Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 721–726

TETRAHEDRON: *ASYMMETRY*

Enzymatic ammonolysis of ethyl (±)-4-chloro-3-hydroxybutanoate. Chemoenzymatic syntheses of both enantiomers of pyrrolidin-3-ol and 5-(chloromethyl)-1,3-oxazolidin-2-one

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Received 15 December 1998; accepted 26 January 1999

Abstract

Lipase B from *Candida antarctica* efficiently catalysed the kinetic resolution of ethyl (±)-4-chloro-3-hydroxybutanoate through an ammonolysis reaction. Using this methodology, both enantiomers of 4-chloro-3-hydroxybutanamide were prepared and converted into pyrrolidin-3-ol and 5-(chloromethyl)-1,3-oxazolidin-2-one by simple processes consisting of a reduction reaction and a Hofmann rearrangement, respectively.

1. Introduction

Lipase-catalysed kinetic resolution of racemates is one of the most frequently used strategies for the preparation of optically active compounds. A great variety of carboxylic acids, esters, alcohols and, in minor extensions, amides and amines have been obtained by esterification, hydrolysis and aminolysis processes. In recent years, we and other authors have shown the efficiency of the lipase B from *Candida antarctica* (CALB) to catalyse ammonolysis reactions. The preparation, under very mild conditions, of different *N*-unsubstituted amides,[1] as well as the resolution and asymmetrisation of esters[2] by means of this methodology have been some of the contributions to this field.

In connection with these studies, we explore here the potential of CALB to catalyse resolution by ammonolysis of ethyl (±)-4-chloro-3-hydroxybutanoate. This substrate has been chosen due to the further applications of the resulting enantiomerically enriched 4-chloro-3-hydroxybutanamide. Thus we also report in this work two new chemoenzymatic methods for the synthesis of both enantiomers of pyrrolidin-3-ol and 5-(chloromethyl)-1,3-oxazolidin-2-one. Enantiomerically enriched pyrrolidin-3-ol

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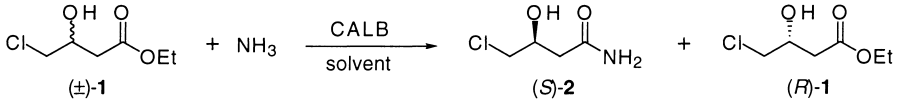
0957-4166/99/$ - see front matter
PII: S0957-4166(99)00034-8

has been employed as a precursor of alkaloids,[3] β-proline derivatives[4] and other compounds of medicinal interest,[5] and, more specifically, its (*S*)-isomer is a constituent of compounds with activity as kappa-receptor agonists.[6] In addition, enantiopure 5-(chloromethyl)-1,3-oxazolidin-2-one has been used as a precursor of building blocks for hydroxyethylene isosters.[7]

2. Results and discussion

Since the solvent can have a remarkable effect on the enantiomeric ratio and the catalytic activity of enzymes,[8] we decided to test the effect of different solvents on the ammonolysis of ethyl (±)-4-chloro-3-hydroxybutanoate (±)-**1**. All the reactions were carried out at room temperature using a freshly prepared solution of ammonia in the appropriate solvent. In order to avoid the competitive enzymatic hydrolysis of (±)-**1**, the ammonia was previously dried using BaO as desiccant. As shown in Table 1, CALB retained its chiral preference in all the solvents used, (*S*)-4-chloro-3-hydroxybutanamide, (*S*)-**2**, being obtained as the major isomer. However, the reaction rate and enantiomeric ratio (*E*)[9] varied markedly. Reaction rate changes can be explained in terms of the different solubility of ammonia in each solvent. Thus, good reaction rates were attained with hydrogen-bond acceptor solvents (Table 1, entries 1, 2 and 5), especially with *tert*-butyl alcohol (TBA). Unfortunately, in this medium the lipase showed the poorest *E*-value (*E*=17). The most suitable solvent was 1,4-dioxane (entry 5), which allowed us to achieve both amide (*S*)-**2** and the remaining ester (*R*)-**1** with high enantiomeric excesses (ee) and yields. By controlling the extent of conversion the ee of (*S*)-**2** could be slightly improved. Thus, at 34% conversion (*S*)-**2** was obtained with 93% ee (entry 6). In addition, when the reaction was carried out at 55% conversion (entry 7), the remaining (*R*)-**1** was obtained with 98% ee.

