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Enzymatic Asymmetrization of *meso*-2-Cycloalken-1,4-diols and Their Diacetates in Organic and Aqueous Media

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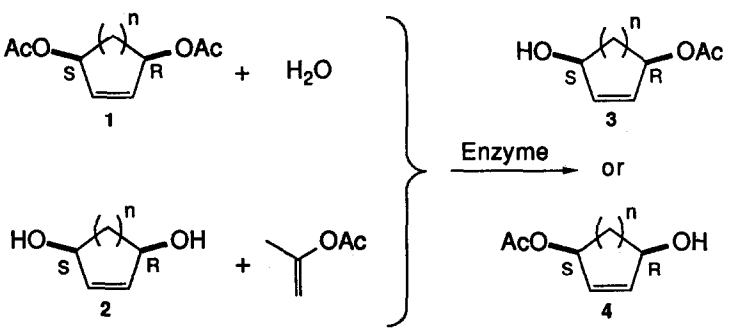
Abstract: *meso*-2-Cycloalken-1,4-diols or the corresponding diacetates with five-, six-, and seven-membered rings were subjected to enzymatic asymmetrizations utilizing a recombinant version of lipase B from *Candida antarctica* (Novo SP-435) in organic or aqueous media.

The synthetic potential of enzymes is fully realized in the asymmetrization of prochiral or *meso* substrates which can be completely processed to a single enantiomer. This laboratory has utilized enzymes to synthesize various enantiomerically pure five-, six-, and seven-membered ring compounds derived from *meso*-diols or the corresponding diacetates.[1-3] A survey of the literature reveals that many enzymes have been studied in the enantioselective hydrolysis of five-, six-, and seven-membered *meso*-2-cycloalken-1,4-diols (Table 1). The five-membered diacetate **1a** has received the most attention and has been successfully hydrolyzed in moderate to good yields and enantioselectivities by many enzymes including α-chymotrypsin (α-CT), pig liver esterase (PLE), *Candida rugosa* lipase (CRL), *Saccharomyces cerevisiae* (baker's yeast), and lipase from *Rhizopus sp.* (RSL).[4,5] It has also been shown that electric eel acetylcholinesterase (EEACE),[7a] porcine pancreatic lipase (PPL),[7b] and *Pseudomonas cepacia* lipase (PCL)[5,8] can provide monoacetate **3a** in high enantiomeric purity.

Scheme 1



The enantioselective hydrolysis of the six-membered diacetate **1b** has not been as successful as the five-membered ring analogue despite numerous efforts. The monoacetate **3b** was produced in moderate yields and with enantiomeric excesses of 72-79% using PCL.[6,9] A recombinant cutinase from *Fusarium solani pisi* (PGL) has provided **4b** in 82%ee (38% yield).[10] Enantioselective hydrolyses with other enzymes including CRL and PLE produced monoacetates with low enantiomeric excesses. The seven-membered ring diacetate **1c** has received very little attention compared to the aforementioned systems. Enantiomerically pure monoacetate **4c** was produced in 39% yield by hydrolysis of diacetate **1c** with EEACE; hydrolysis with CCL produced monoacetate **4c** in 40% yield with low enantioselectivity.[11] This laboratory has shown that monoacetate **4c** can be produced in 80% chemical yield and 35% enantiomeric excess by the hydrolysis of **1c** with PCL.[8]

Table 1. Survey of recent literature on enzymatic hydrolyses of *meso*-diacetates **1a-c.**

Enzyme	Substrate	Product	Chemical Yield	% ee	Ref.
α-CT	**1a**	**4a**	73%	42	4
Baker's yeast	**1a**	**4a**	87%	74	4
PLE	**1a**	**4a**	86%	86	4
CRL	**1a**	**3a**	82%	50	4
RSL	**1a**	**3a**	83%	66	4
EEACE	**1a**	**3a**	94%	96	7a
PCL	**1a**	**3a**	90%	98	8
PPL	**1a**	**3a**	87%	92	7b
CAL-B	**1a**	**3a**	90%	>99	this work
PLE	**1b**	**4b**	59%	49	9
PLE	**1b**	**4b**	82%	53	10
SAM-II	**1b**	**3b**	67%	64	10
PGL	**1b**	**4b**	38%	82	10
PLE	**1b**	**4b**	-	62	6
PCL	**1b**	**3b**	64%	79	9
PCL	**1b**	**3b**	-	72	6
CRL	**1b**	**3b**	-	41	6
EEACE	**1b**	**3b** + **4b**	-	0	6,9
CAL-B	**1b**	**4b**	91%	50	this work
CRL	**1c**	**4c**	40%	44	10
EEACE	**1c**	**4c**	39%	>99	10
PCL	**1c**	**4c**	80%	35	8
CAL-B	**1c**	**4c**	92%	>99	this work

This report focuses on the use of *Candida antarctica* lipase B (CAL-B) supplied as an acrylic supported biocatalyst (Novo SP-435)[12] using enzyme produced by a host organism, *Aspergillus oryzae*, from the genetic code of *Candida antarctica*. The present study describes the enantioselective transesterifications or hydrolyses of five-, six- and seven-membered *meso*-diols or the corresponding diacetates using this new biocatalyst (Scheme 1). The biocatalyst is easily recoverable, an important asset of this chemistry.

