Pergamon

Tetrahedron: *Asymmetry* 13 (2002) 1091–1096

TETRAHEDRON: *ASYMMETRY*

Enzymatic resolution of the chiral auxiliary 2-methoxy-2-phenylethanol

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Received 26 April 2002; accepted 20 May 2002

Abstract—Several lipase-catalyzed processes are evaluated for the resolution of 2-methoxy-2-phenylethanol, a chiral auxiliary in the synthesis of optically active 1,4-dihydropyridines. *Candida antarctica* lipase B (CAL B) catalyzes the enzymatic acylation of the primary alcohol with high enantioselectivity. © 2002 Elsevier Science Ltd. All rights reserved.

1. Introduction

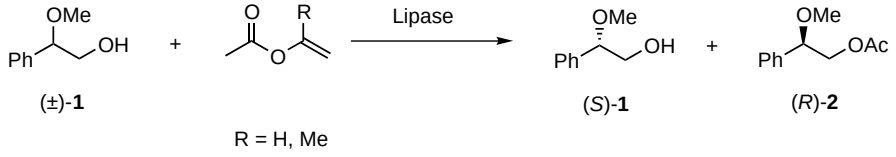
Enantiomerically pure 2-methoxy-2-phenylethanol, **1**, has been described as a useful chiral auxiliary in the synthesis of 1,4-dihydropyridines.[1] Increasing interest in the preparation of these compounds from the pharmaceutical industry means that there is a demand for the development of new synthetic methods for **1** which are amenable to large-scale production and the use of enzymes as catalysts is very suitable for this purpose.[2] While lipase-catalyzed processes in organic solvents have been widely used for the preparation of enantiomerically pure alcohols and the enzymatic resolution of secondary alcohols is now well established,[3] the resolution of primary alcohols has been less studied. Nevertheless, lipases have been successfully used for the preparation of enantiopure primary alcohols.[4] Recently, Kanerva and co-workers have described the resolution of 1-phenylethan-1,2-diol through sequential methanolysis of the mono- or diacetate derivatives.[5] Herein, we describe the application of lipases for the resolution of 2-methoxy-2-phenylethanol.

2. Results and discussion

Our initial experiments were designed to find the most suitable lipase for catalyzing the transesterification of 2-methoxy-2-phenylethanol, (±)-**1**. In a first set of experiments, vinyl acetate was chosen as the acyl donor and also as the reaction solvent. These enzymatic processes were carried out at 30°C (Scheme 1, Table 1).

Lipase B from *C. antarctica* (CAL B) showed the highest enantioselectivity ($E=47$). All of the tested lipases but *C. rugosa* lipase showed the same stereochemical preference, with the (*R*)-enantiomer being preferentially acylated. In view of these promising results, we decided to study the effect of the reaction parameters on the enantioselectivity of the acylation of alcohol (±)-**1**. Taking into account that the temperature can have a marked effect on the enantioselectivity,[7] we lowered to 15°C. Nevertheless, the enantioselectivities were not significantly enhanced (Table 2).

Next, we studied the influence of the organic solvent in the CAL B-catalysed acylation. Table 3 summarizes the



Scheme 1.

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PII: S0957-4166(02)00246-X

Table 1. Lipase-catalyzed acetylation of (±)-**1** with vinyl acetate, at 30°C. R = H

Lipase (mg/mmol substrate)	Time (h)	c[a] (%)	Remaining alcohol **1**		Product **2**		E[c]
			Conf.	e.e.$_s$[b] (%)	Conf.	e.e.$_p$[b] (%)	
CAL B (150)	0.5	49	(*S*)	89	(*R*)	86	47
CAL B-L2 (10)	0.5	59	(*S*)	>99[d]	(*R*)	70	40
CAL-A-L5 (100)	0.2	77	(*S*)	94	(*R*)	27	5
CRL-L3A (10)	7	36	(*R*)	15	(*S*)	27	2
CRL-L3 (200)	1.5	74	(*R*)	61	(*S*)	28	3
PPL (150)	11	36	(*S*)	39	(*R*)	68	8
PPL-L7 (200)	7	27	(*S*)	28	(*R*)	77	10
PSL (150)	22	43	(*S*)	36	(*R*)	46	4
PSL-L6 (50)	3	67	Nd	Nd	Nd	Nd	Nd
MML-L9 (10)	3	49	(*S*)	28	(*R*)	28	2
ASL-L10 (100)	1.5	76	(*S*)	>99[d]	(*R*)	32	11
TLL-L8 (200)	7	12	(*S*)	2	(*R*)	22	2

