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Synthesis of Aromatic 1,2-Amino Alcohols Utilizing a Bienzymatic Dynamic Kinetic Asymmetric Transformation

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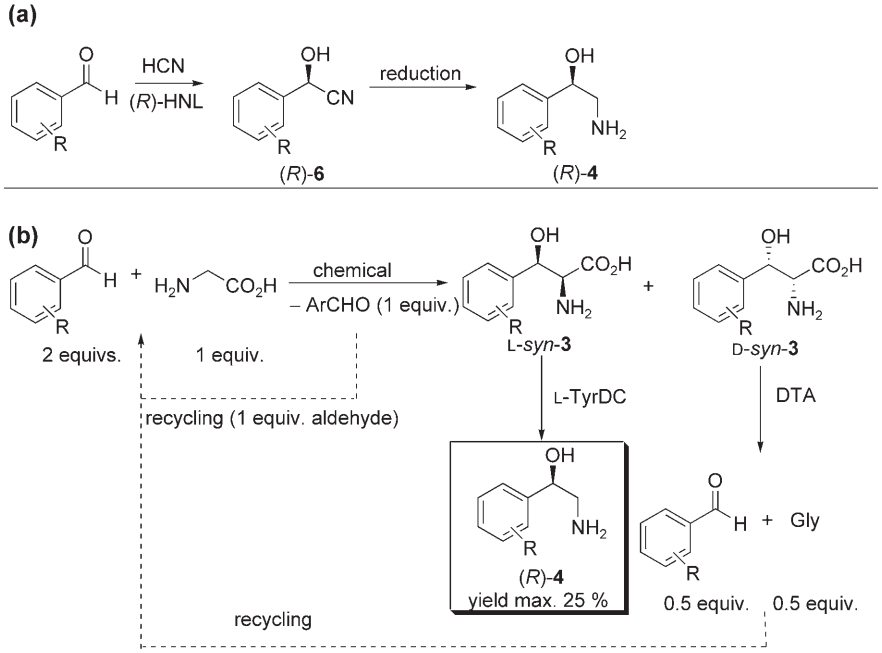
Abstract: The applicability of the recent published bienzymatic protocol for the synthesis of (*R*)-2-amino-1-phenylethanol was tested using L-threonine aldolase from *Pseudomonas putida* and L-tyrosine decarboxylase from either *Enterococcus faecalis* (Efa) or two genes from *Enterococcus faecium* (Efi1, Efi2). In all 21 benzaldehyde derivatives were applied for an initial TLC screening. On a small scale, octopamine and noradrenaline were obtained as (*S*)-enantiomers using Efi1. Three protocols were upscaled yielding enantioenriched (*S*)-octopamine (yield 99%, *ee* 81%), (*R*)-2-amino-1-phenylethanol (yield 61%, *ee* 62%) and (*S*)-noradrenaline (yield 76%, *ee* 79%).

Keywords: aldol reaction; amino alcohols; biocatalysis; lyases; threonine aldolase; tyrosine decarboxylase