The enzymatic hydrolyses of *meso*-diacetates of five- and seven-membered compounds produced monoacetates with high optical purities. The six-membered diacetate gave excellent chemical yield, but poor enantioselectivity (Table 2). It is interesting to note that the acetate at the *(S)* center was hydrolyzed in the five-membered ring, whereas the acetate at the *(R)* center was hydrolyzed in the six- and seven-membered rings.

Table 2. SP-435-catalyzed hydrolyses of *meso*-diacetates **1a-c**

Substrate	Product	Temp. / Time	Chemical Yield	Optical Purity	Optical Rotation
1a	**3a**	25 °C / 2 h	90%	>99% [a]	+68.0 (c 1.02, $CHCl_3$)
1b	**4b**	45 °C / 3 h	91%	50% [b]	-51.3 (c 0.92, $CHCl_3$)
1c	**4c**	50 °C / 24 h	92%	>99% [c]	+6.1 (c 1.44, $CHCl_3$)

[a] Lit. value $[\alpha]_D$ = +66.3°, >98% ee (capillary GC analysis of MTPA derived esters)(ref. 7a). [b] Lit. value $[\alpha]_D$ = -100°, 98% ee (^{19}F and ^1H NMR of MTPA derived esters) (ref. 6). [c] Lit. value $[\alpha]_D$ = -98.3° (c 5.00, $CHCl_3$) for cnone derived from **4a**; found $[\alpha]_D$ = -126.9° (c 1.08, $CHCl_3$) (ref. 11).

The five-membered diacetate **1a** was hydrolyzed with SP-435 lipase (pH 8.0 phosphate buffer, 25 °C for 2 h) to produce monoacetate **3a** in 90% chemical yield. The optical purity was determined to be >99% based on the observed optical rotation.[7] The six-membered diacetate **1b** was hydrolyzed with SP-435 (pH 8.0 phosphate

buffer, 45 °C for 3 h) to produce monoacetate **4b** in 91% chemical yield with an optical purity of 50%;[6] **4b** was produced in 89% chemical yield (50% ee) when the hydrolysis was carried out at 25 °C. Attempted hydrolysis of **1c** at room temperature gave low chemical yields owing to poor solubility and slow reaction rate. Addition of co-solvents (THF, *t*-butyl alcohol) did not increase the rate of hydrolysis. The seven-membered diacetate **1c** was hydrolyzed smoothly at 50 °C (pH 8.0 phosphate buffer, 24 h) to produce the monoacetate **4c** in 92% chemical yield.[13] The optical purity of **4c** was determined to be >99% based on the observed optical rotation of enone derived from monoacetate **4c**.[11]

Similar results were obtained for the SP-435-catalyzed transesterification of *meso* diols with isopropenyl acetate in organic media.[14] The five- and seven-membered diols gave monoacetates with high optical purities, but the six-membered diol gave poor results (Table 3). The five-membered diol **2a** was acetylated with SP-435 (50 °C in isopropenyl acetate for 72 h) to produce the monoacetate **4a** in 48% chemical yield and the diacetate **1a** in 43% yield. The optical purity of **4a** was determined to be >99%. Addition of a nonpolar solvent (*t*-butyl methyl ether) gave similar results (52% yield, >99% ee) and did not increase the rate of acetylation. The six-membered diol **2b** was acetylated with SP-435 in a mixture of *t*-butyl methyl ether (3 parts) and isopropenyl acetate (5 parts) at 50 °C for 89 h to produce the monoacetate **3b** in 25% chemical yield and 59% optical purity, along with 30% recovered unreacted starting material, and 35% diacetate **1b**. Total conversion to the diacetate **1b** was accomplished in 24 h when the reaction was run in isopropenyl acetate (1 part) and hexanes (4 parts). The seven-membered diol **2c** was acetylated with SP-435 (50 °C in isopropenyl acetate) to produce the monoacetate **3c** in 41% chemical yield and the unreacted starting material **2c** in 50% yield. The rate of the reaction was increased by the addition of nonpolar co-solvents (hexane or *t*-butyl methyl ether). The monoacetate was produced in 81% yield in a solvent system of *t*-butyl methyl ether (4 parts) and isopropenyl acetate (1 part) at 50 °C for 5 h. The optical purity was determined to be >99%.[11]

Table 3. SP-435-catalyzed acetylation of *meso*-diols **2a-c** with isopropenyl acetate.

