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Synthesis of Enantiomerically Pure Ring-Substituted L-Pyridylalanines by Biocatalytic Hydroamination

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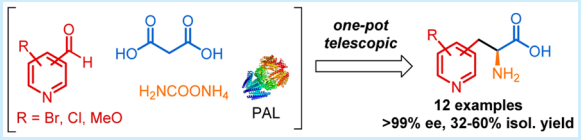
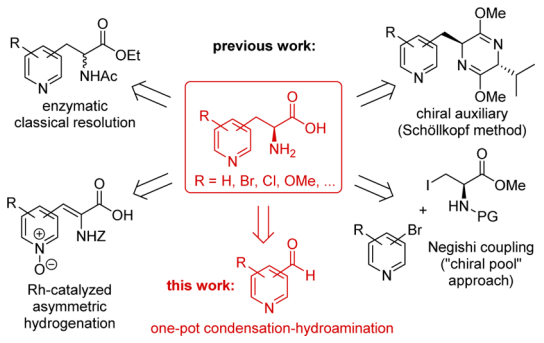
Supporting Information

ABSTRACT: Current routes to nitrogen-containing heteroarylalanines involve complex multistep synthesis and are often reliant on protection/deprotection steps and wasteful chromatographic purifications. In order to complement existing methodologies, a convenient telescopic strategy was developed for the synthesis of L-pyridylalanine analogues (12 examples) and other L-heteroarylalanines (5 examples) starting from the corresponding aldehydes. A phenylalanine ammonia lyase (PAL) from *Anabaena variabilis* was used as the biocatalyst to give conversions ranging between 88 and 95%, isolated yields of 32−60%, and perfect enantiopurity (>99% ee) by employing an additional deracemization cascade where necessary.



Non-natural heteroaromatic amino acids and their derivatives have gained great attention in medicinal chemistry as chiral building blocks and as drug candidates.[1] For example, L-α-azatyrosine, a natural product isolated from *Streptomyces chibansis*[2] and actinobacteria, has been shown to possess antibiotic and anticancer properties[3] and is implicated in the biosynthesis of kedarcidin, an antitumor antibiotic.[4] The biosynthetic origin of L-α-azatyrosine is still unclear, and as a result, access to these compounds and their derivatives from biological sources is limited. Currently, there are four major synthetic routes to these compounds: via classical resolution,[5] chiral auxiliaries,[6] asymmetric hydrogenation,[7] and the "chiral pool" approach[8] (Scheme 1).

Scheme 1. Currently Employed Strategies for the Synthesis of Substituted L-Pyridylalanines



The classical resolution approach is one of the earliest routes proposed, based on selective hydrolysis of racemic pyridylalanine esters by hydrolases such as subtilisin[5a] and α-chymotrypsin.[5b] The nature of this method limits the theoretical yield to 50%, of which 35−45% could be achieved in practice.

The chiral auxiliary approach consists of a diastereoselective alkylation of the Schöllkopf reagent (2,5-diketopiperazine derivative) and subsequent hydrolysis of the resulting azaenolate. Multiple chromatography steps are required, resulting in poor atom efficiency with moderate to good yield.[6]

Asymmetric hydrogenation of *N*-protected dehydroamino acids over a rhodium catalyst is one of the most common routes to enantiopure amino acids,[7] a process well-known for the synthesis of L-DOPA.[9] However, due to catalyst poisoning attributed to the nitrogen on the pyridine ring, the synthesis of pyridylalanines with this method requires first a protection step by forming the *N*-oxide then a reductive deprotection after the asymmetric hydrogenation step. Another strategy includes addition of a noncomplexing acid such as HBAF to protonate the nitrogen to prevent catalyst deactivation.[7]

Finally, a recently developed synthesis employed palladium-catalyzed Negishi coupling between a suitable bromopyridine and protected L-β-iodoalanine, obtained from L-serine ("chiral pool" approach).[8]

All of the synthetic strategies reported to date require lengthy multistep processes, multiple chromatographic stages, and protection/deprotection steps and hence result in relatively low yields for the synthesis of these valuable building blocks.

