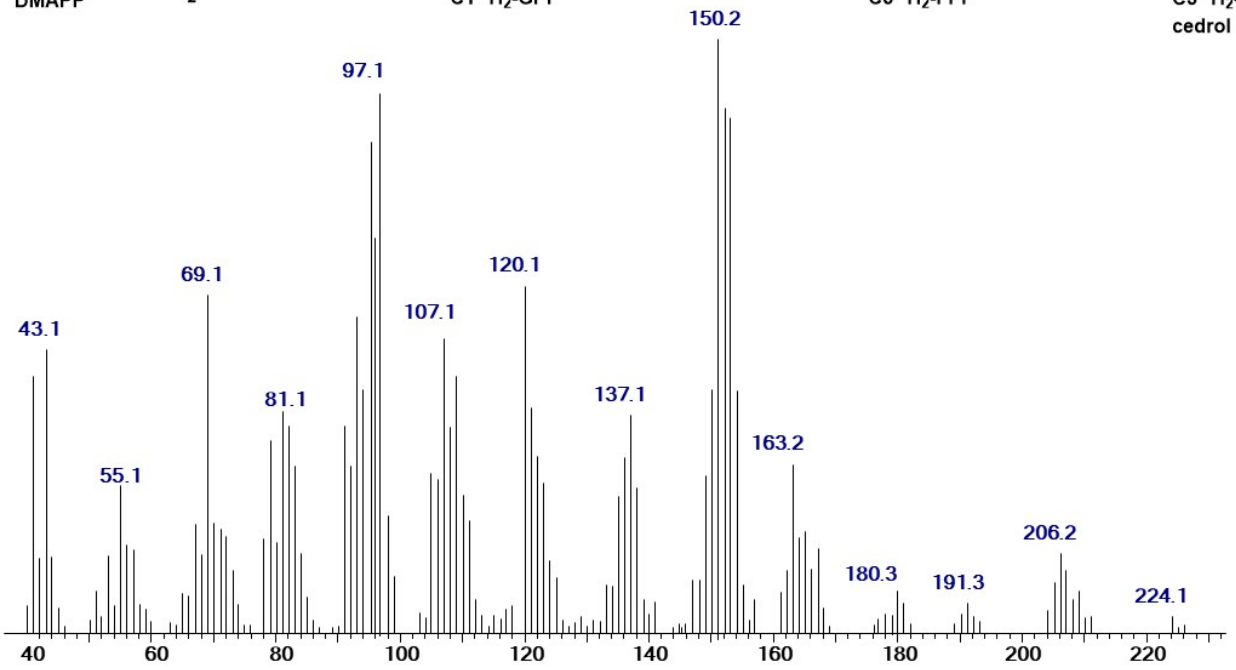
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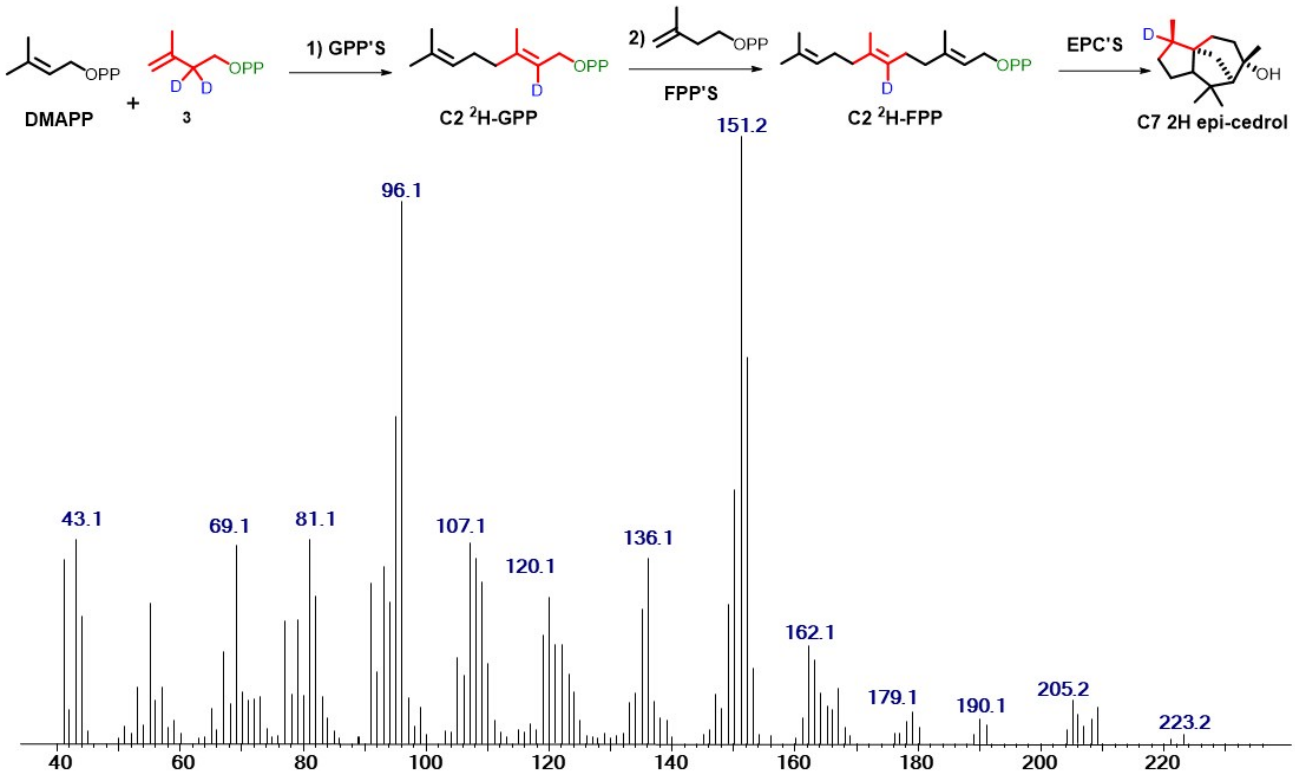
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2)
FPP'S
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EPC'S
C7 2H epi-cedrol
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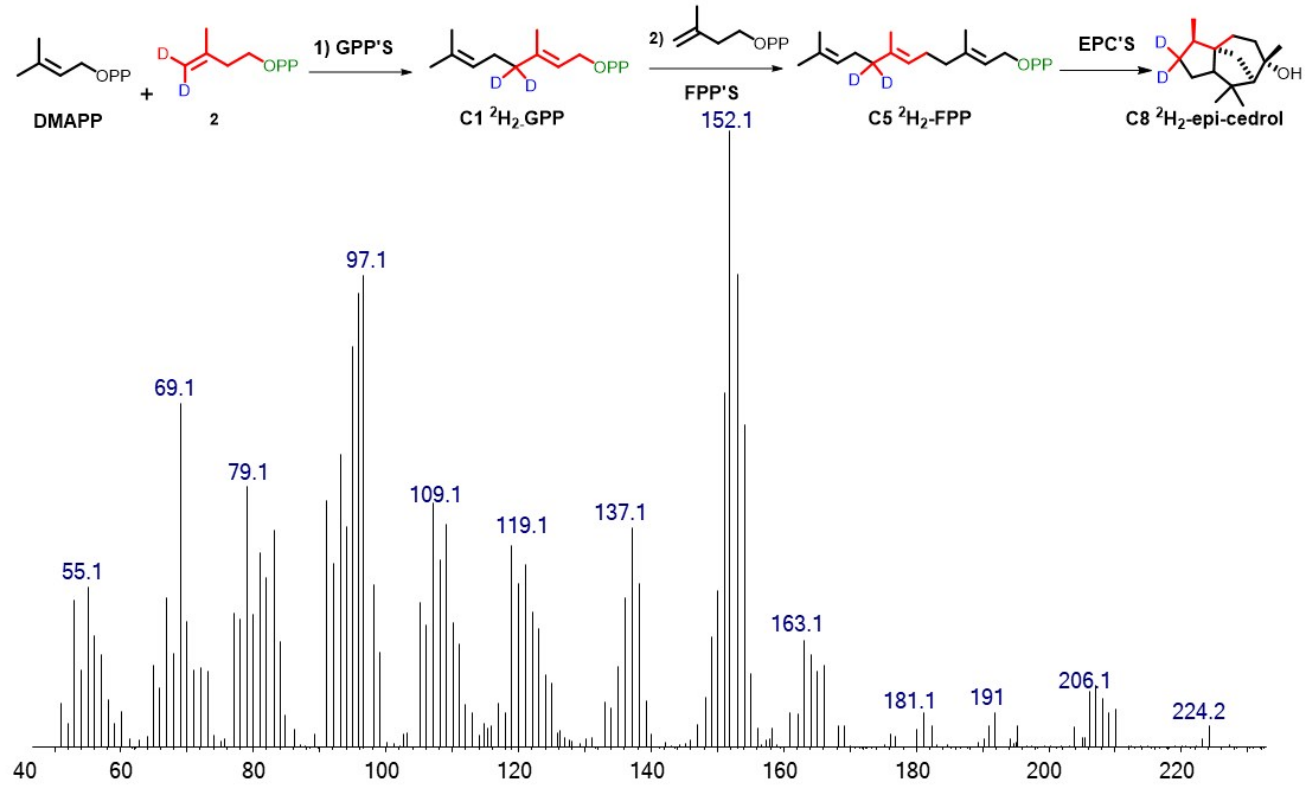
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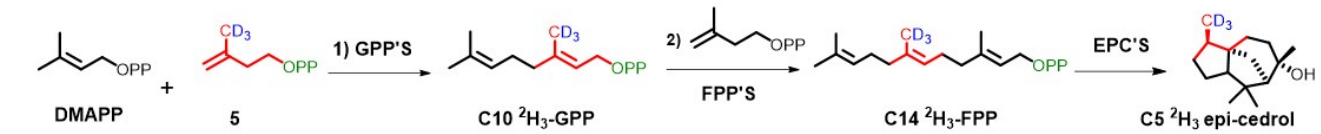
