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Lipase-Involved Strategy to the Enantiomers of 4-Benzyl-β-Lactam as a Key Intermediate in the Preparation of β-Phenylalanine Derivatives

Xiang-Guo Li, Liisa T. Kanerva*

Laboratory of Synthetic Drug Chemistry and Department of Chemistry, University of Turku, Lemminkäisenkatu 5 *C*, FIN-20520 Turku, Finland
Fax: (+358)-2-333-7955, e-mail: lkanerva@utu.fi

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Abstract: A simple chemoenzymatic method for the preparation of the enantiomers of 4-benzyl-β-lactam (4-benzylazetidin-2-one) from allylbenzene has been described. The enantiomers of this key intermediate have been used to produce the corresponding enantiomers of β-phenylalanine and *N*-Boc-protected β-phenylalanine amide through the simple cleavage of the lactam ring by acid-catalyzed hydrolysis and by ammonolysis, respectively. *Burkholderia cepacia* lipase-catalyzed kinetic double resolution techniques were responsible for achieving enantiopurity in the products. This was performed through the acylation of *N*-hydroxymethylated β-lactam followed by the butanolysis of the obtained (*S*)-ester. Direct lipase-catalyzed cleavage of the β-lactam ring has also been studied.

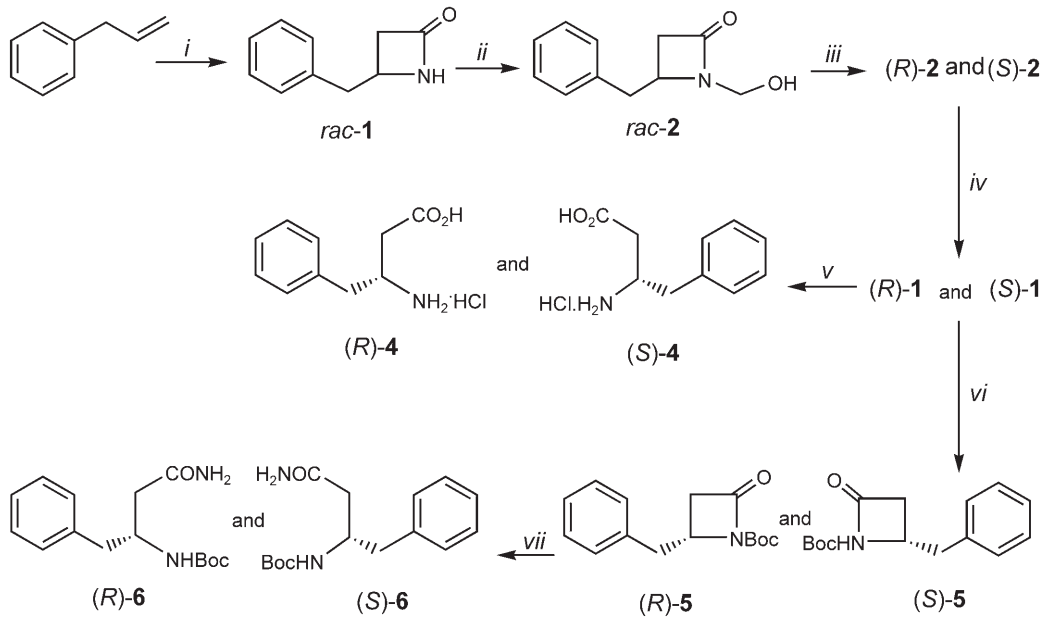
Keywords: β-amino acid; N-Boc-β-lactam; enantioselective acylation; enantioselective alcoholysis; hydrolysis; lipase catalysis

Introduction

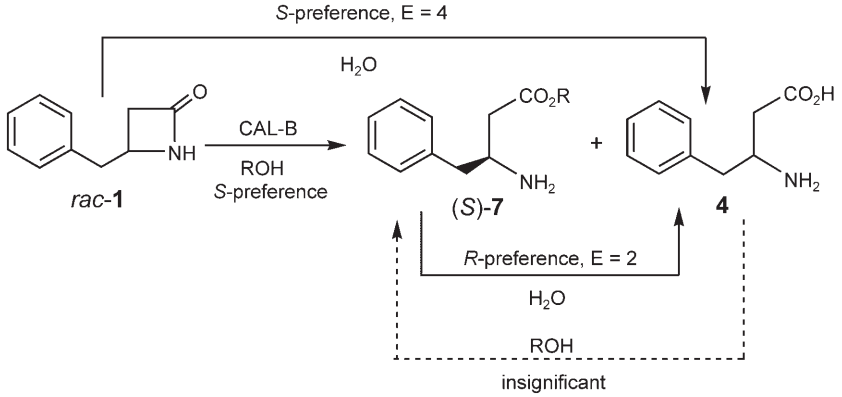
The enantiomers of β-lactams (2-azetidinones) are pharmaceutically interesting compounds as β-lactam antibiotics and applicable for various synthetic purposes as versatile intermediates, including the preparation of β-amino acids.[1–5] Several synthetic methods to construct enantiopure β-lactams are thoroughly described in a number of reviews.[3–8] Enzymatic methods include enantioselective hydrolysis and alcoholysis of *rac*-β-lactam rings by esterase, lipase and lactamase enzymes.[1,9–15] In addition, lipase-catalyzed enantioselective acylation of *N*-hydroxymethylated β-lactams has proved to be highly usable for preparative purposes, allowing both enantiomers of the β-lactam to be prepared simultaneously after the removal of the esterified or free hydroxymethyl tail.[16–20]

The only example for the synthesis of 4-benzyl-β-lactam concerns that of (*S*)-benzyl-β-lactam [(*S*)-**1**] through cyclization of the β^3-amino ester which, in turn, is obtained through the Arndt–Eistert homologation from L-phenylalanine.[2] However, the use of hazardous diazomethane makes the homologation method unattractive for large-scale synthesis. Preparation of (*S*)-3-*tert*-butoxycarbonylamino-4-phenylbutanamide [(*S*)-**6**] has also been previously described from L-phenylalanine.[21] We have long worked with the lipase-catalyzed preparation of the enantiomers of β-amino acid derivatives, and in this context want to focus on sustainable chemoenzymatic synthetic routes. We recently studied such a route for the preparation of the β-tryptophan enantiomers where lipase-catalyzed acylation of the β-amino nitrile was used for introducing enantiopurity in the molecules.[22]

