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Bioinversion/Retention

Biocontrolled Formal Inversion or Retention of L-α-Amino Acids to Enantiopure (*R*)- or (*S*)-Hydroxyacids

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Abstract: Natural L-α-amino acids and L-norleucine were transformed to the corresponding α-hydroxy acids by formal biocatalytic inversion or retention of absolute configuration. The one-pot transformation was achieved by a concurrent oxidation reduction cascade in aqueous media. A representative panel of enantiopure (*R*)- and (*S*)-2-hydroxy acids possessing aliphatic, aromatic and heteroaromatic moieties were isolated in high yield (67–85%) and enantiopure form (>99% *ee*) without requiring chromatographic purification.

Introduction

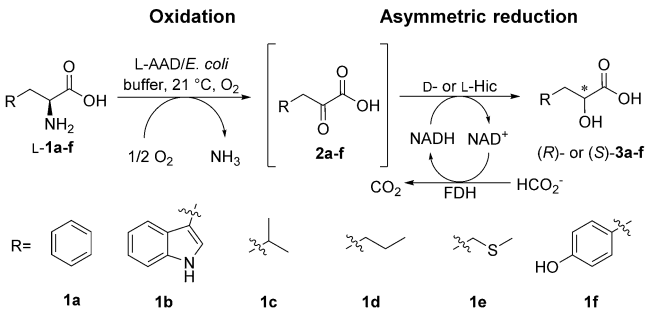
Walden was the first to report on the transformation of an α-amino acid to the corresponding α-hydroxy acid through retention of configuration,[1] which was later also demonstrated for other amino acids with HNO_2.[2] Since then, diazotization has become a common applied method to transform mainly L-amino acids to optically enriched L-α-hydroxy acids.[3] Alternative methods to prepare optically enriched α-hydroxy acids encompass, for instance, asymmetric dihydroxylation,[4] asymmetric reduction of 2-oxo acids,[5] hydroxylation of carboxylic acids,[6] addition of cyanide to aldehydes and nitrile hydrolysis[7] or enzymatic deracemization.[8]

α-Hydroxy acids are found in numerous natural products, as well as in pharmaceutical and plant-protection agents.[9] In particular, α-hydroxy acids are essential constituents of depsipeptides in which they function as mimetics for the corresponding natural amino acids.[10] Diazotization of the natural L-amino acids leads in general to L-hydroxy acids but fails for sensitive amino acids, for example, tyrosine; unfortunately, there is no common employed one-pot option available to prepare the stereocomplementary D-hydroxy acids from the corresponding L-α-amino acids.

To the best of our knowledge, in Nature no enzyme is known to substitute an amino moiety by a hydroxyl group: consequently our aim was to design an artificial biocatalytic cascade to emulate diazotization but giving the option to prepare either the L- or D-hydroxy acid.[11] Biocatalytic cascade reactions have recently attracted significant attention enabling multistep transformations in one-pot thereby circumventing the isolation of (possible unstable) intermediates.[12]

Results and Discussion

The design of the cascade involves oxidation of the L-amino acid substrate by an amino acid deaminase at the expense of molecular oxygen giving the prochiral α-keto acid (Scheme 1). The latter is stereoselectively reduced either to the (*R*)- or (*S*)-α-hydroxy acid depending on the stereopreference of the selected dehydrogenase.



Scheme 1. One-pot simultaneous multi-enzyme system for the synthesis of enantiomerically pure (*R*)- or (*S*)-2-hydroxy acids starting from L-amino acids.

The reverse transformation, the reaction of an alcohol moiety to the corresponding amine, was previously shown to work in the absence of redox reagents.[13] The reason is that in this case the overall reaction is energetically downhill.[13d] In contrast, the formal substitution of an amino moiety by a hydroxy group is energetically uphill; consequently, redox reagents, such as molecular oxygen and formate, are required for the investigated transformation.

