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Development of an Acid-Washable Tag for the Separation of Enantiomers from Bioresolutions

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Abstract:

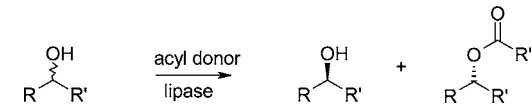
A separation tool involving the use of a vinyl ester amino acid as acyl donor in a bioresolution has been developed. The acid-washable acyl group acts as a removable tag, facilitating separation of the secondary alcohol from the bioresolution product mixture. The use of these acyl donors from cheap, commercially available amino acids has been demonstrated in conjunction with the hydrolase enzyme *Candida antarctica* lipase B in the selective acylation of a variety of secondary alcohols.

Introduction

Despite the dramatic improvements in enantioselective synthesis and chromatographic separation methods, bioresolution still remains one of the most inexpensive and operationally simple methods for producing pure enantiomers on a large scale in the chemical industry.[1] Typically the reactions are carried out under ambient and neutral conditions. For example, hydrolase-catalysed kinetic resolutions have been widely used for the synthesis of enantiopure secondary alcohols as shown in Scheme 1. These are important intermediates for the preparation of active pharmaceutical ingredients (APIs).[2]

Following identification of the biocatalyst used in the selective bioresolution, the separation of the modified enantiomers becomes the processing problem. The kinetic bioresolution of racemic secondary alcohols is usually carried out by a selective transesterification using acyl donors such as the enol esters, vinyl acetate or isopropenyl acetate. The use of these vinyl esters as acyl donors has the drawback of the separation of the ester (product) and the alcohol (substrate) by column chromatography, which can be costly and troublesome especially at large scale. To avoid this problem, an extractive workup using the difference in polarity between the two products, by choosing a long-chain fatty acid (vinyl butyrate, vinyl decanoate) as acyl donor, can be used (neutral workup).[3] An alternative strategy involves cyclic anhydrides, such as succinic anhydride (basic workup).[4] The resulting hemisuccinate product can be separated from the unreacted alcohol by an aqueous base workup. A summary of purification techniques used for the separation of enantiomers from a bioresolution is shown in Figure 1.

Scheme 1. **Selective acylation of a secondary alcohol using a lipase enzyme**



To our knowledge there are no reported acid-washable acyl donors used in the separation of enantiomers from a bioresolution in the literature. The present work describes an easy and efficient method for the separation of products from a bioresolution of secondary alcohols using vinyl ester amino acids as acyl donors which lead to esters that are aqueous acid soluble. This methodology can be applied to the industrial preparation of valuable chiral secondary alcohols where the other techniques fail in the separation workup.

It has been reported that proteases catalyse transesterifications of alcohols with some *N*-protected vinyl ester amino acids.[5] We decided to investigate this biotransformation using *N*-Boc-protected vinyl ester amino acids for the bioresolution of secondary alcohols using the amino moiety as an acid-washable tag as shown in Scheme 2.

Results and Discussion

To develop a process involving the bioresolution of secondary alcohols followed by an acid wash separation of products, the hydrolase enzyme *Candida antarctica* lipase B (CAL-B) was chosen since it is well recognized and accepted as a useful catalyst in the chemical industry.[6]

The vinyl ester amino acids were synthesised from the corresponding *N*-Boc-amino acids by treatment with vinyl acetate, according to the method of Lobell and Schneider,[7] in

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(1) (a) Ou, L.; Xu, Y.; Ludwig, D.; Pan, J.; Xu, J. H. *Org. Process. Res. Dev.* **2008**, *12*, 192–195. (b) Park, O.-J.; Lee, S.-H.; Park, T.-Y.; Chung, W.-G.; Lee, S.-W. *Org. Process. Res. Dev.* **2006**, *10*, 588–591. (c) Yazbeck, D.; Derrick, A.; Panesar, M.; Deese, A.; Gujral, A.; Tao, J. *Org. Process. Res. Dev.* **2006**, *10*, 655–660.

(2) (a) Ghanem, A.; Aboul-Enien, H. Y. *Tetrahedron: Asymmetry* **2004**, *15*, 3331–3351. (b) Gotor-Fernández, V.; Brieva, R.; Gotor, V. *J. Mol. Catal. B: Enzym.* **2006**, *40*, 111–120.

