Available online at www.sciencedirect.com
SCIENCE DIRECT®
Tetrahedron: Asymmetry 15 (2004) 3407–3412
Tetrahedron: Asymmetry

Enzymatic resolution of cyclic *N*-Boc protected β-aminoacids

Cyrille Pousset,[a,b,*] Roland Callens,[b] Mansour Haddad[a] and Marc Larchevêque[a,*]

[a]*Laboratoire de Synthèse Organique associé au CNRS, ENSCP, 11 rue Pierre et Marie Curie, 75231 Paris Cedex 05, France*
[b]*Peptisyntha et Cie S.N.C., 310 rue de Ransbeek, Bruxelles, Belgium*

Received 16 July 2004; accepted 14 September 2004

Abstract—Methyl and ethyl esters of *N*-Boc homoproline, homopipecolic acid and 3-carboxymethyl-morpholine were kinetically resolved by hydrolysis catalysed by *Burkholderia cepacia* lipase to give the corresponding acids and residual esters in enantiomeric excesses better than 99% (E >100).

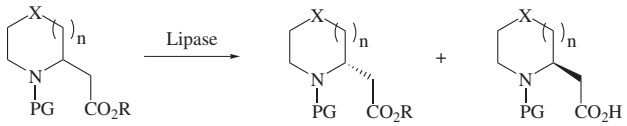
1. Introduction

The synthesis of conformationally constrained amino-acid derivatives has recently received much attention due to their ability to act as conformational probes when incorporated into peptides and peptidomimetics.[1] Among them, optically active β-aminoacids, although of less importance than the parent α-aminoacids, are crucial structural features of numerous biologically active and natural products,[2] with various methods available for the synthesis of linear compounds by asymmetric synthesis or enzymatic resolutions.[3]

In contrast, there are few methods available for the asymmetric synthesis of cyclic β^3-aminoacids. Arndt–Eistert homologation of (*S*)-proline has been used to access to homoproline[4] and homopipecolic acid.[5] More recently, two general approaches to five- and six-membered ring aminoacids have been disclosed using diastereocontrolled Michael additions of chiral amides to α,β-unsaturated esters followed by intramolecular ring closure[6] and diastereoselective reduction of the resulting chiral β-enaminoesters.[7]

Concerning the use of enzyme-catalysed kinetic resolutions, interesting results were obtained for the resolution of linear β-aminoacids by the hydrolysis of *N*-phenylacetamido derivatives using penicillin acylase,[8] by acylation of β-aminoesters catalysed by *Candida antarctica* lipase,[9] and more recently by acylation of *N*-hydroxymethylated β-lactams.[10] However although some results were reported for the resolution of cyclic $\beta^{2,3}$ aminoacids by acylation of *N*-hydroxymethylated β-lactams[11,3b] or by acylation of cyclic 1-2 aminoesters,[12] to the best of our knowledge, the resolution of 2-substituted tetrahydroquinolines was the only example reported for the obtention of optically active heterocyclic β^3-aminoacids (β-homoaminoacids).[13]

Herein we report a novel and highly efficient kinetic resolution of *N*-Boc homoproline, homopipecolic acid and 3-carboxymethylmorpholine by hydrolysis of the corresponding esters catalysed by *Burkholderia cepacia* lipase (Scheme 1).[14]



n = 0, 1; X = CH_2, O

Scheme 1.

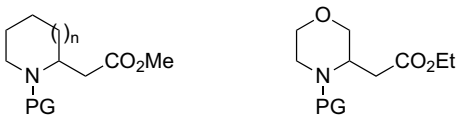
2. Results and discussion

Among all the types of enzyme-catalysed reactions, hydrolytic transformations involving the cleavage of amide or ester bonds with proteases, esterases or lipases are the easiest to perform. In the case of aminoacids, the presence of two different functions offers the possibility of using different strategies such as the hydrolysis of esters, acylation of amines or transesterification. Taking

* Corresponding authors. Fax: +33 22 64 22 25; e-mail: larchm@ext.jussieu.fr

0957-4166/$ - see front matter © 2004 Published by Elsevier Ltd.
doi:10.1016/j.tetasy.2004.09.022

advantage of the recent results concerning the resolution of pipecolic acid by *N*-acylation of its methyl ester catalysed by *C. antarctica* lipase A,[15] a first experiment was attempted with the free homoproline **1** (Fig. 1). This latter was prepared by reaction of monoethyl malonate with 1-pyroline trimer,[16] and the acylation achieved in ethyl acetate as the acyl donor in the presence of *C. antarctica* lipase B (CAL-B, Chirazyme L2). The rate of the reaction was very low and a 40% conversion reached only after 6 days. A better conversion rate was observed with 2,2,2-trifluoroethylacetate (30% after 24 h); however, in these two experiments, both the acylated aminoester and the recovered aminoester were racemic.



