Supporting Information

for

The Structural Basis of Functional Group Activation by Sulfotransferases in Complex Metabolic Pathways

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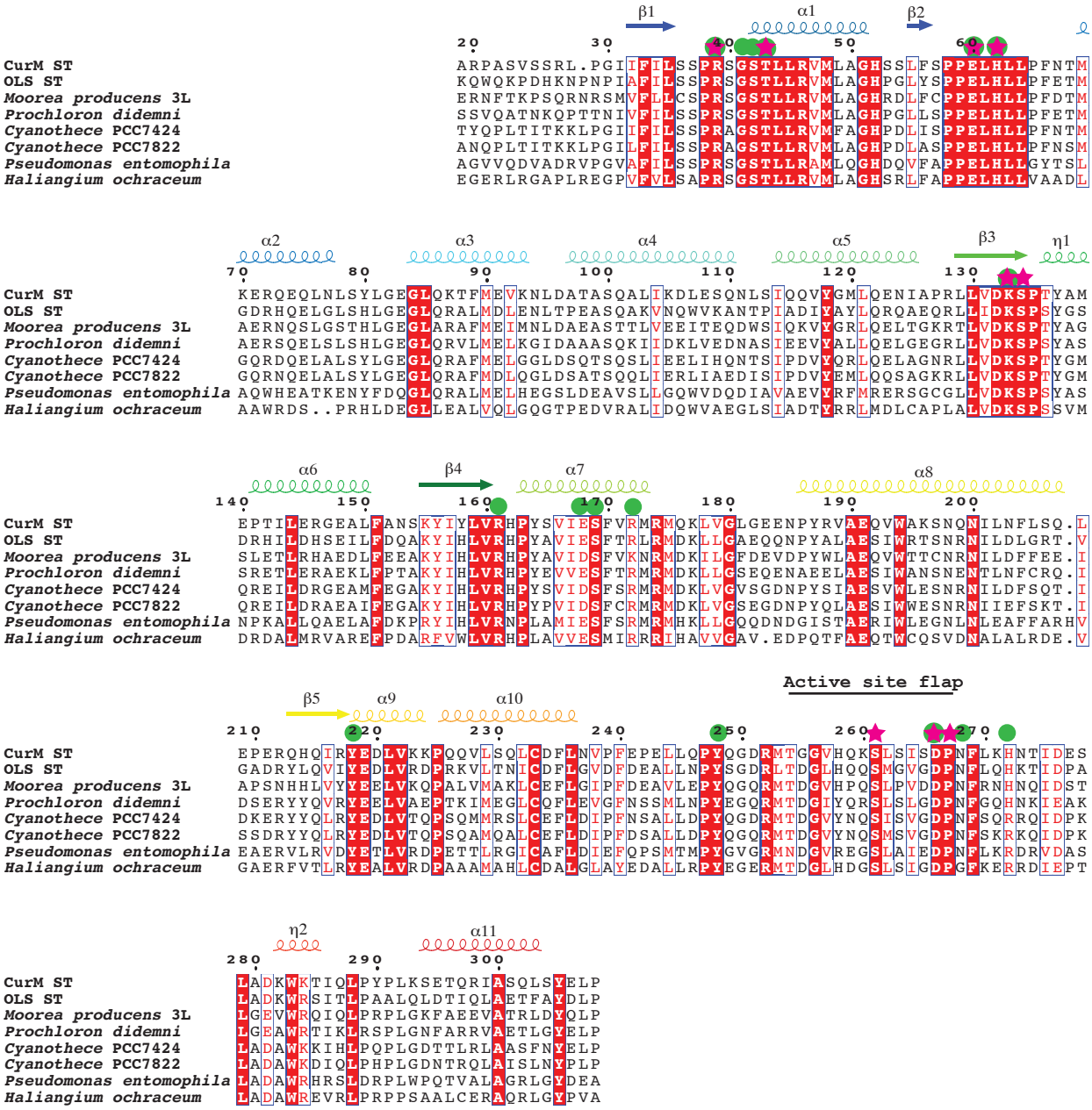
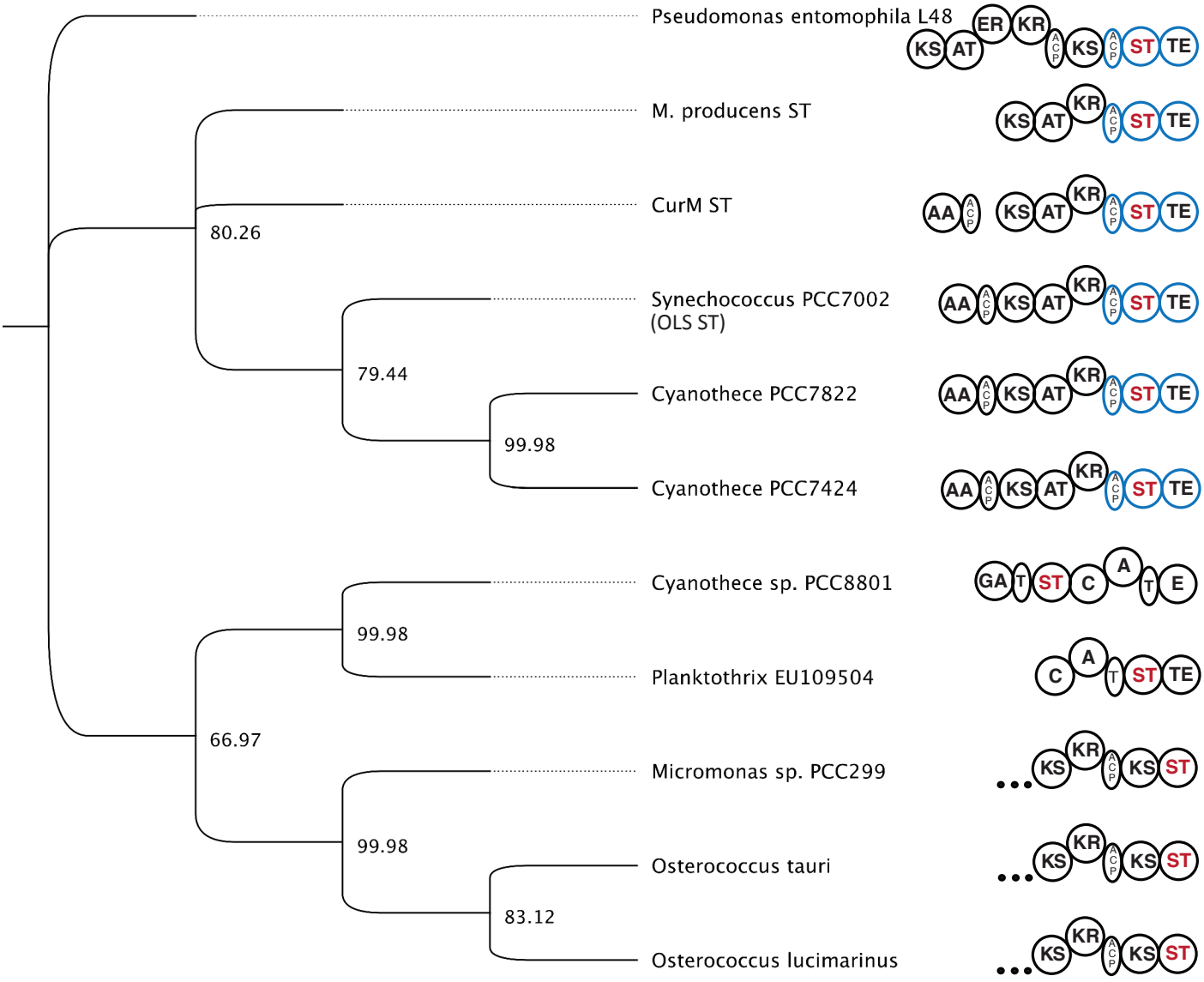
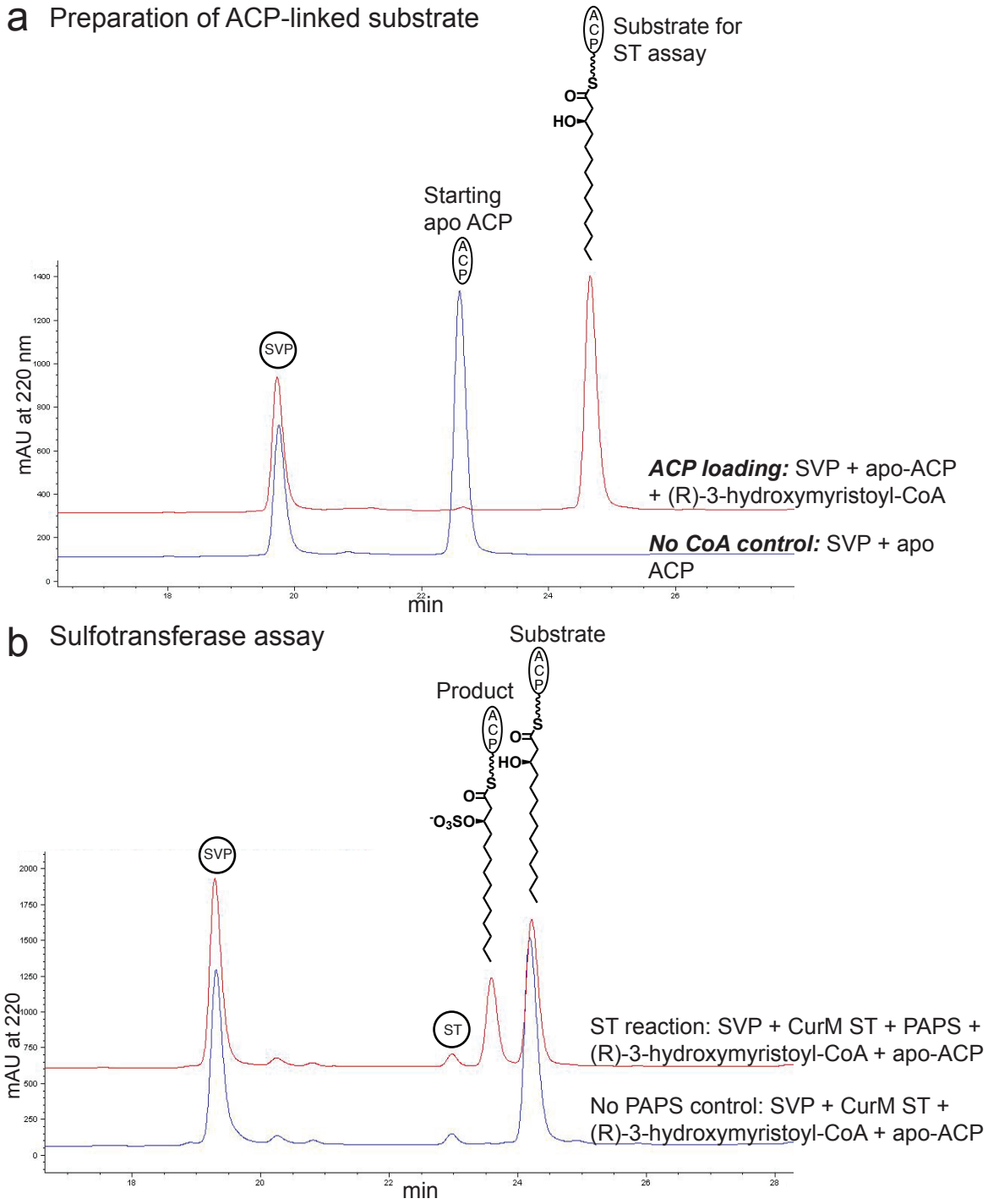


