Pergamon

*Tetrahedron: Asymmetry* Vol 5, No 4, pp. 691-698, 1994
Elsevier Science Ltd
Printed in Great Britain
0957-4166/94 $6.00+0.00

0957-4166(94)E0090-W

# REGIO- AND ENANTIOSELECTIVITY OF *Pseudomonas cepacia* LIPASE IN THE TRANSESTERIFICATION OF 2-SUBSTITUTED-1,4-BUTANEDIOLS

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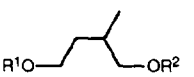
**Abstract.** The transesterification of 2-substituted-1,4-butanediols **1a**, **2a** and **3a** with vinyl acetate catalyzed by the *Pseudomonas cepacia* lipase in organic solvents affords preferentially the 1-acetate, the highest regioselectivity having been found for the epoxydiol **3a**, which is enantioselectively resolved [86% ee for the unreacted (S)-(-)-**3a**].

## Introduction

Recently, we have shown that 2-methyl-1,4-butanediol **1a** can be resolved by transesterification with vinyl acetate catalyzed by the *Pseudomonas fluorescens* (PFL) lipase in organic solvents.[1] A 70% enantiomeric excess (ee) of the unreacted (R)-**1a** (35% yield) was reached when 50% of a mixture of the monoacetates **1b** and **1c** and 15% of the diacetate **1d** were formed.




1

a. $R^1 = R^2 = H$

b. $R^1 = H$, $R^2 = COCH_3$

c. $R^1 = COCH_3$, $R^2 = H$

d $R^1 = R^2 = COCH_3$

e. $R^1 = H$, $R^2 = COC_3H_7$

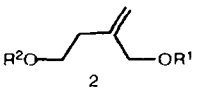
f. $R^1 = COC_3H_7$, $R^2 = H$

g. $R^1 = R^2 = COC_3H_7$

h. $R^1 = H$, $R^2 = COC_7H_{15}$

i. $R^1 = COC_7H_{15}$, $R^2 = H$

In the above conditions the ratio **1b**:**1c** was 7:3 indicating that the 1-hydroxy group was preferentially, but not exclusively acylated. This prompted us to study the regioselectivity of the formation of the 1-acetate **1b** and the 4-acetate **1c**. Similar studies using the *Candida cylindracea* (CCL) lipase, were carried out on 2-thiobenzyl-1,4-butanediol as substrate.[2] Detailed studies have been performed on the regioselective enzymatic acylation of polyhydroxylated compounds such as carbohydrates,[3] but less information is available on open chain polyols. In order to study the regioselectivity of the enzymatic transesterification of 2-substituted 1,4-butanediols, we extended our previous observations on 2-methyl-1,4-butanediol **1a**[1] to 2-methylene-1,4-butanediol **2a** and the corresponding epoxydiol **3a**, considering also the use of other acylating agent.



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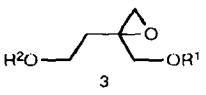
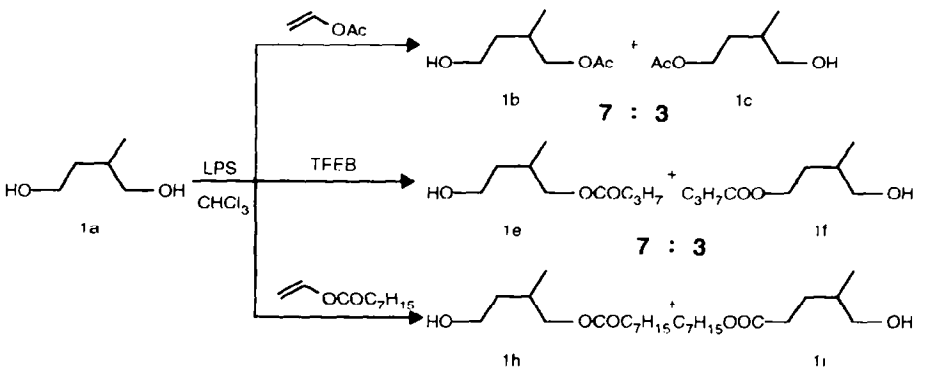