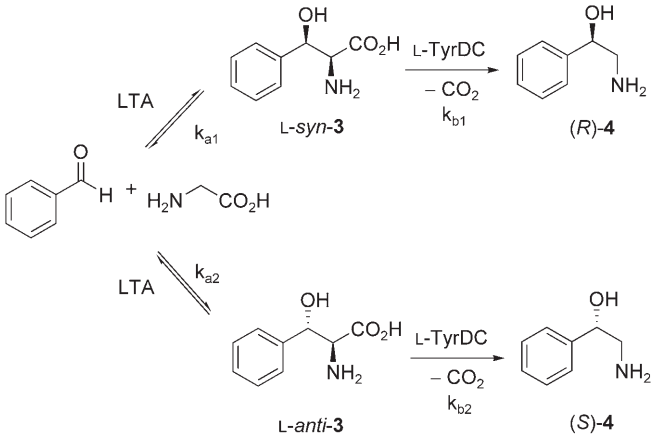
Introduction

The 1,2-amino alcohol moiety is a widespread structural motif in natural and synthetic biologically active molecules. Especially aromatic 1,2-amino alcohols constitute an important class of compounds such as octopamine (**1**),[1] noradrenaline (**2**),[2] levabuterol,[3] terbutaline,[2] and norfenefrine.[4] To date a wealth of synthetic procedures for the production of vicinal amino alcohols has been developed.[5] The classical synthetic route to enantiopure compounds is *via* an HNL-catalyzed synthesis of enantiopure cyanohydrins and further reduction by using $NaBH_4$ or $LiAlH_4$ [Scheme 1 (**a**)].[6] However, the toxicity of HCN/cyanohydrins and the low yield due to the laborious work-up of the final amino alcohol have prevented broad industrial applications. Recently, a novel approach using catalytic hydrogenation of cyanohydrin esters and an ensuing intramolecular acyl-transfer seemed promising especially if *N*-acylated 1,2-amino alcohols were the desired products.[7] Other synthetic strategies comprise the ring-opening of an appropriate enantiopure epoxide[8] and the stereoselective reduction of a corresponding ketone precursor.[9] The decarboxylation of phenylserine derivatives using L-tyrosine decarboxylase (L-TyrDC) has recently been patented.[10] L-Amino acid decarboxylases are known to be highly substrate-specific. Only L-TyrDC tolerates an additional β-hydroxy group, is sufficiently flexible for the bulky aromatic substituents and converts L-phenylserine (L-**3**) to 2-amino-1-phenylethanol (**4**) at high rates.[10] Consequently, L-TyrDC was utilized to degrade chemically synthesized DL-*syn*-**3** mixtures to (*R*)-**4** with a maximum yield of 25%, leaving D-*syn*-phenylserine unchanged.[10] Additionally, D-threonine aldolase (DTA) was utilized in a bienzymatic parallel kinetic resolution protocol of DL-*syn*-**3** to recycle the non-reacting enantiomer (D-*syn*) by cleaving it to benzaldehyde and glycine, which re-enter the chemical production process [Scheme 1 (**b**)].[11]

We recently reported on an alternative one-pot route to (*R*)-**4** applying a novel dynamic kinetic asymmetric transformation (DYKAT)[12] concept starting from cheap compounds – glycine and benzaldehyde (Scheme 2).[13] The low diastereoselectivity and the thermodynamic limitation (moderate yield) of L-threonine aldolase (LTA from *Pseudomonas putida*)[14] catalyzed synthesis of phenylserine derivatives was overcome by the combination with L-TyrDC from *Enterococcus faecalis*[15] (Efa) to give enantioenriched (*R*)-**4**.[13] Herein we report on the successful application of another L-TyrDC from *Enterococcus faecium* (Efi1) and the extension of the substrate spectrum to substituted benzaldehydes.



Scheme 1. Production of (*R*)-**4** using a (**a**) two-step protocol,[6] and (**b**) a bienzymatic parallel kinetic resolution of chemically synthesized L/D-*syn*-**3**.[11]



Scheme 2. Synthesis of **4** applying a one-pot/two-enzymes DYKAT.

Results and Discussion

L-Tyrosine Decarboxylases: Overexpression and Characterization

Biogenic tyramine production has been intensively studied in food due to the toxicological effects derived from their vasoactive and psychoactive properties. The formation of tyramine in foods depends on the concentration of free tyrosine and the presence of microorganisms having tyrosine decarboxylase activity. Thus, in detailed studies tyramine activity was found in archaea,[16] some bacteria belonging to the genera *Enterococcus*,[15a] *Carnobacterium*[17] and *Lactobacillus*[18] as well as higher organisms such as plants,[19] insects[20] and mammals.[20] While L-TyrDCs from eukaryotes have been well characterized, little is known about their counterpart in prokaryotes. So far, only L-TyrDC from *Lactobacillus brevis* IOEB 9809[18] and *Enterococcus faecalis*[15] were purified and characterized. Interestingly, the L-TyrDC activity was described as a differentiation method for Enterococci – only *E. faecalis* should be capable of tyramine production. Recently, this was disproved by Muñoz et al. – also *E. faecium* produces tyramine at high tyrosine concentrations.[21] For our study, three tyrosine decarboxylases were found in Enterococci utilizing sequence comparison: L-TyrDC from *Enterococcus faecalis*[15] V583 (Efa) and two genes from *Enterococcus faecium* (Efi1 and Efi2) with 91 and 76% homology to Efa, respectively (Figure 1). Efa was shown to be selective for L-*syn*-**3** whereas Efi1 and Efi2 showed *anti*-selectivity (Table 1) – implying a broad application for the synthesis of either (*R*)- or (*S*)-amino alcohols starting from the same enantiopure phenylserine diastereomers (L-*syn*/*anti*). The pH optima of Efa and Efi1 were elucidated to be 5.5 whereas Efi2 was active in a lower pH region with an optimum at pH 4.5.

