Steric enforcement of cis-epoxide formation in the radical C–O-coupling reaction by which (*S*)-2-hydroxypropylphosphonate epoxidase (HppE) produces Fosfomycin

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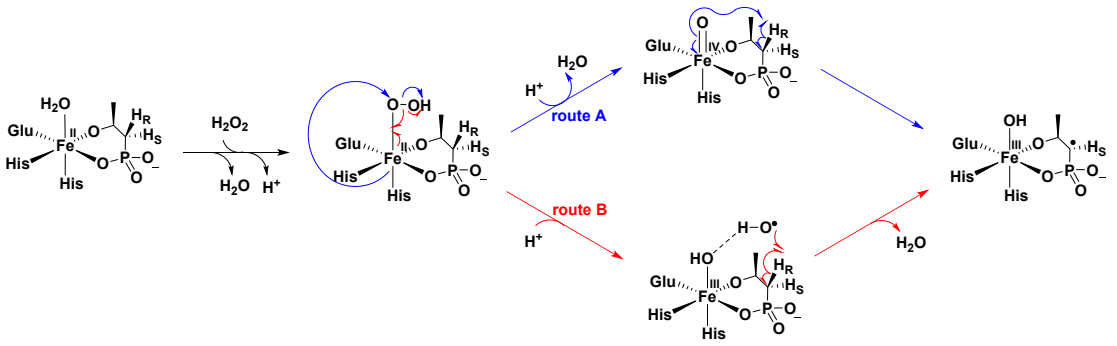
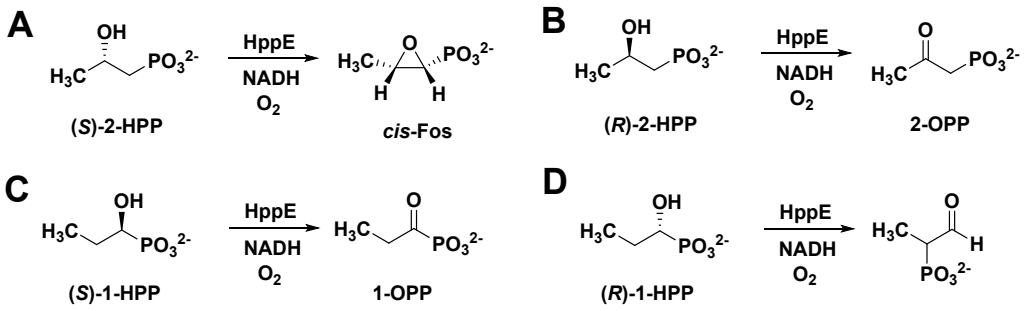
1. General Methods and Background

All chemicals and reagents were obtained from commercial suppliers (Sigma-Adrich, VWR, Alfa Aesar) and used without further purification. Silica gel chromatography was carried out using resin (230-400 mesh, grade 60) obtained from Sorbent Technologies (Norcross, GA). ^{1}H, ^{31}P, ^{19}F and ^{13}C NMR spectra were recorded on Bruker 360, 400 and 500 MHz spectrometers in the Department of Chemistry at The Pennsylvania State University (PSU). Data for ^{1}H NMR are reported as follows: chemical shifts (δ ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, dd = doublet of doublets, td = triplet of doublets, ddd = doublet of doublet of doublets), coupling constant (Hz), and integrated area (relative to that of solvent, $CDCl_3$ or D_2O, unless otherwise noted). High-resolution mass spectra were obtained at the PSU Mass Spectral Facility. Over-expression and preparation of recombinant HppE and variants were carried out as previously described.[1]

Scheme S1. Two possibilities for the identity, and the mechanism of formation, of the *proR* C1-H•-abstracting intermediate in the HppE reaction.

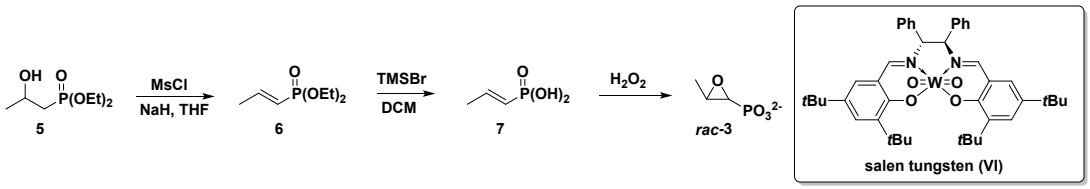


Scheme S2. Distinct oxidation reactions catalyzed by HppE upon its native substrate, (*S*)-2-HPP (***A***), and stereo- and structural isomers thereof (***B–D***).



2. Mixed stereochemistry in the C–O-coupling (cyclization) step of the HppE reaction.

2.1 Synthesis of *rac-trans*-Fos (3).



Diethyl (*E*)-prop-1-en-1-ylphosphonate (**6**). To a solution of **5** (1 g, 5.10 mmol) in THF (20 mL) was added MsCl (700 mg, 6.12 mmol) and NaH (2.4 g, 50.97 mmol). The reaction was stirred at room temperature (rt) for 5 h and quenched by addition of water (80 mL) followed by extraction with ethyl acetate (50 mL × 3). The combined organic layer was concentrated and purified by chromatography [dichloromethane (DCM)/methanol = 40/1] to afford **6** (0.8 g, 88%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 6.84 – 6.69 (m, 1H), 5.73 – 5.59 (m, 1H), 4.10 – 4.02 (m, 4H), 1.91 (dt, *J* = 6.6, 2.2 Hz, 3H), 1.31 (t, *J* = 7.0 Hz, 6H). ^{31}P NMR (202 MHz, $CDCl_3$) δ 18.43.

(*E*)-prop-1-en-1-ylphosphonate (7). Following the published procedure,[1] **7** was obtained as a white solid (0.5 g, 90%) from **6** (0.8 g). ^{1}H NMR (500 MHz, D_2O) δ 6.46 – 6.35 (m, 1H), 5.78 – 5.69 (m, 1H), 1.79 (dt, *J* = 6.5, 2.0 Hz, 3H). ^{13}C NMR (126 MHz, D_2O) δ 142.67 (d, *J* = 4.3 Hz), 123.56 (d, *J* = 177.6 Hz), 19.08 (d, *J* = 22.6 Hz). ^{31}P NMR (202 MHz, D_2O) δ 13.31.

***rac-trans*-(3-methyloxiran-2-yl)phosphonate (3).** *Trans*-1-propenylphosphonate (250 mg, 2 mmol) dissolved in CH_2Cl_2 (50 mL) was added to racemic α-phenylethylamine (250 μL, 2 mmol), as described previously.[2] The salen tungsten (VI) catalyst (0.1 mmol) was subsequently added to the mixture. Following addition of H_2O_2 (1.05 mL, 10 mmol, 30% aqueous), the reaction mixture was stirred at rt for 24 h. The organic layer was removed after the addition of water to dissolve the epoxide, and the aqueous layer was dried to give the product (200 mg, 71%). ^{1}H NMR (500 MHz, D_2O) δ 3.23 – 3.18 (m, 1H), 2.70 (dd, *J* = 24.6, 3.1 Hz, 1H), 1.32 (d, *J* = 5.3 Hz, 3H). ^{13}C NMR (126 MHz, D_2O) δ 54.80 (d, *J* = 182.9 Hz), 54.28, 16.94. ^{31}P NMR (202 MHz, 100 mM NaOD in D_2O) δ 10.49.

2.2 Re-examination of the HppE reaction stereochemistry.

(*S*)-2-HPP (**1**) was synthesized as previously described; the ^{1}H, and ^{13}C NMR spectra of **1** were consistent with those reported in the literature.[1, 3] To a 25 mL solution of 20 mM Tris-HCl buffer (pH 7.5) were added, in an anoxic chamber, Fe^{II} [from $Fe(NH_4)_2(SO_4)_2$] (0.8 mM final concentration), HppE (1 mM final concentration), (*S*)-2-HPP (10 mM final concentration), and sodium *L*-ascorbate (20 mM final concentration). H_2O_2 (10 mM final concentration) was then added dropwise to the solution at 4 ^{o}C, and the reaction was allowed to proceed for an additional 10 min. The pH of the solution was then adjusted to between 3 and 4 using 6 M HCl, and the sample was centrifuged to remove the precipitated protein. The supernatant was diluted with 200 mL of water, and this solution was loaded onto a DEAE anion exchange column (15 mL, Bio-Rad). The column was washed with water (200 mL), and the bound material was then eluted with 30 mL of 100 mM NH_4HCO_3. The eluant was then lyophilized to yield **2** and **3**. The conversion (~100%) and cis:trans product ratio (95/5) were obtained by ^{1}H NMR analysis (**Figure S1**). For both **2** and **3,** the spectroscopic data were found to match those reported in the literature.[4]

(1*R*,2*S*)-1,2-epoxypropylphosphonate (2). ^{1}H NMR (500 MHz, D_2O) δ 3.30 (m, 1H), 2.85 (dd, J = 19.4, 5.1 Hz, 1H), 1.48 (d, J = 5.6 Hz, 3H). ^{13}C NMR (75 MHz, D_2O) δ 56.43 (J = 175.7 Hz), 54.81, 13.89. ^{31}P NMR (202 MHz, 100 mM NaOD in D_2O) δ 9.95 (dd, J = 18.7, 5.3 Hz). The coupling of 5.1 Hz between the vicinal epoxide protons (δ 2.85 and 3.30 ppm) indicates a cis configuration.[5]

(1*S*,2*S*)-1,2-epoxypropylphosphonate (**3**). ^{1}H NMR (500 MHz, D_2O) δ 3.21 – 3.18 (m, 1H), 2.64 (dd, J = 22.2, 2.7 Hz, 1H), 1.36 (dd, J = 5.2, 1.5 Hz, 1H). ^{31}P NMR (202 MHz, 100 mM NaOD in D_2O) δ 10.45 (dd, J = 21.7, 5.1 Hz). The coupling of 2.7 Hz between the vicinal epoxide protons indicates a trans configuration.[4, 6-7]

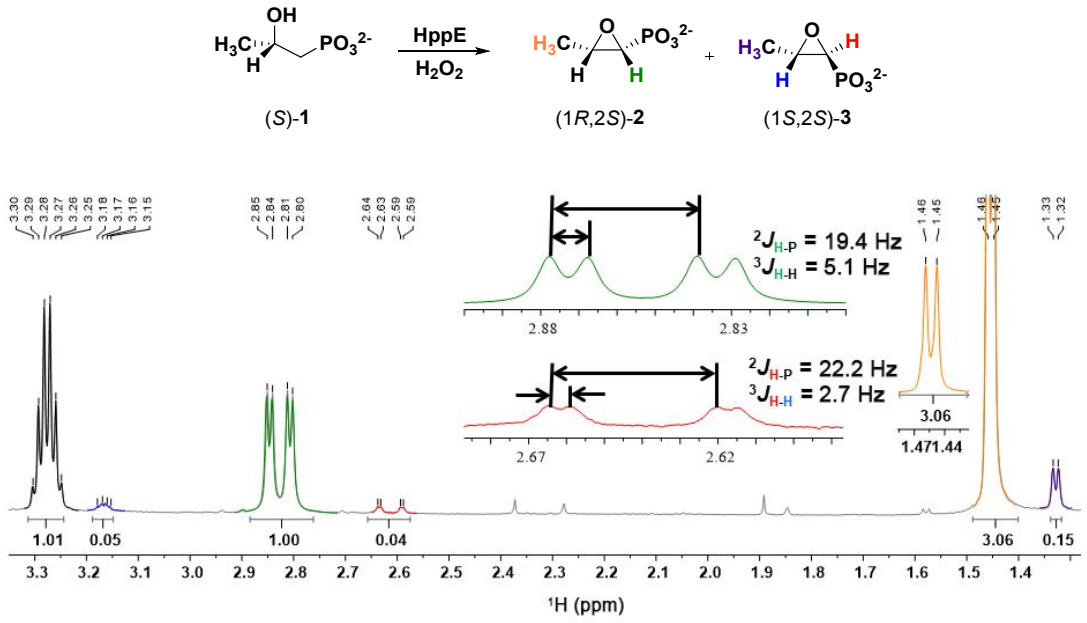


Figure S1. ^{1}H NMR spectrum of products isolated from the HppE reaction.

2.3 Synthesis of 1-*d*-2*S*-HPP diastereomers (1d and 1e). For details, see *Sec 6.1*.

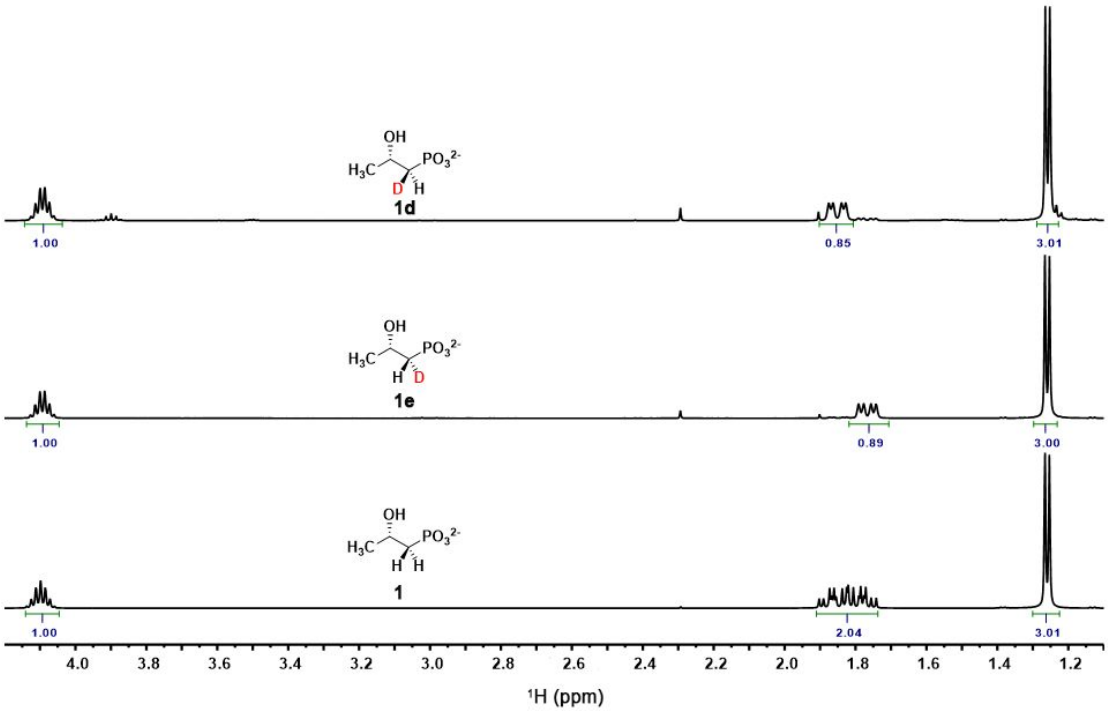


Figure S2. Comparison of the ^{1}H NMR spectra of the two 1-*d*-2*S*-HPP diastereomers (**1d** and **1e**) and unlabeled (*S*)-2-HPP (**1**).

2.4 Reaction of 1-*d*-2*S*-HPP diastereomers with HppE.

Reactions were carried out in 2 mL tubes in a total volume of 0.50 mL. They contained (final concentrations after addition of H_2O_2) 20 mM Tris-HCl buffer (pH 7.5), 0.16 mM Fe^{II} [from $Fe(NH_4)_2(SO_4)_2$], 0.2 mM HppE, 5 mM (*S*)-2-HPP or one of its isotopologs, and 4 mM sodium *L*-ascorbate. These components were mixed in an anoxic chamber, and H_2O_2 (1 equiv. relative to substrate) was added slowly at rt. Reactions were allowed to proceed for an additional 10 min (after the addition of H_2O_2 was complete) before being quenched by addition of 0.10 mL of quench solution **A** (600 mM NaOD, 3 mM sodium propylphosphonate in D_2O). The reaction mixtures were transferred to NMR tubes and subjected to ^{31}P NMR analysis. Each reaction was performed in triplicate. The cis:trans ratios were calculated by integration of the relevant peaks.

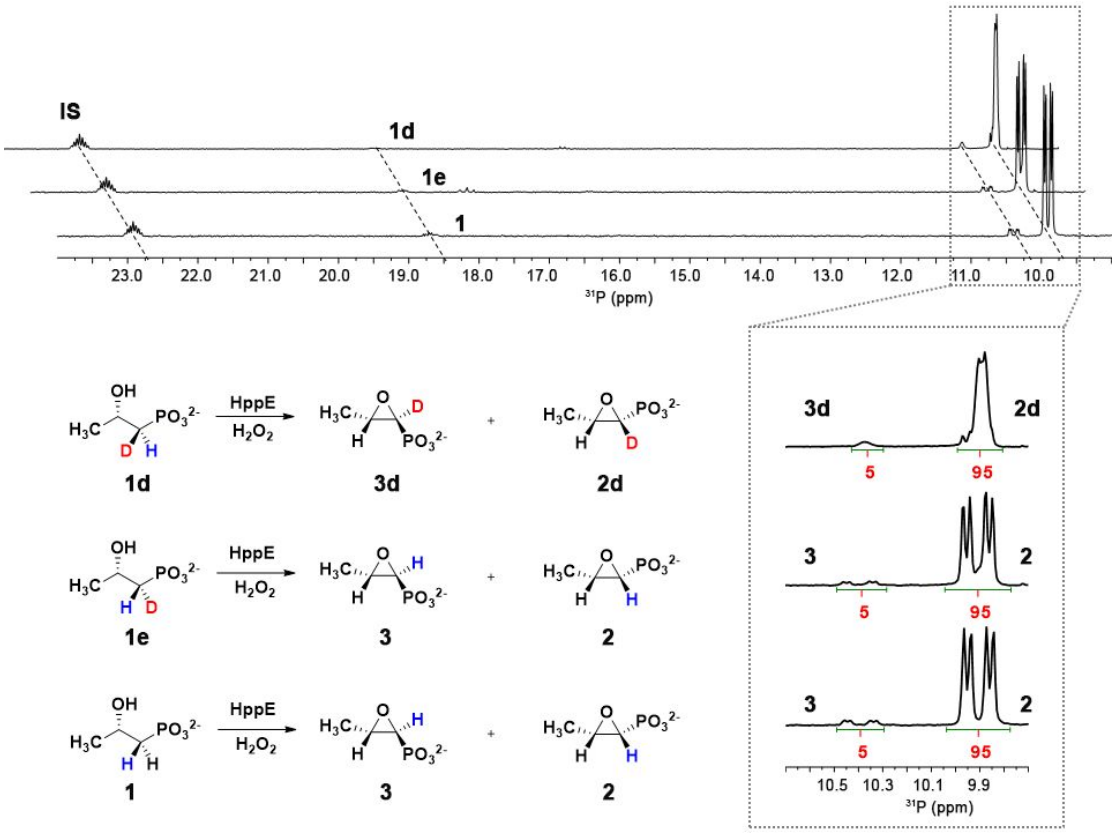


Figure S3. ^{31}P-NMR spectra of reactions of HppE with 1*S*-*d*-2*S*-HPP (**1d**) (***B***, *top line*), 1*R*-*d*-2*S*-HPP (**1e**) (***B***, *middle line*) and (*S*)-2-HPP (**1**) (***B***, *bottom line*). The multiplet at ~23.0 ppm is from the sodium propylphosphonate internal standard. The doublet of doublets ($^2J_{P\text{-}H}$ = ~21, $^3J_{P\text{-}H}$ = ~5 Hz) and doublet ($^3J_{P\text{-}H}$ = ~5 Hz) signals at ~10.4 are from *trans*-Fos (**3**) and *trans*-1-*d*-Fos (**3d**), respectively. The doublet of doublets ($^2J_{P\text{-}H}$ = ~19, $^3J_{P\text{-}H}$ = ~5 Hz) and doublet ($^3J_{P\text{-}H}$ = ~5 Hz) signals at ~9.9 are from *cis*-Fos (**2**) and *cis*-1-*d*-Fos (**2d**), respectively. The Scheme above the figure summarizes the reaction outcomes (***A***).

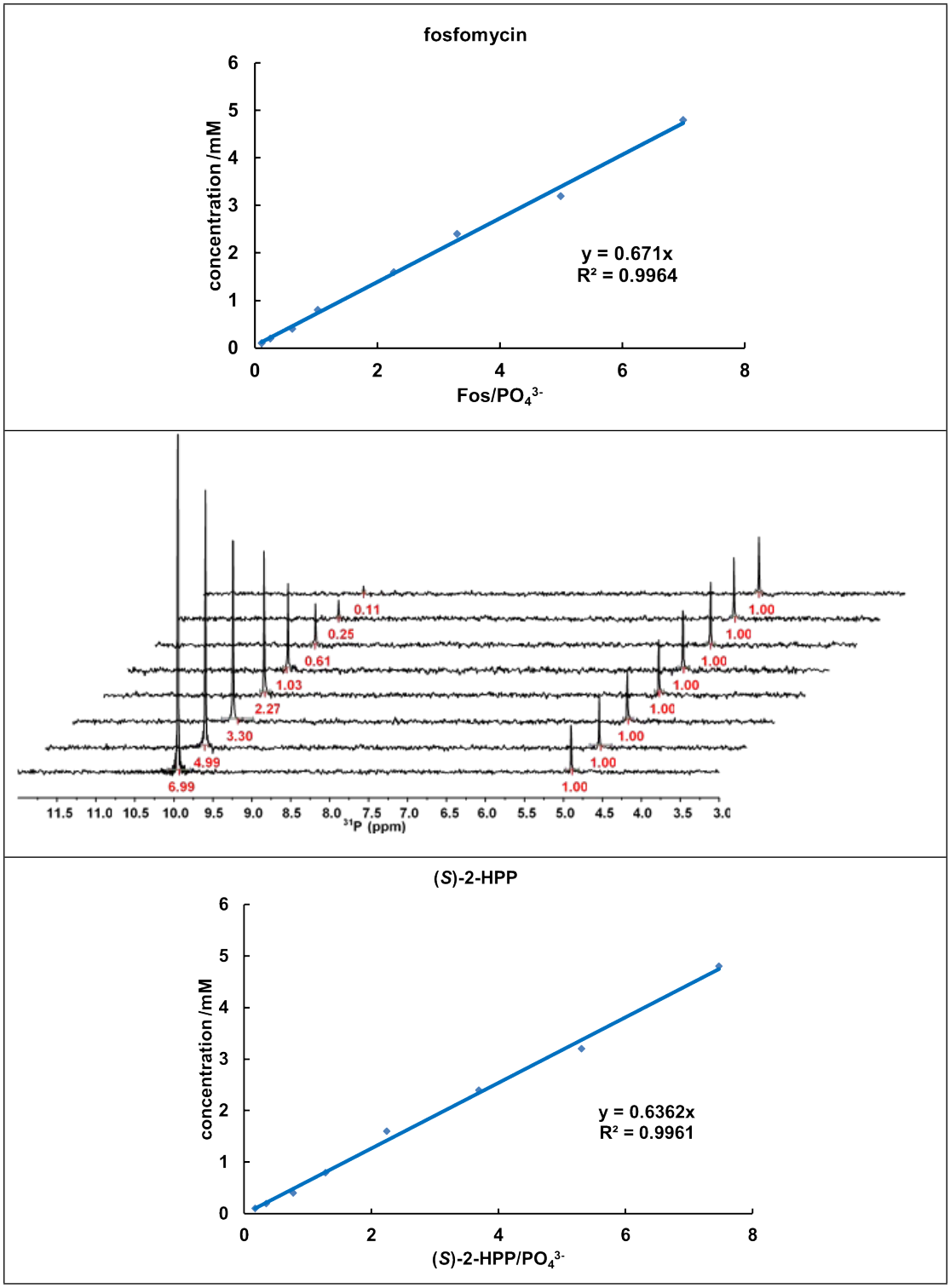
3. Structure-guided mutagenesis to alter cyclization stereochemistry.

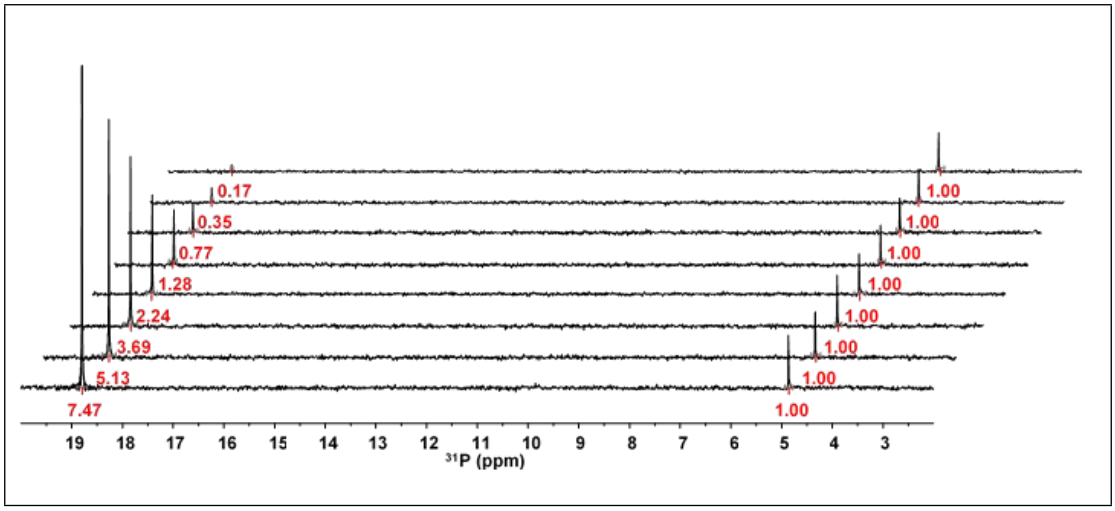
3.1 DNA constructs for overexpression of F182A, L193F, L120F, L193A, L193F/L144F HppE variants. The plasmid encoding HppE from *Streptomyces wedmorensis* was used as a template for inserting single-codon substitutions into the various constructs, except in the case of the construct for the L193F/L144F double variant, which was produced using the plasmid encoding the L193F single variant as the template. The final constructs were verified by DNA sequencing at the PSU Molecular Core Facility. The various L→A, L→F, and F→A substitutions were generated following previously published methods[8] using the primers listed in the table below:

Primer name		sequence
L120F	Forward	5'-**TTT**GTGGTGGACGTGCTGACGG-3'
	Reverse	5'-GGGGACGAGCGAAGGCGC-3'
F182A	Forward	5'-**GCG**ACGGCGGCCAAGGGCACG-3'
	Reverse	5'-GGCGTGCGGCACGTGCTC-3'
L193F	Forward	5'-**TTT**ATCGCCGTCAACTTCTGAAAGC-3'
	Reverse	5'-CTTCGCGGAACCCGTGCCC-3'
L144F	Forward	5'-**TTT**TTCGTGCTCGAGGGCGAG-3'
	Reverse	5'-GAACTCGTTGCCGGCGTGG-3'

3.2 Standard curves for Fos and (*S*)-2-HPP.

Table S1. Standard curves for Fos and (*S*)-2-HPP used in ^{31}P NMR analysis. Representative NMR spectra are shown below each standard curve. All data points represent the average of duplicate trials. The standard curves plot product/substrate concentration in mM (*y*-axis) against the ratio of the integrated NMR peak areas from the product/substrate and the internal standard (*x*-axis). Each assay contained 0.60 mL of 0.1 ~ 4.8 mM substrate or product, 0.5 mM H_3PO_4 as the internal standard, and 100 mM NaOD in D_2O.


fosfomycin
concentration /mM
y = 0.671x
R² = 0.9964
Fos/PO4 3-
0.11
0.25
0.61
1.03
2.27
3.30
4.99
6.99
1.00
31P (ppm)
(S)-2-HPP
concentration /mM
y = 0.6362x
R² = 0.9961
(S)-2-HPP/PO4 3-



3.3 Determination of total turnover number (TTN)

Assays were carried out in a total volume of 0.5 mL in 2 mL tubes. They contained 20 mM Tris-HCl buffer (pH 7.5), Fe^{II} [from $Fe(NH_4)_2(SO_4)_2$, 0.08 mM final concentration], HppE (1.25 equiv. relative to Fe^{II}), (*S*)-2-HPP (5 mM final concentration), and sodium L-ascorbate (25 equiv. relative to Fe^{II}). H_2O_2 (1 equiv. relative to the substrate) was added slowly into the solution at rt in an anoxic chamber. The reaction was then allowed to proceed for additional 10 min before being quenched by the addition of 0.10 mL of solution **B** (600 mM NaOD, 3 mM H_3PO_4 in D_2O). The mixtures were subsequently transferred to NMR tubes and subjected to ^{31}PCPD NMR analysis. Each reaction was performed in triplicate. TTNs were calculated relative to the Fe^{II} concentration.

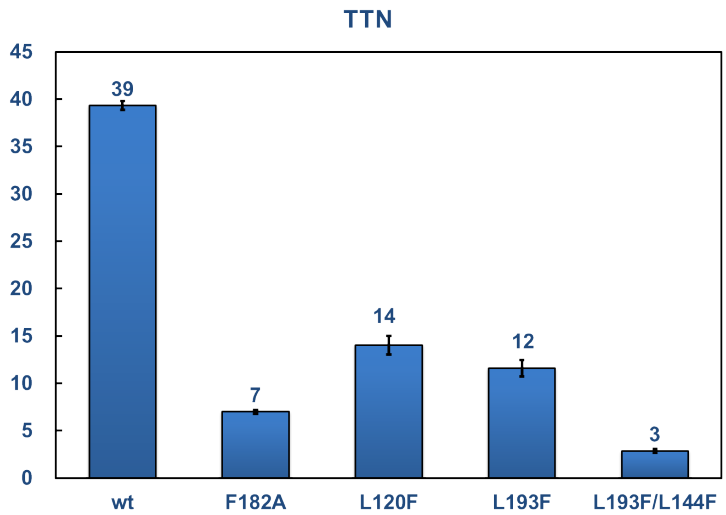
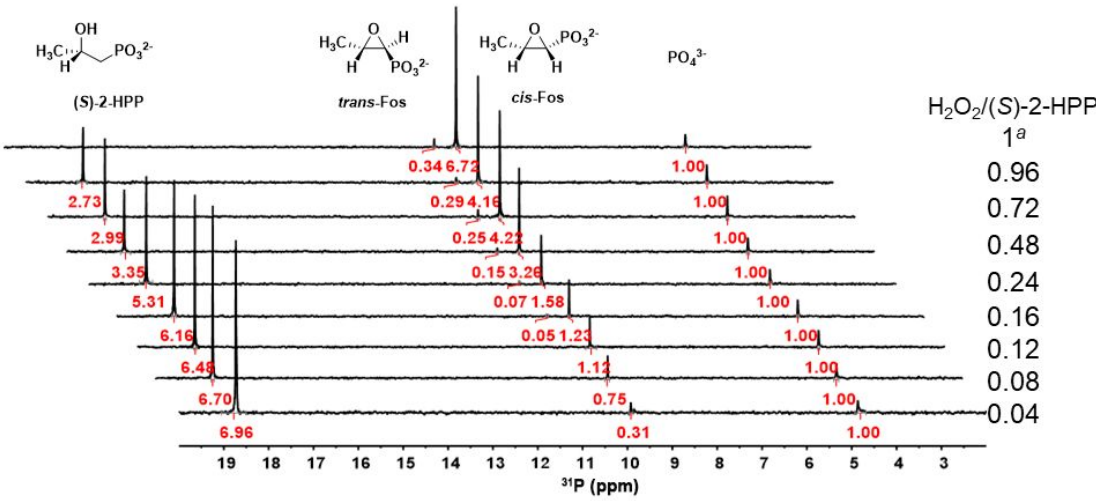


Figure S4. TTNs of reactions of (*S*)-2-HPP with HppE or variants.

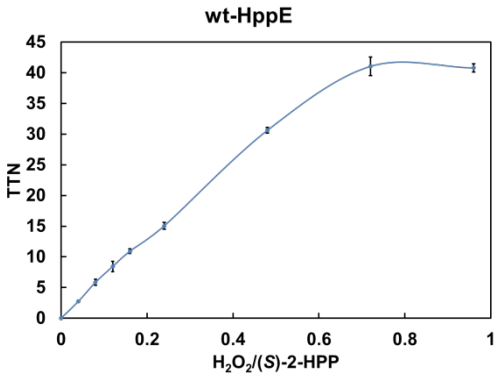
3.4 Fos/H_2O_2 reaction stoichiometries for wild type HppE and variants.

Assays (0.50 mL) were carried out in 2 mL tubes and contained 20 mM Tris-HCl buffer (pH 7.5), Fe^{II} [from $Fe(NH_4)_2(SO_4)_2$, 0.08/0.4/0.3 mM final concentration for HppE/F182A/L193F, respectively], enzyme (1.25 equiv. relative to Fe^{II}), (*S*)-2-HPP (5 mM final concentration), and sodium L-ascorbate (25 equiv. relative to Fe^{II}). Varying quantities of H_2O_2 were added slowly into the solution at rt in an anoxic chamber. The specific reaction conditions for each experiment are given in the Figure legends below. The reaction was allowed to proceed for 10 min after addition of H_2O_2 before being quenched by addition of 0.10 mL of solution **B** (600 mM NaOD, 3 mM H_3PO_4 in D_2O). The samples were subsequently transferred to NMR tubes and subjected to ^{31}PCPD NMR analysis. Each reaction was performed in triplicate.

