Supporting Information

Positioning group-enabled biocatalytic oxidative dearomatization

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Safety Statement: No unusual safety hazards were encountered.

Part I. Substrate Synthesis

All reagents were used as received unless otherwise noted. Reactions were carried out under a nitrogen atmosphere using standard Schlenk techniques unless otherwise noted. Solvents were degassed and dried over aluminum columns on an MBraun solvent system (Model PS-00-3). Reactions were monitored by thin layer chromatography using Millipore 60 F254 precoated silica TLC plates (0.25 mm) which were visualized using UV, p-anisaldehyde, CAM, DNP, or Br-cresol stain. Flash column chromatography was performed using Macherey-Nagel 60 μm (230-400 mesh) silica gel. All compounds purified by column chromatography were sufficiently pure for use in further experiments unless otherwise indicated. ¹H and ¹³C NMR spectra were obtained in CDCl₃ at rt (25 °C), unless otherwise noted, on Varian 400 MHz or Varian 600 MHz spectrometers. Chemical shifts of ¹H NMR spectra were recorded in parts per million (ppm) on the δ scale. High resolution electrospray mass spectra were obtained on an Agilent G6545A quadrupole-time of flight mass spectrometer in positive mode with an Agilent 1290 UPLC system. Solvent A = water with 0.1% formic acid. Solvent B = 95% acetonitrile, 5% water and 0.1% formic acid. IR spectra were recorded on a Perkin-Elmer Spectrum BX FT-IR spectrometer. Optical rotations were measured at rt in MeCN unless otherwise noted, on a Jasco P-2000 polarimeter.

2,4-Dihydroxy-3,5-dimethylbenzoic acid (S1)

Adapted from a report by Larrosa and coworkers.¹ In the glove box, NaH (60% dispersion in mineral oil, 1.43 g, 37.4 mmol, 4.0 equiv) was added to a vial containing 2,4-dimethylbenzene-1,3-diol (1.29 g, 9.34 mmol, 1.0 equiv) and 2,4,6-trimethylphenol (1.27 g, 9.34 mmol, 1.0 equiv). The resulting mixture was heated at 100 °C for 5 min, then cooled to room temperature and ground into powder with a spatula. The vial containing the mixture was taken out of the glove box, purged with CO₂, equipped with a balloon filled with CO₂, and heated at 185 °C for 2 h. After this time, the reaction mixture was cooled to room temperature, carefully quenched with water (5 mL), acidified to pH 4 with aq. HCl (1 M), and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na₂SO₄, evaporated to dryness, and purified by column chromatography (10- 50% EtOAc/hexanes) to yield 732 mg (43%) of salicylic acid S1 as a tan solid. ¹H NMR (400 MHz, CD₃OD) δ 7.44 (s, 1 H), 2.07 (s, 3 H), 2.13 (s, 3 H). All spectra obtained were consistent with literature values.²

(E)-But-2-en-1-yl 2,4-dihydroxy-3,5-dimethylbenzoate (27)

0.50 mL DCM was added to an oven-dried vial equipped with a stir bar containing 2,4-dihydroxy-3,5-dimethylbenzoic acid (S1, 50.0 mg, 0.270 mmol, 1.0 equiv). Oxalyl chloride (35 μL, 0.412 mmol, 1.5 equiv) was slowly added followed by DMF (1 drop, approx 10 μL). The mixture was stirred for 2 h before crotyl alcohol (36 μL, 0.450 mmol, 1.5 equiv mixture of cis and trans 1:19) was added dropwise. After 2 h, the reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na₂SO₄, evaporated to dryness, and purified by column chromatography (0-15% EtOAc/hexanes) to yield 41 mg (65%) of the crotyl ester as a colorless solid. None of the undesired cis isomer was observed by UPLC-PDA. ¹H NMR (400 MHz, CD₃OD) δ 7.42 (s, 1 H), 5.89 (m, 1 H), 5.70 (m, 1 H), 4.70 (d, J = 6.4 Hz, 2H),
2.12 (s, 3H), 2.07 (s, 3H), 1.74 (d, J = 6.4 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 170.3, 159.6, 159.6, 131.1, 127.9, 124.9, 115.8, 110.4, 103.6, 64.9, 16.5, 14.8, 6.8; HRMS (ESI) m/z calculated for C$_{13}$H$_{18}$O$_4$ [M+H]$^+$: 237.1121, found: 237.1121; IR (thin film): 3440, 2919, 1644, 1609, 1402, 1295 cm$^{-1}$; MP: 91-92 °C.

![Image of molecule](image1)

(E)-Pent-2-en-1-yl 2,4-dihydroxy-3,5-dimethylbenzoate (Fig. 2A, Entry 8)
0.50 mL DCM was added to an oven-dried vial equipped with a stir bar containing 2,4-dihydroxy-3,5-dimethylbenzoic acid (S1, 50.0 mg, 0.270 mmol, 1.0 equiv). Oxalyl chloride (35 μL, 0.412 mmol, 1.5 equiv) was slowly added followed by DMF (1 drop, approx 10 μL). The mixture was stirred for 2 h before (E)-pent-2-en-1-ol (28 μL, 0.270 mmol, 1.0 equiv) was added dropwise. After 2 h, the reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na$_2$SO$_4$, evaporated to dryness and purified by column chromatography (0-15% EtOAc/hexanes) to yield 17 mg (25%) of the pentenyl ester as a colorless solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 11.11 (s, 1H), 7.51 (s, 1H), 5.90 (m, 1H), 5.66 (m, 1H), 5.16 (s, 1H), 4.75 (dt, J = 6.5, 1.2 Hz, 2H), 2.18 (s, 3H), 2.12 (s, 3H), 2.09 (m, 2H), 1.03 (t, J = 7.5 Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 170.2, 159.8, 158.0, 138.4, 128.6, 122.6, 114.5, 110.0, 104.9, 65.6, 25.3, 15.3, 13.1, 7.7; HRMS (ESI) m/z calculated for C$_{14}$H$_{19}$O$_4$ [M+H]$^+$: 251.1278, found: 251.1262; IR (thin film): 3460, 2925, 1645, 1611, 1401, 1292 cm$^{-1}$; MP: 60-61 °C.

![Image of molecule](image2)

Butyl 2,4-dihydroxy-3,5-dimethylbenzoate (Fig. 2A, Entry 9)
0.50 mL DCM was added to an oven-dried vial equipped with a stir bar containing 2,4-dihydroxy-3,5-dimethylbenzoic acid (S1, 50.0 mg, 0.270 mmol, 1.0 equiv). Oxalyl chloride (35 μL, 0.412 mmol, 1.5 equiv) was slowly added followed by DMF (1 drop, approx 10 μL). The mixture was stirred for 2 h before butanol (29 μL, 0.270 mmol, 1.0 equiv) was added dropwise. After 2 h, the reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na$_2$SO$_4$, evaporated to dryness, and purified by column chromatography (0-15% EtOAc/hexanes) to yield 10 mg (16%) of the butyl ester as a colorless solid. $^1$H NMR (400 MHz, CD$_3$OD) δ 7.42 (s, 1H), 4.29 (t, J = 6.6 Hz, 2H), 2.13 (s, 3H), 2.07 (s, 3H), 1.74 (m, 2H), 1.48 (h, J = 7.4 Hz, 2H), 0.99 (t, J = 7.4 Hz, 3H); $^{13}$C NMR (100 MHz, CD$_3$OD) δ 170.5, 159.7, 159.6, 127.8, 115.7, 110.4, 103.6, 64.2, 30.5, 18.9, 14.8, 12.6, 6.8; HRMS (ESI) m/z calculated for C$_{13}$H$_{19}$O$_4$ [M+H]$^+$: 239.1278, found: 239.1275; IR (thin film): 3442, 2925, 1642, 1610, 1403, 1288 cm$^{-1}$; MP: 93-94 °C.

![Image of molecule](image3)

Pentyl 2,4-dihydroxy-3,5-dimethylbenzoate (Fig. 2A, Entry 10)
0.50 mL DCM was added to an oven-dried vial equipped with a stir bar containing 2,4-dihydroxy-3,5-dimethylbenzoic acid (S1, 50.0 mg, 0.270 mmol, 1.0 equiv). Oxalyl chloride (35 μL, 0.412 mmol, 1.5 equiv) was slowly added followed by DMF (1 drop, approx 10 μL). The mixture was stirred for 2 h before pentanol (29 μL, 0.270 mmol, 1.0 equiv) was added dropwise. After 2 h, the reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na₂SO₄, evaporated to dryness and purified by column chromatography (0-15% EtOAc/hexanes) to yield 50 mg (74%) of the pentyl ester as a colorless solid. ¹H NMR (400 MHz, CDCl₃) δ 11.17 (s, 1H), 7.48 (s, 1H), 5.27 (s, 1H), 4.30 (t, J = 6.7 Hz, 2H), 2.19 (s, 3H), 2.14 (s, 3H), 1.77 (p, J = 6.9 Hz, 2H), 1.42 (m, 4H), 0.94 (m, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 170.6, 159.8, 158.0, 128.4, 114.6, 110.0, 104.9, 65.1, 28.4, 28.1, 22.3, 15.4, 13.7, 7.6; HRMS (ESI) m/z calculated for C₁₄H₂₂O₄ [M+H]⁺: 253.1434, found: 253.1438; IR (thin film): 3514, 2922, 1735, 1635, 3390, 2924, 1735, 1635, 1590, 1370 cm⁻¹; MP: 67-68 ºC.

N-Butyl-2,4-dihydroxy-3,5-dimethylbenzamide (Fig. 2A, Entry 6)
0.50 mL DCM was added to an oven-dried vial equipped with a stir bar containing 2,4-dihydroxy-3,5-dimethylbenzoic acid (S1, 50.0 mg, 0.270 mmol, 1.0 equiv). Oxalyl chloride (35 μL, 0.412 mmol, 1.5 equiv) was slowly added followed by DMF (1 drop, approx 10 μL). The mixture was stirred for 2 h before butyl amine (29 μL, 0.270 mmol, 1.0 equiv) was added dropwise. After 2 h, the reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na₂SO₄, evaporated to dryness and purified by column chromatography (0-15% EtOAc/hexanes) to yield 23 mg (34%) of the amide as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 12.77 (s, 1H), 6.95 (s, 1H), 6.07 (s, 1H), 5.02 (s, 1H), 3.43 (q, J = 6.6 Hz, 2H), 2.19 (s, 3H), 2.14 (s, 3H), 1.60 (m, 2H), 1.41 (q, J = 7.5 Hz, 2H), 0.96 (t, J = 7.3 Hz, 3H); ¹³C NMR (100 MHz, CDCl₃) δ 170.2, 159.7, 156.5, 123.9, 113.8, 110.8, 106.7, 39.3, 31.6, 20.1, 15.6, 13.7, 7.6; HRMS (ESI) m/z calculated for C₁₃H₂₀N₂O₃ [M+H]⁺: 238.1438, found: 238.1432; IR (thin film): 3390, 2924, 1735, 1635, 1590, 1370 cm⁻¹.