In addition to the three depicted L-TyrDCs (Figure 1) a purchased L-TyrDC from *Streptococcus faecalis* (prior to 1984, *E. faecalis* was known as *Streptococcus faecalis*)[24] (Efa-S from Sigma–Aldrich) was tested on the synthetic applicability for the decarbox-

Figure 1. Sequence alignment of L-tyrosine decarboxylase from *Enterococcus faecalis* (Efa) and two genes from *Enterococcus faecium* (Efi1, Efi2). The alignment was made with Clustal W (http://www.ebi.ac.uk/clustalw/).[22]

Table 1. Characteristics of *Enterococci* L-TyrDCs.[a]

Substrate	pH range	**Efa** 4.0–8.0 (5.5)	**Efi1** 4.5–7.5 (5.5)	**Efi2** 4.0–6.0 (4.5)
L-*syn*-**3**	sp. act. [U/mg]	0.16	0.07	0.01
	K_M [mM]	188	76	71
	v_{max} [U/mg]	0.42	0.08	*ca.* 0.01
L-*anti*-**3**	sp. act. [U/mg]	0.01	0.02	0.01
	K_M [mM]	12	35	35
	v_{max} [U/mg]	0.02	0.05	*ca.* 0.02
E[a]		1.4	1.3	1.7
major		*syn*	*anti*	*anti*

[a] Based on one measurement implying an error margin of 20%.

[b] *Pseudo* E value:[23] $E = (v_{max}/K_M)_{major}/(v_{max}/K_M)_{minor}$.

ylation of DL-*syn*-**3** and DL-*anti*-**3**. All L-TyrDCs catalyzed the decarboxylation of *syn*- and *anti*-**3** (Table 2). Efa showed the highest synthetic *syn*/*anti* ratio (entry 3 vs. 4, conversion after 20 h) of L-*syn*-**3**, and the reaction was complete after 20 h, whereas the initially more active Efi1 (1 h) gave **4** with a conversion of 28% (20 h, entry 5) and lower selectivity (entry 5 *vs.* 6). Efa-S gave lower selectivity (entry 1 *vs.* 2) than our overexpressed Efa while Efi2 showed the lowest *syn*/*anti* discrimination (entry 7 *vs.* 8).

Bienzymatic Synthesis of 1,2-Amino Alcohols

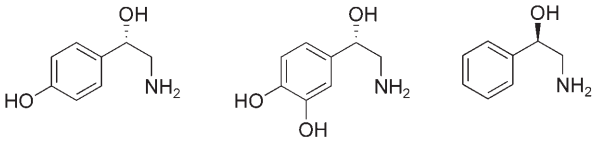
We tested the compatibility and the pH-dependency of the coupled LTA/L-TyrDC (Efa) reaction on the synthesis of (*R*)-**4** (Figure 2). We were able to show that reactions at pH 5.5 gave initially less formation of intermediate L-**3** than at higher pH but an increased conversion of (*R*)-**4** up to 76% with high selectivity after 30 h [*ee* 75% (*R*)]. The overall enantioselectivity of (*R*)-**4** was shown to be pH-independent and stable over a time-course of 58 h. Stable selectivities of the intermediate and product imply that the dynamic system is efficient.

