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Chemoenzymatic Synthesis of Optically Pure L- and D-Biarylalanines through Biocatalytic Asymmetric Amination and Palladium-Catalyzed Arylation

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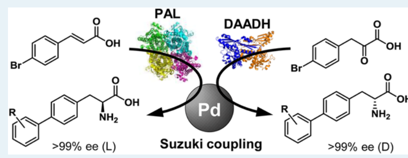
Ⓢ *Supporting Information*

ABSTRACT: A chemoenzymatic approach was developed and optimized for the synthesis of a range of N-protected nonnatural L- and D-biarylalanine derivatives. Starting from 4-bromocinnamic acid and 4-bromophenylpyruvic acid using a phenylalanine ammonia lyase (PAL) and an evolved D-amino acid dehydrogenase (DAADH), respectively, both enantiomers of 4-bromophenylalanine were obtained and subsequently coupled with a panel of arylboronic acids to give the target compounds in high yield and optical purity under mild aqueous conditions.



KEYWORDS: *biocatalysis, biarylalanines, amino acids, amination, Suzuki-Miyaura coupling, cascade reactions*

An increasing number of drugs in development contain nonnatural amino acids in their core motif; in particular, the biarylalanine moiety (Figure 1). For example, L-biarylalanines have been incorporated in dipeptidyl peptidase 4 (DPP IV)[1] inhibitors, $\alpha 4\beta 7$ integrin inhibitors,[2] viral 3C-protease inhibitors,[3] and endothelin-converting enzyme inhibitors,[4] and D-enantiomers are featured in botulinum toxin inhibitors,[5] amyloid-β-peptide aggregation inhibitors,[6] kinesin-14 motor protein KIFC1 inhibitors,[7] and reverse cholesterol transport facilitators.[8]

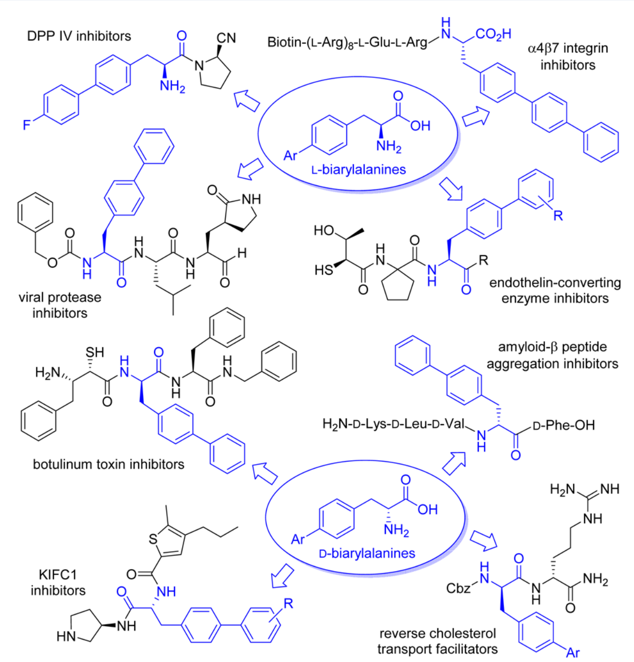
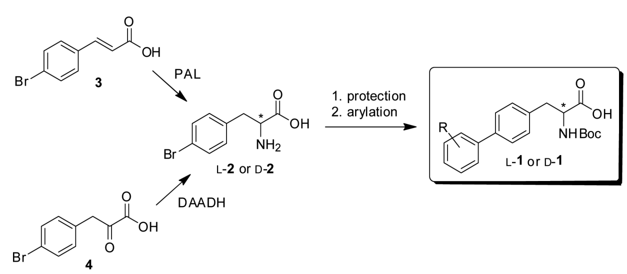


Figure 1. Patented pharmaceuticals containing L- and D-biarylalanines as chiral building blocks.

Although a number of chemical methods have been described in the literature for the synthesis of nonnatural amino acids,[9] to date, no chemoenzymatic approach to biarylalanines has been reported. Herein, we present two efficient biocatalytic strategies (hydroamination of 4-bromocinnamic acid **3** and reductive amination of 4-bromophenylpyruvic acid **4**) for the preparation of both enantiomers of 4-bromophenylalanine **2**, followed by protection and Suzuki–Miyaura coupling with a panel of arylboronic acids, to afford the target compounds L- and D-**1** (Scheme 1).