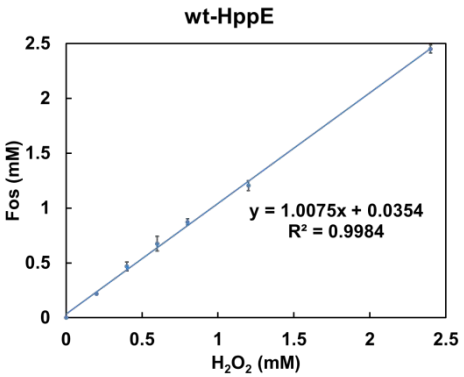
A



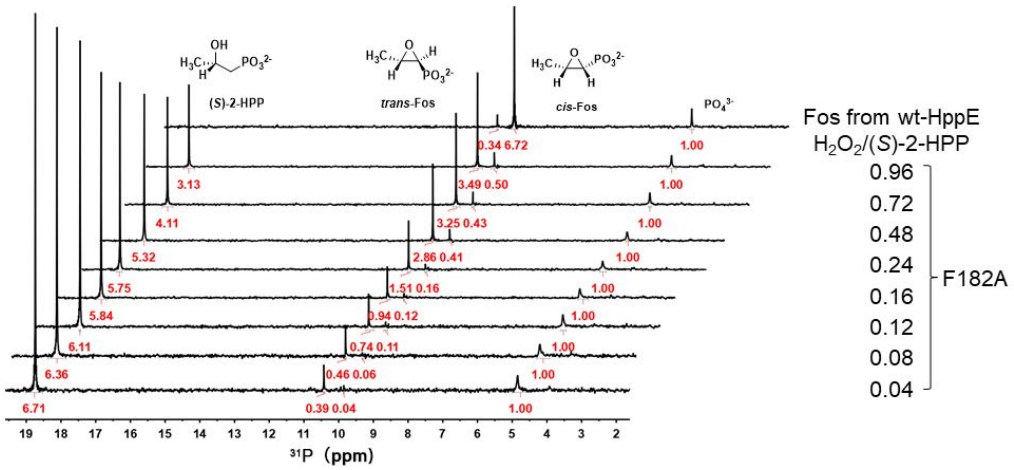
B



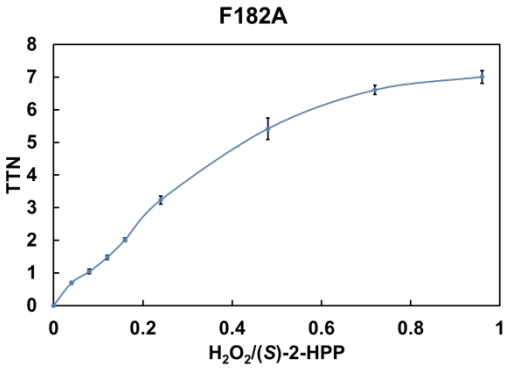
C



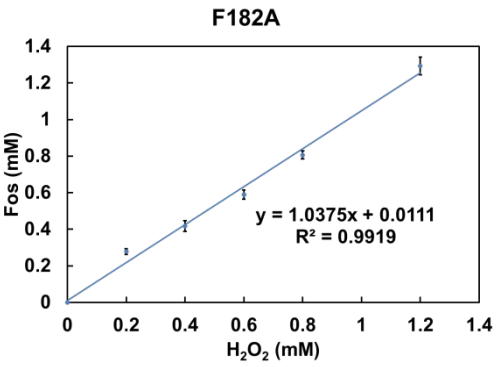
D


(S)-2-HPP
trans-Fos
cis-Fos
PO4 3-
Fos from wt-HppE
H2O2/(S)-2-HPP
0.96
0.72
0.48
0.24
0.16
0.12
0.08
0.04
F182A
31P (ppm)

E


F182A
TTN
H2O2/(S)-2-HPP

F


F182A
Fos (mM)
y = 1.0375x + 0.0111
R² = 0.9919
H2O2 (mM)

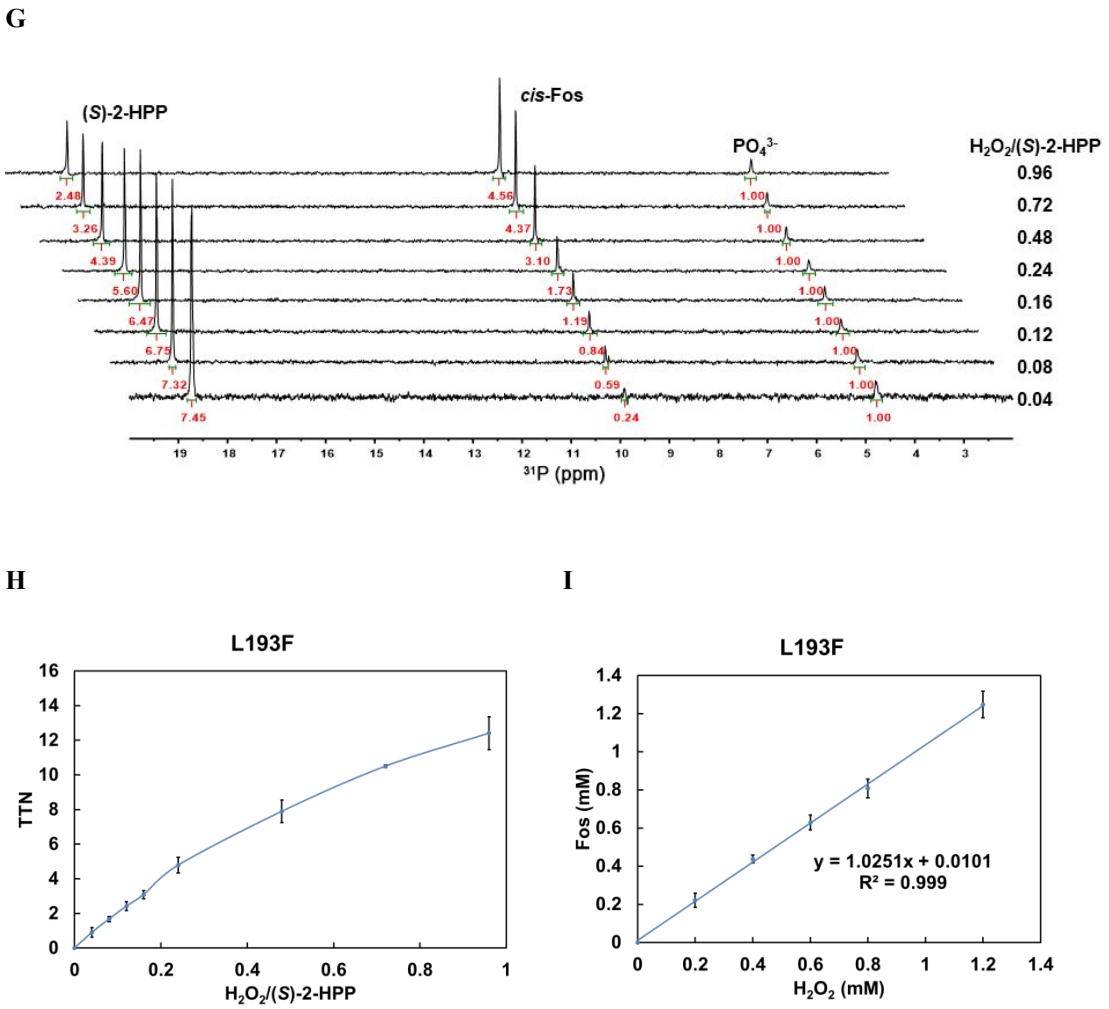


Figure S5. ^{31}P NMR analyses of the reactions of (*S*)-2-HPP with wild-type HppE and its F182A and L193F variants. Panels ***A*-*C*** depict results for the wild-type enzyme, ***D*-*F*** the F182A variant, and ***G*-*I*** the L193F variant. The ^{31}PCPD NMR spectra show single peaks at δ ~18.7 ppm from (*S*)-2-HPP, ~10.4 ppm from *trans*-Fos (**3**), ~9.9 ppm from *cis*-Fos (**2**) and ~4.8 ppm from the internal standard PO_4^{3-}. Panels ***B***, ***E***, and ***H*** plot turnover number (TTN) (*y*-axis) versus the ratio of H_2O_2/(*S*)-2-HPP (*x*-axis) for the three enzymes (in the same order). Panels ***C***, ***F***, and ***I*** plot the sum of the concentrations of the two Fos products versus concentration H_2O_2 added. The slope of these lines yields the Fos/H_2O_2 reaction stoichiometry, which is in all three cases indistinguishable from unity. The error bars are the standard deviations from the mean values of the three measurements performed at each concentration. [a]same condition except using 0.2 mM wt-HppE, 0.16 mM Fe^{II} (***A***, *top line*).

3.5 Analysis of stereochemistry of (*S*)-2-HPP cyclization by HppE and variants.

The specific reaction conditions for each experiment are shown in Table S2 below. The concentrations of products, their cis:trans ratios, and the conversions were calculated by comparison to the standard curves above. TTNs were calculated relative to Fe^{II} concentration. Each reaction was performed in triplicate. Selected spectra can be found in Figure S6.

Table S2. ^{31}P NMR analysis of the reactions of (*S*)-2-HPP with HppE and variants. [Fe-E] = concentration of Fe(II)•enzyme complex. [Sub] = concentration of substrate in the reaction. Sub/IS, trans/IS, and cis/IS are quantities of substrate, *trans*-Fos (**3**) and *cis*-Fos (**2**), respectively, relative to the internal standard as calculated from the integrated intensities of the peaks arising from each compound in the NMR spectra. [Pdt] = calculated concentration of combined products, cis% = cis-product ratio. Conv% = calculated conversion.

Entry	E	[Fe-E] (mM)	[Sub] (mM)	Sub/IS	trans/IS	cis/IS	[Pdt] (mM)	TTN	cis(%)	Conv(%)
1	L193F	0.4	3.6	0.75±0.03	0.00±0.00	4.57±0.80	3.56±0.63	8.9±1.6	100±0.0	85.3±2.4
2	wt	0.16	4.5	0.00±0.00	0.33±0.03	5.94±0.48	4.89±0.39	30.6±2.4	94.7±0.3	100±0
3	L120F	0.4	4.5	2.11±0.34	0.66±0.02	3.02±0.26	2.87±0.19	7.2±0.5	81.9±1.7	63.8±5.4
4	F182A	0.8	4	0.31±0.02	4.21±0.31	0.64±0.07	3.78±0.30	4.7±0.4	12.8±0.6	94.0±0.2

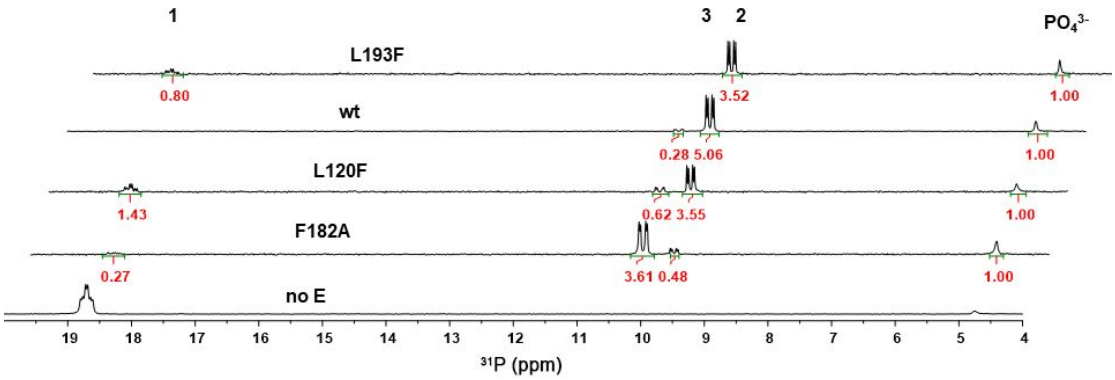


Figure S6. Selected ^{31}P NMR spectra of reactions of (*S*)-2-HPP with HppE and its variants. The doublet of doublet of doublets at ~18.71 ppm (ddd, J = 15.9, 7.0 Hz) is from (*S*)-2-HPP. The doublet of doublets at ~10.4 and ~9.9 ppm are from *trans*-Fos (**3**) and *cis*-Fos (**2**), respectively. The single peak at ~4.8 ppm is from the internal standard (PO_4^{3-}).

3.6 Demonstration that F182A and L193F variants still abstract the *pro-R* hydrogen.

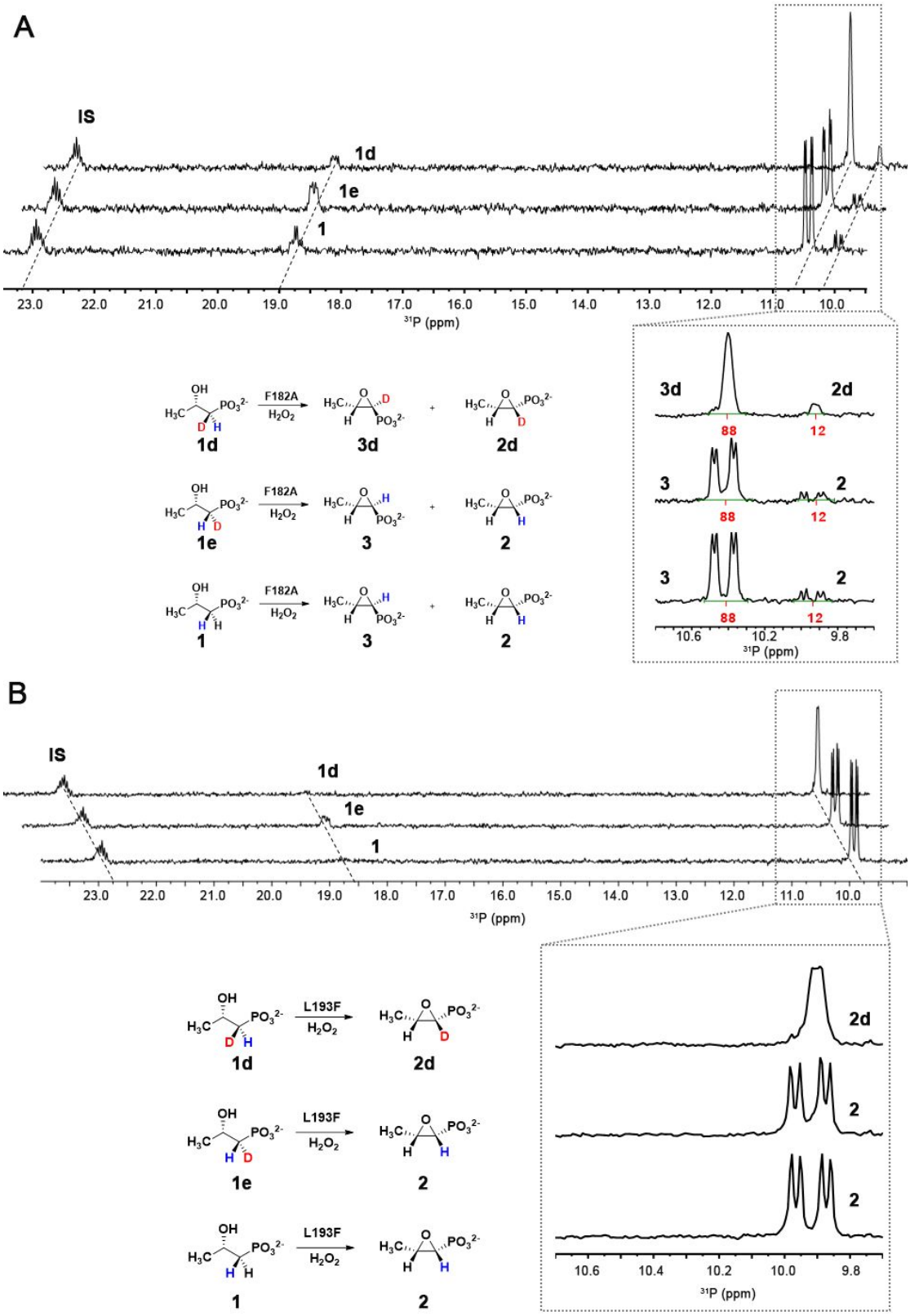


Figure S7. Selected ^{31}P NMR spectra of reactions of the F182A (***A***) and L193F (***B***) variants with 1*S*-*d*-2*S*-HPP (**1d**) (*top lines*), 1*R*-*d*-2*S*-HPP (**1e**) (*middle lines*) and (*S*)-2-HPP (**1**) (*bottom lines*). The reaction

conditions were as described in *Sec 2.4*, but 0.4 mM of each Fe(II)-reconstituted variant protein was used. The multiplet at ~23.0 ppm is from the internal standard sodium propylphosphonate. The doublet of doublet of doublets ($^2J_{P\text{-}H}$ = ~16, $^3J_{P\text{-}H}$ = ~7 Hz) at ~18.6 ppm is from **1**. The doublet of doublets ($^2J_{P\text{-}H}$ = ~16, $^3J_{P\text{-}H}$ = ~7 Hz) at ~18.6 ppm is from **1d** and **1e**. The doublet of doublets ($^2J_{P\text{-}H}$ = ~21, $^3J_{P\text{-}H}$ = ~5 Hz) at ~10.4 ppm is from *trans*-Fos (**3**). The doublet ($^3J_{P\text{-}H}$ = ~5 Hz) at ~10.4 ppm is from *trans*-1-*d*-Fos (**3d**). The doublet of doublets ($^2J_{P\text{-}H}$ = ~19, $^3J_{P\text{-}H}$ = ~5 Hz) at ~9.9 ppm is from *cis*-Fos (**2**), and the doublet ($^3J_{P\text{-}H}$ = ~5 Hz) at ~9.9 ppm is from *cis*-1-*d*-Fos (**2d**). The Scheme in the figures summarizes the reaction outcomes.

4. Use of halogen substitution to distinguish between polar and radicaloid C–O-coupling mechanisms.

4.1 Isolation and characterization of products generated by HppE from (*S*)-3-F_3-HPP (1c).

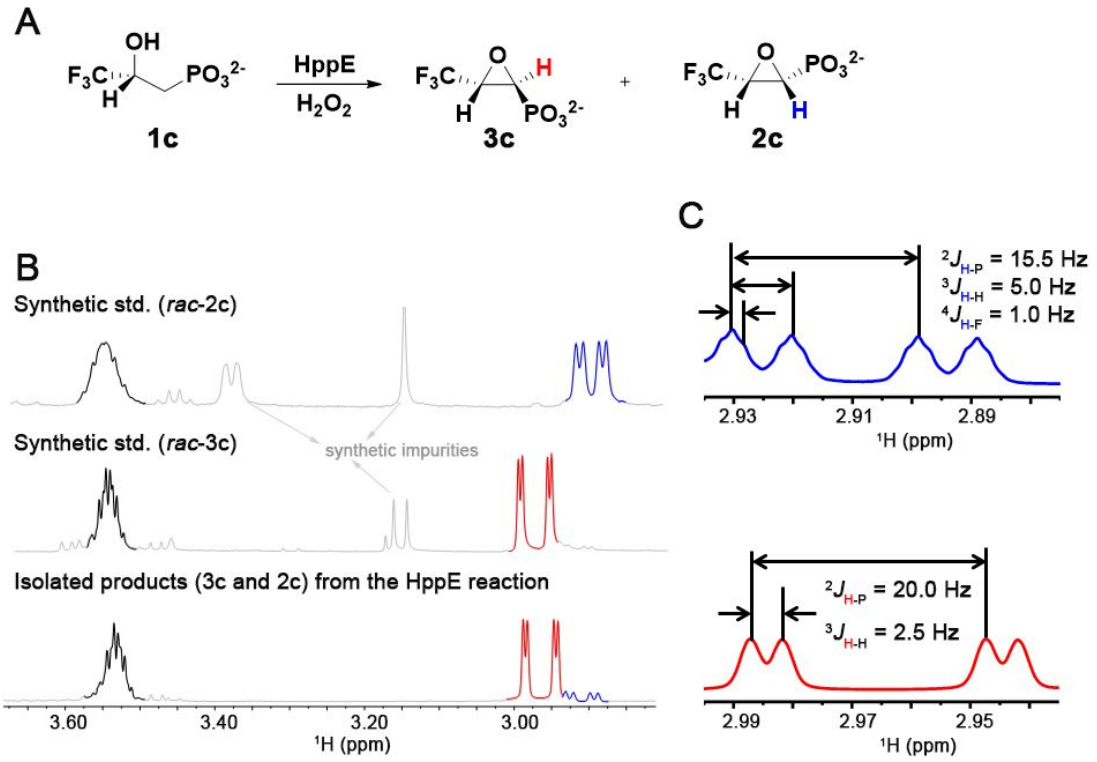


Figure S8. Analysis of the products generated by HppE from (*S*)-3-F_3-2-HPP (**1c**). (**A**) Reaction scheme showing two possible products. (**B**) ^{1}H NMR spectra of isolated *cis*/*trans*-F_3-Fos (**2c**/**3c**) generated by HppE (*bottom*) and the synthetic *trans*-3-F_3-Fos (**3c**; *middle*) and *cis*-3-F_3-Fos (**2c**; *top*) standards. (**C**) Blow-up of the ^{1}H NMR spectra of isolated 3-F_3-Fos products showing vicinal and geminal couplings. $^3J_{H\text{-}H}$ of *cis*-3-F_3-Fos (**2c**) is ~5 Hz, and $^3J_{H\text{-}H}$ of *trans*-F_3-Fos (**3c**) is ~3 Hz. The reaction conditions and isolation procedure are described in *Sec 2.2*, except that the final concentration of substrate was 3 mM.

(1*S*,2*S*)-1,2-epoxy-3,3,3-trifluoropropylphosphate (**3c**): ^{1}H NMR (500 MHz, D_2O) δ 3.67 – 3.62 (m, 1H), 3.08 (dd, J = 20.0, 2.5 Hz, 1H). ^{31}P NMR (202 MHz, D_2O) δ 6.70 (dd, J = 19.9, 4.3 Hz). ^{19}F NMR (471 MHz, D_2O) δ -74.27 (d, J = 4.7 Hz). ^{13}C NMR (126 MHz, D_2O) δ 122.87 (q, J = 275.9 Hz), 52.54 (q, J = 40.5 Hz), 51.25 (d, J = 170.3 Hz). HRMS (ESI): calcd for $C_3H_3F_3O_4P$ ($M\text{-}H^-$) 190.9726, found 190.9732.

(1*R*,2*S*)-1,2-epoxy-3,3,3-trifluoropropylphosphonate (**2c**): ^{1}H NMR (500 MHz, D_2O) δ 3.67 – 3.62 (m, 1H), 3.02 (m, *J* = 15.5, 5.0, 1.0 Hz, 1 H). ^{31}P NMR (202 MHz, D_2O) δ 5.27 (dd, *J* = 15.9, 2.3 Hz). ^{19}F NMR (471 MHz, D_2O) δ -67.31 (d, *J* = 6.7 Hz).

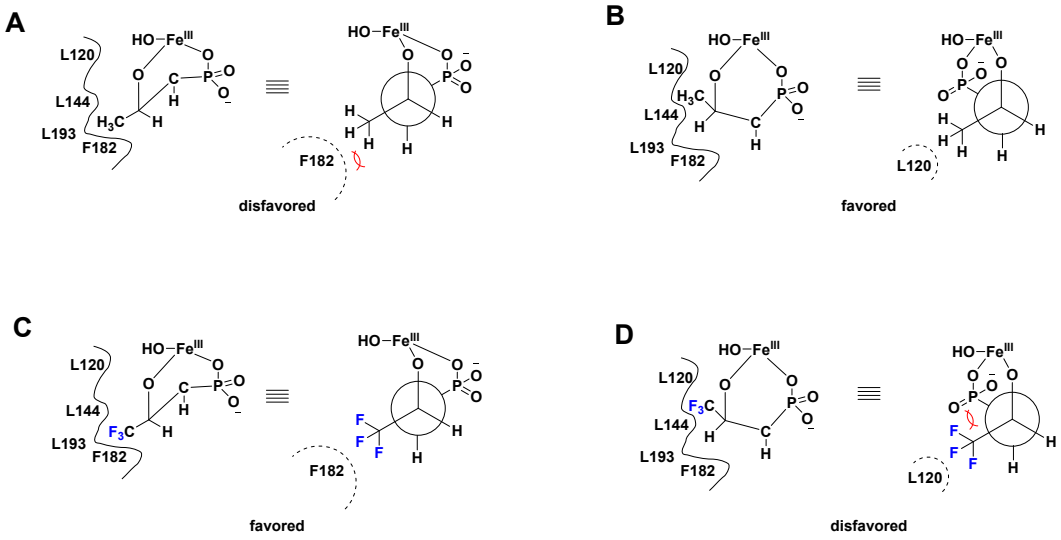
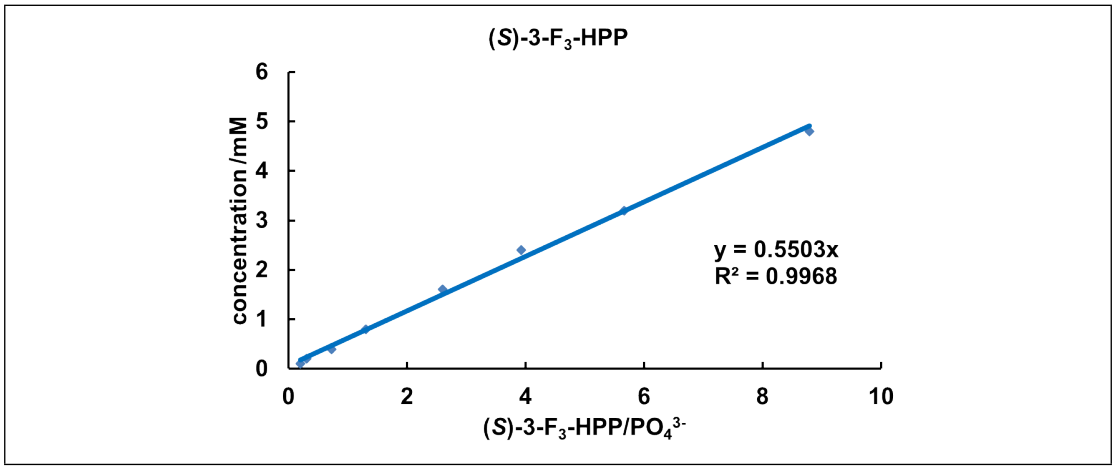


Figure S9. Explanation for the favored transition state (***B***) for *cis*-Fos (**2**) formation by C1 inversion and the favored transition state (***C***) for *trans*-3-F_3-Fos (**3c**) formation by C1 retention in the active pocket of wild-type HppE.

4.2 Stereochemical course of the cyclization of (*S*)-3-F_3-2-HPP (1c) by HppE and variants.

Table S3. The standard curves of (*S*)-3-F_3-HPP by ^{31}P and ^{19}F NMR with representative spectra are shown below. The method is as described in the caption of Table S1.



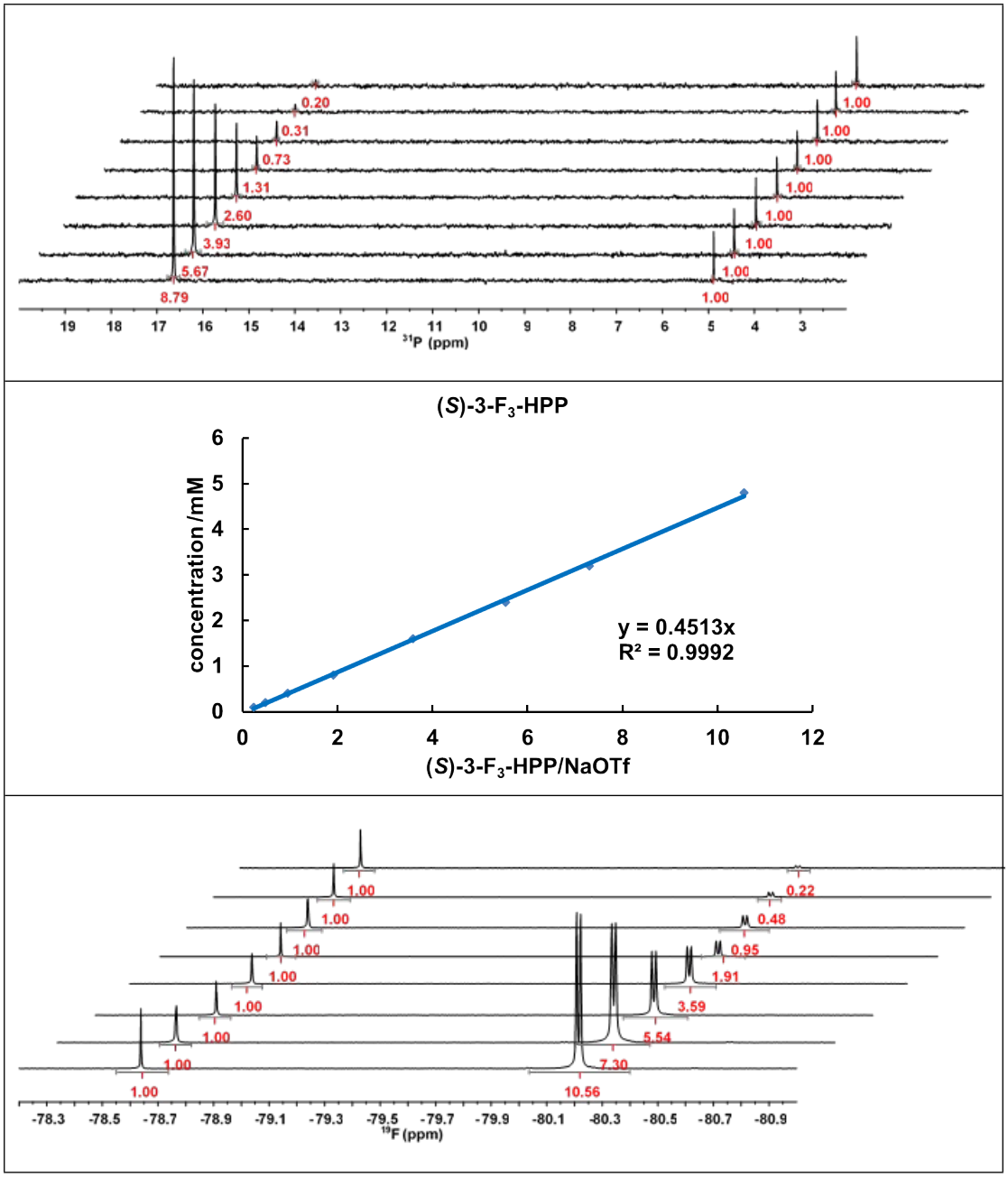


Table S4. ^{31}P NMR analysis of the reactions of (*S*)-3-F_3-2-HPP (**1c**) with HppE and variants. Column headings are as described in the caption of Table S2.

Entry	E	[Fe-E] (mM)	[Sub] (mM)	Sub/IS	trans/IS	cis/IS	[Pdt] (mM)	TTN	cis(%)	Conv(%)
1	L193F/ L144F	0.8	1.5	1.29±0.07	0.12±0.01	1.10±0.17	0.80±0.11	1.0±0.1	90.2±0.8	48.1±2.2
2	L193F	0.4	3	1.44±0.41	1.57±0.23	2.04±0.31	2.38±0.35	6.0±0.9	56.4±0.3	72.9±3.7

3	wt	0.8	3	1.31±0.05	2.49±0.10	0.74±0.02	2.13±0.08	2.7±0.1	22.8±0.2	71.1±0.1
4	L120F	0.4	4	4.63±0.47	1.75±0.10	0.20±0.01	1.28±0.06	3.2±0.2	10.2±0.9	29.9±3.0
5	F182A	0.8	3	0.38±0.02	2.47±0.10	0.00±0.00	1.63±0.06	2.0±0.1	0.0±0.0	86.7±1.1

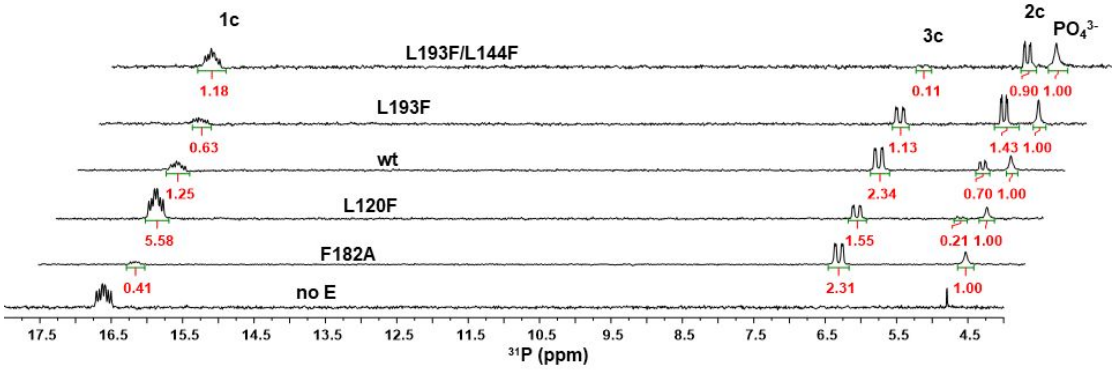


Figure S10. Selected ^{31}P-NMR spectra of the reactions of (*S*)-3-F_3-2-HPP (**1c**) with HppE and variants. The multiplets at ~16.6, ~6.7 and ~5.2 ppm are from (*S*)-3-F_3-2-HPP (**1c**), *trans*-3-F_3-Fos (**3c**) and *cis*-3-F_3-Fos (**2c**), respectively. The single peak at ~4.8 ppm corresponds to the internal standard PO_4^{3-}.

Table S5. ^{19}F NMR analysis of the reactions of (*S*)-3-F_3-2-HPP with HppE and variants. Column headings are as described in the caption of Table S2.

Entry	E	[Fe-E] (mM)	[Sub] (mM)	Sub/IS	trans/IS	cis/IS	[Pdt] (mM)	TTN	cis(%)	Conv(%)
1	L193F/ L144F	0.8	1.5	1.39±0.21	0.14±0.02	1.33±0.12	0.79±0.07	1.0±0.1	90.7±0.4	51.7±6.1
2	L193F	0.4	3	1.46±0.38	1.80±0.04	2.33±0.06	2.23±0.05	5.6±0.1	56.3±0.3	74.6±5.4
3	wt	0.8	3	1.05±0.38	3.32±0.32	0.83±0.03	2.24±0.18	2.8±0.2	20.1±1.3	80.1±6.9
4	L120F	0.4	4	4.15±0.02	1.89	0.18±0.01	1.12±0.01	3.2±0.2	8.7±0.5	33.2±0.1
5	F182A	0.8	3	0.78±0.41	2.15±0.29	0.00±0.00	1.16±0.16	1.4±0.2	0.0±0.0	74.3±12.4

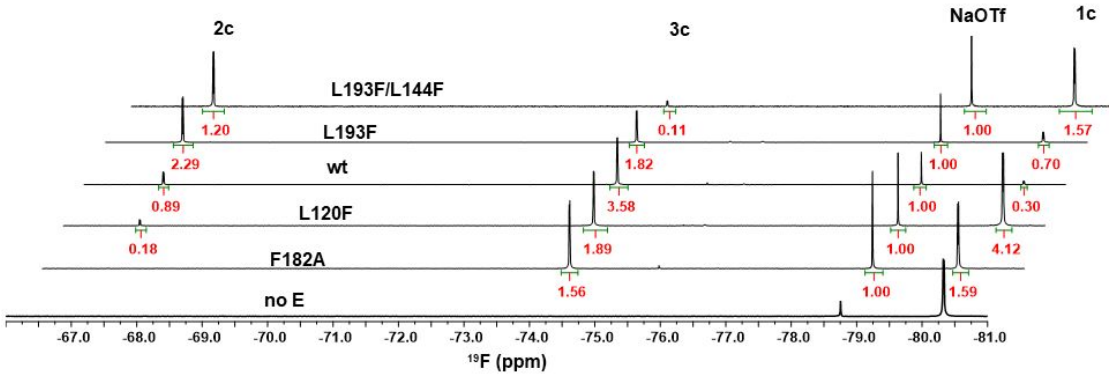


Figure S11. Selected ^{19}F-NMR spectra of the reactions of (*S*)-3-F_3-2-HPP (**1c**) with HppE and variants. The doublets at ~ –67.2, ~ –74.1 and ~ –80.3 are from *cis*-3-F_3-Fos (**2c**), *trans*-3-F_3-Fos (**3c**), and (*S*)-3-F_3-2-HPP (**1c**), respectively. The single peak at ~ –78.7 corresponds to the internal standard, NaOTf.

4.3 Characterization of the products generated by HppE from (*S*)-3-F-2-HPP (1a) and (*S*)-3-F_2-2-HPP (1b).

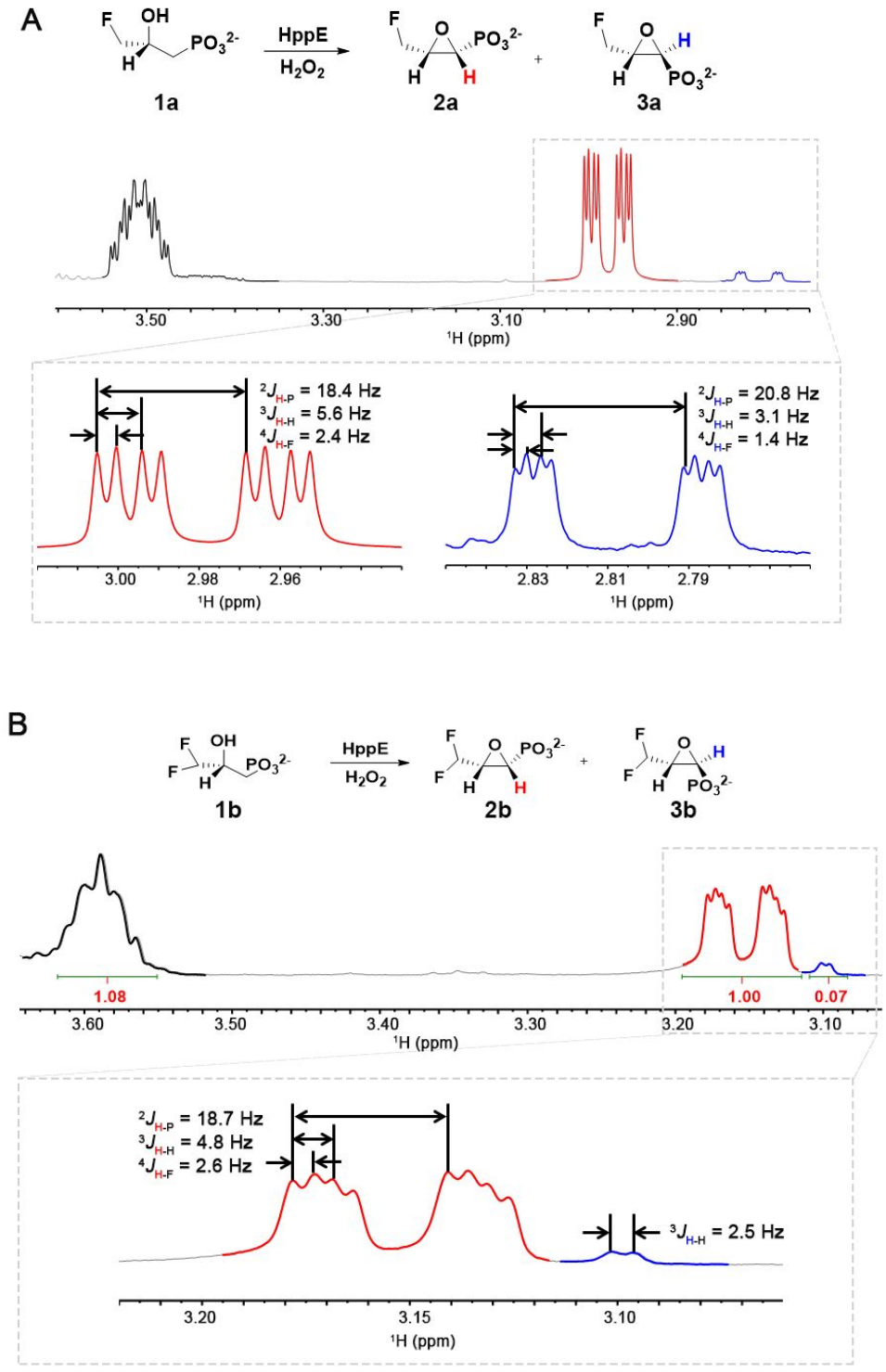


Figure S12. Reaction schemes (*top*) and ^{1}H-NMR spectra of isolated products (*bottom*) for reactions of (**A**) (*S*)-3-F-2-HPP and (**B**) (*S*)-3-F_2-2-HPP by HppE. $^3J_{H-H}$ for the cis epoxide is ~5 Hz, whereas that for the trans epoxide is ~3 Hz.

