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Tetrahedron: Asymmetry
Tetrahedron: *Asymmetry* 19 (2008) 500–511

Chemoenzymatic preparation of enantiopure L-benzofuranyl- and L-benzo[*b*]thiophenyl alanines

Paula Veronica Podea, Monica Ioana Toşa, Csaba Paizs and Florin Dan Irimie*

Department of Biochemistry and Biochemical Engineering, Babeş-Bolyai University, 400028-Arany János 11, Cluj-Napoca, Romania

Received 13 December 2007; accepted 23 January 2008

Abstract—Lipase mediated DKR followed by a chemical and an enzymatic hydrolytic step were combined for the synthesis of enantiopure L-benzofuranyl- and L-benzothienyl alanines.

1. Introduction

Both naturally occurring and unnatural α-arylalanines are frequent constituents of biological and pharmaceutical products. Several non-proteinogenic aminoacids as L-Dopa, L-homophenylalanine or D-2-naphthylalanine are already important pharmaceuticals,[1] while others such as D-phenylglycine, D-*para*-hydroxy-phenylglycine, and 2-thienyl-alanine are useful for the synthesis of certain drugs.[2] Many others serve as building blocks for modern drug discovery research.[3] The enantioselective synthesis of amino acids is an attractive goal. While L-natural aminoacids are obtained by fermentation or by applying natural enzymatic systems, for enantiopure unnatural aminoacid synthesis, chiral chemical catalysts or biocatalysts must be used. The use of ammonia lyases[4,5] or oxidoreductases[1] for the enantioselective asymmetrization of prochiral substrates provides the desired enantiopure amino acids with 100% theoretical yield. However, their use for biocatalysis is rather limited, as they show not only a strict reaction and enantioselectivity but are also specific for one or a few substrates. Therefore, enzymes with broad substrate selectivity, while maintaining other selectivities are the preferred biocatalysts. Hydrolases (acylases,[6] amidases[7] and hydantoinases, esterases,[8] nitrilases or nitrilhydratases[9]) fulfil these criteria and by kinetic resolution of different kinds of racemic substrates are the most important biocatalysts for enantioselective aminoacid synthesis. The main drawbacks of traditional resolution [maximum 50% theoretical yield and the decrease of enantiomeric excess (ee) with the conversion] can be overcome by dynamic kinetic resolution (DKR), whereby a racemic mixture is transformed to one of the enantiomers by in situ racemizing the less reactive enantiomer in the course of the process.

Under such conditions, the starting material is always racemic, allowing the maximum enantiopurity (determined by the E value for the corresponding kinetic resolution) at the theoretical zero conversion to the more reactive enantiomer to be maintained throughout the reaction. This is extremely important for an enzymatic reaction proceeding enantioselectively, since the ee of the product enantiomer tends to decrease with increasing conversion of kinetic resolution especially at E values less than 100.

It was shown that 2-substituted oxazol-5(4*H*)-ones, due to the low pK_a of the C-4 proton[10] and their high reactivity with different nucleophiles, are excellent substrates for enzymatic dynamic kinetic resolution. The enzymatic DKR of 4-substituted 2-phenyloxazol-5(4*H*)-one proved to be an efficient and elegant method for enantiopure L-phenyl-alanine[11] and L-*tert*-leucine synthesis.[12]

Herein, we report the chemoenzymatic synthesis of enantiopure L-2-amino-3-(heteroaryl)propanoic acids starting from the corresponding racemic 2-acetamido-3-(heteroaryl)propanoic acids. Enantiopure L-aminoacids (ee >99%) were produced with high yields (~80%) combining the enzymatic DKR of the corresponding oxazolones with a chemical and an enzymatic hydrolytic step.

* Corresponding author. Tel.: +40 264 593833; fax: +40 264 590818; e-mail: irimie@chem.ubbcluj.ro

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doi:10.1016/j.tetasy.2008.01.031

2. Results and discussion

2.1. Chemical synthesis

Commercially available benzofuran, benzo[*b*]thiophene and 1-(2-hydroxyphenyl)ethanone were used as general starting materials (Scheme 1).

The aldehyde group was selectively introduced at the 2-position of the benzofuran ring via a Vilsmeyer–Haack formylation of benzofuran with phosphoryl trichloride in DMF; however, benzofuran-2-carbaldehyde **4a** was not produced with high yield. The remaining benzofuran was easily recovered because the quantity of the side products was small. Benzo[*b*]thiophene was quantitatively transformed into benzo[*b*]thiophen-2-yl-lithium with butyl lithium at −78 °C, which was then easily transformed into the corresponding aldehyde **4b** with DMF.

The chloromethylation of benzo[*b*]thiophene takes place selectively at the 3-position, but the yield was relatively poor. The synthesis of benzofuran-3-carbaldehyde using benzofuran as a starting material was unsuccessful. For this reason, we chose a synthetic pathway which was based on an intramolecular reaction, yielding a 3 substituted benzofuran which could be transformed further into the desired aldehyde. 1-(2-Hydroxyphenyl)ethanone was transformed in (2-acetyl-phenoxy)acetic acid ethyl ester **1**, followed by the mild hydrolysis of the ester. The intramolecular cyclisation of (2-acetyl-phenoxy)acetic acid **2** followed by the decarboxylation of the intermediate allowed us to isolate 3-methyl-benzofuran **3** by in vacuo distillation. By the oxidation of the latest compound with SeO_2, benzofuran-3-carbaldehyde **4c** was formed (Scheme 1).

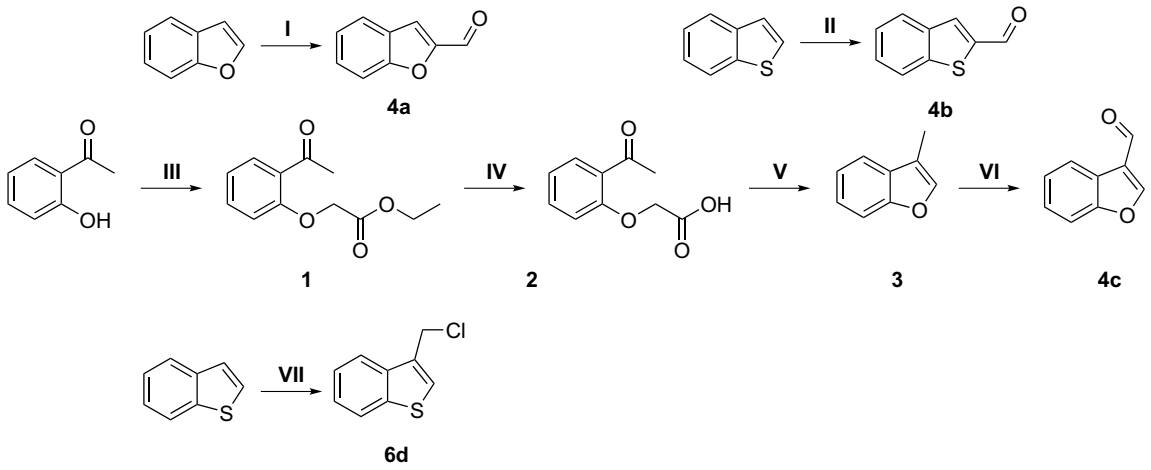
Aldehydes **4a**–**c** and 3-(chloromethyl)benzo[*b*]thiophene **6d** were used as starting materials for the synthesis of racemic amino acids and their derivatives (Scheme 2).

Aldehydes were then transformed into chloromethylene derivatives via the corresponding alcohols. Alcohols **5a**–**c** were synthesized by the reduction of the aldehydes with $NaBH_4$ in methanol, while halogenated compounds **6a**–**c** were prepared with thionylchloride in dichloromethane in the presence of 1*H*-benzo[*d*][1,2,3]triazole. The use of 1*H*-benzo[*d*][1,2,3]triazole overcame the destruction of acid-sensitive compounds, enhancing the yields of the reactions. The coupling of the halogenated compounds **6a**–**d** with diethyl-2-acetamido-malonate afforded the diethyl-2-acetamido-2-((heteroaryl)methyl)malonate **7a**–**d**. By mild basic hydrolysis of the diethyl esters formed, 2-acetamido-2-((heteroaryl)methyl)malonic acids **8a**–**d** were obtained, which were further decarboxylated in boiling toluene. In the final step, 2-acetamido-3-(heteroaryl)propanoic acids *rac*-**9a**–**d** were chemically transformed into the racemates involved in the stereoselective enzymatic reactions. Racemic **9a**–**d** were transformed with alcohols in dry tetrahydrofuran in the presence of carbonyl diimidazole into 2-acetamido-3-(heteroaryl)propanoic esters *rac*-**10a**–**d**; then in the presence of dicyclohexyl carbodiimide (DCC)[13] in dry dichloromethane into the corresponding *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-ones *rac*-**11a**–**d**; the protecting acetyl group of *rac*-**9a**–**d** was removed by acidic hydrolysis and the phenylalanine analogues formed *rac*-**12a**–**d** were isolated by precipitation at their isoelectric point.