[a] Conversion, $c = \text{e.e.}_s/(\text{e.e.}_s + \text{e.e.}_p)$.
[b] Determined by gas chromatography.
[c] Enantiomeric ratio, $E = \ln[(1-c)(1+\text{e.e.}_p)]/\ln[(1-c)(1-\text{e.e.}_p)]$.[6]
[d] Only the (*S*)-enantiomer is detected by gas chromatography, the E value has been calculated taking e.e.$_s$ = 99.9.

Table 2. Lipase-catalyzed acetylation of (±)-**1** with vinyl acetate, at 15°C. R = H

Lipase	Time (h)	c (%)	Remaining alcohol **1**	Product **2**	E
			e.e.$_s$ (%)	e.e.$_p$ (%)	
CAL B	0.6	54	96	85	48
CAL B-L2	0.6	56	99	76	39
CAL-A-L5	0.2	46	52	60	7

Table 3. CAL B-catalyzed acetylation of (±)-**1** in organic solvents

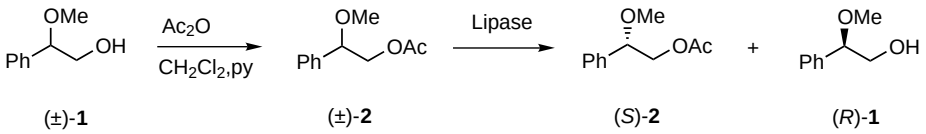
Solvent	R[a]	T (°C)	Time (h)	c (%)	Remaining alcohol **1**	Product **2**	E
					e.e.$_s$ (%)	e.e.$_p$ (%)	
Hexane	Me	50	1.3	48	40	56	5
Hexane	Me	30	23	47	51	59	6
Hexane	H	50	1	59	88	62	12
Hexane	H	30	23	55	73	60	8
Dioxane	Me	50	46	13	Nd	Nd	Nd
Dioxane	Me	30	46	9	Nd	Nd	Nd
^{i}Pr$_2$O	Me	50	24	38	1	2	Nd
^{i}Pr$_2$O	Me	30	26	9	Nd	Nd	Nd
Ethyl acetate	H	30	0.75	75	99	33	11
Acetonitrile	H	30	0.75	54	96	82	39

[a] 2 equiv. of the acyl donor.

results of the experiments at 30 and 50°C. The reactions were carried out using 2 equiv. of vinyl acetate or isopropenyl acetate. Under these conditions, a strong influence of the organic solvent upon the enantioselectivity of the process was observed. It is apparent that acetonitrile was the best solvent for the CAL B catalysed reaction: after only 45 min, 54% conversion was achieved, with an E value of 39. Nevertheless, the use of the acyl donor vinyl acetate also as the reaction solvent is preferable to other organic solvents (see Table 1). Very low reaction rates and enantioselectivities were observed in other tested solvents.

Another common approach for the resolution of racemic alcohols is enzymatic hydrolysis. Taking into account that the best enzyme for the acylation is the CAL B lipase, we examined the hydrolysis of the acetyl derivative (±)-**2** catalyzed by this lipase under different conditions (Scheme 2).