(1*R*,2*S*)-1,2-epoxy-3-fluoropropylphosphonate (2a). ^{1}H NMR (500 MHz, D_2O) δ 4.92-4.80 (m, 2H), 3.52 – 3.44 (m, 1H), 2.95 (ddd, *J* = 18.4, 5.6, 2.4 Hz, 1H). ^{13}C NMR (126 MHz, D_2O) δ 83.85 (d, *J* = 157.8 Hz), 55.45 (d, *J* = 25.1 Hz), 52.91 (dd, *J* = 172.5, 8.6 Hz). ^{31}P NMR (202 MHz, 100 mM NaOD in D_2O) δ 8.25 (ddd, *J* = 18.4, 4.5, 2.5 Hz). ^{19}F NMR (471 MHz, 100 mM NaOD in D_2O) δ -222.34 (m, *J* = 47.6, 11.9, 2.8 Hz). HRMS (ESI): calcd for $C_3H_5FO_4P$ ($M-H^-$) 154.9915, found 154.9919.

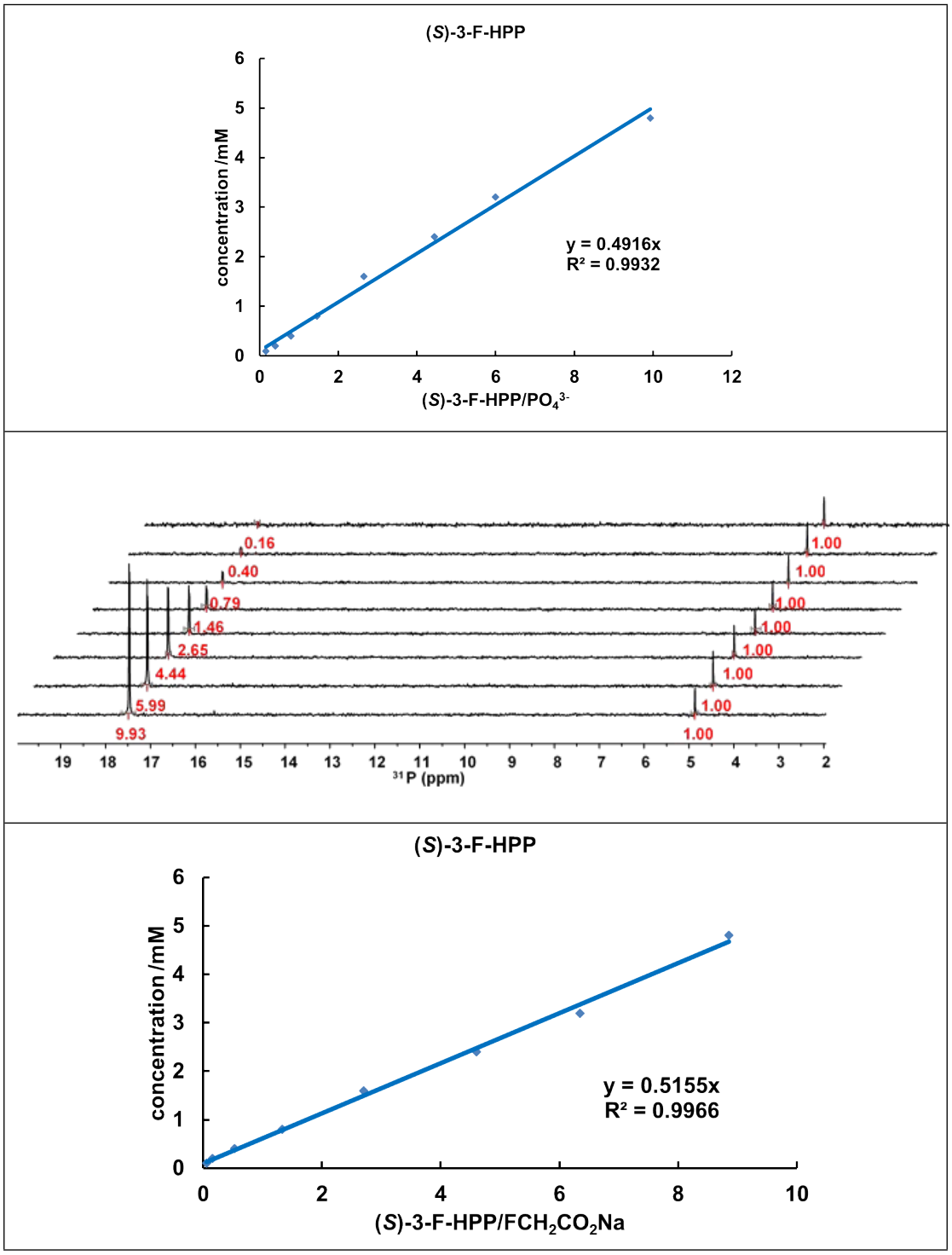
(1*S*,2*S*)-1,2-epoxy-3-fluoropropylphosphonate (3a). ^{1}H NMR (500 MHz, D_2O) δ 4.92-4.80 (m, 2H, overlapped), 3.45 – 3.39 (m, 1H), 2.81 (ddd, *J* = 20.8, 3.1, 1.4 Hz, 1H). ^{31}P NMR (202 MHz, 100 mM NaOD in D_2O) δ 9.10 (ddd, *J* = 20.2, 5.0, 5.0 Hz). ^{19}F NMR (471 MHz, 100 mM NaOD in D_2O) -225.58 (m, *J* = 47.3, 13.7, 4.5 Hz).

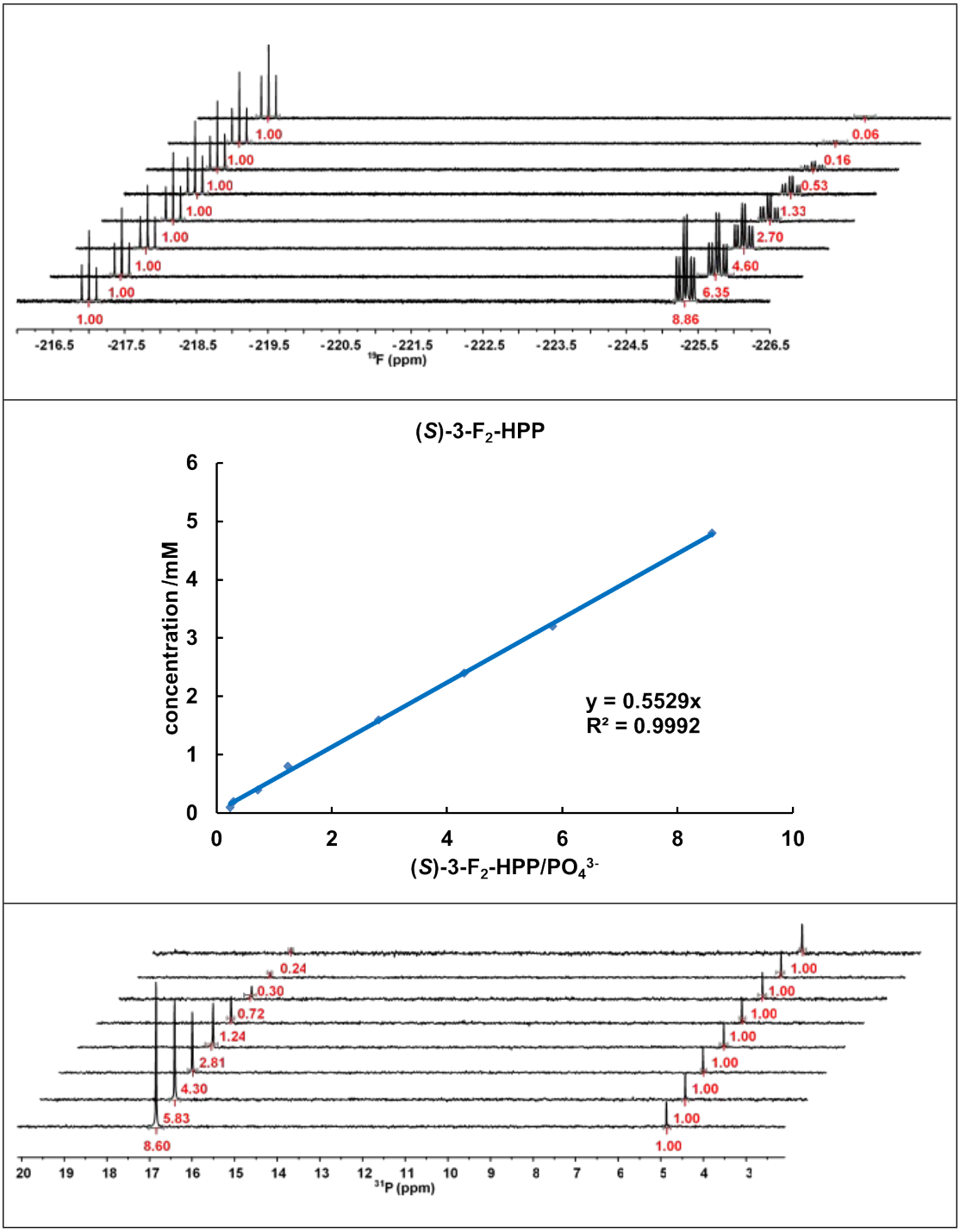
(1*R*,2*S*)-1,2-epoxy-3,3-difluoropropylphosphonate (2b). ^{1}H NMR (500 MHz, D_2O) δ 6.37 – 6.14 (m, H), 3.46 – 3.42 (m, 1H), 3.00 (ddd, *J* = 18.7, 4.8, 2.6 Hz, 1H). ^{13}C NMR (126 MHz, D_2O) δ 114.60 (t, *J* = 235.9 Hz), 54.44 (dd, *J* = 42.6, 30.5 Hz), 52.58 (dd, *J* = 171.0, 7.7 Hz). ^{31}P NMR (202 MHz, 100 mM NaOD in D_2O) δ 7.09 (*J* = m, 17.1, 3.8, 2.0 Hz). ^{19}F NMR (471 MHz, 100 mM NaOD in D_2O) δ -118.26 (m, *J* = 310.4, 57.7, 7.4 Hz), -121.20 (m, *J* = 310.4, 52.9 Hz). HRMS (ESI): calcd for $C_3H_4F_2O_4P$ ($M-H^-$) 172.9820, found 172.9825.

(1*S*,2*S*)-1,2-epoxy-3,3-difluoropropylphosphonate (3b). ^{1}H NMR (500 MHz, D_2O) δ 6.30 – 6.10 (m, 1H, overlapped), 3.61 – 3.56 (m, 1H, overlapped), 3.13 – 3.10 (m, overlapped, 1H). ^{31}P NMR (202 MHz, 100 mM NaOD in D_2O) δ 7.78 (m, *J* = 20.1, 4.9, 2.6 Hz). ^{19}F NMR (471 MHz, 100 mM NaOD in D_2O) -123.94 (m, *J* = 295.6, 54.6, 4.7 Hz), -125.36 (m, *J* = 295.8, 55.2, 9.4, 2.8 Hz).

4.4 Stereochemical course of cyclization of (*S*)-3-F-HPP and (*S*)-3-F_2-HPP by HppE and variants.

Table S6. The standard curves of (*S*)-3-F-HPP and (*S*)-3-F_2-HPP by ^{31}P and ^{19}F NMR with representative spectra are shown below. The method is as described in the caption of Table S1.




1.00
1.00
1.00
1.00
1.00
1.00
1.00
1.00
0.06
0.16
0.53
1.33
2.70
4.60
6.35
8.86
-216.5
-217.5
-218.5
-219.5
-220.5
-221.5
-222.5
-223.5
-224.5
-225.5
-226.5
^{19}F (ppm)
(S)-3-F$_2$-HPP
concentration /mM
y = 0.5529x
R² = 0.9992
(S)-3-F$_2$-HPP/PO$_4^{3-}$
0.24
0.30
0.72
1.24
2.81
4.30
5.83
8.60
1.00
1.00
1.00
1.00
1.00
1.00
1.00
1.00
^{31}P (ppm)

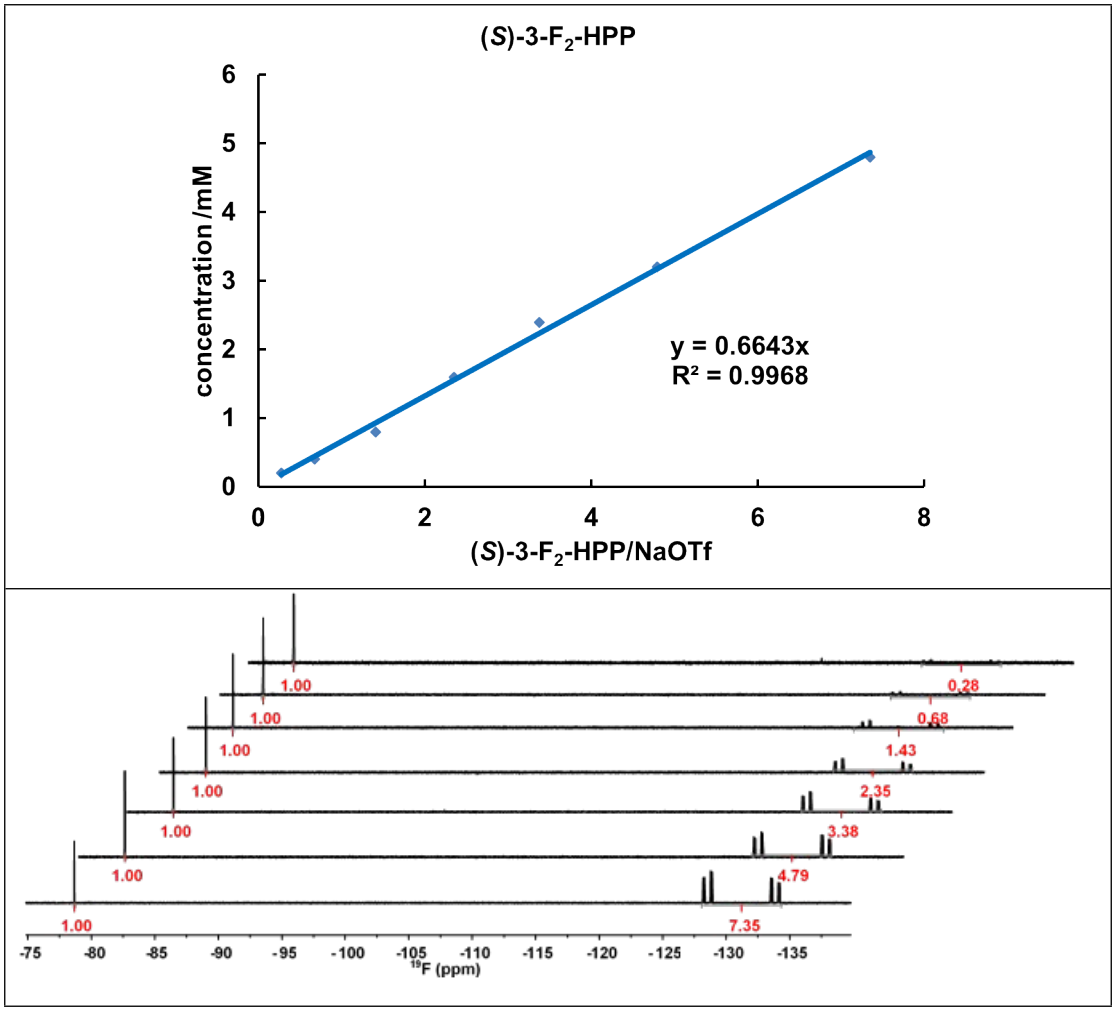


Table S7. ^{31}P NMR analysis of the reactions of (*S*)-3-F-2-HPP with HppE and variants. Column headings are as described in the caption of Table S2.

Entry	E	[Fe-E] (mM)	[Sub] (mM)	Sub/IS	trans/IS	cis/IS	[Pdt] (mM)	TTN	cis(%)	Conv(%)
1	L193F	0.4	1.5	0.44±0.14	0.00±0.00	1.59±0.08	0.95±0.05	2.4±0.1	100±0.0	78.9±5.3
2	wt	0.4	4	1.10±0.01	0.35±0.02	5.01±0.31	3.22±0.39	8.1±0.5	93.4±0.3	83.1±0.5
3	L120F	0.4	3.6	1.64±0.39	0.47±0.02	3.43±0.02	2.34±0.02	5.8±0.0	87.9±0.4	71.0±4.6
4	F182A	0.8	3	1.17±0.05	4.04±0.16	0.11±0.01	2.49±0.09	3.1±0.1	2.7±0.2	78.1±0.3

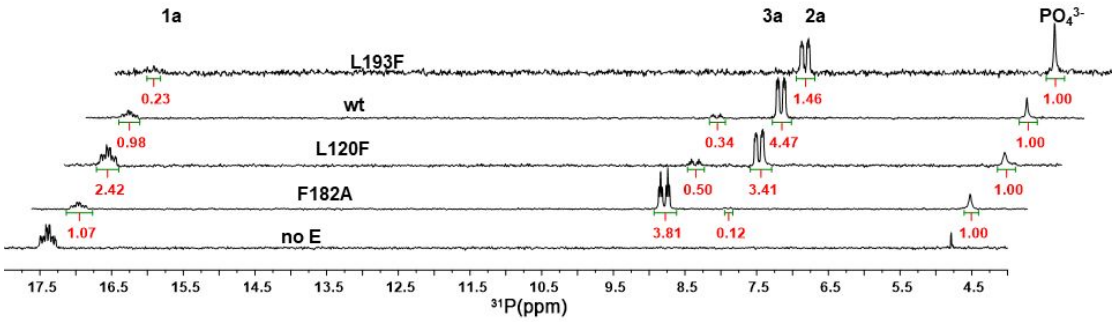


Figure S13. Selected ^{31}P NMR spectra of reactions of (*S*)-3-F-2-HPP (**1a**) with HppE and variants. The multiplet at ~17.4 ppm is from (*S*)-3-F-2-HPP (**1a**). The two signals (doublet of doublet of doublets) at ~9.1 and ~8.2 ppm are from the trans and cis products, respectively. The single peak at ~4.8 ppm arises from the internal standard, PO_4^{3-}.

Table S8. ^{19}F-NMR analysis of the reactions of (*S*)-3-F-2-HPP with HppE and variants. Column headings are as described in the caption of Table S2.

Entry	E	[Fe-E] (mM)	[Sub] (mM)	Sub/IS	trans/IS	cis/IS	[Pdt] (mM)	TTN	cis(%)	Conv(%)
1	L193F	0.8	3.6	3.66±0.89	0.00±0.00	2.76±0.13	1.65±0.08	2.1±0.1	100±0.0	44.9±8.2
2	wt	0.4	4	0.97±0.33	0.33±0.06	5.24±0.35	3.34±0.25	8.4±0.6	94.2±0.8	86.0±3.8
3	L120F	0.4	3.6	1.33±0.08	0.51±0.06	3.50±0.10	2.40±0.09	6.0±0.2	87.4±0.9	75.1±0.4
4	F182A	0.8	3	1.29±0.02	3.85±0.05	0.06±0.03	2.35±0.35	2.9±0.4	1.4±0.7	74.4±3.4

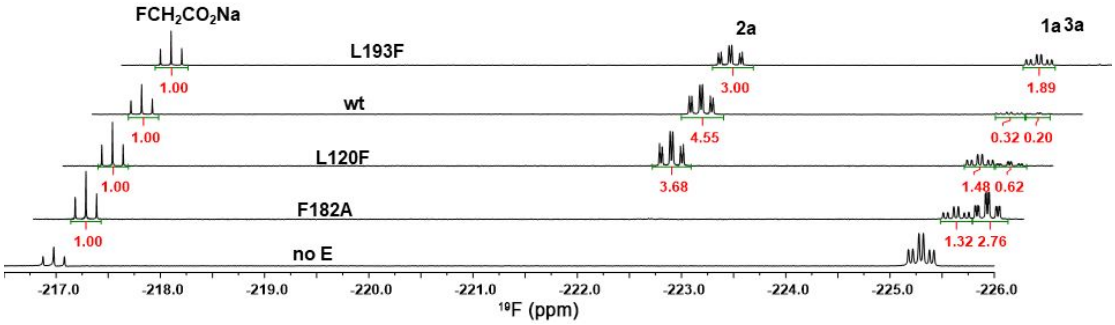


Figure S14. Selected ^{19}F-NMR spectra of reactions of (*S*)-3-F-2-HPP (**1a**) with HppE and variants. The triplet at ~ –217.0 ppm is from the internal standard, FCH_2CO_2Na. The triplets of doublets at ~ –222.3, ~ -225.3 and ~ -225.6 are from *cis*-3-F-Fos (**2a**), (*S*)-3-F-2-HPP (**1a**) and *trans*-3-F-Fos (**3a**), respectively.

Table S9. ^{31}P NMR analysis of the reactions of (*S*)-3-F_2-2-HPP with HppE and variants. Column headings are as described in the caption of Table S2.

Entry	E	[Fe-E] (mM)	[Sub] (mM)	Sub/IS	trans/IS	cis/IS	[Pdt] (mM)	TTN	cis(%)	Conv(%)
1	L193F	0.4	1.5	0.57±0.17	0.00±0.00	1.44±0.22	0.86±0.16	2.6±0.4	100±0.0	73.0±4.0
2	wt	0.7	3.6	0.32±0.00	0.32±0.02	4.21±0.20	3.26±0.14	4.7±0.2	92.9±0.5	93.4±0.2
3	L120F	0.4	4	3.36±0.55	0.44±0.01	1.98±0.12	1.74±0.10	4.3±0.2	82.0±0.4	42.6±5.7
4	F182A	0.8	3	0.43±0.05	3.40±0.35	0.09±0.00	2.51±0.25	3.1±0.5	2.6±0.4	89.0±0.8

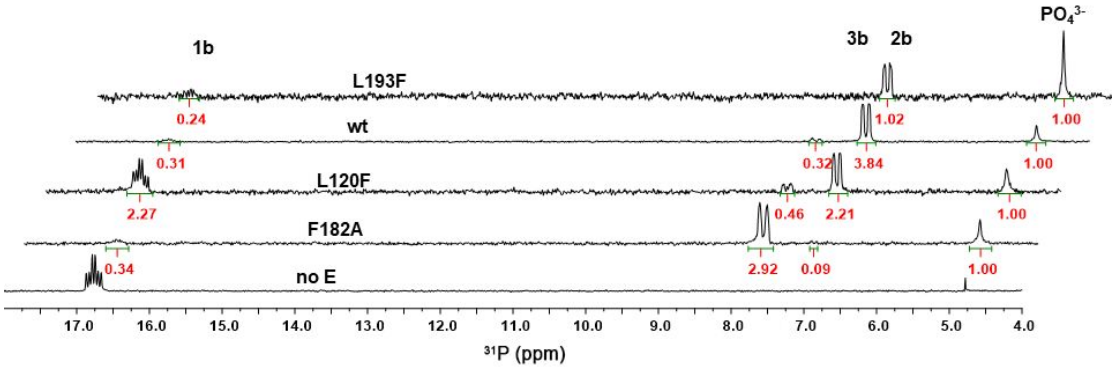


Figure S15. Selected ^{31}P-NMR spectra of the reactions of (*S*)-3-F_2-2-HPP (**1b**) with HppE and variants. The multiplets at ~16.8, ~7.8 and ~7.1 ppm are from (*S*)-3-F_2-2-HPP (**1b**), *trans*-3-F_2-Fos (3b**)**, and *cis*-3-F_2-Fos (**2b**), respectively. The single peak at ~4.8 ppm arises from the internal standard, PO_4^{3-}.

Table S10. ^{19}F-NMR analysis of the reactions of (*S*)-3-F_2-2-HPP (**1b**) with HppE and variants. Column headings are as described in the caption of Table S2.

Entry	E	[Fe-E] (mM)	[Sub] (mM)	Sub/IS	trans/IS	cis/IS	[Pdt] (mM)	TTN	cis(%)	Conv(%)
1	L193F	0.4	4	4.03±0.04	0.00±0.00	1.84±0.03	1.33±0.03	3.3±0.1	99.8±0.2	31.4±0.4
2	wt	0.7	3.6	0.20±0.10	0.21±0.10	4.64±0.07	3.49±0.05	5.0±0.1	95.7±1.9	93.4±0.2
3	L120F	0.4	4	3.89±0.01	0.42±0.00	1.87±0.02	1.64±0.02	4.1±0.0	81.7±0.3	37.0±0.3
4	F182A	0.8	3	0.93±0.45	3.12±0.58	0.06±0.03	2.29±0.44	2.9±0.5	1.6±0.8	76.7±11.9

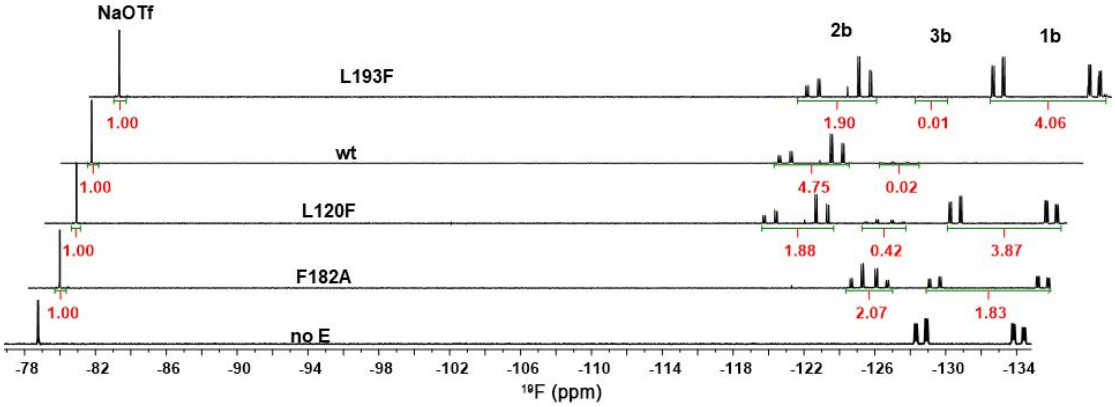


Figure S16. Selected ^{19}F-NMR spectra of the reactions of (*S*)-3-F_2-2-HPP (**1b**) with HppE and variants. The multiplets from ~ –117.7 ppm to ~ –121.4 ppm, ~ –123.6 ppm to ~ –125.7 ppm, and ~ –128.3 ppm to ~ –134.4 ppm, are from *cis*-3-F_2-Fos (**2b**), *trans*-3-F_2-Fos (**3b**) and (*S*)-3-F_2-2-HPP (**1b**), respectively. The single peak at ~ –78.7 ppm corresponds to the internal standard, NaOTf.

4.5 A racemic mixture of the (1*S*,2*S*)- and (1*R*,2*R*)-1-Cl-3-F$_3$-HPP (*rac*-1g) is converted by HppE into a mixture of the 2-ketone (4g), cis-epoxide (2g) and trans-epoxide (3g) products

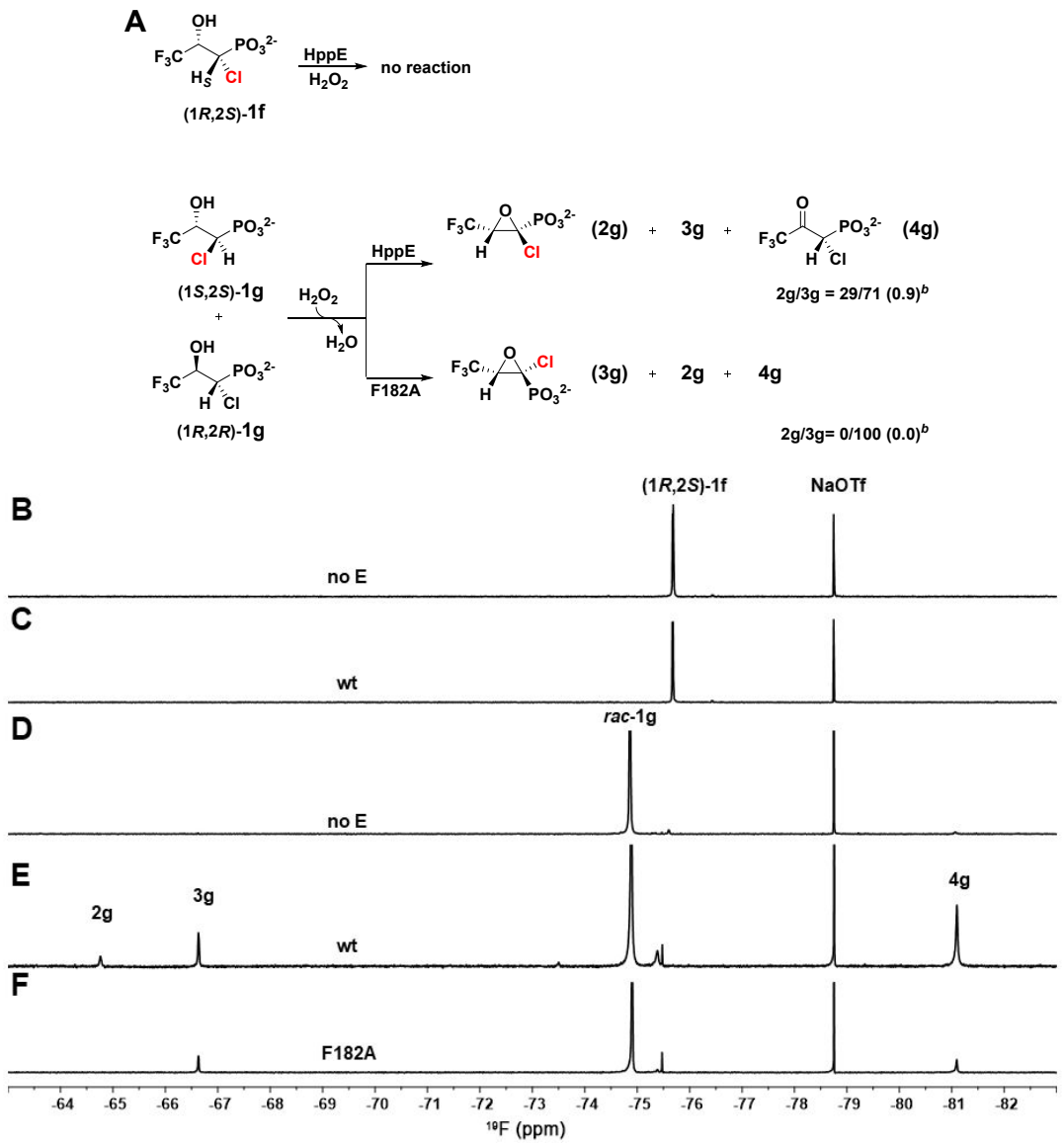
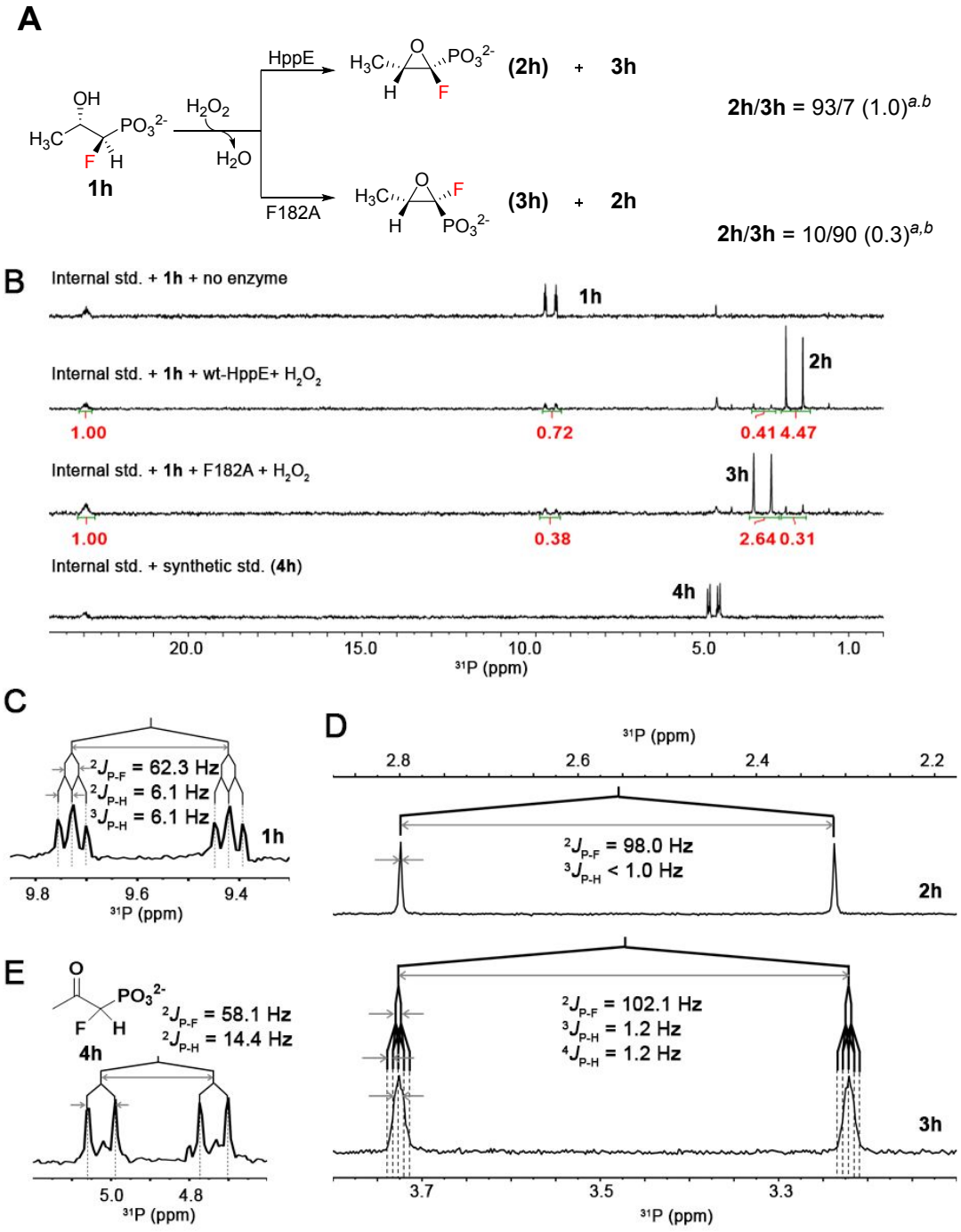


Figure S17. ^{19}F-NMR spectra of the reactions of (1*R*,2*S*)-1-Cl-3-F$_3$-HPP [(1*R*,2*S*)-**1f**] with HppE (***B***-***C***), *rac*-*anti*-1-Cl-3-F$_3$-HPP (*rac*-**1g**) with HppE and the F182 variant (***B***-***F***). The doublets at -64.7 and -66.6 are from *cis*- and *trans*-1-Cl-3-F$_3$-Fos (**2g** and **3g**), respectively. The peaks at -74.9, -75.7, -78.7, and -81.1 are from the substrates [*rac*-**1g** and (1*R*,2*S*)-**1f**], the internal standard NaOTf and 1-Cl-3-F$_3$-2-OPP (**4g**), respectively. Reaction schemes depicting formation of the cis and trans epoxides (**2g** and **3g**, respectively) by the two proteins (***A***).

