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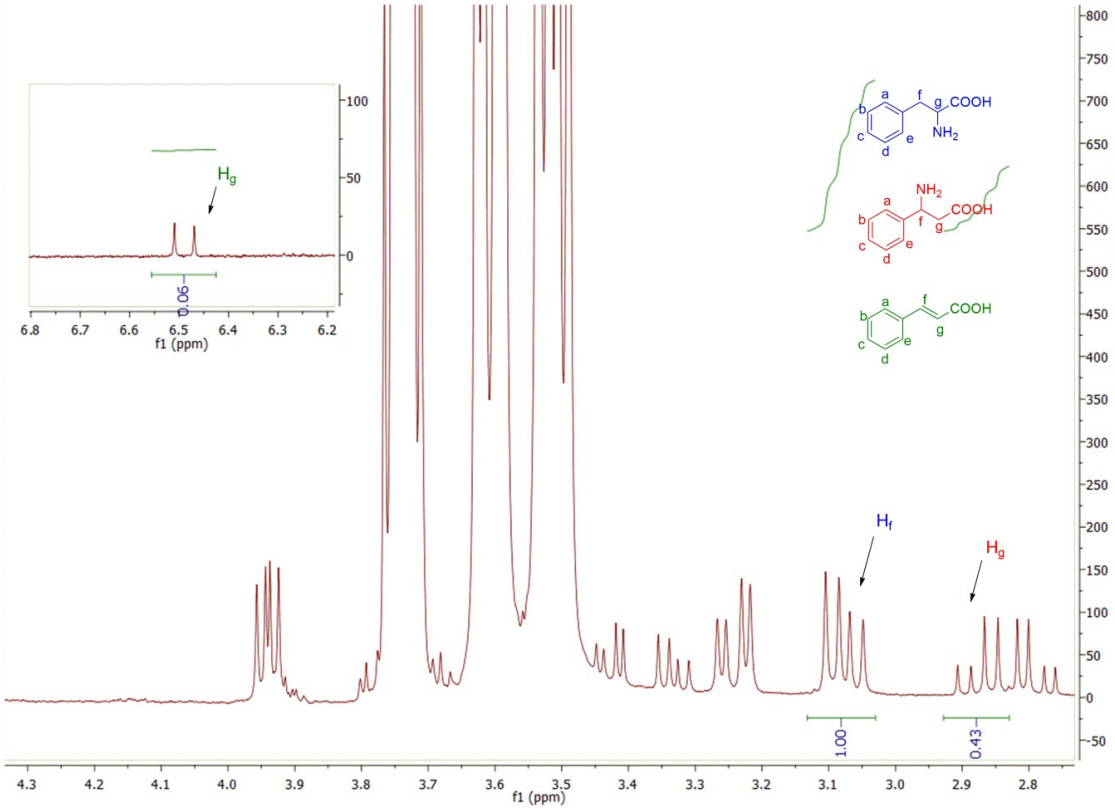
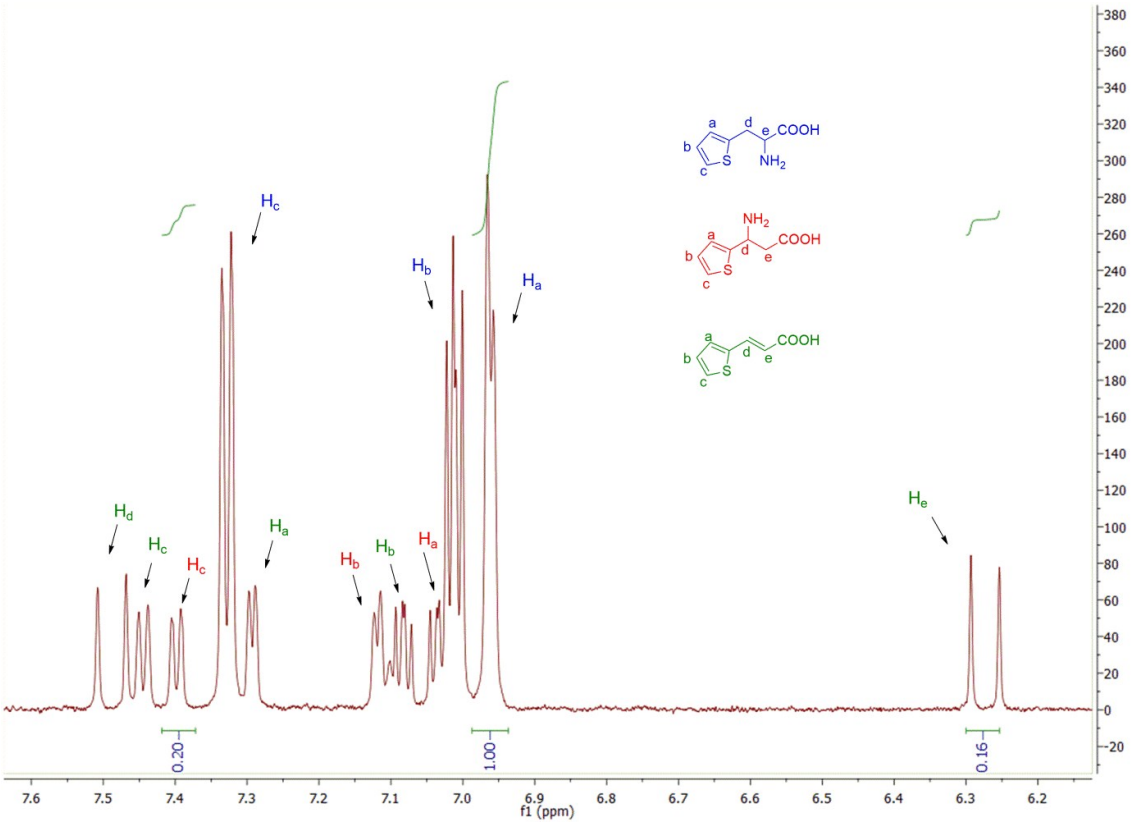
1. ^{1}H- and ^{19}F-NMR spectra of the enzymatic transformations

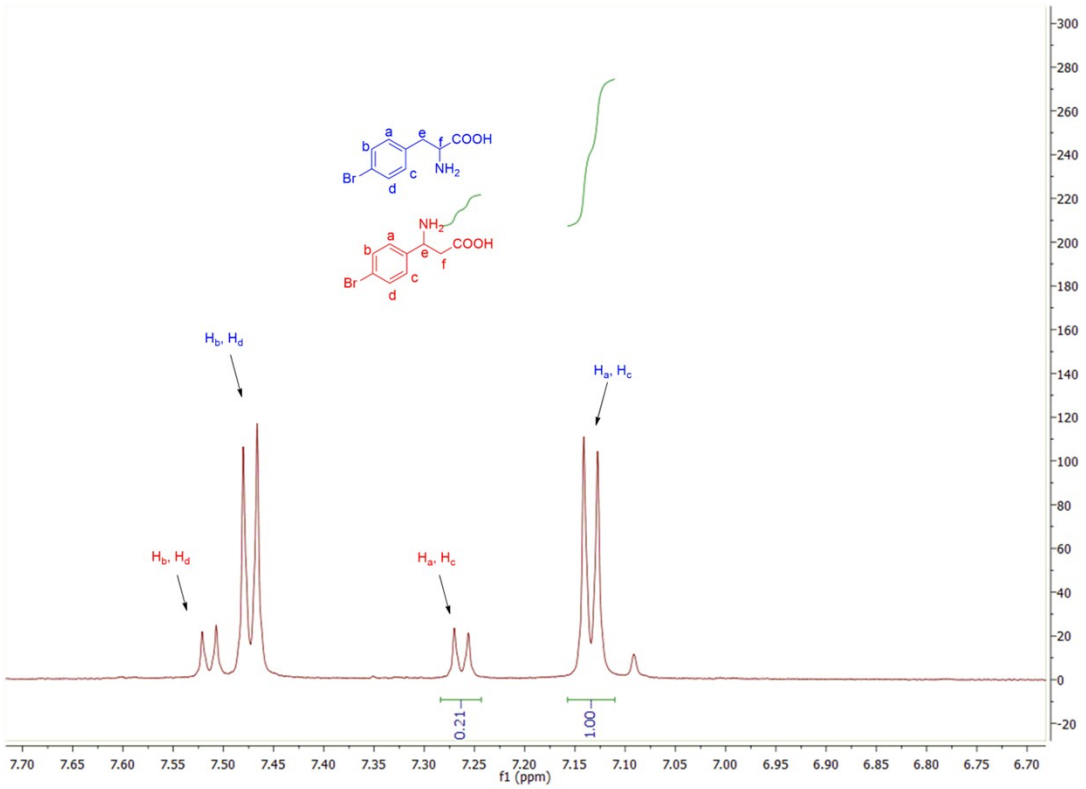
Results presented in Table 1 and Table 2 (see main manuscript) show the relative molar fractions for each component of enzymatic reaction mixtures, calculated from the integral values of the clearly distinguishable ^{1}H- or ^{19}F-NMR signals assigned for each substance.



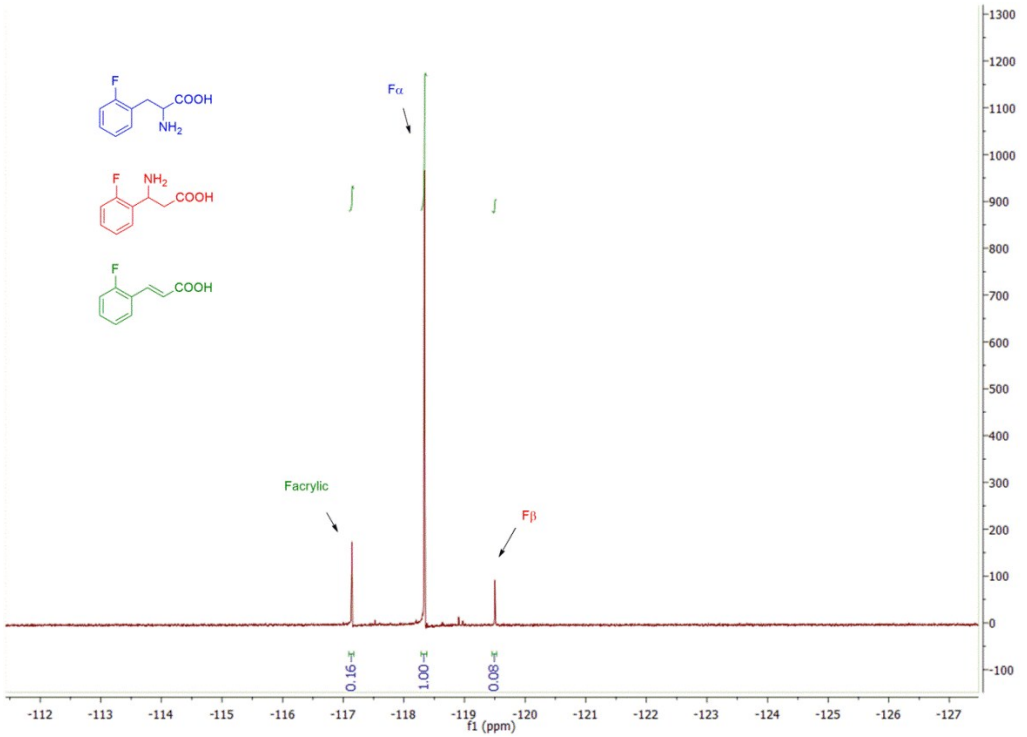
^{1}H-NMR of the products of *Pa*PAM-catalysed transformation of (±)-α-phenylalanine *rac*-**1a**



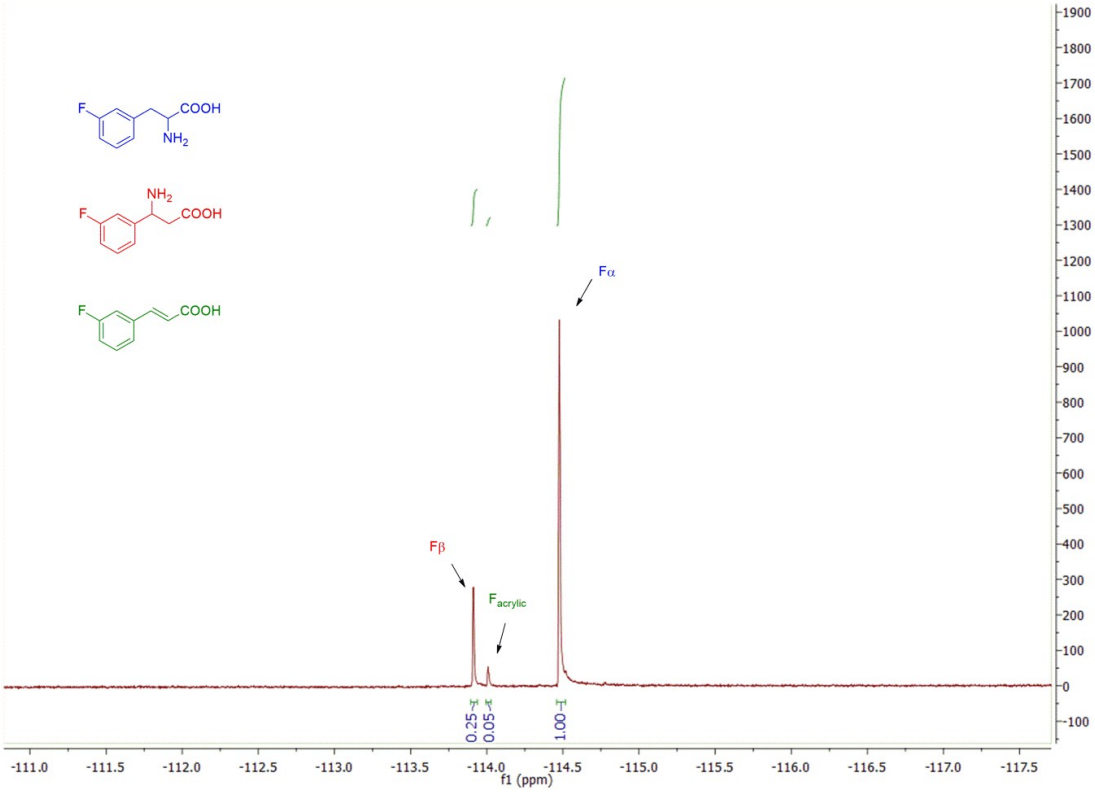
[1]H-NMR of the products of *Pa*PAM-catalysed transformation of (±)-α-(thiophen-2-yl)alanine *rac*-**1b**



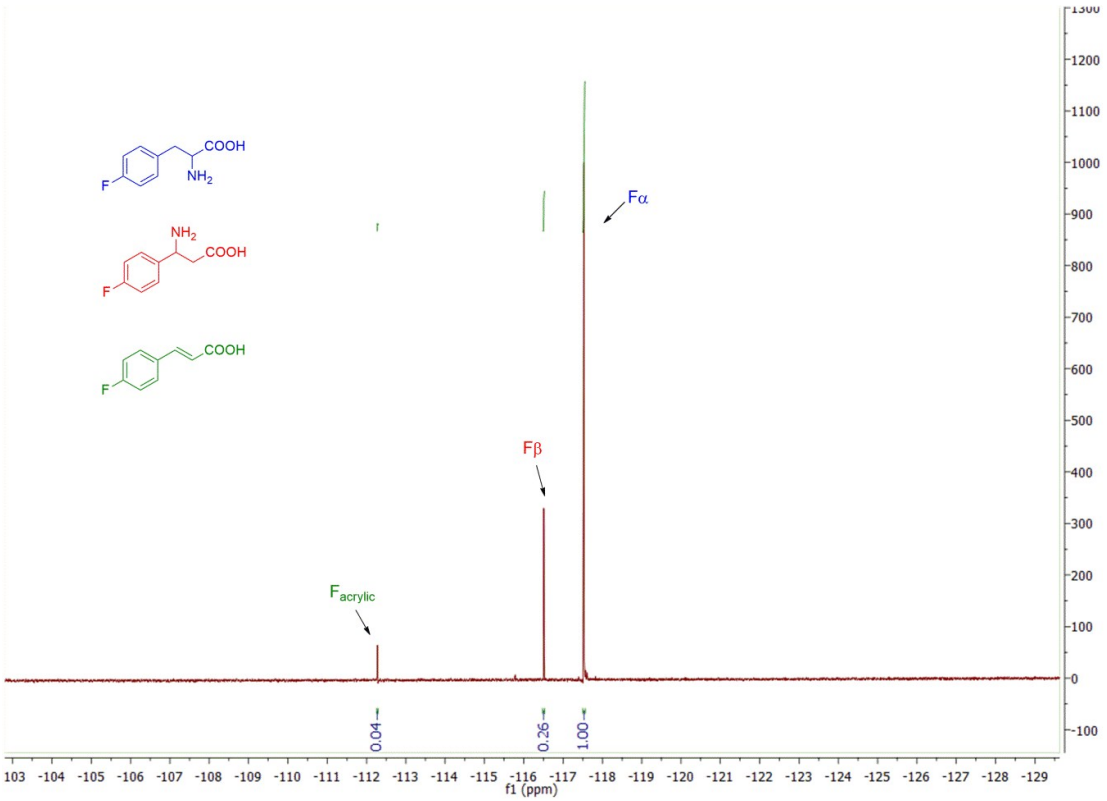
[1]H-NMR of the products of *Pa*PAM-catalysed transformation of(±)-4-bromo-α-phenylalanine *rac*-**1c**



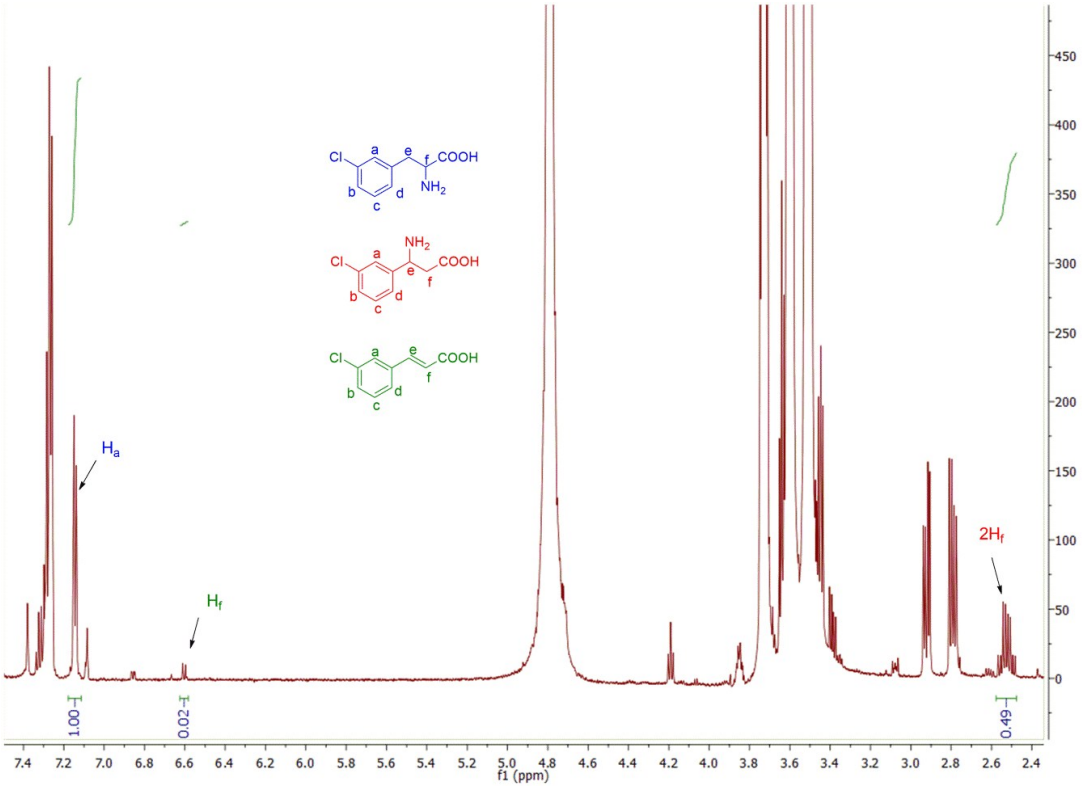
^{19}F-NMR of the products of *Pa*PAM-catalysed transformation of (±)-2-fluoro-α-phenylalanine *rac*-**1d**



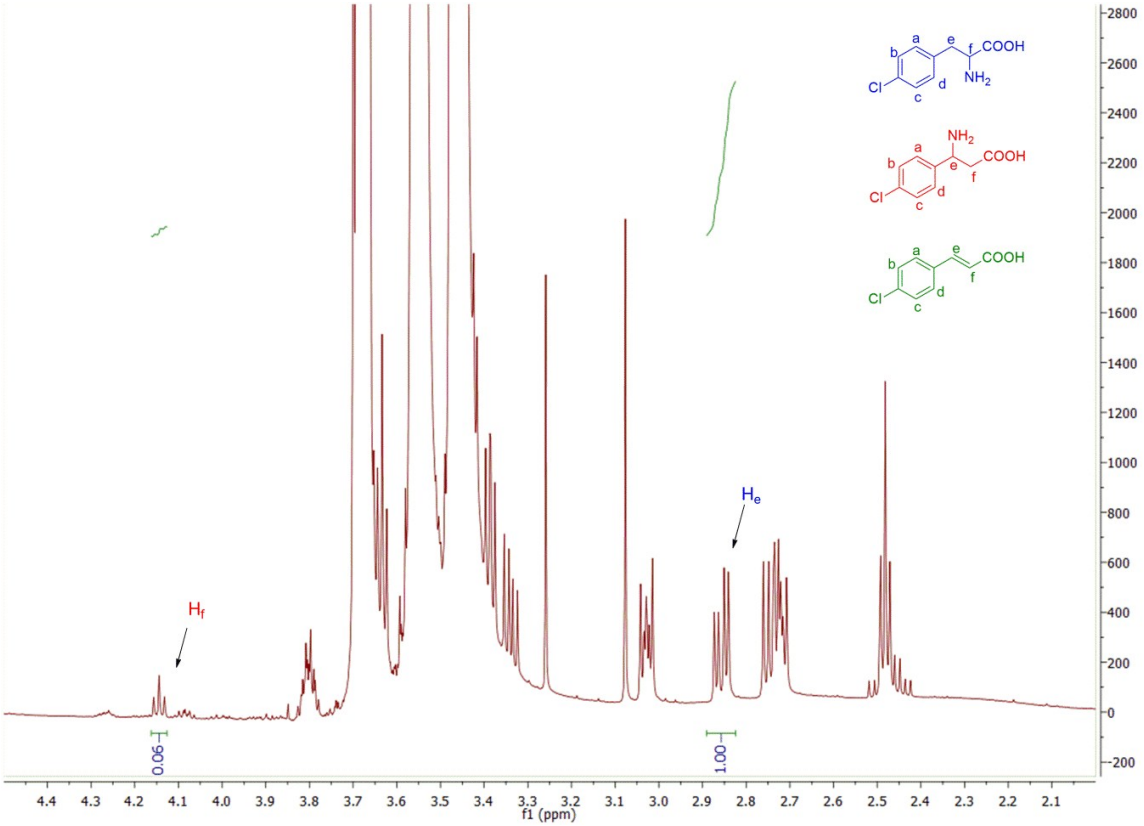
^{19}F-NMR of the products of *Pa*PAM-catalysed transformation of (±)-3-fluoro-α-phenylalanine *rac*-**1e**