TLC Screening

In addition, substituted aromatic aldehydes were screened using simple TLC detection (Table 3). The R_f values of glycine, the intermediate and the final amino alcohol are significantly different. Thus, we showed that 3- and 4-hydroxy-, and 2- and 3-fluorobenzaldehydes gave high conversion, presumably because they are sterically small or mimic the natural substrate tyrosine whereas the conversion of bulky groups such as bromo- or nitro substituents was low. This list gives important hints for the steric and electronic demand on the substrates of L-TyrDC, while most substrates have already been tested on the LTA-catalyzed synthesis of phenylserine derivatives.[17]

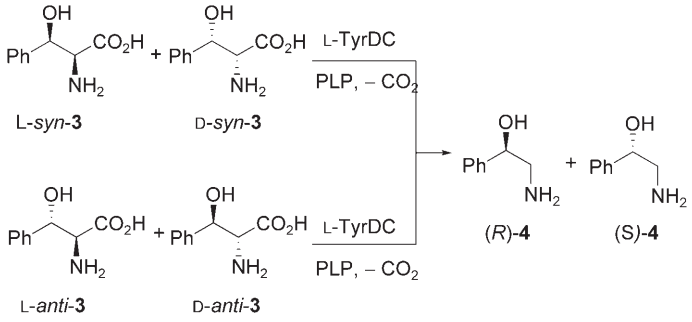
Synthesis of 1, 2 and 4

Octopamine (**1**) is biologically active and an important target for industrial products and therefore we subjected 4-hydroxybenzaldehyde to our bienzymatic process utilizing LTA and L-TyrDC. L-TyrDC from



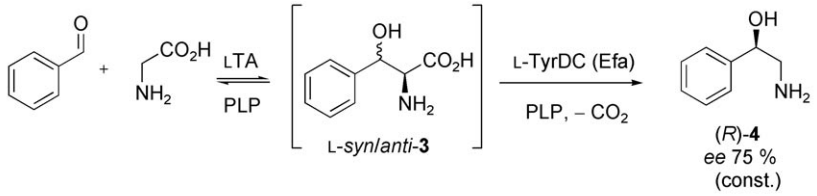
(*S*)-**1** (*S*)-**2** (*R*)-**4**

Table 2. Decarboxylation of **3**[a].



		3	4 [mM],[b] 60 min	4 [mM],[b] 20 h	activity [U/mL][c]	spec. activity [U/mg]	*syn/anti*[d]
1	Efa-S	DL-*syn*	0.56	5.72	0.093	0.009	5.6
2	Efa-S	DL-*anti*	0.18	1.03	0.030	0.003	
3	Efa	DL-*syn*	11.3	46.3	1.85	0.015	9.7
4	Efa	DL-*anti*	0.74	4.75	0.123	0.001	
5	Efi1	DL-*syn*	4.42	28.4	0.737	0.049	3.0
6	Efi1	DL-*anti*	1.82	9.62	0.303	0.020	
7	Efi2[e]	DL-*syn*	0.82	14.4	0.136	0.010	2.0
8	Efi2[e]	DL-*anti*	0.51	7.30	0.085	0.007	

[a] *Conditions:* L-**3** 50 mM, D-**3** 50 mM, PLP 50 μM, pH 5.5, 30 °C.
[b] Determined by HPLC.
[c] Based on one measurement implying an error margin of 20 %.
[d] *syn/anti* (conversion of **4** at 20 h).
[e] pH 4.5.