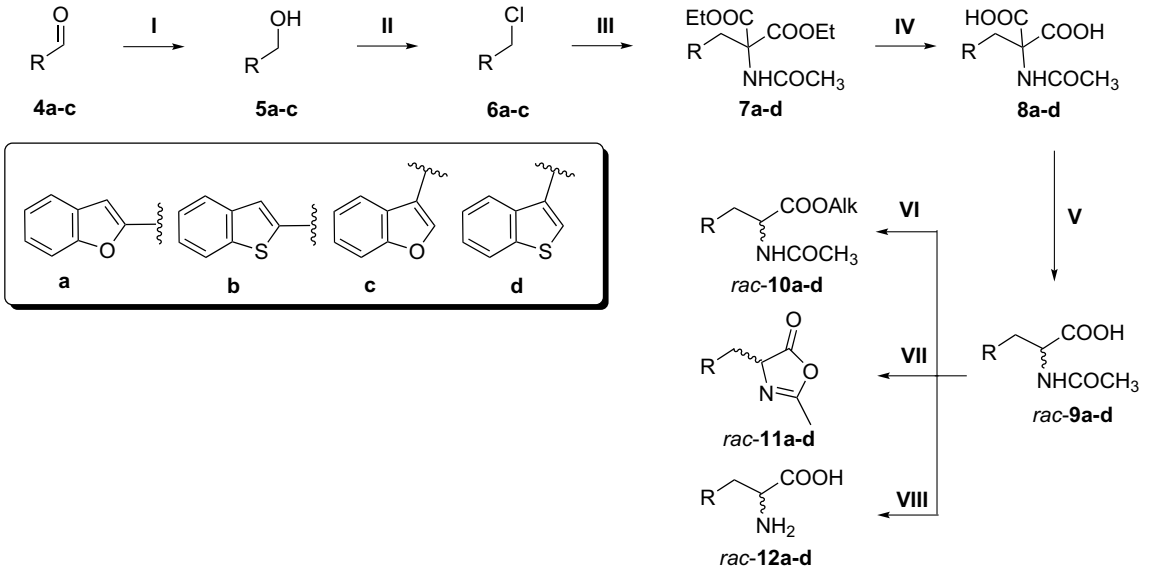
2.2. Enzymatic synthesis

To investigate the stereoselectivity and the conditions of DKR for the lipase mediated alcoholysis of 4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-ones *rac*-**11a**–**d**, the chromatographic separation of the enantiomers of the reaction counterparts *rac*-4-((heteroaryl)methyl)-2-methyloxazol-(4*H*)-ones *rac*-**11a**–**d** and *rac*-2-acetamido-3-(heteroaryl)propanoic esters *rac*-**9a**–**d** was first established (Fig. 1a).

A large number of commercially available lipases exhibit a high degree of substrate tolerance leading to products with various degrees of enantioselectivity.



Scheme 1. Synthesis of heteroaryl aldehydes. Reagents and conditions: (I) $POCl_3$/DMF; (II) (1) BuLi, −78 °C, 1 h, (2) DMF, −10 °C, 2 h; (III) $ClCH_2COOEt$, K_2CO_3/acetone; (IV) K_2CO_3/H_2O; (V) Ac_2O/AcO^-Na^+; (VI) SeO_2/dioxane, reflux; (VII) CH_2O/HCl, 60 °C.



Scheme 2. Synthesis of racemic heteroaryl alanines and their derivatives. Reagents and conditions: (I) $NaBH_4/CH_3OH$; (II) $SOCl_2$, benzotriazole/CH_2Cl_2; (III) NaH, $CH_3CONHCH(COOEt)_2$/DMF, 60 °C; (IV) 10% KOH, 20 h; (V) toluene/xylene, reflux, 2 h; (VI) alcohol, CDI/THF; (VII) DCC/CH_2Cl_2; (VIII) 18% HCl, reflux, 4 h.

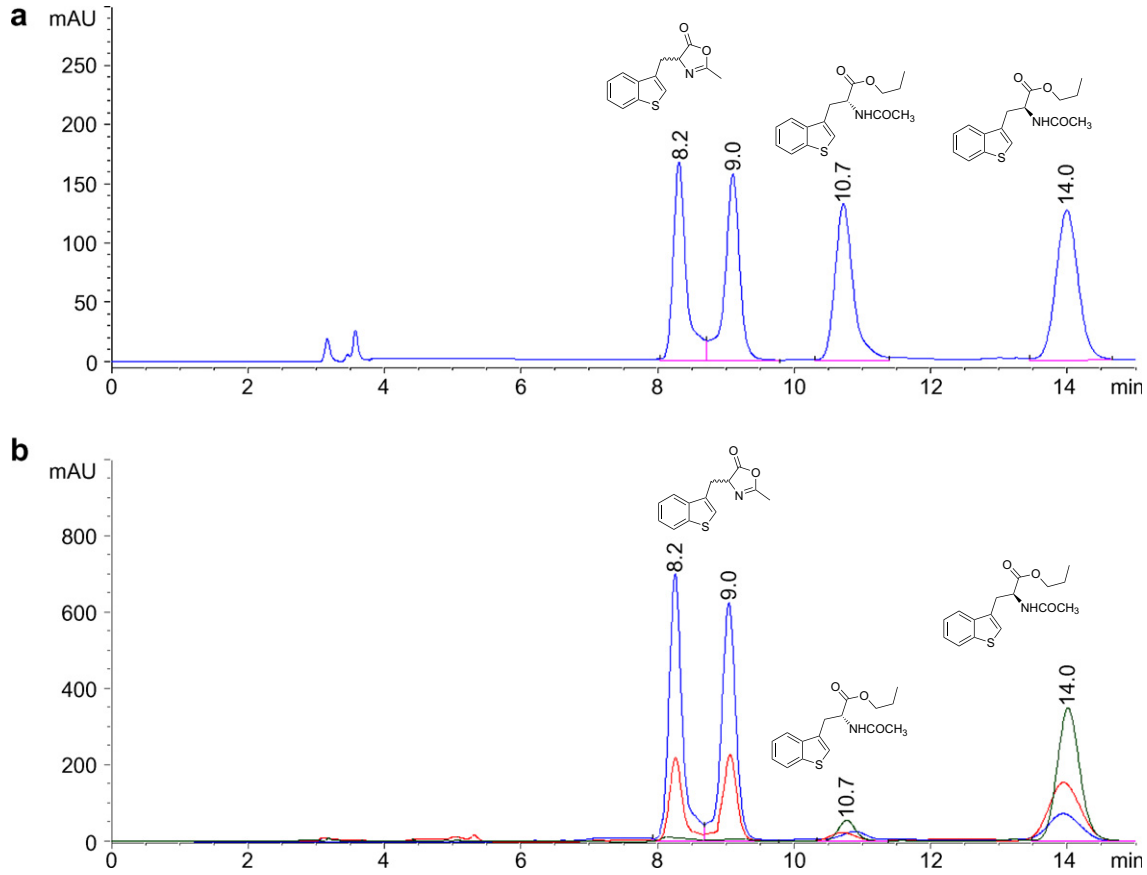


Figure 1. (a) Elution diagram of the mixture of the racemic starting material *rac*-**11d** and racemic product *rac*-**10d** for the lipase catalysed DKR. (b) Elution diagram for the enzymatic DKR of *rac*-4-((benzo[*b*]thiophen-3-yl)methyl)-2-methyloxazol-5(4*H*)-one *rac*-**11d**: after 2 h (blue trace), after 24 h (red trace) and after 48 h (green trace).

To obtain L-2-amino-3-(heteroaryl)propanoic esters L-**10a**–**d** with good yields and high ee several enzymes were tested as potential biocatalysts in neat ethanol as solvent and nucleophile. All lipases, except for lipase F, showed the

same enantiopreference in their alcoholysis, although the behaviour of lipases greatly differed. Lipases AY, PS, CcL and CrL were catalytically inactive; lipase AK (ee 13–17%), lipase F (ee 5–7%) and Lipozyme TL IM (ee 7–11%) gave moderate selectivity and reactivity, whereas Novozyme 435 showed acceptable properties (Table 1, entry 2). Accordingly, Novozyme 435 was used for further studies.

It is known that the nature of the nucleophile and solvent could substantially influence the selectivity of the enzymatic reaction. For Novozyme 435 mediated alcoholysis of *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-ones *rac*-**11a–d** (Scheme 3a), two conditions were compared. The reactions were first performed in alcohols at room temperature. Four different alcohols were used as both solvent and nucleophile.

1-Propanol was found to be the most selective nucleophilic agent for all substrates (Table 1, entry 3). The enzymatic propanolysis was carried out at room temperature in several organic solvents.

Dioxane proved to be the most appropriate solvent for the biocatalytic ring-opening of *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-ones *rac*-**11a–d** (Table 2, entry 1). It was previously[1,11] shown that the conversion of the reaction and the ee of the products in the enzymatic dynamic kinetic resolution of *rac*-4-substituted 2-phenyloxazol-5(4*H*)-ones were strongly influenced by the presence of water and triethylamine and by the nucleophile substrate and the enzyme–substrate ratio. It is worth emphasizing that even small quantities of water caused a decrease in the ee of the enzymatic alcoholysis product. The generated carboxylic acid most likely interacts with the enzyme leading to a change in the conformation that alters the stereocomplementarity between enzyme and the reacting enantiomer of the substrate. To avoid this inconvenience, all enzymatic reactions were carried out in anhydrous conditions. It should be noted that before use, the enzymes were dried or crashed and dried (in case of immobilized enzymes), as described by Turner et al.[11] In contrast to all earlier reported results, *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-ones *rac*-**11a–d** showed spontaneous racemization during the enzymatic reaction (Fig. 1b, blue trace and red trace); the presence of triethylamine in the reaction media was not helpful. Moreover, by favouring the non-enzymatic alcoholysis, even a very small quantity of triethylamine (0.1 equiv) increased the reaction time and

