SUPPORTING INFORMATION:

α,α'-Dihydroxyketone formation using aromatic and heteroaromatic aldehydes with evolved transketolase enzymes

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General methods

Unless otherwise noted, solvents and reagents were reagent grade from commercial suppliers (Sigma-Aldrich) and used without further purification. Dry CH_2Cl_2 was obtained using anhydrous alumina columns.[1] All moisture-sensitive reactions were performed under a nitrogen or argon atmosphere using oven-dried glassware. Reactions were monitored by TLC on Kieselgel 60 F_{254} plates with detection by UV, potassium permanganate and phosphomolybdic acid (PMA) [PMA hydrate (12 g) and ethanol (250 mL)] stains. Flash column chromatography was carried out using silica gel (particle size 40-63 μm). ^{1}H NMR and ^{13}C NMR spectra were recorded at the field indicated using Bruker AMX300 MHz, AMX400 Avance-500 MHz and Avance-600 MHz machines. Coupling constants are measured in Hertz (Hz) and unless otherwise specified, NMR spectra were recorded at 298 K. Mass spectra were recorded on a Thermo Finnegan MAT 900XP and Micro Mass Quattro LC electrospray mass spectrometers VG ZAB 2SE. Infrared spectra were recorded on a Shimadzu FTIR-8700 and Perkin Elmer Spectrum 100 FTIR spectrometer. Optical rotations were recorded on a Perkin Elmer model 343 polarimeter at 589 nm, quoted in deg cm^2 g^{-1} and conc (*c*) in g/100 mL. Chiral HPLC analysis was performed on a Varian Prostar instrument equipped with Chiracel OD or Chiralpak AD chiral columns (Daicel; Chiral Technologies Europe, France) 25 cm × 0.46 cm.

Lithium hydroxypyruvate was synthesised as previously described.[2] 1,3-dihydroxy-1-phenylpropan-2-one **3a** was prepared as previously described.[3]

Synthesis of racemic α,α'-dihydroxyketones. The corresponding aldehyde **2b**–**2f** (1.00 mmol) was added to a solution of Li-**1** (110 mg, 1.00 mmol) and *N*-methylmorpholine (110 μL, 1.00 mmol) in water (20 mL) at pH 8 (adjusted with 10% HCl). The reaction was stirred for 24–48 h at rt and monitored by TLC analysis. Upon concentration *in vacuo*, the crude material was dry loaded and purified using flash silica chromatography.

Chiral HPLC analysis of 3a, 3d-f to determine *ee*s. Compounds **3d** and **3e** were monobenzoylated (dibenzoylated compounds were not separable by chiral HPLC columns used) and the products analysed by chiral HPLC to determine *ee*s.[4] Ketodiols **3a** and **3f** were dibenzoylated for chiral HPLC analysis.[4] HPLC analysis for **3a**, **3d** and **3e** was carried out using a Chiralcel OD column, and for **3f** on a Chiralpak AD column, and the hexane:2-propanol solvent system given.

1,3-Dihydroxy-1-phenylpropan-2-one[3] (3a). Racemic **3a** was dibenzoylated and HPLC analysis of the product (82:18, 1.0 mL min^{-1}) gave retention times of 10.5 min (*R*-isomer) and 13.4 min (*S*-isomer).

1-Furan-2-yl-1,3-dihydroxypropan-2-one (3b). The reaction was carried out for 48 h and the product purified using flash silica chromatography (EtOAc:hexane, 1:1) to give **3b** as a colorless oil (7 mg, 5%); ν_{max}(neat)/cm^{-1} 3391, 1708; ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.39 (2H, s, O*H*), 4.28 (1H, d, *J* 19.5, C*H*HOH), 4.43 (1H, d, *J* 19.5, CH*H*OH), 5.30 (1H, s, HOC*H*), 6.35–6.47 (2H, m), 7.41 (1H, m); ^{13}C NMR (125 MHz; $CDCl_3$) δ 66.1 (*C*H$_2$), 71.0 (*C*HOH), 110.5, 111.0, 143.6, 149.6 (*C*CHOH), 206.9, (*C*=O); *m/z* (HRCI) found MH$^+$ 157.04964. $C_7H_9O_4$ requires 157.05008.

