Supporting Information

Biocatalytic asymmetric construction of secondary and tertiary fluorides from ß-fluoro-α-ketoacids

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Abstract: Fluorine is a critical element for the design of bioactive compounds, but its incorporation with high regio- and stereoselectivity using environmentally friendly reagents and catalysts remains an area of development. Stereogenic tertiary fluorides pose a particular synthetic challenge and are thus present in only a few approved pharmaceuticals such as fluticasone, solithromycin, and sofosbuvir. The aldol reaction of fluorinated donors provides an atom-economical approach to asymmetric C-F motifs via C-C bond formation. Here we report that the type II pyruvate aldolase HpcH and engineered mutants thereof are biocatalysts for carboligation of ß-fluoro-α-ketoacids (including fluoropyruvate, ß-fluoro-α-ketobutyrate, and ß-fluoro-α-ketovalerate) with many diverse aldehydes. The reaction proceeds with kinetic resolution in the case of racemic donors. The reactivity of HpcH towards these new donors, which are non-native in both steric and electronic properties, grants access to enantiopure fragments with secondary or tertiary fluoride stereocenters. In addition to representing the first asymmetric synthesis of tertiary fluorides via biocatalytic carboligation, the afforded products could improve the diversity of fluorinated building blocks and enable the synthesis of fluorinated drug analogs.

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Materials and Methods

Commercial materials. Acetaldehyde, ammonium formate (LC-MS), ammonium persulfate, benzaldehyde, *n*-butyllithium (2.5 M in hexanes), catalase from bovine liver, CHES, chlorotrimethylsilane (TMS-Cl), chromium trioxide, cobalt chloride hexahydrate, copper(II) chloride dihydrate, cyclohexanecarboxaldehyde, DIBAL-H (1 M in toluene), diisopropylamine, dimethoxyacetaldehyde (60 wt.% in H_2O), 2,2-dimethoxypropane, 4-dimethylaminopyridine (DMAP), ethyl bromofluoroacetate, 2-ethylbutyraldehyde, ethyl 2-oxo-4-phenylbutyrate, ß-fluoropyruvic acid sodium salt monohydrate, 13.4 M formaldehyde, furfural, D-glyceraldehyde, L-glyceraldehyde, glycolaldehyde dimer, glyoxylic acid monohydrate, HEPPS (EPPS), HF-pyridine, iron(II) chloride, isobutyraldehyde, isovaleraldehyde, L-lactate dehydrogenase from rabbit muscle (LDH), lithium hydroxide monohydrate, lysozyme, 2-mercaptoethanol, 3 M methanolic HCl, (R)-(+)-α-methoxy-α-trifluoromethylphenylacetic acid ((+)-MTPA), methyl chloroacetate, methyl 2-oxobutanoate, 4-methyl-2-oxovaleric acid, nickel chloride hexahydrate, ß-nicotinamide adenine dinucleotide hydrate (NAD^+), ß-nicotinamide adenine dinucleotide reduced disodium salt hydrate (NADH), oxalyl chloride (2 M in CH_2Cl_2), 2-oxovaleric acid, phenylmethanesulfonyl fluoride (PMSF), polyethyleneimine (PEI), propionaldehyde, 2-pyridinecarboxaldehyde, 3-pyridinecarboxaldehyde, 4-pyridinecarboxaldehyde, Selectfluor, sodium methoxide, sodium phosphate dibasic heptahydrate, sodium pyruvate, sodium dodecyl sulfate, TAPS, TEMED, 2-thiophenecarboxaldehyde, zinc chloride, and zinc dust were purchased from Sigma-Aldrich (St. Louis, MO). Agarose, Bicine, bromophenol blue, calcium chloride dihydrate, CAPS, carbenicillin disodium salt, deoxynucleotides (dNTPs), dithiothreitol (DTT), EDTA disodium salt dihydrate, formic acid (LC-MS), glycerol, glycine, HEPES, 30 wt.% H_2O_2, imidazole, kanamycin sulfate, manganese chloride tetrahydrate, O'GeneRuler 1 kb Plus DNA Ladder, PageRuler Plus Prestained Protein Ladder, PEG 3350, PEG 8000, potassium chloride, sodium phosphate monobasic monohydrate, TCEP, trichloroacetic acid, Tricine, Tris base, and urea were purchased from Thermo Fisher Scientific (Waltham, MA). LB Miller agar, LB Miller broth, magnesium chloride hexahydrate, Terrific broth (TB), and triethylamine were from EMD-Millipore (Burlington, MA). Ethyl 2-fluoropropionate was from TCI (Tokyo, Japan). InstantBlue Protein Stain was from Expedeon (San Diego, CA). Isopropyl ß-D-1-thiogalactopyranoside (IPTG) was from Santa Cruz Biotechnology (Dallas, TX). Acrylamide-bis 30% solution, Bradford reagent, and ethidium bromide were from Bio-Rad (Hercules, CA). Chloroform-*d*, deuterium oxide, and DMSO-d_6 were from Cambridge Isotope Laboratories (Tewksbury, MA). Phusion polymerase, restriction enzymes, Taq ligase, and T5 exonuclease were from New England Biolabs (Ipswitch, MA). Ni-NTA agarose resin and DNA purification kits were from Qiagen (Redwood City, CA). Oligonucleotides and gBlocks gene fragments were synthesized by Integrated DNA Technologies (Coralville, IA). All chemicals were used as purchased.

Bacterial strains. *Escherichia coli* DH10B-T1^R was used for plasmid construction and *E. coli* BL21(DE3)-T1^R was used for protein production. The strains were made chemically competent by the method of [*1*].

Construction of expression plasmids. Plasmids were constructed by Gibson isothermal assembly and verified by sequencing (Quintara Biosciences; South San Francisco, CA and Genewiz; South Plainfield, NJ). *In silico* sequence alignment were performed on Benchling (Benchling, San Francisco, CA). All DNA sequences used in this study are listed in *Table S1*. To construct pET16hp-HpcH, the parent plasmid pET16hp-IMDH was digested with NdeI/BamHI. To construct pSV272.1-HpcH, the parent plasmid pSV272.1 was digested with SfoI/HindIII. Linearized vectors were purified by extraction from 1% agarose gel. The gene encoding HpcH from the *E. coli* C strain was obtained as a codon-optimized gBlock with overhangs homologous to pET16hp, and was resuspended in water to 50 ng/µL. To subclone HpcH into pSV272.1, the gene was amplified from pET16hp-HpcH using primers with overhangs homologous to pSV272.1. PCR primers were reconstituted to 100 µM in water. The 50 µL PCR reaction contained 5× Phusion HF buffer, 0.2 mM dNTPs, 10 ng template, 2 units Phusion HF polymerase, and 0.5 µM each primer. Thermal cycling was as follows: 3 min at 98 ºC, followed by 35 cycles of 30 s at 98 ºC, 30 s at (T_m-5) ºC, and 1 min at 72 ºC, followed by a final extension of 5 min at 72 ºC. A 5 µL sample was visualized on 1% agarose gel and the remaining material was purified by spin column.

Purified vectors and inserts were combined (9:1 *v/v*) in 5 µL volume, to which 15 µL of Gibson master mix, prepared according to [*2*], was added. The mixture was incubated in a thermocycler at 50 ºC for 1 h, then added to chemically competent *E. coli* DH10B-T1^R along with 20 µL of 5× KCM solution (0.5 M KCl, 0.15 M $CaCl_2$, 0.25 M $MgCl_2$) and water to 200 µL total. After incubation on ice for 20 min, the cells were heat-shocked at 42 ºC for 90 s and returned to ice for 2 min. Cells were diluted with 1 mL of LB, recovered with shaking at 37 ºC for 45 min, and pelleted by centrifugation (7,000 × *g*, 1 min). The supernatant was partially removed (0.8 mL), then the cells were resuspended and plated on LB agar with 50 µg/mL antibiotic. Plates were incubated at 37 ºC overnight. Liquid cultures (5 mL LB with 50 µg/mL antibiotic) were inoculated with single colonies and grown at 37 ºC overnight, following which the plasmids were isolated and confirmed by sequencing from the T7 promoter and T7 terminator. Plasmid pSV272.1-HpcH was also sequenced with the sequencing primer HpcH-seqL83-R to confirm MBP fusion.

All mutations of the pET16b-HpcH were introduced by the two-step modification [*3*] of the Agilent Quikchange method. Mutagenic primers were reconstituted to 100 µM in water. Single primer extension reactions (50 µL) were prepared containing 5X Phusion HF buffer, 0.2 mM dNTPs, 20 ng template, 1 unit Phusion HF polymerase, and 0.5 µM forward or reverse primer. Thermal cycling was as follows: 3

min at 98 ºC, followed by 10 cycles of 30 s at 98 ºC, 30 s at 65 ºC, and 3 min at 72 ºC, followed by a final extension of 5 min at 72 ºC. Then, 25 µL of each primer extension reaction was combined and supplemented with 1 unit Phusion HF polymerase. Thermal cycling was continued for 25 cycles. DpnI (2 units) was added to digest the template for 1 h at 37 ºC. *E. coli* DH10B-T1^R was transformed with 5 µL of the mixture (KCM method), then plated on LB agar with 50 µg/mL carbenicillin and incubated at 37 ºC overnight. Liquid cultures were inoculated with single colonies and grown at 37 ºC overnight. The plasmids were isolated and mutations confirmed by sequencing.

Expression and purification of His-tagged proteins. Expression plasmids (10 ng) were transformed into *E. coli* BL21(DE3)-T1^R (KCM method) and a single colony was used to inoculate 25 mL of TB media with 50 µg/mL of the appropriate antibiotic. The seed culture was grown overnight with shaking at 37 ºC (200 RPM). Then, 1 L of TB in an Ultra Yield baffled flask (Thomson Instrument Company; Oceanside, CA) was inoculated with the seed culture and growth was continued to OD600 of 0.8-1.2. The culture was chilled in an ice bath for 15 min and protein expression was induced with IPTG (1 mM). Expression proceeded at 16 ºC overnight, after which the cells were harvested (7,000 × *g*, 5 min, 4 ºC) and either stored at -80 ºC or subjected to protein purification immediately.

Harvested cells were resuspended in 5 mL/(g wet cell wt.) of lysis buffer (20 mM Tris pH 7.5, 500 mM NaCl, 10 mM imidazole, 10% *v/v* glycerol). To the cells was added PMSF (1 mM), lysozyme (1 mg/mL) and β-ME (0.01% *v/v*). After incubation at r.t. for 30 min, the sample was sonicated (QSonica Q700) with the following program: 10 s on, 20 s off, 1 min total process time, amplitude 50. Cell debris was removed by centrifugation (15,000 × *g*, 20 min, 4 ºC). To the supernatant was added PEI (0.05% *w/v*) and the precipitated nucleic acids were removed by centrifugation (15,000 × *g*, 20 min, 4 ºC). Ni-NTA agarose resin (50% suspension in 20% EtOH, 0.2 mL/(g wet cell wt.)) was added to the samples, which were shaken gently on an orbital shaker at r.t. for 30 min and then poured into a glass column. The resin was washed with >20 column volumes (CV) of wash buffer (same as lysis buffer with 20 mM imidazole) until the effluent tested negative by the Bradford dye-binding assay. Protein was eluted with >5 CV of elution buffer (same as lysis buffer with 250 mM imidazole) until the effluent tested negative again. The elution fraction was supplemented with β-ME (0.01% *v/v*) and $MgCl_2$ (1 mM). Fractions were analyzed by SDS-PAGE and all purified proteins are visualized in Figure S2.

The proteins were concentrated in Amicon Ultra-15 centrifugal filter units (EMD Millipore; Burlington, MA) with 10 kDa molecular weight cutoff for HpcH and 30 kDa cutoff for MBP-HpcH (5,000 × *g*, 20 min per round, 25 ºC). (Note that HpcH and its mutants are poorly soluble at 4 ºC). Between each round of centrifugation, the concentration gradient was dispersed by pipetting. Buffer exchange was conducted with PD-10 columns (GE Healthcare; Chicago, IL) into HEPES storage buffer (20 mM HEPES pH 7.5, 100 mM NaCl, 10% *v/v* glycerol, 1 mM TCEP). The desalted samples were supplemented with

$MgCl_2$ (1 mM) and further concentrated to 150 μM. Protein concentrations were determined by the absorbance at 280 nm, using extinction coefficients (wt-HpcH and all mutants except W19A: 32430 $M^{-1}cm^{-1}$, HpcH-W19A: 26930 $M^{-1}cm^{-1}$, MBP-HpcH: 100270 $M^{-1}cm^{-1}$) predicted with ExPASy ProtParam (Swiss Institute of Bioinformatics).

General remarks for 1D-NMR spectroscopy. NMR was performed at the UC Berkeley College of Chemistry NMR Facility. Characterization of isolated compounds (^1H, ^{13}C, and ^{19}F NMR) and ^{19}F NMR aldolase assays were performed on a Bruker AV-600 (600 MHz) equipped with a Z-gradient broadband cryoprobe (supported by NIH S10OD024998). ^1H NMR assays for native activity of aldolases was performed on a Bruker AV-500 (500 MHz) equipped with a Z-gradient broadband probe. All experiments were conducted at 298 K. Chemical shifts were referenced to the solvent and expressed relative to tetramethylsilane (^1H and ^{13}C) or trichlorofluoromethane (^{19}F). The spectral center of ^{19}F NMR was -150 ppm and the spectral width was 300 ppm. In general, the number of ^1H or ^{19}F scans was 8, and the number of ^{13}C scans was >256. For ^{19}F NMR aldolase assays, the number of ^{19}F scans was 1. Data was processed in MestreNova (MestreLab Research). Baseline distortion in the -150 to -200 ppm region of ^{19}F NMR due to fluoropolymer in the probes was corrected with backward linear prediction (Toeplitz) of the first 128 data points, followed by manual phase correction and baseline correction (Whittaker smoother).

General procedures for synthesis of 3-fluoro-2-oxoacids (1, 2a-d). Adapted from [*4*]. A critical optimization was the use of acetonitrile as solvent for all steps, which simplified the silylation workup, solubilized Selectfluor, and prevented fluoride elimination during hydrolysis.

Silylation procedure for synthesis of 2-oxoester silyl enol ethers. To a solution of 2-oxoester (20 mmol) in CH_3CN (40 mL) under N_2 was added TMS-Cl (2.39 g, 2.79 mL, 22 mmol) followed by Et_3N (2.43 g, 3.35 mL, 24 mmol). The mixture was stirred vigorously at r.t. for 4 h, then diluted with H_2O (60 mL) and extracted with hexanes (3 × 60 mL). The organic layer was washed with brine (60 mL), dried over Na_2SO_4 and concentrated. The silyl enol ether was purified by silica gel chromatography using the solvents indicated below.

Fluorination procedure for synthesis of 3-fluoro-2-oxoesters. To a solution of silyl enol ether (20 mmol) in CH_3CN (80 mL) was added finely crushed Selectfluor (7.79 g, 22 mmol). The suspension was stirred at r.t. overnight, then the solvent was evaporated. The product was extracted from the white solids by trituration with Et_2O (2 × 100 mL) which was then filtered and concentrated. The 3-fluoro-2-oxoester was purified by silica gel chromatography using the solvents indicated below. For mixtures of keto and hydrate forms, reported yields are based on the keto molecular weight.

Saponification procedure for synthesis of 3-fluoro-2-oxoacids. To a solution of 3-fluoro-2-oxoester (5 mmol, keto wt.) in CH_3CN (20 mL) was added $LiOH\text{-}H_2O$ (231 mg, 5.5 mmol). The reaction was stirred at r.t. for 1 h, then the solvent was evaporated. The residue was dissolved in a minimal amount of

water. After washing with EtOAc (2 × 30 mL) the aqueous layer was acidified to pH 0 with 6 M HCl and the product was extracted with EtOAc (3 × 30 mL). The organic layer was washed with brine (30 mL), dried over Na_2SO_4 and concentrated. The free acid was weighed, then neutralized according to the following procedure. A solution of the acid in CH_3CN (20 mL) was treated with quantitatively-prepared 3M NaOH (1.0 eq. hydrate wt.) and stirred vigorously for 15 min. The solvent was evaporated and the sodium salt dried under vacuum.

3-Fluoro-2-oxobutanoic acid (FKB, 2a).

TMS-enol ether of methyl 2-oxobutanoate. Methyl 2-oxobutanoate (2.32 g, 20 mmol) was silylated by the general procedure and chromatographed (29:1 hexanes/Et_2O) to give the silyl enol ether as a clear liquid (1.83 g, 49%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.15 (q, *J* = 7.1 Hz, 1H), 3.76 (s, 3H), 1.72 (d, *J* = 7.1 Hz, 3H), 0.24 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.2, 141.4, 118.4, 51.8, 11.3, 0.4.

Methyl 3-fluoro-2-oxobutanoate. The silyl enol ether of methyl 2-oxobutanoate (3.77 g, 20 mmol) was fluorinated by the general procedure and chromatographed (1:1 hexanes/Et_2O) to give the fluoroester as a yellow oil (2.24 g, 84%, keto/hydrate 31:69). ^{1}H NMR (600 MHz, $CDCl_3$) Keto δ 5.53 (dq, *J* = 48.0, 7.0 Hz, 1H), 3.93 (s, 3H), 1.64 (dd, *J* = 23.7, 7.0 Hz, 3H); Hydrate δ 4.87 (dq, *J* = 46.8, 6.4 Hz, 1H), 4.31 (s, 1H), 3.91 (s, 3H), 3.72 (s, 1H), 1.42 (dd, *J* = 24.7, 6.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) Keto δ 190.9 (d, *J* = 23.8 Hz), 160.8 (d, *J* = 1.8 Hz), 89.7 (d, *J* = 181.0 Hz), 53.2, 17.0 (d, *J* = 22.2 Hz); Hydrate δ 171.1 (d, *J* = 1.2 Hz), 93.4 (d, *J* = 25.0 Hz), 90.5 (d, *J* = 174.2 Hz), 53.7, 13.9 (d, *J* = 22.1 Hz). ^{19}F NMR (565 MHz, $CDCl_3$) Keto δ -189.5 (dq, *J* = 47.9, 23.9 Hz); Hydrate δ -188.8 (dq, *J* = 48.9, 24.7 Hz).

3-Fluoro-2-oxobutanoic acid (FKB, **2a***).* Methyl 3-fluoro-2-oxobutanoate (671 mg, 5 mmol) was saponified by the general procedure to give the fluoroacid as a cloudy syrup (273 mg, 40%) which was converted to a sodium salt prior to NMR analysis and experimental use. In aqueous solution the salt becomes ~91% hydrated, and spectral data is given only for the hydrate. ^{1}H NMR (600 MHz, D_2O) δ 4.79 (dq, *J* = 47.3, 6.5 Hz, 1H), 1.26 (dd, *J* = 25.5, 6.4 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 175.7 (d, *J* = 2.8 Hz), 94.4 (d, *J* = 22.2 Hz), 92.3 (d, *J* = 170.9 Hz), 14.1 (d, *J* = 21.8 Hz). ^{19}F NMR (565 MHz, D_2O) δ -188.6 (dq, *J* = 50.5, 25.8 Hz).

