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# Stereoselectivity of an ω-transaminase-mediated amination of 1,3-dihydroxy-1-phenylpropane-2-one

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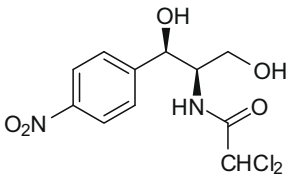
ABSTRACT

The stereoselectivity of a recently isolated ω-transaminase from *Chromobacterium violaceum* in the amination of 1,3-dihydroxy-1-phenylpropan-2-one has been determined. The enzyme is not enantioselective towards a racemic mixture of 1,3-dihydroxy-1-phenylpropan-2-one but is highly stereoselective forming (2*S*)-2-amino-1-phenyl-1,3-propanediols in >99% ee.

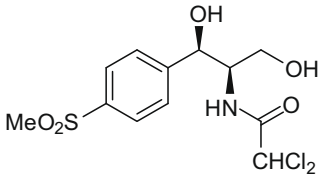


## 1. Introduction

The chiral aminodiol motif is present in many pharmacologically important compounds such as antiviral glycosidase inhibitors[1,2] and sphingolipids.[3,4] Specifically, the broad spectrum antibiotics chloramphenicol **1** and thiamphenicol **2** contain the 2-amino-1-phenyl-1,3-propanediol functionality.[5,6]




chloramphenicol **1**




thiamphenicol **2**

The development of efficient, stereoselective methodology to chiral aminodiols remains a major challenge. There are a wide range of synthetic strategies to chiral amines, however it has recently been highlighted that green chemistry approaches to chiral amines are highly desirable, particularly via ketones or aldehydes.[7] Biocatalytic routes to chiral amines predominantly focus on the kinetic resolution of racemic mixtures. In particular, *N*-acyl amides may be resolved using amidases, lipases or peptidases.[8] Although much of this methodology has been developed for use on an industrial scale, it is limited by the fact that the maximum yield of conversion is 50%. Biocatalytic asymmetric syntheses of amines are therefore highly desirable and a deracemization approach using racemic amines has been reported.[9] Recently, the use of ω-transaminases (TAm) to generate chiral amines has received increasing attention.[10–12] Practical difficulties relating to unfavourable thermodynamic parameters have been addressed and a coupled enzyme system incorporating ω-transaminase, α-amino acid dehydrogenase and formate dehydrogenase has been reported.[13,14]

As part of a multidisciplinary project aiming to develop biocatalytic routes to pharmacologically interesting targets, we are currently investigating the biocatalytic and chemoenzymatic synthesis of aminodiols. We have recently reported the one-pot synthesis of aminoalcohols using an engineered de novo transketolase (TK) and β-alanine TAm pathway in *Escherichia coli*.[15] We have also reported *Chromobacterium violaceum* DSM30191 ω-transaminase (*C. violaceum* TAm) that accepts a range of ketones and aldehydes as well as a ketoalcohol as substrates.[16] Our aim was now to establish the stereoselectivity of *C. violaceum* TAm when using an α,α′-dihydroxyketone, notably the aromatic substrate 1,3-dihydroxy-1-phenylpropan-2-one **3** to give 2-amino-1-phenyl-1,3-propanediol **4** (Scheme 1, four possible diastereoisomers shown). Previous work had indicated that when *C. violaceum* TAm was used with **3** to generate **4**, two products were formed which when analysed by HPLC and LC/MS were believed to be the *syn*- and *anti*-isomers. Based upon previous work describing the selectivity of ω-TAms where (*S*)-α-methylbenzylamine was the amine donor, these were postulated to be the (1*S*,2*S*)- and (1*R*,2*S*)-isomers **4a** and **4d**. However, the 1,3-dihydroxyketone could adopt an alternative orientation in the active site via hydrogen-bonding interactions, or a non-stereoselective amination with TAm could give **4b** and **4d**. These studies set out to establish the absolute stereochemistries resulting from the biocon-