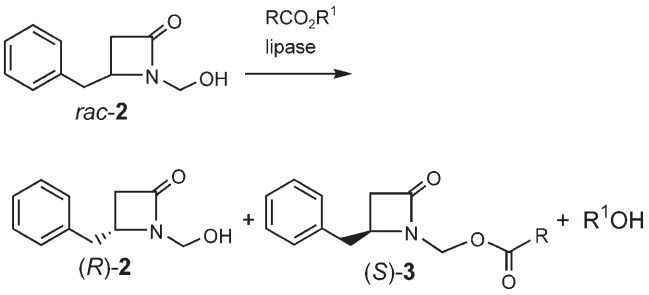
In the present paper, the chemoenzymatic preparation of the enantiomers of 4-benzyl-β-lactam [(*R*)- and (*S*)-**1**] is described. For this purpose, previously introduced lipase-catalyzed methods, lipase-catalyzed ring-opening of the lactam ring in *rac*-**1** (Scheme 2),[10,12] lipase-catalyzed asymmetric acylation of *N*-hydroxymethylated β-lactam *rac*-**2** (Scheme 3)[16] and lipase-catalyzed asymmetric alcoholysis of the corresponding ester *rac*-**3** (Scheme 4),[23] have been studied. The obtained (*R*)- and (*S*)-**1** have been further used as key intermediates for the preparation of enantiopure β^3-amino acids (*R*)- and (*S*)-**4**, *N*-Boc-protected β-lactam products (*R*)- and (*S*)-**5** and *N*-Boc-protected amino amides (*R*)- and (*S*)-**6** (Scheme 1). For instance, the β-lactam products (*R*)- and (*S*)-**5** are valuable intermediates which can be directly exploited in peptide synthesis due to activation caused by *N*-Boc.[24]



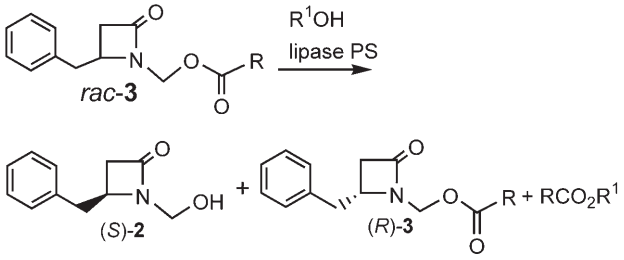
Scheme 1. *i*: CSI, CH_2Cl_2, 30 °C, then Na_2SO_3, K_2CO_3; *ii*: paraformaldehyde, K_2CO_3, H_2O, ultrasound, THF; *iii*: lipase-PS, first $PrCO_2CH{=}CH_2$ then BuOH; *iv*: NH_4OH, methanol; *v*: aqueous HCl (6 N); *vi*: $(Boc)_2O$, NEt_3, DMAP, CH_2Cl_2; *vii*: 25% NH_3 in methanol.



Scheme 2. CAL-B-catalyzed transformations in the reaction of *rac*-**1** with alcohols and water.



Scheme 3. Lipase PS-catalyzed acylation of *rac*-**2**.



Scheme 4. Lipase PS-catalyzed alcoholysis of *rac*-**3**.

Results and Discussion

Synthetic Routes to 3-Benzyl-3-propanic Acid Derivatives

Regio- and stereoselective 1,2-dipolar cycloaddition of chlorosulfonyl isocyanate (CSI) to alkenes has established itself as an effective method for the construction of a β-lactam ring.[12–20] Accordingly, preparation of *rac*-**1** was started by adding CSI slowly to the solution of allylbenzene in dry dichloromethane at 30° (Scheme 1). Reductive hydrolysis of the resulting azetidinone derivative with Na_2SO_3 furnished the reaction with 47% isolated yield. *Rac*-**2** was obtained without difficulty through the transformation of *rac*-**1** with paraformaldehyde under ultrasound.

Based on lipase PS-catalyzed kinetic resolution, the preparation of (*R*)- and (*S*)-**2** was performed as shown in detail later. The transformation of the obtained enantiomers into (*R*)- and (S)-**1** proceeded quantitatively with 25% ammonium hydroxide. Hydrolysis of (*R*)- and (S)-**1** in aqueous HCl afforded the amino acids (*R*)- and (*S*)-**4**, respectively. The enzymatic resolution products of *rac*-**1** [(*R*)-**2** and (*S*)-**3**, Scheme 3] can be also directly hydrolyzed in 18% HCl (not done in this work) with a risk for the formation of a dimerization product under acidic conditions.[16,18] On the other hand, the DMAP-catalyzed *N*-protection of (*R*)- and (S)-**1** with $(Boc)_2O$ yielded (*R*)- and (*S*)-**5** without difficulty. These highly valuable enantiomers with the *N*-Boc activated carbonyl groups are amenable to nucleophilic attack. Accordingly, they are potential substrates for many purposes such as for ammonolysis, leading to the formation of (*R*)-**6** from (*R*)-**5** and (*S*)-**6** from (*S*)-**5** as shown in the Experimental Section.

Enantioselective Cleavage of the β-Lactam Ring by Lipase Catalysis

It is generally accepted that lipases, although capable of forming amide bonds, are unsuitable for cleaving them. This is due to the resonance-stabilized carbonyl carbon. On the other hand, the lack of resonance stabilization in β-lactam rings has induced researchers to study the lipase-catalyzed enantioselective cleavage of the ring. Sih et al. first succeeded to open a *N*-benzoyl-activated β-lactam ring with methanol in *tert*-butyl methyl ether (TBME) in the presence of *Burkholderia cepacia* lipase (formerly lipase from *Pseudomonas cepasia*).[1] Later, lipases have been used for highly enantioselective hydrolyses of various unactivated β-lactams in organic solvents in the presence of small amounts (1 equiv.) of water or in the presence of both water and an alcohol.[12–15] The use of elevated temperature has been a common prerequisite for high enantioselectivity in the studied reactions. For the hydrolysis method, a task to keep the amount of water sufficient for activation in organic solvents can become a problem, especially for large-scale resolutions. For this reason, the ring opening with an alcohol seems more fascinating.