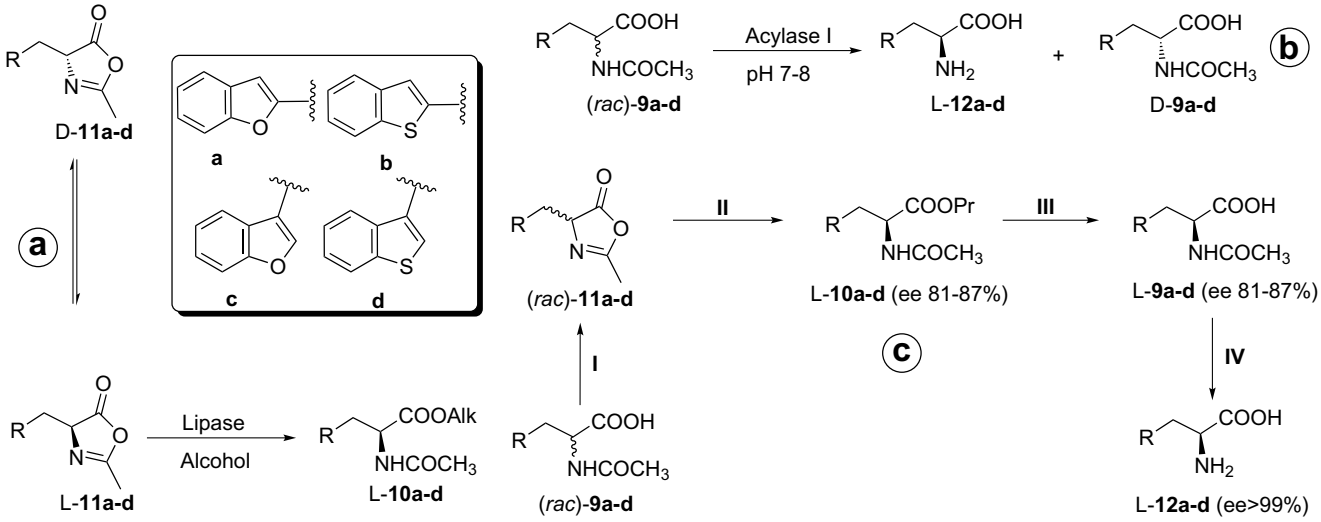
Table 1. Enantiomeric excess for the Novozyme 435 mediated alcoholysis of *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-ones *rac*-**11a–d**

Entry	Type of ester	ee for L-2-amino-3-(heteroaryl)propanoic esters[a,b] (%)			
		L-**10a**	L-**10b**	L-**10c**	L-**10d**
1	Methyl ester	3	3	3	3
2	Ethyl ester	67	65	65	73
3	Propyl ester	71	71	69	79
4	Butyl ester	67	69	67	75

[a] In neat alcohol.
[b] Total conversion after 6 h.

Table 2. Enantiomeric excess for DKR of *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-ones *rac*-**11a–d** in organic solvents

Entry	Solvent	ee for L-2-amino-3-(heteroaryl)-propanoic acid propyl esters (%)			
		L-**10a**	L-**10b**	L-**10c**	L-**10d**
1	Dioxane	75	79	83	86
2	Dichloromethane	39	37	42	54
3	Toluene	69	59	69	79
4	Acetonitrile	61	55	73	83
5	Tetrahydrofuran	71	47	81	83
6	Diethylether	67	71	59	73



Scheme 3. Enantioselective synthesis of L-heteroaryl alanines and their derivatives: (a) DKR of the lipase mediated alcoholysis of oxazolones *rac*-**11a–d**; (b) enantiomer selective hydrolysis of *rac*-**9a–d**; (c) *chemoenzymatic* synthesis of enantiopure L-**12a–d**. Reagents and conditions: (I) DCC/CH_2Cl_2; (II) propanol, Novozyme 435/dioxane; (III) Na_2CO_3, H_2O, reflux; (IV) Acylase I, pH 7–8.

decreased the enantioselectivity of the global transformation. A high nucleophile–substrate ratio (3–10) also decreased the stereoselectivity of the lipase mediated reaction. It was found that 2 equiv of the nucleophile was the optimal amount necessary for total conversion of *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-ones *rac*-**11a–d** (Fig. 1b, green trace), while the concurrent non-enzymatic alcoholysis decreased the ee when the substrate–enzyme ratio was higher than 1:1 (m/m). These results are in good agreement with our previous observation concerning the non-enzymatic alcoholysis of the *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-ones. It was found that in neat alcohols *rac*-4-((heteroaryl)-methyl)-2-methyloxazol-5(4*H*)-ones *rac*-**11a–d** were totally transformed into racemic products in about 1 day, while using triethylamine as a catalyst (0.25 equiv) total conversion of the substrate occurs in 5–6 h.

Starting from the enantiomerically enriched L-2-acetamido-3-(heteroaryl)propanoic acids propyl esters L-**10a–d**, a second chiral selector had to be used in one of the next two hydrolytic steps, to obtain enantiopure L-2-amino-3-(heteroaryl)propanoic acids. Acylase I proved to be a stereospecific catalyst for the kinetic resolution of racemic 2-acetamido-3-(heteroaryl)propanoic acids *rac*-**9a–d** (Scheme 3b). Enantiopure L-heteroaryl-amino acids L-**12a–d** and D-heteroaryl-*N*-acetyl-amino-acids D-**9a–d** were isolated from the reaction mixture.

With these results in hand, the preparative scale synthesis of the enantiopure L-**12a–d** from racemic 2-acetamido-3-(heteroaryl)propanoic acids *rac*-**9a–d** was set up (Scheme 3c).

The progress of the reaction was monitored after each step by HPLC to determine the enantiopurity of the isolated compounds and the conditions for quantitative conversions. The lipase mediated DKR was stopped when the consumption of the *rac*-4-((heteroaryl)methyl)-2-methyloxazol 5(4*H*)-ones *rac*-**11a–d** ceased. The ee of the isolated products was the same as the one found in the case of small scale reactions. Then, by the chemical hydrolysis, without altering the ee (Fig. 2), the enantiomer enriched L-2-acetamido-3-(heteroaryl)propanoic acid propyl esters L-**10a–d** were transformed in good yields (~98%) into L-2-acetamido-3-(heteroaryl)-propanoic acids L-**9a–d**.

After the enantiospecific Acylase I mediated hydrolysis of the latest compounds, the enantiopure final products, L-heteroarylalanines L-**12a–d** were easily isolated on an ion-exchange column. The yields and the specific rotation values of the isolated final products are shown in Table 3.

Besides the yields, which varied between 76% and 85%, the absolute configurations and the enantiomeric purity of the isolated heteroarylalanines were also determined. Figure 3 shows the elution diagram on a chiral column of the L-2-amino-3-(benzo[*b*]thiophene-3-yl)propanoic acid L-**12d** (red trace) produced and that of the racemic 2-amino-3-(benzo[*b*]thiophene-3-yl)propanoic acid *rac*-**12d** (blue trace) as a control.

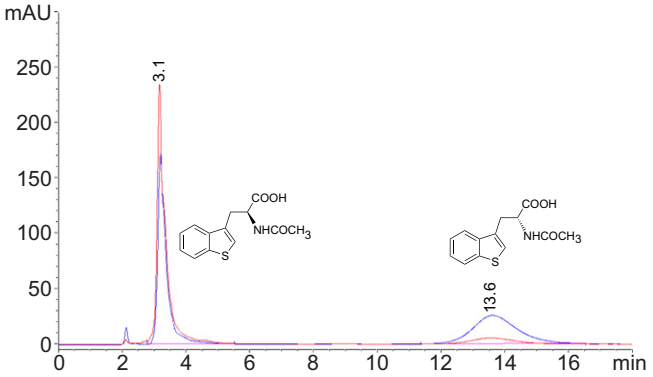


Figure 2. Elution diagram of the isolated product of the chemical hydrolysis, L-2-acetamido-3-(benzo[*b*]thiophene-3-yl)propanoic acid L-**9d** (red trace) and *rac*-2-acetamido-3-(benzo[*b*]thiophene-3-yl)propanoic acid *rac*-**9d** as reference (blue trace).

The expected L-configuration of all heteroaryl alanines was confirmed by measuring their specific rotations, which were consistent with the literature values.

3. Conclusions

The present work describes the usability of a chemo-enzymatic procedure for the preparation of enantiopure heteroaryl alanines starting from racemic 2-acetamido-3-(hetero- aryl)propanoic acids. The first enzyme catalyzed step was the Novozyme 435 mediated dynamic kinetic resolution of the oxazolones which provided the N- and C-protected L-amino acids (ee 76–85%) with theoretical 100% yield. The protecting groups were removed with excellent yields by combining a second chemical (mild hydrolysis of the ester group) and a third enzymatic step (hydrolysis of the amide group). The latest, due to the L-specificity of Acylase I, raised the ee of the final product to more than 99%.

4. Experimental

4.1. Analytical methods

The ^{1}H and ^{13}C NMR spectra were recorded on a Bruker spectrometer operating at 300 MHz and 75 MHz, respectively, at 25 °C. Electron impact mass spectra (EI-MS) were taken on a VG 7070E mass spectrometer operating at 70 eV.

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 the enantiomeric separation of *rac*-**9**,**12a–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*-**10–11a–d**, both at 1 mL/min flow rate. For all chiral compounds, high resolution enantiomeric separation was performed. Retention times for L- and D-**9–12a–d** are presented in Table 4.

Table 3. Yields and specific rotations for the isolated enantiopure L-amino acids L-**12a–d**

Substrate	Product[a]	Yield[b] (%)	$[\alpha]_D^{20}$ (10 mg mL^{-1})
rac-**9a**	L-**12a**	76	−14.5, CH_3COOH (−14.5, CH_3COOH, 20 °C[5])
rac-**9b**	L-**12b**	79	−23.8, CH_3COOH (−23.8, CH_3COOH, 20 °C[5])
rac-**9c**	L-**12c**	83	−8.0, CH_3COOH
rac-**9d**	L-**12d**	85	−26.0, CH_3COOH (−9.2, 0.1 M HCl, 20 °C[14])

[a] ee >99% for all the compounds.
[b] Yields are given for the isolated compounds.

