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Chemoenzymatic One-Pot Synthesis of Enantiopure L-Arylalanines from Arylaldehydes

Csaba Paizs,[a,b] Adrian Katona,[a] and János Rétey*[a]

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Various enantiopure L-arylalanines can be prepared from the corresponding arylaldehydes in one-pot fashion by a combination of chemical and enzymatic reactions. The reagents used were (triphenyl-λ^5-phosphanylidene)ethyl acetate (Wittig reaction), porcine liver esterase (PLE), phenylalanine ammonia lyase (PAL) from parsley and ammonia. As examples the following aryl groups were used: phenyl, 4-chlorophenyl, 3-fluorophenyl and thiophen-2-yl. The pure L-enantiomers of phenyl-, 4-chlorophenyl-, 3-fluorophenyl- and thiophen-2-yl-alanines were obtained after ion-exchange chromatography in 88, 78, 72 and 91 % yields, respectively, calculated from the arylaldehydes.

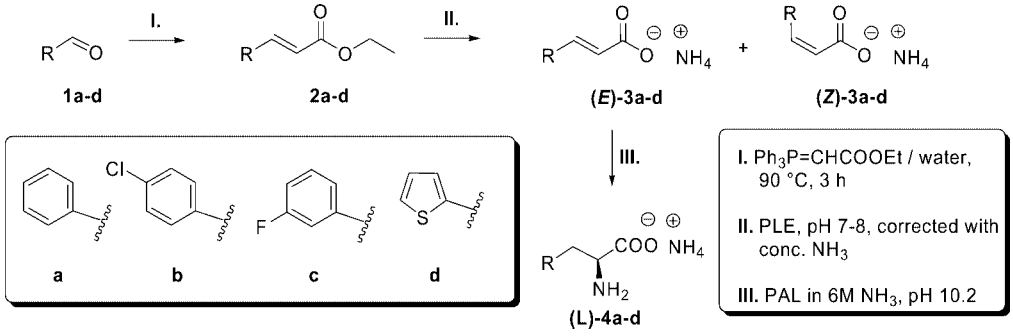
Introduction

Both naturally occurring and unnatural L-arylalanines are frequent constituents of biologically and pharmaceutically important products such as protease inhibitors, an extremely important class of pharmaceuticals for treatment of HIV, influenza or human cytomegalovirus.[1–3] Their enantioselective synthesis by a simple method from commercially available achiral and cheap starting materials is an attractive goal, while the incorporation of unnatural arylamino acids into pharmaceutically active compounds such as antibiotics might circumvent resistance developed against them in certain diseases.

Since most enzymes not only show strict reaction- and enantioselectivity but are also specific to one or a few substrates, their use in biocatalysis is rather limited. Enzymes of broad substrate specificity, while maintaining other specificities, are therefore preferred as biocatalysts. Porcine liver esterase (PLE)[4–7] and phenylalanine ammonia lyase (PAL)[8] fulfil these criteria.

Results and Discussion

Syntheses consisting of several steps usually require time-consuming and expensive purification of the intermediates



Scheme 1. The one-pot synthesis of L-arylalanines.

to remove side products or reagents that would impair the next step. To avoid such problems, practically quantitative conversions and compatible reagents and solvents are required, and in such a case a one-pot procedure should be achievable without purification of the intermediates. There are several reports of chemoenzymatic one-pot syntheses for the preparation of enantiomerically pure compounds such as 4-amino-2-hydroxy acids[9] or natural compounds

[a] Institute of Organic Chemistry and Biochemistry, University of Karlsruhe,
Richard-Willstätter-Allee, 76128 Karlsruhe, Germany
Fax: +49-721-6084823
E-mail: janos.retey@ioc.uka.de
[b] Department of Biochemistry and Biochemical Engineering, Babeş-Bolyai University,
Arany János str. 11, 400028 Cluj-Napoca (Kolozsvár), Romania
Fax: +49-264-590818
E-mail: paizs@chem.ubbcluj.ro