1: PG = H; n = 0
2: PG = CO-Bn; n = 0
3: PG = CO_2Me; n = 0
4: PG = Boc; n = 0
5: PG = Boc; n = 1

6: PG = H
7: PG = Boc

Figure 1.

In another attempt, we tried to hydrolyse ethyl ester **6**. In the presence of Novozym 435®, an immobilised form of CAL-B, the hydrolysis was total in 6 h at pH 7 but again the reaction was unselective and the resulting aminoacid was obtained as a racemate.

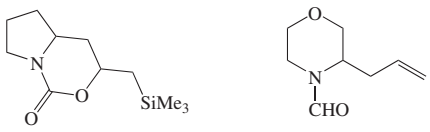
We then turned our attention towards the hydrolysis of *N*-protected aminoesters. First, the enzymatic hydrolysis of the *N*-benzylacetyl homoproline methyl ester **2** was studied in 10^{-2} M phosphate buffer at pH 7 with a penicilline acylase. Indeed, these enzymes are known to cleave the amide bonds of *N*-benzylacetyl amides selectively.[8,17] However, in contrast with linear β-aminoacids, the supported enzyme ChiroCLEC-EC® was unable to catalyse the cleavage of the amide bond of the cyclic tertiary amide **2**, which was recovered unchanged after 3 days. We then turned our attention towards the use of lipases and proteases, a great number of which are commercially available. Among the various enzymes screened, the lipase of *Candida cylindracea* was totally inefficient. In contrast, α-chymotrypsin and *C. antarctica* lipase (CAL B) showed moderate enantioselectivity (Table 1). We then decided to modify the nature of the protecting group and introduce a larger group onto the nitrogen. Following the results recently reported by Schäfer et al. for the enzymatic kinetic resolution of a *N*-Boc unsaturated proline by hydrolysis of the corresponding methyl ester catalysed by Novozyme 435®,[18] we decided to synthesise and test the *N*-Boc protected esters.

The required racemic esters **4** and **5** were easily synthesised by oxidative cleavage of 2-allyl pyrrolidine, piperidine or morpholine. These compounds were prepared by allylation of the *N*-acylated aminals **8** obtained by partial reduction of *N*-protected lactams followed by acid transacetalisation in methanol[19] or better by electrochemical anodic oxidation.[20] The reaction of these *N*-acylaminals with allyltrimethylsilane at a low temperature in the presence of titanium tetrachloride generated acyliminium cations, which were trapped by the allylsilane to give the corresponding *N*-protected 2-allylated heterocycles **10** or **11** in high yields (Scheme 2).[21] However the choice of the protecting group was crucial. Indeed, when the Boc protection was used, the oxidation yields were poor (40% for the *N*-Boc 2-carboxymethylpyrrolidine from the *N*-Boc 2-allylpyrrolidine). Moreover, during the allylation, we observed the formation of bicyclic compound **14** (Fig. 2) resulting from a participation of the *tert*-butoxycarbamate. We then choose the carbomethoxy protection for further work; the required α-methoxy carbamates prepared according to the Shono method were allylated in better than 90% yield and oxidised either by ruthenium catalysed oxidation[22] or by ozonolysis followed by an H_2O_2 oxidative treatment. After the removal of the protecting group with hydrobromic acid in acetic acid and purification on a sulfo-

Table 1. Enzyme-catalysed hydrolysis of cyclic β-aminoesters[a]

Entry	Compound	Enzyme	Time (h)	Residual ester: ee%	Acid ee%	E[b]
1	**2**	*C. cylindracea*	72	—	—	—
2	**2**	α-Chimotrypsin	72	40	47	4
3	**2**	CAL-B[c]	52	17	35	2.5
4	**3**	*B. cepacia*	120	8	34	2
5	**4**	*B. cepacia*	30	99	>99	>100
6	**4**[d]	*B. cepacia*	48	99	94	>100
7	**5**	*B. cepacia*	96	98	>99	>100
8	**5**	*B. cepacia*[e]	66	98.5	>99	>100
9	**7**	α-Chimotrypsin	60	—	—	—
10	**7**	CAL-B	25	50	70	9
11	**7**	*B. cepacia*[f]	10	>99	>99	>100

