Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 891–899

TETRAHEDRON: *ASYMMETRY*

Desymmetrization of *meso*-cyclopenten-*cis*-1,4-diol to 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate by irreversible transesterification using Chirazyme®

Sandeep R. Ghorpade, Rajendra K. Kharul, Rohini R. Joshi, Uttam R. Kalkote * and T. Ravindranathan *

Division of Organic Chemistry, Technology, National Chemical Laboratory, Pune 411 008, India

Received 7 January 1999; accepted 16 February 1999

Abstract

The parameter optimization study for the desymmetrization of *meso*-cyclopenten-1,4-diol **1** through irreversible transesterification using an immobilized lipase from *Mucor meihei*, i.e., Lipozyme®/Chirazyme® is presented. The enzyme was studied for the transesterification of **1** in various organic solvents by varying reaction parameters such as the nature of acyl donor, temperature, enzyme quantity etc., to afford optically active 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate **2** of >98% enantiomeric excess in >60% yield.

1. Introduction

The importance of 4-(*R*)-hydroxycyclopent-2-en-1-(*S*)-acetate **2** in the synthesis of biologically active cyclopentenoid natural products,[1] e.g., prostaglandins, prostacyclins and thromboxanes, has attracted the attention of synthetic chemists. This has resulted in a variety of approaches in its preparation.[2] Enzyme viz. lipase/esterase-catalyzed desymmetrization of *meso*-cyclopenten-1,4-diol **1** by transesterification,[3] or by enzymatic hydrolysis of its diacetate,[4] or kinetic resolution of monoprotected cyclopentenediol[5] appear to be the preferred methods of choice in the preparation of enantiomerically pure **2**. A kinetic resolution suffers from the drawback of throwing half of the material away, whereas, by using the desymmetrization method, 100% yield can be obtained theoretically. In the case of desymmetrization of **4** through enzymatic hydrolysis, most of the efficient enzymes reported, with the exception of PLE,[4c] have pro-*S* preference. Although high enantiomeric excess and good yields are achievable for the enantiomer of **2** by the hydrolysis method, manipulation of this (4*S*)-hydroxy enantiomer through a few chemical steps is required to get the desired (4*R*)-hydroxy configuration.[6] The same enzymes can catalyze the

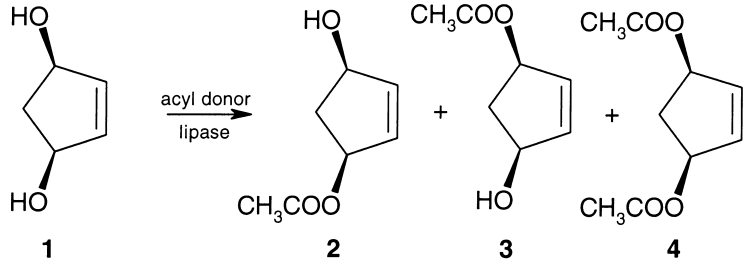
* Corresponding author. E-mail: kalkote@dalton.ncl.res.in

0957-4166/99/$ - see front matter
PII: S0957-4166(99)00068-3

transesterification of **1** to yield **2**. Various enzymes have been attempted for the conversion using a variety of irreversible acyl donors, generally in the THF–Et_3N system.[7] Monoacylated products with a very high enantiomeric purity have been obtained in many cases in 50–60% yield along with remarkable quantities of the diacylated product **4** indicating a low selectivity in the first acylation step which was then compensated for in the second step. Unfortunately, the lipase from the *Mucor* species, which gave the best results (yield 85%, ee >98%),[7c,d] is not available commercially,[3] whereas the lipase from *Mucor meihei*, i.e., Lipozyme IM®/Chirazyme®, which is available commercially in bulk, was found to be inefficient for the conversion (yield <5%) under the conditions attempted by the authors (THF–Et_3N).[7d]

Considering the good market demand for the high-cost intermediate **2**, development of economically viable enzymatic technology for its large-scale preparation has been the major goal of our group. We have already reported on the enantioselective hydrolysis of *meso*-diacetate **4** to **3** using yeast (NCIM 3574).[8]

Enhancement of enzyme efficiency through medium engineering, i.e., optimization of the solvent system and optimization of other parameters such as pH, temperature, etc., has been well reported in several cases.[9] The availability of Chirazyme® in bulk prompted us to consider the study of enhancement in its selectivity towards desymmetrization of **1** by transesterification through medium engineering and optimization of other parameters.