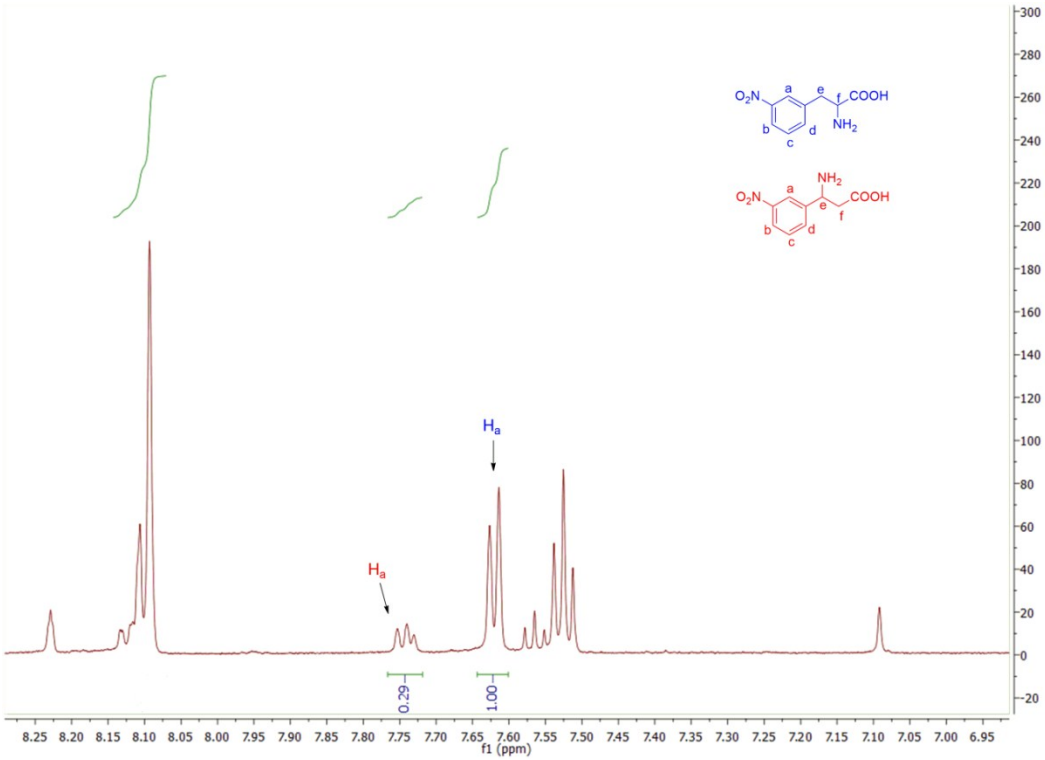
^{19}F-NMR of the products of *Pa*PAM-catalysed transformation of (±)-4-fluoro-α-phenylalanine *rac*-**1f**



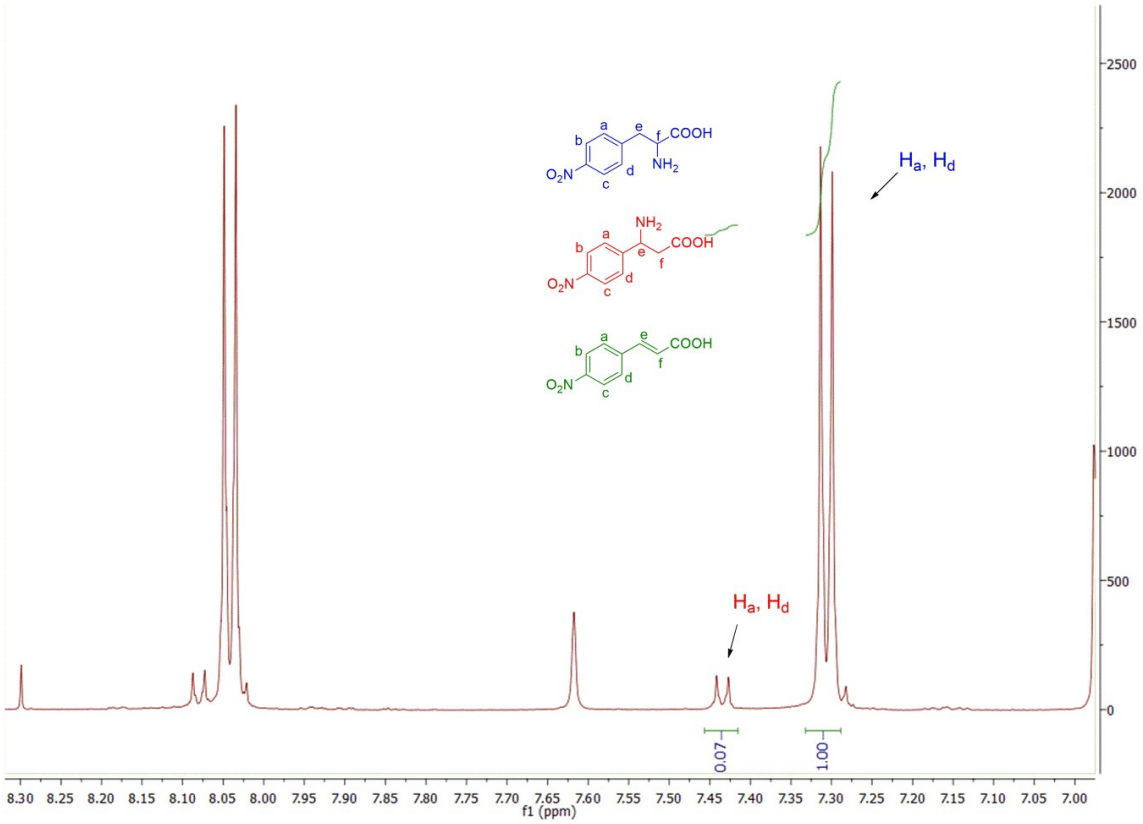
^{1}H-NMR of the products of *Pa*PAM-catalysed transformation of (±)-3-chloro-α-phenylalanine *rac*-**1h**



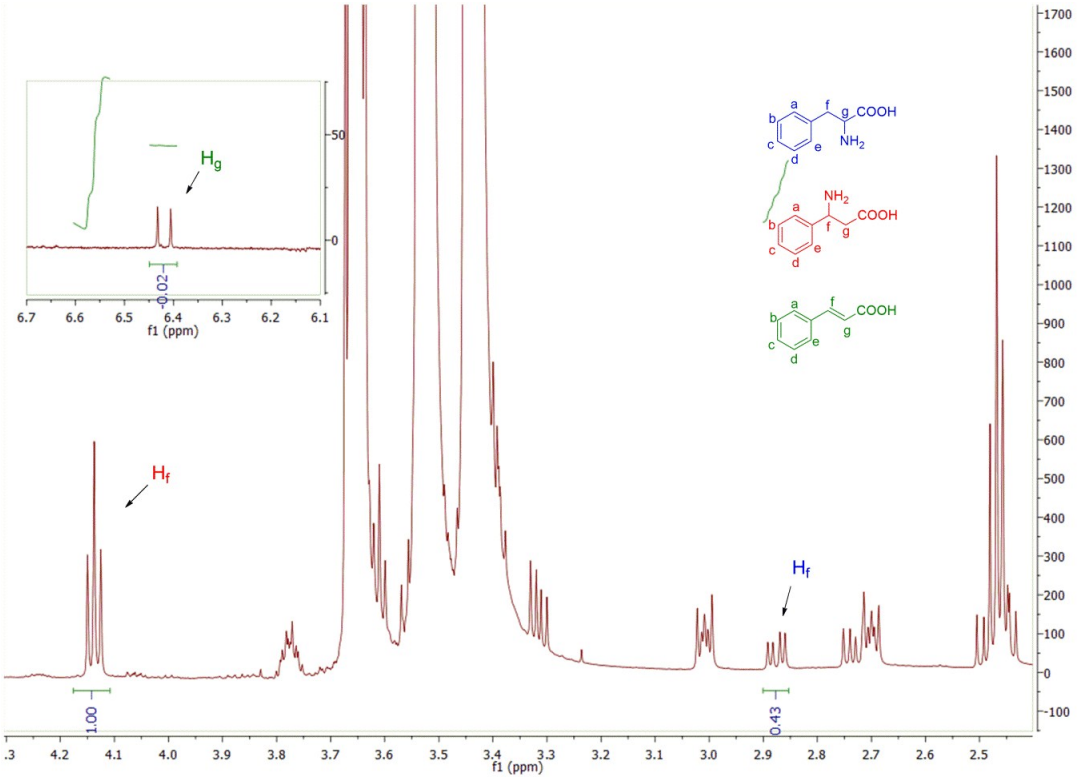
^{1}H-NMR of the products of *Pa*PAM-catalysed transformation of (±)-4-chloro-α-phenylalanine *rac*-**1i**



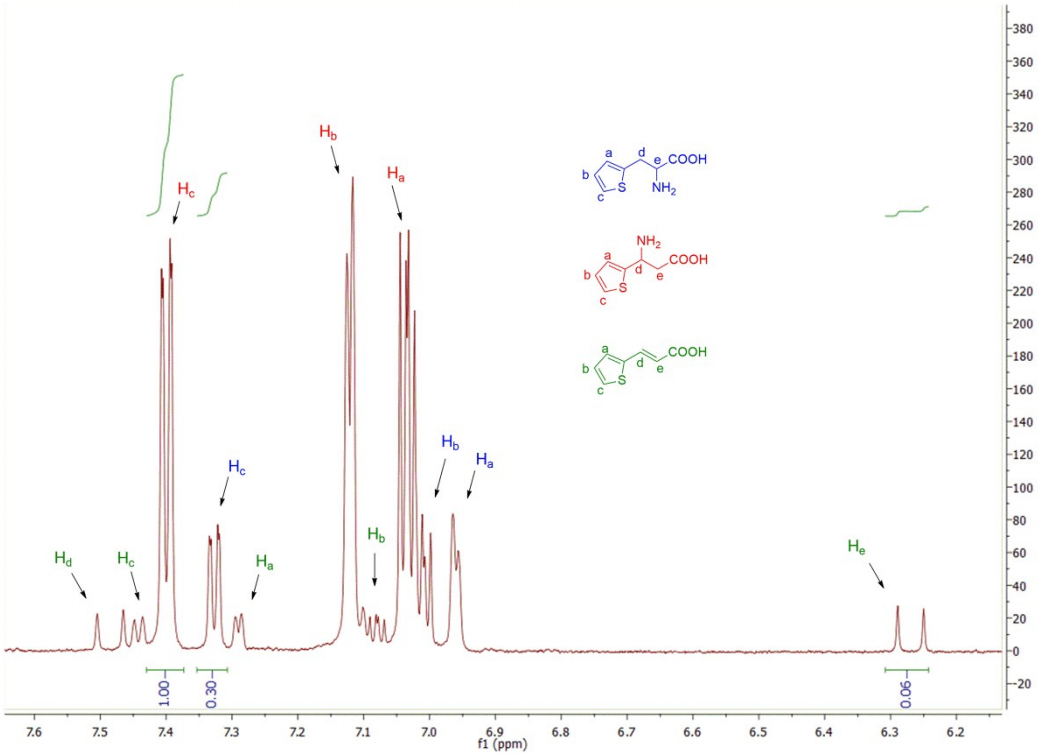
^{1}H-NMR of the products of *Pa*PAM-catalysed transformation of (±)-3-nitro-α-phenylalanine *rac*-**1k**



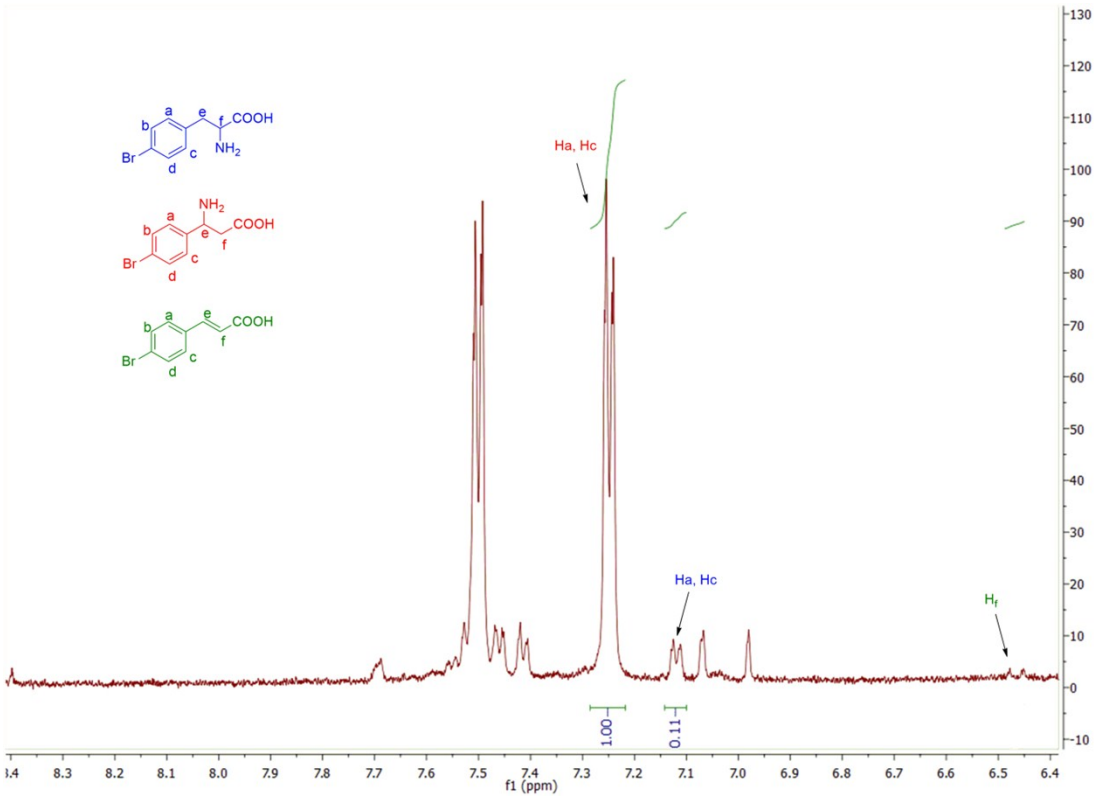
[1]H-NMR of the products of *Pa*PAM-catalysed transformation of (±)-4-nitro-α-phenylalanine *rac*-**1l**



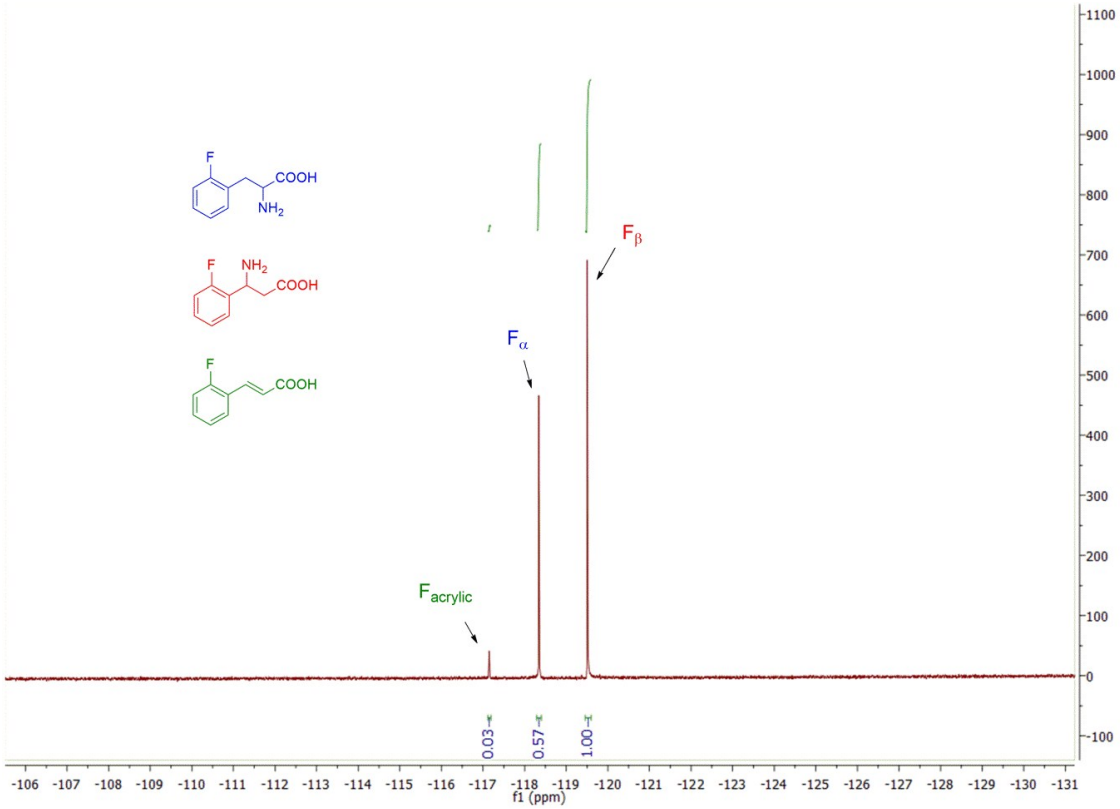
[1]H-NMR of the products of *Pa*PAM-catalysed transformation of (±)-β-phenylalanine *rac*-**2a**



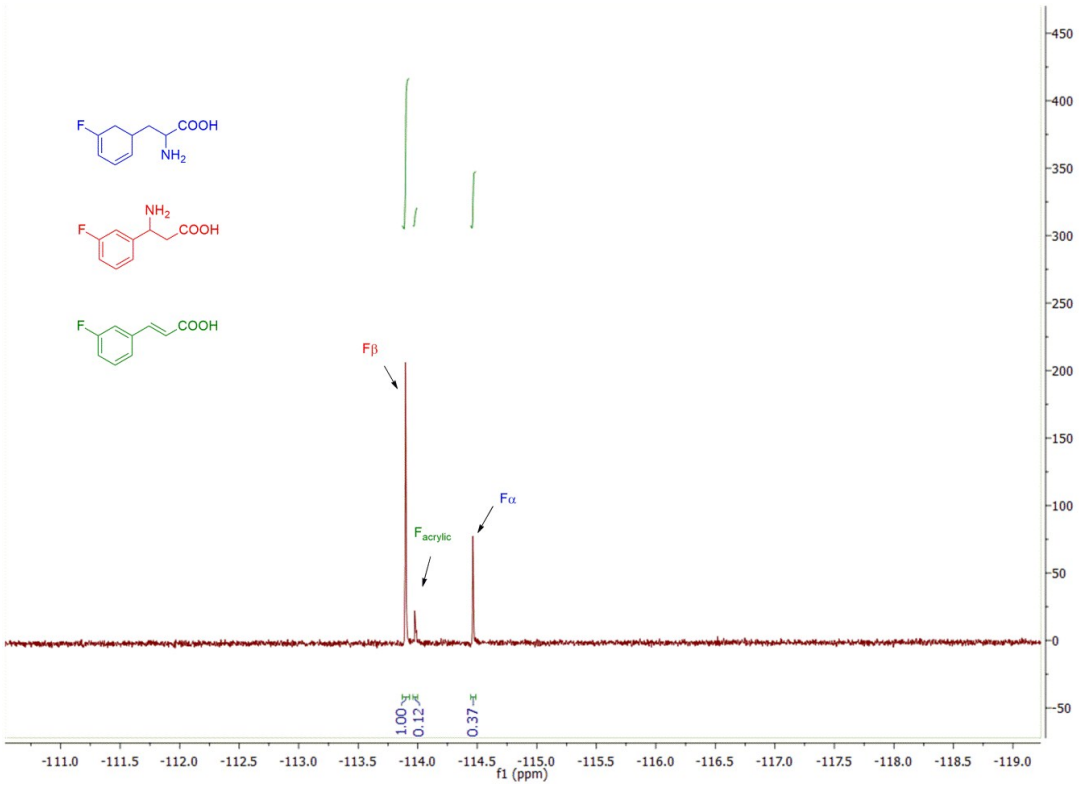
[1]H-NMR of the products of *Pa*PAM-catalysed transformation of (±)-β-(thiophen-2-yl)alanine *rac*-**2b**



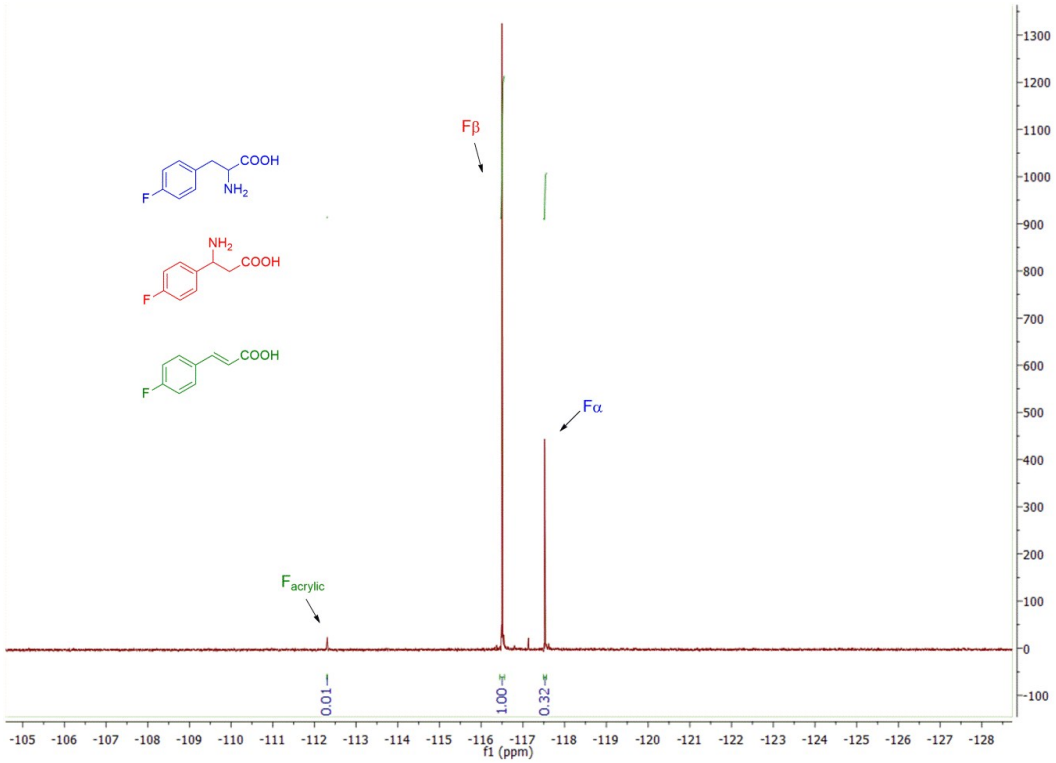
[1]H-NMR of the products of *Pa*PAM-catalysed transformation of (±)-4-bromo-β-phenylalanine *rac*-**2c**