1,3-Dihydroxy-1-(thiophen-2-yl)propan-2-one (3c). The reaction was carried out for 48 h and the product purified using flash silica chromatography (EtOAc:hexane, 1:1) to give **3c** as a brown oil (8 mg, 5%); ν_{max}(neat)/cm^{-1} 3391, 1708, 694; ^{1}H NMR (500 MHz; $CDCl_3$) δ 3.86 (1H, s, O*H*), 4.36 (1H, d, *J* 19.5, C*H*HOH), 4.42 (1H, d, *J* 19.5, CH*H*OH), 5.52 (1H, s, HOC*H*), 7.01–7.10 (2H, m), 7.35 (1H, m); ^{13}C NMR (125 MHz; $CDCl_3$) δ 65.2 (*C*H$_2$), 73.1 (*C*HO), 126.6, 127.1, 127.5, 140.1, 207.8, (*C*=O); *m/z* (HRCI) found M$^+$ 173.02695. $C_7H_9O_3S$ requires 173.02724.

1,3-Dihydroxy-4-phenylbutan-2-one[5] **(3d).** The reaction was carried out for 48 h and the product purified using flash silica chromatography (EtOAc:hexane, 1:1) to give **3d** as a white solid (27 mg, 15%); M.p. 122–130 ºC (EtOAc:hexane); ν_{max}(neat)/cm^{-1} 3262, 2966–2883, 1720; ^{1}H NMR (300 MHz; $CDCl_3$) δ 2.85 (1H, dd, *J* 14.0 and 7.9, CH*H*Ph) 3.08 (1H, dd, *J* 14.0 and 4.6, C*H*HPh), 4.28 (1H, d, *J* 18.0, C*H*HOH), 4.38 (1H, d, *J* 18.0, CH*H*OH), 4.47 (1H, m, C*H*OH), 7.18–7.34 (5H, m, Ar); ^{13}C NMR (125 MHz; $CDCl_3$) δ 40.4 (*C*H$_2$Ph), 66.3 (*C*H$_2$OH), 76.1 (*C*HOH), 127.4, 128.7, 129.3, 135.6, 211.2 (*C*=O); *m/z* (FTMS) found [2M+Na]$^+$ 383.1454. $C_{20}H_{24}O_6Na$ requires 383.1471. Racemic **3d** was monobenzoylated and HPLC analysis of the product (97:3, 0.8 mL min^{-1}) gave retention times of 45.2 min (3*R*-isomer) and 57.0 min (3*S*-isomer).

1,3-Dihydroxy-4-phenyl-pentan-2-one (3e). The reaction was carried out for 48 h and the product purified using flash silica chromatography (EtOAc:hexane, 1:1) to give **3e** as a white solid (25 mg, 13%); M.p. 120–130 ºC (EtOAc:hexane); ν_{max}(neat)/cm^{-1} 3407, 2925–2855, 1720; **Isomer 1** ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.27 (3H, d, *J* 7.1, C*H*$_3$), 3.20 (1H, m, C*H*CH$_3$), 4.19 (1H, d, *J* 19.8, C*H*HOH), 4.27 (1H, d, *J* 19.8, C*H*HOH), 4.33 (1H, d, *J* 4.5, C*H*OH), 7.21–7.37 (5H, m, Ar); ^{13}C NMR (125 MHz; $CDCl_3$) δ 14.3 (*C*H$_3$), 43.6 (*C*HCH$_3$), 127.4, 127.7, 129.0, 142.0, 211.5 (*C*=O); **Isomer 2** ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.42 (3H, d, *J* 7.1, C*H*$_3$) 3.24 (1H, m, C*H*CH$_3$), 4.06 (1H, d, *J* 19.5, C*H*HOH), 4.33–4.40 (2H, m, C*H*HOH and C*H*OH), 7.21–7.37 (5H, m, Ar); *m/z* (FTMS) found MNa$^+$ 217.0836. $C_{11}H_{14}O_3Na$ requires 217.0835. The **3e** isomers were monobenzoylated and HPLC analysis of the product (97:3, 0.8 mL min^{-1}) gave retention times of approximately 20.0 min, 21.4 min, 24.3 min, and 26.2 min. Aldehyde 2*R*-**2e** was synthesised as previously described.[6] When used in the biomimetic reaction above, followed by monobenzoylation, HPLC analysis revealed that

the peaks at retention times of 20.0 min and 26.2 min (peaks 1 and 4) were enhanced significantly indicating these corresponded to **3e**-(3*RS*,4*R*).

