

## **Structural basis of stereospecificity in the bacterial biodegradation of $\beta$ -aryl ether bonds found in lignin**

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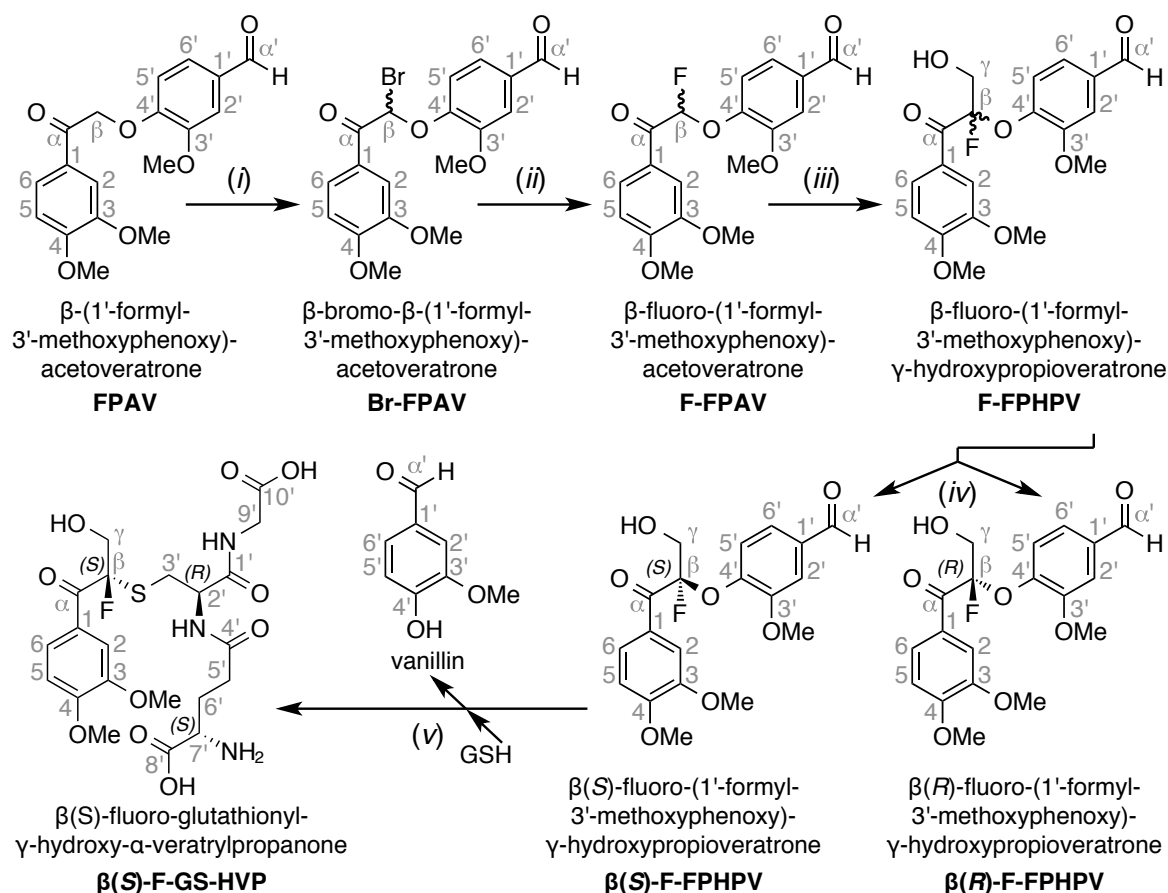
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## SUPPLEMENTARY INFORMATION – CHEMICAL SYNTHESSES AND NMR DATA

### CHEMICAL SYNTHESSES:

**General.** Reagents and chemicals were purchased from Sigma-Aldrich.  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra were recorded on a Bruker Biospin (Billerica, MA) AVANCE 700 MHz spectrometer fitted with a cryogenically cooled 5-mm TXI gradient probe with inverse geometry (proton coils closest to the sample).  $^{19}\text{F}$  NMR spectra were recorded on a Bruker Biospin DMX 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm).  $J$  values are recorded in Hz. The central NMR solvent peaks were used as internal references ( $\delta_{\text{H}}$ : 2.05 ppm and  $\delta_{\text{C}}$ : 29.8 ppm for acetone- $d_6$ ;  $\delta_{\text{H}}$ : 4.79 ppm for (HDO in)  $\text{D}_2\text{O}$  (1,2). Carbon and proton assignments reported for all compounds in the attached spectra were determined via the aid of 2D COSY, HSQC, and HMBC NMR spectra. Merck-EMD Millipore aluminum-backed Silica Gel 60  $\text{F}_{254}$  normal-phase thin-layer chromatography plates were used for small-scale separation of organic compounds using a mixture of hexane and ethyl acetate as the mobile solvent.

Syntheses of  $\beta$ -(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (FPAV, **Supplementary Figure 3**) and racemic  $\beta$ -(1'-formyl-3'-methoxyphenoxy)- $\gamma$ -hydroxypropioveratrone (FPHPV) were carried out as described previously (3,4) according to the method of Adler and Eriksoo (5,6). Biotage KP-Sil silica gel was used for preparative separations of organic compounds by flash chromatography using a CombiFlash  $\text{R}_f$  delivery module using a mixture of hexane and ethyl acetate as the mobile phase.



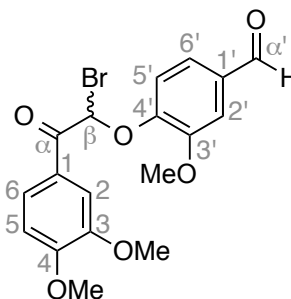
**Supplementary Figure 3.** Scheme for organic synthesis of (i) racemic  $\beta$ -bromo-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (Br-FPAV), (ii) racemic  $\beta$ -fluoro-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (F-FPAV), (iii) racemic  $\beta$ -fluoro-(1'-formyl-3'-methoxyphenoxy)- $\gamma$ -hydroxypropioveratrone (F-FPHPV), (iv) chiral chromatographic separation of enantiomers  $\beta(S)$ -F-FPHPV and  $\beta(R)$ -F-FPHPV, and (v) enzymatic synthesis of  $\beta(S)$ -fluoro-glutathionyl- $\gamma$ -hydroxy- $\alpha$ -veratrylpropanone ( $\beta(S)$ -F-GS-HVP). Reagents and conditions: (i) pyridinium tribromide, EtOAc, 90 min, 68%; (ii) Ag(I)F, acetonitrile, 18 h, 90%; (iii) formaldehyde, K<sub>2</sub>CO<sub>3</sub>, 1,4-dioxane, 18 h, 23%; (iv) chiral chromatography, ethanol/hexane; (v) LigE, glutathione, 25 mM Tris in H<sub>2</sub>O, pH 8.0, 18 h, C<sub>18</sub> chromatography, H<sub>2</sub>O/methanol.

**Synthesis of *racem*- $\beta$ -bromo-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (Br-FPAV), Supplementary Figure 8(i).** Synthetic FPAV served as the starting material in the synthesis of *racem*- $\beta$ -bromo-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (Br-FPAV, Supplementary Figure 8(i)). A solution of ethyl acetate (100 mL), FPAV (2.5 g, 7.6 mmol), and pyridinium tribromide (2.9 g, 9.1 mmol) was prepared in a 250-mL round-bottom flask with stirring. After 90 min, the reaction mixture was washed three times with saturated Na<sub>2</sub>CO<sub>3</sub>, once with H<sub>2</sub>O, and once with brine. The organic layer was then dried over MgSO<sub>4</sub> and the solvent was evaporated *in vacuo*, and the residual oil was identified as Br-FPAV (2.1 g, 68% yield).

***racem*- $\beta$ -bromo-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (Br-FPAV):**

<sup>1</sup>H NMR (700 MHz, acetone-*d*<sub>6</sub>)  $\delta$  10.00 (s, 1H, H $\alpha'$ ); 8.00 (dd, 1H,  $J_{6-5} = 8.5$  Hz,  $J_{6-2} = 2.0$  Hz, H6); 7.77 (d, 1H,  $J_{2-6} = 2.0$  Hz, H2); 7.68 (s, 1H, H $\beta$ ); 7.67 – 7.65 (m, 2H, H6'/H5'); 7.61 (d, 1H,  $J_{2'-6'} = 1.7$  Hz, H2'); 7.14 (d, 1H,  $J_{5'-6'} = 8.5$  Hz, H5); 3.97 (s, 3H, 3'-OMe); 3.95 (s, 3H, 4-OMe); 3.92 (s, 3H, 3-OMe).

<sup>13</sup>C NMR (176 MHz, acetone-*d*<sub>6</sub>)  $\delta$  191.7 (C $\alpha'$ ); 186.4 (C $\alpha$ ); 155.6 (C4); 152.2 (C3'); 150.2 (C3); 149.3 (C4'); 134.9 (C1'); 125.6 (C1); 125.4 (C6); 125.0 (C6'); 119.3 (C5'); 112.7 (C2); 112.3 (C2'); 111.6 (C5); 82.6 (C $\beta$ ); 56.6 (3'-OMe); 56.3 (4-OMe); 56.1 (3-OMe).