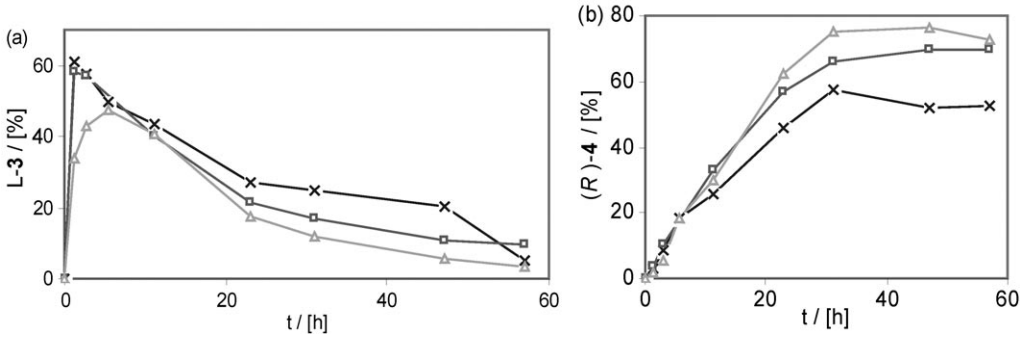
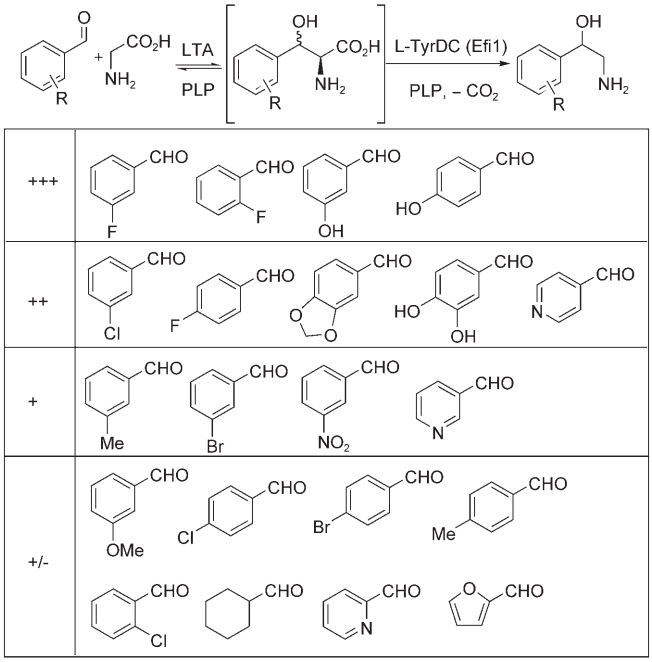


Figure 2. Bienzymatic synthesis of (*R*)-**4** at pH 6.5 (×), 6.0 (□) and 5.5 (△); *conditions:* 1 mL scale, benzaldehyde 100 mM, glycine 1 M, PLP 50 μM, 25 °C, LTA 19 U, L-TyrDC (Efa) 0.4 U; conversion and *ee* determined by HPLC.

Enterococcus faecalis (Efa) showed higher diastereoselectivity for the synthesis of (*R*)-**4** than L-TyrDC from *Enterococcus faecium* (Efi1, Efi2) and, thus, Efa was chosen for initial investigations. 4-Hydroxybenzaldehyde was shown to be a poor substrate for LTA (conversion <20 %) due to its reduced electrophilic properties and steric hindrance.[14b] However, the bienzymatic synthesis of **1** occurred at a rate comparable to benzaldehyde which is a much better substrate for LTA (Figure 3). This can be explained by the additional 4-hydroxy group in **5** which mimics the natural substrate of L-TyrDC and hence, its activity for L-TyrDC is increased as compared to **3**. After 58 h, 67 % of the amino alcohol was detected although the

Table 3. Synthesis of substituted amino alcohols.[a]



[a] *Conditions:* 0.2 mL scale, aldehyde 0.2–0.5 M, glycine 1.3 M, PLP 0.13 mM, pH 6.0, room temperature, LTA 0.6 U, L-TyrDC 0.04 U (Efi1). The outcome of the reaction was classified by a qualitative comparison of spot size and intensity (+++: as benzaldehyde, +/−: faint spot).

concentration of the phenylserine derivative intermediate **5** never exceeded 20%. Finally, three acceptor aldehydes were tested on all three overexpressed L-TyrDCs (Efa, Efi1, Efi2, Table 4). Low conversions of amino alcohols were detected (<5%) when Efi2 was utilized due to its low pH optimum of pH 4.5 accompanied by the reduced activity of LTA at such a low pH.[14b] The bienzymatic process was optimized for the synthesis of **2** focusing on substrate concentrations and utilized enzymes (Table 4).