1,3-Dihydroxy-1-(3-hydroxyphenyl)propan-2-one (3f). The reaction was carried out for 48 h and the product purified using flash silica chromatography (EtOAc:hexane, 1:1) to give **3f** as a colorless oil (10 mg, 4%). ν_{max}(neat)/cm^{-1} 3407, 2925–2855, 1720; ^{1}H NMR (600 MHz; CD_3OD) δ 4.32 (1H, d, *J* 19.0, C*H*HOH), 4.37 (1H, d, *J* 19.0, CH*H*OH), 5.18 (1H, s, C*H*OH), 6.73 (1H, dd, *J* 8.1 and 1.8, 4'-H), 6.84 (1H, br s, 2'-H), 6.87 (1H, d, *J* 7.7, 6'-H), 7.18 (1H, app t, *J* 7.9, 5'-H); ^{13}C NMR (125 MHz; CD_3OD) δ 65.0 (CH_2OH), 77.1 (CHOH), 114.5, 119.9, 123.0, 131.0, 137.7, 156.5, 211.0 (*C*=O); *m/z* (FTMS) found MH^+ 183.06632. $C_9H_{11}O_4$ requires 183.06730. Racemic **3f** was dibenzoylated and HPLC analysis of the product (80:20, 1.0 mL min^{-1}) gave retention times of 12.9 min (3*S*-isomer) and 17.1 min (3*R*-isomer).

Mutant F434A. Site-directed mutagenesis for the F434A TK mutant was performed using the Quikchange kit (Stratagene, Netherlands), forward (5'-CGTACACACCTCCACCGCCCTGATGTTCGTGG-3') and reverse (5'-CCACGAACATCAGGGCGGTGGAGGTGTACG-3') primers,[7] and plasmid template pQR706 which harbours the *E. coli tkta* gene complete with its natural promoter. The resulting pQR780 plasmid was transformed into supercompetent XL1-Blue (Stratagene) *Epicurian coli* cells, and subsequently re-transformed into JM107 *E. coli* cells for activity measurements.

TK conversions. The TK cell free lysates were prepared using an identical procedure to that previously described.[4] The biotransformations were conducted at 50 mM reaction concentrations. ThDP (22 mg, 48 μmol) and $MgCl_2.6H_2O$ (39 mg, 180 μmol) were dissolved in H_2O (10 mL) and the pH adjusted to 7 using 0.1 M NaOH. To this stirred solution, at 25 ºC, was added TK clarified lysate (2 mL, containing approximately 0.3 mg mL^{-1} of TK) and the mixture stirred for 20 min. In another flask, Li-**1** (110 mg, 1.00 mmol) and the aldehyde (1.00 mmol) were dissolved in H_2O (8 mL) and the pH adjusted to 7 with 0.1 M NaOH. Following the 20 min enzyme/cofactor pre-incubation, the Li-**1**/aldehyde mixture (**2a-2f**) was added to the enzyme solution and the mixture stirred at rt for 24 h. During this time, the pH was maintained at 7.0 by addition of 1 M HCl using a pH stat (Stat Titrino, Metrohm) and the reactions were followed by TLC analysis. Silica was added and the reaction mixture concentrated to dryness, dry loaded onto a flash silica gel column, and purified. The *ees* were determined from the mono/dibenzoylated procedure given above.

TK formation of 1,3-dihydroxy-1-phenylpropan-2-one (3a). WT-TK gave no **3a**; D469E-TK gave **3a** (3 mg, 2%) as a racemate and 5% of **4**; D469T-TK gave **3a** (3 mg, 2%) in 70% *ee* (3*R*-isomer) and 5% of **4**; D469K-TK gave **3a** (3 mg, 2%) in 82% *ee* (3*R*-isomer) and 5% of **4**, and F434A-TK generated **3a** (16 mg, 10%) in 82% *ee* (3*R*-isomer) as a colorless oil; $[\alpha]^{20}_D$ = +74.0 (*c*

