Electronic Supplementary Material (ESI) for Chemical Communications

Supporting Information

Gram Scale Synthesis of 3-Fluoro-1-hydroxyacetone Phosphate: A Novel Donor Substrate in Rabbit Muscle Aldolase-Catalyzed Aldol Reactions

Michael Fischer, Hanspeter Kählig, and Walther Schmid*

Department of Organic Chemistry, University of Vienna, Währingerstrasse 38, 1090 Vienna, Austria

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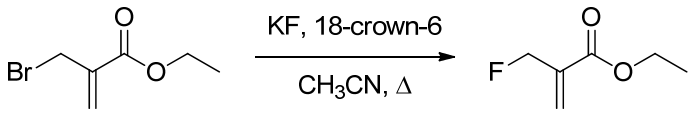
General Considerations. Anhydrous THF was distilled from potassium under argon, anhydrous CH_3CN was distilled of CaH_2. ^1H and ^{13}C NMR spectra were recorded on either 400 MHZ or 600 MHz spectrometer. Unless otherwise stated, all NMR spectra were measured either in $CDCl_3$ solutions and referenced to the residual $CHCl_3$ signal (^1H, δ=7.26 ppm; ^{13}C, δ=77.16 ppm) or in D_2O solutions (HDO, ^1H, δ=4.79 ppm). All ^1H and ^{13}C shifts are given in ppm. Flash chromatography was performed using silica gel 60 (0.004-0.063 mm). TLC monitoring was done on silica plates (silica gel 60 F_{254}), compounds were visualized by treatment with a solution of $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (48g) and $Ce(SO_4)_2$ (2g) in 10% H_2SO_4 (1L), followed by heating, or with an aqueous $KMnO_4$-solution. Ethyl α-bromomethylacrylate was synthesized according to a literature procedure.[1] pH and pD measurements were accomplished using a glass electrode (pD = pH + 0.4).[2] RAMA (lyophilized powder) and acid phosphatase (Type XA: from Sweet Potato) were purchased by Sigma Aldrich.

Ethyl α-fluoromethylacrylate (1):

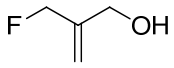


1

To a solution of ethyl α-bromomethylacrylate (48.3 g, 0.25 mol) in dry CH_3CN (200 mL), KF (43.6 g, 0.75 mol) and 18-crown-6 (1 g, 3.75 mmol) were added under argon. The mixture was refluxed until NMR monitoring (small aliquots were taken and measured in $CDCl_3$) showed complete consumption of the starting material (up to 3 days). The reaction was cooled to 0°C and quenched by addition of ice-cold water (75 mL). The aqueous phase was separated and the organic phase was extracted with water (2 x 50 mL). The combined aqueous phases were re-extracted with ether (150 mL) and the combined organic phases were dried over $MgSO_4$, filtered and concentrated under reduced pressure (22°C, 50 Torr). The product was purified by vacuum distillation over a vigreux column to yield ethyl α-fluoromethylacrylate **(1)** (19.8 g, 60 %).

The moderate yield was due to co-evaporation of ethyl α-fluoromethylacrylate **(1)** with CH_3CN and CH_2Cl_2. Spectroscopical data were in accordance with Villieras et al.[2]

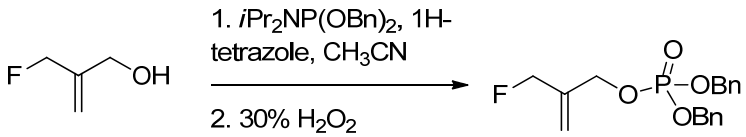
2-(Fluoromethyl)prop-2-en-1-ol (2):



2

To a solution of ethyl α-fluoromethylacrylate (**1**) (5.28 g, 0.04 mol) in dry CH_2Cl_2 (200 mL) at −78°C, DIBAL-H (1M in Hexan, 83.2 mL) was slowly added over 45 min under argon. After stirring the reaction mixture for 2 ½ hours at −65°C and for 45 min at room temperature the mixture was again cooled to −20°C. A saturated solution of Na_2SO_4 (2 mL) was slowly added and the reaction mixture was warmed up to 0°C. After addition of an ice cold saturated solution of NH_4Cl (50 mL) at 0°C, a white precipitate formed at room temperature, which was diluted with CH_2Cl_2 (100 mL) and filtered. The filtrate was carefully washed with CH_2Cl_2, the phases were separated and the combined organic phases were dried over $MgSO_4$, filtered and concentrated at reduced pressure (40°C, 750 Torr). The crude product was purified by flash chromatography (gradient: pentan/ether : 15:1→1:1) to yield 2-(fluoromethyl)prop-2-en-1-ol **(2)** (2.27 g, 63 %). The moderate yield was due to co-evaporation of 2-(fluoromethyl)prop-2-en-1-ol **(2)** with various solvents used. IR (film, cm^{-1}): ν = 3317, 2927, 1438, 1211, 1025; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.29 (m, 1H), 5.24 (m, 1H), 4.93 (m, J = 47.2 Hz, 2H), 4.23 (s, 2H), 1.58 (bs, 1OH). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 144.13 (d, J = 14.6 Hz), 114.5 (d, J = 9.8 Hz), 83.8 (d, J = 165.0 Hz), 63.33 (d, J = 2.9 Hz). ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = –218.04 (dt, J = 47.2, 3.4 Hz). HRMS (EI) calcd for C_4H_7O (M-F): 71.0497, found 71.0495.

(**Dibenzyl (2-(fluoromethyl)allyl) phosphate (3):**

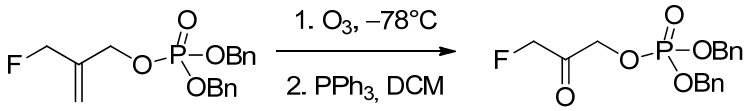


2 **3**

To a solution of 2-(fluoromethyl)prop-2-en-1-ol **(2)** (1.23 g, 13.65 mmol) in dry CH_2Cl_2 (50 mL), dibenzyl *N*,*N*-diisopropylphosphoramidite (6.60 g, 19.11 mmol) was added under argon. The

reaction mixture was cooled to -5°C and tetrazole (~0.45 M in CH_3CN, 51 mL, 22.9 mmol) was added slowly. A white solid precipitated and after the solution was stirred for 10 min at room temperature, a H_2O_2 solution (30% in H_2O, 3.1 mL, 30 mmol) was added. After TLC monitoring (hexane/EtOAc = 2/1) showed complete consumption of the intermediate phosphite (~20 min), H_2O (20 mL) and CH_2Cl_2 (20 mL) was added. The organic phase was separated and the aqueous phase was extracted with CH_2Cl_2 (3 x 20 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated. The crude material was purified by flash chromatography (hexane/EtOAc = 2/1) to yield dibenzyl (2-(fluoromethyl)allyl) phosphate (**3**) (3.53 g, 74 %). IR (film, cm^{-1}): $\nu = 2927$, 1456, 1272, 1012, 738, 696; ^1H NMR (400 MHz, $CDCl_3$): δ = 7.37–7.30 (m, 10H), 5.30–5.25 (m, 2H), 5.06 (dd, J = 11.8, 8.4 Hz, 2H), 5.04 (dd, J = 11.8, 8.4 Hz, 2H), 4.82 (m, J = 46.9 Hz, 2H), 4.52 (d, J = 7.3 Hz, 2H). ^{13}C NMR (600 MHz, $CDCl_3$) δ = 139.5, 135.9 (d, J_{CF} = 6.7 Hz), 128.7, 128.1, 117.3 (d, J_{CF} = 9.5 Hz), 82.7 (d, J_{CF} = 167.0 Hz), 69.6 (d, J_{CP} = 5.5 Hz), 67.1 (dd, J_{CP} = 5.5 Hz, J_{CF} = 2.8 Hz). ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = –219.26 (dt, J = 46.9, 3.3 Hz). HRMS (ESI) calcd for $C_{18}H_{21}O_4FP$ (M+H): 351.1162, found 351.1167.

Dibenzyl (3-fluoro-2-oxopropyl) phosphate (4): 3 → 4

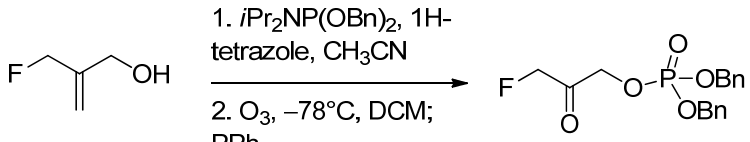


3 **4**

Ozone was bubbled through a solution of compound **3** (3.50 g, 9.99 mmol) in dry CH_2Cl_2 (25 mL) at -78°C until the color of the mixture turned blue. Dry air was bubbled through the solution until the blue color disappeared. After addition of $(CH_3)_2S$ (0.87 g, 14 mmol), the mixture was allowed to reach room temperature by stirring overnight. The solvent was removed under reduced pressure to yield dibenzyl (3-fluoro-2-oxopropyl) phosphate (**4**) (3.34 g; 95 %). IR (film, cm^{-1}): ν = 2927, 1751, 1271, 1009, 737, 696. ^1H NMR (400 MHz, $CDCl_3$): δ = 7.40–7.28 (m, 10H), 5.13 (dd, J = 11.7, 8.6 Hz, 2H) 5.09 (dd, J = 11.7, 9.1 Hz, 2H), 4.89 (d, J = 47.0 Hz, 2H), 4.70 (dd, J = 9.7, 1.7 Hz, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 199.9 (dd, J = 19.0, 5.9 Hz, 1C), 135.6 (d, J =

6.5 Hz, 2C), 128.9 (2C), 128.8 (4C), 128.3 (4C), 84.3 (d, J = 182.5 Hz, 1C), 70.0 (d, J = 5.8 Hz, 2C), 69.3 (dd, J =5.6, 2.6 Hz, 1C). ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = –237.3 (tt, J = 47.0, 1.7 Hz). HRMS (EI) calcd for $C_{17}H_{19}O_5PF$ (M+H): 353.0954, found 353.0945.

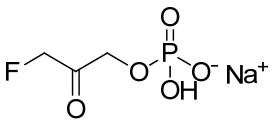
Dibenzyl (3-fluoro-2-oxopropyl) phosphate (4): 2 → 4



2 **4**

To a solution of 2-(fluoromethyl)prop-2-en-1-ol **(2)** (0.45 g, 5 mmol) in dry CH_2Cl_2 (20 mL), dibenzyl *N*,*N*-diisopropylphosphoramidite (2.42 g, 7 mmol) was added under argon. The reaction mixture was cooled to -5°C and tetrazole (~0.45 M in CH_3CN, 18.6 mL) was added slowly. A white solid precipitated and the solution was stirred for 15 min at room temperature. Ozone was bubbled through this solution at -78°C until the color of the mixture turned blue. Dry air was bubbled through the solution until the blue color disappeared. After addition of $(CH_3)_2S$ (0.43 g, 7 mmol), the mixture was allowed to reach room temperature by stirring overnight. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography (toluol/EtOAc = 1/1) to yield dibenzyl (3-fluoro-2-oxopropyl) phosphate (**4**) (914 mg, 52 %).

3-Fluoro-1-hydroxyacetone phosphate mono sodium salt (5):

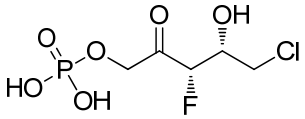


5

Compound **4** (2.04 g, 5.79 mmol) was dissolved in *t*BuOH (60 mL) and hydrogenated in the presence of 10% palladium-on-charcoal (308 mg) with a balloon of hydrogen. After TLC monitoring (tolulol/EtOAc = 1/1) showed consumption of the starting material, the catalyst was removed by filtration and the filter was rinsed with water (30 mL). After addition of NaOH (0.1 M,

5.79 mL,) the solution (pH = 6.5-6.8) was concentrated under reduced pressure (not to dryness), water added and concentrated again to remove *t*BuOH (not to dryness). The mono sodium salt of 3-fluoro-1-hydroxyacetone phosphate (**5**) (1.06 g, 95 %) was obtained after lyophilisation. ^{1}H NMR (400 MHz, D_2O): δ^{keto} = 5.30 (d, *J* = 46.5 Hz, 2H), 4.63 (d, *J* = 7.4 Hz, 2H); $\delta^{hydrate}$ = 4.40 (d, *J* = 46.6 Hz, 2H), 3.86 (dd, *J* = 5.9, 2.2 Hz, 2H) . ^{13}C NMR (400 MHz, D_2O): $\delta^{hydrate}$ = 93.5 (dd, *J* = 19.2, 7.7 Hz, 1C), 84.0 (d, *J* = 172.3 Hz, 1C), 66.4 (dd, *J* = 5.0, 1.8 Hz, 1C); δ^{keto} = 207.3 (from HMBC, 1C), 85.0 (d, *J* = 176.8 Hz, 1C), 67.9 (from HMBC, 1C); ^{19}F NMR (600 MHz, D_2O, coupled): $\delta^{hydrate}$ = –230.9 (tt, *J* = 1.9, 46.6 Hz); δ^{keto} = –237.1 (t, *J* = 46.7 Hz). HRMS (ESI-neg. mode) calcd for $C_3H_5O_5FP$ (M-H): 170.9859 found, 170.9856.

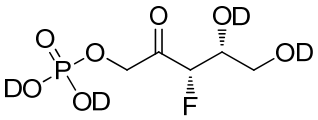
^{19}F-NMR study of RAMA with chloroacetaldehyde in H_2O: 5-Chloro-3,5-dideoxy-3-fluoro-D-*threo*-pent-2-ulose-1-phosphate (6):



6

Good resolved ^{19}F-NMR spectra for compound **6** were obtained by using a BIS-TRIS buffer (2-[bis(2-hydroxyethyl)amino]-2-(hydroxymethyl)propane-1,3-diol). To a solution of FHAP (**5**) (42.5 mg, 0.219 mmol) in H_2O (2 mL), chloroacetaldehyde (~ 50% in H_2O; 33 µL, 0.255 mmol) and BIS-TRIS (2 mg, 0.01 mmol) was added and the pH was adjusted with small quantities of 1 M NaOH solutions to pH = 6.8. RAMA (43.6 U) was added (SI, Figure 3). After slow stirring over night, RAMA (19 U) was repeatedly added (SI, Figure 4).

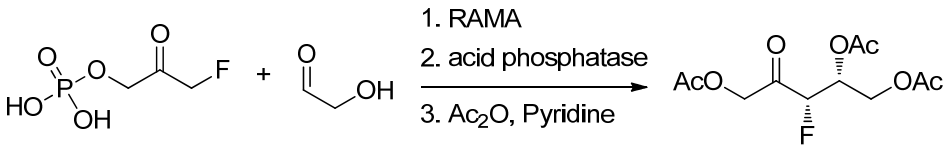
^{19}F- and ^{1}H-NMR study of RAMA with glycolacetaldehyde in D_2O: 3-deoxy-3-fluoro-D-*threo*-pent-2-ulose-1-phosphate (7):



7

To a solution of FHAP (**5**) (4 mg; 0.021 mmol) in D_2O (0.6 mL), glycolaldehyde (1.3 mg, 0.021 mmol) was added and the pD was adjusted with small quantities of 1M NaOD or DCl solutions to pD = 7.3. RAMA (2U) was added and the reaction progress was monitored by ^{1}H-NMR and ^{19}F-NMR (SI, Figure 5).

