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# Lipase Asymmetrization of *cis*-3,7-Dihydroxycycloheptene Derivatives in Organic and Aqueous Media

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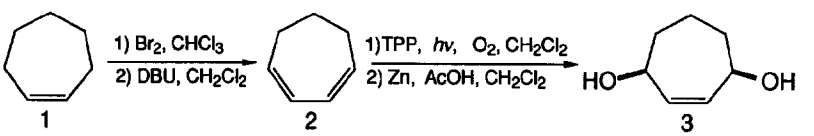
**Abstract:** The *meso*-diol and corresponding diacetates of cycloheptene derivatives were subjected to enzymatic asymmetrizations utilizing *Pseudomonas cepacia* lipase in organic and aqueous media. These biotransformations illustrate the enantio-complementary nature of enzymatic reactions in organic and aqueous media.

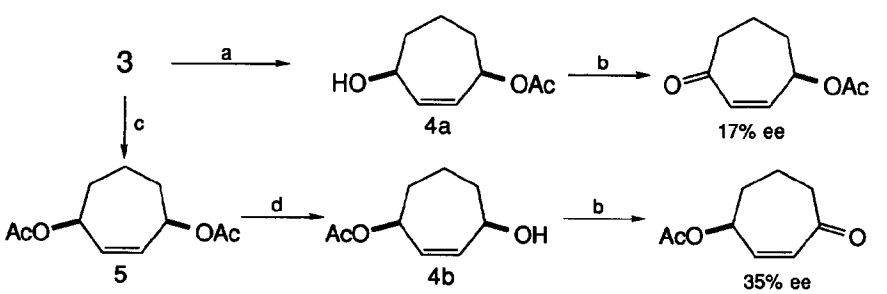
Enzymes have been increasingly important in organic synthesis as a primary means to produce enantiomerically pure molecules.[1] The synthetic potential of enzymes is fully realized in the asymmetrization of *meso* substrates which can produce one enantiomer in 100% theoretical yield. We have previously used enzymes to synthesize enantiomerically pure six and seven membered ring compounds derived from *meso*-diols or diacetate esters.[2] Specifically, this laboratory has shown that *meso*-diol **3** and *meso*-diacetate **5** can be successfully asymmetrized (enantiomeric excess >99%) with a recombinant version of lipase B from *Candida antarctica* (Novo SP-435) in good chemical yield.[3] Pearson has shown that the *meso*-diacetate **5** can be enantioselectively hydrolyzed to form the monoacetate **4b** in low yields with electric eel acetylcholinesterase.[4] Pearson has examined the hydrolysis of ester **5** with a lipase from *Candida cylindracea*; at best, monoacetate **4b** with an enantiomeric excess of 44% was obtained.[5] This report focuses on the asymmetrization of *meso*-diols and -diacetates derived from cycloheptene **1** utilizing *Pseudomonas cepacia* lipase to produce new useful chiral intermediates.

**Scheme 1**




Cycloheptene (**1**) was converted to 1,3-cycloheptadiene (**2**) in ca. 50% yield *via* the dibromide. The diene **2** was oxidized to give the endoperoxide in 97% yield; subsequent reduction with activated zinc and acetic acid produced the diol **3** in 95% yield (Scheme 1).

**Scheme 2**

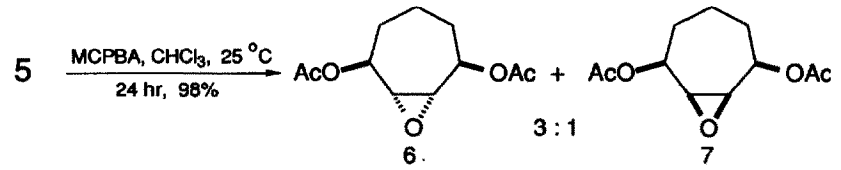



a) Amano P-30 lipase, isopropenyl acetate, *t*-butyl methyl ether, 50 °C, 65% b) PCC, $CH_2Cl_2$, 70%
c) acetic anhydride, pyridine, $CH_2Cl_2$, 99% d) LPL-800 on Azlactone beads, water, pH 8.0, 80%