Scheme 1. Chemoenzymatic Approach to L- or D-1



One of the most attractive biocatalytic routes to optically pure L-arylalanines is the asymmetric hydroamination of arylpropenoic acids catalyzed by phenylalanine ammonia lyases (PALs),[10] with 100% atom economy and no need for cofactor regeneration systems. In nature, PAL catalyzes the deamination of L-phenylalanine to cinnamic acid,[11] and the reverse reaction

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has been exploited in the synthesis of novel L-phenylalanine analogues from substituted cinnamic acids.[12]

The biotransformation of 4-bromocinnamic acid **3** was tested under the standard conditions for PAL aminations (5 mM **3**, 5 M aqueous ammonia, pH 9.6) using three different wild-type PALs overproduced in *Escherichia coli* whole cells: PcPAL from *Petroselinum crispum* (parsley), *Rg*PAL from the red yeast *Rhodotorula glutinis* and *Av*PAL from the cyanobacterium *Anabaena variabilis*. The final conversions obtained are reported in Table 1, with the best results being provided by *Av*PAL,

Table 1. PAL Amination of 3[a]

PAL variant	conv (%)[a]
*Pc*PAL	44
*Rg*PAL	67
*Av*PAL	71
*Av*PAL-F107L	72
*Av*PAL-F107I	75
*Av*PAL-F107A	80

[a]Experimental conditions: 25 mg mL^{-1} lyophilized *E. coli* cells producing PAL, 5 mM **3**, 5 M NH_4OH, pH 9.6, 37 °C, 18 h. [a]Determined by HPLC.

which has recently emerged as a promising candidate for preparative-scale biotransformations because of its broader substrate scope.[13]

Analysis of the active site of *Av*PAL suggested that the conversion might be improved by reducing the steric clash between the bromine atom and the residue F107 in the aromatic binding pocket (Figure 2). We therefore designed

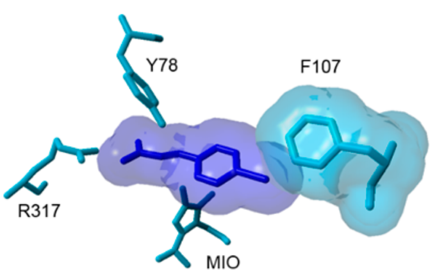


Figure 2. Active site model of **3** bound to AvPAL (MIO = 4-methylideneimidazol-5-one).

variants in which F107 was mutated to smaller hydrophobic residues, that is, F107I, F107L, and F107A. For all three variants, the conversions were found to be higher compared with WT (Table 1), and the highest value was obtained with the variant F107A. HPLC analysis on a chiral stationary phase showed that the phenylalanine product L-**2** was obtained with >99% ee.

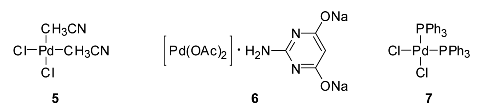
We also tested the PAL-catalyzed hydroamination of 4-phenylcinnamic acid under the same conditions (see Supporting Information). No conversion was observed, proving that our chemoenzymatic approach is essential for the PAL-mediated synthesis of L-**2**.

To access D-**2**, we selected an NADPH-dependent D-amino acid dehydrogenase (DAADH, engineered from a *meso*-diaminopimelate dehydrogenase from *Corynebacterium glutamicum*), which has been previously reported to be a very effective catalyst for the reductive amination of a wide range of aliphatic and aromatic α-ketoacids.[14] The reductive amination of 4-bromophenylpyruvic acid **4** was tested with DAADH cell-free extract (10 mM **4**, 200 mM NH_4^+, 100 mM carbonate buffer, pH 9.0) using methanol as a cosolvent and the glucose/glucose dehydrogenase (GDH) system for the regeneration of the NADPH cofactor, giving complete conversion to D-**2** with >99% ee by HPLC.

Remarkably, the DAADH-catalyzed reductive amination of 4-phenylphenylpyruvic acid under the same conditions (see Supporting Information) afforded 82% conversion, proving once again the broad substrate spectrum of this engineered biocatalyst. However, the strategy based on the synthesis of D-**2** as a gateway intermediate to a wide range of coupled products is more efficient and versatile than optimizing the biotransformation conditions for each different biaryl substrate.