As expected, a high excess of glycine over the aldehyde improved the conversion. A 10- to 20-fold excess of active LTA (7–9 U/mL) compared to L-TyrDC (0.3–1 U/mL) gave the best results. In this upscaled process, Efi1 was found to be the best decarboxylase and the optimized reaction parameters (Table 4) were tested on 3,4-dihydroxybenzaldehyde, benzaldehyde and 4-hydroxybenzaldehyde yielding noradrenaline (**2**), 2-amino-1-phenylethanol (**4**) and octopamine (**1**), respectively. (*R*)-**1** was synthesized using a two-step protocol [see Scheme 1 (**a**)]: synthesis of (*R*)-2-(4-hydroxyphenyl)-2-hydroxyacetonitrile **6** and ensuing reduction to (*R*)-**1**) as analytical reference material. Interestingly, for both hydroxy-substituted amino alcohols (**1** and **2**) the (*S*)-enantiomer was obtained with high *ee* whereas for benzaldehyde the (*R*)-enantiomer was preferred with lower *ee*. The enantiopreference of (*R*) or (*S*) can be explained by altered diastereoselectivities of Efi1. The aldol reaction provides a fast epimerization whereas the decarboxylase prefers one diastereomer over the other. Quantitative conversion of **1** was achieved after only 17 h in an up-scaled process (160 mL). Non-optimized work-up gave (*S*)-octopamine (**1**) with good yield (47%).

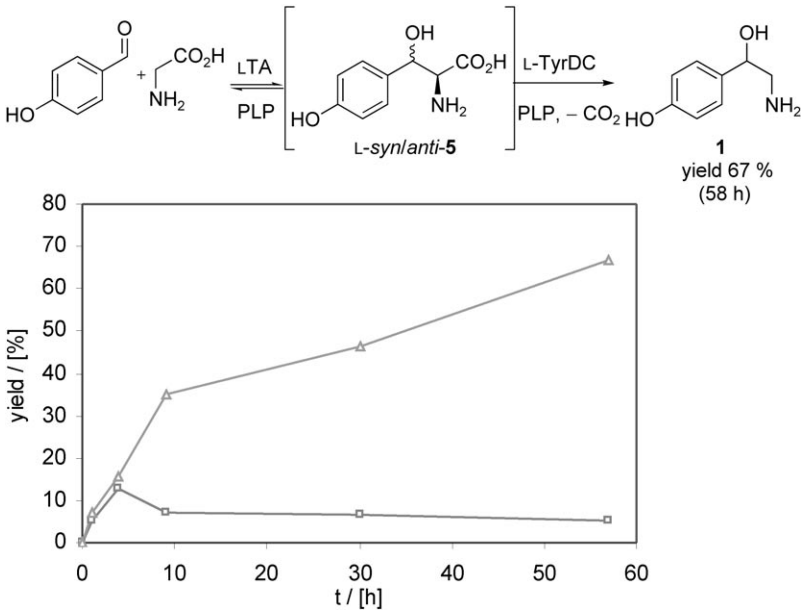
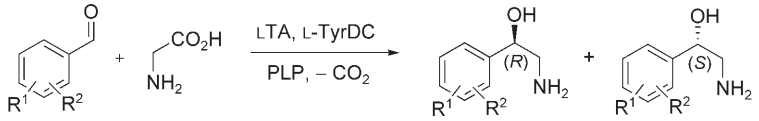


Figure 3. Synthesis of **1** (△) and intermediate **5** (□); *conditions:* 1 mL scale, aldehyde 100 mM, glycine 1 M, PLP 50 μM, pH 5.5, 25 °C, LTA 38 U, L-TyrDC 0.4 U (Efa); conversion determined by HPLC.

Table 4. Optimized conditions for the synthesis of noradrenaline (**2**) and application on the up-scaled synthesis of (*S*)-**2**, (*R*)-**4** and (*S*)-**1** at room temperature.



