STUDIA UBB CHEMIA, LVI, 2, 2011 (p. 155 - 166)

# BIOCATALYTIC PREPARATION OF ENANTIOPURE 2-AMINO-3-(5-ARYL-FURAN-2-YL)PROPANOIC ACIDS

**LAURA ANCUȚA POP***

**ABSTRACT.** The synthesis of both enantiomers of 2-amino-3-(5-aryl-furan-2-yl)propanoic acids acids is presented by two kinetic resolution processes: the enantiomer selective Acylase I mediated hydrolysis of *N*-acetylated amino acids and the enantiomer selective baker's yeast catalysed ester hydrolysis of the double protected amino acids.

***Keywords:*** *Kinetic resolution, Acylase I, baker's yeast, enantioselectivity, amino acids*

## INTRODUCTION

Unnatural amino acids are useful building blocks for the asymmetric synthesis of several anticancer and antiviral compounds[1].They can increase the activity, stability, bioavailability and binding specificity of the peptides and proteins in which they are inserted[2]. Some of them are already important drugs in the treatment of Parkinson's disease, arthritis and high blood pressure. Due to their nature, they can also be used as templates for asymmetric catalysis[3].

While fermentation is widely used for the production of natural α-amino acids, for the synthesis of unnatural α-amino acids several chemical[1-2,4] or chemoenzymatic[5] methods were developed. The chemical asymmetric syntheses are generally multistep procedures requiring special reagents, tedious purification steps and in most of the cases the global yields are low. Several synthetic enzymatic procedures involving aminomutases[6], ammonia lyases[7], transaminases[8] or hydrolases as biocatalysts were developed for the efficient preparative scale synthesis of both L- and D-unnatural amino acids.

Hydrolases are one of the most important biocatalysts for the kinetic resolution of various heterocyclic racemic substrates, including secondary alcohols[9], ethane-1,2-diols[10], α-cyanohydrins[11] and β-hydroxy acids[12].

On one hand, Acylase I (Aminoacylase I, *N*-acyl- L-amino acid amidohydrolase, E.C. 3.5.1.14) as a readily available inexpensive enzyme with relaxed substrate specificity is widely used for the synthesis of L-amino

* *Universitatea Babeş-Bolyai, Facultatea de Chimie şi Inginerie Chimică, Str. Kogălniceanu, Nr. 1, RO-400084 Cluj-Napoca, Romania, lisprava@chem.ubbcluj.ro*

acids[13]. It is also preferred for the industrial preparation of enatiopure L-amino acid by the kinetic resolution of corresponding racemic *N*-acyl derivatives[14].

On the other hand, due to its low cost and broad substrate acceptability, baker's yeast is an useful catalysts for organic synthesis. Since it is a non-pathogenic microorganism, is simple to grow at any scale, is accessible in stable dried form and it can be bought from a local store, baker's yeast was widely used for the regio-, chemo- and stereoselective reduction of various kind of carbonylic compounds[15]. Enantiomerically enriched heteroaryl ethanols[16] or non chiral heteroayl methanols[17] were synthesized with high yields by cellular reduction. Baker's yeast reduction of hydroxymethyl ketones and acetoxymethyl ketones proved to be useful for production of both opposite enantiomeric forms of 1,2-diols with 100% theoretical yield[18]. The kinetic resolution of racemic phenothiazine-based aldehyde sulfoxides by baker's yeast mediated enantioselective reduction was also reported[19].

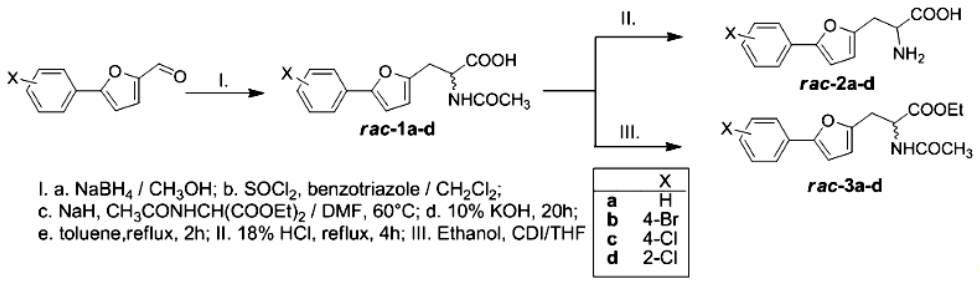
Beside the reductive capacity, the hydrolytic ability of the baker's yeast cells is also known. Some reports already presented the baker's yeast mediated stereoselective hydrolysis of the ester moiety of natural *N*-acetyl amino acid esters[20].

