SUPPORTING INFORMATION

Influence of the aromatic moiety in α- and β-arylalanines on their biotransformation with phenylalanine 2,3-aminomutase from Pantoea agglomerans†

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1. $^1$H- and $^{19}$F-NMR spectra of the enzymatic transformations

Results presented in Table 1 and Table 2 (see main manuscript) show the relative molar fractions for each component of enzymatic reaction mixtures, calculated from the integral values of the clearly distinguishable $^1$H- or $^{19}$F-NMR signals assigned for each substance.

$^1$H-NMR of the products of PaPAM-catalysed transformation of ($\pm$)-$\alpha$-phenylalanine rac-1a
$^1$H-NMR of the products of PaPAM-catalysed transformation of (+)-α-(thiophen-2-yl)alanine rac-$\textbf{1b}$

$^1$H-NMR of the products of PaPAM-catalysed transformation of (+)-4-bromo-α-phenylalanine rac-$\textbf{1c}$
$^{19}$F-NMR of the products of PaPAM-catalysed transformation of (±)-2-fluoro-α-phenylalanine rac-1d

$^{19}$F-NMR of the products of PaPAM-catalysed transformation of (±)-3-fluoro-α-phenylalanine rac-1e
$^{19}$F-NMR of the products of PaPAM-catalysed transformation of (±)-4-fluoro-α-phenylalanine rac-1f

$^1$H-NMR of the products of PaPAM-catalysed transformation of (±)-3-chloro-α-phenylalanine rac-1h
$^1$H-NMR of the products of PaPAM-catalysed transformation of (±)-4-chloro-α-phenylalanine rac-$^{1i}$

$^1$H-NMR of the products of PaPAM-catalysed transformation of (±)-3-nitro-α-phenylalanine rac-$^{1k}$
$^1$H-NMR of the products of PaPAM-catalysed transformation of (±)-4-nitro-α-phenylalanine rac-1l

$^1$H-NMR of the products of PaPAM-catalysed transformation of (±)-β-phenylalanine rac-2a
$^1$H-NMR of the products of PaPAM-catalysed transformation of (±)-β-(thiophen-2-yl)alanine rac-2b

$^1$H-NMR of the products of PaPAM-catalysed transformation of (±)-4-bromo-β-phenylalanine rac-2c
$^{19}$F-NMR of the products of PaPAM-catalysed transformation of (±)-2-fluoro-β-phenylalanine *rac-2d*

$^{19}$F-NMR of the products of PaPAM-catalysed transformation of (±)-3-fluoro-β-phenylalanine *rac-2e*
$^{19}$F-NMR of the products of PaPAM-catalysed transformation of (±)-4-fluoro-β-phenylalanine rac-2f

$^1$H-NMR of PaPAM-catalysed transformation of (±)-2-chloro-β-phenylalanine rac-2g
$^1$H-NMR of PaPAM-catalysed transformation of (±)-2-nitro-β-phenylalanine rac-2j
2. HPLC monitoring of the enzymatic reactions

2.1 Determination of conversion and molar fraction values

In order to determine the conversion and/or molar fractions of the products of PaPAM-catalysed enzymatic transformations, the response factor of each compound was determined by mixtures of known composition of authentic racemic α- and β-amino acids and the corresponding arylacrylate injected onto Gemini NX-C-18 column (150 × 4.6 mm × 5 µm). Mobile phase: A: NH₄OH buffer (0.1 M, pH 9.0) / B: MeOH, flow rate: 0.9 mL/min, measurements performed at 20°C. Reverse phase HPLC analyses were performed on an Agilent 1100 Series system equipped with a G1379A degasser, G1311A quaternary pump, a G1329A autosampler, a G1316A temperature controlled column compartment and a G1315B diode array detector.

