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Naphthyl-L-α-amino acids via chemo-enzymatic dynamic kinetic resolution

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ABSTRACT

A double catalyst system (protease + base) was applied to the dynamic kinetic resolution (DKR) of isomeric 1- and 2-α-naphthyl-glycines and -alanines exploiting the in situ racemization of their thioesters. Due to the different C-acidity of the two sets of compounds, different experimental conditions have been devised to perform the simultaneous resolution/racemization process.

In all cases, the racemic *N*-Boc-thioesters were converted into the aminoacids with an L-configuration almost quantitatively and with complete enantioselectivity.

1. Introduction

Non-proteinogenic α-amino acids, because of their absolute configuration or their chemical structure, are often defined as non-natural even though some of them occur in nature.[1–5] They belong to a group of building blocks that are required for the preparation of peptide mimetics and new bio active compounds.[6–12] They are often chiral and, although they can be obtained by an asymmetric synthesis,[13–16] the most commonly used methods require the preparation of the racemate followed by a kinetic resolution step.[17–29] Naphthyl amino acids (Fig. 1) form a special group of non-natural α-amino acids because of their resemblance to tryptophan, phenylalanine, and tyrosine. Moreover, they are ideal candidates as enzyme inhibitors, components of new drugs or analogues of peptide based existing drugs,[30–41] chiral auxiliaries, components in analytical devices, and in asymmetric synthesis.[42–45] They can be obtained by asymmetric synthesis,[46–48] but many of the approaches also rely on enzymatic catalysis, either via kinetic resolution methods[49,54] or enzymatic deracemization through microbial dynamic kinetic resolution (DKR)[50] or multistep enzyme catalyzed stereoinversion.[51]

We have recently accomplished a multistep cascade deracemization of 3-(2-naphthyl)-L-alanine **3**, by tuning the substrate specificity of the key enzyme D-amino acid oxidase from the yeast *Rhodotorula gracilis* (RgDAAO) by directed evolution to accept this non-natural amino acid.[51] The three enzyme-catalyzed system is summarized in Scheme 1: RgDAAO oxidizes the D-enantiomer to the corresponding ketoacid (2-naphthyl pyruvate 2-NPA), which in turn is transformed into the L-enantiomer by the L-specific aspartate amino transferase (L-AspAT). The amino donor cysteine sulfinic acid (L-CSA) decomposes into pyruvate and sulfur dioxide, thus shifting the equilibrium toward the product. One advantage

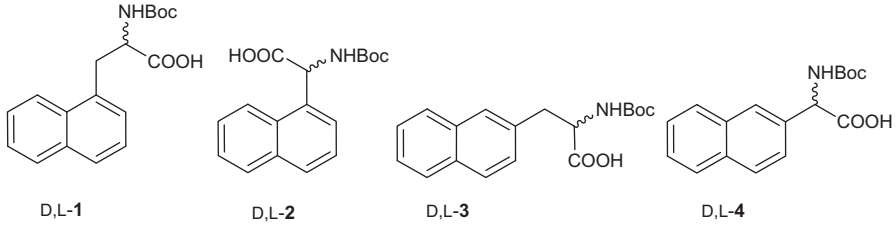

Figure 1. Structure of naphthyl amino acids **1**–**4**.

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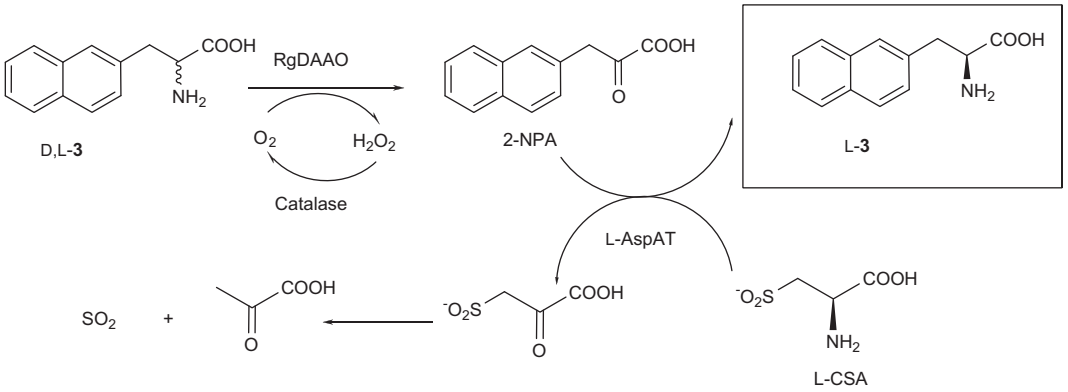


Scheme 1. Multistep cascade deracemization of 3-(2-naphthyl)-L-alanine D,L-**3**.

of this enzymatic cascade reaction is the fact that the reaction runs in one single reactor and that, compared with processes in which the α-ketoacid is directly employed as a substrate for the L-AspAT, the starting material already contains half of the end product and therefore the E-factor[52] is much more favorable. However, one drawback to this system is the extremely low water solubility of the substrate in water.

Such low water solubility was exploited in an enzyme-catalyzed process driven by the crystallization of the product formed.[53] In this approach, 3-(2-naphthyl)-L-alanine was obtained by transamination between its corresponding α-keto acid, 3-(2-naphthyl)-pyruvate and L-glutamate (Glu) at 70 °C, using a thermostable L-amino transferase and L-glutamate as an amino donor. The relevant difference in the solubility of the keto acid compared with the aminoacid product forces the latter to precipitate and pushes the reaction toward completion. We have also used the oxidative enzyme DAAO in a kinetic resolution process, this time on both naphthylglycines and naphthylalanines.[54] In that case a significant improvement in the bioconversion was obtained using a single-point mutant enzyme designed by a rational approach. By employing this D-aminoacid oxidase variant, the complete resolution of all four unnatural amino acids tested was obtained.