Table 1
Ammonolysis of (±)-**1**



(±)-**1** (*S*)-**2** (*R*)-**1**

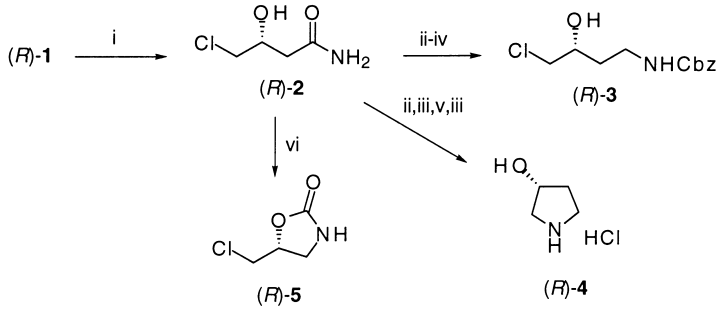
Entry	solvent	t, h	c,[a] %	ee, % (*S*)-**2**	ee, % (*R*)-**1**	*E*[a]
1	THF	3.5	30	92	40	36
2	TBA	1	51	75	79	17
3	CH_2Cl_2	5.0	13	95	14	45
4	toluene	20	48	80	76	20
5	1,4-dioxane	4	48	89	82	44
6	1,4-dioxane	1.5	34	93	48	
7	1,4-dioxane	6.0	55	81	98	

[a] Conversion, see ref. 9

Amide **2** was assigned the *S*-configuration according to the configuration of the remaining ester **1**, for which the *R*-configuration was established by comparison of its specific rotation with the reported data for (*S*)-(−)-**1**.[10] Enantiomeric excesses for (*R*)-**1** and (*S*)-**2** were determined by chiral HPLC analysis of their *O*-benzoyl and *O*-*tert*-butyldiphenylsilyl derivatives, respectively (see experimental section).

With the aim of obtaining the amide (*R*)-**2**, we tried the conventional ammonolysis (NH_3/MeOH, 4°C) of the enantiomerically enriched ester (*R*)-**1** (98% ee), but after several days of reaction amide (*R*)-**2** was not detected, (*R*)-**1** being transformed into a mixture of compounds which were not identified. For this reason, we decided to use CALB to perform the ammonolysis of (*R*)-**1** (Scheme 1). From the results

collected in Table 1 it is deduced that, for this purpose, the best solvent is *tert*-butyl alcohol, because in this medium the enzyme showed very high activity and low *E*-value. As expected, the ammonolysis of (*R*)-**1** was slower than that of the racemic substrate, but, after three days of reaction, amide (*R*)-**2** was obtained with very high yield and without racemisation as determined by chiral HPLC.



Scheme 1. Reagents and conditions: (i) NH_3, CALB, Bu^{t}OH, rt; (ii) $BH_3 \cdot THF$, reflux; (iii) 6 N HCl; (iv) CbzCl, K_2CO_3, CH_2Cl_2, H_2O; (v) TMEDA, MeOH; (vi) PIDA, THF, MeOH

Transformation of (*R*)-**2** into (*R*)-pyrrolidin-3-ol (Scheme 1) was accomplished by reduction of the amide group with $BH_3 \cdot THF$ and subsequent intramolecular nucleophilic subtitution of the chlorine by the resulting amino group. In order to achieve the intermediate 4-amino-1-chlorobutan-2-ol, the crude material obtained from the acid hydrolysis of the reduction process was treated with benzyl chloroformate, thus obtaining the highly functionalised benzyl carbamate (*R*)-**3**.[†] In addition, when the residue of the acid hydrolysis step was treated with TMEDA, (*R*)-4-amino-1-chlorobutan-2-ol was converted into the desired cyclic (*R*)-pyrrolidin-3-ol, which was isolated as its hydrochloride (*R*)-**4**. Similarly, compound (*S*)-**2** was converted into the corresponding (*S*)-**3** and (*S*)-**4**.

