Pergamon

Tetrahedron: *Asymmetry* 9 (1998) 429–435

TETRAHEDRON: *ASYMMETRY*

Enzymatic resolution of methyl *N*-alkyl-azetidine-2-carboxylates by *Candida antarctica* lipase-mediated ammoniolysis

Wim A. J. Starmans, Roy G. Doppen, Lambertus Thijs and Binne Zwanenburg *

Department of Organic Chemistry, NSR-Center for Molecular Structure, Design and Synthesis, University of Nijmegen, Toernooiveld 1, 6525 ED Nijmegen, The Netherlands

Received 10 November 1997; accepted 15 December 1997

Abstract

A facile method for the synthesis of optically active azetidine-2-carboxylic acid derivatives is presented. Racemic *N*-alkylated azetidine esters are resolved by lipase from *Candida antarctica* in an ammoniolysis reaction, and both the *S*-amide and the *R*-ester are obtained with excellent stereoselectivity. © 1998 Elsevier Science Ltd. All rights reserved.

1. Introduction

Optically active azetidines substituted with one or two carboxyl groups are interesting nonproteinogenic amino acids. They were applied in the synthesis of peptides[1] and natural products.[1,2] Moreover, optically active 2- and 2,4-substituted azetidines have been employed as chiral auxiliaries in asymmetric synthesis.[3–5] Because of our interest in *N*-alkylated azetidines in chirality transfer reactions, we sought a method for obtaining both antipodes in enantiopure form. Only azetidine-(2*S*)-carboxylic acid is commercially available and a satisfactory route to the enantiomer is not yet available. An enzymatic resolution of racemic azetidine-2-carboxylic acid derivatives would in principle be a suitable method to obtain both antipodes. For this purpose several enzymes were investigated for the enantioselective hydrolysis of racemic methyl-*N*-benzyl-azetidine-2-carboxylate and *N*-benzyl-azetidine-2-carboxamide. However, these resolutions were unsuccessful. We therefore turned our attention to biocatalytic transacylation reactions.

Lipases usually are effective in the transacylation of a wide variety of nucleophiles such as hydrogen peroxide, amines, hydrazines and oximes.[6–10] The enzymatic formation of carboxamides from carboxylic esters by *Candida antarctica*, whereby ammonia serves as the nucleophile, was successfully accomplished with esters, such as β-ketoesters,[11,12] α,β-unsaturated esters,[13] dimethyl succinate[14] and fatty

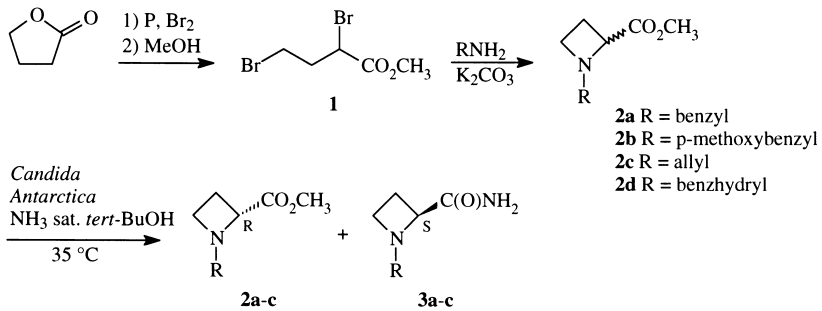
* Corresponding author. E-mail: zwanenb@sci.kun.nl

PII: S0957-4166(97)00632-0

esters.[15,16] Amino acid esters have only been reported once as substrates in this reaction.[17] However, the results reported were promising and this prompted us to investigate the lipase catalyzed ammoniolysis of azetidine-2-carboxylic esters for the purposes indicated above.

2. Results

Starting from γ-butyrolactone, methyl 2,4-dibromobutyrate **1** was obtained by a Hell–Volhard–Zelinski bromination. Several primary amines were then used to synthesize racemic azetidine-2-carboxylic esters **2** following modified literature procedures,[18,19] *i.e.* one equivalent amine and potassium carbonate as a co-base were used in refluxing acetonitrile/water instead of three equivalents of amine in refluxing acetonitrile.[18] The esters **2** were then subjected to ammoniolysis by *Candida antarctica* lipase in *tert*-butyl alcohol saturated with ammonia (Scheme 1).