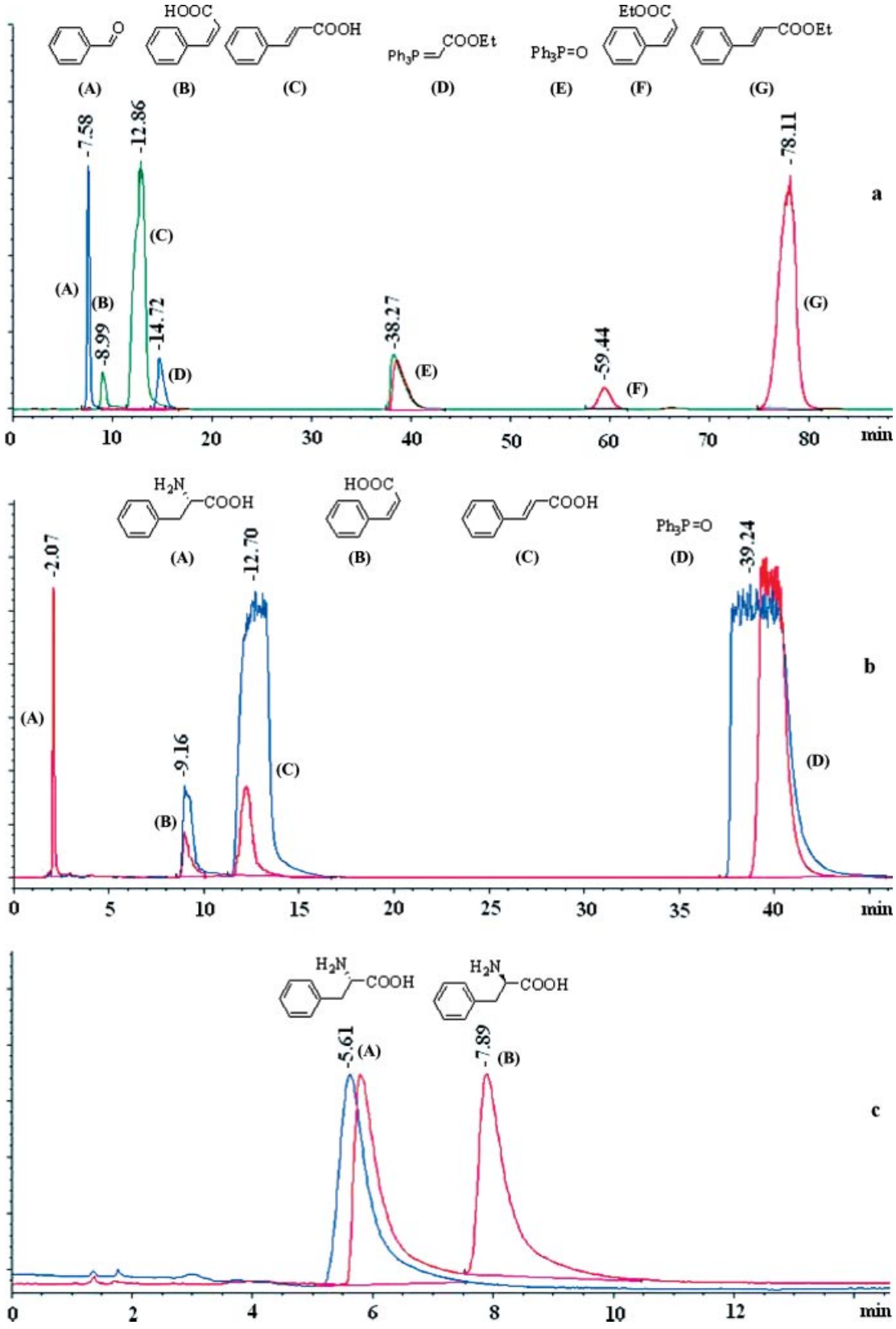


Figure 1. (a) Elution diagram of the starting materials (blue trace) and the products of the Wittig (red trace) and PLE-mediated reactions (green trace). (b) Elution diagram of the products of the two enzymatic steps of the one-pot reaction: PLE-mediated (blue trace) and PAL-catalysed (red trace). (c) Elution diagram of the isolated product of the one-pot reaction, L-phenylalanine (blue trace) and (*rac*)-phenylalanine as reference (red trace).

such as (*R*)- and (*S*)-tembamide,[10] but no chemoenzymatic one-pot syntheses of amino acids have so far been reported.

Since Wittig reactions between aryl aldehydes and (triphenyl-λ^5-phosphanylidene)ethyl acetate commonly take place[11] in quantitative yields in aqueous solutions, subsequent conversions by PLE and PAL in the same pot might be hoped to afford enantiopure L-arylalanines in excellent yields. Here we demonstrate with four examples that this is indeed a powerful and quick procedure.

The reaction sequence and the aryl groups (**a**–**d**) chosen are shown in Scheme 1. The progress of the reaction was monitored after each step by HPLC to determine the best conditions for quantitative conversions. When this had occurred, the conditions (dilution, temperature, pH, ammonia concentration) were adjusted for the reagents catalysing the next step. For clarity the starting materials (blue trace), the Wittig products (red trace) and the products after hydrolysis by PLE (green trace) are shown separately in Figure 1a. It so happens that the Wittig reaction is not completely stereospecific, the (*E*) and (*Z*) diastereomers being produced in a ratio of 95:5. PLE is not specific for either one of these diastereomers, as can be seen from the green trace, but (*Z*)-cinnamic acid is inert to PAL. Figure 1b shows the HPLC analysis after PLE-mediated hydrolysis (blue trace) and after the PAL-catalysed ammonia addition (red trace). The elution diagrams are monitored at 205 nm; since the absorption of phenylalanine at this wavelength is much less than those of (*E*)- and (*Z*)-cinnamic acids the areas under the lines do not correspond to the real amounts of the products. The PAL reaction was stopped when the consumption of the (*E*)-cinnamate ceased. The final product, L-phenylalanine was easily isolated on an ion-exchange column. The procedure for the other arylalanines was similar and the yields on the isolated arylalanines are shown in Table 1. Beside the yields, which varied between 72 and 91%, the absolute configurations and the enantiomeric purities of the isolated arylalanines were also determined. Figure 1c shows the elution diagram from a chiral column of the produced phenylalanine (blue trace) together with that of racemic phenylalanine as a control (red trace). The expected L configurations and the enantiopurities of all the arylalanines were confirmed by their optical rotations (data not shown), which were consistent with literature values.[12–15]