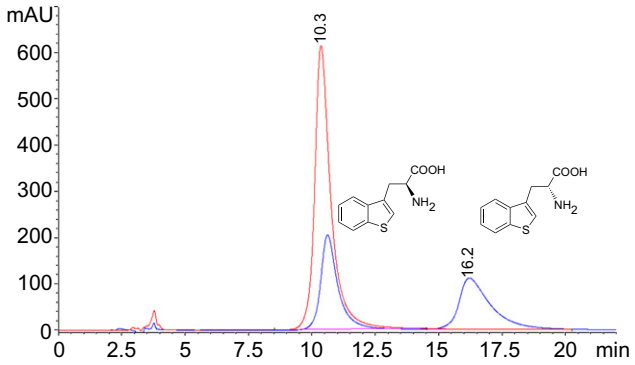


Figure 3. Elution diagram of the isolated enantiopure L-2-amino-3-(benzo[*b*]thiophene-3-yl)propanoic acid L-**12d** (red trace) and *rac*-2-amino-3-(benzo[*b*]thiophene-3-yl)propanoic acid *rac*-**12d** as reference (blue trace).

Table 4. Retention times of the enantiomers of *rac*-**9**–**12a–d**

rt (min)							
L-**9a**	D-**9a**	L-**9b**	D-**9b**	L-**9c**	D-**9c**	L-**9d**	D-**9d**
2.8	11.6	2.9	8.7	2.8	11.0	3.0	13.1
D-**10a**	L-**10a**	D-**10b**	L-**10b**	D-**10c**	L-**10c**	D-**10d**	L-**10d**
12.5[a]	14.4[a]	12.0[a]	13.6[a]	8.1[a]	10.9[a]	12.0[a]	15.0[a]
10.4[b]	12.2[b]	9.3[b]	12.3[b]	11.2[b]	12.9[b]	10.5[b]	13.5[b]
10.6[c]	12.6[c]	11.5[c]	15.8[c]	11.5[c]	13.0[c]	10.7[c]	14.0[c]
9.5[d]	11.0[d]	10.2[d]	13.2[d]	11.2[d]	12.9[d]	9.4[d]	11.8[d]
11a		**11b**		**11c**		**11d**	
8.9	12.3	11.3	11.5	7.5	8.1	8.2	9.0
L-**12a**	D-**12a**	L-**12b**	D-**12b**	L-**12c**	D-**12c**	L-**12d**	D-**12d**
8.8	14.9	10.1	15.2	8.9	13.5	10.5	15.7

[a] Methyl ester.
[b] Ethyl ester.
[c] Propyl ester.
[d] Butyl ester.

Thin layer chromatography (TLC) was carried out using Merck Kieselgel 60F_{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 a 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}.

4.2. Reagents and solvents

Benzofuran, benzo[*b*]thiophene, 1-(2-hydroxyphenyl)-ethanone, ethyl 2-chloroacetate, all inorganic reagents and solvents were produced from Aldrich or Fluka. All solvents were purified and dried by standard methods as required. Lipases AY, AK, PS and F were from Amano, Europe, England. Lipases from *Candida rugosa* (CrL), *Candida cylindracea* (CcL) and Acylase I were purchased from Fluka. Lipase B (CAL-B, Novozyme 435) from *Candida antarctica* and Lypozyme TL IM were purchased from Novozymes, Denmark.

4.3. Chemical synthesis of racemic heteroaryl alanines and their derivatives

4.3.1. Synthesis of benzofuran-2-carbaldehyde 4a. Phosphoryl trichloride (132 g, 80 mL, 0.86 mol) was added in small portions with slight warming into a solution of benzofuran (92 g, 0.78 mol) in anhydrous dimethylformamide (150 g, 158 mL, 2.05 mol). The mixture was heated for 10 h at 100 °C, then cooled to room temperature and treated with an extra portion of dimethylformamide (50 g, 52.7 mL, 0.68 mol) and phosphoryl trichloride (40 g, 24.3 mL, 0.26 mol). The mixture was heated for another 10 h. The cooled mixture was treated with saturated sodium acetate solution in water until the pH rose to 6. The organic compounds were extracted with Et_2O (3 × 100 mL). The combined organic layers were washed with saturated $KHCO_3$ solution (3 × 50 mL) and water (100 mL), and finally dried over anhydrous $MgSO_4$. The solvent was removed and the residue distilled in vacuo. Yield = 62%; bp 135–136 °C/18 mmHg (135–136 °C/18 mmHg[15]); HRMS: M^+ found (M^+ calculated for $C_9H_6O_2$): 146.03701 (146.03678); MS: *m*/*z* (%) = 148 (1.53, M+2), 147 (19.73, M+1), 146 (100, M), 145 (87.96), 138 (0.44), 131 (0.36), 121 (0.29), 120 (0.71), 119 (1.18), 118 (15.48), 117 (0.68), 116 (0.39), 102 (0.40), 101 (1.13), 98 (0.33), 92 (0.64); ^{1}H NMR ($CDCl_3$): δ = 7.24–7.28 (m, 1H), 7.42–7.46 (m, 1H), 7.51 (s, 1H), 7.52 (d, 1H), 7.67 (d, 1H), 9.79 (s, 1H); ^{13}C NMR ($CDCl_3$): δ = 112.7, 117.9, 123.7, 124.2, 126.7, 129.3, 152.7, 156.3, 179.8.

4.3.2. Synthesis of benzo[*b*]thiophene-2-carbaldehyde 4b. Into a cooled solution (−78 °C) of benzo[*b*]thiophene (3.24 g, 2.17 mL, 20 mmol) in anhydrous THF (120 mL), 2.7 M *n*-BuLi in heptane (~8.15 mL, 1.1 equiv) was added dropwise under nitrogen, and the mixture was stirred for 1 h at −78 °C. Then, anhydrous DMF (4.38 g, 4.62 mL, 60 mmol) was added and the solution was stirred for 2 h

while warming the reaction mixture to −10 °C. The mixture was hydrolyzed with saturated NH_4Cl solution (30 mL) and diluted with water (100 mL). After the separation of the phases, the water phase was extracted with Et_2O (3 × 50 mL). The combined organic layers were washed with water and brine, dried over anhydrous $MgSO_4$ and concentrated in vacuo. The crude product was purified by column chromatography on silica gel using dichloromethane as eluent. Yield = 62%; mp 34–35 °C from ethanol (34–34.3 °C from ethanol[16]) HRMS: M^+ found (M^+ calculated for C_9H_6OS): 162.01418 (162.01394); MS: *m*/*z* (%) = 164 (15.42, M+2), 163 (56.40, M+1), 162 (100, M), 161 (97.75), 147 (0.89), 136 (2.06), 135 (6.49), 134 (58.68), 133 (61.29), 132 (5.52), 108 (5.84), 107 (1.25), 106 (3.02), 105 (1.69), 102 (2.94), 98 (1.58); ^1H NMR ($CDCl_3$): δ = 7.34–7.43 (m, 1H), 7.43–745 (m, 1H), 7.82 (d, 1H), 7.87 (d, 1H), 7.95 (s, 1H), 10.03 (s, 1H); ^{13}C NMR ($CDCl_3$): δ = 122.3, 124.3, 125.3, 127.2, 133.5, 137.5, 141.7, 142.3, 183.7.

4.3.3. Synthesis of (2-acetyl-phenoxy)acetic acid ethyl ester 1. A mixture of 1-(2-hydroxyphenyl)ethanone (110 g, 97.26 mL, 0.80 mol), ethyl 2-chloroacetate (122.5 g, 107 mL, 1.0 mol) and anhydrous K_2CO_3 (110.5 g, 0.80 mol) in acetone (600 mL) was refluxed for 58 h. After cooling, the precipitate was filtered off and washed with acetone (3 × 150 mL). The acetone was removed by distillation from the combined solutions and the crude product was distilled under reduced pressure affording **1**. Yield: 58%, bp 185 °C/5 mmHg (185 °C/5 mmHg[17]); HRMS: M^+ found (M^+ calculated for $C_{12}H_{14}O_4$): 222.08889 (222.08921); MS: *m*/*z* (%) = 224 (1.14, M+2), 223 (9.67, M+1), 222 (85.33, M), 208 (3.89), 207 (39.67), 204 (1.15), 193 (1.26), 180 (1.72), 179 (9.33), 177 (1.70), 176 (2.98), 153 (1.19), 152 (5.68), 151 (85.63), 150 (8.08), 149 (100), 148 (12.94), 147 (1.40), 137 (0.84), 136 (1.78), 135 (6.10), 134 (1.48), 133 (5.88), 132 (1.10), 131 (5.12), 124 (0.76), 123 (9.80), 122 (4.17), 121 (62.24), 120 (7.44), 119 (1.31), 118 (1.25), 107 (5.43), 106 (2.13), 105 (26.11), 104 (1.70), 103 (1.91), 101 (3.35), 95 (1.13); ^1H NMR ($CDCl_3$): δ = 1.23 (t, 3H), 2.65 (s, 3H), 4.21 (q, 2H), 4.65 (s, 2H), 6.75 (d, 1H), 6–6.99 (m, 1H), 7.35–7.39 (m, 1H), 7.69 (d, 1H); ^{13}C NMR ($CDCl_3$): δ = 13.1, 31.0, 60.5, 64.3, 111.2, 120.7, 127.8, 129.7, 132.5, 155.9, 167.1, 198.7.

4.3.4. Synthesis of (2-acetyl-phenoxy)acetic acid 2. Into the vigorously stirred solution of sodium carbonate (53 g, 0.50 mol) in water (800 mL), (2-acetyl-phenoxy)acetic acid ethyl ester (100 g, 0.45 mol) was added and the mixture was gently refluxed. After 1 h of heating the solution was cooled to 5 °C and acidified carefully with concentrated HCl solution. The deposited precipitate was filtered off, washed several times with cold water. Yield = 97%; mp 115 °C from water (114–114.3 °C from water[16]); HRMS: M^+ found (M^+ calculated for $C_{10}H_{10}O_4$): 194.05708 (194.05791); MS: *m*/*z* (%) = 195 (1.42, M+1), 194 (12.32, M), 180 (0.76), 179 (7.63), 176 (0.92), 153 (5.12), 152 (2.66), 151 (25.10), 150 (33.02), 149 (6.50), 148 (6.88), 137 (1.06), 136 (11.71), 135 (56.58), 134 (1.45), 133 (7.52), 132 (3.50), 131 (2.83), 124 (0.89), 123 (8.21), 122 (7.53), 121 (100), 120 (4.40), 119 (0.84), 118 (1.01), 108 (1.58), 107 (10.66), 106 (3.26), 105 (18.51), 104 (2.74), 103 (1.26), 95 (1.75); ^1H NMR ($CDCl_3$): δ = 2.61 (s, 3H), 4.71 (s, 2H), 6.89 (d, 1H), 7.04–7.08 (m, 1H), 7.44–7.48 (m, 1H), 7.72 (d, 1H); ^{13}C NMR ($CDCl_3$): δ = 29.1, 65.8, 113.7, 121.5, 126.5, 130.3, 133.6, 155.8, 169.8, 199.5.