Herein we describe the synthesis of both optically pure enantiomers of various 2-amino-3-(5-aryl-furan-2-yl)propanoic acids by enzymatic kinetic resolution of the racemic substrates using as biocatalyst two enzymes acting on different functional groups: Acylase I and esterases from baker's yeast.

## RESULTS AND DISCUSSION

### *1. Chemical synthesis*

The racemic amino acids and their derivatives (*N*-acetyl amino acids and *N*-acetyl amino esters) were synthesized by a known procedure starting from their corresponding aldehydes[21] (Scheme 1).




*Scheme 1*

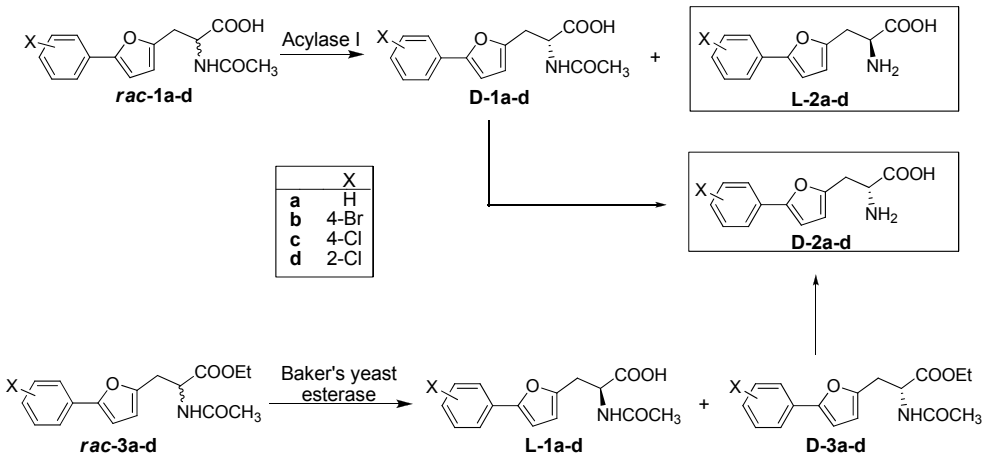
*2. Enzyme catalysed synthesis*

For the synthesis of enantiopure **L**-alanines two distinct enzymatic kinetic resolution methods were tested.

In the first method, the racemic *N*-acetyl alanines were hydrolyzed in presence of Acylase I at a pH 7-8 obtaining the optically enriched L-2-amino-(5-aryl-furan-2-yl)propanoic acids **L-2a-d** and their *N*-acetylated counterparts, D-2-acetamido-3-((5-aryl-furan-2-yl) propanoic acids **D-1a-d** (Scheme 2).

In the second method, exploiting the stereoselective esterase activity of the baker's yeast cells, the racemic ethyl 2-acetamido-3-(5-phenyl-furan-2-yl)propanoates (*rac*-**3a-d**) were transformed into D-ethyl-2-acetamido-3-(5-phenyl-furan-2-yl)propanoates **D-3a-d** and L-2-acetamido-3-((5-phenyl-furan-2-yl) propanoic acids **L-1a-d** (Scheme 2).

The obtained D-2-acetamido-3-((5-aryl-furan-2-yl) propanoic acids **D-1a-d** and D-ethyl-2-acetamido-3-(5-phenyl-furan-2-yl)propanoates **D-3a-d** can be further hydrolyzed into the corresponding D-alanines in accordance with the known procedure which states that *N*-acetyl amino acid esters can be deprotected by the sequential hydrolysis of the ester in weak basic medium followed by the acidolysis of the amidic bond (Scheme 2).




*Scheme 2*

In order to find the optimal conditions (high reaction rates and high stereoselectivity) first the analytical scale experiments were performed. The optimal Aminoacylase I catalyzed transformation occurred in water, pH 7-8 in presence of $CoCl_2 \times 6H_2O$ as additive. The reactions were stopped after 24 h, at approx. 50% conversion. Best result for the baker's yeast mediated enantiomer selective hydrolysis were found when reactions were performed without additives in presence of non fermenting water suspended cells.

