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Interfacing Whole Cell Biocatalysis with a Biocompatible Pictet-Spengler Reaction for One-Pot Syntheses of Tetrahydroisoquinolines and Tryptolines**

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Biocatalytic processes are highly selective and specific. However, their utility is limited by the comparatively narrow scope of enzyme-catalysed transformations. To expand product scope, we are developing biocompatible processes that combine biocatalytic reactions with chemo-catalysis in single-flask processes. Here, we show that a chemocatalysed Pictet-Spengler annulation can be interfaced with biocatalysed alcohol oxidation. This two-step, one-pot cascade reaction converts tyramine and aliphatic alcohols to tetrahydroisoquinoline alkaloids in aqueous buffer at mild pH. Tryptamine derivatives are also efficiently converted to tryptolines. Optimization of stoichiometry, pH, reaction time, and whole-cell catalyst deliver the tetrahydroisouinolines and tryptolines in >90% and >40% isolated yield, respectively, with excellent regioselectivity.

Introduction

Biocatalysis offers several advantages over traditional synthesis. Microbes can upgrade renewable feedstocks and perform multi-step transformations with built-in co-factor regeneration,[1] while enzymes provide exquisite chemo-, regio-, and stereo-control.[2] These transformations are typically carried out in mild, aqueous conditions. Yet broad application of biocatalysis is limited by the narrow scope of enzyme and whole-cell catalysed transformations. Enzymes are inherently selective in what substrates they will accommodate and transform to minimize off-target reactivity and achieve high levels of control, while whole-cell catalysts have mass transfer and toxicity limitations.[3] These and other factors limit the scope of products that can be accessed through biosynthetic means alone.

To expand product scope, recent efforts have sought to combine biocatalytic reactions with chemo-catalysis in single-flask processes.[4–7] This one-pot approach expands the limited scope of biocatalysis alone while avoiding organic solvents and harsh conditions. Some tandem systems use engineered microbes to produce non-native metabolites for use in subsequent biocompatible reactions.[8–10] In these systems, the microbe is tailored to produce the necessary substrate of the abiotic reaction. However, the reliance on transition metal catalysts and designer microbes makes them less sustainable and less accessible. Alternatively, the chemical transformation can be adapted to accommodate an existing bioconversion process or metabolite. This approach involves re-designing classic synthetic chemical transformations to function in the presence of live cells or enzymes.

We have initiated a program that upgrades alcohol substrates in cellular-compatible conditions. Fermentation affords easy access to diverse alcohols from inexpensive feedstocks.[11–13] These alcohol products generally require distillation before they are used elsewhere. However, results from our group and others suggest that native metabolites can potentially be upgraded directly in the fermentation broth, obviating the need for energy-intensive distillation. Molinari and co-workers have shown that *Gluconobacter oxydans* oxidizes primary alcohols to aldehydes, which can undergo a biocompatible condensation with hydroxylamine to form oximes.[14] Our group has shown that *G. oxydans* and *Komagataella pastoris* oxidize alcohols to aldehydes, which are then upgraded in the same flask with lysine organocatalysis to afford α,β-unsaturated aldehydes.[15,16] A recent comprehensive screen by Dennis *et al.* revealed that tyramine is a more effective organocatalyst than lysine for biocompatible aldol condensations.[17] We have also expanded the scope of biocompatible reactions by coupling biocatalysed alcohol oxidation to a three-step Henry-dehydration-Michael cascade to deliver 1,3-dinitroalkanes.[18] Nevertheless, despite this recent progress, there are a dearth of reactions and guiding principles to adapt synthetic transformations to biocompatible conditions.

The Pictet-Spengler reaction is a key synthetic transformation for the synthesis of annulated hetereocycles, which form the core of a range of natural metabolites, antibiotics, and drug molecules. In the Pictet-Spengler reaction, β-arylethylamines condense with an aldehyde or ketone to yield an imine intermediate, followed by nucleophilic attack from an electron-rich aromatic ring to yield an annulated product. This reaction

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is commonly used to convert tryptamines and tyramines to furnish tryptolines and tetrahydroisoquinolines, respectively. Often, acid catalysts are used to protonate the imine to form an iminium, which is sufficiently electrophilic to promote ring closure.[19] Additionally, Pictet-Spengler reactions typically use organic solvents and high temperatures, neither of which are appropriate for biocompatible conditions.

