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Enzymatic approach to both enantiomers of *N*-Boc hydrophobic amino acids

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Abstract—Protease catalysed hydrolysis of *N*-Boc-amino acid esters allows us to obtain *N*-Boc L-acids and D-esters of amino butanoic acid, nor-leucine, nor-valine, leucine and t-leucine in excellent ee. The reaction occurs in short reaction times and high concentrations. When a biphasic system (buffer–MTBE) is employed, a strong solvent effect is observed. This method could be of significance for the preparation of D-t-leucine, for which a practical method is currently unavailable.

1. Introduction

Nonnatural amino acids are compounds of growing interest for incorporation in nonribosomal peptides (NRPs), a class of microbial natural products which includes several valuable small molecule therapeutics, such as antibiotics and immunosuppressants, for the preparation of new cyclic and linear peptidic drugs, for the synthesis of peptide segments with predictable folding properties and compounds not subject to protease action.[1a] Among them, C-4 to C-6 hydrophobic amino acids have found several applications in both enantiomeric forms in the synthesis of peptidomimetics,[1b,1c] protease inhibitors,[2] conformationally restricted cyclosporine β-turn analogues,[3] and in the total synthesis of compounds with pharmacological activity.[4–7]

In research towards new drugs, both enantiomeric forms of these compounds are usually needed. Biocatalysis is probably the synthetic methodology more suited for the preparation of nonnatural AAs in both enantiomeric forms. This can be achieved by asymmetric synthesis for the D-forms (hydantoinases, amino transferases, etc.) and for the L-forms, although kinetic resolution remains the more often applied method: the advantages of this approach are the wide library of hydrolytic enzymes available and the ease of racemisation of the eventually unwanted enantiomer. Racemic AAs esters have often been used as starting materials for this procedure: the recovery of both enantiomers in the case of aliphatic AAs can be effected by solvent extraction.[8] N-protected esters have the advantage of affording compounds ready for further synthetic manipulation and improved solvent solubility.

Herein, we report the kinetic resolution of *N*-Boc esters of hydrophobic amino acids in water or biphasic systems using subtilisin and other proteases as biocatalysts. Appropriate reaction conditions allow the obtention of both enantiomers with high yields and enantiomeric excesses. A strong solvent effect, influencing reaction rate and selectivity, was observed.

2. Results and discussion

2.1. General procedure

N-Boc AAs-esters **1**–**5** (Fig. 1) were prepared by standard procedures.[16] Ester hydrolysis was performed in either water suspensions or in biphasic mixtures with water/immiscible organic solvents (Tol, MTBE). Formal concentrations were 10–300 mM, pH 7.7, 36 °C. The reaction was titrated with NaOH solution and extracted at around 48–52% conversion. Solvent extraction allowed the separation of the unreacted D-ester from the *N*-Boc-AA in the L-form.

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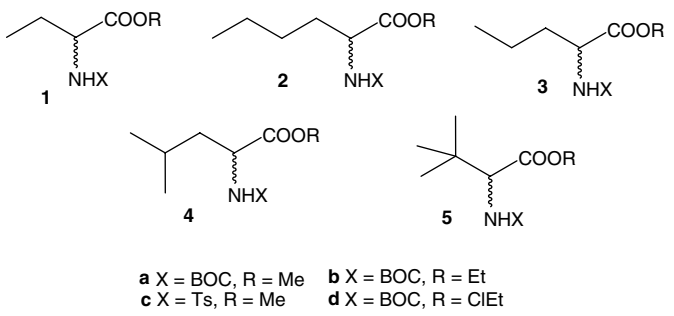

Figure 1.

The latter was then extracted after acidification. Subtilisin from commercial sources, either free or immobilised, was used in a ratio of 200 U/g of derivatised AA. Several commercial proteases are available on a large scale at low costs. Alcalase from Novozymes, Pronase-N-, Proleather, from Amano can be used for this work; results are reported using protease from *Bacillus licheniformis* from Fluka catalogue, unless specified.