Having access to both enantiomers of **2** through different biocatalytic routes, we turned our attention to the development of the arylation step. Three palladium catalysts (**5**−**7**) were shown to be active under aqueous conditions (Scheme 2),[15−17] and to investigate their suitability, a model reaction was set up between 4-bromobenzoic acid **4** and phenylboronic acid **9a**.

Scheme 2. Catalysts for Aqueous Suzuki Coupling



Catalyst **5**, in a water/ethanol mixture as solvent, gave a good yield of compound **10** (Table 2, entry 1), whereas in neat water, the conversion dropped considerably to 24% (entry 2). Phenol and biphenyl were identified as the predominant side products; however, with exclusion of oxygen, the conversion increased to 36% (entry 3). In an oxidative environment, palladium can react with oxygen to form a palladium−peroxo intermediate, which catalyzes the homocoupling and the oxidation of the boronic acid.[20] By increasing catalyst concentration and temperature, up to 86% yield of **10** in neat water was obtained (entry 6) without the need for a nitrogen atmosphere.

Davis et al. have reported the use of catalyst **6** in the bioconjugation of proteins and peptides via a Suzuki−Miyaura cross-coupling reaction;[18] however, the reaction with **6** was slow, and a maximum yield of 41% was obtained (entry 7). We applied the best conditions from catalyst **5** to catalyst **6** (entry 8): although the yield increased, it was still lower than with **5**. Catalyst **7** was completely insoluble under the conditions tested (entry 9), so it was not pursued any further. Na_2CO_3 as a less expensive alternative to Cs_2CO_3 (entry 10) gave lower conversion. To reduce the reaction times (24 h), microwave irradiation was employed, affording similar yields after only 20 min (entry 11).

The optimized conditions from the model reaction (entry 11) were applied to the coupling of *N*-Boc-4-bromo-L-phenylalanine, L-**11**, with **9a**, affording the final biaryl product, L-**1a**, in 93% conversion and >99% ee by HPLC (Scheme 3).

Addition of Boc_2O after the PAL biotransformation formed the desired product L-**11**, but also side products *tert*-butyl carbamate **12** (by reaction with free ammonia) and 4-phenylcinnamic acid **13** (from the coupling between **3** and **9a**). The coupling reaction performed in the presence of varying amounts of **12** and **3** led to lower yields (Figure S1a, Supporting Information). Furthermore, protection and coupling attempts on the crude PAL biotransformation mixture yielded no product due to the high ammonia concentration in

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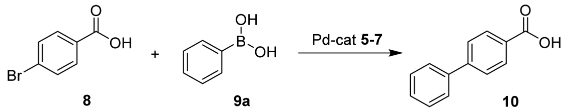
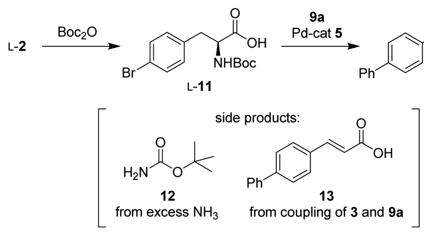
Table 2. Optimisation of the Reaction between 8 and 9a under Aqueous Conditions



entry	cat. (mol %)	solvent	conditions	temp (°C)	base	conv (%)[a]
1	**5** (2)	H_2O/EtOH 2:1	4 h, air	50	Cs_2CO_3	62
2	**5** (2)	H_2O	4 h, air	50	Cs_2CO_3	24
3	**5** (2)	H_2O	4 h, N_2	50	Cs_2CO_3	36
4	**5** (10)	H_2O	4 h, N_2	50	Cs_2CO_3	71
5	**5** (10)	H_2O	4 h, air	50	Cs_2CO_3	43
6	**5** (10)	H_2O	24 h, air	80	Cs_2CO_3	86
7	**6** (4)	H_2O	4 h, air	50	KP_i buffer pH 7.5	41
8	**6** (10)	H_2O	24 h, air	80	Cs_2CO_3	56
9	**7** (2−10)	H_2O	4 h, air	50	Cs_2CO_3	< 5
10	**5** (10)	H_2O	24 h, air	80	Na_2CO_3	68
11	**5** (10)	H_2O	20 min, air, MW	120	Cs_2CO_3	84

[a]Determined by HPLC.