3-Fluoro-2-oxopentanoic acid (FKV, 2b).

Methyl 2-oxopentanoate. 2-Oxovaleric acid (2.32 g, 20 mmol) was dissolved in 2,2-dimethoxypropane (40 mL) and MeOH (10 mL). TMS-Cl (217 mg, 254 µL, 2 mmol) was added and the mixture was stirred at r.t. overnight. The solvent was evaporated and the residue purified by silica gel chromatography (4:1 hexanes/Et_2O) to give the methyl ester as a yellow liquid (2.34 g, 90%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.88 (s, 3H), 2.84 (t, *J* = 7.2 Hz, 2H), 1.69 (sext, *J* = 7.3 Hz, 2H), 0.98 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 194.2, 161.6, 52.8, 41.2, 16.5, 13.5.

TMS-enol ether of methyl 2-oxopentanoate. Methyl 2-oxopentanoate (2.60 g, 20 mmol) was silylated by the general procedure and chromatographed (29:1 hexanes/Et_2O) to give the silyl enol ether as a clear liquid (2.79 g, 69%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 6.07 (t, *J* = 7.5 Hz, 1H), 3.77 (s, 3H), 2.19 (quint, *J* = 7.5 Hz, 2H), 1.04 (t, *J* = 7.6 Hz, 3H), 0.23 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.4, 139.9, 125.4, 51.8, 19.2, 13.2, 0.4.

Methyl 3-fluoro-2-oxopentanoate. The silyl enol ether of methyl 2-oxopentanoate (4.05 g, 20 mmol) was fluorinated by the general procedure and chromatographed (4:1 hexanes/Et_2O) to give the fluoroester as a yellow liquid (2.23 g, 75%, keto/hydrate 53:47.). ^{1}H NMR (600 MHz, $CDCl_3$) Keto δ 5.37 (ddd, *J* = 48.7, 7.6, 4.2 Hz, 1H), 3.93 (s, 3H), 2.14-1.88 (m, 2H), 1.09 (t, *J* = 3.8 Hz, 3H); Hydrate δ 4.60 (ddd, *J* = 47.4, 9.8, 2.9 Hz, 1H), 4.17 (s, 1H), 3.91 (s, 3H), 3.45 (s, 1H), 1.87-1.66 (m, 2H), 1.07 (t, *J* = 3.8 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) Keto δ 190.9 (d, *J* = 23.9 Hz), 161.0 (d, *J* = 2.1 Hz), 94.0 (d, *J* = 184.1 Hz), 53.1, 24.7 (d, *J* = 21.4 Hz), 8.7 (d, *J* = 4.0 Hz); Hydrate δ 171.0 (d, *J* = 1.5 Hz), 95.4 (d, *J* = 176.8 Hz), 93.3 (d, *J* = 25.3 Hz), 53.7, 21.5 (d, *J* = 21.1 Hz), 9.7 (d, *J* = 3.9 Hz). ^{19}F NMR (565 MHz, $CDCl_3$) Keto δ -198.8 (ddd, *J* = 49.9, 27.8, 23.2 Hz); Hydrate δ -197.8 (ddd, *J* = 47.2, 39.5, 15.7 Hz).

3-Fluoro-2-oxopentanoic acid (FKV, **2b***).* Methyl 3-fluoro-2-oxopentanoate (741 mg, 5 mmol) was saponified by the general procedure to give the fluoroacid as a cloudy syrup (403 mg, 53%) which was converted to a sodium salt prior to NMR analysis and experimental use. In aqueous solution the salt becomes ~68% hydrated. ^{1}H NMR (600 MHz, D_2O) Keto δ 5.46 (ddd, *J* = 49.0, 6.9, 4.4 Hz, 1H), 2.06-1.77 (m, 2H), 0.93 (t, *J* = 7.4 Hz, 3H); Hydrate δ 4.53 (ddd, *J* = 47.9, 8.9, 3.7 Hz, 1H), 1.66-1.47 (m, 2H), 0.95 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, D_2O) Keto δ 202.2 (d, *J* = 20.6 Hz), 168.6 (d, *J* = 8.3 Hz), 95.3 (d, *J* = 180.0 Hz), 24.2 (d, *J* = 20.9 Hz), 7.9 (d, *J* = 3.9 Hz). Hydrate δ 175.7 (d, *J* = 2.9 Hz), 97.4 (d, *J* = 174.0 Hz), 94.3 (d, *J* = 22.1 Hz), 21.9 (d, *J* = 20.9 Hz), 9.2 (d, *J* = 3.8 Hz). ^{19}F NMR (565 MHz, D_2O) Keto δ -198.8 (dt, *J* = 52.2, 27.2 Hz); Hydrate δ -198.4 (m).

3-Fluoro-4-methyl-2-oxopentanoic acid (FKMV, 2c).

Methyl 4-methyl-2-oxopentanoate. 4-Methyl-2-oxovaleric acid (2.60 g, 20 mmol) was dissolved in 2,2-dimethoxypropane (40 mL) and methanol (10 mL). Chlorotrimethylsilane (217 mg, 254 μL, 2 mmol) was added and the mixture was stirred at r.t. overnight. The solvent was evaporated and the residue purified by silica gel chromatography (9:1 hexanes/Et_2O) to give the methyl ester as a yellow liquid (2.63 g, 91%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.88 (s, 3H), 2.73 (d, *J* = 6.9 Hz, 2H), 2.21 (non, *J* = 6.7 Hz, 1H), 0.98 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 194.0, 161.7, 52.9, 47.9, 24.2, 22.4.

TMS-enol ether of methyl 4-methyl-2-oxopentanoate. Methyl 4-methyl-2-oxopentanoate (2.88 g, 20 mmol) was silylated by the general procedure and chromatographed (29:1 hexanes/Et_2O) to give the silyl enol ether as a clear liquid (2.95 g, 68%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.92 (d, *J* = 9.6 Hz, 1H), 3.77 (s,

3H), 2.77 (dsept, J = 9.7, 6.7 Hz, 1H), 1.03 (d, J = 6.7 Hz, 6H), 0.24 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 165.6, 138.6, 130.5, 51.8, 25.4, 22.1, 0.4.

Methyl 3-fluoro-4-methyl-2-oxopentanoate. The silyl enol ether of methyl 4-methyl-2-oxopentanoate (4.33 g, 20 mmol) was fluorinated by the general procedure and chromatographed (9:1 hexanes/Et_2O) to give the fluoroester as a yellow liquid (2.59 g, 80%, keto/hydrate 88:12). The spectral data is given only for the keto form. ^{1}H NMR (600 MHz, $CDCl_3$) δ 5.22 (dd, J = 48.6, 4.1 Hz, 1H), 3.93 (s, 3H), 2.39 (m, 1H), 1.14 (d, J = 6.9 Hz, 3H), 0.99 (d, J = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 191.2 (d, J = 24.5 Hz), 161.4 (d, J = 2.2 Hz), 96.8 (d, J = 186.5 Hz), 53.1, 30.4 (d, J = 20.5 Hz), 18.5 (d, J = 3.9 Hz), 15.9 (d, J = 5.3 Hz). ^{19}F NMR (565 MHz, $CDCl_3$) δ -207.0 (dd, J = 48.6, 26.6 Hz).

3-Fluoro-4-methyl-2-oxopentanoic acid (FKMV, **2c***).* Methyl 3-fluoro-4-methyl-2-oxopentanoate (811 mg, 5 mmol) was saponified by the general procedure to give the fluoroacid as a cloudy syrup (636 mg, 77%) which was converted to a sodium salt prior to NMR analysis and experimental use. In aqueous solution the salt becomes ~25% hydrated. ^{1}H NMR (600 MHz, D_2O) Keto δ 5.38 (dd, J = 48.9, 2.6 Hz, 1H), 2.36-2.23 (m, 1H), 1.04 (d, J = 7.0 Hz, 3H), 0.84 (d, J = 7.0 Hz, 3H); Hydrate δ 4.38 (dd, J = 46.5, 6.4 Hz, 1H), 1.99-1.88 (m, 1H), 0.94 (d, J = 6.9 Hz, 3H), 0.91 (d, J = 6.9 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) Keto δ 202.3 (d, J = 19.9 Hz), 168.8 (d, J = 2.3 Hz), 98.1 (d, J = 183.5 Hz), 29.8 (d, J = 20.1 Hz), 17.9 (d, J = 3.3 Hz), 14.9 (d, J = 5.6 Hz); Hydrate δ 175.8 (d, J = 3.3 Hz), 99.6 (d, J = 176.3 Hz), 94.5 (d, J = 22.2 Hz), 28.2 (d, J = 20.5 Hz), 19.0 (d, J = 6.5 Hz), 17.1 (d, J = 7.5 Hz). ^{19}F NMR (565 MHz, D_2O) Keto δ -208.6 (dd, J = 48.9, 30.6 Hz); Hydrate δ -200.7 (dd, J = 46.2, 19.0 Hz).

3-Fluoro-2-oxo-4-phenylbutanoic acid (FKPB, 2d).

TMS-enol ether of ethyl 2-oxo-4-phenylbutanoate. Ethyl 2-oxo-4-phenylbutanoate (4.12 g, 20 mmol) was silylated by the general procedure with the modification that the product was extracted with 1:1 hexanes/toluene, then chromatographed (20:9:1 hexanes/toluene/Et_2O) to give the silyl enol ether as a clear liquid (3.57 g, 64%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.35-7.16 (m, 5H), 6.25 (t, J = 7.6 Hz, 1H), 4.24 (q, J = 7.1 Hz, 2H), 3.54 (d, J = 7.6 Hz, 2H), 1.33 (t, J = 7.1 Hz, 3H), 0.28 (s, 9H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 164.8, 141.0, 139.7, 128.53, 128.47, 126.2, 121.3, 61.0, 32.0, 14.2, 0.6.

Ethyl 3-fluoro-2-oxo-4-phenylbutanoate. The silyl enol ether of ethyl 2-oxo-4-phenylbutanoate (5.57 g, 20 mmol) was fluorinated by the general procedure and chromatographed (1:1 hexanes/Et_2O) to give the fluoroester as bright yellow syrup (3.75 g, 84%, keto/hydrate 19:81). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.38-7.21 (m, 5H), 5.64 (ddd, J = 48.4, 8.3, 4.0 Hz, 1H keto), 4.92 (ddd, J = 46.9, 8.6, 4.1 Hz, 1H hydrate), 4.40-4.24 (m, 2H), 4.33 (s, 1H hydrate), 3.49 (s, 1H hydrate), 3.39-2.96 (m, 2H), 1.39 (t, J = 7.2 Hz, 3H keto), 1.35 (t, J = 7.2 Hz, 3H hydrate). ^{13}C NMR (151 MHz, $CDCl_3$) Keto δ 190.3 (d, J = 23.3 Hz), 160.4, 134.6 (d, J = 1.6 Hz), 129.4, 128.8, 127.4, 93.2 (d, J = 186.4 Hz), 62.9, 37.8 (d, J = 21.0 Hz), 13.9; Hydrate δ 170.3, 136.5 (d, J = 2.7 Hz), 129.4, 128.6, 126.8, 94.3 (d, J = 179.4 Hz), 93.1 (d, J = 25.4 Hz),

63.3, 34.8 (d, J = 21.2 Hz), 14.0. ^{19}F NMR (565 MHz, $CDCl_3$) Keto δ -193.9 (ddd, J = 48.4, 30.8, 22.1 Hz); Hydrate δ -193.7 (ddd, J = 46.8, 35.0, 19.3 Hz).

3-Fluoro-2-oxo-4-phenylbutanoic acid (FKPB, **2d***).* Ethyl 3-fluoro-2-oxo-4-phenylbutanoate (1.12 g, 5 mmol) was saponified by the general procedure to give the fluoroacid as a yellow paste-like solid (825 mg, 77%) which was converted to a sodium salt prior to NMR analysis and experimental use. In aqueous solution the salt becomes ~90% hydrated, and spectral data is given only for the hydrate. ^{1}H NMR (600 MHz, D_2O) δ 7.39-7.23 (m, 5H), 4.89-4.75 (m, 1H), 3.03-2.85 (m, 2H). ^{13}C NMR (151 MHz, D_2O) δ 175.6, 137.6, 129.3, 128.8, 126.8, 96.3 (d, J = 176.5 Hz), 94.2 (d, J = 22.6 Hz), 34.9 (d, J = 20.9 Hz). ^{19}F NMR (565 MHz, D_2O) δ -196.2 (m).

3-Fluoro-3-phenylpyruvic acid (FPP, 2e). Adapted from [*5*].

Methyl phenylglycidate. To a mixture of benzaldehyde (4.24 g, 4.07 mL, 40 mmol) and methyl chloroacetate (8.68 g, 7.01 mL, 80 mmol) was slowly added a suspension of sodium methoxide (6.48 g, 120 mmol) in methanol (60 mL). The thick mixture was stirred vigorously for 4 h, then diluted with water (150 mL). The product was extracted with EtOAc (3 × 70 mL), washed with brine (70 mL), dried over Na_2SO_4, and concentrated. Purification by silica gel chromatography (9:1 hexanes/EtOAc) gave the product as a clear liquid (1.69 g, 24%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.41-7.36 (m, 3H), 7.34-7.30 (m, 2H), 4.13 (d, J = 1.8 Hz, 1H), 3.86 (s, 3H), 3.54 (d, J = 1.8 Hz, 1H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 168.6, 134.9, 129.0, 128.7, 125.8, 58.0, 56.6, 52.6.

Methyl 3-fluoro-3-phenyllactate. In a polypropylene tube, methyl phenylglycidate (1.78 g, 10 mmol) was dissolved in dichloromethane (20 mL), then 70% HF/pyridine (0.91 mL, ~35 mmol HF) was added dropwise. After stirring 1 h the mixture was diluted with EtOAc (60 mL). The organic layer was washed with sat. $NaHCO_3$ (2 × 40 mL), water (40 mL), and brine (40 mL), then dried over Na_2SO_4 and concentrated. Purification by silica gel chromatography (4:1 hexanes/EtOAc) gave the product (3:1 syn/anti mixture of diastereomers) as a yellow oil (1.42 g, 72%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.46-7.33 (m, 5H), 5.81 (dd, J = 45.1, 2.3 Hz, 1H syn), 5.75 (dd, J = 44.9, 3.8 Hz, 1H anti), 4.69 (ddd, J = 15.0, 7.3, 3.8 Hz, 1H anti), 4.47 (ddd, J = 27.0, 6.8, 2.3 Hz, 1H syn), 3.90 (s, 3H syn), 3.79 (s, 3H anti), 3.03 (d, J = 6.9 Hz, 1H syn), 2.87 (d, J = 7.5 Hz, 1H anti). ^{13}C NMR (151 MHz, $CDCl_3$) Syn δ 171.8 (d, J = 2.8 Hz), 135.6 (d, J = 20.9 Hz), 128.8 (d, J = 1.2 Hz), 128.4, 125.9 (d, J = 7.6 Hz), 93.2 (d, J = 180.2 Hz), 73.6 (d, J = 23.7 Hz), 53.1; Anti δ 171.3 (d, J = 9.3 Hz), 134.7 (d, J = 21.0 Hz), 129.0 (d, J = 1.2 Hz), 128.4, 126.1 (d, J = 7.2 Hz), 93.7 (d, J = 180.7 Hz), 73.7 (d, J = 25.9 Hz), 52.8. ^{19}F NMR (565 MHz, $CDCl_3$) Syn δ -194.5 (dd, J = 45.1, 27.0 Hz); Anti δ -188.0 (dd, J = 45.3, 15.3 Hz).

Methyl 3-fluoro-3-phenylpyruvate. The 3-fluoro-3-phenyllactate (1.39 g, 7 mmol) was dissolved in acetone (10 mL) followed by addition of Jones reagent (10 mL, 2.5 M CrO_3 in 25% aq. H_2SO_4). The mixture was stirred overnight, quenched with isopropanol (2 mL), and diluted with water (60 mL). The

product was extracted with EtOAc (3 × 40 mL), washed with sat. $NaHCO_3$ (40 mL) and brine (40 mL), then dried over Na_2SO_4 and concentrated. Purification by silica gel chromatography (4:1 hexanes/EtOAc) gave the product as a yellow oil (437 mg, 32%, keto/hydrate 66:34). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.52-7.40 (m, 5H), 6.48 (d, *J* = 47.1 Hz, 1H keto), 5.69 (d, *J* = 44.6 Hz, 1H hydrate), 3.94 (s, 3H hydrate), 3.84 (s, 3H keto). ^{13}C NMR (151 MHz, $CDCl_3$) Keto δ 187.0 (d, *J* = 24.9 Hz), 160.1 (d, *J* = 3.2 Hz), 131.2 (d, *J* = 20.4 Hz), 130.3 (d, *J* = 2.5 Hz), 129.2, 128.0 (d, *J* = 5.1 Hz), 93.2 (d, *J* = 187.0 Hz), 53.2; Hydrate δ 170.6, 132.9 (d, *J* = 20.6 Hz), 129.5 (d, *J* = 1.2 Hz), 128.2, 127.6 (d, *J* = 7.0 Hz), 93.3 (d, *J* = 181.2 Hz), 93.2 (d, *J* = 28.7 Hz), 53.8. ^{19}F NMR (565 MHz, $CDCl_3$) Keto δ -183.6 (d, *J* = 47.0 Hz); Hydrate δ -189.2 (d, *J* = 44.4 Hz).

3-Fluoro-3-phenylpyruvic acid (FPP, **2e***).* Methyl 3-fluorophenylpyruvate (588 mg, 3 mmol) was saponified by the general procedure with lithium hydroxide monohydrate (138 mg, 3.3 mmol) to give the fluoroacid as a cloudy yellow syrup (508 mg, 85%), which was converted to a sodium salt prior to NMR analysis and experimental use. In aqueous solution the salt becomes ~80% hydrated, and spectral data is given only for the hydrate. ^{1}H NMR (600 MHz, D_2O) δ 7.48-7.35 (m, 5H), 5.63 (d, *J* = 44.8 Hz). ^{13}C NMR (151 MHz, D_2O) δ 175.31 (d, *J* = 2.2 Hz), 134.5 (d, *J* = 20.2 Hz), 129.1 (d, *J* = 1.7 Hz), 128.1, 127.4 (d, *J* = 7.3 Hz), 95.1 (d, *J* = 176.3 Hz), 94.5 (d, *J* = 26.1 Hz). ^{19}F NMR (565 MHz, D_2O) δ -189.8 (d, *J* = 44.3 Hz).