2.2. D- and L-Aminobutanoic acid

N-Boc-amino butanoic acid in either the L- or D-form is incorporated in peptides and peptidomimetics with pharmacological activity.[9]

A number of derivatives of D,L-amino butanoic acid were tested as substrates of proteases and D,L-*N*-Boc-aminobutanoic acid OMe **1a** proved to be the most convenient substrate for protease hydrolysis. Racemic **1a** was suspended/dissolved at a concentration of up to 60 g/L at 30 °C in phosphate buffer at pH 7.5 and the mixture kept at this pH value with the automatic addition of 1 M NaOH. The protease was then added (200 U/g substrate) and the hydrolysis followed from the titration curve.

Figure 2 shows the course of the hydrolysis of compound **1a** in a water suspension and in a biphasic system. A blank reaction shows that there is no competing chemical hydrolysis at the same pH, temperature and concentration. When the reaction was run in water, the obtention of the L-acid in its enantiomerically pure form was only possible at limited conversions (40%), while the D-form could be obtained at higher conversion. As can be easily seen from curve A, the hydrolysis continued even at a conversion higher than 50%. Curve B describes the course of hydrolysis of compound **1a** at the same water concentrations, in the presence of an immiscible solvent (MTBE). Under these conditions, the hydrolysis completely stopped at 50% conversion, allowing the obtention of both enantiomers in ee higher than 98%. A similar effect was also observed with other solvents. The effect of a (co)-solvent has been shown to influence both the rate of conversion and selectivity when hydrolytic enzymes are used in kinetic resolutions.[10] In the (almost) complete absence of water, solvent effects have been attributed to substrate diffusional limitations or to protein structural modifications,[11] while in the presence of bulk water, solvent effects have been related to the degree of preferential solvation of different parts of the molecule and hence with solvent log *P*.[12]

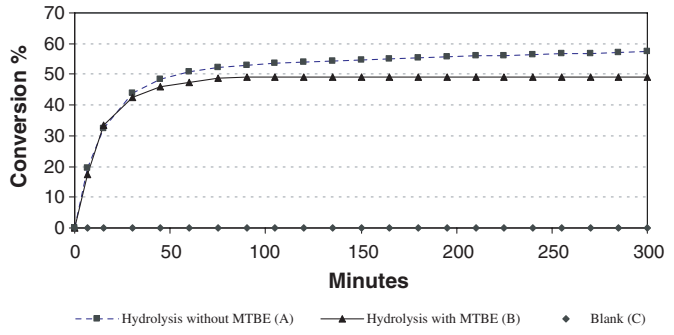

Figure 2. Protease catalysed hydrolysis of compound **1a** in a water suspension and in a biphasic system.

In the case of crude enzyme preparations, a solvent effect is usually attributed to selective deactivation of iso-forms. Since the observed change in selectivity was not observed with all the substrates tested, this hypothesis should be discarded. Since we compare a hydrophobic substrate in water solution and a biphasic system with the substrate soluble in the organic phase, we believe that the different selectivity can be related to mass transport limitations.

2.3. D- and L- nor-Valine and nor-Leucine

D,L-*N*-Boc nor-valine-OMe **3a** was submitted to enzymatic hydrolysis in a water solution and in a biphasic system. *E*-values were calculated under both conditions. When the protease from *B. licheniformis* in water was used, an *E*-value of 12 was calculated, while in a biphasic system (water/MTBE) $E = 113$ was observed. The solvent effect is similar when protease N (Amano) was used on the same substrate (E_{water} was 44 while $E_{\text{biphasic}} = 170$). The resolution of the *N*-Boc-methyl esters allowed us to obtain both the D-ester and L-acid in high ee and short reaction times (2 h). A similar procedure was also applied to the resolution of D,L-*N*-Boc-nor-leucine-OMe **2a**. In this case, the best results in terms of reaction rates and ee were obtained with a 2:1 water–MTBE ratio. The reaction is considerably slower than with compounds **1** and **3** (50% conversion in 15 h).