Enzymatic synthesis of 1,4,5-tri-*O*-acetyl-3-deoxo-3-fluoro-D-*threo*-pent-2-ulose (8):

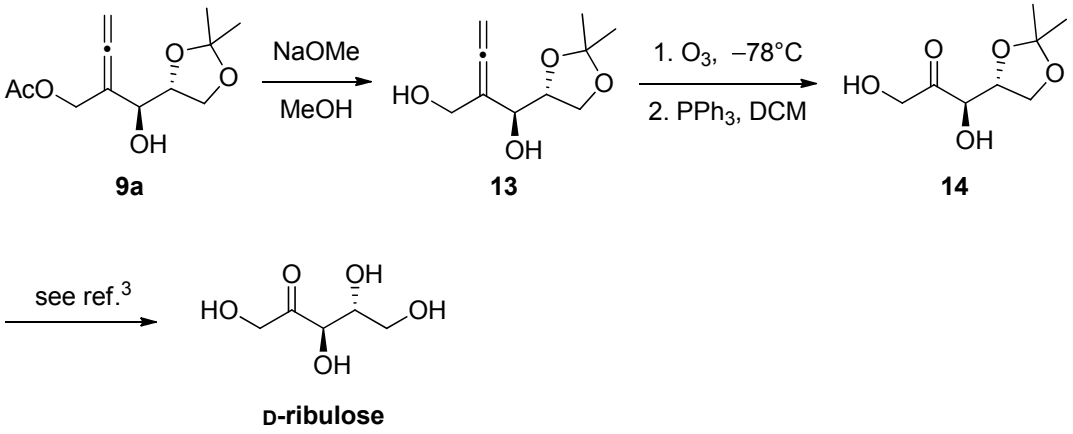


5 **8**

To a solution of FHAP mono sodium salt (100 mg, 0.52 mmol) in H_2O (30 mL), glycolaldehyde (36 mg, 0.6 mmol) and BIS-TRIS (31 mg, 0.15 mmol) was added. After the pH was adjusted with small quantities of 1 M NaOH solutions to pH = 6.8, RAMA (95 U) was added. The solution was slowly stirring over night and RAMA (47.5 U) was added again. After 2 days the pH was adjusted to pH = 5.1 with 1 N HCl solution, acid phosphatase (110 U) was added. The reaction mixture was slowly stirred over night and the pH was adjusted to 6.85 with 1N NaOH. After lyophilization the crude product was purified by flash chromatography (CHCl/MeOH = 9/1). Since a structural determination was not possible, the fractions with Rf between 0.56 and 0.30 were pooled together, evaporated and suspended in dry pyridine (2 mL). Dimethylaminopyridine (11 mg, 0.09 mmol) was added and the solution was cooled to 0°C under argon. Acetanhydride (191 mg, 1.87 mmol) was added and the solution was stirred over night. The reaction mixture was quenched by the addition

of H_2O (1 mL) at 0°C and the aqueous phase was extracted with EtOAc (3 x 5ml). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and evaporated *in vacuo*. The product was purified by flash chromatography (hexane/EtOAc = 4/1) to yield 1,4,5- tri- *O*-acetyl-3-deoxy-3-fluoro-D-*threo*-pent-2-ulose (**8**) (10 mg, 7 %). $[\alpha]_D^{20}$ = -24.5 (*c* 0.5, CH_2Cl_2); IR (film, cm^{-1}): ν = 2925, 2855, 1744, 1372, 1227, 1048; ^1H NMR (400 MHz, $CDCl_3$,): δ = 5.48 (ddt, *J* = 27.8, 6.5, 2.4 Hz, 1H), 5.14 (dd, *J* = 47.5, 2.5 Hz, 1H), 5.05 (dd, *J* = 18.2, 2.1 Hz, 1H) 4.92 (dd, *J* = 18.2, 2.1 Hz, 1H), 4.31 (dd, *J* = 11.4, 6.6 Hz, 1H), 4.24 (ddd, *J* = 11.5, 6.2, 1.0 Hz, 1H), 2.17 (s, 3H), 2.12 (s, 3H), 2.08 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 199.6 (d, *J* = 26.5 Hz), 170.3, 170.1, 170.0, 93.3 (d, *J* = 191.3 Hz), 69.7 (d, *J* = 17.8 Hz), 67.2 (d, *J* = 4.6 Hz), 60.6 (d, *J* = 6.0 Hz), 20.7, 20.6, 20.5. ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = –215.12 (ddt, *J* = 47.3, 27.8, 2.4 Hz). HRMS (ESI) calcd for $C_{11}H_{15}O_7FNa$ (M+Na): 301.0700, found 301.0695.

Determination of the absolute stereochemistry of compound 9a:



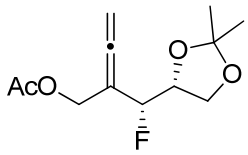
9a **13** **14**

D-ribulose

To a suspension of compound **9a**[3] (9 mg, 0.037 mmol) in dry MeOH under argon, NaOMe (0.5 mg, 9 µmol) was added at 0°C. The reaction mixture was stirred at room temperature until TLC monitoring (H/EtOAC = 1/1) showed conversion of the starting material. Then Dowex 50 H^+ was added and the mixture was stirred for 5 min. After neutralization the resin was removed by filtration, washed in with MeOH and the filtrate concentrated *in vacuo* to yield compound **13**.[3] The crude material was dissolved in dry CH_2Cl_2 (1 mL) and cooled to –78°C. Ozone was bubbled through this solution at -78°C until the color of the mixture turned blue. Dry air was bubbled

through the solution until the blue color disappeared. After addition of PPh_3 (12 mg, 0.044 mmol), the mixture was allowed to reach room temperature by stirring overnight. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography (hexane/EtOAc = 1/1) to yield 4,5-*O*diisopropylidine-D-*erythro*-pent-2-ulose (**14**) (6.4 mg, 91 %).[3] Compound **14** was transformed in D-ribulose as previously published.[3] The spectroscopical data for D-ribulose were identical with those reported.[4]

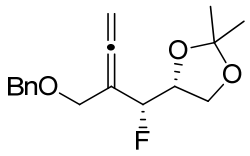
(*2R,3R*)-4-(Acetyloxymethyl)-3-fluoro-1,2-*O*-isopropylidene-hexa-4,5-dien-1,2-diol (10a):



10a

To a suspension of compound **9a**[3] (34 mg, 0.14 mmol) in dry CH_2Cl_2 (4 mL) under argon, collidine (34 mg, 0.281 mmol) was added and the reaction mixture cooled to −78°C. After dropwise addition of DAST (95 %) (47 mg, 0.281 mmol) the solution was stirred for 15 min at −78°C and then warmed up to −30°C. After TLC monitoring showed conversion of the starting material, the reaction was quenched by the addition of a $NaHCO_3$-solution (10%, 0.5 mL). The solution was brought to room temperature and the aqueous phase was extracted with CH_2Cl_2 (3 x 2 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated *in vacuo*. Flash chromatography on silica gel in hexane/EtOAc = 8/1 afforded compound **10a** (12 mg, 35 %). $[\alpha]_D^{20}$ = +35.1 (*c* = 0.2, CH_2Cl_2); IR (film, cm^{-1}): ν = 2935, 1963, 1745, 1365, 1225; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 5.1–5.0 (m, 2H), 4.88 (ddt, *J* = 48.7, 7.8, 1.5 Hz, 1H), 4.69–4.64 (m, 2H), 4.47–4.34 (m, 1H), 4.06 (ddd, *J* = 8.8, 6.9, 2.1 Hz, 1H), 3.69 (ddd, *J* = 8.6, 6.6, 0.9 Hz, 1H), 2.08 (s, 3H), 1.47 (s, 3H), 1.39 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 208.1 (d, *J* = 8.5 Hz), 170.5, 110.7, 97.2 (d, *J* = 21.3 Hz), 91.8 (d, *J* = 180.5 Hz) 79.5 (d, *J* = 2.7 Hz), 76.5 (d, *J* = 20.0 Hz), 65.7 (d, *J* = 5.9 Hz), 61.7 (d, *J* = 0.7 Hz), 26.7, 25.4, 21.0. ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = −182.0 (dm, *J* = 48.8 Hz). HRMS (EI) calcd for $C_{11}H_{14}O_4F$ (M-CH_3): 229.0876, found 229.0875.

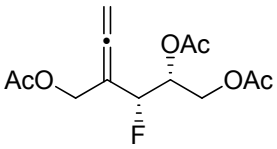
(*2R,3R*)-4-(Benzylloxymethyl)-3-fluoro-1,2-*O*-isopropylidene-hexa-4,5-dien-1,2-diol (10b):



10b

To a suspension of compound **9b**[3] (73 mg, 0.25 mmol) in dry CH_2Cl_2 (6 mL) under argon, collidine (61 mg, 0.5 mmol) was added and the reaction mixture cooled to −78°C. After dropwise addition of DAST (95 %) (84 mg, 0.5 mmol) the solution was stirred for 15 min at −78°C and then warmed up to −30°C. After TLC monitoring showed conversion of the starting material, the reaction was quenched by the addition of a $NaHCO_3$-solution (10%, 1 mL). The solution was brought to room temperature and the aqueous phase was extracted with CH_2Cl_2 (3 x 3 mL). The combined organic phases were dried over $MgSO_4$, filtered and evaporated *in vacuo*. Flash chromatography on silica gel in hexane/EtOAc = 8/1 afforded compound **10b** (18 mg, 25 %). IR (film, cm^{-1}): ν = 2988, 2924, 2854, 1958, 1372, 1069, 740, 699; ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.33–7.27 (m, 5H), 5.04–4.82 (m, 2H), 4.92 (ddt, *J* = 49.1, 8.0, 1.3 Hz, 1H), 4.54 (d, *J* = 11.5 Hz, 1H), 4.51 (d, *J* = 11.8 Hz, 1H), 4.51–4.40 (m, 1H), 4.14-4.11 (m, 2H), 4.03 (ddd, *J* = 8.6, 7.0, 2.2 Hz, 1H), 3.67 (ddd, *J* = 8.6, 6.6, 1.1 Hz, 1H), 1.46 (s, 3H), 1.38 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 208.3 (d, *J* = 8.5 Hz), 137.9, 128.6, 127.97, 127.96, 110.5, 98.0 (d, *J* = 21.0 Hz), 92.2 (d, *J* = 179.2 Hz), 78.3 (d, *J* = 2.9 Hz), 76.6 (d, *J* = 20.2 Hz), 72.5, 67.9, 65.8 (d, *J* = 6.4 Hz), 26.8, 25.5. ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = −180.2 (dm, *J* = 49.2 Hz). HRMS (EI) calcd for $C_{16}H_{18}O_3F$ (M-CH_3): 277.1240, found 277.1233.

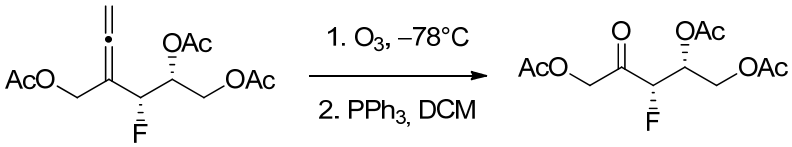
(*2R,3R*)-1,2-Di-*O*-acetyl-4-(acetyloxymethyl)-3-fluoro-hexa-4,5-dien-1,2-diol (11a):



11a

To a suspension of compound **10a** (11 mg, 0.045 mmol) in 2 mL of a mixture of water/THF (2:1), 10 mg of Amberlyst 15 (H^+-form) were added and the mixture was stirred at room temperature. After TLC monitoring showed conversion of the starting material (~4 h), the resin was removed by filtration, washed with methanol and the solvent was removed under reduced pressure. The crude product was suspended in dry pyridine (0.5 mL), dimethylaminopyridine (0.5 mg, 4 µmol) was added and the solution was cooled to 0°C under argon. Acetanhydride (18 mg, 0.176 mmol) was added and the solution was stirred over night. The reaction mixture was quenched by addition of H_2O (200 µL) at 0°C and the aqueous phase was extracted with EtOAc (3 x 1ml). The combined organic phases were washed with brine, dried over $MgSO_4$, filtered and evaporated *in vacuo*. The product was purified by flash chromatography (hexane/EtOAc = 4/1) to yield (*2R,3R*)-3-fluoro-1,2,5-*O*-triacetyl-4-vinylidene-pentan-1,2,5-triol (**11a**) (10 mg, 80 %). $[\alpha]_D^{20}$ = +28.0 (*c* 0.1, CH_2Cl_2); IR (film, cm^{-1}): ν = 2922, 1973, 1744, 1374, 1222, 1047; ^1H NMR (400 MHz, $CDCl_3$): δ = 5.39 (dddd, *J* = 17.8, 5.7, 3.9, 1.9 Hz, 1H), 5.14 (ddt, *J* = 47.2, 5.9, 1.7 Hz, 1H), 5.10–5.03 (m, 2H), 4.67 (m, 2H), 4.36 (dd, *J* = 11.9, 3.8 Hz, 1H), 4.16 (dd, *J* = 12.0, 5.9 Hz, 1H) 2.12 (s, 3H) 2.09 (s, 3H) 2.02 (s, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ = 208.06 (d, *J* = 8.0 Hz), 170.60, 170.59, 170.2, 96.4 (d, *J* = 22.0 Hz), 88.8 (d, *J* = 182.0 Hz), 79.7 (d, *J* = 1.8 Hz), 70.8 (d, *J* = 20.9 Hz), 62.33 (d, *J* = 6.0 Hz), 61.6 (d, *J* = 1.7 Hz), 21.0, 20.9, 20.8. ^{19}F NMR (600 MHz, $CDCl_3$, coupled): δ = –191.7 (ddt, *J* = 46.9, 17.5, 7. 8 Hz). HRMS (ESI) calcd for $C_{13}H_{17}O_6FNa$ (M+Na): 311.0907, found 311.0912.

Chemical synthesis of 1,4,5-tri-*O*-acetyl-3-deoxy-3-fluoro-D-*threo*-pent-2-ulose (8):

