ChemPubSoc Europe

DOI: 10.1002/ejoc.201700174

EurJOC European Journal of Organic Chemistry

Full Paper

Enzymatic Hydrolysis

Covalently Immobilized Lipases are Efficient Stereoselective Catalysts for the Kinetic Resolution of *rac*-(5-Phenylfuran-2-yl)-β-alanine Ethyl Ester Hydrochlorides

Botond Nagy,[a,b] Zsolt Galla,[a] László Csaba Bencze,[b] Monica Ioana Toşa,[b] Csaba Paizs,[b] Enikő Forró,[a] and Ferenc Fülöp*[a]

Abstract: Lipase-catalyzed enzymatic resolution of several new, exotic and variously substituted *rac*-(5-phenylfuran-2-yl)-β-alanine ethyl esters was investigated. Given the structural instability of unsubstituted *rac*-(5-phenylfuran-2-yl)-β-alanine ethyl ester, we used the stable hydrochloride salt of this *rac*-heteroaryl-3-aminopropanoic acid ethyl ester as potential substrate to increase the scope of the reaction. Optimization experiments revealed an efficient procedure for both analytical- and preparative-scale (*S*)-selective hydrolysis of several racemic β-amino ester hydrochlorides in NH_4OAc buffer (20 mM, pH 5.8) at 30 °C. Enzymatic resolutions were performed with covalently bound lipase AK from *Pseudomonas fluorescens* and lipase PS from *Burkholderia cepacia* on Immobead T2-150 as catalyst. Seven out of eight new resolution products were successfully isolated and appropriately characterized.

Introduction

Natural and unnatural β-amino acids are valuable and versatile building blocks of pharmaceutically important compounds and biologically active natural products,[1] making them especially valuable for drug research and development.[2] Chiral β-amino acids are present in the structure of anticancer agents taxol[3] and bleomycin,[4] dolastatins and many others.[5] Furthermore, they are key structural elements of β-peptides and peptidomimetics[6] because peptides containing β-amino acids show remarkable stability against protease-type hydrolases.[7]

A large number of chemical and biochemical methods have been developed for the asymmetric synthesis of β-amino acids.[8] Ammonia lyases and aminomutases,[9] pyrimidine-catabolism enzymes,[10] hydantoinases,[11] β-transaminases,[12] and ω-transaminases[13] have been reported to be efficient biocatalysts for the preparation of enantiomerically pure β-amino acids. Lipases, however, have gained greater popularity and become the most widely used group of biocatalysts because of their ability to accept a wide range of substrates and their stability in organic solvents.[14] Indeed, they catalyze several asymmetric transformations of non-natural amino acid derivatives including enantioselective ring cleavage of β-lactams,[15] hydrolysis of N-protected- and non-protected β-amino esters,[16] N-acylation,[17] and transesterification reactions.[18]

Phenylfuran-2-yl moieties are involved as building blocks in the structure of mPGES-1 inhibitors based on dihydropyrimidin-2(1*H*)-one,[19] CXCR2/CXCR1 receptor antagonists,[20] and anthrax lethal factor inhibitors.[21] They also exhibit radical-scavenging activity and cytoprotective effects against oxygenases.[22] These heteroaromatic structures are not unknown to our research group. We have already described highly stereoselective, lipase- or baker's yeast-mediated procedures for the kinetic resolution of racemic 1-(5-phenylfuran-2-yl)ethanols, ethane-1,2-diols, and ethanones.[23] Recently, L-(5-phenylfuran-2-yl)-α-alanines were also synthesized by a sequential multienzyme process.[24]

In this work, we disclose our results on the lipase-catalyzed resolution of several new, exotically substituted phenylfuran-based β-amino esters (*rac*-**3a**–**d**), by applying enantioselective hydrolysis. The real challenge to overcome has been the low solubility of substrates in organic solvents.

