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Lipase-mediated kinetic resolution of allylic(hydroxymethyl)methylenecyclopentane building blocks

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Abstract

Enzymatic-mediated kinetic acylation of allylic(hydroxymethyl)methylenecyclopentane building blocks (±)-**1a**,**b** is reported using various commercially available lipases. Lipase Amano AK (*Pseudomonas* sp.) proved to be the best lipase in the case of (–)-**1b** which was obtained with a 96% enantiomeric excess. Single crystal X-ray diffraction analysis, using the (1*S*,4*R*)-camphanate derivative of (–)-**1b**, helped us to assign the *S* absolute configuration of (–)-**1b** and the enantiomeric specificity of the tested lipases. © 2000 Elsevier Science Ltd. All rights reserved.

1. Introduction

Allylic hydroxymethyl derivatives of the methylenecycloalkane moieties have emerged as highly attractive and versatile building blocks for the synthesis of several natural products.[1,2]

We have recently reported a new one-pot access to methylenecyclopentane and methylenecyclohexane core targets which made use of a controlled H-ene/protodesilylation sequence using two different Lewis acids and starting from readily available isocyclic allylsilanes and paraformaldehyde[3] (Fig. 1).

Enzymatic reactions are characterized by a number of advantageous properties for their preparative applications because of the mild reaction conditions and the selectivity of the enzymes.[4–9]

Being currently involved in the preparation of enantiomerically pure allylic (hydroxymethyl)-methylenecyclopentane building blocks,[10] we report here a study of the resolution of the racemic derivatives (±)-**1a**,**b** via an irreversible lipase-mediated acylation in organic media (Scheme 1).

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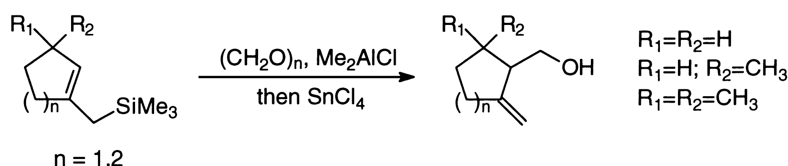
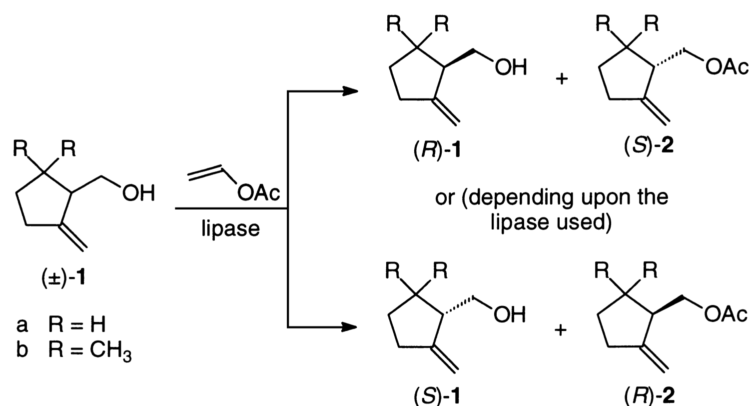


Figure 1.



Scheme 1.

2. Results and discussion

Screening experiments were made using six commercially available lipases. The resolution procedure is as follows: a mixture of (±)-**1a**,**b** (100 mg), lipase (100 mg) and vinyl acetate (5 mL) was stirred at 30°C. The reaction progress was monitored by removing aliquots from the supernatant and analyzing directly by both TLC and GC (see Experimental). The reaction was stopped at the conversion rate indicated in Tables 1 and 2.

Enantiomeric excess (ee) was determined by the ratio of the peak areas obtained by GC separation using a chiral phase (see Experimental). The enantiomers of both the remaining alcohol and the acetate produced gave rise to baseline-separated peaks, but they were partially overlapping each other in the crude product. Accordingly, the enantiomeric purity was determined after separation of the acetate and the unreacted alcohol by silica gel column chromatography.

