SUPPORTING INFORMATION:

A Multi-Disciplinary Approach Toward the Rapid and Preparative Scale Biocatalytic Synthesis of Chiral Amino Alcohols: A Concise Transketolase / ω-Transaminase-Mediated Synthesis of (2*S*,3*S*)-2-Aminopentane-1,3-diol

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1. General experimental

Unless otherwise noted, solvents and reagents were reagent grade from commercial suppliers (Sigma-Aldrich) and used without further purification. 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 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, 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 spectrometer.

Racemic 1,3-dihydroxypentan-2-one was prepared as previously described using the biomimetic TK reaction,[1] and 1,3-diacetoxyhydroxypentan-2-one was prepared for GC analysis as previously reported.[2]

2. Four diastereomer mixture of 2-aminopentane-1,3-diol hydrochloride

2.1 2-Benzylaminopentane-1,3-diol. $NaCNBH_3$ (543 mg, 8.64 mmol) was added to a solution of *rac*-1,3-dihydroxypentan-2-one (340 mg, 2.88 mmol) and benzylamine (629 μL, 5.76 mmol) in MeOH (10 mL). The pH was adjusted to 6 using glacial acetic acid and the reaction stirred at RT overnight. The reaction mixture was concentrated to dryness *in vacuo* and the residue partitioned between CH_2Cl_2 (100 mL) and $NaHCO_3$ (sat) (100 mL). The aqueous was extracted with further CH_2Cl_2 (2 × 100 mL) and the combined organics dried over $MgSO_4$. The organics were concentrated *in vacuo* and the residue purified by flash silica chromatography (MeOH/EtOAc, 1:9) to give the *titled compound* as an oil (330 mg, 55%) and a 55:45 mixture of *anti:syn* isomers. ^{1}H NMR (400 MHz; d_4-MeOD) δ 0.93 (1.35H, t, *J* 7.4, CH_3), 0.96 (1.65H, t, *J* 7.4, CH_3), 1.32–1.70 (2H, m, CH_2CH$_3$), 2.55 (0.45H, dt, *J* 6.0 and 4.9, C*H*HN), 2.62 (0.55H, dt, *J* 6.2 and 4.5, CH*H*N), 3.47–3.94 (5H, m, CH_2OH, C*H*OH and CH_2Ph), 7.20–7.37 (5H, m, ArH); ^{13}C NMR (100 MHz; d_4-MeOD) δ 10.7 and 11.0 (*C*H$_3$), 27.3 and 27.5 (*C*H$_2$CH$_3$), 52.5 and 53.0 (*C*H$_2$N), 61.6 and 61.4 (*C*H$_2$O), 63.2 and 63.7 (*C*HN), 73.6 and 73.7 (*C*HOH), 128.2, 129.5 (signals superimposed), 141.1 and 141.3.

2.2 2-Aminopentane-1,3-diol hydrochloride. 2-Benzylamino-pentane-1,3-diol (140 mg, 0.67 mmol) was dissolved in MeOH (5 mL) and the pH adjusted to 1-2 using 1 M HCl (aq). The mixture was concentrated to dryness *in vacuo* and redissolved in MeOH (5 mL). Pd/C (100 mg) was added and the mixture subjected to H_2 (1 atm.) for 48 h. The reaction was then filtered and concentrated to yield the titled compound as an oil (103 mg, 99%), and a 55:45 mixture of *anti*[3]*:syn* isomers. ^{1}H NMR (300 MHz; d_4-MeOD) δ 0.99 (1.35H, t, *J* 7.3, CH_3), 1.00 (1.65H, t, *J* 7.3, CH_3), 1.49 (2H, m, CH_2CH$_3$), 3.07 (0.45H, dt, *J* 6.8 and 4.1, C*H*NH$_3$Cl), 3.22 (0.55H, dt, *J* 8.5 and 4.0, C*H*NH$_3$Cl), 3.57–3.86 (3H, m, CH_2OH and C*H*OH); ^{13}C NMR

(75 MHz, d_4-MeOD) δ 9.9 and 10.7 (*C*H_3), 27.2 and 27.7 (*C*H_2CH$_3$), 58.2, 58.8, 58.9, 60.5, 70.4 and 71.8 (*C*HOH); *m/z* (+HRCI) [M-Cl] calcd for $C_5H_{14}NO_2$ 120.10245; found 120.10231.