In the present work, *rac*-**1** was subjected to alcoholysis with methanol and *rac*-2-octanol in diisopropyl ether (DIPE) at 45–65 °C (Scheme 2, Table 1) in the presence of *Candida antarctica* lipase B (CAL-B, known as Novozyme 435 preparation). CAL-B and DIPE were chosen because highly enantioselective ring openings of unactivated β-lactam rings were reported earlier.[12–15] The enantiomerically enriched ester product (*S*)-**7** was obtained in each case but the amount of the ester was less than expected on the basis of conversion (entries 4–7). CAL-B easily enables hydrolysis of hydrolysable substances.[25] The water for hydrolysis can originate from the Novozyme 435 preparation where CAL-B is adsorbed on lipophilic material (divinylbenzene-cross-linked polymer based on methyl and butyl methacrylic

Table 1. CAL-B (30 mg/mL)-catalyzed alcoholysis of *rac*-**1** (0.05 M) in DIPE.[a]

Entry	Alcohol [M]	Conversion [%]	Time [h]	Temp. [°C]	ee^1 [%][b]	ee^7 [%][b]	ee^4 [%][c]	Ratio of [**7**]:[**4**]
1	no alcohol	44	3	45	36 (*R*)	–	46 (*S*)	–
2	no alcohol	65	2	65	45 (*R*)	–	45 (*S*)	–
3	H_2O (1 equiv.)	48	3	45	22 (*R*)	–	24 (*S*)	–
4	MeOH (0.2)	40	3	45	26 (*R*)	81 (*S*)	59 (*R*)	1.1:1
5	MeOH (0.4)	49	3	45	12 (*R*)	76 (*S*)	55 (*R*)	1.1:1
6	MeOH (0.4)	44	2	55	23 (*R*)	81 (*S*)	7 (*R*)	0.7:1
7	MeOH (0.4)	53	2	65	48 (*R*)	79 (*S*)	16 (*S*)	0.7:1
8	MeOH (0.4)[d]	5	19	45	2 (*R*)	–	37 (*S*)	–
9	*rac*-2-octanol (0.4)	32	3	45	23 (*R*)	–	–	1:2.3

[a] The amounts of r*ac*-**1** and the alcoholysis product **7** in the samples were obtained by calibration; the amount of **4** was obtained by subtracting the amount of **7** from **1**.
[b] ee determined by GC.
[c] The amounts of *R*- and *S*-enantiomers for ee were obtained by subtraction like in [a].
[d] CAL-B replaced with lipase PS preparation (30 mg/mL).

esters),[26] allowing the attached water to be released into organic solvents. *S*-Selective hydrolysis was seen clearly when *rac*-**1** was subjected to the enzymatic reaction conditions in the absence of an added competitive nucleophile (entries 1 and 2). Interestingly, when *rac*-**7** reacted under the same conditions the reaction was slightly *R*-selective. Thus, significant hydrolysis of *rac*-**1** and enantiomerically enriched (*S*)-**7** in the formation of acid **4** (directly undetectable by the GC method) gives a reasonable explanation for the observed incompatibility between conversion and the amount of the ester product even in the excess of alcohols.

The chiral GC method gives a good baseline separation for the enantiomers of **1** and **7**. Moreover, the initial amount of *rac*-**1** is known. Thus, expecting that the CAL-B-catalyzed reaction of *rac*-**1** in the presence of methanol (or *rac*-2-octanol) leads exclusively to products **7** and **4** the amounts of the enantiomers of **4** (and ee values) can be calculated in the sample. According to the results, (*R*)-**4** is obtained in excess at 45 and 55 °C, the proportion of the *S*-enantiomer increasing with temperature (entries 5–7). Evidently, *S*-selective hydrolysis of *rac*-**1** becomes more prominent than its *S*-selective methanolysis at higher temperatures. Still the enantiopuritity of (*S*)-**7** is unexpectedly high and reflects enantiomeric purification through the *R*-selective hydrolysis step from **7** to **4**.

As a conclusion, the moderate ee values in Table 1 indicate low enantioselectivity for both hydrolysis and alcoholysis of *rac*-**1**, making the method unusable for the preparation of enantiopure (*R*)-**1** [and (*S*)-**4** or (*S*)-**7**]. Even lower enantioselectivity ($ee^1 = 22$ at 48% conversion) is evident in the presence of 1 equivalent of water (Table 1, entry 3). In the case of methanolysis, the reaction turns very slow when lipase PS preparation is used as a catalyst in the place of CAL-B (entry 8).

Enzymatic Kinetic Resolution of *rac*-2 by Acylation

A decade ago the first studies in this laboratory on enzymatic acylation of *N*-hydroxymethylated β-lactams indicated lipase PS and lipase AK (from *Pseudomonas fluorescens*) in dry acetone as highly applicable for the present kind of kinetic resolutions.[16] It was proposed already in this early work and more precisely shown in a more recent work that water in the seemingly dry enzyme preparation can cause hydrolysis of the acylated product [such as (*S*)-**3**] and acyl donor (such as $RCO_2CH{=}CH_2$), leading to the formation of an acid (RCO_2H).[18] The system ends up in a complicated equilibrium involving the water produced when RCO_2H reacts with the serine hydroxy at the active site (the formation of an acyl-enzyme intermediate) and the consumption of this water through the hydrolysis of the acylated product enantiomer. As a result, the reaction tends to cease and enantiopurities drop with time. Initial tests for the acylation of *rac*-**2** with these two lipases in dry acetone indicated moderate enantioselectivities as shown by the values of enantiomer ratios "*E*" (Scheme 2, Table 2). Since the reaction ceased in the presence of two equivalents of vinyl butanoate (entry 1), the concentration of the acyl donor was set to 0.6 M (entries 2–6). Vinyl butanoate, vinyl acetate and 2,2,2-trifluoroethyl butanoate as acyl donors behaved in a similar manner (entries 2–4) except that the reaction was slow in the latter case. Lipase PS preparation was chosen for further studies. The *E* values although calculated at early reaction stages should be taken with caution due to possible hydrolysis as a side reaction. For this reason the *E* values are given as "*E*".

Solvent effects often play a pivotal role on enantioselectivity. This is clearly the case for acylation of *rac*-**2** (0.05 mmol/mL) with vinyl butanoate (0.6 mmol/mL) and lipase PS preparation (30 mg/mL) in dry organic solvents (Table 3). The reaction in toluene [containing 5% (v/v) acetone to dissolve the substrate] with "*E*" = 53 (entry 1) was the choice for the present application. As shown in the Table, enantioselectivity ("*E*" close to 50) was independent of vinyl butanoate concentration over the range 0.1 to 0.6 M (Table 4, entries 3–5). Although the acylation reaction seemed to proceed well in toluene the enantiopurity drops of the less reactive enantiomer, in particular, indicated enzymatic ester hydrolysis as a side reaction at higher conversions. The lipase PS-catalyzed acylation of *rac*-**2** with butanoic acid (0.05 M) in toluene proceeded slowly compared to the desired acylation with 0.1–0.6 M vinyl butanoate (en-

Table 2. Acylation of *rac*-**2** (0.05 M) with achiral acyl donors in dry acetone (2 mL) in the presence of the enzyme preparation (30 mg/mL) at 23 °C.

Entry	Enzyme preparation	Acyl donor [M]	Time [h]	Conversion [%]	"*E*"
1	lipase PS	$PrCO_2CH{=}CH_2$ (0.1)	5	19[a]	15
2	lipase PS	$PrCO_2CH{=}CH_2$ (0.6)	5	36	15 ± 1
3	lipase PS	$PrCO_2CH_2CF_3$ (0.6)	12	23	17 ± 2
4	lipase PS	$CH_3CO_2CH{=}CH_2$ (0.6)	5	35	12 ± 1
5	lipase PS-C II	$PrCO_2CH{=}CH_2$ (0.6)	2	48	6 ± 1
6	lipase AK	$PrCO_2CH{=}CH_2$ (0.6)	2	48	5 ± 1

[a] Equilibria in the enzymatic reaction mixture tend to stop the reaction.