0.5, $CHCl_3$). Data for **1,3,4,5-Tetrahydroxy-3-phenylpentan-2-one (4, R = Ph).** The biotransformation side product (**4**) was generated using the D469-TK mutants as a mixture of isomers (11 mg, 5%) as an oil. ν_{max}(neat)/cm^{-1} 3391, 1684; ^{1}H NMR (500 MHz; CD_3OD) δ 3.05 (0.6H, d, *J* 11.1, C*H*HOH), 3.73–3.78 (1.4H, m, C*H*HOHs and CH*H*OHs), 3.96 (0.4H, d, *J* 11.1, C*H*HOH), 4.34 (0.4H, d, *J* 11.1, CH*H*OH), 4.60 (0.6H, d, *J* 19.4, C*H*HOH), 4.65 (0.6H, d, *J* 19.4, CH*H*OH), 4.81 (1H, m, C*H*OH), 7.24–7.32 (3H, m, Ph), 7.40 (2H, dd, *J* 8.0 and 1.2, Ph); ^{13}C NMR (150 MHz; CD_3OD) δ 67.0 and 67.4 (*C*H_2), 68.8 and 69.1(*C*H_2), 76.6 and 76.7 (*C*H), 86.2 and 86.8, 128.8–129.0 (signals superimposed), 141.2 and 141.3, 214.6 and 215.4 (*C*=O); *m/z* (HRCI) found MH$^+$ 227.09110. $C_{11}H_{15}O_5$ requires 227.09195.

The absolute stereochemistry of **3a** generated using F434A-TK was determined using the Mosher's derivatisation method (both Mosher's esters formed).[8]

(2*S*,3'*R*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3'-hydroxy-2'-oxo-3'-phenyl-propyl ester. The reaction was carried out under anhydrous conditions. To a stirred solution of F434A-TK generated **3a** (0.008 g, 0.05 mmol) in CH_2Cl_2 (1 mL) was added triethylamine (34 μL, 0.25 mmol) and (*R*)-MTPA chloride (10 μL, 0.04 mmol) the reaction was stirred for 12 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography (EtOAc:hexane, 1:4) to afford the titled compound as a colourless oil (0.016 g, 84%). R_f 0.40 (EtOAc:hexane, 1:4); $[\alpha]^{25}_D$ = +12.0 (*c* 0.25, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3420, 2930, 2855, 1734; ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.61 (3H, s, OC*H*$_3$), 4.70 (0.17H, d, *J* 17.0, C*H*HO (2*R*,3'R)), 4.84 (0.83H, d, *J* 17.0, CH*H*O (2*S*,3'*R*)), 4.92 (0.83H, d, *J* 17.0, CH*H*O (2*S*,3'*R*)), 5.02 (0.17H, d, *J* 17.0, CH*H*O (2*R*,3'R)), 5.26 (1H, s, C*H*OH), 7.32–7.43 (8H, m, Ar), 7.58 (2H, m, Ar); ^{13}C NMR (150 MHz; $CDCl_3$) δ 55.9 (O*C*H$_3$), 66.5, 78.1 (*C*HOH), 127.4, 127.5, 128.6, 129.5, 129.6, 130.0, 131.7, 136.8, 166.2 (*C*=O ester), 201.6 (*C*=O, ketone); ^{19}F NMR (282 MHz; $CDCl_3$) δ –72.2; *m/z* (HRCI) found MH$^+$ 383.11154. $C_{19}H_{18}F_3O_5$ requires 383.11063.

(2*R*,3'*R*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3'-hydroxy-2'-oxo-3'-phenyl-propyl ester. The reaction was carried out under anhydrous conditions. To a stirred solution of F434A-TK generated **3a** (0.008 g, 0.05 mmol) in CH_2Cl_2 (1 mL) was added triethylamine (34 μL, 0.25 mmol) and (*R*)-MTPA chloride (10 μL, 0.04 mmol) the reaction was stirred for 12 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography (EtOAc:hexane, 1:4) to afford the titled compound as a colourless oil (0.016 g, 84%). R_f 0.40 (EtOAc:hexane, 1:4); $[\alpha]^{25}_D$ = +30.0 (*c* 0.5, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3420, 2930, 2855, 1734; ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.61 (3H, s, OC*H*$_3$), 4.69 (0.93H, d, *J* 17.0, C*H*HO (2*R*,3'R)), 4.84 (0.07H, d, *J* 17.0, CH*H*O (2*S*,3'*R*)), 4.91 (0.07H, d, *J* 17.0, CH*H*O (2*S*,3'*R*)), 5.02 (0.93H, d, *J* 17.0, CH*H*O (2*R*,3'R)), 5.25 (1H, s, C*H*OH), 7.25–7.60 (8H, m, Ar), 7.59 (2H, m, Ar); ^{13}C NMR (150 MHz; $CDCl_3$) δ 55.9

(O*C*H$_3$), 66.5, 78.1 (*C*HOH), 127.4, 127.5, 128.6, 129.5, 129.6, 130.0, 131.8, 136.7, 166.3 (*C*=O ester), 201.7 (*C*=O, ketone); ^{19}F NMR (282 MHz; $CDCl_3$) δ –72.2; *m/z* (HRCI) found MH^+ 383.11154. $C_{19}H_{18}F_3O_5$ requires 383.11063.