(3) Halle, R.; ter; Bernet, Y.; Billard, S.; Bufferne, C.; Carlier, P.; Delatre, C.; Flouzat, C.; Humblot, G.; Laigle, J. C.; Lombard, F.; Wilmouth, S. *Org. Process. Res. Dev* **2004**, *8*, 283–286.

(4) (a) Goswami, A.; Howell, J. M.; Hua, E. Y.; Mirfakhrae, K. D.; Soumeillant, M. C.; Swaminathan, S.; Qian, X.; Quiroz, F. A.; Vu, T. C.; Wang, X.; Zheng, B.; Kronenthal, D. R.; Patel, R. N. *Org. Process. Res. Dev* **2001**, *5*, 415–420. (b) Bouzemi, N.; Debbeche, H.; Aribi-Zouiou, L.; Fiaud, J.-C. *Tetrahedron Lett.* **2004**, *45*, 627–630.

(5) (a) Lloyd, R. C.; Dickman, M.; Jones, J. B. *Tetrahedron: Asymmetry* **1998**, *9*, 551–561. (b) Davis, B. G.; Fairbanks, A. J. *Method for preparing ester linked peptide-carbohydrate conjugates. US* **2004/0208883**, *A1*, 2004.

(6) (a) Vaidyanathan, R.; Hesmondhalgh, L.; Hu, S. *Org. Process. Res. Dev.* **2007**, *11*, 903–906. (b) Gotor, V. *Org. Process. Res. Dev.* **2002**, *6*, 420–426. (c) Reigada, J. B.; Tcacenco, C. M.; Andrade, L. H.; Kato, M. J.; Porto, A. L. M.; Lago, J. H. G. *Tetrahedron: Asymmetry* **2007**, *18*, 1054–1058.

(7) Lobell, M.; Schneider, M. P. *Synthesis* **1994**, 375–377.

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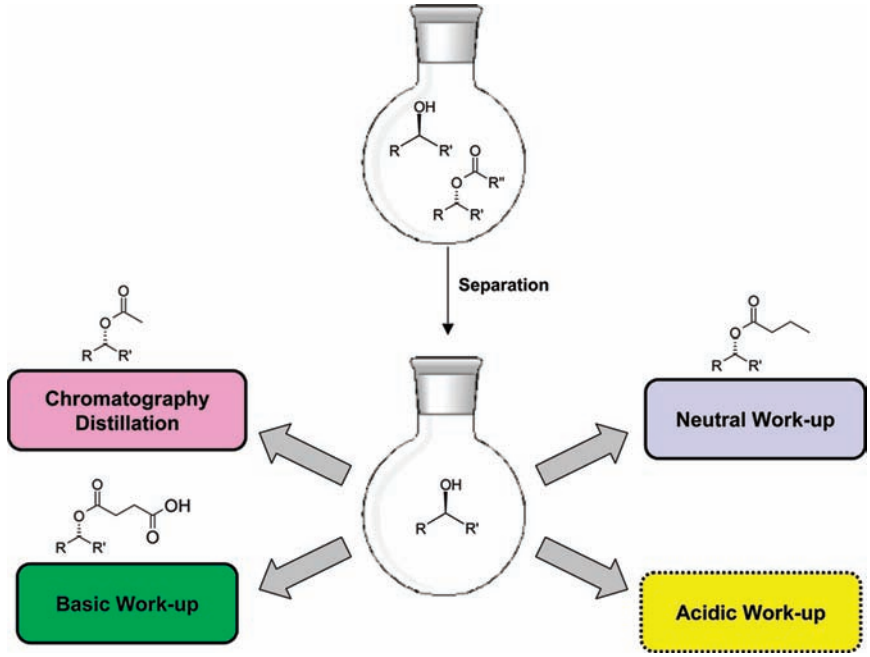
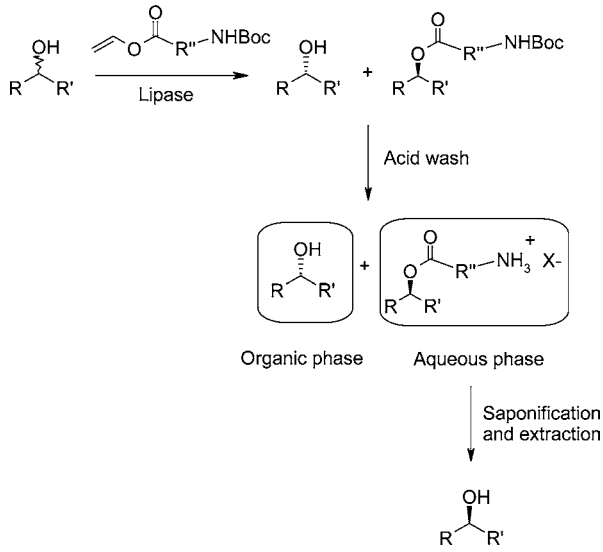
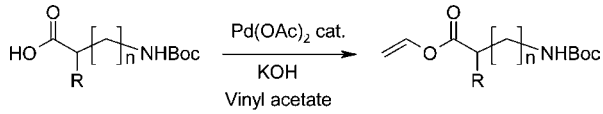


