Tetrahedron: *Asymmetry* 20 (2009) 1641–1645

Contents lists available at ScienceDirect

Tetrahedron: *Asymmetry*

journal homepage: www.elsevier.com/locate/tetasy

Enzymatic synthesis of carnosine derivatives catalysed by *Burkholderia cepacia* lipase

Paola D'Arrigo [a,*], Liisa T. Kanerva [b], Xiang-Guo Li [b], Caterina Saraceno [a], Stefano Servi [a], Davide Tessaro [a]

[a] *Dipartimento di Chimica, Materiali ed Ingegneria Chimica 'Giulio Natta', Politecnico di Milano and Centro Interuniversitario di Ricera in Biotecnologie Proteiche "The Protein Factory", Politecnico di Milano and Universita degli Studi dell' Insubria, Via Mancinelli 7, 20131 Milano, Italy*

[b] *Pharmacology, Drug Development and Therapeutics/Laboratory of Synthetic Drug Chemistry and Department of Chemistry, University of Turku, 20520-Turku, Finland*

ARTICLE INFO

Article history:
Received 6 April 2009
Accepted 12 May 2009
Available online 29 July 2009

ABSTRACT

A new enzymatic synthesis of α,β-dipeptides has been developed with particular focus on the preparation of carnosine (β-alanyl-α-histidine) and analogues. The lipase PS-D from *Burkholderia cepacia* has been used as a catalyst for the formation of the peptide bond starting from a β-lactam and a protected α-amino acid.

1. Introduction

Over the last few years, interest in β-amino acids and their use in the synthesis of β-dipeptides have increased significantly. This is due to the fact that this family of small molecules is of interest for both their pharmaceutical and chemical aspects. β-Peptides seem to function in ways similar to the corresponding α-peptides but with a lower affinity for blood cells and higher stability towards protease catalysed hydrolysis. For these reasons, they present interesting activities such as peptidomimetics, protease inhibitors and hormonal signals.[1] The chemical synthesis of α-peptides has been thoroughly reported and reviewed.[2] It has the drawback of using condensing agents (DCC for example) and repetitive protection–deprotection steps. Enzymatic approaches to obtain peptides, especially α-peptides, have been well investigated: in fact proteases are widely studied as catalysts for peptide bond formation, even if they show many limiting factors (hydrolysis of the peptide bond, substrate specificity). On the other hand, publications about the enzymatic synthesis of β-peptides are rare.[3,4]

Herein, we report an enzymatic α,β-dipeptide bond formation focused on the synthesis of carnosine. This is a simple endogenous α,β-dipeptide formed by β-alanine and L-histidine and was first identified over 100 years ago (see Fig. 1).[5] Carnosine is present in innervated tissues, muscles, cerebrospinal fluid, olfactory bulbs and lenses. This dipeptide is well known to act as a physiological buffer, metal ion chelator, immunomodulator, neurotransmitter and anti-tumour agent and to show antioxidant and free-radical scavenging activities,[6,7] even though the proper function is not fully understood. It is also a possible modulator of diabetic complications, atherosclerosis and Alzheimer's disease. Recent studies have shown that carnosine behaves as a multifunctional antioxidant acting as a natural endogenous scavenger of toxic aldehydes (from the slightly reactive glucose to the highly deleterious malondialdehyde).[8] In fact, reactive carbonyl species generated by lipid peroxidation, such as 4-hydroxy-*trans*-2-nonenal (HNE), acrolein, malondialdehyde and glyoxal, lead to covalently modified proteins contributing to oxidative damage.[9,10] It has been demonstrated that carnosine is able to quench HNE derived from lipid peroxidation and is involved in the pathogenesis of Alzheimer's disease.[11] These data also suggest that carnosine reduces the effect of α,β-unsaturated aldehydes on cellular macromolecules protecting them and reducing formation of products involved in processes such as cardiovascular ischaemic damage and inflammatory diseases where these aldehydes are highly involved.[12] The physiological and antioxidant activities of carnosine in vivo are limited by its susceptibility to hydrolysis by human cytosolic and serum carnosinases.[13] Structural modification of carnosine strongly modifies its biological properties.[14,15] Therefore there is considerable interest in the synthesis of substituted carnosines with higher biological stability in order to study their possible therapeutic applications.[16]

