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But-2-ene-1,4-diamine and But-2-ene-1,4-diol as Donors for Thermodynamically Favored Transaminase- and Alcohol Dehydrogenase-Catalyzed Processes

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Abstract: Both *cis*- and *trans*-but-2-ene-1,4-diamines have been prepared and efficiently applied as sacrificial cosubstrates in enzymatic transamination reactions. The best results were obtained with the *cis*-diamine. The thermodynamic equilibrium of the stereoselective transamination process is shifted to the amine formation due to tautomerization of 5*H*-pyrrole into 1*H*-pyrrole, achieving high conversions (78–99%) and enantiomeric excess (up to >99%) by using a small excess of the amine donor. Furthermore, when the reaction proceeded, a strong coloration was observed due to polymerization of 1*H*-pyrrole. A structurally related compound, *cis*-but-2-ene-1,4-diol, has been utilized as cosubstrate in different alcohol dehydrogenase (ADH)-mediated bioreductions. In this case, high conversions (91–99%) were observed due to a lactonization process. Both strategies are convenient from both synthetic and atom economy points of view in the production of valuable optically active products.

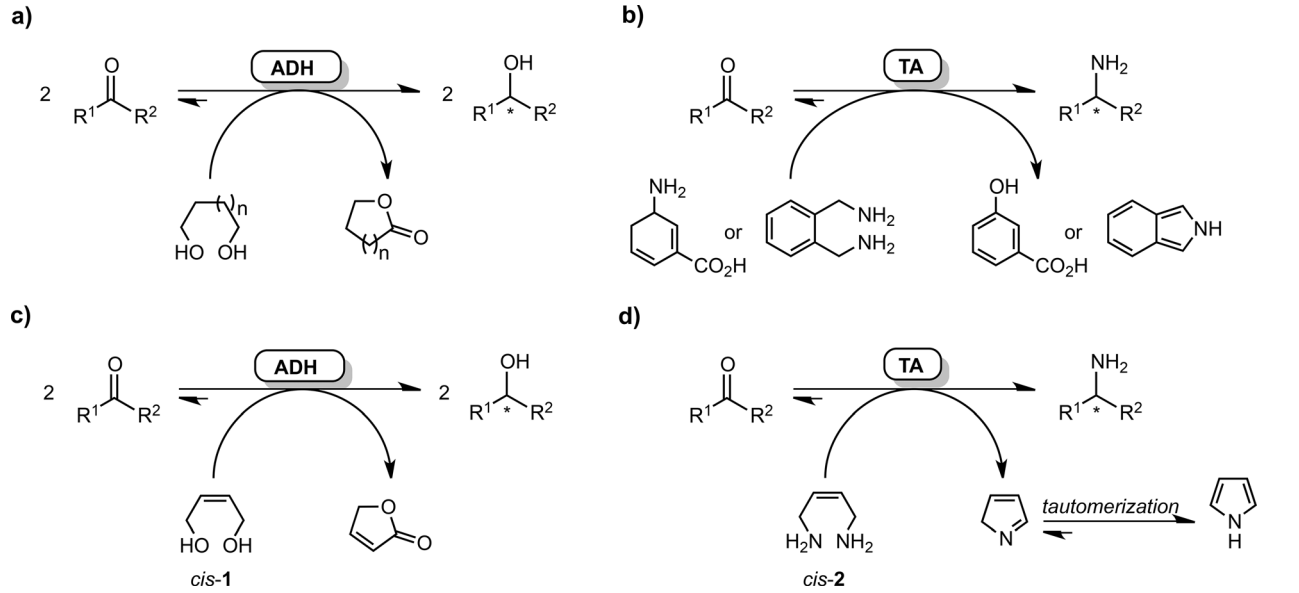
Keywords: alcohol dehydrogenases; atom economy; biocatalysis; cofactor recycling; transaminases