4-Hexyl-2,6-dimethylbenzene-1,3-diol (Fig. 2A, Entry 3)
A solution of 1-(2,4-dihydroxy-3,5-dimethylphenyl)hexan-1-one (115 mg, 0.692 mmol, 1.0 equiv) and Zn dust (452 mg, 6.92 mmol, 10 equiv) in EtOH (2.1 mL) was added to a round-bottom flask equipped with a stir bar and cooled to 0 ºC. HCl conc. aq. (2.1 mL) was added dropwise over 15 min. The mixture slurry was then stirred at 0 ºC for 20 min before filtration. The filtrate was quenched by the slow addition of saturated sodium bicarbonate (1 mL) and extracted with EtOAc (3 x 50 mL). The combined organic layers were dried over anhydrous Na₂SO₄, evaporated to dryness and purified by column chromatography silica gel (0-20% EtOAc/hexanes) to afford 103 mg (98% yield) of the title compound as a white solid. ¹H NMR (600 MHz, CDCl₃) δ 6.72 (s, 1H), 4.55 (s, 1H), 4.53 (s, 1H), 2.49 (t, J = 8.1 Hz, 2H), 2.17 (overlapping s, 6H), 1.56 (m, 2H), 1.38 (m, 2H), 1.32 (m, 4H), 0.89 (t, J = 6.5, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 150.6, 150.3, 128.2, 119.6, 114.4, 109.6, 31.8, 30.2, 29.8, 29.3, 22.6,
15.4, 14.1, 8.5; \textbf{HRMS (ESI)} m/z calculated for C$_{14}$H$_{23}$O$_2$ [M+H]$^+$: 223.1693, found: 223.1694; IR (thin film): 3512, 2923, 1611, 1468, 1377, 1191 cm$^{-1}$; \textbf{MP}: 84-86 °C.

1-(2,4-dihydroxy-3,5-dimethylphenyl)hexan-1-one (Fig. 2A, Entry 2)
Prepared as previously reported.$^3$

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\begin{align*}
\text{Sorbicillin (13)} \\
\text{Prepared as previously reported.}^3
\end{align*}
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\begin{align*}
\text{(E)-But-2-en-1-yl 2,4-dihydroxy-3,5,6-trimethylbenzoate (28)} \\
\text{Adapted from a protocol developed by Giannis.}^5 \\
\text{Methyl 2,4-dihydroxy-3,5,6-trimethylbenzoate (180 mg, 1.20 mmol, 1.0 equiv) was dissolved in crotyl alcohol (2.4 mL) in a flame-dried vial. After the addition of dibutyltin oxide (71.0 mg, 0.120 mmol, 10 mol %) the mixture was heated at reflux for 12 h. The reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine (10 mL), dried over anhydrous Na$_2$SO$_4$, evaporated to dryness and purified by column chromatography (0-15% EtOAc/hexanes) to yield 140 mg (47%) of the crotyl ester as a colorless solid. $^1$H NMR (600 MHz, CDCl$_3$) δ 11.42 (s, 1H), 5.86 (m, 1H), 5.7 (m, 1H), 5.11 (s, 1H), 4.76 (d, $J$ = 6.5 Hz, 2H), 2.43 (s, 3H), 2.12 (overlapping s, 6H), 1.74 (d, $J$ = 6.5 Hz, 3H); $^{13}$C NMR (150 MHz, CDCl$_3$) δ 172.0, 159.5, 156.5, 137.6, 131.8, 124.7, 114.7, 107.2, 106.3, 65.9, 18.9, 17.8, 11.8, 8.0; HRMS (ESI) m/z calculated for C$_{14}$H$_{19}$O$_4$ [M+H]$^+$: 251.1278, found: 251.1277; IR (thin film): 3464, 3176, 2949, 2930, 1641, 1611, 1188 cm$^{-1}$; MP: 77-78 °C.
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\begin{align*}
\text{(E)-But-2-en-1-yl 5-ethyl-2,4-dihydroxy-3-methylbenzoate (29)} \\
\text{Adapted from a report by Larrosa and coworkers.}^4 \\
\text{In the glove box, NaH (60% wt/wt dispersion in mineral oil, 1.43 g, 37.4 mmol, 4.0 equiv) was added to a vial containing 4-ethyl-2-methylbenzene-1,3-diol (1.29 g, 9.34}
\end{align*}
\]
mmol, 1.0 equiv) and 2,4,6-trimethylphenol (1.27 g, 9.34 mmol, 1.0 equiv). The resulting mixture was heated at 100 °C for 5 min, cooled to room temperature and ground into powder with a spatula. The vial containing the mixture was taken out of the glove box, purged with CO₂ and equipped with a balloon filled with CO₂ at 185 °C for 2 h. After this time, the reaction mixture was cooled to room temperature, carefully quenched with water (5 mL), acidified to pH 4 with aq. HCl (1 M), and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na₂SO₄, evaporated to dryness and purified by column chromatography (30-50% EtOAc/hexanes) to yield 732 mg (43%) of salicylic acid S2 as a tan solid. DCM (10 mL) was added to an oven-dried vial equipped with a stir bar containing 5-ethyl-2,4-dihydroxy-3-methylbenzoic acid (S2, 400 mg, 2.04 mmol, 1.0 equiv). Oxalyl chloride (192 μL, 2.24 mmol, 1.1 equiv) was slowly added followed by DMF (1 drop, approx 10 μL). The mixture was stirred for 2 h before crotyl alcohol (348 μL, 4.08 mmol, 2.0 equiv) was added dropwise. After 2 h, the reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na₂SO₄, evaporated to dryness and purified by column chromatography (0-15% EtOAc/hexanes) to yield 23 mg (34%) of the crotyl ester as a colorless solid. 

**(E)-But-2-en-1-yl 3-ethyl-2,4-dihydroxy-5-methylbenzoate (30)**

Adapted from a report by Larrosa and coworkers. In the glove box, NaH (60% dispersion in mineral oil, 268 mg, 11.2 mmol, 4.0 equiv) was added to a vial containing 2-ethyl-4-methylbenzene-1,3-diol (380 mg, 2.79 mmol, 1.0 equiv) and 2,4,6-trimethylphenol (425 mg, 2.79 mmol, 1.0 equiv). The resulting mixture was heated at 100 °C for 5 min, cooled to room temperature and ground into powder with a spatula. The vial containing the mixture was taken out of the glove box, purged with CO₂ and equipped with a balloon filled with CO₂ at 185 °C for 2 h. The reaction mixture was cooled to room temperature, carefully quenched with water (5 mL), acidified to pH 4 with aq. HCl (1 M), and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na₂SO₄, evaporated to dryness and purified by column chromatography (30-50% EtOAc/hexanes) to yield 305 mg (56%) of salicylic acid S3 as a tan solid.

3-ethyl-2,4-dihydroxy-5-methylbenzoic acid (S3, 305 mg, 1.55 mmol, 1.0 equiv) was stirred in DCM (9 mL) in a flame-dried round-bottom flask. Oxalyl chloride (147 μL, 1.71 mmol, 1.1 equiv) was slowly added followed by DMF (1 drop, approx 10 μL). The mixture was stirred for 2 h before crotyl alcohol (264 μL, 3.10 mmol, 2.0 equiv) was added dropwise. After 2 h, the reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na₂SO₄, evaporated to dryness, and purified by column chromatography (0-15% EtOAc/hexanes) to yield 94 mg (24%) of the crotyl ester as a colorless solid. 

**H NMR** (600 MHz, CDCl₃) δ 11.07 (s, 1H), 7.49 (s, 1H), 5.86 (m, 1H), 5.68 (m, 1H), 5.23 (s, 1H), 4.72 (d, J = 6.5 Hz, 1H), 2.67 (q, J = 7.5 Hz, 2H), 2.17 (s, 3H), 1.75 (d, J = 6.5 Hz, 3H), 1.14 (t, J = 7.5 Hz, 3H); **C NMR** (150 MHz, CDCl₃) δ 170.3, 159.7, 157.6, 131.8, 128.7, 124.8, 116.4, 114.6, 104.9, 65.5, 17.8, 16.2, 15.3, 13.2; **HRMS** (ESI) m/z calculated for C₁₄H₁₉O₄ [M+H]^+ : 251.1278, found: 251.1276; **IR** (thin film): 3570, 2952, 1746, 1641, 1196 cm⁻¹; **MP:** 89-90 °C.
4-isopentyl-2-methylbenzene-1,3-diol (S5)
1-iodo-2,4-dimethoxy-3-methylbenzene (1.00 g, 3.60 mmol, 1.0 equiv) was added to a flame-dried round-bottom flask and dissolved in THF (25 mL). The mixture was cooled to 0 °C and nBuLi (1.73 mL, 4.32 mmmol, 1.2 equiv, 2.5 M in hexanes) was slowly added. The mixture was stirred at 0 °C for 30 min, before 1-bromo-3-methylbutane (1.29 mL, 10.8 mmol, 3.0 equiv) was added. The mixture was then warmed to rt and stirred for 30 min at which time the reaction mixture was quenched by the slow addition of water and diluted with EtOAc (10 mL). The resulting layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford a brown oil. The crude material was transferred to a flame-dried vial, dissolved in DCM (45 mL) and cooled to -78 °C. BBr₃ (7.20 mL, 7.20 mmol, 1 M in DCM, 2.0 equiv) was added dropwise. The mixture was warmed to rt and stirred for 16 h at which time the reaction mixture was quenched by the slow addition of water. The resulting solution was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford a dark brown solid. Purification on silica gel (100% hexanes to 15% EtOAc in hexanes) afforded 650 mg (93% yield over two steps) of S5 as a tan crystalline solid. ¹H NMR (400 MHz, CDCl₃) δ 6.81 (d, J = 8.1 Hz, 1H), 6.34 (d, J = 8.1 Hz, 1H), 4.76 (s, 1H), 4.72 (s, 1H), 2.51 (m, 2H), 2.14 (s, 3H), 1.58 (m, 1H), 1.46 (m, 2H), 0.94 (d, J = 6.6 Hz, 6H); ¹³C NMR (150 MHz, CDCl₃) δ 152.5, 152.4, 126.9, 126.7, 120.4, 109.9, 107.1, 39.2, 27.7, 27.6, 22.5, 8.2; HRMS (ESI) m/z calculated for C₁₂H₁₉O₂ [M+H]⁺: 195.1385, found: 195.1384; IR (thin film): 3345, 2955, 1600, 1455, 1188 cm⁻¹; MP: 56-58 °C.