Substrate	Product	Temp. / Time	Chemical Yield	Optical Purity	Optical Rotation
2a	**4a**	50 °C / 72 h	48%	>99% [a]	-69.6 (c 1.00, $CHCl_3$)
2b	**3b**	50 °C / 89 h	25%	59% [b]	+59.8 (c 0.94, $CHCl_3$)
2c	**3c**	50 °C / 89 h	81%	>99% [c]	-6.2 (c 0.87, $CHCl_3$)

[a] Lit. value $[\alpha]_D$ -66° (c 0.63, $CHCl_3$), >98% ee (capillary GC analysis of MTPA derived esters) (ref. 7c). [b] Lit. value $[\alpha]_D$ -100°, 98% ee (^{19}F and ^1H NMR of MTPA derived esters) (ref. 6). [c] Lit. value $[\alpha]_D$ = -98.3° (c 5.00, $CHCl_3$) for enone derived from **4a**; found for enantiomer $[\alpha]_D$ = +107.4° (c 0.62, $CHCl_3$) (ref.11).

The enzyme studied here gave excellent results for the five- and seven-membered ring systems, and poor results for the six. The -CH=CH- and $-CH_2CH_2-$ in the six-membered case are too similar in size and cannot be fully distinguished by the enzyme, whereas in the five- and seven-membered ring, the groups flanking the diol or diacetate system can be differentiated by the enzyme. In the cases herein reported, the hydrolysis of diacetates of these ring systems with SP-435 were more efficient then their acetylation counterpart done in organic media. This should not be taken as a generality. Ongoing studies in our laboratory indicate that this new biocatalyst is likely to become a standard and highly useful tool in organic synthesis.[15]

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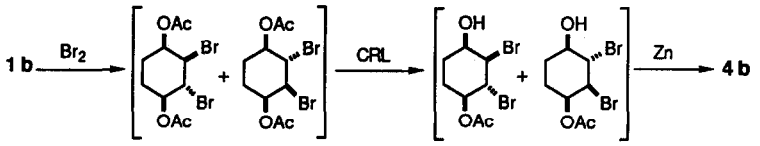
(6) Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org.Chem.***1991**, *56*, 2656. Enantiomerically pure **4b** was produced by enzymatic hydrolysis of the racemic dibromodiacetate from **1b** using *Candida rugosa* lipase (CRL).



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(9) Sih, C. J.; Girdaukas, G.; Shih, Y-E.; Gu, Q-M.; Harris, K. J. *Tetrahedron Lett.* **1991**, *32*, 3941. Transesterification of the *meso*-diol **2b** in isopropenyl acetate as the acyl donor and solvent using *Pseudomanas cepacia* lipase produced monoacetate **4b** in 51% chemical yield and 95% enantiomeric excess.

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(12) Biocatalyst supplied by Novo Nordisk, Novo Allé, D-2880 Bagsvaerd, Denmark.

(13) The enzymatic hydrolysis of **1c** was conducted as follows: The diacetate **1c** (202 mg, 0.95 mmol) and the catalyst SP-435 (40 mg) were added to a round-bottomed flask charged with pH 8.0 phosphate buffer (12.0 mL, phosphate buffer was produced by dissolving one Metrepak pHydrion buffer capsule [pH 8.00 ±0.02 at 25 °C] into distilled water [100 mL]). The suspension was stirred at 50 °C for 24 h. The catalyst was removed by filtration and rinsed with ethyl acetate (10 mL). The filtrate was saturated with NaCl and extracted with ethyl acetate (2 x 20 mL). The ethyl acetate was dried over $MgSO_4$, filtered, and the solvent removed under reduced pressure. The crude monoacetate was purified by column chromatography to produce monoacetate **4c** (149 mg, 92%) as a white crystalline solid: mp 43-45 °C; $[\alpha]_D^{25}$ = +6.1°(c 1.44, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$) δ 5.75(d, *J* = 12.2 Hz, 1 H), 5.57(d, *J* = 12.2 Hz, 1 H), 5.28(d, *J* = 9.7 Hz, 1 H), 4.34(d, *J* = 10.1 Hz, 1H), 2.18(m, 1 H), 2.04(s, 3 H), 2.00-1.49(m, 6 H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.19, 23.40, 32.38, 36.07, 71.61, 73.94, 131.27, 136.71, 170.32. Anal. Calcd. for $C_9H_{14}O_3$ C,63.51; H, 8.29. Found: C, 63.37; H, 8.32.

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