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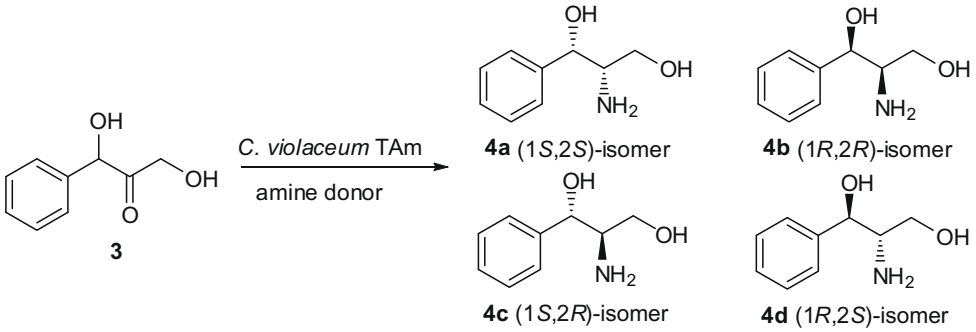



**Scheme 1.** ω-TAm-catalysed conversion of **3** to diastereoisomers **4a**–**4d**.

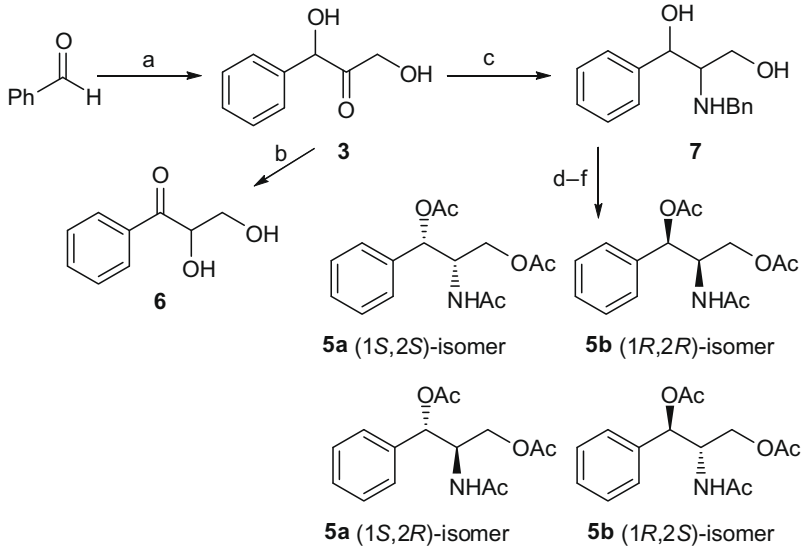
version, which is crucial for synthetic applications with this enzyme. Notably, it was important to determine whether one of the enantiomers of **3** is non-stereoselectively transformed to **4**, or if both enantiomers of **3** are stereoselectively converted to **4**.

