Pergamon

Tetrahedron: Asymmetry Vol. 6, No. 2, pp. 519-524, 1995

0957-4166/95 $9.50+0.00

0957-4166(95)00035-6

Desymmetrization of *cis*-1,2-Dihydroxycycloalkanes by Stereoselective Lipase Mediated Esterification

Giovanni Nicolosi ,* Angela Patti, Mario Piattelli and Claudia Sanfilippo

Istituto del CNR per lo Studio delle Sostanze Naturali di Interesse Alimentare e Chimico-farmaceutico

Via dei Santuario 110, 95028 Valverde CT, Italy

Abstract: *Meso*-compounds 1,2-dihydroxycyclopentane (**1**), 1,2-dihydroxycyclohexane (**2**) and 1,2-dihydroxycycloheptane (**3**) were desymmetrised by enantiotoposelective esterification with vinyl acetate in *tert*-butyl methyl ether catalysed by lipases from *Pseudomonas cepacia*, *Candida antarctica* (Novozym 435®) and *Mucor miehei* (Lipozyme TM®). Both of the lipases from *P. cepacia* and *M. miehei* afforded the (*1S,2R*)-acetate (+)-**1a** and (*1R,2S*)-acetates (–)-**2a** and (+)-**3a** in good enantiomeric excesses and chemical yield.

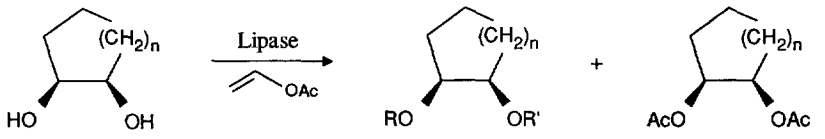
INTRODUCTION

An enantiomerically pure compound can be obtained from the corresponding *meso*-form by a transformation capable of discriminating between enantiotopic groups linked to centres of opposite configuration within the same molecule. With this methodology the yield of the wanted enantiomer can reach 100%, at least in principle, as opposed to the resolution methods whose maximum yield is 50%. The ability of enzymes to catalyse enantiotoposelective reactions has been exploited in the asymmetrization of *meso*-compounds.[1a-d] For instance, lipases have been used successfully in the case of primary or secondary *meso*-diols.[2a-o] Among these molecules, 1,2-dihydroxycycloalkanes deserve particular attention owing to their possible use as chiral auxiliaries for asymmetric conjugate addition[3] or as chiral bifunctional building blocks. Crout *et al.*[4] have reported that, while (±)-*trans*-1,2-diacetoxycycloalkanes are hydrolysed in the presence of porcine liver esterase to give the corresponding monoacetates with moderate to good enantiomeric excesses, the *cis*-isomers in the same conditions are converted exclusively into the monoacetates, however racemic, and a rapid acyl migration during work-up has been suggested to explain this result. Subsequent work by Xie *et al.*[5,6] has shown that hydrolysis of cyclic acetates catalysed by *Pseudomonas fluorescens* lipase occurs with high enantioselectivity in the case of five-membered diacetates, while less satisfying e.e. values are obtained with six- and seven-membered ring diacetates (70 and 2%, respectively).

In light of this we decided to explore, as a part of an investigation on the biocatalysed desymmetrization of *meso*-diols,[7a,b] the possibility to obtain optically active monoacetates of the cyclic *cis*-compounds **1-3** following the more direct route of the transesterification of the free diols using vinyl acetate as acyl donor.