Both enantiomers of 5-(chloromethyl)-1,3-oxazolidin-2-one (*R*)-**5** and (*S*)-**5** were obtained from (*R*)-**2** and (*S*)-**2**, respectively, by means of a Hofmann rearrangement. Reaction of (*R*)-**2** with $Hg(OAc)_2$ and NBS in DMF led to (*R*)-**5** in a moderate yield (63%). Nevertheless, a remarkable improvement in the yield of (*R*)-**5** was reached when the rearrangement was accomplished with iodosobenzene diacetate (PIDA).[11] In a similar manner, treatment of (*S*)-**2** with PIDA yielded (*S*)-**5**.

Optical purities of compounds **4** and **5** were established by comparison of their specific rotations with the reported data for these compounds (see experimental), showing that no racemisation had taken place. As compound **4** was obtained without racemisation, compound **3** was assumed to have the same ee as its precursor compound **2**.

In conclusion, we have demonstrated the utility of lipase B from *Candida antarctica* in the preparation of (*R*)- and (*S*)-4-chloro-3-hydroxybutanamide **2** from (±)-**1**. Starting from (*R*)- and (*S*)-**2**, both enantiomers of pyrrolidin-3-ol and 5-(chloromethyl)-1,3-oxazolidin-2-one have effectively been obtained. The chemoenzymatic syntheses described here constitute an interesting alternative to other multistep syntheses of these compounds.[12]

3. Experimental

Lipase B from *Candida antarctica* (CALB), Novozym 435, was a gift from Novo Nordisk Co. All reagents were of commercial quality and were purchased from Aldrich Chemie. Hydroxy ester

[†] In this process a small amount (5%) of *N*-benzyloxycarbonylpyrrolidin-3-ol was also obtained.

(±)-**1** was obtained by reduction ($NaBH_4$, EtOH) of the commercially available ethyl 4-chloro-3-oxobutanoate. Solvents were distilled over an adequate desiccant and stored under nitrogen. For column chromatography, Merck silica gel 60/230–400 mesh was used. Melting points were taken using a Gallenkamp apparatus and are uncorrected. Optical rotations were measured using a Perkin–Elmer 241 polarimeter. IR spectra were recorded on a Perkin–Elmer 1720-X FT infrared spectrometer. ^1H and ^{13}C NMR spectra were obtained on a Bruker AC-300 (^1H 300 MHz and ^{13}C 75.5 MHz) and a Bruker AC-200 (^1H 200 MHz and ^{13}C 50.3 MHz) spectrometer, using TMS as internal standard. Mass spectra were recorded on a Hewlett–Packard 5987 A spectrometer. Microanalyses were performed on a Perkin–Elmer 240B elemental analyser.

*3.1. General procedure for the ammonolysis of ethyl (±)-4-chloro-3-hydroxybutanoate [(±)-**1**]*

Dry ammonia was bubbled through the appropriate solvent (22 mL) for 10 min at room temperature. Then, ester (±)-**1** (10 mmol) and CALB (1 g) were added and the resulting mixture was shaken at rt and 250 r.p.m. during the time shown in Table 1. Afterwards, the enzyme was filtered, washed with dichloromethane and the organic solvents were evaporated. Column chromatography (ethyl acetate as eluent) of the residue yielded (*R*)-**1** and (*S*)-**2**. In all cases (*R*)-**1** was recovered with >95% yield (calculated taking into account the conversion value).

*3.1.1. (*S*)-4-Chloro-3-hydroxybutanamide (*S*)-**2***

Yield, 85% (reaction with 1,4-dioxane at conv.=48%); mp 66–67°C; $[\alpha]_D^{21}$ −18.7 (*c* 0.6, CH_3OH, 93% ee); IR (KBr) 1615, 1659, 3400 cm^{-1}; ^1H NMR (CD_3OD) δ (ppm) 2.54–2.76 (m, 2H, CH_2), 3.69–3.85 (m, 2H, CH_2Cl), 4.38 (m, 1H, CH); ^{13}C NMR (CD_3OD) δ (ppm) 41.3 (CH_2), 49.9 (CH_2), 69.8 (CH), 176.3 (C=O); MS (CI, CH_4) m/z 138 [$(M+1)^+$, 1.6], 140 [$(M+3)^+$, 0.5], 88 (100). Anal. calcd for $C_4H_8ClNO_2$: C, 34.92; H, 5.86; N, 10.18. Found: C, 34.60; H, 5.62; N, 9.79.