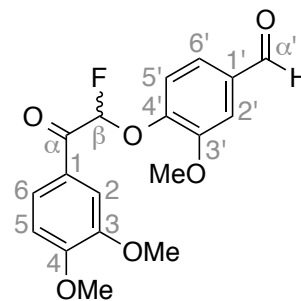
**Synthesis of *racem*- $\beta$ -fluoro-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (F-FPAV), Supplementary Figure 8(ii).** To a magnetically stirred solution of Br-FPAV (2.0 g, 5.0 mmol) dissolved in anhydrous acetonitrile (50 mL) in a 250-mL round-bottom flask maintained under an inert atmosphere at 60 °C, silver (I) fluoride (Ag(I)F) was added (3.2 g, 25.0 mmol). After 18 h, inorganics (7) were removed by filtration and the filtrate was evaporated *in vacuo*. The resulting residue was taken up with ethyl acetate and washed once with aqueous NH<sub>4</sub>Cl, three times with H<sub>2</sub>O, and once with brine. The organic layer was then dried over MgSO<sub>4</sub> and the solvent was again evaporated *in vacuo*. The product oil was further purified by flash chromatography using a mobile phase of Hexane/EtOAc = 1/1 (R<sub>f</sub> = 0.36). The product was then dissolved in hot ethyl acetate, hexane was added slowly and the mixture allowed to cool, affording crystalline *racem*- $\beta$ -fluoro-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (F-FPAV, 1.6 g, 90% yield).

**racem-β-fluoro-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (F-FPAV):**

<sup>1</sup>H NMR (700 MHz, acetone-*d*<sub>6</sub>) δ 9.99 (s, 1H, H<sub>α'</sub>); 7.91 (dd, 1H, *J*<sub>6-5</sub> = 8.5 Hz, *J*<sub>6-2</sub> = 1.8 Hz, H<sub>6</sub>); 7.67 (d, 1H, *J*<sub>2-6</sub> = 1.8 Hz, H<sub>2</sub>); 7.63 (dd, 1H, *J*<sub>6'-5'</sub> = 8.2 Hz, *J*<sub>6'-2'</sub> = 1.8 Hz, H<sub>6'</sub>); 7.61 (d, 1H, *J*<sub>2'-6'</sub> = 1.8 Hz, H<sub>2'</sub>); 7.55 (d, 1H, *J*<sub>5-6</sub> = 8.2 Hz, H<sub>5</sub>); 7.15 (d, 1H, *J*<sub>5'-6'</sub> = 8.5 Hz, H<sub>5'</sub>); 6.88 (d, 1H, *J*<sub>β-F</sub> = 59.6 Hz, H<sub>β</sub>); 3.96 (s, 3H, 3'-OMe); 3.94 (s, 3H, 4-OMe); 3.90 (s, 3H, 3-OMe).

<sup>13</sup>C NMR (176 MHz, acetone-*d*<sub>6</sub>) δ 191.6 (C<sub>α'</sub>); 187.4 (d, *J*<sub>α-βF</sub> = 25.7 Hz, C<sub>α</sub>); 155.8 (C<sub>4</sub>); 151.8 (C<sub>3'</sub>); 150.3 (C<sub>3</sub>); 150.1 (C<sub>4'</sub>); 134.8 (C<sub>1'</sub>); 126.4 (C<sub>1</sub>); 125.7 (C<sub>6</sub>); 125.2 (C<sub>6'</sub>); 119.5 (C<sub>5'</sub>); 112.3 (C<sub>2'</sub>); 112.2 (C<sub>2</sub>); 111.6 (C<sub>5</sub>); 106.1 (d, *J*<sub>β-βF</sub> = 232.9 Hz, C<sub>β</sub>); 56.5 (3'-OMe); 56.3 (4-OMe); 56.1 (3-OMe).

<sup>19</sup>F NMR (376.5 MHz, acetone-*d*<sub>6</sub>) δ -132.3 (d, *J*<sub>βF-βH</sub> = 59.6 Hz, F<sub>β</sub>).



**Synthesis of racem-β-fluoro-(1'-formyl-3'-methoxyphenoxy)-γ-hydroxypropioveratrone (F-FPHPV) racemate, Supplementary Figure 8 (iii–iv).** To a magnetically stirred solution of 1,4-dioxane (40 mL), F-FPAV (0.8 g, 2.3 mmol), and formaldehyde (136 mg, 4.5 mmol, 340 μL of 37% formaldehyde in H<sub>2</sub>O) in a 100-mL round-bottom flask, anhydrous K<sub>2</sub>CO<sub>3</sub> (0.4 g, 22.7 mmol) was added and the reaction was set to 60 °C. After 18 h, the reaction was cooled to room temperature, carbonates were removed by filtration, and 1,4-dioxane was evaporated *in vacuo*. The product was dissolved in ethyl acetate and washed three times with H<sub>2</sub>O and once with brine. The organic layer was dried over MgSO<sub>4</sub> and the solvent evaporated *in vacuo*. The residue was further purified by flash chromatography using a mobile phase of Hexane/EtOAc = 1/3 (R<sub>f</sub> = 0.19). Racemic β-fluoro-(1'-formyl-3'-methoxyphenoxy)-γ-hydroxypropioveratrone (F-FPHPV) was recovered from chromatographic fractions as an oil (0.2 g, 23% yield).

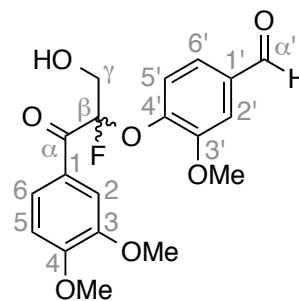
The chiral enantiomers of racemate F-FPHPV (*i.e.*, β(*S*)-F-FPHPV and β(*R*)-F-FPHPV) were purified via chiral chromatography where racemic F-FPHPV (5 mg, 13.2 mmol) was dissolved in ethanol (5 mL) and injected into a Beckman 125NM solvent module HPLC system equipped with a Diacel Chemical Industries CHIRALPAK AY-H column (10 by 250 mm) and Beckman 168 UV detector. A mixture of ethanol and hexane was used as the mobile phase at a flow rate of 2.0 mL min.<sup>-1</sup> The ethanol fraction of the total flow (with hexane as the remainder) was adjusted over a gradient as follows: 0–10 min, 10% ethanol; 10–60 min, gradient from 10–40% ethanol; 60–71 min, 40% ethanol; 71–72 min, gradient from 40–10% ethanol; 72–80 min, 10% ethanol. β(*S*)-F-FPHPV and β(*R*)-F-FPHPV eluted from the column after retention times (*t*<sub>R</sub>) of 46.1 min (R<sub>f</sub> = 0.42) and 53.9 min (R<sub>f</sub> = 0.36), respectively, and their fractions were collected, pooled, and solvents were dried *in vacuo*.

**racem-β-fluoro-(1'-formyl-3'-methoxyphenoxy)-γ-hydroxypropioveratrone (F-FPHPV):**

<sup>1</sup>H NMR (700 MHz, acetone-*d*<sub>6</sub>) δ 9.85 (s, 1H, H<sub>α'</sub>); 7.93 (dd, 1H, *J*<sub>6-5</sub> = 8.6 Hz, *J*<sub>6-2</sub> = 2.0 Hz, H<sub>6</sub>); 7.64 (d, 1H, *J*<sub>2-6</sub> = 2.0 Hz, H<sub>2</sub>); 7.48 (d, 1H, *J*<sub>2'-6'</sub> = 1.8 Hz, H<sub>2'</sub>); 7.41 (dd, 1H, *J*<sub>6'-5'</sub> = 8.3 Hz, *J*<sub>6'-2'</sub> = 1.8 Hz, H<sub>6'</sub>); 7.28 (d, 1H, *J*<sub>5-6</sub> = 8.3 Hz, H<sub>5</sub>); 7.04 (d, 1H, *J*<sub>5'-6'</sub> = 8.6 Hz, H<sub>5'</sub>); 4.81 (dd, 1H, *J*<sub>γOH-γa</sub> = 7.6 Hz, *J*<sub>γOH-γb</sub> = 6.0 Hz, γ-OH); 4.27 (ddd, 1H, *J*<sub>γa-βF</sub> = 23.2 Hz, *J*<sub>γa-γb</sub> = 12.4 Hz, *J*<sub>γa-γOH</sub> = 7.6 Hz, H<sub>γa</sub>); 4.19 (ddd, 1H, *J*<sub>γb-βF</sub> = 12.5 Hz, *J*<sub>γb-γa</sub> = 12.4 Hz, *J*<sub>γb-γOH</sub> = 6.1 Hz, H<sub>γb</sub>); 3.97 (s, 3H, 3'-OMe); 3.89 (s, 3H, 4-OMe); 3.85 (s, 3H, 3-OMe).

<sup>13</sup>C NMR (176 MHz, acetone-*d*<sub>6</sub>) δ 191.4 (C<sub>α'</sub>); 190.3 (d, *J*<sub>α-βF</sub> = 27.6 Hz, C<sub>α</sub>); 155.3 (C<sub>4</sub>); 151.6 (C<sub>3'</sub>); 149.8 (C<sub>3</sub>); 148.7 (C<sub>4'</sub>); 134.0 (C<sub>1'</sub>); 127.2 (C<sub>1</sub>); 125.6 (C<sub>6</sub>); 125.1 (C<sub>6'</sub>); 119.3 (C<sub>5'</sub>); 114.3 (d, *J*<sub>β-βF</sub> = 241.8 Hz, C<sub>β</sub>); 112.8 (C<sub>2</sub>); 111.8 (C<sub>2'</sub>); 111.4 (C<sub>5</sub>); 66.2 (d, *J*<sub>γ-βF</sub> = 28.5 Hz C<sub>γ</sub>); 56.4 (3'-OMe); 56.2 (4-OMe); 56.0 (3-OMe).