RESULTS AND DISCUSSION

A preliminary screening of six lipases [*Candida antarctica* lipase (Novozym 435®), *C. cylindracea* lipase (CCL), *Mucor miehei* lipase (Lipozyme TM®), *Pseudomonas cepacia* lipase (PSL) *Rhizopus javanicus* lipase and porcine pancreas lipase (PPL)[8]] revealed that PSL, Novozym 435® and Lipozyme TM® were the best candidates in terms of reaction speed and optical purity of the product.



n=1	**1**	R=Ac R'=H	(1*S*,2*R*)-**1a**	**1b**
n=2	**2**	R=H R'=Ac	(1*R*,2*S*)-**2a**	**2b**
n=3	**3**	R=H R'=Ac	(1*R*,2*S*)-**3a**	**3b**

Reagents and conditions: Substrate 50mM, solvent *tert*-butylmethyl ether, enzyme 10 mg/mL, vinyl acetate (5 eqv.), 45 °C, 300 rpm, *Pseudomonas cepacia* lipase for **1** and **3**; Lipozyme TM® for **2**.

When *cis*-1,2-dihydroxycyclopentane **1** was esterified in the presence of PSL, after a reaction period of 0.5 h the (1*S*,2*R*)-monoester **1a** was obtained with 82% chemical yield and 79% e.e (Table 1, entry 1). An increase of the reaction time to 8 h resulted, due to kinetic amplification, in an improvement of the e.e. (88%), however at the expense of the chemical yield (73%, entry 2). Comparable results were obtained with Lipozyme TM®, that after 5 h gave **1a** of good e.e. (81%, entry 5) with acceptable chemical yield (75%). Less satisfying results were obtained with the use of Novozym 435® (entry 3) that showed the same stereopreference as the other two enzymes.

When *cis*-1,2-dihydroxycyclohexane **2** was the substrate, acetylation catalysed by Lipozyme TM® afforded, after 7 h, (1*R*,2*S*)-monoester **2a** with high e.e. (95%, entry 9). This value was further enhanced and homochiral monoacetate (e.e. >98) was formed after 12h, along with a moderate amount of diester **2b** (6%, entry 10). PSL also gave a monoester of good e.e. (87%, entry 6). In this case, however, kinetic amplification was not effective due to a lack of diester (**2b**) formation, so an extension of the reaction time did not modify its value. The use of Novozym 435® resulted in the formation of **2a** with decidedly lower e.e. The three enzymes PSL, Novozym 435® and Lipozyme TM®, all showed the same *R* stereopreference, which is therefore opposite to that found for the five-membered-ring substrate.

Enantiotoposelectivity was definitely poorer in the transesterification of *cis*-1,2-dihydroxycycloheptane **3**. The best results in terms of enantiomeric excess were obtained with PSL, that yielded monoester **3a** with an e.e. value of ca. 70%, not liable to remarkable improvement by the use of kinetic amplification (entries 11 and 12). The results with Lipozyme TM® and Novozym 435® were even less encouraging (entries 13 and 14). In any case, all three enzymes gave a monoester of the same positive sign of optical rotation indicating, according to the literature, the (*1R*,*2S*)-configuration.

Table 1. Transesterification of 1,2-cycloalkanediols **1-3** mediated by lipases in *tert*-butyl-metyl ether [a]

Entry	Sub-strate	Enzyme	Time (h)	%Diol[b]	% Mono-ester[b]	ee[c]	Stereopre-ference	% Diester[b]
1	**1**	PSL	0.5	16	82	*79*	1*S*,2*R*	2
2			8		73	*88*		27
3		Novozym 435®	3	-	86	*55*	1*S*,2*R*	14
4		Lipozyme TM®	0.5	45	55	*60*	1*S*,2*R*	-
5			5		75	*81*		25
6	**2**	PSL	1.5	6	94	*87*	*1R,2S*	-
7			7	-	100	*88*		-
8		Novozym 435®	5	-	95	*40*	*1R,2S*	5
9		Lipozyme TM®	7	7	90	*95*	*1R,2S*	3
10			12	-	94	>98		6
11	**3**	PSL	6	9	91	*68*	*1R,2S*	-
12			24	-	85	*80*		15
13		Novozym 435®	6	-	82	*28*	*1R,2S*	18
14		Lipozyme TM®	24	9	59	*60*	*1R,2S*	32

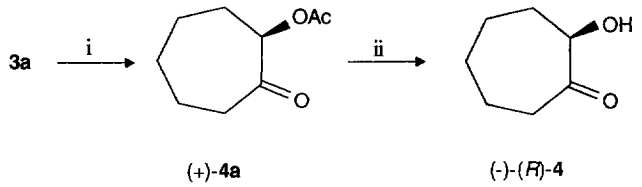
[a]Substrate 50 mM, enzyme 10 mg/mL, vinyl acetate (5 eqv.), 45 °C, 300 rpm.