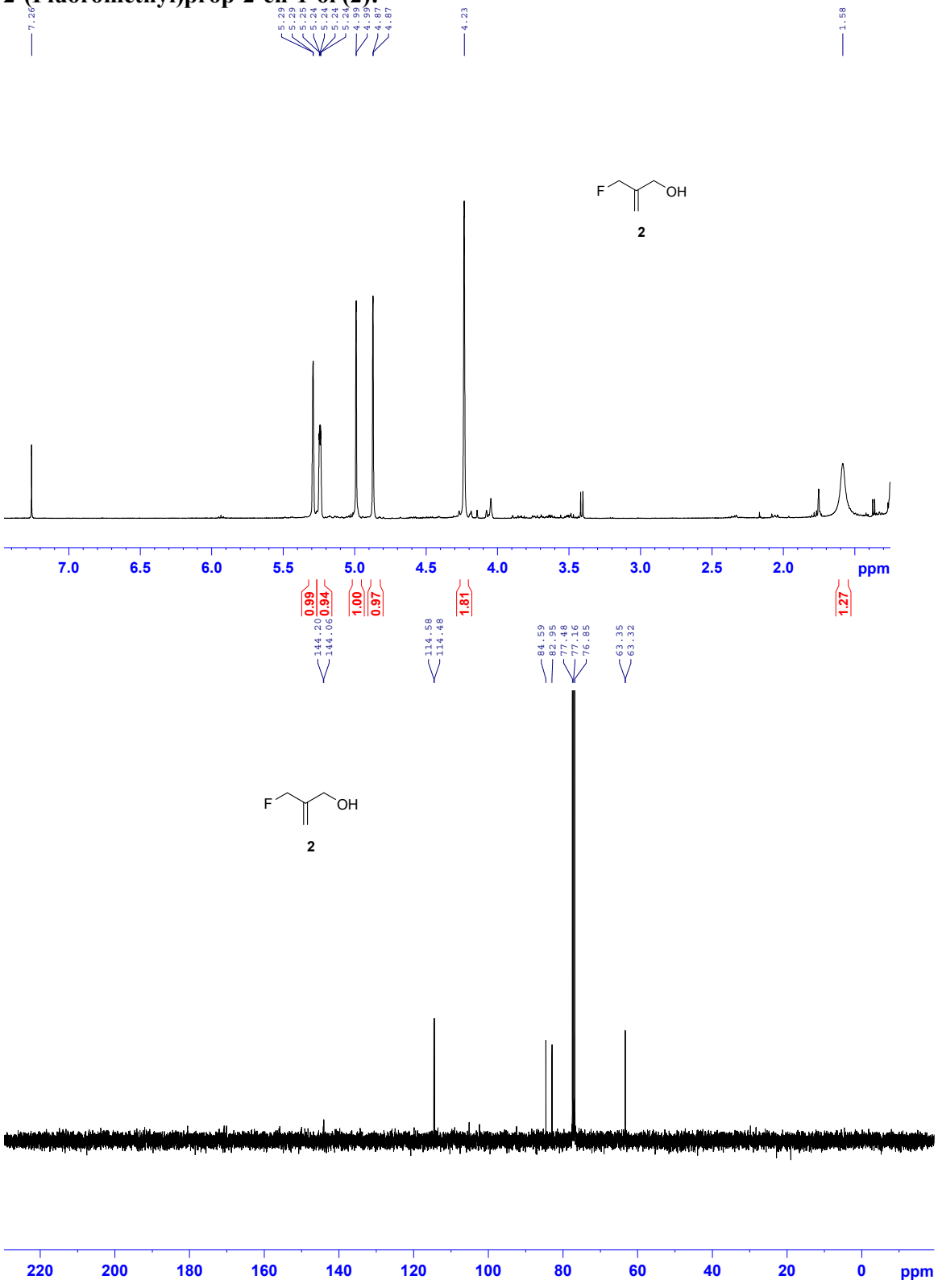


11a **8**

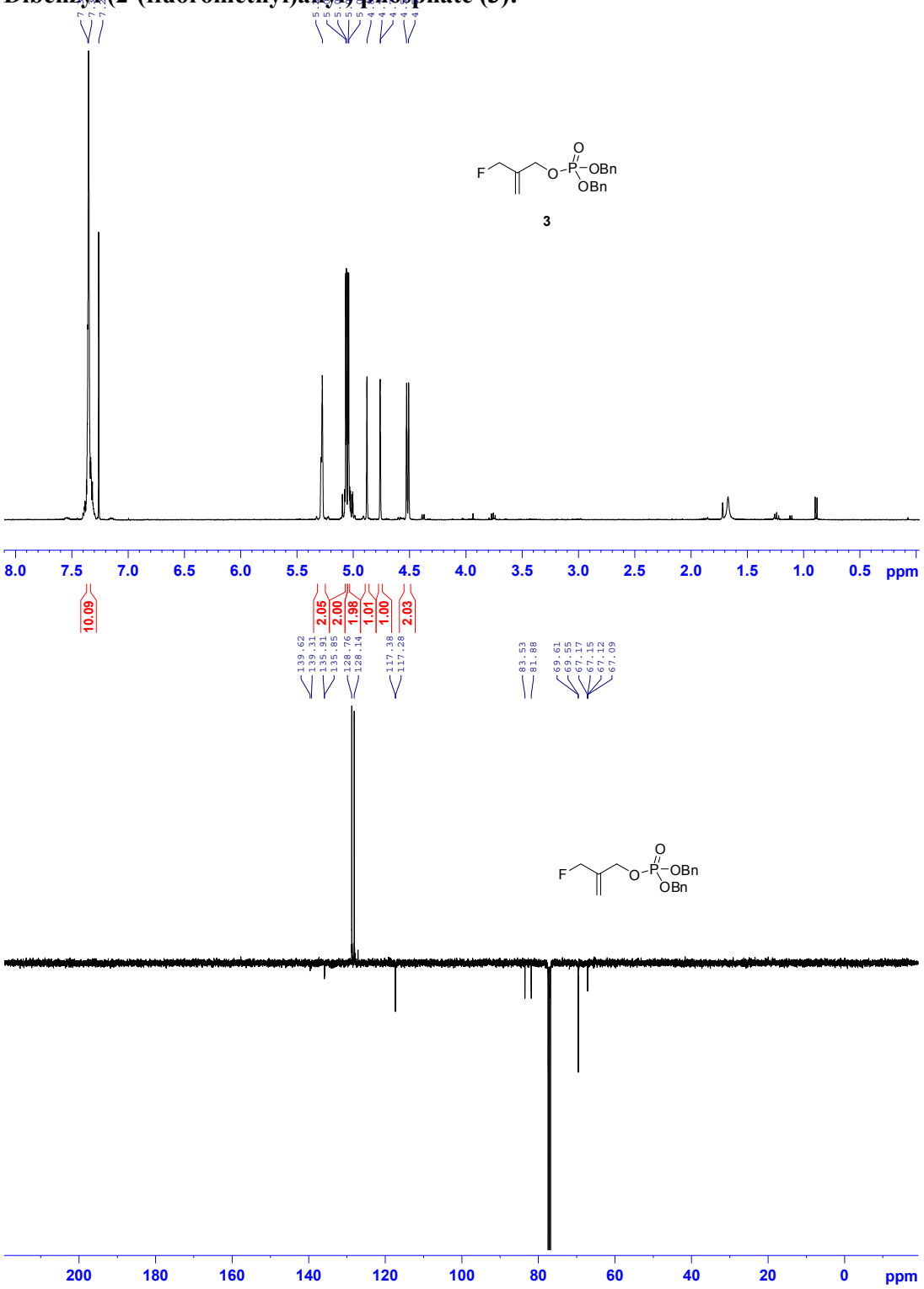
Ozone was bubbled through a solution of compound **11a** (3 mg, 10.4 µmol) in dry CH_2Cl_2 (1.5 mL) at -78°C until the color of the mixture turned blue. Dry air was bubbled through the solution until the blue color disappeared. After addition of PPh_3 (4 mg, 15 µmol), the mixture was allowed to

reach room temperature by stirring overnight. The solvent was removed under reduced pressure and the crude material was purified by flash chromatography (hexane/ethyl acetate = 3:1) to yield compound **8** (2.7 mg; 95 %). $[\alpha]_D^{20}$ = -26.5 (*c* 0.15, CH_2Cl_2). Spectroscopical data were identical with the data obtained from the enzymatic synthesis.

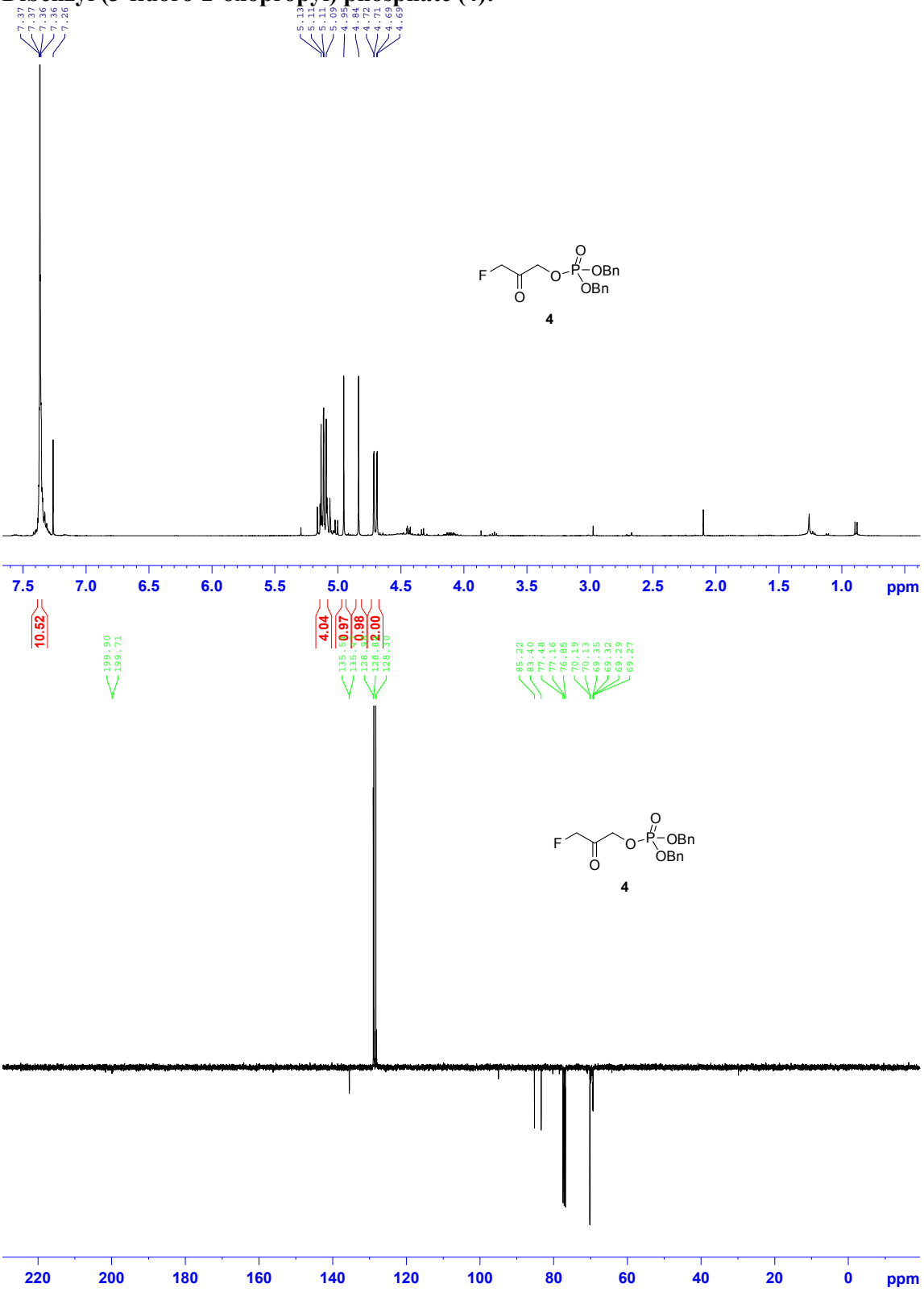
2-(Fluoromethyl)prop-2-en-1-ol (2):



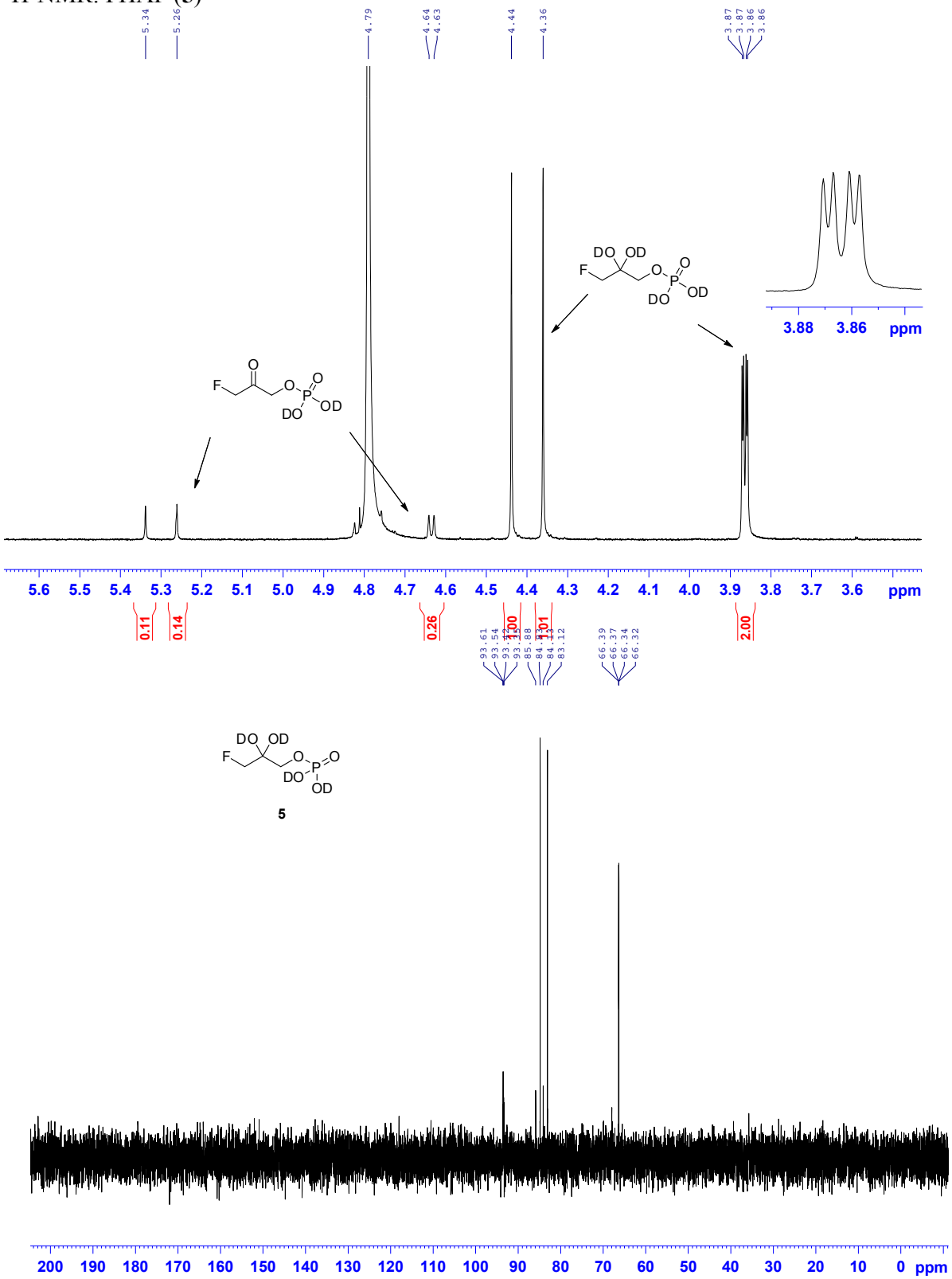
Dibenzyl (2-(fluoromethyl)allyl) phosphate (3):

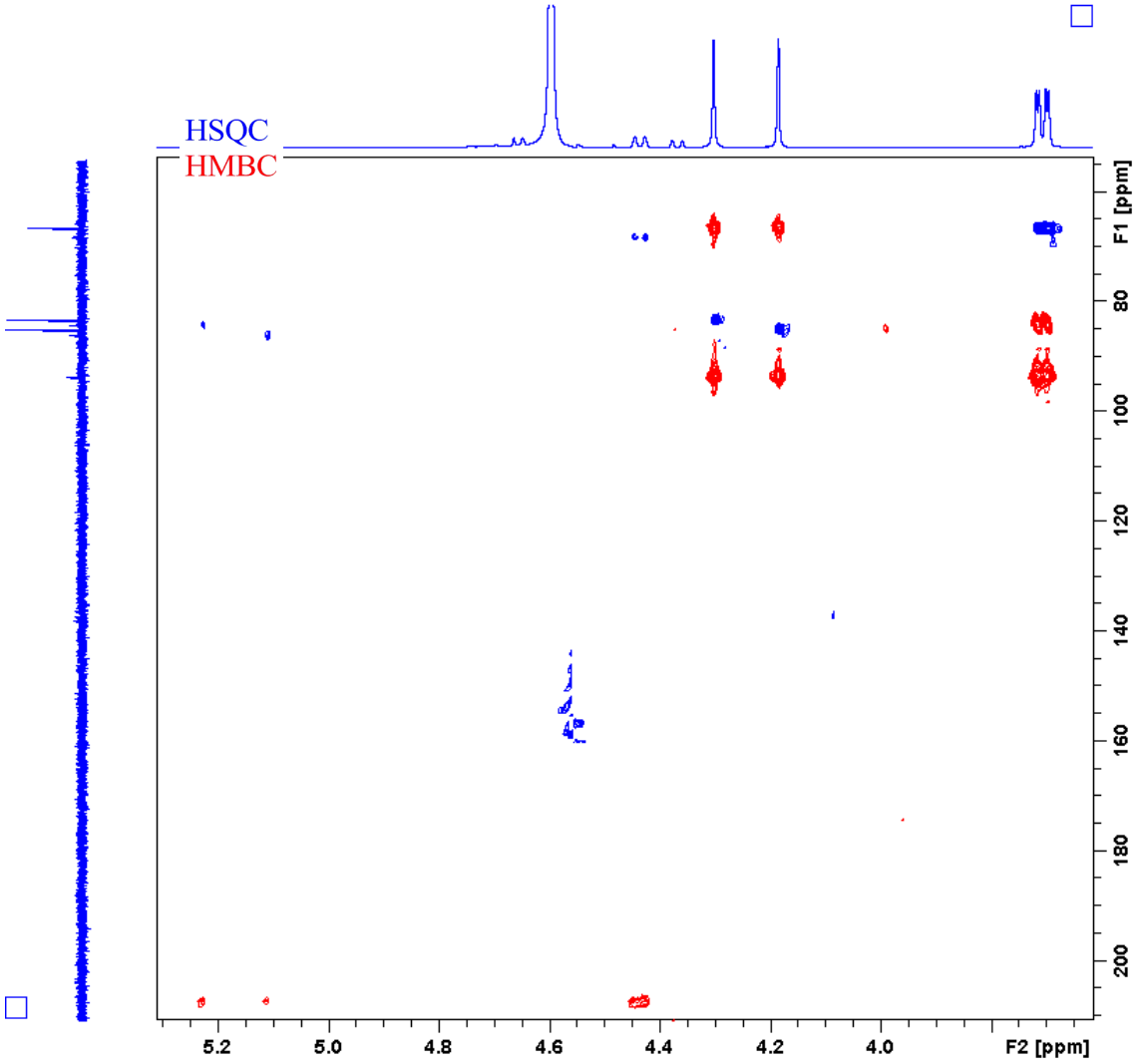


Dibenzyl (3-fluoro-2-oxopropyl) phosphate (4):