Introduction

Transaminase (TA)- and alcohol dehydrogenase (ADH)-catalyzed transformations are prominent biocatalytic examples at academic and industrial levels to obtain enantiopure target molecules.[1] Both biocatalyst types can act over carbonyl compounds to accomplish desymmetrization processes (ideally in quantitative yield and selectivity), affording (chiral) amines[2] and alcohols,[3] respectively. Their mechanisms are different, but they have as a common feature the necessity of a second molecule (cofactor) to accomplish these transformations.[4] These coenzymes, nicotinamide [NAD(P)H] for ADHs and pyridoxal 5′-phosphate (PLP) for TAs, must usually be employed in catalytic amounts due to their high costs and inhibition issues. To afford this, excellent cofactor recycling strategies that can overcome the use of stoichiometric quantities of the coenzyme together with a driving of the thermodynamic equilibrium of the process have been described.[5]

For ADHs, among the different existing methodologies, the use of a cheap sacrificial alcohol such as 2-propanol has been commonly described in a 'coupled-substrate' approach, generally obtaining excellent results.[6] For TAs, a similar methodology can be successfully applied using an auxiliary amine donor such as isopropylamine.[7] Unfortunately, these cosubstrates must be utilized in a huge molar excess regarding the carbonyl compound to adequately force the reaction into the reduced derivatives. This obviously leads to a poorer atom economy of the process.

Recently, the application of cosubstrates that can greatly reduce the equivalents utilized in biocatalyzed transformations mediated by ADHs and TAs, is gaining more relevance. Hence, the use of diols such as 1,4-butanediol[8] or 1,6-hexanediol[9] has allowed 'substrate-coupled' redox processes in close to stoichiometric relation regarding the target substrate. This is due to the thermodynamically favored ring closure of the ω-hydroxy aldehyde intermediate, thus forming the corresponding hemiacetal, which in a subsequent oxidative step renders the final lactone co-product



Scheme 1. Strategies to drive the thermodynamic equilibria in 'coupled-substrate' approaches: a) ADH-catalyzed reductions *via* lactonization; b) TA-catalyzed aminations *via* aromatization; c) and d) ADH- and TA-catalyzed processes using *cis*-1,4-but-2-ene-diol and *cis*-1,4-but-2-ene-diamine, respectively (this study).

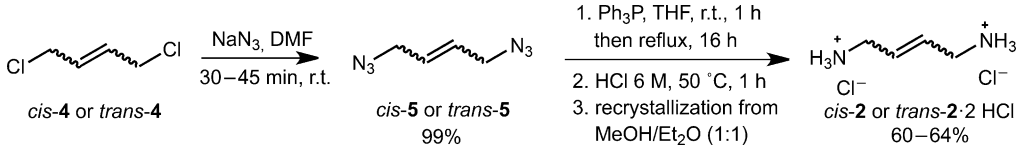
(Scheme 1a). On the other hand, a cyclic amine[10] or a diamine[11] has been described as amine donor that can highly displace the equilibria in TA-catalyzed protocols due to ring aromatization (Scheme 1b). However, the high cost of these tailored amines and difficulties in separating the excess of the donors from the products make interesting the study of other alternatives.

Herein, we have studied the application of two related compounds, *cis*-but-2-ene-1,4-diol (*cis*-**1**) and *cis*-but-2-ene-1,4-diamine (*cis*-**2**), as cosubstrates in ADH- and TA-mediated transformations, respectively (Scheme 1c and d). Thus, after optimization of the reaction conditions, we demonstrate that these derivatives can be employed at much lower levels than conventional cosubstrates to obtain enantioenriched alcohol and amine products through enzyme-catalyzed reactions, with high purity after a simple extraction.

Based on previously described protocols that make use of designed cosubstrates to favor TA-mediated processes,[10,11] we envisaged that simple 1,4-diamines could be used for this purpose. Once the ω-amino aldehyde intermediates were formed, they could intramolecularly cyclize, thus providing the driving force that could transform quantitatively the carbonyl compound of interest. With this in mind, diamines *cis*-**2** and 1,4-butanediamine **3** were envisioned as perfect candidates. Moreover, in the case of *cis*-**2**, after cyclization to 5*H*-pyrrole, it could tautomerize into 1*H*-pyrrole (Scheme 1d).