Scheme 1 outlines the strategy that we have followed: a lipase catalysed peptide bond formation namely the *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) lipase (lipase PS-D)-catalysed enantioselective ring opening of β-lactams (2-azetidinones) according to the recent synthesis of β-dipeptides and β-aminoamides.[17] In fact, β-lactams are versatile intermediates in various

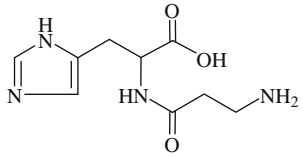
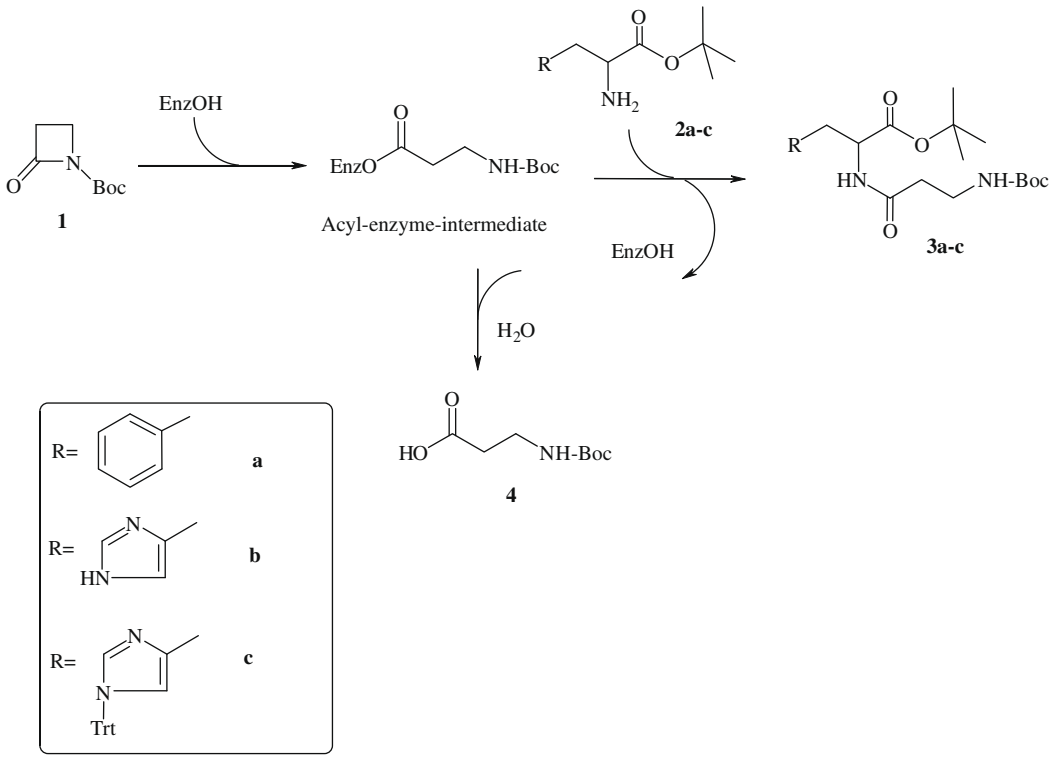


Figure 1. Structure of the dipeptide carnosine.

* Corresponding author. Tel.: +39 0223993075; fax: +39 0223993080.
E-mail address: paola.d'arrigo@polimi.it (P. D'Arrigo).

0957-4166/$ - see front matter © 2009 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tetasy.2009.05.040



Scheme 1. Mechanism of lipase-catalysed dipeptide formation.

types of synthetic products, for example, as acylating agents for coupling reactions with O- and N-nucleophiles.[18] In the present work, *N*-Boc-protected-2-azetidinone **1** was submitted to nucleophilic attack by α-amino acids, D- and L-phenylalanine D-**2a** and L-**2a** and D- and L-histidine D-**2b** and L-**2b**. *t*-Butyl esters were used in order to avoid collateral condensation between aminoacids and to prevent an interesterification-type reaction[19] between the β-lactam and the amino esters of small *n*-alcohols (methyl or ethyl esters). Boc-protection as a common N-protective group in peptide chemistry accomplishes activation of the carbonyl carbon of the β-lactam to ring opening.