Stopping the reaction by extracting the products with dichloromethane at approx. 50% conversion, highly enatiomerically enriched products (ee 99%) were formed. With these results in our hands, the preparative scale experiments were performed. All the dilutions, substrate-biocatalysts *ratio* and reaction conditions were the same with those found as optimal for the analytical scale reactions and no significant differences for yield and enantiomeric excesses of the products were observed, compared to those found for the analytical scale biotransformations. The enantiomeric excess, optical rotation and yields for each individual enantiomer is presented in Table 1.

**Table 1.** Enantiomeric excess, optically rotatory power and yield of each compound from the preparative scale experiments

| Compound | ee% | $[\alpha]_D$ | Yield (%) |
|---|---|---|---|
| **L-1a** | 99 | -25.8[a] | 46.3 |
| **L-1b** | 99 | -12.9[a] | 49.1 |
| **L-1c** | 99 | -21.8[a] | 44.2 |
| **L-1d** | 99 | -20.8[a] | 40.3 |
| **L-2a** | 99 | +28.2[b] | 38.5 |
| **L-2b** | 99 | +10.8[b] | 45.2 |
| **L-2c** | 99 | +17.8[b] | 42.8 |
| **L-2d** | 99 | +16.7[b] | 44.5 |
| **D-3a** | 99 | +17.2[c] | 40.5 |
| **D-3b** | 99 | +19.9[c] | 45.3 |
| **D-3c** | 99 | +18.3[c] | 43.4 |
| **D-3d** | 98 | +15.5[c] | 44.9 |

[a-] in MeOH; [b] in $CH_3COOH$; [c] in $CHCl_3$,

## CONCLUSIONS

The present work describes the usability of the enzymatic kinetic resolution process for obtaining both highly enantiomerically enriched 2-amino-3-((5-aryl-furan-2-yl)propanoic acids using two kind of biocatalysts (Acylase I from *Aspergillus meleus* and esterases from baker's yeast) showing the same enantio-preference. Besides the high stereoselectivity, both procedures provide good yields for the pure isolated products.

## EXPERIMENTAL SECTION

*1. Analytical methods*

The $^1$H- and $^{13}$C-NMR spectra were recorded on a Bruker Advance spectrometer operating at 300 and 75 MHz, respectively at 25 °C in DMSO-$d_6$ or $CDCl_3$. Electron impact mass spectra (EI-MS) were taken on a LCQ advantage

by ThermoFisher spectrometer operating at 1950-2050V, the samples were dissolved in MeOH/$H_2O$=50/50 (v/v), or MeOH/$CH_3CN$=50/50.

High Performance Liquid Chromatography (HPLC) analyses were conducted with a HP 1200 instrument using an Astec Chirobiotic-Tag column (4.6×250 mm) and a mixture of methanol and TEAA buffer, pH 4.1 (80:20, v/v) as eluent for enantiomeric separation of *rac*-**1,2 a-d** and a Chiralpak IA column (4.6×250 mm) and a mixture of hexane and 2-propanol (90:10, v/v) as eluent for enantiomeric separation of *rac*-**3a,c,d**, and a Chiralpak IC column (4.6x250mm) and a mixture of *n*-hexane: 2-propanol (90:10, v/v) as eluent for enantiomeric separation of *rac*-**3b** at 1 mL/min flow rate in all cases. For all chiral compounds, high resolution enantiomeric separation was performed. Retention times for **L-** and **D-1-3a-d** are presented in Table 2.

Thin Layer Chromatography (TLC) was carried out using Merck Kieselgel 60$F_{254}$ sheets. Spots were visualized by treatment with 5% ethanolic phosphomolybdic acid solution and heating. Preparative chromatographic separations were performed using column chromatography on Merck Kieselgel 60 (63-200 μm). Melting points were determined by hot plate method and are uncorrected. Optical rotations were determined on a Perkin-Elmer 201 polarimeter and $[\alpha]_D^{20}$ values are given in units of $10^{-1}$ deg $cm^2$ $g^{-1}$.