4.6 (1*R*,2*S*)-1-F-HPP (1h) is readily cyclized by HppE and its F182A variant.



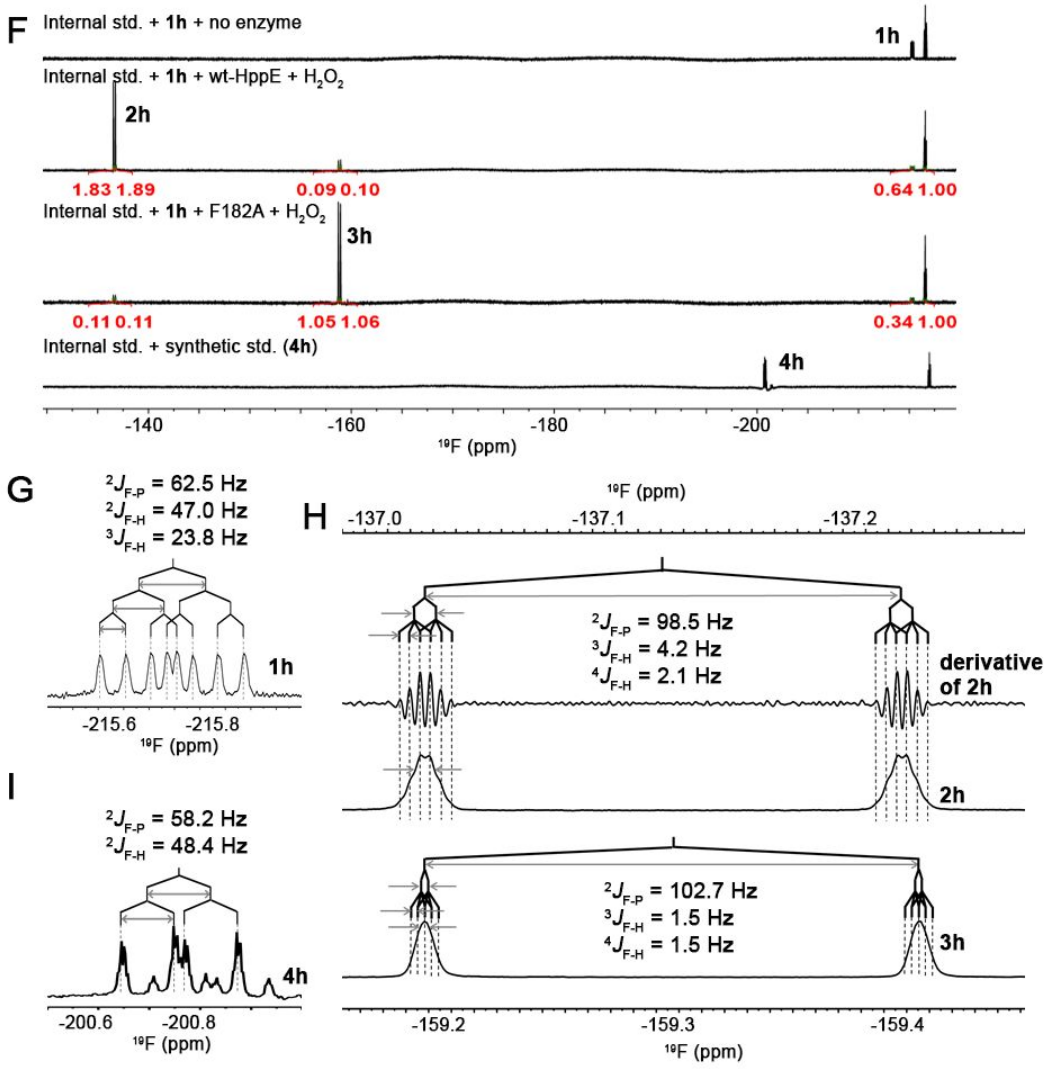


Figure S18. Analysis of the transformation of (*1R*,2*S*)-1-F-2-HPP (**1h**) by wild-type HppE and its F182A variant. (**A**) Reaction scheme depicting formation of the cis and trans epoxides (**2h** and **3h**, respectively) by the two proteins. (**B**) ^{31}P-NMR spectra of the reactions of **1h** with the wild-type and F182 proteins (*second and third spectra from top*) along with the spectra of the control reaction sample lacking any enzyme (*top*) and a sample of the synthetic 2-ketone standard (**4h**, *bottom*). (**C-E**) Blow-ups of the regions of the spectra in **B** showing: the ddd ($^2J_{P\text{-}F}$ = 62.3, $^2J_{P\text{-}H}$ = 6.1, $^3J_{P\text{-}H}$ = 6.1 Hz) features of **1h** at ~ 9.6 ppm (**C**); the doublet features of the cis (**2h**, *top*) and trans (**3h**, *bottom*) epoxide products at ~ 2.6 ppm ($^2J_{P\text{-}F}$ = 98.0, $^3J_{P\text{-}H}$ < 1.0 Hz) and ~ 3.5 ppm ($^2J_{P\text{-}F}$ = 102.1 Hz, $^3J_{P\text{-}H}$, $^4J_{P\text{-}H}$ = 1.2 Hz) (**D**); and the dd ($^2J_{P\text{-}F}$ = 58.1, $^2J_{P\text{-}H}$ =14.4 Hz) feature of the 2-ketone (**4h**) at ~ 4.9 ppm (**E**). (**F**) ^{19}F-NMR spectra of the reactions of **1h** with the wild-type and F182 proteins (*second and third spectra from top*) along with the spectra of the control reaction sample lacking any enzyme (*top*) and a sample of the synthetic 2-ketone

standard (**4h**, *bottom*). (**G-I**) Blow-ups of the regions of the spectra in **F** showing: the doublet of doublet of quartets of the cis (**2h**, *top*) and trans (**3h**, *bottom*) epoxide products at ~ –137.1 ppm ($^{2}J_{F\text{-}P}$ = 98.5, $^{3}J_{F\text{-}H}$ = 4.2, $^{4}J_{F\text{-}H}$ = 2.1) and ~ –159.3 ppm ($^{2}J_{F\text{-}P}$ = 102.5, $^{3}J_{F\text{-}H}$, $^{4}J_{F\text{-}H}$ = 1.5) (**G**); the dd feature ($^{2}J_{P\text{-}F}$ = 58.2, $^{2}J_{F\text{-}H}$ = 48.4 Hz) of the 2-ketone (**4h**) at ~ –200.8 ppm (**H**); and the ddd feature ($^{2}J_{F\text{-}P}$ = 62.5, $^{2}J_{F\text{-}H}$ = 47.0, $^{3}J_{F\text{-}H}$ = 23.8 Hz) of **1h** at ~ –215.7 ppm (**I**). The multiplet at 22.9 ppm in the ^{31}P-NMR spectrum and the triplet at –217 ppm in the ^{19}F-NMR spectrum arise from the internal standards, sodium propylphosphonate and sodium fluoroacetate, respectively.

5. Antimicrobial potencies of the (halogenated) cis- and trans-epoxide products

A bioautography assay[9-10] was used for the evaluation of antimicrobial potencies of the cis- and trans-epoxide products. As described above, *cis*-F_n-Fos (n = 1-2) and *trans*-F_n-Fos (n = 1-3) compounds were produced by the L193F and F182A variants, respectively. The *cis*-F_3-Fos was produced by the L193F/L144F variant. The concentration of each compound was quantified by NMR. Each compound was then diluted or concentrated to 1.5 mM. Agar plates were spread with 10^7 colony-forming units (cfu) of *Escherichia coli* DH5α (K12), a strain that is susceptible to Fos, and 5-mm filter discs were placed on top. A 10 μL aliquot of each 1.5 mM stock was used to wet one of the filter discs. Fos (1.5 mM) was used as the positive control, while the relevant substrate (1.5 mM) served as the negative control. The discs were subsequently incubated overnight at 37 °C. The antimicrobial potencies of the Fos analogs were normalized by comparing the zones of inhibition of the product group and positive control (Figure S20, 21). Each assay was performed in triplicate. The error bars correspond to the standard errors from the mean values of the three experiments. Experiments with 10 μL of 0.15, 0.375, 0.75, 1.125, 1.5 and 2.0 mM commercial Fos were used for quantification of the zone of inhibition (Figure S19).

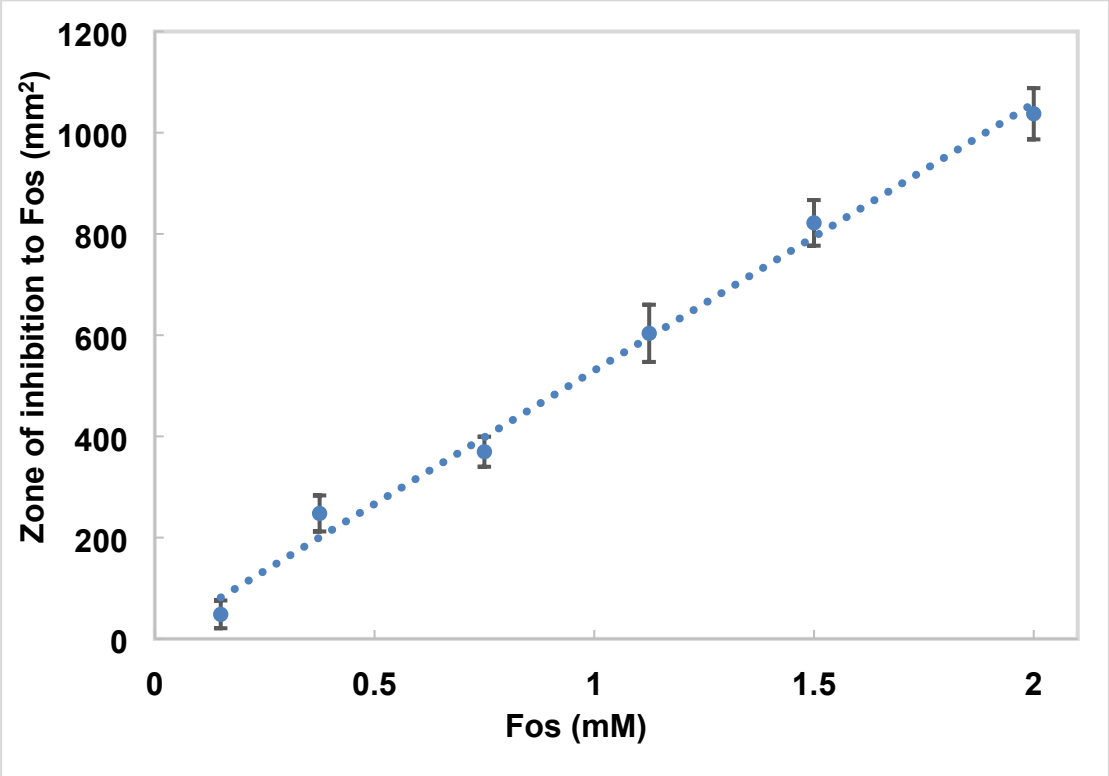
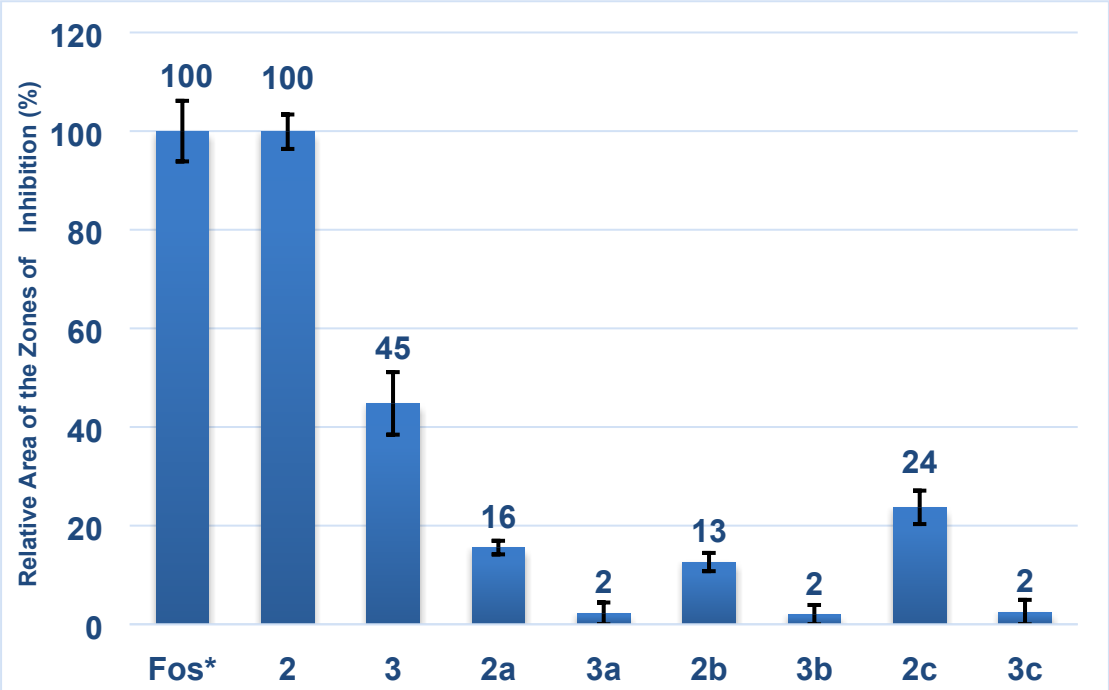
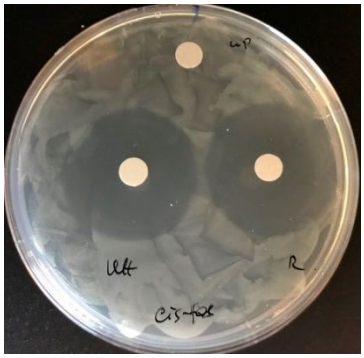


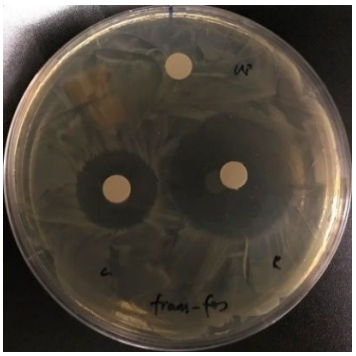
Figure S19. Standard curve relating area of the zone of inhibition to [Fos] (commercial compound).



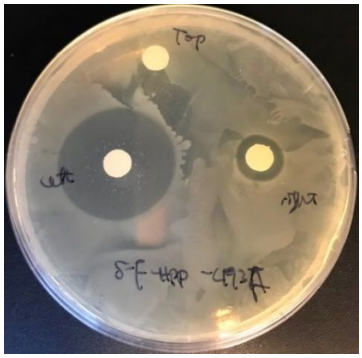
Figures S20. The relative areas of the zones of inhibition of *cis*/*trans*-F_n-Fos compounds relative to that generated by the same concentration of commercial Fos*.



(*S*)-HPP (top); Fos control (right); *cis*-Fos (**2**) (left)

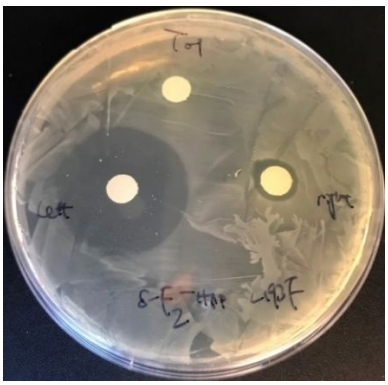


(*S*)-HPP (top); Fos control (right); *trans*-Fos (**3**) (left)

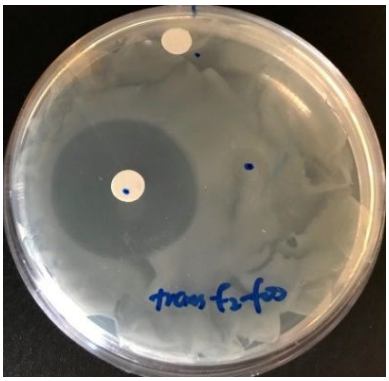


(*S*)-F-HPP (top); Fos control (left); *cis*-F-Fos (**2a**) (right)

(*S*)-F-HPP (top); Fos control (left); *trans*-F-Fos (**3a**) (right)



(*S*)-F_2-HPP (top); Fos control (left); *cis*-F_2-Fos (**2b**) (right)



(*S*)-F_2-HPP (top); Fos control (left); *trans*-F_2-Fos (**3b**) (right)

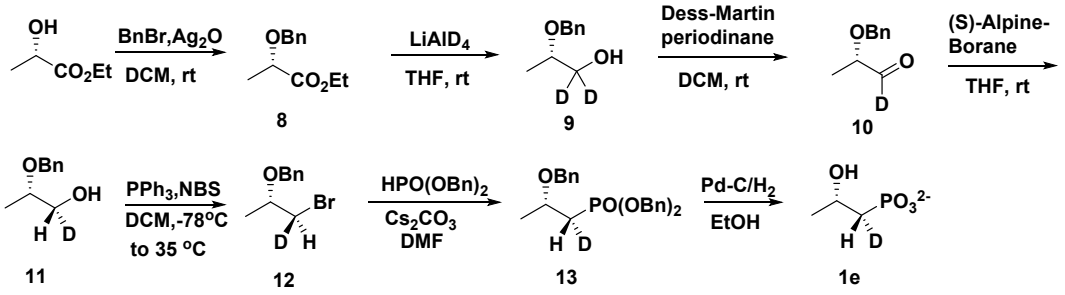
(*S*)-F_3-HPP (top); Fos control (left); *cis*-F_3-Fos (**2c**) (right)

(*S*)-F_3-HPP (top); Fos control (left); *trans*-F_3-Fos (**3c**) (right)

Figure S21. Representative agar plates for measuring the zones of inhibition.

6. Synthesis and characterization of the substrate and product standards.

6.1 Synthesis of 1-*d*-2*S*-HPP diastereomers (1d and 1e) using adaptations of previously published methods.[11-12]



Ethyl (*S*)-2-(benzyloxy)propanoate (8). To a solution of (*L*)-ethyl lactate (10 g, 84.65 mmol) in dry ether (100 mL) were added Ag_2O (29.42 g, 126.98 mmol) and BnBr (17.37 g, 101.58 mmol) at rt. The reaction was subsequently stirred for 16 h at 40 °C. After completion, it was filtered through a Celite pad and concentrated to obtain the crude compound. The material was purified via silica gel column chromatography (hexanes/ethyl acetate = 10/1) to afford compound **8** (13 g, 74%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.40 – 7.25 (m, 5H), 4.70 (d, *J* = 11.6 Hz, 1H), 4.45 (d, *J* = 11.6 Hz, 1H), 4.26 – 4.16 (m, 2H), 4.05 (q, *J* = 6.8 Hz, 1H), 1.44 (d, *J* = 6.9 Hz, 3H), 1.29 (t, *J* = 7.1 Hz, 3H).

(*S*)-2-(benzyloxy)propan-1,1-*d*$_2$-1-ol (9). Into a mixture of $LiAlD_4$ (1.13 g, 26.89 mmol) suspended in anhydrous THF (30 mL) was dripped a solution of ethyl (*S*)-2-(benzyloxy)propanoate (7 g, 33.61 mmol) in THF (30 mL) at rt. The reaction was then stirred for 1 h. After complete consumption of the substrate, water was dripped in to quench the reaction until no bubbles were generated. The mixture was subsequently filtered through a Celite pad and concentrated before purification by chromatography (hexanes/ethyl acetate = 1/1) to afford **9** (4 g, 72%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.27 (m, 5H), 4.65 (d, *J* = 11.6 Hz, 1H), 4.49 (d, *J* = 11.6 Hz, 1H), 3.67 (q, *J* = 6.3 Hz, 1H), 2.28 (s, 1H), 1.18 (d, *J* = 6.3 Hz, 3H).

(*S*)-2-(benzyloxy)propanal-1-*d* (10). To a stirred solution of (*S*)-2-(benzyloxy)propan-1,1-*d*$_2$-1-ol (4 g, 23.78 mmol) in DCM was added Dess martin periodinane (15.13 g, 35.66 mmol), and the reaction was stirred at rt for 3 hours. After completion, the reaction was washed thoroughly with saturated aqueous sodium thiosulfate, saturated aqueous sodium bicarbonate and brine. The organic layer was dried over

anhydrous sodium sulfate and concentrated *in vacuo*, and the resulting residue was used in the next step without further purification.

(1*S*,2*R*)-2-(benzyloxy)propan-1-*d*-1-ol (11). To a stirred solution of **10** (1.4 g, 8.47 mmol) in THF (30 mL) was added (*S*)-Alpine-Borane (25.4 mL, 12.71 mmol, 0.5 M solution in THF), and the resulting reaction mixture was stirred at rt overnight. Acetaldehyde (1.44 mL, 25.42 mmol) and ethanolamine (1.22 mL, 20.34 mmol) were subsequently added to quench the reaction. The resulting mixture was stirred at rt for 30 min and then diluted with ethyl acetate (150 mL). The organic layer was then washed thoroughly with 1M HCl, saturated aqueous sodium bicarbonate and brine before being dried over anhydrous sodium sulfate and concentrated *in vacuo*. The resulting residue was purified by silica gel chromatography (hexanes/ethyl acetate = 1/1), giving 1.2 g of the alcohol, **11**, as a colorless oil with a yield of 85%. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.37 – 7.29 (m, 5H), 4.65 (d, *J* = 11.6 Hz, 1H), 4.50 (d, *J* = 11.6 Hz, 1H), 3.70 – 3.62 (m, 1H), 3.58 (br, 1H), 2.44 (br, 1H), 1.18 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.50, 128.48, 127.77, 127.72, 75.55, 70.82, 65.92 (t, *J* = 21.78 Hz), 15.93.

((((1*S*,2*S*)-1-bromopropan-2-yl-1-*d*)oxy)methyl)benzene (12). Into a solution of *N*-bromosuccinimide (1.53 g, 8.61 mmol) in DCM (10 mL) was dripped a solution of triphenyl phosphine (2.26 g, 8.61 mmol) in DCM (15 mL) at -78 °C in the dark. The reaction was stirred at the same temperature for 10 min, while a solution of **11** (1.2 g, 7.18 mmol) in DCM (9 mL) was dripped in. Following removal of the cooling bath, the reaction was first stirred first at rt for 1 h and subsequently at 35 °C for an additional 30 min. The mixture was subsequently concentered *in vacuo* and purified by silica gel chromatography (hexanes/DCM = 1/1) to afford the bromide, **12** (1.35g, 82%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.49 – 7.28 (m, 5H), 4.61 (br, 2H), 3.79 – 3.74 (m, 1H), 3.48 – 3.46 (m, 1H), 1.34 (d, *J* = 6.2 Hz, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 138.19, 138.18, 128.49, 127.79, 127.76, 74.14, 71.13, 36.33(t, *J* = 23.11 Hz), 18.95.

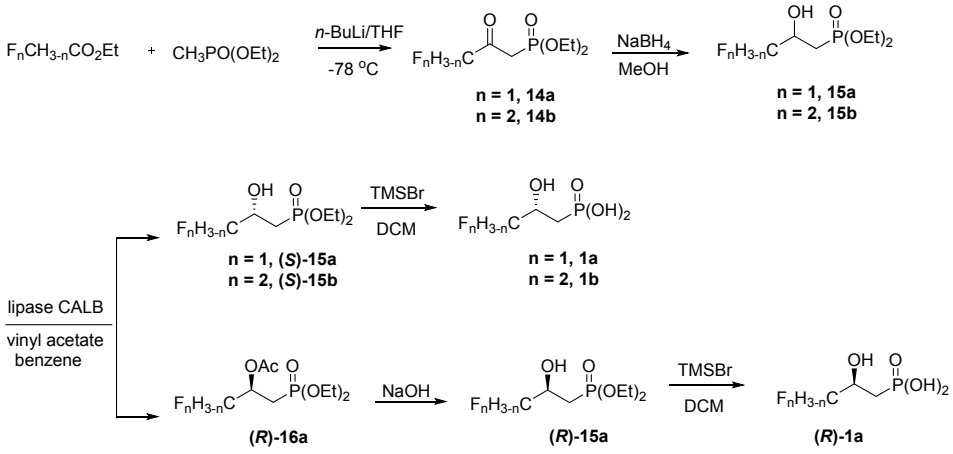
Dibenzyl ((1*R*,2*S*)-2-(benzyloxy)propyl-1-*d*)phosphonate (13). To a stirred solution of dibenzyl phosphonate (0.957 g, 3.65 mmol) and **12** (0.7 g, 3.04 mmol) in DMF (20 mL) was added cesium carbonate (1.98 g, 6.08 mmol). The resulting suspension was the stirred at rt for 5 h before being quenched by addition of water (100 mL). The reaction mixture was subsequently extracted with EtOAc (100 mL × 3), the organic phase was concentrated *in vacuo*, and the resulting residue was purified by

silica gel chromatography (hexanes/ethyl acetate = 1/1) to afford **13** (0.8 g, 64%) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.41 – 7.24 (m, 15H), 5.11 – 4.90 (m, 4H), 4.53 (d, *J* = 11.4 Hz, 1H), 4.46 (d, *J* = 11.4 Hz, 1H), 4.00 – 3.90 (m, 1H), 1.99 (dd, *J* = 18.2, 6.7 Hz, 1H), 1.34 (d, *J* = 6.1 Hz, 3H). ^{31}P NMR (202 MHz, $CDCl_3$) δ 29.81.

((1*R*,2*S*)-2-hydroxypropyl-1-*d*)phosphonate (1e). Compound **13** (440 mg, 1.07 mmol) and Pd/C (50 mg, 10% palladium on carbon) were suspended in ethanol (30 mL) and stirred under a hydrogen atmosphere overnight. After completion, the Pd/C was removed via filtration through a Celite pad. Sodium hydroxide (213 μL of 10 M in water, 2.13 mmol) was then added to the filtrate. The resulting mixture was stirred at rt for 30 min and dried to afford the product (150 mg, 76%).^{1}H NMR (500 MHz, D_2O) δ 4.08 (m, *J* = 6.6 Hz, 1H), 1.75 (dd, *J* = 17.4, 7.2 Hz, 1H), 1.25 (d, *J* = 6.2 Hz, 3H) (**Figure S2**). ^{13}C NMR (126 MHz, D_2O) δ 64.31, 37.14 (dt, *J* = 129.0, 19.0 Hz), 23.03 (d, *J* = 8.3 Hz). HRMS (ESI): calcd for $C_3H_7DO_4P$ ($M\text{-}H^-$) 140.0228, found 140.0234.

((1*S*,2*S*)-2-hydroxypropyl-1-*d*)phosphonate (1d) was synthesized following the same procedure except using (*R*)-alpine borane instead of the (*S*) enatntiomer. ^{1}H NMR (500 MHz, D_2O) δ 4.14 – 4.07 (m, 1H), 1.86 (dd, *J* = 18.0, 6.1 Hz, 1H), 1.27 (d, *J* = 6.2 Hz, 3H) (**Figure S2**). ^{13}C NMR (126 MHz, D_2O) δ 64.33, 37.15 (dt, *J* = 129.0, 18.8 Hz), 23.03 (d, *J* = 8.4 Hz). HRMS (ESI): calcd for $C_3H_7DO_4P$ ($M\text{-}H^-$) 140.0228, found 140.0234.

6.2 Synthesis of (*S*)-3-F-2-HPP (1a) and (*S*)-3,3-F_2-2-HPP (1b).



Diethyl (3-fluoro-2-oxopropyl)phosphonate (14a). To a mixture of THF (30 mL) and diethyl

methylphosphonate (5 g, 32.87 mmol) cooled to -78 °C was gradually added *n*-BuLi (19 mL, 49.30 mmol, 2.6 M in *n*-hexane) and ethyl 2-fluoroacetate (3.49 g, 32.87 mmol). The reaction temperature was kept under -65 °C while the mixture was then stirred for an additional 3 h, before quenching via addition of 1 *N* HCl (30 mL). The mixture was extracted with ether, washed with aqueous $NaHCO_3$, and dried over Na_2SO_4. Subsequent concentration under reduced pressure yielded an oily residue that was purified by flash chromatography on silica gel with hexanes/ethyl acetate (1/1) as the eluting solvent to give **14a** (3.8 g, 54%). ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.91 (d, J = 47.3 Hz, 2H), 4.15 (p, J = 7.6, 7.1 Hz, 4H), 3.19 (dd, J = 22.9, 3.5 Hz, 2H), 1.33 (t, J = 7.0 Hz, 6H). ^{31}P NMR (146 MHz, $CDCl_3$) δ 18.04. ^{19}F NMR (282 MHz, $CDCl_3$) δ -225.01 (t, J = 47.2 Hz).

Diethyl (3-fluoro-2-hydroxypropyl)phosphonate (*rac*-15a). To a solution of **14a** (2 g, 9.43 mmol) in methanol (20 mL), which was maintained at ~0-5° C via the use of an ice bath, was gradually added $NaBH_4$ (1.07 g, 28.28 mmol). After 10 min, the reaction mixture was quenched by addition of saturated NH_4Cl (30 mL) and extracted by DCM (3 ×50 mL). The organic layer was subsequently concentrated and purified by chromatography to afford **15a** as a colorless oil (1.90 g, 94%). ^{1}H NMR (360 MHz, $CDCl_3$) δ 4.39 (dd, J = 47.1, 4.8 Hz, 2H), 4.30 – 3.96 (m, 5H), 3.03 (br, 1H), 2.15 (dd, J = 17.9, 5.9 Hz, 2H), 1.33 (t, J = 7.0 Hz, 6H). ^{19}F NMR (471 MHz, $CDCl_3$) δ -228.67 (tdd, J = 47.1, 17.5, 2.9 Hz). ^{31}P NMR (146 MHz, $CDCl_3$) δ 29.03.

Diethyl (*S*)-(3-fluoro-2-hydroxypropyl)phosphonate ((*S*)-15a) and (*R*)-1-(diethoxyphosphoryl)-3-fluoropropan-2-yl acetate ((*R*)-16a). Following published procedures,[3] *Candida antarctica*: lipase B (Novozyme 435) (2.0 g) and vinyl acetate (5.2 mL, 46.69 mmol) were added to a solution of **15a** (1.0 g, 4.57 mmol) in 40 mL benzene. The reaction was then stirred at rt, and the progress of the reaction was monitored by ^{31}P NMR spectroscopy. Once the reaction reached ~ 50% conversion, the reaction mixture was subsequently filtered through a Celite pad. Concentration under reduced pressure yielded an oily residue that was purified and separated by silica gel chromatography with DCM/acetone (4/1~1/1) as the eluting solvent to give a mixture of (*S*)-**15a** (0.45 g, 45%) and (*R*)-**16a** (0.55 g, 46%) as a colorless oil.

(*S*)-**15a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.48 – 4.31 (m, 2H), 4.29 – 4.03 (m, 5H), 3.67 (br, 1H), 2.05 – 1.97 (m, 2H), 1.33 (td, J = 7.0, 1.6 Hz, 6H). ^{31}P NMR (202 MHz, $CDCl_3$) δ 28.98. ^{19}F NMR (471 MHz,

$CDCl_3$) δ -228.86 (tdd, *J* = 47.1, 17.2, 3.0 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 86.09 (dd, *J* = 172.0, 16.7 Hz), 65.61 (dd, *J* = 21.1, 4.4 Hz), 62.25 (dd, *J* = 14.8, 6.5 Hz), 29.26 (dd, *J* = 141.1, 5.6 Hz), 16.51 (dd, *J* = 6.0, 3.0 Hz). The e.e. of (*S*)-**15a** was determined to be 99% (**Figure S22**).

(*R*)-**16a**. ^{1}H NMR (500 MHz, $CDCl_3$) δ 5.38 – 5.17 (m, 1H), 4.54 (dd, *J* = 47.4, 4.9, 3.3 Hz, 2H), 4.15 – 4.06 (m, 4H), 2.17 (ddd, *J* = 19.2, 6.9, 3.6 Hz, 2H), 2.09 (s, 3H), 1.33 (t, *J* = 7.1 Hz, 6H). ^{31}P NMR (202 MHz, $CDCl_3$) δ 25.29. ^{19}F NMR (471 MHz, $CDCl_3$) δ -231.17 (td, *J* = 47.1, 22.4 Hz). ^{13}C NMR (125 MHz, $CDCl_3$) δ 170.12, 83.38 (dd, *J* = 174.7, 7.6 Hz), 67.88 (d, *J* = 19.9 Hz), 62.21 (dd, *J* = 6.4, 2.1 Hz), 26.72 (dd, *J* = 141.7, 6.4 Hz), 21.11, 16.50 (d, *J* = 6.1 Hz).

(*R*)-15a. A solution of (*R*)-**16a** (0.2 g, 0.78 mmol) in NH_3/MeOH (2 M, 7 mL) was stirred for 48 h at rt.[13] The solvent was subsequently removed under reduced pressure, and the remaining residue was purified on silica gel (DCM/acetone = 1/1) to afford (*R*)-**15a** as a colorless oil in 92% yield. The ^{1}H, ^{31}P, ^{19}F, and ^{13}C NMR spectra of (*R*)-**15a** are identical to those of (*S*)-**15a**. The e.e. of (*R*)-**15a** was determined to be 92% (**Figure S22**).

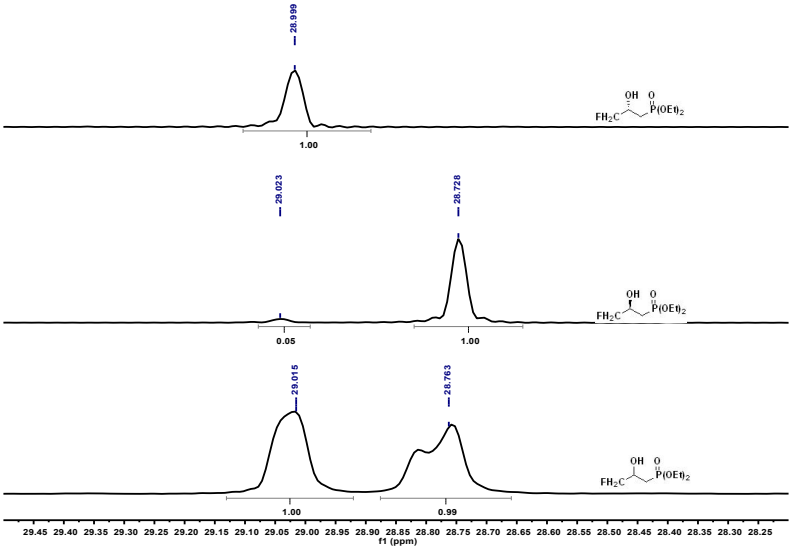


Figure S22. Determination of the e.e. of (*R*)-**15a** (92%, middle spectrum) and (*S*)-**15a** (99%, top spectrum) by ^{31}P NMR, utilizing quinine as a chiral solvating agent[14-15]. Each sample contained 50 mg of quinine and 15 mg of substrate dissolved in 0.6 mL of $CDCl_3$.

(*S*)-(3-fluoro-2-hydroxypropyl)phosphonate (1a). TMSBr (0.71 g, 4.62 mmol) and allyl

trimethylsilane (0.27 g, 2.38 mmol) were added to a solution of (*S*)-**15a** (0.3 mg, 1.4 mmol) in CH_2Cl_2 (20 mL) at rt, and the solution was stirred overnight. The solvent was then removed under reduced pressure, and the resulting residue was resuspended in $CHCl_3$ (20 mL) and water (20 mL) before being neutralized with NH_4HCO_3. The aqueous layer was subsequently collected and lyophilized to afford (*S*)-**1a** as a white solid (0.18 g, 80%). ^{1}H NMR (500 MHz, D_2O) δ 4.56 (ddd, *J* = 46.9, 10.0, 2.6 Hz, 1H), 4.40 (ddd, *J* = 47.5, 10.0, 6.2 Hz, 1H), 4.23 – 4.12 (m, 1H), 1.91 – 1.78 (m, 2H). ^{31}P NMR (202 MHz, 100 mM NaOD in D_2O) δ 17.39 (m, *J* = 16.3, 8.3, 2.5 Hz). ^{19}F NMR (471 MHz, 100 mM NaOD in D_2O) δ -227.29 (m, *J* = 47.1, 20.8, 3.3 Hz). ^{13}C NMR (126 MHz, D_2O) δ 86.76 (dd, *J* = 167.2, 10.5 Hz), 66.64 (d, *J* = 19.3 Hz), 30.77 (dd, *J* = 131.4, 7.1 Hz). The ^{1}H, ^{31}P, ^{19}F, and ^{13}C NMR spectra of (*R*)-**1a** were identical to those of (*S*)-**1a**. HRMS (ESI): calcd for $C_3H_7FO_4P$ ($M\text{-}H^-$) 157.0071, found 157.0076.