Lipases (triacylglycerol-hydrolases, EC 3.1.1.3) are serine hydrolases which in Nature catalyse the hydrolysis and synthesis of long-chain triacylglycerides. Lipases work with a double displacement mechanism via an acyl-enzyme intermediate at the water–lipid interface, and an interfacial activation of the lipase is usually necessary. Lipases are generally stable and active in organic solvents and function without cofactors. These features have made lipases attractive in industrial use. In addition to their physiological substrates, lipases have broad substrate specificity, and although they catalyse peptide bond formation they rarely have been shown to possess amidase activity as opposed to serine proteases (which act with the same mechanism) such as chymotrypsin and subtilisin which hydrolyse both amides and esters.[20]

2. Results and discussion

The principle of the use of lipases in organic solvents is based on the formation of an acyl-enzyme intermediate in the reaction of an acyl donor (β-lactam **1** in the present case) with the serine residue at the catalytic centre followed by its reaction with any nucleophile [one of the amino esters **2a–c** in the present case; Scheme 1]. As shown in Scheme 1, the difference to the normal lipase-catalysed reaction, where two substrates react to two new products, is that there is no product release in the formation of the intermediate resulting in the dipeptide [one of **3a–c**] as the only product. When the nucleophile is an α-amino acid, an α/β-dipeptide is formed. On the basis of previous works, we expected to see two competitive side-reactions in the formation of the desired α/β-dipeptide: the hydrolysis of the acyl-enzyme intermediate by water in the seemingly dry lipase PS-D preparation yielded **4** and the chemical dipeptide.[19,20] Amino esters **2a–c** have been used in excess in order to favour the aminolysis over the hydrolysis of the β-lactam, and have been used as pure enantiomers to ensure the formation of enantiopure **3a–c** in particular *t*-butyl esters, in order to prevent interesterification[21] between **1** and simple amino esters.

This strategy was first applied to the synthesis of a model dipeptide: *N*-Boc-2-azetidinone **1** was subjected to reaction with L- and D-**2a** in diisopropyl ether (DIPE) in the presence of lipase PS-D (see Scheme 1 and Table 1). Reactions were monitored by HPLC and the conversion of the β-lactam **1** was measured towards a standard reference (homoveratronitrile or naphthalene). Compound 1 disappeared completely within 6 h (see Fig. 2).

Table 1
Specific rotations {$[\alpha]_D^{24}$ (*c*, $CHCl_3$)} of α-amino esters and α/β-dipeptides and isolated yields of **3** for the lipase PS-D-catalysed reaction between **1** and **2a–c**

Entry	α-Amino ester[a]	**2**, $[\alpha]_D^{24}$ (*c*)[b]	Dipeptide **3**	**3**, Yield (%)	**3**, $[\alpha]_D^{24}$ (*c*)[b]
1	L-**2a**	+10.1 (1.01)	L-**3a**	57	+26.4 (0.72)
2	D-**2a**	−10.5 (1.01)	D-**3a**	54	−25.9 (0.97)
3	L-**2b**	+4.05 (1.08)	L-**3b**	53	+5.5 (1.25)
4	D-**2b**	−3.96 (1.03)	D-**3b**	57	−6.1 (1.05)
5	L-**2c**	−4.02 (1.09)	L-**3c**	52	+9.6 (1.46)

[a] ee 99% or higher.
[b] mg/mL.

Since chemical dipeptide formation can be significant in addition to the enzymatic one, the reaction was also studied in the absence of the enzyme. As can be seen from results in Figure 2, the chemical reaction is insignificant with respect to the reaction in

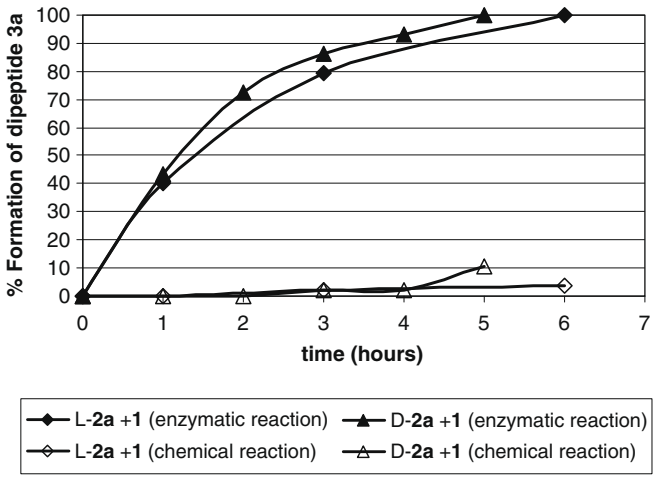


Figure 2. Formation of dipeptides L- and D-**3a** in the reaction of **1** with L-**2a** and D-**2a**. The enzymatic and the chemical reactions are reported.