We recently found that a combined catalytic system composed of a hydrolytic enzyme and a suitable organic base constituted an efficient and high yielding process for the dynamic kinetic resolution of α-AA derivatives provided that the substrate could be racemized in the reaction conditions in the presence of the base while the acid formed is not. We set up a protocol in which arylglycine thioesters were prone to a DKR when using trioctylamine (TOA) as a base and subtilisin as the enzymatic catalyst in a biphasic system water/MTBE.[55] Herein we report how that system, whose applicability was limited to arylglycines, can eventually be transferred to the DKR of racemic naphthyl-alanines **1**,**3** and -glycines **2**,**4** allowing us to reach an efficient protocol for the production of the four amino acids in an enantiomerically pure L-form, in yields higher than 90% and ees of around 99%.

2. Results and discussion

Thio-esters of carboxylic acids are reactive species, which mimick the activation that occurs in nature during the transfer of acyl moieties. They behave as excellent acyl donors in transesterification and hydrolysis reactions, catalyzed by hydrolytic enzymes, lipases, and proteases. Many examples of lipase-catalyzed kinetic resolutions employing thioesters as acyl donors both in hydrolysis and transesterification reactions have been published.[56–60]

Thio-esters have another peculiar chemical reactivity: they are considerably more C-acidic than the corresponding carboxylates or their oxo-esters.[61,62] The combination of these two factors, that is, being excellent acyl donors in enzyme-catalyzed reactions and having high C-acidity, both derived from the poor donation of sulfur lone pairs into the π* C=O, makes these types of substrates very suitable for dynamic kinetic resolutions, where they are continuously racemized by an organic base and selectively transformed into enantiomerically pure products by a selective enzyme.[63,64]

Thus, we applied this concept to the DKR of a series of *N*-Boc-α-AA-thioesters, using subtilisin as a hydrolytic biocatalyst and a tertiary amine as a racemizing base. Although the nature of the thioester strongly influences the carbon acidity, which allows racemization to occur at a useful rate (thiobenzyl > thioethyl), a larger effect is associated with the presence of functional groups stabilizing the intermediate anion present at the α-position to the stereogenic

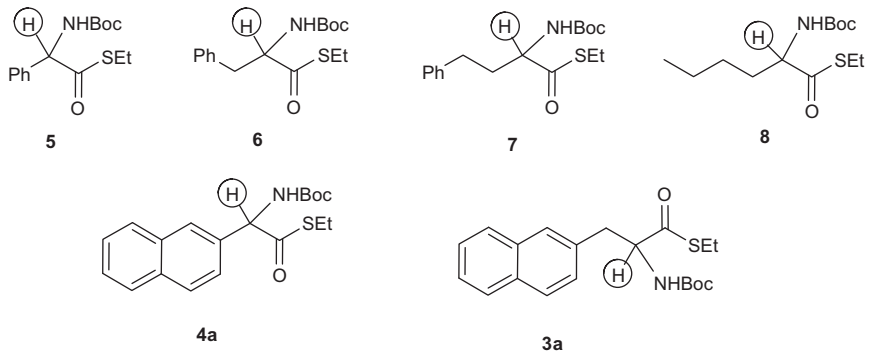


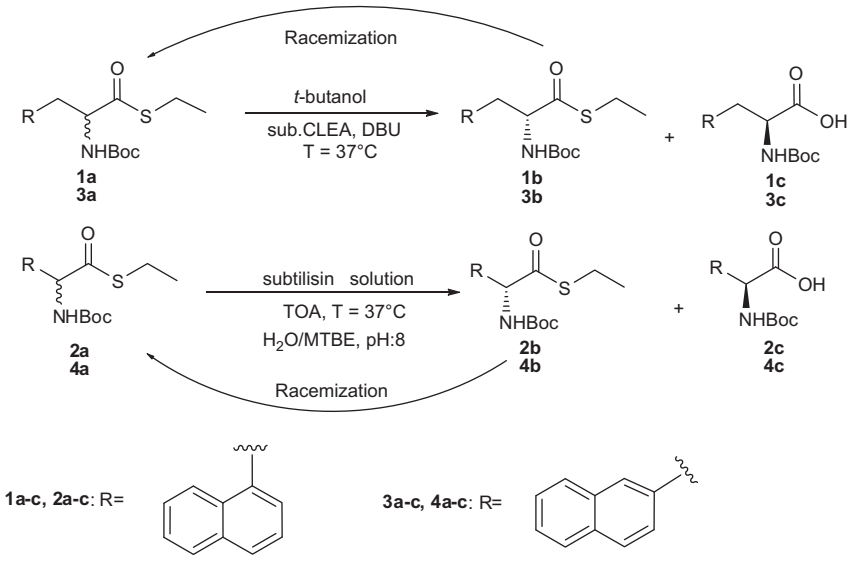
Fig. 2. Series of *N*-Boc-α-AA-thioesters submitted to DKR.

C-atom.[64] The racemization kinetic constants k_{rac} for compounds **5**–**8** were measured, and their relative values proved to be quite useful in evaluating the relative acidity of the α-protons, thus allowing us to select a strong enough base to effect a practical racemization. Using compound **5** as a reference (Fig. 2), the ratio between the kinetic constants were correlated with the structural features of the thioesters. Compound **5** was racemized 58 times faster than **6**, 580 times faster than **7**, and 950 times when compared to thioester **8**. Thus, while compound **5,** due to the presence of an aromatic ring on the α-C can be racemized with trialkylamine, stronger bases are required to readily racemize compounds **6**–**8**.[65]

If we use these numbers to draw similar conclusions for naphthyl aminoacid thioesters, we expect that compound **4a** and its isomer **2a** will be racemized in the presence of TOA, while for compound **3a** and its isomer **1a**, a stronger base will be required.