However, milder conditions exist. Bates and coworkers studied the aqueous Pictet-Spengler reactions of formaldehyde and acetaldehyde with 3-hydroxyphenylethylamines.[20–22] These substrates deliver tetrahydroisoquinoline products under acidic to mildly-alkaline conditions and at 20 °C, albeit with excess (800 mol%) aldehyde. An intriguing finding was that these reactions occur through two pH-dependent mechanisms. Under acidic conditions, the 3-hydroxy group stabilizes the positive charge upon attack of the iminium. This mechanism strictly directs ring closure to the position *para* the hydroxy group. Under mildly-acidic to mildly-alkaline conditions, 3-hydroxyphenylethylamines are instead activated by their deprotonated 3-hydroxy group, which enables ring closure at both the *para* and *ortho* positions. The *ortho* position is presumably made accessible via the coulombic attraction between the iminium cation and phenolate anion. The rate of this latter process increases with deprotonation and, by extension, alkalinity. Thus, pH influences both the rate and product distribution of these reactions. Alternatively, plants use norcoclaurine synthase for the regio- and stereo-selective Pictet-Spengler annulation of dopamine and 4-hydroxyphenylacetaldehyde.[23] Inspired by norcoclaurine synthase, the Hailes group developed an aqueous, biomimetic protocol for the Pictet-Spengler reaction of 3-hydroxyphenylethylamines with aldehydes[24] and ketones.[25] Where traditional methods use acid catalysts, forcing conditions, and organic solvents, this methodology uses mixed organic/aqueous buffer pH 6, 50 °C with a potassium phosphate (KPi) catalyst. KPi not only accelerates the reaction but also leads to improved regioselectivity, biasing attack from the *para* position.[26] We thus anticipated that this methodology could be adapted to conditions compatible with one-pot biocatalysis, yielding a multi-step cascade reaction under biocompatible conditions.

Here, we present a biocompatible Pictet-Spengler annulation that merges a whole-cell biocatalysed alcohol oxidation with a one-pot KPi-catalysed Pictet-Spengler annulation to deliver tetrahydroisoquioline and tryptoline products (Figure 1). Careful optimization of substrate concentration, reaction time, stoichiometry and biocatalyst deliver near-quantitative conversion to annulation products through this tandem bio-hybrid cascade. A comprehensive substrate scope reveals that C_2-C_5 linear and branched alcohols and both 3-hydroxyphenylethylamines and tryptamines are all suitable substrates for this methodology. This tandem protocol uses native microbes and inexpensive phosphate catalysis to upgrade alcohols at low concentrations and in mild, aqueous conditions.

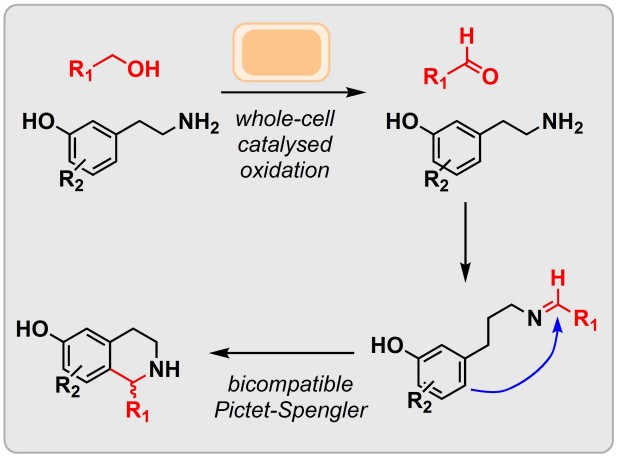


Figure 1. We envisioned a multi-catalytic cascade could be used to deliver annulated heterocycles, in which whole-cell catalysis would chemoselectively oxidize alcohol substrates to aldehydes while a biocompatible catalyst could execute a subsequent Pictet-Spengler annulation.

Results and Discussion

We identified *Komagataella pastoris* and *Gluconobacter oxydans* as promising whole-cell biocatalysts. *K. (Pichia) pastoris* are methylotrophic yeast that use soluble alcohol oxidases within peroxisomes to oxidize alcohols to aldehydes with commensurate reduction of O_2 to hydrogen peroxide.[27] *G. oxydans* are aerobic bacteria that use membrane-bound alcohol dehydrogenases to oxidize alcohols to aldehydes.[28] An unusual characteristic of certain acetic acid bacteria compared to other bacteria is that the aldehyde intermediate persists long enough that it may be isolated or intercepted.[15] Previous work has shown that both *K. pastoris* and *G. oxydans* can oxidize aliphatic and select aryl alcohols to aldehydes under air and in phosphate buffer,[27,29–31] with precedent in tandem systems.[14–16]

K. pastoris and *G. oxydans* have differences that justify exploring both. Both whole cell biocatalysts oxidize C_1-C_6 *n*-alcohols.[27,29,30] Beyond this, *G. oxydans* oxidize some branched alcohols and 2-phenylethanol,[29] while *K. pastoris* oxidizes benzyl alcohol.[31] *G. oxydans* convert alcohols to aldehydes more quickly, but, without subsequent aldehyde conversion or isolation, *G. oxydans* over oxidize aldehydes to carboxylic acids at extended reaction times.[15] *K.* pastoris oxidizes alcohols more slowly but are more tolerant of higher alcohol concentrations. Therefore, we decided to explore both whole-cell biocatalysts in the tandem system.