3

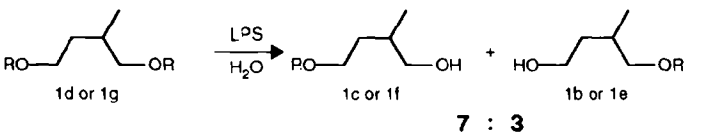
a $R^1 = R^2 = H$ b. $R^1 = COCH_3$, $R^2 = H$ c. $R^1 = H$, $R^2 = COCH_3$ d $R^1 = R^2 = COCH_3$

**Regioselectivity of the Enzymatic Acylation of 2-Methyl -1,4-butanediol 1a**

The enzymatic transesterification of the diol **1a** with vinyl acetate in an organic solvent previously reported[1] had been carried out in the presence of PFL (SAM-2, Fluka, Switzerland). We repeated the reaction in the presence of the lipase PS from *Pseudomonas cepacia*[4] (LPS, Amano, Japan) and found the same results, and therefore decided to use the Amano lipase LPS for the study reported in this paper.[5] The transesterification of **1a** with LPS was monitored at intervals of time and we found that from the beginning the ratio **1b**:**1c** was 7:3 and was maintained through the whole reaction. When all the diol **1a** had reacted (24 h), 40% of the diacetate **1d** and 60% of the mixture of the monoacetates **1b** and **1c** (ratio 7:3) was obtained. Continuing our investigation on the regioselectivity of the LPS-catalyzed reaction, we kept constant the enzyme/substrate ratio (312 U/mmol) independently from the nature of the substituent at carbon 2 of the examined butanediol. An alternative procedure for the enzymatic transesterification is the use of trifluoroethyl butyrate (TFEB) as acylating agent[6] and TFEB was used in chloroform with LPS and the diol **1a**. After 30 h still 37% of unreacted diol **1a** was present and the ratio of 1- and 4-monobutanoates was the same as for the monoacetates (**1e**:**1f**, 7:3). For the transesterification of the diol **1a** to octanoates, vinyl octanoate[7] in chloroform was used and the 1- and 4-octanoates **1h** and **1i** were formed with less regioselectivity than the corresponding acetates and butanoates (**1h**:**1i**, 53:47).

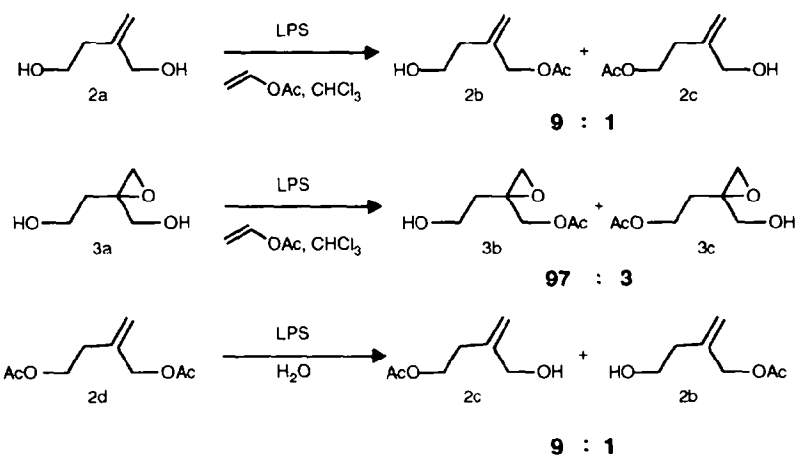


The LPS-catalyzed aqueous hydrolysis of the diacetate **1d** and dibutanoate **1g** showed a reversed regioselectivity with respect to the transesterification, since the main products were the 4-acetate **1c** and the 4-butanoate **1f**. Interestingly, the ratio between the 1- and 4-monoesters was similar to the value obtained for the transesterification process (**1c**:**1b** and **1f**:**1e**, 7:3).