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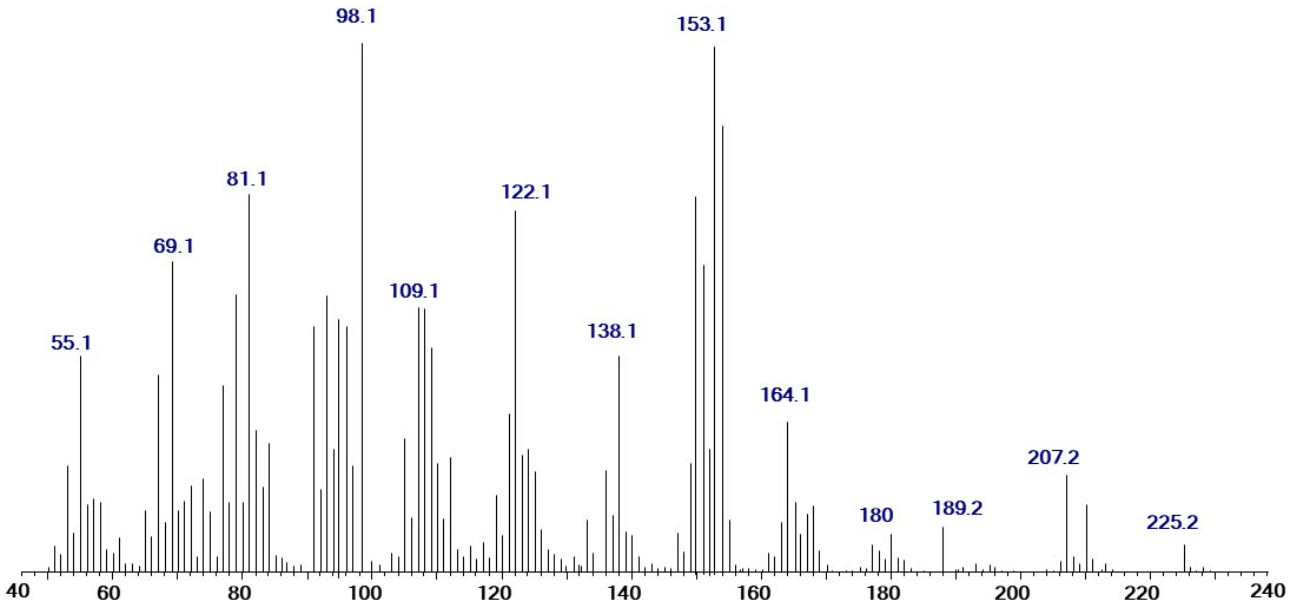
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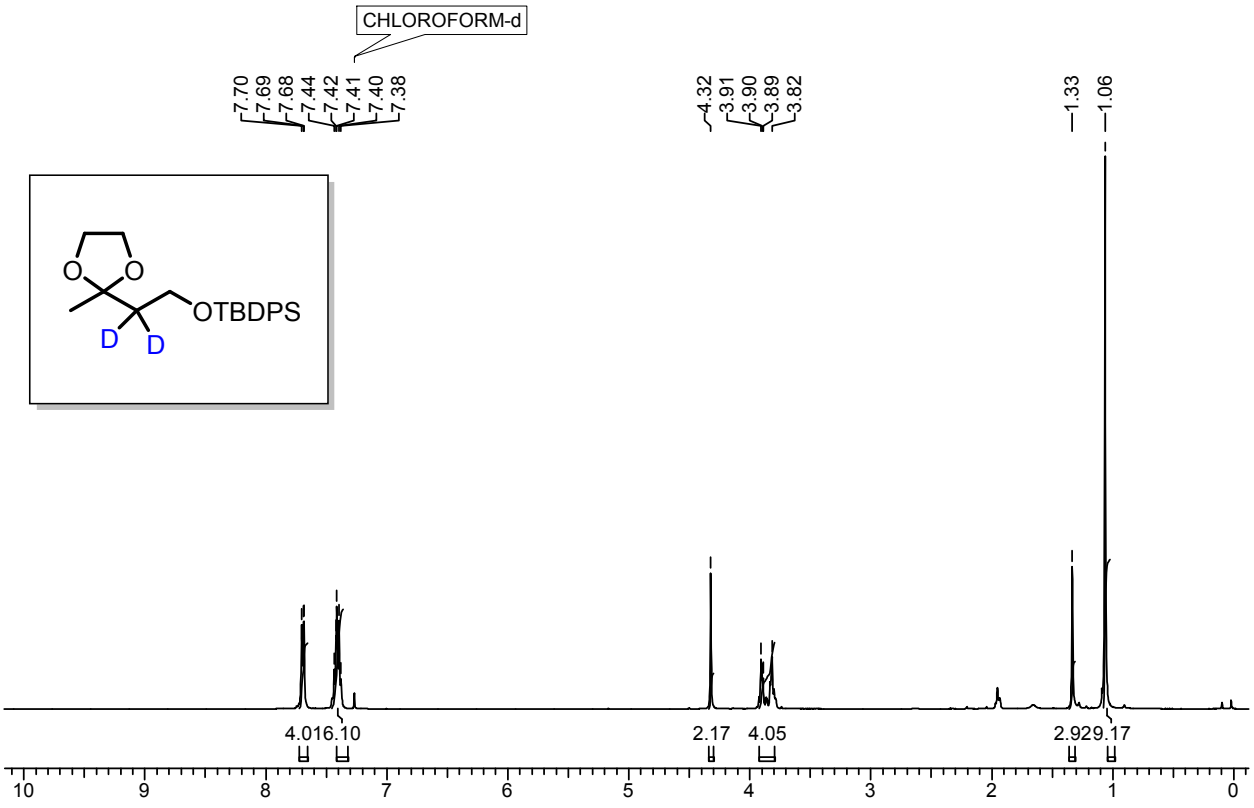
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4.05
2.92
9.17
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5
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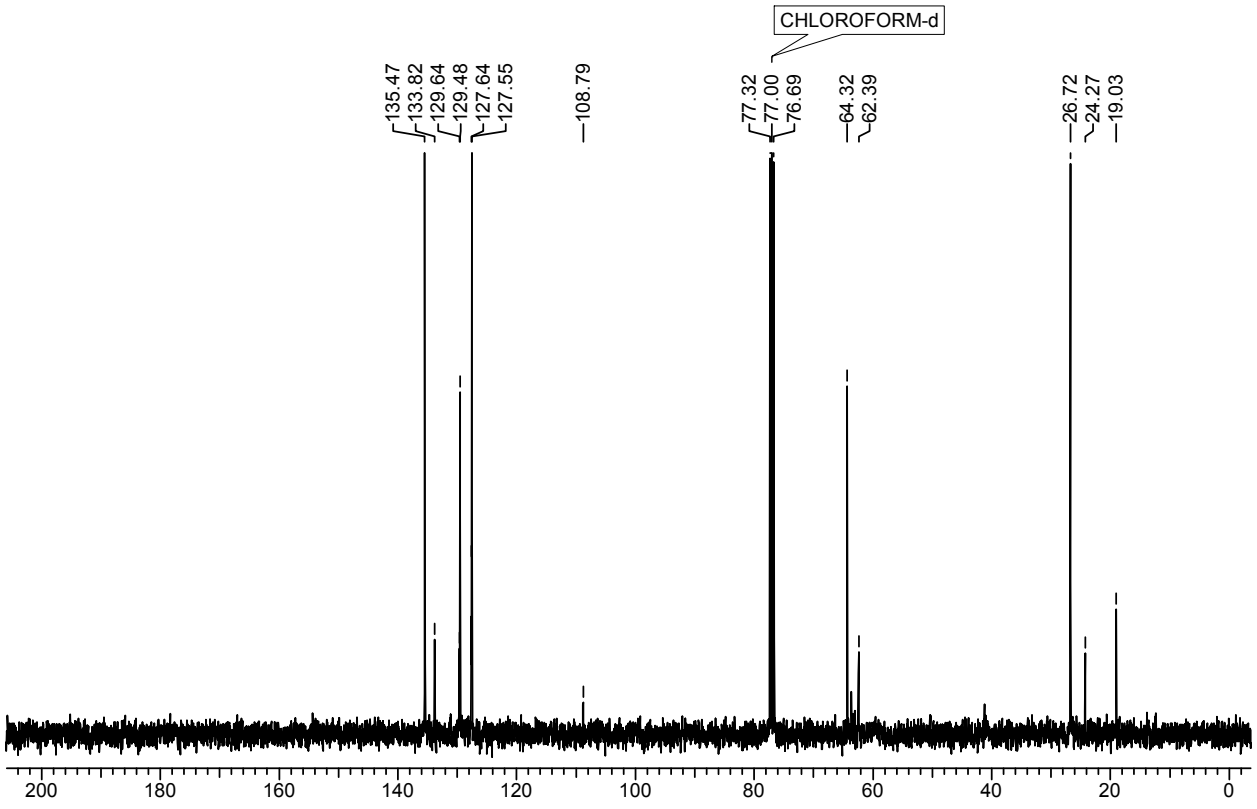