<sup>19</sup>F NMR (376.5 MHz, acetone-*d*<sub>6</sub>) δ -117.7 (dd, *J*<sub>βF-γHa</sub> = 23.2 Hz, *J*<sub>βF-γHb</sub> = 12.5 Hz, F<sub>β</sub>).

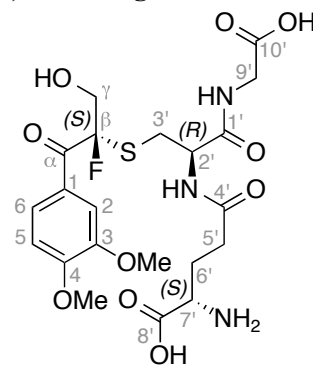


**Enzymatic synthesis of  $\beta(S)$ -fluoro-glutathionyl- $\gamma$ -hydroxy- $\alpha$ -veratrylpropanone ( $\beta(S)$ -F-GS-HVP), Supplementary Figure 8(v).** *In vitro* enzymatic synthesis with  $\beta$ -etherase LigE ( $360 \mu\text{g mL}^{-1}$ ) as a catalyst was conducted in 4 mL of an aqueous assay buffer [1.5 mM  $\beta(S)$ -F-FPHPV, 25 mM Tris, 2.5% DMSO, 5 mM glutathione (GSH), pH 8.0]. Reagents were added as follows: 2.3 mg  $\beta(S)$ -F-FPHPV, 100  $\mu\text{L}$  DMSO, 3500  $\mu\text{L}$  of 25.6 mM Tris (pH 9.0), 200  $\mu\text{L}$  of 100 mM GSH in 25.6 mM Tris, 200  $\mu\text{L}$  of a  $7.2 \mu\text{g mL}^{-1}$  stock of LigE. Prior to addition of LigE, the pH of the assay mixture was measured at pH 8.0 using pH paper. After 18 h, the 4 mL reaction was filtered through a 10,000 MWCO filter for the removal of protein and six ethyl acetate extractions were conducted for the removal of vanillin and residual  $\beta(S)$ -F-FPHPV. The aqueous fraction was then loaded onto a pre-packed Biotage KP-C<sub>18</sub> (100 g) reversed phase column using a Beckman 125NM solvent delivery module equipped with a Beckman 168 UV detector. A mixture of water and methanol was used for the mobile phase at a flow rate of 10 mL/min. The proportions of the total flow made up by each buffer were adjusted over a gradient: 0–15 min, 0% methanol; 15–20 min, gradient from 0-100% methanol; 20–35 min, 100% methanol; 35–40 min, gradient from 100-0% methanol; 40–50 min, 0% methanol. After elution of the perceived GSH-conjugated reaction product ( $t_R = 31.0$  min), fractions with UV absorption at 280 nm were collected, pooled, dried over a gentle stream of nitrogen gas overnight, and then analyzed by NMR. The <sup>1</sup>H, COSY, HSQC, and HMBC NMR spectra of the isolated product from the LigE-catalyzed reaction were consistent with the identity of  $\beta(S)$ -fluoro-glutathionyl- $\gamma$ -hydroxy- $\alpha$ -veratrylpropanone ( $\beta(S)$ -F-GS-HVP, Figure S8A). Because  $\beta(S)$ -F-GS-HVP was recovered from the reaction in low quantity, a <sup>13</sup>C NMR spectrum could not be obtained directly. Rather, the <sup>13</sup>C NMR spectral data reported was determined from the <sup>1</sup>H, COSY, HSQC, and HMBC NMR spectra.

**$\beta(S)$ -fluoro-glutathionyl- $\gamma$ -hydroxy- $\alpha$ -veratrylpropanone ( $\beta(S)$ -F-GS-HVP), from LigE:**

<sup>1</sup>H NMR (700 MHz, D<sub>2</sub>O)  $\delta$  7.89 (dd, 1H,  $J_{6-5} = 8.6$  Hz,  $J_{6-2} = 2.0$  Hz, H6); 7.59 (d, 1H,  $J_{2-6} = 2.0$  Hz, H2); 7.01 (d, 1H,  $J_{5-6} = 8.6$  Hz, H5); 4.47 (dd, 1H,  $J_{2'-3b'} = 8.1$  Hz,  $J_{2'-3a'} = 4.9$  Hz, H2'); 4.21 (dd, 1H,  $J_{\gamma a-\beta F} = 27.3$  Hz,  $J_{\gamma a-\gamma b} = 13.2$  Hz, H $\gamma_a$ ); 4.07 (dd, 1H,  $J_{\gamma b-\beta F} = 15.8$  Hz,  $J_{\gamma b-\gamma a} = 13.2$  Hz, H $\gamma_b$ ); 3.84 (s, 3H, 4-OMe); 3.80 (s, 3H, 3-OMe); 3.62 (d, 1H,  $J_{9a'-9b'} = 17.2$  Hz, H9'a); 3.60 (t, 1H,  $J_{7'-6'} = 6.3$  Hz, H7'); 3.48 (d, 1H,  $J_{9b'-9a'} = 17.2$  Hz, H9'b); 3.05 (dd, 1H,  $J_{3a'-3b'} = 14.1$  Hz,  $J_{3a'-2'} = 4.9$  Hz, H3'a); 2.90 (dd, 1H,  $J_{3b'-3a'} = 14.1$  Hz,  $J_{3b'-2'} = 8.1$  Hz, H3'b); 2.34 – 2.28 (m, 2H, H5'a/b); 2.00 – 1.94 (m, 2H, H6'a/b).

<sup>13</sup>C NMR (176 MHz, D<sub>2</sub>O)  $\delta$  194.1 (d,  $J_{\gamma-\beta F} = \sim 25$  Hz, C $\alpha$ ); 176.1 (C10'); 175.2 (C4'); 174.6 (C8'); 171.5 (C1'); 154.0 (C4); 148.3 (C3); 127.0 (C1); 126.0 (C6); 112.6 (C2); 111.0 (C5); 107.7 (d,  $J_{\beta-\beta F} = \sim 240$  Hz, C $\beta$ ); 65.7 (d,  $J_{\gamma-\beta F} = \sim 25$  Hz, C $\gamma$ ); 56.0 (4-OMe); 55.9 (3-OMe); 54.1 (C7'); 53.0 (C2'); 43.2 (C9'); 31.4 (C5'); 30.2 (C3'); 26.1 (C6').

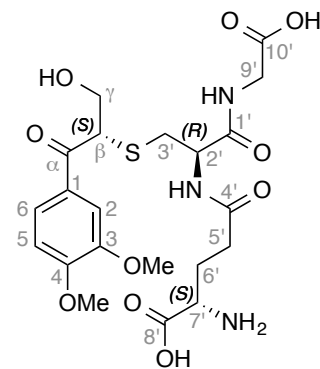


**Enzymatic synthesis of  $\beta(S)$ -glutathionyl- $\gamma$ -hydroxy- $\alpha$ -veratrylpropanone ( $\beta(S)$ -GS-HVP).** With  $\beta(R)$ -F-FPHPV used as the reaction substrate, rather than  $\beta(S)$ -F-FPHPV, the same procedure was used to isolate the product  $\beta(S)$ -GS-HVP from a LigE-catalyzed reaction, as previously described (3).

**$\beta$ (S)-glutathionyl- $\gamma$ -hydroxy- $\alpha$ -veratrylpropanone ( $\beta$ (S)-GS-HVP), from LigE:**

$^1\text{H}$  NMR (700 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.68 (dd, 1H,  $J_{6-5} = 8.4$  Hz,  $J_{6-2} = 2.0$  Hz, H6); 7.48 (d, 1H,  $J_{2-6} = 2.0$  Hz, H2); 7.02 (d, 1H,  $J_{5-6} = 8.4$  Hz, H5); 4.54 (dd, 1H,  $J_{\beta-\gamma_a} = 7.9$ ,  $J_{\beta-\gamma_b} = 5.9$  Hz, H $\beta$ ); 4.27 (dd, 1H,  $J_{2'-3b'} = 8.8$  Hz,  $J_{2'-3a'} = 5.1$  Hz, H2'); 3.98 (dd, 1H,  $J_{\gamma_a-\gamma_b} = 11.6$  Hz,  $J_{\gamma_a-\beta} = 7.9$  Hz, H $\gamma_a$ ); 3.84 (s, 3H, 4-OMe); 3.83 - 3.78 (m, 1H, H $\gamma_b$ ); 3.80 (s, 3H, 3-OMe); 3.57 (d, 1H,  $J_{9a'-9b'} = 17.3$  Hz, H9'a); 3.50 (d, 1H,  $J_{9b'-9a'} = 17.3$  Hz, H9'b); 3.49 (t, 1H,  $J_{7'-6'} = 6.4$  Hz, H7'); 2.94 (dd, 1H,  $J_{3a'-3b'} = 14.2$  Hz,  $J_{3a'-2'} = 5.1$  Hz, H3'a); 2.77 (dd, 1H,  $J_{3b'-3a'} = 14.2$  Hz,  $J_{3b'-2'} = 8.8$  Hz, H3'b); 2.26 - 2.16 (m, 2H, H5'a/b); 1.90 - 1.80 (m, 2H, H6'a/b).