Attempts to convert the diol **3** into the enantiomerically pure monoacetate ester **4a** *via* enzymatic asymmetrization utilizing crude *Pseudomonas cepacia* lipase (Amano P-30) in isopropenyl acetate[6a] were unsuccessful, giving an enantiomeric excess of 17%. Conversion of the diol **3** into the diacetate ester **5** furnished another substrate for the asymmetrization. Attempted enantioselective hydrolysis of the diacetate ester to the

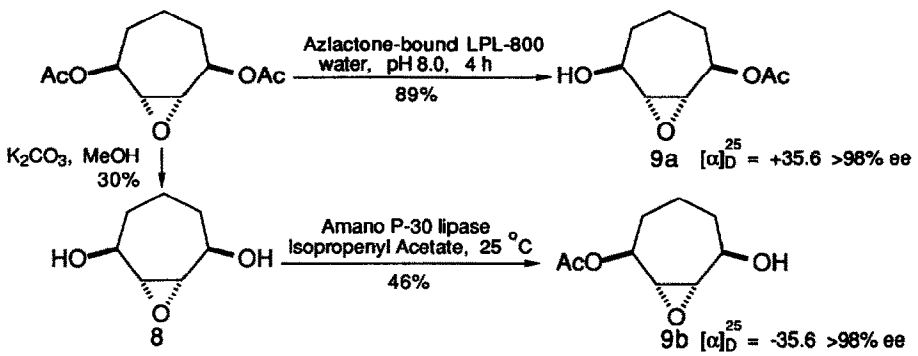
monoacetate **4b** using purified *Pseudomonas cepacia* lipase (Amano LPL-800) immobilized onto azlactone polymer beads[7] gave an enantiomeric excess of 35% (Scheme 2).

**Scheme 3**



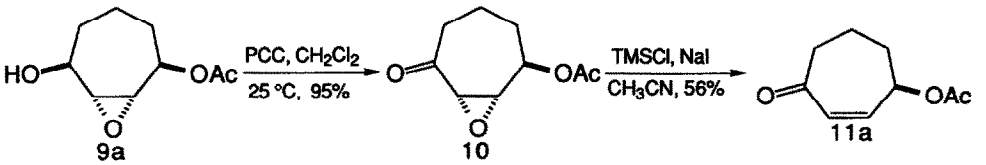

A possible reason for the unsuccessful asymmetrization of the diol **3** or diacetate **5** with the lipase is that the enzyme is unable to sterically distinguish between the CH=CH and the $CH_2CH_2$ units flanking the stereogenic centers. The geometry of the active site allows the molecule to position itself into the enzyme in either orientation causing indiscriminate hydrolysis or acetylation of both enantiotopic groups leading to low enantioselectivity. By converting the olefin into a larger functionality, the enzyme may be able to better distinguish enantiotopic sites. The strategy of amplifying stereodifferences has been successfully employed by a number of groups, e.g. CH=CH ⇀ CHBr-CHBr,[5] CH=CH ⇀ CHBr-$CH_2$,[8a] $CH_2OH$ ⇀ $CH_2OSiR_3$,[8b] $CH_2OH$ ⇀ $CH_2O$Trityl.[8c] To test this strategy in the present system, the olefin **5** was converted into a 3:1 mixture of the *trans*-**6** and *cis*-**7** epoxides by treatment with *m*-chloroperoxybenzoic acid (MCPBA) in chloroform (Scheme 3).