Compound (*S*)-3,3-F_2-2-HPP (**1b**) was prepared using the same procedure as for **1a**, except that ethyl 2,2-difluoroacetate was used as the starting material. ^{1}H NMR data for intermediates and product are summarized below.

Diethyl (3,3-difluoro-2-oxopropyl)phosphonate (14b). ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.88 (t, *J* = 53.7 Hz, 0.5 H), 5.54 (t, *J* = 55.8 Hz, 0.5 H), 4.19 – 4.12 (m, 4H), 3.32 (d, *J* = 22.6 Hz, 1H), 2.21 (d, *J* = 18.8 Hz, 1H), 1.32 (t, *J* = 7.0 Hz, 6H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -128.60 (d, *J* = 53.7 Hz), -134.23 (dd, *J* = 56.0, 6.9 Hz). ^{31}P NMR (146 MHz, $CDCl_3$) δ 27.76, 17.37.

Diethyl (3,3-difluoro-2-hydroxypropyl)phosphonate (*rac*-15b). ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.75 (t, *J* = 55.9 Hz, 1H), 4.19 – 4.08 (m, 5H), 3.67 (br, 1H), 2.14 – 1.95 (m, 2H), 1.33 (t, *J* = 7.1 Hz, 6H). ^{31}P NMR (146 MHz, $CDCl_3$) δ 28.73. ^{19}F NMR (282 MHz, $CDCl_3$) δ -128.58 (ddd, *J* = 285.0, 55.6, 8.5 Hz), -133.37 (ddd, *J* = 285.1, 56.5, 13.4 Hz).

Diethyl (*S*)-(3,3-difluoro-2-hydroxypropyl)phosphonate ((*S*)-15b). Following the same procedure for the kinetic resolution of (*S*)-**15a**, (*S*)-**15b** was obtained in 45% yield and 99% e.e. (**Figure S23**).

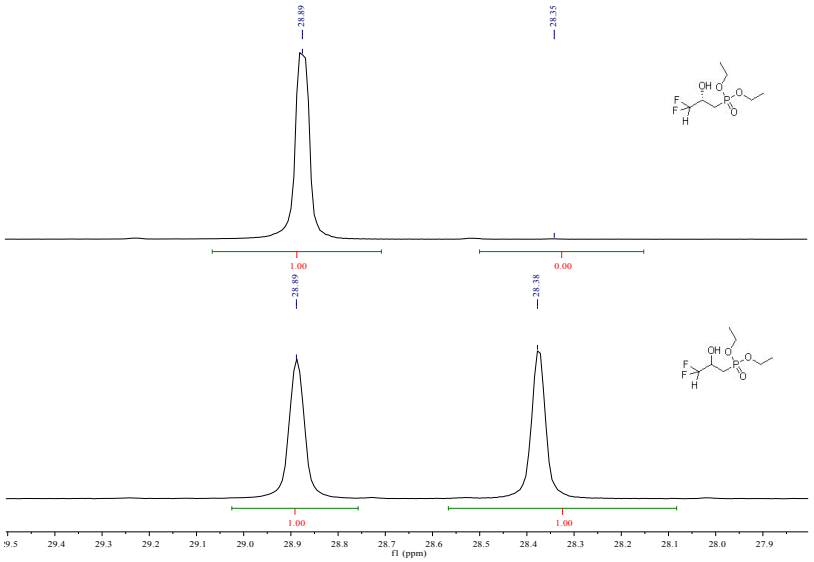
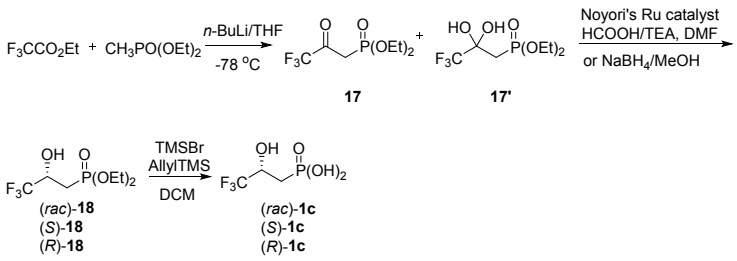


Figure S23. Determination of the e.e. of (*S*)-**15b** (99%, top spectrum) by ^{31}P NMR with quinine as a chiral solvating agent.[14-15] Each sample contained 50 mg of quinine and 15 mg of substrate dissolved in 0.6 mL of $CDCl_3$.

(*S*)-(3,3-difluoro-2-hydroxypropyl)phosphonate (1b). Analogously to the preparation of (*S*)-**1a**, (*S*)-**1b** was obtained in 87% yield. ^{1}H NMR (500 MHz, D_2O) δ 5.91 (m, *J* = 55.2, 2.4 Hz, 1H), 4.20 – 4.03 (m, 1H), 2.03 – 1.79 (m, 2H). ^{19}F NMR (471 MHz, 100 mM NaOD in D_2O) δ -129.12 (ddd, *J* = 279.5, 54.9, 8.9 Hz), -134.11 (ddd, *J* = 279.8, 55.3, 16.1 Hz). ^{31}P NMR (202 MHz, 100 mM NaOD in D_2O) δ 16.77 (m, *J* = 16.6, 8.3 Hz). ^{13}C NMR (126 MHz, D_2O) δ 115.72 (td, *J* = 242.2, 14.1 Hz), 66.89 (td, *J* = 22.6, 2.7 Hz), 29.06 (ddd, *J* = 133.3, 4.0, 2.2 Hz). HRMS (ESI): calcd for $C_3H_6F_2O_4P$ ($M\text{-}H^-$) 174.9977, found 174.9983.

6.3 Synthesis of (*S*)-3,3,3-F_3-HPP (1c).



Diethyl (3,3,3-trifluoro-2-oxopropyl)phosphonate (17) and diethyl (3,3,3-trifluoro-2,2-dihydroxypropyl)phosphonate (17'). The published procedures of Yuan *et al.* were followed.[16] To a mixture of THF (60 mL) and diethyl methylphosphonate (15 g, 98 mmol) cooled to -78 °C were gradually

added *n*-BuLi (1.6 mol) in *n*-hexane (67 mL) and ethyl trifluoroacetate (14.5 g, 105 mmol). The reaction temperature was kept under -65 °C while the mixture was stirred for an additional 3 h before it was quenched by addition of 1*N* HCl (30 mL). The reaction mixture was then extracted with ether, washed with aqueous $NaHCO_3$ solution, and dried over Na_2SO_4. Subsequent concentration under reduced pressure gave an oily residue that was purified by flash chromatography on silica gel with hexanes/ethyl acetate (1/1) as the eluting solvent to yield **17** and **17'** in a ratio of 13/87 as crystalline product (8.5 g, 70%). ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.70 (br, s, 2H, $C(OH)_2CH_2P$), 4.18 (q, 4H, $P(OCH_2CH_3)_2$), 3.37 (d, J = 22.7 Hz, 2H, $COCH_2P$), 2.30 (d, J = 19.3 Hz, 2H, $C(OH)_2CH_2P$), 1.34 (t, J = 7.1 Hz, 6H, $P(OCH_2CH_3)_2$). ^{19}F NMR (282 MHz, $CDCl_3$) δ -79.20, -87.71($CF_3C(OH)_2$). ^{31}P NMR (146 MHz, $CDCl_3$) δ 26.16 ($C(OH)_2CH_2P$), 15.75.

Diethyl (*S*)-(3,3,3-trifluoro-2-hydroxypropyl)phosphonate ((*S*)-18). The synthesis was carried out in accordance with published procedures.[17-18] To an 8-mL DMF solution of RuCl(*p*-cymene)[(*R*,*R*)-Ts-DPEN] (100 mg, 4%) was added the **17** and **17'** (1 g, 4 mmol), followed by addition of HCO_2H/Et_3N (v/v = 5/2, 2.08 mL). The mixture was then stirred at 28 °C under N_2, and the evolution of the reaction was monitored by TLC. The reaction mixture was subsequently diluted with water (40 mL) and extracted with ethyl acetate (40 mL × 3). The combined organic layer was then dried over $MgSO_4$ and concentrated before the crude product was purified by flash chromatography on silica gel (elution gradient from hexanes/ethyl acetate =1/1 to 100% ethyl acetate) to afford (*S*)-**18** (700 mg, 69%) as an oil with 99% enantiomeric excess (e.e.) as determined by ^{31}P NMR using quinine as chiral solvating agent[14-15] (**Figure S24**), ^{31}P NMR (146 MHz, $CDCl_3$) δ 27.95. ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.21 (d, J = 5.1 Hz, 1H), 4.40 – 4.30 (m, 1H), 4.19 – 4.06 (m, 4H), 2.16 – 2.00 (m, 2H), 1.36 – 1.31 (m, 6H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -81.16 (d, J = 6.5 Hz).

Diethyl (*R*)-(3,3,3-trifluoro-2-hydroxypropyl)phosphonate ((*R*)-18). (*R*)-**18** was synthesized following the same procedure as for (*S*)-**18**, except RuCl(*p*-cymene)[(*S*,*S*)-Ts-DPEN] was used as the catalyst. ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.24 (br, 1H), 4.40 – 4.31 (m, 1H), 4.21 – 4.08 (m, 4H), 2.18 – 2.04 (m, 2H), 1.38 – 1.31 (m, 6H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -81.11 (d, J = 6.4 Hz). The e.e. of (*R*)-**18** was determined to be 99% (**Figure S24**), ^{31}P NMR (146 MHz, $CDCl_3$) δ 27.06.

Diethyl (*rac*)-(3,3,3-trifluoro-2-hydroxypropyl)phosphonate ((*rac*)-18). To a solution of the mixture of **17** and **17'** (0.5 g, 2.02 mmol) in methanol (10 mL), maintained at ~0-5° C via the use of an ice bath, was gradually added $NaBH_4$ (0.23 g, 6.05 mmol). After 5 min, the reaction mixture was concentrated and purified by chromatography to afford 0.49 g of (*rac*)-**18** as a colorless oily liquid with a yield of 97.5%. ^{1}H NMR ($CDCl_3$) δ 5.72 (br, 1H), 4.41 – 4.30 (m, 1H), 4.16 – 4.06 (m, 4H), 2.21 – 2.02 (m, 2H), 1.34 – 1.29 (m, 6H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -81.10 (d, J = 6.1 Hz). ^{31}P NMR (146 MHz, $CDCl_3$) δ 27.44.

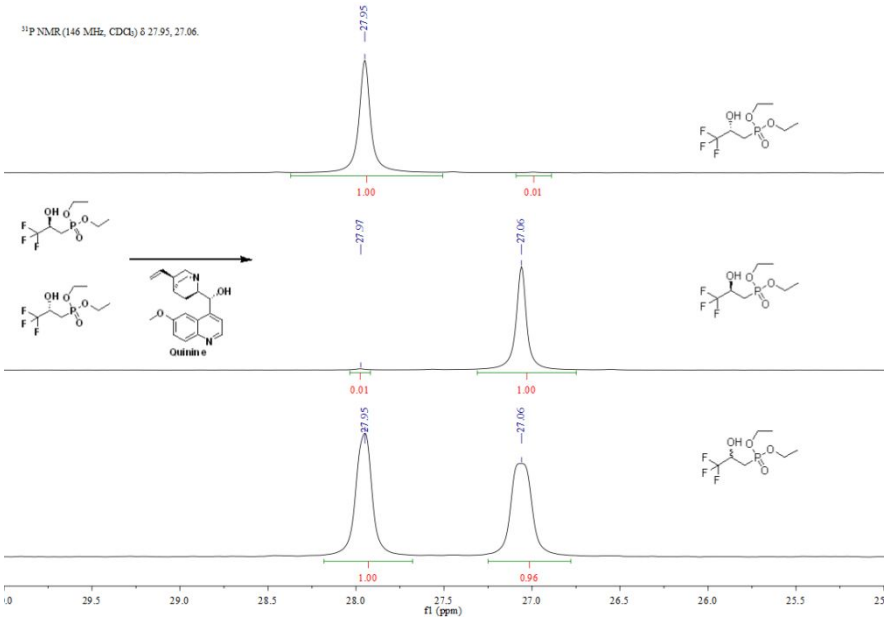


Figure S24. Determination of the e.e. of (*S*)-**18** (99%, top spectrum) and (*R*)-**18** (99%, middle spectrum) by ^{31}P NMR with quinine as a chiral solvating agent.[14-15] Each sample contained 50 mg of quinine and 15 mg of substrate dissolved in 0.6 mL of $CDCl_3$.

(*S*)-(3,3,3-trifluoro-2-hydroxypropyl)phosphonic acid ((*S*)-1c). TMSBr (0.61 g, 3.96 mmol) and allyl trimethylsilane (0.23 g, 2.04 mmol) were added to a solution of (*S*)-**18** (0.3 mg, 1.20 mmol) in CH_2Cl_2 (20 mL) at rt, and the solution was stirred overnight. The solvent was subsequently removed under reduced pressure. The residue was resuspended in a solution of $CHCl_3$ (20 mL) and water (20 mL). Following neutralization with ammonium bicarbonate, the aqueous layer was collected and lyophilized to afford (*S*)-**1c** as a white solid (0.22 g, 80%). The e.e. was determined to be 99% using α-cyclodextrin as chiral solvating agent[19-20] (**Figure S25**). ^{1}H NMR (360 MHz, D_2O) δ 4.28 – 4.16 (m, 1H), 1.85 – 1.57 (m, 2H). ^{13}C NMR (75 MHz, D_2O) δ 125.49 (qd, J = 281.2, 19.6 Hz), 66.88 (qd, J =

32.1, 4.1 Hz), 28.78 (d, J = 134.7 Hz). ^{19}F NMR (282 MHz, $CDCl_3$) δ -81.02 (d, J = 6.7 Hz). ^{31}P NMR (146 MHz, $CDCl_3$) δ 16.79. HRMS (ESI): calcd for $C_3H_5F_3O_4P$ ($M-H^-$) 192.9883, found 192.9890.

(*R*)-(3,3,3-trifluoro-2-hydroxypropyl)phosphonic acid ((*R*)-1c). The ^{1}H, ^{31}P, ^{19}F and ^{13}C NMR spectra of (*R*)-**1c** were identical to those of (*S*)-**1c**, and the e.e. of (*R*)-**1c** was likewise determined to be 99% (**Figure S25**).[19-20]

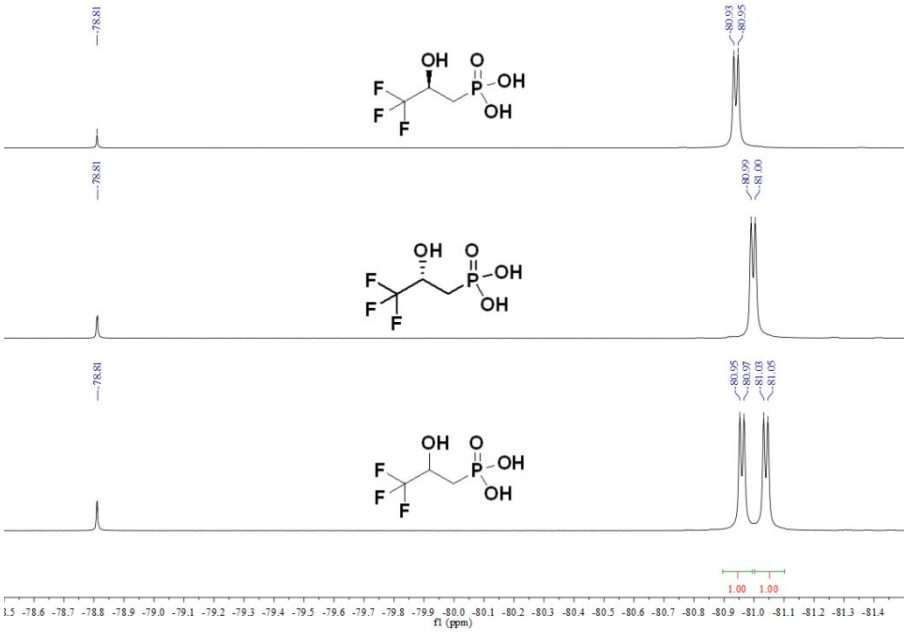
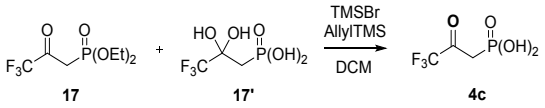


Figure S25. Determination of the e.e. of (*S*)-**1c** (99%, middle spectrum) and (*R*)-**1c** (99%, top spectrum) by ^{19}F NMR utilizing α-cyclodextrin as a chiral solvating agent[19-20] and NaOTf as an internal standard. Each sample contained 40 mg of α-cyclodextrin and 3.6 mM of substrate dissolved in 0.5 mL of D_2O.

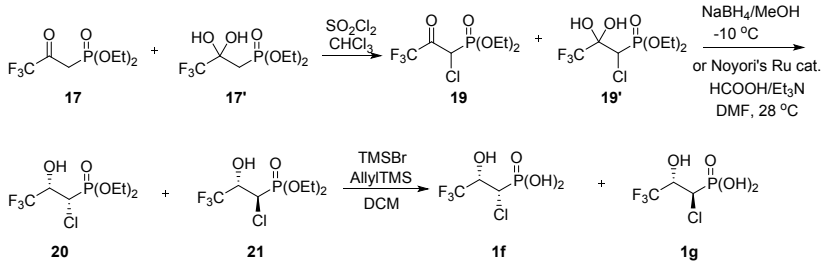
6.4 Synthesis of 3,3,3-F_3-2-OPP (4c).



17 **17'** **4c**

(3,3,3-trifluoro-2,2-dihydroxypropyl)phosphonate (4c). Following the same procedure as for the synthesis of (*S*)-**1a**, compound **4c** was obtained in 70% yield using the mixture of **17** and **17'** as the starting material. ^{1}H NMR (360 MHz, D_2O) δ 2.02 (d, J = 17.2 Hz, 2H). ^{31}P NMR (146 MHz, D_2O) δ 16.02. ^{19}F NMR (282 MHz, D_2O) δ -87.28. ^{13}C NMR (75 MHz, D_2O) δ 123.23 (qd, J = 285.6, 15.9 Hz), 92.34 (qd, J = 33.7, 8.0 Hz), 31.03 (d, J = 122.9 Hz). HRMS (ESI): calcd for $C_3H_3F_3O_4P$ ($M-H^-$) 190.9726, found 190.9733.

6.5 Synthesis of (1*R*,2*S*)-1-Cl-3,3,3-F_3-HPP (1f) and *anti*-1-Cl-3,3,3-F_3-HPP (1g).



Diethyl (1-chloro-3,3,3-trifluoro-2-oxopropyl)phosphonate (19) and diethyl (1-chloro-3,3,3-trifluoro-2,2-dihydroxypropyl)phosphonate (19'). Following published procedures of Marocco *et al.*,[4] sulfuryl chloride (977 μL, 12.09 mmol) was dripped into a solution of **17** and **17'** (3 g, 12.09 mmol) in 24 mL of $CHCl_3$ at 0 °C. The reaction was then allowed to stir for 4 h at rt before being quenched by the addition of water (25 mL) and extracted with methylene chloride (3 × 25 mL). The combined organic layers were subsequently dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude oil obtained was purified by flash chromatography (DCM/acetone = 8/1) to give 2.5 g of **19** and **19'** as an oil in a ratio of 1/10 and 73% overall yield. ^{1}H NMR (360 MHz, $CDCl_3$) δ 6.44 (br, 1H, $C(OH)_2CHClP$), 5.48 (br, 1H, $C(OH)_2CHClP$), 5.04 (d, J = 17.7 Hz, $COCHClP$), 4.38 – 4.20 (m, 4H, $P(OCH_2CH_3)_2$), 4.16 (d, J = 14.1 Hz, 1H, $C(OH)_2CHClP$), 1.40 – 1.35 (m, 6H, $P(OCH_2CH_3)_2$). ^{19}F NMR (282 MHz, $CDCl_3$) δ -75.68, -81.84 ($CF_3C(OH)_2CHClP$). ^{31}P NMR (146 MHz, $CDCl_3$) δ 17.23 ($CF_3C(OH)_2CHClP$), 9.35.

Diethyl ((1*R*,2*S*)-1-chloro-3,3,3-trifluoro-2-hydroxypropyl)phosphonate ((1*R*,2*S*)-**20) and Diethyl ((1*S*,2*S*)-1-chloro-3,3,3-trifluoro-2-hydroxypropyl)phosphonate (**(1*S*,2*S*)-**21)**. The synthesis was carried out in accordance with published procedures.[17-18] To an 8-mL DMF solution of RuCl(*p*-cymene)[(*R*,*R*)-Ts-DPEN] (89.77 mg, 4%) was added the **19** and **19'** (1 g, 3.54 mmol) followed by the addition of HCO_2H/Et_3N (v/v = 5/2, 1.83 mL). The mixture was then stirred at 28 °C under N_2, and the evolution of the reaction was monitored by TLC. The reaction solution was diluted with water (40 mL) and extracted with ethyl acetate (40 mL × 3). The combined organic layer was subsequently dried over $MgSO_4$ and concentrated. The crude product was purified by flash chromatography on silica gel (elution gradient from hexanes/ethyl acetate =1/1 to 100% ethyl acetate) to afford (1*R*,2*S*)-**20** and (1*S*,2*S*)-**21** (350 mg, 35%) in a ratio (d.r.) of 92/8 as an oil. The e.e. value (98%) of (1*R*,2*S*)-**20** was determined by ^{31}P NMR with quinine as the chiral solvating agent[14-15] (**Figure S26**). ^{1}H NMR (360 MHz, $CDCl_3$) δ 5.11

(br, 1H), 4.65 (p, J = 6.7 Hz, 1H), 4.34 – 4.15 (m, 5H), 1.36 (td, J = 7.1, 2.6 Hz, 6H). ^{19}F NMR (282 MHz, $CDCl_3$) δ -76.19 (d, J = 6.1 Hz, (1*S*,2*S*)-**21**), -76.37 (dd, J = 6.6, 2.7 Hz, (1*R*,2*S*)-**20**). ^{31}P NMR (146 MHz, $CDCl_3$) δ 16.83 ((1*S*,2*S*)-**21**), 16.39((1*R*,2*S*)-**20**).

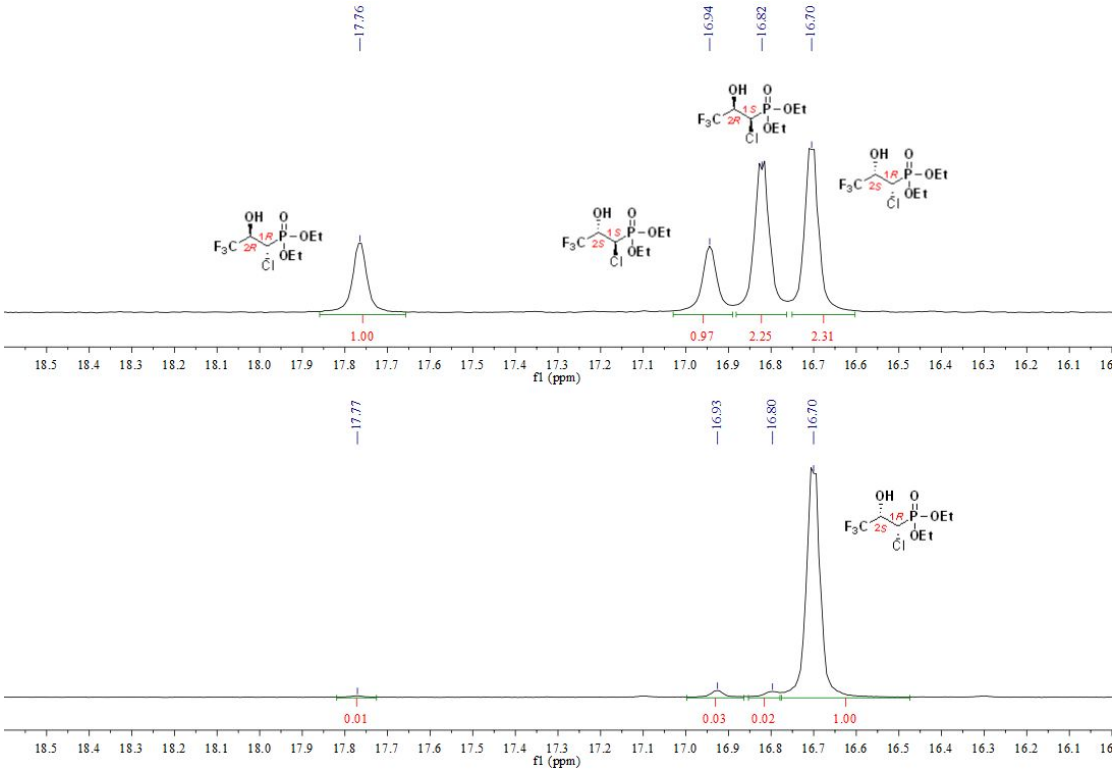


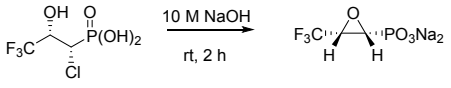
Figure S26. Determination of the e.e. of (1*R*,2*S*)-**20** (98%, bottom spectrum) via ^{31}P NMR, with racemic *anti*-**21** and *syn*-**20** (top spectrum) as controls. Quinine was added as a chiral solvating agent.[14-15] Each sample contained 50 mg of quinine and 15 mg of substrate dissolved in 0.6 mL of $CDCl_3$.

((1*R*,2*S*)-1-chloro-3,3,3-trifluoro-2-hydroxypropyl)phosphonate (1f). TMSBr (0.46 g, 3.48 mmol) and allyl trimethylsilane (0.28 g, 1.79 mmol) were added to a solution of (1*R*,2*S*)-**20** (0.3 mg, 1.05 mmol) in CH_2Cl_2 (20 mL) at rt, and the solution was stirred for 48 h. The solvent was subsequently removed under reduced pressure, and the residue was dissolved in $CHCl_3$ (20 mL) before being extracted with 30 mL of an aqueous 0.2 M ammonium acetate solution. The aqueous layer was then collected and lyophilized to afford (1*R*,2*S*)-**1f** as a white solid (0.18 g, 75%). ^{1}H NMR (500 MHz, D_2O) δ 4.68 (p, J = 6.9 Hz, 1H), 4.03 (d, J = 12.8 Hz, 1H). ^{31}P NMR (202 MHz, D_2O) δ 11.13. ^{19}F NMR (471 MHz, D_2O) δ -75.74 (d, J = 7.1 Hz). ^{13}C NMR (126 MHz, D_2O) δ 124.28 (qd, J = 281.8, 16.4 Hz), 69.04 (q, J = 31.2 Hz), 51.90 (d, J = 131.3 Hz). HRMS (ESI): calcd for $C_3H_4ClF_3O_4P$ (M-H^-) 226.9493, found 226.9501.

***rac*-Diethyl (1-chloro-3,3,3-trifluoro-2-hydroxypropyl)phosphonate (21)**. Following the synthesis of *rac*-**15**, $NaBH_4$ reduction at rt gave *rac*-**21** (0.6 g, 59 %, d.r. 32/68) as a colorless oily liquid. By contrast, reduction at -10 °C afforded *rac*-**21** with a d.r. of 59/41.

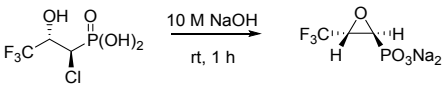
***rac-anti*-(1-chloro-3,3,3-trifluoro-2-hydroxypropyl)phosphonate (1g)**. TMSBr (0.92 g, 6.96 mmol) and allyl trimethylsilane (0.56 g, 3.58 mmol) were added to a solution of **21** (0.6 mg, 2.1 mmol, d.r. 59/41) in CH_2Cl_2 (20 mL) at rt, and the solution was stirred for 48 h. The solvent was subsequently removed under reduced pressure, and the residue was dissolved in $CHCl_3$ (20 mL) before being extracted with 30 mL of an aqueous 0.2 M ammonium bicarbonate solution. The aqueous layer was then collected and evaporated under vacuum to afford **1g** as a white solid (0.36 g, 75%). Further purification was achieved when the mixture of *anti*-**1g** and *syn*-**1f** was resuspended in 5 mL of ethanol, stirred for 0.5 h, and then filtered. Evaporation of the filtrate under vacuum afforded relatively pure *anti*-**1g**. ^{1}H NMR (500 MHz, D_2O) δ 4.49 – 4.42 (m, 1H), 4.03 (dd, *J* = 12.2, 7.4 Hz, 1H). ^{31}P NMR (202 MHz, D_2O) δ 11.16, 11.36. ^{19}F NMR (471 MHz, D_2O) δ -74.46 (d, *J* = 6.6 Hz). ^{13}C NMR (126 MHz, D_2O) δ 123.24 (qd, *J* = 282.7, 12.6 Hz), 70.64 (q, *J* = 30.1 Hz), 50.57 (d, *J* = 133.5 Hz). HRMS (ESI): calcd for $C_3H_4ClF_3O_4P$ ($M-H^-$) 226.9493, found 226.9502.

6.6 Chemical synthesis of *cis*- and *trans*-3,3,3-F_3-Fos (2c and 3c) standards.



(1*R*,2*S*)-1f **cis-3,3,3-F_3-Fos 2c**

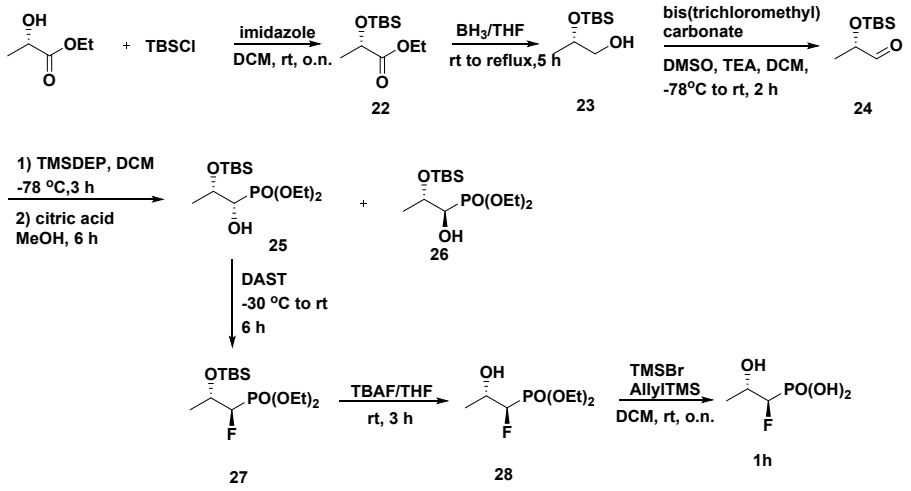
(1*R*,2*S*)-1,2-epoxy-3,3,3-trifluoropropylphosphonate (2c). Published procedures of Marocco *et al.* were followed.[4] To a solution of 10 M NaOH (200 µL) was added (1*R*,2*S*)-**1f** (20 mg, 0.087 mmol). The reaction was stirred for 2 h and diluted with 400 µL of D_2O, before being transferred to an NMR tube. (1*R*,2*S*)-**2c** was generated in a 30% calculated yield. ^{1}H NMR (500 MHz, 2 M NaOH in D_2O) δ 3.58 – 3.53 (m, 1H), 2.91 (dd, *J* = 15.5, 5.0 Hz, 1H). ^{19}F NMR (471 MHz, 2 M NaOH in D_2O) δ -67.83 (d, *J* = 6.5 Hz). ^{31}P NMR (202 MHz, 2 M NaOH in D_2O) δ 5.32 (d, *J* = 16.0 Hz).



anti-**1g** *trans*-**3c**

***Trans*-1,2-epoxy-3,3,3-trifluoropropylphosphonate (3c)** To a solution of 10 M NaOH (200 µL) was added *anti*-**1g** (20 mg, 0.087 mmol). The reaction was subsequently stirred for 1 h before being diluted with 400 µL D_2O and transferred to an NMR tube. Compound ***trans*-3c** was generated in a 90% calculated yield. ^{1}H NMR (500 MHz, 2 M NaOH in D_2O) δ 3.58 – 3.54 (m, 1H), 2.99 (dd, *J* = 19.8, 2.8 Hz, 1H). ^{19}F NMR (471 MHz, 2 M NaOH in D_2O) δ -74.13 (d, *J* = 5.0 Hz). ^{31}P NMR (202 MHz, 2 M NaOH in D_2O) δ 6.75 (dd, *J* = 19.8, 4.4 Hz).

6.7 Synthesis of (1*R*,2*S*)-1-F-HPP (1h) and 1-F-2-OPP (4h) using adaptions of previously published methods.[21-23]



Ethyl (*S*)-2-((tert-butyldimethylsilyl)oxy)propanoate (22). To a stirred solution of (−)-ethyl *L*-lactate (5.00 ml, 44.1 mmol) and imidazole (7.51 g, 110.26 mmol, 2.5 equiv) in CH_2Cl_2 (100 mL) was added *tert*-butyldimethylsilyl chloride (7.98 g, 52.92 mmol, 1.2 equiv) at rt under nitrogen atmosphere. After 18 h, the reaction mixture was quenched by addition of water (100 mL). The aqueous phase was then extracted 3 times with CH_2Cl_2, washed with brine, dried over $MgSO_4$, filtered, and concentrated before being purified by chromatography (hexanes/ethyl acetate = 20/1) to yield **22** (10.25 g, 43.1 mmol, quant. yield) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.28 (q, *J* = 6.8 Hz, 1H),4.20 – 4.10 (m, 2H), 1.37 (d, *J* = 6.7 Hz, 3H), 1.25 (t, *J* = 7.2 Hz, 3H), 0.88 (s, 9H), 0.07 (s, 3H), 0.04 (s, 3H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 174.05, 68.42, 60.68, 25.68, 21.27, 18.29, 14.16, -4.98, -5.31. ^{13}C NMR (126 MHz, $CDCl_3$) δ 69.06, 68.15, 25.81, 19.82, 18.05, -4.41, -4.81.

(*S*)-2-((tert-butyldimethylsilyl)oxy)propan-1-ol (23). Into a solution of (*S*)-ethyl 2-((tert-butyldimethylsilyl)oxy)propanoate (10.25 g, 44.10 mmol) was dripped BH_3·THF (88.2 mL, 88.2 mmol, 1M). The reaction mixture was subsequently heated to reflux for 5 h before water was slowly added. The aqueous layer was then extracted with CH_2Cl_2 (2×500 mL), dried over $MgSO_4$, concentrated, and purified by chromatography (hexanes/ethyl acetate = 10/1) to afford **23** (8.40 g, 44.10 mmol, quant. yield) as a colorless oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.98 – 3.84 (m, 1H), 3.50 – 3.46 (m, 1H), 3.39 – 3.30 (m, 1H), 2.08 – 1.99 (m, 1H), 1.10 (d, *J* = 6.2 Hz, 3H), 0.89 (s, 9H), 0.07 (s, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 69.06, 68.15, 25.81, 19.82, 18.05, -4.41, -4.81.

(*S*)-2-((tert-butyldimethylsilyl)oxy)propanal (24). To a stirred solution of bis(trichloromethyl) carbonate (3.12 g, 10.51 mmol) in CH_2C1_2 (30 mL) at -78 °C was added DMSO (4.5 mL, 63.04 mmol). The reaction mixture was stirred for 15 min before a solution of **23** (5 g, 26.27 mmol) in CH_2C1_2 (20 mL) was slowly added at the same temperature. After 15 min of stirring, triethylamine (10.27 mL, 73.55 mmol) in CH_2C1_2 (40 mL) was dripped in while the temperature was maintained below -70 °C. After this addition, the resulting suspension was stirred at -78 °C for another 5 min before the acetone-dry ice bath was removed. The reaction mixture was subsequently stirred at rt for 2 h and then was washed with 1 *N* HCl (100 mL × 2). Evaporation of the organic solvent under reduced pressure produced a residue, which was purified by a short column (hexanes/ethyl acetate = 20/1) to afford **24** (4.3g, 82%): ^{1}H NMR (500 MHz, $CDCl_3$) δ 9.59 (s, 1H), 4.07 (q, *J* = 6.8 Hz, 1H), 1.25 (d, *J* = 6.9 Hz, 3H), 0.90 (s, 9H), 0.08 (d, *J* = 6.1 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 204.06, 73.80, 25.71, 18.47, 18.14, -4.78, -4.84.