Figure 1. **Purification techniques used for the separation of enantiomers after a bioresolution.**

Scheme 2. **Bioresolution of secondary alcohols using vinyl ester amino acids as acyl donors**



Scheme 3. **Synthesis of the vinyl ester amino acids from *N*-Boc-protected amino acids**



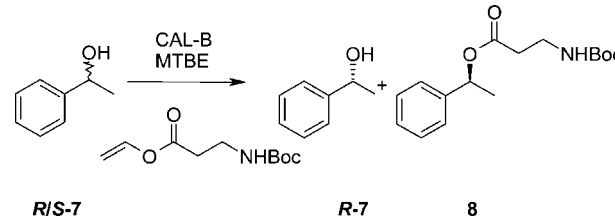
1, R=H, n=0
2, R=H, n=1
3, R=CH(CH$_3$)$_2$, n=0

4, R=H, n=0
5, R=H, n=1
6, R=CH(CH$_3$)$_2$, n=0

the presence of potassium hydroxide and a catalytic amount of palladium acetate as shown in Scheme 3.

The acyl donors selected for investigation were derived from cheap, commercially available amino acids glycine **1**, β-alanine **2** and valine **3**, yielding the corresponding acyl donors **4**, **5**, and **6**. Initially, the CAL-B-catalysed transesterification of racemic 1-phenylethanol (***R*/*S*)-7** in MTBE was investigated to test the methodology and the bioresolution showed high activity and selectivity using *N*-Boc-β-alanine vinyl ester **5** with an *E*-value of >200 as shown in Scheme 4.

Scheme 4. **Bioresolution of 1-phenylethanol (*R*/*S*)-7 using CAL-B and *N*-Boc-β-alanine vinyl ester 5 as acyl donor**



***R*/*S*-7** ***R*-7** **8**

Table 1. **Bioresolution of (*R*/*S*)-7 using acyl donors 4, 5, and 6**

acyl donor	(*S*)-alcohol (% ee)[a]	(*R*)-ester (% ee)[a]	conversion (%)[b]	*E*[b]
5	89	99.3	47	>200
4	48.5	96.8	33	105
6	4.5	100	5	>200

[a] Determination of ee by HPLC using CHIRALCEL OJ-H column. Determination of the ee of the product was achieved by acidic hydrolysis in MeOH using 5 M aqueous HCl and saponification followed by extraction of the reaction mixture. [b] Calculated from $ee_{alcohol}$ and ee_{ester} after 16 h reaction.

The bioresolution of (***R*/*S*)-7** using acyl donors **4**, **5**, and **6** is shown in Table 1. The use of valine **6** as the acyl donor resulted in a sluggish reaction, potentially due to steric hindrance of this acyl donor.

From these results we decided to investigate the solvent tolerance for the acylation using **5** as the acyl donor. The bioresolution of (***R*/*S*)-7** showed high selectivity for all solvents screened with *E*-values >90 as shown in Table 2.

These results show that this bioresolution can be performed in a wide range of organic solvents with good *E*-values. Temperature optimisation studies were carried out from 20 to 70 °C as shown in Figure 2, and as expected, the bioresolution showed a temperature profile with maximum activity being achieved at 50 °C with an *E*-value of >200.