4.3.5. Synthesis of benzofuran-3-carbaldehyde 4c. A mixture of (2-acetyl-phenoxy)acetic acid (77.5 g, 0.40 mol) and anhydrous sodium acetate (141 g, 1.7 mol) in acetic anhydride (285 g, 2.8 mol) was heated at 160 °C for 3 h. After cooling, the mixture was poured into 900 mL water and then extracted with Et_2O (3 × 300 mL). The combined organic layers were washed with 10% Na_2CO_3 solution (3 × 150 mL) and then with water (3 × 100 mL) and finally dried over $MgSO_4$. The solvent was removed and 3-methylbenzofuran was isolated by distillation. Then a mixture of 3-methyl-benzofuran (10.02 g, 75.8 mmol) and selenium dioxide (9.70 g, 87.4 mmol) in dry 1,4-dioxane (100 mL) was refluxed for 48 h. The resulting black precipitate was filtered off and the crude product fractionated by distillation under reduced pressure.Yield = 77%; bp 69 °C/0.4 mmHg (68–69 °C/0.4 mmHg[18]); HRMS: M^+ found (M^+ calculated for $C_9H_8O_2$): 146.03705 (146.03678); MS: *m*/*z* (%) = 148 (0.62, M+2), 147 (6.48, M+1), 146 (82.02, M), 145 (100), 144 (0.15), 136 (0.20), 131 (0.15), 121 (0.63), 119 (0.15), 118 (1.52), 117 (1.41), 116 (0.16), 109 (0.14), 107 (0.28), 106 (0.16), 105 (0.19), 102 (0.16), 101 (0.26), 98 (0.14); ^1H NMR ($CDCl_3$): δ = 7.27–7.34 (m, 2H), 7.45 (d, 1H), 8.10 (d, 1H), 8.17 (s, 1H), 10.07 (s, 1H); ^{13}C NMR ($CDCl_3$): δ = 111.7, 122.6, 122.9, 123.7, 124.9, 126.3, 155.4, 156.0, 184.8.

4.3.6. Synthesis of 3-chloromethyl-benzo[*b*]thiophene 6d. A rapid stream of HCl gas was passed into a vigorously stirred mixture of 37% formaldehyde (9.7 g, 0.12 mol), concentrated hydrochloric acid (9.7 mL, 0.11 mol) and benzo[*b*]thiophene (13.05 g, 0.97 mol) until the mixture was saturated with HCl. During this time, the temperature of the mixture rose to 65 °C. This temperature was maintained for 1 h, while a slow stream of hydrogen chloride gas was passed into the mixture. After the reaction was completed, water (100 mL) was added to the cold reaction mixture, the organic layer was separated and the water phase extracted with Et_2O (3 × 50 mL). The combined organic layers were washed successively with water (3 × 50 mL), saturated Na_2CO_3 solution (3 × 25 mL) and finally with water (3 × 50 mL). The organic layer was dried over $MgSO_4$, and then the ether was evaporated. The crude product was fractionated in vacuo. Yield 74.3%; bp 125–127 °C/2 mmHg (125–127 °C/2 mmHg[19]).

4.3.7. Reduction of aldehydes 4a–c. To a stirred solution of aldehyde **5a–c** (4 g) in methanol (40 mL), $NaBH_4$ was added in small portions at room temperature, until the entire amount of the aldehyde was transformed. The progress of the reaction was followed by thin layer chromatography, using dichloromethane as eluent. Then the reaction mixture was treated with 1M HCl (4 mL) and the methanol was removed in vacuo. The crude product was treated with a mixture of dichloromethane–water = 1:1 (v/v), the organic layer was separated, dried over anhydrous $MgSO_4$ and the dichloromethane was removed in vacuo. The crude product was purified by column chromatography, using

dichloromethane–acetone = 9:1 (v/v) as eluent. The pure compound was immediately used in the next step.

4.3.8. Synthesis of chloromethylene derivatives 6a–c. A stock solution (1.5 M) was prepared by mixing up a volume of a viscous clear solution of thionyl chloride (5.46 mL, 0.075 mol) and 1*H*-benzo[*d*][1,2,3]triazole (8.93 g, 0.075 mol) with dry dichloromethane up to 50 mL and transferred to a 50 mL burette. The reaction was started by adding the stock solution (1.25 mmol equiv) intermittently from the burette to a stirred solution of the alcohol **5a–c** (1 mmol) in dry dichloromethane (20 mL). Before the addition was complete, 1*H*-benzo[*d*][1,2,3]triazole hydrochloride started to precipitate. The reaction mixture was then stirred further for 5–10 min. At the end the solid was filtered off and washed with dry dichloromethane (2 × 25 mL). The filtrate was washed with 10% HCl (25 mL), water (25 mL), followed by 2% NaOH solution (25 mL). The organic layer was separated and dried over anhydrous $MgSO_4$. The crude product was purified by distillation under reduced pressure and the distillate was immediately used in the next step.

4.3.9. Synthesis of malonic acid diethyl esters 7a–d. 55% NaH oil suspension (0.85 g, 20 mmol) was added into dry dimethylformamide (20 mL) and vigorously stirred for 30 min under argon at room temperature. Subsequently, 2-acetylamino-malonic acid diethyl ester (19.5 mmol) was added to the suspension and then the reaction mixture was stirred for 30 min. Chloromethylene derivative (19 mmol) **6a–d** was then added, after which the mixture was stirred for 3 h at room temperature and for another 4 h at 60 °C. After cooling the reaction product was poured into a water–ice mixture (100 g). The resulting precipitate was filtered off, dried in vacuo at room temperature and finally purified by column chromatography on silica gel, using as eluent, dichloromethane–CH_3OH = 99:1 (v/v).

4.3.9.1. Diethyl 2-acetamido-2-((benzofuran-2-yl)-methyl)-malonate 7a. Yield = 80%; mp 93–94 °C from ethanol; HRMS: M^+ found (M^+ calculated for $C_{18}H_{21}NO_6$): 347.13130 (347.13689); MS: *m*/*z* (%) = 349 (1.36, M+2), 348 (9.56, M+1), 347 (49.88, M), 303 (1.73), 302 (10.36), 290 (7.84), 289 (54.75), 288 (100), 274 (3.22), 260 (3.89), 259 (1.60), 244 (3.02), 243 (16.04), 233 (3.95), 232 (36.13), 231 (6.49), 228 (8.13), 217 (3.64), 216 (32.49), 215 (7.72), 214 (16.25), 213 (4.26), 203 (6.80), 199 (2.32), 198 (9.85), 188 (4.90), 187 (2.46), 186 (5.86), 175 (3.70), 174 (23.31), 172 (1.44), 171 (7.01), 170 (15.81), 162 (1.52), 160 (3.94), 159 (12.16), 158 (16.07), 157 (14.83), 156 (1.98), 147 (3.28), 146 (22.91), 145 (1.53), 144 (3.43), 142 (6.83), 133 (1.58), 132 (7.82), 131 (66.22), 130 (3.55), 118 (5.04), 115 (3.73), 103 (4.25), 102 (3.67), 77 (7.62); ^{1}H NMR ($CDCl_3$): δ = 7.21 (t, 6H), 2.01 (s, 3H), 3.89 (s, 2H), 4.20 (q, 4H), 6.72 (s, 1H, NH), 6.89 (s, 1H), 7.17–7.25 (m, 1H), 7.59 (d, 1H), 7.67 (d, 1H); ^{13}C NMR ($CDCl_3$): δ = 14.0, 23.2, 33.5, 63.0, 66.9, 122.1, 123.1, 124.1, 124.2, 124.3, 137.7, 139.5, 140.0, 167.1, 169.4.

4.3.9.2. Diethyl 2-acetamido-2-((benzo[*b*]thiophen-2-yl)-methyl)malonate 7b. Yield = 79%; mp 96 °C from ethanol; HRMS: M^+ found (M^+ calculated for $C_{18}H_{21}NO_5S$): 363.11375 (363.11404); MS: *m*/*z* (%) = 365 (3.41, M+2), 364 (10.40, M+1), 363 (51.63, M), 319 (1.00), 318 (5.38), 307 (2.19), 306 (13.20), 305 (45.85), 304 (1.00), 290 (1.49), 276 (1.76), 260 (1.21), 259 (6.45), 249 (2.46), 248 (17.11), 247 (3.26), 244 (4.34), 233 (1.75), 232 (12.49), 231 (5.42), 230 (27.91), 229 (2.64), 219 (2.66), 216 (1.21), 215 (3.15), 214 (21.84), 204 (1.51), 203 (1.38), 202 (3.75), 188 (1.28), 187 (4.31), 186 (13.03), 176 (4.45), 175 (13.91), 174 (34.85), 173 (8.01), 172 (2.73), 163 (1.06), 162 (1.07), 161 (1.41), 160 (2.70), 159 (2.85), 158 (11.70), 149 (4.96), 148 (10.01), 147 (61.04), 146 (4.71), 145 (2.30), 134 (3.04), 118 (1.25), 115 (4.37), 103 (2.73), 102 (1.74), 89 (1.16); ^{1}H NMR ($CDCl_3$): δ = 1.24 (t, 6H), 1.93 (s, 3H), 3.81 (s, 2H), 4.24 (q, 4H), 6.36 (s, 1H), 6.66 (s, 1H, NH), 7.08–7.16 (m, 2H), 7.26 (d, 1H), 7.40 (d, 1H); ^{13}C NMR ($CDCl_3$): δ = 14.0, 23.0, 31.9, 63.0, 65.6, 105.8, 110.9, 120.7, 122.7, 123.9, 128.3, 153.0, 154.9, 167.2, 169.4.