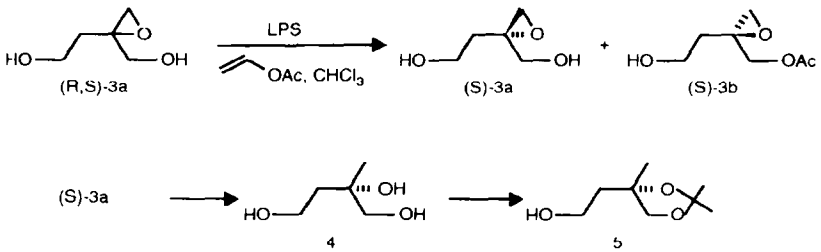

**LPS-Catalyzed Transesterification of Diols 2a, 3a and Hydrolysis of the Diacetate 2d**

We continued our study on the LPS-catalyzed reaction with vinyl acetate using as substrates 2-methylene-1,4-butanediol **2a** and the corresponding epoxide **3a**. The compound **2a** was obtained by reduction of diethyl itaconate with a solution of diisobutylaluminum hydride (DIBAH) in hexane.[8] For the preparation of the compound **3a**, the Sharpless procedure of epoxidation of allyl alcohols (vanadium acetylacetonate, *t*-butyl hydroperoxide)[9] was satisfactory. The results from the enzymatic reaction show that, compared to 2-methyl-1,4-butanediol **1a**, the regioselectivity of the 1- versus the 4-hydroxy group was higher in the transesterification of the 2-methylene-1,4-butanediol **2a** (1-acetate **2b**: 4-acetate **2c**, 9:1). Interestingly, the aqueous enzymatic hydrolysis of the diacetate **2d** afforded the 2- and 4-acetates **2b** and **2c** in the reversed ratio of 1:9. In the case of the 2-epoxy analogue **3a**, the regioselectivity of the LPS-catalyzed acylation was improved (1-acetate **3b**: 4-acetate **3c**, 97:3).



**Enantioselectivity of the Enzymatic Resolution of the Racemic Epoxyalcohol 3a**

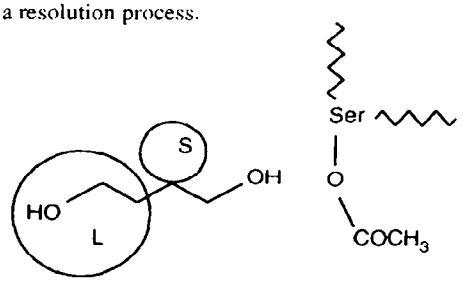
The above results indicate that the nature of the group at the position 2 was important to direct the regioselectivity of the enzymatic reaction. The highest regioselectivity was observed for the compound **3a**, for which almost an exclusive acylation takes place at the C-1 hydroxy group. Due to the absence of reactivity of the 4-hydroxy group, we could study also the enantioselectivity of the resolution of the racemic diol **3a** during the process of transesterification to the acetate **3b**. As for other similar compounds,[10] it was conceivable to achieve the highest ee for the acetate **3b** if the conversion was stopped at 40%. In a separate experiment, the most enantioselective preparation of the epoxydiol **3a** could be reached if 60% of the acetate **3b** was formed. The resolution of the racemic **3a** afforded the (-)-unreacted diol **3a** and the (+)-1-acetate **3b** (contaminated by 3% of the 4-acetate **3c**). For the assignment of the configuration, the diol **3a** was reduced by $LiAlH_4$ in tetrahydrofuran to the triol **4**, which was converted into the known (S)-(-)-acetonide **5**.[11]




The optical rotation of the above compound **5** and the analysis of the $^1$H-NMR (500 MHz) of the di-MTPA ester[12] of the (S) (-)-**3a** showed an enantiomeric excess of 86%. This result is in contrast with the absence of enantioselectivity reported for the CCL-catalyzed resolution of the 2-thiobenzyl analogue.[2]

**Conclusions**

The results so far obtained for the 2-substituted-1,4-butanediols examined by us may be discussed in terms of steric demand of the enzyme active site. X Ray studies for several lipases have provided important structural informations on the active site of these hydrolases.[13] In absence of such data for the *Pseudomonas* lipases, two-dimensional[14] and three-dimensional[15] models have been proposed in order to suggest a topology for the *Pseudomonas* lipases active site, which can allow the prediction of the stereodifferentiation for a resolution process.