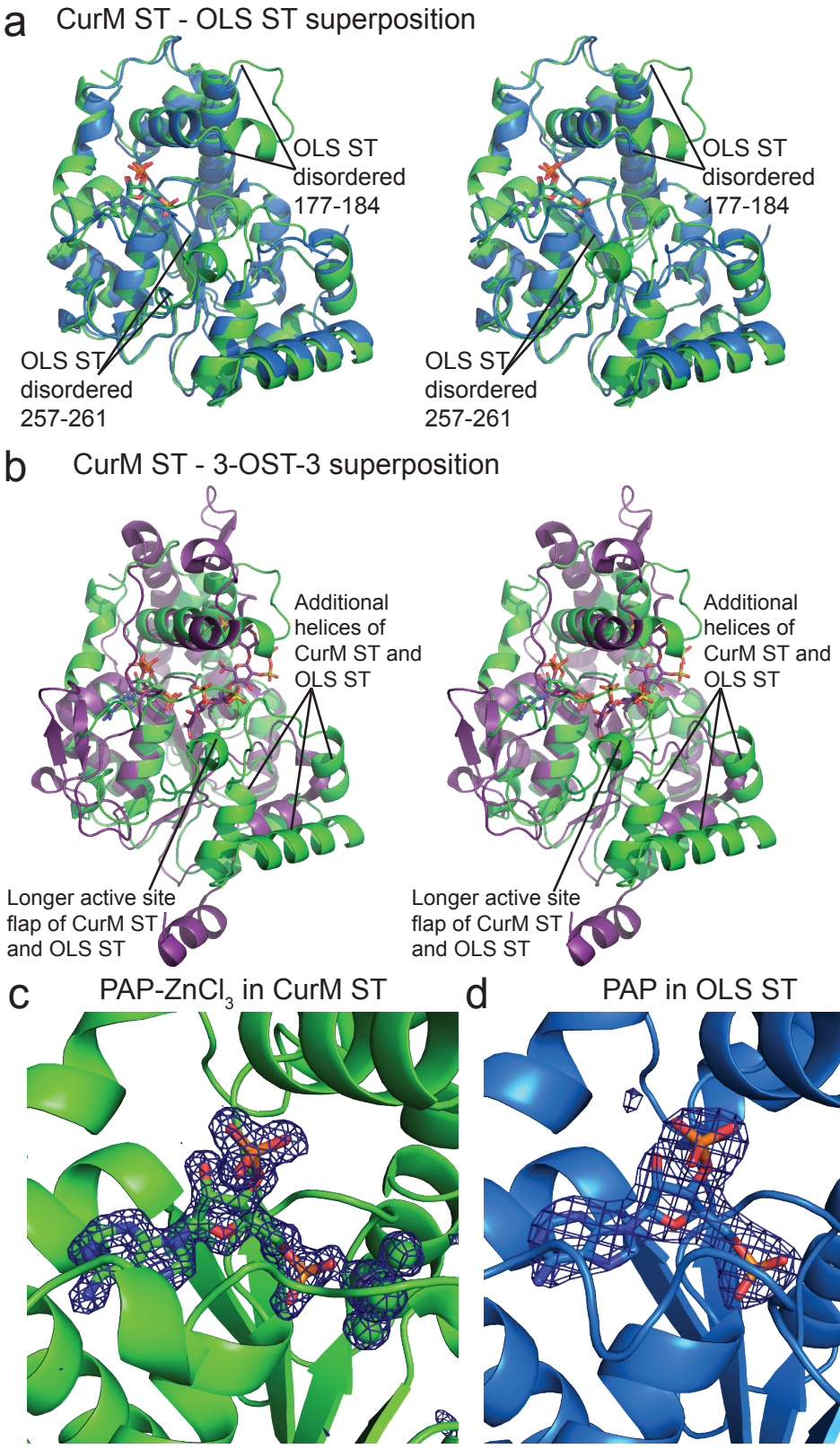
Supplementary Figure 1 Sequence alignment of activating STs. CurM ST and OLS ST are aligned with putative STs from open reading frames containing tandem ACP-ST-TE tridomains. Invariant amino acids are highlighted in red, conserved residues are printed in red. Green circles indicate amino acids in the active site and magenta stars indicate those probed by site-directed mutagenesis. Secondary structures are indicated above the alignment. Sequence alignment was performed by MUSCLE (17) and the figure was prepared using ESPript (24). GenBank entries are: *Moorea producens* (CurM_ST) (GenBank ACV42478), *Synechococcus* PCC 7002 (OLS ST) (YP_001734428), *Cyanothece* PCC 7424 (YP_002377174), *Cyanothece* PCC 7822 (ZP_03153601), *Moorea producens* 3L (ZP_08425908), *Prochloron didemni* (AEH57210), *Pseudomonas entomophila* L48 (YP_610919), *Haliangium ochraceum* DSM 14365 (YP_003265308).