[b]Determined by GC or by ^{1}H NMR analysis of the mixture.

[c]Determined by GC on chiral phase (entries 6-10) or by ^{1}H NMR analysis of the mixture in the presence of Eu(hfc)$_3$ (entries 1-5 and 11-14).

However, taking into account the low value of the specific optical rotation and the extremely low e.e. (2%) of the reported sample,[6] we considered a confirmation necessary. To this end monoester **3a,** obtained from **3** by transesterification catalysed by PSL, was oxidised with CrO_3/Py to give ketoester (+)-**4a** that was subjected to alcoholysis catalysed by PSL thus affording the known hydroxyketone (–)-(*R*)-2-hydroxycycloheptanone (**4**) (see scheme 1).

The data above show that enantiotoposelectivity in the asymmetrization of the *cis*-dihydroxycycloalkanes **1-3** *via* enzymatic transesterification catalysed by Lipozyme TM®, Novozym 435® and PSL is a function of both dimension of the cycle and enzyme. In brief, the six-membered-ring diol is the most efficiently asymmetrized to give a monoacetate of high e.e. with PSL and homochiral with Lipozyme TM®. Enantiotoposelectivity is a little less marked in the case of the five-membered-ring compound, but in this case also it is possible to obtain a monoacetate with very good e.e. using PSL or Lipozyme TM® in conditions of kinetic amplification.



(+)-**4a** (-)-(*R*)-**4**

Scheme 1. i) CrO_3/Py; ii) *Pseudomonas cepacia* lipase, *n*-butanol

The seven-membered-ring diol **3** gave the less satisfying results and in none of the conditions tested did the e.e. exceed 80%. The inversion of configuration passing from the cyclopentane to the cyclohexane and cycloheptane derivatives is remarkable. Analogous inversion of stereopreference as a function of ring dimension has previously been observed in the partial hydrolysis of cycloalkane-1,2-diacetates in the presence of *P. fluorescens* lipase[6] or porcine liver esterase,[4] and it has been suggested that it may be attributable to acetyl-migration catalysed by the enzyme. This explanation seems inapplicable to the case in hand, since racemic monoacetate **1a** after treatment with PSL in conditions comparable to those used in the enzymatic acylation was recovered chemically and optically unaltered. Finally, it is to be observed that monoacetates **1a-3a** suffer an appreciable decrease in enantiomeric excess following work-up of the reaction mixture. Therefore when they are needed for successive transformations, at least in the cases in which the chemical purity is high (as for instance in entry 10), it is advisable to use the crude material and purify the final product.

EXPERIMENTAL SECTION

^1H and ^{13}C NMR spectra were recorded in $CDCl_3$ at 250.13 and 62.9 MHz respectively on a Bruker AC 250 instrument. Chemical shifts are in ppm (δ) downfield from TMS and coupling constants (*J*) are in Hz. Europium (III) tris[3-(heptafluoropropyl)hydroxymethylene]-(+)-camphorate [Eu(hfc)$_3$] was used as chiral shift reagent to determine enantiomer ratio of **1a** and **3a**. Optical rotations were measured in $CHCl_3$ solutions on a DIP 135 JASCO instrument. GC analyses were carried out using HP-5 (5% phenylmethylsilicone) or Chiraldex G-DA (dialkyl γ-cyclodextrin) capillary column.