If we adopt these models for a tentative explanation of our results on the regioselectivity during the acylation process of the diols **1a**, **2a** and **3a**, we should suggest that the C-1 primary alcohol, better than the C-4 moiety may approach the acylated amino acid (serine) that is supposed to be formed in the active site.[16] In other words, the factor which governs the regioselectivity may be the distinction between the large $CH_2CH_2OH$ group (L) and the small groups S (**1a** = $CH_3$, **2a** = methylene, **3a** = oxirane ring). In the diol **1a**, the free-rotating C-2 methyl group induces less regioselection than the two more rigid methylene (diol **2a**) and oxirane (diol **3a**) groups. In particular, the diol **3a** can adequately fit in the active site, so that a good enantioselectivity can also be observed in the resolution process.

**Experimental Section**

All the chemicals were purchased from Fluka (Switzerland) and the *Pseudomonas cepacia* lipase (LPS, 30 U/mg) was a gift from Amano (Japan). The enzyme was used without further purification and the enzymatic reactions were carried out at 30 °C under stirring. The 60 MHz $^1$H-NMR spectra were recorded on a Varian EM 360 L spectrometer for solution in $CDCl_3$ ($SiMe_4$as internal standard). The 500 MHz $^1$H-NMR spectra were recorded on a Bruker instrument (AM 500)). Optical rotations were measured at 25 °C on a Perkin-Elmer polarimeter (Model 241). Distillations for analytical purposes were performed on a glass tube oven Büchi GKR-50. TLC analyses were carried out on silica gel Merck 60 F254 plates and column chromatographies were performed on silica gel Merck 60 (230-400) mesh), unless otherwise stated. As a standard work-up procedure, the final organic solution was dried over sodium sulfate, which was filtered off and the solvent removed at reduced pressure.

***2-Methyl-1,4-butanediol, 1,4-diacetate 1d.*** - To a solution of 2-methyl-1,4-butanediol **1a**[1] (0.515 g, 4.95 mmol) in dry pyridine (3.25 mL) acetic anhydride (4.87 mL, 51.5 mmol) was added and the solution was kept at room temperature (18 h). Addition of water (10 mL), extraction with dichloromethane (3 x 10 mL) and the final work up afforded a residue of practically pure diacetate **1d** (0.85 g, 91%). B. p. 160 °C (6 mmHg); $\delta_H$ 0.95 (d, 3 H, $CH_3$CH), 1.40 - 2.15 (m+s, 9 H, $CH_2$CH, $CH$CH$_2$, and $CH_3$CO), 3.90 - 4.45 (d+t, 4 H, $CH_2$OAc). $C_9H_{16}O_4$: Anal. found: C, 57.52; H, 8.63. Calc.: C, 57.45; H, 8.51%.

***2-Methyl-1,4-butanediol, 1,4-dibutanoate 1g.*** - To a solution of 2-methyl-1,4-butanediol **1a**[1] (1.5 g, 14.4 mmol) in dry pyridine (16 mL) at 4 °C butyryl chloride (3.64 mL, 34.7 mmol) was added dropwise and the solution was kept at room temperature (18 h). After addition of water (20 mL), the product was recovered by extraction with dichloromethane (3 x 20 mL) and the final work-up afforded a residue of crude dibutanoate **1g**. Column chromatography (hexane/ethyl acetate, 9:1) afforded the dibutanoate **1g** (2.06, 59%). B. p. 115-120 °C (3 mmHg); $\delta_H$ 0.75 - 1.20 (t+d, 9 H, $CH_3$CH and $CH_3CH_2$), 1.30 - 2.10 (m, 7 H, $CH_2$CH, $CH$CH$_2$, and $CH_2CH_2$), 2.40 (t, 4 H, $CH_2$CO), 3.95 - 4.45 (d+t, 4 H, $CH_2$OCO). $C_{13}H_{24}O_4$: Anal. found: C, 64.02; H, 9.93. Calc.: C, 63.93; H, 9.84%.