In the case of alcohol **1a** (Scheme 1, R = H) the lipase-mediated acylation was always very fast but, except for PPL leading to 72% ee (Table 1, entry 3), the molecular recognition for the enantiomers of the substrate was low and the tested lipases proved to be poor enantioselective catalysts for the reaction. The results are summarized in Table 1.

Using the sterically hindered alcohol **1b** (Scheme 1, R = CH_3) and the same set of enzymes, the results were quite different. *Pseudomonas* species lipases were good biocatalysts for the reaction to occur (Table 2).

The use of lipase PS (from *Pseudomonas cepacia*, Amano PS) as a catalyst assured enantiospecific acylation with a good selectivity for the remaining alcohol (86% ee, entry 5). Moreover, when *Pseudomonas* sp. lipase (Amano AK) was used, it is especially worth noting that the

Table 1
Lipase screening performed on (±)-**1a**

Entry	Lipase	Reaction Time	Conversion %[a]	Remaining alcohol ee %[b]	Produced acetate ee %[b]
1	*Mucor Miehei*	1 h	40	36	53
2	*Candida Rugosa*	6 h	40	3	4
3	Porcine pancreatic	1 h	72	72	31
4	*Candida antartica*	1 h	100	–	0
5	Amano PS	1 h	68	14	31
6	Amano AK	1 h	100	–	0

[a] Measured by GC on a capillary column (CP-Wax-52).

[b] Measured by chiral GC (megadex DETTBSβ) after isolation by column chromatography.

Table 2
Lipase screening performed on (±)-**1b**

Entry	Lipase	Reaction Time	Conversion %[a]	Remaining alcohol		Produced acetate	
				ee %[b]	Abs. Conf.	ee %[b]	Abs. Conf.
1	*Mucor Miehei*	6 days	0	–	–	–	–
2	*Candida Rugosa*	1 h	75	0	–	0	–
3	Porcine pancreatic	29 h	52	14	*R*[c]	13	*S*
4	*Candida antartica*	28 h	65	35	*R*[c]	23	*S*
5	Amano PS	26 h	65	86	*S*[c]	51	*R*
6	Amano AK	7 h	62	96	*S*	62	*R*

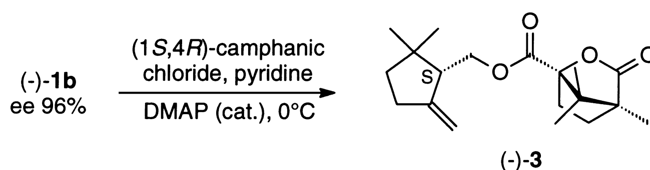
[a] Measured by GC on a capillary column (CP-Wax-52).

[b] Measured by chiral GC (megadex DETTBSβ) after isolation by column chromatography.

[c] Assigned by comparison with the t_R of the established (*S*)-**1b** (entry 6) using chiral GC chromatogram.

enantiomeric purity of the recovered alcohol was very high (96% ee, entry 6). Following from this, and in order to establish the enantiomeric specificity of the different lipases, the absolute configuration of this alcohol was determined. For all that, the crystalline ester (–)-**3** was synthesized using (1*S*,4*R*)-camphanic chloride[11,12] as a chiral auxiliary (Scheme 2) and submitted to X-ray crystallography.

The ORTEP[13] drawing of (–)-**3** is shown in Fig. 2. Because the absolute configuration of the camphanic moiety is (1*S*,4*R*), the absolute configuration of the starting alcohol (–)-**1b** at the stereogenic center was determined as *S*. This alcohol agrees with the second chiral GC peak. With this result in hand, assigning the enantiomeric specificity of each lipase has been possible, based on the retention time in the chiral GC chromatogram of the remaining alcohols (Table 2).



Scheme 2.