To ensure whole-cell biocatalyst activity, we chose a fully aqueous KPi buffered system at pH at 28 °C. We chose 1-butanol and *m*-tyramine as model substrates. Compared with shorter alcohols, 1-butanol is less volatile and installs a longer alkyl chain in the annulation product, which aids in purification. Moreover, 1-butanol is well-tolerated and efficiently converted by both biocatalysts. Dopamine, *m*-tyramine and 5-hydroxydopamine are suitable β-arylethylamine substrates for the phosphate-catalysed Pictet-Spengler reaction.[24] These sub-

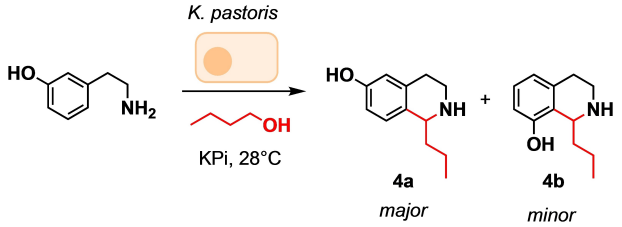
strates all contain the critical 3-hydroxyphenylethylamine motif where the *m*-hydroxy group activates the two positions *ortho* to the ethylamine towards electrophilic substitution. Because it lacks an activating *m*-hydroxy group, *p*-tyramine is not a competent substrate of this reaction.[22,24] Our initial experiments revealed that dopamine rapidly forms black precipitates under our conditions (Figure S1), presumably via oxidative polymerization.[32] Indeed, Pesnot *et al.* reported that dopamine instability was the main synthetic challenge of this reaction.[24] This poses a particular challenge in the aerobic conditions demanded by our biocatalyst. Thus, dopamine and 5-hydroxydopamine were not suitable substrates of our methodology. Because *m*-tyramine is less prone to oxidation than its catechol counterparts, it was used as the model β-arylethlamine. Gratifyingly, both biocatalysts gave annulation products in the model tandem reaction in these preliminary conditions.

We optimized the *K. pastoris* tandem system with respect to stoichiometry, buffer concentration, and buffer pH, with HPLC-UV conversion and product distribution (regioisomeric ratio, r.r.) as metrics (Table 1). *m*-Tyramine (30 mM) and 1-butanol (2 equivalents) in 200 mM pH 6.3 phosphate buffer gave the best balance of conversion and regioselectivity. We attributed the poor conversion of 60 mM and 90 mM *m*-tyramine samples to butanol toxicity and cell inhibition. 1.2, 1.5, and 2.0 equivalents butanol performed comparably, prompting the use of 2.0 equivalents of alcohol to proactively compensate for evaporation of shorter alcohols/aldehyde intermediates. Higher buffer concentrations improved both conversion and regioselectivity. The effect of pH on product yield and regioisomeric ratio was less clear, potentially reflecting competing phosphate-mediated (regioselective) and general (non-regioselective) annulation mechanisms as well as pH effects on butanol oxidation.[24] Under the optimized reaction conditions (200 mM KPi, 30 mM *m*-tyramine, 60 mM 1-butanol, pH 6.3), we saw 73% conversion with a 28:1 regioisomeric ratio in favour of attack from the *para*-position. A time-course experiment revealed that the optimized reaction took 48 h to plateau (Figure S2).

We next surveyed the alcohol scope for the *K. pastoris* tandem system. Of the seven C_1-C_6 primary alcohols surveyed, only ethanol, *n*-propanol, and *n*-butanol gave annulation products (Table 2). Isobutyl alcohol, n-hexanol, and isoamyl alcohol were likely outside the narrow substrate scope of *K. pastoris* and were thus not oxidized to their corresponding aldehyde intermediates. In contrast, *K. pastoris* can use methanol as their principal carbon and energy source, assimilating its carbon or oxidizing it completely to water and carbon dioxide via a short-lived formaldehyde intermediate.[27] Thus, methanol–despite being the native substrate of alcohol oxidase in *K. pastoris*–apparently did not accumulate enough formaldehyde to produce measurable Pictet-Spengler product.