2.4. D-Leucine

In order to obtain the D-enantiomer from the racemic compound, a specific solution was found for this AA: compounds *rac*-**4a**, *rac*-**4b** and *rac*-**4d** were hydrolysed with proteases in water and the biphasic system, but the reaction rates proved much slower for all compounds than for the previous ones. However, the *N*-tosyl derivative *rac*-**4c** was readily hydrolysed leaving behind the D-methyl ester >99% ee in 5 h.

2.5. D- and L-t-Leucine

Due to its bulky and hydrophobic *tert*-butyl side chain, L-t-leucine has found increased use as a building block for the synthesis of chiral auxiliaries and biologically active compounds.[13]

Several preparative methods have been reported, both by resolution of racemic mixtures and by enantioselective synthesis using the most useful enzymatic systems available for kinetic resolution, namely hydantoinases, penicillin acylases and lipases.[14] However, L-t-leucine is prepared on an industrial scale by enzymatic reductive amination of the corresponding keto-acid via a coupled enzyme system.[15]

This method cannot be extended to the preparation of the D-enantiomer due to the lack of enzymes with an opposite selectivity. The hydantoinase process is the privileged system for D-amino acid production, but has the main limitation of requiring a carbamoylase for the complete hydrolysis. These second enzymes often have a substrate specificity different from the preceding one. The production of D-t-leucine with this method suffers from this limitation.

Recently, a chemoenzymatic approach to synthesise D-t-leucine has been described[13] utilizing the enantioselective cleavage of *N*-acetyl-t-leucine esters by a *B. licheniformis* protease, allowing the obtention of D-*N*-Ac-t-leucine-OH of high ee. Although the method has been applied on a multikilogram scale,[14d,14e] the utility is hampered by the low reaction rates (5 days) and from the fact that the acetyl group is not a useful protective group in peptide synthesis. *rac*-*N*-Boc-t-leucine-OMe is an excellent substrate for the protease catalysed hydrolysis in water. The reaction is relatively fast, requiring reaction times of 18 h and giving access to D-*N*-Boc-t-leucine-OH in high yield and excellent ee after separation from the L-acid and chemical hydrolysis. The resolution process was possible in a water suspension at a formal concentration of >50 g/L. Thus the method seems appropriate for a large scale preparation of D-t-leucine. The use of an immiscible solvent was of no advantage in this case requiring much longer reaction times (Table 1).

3. Conclusions

N-Boc-esters of *rac*-hydrophobic amino acids can be resolved into the enantiomeric forms with commercially available proteases. The control of the conversion allows us to obtain both enantiomers in excellent ee. The enzyme catalysed reaction in a biphasic system allows us to limit the hydrolysis at 50% of the L-forms of compounds **1**–**4**. The improved selectivity derived from the solvent effect can be attributed to the control of substrate concentration in the water phase. The solvent addition is however not required in the resolution of compound **4**. In fact D,L-*N*-Boc-t-Leu-esters can be resolved in a reasonable reaction time with excellent selectivity in a water suspension with a formal concentration of 50 g/L. This constitutes as a valuable approach to D-*N*-Boc-t-Leu-OH, a compound currently unavailable with a more convenient approach.

Table 1. Subtilisin catalysed resolution of D,L-*N*-Boc-t-Leu-OMe

Substrate	Conversion (%)	*t* (h)	ee[a] L (%)	ee[a] D (%)
D,L-*N*-Boc-t-Leu-OMe	58	18	91 (90)	99 (77)
D,L-*N*-Boc-t-Leu-OMe	49	15	96 (91)	78 (86)

[a] As free amino acid.

4. Experimental

Protease type VIII from *B. licheniformis* was from Sigma. Protease N and pronase were from Amano.