In order to assess the feasibility of this approach, we first confirmed that in the presence of a base and water, the *N*-Boc-thioesters **2a** and **4a** were not prone to spontaneous base catalyzed hydrolysis and could be racemized at a rate comparable with the rate of the enzyme catalyzed hydrolysis. In fact both naphthyl glycines racemized promptly in water/TOA with a $t_{1/2}$ of 106 min under the reaction conditions while they were completely stable to chemical hydrolysis. A commercial soluble preparate of the protease subtilisin was used in the enzymatic hydrolysis, which occurred promptly at pH 8 and 35 °C.[64] As expected, compounds **1a** and **3a** proved to be configurationally stable under the same conditions. 1,8-Diazabicyclo-[5.4.0]undec-7-ene (DBU) was previously identified as a stronger base that might be applied as a racemization catalyst. Compounds L-**1a** and L-**3a**, obtained via a protease-catalyzed kinetic resolution of the corresponding racemates, were submitted to racemization in *t*-butanol (*t*-BuOH) with DBU as the catalyst. Kinetic constants were in agreement with the data previously obtained with compound **6.** The apparent racemization kinetics were determined by measuring the decrease in rotation values of the L-form in the presence of a base. Thus, approximately 20 mg of substrate was dissolved in 2 mL of 2-propanol or *t*-BuOH in a polarimetric tube and the rotation value was recorded. Next, 0.5 equiv of DBU was then added at 40 °C and a decrease in the specific rotation value was observed over 30 min.

Control experiments proved that no change in the rotation values in time was observed when the substrate was the N-Boc-amino acid or with the thioesters in the absence of DBU. We found that TOA was not effective in promoting racemization on these compounds. Attempts to use DBU in aqueous media proved that chemical hydrolysis strongly interfered with the enzyme catalyzed reaction. Therefore, the use of this base forced us to apply an organic solvent containing slightly more than the stoichiometric amount of water, and the protease in a suitable, immobilized, form. Scheme 2 outlines the two different sets of the reaction conditions



Scheme 2. DKR of thioesters of naphthyl derivatives **1**–**4a** by subtilisin.

for the protease/base-catalyzed DKR, of the two groups of compounds **1**–**3** and **2**–**4**.

C compounds **2a** and **4a** in a biphasic system H_2O/MTBE with TOA as a racemization catalyst were hydrolyzed using the protease subtilisin in a water soluble form. In these conditions the L-form was hydrolyzed exclusively, while the D-form was rapidly racemized, restoring the L-thioester for further hydrolysis. As an outcome of the process, a DKR of 2-(2-naphthyl)-glycine derivative **4a** was achieved to yield the L-acid **4c** in yields of higher than 90% and complete enantioselectivity. The sterically hindered α-naphthyl isomer L-**2c** could be obtained in high yields and enantioselectively although at a much reduced rate (Table 1).

Table 1
DKR of compounds **1a**–**4a**

Aminoacid thioester	Product	Time	Conversion (%)	Yield (%)	ee%
1a	L-**1c**	6 days	>95	>92	>99
2a	L-**2c**	29 days	>95	>90	>98
3a	L-**3c**	27.5 h	>98	>93	>99
4a	L-**4c**	9 days	>99	>91	>99

In our previous study on the base-catalyzed racemization of aminoacid thioesters[65] we observed that compounds with the α-C not directly linked to the aromatic ring could not be racemized with TOA due to their reduced acidity. The process for the DKR of compounds **1a** and **3a** was thus based on DBU as the racemization catalyst.

The best conditions for the DKR of **1a** and **3a** were obtained with *t*-butanol containing 0.1% of water as a solvent,[66] and DBU as the racemization catalyst. In the presence of more than a stoichiometric amount of water, the rate of the base-catalyzed ester hydrolysis increased while the acid ee decreased at the same time. From the different subtilisin forms suitable to work in an organic solvent, CLEA-subtilisin was used. Under these conditions, the deracemization of compounds **1a** and **3a** to the corresponding L-acids L-**1c** and L-**3c** was excellent. Other immobilized forms of subtilisin were much less effective.[67] The CLEA catalyst was recovered by filtration, washed with a phosphate buffer (100 mM, pH 8), dried, and reused for at least 15 times. The large difference in kinetics of the four compounds toward enzymatic hydrolysis, in some ways parallels the apparent accessibility of the functional group from the enzyme active site.

3. Conclusion

α-Naphthyl aminoacids in enantiomerically pure form are interesting intermediates for practical synthetic and catalytic applications. The L-enantiomers are available via a number of methods employing enzymes as catalysts, such as classical kinetic resolutions[49,54] or deracemizations through microbial DKR[50] or stereo-inversion.[51] Herein we have devised a method for the DKR of racemic N-protected-naphthylaminoacid-thioesters allowing us to obtain all four of the projected compounds in excellent yields and nearly complete stereoselectivity. Two different processes have been adapted according to the structural features of the substrates **1**–**2** versus **3**–**4**. The nature of the base as a racemization catalyst, of the solvent system to minimize unwanted chemical reactions (hydrolysis) and of the suitable form of the enzyme catalyst has been carefully selected. While in a biphasic system a soluble form of subtilisin is effective, in an organic solvent (*t*-BuOH) with a controlled water amount, the protease is required as a cross linked aggregate (CLEA) for efficient reaction and effective recycling of the catalyst. This method compares favorably with previously published procedures for obtaining high chemical yields and high ees of the products. The results with these types of yields and selectivities are otherwise difficult to reach with methods not based on a DKR process.[68]

4. Experimental

4.1. General

All chemicals were purchased from Sigma–Aldrich. All solvents were of analytical grade. Crosslinked subtilisin from *Bacillus subtilis* CLEA® was from CLEA technologies (450 U/g) and subtilisin solution Alcalase 2.5L DX from Novozymes (50 U/mL). 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 analysis was performed on a Merck apparatus equipped with an UV detector and fitted with a Chiralcel OD 5 μm column, length internal diameter = 25/4.6 or with a Crownpak CR+ 5 μm column, length/internal diameter = 15/4.6. ^{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 the internal standard. All spectra were recorded in DMSO-d_6 at 305 K. 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. Hydrolysis reactions were monitored at 37 °C using a Radiometer analytical Titralab® 854.

Analytical evaluation of the enantiomeric excess of the DKR products was obtained using different procedures:

(a) Direct analysis of the L-*N*-Boc-AA-OH (compounds type **c**).