To improve on the alcohol scope, we next shifted focus to a *G. oxydans* whole-cell biocatalyst to accommodate more diverse alcohol substrates. As before, we optimized the tandem system with respect to buffer concentration and pH (Table S1). Neither optima differed significantly from those of the *K. pastoris*

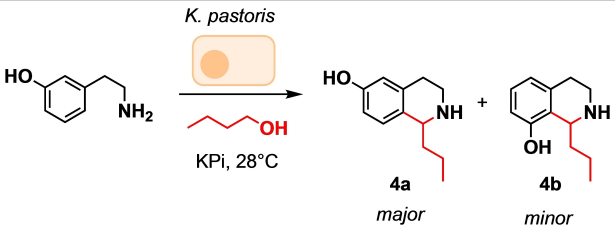
Table 1. Optimizing the *K. pastoris* tandem system.



[KPi] (mM)	[*m*-tyramine] (mM)	[*n*-butanol] (equiv.)	pH	% conv.	r.r.
100	10	2	6.4	73	19
100	30	2	6.3	70	22
100	60	2	6.3	42	23
100	90	2	6.2	11	25
100	30	1	6.3	63	21
100	30	1.2	6.3	74	22
100	30	1.5	6.3	68	9
10	30	2	6.4	46	16
50	30	2	6.4	70	22
200	**30**	**2**	**6.3**	**73**	**28**
100	30	2	4.6	48	23
100	30	2	5.3	70	22
100	30	2	5.8	60	18
100	30	2	6.8	74	9
100	30	2	7.5	49	8
100	30	2	7.8	70	22

Reaction conditions: *K. pastoris* in KPi (10 mL), *m*-tyramine·HCl, and 1-butanol at the indicated concentrations and pH were shaken at 180 rpm and 28 °C for 48 h. Conversion (% conv.) and regioisomeric ratio (r.r. = a/b) were determined by HPLC.

Table 2. *K. pastoris* tandem alcohol scope.



alcohol	R	product	% conv.	r.r.
methanol	H	**1**	0	–
ethanol	Me	**2 a/b**	65	54
1-propanol	Et	**3 a/b**	74	32
1-butanol	Pr	**4 a/b**	76	29
1-hexanol	Pe	**6 a/b**	0	–
isobutyl alcohol	iPr	**7 a/b**	0	–
isoamyl alcohol	iBu	**8 a/b**	0	–

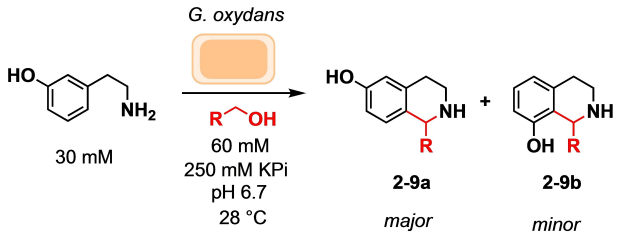
Reaction conditions: *K. pastoris* in KPi (10 mL, 200 mM, pH 6.3), *m*-tyramine·HCl (30 mM), and the indicated alcohol (60 mM) were shaken at 180 rpm and 28 °C for 48 h. Conversion (% conv.) and regioisomeric ratio (r.r. = a/b) were determined by HPLC.

system; however, under these optimized conditions, the *G. oxydans* tandem system delivered near-quantitative conversion to **4**. The optical rotation of the isolated product was $[a]_D^{20} = -0.3 \pm 0.3°$, indicating the product is a racemate. To understand the better performance of *G. oxydans* over *K. pastoris*, we completed a full suite of constituent toxicity toward each biocatalyst (Figure S3). *K. pastoris* viability was unaffected by n-butanol (60 mM) or *m*-tyramine (30 mM), but in the full reaction containing both n-butanol and *m*-tyramine, we observed a >95% decrease in *K. pastoris* colony forming units (CFUs/mL). By contrast, n-butanol prompted a ~75% decrease cell viability in *G. oxydans*, indicating that *G. oxydans* is more susceptible to n-butanol-induced toxicity. Notably, only a mild further decrease in cell viability was observed in the full reaction with *G. oxydans*. Hypothesizing that the THIQ product was more toxic to the yeast biocatalyst than to the bacterial biocatalyst, we next synthesized THIQ **4**, isolated it, and measured its toxicity against both biocatalysts. Again, a 95% decrease in cell viability was observed against *K. pastoris* while only a 40% decrease in cell viability was observed against *G. oxydans*. We thus hypothesize that one origin of the better *G. oxydans* performance is its lower susceptibility to product-induced toxicity. This result reveals a key advantage to having multiple biocatalysts available. In this case, moving from the fungi kingdom to the bacteria kingdom helps minimize product-induced toxicity and contributes to system efficiency.