4.3.9.3. Diethyl 2-acetamido-2-((benzofuran-3-yl)-methyl)-malonate 7c. Yield = 81%; mp 99 °C from ethanol; HRMS: M^+ found (M^+ calculated for $C_{18}H_{21}NO_6$): 347.13678 (347.13689); MS: *m*/*z* (%) = 348 (5.93, M+1), 347 (26.47, M), 302 (4.11), 289 (14.65), 288 (87.84), 259 (2.87), 243 (3.96), 242 (14.60), 232 (17.39), 228 (5.49), 215 (5.53), 214 (38.40), 213 (3.96), 198 (8.10), 186 (6.68), 185 (6.84), 174 (16.51), 171 (11.52), 170 (4.41), 160 (3.14), 159 (6.60), 158 (10.08), 157 (4.11), 153 (8.49), 149 (6.22), 146 (14.32), 136 (4.96), 132 (5.30), 131 (39.14), 125 (2.83), 115 (3.42), 111 (4.64), 107 (7.48), 106 (4.45), 105 (5.52), 103 (7.18), 102 (4.31), 101 (5.98), 98 (3.65); ^{1}H NMR ($CDCl_3$): δ = 1.21 (t, 6H), 1.92 (s, 3H), 3.72 (s, 2H), 4.18 (q, 4H), 6.64 (s, 1H, NH), 7.11–7.15 (m, 1H), 7.17–7.21 (m, 1H), 7.25 (s, 1H), 7.34–7.37 (m, 2H); ^{13}C NMR ($CDCl_3$): δ = 14.0, 23.2, 26.5, 62.8, 66.9, 111.6, 114.2, 119.4, 122.6, 124.4, 128.4, 142.9, 155.0, 167.5, 169.4.

4.3.9.4. Diethyl 2-acetamido-2-((benzo[*b*]thiophen-3-yl)-methyl)malonate 7d. Yield = 80%; mp 109–110 °C/from ethanol (109–110 °C from benzene–petroleum ether[20]); HRMS: M^+ found (M^+ calculated for $C_{18}H_{21}NO_5S$): 363.11455 (363.11404); MS: *m*/*z* (%) = 364 (5.24, M+1), 363 (23.50, M), 306 (6.89), 305 (19.17), 304 (100), 259 (17.39), 256 (6.30), 248 (7.51), 230 (15.43), 228 (9.76), 214 (22.20), 191 (7.73), 187 (22.95), 186 (15.74), 182 (5.30), 178 (5.19), 174 (14.91), 158 (8.13), 154 (5.11), 153 (23.55), 149 (12.89), 148 (6.65), 147 (52.51), 140 (16.37), 136 (14.19), 125 (8.68), 115 (11.75), 112 (6.83), 111 (13.70), 108 (6.81), 107 (15.22), 106 (12.05), 105 (11.22), 101 (5.96), 98.1 (8.01); ^{1}H NMR ($CDCl_3$): δ = 1.21 (t, 6H), 1.88 (s, 3H), 3.87 (s, 2H), 4.17 (q, 4H), 6.54 (s, 1H, NH), 7.22–7.29 (m, 2H), 7.60–7.64 (m, 1H), 7.73–7.77 (m, 1H); ^{13}C NMR ($CDCl_3$): δ = 14.0, 23.1, 30.5, 62.9, 67.1, 121.7, 122.8, 124.0, 124.3, 124.4, 130.2, 139.5, 140.0, 167.5, 169.5.

4.3.10. Synthesis of *rac*-2-acetamido-3-(heteroaryl)-propanoic acids *rac*-9a–d. The diethyl 2-acetamido-2-((heteroaryl)methyl)malonate **7a–d** (0.75 g) dissolved in methanol (2.5 mL) was added in one portion into the 10% NaOH solution (2.5 mL), after which the reaction mixture was stirred for 20 h at room temperature. Subsequently, the methanol was removed in vacuo and the pH of the solution was adjusted to 1 with concentrated HCl, at −10 °C, giving

a white precipitate, which was filtered and dried in vacuo at room temperature. The formed dicarboxylic acids **8a–d** were suspended in toluene (10 mL) and heated at reflux for 2 h. The solvent was removed in vacuo, affording the pure product.

4.3.10.1. *rac*-2-Acetamido-3-(benzofuran-2-yl)propanoic acid *rac*-9a. Yield = 81%; mp 115 °C from ethyl acetate–hexane; HRMS: M^+ found (M^+ calculated for $C_{13}H_{13}NO_4$): 247.08482 (247.08446); MS: *m/z* (%) = 248 (2.12, M+1), 247 (14.31, M), 229 (0.51), 216 (0.63), 204 (1.88), 201 (0.67), 190 (1.08), 189 (11.04), 188 (100.00), 187 (0.74), 171 (2.41), 170 (0.56), 167 (0.55), 161 (0.91), 160 (7.32), 159 (2.84), 158 (0.63), 149 (1.02), 147 (2.51), 146 (5.42), 145 (0.62), 144 (2.93), 143 (0.60), 142 (1.30), 134 (0.74), 133 (1.42), 132 (7.61), 131 (52.11), 130 (1.02), 129 (0.46), 118 (1.63), 116 (0.54), 115 (2.74), 114 (0.46), 105 (1.22), 104 (0.63), 103 (3.11), 102 (2.04), 101 (0.55), 97.1 (0.60); ^{1}H NMR (DMSO-d_6): δ = 1.80 (s, 1H), 3.05–3.27 (m, 2H), 4.43–4.49 (m, 1H), 6.60 (s, 1H), 7.15–7.23 (m, 2H), 7.47 (d, 1H), 7.53 (d, 1H), 8.08 (d, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 22.5, 30.7, 51.6, 103.4, 110.6, 120.4, 122.5, 123.3, 128.5, 154.0, 156.0, 169.0, 172.9.

4.3.10.2. *rac*-2-Acetamido-3-(benzo[*b*]thiophen-2-yl)-propanoic acid *rac*-9b. Yield = 83%; mp 208 °C from ethyl acetate–hexane (decomp.); HRMS: M^+ found (M^+ calculated for $C_{13}H_{13}NO_3S$): 263.06191 (263.06161); MS: *m/z* (%) = 263 (20.59. M), 206 (6.23), 205 (14.06), 204 (97.76), 187 (3.79), 176 (6.23), 163 (2.85), 162 (7.50), 161 (4.60), 160 (4.30), 154 (2.51), 153 (17.42), 151 (5.57), 150 (4.97), 149 (9.39), 148 (13.28), 147 (100.00), 136 (7.52), 135 (2.57), 134 (8.23), 121 (4.74), 116 (2.67), 115 (11.09), 108 (5.79), 107 (19.76), 106 (14.60), 105 (7.02), 104 (2.54), 103 (7.03), 102 (3.71), 91 (18.08), 90 (2.56); ^{1}H NMR (DMSO-d_6): δ = 1.84 (s, 3H), 3.18–3.39 (m, 2H), 4.47–4.32 (m, 1H), 7.20 (s, 1H), 7.26–7.35 (m, 2H), 7.75 (d, 1H), 7.88 (d, 1H), 8.33 (d, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 22.5, 32.0, 53.2, 122.1, 122.7, 123.0, 123.8, 124.2, 139.0, 139.5, 141.0, 169.4, 172.5.

4.3.10.3. *rac*-2-Acetamido-3-(benzofuran-3-yl)propanoic acid *rac*-9c. Yield = 86%; mp 156 °C from ethanol–water (155–156 °C from water[21]); HRMS: M^+ found (M^+ calculated for $C_{13}H_{13}NO_4$): 247.08531 (247.08446); MS: *m/z* (%) = 249 (0.49, M+2), 248 (4.27, M+1), 247 (29.44, M), 232 (0.25), 229 (0.87), 216 (0.79), 206 (0.26), 205 (0.72), 204 (3.99), 203 (1.60), 202 (0.55), 201 (0.65), 190 (1.03), 189 (10.04), 188 (100.00), 187 (3.50), 186 (0.54), 185 (0.37), 172 (0.39), 171 (2.19), 170 (0.24), 162 (0.32), 161 (1.07), 160 (8.72), 159 (4.34), 158 (1.60), 149 (0.28), 148 (0.38), 147 (3.17), 146 (25.27), 145 (2.05), 144 (6.47), 143 (0.37), 142 (0.55), 134 (0.52), 133 (1.23), 132 (8.96), 131 (79.93), 130 (1.62), 121 (0.74), 119 (0.31), 118 (2.73), 117 (0.45), 116 (0.71), 115 (4.11), 114 (0.39), 105 (1.18), 104 (1.09), 103 (7.21), 102 (1.78), 101 (0.24), 91.1 (0.49); ^{1}H NMR (DMSO-d_6): δ = 1.86 (s, 3H), 3.01–3.21 (m, 2H), 4.35–4.61 (m, 1H), 7.30–7.39 (m, 2H), 7.60 (d, 2H), 7.71 (d, 1H), 7.81 (s, 1H), 8.33 (d, 1H, NH); ^{13}C NMR (DMSO-d_6): δ = 22.3, 25.3, 51.8, 111.2, 116.0, 119.6, 122.5, 124.2, 127.7, 142.9, 154.4, 169.3, 173.9.