**Table 2.** Retention times of the enantiomers of *rac*-**1-3a-d**.

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | | $R_t$ **(min.)** | | | |
| **L-1a** | **D-1a** | **L-1b** | **D-1b** | **L-1c** | **D-1c** | **L-1d** | **D-1d** |
| 10.9 | 3.2 | 13.6 | 3.3 | 12.7 | 3.2 | 12.9 | 3.7 |
| **L-2a** | **D-2a** | **L-2b** | **D-2b** | **L-2c** | **D-2c** | **L-2d** | **D-2d** |
| 9.0 | 13.9 | 9.7 | 16.2 | 9.2 | 15.4 | 10.2 | 15.4 |
| **L-3a** | **D-3a** | **L-3b** | **D-3b** | **L-3c** | **D-3c** | **L-3d** | **D-3d** |
| 13.0 | 9.6 | 10.4 | 9.3 | 15.8 | 10.1 | 10.8 | 8.5 |

*2. Reagents and solvents*

All inorganic reagents and solvents were products of Aldrich or Fluka. All solvents were purified and dried by standard methods as required. Acylase I was purchased from Fluka and baker's yeast Budafok Ltd, Hungary was purchased from a local store.

*3. Chemical synthesis of racemic alanines and their derivatives*

*3.1. Synthesis of rac-2-acetamido-3-((5-aryl-furan-2-yl)propanoic acids rac-1a-d.* The racemic 2-acetamido-3-aryl-propanoic acids *rac*-**1a-d** were synthesized from the corresponding 5-phenylfuran-2-carbaldehydes prepared using a known procedure[21]. Aldehydes were transformed in chloromethylene derivatives *via* the corresponding alcohols. Further the coupling of the halogenated compounds with diethyl-2-acetamido-malonate afforded the diethyl-

2-acetamido-2-((aryl-furan-2-yl)-methyl)malonates which generate the diethyl esters by mild basic hydrolysis. These compounds were further decarboxylated in boiling toluene into the corresponding 2-acetamido-3-(arylfuran-2-yl)propanoic acids *rac*-**1a-d**.

*3.1.1. rac-2-Acetamido-3-(5-phenylfuran-2yl)propanoic acid (rac-1a)* Yield 47%, m.p. 197 °C; $^{1}$H-NMR (300 MHz, DMSO-$d_6$): δ = 1.83 (3H, s); 3.03 (1H, dd, *J*=8.47Hz, *J*=15.25Hz); 3.12(1H, dd, *J*=5.08Hz, *J*=15.25Hz); 4.52 (1H, ddd, *J*=5.27Hz, *J*=8.28Hz, *J*=8.1Hz), 6.26 (1H, d, *J*=3.3Hz), 6.81 (1H, d, *J*=3.3Hz); 7.24(2H, dd, *J*=7.65Hz, *J*=7.35Hz); 7.39(1H, dd, *J*=7.35Hz, *J*=7.65Hz); 7.26(2H, d, *J*=7.35Hz); 8.31(1H, d, *J*=8.1Hz); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): 22.40; 29.90; 51.13; 106.56; 109.46; 123.14; 127.13; 128.82; 130.48, 151,48; 151.98; 169.33; 172.62; ESI$^{+}$-MS: m/z: 296.0890 [M+Na$^{+}$].

*3.1.2. rac-2-Acetamido-3-(5-(4-bromophenyl)furan-2-yl)propanoic acid (rac-1b)* Yield 54%, m.p. 194°C; $^{1}$H-NMR: (300 MHz, DMSO-$d_6$): δ = 1.83 (3H, s); 2.69 (1H, dd, *J*=8.57Hz, *J*=15.30Hz); 2.78 (1H, dd, *J*=5.08Hz, *J*=15.30Hz); 4.51 (1H, ddd, *J*=5.27Hz, *J*=8.28Hz, *J*=8.1Hz); 6.27 (1H, d, *J*=3.3Hz); 6.88 (1H, d, *J*=3.3Hz); 7.58-7.95 (4H, m); 8.29(1H, d, *J*=8.1Hz); 12.84(1H, s); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): 22,37; 29.87; 51.03; 107.49; 109,66; 119.90; 125.08; 129.63; 131.74; 150.88; 151.99; 169.28; 172.57; ESI$^{-}$-MS: m/z (abundance): 350.0027 (100%); 352.0017 (98.13%) [M-H$^{-}$].