1 **2** **3** **4**

2. Results and discussion

2.1. Effect of solvent variation

The effect of solvent on activity and specificity of various enzymes has been well documented in the literature.[9,10] Attempts have been made to derive correlations between enantioselectivity and physicochemical characteristics of the solvent such as hydrophobicity or dielectric constant etc.[10,11] Initially, we carried out transesterification of *meso*-diol **1** with vinyl acetate in various organic solvents using Chirazyme® as catalyst. The results are indicated in Table 1. Although we cannot conclude on correlation of enzyme efficacy and physicochemical properties of the solvent with available data, the nature of the solvent was found to have a profound effect on enzyme efficacy. In general, enzyme activity was good in all the ether solvents tested. Enzyme efficacy in terms of yield and enantiopurity of product **2** was maximum in diethyl ether (entry 5, yield 45%, enantiomeric excess 88%) and *tert*-butyl methyl ether (TBME) (entry 7, yield 35%, ee 93%). The reaction in ethyl acetate and butyl acetate afforded **2** in high enantiomeric excess, but in lower yields (entries 8 and 13, respectively). In the case of THF and acetonitrile, although the second acylation step was inhibited, the enantioselectivity of the monoacylation step was rather poor. TBME was found to have a potentiating effect on enzyme activity, thus exhibiting fast reaction rates (2 h only) and good enantioselectivity (ee of **2**=93%).

Table 1
Various solvents attempted using vinyl acetate as acyl donor[a]

NO.	Solvent	Time hr	Yield of **2** (%)	$[\alpha]_D$ of **2** [b]	e.e.[c] of **2** (%)	Yield of **4** (%)
1.	-	2	40.0	-55.4	80.0	40
2.	THF	12	20.0	-62.0	89.0	65
3.	Dioxan	10	84.5	-15.5	22.0	-
4.	Dioxan	34	70.0	-26.4	38.0	Traces
5.	Ether	10	45.0	-61.2	88.0	35
6.	Diisoproyl ether	10	32.0	-15.1	23.8	66
7.	TBME	2	35.0	-64.8	93.0	60
8.	Ethyl acetate	12	26.0	-68.0	>98.0	70
9.	Acetone	28	30.0	-54.8	79.0	54
10.	Acetonitrile	60	36.0	-35.0	50.0	Traces
11.	Toluene	No reaction				
12.	Hexane	No reaction				
13.	Butyl acetate	12	22.0	-66.0	95.0	72

a : 1mmol of **1** was reacted with 5 eqv. vinyl acetate & 0.2g enzyme in 5 mL of solvent

b: Lit[7f] $[\alpha]_D$ = -69.3 (c =1, $CHCl_3$) ee > 99%, c: e.e. = enantiomeric excess

2.2. *Effect of an acyl donor*

The effect of an acyl donor on enantioselectivity of the lipase-catalyzed transesterification reaction has been well demonstrated by Ema et al.[12] In order to study the effect of an acyl donor, we carried out the reaction with different acyl donors (Table 2). The reaction proceeded slowly with low enantioselectivity with the acyl donors, except for vinyl acetate. The inefficient reaction with isopropenyl acetate may be attributed to steric reasons. Thus, vinyl acetate was the only suitable acyl donor for the conversion and was used for further parameter optimization.