A time-course experiment showed that the reaction with *G. oxydans* plateaued within just 5 h (Figure S4), allowing us to reduce the reaction time to 12 h in subsequent experiments. Gratifyingly, the use of *G. oxydans* as a biocatalyst in the tandem system improved the alcohol scope (Table 3). Ethanol, 1-propanol, 1-butanol, 1-pentanol, isoamyl alcohol, and 2-methoxyethanol all reacted well with up to quantitative conversion with high regioisomeric excess, delivering the tetrahydroisoquiolines in 57–91% isolated yield. *G. oxydans* can also convert 2-phenylethanols to phenylacetaldehydes,[33] which are suitable aldehyde substrates for the Pictet-Spengler reaction.[24] However, neither 2-phenylethanol nor 2-(4-hydroxyphenyl)ethanol gave detectable conversion to benzylisoquinoline alkaloid products in the tandem reaction. By isolating the oxidation step of the tandem reaction and tracking product yield by ^{1}H NMR, we observed that *G. oxydans* was not producing phenylacetaldehyde at high enough concentration for effective Pictet-Spengler annulation under these conditions (Figure S5).

We next sought to probe the amine scope by exploring the reactivity of tryptamines. Callaway and co-workers showed that tryptamine, serotonin, and 5-methoxytryptamine undergo the Pictet-Spengler reaction with acetaldehyde in physiological conditions.[34] They used KPi buffer for pH control, but did not discuss its possible catalytic role in the annulation. We first assessed the role of KPi in the Pictet-Spengler reaction of tryptamines by monitoring conversion in the reaction between butyraldehyde and 5-methoxytryptamine in HEPES buffer (control) and with KPi at several concentrations (Figure S6). While HEPES buffer delivered less than 1% conversion after 8 h, KPi achieved up to 45% conversion over the same interval, confirming the need for a phosphate-based catalyst.

Table 3. *G. oxydans* tandem alcohol scope.

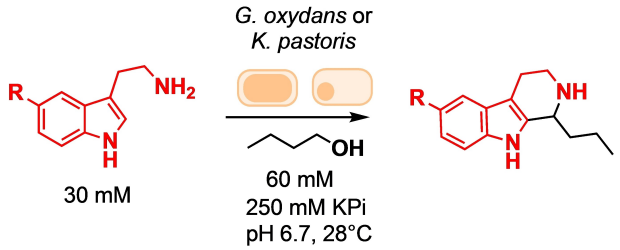


alcohol	R	product	% conv. (isolated % yield)	r.r.
tableh	Me	**2a/b**	100 (63)	63
1-propanol	Et	**3a/b**	92 (91)	32
1-butanol	Pr	**4a/b**	97 (62)	30
1-pentanol	Bu	**5a/b**	100 (70)	29
1-hexanol	Pe	**6a/b**	3	–
isobutyl alcohol	iPr	**7a/b**	11	–
isoamyl alcohol	iBu	**8a/b**	100 (74)	28
2-methoxyethanol	MeOMe	**9a/b**	65 (57)	5
benzyl alcohol	Ph	**10a/b**	0	–
2-phenylethanol	Bn	**11a/b**	0	–
2-(4-hydroxyphenyl)ethanol	4-OHBn	**12a/b**	0	–

Reaction conditions: G. oxydans in KPi (10 mL, 250 mM, pH 6.7), m-tyramine·HCl (30 mM), and the indicated alcohol (60 mM) were shaken at 180 rpm and 28 °C for 12 h. Conversion (% conv.) and regioisomeric ratio (r.r. = a/b) were determined by HPLC-UV.

Tryptamine, serotonin, and 5-methoxytryptamine were used in the tandem *G. oxydans* system with 1-butanol but gave less than 40% conversion in every case (Table 4). This poor conversion resulted from the slow annulation of tryptamines:

Table 4. Tandem tryptamine scope.



Substrate	R	product	*G. oxydans* % conv.	*K. pastoris* % conv. (isolated % yield)
tryptamine	H	**13**	0	12 (11)
serotonin	OH	**14**	21	53 (44)
5-methoxytryptamine	OMe	**15**	40	56 (41)

Reaction conditions: *G. oxydans* or *K. pastoris* as indicated in KPi (10 mL, 250 mM, pH 6.7), the indicated amine·HCl (30 mM), and 1-butanol (60 mM) were shaken at 180 rpm and 28 °C for 12 h. Conversion (% conv.) was determined by HPLC.

KPi-catalysed annulations of tryptamines are slower than those of *m*-tyramines, achieving only 90% conversion after 48 h under the same conditions that deliver up to quantitative conversion from *m*-tyramine substrates in the tandem system (Figure S7).