3. Chiral HPLC assay for the determination of *e.e.* and *d.e.* of transaminase-derived 2-aminopentane-1,3-diol

To facilitate chiral HPLC assay development, benzoylated samples of both the four diastereomer mixture and the transaminase-derived 2-aminopentane-1,3-diol were synthesised.

3.1 2-Benzamido-3-hydroxypentanol dibenzoate ester. Benzoyl chloride (120 μL, 1.03 mmol) was added to a stirred solution of 2-aminopentane-1,3-diol hydrochloride (four diastereomer mixture) (40 mg, 0.257 mmol) in pyridine (3 mL) at 0 ºC. The mixture was warmed to RT and stirred for 17 h. The final suspension was concentrated to dryness *in vacuo* and the residue partitioned between EtOAc (50 mL) and 0.3 M $KHSO_4$ (50 mL). The organic phase was washed with $NaHCO_3$ (sat.) (50 mL) and dried ($MgSO_4$). Concentration *in vacuo* gave a residue which was purified by flash silica chromatography (EtOAc/hexane, 1:2) to yield the *titled compound* as a white solid (111 mg, 100%). ^{1}H NMR (300 MHz; $CDCl_3$) δ 1.06 (1.35H, t, *J* 7.4, CH_3), 1.07 (1.65H, t, *J* 7.4, CH_3), 1.85–2.04 (2H, m, C*H*$_2$CH$_3$), 4.45–4.68 (2H, m, C*H*$_2$OH), 4.86–4.96 (1H, m, C*H*N), 5.33 (0.55H, dt, *J* 7.6 and 4.9, C*H*OH), 5.47 (0.45H, dt, *J* 6.6 and 4.8, C*H*OH), 6.65 (0.45H, d, *J* 9.2, NH), 6.65 (0.55H, d, *J* 8.7, NH), 7.34–7.59 (9H, m, ArH), 7.72–7.79 (2H, m, ArH), 7.94–8.05 (4H, m, ArH); ^{13}C NMR (150 MHz; $CDCl_3$) δ 9.9 and 10.1 (*C*H_3), 24.9 and 25.4 (*C*H_2CH$_3$), 51.1 and 51.4 (*C*N), 63.3 and 64.5 (*C*H_2O), 75.0 and 76.9 (*C*HO), 127.0, 127.1, 128.50, 128.54, 128.62, 128.65, 128.75, 128.78, 129.5, 129.6, 129.7, 129.76, 129.78, 129.9, 131.79, 131.82, 133.29, 133.33, 133.48, 133.51, 134.2, 166.60, 166.64, 166.86, 166.93, 167.3, 167.6; *m/z* (+HRCI) MH calcd for $C_{26}H_{26}NO_5$ 432.18110; found 432.18045.

The same procedure was used to obtain a sample of (2*S*,3*S*) and (2*S*,3*R*)-2-benzamido-3-hydroxypentanol dibenzoate ester from the transaminase-derived 2-aminopentane-1,3-diol.

3.2 Chiral HPLC assay for the determination of *ee* and *de* of transaminase derived 2-aminopentane-1,3-diol. The HPLC assay was developed using the four diastereomer sample of 2-benzamido-3-hydroxypentanol dibenzoate. Analysis was performed on a Varian Prostar instrument equipped with a Chiracel OD chiral column (Daicel, 25 cm x 0.46 cm). HPLC conditions: injection volume, 10 μL; mobile phase, ^{i}PrOH:Hexane, 5:95; Flow rate, 0.8 mL/min; detection, 210 nm.

Retention times for the four-isomer mixture of 2-benzamido-3-hydroxypentanol dibenzoate ester: 26.7 min, 31.5 min, 40.6 min, 47.8 min. The transaminase-derived 2-aminopentane-1,3-diol which was benzoylated to give (2*S*,3*S*) and (2*S*,3*R*)-2-benzamido-3-hydroxypentanol dibenzoate ester gave products with retention times of 31.5 min for the (2*S*,3*S*) *syn*-isomer and 40.6 min for the (2*S*,3*R*)-*anti*-isomer (61% *de*). The remaining peaks in the four isomer mixture were then tentatively assigned as 26.7 min - the (2*R*,3*S*) *anti*-isomer and 47.8 min - the (2*R*,3*R*) *syn*-isomer.

4. References

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