4-benzyl-2-methylbenzene-1,3-diol (S6)
1-iodo-2,4-dimethoxy-3-methylbenzene (1.00 g, 3.60 mmol, 1.0 equiv) was added to a flame-dried round-bottom flask and dissolved in THF (25 mL). The mixture was cooled to 0 °C and nBuLi (1.73 mL, 4.32 mmmol, 1.2 equiv, 2.5 M in hexanes) was slowly added. The mixture was stirred at 0 °C for 30 min, before benzylbromide (1.28 mL, 10.8 mmol, 3.0 equiv) was added. The mixture was then warmed to rt and stirred for 30 min at which time the reaction mixture was quenched by the slow addition of water and diluted with EtOAc (10 mL). The resulting layers were separated, and the aqueous layer was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford a brown oil. The crude material was transferred to a flame-dried vial, dissolved in DCM (45 mL) and cooled to -78 °C. BBr₃ (7.20 mL, 7.20 mmol, 1 M in DCM, 2.0 equiv) was added slowly. The mixture was warmed to rt and stirred for 16 h at which time the reaction mixture was quenched by the slow addition of water. The resulting solution was extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford a dark brown solid. Purification on silica gel (100% hexanes to 30% EtOAc in hexanes) afforded 563 mg (73% yield over two steps) of S6 as a tan
crystalline solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 7.25 (m, 5H), 6.85 (d, $J = 8.2$ Hz, 1H), 6.38 (d, $J = 8.2$ Hz, 1H), 3.93 (s, 2H), 2.12 (s, 3H). All values obtained were consistent with literature values.\(^4\)

\[\text{(E)-but-2-en-1-yl 2,4-dihydroxy-5-methyl-3-propylbenzoate (31)}\]
Adapted from a report by Larrosa and coworkers.\(^1\) In the glove box, NaH (60% dispersion in mineral oil, 1.43 g, 37.4 mmol, 4.0 equiv) was added to a vial containing 4-methyl-2-propylbenzene-1,3-diol (1.29 g, 9.34 mmol, 1.0 equiv) and 2,4,6-trimethylphenol (1.27 g, 9.34 mmol, 1.0 equiv). The resulting mixture was heated at 100 °C for 5 min, cooled to room temperature and ground into powder with a spatula. The vial containing the mixture was taken out of the glove box, purged with CO$_2$ and equipped with a balloon filled with CO$_2$ at 185 °C for 2 h. After this time, the reaction mixture was cooled to room temperature, carefully quenched with water (5 mL), acidified to pH 4 with aq. HCl (1 M), and extracted with EtOAc (3 x 20 mL). DCM (2.5 mL) was added to an oven-dried vial equipped with a stir bar containing 2,4-dihydroxy-5-methyl-3-propylbenzoic acid (S12, 92.0 mg, 0.438 mmol, 1.0 equiv). Oxaly chloride (45.0 μL, 0.525 mmol, 1.2 equiv) was slowly added followed by DMF (1 drop, approx 10 μL). The mixture was stirred for 2 h before crotyl alcohol (75.0 μL, 0.876 mmol, 2.0 equiv) was added dropwise. After 2 h, the reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na$_2$SO$_4$, evaporated to dryness and purified by column chromatography (0-15% EtOAc/hexanes) to yield 78 mg (67%) of the crotyl ester as a colorless solid. $^1$H NMR (400 MHz, CDCl$_3$) δ 11.07 (s, 1H), 7.50 (s, 1H), 5.86 (dd, $J = 6.0$ Hz, 1H), 5.70 (m, 1H), 5.26 (s, 1H), 4.73 (d, $J = 5.4$ Hz, 2H), 2.63 (t, $J = 6.2$ Hz, 3H), 2.17 (s, 3H), 1.67 (d, $J = 6.5$ Hz, 2H), 1.58 (m, 2H), 0.98 (t, $J = 6.0$ Hz, 3H); $^{13}$C NMR (100 MHz, CDCl$_3$) δ 170.3, 159.9, 157.9, 131.8, 128.8, 124.9, 114.9, 114.6, 104.9, 65.5, 24.9, 21.9, 17.8, 15.4, 14.1; HRMS (ESI) m/z calculated for C$_{15}$H$_{21}$O$_4$ [M+H]$^+$: 265.1434, found: 265.1433; IR (thin film): 3404, 2952, 1744, 1637, 1196, 1397, 1194 cm$^{-1}$; MP: 93-95 °C.

\[\text{(E)-but-2-en-1-yl 2,4-dihydroxy-5-isopentyl-3-methylbenzoate (32)}\]
Adapted from a report by Larrosa and coworkers.\(^1\) In the glove box, NaH (60% wt/wt dispersion in mineral oil, 381 mg, 9.92 mmol, 4.0 equiv) was added to a vial containing 4-isopentyl-2-methylbenzene-1,3-diol (482 mg, 2.48 mmol, 1.0 equiv) and 2,4,6-trimethylphenol (338 mg, 2.48 mmol, 1.0 equiv). The resulting mixture was heated at 100 °C for 5 min, cooled to room temperature and ground into powder with a spatula. The vial containing the mixture was taken out of the glove box, purged with CO$_2$ and equipped with a balloon filled with CO$_2$ at 185 °C for 2 h. After this time, the reaction mixture was cooled to room temperature, carefully quenched with water (5 mL), acidified to pH 4 with aq. HCl (1 M), and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na$_2$SO$_4$, evaporated to dryness and purified by column chromatography (10-50% EtOAc/hexanes) to yield 228 mg (45%) of salicylic acid S7 as a tan solid. 5 mL DCM was added to an oven-dried vial equipped with a stir bar containing 2,4-dihydroxy-5-isopentyl-3-methylbenzoic acid (S7, 228 mg, 1.11...
mmol, 1.0 equiv). Oxalyl chloride (105 µL, 1.22 mmol, 1.1 equiv) was slowly added followed by DMF (1 drop, approx 10 µL). The mixture was stirred for 2 h before crotyl alcohol (190 µL, 2.22 mmol, 2.0 equiv) was added dropwise. After 2 h, the reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous Na₂SO₄, evaporated to dryness and purified by column chromatography (0-15% EtOAc/hexanes) to yield 201 mg (62%) of the crotyl ester as a colorless solid. ¹H NMR (600 MHz, CDCl₃) δ 11.13 (s, 1H), 7.48 (s, 1H), 5.87 (m, 1H), 5.70 (m, 1H), 5.24 (s, 1H), 4.73 (d, 2H), 2.53 (m, 2H), 2.13 (s, 3H), 1.75 (d, J = 6.6, Hz, 3H), 1.60 (m, 1H), 1.55 (m, 2H), 0.94 (d, J = 6.5 Hz, 6H); ¹³C NMR (150 MHz, CDCl₃) δ 170.3, 159.6, 157.7, 131.7, 127.8, 124.9, 119.9, 110.0, 104.9, 65.6, 39.0, 28.0, 27.6, 22.52, 17.8, 7.7; HRMS (ESI) m/z calculated for C₁₇H₂₅O₄ [M+H]⁺: 293.1747, found: 293.1721; IR (thin film): 3464, 3178, 2963, 1639, 1610, 1449, 1187 cm⁻¹; MP: 52-53 °C.

(E)-but-2-en-1-yl 3-chloro-2,4-dihydroxy-5-methylbenzoate (33)
2 mL MeCN was added to a flame-dried vial containing 2,4-dihydroxy-5-methylbenzoic acid (45.0 mg, 0.268 mmol, 1.0 equiv) and NCS (35.8 mg, 0.268 mmol, 1.0 equiv). The reaction was stirred at 40 °C for 3 h. After this time, the reaction mixture was cooled to room temperature, quenched with water (3 mL), and extracted with EtOAc (3 x 10 mL). The organic layers were dried over anhydrous Na₂SO₄ and concentrated to dryness. 1.00 mL DCM was added to an oven-dried vial equipped with a stir bar containing 3-chloro-2,4-dihydroxy-5-methylbenzoic acid (S10, 65.0 mg, 0.253 mmol, 1.0 equiv). Oxalyl chloride (24 µL, 0.28 mmol, 1.1 equiv) was slowly added followed by DMF (1 drop, approx 10 µL). The mixture was stirred for 2 h before crotyl alcohol (43 µL, 0.51 mmol, 2.0 equiv) was added dropwise. After 2 h, the reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 10 mL). The organic layers were dried over anhydrous Na₂SO₄, evaporated to dryness and purified by column chromatography (0-10% EtOAc/hexanes) to yield 30 mg (46%) of the crotyl ester as a colorless solid. ¹H NMR (400 MHz, CDCl₃) δ 11.41 (s, 1H), 7.55 (s, 1H), 6.14 (s, 1H), 5.88 (dq, J = 13.4, 6.6 Hz, 1H), 5.68 (dt, J = 14.7, 6.6 Hz, 1H), 4.74 (d, J = 6.5 Hz, 2H), 2.20 (s, 3H), 1.76 (d, J = 6.6 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 169.6, 156.7, 155.4, 132.4, 129.4, 124.4, 116.3, 107.1, 105.6, 66.1, 17.8, 15.5; HRMS (ESI) m/z calculated for C₁₂H₁₄ClO₄ [M+H]⁺: 257.0575, found: 257.0575; IR (thin film): 3464, 3176, 2949, 2930, 1641, 1611, 1188 cm⁻¹.

(E)-but-2-en-1-yl 3-bromo-2,4-dihydroxy-5-methylbenzoate (34)
2 mL MeCN was added to a flame-dried vial containing 2,4-dihydroxy-5-methylbenzoic acid (45.0 mg, 0.268 mmol, 1.0 equiv) and NBS (35.8 mg, 0.268 mmol, 1.0 equiv). The reaction was stirred at 40 °C for 3 h. After this time, the reaction mixture was cooled to room temperature, quenched with water (3 mL) then extracted with EtOAc (3 x 10 mL). The combined organic layers were washed with brine (5 mL), dried over anhydrous Na₂SO₄ and concentrated to dryness. 0.50 mL DCM was added to an oven-dried vial equipped with a stir bar containing 3-bromo-2,4-dihydroxy-5-methylbenzoic acid (S9, 31.3 mg, 0.155 mmol, 1.0 equiv). Oxalyl chloride (15 µL, 0.17 mmol, 1.1 equiv) was slowly added followed by DMF (1 drop, approx 10 µL). The mixture was stirred for 2 h before crotyl alcohol (26 µL, 0.31 mmol, 2.0 equiv) was added dropwise. After 2 h, the reaction was quenched by addition of water (5 mL) and extracted with EtOAc (3 x 10 mL). The organic layers were dried over anhydrous
Na₂SO₄, evaporated to dryness and purified by column chromatography (0-15% EtOAc/hexanes) to yield 9.9 mg (21%) of the crotyl ester as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 11.56 (s, 1H), 7.60 (s, 1H), 6.14 (s, 1H), 5.90 (m, 1H), 5.65 (m, 1H), 4.76 (d, J = 6.6 2H), 2.23 (s, 3H), 1.77 (d, J = 6.5 Hz, 3H); ¹³C NMR (150 MHz, CDCl₃) δ 169.6, 157.8, 156.4, 132.4, 130.3, 124.4, 116.8, 105.8, 98.5, 66.1, 17.8, 15.8; HRMS (ESI) m/z calculated for C₁₂H₁₀BrO₄ [M+H]+: 301.0070, found: 301.0072; IR (thin film): 3464, 2957, 2923, 2854, 1739, 1639, 1455 cm⁻¹.