Table 3. Acylation of *rac*-**2** (0.05 M) with vinyl butanoate (0.6 M) in organic solvents in the presence of the lipase PS preparation (30 mg/mL) at 23 °C.

Entry	Solvent	Time [h]	Conversion [%]	ee$^{(R)\text{-}2}$ [%]	ee$^{(S)\text{-}3}$ [%]	"E"
1	Toluene[a]	1.5	51	93	88	53±1
2	Methoxybenzene	1.5	35	48	90	32±0.4
3	Fluorobenzene	1.5	47	73	82	22±0.2
4	Acetone	1.5	14	14	87	17±1
5	TBME	1.5	50	80	79	21±7
6	DIPE/acetone (5:1)	1.5	49	85	88	38±2
7	DIPE/CH_2Cl_2 (2:1)	1	26	31	89	20±1
8	Hexane	1	46	68	81	19±1

[a] Containing acetone 5% (v/v).

tries 9 and 10 compared to 3–5). Thus, the water originally present in the enzyme preparation is evidently more harmful than the water from esterification. Addition of triethylamine in order to neutralize the formed butanoic acid (entry 6) or lowered temperature in order to enhance enantioselectivity (entry 1) failed.

As a conclusion, lipase PS-catalyzed kinetic resolution of *rac*-**2** in toluene allows the separation of the enantiomers, vinyl butanoate (2 equivs.) as an acyl donor being most applicable (Table 4, entry 3). However, enzymatic kinetic resolution with "E" = 58 has to be stopped at *ca.* 30% conversion in order to obtain (*S*)-**3** and somewhat over 50% conversion in order to obtain the unreacted (*R*)-**2** at ee = 95%.

Enzymatic Kinetic Resolution of *rac*-3 by Alcoholysis

For the preparation of the enantiomers (*R*)- and (*S*)-**2**, lipase-catalyzed alcoholysis of *rac*-**3** is an alternative method to acylation of *rac*-**2** (Scheme 4). Lipase-catalyzed alcoholysis can also be used as a mild ester-bond cleaving method in order to liberate (*S*)-**2** from (*S*)-**3** and at the same time to enhance enantiopurity. Enzymatic hydrolysis of *rac*-**3** as a side reaction is not harmful (when enantioselective enough) in the connection of alcoholysis because the two parallel reactions then lead to the formation of the same (*S*)-**2**.

Alcoholysis of *rac*-**3** with propanol in various organic solvents was conducted in the presence of lipase PS preparation (Table 5). The further optimization was performed in DIPE (Table 6). At low alcohol concentrations (0.05 M), enantioselectivity and reactivity were hardly affected by the nature of an alcohol (entries 2, 9, 12 and 13). Thus, basically any of them can be used. In the next experimental set, methanol was used as a nucleophile and its concentration effect was studied. Both reactivity and enantioselectivity notably decreased with increasing alcohol contents of the system (entries 2–8). The trend was similar in the presence of ethanol (entries 9 and 10) and 1-butanol (entries 13–15). The favorable effect of low temperature (23 °C) on enantioselectivity was clear according to the results in Table 6 (entries 5 and 6 and 10 and 11).

It is worth noting that lipase PS-catalyzed hydrolysis of *rac*-**3** with the water adsorbed in the enzyme preparation was significant when there was no competing nucleophile in the system (Table 6, entry 16). In this reaction, the formation of butanoic acid as a hydrolysis product was naturally observed in the chiral GC method and

Table 4. Acylation of *rac*-**2** (0.05 M) **with** achiral acyl donors in toluene containing acetone [5% (v/v)] in the presence of lipase PS preparation (30 mg/mL).

Entry	Acyl donor [M]	Temp. [°C]	Time [h]	Conversion [%]	ee$^{(R)\text{-}2}$ [%]	ee$^{(S)\text{-}3}$ [%]	"E"
1	$PrCO_2CH{=}CH_2$ (0.1)	6	1	17	19	95	40±2
2	$PrCO_2CH{=}CH_2$ (0.1)[a]	23	8	45	70	87	29±2
3	$PrCO_2CH{=}CH_2$ (0.1)	23	4	55	97	81	58±2
4	$PrCO_2CH{=}CH_2$ (0.2)	23	1.5	51	90	88	49±1
5	$PrCO_2CH{=}CH_2$ (0.6)	23	1	48	83	91	52±1
6	$PrCO_2CH{=}CH_2$ (0.6)[b]	23	1.5	49	81	84	29±4
7	$PrCO_2CH{=}CH_2$ (3.0)	23	1	48	79	87	35±1
8	$PrCO_2CH_2CF_3$ (0.6)	23	6	41	64	92	48±1
9	$PrCO_2H$ (0.05)	23	1	–	–	–	–
10	$PrCO_2H$ (0.05)	23	69	9	10	93	29

[a] 10 mg/mL of lipase PS on celite.
[b] NEt_3 (0.2 M) added.

Table 5. Alcoholysis of *rac*-**3** (0.05 M) with PrOH (0.05 M) in the presence of lipase PS preparation (30 mg/mL) in different solvents at 23 °C.

Entry	Solvent	Time [h]	Conversion [%]	ee$^{(R)\text{-}3}$ [%]	ee$^{(S)\text{-}2}$ [%]	E
1	DIPE	5	51	94	92	84±4
2	TBME	4	51	93	89	55±3
3	THF	74	3	2	55	4
4	Toluene	17	20	24	94	39
5	Acetone	74	16	17	86	15
6	Acetonitrile	74	18	17	76	9

Table 6. Deacylation of *rac*-**3** (0.05 M) with an alcohol in DIPE in the presence of lipase PS preparation (30 mg/mL).