CHLOROFORM-d
135.47
133.82
129.64
129.48
127.64
127.55
108.79
77.32
77.00
76.69
64.32
62.39
26.72
24.27
19.03
200
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140
120
100
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60
40
20
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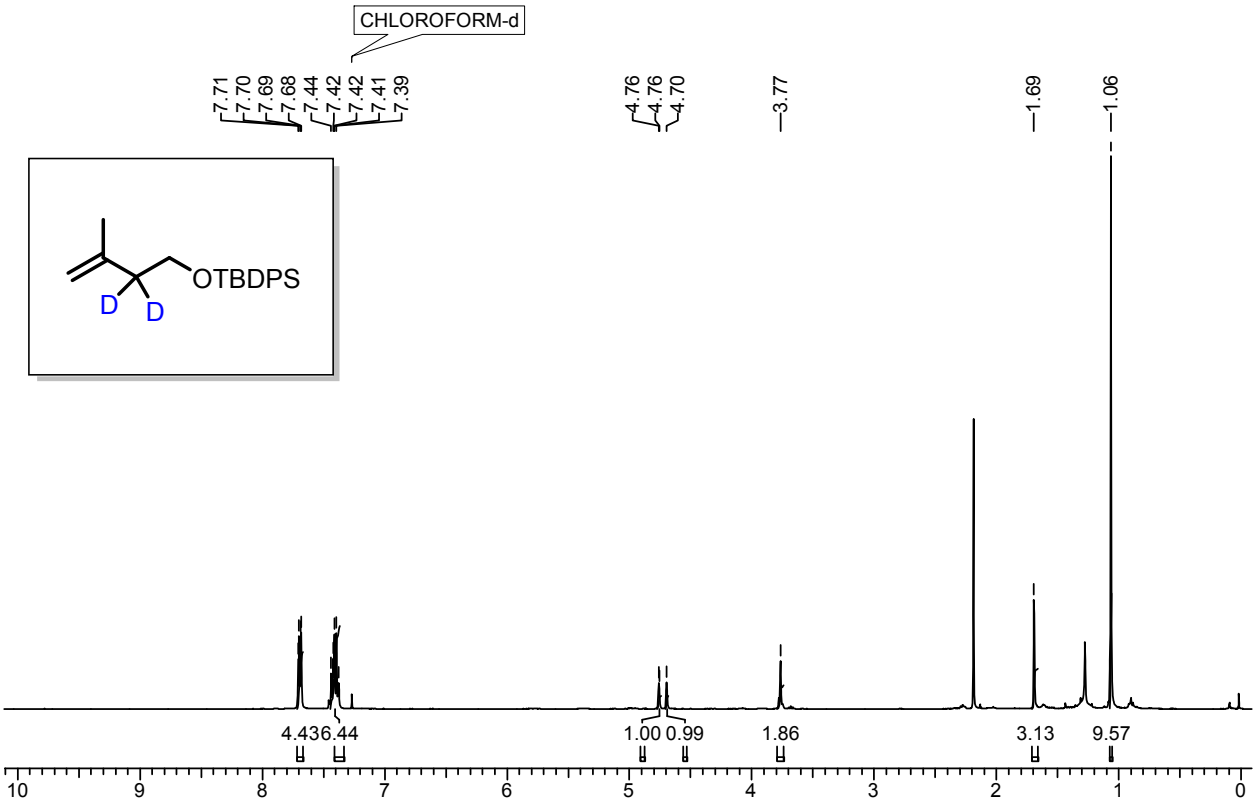
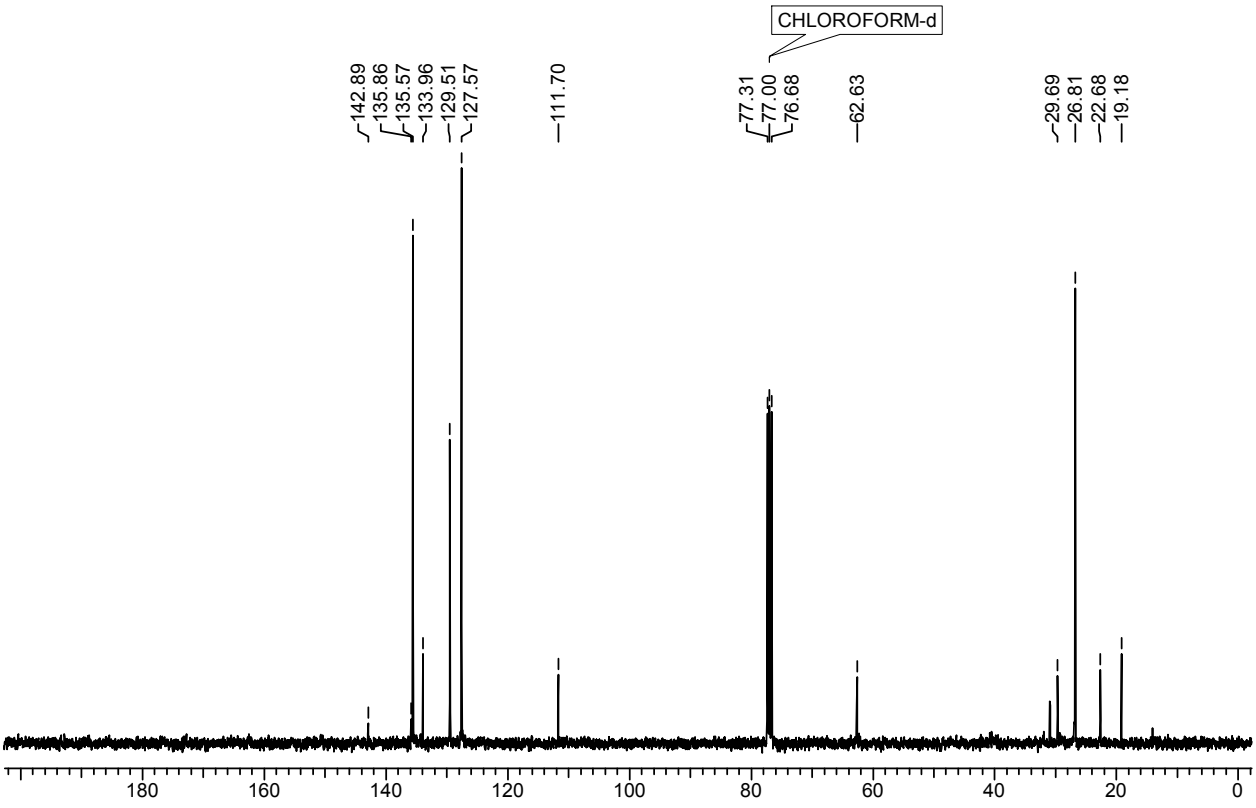
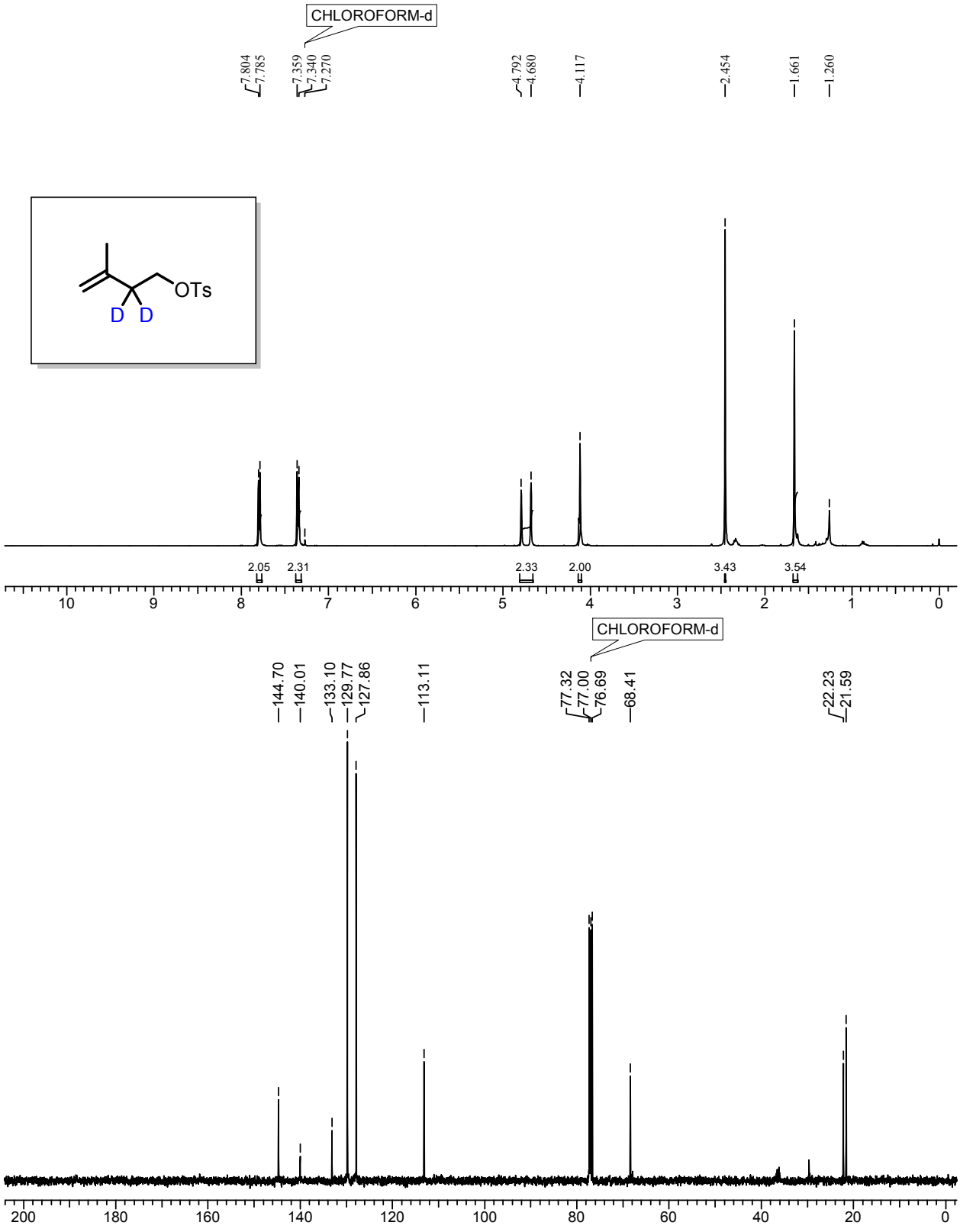