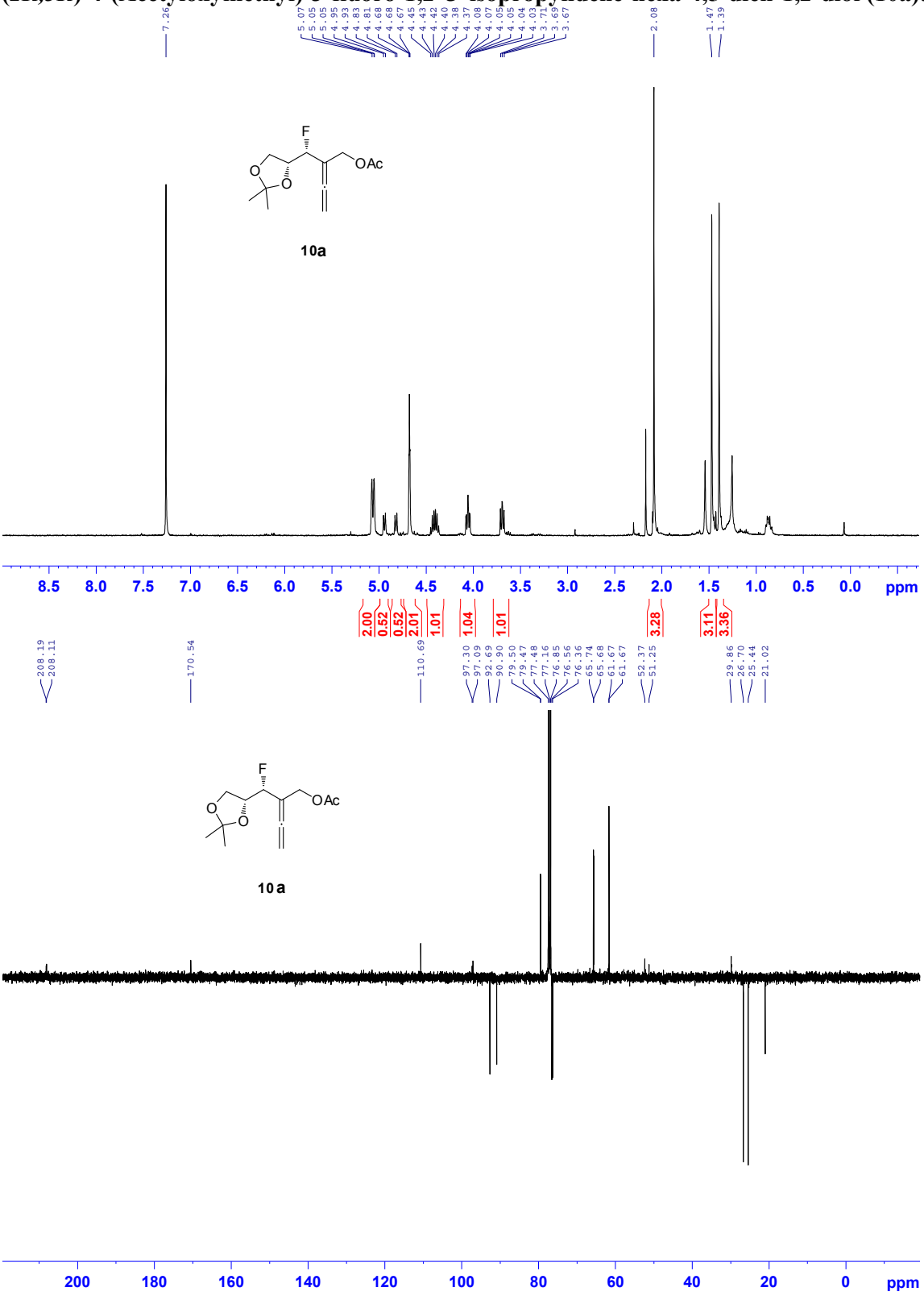


^{1}H-NMR: FHAP (**5**)

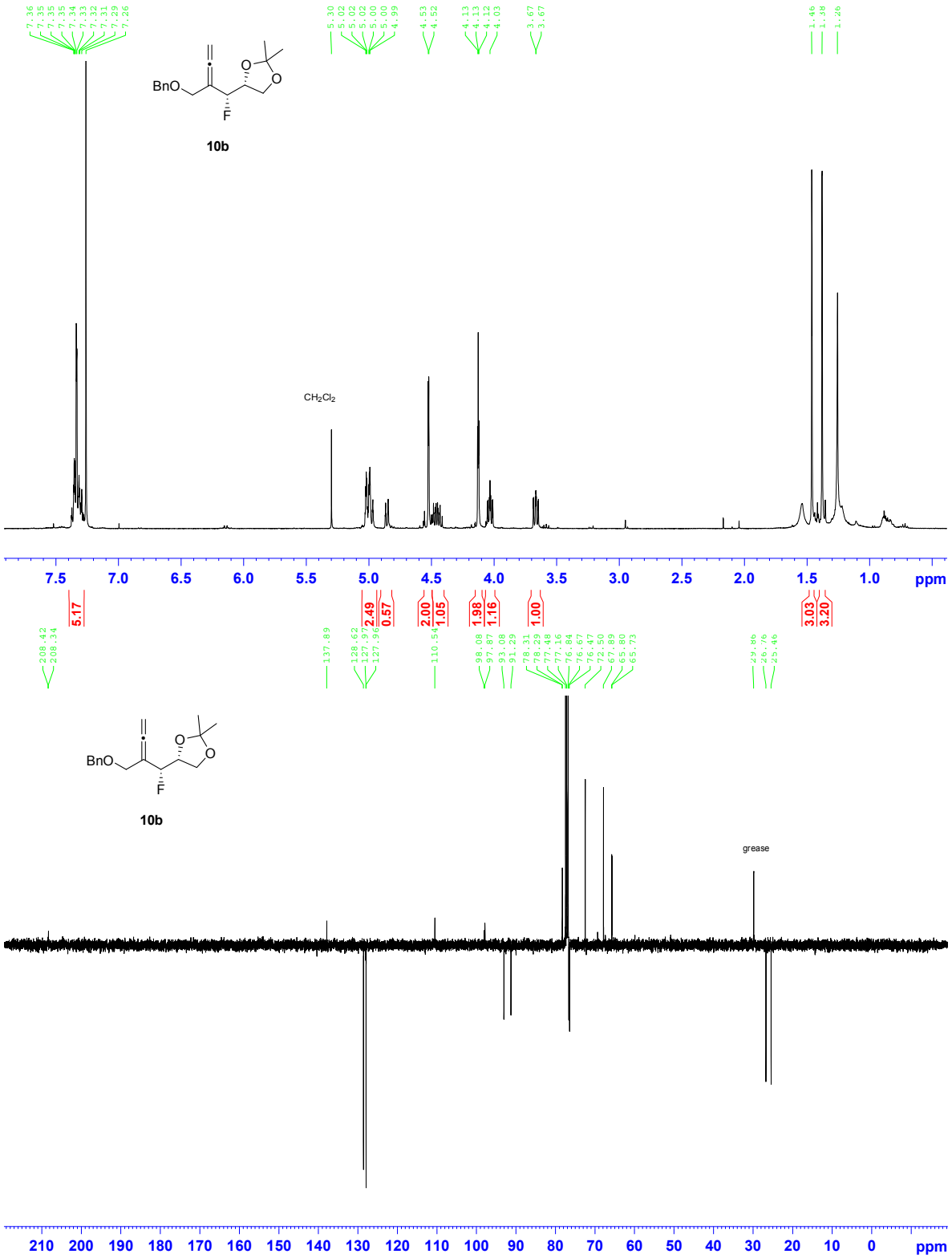

5.34
5.26
4.79
4.64
4.63
4.44
4.36
3.87
3.87
3.86
3.86
DO OD
F
O
O
P
DO
OD
3.88
3.86
ppm
O
F
O
O
P
DO
OD
5.6 5.5 5.4 5.3 5.2 5.1 5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 ppm
0.11
0.14
0.26
1.00
1.01
2.00
93.61
93.54
93.46
93.12
85.88
84.13
84.03
83.12
66.39
66.37
66.34
66.32
DO OD
F
O
O
P
DO
OD
5
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 ppm


HSQC
HMBC
F1 [ppm]
80
100
120
140
160
180
200
5.2
5.0
4.8
4.6
4.4
4.2
4.0
F2 [ppm]

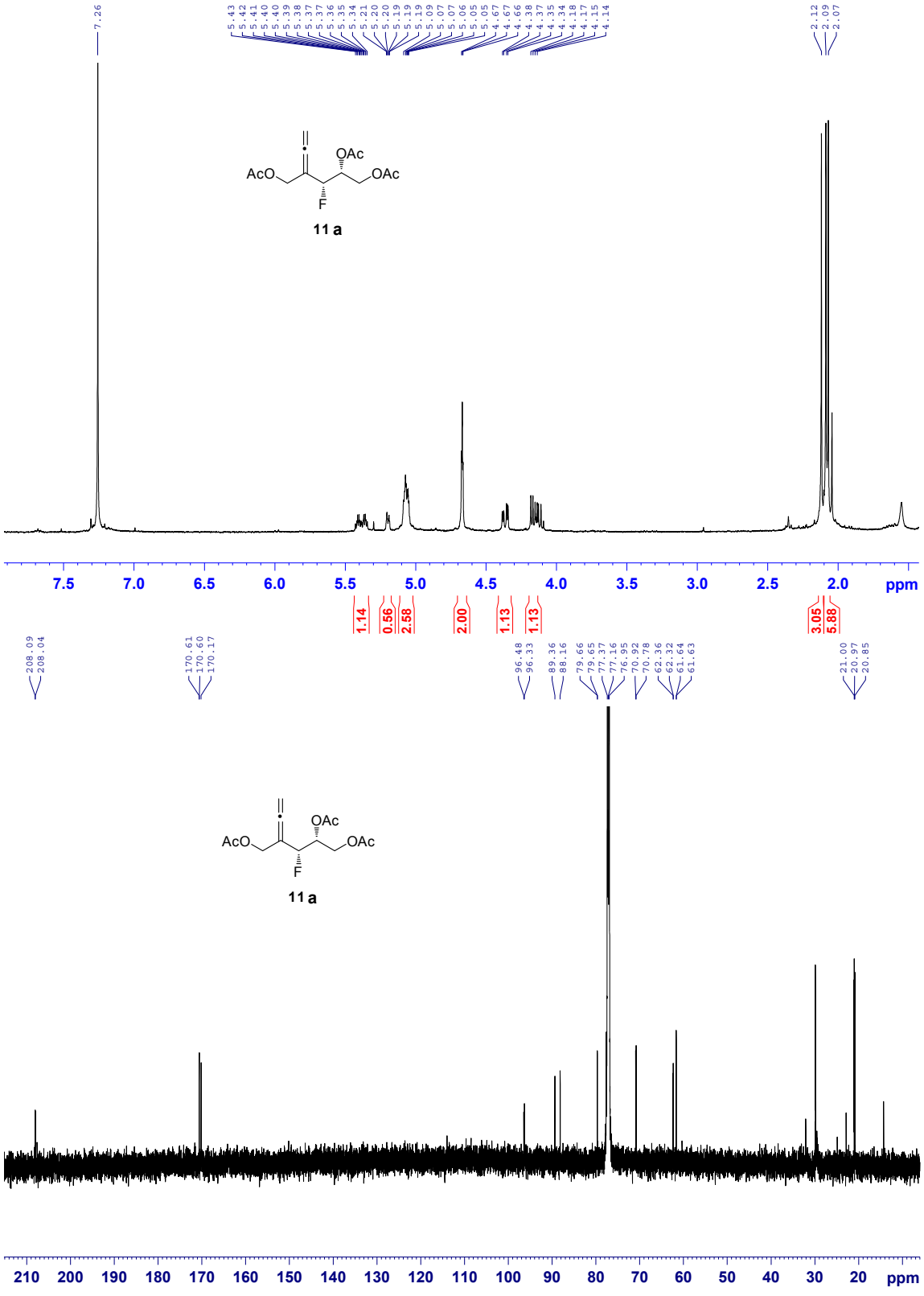
(*2R,3R*)-4-(Acetyloxymethyl)-3-fluoro-1,2-*O*-isopropylidene-hexa-4,5-dien-1,2-diol (10a):



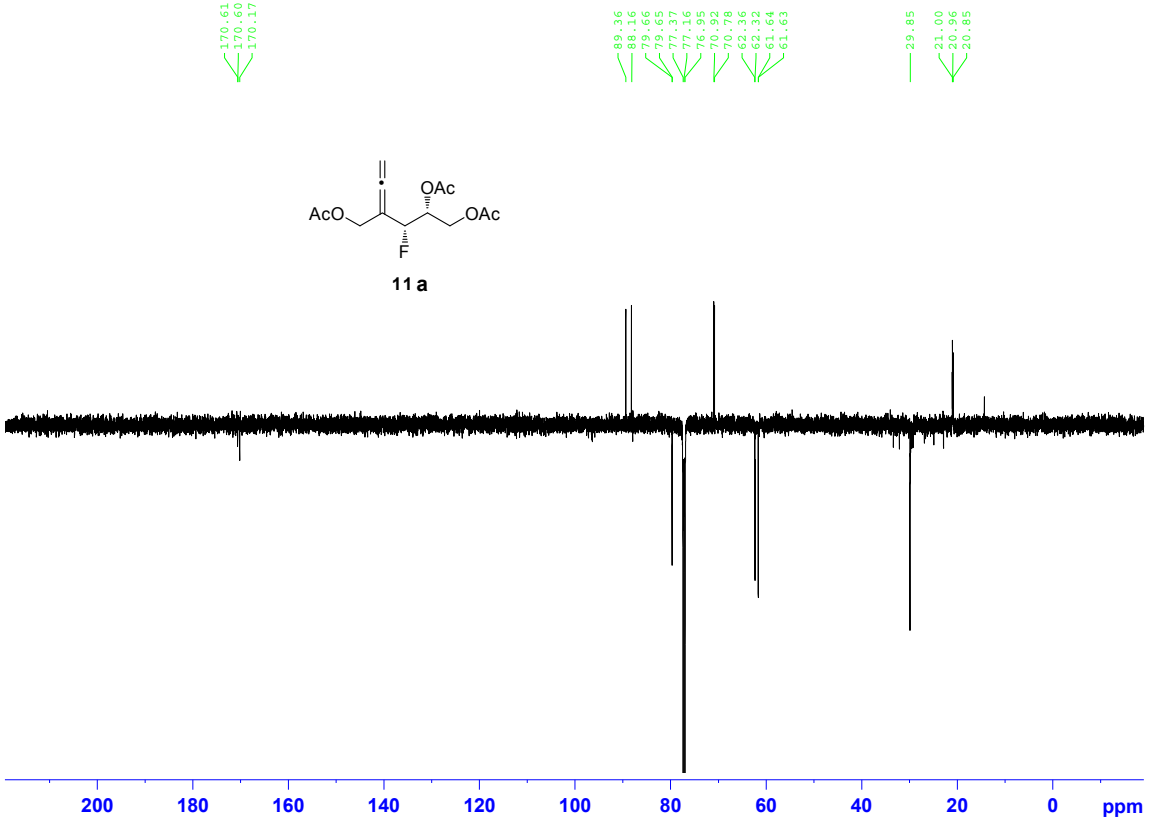
(*2R,3R*)-4-(Benzyloxymethyl)-3-fluoro-1,2-*O*-isopropylidene-hexa-4,5-dien-1,2-diol (10b):