Entry	Alcohol [M]	Temp. [°C]	Time [h]	Conversion [%]	ee$^{(R)\text{-}3}$ [%]	ee$^{(S)\text{-}2}$ [%]	E
1	MeOH (0.05)	23	2	48[a]	85	93	79±4
2	MeOH (0.05)	23	5	50	91	92	75±1
3	MeOH (0.1)	23	7	50	91	92	68±1
4	MeOH (0.2)	23	7	50	91	92	73±5
5	MeOH (0.4)	23	9	50	90	91	65±6
6	MeOH (0.4)	45	5	51	89	86	40±1
7	MeOH (0.6)	23	12	49	88	90	59±9
8	MeOH (0.8)	23	22	51	92	89	53±2
9	EtOH (0.05)	23	5	49	89	92	74±2
10	EtOH (1.6)	23	50	37	54	93	45±1
11	EtOH (1.6)	45	50	25	29	86	23±4
12	PrOH (0.05)	23	5	51	94	92	84±2
13	BuOH (0.05)	23	5	51	94	91	73±3
14	BuOH (0.1)	23	5	51	96	91	82±2
15	BuOH[b]	45	128	2	1	70	6
16	H_2O[c]	23	5	35	45	83	17

[a] 75 mg/mL of lipase PS on celite.
[b] BuOH as a solvent.
[c] Hydrolysis caused by the water in the seemingly dry enzyme preparation.

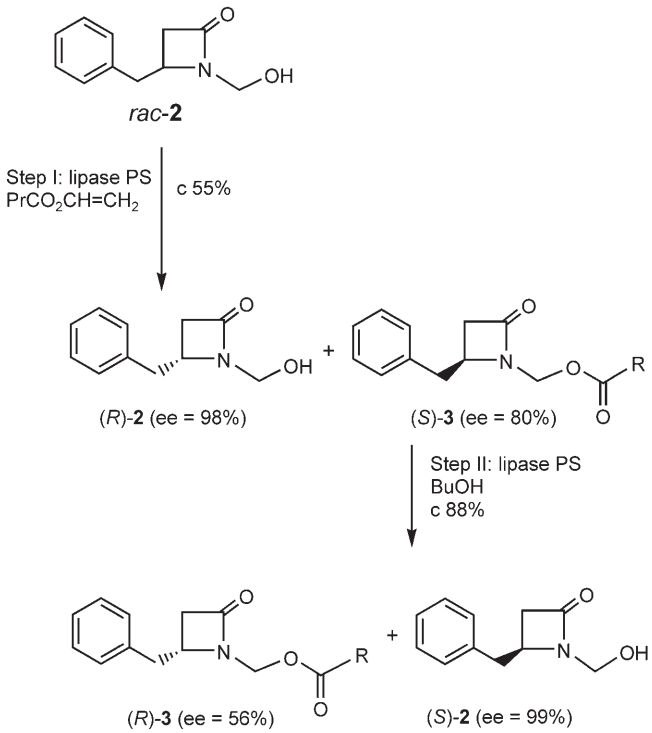
its amount was in full accordance with the disappearance of **3**. However, in the presence of an alcohol the hydrolysis was suppressed to a negligible level. For example, butanoic acid was not observed when BuOH (0.1 M) was present (entry 15) and the amount of the formed $PrCO_2Bu$ was approximately what was expected on stoichiometric basis.

As a conclusion, lipase PS-catalyzed kinetic resolution of *rac*-**3** with primary alcohols in DIPE allows the separation of the enantiomers with E close to 80. As with enzymatic acylation, conversion needs to be controlled according to which enantiomer is wanted. The use of 1-butanol with $E=82$ (Table 6, entry 14) is beneficial due to the easy detection of the formed butyl butanoate together with the resolution products in the GC method.

Gram-Scale Preparation of the Enantiomers of 2

It is clear according to the above results that (*R*)-**2** as an enantiopure intermediate can be produced as the less reactive enantiomer using the lipase PS-catalyzed acylation of *rac*-**2** in toluene (Scheme 3). When (*S*)-**2** is the desired enantiomer the lipase PS-catalyzed alcoholysis with 1-butanol (or some other primary alcohol) in DIPE leads to the goal (Scheme 4). For the preparation of both (*R*)-and (*S*)-**2**, a double resolution technique is most reasonable. A method with minimized number of reaction steps is then worth considering. This is most conveniently carried out by performing first enzymatic acylation of *rac*-**2** followed by enzymatic alcoholysis of (*S*)-**3** according to Scheme 5.

Lipase PS-catalyzed acylation of *rac*-**2** with vinyl butanoate in toluene was stopped at 55% conversion by filtering off the enzyme. The separation of the resolution products on a silica gel column yielded the unreacted (*R*)-**2** (ee = 98%) and the butanoate (*S*)-**3** (ee = 80%). The separated (*S*)-**3** was then subjected to lipase PS-catalyzed alcoholysis with 1-butanol. The reaction was stopped at 88% conversion yielding (*S*)-**2** (99% ee). As a total outcome from the double resolution, *rac*-**2** was transformed into (*R*)-**2** at 45% and into (*S*)-**2** at 46% isolated yields, corresponding closely to the theo-



Scheme 5. Gram-scale preparation of (*R*)- and (*S*)-**2**.

retical 50% proportions of the enantiomers in the racemate.

Absolute configurations of the enantiomeric products were designated by comparing the optical rotation of (*S*)-**6** $[\alpha]_D^{25}$: −20.6 (*c* 0.9, $CHCl_3$) with the literature data $[\alpha]_D^{20}$: −18.96 (*c* 2.6, $CHCl_3$).[21] Accordingly, for acylation of *rac*-**2** lipase PS favors the reaction of the *S*-enantiomer in the formation of (*S*)-**3**. This is further transformed into (*S*)-**2**, (*S*)-**1**, (*S*)-**5** and (*S*)-**6** (Scheme 1). The places of the peaks at the chromatograms thereafter allow the absolute configurations of the reaction products to be known.

Conclusion

The present research describes a simple chemoenzymatic method to the enantiomers of 4-benzyl-β-lactam ((*R*)- and (*S*)-**1**) starting from allylbenzene. The enantiomers of this key intermediate serve to produce the corresponding enantiomers of β-phenylalanine [(*R*)- and (*S*)-**4**] and *N*-Boc-protected β-phenylalanine amide [(*R*)- and (*S*)-**6**] through simple cleavage of the lactam ring by acid-catalyzed hydrolysis and by ammonolysis after the Boc-protection, respectively.

The present work indicates the subtle applicability of lipases in the preparation of enantiopure β-amino acids and their derivatives. Although the lipase-catalyzed cleavage of the β-lactam ring of *rac*-**1** with water, methanol or *rac*-2-octanol was not enantioselective enough for introducing enantiopurity on the chemoenzymatic reaction route, it was possible to produce enantiopurity using *N*-hydroxymethylated derivatives as substrates. Accordingly, lipase PS-catalyzed asymmetric acylation of *rac*-**2** in toluene followed by lipase PS-catalyzed deacylation of the esterified product (*S*)-**3** with 1-butanol provided us with enantiopure (*R*)- and (*S*)-**2** (ee ≥ 98%) close to quantitative chemical yields from the racemate.