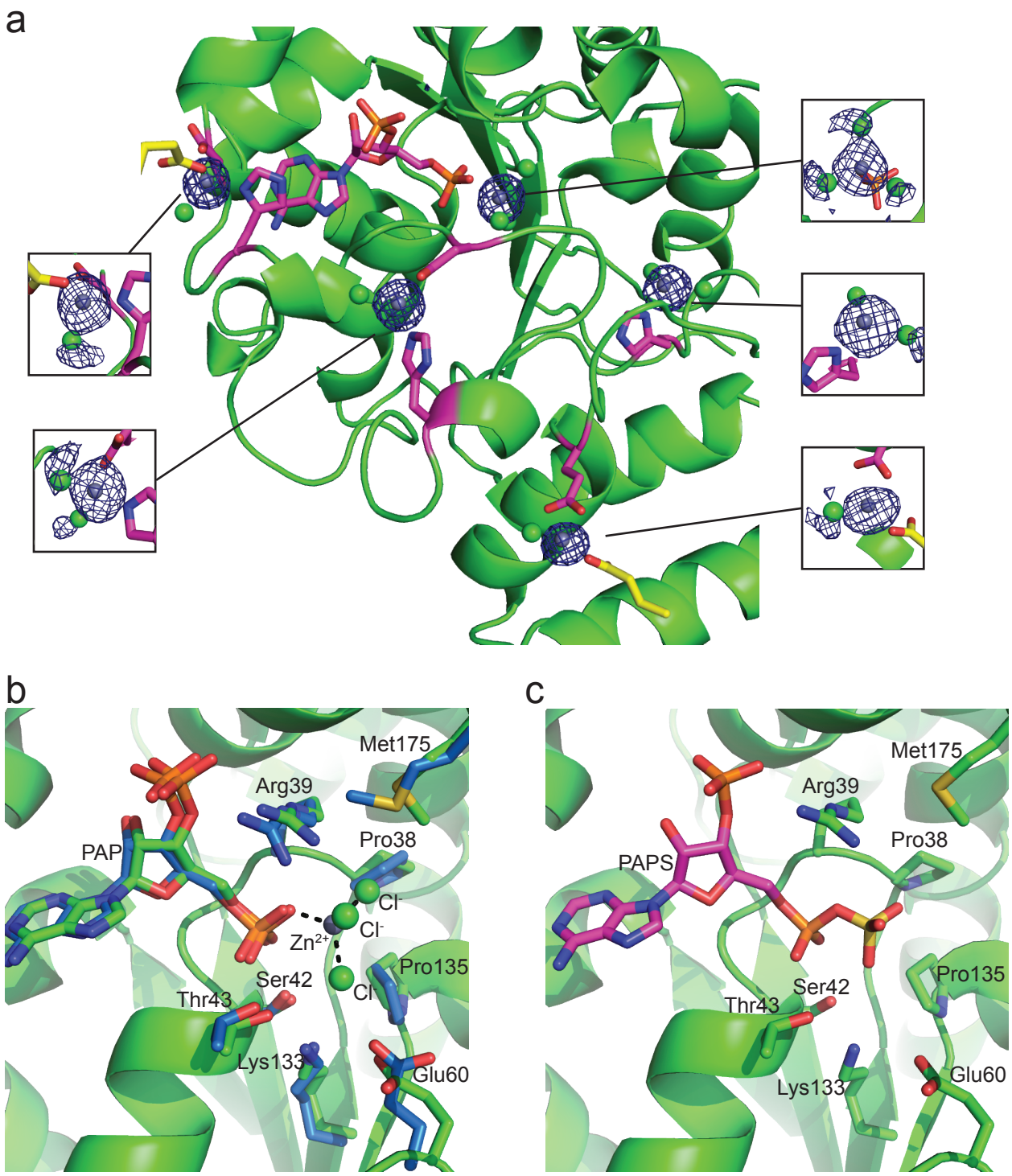
Supplementary Figure 2 Phylogenetic analysis of activating STs and close homologs. ST domains are depicted in red and activating ACP-ST-TE tridomains are highlighted in blue. Domains neighboring the ST are depicted: ketosynthase (KS), acyl transferase (AT), ketoreductase (KR), acyl carrier protein (ACP), sulfotransferase (ST), thioesterase (TE), acyl activating (AA), glyceric acid loading (GA), thiolation (T), condensation (C), and adenylation (A). GenBank accession numbers for sequences not listed in Supplementary Figure 1 are: *Planktothrix agardhii* NIVA-CYA 116 (ABI26077), *Cyanothece* PCC 8801 (YP_002372038), *Ostreococcus lucimarinus* CCE9901 (XP_001416378), *Micromonas* RCC299 (XP_002507643), *Ostreococcus tauri* COG3321 (XM_003074782).



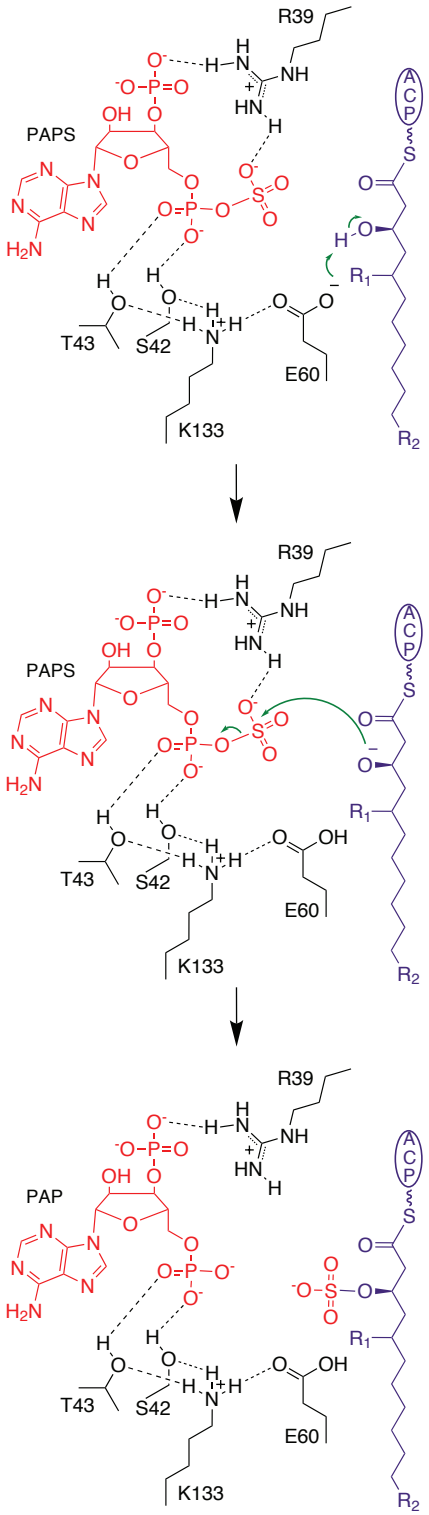
Supplemental Figure 3 HPLC detection of ST substrates and products. a) The ACP loading reaction analyzed by reverse-phase HPLC. Svp was combined with apo-ACP and (R)-3-hydroxymyristoyl-CoA to load the ACP with (R)-3-hydroxymyristoyl-phosphopantethiene. The lower trace is a CoA-free control. b) The ST reaction analyzed by HPLC. The one-pot assay mixture was separated by reverse-phase HPLC resulting in peaks for Svp, (R)-3-hydroxymyristoyl-ACP, and the product, (R)-3-sulfomyristoyl-ACP.