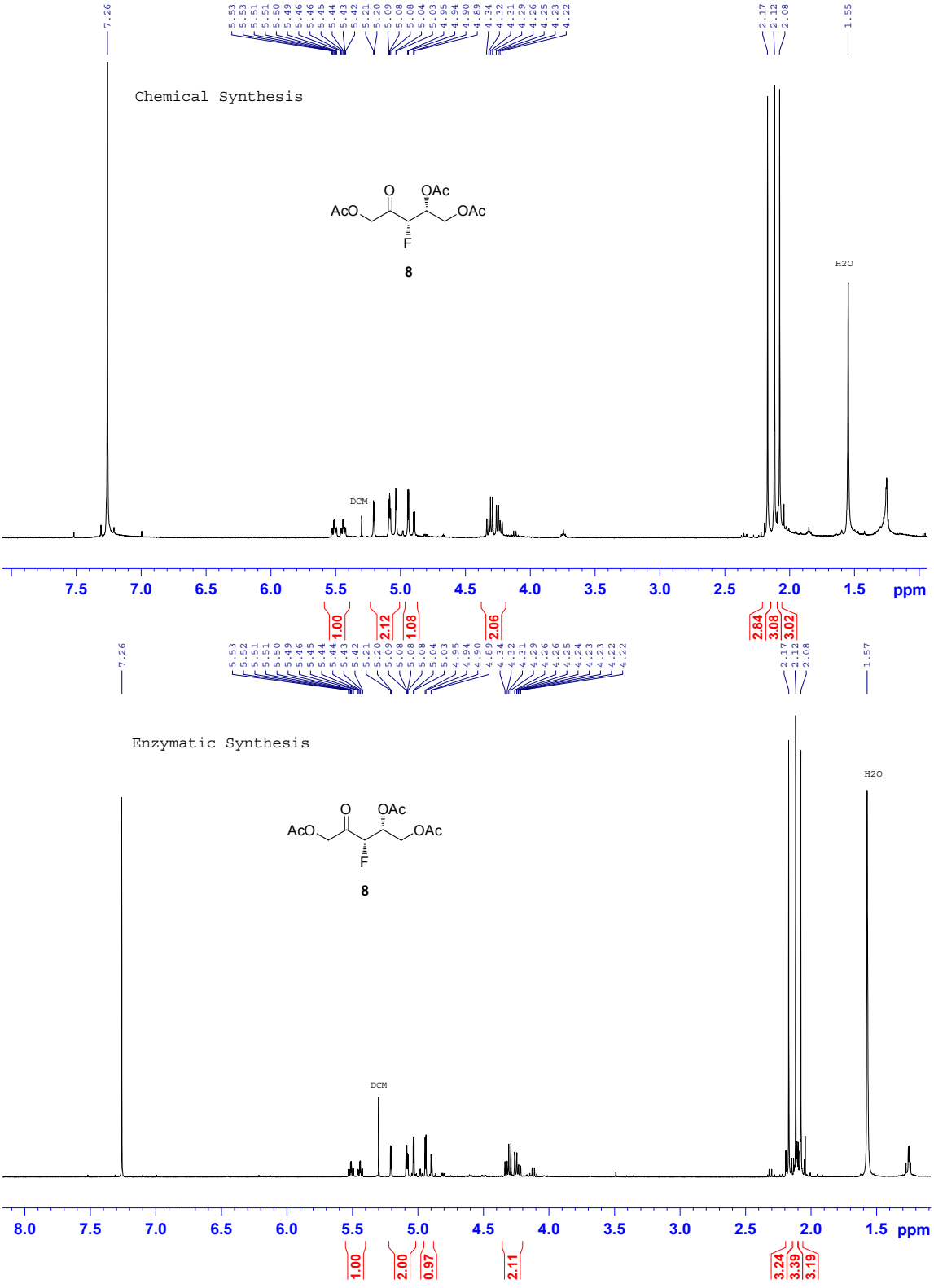
(*2R,3R*)-1,2-Di-*O*-acetyl-4-(acetyloxymethyl)-3-fluoro-hexa-4,5-dien-1,2-diol (11a):



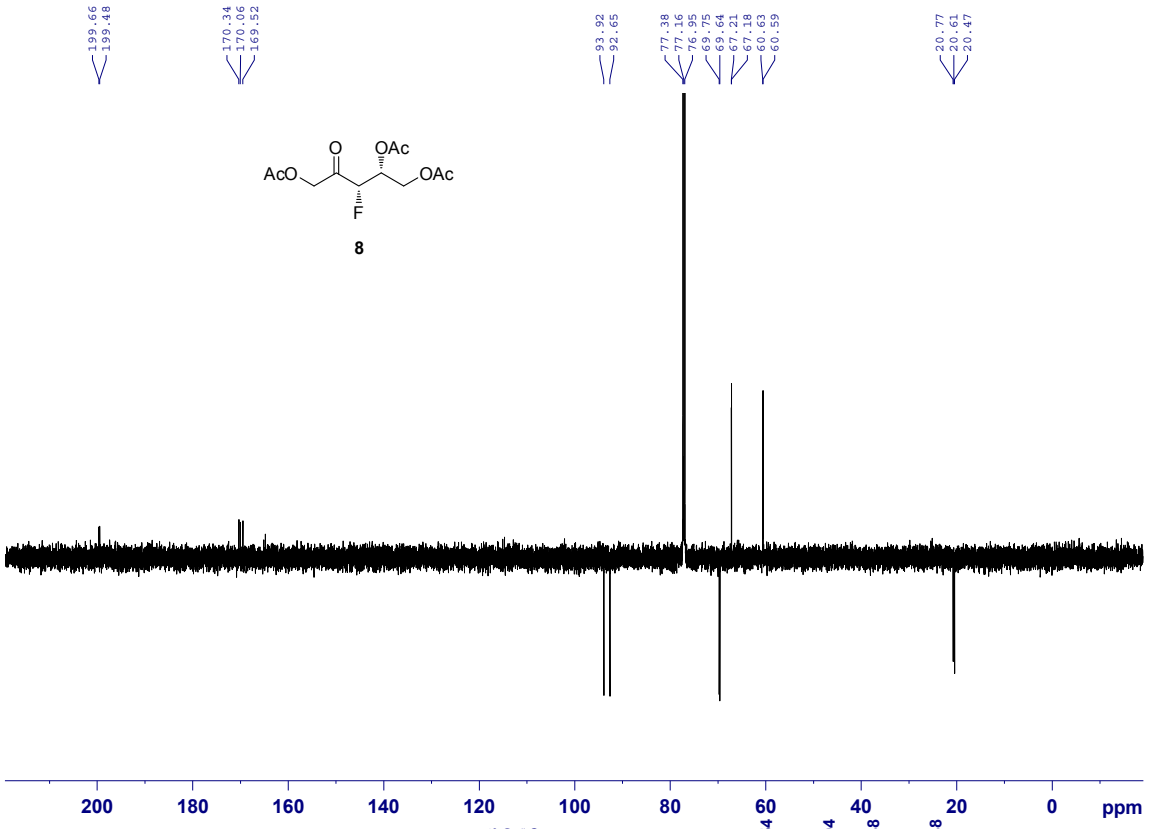
^{13}C NMR (JMOD): For the quaternary carbon of compound **11a** see above.



1,4,5-Tri-*O*-acetyl-3-deoxy-3-fluoro-D-*threo*-pent-2-ulose (8):



^{13}C-NMR:



^{19}F NMR (coupled)

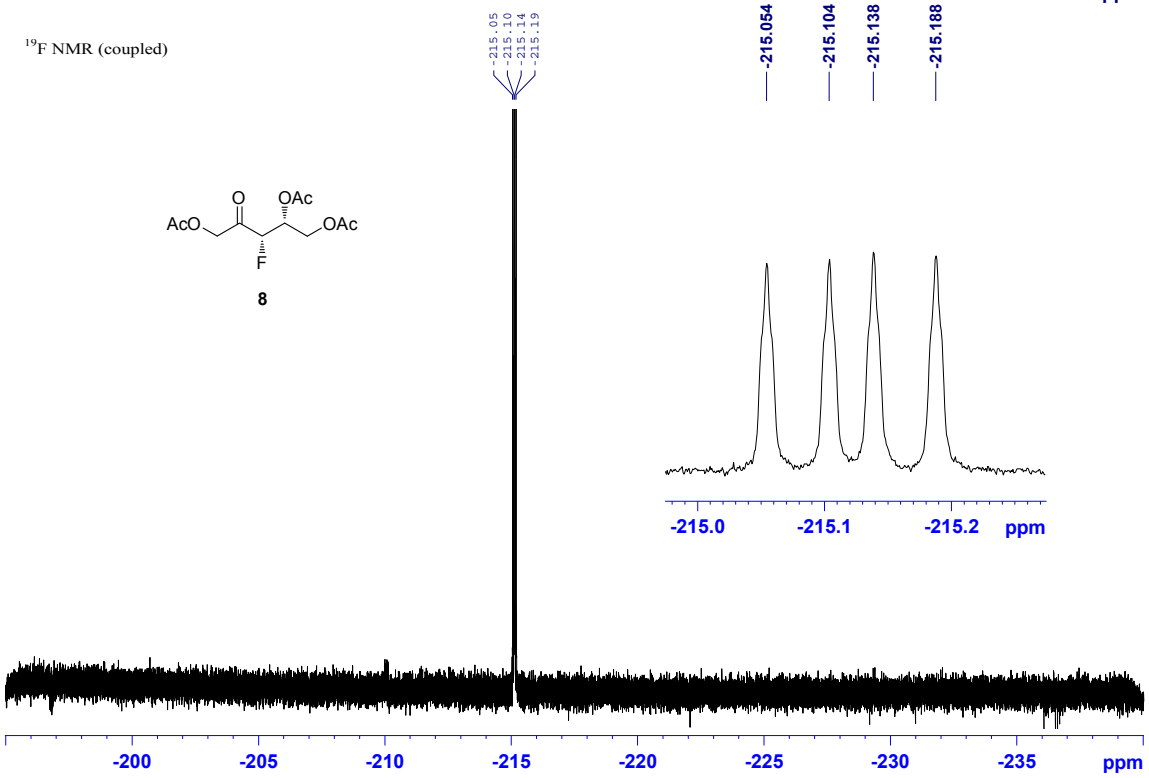
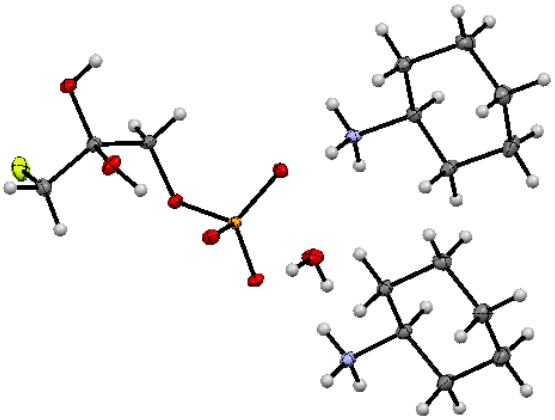


Figure 1. Crystal structure of biscyclohexylammonium salt of FHAP (**5**): The keto functionality is hydrated)[5]



Fluorine: green

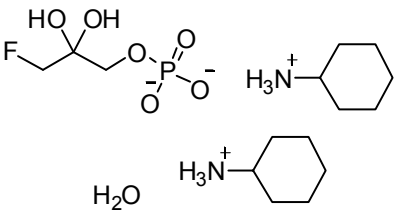
Oxygen: red

Phosphor: orange

Nitrogen: blue

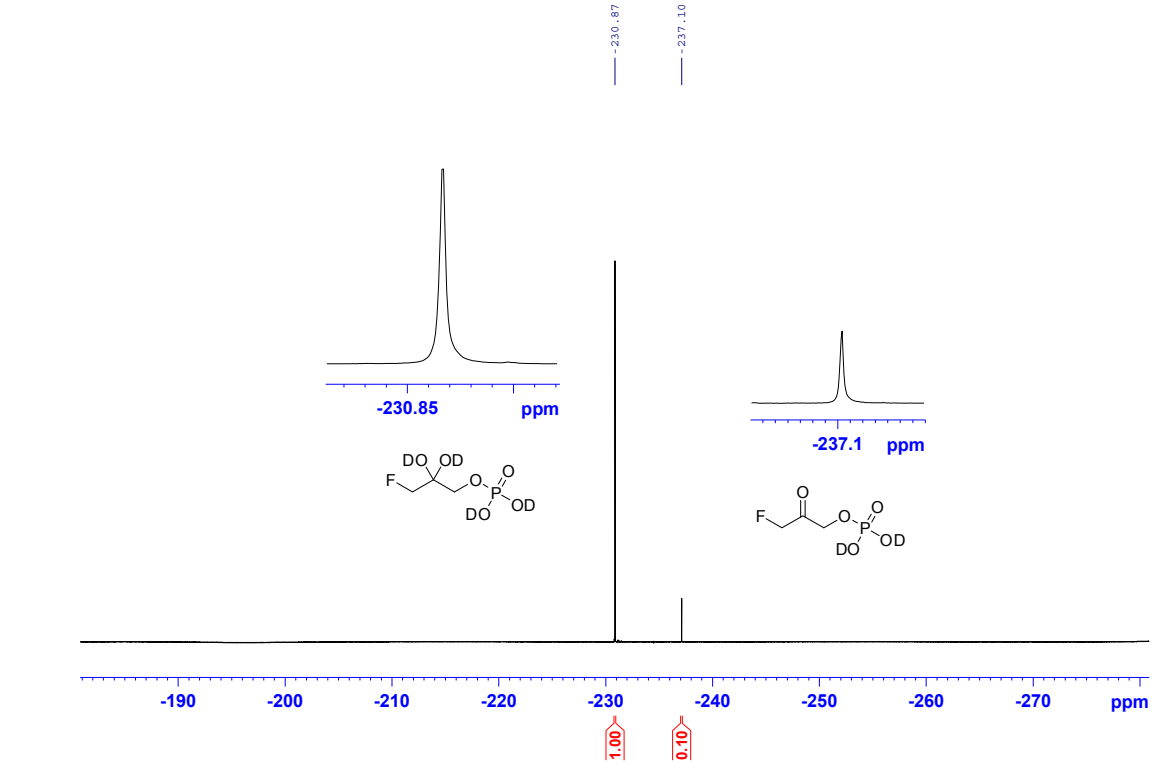
Carbon: black

Hydrogen: gray-white



CCDC-809382 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Figure 2. ^{19}F-NMR spectrum (decoupled): FHAP (**5**) in D_2O