Table 2. **Solvent screen for the bioresolution of (*R/S*)-7 using acyl donor 5**

solvent	(*S*)-alcohol (% ee)[a]	(*R*)-ester (% ee)[a]	conversion (%)[b]	*E*[b]
MTBE	98.6	93.6	51	170
hexane	92.7	96.7	49	>200
heptane	100	94.3	52	>200
THF	58.6	100	37	>200
MeTHF	68.5	96.8	41	134
toluene	99.9	84.4	54	90
acetonitrile	98.8	100	5	>200
acetone	38.9	99	28	>200
cyclohexane	99.6	89.8	53	141

[a] Determination of ee by HPLC using CHIRALCEL OJ-H column. Determination of the ee of the product was achieved by acidic hydrolysis in MeOH using 5 M aqueous HCl, saponification followed by extraction of the reaction mixture. [b] Calculated from $ee_{alcohol}$ and ee_{ester} after 16 h reaction.

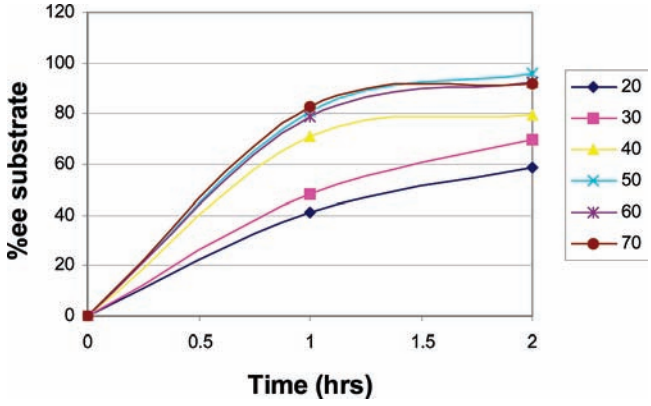


Figure 2. **Temperature profile for the bioresolution of (*R/S*)-7 with acyl donor 5 in MTBE.**

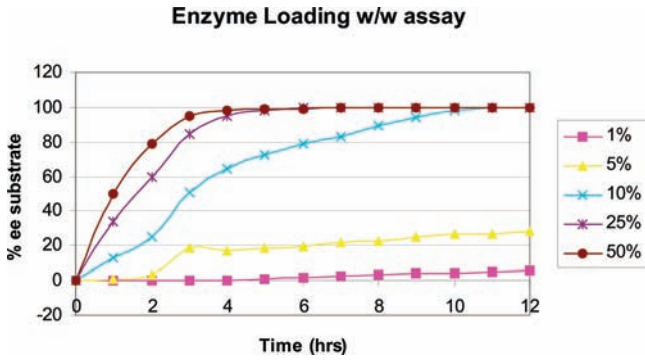


Figure 3. **Bioresolution profile of (*R/S*)-7 at different enzyme loadings with acyl donor 5 in MTBE.**

With increasing pressures to lower costs, the enzyme loading was also investigated as shown in Figure 3. The bioresolution was possible at a 10% wt/wt loading of CAL-B with reaction completion in less than 12 h.

The methodology was expanded to other secondary alcohol substrates **9**−**12** as shown in Figure 4. The bioresolutions were carried out at an enzyme loading of 50% wt/wt for rapid turn around of results with *N*-Boc-*β*-alanine vinyl ester **5** as acyl donor in MTBE.

A summary of the *E*-values are shown in Table 3. To our delight the selectivity of the enzyme was maintained to >125 for the different substrates **7**−**12**.

Scale-Up and Separation. To test the scalability of the biotransformation, the bioresolution of (***R/S*)-7** was scaled to

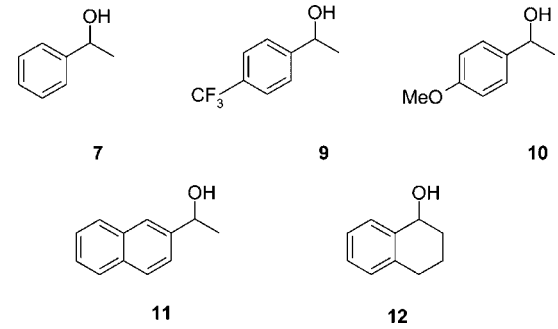


Figure 4. **Secondary alcohol substrates used to demonstrate the bioresolution with acyl donor 5.**

Table 3. **Bioresolution of 7, 9−12 with acyl donor 5**

substrate	(*S*)-alcohol (% ee)[a]	(*R*)-ester (% ee)[a]	conversion (%)[b]	*E*[b]
7	80.0	99.0	45	>200
9	66.2	99.6	40	>200
10	72.4	99.0	42	>200
11	99.0	92.0	52	125
12	54.2	99.0	44	>200

[a] Determination of ee by HPLC using CHIRALCEL OJ-H column. Determination of the ee of the product was achieved by acidic hydrolysis in MeOH using 5 M aqueous HCl and saponification followed by extraction of the reaction mixture. [b] Calculated from $ee_{alcohol}$ and ee_{ester} after 16 h reaction.