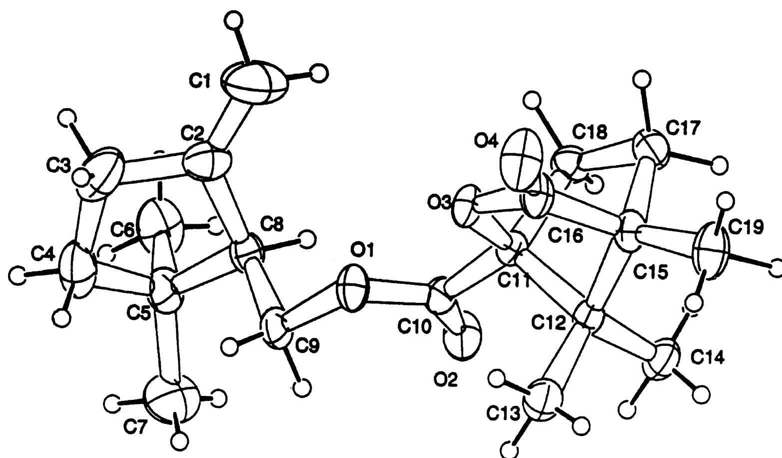


Figure 2. ORTEP of the (1*S*,4*R*)-camphanate derivative (–)-**3**

3. Experimental

^{1}H and ^{13}C NMR spectra were recorded in $CDCl_3$ on a Bruker AM-200 spectrometer. Infrared spectra were obtained as a film using a Perkin–Elmer 257 infrared spectrometer. Routine monotoring of reactions was performed using Merck 60F 254 silica gel, aluminum-supported TLC plates. Column chromatographies were performed using silica gel 60 (230–400 mesh) and petroleum ether/ether (gradients) as eluent. Conversion rate of the resolution experiments was measured by GC analysis using a WCOT fused silica column (25 m×0.32 mm i.d.; CP-Wax-52 CB stationary phase; N_2 carrier gas: 50 kPa). Enantiomeric excess determinations were carried out using a MEGADEX DETTBSβ fused silica column (25 m×0.32 mm i.d.; N_2 carrier gas: 70 kPa). Optical rotations were measured on a Perkin–Elmer 341 polarimeter. Melting points are uncorrected. Microanalyses were performed on a Technicon CHN analyzer at our University. Porcine pancreatic lipase (PPL, type II) and *Candida rugosa* lipase (CRL, type VII) were purchased from Sigma. *Mucor miehei* lipase and *Candida antartica* lipase were from Novo Nordisk A/S. *Pseudomonas* sp. lipase (Amano AK) and *Pseudomonas cepacia* lipase (Amano PS) were from Amano Pharmaceuticals.

3.1. *General procedure for lipase screening acylation of (±)-**1a**,**b***

To a solution of (±)-**1a**,**b** (100 mg) in vinyl acetate (5 mL) was added the lipase (100 mg). The mixture was stirred magnetically in a hermetically stoppered one-neck flask. The course of the reaction was monitored by TLC and GC. After the period indicated in Tables 1 and 2, the reaction

mass was filtered through a thin pad of Celite, and the cake was washed with dry Et_2O. The filtrate was concentrated in vacuo to give an oil. After a capillary GC analysis, the oil was chromatographed on a silica gel column. The first eluted fractions provided the acetate derivatives **2a**,**b** and the last eluted fractions afforded the unreacted starting alcohols **1a**,**b** to be analyzed on the chiral GC column. Detailed results of the lipase-mediated acylations are reported in Tables 1 and 2.

2-Methylidene-cyclopentanemethyl acetate **2a**: IR (neat): ν 3030, 1730, 1650, 1040, 890 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 4.78 (s, 1H), 4.67 (s, 1H), 4.10 (d, J=5.2 Hz, 2H), 2.35–2.13 (m, 3H), 2.03 (s, 3H), 1.75–1.44 (m, 4H). Anal. calcd for $C_9H_{14}O_2$: C, 70.10; H, 9.15. Found: C, 69.92; H, 9.13.