^{19}F-NMR spectrum (coupled): FHAP (**5**) in D_2O

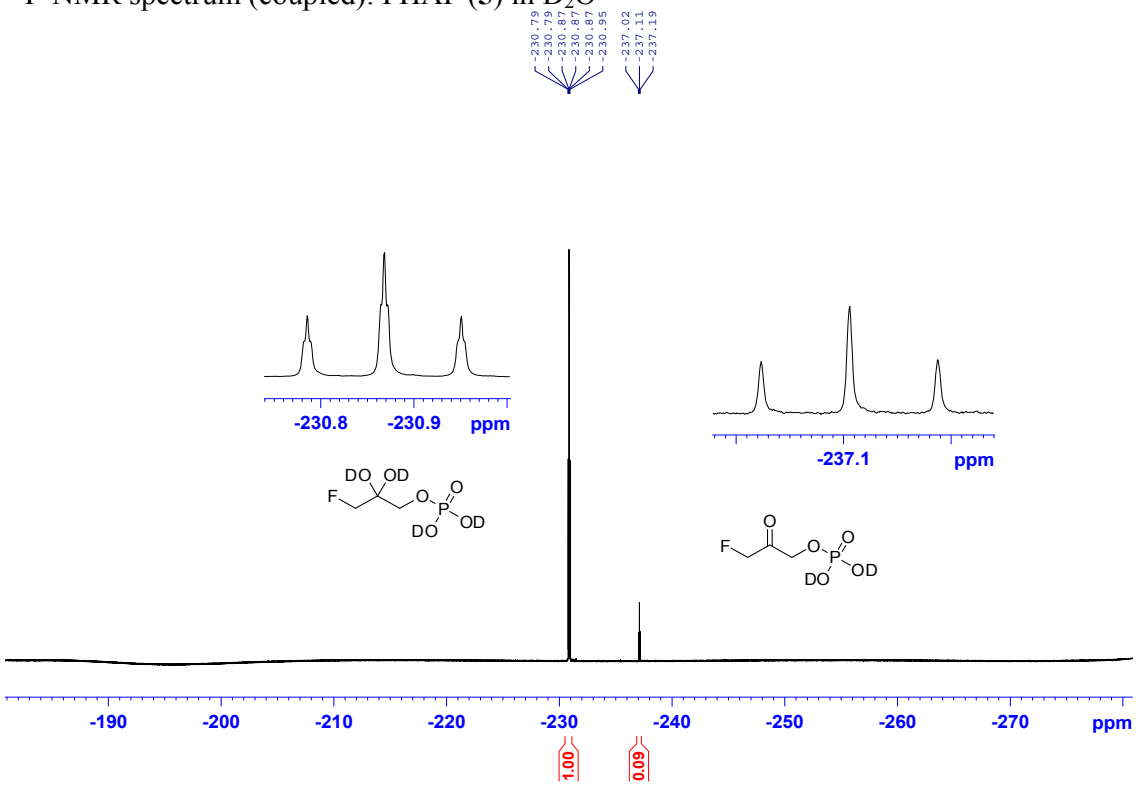


Figure 3. ^{19}F-NMR spectra: RAMA catalyzed formation of 5-chloro-3,5-dideoxy-3-fluoro-D-*threo*-pentulose 1-phosphate **(6)** in H_2O after 20 hours.

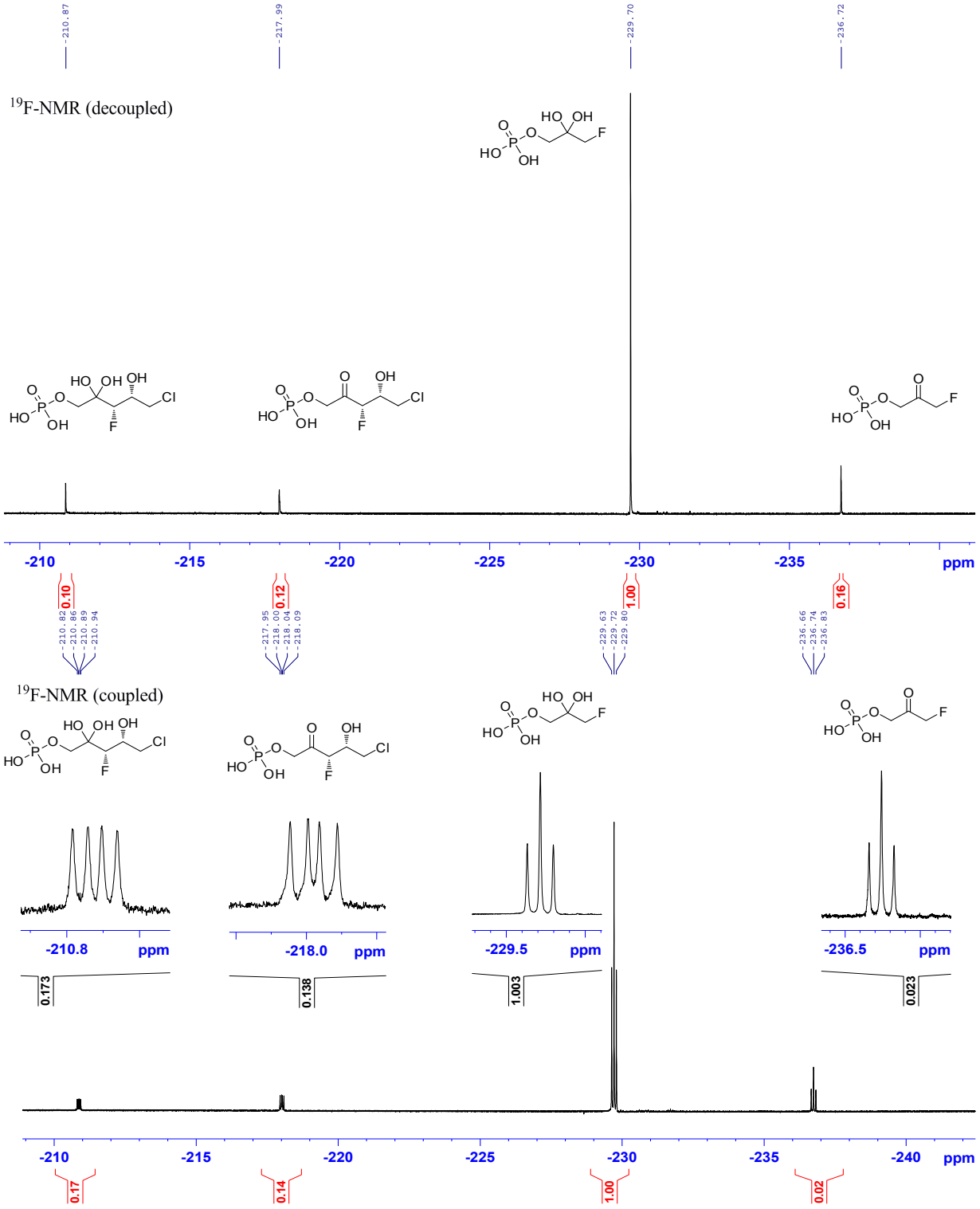


Figure 4. ^{19}F-NMR spectra (coupled): RAMA catalyzed formation of 5-chloro-3,5-dideoxy-3-fluoro-D-*threo*-pentulose 1-phosphate **(6)** in D_2O after repeated addition of RAMA over 3 days.

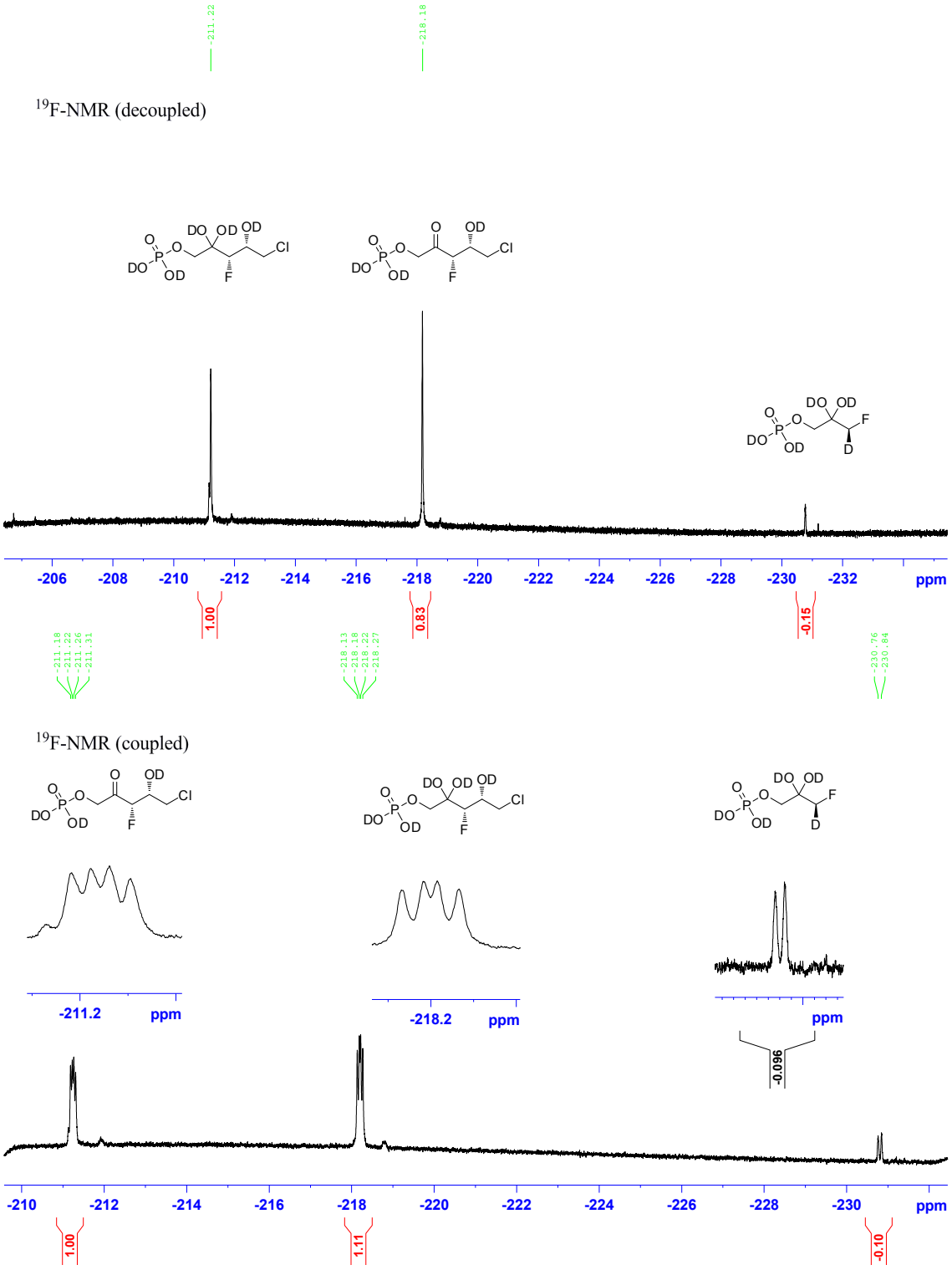


Figure 5. ^{19}F-NMR spectra: RAMA catalyzed reaction of FHAP with glycolaldehyde in D_2O: 3d after repeated addition of RAMA

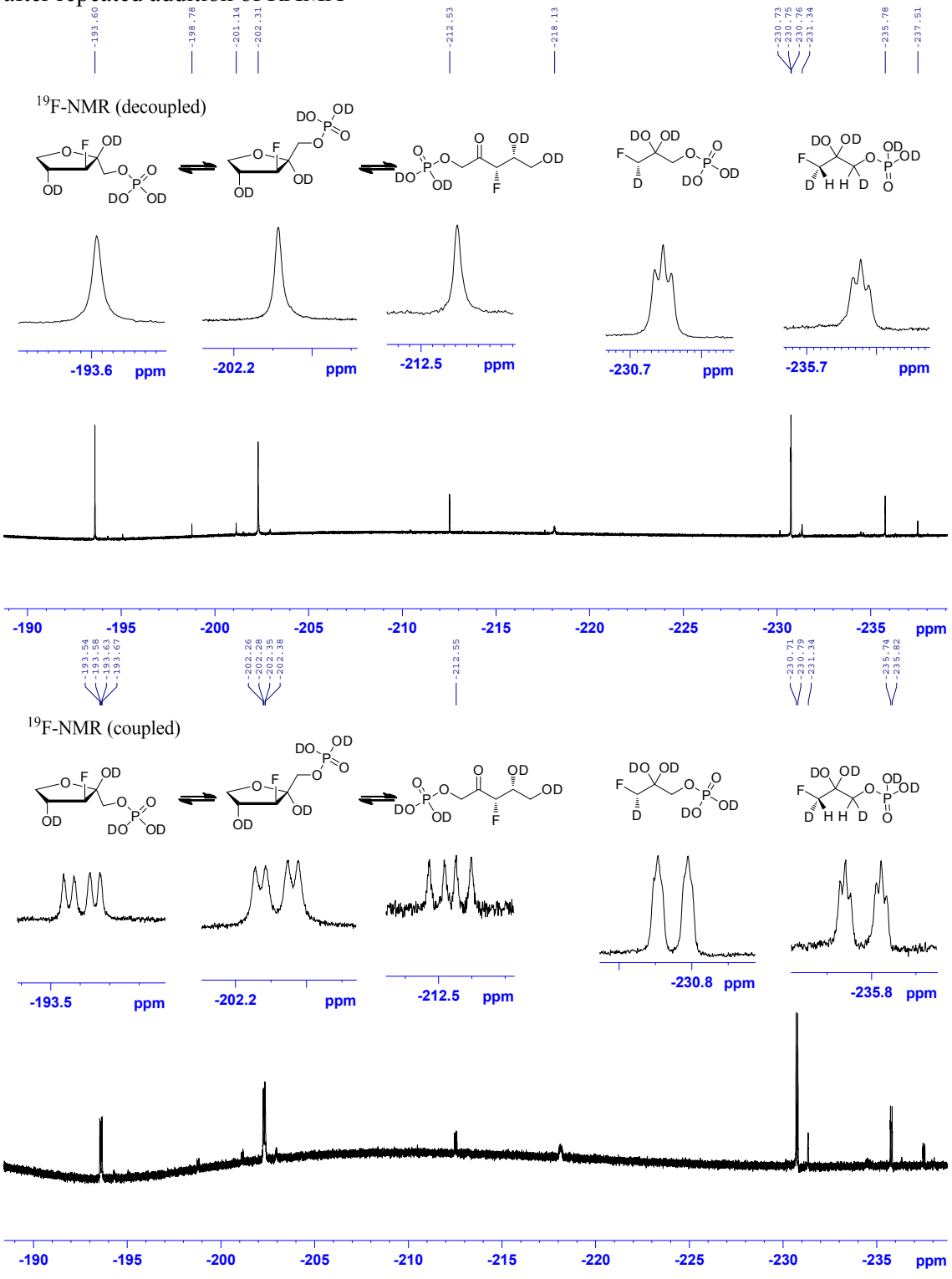


Figure 6. ^{1}H-NMR (CD_3OD): RAMA catalyzed reaction of FHAP with glycolaldehyde in D_2O after dephosphorylation with acid phosphatase.