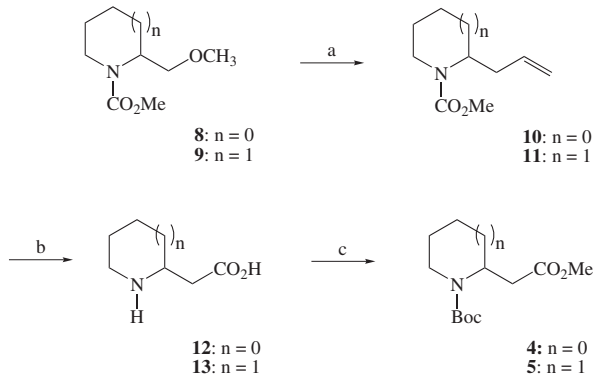
[a] All reactions were carried out by stirring a mixture of substrate (1 mmol) and catalyst (200 mg) in a mixture H_2O/THF (5/1) at 25 °C and at pH 7 (controlled with a pH Stat and addition of 0.1 M aqueous NaOH).
[b] $E = \ln[ee_p(1 - ee_s)]/(ee_p + ee_s)/\ln[ee_p(1 + ee_s)]/(ee_p + ee_s)$.[23]
[c] *Candida antarctica* lipase (MeOH as cosolvent).
[d] The ethyl ester was used.
[e] Reaction achieved at 35 °C.
[f] 140 mg of lipase was used.



14 15

Figure 2.

nated resin, the free aminoacids were esterified in acidic methanol and *N*-Boc protected with di-*tert*-butoxypyrocarbonate.



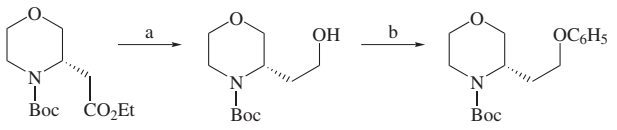
Scheme 2. Reagents and conditions: (a) allyltrimethylsilane, 2.7 equiv, $TiCl_4$, 1.4 equiv, CH_2Cl_2, −30 to 0 °C, 1 h; (b) $NaIO_4$, 4 equiv, $RuCl_3$–H_2O_n, 0.05 equiv, CCl_4–CH_3CN–H_2O (2-2-3), 2 h then 33% HBr–CH_3CO_2H neat, 16 h, rt; (c) MeOH, CH_3COCl, 0.05 equiv, 15 h then Boc_2O, 1.1 equiv, Et_3N, CH_2Cl_2.

It should be noted that for the morpholino compound we used a formyl protection, which was easier to cleave in acidic medium than the corresponding carbomethoxy group. Allylated amide **15** was thus obtained in 85% yield from the corresponding aminal.

The kinetic hydrolysis of racemic ethyl ester **7** was then carried out with CAL-B in water/THF, but the selectivity factor was low (Table 1, entry 10). We then screened some other lipases and were pleased to find that by using *B. cepacia* lipase (Amano PS), a 50% conversion of the racemic ester was obtained after 10 h to give the corresponding acid with an ee better than 99% (entry 11). The enantiomeric excesses were measured by GC with a chiral stationary phase directly for the residual ester or after esterification with diazomethane for the acid. This selective hydrolysis was then extended to the esters **4** and **5** and high enantioselectivities also observed (entries 5 and 8). The homoproline and the homopipecolic acid were thus obtained in high enantiomeric excesses and chemical yields. It is interesting to note that decreasing the size of the nitrogen substituent by using a methyl carbamate strongly decreased the rate of the reaction and the enantioselectivity (entry 4).

2.1. Absolute configuration of the products

The absolute configurations of the products were determined by comparison with literature data for the pyrrolidino and piperidino derivatives. In order to determine the configuration of the morpholino derivatives not previously described, we used a chemical correlation. Residual ester **7** was reduced into the corresponding alcohol and transformed into ether **16** using a Mitsunobu substitution (Scheme 3).



Scheme 3. Reagents and conditions: DIBAL-H 1 M in toluene, 2 equiv, Et_2O, 0 °C, 1 h, 80%; (b) phenol 1.5 equiv $(C_6H_5)_3P$, 1.5 equiv DIAD, 1.5 equiv, THF, 0 °C, 2.5 h, 63%.

Comparison of the rotatory power of this ether with the one previously reported for the same compound prepared from serine[24] allowed us to assign the (*S*)-configuration to the ester isolated after resolution. It should be noted that the three residual esters present the same spatial arrangement (*R* for **4** and **5**, *S* for **7** due to an inversion in the group priority) and that they all have a shorter retention time than their enantiomers in GC.