Native activity assay of pyruvate aldolases. Reactions of 0.6 mL total volume were prepared containing 50 mM sodium pyruvate (from 1 M in H_2O, 30 μL), 100 mM glycolaldehyde (from 2 M in H_2O, 30 μL), 0.1 mol% aldolase (from 150 μM solution in storage buffer, 200 μL), and 20 mM HEPES, 1 mM $MgCl_2$, pH 7.5 (340 μL). Reactions proceeded at r.t. for 1 h and were quenched by decarboxylation with 60 μL of 30 wt.% H_2O_2 and incubation for 30 min. After removal of precipitated proteins by centrifugation (15,000 × *g*, 10 min), the samples were frozen at -80 ºC and lyophilized in a vacuum concentrator, and remaining solids were resuspended in D_2O (700 μL) for ^{1}H NMR analysis. The extent of conversion was obtained from integration of acetate methyl protons (2.81 ppm) and 3,4-dihydroxybutanoate methylene protons (2.23 ppm). The native activity of HpcH aldolase, all studied HpcH mutants, and MBP-HpcH was confirmed by observation of >90% conversion. In the absence of enzyme (volume replaced with storage buffer), 0% conversion was observed.

Aldol addition assay of fluorinated donor substrates. Reactions of 0.6 mL total volume were prepared containing 50 mM fluoro-donor substrate, 100 mM acceptor substrate (30 μL of a 2 M stock solution in most cases), 0.1 mol% aldolase (from 150 μM solution in storage buffer, 200 μL), and 20 mM HEPES, 1 mM $MgCl_2$, pH 7.5 (370 μL). The desired amount of fluoro-donor was weighed and dissolved in reaction buffer, and this solution was aliquoted before adding acceptors or enzymes. Formaldehyde, acetaldehyde, propionaldehyde, D-glyceraldehyde, glycolaldehyde, dimethoxyacetaldehyde, glyoxylic

acid, 2-, 3-, and 4-pyridinecarboxaldehyde were added from 2 M solutions in H_2O. The solution of glyoxylic acid was neutralized with 3 M NaOH prior to use. The expensive acceptor L-glyceraldehyde was applied at only 50 mM, and was added from a 1 M solution in H_2O. The acceptors 2-ethylbutyraldehyde, cyclohexanecarboxaldehyde, benzaldehyde, and 2-thiophenecarboxaldehyde were added from 2 M solutions in DMSO, and thus the reactions contained 5% DMSO *v/v* as co-solvent. Isobutyraldehyde, isovaleraldehyde, and furfural were added as pure liquids (60 µmol) followed by supplementary H_2O (to 30 µL). Reactions proceeded at r.t. for 1 h, 4 h, 16 h, 48 h, or 7 d, and were quenched by decarboxylation with 60 µL of 30 wt.% H_2O_2 and incubation for 30 min. After removal of precipitated proteins by centrifugation (15,000 × *g*, 10 min), D_2O (100 µL) was added for ^{19}F NMR analysis. The extent of conversion was obtained from integration of the resulting 2-fluoroacids. The chemical shift of the decarboxylated fluoro-donor was referenced to its average value across all samples (fluoroacetate: -216.979 ppm, 2-fluoropropionate: -173.288 ppm, 2-fluorobutyrate: -183.244 ppm).

In control reactions during HpcH mutant screening, the volume of enzyme was replaced with storage buffer. In the secondary screen of HpcH mutants at 0.3 mol%, enzyme was added from stocks that had been concentrated three-fold (450 µM solution in storage buffer, 200 µL). In reactions of fluoropyruvate accelerated by basic pH, the reaction buffer was 20 mM CHES, 1 mM $MgCl_2$, pH 9.3. The combination of this buffer with the enzyme solution gives a final pH of 8.5. Various other buffers (HEPPS pH 8.5, Bicine pH 8.5, Tricine pH 8.5, TAPS pH 8.5, CAPS pH 10.5, sodium carbonate pH 10.5, sodium phosphate pH 12.5) and other divalent metals (Mn, Fe, Co, Ni, Cu, Zn) were also tested for their effect on promoting background aldol addition. These non-enzymatic reactions contained only the fluoro-donor and formaldehyde dissolved in the buffer of interest (570 µL) plus metal dichloride (1 mM).

High resolution LC-MS analysis. Verification of all products observed in ^{19}F NMR analysis was performed by electrospray ionization (ESI) high-resolution LC-MS (see Figure S5). A small portion (10 µL) of each NMR sample was mixed with water (80 µL) and 100% *w/v* trichloroacetic acid (10 µL), and incubated at 4 ºC for 30 min to precipitate residual proteins. The samples were clarified by centrifugation (15,000 × *g*, 20 min, 4 ºC) and diluted 100-fold into either 0.1% formic acid (for reverse-phase chromatography) or 90% acetonitrile (for HILIC-phase chromatography). An Agilent 1290 Infinity LC system was equipped with an Agilent Poroshell 120 EC-C18 column (2.7 µm, 2.1 × 50 mm) or an Ascentis Express HILIC column (2 µm, 2.1 × 50 mm) and coupled to an Agilent 6530 Q-TOF MS operated in negative full-scan mode (capillary voltage 3500 V, nozzle voltage 500 V, fragmentor voltage 175 V, scan range 100-1700 *m/z*). Reverse-phase chromatography conditions were as follows. Solvent A: 0.1% *v/v* formic acid; solvent B: acetonitrile; flow rate 0.6 mL/min; maximum pressure 600 bar; timetable (%B): 0% at 0 min, 0% at 1 min, 100% at 6 min, 100% at 6.5 min, 0% at 7.5 min. HILIC-phase chromatography conditions were as follows. Solvent A: 10% acetonitrile, 10 mM ammonium formate; solvent B: 90%

acetonitrile, 10 mM ammonium formate; flow rate 0.4 mL/min; maximum pressure 600 bar; timetable (%A): 0% at 0 min, 0% at 1 min, 75% at 6 min, 75% at 6.5 min, 0% at 7.5 min.

Characterization of kinetic resolution by enzymatic reduction. Enzymatic aldol reactions of 3-fluoro-2-oxobutanoate (0.3 mL total volume) were prepared by the method above, using formaldehyde, propionaldehyde, glycolaldehyde, or 2-pyridinecarboxaldehyde as acceptors. A sample with no aldolase and no aldehyde served as control. After 48 h of incubation, to the samples was added 100 mM EDTA pH 9.0 (100 µL) to inactivate the aldolase, followed 10 min later by NADH (200 µL of a 100 mM stock in 10 mM NaOH) and LDH (100 µL of 2 mg/mL stock in 50 mM sodium phosphate pH 7.5). The enzymatic reduction reactions were incubated at r.t. for 16 h followed by addition of D_2O (100 µL) for ^{19}F NMR analysis (see Figure S4).

General procedure for synthesis of 2-fluoroesters (7a-c) from formaldehyde. In a plastic tube, the starting 3-fluoro-2-oxoacid sodium salt (0.6 mmol) was dissolved in the specified enzymatic reaction buffer (7.4 mL). Formaldehyde solution (2 M in H_2O, 0.6 mL, 1.2 mmol) was added, followed by the specified preparation of HpcH aldolase or mutant thereof (4 mL in HEPES storage buffer containing 1 mM $MgCl_2$, pH 7.5). The reaction mixture (12 mL) was incubated without stirring at r.t. for the specified duration, then was quenched with H_2O_2 (30% w/w, 1.2 mL) and stirred for 1 h for decarboxylation. Excess H_2O_2 was decomposed with catalase (1 mg) and stirring for 30 min. The mixture was concentrated to near dryness by co-evaporation with CH_3CN (50 mL), then the residue was dissolved in methanolic HCl (1 M, 30 mL) and stirred at r.t. overnight for esterification. The solvent was evaporated and replaced with saturated aqueous $NaHCO_3$ (20 mL), from which the product was extracted with EtOAc (6 × 20 mL). The organic layer was washed with brine (20 mL), dried over Na_2SO_4, and concentrated. Products were purified by silica gel chromatography with the specified solvents. Racemic standards were prepared by replacing the volume of enzymatic reaction buffer and aldolase solution with the specified racemic reaction buffer containing $CoCl_2$ (11.4 mL). The mixture was incubated without stirring at r.t. for the specified duration. All other aspects of the reaction and product isolation remain identical.

Methyl (*S*)- or (*RS*)-2-fluoro-3-hydroxypropanoate (7a or *rac*-7a). Sodium fluoropyruvate (88 mg, 0.6 mmol) was treated by the general procedure above, with the following details. Enzymatic reaction buffer: 20 mM CHES-NaOH, 1 mM $MgCl_2$, pH 9.3. Enzyme preparation: WT HpcH, 4.65 mg/mL (150 µM), 0.1 mol% catalyst load. Enzymatic reaction time: 4 h. Note the final mixture has pH 8.5. A racemic standard (*RS*) was prepared with the following details. Racemic reaction buffer: 20 mM HEPPS-NaOH, 1 mM $CoCl_2$, pH 8.5. Racemic reaction time: 48 h. Chromatography with 1:1 hexanes/EtOAc provided the stereopure product (35 mg, 48%) or the racemic product (16 mg, 22%) as a colorless oil. ^1H NMR (600 MHz, $CDCl_3$) δ 5.03 (ddd, J = 48.2, 4.5, 2.9 Hz, 1H), 4.11-3.98 (m, 2H), 3.86 (s, 3H), 2.11 (s, 1H).

^{13}C NMR (151 MHz, $CDCl_3$) δ 168.2 (d, *J* = 24.2 Hz), 89.5 (d, *J* = 185.4 Hz), 62.8 (d, *J* = 21.3 Hz), 52.6. ^{19}F NMR (565 MHz, $CDCl_3$) δ -199.8 (ddd, *J* = 48.5, 28.5, 20.8 Hz).

Methyl (*S*)- or (*RS*)-2-fluoro-3-hydroxy-2-methylpropanoate (7b or *rac*-7b). Sodium 3-fluoro-2-oxobutanoate (96 mg, 0.6 mmol) was treated by the general procedure above, with the following details. Enzymatic reaction buffer: 20 mM HEPES-NaOH, 1 mM $MgCl_2$, pH 7.5. Enzyme preparation: WT HpcH, 4.65 mg/mL (150 μM), 0.1 mol% catalyst load. Enzymatic reaction time: 48 h. A racemic standard (*RS*) was prepared with the following details. Racemic reaction buffer: 20 mM Na_2HPO_4-NaOH, 1 mM $CoCl_2$, pH 12.5. Racemic reaction time: 48 h. Chromatography with 2:1 hexanes/EtOAc provided the stereopure product (15 mg, 37%, theoretical yield 0.3 mmol) or the racemic product (10 mg, 13%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.96-3.81 (m, 2H), 3.85 (s, 3H), 2.00 (s, 1H), 1.57 (d, *J* = 21.4 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 171.1 (d, *J* = 25.5 Hz), 95.5 (d, *J* = 184.7 Hz), 67.0 (d, *J* = 23.7 Hz), 52.8, 19.8 (d, *J* = 23.5 Hz). ^{19}F NMR (565 MHz, $CDCl_3$) δ -164.8 (m).

Methyl (*S*)- or (*RS*)-2-fluoro-2-(hydroxymethyl)butanoate (7c or *rac*-7c). Sodium 3-fluoro-2-oxopentanoate (104 mg, 0.6 mmol) was treated by the general procedure above, with the following details. Enzymatic reaction buffer: 20 mM HEPES-NaOH, 1 mM $MgCl_2$, pH 7.5. Enzyme preparation: HpcH F170V, 13.95 mg/mL (450 μM), 0.3 mol% catalyst load. Enzymatic reaction time: 1 week. A racemic standard (*RS*) was prepared with the following details. Racemic reaction buffer: 20 mM Na_2HPO_4-NaOH, 1 mM $CoCl_2$, pH 12.5. Racemic reaction time: 48 h. Chromatography with 2:1 hexanes/EtOAc provided the stereopure product (17 mg, 38%, theoretical yield 0.3 mmol) or the racemic product (22 mg, 24%) as a colorless oil. ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.96-3.83 (m, 2H), 3.85 (s, 3H), 2.02 (s, 1H), 1.97-1.82 (m, 2H), 0.98 (t, *J* = 7.5 Hz, 3H). ^{13}C NMR (151 MHz, $CDCl_3$) δ 170.6 (d, *J* = 26.5 Hz), 98.8 (d, *J* = 187.0 Hz), 66.2 (d, *J* = 23.6 Hz), 52.6, 26.6 (d, *J* = 22.2 Hz), 7.2 (d, *J* = 4.4 Hz). ^{19}F NMR (565 MHz, $CDCl_3$) δ -175.2 (tt, *J* = 28.6, 14.8 Hz).

Ethyl (2*S*,3*RS*)-2-fluoro-3-hydroxy-3-phenylpropanoate (8a). In a plastic tube, sodium fluoropyruvate (88 mg, 0.6 mmol) was dissolved in 20 mM CHES-NaOH, 1 mM $MgCl_2$, pH 9.3 (7.4 mL). A solution of benzaldehyde (2 M in DMSO, 0.6 mL, 1.2 mmol) was added, followed by the enzyme preparation: MBP-HpcH, 10.89 mg/mL (150 μM), 0.1 mol% catalyst load, in 4 mL of HEPES storage buffer containing 1 mM $MgCl_2$, pH 7.5. Note the final mixture has pH 8.5. The reaction mixture (12 mL) was stirred very slowly (~60 rpm) at r.t. for 16 h, then was quenched with H_2O_2 (30% w/w, 1.2 mL) and stirred for 1 h for decarboxylation. Excess H_2O_2 was decomposed with catalase (1 mg) and stirring for 30 min. The mixture was diluted with water (40 mL), acidified to pH 0 with 6 M HCl, and then extracted with methyl *tert*-butyl ether (3 × 30 mL). The organic layers were washed with brine (30 mL) and concentrated, then the residue was dissolved in ethanol (30 mL) and thionyl chloride (1 mL) added slowly. The esterification reaction was stirred at r.t. overnight, then the solvent was evaporated and replaced with

saturated aqueous $NaHCO_3$ (30 mL), from which the product was extracted with EtOAc (3 × 30 mL). The organic layer was washed with brine (30 mL), dried over Na_2SO_4, and concentrated. Purification by silica gel chromatography (9:1 then 4:1 hexanes/EtOAc) afforded the product (61:39 syn/anti mixture of diastereomers) as a colorless oil (55 mg, 43%). ^{1}H NMR (600 MHz, $CDCl_3$) δ 7.44-7.34 (m, 5H), 5.16 (dd, *J* = 22.1, 3.7 Hz, 1H syn), 5.15 (dd, *J* = 12.1, 4.3 Hz, 1H anti), 5.07 (dd, *J* = 48.2, 5.0 Hz, 1H anti), 5.04 (dd, *J* = 48.1, 3.8 Hz, 1H syn), 4.28-4.20 (m, 2H), 1.25 (t, *J* = 7.2 Hz, 3H syn), 1.22 (t, *J* = 7.2 Hz, 3H anti). ^{13}C NMR (151 MHz, $CDCl_3$) Syn δ 167.68 (d, *J* = 24.3 Hz), 137.9 (d, *J* = 2.8 Hz, syn), 128.62, 128.5, 126.5, 91.5 (d, *J* = 191.9 Hz), 74.0 (d, *J* = 20.0 Hz), 61.9, 13.98; Anti δ 167.69 (d, *J* = 23.2 Hz), 137.6 (d, *J* = 2.8 Hz) 128.65, 128.62, 126.8, 90.9 (d, *J* = 191.1 Hz), 73.7 (d, *J* = 22.0 Hz), 61.8, 13.96. ^{19}F NMR (565 MHz, $CDCl_3$) Syn δ -202.7 (dd, *J* = 48.5, 22.3 Hz); Anti δ -197.7 (dd, *J* = 48.2, 16.2 Hz).

Ethyl (2*RS*,3*RS*)-2-fluoro-3-hydroxy-3-phenylpropanoate (*rac*-8a). A racemic standard of the previous product was prepared by Reformatsky reaction of benzaldehyde (531 mg, 508 µL, 5 mmol) and ethyl bromofluoroacetate (1.29 g, 827 µL, 7 mmol). The procedure was adapted from [*6*]. To a suspension of zinc dust (588 mg, 9 mmol) in THF (10 mL) under nitrogen gas was added DIBAL-H (1 M in toluene, 0.1 mL, 2 mol%) and 5% of the alkyl bromide. Zinc was activated by vigorous stirring for 15 min, then the rest of the alkyl bromide and the benzaldehyde were added successively. The reaction was stirred at r.t. for 16 h, then diluted with EtOAc (30 mL) and filtered through Celite. After thorough shaking with 1 M NH_4Cl (30 mL), the layers were separated and the aqueous layer was extracted with EtOAc (3 × 30 mL). The organic layers were washed with brine (30 mL), dried over Na_2SO_4, and concentrated. Purification by silica gel chromatography (9:1 then 4:1 hexanes/EtOAc) afforded the product (55:45 syn/anti mixture of diastereomers) as a colorless oil (603 mg, 57%). Spectral data was identical to the previous section aside from the diastereomeric ratio.

Ethyl (2*S*,3*RS*)-2-fluoro-3-hydroxy-2-methyl-3-(2-pyridyl)propanoate (8b). In a plastic tube, sodium 3-fluoro-2-oxobutanoate (96 mg, 0.6 mmol) was dissolved in 20 mM HEPES-NaOH, 1 mM $MgCl_2$, pH 7.5 (7.4 mL). A solution of 2-pyridinecarboxaldehyde (2 M in H_2O, 0.6 mL, 1.2 mmol) was added, followed by the enzyme preparation: WT HpcH, 4.65 mg/mL (150 µM), 0.1 mol% catalyst load, in 4 mL of HEPES storage buffer containing 1 mM $MgCl_2$, pH 7.5. The reaction mixture (12 mL) was incubated without stirring at r.t. for 48 h, then was quenched with H_2O_2 (30% w/w, 1.2 mL) and stirred for 1 h for decarboxylation. Excess H_2O_2 was decomposed with catalase (1 mg) and stirring for 30 min. The mixture was concentrated to near dryness by co-evaporation with CH_3CN (50 mL), then the residue was dissolved in ethanol (30 mL) and thionyl chloride (1 mL) added slowly. The esterification reaction was stirred at r.t. overnight, then the solvent was evaporated and replaced with saturated aqueous $NaHCO_3$ (30 mL), from which the product was extracted with EtOAc (3 × 30 mL). The organic layer was washed with brine (30 mL), dried over Na_2SO_4, and concentrated. The residue was passed through a short column

of silica gel (eluted with 1:1 hexanes/EtOAc), then concentrated and suspended in 1 M $NaHSO_3$ (10 mL) to remove residual 2-pyridinecarboxaldehyde. Extraction with CH_2Cl_2 (3 × 20 mL) followed by washing with brine (20 mL), drying over Na_2SO_4 and concentration provided a yellow oil, containing the product (21:79 syn/anti mixture of diastereomers) and the diethyl acetal of 2-pyridinecarboxaldehyde. Due to difficulty in thoroughly removing the latter impurity, relative integrals from ^{1}H NMR were used to subtract its weight, giving a corrected yield of product (31 mg, 45%, theoretical yield 0.3 mmol). ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.63-8.61 (m, 1H anti), 8.61-8.60 (m, 1H syn), 7.81-7.78 (m, 1H syn), 7.78-7.75 (m, 1H anti), 7.47-7.44 (m, 1H syn), 7.36-7.32 (m, 1H), 5.14 (d, *J* = 15.3 Hz, 1H anti), 5.01 (d, *J* = 15.5 Hz, 1H syn), 4.33-4.27 (m, 2H anti), 4.22-4.18 (m, 2H syn), 1.67 (d, *J* = 22.0 Hz, 3H syn), 1.53 (d, *J* = 22.4 Hz, 3H anti), 1.32 (t, *J* = 7.1 Hz, 3H anti), 1.23 (t, *J* = 7.1 Hz, 3H syn). ^{13}C NMR (151 MHz, $CDCl_3$) Syn δ 169.9 (d, *J* = 25.3 Hz), 155.6, 147.60, 137.2, 123.65, 123.2 (d, *J* = 3.0 Hz), 96.2 (d, *J* = 190.2 Hz), 75.5 (d, *J* = 24.8 Hz), 61.7, 20.7 (d, *J* = 23.0 Hz), 14.0; Anti δ 170.3 (d, *J* = 23.4 Hz), 155.4, 147.57, 137.3, 123.59, 122.5 (d, *J* = 2.8 Hz), 96.3 (d, *J* = 189.7 Hz), 73.9 (d, *J* = 25.2 Hz), 61.9, 18.5 (d, *J* = 23.2 Hz), 14.1. ^{19}F NMR (565 MHz, $CDCl_3$) Syn δ -165.11 (quint, *J* = 19.9, 19.2 Hz); Anti δ -163.75 (quint, *J* = 21.6 Hz).