Diethyl ((4*S*,5*S*)-2,2,5,7,7,8,8-heptamethyl-3,6-dioxa-2,7-disilanonan-4-yl)phosphonate (25). To a stirred solution of silylphosphonic ester (TMSDEP, 23.36 mmol) [prepared *in situ* in CH_2Cl_2 (200 mL) from DEP (3.23 g, 23.36 mmol), TEA (2.79g, 27.61 mmol), TMSC1 (3 g, 27.61 mmol) under an argon atmosphere at 0 °C for 30 min)], (*S*)-lactaldehyde **24** (4 g, 21.24 mmol) in 80 mL CH_2Cl_2 was slowly added at -78°C, and the reaction mixture was stirred at the same temperature for 3 h. Water was added to quench the reaction, and the mixture was warmed to 0 °C. The mixture was then extracted with CH_2Cl_2 and washed with brine. Concentration *in vacuo* yielded the crude adducts (*syn*/*anti* = 3/1, as determined by ^{31}P-NMR). Exposure of the crude mixture to citric acid (2 eq) in methanol (300 mL) at rt for 6 h followed by silica gel chromatography (CH_2Cl_2/acetone = 16/1) gave the hydroxyphosphonic esters, **25**

and **26** (3 g, total yield 43%, **25**/**26** = 3/1).

Diethyl ((1*S*,2*S*)-2-((tert-butyldimethylsilyl)oxy)-1-hydroxypropyl)phosphonate (25). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.23 – 4.13 (m, 5H), 3.62 – 3.58 (m, 1H), 2.80 (dd, *J* = 12.4, 6.5 Hz, 1H), 1.35 – 1.26 (m, 9H), 0.90 (s, 9H), 0.11 (d, *J* = 4.6 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 72.57 (d, *J* = 162.2 Hz), 67.75 (d, *J* = 4.3 Hz), 62.40 (dd, *J* = 49.7, 6.9 Hz), 25.77, 21.23 (d, *J* = 7.8 Hz), 17.99, 16.46 (dd, *J* = 5.6, 2.1 Hz), -4.21, -4.83. ^{31}P NMR (202 MHz, $CDCl_3$) δ 22.17.

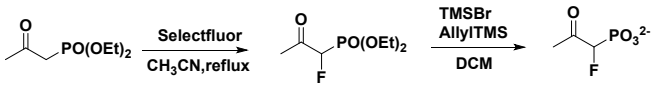
Diethyl ((1*R*,2*S*)-2-((tert-butyldimethylsilyl)oxy)-1-hydroxypropyl)phosphonate (**26**). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.24 – 4.10 (m, 5H), 3.92 – 3.88 (m, 1H), 2.71 – 2.63 (m, 1H), 1.38 – 1.26 (m, 9H), 0.89 (s, 9H), 0.08 (d, *J* = 2.0 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 72.70 (d, *J* = 159.4 Hz), 68.82 (d, *J* = 6.3 Hz), 62.49 (dd, *J* = 30.2, 6.9 Hz), 25.78, 18.64 (d, *J* = 2.7 Hz), 18.04, 16.47 (dd, *J* = 5.9, 2.6 Hz), -4.52, -4.88. ^{31}P NMR (202 MHz, $CDCl_3$) δ 21.79.

Diethyl ((1*R*,2*S*)-2-((tert-butyldimethylsilyl)oxy)-1-fluoropropyl)phosphonate (27). To a solution of **25** (0.4 g, 1.23 mmol) in anhydrous DCM (20 mL), DAST (1.13 mL, 8.58 mmol) was dripped in at −30 °C. The mixture was then warmed to rt and stirred for 6 h before being cooled back down to −30 °C and quenched with ice-cold water. The aqueous layer was extracted with DCM (100 mL × 2), and the combined organic extracts were subsequently dried over Na_2SO_4, and concentrated under reduced pressure. The resultant residue was purified by silica gel column chromatography (hexanes/ethyl acetate = 4/1) to give fluorinated product, **27** (100 mg, 25%) as an oil. ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.61 (ddd, *J* = 46.0, 5.6, 3.3 Hz, 1H), 4.35 – 4.13 (m, 5H), 1.40 – 1.32 (m, 9H), 0.89 (s, 9H), 0.09 (d, *J* = 2.2 Hz, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 92.52 (dd, *J* = 187.6, 164.3 Hz), 67.78 (dd, *J* = 21.2, 6.3 Hz), 62.84 (dd, *J* = 82.9, 6.9 Hz), 25.76, 18.69 (dd, *J* = 7.4, 2.9 Hz), 18.09, 16.43 (t, *J* = 6.3 Hz), -4.64, -4.83. ^{31}P CPD NMR (202 MHz, $CDCl_3$) δ 15.87 (d, *J* = 76.6 Hz). ^{19}F NMR (471 MHz, $CDCl_3$) δ -217.06 (ddd, *J* = 76.5, 46.0, 22.1 Hz).

Diethyl ((1*R*,2*S*)-1-fluoro-2-hydroxypropyl)phosphonate (28). To a solution of **27** (50 mg, 0.15 mmol) in THF (10 mL) was added TBAF (304 μL, 1M in THF). The mixture was stirred at rt for 3 h and concentrated *in vacuo* before being purified by silica gel column chromatography (DCM/acetone = 4/1) to give **28** (25 mg, 76%). ^{1}H NMR (500 MHz, $CDCl_3$) δ 4.48 (ddd, *J* = 45.9, 7.0, 2.5 Hz, 1H), 4.29 – 4.14

(m, 5H), 3.19 (d, J = 3.8 Hz, 1H), 1.37 (td, J = 7.2, 2.2 Hz, 9H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 90.61 (dd, J = 188.9, 162.2 Hz), 66.13 (dd, J = 21.4, 2.1 Hz), 63.42 (dd, J = 98.8, 6.8 Hz), 18.70 (dd, J = 9.0, 4.6 Hz), 16.42 (dd, J = 8.7, 5.6 Hz). ^{31}P NMR (202 MHz, $CDCl_3$) δ 17.09 (d, J = 72.3 Hz). ^{19}F NMR (471 MHz, $CDCl_3$) δ -212.05 (ddd, J = 72.1, 46.2, 10.9 Hz).

((1*R*,2*S*)-1-fluoro-2-hydroxypropyl)phosphonate (1h). To a solution of **28** (20 mg, 0.094 mmol) in DCM (10 mL) was added TMSBr (500 μL) and allylTMS (300 μL). The mixture was stirred at rt overnight, and the solvent removed *in vacuo*. The concentrated product was neutralized by addition of aqueous NH_4HCO_3 and washed with $CHCl_3$. The aqueous layer was then separated, concentrated, and purified by a P2 column to give **1h** (15 mg, 84%). ^{1}H NMR (500 MHz, D_2O) δ 4.52 (ddd, J = 47.1, 6.5, 3.5 Hz, 1H), 4.25 – 4.07 (m, 1H), 1.30 (dd, J = 6.5, 1.3 Hz, 3H). ^{13}C NMR (126 MHz, D_2O) δ 95.05 (dd, J = 178.8, 147.7 Hz), 67.37 (dd, J = 20.3, 6.5 Hz), 16.58 (dd, J = 6.4, 2.7 Hz). ^{19}F NMR (471 MHz, 100 mM NaOD in D_2O) δ -215.70 (ddd, J = 62.1, 46.7, 23.5 Hz). ^{31}P NMR (202 MHz, 100 mM NaOD in D_2O) δ 9.57 (ddd, J = 62.2, 6.1, 6.1 Hz). HRMS (ESI): calcd for $C_3H_7FO_4P$ ($M\text{-}H^-$) 157.0071, found 157.0078.



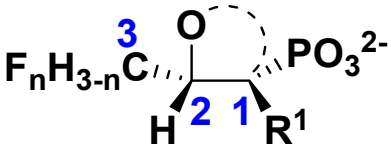
29 4h

Diethyl (1-fluoro-2-oxopropyl)phosphonate (29). To a stirred solution of the β-ketophosphonate (1 g, 5.15 mmol) in dry acetonitrile (20 mL) was added Selectfluor (3.5 g, 2.0 mmol) at rt. The mixture was heated to reflux for 24 h. The reaction solution was cooled back to rt, and EtOAc (30 mL) and saturated aqueous NH_4Cl (20 mL) were added in succession. The layers were then separated, and the organic layer was washed with brine (2 ×15 mL) before being concentrated and purified by column chromatography (hexanes/EtOAc = 4/1) to afford **29** (0.3 g, yield 27%).^{1}H NMR (500 MHz, $CDCl_3$) δ 5.10 (dd, J = 47.9, 14.3 Hz, 1H), 4.23 – 4.09 (m, 4H), 2.30 (d, J = 4.3 Hz, 3H), 1.33 – 1.26 (m, 6H). ^{13}C NMR (126 MHz, $CDCl_3$) δ 200.56 (d, J = 20.3 Hz), 91.60 (dd, J = 197.5, 152.6 Hz), 64.15 (t, J = 6.3 Hz), 26.72, 16.26 (dd, J = 5.9, 2.6 Hz). ^{19}F NMR (471 MHz, $CDCl_3$) δ -207.25 – -207.73 (m).

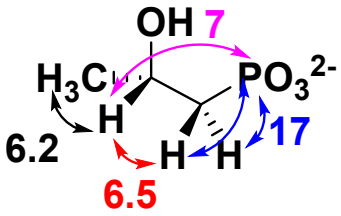
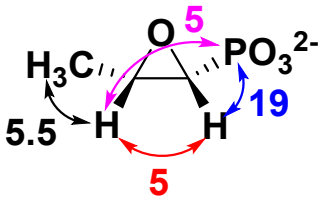
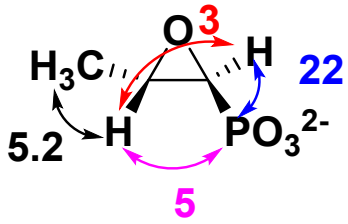
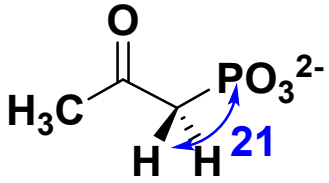
(1-fluoro-2-oxopropyl)phosphonate (4h). ^{1}H NMR (500 MHz, D_2O) δ 5.23 (dd, J = 48.2, 14.0 Hz, 1H), 2.28 (d, J = 3.0 Hz, 3H). ^{13}C NMR (126 MHz, D_2O) δ 208.92 (d, J = 15.4 Hz), 96.88 (dd, J = 187.6,

125.3 Hz), 26.31. ^{31}P NMR (202 MHz, D_2O) δ 4.95 (dd, J = 58.6, 14.0 Hz). ^{19}F NMR (471 MHz, D_2O) δ -201.02 – -201.35 (ddd, J = 59.8, 48.3, 3.9 Hz). HRMS (ESI): calcd for $C_3H_5FO_4P$ ($M-H^-$) 154.9915, found 154.9921.

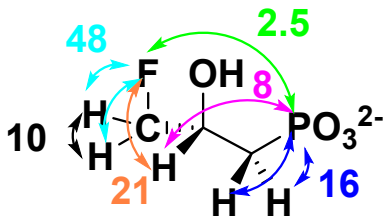
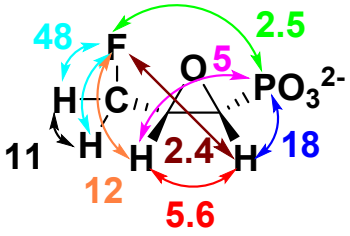
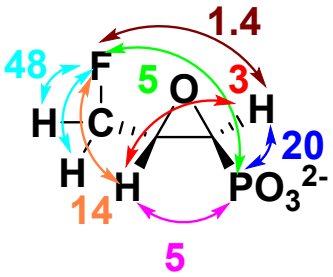
Table S11. Assignment of the chemical shift and corresponding coupling constant of compounds **1-4**, **1a-3a**, **1b-3b**, **1c-4c**, and **1f-4f**.



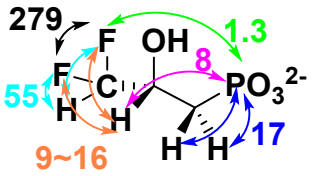
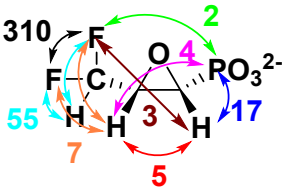
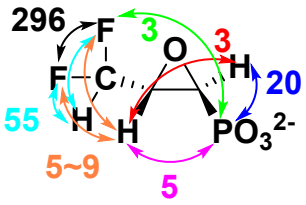
n = 0-3 **R^1 = H,F**

Compd. No.	Structures with coupling constants	Chemical shift (δ, ppm) with coupling constants (J, Hz)			
		C3-H	C2-H	C1-H	C1-P
1		1.18 (d, J = 6.2 Hz, 3H).	4.02 (m, J = 6.5 Hz, 1H),	1.74 (m, J = 17.6, 14.9, 6.7 Hz, 2H),	18.72 (m, J = 16.0, 7.2 Hz),
2		1.48 (d, J = 5.6 Hz, 3H)	3.30 (m, J = 5.5 Hz, 1H)	2.85 (dd, J = 19.4, 5.1 Hz, 1H)	9.95 (dd, J = 18.7, 5.3 Hz)
3		1.36 (dd, J = 5.2, 1.5 Hz, 3H)	3.20 (m, J = 5.2, 3.0 Hz, 1H),	2.64 (dd, J = 22.2, 2.7 Hz, 1H),	10.45 (dd, J = 21.7, 5.1 Hz)
4		2.16 (s, 3H)[13]		2.81 (d, J = 21.0, 2H),[13]	9.74 (t, J = 20.6 Hz).

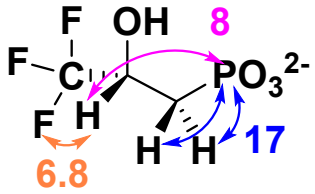
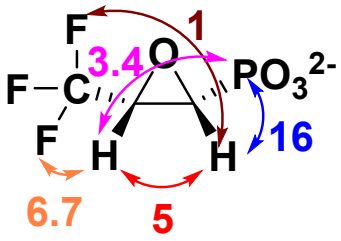
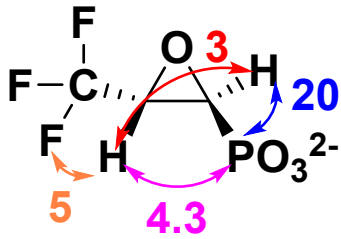
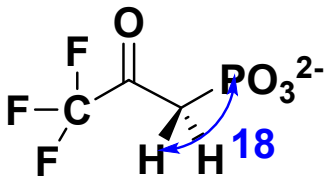
continued

Compd. No.	Structures with coupling constants	Chemical shift (δ, ppm) with coupling constants (*J*, Hz)				
		C3-F	C3-H_2	C2-H	C1-H	C1-P
1a		-225.09 – -225.44 (m, *J* = 48.5, 20.6, 3.3 Hz),	4.49 (ddd, *J* = 46.9, 10.0, 2.5 Hz, 1H), 4.30 (ddd, *J* = 47.8, 9.9, 6.5 Hz, 1H)	4.16 – 4.01 (m, 1H),	1.76 – 1.56 (m, 2H).	17.39 (m, *J* = 16.3, 8.3, 2.5 Hz)
2a		-222.34 (m, *J* = 47.7, 12.0, 2.8 Hz),	4.95 – 4.82 (m, 47.8, 11.1, 2.0 Hz, 2H),	3.51 – 3.44 (m, 1H),	2.98 (ddd, *J* = 18.4, 5.6, 2.4 Hz, 1H)	8.25 (ddd, *J* = 18.6, 4.5, 2.5 Hz).
3a		-225.58 (m, *J* = 47.6, 13.7, 4.5 Hz).	4.92 – 4.80 (m, 2H, overlapped)	3.45 – 3.40 (m)	2.81 (ddd, *J* = 20.8, 3.1, 1.4 Hz, 1H)	9.10 (ddd, *J* = 20.2, 5.0, 5.0 Hz).

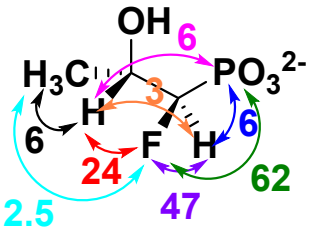
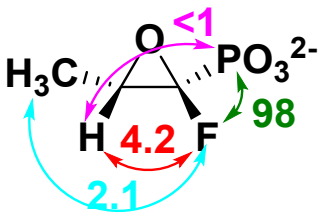
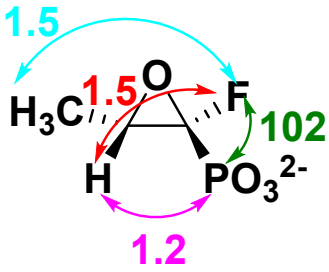
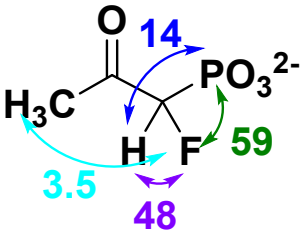
continued

Compd. No.	Structures with coupling constants	Chemical shift (δ, ppm) with coupling constants (*J*, Hz)				
		C3-F	C3-H	C2-H	C1-H	C1-P
1b		-129.12 (ddd, *J* = 279.5, 55.0, 9.0 Hz), -134.11 (ddd, *J* = 279.7, 55.2, 16.1 Hz).	5.91 (m, *J* = 55.2, 2.4 Hz, 1H),	4.20 – 4.03 (m, 1H),	2.03 – 1.79 (m, 2H).	16.77 (m, *J* = 16.6, 8.3, 1.3 Hz)
2b		-118.26 (ddd, *J* = 310.4, 57.7, 7.4 Hz), -121.20 (dd, *J* = 310.4, 52.9 Hz).	6.25 (ddd, *J* = 57.8, 52.9, 7.0 Hz, 1H),	3.44 (m, *J* = 6.0 Hz, 1H),	3.00 (ddd, *J* = 18.7, 4.8, 2.6 Hz, 1H)	7.09 (*J* = m, 17.1, 3.8, 2.0 Hz)
3b		-123.94 (ddd, *J* = 295.6, 54.6, 4.7 Hz), -125.36 (ddd, *J* = 295.8, 55.2, 9.4 Hz).	6.30 –6.10 (m, 1H, overlapped)	3.46 –3.42 (m, 1H, overlapped)	3.14 – 3.12 (m, 1H, 2.6 Hz)	7.78 (*J* = m, 20.1, 4.9, 2.6 Hz)

continued

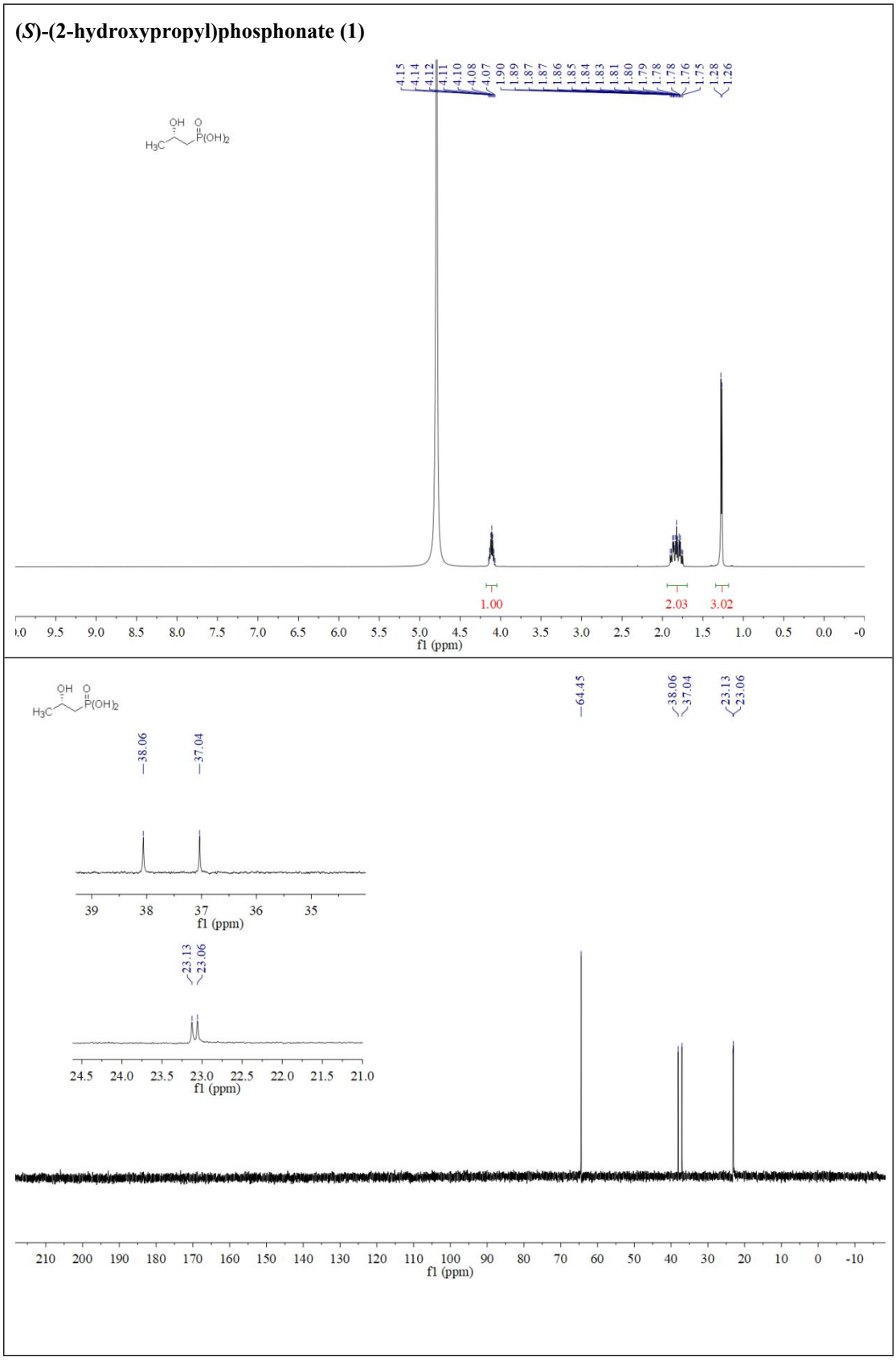
Compd. No.	Structures with coupling constants	Chemical shift (δ, ppm) with coupling constants (*J*, Hz)			
		C3-F	C2-H	C1-H	C1-P
1c		-80.32 (d, *J* = 6.8 Hz).	4.32 – 4.10 (m, 1H),	1.88 – 1.56 (m, 2H).	16.59 (m, *J* = 16.6, 8.1 Hz).
2c		-67.17 (d, *J* = 6.3 Hz),	3.68 – 3.61 (m, 1H),	3.02 (m, *J* = 15.6, 4.9, 1.0 Hz, 1H).	5.29 (dd, *J* = 15.9, 3.4 Hz).
3c		-74.11 (d, *J* = 5.0 Hz),	3.68 – 3.61 (m, 1H),	3.08 (dd, *J* = 20.0, 2.7 Hz, 1H),	6.76 (dd, *J* = 19.9, 4.3 Hz),
4c		-87.28 (s)		2.02 (d, *J* = 17.2, 2H)	16.02 (d, *J* = 18.0 Hz)

continued

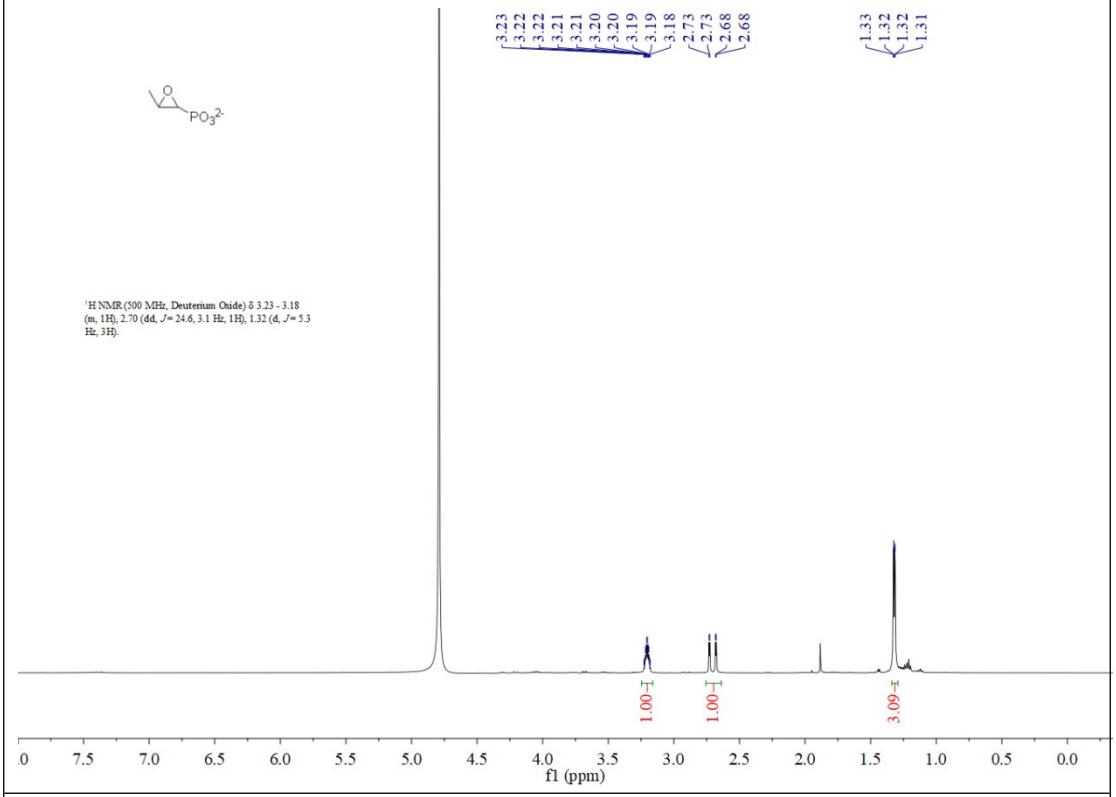
Compd. No.	Structures with coupling constants	Chemical shift (δ, ppm) with coupling constants (*J*, Hz)				
		C3-H	C2-H	C1-H	C1-P	C1-F
1h		1.30 (dd, *J* = 6.5, 1.3 Hz, 3H).	4.26 – 4.09 (m, *J* = 25, 6.5 Hz, 1H),	4.52 (ddd, *J* = 47.1, 6.5, 3.5 Hz, 1H),	9.57 (ddd, *J* = 62.3, 6.1, 6.1 Hz)	-215.70 (m, *J* = 62.4, 47.2, 24.2, 2.5 Hz)
2h					2.56 (d, *J* = 98.6, <1 Hz)	-137.12 (m, *J* = 98.3, 4.2, 2.1 Hz),
3h					3.48 (m, *J* = 102.1, 1.2, 1.2 Hz)	-159.24 (d, *J* = 102.5, 1.5.1.5 Hz)
4h		2.28 (d, *J* = 3.0 Hz, 3H).		5.23 (dd, *J* = 48.2, 14.0 Hz, 1H),	4.95 (dd, *J* = 58.6, 14.0 Hz).	-201.02 – -201.35 (ddd, *J* = 59.8, 48.3, 3.9 Hz)

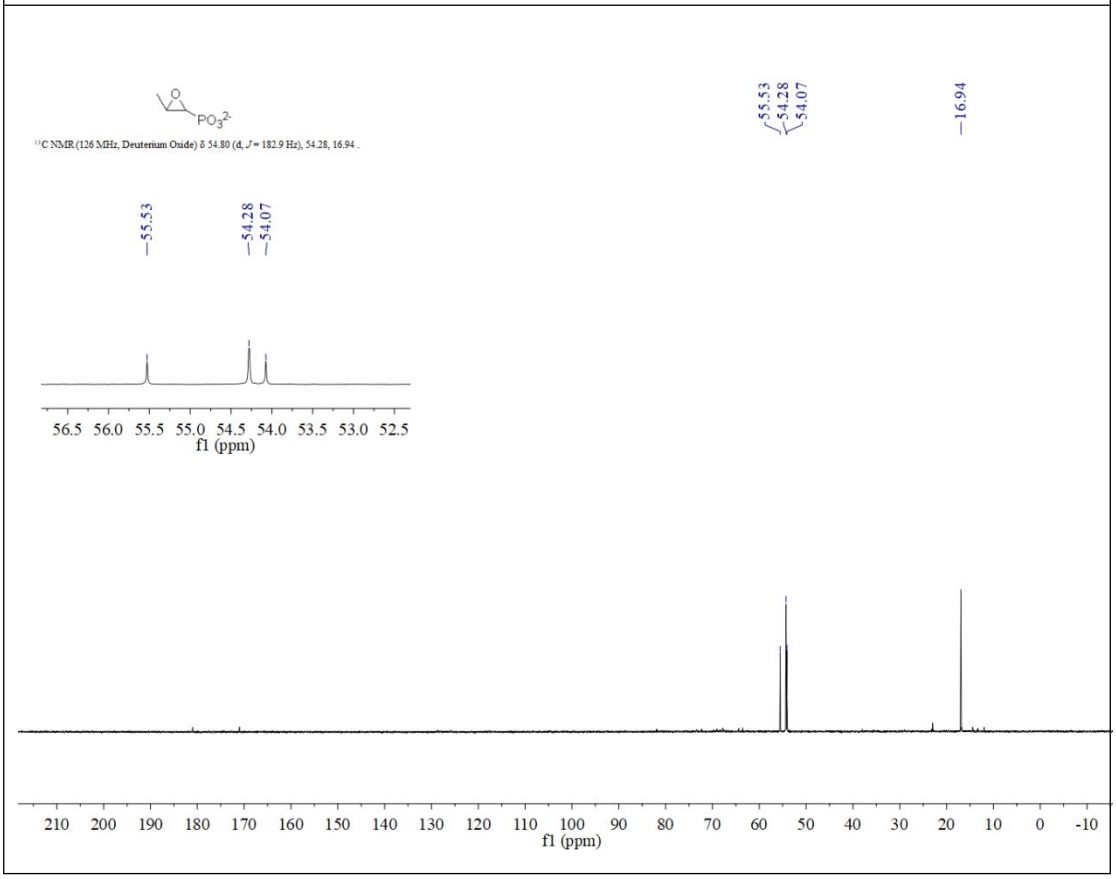
7. Copies of NMR and HRMS spectra

(*S*)-(2-hydroxypropyl)phosphonate (1)

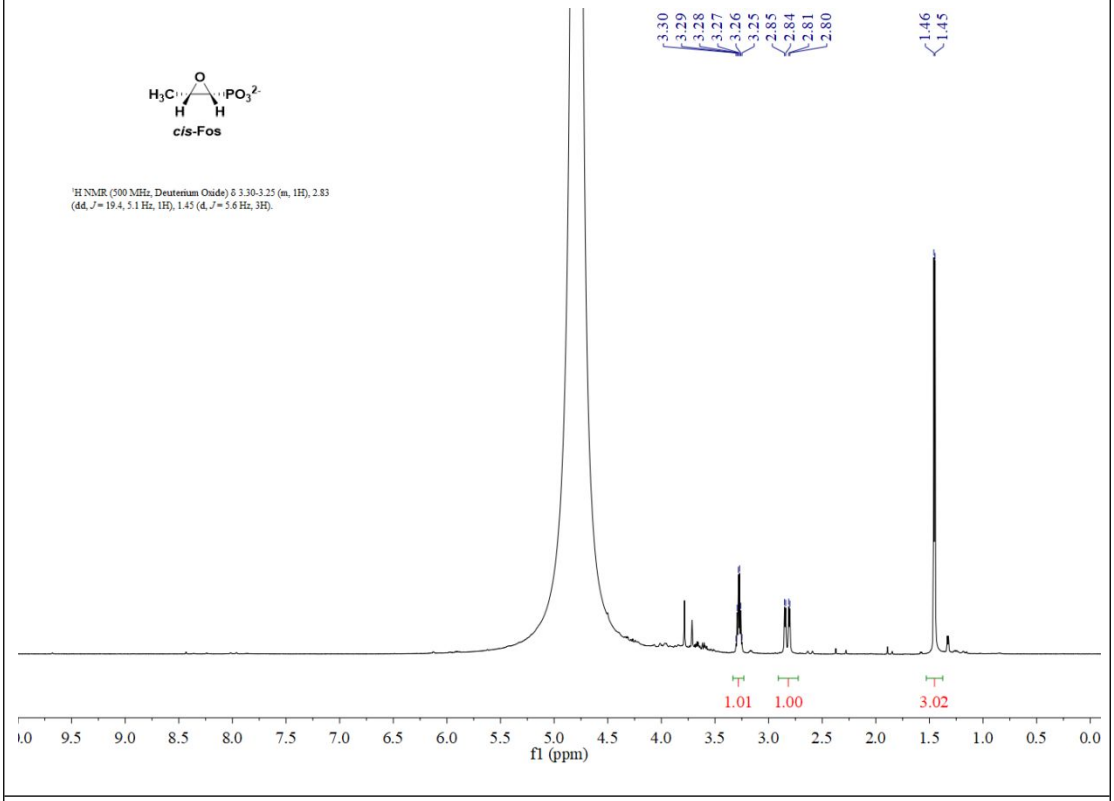


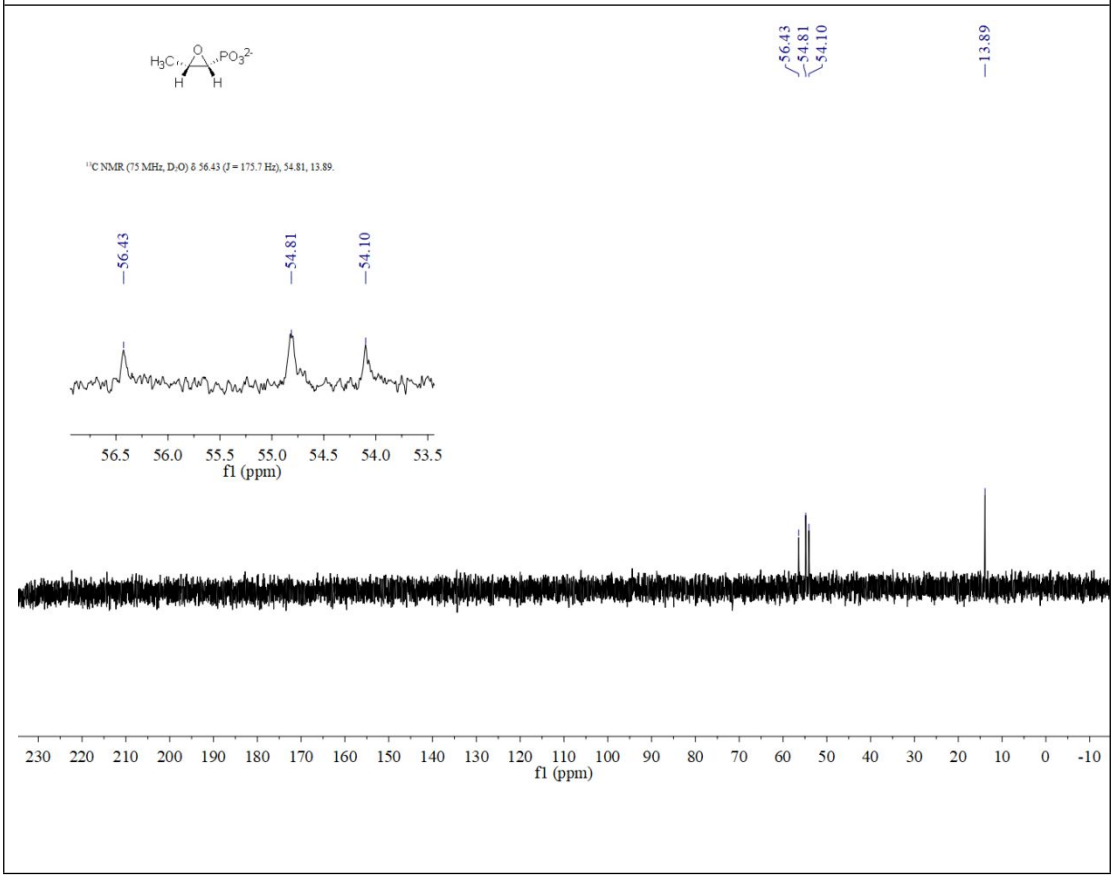
***rac-trans*-(3-methyloxiran-2-yl)phosphonate (*rac*-3)**