Our methodology with a *G. oxydans* biocatalyst does not afford enough time for tryptamine substrates to fully react prior to biocatalyst-mediated conversion of the aldehyde to its corresponding carboxylic acids. We considered that the slow oxidation of *K. pastoris* would satisfy the slow Pictet-Spengler of tryptamines while avoiding over oxidation. Gratifyingly, *K. pastoris* delivered better conversion than *G. oxydans* for all three tryptamine substrates under identical conditions (Table 4). This result highlights the importance of matching the rates of each reaction step in multi-step reaction cascades to maximize conversions and yields.

Conclusions

To close, we have interfaced whole-cell alcohol oxidation with the phosphate-catalysed Pictet-Spengler reaction of β-arylethylamines. Live *G. oxydans* or *K. pastoris* convert C_2-C_5 alcohols to their corresponding aldehydes, which condense with *m*-tyramine or tryptamine substrates to form imine intermediates. Aqueous phosphate promotes annulation of these imine adducts to furnish tetrahydroisoquinoline and tryptoline products. This tandem protocol uses native microbes and an inexpensive phosphate catalyst to upgrade alcohols to annulated products in mild, aqueous conditions. Our work sets the stage for expanding this methodology to kinetic resolution for the synthesis of chiral products.[35]

Experimental

General methods. Unless otherwise noted, all chemicals and solvents were used as received from commercial sources. HPLC with in-line UV detection and mass spectroscopy was carried out on an Agilent 1260 Infinity II equipped with an Agilent Poroshell 120 EC–C18 Threaded Column and paired with an Agilent InfinityLab LC/MSD iQ Mass Spectrometer in ESI^+ mode.

NMR spectra were acquired with a JEOL ECA-500 spectrometer operating at 500 MHz for proton spectra (^{1}H NMR) and 125 MHz for carbon-13 (^{13}C NMR).

Media, strains, culture conditions. YDP media was prepared as described by Stewart et al. (2020)[16] from 20 g/L peptone, 10 g/L yeast extract, and 20 g/L dextrose in de-ionized water, adjusted to pH 5.6 with HCl, and autoclaved. Methanol media was prepared as described by Stewart *et al.* (2022)[16] from 2.6 g/L monobasic potassium phosphate, 0.3 g/L dibasic potassium phosphate, 1.5 g/L ammonium sulfate, 0.3 g/L magnesium sulfate heptahydrate, 1 mg/L ferrous sulfate heptahydrate, 7 μg/L cupric sulfate pentahydrate, 10 μg/L manganese sulfate monohydrate, 120 μg/L zinc sulfate heptahydrate, 10 μg/L boric acid, and 1 g/L yeast extract in de-ionized water, adjusted to pH 5.6 with 1 M HCl, and autoclaved. After the solution had cooled, 10 g/L methanol was added. Glycerol media was prepared as described by Stewart *et al.* (2020)[15] from 1.4 g/L monobasic potassium phosphate, 10 g/L yeast extract, and 25 g/L glycerol in de-ionized water. pH was adjusted to 5.0 with 1 M HCl and 1 M NaOH. The solution was autoclaved at 121 °C for 40 minutes.

Freeze-dried *Komagataella pastoris* ATCC® 28485TM was cultured in YDP media under air with ample headspace at 30 °C to mid-exponential phase (~48 hours). The *K. pastoris* cultures were diluted 1:1 with 20% glucose then left to rest for 15 minutes. The diluted cell cultures were then divided into 1 mL aliquots in cryogenic tubes, placed in an insulated Styrofoam cooler and frozen at −80 °C. Frozen *K. pastoris* were cultured in methanol media to induce alcohol oxidase expression. Freeze-dried *Gluconobacter oxydans* ATCC® 621 were cultured directly in glycerol media to induce alcohol dehydrogenase expression.

***K. pastoris* tandem system**. *K. pastoris* was cultured from frozen stock in ~125 mL methanol media with ~875 mL air headspace in a shaker incubator at 28 °C and 180 rpm. After 72 hours, 10 mL cell cultures were pelleted at 500×g for 10 min, re-suspended in 10 mL KPi buffer, pelleted again, and resuspended once more in 10 mL KPi buffer. The 10 mL cell suspensions were transferred to 125 mL screw-cap flasks. The amine substrate was added, followed by the alcohol. The flasks were capped and incubated in a shaker incubator at 28 °C and 180 rpm. After 48 h, reactions were sampled for HPLC analysis. HPLC samples were prepared by filtering ~10 uL reaction media through 0.22 μm Luer lock syringe filters and then diluting the filtrate 100-fold into HPLC-grade water.