## 2. Results and discussion

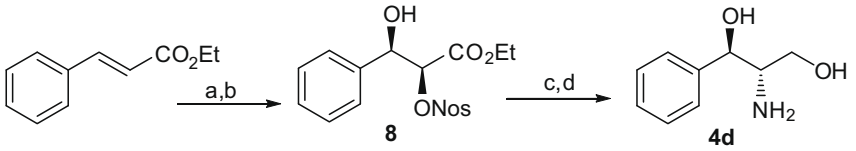
In order to establish the selectivity of the *C. violaceum* TAm, chemically derived analytical standards were required. Both **4a** (the (1*S*,2*S*)-isomer) and **4b** (the (1*R*,2*R*)-isomer) are commercially available and samples of both were readily triacetylated in quantitative yield to give (1*S*,2*S*)-**5a** and (1*R*,2*R*)-**5b** for chiral HPLC method development.[17] A sample of all four of the possible diastereoisomers **4a**–**4d** was synthesised which could be conveniently prepared from the dihydroxyketone **3** (Scheme 2). Racemic **3** was synthesised via the tertiary amine-catalysed condensation of benzaldehyde with lithium hydroxypyruvate (LiHPA) which has been reported recently in our laboratory.[18] In addition it was noted that if extended reaction times were used, or the product **3** was stored in water for several days, a new product was generated which was postulated to be **6**, formed by the tautomerisation of **3**. The rearranged product was confirmed to be 2,3-dihydroxy-1-phenylpropan-1-one **6**, which was readily prepared via a crossed acyloin condensation between glycolaldehyde and benzaldehyde with catalytic 3-benzyl-5-(2-hydroxyethyl)-4-methyl thiazolium chloride.[19] It was therefore decided to investigate the regioselectivity of the TAm reaction by establishing whether **6** was also a substrate which would generate an isomer of **4**, the 1-amino-2,3-diol.

For synthesis of the triacetylated 2-amino-1,3-diol **5**, the reductive amination of **3** was achieved using sodium cyanoborohydride to give compound **7** in 63% yield, which was converted to the hydrochloride salt. Hydrogenation of the hydrochloride salt gave the desired four diastereoisomers mixture of **4** as a 2:5 mixture of *syn*-**4a** and *syn*-**4b**: *anti*-**4c** and *anti*-**4d** isomers. These were directly acetylated as before to give the mixture **5a**–**5d**, which was used for chiral HPLC method development.

To establish the absolute stereochemistry of reaction products, synthesis of one of the *anti*-isomers was also required. Compound **4d**, the (1*R*,2*S*)-isomer was synthesised (Scheme 3) via modification of a procedure described by Nicolaou et al.[20] Sharpless asymmetric dihydroxylation of ethyl cinnamate yielded the *syn*-diol (2*S*,3*R*)-ethyl-2,3-dihydroxy-3-phenylpropanoate. This was selectively converted to the mononosylate (2*S*,3*R*)-**8** using stoichiometric quantities of nosyl chloride. Although some epimerisation and generation of the (2*R*,3*R*)-isomer of **8** was observed (70% de by NMR spectroscopy) this was not problematical as the material was required for analytical purposes. Subsequent $S_N2$ displacement with $NaN_3$ yielded the azide (in 70% de), then simultaneous reduction of both azide and ester functionalities using $LiAlH_4$ gave the desired *anti*-isomer **4d** in 44% yield over the four steps and in 70% de (1*R*,2*R*-diastereoisomer **4b** also present). A sample of **5d**-(1*R*,2*S*) was obtained by acetylation in 70% de.[17]




**Scheme 2.** Synthesis of the four diastereoisomers of **4**. Reagents and conditions: (a) LiHPA, 3-(4-morpholino)propanesulfonic acid (MOPS), 25%; (b) extended reaction time for (a) or slow conversion in water; (c) $BnNH_2$, $NaBH_3CN$, AcOH, MeOH, 17 h, 63%; (d) 1 M HCl, MeOH, 100%: (e) $H_2$, Pd/C, MeOH, 48 h, 71%; (f) $Ac_2O$, pyridine, 100%.




**Scheme 3.** Synthesis of **4d**. Reagents and conditions: (a) AD-β, $MeSO_2NH_2$, *t*BuOH/$H_2O$ (1:1), rt, 18 h, 72%; (b) NosCl, $Et_3N$, $CH_2Cl_2$, 0 °C, 5 h, 82%; (c) $NaN_3$, DMF, 55 °C, 17 h, 75%; (d) $LiAlH_4$, THF, reflux, 5 h, 100%.