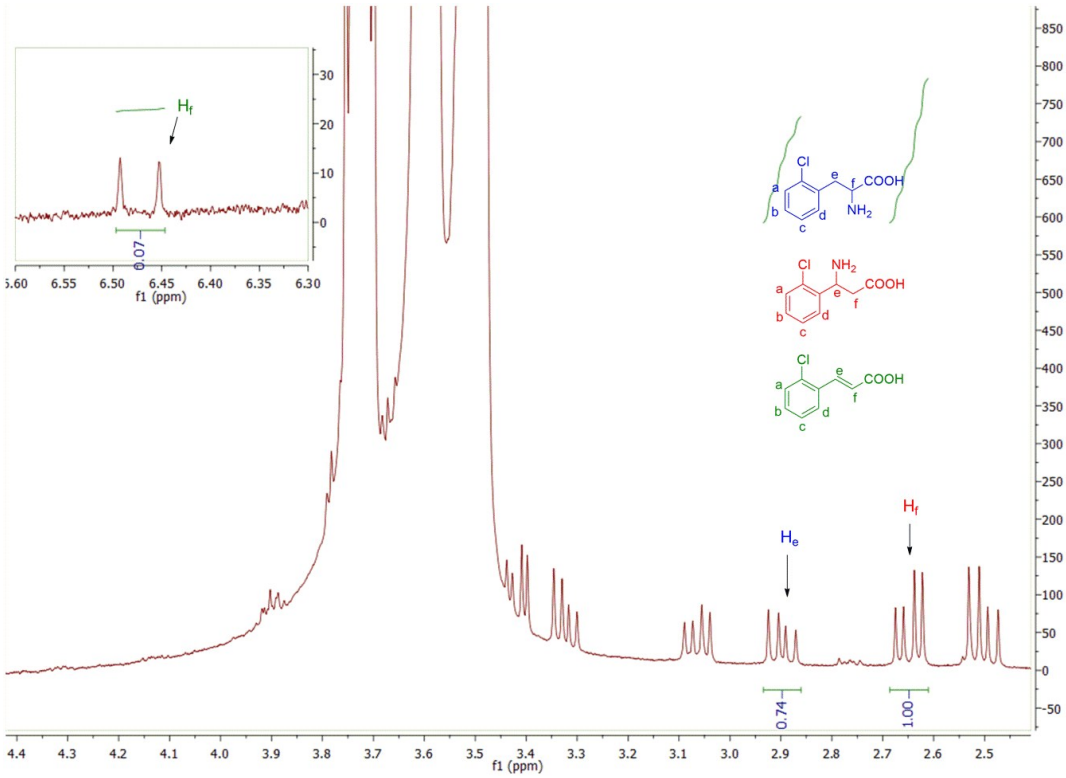
^{19}F-NMR of the products of *Pa*PAM-catalysed transformation of (±)-2-fluoro-β-phenylalanine *rac*-**2d**



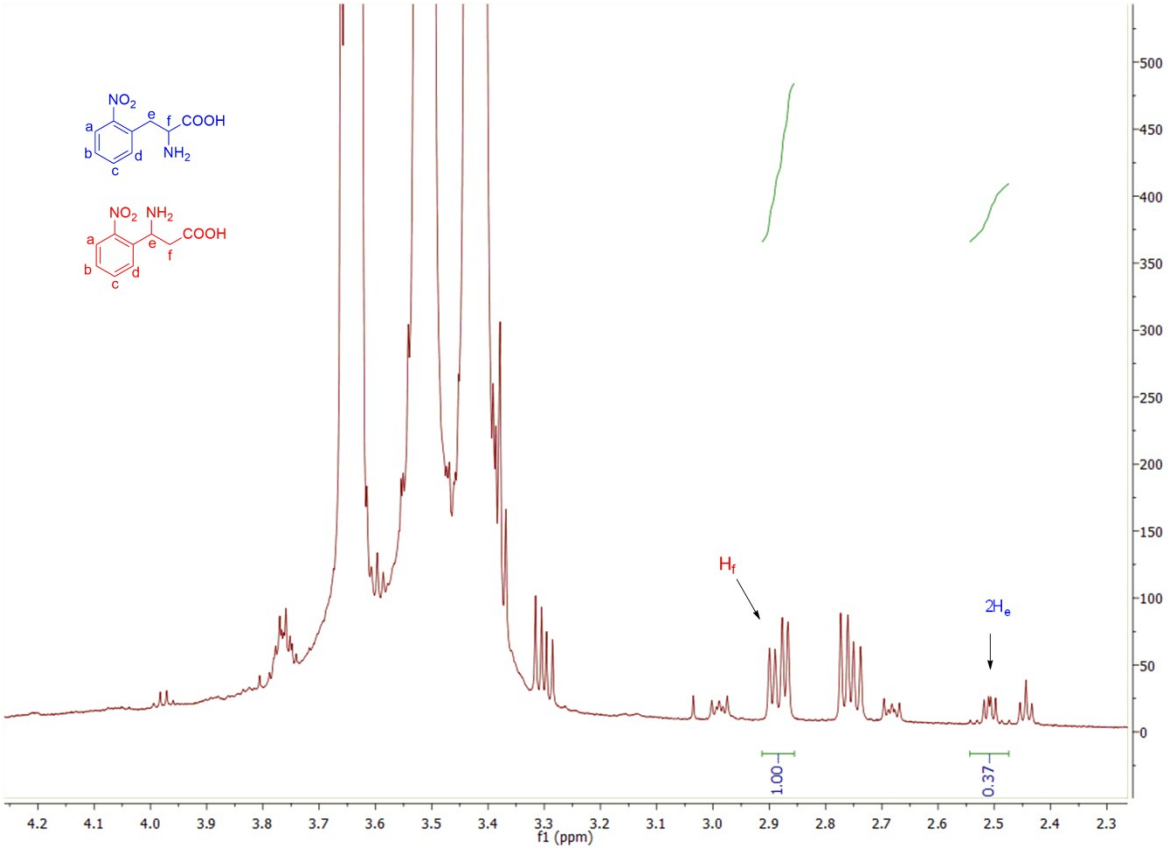
^{19}F-NMR of the products of *Pa*PAM-catalysed transformation of (±)-3-fluoro-β-phenylalanine *rac*-**2e**



^{19}F-NMR of the products of *Pa*PAM-catalysed transformation of (±)-4-fluoro-β-phenylalanine *rac*-**2f**



^{1}H-NMR of *Pa*PAM-catalysed transformation of (±)-2-chloro-β-phenylalanine *rac*-**2g**



^{1}H-NMR of *Pa*PAM-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 *Pa*PAM-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: NH_4OH 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

Aryl moiety in 1,2,3	Eluent* [% B]	λ [nm]	Response factor**		
			2 *vs***1**	**3** *vs***1**	**3***vs***2**
Phenyl	10 to 39 in 12 min	220	1.988	0.161	0.084
Thiophen-2-yl	10 to 39 in 12 min	250	1.146	0.413	0.387
4-Bromophenyl	20 to 54 in 12min	220	1.857	0.372	0.222
2-Fluorophenyl	10 to 39 in 12 min	220	1.249	0.139	0.119
3-Fluorophenyl	10 to 39 in 12min	220	3.039	0.156	0.05
4-Fluorophenyl	10 to 39 in 12 min	220	1.388	0.123	0.095
2-Chlorophenyl	10 to 39 in 12 min	220	–	–	0.386
3-Chlorophenyl	15 to 50 in 15 min	220	0.817	0.265	–
4-Chlorophenyl	15 to 50 in 15 min	220	0.948	0.679	–
2-Nitrophenyl	10 to 50 in 15 min	260	–	–	0.019
3-Nitrophenyl	10 to 50 in 15 min	260	2.238	0.144	–
4-Nitrophenyl	10 to 50 in 15 min	220	1.138	1.228	–

*Eluent **A**: NH_4OH 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

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

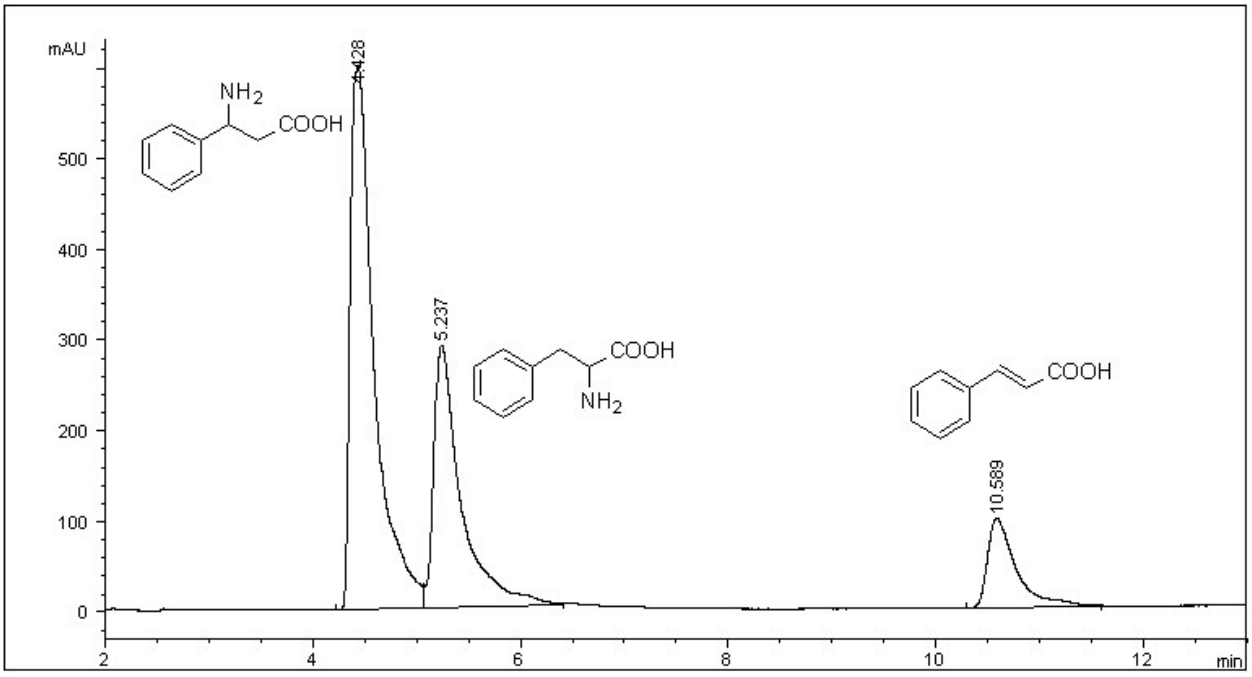
x_2, $x_{(S)\text{-}1}$ and x_3 represent the relative molar fractions of the reaction components as determined by HPLC on Gemini NX-C-18 column

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

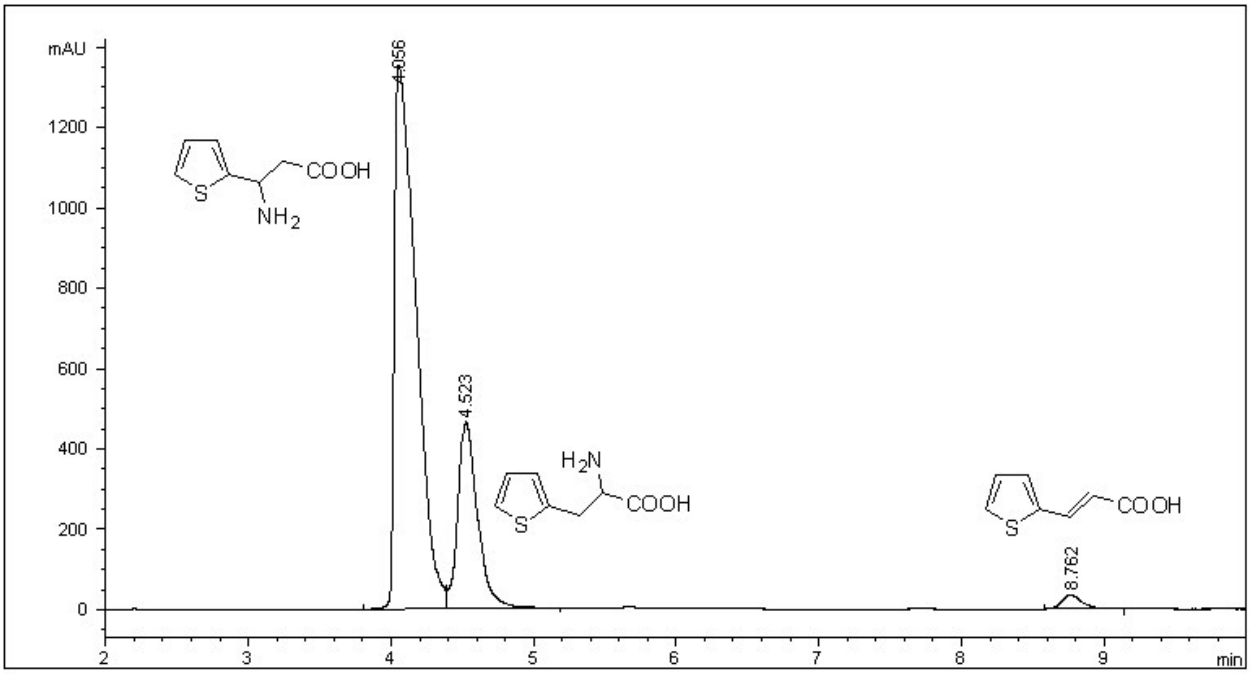
Aryl moiety in 1,2,3	x_2 [%]	$x_{(S)\text{-}1}$ [%]	x_3 [%]
Phenyl	68.3	28.9	2.8
Thiophenyl-2-yl	76.1	17.7	6.2
4-Bromophenyl	82.9	17.1	0
2-Fluorophenyl	88.3	9.3	2.4
3-Fluorophenyl	60.9	37.9	1.2
4-Fluorophenyl	75.5	23.3	1.2
3-Chlorophenyl	82.8	16.0	1.2
4-Chlorophenyl	83.9	16.1	0
3-Nitrophenyl	76.8	23.0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

x_1, $x_{(S)\text{-}2}$ and x_3 represent the relative molar fractions of the reaction components as determined by HPLC on Gemini NX-C-18 column

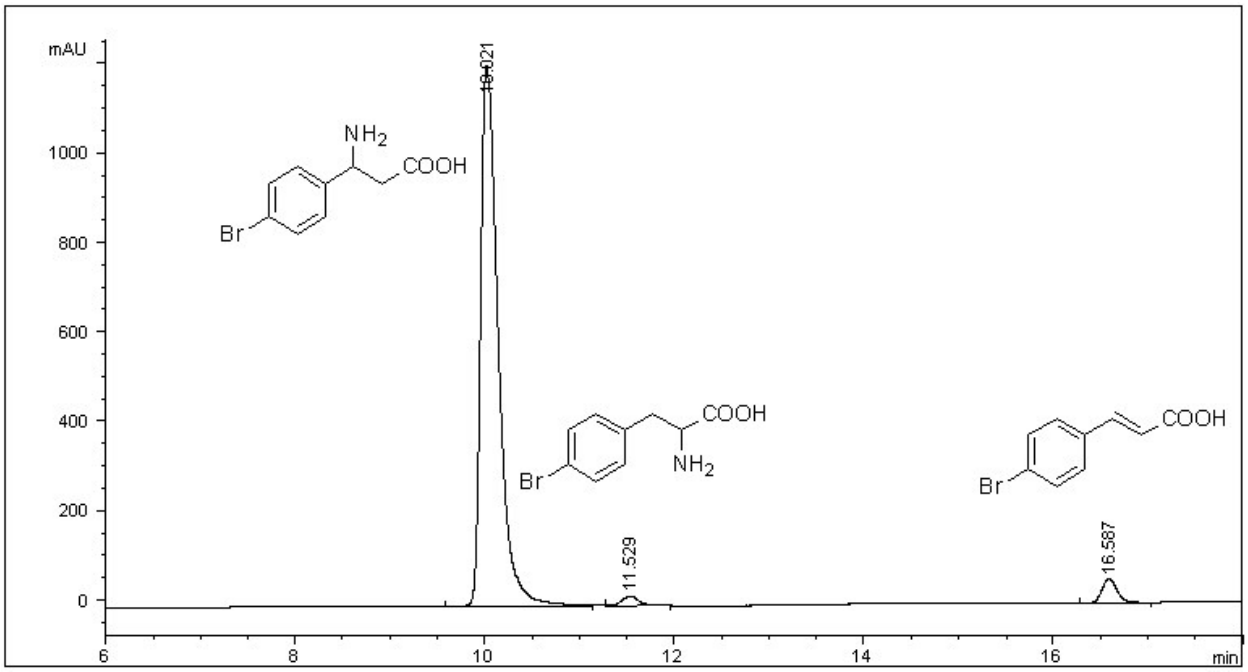
2.2 HPLC analysis of the PaPAM-catalysed reactions after 20 h



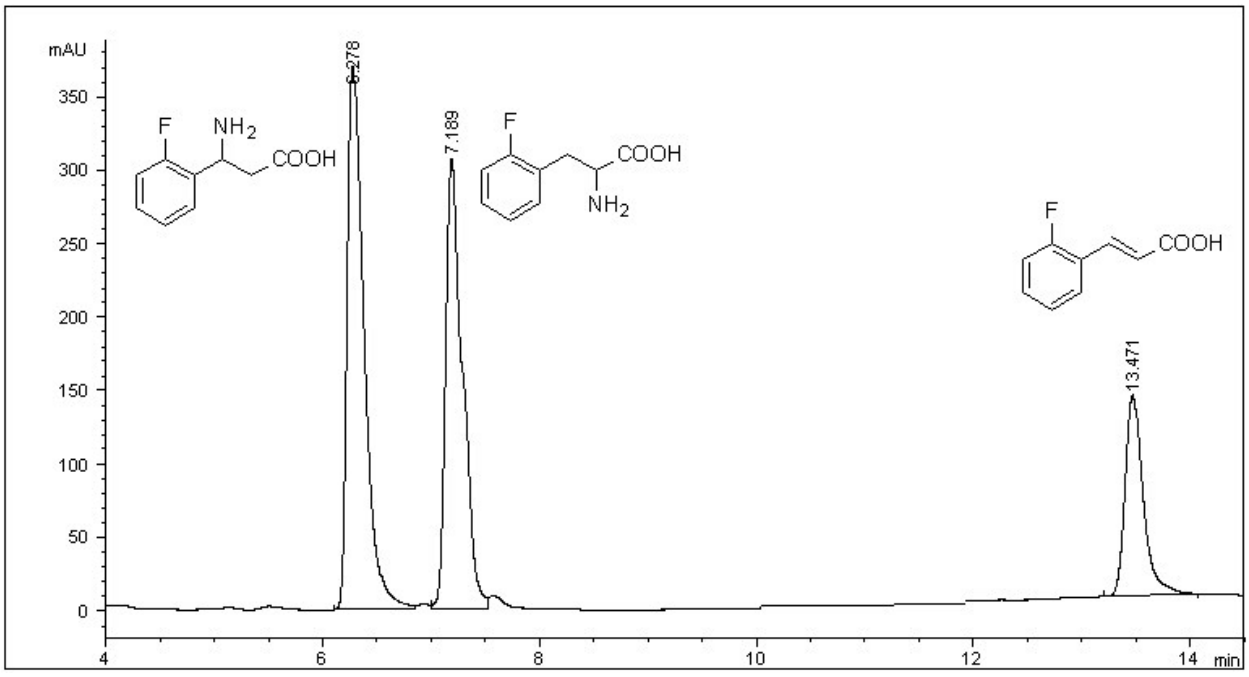
Products from (±)-β-phenylalanine (*rac*-**2a**) by *Pa*PAM after 20 h.



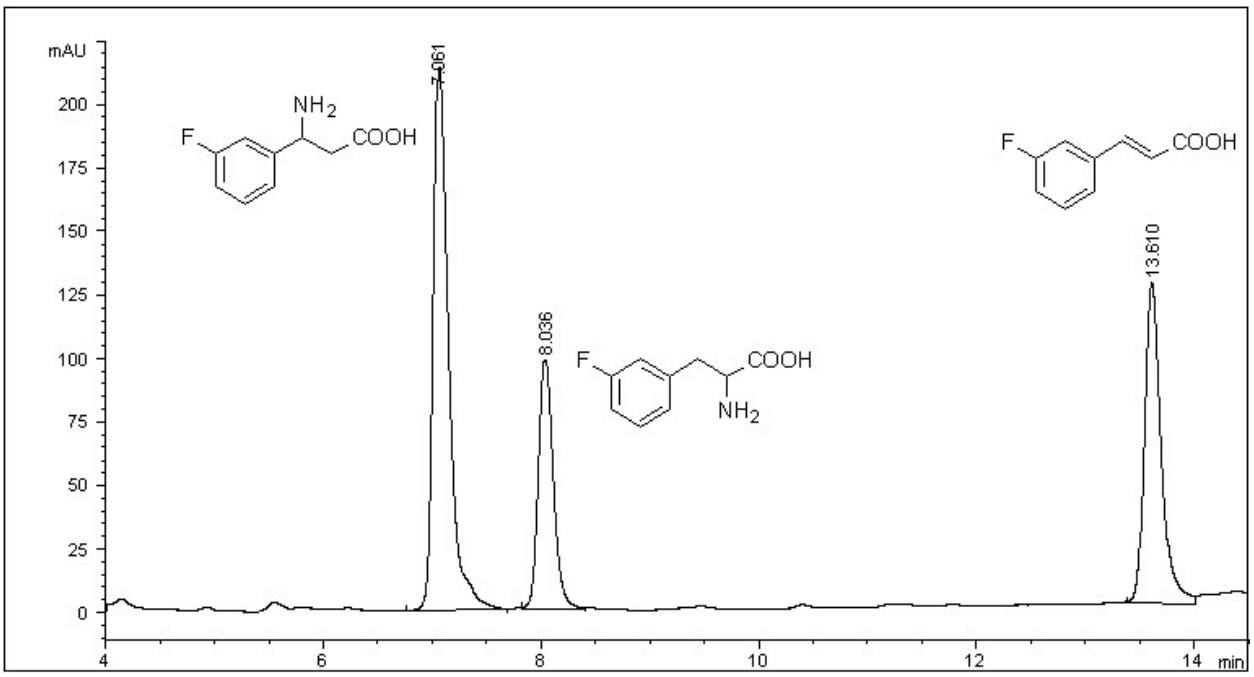
Products from (±)-β-(thiophen-2-yl)alanine (*rac*-**2b**) by *Pa*PAM after 20 h.