CHLOROFORM-d
7.71
7.70
7.69
7.68
7.44
7.42
7.42
7.41
7.39
4.76
4.76
4.70
3.77
1.69
1.06
OTBDPS
D
D
4.43
6.44
1.00
0.99
1.86
3.13
9.57
10
9
8
7
6
5
4
3
2
1
0


CHLOROFORM-d
142.89
135.86
135.57
133.96
129.51
127.57
111.70
77.31
77.00
76.68
62.63
29.69
26.81
22.68
19.18
180
160
140
120
100
80
60
40
20
0


CHLOROFORM-d
7.804
7.785
7.359
7.340
7.270
4.792
4.680
4.117
2.454
1.661
1.260
OTs
D D
2.05
2.31
2.33
2.00
3.43
3.54
10
9
8
7
6
5
4
3
2
1
0
CHLOROFORM-d
144.70
140.01
133.10
129.77
127.86
113.11
77.32
77.00
76.69
68.41
22.23
21.59
200
180
160
140
120
100
80
60
40
20
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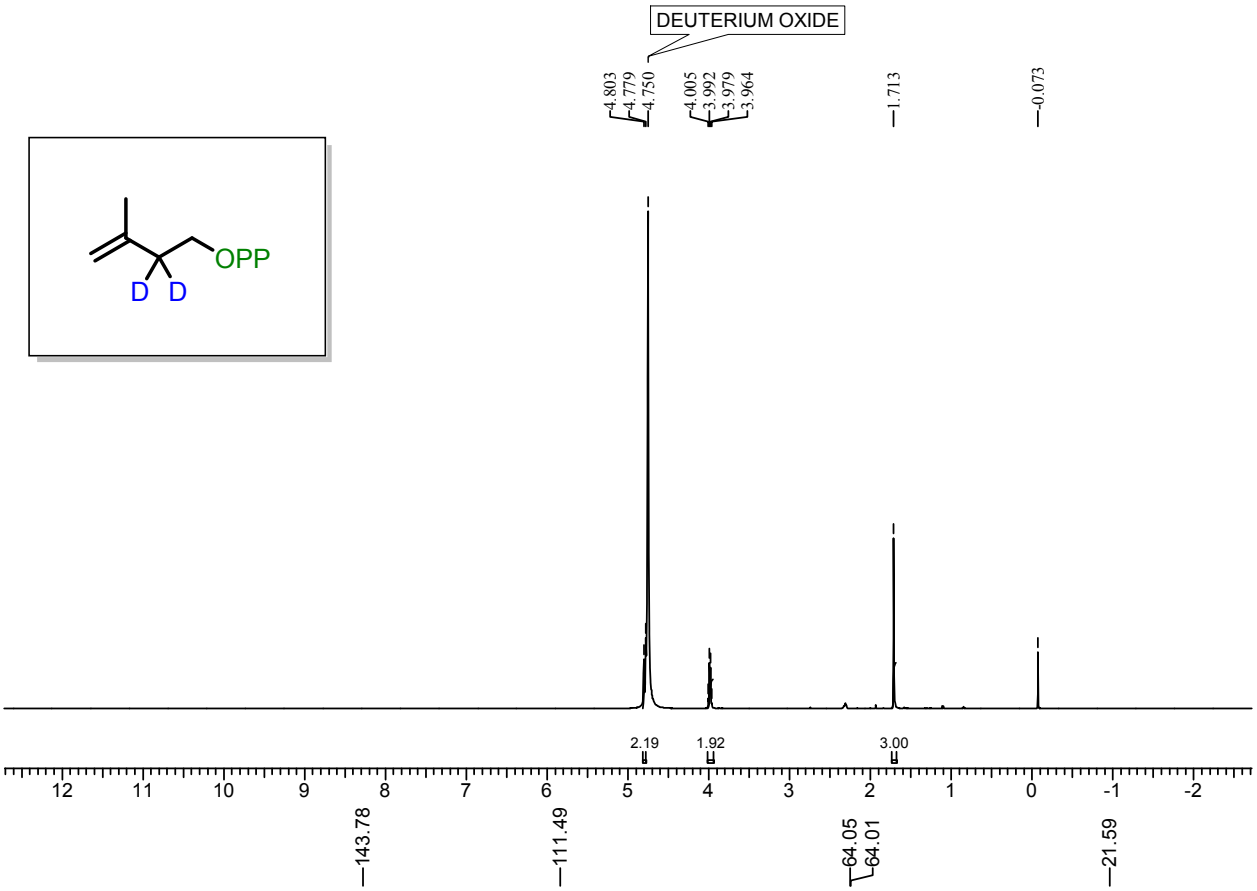
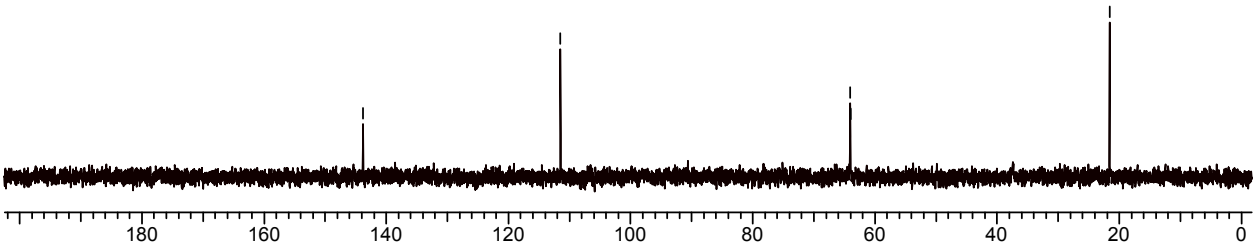
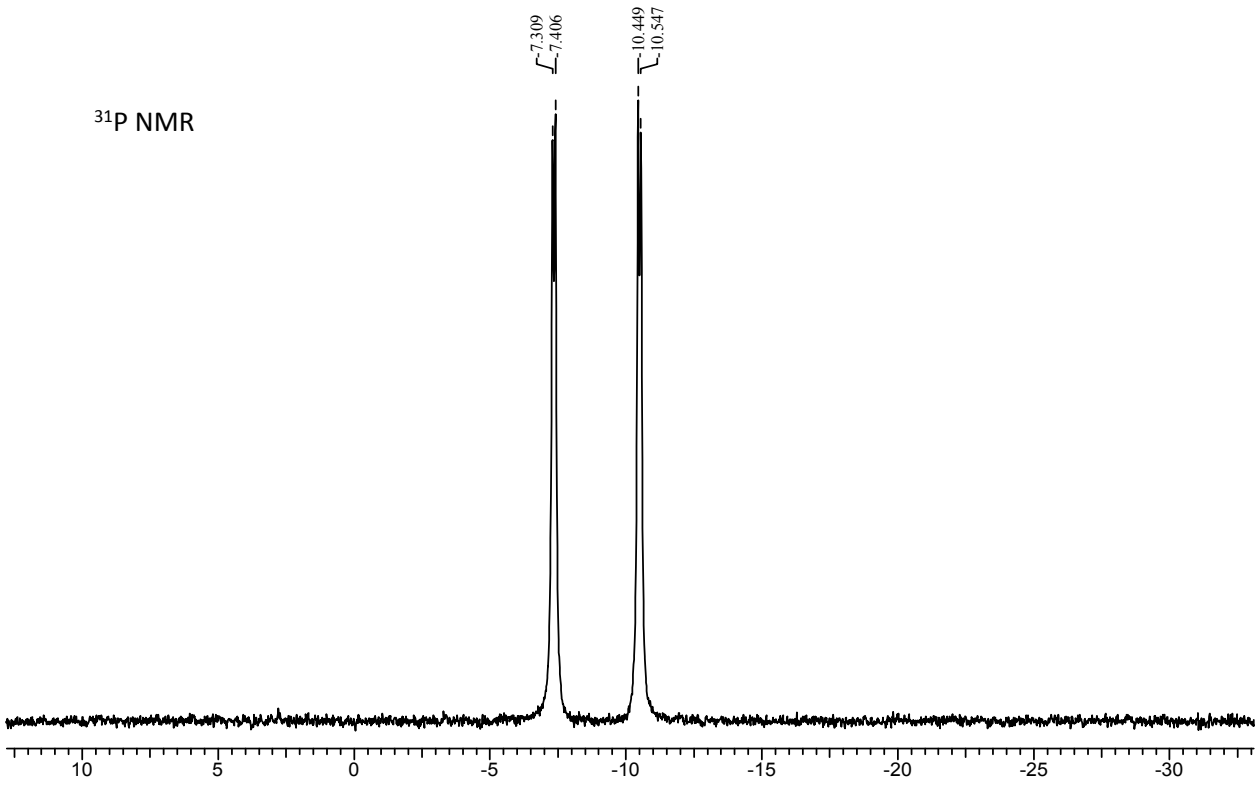
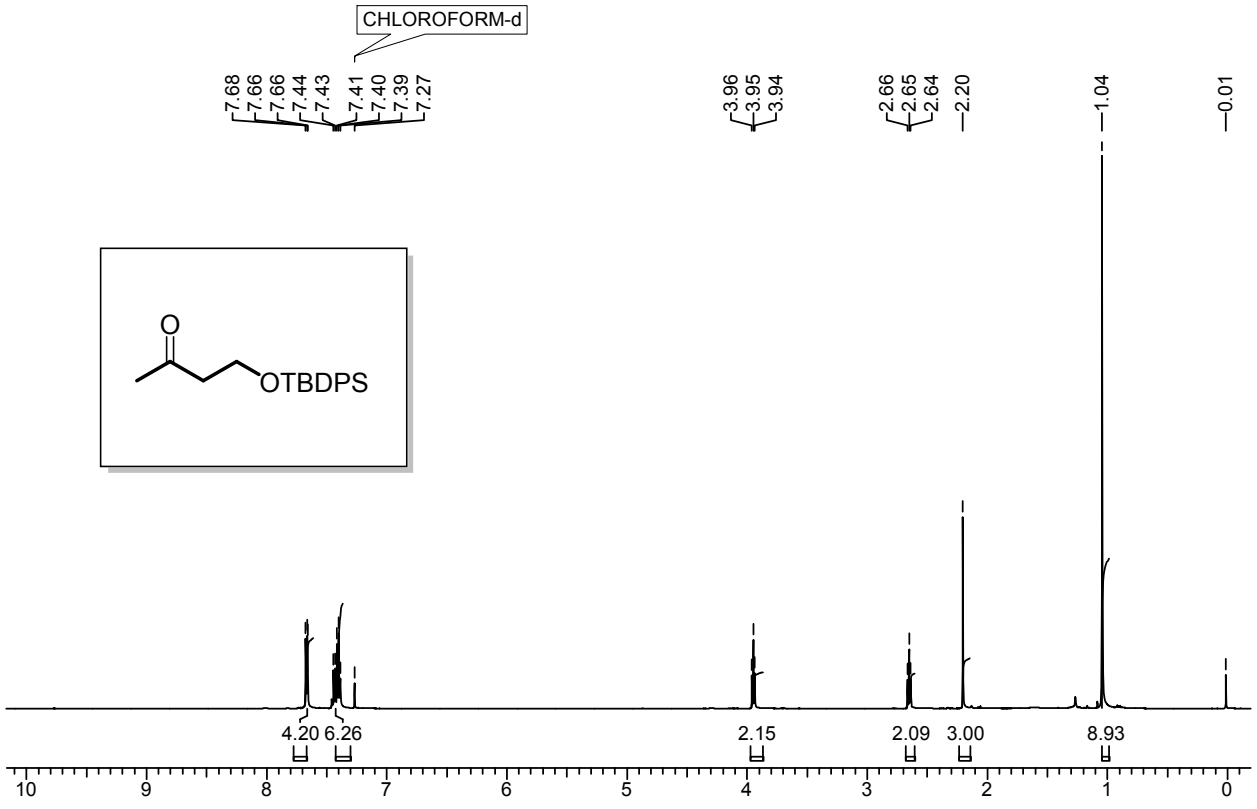
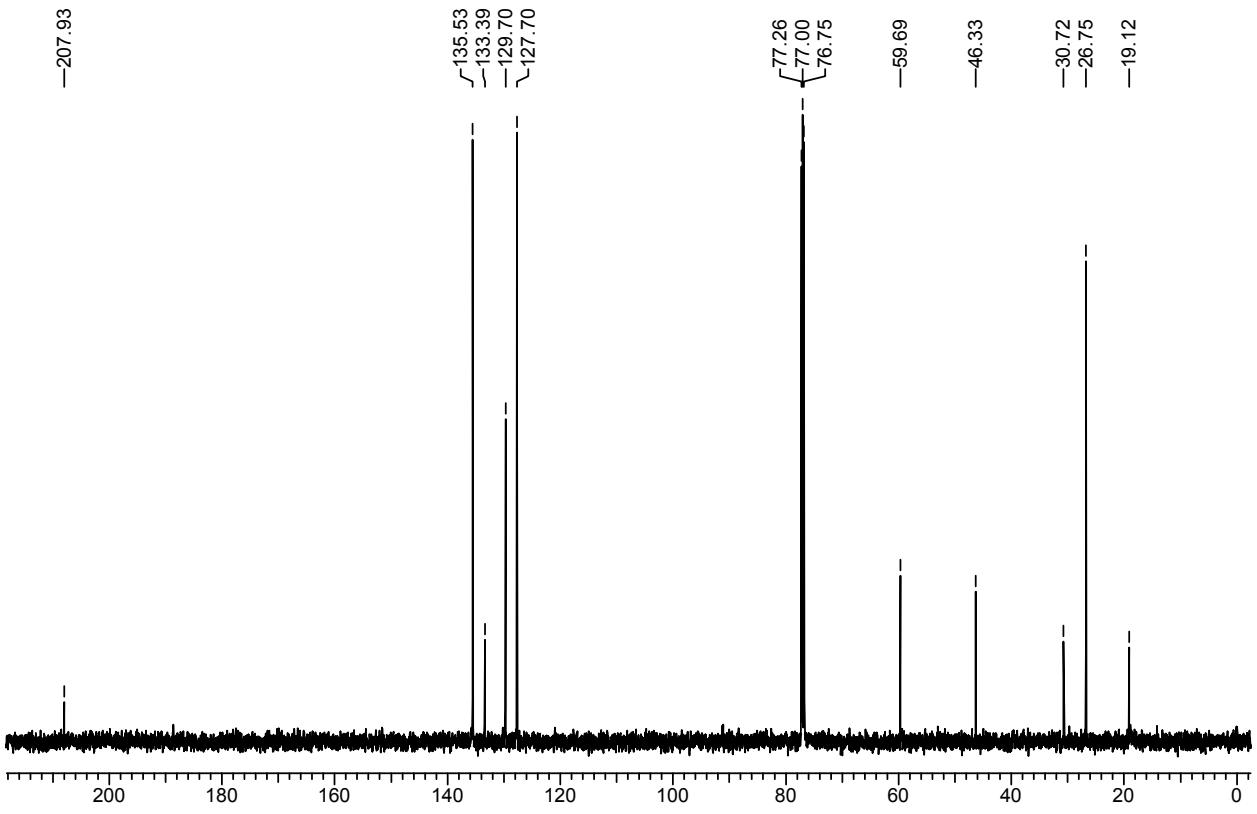
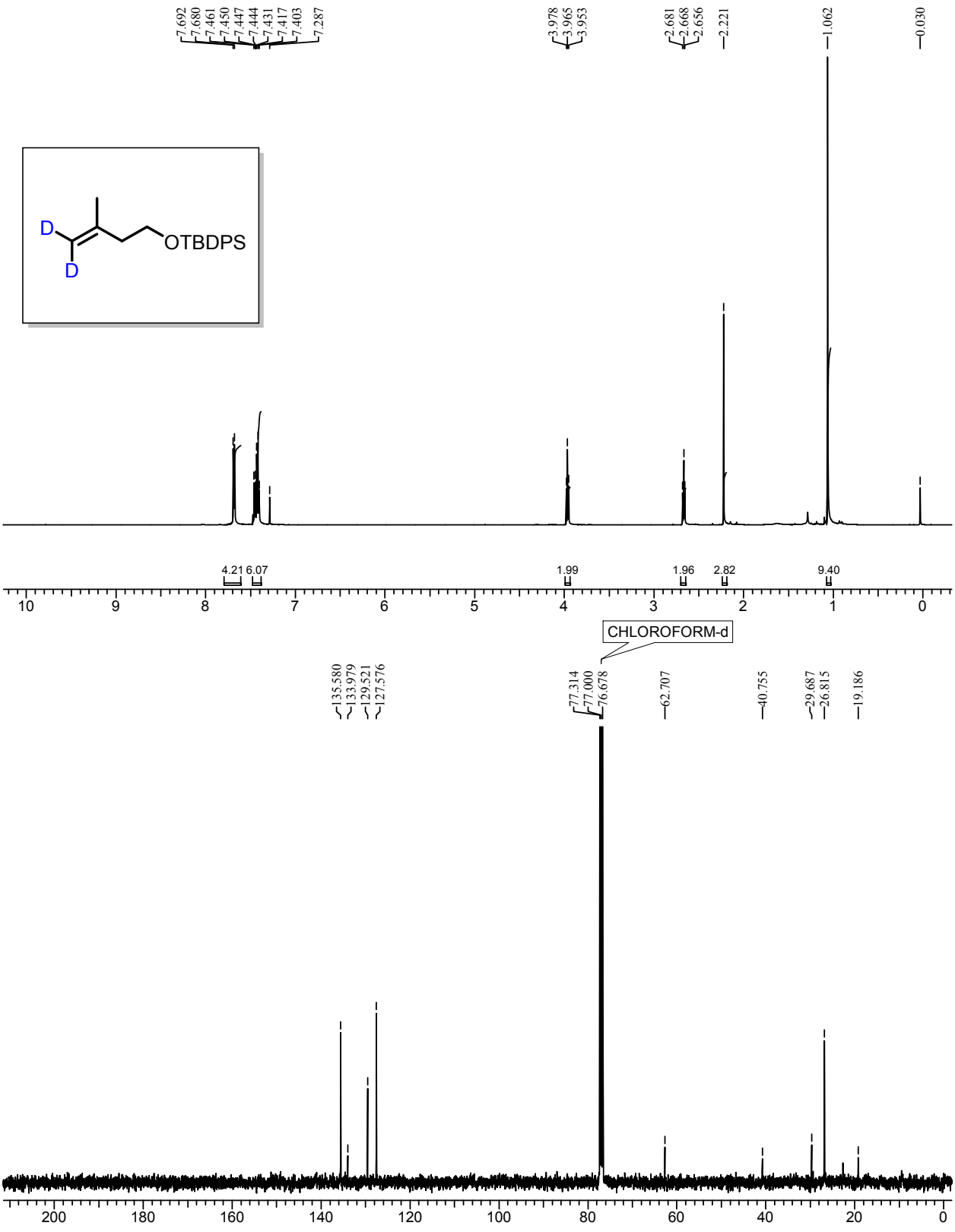