4.3.10.4. *rac*-2-Acetamido-3-(benzo[*b*]thiophen-3-yl)-propanoic acid *rac*-9d. Yield = 85%; mp 154 °C from toluene (153 °C from benzene–petroleum ether[14]); HRMS: M^+ found (M^+ calculated for $C_{13}H_{13}NO_3S$): 263.06180 (263.06161); MS: *m/z* (%) = 263 (22.57, M), 206 (5.38), 205 (9.72), 204 (80.60), 195 (3.92), 191 (4.19), 181 (4.36), 176 (4.69), 175 (4.37), 167 (5.28), 165 (4.75), 163 (4.22), 162 (7.43), 161 (4.47), 160 (4.29), 153 (7.79), 151 (6.44), 149 (10.58), 148 (10.67), 147 (100.00), 141 (4.07), 139 (4.49), 137 (7.13), 136 (4.06), 135 (4.72), 134 (4.88), 127 (4.87), 126 (4.02), 125 (10.91), 124 (4.15), 123 (8.97), 121 (4.98), 115 (7.52), 113 (5.17), 112 (4.43), 111 (16.32), 110 (5.23), 109 (11.35), 107 (4.75), 103 (4.43), 99 (6.05); ^{1}H NMR (DMSO-d_6): δ = 1.75 (s, 3H), 3.36–3.40 (m, 2H), 4.38–4.41 (m, 1H), 7.32–7.35 (m, 2H), 7.39 (s, 1H), 7.82 (d, 1H), 7.91 (d, 1H); ^{13}C NMR (DMSO-d_6): δ = 22.7, 30.9, 53.9, 121.7, 122.6, 122.8, 123.8, 123.9, 133.6, 139.2, 139.3, 168.7, 175.5.

4.3.11. Synthesis of *rac*-propyl 2-acetamido-3-(heteroaryl)propanoates *rac*-10a–d. Into a solution of carbonyldiimidazole (90 mg, 0.55 mmol) and *rac*-2-acetamido-3-(heteroaryl)propanoic acid *rac*-**9a–d** (0.5 mmol) in anhydrous THF (2.5 mL), 1-propanol (45 mg, 56 μL, 0.75 mmol) was added in one portion at room temperature. After the reaction was complete (checked by TLC), the solvent was distilled off in vacuo and the crude product was purified with column chromatography on silica gel using as eluent dichloromethane–acetone 90:10 (v/v). Methyl, ethyl and butyl 2-acetamido-3-(heteroaryl)propanoates were prepared in the same manner.

4.3.11.1. *rac*-Propyl 2-acetamido-3-(benzofuran-2-yl)-propanoate *rac*-10a. Yield = 89%; semisolid, HRMS: M^+ found (M^+ calculated for $C_{16}H_{19}NO_4$): 289.13163 (289.13141); MS: *m/z* (%) = 291 (1.8, M+2), 290 (18, M+1), 289 (100, M), 288 (17), 287 (12), 284 (11.5), 279 (20.5), 278 (20.5), 268 (11.3), 264 (20.8), 258 (27.1), 257 (15.2), 248 (58.4), 242 (17.3), 231 (18.5), 229 (12.4), 225 (12.7), 215 (12.3), 212 (13.6), 209 (14.9), 193 (22.1), 176 (12.2), 159 (20.1), 129 (14.6); ^{1}H NMR ($CDCl_3$): δ = 0.92 (t, 3H), 1.60–1.74 (m, 2H), 1.99 (s, 3H), 3.33 (d, 2H), 4.05–4.18 (m, 2H), 4.92–4.98 (m, 1H), 6.47 (1H, NH), 6.49 (s,1H), 7.16–7.26 (m, 2H), 7.39 (d, 1H), 7.49 (d, 1H); ^{13}C NMR ($CDCl_3$): δ = 10.3, 21.8, 23.0, 31.0, 51.2, 67.4, 104.9, 110.8, 120.6, 122.7, 123.8, 128.3, 153.5, 154.9, 170.0, 171.2.

4.3.11.2. *rac*-Propyl 2-acetamido-3-(benzo[*b*]-thiophen-2-yl)propanoate *rac*-10b. Yield = 88%; semisolid, HRMS: M^+ found (M^+ calculated for $C_{16}H_{19}NO_3S$): 305.10796 (305.10856); MS: *m/z* (%) = 307 (9.8, M+2), 306 (23.1, M+1), 305 (100, M), 304 (32.9), 303 (31.6), 302 (13.9), 301 (14.0), 300 (35.6), 290 (64.1), 289 (69.6), 288 (33.4), 287 (36.8), 286 (39.1), 285 (16.2), 275 (20.1), 271 (17.4), 270 (70.4), 260 (16.6), 257 (15.4), 256 (26.7), 247 (16.6), 225 (24.4), 219 (15.2), 213 (23.5), 201 (8.9), 185 (11.3), 170(6.7), 144 (2.3), 127 (6.8); ^{1}H NMR(300 MHz, $CDCl_3$): δ = 0.94 (t, 3H), 1.63–1.74 (m, 2H), 2.03 (s, 3H), 3.38–3.52 (m, 2H), 4.12 (t, 2H), 4.92–4.98 (m, 1H), 6.43 (1H, NH), 7.01 (s, 1H), 7.25–7.35 (m, 2H), 7.69 (d, 1H), 7.76 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): δ = 10.3, 21.8, 23.1,

32.8, 52.8, 67.4, 122.1, 123.0, 123.4, 124.0, 124.3, 138.6, 139.7, 139.8, 169.9, 171.0.

4.3.11.3. *rac*-Propyl 2-acetamido-3-(benzofuran-3-yl)propanoate *rac*-10c. Yield = 91%; semisolid, HRMS: M^+ found (M^+ calculated for $C_{16}H_{19}NO_4$): 289.13158 (289.13141); MS: *m*/*z* (%) = 291 (1.8, M+2), 290 (18, M+1), 289 (100, M), 288 (57), 287 (35), 284 (17.1), 280 (11.2), 279 (33.5), 278 (35.6), 277 (44.1), 276 (24.8), 264 (19.2), 259 (12.2), 254 (13.9), 252 (16.2), 249 (29.4), 248 (48.1), 247 (65.7), 246 (98), 219 (17.6), 202 (14.5), 135 (18.5), 129 (14.8); ^{1}H NMR (300 MHz, $CDCl_3$): $\delta = 0.87$ (t, 3H), 1.54–1.66 (m, 2H), 1.98 (s, 3H), 3.15–3.30 (m, 2H), 3.96–4.11 (m, 2H), 4.92–4.98 (m, 1H), 6.40 (1H, NH), 7.21–7.32 (m, 2H), 7.42–7.53 (m, 3H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 10.2$, 21.7, 23.1, 26.2, 52.3, 67.3, 111.5, 114.9, 119.4, 122.6, 124.5, 128.0, 142.4, 155.1, 169.9, 171.7.

4.3.11.4. *rac*-Propyl 2-acetamido-3-(benzo[*b*]-thiophen-3-yl)propanoate *rac*-10d. Yield = 84%; semisolid, HRMS: M^+ found (M^+ calculated for $C_{16}H_{19}NO_3S$): 305.10879 (305.10856); MS: *m*/*z* (%) = 307 (9.8, M+2), 306 (23.1, M+1), 305 (100, M), 304 (47.2), 303 (13.4), 289 (2.3), 269 (3.3), 265 (5.2), 264 (4.9), 263 (12.2), 261 (1.4), 246 (3.0), 245 (7.5), 233 (1.9), 226 (2.3), 222 (5.9), 221 (9.1), 220 (1.8), 217 (2.7), 214 (2.1), 211 (4.6), 150 (2.0), 123 (5.3); ^{1}H NMR(300 MHz, $CDCl_3$): $\delta = 0.85$ (t, 3H), 1.53–1.60(m, 2H), 1.95 (s, 3H), 3.31–3.45 (m, 2H), 3.92–4.09 (m, 2H), 4.96–5.02 (m, 1H), 6.40 (1H, NH), 7.15 (s, 1H), 7.31–7.41 (m, 2H), 7.76 (d, 1H), 7.85 (d, 1H); ^{13}C NMR (75 MHz, $CDCl_3$): $\delta = 10.2$, 21.7, 23.0, 30.8, 52.5, 67.2, 121.5, 122.8, 123.7, 124.1, 124.4, 130.8, 138.9, 140.2, 170.0, 171.8.

4.3.12. Synthesis of *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-ones *rac*-11a–d. Into a solution of *rac*-2-acetamido-3-(heteroaryl)propanoic acid *rac*-**9a**–**d** (0.5 mmol) in anhydrous dichloromethane (5 mL), a solution of dicyclohexylcarbodiimide (135 mg, 0.6 mmol) in anhydrous dichloromethane (2 mL) was added dropwise at 0 °C. Before the addition was completed, 1,3-dicyclohexylurea started to precipitate. The reaction mixture was stirred further for 5–10 min. At the end, the solid was filtered off and dichloromethane was removed under reduced pressure at room temperature yielding the desired product. The obtained *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-one *rac*-**11a**–**d** was immediately used in the enzymatic reactions without further purification.

4.3.13. Synthesis of *rac*-2-amino-3-(heteroaryl)-propanoic acids *rac*-12a–d. The *rac*-2-acetamido-3-(heteroaryl)propanoic acid **9a-d** (1 g) was suspended in half concentrated HCl (10 mL) and the mixtures were 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 and the pH was adjusted to 5.6, stirred for 30 min, filtered and dried at room temperature.