Testing first each step separately, L-phenyl alanine [(*S*)-**1a**, 50 mM] and phenylpyruvic acid (**2a**, 10–100 mM), were used as model substrates. The oxidation of (*S*)-**1a** was investigated

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using L-amino acid oxidases[14] and deaminases (L-AAO/L-AADs) employed as freeze-dried *E. coli* cell preparations containing the overexpressed enzyme. Possible formed H_2O_2 will be disproportionated by the catalase(s) present in *E. coli*. Using air as oxygen source, best results were obtained for the L-AAD from *Proteus myxofaciens* (56% conversion, 18 h), which produces ammonia as a side product.[15] Improved conversion (95%) was achieved when the reaction was conducted under 2 bar of oxygen pressure.

For the asymmetric reduction of **2a** different *S* and *R* selective reductases were tested. Best results were obtained employing the stereocomplementary L- and D-isocaproate reductases (HicDHs) from *Lactobacillus paracasei* DSM 20008 (L-Hic)[16] and *Lactobacillus confuses* DSM 20196 (D-Hic).[17] Applying these reductases, the *S* as well as the *R* enantiomer of **3a** were accessible in enantiopure form (>99% *ee*) at substrate concentrations of up to 100 mM.

In a next step, the oxidation and reduction steps were run simultaneously. To identify suitable reaction conditions, the amount of L-AAD was varied while the other reaction parameters were kept constant. Stopping the reaction after one hour, the amount of final product (*S*)-**3a** increased with higher amount of the deaminase (Figure 1). In all these experiments, the amount of the intermediate ketoacid **2a** remained below 5%, demonstrating the ideal coupling of the system and indicating that the oxidation is the limiting step of the set up.

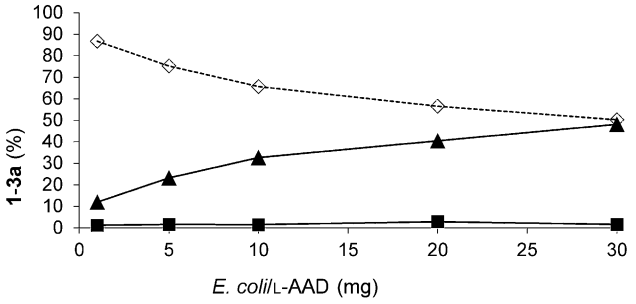


Figure 1. Percentage of **1**–**3a** versus the amount of L-AAD (◇: **1a**; ■: **2a**; ▲: **3a**). Reaction conditions: (*S*)-**1a** (50 mM), O_2 (2 bar), L-AAD, L-Hic (15 mg, 6.6 U), FDH (2 mg, 4.2 U), HCO_2NH_4 (150 mM, pH 7), 1 h, 21 °C, 170 rpm.

Consequently, the O_2 pressure was investigated showing that at 1 bar O_2 comparable results were obtained to those at 2 bar (Supporting Information Figure S3). Consequently, one bar O_2 was used for further experiments.

Following the biocatalytic transformation under these conditions over time, the transformation of (*S*)-**1a** to (*S*)-hydroxy acid (*S*)-**3a** went to completion within 7 h without formation of any side products (Figure 2). Notably, the amount of keto acid **2a** remained constant and below 3% along the reaction.

Preparative inversion as well as retention of L-amino acid (*S*)-**1a** (66 mg, 0.40 mmol, 50–200 mM) was achieved by combining the L-AAD with the L- or D-selective HicDHs at 50–200 mM concentration (Table 1), leading to (*S*)- or (*R*)-**3a** with complete conversion and in enantiopure form (>99% *ee*). At 100–200 mM substrate concentration the hydroxy acids (*S*)- and (*R*)-