2.3. *Effect of temperature*

It is widely believed that enzymes, like other catalysts, generally exhibit their highest selectivity at low temperatures. This assumption has been supported by several experimental observations, not only with hydrolases[9] but also with dehydrogenase. Sakai et al., in their lipase PS-catalyzed kinetic resolution studies,[13] examined temperature variation effects ranging from 30 to −60°C on the enantioselectivity of

Table 2
Other acyl donors[a]

No.	Acyl donor	Solvent	Temp	Time	Yield of **2** (%)	$[\alpha]_D$ of **2**	e.e. of **2** (%)	Yield of **4** (%)
1.	Isopropenyl acetate	-	RT	7 days	35.0	-33.3	48	16
2.	Isopropenyl acetate	TBME	RT	6 days	22.5	-42.8	64	10
3.	Isopropenyl acetate	THF	RT	No reaction				
4.	Ethyl acetate	-	RT	48 hr	21.0	-26.6	38	Traces
5.	Butyl acetate	-	RT	24 hr	46.4	-31.6	45	Traces
6.	Isopropyl acetate	-	RT	very slow reaction				
7.	n-Hexyl acetate	-	RT					

a: 1mmol of **1** was reacted with 5 eqv. acyl donor & 0.2g enzyme in 5 mL of solvent & in case of neat reactions 5 mL of acyl donor was used.

the reaction and have found a linear increase in enantioselectivity up to −40°C. Therefore, we studied the reaction at lower temperatures in several solvents (Table 3). Lower temperatures were found to have a very much more beneficial effect on enzyme efficacy in terms of chemical yields and enantiopurity of products. Here again, diethyl ether and TBME turned out to be the best choice, affording **2** of >95% ee in 65% and 52% yield, respectively (entries 3 and 6) at 4°C. Again, reaction rates were much faster in TBME than in any other solvent. Next, we turned our attention to optimization of the ratio of lipase to substrate and ratio of acyl donor to substrate.

2.4. Ratio of enzyme to substrate

Table 4 indicates a surprising result showing that a reduction in enzyme to substrate ratio from 2:1 to 1:1 has a beneficial effect in both solvents, thus affording product **2** of >99% ee in >40% yield for both solvents (entries 2 and 7). But at lower temperatures, the yields and ees dropped in ether with a 1:1 enzyme:substrate ratio (entry 3); a reason may be that the reaction is much slower at the lower temperature with lower amounts of the enzyme, as indicated by lower yields of monoacetate **2** as well as diacetate **4**; whereas reaction in TBME with a 1:1 enzyme:substrate ratio at 4°C afforded **2** of >98% ee in >60% yield (entry 9).

2.5. Ratio of vinyl acetate to substrate

The quantity of vinyl acetate acyl donor was varied from 5 equiv. to 1.5 equiv., using ether and TBME as the solvent with 0.1 g of enzyme. Table 5 indicates that a minimum of 5 equiv. of acyl donor is

Table 3
Variations in temperature[a]

No.	Solvent	Temp (°C)	Time (hr)	Yield of **2** (%)	$[\alpha]_D$ of **2**	e.e. of **2** (%)	Yield of **4** (%)
1.	Ether	RT	10	45	-61.2	88.0	35
2.	Ether	15	15	52	-66.0	95.2	30
3.	Ether	4	24	65	-67.0	96.7	37
4.	TBME	RT	2	35	-64.8	93.0	60
5.	TBME	15	3	46	-68.6	>98.0	40
6.	TBME	4	4	52	>-69.0	>99.0	35
7.	Ethyl acetate	RT	12	26	-68.0	98.0	70
8.	Ethyl acetate	4	22	63	-62.0	89.0	20
9.	THF	RT	12	20	-62.0	89.0	65
10.	THF	10	33	37	-36.5	52.0	62
11.	THF	0	29	67	-37.1	52.0	30

a: 1mmol of **1** was reacted with 5 eqv. vinyl acetate & 0.2g enzyme in 5 mL of solvent

required for an efficient reaction. The reason may be explained as follows. The first acylation, which is the desymmetrization step, would require some excess of acyl donor. The pro-*S* selectivity of the enzyme is not very high; thus **2** and **3** are both formed in unequal quantities in the first step, **2** being the major one. The second acylation is a kinetic resolution where the enzyme is still more selective for the pro-*S*-OH group; therefore, it acylates **3** much faster than **2**, thus enriching the enantiomeric excess of **2**. Thus, to have **2** with a desired enantiomeric excess of >98%, formation of diacetate **4** in a sufficient quantity is required which in turn demands an excess quantity of acyl donor.