$^{13}\text{C}$  NMR (176 MHz,  $\text{D}_2\text{O}$ )  $\delta$  198.4 (C $\alpha$ ); 176.8 (C10'); 175.5 (C4'); 175.4 (C8'); 172.1 (C1'); 154.4 (C4); 149.0 (C3); 128.9 (C1); 125.3 (C6); 111.8 (C2); 111.8 (C5); 61.6 (C $\gamma$ ); 56.7 (4-OMe); 56.5 (3-OMe); 54.9 (C7'); 53.8 (C2'); 48.6 (C $\beta$ ); 44.0 (C9'); 32.2 (C5'); 32.1 (C3'); 27.3 (C6').

**SUPPLEMENTARY REFERENCES**

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## SUPPLEMENTARY INFORMATION - <sup>1</sup>H AND <sup>13</sup>C NMR SPECTRA OF MODEL COMPOUNDS AND REACTION PRODUCTS

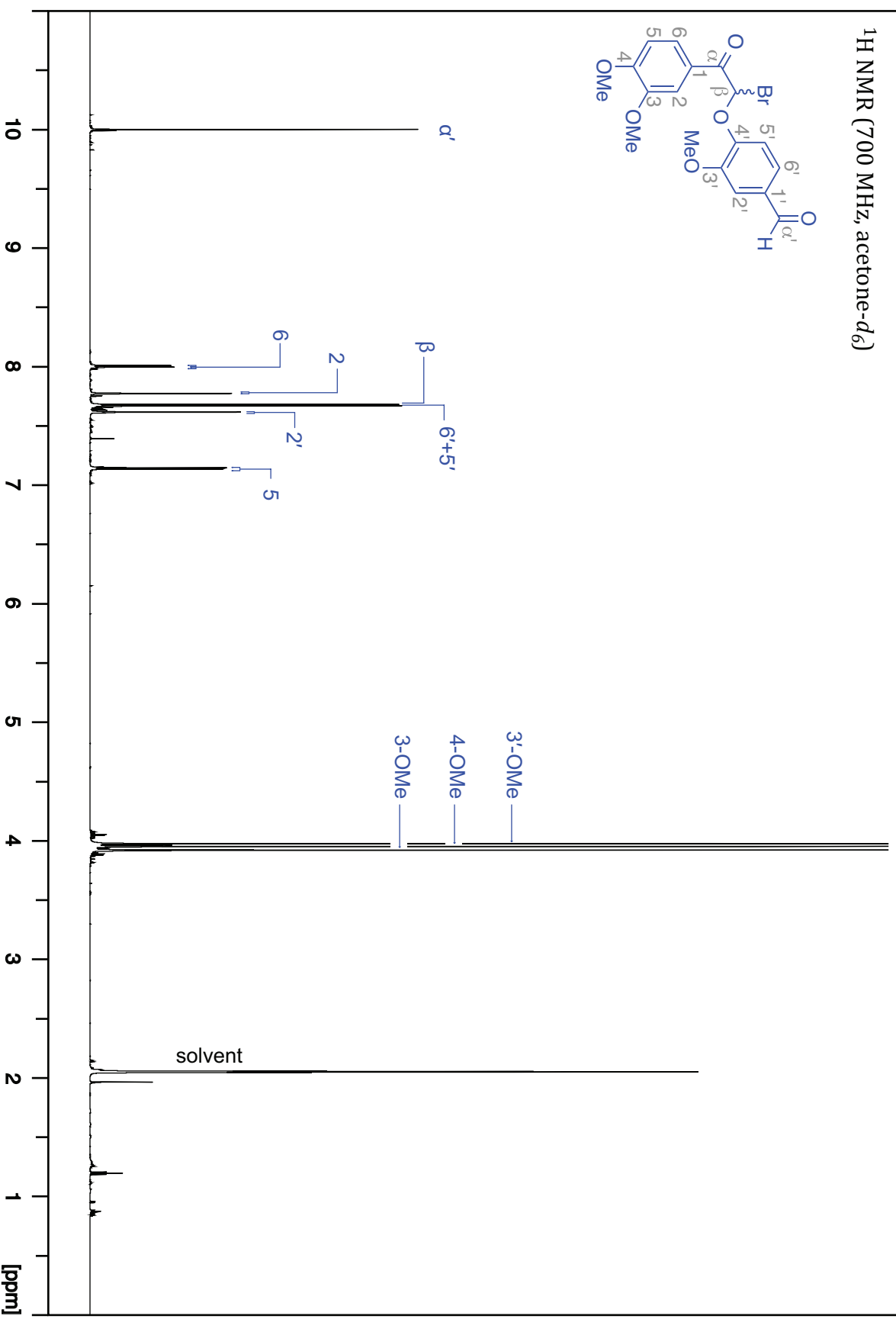
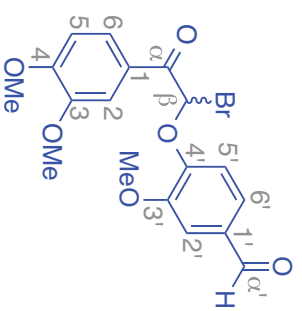
COMPOUND:	Source: <sup>a</sup>	Spectrum:	Solvent: <sup>b</sup>	Page:
<i>racem</i> -β-bromo-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (Br-FPAV)	synthetic	<sup>1</sup> H	acetone- <i>d</i> <sub>6</sub>	S-18
		<sup>13</sup> C	acetone- <i>d</i> <sub>6</sub>	S-19
<i>racem</i> -β-fluoro-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (F-FPAV)	synthetic	<sup>1</sup> H	acetone- <i>d</i> <sub>6</sub>	S-20
		<sup>13</sup> C	acetone- <i>d</i> <sub>6</sub>	S-21
		<sup>19</sup> F	acetone- <i>d</i> <sub>6</sub>	S-22
<i>racem</i> -β-fluoro-(1'-formyl-3'-methoxyphenoxy)-γ-hydroxypropio- veratrone (F-FPHPV)	synthetic	<sup>1</sup> H	acetone- <i>d</i> <sub>6</sub>	S-23
		<sup>13</sup> C	acetone- <i>d</i> <sub>6</sub>	S-24
		<sup>19</sup> F	acetone- <i>d</i> <sub>6</sub>	S-25
β( <i>S</i> )-fluoro-glutathionyl-γ-hydroxy-α-veratrylpropanone (β( <i>S</i> )-F-GS-HVP)	LigE	<sup>1</sup> H	D <sub>2</sub> O	S-26
β( <i>S</i> )-glutathionyl-γ-hydroxy-α-veratrylpropanone (β( <i>S</i> )-GS-HVP)	LigE	<sup>1</sup> H	D <sub>2</sub> O	S-27
		<sup>13</sup> C	D <sub>2</sub> O	S-28

<sup>a</sup> Compounds were obtained either via organic synthesis or from LigE β-etherase-catalyzed reaction products.

<sup>b</sup> <sup>1</sup>H, <sup>13</sup>C, and <sup>19</sup>F NMR spectra were analyzed in either acetone-*d*<sub>6</sub> or D<sub>2</sub>O as NMR solvents.