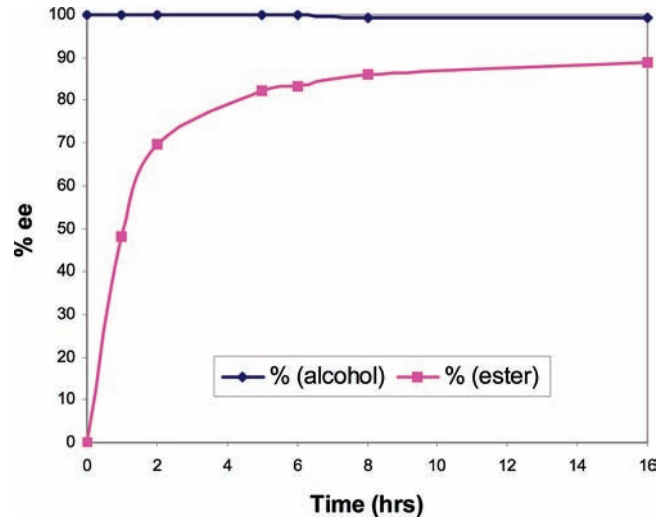


Figure 5. **Reaction profile for the bioresolution of 1-phenylethanol (*R/S*)-7.**

30 g, using the acid-washable acyl donor **4**, which was readily available to us and gave good *E*-value for the bioresolution. The reaction profile is shown in Figure 5. The bioresolution was stopped after 16 h with ee of alcohol and ester being 91.5% and 98.9%, respectively.

The reaction mixture was filtered to remove the enzyme followed by the addition of 10% v/v of methanol and 5 M HCl (5 volumes). The mixture was stirred until complete disappearance of *N*-Boc-protected amino ester due to its hydrolysis to the amino ester. The reaction mixture was then separated and the organic layer yielded (*S*)-1-phenylethanol (14.1 g, 47% yield) with 91.5% ee. The acidic aqueous layer was then adjusted to pH 12 using 5 M NaOH and stirred until complete saponification had occurred. The mixture was then extracted,

and the organic layer yielded (*R*)-1-phenylethanol (12.7 g, 42% yield) with 98.9% ee.

Conclusion

This work has resulted in a new methodology that can be added to the chemists' tool-box for the separation of enantiomers after a bioresolution. The acid-soluble tag fully complements the other workup techniques and can be considered as a potential workup solution where the other techniques fail. We are currently investigating other protecting groups that can be cleaved under a range of conditions so that the acyl donor can be tuned to downstream chemistry to minimise processing time. In addition, the presence of the amino moiety has the added advantage for the potential ee polish through crystallization of a corresponding salt. This work is currently under investigation.

Experimental section

Chemicals and Enzymes. Chemicals were purchased from Alfa Aesar. Hydrolase enzyme CAL-B (Novozym 435) was obtained from Enzagen Ltd.

Analytical Methods. ^{1}H NMR spectra were recorded at 500 MHz on a Bruker AV-500 spectrometer; shifts are relative to internal TMS.

The enantiomeric excesses were measured by chiral stationary phase HPLC on Chiracel OJ-H column (250 mm × 4.6 mm × 10 μm, Daicel Chemical Industries) with UV detection (λ = 210 nm). For substrate **7** 1-phenylethanol, eluent hexane/2-propanol (9:1); flow rate 0.5 mL/min; typical retention times were 17.0 min (*S*)-1-phenylethanol, 19.3 min (*R*)-1-phenylethanol. For substrate **9** 1-(4-trifluoromethylphenyl)ethanol eluent hexane/2-propanol (9:1); flow rate 0.25 mL/min; typical retention times were 20.0 min (*S*), 21.2 min (*R*). For substrate **10** 1-(4-methoxyphenyl)ethanol eluent hexane/2-propanol (9:1); flow rate 0.8 mL/min; typical retention times were 18.1 min (*S*), 19.2 min (*R*). For substrate **11** 1-(naphthalen-2-yl)ethanol eluent hexane/2-propanol (9:1); flow rate 1 mL/min; typical retention times were 16.5 min (*S*), 21.3 min (*R*). For substrate **12** 1-(1,2,3,4-tetrahydronaphthalen-2-yl)ethanol eluent hexane/2-propanol (9:1); flow rate 0.5 mL/min; typical retention times were 14.0 min (*S*), 17.1 min (*R*).