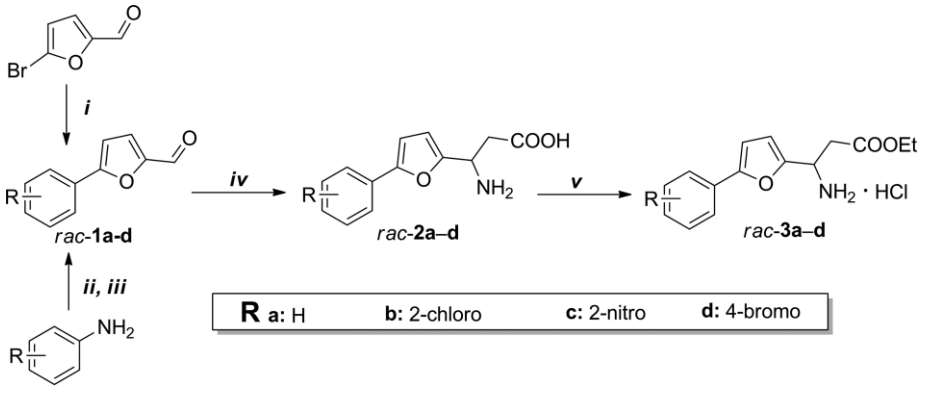
Results and Discussion

Synthesis of 3-Amino-3-(5-phenylfuran-2-yl)propionic Acid Ethyl Esters *rac*-3a–d

Racemic 3-amino-3-(5-phenylfuran-2-yl)propionic acid ethyl ester hydrochlorides (*rac*-**3a**–**d**·HCl) were prepared according to Scheme 1. 5-Phenylfuran-2-carbaldehyde **1a** was obtained by Suzuki–Miyaura coupling reaction between phenylboronic acid and 5-bromofuran-2-carbaldehyde in the presence of a catalytic amount of tetrakis(triphenylphosphine)palladium(0). Substituted phenylfuran derivatives **1b**–**d**, in turn, were synthesized

[a] *Institute of Pharmaceutical Chemistry, University of Szeged 6701 Szeged, Eötvös u. 6, Hungary*
E-mail: fulop@pharm.u-szeged.hu
http://www2.pharm.u-szeged.hu/gyki/index.php/en/
[b] *Faculty of Chemistry and Chemical Engineering, Biocatalysis and Biotransformation Research Centre 400028 Cluj-Napoca, Arany János str. 11, Romania*
https://www.researchgate.net/profile/Csaba_Paizs

Supporting information for this article is available on the WWW under https://doi.org/10.1002/ejoc.201700174.



Scheme 1. Chemical synthesis of racemic substrate molecules. Reagents and conditions: (i) Phenylboronic acid, $Pd(PPh_3)_4$, Na_2CO_3, toluene/EtOH; (ii) $NaNO_2$, HCl, 0–5 °C; (iii) Furan-2-carbaldehyde, $CuCl_2$, room temp., overnight; (iv) Ammonium acetate, $CH_2(COOH)_2$, AcOH, reflux; (v) $SOCl_2$, EtOH, –10 °C.

by Meerwein arylation, coupling furan-2-carbaldehyde with the corresponding aryl-diazonium halides in the presence of $CuCl_2$. The slightly modified Rodionov-type transformation of aldehydes into β-amino acids and a final esterification step of the latter compounds with ethanol induced by thionyl chloride afforded the desired *rac*-**3a**–**d**·HCl.

It is important to note that the liberation of *rac*-**3a**–**d** from the synthesized *rac*-**3a**–**d**·HCl was detected. However, the subsequent isolation and purification procedures resulted in the complete degradation of *rac*-**3a**–**d**, impairing their further chemical transformation or their use as substrate in enzymatic kinetic resolutions.

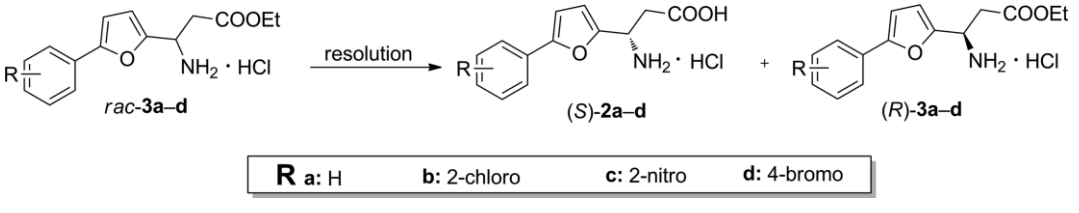
Enzymatic Kinetic Resolution through Enantioselective Hydrolysis

Because of the structural instability of the substrates, we investigated the lipase-mediated biotransformation of the more stable racemic phenylfuran-2-yl-β-alanine hydrochlorides *rac*-**3a**–**d**·HCl. This decision was based on our very recent results on enzyme-catalyzed dynamic kinetic resolution of racemic hydrochloric salts of 1,2,3,4-tetrahydroisoquinoline-1-carboxylates.[25] Specifically, our attention focused on the development of an efficient enzyme-assisted enantioselective ester hydrolysis of *rac*-**3a**–**d**·HCl (Scheme 2).