TK formation of 1-furan-2-yl-1,3-dihydroxypropan-2-one (3b). WT-TK gave no **3b**; D469E-TK gave **3b** (8 mg, 5%) and **5** (R = furyl) in <2%; D469T-TK gave **3b** (5 mg, 3%) and **5** (R = furyl) in <2%; D469K-TK gave **3b** (5 mg, 3%) and **5** (R = furyl) in <2%, and F434A-TK generated **3b** (2 mg, 1%) as a colorless oil and **5** (R = furyl) in <2%. $[\alpha]^{20}_D$ of **3b** = +54.0 (*c* 0.4, $CHCl_3$).

Data for **1-Furan-2-yl-2,3-dihydroxy-propan-1-one (5, R = furyl).** The biotransformation side product (**5**) was generated using the D469 and F434-TK mutants (3 mg, 2%) as an oil. ν_{max}(neat)/cm^{-1} 3391, 1708; ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.93 (1H, dd, *J* 11.8 and 4.5, C*H*HOH), 4.06 (1H, dd, *J* 11.8 and 3.3, CH*H*OH), 4.91 (1H, m, C*H*OH), 6.60 (1H, m, Ar), 7.37 (1H, m, Ar), 7.64 (1H, m, Ar); ^{13}C NMR (150 MHz; CD_3OD) δ 64.9 (*C*H$_2$), 74.8 (*C*HO), 112.8, 119.5, 147.4, 150.3, 187.7 (*C*=O); *m/z* (HRCI) found M^+ 157.04964. $C_7H_9O_4$ requires 157.05008.

TK formation of 1,3-dihydroxy-1-(thiophen-2-yl)propan-2-one (3c). WT-TK gave no **3c**; D469E-TK gave **3c** (4 mg, 2%) and **5** (R = thienyl) in <2%; D469T-TK gave **3c** (5 mg, 3%) and **5** (R = thienyl) in <2%; D469K-TK gave **3c** (4 mg, 2%) and **5** (R = thienyl) in <2%, and F434A-TK gave **3c** (2 mg, 1%) as an oil and **5** (R = furyl) in <2%. $[\alpha]^{20}_D$ of **3c** = +26.0 (*c* 0.15, $CHCl_3$).

Data for **2,3-Dihydroxy-1-thiophen-2-yl-propan-1-one (5, R = thienyl).** The biotransformation side product (**5**) was generated using the D469 and F434-TK mutants (3 mg, 2%) as an oil. ν_{max}(neat)/cm^{-1} 3391, 1708; ^{1}H NMR (300 MHz; $CDCl_3$) δ 3.86 (1H, dd, *J* 11.7 and 5.1, C*H*HOH), 4.05 (1H, dd, *J* 11.7 and 3.3, CH*H*OH), 4.97 (1H, m, C*H*OH), 7.20 (1H, app t, *J* 4.5, Ar), 7.76 (1H, d, *J* 5.0, Ar), 7.82 (1H, d, *J* 4.0, Ar); ^{13}C NMR (150 MHz; CD_3OD) δ 66.2 (*C*H$_2$), 75.3 (*C*HO), 128.6, 133.5, 135.5, 139.7, 191.8 (*C*=O); *m/z* (HRCI) found M^+ 173.02695. $C_7H_9O_3S$ requires 173.02724.

TK formation of 1,3-dihydroxy-4-phenylbutane-2-one (3d). WT-TK gave **3d** (9 mg, 5%) in 93% *ee* (3*S*-isomer); D469E-TK gave **3d** (77 mg, 43%) in 90% *ee* (3*S*-isomer); D469T-TK gave **3d** (90 mg, 50%) in 96% *ee* (3*S*-isomer); D469K-TK gave **3d** (81 mg, 45%) in 95% *ee* (3*S*-isomer), and F434A-TK generated **3d** (86 mg, 48%) in 97% ee (3*S*-isomer) as a white solid. M.p. 122-130 °C; $[\alpha]^{20}_D$ = +30.0 (*c* 0.5, $CHCl_3$).