***racem*- $\beta$ -bromo-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (Br-FPAV)**

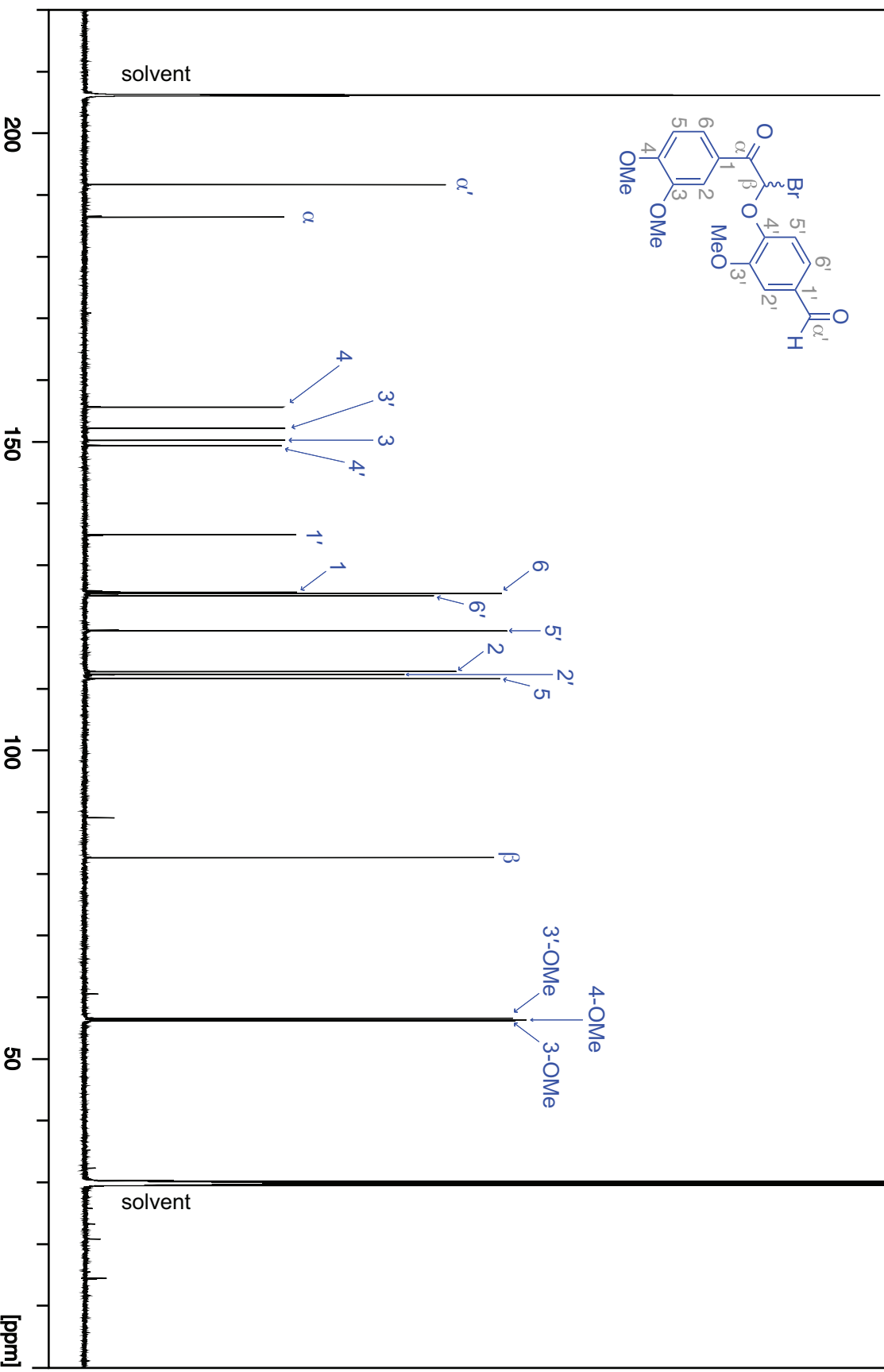
$^1\text{H}$  NMR (700 MHz, acetone- $d_6$ )





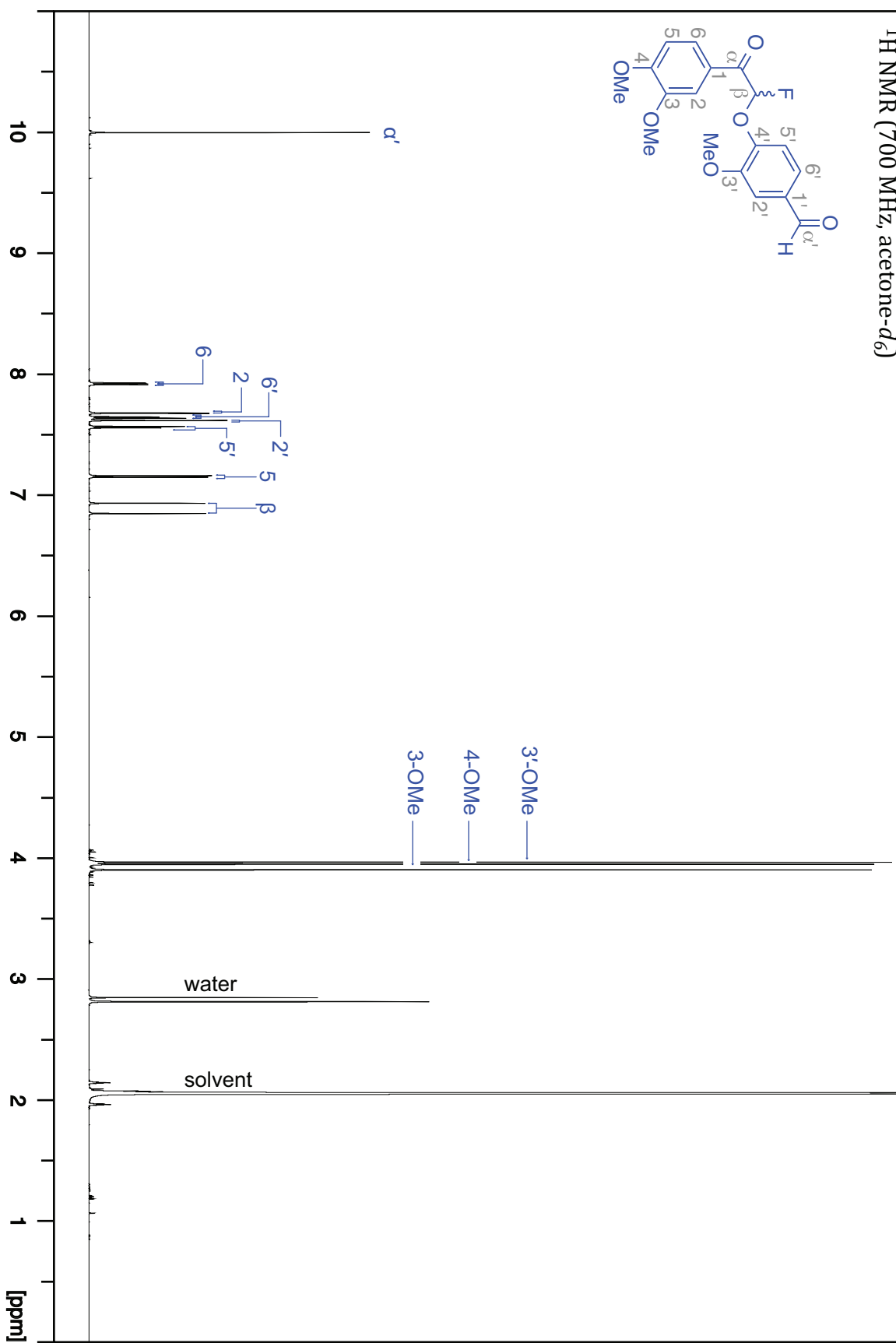
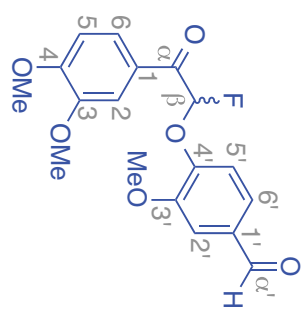
***racem*- $\beta$ -bromo-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (Br-FPAV)**

$^{13}\text{C}$  NMR (176 MHz, acetone- $d_6$ )



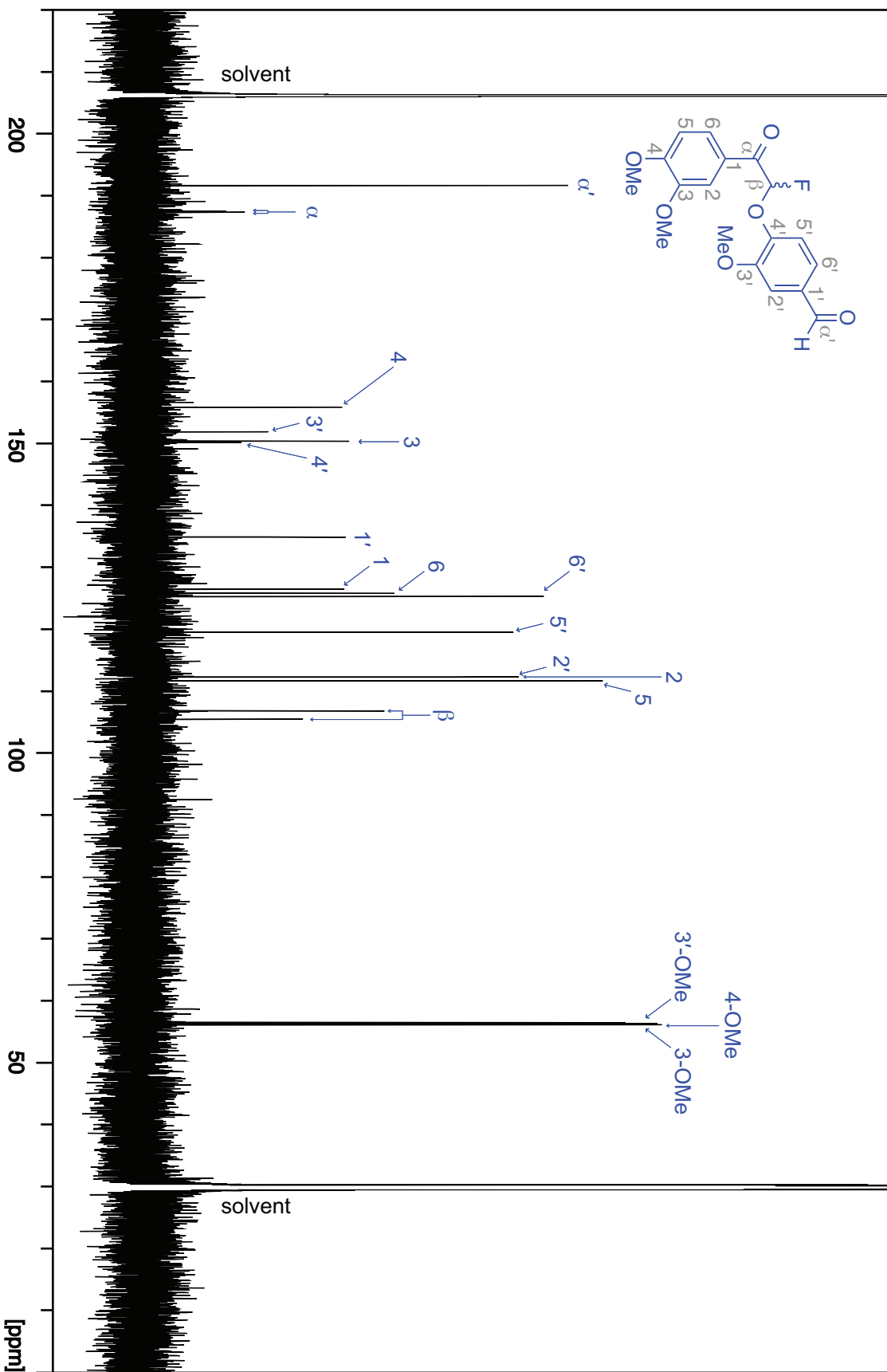
**racem- $\beta$ -fluoro-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (F-FPAV)**