General Method for the Preparation of the *N*-Boc-vinyl Ester Amino Acids. A mixture of *N*-Boc-amino acid (2.65 mmol), $Pd(OAc)_2$ (0.0265 mmol, 0.01 equiv), KOH (0.265 mmol, 0.1 equiv) and 5 mL of vinyl acetate (10 vol) was stirred for 24 h at room temperature under N_2. The reaction mixture was filtered on Celite, and the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography (95:5 EtOAc/hexane) to give the corresponding *N*-Boc-vinyl ester amino acid.

***N*-Boc-glycine vinyl ester (4):** yellow oil. 80% yield. ^{1}H NMR (DMSO, 500 MHz) δ 7.31 (1H, t, J = 5.0, NH), 7.20 (1H, dd, J_1 = 5.0, J_2 = 15.0, CH_2=CH−O), 4.93 (1H, dd, J_1 = 1.4, J_2 = 15.0, CH_2=CH), 4.70 (1H, dd, J_1 = 1.4, J_2 = 5.0, CH_2=CH), 3.80 (2H, d, J = 5.0, CH_2−NH), 1.40 (9H, s, $(CH_3)_3$).

***N*-Boc-*β*-alanine vinyl ester (5):** yellow oil. 85% yield. ^{1}H NMR (DMSO, 500 MHz) δ 7.20 (1H, dd, J_1 = 6.3, J_2 = 14.0, CH_2=CH−O), 6.92 (1H, s, NH), 4.35 (1H, dd, J_1 = 1.5, J_2 = 14, CH_2=CH), 4.66 (1H, dd, J_1 = 1.5, J_2 = 6.3, CH_2=CH), 3.20 (2H, t, J = 6.8, CH_2−CH_2−NH), 2.56 (2H, t, J = 6.8, CH_2−CH_2−NH), 1.38 (9H, s, $(CH_3)_3$).

***N*-Boc-valine vinyl ester (6):** yellow oil. 77% yield. ^{1}H NMR (DMSO, 500 MHz) δ 7.34 (1H, d, J = 5.0, NH), 7.22 (1H, dd, J_1= 5.0, J_2 = 15.0, CH_2=CH−O), 4.93 (1H, dd, J_1 = 1.4, J_2 = 15.0, CH_2=CH), 4.71 (1H, dd, J_1= 1.4, J_2 = 5.0, CH_2=CH), 3.90 (1H, m, CH−NH), 2.05 (1H, m, CH−CH_3), 1.40 (9H, s, $(CH_3)_3$), 0.90 (6H, m, 2 × CH_3).

Large-Scale Bioresolution of 1-Phenylethanol Using *N*-Boc-glycine Vinyl Ester. To a stirred (±400 rpm) solution of 30 g (0.24 mol) of 1-phenylethanol and 36 g (0.18 mol) of *N*-Boc-glycine vinyl ester in 600 mL of MTBE (20 vol) was added 7.5 g of CAL-B (25 wt %/wt). After stirring for 16 h at 30 °C, a sample was taken and HPLC analysis indicated 91.5% ee for the alcohol. After filtering off the enzyme, 60 mL (10% v/v) of methanol and 150 mL of 5 M HCl (5 vol) were added to the solution. The solution was stirred at 30 °C for 1 h. The disappearance of the Boc-amino ester was checked by TLC (8:2 hexane/EtOAc). At the end of the Boc-deprotection, the remaining 1-phenylethanol was extracted three times with 150 mL (5 vol) of MTBE. The solvent was removed to yield (*S*)-1-phenylethanol (14.1 g, 47% yield) with 91.5% ee (97% purity by NMR). The pH of the aqueous phase was then adjusted to pH 12 using 5 M NaOH. The solution was stirred at 30 °C for 1 h, resulting in complete saponification. The mixture was then extracted two times with 150 mL (5 vol) of MTBE. The organic phase was dried over Na_2SO_4, filtered, and solvent evaporated to afford 12.7 g (42% yield) of (*R*)-1-phenylethanol with 98.9% ee (98% purity by NMR).[8]

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(8) No trace of diketopiperazine was observed.