The absolute stereochemistry of **3d** generated using D469E-TK was determined using the Mosher's derivatisation method.[8]

(2*S*,3'*S*)-3,3,3-Trifluoro-2-methoxy-2-phenyl propionic acid 3'-hydroxy-2'-oxo-4'-phenylbutyl ester. The reaction was carried out under anhydrous conditions. To a stirred solution of synthesized

D469E **3d** (0.01 g, 0.04 mmol) in CH_2Cl_2 (1 mL) was added triethylamine (34 μL, 0.25 mmol) and (*R*)-MTPA chloride (10 μL, 0.04 mmol) and the reaction was stirred for 12 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography (EtOAc:hexane, 1:4) to afford the titled compound as a colourless oil (0.015 g, 85%). R_f 0.40 (EtOAc:hexane, 1:4); $[\alpha]^{25}_D$ = –30.0 (*c* 0.3, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3600, 2932, 1759, 1737; ^{1}H NMR (300 MHz; $CDCl_3$) δ 2.59 (1H, d, *J* 4.0 Hz, O*H*) 2.91 (1H, dd, *J* 14.1 and 8.6, PhC*H*H,) 3.17 (1H, dd, *J* 14.1 and 4.4, PhCH*H*) 3.66 (1H, s, OCH_3), 4.50 (1H, m, C*H*OH) 4.95 (0.95H, d, *J* 17.4, C*H*HO (2*S,*3'*S*)), 5.03 (0.05H, d, *J* 17.4, C*H*HO (2*R*,3'*S*)), 5.09 (0.05H, d, *J* 17.4, CH*H*O (2*R*,3'*S*)), 5.16 (0.95H, d, *J* 17.4, CH*H*O (2*S,*3'*S*)), 7.21–7.42 (5H, m), 7.43 (3H, m), 7.63 (2H, m); ^{13}C NMR (125 MHz; $CDCl_3$) δ 40.2 (*C*H_2Ar), 55.8 (O*C*H_3), 67.8 (CH_2O), 76.7 (*C*HOH), 127.5, 127.6, 128.5, 129.0, 129.5, 129.9, 131.9, 135.6, 166.3 (*C*=O ester), 203.5 (*C*=O, ketone); ^{19}F NMR (282 MHz; $CDCl_3$) δ -72.2; *m/z* (-HRES) found [M-H] 395.1119. $C_{20}H_{19}F_3O_5$ requires 395.1106.

TK formation of 1,3-dihydroxy-4-phenylbutane-2-one (3e). Using racemic **2e** WT-TK gave **3e** (68 mg, 35%) with 88% (3*S*,4*R*) and 12% (3*S*,4*S*); D469E-TK gave **3e** (59 mg, 30%) with 95% (3*S*,4*R*) and 5% (3*S*,4*S*); D469T-TK gave **3e** (78 mg, 40%) with 95% (3*S*,4*R*) and 5% (3*S*,4*S*); D469K-TK gave **3e** (73 mg, 38%) with 95% (3*S*,4*R*) and 5% (3*S*,4*S*), and F434A-TK gave **3e** (68 mg, 35%) with 85% (3*S*,4*R*) and 15% (3*S*,4*S*) as a white solid. M.p. 120-130 °C; $[\alpha]^{25}_D$ = +30.9 (*c* 0.22, $CHCl_3$). Major isomer by ^{1}H NMR is isomer 1 from the biomimetic reaction. (2*R*)-**2e** was readily accepted by D469T to give one product in 40% yield, the (3*S*,4*R*)-isomer (with a retention time of approximately 26.2 min (peak 4) using the same HPLC condition as for the biomimetic reaction.