(Ε)-but-2-en-1-yl 5-benzyl-2,4-dihydroxy-3-methylbenzoate (35)
Adapted from a report by Larrosa and coworkers. In the glove box, NaH (60% wt/wt dispersion in mineral oil, 1.43 g, 37.4 mmol, 4.0 equiv) was added to a vial containing 4-benzyl-2-methylbenzene-1,3-diol (1.27 g, 9.34 mmol, 1.0 equiv) and 2,4,6-trimethylphenol (1.27 g, 9.34 mmol, 1.0 equiv). The resulting mixture was heated at 100 °C for 5 min, cooled to room temperature and ground into powder with a spatula. The vial containing the reaction mixture was taken out of the glove box, purged with CO₂ and equipped with a balloon filled with CO₂ at 185 °C for 2 h. After this time, the reaction mixture was cooled to room temperature, carefully quenched with water (5 mL), acidified to pH 4 with aq. HCl (1 M), and extracted with EtOAc (3 x 20 mL). The organic layers were dried over anhydrous MgSO₄, evaporated to dryness and purified by column chromatography (0-15% EtOAc/hexanes) to yield 9.9 mg (34%) of the crotyl ester as a colorless oil. ¹H NMR (400 MHz, CDCl₃) δ 11.18 (s, 1H), 7.58 (s, 1H), 7.20 (m, 5H), 5.86 (m, 1H), 5.70 (m, 1H), 5.12 (d, J = 1.2 Hz, 2H), 4.74 (m, 2H), 3.94 (s, 1H), 2.11 (s, 3H), 1.76 (d, J = 6.2 Hz, 2H); ¹³C NMR (150 MHz, CDCl₃) 170.2, 160.4, 158.1, 139.4, 131.8, 129.1, 128.8, 128.4, 126.6, 124.8, 117.8, 111.0, 105.1, 65.6, 36.4, 17.8, 7.8; HRMS (ESI) m/z calculated for C₁₉H₁₄O₄ [M+H]+: 313.1434, found: 313.1438; IR (thin film): 3546, 2957, 2923, 2854, 1739, 1578, 1564, 1324, 1303, 1244, 116.8, 105.8, 98.5, 66.1, 17.8, 15.8; HRMS (ESI) m/z calculated for C₁₂H₁₀BrO₄ [M+H]+: 301.0070, found: 301.0072; IR (thin film): 3464, 2957, 2923, 2854, 1739, 1639, 1455 cm⁻¹.

4-methyl-2-propylbenzene-1,3-diol (S11)
2,4-dimethoxy-1-methylbenzene (874 mg, 5.74 mmol, 1.0 equiv) was added to a flame-dried round-bottom flask and dissolved in THF (12 mL). The mixture was cooled to 0 °C and nBuLi (3.44 mL, 8.61 mmol, 1.5 equiv, 2.5 M in hexanes) was slowly added. The mixture was stirred at 0 °C for 30 min, before 1-bromopropane (1.60 mL, 17.2 mmol, 3.0 equiv) was added. The mixture was then warmed to rt and stirred for 30 min at which time the reaction mixture was quenched by the slow addition of water (10 mL) and diluted with EtOAc (10 mL). The resulting layers were separated, and the aqueous layer was extracted with EtOAc (3 x 20 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford a brown oil. The crude material was transferred to a flame-dried vial, dissolved in DCM (70 mL) and cooled to -78 °C. BBr₃ (11.5 mL, 11.5 mmol, 1 M in DCM, 2.0 equiv) was added slowly. The mixture was then warmed to rt and stirred for 16 h at which time the reaction mixture was quenched by the slow addition of water.
The resulting solution was extracted with EtOAc (3 x 100 mL). The combined organic layers were washed with brine, dried over anhydrous Na₂SO₄ and concentrated under reduced pressure to afford a dark brown solid. Purification on silica gel (100% hexanes to 30% EtOAc in hexanes) afforded 897 mg (94% yield over two steps) of S11 as a tan crystalline solid. 

\[ \text{Purification on silic} \text{a gel (100% hexanes to 30% EtOAc in hexanes) afforded 897 mg (94% yield over two steps)} \]

\[ \text{S11 as a tan crystalline solid.} \]

\[ \text{1H NMR (400 MHz, CDCl}_3\text{)} \delta 6.81 (d, J = 8.0 \text{ Hz, 1H}), 6.32 (d, J = 8.0 \text{ Hz, 1H}), 4.67 (s, 1H), 4.63 (s, 1H), 2.62 (t, J = 8.4, 2H), 2.17 (s, 3H), 1.59 (h, J = 7.4 \text{ Hz, 2H}), 0.99 (t, J = 7.4 \text{ Hz, 3H}); \]

\[ \text{13C NMR (150 MHz, CDCl}_3\text{)} \delta 152.7, 152.6, 127.8, 115.1, 114.7, 107.1, 25.4, 22.3, 15.5, 14.2; \]

\[ \text{HRMS (ESI) m/z calculated for C}_{10}\text{H}_{15}\text{O}_2 [M+H]^+: 167.1067, found: 167.1066; IR (thin film): 3345, 2955, 1600, 1455, 1188 cm}^{-1}; \]

\[ \text{MP: 95-97 °C.} \]

**General procedure for lead tetraacetate (LTA)-mediated oxidative dearomatization:** Adapted from a protocol developed by Nicolau.\(^2\) Substrate (50 mg, 1 equiv) was dissolved in AcOH and DCM (5:1, 0.04 M). LTA (1.2 equiv) was added and the reaction was allowed to stir at rt for 1 h. The reaction was quenched by addition of water (10 mL) and the mixture was extracted with EtOAc (3 x 15 mL). The combined organic layers were washed with water (1 x 20 mL), brine (1 x 20 mL), dried over sodium sulfate, and concentrated under reduced pressure to afford a yellow oil. Purification on silica gel afforded the O-acylated o-quinol product. Full characterization given for enzymatically generated material.

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**((E))-but-2-en-1-yl 3-acetoxy-6-hydroxy-3,5-dimethyl-4-oxocyclohexa-1,5-diene-1-carboxylate (±37)**

The title compound was prepared according to the general procedure for LTA-mediated oxidative dearomatization. Purification on silica gel (20-40% EtOAc in hexanes) afforded 10 mg (16% yield) of the o-quinol as a yellow oil. 

\[ \text{1H NMR (400 MHz, CDCl}_3\text{)} \delta 10.82 (s, 1H), 7.38 (s, 1H), 5.90 (dd, J = 15.0, 7.0 \text{ Hz, 1H}), 5.66 (m, 1H), 4.71 (d, J = 6.8 \text{ Hz, 2H}), 2.12 (s, 3H), 1.87 (s, 3H), 1.77 (d, J = 6.4 \text{ Hz, 3H}), 1.46 (s, 3H). \]

**((E))-but-2-en-1-yl 3-acetoxy-3-ethyl-6-hydroxy-5-methyl-4-oxocyclohexa-1,5-diene-1-carboxylate (±39)**

The title compound was prepared according to the general procedure for LTA-mediated oxidative dearomatization. Purification on silica gel (20-40% EtOAc in hexanes) afforded 4.9 mg (8.0% yield) of the o-quinol as a yellow oil. 

\[ \text{1H NMR (400 MHz, CDCl}_3\text{)} \delta 10.78 (s, 1H), 7.37 (s, 1H), 5.90 (dd, J = 15.0, 7.0 \text{ Hz, 1H}), 5.63 (m, 1H), 4.71 (d, J = 6.9 \text{ Hz, 2H}), 2.12 (s, 3H), 1.85 (s, 3H), 1.84 (m, 2H), 1.76 (d, J = 6.7 \text{ Hz, 3H}), 0.91 (t, J = 7.5 \text{ Hz, 3H}). \]
The title compound was prepared according to the general procedure for LTA-mediated oxidative dearomatization. Purification on silica gel (20-40% EtOAc in hexanes) afforded 1.8 mg (3.0% yield) of the \( \text{o}-\)quinol as a yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 10.77 (s, 1H), 7.37 (s, 1H), 5.89 (m, 1H), 5.64 (m, 1H), 4.71 (d, \( J = 3.5 \) Hz, 2H), 2.41 (q, \( J = 6.7 \) Hz, 2H), 2.12 (s, 3H), 1.77 (d, \( J = 6.5 \) Hz, 3H), 1.45 (s, 3H), 1.00 (t, \( J = 7.4 \) Hz, 3H).

The title compound was prepared according to the general procedure for LTA-mediated oxidative dearomatization. Purification on silica gel (20-40% EtOAc in hexanes) afforded 10 mg (16% yield) of the \( \text{o}-\)quinol as a yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 10.75 (s, 1H), 7.36 (s, 1H), 5.88 (m, 1H), 5.60 (m, 1H), 4.70 (t, \( J = 7.4 \) Hz, 2H), 2.36 (m, 2H), 2.10 (s, 3H), 1.75 (d, \( J = 6.7 \) Hz, 3H), 1.43 (m, 5H), 0.88 (t, \( J = 7.1 \) Hz, 3H).

The title compound was prepared according to the general procedure for LTA-mediated oxidative dearomatization. Purification on silica gel (20-40% EtOAc in hexanes) afforded 3.3 mg (6% yield) of the \( \text{o}-\)quinol as a yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \( \delta \) 10.96 (s, 1H), 5.87 (m, 1H), 5.64 (dt, \( J = 15.1, 7.0 \) Hz, 1H), 4.74 (t, \( J = 6.7 \) Hz, 2H), 2.20 (s, 3H), 2.14 (s, 3H), 1.85 (s, 3H), 1.75 (d, \( J = 6.5 \) Hz, 3H), 1.44 (s, 3H).
Part II. Plasmids and proteins

Plasmids: A gene encoding sorbC (XP_002567552) was codon-optimized for overexpression in *E. coli* and synthesized by GeneArt (Thermofisher). The synthesized sequence was cloned by GeneArt into pET151 vectors conferring the T7 expression system, ampicillin resistance, and N-terminal 6×His-tag encoded upstream of the insert gene.

Codon-Optimized *sorbC* Sequence

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ATGACCCGTAGCCCAAATAGCCCTTTGAAATTGGTGTGATTACCAGGTCTGGCACTGG
CACGATGCTCTGAAACATGACGTGGCATTGTGTTCTGGCAGATTTAAGGTTGTTTAAAG
GCACGATCCGGCTAGCTACCCAGCAGCGGTGGTGGTACGCTGCAACCGCGAGCG
CGTGGTCTGGCTGAAGCACCGAATGTTAGCGGTATTGTTGCAAGCTTATAGCGAAGT
TCGTGTTATGAACGCAGCAGTGGCTGGTGCGTAGCAGCCGTACGTACAGGTGAACT
GTGCACCTGGAAAGAATGGCCTGAA
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SorbC Protein Sequence

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MTRSANSPFEVAIVGGGITGLALAVGLLKRNVSFTIYERAENFGELGVGITFTPNAQRAMEALDPCVLQSFTNV
ASAPSGGTINFVDGVREQGSEDPRSTAAALFQLHVKGYYKACRRCDVFQIVQHIPDCVQYRKLWLSIETD
HESGRAVLKFRDEGIAVIVGIDQVRASMFPGTDELCPRAQYSHQLGNYMRGLMVPAQTAVLGPETS
SAVLHPTGAFVLTIPALEVHMIEAFIMDKEEPEVQTSSTDRTKSLYVLPATRNATKAFÆGPFTVRSASMF
PEKLEKWAFDMLEAPVFTAKFRGVMRAHASTPNQGGGAFGIEEDAVLAEVLAELAEAPNVNGIVASE
ALAVYSEVRYESQWLVRSSRTGERLCTWKDRDWGLAAEELSRISRSHQLWDHTAGMVSDALAILGERV
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Protein overexpression and purification: A plasmid containing *sorbC* was transformed using standard heat-shock protocols into chemically competent *E. coli* BL21(DE3) cells. Overexpression of SorbC was achieved in 500 mL Terrific Broth (TB) (4% glycerol (v/v)) in 2.8 L flasks. 500 mL portions of media were inoculated with 5 mL overnight culture prepared from a single colony in Luria Broth (LB) and 100 μg/mL ampicillin (Gold Biotechnology). Cultures were grown at 37 °C and 250 rpm until the optical density at 600 nm reached 0.8. The cultures were then cooled to 20 °C for 1 h and protein expression was induced with 0.1 mM isopropyl-β-D-1-thiogalactopyranoside (IPTG, Gold Biotechnology). Expression continued at 20 °C overnight (approx. 18 h) at 200 rpm. The typical yield of cell pellet for one 500 mL culture was ~25 g.