Previously, **2** has been prepared by the kinetic resolution of monoprotected cyclopentendiol using pancreatin and Lipozyme IM® in 48% and 37% yield, respectively.[5] Our results are superior to these as we have obtained **2** of >98% ee in 64% yield (entry 9, Table 4); also extra protection–deprotection steps are avoided, thus avoiding further losses in overall yield.

2.6. Effect of various additives

Addition of certain additives such as water, amines, DMF and DMSO in small percentages have been reported to improve selectivity of hydrolytic enzymes in several cases.[14] Especially the intrinsic water content (more precisely, the water activity, a_w) has been found to have an influence on the enzyme

Table 4
Variations in enzyme:substrate ratio[a]

No.	Solvent	Enzyme : substrate ratio	Temp (°C)	Time (hr)	Yield of **2** (%)	$[\alpha]_D$ of **2**	e.e. of **2** (%)	Yield of **4** (%)
1.	Ether	2:1	RT	10.00	45	-61.2	88.0	35
2.	Ether	1:1	RT	10.00	43	>-69.0	>99.0	45
3.	Ether	0.5:1	RT	10.00	51	-59.4	85.7	30
4.	Ether	1:1	15	15.00	40	-62.3	89.8	25
5.	Ether	1:1	4	26.00	52	-51.0	73.6	25
6.	TBME	2:1	RT	2.00	35	-64.8	93.0	35
7.	TBME	1:1	RT	2.00	46	-69.3	>99.0	50
8.	TBME	0.5:1	RT	2.00	43	-61.3	88.4	40
9.	TBME	1:1	4	4.75	64	-68.1	>98.0	28

a: 1mmol of **1** was reacted with 5 eqv. vinyl donor in 5 mL of solvent

Table 5
Variations in ratio of vinyl acetate[a]

No.	Solvent	Equi. of vinyl acetate	Temp ^{0}C	Time (hr)	Yield of **2** (%)	$[\alpha]_D$ of **2**	e.e. of **2** (%)	Yield of **4** (%)
1.	Ether	5.0	RT	10.0	43.0	-69.0	>99.0	45.0
2.	Ether	3.0	RT	12.0	30.0	-40.0	58.0	traces
3.	Ether	1.5	RT	24.0	17.6	-30.0	43.0	traces
4.	TBME	5.0	RT	2.0	46.0	-69.3	>99.0	50.0
5.	TBME	3.0	RT	3.0	46.5	-66.3	95.7	44.3
6.	TBME	1.5	RT	5.5	52.8	-50.0	72.0	28.3

a: 1mmol of **1** was reacted with denoted eqv. vinyl acetate & 0.1g enzyme.

Table 6
Effect of various additives[a]

No.	Solvent	Additive	Quantity of additive (%)	Time	Yield of **2** (%)	$[\alpha]_D$ of **2**	e.e.of **2** (%)	Yield of **4** (%)
1.	THF	Water	1	5 days	20	-24.0	34.6	-
2.	Dioxan	Water	1	5 days	51	-28.0	40.0	-
3.	THF	Et_3N	10	15 hr	50	-43.0	62.0	traces
4.	TBME	Et_3N	10	24 hr	35	-52.0	75.0	traces
5.	TBME	DMF	10	24 hr	31	-43.0	62.0	traces
6.	TBME	DMSO	10	No reac				
7.	TBME	CH_3CN	50	10 hr	45	-43.4	62.0	30
8.	Ether	CH_3CN	50	19 hr	53	-48.7	70.0	27
9.	TBME	Et_3N	20	43 hr	43	-37.0	53.0	traces

a: 1mmol of **1** was reacted with 5 eqv. vinyl acetate & 0.1g enzyme in 5 mL of solvent

selectivity in several cases.[10,14g,15] Our results with various additives in the reaction are presented in Table 6. Unfortunately, none of the additives attempted were found to have any beneficial effect on enzyme efficacy, on the contrary yields and ee had badly deteriorated in most of the cases. The commercial enzyme preparation has a 2–3% moisture content which seems to be optimal in this case. Added extra water was found to be detrimental to the reaction.