$^1\text{H}$  NMR (700 MHz, acetone- $d_6$ )



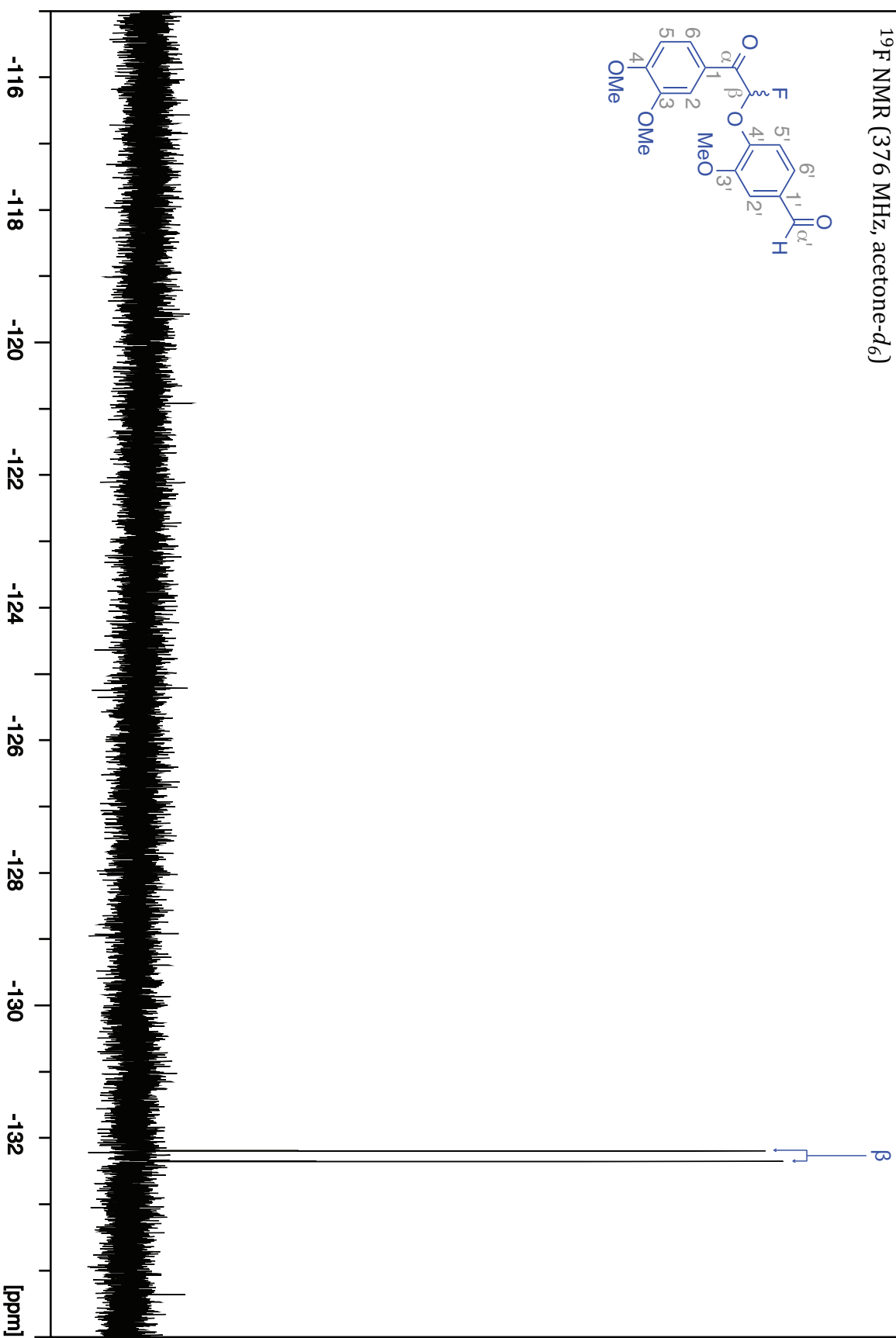
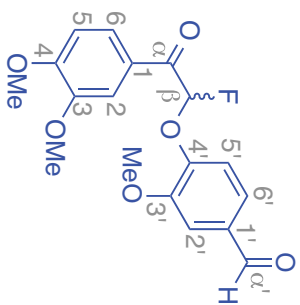
***racem*- $\beta$ -fluoro-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (F-FPAV)**

$^{13}\text{C}$  NMR (176 MHz, acetone- $d_6$ )



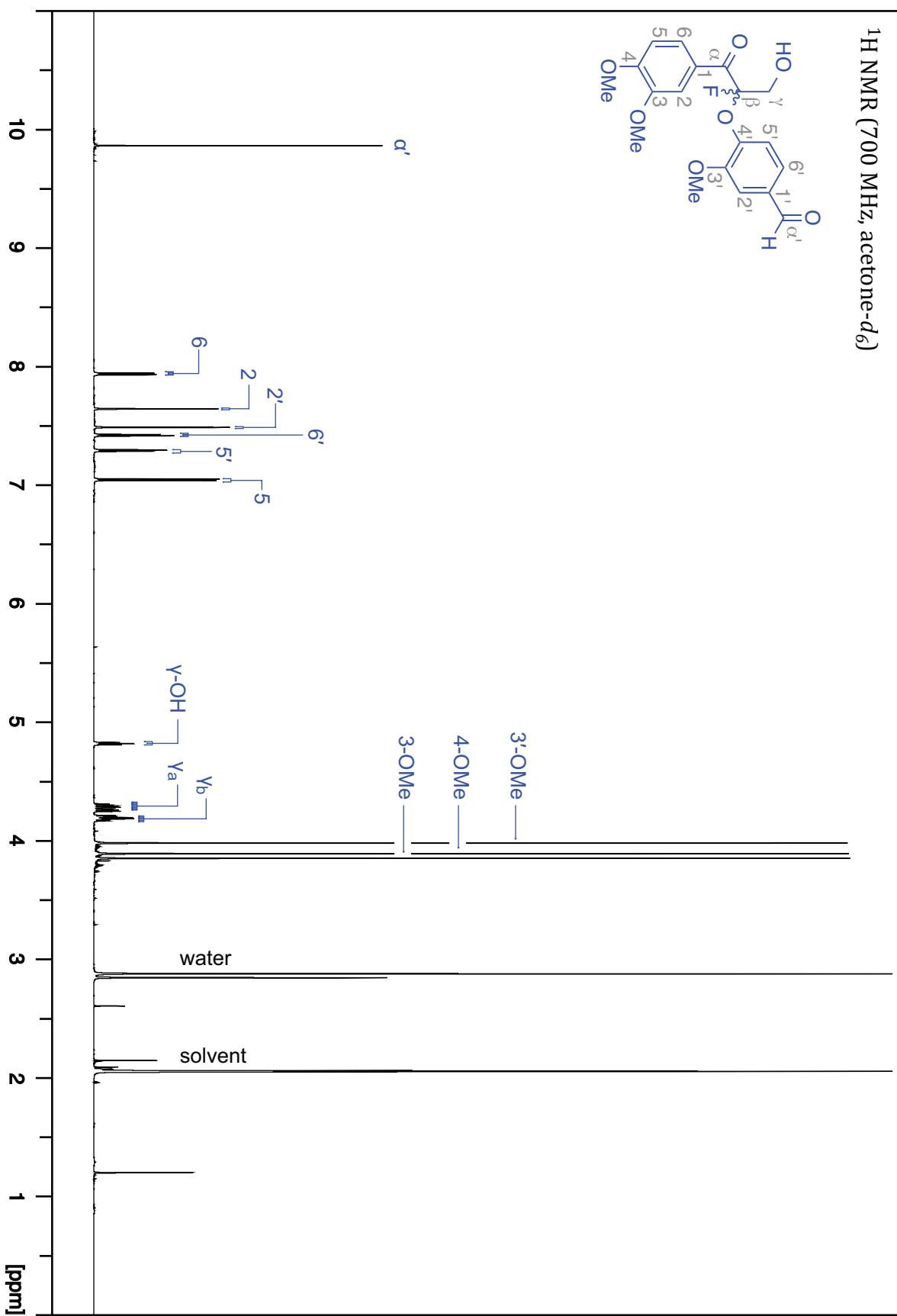
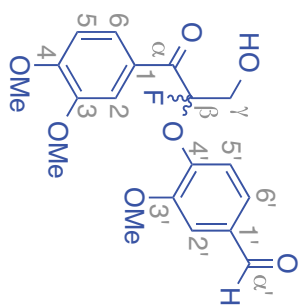
***racem*- $\beta$ -fluoro-(1'-formyl-3'-methoxyphenoxy)-acetoveratrone (F-FPAV)**