*3.2. (*R*)-4-Chloro-3-hydroxybutanamide (*R*)-**2***

Compound (*R*)-**2** was obtained from (*R*)-**1** (98% ee) following the procedure described for (±)-**1**, using as solvent *tert*-butyl alcohol. Reaction time, 72 h; yield, 90%; $[\alpha]_D^{23}$ +20.4 (*c* 0.8, CH_3OH, 98% ee).

*3.3. Benzyl (*S*)-N-(4-chloro-3-hydroxybutyl)carbamate (*S*)-**3***

$BH_3 \cdot THF$ complex (1.0 M solution in THF) (1.0 mL) was added dropwise under nitrogen to a solution of amide (*S*)-**2** (0.69 mmol) in THF (19 mL) at rt. After being refluxed for 14 h, 6 N HCl (0.15 mL) was added at rt. The reaction mixture was stirred for 4 h and the solvents were evaporated. The residue was dissolved in water (3.0 mL) and K_2CO_3 (1.9 mmol) was added. Immediately afterwards, dichloromethane (3.0 mL) and benzyl chloroformate (1.0 mmol) were added at 4°C, and the resulting reaction mixture was stirred at rt for 14 h. Then, the organic layer was separated and the aqueous layer was extracted again with dichloromethane. The combined organic extracts were dried and evaporated. Column chromatography of the residue (hexane:ethyl acetate 2:1) gave 0.45 mmol (65%) of (*S*)-**3**. Oil; $[\alpha]_D^{20}$ −17.2 (*c* 1.0, MeOH); IR (KBr) 3416, 3346, 1691 cm^{-1}; ^1H NMR ($CDCl_3$) δ (ppm) 1.54–1.66 (m, 1H, C*H*H), 1.69–1.79 (m, 1H, CH*H*), 3.20–3.30 (m, 1H, C*H*H), 3.38–3.57 (m, 3H, CH*H*, CH_2), 3.67 (bs, 1H, OH), 3.87 (m, 1H, CH), 5.08 (s, 2H, CH_2), 5.45 (m, 1H, NH), 7.33 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$) δ (ppm) 33.9 (CH_2), 37.5 (CH_2), 49.3 (CH_2), 66.6 (CH_2), 69.0 (CH), 127.8 (CH), 127.9 (CH), 128.3 (CH), 136.2 (C), 156.9 (C=O); MS (70 eV) m/z 257 (M^+, 4), 259 [$(M+2)^+$, 1.5], 108 (82), 91 (100).

3.4. Benzyl (R)-N-(4-chloro-3-hydroxybutyl)carbamate (R)-3

Compound (*R*)-**3** was prepared from (*R*)-**2** as described for (*S*)-**3**. $[\alpha]_D^{20}$ +18.8 (*c* 1.0, MeOH).

3.5. (S)-Pyrrolidin-3-ol hydrochloride (S)-4

$BH_3 \cdot THF$ complex (1.0 M solution in THF) (2.3 mL) was added dropwise under nitrogen to a solution of (*S*)-**2** (1.5 mmol) in THF (41 mL) at rt. After being refluxed for 14 h, 6 N HCl (0.30 mL) was added at rt. The reaction mixture was stirred for 4 h and the solvent was evaporated. The residue was dissolved in methanol (2.0 mL) and TMEDA (1.7 mmol) was added. After stirring at rt for 18 h the solvents were evaporated. Column chromatography of the crude product (dichloromethane:methanol:30% aq. ammonia 2:2:0.1) gave 1.33 mmol (87%) of pyrrolidin-3-ol, which was dissolved in methanol (2.0 mL) and subsequently treated with 6 N HCl (0.4 mL). After stirring the acid solution at rt for 30 min, the solvent was evaporated yielding (*S*)-**4** as a hygroscopic solid. $[\alpha]_D^{26}$ +8.3 (*c* 1.9, CH_3OH); IR (KBr) 3412, 3005, 1628 cm^{-1}; ^1H NMR (CD_3OD) δ (ppm) 2.21–2.38 (m, 2H, CH_2), 3.37–3.45 (m, 2H, CH_2), 3.56–3.70 (m, 2H, CH_2), 4.74 (m, 1H, CH); ^{13}C NMR (CD_3OD) δ (ppm) 34.4 (CH_2), 45.0 (CH_2), 54.3 (CH_2), 70.8 (CH); MS (NBA matrix) m/z 88 [$(M+1)^+$ 100], 70 (9).