Ethyl (2*RS*,3*RS*)-2-fluoro-3-hydroxy-3-(2-pyridyl)propanoate (*rac*-8b). A racemic standard of the previous product was prepared by LDA-promoted aldol reaction of 2-pyridinecarboxaldehyde (643 mg, 570 µL, 6 mmol) and ethyl 2-fluoropropionate (601 mg, 5 mmol). To a solution of diisopropylamine (607 mg, 847 µL, 6 mmol) in THF (10 mL) under nitrogen gas at -78 ºC was added n-BuLi (2.5 M in hexanes, 2.2 mL, 5.5 mmol). After stirring 1 min the ethyl 2-fluoropropionate was added and the solution stirred for 15 min. Then, the 2-pyridinecarboxaldehyde was added and stirring was continued at -78 ºC for 30 min. The reaction was quenched at -78 ºC with 1 M NH_4Cl (5 mL), then diluted with sat. $NaHCO_3$ (40 mL) and warmed to room temperature. The product was extracted with EtOAc (3 × 30 mL), washed with brine (30 mL), dried over Na_2SO_4 and concentrated. The residue was passed through a short column of silica gel (eluted with 1:1 hexanes/EtOAc), then concentrated and suspended in 1 M $NaHSO_3$ (10 mL) to remove residual 2-pyridinecarboxaldehyde. Extraction with CH_2Cl_2 (3 × 20 mL) followed by washing with brine (20 mL), drying over Na_2SO_4 and concentration provided the product (35:65 syn/anti mixture of diastereomers) as a yellow oil (277 mg, 24%). Spectral data was identical to the previous section aside from the diastereomeric ratio.

Chiral derivatization with Mosher's acid. Procedure was adapted from [*7*]. To a solution of (+)-MTPA (0.1 mmol, 23.4 mg) in dry hexanes (2 mL) was added catalytic amount of DMF (~1 µL) followed by oxalyl chloride (2 M in CH_2Cl_2, 0.5 mmol, 0.25 mL). After stirring at r.t. for 1 h, the solvent was evaporated. The acid chloride was dissolved in dry CH_2Cl_2 (2 mL), to which was added the alcohol to be derivatized (0.08 mmol), triethylamine (0.3 mmol, 42 µL), and catalytic amount of DMAP (~1 mg). The

mixture was stirred overnight, then diluted with ether (20 mL) and washed with water (2 × 10 mL) and brine (10 mL). The organic layer was dried over Na_2SO_4 and concentrated. The residue was dissolved in $CDCl_3$ (0.7 mL) for analysis by ^{19}F NMR spectroscopy (see Figure S14).

Ammonium (2*S*,3*S*,4*R*)-2-fluoro-3,4,5-trihydroxy-2-methylpentanoate (5j-NH_4). In a plastic tube, sodium 3-fluoro-2-oxopentanoate (26 mg, 0.15 mmol) was dissolved in 20 mM HEPES-NaOH, 1 mM $MgCl_2$, pH 7.5 (1.85 mL). A solution of D-glyceraldehyde (2 M in H_2O, 0.15 mL, 0.3 mmol) was added, followed by the enzyme preparation: WT HpcH, 4.65 mg/mL (150 μM), 0.1 mol% catalyst load, in 1 mL of HEPES storage buffer containing 1 mM $MgCl_2$, pH 7.5. The reaction mixture (4 mL) was incubated without stirring at r.t. for 48 h, then was quenched with H_2O_2 (30% w/w, 0.4 mL) and stirred for 1 h for decarboxylation. Excess H_2O_2 was decomposed with catalase (1 mg) and stirring for 30 min. After removing the precipitate by centrifugation (13,000 × *g*, 5 min), the sample was lyophilized in a vacuum concentrator to reduce the volume to 2 mL and then filtered through a 0.22 μm syringe filter. The product was purified semi-preparative HPLC on an Agilent 1200 Series HPLC System equipped with a SeQuant ZIC-HILIC column (5 μm, 200 Å, 21.2 × 150 mm). Chromatography conditions were as follows. Solvent A: 10% acetonitrile, 10 mM ammonium formate; solvent B: 90% acetonitrile, 10 mM ammonium formate; flow rate 5 mL/min; maximum pressure 400 bar; timetable (%A): 0% at 0 min, 0% at 10 min, 75% at 70 min, 100% at 80 min, 100% at 90 min, 0% at 100 min, 0% at 120 min; fraction collection: 384 fractions in deep 2 mL 96-well plates. Without dilution, the fractions were directly screened by electrospray ionization (ESI) LC-MS. Agilent 1290 Infinity LC system was equipped with an Ascentis Express HILIC column, and chromatography conditions were identical to those described previously in the section "*High resolution LC-MS analysis*". Agilent 6460 Triple-Quad MS was operated in negative mode with single-ion monitoring of the 181.0518 *m/z* ion. Fractions containing the product were combined and concentrated by rotary evaporation to ~10 mL, then lyophilized in a vacuum concentrator to reveal white solids, consisting of ammonium formate and the product (5:95 syn/anti mixture of diastereomers). The amount of product was estimated by addition of DMSO (37.5 μmol) as internal standard to the NMR sample (2.4 mg, 16%, theoretical yield 75 μmol). Spectral data is given for the *anti* diastereomer only. ^1H NMR (600 MHz, D_2O) δ 3.86-3.80 (m, 1H), 3.77 (dd, J = 10.9, 5.9 Hz, 1H), 3.67-3.55 (m, 2H), 1.35 (d, J = 22.8 Hz, 3H). ^{13}C NMR (151 MHz, D_2O) δ 94.0 (d, J = 178.2 Hz), 71.2 (d, J = 19.2 Hz), 64.6 (d, J = 2.2 Hz), 60.3, 14.8 (d, J = 24.2 Hz). ^{19}F NMR (565 MHz, D_2O) δ -172.1 (quint, J = 23.5 Hz). The carboxylate peak in ^{13}C NMR was not assigned due to presence of a large formate peak.

Ammonium (2*S*,3*RS*,4*S*)-2-fluoro-3,4,5-trihydroxy-2-methylpentanoate (5k-NH_4). The previous procedure was repeated using L-glyceraldehyde (1 M in H_2O, 0.15 mL, 0.15 mmol) as acceptor. All other aspects of the reaction and product isolation remained identical, providing a sample of white solids, consisting of ammonium formate and the product (33:67 syn/anti mixture of diastereomers). The amount

of product was estimated by addition of DMSO (37.5 μmol) as internal standard to the NMR sample (1.9 mg, 13%, theoretical yield 75 μmol). ^{1}H NMR (600 MHz, D_2O) δ 4.22-4.09 (m, 1H), 3.99-3.94 (m, 1H), 3.84-3.79 (m, 1H), 3.70-3.61 (m, 1H), 1.45 (d, J = 23.2 Hz, 3H anti), 1.32 (d, J = 23.5 Hz, 3H syn). ^{13}C NMR (151 MHz, D_2O) Syn δ 104.0 (d, J = 200.9 Hz), 80.97, 72.7 (d, J = 17.2 Hz), 60.8, 15.8 (d, J = 25.5 Hz); Anti δ 101.5 (d, J = 179.0 Hz), 80.95, 72.9 (d, J = 17.4 Hz), 61.5, 15.4 (d, J = 24.4 Hz). ^{19}F NMR (565 MHz, D_2O) Syn δ -173.5 (quint, J = 23.3 Hz); Anti δ -172.3 (quint, J = 23.8 Hz). The carboxylate peak in ^{13}C NMR was not assigned due to presence of a large formate peak.

Supplementary Results

Figure S1. Synthesis of 3-fluoro-2-oxoacid donor substrates. (A) Synthesis of 3-fluoro-2-oxoacid sodium salts that contain an aliphatic substitution at C-3. Procedure was adapted from [*4*]. (B) Synthesis of 3-fluoro-3-phenylpyruvic acid sodium salt. Procedure was adapted from [*5*].

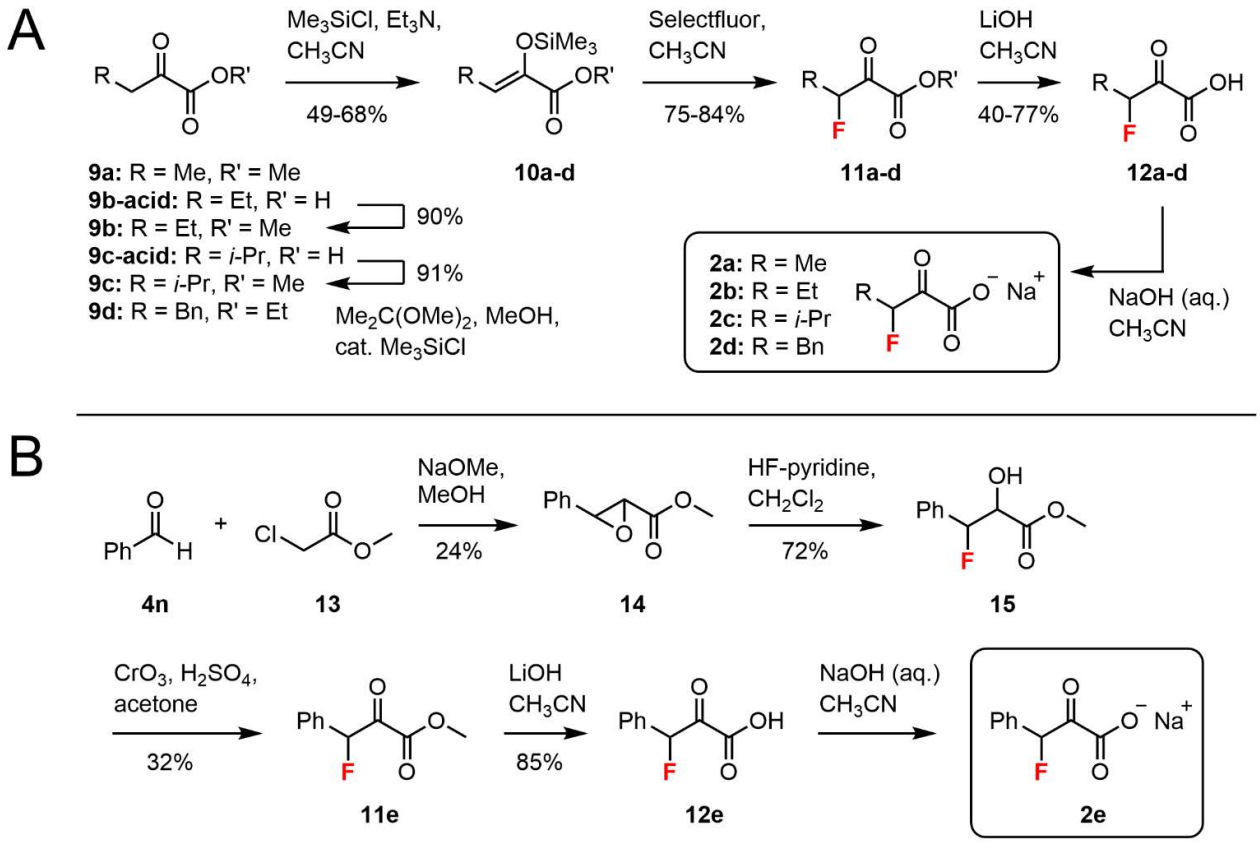


Table S1. Plasmids and DNA sequences used in this study.

A. Plasmids

Plasmid	Notes	Genotype
pET16hp-IMDH	Parent vector	ColE1, Ap^R, *lacI*, pT7 His_{10}-PreSc-IMDH T7t
pSV272.1	Parent vector for MBP fusion	ColE1, Km^R, *lacI*, pT7 His_6-MBP-[MCS] T7t
pET16hp-HpcH	EcHpcH, Uniprot B1IS70	ColE1, Ap^R, *lacI*, pT7 His_{10}-PreSc-EcHpcH T7t
pET16hp-HpcH-W19A	Single mutant	- W19A
pET16hp-HpcH-F170A	Single mutant	- F170A
pET16hp-HpcH-L212A	Single mutant	- L212A
pET16hp-HpcH-F170A-L212A	Double mutant	- F170A/L212A
pET16hp-HpcH-F170V	Single mutant	- F170V
pET16hp-HpcH-F170V-L212A	Double mutant	- F170V/L212A
pET16hp-HpcH-F170L	Single mutant	- F170L
pET16hp-HpcH-F170I	Single mutant	- F170I
pET16hp-HpcH-L212I	Single mutant	- L212I
pET16hp-HpcH-L212V	Single mutant	- L212V
pSV272.1-HpcH	MBP-fused EcHpcH	ColE1, Km^R, *lacI*, pT7 His_6-MBP-EcHpcH T7t

B. Oligonucleotides & gBlocks

Oligonucleotide	Sequence (mutated codon underlined, vector homology regions in lowercase)
HpcH-W19A-F	GGCCGCCCGCAGATCGGATTAGCGCTGGGGCTGAGTAGCAGCTAC
HpcH-W19A-R	GTAGCTGCTACTCAGCCCCAGCGCTAATCCGATCTGCGGGCGGCC
HpcH-F170A-F	GAAGGCGTCGACGGCGTGGCGATCGGCCCGGCGGATCTG
HpcH-F170A-R	CAGATCCGCCGGGCCGATCGCCACGCCGTCGACGCCTTC
HpcH-L212A-F	GGCAAAGCGCCGGGGATCGCGATCGCCAATGAGCAACTG
HpcH-L212A-R	CAGTTGCTCATTGGCGATCGCGATCCCCGGCGCTTTGCC
HpcH-F170V-F	GAAGGCGTCGACGGCGTGGTGATCGGCCCGGCGGATCTG
HpcH-F170V-R	CAGATCCGCCGGGCCGATCACCACGCCGTCGACGCCTTC
HpcH-F170L-F	GAAGGCGTCGACGGCGTGCTGATCGGCCCGGCGGATCTG
HpcH-F170L-R	CAGATCCGCCGGGCCGATCAGCACGCCGTCGACGCCTTC
HpcH-F170I-F	GAAGGCGTCGACGGCGTGATTATCGGCCCGGCGGATCTG
HpcH-F170I-R	CAGATCCGCCGGGCCGATAATCACGCCGTCGACGCCTTC
HpcH-L212I-F	GGCAAAGCGCCGGGGATCATTATCGCCAATGAGCAACTG
HpcH-L212I-R	CAGTTGCTCATTGGCGATAATGATCCCCGGCGCTTTGCC
HpcH-L212V-F	GGCAAAGCGCCGGGGATCGTGATCGCCAATGAGCAACTG
HpcH-L212V-R	CAGTTGCTCATTGGCGATCACGATCCCCGGCGCTTTGCC
HpcH-seqL83-R	CAGCAGTTGTTTGATTTGCACCGGATC
pSV272.1-HpcH-F	ggatcgaggaaaacctgtattttcagggcATGGAAAACAGTTTTAAAGCGGCGCTGAAAG
pSV272.1-HpcH-R	tggctggctagcccgtttgatctcgagtgcggccgcaTTAATACACGCCGGGCTTCACGG
pET16hp-HpcH (gBlock)	ctagaagtgctttttcagggcccgcatATGGAAAACAGTTTTAAAGCGGCGCTGAAAGCAGGCCGCC CGCAGATCGGATTATGGCTGGGGCTGAGTAGCAGCTACAGCGCAGAGTTACTGGCCGGAGCAGGATT CGACTGGTTATTGATCGACGGTGAGCACGCGCCGAATAACGTGCAAACCGTGCTCACCCAGCTACAG GCGATTGCGCCCTACCCCAGCCAGCCGGTGGTACGTCCGTCGTGGAACGATCCGGTGCAAATCAAAC AACTGCTGGACGTCGGCACACAAACCTTGCTGGTGCCGATGGTACAAAACGCTGACGAAGCCCGTGA AGCGGTACGCGCCACCCGTTATCCCCCCGCCGGTATTCGCGGTGTGGGCAGTGCGCTGGCCCGAGCC TCGCGCTGGAATCGTATTCCTGATTACCTGCAAAAAGCCAACGATCAAATGTGCGTGCTGGTGCAGA TCGAAACGCGTGAGGCAATGAAGAACTTACCGCAGATTCTGGACGTGGAAGGCGTCGACGGCGTGTT TATCGGCCCGGCGGATCTGAGCGCCGATATGGGTTATGCCGGTAATCCGCAGCACCCGGAAGTACAG GCCGCCATTGAGCAGGCGATCGTGCAGATCCGTGAATCGGGCAAAGCGCCGGGGATCCTGATCGCCA ATGAGCAACTGGCAAAACGCTATCTGGAACTGGGCGCGCTGTTTGTCGCCGTCGGCGTTGACACCAC CCTGCTCGCCCGCGCCGCTGAAGCGCTGGCAGCACGGTTTGGTGCGCAGGCCACCGCCGTGAAGCCC GGCGTGTATTAAggatccggctgctaacaaagcccgaaag

Figure S2. SDS-PAGE of HpcH aldolase and its variants, after expression in *E. coli* and purification by Ni-NTA chromatography. (A) N-terminal His_{10}-tagged HpcH, from left to right: wild-type, W19A, F170A, L212A, F170A/L212A, F170V, F170V/L212A, molecular weight marker. (B) N-terminal His_{10}-tagged HpcH, from left to right: F170L, F170I, L212I, L212V, molecular weight marker. (C) N-terminal His_6-MBP tagged HpcH aldolase (maltose-binding protein fusion) and molecular weight marker.