CAL B induces a high reaction rate in the hydrolysis of the substrate in aqueous media (phosphate buffer, pH 7) and 41% conversion was obtained after only 30 min. However, the enantioselectivity of this process is relatively low (E = 10). When the reaction was carried out



Scheme 2.

in organic solvents with a small amount of water, only the reaction in water–saturated hexane shows a moderate reaction rate, 41% conversion was obtained after 7 h, but again the enantioselectivity of the process is low ($E=9$). When other organic solvents were used as reaction media for the enzymatic hydrolysis, very low conversions were obtained.

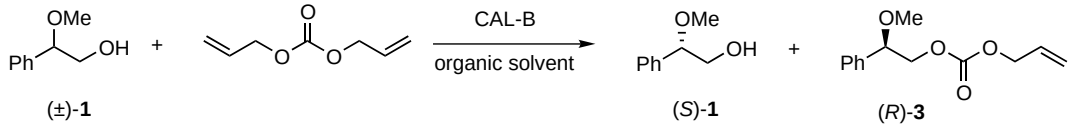
Enzymatic alkoxycarbonylation or the enzymatic hydrolysis of carbonates are also useful procedures for the resolution of racemic alcohols.[8] We first studied the influence of the organic solvent in the alkoxycarbonylation of 2-methoxy-2-phenylethanol, (±)-**1** with diallyl carbonate in the presence of CAL B (Scheme 3).

Table 4 summarizes the results of these experiments. The best enantiomeric ratio was obtained in THF ($E=30$), with a moderate reaction rate (20% conversion after 32 h). In order to improve the enantioselectivity and the reaction rate we carried out the process at different temperatures. At 15°C the reaction rate decreased considerably, but we did not observe an improvement in enantioselectivity. At 60°C the process is faster, but the enantiomeric ratio decreases ($E=10$). We also studied the effect of a small amount of triethyl amine in the reaction media, but the enantioselectivity of this process was lower than in the absence of the additive. Finally, the best biocatalytic conditions found for the alkoxycarbonylation with diallyl carbonate were applied to the process with different carbonates (dibenzyl carbonate, vinylacetoxime carbonate and phenyl *p*-nitrophenyl carbonate), but in all cases the reaction rates and enantiomeric ratios were very low.

As in the case of the acylation, we examined the CAL B-catalyzed hydrolysis of the carbonate derivative (±)-**3** (Scheme 4). The experiments were carried out in different organic solvents, and the results obtained are summarized in Table 5. The lipase induces a low to moderate reaction rate depending on the organic solvent used. Unfortunately, the enantioselectivity of these hydrolytic processes was low in all cases.

3. Conclusions

We have described the resolution of 2-methoxy-2-phenylethanol, (±)-**1** via a CAL B-catalyzed acylation and alkoxycarbonylation. High enantioselectivities can be achieved by an appropriate selection of the reaction parameters. In general, the best conditions for CAL B acylation involved the use of vinyl acetate as an acyl donor and also as the solvent. On the other hand,

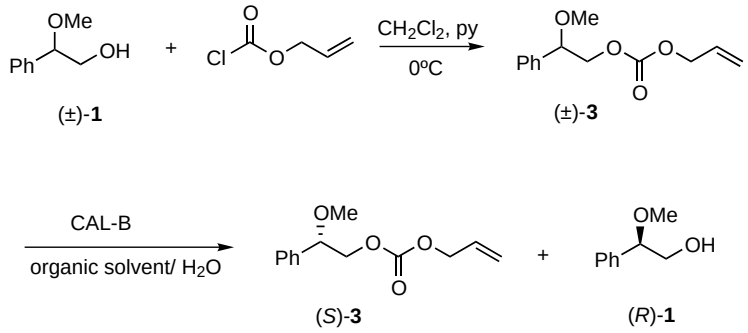


Scheme 3.