Using the standards synthesised, both standard reverse-phase (RP)-HPLC and chiral HPLC assays were established using **4** (*syn*- and *anti*-isomers) and **5a**–**5d**. Transamination reactions were performed, using *C. violaceum* TAm and cofactor pyridoxal 5'-phosphate (PLP), with ketodiols **3** and **6**, and (*S*)-(α)-methylbenzylamine as the amine donor. Interestingly, no conversion of **6** to 1-amino-2,3-dihydroxy-1-phenylpropane was observed, highlighting the regioselectivity of the transaminase for 1,3-dihydroxy-1-phenylpropan-2-one **3** rather than for 2,3-dihydroxy-1-phenylpropan-1-one **6**. This was also interesting because acetophenone is aminated, and suggested that the 2,3-hydroxy group is unable to fit into one of the binding pockets.

Compound **3** was readily biotransformed and the reactions were monitored by RP-HPLC and LC–MS. After 24 h, the reaction was complete and two products were evident by RP-HPLC in a 1:1 ratio. A series of RP-HPLC experiments with the synthesised analytical standard **4d** and commercially available **4a** and **4b** confirmed that the two products were *syn*- and *anti*-**4**. The transaminase reaction mixture was purified by HPLC to give a sample of *syn*- and *anti*-**4**. Triacetylation as previously described gave **5**, and analysis by chiral HPLC and comparison to **5a**, **5b** and **5d** showed the TAm products to be exclusively (1*S*,2*S*)-**5a** and (1*R*,2*S*)-**5d**, establishing the TAm products as **4a** and **4d** (Scheme 4). No **5b** or **5c** was detectable by chiral HPLC. This indicated that the transaminase could accept either the (*R*)- or (*S*)-1,3-dihydroxy ketone but formed the (2*S*)-amine (in >99% ee by HPLC) in **4**. This is consistent with previous work describing the stereoselectivity of a pyruvate TAm, for which a two-binding site model was proposed, where (*S*)-α-methylbenzylamine was the amine donor.[21]

## 3. Conclusion

Using chemically derived standards we have shown that a recently reported ω-transaminase, isolated from *C. violaceum*, exhibits very high (>99% ee) (*S*)-stereoselectivity in transforming 1,3-dihydroxy-1-phenylpropan-2-one **3** to 2-amino-1-phenyl-1,3-propanediol **4**. The enzyme was not enantioselective for either (*R*)- or (*S*)-**3**, but both enantiomers were readily bioconverted to give a mixture of **4a** and **4d**. The TAm was also regioselective for **3** rather than for **6**. These results are important for applications of the ω-TAm with other α,α′-dihydroxyketones.

The versatility of *C. violaceum* to accept either 1,3-dihydroxyketone enantiomer will also enhance its potential use in applications with enantiomeric precursors such as for two-enzyme step TK-TAm applications.[15] Indeed we have recently reported TK single-point active site mutants that have improved substrate specificities towards propanal, and generate (3*R*)- and (3*S*)-1,3-dihydroxypentan-2-one in high ees.[22,23] Such substrates could be used with TAm to give *syn*- and *anti*-aminodiols in high optical purities. Further studies are now underway using TAm with alternative substrates.

## 4. Experimental

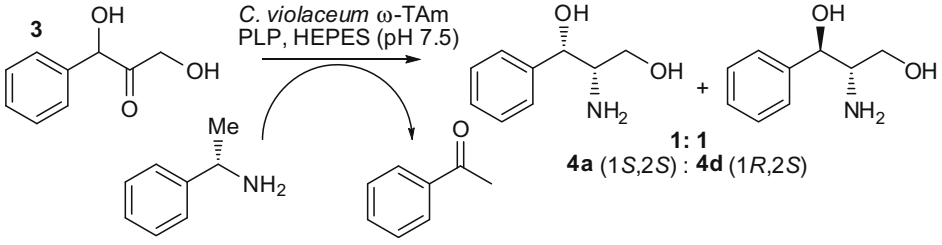
### 4.1. General information

Unless otherwise noted, solvents and reagents were of reagent grade from commercial suppliers (Sigma–Aldrich) and were used without further purification. Benzaldehyde was distilled prior to use. Dry THF was obtained using anhydrous alumina columns.[24] 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 a Bruker AMX300 MHz and AMX500 MHz machine. 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. Infrared spectra were recorded on a Shamadazu FTIR-8700 infrared spectrophotometer. Optical rotations were recorded on an Optical Activity Limited PolAAR2000 polarimeter at 589 nm, and were quoted in deg cm$^2$ g$^{-1}$ and conc (*c*) was quoted in g/100 mL.