Reaction parameters	Optimized	R^1=3-OH, R^2=4-OH	R^1=R^2=H	R^1=4-OH, R^2=H
25–200 mM aldehyde	50 mM	25 mL	25 mL	160 mL
0.25-2.5 M glycine	2.5 M	*t* 89 h	*t* 89 h	*t* 17 h
1.5–6.0 U mL^{-1} LTA	7–9 U/mL	**2**	**4**	**1**4
Efa or Efi1	Efi1	analytical yield 76%	analytical yield 61%	analytical yield 99%
0.5–3.0 U mL^{-1} L-TyrDC	0.3–1 U/mL	*ee* 79% (*S*)	*ee* 61% (*R*)	*ee* 81% (*S*)
cosolvent: DMSO	10% (v/v)			isolated yield 47%

Conclusions

The TLC screening of 21 substituted benzaldehydes showed the broad applicability of this bienzymatic protocol. In more detailed investigations octopamine (**1**) and noradrenaline (**2**) were obtained as (*S*)-enantiomers whereas 2-amino-1-phenylethanol (**4**) was obtained in the (*R*)-form. This protocol was successfully up-scaled to 160 mL. These results clearly show that the combination of LTA and LTyrDG provides enantioenriched amino alcohols at high conversion and thus, other benzaldehyde derivatives will be investigated in the near future.

Experimental Section

General Experimental Procedures

All reagents including *rac*-**1**, (*R*)-**2**, (*S*)-**2**, *syn*-**3**, (*R*)-**4**, (*S*)-**4** and solvents were obtained from commercial sources and appropriately purified, if necessary. ^{1}H and ^{13}C NMR spectra were recorded on a Varian INOVA 500 (^{1}H 499.82 MHz, ^{13}C 125.69 MHz) or on a Varian GEMINI 200 (^{1}H 199.98 MHz, ^{13}C 50.29 MHz) using the residual peaks of $CDCl_3$ (^{1}H: δ = 7.26, ^{13}C δ = 77.0) or D_2O (^{1}H: δ = 4.79) as references. Analytical HPLC was carried out with a Hewlett Packard Series 1100 HPLC using a G1315A diode array detector. 2-Amino-1-phenylethanol (**4**) and its derivatives (**1** and **2**) were analyzed on a Crownpack® Cr (+) (150 mm, 5 μm) column under standard conditions ($HClO_4$ solution pH 1.0, 114 mM, 1.0 mL/min, 15 °C).[13] DL-*anti*-**3** was synthesized as reference material using published methods.[25] Optical rotations were measured on a Perkin–Elmer 341 polarimeter. GC analyses were performed according to published data[26] using a Hewlett Packard 6890 instrument equipped with a gammaTFA column and an FID. All amino alcohols are known compounds and thus, no characterization using high-resolution MS or microanalysis was undertaken.

General Method for a Bienzymatic DYKAT: Synthesis of 4

To a solution of benzaldehyde (10 mg, 0.1 mmol), glycine (75 mg, 1.0 mmol) and pyridoxal 5′-phosphate (13 ng, 50 nmol) in 1.0 mL buffer (KH_2PO_4, 50 mM, pH 5.5) LTA (38 U) and L-TyrDC (Efa: 0.4 U) were added. The reaction mixture was stirred at 25 °C; conversion, *de* and *ee* were determined by HPLC.

Synthesis of Substituted Amino Alcohols: TLC Screening

To a sodium phosphate buffer (180 μL, pH 6.0, 0.27 M) containing PLP (0.13 mM) and glycine (19 mg, 0.25 mmol) was added an aldehyde solution (20 μL, 0.25–0.5 M in DMSO). The reaction was started by addition of LTA (0.6 U) and L-TyrDC (Efi1, 0.04 U). The solutions were stirred for 1–3 days at room temperature and formation of the corresponding substituted phenylserines and amino alcohols was monitored by thin-layer chromatography on silica-coated glass plates. The substituted amino alcohols were synthesized as reference materials using a two-step approach.[6] R_f values: amino alcohols at R_f = 0.6–0.7, substituted phenylserines at R_f = 0.2–0.3, glycine at R_f = 0 (eluent: dichloromethane/methanol/25% aqueous ammonia 75/20/5 (v/v/v); ninhydrin staining).