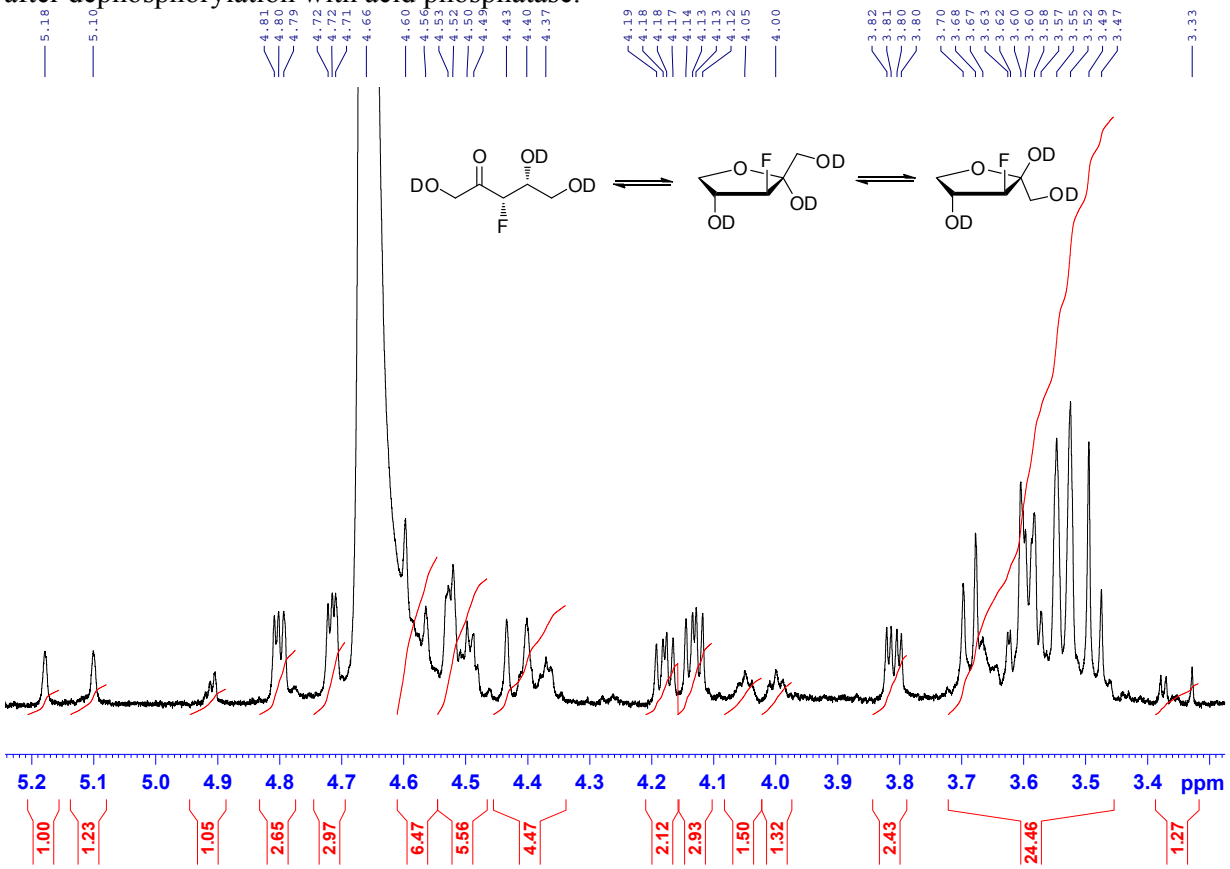


Figure 7. ^{19}F-NMR (decoupled; CD_3OD): RAMA catalyzed reaction of FHAP with glycolaldehyde in D_2O after dephosphorylation with acid phosphatase.

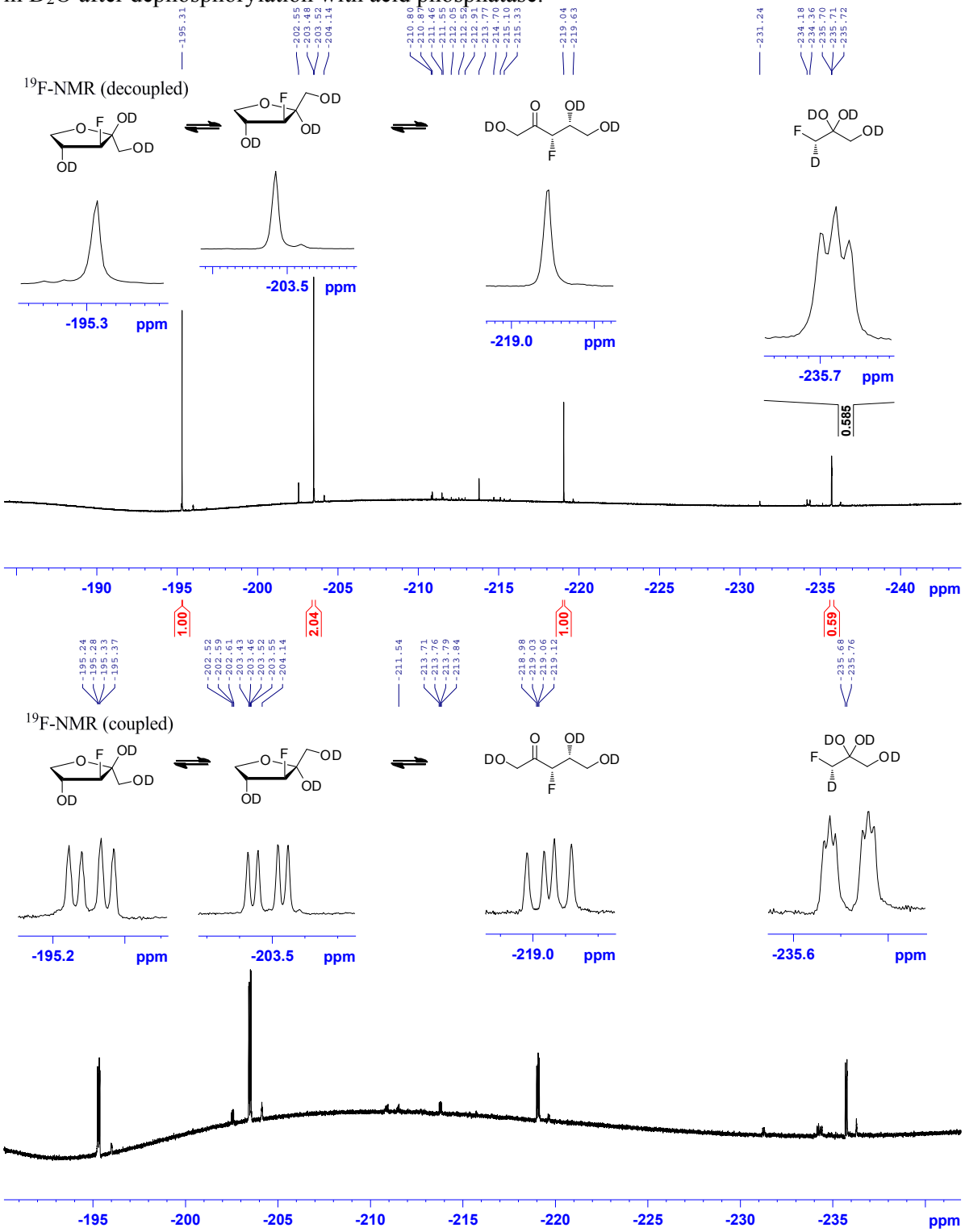
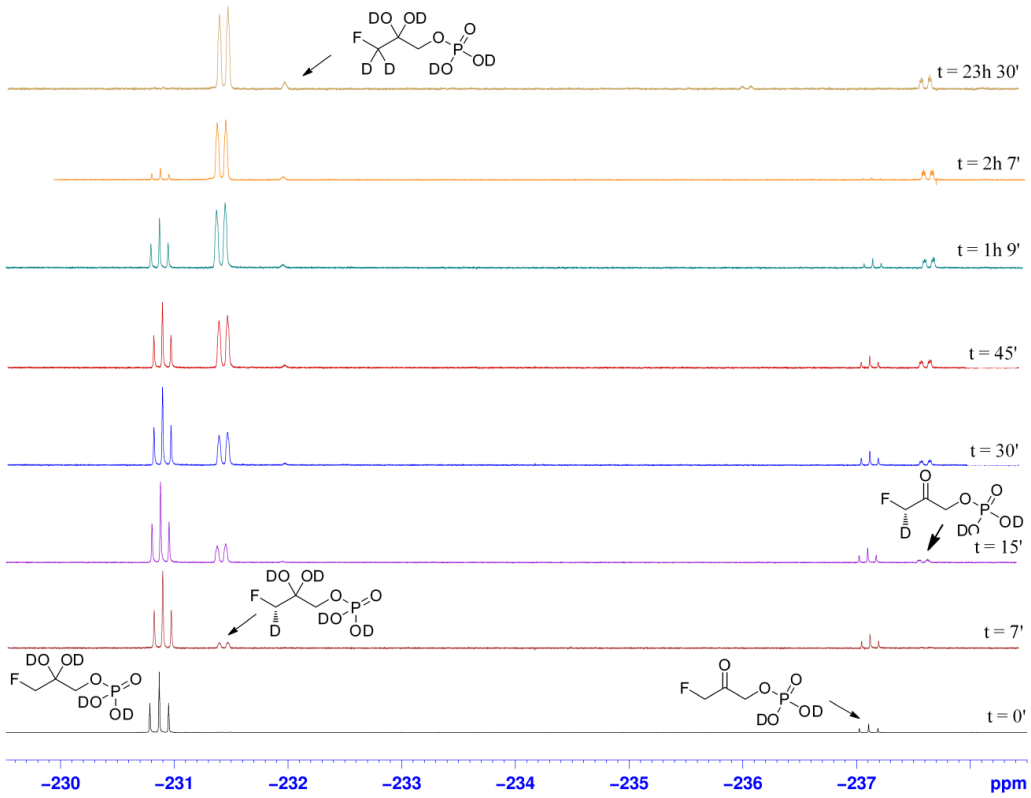


Figure 8. ^{19}F-NMR spectra: RAMA catalyzed incorporation of deuterium from D_2O into FHAP[a]



[a]experimental conditions: A solution of FHAP mono sodium salt (4 mg, 0.021 mmol) in D_2O (0.6 mL) was adjusted with small quantities of 1M NaOD to pD = 7.3. RAMA (4 U) was added and the reaction progress was monitored by ^{1}H-NMR and ^{19}F-NMR. (*S*)-3-D-FHAP: ^{1}H NMR ($CDCl_3$, 400 MHz): δ^{keto} 5.32 (d, J = 46.4 Hz, 1H) 4.53 (d, J = 7.0 Hz, 2H) $\delta^{hydrate}$ 4.36 (d, J = 46.8 Hz, 1H) 3.84 (dd, J = 8.1, 2.2 Hz, 2H). ^{19}F NMR: $\delta^{hydrate}$ –230.7 (dt, J = 46.7, 6.3 Hz); δ^{keto} –237.5 (dt, J = 46.4, 6.3 Hz). HRMS (ESI-neg. mode) calcd for $C_3H_3O_5D_2FP$ (M-D): 172.9984 found, 172.9988 (Sample was measured in D_2O).

Figure 9. ^{1}H NMR: RAMA catalyzed incorporation of deuterium from D_2O into FHAP: 2h 17' after addition of RAMA

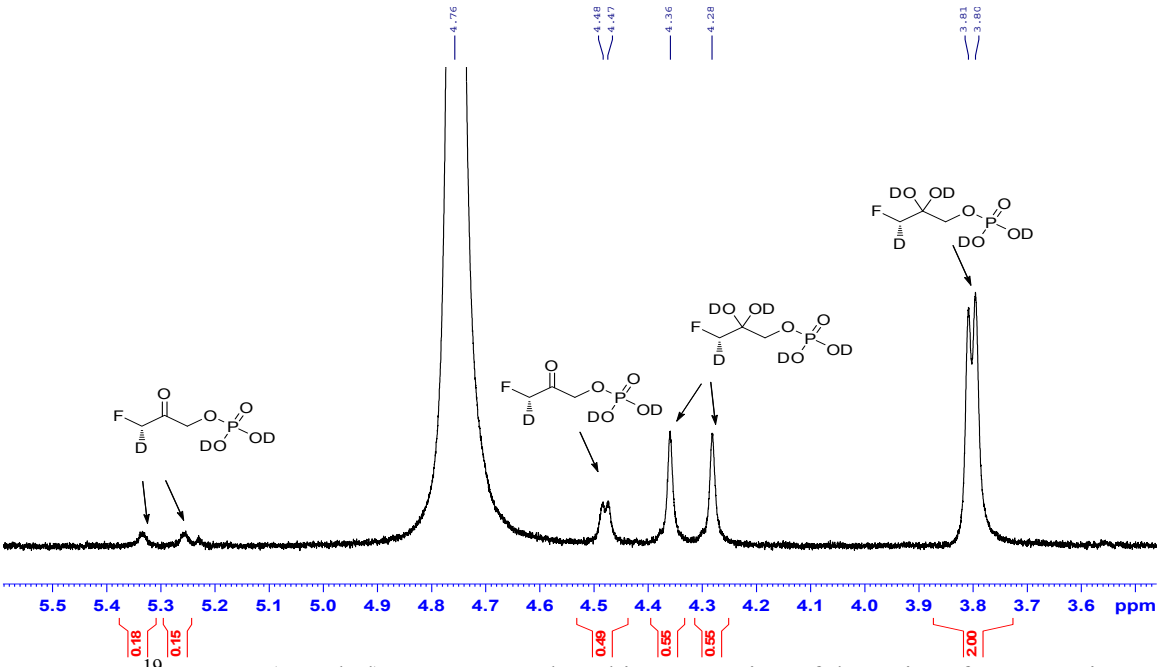


Figure 10. ^{19}F-NMR (coupled): RAMA catalyzed incorporation of deuterium from D_2O into FHAP: 2h 7' after addition of RAMA

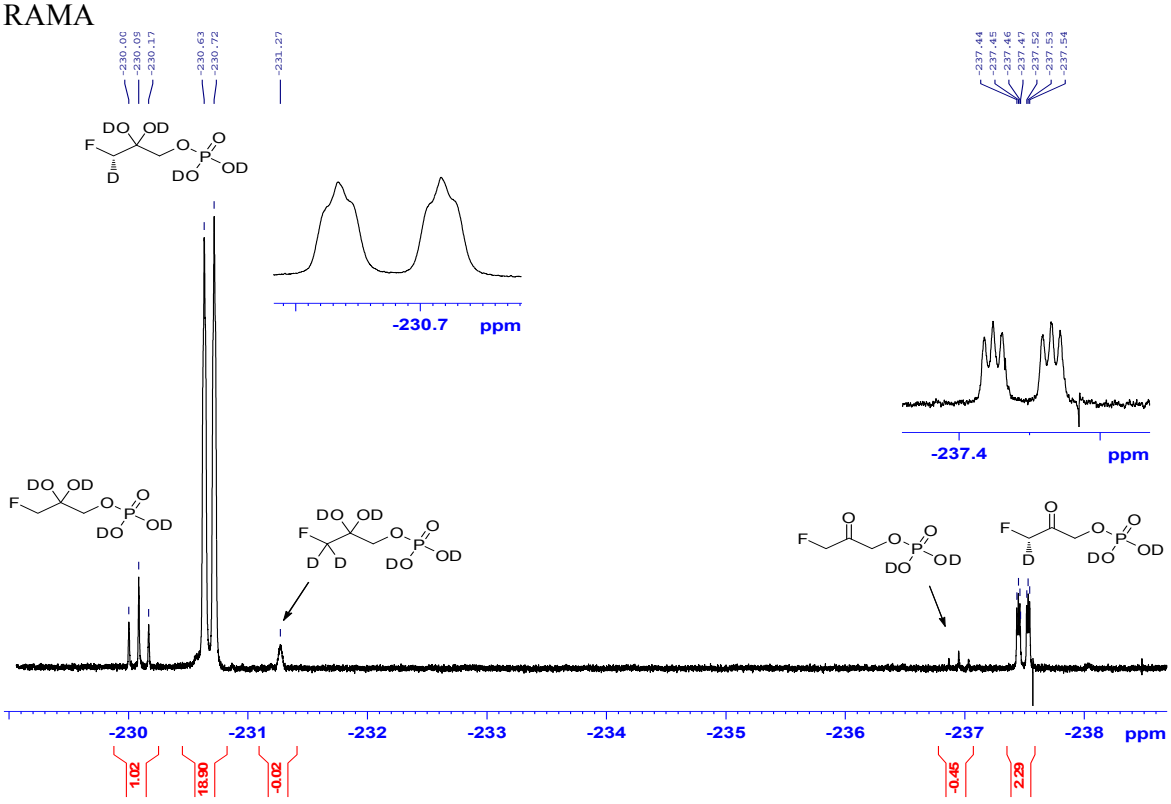


Figure 11. ^{19}F-NMR (decoupled): RAMA catalyzed incorporation of deuterium from D_2O into FHAP: 2h 17' after addition of RAMA