*3.1.3. rac-2-Acetamido-3-(5-(4-chlorophenyl) furan-2-yl)propanoic acid (rac-1c)*. Yield 43%, m.p.196°C; $^{1}$H-NMR: (300 MHz, DMSO-$d_6$): δ = 1.82 (3H, s); 3.02 (1H, dd, *J*=8.57Hz, *J*=15.30Hz); 3.11(1H, dd, *J*=8.57Hz, *J*=15.30Hz); 4.48 (1H, ddd, *J*=5.27Hz, *J*=8.28Hz, *J*=8.1Hz); 6.27 (1H,d, *J*=3.2Hz); 6.87 (1H,d, *J*=3.2Hz); 7.46 (2H,d, *J*=8.47Hz); 7.66 (2H,d, *J*=8.47Hz); 8.31 (1H,d, *J*=7.72Hz); $^{13}$C-NMR (75 MHz, DMSO- $d_6$): 22.39; 29.88; 51.09; 107.42; 109.66; 124.83; 128.88; 129.33; 131.41; 150.87; 151.97; 169.34; 172.59; ESI$^{+}$-MS: m/z (abundance): 330.0506 (100%); 332.0494 (34.51%) [M+Na$^{+}$].

*3.1.4. rac-2-Acetamido-3-(5-(2-chlorophenyl) furan-2-yl)propanoic acid (rac-1d)*. Yield 36%, m.p. 130°C; $^{1}$H-NMR: (300 MHz, DMSO-$d_6$): δ = 1.82 (3H, s); 3.04 (1H, dd, *J*=8.47Hz, *J*=15.25Hz); 3.15 (1H, dd, *J*=4.99Hz, *J*=15.25Hz); 4.51 (1H, ddd, *J*=5.27Hz, *J*=8.28Hz, *J*=8.1Hz), 6.32 (1H, d, *J*=3.3Hz); 7.01 (1H, d, *J*=3.3Hz); 7.26 (1H, dd, *J*=7.53Hz, *J*=1.2Hz ); 7.38 (1H, dd, *J*=7.53Hz, *J*=8.1Hz); 7.43 (1H, d, *J*=7.91Hz); 7.81 (1H, d, *J*=7.91Hz); 8.23 (1H, d, J=7.91Hz); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): 22.49; 30.00; 51.38; 109.45; 112.00; 127.54; 128.44; 128.53; 128.73; 130.71; 148.05; 152.30; 169.25; 172.80; ESI$^{+}$-MS: m/z (abundance): 330.0515 (100%); 332.0501 (35.72%) [M+Na$^{+}$].

*3.2. Synthesis of rac-2-amino-3-((5-aryl-furan-2-yl)propanoic acids (rac-2a-d)*. *rac*-2-acetamido-3-aryl-propanoic acid *rac*-**1a-d** (1 g) was suspended in half concentrated HCl (10 mL) and the mixtures was refluxed for 4 h. After that the solvent was removed *in vacuo*. The crude product was washed with diethylether and dried yielding the pure product.

*3.2.1. rac-2-Amino-3-(5-phenylfuran-2-yl)propanoic acid (rac-2a).* Yield 61%, m.p.= 170°C. $^1$H-NMR: (300 MHz, DMSO-$d_6$): δ = 3.24 (2H, dd, *J*=5.7Hz, *J*=7.5Hz); 4.14 (1H, t, *J*=5.4Hz); 6.46 (1H,d, *J*=2.7Hz); 6.89 (1H, d, *J*=2.7Hz); 7.52-7.92(5H, m); 8.55(3H, s, $NH_3^+$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): *δ* = 28.5; 50.9; 106.6; 111.0; 123.7; 127.9; 128.6; 130.3; 153.5; 153.4; 170.14; HRMS ESI$^+$: m/z: 232.1087 [M+H$^+$].

*3.2.2. rac-2-Amino-3-(5-(4-bromophenyl)furan-2-yl)propanoic acid (rac-2b).* Yield 68%, m.p.=175°C. $^1$H-NMR: (300 MHz, DMSO-$d_6$): δ = 3.22 (2H,dd, *J*=5.7Hz, *J*=7.5Hz); 4.14 (1H, t, *J*=5.4Hz); 6.46 (1H,d, *J*=3Hz); 6.87 (1H,d, *J*=3Hz); 7.72 (2H, d, *J*=8.4Hz); 7.89 (2H,d, *J*=8.4Hz ); 8.45 (3H, s, $NH_3^+$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): *δ* = 28.5; 50.8; 107.4; 111.2; 120.0; 125.2; 129.4; 131.7; 149.0; 151.5; 169.9; HRMS ESI$^+$: m/z (abundance): 310.0183 (100%); 312.0176 (99.12%) [M+H$^+$].