^{1}H NMR analyses were carried out on a 400 MHz *Varian EMX* instrument. EI-MS were recorded on a *TSQ mass spectrometer*. Optical rotation data were measured with a *Propol automatic digital polarimeter*.

4.1. Resolution of D,L-*N*-Boc-aminobutanoic acid-OMe

D,L-*N*-Boc-aminobutanoic acid-OMe (2 g, 9.2 mmol) was suspended in 15 mL of water and 15 mL of MTBE, and the mixture was then stirred at 30 °C. The aqueous phase was adjusted to pH 7.5 and 400 U of protease was added. The pH was kept at the initial value with the automatic addition of 1 M NaOH. After 4 h the reaction was complete. The pH was adjusted with $NaHCO_3$ to 8.4 and the unreacted substrate recovered by phase separation. Drying over sodium sulfate and evaporation of the solvent gave 970 mg of D-methyl ester as a pale oil (yields 97%).

^{1}H NMR ($CDCl_3$): δ 0.93 (t, $J = 7.5$ Hz, 3H): δ 1.45 (s, 9H), δ 1.59–1.91 (m, 2H), δ 3.74 (s, 3H), δ 4.26 (br s, 1H), δ 5.01 (br s, 1H). $[\alpha]_D = +39$ (*c* 2.22 g/100 mL; 25 °C; 589 nm; MeOH).

The acidic product was recovered by extraction of the water phase with ethyl acetate (3×15 mL) at pH 3. Drying and evaporation of the solvent gave 920 mg of L-acid as a pale oil (yields 96%). ^{1}H NMR (2:1 rotamer ratio, the asterisk denotes minor rotamer peaks, $CDCl_3$): δ 0.98 (t, $J = 7.4$ Hz, 3H), δ 1.45 (s, 9H), δ 1.64–1.96 (m, 2H), δ 4.09* (br s, 1H), δ 4.28 (br s, 1H), δ 5.05 (br s, 1H), δ 6.19* (br s, 1H). $[\alpha]_D = -17.8$; (*c* 2.00 g/100 mL; 25 °C; 589 nm; MeOH): (lit. -18.8[17]).

4.2. Resolution of D,L-*N*-Boc-nor-valine-OMe

D,L-*N*-Boc-nor-valine-OMe (2 g, 8.65 mmol) was dissolved in 10 mL of water and 10 mL of MTBE and the mixture was stirred at 30 °C. The aqueous phase was adjusted at pH 7.5 and 400 U of protease was added. The pH was kept at the initial value with automatic addition of 1 M NaOH. After 4 h, the reaction was complete (50% of hydrolysis was reached). After this time, the pH was adjusted with $NaHCO_3$ to 8.4 and the unreacted substrate recovered by phase separation. The aqueous phase was again extracted with diethyl ether (3×10 mL). The combined organic layers were dried over sodium sulfate and evaporation of the solvent gave 853 mg of D-methyl ester as a pale oil (yield 85% of D-enantiomer). ^{1}H NMR ($CDCl_3$): δ 0.93 (t, $J = 7.2$ Hz, 3H), δ 1.29–1.41 (m, 2H), δ 1.44 (s, 9H), δ 1.54–1.82 (m, 2H), δ 3.73 (s, 3H), δ 4.28 (br s, 1H), δ 4.98 (br s, 1H). $[\alpha]_D = +32$ (*c* 1.01 g/100 mL; 25 °C; 589 nm; MeOH) (lit. $+32$[8f]). Ee D-*N*-Boc-nor-valine-OMe >99.9%. (HPLC: Chiralcel OD, esano-IPA 99-1, flow 1 mL/min, 210 nm.)