Lithium hydroxypyruvate was synthesised as previously described.[25] The ω-transaminase from *C. violaceum* was cloned, over-expressed in *E. coli* and obtained as a clarified lysate as reported elsewhere.[16] Compounds **4a** and **4b** were commercially available. The acetylation of compound **4** was carried out as previously described.[17] Compounds **3**,[18] **6**[19] and (2*S*,3*R*)-ethyl-2,3-dihydroxy-3-phenylpropanoate[26] were prepared as previously reported.

### 4.2. (1*S*,2*S*)- and (1*R*,2*R*)-2-Acetamido-1-phenylpropane-1,3-diyl diacetates 5a and 5b

Compounds **4a** and **4b** were acetylated as previously described[17] to give **5a** and **5b** in quantitative yield for HPLC analysis.




**Scheme 4.** Formation of **4a** and **4d** from **3** using *C. violaceum* TAm, PLP and (*S*)-(α)-methylbenzylamine as the amine donor.

Compound **5a** $[\alpha]_D^{20} = +47.1$ (*c* 2.0, $CHCl_3$); **5b** $[\alpha]_D^{20} = -61.1$ (*c* 2.0, $CHCl_3$); $^1$H NMR (300 MHz; $CDCl_3$) $\delta$ 1.92 (3H, s, NH*Ac*), 2.01 (3H, s, $CH_2OAc$), 2.06 (3H, s, CHO*Ac*), 3.82 (1H, dd, *J* 11.5 and 5.0, CH*H*OAc), 4.03 (1H, dd, *J* 11.5 and 4.8, C*H*HOAc), 4.62 (1H, m, C*H*NHAc), 5.90 (1H, d, *J* 7.1, C*H*OAc), 6.03 (1H, d, *J* 9.2, N*H*), 7.30 (5H, m, Ph); $^{13}$C NMR (75 MHz; $CDCl_3$) $\delta$ 20.6, 20.9 and 23.0 (O*Ac* and NH*Ac*), 52.0 (*C*HNHAc), 63.1 ($CH_2OAc$), 74.1 (*C*HOAc), 126.7, 128.6, 136.5, 149.4, 169.9 (*C*=O), 170.2 (*C*=O), 170.6 (*C*=O); *m/z* (+HRFAB) [MNa] calcd for $C_{15}H_{19}NNaO_5$, 316.11609; found 316.11550.