**Scheme 4**



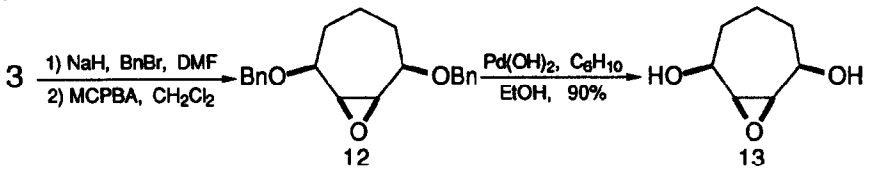

Treatment of the *trans* epoxide **6** with $K_2CO_3$ and MeOH resulted in many side products and diol **8** was obtained in only 30% yield. Exposure of diol **8** to Amano P-30 lipase in isopropenyl acetate and *t*-butyl methyl ether at 25 °C yielded the monoacetate ester **9b** in 46% yield and >98% enantiomeric excess (determined from the $^{19}F$ NMR of the MTPA ester of monoacetate **9b** and its racemate)[9] and diacetate **6** in 47% yield. Treatment of the diacetate ester **6** with the LPL-800 immobilized on azlactone polymer beads in water at pH 8.0 for 4 h resulted in the formation of the monoacetate **9a** in 89% yield and 98% enantiomeric excess (Scheme 4).

**Scheme 5**



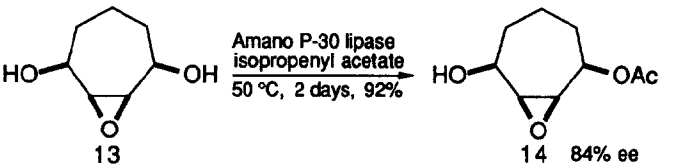

The absolute configuration was determined by synthesis of the acetoxy-enone **11a**. Monoacetate **9a** was oxidized with pyridinium chlorochromate (PCC) buffered with NaOAc to produce the epoxy-ketone **10** in 95% yield. The epoxy-ketone **10** was transformed into the enone **11a** by treatment with chlorotrimethylsilane and sodium iodide in acetonitrile[10] at 0 °C to produce the enone in 56% yield, $[\alpha]_D^{25}$ = +106.9 (c 1.00, $CHCl_3$). The previously published value of $[\alpha]_D^{23}$ = -98.3 (c 5.00, $CHCl_3$)[4] was assigned the (*S*) configuration at $C_4$, thus enone **11a** has the (*R*) configuration at $C_4$ (Scheme 5).

**Scheme 6**




Enzymatic asymmetrization of the *cis* epoxide **7** was also attempted. A more efficient route to the *cis* epoxide was achieved by dibenzylation of the *meso*-diol **3**, followed by oxidation of the olefin with MCPBA to furnish exclusively the *cis* epoxide **12** in 86% yield for the two steps. Removal of the benzyl groups produced the all *cis* epoxy-diol **13** in 90% yield (Scheme 6). The *cis* epoxy-diol **13** was unreactive toward acetylation at room temperature when treated with Amano P-30 lipase in isopropenyl acetate for 48 h. When the temperature was increased to 50 °C for 48 hours, the monoacetate **14** was produced in 88% yield . The enantiomeric excess was determined to be 77% from the $^{19}F$ NMR of the MTPA ester of monoacetate **14** and its racemate.[6,11] Identical reaction conditions provided product **14** in 92% chemical yield with an enantiomeric excess of 84% (Scheme 8). The absolute stereochemisty was determined by conversion of alcohol **14** to enone **11a**.[12]

**Scheme 8**




In conclusion, this report shows illustrates the chemoenzymatic syntheses of enantiomerically pure intermediates from achiral cycloalkenes. We report here the synthesis of epoxy-alcohol **9a** from cycloheptene in high chemical yield and excellent enantioselectivity and epoxy-alcohol **14** in moderate enantioselectivity and high chemical yield. These biotransformations illustrate the enantio-complementary nature of enzymatic reactions in organic and aqueous media.[6b]

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(5) *Candida rugosa* was previously classified as *Candida cyclindracea*. Likewise *Pseudomonas fluorescens* has been reclassified as *Pseudomonas cepacia*. CRL is used to designate the lipase previously known as CCL and PCL is now used in place of PFL (see Kazlauskas, R. J.; Weissfloch, A. N. E.; Rappaport, A. T.; Cuccia, L. A. *J. Org. Chem.* **1991**, *56*, 2656).