Table 1. Yields and reaction times for the one-pot synthesis of L-arylalanines.

Subst.	Prod.[a]	Yield (%)[b]	Time (days)
1a	L-**4a**	88	2
1b	L-**4b**	78	2
1c	L-**4c**	72	3
1d	L-**4d**	91	4

[a] *ee* > 98% for all the compounds. [b] Yields given are for the isolated compounds.

In conclusion, we present a one-pot, high-yielding chemoenzymatic synthesis of enantiopure L-arylalanines from the corresponding arylaldehydes. From earlier literature[16–19] on the substrate specificity of PAL we estimate that at least 37 arylalanines can be prepared by the new method.

Experimental Section

Materials and Methods: The NMR spectra were recorded on a Bruker spectrometer operating at 400 MHz and 100 MHz at 25 °C. EI-MS spectra were taken on a VG 7070E mass spectrometer operating at 70 eV. HPLC analyses were conducted with a HP 1050 instrument and a Nucleodur 100–5 C8 ec column (0.46 × 25 cm), the eluent was HCl (20 mM) in water/acetonitrile (70:30, *v*/*v*) isocratic at a 1 mL min^{-1} flow rate. For each analysis 50 μL of the reaction mass suspension was diluted with 450 μL of a water/methanol mixture (50:50, *v*/*v*), dissolving the solid triphenylphosphane oxide. A Chirobiotic-T column (0.46 × 25 cm) was used for examination of the enantiomeric compositions of the isolated amino acids **4a**–**d** with a methanol/water mixture (80:20, *v*/*v*) as eluent at 1 mL min^{-1} flow rate. Baseline enantiomeric separation of all racemic amino acids **4a**–**d** was performed. Melting points were determined by the hot plate method and are uncorrected. Optical rotations were determined on a Perkin–Elmer 201 polarimeter. All analytical and physical data for the isolated and purified L-amino acids are in good agreement with the literature data.[14,20,21]

Aldehydes **1a**–**d**, (triphenyl–λ^5–phosphanylidene)ethyl acetate, porcine liver esterase (PLE) and ion-exchange resin (Dowex 50X8) were products of Aldrich, Fluka or Sigma.

Recombinant PAL

Wild-type phenylalanine ammonia lyase was overexpressed in *E. coli* and purified as described, first according to Schuster and Rétey[22] and later with the improved method of Baedeker and Schulz.[23]

Synthesis of L-Amino Acids from the Corresponding Aldehydes by a One-Pot, Three-Step Reaction: A mixture of one of the aldehydes **1a**–**d** (5 mmol) and (triphenyl-λ^5-phosphanylidene)acetic acid ethyl ester (1.91 g, 5.5 mmol) in water (15 mL) was vigorously stirred at 90 °C until the aldehyde had been completely transformed into the cinnamate [mostly (*E*)-cinnamate (approx. 3 h, checked with HPLC); in each case 3–5% (*Z*) isomer was also formed]. After the mixture had cooled to room temperature the pH of the solution was adjusted to 8 with conc. ammonia solution and PLE (2 mg, 28 iU) was added. The suspension was stirred and sonicated at 25–30 °C while the pH of the solution was kept between 7 and 9 by addition of conc. ammonia. After completion of the hydrolysis of the cinnamic acid ester (approx. 6 h, checked with HPLC), water and conc. ammonia were added to produce a 6 M ammonia solution and a 2 mM cinnamate concentration. CO_2 was bubbled through the system until the pH of the solution had been adjusted to 10.2. After the addition of PAL (1 iU) the reaction mixture was stirred under argon at 30 °C. The progress of the reaction was followed by HPLC and when the formation of the L-amino acid had ceased the reaction mixture was degassed under reduced pressure, the pH was adjusted to 1.5 with 5% HCl, and the solution was filtered, heated to 90 °C for 10 min, cooled to room temperature, filtered again and applied to a Dowex 50X8 cation-exchange resin column.

Elution of the pure L-enantiomers of the amino acids **4a**–**d** was achieved with 2 M ammonia solution.

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