***LPS-Catalyzed Transesterification of Diol 1a.***

***a. With TFEB.*** - To a solution of the diol **1a** (0.33 g, 3.17 mmol) in chloroform (6.3 mL), trifluoroethyl butanoate (TFEB, 1.45 mL, 9.68 mmol) and LPS (33.6 mg) were added. The reaction progress was monitored by GLC (130 °C), revealing that after 30 h the unreacted diol **1a** was present (37%) together with a 7:3 mixture of monobutanoates (63%) [retention times ($T_R$) of 12.5 and 12.7 min, respectively] which was isolated (0.309 g, 55%) after a column chromatography (hexane/ethyl acetate, 7:3). A fraction of the above chromatography furnished the pure monobutanoate with $T_R$ 12.7 min (0.03 g, 5 %). The identity of this compound as the 4-butanoate **1f** was established by $^1$H-NMR (60 MHz). $\delta_H$ 0.80 - 1.20 (m, 6 H, $CH_3$CH and $CH_3CH_2$), 1.25 - 2.00 (m, 4 H, $CH_2$CH and $CH_2CH_3$), 2.00 - 2.60 (m, 4 H, $CH$CH$_3$, $CH_2$CO, and an exchangeable hydrogen), 3.60 (d, 2 H, $CH_2$OH), 4.30 (t, 2 H, $CH_2$OCO). Another fraction

with the same eluant (hexane/ethyl acetate, 7:3) contained nearly pure 1-butanoate **1e** ($T_R$ 12.5 min), which showed by NMR the following significant resonances: 3.80 (t, 2 H, $CH_2OH$) and 4.10 (d, 2 H, $CH_2OCO$).

***b. With Vinyl Octanoate.*** - To a solution of the diol **1a** (0.5 g, 4.8 mmol) in chloroform (9.5 mL), vinyl octanoate[7] (1.79 mL) and LPS (52.5 mg) were added. The reaction progress was monitored by GLC (200 °C), revealing that after 72 h the diol **1a** and the dioctanoate were not present and a 53:47 mixture of monooctanoates [retention times ($T_R$) of 8.64 and 8.74 min, respectively] was formed. The mixture was purified by column chromatography (hexane/ethyl acetate, 6:4) and a fraction consisting of a mixture enriched of the monooctanoate with $T_R$ 8.74 min (0.09 g, 8%) was isolated. The identity of this compound as the 4-octanoate **1i** was established by $^1$H-NMR (60 MHz). $\delta_H$ 0.75 - 1.15 (m, 6 H, $CH_3CH$ and $CH_3CH_2$), 1.15 - 2.05 (m, 13 H, $CH_2CH$ and $(CH_2)_5CH_3$ and $CH$), 2.40 (t, 2 H, $CH_2CO$), 3.60 (d, 2 H, $CH_2OH$), 4.15 (t, 2 H, $CH_2OCO$), 4.25 (m, 1 H, exchangeable). Another fraction with the same eluant (hexane/ethyl acetate, 6:4) contained nearly pure 1-octanoate **1h** ($T_R$ 8.64 min), which showed by NMR the following significant resonances: 3.80 (t, 2 H, $CH_2OH$) and 4.05 (d, 2 H, $CH_2OCO$).

***Hydrolysis of Diesters of the Diol 1a.***

***a. Hydrolysis of the Diacetate 1d.*** - A mixture of the diacetate **1d** (0.56 g, 2.98 mmol) and LPS (65 mg) in a phosphate buffer (pH 7, 6.4 mL)) was stirred, keeping the pH constant by addition of 1 N sodium hydroxide (2.38 mL, corresponding to 80% conversion). After 3 h, a mixture of the monoacetates **1c** and **1b** were formed in the ratio 7:3, as established by GLC (*vide supra*). The amount of the diol **1a** was not determined, since it was not extracted and remained in the aqueous phase.

***b. Hydrolysis of the Dibutanoate 1g.*** - A mixture of the dibutanoate **1g** (0.142 g, 0.58 mmol) and LPS (12.8 mg) in a phosphate buffer (pH 7, 1.25 mL)) was stirred, keeping the pH constant by addition of 1 N sodium hydroxide (0.46 mL, corresponding to 80% conversion). After 2 h, the starting dibutanoate (20%), the diol **1a** (3%), and a mixture of the monoacetates **1f** and **1e** were formed in the ratio 7:3 (by GLC).