The acidic product was recovered by extraction of the water phase with ethyl acetate (3×15 mL) at pH 3. Drying

and evaporation of the solvent gave 935 mg of L-acid as a pale oil (98% yield). ^{1}H NMR (4:1 rotamer ratio, the asterisk denotes minor rotamer peaks, $CDCl_3$): δ 0.95 (t, $J = 7.37$ Hz, 3H), δ 1.35–1.45 (m, 2H), δ 1.44 (s, 9H), δ 1.59–1.89 (m, 2H), δ 4.15* (br s, 1H), δ 4.30 (br s, 1H), δ 4.99 (br s, 1H), δ 5.95* (br s, 1H). $[\alpha]_D = -14.1$ (*c* 2.1 g/100 mL; 25 °C; 589 nm; MeOH) (lit. −14[8f]): Ee of L-*N*-Boc-nor-valine-OH 97% (the sample was treated with diazomethane to obtain the methyl ester derivative: HPLC: Chiralcel OD, esano-IPA 99-1, flow 1 mL/min, 210 nm).

4.3. Resolution of D,L-*N*-Boc-nor-leucine-OMe

To a solution of 70 g of D,L-*N*-Boc-nor-leucine-OMe (0.26 mol) in 150 mL of MTBE, 300 mL of distilled water was added. The pH was then adjusted up to 8.0 with NaOH (1 M) and 1300 U of protease was added. The reaction was kept at pH 8.0 by the automatic addition of NaOH (1 M), at 37 °C, under a vigorous mechanical stirrer. After 15 h, the reaction was complete and the D-*N*-Boc-nor-leucine-OMe recovered by phase separation (pH 8). The water phase was extracted with MTBE (2×200 mL). The combined organic layers were washed with 300 mL of distilled water and after drying on sodium sulfate and evaporation of the solvent, 30.3 g of a green oil was isolated (yield 95%). ^{1}H NMR ($CDCl_3$): δ 0.9 (t, $J = 6.9$ Hz, 3H), δ 1.24–1.37 (m, 4H), δ 1.44 (s, 9H), δ 1.54–1.84 (m, 2H), δ 3.73 (s, 3H), δ 4.27 (br s, 1H), δ 4.98 (br s, 1H).

The acidic product (L-*N*-Boc-nor-Leu-OH) was recovered by extraction of the water phase with ethyl acetate (3×200 mL) to pH 3. Drying and evaporation of the solvent gave 27.2 g of the L-*N*-Boc-nor-Leu-OH as a yellow oil (yield 90%).

^{1}H NMR (1:2 rotamer ratio, the asterisk denotes minor rotamer peaks, $CDCl_3$): δ 0.90 (t, $J = 6.94$ Hz, 3H), δ 1.28–1.41 (m, 4H), δ 1.45 (s, 9H), δ 1.60–1.93 (m, 2H), δ 4.1* (br s, 1H), δ 4.3 (br s, 1H), δ 5.0 (br s, 1H), δ 6.09* (br s, 1H). The ee value was calculated by HPLC (Chirobiotic T, H_2O–MeOH 80–20, 1 mL/min, 25 °C, 210 nm) on free amino acid after standard deprotection of the isolated end products: L-nor-leu >99.9; $[\alpha]_D = +23.2$ (*c* 5% in HCl 6 M; 25 °C; 589 nm).

Ee D-nor-Leu >99.9, $[\alpha]_D = -23.1$ (*c* 5% in HCl 6 M; 25 °C; 589 nm).

4.4. Resolution of D,L-*N*-tosyl-leucine-OMe 4c

rac-**4c** (4 g) was dissolved in 100 mL of a 3:2 water–acetone mixture at 39 °C. Protease (400 U) was added and the pH kept at 7.8 with the automatic addition of 1 M NaOH. The reaction was complete after 5 h reaction time. The unreacted ester was extracted with ethyl acetate giving 2 g of crude material. HPLC on Chiralcel OD proved the compound to be >99% ee.

The L-acid was precipitated from the aqueous solution and esterified with diazomethane to give a product of 88% ee by HPLC.