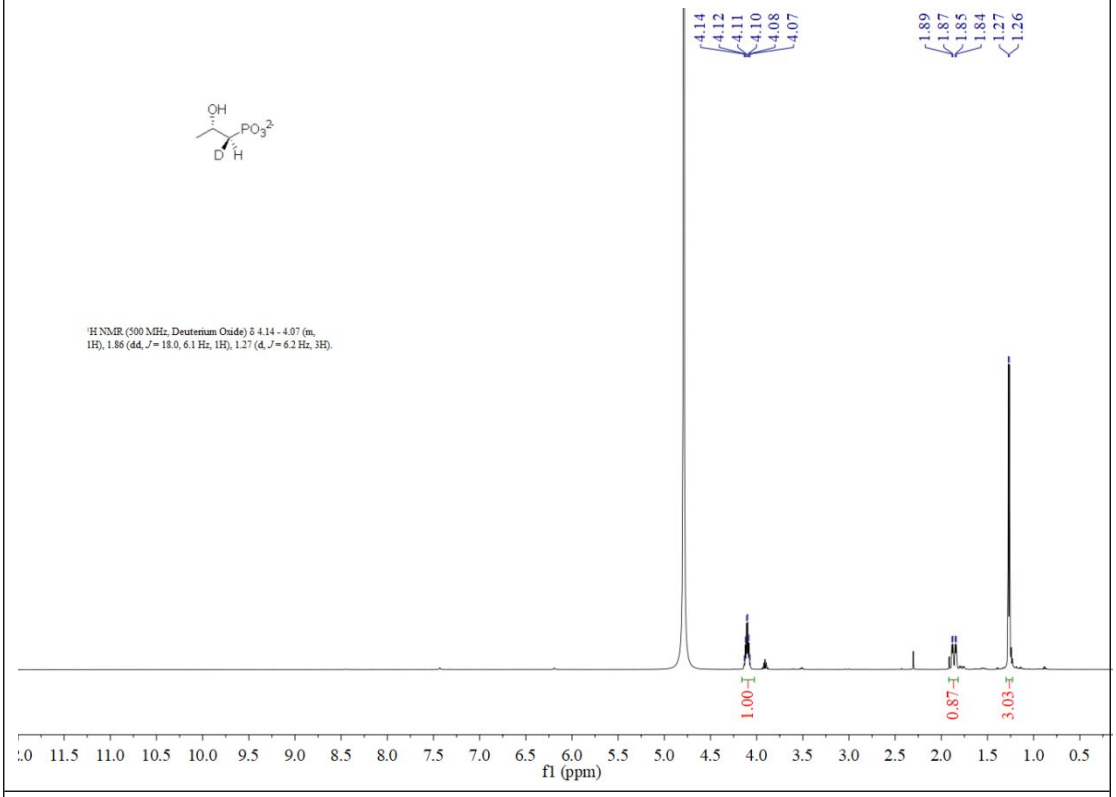


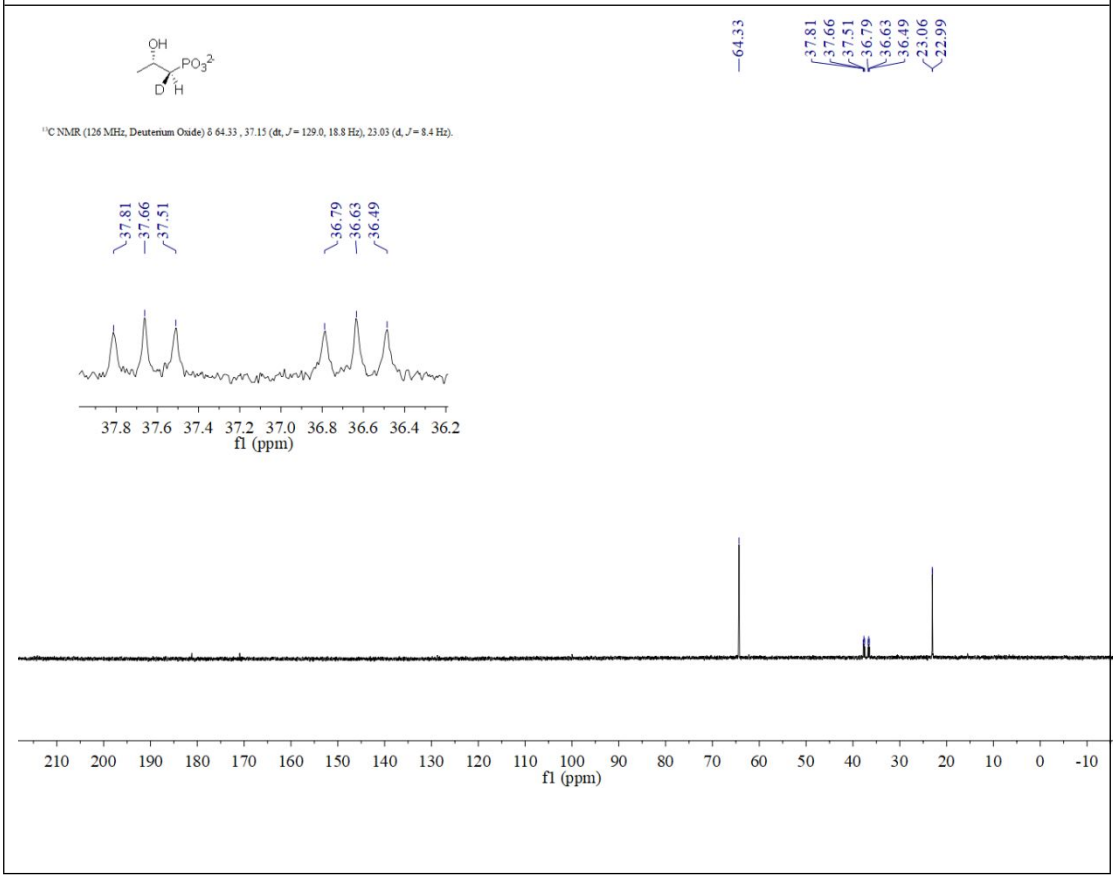
(1*R*,2*S*)-1,2-epoxypropylphosphonate (2)



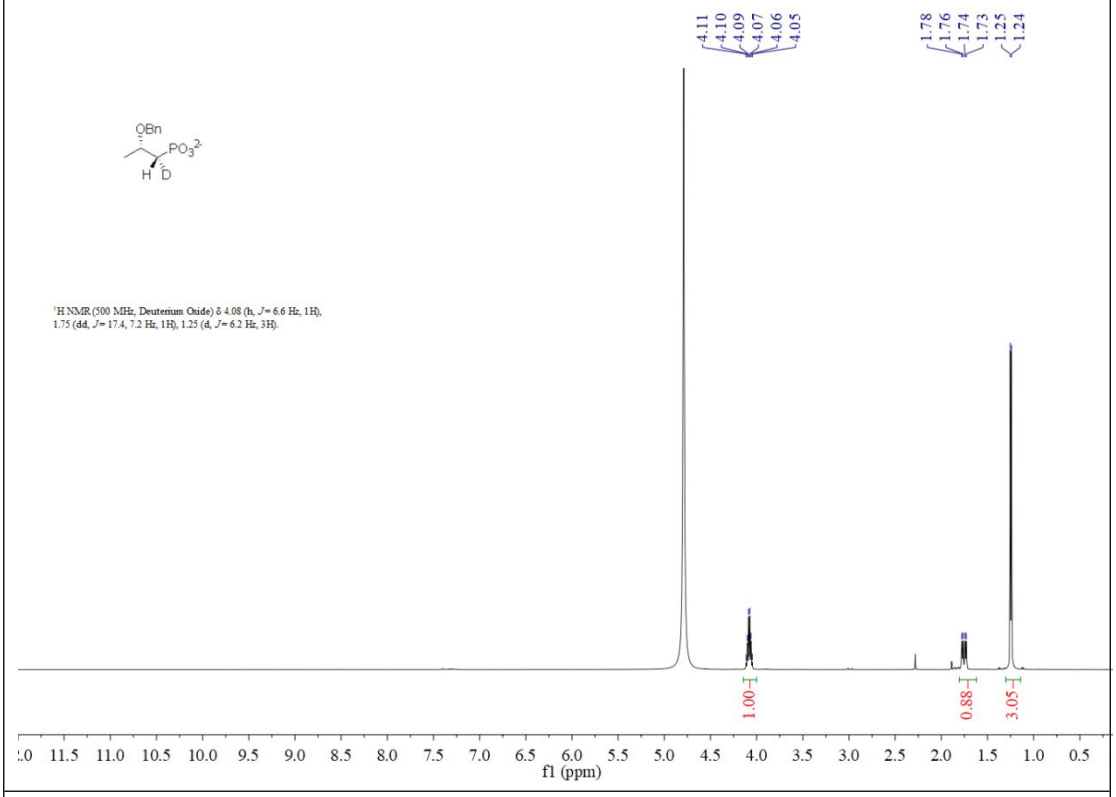


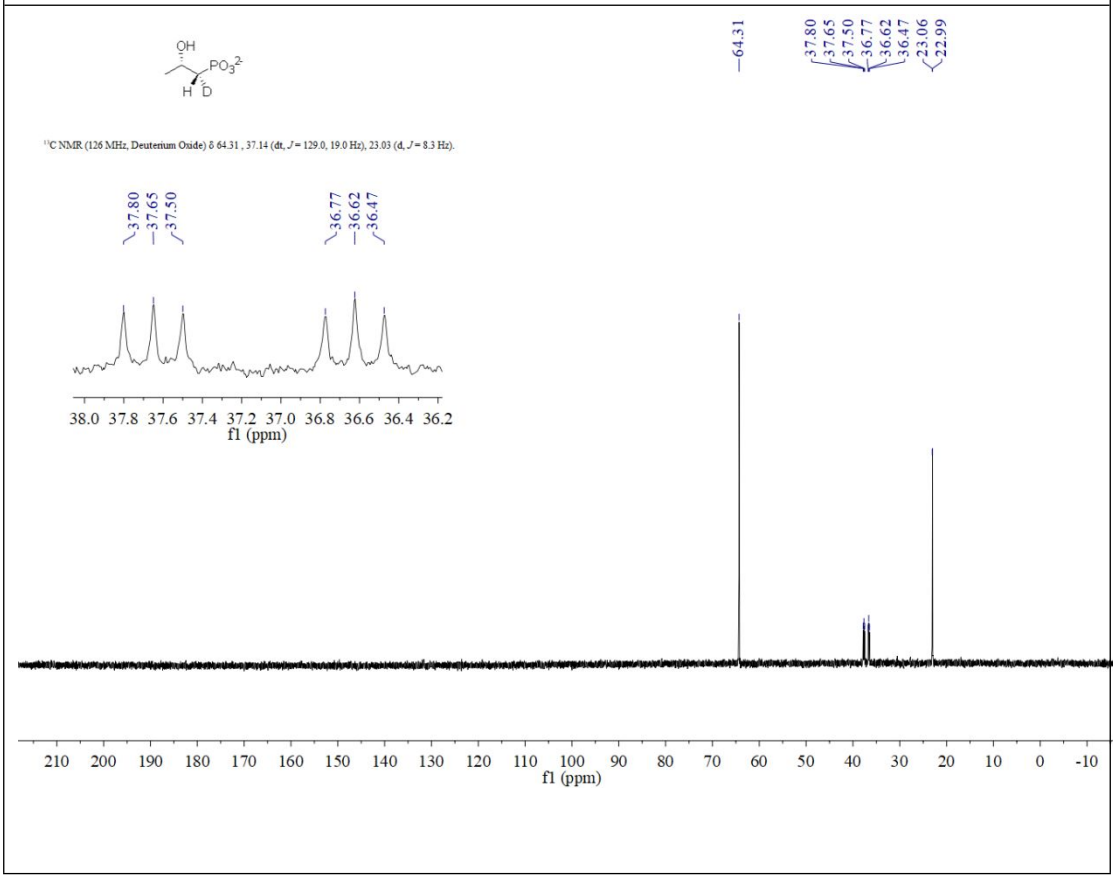
((1*S*,2*S*)-2-hydroxypropyl-1-*d*)phosphonate (1d)



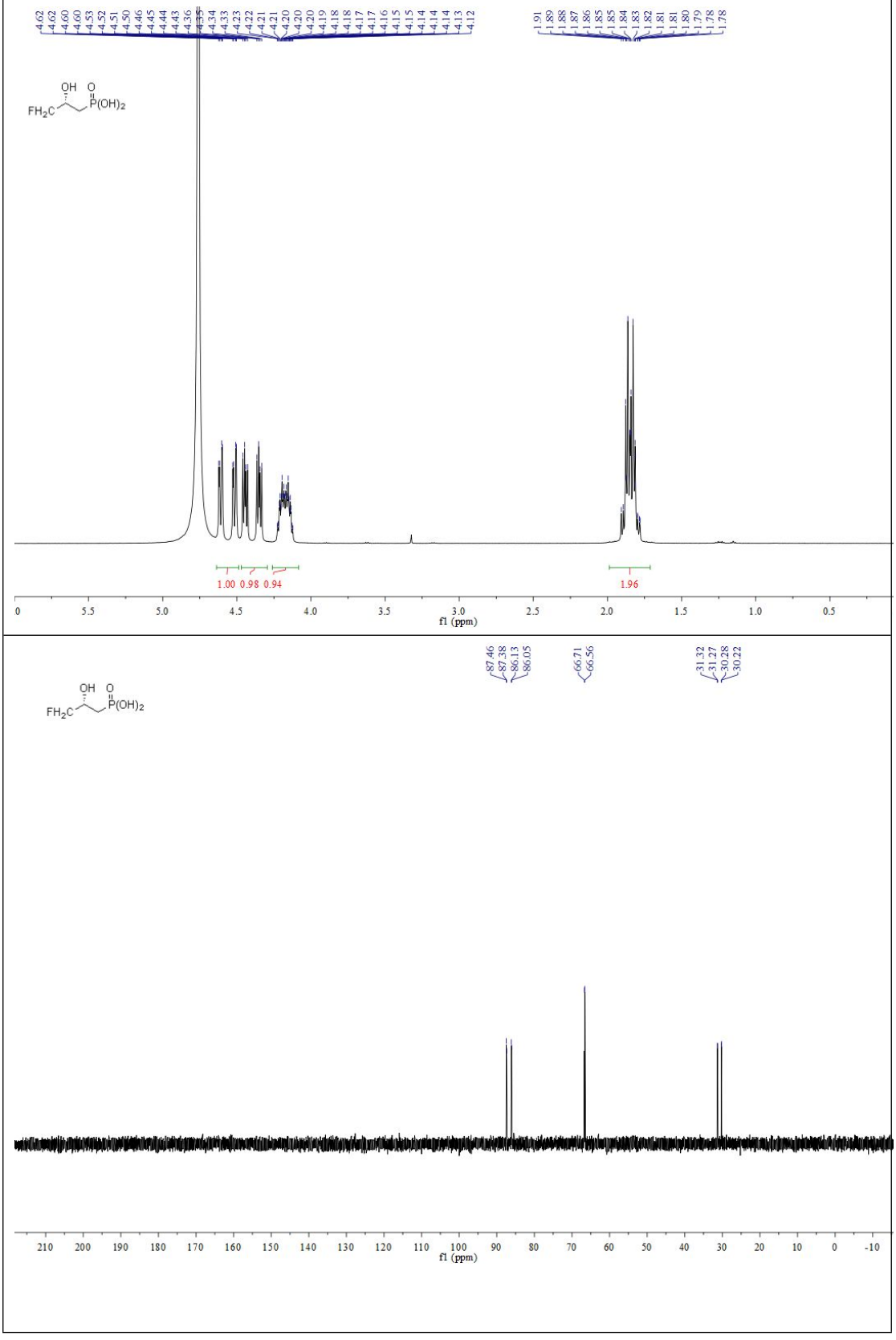


((1*R*,2*S*)-2-hydroxypropyl-1-*d*)phosphonate (1e)

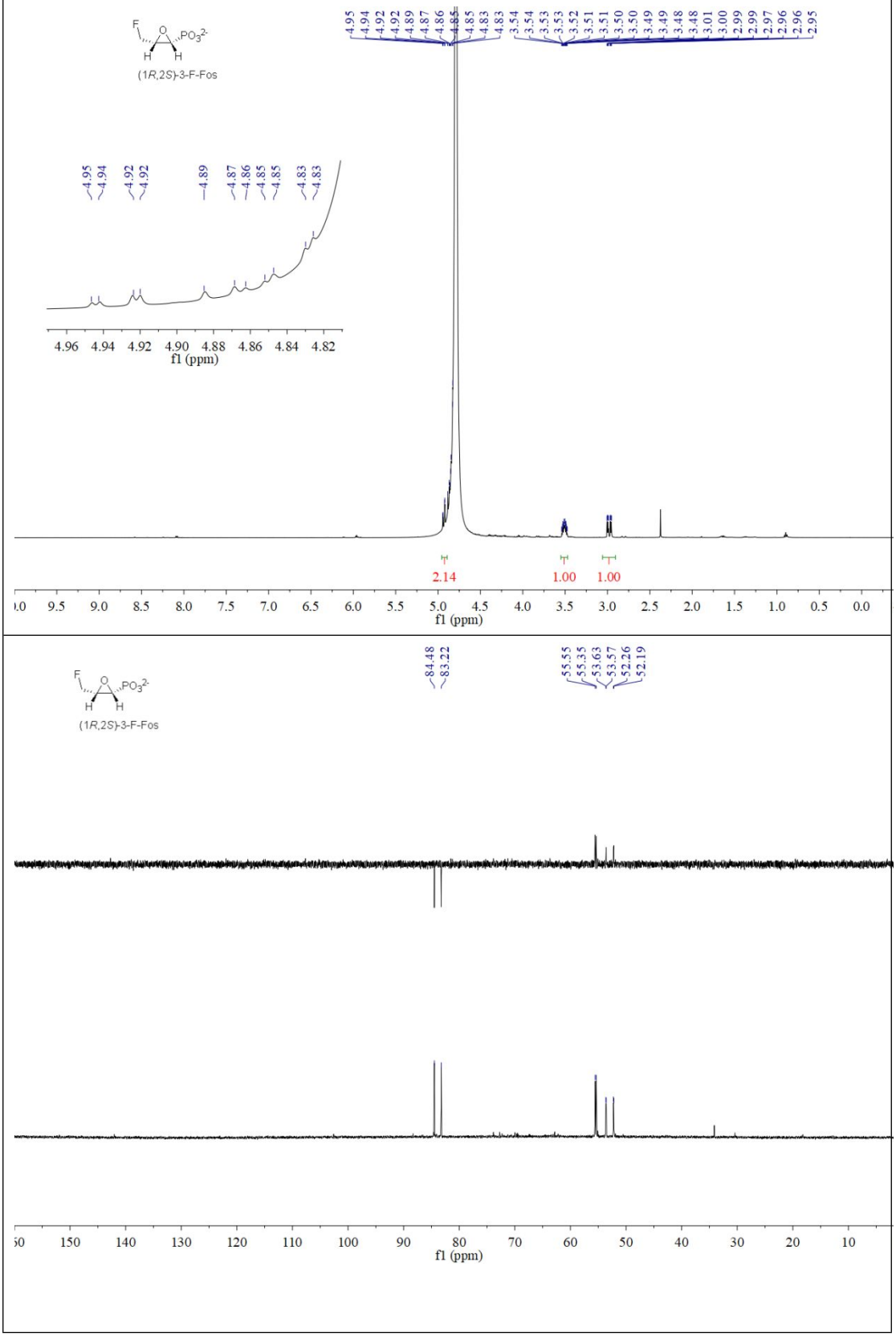




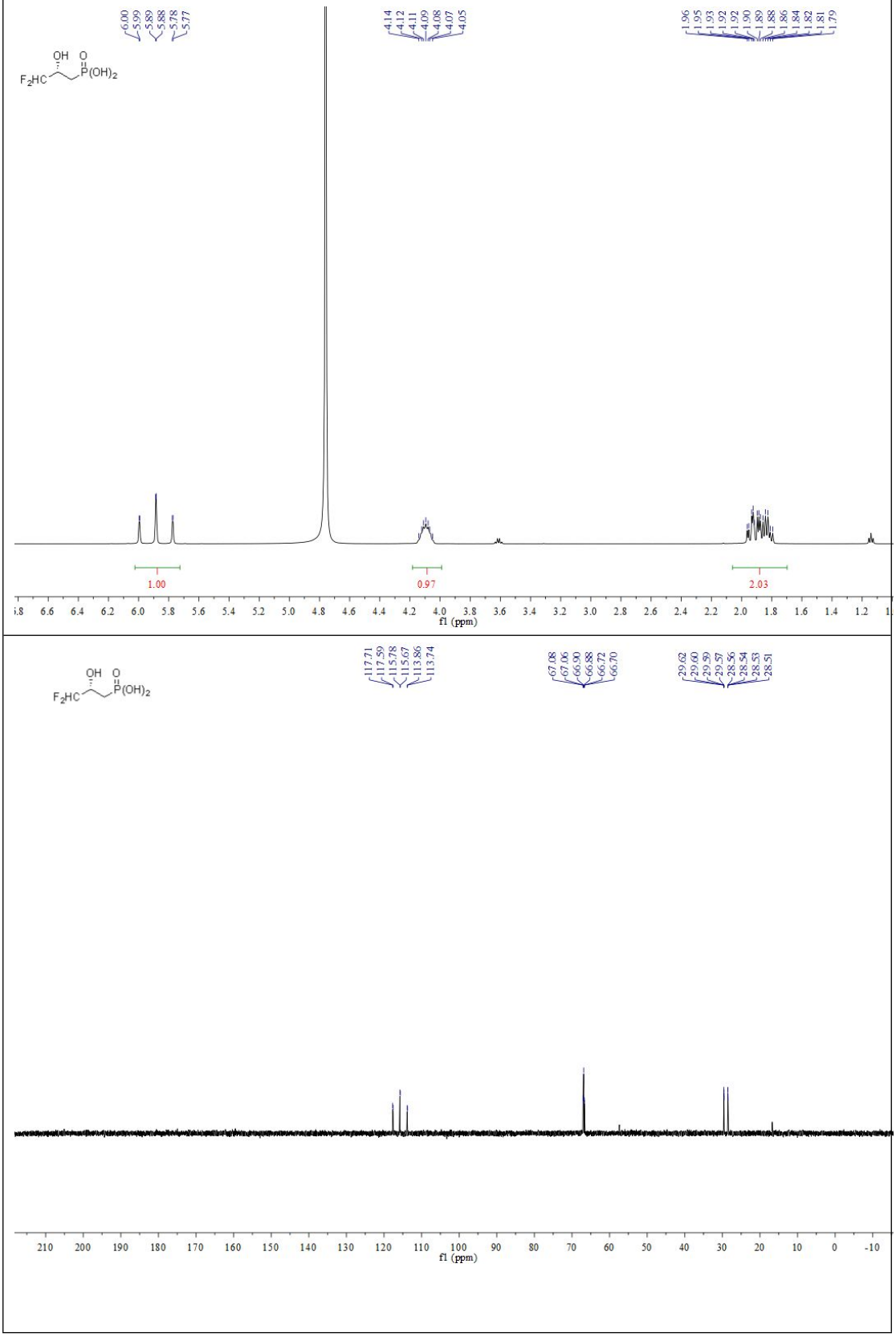
(*S*)-(3-fluoro-2-hydroxypropyl)phosphonate (1a)



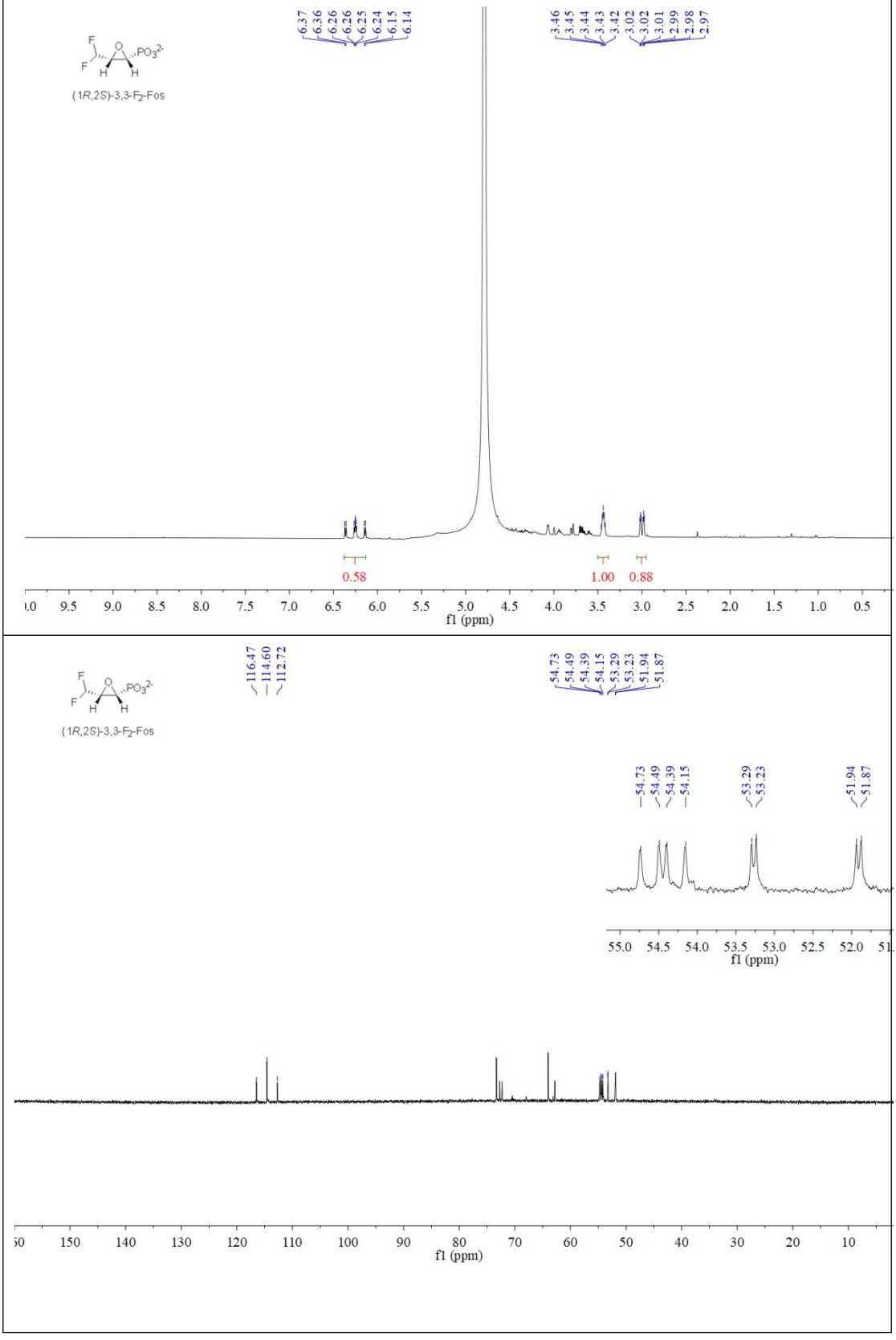
(1*R*,2*S*)-1,2-epoxy-3-fluoropropylphosphonate (2a)



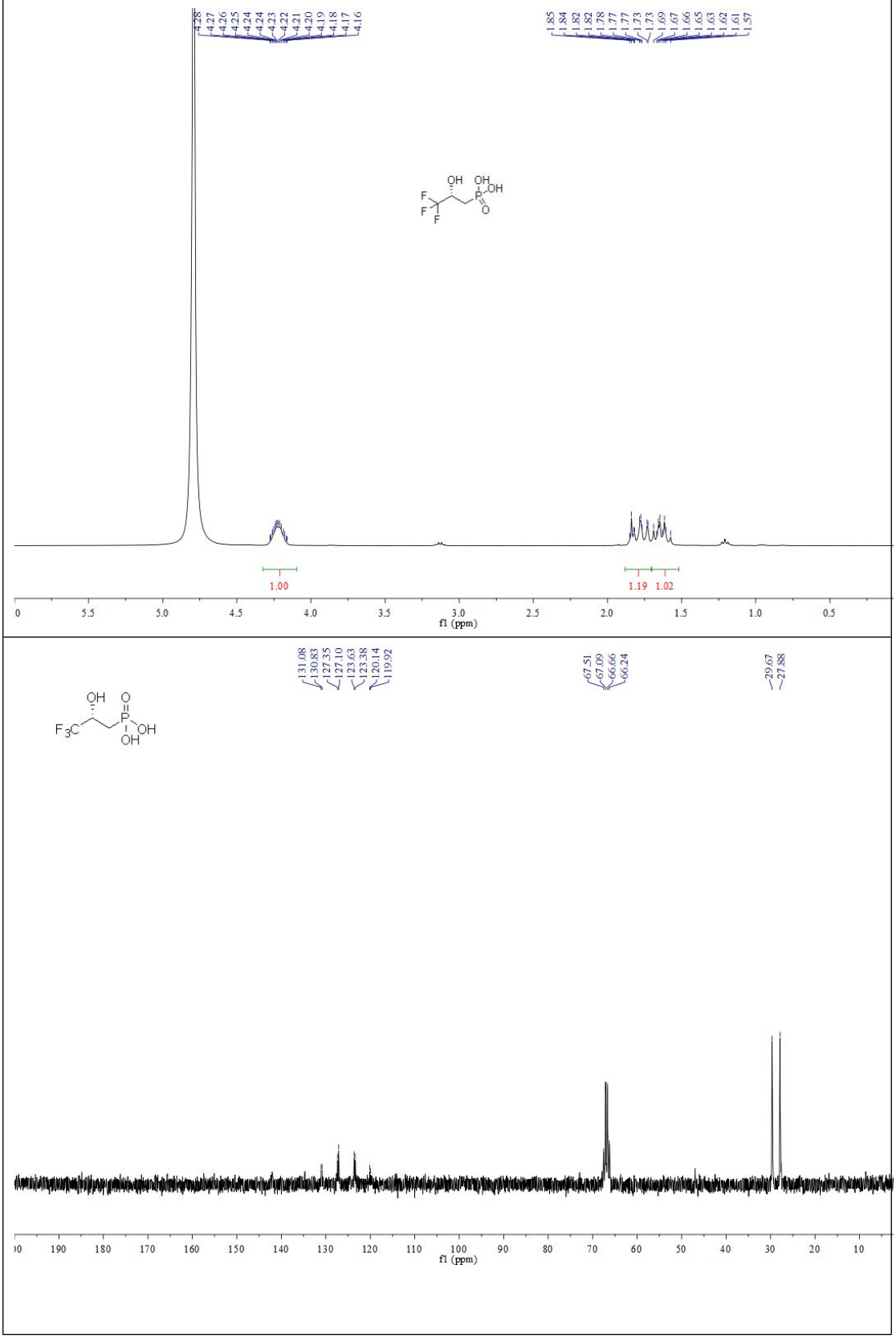
(*S*)-(3,3-difluoro-2-hydroxypropyl)phosphonate (1b)



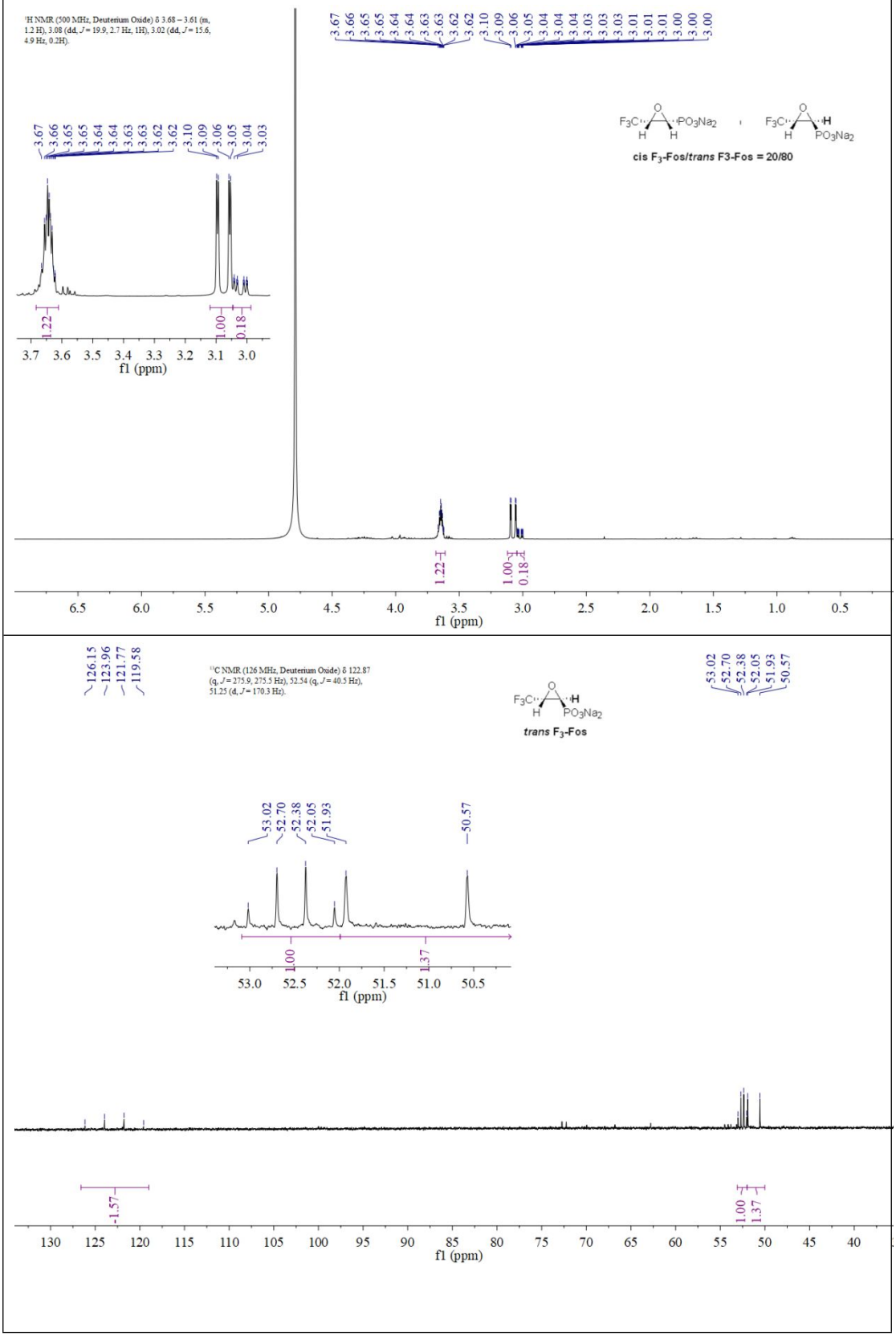
(1*R*,2*S*)-1,2-epoxy-3,3-difluoropropylphosphonate (2b)