$^{19}\text{F}$  NMR (376 MHz, acetone- $d_6$ )



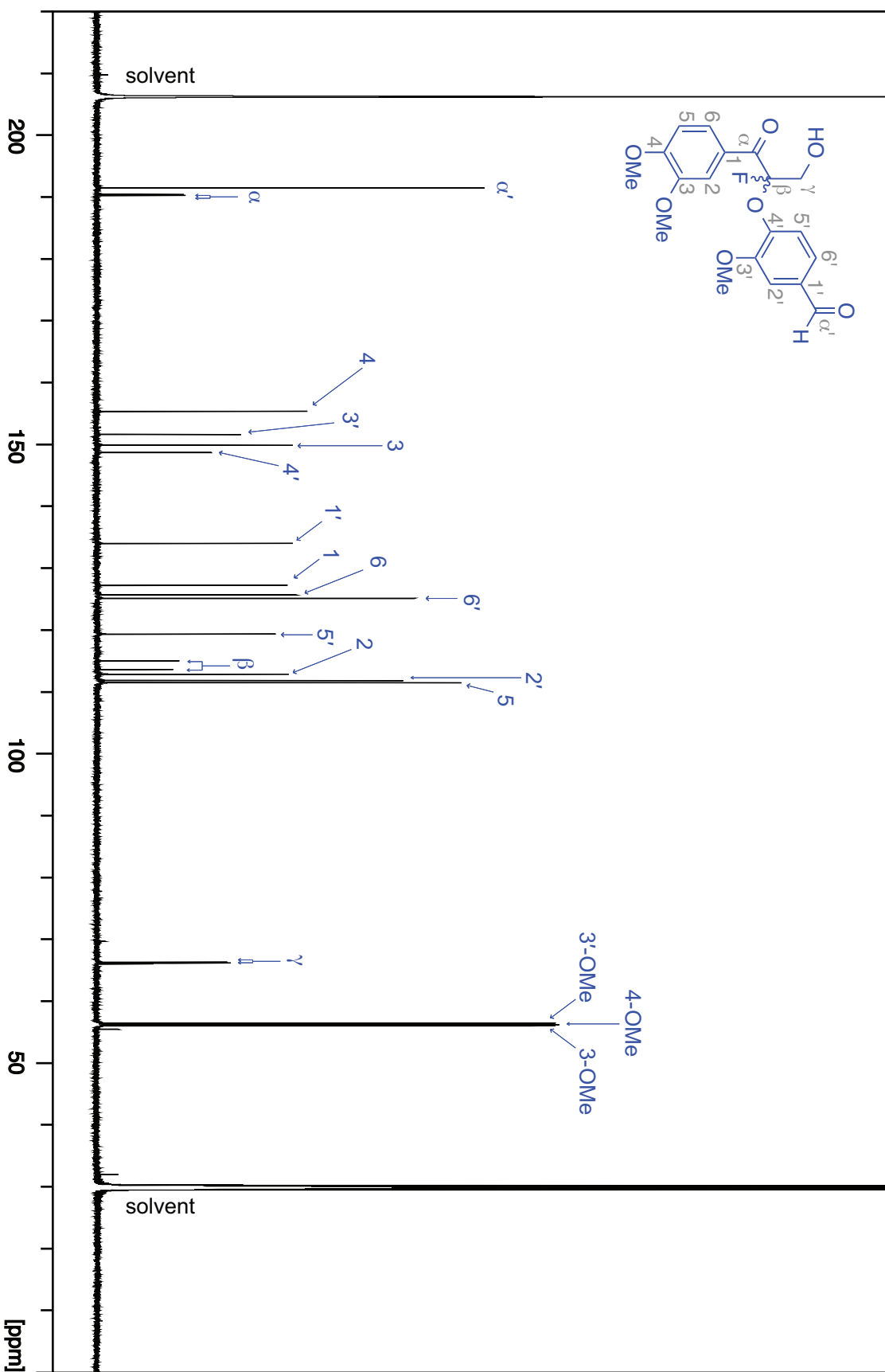
**racem-β-fluoro-(1'-formyl-3'-methoxyphenoxy)-γ-hydroxypropioveratrone (F-FRHPV)**

<sup>1</sup>H NMR (700 MHz, acetone-d<sub>6</sub>)



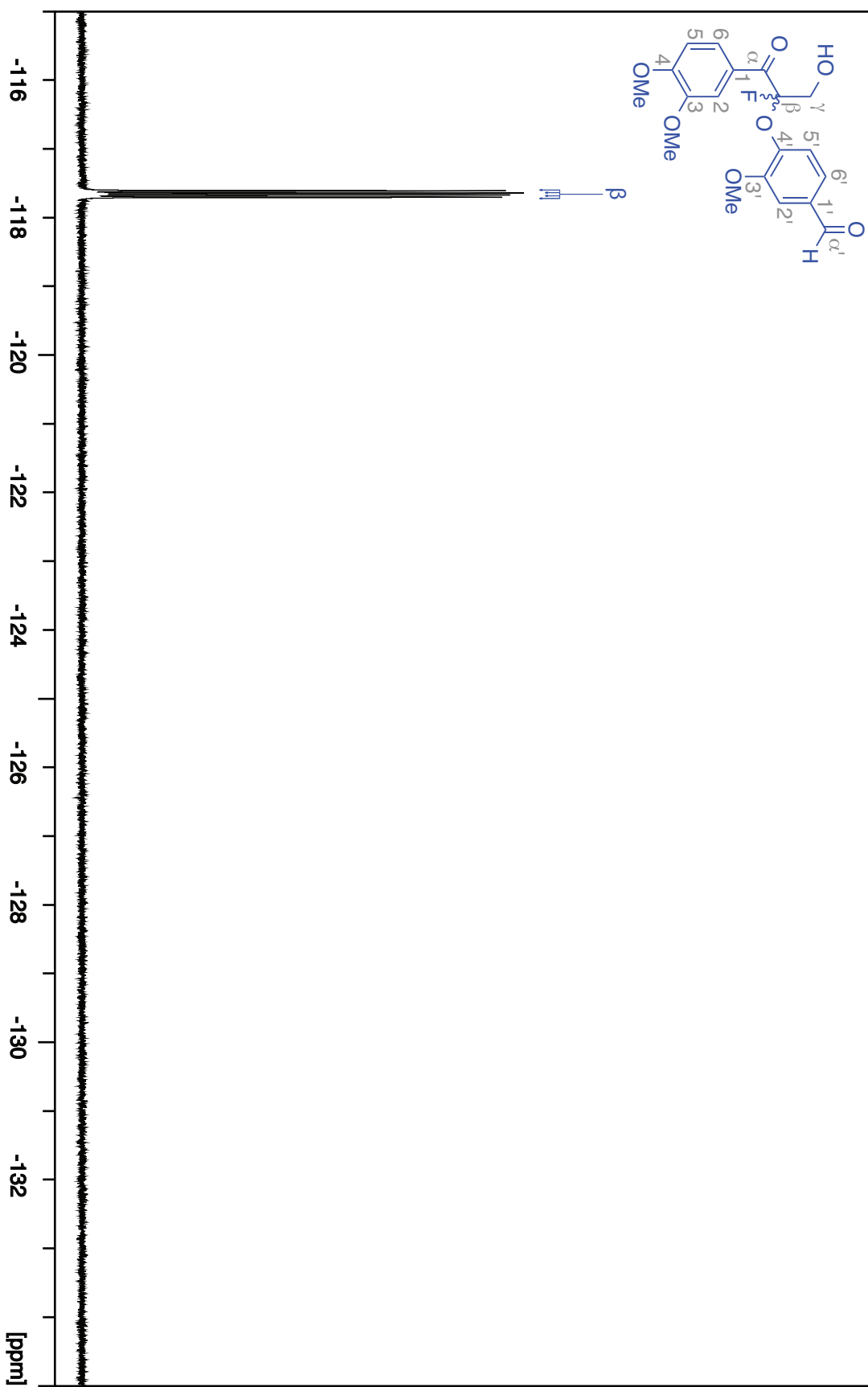
***racem*- $\beta$ -fluoro-(1'-formyl-3'-methoxyphenoxy)- $\gamma$ -hydroxypropioveratrone (F-FPHPV)**

$^{13}\text{C}$  NMR (176 MHz, acetone- $d_6$ )