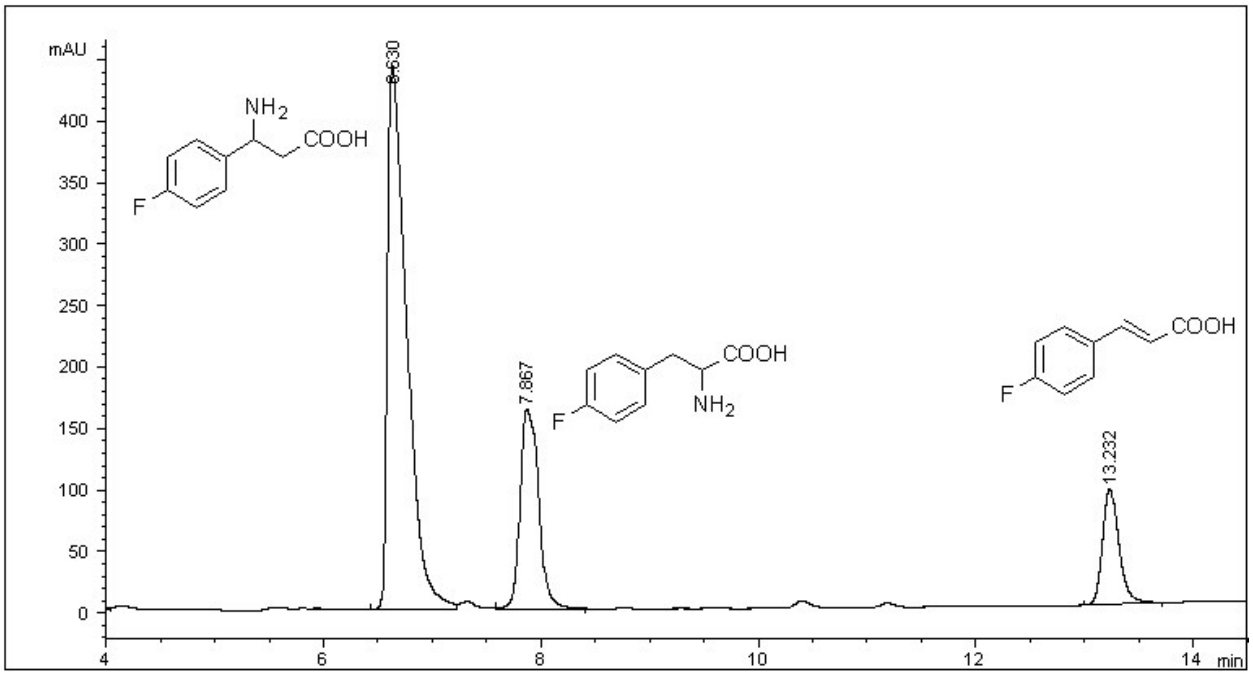
Products from (±)-4-bromo-β-phenylalanine (*rac*-**2c**) by *Pa*PAM after 20 h.



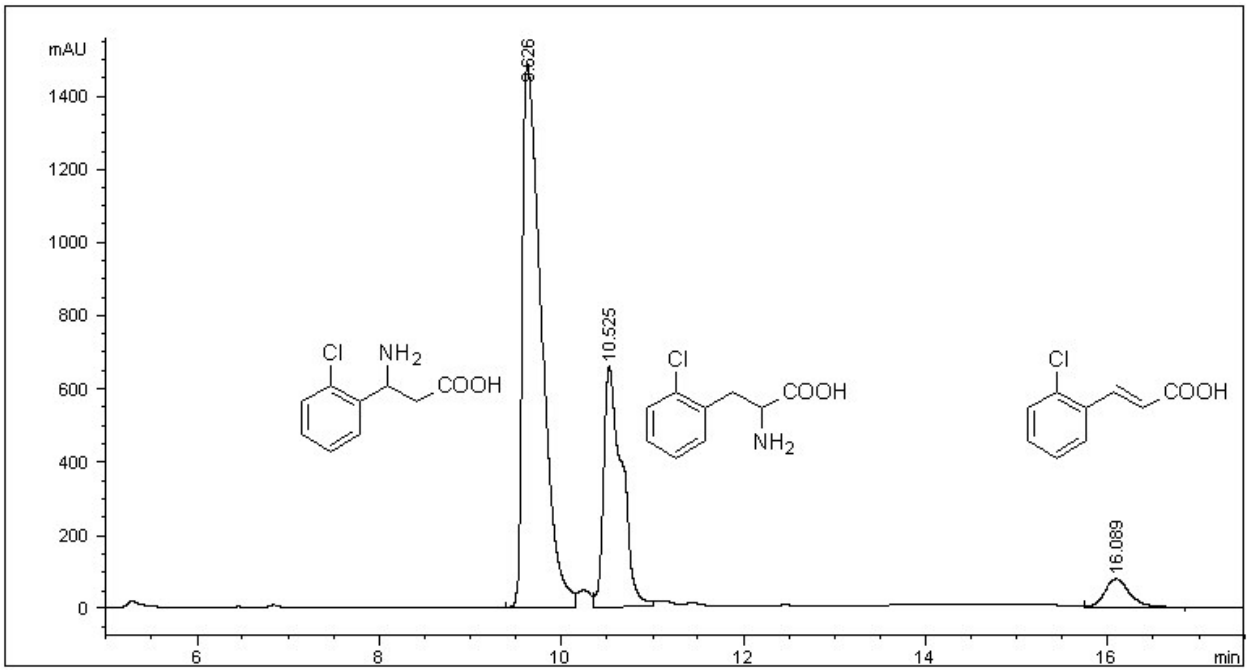
Products from (±)-2-fluoro-β-phenylalanine (*rac*-**2d**) by *Pa*PAM after 20 h.



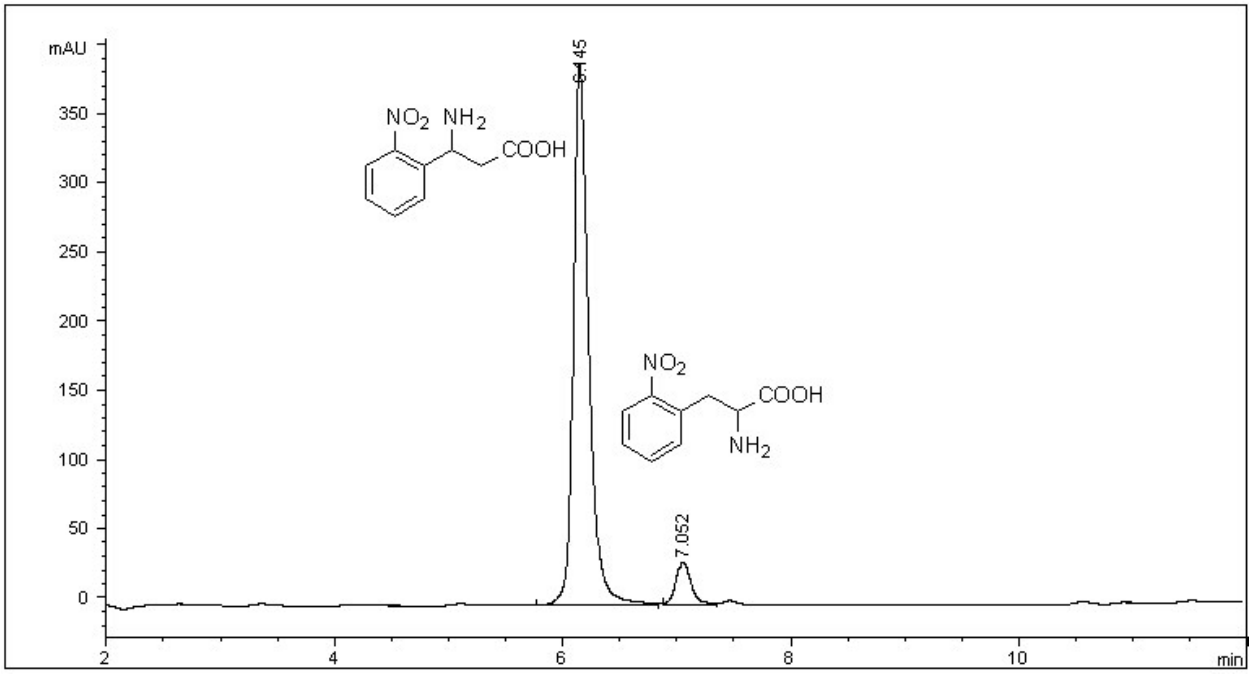
Products from (±)-3-fluoro-β-phenylalanine (*rac*-**2e**) by *Pa*PAM after 20 h.



Products from (±)-4-fluoro-β-phenylalanine (*rac*-**2f**) by *Pa*PAM after 20 h.



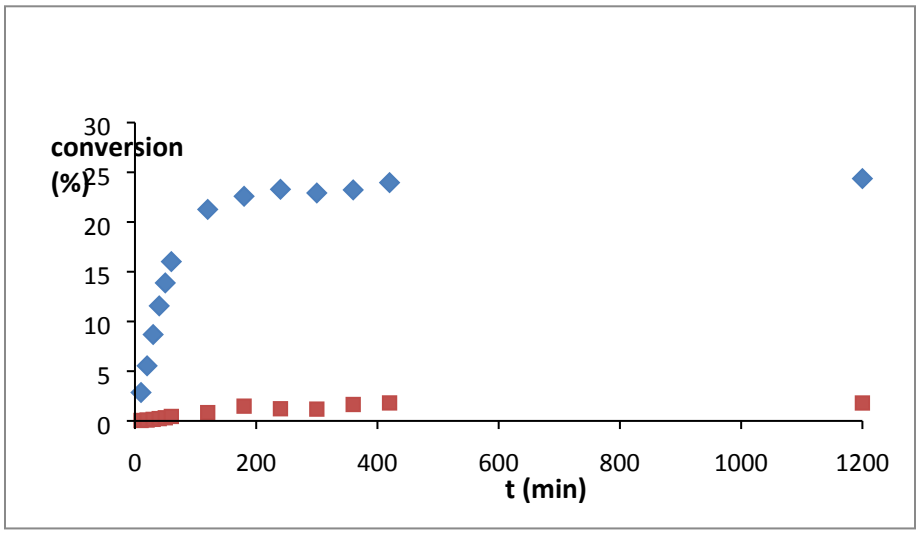
Products from (±)-2-chloro-β-phenylalanine (*rac*-**2g**) by *Pa*PAM after 20 h.



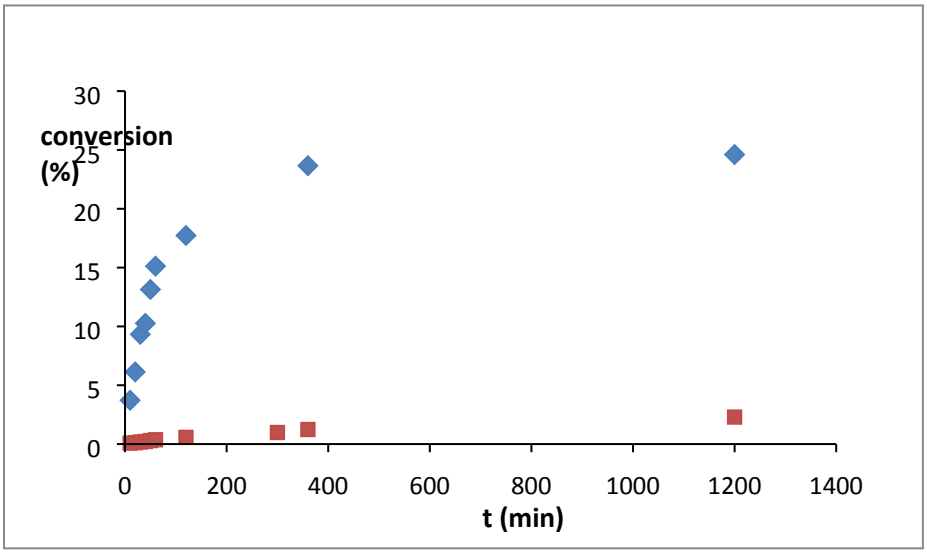
Products from (±)-2-nitro-β-phenylalanine (*rac*-**2j**) by *Pa*PAM after 20 h.

3. Time-course profiles of the PaPAM-catalysed reactions

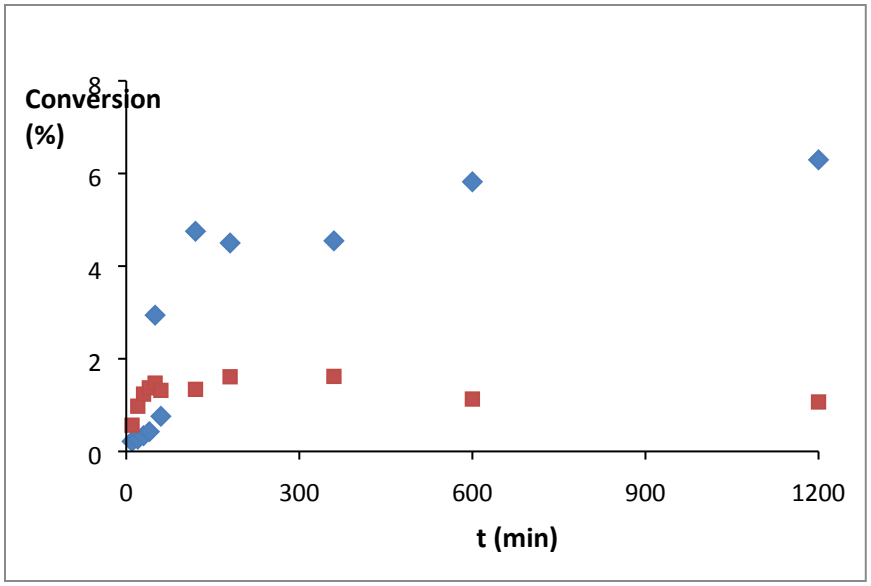
Into the solution of the substrate (*rac*-**1a-f,h,i,k,l**or*rac*-**2a-g,j,** 4 mg) in $(NH_4)_2CO_3$ buffer (100 mM, pH 8.0, 2 mL), *Pa*PAM (1.6 mg) was added and the reaction mixture was stirred at room temperature. Sample preparations and HPLC measurements were performed as described in experimental section in main manuscript.



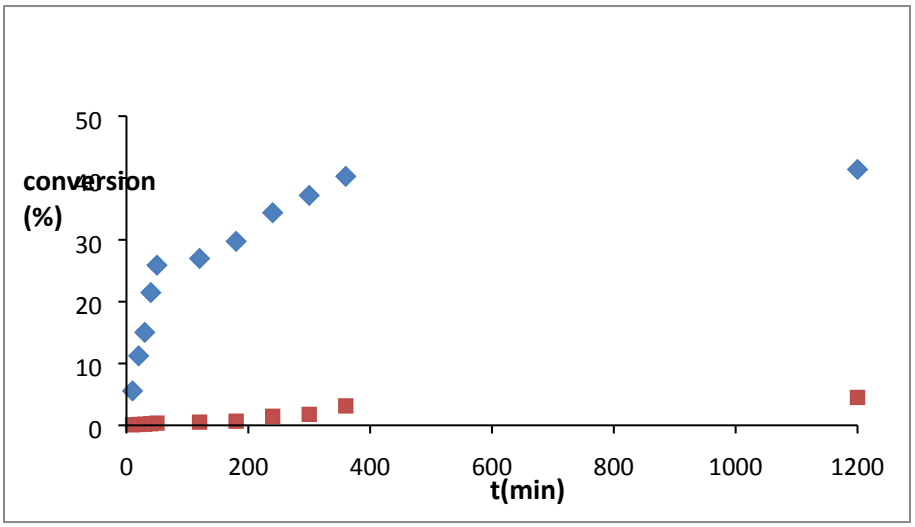
Time-course profile of the conversion of (±)-β-phenylalanine (*rac*-**2a**, ◆) to (*S*)-α-phenylalanine [(*S*)-**1a**] and (*E*)-cinnamic acid (**3a**, 🕘)



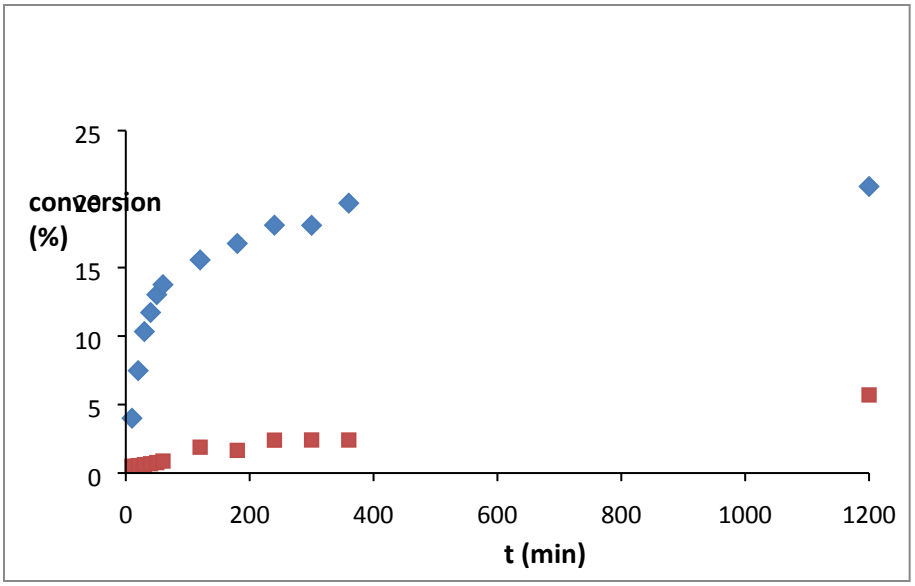
Time-course profile of the conversion of (±)-β-(thiophen-2-yl)alanine (*rac*-**2b**, ◆) to (*S*)-α-(thiophen-2-yl)alanine [(*S*)-**1b**)] and (*E*)-3-(thiophen-2-yl)acrylic acid (**3b**, 🕘)



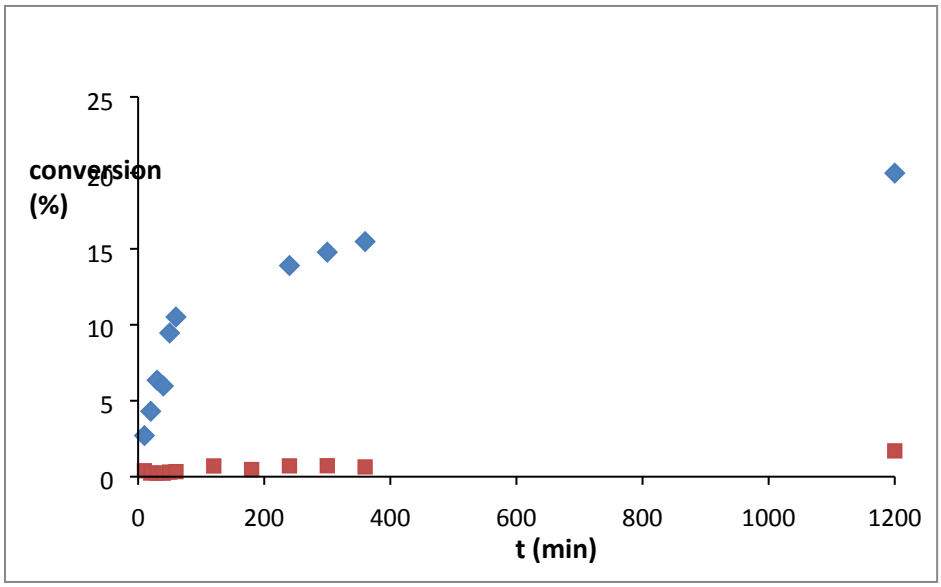
Time-course profile of the conversion of (±)-4-bromo-β-phenylalanine (*rac*-**2c**, ◆) to (*S*)-4-bromo-α-phenylalanine [(*S*)-**1c**)] and (*E*)-4-bromocinnamic acid (**3c**, 🕘)



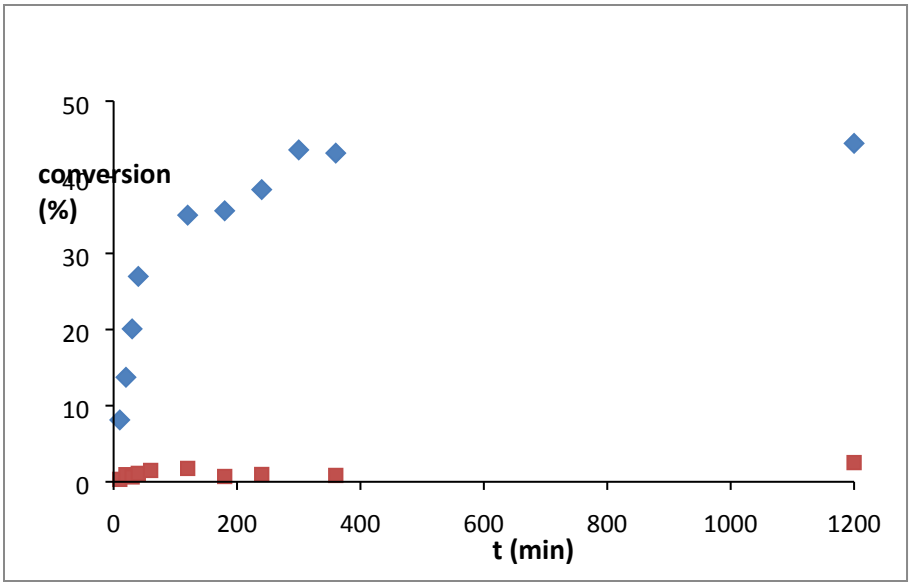
Time-course profile of the conversion of (±)-4-fluoro-β-phenylalanine (*rac*-**2d**, ◆) to (*S*)-4-fluoro-α-phenylalanine [(*S*)-**1d**)] and (*E*)-4-fluorocinnamic acid (**3d**, 🕘)



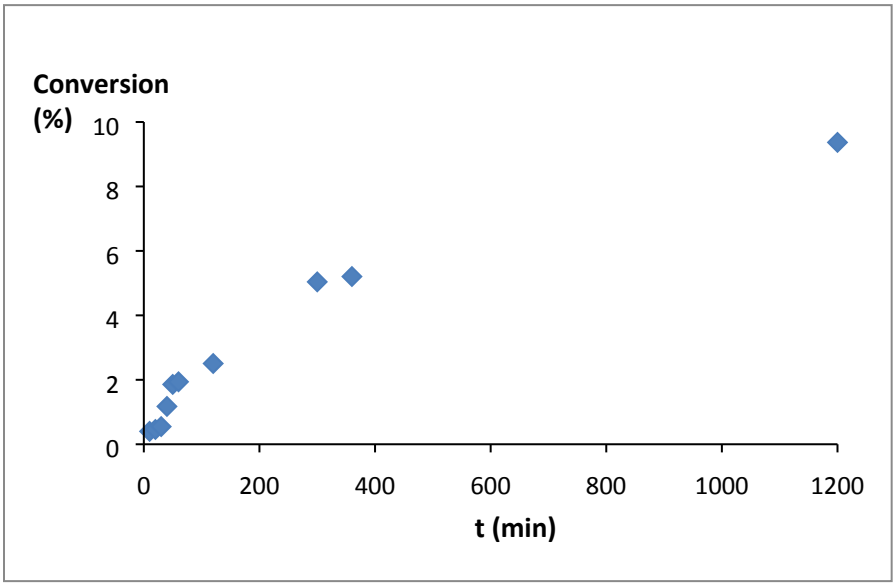
Time-course profile of the conversion of (±)-3-fluoro-β-phenylalanine (*rac*-**2e**, ◆) to (*S*)-3-fluoro-α-phenylalanine [(*S*)-**1e**)] and (*E*)-4-fluorocinnamic acid (**3e**, 🕘)



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**, 🕘)