the presence of lipase PS-D. Thus, we can conclude that the ring-opening reaction is enzyme-catalysed. The yields of the isolated dipeptides L- and D-**3a** are around 55% (see Table 1, entry 1 and entry 2). Since the reaction produces practically the same amount of dipeptide regardless of the amino ester absolute configuration, we can also conclude that the enzymatic peptide formation is not enantioselective with respect to the nucleophile.

The same procedure was applied to the synthesis of carnosine derivative L-**3c** (Scheme 1). The reactions were carried out in the presence of lipase PS-D in *t*-butyl methyl ether (MTBE). The kinetics of the two enantiomers L-**2b** and D-**2b** were again quite similar. The reaction with L-**2b** completed in 22 h while the chemical reaction at the same time reached ca. 60% conversion. Reactions on larger scales were then performed leading to final yields of 57% for L-**3b** and 56% for D-**3b**.

Since **2b** was not completely soluble in MTBE, the possibility of using a cosolvent in order to facilitate substrate dissolution was considered. *t*-Amyl alcohol was employed as a cosolvent in MTBE. However, the enzymatic reaction then stopped at 75% conversion in less than 10 h. In order to improve the solubility in an organic medium, a more hydrophobic substrate in the **c** series was obtained by protecting the so-called τ-nitrogen of histidine with the triphenylmethyl (Trt) group (L-**2c**). When the enzymatic reaction of **1** was carried out using L-**2c** as a nucleophile in MTBE 100% conversion was reached in 24 h while the non-enzymatic reaction proceeded to only 27% conversion. From the results of the enzymatic reaction, L-**3c** was isolated in 52% yield after a reaction time of 24 h. The effect of the substrate modification is thus significant in modifying the enzymatic versus non-enzymatic peptide bond formation. The definition of a structure–reactivity relationship requires more investigation.

3. Conclusion

Herein, we have reported a new enzymatic synthesis of α,β-dipeptides using lipase PS-D from *B. cepacia* as a catalyst for the formation of a peptide bond. The method is based on the reaction of the activated azetidinone in the form of the *N*-Boc derivative **1** with nucleophiles (protected α-amino acids) **2a–c** in an organic media (DIPE or MTBE) with a cosolvent (*t*-amyl alcohol) to improve the solubility of the nucleophile. The activated azetidinone **1** is also prone to hydrolysis (non anhydrous conditions) or to non-enzyme-catalysed reaction with the amino acid derivative. The enzyme-catalysed reaction is not selective for the stereochemistry of the incoming nucleophile. Although the enzyme-catalysed reaction and the spontaneous chemical one afford the same product it is interesting to evaluate the increase in reaction rate in the enzymatic reaction. This is very large in the case of the preparation of **3a**, while it is reduced for **3b** and **3c**. The method is useful for the synthesis of carnosine derivatives of both absolute configurations.

4. Experimental

4.1. General experimental details

All chemicals were purchased from Sigma–Aldrich. Trityl-Histidine was a gift from Flamma s.p.a., Chignolo d'Isola, Italy. Solvents were of analytical grade. Lipase PS-D from *B. cepacia* immobilised on diatomite particles was from Amano Europe, England. ^{1}H NMR spectra were recorded on Bruker ARX 400 instrument operating at the ^{1}H resonance frequency of 400 MHz. Chemical shifts (δ, ppm) are reported relative to tetramethylsilane (TMS) as an internal standard. All spectra were recorded at 305 K. Mass spectra were recorded on a ESI/MS Bruker Esquire 3000 by direct infusion of methanol solution of compounds. Silica Gel 60 F_{254} plates (Merck) were used for analytical TLC. Detection was achieved with UV light followed by I_2 staining or ninhydrin or potassium permanganate.