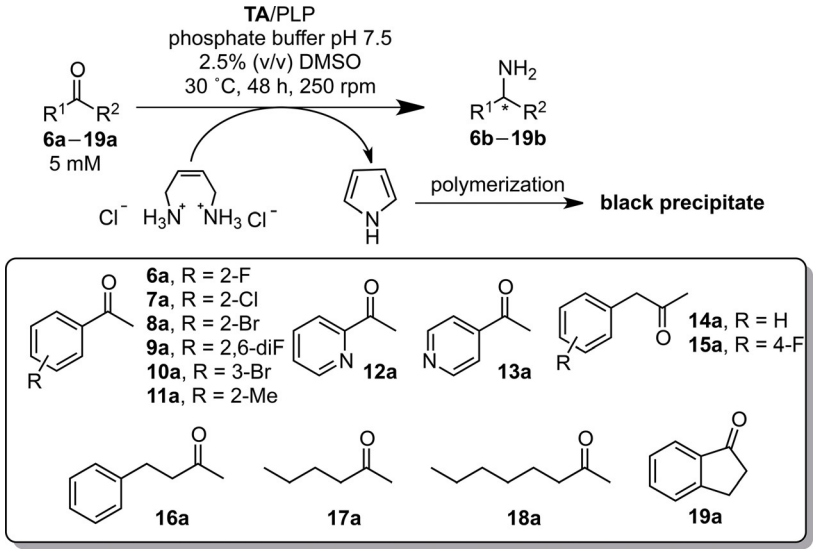
Results and Discussion

While diamine **3** is commercially available, we synthesized *cis*-**2**. As a first approach, we used diol *cis*-**1** as starting material.[12] Different conditions such as Mitsunobu reaction or alcohol activation through *O*-tosylation were tried, but unfortunately these protocols did not work out. Hence, we employed a slightly modified method proposed by Delcros and co-workers,[13] starting from commercially accessible *cis*-1,4-dichlorobut-2-ene (*cis*-**4**, Scheme 2). In a first step, both halogen atoms were substituted by azides using a typical nucleophilic substitution protocol and, in a second step, the reduction of diazido compound *cis*-**5** was carried out under Staudinger conditions with triphenylphosphine, followed by acidic hydrolysis affording *cis*-**2** as dichlorohydrate salt. During this synthetic pathway an alkene isomerization into the *trans*-diamine



Scheme 2. Synthetic pathway to obtain diamines *cis*-**2** and *trans*-**2**.

Table 1. Amination of ketones **6a**–**19a** using *cis*-**2** (3 equiv.) as amine donor.[a]



Entry	Ketone	Enzyme	*c* [%][b]	*ee* [%][c]
1	**6a**	ATA-033	96	>99 (*R*)
2	**6a**	TA-P1-G06	97	>99 (*S*)
3	**7a**	ATA-033	98	>99 (*R*)
4	**7a**	TA-P1-G06	99	>99 (*S*)
5	**8a**	ATA-025	99	>99 (*R*)
6	**8a**	TA-P1-A06	99	>99 (*S*)
7	**9a**	ATA-012	96	>99 (*R*)
8	**10a**	ATA-033	79	>99 (*R*)
9	**11a**	ATA-024	82	>99 (*R*)
10	**12a**	ATA-025	93	>99 (*R*)
11	**12a**	TA-P1-A06	93	99 (*R*)
12	**13a**	ATA-033	98	>99 (*R*)
13	**14a**	TA-P1-A06	78	>99 (*S*)
14	**15a**	TA-P1-A06	99	92 (*S*)
15	**16a**	TA-P1-A06	92	95 (*S*)
16	**17a**	ATA-113	80	82 (*S*)
17	**18a**	TA-P1-A06	82	88 (*S*)
18	**19a**	ATA-256	89	26 (*S*)

[a] For *reaction conditions*, see the Supporting Information.
[b] Measured by GC analysis.
[c] Measured by chiral GC or HPLC analysis.

(*trans*-**2**) was observed, therefore recrystallization in a methanol/diethyl ether (1:1) mixture was performed, providing the desired compound with high *cis*/*trans* selectivity (approx. 95:5). To check the effect of the double bond stereochemistry, diamine *trans*-**2** was also synthesized following the same reaction route (Scheme 2).