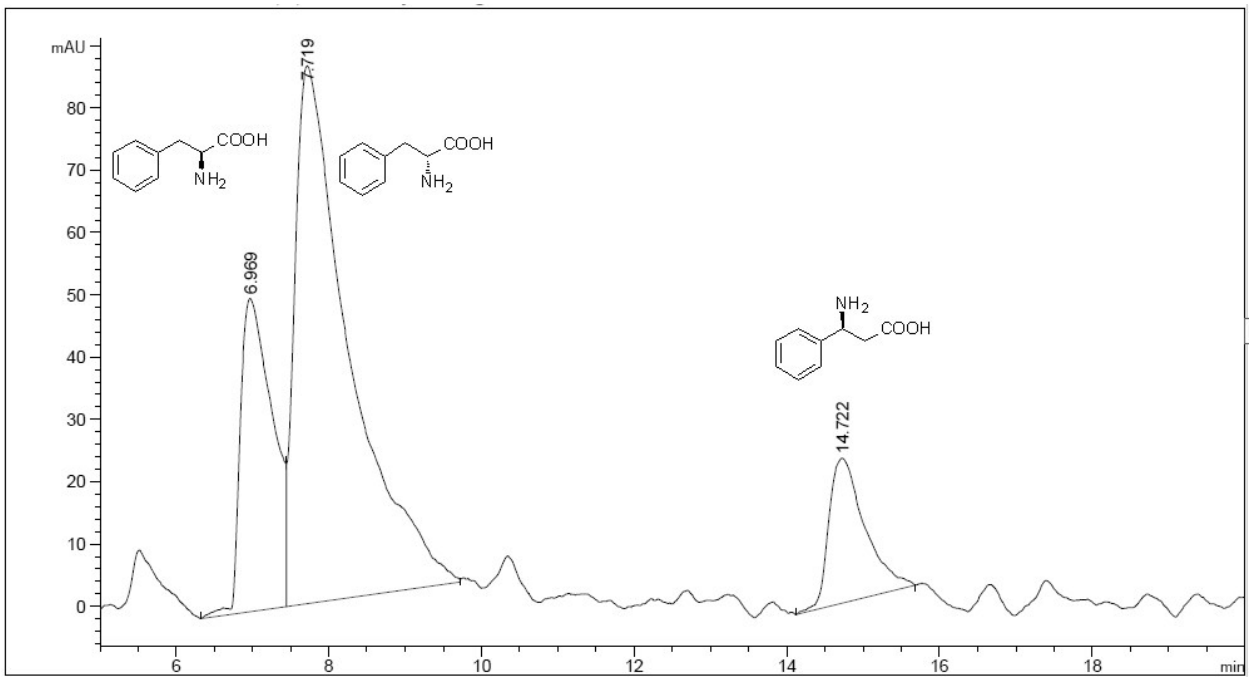
Time-course profile of the conversion of (±)-2-chloro-β-phenylalanine (*rac*-**2g**, ◆) to (*S*)-2-chloro-α-phenylalanine [(*S*)-**1g**)] and (*E*)-2-chlorocinnamic acid (**3g**, 🕘)



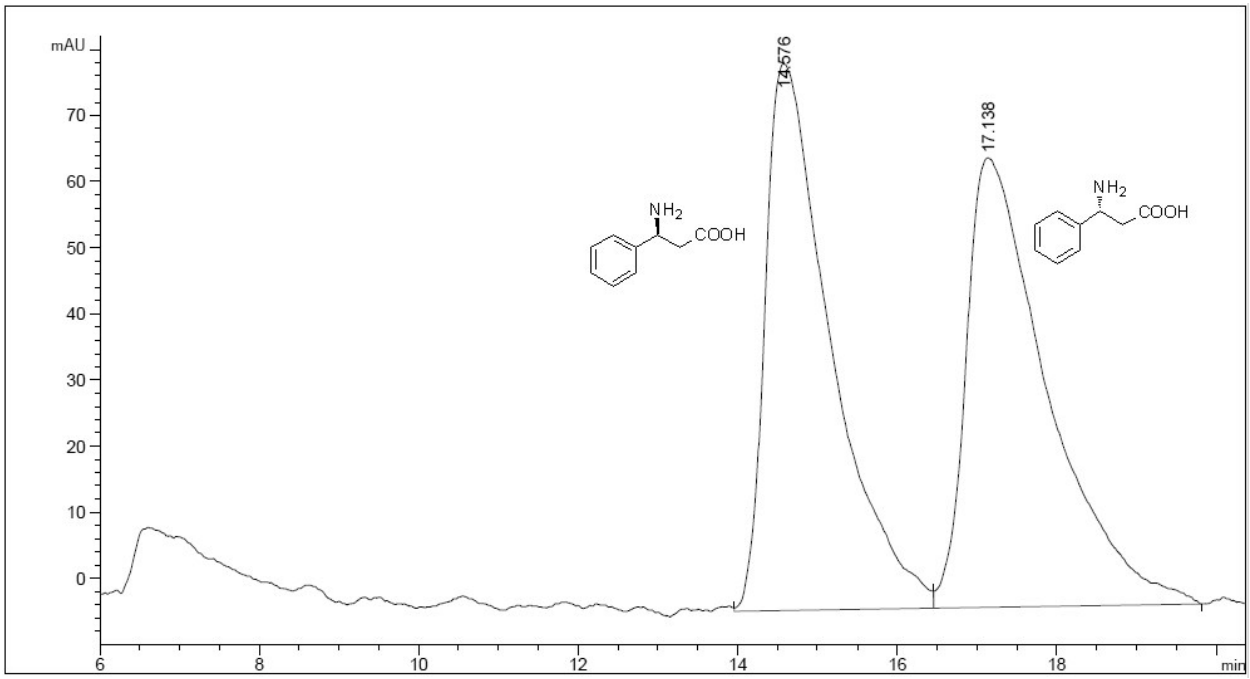
Time-course profile of the conversion of (±)-2-nitro-β-phenylalanine (*rac*-**2j**) to (*S*)-2-nitro-α-phenylalanine [(*S*)-**1j**, ◆]

4. HPLC determination of the enantiomeric compositions

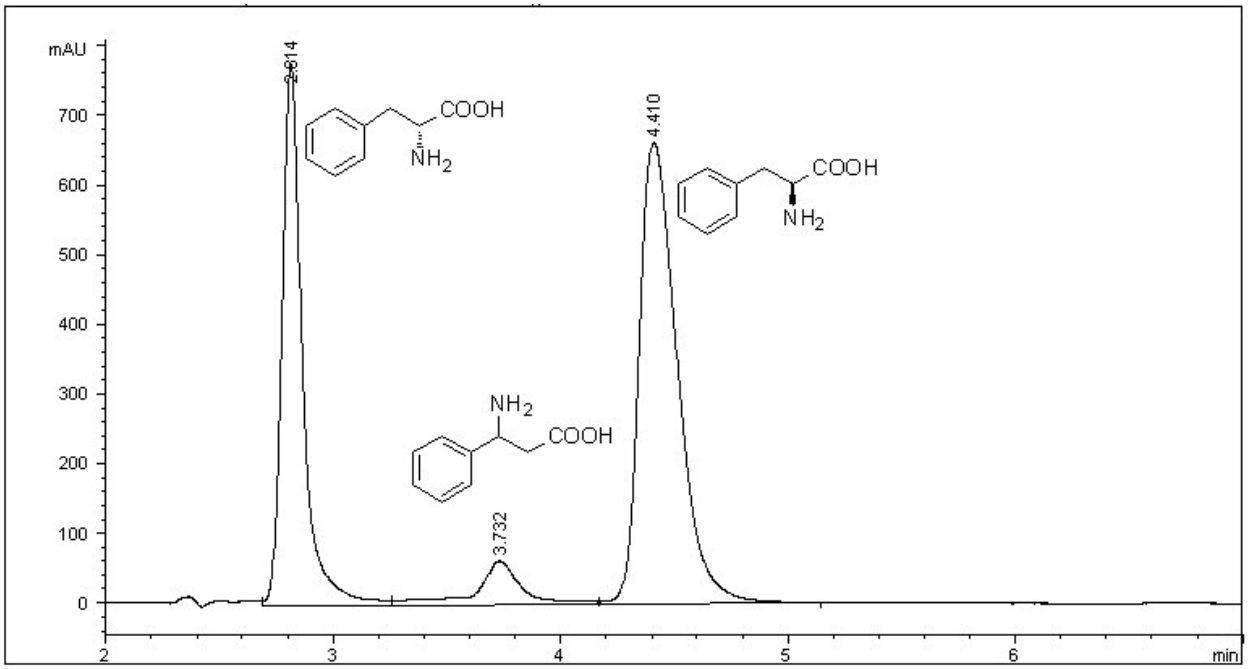
Samples taken from the enzymatic reactions after 20 h as described in section 2 were injected onto Crownpak® CR-I(+)column (150 × 3.0 mm × 5 µm) using $HClO_4$ solution (pH 1.5) : acetonitrile as mobile phase, flow rate: 0.4mLmin^{-1}or onto Chiralpak® ZWIX (+) column (250 × 4.6 mm × 3 µm) using MeOH (50 mM diethylamine, 100 mM formic acid) : acetonitrile : H_2O, 49:49:2 as mobile phase, flow rate: 1 mL/min.



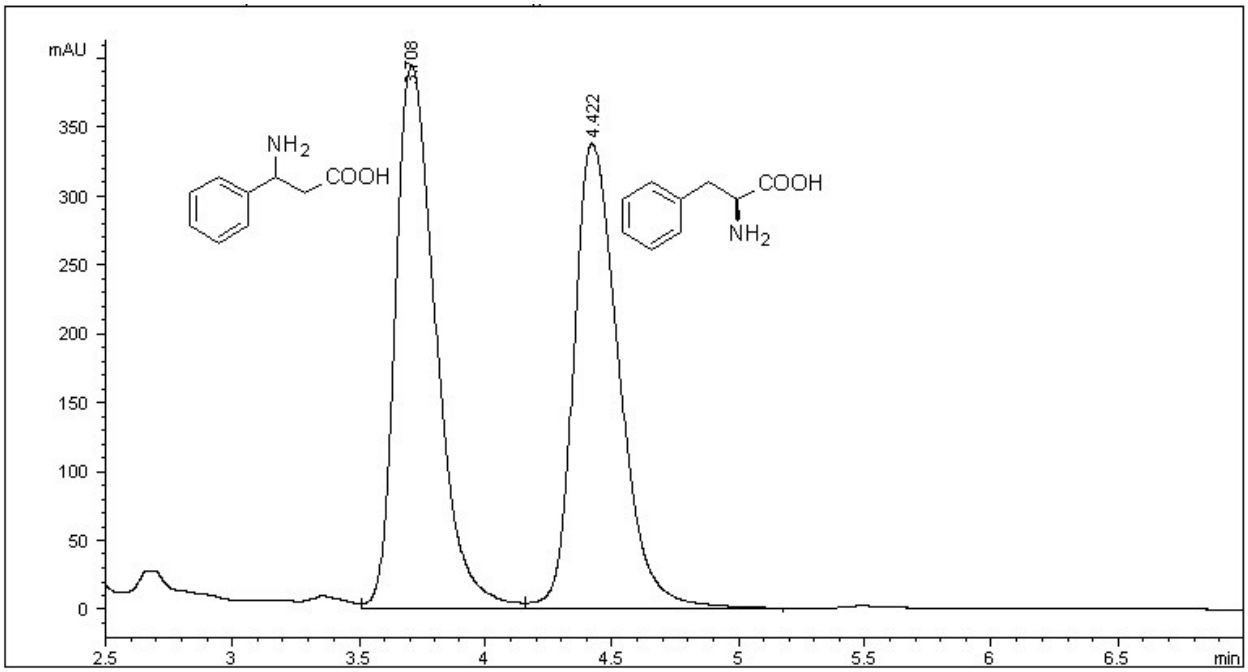
Products from (±)-α-phenylalanine (*rac*-**1a**) by *Pa*PAM after 20 h [Chiralpak ZWIX (+) column]



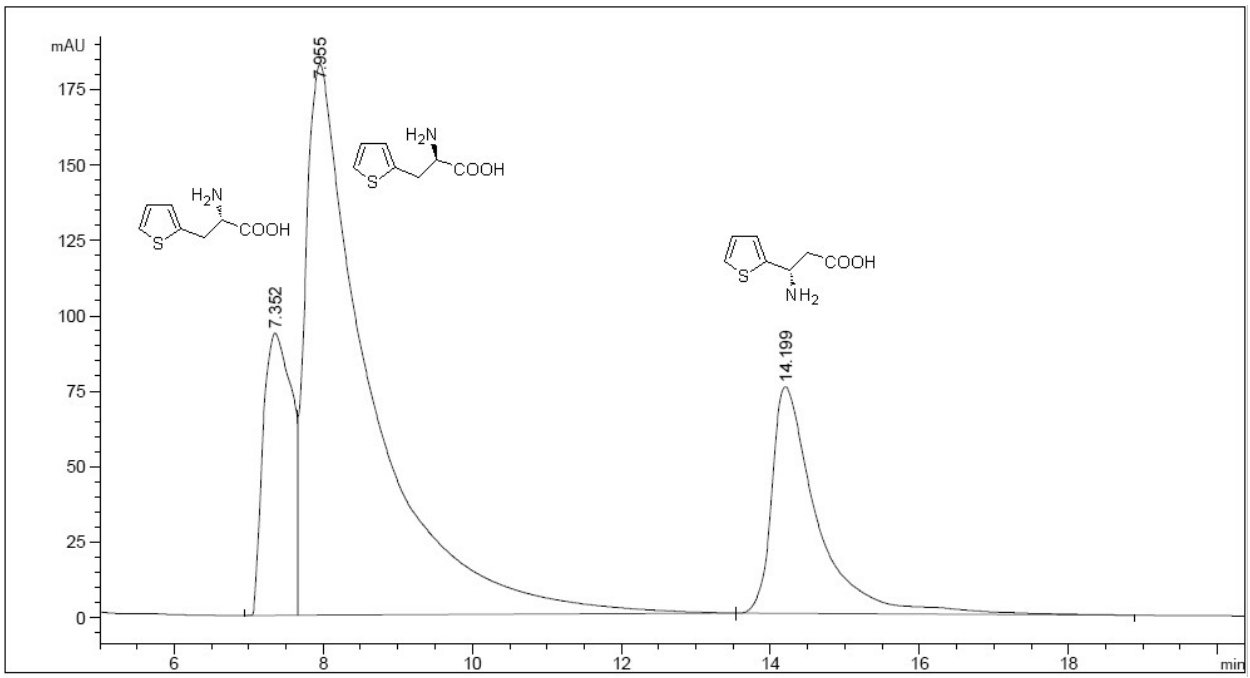
Enantioseparation of authentic (±)-β-phenylalanine (*rac*-**2a**) on Chiralpak ZWIX(+) column



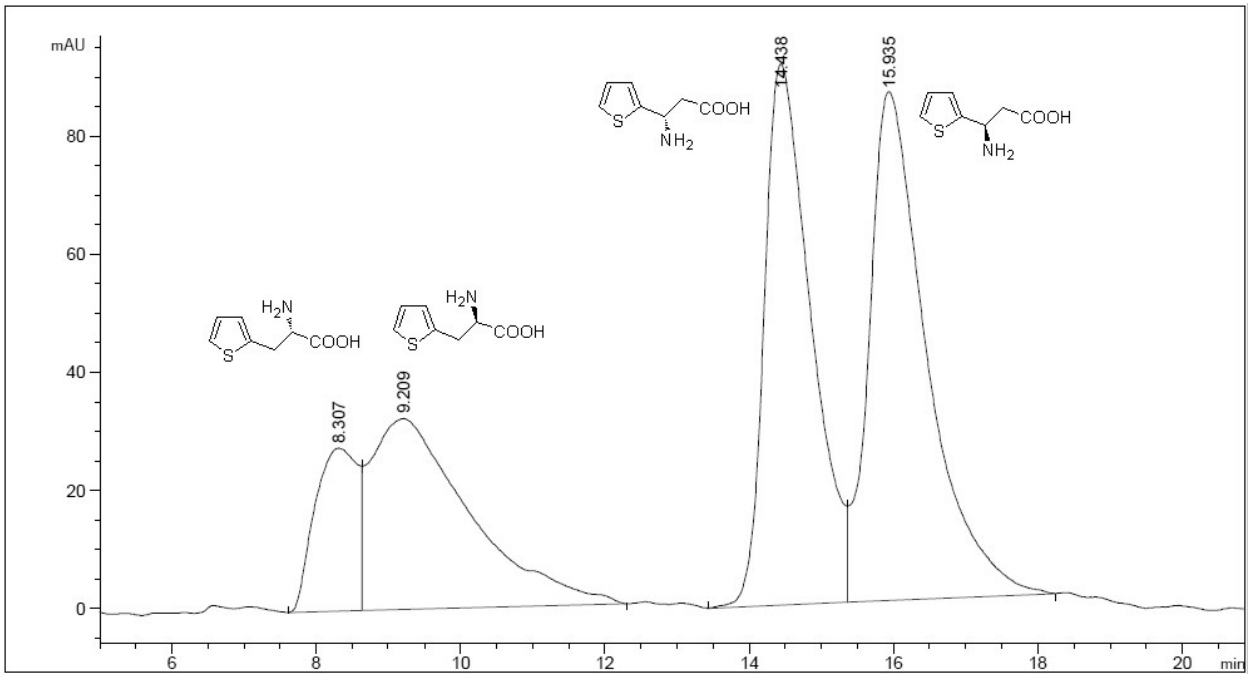
Products from (±)-α-phenylalanine (*rac*-**1a**) by *Pa*PAM after 20 h
[Crownpak CR-I(+) column]



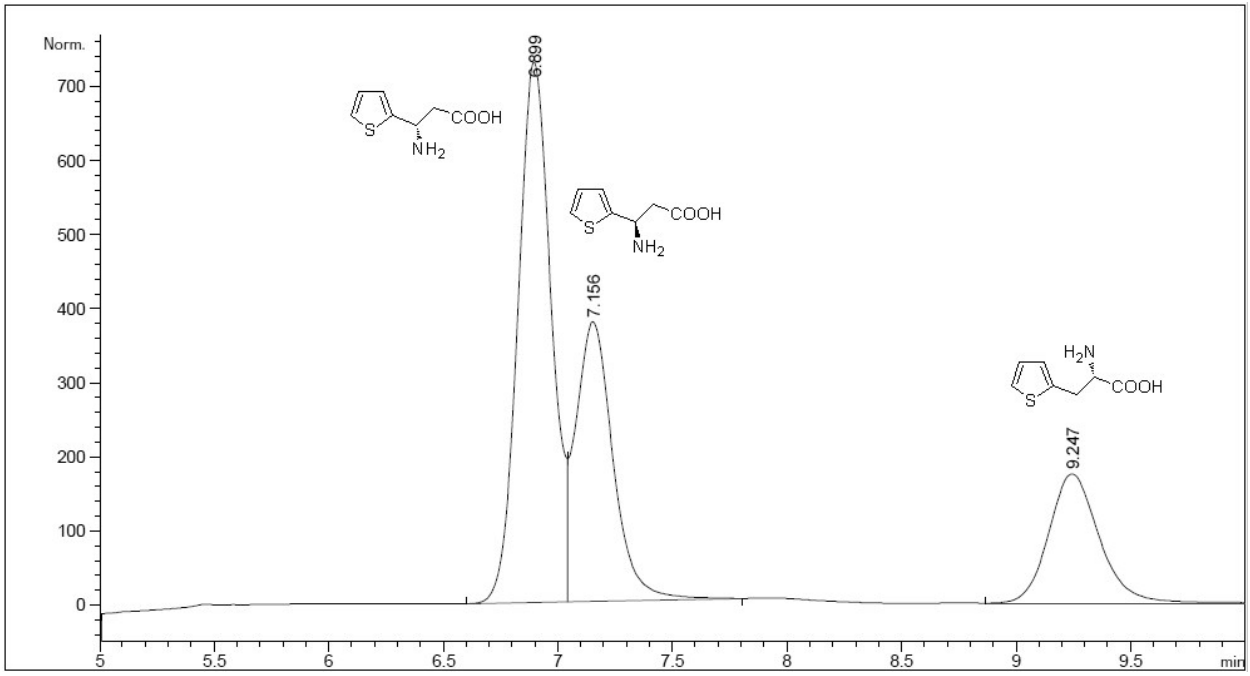
Enantioseparation of authentic (±)-β-phenylalanine (*rac*-**2a**) on Crownpak CR-I(+) column



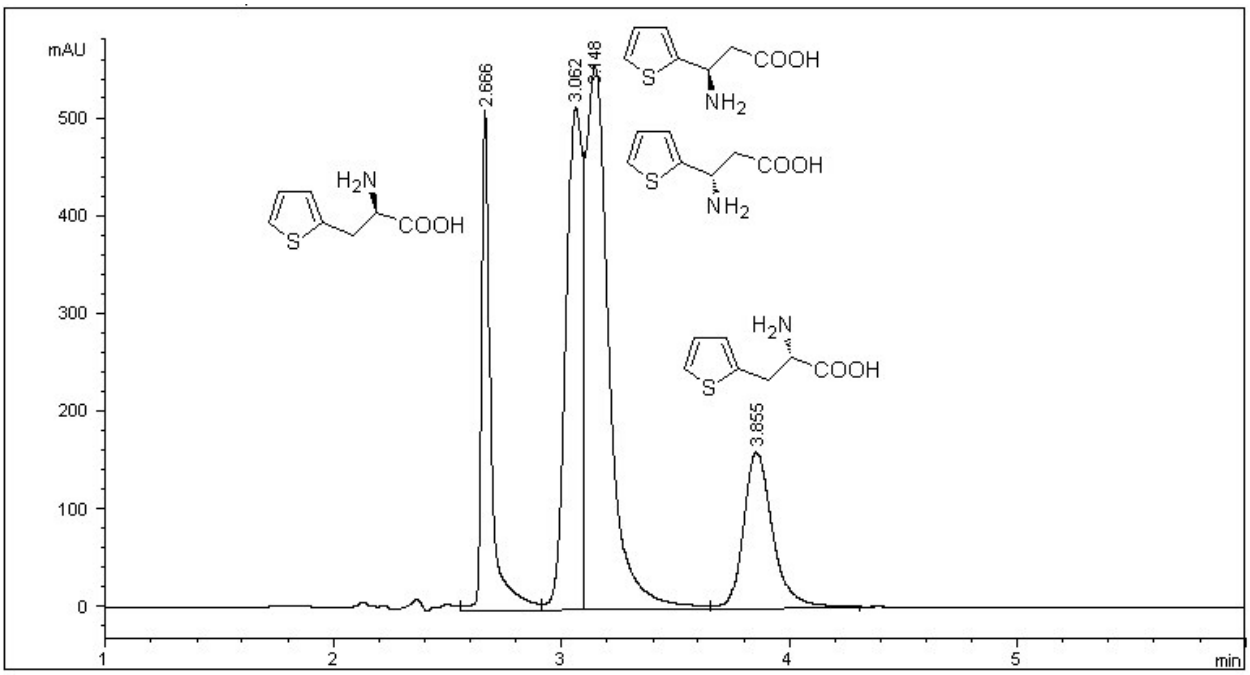
Products from (±)-α-(thiophen-2-yl)alanine (*rac*-**1b**) by *Pa*PAM after 20 h
[Chiralpak ZWIX(+) column]