Table S1. HPLC conditions and response factors

<table>
<thead>
<tr>
<th>Aryl moiety in 1,2,3</th>
<th>Eluent* [% B]</th>
<th>λ [nm]</th>
<th>Response factor**</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>2 vs 1</td>
</tr>
<tr>
<td>Phenyl</td>
<td>10 to 39 in 12 min</td>
<td>220</td>
<td>1.988</td>
</tr>
<tr>
<td>Thiophen-2-yl</td>
<td>10 to 39 in 12 min</td>
<td>250</td>
<td>1.146</td>
</tr>
<tr>
<td>4-Bromophenyl</td>
<td>20 to 54 in 12 min</td>
<td>220</td>
<td>1.857</td>
</tr>
<tr>
<td>2-Fluorophenyl</td>
<td>10 to 39 in 12 min</td>
<td>220</td>
<td>1.249</td>
</tr>
<tr>
<td>3-Fluorophenyl</td>
<td>10 to 39 in 12 min</td>
<td>220</td>
<td>3.039</td>
</tr>
<tr>
<td>4-Fluorophenyl</td>
<td>10 to 39 in 12 min</td>
<td>220</td>
<td>1.388</td>
</tr>
<tr>
<td>2-Chlorophenyl</td>
<td>10 to 39 in 12 min</td>
<td>220</td>
<td>—</td>
</tr>
<tr>
<td>3-Chlorophenyl</td>
<td>15 to 50 in 15 min</td>
<td>220</td>
<td>0.817</td>
</tr>
<tr>
<td>4-Chlorophenyl</td>
<td>15 to 50 in 15 min</td>
<td>220</td>
<td>0.948</td>
</tr>
<tr>
<td>2-Nitrophenyl</td>
<td>10 to 50 in 15 min</td>
<td>260</td>
<td>—</td>
</tr>
<tr>
<td>3-Nitrophenyl</td>
<td>10 to 50 in 15 min</td>
<td>260</td>
<td>2.238</td>
</tr>
<tr>
<td>4-Nitrophenyl</td>
<td>10 to 50 in 15 min</td>
<td>220</td>
<td>1.138</td>
</tr>
</tbody>
</table>

*Eluent A: NH₄OH buffer (0.1 M, pH 9.0); B: MeOH

** 1– rac-α-arylalanine; 2– rac-β-arylalanine; 3 – arylacrylate
Table S2. Relative molar fractions of the products in the enzymatic reactions of rac-β-arylalanines after 20 h

<table>
<thead>
<tr>
<th>Aryl moiety in 1,2,3</th>
<th>$x_2$ [%]</th>
<th>$x_{(S)-1}$ [%]</th>
<th>$x_3$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenyl</td>
<td>73.9</td>
<td>24.3</td>
<td>1.8</td>
</tr>
<tr>
<td>Thiophenyl-2-yl</td>
<td>73.1</td>
<td>24.6</td>
<td>2.3</td>
</tr>
<tr>
<td>4-Bromophenyl</td>
<td>92.8</td>
<td>6.2</td>
<td>1</td>
</tr>
<tr>
<td>2-Fluorophenyl</td>
<td>54</td>
<td>41.5</td>
<td>4.5</td>
</tr>
<tr>
<td>3-Fluorophenyl</td>
<td>74.8</td>
<td>20.3</td>
<td>4.9</td>
</tr>
<tr>
<td>4-Fluorophenyl</td>
<td>78.3</td>
<td>20</td>
<td>1.7</td>
</tr>
<tr>
<td>2-Chlorophenyl</td>
<td>53.5</td>
<td>44.4</td>
<td>2.5</td>
</tr>
<tr>
<td>2-Nitrophenyl</td>
<td>90.8</td>
<td>9.2</td>
<td>0</td>
</tr>
</tbody>
</table>

$x_2$, $x_{(S)-1}$ and $x_3$ represent the relative molar fractions of the reaction components as determined by HPLC on Gemini NX-C-18 column.

Table S3. Relative molar fractions of the products in the enzymatic reactions of rac-α-arylalanines after 20 h

<table>
<thead>
<tr>
<th>Aryl moiety in 1,2,3</th>
<th>$x_2$ [%]</th>
<th>$x_{(S)-2}$ [%]</th>
<th>$x_3$ [%]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenyl</td>
<td>68.3</td>
<td>28.9</td>
<td>2.8</td>
</tr>
<tr>
<td>Thiophenyl-2-yl</td>
<td>76.1</td>
<td>17.7</td>
<td>6.2</td>
</tr>
<tr>
<td>4-Bromophenyl</td>
<td>82.9</td>
<td>17.1</td>
<td>0</td>
</tr>
<tr>
<td>2-Fluorophenyl</td>
<td>88.3</td>
<td>9.3</td>
<td>2.4</td>
</tr>
<tr>
<td>3-Fluorophenyl</td>
<td>60.9</td>
<td>37.9</td>
<td>1.2</td>
</tr>
<tr>
<td>4-Fluorophenyl</td>
<td>75.5</td>
<td>23.3</td>
<td>1.2</td>
</tr>
<tr>
<td>3-Chlorophenyl</td>
<td>82.8</td>
<td>16.0</td>
<td>1.2</td>
</tr>
<tr>
<td>4-Chlorophenyl</td>
<td>83.9</td>
<td>16.1</td>
<td>0</td>
</tr>
<tr>
<td>3-Nitrophenyl</td>
<td>76.8</td>
<td>23.0</td>
<td>0.2</td>
</tr>
<tr>
<td>4-Nitrophenyl</td>
<td>94.3</td>
<td>5.7</td>
<td>0</td>
</tr>
</tbody>
</table>

$x_2$, $x_{(S)-2}$ and $x_3$ represent the relative molar fractions of the reaction components as determined by HPLC on Gemini NX-C-18 column.
2.2 HPLC analysis of the PaPAM-catalysed reactions after 20 h

Products from (±)-β-phenylalanine (rac-2a) by PaPAM after 20 h.