4.5. Resolution of D,L-*N*-Boc-t-leucine-OMe

4.5.1. General procedure. A suspension of the substrate in phosphate buffer at pH 7.5 was heated at 35 °C. The protease was then added and the reaction mixture kept at pH 7.5 by automatic titration with 0.5 M NaOH. When the consumption of NaOH reached about 50% (less or more to isolate L-*N*-Boc-t-leucine-OH or D-*N*-Boc-t-leucine-OMe), the reaction was stopped and the unreacted substrate recovered by extraction with ethyl acetate at pH 7.5. The acidic product could be recovered by extraction with ethyl acetate at pH 3.

4.5.2. D-t-Leucine. D,L-*N*-Boc-t-leucine-OMe (2 g) was dissolved in 100 mL of phosphate buffer. Protease (9 kU) was added and the pH kept at 7.8. The reaction was stopped after 18 h (58% of sodium hydroxide consumption). After extraction, 820 mg of D-*N*-Boc-t-leucine-OMe was obtained as a pale oil (yield 82%). ^{1}H NMR ($CDCl_3$): δ 0.96 (s, 9H), δ 1.45 (s, 9H), δ 3.71 (s, 3H), δ 4.05 (d br, 1H, $J = 7.2$ Hz,), δ 5.08 (br s, 1H).

To a solution of 500 mg of D-*N*-Boc-t-leucine-OMe in 5 mL of distilled water, 2 mL of NaOH (1 M) was added. After 12 h, the D-*N*-Boc-t-leucine-OH was extracted to afford 440 mg (yield 89%) of pale oil, which was immediately deprotected with dioxane saturated with HCl. After evaporation of the solvent, the D-t-leucine was purified with ionic exchange resin (Dowex 50 W × 8). 240 mg of D-t-leucine was obtained as a white solid (yield 96%). ^{1}H NMR (DMSO + TFA) δ 1.02 (s, 9H), δ 3.6 (s, 1H), δ 8.17 (br s, 2H). Ee D-t-leucine >99% (HPLC: Chirobiotic Tag, TEAA:MeOH 7–3, flow 0.5 mL/min, 215 nm). $[\alpha]_D = -31.1$ (*c* 1.01 g/100 mL; 25 °C; 589 nm); acetic acid: (lit.: $[\alpha]_D = +31.2$ for the L-enantiomer).[18]

4.5.3. L-t-Leucine. D,L-*N*-Boc-t-leucine-OMe (2.5 g) was dissolved in 50 mL of phosphate buffer. Protease (9 kU) was added and the reaction stopped after 23 h (49% of sodium hydroxide consumption). After extraction of the acidic product, 1.1 g of L-*N*-Boc-t-leucine-OH was obtained as a pale oil (yield 93%).

^{1}H NMR ($CDCl_3$): δ 1.02 (s, 9H), δ 1.44 (s, 9H), δ 5.12 (br s, 1H).

L-*N*-Boc-t-leucine-OH (1.0 g) was deprotected with dioxane saturated with HCl. After evaporation of the solvent, L-t-leucine was purified with ionic exchange resin (Dowex 50 W × 8). L-t-leucine (560 mg) was obtained as a white solid (yield 98%). ^{1}H NMR (DMSO + TFA) δ 1.02 (s, 9H), δ 3.6 (s, 1H), δ 8.17 (br s, 2H). Ee L-t-leucine 97% (HPLC: Chirobiotic Tag, TEAA:MeOH 7-3, flow 0.5 mL/min, 215 nm) $[\alpha]_D = +31.4$ (*c* 1.01 g/100 mL; 25 °C; 589 nm; acetic acid) (lit.: $[\alpha]_D = +31.2$).[18]

References

1. (a) Wang, L.; Schultz, P. G. *Angew. Chem., Int. Ed.* **2005**, *44*, 34–66; (b) Sun, H.; Zaneta, C. J.; 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; (c) 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.