2,2-Dimethyl-5-methylidene-cyclopentanemethyl acetate **2b**: IR (neat): ν 3020, 1735, 1650, 1040, 890 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 4.87 (m, 2H), 4.10 (m, 2H), 2.33 (m, 3H), 2.02 (s, 3H), 1.46 (t, J=7.3 Hz, 2H), 1.06 (s, 3H), 0.79 (s, 3H). Anal. calcd for $C_{11}H_{18}O_2$: C, 72.49; H, 9.95. Found: C, 72.05; H, 9.91.

3.2. *(–)-(1S)-2,2-Dimethyl-5-methylidene-cyclopentane methanol (–)-**1b***

To a solution of (±)-**1b** (1.3 g, 9.27 mmol) in vinyl acetate (25 mL) was added 700 mg of *Pseudomonas* sp. lipase (Amano AK). The mixture was stoppered under argon atmosphere and stirred magnetically. The course of the reaction was monitored by GC. After 12 h (61% conversion rate), the reaction mixture was filtered through a thin pad of Celite, the cake on the filter was washed with dry ether (40 mL) and the filtrate was concentrated in vacuo. The crude residue was subjected to silica gel column chromatography to afford 996 mg of acetate (+)-**2b** (yield 59%, ee 61%) and 481 mg of unreacted (–)-**1b** (yield 37%, ee 96%).

Compound (–)-**1b**: $[\alpha]_D^{25}$=–1.7 (c 1.0, $CHCl_3$); IR (neat): ν 3350, 3070, 1660, 1040, 890 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 4.95 (m, 2H), 3.71 and 3.52 (ABX, J=–10.7, 7.7, 5.7 Hz, 2H), 2.36 (t, J=7.6 Hz, 2H), 2.12 (t, J=6.5 Hz, 1H), 1.58–1.40 (m, 2H), 1.02 (s, 3H), 0.87 (s, 3H). Anal. calcd for $C_9H_{16}O$: C, 77.09; H, 11.50. Found: C, 77.05; H, 11.58.

3.3. *Reaction of (–)-**1b** with (–)-(1S,4R)-camphanic chloride*

To a solution of (–)-**1b** (420 mg, 3.0 mmol) and DMAP (37 mg, 0.3 mmol) in pyridine (15 mL) at 0°C under argon atmosphere was added (–)-(1*S*,4*R*)-camphanic chloride (650 mg, 3.0 mmol) in small portions over a period of 10 min. The cooling bath was removed and the solution was stirred at room temperature. The reaction was monitored by TLC and was complete within 1 h. The mixture was diluted with CH_2Cl_2 (50 mL) and was sequentially washed with water (3×25 mL), 2N HCl (10 mL portions) until pH 2 (paper indicator), 1 M $NaHCO_3$ (20 mL), saturated $CuSO_4\cdot5H_2O$ (2×10 mL) and brine (2×20 mL). The organic layer was dried over $MgSO_4$, filtered and concentrated in vacuo. The crude mixture was subjected to column chromatography and afforded the camphanate derivative (–)-**3** (893 mg, 93%). Crystallization from *n*-hexane afforded white crystals.

Compound (–)-**3**: $[\alpha]_D^{25}$=–8.6 (c 1.0, $CHCl_3$); mp=51–52°C; IR (neat): ν 3067, 1787, 1752, 1722, 1657, 1468, 1268, 1167, 1097, 1061, 931, 890 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 4.90 (m, 2H), 4.27 (d, J=6.6 Hz, 2H), 2.50–2.26 (m, 4H), 2.09–1.80 (m, 2H), 1.66 (m, 1H), 1.49 (t, J=7.9 Hz, 2 H), 1.09 (s, 6H), 1.03 (s, 3H), 0.94 (s, 3H), 0.82 (s, 3H). Anal. calcd for $C_{19}H_{28}O_4$: C, 71.22; H, 8.81. Found: C, 71.33; H, 8.84.

Acknowledgements

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