Commercially available *cis*-1,2-dihydroxycyclopentane **1** and *cis*-1,2-dihydroxycyclohexane **2** were used as received. *cis*-1,2-Dihydroxycycloheptane **3** was obtained from cycloheptene by treatment with silver acetate/acetic acid according to Brutcher and Evans[9]. Vinyl acetate was distilled prior to use. Novozym 435® (immobilized lipase from *Candida antarctica*) and Lipozyme TM® (immobilized lipase from *Mucor miehei*) are registered marks from Novo Nordisk. Crude lipases from *Pseudomonas cepacia* (PSL) and *Rhizopus javanicus* were obtained from Amano. Lipases from *Candida cilyndracea* and from porcine pancreas were purchased from Sigma. Column chromatography was performed on Silica gel; analytical TLC was performed on Merck silica gel 60-F_{254} precoated glass plates and compounds were visualized by spraying with molybdophosphoric acid.

Standard procedure for lipase mediated esterification of diols 1- 3

Enzyme (10 mg/mL) and vinyl acetate (30 mL/mL) were added to a 50 mM solution of diol in *tert*-butylmethyl ether. The suspension was stirred at 300 rpm and 45 °C and the reaction monitored by GC. At the

given time the reaction was quenched filtering off the enzyme and the solution was taken to dryness. Enantiomeric excess of the monoacetate was determined by GC on chiral phase or by ^{1}H NMR analysis of the mixture in the presence of Eu(hfc)$_3$.

Enzymatic preparation of 1a

A suspension of **1** (100 mg, 0.98 mmol), vinyl acetate (0.40 mL, 5.0 mmol) and PSL (800 mg) in *tert*-butylmethyl ether (20 mL) was shaken (300 rpm) at 45 °C for 3.5 h. After filtration of the enzyme, the solvent was evaporated under reduced pressure and the residue chromatographed to give **1a.**

(+)-(1S,2R)-1-Acetoxy-2-hydroxycyclopentane (**1a**): yield 112 mg (80%); e.e. 73% ; $[\alpha]_D$ +3.09 (c = 5.5).^{1}H NMR δ = 1.50-1.90 (m, 6H), 2.09 (s, 3H, -OCOCH$_3$), 4.17 (m, 1H, -C*H*OH), 4.97 (m, 1H, -C*H*OCOCH$_3$). ^{13}C NMR δ = 19.36 (OCO*C*H$_3$), 21.06 (C-4), 28.02 (C-3), 30.72 (C-5), 72.98 (C-2), 76.80 (C-1), 170.90 (O*C*OCH$_3$). Anal. Calcd for $C_7H_{12}O_3$: C 58.32, H 8.33; found C 58.10, H 8.30.

Enzymatic preparation of 2a

A suspension of **2** (100 mg, 0.86 mmol), vinyl acetate (0.36 mL, 4.5 mmol) and Lipozyme TM® (720 mg) in *tert*-butylmethyl ether (18 mL) was shaken at 300 rpm at 45 °C for 7 h. The reaction was quenched filtering off the enzyme, the solvent evaporated under reduced pressure and the residue purified by chromatography to give **2a.**

(−)-(1R,2S)-1-Acetoxy-2-hydroxycyclohexane (**2a**) : yield 130 mg (95 %); e.e. 70% ; $[\alpha]_D$ −1.9 (c = 1.76). ^{1}H NMR δ = 1.36 (m, 2H), 1.68-1.85 (m, 6H) 2.09 (s, 3H, -OCOCH$_3$), 3.88 (m, 1H, -C*H*OH), 4.91 (ddd, 1H, J= 8.0, 3.2 and 3.0 Hz, -C*H*OCOCH$_3$). ^{13}C NMR δ =20.94, 21.11 (OCO*C*H$_3$), 21.83, 26.80, 30.13, 68.92 (C-2), 74.04 (C-1), 170.72 (O*C*OCH$_3$). Anal. Calcd for $C_8H_{14}O_3$: C 60.74, H 8.86; found C 60.61, H 8.89.