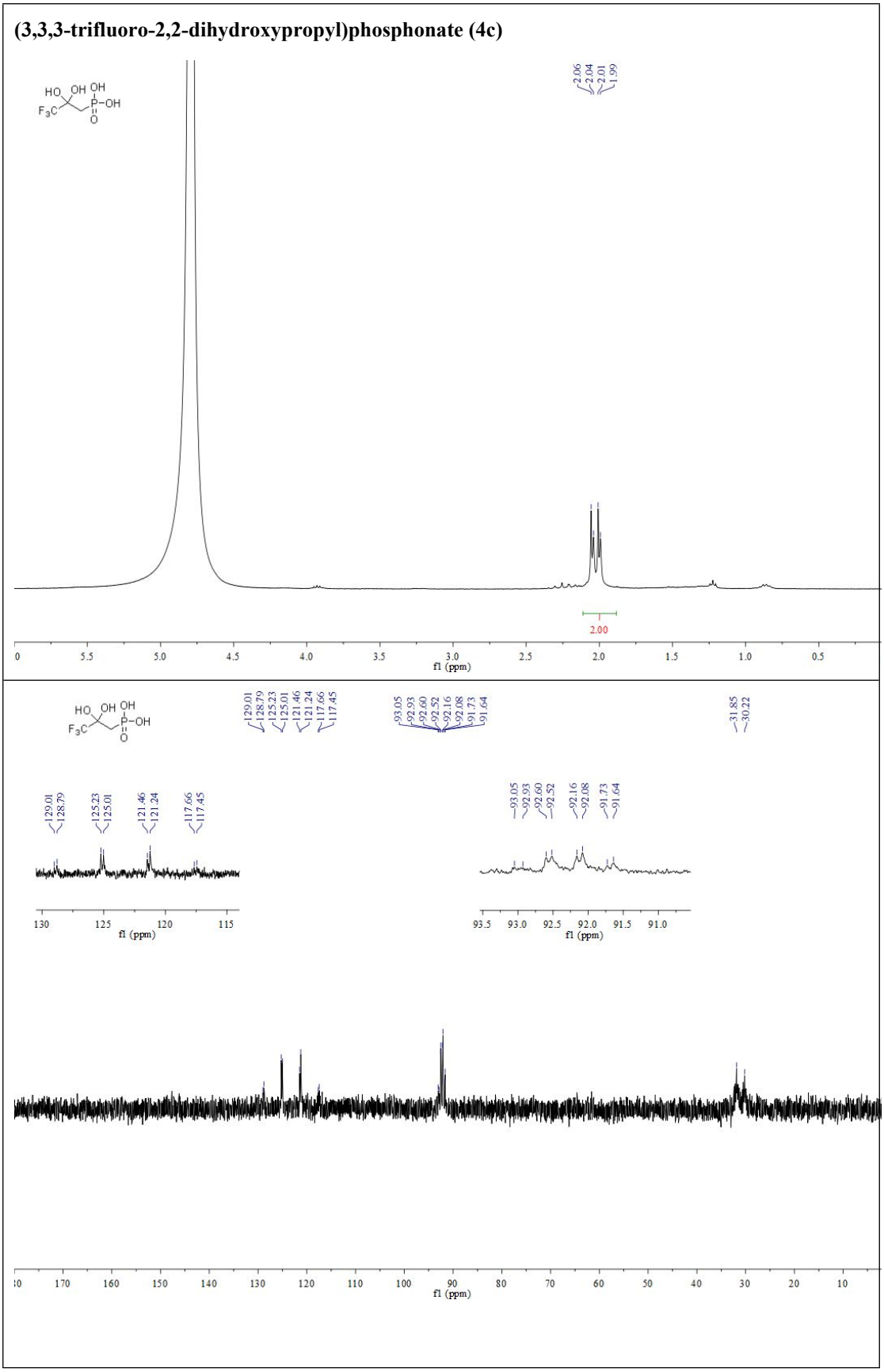
(*S*)-(3,3,3-trifluoro-2-hydroxypropyl)phosphonate (1c)



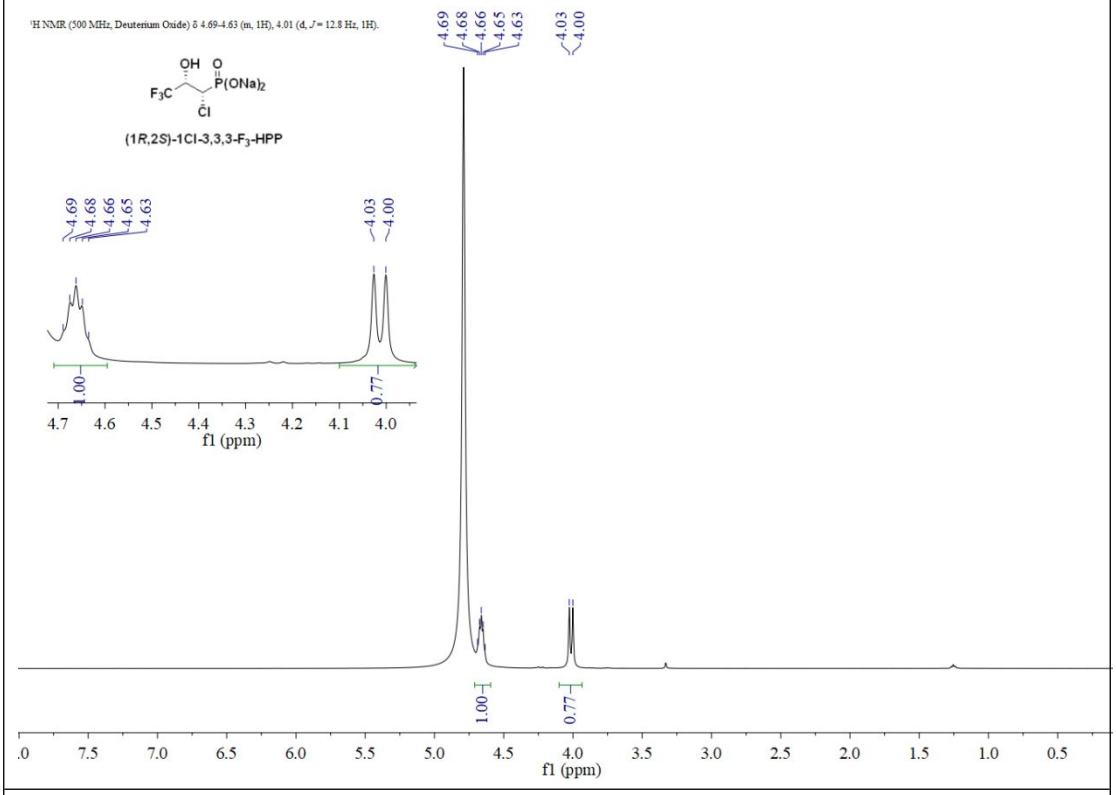
(1*S*,2*S*)-1,2-epoxy-3,3,3-trifluoropropylphosphonate (3c)

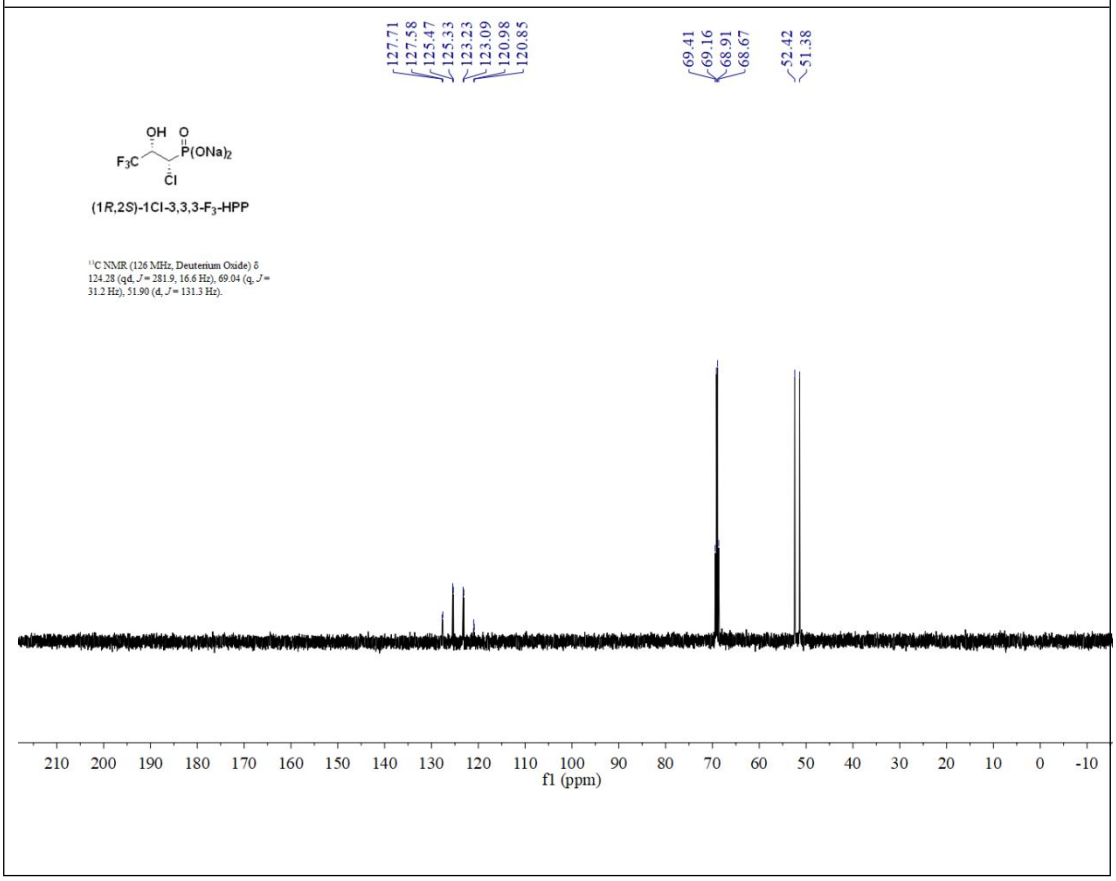


(3,3,3-trifluoro-2,2-dihydroxypropyl)phosphonate (4c)

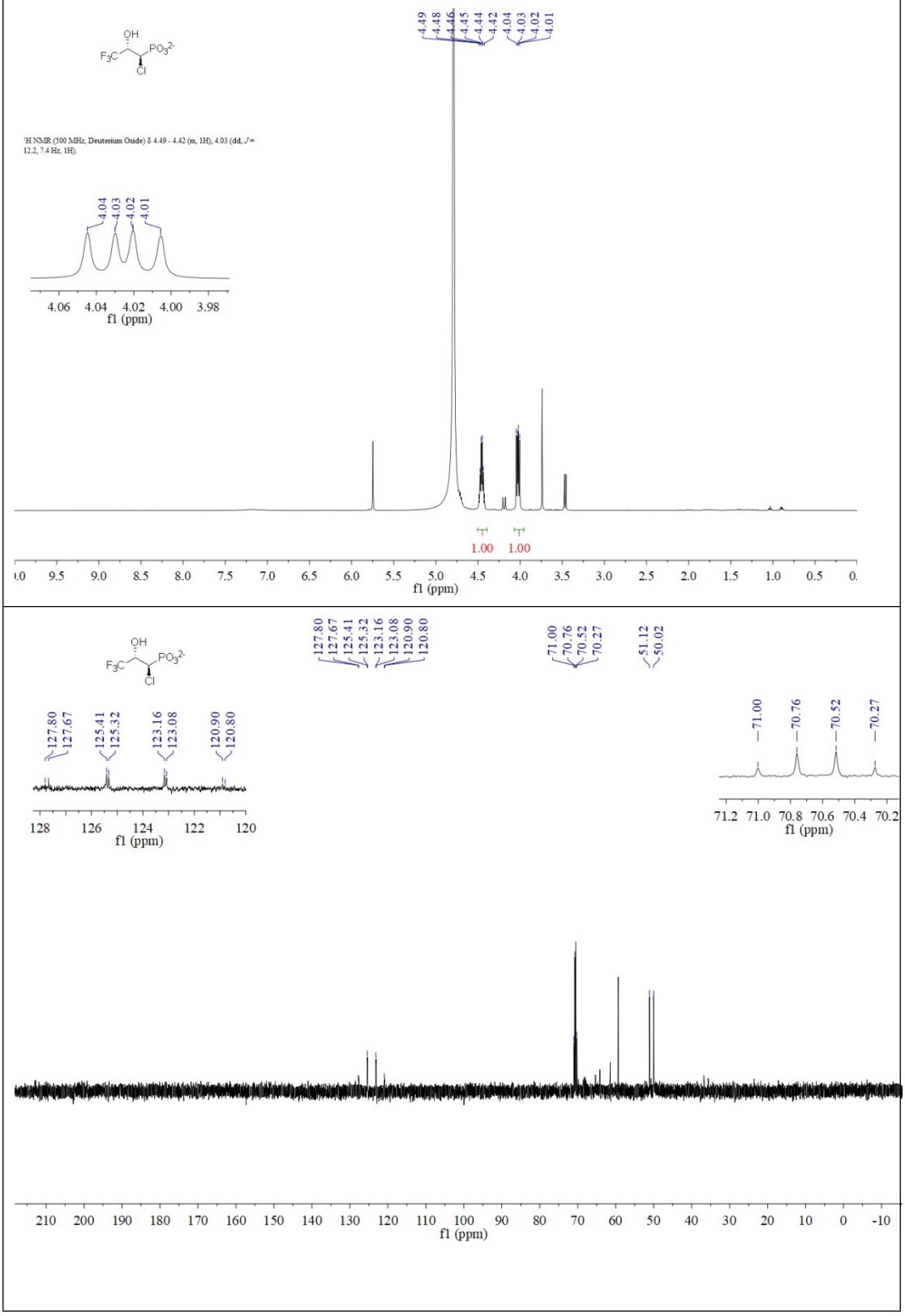


((1*R*,2*S*)-1-chloro-3,3,3-trifluoro-2-hydroxypropyl)phosphonate (1f)

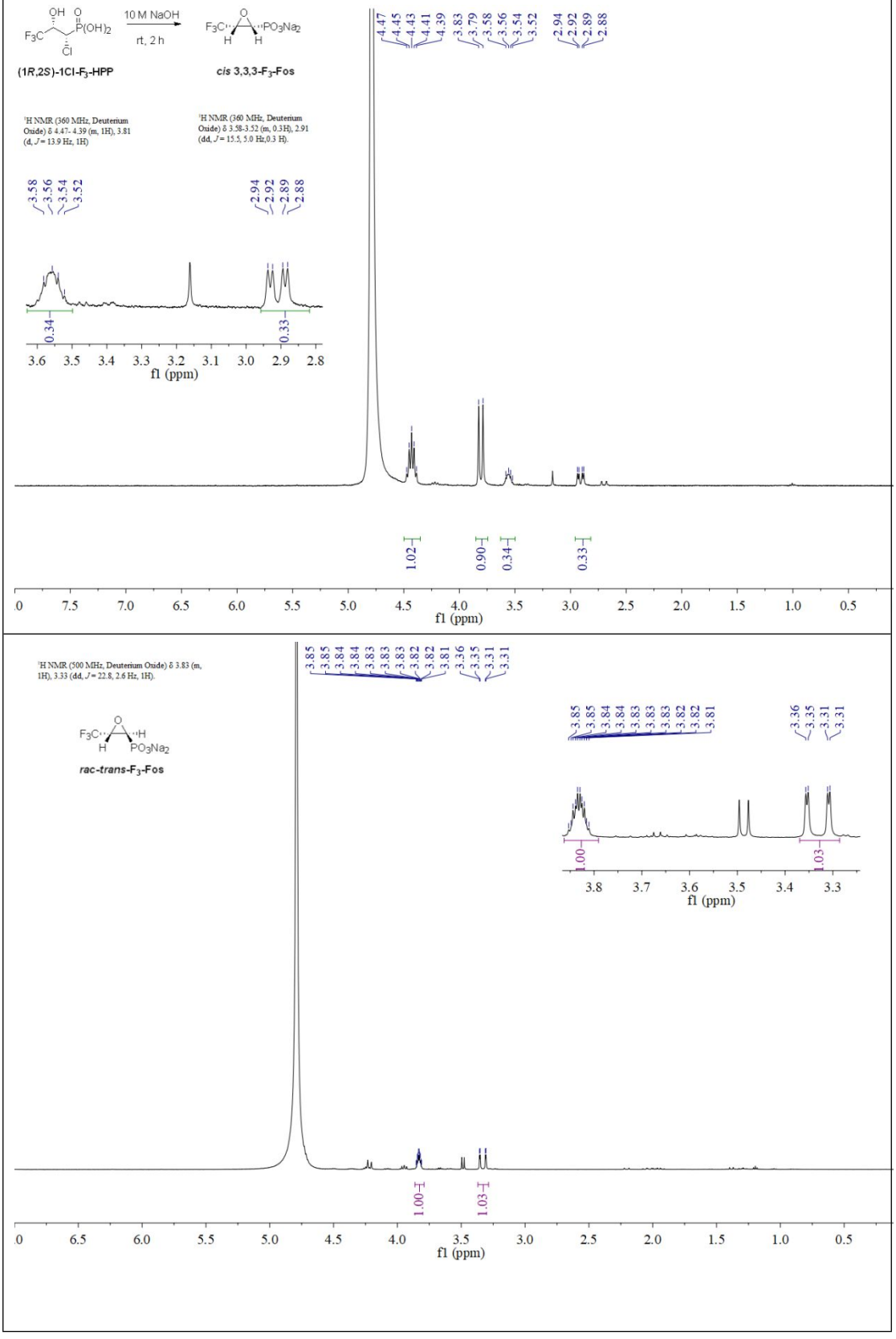




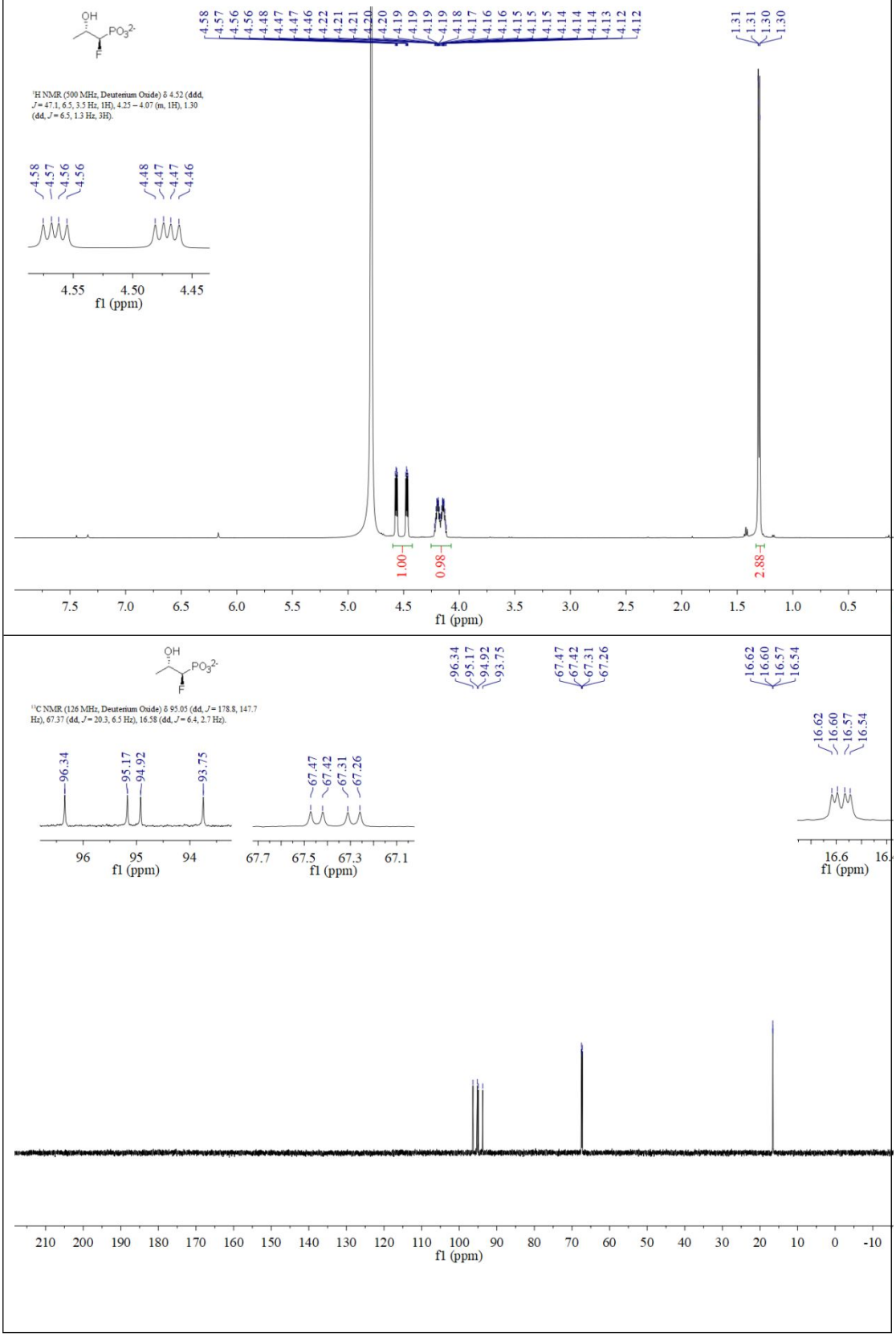
***rac-anti*-1-chloro-3,3,3-trifluoro-2-hydroxypropyl)phosphonate (1g)**



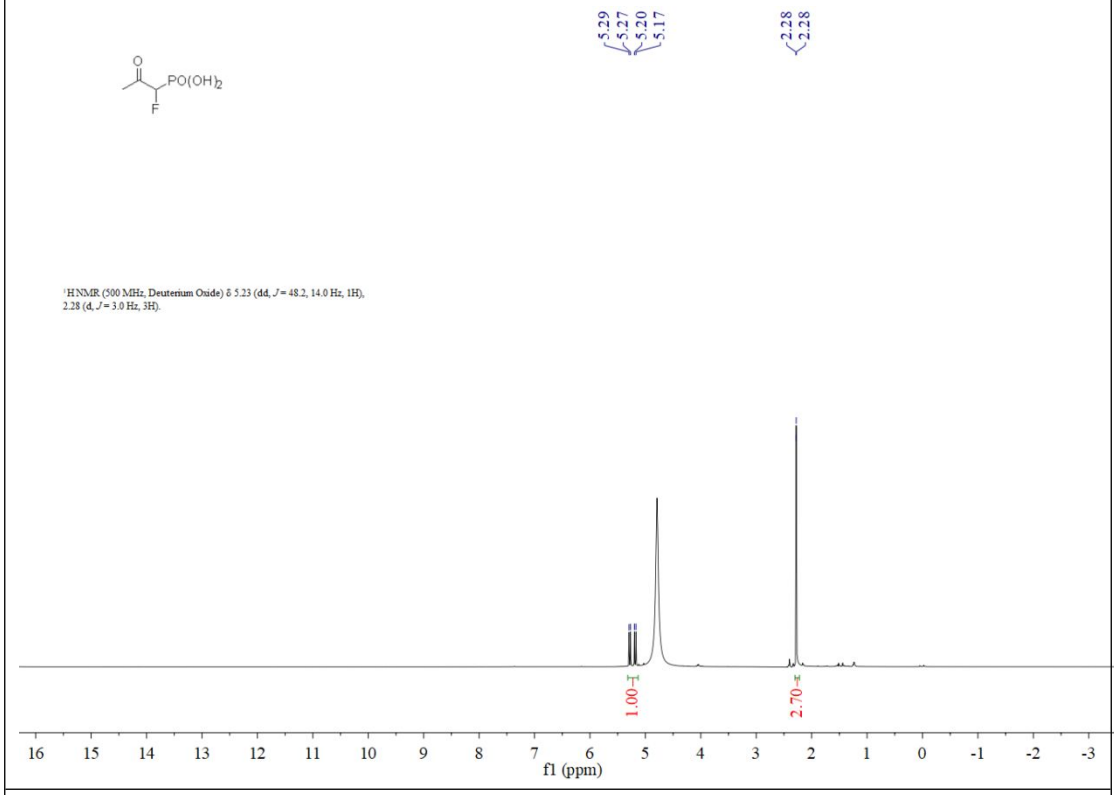
Cis- and _trans_-3,3,3-F_3-Fos (2c and 3c)



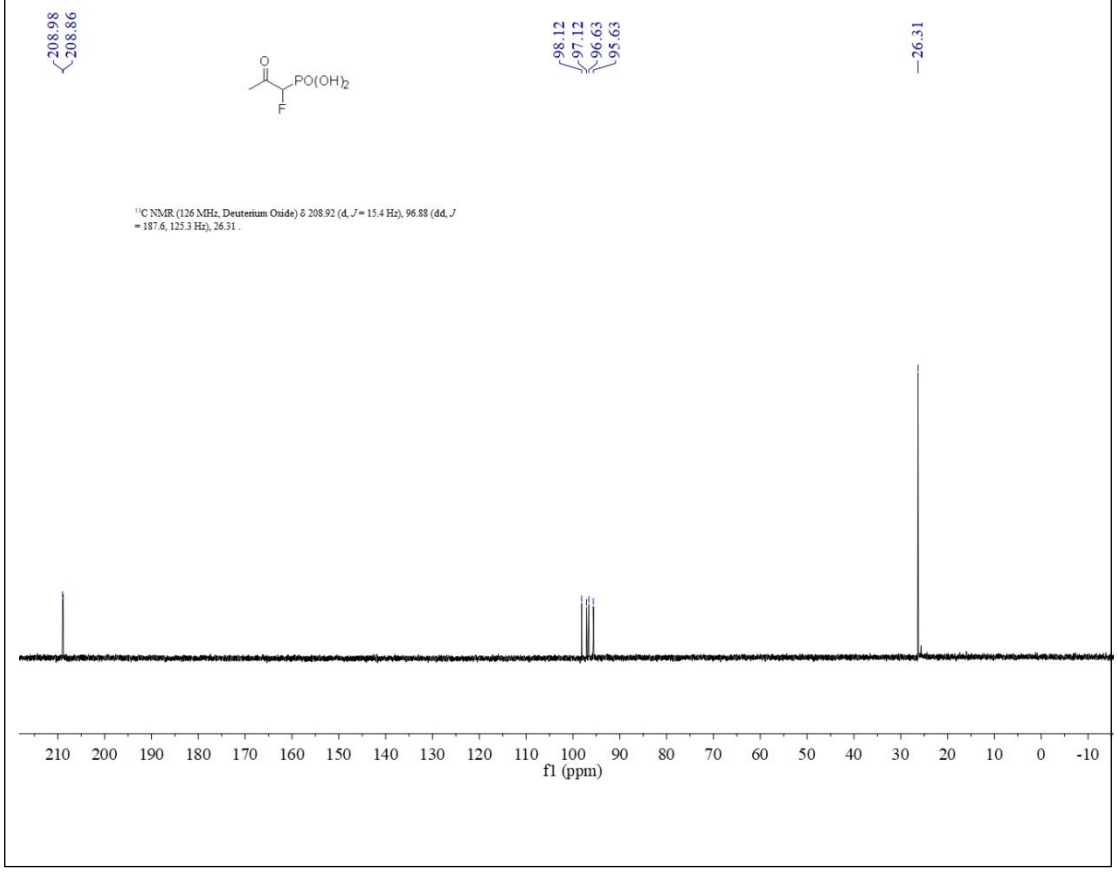
((1*R*,2*S*)-1-fluoro-2-hydroxypropyl)phosphonate (1h)



(1-fluoro-2-oxopropyl)phosphonate (4h)

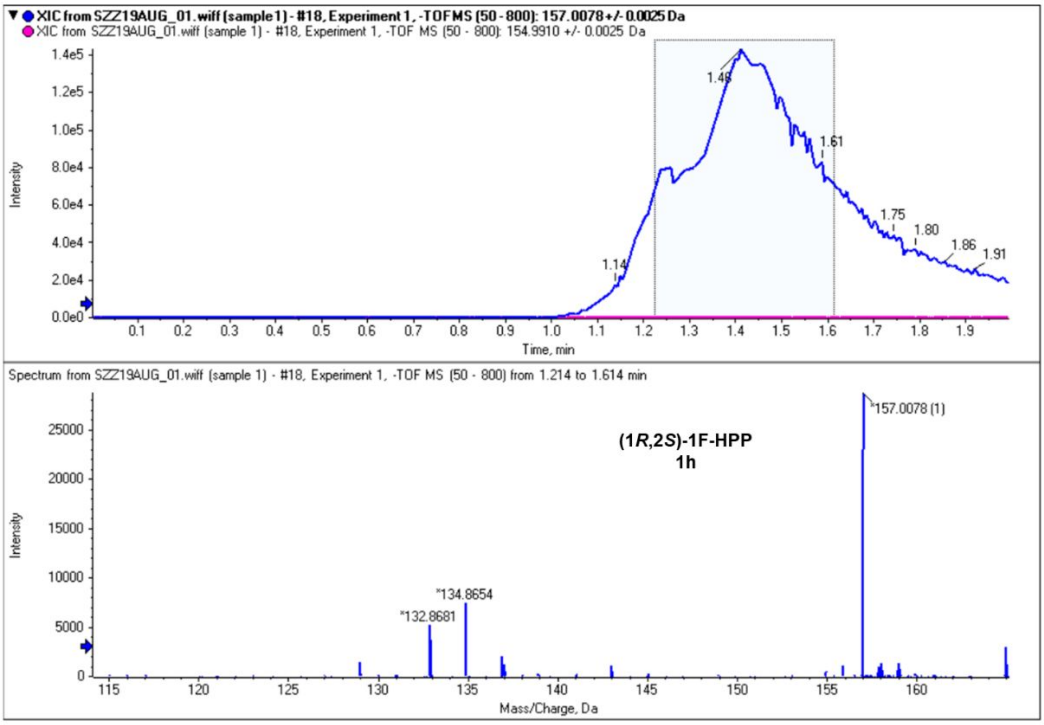


^{1}H NMR (500 MHz, Deuterium Oxide) δ 5.23 (dd, J = 48.2, 14.0 Hz, 1H), 2.28 (d, J = 3.0 Hz, 3H).

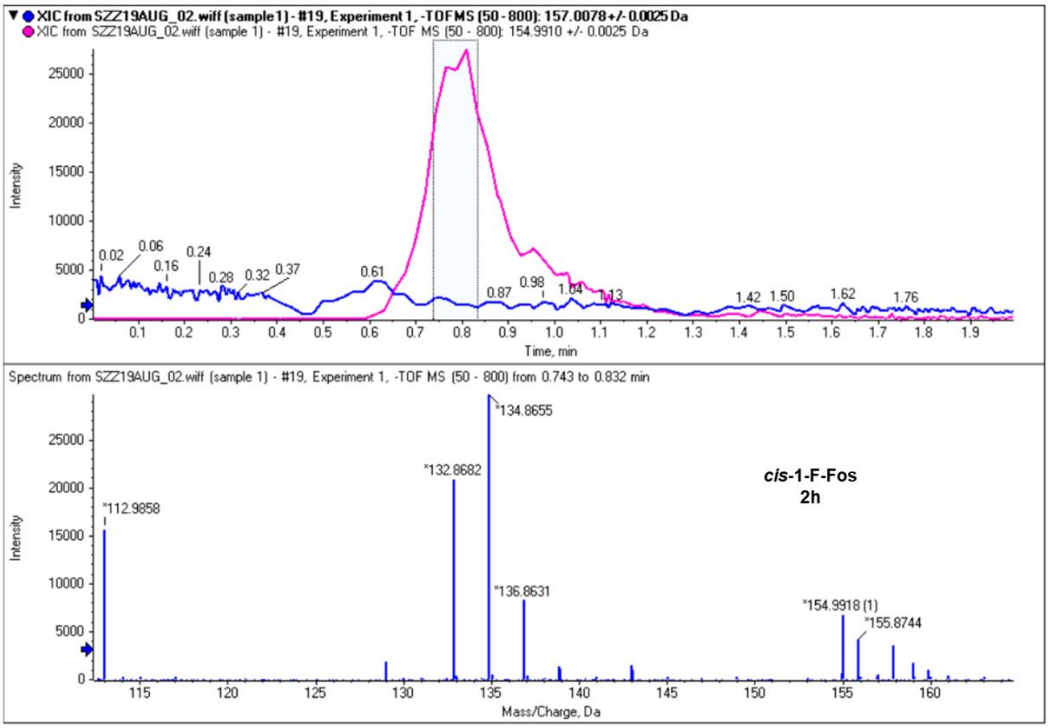


^{13}C NMR (126 MHz, Deuterium Oxide) δ 208.92 (d, J = 15.4 Hz), 96.88 (dd, J = 187.6, 125.3 Hz), 26.31 .

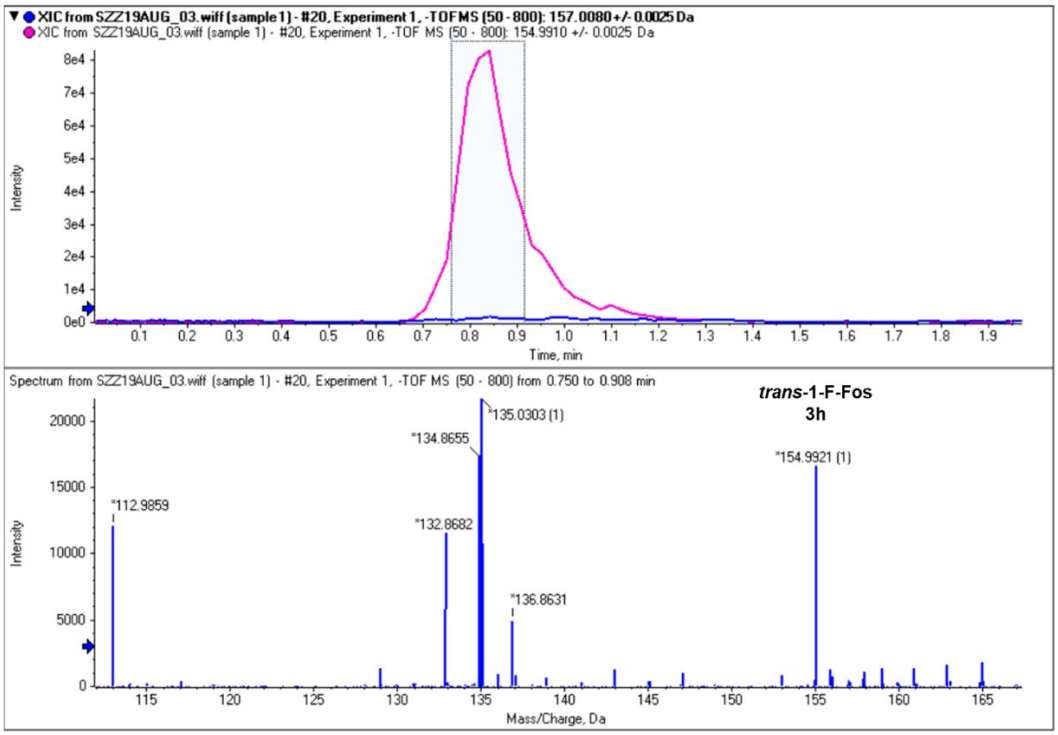
HRMS analysis of 1h



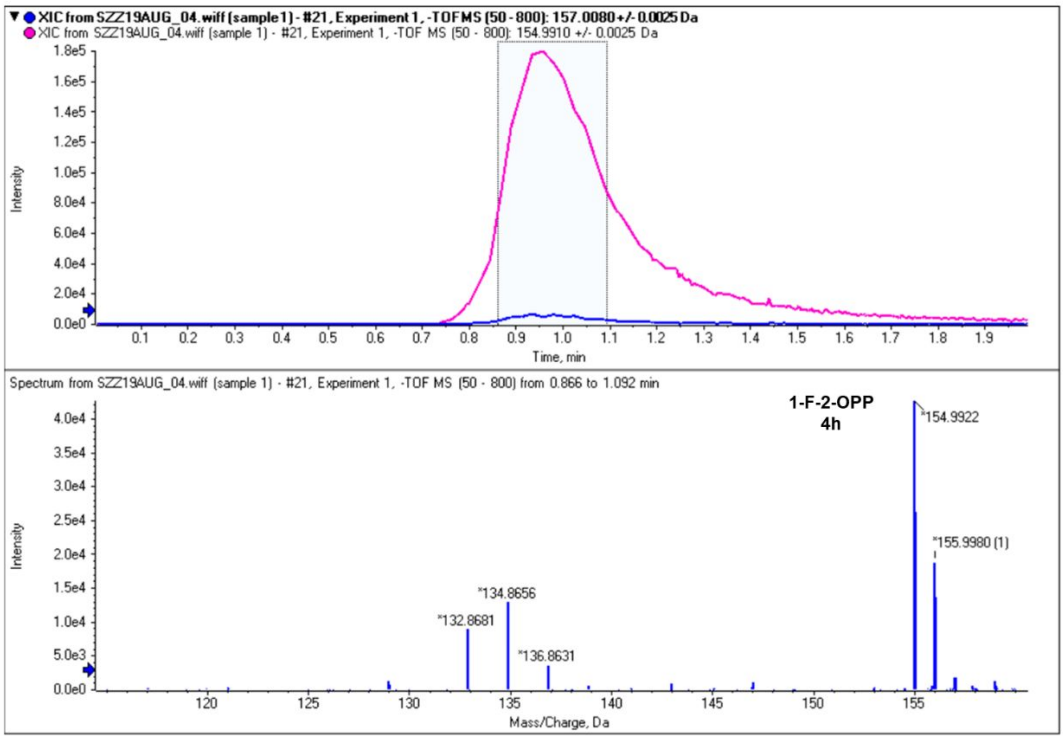
HRMS analysis of 2h



HRMS analysis of 3h



HRMS analysis of 4h



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