3.6. (R)-Pyrrolidin-3-ol hydrochloride (R)-4

Compound (*R*)-**4** was prepared from (*R*)-**2** as described for (*S*)-**4**. $[\alpha]_D^{26}$ −8.5 (*c* 1.5, CH_3OH); lit.[12a] $[\alpha]_D^{20}$ −7.6 (*c* 3.45, CH_3OH, 100% ee).

3.7. General procedure for the Hofmann rearrangement of (R)- and (S)-4-chloro-3-hydroxybutanamide 2

Iodosobenzene diacetate (0.67 mmol) was added to a solution of amide **2** (0.51 mmol) in THF:MeOH 2.5:1 (5 mL) at rt. After stirring for 4 h the temperature was raised to 60°C and the reaction was stirred for 15 min. Immediately afterwards the solvent was evaporated. Column chromatography of the residue (hexane:ethyl acetate 1:1) gave the corresponding product **5**.

3.7.1. (S)-5-(Chloromethyl)-1,3-oxazolidin-2-one (S)-5

Obtained from (*S*)-**2**. Yield, 92%; $[\alpha]_D^{25}$ +16.9 (*c* 0.8, CH_2Cl_2); lit.[12b] $[\alpha]_D^{25}$ +19.1 (*c* 3.3, CH_2Cl_2, 100% ee). The spectral data for (*S*)-**5** were in accordance with literature[12b] values: ^1H NMR ($CDCl_3$) δ (ppm) 3.50–3.81 (m, 4H, $2CH_2$), 4.86 (m, 1H, CH), 6.45 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ (ppm) 43.5 (CH_2), 44.4 (CH_2), 74.6 (CH), 159,2 (C=O); MS (CI, CH_4) m/z 135 (M^+, 22), 136 [$(M+1)^+$, 8], 137 [$(M+2)^+$, 8], 138 [$(M+3)^+$, 3], 86 (100).

3.7.2. (R)-5-(Chloromethyl)-1,3-oxazolidin-2-one (R)-5

Obtained from (*R*)-**2**. $[\alpha]_D^{25}$ −18.1 (*c* 0.7, CH_2Cl_2); lit.[12b] $[\alpha]_D^{25}$ −18.7 (*c* 3.2, CH_2Cl_2, 100% ee).

3.8. Determination of the ee of 1

To a solution of **1** (0.36 mmol) in THF (1.5 mL), pyridine (2.0 mmol) and benzoyl chloride (0.5 mmol) were added at 0°C. After 12 h at room temperature, the solvent was evaporated and CH_2Cl_2 was added to the residue. The organic solution was successively washed with 2 N HCl and water, and then evaporated

to yield *O*-benzoyl-**1**, which was purified by column chromatography (CH_2Cl_2:hexane 1:1 as eluent). Chiral HPLC analysis (Chiralcel-OD, hexane:Pr^{i}OH 95:5, 0.4 mL/min): (*R*)-*O*-benzoyl-**1**, t_R 20.2 min; (±)-*O*-benzoyl-**1**, two peaks, t_R 18.1 and 20.2 min; Rs 2.5.

3.9. Determination of the ee of ***2***

A solution of **2** (0.36 mmol) in THF (1.5 mL) was treated with TBDPSCl (0.73 mmol) and imidazole (1.46 mmol). When the reaction was finished (12 h, rt), the solvent was evaporated and AcOEt was added to the residue. The organic solution was washed with 2 N HCl and water, dried and then evaporated to yield *O*-TBDPS-**2**, which was purified by column chromatography (AcOEt:hexane 1:1 as eluent). Chiral HPLC analysis (Chiralcel-OD, hexane:Pr^{i}OH 95:5, 0.8 mL/min): (*S*)-*O*-TBDPS-**2**, t_R 28.5 min; (±)-*O*-TBDPS-**2**, two peaks, t_R 24.8 and 28.2 min; Rs 2.1.

Acknowledgements

We are grateful to the CICYT (Project BIO 98-0770) for financial support, to Novo Nordisk Co. for the generous gift of the CA lipase, and E. G.-U. thanks the Ministerio de Educación y Ciencia for a predoctoral fellowship.

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