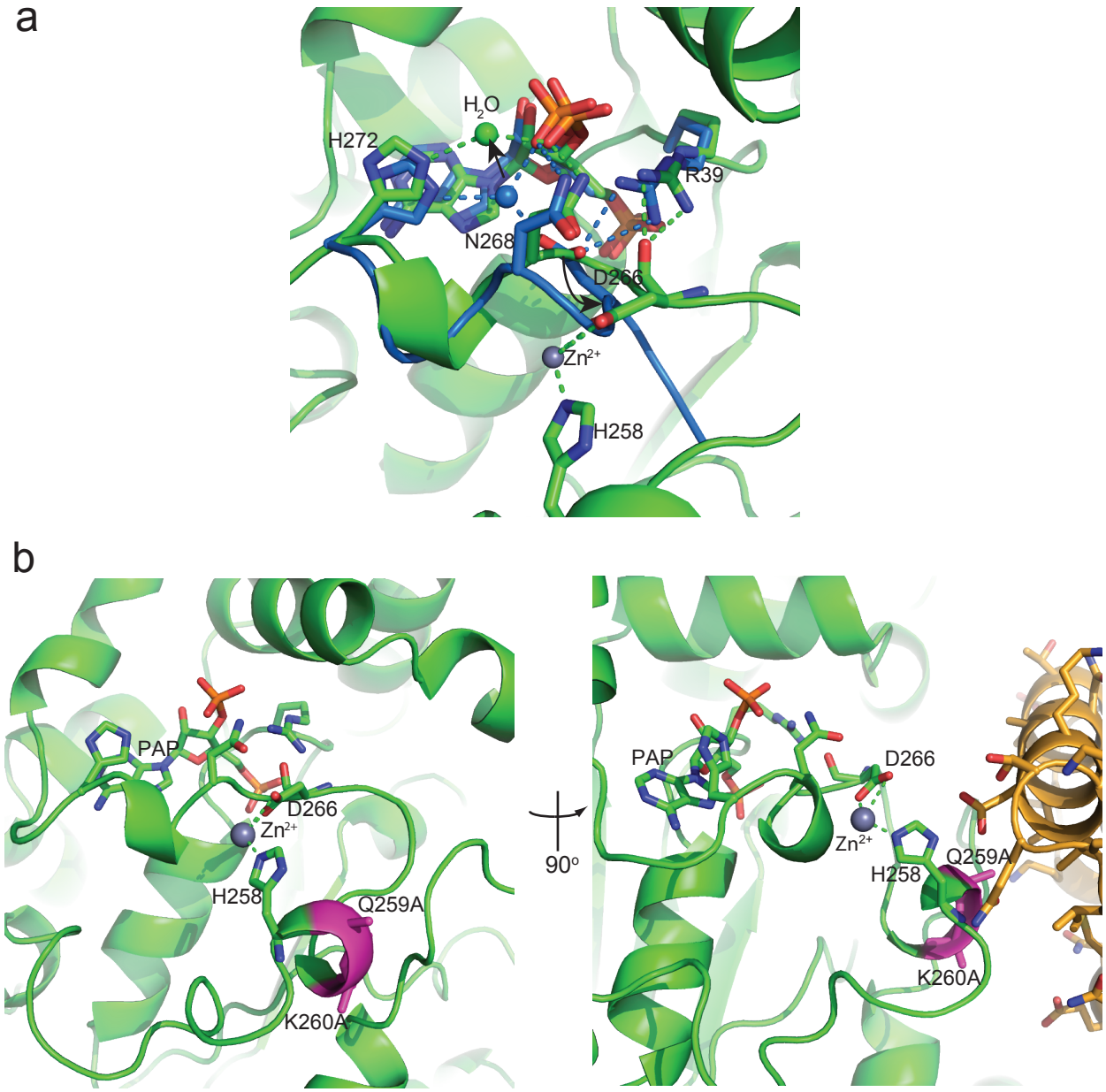
Supplemental Figure 4 Comparison of ST structures. a) Structure alignment of CurM ST (green) to OLS ST (blue) (RMSD = 0.785 Å for 228 Cα atoms). PAP is shown in stick. b) Structure alignment of CurM ST (green) to 3-OST (1T8U (20)) (purple) (RMSD = 1.47 Å for 114 Cα atoms). PAP is shown in stick for CurM ST (green C). PAP and the tetrasaccharide substrate of 3-OST are also shown in stick (purple C). Both parts A and B are shown in stereo. c) Electron density of PAP and $ZnCl_3^-$ bound to CurM ST. d) Electron density of PAP bound to OLS ST. In both parts C and D, electron density is from an omit Fo-Fc map contoured to 4.0 sigma after removing ligands from the atomic model and running one round of refinement.