Scheme 1. Synthetic route to optically active azetidines by enzymatic ammoniolysis

The enzymatic reactions were monitored in time and the selectivity was determined as a function of conversion (Fig. 1). The very high substrate specificity of *Candida antarctica* for **2c** is demonstrated by the almost straight line for the conversion versus the e.e., with only minimal levelling off.

The results of the enzymatic resolution are collected in Table 1. Blank experiments showed that no appreciable ammoniolysis takes place in the absence of the enzyme. The enantiomeric excess of the esters and amides were determined by chiral capillary GC and capillary electrophoresis. Furthermore, the specific rotation of enantiopure (2*R*)-**2a** was compared with that reported by Seebach and coworkers[1] for the antipode: the enantiopure ester obtained after enzymatic resolution showed $[\alpha]_D^{20}$: +125.2 (c=1, CH_2Cl_2), whereas the literature reports $[\alpha]_D^{20}$: −115 (c=1.3, CH_2Cl_2). This suggests that Seebach's route

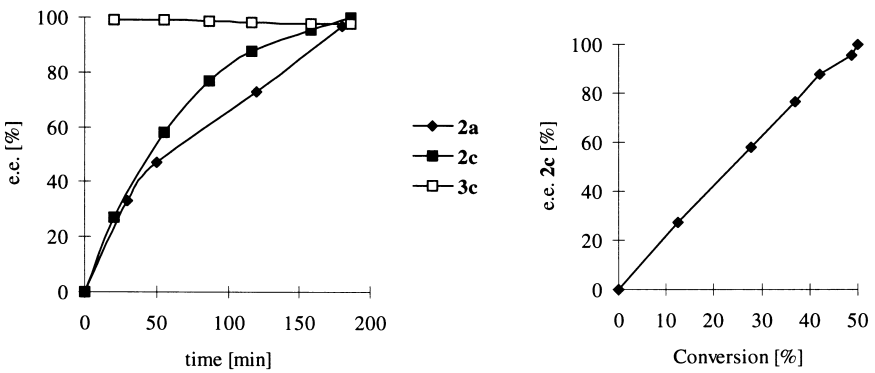


Fig. 1. Selectivities of *Candida antarctica* mediated ammoniolysis versus time and conversion

Table 1
Selectivities of *C. antarctica* mediated ammoniolysis of *N*-alkylated azetidine esters

R	Conversion	ee ester [%]	$[\alpha]_D^{20}$ ester[a]	ee amide [%]
Benzyl	56 %	> 99 (R)[b]	+125.2	80 (S)[c] (> 99[d])
p-Methoxybenzyl	54 %	> 99[b]	+70.9	84[c]
Allyl	50 %	> 99[b]	+158.4	97[b]
Benzhydryl	0 %[e]	-	-	-

a optical rotation: (c=1, CH_2Cl_2)

b determined by chiral capillary GC using a Beta-DEX™ 120 column by Supelco

c determined by chiral capillary electrophoresis

d value between parentheses after one crystallization

e not accepted by *Candida Antarctica* lipase

toward optically active (2*S*)-**2a**, using base induced alkylation of the nitrogen atom, is accompanied by partial racemization.

3. Conclusions

The azetidine ester **2d**, with the bulky benzhydryl group, was not accepted by *Candida antarctica*, probably due to steric factors. The other three substrates were converted with a remarkably high selectivity to the primary amides. This enzymatic resolution leads to the formation of *N*-alkylated azetidine-(2*S*) and (2*R*)-carboxylic acid derivatives of high enantiopurity.

4. Experimental

4.1. General remarks

Melting points were determined using a Reichert thermopan microscope and are uncorrected. Optical rotations were measured with a Perkin–Elmer automatic polarimeter, model 241 MC, using concentrations c in g/100 ml at 20°C in the solvents indicated. ^{1}H and ^{13}C NMR spectra were recorded with a Bruker AC 100 (100 MHz, FT) or a Bruker AM-400 (400 MHz, FT) spectrometer. IR spectra were recorded on a Perkin–Elmer 298 spectrophotometer. For (high resolution) mass spectra a double focussing VG7070E mass spectrometer was used. GC–MS were measured using a Varian Saturn II GC–MS by on-column injection (DB-1 column, length 30 m, internal diameter 0.25 mm, film thickness 0.25 μm). Elemental analyses were performed using a Carlo Erba Instruments CHNS-O EA 1108 element analyzer. Capillary electrophoresis was performed on a Hewlett–Packard CE apparatus, using β-cyclodextrine saturated Tris.HCl/phosphate buffer (pH 2.5) at 30 kV and 10°C, while detection was at 254 nm. Chiral capillary GC was performed using a Beta-DEX™ 120 fused silica column (length 50 m, internal diameter 0.25 mm, film thickness 0.25 μm) by Supelco.