***2-Methylene-1,4-butanediol 2a.*** - To a solution of diethyl itaconate (2 g, 10.75 mmol) in dry tetrahydrofuran (70 mL) diisobutylaluminum hydride (DBAH, 1 M in hexane, 46.8 mL) was added dropwise at -20 °C under a nitrogen atmosphere. After 1 h, 5 N sulfuric acid was added to reach pH 2 and the precipitate was removed by filtration onto Celite. After neutralization ($NaHCO_3$), the solvent was evaporated at reduced pressure and to the residue dichloromethane was added (50 mL) and the aqueous phase separated. The organic solution was worked-up as usually, affording the title diol **2a** (0.85 g, 78%) with chemico-physical characteristics in agreement with the literature data.[17]

***2-Methylene-1,4-butanediol oxide 3a.*** - To a solution of vanadyl acetoacetonate (11 mg) and 2-methylene-1,4-butanediol **2a** (0.85 g, 8.33 mmol) in dry benzene (43 mL), anhydrous *tert*-butyl hydroperoxide (1.4 mL prepared according to a published procedure[18]) in benzene (3.8 mL) was added. The solution was kept under nitrogen at room temperature (18 h) and then concentrated under nitrogen stream. The mixture was purified by column chromatography (Florisil, dichloromethane/methanol, 9:1) affording the pure epoxydiol **3a** (0.54 g, 55%). B. p. 240 - 245 °C (10 mm Hg); $\delta_H$ 1.95 (t, 2 H, $CH_2CH_2$), 2.90 (m, 2 H, oxirane $CH_2O$), 3.35 - 4.05 (m, 6 H, $CH_2OH$ and exchangeable). $C_5H_{10}O_3$: Anal. found: C, 50.90; H, 8.58. Calc.: C, 50.83; H, 8.53%.

***LPS-Catalyzed Transesterification of the Diol 2a and Hydrolysis of the Diacetate 2d.***

***a. Transesterification of the Diol 2a.*** - To a solution of the diol **2a** (0.14 g, 1.37 mmol) in chloroform (2.4 mL), vinyl acetate (0.44 mL) and LPS (18 mg) were added. The reaction progress was monitored by GLC (130 °C), revealing that after 2 h the unreacted diol **2a** was present (0.057 g, 40 %) together with the diacetate (0.051 g, 20%), and a 9:1 mixture of monoacetates (40%) [retention times ($T_R$) of 5.56 and 5.73 min, respectively] which was isolated (0.069 g, 35%) after a column chromatography (hexane/ethyl acetate, 7:3). A fraction of the above chromatography furnished the pure main monoacetate with $T_R$ 5.56 min (0.015 g, 7.6%). The identity of this compound as the 1-acetate **2b** was established by $^1$H-NMR (60 MHz). $\delta_H$ 1.85 - 2.00 (m, 1 H, exchangeable), 2.10 (s, 3 H, $CH_3CO$), 2.45 (t, 2 H, $CH_2CH_2O$), 3.85 (t, 2 H, $CH_2OH$), 4.70 (s, 2 H, $CH_2OCO$), 5.20 (s, 1 H, *CH*=), 5.30 (s, 1 H, *CH*=).

***b. Hydrolysis of the Diacetate 2d.*** - The diacetate **2d** was prepared by acetylation (pyridine/acetic anhydride) of the diol **2a** and a mixture of the diester **2d** (0.172 g, 0.92 mmol) and LPS (22 mg) in a phosphate buffer (pH 7, 2.16 mL) was stirred, keeping the pH constant by addition of 1 N sodium hydroxide (0.8 mL, 1.5 h for 90% conversion). Dichloromethane (3 mL) was added and the products obtained after the usual work-up were examined by GLC (130 °C). Only the 1:9 mixture of the monoacetates **2b** and **2c** was present (the diacetate **2d** was 1%) and the NMR spectrum corresponded essentially to the 4-monoacetate **2c**: $\delta_H$ 1.90 - 2.35 (m, 1 H, exchangeable), 2.10 (s, 3 H, $CH_3CO$), 2.50 (t, 2 H, $CH_2CH_2O$), 4.20 (s, 2 H, $CH_2OH$), 4.30 (t, 2 H, $CH_2OCO$), 5.10 (s, 1 H, *CH*=), 5.25 (s, 1 H, *CH*=).