3. Conclusion

In summary, the resolution of homoproline, homopipecolic acid and 3-carboxymethylmorpholine by hydrolysis of the corresponding methyl or ethyl esters in the presence of *B. cepacia* lipase allowed us to obtain these cyclic β^3-aminoacids in high enantiomerical purities.

4. Experimental

4.1. General methods

Products were purified by distillation or by medium pressure liquid chromatography on a Jobin–Yvon Modulprep (Kieselgel 60H Merck) or by flash chromatography (Kieselgel 60 Merck: 230–400 Mesh; solvent: cyclohexane/EtOAc) and analysed by GC (Chrompack CP-Sil 8CB, 50 m capillary column) or by TLC (Merck silica gel 60F 254). NMR spectra were recorded on a Bruker AC at 200 MHz for ^1H and 50 MHz for ^{13}C NMR or on a Bruker 400 Avance for ^1H. $CDCl_3$ was used as the solvent with TMS as the internal standard. IR spectra were recorded on a Perkin–Elmer 599 spectrophotometer. Mass spectra were recorded on a Ribermag R10-10C instrument at 70 eV ionising voltage; ammonia was used for chemical ionisation. Optical rotations were measured on a Jasco P-1010 polarimeter. The enantiomeric excesses were measured on a 25 m Chirasil-DEX CB column (Chrompack-Varian). CAL B (chirazyme L-2 f-2) was purchased from Boehringer (Mannheim), *C. cylindracea* lipase immobilised in Sol-Gel-AK from Fluka and *B. cepacia* lipase from Amano Pharmaceutical Co.

4.2. 2-Allyl-1-methoxycarbonylpyrrolidine 10

Under argon, $TiCl_4$ (3.19 g, 1.7 mmol) in CH_2Cl_2 (6 mL) was slowly added at −30 °C to a stirred solution of 2-methoxy-1-methoxycarbonylpyrrolidine[20b] (1.91 g, 1.2 mmol) and allylsilane (3.69 g, 3.3 mmol) in CH_2Cl_2. After stirring for 15 min at −30 °C, the solution was slowly warmed up to 0 °C and stirred for 2 h then hydrolysed with brine. After extraction (CH_2Cl_2, 3 × 20 mL), the organic phase was dried over $MgSO_4$ and evaporated in vacuo. The crude product was chromatographed on silicagel (cycloheane/AcOEt: 3/2) to give 1.94 g of a colourless oil (94%). ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.76 (m, 4H), 2.09 (m, 1H), 2.45 (m, 1H), 3.31 (m, 2H), 3.62 (s, 3H), 3.80 (m, 1H), 4.9–5.1 (m, 2H), 4.95–5.05 (m, 1H); ^{13}C NMR: δ 23.6, 29.6, 38.6, 46.7, 52.2, 57.1, 117.3, 135.1, 155.7; MS (CI NH_3) *m/z* 183 ($M+NH_4^+$, 100%), 170 ($M+H^+$, 22%), 156 (15%); Anal. Calcd for $C_9H_{15}NO_2$: C, 63.88; H, 8.93; N, 8.28. Found: C, 63.69; H, 8.81; N, 8.33.

4.3. (±)-2-Carbomethoxymethyl-1-methoxycarbonylpyrrolidine 4

Allylpyrrolidine **10** (1.69 g, 10 mmol) was added to a suspension of $NaIO_4$ (8.56 g, 0.04 mol) and $RuCl_3{\cdot}nH_2O$ (0.5 mmol, 104 mg) in a 1/1/2 mixture of $CCl_4/CH_3CN/H_2O$ (70 mL). The temperature was maintained at 20 °C. After stirring for 2.5 h, the suspension was filtered on Celite and extracted with CH_2Cl_2 The aqueous phase was dried over $MgSO_4$ and the solvents removed in vacuo. The resulting oil was quickly chromatographed on silica gel (cyclohexane/AcOEt/HCO_2H: 45/54/1) to give 1.50 g of acid (80%). A 33% solution of HBr in acetic acid (10 mL) was added to this crude acid (1.50 g, 8 mmol) and the resulting mixture stirred for 16 h at room temperature. The solvent was then removed and the residual solid purified on a H^+ 50X8 Dowex resin and eluted with 1 M ammonia. After lyophilisation, the crude aminoacid was added to a solution of anhydrous HCl in methanol prepared by the addition of acetyl chloride (1.88 g, 24 mmol) to methanol (50 mL) and stirred for 36 h. The methanol was then removed in vacuo and the residual oil stirred with Boc_2O (1.2 equiv) and Et_3N (3 equiv) in CH_2Cl_2 (15 mL) for 20 h at room temperature and then heated to 40 °C for 4 h. After hydrolysis, the solution was extracted with CH_2Cl_2, washed with a saturated solution of sodium hydrogenocarbonate and evaporated. The solvent was removed in vacuo and the residual oil chromatographed (cyclohexane/AcOEt: 3/1) to give 1.38 g of pure **4** (71% yield from the acid). IR (KBr) cm^{-1}: 2955, 1737, 1698, 1452, 1385, 1194; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.43 (s, 9H); 1.79 (m, 3H), 2.0 (m, 1H), 2.78 (dd, 1H, J = 9.8 and 16.2 Hz), 2.85 (m, 1H), 3.30 (m, 2H), 3.64 (s, 3H), 4,10 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): 22.7, 28.4, 31.0, 39.0, 46.2, 51.5, 53.9, 77.7, 154.2, 171.9; MS ($CINH_3$) *m/z* 244 (M+1, 100%), 205 (23%), 188 (52%).