4.2. Methyl 2,4-dibromobutyrate **1**

To a cooled suspension of γ-butyrolactone (40.00 g, 0.47 mol) and red phosphorus (4.00 g, 0.13 mol) approximately 10 ml of bromine was carefully added dropwise. The resulting mixture was then heated to about 100°C and the remaining part of the bromine (total amount 52.50 ml, 1.03 mol) was gradually added. After an additional 3 h of heating, the reaction mixture was cooled to room temperature and flushed with a nitrogen flow for 30 min. The suspension was then cooled to 0°C and an excess of methanol (50 ml) was carefully added. The resulting solution was set aside for 19 h, after which it was concentrated *in vacuo*. The residue was taken up in diethyl ether and $Na_2S_2O_3$ solution was added, the layers separated and the aqueous solution extracted once with ether. The combined organic layers were successively washed with $Na_2S_2O_3$ solution and saturated NaCl solution, dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting yellow oil was purified by vacuum distillation.

Yield: 102.70 g, 85%. Bp.: 53°C (0.03 mmHg). ^{1}H NMR ($CDCl_3$): δ 4.54 (t, J=7.1 Hz, 1H, CHBr), 3.81 (s, 3H, $COOCH_3$), 3.55 (t, J=6.2 Hz, 2H, CH_2Br), 2.53 (dt, J=6.3 Hz and J=9.4 Hz, 2H, CH_2). IR (CCl_4): σ 1742 (s, $COOCH_3$). GC–MS: *m/z* 227 ($M^+ - OCH_3$, isotope pattern of 2 bromines), 199 ($M^+ - COOCH_3$, isotope pattern of 2 bromines), 179 ($M^+ - {}^{79}Br$, isotope pattern of 1 bromine).

4.3. General procedure for the synthesis of racemic azetidine esters

To a refluxing solution of **1** in acetonitrile:distilled water (25:1, *v/v*) containing one equivalent of potassium carbonate, a solution of 1.1 equivalent primary amine in acetonitrile was added dropwise. When no more **1** was present, as was determined by GC, the reaction was stopped by pouring the mixture into chloroform/water. The layers were separated and the organic layer acidified with 6 N HCl solution until pH>4. The layers were separated, the aqueous layer neutralized with 6 N NaOH solution until pH<8 and extracted three times with chloroform. The latter organic extracts were then dried over $MgSO_4$, filtered and concentrated *in vacuo*. The resulting yellow oil was purified by distillation *in vacuo* or flash chromatography.

4.4. (±)-Methyl 1-benzylazetidine-2-carboxylate **2a**

Yield: 45%. Bp.: 107–109°C (1.0 mmHg). ^{1}H NMR ($CDCl_3$): δ 7.27 (m, 5H, Ph), 3.88–3.51 (m, 3H, NCH and CH_2Ph), 3.63 (s, 3H, $COOCH_3$), 3.32 and 2.95 (dt, J=2.9 Hz and J=7.2 Hz, and dt, J=8.4 Hz and J=6.7 Hz, 2H, NCH_2), 2.46–2.15 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$): δ 173.0 (s, C(O)O), 139.0 (s, aromatic C), 129.1, 128.3, 127.2 (d, aromatic C), 64.3 (d, NCH), 62.4 (t, CH_2Ph), 51.7 (q, OCH_3), 50.8 (t, NCH_2), 21.6 (t, CH_2). IR (CCl_4): σ 1740 (s, $COOCH_3$), 700 (s, monosubstituted phenyl). Mass (EI): *m/z* 205 (M^+), 91 (tropilium). HR-MS: calculated for $C_{12}H_{15}NO_2$ 205.11028, found 205.11025.