First, an enzyme screening was conducted in toluene containing 0.5 equiv. water with *rac*-**3a**·HCl as a model compound. NEt_3 (2–10 equiv.) was also added into the reaction mixture for the in situ deprotonation of *rac*-**3a**·HCl. Various enzymes, namely lipase A from *Candida antarctica* immobilized on Celite, lipase B from *Candida antarctica* immobilized by adsorption on hydrophobic acrylic resin (Novozyme 435), lipase PS from *Burkholderia cepacia* immobilized on diatomaceous earth (LPS-Diat), lipase from *Mucor miehei*, pancreatic porcine lipase PPL, lipase AK from *Pseudomonas fluorescens* immobilized on Celite (LAK-Cel), and lipase from *Candida rugosa* (CRL) were tested at 45 °C for the kinetic resolution of the in situ released *rac*-**3a**. In 19-hour reactions, lipase A from *Candida antarctica* and lipase from *Mucor miehei* proved to be inactive. PPL, Novozyme 435 and CRL, in turn, showed some activities (conversions between 35–40 %) but very low enantioselectivities (enantiomeric excesses below 70 % in all cases). Whereas LAK-Cel (4.8 % w/w protein content) displayed satisfactory activity and moderate enantioselectivity, to our delight, however, excellent enantioselectivities (E >> 200) were observed for LPS-Diat (6.7 % w/w protein content), as shown in Table 1, entries 1 and 6. Therefore, LPS-Diat was selected for further studies.

Distilled water and several organic solvents, such as methyl *tert*-butyl ether (MTBE), diisopropyl ether (DIIPE), 1,4-dioxane and 2-methyltetrahydrofuran were investigated as potential reaction media. Whereas excellent reaction rates, but very low enantioselectivities were observed in acyclic ethers (Table 1, entries 3 and 4), no catalytic activities were found when cyclic ethers were used. Although the biocatalytically formed (*S*)-**2a** precipitated as (*S*)-**2a**·HCl, the reproducibility of the small-scale enzymatic reactions was unsatisfactory because of the multiple heterogeneity of the reaction mixture.

Given that all β-amino acid hydrochlorides *rac*-**2a**–**d**·HCl and β-amino ester hydrochlorides *rac*-**3a**–**d**·HCl are water-soluble compounds, we turned our attention to the development of a reliable enzymatic kinetic resolution of *rac*-**3a**–**d**·HCl in aqueous media. Promising results were obtained for the unsubstituted



Scheme 2. Lipase-catalyzed enantioselective hydrolysis of (±)-**3a**–**d**.

Table 1. Preliminary results for the enzymatic hydrolysis of β-amino esters (±)-**3a**–**d**.[a]

Entry	Substrate[a]	Enzyme[a]	Solvent[a]	Temp. [°C]	Time [h]	ee_s [%][c]	ee_p [%][c]	Conv. [%]	*E*	Specific enzyme activity $\mu mol_{substrate}/(mg_{protein} \times min)$
1	*rac*-**3a**	LPS-Diat	toluene[b]	45	7	90	99	48	>>200	8.6×10^{-3}
2	*rac*-**3a**	LAK-Cel	toluene[b]	45	11	25	99	20	254	3.2×10^{-3}
3	*rac*-**3a**	LPS-Diat	MTBE[b]	45	1.5	99	27	78	7	65×10^{-3}
4	*rac*-**3a**	LPS-Diat	DIIPE[b]	45	1.5	99	45	69	12	57.5×10^{-3}
5	*rac*-**3a**	LPS-Diat	H_2O	45	1	95	95	50	146	62.5×10^{-3}
6	*rac*-**3a**	LPS-Diat	H_2O	30	2	96	99	49	>>200	30.6×10^{-3}
7	*rac*-**3b**	LAK-Cel	H_2O	30	4	83	96	47	102	20.4×10^{-3}
8	*rac*-**3c**	LAK-Cel	H_2O	30	8	40	78	34	12	7.4×10^{-3}
9	*rac*-**3d**	LAK-Cel	H_2O	30	8	25	85	22	16	4.8×10^{-3}

[a] Reaction conditions: substrate (0.015 mmol/mL), enzyme preparation (30 mg/mL), solvent (1 mL). [b] 5 equiv. NEt_3, 0.5 equiv. H_2O as reactant. [c] Based on HPLC analysis (see the Supporting Information).

ester (±)-**3a**·HCl with LPS-Diat in water at 45 °C (Table 1, entries 5 and 6). The poor enantiomeric excess of product (*S*)-**2a**·HCl at 45 °C can be attributed to the chemical hydrolysis of the amino ester, which can occur at high reaction temperature. Indeed, aqueous solutions of (±)-**3a**·HCl incubated at 4, 15, 20, 25, 30, and 45 °C without enzyme revealed that approximately 6 % chemical hydrolysis product was generated above 30 °C after 1 h incubation time. For this reason, 30 °C was selected as the standard reaction temperature for further studies.