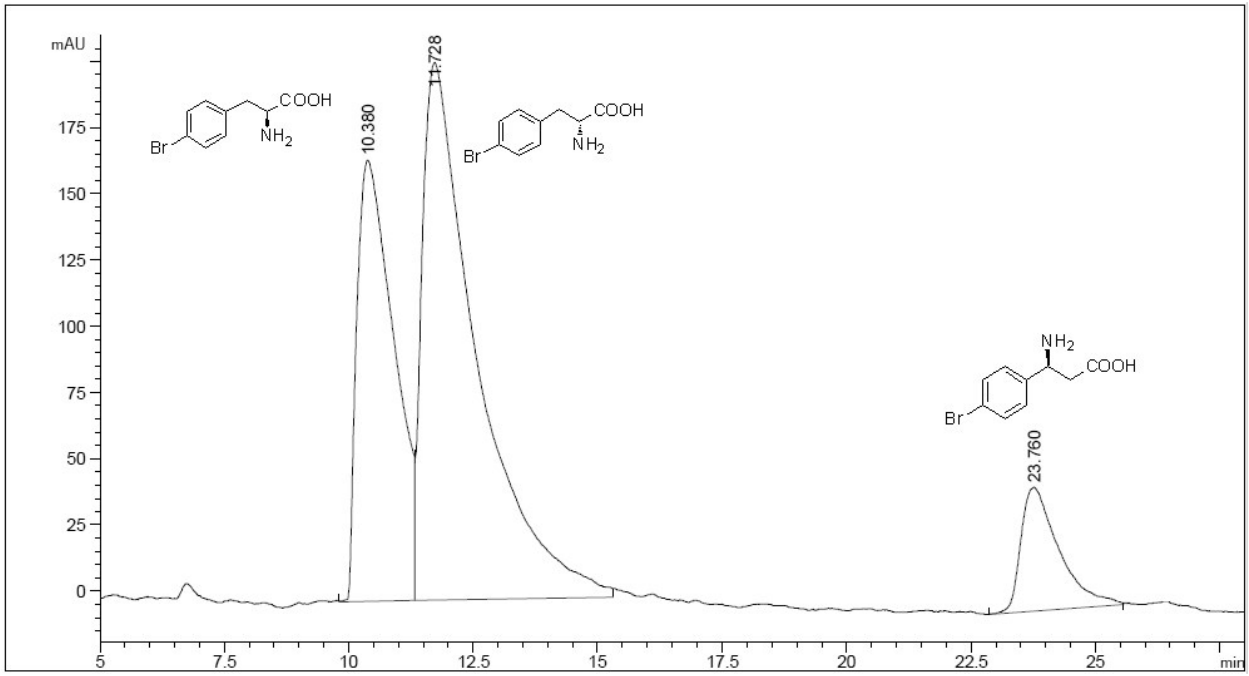
Enantioseparation of authentic (±)-β-(thiophen-2-yl)alanine (*rac*-**2b**) and
(±)-α-(thiophen-2-yl)alanine (*rac*-**1b**) on Chiralpak ZWIX(+) column



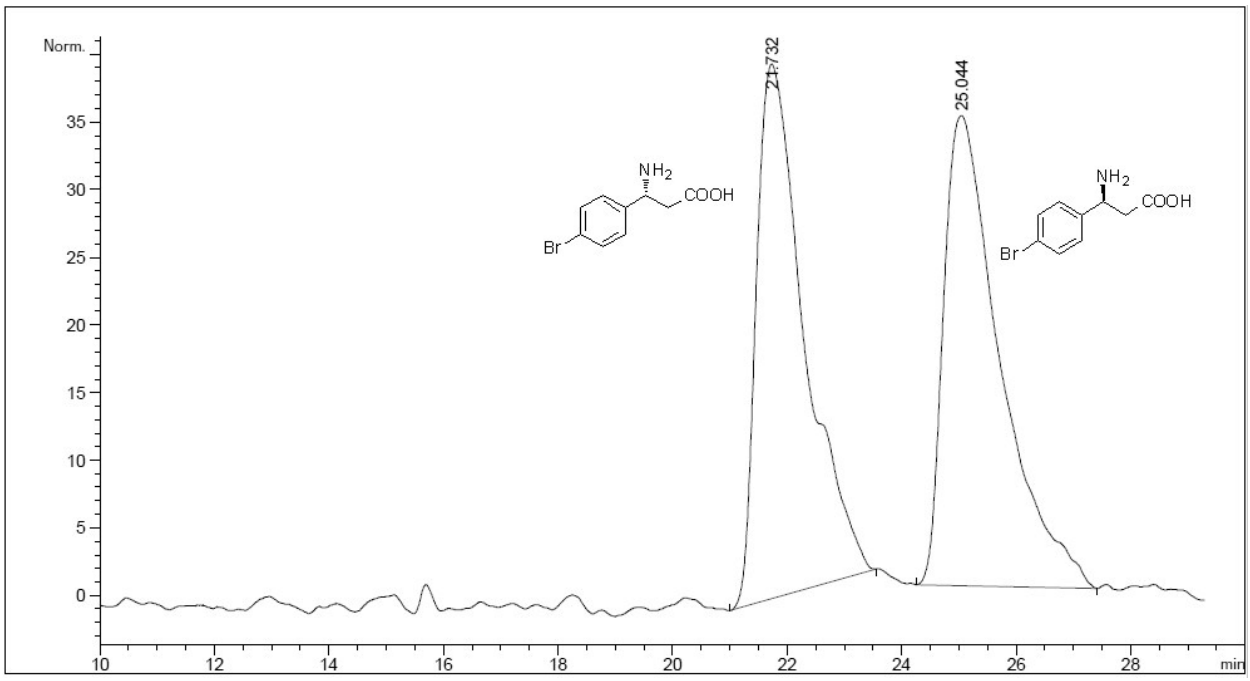
Products from (±)-β-(thiophen-2-yl)alanine (*rac*-**2b**) by *Pa*PAM after 20 h
[Crownpak CR-I(+)column]



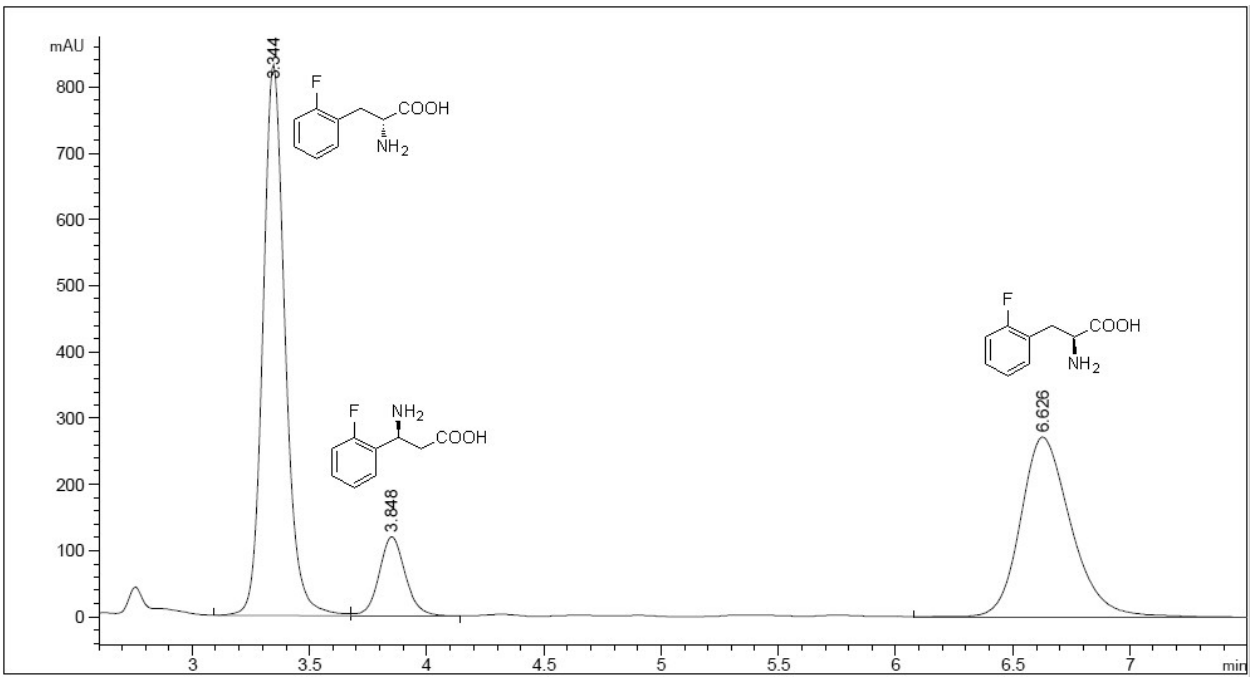
Enantioseparation of authentic (±)-β-(thiophen-2-yl)alanine (*rac*-**2b**) and
(±)-α-(thiophen-2-yl)alanine (*rac*-**1b**) on Crownpak CR-I(+) column



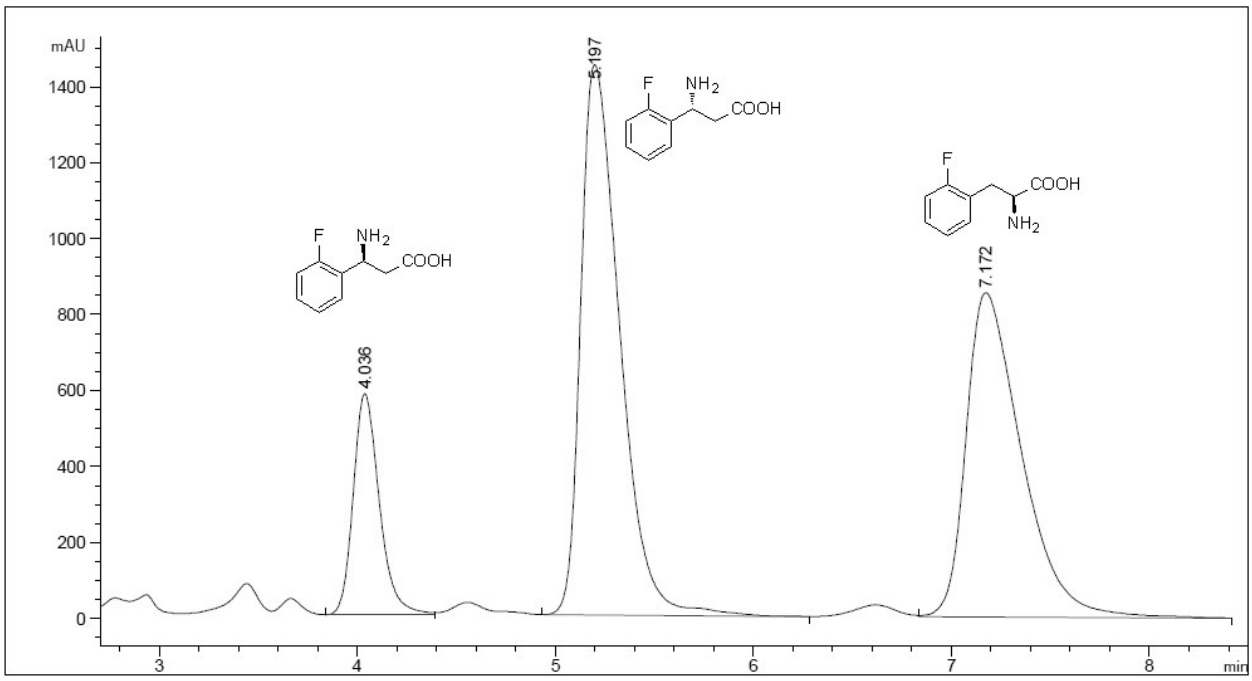
Products from (±)-4-bromo-α-phenylalanine (*rac*-**1c**) by *Pa*PAM after 20 h
[Chiralpak ZWIX(+) column]



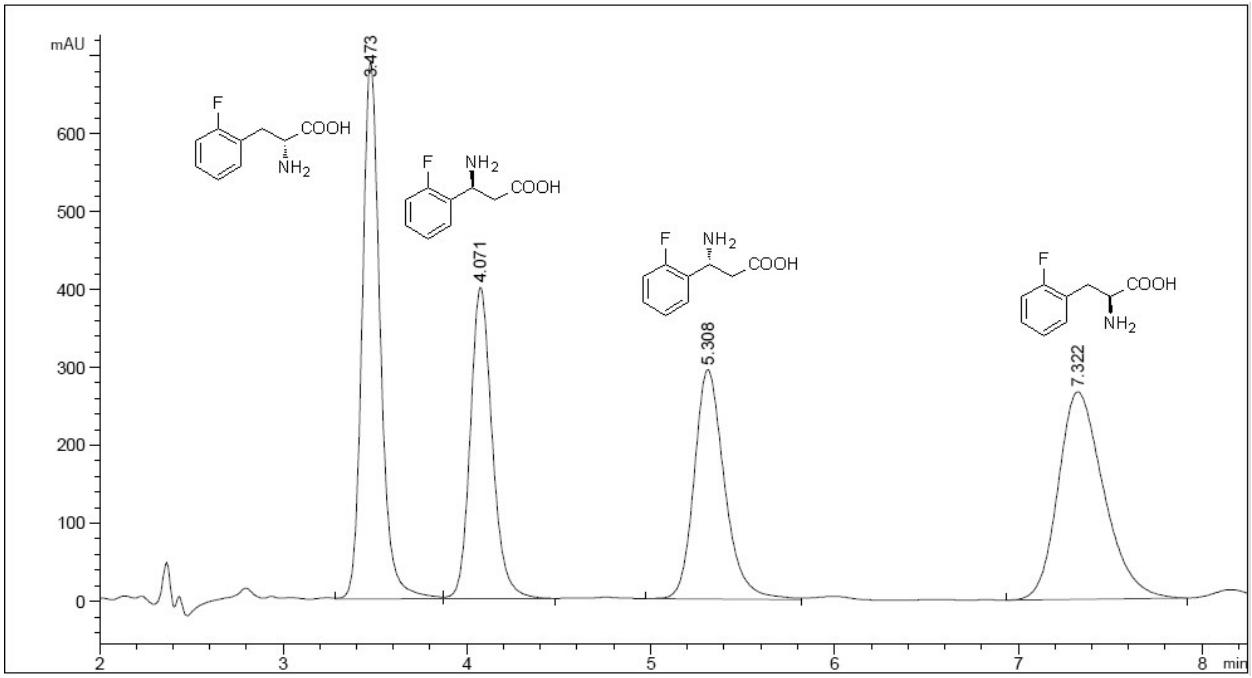
Enantioseparation of authentic (±)-4-bromo-α-phenylalanine (*rac*-**1c**)
on Chiralpak ZWIX(+) column



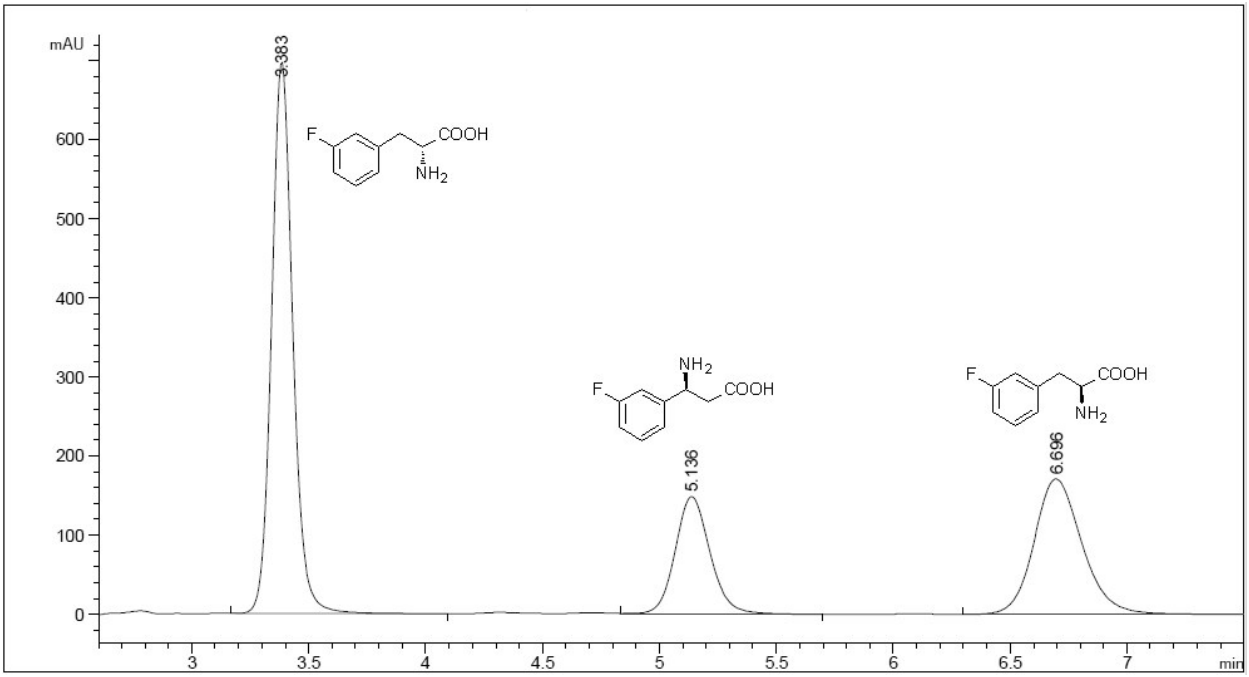
Products from (±)-4-fluoro-α-phenylalanine (*rac*-**1d**) by *Pa*PAM after 20 h
[Crownpak CR-I(+)column]



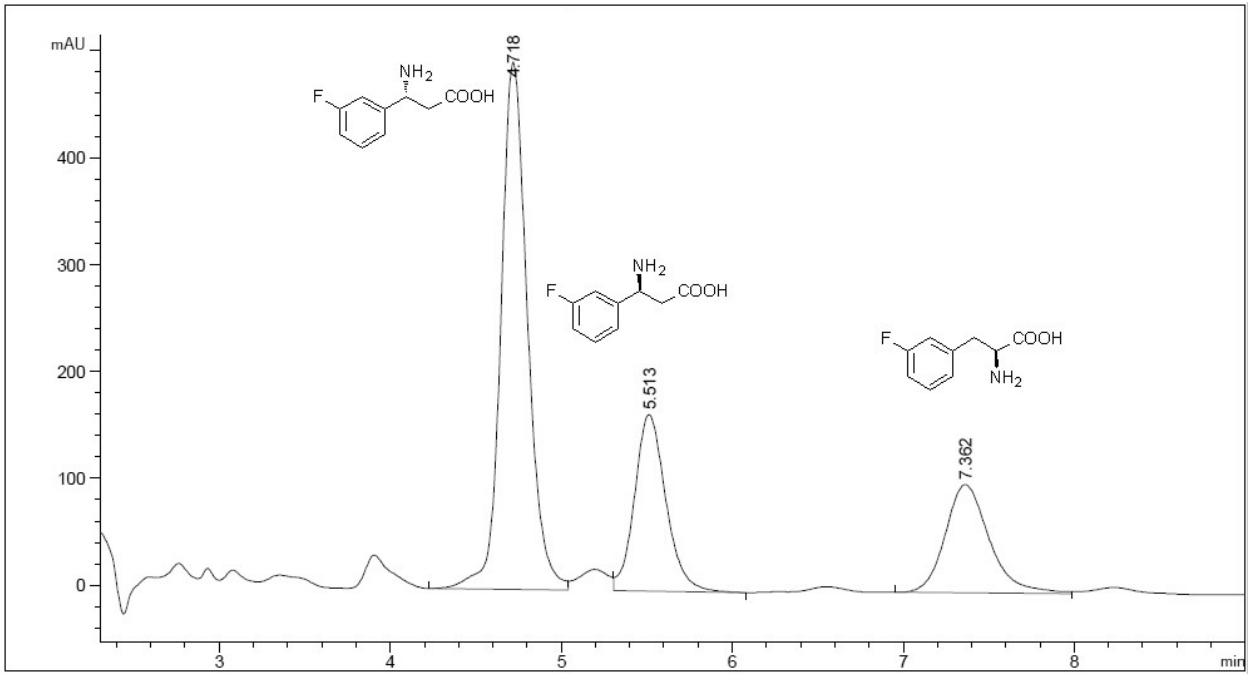
Products from (±)-4-fluoro-β-phenylalanine (*rac*-**2d**) by *Pa*PAM after 20 h
[Crownpak CR-I(+)column]



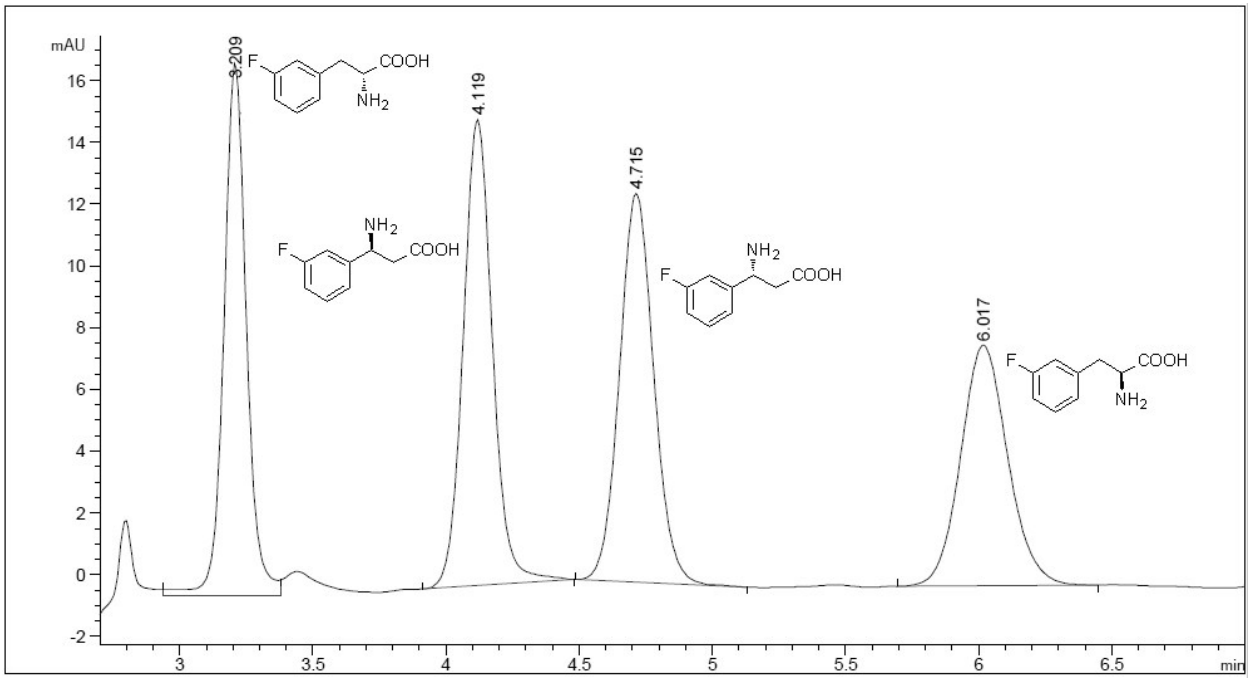
Enantioseparation of authentic (±)-4-fluoro-α-phenylalanine and (±)-4-fluoro-β-phenylalanine on Crownpak CR-I(+)column