DEUTERIUM OXIDE
4.803
4.779
4.750
4.005
3.992
3.979
3.964
1.713
0.073
OPP
D D
2.19
1.92
3.00
12
11
10
9
8
7
6
5
4
3
2
1
0
-1
-2
143.78
111.49
64.05
64.01
21.59

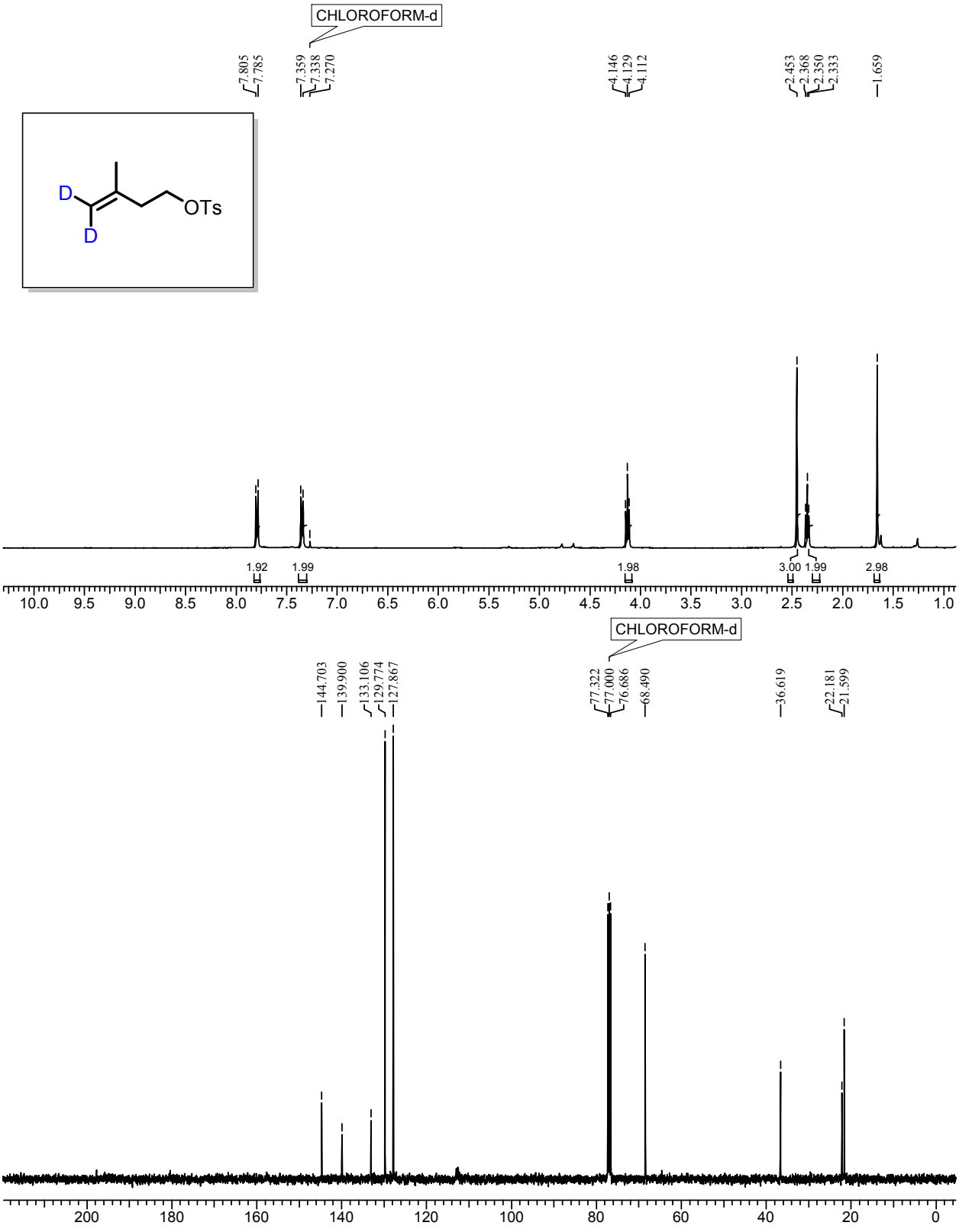

180
160
140
120
100
80
60
40
20
0

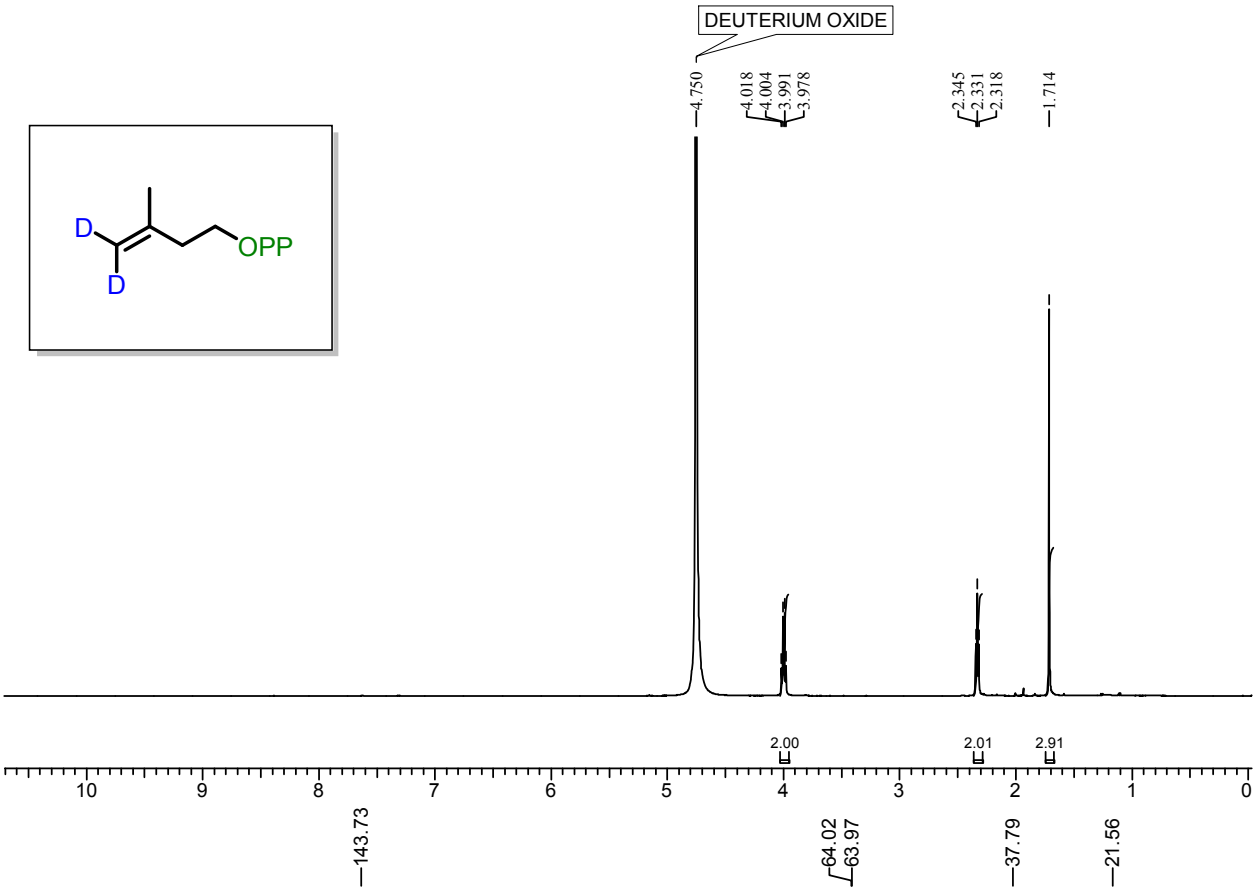

31P NMR
7.309
7.406
10.449
10.547
10
5
0
-5
-10
-15
-20
-25
-30

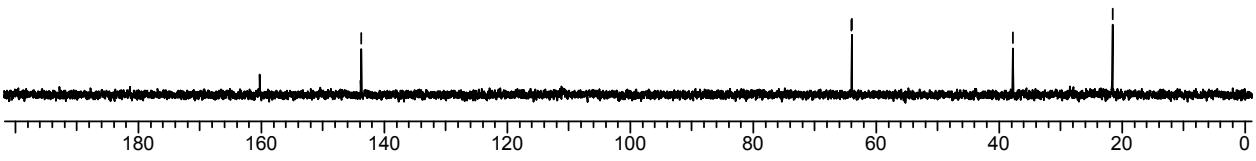

CHLOROFORM-d
7.68
7.66
7.66
7.44
7.43
7.41
7.40
7.39
7.27
3.96
3.95
3.94
2.66
2.65
2.64
2.20
1.04
0.01
O
OTBDPS
4.20
6.26
2.15
2.09
3.00
8.93
10
9
8
7
6
5
4
3
2
1
0

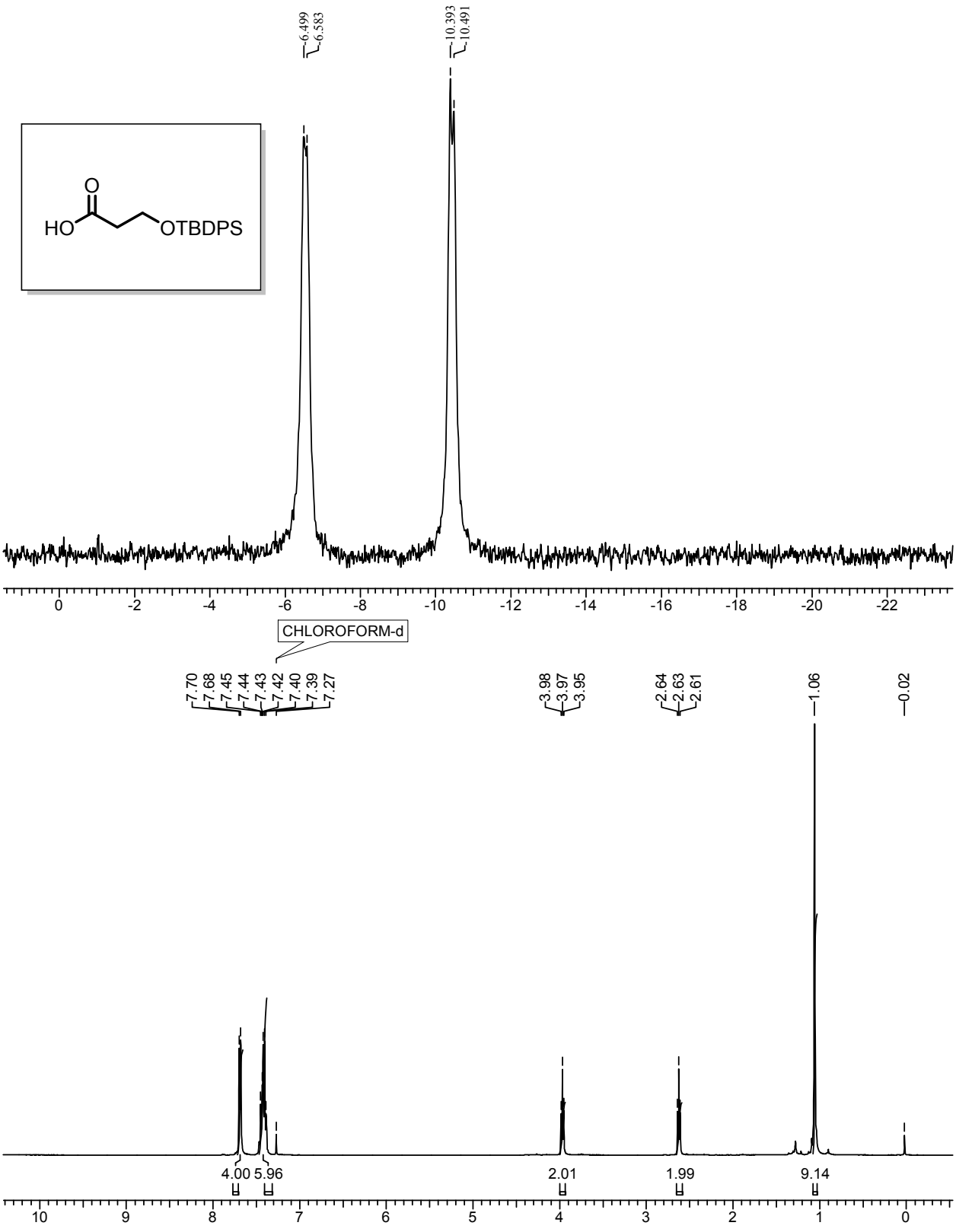

207.93
135.53
133.39
129.70
127.70
77.26
77.00
76.75
59.69
46.33
30.72
26.75
19.12
200
180
160
140
120
100
80
60
40
20
0

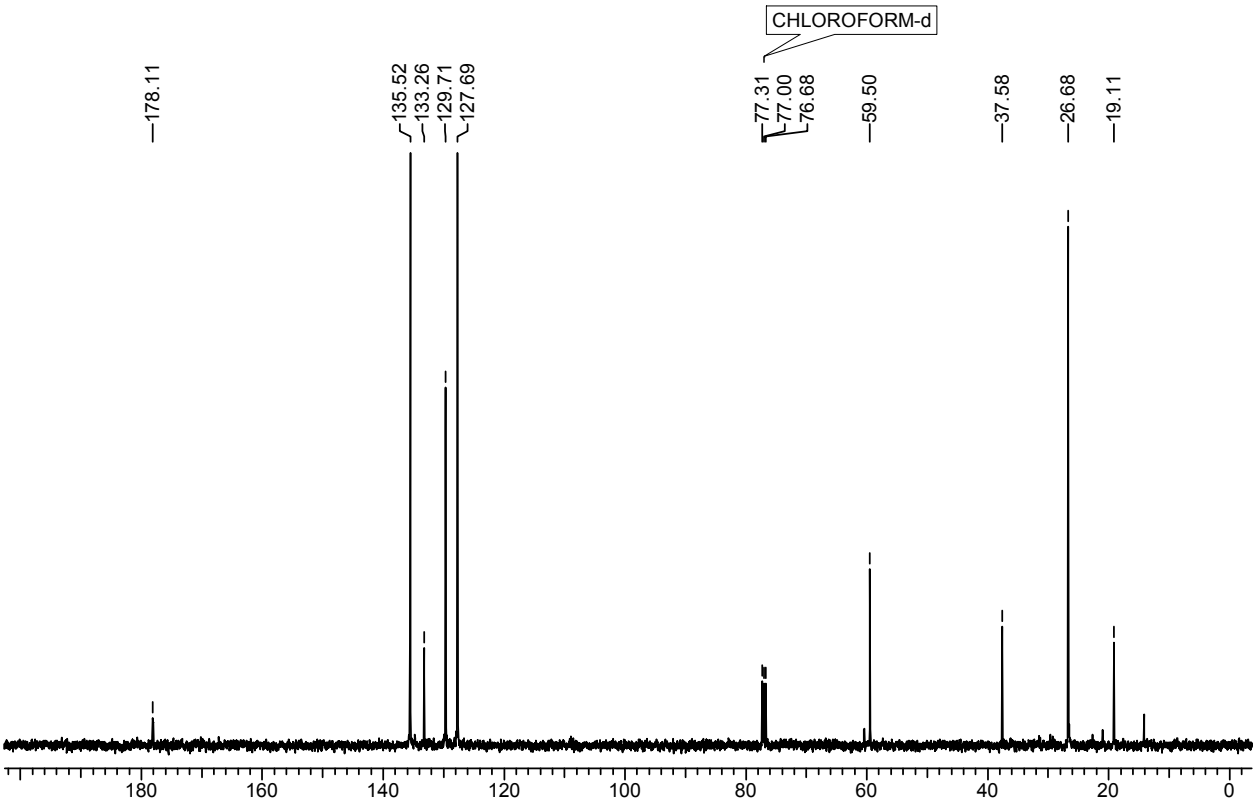

7.692
7.680
7.461
7.450
7.447
7.444
7.431
7.417
7.403
7.287
3.978
3.965
3.953
2.681
2.668
2.656
2.221
1.062
0.030
D
D
OTBDPS
4.21
6.07
1.99
1.96
2.82
9.40
CHLOROFORM-d
135.580
133.979
129.521
127.576
77.314
77.000
76.678
62.707
40.755
29.687
26.815
19.186