At first, L-*N*-Boc-AA-OH was reacted with diazomethane and the enantiomeric excess of the corresponding methyl-ester was evaluated by chiral HPLC method A (Chiralcel OD column, hexane/2-propanol = 400/1.2, flow = 1.2 mL/min, T = 308 K, λ = 254 nm);

(b) Determination on the L-AA-OH.

At first, L-*N*-Boc-AA-OH (compounds type **c**) was deprotected with dioxane saturated with HCl at 60 °C to give L-AA-OH·HCl as a white solid. The enantiomeric excess of L-AA-OH·HCl was evaluated by chiral HPLC method B (Crownpak CR+ column, aqueous perchloric acid (pH: 1.5)/MeOH: 9/1 flow = 1.1 mL/min, T = 308 K, λ = 210 nm).

4.2. Racemization procedure

Approximately 20 mg of substrate was dissolved in 2 mL of 2-propanol in a polarimetric tube and the rotation value was recorded. Next, 0.5 equiv of DBU was added at 40 °C and a decrease in the specific rotation value was recorded over 30 min. Control experiments proved that no change in the rotation values over time was observed when the substrate was the *N*-Boc-amino acid or with the thioesters in the absence of a base.

4.3. General procedure for *N*-Boc-aminoacid thioethyl ester synthesis

To a suspension of D,L-*N*-Boc-AA (19 mmol) in 38 mL of CH_2Cl_2 and 4.6 mL of TEA (33 mmol) *iso*-butylchloroformate (4 g, 33 mmol) is added at 0 °C and the mixture was stirred over 10 min. Ethane thiol (2.6 g, 41 mmol) was then added in CH_2Cl_2 (2 mL). After 60 min, 12 mL of ethylether was added and the TEA·HCl filtered, and rinsed with a solvent. The organic phase was washed with 0.1 M HCl (2 × 12 mL), water (3 mL) and brine, dried over sodium sulfate, and the solvent removed in vacuo. The crude material was mixed with ethyl acetate/hexane and obtained as a solid in quantitative yield. The L-enantiomers were prepared from the optically active material of commercial origin.

4.4. (*RS*)-*S*-ethyl 2-(*tert*-butoxycarbonylamino)-3-(naphthalen-1-yl)propanethioate

4.4.1. (D,L-*N*-Boc-1-naphthyl Ala-SEt) 1a

White solid (from 2-propanol), mp: 114–116 °C; ^{1}H NMR δ = 1.182 (br s, 3H), 1.347 (s, 9H), 2.842 (m, 2H), 3.328 (br s, 1H), 3.700 (br s, 1H), 4.740 (br s, 1H), 5.018 (br s, 1H), 7.274–8.080 (m, 7H). ^{13}C NMR, δ = 200.915, 154.822, 133.867, 132.259, 132.057, 128.767, 127.766, 127.468, 126.280, 125.616, 125.176, 123.156, 80.059, 60.869, 35.577, 28.147, 23.217, 14.266. ESI/MS: $[M+Na]^+$ = 382.2, $[M+H]^+$ = 360.1.

4.5. (*S*)-*S*-Ethyl 2-(*tert*-butoxycarbonylamino)-3-(naphthalen-1-yl)propanethioate

4.5.1. (L-*N*-Boc-1-naphthyl Ala-SEt) L-1a

$[\alpha]_D^{20} = +58.5$ (20.1 mg/2 mL 2-PrOH). The $[\alpha]_D^{20}$ value of 20.1 mg of L-**1a** in 2 mL of 2-propanol in a polarimeter was measured and 4.18 μL of DBU added at 45 °C. The time for the complete racemization was recorded and the kinetic constant calculated. $k_{rac} = 3.2 \times 10^{-4}$ mM^{-1} min^{-1}.

4.6. (*RS*)-*S*-Ethyl 2-(*tert*-butoxycarbonylamino)-3-(naphthalen-2-yl)propanethioate

4.6.1. (D,L-*N*-Boc-2-naphthyl Ala-SEt) 3a

White solid mp: 116–118 °C. ^{1}H NMR δ = 1.169 (t, J = 7.279 Hz, 3H), 1.321 (s, 9H), 2.826 (q, J = 7.279, 2H), 3.160 (br s, 1H) 3.259 (br s, 1H), 4.674 (br s, 1H), 4.941 (br s, 1H), 7.225–7.744 (m, 7H). ^{13}C NMR, δ = 200.898, 154.913, 133.383, 132.442, 132.390, 128.177, 128.042, 127.559, 127.498, 127.315, 126.036, 125.637, 80.115, 60.981, 38.514, 28.171, 23.233, 14.358. ESI/MS: $[M + Na]^+$ = 382.1.

4.7. (*S*)-*S*-Ethyl 2-(*tert*-butoxycarbonylamino)-3-(naphthalen-2-yl)propanethioate

4.7.1. (L-*N*-Boc-2-naphthyl Ala-SEt) L-3a

$[\alpha]_D^{20} = -39.75$ (20.1 mg/2 mL 2-PrOH). The $[\alpha]_D^{20}$ value of 20.1 mg of L-**1a** in 2 mL of 2-propanol in a polarimeter was measured and 4.18 μL of DBU added at 45 °C. The time for the complete racemization was recorded and the kinetic constant calculated. $k_{rac} = 2.3 \times 10^{-4}$ mM^{-1} min^{-1}.

4.8. (*RS*)-*S*-Ethyl 2-(*tert*-butoxycarbonylamino)-2-(naphthalen-1-yl)propanethioate

4.8.1. (D,L-*N*-Boc-1-naphthyl Gly-Set) 2a

White solid (2-PrOH) mp: 89–90 °C. ^{1}H NMR, δ = 1.162 (t, *J* = 7.279 Hz, 3H), 1.383 (s, 9H), 2.846 (m, 2H), 5.508 (br s, 1H), 6.087 (br s, 1H), 7.339–8.050 (m, 7H). ^{13}C NMR, δ = 199.564, 154.870, 134.079, 133.049, 131.030, 129.358, 128.936, 126.872, 126.255, 125.997, 125.240, 123.156, 80.330, 61.718, 28.223, 23.546, 14.264. ESI/MS: $[M+Na]^+$ = 368.1.