4.3.13.1. *rac*-2-Amino-3-(benzofuran-2-yl)propanoic acid *rac*-12a. Yield = 93%; mp 205 °C from water–ethanol; HRMS: M^+ found (M^+ calculated for $C_{11}H_{11}NO_3$): 205.07366 (205.07389); MS: *m*/*z* (%) = 206 (5.14, M+1), 205 (30.38, M), 160 (5.34), 159 (4.48), 149 (2.42), 148 (3.31), 147 (16.70), 144 (2.54), 133 (5.48), 132 (41.90), 131 (100), 130 (3.62), 115 (5.96), 105 (5.59), 104 (2.73), 103 (9.94), 102 (6.74), 89 (6.14); ^{1}H NMR (DMSO-d_6): $\delta = 3.43$ (d, 2H), 4.27 (t, 1H), 6.79 (s, 1H), 7.20–7.29 (m, 2H), 7.50 (d, 1H), 7.59 (d, 1H), 8.70 (s, 3H, NH_3^+); ^{13}C NMR (DMSO-d_6): $\delta = 28.8$, 50.7, 105.7, 110.9, 120.8, 122.7, 123.9, 128.2, 152.1, 154.4, 169.8.

4.3.13.2. *rac*-2-Amino-3-(benzo[*b*]thiophen-2-yl)-propanoic acid *rac*-12b. Yield = 78%; mp 258 °C from water–ethanol (258 °C from water–ethanol[22]); HRMS: M^+ found (M^+ calculated for $C_{11}H_{11}NO_2S$): 221.05089 (221.05105); MS: *m*/*z* (%) = 222 (2.05, M+1), 221 (12.61, M), 204 (0.92), 176 (2.72), 175 (1.49), 174 (0.78), 165 (1.85), 160 (1.13), 150 (2.48), 149 (8.44), 148 (27.07), 147 (100.00), 146 (1.69), 145 (2.25), 143 (1.76), 134 (4.91), 121 (3.03), 116 (1.64), 115 (8.00), 114 (1.11), 108 (0.98), 104 (1.00), 103 (5.37), 102 (2.87), 101 (1.17), 95 (1.10); ^{1}H NMR (DMSO-d_6): $\delta = 3.45$–3.58 (m, 2H), 4.28 (t, 1H), 7.31–7.39 (m, 3H), 7.80 (d, 1H), 7.93 (d, 1H), 8.57 (s, 1H, NH_3^+); ^{13}C NMR (DMSO-d_6): $\delta = 30.6$, 52.6, 122.2, 123.3, 124.2, 124.4, 124.7, 137.2, 139.4, 139.5, 169.8.

4.3.13.3. *rac*-2-Amino-3-(benzofuran-3-yl)propanoic acid *rac*-12c. Yield = 92%; mp 240 °C from water (240 °C from water[20]); HRMS: M^+ found (M^+ calculated for $C_{11}H_{11}NO_3$): 205.07415 (205.07389); MS: *m*/*z* (%) = 206 (2.60, M+1), 205 (16.11, M), 160 (5.37), 133 (3.82), 132 (41.68), 131 (100.00), 130 (5.95), 118 (0.65), 115 (12.99), 107 (1.99), 106 (0.73), 105 (6.78), 104 (6.70), 103 (31.70), 102 (11.02), 101 (1.55), 98 (1.80); ^{1}H NMR (DMSO-d_6): $\delta = 3.30$ (d, 2H), 4.22 (t, 1H), 7.26–7.36 (m, 2H), 7.58 (d, 1H), 7.72 (d, 1H), 7.88 (s, 1H), 8.56 (s, 3H, NH_3^+); ^{13}C NMR (DMSO-d_6): $\delta = 24.2$, 51.5, 111.3, 113.4, 119.9, 122.6, 124.3, 127.3, 144.3, 154.6, 170.4.

4.3.13.4. *rac*-2-Amino-3-(benzo[*b*]thiophen-3-yl)-propanoic acid *rac*-12d. Yield = 75%; mp 280 °C from water (279–280 °C[23]); HRMS: M^+ found (M^+ calculated for $C_{11}H_{11}NO_2S$): 221.05133 (221.05105); MS: *m*/*z* (%) = 222 (2.55, M+1), 221 (15.82, M), 185 (4.76), 178 (2.08), 177 (1.58), 176 (4.81), 175 (6.03), 174 (4.02), 173 (2.11), 172 (2.38), 157 (1.31), 153 (1.25), 150 (1.79), 149 (8.52), 148 (24.05), 147 (100), 146 (1.52), 145 (2.38), 144 (1.36), 143 (3.20), 134 (4.08), 131 (1.19), 129 (1.99), 128 (1.97), 127 (1.61), 121 (1.95), 116 (1.85), 115 (7.48), 114 (1.83), 103 (5.49), 102 (2.41), 101 (1.15), 98 (1.38); ^{1}H NMR (DMSO-d_6): $\delta = 3.42$ (d, 2H), 4.14 (t, 1H), 7.38–7.45 (m, 2H), 7.64 (s, 1H), 7.93 (d, 1H), 8.00 (d, 1H), 8.58 (s, 3H, NH_3^+); ^{13}C NMR (DMSO-d_6): $\delta = 28.9$, 51.9, 121.7, 122.9, 124.2, 124.4, 125.8, 129.5, 138.3, 139.7, 170.3.

4.4. Enzymatic small scale reactions

4.4.1. Dynamic kinetic resolution of *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-ones *rac*-11a–d. In a typical small scale experiment, lipase (20 mg) was added to a solution of *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-one *rac*-**11a**–**d** (20 mg) in different alcohols

(methanol, ethanol, 1-propanol, 1-butanol) (1 mL) and the mixture was stirred at the room temperature. Samples (10 μL) were taken at intervals, diluted with hexane–isopropyl alcohol (9:1) (990 μL) and analyzed by HPLC using a Chiralpak IA column.

For improving the ee, different solvents were used as reaction media.

In a typical small scale experiment, Novozyme 435 (20 mg) was added into a solution of *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-one *rac*-**11a–d** (20 mg) and 2 equivalents of propanol in different kinds of solvents (1 mL) were added. The mixture was stirred at room temperature and samples (10 μL) were taken, diluted with hexane–isopropyl alcohol (9:1) (990 μL) and analyzed by HPLC using a Chiralpak IA column.

4.4.2. Kinetic resolution of *rac*-2-acetamido-3-(heteroaryl)propanoic acids *rac*-9a–d. *rac*-2-Acetamido-3-(heteroaryl)propanoic acid *rac*-**9a-d** (1 mmol) was suspended in water (10 mL). By adjusting the pH to 8 with LiOH solution (1.25 M), the suspension merged into solution. Then Acylase I (5 units, 12 mg) and $CoCl_2 \times 6H_2O$ (5 mg, 0.02 mmol) were added and the reaction mixture was stirred at 37 °C, while by the addition of LiOH solution (1.25 M), the pH of the solution was permanently kept between 7 and 8. After completion of L-2-acetamido-3-(heteroaryl)propanoic acid L-**9a–d** hydrolysis (approx. 24 h, checked by HPLC using an Astec Chirobiotic-Tag column with methanol and TEAA-buffer, pH 4.1, 80:20 (v/v) as eluent) the pH was adjusted to 1.5 with 5% HCl. The untransformed enantiopure D-2-acetamido-3-(heteroaryl)propanoic acid D-**9a–d** was filtered off and washed with deionized water (3 × 1 mL). The filtrate was heated with active charcoal (10 mg) to 90 °C for 2 min, cooled to room temperature, filtered, and applied to a Dowex 50X8 cation exchange resin column. Elution of the pure L-enantiomer of the 2-amino-3-(heteroaryl)propanoic acid L-**12a–d** occurred with 2 M ammonia solution.

4.5. Large scale enzymatic preparation of enantiopure L-2-amino-3-(heteroaryl)propanoic acids L-12a–d

Into a solution of *rac*-2-acetamido-3-(heteroaryl)propanoic acid *rac*-**9a–d** (5 mmol) in anhydrous dichloromethane (50 mL), a solution of dicyclohexylcarbodiimide (1350 mg, 6 mmol) in anhydrous dichloromethane (50 mL) was added dropwise at 0 °C. The reaction mixture was stirred for 5–10 min, the solid formed was filtered off and dichloromethane was removed under reduced pressure at room temperature. The formed *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-one *rac*-**11a–d** was dissolved in dioxane (65 ml) followed by the addition of Novozyme 435 (1300 mg) and propanol (600 mg, 750 μL, 10 mmol).

The mixture was stirred at the room temperature. After completion of the *rac*-4-((heteroaryl)methyl)-2-methyloxazol-5(4*H*)-one *rac*-**11a–d** propanolysis (reaction followed by HPLC as described in Section 4.4.1), the enzyme was filtered off and the solvent was removed under reduced pressure. The isolated L-**10a–d** as a semisolid was suspended into a vigorously stirred solution of sodium carbonate (0.053 g, 5 mmol) in water (8 mL) and the mixture was gently refluxed. After 2 h of heating, the solution was cooled to 5 °C and extracted with dichloromethane (3 × 10 mL). The aqueous phase was then acidified carefully with concentrated HCl solution. The deposited precipitate was filtered off and washed several times with cold water. The isolated L-2-acetamido-3-(heteroaryl)propanoic acid L-**9a–d** was then dissolved in water (10 mL) by adjusting the pH to 8 with LiOH solution (1.25 M). Then Acylase I (22 units, 60 mg) and $CoCl_2 \times 6H_2O$ (50 mg 0.2 mmol) were added and the reaction mixture was stirred at 37 °C, while by the addition of LiOH solution (1.25 M), the pH of the solution was kept between 7 and 8. After completion of the L-2-acetamido-3-(heteroaryl)-propanoic acid L-**9a–d** hydrolysis, the pH was adjusted to 1.5 with 5% HCl. The suspension formed was extracted with dichloromethane (3 × 10 mL). The aqueous phase was heated with active charcoal (50 mg) to 90 °C for 2 min, cooled at room temperature, filtered and applied to a Dowex 50X8 cation exchange resin column. Elution of the pure L-enantiomers of the 2-amino-3-(heteroaryl)propanoic acids L-**12a–d** occurred with 2 M ammonia solution.

Acknowledgements

Financial support from the Romanian Ministry of Education and Research (CEEX Contract No. 1480-6/07.04.2006) is gratefully acknowledged.

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