General purification procedure: 25–30 g of cell pellet was resuspended in 100 mL of lysis buffer containing 50 mM Tris HCl pH 7.4, 300 mM NaCl, 10 mM imidazole, and 10% glycerol. Approximately 1 mg/mL lysozyme was added prior to incubating on a rocker at 4 °C for 4 h. Cells were lysed by passing the total cell lysate
through an Avestin pressure homogenizer at 15000 psi. The total lysate was centrifuged at 40,000 x g for 30 min and the supernatant was filtered through 0.45 µm syringe filters. The crude cell lysate was loaded onto a 5 mL HisTrap HP column (General Electric) on an AKTA Pure FPLC system (General Electric) at a flow rate of 2.5 mL/min. Buffer A = the lysis buffer listed above, and Buffer B = 50 mM Tris HCl pH 7.4, 300 mM NaCl, 10% glycerol, and 400 mM imidazole. The column was washed with 25 mM imidazole (6.3% Buffer B) for 6 CV and eluted in a gradient to 100% Buffer B over 8 CV. Fractions containing SorbC were visibly yellow and pooled for desalting by dialysis. SorbC was dialyzed into 50 mM Tris HCl pH 7.4 and 10% glycerol buffer. Average yields of 100 mg of SorbC were obtained from 1 L fermentations of the expression strain. Molecular weights including the 6xHis-tags for SorbC was estimated by the ProtParam tool on the Expasy server to be 49.2 kDa. This molecular weight is consistent with the protein band observed by SDS-PAGE analysis (Figure S1). The purified protein (typically 62 µM) was aliquoted into 0.6 mL tubes and frozen in liquid nitrogen for long-term storage at -80 °C.

**Supplementary Figure S1.** SDS-PAGE gel of SorbC. Lane 1 ladder, lane 2 crude lysate, lane 3 insoluble fraction, lane 4 purified protein.

**Determination of flavin incorporation and extinction coefficients:** Samples of protein were diluted to 10 µM in 1 mL using storage buffer for UV-vis analysis using a disposable poly(methyl 2-methylpropenoate) cuvette. The absorbance spectrum for the protein was taken from 300 nm to 700 nm in 2 nm increments (blue trace in Figure S2). A 20 µL aliquot of fresh 10% sodium dodecyl sulfate (w/v) was added to each 1 mL solution and mixed. Samples were incubated at room temperature for 10 min before reading the absorbance spectra again under the same conditions (red trace in Figure S2). The absorbance at 450 nm for the denatured protein and the extinction coefficient of free FAD (11300 M⁻¹ cm⁻¹) was used to calculate the concentration of FAD in protein using Beer’s law. The typical FAD incorporation was 89% for SorbC. The extinction coefficient was calculated using the concentration of free flavin obtained and the absorbance at 450 nm of SorbC. At 450 nm, the extinction coefficients of SorbC is 11970 M⁻¹ cm⁻¹.

**Supplementary Figure S2.** Native enzyme absorbance spectra compared to denatured protein absorbance spectra exposing free FAD to solution.
Binding titration of SorbC with substrates. All spectral binding measurements were carried out on a UV-2501PC spectrophotometer (Shimazu). Spectral binding titrations of substrates were performed at 4 °C in 50 mM phosphate buffer pH 8.0 using 1 mL volume samples containing 20 µM enzyme in a 1 cm pathlength cuvette. Substrate stock solutions were made at 3-14 mM in 100 mM phosphate buffer pH 8.0. Titrations were performed using stepwise additions of the substrate. After each addition of substrate, the cuvette was sealed with a Teflon stopper and slowly inverted 5 times to allow for thorough mixing. UV-Vis spectra (300–700 nm) were recorded for the ligand-free enzyme and following each addition of substrate. Substrate additions were continued until no further shifts in the spectrum occurred or 500 µM substrate was reached. A turbidity correction was applied using the Rayleigh scattering equation, eq. 1.

\[ \text{scatter} = \frac{1}{\lambda^4} + \text{constant} \]

A difference spectrum was generated at each stage in the titration by subtraction of the ligand-free enzyme spectrum from each ligand-bound form of substrate [Substrate:FAD-dependent monooxygenase] spectrum produced. The wavelengths of the absorbance minimum and maximum were identified from the difference spectra. A maximal induced absorbance change (ΔA\text{max}) at each point in the titration was determined by subtracting the absorbance at the wavelength minimum from the absorbance maximum. The same wavelength pair was used for each concentration of substrate. ΔA\text{max} values were plotted against ligand concentration, and data were fitted using a cooperative binding (The Hill equation) function, to determine dissociation constants (K\text{d} values), as described previously. All data fitting was done using GraphPad Prism software.

Supplementary Figure S3. (A) Binding titration of SorbC with 13. Colored lines show spectra collected after stepwise additions of substrate 13. (B) The plot of substrate-induced absorbance difference against the relevant concentration of substrate (13) at 380 nm. At higher concentrations of substrate, absorption due to substrate became too intense to distinguish changes in flavin absorbance.
Supplementary Figure S4. Binding titration of SorbC (A) Binding titration of SorbC with Fig. 2C, Entry 2. Colored lines show spectra collected after stepwise additions of substrate Fig. 2C, Entry 2. (B) The substrate-induced absorbance difference against the relevant concentration of substrate (Fig. 2C, Entry 2) at 450 nm.
Supplementary Figure S5. (A) Binding titration of SorbC with 26. Colored lines show spectra collected after stepwise additions of substrate 26. (B) The substrate-induced absorbance difference against the relevant concentration of substrate 26 at 450 nm.

Supplementary Figure S6. (A) Binding titration of SorbC with 27. Colored lines show spectra collected after stepwise additions of substrate 27. (B) The substrate-induced absorbance difference against the relevant concentration of substrate 27 at 450 nm.
Part III. Biocatalytic Reactions

**Stock solutions:** Stock solutions of each substrate (50 mM) were prepared by dissolving the substrate in DMSO (analytical grade). Stock solutions of NADP+ (100 mM) and glucose-6-phosphate (G6P, 500 mM) were stored at -20 °C. Aliquots of SorbC (62 µM) and glucose-6-phosphate dehydrogenase (G6PDH, 100 U/mL) were stored at -80 °C. Analytical-scale reactions: Each reaction contained 25 µL 100 mM potassium phosphate buffer, pH 8.0, 2.5 mM substrate (2.5 µL of a 50 mM stock solution in DMSO), 2.5 µM SorbC, 5 mM G6P (0.5 µL, 500 mM), 1 mM NADP+ (0.5 µL, 100 mM), 1 U/mL G6P-DH (0.5 µL, 100 U/mL), and Milli-Q water to a final volume of 50 µL. The reaction was carried out at 30 °C for 1 h and quenched by addition of 75 µL acetonitrile with 25 mM pentamethylbenzene as an internal standard. Precipitated biomolecules were pelleted by centrifugation (16,000 x g, 12 min). The supernatant was analyzed by UPLC-DAD and conversion obtained by comparison to calibration curves of each substrate. Determination of total turnover number (TTN): Total turnover number was determined by analyzing (# of moles of starting material consumed)/(# of moles of enzyme) under the following conditions: 2.5 µM SorbC, 2.5 mM substrate, 1 mM NADP+, 0.05 U G6PDH, and 5 mM G6P for NADPH regeneration in 50 µL of reaction buffer (50 mM potassium phosphate, pH 8.0). The reaction was carried out at 30 °C for 1 h and quenched by addition of 75 µL acetonitrile with 2.5 mM pentamethylbenzene as an internal standard. Precipitated biomolecules were pelleted by centrifugation (16,000 x g, 12 min). The subsequent liquid chromatography PDA spectrometry (UPLC) analysis was performed on a Waters Aquity H-Class UPLC-PDA using a Phenomenex Kinetex 1.7 µm C18, 2.1x150 mm column under the following conditions: Method A: mobile phase (A = deionized water + 0.1% formic acid, B = acetonitrile + 0.1% formic acid), 5% to 100% B over 1.5 min, 100% B for 1.0 min; flow rate, 0.5 mL/min; Method B: mobile phase (A = deionized water + 0.1% formic acid, B = acetonitrile + 0.1% formic acid), 5% to 100% B over 2 min, 100% B for 1 min; flow rate, 0.5 mL/min. Based on calibration curves of the starting materials, the percent conversion of the substrate to dearomatized product was calculated with AUCsubstrate/AUCinternal standard at 270 nm. All reactions were performed and analyzed in triplicate.

**General procedure for in vitro milligram-scale reactions:** Preparative–scale enzymatic reactions were conducted on 10 mg of each substrate under the following conditions: 2.5 µM SorbC, 2.5 mM substrate, 1 mM NADP+, 1 U/mL G6PDH, and 5 mM G6P for NADPH generation in reaction buffer (50 mM potassium phosphate buffer, pH 8.0). The reaction mixture was added to a crystallizing dish and incubated at 30 °C for 2 h. The increased surface area afforded by the crystallizing dish as reaction vessel allowed for higher conversions with some substrates due to increased oxygen concentration. After 2 h, a 50 µL aliquot was removed and processed in an identical manner to the analytical-scale reactions described above to determine substrate conversion. The remaining reaction mixture was diluted with acetonitrile (2 x total reaction volume). Precipitated biomolecules were pelleted by centrifugation (4,000 x g, 12 min). The reaction mixture was cooled to 0 °C and pyridine (14.0 µL, 0.173 mmol) was added followed by Ac2O (10.4 µL, 0.110 mmol). The resulting mixture was stirred at rt for 2 h. The reaction was then acidified to pH 2 and the mixture was extracted with EtOAc (3 x 70 mL). The combined organic layers were washed with brine (1 x 50 mL), dried over sodium sulfate and concentrated under reduced pressure. Purification on silica gel (30% hexanes to 50% EtOAc in hexanes) afforded the acylated o-quinol products.

**Steady-state kinetics of SorbC with 13 and 27.** To determine the time at which initial rates could be recorded, duplicate 50 µL reactions with 10 µM 13, or 27 were performed and analyzed compared to an internal standard (300 µM) by UPLC. The resulting timecourse demonstrated that 1 min is the optimal time to capture the initial reaction rates for a variety of concentrations of 13 or 27.

To determine the steady-state kinetic parameters of the SorbC with sorbicillin (13) reactions were conducted on 50 µL scale with substrate ranging 10 µM – 200 µM in duplicate with 12.5 nM SorbC and 50 mM KP, pH 8.0 buffer in a 96-well plate. Reactions were initiated by addition of 500 µM NADH (10 µL distributed by multichannel pipette). Reactions were quenched after 30 s by addition of 100 µL acetonitrile. 96-well plates were centrifuged at 2000 x g for 2 min, then 100 µL of the centrifuged mixture was added to 100 µL of a dilution mix containing 450 µM in acetonitrile in a clean 96-well 0.22 µm filter plate. The plate was centrifuged over a clean 96-well plate at 2000 x g for 2 min. 2 µL each sample was injected on the TOF LC/MS. The resulting Michaelis-Menten curves
for each substrate and standard curves used to quantify substrate are shown in Fig S7-S8.