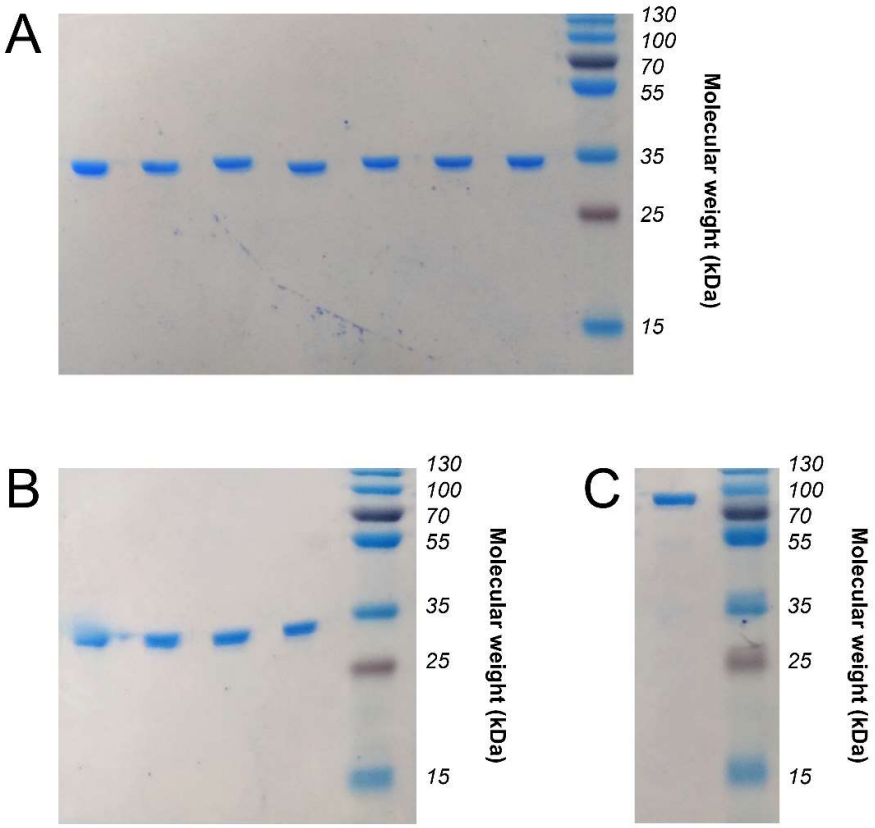


Figure S3. Active site architecture of HpcH aldolase. The active site of HpcH from the crystal structure (RCSB PDB 4B5U) [*8*] is shown below. The non-catalytic residues W19, F170, and L212, which form a pocket around the pyruvate ß-carbon, were targeted for mutation.

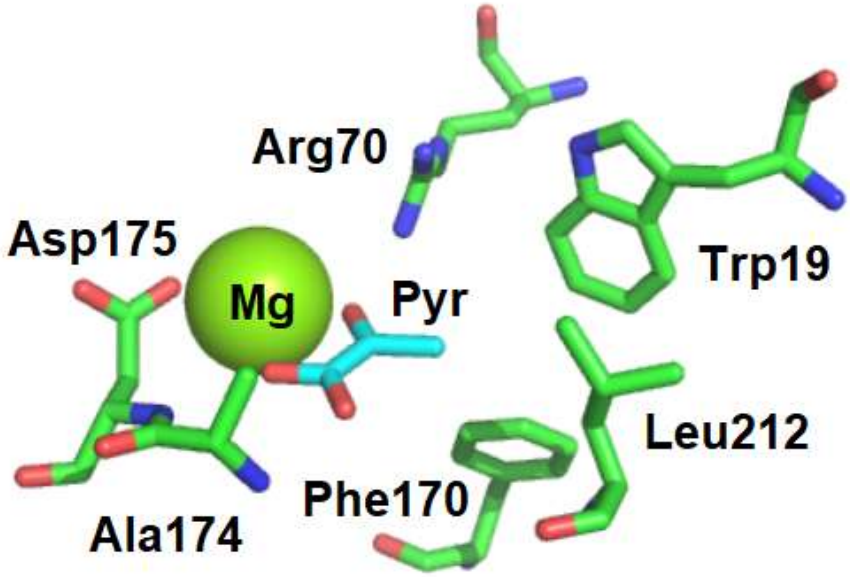
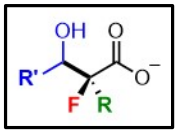


Table S2. ^{19}F NMR spectral data of 2-fluoroacid products.



			anti product		*syn* product	
	R	R'	δ (ppm)	J_{F-H} (Hz)	δ (ppm)	J_{F-H} (Hz)
4a	H	H	-188.62 (dt)	51.1, 27.1	-	-
4b	H	Me	-196.37 (dd)	49.6, 23.7	-198.91 (dd)	52.2, 27.1
4c	H	Et	-196.72 (dd)	51.4, 27.2	-198.58 (dd)	49.4, 26.3
4d	H	*i*-Pr	-193.85 (dd)	50.6, 26.4	-200.35 (dd)	48.8, 28.9
4e	H	*i*-Bu	-197.36 (dd)	52.3, 27.5	-197.67 (dd)	49.6, 25.9
4f	H	$CH(Et)_2$	-193.01 (dd)	50.8, 26.5	-199.13 (dd)	49.6, 28.4
4g	H	*c*-Hex	-193.50 (dd)	50.1, 26.6	-200.30 (dd)	49.1, 29.5
4h	H	2-Furyl	-194.38 (dd)	49.3, 23.5	-195.88 (dd)	50.7, 27.2
4i	H	Thiophen-2-yl	-194.37 (dd)	49.1, 24.3	-196.27 (dd)	51.4, 28.3
4j	H	(*R*)-$CH(OH)CH_2OH$	-189.92 (dd)	49.9, 20.1	-202.51 (dd)	48.4, 28.9
4k	H	(*S*)-$CH(OH)CH_2OH$	-193.78 (dd)	49.8, 25.3	-203.91 (dd)	51.1, 30.7
4l	H	CH_2OH	-194.78 (dd)	50.5, 24.9	-200.47 (dd)	49.3, 27.1
4m	H	$CH(OMe)_2$	-196.51 (dd)	49.5, 26.3	-200.83 (dd)	49.0, 28.7
4n	H	Ph	-191.99 (dd)	50.6, 23.4	-196.97 (dd)	48.9, 26.5
4o	H	2-Pyridyl	-195.47 (dd)	49.2, 24.5	-196.55 (dd)	51.7, 26.9
4p	H	3-Pyridyl	-195.41 (dd)	51.4, 26.2	-196.80 (dd)	48.9, 25.8
4q	H	4-Pyridyl	-194.98 (dd)	49.4, 24.6	-196.01 (dd)	51.7, 26.9
5a	Me	H	-155.75 (h)	22.3	-	-
5b	Me	Me	-163.29 (p)	20.6	-166.33 (p)	21.7
5c	Me	Et	-160.33 (p)	22.0	-164.59 (p)	22.9
5e	Me	*i*-Bu	-160.32 (p)	20.0	-164.95 (p)	22.8
5j	Me	(*R*)-$CH(OH)CH_2OH$	-171.95 (p)	23.3	-	-
5k	Me	(*S*)-$CH(OH)CH_2OH$	-172.18 (p)	23.8	-173.46 (p)	23.5
5l	Me	CH_2OH	-162.76 (p)	22.2	-	-
5m	Me	$CH(OMe)_2$	-159.45 (p)	21.0	-165.14 (p)	23.2
5o	Me	2-Pyridyl	-161.43 (p)	21.6	-163.79 (p)	21.3
5p	Me	3-Pyridyl	-163.84 (p)	20.8	-164.63 (p)	22.6
5q	Me	4-Pyridyl	-161.93 (p)	20.7	-163.45 (p)	22.5
6a	Et	H	-166.89 (p)	22.4	-	-
6b	Et	Me	-175.29 (m)	-	-177.71 (m)	-
6c	Et	Et	-172.52 (m)	-	-175.90 (m)	-
6e	Et	*i*-Bu	-172.82 (m)	-	-176.34 (m)	-
6o	Et	2-Pyridyl	-173.35 (m)	-	-175.28 (m)	-
6p	Et	3-Pyridyl	-175.34 (m)	-	-176.10 (m)	-
6q	Et	4-Pyridyl	-174.24 (m)	-	-175.40 (m)	-

Table S3. ^{19}F NMR conversion data for enzymatic reactions of fluoropyruvate. Enzyme loading was 0.1 mol%. Notation: *tr.* = trace product of <1%; *n.d.* = not detected. The ratio of diastereomers is expressed as % *syn*, thus % *anti* = 100-(% *syn*). Bolded conditions were further investigated with other aldehydes. (A) Optimization of reactions on formaldehyde. The rate was accelerated when the pH was raised from 7.5 to 8.5. (B) Reactions with other aldehydes using the high pH modification. (C) Competition experiments of fluoropyruvate with other 3-fluoro-2-oxoacids, both applied at 50 mM. No effect of inhibition on the conversion of fluoropyruvate was observed.

A

HpcH	Aldehyde	pH	Time (h)	Conv. (%)
No enzyme	Formaldehyde	7.5	4	*tr.*
No enzyme	Formaldehyde	7.5	16	4
No enzyme	Formaldehyde	8.5	4	*tr.*
WT	Formaldehyde	7.5	4	69
W19A	Formaldehyde	7.5	4	9
F170A	Formaldehyde	7.5	4	5
L212A	Formaldehyde	7.5	4	7
F170A/L212A	Formaldehyde	7.5	4	2
F170V	Formaldehyde	7.5	4	6
F170V/L212A	Formaldehyde	7.5	4	4
F170L	Formaldehyde	7.5	4	30
F170I	Formaldehyde	7.5	4	7
L212I	Formaldehyde	7.5	4	57
L212V	Formaldehyde	7.5	4	33
WT	Formaldehyde	7.5	16	95
WT	Formaldehyde	8.5	1	54
WT	**Formaldehyde**	**8.5**	**4**	**98**
MBP-fused	Formaldehyde	7.5	4	76
MBP-fused	Formaldehyde	7.5	16	97
MBP-fused	**Formaldehyde**	**8.5**	**4**	**96**

B

HpcH	Aldehyde	pH	Time (h)	Conv. (%)	% *syn*
WT	Acetaldehyde	8.5	1	54	57
WT	Acetaldehyde	8.5	4	87	42
WT	Propionaldehyde	8.5	1	81	66
WT	Isobutyraldehyde	8.5	1	52	80
WT	Isobutyraldehyde	8.5	4	61	80
WT	Isobutyraldehyde	8.5	16	67	80
WT	Isobutyraldehyde	8.5	48	68	81
WT	Isovaleraldehyde	8.5	1	58	66
WT	Isovaleraldehyde	8.5	4	92	65
MBP-fused	2-Ethylbutyraldehyde	8.5	1	17	80
MBP-fused	2-Ethylbutyraldehyde	8.5	4	21	81
MBP-fused	2-Ethylbutyraldehyde	8.5	16	29	81
MBP-fused	2-Ethylbutyraldehyde	8.5	48	30	84
MBP-fused	Cyclohexanecarboxaldehyde	8.5	1	49	79
MBP-fused	Cyclohexanecarboxaldehyde	8.5	4	72	82
WT	Furfural	8.5	1	56	39
WT	Furfural	8.5	4	59	39
WT	Furfural	8.5	16	61	39
WT	Furfural	8.5	48	65	39
WT	2-Thiophenecarboxaldehyde	8.5	1	24	43
WT	2-Thiophenecarboxaldehyde	8.5	4	27	43
WT	2-Thiophenecarboxaldehyde	8.5	16	30	42
WT	2-Thiophenecarboxaldehyde	8.5	48	36	42
WT	D-Glyceraldehyde	8.5	1	71	16
WT	L-Glyceraldehyde	8.5	1	95	0
WT	Glycolaldehyde	8.5	1	85	9
WT	Dimethoxyacetaldehyde	8.5	1	83	14
MBP-fused	Benzaldehyde	8.5	1	14	35
MBP-fused	Benzaldehyde	8.5	4	36	39
MBP-fused	Benzaldehyde	8.5	16	54	39
MBP-fused	Benzaldehyde	8.5	48	55	39
WT	2-Pyridinecarboxaldehyde	8.5	1	80	61
WT	3-Pyridinecarboxaldehyde	8.5	1	84	39
WT	4-Pyridinecarboxaldehyde	8.5	1	82	64

C

HpcH	Competitor	Aldehyde	pH	Time (h)	Conv. (%)
WT	FKB (**2a**)	Formaldehyde	7.5	4	78
WT	FKB (**2b**)	Formaldehyde	7.5	4	64
WT	FKMV (**2c**)	Formaldehyde	7.5	4	64
WT	FKPB (**2d**)	Formaldehyde	7.5	4	74

Table S4. ^{19}F NMR conversion data for enzymatic reactions of extended 3-fluoro-2-oxoacids. Enzyme loading was 0.1 mol% unless otherwise noted. Notation: *tr.* = trace product of <1%; *n.d.* = not detected. Due to kinetic resolution, 100% conversion is equal to 50% consumption of the racemic donor. The ratio of diastereomers is expressed as % *syn*, thus % *anti* = 100-(% *syn*). Bolded conditions were further investigated with other aldehydes. (A) Optimization of reactions of FKB (**2a**) and FKV (**2b**) on formaldehyde. (B) Final optimization of FKV with higher enzyme loading. (C) Reactions of FKB and FKV with other aldehydes. (D) Unsuccessful reactions of FKMV (**2c**) and FKPB (**2d**) even under forcing conditions; unsuccessful reactions of FPP (**2e**) which reacted non-enzymatically.

A

Donor	HpcH	Aldehyde	pH	Time (h)	Conv. (%)
FKB	No enzyme	Formaldehyde	7.5	16	*n.d.*
FKB	No enzyme	Formaldehyde	7.5	48	*n.d.*
FKB	No enzyme	Formaldehyde	8.5	16	*n.d.*
FKV	No enzyme	Formaldehyde	7.5	48	*n.d.*
FKB	WT	Formaldehyde	7.5	16	50
FKB	W19A	Formaldehyde	7.5	16	*tr.*
FKB	F170A	Formaldehyde	7.5	16	2
FKB	L212A	Formaldehyde	7.5	16	16
FKB	F170A/L212A	Formaldehyde	7.5	16	*tr.*
FKB	F170V	Formaldehyde	7.5	16	*tr.*
FKB	F170V/L212A	Formaldehyde	7.5	16	*tr.*
FKB	F170L	Formaldehyde	7.5	16	24
FKB	F170I	Formaldehyde	7.5	16	2
FKB	L212I	Formaldehyde	7.5	16	*tr.*
FKB	L212V	Formaldehyde	7.5	16	*n.d.*
FKB	**WT**	**Formaldehyde**	**7.5**	**48**	**82**
FKB	WT	Formaldehyde	8.5	16	32
FKV	WT	Formaldehyde	7.5	48	2
FKV	W19A	Formaldehyde	7.5	48	*tr.*
FKV	F170A	Formaldehyde	7.5	48	6
FKV	L212A	Formaldehyde	7.5	48	10
FKV	F170A/L212A	Formaldehyde	7.5	48	2
FKV	F170V	Formaldehyde	7.5	48	10
FKV	F170V/L212A	Formaldehyde	7.5	48	2
FKV	F170L	Formaldehyde	7.5	48	13
FKV	F170I	Formaldehyde	7.5	48	13
FKV	L212I	Formaldehyde	7.5	48	*tr.*
FKV	L212V	Formaldehyde	7.5	48	*tr.*

B

Donor	HpcH	Aldehyde	pH	Time (h)	Conv. (%)
FKV	WT (0.3%)	Formaldehyde	7.5	48	3
FKV	F170L (0.3%)	Formaldehyde	7.5	48	22
FKV	F170V (0.3%)	Formaldehyde	7.5	48	29
FKV	F170A (0.3%)	Formaldehyde	7.5	48	29
FKV	L212A (0.3%)	Formaldehyde	7.5	48	20
FKV	WT (0.3%)	Formaldehyde	7.5	1 week	3
FKV	F170L (0.3%)	Formaldehyde	7.5	1 week	22
FKV	**F170V (0.3%)**	**Formaldehyde**	**7.5**	**1 week**	**40**
FKV	F170A (0.3%)	Formaldehyde	7.5	1 week	32
FKV	L212A (0.3%)	Formaldehyde	7.5	1 week	27

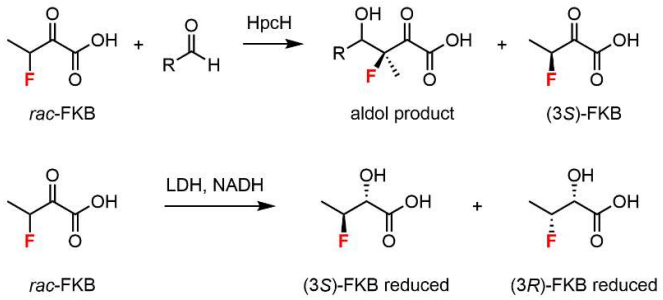
C

Donor	HpcH	Aldehyde	pH	Time (h)	Conv. (%)	% *syn*
FKB	WT	Acetaldehyde	7.5	48	87	44
FKB	WT	Propionaldehyde	7.5	48	84	50
FKB	WT	Isovaleraldehyde	7.5	48	71	54
FKB	WT	D-Glyceraldehyde	7.5	48	63	4
FKB	WT	L-Glyceraldehyde	7.5	48	85	31
FKB	WT	Glycolaldehyde	7.5	48	77	18
FKB	WT	Dimethoxyacetaldehyde	7.5	48	88	81
FKB	WT	2-Pyridinecarboxaldehyde	7.5	48	87	22
FKB	WT	3-Pyridinecarboxaldehyde	7.5	48	77	38
FKB	WT	4-Pyridinecarboxaldehyde	7.5	48	86	39
FKV	F170V (0.3%)	Acetaldehyde	7.5	1 week	98	50
FKV	F170V (0.3%)	Propionaldehyde	7.5	1 week	98	50
FKV	F170V (0.3%)	Isovaleraldehyde	7.5	1 week	89	56
FKV	F170V (0.3%)	2-Pyridinecarboxaldehyde	7.5	1 week	50	55
FKV	F170V (0.3%)	3-Pyridinecarboxaldehyde	7.5	1 week	98	37
FKV	F170V (0.3%)	4-Pyridinecarboxaldehyde	7.5	1 week	98	37

D

Donor	HpcH	Aldehyde	pH	Time (h)	Conv. (%)
FKMV	No enzyme	Formaldehyde	7.5	48	*n.d.*
FKPB	No enzyme	Formaldehyde	7.5	48	*n.d.*
FPP*	No enzyme*	Formaldehyde	7.5	48	30*
**Conversion expressed relative to total donor (not kinetic resolution). Identical outcome observed with inclusion of any enzyme, due to the background rate.*					
FKMV	WT	Formaldehyde	7.5	48	*n.d.*
FKMV	W19A	Formaldehyde	7.5	48	*n.d.*
FKMV	F170A	Formaldehyde	7.5	48	*n.d.*
FKMV	L212A	Formaldehyde	7.5	48	*n.d.*
FKMV	F170A/L212A	Formaldehyde	7.5	48	*n.d.*
FKMV	F170V	Formaldehyde	7.5	48	*n.d.*
FKMV	F170V/L212A	Formaldehyde	7.5	48	*n.d.*
FKMV	F170L	Formaldehyde	7.5	48	*n.d.*
FKMV	F170I	Formaldehyde	7.5	48	*n.d.*
FKMV	L212I	Formaldehyde	7.5	48	*n.d.*
FKMV	L212V	Formaldehyde	7.5	48	*n.d.*
FKPB	WT	Formaldehyde	7.5	48	*n.d.*
FKPB	W19A	Formaldehyde	7.5	48	*n.d.*
FKPB	F170A	Formaldehyde	7.5	48	*n.d.*
FKPB	L212A	Formaldehyde	7.5	48	*n.d.*
FKPB	F170A/L212A	Formaldehyde	7.5	48	*n.d.*
FKPB	F170V	Formaldehyde	7.5	48	*n.d.*
FKPB	F170V/L212A	Formaldehyde	7.5	48	*n.d.*
FKPB	F170L	Formaldehyde	7.5	48	*n.d.*
FKPB	F170I	Formaldehyde	7.5	48	*n.d.*
FKPB	L212I	Formaldehyde	7.5	48	*n.d.*
FKPB	L212V	Formaldehyde	7.5	48	*n.d.*
FKMV	WT (0.3%)	Glycolaldehyde	7.5	48	*n.d.*
FKMV	F170L (0.3%)	Glycolaldehyde	7.5	48	*n.d.*
FKMV	F170V (0.3%)	Glycolaldehyde	7.5	48	*n.d.*
FKMV	F170A (0.3%)	Glycolaldehyde	7.5	48	*n.d.*
FKMV	L212A (0.3%)	Glycolaldehyde	7.5	48	*n.d.*
FKPB	WT (0.3%)	Glycolaldehyde	7.5	48	*n.d.*
FKPB	F170L (0.3%)	Glycolaldehyde	7.5	48	*n.d.*
FKPB	F170V (0.3%)	Glycolaldehyde	7.5	48	*n.d.*
FKPB	F170A (0.3%)	Glycolaldehyde	7.5	48	*n.d.*
FKPB	L212A (0.3%)	Glycolaldehyde	7.5	48	*n.d.*

Figure S4. Characterization of kinetic resolution by enzymatic reduction. After performing the aldol reaction of FKB (**2a**) with various aldehydes, HpcH aldolase was inactivated with EDTA and the remaining starting material was reduced with lactate dehydrogenase (LDH). While the control sample displayed both diastereomers in equal amounts of the product 3-fluoro-2-hydroxybutanoate, all enzymatic reactions were highly enriched in one diastereomer, corresponding to the enantiomer of 3-fluoro-2-oxobutanoate that was unreactive towards HpcH aldolase.