4.9. (*S*)-*S*-Ethyl 2-(*tert*-butoxycarbonylamino)-2-(naphthalen-1-yl)propanethioate

4.9.1. (L-*N*-Boc-1-naphthyl Gly-SEt) L-2a

$[\alpha]_D^{20} = -141.9$ (20.0 mg/2 mL 2-PrOH).

The $[\alpha]_D^{20}$ value of 20.0 mg of L-**2a** in 2 mL of 2-propanol in a polarimeter was measured and 4.33 μL of DBU added at 45 °C. The time for the complete racemization was recorded and the kinetic constant calculated. $k_{rac} = 8.7 \times 10^{-3}$ mM^{-1} min^{-1}.

4.10. (*RS*)-*S*-Ethyl 2-(*tert*-butoxycarbonylamino)-2-(naphthalen-2-yl)ethanethioate

4.10.1. (D,L-*N*-Boc-2-naphthyl Gly-SEt) 4a

White solid (2-PrOH) mp: 77–78 °C. ^{1}H NMR δ = 1.185 (t, *J* = 7.279 Hz, 3H), 1.426 (s, 9H), 2.866 (m, 2H), 5.569 (br s, 1H), 5.738 (br s, 1H), 7.411–7.825 (m, 7H). ^{13}C NMR, δ = 198.471, 154.655, 134.170, 133.230, 133.135, 128.802, 127.993, 127.588, 127.029, 126.357, 124.616, 80.271, 64.723, 28.218, 23.490, 14.222. ESI/MS: $[M+Na]^+$ = 368.1, $[M+H]^+$ = 346.1.

4.11. (*S*)-*S*-Ethyl 2-(*tert*-butoxycarbonylamino)-2-(naphthalen-2-yl)ethanethioate

4.11.1. (L-*N*-Boc-2-naphthyl Gly-SEt) L-4a

$[\alpha]_D^{20} = -104.65$ (23.2 mg/2 mL 2-PrOH). The $[\alpha]_D^{20}$ value of 20.5 mg of L-4a in 2 mL of 2-propanol in a polarimeter was measured and 4.44 μL DBU added at 45 °C. The time for the complete racemization was recorded and the kinetic constant calculated. $k_{rac} = 8.4 \times 10^{-2}$ mM^{-1} min^{-1}.

4.12. General procedure of DKR of *N*-Boc-naphthylalanines thioesters 1a and 3a by subtilisin CLEA

The DKR of **1a** and **3a** was carried out in a monophasic system. The thioester **1a** or **3a** was dissolved in *t*-butanol, and the resulting solution was poured into a 50 mL polypropylene tube, which was inserted into an orbital shaker thermostated at 37 °C. Next, DBU (2 equiv/equiv thioester) and naphthalene (as an internal std. 2 mg) were added. An aliquot was taken from the solution, constituting the t_0 sample for the HPLC analysis (hexane/2-propanol, flow: 1.2 mL/min). Next, subtilisin Alcalase CLEA® (450 U/g) was added in 1/1 w/w ratio with a substrate. Aliquots were taken at pre-determined intervals in order to monitor the conversion. At the end of the reaction (t_n), the enzyme was filtered and the *t*-butanol was evaporated. The residue was dissolved in water (at pH 8), and the water phase washed with ethyl ether (3 times). Next, L-*N*-Boc-AA-OH was recovered by extraction of the water phase with ethyl acetate (50 mL × 3 times) at pH 3. Drying and evaporation of the solvent gave a quantitative amount of L-*N*-Boc-AA-OH. The enantiomeric excess was analyzed by chiral HPLC (method B) and polarimetric analysis.

4.13. (*S*)-2-(*tert*-Butoxycarbonylamino)-3-(naphthalen-1-yl)propanoic acid

4.13.1. (L-*N*-Boc-1-naphthyl Ala-OH) L-1c

Thioester **1a** (800 mg, 2.23 mmol), naphthalene (30 mg), DBU (2 equiv, 4.45 mmol, 0.656 mL), and subtilisin CLEA (800 mg) were used. The conversion was monitored by chiral HPLC analysis (method A): Peaks D: 31 min; L: 53.5 min; naphthalene: 4.5 min. L-**1c**: white solid (crystallized from isopropylether), yield 92.4% (2.061 mmol, 650 mg); mp = 76–8 °C; $[\alpha]_D^{20} = -30.9$ (17.7 mg/2 mL 2-PrOH). ^{1}H NMR δ = 1.330 (s, 9H), 3.120 (br s, 0,5H), 3.359 (br s, 0.5 H), 3.695 (br s, 0.5 H), 3.818 (br s, 0.5 H), 4.656 (br s, 1 H), 5.012 (br s, 0.5 H), 6.916 (br s, 0.5 H), 7.308–8.087(m, 7H). ^{13}C NMR δ = 175.750, 156.647, 133.927, 132.893, 132.185, 128.812, 128.144, 127.739, 127.556 126.254, 125.612, 123.370, 81.136, 55.077, 37.852, 28.237. ESI/MS: $[M+Na]^+$ = 338.2, $[M+H]^+$ = 316.1. Ee of (L-1-naphthyl Ala-OH·HCl): 99.8%; chiral HPLC analysis (method B): Peak L: 21.1 min (D: 16.4 min). Polarimetric analysis for (L-1-naphthyl Ala-OH·HCl): $[\alpha]_D^{25} = -15$ (*c* 1, H_2O); lit.[71]: $[\alpha]_D^{25} = -14.4$ (*c* 1, H_2O).