With the advent of biotechnology and the use of enzymes in organic synthesis, the need for expensive catalysts, toxic solvents, heavy metals, and protecting groups has been mitigated. In many cases, biocatalysis has become the strategy of choice for the regioselective and stereoselective synthesis of high value chiral building blocks for pharmaceutical and agrochemical applications. Previous attempts to develop asymmetric biocatalytic approaches to pyridylalanines (to circumvent the limitation of 50% maximum theoretical yield of enzymatic resolution) have

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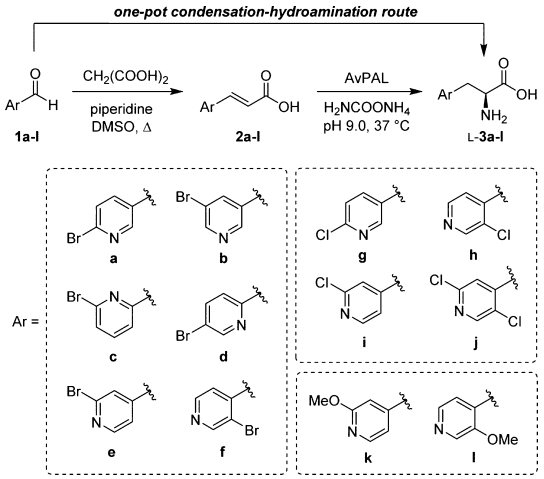
had limited success. For example, tyrosine phenol lyase (TPL), a PLP-dependent enzyme, has been used to synthesize L-azatyrosine isomers after incubation with hydroxypyridines and ammonium pyruvate for 5 days with isolated yields of 10−13%.[10]

The enzyme phenylalanine ammonia lyase (PAL) has recently emerged as a biocatalyst for the synthesis of non-natural amino acids;[11] PAL catalyzes the highly enantioselective cofactor-free addition of ammonia to inexpensive arylacrylic acid derivatives. This allows access to substituted L-phenylalanines with perfect atom economy, no protection/deprotection steps, and without the need for expensive cofactor recycling systems (common for many enzymatic reactions).[12] The anti-Michael addition of a nucleophile to an α,β-unsaturated acid is difficult to achieve chemically and highlights the synthetic utility of this enzyme. The synthesis of unsubstituted pyridylalanines has been demonstrated by Retey and co-workers, yielding 60−75% of the isolated product using a PAL from *Petroselinum crispum* (PcPAL).[13] Kinetic data for the natural reaction (amino acid to acrylic acid) have also been reported for pyridine and pyrimidine substrates.[14] However, there are no reports on substituted pyridylacrylic acids, bearing functional handles for further manipulation, being substrates for PALs in the context of synthetic applications. Herein, we present an efficient one-pot synthetic route to the target mono- and disubstituted L-pyridylalanines in high yield and ee based on PAL-mediated asymmetric hydroamination as summarized in Scheme 2.

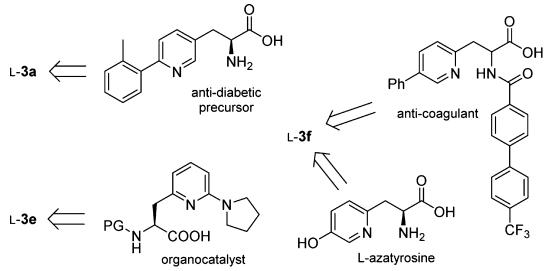
Scheme 2. One-Pot Telescopic Synthesis of L-Pyridylalanines L-3a−l by Knoevenagel−Doebner Condensation and Biocatalytic Hydroamination



Pyridylacrylic acid substrates **2a−l** can be synthesized easily from the corresponding benzaldehydes **1a−l** and malonic acid via the Knoevenagel−Doebner condensation[15] and then submitted to biocatalytic hydroamination to produce L-**3a−l**. The procedure for the latter step utilizes the bacterial PAL from *Anabaena variabilis* (AvPAL), expressed recombinantly in *Escherichia coli* BL21(DE3) cells, and an unadjusted NH_2COONH_4 reaction buffer. The biocatalyst, used as lyophilized whole cells, can easily be prepared in high yield (6 g L^{-1} of culture medium) and has been used in intensified preparative processes.[16]