***LPS-Catalyzed Transesterification of the Epoxydiol 3a.***

***a. Regioselectivity of the Enzymatic Reaction.*** - To a solution of the epoxydiol **3a** (0.26 g, 2.2 mmol) in chloroform (3.8 mL) vinyl acetate (0.7 mL) and LPS (28.8 mg) were added. After 2 h at 31 °C, the enzyme was removed by filtration, the solvent evaporated, and in the crude mixture analyzed by GLC (130 °C) the unreacted diol **3a** was undetectable, whereas the diacetate **3d** (37%) and a 3:97 ratio of the monoacetates (63%) were observed ($T_R$ 10.071 and 10.678 min). A column chromatography allowed the purification of the monoacetates [hexane/diethyl ether (1:1), 60%] from the diacetate (34%) [hexane/diethyl ether (9:1), 34%]. Diacetate **3d**: $\delta_H$ 1.90 - 2.30 (m, 8 H, $CH_2CH_2O$ and $CH_3CO$), 2.80 - 2.95 (m, 2 H, $CH_2O$), 4.10 - 4.55 (m, 4 H, $CH_2$ OCO). Monoacetate **3b**: 1.90 - 2.30 (t+s, 5 H, $CH_2CH_2O$ and $CH_3CO$), 2.50 - 2.60 (m, 1 H, exchangeable), 2.80 - 3.00 (m, 2 H, $CH_2O$), 3.80 (t, 2 H, $CH_2OH$), 4.25 (dd, 2 H, $CH_2$ OCO).

***b. Resolution of the Epoxydiol 3a.*** - The reaction was carried out as above, and 60% conversion of the epoxy diol **3a** to the monoacetate **3b** (the amount of **3c** was estimated less than 3%) was reached in 25 min. The epoxy diol **3a** was purified as previously described and isolated (0.084 g, 32%), showing $[\alpha]_D$ -4.2 (*c* 1 in chloroform). For the determination of the ee, the diMTPA ester was prepared from racemic and optically active **3a** and (R)-MTPA chloride.[12] The derivative from racemic **3a** showed at 500 MHz $^1$H-NMR analysis the signals of the two hydrogens of the -$CH_2OCO$ group at position 1 as four doublets between 4.044 and 4.477 ppm. The ratio of the four doublets for the derivative prepared from optically active **3a** was 93:7.

***Assessment of the Configuration of the Enzymatically Formed (-)-Epoxydiol 3a.*** - A solution of the (-)-epoxydiol **3a** (0.1 g, 0.85 mmol) in dry tetrahydrofuran (2 mL) was added to a suspension of lithium

aluminum hydride (0.094 g, 2.48 mmol) in the same solvent (2 mL). When the starting material disappeared, water (0.1 mL), 15% NaOH (0.1 mL), and water (0.3 mL) were sequentially added. The precipitate was removed by filtration and the solvent evaporated at reduced pressure to afford a crude mixture (0.054 g) that was dissolved into acetone (5 mL). A catalytic amount of *p*-toluenesulfonic acid was added and the reaction was kept at room temperature (18 h). A saturated sodium hydrogencarbonate solution was added to neutrality, the solvent evaporated and the products were purified by column chromatography (neutral alumina grade III, elution with hexane/diethyl ether, 8:2). The acetonide **5** was isolated (0.050 g, 37%) and showed chemico-physical characteristics in agreement with published data[19]; $[\alpha]_D$ -8.2 (*c* 1.67 in chloroform). The ee of the optically active acetonide **5** was established by $^1$H-NMR 500 MHz of the corresponding (S)-MTPA ester.[12] For the racemic acetonide, the signals due to the protons of the methylene group of the acetonide ring consisted of four doublets between 3.575 and 3.755 ppm, whereas in the optically active compound, the four doublets were in a 92:8 ratio.

***Acknowledgements.*** We thank Ministero della Università e Ricerca Scientifica e Tecnologica (MURST) and Consiglio Nazionale delle Ricerche (CNR) for financial support.

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4. Apparently the two strains of *Pseudomonas* are the same and *P. fluorescens* has been only renamed *P. cepacia*.
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(*Received in UK* 31 *January* 1994)