4.5. (±)-Methyl 1-(4-methoxybenzyl)azetidine-2-carboxylate **2b**

Yield: 39%. Bp.: 85°C (0.07 bar). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.20 (d, J_{ortho}=9.9 Hz, 2H, aromatic), 6.84 (d, J_{ortho}=9.6 Hz, 2H, aromatic), 3.79 (s, 3H, OCH_3), 3.73 and 3.70 (AB quartet, J=8.7 Hz and J=12.3 Hz, 2H, benzylic CH_2), 3.64 (s, 3H, $COOCH_3$), 3.54 (d, J=12.4 Hz, 1H, NCH), 3.29 (dt, J=2.3 Hz and J=7.4 Hz, 1H, NCH_2), 2.93 (q, J=8.0 Hz, 1H, NCH_2), 2.36 (m, 1H, CH_2), 2.21 (m, 1H, CH_2). ^{13}C NMR ($CDCl_3$): δ 173.0 (s, C(O)O), 158.9 (s, aromatic C), 130.3 (d, aromatic C), 129.1 (s, aromatic C), 113.7 (d, aromatic C), 64.2 (d, NCH), 61.7 (t, CH_2Ph), 55.2 (q, OCH_3), 51.7 (q, $COOCH_3$), 50.5 (t, NCH_2),

21.6 (t, CH_2). IR ($CHCl_3$): σ 1720 (s, $COOCH_3$). GC–MS: *m/z* 236 (M^++1), 176 ($M^+ - COOCH_3$), 121 (methoxytropilium). HR–MS: calculated for $C_{13}H_{17}NO_3$ 235.12084, found 235.12027.

4.6. (±)-Methyl 1-allylazetidine-2-carboxylate **2c**

Yield: 64%. Bp.: 47–48°C (1.0 mmHg). ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.86–5.75 (m, 1H, $CH_2CH{=}CH_2$), 5.20 and 5.11 (d, 17.1 Hz, and d, J=10.1 Hz, 2H, $CH_2CH{=}CH_2$), 3.73 (s, 3H, $COOCH_3$), 3.66 (t, J=8.5 Hz, 1H, NCH), 3.38 and 2.90 (dt, J=2.5 Hz and J=7.5 Hz, and q, J=8.0 Hz, 2H, NCH_2), 3.26 and 3.07 (dd, J=6.4 Hz and 12.9 Hz, and dd, J=6.7 Hz and J=12.9 Hz, 2H, $CH_2CH{=}CH_2$), 2.40–2.31 and 2.27–2.20 (m and m, 2H, CH_2). ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.8 (s, C(O)O), 133.4 (d, $CH_2CH{=}CH_2$), 117.5 (t, $CH_2CH{=}CH_2$), 64.0 (d, NCH), 61.0 (t, $CH_2CH{=}CH_2$), 51.4 (q, $COOCH_3$), 50.2 (t, NCH_2), 21.3 (t, CH_2). IR ($CHCl_3$): σ 1718 (s, $COOCH_3$). GC–MS: *m/z* 156 (M^++1), 96 ($M^+ - COOCH_3$). HR–MS: calculated for $C_8H_{13}NO_2$ 155.09463, found 155.09416.

4.7. (±)-Methyl 1-benzhydrylazetidine-2-carboxylate **2d**

Yield: 50%. Bp.: 130°C (0.1 mmHg). ^{1}H NMR ($CDCl_3$): δ 7.52–7.09 (m, 10H, aromatic benzhydryl), 4.46 (s, 1H, benzylic CH), 3.72 (t, J=8.1 Hz, 1H, NCH), 3.50–3.34 (m, 1H, NCH_2), 3.34 (s, 3H, $COOCH_3$), 2.90 (dt, J=7.7 Hz and J=8.4 Hz, 1H, NCH_2), 2.49–2.0 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$): δ 172.9 (s, C(O)O), 141.4, 141.2 (s, aromatic C), 128.5, 128.3, 128.2, 127.5, 127.2 (d, aromatic C), 77.5 and 64.6 (d, $CHPh_2$ and NCH), 51.4 (q, $COOCH_3$), 51.4 (t, NCH_2), 20.9 (t, CH_2). IR ($CHCl_3$): σ 1715 (s, $COOCH_3$). GC–MS: *m/z* 282 (M^++1), 222 ($M^+ - COOCH_3$), 167 (Ph–CH^+–Ph). HR–MS: calculated for $C_{18}H_{19}NO_2$ 281.14158, found 281.14091.

4.8. General procedure for the synthesis of racemic azetidine carboxamides

A solution of azetidine ester **2** (4.87 mmol) in concentrated aqueous ammonia (20 ml) was stirred for 3 h and then extracted three times with chloroform to provide the corresponding carboxamide as a white solid.