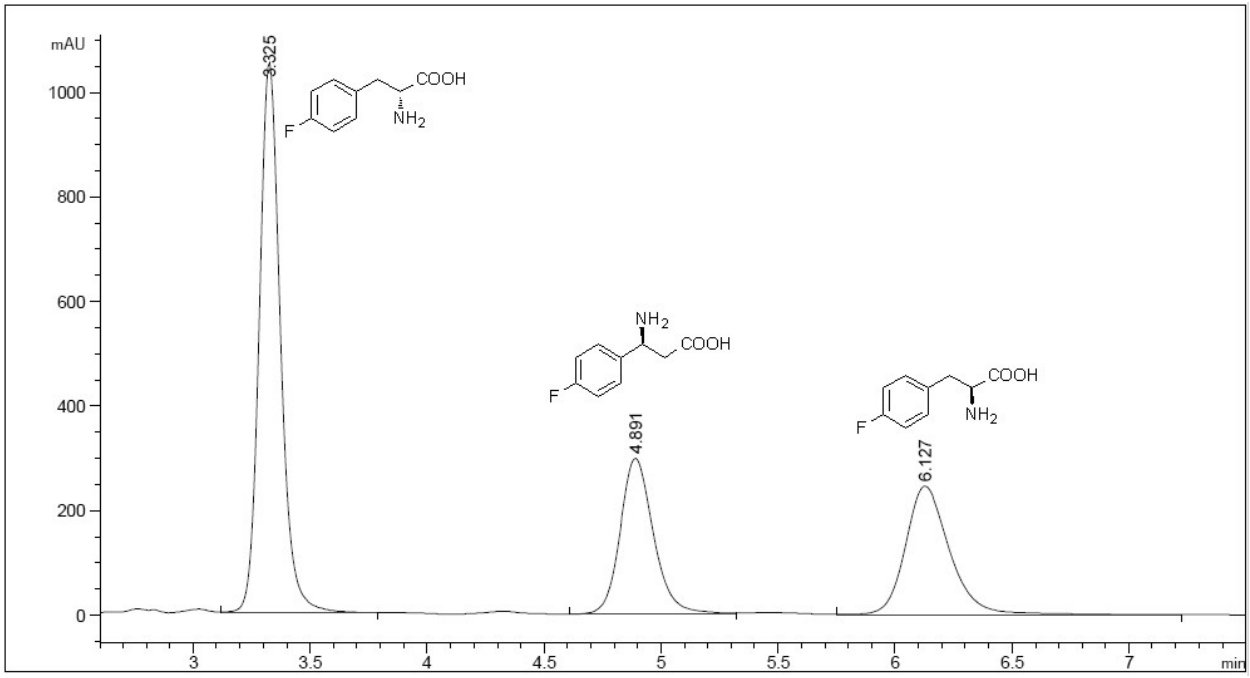
Products from (±)-3-fluoro-α-phenylalanine (*rac*-**1e**) by *Pa*PAM after 20 h [Crownpak CR-I(+)column]



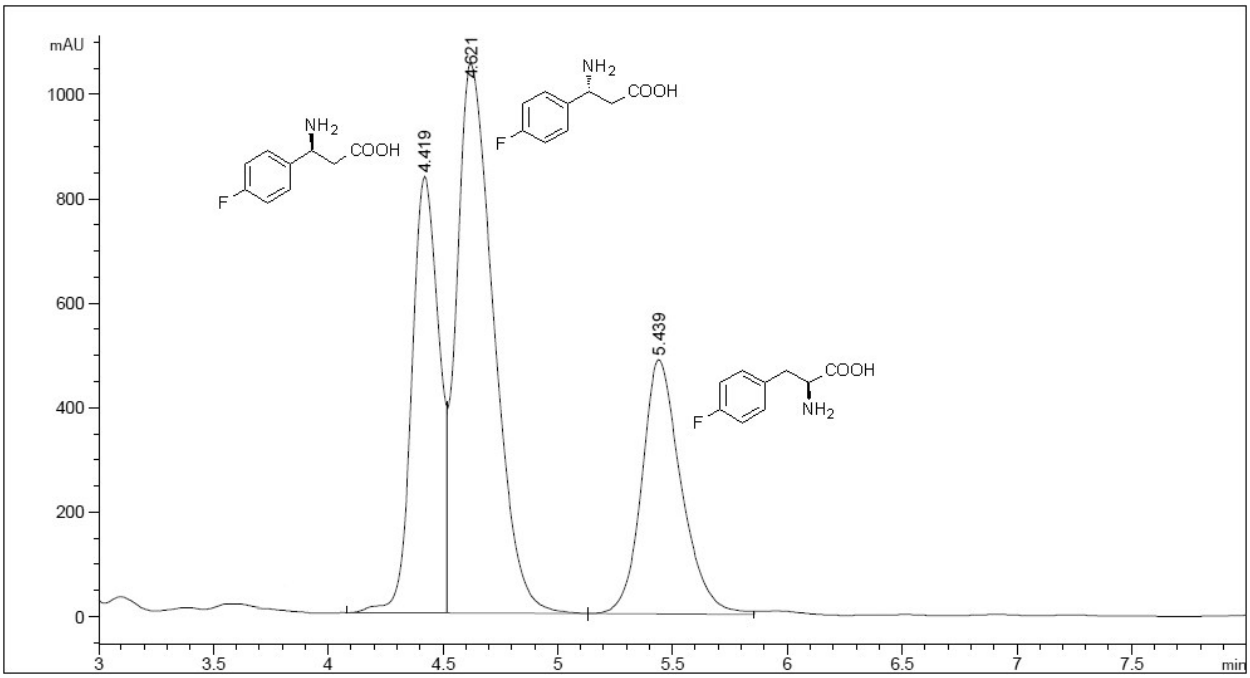
Products from (±)-3-fluoro-β-phenylalanine (*rac*-**2e**) by *Pa*PAM after 20 h
[Crownpak CR-I(+) column]



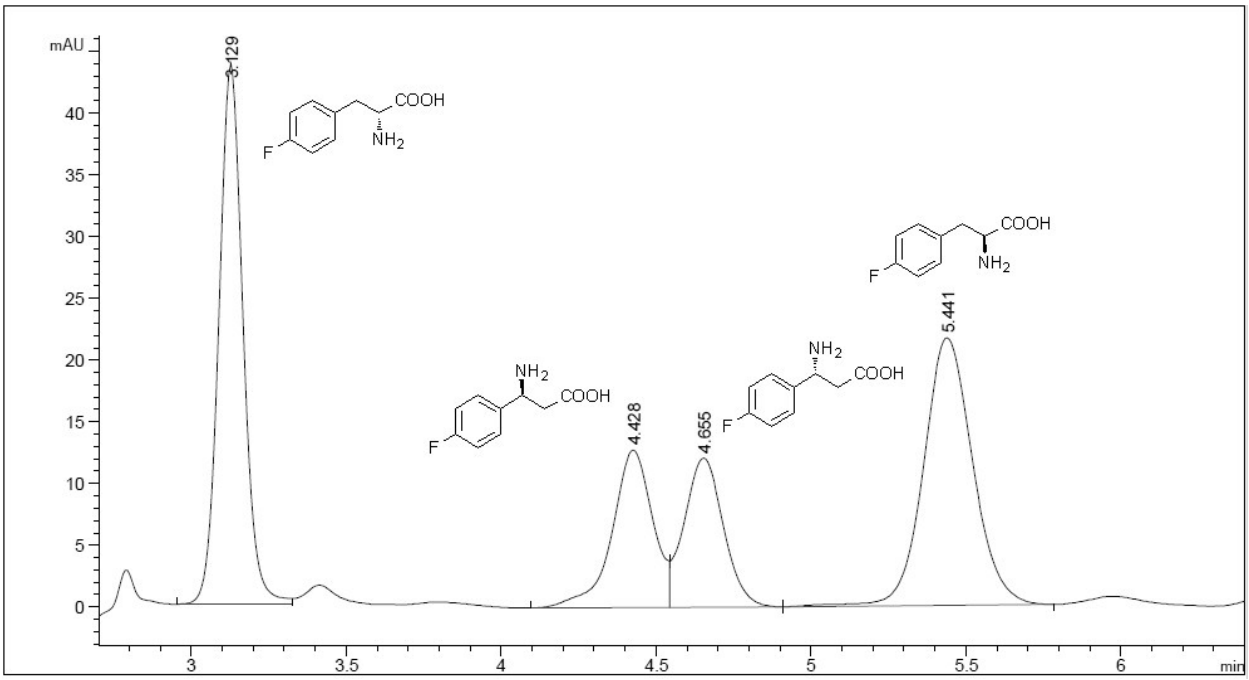
Enantioseparation of authentic (±)-3-fluoro-α-phenylalanine (*rac*-**1e**) and
(±)-3-fluoro-β-phenylalanine (*rac*-**2e**) on Crownpak CR-I(+)column



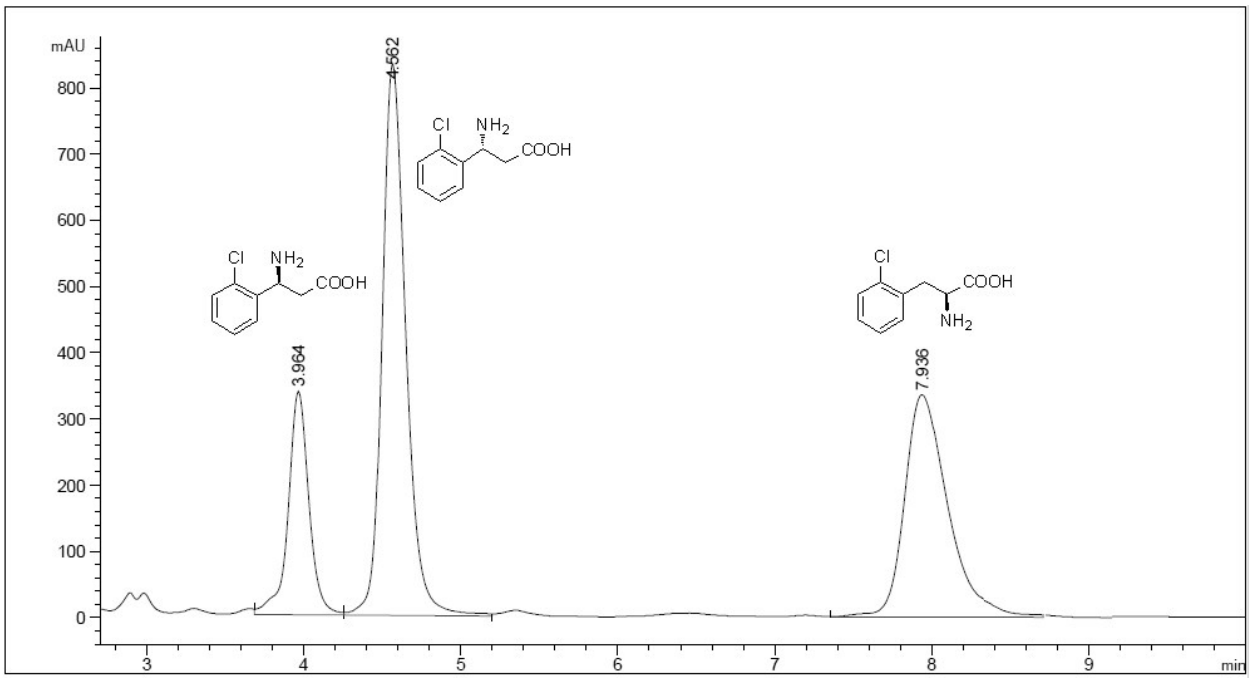
Products from (±)-4-fluoro-α-phenylalanine (*rac*-**1f**) by *Pa*PAM after 20 h
[Crownpak CR-I(+)column]



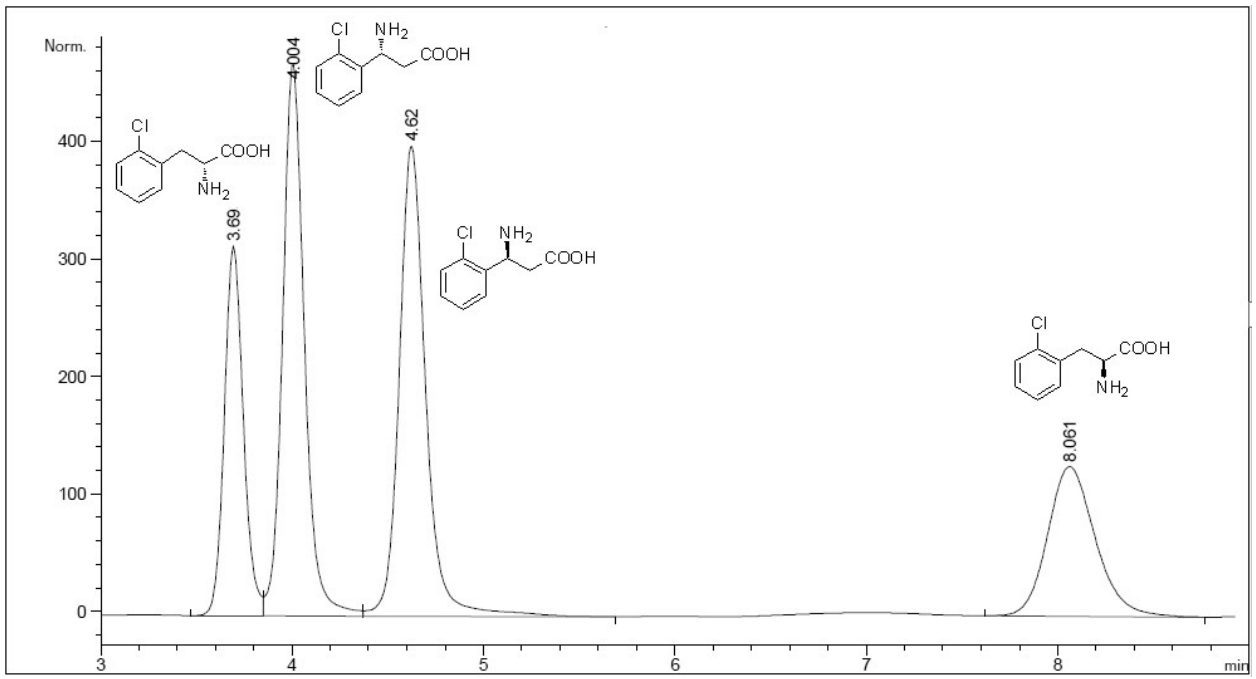
Products from (±)-4-fluoro-β-phenylalanine (*rac*-**2f**) by *Pa*PAM after 20 h
[Crownpak CR-I(+) column]



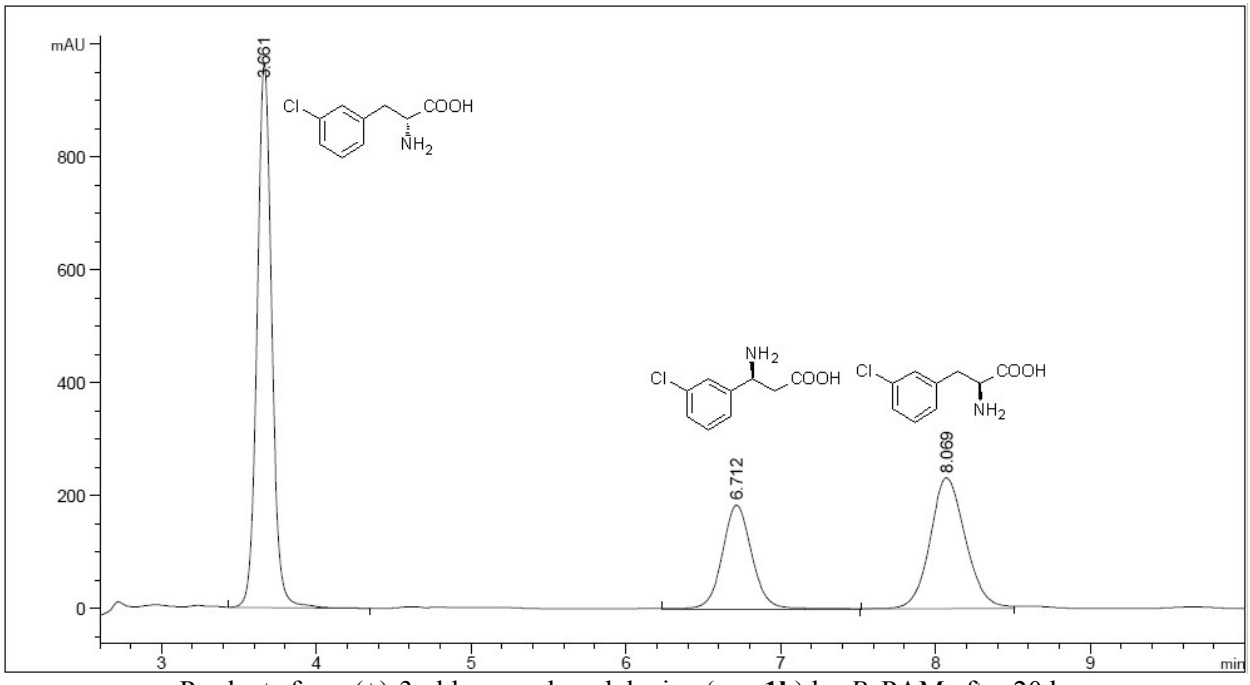
Enantioseparation of authentic (±)-4-fluoro-α-phenylalanine (*rac*-**1f**) and (±)-4-fluoro-β-phenylalanine (*rac*-**2f**) on Crownpak CR-I(+)column



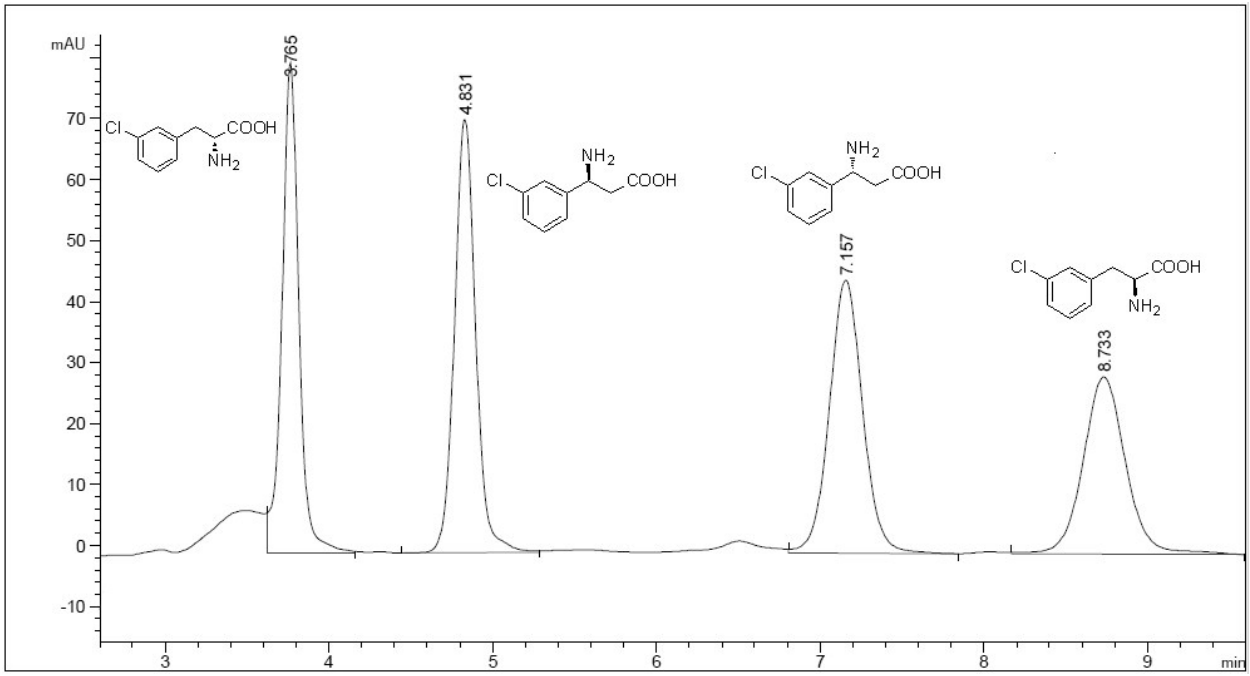
Products from (±)-2-chloro-β-phenylalanine (*rac*-**2g**) by *Pa*PAM after 20 h [Crownpak CR-I(+) column]



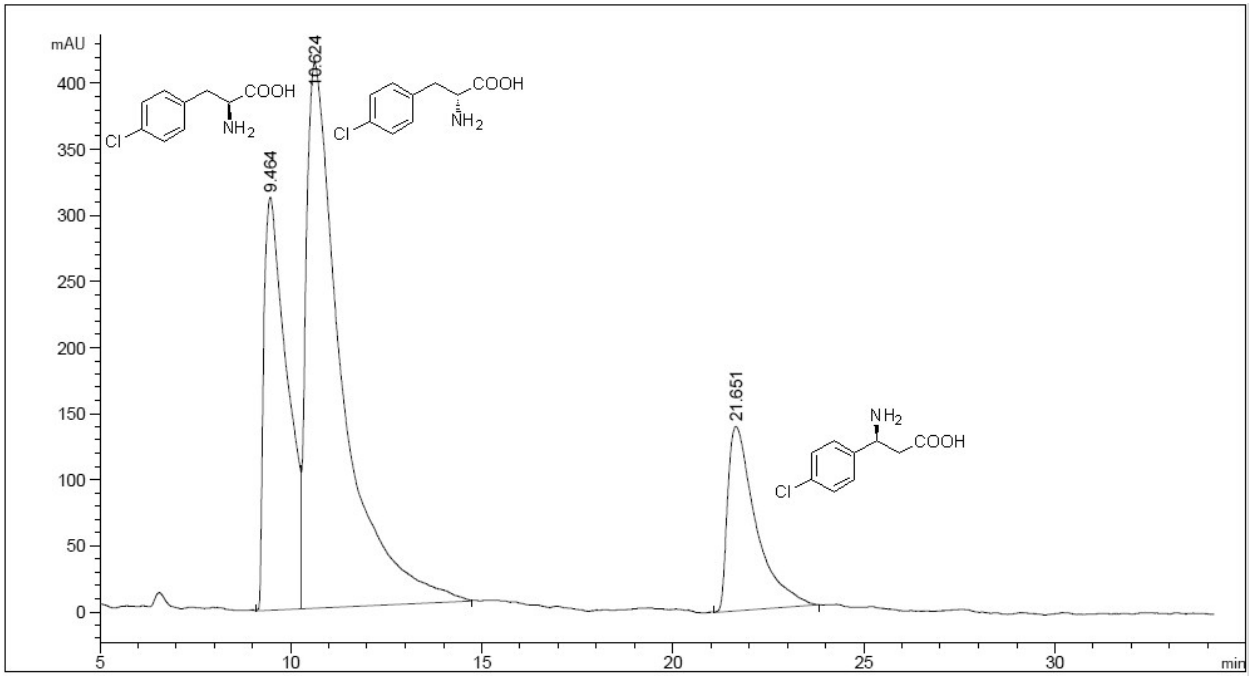
Enantioseparation of authentic (±)-2-chloro-α-phenylalanine (*rac*-**1g**) and (±)-2-chloro-β-phenylalanine (*rac*-**2g**) on Crownpak CR-I(+) column