Optimization reactions used *m*-tyramine and *n*-butanol substrates at varied stoichiometries and a range of KPi buffer concentrations and pH (Table 1). The time-course reaction (Figure S2) used *m*-tyramine (300 μmol) and 1-butanol (600 μmol) in 10 mL pH 6.3, 200 mM KPi. Alcohol scope reactions used *m*-tyramine·HCl (300 μmol) and methanol, ethanol, 1-propanol, 1-butanol, 1-hexanol, isobutyl alcohol, or isoamyl alcohol (600 μmol) in 10 mL pH 6.3 200 mM KPi (Table 2). Tryptamine scope reactions used tryptamine·HCl, serotonin·HCl, or 5-methoxytryptamine·HCl (300 μmol) and 1-butanol (600 μmol) in 10 mL pH 6.7 250 mM KPi (Table 6).

***G. oxydans* tandem system**. *G. oxydans* was cultured from freeze-dried pellet in ~125 mL glycerol media with ~875 mL air headspace in a shaker incubator at 28 °C and 180 rpm. After 48 hours, 10 mL cell cultures were pelleted at 6000×g for 10 min, re-suspended in 10 mL KPi buffer, pellet again, and resuspended once more in 10 mL KPi buffer. The 10 mL cell suspensions were transferred to 125 mL screw-cap flasks. The amine substrate was added, followed by the alcohol. The flasks were capped and incubated in a shaker incubator at 28 °C and 180 rpm. After some time, reactions were sampled for HPLC analysis. HPLC samples were prepared by filtering ~10 uL reaction media through celite on a cotton plug and then diluting the filtrate 100-fold into HPLC-grade water.

Synthesis and isolation. Tetrahydroquinoline products: The β-arylethylamine·HCl salt (300 μmol) and alcohol (600 μmol) were added to live G. *oxydans* cells suspended in 10 mL 250 mM pH 6.7 KPi in a 250 mL flask under air. The flask was capped and incubated at 28 °C and 180 rpm for 48 h. At 12 h, the reaction was sampled for HPLC and then 3 g NaCl, 1 g Na_2SO_3, and 1 g K_2CO_3 were added. The aqueous mixture was extracted with 3×10 mL ethyl acetate. Centrifugation (20,000 g×5 min) was used to break the emulsion at each extraction. The combined ethyl acetate layers were washed with 3:1:1 NaCl/Na_2SO_3/K_2CO_3 brine. K_2CO_3 and Na_2SO_3 provided basic and reducing conditions, respectively, while NaCl comprised the bulk of the dissolved salt and provided ionic strength. The solvent was removed with high-vacuum on a Schlenk line, affording the isolated product as mixed regioisomers.

Tryptoline products: The tryptamine·HCl salt (300 μmol) and 1-butanol (600 μmol, 55 μL) were added to live *K. pastoris* cells

suspended in 10 mL 200 mM pH 6.3 KPi in a 250 mL flask under air. The flask was capped and incubated at 28 °C and 180 rpm for 48 h. At 48 h the reaction was sampled for HPLC. To the reaction mixture, 3 g NaCl, 1 g Na_2SO_3, and 1 g K_2CO_3 were added. The aqueous mixture was extracted with 3×10 mL ethyl acetate. Centrifugation (20,000 g x 5 min) was used to break the emulsion at each extraction. The ethyl acetate layers were combined and the solvent removed by rotary evaporation at 45 °C to yield the crude product as a yellow oil. The crude product was taken up into 10% methanol in methylene chloride and then purified by flask chromatography on silica, eluting with 10% methanol in methylene chloride. The solvent was removed with high-vacuum on a Schlenk line, affording the product.

Author Contributions

Campbell M. Andersen: Formal analysis; Investigation; Methodology; Writing – original draft preparation; Writing – Review & Editing. Luke D. Knudson: Formal analysis; Investigation; Methodology. Dylan W. Domaille: Conceptualization; Funding Acquisition; Project Administration; Supervision; Writing - Review & Editing.