(6) (a) For use of isopropenyl acetate as an acyl transfer agent see Wang, Y.-F; Lalonde, J. J.; Momongan, M.; Bergbreiter, D. E.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 7200. (b) For a general discussion of the use of enzymes in organic solvents see Klibanov, A. M. *Acc. Chem. Res.* **1990**, *23*, 11.

(7) This experimental material was provided by the 3M Company. Details of its use in enzyme immobilization will be the subject of a future publication.

(8) (a) Gupta, A. K.; Kazlauskas, R. J., *Tetrahedron Asym.*, this issue. (b) Goergins, U.; Schneider, M. P. *J. Chem. Soc., Chem. Commun.* **1991**, 1066. (c) Kim, M. J.; Choi, Y. K. *J. Org. Chem.* **1992**, *57*, 1605.

(9) Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem.* **1969**, *34*, 2543. The $^{19}F$ NMR showed two signals for the racemic MTPA derivative at -70.611 and -70.636 ppm. The MTPA ester of monoacetate **11b** had only one signal in the $^{19}F$ spectra at -70.639 ppm.

(10) Caputo, R.; Mongoni, L.; Neri, O.; Palumbo, G. *Tetrahedron Lett.* **1981**, *22*, 3551.

(11) The $^{19}F$ NMR showed two signals for the racemic MTPA derivative at -70.668 and -70.817 ppm. The MTPA ester of monoacetate **14** also had two signals in the $^{19}F$ spectra at -70.636 and -70.793 ppm in a ratio of 7.77 to 1.00 which corresponds to an enantiomeric excess of 77% for alcohol **14**.

(12) The enone possessed an $[\alpha]_D^{25} = +86.0$ (c 1.23, $CHCl_3$) which corresponds to an 80% enantiomeric excess and a (*R*) configuration at $C_4$.

**Physical Data:**

**3** mp 97-98 °C. $^1H$ NMR δ 5.76 (s, 2 H), 4.30 (dm, *J* = 8.3 Hz, 2 H), 1.25-2.10 (m, 8 H); $^{13}C$ NMR δ 23.0, 36.0, 71.4, 135.7. Anal. calcd. for $C_7H_{12}O_2$: C, 65.60; H, 9.44. Found: C, 65.57; H, 9.61.

**4** mp 43-45 °C. $^1H$ NMR 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 δ 21.2, 23.4, 32.4, 36.1, 71.61, 73.9, 131.3, 136.7, 170.3; MS {CI} *m/z* 67 (5), 81 (12), 93 (50), 110 (100), 153 (25), 171 [M+H] (1). Anal. calcd. for $C_9H_{14}O_3$: C,63.51; H, 8.29. Found: C, 63.37; H,8.39.

**5** mp 78-79 °C. $^1H$ NMR δ 5.66 (s, 2 H) 5.35 (dm, *J* = 10.3 Hz, 2 H), 2.05 (s, 6 H), 1.55-2.02 (m, 6 H); $^{13}C$ NMR δ 21.2, 23.0, 32.3, 73.6, 132.5, 170.1. Anal. calcd. for $C_{11}H_{16}O_4$: C, 62.25; H, 7.60. Found: C, 62.44; H, 7.83.

**6** mp 49-51 °C. $^1H$ NMR δ 5.09 (dd, *J* = 11.1, 3.0 Hz, 2 H), 3.21 (s, 2 H), 2.09(s, 6 H), 1.80-1.55 (m, 6 H); $^{13}C$ NMR δ 20.7, 21.1, 29.4, 56.2, 73.1, 170.3. Anal. calcd. for $C_{11}H_{16}O_5$: C, 57.89; H, 7.07. Found: C, 57.79; H, 7.08.

**8** mp 149-151 °C. $^1H$ NMR ($CD_3OD$) δ 3.85 (dd, *J* = 11.1, 3.3 Hz, 2 H), 3.18 (s, 2 H), 1.76-1.44 (m, 5 H), 1.08-0.96 (m, 1 H); $^{13}C$ NMR ($CD_3OD$) δ 22.4, 34.2, 60.2, 72.3. Anal. calcd. for $C_7H_{12}O_3$: C, 58.32; H, 8.39. Found: C, 58.03; H, 8.41.