**Steady-state kinetics of SorbC with sorbicillin (13) and crotyl ester 27.** SorbC was prepared to a final concentration of 120 nM. To determine the steady-state kinetic parameters of SorbC with sorbicillin (13), reactions were conducted on 50 µL scale, in duplicate, under the following conditions: 10–250 µM substrate, 120 nM SorbC, 50 mM KP, pH 8.0 buffer in a 96-well plate. For crotyl ester 27, the substrate range was 10 µM–150 µM with all other reaction components the same as the sorbicillin (13) reactions. Reactions were initiated by the addition of 500 µM NADH (10 µL total volume added by multichannel pipette). Reactions were quenched after 1 min by addition of 150 µL acetonitrile containing 300 µM trimethoxy benzene as internal standard. 96-well plates were centrifuged at 2000 x g for 2 min, then 100 µL of the centrifuged mixture was added to a 96-well 0.22 µm filter plate. The plate was centrifuged over a 96-well plate at 2000 x g for 2 min. Product standards were prepared in the same manner. Samples were analyzed by TOF LC/MS. The resulting Michaelis-Menten curves for each substrate are shown in Fig S8 and S9.

**Supplementary Figure S7.** A) Michaelis-Menten plot of SorbC reactions with native substrate sorbicillin (13). B) Standard curve of sorbicillin (13).
**Supplementary Figure S8.** A) Michaelis-Menten plots of SorbC reactions with substrate 27. B) Standard curve substrate 27.

(E)-but-2-en-1-yl (S)-3-acetoxyl-6-hydroxy-3,5-dimethyl-4-oxocyclohexa-1,5-diene-1-carboxylate (37)
The title compound was prepared according to the general procedure for milligram-scale in vitro enzymatic oxidative dearomatization. Purification on silica gel (20-40% EtOAc in hexanes) afforded 1.0 mg (8.0% yield) of the o-quinol as a yellow oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.82 (s, 1H), 7.38 (s, 1H), 5.90 (dd, $J$ = 15.0, 7.0 Hz, 1H), 5.66 (m, 1H), 4.71 (d, $J$ = 6.8 Hz, 2H), 2.12 (s, 3H), 1.87 (s, 3H), 1.77 (d, $J$ = 6.4 Hz, 3H), 1.46 (s, 3H); $^{13}$C NMR (150 MHz, CDCl$_3$) δ 195.4, 169.8, 167.4, 160.3, 151.9, 133.9, 123.5, 118.7, 111.7, 78.0, 67.3, 23.9, 20.4, 17.8, 7.3; HRMS (ESI) m/z calculated for C$_{15}$H$_{19}$O$_6$ [M+H]$^+$: 295.1176, found: 295.1180; IR (thin film): 3426, 2923, 1736, 1699, 1659, 1396, 1194 cm$^{-1}$; $[\alpha]_D^{25}$ +65 (c 0.12, MeCN).

(E)-but-2-en-1-yl (S)-3-acetoxyl-6-hydroxy-2,3,5-trimethyl-4-oxocyclohexa-1,5-diene-1-carboxylate (38)
The title compound was prepared according to the general procedure for milligram-scale in vitro enzymatic oxidative dearomatization. Purification on silica gel (20-40% EtOAc in hexanes) afforded 8.0 mg (59% yield) of the o-quinol as a yellow oil. $^1$H NMR (400 MHz, CDCl$_3$) δ 10.96 (s, 1H), 5.87 (m, 1H), 5.64 (dt, $J$ = 15.1, 7.0 Hz, 1H), 4.74 (t, $J$ = 6.7 Hz, 2H), 2.20 (s, 3H), 2.14 (s, 3H), 1.85 (s, 3H), 1.75 (d, $J$ = 6.5 Hz, 3H), 1.44 (s, 3H); $^{13}$C NMR (150 MHz, CDCl$_3$) δ 195.2, 169.7, 169.1, 164.4, 161.4, 133.9, 123.5, 116.3, 109.7, 81.9, 67.4, 24.9, 20.5, 17.8, 17.1, 7.4; HRMS (ESI) m/z calculated for C$_{16}$H$_{21}$O$_6$ [M+H]$^+$: 309.1333, found: 309.1332; IR (thin film): 3165, 2963, 1737, 1698, 1654, 1396, 1235 cm$^{-1}$; $[\alpha]_D^{25}$ +27 (c 0.06, MeCN).
(E)-but-2-en-1-yl 3-acetoxy-3-ethyl-6-hydroxy-5-methyl-4-oxocyclohexa-1,5-diene-1-carboxylate (39)

The title compound was prepared according to the general procedure for milligram oxidative dearomatization. Purification on silica gel (20-40% EtOAc in hexanes) afforded 1.0 mg (8.0% yield) of the o-quinol as a yellow oil. ^1H NMR (400 MHz, CDCl₃) δ 10.78 (s, 1H), 7.37 (s, 1H), 5.88 (dd, J = 14.9, 6.9 Hz, 1H), 5.63 (m, 1H), 4.71 (d, J = 6.9 Hz, 2H), 2.12 (s, 3H), 1.85 (s, 3H), 1.84 (m, 2H), 1.76 (d, J = 6.7 Hz, 3H), 0.91 (t, J = 7.5 Hz, 3H); ^13C NMR (150 MHz, CDCl₃) δ 195.4, 169.9, 167.3, 160.3, 151.3, 133.8, 123.5, 119.6, 112.4, 80.9, 67.3, 32.0, 20.5, 17.8, 7.5, 7.1; HRMS (ESI) m/z calculated for C₁₆H₂₁O₆ [M+H]^+: 309.1333, found: 309.1335; IR (thin film): 3165, 2963, 1738, 1697, 1654, 1396, 1233 cm⁻¹; [α]_D25 +57 (c 0.08, MeCN).

(E)-but-2-en-1-yl 3-acetoxy-5-ethyl-6-hydroxy-3-methyl-4-oxocyclohexa-1,5-diene-1-carboxylate (40)

The title compound was prepared according to the general procedure for milligram oxidative dearomatization. Purification on silica gel (20-40% EtOAc in hexanes) afforded 3.8 mg (28% yield) of the o-quinol as a yellow oil. ^1H NMR (400 MHz, CDCl₃) δ 10.77 (s, 1H), 7.37 (s, 1H), 5.89 (m, 1H), 5.64 (m, 1H), 4.71 (d, J = 3.5 Hz, 2H), 2.41 (q, J = 6.7 Hz, 2H), 2.12 (s, 3H), 1.77 (d, J = 6.5 Hz, 3H), 1.45 (s, 3H), 1.00 (t, J = 7.4 Hz, 3H); ^13C NMR (150 MHz, CDCl₃) δ 194.8, 169.8, 167.5, 159.9, 152.0, 133.8, 123.5, 118.8, 117.7, 78.0, 67.3, 23.8, 20.5, 17.8, 15.5, 12.6; HRMS (ESI) m/z calculated for C₁₆H₂₀O₆ [M+H]^+: 309.1333, found: 309.1337; IR (thin film): 3162, 2928, 1738, 1697, 1652, 1369, 1235 cm⁻¹; [α]_D25 +41 (c 0.06, MeCN).

(E)-but-2-en-1-yl (S)-3-acetoxy-6-hydroxy-3-methyl-4-oxo-5-propylcyclohexa-1,5-diene-1-carboxylate (41)

The title compound was prepared according to the general procedure for milligram-scale in vitro enzymatic oxidative dearomatization. Purification on silica gel (20-40% EtOAc in hexanes) afforded 4.1 mg (35% yield) of the o-quinol as a yellow oil. ^1H NMR (400 MHz, CDCl₃) δ 10.75 (s, 1H), 7.36 (s, 1H), 5.88 (m, 1H), 5.60 (m, 1H), 4.70 (t, J = 7.4 Hz, 2H), 2.36 (m, 2H), 2.10 (s, 3H), 1.75 (d, J = 6.7 Hz, 3H), 1.43 (m, 5H), 0.88 (t, J = 7.1 Hz, 3H); ^13C NMR (150 MHz, CDCl₃) δ 195.0, 169.8, 167.5, 160.2, 152.0, 133.8, 123.5, 118.7, 116.2, 78.0, 67.3, 24.0, 23.9, 21.3, 20.4, 17.8, 13.9; HRMS (ESI) m/z calculated for C₁₇H₂₂O₆ [M+H]^+: 323.1489, found: 323.1494; IR (thin film): 3167, 2960, 2925, 1738, 1697, 1654, 1369, 1236 cm⁻¹; [α]_D25 +45 (c 0.06, MeCN).
(S)-3-acetoxy-6-hydroxy-3,5-dimethyl-4-oxocyclohexa-1,5-diene-1-carboxylic acid (42)

o-quinol 29 (37.3 mg, 0.127 mmol, 1.0 equiv) and Pd(PPh$_3$)$_4$ (10.3 mg, 0.07 equiv, 0.0088 mmol) were added to a flame-dried vial equipped with a stir bar and dissolved in anhydrous THF (2.5 mL). Morpholine (33.0 µL, 0.381 mmol, 3.0 equiv) is then added and the reaction is stirred at rt. After 2 h, the reaction was quenched by addition of 1 M HCl, aq (1 mL) and extracted with EtOAc (3 x 5 mL). The organic layers were dried over anhydrous Na$_2$SO$_4$, evaporated to dryness and purified by column chromatography (30-100% EtOAc/hexanes) to yield 18 mg (60%) of the acid as a colorless solid. **1H NMR** (600 MHz, CD$_3$OD) δ 7.55 (s, 1H), 2.07 (s, 3H), 1.78 (s, 3H), 1.44 (s, 3H); **13C NMR** (300 MHz, CD$_3$OD) δ 169.3, 152.5, 119.3, 110.0, 77.4, 56.0, 22.9, 18.8, 5.77; **HRMS (ESI) m/z** calculated for C$_{11}$H$_{13}$O$_6$ [M+H]$^+$: 241.0707, found: 241.0706; **IR** (thin film): 3263, 2945, 1710, 1650, 1614, 1347 cm$^{-1}$; **MP**: 87 – 90 °C.

(E)-but-2-en-1-yl,(4S,5S,7R,9S)-9-hydroxy-7,9-dimethyl-1,3,6,8-tetraoxooctahydro-1H-4,7-ethanoisoindole-5-carboxylate (45)

The title compound was prepared according to the general procedure for milligram-scale in vitro enzymatic oxidative dearomatization, followed by addition of maleimde (25 mM in DMSO, 5 equiv). Purification on silica gel (20-100% EtOAc in hexanes) afforded 10.3 mg (23% yield) of the cycloadduct as a yellow oil. **1H NMR** (600 MHz, CDCl$_3$) δ 11.60 (s, 1H), 8.20 (s, 1H), 5.83 (dq, $J = 13.4, 6.5$ Hz, 1H), 5.59 (dd, $J = 14.7, 7.4$ Hz, 1H), 4.67 (dd, $J = 12.6, 6.4$ Hz, 1H), 4.60 (m, 1H), 3.87 (d, $J = 3.3$ Hz, 1H), 3.76 (dd, $J = 8.5, 3.2$ Hz, 1H), 2.94 (d, $J = 8.4$ Hz, 1H), 1.74 (d, $J = 6.6$ Hz, 3H), 1.58 (s, 3H), 1.31 (s, 3H); **13C NMR** (300 MHz, CDCl$_3$) δ 207.8, 176.6, 173.7, 168.6, 168.5, 132.0, 124.2, 100.0, 71.9, 65.9, 54.2, 47.0, 42.1, 41.3, 24.5, 17.8, 9.5. **HRMS (ESI) m/z** calculated for [M+Na]$^+$ C$_{17}$H$_{19}$NO$_7$Na [M+Na]$^+$: 372.1054, found: 372.1048; **IR** (thin film): 2928, 1737, 1705, 1622, 1412, 1378 cm$^{-1}$; [α]$_D^{25}$ +62 (c 0.1, MeCN).
Part V. Substrate Calibration Curves.