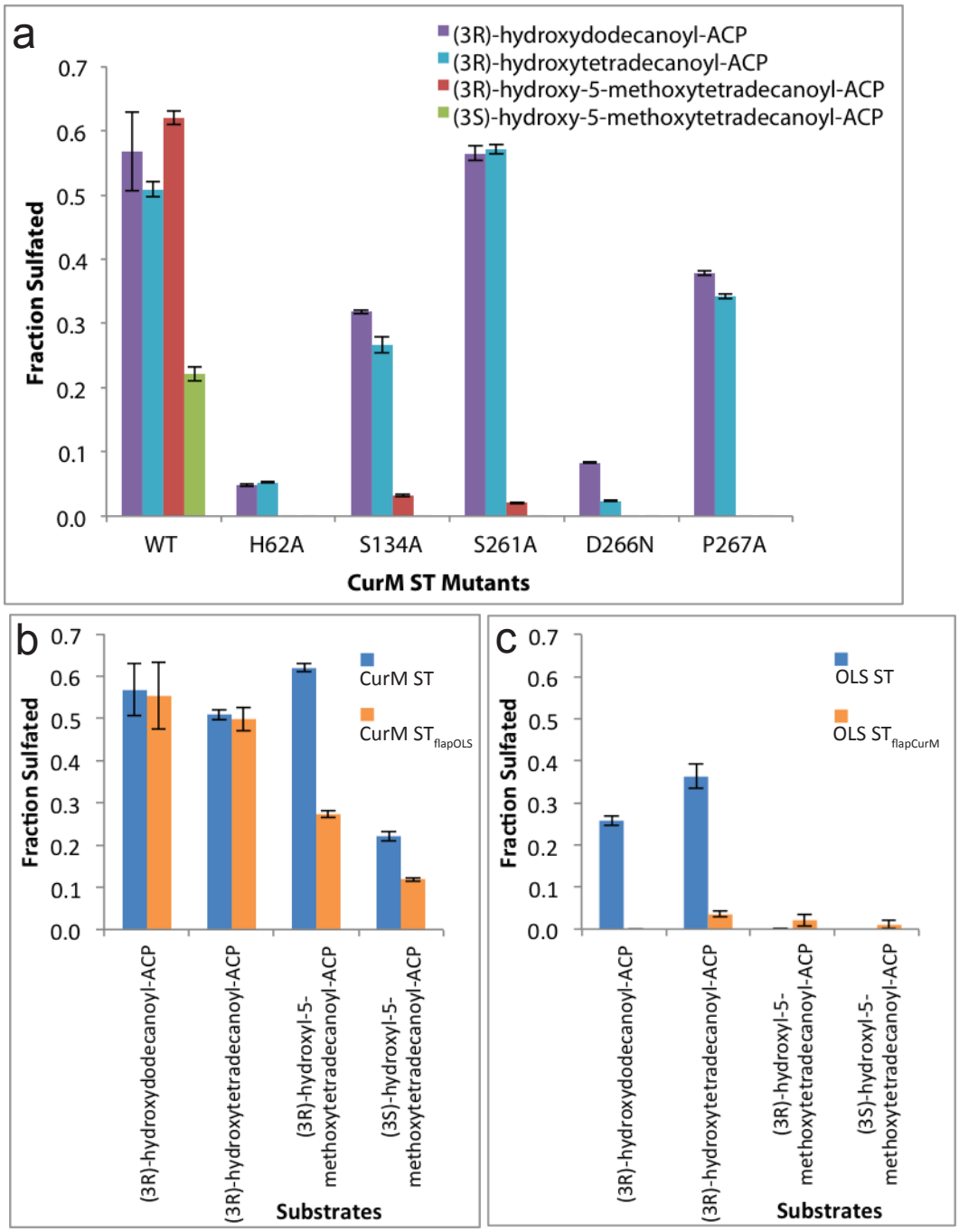
Supplemental Figure 5 Zinc binding in CurM ST crystals. a) An anomalous difference Fourier map contoured at 5σ from a dataset collected at 10.000 keV (1.23984 Å), 341 eV above the Zn K absorption edge. Peaks for the five zinc bound to CurM ST are shown. Amino acid ligands are shown in stick (magenta C, and yellow C for residues from a neighboring molecule in the crystal lattice). Small panels show anomalous difference density contoured at 2.5σ. Peaks adjacent to the zinc sites were interpreted as chloride ligands (green). b) Zinc bound to PAP in CurM ST superimposed with OLS ST. Zn^{2+} (gray) coordinates three Cl^- ligands (green) and one PAP 5'-phosphate oxygen (green C, red O, blue N, orange P). Residues surrounding the $ZnCl_3^-$ are shown in stick for CurM ST (green C) and OLS ST (blue C). c) PAPS in the CurM ST active site. View as in B with modeled PAPS (magenta C), using PAP-$ZnCl_3^-$ as a guide.



Supplemental Figure 6 Proposed mechanism of catalysis for activating STs.



Supplemental Figure 7 Features of the CurM ST crystallization that contribute to the well ordered conformation of the active-site flap. a) Differences in CurM ST and OLS ST PAP-binding residues. Residues in CurM ST (green C) and OLS ST (blue C) are shown in stick. Asp266 and His258 coordinate a bound Zn^{2+} ion in CurM ST. In OLS ST, Asp266 points into the active site and contacts Arg39 and an ordered water. In CurM ST, Arg39 instead interacts with a backbone carbonyl. b) Position of the surface entropy reduction (SER) mutations that enabled crystallization of CurM ST. SER Ala substitutions are shown as magenta sticks. The crystal contact mediated by the SER mutations is shown (gold C, red O, blue N).



Supplemental Figure 8 Substrate preference for ST variants. a) CurM ST single amino acid substitutions. Raw HPLC chromatogram peak areas for the substrate and sulfonated product after 5-min reactions were used to calculate the fraction of substrate sulfonated. Mean ± standard deviation from duplicate experiments is shown. b) Substrate preference for CurM ST chimera with OLS ST flap. Experiment was performed as in A. Mean ± standard deviation from triplicate experiments is shown. CurM $ST_{flapOLS}$ shows similar activity to CurM ST with simple alkyl substrates but decreased activity with C5-methoxy substrates. c) Substrate preference for OLS ST chimera with CurM ST flap. Raw HPLC chromatogram peak areas for the substrate and sulfonated product after 3 h reactions were used to calculate the fraction of substrate sulfonated. Mean ± standard deviation from triplicate experiments is shown. OLS $ST_{flapCurM}$ shows very low overall activity.