4.9. (±)-1-Benzylazetidine-2-carboxamide **3a**

Yield: 81%. Mp.: 104–105°C (ethanol (96%)). ^{1}H NMR (CD_3OD): δ 7.24 (s, 5H, Ph), 4.92 (t, J=9.2 Hz, 1H, NCH), 4.20 (s, 2H, benzylic CH_2), 3.97 and 3.73 (t, J=9.4 Hz, and dt, J=4.3 Hz and J=9.5 Hz, 2H, NCH_2), 2.8–2.15 (m, 2H, CH_2). IR (CCl_4): σ 3500 and 3430 (s, NH_2 amide), 1685 (s, CO amide), 700 (s, monosubstituted phenyl). GC–MS: *m/z* 191 (M^++1), 146 ($M^+ - CO(NH_2)$), 91 (tropilium). Elemental analysis: calculated for $C_{11}H_{14}N_2O$ % C 69.45, % H 7.42, % N 14.72. Found % C 69.43, % H 7.10, % N 14.45.

4.10. (±)-1-(4-Methoxybenzyl)azetidine-2-carboxamide **3b**

Mp.: 109.5–111°C. ^{1}H NMR ($CDCl_3$): δ 7.18 (d, J_{ortho}=8.6 Hz, 2H, aromatic), 6.84 (d, J_{ortho}=8.6 Hz, 2H, aromatic), 5.71 (broad s, $CONH_2$), 3.79 (s, 3H, OCH_3), 3.78 (t, J=9 Hz, 1H, NCH), 3.57 (d, J=4.5 Hz, 2H, benzylic CH_2), 3.33 and 3.49 (dt, J=2.5 Hz and J=7.8 Hz, and dt, J=7.6 Hz and J=7.9 Hz, 2H, NCH_2), 2.55–2.04 (m, 2H, CH_2). ^{13}C NMR ($CDCl_3$): δ 176.5 (s, $CONH_2$), 129.6 (d, aromatic C), 129.4 (s, aromatic C), 114.0 (d, aromatic C), 66.0 (d, NCH), 61.7 (t, benzylic CH_2), 55.3 (q, OCH_3),

50.6 (t, NCH_2), 22.9 (t, CH_2). IR (KBr): σ 3370 (s, NH), 1620 (s, $CONH_2$). GC–MS: *m/z* 221 (M^++1), 176 ($M^+ - CO(NH_2)$), 121 (methoxytropilium). HR–MS: calculated for $C_{12}H_{16}N_2O_2$ 220.12118, found 220.12129.

*4.11. (±)-1-Allylazetidine-2-carboxamide **3c***

Mp.: 49–52°C. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.17 and 5.9 (broad s, 2H, $CONH_2$), 5.81–5.67 (m, 1H, $CH_2CH{=}CH_2$), 5.19 and 5.11 (d, J=17.1 Hz and d, J=10.2 Hz, 2H, $CH_2CH{=}CH_2$), 3.54 (t, J=8.6 Hz, 1H, NCH), 3.39 and 2.94 (dt, J=2.7 Hz and J=7.7 Hz, and dt, J=7.9 Hz and J=8.5 Hz, 2H, NCH_2), 3.16 and 3.06 (dd, J=6.1 Hz and J=13.3 Hz, and dd, J=6.4 Hz and J=13.2 Hz, 2H, $CH_2CH{=}CH_2$), 2.46–2.34 and 2.22–2.10 (m and m, 2H, CH_2). ^{13}C NMR ($CDCl_3$): δ 176.8 (s, $CONH_2$), 133.7 (d, $CH_2CH{=}CH_2$), 117.9 (t, $CH_2CH{=}CH_2$), 65.8 (d, NCH), 60.9 (t, $CH_2CH{=}CH_2$), 50.6 (t, NCH_2), 22.7 (t, CH_2). IR ($CHCl_3$): σ 1670 (s, $CONH_2$). GC–MS: *m/z* 141 (M^++1), 96 ($M^+ - CO(NH_2)$). HR–MS: calculated for $C_7H_{12}N_2O$ 140.09496, found 140.09492.

4.12. General procedure for the ammoniolysis of racemic azetidine-2-carboxylic esters

A stock solution of NH_3 saturated *tert*-butyl alcohol was prepared by bubbling NH_3 gas through 20 ml of *tert*-butyl alcohol for approximately 60 min. while stirring. A reference line was made by GC-analysis of solutions of the racemic azetidine ester and 2-bromonaphthalene (internal standard) with different weight ratios.