Experimental Section

General Remarks

Allylbenzene, chlorosulfonyl isocyanate (CSI), paraformaldehyde, vinyl butanoate, vinyl acetate and the solvents were products of Aldrich or Fluka. 2,2,2-Trifluoroethyl butanoate was prepared from butanoyl chloride and 2,2,2-trifluoroethanol. All solvents were of the highest analytical grade and were dried by standard methods when necessary. Lipase from *Burkholderia cepacia* (formerly *Pseudomonas cepacia*) as a native enzyme (lipase PS) and as immobilized on ceramic particles (lipase PS-C II) and from *Pseudomonas fluorescence* (lipase AK) as a native enzyme were purchased from Amano Europe, England. Before use, native lipases were adsorbed on celite by dissolving the enzyme (5 g) and sucrose (3 g) in Tris-HCl buffer (250 mL, 20 mM, pH = 7.9) followed by the addition of celite (17 g). The mixture was dried by letting the water evaporate. The lipase preparation containing 20% (w/w) of the enzyme was thus obtained. *Candida antarctica* lipase B (CAL-B, Novozyme 435) was a generous gift from Novo Nordisk. Preparative chromatographic separations were performed by column chromatography on Merck Kieselgel 60 (0.063–0.200 μm). TLC was carried out with Merck Kieselgel $60F_{254}$ sheets. If not otherwise stated, all enzymatic reactions were performed at room temperature (23 °C). Melting points were measured on a Mettler FP80 instrument at a heating rate of 2 °C/min.

The ^1H and ^{13}C NMR spectra were recorded on a Bruker 500 spectrometer with tetramethylsilane (TMS) as an internal standard. ^1H-^1H COSY, ^1H-^{13}C HQSC and ^1H-^{13}C HMBC spectra were used for the assignments of the chemical shifts. Mass spectra were taken on a VG 7070E mass spectrometer. Optical rotations were determined with a Perkin-Elemer polarimeter, and $[\alpha]_D$ values are given in units of 10^{-1} deg cm^2 g^{-1}. The determination of *E* was based on the equation $E = \ln[(1-c)(1-ee_s)]/\ln[(1-c)(1+ee_s)]$ with the use of linear regression, *E* being the slope of a line $\ln[(1-c)(1-ee_s)]$ *vs.* $\ln[(1-c)(1+ee_s)]$.[27]

Typical Small-Scale Experiment

The lipase preparation (usually 30 mg/mL) was added to the solution of *rac*-**2** (0.05 M) and an acyl donor (0.1–3.0 M) or to the solution of *rac*-**3** (0.05 M) and an alcohol (0.05 M to neat alcohol) in an organic solvent (2 mL). The progress of the reactions and the ee values were followed by taking samples (0.1 mL) at intervals and analyzing them by gas chromatography. The GC was equipped with a Chrompack CP-Chira-

sil-DEX CB column. Calibration in the GC method was based on the use of dihexyl ether as an internal standard.

Preparation of 4-Benzylazetidin-2-one (*rac*-1)

A solution of chlorosulfonyl isocyanate (CSI) (3.96 g, 28.0 mmol) in dichloromethane (3 mL) was added dropwise to a solution of allylbenzene (3.30 g, 28.0 mmol) in dry dichloromethane (11 mL) at 0 °C. The reaction mixture was stirred at 30 °C for 6 days. The resulting mixture was added to a vigorously stirred solution of Na_2SO_3 (0.35 g, 2.8 mmol) and K_2CO_3 (8.11 g, 58.8 mmol) in H_2O (73 mL) during two days. The organic layer was separated and the aqueous layer was extracted with dichloromethane (3 × 15 mL). The combined organic phases were dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified on a silica gel column eluting with petroleum ether/ethyl acetate (1:3), affording a white solid product; yield: 2.13 g (13.2 mmol, 47%); mp 52–54 °C. HR-MS: M^+ found (M^+ calculated for $C_{10}H_{11}NO$): 161.08430 (161.08406); MS: *m*/*z* (relative intensity) = 161 (16), 133 (8), 118 (100), 103 (4), 92 (18), 91 (69), 77 (5); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.69–2.71 (d, *J* = 15.0 Hz, 1H), 2.86–2.90 (dd, *J* = 13.5, 8.0 Hz, 1H), 2.94–2.98 (dd, *J* = 13.5, 6.0 Hz, 1H), 3.05–3.09 (d, *J* = 15.0 Hz, 1H), 3.84–3.87 (m, 1H), 6.32 (br. s, 1H), 7.19–7.36 (m, 5H); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 41.82, 43.26, 48.94, 126.89, 128.79, 128.82, 137.56, 167.81.

Preparation of 4-Benzyl-1-hydroxymethylazetidin-2-one (*rac*-2)

Racemic **1** (0.85 g, 5.3 mmol) was dissolved in tetrahydrofuran (12 mL) and paraformaldehyde (0.18 g, 6.0 mmol), K_2CO_3 (0.07 g, 0.5 mmol) and H_2O (0.5 mL) were added. The suspension was sonicated for 6 h. The solvent was evaporated off and the residue was dissolved in diethyl ether (20 mL). The solution was dried over anhydrous Na_2SO_4, filtered and evaporated. The residue was purified on a silica gel column eluting with ethyl acetate/petroleum ether (2:1), affording a semisolid product; yield: 0.99 g (5.2 mmol, 98%). HR-MS: M^+ found (M^+ calculated for $C_{11}H_{13}NO_2$): 191.09530 (191.09463); MS: *m*/*z* (relative intensity) = 191 (11), 163 (7), 146 (25), 132 (7), 118 (62), 100 (100), 91 (71), 77 (8); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 2.66–2.70 (dd, *J* = 15.0, 2.5 Hz, 1H, CH_2CON), 2.80–2.85 (dd, *J* = 14.0, 8.5 Hz, 1H, $ArCH_2$), 2.95–2.99 (dd, *J* = 15.0, 5.0 Hz, 1H, CH_2CON), 3.15–3.19 (dd, *J* = 14.0, 5.5 Hz, 1H, $ArCH_2$), 4.03–4.07 (m, 1H, CH_2CHN), 4.42–4.44 (d, *J* = 11.5 Hz, 1H, CH_2OH), 4.77–4.80 (d, *J* = 11.5 Hz, 1H, CH_2OH), 7.19–7.35 (m, 5H, arom); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 39.74 ($ArCH_2$), 42.50 (CH_2CON), 51.50 (CH_2CHN), 63.84 (CH_2OH), 126.95, 128.80, 128.88, 136.88, 167.75 (*C*O).