Entry 3

\[ Y = 16.86X + 1.475 \quad 0.9827 \]

Entry 6

\[ Y = 5.866X + 0.4234 \quad 0.9967 \]

Entry 8

\[ Y = 20.57X + 0.4554 \quad 0.9991 \]

Entry 9

\[ Y = 12.72X - 0.04262 \quad 0.9989 \]

Entry 10

\[ Y = 7.615X + 0.2091 \quad 0.9993 \]

Entry 27

\[ Y = 15.25X + 0.9857 \quad 0.9911 \]

Entry 28

\[ Y = 11.15X + 0.1876 \quad 0.9998 \]

Entry 29

\[ Y = 18.37X + 0.3762 \quad 0.9996 \]
30

\[ Y = 14.69X + 0.12 \]

31

\[ Y = 12.12X + 0.3368 \]

0.9995

32

\[ Y = 12.42X + 0.2258 \]

0.9999

33

\[ Y = 15.82X + 0.4901 \]

0.999

34

\[ Y = 16.31X + 0.2928 \]

0.9998

35

\[ Y = 14.06X + 0.2799 \]

0.9998
Part VI. UPLC Traces of Biocatalytic Reactions.

**Abbreviations:** NEC = no enzyme control, P = product, SM = starting material, IS = internal standard.

**Supplementary Figure S9.** UPLC-PDA trace of SorbC reactions with Entry 3 and NEC.

Supplementary Figure S9: UPLC-PDA trace of SorbC reactions with Entry 3 and NEC.

at 270 nm

| Retention Time | Area   | % Area | Height |
|----------------|--------|--------|--------|
| 1              | 2.506  | 11046  | 4.40   | 5286   |
| 2              | 2.681  | 132681 | 52.79  | 102077 |
| 3              | 2.950  | 107590 | 42.81  | 72312  |

NEC Table 1 Entry 3 at 270 nm

| Retention Time | Area   | % Area | Height |
|----------------|--------|--------|--------|
| 1              | 2.686  | 159130 | 59.40  | 127839 |
| 2              | 2.955  | 108773 | 40.60  | 74266  |
Supplementary Figure S10. UPLC-PDA trace of SorbC reactions with Fig 2C, Entry 6 and NEC. Impurities in the starting material denoted with a #.

SorbC Fig 2C, Entry 6 at 270 nm

| Retention Time | Area     | % Area | Height  |
|----------------|----------|--------|---------|
| 1              | 2.291    | 164448 | 42.80   |
| 2              | 2.577    | 109187 | 28.42   |
| 3              | 2.951    | 110552 | 28.78   |

NEC Fig 2C, Entry 6 at 270 nm

| Retention Time | Area     | % Area | Height  |
|----------------|----------|--------|---------|
| 1              | 2.516    | 707887 | 87.49   |
| 2              | 2.955    | 101262 | 12.51   |
Supplementary Figure S11. UPLC-PDA trace of SorbC reactions with 27 and NEC. Byproduct annotated with an asterisk. See Supplementary Figure S2 for discussion of byproduct.

SorbC and 27 at 240 nm

| Retention Time | Area     | % Area | Height    |
|----------------|----------|--------|-----------|
| 1              | 2.374    | 284975 | 16.57     |
| 2              | 2.706    | 423835 | 24.64     |
| 3              | 2.984    | 1011156| 58.79     |

SorbC and 27 at 270 nm

| Retention Time | Area     | % Area | Height    |
|----------------|----------|--------|-----------|
| 1              | 2.683    | 1423901| 94.40     |
| 2              | 2.951    | 84541  | 5.60      |
Supplementary Figure S12. UPLC-PDA trace of SorbC reactions with Fig 2C, Entry 8 and NEC. Byproduct annotated with an asterisk.

SorbC Fig 2C, Entry 8 at 240 nm

| Retention Time | Area  | % Area | Height  |
|----------------|-------|--------|---------|
| 1              | 2.440 | 205923 | 12.80   |
| 2              | 2.765 | 326172 | 20.27   |
| 3              | 2.954 | 1076817| 66.93   |

SorbC Fig 2C, Entry 8 at 270 nm

| Retention Time | Area  | % Area | Height  |
|----------------|-------|--------|---------|
| 1              | 2.503 | 210.9  |         |
| 2              | 2.625 | 316.5  |         |
| 3              | 2.835 | 264.7  |         |

NEC Fig 2C, Entry 8

| Retention Time | Area  | % Area | Height  |
|----------------|-------|--------|---------|
| 1              | 2.839 | 1986512| 94.81   |
| 2              | 2.956 | 108837 | 5.19    |
Supplementary Figure S13. UPLC-PDA trace of SorbC reactions with Fig 2C, Entry 9 and NEC. Byproduct annotated with an asterisk.

SorbC Fig 2C, Entry 9 at 240 nm

| Retention Time | Area   | % Area | Height |
|----------------|--------|--------|--------|
| 1              | 2.413  | 301350 | 19.65  |
| 2              | 2.752  | 220513 | 14.38  |
| 3              | 2.954  | 1011612| 65.97  |

SorbC Fig 2C, Entry 9 at 270 nm

NEC Fig 2C, Entry 9

| Retention Time | Area   | % Area | Height |
|----------------|--------|--------|--------|
| 1              | 2.752  | 988494 | 90.71  |
| 2              | 2.955  | 101253 | 9.29   |
Supplementary Figure S14. UPLC-PDA trace of SorbC reactions with Fig 2C, Entry 10 and NEC. Byproduct annotated by an asterisk.

\[
\begin{align*}
\text{SorbC} & \quad \text{and} \quad \text{Fig 2C, Entry 10 at 240 nm} \\
\text{SorbC} & \quad \text{and} \quad \text{Fig 2C, Entry 10 at 270 nm} \\
\text{NEC} & \quad \text{Fig 2C, Entry 10 at 270 nm}
\end{align*}
\]

| Retention Time | Area  | % Area | Height  |
|----------------|-------|--------|---------|
| 1              | 2.441 | 77178  | 10.25   | 58829   |
| 2              | 2.558 | 230373 | 30.59   | 158864  |
| 3              | 2.763 | 344260 | 45.71   | 261422  |
| 4              | 2.952 | 101360 | 13.46   | 69715   |

\[
\begin{align*}
\text{Retention Time} & \quad \text{Area}  \\
1 & \quad 2.767  \\
2 & \quad 2.956
\end{align*}
\]

\[
\begin{align*}
\text{Retention Time} & \quad \text{Area}  \\
1 & \quad 2.767  \\
2 & \quad 2.956
\end{align*}
\]
Supplementary Figure S15. UPLC-PDA trace of SorbC reactions with 28 and NEC. Byproduct annotated with an asterisk.

**SorbC and 28 at 240 nm**

| Retention Time | Area     | % Area | Height   |
|----------------|----------|--------|----------|
| 1              | 2.560    | 113288 | 15.76    |
| 2              | 2.758    | 437320 | 60.85    |
| 3              | 2.965    | 168037 | 23.38    |

**SorbC and 28 at 270 nm**

| Retention Time | Area     | % Area | Height   |
|----------------|----------|--------|----------|
| 1              | 2.411    | 1490565| 93.12    |
| 2              | 2.948    | 110155 | 6.88     |

**NEC 28 at 270 nm**

| Retention Time | Area     | % Area | Height   |
|----------------|----------|--------|----------|
| 1              | 2.747    | 1490565| 93.12    |
| 2              | 2.948    | 110155 | 6.88     |
**Supplementary Figure S16.** UPLC-PDA trace of SorbC reactions with 29 and NEC. Byproduct annotated with an asterisk.

**SorbC and 29 at 230 nm**

| Retention Time | Area    | % Area | Height   |
|----------------|---------|--------|----------|
| 1              | 2.447   | 153081 | 17.24    |
| 2              | 2.553   | 83030  | 9.35     |
| 3              | 2.753   | 549538 | 61.90    |
| 4              | 2.951   | 102195 | 11.51    |

**SorbC and 29 at 270 nm**

| Retention Time | Area    | % Area | Height   |
|----------------|---------|--------|----------|
| 1              | 2.752   | 1794595| 94.76    |
| 2              | 2.951   | 99228  | 5.24     |

**NEC of 29 at 270 nm**

| Retention Time | Area    | % Area | Height   |
|----------------|---------|--------|----------|
| 1              | 2.751   | 1416054|          |
| 2              | 2.951   | 45478  |          |
Supplementary Figure S17. UPLC-PDA trace of SorbC reactions with 30 and NEC.

SorbC with 30 at 270 nm

| Retention Time | Area   | % Area | Height  |
|----------------|--------|--------|---------|
| 1              | 2.527  | 120391 | 12.95   |
| 2              | 2.823  | 708205 | 76.19   |
| 3              | 2.949  | 100905 | 10.86   |

NEC 30 at 270 nm

| Retention Time | Area   | % Area | Height  |
|----------------|--------|--------|---------|
| 1              | 2.824  | 1609098| 94.60   |
| 2              | 2.950  | 91833  | 5.40    |
Supplementary Figure S18. UPLC-PDA trace of SorbC reactions with 31 and NEC. Byproduct annotated with an asterisk.

SorbC and 31 at 240 nm

| Retention Time | Area     | % Area | Height |
|----------------|----------|--------|--------|
| 1              | 2.249    | 60565  | 10.20  |
| 2              | 2.719    | 509107 | 85.75  |
| 3              | 2.962    | 24021  | 4.05   |

SorbC and 31 at 270 nm

NEC 31 at 270 nm
Supplementary Figure S19. UPLC-PDA trace of SorbC reactions with 32 and NEC.

2.5 μM SorbC, O₂
NADP⁺, G6P, G6PDH
15% DMSO, KPi buffer pH 8.0

SorbC and 32 at 270 nm

| Retention Time | Area   | % Area | Height  |
|----------------|--------|--------|---------|
| 1              | 2.364  | 39944  | 2.99    | 29879   |
| 2              | 2.900  | 97083  | 7.28    | 78987   |
| 3              | 2.968  | 1196964| 89.73   | 846065  |

NEC 32 at 270 nm

| Retention Time | Area   | % Area | Height  |
|----------------|--------|--------|---------|
| 1              | 2.899  | 153450 | 10.44   | 123568  |
| 2              | 2.967  | 1315706| 89.56   | 941069  |
**Supplementary Figure S20.** UPLC-PDA trace of SorbC reactions with 33 and NEC. Impurity from starting material denoted with a #.