As a substrate panel for our investigation, we selected six bromopyridine carboxaldehydes **1a−f** (Scheme 2) because of the practical applications of the corresponding arylalanines as chiral building blocks (Scheme 3). For instance, they are found in

Scheme 3. Applications of Products L-3a,e,f to the Synthesis of APIs, Natural Products, and Organocatalysts



anticoagulants,[17a] DPPI inhibitors,[17b] leukocyte adhesion inhibitors,[17c] azaindoline anticancer agents,[17d] and antidiabetics.[17e] Furthermore, molecules of this class have also been used as core structures of organocatalysts.[8] In many cases, the key chiral center is the pyridylalanine motif, highlighting the importance of stereoselective synthesis of these compounds by employing an inexpensive and simple process.

In order to test the robustness of the method, we expanded our panel including chloro- (**1g−i**), dichloro- (**1j**), and methoxy-substituted (**1k,l**) pyridine carboxaldehydes to provide access to the novel uncharacterized compounds **3g−l**. We elected to cover all the three isomeric classes of substituted pyridines: the nicotinaldehyde series (*meta*-relationship, **1a,b,g**), the picolinaldehyde series (*ortho*-relationship, **1c,d**), and the isonicotinaldehyde series (*para*-relationship, **1e,f,h−l**).

The Knoevenagel−Doebner condensation carried out in DMSO at 100 °C for 16 h[15c] afforded full conversion to acrylic acids **2a−l** (confirmed by ^{1}H NMR). The usual workup procedure involves quenching the reaction mixture with HCl; however, with the presence of a nitrogen atom in the ring, the isolation proved troublesome due to the high solubility of the target products **2a−l**. Addition of the condensation mixture to deionized water at neutral pH caused precipitation of cinnamic acids **2a−d,g−j,l** in high purity but with low yield. A different workup procedure was adopted by extracting the product with ethyl acetate followed by purification by chromatography, affording higher yields but unsatisfactory purity (by ^{1}H NMR). Surprisingly, acrylic acids **2e,f,k** partially precipitated out of solution when cooled to room temperature (without any workup), with good isolated yields obtained by addition of water and cooling the solution at 4 °C.

Due to the low yields obtained for the isolation of compounds **2a−l**, we envisaged a one-pot telescopic route[18] starting from the aldehydes **1a−l** to access pyridylalanines **3a−l**. A solution of the aldehyde (1 M), malonic acid, and piperidine in DMSO was heated, the condensation mixture was diluted in saturated aqueous ammonium carbamate (50 mM final concentration of **2a−l**), and lyophilized *E. coli* cells producing AvPAL (25 mg mL^{-1}) were added as the biocatalyst. Biotransformation mixtures were incubated at 37 °C for 2−30 h (Table 1). Very high conversions were achieved, and the products could be readily purified by adsorption on ion-exchange resin (Dowex 50WX8) to afford white solids of excellent purity (by ^{1}H NMR and HPLC) in good yields.

Compounds with electron-donating groups (EDGs) generally lead to low conversion with PAL enzymes, whereas those with electron-withdrawing groups (EWGs) are accepted with high conversion, provided that the substrate is not sterically hindered.[11] The presence of an electron-poor pyridine ring enhances the rate of catalysis, hence leading to higher

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Table 1. One-Pot Telescopic Synthesis of Compounds L-3a–l

product	time (h)	conv[a] (%)	ee[b] (%)	final ee[c] (%)	isol yield[d] (%)
3a	22	97	>99	*e*	51
3b	6	97	93	>99	47
3c	6	95	90	>99	53
3d	30	88	61	>99	44
3e	4	99	80	>99	52
3f	4	99	84	>99	48
3g	22	99	>99	*e*	45
3h	2	99	92	>99	40
3i	2	97	92	>99	60
3j	2	99	96	>99	32
3k	30	99	>99	*e*	46
3l	22	99	>99	*e*	56