*3.2.3. rac-2-Amino-3-(5-(4-chlorophenyl)furan-2-yl)propanoic acid (rac-2c).* Yield 60%, m.p.=180°C. $^1$H-NMR: (300 MHz, DMSO-$d_6$): δ = 3.23 (2H,dd, *J*=5.7Hz, *J*=7.5Hz); 4.13(1H, t, *J*=5.4Hz); 6.65(1H,d, *J*=3Hz); 6.95 (1H,d, *J*=3Hz); 7.58 (2H,d, *J*=8.7Hz ); 7.97 (2H,d, *J*=8.7Hz); 8.54 (3H, s, $NH_3^+$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): *δ* = 28.4; 50.8; 108.3; 111.2; 125.0; 128.8; 129.8; 135,0; 152.5; 154.7; 170.1; HSMS ESI$^-$ : m/z (abundance): 282.0521 (100%), 284.508 (35.66%) [M+OH$^-$].

*3.2.4. rac-2-Amino-3-(5-(2-chlorophenyl)furan-yl)propanoic acid (rac-2d).* Yield 65%, m.p.=210°C. $^1$H-NMR: (300 MHz, DMSO-$d_6$): δ = 3.35 (2H,dd, *J*=5.7Hz, *J*=7.5Hz); 4.20 (1H, t, *J*=5.4Hz); 6.46 (1H,d, *J*=2.7Hz); 7.04 (1H,d, *J*=3Hz); 7.29-7.84 (4H,m); 8.72(3H, s, $NH_3^+$); $^{13}$C-NMR (75 MHz, DMSO-$d_6$): 28.60; 51.01; 111.23; 112.07; 127.55; 127.94; 128.38; 128.65; 128.73; 130.72; 148.89; 149.07; 170.01; HRMS ESI$^+$: m/z (abundance): 266.0579 (100%); 268.0549 (33.02%) [M+H$^+$]; HRMS ESI$^-$: m/z (abundance):264.0438(100%); 266.0415(34.41%) [M-H$^-$]

*3.3. Synthesis of racemic ethyl 2-acetamido-3-(5-aryl-furan-2-yl) propanoates rac-3a-d.* Into a solution of *N,N'*-carbonyldiimidazole (90 mg, 0.55 mmol) and *rac*-2-acetamido-3-((5-phenyl-furan-2-yl)propanoic acid *rac*-**1a-d** (0.5 mmol) in anhydrous THF (2.5 mL), ethanol (45 mg, 56 µL, 0.75 mmol) was added in one portion at room temperature. After the reaction was completed (checked by TLC), the solvent was distilled off *in vacuo* and the crude product was purified by column chromatography on silica gel using as eluent dichloromethane:acetone (90:10, v/v).

*3.3.1. rac-ethyl 2-acetamido-3-(5-phenylfuran-2-yl)propanoate (rac-3a).* Yield 24.58%. $^1$H-NMR: (300 MHz, $CDCl_3$): δ = 1.17 (3H, t, *J*=8.4Hz); 1.92 (3H, s); 3.15 (1H, dd, *J*=4.8Hz, *J*=2.63Hz); 3.17( 1H, dd, *J*=3.01Hz, *J*=4.8Hz); 4.15 (2H, q, J=8.4Hz); 4.76 (1H, dd, *J*=5.4Hz, *J*=6.15Hz,); 6.05 (1H, d, *J*=2.4Hz); 6.43 (1H, dd, *J*=2.4Hz); 7.16 (2H, dd, *J*=7.8Hz, *J*=7.8Hz); 7.28 (2H, dd, *J*=7.8Hz, *J*=6.9Hz); 7.45(1H, d, *J*=7.8Hz); 7.50 (1H, s). $^{13}$C-NMR (75 MHz,

$CDCl_3$): 14.30; 23.34; 30.92; 51.65; 61.93; 105.88; 110.27; 123.59; 127.38; 128.83; 130.86; 150.16; 153.65; 169.88; 171.43; $ESI^+$-MS: m/z: 324.1194 [$M+Na^+$]; 365.1459 [$M+CH_3CN+Na^+$].