3. Conclusion

Desymmetrization of **1** has been demonstrated successfully through irreversible transesterification using Chirazyme® by a parameter optimization approach. Thus *meso*-cyclopentene-1,4-diol was monoacylated with vinyl acetate in the presence of Chirazyme® in TBME at 4°C to afford **2**, an important prostaglandin intermediate in >60% yield with >98% ee. Further studies regarding enzyme recycling and scale-up of the process are in progress. The results indicate a strong possibility of exploitation of Chirazyme® for the development of economically-viable technology for large-scale production of **2**.

4. Experimental

4.1. General

Optical rotations were recorded on a Jasco Dip-181 digital polarimeter using sodium vapor lamp. Enantiomeric excesses (ee) were determined by comparing the specific rotation value $[\alpha]_D$ with the

literature value. All the solvents and reagents were of LR quality and used without further purification. Chirazyme® was obtained as a gift sample from Boehringer Mannheim, Germany.

4.2. Typical example of desymmetrization experiment

In a typical experiment, *meso*-diol **1** (0.1 g, 1 mmol), vinyl acetate (0.430 g, 5 mmol, 0.46 mL) in 5 mL TBME was stirred at 4°C for half an hour. Chirazyme® (0.1 g) was added to the reaction mixture. The mixture was stirred at 4°C for 4.75 h. Filtered solvent was evaporated under reduced pressure. Residue was chromatographed on silica gel (No. 60-120) column using ethyl acetate/petroleum ether as an eluent to separate diacetate **4** (yield=0.051 g, 28%) and monoacetate **2** (yield=0.091 g, 64%; $[\alpha]_D$=−68.1 (*c* 1, $CHCl_3$), ee >98%, lit.[7f] −69.3 (*c* 1, $CHCl_3$), ee >99%).

Acknowledgements

The authors are thankful to Boehringer Mannheim, Germany for the gift sample of Chirazyme®. S.R.G. and R.K.K. are thankful to CSIR, New Delhi for financial support.