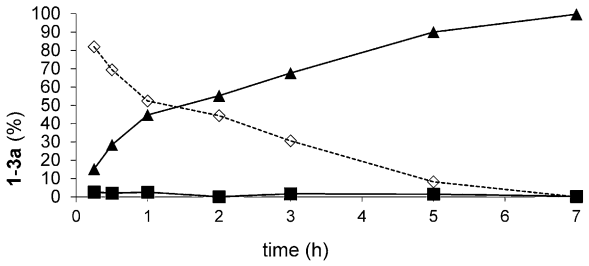


Figure 2. Percentage of **1**–**3a** versus time (◇: **1a**; ■: **2a**; ▲: **3a**). Reaction conditions: (*S*)-**1a** (50 mM), O_2 (1 bar), L-AAD (20 mg, 1 U), L-Hic (15 mg, 6.6 U), FDH (2 mg, 4.2 U), HCO_2NH_4 (150 mM, pH 7), 21 °C, 170 rpm.

3a were isolated with high yields (78–84%) after a simple extraction step without requiring chromatographic purification.

To demonstrate the scope of the method a representative panel of natural amino acids and L-norleucine (*S*)-**1b**–**f** was transformed possessing different functionalities, such as aliphatic, aromatic and heteroaromatic moieties. The substrate concentration was optimized in each case considering the solubility of the amino acid aiming to obtain complete conversion (see Supporting Information Table S2 for details). For instance, excellent results were obtained for tryptophan (**1b**) bearing a sensitive indole moiety. At 50 mM substrate concentration the hydroxy acids (*R*)- as well as (*S*)-**3b** were isolated in enantiopure form (>99% *ee*) and high isolated yield (83%, Table 2, entries 1 and 2). The transformation was also successfully performed for amino acids bearing aliphatic chains (**1c**–**d**, entries 3–6). In these cases the reactions were followed by NMR spectroscopy (see the Supporting Information for details). At 100 mM substrate concentration enantiopure (*R*)- as well as (*S*)-**3c**–**d** were successfully isolated with high yields. In the case of (*R*)-**3c**, the biocatalytic inversion was performed with 0.5 g of substrate (entry 4) demonstrating the scalability of the methodology. Using methionine (*S*)-**1e** as starting material, the bioretention and inversion were performed at 200 mM substrate concentration allowing the isolation of enantiopure (*S*)- as well

Table 1. Simultaneous oxidation and asymmetric reduction for the inversion and retention of (*S*)-**1a** to yield enantiopure hydroxy acids (*S*)- or (*R*)-**3a**.

Entry	Hic	[**1a**][a]	*c* [%][b]	**2a** [%][b]	**3a** [%][b]	*ee* **3a** [%][c]
1	L-Hic	50	>99	3	97	>99 (*S*)
2	L-Hic	100	>99	2	98 (81)[d]	>99 (*S*)
3	L-Hic	200	>99	1	99 (78)[d]	>99 (*S*)
4	D-Hic	50	>99	3	97	>99 (*R*)
5	D-Hic	100	>99	1	99 (84)[d]	>99 (*R*)
6	D-Hic	200	>99	1	99 (79)[d]	>99 (*R*)

Reaction conditions: (*S*)-**1a** (50–200 mM), HCO_2NH_4 (3 equiv, pH 7), NAD^+ (1 mM), FDH (42 U mmol^{-1}), 1 bar O_2, 7 h, 21 °C, 170 rpm; L-AAD: 15 U mmol^{-1}. Hic: 66 U mmol^{-1} for L-Hic and 96 U mmol^{-1} for D-Hic. [a] Concentration of starting material. [b] Determined by HPLC, reverse phase. [c] Determined by HPLC on a chiral phase. [d] Isolated yields in brackets.

as (*R*)-**3e** without detecting any side reaction due to oxidation of the sulfur atom. In all experiments the enantiopure (*R*)- or (*S*)-hydroxy acids were isolated in pure form after a simple extraction step without requiring chromatographic purification.

Table 2. Biocatalytic redox inversion/retention for the preparation of (*S*)- or (*R*)-**3b–e** employing the combination of a L-AAD with L- or D-Hic.