2. (a) Perni, R. B.; Pitlik, J.; Britt, S. D.; Court, J. J.; Courtney, L. F.; Deininger, D. D.; Farmer, L. J.; Gates, C. A.; Harbeson, S. L.; Levin, R. B.; Lin, C.; Lin, K.; Moon, Y.-C.; Luong, Y.-P.; O'Malley, E. T.; Rao, B. G.; Thomson, J. A.; Tung, R. D.; Van Drie, J. H.; Wei, Y. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 1441–1446; (b) Llinas-Brunet, M.; Bailey, M. D.; Ghiro, E.; Gorys, V.; Halmos, T.; Poirier, M.; Rancourt, J.; Goudreau, N. *J. Med. Chem.* **2004**, *47*, 6584–6594; (c) Glenn, M. P.; Pattenden, L. K.; Reid, R. C.; Tyssen, D. P.; Tyndall, J. D. A.; Birch, C. J.; Fairlie, D. P. *J. Med. Chem.* **2002**, *45*, 371–381.
3. Lee, J. P.; Dunlap, B.; Rich, D. H. *Int. J. Pept. Prot. Res.* **1990**, *35*, 481–494.
4. Ley, S. V.; Priour, A. *Eur. J. Org. Chem.* **2002**, 3995–4004.
5. Oka, T.; Yasusa, T.; Ando, T.; Watanabe, M.; Yoneda, F.; Ishida, T.; Knoll, J. *Bioorg. Med. Chem.* **2001**, *9*, 1213–1219.
6. (a) Jefford, C. W.; Tang, Q.; Zaslona, A. *J. Am. Chem. Soc.* **1991**, *113*, 3513–3518; (b) Nieman, J. A.; Coleman, J. E.; Wallace, D. J.; Piers, E.; Lim, L. Y.; Roberge, M.; Andersen, R. J. *J. Nat. Products* **2003**, *66*, 183–199.
7. (a) Senten, K.; Van der Veken, P.; De Meester, I.; Lambeir, A.-M.; Scharpe, S.; Haemers, A.; Augustyns, K. *J. Med. Chem.* **2003**, *46*, 5005–5014; (b) Sheppard, G. S.; Kawai, M.; Craig, R. A.; Davidson, D. J.; Majest, S. M.; Bell, R. L.; Henkin, J. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 965–966; (c) Fray, M. J.; Dickinson, R. P.; Huggins, J. P.; Occleston, N. L. *J. Med. Chem.* **2003**, *46*, 3514–3525.
8. (a) Chenault, H. K.; Dahmer, J.; Whitesides, G. M. *J. Am. Chem. Soc.* **1989**, *111*, 6354–6364; (b) Miyazawa, T.; Minowa, H.; Miyamoto, T.; Imagawa, K.; Yanagihara, R.; Yamada, T. *Tetrahedron: Asymmetry* **1997**, *8*, 367–370; (c) Chen, S.-T.; Huang, W.-H.; Wang, K.-T. *J. Org. Chem.* **1994**, *59*, 7580–7581; (d) Chen, S.-T.; Huang, W.-H.; Wang, K.-T. *Chirality* **1994**, *6*, 572–576; (e) Pugniere, M.; Domergue, N.; Castro, B.; Previero, A. *Chirality* **1994**, *6*, 472–478; (f) Sasaki, N. A.; Hachimoto, C.; Potier, P. *Tetrahedron Lett.* **1987**, *48*, 6069–6072; (g) Miyazawa, T. *Amino Acids* **1999**, *16*, 191–213.
9. (a) Sun, H.; Zaneta, C. J.; Yang, C. Y.; Tomita, Y.; Pan, H.; Yoshioka, Y.; Krajewski, K.; Roller, P. P.; Wang, S. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 793–797; (b) Sun, H.; Zaneta, C. J.; 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; (c) Shioiri, T.; Hughes, R. J. *Heterocycles* **2003**, *61*, 23–29; (d) Oscarsson, K.; Poliakov, A.; Oscarson, S.; Danielson, U. H.; Hallberg, A.; Samuelsson, B. *Bioorg. Med. Chem.* **2003**, *11*, 2955–2963; (e) Ley, S. V.; Priour, A. *Eur. J. Org. Chem.* **2002**, 3995–4004.