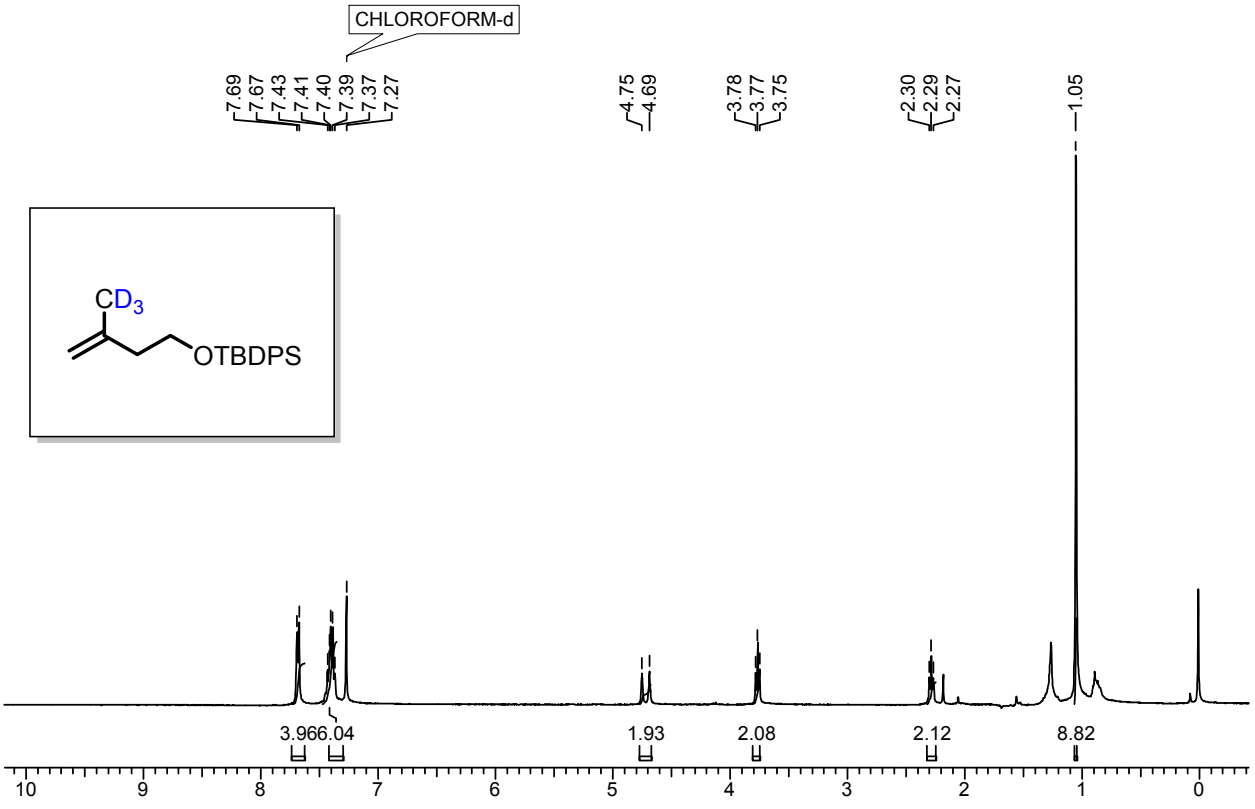

CHLOROFORM-d
7.805
7.785
7.359
7.338
7.270
4.146
4.129
4.112
2.453
2.368
2.350
2.333
1.659
D
D
OTs
1.92
1.99
1.98
3.00
1.99
2.98
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
CHLOROFORM-d
144.703
139.900
133.106
129.774
127.867
77.322
77.000
76.686
68.490
36.619
22.181
21.599
200
180
160
140
120
100
80
60
40
20
0


DEUTERIUM OXIDE
4.750
4.018
4.004
3.991
3.978
2.345
2.331
2.318
1.714
D
D
OPP
2.00
2.01
2.91
10
9
8
7
6
5
4
3
2
1
0
143.73
64.02
63.97
37.79
21.56


180
160
140
120
100
80
60
40
20
0


6.499
6.583
10.393
10.491
HO
O
OTBDPS
CHLOROFORM-d
7.70
7.68
7.45
7.44
7.43
7.42
7.40
7.39
7.27
3.98
3.97
3.95
2.64
2.63
2.61
1.06
0.02
4.00
5.96
2.01
1.99
9.14


CHLOROFORM-d
178.11
135.52
133.26
129.71
127.69
77.31
77.00
76.68
59.50
37.58
26.68
19.11
180
160
140
120
100
80
60
40
20
0


CHLOROFORM-d
7.69
7.67
7.43
7.41
7.40
7.39
7.37
7.27
4.75
4.69
3.78
3.77
3.75
2.30
2.29
2.27
1.05
CD3
OTBDPS
3.96
6.04
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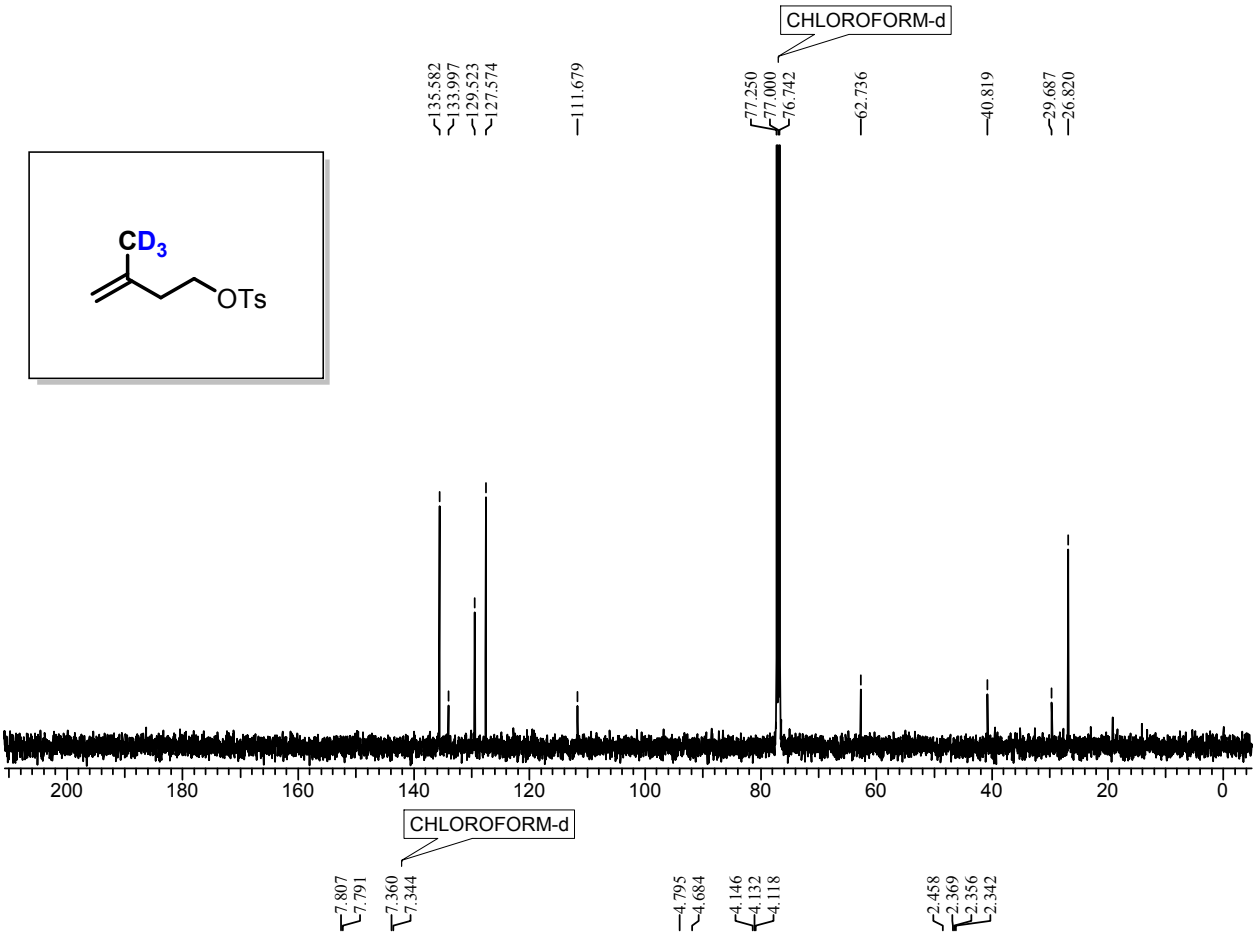
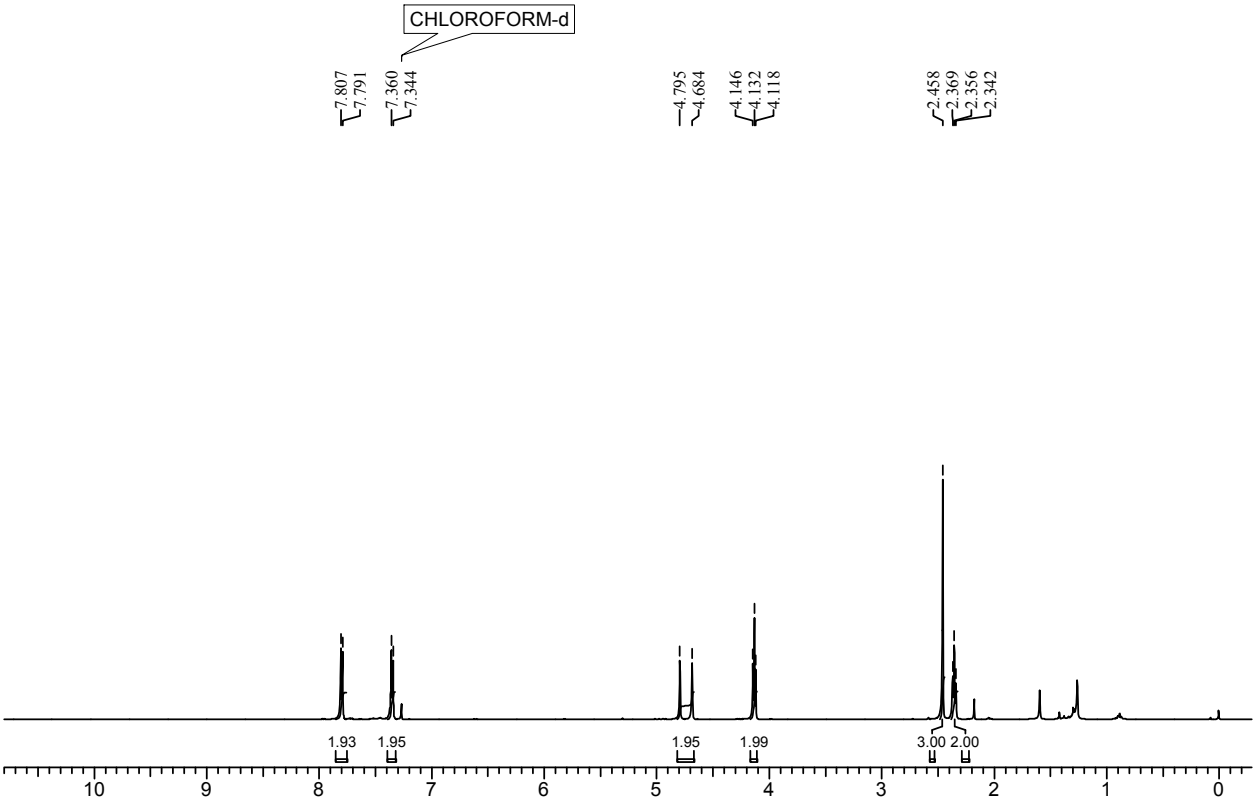
2.08
2.12
8.82
10
9
8
7
6
5
4
3
2
1
0