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Conflict of Interests

There are no conflicts to declare.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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[1] R. A. Sheldon, J. M. Woodley, *Chem. Rev.* **2018**, *118*, 801–838.
[2] J. Albarrán-Velo, D. González-Martínez, V. Gotor-Fernández, *Biocatal. Biotransform.* **2017**, *36*, 102–130.
[3] R. A. Sheldon, D. Brady, *ChemSusChem* **2019**, *12*, 2859–2881.
[4] Y. Wang, H. Zhao, *Catalysts* **2016**, *6*, 194.
[5] K. N. Stewart, D. W. Domaille, *ChemBioChem* **2021**, *22*, 469–477.
[6] S. Wallace, E. P. Balskus, *Curr. Opin. Biotechnol.* **2014**, *30*, 1–8.
[7] J. C. Sadler, J. A. Dennis, N. W. Johnson, S. Wallace, *RSC Chem. Biol.* **2021**, *2*, 1073–1083.
[8] G. Sirasani, L. Tong, E. P. Balskus, *Angew. Chem.* **2014**, *126*, 7919–7922.
[9] S. Wallace, E. P. Balskus, *Angew. Chem.* **2016**, *128*, 6127–6131.
[10] S. Wu, Y. Zhou, D. Gerngross, M. Jeschek, T. R. Ward, *Nat. Commun.* **2019**, *10*, 5060.
[11] T. C. Ezeji, N. Qureshi, H. P. Blaschek, *Bioprocess Biosyst. Eng.* **2013**, *36*, 109–116.
[12] T. Walther, J. M. François, *Biotechnol. Adv.* **2016**, *34*, 984–996.
[13] E. Lamsen, S. Atsumi, *Front. Microbiol.* **2012**, *3*, 196.
[14] P. Zambelli, A. Pinto, D. Romano, E. Crotti, P. Conti, L. Tamborini, R. Villa, F. Molinari, *Green Chem.* **2012**, *14*, 2158–2161.
[15] K. N. Stewart, E. G. Hicks, D. W. Domaille, *ACS Sustainable Chem. Eng.* **2020**, *8*, 4114–4119.
[16] K. N. Stewart, D. W. Domaille, *React. Chem. Eng.* **2022**, *7*, 1328–1334.
[17] J. A. Dennis, J. C. Sadler, S. Wallace, *ChemBioChem* **2022**, *23*, e202200238.
[18] K. N. Stewart, K. G. Hawkins, C. M. Andersen, D. W. Domaille, *React. Chem. Eng.* **2023**, *8*, 1152–1158.
[19] W. M. Whaley, T. R. Govindachari in *Organic Reactions*, John Wiley & Sons, Hoboken, **2011**, pp. 151–190.
[20] H. A. Bates, *J. Org. Chem.* **1983**, *48*, 1932–1934.
[21] H. A. Bates, *J. Org. Chem.* **1981**, *46*, 4931–4935.
[22] H. A. Bates, K. Bagheri, P. M. Vertino, *J. Org. Chem.* **1986**, *51*, 3061–3063.
[23] A. Bonamore, M. Barba, B. Botta, A. Boffi, A. Macone, *Molecules* **2010**, *15*, 2070–2078.
[24] T. Pesnot, M. C. Gershater, J. M. Ward, H. C. Hailes, *Chem. Commun.* **2011**, *47*, 3242–3244.
[25] J. Zhao, D. Méndez-Sánchez, J. M. Ward, H. C. Hailes, *J. Org. Chem.* **2019**, *84*, 7702–7710.
[26] R. D. Parra, J. Maresh, *Comput. Theor. Chem.* **2016**, *1082*, 1–10.
[27] S. J. B. Duff, W. D. Murray, *Ann. N. Y. Acad. Sci.* **1988**, *542*, 428–433.
[28] G. A. R. da Silva, S. S. de S. Oliveira, S. F. Lima, R. P. do Nascimento, A. R. de S. Baptista, S. B. Fiaux, *World J. Microbiol. Biotechnol.* **2022**, *38*, 134.
[29] F. Molinari, R. Villa, M. Manzoni, F. Aragozzini, *Appl. Microbiol. Biotechnol.* **1995**, *43*, 989–994.
[30] W . Murray, S. J. B. Duff, *Appl. Microbiol. Biotechnol.* **1990**, *33*, 202–205.
[31] S. J. B. Duff, W. D. Murray, *Biotechnol. Bioeng.* **1989**, *34*, 153–159.
[32] R. Lakshminarayanan, S. Madhavi, C. P. C. Sim, *Dopamine - Health and Disease* (Ed. S. C. Yenisetti), InTech, London, **2018**, pp. 113–132.
[33] R. Villa, A. Romano, R. Gandolfi, J. V. S. Gago, F. Molinari, *Tetrahedron Lett.* **2002**, *43*, 6059–6061.
[34] J. C. Callaway, J. Gyntber, A. Poso, M. M. Airaksinen, J. Vepsäläinen, *J. Heterocycl. Chem.* **1994**, *31*, 431–435.
[35] N. Richter, M. Neumann, A. Liese, R. Wohlgemuth, T. Eggert, W. Hummel, *ChemBioChem* **2009**, *10*, 1888–1896.

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