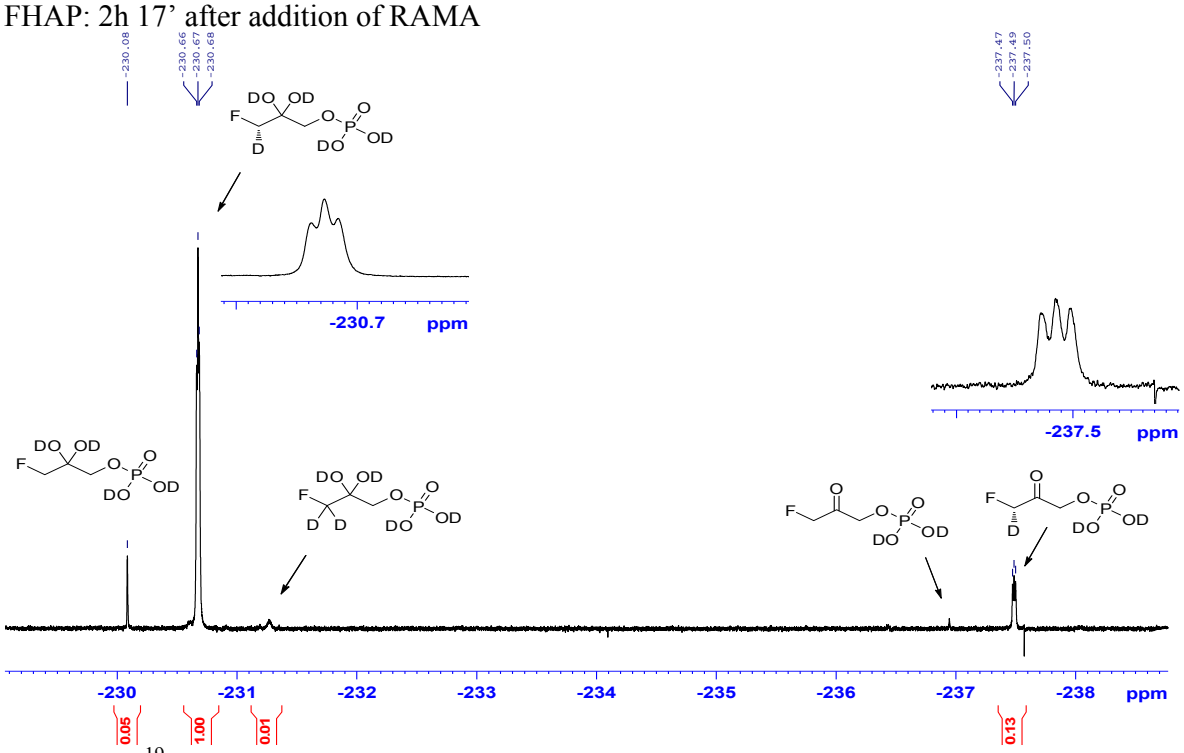


Figure 12. ^{19}F-NMR (coupled): RAMA catalyzed incorporation of deuterium from D_2O into FHAP: 23h 33' after addition of RAMA

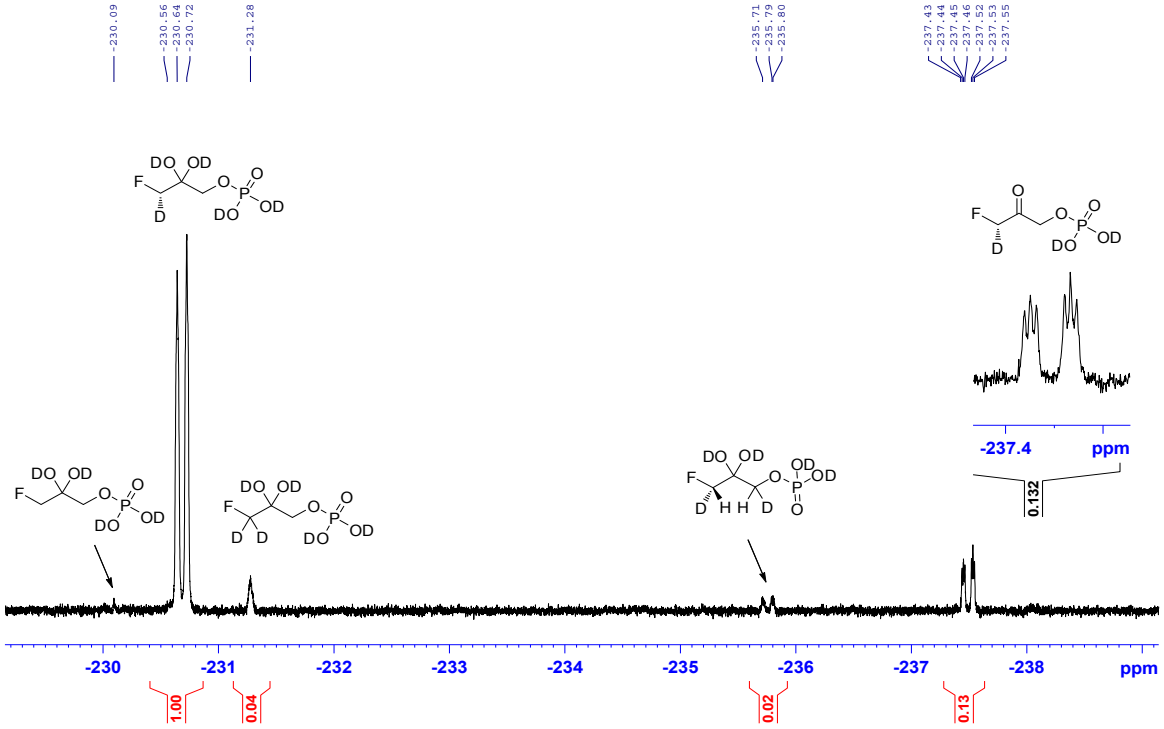


Figure 13. ^{19}F-NMR (decoupled): RAMA catalyzed incorporation of deuterium from D_2O into FHAP: 3 days after repeated addition of RAMA

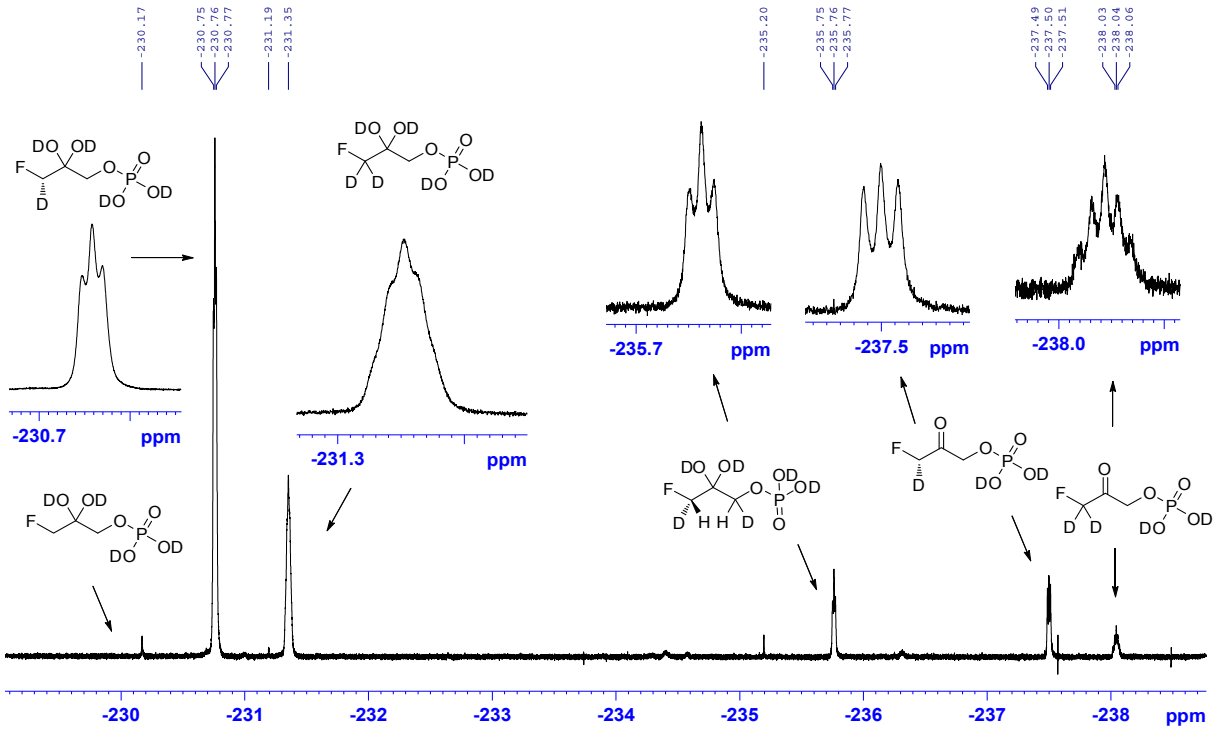
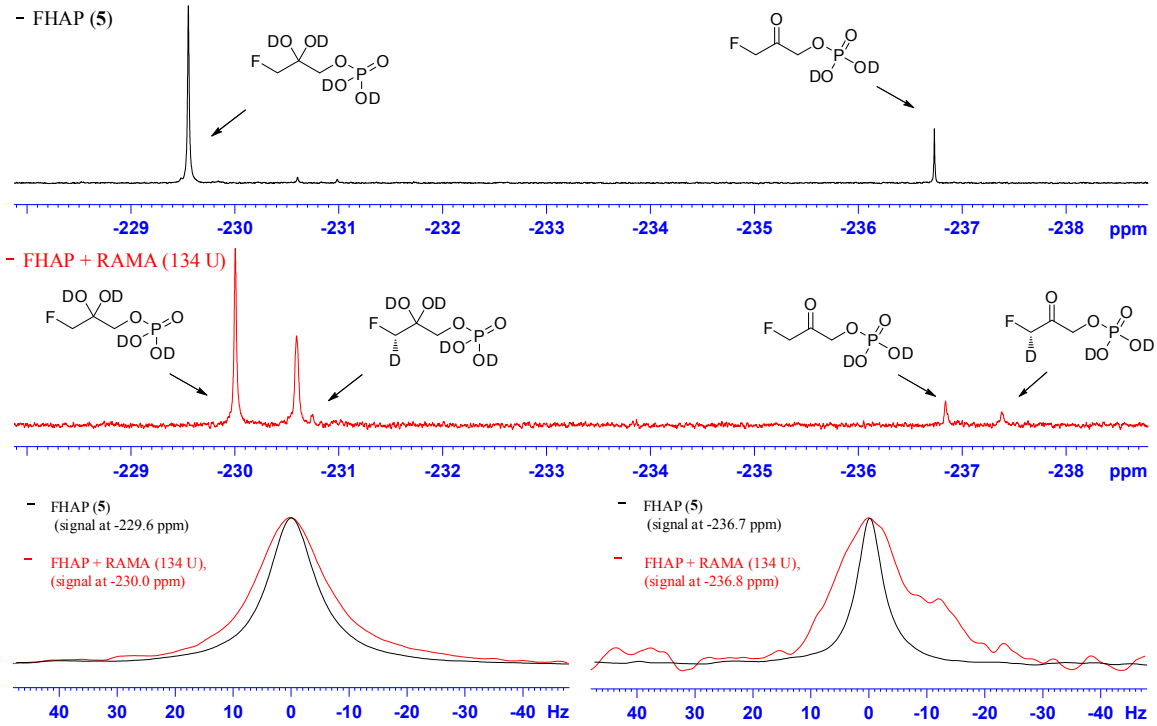
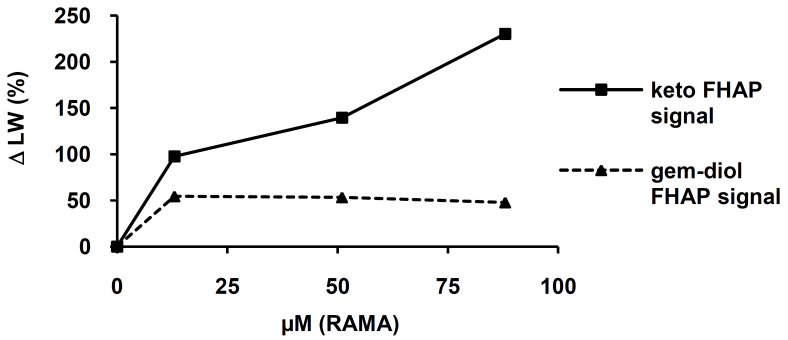


Figure 14. ^{19}F-NMR spectra (decoupled): ^{19}F-NMR binding study of FHAP with RAMA: Change of line width of ^{19}F NMR signals[a]



[a]experimental conditions: FHAP (0.7 mg, 3.6 µmol) was dissolved in 450 µL H_2O and 50 µL D_2O and the pH was adjusted to 7.3 by 1 M NaOH (black line). RAMA was repeatedly added [1st addition: 1.2 mg, 14 U, in 70 µL D_2O; 2nd addition: 4.3 mg, 49 U in 100 µL D_2O; 3rd addition: 6.2 mg, 71 U, in 150 µL D_2O (red line)]



^{19}F-NMR binding study of FHAP with RAMA: Changes in line width (LW) of ^{19}F-NMR resonances – graphical representation of data from table 1

Table 1. ^{19}F-NMR binding study of FHAP with RAMA: Change of line width of ^{19}F NMR signals[a]

FHAP (mg)	RAMA (µM)	Line width (Hz) *FHAP (gem-diol)*	ΔLW (%)	Line width (Hz) *FHAP (keto)*	ΔLW (%)
0,7	0	9,0	-	4,3	-
0,7	13	13,9	54	8,5	98
0,7	51	13,8	53	10,3	140
0,7	88	13,3	48	14,2	230

References:

[1] J. Villieras, M. Rambaud, *Synthesis* **1982**, 924–926.

[2] T. H. Fife, T. C. Bruice, *J. Phys. Chem.* **1961**, *65*, 1079–1080.

[3] The spectroscopical data were identical with those reported previously: Fischer, M; Schmölzer, S.; Nowikow, T.; Schmid., W. *Eur. J. Org. Chem.* **2011**, *9*, 1645–1651.

[4] ^{1}H-NMR, ^{13}C-NMR and HRMS were in full agreement with previously published data: a) T. Vuorinen, A. S. Serianni, *Carbohydr. Res.* **1990**, *209*, 13–31. b) D. de Wit, F. van Rantwijk, L. Maat, A. P. G. Kieboom, *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 271–274.

[5] Crystal Structure Visualisation: "*Mercury CSD 2.0 - New Features for the Visualization and Investigation of Crystal Structures"* C. F. Macrae, I. J. Bruno, J. A. Chisholm, P. R. Edgington, P. McCabe, E. Pidcock, L. Rodriguez-Monge, R. Taylor, J. van de Streek and P. A. Wood, *J. Appl. Cryst.* **2008**, *41*, 466–470.