(*R*)-2-(4-Hydroxyphenyl)-2-hydroxyacetonitrile [(*R*)-6]

4-Hydroxybenzaldehyde (1.8 g, 15 mmol) was dissolved in 2.1 mL TBME and (*R*)-HNL from *Prunus amygdalus* 1780 U, activity assay[26]) was added. Then the solution was set to a pH of 3.4 with citric acid. After addition of 1.2 mL of freshly prepared HCN (0.8 g, 31 mmol) the solution was stirred at 0 °C for six hours. The reaction mixture was evaporated to give crude product (*R*)-**6**; yield: 883 mg (40%); NMR data was consistent with those reported;[27] GC (gammaTFA) 1 bar H_2, 175 °C isotherm, t_R = 3.56 min, t_S = 3.75 min., *ee* 79% (GC); $[\alpha]_D^{20}$: +30.8 (*c* 1.0, EtOH); lit.[28] $[\alpha]_D^{20}$: +26.0 (*c* 5.12, AcOEt).

(*R*)-Octopamine [(*R*)-1]

(*R*)-**6** (800 mg, 5.4 mmol) was dissolved in dry THF (34 mL) and $LiAlH_4$ (307 mg, 8.1 mmol) was added, the mixture was stirred at room temperature overnight. The ice-cooled reaction mixture was quenched with Na_2SO_4 solution (saturated, 5 mL), the pH brought to >10 and the precipitate filtered off. The filtrate was evaporated and purified by column chromatography with dichloromethane/methanol/25% aqueous NH_3 in a ratio of 75/20/5 (v/v/v) as eluent to give solid (*R*)-**1**; yield: 128 mg (16%). (*R*)- and (*S*)-**1** were not separated on a Crownpack® Cr (+) (150 mm, 5 μm) column and thus, for *ee* determination a Chirosil® RCA (+) crown ether column was utilized under standard conditions [10 mM CH_3COOH in water/CH_3CN with 10 mM CH_3COOH = 90/10 (v/v%), 1.6 mL/min, 50 °C]; HPLC: $t_{(R)\text{-}1}$ = 7.3 min, $t_{(S)\text{-}1}$ = 8.4 min; $[\alpha]_D^{20}$: −36.1 (*c* 0.56, H_2O); lit.[29] $[\alpha]_D^{20}$: −37.6 (*c* 0.56, H_2O); NMR data were consistent with those reported.[29]

(*S*)-Octopamine [(*S*)-1]

4-Hydroxybenzaldehyde (0.977 g, 8.0 mmol) was dissolved in DMSO (16 mL) and mixed with glycine (30 g, 0.4 mol) together with LTA (1400 U) and L-TyrDC (Efi1, 40 U) in citrate/phosphate buffer pH 6.0 (37 mM citric acid, 126 mM Na_2HPO_4). The mixture was incubated in a 250 mL round-bottom flask with stirring at room temperature. The reaction mixture was acidified to pH 1–2, and precipitated protein was removed by centrifugation. After titration to pH 3 an ultrafiltration was applied (Amicon 8050 stirred cell, YM-10 membrane, Millipore). The ultrafiltrate was concentrated to 0.1 L under vacuum, acetone was added, and the mixture was stored at −20 °C for 1 h. Precipitated glycine was filtered off, and the filtrate was concentrated to a volume of 40 mL. After adjusting to pH 10.5 with aqueous NaOH (30%), the solution was evaporated leaving a liquid residue that was treated with ethyl acetate. Precipitated solids were filtered off, and the filtrate was evaporated under vacuum. The remaining liquid was purified by column chromatography with dichloromethane/methanol/25% aqueous NH_3 in a ratio of 75/20/5 (v/v/v) as eluent to give solid (*S*)-**1**; yield: 574 mg (47%); $[\alpha]_D^{20}$: +27.7 (*c* 0.55, H_2O); for (*R*)-**1**: lit.[29] $[\alpha]_D^{20}$: −37.6 (*c* 0.56, H_2O). This corresponds to an *ee* of 74% for (*S*)-**1**, which is in agreement with the *ee* value determined by chiral HPLC analysis of 81%. NMR data were consistent with those reported.[29]

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