The absolute stereochemistry of **3e** at C-3 generated using D469E-TK was determined using the Mosher's derivatisation method.[8]

(2S,3'S,4'S)-3'-hydroxy-2'-oxo-4'-phenylpentyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate. The reaction was carried out under anhydrous conditions. To a stirred solution of synthesized D469E **3e** (0.012 g, 0.06 mmol) in CH_2Cl_2 (1 mL) was added triethylamine (34 μL, 0.25 mmol) and (*R*)-MTPA chloride (10 μL, 0.04 mmol) the reaction was stirred for 12 h at rt. The product was dry loaded onto silica gel and purified using flash chromatography (hexane: EtOAc, 4:1) to afford the titled compound as a colourless oil (0.018 g, 75%), R_f 0.45 (hexane: EtOAc, 4:1);); $[\alpha]^{25}_D$ = –37.2 (*c* 0.25, $CHCl_3$); ν_{max}(neat)/cm^{-1} 3471, 2954, 1759, 1735, 1170; ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.32 (3H, d, *J* 7.1, CHCH_3), 1.59 (1H, br, O*H*), 3.27 (1H, m, C*H*CH_3), 3.65 (1H, s, OCH_3), 4.40 (1H, d, *J* 3.9, C*H*OH) 4.87 (1H, d, *J* 17.0, C*H*HO (2*S,*3'*S*)), 4.96 (trace 2*S,*3'*R*)-isomer), 5.04 (1H, d, *J* 17.0, CH*H*O (2*S*,3'*S*)), 7.21–7.42 (5H, m), 7.43 (3H, m, Ph), 7.63 (2H, m, Ph); ^{13}C NMR (125 MHz; $CDCl_3$) δ 13.8, 43.2, 55.8 (O*C*H_3), 68.5 (*C*H_2), 80.1 (*C*HOH), 127.4,

127.8, 128.5, 129.0, 129.9, 129.9, 131.9, 142.0, 166.2 (*C*=O ester), 203.7 (*C*=O, ketone); ^{19}F NMR (282 MHz; $CDCl_3$) δ –72.3; *m/z* (HRMS) found MH^+ 433.1234. $C_{21}H_{21}F_3O_5$ requires 433.1239.

TK formation of 1,3-dihydroxy-1-(3-hydroxyphenyl)propan-2-one (3f). WT-TK and D469E-TK gave no **3f**; D469T-TK gave **3f** (7 mg, 4%) as a racemate, and F434A-TK gave **3f** (11 mg, 6%) in 53% *ee* (3*R*-isomer) as a colorless oil. $[\alpha]^{25}_D$ = +99.3 (*c* 0.08, MeOH).

The absolute stereochemistry of **3f** using F434A-TK was determined using the Mosher's derivatisation method.[8]

(2*R*,3'*R*)-3-(1-Hydroxy-2-oxo-3-[((2*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropanoyl)oxy)-propyl]phenyl-3,3,3-trifluoro-2-methoxy-2-phenylpropanoate. To a stirred solution of 1,3-dihydroxy-1-(3-hydroxyphenyl)propan-2-one (3 mg, 0.02 mmol) in CH_2Cl_2 (2 mL) was added 2,4,6-collidine (10 μL, 0.08 mmol) and (*S*)-MTPA chloride (10 μL, 0.04 mmol). The reaction was stirred for 36 h at rt. The product was dry loaded onto silica gel and purified using flash silica chromatography (EtOAc:hexane, 3:7) to afford the titled compound as a colourless oil (1 mg, 8%). R_f 0.14 (EtOAc:hexane, 3:7). ν_{max}(neat)/cm^{-1} 3420, 2930, 2855, 1734; ^{1}H NMR (600 MHz; $CDCl_3$) δ 3.62 (6H, s, OC*H*$_3$), 4.74 (0.4H, d, *J* 16.9, C*H*HO (2*R*,3'*R*,2"*R*)), 4.90 (0.2H, m, C*HH*O (2*R*,3'*S*,2"*R*)), 5.02 (0.4H, d, *J* 16.9, CH*H*O (2*R*,3'*R*,2"*R*)), 5.20 (1H, d, *J* 3.8, C*H*OH), 7.25–7.60 (14H, m, Ar); ^{13}C NMR (150 MHz; $CDCl_3$) δ 55.9 (O*C*H$_3$), 66.5 (*C*H$_2$), 77.1 (*C*HOH), 127.3, 127.5, 128.6–129.0 (signals superimposed(, 130.0, 130.9, 138.5, 156.4, 166.6 (*C*=O esters), 201.8 (*C*=O, ketone); ^{19}F NMR (282 MHz; $CDCl_3$) δ –72.2; *m/z* (ES+) found MH^+ 615.09. $C_{29}H_{25}F_6O_8$ requires 615.13.

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