#### 4.2.1. 2-Benzylamino-1-phenyl-1,3-propanediol 7

Compound **3**[18] (85 mg, 0.512 mmol) and benzylamine (112 μL, 1.024 mmol) were dissolved in MeOH (5 mL). $NaCNBH_3$ (96 mg, 1.536 mmol) was added, the pH was adjusted to 6 with acetic acid and the reaction mixture was stirred at rt for 17 h. The reaction mixture was concentrated to dryness in vacuo and the residue was partitioned between $CH_2Cl_2$ (50 mL) and $NaHCO_3$ (satd) (50 mL). The product was extracted with further $CH_2Cl_2$ (2 × 50 mL), dried ($MgSO_4$) and concentrated, and was then purified using flash silica chromatography (EtOAc) to yield **7** (83 mg, 63%) as a colourless oil. The product was obtained as a 2.6:1 mixture of *anti:syn*[27] isomers. $^1$H NMR (300 MHz; $CDCl_3$) $\delta$ 2.65 (3H, br s, N*H* and O*H*), 2.80 (0.28H, dt, *J* 7.1 and 3.7, C*H*$CH_2$OH), 2.86 (0.72H, m), 3.38 (0.28H, dd, *J* 11.2 and 3.7, C*H*HOH), 3.50 (0.72H, dd, 11.2 and 4.1, C*H*HOH), 3.60 (0.72H, dd, 11.2 and 5.3, CH*H*OH), 3.67 (0.28H, m, CH*H*OH), 3.83 (2H, s, $CH_2$Ph), 4.66 (0.28H, d, *J* 7.1, C*H*OHPh), 4.88 (0.72H, d, *J* 5.0, C*H*OHPh), 7.31 (10H, m, 2 × Ph); $^{13}$C NMR (75 MHz; $CDCl_3$) $\delta$ 51.3 and 51.5 ($CH_2$Ph), 60.0 and 60.3 ($CH_2OH$), 62.7 and 64.0 (*C*H$CH_2$OH), 73.4 and 73.6 (*C*HOHPh), 125.9, 126.6, 127.3, 127.7, 127.8, 128.1, 128.5, 128.6, 139.6, 139.7, 141.3, 141.9; *m/z* (+HRFAB) [MH] calcd for $C_{16}H_{19}NO_2$, 258.149400; found 258.149645.

### 4.3. Diastereoisomeric mixture of 5a–d

Diol **7** (83 mg, 0.323 mmol) was dissolved in MeOH (5 mL) and the pH was adjusted to 1 by the addition of 1 M HCl. The mixture was concentrated to dryness in vacuo to yield a foam which was redissolved in fresh MeOH (5 mL). Pd/C (50 mg, 10% wt) was added and the mixture was subjected to hydrogenation for 2 days. The catalyst was removed by filtration through Celite and the organics were concentrated to yield crude 2-amino-1-phenyl-1,3-propanediol hydrochloride salt which was used without further purification. The crude salt was dissolved in pyridine (3 mL) and $Ac_2O$ (1 mL) and then DMAP (cat.) was added. The reaction mixture was stirred at rt for 17 h, and was then concentrated to dryness in vacuo. The residue was partitioned between EtOAc (20 mL) and 0.3 M $KHSO_4$ (20 mL), washed with $NaHCO_3$ (satd; 20 mL) and dried ($MgSO_4$). Concentration in vacuo gave a residue which was purified using flash silica chromatography (EtOAc/hexane, 1:1) to yield **5a–d** as a colourless oil (67 mg, 71% over 3 steps). The product was obtained as a 2.6:1 mixture of *anti*:*syn* isomers and the spectral data were in agreement with those reported for **5** above and in the literature.[17b]

### 4.4. (2*S*,3*R*) Ethyl 3-hydroxy-2-(4-nitrophenylsulfonyloxy)-3-phenylpropanoate 8

To a solution of (2*S*,3*R*) ethyl-2,3-dihydroxy-3-phenylpropanoate (4.23 g, 20.1 mmol) in $CH_2Cl_2$ (100 mL) at 0 °C were added triethylamine (5.60 mL, 40.2 mmol) and then 4-nitrobenzenesulfonyl chloride (4.45 g, 20.1 mmol). The reaction mixture was stirred for 5 h and was then quenched with the addition of saturated $NH_4Cl$ (18 mL). The organic layer was washed with 1 M HCl (35 mL), water (35 mL) and saturated NaCl solution (35 mL) and was dried ($MgSO_4$). Concentration in vacuo yielded a residue which was purified using flash silica chromatography (EtOAc/petroleum ether 60-80, 1:4 to 1:1 gradient) to give the desired compound (6.53 g, 82%). $[\alpha]_D^{24} = -42.5$ (*c* 3.9, $CHCl_3$); $^1$H NMR (300 MHz; $CDCl_3$) $\delta$ 1.19 (3H, t, *J* 7.2, $CH_3$), 2.58 (1H, d, *J* 5.8, OH), 4.18 (2H, q, *J* 7.2, $CH_2$), 5.02 (1H, d, *J* 3.9, C*H*ONs), 5.22 (1H, dd, *J* 5.8 and 3.9, C*H*OH), 7.22 (5H, m, ArH), 7.81 (2H, d, *J* 9.0, ArH), 8.20 (2H, d, *J* 9.0, ArH); $^{13}$C NMR (125 MHz; $CDCl_3$) $\delta$ 13.9 ($CH_2CH_3$), 62.7 ($CH_2CH_3$), 73.6 (*C*HOH), 82.4 (*C*HONs), 124.2, 126.2, 128.5, 128.7, 129.1, 137.6, 141.5, 150.6, 166.5 (*C*=O); *m/z* (+HRCI) [$MC_2H_5$] calcd for $C_{19}H_{22}NO_8S$, 424.10661; found 424.10582.