4.14. (*S*)- 2-(*tert*-Butoxycarbonylamino)-3-(naphthalen-2-yl)propanoic acid

4.14.1. (L-*N*-Boc-2-naphthyl Ala-OH) L-3c

Thioester **3a** (200 mg, 0.56 mmol), naphthalene (10 mg), DBU (2 equiv, 1.1 mmol, 0.166 mL), and subtilisin CLEA (200 mg) were used. The conversion has been monitored by chiral HPLC analysis (method A): Peaks: D: 44.5 min; L: 48.5 min; naphthalene: 6 min. L-**3c**: yield: 93.4% (0.523 mmol, 165 mg), mp = 95 °C; $[\alpha]_D^{20} = +45$ (*c* 1, CH_3OH). ^{1}H NMR δ = 1.343 (s, 9H), 3.011–3.286 (m, 2H), 4.432 (br s, 0.3 H), 4.656 (br s, 0.7 H), 4.980 (br s, 0.7 H), 6.372 (br s, 0.3 H), 7.273–7.748 (m, 7H).

^{13}C NMR δ = 176.369, 155.510, 133.660, 133,493, 132.536, 128,250, 128.199, 127.642, 127.384, 126.108, 125.697, 80.320, 54.315, 37.996, 27.968. ESI/MS: $[M+Na]^+$ = 338.2, $[M+H]^+$ = 316.1. Ee for (L-2-naphthyl Ala-OH·HCl): 99.5%; chiral HPLC analysis (method B): Peak L: 38.9 min (D: 29.9 min). Polarimetric analysis for (L-2-naphthyl Ala-OH·HCl): $[\alpha]_D^{20} = -14.5$ (*c* 1, H_2O), lit.[72] $[\alpha]_D^{25} = -14.2$ (*c* 1.08, H_2O).

4.15. General procedure for DKR of *N*-Boc-AA-thioesters with a subtilisin solution

The DKR of **2a** and **4a** was carried out in a biphasic system. The reaction was carried out in an automatic titrator. To a solution of D,L-*N*-Boc-AA-SEt (6.5 mmol) in 50 mL of MTBE, water (100 mL) and trioctylamine (3.25 mmol) were added. The pH was adjusted to 8 with NaOH (0.5 M) and 500 U of subtilisin was added. The reaction was kept at pH 8 by the automatic addition of NaOH (0.5 M), at 37 °C with vigorous mechanical stirring. After 2 days, the consumption of NaOH 0.5 M reached 100% of the theoretical. The water phase was separated from the organic layer and again extracted (at pH 8) with 30 mL of diethyl ether. The aqueous phase was brought to pH 3 by the dropwise addition of 6 M HCl. Next, 30 mL of ethyl acetate was added, and the resulting mixture was suction-filtered through a Celite bed. The organic phase was separated, and the aqueous layer was extracted twice with further 30 mL of ethyl acetate. The organic fractions were combined, dried, and evaporation of the solvent gave a quantitative amount of L-*N*-Boc-AA-OH. Enantiomeric excess analyzed by chiral HPLC was >99%. L-*N*-Boc-AA-OH was deprotected with dioxane saturated with HCl at 60 °C to give of L-AA-OH·HCl as a white solid.

4.16. (*S*)-2-(*tert*-Butoxycarbonylamino)-2-(naphthalen-1-yl) ethanoic acid

4.16.1. (L-*N*-Boc-1-naphthyl-Gly-OH) L-2c

White solid (isopropylether), mp = 180–181 °C (dec. 183 °C), lit.[73] mp = 182–183 °C. Polarimetric analysis: $[\alpha]_D^{25} = +90.7$ (15.7 mg in 2 mL 2-propanol). ^{1}H NMR ($CDCl_3$) δ = 1.452 (s, 9H), 5.518 (br s, 1H), 5.943 (br s, 0.5H), 6.121 (br s, 0.5H), 7.343–8.344 (m, 7H); ^{13}C NMR ($CDCl_3$) δ = 175.596, 156.679, 134.572, 134.089, 133.914, 129.505, 128.758, 127.004, 126.532, 126.113, 125.624, 123.340, 81.528, 55.544, 28.308. ESI/MS: $[M+Na]^+$ = 324.1. Ee as (*S*)-2-amino-2-(naphthalen-1-yl)ethanoic acid hydrochloride (L-1-naphthyl Gly-OH·HCl): 96.7%; chiral HPLC analysis (method B): Peak: L: 53.5 min (D: 22.4 min). Polarimetric analysis for (L-1-naphthyl Gly-OH·HCl): $[\alpha]_D^{20} = +26.6$ (*c* 1.0, H_2O).

4.17. (*S*)-2-(*tert*-Butoxycarbonylamino)-2-(naphthalen-2-yl) ethanoic acid

4.17.1. (L-*N*-Boc-2-naphthyl-Gly-OH) L-4c

Thioester D,L-**4a** (400 mg, 1.16 mmol), naphthalene (30 mg), TOA (0.5 equiv, 0.58 mmol, 0.253 mL), and 2 mL subtilisin solution were used. The conversion was monitored both by a pH-stat device (2.4 mL of 0.5 M NaOH was added to biphasic system water/MTBE) and by chiral HPLC analysis (method A): Peak D at 35 min; L at 37 min, naphthalene at 6 min. **4c**: white solid (crystallized from isopropylether), yield: 91.5% (1.062 mmol, 320 mg), mp = 77–78 °C; Polarimetric analysis: $[\alpha]_D^{20} = +158.3$ (19.1 mg/2 mL 2-PrOH). ^{1}H NMR δ = 1.364 (s, 9H), 5.275 (br s, 1 H), 5.474 (br s, 0.5 H), 5.633 (br s, 0.5 H), 7.391–8.062 (m, 7H). ^{13}C NMR δ = 173.542, 156.980, 135.700, 133.260, 133.155, 128.303, 128.026, 127.651, 126.346, 126.195, 126.069, 125.023, 81.726, 59.035, 27.962. ESI/MS: $[M+Na]^+$ = 324.1 Ee as (*S*)-2-amino-2-(naphthalen-2-yl)ethanoic acid hydrochloride (L-2-naphthyl Gly -OH∗HCl): 99.7%; chiral HPLC analysis (method B): Peak L: 32 min (D: 12 min). Polarimetric analysis for (L-naphthyl Gly-OH·HCl): $[\alpha]_D^{25} = -8$ (*c* 0.5, H_2O).