Preparation of 4-Benzyl-1-butanoyloxymethylazetidin-2-one (*rac*-3)

NEt_3 (0.65 g, 6.4 mmol) and butanoic anhydride (0.76 g, 4.8 mmol) were added dropwise to a solution of *rac*-**2** (0.61 g, 3.2 mmol) in CH_2Cl_2 (20 mL) at 0 °C. The reaction mixture was stirred for 20 hours before the solvent was evaporated under vacuum. The residue was purified on silica eluting with ethyl acetate/petroleum ether (2:1), affording *rac*-**3** as an oil; yield: 0.79 g (3.0 mmol, 94%); MS: *m*/*z* (relative intensity) = 261 (0.02), 173 (57), 145 (13), 132 (15), 117 (28), 100 (11), 91 (17), 71 (100); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 0.98 (t, *J* = 7.5 Hz, 3H, CH_3), 1.65–1.72 (m, 2H), 2.34 (t, *J* = 7.2 Hz, 2H, $COCH_2$), 2.72–2.80 (m, 2H), 2.99–3.03 (dd, *J* = 15.2, 5.1 Hz, 1H), 3.28–3.32 (dd, *J* = 13.5, 4.5 Hz, 1H), 3.93–3.96 (m, 1H, CH_2CHN), 5.11–5.21 (q, *J* = 11.5 Hz, 2H), 7.20–7.35 (m, 5H, arom); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 13.64, 18.25, 35.89, 39.35, 42.98, 53.06, 63.02, 126.96, 128.77, 128.95, 136.50, 167.26, 173.54.

Gram-Scale Preparation of (*R*)- and (*S*)-2 by Kinetic Resolution

For acylation (step I), *rac*-**2** (1.06 g, 5.6 mmol) was dissolved in toluene (110 mL) and acetone (5 mL), lipase PS preparation (30 mg/mL) and vinyl butanoate (1.26 g, 11.1 mmol) were added. The reaction was stopped by filtering off the enzyme at 55% conversion with ee = 98% for the unreacted (*R*)-**2** and 80% ee for the produced (*S*)-**3**. The solvent was evaporated and the residue was purified on a silica gel column. (*S*)-**3** {0.79 g, 3.0 mmol; $[\alpha]_D^{25}$: −11.4 (*c* 1, $CHCl_3$)} was obtained first eluting with ethyl acetate/petroleum ether (1:2) in 99% yield. Elution with petroleum ether/ethyl acetate (1:3) afforded the unreacted (*R*)-**2** {0.47 g, 2.5 mmol; $[\alpha]_D^{25}$: −73.6 (*c* 1, $CHCl_3$)} as a semisolid in 98% yield.

For alcoholysis (step II), (*S*)-**3** (0.79 mg, 3.0 mmol, 80% ee) was dissolved in DIPE (61 mL), and BuOH (0.45 g, 6.1 mmol) and lipase PS preparation (30 mg/mL) were added. After 2.5 hours, the reaction was stopped by filtering off the enzyme at 88% conversion with 99% ee for the produced (*S*)-**2** and 56% ee for the unreacted (*R*)-**3**. The column separation proceeded as above. (*S*)-**2** {0.50 g, 2.6 mmol; $[\alpha]_D^{25}$: +73.3 (*c* 1, $CHCl_3$)} was obtained in 98% yield. (*R*)-**3** {0.09 g, 0.3 mmol; $[\alpha]_D^{25}$: +8.2 (*c* 1, $CHCl_3$)} was isolated as an oil.

Transformations of (*R*)- and (*S*)-2 into (*R*)- and (*S*)-1

Aqueous NH_4OH (25%, 1 mL) was added dropwise to the solution of (*R*)-**2** (0.19 g, 1.0 mmol) in methanol (2 mL) at 0 °C. Two hours later, the starting material was totally consumed as indicated by TLC. The solvent was evaporated under vacuum giving (*R*)-**1** {ee = 98%; $[\alpha]_D^{25}$: +30.1 (*c* 0.65, $CHCl_3$)} in quantitative yield.

Similarly, (*S*)-**1** was obtained from (*S*)-2 {ee = 99%; $[\alpha]_D^{25}$: −30.7 (*c* 1, $CHCl_3$)} in quantitative yield.

Transformations of (*R*)- and (*S*)-1 into (*R*)- and (*S*)-4

(*S*)-**1** (0.10 g, 0.62 mmol) was added to aqueous HCl (6 N, 3.5 mL). The mixture was gently refluxed for 5 hours before the solvent was evaporated, affording (*S*)-**4**; yield: 0.13 g (0.62 mmol, quantitative yield); mp 176–178 °C; ee = 99%. HR-MS was not obtained due to too low intensity. MS: *m*/*z* (relative intensity) = 118 (4), 88 (100), 77 (3), 70 (47); ^{1}H NMR (D_2O, 500 MHz): δ = 2.52–2.58 (dd, *J* = 17.5, 7.5 Hz, 1H, CH_2CO_2H), 2.63–2.67 (dd, *J* = 18.0, 5.0 Hz, 1H, CH_2CO_2H), 2.88–2.89 (d, *J* = 7.5 Hz, 2H, $ArCH_2$), 3.74–

3.78 (m, 1H, C*H*NH_2), 7.19–7.36 (m, 5H, aroma.); ^{13}C NMR (D_2O, 126 MHz): δ = 35.52, 37.85, 49.43, 127.69, 129.11, 129.46, 135.02, 173.98. Similarly, (*R*)-**4** (ee = 98%) was obtained from (*R*)-**1** in quantitative yield.

Transformations of (*R*)- and (*S*)-1 into (*R*)- and (*S*)-5

Boc_2O (0.54 g, 2.5 mmol) in CH_2Cl_2 (3 mL) was added to a solution of (*R*)-**1** (0.20 g, 1.2 mmol) and DMAP (0.015 g, 0.12 mmol) in CH_2Cl_2 (5 mL). After 10 hours, the starting material was totally consumed as indicated by TLC. The solvent was evaporated and the residue was purified on a silica gel column eluting with ethyl acetate/petroleum ether (1:5). (*R*)-**5** {0.32 g, 1.2 mmol; mp 75–77 °C; ee = 98%; $[\alpha]_D^{25}$: −104.2 (*c* 1, $CHCl_3$)} was obtained in 99% yield. HR-MS: M^+ found (M^+ calculated for $C_{15}H_{19}NO_3$): 261.13690 (261.13649); MS: *m*/*z* (relative intensity) = 261 (0.1), 205 (57), 187 (5), 145 (20), 118 (40), 91 (20), 57 (100); ^{1}H NMR ($CDCl_3$, 500 MHz): δ = 1.56 [s, 9H, C(CH_3)$_3$], 2.66–2.69 (dd, *J* = 16.0, 3.0 Hz, 1H), 2.83–2.88 (dd, *J* = 13.5, 8.5 Hz, 1H), 2.92–2.96 (d, *J* = 16.0, 6.0 Hz, 1H), 3.41–3.44 (dd, *J* = 13.5, 2.5 Hz, 1H), 4.15–4.18 (m, 1H, CH_2C*H*N), 7.16–7.33 (m, 5H, arom); ^{13}C NMR ($CDCl_3$, 126 MHz): δ = 28.11, 38.42, 41.35, 51.71, 83.31, 127.05, 128.79, 129.24, 135.94, 148.02, 164.27.