LPS-Diat displayed low and continuously decreasing activities towards phenyl-substituted derivatives (±)-**3b**–**d**·HCl (conversion values were below 20 % in all cases after 24 h reaction time; data not shown). Therefore, a second enzyme-screening test was performed in water, and immobilized lipase AK on Celite (LAK-Cel) was found to be the most promising biocatalyst (Table 1, entries 7–9), although the stability of the enzyme preparation still diminished in time.

The significant decrease in the enzyme activity noted above can be ascribed to the structural instability of the enzyme preparations. LPS and LAK immobilized on the surface of Diatomite or Celite by adsorption are susceptible to desorption in aqueous media; indeed, water-soluble proteins were spectrophotometrically detected in the reaction mixtures by using the Bradford method. Moreover, free LAK and LPS tested in small-scale experiments proved to be completely inactive.

To enhance the stability of LPS and LAK, both enzymes were covalently bonded on Immobead T2-150 (see the Experimental Section), which is a hydrophobic support of methacrylate copolymers with epoxy groups having an average particle size of 0.15–0.30 mm. Multipoint covalent attachment between the oxirane groups and the free amino groups on the enzyme surface provides high stability for the enzyme preparation. The obtained LPS-IM and LAK-IM enzyme preparations, both with 9.4 % protein content, were found to be both highly stereoselective and stable biocatalysts. Small-scale reusability tests for LPS-IM and LAK-IM revealed that after 12 reaction cycles, both biocatalysts retained more than 90 % of their initial activity.

It is well known that enzyme stability, activity, and selectivity are strongly influenced by the ionic strength and pH of the solution.[26] Therefore, several buffers in various concentrations were tested as reaction medium instead of distilled water to improve the enzymatic kinetic resolution of *rac*-**3a**–**d**·HCl. Phosphate-citrate buffers and acetate at different concentration (20–500 mM) in the pH range of 4–6 were tested to enhance the operational parameters of LPS-IM and LAK-IM. It is important to note that in solutions with pH >6, deprotonation of *rac*-**3a**–**d**·HCl and subsequent decomposition of the released *rac*-**3a**–**d** was observed. Optimal biotransformation of *rac*-**3a**–**d**·HCl occurred in 20 mM ammonium acetate/acetic acid buffer at pH 5.8, as shown in Table 2.

Enzymatic reaction velocities calculated as $\mu mol_{substrate}/(mg_{protein} \times min)$[27] generally revealed a slightly increased enzymatic activity for covalently immobilized enzyme preparations (LAK-IM and LPS-IM) compared with physically adsorbed biocatalysts (LAK-Cel and LPS-Diat) as shown in Table 1 and Table 2. Nevertheless, the low structural stability of LAK-Cel and LPS-Diat in water impaired their efficacy for preparative-scale biotransformation of (±)-**3a**–**d**. Except for *ortho*-nitro derivative (±)-**3c**, covalently immobilized lipase AK from *Pseudomonas fluorescens* (LAK-IM) provides high selectivities, conversions and enantiomer excesses for all tested compounds (Table 2, entries 2, 4 and 6 vs. Table 1, entries 2, 7, 8 and 9). Lipase LPS, bonded

Table 2. LPS- and AK-catalyzed hydrolysis of substituted 5-phenylfuran-β-amino esters (±)-**3a**–**d**.[a]

Entry	Substrate	Enzyme[b]	Time [h]	ee_s [%][b]	ee_p [%][b]	Conv. [%]	*E*	Specific enzyme activity $\mu mol_{substrate}/(mg_{protein} \times min)$
1	*rac*-**3a**	LPS-IM	6	99	95	51	206	75.4×10^{-3}
2	*rac*-**3b**	LAK-IM	6	99	93	51	145	75.4×10^{-3}
3	*rac*-**3b**	LPS-IM	2	99	94	51	170	226.1×10^{-3}
4	*rac*-**3c**	LAK-IM	6	65	93	41	54	60.6×10^{-3}
5	*rac*-**3c**	LPS-IM	6	72	88	45	34	66.5×10^{-3}
6	*rac*-**3d**	LAK-IM	9	99	95	51	206	50.2×10^{-3}
7	*rac*-**3d**	LPS-IM	6	99	95	51	206	75.4×10^{-3}

[a] Reaction conditions: substrate (0.015 mmol/mL), enzyme preparation (3 mg/mL), 20 mM NH_4OAc, pH 5.8, 30 °C. [b] Based on HPLC analysis (see the Supporting Information).