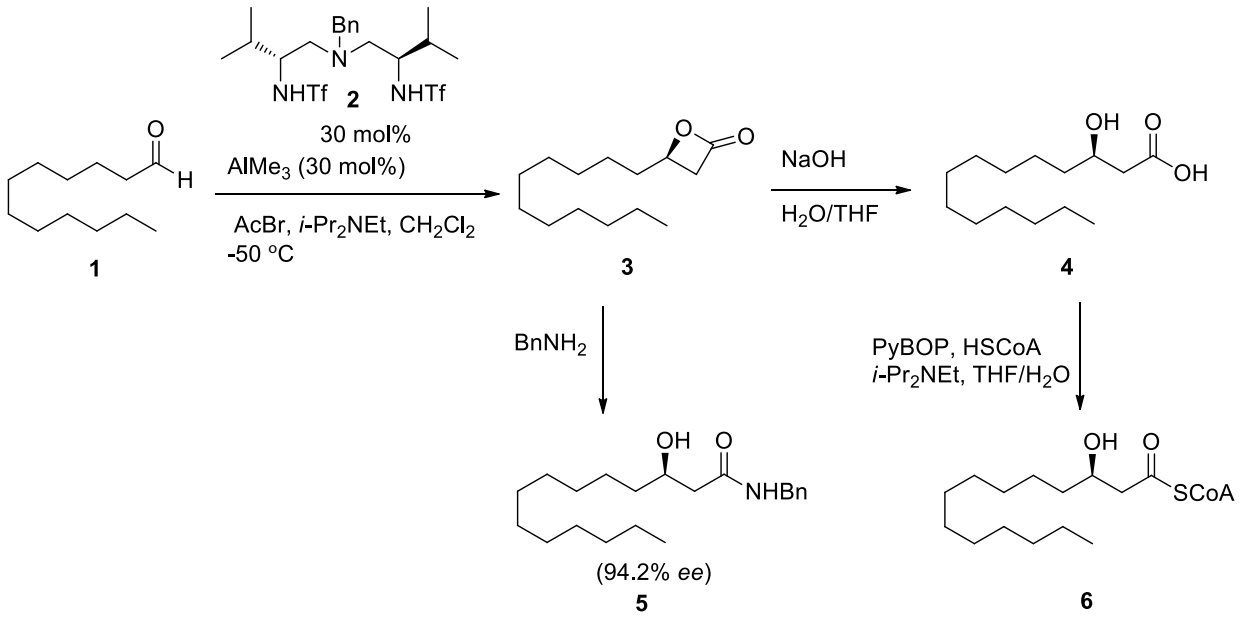
Supplemental Table 1

Effect of Zn^{2+} and Gln259/Lys260 Ala Substitutions on CurM ST Activity

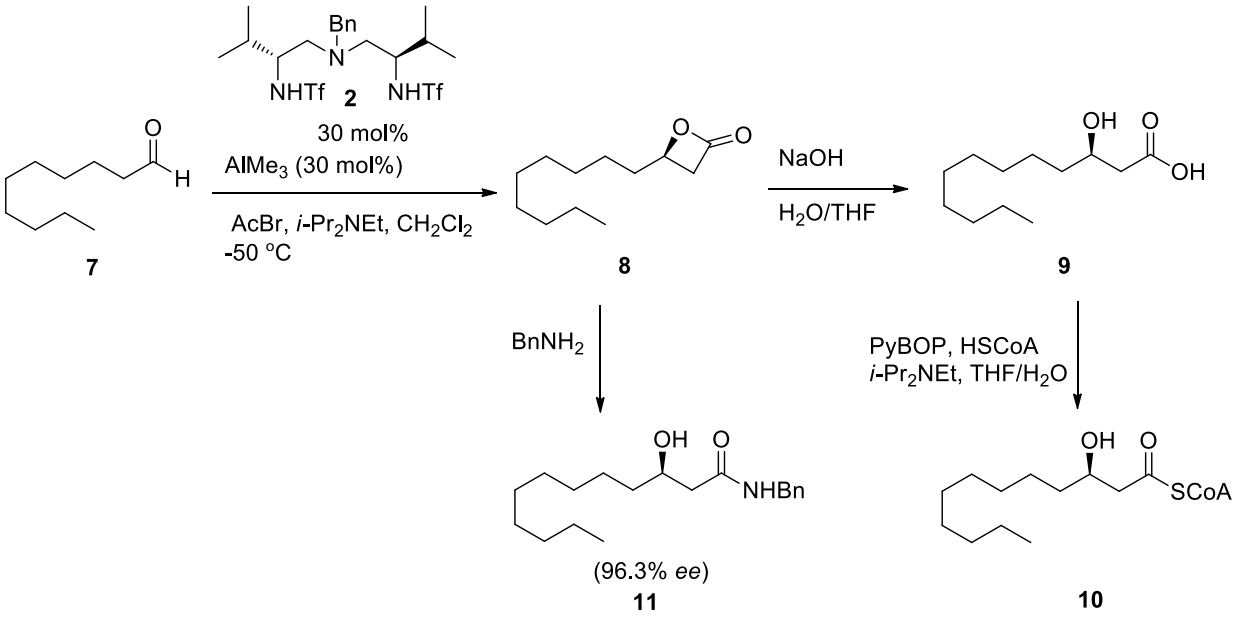
	% WT
CurM ST WT	100.0[a]
100 μM Zn^{2+}	52.9
10 μM Zn^{2+}	74.3
1 μM Zn^{2+}	73.9
Q259A/K260A	34.6

[a]Raw HPLC chromatogram peak area for the 3-*R*-hydroxymyristoyl-ACP substrate and sulfated product were used to calculate the fraction of substrate sulfated and then normalized to a wild type zinc-free control.

Overview of Synthesis Protocols



Scheme 1. Synthesis of 3-(*R*)-tetradecanoylCoA (**6**) and amide **5**

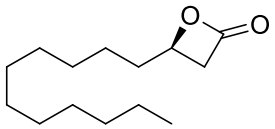


Scheme 1. Synthesis of 3-(*R*)-dodecanoylCoA (**10**) and amide **11**

Chemical Synthesis

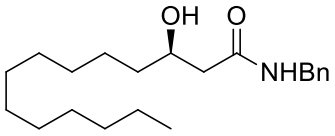
Contents:

General. All reactions were performed under an N_2 atmosphere and all glassware was flame dried prior to use. CH_2Cl_2 and toluene were dried by passing through a column of activated alumina and degassed prior to use by freeze-pump-thaw method. Reactions carried out at -78 °C employed a CO_2/acetone bath. THF and Et_2O were distilled over sodium/benzophenone ketyl. Reactions were monitored by TLC analysis (pre-coated silica gel 60 F254 plates, 250 μm layer thickness) and visualization was accomplished with a 254 nm UV light and by staining with a PMA solution (5 g of phosphomolybdic acid in 100 mL of 95% EtOH), *p*-anisaldehyde solution (2.5 mL of *p*-anisaldehyde, 2 mL of AcOH, and 3.5 mL of conc. H_2SO_4 in 100 mL of 95% EtOH), Vaughn's reagent (4.8 g of $(NH_4)_6Mo_7O_{24}•4H_2O$ and 0.2 g of $Ce(SO_4)_2$ in 100 mL of a 3.5 N H_2SO_4 solution) or a $KMnO_4$ solution (1.5 g of $KMnO_4$ and 1.5 g of K_2CO_3 in 100 mL of a 0.1% NaOH solution). Flash chromatography on SiO_2 was used to purify the crude reaction mixtures. ^{1}H NMR spectra were obtained at 300 or 400 MHz in $CDCl_3$ unless otherwise noted. Chemical shifts were reported in parts per million with the residual solvent peak used as an internal standard. ^{1}H NMR spectra were obtained and are tabulated as follows: chemical shift, multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet), number of protons, and coupling constant(s). ^{13}C NMR spectra were obtained at 76 or 100 MHz using a proton-decoupled pulse sequence with a d1 of 3 sec, and are tabulated by observed peak. IR spectra were obtained on a IdentifyIR-ATR spectrometer.



3

(*R*)-4-Undecyloxetan-2-one (3). To a flame-dried, N_2-cooled flask equipped with a stir bar was added a solution of the triamine ligand **2**[1] (0.371 g, 0.684 mmol, 30 mol%) in CH_2Cl_2 (12 mL). The mixture was stirred at room temperature and treated slowly with a solution of $AlMe_3$ (49.0 mg, 0.684 mmol, 30 mol%) in CH_2Cl_2 (3 mL). The reaction mixture was stirred at room temperature for 2 h, cooled to -50 °C and treated with *i*-Pr_2NEt (0.680 mL, 3.87 mmol), freshly distilled acetyl bromide (0.320 mL, 4.33 mmol) and dodecanal (0.550 mL, 2.28 mmol). The resultant pale yellow solution was stirred for 14 h at -45 °C, warmed to room temperature, and poured into a separatory funnel containing 0.1 N HCl (25 mL). The organic layer was successively washed with sat. $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a yellow oil that was purified by chromatography on SiO_2 (hexanes:EtOAc, 9:1) to provide **3**[2] (0.351 g, 68% yield) as a colorless oil: $[\alpha]_D$ +15.0 (*c* 1.55, $CHCl_3$); IR (ATR) 2921, 2851, 1825, 1446, 1144, 1123 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz) δ 4.54-4.46 (m, 1 H), 3.50 (dd, 1 H, *J* = 5.7, 16.2 Hz), 3.05 (dd, 1 H, *J* = 4.5, 16.2 Hz), 1.89-1.68 (m, 2 H), 1.26 (app s, 20 H), 0.87 (t, 3 H, *J* = 6.9 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 168.6, 71.4, 42.9, 34.7, 32.0, 29.7, 29.6, 29.5, 29.4, 29.2, 25.0, 22.8, 14.2.



5

(*R*)-*N*-Benzyl-3-hydroxytetradecanamide (5). A mixture of lactone **3** (53 mg, 0.23 mmol) and benzylamine (0.13 mL, 1.2 mmol) was stirred at room temperature for 16 h. The reaction mixture was diluted with EtOAc (10 mL) and washed with 1 N HCl (2 x 10 mL), water (10 mL)

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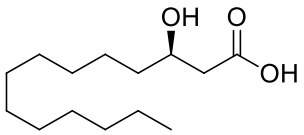
[1] Nelson, S. G.; Peelen, T. J.; Wan, Z., "Catalytic asymmetric acyl halide-aldehyde cyclocondensations. A strategy for enantioselective catalyzed cross aldol reactions." *J. Am. Chem. Soc.* **1999**, *121*, 9742-9743.