Table 4. CAL B-catalyzed alkoxycarbonylation of (±)-**1** in organic solvents[a]

Solvent	T (°C)	Time (h)	c (%)	Remaining alcohol **1**	Product **3**	E
				e.e.$_s$[b] (%)	e.e.$_p$[c] (%)	
1,4-Dioxane	30	24	32	38	83	15
^{t}BuOMe	30	45	41	7	10	1
Toluene	30	34	30	30	69	7
^{i}Pr$_2$O	30	48	67	Nd	Nd	Nd
Et$_2$O	30	96	47	9	10	1
Acetonitrile	30	96	42	50	68	9
CH_2CCl_2	30	96	25	29	86	17
THF	15	96	28	35	90	26
THF	30	32	20	23	93	30
THF	60	27	28	30	77	10
THF[d]	30	96	35	47	86	21

[a] 2 equiv. diallyl carbonate.
[b] Determined by HPLC.
[c] Determined by ^{1}H NMR in the presence of Eu(hfc)$_3$.
[d] 0.1 equiv. of Et$_3$N were added to the reaction media.



Scheme 4.

Table 5. CAL B-catalyzed hydrolysis of carbonate (±)-**3** in water–organic solvents, at 30°C[a]

Solvent	Time (days)	*c* (%)	Remaining carbonate **3**	Product **1**	*E*
			e.e.$_s$ (%)	e.e.$_p$ (%)	
Dioxane	4	34	41	81	14
Acetonitrile	7	13	13	85	15
THF	9	14	13	77	9
Hexane	3	39	47	71	10

[a] 10 equiv. H_2O.

alkoxycarbonylation catalyzed by CAL B afforded moderated enantioselectivities using diallyl carbonate as the alkoxycarbonylating agent in THF, at 30°C. The enzymatic acylation is preferable to the alkoxycarbonylation. Taking into account the simplicity and ease of scale-up in lipase-catalyzed reactions, the method has great potential applicability within the pharmaceutical industry.

4. Experimental

4.1. General

Enzymatic reactions were carried out in a Gallenkamp incubatory orbital shaker. Immobilized *C. anctarctica* lipase B (CAL B), Novozym 435, was a gift from Novo Nordisk. *Pseudomonas cepacia* lipase lyophilized (PSL) was purchased from Amano Pharmaceutical Co. Porcine pancreas lipase (PPL) is a product of Sigma. *C. antarctica* B (CALB-L2), *Candida rugosa* (CRL, L3 y L3A –purified–, *Candida antarctica* A (CALA-L5), *Pseudomonas* sp. (PSL-L6), porcine pancreas (PPL-L7), *Thermomyces lanuginosa* (TLL-L8), and *Mucor Miehei* (MML-L9) lipases were obtained from Roche Diagnostics.

Melting points were taken using a Gallenkamp apparatus and were uncorrected. Optical rotations were measured using a Perkin–Elmer 241 polarimeter and are quoted in units of 10^{-1} deg cm^2 g^{-1}. ^{1}H and ^{13}C NMR were obtained with TMS (tetramethylsilane) as internal standard using a Bruker AC-300 (^{1}H–300 MHz and ^{13}C–75.5 MHz) spectrometer. Mass spectra were recorded on a Hewlett–Packard 5987A and Finigan MAT/95 spectrometers. Microanalyses were performed on a Perkin–Elmer 240B elemental analyzer. All reagents were purchased from Aldrich Chemie. Solvents were distilled over an adequate desiccant and stored under nitrogen. Flash chromatography was performed using Merck silica gel 60 (230–400 mesh).

4.2. Lipase-catalyzed acetylation of (±)-2-methoxy-2-phenylethanol with vinyl acetate. General procedure

The reaction mixture contained alcohol (±)-**1** (0.985 mmol), vinyl acetate (1.971 mmol) and the lipase (amount indicated in Table 1) in the corresponding organic solvent (7 mL) (when vinyl acetate itself was also the solvent of the reaction, 49.25 mmol of this reactant were used). The mixture was shaken at 250 rpm in a rotary shaker. The progress of the reaction was monitored by TLC (hexane/ethyl acetate, 7:3). The enzyme was removed by filtration and washed with ethyl acetate. The solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/ethyl acetate, 4:1) to afford compound (*R*)-(−)-**2** and the corresponding enantiomer of the remaining substrate (*S*)-(+)-**1**. The purification procedure affords both compounds quantitatively, and the yields obtained are in accordance with the corresponding conversion shown in Tables 1–3.