HPLC analyses were performed on an Agilent 1100 series apparatus with a UV detector. The columns which have been used are a Phenomenex Luna C18 column, length/internal diameter = 125/4, temperature 30 °C, mobile phase $CH_3COONEt_3$ (0.1%) pH 4.53/CH_3CN from 6/4 to 8/2; 1 mL min^{-1} and a Chiracel OD column with hexane/2-propanol from 95/5 to 98/2 with 0.1% of diethylamine as basicity corrector, 0.9 mL min^{-1}, room temperature. The 20 μL samples of CH_3OH solution of 1 mg mL^{-1} were injected into the column at a mobile phase flow rate of 1 ml/min. Optical rotations were determined with a Propol Digital Polarimeter Dr. Kenchen, and $[\alpha]_D$ values are given in units of deg cm^2 g^{-1} at 25 °C.

4.2. Synthesis

4.2.1. *N*-Boc-2-azetidinone 1

2-Azetidinone (200 mg, 2.81 mmol) was dissolved in CH_2Cl_2 (5 mL) and DMAP (34.4 mg, 0.281 mmol) was added. A solution of Boc_2O (921 mg, 4.22 mmol) in CH_2Cl_2 (2 mL) was added to the mixture while stirring at room temperature. The reaction was stopped after 2 h, after which the solvent was removed under vacuum and the oily residue obtained was applied to a column for chromatographic purification, using a mixture of hexane/ethyl acetate (8/2, v/v) as an eluent. A colourless oil was isolated as product **1** (327 mg) in 68% yield. ^{1}H NMR ($CDCl_3$): δ = 3.53 (t, 2H J = 5.08 Hz,), 2.96 (t, 2H J = 5.08 Hz), 1.50 (s, 9H). ESI/MS: $[M+Na]^+$ = 193.8.

4.2.2. L- and D-Phenylalanine *t*-butyl esters L-2a and D-2a

L- or D-Phenylalanine (1.00 g, 6.053 mmol) was charged in a round-bottomed flask equipped with a magnetical stirrer and added to AcO^tBu (25 mL). The obtained suspension was treated with 70% $HClO_4$ (d = 1.664 g mL; 0.548 ml, 0.912 g, 9.080 mmol) to give a homogeneous solution. The reaction was stirred overnight at room temperature and then extracted with water (3 × 30 mL) and HCl 0.5 M (2 × 30 mL). The combined aqueous layers were added to solid K_2CO_3 at pH 9 and then extracted with CH_2Cl_2 (3 × 80 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to dryness to afford the product **2a** as a yellow oil (0.85 g, 55% yield).

L-Phenylalanine *t*-butyl ester L-**2a**: ^{1}H-NMR ($CDCl_3$): δ 7.30–7.20 (m, 5H), 3.66 (dd, J = 1.6 Hz, 5.78 Hz, 1H), 3.04 (dd, J = 13.6 Hz, 5.78 Hz, 1H), 2.91 (dd, J = 13.7 Hz, 7.71 Hz, 1H), 2.41 (br s, 2H),

1.42 (s, 9H); $[\alpha]_D^{24} = +10.1$ (*c* 1.01, $CHCl_3$); ESI/MS: $[M+H]^+$ = 221.9 $[M+Na]^+$ = 243.9.

D-Phenylalanine *t*-butyl ester D-**2a**: $[\alpha]_D^{24} = -10.5$ (*c* 1.01, $CHCl_3$).

4.2.3. L- and D-Histidine *t*-butyl ester L-2b and D-2b

L- or D-Histidine (0.50 g, 3.22 mmol) was charged in a round-bottomed flask equipped with a magnetic stirrer and suspended in AcO^{t}Bu (25 mL). The suspension was treated with $HClO_4$ (0.274 mL, 0.456 g, 4.54 mmol) while stirring to give a homogeneous solution. The reaction was stirred overnight at room temperature and then extracted with water (3 × 30 ml) and HCl 0.5 M (2 × 30 mL). The combined aqueous layers were added to solid K_2CO_3 at pH 9 and then extracted with CH_2Cl_2 (3 × 80 mL). The combined organic layers were dried over Na_2SO_4 and evaporated to dryness to afford the product **2b** as a yellow oil (0.330 g; 48.4% yield).