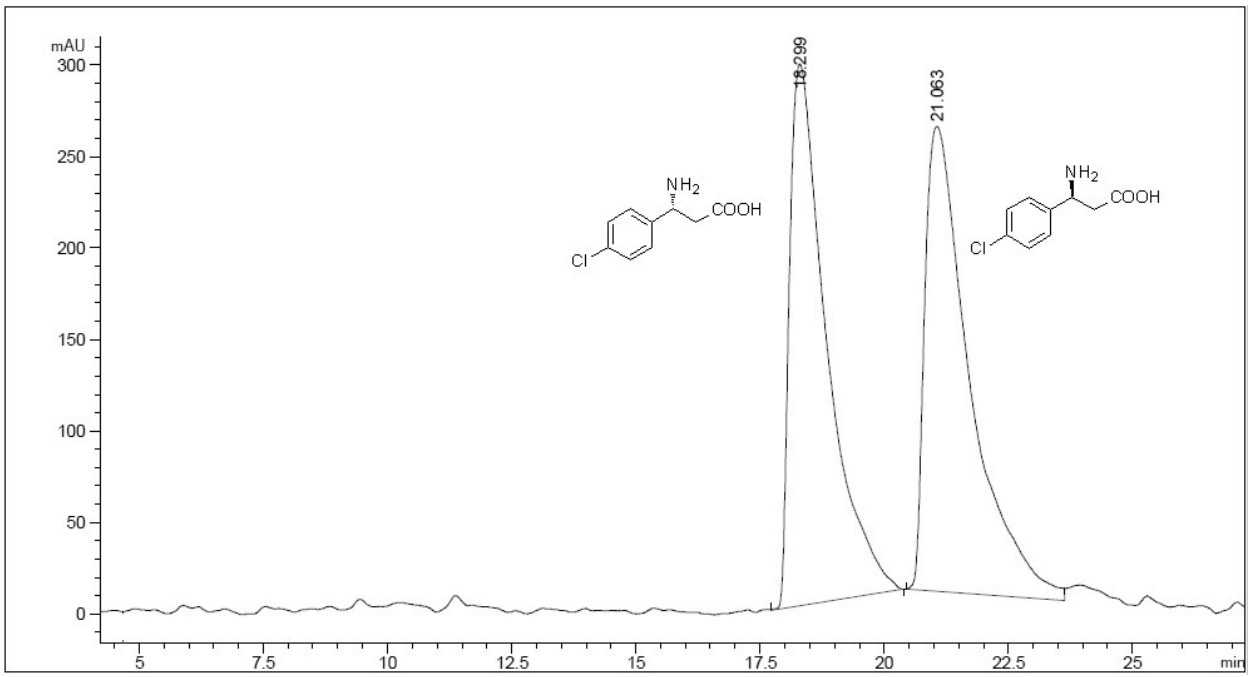
Products from (±)-3-chloro-α-phenylalanine (*rac*-**1h**) by *Pa*PAM after 20 h [Crownpak CR-I(+)column]



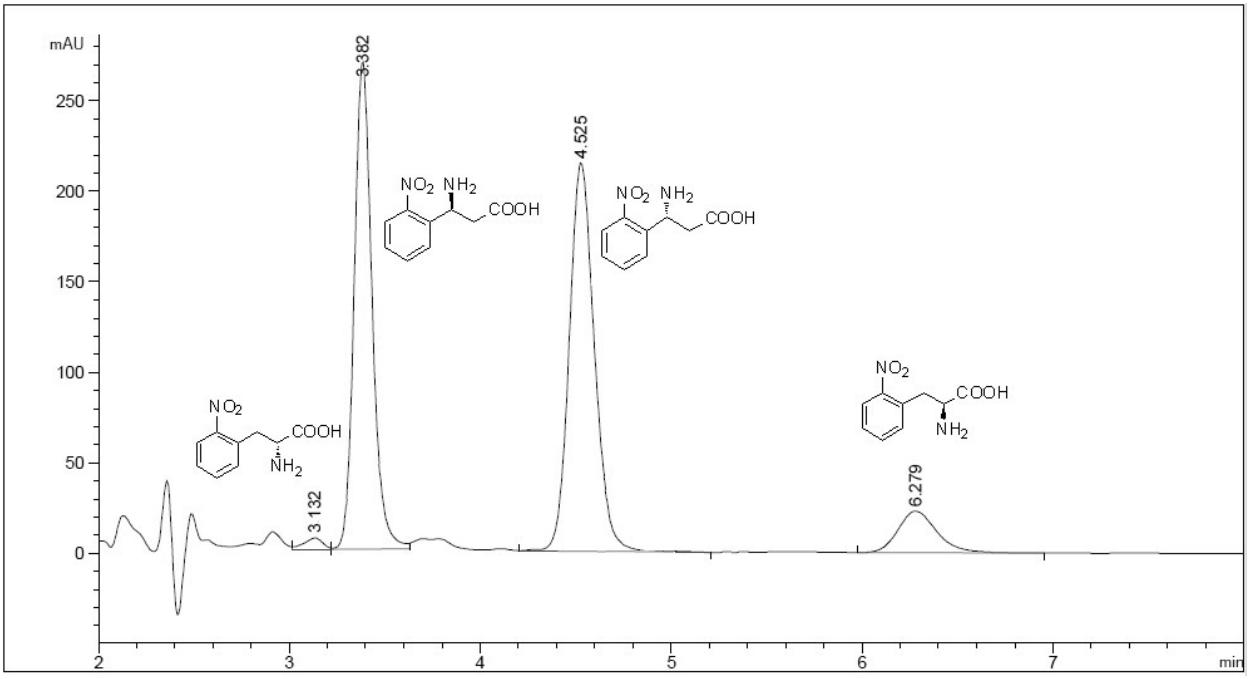
Enantioseparation of authentic (±)-3-chloro-α-phenylalanine (*rac*-**1f**) and (±)-3-chloro-β-phenylalanine (*rac*-**2h**) on Crownpak CR-I(+) column



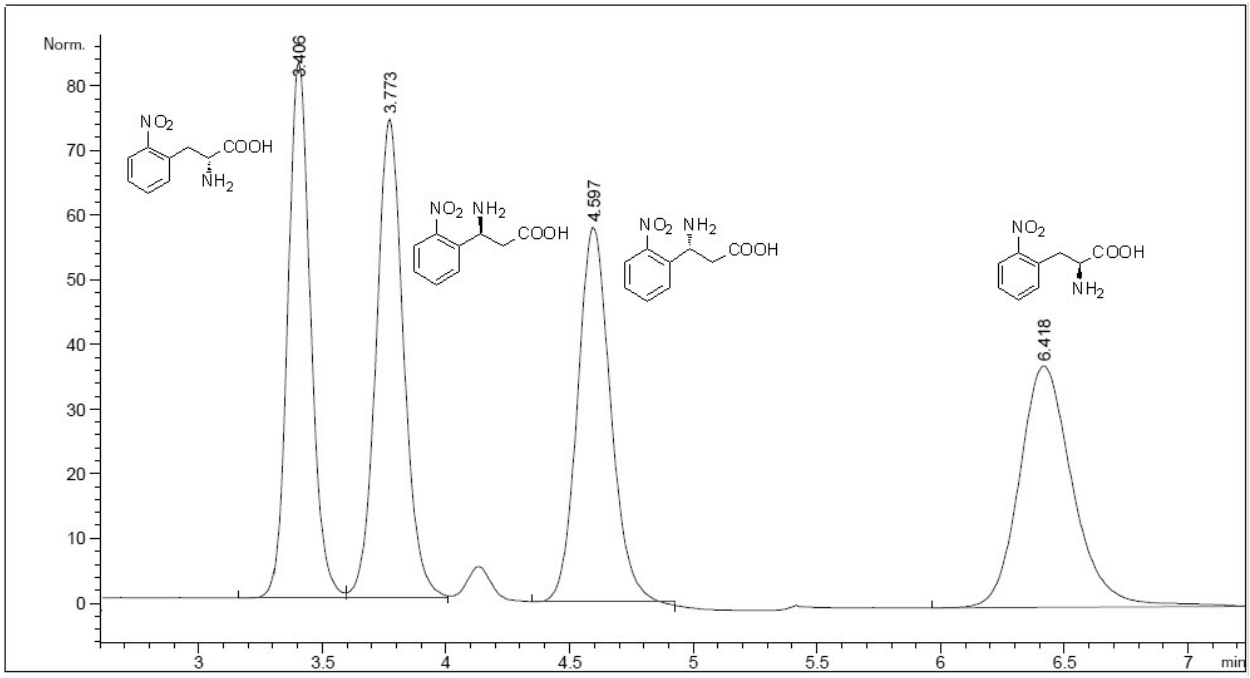
Products from (±)-4-chloro-α-phenylalanine (*rac*-**1i**) by *Pa*PAM after 20 h [Chiralpak ZWIX (+) column]



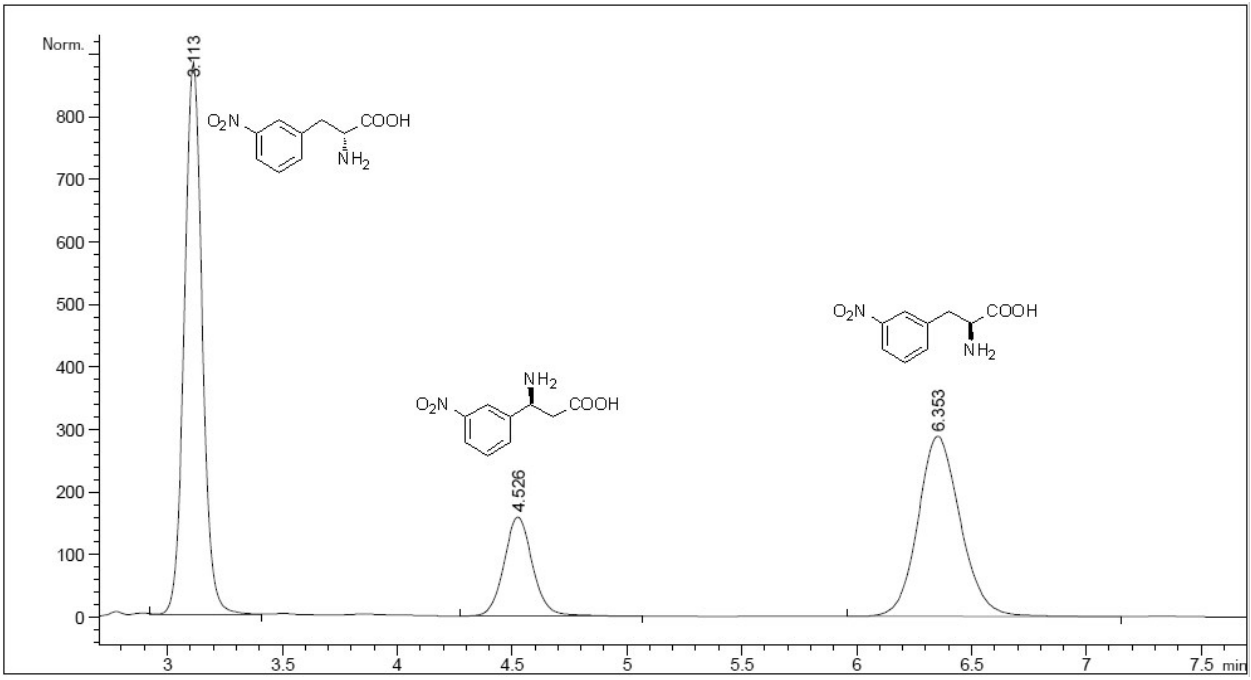
Enantioseparation of authentic (±)-4-chloro-α-phenylalanine (*rac*-**2i**) on Chiralpak ZWIX(+) column



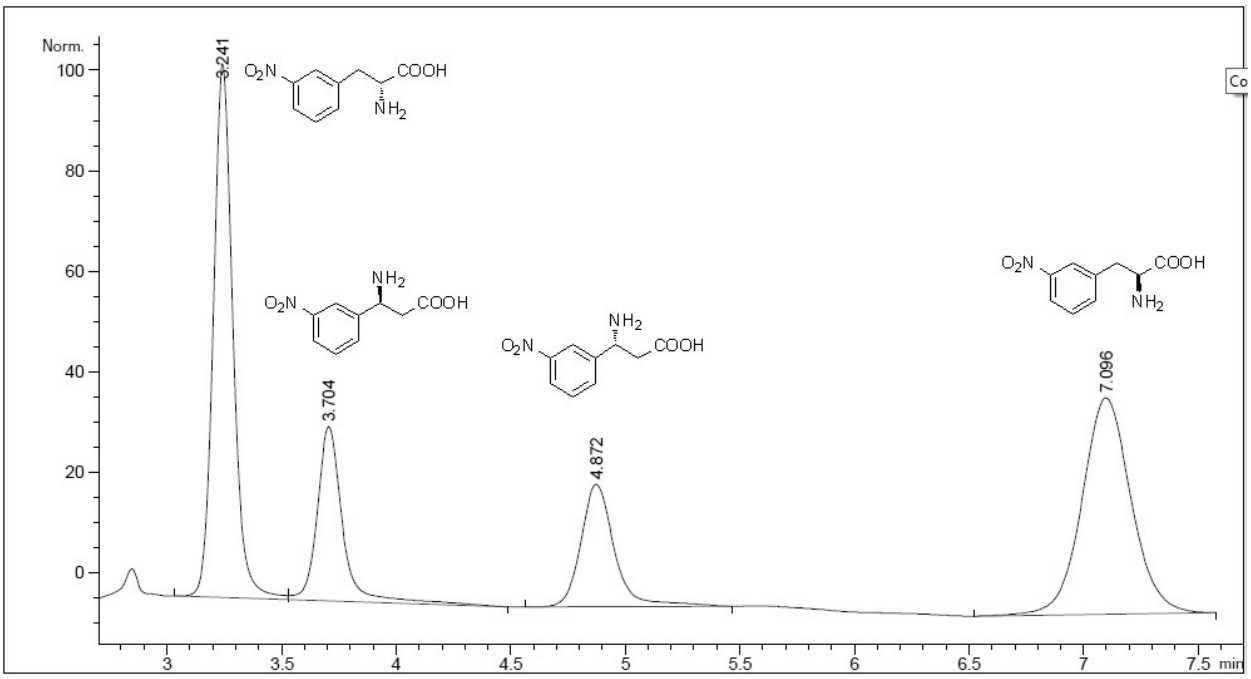
Products from (±)-4-nitro-β-phenylalanine (*rac*-**2j**) by *Pa*PAM after 20 h [Crownpak CR-I(+)column]



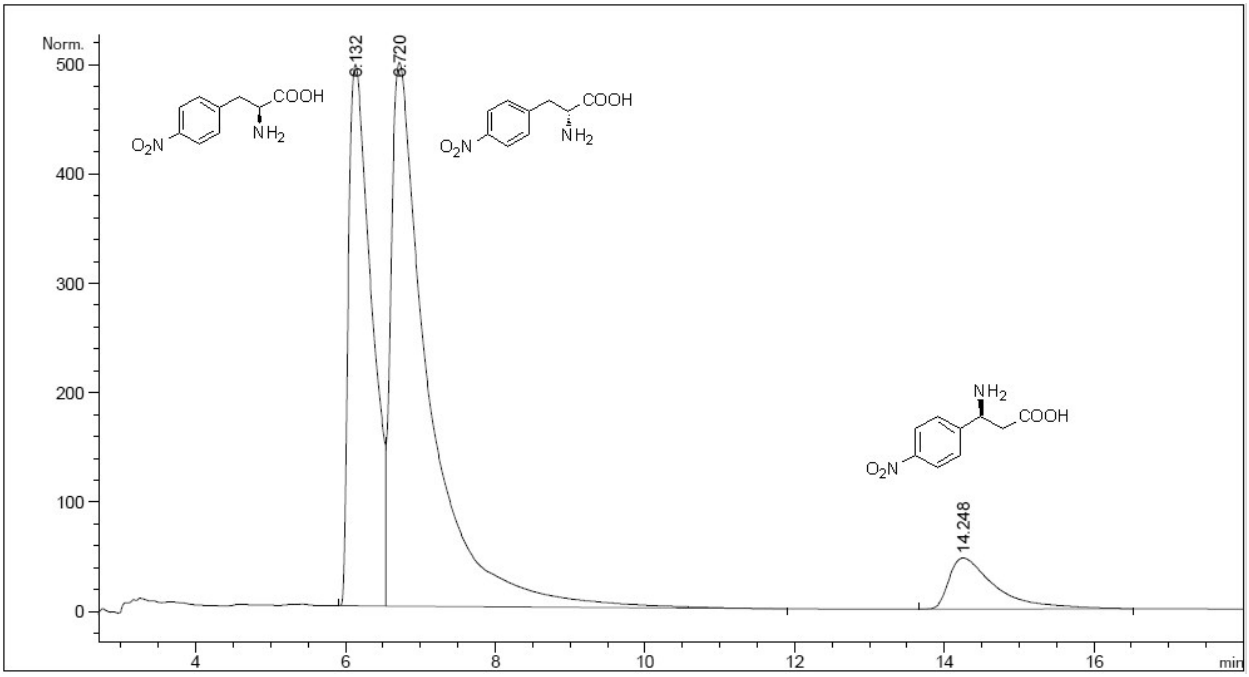
Enantioseparation of authentic (±)-4-nitro-α-phenylalanine (*rac*-**1j**)
and (±)-4-nitro-β-phenylalanine (*rac*-**2j**) onCrownpak CR-I(+) column



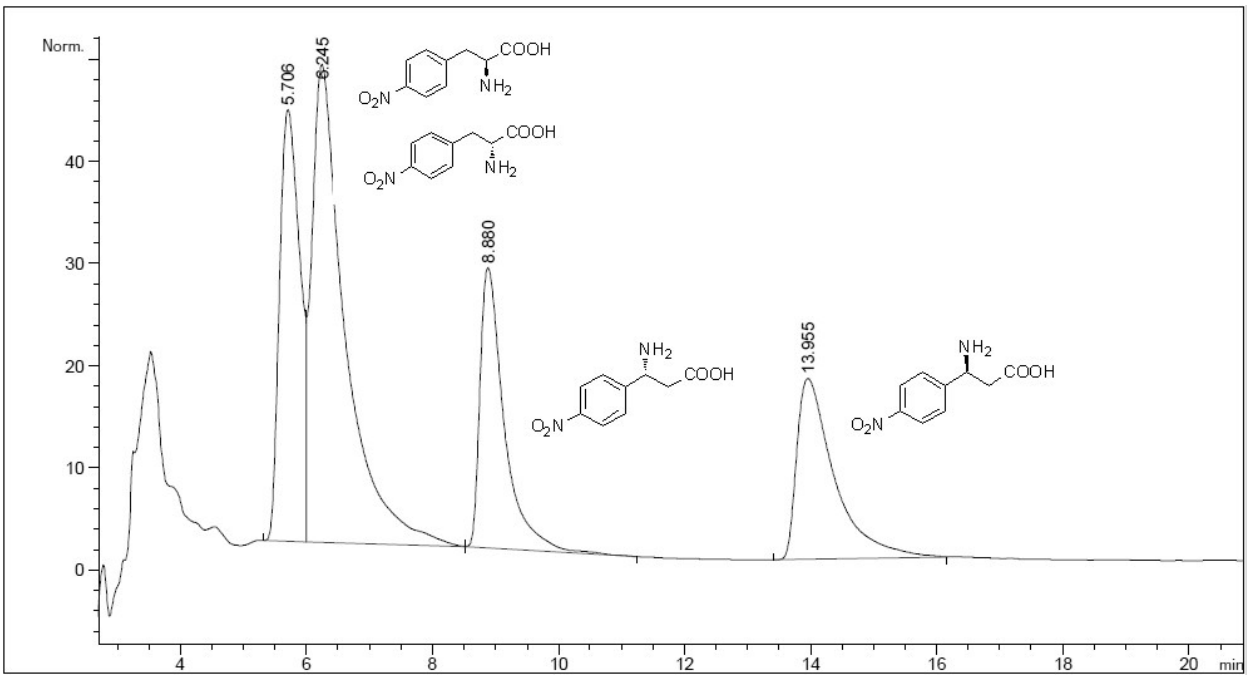
Products from (±)-3-nitro-α-phenylalanine (*rac*-**1k**) by *Pa*PAM after 20 h
[Crownpak CR-I(+) column]



Enantioseparation of authentic (±)-3-nitro-α-phenylalanine (*rac*-**1k**)
and (±)-3-nitro-β-phenylalanine (*rac*-**2k**) on Crownpak CR-I(+) column



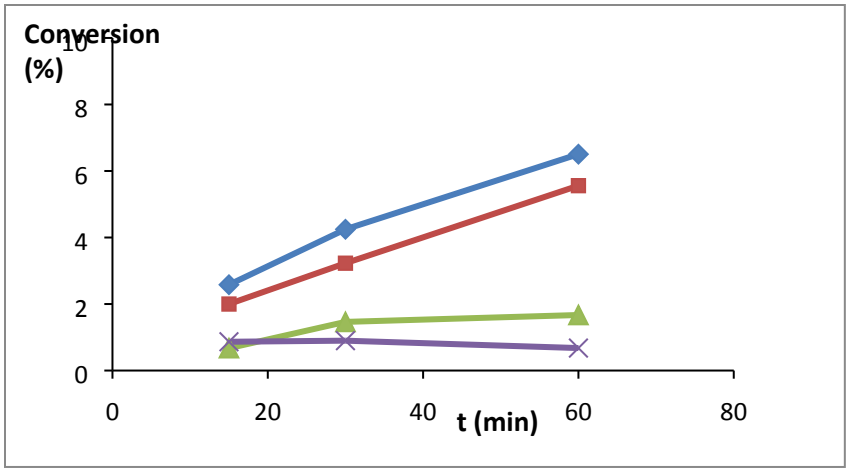
Products from (±)-4-nitro-α-phenylalanine (*rac*-**1l**) catalyzed by *Pa*PAM, after 20 h
[Chiralpack ZWIX(+) column]



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 *Pa*PAM 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**, ①); (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 *Pa*PAM enzyme

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

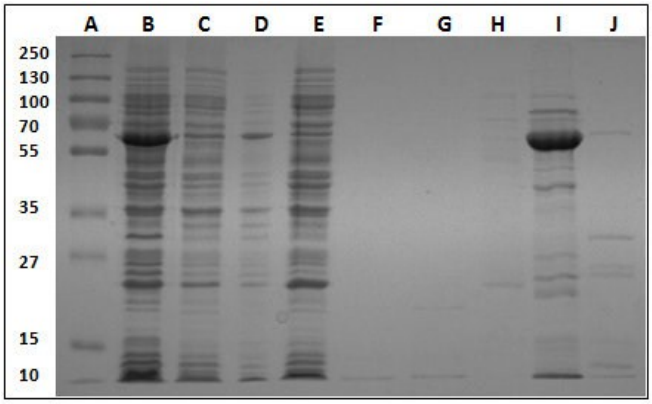


Figure S1. Purificationof *Pa*PAM with Ni-NTA. Lane **A**: protein ladder, Lane **B**: supernatant, Lane **C**:flow through, Lane **D**: pellet, Lane **E**: LS1, Lane **F**: HS, Lane **G**:LS2, Lane **H**: 20 mM Imidazole, Lane **I**: 350 mM Imidazole, Lane **J**: 1 mM Imidazole. The samples were prepared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