References

1. Cane, D. E.; Walsh, C. T.; Khosla, C. *Science* **1998**, *282*, 63–68.
2. Strieker, M.; Marahiel, M. A. *ChemBioChem* **2009**, *10*, 607–616.
3. Caboche, S.; Pupin, M.; Leclere, V.; Jacques, P.; Kucherov, G. *BMC Struct. Biol.* **2009**, *9*, 15. http://dx.doi.org/10.1186/1472-6807-9-15.
4. Rouhi, A. M. *Chem. Eng. News* **2004**, *82*, 47–62.
5. Schmid, A.; Dordick, J. S.; Hauer, B.; Kiener, A.; Wubbolt, M.; Witholt, B. *Nature* **2001**, *409*, 258–268.
6. Schoemake, H. E.; Mink, D.; Wubbolts, M. G. *Science* **2003**, *299*, 1694–1697.
7. Loffet, A. J. *Pep. Sci.* **2002**, *8*, 1–7.
8. Kee, S.; Jois, S. D. S. *Curr. Pharm. Design* **2003**, *9*, 1209–1224.
9. Hanessian, S.; McNaughton Smith, G.; Lombart, H. G. *Tetrahedron* **1997**, *53*, 12789–12854.
10. Wang, L.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 34–66.
11. Sun, H.; Nikolovska-Coleska, Z.; Yang, C. Y.; Xu, L.; Liu, M.; Tomita, Y.; Pan, H.; Yoshioka, Y.; Krajewski, K.; Roller, P. P.; Wang, S. *J. Am. Chem. Soc.* **2004**, *126*, 16686–16687.
12. Oost, T. K.; Sun, C.; Armstrong, R. C.; Al-Assaad, A. S.; Betz, S. F.; Deckwerth, T. L.; Ding, H.; Elmore, S. W.; Meadows, R. P.; Olejniczak, E. T.; Oleksijew, A.; Oltersdorf, T.; Rosenberg, S. H.; Shoemaker, A. R.; Tomaselli, K. J.; Zou, H.; Fesik, S. W. *J. Med. Chem.* **2004**, *47*, 4417–4426.
13. (a) Williams, R. M. *Synthesis of Optically Active α-Amino Acids*; Pergamon: Oxford, 1989; (b) Wirth, T. *Angew. Chem., Int. Ed.* **1997**, *36*, 225–227.
14. Ma, J. A. *Angew. Chem., Int. Ed.* **2003**, *42*, 4290–4299.
15. Burk, M. J. *Acc. Chem. Res.* **2000**, *33*, 363–372.
16. Noyori, R.; Ohkuma, T. *Angew. Chem., Int. Ed.* **2001**, *40*, 40.
17. Chenault, H. K.; Dahmer, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 6354–6364.
18. Miyazawa, T.; Minowa, H.; Miyamoto, T. K.; Imagawa, R.; Yanagihara, T.; Yamada, Y. *Tetrahedron: Asymmetry* **1997**, *8*, 367–370.
19. Chen, S.-T.; Huang, W.-H.; Wang, K. T. *J. Org. Chem.* **1994**, *59*, 7580–7581.
20. Chen, S. T.; Huang, W. H.; Wang, K. T. *Chirality* **1994**, *6*, 572–576.
21. Pugniere, M.; Domergue, N.; Castro, B.; Previero, A. *Chirality* **1994**, *6*, 472–478.
22. Sasaki, N. A.; Hachimoto, C.; Potier, P. *Tetrahedron Lett.* **1987**, *48*, 6069–6072.
23. Miyazawa, T. *Amino Acids* **1999**, *16*, 191–213.
24. Agosta, E.; Caligiuri, A.; D'Arrigo, P.; Servi, S.; Tessaro, D.; Canevotti, R. *Tetrahedron: Asymmetry* **2006**, *17*, 1995–1999.
25. Gonalves, L. P. B.; Antunes, O. A. C.; Pinto, G. F.; Oestreicher, E. G. *Tetrahedron: Asymmetry* **2000**, *11*, 1465–1468.
26. Li, T.; Kootstra, A. B.; Fotheringham, I. G. *Org. Proc. Res. Dev.* **2002**, *6*, 533–538.
27. Stewart, J. D. *Curr. Opin. Chem. Biol.* **2001**, *5*, 120–129.
28. Taylor, P. P.; Pantaleone, D. P.; Senkpeil, R. F.; Fotheringham, I. G. *Trends Biotechnol.* **1998**, *16*, 412–418.
29. Hovhannisyan, N.; Harutyunyan, S.; Hovhannisyan, A.; Hambardzumyan, A.; Chitchyan, M.; Melkumyan, M.; Oganezova, G.; Avetisyan, N. *Amino Acids* **2009**, *37*, 531–536.
30. Chrisp, P.; Goa, K. L. *Drugs* **1990**, *39*, 523–551.
31. Hirayama, R.; Yamamoto, M.; Tsukida, T.; Matsuo, K.; Obata, Y.; Sakamoto, F.; Ikeda, S. *Bioorg. Med. Chem.* **1997**, *5*, 765–777.
32. Cooper, R.D.G.; Blaszczak, L.C.; Turner, J.R.; Conrad, P.C.; Daugherty, B.W.; Wade, B. US 83–484128 19830412 CAN 102:148986.
33. Rivier, J. E.; Porter, J.; Rivier, C. L.; Perrin, M.; Corrigan, A.; Hook, W. A.; Siraganian, R. P.; Vale, W. W. *J. Med. Chem.* **1986**, *29*, 1846–1851.
34. Yabe, Y.; Morita, A.; Miura, C.; Kobayashi, S.; Baba, Y. *Chem. Pharm. Bull.* **1977**, *25*, 2731–2734.
35. Prochazka, Z.; Slaninova, J. *Collect. Czech. Chem. Commun.* **1995**, *60*, 2170–2177.
36. Hohsaka, T.; Kajihara, D.; Ashizuka, Y.; Murakami, H.; Sisido, M. *J. Am. Chem. Soc.* **1999**, *121*, 34–40.
37. Tran, T. A.; Mattern, R. H.; Morgan, B. A.; Taylor, J. E.; Goodman, M. *J. Pept. Sci.* **1999**, *5*, 113–130.
38. Haug, B. E.; Skar, M. L.; Svendsen, J. S. *J. Pept. Sci.* **2001**, *7*, 425–432.
39. Ranjalahy-Rasoloarijao, L.; Lazaro, R.; Daumas, P.; Heitz, F. *Int. J. Pep. Prot. Res.* **1989**, *33*, 273–280.
40. Sobocinska, M.; Lempicka, E.; Konieczna, E.; Derdowska, L.; Lammek, B.; Melhem, S.; Kozik, W.; Janecka, J.; Janecki, M.; Trzeciak, H. I. *J. Pharm. Pharmacol.* **2000**, *52*, 1105–1112.
41. Tamamura, H.; Omagari, A.; Hiramatsu, K.; Oishi, S.; Habashita, H.; Kanamoto, T.; Gotoh, K.; Yamamoto, N.; Nakashima, H.; Otaka, A.; Fujii, N. *Bioorg. Med. Chem.* **2002**, *10*, 1417–1426.
42. Daumas, P.; Heitz, F. *Biochemie* **1989**, *71*, 77–81.
43. Lingen, H. L.; Van de Mortel, J. K. W.; Hekking, K. F. W.; Van Delft, F. L.; Sonke, T.; Rutjes, F. P. J. T. *J. Eur. Org. Chem.* **2003**, 317–324.
44. Zhang, X.; Ouyang, J.; Baeyens, W. R. G.; Zhai, S.; Yang, Y.; Huang, G. *J. Pharm. Biomed. Anal.* **2003**, *31*, 1047–1057.
45. Zhang, X.; Ouyang, J.; Yang, Y. *Anal. Lett.* **2001**, *34*, 1851–1864.
46. Williams, R. M.; Hendrix, J. A. *J. Org. Chem.* **1990**, *55*, 3723–3728.
47. Reimann, E.; Voss, D. *Arch. Pharmazie* **1976**, *309*, 978–983.
48. Dave, H. R.; Hosangadi, B. D. *Tetrahedron* **1999**, *55*, 11295–11308.
49. Pugniere, M.; Castro, B.; Previero, A. *Chirality* **1991**, *3*, 170–173.
50. Syldtak, C.; Voelkel, D.; Bilitewski, U.; Krohn, K.; Hoeke, H.; Wagner, F. *Biotechnol. Lett.* **1992**, *14*, 105–110.
51. Caligiuri, A.; D'Arrigo, P.; Gefflaut, T.; Molla, G.; Pollegioni, L.; Rosini, E.; Rossi, C.; Servi, S. *Biocat. Biotrans.* **2006**, *24*, 409–413.
52. Sheldon, R. A. *Chem. Ind. (London)* **1992**, 903.
53. Hanzawa, S.; Oe, S.; Tokuhisa, K.; Kawano, K.; Kobayashi, T.; Kudo, T.; Kakidani, H. *Biotechnol. Lett.* **2001**, *23*, 589–591.
54. Caligiuri, A.; D'Arrigo, P.; Rosini, E.; Molla, G.; Servi, S.; Tessaro, D.; Pollegioni, L. *Adv. Synth. Catal.* **2006**, *348*, 2183–2190.
55. D'Arrigo, P.; Cerioli, L.; Servi, S.; Tessaro, D.; Viani, F. submitted for publication.