In a first set of experiments, three different ketones (**6a**–**8a**, see Table 1) were used as suitable substrates with two commercially available transaminases (Figure 1). Thus, a small excess of the amine donor (1.5 equivalents) was applied. Gladly, TAs could accept diamine *cis*-**2** as donor, affording the corresponding amines **6b**–**8b** with high to excellent conversions after 48 h at 30 °C. Although in a minor extent,

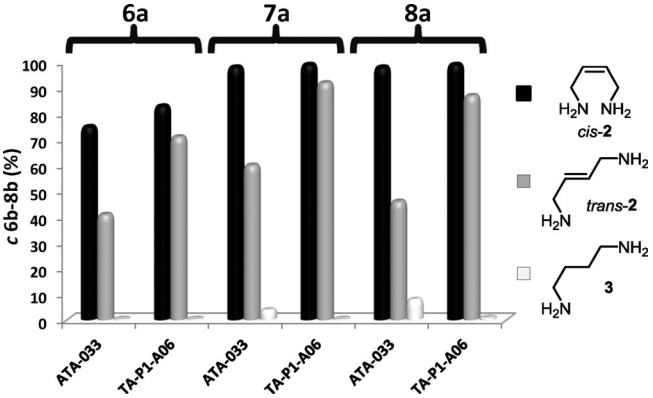
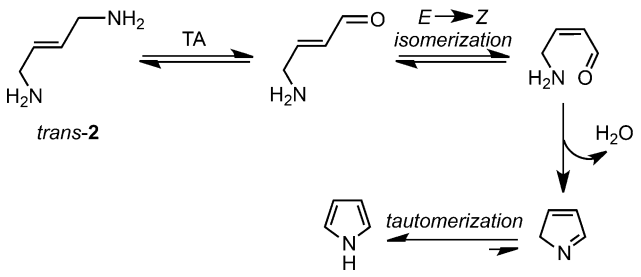


Figure 1. Effect of the amine donor in TA-catalyzed reactions using 1.5 equiv. of *cis*-**2**, *trans*-**2**, and **3**.

trans-**2** could also provide moderate to high conversions, and the saturated diamine **3** appeared as a poor substrate for these enzymes. These results illustrate the relevance of the presence and stereochemistry of the double bond in the structure of the diamine, which is the key to provide the ring aromatization.

Interestingly, when the reaction proceeded with *cis*-**2** and *trans*-**2**, the formation of black colored precipitates was observed. This can be ascribed to the polymerization of the pyrrole by-product resulting in the formation of polypyrrole.[14] In fact, when we incubated pyrrole in the reaction medium in the absence of enzyme and PLP, we did not observe coloration, but when we accomplished the same experiment adding only PLP, a quick formation of the black polymers was detected. It seems that PLP mediates the polymerization of pyrrole acting as an acidic catalyst. This phenomena is also in agreement with that previously described by Turner and co-workers,[11] where the isoindole co-product (Scheme 1b) polymerized under similar conditions affording intense colored derivatives. As in their case, this observation can potentially lead to a sensitive and operationally simple colorimetric assay to identify TA activity.[11,15]

The formation of pyrrole was also demonstrated by NMR experiments by performing the enzymatic reactions in deuterium oxide (see the Supporting Information for more details). Surprisingly, when we studied the transformation using *trans*-**2** as amine donor, the presence of pyrrole was also observed in the reaction crude. This fact can explain why in the presence of this diamine the aspect of the medium was similar. If pyrrole is formed, at some point there must be a *cis*/*trans* isomerization of the double bond, otherwise cyclization would not be possible. When we incubated *trans*-**2** in the reaction medium without any ketone substrate, isomerization was not observed. Therefore, we speculate that the *trans*-ω-amino aldehyde intermediate formed after deamination of *trans*-**2** must be the species which undergoes this process (Scheme 3). This is not surprising as it has been described that similar α,β-unsaturated aldehydes can isomerize in the presence of pyridine derivatives, which can achieve a reversible 1,4-addition.[16] In our case, PLP could act as the isomerization agent.



Scheme 3. Transformation of diamine *trans*-**2** into pyrrole through the isomerization of the *trans*-ω-amino aldehyde intermediate.