Products from (±)-β-(thiophen-2-yl)alanine (rac-2b) by PaPAM after 20 h.
Products from (±)-4-bromo-β-phenylalanine (*rac*-2c) by PaPAM after 20 h.

Products from (±)-2-fluoro-β-phenylalanine (*rac*-2d) by PaPAM after 20 h.
Products from (±)-3-fluoro-β-phenylalanine (rac-2e) by PaPAM after 20 h.

Products from (±)-4-fluoro-β-phenylalanine (rac-2f) by PaPAM after 20 h.
Products from (±)-2-chloro-β-phenylalanine (rac-2g) by PaPAM after 20 h.

Products from (±)-2-nitro-β-phenylalanine (rac-2j) by PaPAM after 20 h.
3. Time-course profiles of the PaPAM-catalysed reactions

Into the solution of the substrate \((rac-1a-f,h,i,k,l,or rac-2a-g,j, 4 \text{ mg})\) in \((\text{NH}_4)_2\text{CO}_3\) buffer (100 mM, pH 8.0, 2 mL), PaPAM (1.6 mg) was added and the reaction mixture was stirred at room temperature. Sample preparations and HPLC measurements were performed as described in experimental section in main manuscript.
Time-course profile of the conversion of (±)-4-bromo-β-phenylalanine (rac-2c, ◆) to (S)-4-bromo-α-phenylalanine ([S]-1c) and (E)-4-bromocinnamic acid (3c, ◇).

Time-course profile of the conversion of (±)-4-fluoro-β-phenylalanine (rac-2d, ◆) to (S)-4-fluoro-α-phenylalanine ([S]-1d) and (E)-4-fluorocinnamic acid (3d, ◇).
Time-course profile of the conversion of (±)-3-fluoro-β-phenylalanine (rac-2e, ◆) to (S)-3-fluoro-α-phenylalanine [(S)-1e]] and (E)-4-fluorocinnamic acid (3e, ◆)

Time-course profile of the conversion of (±)-4-fluoro-β-phenylalanine (rac-2f, ◆) to (S)-4-fluoro-α-phenylalanine [(S)-1f]] and (E)-4-fluorocinnamic acid (3f, ◆)
Time-course profile of the conversion of (±)-2-chloro-β-phenylalanine (rac-2g, ■) to (S)-2-chloro-α-phenylalanine [(S)-1g]) and (E)-2-chlorocinnamic acid (3g, ○).

Time-course profile of the conversion of (±)-2-nitro-β-phenylalanine (rac-2j) to (S)-2-nitro-α-phenylalanine [(S)-1j, ■].
4. HPLC determination of the enantiomeric compositions

Samples taken from the enzymatic reactions after 20 h as described in section 2 were injected onto Crownpak® CR-I(+) column (150 × 3.0 mm × 5 µm) using HClO₄ solution (pH 1.5): acetonitrile as mobile phase, flow rate: 0.4 mL min⁻¹ or onto Chiralpak® ZWIX (+) column (250 × 4.6 mm × 3 µm) using MeOH (50 mM diethylamine, 100 mM formic acid) : acetonitrile : H₂O, 49:49:2 as mobile phase, flow rate: 1 mL/min.
Products from (+)-α-phenylalanine (rac-1a) by PaPAM after 20 h
[Crownpak CR-I(+) column]

Enantioseparation of authentic (+)-β-phenylalanine (rac-2a) on Crownpak CR-I(+) column
Products from (±)-α-(thiophen-2-yl)alanine (rac-1b) by PaPAM after 20 h
[Chiralpak ZWIX(+) column]

Enantioseparation of authentic (±)-β-(thiophen-2-yl)alanine (rac-2b) and
(±)-α-(thiophen-2-yl)alanine (rac-1b) on Chiralpak ZWIX(+) column
Products from (+)-ß-(thiophen-2-yl)alanine \((rac-2b)\) by PaPAM after 20 h
[Crownpak CR-I(+) column]

Enantioseparation of authentic (+)-ß-(thiophen-2-yl)alanine \((rac-2b)\) and
(+)-ß-(thiophen-2-yl)alanine \((rac-1b)\) on Crownpak CR-I(+) column
Products from (+)-4-bromo-α-phenylalanine (rac-1c) by PaPAM after 20 h
[Chiralpak ZWIX(+) column]

Enantioseparation of authentic (+)-4-bromo-α-phenylalanine (rac-1c)
on Chiralpak ZWIX(+) column
Products from (±)-4-fluoro-α-phenylalanine (rac-1d) by PaPAM after 20 h [Crownpak CR-I(+)column]