4.4. 2-Allyl-1-methoxycarbonylpiperidine 11

Using the previously described procedure an 88% yield was obtained. ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.4–1.6 (m, 8H), 2.29 (m, 2H), 2.78 (t, 1H, J = 15 Hz), 3.61 (s, 3H), 3.95 (d, 1H, J = 14 Hz), 4.25 (m, 1H), 4.9–5.1 (m, 2H), 5.67 (m, 1H); ^{13}C NMR: δ 18.5, 25.3, 26.7, 27.3, 39.0, 50.1, 52.2, 116.6, 135.1, 156.0; MS ($CINH_3$) *m/z* 201 ($M+NH_4^+$, 28%), 184 ($M+H^+$, 100%), 142 (18%); Anal. Calcd for $C_{10}H_{17}NO_2$: C, 65.54; H, 9.35; N, 7.64. Found: C, 65.50; H, 9.21; N, 7.72.

4.5. (±)-2-Carbomethoxymethyl-1-methoxycarbonylpiperidine 5

From compound **11** (915 mg, 5 mmol), 0.82 g of crude acid was obtained after oxidation (85%). Compound **5** was prepared as previously described and isolated in 80% from the acid. IR (KBr) cm^{-1} 2976, 1743, 1694, 1413, 1392, 1163; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.38 (s, 9H), 1.56 (m, 6H), 2.48 (dd, 2H, J = 7.2 and 7.6 Hz), 2.70 (m, 1H), 3.59 (dm, 1H), 4.60 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.8, 25.2, 27.4; 28.3, 35.0, 39.1, 47.8, 51.6, 79.5, 154.7, 171.8; MS (EI) *m/z* 257 (1%), 201 (5%), 184 (4%), 170 (5%), 156 (23%), 142 (19%), 128 (47%), 84 (100%), 57 (67%).

4.6. 3-Allyl-4-formylmorpholine 15

Using the previously described procedure, an 85% yield was obtained. IR (KBr) cm^{-1} 3600, 1681, 1666; ^{1}H NMR (200 MHz, $CDCl_3$) two conformers: δ 2.4–4.3 (m, 9H), 5.0–5.2 (m, 2H), 5.6–5.8 (dm, 1H), 8.02 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 33.0–33.8, 36.5–41.8, 47.6–53.8, 66.4–67.1, 68.1–69.4, 117.8–118.8, 133.2–134.0, 161.0; MS (EI) *m/z* 155 (1%), 127 (4%), 114 (100%), 86 (12%), 68 (4%), 58 (14%), 41 (9%), 39 (5%). Anal. Calcd for $C_8H_{13}NO_2$: C, 61.91; H, 8.44; N, 9.03. Found: C, 61.94; H, 8.52; N, 8.92.