Subsequently, *cis*-**2** was applied as amine donor in phosphate buffer and in the presence (2.5% v/v) of dimethyl sulfoxide (DMSO) as solubilizing agent with ketones **6a**–**19a** (Table 1). This selection was made based on a previous study performed in our group[17] due to the relevance of the obtained amines as precursors of interesting derivatives.[18] Aromatic (entries 1–9), heteroaromatic (entries 10–12), and aliphatic substrates (entries 13–17) could be transformed into the corresponding enantioenriched amines with excellent conversions and enantiomeric excess. Interestingly, TAs with opposite stereopreference can work under these conditions, therefore providing both antipodes for some of the selected examples. ATA-025 and ATA-033 showed the best results in terms of *R*-selectivity, and TA-P1-A06 and TA-P1-G06 were in general the best candidates to obtain the *S*-enantiomers. We also carried out a study on a cyclic ketone such as 1-indanone (**19a**, see the Supporting Information). We were pleased to observe that meanwhile 1-indanone was slightly converted for most of the enzymes tested using isopropylamine as amine donor, in some cases conversions dramatically increased using *cis*-**2** to afford the corresponding amine with modest enantiomeric excess (entry 18).

It has been previously described that similar cosubstrates can also favor these processes from a kinetic point of view regarding conventional donors.[8c] In this case, the initial rates of TA-catalyzed reactions using isopropylamine, *cis*-**2**, and *trans*-**2** were measured. We found that isopropylamine reacted faster than both diamines (especially than the *trans* isomer), leading to the highest initial rate (see the Supporting Information). This fact shows that these transformations are mainly thermodynamically (but not kinetically) driven.

The use of this diamine is particularly advantageous, since after a simple extraction protocol, it is possible to obtain the desired amines with high purity, as diamine *cis*-**2** is completely soluble in water and the excess of this compound remains in the aqueous phase even after extraction under basic conditions. Moreover, polymers coming from pyrrole can be easily removed by centrifugation.

As a proof, we applied this transformation concept on a preparative scale. Thus, 100 mg of ketones **7a** and **8a** were transformed into the corresponding (*R*)-amines (>99% *ee*) using ATA-033 and *cis*-**2** (1.5 equiv.) in 74–83% yield after 48 h of reaction.

Finally, we have compared the conventional methodology using an excess of isopropylamine, with thermodynamically favored strategies employed for TA-catalyzed reactions, calculating the theoretical value of waste generated according to the reaction equation.[19] To perform this, we have used the EATOS

Table 2. Quantity of waste generated *per* kg of amine (**6b**) synthesized depending on the amine donor.[a]

Entry	Amine donor	Molar excess[b]	Waste ($kg\,kg^{-1}$ product)
1	IPA[c]	40	17
2	cyclic amine[d]	1.05	1.04
3	diamine[e]	1.1	1.07
4	*cis*-**2**	1.5	0.92

[a] Calculated using the EATOS program. Quantitative conversion was assumed.
[b] Regarding the ketone substrate.
[c] Isopropylamine.
[d] Amine donor described by Berglund and co-workers (see Scheme 1b).[10]
[e] Amine donor described by Turner and co-workers (see Scheme 1b).[11]

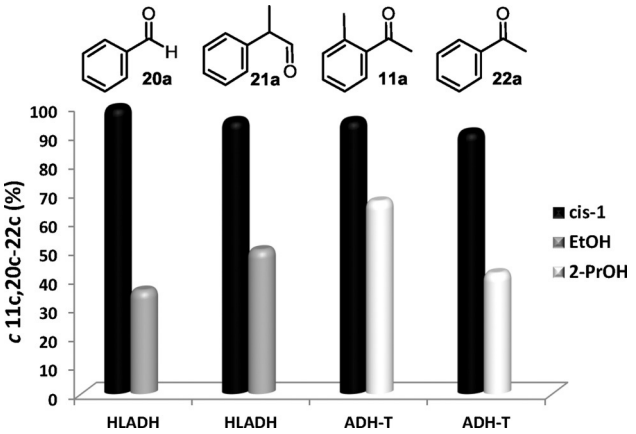


Figure 2. Effect of the hydrogen donor in ADH-catalyzed reactions using 0.5 equiv. (for HLADH) or 3 equiv. (for ADH-T) of *cis*-**1**, EtOH (HLADH) and 2-PrOH (ADH-T).

program,[20] assuming a cosubstrate molar excess as described by the corresponding authors,[10,11] and a quantitative conversion of a model ketone (**6a**, Table 2). As can be seen, our methodology (entry 4) was perfectly comparable to similar previously described strategies (entries 2 and 3), while greatly improving the standard conditions using isopropylamine (entry 1). The method presented here upgrades the previous ones as the coproduct obtained (pyrrole), displays a substantially lower molecular weight.