*3.3.2. rac-ethyl 2-acetamido-3-(5-(4-bromophenyl) furan-2-yl)propanoate (rac-3b).* Yield 22%. $^1$H-NMR: (300 MHz, $CDCl_3$): δ = 1.24 (3H, t, *J*=7.05Hz); 2.05 (3H, s); 3.23 (1H, dd, *J*=2.4Hz, *J*=4.8Hz); 3.51(1H, dd, *J*=3Hz, *J*=4.8Hz); 4.18 (2H, q, J=7.1Hz ), 4.83 (1H, ddd, *J*=5.4Hz, *J*=7.5Hz, *J*=5.4Hz); 6.14 (1H, d, *J*=3Hz); 6.52 (1H, d, *J*=3Hz); 7.40 (2H, d, *J*=8.7Hz); 7.46(2H,d, *J*=8.7Hz); 7.85 (1H, s); $^{13}$C-NMR (75 MHz, $CDCl_3$): 14.28; 23.30; 30.93; 51.55; 61.92; 106.48; 110.37; 121.05; 125.06; 129.75; 131.94; 150.61; 152.54; 169.80; 171.39; $ESI^+$-MS: m/z (abundance): 443.0590 (100%); 445.0573 (99.95%) [$M+CH_3CN+Na^+$]; 402.0319 (57.38%); 404.0296 (58.22%) [$M+Na^+$].

*3.3.3. rac-ethyl 2-acetamido-3-(5-(4-chlorophenyl) furan-2-yl)propanoate (rac-3c).* Yield 20%. $^1$H-NMR: (300 MHz, $CDCl_3$): δ = 1.23 (3H, t, *J*=7.05Hz); 2.00 (3H, s); 3.23 (1H, dd, *J*=2.4Hz, *J*=4.8Hz); 3.24 (1H, dd, *J*=3Hz, *J*=4.8Hz) ; 4.18 (2H, q, *J*=7.1Hz ); 4.82(1H,ddd, *J*=5.27Hz, *J*=5.53Hz, *J*=7.5Hz); 6.14 (1H,d, *J*=2.4Hz ); 6.51 (1H,d, *J*=2.4Hz); 7.30 (2H,d, *J*=8.4Hz); 7.47 (2H,d, *J*=8.4Hz); 8.00 (1H,s); $^{13}$C-NMR (75 MHz, $CDCl_3$): 14.27; 23.28; 30.89; 51.59; 61.93; 106.36; 110.35; 121.97; 124.78; 129.08; 132.96; 150.51; 152.54; 169.94; 171.39; $ESI^+$-MS: m/z (abundance): 358.0831 (100%); 360.0807 (34.95%) [$M+Na^+$]; 399.1097 (100%); 401.1077 (35.57%) [$M+CH_3CN+Na^+$].

*3.3.4. rac-ethyl 2-acetamido-3-(5-(2-chlorophenyl) furan-2-yl)propanoate (rac-3d).* Yield 79.96%. $^1$H-NMR: (300 MHz, $CDCl_3$): δ = 1.24 (3H, t, *J*=6.15Hz); 2.02 (3H, s); 3.26 (1H,dd, *J*=2.4Hz, *J*=4.8Hz); 3.28(1H, dd, *J*=3Hz, *J*=4.8Hz); 4.00 (2H, q, *J*=6.2Hz); 4.18 (1H, ddd, *J*=5.27Hz, *J*=7.5Hz, *J*=5.53Hz); 5.29 (1H, d, *J*=3Hz); 6.20 (1H, d, *J*=3Hz); 7.17 (1H, d, *J*=7.8Hz); 7.29(2H, dd, *J*=7.8Hz, *J*=8.1Hz); 7.4( 1H, d, *J*=8.1Hz); 8.09(1H, s); $^{13}$C-NMR (75 MHz, $CDCl_3$): 14.27; 23.31; 30.84; 51.58; 61.97; 110.37; 111.83; 126.99; 127.71; 128.10; 129.19; 130.03; 130.88; 149.97; 150.31; 170.01; 171.38; $ESI^+$-MS: m/z (abundance): 358.0818 (100%); 360.0795 (32.5%) [$M+Na^+$].