**9** mp 64.5-66 °C. $^1H$ NMR δ 5.03 (dd, *J* = 11.2, 3.6 Hz, 1 H), 3.91 (dm, *J* = 10.9 Hz, 1 H), 3.28 (d, *J* = 5.2 Hz, 1 H), 3.17 (d, *J* = 5.2 Hz, 1 H), 2.94 (s, 1H), 2.05 (s, 3H), 1.83-1.48 (m, 5 H), 1.30-0.50 (m, 1H); $^{13}C$ NMR δ 20.8, 21.1, 29.5, 33.1, 56.6, 58.8, 71.1, 73.4, 170.5. Anal. calcd. for $C_9H_{14}O_4$: C, 58.05; H, 7.58. Found: C, 58.06; H, 7.60.

**10** colorless oil. $^1H$ NMR δ 5.12 (dd, *J* = 11.4, 3.0 Hz, 1 H), 3.45 (s, 2 H), 2.70 (ddd, *J* = 14.2, 11.5, 3.4 Hz, 1 H), 2.35 (m, 1 H), 2.12 (s, 3 H), 2.11-1.83 (m, 3 H), 1.3 (m, 1 H); $^{13}C$ NMR δ 20.9, 21.8, 29.0, 40.5, 56.4, 57.4, 72.4, 169.9, 208.3. HRMS Calcd. for $C_9H_{12}O_4$: 184.0735$_5$, Found 184.0735.

**11** colorless oil. $^1H$ NMR δ 6.42 (ddd, *J* = 12.7, 3.0, 0.9 Hz, 1 H), 6.00 (dd, *J* = 12.5, 2.1 Hz, 1 H), 5.57 (m,1 H) 2.61 (m, 2 H), 2.20--2.13 (m, 1 H), 2.08 (s, 3 H), 1.91-1.80 (m, 3 H); $^{13}C$ NMR δ 18.0, 21.0, 31.6, 42.8, 72.0, 131.3, 144.4, 170.0, 202.4. Anal. calcd. for $C_9H_{12}O_3$: C, 64.27; H, 7.19. Found: C, 64.11; H, 7.17.

**12** mp 70-72 °C. $^1H$ NMR δ 7.46-7.30 (m, 10 H), 4.82 (ABq, *J* = 11.7 Hz, 2 H), 4.61 (ABq, *J* = 11.7 Hz, 2 H), 3.25-3.16 (m, 4 H), 1.98-1.85 (m, 3 H), 1.64 (m, 2 H), 1.61 (m, 1 H); $^{13}C$ NMR δ 24.3, 31.3, 57.3, 71.0, 80.3, 127.6, 127.7, 128.3, 138.0. Anal. calcd. for $C_{21}H_{24}O_3$: C, 77.75; H, 7.46. Found: C, 77.55; H, 7.40.

**13** mp 70-72 °C. $^1H$ NMR ($CD_3OD$) δ 3.38 (d, *J* = 4.4 Hz, 2 H), 3.00 (d, *J* = 4.4 Hz, 2 H), 1.09-1.35 (m, 6 H); $^{13}C$ NMR ($CD_3OD$) δ 24.9, 34.8, 60.1, 74.1. Anal. calcd. for $C_7H_{12}O_3$: C, 58.32; H, 8.39. Found: C, 58.23; H, 8.29.

**14** mp 49-51 °C. $^1H$ NMR δ 4.48 (ddd, *J* = 10.8, 5.4, 1.0 Hz, 1 H), 3.54 (ddd, *J* = 10.5,5.7, 1.5 Hz, 1 H), 3.36 (bs, 1 H), 3.15-3.07 (m, 2 H), 2.03 (s, 3 H), 1.83-1.40 (m, 6 H); $^{13}C$ NMR δ 21.0, 22.9, 30.7, 33.1, 56.3, 59.0, 73.0, 75.4, 170.1. HRMS Calcd. for $C_9H_{14}O_4$+H: 187.0970$_2$, found 187.0966.