Enzymatic preparation of 3a

A suspension of **3** (100 mg, 0.77 mmol), vinyl acetate (0.32 mL, 4.0 mmol) and PSL (650 mg) in *tert*-butylmethyl ether (16 mL) was shaken (300 rpm) at 45 °C for 3 h. The reaction was quenched filtering off the enzyme, the solvent evaporated under reduced pressure and the residue chromatografed to afford **3a.**

(+)-(1R,2S)-1-Acetoxy-2-hydroxycycloheptane (**3a**): yield 120 mg (91%); e.e. 65% ; $[\alpha]_D$ + 7.9 (c = 6). ^{1}H NMR δ = 1.45-1.62 (m, 4H), 1.65-1.78 (m, 4H), 1.94 (m, 2H), 2.08 (s, 3H, OCOCH$_3$), 3.97 (m, 1H, -C*H*OH), 4.94 (ddd, 1H, J= 8.9, 3.5 and 2.7 Hz, -C*H*OCOCH$_3$). ^{13}C NMR δ = 21.16 (OCO*C*H$_3$), 21.66, 22.56, 26.91, 27.51 31.42, 72.09 (C-2), 77.70 (C-1), 170.70 (O*C*OCH$_3$). Anal. Calcd for $C_9H_{16}O_3$: C 62.77, H 9.30; found C 62.87, H 9.31.

Oxidation of (+)-3a to obtain (+)-4a

Crude reaction mixture (38 mg) of enzymatic acylation of **3** [containing 95% of (+)-**3a**, 75% e.e.and 5% of **3b**] was added to a solution of CH_2Cl_2 (5 mL) containing pyridine (500 mL) and CrO_3 (350 mg) and the resulting mixture stirred at r.t. for 2h. After addition of aq. HCl, the organic layer was extracted and evaporated to afford, after purification by chromatography, (+)-**4a.**

(+)-(R)-2-Acetoxycycloheptanone (**4a**): yield 30 mg (87%); e.e. 75%; $[\alpha]_D$ + 12.3 (c = 1.1). NMR data were identical with those reported for (±)-**4a**.[10]

Alcoholysis of (+)-4a to obtain (−)-4

A suspension of (+)-**4a** (100 mg, 0.58 mmol) and PSL (200 mg) in *tert*-butylmethyl ether (10 mL) containing *n*-butanol (1 mL) was shaken (300 rpm) at 45 °C for 24 h. The reaction mixture was filtered and the solvent evaporated under reduced pressure to afford (-)-**4.**

(–)-(R)-2-Hydroxycycloheptanone (**4**): yield 70 mg (95%); e.e. 75% $[\alpha]_D$ –57.6 (*c* = 2.5) ^{1}H NMR δ = 1.21-1.42 (m, 2H), 1.56-1.88 (m, 5H), 2.00-2.13 (m, 1H), 2.46 (ddd, 1H, J=17.3, 10.9 and 3.5 Hz), 2.66-2.75 (m, 1H), 4.30 (m, 1H). ^{13}C NMR = 23.49, 26.65, 29.56, 33.82, 40.10, 77.07 (-CHOH), 213.81 (-CO-).

Investigation on internal transacylation of (±)-1a

Monoacetate (±)-**1a** (20 mg, 0.14 mmol), prepared by conventional chemical acetylation of **1**, was dissolved in *tert*-butylmethyl ether (3 mL) and shaken (300 rpm) at 45 °C in the presence of PSL lipase (30 mg). After 1 h the reaction was stopped filtering off the enzyme, the solvent evaporated under reduced pressure and the residue analysed by ^{1}H-NMR using a chiral shift reagent. No modification in the enantiomer ratio of **1a** was observed.

Acknowledgements. Support for this research was provided by the Italian National Council of Research (CNR - Roma) under the scheme "Tecnologie Chimiche Innovative". Authors are grateful to Amano International Enzyme Co and Novo Nordisk Co for the kind supply of lipases.