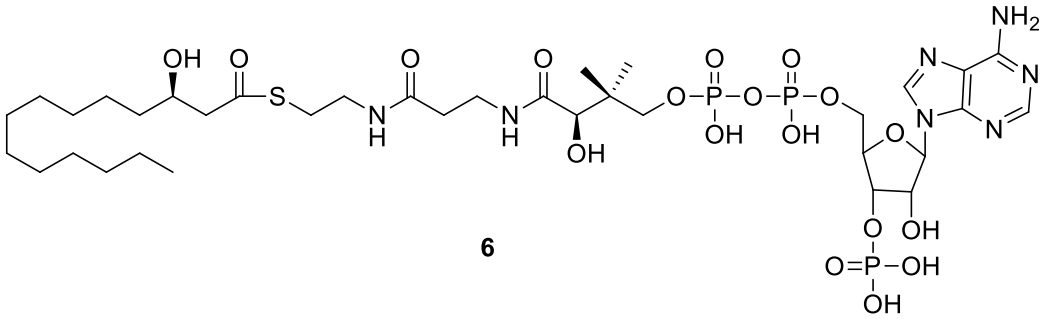
[2] The enantiomeric excess of lactone **3** was determined by chiral HPLC analysis of amide **5**, obtained by ring opening of **3** with benzyl amine.

and brine (10 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a brown residue, which was purified by chromatography on SiO_2 (hexanes:EtOAc, 1:1) to give **5** (59 mg, 76%) as a white solid. The enantiomeric excess of the **5** was determined to be 94.2% *ee* by chiral HPLC (ChiralCel OD, 4.6 mm X 250 mm, hexane/isopropanol (90/10, 30 min), 1 mL/min, λ = 221 nm, $t_R$major = 9.71 min, t_R minor = 12.33 min): Mp 90.4-92.6 °C (CH_2Cl_2); $[\alpha]_D$ -14.9 (*c* 1.06, $CHCl_3$); IR (ATR) 3295, 2924, 2850, 1644, 1567, 1424 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.33-7.24 (m, 5 H), 6.45 (s, 1 H), 4.41 (d, 2 H, *J* = 5.7 Hz), 3.97 (br s, 1 H), 3.69 (br s, 1 H), 2.37 (dd, 1 H, *J* = 2.6, 15.3 Hz), 2.26 (dd, 1 H, *J* = 9.0, 15.2 Hz), 1.53-1.35 (m, 3 H), 1.25 (app s, 17 H), 0.88 (t, 3 H, *J* = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5, 138.1, 128.8, 127.8, 127.6, 68.8, 43.5, 42.5, 37.1, 32.0, 29.7, 29.7, 29.7, 29.7, 29.6, 29.4, 22.8, 14.2; HRMS (TOF MS ES+) *m/z* calcd for $C_{21}H_{35}NO_2Na$ (M+Na) 356.2564, found 356.2594.



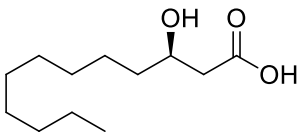
4

(*R*)-3-Hydroxytetradecanoic acid (4). To a stirred solution of **2**[1] (200 mg, 0.884 mmol) in THF (1.5 mL) at room temperature was added a solution of NaOH (117 mg, 2.92 mmol) in H_2O (1 mL). The reaction mixture was stirred at room temperature for 2 h, diluted with H_2O (5 mL) and washed with Et_2O (20 mL) to remove organic impurities. The ether layer was discarded and the aqueous layer was acidified with 2 N HCl (3 mL). The resulting suspension was extracted with Et_2O (2 x 15 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed under reduced pressure to give **4** (188 mg, 87%) as a white solid: Mp 71.3-73.2 °C (CH_2Cl_2); $[\alpha]_D$ -14.4 (*c* 1.15, $CHCl_3$); IR (ATR) 3560, 2918, 2844, 1681, 1466, 1293, 1224 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.05-4.00 (m, 1 H), 2.57 (dd, 1 H, *J* = 3.1, 16.6 Hz), 2.47 (dd, 1 H, *J* = 9.0, 16.6 Hz), 1.58-1.43 (m, 3 H), 1.27 (app s, 17 H), 0.88 (t, 3 H, *J* = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.1, 68.2, 41.2, 36.6, 32.0, 29.8, 29.7, 29.7, 29.7, 29.6, 29.5, 25.6, 22.8, 14.2; HRMS (TOF MS ES-) *m/z* calcd for $C_{28}H_{55}O_6$ (2M-H) 487.3999, found 487.4014.



6

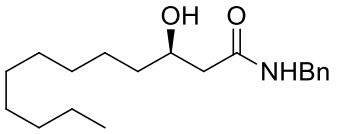
(*R*)-3-Hydroxytetradecanoyl-CoA (6). To a solution of acid **4** (1.59 mg, 0.00651 mmol) in THF (0.2 mL) was added a solution of PyBOP (13.5 mg, 0.0260 mmol) and Hünig's base (9.00 μL, 0.0521 mmol). To this mixture was added a solution of Coenzyme A free acid trihydrate (10.0 mg, 0.0131 mmol) in H_2O (0.2 mL). The resulting solution was stirred at room temperature for 5 h, lyophilized, and the resulting white solid was washed with THF to remove organic impurities. The residue was purified by reverse phase HPLC (Dynamax column (10 X 250 mm) with a gradient from 60% MeOH/40% H_2O (10 mM NH_4OAc) to 90% MeOH/10% H_2O (10 mM NH_4OAc), over 20 min and hold for 10 min, at a flow rate of 1 mL/min, t_R = 21.45 min). The solvent was evaporated under reduced pressure. The residue was dissolved in water and lyophilized to give **6** (3.8 mg, 59%) as a white solid: ^{1}H NMR (D_2O, 400 MHz) δ 8.53 (s, 1 H), 8.23 (s, 1 H), 6.13 (d, 1 H, *J* = 6.8 Hz), 4.54 (br s, 1 H), 4.23-4.19 (m, 2 H), 4.09-3.98 (m, 2 H), 3.79 (dd, 1 H, *J* = 4.8, 9.8 Hz), 3.51 (dd, 1 H, *J* = 4.7, 9.7 Hz), 3.41 (t, 2 H, *J* = 6.6 Hz), 3.31 (t, 2 H, *J* = 6.0 Hz), 3.04-2.93 (m, 2 H), 2.79-2.67 (m, 2 H), 2.39 (t, 2 H, *J* = 6.5 Hz), 1.44-1.40 (m, 2 H), 1.24-1.18 (m, 19 H), 0.84 (s, 3 H), 0.81 (t, 3 H, *J* = 6.7 Hz), 0.70 (s, 3 H); ^{13}C NMR (CD_3OD, 150 MHz) δ 200.5, 175.6, 174.3, 156.3, 155.1, 153.5, 152.2, 150.1, 140.9, 87.8, 84.3, 75.0, 72.9, 69.3, 66.3, 55.2, 51.2, 43.5, 39.7, 39.4, 37.9, 36.5, 36.3, 32.3, 30.8, 30.7 (2C), 30.4, 29.1, 26.5, 23.6, 22.0, 19.1, 18.7, 17.3, 14.9, 13.1; HRMS (TOF MS ES+) *m/z* calcd for $C_{35}H_{62}N_7O_{18}P_3SK$ (M+K) 1032.2722, found 1032.2648.