Similarly, (*S*)-**5** {ee = 99%; $[\alpha]_D^{25}$: +104.4 (*c* 1, $CHCl_3$)} was obtained from (*S*)-**1** in 99% yield.

Transformations of (*R*)- and (*S*)-5 into (*R*)- and (*S*)-6

(*R*)-**5** (0.29 g, 1.1 mmol) was added to 25% NH_3 in methanol (3 mL) at 0 °C and the mixture was stirred for 4 hours at 0 °C. The solvent was evaporated and the residue was washed with hexane (6 mL) and CH_2Cl_2 (6 mL), affording (*R*)-**6** {0.31 g, 1.1 mmol; mp 128–131 °C; $[\alpha]_D^{25}$: +20.6 (*c* 1.0, $CHCl_3$)} in 99% yield. HR-MS was not obtained due to too low intensity. MS: *m*/*z* (relative intensity) = 280 (M^+ + 2, 0.1), 205 (12), 187 (15), 161 (7), 146 (26), 118 (70), 100 (100), 91 (80), 77 (8), 58 (70); ^{1}H NMR (DMSO-d_6, 500 MHz): δ = 1.31 [s, 9H, C(CH_3)$_3$], 2.14–2.18 (dd, *J* = 14.5, 7 Hz, 1H, CH_2CONH$_2$), 2.20–2.24 (dd, *J* = 14.5, 7.0 Hz, 1H, CH_2CONH$_2$), 2.63–2.67 (dd, *J* = 13.5, 8.0 Hz, 1H, ArCH_2), 2.68–2.72 (dd, *J* = 13.0, 5.5 Hz, 1H, ArCH_2), 3.90–3.94 (m, 1H, CH_2C*H*NH), 6.84 (br s, 1H, N*H*), 7.15–7.32 (m, 5H, arom); ^{13}C NMR (DMSO-d_6, 126 MHz): δ = 28.70 [C(*C*H_3)$_3$], 39.48, 40.48, 49.68 (CH_2*C*HNH), 77.92 [*C*(CH$_3$)$_3$], 126.39, 128.51, 129.66, 139.41, 155.26, 172.75.

Similarly, (*S*)-**6** {mp 128–131 °C; lit. mp 125–129 °C;[21] $[\alpha]_D^{25}$: −20.6 (*c* 0.9, $CHCl_3$); lit. $[\alpha]_D^{20}$: −18.96 (*c* 2.6, $CHCl_3$)}[21] was obtained from (*S*)-5 in 96% yield.

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References

[1] R. Brieva, J. Z. Crich, C. J. Sih, *J. Org. Chem.* **1993**, *58*, 1068–1075.
[2] C. Yoakim, W. W. Ogilvie, D. R. Cameron, C. Chabot, I. Guse, B. Haché, J. Naud, J. A. O'Meara, R. Plante, R. Déziel, *J. Med. Chem.* **1998**, *41*, 2882–2891.
[3] G. S. Singh, *Mini-Rev. Med. Chem.* **2004**, *4*, 69–92.
[4] G. S. Singh, *Mini-Rev. Med. Chem.* **2004**, *4*, 93–109.
[5] C. Palomo, J. M. Aizpurua, I. Ganboa, M. Oiarbide, *Synlett* **2001**, 1813–1826.
[6] C. Palomo, J. M. Aizpurua, I. Ganboa, M. Oiarbide, *Eur. J. Org. Chem.* **1999**, 3223–3235.
[7] M. Benaglia, M. Cinquini, F. Cozzi, *Eur. J. Org. Chem.* **2000**, 563–572.
[8] E. Forró, F. Fülöp, *Mini-Rev. Org. Chem.* **2004**, *1*, 93–102.
[9] C. Evans, C. R. McCauge, S. M. Roberts, A. G. Sutherland, R. Wisdom, *J. Chem. Soc. Perkin Trans. 1* **1991**, 2276–2277.
[10] M. Jones, M. I. Page, *J. Chem. Soc. Chem. Commun.* **1991**, 316–317.
[11] W. Adam, P. Groer, H.-U. Humpf, C. R. Saha-Möller, *J. Org. Chem.*, **2000**, *65*, 4919–4922.
[12] S. Park, E. Forró, H. Grewal, F. Fülöp, R. J. Kazlauskas, *Adv. Synth. Catal.* **2003**, *345*, 986–995.
[13] E. Forró, F. Fülöp, *Org. Lett.* **2003**, *5*, 1209–1212.
[14] E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2004**, *15*, 573–575.
[15] E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2004**, *15*, 2875–2880.
[16] P. Csomós, L. T. Kanerva, G. Bernáth, F. Fülöp, *Tetrahedron: Asymmetry* **1996**, *7*, 1789–1796.
[17] Zs. Cs. Gyarmati, A. Liljeblad, M. Rintola, G. Bernáth, L. T. Kanerva, *Tetrahedron: Asymmetry*, **2003**, *14*, 3805–3814.
[18] Zs. Cs. Gyarmati, A. Liljeblad, G. Argay, A. Kálmán, G. Bernáth, L. T. Kanerva, *Adv. Synth. Catal*, **2004**, *346*, 566–572.
[19] J. Kámán, E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2000**, *11*, 1593–1600.
[20] E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2001**, *12*, 2351–2358.
[21] P. W. Sutton, A. Bradley, J. Farràs, P. Romea, F. Urpì, J. Vilarrasa, *Tetrahedron* **2000**, *56*, 7947–7958.
[22] X.-G. Li, L. T. Kanerva, *Tetrahedron: Asymmetry* **2005**, *16*, 1709–1714.
[23] J. Kámán, E. Forró, F. Fülöp, *Tetrahedron: Asymmetry* **2001**, *12*, 1881–1886.
[24] C. Palomo, M. Oiarbide, S. Bindi, *J. Org. Chem.* **1998**, *63*, 2469–2474.
[25] L. Veum, L. T. Kanerva, P. J. Halling, T. Maschmeyer, U. Hanefeld, *Adv. Synth. Catal.* **2005**, *347*, 1015–1021.
[26] N. W. J. T. Heinsman, C. G. P. H. Schroên, A. van der Padt, M. C. R. Franssen, R. M. Boom, K. van't Riet, *Tetrahedron: Asymmetry* **2003**, *14*, 2699–2704.
[27] C.-S., Chen, Y. Fujimoto, G. Girdaukas, C. J. Sih, *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.