[a]Conversion measured by reversed-phase HPLC on a nonchiral phase (compounds **3a–f**) or by ^{1}H NMR (compounds **3g–l**). [b]Enantiomeric excess after the telescopic condensation–hydroamination, measured by reversed-phase HPLC on a chiral phase. [c]Enantiomeric excess after the subsequent deracemization step (Scheme 4), measured by reversed-phase HPLC on a chiral phase. [d]Isolated yield calculated from **1a–l**. [e]Not required due to the high optical purity of the product.

conversions. Remarkably, while methoxy-substituted phenylalanines have previously shown no conversion with PAL enzymes, compounds **3k** and **l** (with a hybrid system of EDG/EWG) gave full conversion with AvPAL.[19]

As a general trend, isonicotine substrates (nitrogen at the *para*-position) were converted faster compared to the other isomers, while substrates with bulky substituents at the *para*-position (**2a,d,g**) required longer reaction times.

It is worth noting that for some of the substrates (**2h–l**) accumulation of small amounts of impurities was apparent in the ^{1}H NMR after prolonged incubation with the biocatalyst. As such, biotransformations were monitored by HPLC and/or ^{1}H NMR in order to identify the optimal reaction time (Table 1), thus eliminating the formation of side products and giving the best compromise between conversion, ee, and purity.

Concerning the optical purity of the products, we observed that substrates with higher rates of amination by AvPAL, in general, gave imperfect ee. In the bromopyridyl subset, compounds **3c–f** (nitrogen in the *ortho*- or *para*-position) were found to give a significant drop in ee over time, while compounds **3a–b** (nitrogen in the *meta*-position) did not show any appreciable decrease in optical purity. The evolution of ee over time is shown in Figure 1a. A similar trend was observed with chloro substituents, with the ee decreasing for the isonicotine isomers (**3h–j**) and being constant for the nicotine isomer (**3g**). This behavior can be rationalized in the light of previous work on the mechanism of the PAL-mediated hydroamination reaction (Figure 1b). The stereoselective addition requires a 4-methylidene-5-imidazolone (MIO) prosthetic group to bind ammonia before transferring it to the α-position of the substrate and an essential tyrosine residue to protonate the intermediate.[11] However, with electron-deficient aromatic systems, there is evidence of an alternative enzyme-catalyzed MIO-independent pathway to afford the racemic product, as we have previously reported.[20] This alternative pathway has been shown to be more prevalent for EWG-bearing substrates due to the higher partial positive charge at the α-position. Therefore, the caveat when using substrates bearing EWGs is the potential loss of optical purity due to this alternative pathway (as seen for some of the substrates in this work, Table 1). The extent of racemization can be linked to the position of the nitrogen atom (*o*-, *p*- ≫ *m*-): for the picoline and isonicotine series, the delocalization of the negative charge of the carbanion-like intermediate on the nitrogen is more effective, making the substrate more susceptible to addition of free ammonia.