### 4.5. (1*R*,2*S*)-2-Amino-1-phenyl-1,3-propanediol (1*R*,2*S*)-4d

Nosylate **8** (8.94 g, 22.6 mmol) was dissolved in DMF (90 mL) and sodium azide was (2.20 g, 33.9 mmol) added. The mixture was stirred at 55 °C for 17 h and was then cooled, diluted with water (200 mL) and extracted with EtOAc (3 × 200 mL). The combined organics were washed with water (200 mL) and then with saturated NaCl solution (200 mL) and were dried ($MgSO_4$). Concentration in vacuo yielded a residue which was purified using flash silica chromatography (EtOAc/petroleum ether 40–60, 5:95 to 1:4 gradient) to give the azide as a yellow oil (4.01 g, 75%) which was directly used in the next step. $[\alpha]_D^{24} = +9.4$ (*c* 11.1, $CHCl_3$); $^1$H NMR (300 MHz; $CDCl_3$) $\delta$ 1.24 (3H, t, *J* 7.1, $CH_3$), 3.21 (1H, br s, OH), 4.08 (1H, d, *J* 6.9, C*H*$CO_2$Et), 4.22 (2H, q, *J* 7.1, $CH_2CH_3$), 5.00 (1H, dd, *J* 6.9 and 4.7, C*H*OH), 7.33 (5H, m, ArH); $^{13}$C NMR (75 MHz; $CDCl_3$) $\delta$ 14.0, 62.2, 66.8, 74.1, 126.7, 128.6, 128.7, 139.1, 168.9 (*C*=O). To lithium aluminium hydride (0.820 g, 21.7 mmol) in THF (100 mL) was added the azide (0.850 g, 3.61 mmol) in THF (50 mL) cautiously. The reaction mixture was heated under reflux for 5 h before being quenched with water and filtered. The solvent was removed in vacuo to give **4d** as a yellow oil (0.600 g, 100%). $[\alpha]_D^{24} = -14.9$ (*c* 2.0, MeOH); $^1$H NMR (300 MHz; $D_2O$) $\delta$ 3.04 (1H, m, C*H*$NH_2$), 3.51 (1H, dd, *J* 11.2 and 6.8, $CH_2OH$), 3.70 (1H, dd, *J* 11.2 and 3.4, $CH_2OH$), 4.46 (1H, d, *J* 7.8, C*H*OHPh); $^{13}$C NMR (75 MHz; $D_2O$) 56.9 (*C*H$NH_2$), 63.4 ($CH_2OH$), 75.2 (*C*HPh), 126.9, 128.7, 129.1, 141.1; *m/z* (+HRES) [MNa] calcd for $C_9H_{13}NO_2Na$, 190.0844; found 190.0917.