Likewise, diol *cis*-**1**, previously tried as synthetic precursor of diamine *cis*-**2**, was envisioned as a suitable cosubstrate for ADH-mediated bioreductions in a similar way as shown by Hollmann and co-workers with the saturated derivatives.[8,9]

After a first enzymatic screening, we observed that horse liver ADH (HLADH) and *Thermoanaerobacter* sp. ADH (ADH-T) overexpressed in *E. coli*, were able to accept this diol as hydrogen donor. Then, it was demonstrated that *cis*-**1** can drive the equilibrium into the reduction mode with higher efficiency than the usual donors such as EtOH (for HLADH) or 2-PrOH (for ADH-T), since by simple addition of an equimolar or a slight excess amount of the diol, excellent conversions (and *ee* for **11c**, **21c** and **22c**) into the corresponding alcohols were achieved (Figure 2). The formation of the unsaturated lactone furan-2(5*H*)-one coproduct was confirmed by GC analysis (see the Supporting Information).

Conclusions

Herein we have shown the use of two related compounds, diamine *cis*-**2** and diol *cis*-**1**, in order to drive the equilibrium of TA- and ADH-catalyzed processes by using a 'coupled-substrate' approach. A tautomerization of co-product 5*H*-pyrrole into 1*H*-pyrrole and a lactonization reaction, respectively, are responsible of the excellent conversions attained using a small excess of these cosubstrates. Furthermore, in the case of the diamine *cis*-**2**, a strong coloration of the enzymatic transformation was observed when the reaction proceeded due to the pyrrole polymerization. The excess of both cosubstrates and coproducts formed can be easily removed from the products of interest, making these compounds highly appealing for ADH- and TA-catalyzed transformations, enhancing their possible applications from both synthetic and atom economy points of view. Thus, the amount of waste generated due to the amine donor employed, was comparable with current existing transaminase-catalyzed methods to shift the reaction equilibrium towards the desired product, improving the traditional methodology using an excess of isopropylamine.

Experimental Section

Synthesis of *cis/trans*-But-2-ene-1,4-diamine Dihydrochloride (*cis/trans*-2)

Diazide intermediates *cis*/*trans*-**5** were obtained from the respective *cis/trans*-**4** using a nucleophilic substitution reaction. Dichlorinated compound *cis*/*trans*-**4** (4.75 mmol, 0.5 mL) was added to a stirred solution of NaN_3 (42.7 mmol, 2.77 g) in DMF (7 mL) and the magnetic stirring was continued until disappearance of the starting material (30–45 min). Then, the reaction was quenched by addition of water (10 mL) and extracted with EtOAc (3 × 15 mL). The organic layer was dried with Na_2SO_4, filtered off and concentrated. The residue was purified by flash column chromatography (20% EtOAc/hexane) on silica gel to remove the DMF still remaining after the extraction, leading to the corresponding diazide compounds *cis*/*trans*-**5** as orange oil in quantitative yields.

The desired diamines were prepared as dihydrochloride salts through the reduction of the previous diazides *cis*/*trans*-**5** and subsequent acidification. To a solution of *cis*/

trans-**5** (4.70 mmol, 649 mg) in dry THF (9 mL), a solution of Ph_3P (9.45 mmol, 2.48 g) in dry THF (6 mL) was added. This mixture was stirred at room temperature during the first hour. After this time, the reaction mixture was heated at 50 °C for 16 h. Then, the hydrolysis was carried out by the addition of an aqueous HCl solution 6 M (1 mL), maintaining the reaction at 50 °C during one additional hour. Finally, water (3 mL) and concentrated HCl (500 μL) were added and THF was removed under vacuum. To the obtained residue, water (10 mL) was added, and the aqueous layer was extracted with EtOAc (5 × 10 mL). The water was removed under reduced pressure (50 °C), affording a brown crude. The brown crude, obtained by evaporating the aqueous layer, was recrystallized from a mixture of MeOH-Et_2O (1/1, v/v), leading to the desired diamine dihydrohloride *cis*-**2** as a white solid (yield: 60%), with a small amount of the unwished isomer *trans*-**2**, just observed in the ^{1}H NMR spectra (ratio *cis*/*trans*: 95:5) or to diamine *trans*-**2** in high purity as a white solid (yield: 64%).