Table 3. Preparative-scale transformations.

Entry	Substrate	Time [h]	Conv. [%]	(*R*)-**2**·HCl Yield [%][a]	*ee* [%][b]	$[\alpha]_D^{22}$	(*S*)-**2**·HCl Yield [%][a]	*ee* [%][b]	$[\alpha]_D^{22}$
1	(±)-**3a**	6	50	–	–	–	57	95	–15.5 (*c* 6; 5 % HCl[c])
2	(±)-**3b**	6	50	67	93	+2.7 (*c* 3; 5 % HCl[c])	93	94	–3.5 (*c* 2; 5 % HCl[c])
3	(±)-**3c**	6	48	30	80	+60 (*c* 1.5; CH_3COOH)	36	86	–77 (*c* 2; CH_3COOH)
4	(±)-**3d**	9	50	52	94	+9.7 (*c* 2; CH_3COOH)	72	95	–6.1 (*c* 1.6; CH_3COOH)

[a] Isolated yield calculated as percentage of the 50 % theoretical yield of the kinetic resolution. [b] Based on HPLC analysis (see the Supporting Information). [c] 5 % aqueous HCl solution.

on Immobead T2-150 (LPS-IM), also afforded significantly increased activity toward substituted 5-phenylfuran-2-yl β-amino esters (±)-**3b**–**d**·HCl in comparison with the LPS-Diat.

Preparative-Scale Biotransformations of Compounds (±)-3a–d·HCl

Preparative-scale hydrolysis of (±)-**3a**–**d**·HCl were carried out under the optimal conditions developed for the small-scale biotransformation (0.015 mg/mL substrate, 3 mg/mL enzyme, 20 mM NH_4OAc pH 5.8, 30 °C). The reactions were stopped at the time indicated in Table 3, by removing the enzyme through filtration. The unreacted (*R*)-**3a**–**d**·HCl and the formed (*S*)-**2a**–**d**·HCl were separated by using preparative C18 HPLC column (see the Experimental Section) and characterized as their hydrochloride salt. As an exception, purification of compound (*R*)-**2a** failed. Isolated yields, conversions, enantiomeric excesses and specific rotation values for the enantiomers are given in Table 3. For stability reasons, (*R*)-**3a**–**d**·HCl esters were hydrolyzed by heating in aqueous hydrochloric acid solution and characterized as amino acid hydrochloride salts.

The Absolute Configuration of the Resolution Products

The absolute configuration of the novel enantiopure β-amino ester (+)-**3d** was determined by a detailed ^1H NMR study of both diastereomers formed with (*R*)- and (*S*)-Mosher acids. Thus, the unreacted ethyl 3-amino-3-[5-(4-bromophenyl)furan-2-yl]-propanoate (+)-**3d** was N-acylated with (*R*)- and (*S*)-MTPA-Cl and the resulting diastereomers were differentiated by their ^1H NMR spectra (see the Supporting Information). Hereby, the (*R*)-(+)-**3d** absolute configuration was assigned for the unreacted enantiomer in the enzyme-catalyzed hydrolysis of *rac*-**3d**.

Based on the same sense of their specific rotation with (*R*)-(+)-**3d** and (*S*)-(–)-**2d**, the absolute configurations for the other enzymatic resolution products were assigned as (*R*)-(+)-**3a**–**c** and (*S*)-(–)-**2a**–**c**.

Conclusions

A synthetic method for the preparation of four novel, very exotic phenylfuran-2-yl-β-alanine esters was developed. The obtained stereoisomers were successfully resolved in their hydrochloride salt form through lipase-catalyzed enantioselective hydrolysis in aqueous media. The covalently immobilized LPS-IM and/or LAK-IM enzyme preparations proved to be excellent, (*S*)-selective biocatalysts for the asymmetric hydrolysis of (±)-**3a**–**d**·HCl in NH_4OAc buffer (20 mM, pH 5.8) at 30 °C with high enantioselectivities ($E > 145$), leading to enantiomers (*R*)-**2b**–**d** (*ee* > 99 %) and (*S*)-**2a**–**d** (*ee* = 93 %). The stable hydrochloride salts of (±)-**3a**–**d** were reliable substrates for their stereoselective biocatalytic hydrolysis in water, offering new possibilities for exploring enzymatic kinetic resolution of other unstable racemates.