Entry	**1b–e** [mM][a]	Hic	*c* [%][b]	**2** [%][b]	**3** [%][b]	*ee* **3** [%][c]
1	**1b** (50)	L-Hic	>99	1	99 (83)[d]	>99 (*S*)
2	**1b** (50)	D-Hic	>99	2	98 (83)[d]	>99 (*R*)
3	**1c** (100)	L-Hic	>99	<1	99 (81)[d]	>99 (*S*)
4	**1c** (100)	D-Hic	>99	<1	99 (85)[d]	>99 (*R*)
5	**1d** (100)	L-Hic	>99	<1	99 (79)[d]	>99 (*S*)
6	**1d** (100)	D-Hic	>99	<1	99 (82)[d]	>99 (*R*)
7	**1e** (200)[e]	L-Hic	>99	<1	99 (77)[d]	>99 (*S*)
8	**1e** (200)[e]	D-Hic	>99	<1	99 (73)[d]	>99 (*R*)

Reaction conditions: (*S*)-**1b–e** (50–200 mM), HCO_2NH_4 (3 equiv, pH 7), NAD^+ (1 mM), FDH (42 U mmol^{-1}), 1 bar O_2, 7 h, 21 °C, 170 rpm; L-AAD: 30 U mmol^{-1} for **1b**; 15 U mmol^{-1} for **1c–e**. Hic: 132 U mmol^{-1} for L-Hic and 192 U mmol^{-1} for D-Hic for **1b**; 66 U mmol^{-1} for L-Hic and 96 U mmol^{-1} for D-Hic for **1c–e**. [a] Concentration of starting material. [b] Determined by HPLC, reverse phase for **1b**; determined by NMR spectroscopy for **1c–e**. [c] Determined by HPLC on a chiral phase for **3b**; determined by GC on a chiral phase for **3c–e**. [d] Isolated yields in brackets. [e] Reaction time 14 h.

Enantiopure 4-hydroxyphenyl lactic acid [(*R*)- or (*S*)-**3f**], is a building block for the preparation of various biologically active compounds, such as Aeruginosins 298A,[18] peroxime proliferator activated receptor α/γ agonists,[19] Microcin SF608,[20] etc. Moreover, (*S*)-**3f** can be used as a key starting material for the synthesis Saroglitazar (*S*)-**4** (Lypaglyn®),[21] which is used for the treatment of diabetes Type II, joining lipid and glucose lowering effects in one single drug. Although numerous synthetic approaches have been reported for the preparation of hydroxy acid (*S*)-**3f**, most of them require long reaction sequences including protection/deprotection strategies due to the reactivity of the phenol group to oxidation.[22]

Employing the here-presented bioretention/inversion cascade, L-tyrosine (*S*)-**1f** was successfully transformed at high substrate concentration (200 mM) and 100 mg scale (Scheme 2). The pharmacologically relevant *S* isomer was obtained in 80% isolated yield and enantiopure form (>99% *ee*). It is remarkable that the transformation worked so well for this substrate considering that (*S*)-**1f** is barely soluble in buffer (2.5 mM) thus at 200 mM concentration most of the amino acid was present as suspended solid.