4.7. (±)-4-*tert*-Butoxycarbonyl-3-carbethoxymethylmorpholine 7

Ozone was bubbled for 10 h through a solution of 3-allyl-4-formylmorpholine (2.6 g, 16.8 mmol) in 2/3 MeOH–CH_2Cl_2 cooled to −78 °C. The excess of ozone was then eliminated with argon and the solvents removed in vacuo. 30% H_2O_2 (5.6 mL) and formic acid (11 mL) were then added to the crude ozonide, the mixture refluxed for 1/2 h and then stirred at room temperature for 12 h. After elimination of the solvents, the residual oil was dissolved in dioxane (10 mL) and 2 M HCl (10 mL), and refluxed for 3 h. The resulting free aminoacid was then purified on a Dowex H^+ 50X8 resin to give, after lyophilisation, 1.66 g (68%) of a white solid. This aminoacid was then esterified with acetyl chloride in ethanol and *N*-Boc protected as previously described. IR (KBr) cm^{-1} 2980, 1735, 1698; ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.25 (t, 3H, J = 7.1 Hz), 1.44 (s, 9H), 2.54 (dd, 1H, J = 5.5 and 15.0 Hz), 2.81 (dd, 3H, J = 8.8 and 15 Hz), 4.11 (q, 2H, J = 7.1 Hz), 4.36 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 14.2, 28.4, 33.8, 39.5, 48.1, 60.7, 66.9, 68.9, 80.3, 154.5, 171.3; MS (EI) *m/z* 273 (1%), 217 (5%), 200 (3%), 172 (24%), 142 (43%), 130 (32%), 86 (46%), 57 (100%), 41 (26%). Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.06; H, 8.63; N, 5.04.

4.8. General procedure for lipase-catalysed hydrolysis

A solution of ester (1 mmol) in THF (2 mL) was added to 10^{-2} M phosphate buffer (8 mL) and water (8 mL) at 25 °C. The pH was adjusted to 7 with 0.1 M HCl and the lipase (250 mg) added in one portion. The resulting suspension was stirred at 150 rpm with the pH controlled by the addition of a 0.1 M solution of NaOH using a pH Stat. When the theoretical quantity of NaOH was added, the suspension was filtered through a small pad of Celite to remove the enzyme. The residual ester was removed by extraction with Et_2O at pH 8. After acidification until pH 2, the acid was then extracted (Et_2O) from the solution.

4.8.1. (2*R*)-1-*tert*-Butoxycarbonyl-2-carbomethoxymethylpyrrolidine. $[\alpha]_D^{20} = +40.1$ (*c* 12.5, MeOH), ee: 99.1 {lit.[25] (*S*) compound: $[\alpha]_D^{20} = -38.8$ (*c* 16, MeOH)}.

4.8.2. (2*S*)-1-*tert*-Butoxycarbonyl-2-carboxymethylpyrrolidine. White solid: mp 98–99 °C (hexane); $[\alpha]_D^{20} = -38.6$ (*c* 1.41, DMF) ee: 99.4% {lit.[26] 99–101 °C; $[\alpha]_D^{20} = -39.5$ (*c* 1.9, DMF)} IR (KBr) cm^{-1}: 3700–2800, 1735, 1655; ^1H NMR (200 MHz, $CDCl_3$): δ 1.39 (s, 9H), 1.76 (m, 3H), 2.0 (m, 1H), 2.28 (dd, 1H, $J = 10.1$ and 16.0 Hz), 2.80 (m, 1H), 3.30 (m, 2H), 4.10 (br s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 23.5, 28.4, 31.2, 39.1, 46.3, 53.9, 79.9, 156.0, 177; MS (EI) *m*/*z* 229 (1%), 173 (5%), 156 (6%), 128 (13%), 114 (30%), 70 (98%), 57 (100%).

4.8.3. (2*R*)-1-*tert*-Butoxycarbonyl-2-carbomethoxymethylpiperidine. $[\alpha]_D^{20} = +8$ (*c* = 3,96; $CHCl_3$), ee: 98% (lit.[5] (*S*) compound $[\alpha]_D^{20} = -8.3$ (*c* = 2,01; MeOH)).

4.8.4. (2*S*)-1-*tert*-Butoxycarbonyl-2-carboxymethylpiperidine. White solid: mp 95 °C (hexane), $[\alpha]_D^{20} = -8$ (*c* 1.53, MeOH) ee: 99.3% (lit.[5] 93 °C) IR (KBr) cm^{-1} 3500–3200, 3011, 2980, 1736, 1639, 1433, 1185; ^1H NMR (200 MHz, $CDCl_3$): δ 1.37 (s, 9H), 1.56 (m, 6H), 2.51 (dd, 2H, $J = 3.2$ and 8.5 Hz), 2.70 (m, 1H), 3.90 (dm, 1H), 4.63 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 18.8, 25.2, 27.4, 28.3, 35.0, 39.1, 47.8, 51.6, 79.5, 154.7, 171.8; MS (EI) *m*/*z* 243 (2%), 187 (5%), 184 (3%), 142 (12%), 128 (42%), 84 (100%), 57 (89%).

4.8.5. (3*S*)-4-*tert*-Butoxycarbonyl-3-carbethoxymethylmorpholine. $[\alpha]_D^{20} = +35.6$ (*c* 1.15; CH_2Cl_2), ee: 99%.