Experimental Section

Materials and Methods: All starting materials and reagents were purchased from Sigma–Aldrich or Alfa-Aesar and used as received. Lipases from *Pseudomonas fluorescens* (LAK), from *Burkholderia cepacia* (LPS), from *Candida rugosa* (CRL), from *Mucor miehei*, from porcine pancreas (PPL) and lipase A from *Candida antarctica* were obtained from Sigma–Aldrich. Lipases from *Pseudomonas fluorescens* immobilized on Celite (LAK-Cel) was prepared by us.[28] Lipase from *Burkholderia cepacia* immobilized on diatomaceous earth (LPS-Diat) was a gift from Amano Enzyme Europe Ltd. Lipase B from *Candida antarctica* (Novozyme 435) was the product of Novozymes, Denmark. Immobead T2-150 was from ChiralVision, The Netherlands.

Covalent Immobilization of Lipase PS from *Burkholderia cepacia* and Lipase AK from *Pseudomonas fluorescens* on Immobead T2-150: Into a solution of Amano lipase from *Burkholderia cepacia* (LPS, 40 mg, 56 μg enzyme in 1 mg of liophylized preparation) or Amano lipase from *Pseudomonas fluorescens* (LAK, 40 mg, 53 μg enzyme in 1 mg of liophylized preparation) and Tween-80 (6.5 μL) in PBS buffer (20 mM Na_2HPO_4, 150 mM NaCl, pH 7, 6 mL) Immobead T2-150 (20 mg) was added and the mixture was shaken overnight at room temperature at 1350 rpm. The mixture was filtered and the biocatalyst was washed with water (3 × 10 mL) until no protein trace was detected in the filtrate. Finally, the immobilized enzymes were freeze-dried. The amount of immobilized enzyme on the support was determined as the difference between the total mass of the pure enzyme in the solution before immobilization (2.12 mg of LAK in 40 mg of liophylized powder and 2.24 mg of LPS in 40 mg of liophylized powder) and in the unified filtrates after immobilization (0.043 mg of LAK and, 0.16 mg of LPS respectively) determined spectrophotometrically by using the Bradford method. Immobilization yield: 92.9 % for LAK and 97.9 % for LPS, enzyme loading: ca. 94 μg protein/mg immobilized preparation for both, LPS-IM and LAK-IM.

Analytical-Scale Enzymatic Hydrolysis: A mixture of racemic substrate (0.015 mol) and lipase (3 mg of enzyme preparation/mL) was shaken in 20 mM NH_4OAc pH 5.8 buffer solution (1 mL) at 30 °C. Samples (6 μL) taken periodically were diluted with the mobile

phase (30 μL), filtered and injected on chiral CROWNPAK CR (+) HPLC column by using a mixture of $HClO_4$ aqueous solution (pH 2.3) and ACN 70:30 (v/v) as mobile phase (except when indicated otherwise; see the Supporting Information).

Preparative-Scale Enzymatic Hydrolysis: Racemic compounds **3a**–**d** (0.6 mmol) were dissolved in 20 mM NH_4OAc pH 5.8 buffer solution (40 mL). LPS-IM for (±)-**3a** (120 mg) and LAK-IM for (±)-**3b**–**d** (120 mg), were added and the mixture was shaken at 30 °C. After completion of the reaction (times indicated in Table 3), the immobilized enzyme was filtered out and the solvent was removed by freeze drying. The unreacted β-amino ester enantiomers (*R*)-**3a**–**d** and the obtained β-amino acid enantiomers (*S*)-**2a**–**d** were separated on achiral ZORBAX SB-C18 preparative column (21.2 × 250 mm) by using a mixture of aqueous HCl solution (100 mM) and MeOH as eluent (see Table 3 and the Supporting Information).

Acknowledgments

B. N. acknowledges financial support from the Székely Forerunner Fellowship Award and from the Sectorial Operational Program for Human Resources Development 2007-2013, cofinanced by the European Social Fund (project POSDRU/159/1.5/S/132400). L. C. B. thanks the Romanian National Authority for Scientific Research and Innovation, CNCS-UEFISCDI, project number PN-II-RU-TE-2014-4-1668 for financial support. The authors also acknowledge the receipt of OTKA Grants K-108943 and K-115731 for financial support.