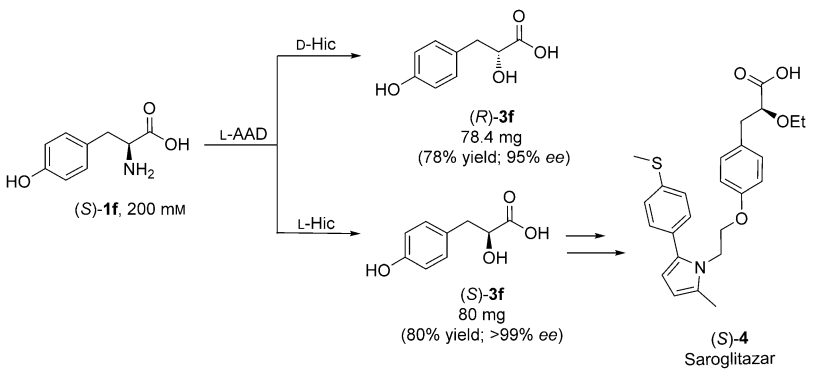
Conclusion

In summary, natural L-α-amino acids and L-norleucine were transformed to the corresponding valuable α-hydroxy acids[23] by a biocatalytic simultaneous oxidation/reduction cascade at the expense of molecular oxygen and formate. In contrast to the established diazotization, inversion as well as retention of the stereocenter could be achieved simply by the choice of the appropriate reductase. Substrate concentrations were optimized finding optimal values between 50 and 200 mM depending on the substrate. The α-hydroxy acids were obtained generally in optically pure form (>99% *ee*) with high isolated yields (73–85%) without requiring chromatographic purification. Due to the very mild reaction conditions in buffer the formation of side products was avoided even for sensitive amino acids, such as tryptophan or tyrosine, circumventing the need for protecting groups. This protocol offers a mild, eco-improved alternative for an established method extending the flexibility to access both product enantiomers and tolerating sensitive functionalities, representing a step towards cleaner and more selective organic transformations.

Experimental Section

General procedures

Chemical reagents were purchased from different commercial sources and used without further purification. Formate dehydrogenase (2.1 U mg^{-1}) was purchased from Evocatal (Evo 1.1.230). Melting points were taken on samples in open capillary tube and are uncorrected. ^{1}H and ^{13}C NMR spectra were obtained using Bruker spectrometer (^{1}H, 300.13 MHz; ^{13}C 75.5 MHz). The chemical shifts are given in delta (δ) and the coupling constants in Hertz (Hz). Mass spectra experiments (MS) were carried out by ESI, using Agilent 1260 HPLC coupled with by a 6120 quadrupole. Conversions for aromatic substrates were determined with a Shimadzu chromatograph UV detector at different wavelengths using a Luna C18 (25 cm×4.6 mm I.D.). Conversions for aliphatic substrates were determined by NMR spectroscopy. Chiral high performance liquid chromatography (HPLC) analyses were carried out in a Shimadzu chromatograph UV detector at different wavelengths using a Chiralcel OJ (25 cm×4.0 mm I.D.) for the determination of enantiomeric excesses ratio. Chiral gas chromatography analyses (GC) were car-



Scheme 2. Biocatalytic cascade for the synthesis of (*R*)- or (*S*)-**3f**, a valuable building block for the preparation of Saroglitazar.

ried out using a flame ionization detector (FID) using Chiralsil-Dex-CB column (25 m×0.32 mm I.D., film 0.25 μm).

Representative procedure for the synthesis of (*R*)-2-hydroxy-4-methylpentanoic acid [(*R*)-3c]

FDH (76 mg, 160 U), L-AAD (1.13 g freeze-dried *E. coli* cells containing overexpressed L-AAD, 56.5 U), D-Hic (0.57 g freeze-dried *E. coli* cells containing overexpressed D-Hic, 365 U) were added to a K-phosphate buffer (38 mL, 100 mM, pH 7.0) containing the substrate (500 mg, 3.81 mmol, 100 mM), NAD^+ (1 mM) and NH_4HCOO (300 mM). The suspension was shaken at 170 rpm, 21 °C and 1 bar of oxygen pressure for 8 h. After this time, the reaction was acidified to pH 1 with aqueous HCl 4 M (1 mL), and extracted with EtOAc (3×50 mL). The organic phases were combined, dried over Na_2SO_4, and the resulting solid washed with *n*-heptane to remove minor impurities coming from the enzyme (3×50 mL) affording (*R*)-**3c** as a white solid (0.417 g, 83% yield >99% *ee*).

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Keywords: asymmetric catalysis · biocatalysis · cascades · inversion · retention

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[23] For instance; hydroxy acids (*R*)- and (*S*)-**3c** are, respectively, 1286 and 86 times more expensive than the starting material L-**1c** (see Supporting Information Table S1 for additional details).

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