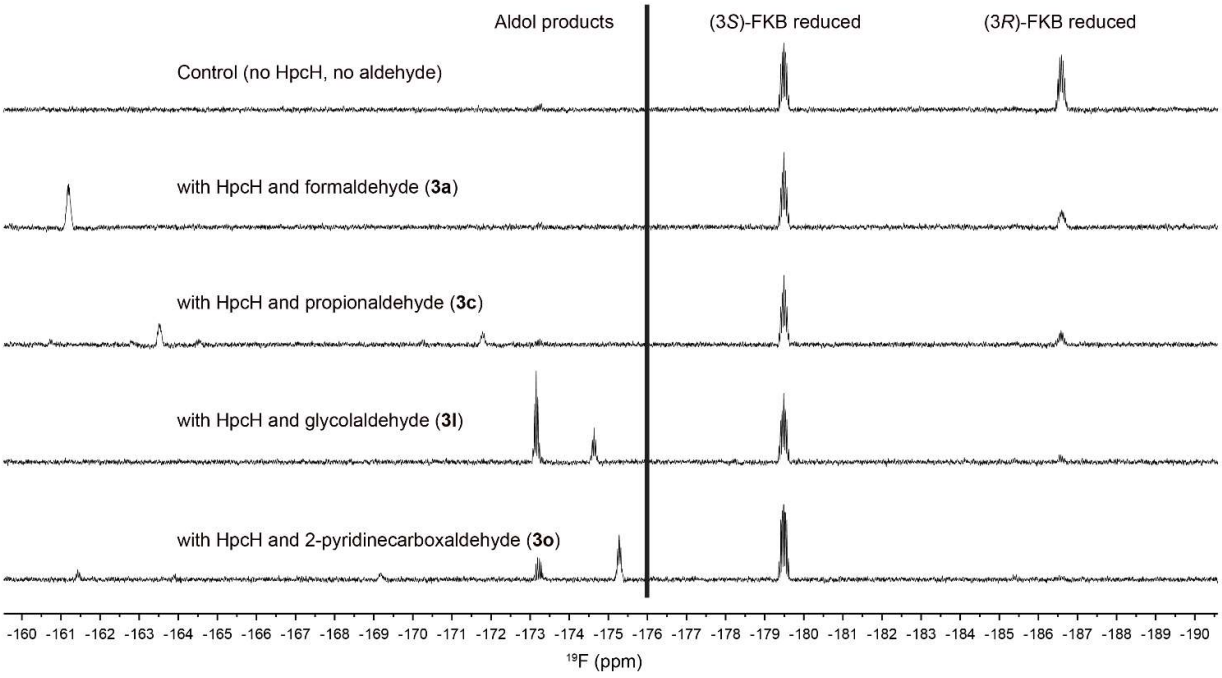
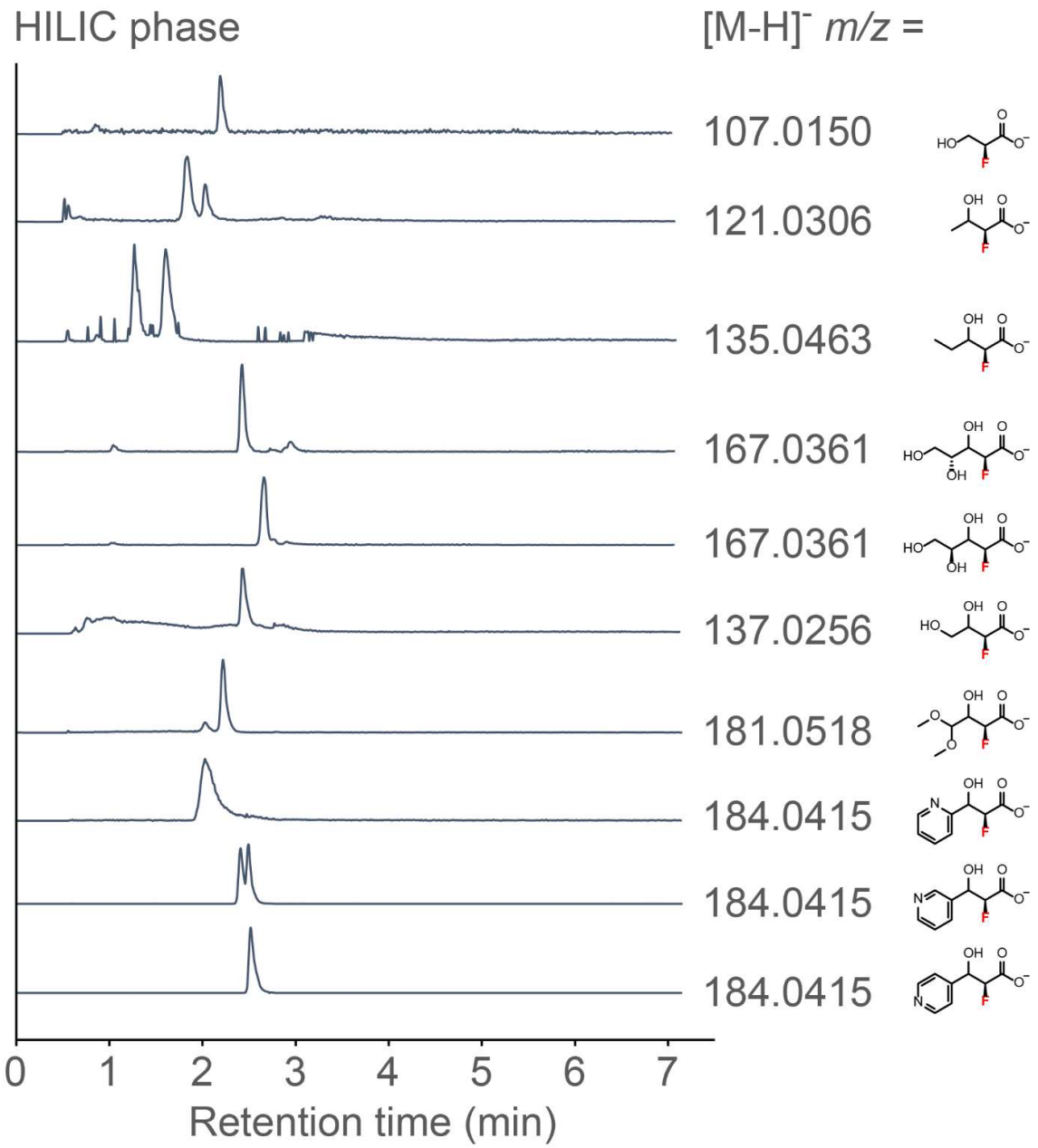
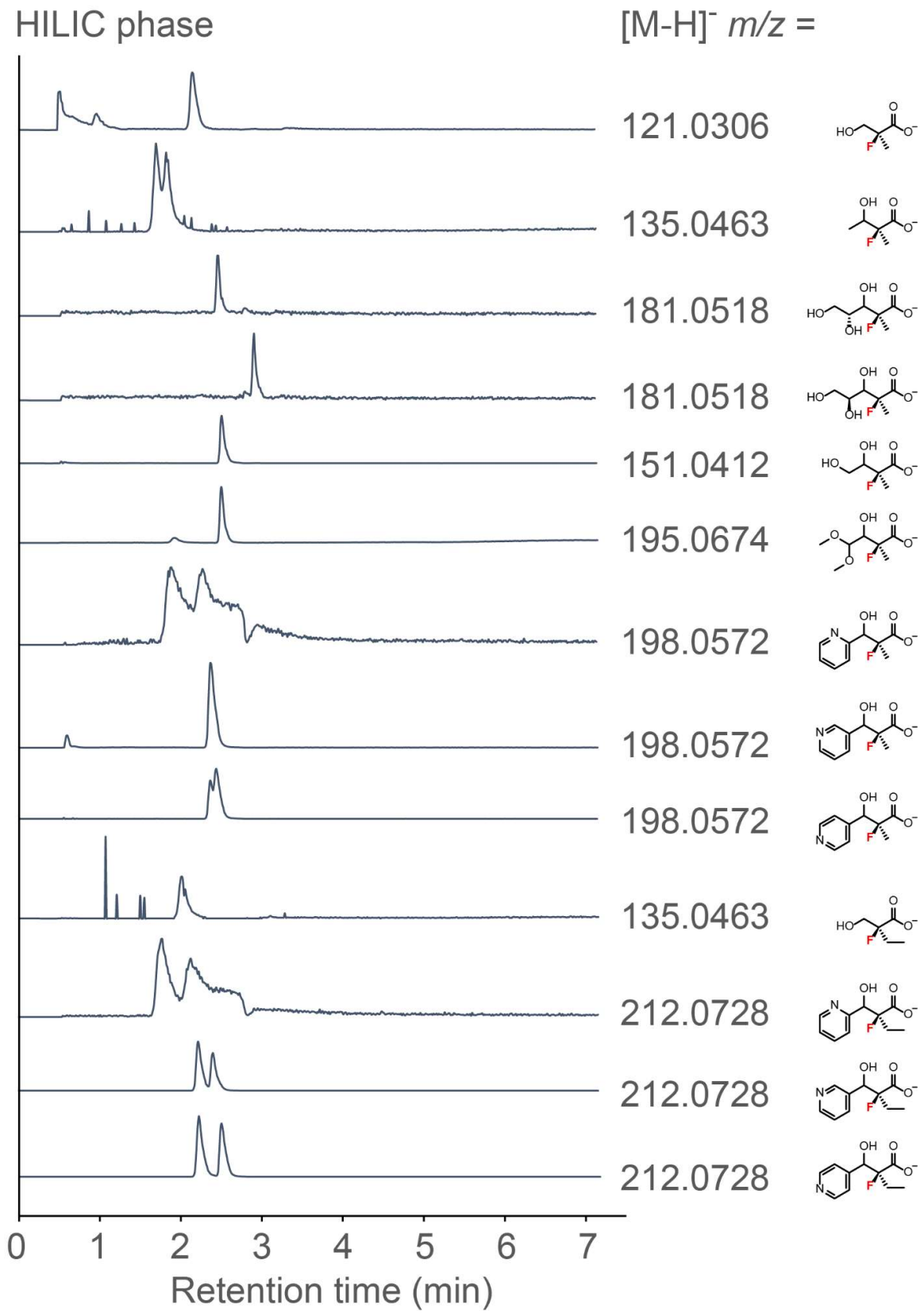


Figure S5. High resolution LC-MS analysis of fluorinated products. Peak height is approximately normalized to the highest intensity within each extracted ion chromatogram. (A) Hydrophilic products derived from fluoropyruvate, separated with a HILIC column. (B) Hydrophilic products derived from FKB and FKV, separated with a HILIC column. (C) Hydrophobic products derived from fluoropyruvate, FKB, and FKV, separated with a reverse-phase C18 column.

A



B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[M-H]- m/z =
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135.0463
181.0518
181.0518
151.0412
195.0674
198.0572
198.0572
198.0572
135.0463
212.0728
212.0728
212.0728
0
1
2
3
4
5
6
7
Retention time (min)

C

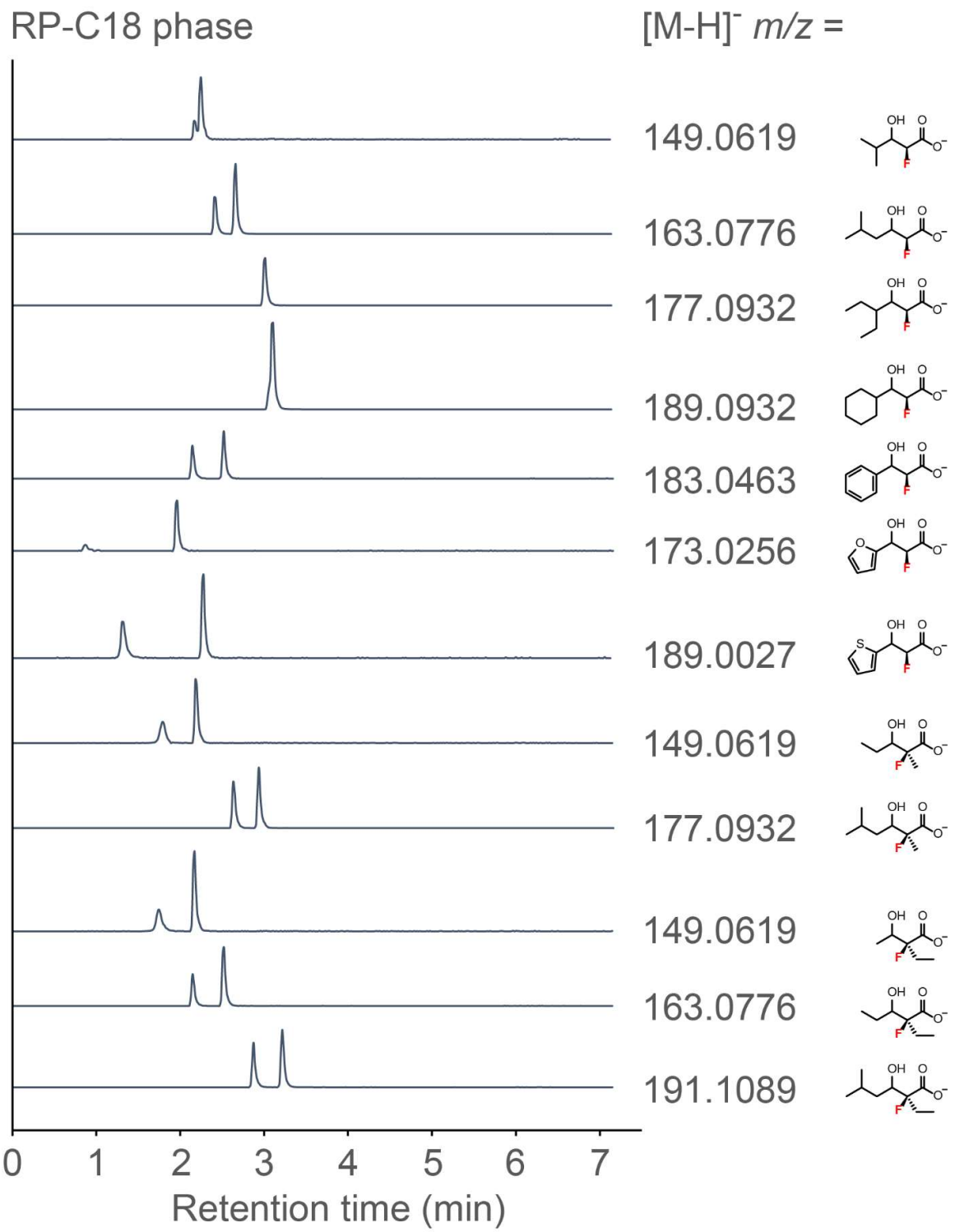

RP-C18 phase
[M-H]- m/z =
149.0619
163.0776
177.0932
189.0932
183.0463
173.0256
189.0027
149.0619
177.0932
149.0619
163.0776
191.1089
0
1
2
3
4
5
6
7
Retention time (min)

Table S5. ^{19}F NMR conversion data for non-enzymatic reactions of fluoro-donors with formaldehyde, catalyzed by buffer or metals. Inclusion of a secondary amine buffer (Tricine, TAPS, or CHES) suppressed non-enzymatic reactions at the higher pH of 8.5. Notation: *tr.* = trace product of <1%; *n.d.* = not detected. Reactions of FKB and FKV in presence of cobalt or nickel were accompanied by ~5% fluoride elimination unless otherwise specified. Bolded conditions were employed for the synthesis of racemic standards of 2-fluoroesters derived from formaldehyde.