CHLOROFORM-d
135.582
133.997
129.523
127.574
111.679
77.250
77.000
76.742
62.736
40.819
29.687
26.820
CD3
OTs
200
180
160
140
120
100
80
60
40
20
0


CHLOROFORM-d
7.807
7.791
7.360
7.344
4.795
4.684
4.146
4.132
4.118
2.458
2.369
2.356
2.342
1.93
1.95
1.95
1.99
3.00
2.00
10
9
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7
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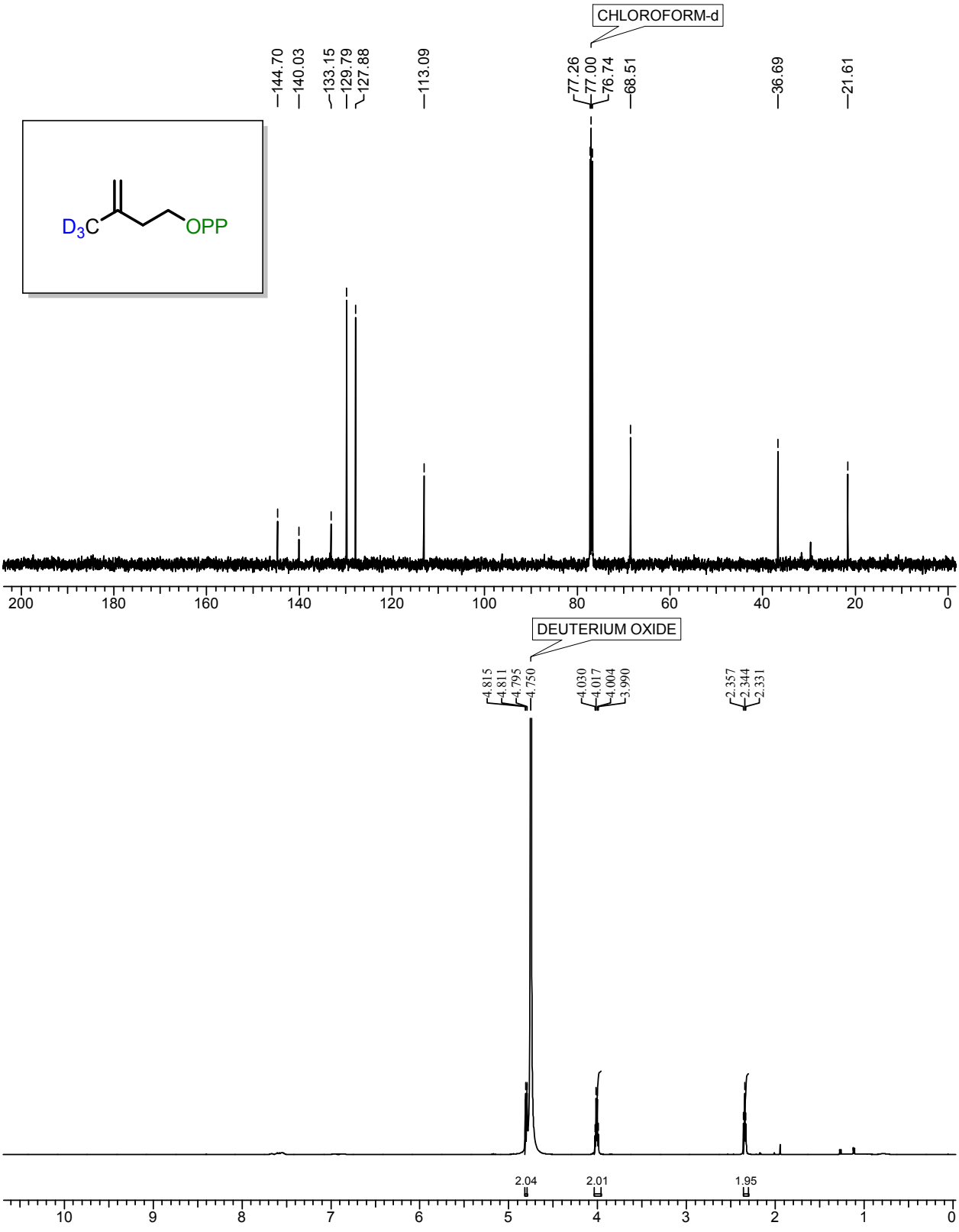
4
3
2
1
0


CHLOROFORM-d
144.70
140.03
133.15
129.79
127.88
113.09
77.26
77.00
76.74
68.51
36.69
21.61
D3C
OPP
200
180
160
140
120
100
80
60
40
20
0
DEUTERIUM OXIDE
4.815
4.811
4.795
4.750
4.030
4.017
4.004
3.990
2.357
2.344
2.331
2.04
2.01
1.95
10
9
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5
4
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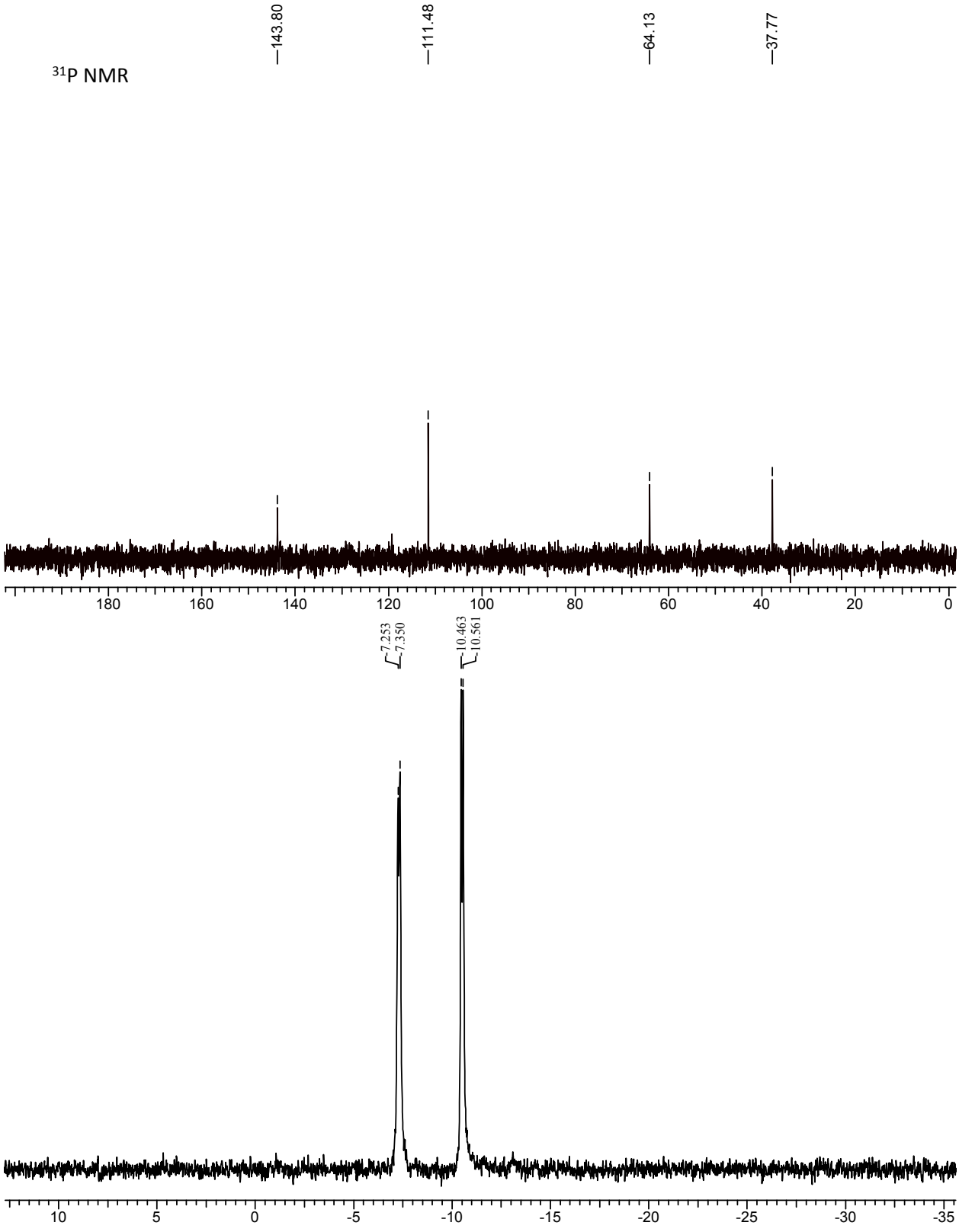
2
1
0

^{31}P NMR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64.13
37.77
180
160
140
120
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80
60
40
20
0
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10.463
10.561
10
5
0
-5
-10
-15
-20
-25
-30
-35

EP #275 RT: 1.23 AV: 1 NL: 2.33E6
T: FTMS + p ESI Full ms [100.0000-1500.0000]