L-Histidine *t*-butyl ester L-**2b**: ^{1}H NMR ($CDCl_3$): δ: 7.53 s, 1H, 6.84 (s, 1H), 4.96 (br s, 2H), 3.66 (dd, *J* = 4.04 Hz, 3.67 Hz, 1H), 3.05 (dd, *J* = 14.76 Hz, 3.85 Hz, 1H), 2.85 (dd, *J* = 14.73 Hz, 8.03 Hz, 1H), 1.44 (s, 9H); $[\alpha]_D^{24} = +4.05$ (*c* 1.08, $CHCl_3$); ESI/MS: $[M+H]^+$ = 221.9 $[M+Na]^+$ = 243.9.

D-histidine *t*-butyl ester D-**2b:** $[\alpha]_D^{24} = -4.0$ (*c* 1.03, $CHCl_3$).

4.2.4. L-Trt-Histidine *t*-butyl ester L-2c

Compound L-**2c** was prepared as described for **2b** starting from Trt-His (1 g; 2.125 mmol). The obtained residue was purified by chromatography using a mixture of CH_2Cl_2/CH_3OH (9/1, v/v) as an eluent. A pale yellow foam was isolated as product **2c** (0.183 g) in 20% yield. ^{1}H NMR ($CDCl_3$): δ 7.35 s, 1H, 7.32 m, 9H), 7.12 (m, 6H), 6.62 (s, 1H), 3.67 (dd, *J* = 5.14 Hz, 4.0 Hz, 1H), 2.91 (dd, *J* = 14.53 Hz, 4.04 Hz, 1H), 2.70 (dd, *J* = 14.31 Hz, 7.72 Hz, 1H), 1.91 (br s, 2H), 1.39 (s, 9H). $[\alpha]_D^{24} = -4.0$ (*c* 1.09, $CHCl_3$). ESI/MS: $[M+H]^+$ = 454.0 $[M+Na]^+$ = 476.1.

4.2.5. Enzymatic synthesis of peptides using Lipase PS-D

N-Boc-2-azetidinone **1** (51.4 mg, 0.25 mmol,) and α-amino acid **2a**–**c** (0.5 mmol) were dissolved in 5 mL of solvent (DIPE or MTBE mixed with 5% of *t*-amyl alcohol in case of reactions involving L- and D-histidine *t*-butyl ester L-**2b** or D-**2b**). The obtained solution was added to lipase PS-D (60 mg/mL). The mixture was stirred at 37 °C, with samples taken in order to measure the conversion of substrates. After the reaction was completed, the enzyme was filtered off and the solvent was evaporated under vacuum. The residue obtained was purified by chromatographic column.

4.2.6. *N*-Boc-β-alanyl-L-phenylalanine *t*-butyl ester L-3a

N-Boc-2-azetidinone **1** (51.4 mg, 0.25 mmol,) and α-amino acid L-**2a** (110.6 mg, 0.5 mmol) were dissolved in 5 mL of solvent (DIPE). The obtained solution was added to Lipase PS-D (60 mg/mL). The mixture was stirred at 37 °C, with samples taken in order to measure the conversion of substrates. After the reaction was completed, the enzyme was filtered off and the solvent was evaporated under vacuum. The obtained residue was purified by chromatographic column using hexane/ethylacetate (1/1, v/v) as an eluent and afforded 44.5 mg of L-**3a** (56.7% yield). ^{1}H NMR ($CDCl_3$): δ 7.23–7.06 (m, 5H), 5.92 (br s, NH), 4.67 (dd, *J* = 5.88 Hz, 13.80 Hz, 1H), 3.29 (dd, *J* = 5.88 Hz, 6.25 Hz, 2H), 3.00 (dd, *J* = 6.25 Hz, 13.95 Hz, 2H), 2.29 (t, *J* = 6.25 Hz, 2H,), 1.36 (s, 9H), 1.34 (s, 9H). $[\alpha]_D^{24} = +26.4$ (*c* 0.72, $CHCl_3$). ESI/MS: $[M+H]^+$ = 393.1 $[M+Na]^+$ = 415.1.