9

(*R*)-3-Hydroxydodecanoic acid (9). To a flame-dried, N_2-cooled flask equipped with a stirbar was added a solution of triamine ligand **2**[1] (475 mg, 0.877 mmol, 30 mol%) in CH_2Cl_2 (12 mL),

followed slowly by a solution of $AlMe_3$ (63.0 mg, 0.877 mmol, 30 mol%) in CH_2Cl_2 (5 mL). The resulting solution was stirred at room temperature for 2 h, cooled to -50 °C and treated with Hünig's base (0.870 mL, 4.97 mmol), freshly distilled acetyl bromide (0.410 mL, 5.56 mmol) and a solution of decanal (457 mg, 2.92 mmol) in CH_2Cl_2 (5 mL). The pale yellow reaction mixture was stirred for 14 h at -50 °C, warmed to room temperature, and poured into a separatory funnel containing 0.1 N HCl (25 mL). The organic layer was washed with sat. $NaHCO_3$ and brine. The organic phase was dried (Na_2SO_4) and the solvent was removed *in vacuo* to afford a yellow oil, which was used in the following step without further purification.[3] To a stirred solution of above crude material in THF (6 mL) at room temperature was added a solution of NaOH (385 mg, 9.63 mmol) in H_2O (3 mL). The reaction mixture was stirred at room temperature for 2 h, diluted with H_2O (10 mL) and washed with Et_2O (3 x 20 mL) to remove organic impurities. The ether layer was discarded and to the aqueous layer was added 2 N HCl (10 mL). The resulting suspension was extracted with Et_2O (3 x 30 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed under reduced pressure to give **9** (413 mg, 65% yield, 2 steps) as a white solid: Mp 59.8-62.0 °C (CH_2Cl_2); $[\alpha]_D$ -15.9 (*c* 1.00, $CHCl_3$); IR (ATR) 3560, 2919, 2844, 1676, 1466, 1293 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 4.05-4.01 (m, 1 H), 2.56 (dd, 1 H, *J* = 3.1, 16.5 Hz), 2.46 (dd, 1 H, *J* = 9.0, 16.5 Hz), 1.58-1.43 (m, 3 H), 1.26 (app s, 13 H), 0.87 (t, 3 H, *J* = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 178.1, 68.2, 41.2, 36.6, 32.0, 29.7, 29.7, 29.6, 29.4, 25.6, 22.8, 14.2.

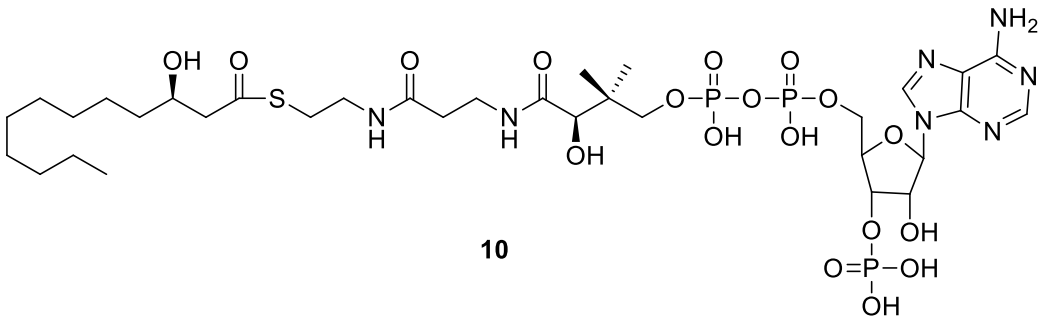


11

(*R*)-N-Benzyl-3-hydroxydodecanamide (11). A mixture of crude lactone **8** (50 mg) and benzylamine (0.140 mL, 1.26 mmol) was stirred at room temperature for 16 h. The reaction mixture was diluted with EtOAc (10 mL) and washed with 1 N HCl (2 x 10 mL), water (10 mL) and brine (10 mL). The organic layer was dried (Na_2SO_4) and concentrated under reduced

[3] The enantiomeric excess of lactone **8** was determined by chiral HPLC analysis of amide **11**, obtained by ring opening of **8** with benzyl amine.

pressure to give a brown residue, which was purified by chromatography on SiO_2 (hexanes:EtOAc, 1:1) to give **11** (42 mg) as a white solid. The enantiomeric excess of **11** was determined to be 96.3% *ee* by chiral HPLC (ChiralCel OD, 4.6 mm X 250 mm, hexane/isopropanol (90/10, 30 min), 1 mL/min, λ = 254 nm, $t_R$major = 9.97 min, t_R minor = 12.99 min): Mp 83.4-84.3 °C (hexanes:EtOAc, 1:1); $[\alpha]_D$ -15.8 (*c* 1.04, $CHCl_3$); IR (ATR) 3920, 2919, 2850, 1644, 1560, 1446 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz) δ 7.33-7.24 (m, 5 H), 6.44-6.40 (m, 1 H), 4.41 (d, 2 H, *J* = 5.7 Hz), 4.00-3.95 (m, 1 H), 3.67 (br s, 1 H), 2.37 (dd, 1 H, *J* = 2.6, 15.3 Hz), 2.26 (dd, 1 H, *J* = 9.0, 15.2 Hz), 1.53-1.35 (m, 3 H), 1.25 (app s, 14 H), 0.88 (t, 3 H, *J* = 6.6 Hz); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 172.5, 138.1, 128.8, 127.8, 127.6, 68.8, 43.5, 42.6, 37.0, 32.0, 29.7, 29.6, 29.4, 25.6, 22.8, 14.2.



10

(*R*)-3-Hydroxydodecanoyl-CoA (10). To a stirred solution of acid **9** (1.89 mg, 0.00876 mmol) in THF (0.2 mL) was added PyBOP (18.2 mg, 0.0350 mmol) and Hünig's base (13.0 μL, 0.0701 mmol), followed by a solution of coenzyme A free acid trihydrate (14.4 mg, 0.0175 mmol) in H_2O (0.2 mL). The reaction mixture was stirred at room temperature for 5 h, lyophilized, and the resulting white solid was washed with THF to remove organic impurities. The white residue was purified by RP-HPLC (Dynamax C-18 (10 X 250 mm), gradient from 60% MeOH/40% H_2O (10 mM NH_4OAc) to 90% MeOH/10% H_2O (10 mM NH_4OAc) over 20 min and held for 10 min, at a flow rate of 1 mL/min, t_R = 20.18 min). The solvent was evaporated under reduced pressure. The residue was dissolved in water and lyophilized to give **10** (4.2 mg, 50%) as a white solid: ^1H NMR (D_2O, 400 MHz) δ 8.46 (s, 1 H), 8.12 (s, 1 H), 6.07 (d, 1 H, *J* = 6.0 Hz), 4.49 (br s, 1 H), 4.14 (br s, 2 H), 3.97-3.92 (m, 2 H), 3.74 (dd, 1 H, *J* = 4.8, 9.6 Hz), 3.55 (dd, 1 H, *J* = 4.8, 9.6 Hz), 3.34 (t, 2 H, *J* = 5.2 Hz), 3.24 (t, 2 H, *J* = 6.0 Hz), 2.93-2.89 (m, 2 H), 2.70-2.59 (m, 2 H), 2.32 (t, 2 H, *J* = 6.8 Hz), 1.35-1.32 (m, 2 H), 1.23-1.09 (m, 17 H), 0.79 (s, 3 H), 0.76 (t, 3 H, *J* = 6.4 Hz), 0.66 (s, 3 H); ^{13}C NMR (CD_3OD, 150 MHz) δ 201.3, 175.6, 174.6, 154.9, 151.7, 141.6,

87.8, 84.3, 82.1, 78.4, 75.0 (2C), 72.9, 69.4, 66.2, 51.8, 39.6, 39.3, 37.5, 36.4, 36.3, 32.6, 30.2, 30.1, 30.0, 29.1, 26.1, 23.4, 22.0, 19.1, 14.8.