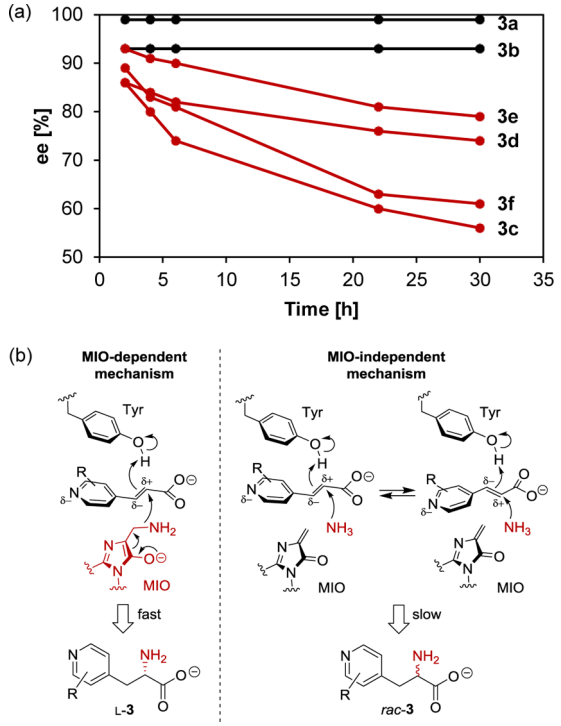
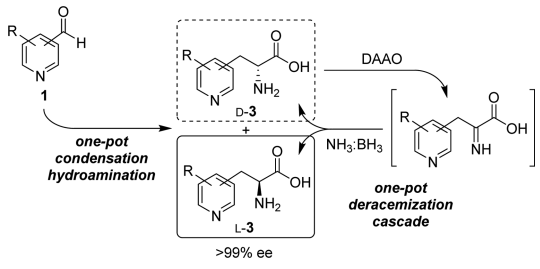


Figure 1. (a) Evolution of the ee over time for products L-**3a–f**. (b) Mechanism of PAL-mediated hydroamination and alternative mechanistic pathway accounting for the formation of the D-product.

Interestingly, while halogen substituents enhance this effect by decreasing the electron density of the ring, EDGs can mitigate it (Table 1): methoxy-substituted compounds (**3k,l**) gave a final ee >99%, which indicates the electron-donating power of the methoxy group counteracts the electron-withdrawing capability of the nitrogen atom.

In order to obtain high ee values, we employed an additional chemoenzymatic cascade system to produce enantiomerically pure L-enantiomer in a deracemization process (Scheme 4).[21] D-Amino acid oxidase (DAAO) selectively oxidizes the D-enantiomer to the imino acid followed by reduction with ammonia–borane in a nonselective manner. Complete consumption of the D-amino acid was observed after 2 h to give L-**3b–j** with >99% ee (Table 1).

Scheme 4. Chiral Polishing of Products L-3b–j by a Chemoenzymatic Deracemization Cascade



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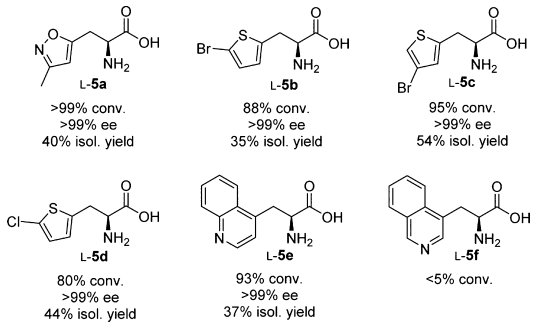
Finally, to demonstrate the broad applicability of our methodology, we tested a panel of six substituted aromatic aldehydes bearing different heterocycle moieties (isoxazole, thiophene, quinoline, and isoquinoline). Complete conversions were obtained with all of the substrates for the condensation step, while only isoxazole, thiophene, and quinoline derivatives were accepted by the enzyme, yielding amino acids L-**5a−e**. The synthesis of the isoquinolinylalanine L-**5f** was unsuccessful due to the poor acceptance of the corresponding acrylic acid by AvPAL. The dramatic difference between quinoline and isoquinoline substrates can be attributed to the different electronic properties of the two systems, i.e., the position of the nitrogen atom (*p*- ≫ *m*-). Amino acids L-**5a−e** were obtained with >99% ee without additional deracemization in moderate to good isolated yield (Scheme 5).

Scheme 5. Examples of Additional Heteroarylalanines Obtained by One-Pot Condensation−Hydroamination



In summary, we have developed a one-pot telescopic route to afford L-pyridylalanine analogues in high conversions, good isolated yields, and excellent purity from the corresponding aldehydes, implementing an additional chemo-enzymatic cascade to give >99% ee for compounds which gave low enantiopurity.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02559.

Experimental details, characterization data of all compounds, and ^{1}H NMR, ^{13}C NMR, and HRMS spectra (PDF)

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Notes

The authors declare no competing financial interest.

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