4.8.6. (3*R*)-4-*tert*-Butoxycarbonyl-3-carboxymethylmorpholine. White solid: mp 82 °C (hexane: *i*-$Prop_2O$, 8/2), $[\alpha]_D^{20} = -35.7$ (*c* 1.94, CH_2Cl_2) ee: 99%; IR (KBr) cm^{-1} 3700–2500, 1713, 1694; ^1H NMR (200 MHz, $CDCl_3$): δ 1.47 (s, 9H), 2.56 (dd, 1H, $J = 6.0$ and 15.4 Hz), 2.83 (dd, 1H, $J = 8.5$ and 15.4 Hz), 2.9–3.9 (m, 6H), 4.34 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ 28.2, 33.4, 39.4, 48.0, 66.7, 68.8, 80.5, 154.5, 175.9; MS (EI) m/z 245 (1%), 190 (2%), 172 (3%), 130 (14%), 114 (13%), 100 (5%), 86 (33%), 70 (13%), 57 (100%). Anal. Calcd for $C_{11}H_{19}NO_5$: C, 53.87; H, 7.81; N, 5.71. Found: C, 53.95; H, 7.91; N, 5.59.

Acknowledgements

The authors are grateful to the Boehringer company (Mannheim) for the generous gift of various lipases. Moreover, C. Pousset is very grateful to Peptisyntha S.A. for financial support.

References

1. (a) Dado, G. P.; Gellman, S. H. *J. Am. Chem. Soc.* **1994**, *116*, 1054–1062; (b) Crisma, M.; Formaggio, F.; Pantano, M.; Valle, G.; Bonora, G. M.; Toniolo, C.; Schoemaker, H. E.; Kamphuis, J. *J. Chem. Soc., Perkin Trans. 2* **1994**, 1735–1742; (c) Seebach, D.; Overhand, M.; Kühnle, F. N. M.; Martinoni, B.; Oberer, L.; Hommel, U.; Widmer, H. *Helv. Chim. Acta* **1996**, *79*, 913–941; (d) Seebach, D.; Matthews, J. L. *Chem. Commun.* **1997**, 2015–2022; (e) Gellman, S. H. *Acc. Chem. Res.* **1998**, *31*, 173–180.
2. (a) Sewald, N. Bioorganic Chemistry, Highlights and New Aspects. In Diederichsen, U., Lindhorst, T. K., Westermann, B., Wessjohann, L. A., Eds.; Wiley-VCH: Weinheim, Germany, 1999; Chapter 4.2, p 193; (b) Sasaki, N. A.; Dockner, M.; Chiaroni, A.; Riche, C.; Potier, P. *J. Org. Chem.* **1997**, *62*, 765–770; (c) Hammer, K.; Undheim, K. *Tetrahedron: Asymmetry* **1998**, *9*, 2359–2368; (d) Rutjes, F. P. J. T.; Schoemaker, H. E. *Tetrahedron Lett.* **1997**, *38*, 677–680.
3. (a) Juaristi, E. In *Enantioselective Synthesis of β-Amino Acids*; Wiley VCH: New York, 1997; (b) Liu, M. L.; Sibi, M. P. *Tetrahedron* **2002**, *58*, 7991–8035; (c) Park, K.-H.; Kurth, M. *Tetrahedron* **2002**, *58*, 8629–8659; (d) Sewald, N. *Angew. Chem., Int. Ed.* **2003**, *42*, 5794–5795.
4. Balaspiri, L.; Penke, B.; Papp, G.; Dombi, G.; Kovacs, K. *Helv. Chim. Acta* **1975**, *58*, 969–973.
5. Morley, C.; Knight, D. W.; Share, A. C. *J. Chem. Soc., Perkin Trans. 1* **1994**, *6*, 2903–2907.
6. (a) Enders, D.; Wiedemann, J. *Liebigs Ann.* **1997**, 699–706; (b) O'Brien, P.; Porter, D. W.; Smith, N. M. *Synlett* **2000**, 1336–1338; (c) Baenziger, M.; Gobbi, L.; Riss, B. P.; Schaefer, F.; Vaupel, A. *Tetrahedron: Asymmetry* **2000**, *11*, 2231–2237; (d) Davies, S. G.; Iwamoto, K.; Smethurst, C. A. P.; Smith, A. D.; Rodriguez-Solla, H. *Synlett* **2002**, 1146–1148.
7. (a) Nikiforov, T.; Stanchev, S.; Milenkov, B.; Dimitrov, B. *Heterocycles* **1986**, *24*, 1825–1829; (b) Bardou, A.; Célérier, J.-P.; Lhommet, G. *Tetrahedron Lett.* **1997**, *38*, 8507–8510.
8. Soloshokok, V. A.; Fokina, N. A.; Rybakova, A. B.; Shishkina, I. P.; Galushko, S. V.; Alexander, E.; Sorochinski, A. E.; Kuhar, V. P.; Savchenko, M. V.; Svedas, V. K. *Tetrahedron: Asymmetry* **1995**, *6*, 1601–1610.
9. (a) Toone, E. J.; Jones, J. B. *Can. J. Chem.* **1987**, *65*, 2722–2726; (b) Sanchez, V. M.; Rebolledo, F.; Gotor, V. *Tetrahedron: Asymmetry* **1997**, *8*, 37–40; (c) Gedey, S.; Liljeblad, A.; Lazar, L.; Fülöp, F.; Kanerva, L. *Tetrahedron: Asymmetry* **2001**, *12*, 105–110.
10. (a) Nagai, H.; Shiozawa, T.; Achiwa, K.; Terao, Y. *Chem. Phar. Bull.* **1992**, *40*, 2227–2233; (b) Yamanaka, T.; Ohkubo, M.; Takahashi, F.; Kato, M. *Tetrahedron Lett.* **2004**, *45*, 2843–2845.
11. (a) Csomos, P.; Kanerva, L. T.; Bernath, G.; Fülöp, F. *Tetrahedron: Asymmetry* **1996**, *7*, 1789–1796; (b) Theil, F.; Ballschuh, S. *Tetrahedron: Asymmetry* **1996**, *7*, 3565–3572; (c) Kaman, J.; Forro, E.; Fülöp, F. *Tetrahedron: Asymmetry* **2000**, *11*, 1593–1600.
12. (a) Kanerva, L. T.; Csomos, P.; Sundholm, O.; Bernath, G.; Fülöp, F. *Tetrahedron: Asymmetry* **1996**, *7*, 1705–1716; (b) Stürmer, R.; Ditrich, K.; Siegel, W. US 6063615,