REFERENCES AND NOTES

1. a) Davies, H.G.; Green, R. H.; Kelly, D.R.; Robert, S. M. In *Biotransformations in Preparative Organic Chemistry*. Academic Press Ed.; New York, 1989. b) Faber, K. In *Biotransformations in Organic Chemistry*. Springer Verlag Ed.; New York, 1992. c) Zhu, L.-M.; Tedford, M.C. *Tetrahedron* **1990**, *46*, 6587. d) Mori, K.; Amaike, M.; Oliver, J.E. *Liebgs Ann. Chem.* **1992**, 1185.
2. a) Johnson, C.R.; Golebiowski, A.; McGill, T. K.; Steensma, D. H. *Tetrahedron Lett.* **1991**, *32*, 2597. b) Harris, J. K.; Gu, Q.-M.; Shih, Y.-R.; Girdaukas, G.; Sih, C. H. *Tetrahedron Lett.* **1991**, *32*, 3944. c) Burgess, K.; Henderson, I. *Tetrahedron Lett.* **1991**, *32*, 5701. d) Santaniello, E.; Ferraboschi, P.; Grisenti, P.; Manzocchi, A. *Chem. Rev.* **1992**, *92*, 1071 and refs. therein. e) Faber, K.; Riva, S. *Synthesis* **1992**, *895* and refs. therein. f) Murata, M.; Uchida, H.; Achiwa, K. *Chem. Pharm. Bull.* **1992**, *40*, 2610. g) Gais, H.-J.; Hemmerle, H.; Kossek, S. *Synthesis*, **1992**, 169. h) Bonini, C.; Racioppi, R.; Righi, G.; Viggiani, L. *J. Org. Chem.* **1993**, *58*, 802. i) Bis, S.J.; Whitaker, D.T.; Johnson, C.R. *Tetrahedron: Asymmetry*, **1993**, 4, 875. l) Naemura, K.; Fukuda, R.; Takahashi, N.; Konishi, M.; Hirose, Y.; Tobe, Y. *Tetrahedron: Asymmetry*, **1993**, *4*, 911. m) Xie, Z.-F.; Suemune, H.; Sakai, K. *Tetrahedron: Asymmetry*, **1993**, *4*, 973. n) Tanaka, M.; Yoshioka, M.; Sakai, K. *Tetrahedron: Asymmetry*, **1993**, *4*, 981. o) Guanti, G.; Banfi, L.; Riva, R. *Tetrahedron: Asymmetry*, **1994**, *5*, 9.
3. Suemune, H., Watanabe, K.; Kato, K., Sakai, K. *Tetrahedron: Asymmetry* **1993**, *4*, 1767.
4. Crout, D.H.G.; Gaudet, V.S.B.; Laumen, K. Schneider, M.P. *J. Chem. Soc., Chem. Commun.* **1986**, 808.
5. Xie, Z-F.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.* **1987**, 838.
6. Xie, Z-F.; Nakamura, I.; Suemune, H.; Sakai, K. *J. Chem. Soc., Chem. Commun.* **1988**, 966.
7. a) Nicolosi, G.; Morrone, R.; Patti, A.; Piattelli, M. *Tetrahedron: Asymmetry* **1992**, *3*, 753. b) Nicolosi, G.; Patti, A.; Piattelli , M.; Sanfilippo, C. *Tetrahedron: Asymmetry*, **1994**, *5*, 283.
8. Esterification of **2** mediated by immobilized PPL to afford (-)-**2a** with 84%ee has been reported by H. Hemmerle and H.-J. Gais., *Tetrahedron Lett.* **1987**, *28*, 3471.
9. Brutcher, F.; Evans III, G. *J. Am. Chem. Soc.* **1957**, *23*, 618.
10. Rubottom, G. M.; Mott, R. C.; Juve, H. D. *J. Org. Chem.*, **1981**, *46*, 2717.

(*Received in UK* 22 *November* 1994; *accepted* 5 *January* 1995)