4.2.7. *N*-Boc-β-alanyl-D-phenylalanine *t*-butyl ester D-3a

N-Boc-2-azetidinone **1** (51.4 mg, 0.25 mmol,) and α-amino acid D-**2**a (110.6 mg, 0.5 mmol) were dissolved in 5 mL of solvent (DIPE). The obtained solution was added to Lipase PS-D (60 mg/mL). The mixture was stirred at 37 °C, taking samples to measure conversion of substrates. After the reaction was completed, the enzyme was filtered off and the solvent was evaporated under vacuum. The obtained residue was purified by chromatographic column using hexane/ethylacetate (1/1; v/v) as an eluent and afforded 42.4 mg of D-**3a** (54% yield). ^{1}H NMR: ($CDCl_3$) δ 7.22–7.06 (m, 5H), 5.91 (br s, 1H, NH), 4.74 (q, *J* = 6.25 Hz, 13.60 Hz, 1H), 3.36 (q, *J* = 5.88 Hz, 2H), 3.08 (dd, 2H, *J* = 5.88 Hz, 6.25 Hz), 2.36 (t, 2H, *J* = 5.88), 1.36 (s, 9H), 1.34 (s, 9H). $[\alpha]_D^{24} = -25.9$ (*c* 0.97, $CHCl_3$). ESI/MS: $[M+H]^+$ = 393.1 $[M+Na]^+$ = 415.1.

4.2.8. *N*-Boc-β-alanyl-L-histidine *t*-butyl ester (*N*-Boc-L-carnosine *t*-butyl ester) L-3b

N-Boc-2-azetidinone **1** (51.4 mg, 0.25 mmol,) and α-amino acid L-**2b** (105.6 mg, 0.5 mmol) were dissolved in 5 mL of solvent (DIPE or MTBE mixed with 5% of *t*-amyl alcohol). The solution obtained was added to Lipase PS-D (60 mg/mL). The mixture was stirred at 37 °C, and samples were taken in order to measure the conversion of the substrates. After the reaction was completed, the enzyme was filtered off and the solvent was evaporated under vacuum. The obtained residue was purified by chromatographic column using as eluent a mixture of CH_2Cl_2/MeOH from 95/5 to 9/1 to afford 65 mg of L-**3b** (56% yield). ^{1}H NMR ($CDCl_3$): δ 7.63 (s, 1H), 6.99 (br s, 1H), 6.83 (s, 1H), 5.54 (br s, 1H), 4.68 (dd, *J* = 12.01 Hz, 5.08 Hz, 1H), 3.41 (m, 2H), 3.09 (t, *J* = 5.2 Hz, 2H), 2.42 (t, *J* = 5.72 Hz), 1.43 (s, 9H), 1.42 (s, 9H). $[\alpha]_D^{24} = +5.5$ (*c* 1.25, $CHCl_3$). ESI/MS: $[M+Na]^+$ = 405.1 $[M+K]^+$ = 421.1.

4.2.9. *N*-Boc-β-Alanyl-D-histidine *t*-butyl ester (*N*-Boc-D-carnosine *t*-butyl ester) D-3b

N-Boc-2-Azetidinone **1** (51.4 mg, 0.25 mmol,) and α-amino acid D-**2b** (105.6 mg, 0.5 mmol) were dissolved in 5 mL of solvent (DIPE or MTBE mixed with 5% of *t*-amyl alcohol). The obtained solution was added to Lipase PS-D (60 mg/mL). The mixture was stirred at 37 °C, and samples were taken in order to measure the conversion of the substrates. After the reaction was completed, the enzyme was filtered off and the solvent was evaporated under vacuum. The obtained residue was purified by chromatographic column using as eluent a mixture of CH_2Cl_2/MeOH from 95/5 to 9/1 to afford 65 mg of D-**3b** (56,7% yield). ^{1}H-NMR: ($CDCl_3$) δ 7.57 s, 1H), 6.97 (br s, 1H), 6.80 (s, 1H), 5.56 (br s, 1H), 4.68 (q, *J* = 5.40 Hz, 12.6 Hz, 1H), 3.41 (m, 2H), 3.08 (t, *J* = 4.70 Hz, 2H), 2.40 (t, *J* = 5.72 Hz, 2H), 1.43 (s, 9H), 1.41 (s, 9H). $[\alpha]_D^{24} = -6.1$ (*c* 1.05, $CHCl_3$). ESI/MS: $[M+H]^+$ = 383.1 $[M+Na]^+$ = 405.1.