Products from (±)-4-fluoro-β-phenylalanine (rac-2d) by PaPAM after 20 h [Crownpak CR-I(+)column]
Enantioseparation of authentic (±)-4-fluoro-α-phenylalanine and (±)-4-fluoro-β-phenylalanine on Crownpak CR-I(+) column

Products from (±)-3-fluoro-α-phenylalanine (rac-1e) by PaPAM after 20 h
[Crownpak CR-I(+)column]
Products from (±)-3-fluoro-β-phenylalanine (rac-2e) by PaPAM after 20 h [Crownpak CR-I(+) column]

Enantioseparation of authentic (±)-3-fluoro-α-phenylalanine (rac-1e) and (±)-3-fluoro-β-phenylalanine (rac-2e) on Crownpak CR-I(+) column
Products from (±)-4-fluoro-α-phenylalanine (rac-1f) by PaPAM after 20 h
[Crownpak CR-I(+)column]

Products from (±)-4-fluoro-β-phenylalanine (rac-2f) by PaPAM after 20 h
[Crownpak CR-I(+) column]
Enantioseparation of authentic (±)-4-fluoro-α-phenylalanine (rac-1f) and (±)-4-fluoro-β-phenylalanine (rac-2f) on Crownpak CR-I(+) column

Products from (±)-2-chloro-β-phenylalanine (rac-2g) by PaPAM after 20 h
[Crownpak CR-I(+) column]
Enantioseparation of authentic (+)-2-chloro-α-phenylalanine (rac-1g) and (+)-2-chloro-β-phenylalanine (rac-2g) on Crownpak CR-I(+) column

Products from (+)-3-chloro-α-phenylalanine (rac-1h) by PaPAM after 20 h [Crownpak CR-I(+)column]
Enantioseparation of authentic (±)-3-chloro-α-phenylalanine (rac-1f) and (±)-3-chloro-β-phenylalanine (rac-2h) on Crownpak CR-I(+) column

Products from (±)-4-chloro-α-phenylalanine (rac-1i) by PaPAM after 20 h [Chiralpak ZWIX (+) column]
Enantioseparation of authentic (±)-4-chloro-α-phenylalanine (rac-2i) on Chiralpak ZWIX(+) column

Products from (±)-4-nitro-β-phenylalanine (rac-2j) by PaPAM after 20 h [Crownpak CR-I(+)column]
Enantioseparation of authentic $(\pm)$-4-nitro-$\alpha$-phenylalanine ($rac$-$1j$) and $(\pm)$-4-nitro-$\beta$-phenylalanine ($rac$-$2j$) on Crownpak CR-I(+) column

Products from $(\pm)$-3-nitro-$\alpha$-phenylalanine ($rac$-$1k$) by PaPAM after 20 h [Crownpak CR-I(+) column]
Enantioseparation of authentic (±)-3-nitro-α-phenylalanine (rac-1k) and (±)-3-nitro-β-phenylalanine (rac-2k) on Crownpak CR-I(+) column

Products from (±)-4-nitro-α-phenylalanine (rac-II) catalyzed by PaPAM, after 20 h [Chiralpack ZWIX(+) column]
Enantioseparation of authentic (±)-4-nitro-α-phenylalanine (rac-1l) and (±)-4-nitro-β-phenylalanine (rac-2l) on Chiralpak ZWIX(+) column.
5. Enzymatic reaction starting from (±)-β-phenylalanine 2a and (S)-β-phenylalanine (S)-2a

The time course profiles of the product formation in PaPAM catalysed reactions from (S)-β-phenylalanine and (±)-β-phenylalanine were determined HPLC on Gemini NX-C-18 column (see Section 2.2 in the Supplementary material).

Time course profiles: (A) conversion of (S)-β-phenylalanine [5 mM, (S)-2a] into (S)-α-phenylalanine [(S)-1a, ◆] and cinnamic acid (2a, ◊); (B) conversion of (±)-β-phenylalanine (10 mM, 2a) into (S)-α-phenylalanine [(S)-1a, ⊙] and cinnamic acid (2a, ×)
6. SDS-PAGE analysis of the purified PaPAM enzyme

The purity of the PaPAM was verified by SDS-PAGE analysis. The samples were boiled for 5 min in Laemmli buffer, and were loaded on a 12% SDS-PAGE.

![SDS-PAGE Image](image)

**Figure S1.** Purification of PaPAM with Ni-NTA. Lane A: protein ladder, Lane B: supernatant, Lane C: flow through, Lane D: pellet, Lane E: LS1, Lane F: HS, Lane G: LS2, Lane H: 20 mM Imidazole, Lane I: 350 mM Imidazole, Lane J: 1 mM Imidazole. The samples were prepared as described in experimental section.