**SorbC and 33 at 240 nm**

| Retention Time | Area      | % Area | Height   |
|----------------|-----------|--------|----------|
| 1              | 2.286     | 32.64  | 816017   |
| 2              | 2.713     | 13.18  | 314630   |
| 3              | 2.959     | 54.18  | 956640   |

**SorbC and 33 at 270 nm**

| Retention Time | Area      | % Area | Height   |
|----------------|-----------|--------|----------|
| 1              | 2.523     | 312.1  | 956640   |
| 2              | 2.712     | 4.45   | 44282    |

**NEC of 33 at 270 nm**

| Retention Time | Area      | % Area | Height   |
|----------------|-----------|--------|----------|
| 1              | 2.708     | 95.55  | 1036838  |
| 2              | 2.950     | 4.45   | 44282    |
Supplementary Figure S21. UPLC-PDA trace of SorbC reactions with 34 and NEC.

SorbC and 34 at 270 nm

| Retention Time | Area    | % Area  | Height   |
|----------------|---------|---------|----------|
| 1              | 2.633   | 90558   | 5.29     |
| 2              | 2.822   | 1530254 | 89.33    |
| 3              | 2.950   | 92228   | 5.38     |

NEC of 34 at 270 nm

| Retention Time | Area    | % Area  | Height   |
|----------------|---------|---------|----------|
| 1              | 2.823   | 1690490 | 94.21    |
| 2              | 2.952   | 103864  | 5.79     |
**Supplementary Figure S22.** UPLC-PDA trace of SorbC reactions with 35 and NEC. Impurity from starting material denoted with a #.

**SorbC and 35 at 270 nm**

| Retention Time | Area    | % Area | Height     |
|----------------|---------|--------|------------|
| 1              | 2.255   | 2.93   | 29745      |
| 2              | 2.820   | 88.62  | 1327825    |
| 3              | 2.955   | 8.44   | 101574     |

**NEC of 35 at 270 nm**

| Retention Time | Area    | % Area | Height     |
|----------------|---------|--------|------------|
| 1              | 2.817   | 95.17  | 997950     |
| 2              | 2.950   | 4.83   | 45787      |
Supplementary Figure S23. With a subset of substrates, in addition to the expected product a second peak with the same m/z was observed (See Supplementary Figure S11 and S14). This byproduct, formed in SorbC reactions, was not found in reactions with IBX or LTA (see traces below). Attempts to isolate and characterize the material corresponding to the peak marked with an asterisk either directly from enzymatic reactions or following acylation were unsuccessful did not produce meaningful quantities of material. Preparative HPLC of the crude reaction mixture did allow for isolation of the material, as judged by UPLC-PDA analysis of the resulting fractions; however, this material accounted for little of the mass balance of the reaction mixture (<0.1 mg isolated from 10 mg-scale reactions) insufficient material was obtained for NMR analysis.
LC-MS trace comparing reaction of 27 with SorbC and IBX.

MS/MS spectra comparing the products of the SorbC reaction and the IBX reaction. Note the second IBX product does not correspond to the more nonpolar SorbC product.
Note the appearance of a more polar peak which corresponds to overoxidation (+32 from sm) of the desired product by hydrogen peroxide produced by uncoupling in the SorbC-catalyzed reaction. Addition of catalase prevents formation of this byproduct.
LC-MS traces of the SorbC reaction with 27 followed by acylation conditions and LTA reaction with SorbC.
Part VII. Determination of Enantiomeric Excess
Representative Supercritical Fluid Chromatography traces

Supplementary Figure S24. SFC-UV-Vis trace of Pd(OAc)$_4$ generated ±37.

SFC-UV-Vis trace of SorbC generated 37.
Supplementary Figure S25. SFC-UV-Vis trace of Pb(OAc)$_4$ generated ±38.

SFC-UV-Vis trace of SorbC generated 38.
Supplementary Figure S26. SFC-UV-Vis trace of Pb(OAc)₄ generated ±39.

SFC-UV-Vis trace of SorbC generated 39.
Supplementary Figure S27. SFC-UV-Vis trace of Pb(OAc)$_4$ generated ±40.

SFC-UV-Vis trace of SorbC generated 40.
Supplementary Figure S28. SFC-UV-Vis trace of Pb(OAc)$_4$ generated ±41.

SFC-UV-Vis trace of SorbC generated 41.
Part VII. Molecular Modeling

Comparative Model of SorbC

The SorbC structure was modeled using the template TropB structure and a pairwise sequence alignment between TropB and SorbC (shown in Figure S1). The pairwise alignment was extracted from a larger multiple sequence alignment of 280 fungal flavin-dependent hydroxylase sequences initially aligned using MUSCLE and subsequently manually adjusted using both the TropB structure and conserved motifs as a guide. The conserved motifs were identified using the expectation maximization algorithm in MEME and visualized using Genedoc. The alignment and structure served as input to MODELLER version 9.11. The model with the most favorable statistical potential DOPE energy (out of 10 models) was selected for further simulation.

SorbC and TropB share 34% sequence identity (53% sequence similarity). The alignment contains indels only in loop regions suggesting that all of the secondary structure elements in TropB are conserved in SorbC. The sequence alignment between SorbC and TropB is shown with conserved segments (motifs from MEME) colored from red (very low E values) to purple (less significant E values). TropB has a nine-residue insertion from Ala74-Pro82 resulting in a longer loop region near the substrate entryway. SorbC has a nine-residue insertion from Gln92-Ser100 resulting in a longer loop region (begins with Glu104 in TropB) that connects two beta-strands. A conserved motif of 21 residues separates these two indel events. SorbC also has a six-residue insertion from Ser260-Arg265 that lies between two conserved motifs and may have some minimal or transient interaction with the adenine moiety of the flavin cofactor. Finally, TropB has a five-residue insertion from Phe353-Arg357 resulting in longer loop region (begins with Leu353 in SorbC) that connects two helices far removed from the active site. All indel events occur in loop regions removed from the active site and thus our initial comparative model should facilitate predictions related to productive substrate binding and catalytic properties. Experiments have shown that substrate binding occurs after FAD binding and facilitates the hydride transfer step from NADPH to FAD before progressing on to the reaction between FADH\textsubscript{2} and O\textsubscript{2} to form C4a-hydroperoxy-FADH\textsubscript{2} intermediate that subsequently hydroxylates the substrate. Thus, substrates may not be converted to hydroxylated products even if the substrate favorably binds to the enzyme.

Figure S29: Pairwise sequence alignment between SorbC and TropB with colored section representing the conserved motifs.

A model of the flavin adenine dinucleotide (FAD) cofactor in a reduced state was then added to the SorbC comparative model using the coordinates from the TropB-FAD crystal structure. This complex was then geometry optimized for 1000 steepest descent steps using the CHARMM all36 protein force field and FAD parameters from ParamChem. The generalized born SW implicit solvent model was used to represent the surrounding solvent. Temperature replica exchange simulations using 16 replicas spanning a temperature range of 260K-500K incremented every 10K was performed for 20 ns with exchanges attempted every 500 steps (2 femtosecond timesteps). The harmonic restraints maintained the conserved secondary structure elements allowing the sidechains to relax. The loop regions that involved an indel event were all unrestrained but only
showed movements that packed the loop closer to the bulk structure of the enzyme. The final structure from 260K was used as input for substrate docking calculations.

Figure S30: SorbC homology model (grey ribbon) with FAD bound (yellow sticks).

Substrate docking using the CDOCKER protocol

Each substrate was constructed using Avogadro\textsuperscript{14} outputting a mol2 formatted file that was given as input to the cgenff program\textsuperscript{15} in order to obtain molecular mechanical force field parameters. The computational docking of the substrate to the SorbC-FAD complex was performed using CHARMM version 41.\textsuperscript{16} Specifically, the CDOCKER method as described by Gagnon et al.\textsuperscript{17} was followed. Briefly, this method first involves random rotation around any rotatable bonds within the substrate followed by random rotational and translational motions of the substrate within a cube defined by the user. In our calculations, this cube was centered between the Thr51 Cβ atom and the Ile234 Cβ atom; two atoms that lie near the substrate entryway. The cube was 20 Å\textsuperscript{3}. Geometry optimizations were performed replacing the full MM protein representation with a “soft” grid potential that allows the geometry to equilibrate even if the initial geometry contains strong repulsive interactions between substrate and receptor. Two “soft” grids were used that differed in the strength of the potential. Geometry optimizations were followed by simulated annealing molecular dynamics in the presence of these grids and then geometry optimization in the presence of the full MM potential energy function for the receptor. Two hundred separate docking calculations were performed for each substrate.

Ligand docking to SorbC reveals functional insights

The native substrate. The MM interaction energy using a distance dependent dielectric function to mimic the surroundings/water was calculated for all 200 structures. Structures with favorable interaction energies and that placed C4a of FAD in close proximity (less than 4.5 Å) to C5 of the substrate (the site of hydroxylation in SorbC) also placed the C1 ketone substituent along the substrate entryway. Thus, the lowest energy structure that also corresponded with these geometric criteria was selected as the reference structure for substrates that were reactive. In the case of the unreactive substrates, the lowest energy structure served as the reference. Root-mean-square displacements (RMSDs) were calculated between these reference structures and all associated docking results. The indiscriminate binding energies agree with experimental data which shows that
many substrates (even unreactive ones) have millimolar binding affinity. The large extended size of the active site and entryway likely contributes to this experimental finding.

The C1 ketonic-substituent binds to the substrate entryway of the SorbC active site. The entryway consists of primarily hydrophobic residues (Phe71, Ile83, Tyr114, Ala116, Ala221, and Pro235). Moving towards the FAD cofactor along the entryway reveals that Thr51 is positioned to hydrogen bond with the ketone oxygen of the substrate. Threonine is unique to SorbC at this position though several sequences contain a serine residue (alanine in TropB). A secondary binding pocket also emerges through these calculations. For reasons that will become apparent, we designate this the “C3 binding pocket”. The C3 binding pocket consists of both hydrophobic and polar residues including Ile83 (makes contributions to both C1 and C3 surfaces), Leu108, Gln332, Thr389, Ile411, Ser415, and Trp419. As can be seen in the sequence alignment, several of these residues are identical in TropB and none of the remaining residues are significantly different in size or polarity between SorbC and TropB. Thus, this secondary binding pocket may be a common feature of fungal aromatic FdHs.

These docking calculations also reveal that when i) the C1 ketone substituent binds in the substrate entryway with ii) the C3-methyl substituent placed in the “C3”-binding pocket and iii) C5 (the site of hydroxylation) in proximity to FAD, Glu245 can form a hydrogen bond with the C4-hydroxyl group. Activating the substrate would simply require a proton transfer from the C4 hydroxyl group to Glu245. This uncommon feature of SorbC was noted early on in our bioinformatic analysis. In our set of 280 fungal FdHs, most enzymes contain a valine at this position. None, except SorbC and two closely related enzymes, contain a residue capable of directly activating the substrate through proton abstraction. This result could explain the low activity of SorbC towards substrates with pKas lower than ~7.5. Substrates that are naturally anionic would experience electrostatic repulsion with Glu245 and thus be less reactive.

Finally, the facial selectivity seen in SorbC is likely a consequence of all these binding interactions.

Figure S31: (left) Plot of the interaction energy between substrate 27 versus rmsd from lowest energy docked structure. (right) Lowest energy docked structure showing the C3- ethyl group in the C3 binding pocket and the crotyl ester in the hydrophobic substrate entryway.
Part VIII. NMR Spectra of Synthetic Compounds

[Chemical structure image]

S1
Entry 8

H₂O

Entry 8

water
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