4.2.10. *N*-Boc-β-alanyl-L-Trt-histidine *t*-butyl ester (*N*-Boc-L-Trt-carnosine *t*-butyl ester) L-3c

N-Boc-2-azetidinone **1** (51.4 mg, 0.25 mmol,) and α-amino acid L-**2c** (110.6 mg, 0.5 mmol) were dissolved in 5 mL of solvent (DIPE). The solution obtained was added to Lipase PS-D (60 mg/mL). The mixture was stirred at 37 °C, and samples were taken in order to measure the conversion of the substrates. After the reaction was completed, the enzyme was filtered off and the solvent was evaporated under vacuum. The obtained residue was purified by chromatographic column using as eluent a mixture of CH_2Cl_2/MeOH 98/2 and afforded 81 mg of desired L-**3c** (52% yield). ^{1}H NMR ($CDCl_3$): δ 7.39 (s, 1H), 7.32 (m, 9H), 7.15 (m, 6H), 6.57 (s, 1H), 6.02 (br s, 1H), 4.68 (dd, *J* = 11.99 Hz, 4.41 Hz, 1H), 3.41 (dd, *J* = 5.88 Hz, 5.51 Hz, 2H), 3.00 (dd, *J* = 13.98 Hz, 4.78 Hz, 2H), 2.40 (dd, J = 5.51, 5.88 Hz, 2H), 1.34 (s, 9H), 1.26 (s, 9H). $[\alpha]_D^{24} = +9.6$ (*c* 1.46, $CHCl_3$) ESI/MS: $[M+H]^+$ = 652.2, $[M+Na]^+$ = 675.2.

References

1. Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodivers.* **2004**, *1*, 1111–1139.
2. Kimmerlin, T.; Seebach, D. *J. Peptide Res.* **2005**, *65*, 229–260.

3. Heck, T.; Kohler, H. P. E.; Limbach, M.; Flogel, O.; Seebach, D.; Geueke, B. *Chem. Biodivers.* **2007**, *4*, 2016–2030.
4. Liljeblad, A.; Kanerva, L. T. *Tetrahedron* **2006**, *62*, 5831–5854.
5. Gulewitsch, V. S.; Amiradzibi, S. *Ber. Dtsch. Chem. Ges.* **1900**, *33*, 1902–1908.
6. Quin, P. J.; Boldyrev, A. A.; Formazuyk, V. E. *Mol. Aspects Med.* **1992**, *13*, 379–444.
7. Hipkiss, A. R.; Preston, J. E.; Himswoth, D. T. M.; Worthington, V. C.; Abbot, N. J. *Neurosci. Lett.* **1997**, *238*, 135–138.
8. Dukic-Stefanovic, S.; Schinzel, R. *Biogerontology* **2001**, 19–34.
9. Carini, M.; Aldini, G.; Beretta, G.; Arlandini, E.; Maffei Facino, R. *J. Mass Spectrom.* **2003**, *38*, 996–1006.
10. Romero, F. J.; Bosch-Morell, F.; Romero, M. J.; Jareno, E. J.; Romero, B.; Marin, N.; Roma, J. *Environ. Health Perspect.* **1998**, *106*, 1229–1234.
11. Guiotto, A.; Ruzza, P.; Babizhayev, M. A.; Calderan, A. *Bioorg. Med. Chem.* **2007**, *15*, 6158–6163.
12. Aldini, G.; Carini, M. *Biochem. Biophys. Res. Commun.* **2002**, *298*, 699–706.
13. Zhou, S.; Decker, E. A. J. *Agric. Food Chem.* **1999**, *47*, 51–55.
14. Pegova, A.; Abe, H.; Boldyrev, A. *Comp. Biochem. Physiol. Part B* **2000**, *127*, 443–446.
15. Boldyrev, A.; Abe, H. *Cell. Mol. Neurobiol.* **1999**, *19*, 163–175.
16. Cacciatore, I.; Cocco, A.; Costa, M.; Fontana, M.; Lucente, G.; Pecci, L.; Pinnen, F. *Amino Acids* **2005**, *28*, 77–83.
17. Li, X.-G.; Lahititie, M.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2008**, *19*, 1857–1861.
18. Palomo, C.; Aizpurua, J. M.; Ganboa, I.; Oiarbide, M. *Synlett* **2001**, 1813–1826.
19. Li, X.-G.; Lähitie, M.; Päiviö, M.; Kanerva, L. T. *Tetrahedron: Asymmetry* **2007**, *18*, 1567–1573.
20. Henke, E.; Bornscheuer, U. T. *Anal. Chem.* **2003**, *75*, 255–260.
21. Li, X.-G.; Kanerva, L. T. *Adv. Synth. Catal.* **2006**, *348*, 197–205.