Keywords: Hydrolases · β-Amino acid · Hydrolysis · Kinetic resolution · Enzyme catalysis

[1] a) J. Kimura, Y. Takada, T. Inayoshi, Y. Nakao, G. Goetz, W. Y. Yoshida, P. J. Scheuer, *J. Org. Chem.* **2002**, *67*, 1760–1767; b) P. Crews, L. V. Manes, M. Boehler, *Tetrahedron Lett.* **1986**, *27*, 2797–2800; c) P. Spiteller, F. Von Nussbaum, in *Enantioselective Synthesis of β-Amino Acids*, 2nd ed., Wiley-VCH, Weinheim, **2005**, pp. 19–91.

[2] a) H. H. Wasserman, H. Matsuyama, R. P. Robinson, *Tetrahedron* **2002**, *58*, 7177–7190; b) *Enantioselective Synthesis of β-Amino Acids*, 2nd ed., (Eds.: R. Juaristi, V. A. Soloshonok), Wiley-VCH, Weinheim, **2005**.

[3] M. C. Wani, H. L. Taylor, M. E. Wall, P. Coggon, A. T. McPhail, *J. Am. Chem. Soc.* **1971**, *93*, 2325–2327.

[4] H. Umezawa, K. Maeda, T. Takeuchi, Y. Okami, *J. Antibiot.* **1966**, *19*, 200–209.

[5] E. Juaristi, H. López-Ruiz, *Curr. Med. Chem.* **1999**, *6*, 983–1004.

[6] a) D. L. Steer, R. A. Lew, P. Perlmutter, A. I. Smith, M. I. Aguilar, *Curr. Med. Chem.* **2002**, *9*, 811–822; b) D. Seebach, T. Kimmerlin, R. Sebesta, M. A. Campo, A. K. Beck, *Tetrahedron* **2004**, *60*, 7455–7506; c) T. A. Martinek, F. Fülöp, *Chem. Soc. Rev.* **2012**, *41*, 687–702.

[7] a) P. Zubrzak, H. Williams, G. M. Coast, R. E. Isaac, G. Reyes-Rangel, E. Juaristi, J. Zabrocki, R. J. Nachman, *Pept. Sci.* **2007**, *88*, 76–82; b) J. Frackenpohl, P. I. Arvidsson, J. V. Schreiber, D. Seebach, *ChemBioChem* **2001**, *2*, 445–455.

[8] a) F. Fülöp, *Chem. Rev.* **2001**, *101*, 2181–2204; b) A. Liljeblad, L. T. Kanerva, *Tetrahedron* **2006**, *62*, 5831–5854; c) L. Kiss, F. Fülöp, *Chem. Rev.* **2014**, *114*, 1116–1169.

[9] a) N. J. Turner, *Curr. Opin. Chem. Biol.* **2011**, *15*, 234–240; b) L. Poppe, C. Paizs, K. Kovács, F. D. Irimie, B. Vértessy, *Meth. Mol. Biol.* **2012**, *794*, 3–19; c) M. M. Heberling, B. Wu, S. Bartsch, D. B. Janssen, *Curr. Opin. Chem. Biol.* **2013**, *17*, 250–260.

[10] A. I. Martinez-Gomez, J. M. Clemente-Jimenez, F. Rodriguez-Vico, L. T. Kanerva, X. G. Li, F. J. Las Heras-Vazquez, S. Martinez-Rodriguez, *Process Biochem.* **2012**, *47*, 2090–2096.

[11] M. O'Neill, B. Hauer, N. Schneider, N. J. Turner, *ACS Catal.* **2011**, *1*, 1014–1016.

[12] J. Kim, D. Kyung, H. Yun, B. K. Cho, J. H. Seo, M. Cha, B. G. Kim, *Appl. Environ. Microbiol.* **2007**, *73*, 1772–1782.

[13] a) D. Koszelewski, K. Tauber, K. Faber, W. Kroutil, *Trends Biotechnol.* **2010**, *28*, 324–332; b) H. S. Bea, H. J. Park, S. H. Lee, H. Yun, *Chem. Commun.* **2011**, *47*, 5894–5896.

[14] E. Busto, V. Gotor-Fernández, V. Gotor, *Chem. Soc. Rev.* **2010**, *39*, 4504–5423.

[15] E. Forró, F. Fülöp, *Mini-Rev. Org. Chem.* **2004**, *1*, 93–102.