4.2.1. (*R*)-(−)-2-Methoxy-2-phenylethyl acetate, (*R*)-2. Colorless oil; $[\alpha]_D^{25}$ −82 (c=1.0, $CHCl_3$), e.e. 95%; ^{1}H NMR ($CDCl_3$) δ (ppm): 2.08 (s, 3H, CH_3), 3.29 (s, 3H, CH_3), 4.16–4.2 (m, 2H, CH_2), 4.40–4.44 (m, 1H, CH), 7.34–7.35 (m, 5H, CH); ^{13}C NMR ($CDCl_3$) δ (ppm): 20.9 (CH_3), 56.9 (CH_3), 67.8 (CH_2), 81.46 (CH), 126.8 (CH), 128.2 (CH), 129.5 (CH), 137.7 (C), 170.8 (C=O); MS (EI) m/z 194 (M^+, <1), 77 (18), 91 (17), 105 (6), 121 (100), 122 (25), 134 (11). HRMS calcd for $C_9H_{10}NO$ [M^+–($OCOCH_3$)–H] 134.07309, found 134.0731.

4.3. Synthesis of (±)-2-methoxy-2-phenylethyl acetate, (±)-2

Acetic anhydride (11.82 mmol) was added under nitrogen to a 0°C solution of alcohol (±)-**1** (9.85 mmol) in 25 mL of CH_2Cl_2, pyridine (11.82 mmol) and a catalytic amount of dimethylaminopyridine. The resulting solution was stirred for 4 h. The resulting mixture was extracted with HCl (1N) and CH_2Cl_2. The organic layers were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel with hexane/ethyl acetate (4:1) to afford compound (±)-**2** in quantitative yield.

4.4. Lipase-catalyzed hydrolysis of (±)-2-methoxy-2-phenylethyl acetate

Two procedures could be used.

Procedure 1: A mixture of (±)-2-methoxy-2-phenylethyl acetate (0.77 mmol) and CAL B lipase (150 mg) suspended in phosphate buffer at pH 7 (0.05 M, 4 mL), was shaken at 250 rpm. The enzyme was removed by filtration and washed with water and CH_2Cl_2. The mixture was extracted with CH_2Cl_2 and the combined organic fractions were dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was purified by flash chromatography on silica gel with hexane/AcOEt (4:1).

Procedure 2: The reaction mixture contained (±)-2-methoxy-2-phenylethyl acetate (0.77 mmol), CAL B lipase (150 mg) in the corresponding water–saturated organic solvent (7 mL). The mixture was shaken at 30°C and 250 rpm in a rotary shaker. The progress of the reaction was monitored by TLC (hexane/ethyl acetate, 4:1). After removal of the enzyme by filtration, the filtrate was washed with CH_2Cl_2 and the solvents were evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (hexane/ethyl acetate, 4:1) to afford (*R*)-(−)-**1** and the corresponding enantiomer of the remaining substrate (*S*)-(+)-**2**.

4.5. Lipase-catalyzed alkoxycarbonylation of (±)-2-methoxy-2-phenylethanol. General procedure

The reaction mixture contained alcohol (±)-**1** (0.985 mmol), the corresponding carbonate (1.971 mmol) and the lipase in the chosen organic solvent (7 mL) (Table 4). The mixture was shaken at 250 rpm in a rotary shaker. The progress of the reaction was monitored by TLC using the solvent system hexane/ethyl acetate (7:3). The enzyme was removed by filtration and washed with ethyl acetate. The solvent was evaporated under reduced pressure and the crude residue was purified by flash chromatography on silica gel (hexane/ethyl acetate, 9.5:0.5) to afford compound (*R*)-(−)-**3** and the corresponding enantiomer of the remaining substrate (*S*)-(+)-**1**. The purification procedure affords both compounds quantitatively and the yields obtained are in accordance with the corresponding conversion showed in Table 4.