### 4.6. (1*R*,2*S*)-2-Acetamido-1-phenylpropane-1,3-diyl diacetate 5d

Compound **4d** was acetylated as described above to give **5d** in quantitative yield. $[\alpha]_D^{20} = -19.4$ (*c* 1.0, $CHCl_3$); $^1$H NMR (300 MHz; $CDCl_3$) $\delta$ 1.88 (3H, s, NH*Ac*), 2.01 (3H, s, $CH_2OAc$), 2.10 (3H, s, CHO*Ac*), 3.98 (1H, dd, *J* 11.6 and 4.1, $CH_2OAc$), 4.32 (1H, dd, *J* 11.6 and 6.4, $CH_2OAc$), 4.65 (1H, m, C*H*NHAc), 5.80 (1H, d, *J* 9.1, N*H*), 5.88 (1H, d, *J* 5.8, C*H*OAc);[17b] $^{13}$C NMR (75 MHz; $CDCl_3$) $\delta$ 20.8, 21.0, 23.2 (O*Ac* and NH*Ac*), 51.6 (CHNHAc), 62.4 ($CH_2OAc$), 74.6 (CHOAc), 126.6, 128.6, 136.5, 141.8, 169.7, 169.8, 171.0 (*C*=O); Found (+HRFAB) $MNa^+$, 316.11654. $C_{15}H_{19}NNaO_5$ requires 316.11609.

### 4.7. RP HPLC assay for TAm-mediated synthesis of 4

The transaminase reaction was analysed using an ACE 5 C18 reverse phase column (150 mm × 4.6 mm, 5 μm particle size; Advanced Chromatography techniques, Aberdeen). A gradient was used from 5% $CH_3CN$/95% 0.1% (v/v) TFA in water to 20% $CH_3CN$/80% 0.1% (v/v) TFA in water, over 8 min followed by a re-equilibration step for 2 min (oven temperature 30 °C, flow rate 1 mL/min). UV detection was carried out at 210 and 250 nm. The retention times were: **4a/4b** 5.73 min; **4c/4d** 5.25 min. All samples were quenched with 0.2% TFA and were briefly centrifuged prior to HPLC analysis.

### 4.8. Chiral HPLC assay for 5

HPLC analysis was performed on a Varian Prostar instrument equipped with a Chiracel AD chiral column (Daicel, 25 cm × 0.46 cm). HPLC conditions: injection volume, 10 μL; mobile phase, $^{i}$PrOH–hexanes, 3:97; flow rate, 0.6 mL/min; detection, 210 nm. Retention times of **5**: (1*R*,2*R*)-**5b**, 74.4 min; (1*S*,2*S*)-**5a**, 77.1 min; (1*R*,2*S*)-**5d**, 99.8 min; (1*S*,2*R*)-**5c**, 105.4 min.

### 4.9. Transaminase-mediated synthesis of 4

1,3-Dihydroxy-1-phenylpropan-2-one[18] (33 mg, 0.20 mmol) and (*S*)-α-methylbenzylamine (24 mg, 0.20 mM) were dissolved in HEPES buffer containing pyridoxal-5′-phosphate (7.5 mL, HEPES 100 mM, PLP 0.2 mM, pH 7.5). To this was added *C. violaceum* TAm clarified lysate (2.5 mL, TAm 8.5 mg/mL, HEPES 50 mM, PLP 0.2 mM, pH 7.5) and the reaction mixture was incubated for 24 h at 37 °C without agitation. A portion of the product mixture was purified by C18 RP-HPLC to yield the desired product as a white solid (1 mg) (*m/z* (+HRCI) [MH] calcd for $C_9H_{14}NO_2$, 168.10245; found 168.10212). The product **4** was redissolved in pyridine (600 μL) and $Ac_2O$ (200 μL) and then DMAP (cat.) was added. After 17 h, the solvent was removed in vacuo and the sample of **5** was analysed by chiral HPLC without further purification. The product was shown to be exclusively a 1:1 mixture of (1*S*,2*S*)-**5a** and (1*R*,2*S*)-**5d**.

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