Donor	Buffer	Metal	Time (h)	Conv. (%)	Notes
FP	HEPES, pH 7.5	none	16	2	
FP	HEPPS, pH 8.5	none	16	15	
FP	Bicine, pH 8.5	none	16	7	
FP	Tricine, pH 8.5	none	16	*n.d.*	
FP	TAPS, pH 8.5	none	16	*n.d.*	
FP	CHES/HEPES, pH 8.5	none	16	2	CHES pH 9.3, HEPES pH 7.5, 2:1 mixture
FP	HEPPS, pH 8.5	Mg	16	28	Trace FP dimerization
FP	HEPPS, pH 8.5	Mn	16	25	Line broadening in ^{19}F NMR
FP	HEPPS, pH 8.5	Fe	16	10	
FP	HEPPS, pH 8.5	Co	16	44	
FP	HEPPS, pH 8.5	Ni	16	50	Trace FP dimerization and double aldol product
FP	HEPPS, pH 8.5	Cu	16	10	
FP	HEPPS, pH 8.5	Zn	16	35	Trace FP dimerization
FP	**HEPPS, pH 8.5**	**Co**	**48**	**77**	Trace FP dimerization and double aldol product
FKB	HEPPS, pH 8.5	Co	48	*tr.*	
FKB	CAPS, pH 10.5	Co	48	*tr.*	
FKB	Na-carbonate, pH 10.5	Co	48	9	
FKB	**Na-phosphate, pH 12.5**	**Co**	**48**	**44**	17% F-elimination; 14% unidentified side pdt
FKB	HEPPS, pH 8.5	Ni	48	*tr.*	
FKB	CAPS, pH 10.5	Ni	48	3	
FKB	Na-carbonate, pH 10.5	Ni	48	11	
FKB	Na-phosphate, pH 12.5	Ni	48	24	14% F-elimination; 47% unidentified side pdt
FKV	**Na-phosphate, pH 12.5**	**Co**	**48**	**83**	8% unidentified side pdt

Figure S6. Synthesis of 2-fluoroesters and racemic standards. For 2-fluoroesters derived from formaldehyde, racemic standards were prepared in water solvent with conditions similar to the corresponding asymmetric enzymatic procedure, except that the enzyme was absent and the buffer was altered. For 2-fluoroesters derived from aromatic aldehydes, racemic standards were prepared by traditional synthesis in anhydrous conditions. Reformatsky reaction of ethyl bromofluoroacetate [*6*] was employed to avoid the use of extremely toxic ethyl fluoroacetate.

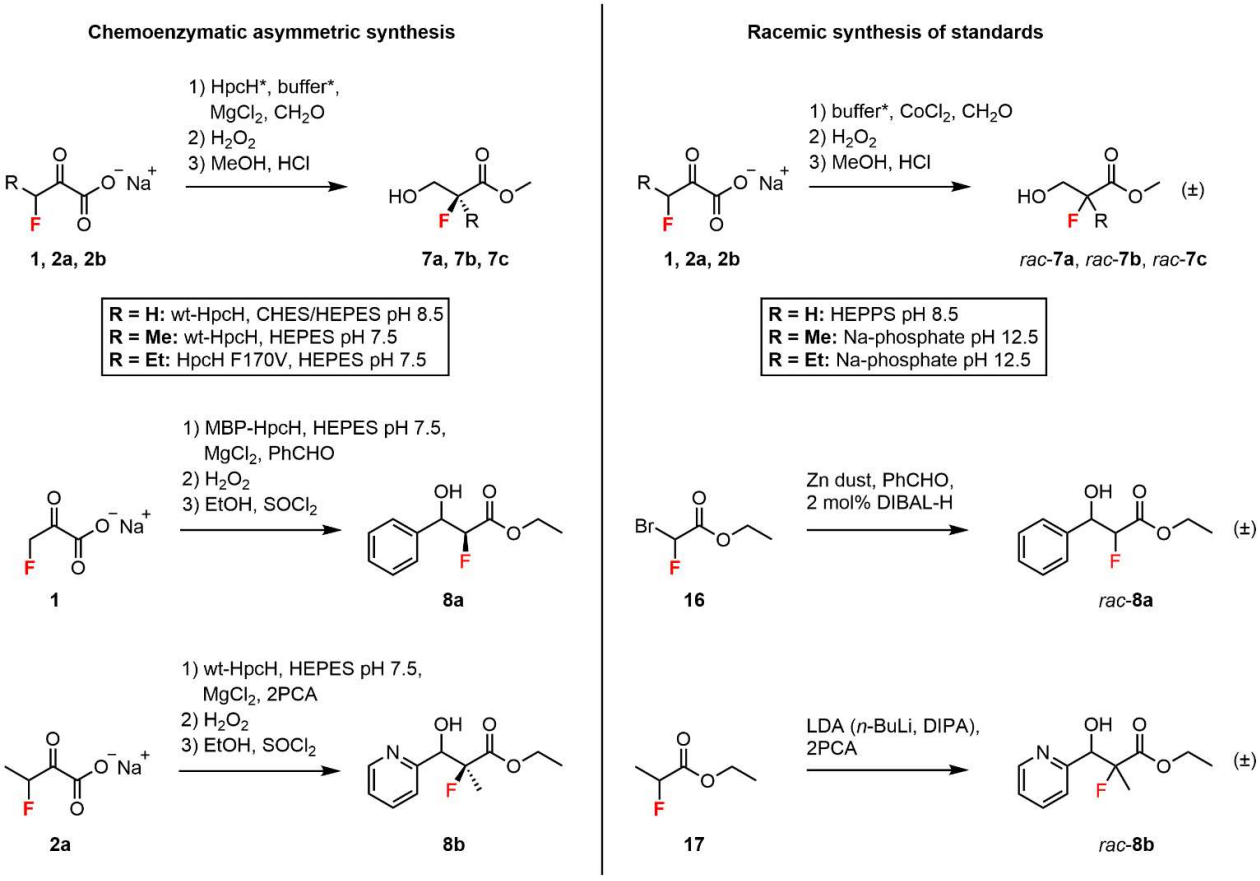


Figure S7. NMR spectra of methyl (*S*)-2-fluoro-3-hydroxypropanoate (7a). ^{1}H, ^{13}C, and ^{19}F spectra. Asterisk indicates peaks due to residual ethyl acetate.

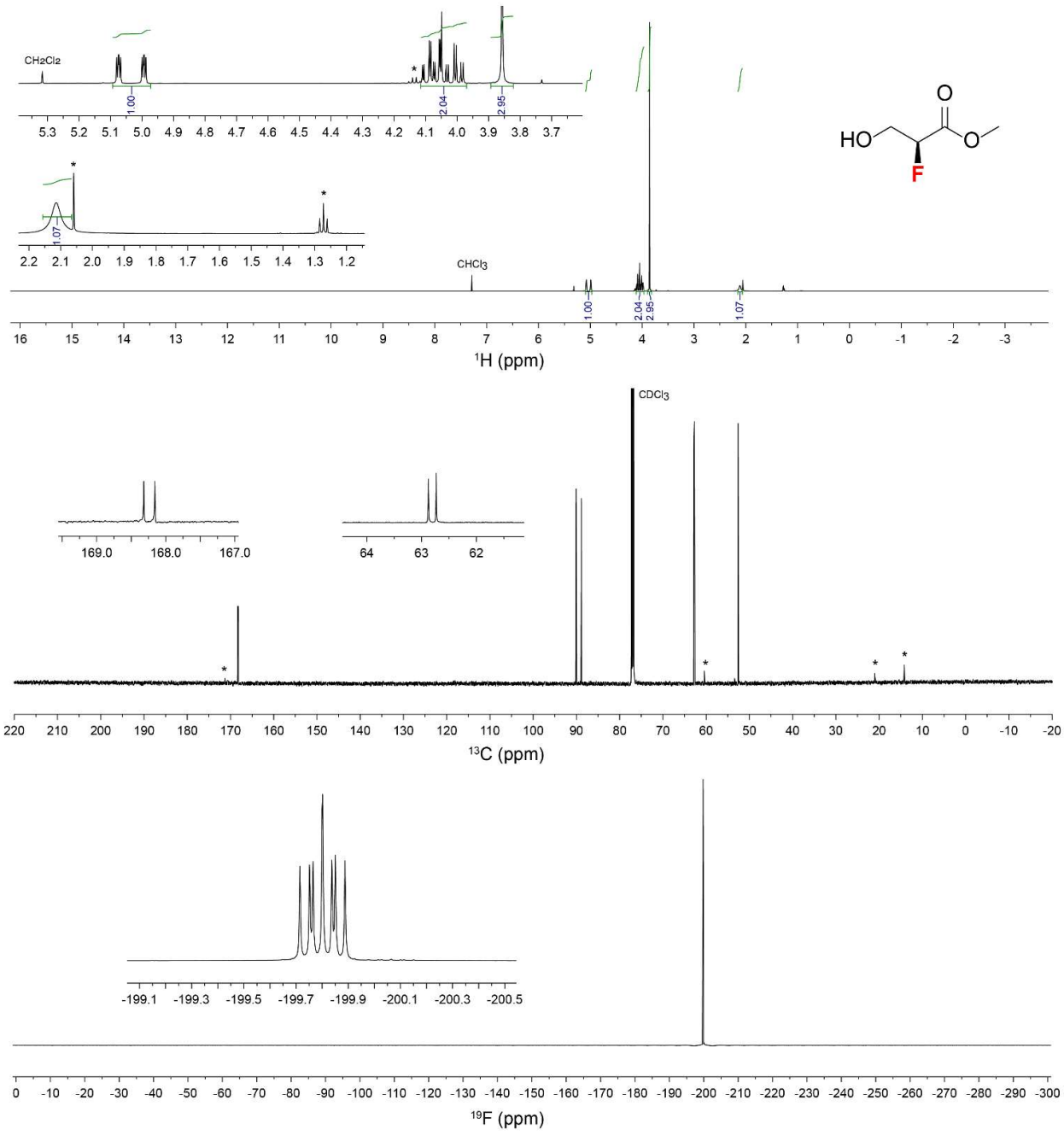


Figure S8. NMR spectra of methyl (*S*)-2-fluoro-3-hydroxy-2-methylpropanoate (7b). ^{1}H, ^{13}C, and ^{19}F spectra. Asterisk indicates peaks due to residual ethyl acetate.

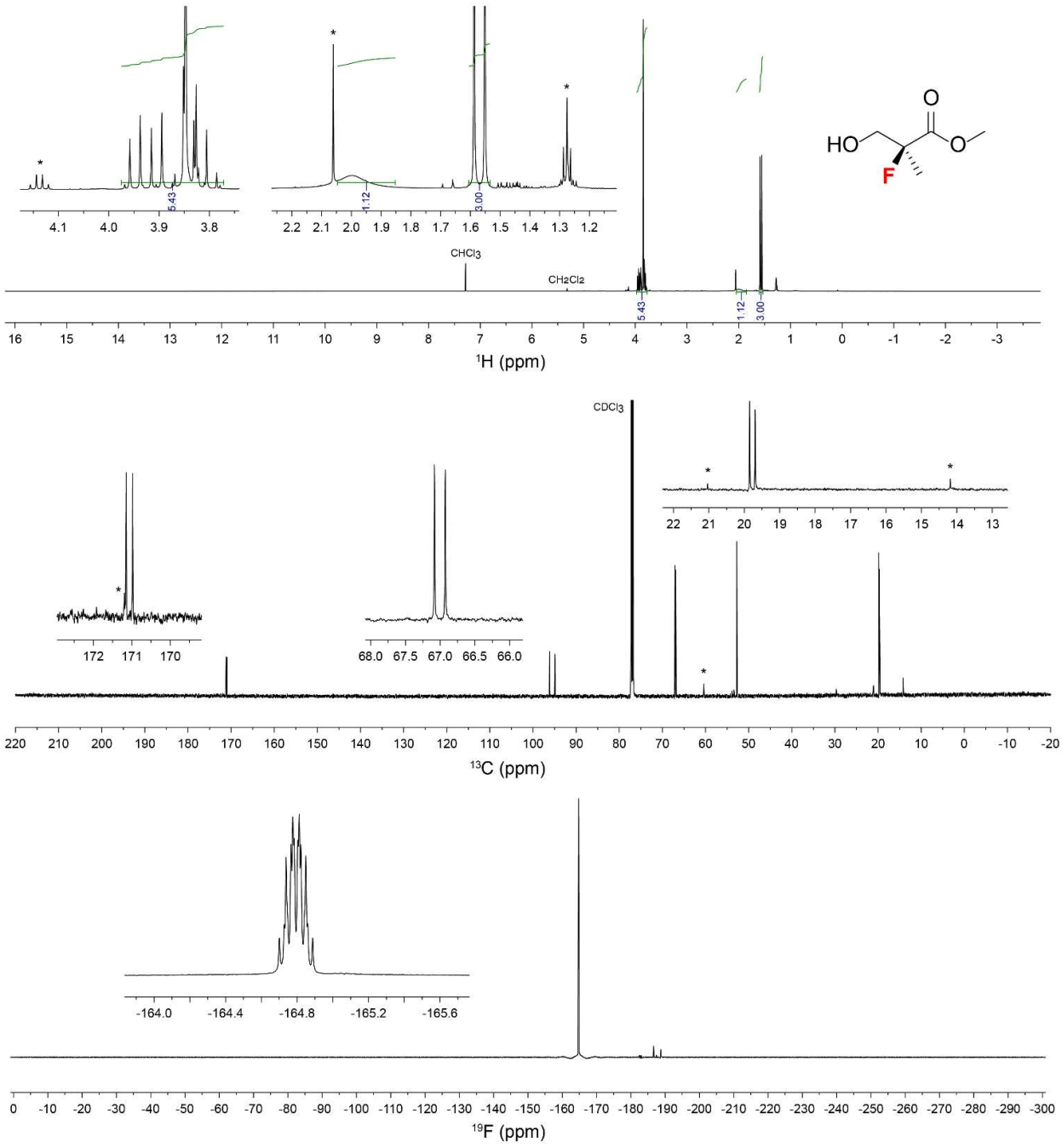


Figure S9. NMR spectra of methyl (*S*)-2-fluoro-2-(hydroxymethyl)butanoate (7c). ^{1}H, ^{13}C, and ^{19}F spectra. Asterisk indicates peaks due to residual ethyl acetate.

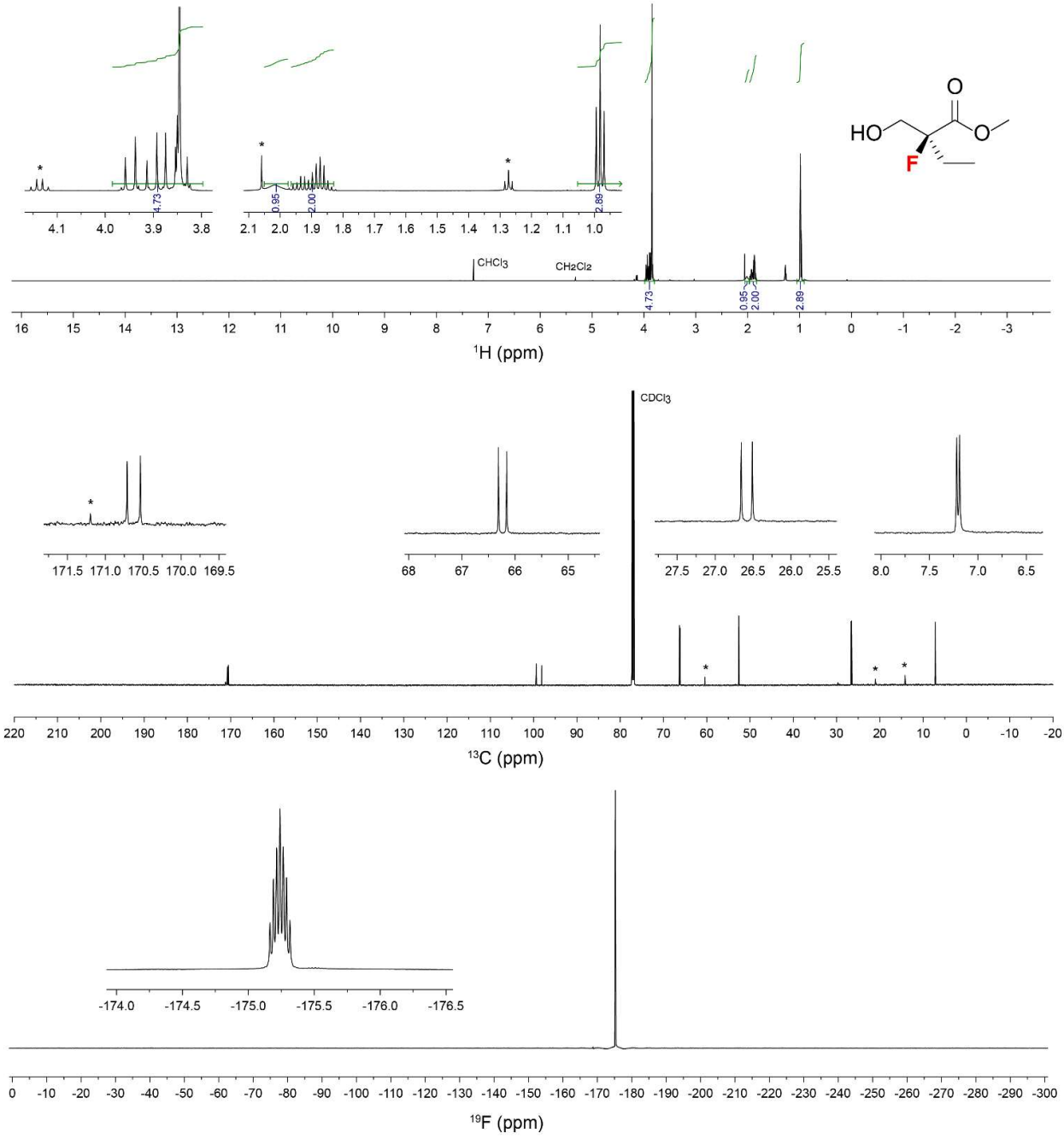


Figure S10. NMR spectra of ethyl (2*S*,3*RS*)-2-fluoro-3-hydroxy-3-phenylpropanoate (8a). ^{1}H, ^{13}C, and ^{19}F spectra (61:39 *syn/anti* ratio). Asterisk indicates peaks due to residual ethyl acetate.