2000; *Chem Abstr.* **1999**, *130*, 66802x, for a recent review, see: (c) Van Rantwijk, F.; Sheldon, R. A. *Tetrahedron* **2004**, *60*, 501–519.
13. Katayama, S.; Ae, N.; Nagata, R. *Tetrahedron: Asymmetry* **1998**, *9*, 4295–4299.
14. Patent: Callens, R.; Larchevêque, M.; Pousset, C. E.P. 2004/003688.
15. Liljeblad, A.; Lindborg, J.; Kanerva, A.; Katajisto, J.; Kanerva, L. T. *Tetrahedron Lett.* **2002**, *43*, 2471–2474.
16. Fukawa, H.; Terao, Y.; Achiwa, K.; Sekiya, M. *Chem. Lett.* **1982**, 231–232.
17. (a) Kaufman, W.; Bauer, K. *Naturwissenschaften* **1960**, *47*, 474–479; (b) Fuganti, C.; Grasseli, P.; Servi, S.; Lazzarini, A.; Casati, P. *Tetrahedron* **1988**, *44*, 2575–2582; (c) Waldmann, H. *Tetrahedron Lett.* **1989**, *30*, 3057–3058.
18. Stürmer, R.; Schäfer, B.; Wolfart, V.; Stahr, H.; Kazmaier, U.; Helmchen, G. *Synthesis* **2001**, 46–48.
19. Okitsu, O.; Suzuki, R.; Kobayashi, S. *J. Org. Chem.* **2001**, *66*, 809–823.
20. (a) Nyberg, K.; Servin, R. *Acta Chem. Scand. B* **1976**, *30*, 640–648; (b) Shono, T. *Tetrahedron* **1984**, *40*, 811–850.
21. (a) Renaud, P.; Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1986**, *25*, 843–844; (b) Renaud, P.; Seebach, D. *Helv. Chim. Acta* **1986**, *69*, 1704–1710.
22. Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936–3938.
23. Chen, C. S.; Fujimoto, Y.; Girdaukas, G.; Sih, C. J. *J. Am. Chem. Soc.* **1982**, *104*, 7294–7299.
24. Brown, G. R.; Foubister, A. J.; Stribling, D. *J. Chem. Soc., Perkin Trans. 1* **1987**, 547–551.
25. Mahboobi, S.; Popp, A.; Burgemeister, T.; Schollmeyer, D. *Tetrahedron: Asymmetry* **1998**, *9*, 2369–2376.
26. Cassal, J.; Fürst, A.; Meier, W. *Helv. Chim. Acta* **1976**, *59*, 1917–1924.