10. (a) Wescott, C. R.; Klibanov, A. M. *Biochim. Biophys. Acta* **1994**, *1206*, 1–9; (b) Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc.* **1994**, *113*, 3166–3171; (aa) Bosetti, A.; Bianchi, D.; Cesti, P.; Golini, P. *Biocatalysis* **1994**, *9*, 71–77; (bb) Carrea, G.; Ottolina, G.; Riva, S. *Trends Biotechnol.* **1995**, *13*, 63–70; (cc) Rivard, M.; Malon, P.; Cerovsky, V. *Amino Acids* **1998**, *15*, 389–392; (dd) Lee, W. H.; Kim, K. J.; Kim, M. G.; Lee, S. B. *J. Ferment. Bioeng.* **1995**, *80*, 613–615; (ee) Kise, H.; Tomiuchi, Y. *Biotechnol. Lett.* **1991**, *13*, 317–322; (ff) Kawashiro, K.; Sugahara, H.; Sugiyama, S.; Hayashi, H. *Biotechnol. Bioeng.* **1997**, *53*, 26–31; (gg) Watanabe, K.; Yoshida, T.; Ueji, S. *Biorg. Chem.* **2004**, *32*, 504–515.
11. Rotticci, D.; Norin, T.; Hult, K. *Org. Lett.* **2003**, *2*, 1373–1376.
12. Savile, C. K.; Kazlauskas, R. J. *J. Am. Chem. Soc.* **2005**, *127*, 12228–12229.
13. Bommarius, A. S.; Schwarm, M.; Stingl, K.; Kottenhahn, M.; Huthmacher, K.; Drauz, K. *Tetrahedron: Asymmetry* **1995**, *6*, 2851.
14. (a) Grabley, S.; Keller, R.; Schlingmann, M.; (Hoechst) Vol. EP 0141 223, EP 0141 223, 1987; (b) Bommarius, A. S.; Drauz, K.; Kottenhahn, M.; (Degussa) Pat. Appl. DE 195 29 211.1, 1995; (c) Turner, N. J.; Winterman, J. R.; McCague, R.; Parratt, J. S.; Taylor, S. J. C. *Tetrahedron Lett.* **1995**, *36*, 1113; (d) Laumen, K.; Ghisalba, O.; Auer, K. *Biosci. Biotechnol. Biochem.* **2001**, *65*, 1977; (e) Laumen, K.; Kittelmann, M.; Ghisalba, O. *J. Mol. Catal. B: Enzym.* **2002**, *19*, 55.
15. (a) Wandrey, C.; Bossow, B. *Biotechnol. Bioind.* **1986**, *3*, 8; (b) Bommarius, A. S.; Schwarm, M.; Drauz, K. *Chimia* **2001**, *55*, 50; (c) Shaked, Z.; Whitesides, G. M. *J. Am. Chem. Soc.* **1980**, *102*, 7104; (d) Wichmann, R.; Wandrey, C.; Bückmann, A. F.; Kula, M.-R. *Biotechnol. Bioeng.* **1981**, *23*, 2789.
16. Wieland, T.; Birr, C.; von Durgen, A. *Ann. Chem.* **1971**, *747*, 207–210.
17. Travins, J. M.; Etzkorn, F. A. *Tetrahedron Lett.* **1998**, *39*, 9389–9392.
18. (a) Viret, J.; Patzelt, H.; Collet, A. *Tetrahedron Lett.* **1986**, *27*, 5865; (b) Yamada, S.-Y.; Mori, Y.; Morimatsu, K.; Ishizu, Y.; Ozaki, Y.; Yoshioka, R.; Nakatani, T.; Seko, H. *J. Org. Chem.* **1996**, *61*, 8586–8590.