Scheme 3. One-Pot Protection and Arylation of L-2

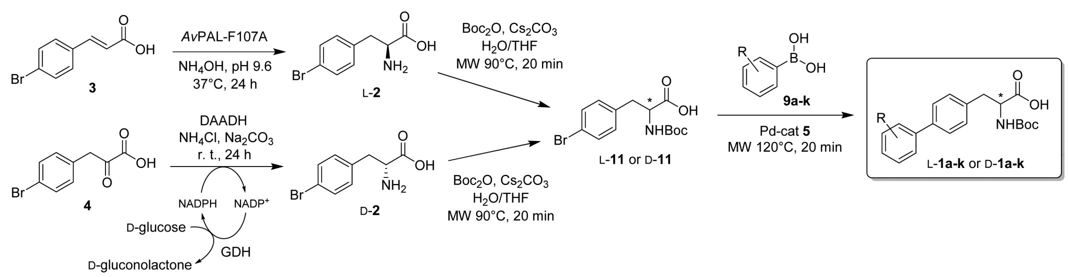


the buffer, as demonstrated by control experiments with increasing NH_4^+ concentration (Figure S1b).

Therefore, for the PAL-mediated synthesis of L-**1a**, the removal of ammonium salts and unreacted **3** from the reaction mixture was performed by adsorption on an ion-exchange resin, affording quantitative recovery of pure L-**2** ready for use in the following step. Boc protection and cross-coupling could be performed in one pot to afford compound L-**1a** (>99% ee) in 70% isolated yield, resulting in an overall yield of 53% from **3** (Table 3).

In the case of the DAADH biotransformation, the substantially lower ammonia concentration and the complete consumption of the starting material prevent competing side reactions and catalyst deactivation. Therefore, it was possible to successfully run the whole sequence as one-pot system, giving

Table 3. Chemoenzymatic Synthesis of Compounds L-1a−k[a] and D-1a−k[b]



		L-1a−k			D-1a−k	
product	R	conv. from L-2 (%)[c]	isol. yield from L-2 (%)[d]	overall isol. yield from 3 (%)[e]	overall conv. from 4 (%)[f]	overall isol. yield from 4 (%)[g]
1a	H	75	70	53	86	57
1b	2-F	99	81	60	99	56
1c	3-F	65	55	41	99	66
1d	4-F	85	70	40	99	64
1e	4-Cl	95	85	63	98	66
1f	3-Cl	80	58	33	74	47
1g	2-MeO	65	61	42	95	62
1h	3-MeO	99	89	65	99	61
1i	4-MeO	99	90	64	97	68
1j	3,4-methylenedioxy	99	80	58	99	70
1k	4-Ph	82	51	39	95	40

[a]Via hydroamination followed by one-pot protection and coupling. [b]Via one-pot reductive amination, protection and coupling. [c]Analytical conversion of L-**2** to L-**1** (determined by HPLC). [d]Isolated yield of L-**1** from L-**2**. [e]Overall isolated yield of L-**1** from **3**. [f]Analytical conversion of **4** to D-**1** (determined by HPLC). [g]Overall isolated yield of D-**1** from **4**.

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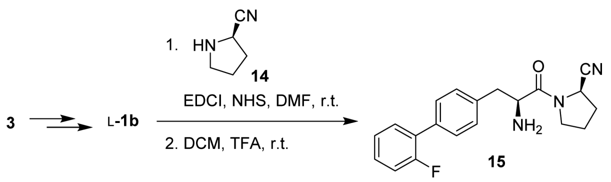
D-**1a** (>99% ee) with overall conversion of 86% and isolated yield of 57% from **4** (Table 3).

To demonstrate the generality of our approach, using the optimized conditions for the chemoenzymatic synthesis of L- and D-**1a**, we employed a panel of substituted phenylboronic acids **9b−k** to afford L- and D-biarylalanine derivatives L- and D-**1b−k** in high yield and >99% ee (Table 3).

As an example of the practical relevance of these building blocks, we exploited our chemoenzymatic approach to L-**1d** in the synthesis of the DPP IV inhibitor **15**[1] (Scheme 4) in 30% overall yield from **3**.

Scheme 4. Chemoenzymatic Synthesis of DPP IV Inhibitor 15



In summary, we designed a green, efficient route to a range of biarylalanines through the marriage of two enantiocomplementary enzymatic transformations with a combinatorial chemocatalytic coupling. The modular independence of the bio- and chemocatalytic conversions shown here may be more broadly applicable in the field of medicinal chemistry, allowing similar expansion to the product range of other biocatalysts.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acscatal.5b01132.

Figure S1, experimental section, and copies of spectra (PDF)

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Notes

The authors declare no competing financial interest.

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