*4. Enzymatic analytical scale reactions*

*4.1. Enzymatic kinetic resolution of racemic 2-acetamido-3-(5-aryl-furan-2-yl)propanoic acids (rac-1a-d) by Acylase I mediated hydrolysis.* *rac*-2-Acetamido-3-((5-aryl-furan-2-yl)propanoic acid (*rac*-**1a-d**) (0.5 mmol) was suspended in water (5mL). By adjusting the pH to 7.5- 8 with LiOH solution (1.25 M), the suspension merged into solution. Acylase I (2.5 units, 6 mg) and $CoCl_2 \times 6H_2O$ (2.5 mg, 0.02 mmol) were added and the reaction mixture was stirred at 37 °C, while by additions of LiOH solution (1.25 M), the pH of the solution was permanently kept between 7 and 8. After the completion of L-2-acetamido-3-arylfuryl-propanoic acid (**L-1a-d**) hydrolysis (approx. 24 h, checked by HPLC) the pH was adjusted to 1.5 with 5% HCl solution. The untransformed enantiopure D-2-acetamido-3-arylfuryl-propanoic acid (**D-1a-d**)

was filtered off and washed with ultrapure water (3×1 mL). The filtrate was heated with active charcoal (10 mg) to 50 °C for 1 min., cooled to room temperature, filtered, and the solvent was evaporated *in vacuo* to obtain the amino acid hydrochloride. The latest was dissolved in the minimal amount of water and the pH was adjusted to 6.5 to obtain **L-2a-d.**

*4.2. Enzymatic kinetic resolution of racemic ethyl 2-acetamido-3-(5-aryl-furan-2-yl)propanoates (rac-3a-d) by baker's yeast esterase mediated hydrolysis.* Baker's yeast (5g) was suspended in 50 ml water. After 1h, the *rac*-ethyl 2-acetamido-3-(5-phenyl-furan-2-yl)propanoates (*rac*-**3a-d**) (0.2 mmol) in 0.5 mL ethanol was added under stirring and left at room temperature for 48 h. The reaction mixture was centrifuged at 5000 rpm for 20 minutes. The supernatant was extracted with $CH_2Cl_2$ (3×30mL) and the organic layer was dried over $Na_2SO_4$, filtered and evaporated *in vacuo* affording the D-ethyl 2-acetamido-3-(5-phenyl-furan-2-yl)propanoates. The aqueous layer was concentrated in *vacuo*. The remained water trace from pure **L-1a-d** was eliminated in under high vacuum (freze drying).

*4.3. Chemical hydrolysis of D-acetamido-3-(5-aryl-furan-2-yl)propanoic acids (D-1a-d) and ethyl-D-2-acetamido-3-(5-aryl-furan-2-yl)propa-noates (D-3a-d).* D-2-acetamido-3-(5-aryl-furan-2-yl)propanoic acid **D-1a-d** (0.5 mmol) was suspended in half concentrated HCl (5 mL) and the mixture was refluxed for 4 h, cooled to room temperature, obtaining the product as a white precipitate, which was filtered, dried and finally washed with diethyl ether. The precipitate was resuspended in water, the pH was adjusted to 6.5, stirred for 30 min, filtered and dried at room temperature yielding the pure enantiomerically enriched **D-2a-d**.

The ethyl D-2-acetamido-3-(5-aryl-furan-2-yl)propanoates **D-3a-d** (1 mmol) was suspended into a vigorously stirred solution of sodium carbonate (0.053 g, 5 mmol) in water (8 mL) and the mixture was gently heated to reflux. After 2 h of heating, the solution was cooled to 5 °C and extracted with dichloromethane (3×10 mL). The aqueous layer was then acidified carefully with concentrated HCl solution. The deposited precipitate was filtered off and washed several times with cold water. The isolated D-2-acetamido-3-(5-aryl-furan-2-yl)propanoic acids **D-2a-d** were further hydrolyzed as described above.

**ACKNOWLEDGMENTS**

Laura Ancuța Pop wishes to thank for the financial support provided from programs co-financed by The SECTORAL OPERATIONAL PROGRAMME HUMAN RESOURCES DEVELOPMENT, Contract **POSDRU 6/1.5/S/3** – „Doctoral studies: through science towards society". Sincere thanks to Prof. Florin Dan Irimie and all the members of the Biochemistry and Biochemical Engineering Department of the Faculty of Chemistry and Chemical Engineering, Cluj Napoca, for all their help, support and guidance during this work.

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