General Procedure for Enzymatic Transamination Reactions using *cis*-2 as Amine Donor

In a 1.5-mL Eppendorf vial, substrate **6a**–**19a** (5 mM, 12.5 μL from 200 mM stock in DMSO), was dissolved in phosphate buffer (500 μL, 100 mM, pH 7.5) containing PLP (2 mM) and *cis*-**2** (7.5 mM, 1.5 equiv. or 15 mM, 3 equiv.). Finally, the commercially available transaminase (2 mg) was added. The initial slightly yellow reaction mixture was shaken at 30 °C and 250 rpm for 48 h (observing after this time that the reaction media was completely black with the appearance of a solid at the bottom of the vial), and then stopped by the addition of an aqueous 10 M NaOH solution (250 μL). Then, the mixture was extracted with EtOAc (2 × 0.5 mL), the organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions were determined by GC and enantiomeric excess by chiral GC or HPLC (see Table 1).

Scaling-Up Reactions

In an Erlenmeyer flask (100 mL), ketone **7a** or **8a** (25 mM, 100 mg) was dissolved in phosphate buffer (100 mM, pH 7.5) containing PLP (2 mM), MeCN (2.5% v/v) as organic cosolvent and *cis*-**2** (37.5 mM, 1.5 equiv.) as amine donor. Finally, ATA-033 (75 mg) was added. Then, the initial slightly yellowish reaction was shaken at 30 °C and 250 rpm for 48 h. After that time, the reaction media was completely black. The conversion was determined by taking a sample (200 μL) of the reaction mixture which was basified with an aqueous NaOH solution 10 M (100 μL) and extracted as shown above, leading to 78% and 89% conversions for **7a** and **8a**, respectively (measured by GC). At this point, the solution was centrifuged at 4,900 rpm for 7 min. The supernatant was decanted, acidified with an aqueous HCl solution 3 M (5 mL) and extracted with Et_2O (3 × 25 mL) discarding the organic layer, in order to remove the small amount of the starting material. Then, the aqueous phase was basified with an aqueous NaOH solution 10 M (8 mL) and extracted with Et_2O (3 × 35 mL). The organic layers were combined, dried over Na_2SO_4 and the solvent was evaporated under reduced pressure, providing (*R*)-**7b** (yield: 74%, >99% *ee*) and (*R*)-**8b** (yield: 83%, >99% *ee*).

General Procedure for Alcohol Dehydrogenase-Catalyzed Reductions using *cis*-But-2-ene-1,4-diol (*cis*-1) as Cosubstrate

For *E. coli*/HLADH: In a 1.5-mL Eppendorf vial, aldehyde **20a** or **21a** (0.022 mmol, 32 mM) was dissolved in phosphate buffer (700 μL, 50 mM, pH 8), containing NADH (1 mM) and *cis*-**1** (0.5 equiv., 16 mM) as cosubstrate. Finally *E. coli*/HLADH (1.5 mg) was added. The reaction was shaken at 30 °C and 250 rpm for 22 h and extracted with EtOAc (2 × 0.5 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions and enantiomeric excess (for **21c**) were determined by GC and HPLC, respectively (see the Supporting Information, Table S4). The presence of furan-2(5*H*)-one in the reaction media was confirmed by GC analysis.

For *E. coli*/ADH-T: In a 1.5-mL Eppendorf vial, the corresponding ketone **11a** or **22a** (0.017 mmol, 34 mM) was dissolved in Tris·HCl buffer (500 μL, 50 mM, pH 7.5), containing NADPH (1 mM) and *cis*-**1** (102 mM, 3 equiv.) as cosubstrate. Finally *E. coli*/ADH-T (15 mg) was added. The reaction mixture was shaken at 30 °C and 250 rpm for 24 h and then extracted with EtOAc (2 × 0.5 mL). The organic layer was separated by centrifugation (2 min, 13000 rpm) and dried over Na_2SO_4. Conversions and enantiomeric excess were determined by GC analysis (see the Supporting Information, Table S5). The presence of furan-2(5*H*)-one in the reaction media was confirmed by GC analysis.

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