[16] a) E. Forró, F. Fülöp, *Curr. Med. Chem.* **2012**, *19*, 6178–6187; b) H. Rangel, M. Carillo-Morales, J. M. Galindo, E. Castillo, A. Obregón-Zúñiga, E. Juaristi, J. Escalante, *Tetrahedron: Asymmetry* **2015**, *26*, 325–332; c) Y. Li, W. Wang, Y. Huang, Q. Zou, G. Zheng, *Process Biochem.* **2013**, *48*, 1674–1678; d) E. Forró, G. Tasnádi, F. Fülöp, *J. Mol. Catal. B* **2013**, *93*, 8–14.

[17] a) M. Shakeri, K. Engström, A. G. Sandström, J. E. Bäckvall, *ChemCatChem* **2010**, *2*, 534–538; b) M. Rodríguez-Mata, E. García-Urdiales, V. Gotor-Fernández, V. Gotor, *Adv. Synth. Catal.* **2010**, *352*, 395–406; c) M. Fitz, E. Forró, E. Vigóczki, L. Lázár, F. Fülöp, *Tetrahedron: Asymmetry* **2008**, *19*, 1114–1119.

[18] P. Flores-Sánchez, J. Escalante, E. Castillo, *Tetrahedron: Asymmetry* **2005**, *16*, 629–634.

[19] a) S. Terracciano, G. Lauro, M. Strocchia, K. Fischer, O. Werz, R. Riccio, I. Bruno, G. Bifulco, *ACS Med. Chem. Lett.* **2015**, *6*, 187–191; b) G. Lauro, M. Strocchia, S. Terracciano, I. Bruno, K. Fischer, C. Pergola, O. Werz, R. Riccio, G. Bifulco, *Eur. J. Med. Chem.* **2014**, *80*, 407–415; c) M. Strocchia, S. Terracciano, M. G. Chini, A. Vassallo, M. C. Vaccaro, F. D. Piaz, R. Riccio, I. Bruno, G. Bifulco, *Chem. Commun.* **2015**, *51*, 3850–3853.

[20] Y. Yu, M. P. Dwyer, J. Chao, C. Aki, J. Chao, B. Purakkattle, D. Rindgen, R. Bond, R. Mayer-Ezel, J. Jakway, H. Qiu, R. W. Hipkin, J. Fossetta, W. Gonsiorek, H. Bian, X. Fan, C. Terminelli, J. Fine, D. Lundell, J. R. Merritt, Z. He, G. Lai, M. Wu, A. Taveras, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 1318–1322.

[21] I. A. Schepetkin, A. I. Khlebnikov, L. N. Kirpotina, M. T. Quinn, *J. Med. Chem.* **2006**, *49*, 5232–5244.

[22] K. Nishio, A. Fukuhara, Y. Omata, Y. Saito, S. Yamaguchi, H. Kato, Y. Yoshida, E. Niki, *Bioorg. Med. Chem.* **2008**, *16*, 10332–10337.

[23] a) P. Hara, M. C. Turcu, R. Sundell, M. Toşa, C. Paizs, F. D. Irimie, L. T. Kanerva, *Tetrahedron: Asymmetry* **2013**, *24*, 142–150; b) L. C. Bencze, C. Paizs, M. I. Toşa, F. D. Irimie, *Tetrahedron: Asymmetry* **2010**, *21*, 356–364; c) L. C. Bencze, C. Paizs, M. I. Toşa, F. D. Irimie, *Tetrahedron: Asymmetry* **2011**, *22*, 675–683.

[24] L. C. Bencze, B. Komjáti, L. A. Pop, C. Paizs, F. D. Irimie, J. Nagy, L. Poppe, M. I. Toşa, *Tetrahedron: Asymmetry* **2015**, *26*, 1095–1101.

[25] E. Forró, R. Megyesi, T. A. Paál, F. Fülöp, *Tetrahedron: Asymmetry* **2016**, *27*, 1213–1216.

[26] T. P. Bennett, E. Frieden, *Modern Topics in Biochemistry*, Macmillan, London, **1969**.

[27] L. Gardossi, P. B. Poulsen, A. Ballesteros, K. Hult, V. K. Svedas, Đ. Vasić-Rački, G. Carrea, A. Magnusson, A. Schmid, R. Wohlgemuth, P. J. Halling, *Trends Biotechnol.* **2010**, *28*, 171–180.

[28] J. Brem, M. C. Turcu, C. Paizs, K. Lundell, M. I. Toşa, F. D. Irimie, L. T. Kanerva, *Proc. Biochem.* **2012**, *47*, 119–126.

Received: February 6, 2017