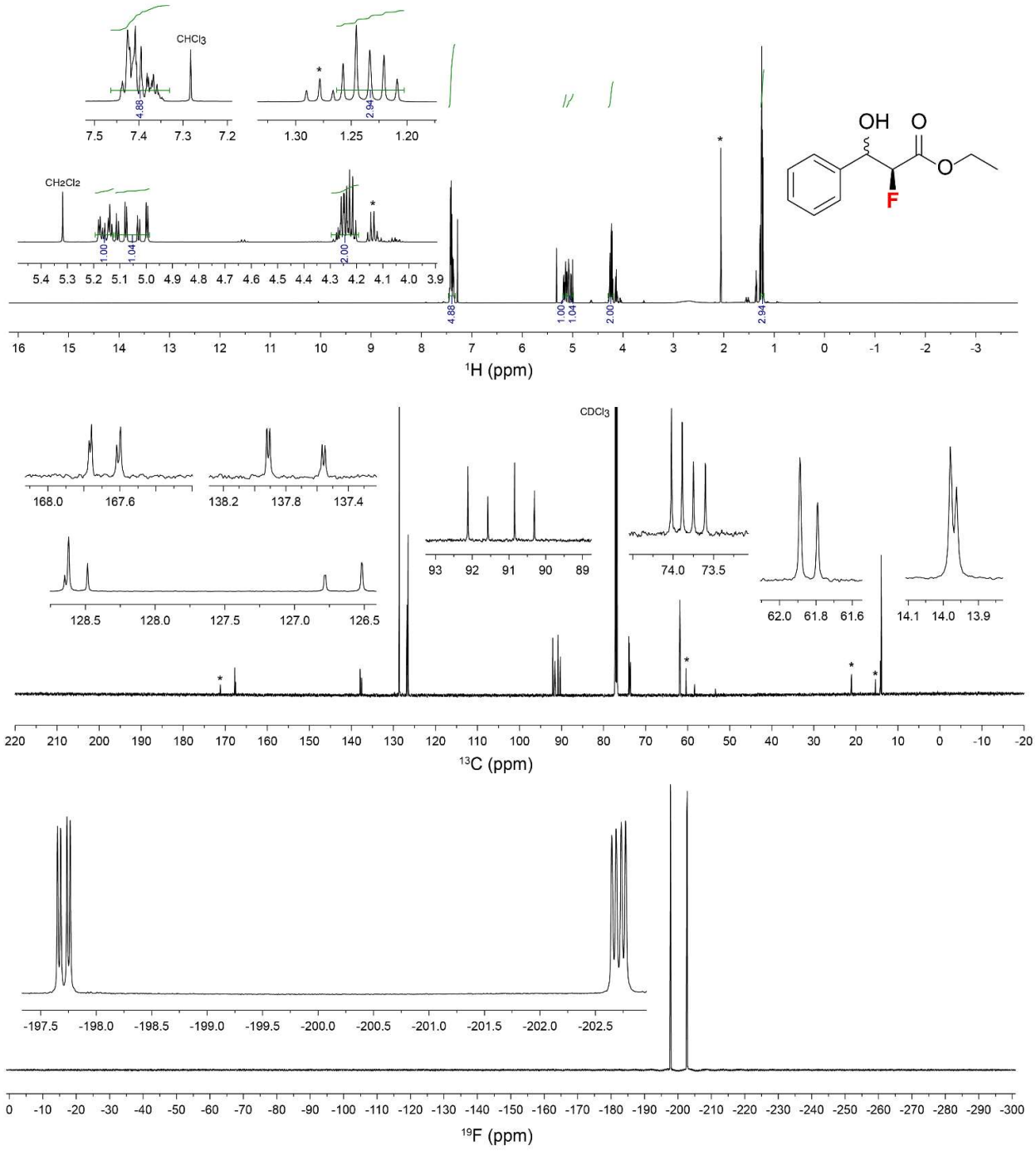


Figure S11. NMR spectra of ethyl (2*S*,3*RS*)-2-fluoro-3-hydroxy-2-methyl-3-(2-pyridyl)propanoate (8b). ^{1}H, ^{13}C, and ^{19}F spectra (21:79 *syn/anti* ratio). Asterisk indicates peaks from the co-purified diethyl acetal of 2-pyridinecarboxaldehyde.

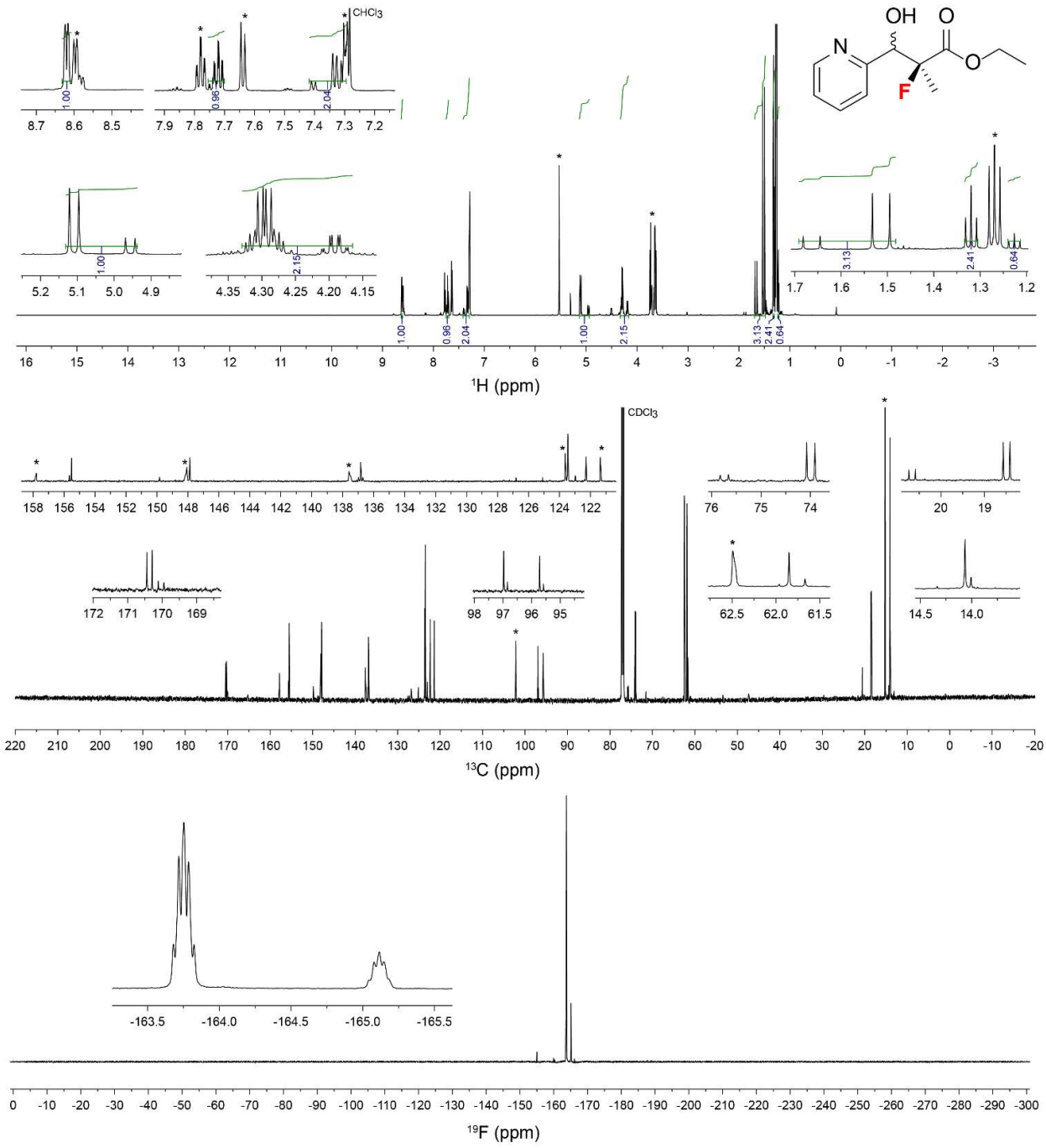


Figure S12. NMR spectra of ammonium (2*S*,3*S*,4*R*)-2-fluoro-3,4,5-trihydroxy-2-methylpentanoate (5j-NH$_4$). ^{1}H, ^{13}C, and ^{19}F spectra (5:95 *syn/anti* ratio, only the *anti* product is annotated).

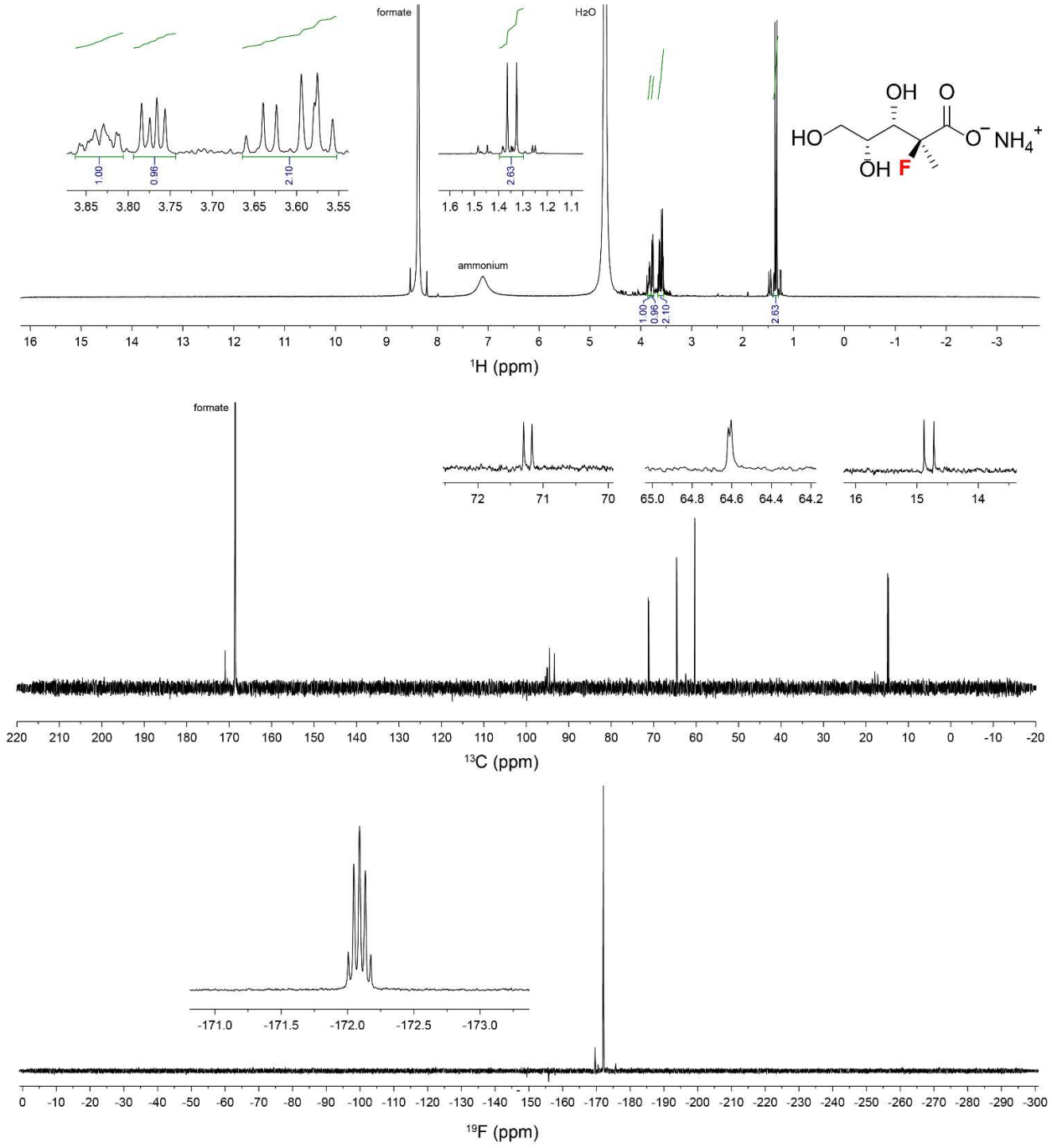


Figure S13. NMR spectra of ammonium (2*S*,3*RS*,4*S*)-2-fluoro-3,4,5-trihydroxy-2-methylpentanoate (5k-NH_4). ^{1}H, ^{13}C, and ^{19}F spectra (33:67 *syn/anti* ratio).

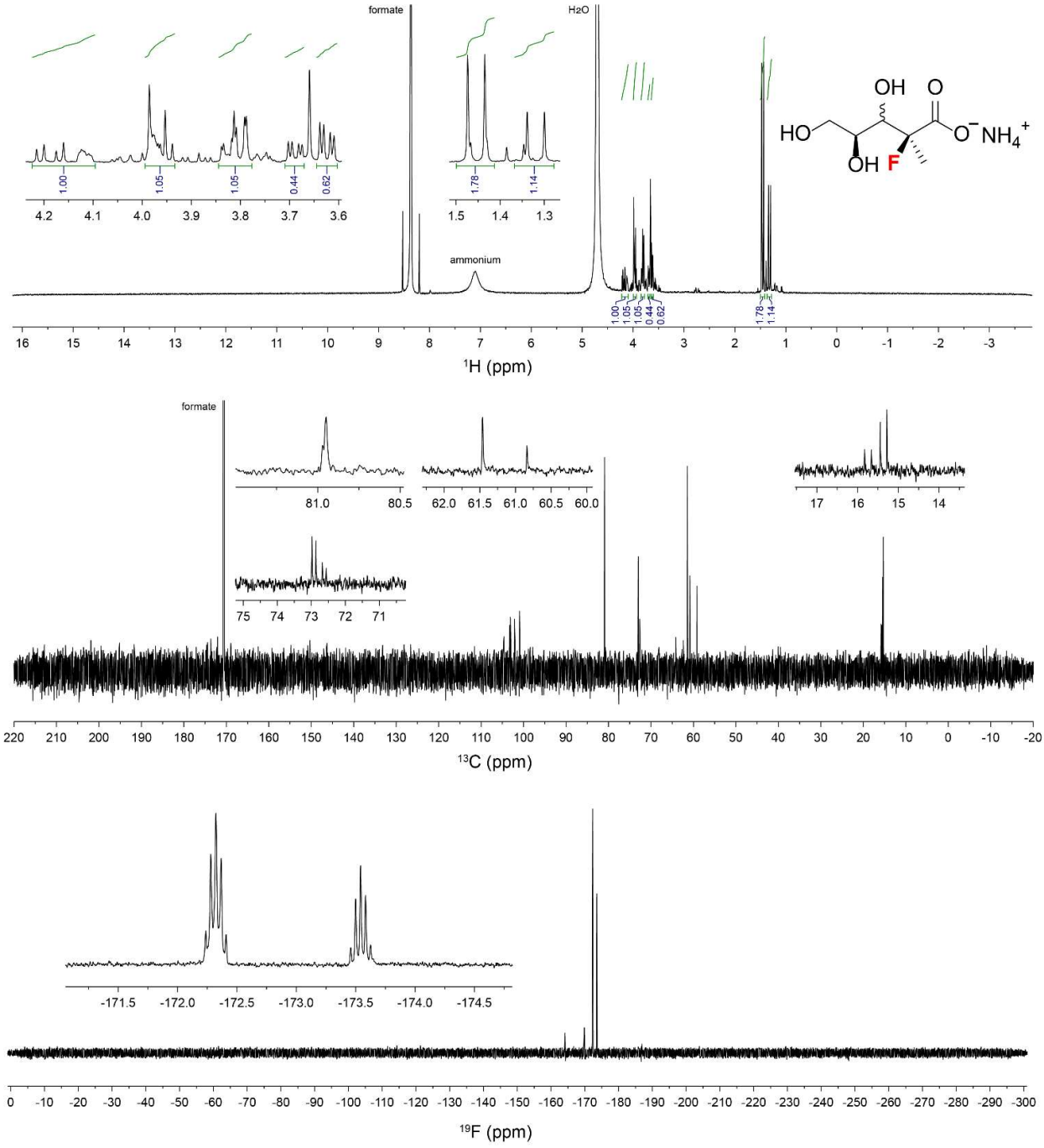
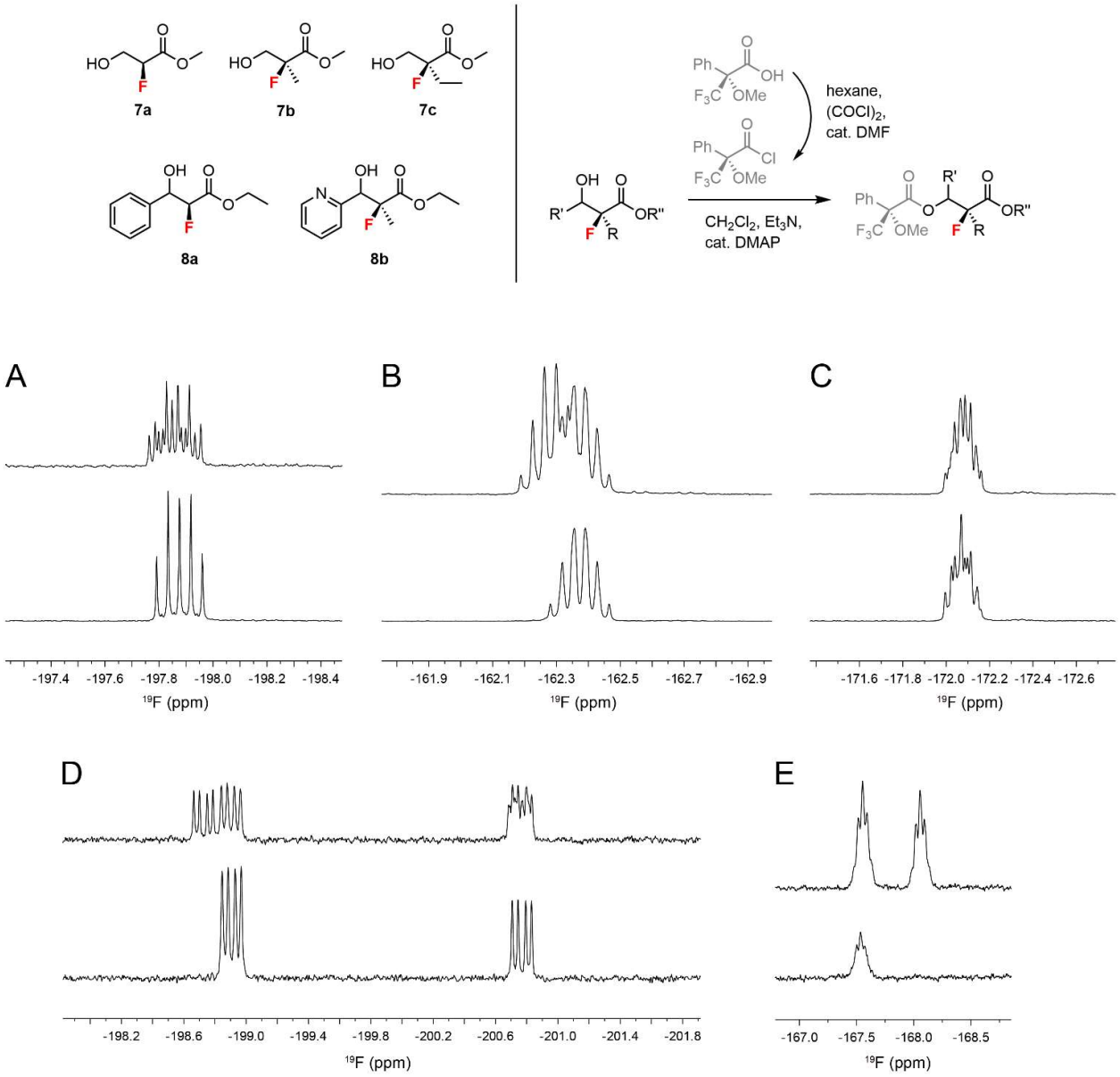


Figure S14. Chiral analysis with (+)-Mosher's acid. Procedure was adapted from [*7*]. Comparative ^{19}F NMR was performed on the derivatized racemic standards (upper spectra, doubled peaks) and the derivatized enzymatic products (lower spectra, single peaks). (A) Results for compound **7a**. (B) Results for compound **7b**. (C) Results for compound **7c**. (D) Results for compound **8a** (*syn* and *anti* diastereomers). (E) Results for compound **8b** (shown for the *syn* diasteromer; the *anti* diastereomer is poorly derivatized due to strong steric hindrance of the alcohol).



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Author Contributions

J. Fang was responsible for designing experiments, performing experiments, collecting data, analyzing data, and writing the manuscript. L. E. Turner was responsible for performing experiments, collecting data, and analyzing data. M. C. Y. Chang was responsible for administering the project, designing experiments, and validating data. All authors were involved in editing the manuscript.