4.5.1. (*R*)-(−)-2-Methoxy-2-phenylethyl allyl carbonate, (*R*)-3. Colorless oil; $[\alpha]_D^{25}$ −63 ($c=1.0$, $CHCl_3$), e.e. 93%; ^{1}H NMR ($CDCl_3$) δ (ppm), J (Hz): 3.29 (s, 3H, CH_3), 4.16–4.32 (m, 2H, CH_2), 4.45–4.49 (m, 1H, CH), 4.62–4.65 (dd, 2H, CH_2, $^3J_{HH}=5.6$, $^3J_{HH}=1.3$), 5.25–5.39 (m, 2H, CH+CH), 5.87–6.00 (m, 1H, CH), 7.38–7.39 (m, 5H, CH); ^{13}C NMR ($CDCl_3$) δ (ppm): 56.9 (CH_3), 68.4 (CH_2), 70.9 (CH_2), 81.3 (CH), 118.7 (CH), 126.8 (CH), 128.3 (CH), 128.5 (CH), 131.4 (CH), 137.4 (C), 154.8 (C=O); MS (ESI$^+$) m/z (%): 259.1 [(M+Na)$^+$, 25].

4.6. Synthesis of (±)-2-methoxy-2-phenylethyl allyl carbonate, (±)-3

Allyl chloroformate (19.7 mmol) was slowly added to a solution of the alcohol (±)-**1** (9.85 mmol) and pyridine (19.7 mmol) in CH_2Cl_2 under nitrogen at 0°C. The solution was stirred for 5 h and extracted with 1N HCl and CH_2Cl_2. The organic fraction was dried over Na_2SO_4, filtered and evaporated under reduced pressure. The residue was subjected to flash chromatography on silica using hexane/AcOEt (4:1) to afford the racemic carbonate (±)-**3** (95% yield).

4.7. Lipase-catalyzed hydrolysis of (±)-2-methoxy-2-phenylethyl allyl carbonate, (±)-3

The reaction mixture contained the carbonate (±)-**3** (0.42 mmol), water (4.2 mmol) and the lipase (150 mg) in the corresponding organic solvent (7 mL). The mixture was shaken at 30°C and 250 rpm in a rotary shaker. The progress of the reaction was monitored by TLC (hexane/ethyl acetate, 9:1). After removal of the enzyme by filtration, the filtrate was washed with CH_2Cl_2 and the solvents evaporated under reduced pressure. The crude residue was purified by flash chromatography on silica gel (hexane/ethyl acetate, 9.5:0.5) to afford (*R*)-(−)-**1** and the corresponding enantiomer of the remaining substrate (*S*)-(+)-**3**.

4.8. Determination of the enantiomeric excess of (*R*)-1, (*S*)-1, (*R*)-2 and (*S*)-2

The enantiomeric excesses of (*R*)-**1**, (*S*)-**1**, (*R*)-**2** and (*S*)-**2** were determined by direct analysis on a chiral GC RtβDEXse column (30 m×0.25 mm; Restek). Column temperature: 95°C. Sample concentration 0.5 mg/mL. Two peaks (t_R 49.4 and 51.1 min) for (*R*)-**1** and (*S*)-**1** and two peaks (69.2 and 71 min) for (*R*)-**2** and (*S*)-**2**, respectively, were resolved.

4.9. Determination of the enantiomeric excess of (*R*)-3 and (*S*)-3

The enantiomeric excesses of (*R*)-**3** and (*S*)-**3** were determined by ^{1}H NMR analysis in the presence of the chiral shift reagent Eu(hfc)$_3$.

Acknowledgements

This work was supported by the Ministerio de Ciencia y Tecnología (FEDER project 1FD97-1255-C02-01). We express our appreciation to Novo Nordisk Co. for the generous gift of the lipase CAL B.

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