A vial was charged with azetidine ester (approximately 1.0 mmol), 2-bromonaphthalene (100 mg, 0.48 mmol), immobilized lipase from *Candida antarctica* (50 mg, Novozym 435) and NH_3 saturated *tert*-butyl alcohol (5.0 ml, 12 mmol NH_3), capped with a septum and shaken in a thermostatized box at 35°C. At regular time intervals 0.1 ml samples were taken with a syringe, diluted with dichloromethane (0.4 ml) after which the conversion was measured by GC-analysis (apolar column, 130°C isothermic). At approximately 50% conversion the reaction was stopped by filtration. The immobilized enzyme was washed with methanol and the combined organic filtrates were concentrated *in vacuo*. The residue was dissolved in water/hexane and the layers were separated. The aqueous layer was extracted twice with hexane, the combined organic layers dried with $MgSO_4$, filtered and concentrated *in vacuo*. The optical purity of the remaining azetidine ester was determined by chiral capillary GC, or when purified by bulb-to-bulb distillation, by optical rotation. The aqueous layer containing the corresponding amide was concentrated *in vacuo* after which the enantiomeric excess of the amide was determined by chiral capillary GC or chiral capillary electrophoresis.

Acknowledgements

We wish to thank Novo Nordisk A/S for the generous gift of immobilized *Candida antarctica* (Novozym 435).

References

1. D. Seebach, T. Vettiger, H.-M. Müller, D. A. Plattner and W. Petter, *Liebigs Ann. Chem.*, **1990**, 687.
2. A. Duréault, M. Portal, F. Carreaux and J. C. Depezay, *Tetrahedron*, **1993**, *49*, 4201.
3. W. Behnen, C. Dauelsberg, S. Wallbaum and J. Martens, *Synth. Commun.*, **1992**, *22*, 2143.

4. W. Behnen, T. Mehler and J. Martens, Tetrahedron: *Asymmetry*, **1993**, *4*, 1413.
5. J. Hoshino, J. Hiraoka, Y. Hata, S. Sawada and Y. Yamamoto, *J. Chem. Soc., Perkin Trans. 1*, **1995**, 693.
6. M. C. de Zoete, F. van Rantwijk and R. A. Sheldon, *Catalysis Today*, **1994**, *22*, 563.
7. I. Alfonso, C. Astorga, F. Rebolledo and V. Gotor, *Chem. Commun*., **1996**, 2471.
8. D. T. Chapman, D. H. G. Crout, M. Mahmoudian, D. I. C. Scopes and P. W. Smith, *Chem. Commun*., **1996**, 2415.
9. S. Puertas, F. Rebolledo and V. Gotor, *J. Org. Chem*., **1996**, *61*, 6024.
10. V. M. Sánchez, F. Rebolledo and V. Gotor, *Tetrahedron: Asymmetry*, **1997**, *8*, 37.
11. M. J. García, F. Rebolledo and V. Gotor, *Tetrahedron Lett*., **1993**, *34*, 6141.
12. M. J. García, F. Rebolledo and V. Gotor, *Tetrahedron*, **1994**, *50*, 6935.
13. V. Sánchez, F. Rebolledo and V. Gotor, *Synlett*, **1994**, 529.
14. S. Puertas, F. Rebolledo and V. Gotor, *Tetrahedron*, **1995**, *51*, 1495.
15. M. C. de Zoete, A. C. Kock-van Dalen, F. van Rantwijk and R. A. Sheldon, *J. Chem. Soc., Chem. Commun*., **1993**, 1831.
16. M. C. de Zoete, A. C. Kock-van Dalen, F. van Rantwijk and R. A. Sheldon, *Biocatalysis*, **1994**, *10*, 307.
17. M. C. de Zoete, A. A. Ouwehand, F. van Rantwijk and R. A. Sheldon, *Recl. Trav. Chim. Pays-Bas*, **1995**, *114*, 171.
18. R. M. Rodebaugh and N. H. Cromwell, *J. Heterocycl. Chem*., **1968**, *5*, 309.
19. R. de Gelder, J. M. M. Smits, W. A. J. Starmans, L. Thijs and B. Zwanenburg, *J. Chem. Crystall*., **1996**, *26*, 639.