References

1. Harre, M.; Raddatz, P.; Walenta, R; Winterfeld E. *Angew. Chem., Int. Ed. Engl*. **1982**, *94*, 480.
2. (a) Ogura, K.; Yamashita, M.; Tsuchihashi, G. *Tetrahedron Lett*. **1976**, *10*, 759. (b) Nara, M.; Terashima, S.; Yamada, S. *Tetrahedron* **1980**, *36*, 3161. (c) Noyori, R.; Tomino, I.; Yamada, M.; Nishizawa, M. *J. Am. Chem. Soc*. **1984**, *106*, 6717. (d) Khanapure, S. P.; Najafi, N.; Manna, S.; Yang, J. J.; Rokach, J. *J. Org. Chem.* **1995**, *60*, 7548. (d) Deardorff, D. R.; Myles, D. C., MacFerrin *Tetrahedron Lett*. **1985**, *26*, 5615. (e) Asami, M. *Tetrahedron Lett*. **1985**, *26*, 5803.
3. Theil, F. *Catalysis Today* **1994**, *22*, 517.
4. (a) Miura, S.; Kurozumi, S.; Toru, T.; Tanaka, T.; Kobayashi, M.; Matsubara, S.; Ishimoto, S. *Tetrahedron* **1976**, *32*, 1893. (b) Takano, S.; Tanigawa, K.; Ogasawaara, K. *Chem. Lett*. **1976**, 189. (c) Wang, Y.-F.; Chen, C.-S.; Gidaukas, G.; Sih, C. J. *J. Am. Chem. Soc*. **1984**, *106*, 3695. (d) Laumen, K.; Schneider, M. *Tetrahedron Lett*. **1984**, *25*, 5875. (e) Laumen, K.; Reimerdes, E. H.; Schneider, M. *Tetrahedron Lett*. **1985**, *26*, 407. (f) Laumen, K.; Schneider, M. *J. Chem. Soc., Chem. Commun*. **1986**, 1298. (g) Deardorff, D. R.; Matthews, A. J.; MacMeeken, D. S.; Cranely, C. L. *Tetrahedron Lett*. **1986**, *27*, 1255. (h) Sugai, T.; Mori, K. *Synthesis* **1988**, 19. (I) Deardorff, D. R.; Windham, C. Q.; Craney, C. L. *Org. Synth*. **1995**, 73.
5. (a) Biadatt, T.; Esker, J. L.; Johnson, C. R. *Tetrahedron: Asymmetry* **1996**, *7*, 2313. (b) Curran, T. T.; Hay, D. A. *Tetrahedron: Asymmetry* **1996**, *7*, 2791.
6. (a) Nokami, J.; Matsuura, H.; Nakasima, K.; Shibata, S. *Chemistry Lett*. **1994**, 1071. (b) Myers, A. G.; Hammond, M.; Wu, Y. *Tetrahedron Lett*. **1996**, *37*, 3083.
7. (a) Theil, F.; Ballschuh, S.; Schick, H.; Haupt, M.; Hafner B.; Schwarz, S. *Synthesis* **1988**, 540. (b) Theil, F.; Schick, H.; Lapitskaya, M. A.; Pivnitsky, K. K. *Liebigs Ann. Chem.* **1991**, 195. (c) Theil, F.; Schick, H.; Weichert, D.; Tannenberger, K.; Ktappach, G. *J. Prakt. Chem*. **1991**, *333*, 497. (d) Theil, F.; Schick, H.; Winter, G.; Reck, G.; *Tetrahedron* **1991**, *47*, 759. (e) Jommi, G.; Orsini, F.; Sisti, M.; Verotta, L. *Gazz. Chim. Ital*. **1988**, *118*, 863. (f) Johnson, C. R.; Bis, S. J. *Tetrahedron Lett*. **1992**, *33*, 7287.
8. Kalkote, U. R.; Joshi, R. R.; Joshi, R. A.; Ravindranathan, T.; Bastawade, K. B.; Gokhale, D. V.; Patil, S. G. *Biotechnology Lett*. **1992**, *14*, 785.
9. Faber, K. *Biotransformations in Organic Chemistry* **1996**, 3rd edn. Graz, Austria.
10. Kvittingen, L. *Tetrahedron* **1994**, *50*, 8253.
11. Fitzpatrick, P. A.; Klibanov, A. M. *J. Am. Chem. Soc*. **1991**, *113*, 3166.
12. Ema, T.; Maeno, S.; Takaya, Y.; Sakai, T.; Utaka, M. *Tetrahedron: Asymmetry* **1996**, *7*, 625.
13. Sakai, T.; Kawabata, I.; Kishimato, T.; Ema, T.; Utaka, M. *J. Org. Chem*. **1997**, *62*, 4906.

14. (a) Bjorklig, F.; Boutelje, J.; Gatenbeck, S.; Hult, K.; Norin, T. *Bioorg. Chem.* **1986**, *14*, 176. (b) Guanti, G.; Banfi, L.; Narisano, E.; Riva, R.; Thea, S. *Tetrahedron Lett.* **1986**, *27*, 4639. (c) Santaniello, E.; Ferrabschi, P.; Grisenti, P.; Aragozzini, F.; Maconi, E. *J. Chem. Soc., Perkin Trans. 1* **1991**, 601. (d) Bjorkling, F.; Boutelje, J.; Gatensenbeck, S.; Halt, K.; Norin, T. *Tetrahedron Lett.* **1985**, *26*, 4957. (e) Guo, Z.-W.; Sih, C. J. *J. Am. Chem. Soc.* **1989**, *111*, 6836. (f) Turner, N. J.; Winterman, J. R., McCague, R.; Parratt, J. S.; Taylor, S. J. C. *Tetrahedron Lett.* **1995**, *36*, 1113. (g) Parker, M.-C.; Brown, S. A.; Robertson, L.; Turner, N. J. *Chem. Commun.* **1998**, 2247.
15. Kitaguchi, H.; Itoh, I.; Ono, M. *Chem. Lett.* **1990**, 1203.