56. Iriuchijima, S.; Kojima, N. *J. Chem. Soc., Chem. Commun* **1981**, 185.
57. Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192–3196.
58. Bianchi, D.; Cesti, P. *J. Org. Chem.* **1990**, *55*, 5657–5659.
59. Frykman, H.; Ohrner, N.; Norin, T.; Hult, K. *Tetrahedron Lett.* **1993**, *8*, 1367–1370.
60. Caussette, M.; Marty, A.; Combes, D. J. *Chem. Tech. Biotechnol.* **1997**, *68*, 257–262.
61. Sproull, K. C.; Bowman, G. T.; Carta, G.; Gainer, J. L. *Biotechnol. Progress* **1997**, *13*, 71–76.
62. Lienhard, G. E.; Wang, T. C. *J. Am. Chem. Soc.* **1968**, *90*, 3781–3787.
63. Um, P. J.; Drueckhammer, D. G. *J. Am. Chem. Soc.* **1998**, *120*, 5605–5610.
64. Arosio, D.; Pedrocchi-Fantoni, G.; Rossi, C.; Saraceno, C.; Servi, S.; Tessaro, D. *Adv. Synth. Catal.* **2007**, *349*, 1345–1348.
65. D'Arrigo, P.; Arosio, D.; Cerioli, L.; Moscatelli, D.; Servi, S.; Viani, F.; Tessaro, D. *Tetrahedron: Asymmetry* **2011**, *22*, 851–856.
66. From a KF analysis the water content in commercial *t*-BuOH was 0.1% but after the addition of the enzymatic preparate the water content raised to 1.5% corresponding to 20 times the stoichiometric amount required for the hydrolysis reaction.
67. Unpublished observations.
68. C.J. Sih has observed that a kinetic resolution approach with in situ racemization of the non-transformed enantiomer, not only is more productive in overcoming the limit of 50%, but the enantiomeric excess of the product becomes independent of the extent of conversion, and the process becomes more enantiospecific.[69,70]
69. Fülling, G.; Sih, C. J. *J. Am. Chem. Soc.* **1987**, *109*, 2845–2846.
70. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294.
71. Atsuumi, S.; Nakano, K.; Masato, Y.; Tanaka, S.; Matsuyama, K. *Chem. Pharm. Bull.* **1992**, *40*(2), 364–370.
72. Rodriguez, M.; Bernad, N.; Galas, M. C.; Lignon, M. F.; Laur, J. *Eur. J. Med. Chem.* **1991**, *26*(3), 245–253.
73. Medina, E.; Vidal-Ferran, A.; Moyano, A.; Pericas, M. A.; Riera, A. *Tetrahedron: Asymmetry* **1997**, *8*, 15581–15586.