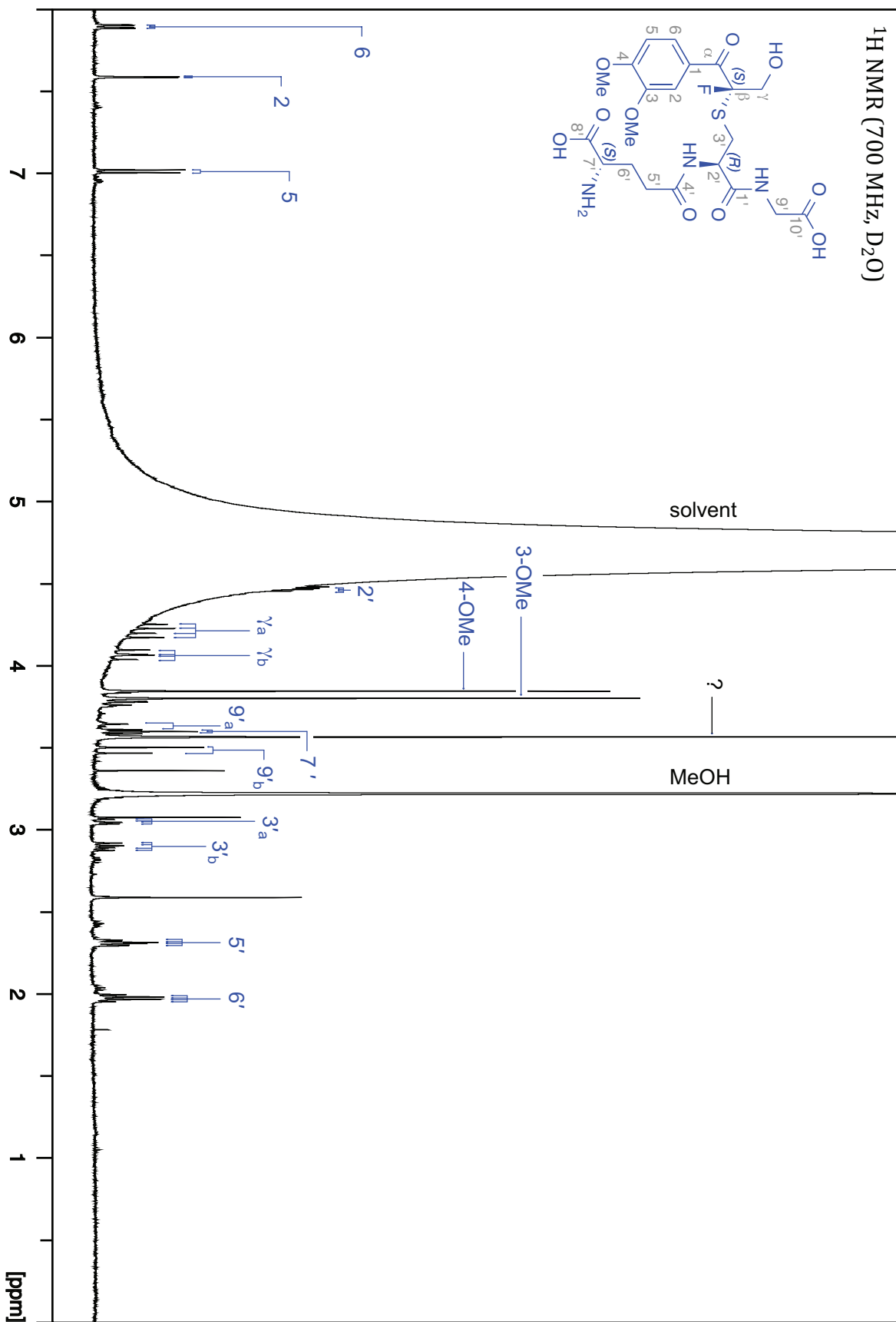
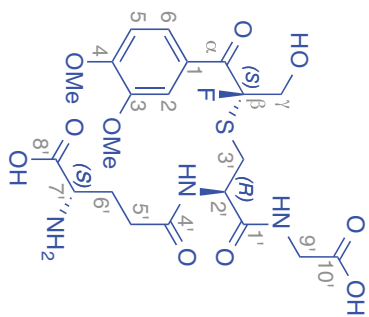


***racem*- $\beta$ -Fluoro-(1'-formyl-3'-methoxyphenoxy)- $\gamma$ -hydroxypropioveratone (F-FRHPV)**

$^{19}\text{F}$  NMR (376 MHz, acetone- $d_6$ )



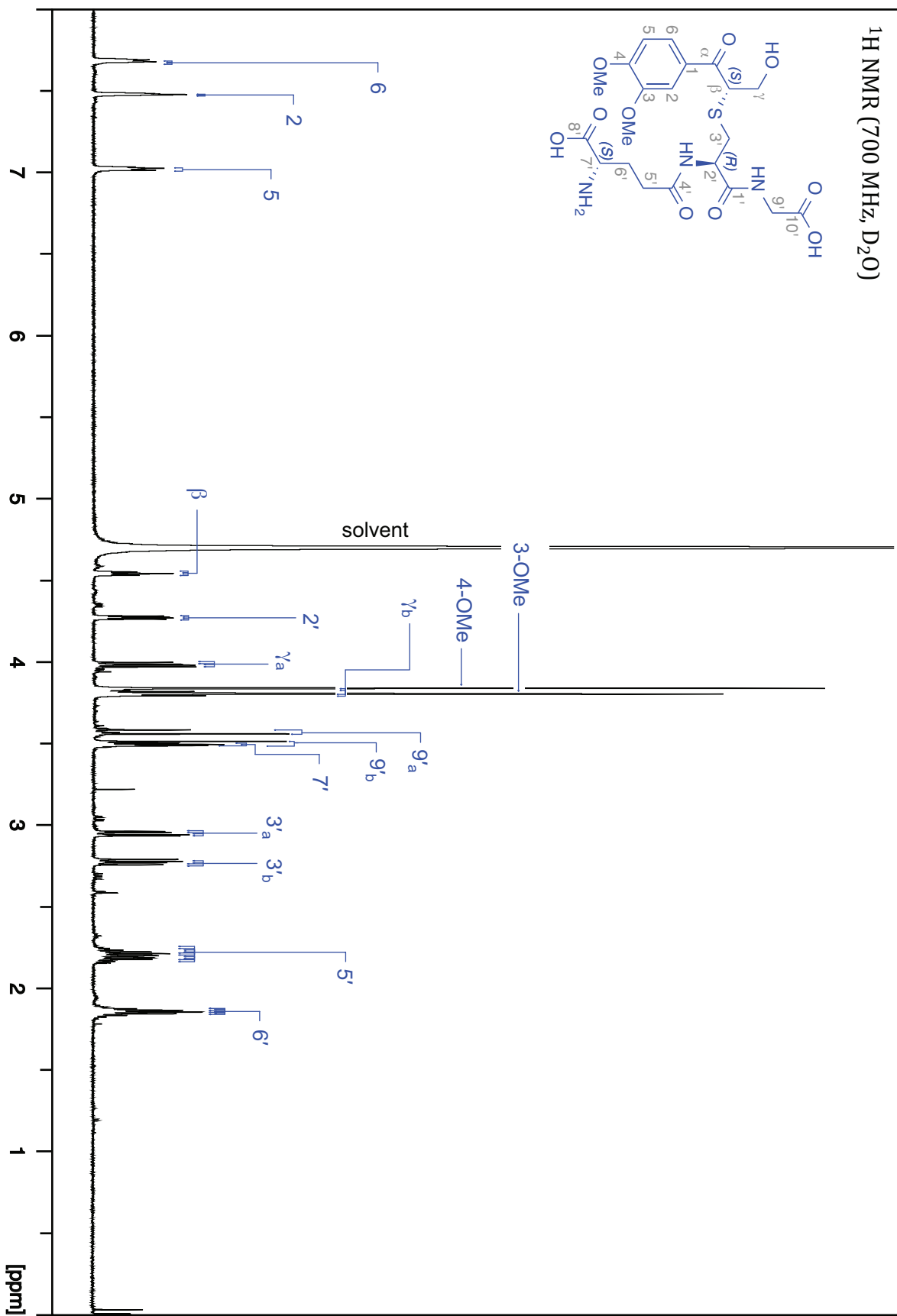
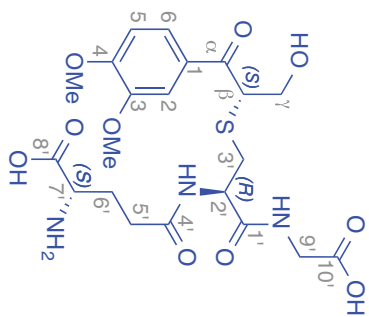
**$\beta$ (S)-fluoro-glutathionyl- $\gamma$ -hydroxy- $\alpha$ -veratrylpropanone ( $\beta$ (S)-F-GS-HVP), from Lige**  
 $^1\text{H}$  NMR (700 MHz,  $\text{D}_2\text{O}$ )





**$\beta$ (S)-glutathionyl- $\gamma$ -hydroxy- $\alpha$ -veratrylpropanone ( $\beta$ (S)-GS-HVP), from LigE**

$^1\text{H}$  NMR (700 MHz,  $\text{D}_2\text{O}$ )



**$\beta(S)$ -glutathionyl- $\gamma$ -hydroxy- $\alpha$ -veratrylpropanone ( $\beta(S)$ -GS-HVP), from Lige**

$^{13}\text{C}$  NMR (176 MHz,  $\text{D}_2\text{O}$ )

